TREATMENT OF ANAEROBICALLY DIGESTED PALM OIL MILL EFFLUENT (POME) USING JERING (*ARCHIDENDRON JIRINGA*) SEEDS AS A NOVEL PLANT-BASED COAGULANT

by

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Abstract

The main aim of this work is to investigate the potential of unmodified plant-based coagulants and flocculants in the treatment of anaerobic POME. The focus of this research is to study jering (*archidendron jiringa*) seeds and their potential as a coagulant in the treatment of anaerobic POME.

The use of synthetic coagulants in the industry has caused detrimental effects to the environment. Therefore, an environmental friendly and cost-effective material is required as a replacement. Since the effectiveness of jering seeds as a coagulant has not been evaluated previously, this study represents the discovery of a plant-based coagulant applicable in anaerobic POME treatment. Using technologies such as SEM, BET, FTIR, Zeta potential and EDX, the characteristics and surface properties of the jering seeds have been successfully identified and compared with the more established coagulant, *moringa oleifera* seeds in order to have a clearer observation on the coagulating properties of jering seeds. Coagulation and flocculation studies were carried out by means of a jar test.

The optimised coagulant dosage of 1.0g/L, flocculant dosage of 0.1g/L, pH of 5, flocculation mixing rate of 20rpm and coagulation mixing rate of 300rpm resulted in a maximum reduction in total suspended solids (TSS) removal (73.3%) and turbidity removal (71.6%). Data selected for all the experiments conducted in this study were determined using the response surface D-optimal model in the Design-Expert software (version 10.0.2, Stat-Ease Inc., Minneapolis, USA).

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Contents

TREATMENT OF ANAEROBICALLY DIGESTED PALM OIL MILL EFFLUENT (POME) USING JERING (*ARCHIDENDRON JIRINGA*) SEEDS AS A NOVEL BIOCOAGULANT I

Abstract **ii**

Acknowledgements **iii**

Contents **iv**

List of Figures **vii**

List of Tables **xi**

Nomenclature **xii**

CHAPTER 1: INTRODUCTION **1**

1.1 Problem Statement 2

1.2 Scope of study 2

1.3 Objectives 4

CHAPTER 2: LITERATURE REVIEW **5**

2.1 Introduction 5

2.2 Characteristics of anaerobic POME 5

2.3 Current palm oil mill effluent (POME) treatments 6

2.4 Coagulation and Flocculation Method 9

2.4.1 Colloids 10

2.4.2 Coagulation 11

2.4.3 Flocculation 12

2.5 Plant-based polymers as coagulants and flocculants 15

2.5.1 Characteristics of plant-based polymers 15

2.5.2 Current applications of plant-based polymers 16

2.5.3 Jering (*Archidendron jiringa*) seeds as coagulant 18

2.5.4 Okra (*Abelmoschus esculentus*) mucilage as flocculant 20

CHAPTER 3: EXPERIMENTAL PLANNING 23

3.1 Introduction 23

3.2 Design of Experiment (DoE) 23

3.2.1 Response Surface Methodology (RSM) 24

3.2.2 D-Optimal Model 25

3.2.2 Experimental Design 28

3.3 Materials preparation 32

3.3.1 Anaerobic POME collection 32

3.3.2 Jering seeds extraction 33

3.3.3 Okra mucilage extraction 33

3.4 Jar test procedure 34

3.5 Measurement methods 36

3.5.1 Chemical oxygen demand (COD) 36

3.5.2 Total suspended solids (TSS) 37

3.5.3 Turbidity 37

3.5.4 pH Measurement 37

CHAPTER 4: RESULTS AND DISCUSSION 39

4.1 Introduction 39

4.2 Optimisation studies 39

4.2.1 Analysis of variance (ANOVA) 41

4.2.2 Model verification 42

4.2.3 Effect of jering seeds coagulant dosage 45

4.2.4 Effect of okra mucilage flocculant dosage 45

4.2.5 Effect of initial pH 46

4.2.6 Effect of flocculation mixing rate 46

4.2.7 Effect of coagulation mixing rate 47

4.3 Response surface methodology (RSM) results 48

4.3.1 Effect of initial pH and coagulation mixing rate 48

4.3.2 Effect of flocculation mixing rate and coagulant dosage 49

4.3.3 Effect of coagulant dosage and flocculant dosage 49
4.4 Analytical tests 50
4.4.1 Fourier Transform Infrared Spectroscopy (FTIR) 50
4.4.2 Scanning Electron Microscopy (SEM) 52
4.4.3 Energy Dispersive X-ray Spectroscopy (EDX) 54
4.4.4 Brunauer-Emmett-Teller (BET) Analysis 54
4.4.5 Zeta potential test 55
4.5 Cost evaluation 56

CHAPTER 5: GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK 58

APPENDIX A: Optimisation results 60

APPENDIX B: SEM Images 68

APPENDIX C: Jar test results 70

APPENDIX D: FTIR results 78

APPENDIX E: Zeta potential test results 80

REFERENCES 801

List of Figures

2.1 Coagulation of colloidal pollutants into microflocs

2.2 Flocculation of coagulated pollutants

2.3 *Archidendron jiringa* (Jering) seeds

2.4 Okra fruit and plant

3.1 Design with Multilevel Qualitative Factors based on Eriksson et al.

3.2 Untreated anaerobic POME, a clear layer of flocs at the bottom of the beaker, treated anaerobic POME.

4.1 Surface morphology of jering seeds

A1 Predicted vs. Actual values plot for final pH

A2 Predicted vs. Actual values plot for COD removal

A3 Predicted vs. Actual values plot for TSS removal

A4 Predicted vs. Actual values plot for turbidity removal

A5 Effects of coagulation mixing rate and initial pH on chemical oxygen demand (COD) removal

A6 Effects of coagulant dosage and flocculation mixing rate on chemical oxygen demand (COD) removal

A7 Effects of flocculant dosage and coagulant dosage on chemical oxygen demand (COD) removal

A8 Effects of coagulation mixing rate and initial pH on total suspended solids (TSS) removal

A9 Effects of coagulant dosage and flocculation mixing rate on total suspended solids (TSS) removal

A10 Effects of flocculant dosage and coagulant dosage on total suspended solids (TSS) removal

A11 Effects of coagulation mixing rate and initial pH on final pH

A12 Effects of coagulant dosage and flocculation mixing rate on final pH

A13 Effects of flocculant dosage and coagulant dosage on final pH

A14 Effects of coagulation mixing rate and initial pH on turbidity removal

A15 Effects of coagulant dosage and flocculation mixing rate on turbidity removal

A16 Effects of flocculant dosage and coagulant dosage on turbidity removal

B1 SEM image [1] of jering seed powder at 10kV and 5000x magnification

B2 SEM image [2] of jering seed powder at 10kV and 5000x magnification

B3 SEM image of jering seed powder at 10kV and 2500x magnification

B4 SEM image of jering seed powder at 10kV and 500x magnification

C1 Effect of coagulant dosage (g/L) on TSS removal (%)

C2 Effect of coagulant dosage (g/L) on turbidity removal (%)

C3 Effect of coagulant dosage (g/L) on final pH

C4 Effect of coagulant dosage (g/L) on COD removal (%)

C5 Effect of flocculant dosage (g/L) on TSS removal (%)

C6 Effect of flocculant dosage (g/L) on turbidity removal (%)

C7 Effect of flocculant dosage (g/L) on final pH

C8 Effect of flocculant dosage (g/L) on COD removal (%)

C9 Effect of initial pH on TSS removal (%)

C10 Effect of initial pH on turbidity removal (%)

C11 Effect of initial pH on final pH

C12 Effect of initial pH on COD removal (%)

C13 Effect of flocculation mixing rate (rpm) on TSS removal (%)

C14 Effect of flocculation mixing rate (rpm) on turbidity removal (%)

C15 Effect of flocculation mixing rate (rpm) on final pH

C16 Effect of flocculation mixing rate (rpm) on COD removal (%)

C17 Effect of coagulation mixing rate (rpm) on TSS removal (%)

C18 Effect of coagulation mixing rate (rpm) on turbidity removal (%)

C19 Effect of coagulation mixing rate (rpm) on final pH

C20 Effect of coagulation mixing rate (rpm) on COD removal (%)

D1 FTIR results for jering seeds

D2 FTIR results for *moringa oleifera* seeds

E1 Zeta potential results for jering seeds

F1 Particle size analysis results for jering seeds

List of Tables

2.1 Characteristics of fresh raw POME, anaerobic POME and the discharge limits by the MPOB

2.2 Advantages and disadvantages between different types of POME treatment

2.3 Comparison of the performance of plant-based materials in industrial wastewater treatment based on TSS and turbidity removals

2.4 Proximate compositions of jering seeds flour

3.1 Minimum number of design runs for optimization designs

3.2 Experimental factors and their respective levels

4.1 Experimental variables and their responses

4.2 ANOVA results for response parameters

4.3 Optimum values of process variables

4.4 Comparison of FTIR spectroscopy results

4.5 EDX Results for jering seeds and *moringa oleifera* seeds

4.6 BET Results on jering seeds and *moringa oleifera* seeds

4.7 Zeta potential values for coagulants

C1 Jar test results from preliminary experiments

Nomenclature

x	coded value of an independent variable
y	responding variable
i	ith independent variable
X	combined input variables
Y	combined responding variables
R^2	coefficient of determination°

Greek symbols

ϵ	experimental error
ΔX_i	value of step change
β	regression coefficient

Abbreviations

POME	Palm Oil Mill Effluent
DoE	Design of Experiment
FTIR	Fourier Transform Infrared Spectroscopy
SEM	Scanning Electron Microscopy
BET	Brunauer-Emmett-Teller
EDX	Energy Dispersive X-ray
3D	Three-dimensional
MPOB	Malaysian Palm Oil Board

CHAPTER 1: INTRODUCTION

Malaysia is the second largest exporter of palm oil in the world next to Indonesia (The Star, 2016). Moreover, for each tonne of crude palm oil that is produced, approximately 5 to 7.5 tonnes of water is required and 50% of the water is discharged as palm oil mill effluent (POME) (Wong, 1980). The POME discharged from the palm oil mills is often treated with a ponding system due to its low cost and simple operating system. However, this leads to river water pollution because of its high organic content. Though aerobic and anaerobic digestion treatments have been introduced to reduce the organic characteristics of POME, it still does not meet the required standards set by the Malaysian Palm Oil Board (MPOB). Alternative systems such as the membrane filtration method have become popular since the emergence of this problem. However, the initial investment required for this system is a large financial burden, especially for small and medium-scale palm oil manufacturers.

Therefore, the focus of this research study is to utilise a novel and plant-based polymer that can be applied in the removal of pollutants in anaerobic POME. The purpose of using plant-based polymeric materials is to reduce the amounts of synthetic chemicals used as well as to reduce the effects of the synthetic polymers on the environment. The mechanism applied to carry out this study is the coagulation-flocculation method. This method is a form of water treatment where polluting particles are combined to form larger groups (flocs), followed by sedimentation. Furthermore, this method has attracted many palm oil manufacturers due to its high removal efficiency and relatively low cost. The effectiveness of the plant-based materials in this method was determined by testing the initial and final pH, suspended solids, turbidity and chemical oxygen demand (COD) content. The results from these tests will then be

compared with other established biocoagulants. For the sake of simplicity, the term 'anaerobically digested POME' will be addressed as 'anaerobic POME' throughout this study.

1.1 Problem Statement

The environmental effects resulting from the effluent of the palm oil mills has caused the industry to be the largest contributor to the pollution loads released into the rivers throughout the country (Azmi & Yunos, 2014). Environmentalists and palm oil mill owners alike are urging the treatment of POME to curb the said problem. However, inorganic coagulants such as alum (aluminium sulphate) and PAC (polyaluminium chloride) are synthetic materials which also contributes to the said pollutions. Alum is the most widely used coagulant in wastewater treatment. These materials are often required in high dosages for effective coagulation due to poor efficiency in attracting organic solids in the wastewater. Consequentially, high levels of aluminium concentrations in the treated water has shown to cause severe detriments to the environment as well as the human health (Bhatia, 2003).

As of February 2016, the benchmark price for crude palm oil (CPO) has increased by 4.5% (The Star, 2016). This is because of the scorching heat weather pattern in Southeast Asia that leads to reduced plant yields and cutting output. Though Malaysia still holds the position for second largest exporter in the world for CPO, local refiners are losing market to the top palm oil producer in the world; Indonesia. This is because Malaysia imposes a CPO export tax at 4.5% to 8.5% according to the purchase quota hence causing most of the world market to choose Indonesia's cheaper prices over Malaysia (The Star, 2016).

This has been an ongoing detriment to the Malaysian palm oil market and local refiners are struggling to keep up with the market

changes. In addition, a detailed cost calculation has also shown that the conventional system of POME treatment, such as the ponding system, is not only the system with the highest environmental pollution and the lowest utilization of renewable resources, but also the system giving rise to the lowest profit (Schuhardt, Wulfert, & Darnoko, 2005). Therefore, local refiners are trying to reduce their overall cost production in order to maintain their profit margin.

As reported by the MPOB in 2014, raw POME has very high concentrations of biochemical oxygen demand (25,000 mg/L), chemical oxygen demand (51,000 mg/L) and suspended solids (18,000 mg/L). Such high concentrations result from the degradable organic matter that retains in the raw POME upon release from the unrecovered palm oil.

The palm oil industry is facing a crucial responsibility to uphold its global status in terms of the environmental protection, economic viability as well as sustainable development. In this context, an environmental friendly coagulant proves to be a viable alternative for treatment of anaerobic POME.

1.2 Scope of study

This research aims to develop a novel and cost-effective plant-based coagulant as a replacement for the synthetic coagulants such as alum and PAC that contributes to the water pollution in rivers. Recent developments have found high protein content vegetable pods and seeds such as *moringa oleifera* seeds and fenugreek seeds to be effective coagulants. The plant-based coagulant researched on for this study are jering (*archidendron jiringa*) seeds which was found to have high protein content, anti-fungal and anti-microbial properties. The aim of this research is to verify the use of jering seeds as a plant-based coagulant in the treatment of anaerobic POME. Two types of plant-based polymers: jering seeds and okra

mucilage are studied, where jering seeds will be applied as the coagulant and okra mucilage as the flocculant. This research will include the study of the optimum conditions for coagulation and flocculation to be carried out using the selected materials by varying the five input variables: initial pH, coagulation mixing rate, flocculation mixing rate, flocculant dosage and coagulation dosage to obtain the optimum values of four responding variables: final pH, COD removal, TSS removal and turbidity removal. Five different types of analytical tests: FTIR, SEM, EDX, BET and Zeta Potential Test will be conducted to understand the elemental and surface characteristics of jering seeds which will further verify the effectiveness of jering seeds as a coagulant.

1.3 Objectives

The main objective of this study is to develop an environmental friendly plant-based coagulant as a replacement for synthetic coagulants in the treatment of anaerobic POME.

The individual objectives for this research are as follows:

i. Develop a novel, cost-effective and plant-based coagulating agent for the treatment of anaerobic POME.
ii. Determine the optimum coagulant and flocculant dosages, pH and mixing rates for jering seeds and okra mucilage that results in maximum removals in total suspended solids (TSS) and turbidity in anaerobic POME; using response surface methodology (RSM) and D-Optimal models as well as analysis of variance (ANOVA) method.
iii. Investigate the characteristics and properties of jering seeds, okra mucilage and POME using FTIR spectroscopy, SEM morphology, BET, EDX and Zeta potential tests in order to understand the coagulation and flocculation properties of the selected materials.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Generally, the palm oil milling process can be categorised into two parts that are the dry and wet milling processes. In Malaysia, the wet milling process is the most extensively applied method to extract crude palm oil (CPO) from the fresh fruit bunches (FFB) (T. Y. Wu, Mohammad, Jahim, & Anuar, 2009). During the wet milling process, half of the water used during the process will end up as palm oil mill effluent (POME), which is produced at nearly threefold the amount of CPO (Shak & Wu, 2014). Moreover, direct discharge of POME into the environment is illegal due to the high concentration of organic content in POME. Therefore, the additional cost is required to treat POME in order to meet the standards set by the Malaysian Palm Oil Board (MPOB) before discharging into the rivers.

2.2 Characteristics of anaerobic POME

The characteristics of anaerobic POME depend on the quality of the raw material and palm oil production processes in palm oil mills. The extraction of CPO from FFB requires large amounts of water. Therefore, POME itself is mainly generated from sterilization of FFB, clarification of the extracted CPO and hydrocyclone separation of the kernel in which large amounts of steam and hot water are used (Sethupathi, 2004).

POME is a highly polluting viscous liquid with fine suspended solids that would cause severe environmental pollution if released into watercourses without prior treatment. The chemical characteristics of POME vary throughout the year depending on mill operations and seasonal cropping (Latif, Ghufran, Wahid, & Ahmad, 2011). Upon fresh release, POME is in the form of a concentrated

brownish liquid with a distinct offensive odour and is characterised by high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) values (Chin, Poh, Tey, Chan, & Chin, 2013). Moreover, POME is acidic in nature, contains high suspended solids and high oil and grease contents (Mohammed & Chong, 2014).

Besides, POME is a non-toxic wastewater. This is because no chemical is added during the oil extraction process (S. Hassan, Kee, & Al-Kayiem, 2013). However, POME is still a threat to the environment due to its large oxygen depleting capability caused by the high organic and nutrient contents. Apart from that, POME also consists of high amounts of suspended solids and dissolved solids resulting from anaerobic digestion.

Parameters	Fresh raw POME	Anaerobic POME	Discharge limits
Moisture (%)	98.21 ± 0.2	94.03 ± 2.3	-
pH	4.33 ± 0.3	7.41 ± 0.2	5-9
Oil and grease (mg/L)	2151 ± 50.1	183 ± 10.1	50
COD (mg/L)	113,191	40,563	-
BOD (mg/L)	35,580	15,580	100
Total suspended solids (mg/L)	18,980	34,720	400

Table 2.1 Characteristics of fresh raw POME, anaerobic POME and the discharge limits by the MPOB (S. Hassan et al., 2013).

In contrast with raw POME, anaerobic POME has slightly different characteristics as demonstrated in Table 2.1. Though anaerobic POME is a significant improvement from fresh raw POME, it still does not meet the required discharge standards.

2.3 Current palm oil mill effluent (POME) treatments

Over the past few decades, new technologies have been surfacing with the aim to improve treatment efficiencies within the palm oil

mills in Malaysia. Among the listed treatment technologies are physicochemical and biological treatments (Vijayaraghavan, Ahmad, & Ezani Bin Abdul Aziz, 2007). In addition, treatment of POME has also been tried using evaporation technology (Ma & Ong, 1985) and a clarification system coupled with filtration and aeration (Larderel, 1994). Apart from that, pre-treatment of POME is commonly carried out to remove the suspended solids and residual oil. This could be done using coagulation and flocculation, solvent extraction, adsorption and membrane separation processes (Abdul Latif Ahmad et al., 2003). The key advantages and disadvantages of the four most commonly applied treatment methods are compared in Table 2.2.

Among the listed treatments for POME, the most frequently used method is the biological treatment or more commonly known as the ponding system which consists of anaerobic, aerobic and facultative processes (Wong, 1980). Furthermore, close to 85% of the total palm oil mills in Malaysia have adopted the ponding system to treat POME due to its low cost and easy maintenance (Ma & Ong, 1985). Anaerobic digestion has been the most widely applied treatment method compared to any other method for POME. This is because anaerobic digestion not only digests the high organic contents in the wastewater but it also generates renewable energy in the form of biogas (Wang et al., 2015).

Following the anaerobic process, the POME is treated aerobically before the final phase in the facultative ponds. In addition, open digesting tanks and facultative ponds are the most commonly employed designs in the biological treatment system because it requires a low capital investment and operational energy (Y. Zhang et al., 2008). However, there are notable drawbacks of the anaerobic digestion treatment method such as long retention time, low treatment efficiency and extensive land area requirement.

Treatment types	Advantages	Disadvantages	References
Coagulation and flocculation	Removes organic colloids, low capital cost, low energy requirements, simple operation and maintenance (does not require complex machinery)	Addition of polymers required	(A. L. Ahmad, Ismail, & Bhatia, 2005; Oladoja, 2015)
Anaerobic	Low energy requirements (no aeration), methane gas production (valuable by-product)	Long retention time, slow start-up, large land area required	(Borja, Banks, & Sánchez, 1996; Metcalf & Eddy, 2003)
Aerobic	Shorter retention time, more effective in handling toxic wastes	High energy requirement (aeration), unsuitable for land applications	(Doble & Kumar, 2005)
Membrane separation	Produce consistent and good water quality after treatment, smaller space required for membrane treatment plants, can disinfect treated water	Short membrane life, membrane fouling, expensive compared to conventional treatment	(Abdul Latif Ahmad et al., 2003; Metcalf & Eddy, 2003)
Evaporation	Solid concentrate from process can be utilized as feed material for fertilizer manufacturing	High energy consumption	(Ma, Tajima, Asahi, & Hanif, 1997)

Table 2.2 Advantages and disadvantages between different types of POME treatment.

Furthermore, the collection of the released biogas (carbon dioxide and methane) for future use is difficult and has a high tendency to escape into the environment in large amounts and cause the greenhouse effect which would lead to global warming. In addition, large quantities of undigested suspended solids settle to the bottom of the ponds and occupy most of the pond space which eventually leads to low treatment efficiencies (Wong, 1980). These detriments are the reasons why the palm oil mills fail to meet the discharge limits and therefore, require further treatment.

2.4 Coagulation and Flocculation Method

Today, coagulation and flocculation method is one of the most widely used wastewater treatment methods and is recognised as a well-established technology. Generally, coagulation and flocculation are two common terminologies that are often interchanged and used to suggest a single process (Ranade & Bhandari, 2014). However, it is important to note that the principal process is coagulation, and flocculation is a default part of the coagulation process as a whole. Coagulation and flocculation is typically employed as a post-treatment or secondary treatment step depending on the nature of the POME.

Coagulation and flocculation : Coagulation and flocculation is a process that can effectively remove soluble, colloidal and suspended particles through induced aggregation of both micro and macro-particulates into larger-sized ones, followed by sedimentation (Oladoja, 2015).

In this case, the coagulation and flocculation method is applied as a post-treatment for anaerobic POME in order to remove pollutants up to the desired levels. This method was selected as the optimum method following the evaluation on the nature of the pollutants, their concentrations, as well as the COD concentrations

of the anaerobic POME. Furthermore, this process does not require complex machinery and zero energy consumption (Wong, 1980).

2.4.1 Colloids

Physicochemical treatment plays an important role in the further treatment of anaerobic POME especially for the recovery of suspended solids. This takes place through the physical liquid-solid separation process. However, before the separation process takes place, the POME suspension has to be broken down in order for the particles to flocculate and form larger aggregates (Ng, Goh, & Tay, 1987).

Colloid: A colloid is a heterogeneous mixture where the particles of a colloid are in the range of 0.001-1μm, representing the range between a molecule and a bacterium (Petrucci, Harwood, Herring, & Madura, 2013).

Unlike in a suspension, the particles in a colloid do not separate into two phases when standing. For colloids, surface properties dominate and cause particles to repel each other. Therefore, the primary objective of is to modify the surface properties through charge neutralization or modification so that they agglomerate to a larger size and are separated from the solution (Ranade & Bhandari, 2014). Through the separation and removal of the pollutants, reduction in colour, turbidity, and COD levels may be observed. A similar research reported results on raw POME that was coagulated with *moringa oleifera* seeds and okra mucilage at a pH of 5 resulting in 87 wt.% sludge recovery (A. L. Ahmad et al., 2005).

Furthermore, the stability of colloids depends on whether the colloidal particles are hydrophilic or hydrophobic. Hydrophilic particles interact strongly with water resulting in a shell of tightly bound water molecules that prevents the particles from aggregating

when they collide (Petrucci et al., 2013). On the other hand, hydrophobic particles are classified as thermodynamically unstable and the dispersion is stabilised by electrostatic repulsion, hence, if enough time is allowed, the particles will aggregate (Ranade & Bhandari, 2014).

In anaerobic POME, we deal primarily with hydrophobic colloids (Ranade & Bhandari, 2014). Therefore, by increasing the kinetics of aggregation, an effective treatment method can be achieved. The removal of hydrophobic colloids in anaerobic POME involves two steps; coagulation followed by flocculation.

2.4.2 Coagulation

Many of the contaminants in anaerobic POME contain matter in the colloidal form which results in a stable suspension such that gravitational forces will not cause precipitation of these colloidal particles (Poh, Ong, Lau, & Chong, 2014). In addition, fine aggregate particles in anaerobic POME naturally have a negative surface charge (Pillai, Ph, & Company, 1997). This is because of the anaerobic digestions that takes places and causes a change in surface charges during the digestion. In order to bring these particles together to form larger flocs, the surface charges need to be neutralized.

Coagulation: Coagulation is the destabilization of colloids (Ranade & Bhandari, 2014). It is a process of charge neutralization and bonding of particles to form microflocs (Pillai et al., 1997).

The coagulation process as a whole represents a physicochemical process where removal of colloidal charged matter takes place through charge modification and charge neutralization (Ranade & Bhandari, 2014).

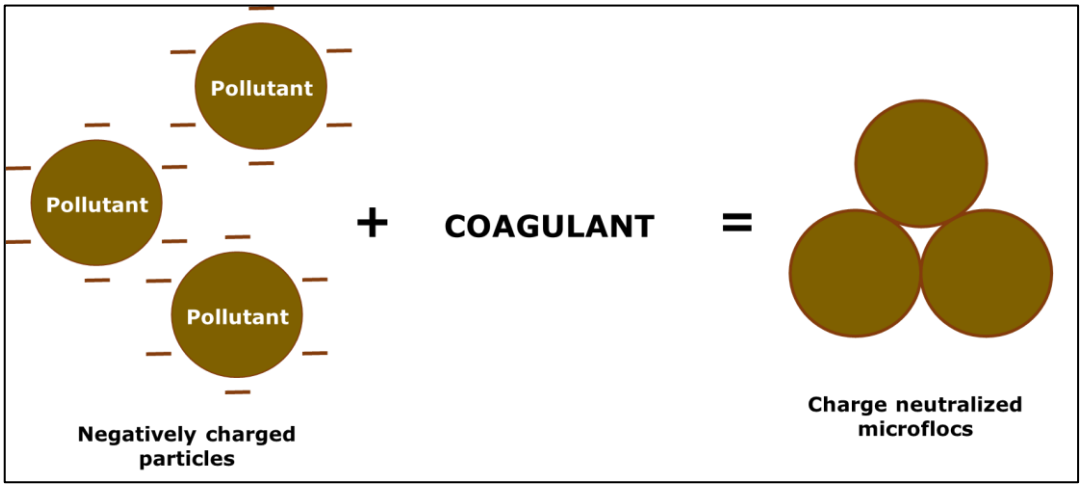


Figure 2.1 Coagulation of colloidal pollutants into microflocs.

As observed in Figure 2.1, charge neutralization is achieved by the addition of a coagulant that neutralizes the negatively charged particles by its positively charged surfaces (Pillai et al., 1997). Following that, the neutralized and coagulated particles are aggregated to larger flocs and are allowed to settle by the addition of a flocculating agent or more commonly known as a flocculant.

There are a few factors that affect the coagulation process such as particle size and surface charge. The smaller the particle size, the larger the total surface area. Therefore, a higher coagulant dosage would be required to coagulate the fine particles. Besides, as the surface charge increases in density, higher coagulant dosage is required for charge neutralization. However, coagulant dosages have to be controlled wisely. This is because an overdose of the coagulant would result in a reverse effect and eventually result in inter-particle repulsion (Pillai et al., 1997).

2.4.3 Flocculation

Generally, the flocculation mechanism in the development of flocs formation could be summarized sequentially as seen in Figure 2.2.

Flocculation: Flocculation is the process of combining charge neutralized microflocs together to allow them to aggregate to a size where they will settle by gravitational forces.

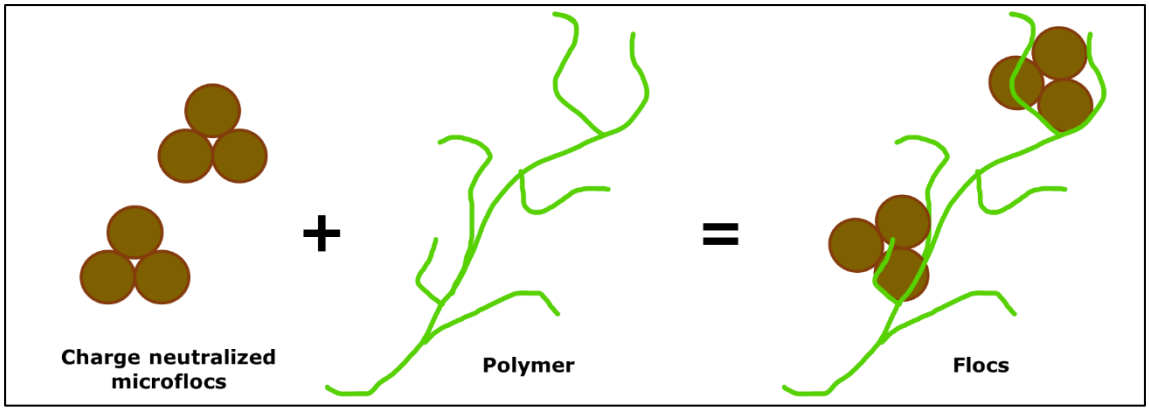


Figure 2.2 Flocculation of coagulated pollutants.

The basic steps in the flocculation mechanism as described by Lee et al. (2014) are as follows:

a) Dispersion of the flocculant in the solution.
b) Diffusion of the flocculant in the solid-liquid interface.
c) Adsorption of the polymer onto the surface of the charge neutralized microflocs.
d) The collision of particles carrying an adsorbed flocculant with other particles.
e) Adsorption of the flocculant onto other particles in order to form microflocs.
f) The growth of the microflocs to larger and stronger flocs by successive collision and adsorption.

There are two types of flocculation processes starting with the perikinetic flocculation followed by orthokinetic flocculation. During perikinetic flocculation, agitation takes place through a plant-based random process (Brownian motion) (Pillai et al., 1997). In addition, perikinetic flocculation occurs immediately after the destabilization and is complete within the duration of a few seconds. This is because the Brownian motion has no effect on the limiting or maximum floc size (Shak & Wu, 2014).

Moving on to the second type, orthokinetic flocculation takes place through induced velocity gradients in the mixture. In this

study, orthokinetic flocculation is applied in all flocculation procedures. Therefore, the velocity gradient may be introduced through mechanical agitation using impeller shafts. The purpose of introducing the velocity gradient is to increase contact between the microflocs and the flocculant (Pillai et al., 1997).

In addition, the intensity of the velocity gradient and time allowed for flocculation are two parameters that influence the rate of particle aggregation (Bhatia, Othman, & Ahmad, 2007). Therefore, the greater the velocity gradients, the higher the contact between particles. However, the greater the velocity gradients, the smaller the floc size. This is due to the continuous breakdown of the larger flocs. Hence, a limiting flocculation time is important for any velocity gradient in order to fix the time beyond which the flocs will not grow any further.

Among the important factors affecting the flocculation process are pH and polymer type. The initial pH of the wastewater plays an important role in the performance of a flocculant. This is because as the pH increases, the required flocculant would have to be highly anionic.

A research study on the performance of nonionic, cationic and anionic flocculants found that nonionic flocculants perform better at lower pH values (acidic). However, anionic flocculants showed very weak activity when tested at low pH values. Apart from that, at pH levels over 9.5, highly anionic flocculants were found to be at the peak of their activity. Besides, polymer type also significantly affects the flocculation efficiency. There are two main functional groups in a flocculant which give the aggregating properties. Those functional groups are the amide group (adsorbing group) and carboxylate group (molecule extension). Therefore, the ideal flocculant would have a balance of these two groups. This is

because the ratio of these two groups determines the degree of ionicity in the polymer.

2.5 Plant-based polymers as coagulants and flocculants

For over 100 years, the use of plant-based coagulants and flocculants has been documented and developed as the primary coagulant and flocculant in water treatment operations (Pillai et al., 1997). Moreover, the advent of green technology has opened a new door to research and development in the utilization of plant-based plant-based polymers as effective coagulants and flocculants in the wastewater treatment industry.

2.5.1 Characteristics of plant-based polymers

In this research, the coagulants and flocculants reviewed are green biobased materials that have the potential to substitute the conventional metal based coagulants in the purification of anaerobic POME. There are a few requirements to meet in the selection of a plant-based polymeric coagulant. Among the more important factors are availability, cost, protein solubility, nature of wastewater and treatment objective.

Coagulant: Coagulants are organic or inorganic chemicals that are used to assist with the removal of colour and turbidity present in untreated, raw water or wastewater (Gebbie, 2006).

Polymeric coagulants: There are 3 types of polymeric coagulants which are non-ionic, cationic and anionic. Anionic and non-ionic polymeric coagulants are polyelectrolytes (Muthuraman, Sasikala, & Prakash, 2013). In addition, plant-based polymeric coagulants are usually polysaccharides or proteins (Hjorth & Jorgensen, 2012).

Flocculant: The function of a flocculant is to bring together coagulated particles into larger aggregates hence allowing sedimentation to take place (Pillai et al., 1997).

Depending on the chemical characteristics, the charge of a flocculant could be anionic, cationic or neutral (Pillai et al., 1997). For coagulation and flocculation processes, a cationic coagulant is normally coupled with neutral or anionic flocculants (Ersoy, 2005). A recent study on the treatment of textile wastewater by (Anastasakis, Kalderis, & Diamadopoulos, 2009) found that a plant-based anionic polysaccharide was found to be a very effective flocculant and capable of suspended solids removals up to 90% and total dissolved solids up to 68%.

2.5.2 Current applications of plant-based polymers

Having discussed the kinetics and mechanisms in the previous section, a discussion on the selection and application of coagulants and flocculants can be done. Along the same objective, a comparison on the performance of the most recent applications of plant-based materials in the treatment of industrial wastewater is summarized in Table 2.3. The data presented is based on TSS and turbidity removals which is a viable representation for the performance of the coagulation and flocculation process.

The advantages of using plant-based and unmodified plant-based coagulants and flocculants are many such as its low cost, it does not affect the pH of the wastewater and it produces a lower sludge volume (Noraini Bunawan, Bunawan, Dusik, & Mat Amin, 2013).

Type of wastewater	Plant-based material	Optimum dosage	TSS removal (%)	Turbidity removal (%)	References
Synthetic turbid water	Algal alginate	0.02 mg/L	-	>98	(Devrimci, Yuksel, & Sanin, 2012)
Concentrated latex effluent	Dragon fruit foliage	-	88.9	99.7	(Idris et al., 2012)
Turbid river water	*J. curcas*	-	-	98.1	(Khodapanah, Ahamad, & Idris, 2013)
Synthetic wastewater	Mallow + $A13^{+}$	12 mg/L	-	96.3-97.4	(Anastasakis et al., 2009)
	Okra + $A13^{+}$	5 mg/L	-	97.3	
Palm oil mill effluent	**Jering seeds**	**1.0 g/L**	**94.9**	**89.0**	**Current study**
Synthetic turbid water	*S. potatorum* seeds	-	-	>95	(Muthuraman et al., 2013)
	P. vulgaris seed	-	-	>85	
Tannery effluent	Fenugreek mucilage	0.08 mg/L	85	-	(A Mishra, Yadav, Agarwal, & Bajpai, 2004)
Synthetic water with kaolin	*Prosopis juliflora*	20-40 mg/L	-	>83.33	(Diaz et al., 1999)
Plant-based turbid water	*Trigonella foenum-graecum* seeds	12 mL/L	-	~81	(Ramamurthy, 2012)
Kaolinite turbid water	*M.oleifera*	32mL/L	-	78	(Tetsuji Okuda, Baes, Nishijima, & Okada, 1999)
Model textile wastewater	Plantago psyllium mucilage	10 mg/L	-	71.4	(Anuradha Mishra & Bajpai, 2005)

Table 2.3 Comparison of the performance of plant-based materials in industrial wastewater treatment based on TSS and turbidity removals.

Apart from that, there are also limitations to be noted such as the release of organic matter and nutrient to the water (T. Okuda, Baes, Nishijima, & Okada, 2001). However, the organic and nutrient release can be avoided by purifying the coagulant component or by removing the released substances from the water (Ghebremichael, Gunaratna, Henriksson, Brumer, & Dalhammar, 2005).

2.5.3 Jering (*Archidendron jiringa*) seeds as coagulant

In Malaysia, *Archidendron jiringa* (Jack) I.C Nielsen, is commonly known as jering seeds and is consumed traditionally throughout the Southeast Asia region for its therapeutic value and blood purification properties (Ong & Norzalina, 1999). *Archidendron jiringa* is also identified by various other names such as *djengkol* in Indonesia, *krakos* in Cambodia and *niang-yai* in Thailand (Sridaran, Karim, & Bhat, 2012).

Furthermore, jering seeds belong to the legume family and they grow in pods (3-9 beans/pod) on the tree which is about 25 to 30m in height and emits a faint sulphurous odour (Barceloux, 2009). In addition, recent pharmacological studies have reported that jering seeds have antimicrobial, antioxidant, anti-gastric and antidiabetic effects (Noraini Bunawan et al., 2013). The appearance of a jering seed is round shaped with a dark brown coloured inner surface and a green coloured edible cotyledon inside as observed in Figure 2.3. In this study, the cotyledon part of the seed is extracted and used as a coagulant in the treatment of anaerobic POME.

Jering seeds are available throughout the year and are sold widely in wet markets throughout Malaysia. Besides, jering seeds have a higher bulk density (0.69 ±0.04 g/ml) compared to other legume seeds such as kidney beans (Kaur, Singh, Sodhi, & Rana, 2009) and soybeans (Oomah, Ward, & Balasubramanian, 2010). The higher the bulk density is beneficial because it allows higher

amounts of extraction for experiments. However, the jering seeds are underutilized because it has only been used for traditional uses and nutritional consumption so far. To date, there are no reports of this species being used as a plant-based coagulant in the treatment of anaerobic POME. Therefore, the primary objective in the selection of jering seeds as a novel biocoagulant is to investigate its physicochemical properties in the treatment of anaerobic POME.



Figure 2.3 *Archidendron Jiringa* (Jering) seeds (O. Hassan, 2007).

A study conducted by (Sridaran et al., 2012) on the functional properties of jering seeds using the proximate composition analysis using a thermogravimetric analyser (TGA) for potential food applications found high moisture and crude protein contents as summarized in Table 2.4.

Among other reasons why jering seeds were chosen is the protein solubility of the seeds. Previous studies have suggested that there is a strong correlation between protein solubility and removal of turbidity and suspended solids in wastewater (Fahmi, Umi Fazara, Hamidin, Abidin, & Hatim, 2014).

Parameter	Percentage (%)
Moisture	58.55 ± 0.58
Crude protein (N x 6.25)	14.19 ± 0.39
Crude fat	1.45 ± 0.24
Crude fibre	1.76 ± 0.10
Ash	0.50 ± 0.06
Carbohydrate	25.67 ± 0.43

Table 2.4 Proximate compositions of jering seeds flour (Sridaran et al., 2012).

Protein solubility is influenced by the hydrophilic or hydrophobic equilibrium, which depends on the amino acid composition especially at the protein surface (Sridaran et al., 2012). The changes that occur in the hydrophilic and hydrophobic equilibrium has a direct effect on the protein solubility due to denaturation (Moure, Sineiro, Domínguez, & Parajó, 2006).

In addition, the protein solubility has been reported to decrease with increasing pH until an isoelectric point is reached at pH 4 (Lawal, Adebowale, & Adebowale, 2007). The increase in protein solubility after pH 4 is because of increased electrostatic repulsion between the molecules. Hence, at pH levels above and below the isoelectric point, the electrostatic repulsion together with the scarcity of hydrophobic residues can cause the protein solubility to be higher (Moure et al., 2006). Therefore, with high protein solubility levels, it is highly probable that jering seeds are an effective coagulating agent in the treatment of anaerobic POME.

2.5.4 Okra (*Abelmoschus esculentus*) mucilage as flocculant

Mucilage and gums are plant-based water soluble polymers (polysaccharides) found in various plants and also in microorganisms (Zannoni, 1989). Furthermore, mucilage is built of sugars and uronic acid units and have the potential to be used in

many pharmaceutical applications such as thickeners, binding agents, and water retention agents (Nayak, Pal, Pradhan, & Ghorai, 2012). In addition, plant-based polymers, mainly polysaccharides have been growing in popularity in industrial wastewater treatment due to its biodegradable, non-toxic, shear stable and wide availability (Agarwal, Rajani, Mishra, & Rai, 2003).

One of the plants which exhibits such properties is the okra plant which produces mucilaginous okra fruit. Besides, a comparison study between plant-based flocculants and conventional flocculants such as Purifloc C-31, Hercofloc and Nalco 610 found that okra mucilage achieved higher turbidity removals (61-74% compared to 50-62% of the conventional flocculants) (Ogedengbe, 1976; Rebhun, Narkis, & Wachs, 1969).

Okra, or scientifically termed as *Abelmoschus esculentus* is a bulky plant widely farmed and produced all over the world especially in Southeast Asia, Ethiopian, and West African countries. Furthermore, okra belongs to the family of *Malvaceae* and is also commonly known by several other vernacular names such as ladies fingers, *kacang bendi*, *bhindi*, *bamia* or *gumbo* (Sangwan, Sngwan, Jalwal, Murti, & Kaushik, 2011).



Figure 2.4 Okra fruit and plant (Kumar, 2014).

Okra is widely consumed as a fresh vegetable in both tropical and non-tropical countries (Camciuc, Deplagne, Vilarem, & Gaset, 1998). In wastewater treatment, okra mucilage is widely applied as a plant-based flocculant. The extraction of the mucilaginous part of the fruit is the primary source of the polysaccharides needed during the flocculation phase in the treatment of anaerobic POME. The main purpose for the addition of flocculants is to increase the efficiency of the separation process through improved floc quality, hence increasing the settling of flocs and pollutant removals (Ranade & Bhandari, 2014).

According to (Karakoltsidis & Constantinides, 1975), the amino acid composition of okra protein is similar to soybean and the protein efficiency ratio is higher than that of soybean. Besides, the optimum flocculation occurs when polyelectrolyte dosages achieve the level needed to neutralise the particle charge or in other words, give a zeta potential close to zero (isoelectric point) (Lee et al., 2014). Therefore, okra mucilage is selected as the plant-based flocculating agent in the treatment of anaerobic POME due to its high polysaccharides content and strong flocculating properties.

CHAPTER 3: EXPERIMENTAL PLANNING

3.1 Introduction

The coagulation and flocculation method is the primary water treatment method applied in the evaluation and treatment of anaerobic POME. However, a performance evaluation on jering seeds as a coagulant must be conducted. The effect of coagulant dosage, flocculant dosage, initial pH and mixing rates on the treatment process must be identified and optimised. Through successful optimisation, the maximum removal efficiencies for COD, suspended solids and turbidity removals can be determined accurately.

3.2 Design of Experiment (DoE)

Traditionally, experimental methods are carried out using the one-variable-at-a-time approach. However, the traditional way is a weak method especially in making connections between the input factors and output responses (Bhatia et al., 2007). Furthermore, the traditional method is more time and energy consuming and the prediction for other conditions is uncertain because the results are valid only under fixed experimental conditions (Robinson, 2000). To solve this problem, the DoE method proves to be a better alternative to study the effect of variables and their responses with a minimum number of experiment runs.

Several papers have reported on the application of DoE in industrial wastewater treatment such as the application of the central composite design (CCD) and response surface methodology (RSM) in the advanced treatment of olive oil mill wastewater using Fenton's peroxidation (Ahmadi, Vahabzadeh, Bonakdarpour, Mofarrah, & Mehranian, 2005), empirical modelling of *Eucalyptus* wood processing (Parajó, Alonso, Lage, & Vázquez, 1992), CCD and

RSM in the optimization study of *moringa oleifera* seeds in POME treatment (Bhatia et al., 2007) and the optimization of ozone treatment for colour and COD removals of acid dye effluent using CCD (Muthukumar, Sargunamani, Selvakumar, & Venkata Rao, 2004).

3.2.1 Response Surface Methodology (RSM)

For the treatment of anaerobic POME, the response surface methodology (RSM) is chosen so that the optimum responses can be achieved with a minimum number of experiments without having to analyse all the possible factor combinations. Besides, the input levels of the different factors for a particular level of response can also be determined.

The objective of this optimization study is to determine the optimum values of the process parameters in the treatment of anaerobic POME using RSM. Therefore, the design of experiment, in this case, is used to study the effects of pH, coagulant dosage, flocculant dosage, coagulation mixing rate and flocculation mixing rate as the process variables on the treatment of anaerobic POME. The RSM model was adopted to optimize the responses of final pH, COD removal, suspended solids removal and turbidity removal against five input factors.

RSM is a collection of statistical and mathematical methods for designing experiments, building models, evaluating the effects of factors and searching for the optimum conditions of the factors (A.L. Ahmad, Chan, Abd Shukor, & Mashitah, 2010). Hence, DOE could be used in POME wastewater treatment with minimum numbers of experimental runs, and to determine the optimum conditions using RSM. In order to determine the relationship between the factors and the response variables investigated, the data collected must be analysed in a statistical manner using

regression (Lazic, 2004). In addition, an estimate of the parameters for the factors is also required. In developing the regression equation, the test factors were coded according to Equation [1].

$$x_i = \frac{X_i - X_i^x}{\Delta X_i} \qquad [1]$$

where x_i is the coded value of the ith independent variable, X_i, the natural value of the ith independent variable, X_i^x the natural value of the ith independent variable at the centre point, and ΔX_i is the value of step change (A. L. Ahmad et al., 2005).

3.2.2 D-Optimal Model

In any optimization design, it is unlikely to design a model that is 100% accurate. However, the right approach in designing a model is to utilize the complexity of the experiment and apply it into an equation (Eriksson, 2000). One of the basic equations is the linear model where the input factors $x_1, x_2, \dots x_n$ influence the response, y as seen in Equation [2] below.

$$y = \beta_0 + \beta_1 x_1 + \cdots + \beta_n x_n + \epsilon \qquad [2]$$

In this model, $\beta_1, \beta_1, \dots, \beta_n$ represents the regression coefficients and ϵ is the random part of the model which is assumed to be normally distributed with mean 0 and variance σ^2 (C. F. J. Wu & Hamada, 2000). Equation [2] can be further extended to accommodate cases where the number of responses is more than one. The number of responses is represented by the symbol N as seen in Equation [3] below.

$$y_i = \beta_0 + \beta_1 x_{i1} + \cdots + \beta_n x_{in} + \epsilon_i, \quad i = 1, \dots, N \qquad [3]$$

where y_i is the ith response with the input factors $x_{i1}, x_{i2}, \dots x_{in}$. Equation [3] can be written in simplified notation that would form Equation [4].

$$Y = X\beta + \epsilon \qquad [4]$$

where the symbol X contains all factors for the responses and Y and ϵ are the symbols for the combined variables from the ith to the Nth variables. In this model, the regression coefficient β is the unknown parameter (C. F. J. Wu & Hamada, 2000).

Apart from the linear model, there are other mathematical models such as the quadratic and interaction models which are widely applied. For the treatment of anaerobic POME, the quadratic model is chosen because it is an extension of the linear and interaction models with additional quadratic terms for each factor (Triefenbach, 2008).

Besides, quadratic models are the most complex among the three basic models which make it a common option for optimization processes because it provides a more accurate optimisation model. A quadratic term is the square of a factor x_i with the regression coefficient, β_{11} (Triefenbach, 2008). Similar to the linear model discussed previously, a quadratic model with five input factors has the following equation [5]:

$$\begin{aligned} y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_{11} x_1^2 + \beta_{22} x_2^2 \\ + \beta_{33} x_3^2 + \beta_{44} x_4^2 + \beta_{55} x_5^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 \\ + \beta_{14} x_1 x_4 + \beta_{15} x_1 x_5 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 \\ + \beta_{25} x_2 x_5 + \beta_{34} x_3 x_4 + \beta_{35} x_3 x_5 + \beta_{45} x_4 x_5 + \epsilon \end{aligned} \qquad [5]$$

An example is shown in Figure 3.1 for an investigation with two qualitative factors (Variables A and B) and one quantitative factor (Variable C). The discrete values for the qualitative factors

are 3 and 4 resulting in a full factorial design of 24 runs to solve the experiment. However, the D-optimal approach reduces this number to only 12 experimental runs (Eriksson, 2000). These experiments are shown in Figure 3.1 as filled circles which clearly demonstrate a balanced design that is equally placed over the whole experimental region.

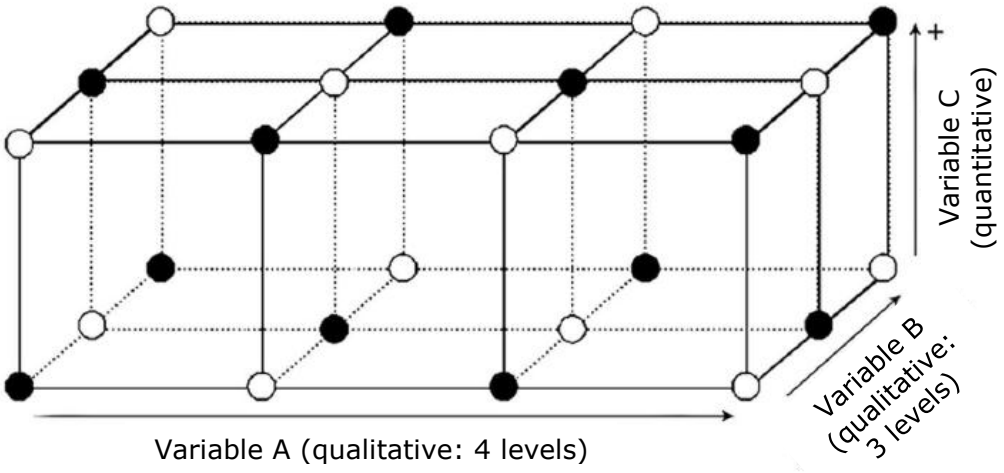


Figure 3.1: Design with Multilevel Qualitative Factors based on Eriksson et al. (2000, p.214, fig. 18.4): *This picture shows the experimental region for a screening investigation with 3 variables. Variables B and A are qualitative and have 3 and 4 discrete levels. Variable C is a normal quantitative factor with values between -1 and +1. The filled circles represent the point chosen by a D-optimal algorithm. A full 4*3*2 factorial design would select all 24 points.*

Since the five input factors have continuous values, the number of designs for a normal run increases drastically. Therefore, the D-optimal approach is adopted to this design to reduce the number of experimental runs at the same time forming a balanced design that includes the whole experimental scope (Triefenbach, 2008).

In comparison to other classical designs such as the full factorial and fractional factorial designs, D-optimal design always

produces a lower number of experimental runs that do not increase rapidly with the growing number of factors as demonstrated in Table 3.1.

Factors	Full factorial	Fractional factorial	D-Optimal
5	32	16	16
6	64	32	28
7	128	64	35
8	256	64	43
9	512	128	52

Table 3.1: Minimum number of design runs for optimization designs (Umetrics, 2006).

Apart from that, the D-optimal design allows modification of the underlying models in various ways. As demonstrated in Equation [6], it is possible to delete selected terms if the data was found to be inadmissible or unimportant for the response (Triefenbach, 2008). Therefore, the number of runs can be successfully reduced without causing a major influence on the overall experimental design.

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_{12} + \cancel{\beta_{13} x_{13}} + \beta_{23} x_{23} + \epsilon \quad [6]$$

where y is the response of the model, β_i is the coefficient of the model, x_i is the single variable in the experiment and ϵ is the experimental error (Eriksson, 2000).

3.2.3 Experimental Design

Response surface methodology (RSM) has been selected to analyse the relationship between the input variables and the output responses. Therefore, the five input variables in this study are initial pH, coagulant dosage (mg/L), flocculant dosage (mg/L), flocculation mixing rate (rpm) and coagulation mixing rate (rpm). The range and levels for these variables are tabulated in Table 3.2. It was

found in preliminary studies that the coagulation and flocculation efficiency decreased when the pH was less than 5 and more than 8. Therefore, the pH range studied in this case was set between 5 and 8. The ranges for the remaining four input variables were determined based on preliminary experiments. The experiments were conducted using the jar test method which is explained in section 3.4 of this chapter. The data for the preliminary experiments can be found in Appendix C, Table C1: Jar test results for preliminary experiments.

Factors	Name	Units	Type	Low Level (-1)	High Level (+1)
A	pH	-	Continuous	5	8
B	Coagulation mixing rate	rpm	Continuous	200	300
C	Flocculation mixing rate	rpm	Continuous	20	90
D	Coagulant dosage	mg/L	Continuous	0.1	1
E	Flocculant dosage	mg/L	Continuous	0	0.1

Table 3.2 Experimental factors and their respective levels.

The basis of selection for these design parameters were determined from the data obtained by conducting the jar test method on varying input factors that were found to provide the best results. From these data, the RSM from the DoE method is utilised to further refine and optimise the preliminary data selection.

From Equations [4] and [5] as discussed in the previous section, the quadratic model could be summarized and written as seen in Equation [7]. In order to determine the optimum values, the quadratic equation model for predicting the optimal conditions can be determined using equation [7].

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{jj} {X_j}^2 + \sum_{i=1}^{k} \beta_{ij} X_i X_j + \cdots + \epsilon \qquad [7]$$

where i is the linear coefficient, j is the quadratic coefficient, β is the regression coefficient, k is the number of factors studied and optimized in the experiment and ϵ is the random error.

A randomized, quadratic, second-order polynomial model indicated that 31 experimental runs are required as presented in Table 3.3. Therefore, the central values (zero level) chosen for the experimental design were pH of 6.5, coagulation mixing rate of 100rpm, flocculation mixing rate of 55rpm, coagulant dosage of 0.55mg/L and flocculant dosage of 0.05mg/L.

Run	A: pH	B: Coagulation mixing rate	C: Flocculation mixing rate	D: Coagulant dosage	E: Flocculant dosage
1	-1.000	1.000	1.000	1.000	-1.000
2	-0.667	0.140	0.114	0.111	1.000
3	-1.000	1.000	-1.000	1.000	1.000
4	1.000	1.000	-1.000	1.000	-1.000
5	-1.000	-1.000	-1.000	-0.333	0.200
6	1.000	1.000	1.000	1.000	1.000
7	-1.000	1.000	1.000	-1.000	1.000
8	1.000	-0.060	0.000	0.111	-1.000
9	-1.000	-1.000	1.000	-1.000	-1.000
10	1.000	-0.060	0.000	0.111	-1.000
11	1.000	-1.000	1.000	1.000	-1.000
12	0.000	0.000	1.000	1.000	0.000
13	-0.667	0.140	0.114	0.111	1.000
14	-0.333	-1.000	-0.286	-1.000	1.000
15	1.000	-1.000	-1.000	1.000	1.000
16	-1.000	-1.000	1.000	1.000	1.000
17	-1.000	-1.000	-1.000	1.000	-1.000
18	1.000	1.000	1.000	-1.000	-1.000
19	-0.333	0.740	0.400	-0.333	-0.400
20	-0.333	0.740	0.400	-0.333	-0.400
21	0.333	1.000	-0.200	1.000	0.200
22	0.333	1.000	-1.000	-0.111	1.000
23	0.333	-0.900	1.000	-0.333	-0.400
24	1.000	-1.000	1.000	-1.000	1.000
25	0.667	-1.000	-1.000	1.000	-0.800
26	1.000	-1.000	-1.000	-1.000	-1.000
27	1.000	1.000	-0.114	-1.000	0.200
28	0.000	0.000	1.000	1.000	0.000
29	-1.000	1.000	-1.000	-1.000	-1.000
30	-1.000	-1.000	-1.000	-0.333	0.400
31	1.000	0.140	-1.000	-1.000	1.000

Table 3.3 Input variables in coded values.

3.3 Materials preparation

There are several aspects that have been standardised throughout the materials preparation procedure. A study by Ramamurthy (Ramamurthy, 2012) found that extraction of coagulants can be done using distilled water, salt solution, buffer solutions or organic solvents. Several studies have reported that the extracts obtained from the use of distilled water as the solvent showed higher values of protein concentration and higher coagulative effects than that of salt solution (Ghebremichael et al., 2005). Therefore, distilled water was used as the solvent in the extraction of jering seeds and okra mucilage. Besides, all procedures were carried out in a chemical laboratory at room temperature (28°C ± 2).

3.3.1 Anaerobic POME collection

Anaerobic POME was sourced from the Seri Ulu Langat Palm Oil Mill in Dengkil, Selangor. The samples were procured at room temperature (28 ± 2 °C) from the first out of the six operating anaerobic ponds at the mill. On a side note, the characteristics of the sample changes depending on the processing facilities of palm oil, climate, season and environmental conditions (Parthasarathy, Mohammed, Fong, Gomes, & Manickam, 2016). In order to minimize the effects of varying characteristics in the sample caused by seasonal changes, all the experiments were conducted on the same batch of POME sample collection to obtain accurate results.

In order to prevent the wastewater from undergoing biodegradation due to microbial action, the samples were preserved at a temperature of 4°C in 10L airtight containers away from sunlight exposure. Prior to using the sample for experiments, the required volume was thawed to room temperature at 28°C. Following that, a portion of the thawed sample was withdrawn and analysed for their initial turbidity, TSS, COD and pH properties.

Samples of 300mL were measured by means of a measuring cylinder before transferring the effluent to 500mL glass beakers.

3.3.2 Jering seeds extraction

Jering seeds were purchased from a local wet market in Semenyih town about 15 minutes away from the University of Nottingham, Malaysia. The seed pods were pried open and the green edible cotyledons were removed from the pods. The whole cotyledons were chopped into smaller pieces of approximately 1-2cm in length and dried in the oven for 14 hours at 60°C. This was done to ensure even drying over the total surface area of the seeds.

Following that, the dried material was blended in a rotor beater mill (Retsch SR-300) at various intensities until a fine powder with a pore size of fewer than 250 microns was obtained. The fine powder was then utilised in the preparation of a 1% stock solution (1g in 100mL distilled water). The solution was shaken vigorously in a tightly capped glass bottle for a duration of 3 minutes to ensure maximum dissolution of the powder in the distilled water. A duration any longer than 3 minutes was found to produce the same effect on the dissolution of the powder in distilled water. Therefore, the optimum duration in this context was determined as 3 minutes. The solution was then filtered using a Filtres Fioroni 601 filter paper to obtain the pure extract. Fresh batches of stock solution were prepared weekly in order to avoid microbial degradation of the coagulant solution.

3.3.3 Okra mucilage extraction

Okra fruits were purchased from a local supermarket in Semenyih town about 10 minutes away from the University of Nottingham, Malaysia. The okra was washed thoroughly with water and chopped into equal-sized pieces of approximately 1 inch in length and the unwanted seeds were removed. The okra pieces were then

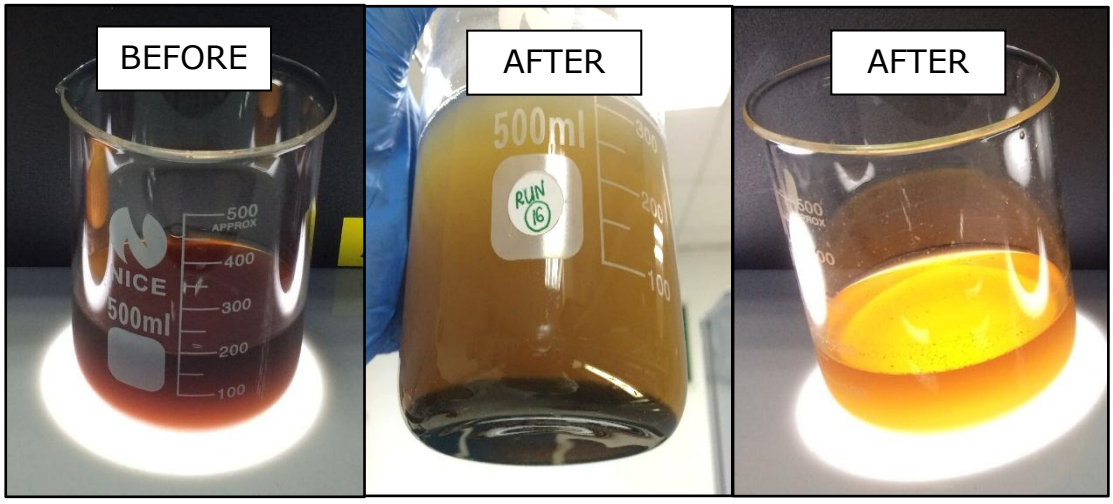
immersed in a beaker of distilled water in a 1:1 ratio of inner okra gum to distilled water which was then left in a cool and dark room for 10 hours to extract the mucilage. The immersion time was decided based on bi-hourly observations on the immersion fluid. It was found that 10 hours was the optimum extraction time for the mucilaginous extract to develop to the desired conditions. A duration longer than 10 hours would result in microbial degradation which is an undesirable outcome. Following that, the mucilage was squeezed through a muslin cloth filter to obtain the pure extract in the form of a sticky liquid. This resulting liquid was applied as a flocculant. Fresh flocculant solution was prepared weekly in order to avoid microbial degradation of the solution.

3.4 Jar test procedure

In physicochemical industrial wastewater treatment (coagulation and flocculation method), jar test is a well-established tool to evaluate the efficiency of the material selection. Therefore, jar test results show the treatment efficiency in terms of suspended solids and organic matter removals (Aragonés-Beltrán, Mendoza-Roca, Bes-Piá, García-Melón, & Parra-Ruiz, 2009). Experiments were carried out using a jar test apparatus, manufactured by Lovibond. The apparatus consists of 6 impeller shafts that rotate simultaneously.

Prior to the jar test procedure, the total suspended solids (TSS) and turbidity of the raw effluent were measured using the HACH DR2800 spectrophotometer and MARTINI Mi415 equipment respectively. 300mL of anaerobic POME were filled into six 500mL beakers and are labelled accordingly. Out of the 6 beakers, one is utilised as the control. The pH values for each beaker are adjusted according to the DoE experimental design simulated by the Design Expert 10.0 software.

Following that, the impeller shafts were lowered into the beakers until the shafts were approximately 1 inch away from the bottom of the beakers. Stirring of the effluent was commenced at coagulation mixing rates according to the DoE experiment design, and simultaneously, the jering seeds extract was carefully added. The rapid mixing was carried out for a total duration of 3 minutes to ensure even distribution of the coagulant extract in the wastewater. This is to provide enough contact time to allow maximum collisions between the suspended particles and the coagulant, hence, effective agglomeration is ensured. Following that, stirring at flocculation mixing rate according to the DoE experiment design was allowed for a duration of 15 minutes. At this stage, the microflocs grew into larger flocs and began to settle. As the flocs become denser, they overcame the buoyancy and drag forces applied by the effluent (Abdul Hamid, Lananan, Khatoon, Jusoh, & Endut, 2016). Therefore, sedimentation began to take place and a layer of flocs was formed at the bottom of the beaker as observed in Figure 3.2.



Figures 3.2 Untreated anaerobic POME, a clear layer of flocs at the bottom of the beaker, treated anaerobic POME.

The settling time was 6 hours for each test. 10mL of the supernatant from each beaker was extracted every hour for 6 hours

using a 12mL capacity syringe from approximately 1cm below the solution surface. The extracted supernatant was then tested for suspended solids and turbidity content using the spectrophotometer. At the 6th hour, the supernatant extracted was tested for the final COD content and final pH reading. Experiments were repeated thrice to obtain the average readings. The resulting data is presented in Table C1 in Appendix C.

3.5 Measurement methods

In order to understand the physical and elemental characteristics of the jering seeds, several characterization tests have been carried out namely, FTIR, SEM, EDX, BET and Zeta potential test. A detailed discussion on the applications of these methods can be found in subsection 4.4 of Chapter 4. Besides, the analytical methods applied to test the four responding variables in this study are as listed below.

3.5.1 Chemical oxygen demand (COD)

In this study, all COD measurements were carried out according to the APHA Standard Method 8000 using the colorimetric method. Following the standard guideline, 2ml of the sample was added into the COD vial (HR 20-1500 mg/L) and digested for 2 hours in the HACH digester (DRB200, Loveland, CO). Following that, the COD concentration reading in mg/L was measured using the HACH spectrophotometer (DR2800, Loveland, CO). The results in mg/L COD are defined as the milligrams of O_2 consumed per litre of sample. The reagent in the COD vials contains silver and mercury ions where silver is a catalyst and mercury is used to complex chloride interferences.

3.5.2 Total suspended solids (TSS)

Similar to the COD measurement, the TSS content in the sample was measured according to the APHA Standard Method 8006 using the same equipment, HACH spectrophotometer (DR2800, Loveland, CO). 10mL of the sample was measured and poured into a square quartz sample cell which was provided along with the equipment. The TSS reading in mg/L was immediately obtained upon inserting the sample cell into the spectrophotometer. This method of determining the suspended solids content is a direct measurement which does not require filtration that common gravimetric procedures do.

3.5.3 Turbidity

The turbidity content in the sample was measured using the absorptiometric method according to the APHA Standard Method 8237. 10mL of the sample was poured into a sample cell and placed in the cell holder in the HACH Colorimeter (DR800, Loveland, CO). Following that, the turbidity reading was obtained in Formazin Attenuation Unit (FAU) unit (1 NTU = 1 FTU = 1 FAU). This turbidity test was measured based on an optical property of the sample which resulted from scattering and absorption of light by particles in the sample.

3.5.4 pH Measurement

The degree of acidity in the sample was measured using the HACH SensION+ Portable Meter (MM110 portable pH/ORP) according to the APHA Standard Method 8156. The probe was inserted into a beaker containing the sample to be tested and the pH reading was obtained. The pH is the hydrogen ion activity in a solution and is defined as $-\log_{10}a(H^+)$, where $a(H^+)$ is the activity of the hydrogen ion. The accuracy of the measurements is dependent on many

factors that are related to the overall system, which includes the meter, the probe, and calibration solutions.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Introduction

The regression and graphical analysis for the optimisation were done using Design-Expert software (version 10.0.2, Stat-Ease Inc., Minneapolis, USA). In order to visualize the relationship between the experimental variables and responses, the response surface and contour plots were generated from the models. Following that, the optimum values of the process variables were obtained from the response surface.

4.2 Optimisation studies

The final suspended solids content, turbidity levels and COD levels, as well as the final pH, were measured for each set of the experimental run. By using multiple regression analysis, 5 variables were correlated with 4 response variables according to the polynomial equation [7] as explained in section 3.2.2. Following that, a simpler understanding on the effect of process variables on the responses is discussed by plotting graphs based on the jar test results to obtain the trend lines. Using Microsoft Excel 2016, 20 graphs were plotted to study the correlation between the 5 input variables and 4 output responses. Table 4.1 shows the coded experimental variables for each experimental run along with their respective responses where A, B, C, D and E where the coded values representing the initial pH (A), coagulation mixing rate (B), flocculation mixing rate (C), coagulant dosage (D) and flocculant dosage (E) are the independent input variables whereas final pH (Y_1), chemical oxygen demand (COD) removals (Y_2), turbidity removals (Y_3) and total suspended solids (TSS) removals (Y_4) are the responding variables. A similar table containing the actual values can be found in Table C1 in Appendix C.

Run	Experimental variables					Responses			
	A	**B**	**C**	**D**	**E**	Y_1	Y_2	Y_3	Y_4
1	-1	1	1	1	-1	5.9	1.6	56.9	68.0
2	-1	0	0	0	1	6.3	-4.4	70.0	77.4
3	-1	1	-1	1	1	5.8	1.7	83.4	82.6
4	1	1	-1	1	-1	7.9	13.2	-6.3	18.4
5	-1	-1	-1	0	0	5.0	39.2	42.7	57.4
6	1	1	1	1	1	7.7	0.5	55.3	35.7
7	-1	1	1	-1	1	5.5	1.1	89.0	53.6
8	1	0	0	0	-1	8.0	-3.7	4.9	18.5
9	-1	-1	1	-1	-1	5.8	29.3	17.0	34.9
10	1	0	0	0	-1	8.1	7.4	4.90	30.4
11	1	-1	1	1	-1	8.1	-4.0	0.0	23.7
12	0	0	1	1	0	6.7	0	18.7	99.0
13	-1	0	0	0	1	6.4	-0.2	73.8	67.5
14	0	-1	0	-1	1	6.3	-12.3	0.0	1.6
15	1	-1	-1	1	1	7.5	-3.3	30.2	19.1
16	-1	-1	1	1	1	5.5	40.1	49.9	71.1
17	-1	-1	-1	1	-1	5.3	32.2	7.6	35.5
18	1	1	1	-1	-1	8.1	1.3	0.0	21.7
19	0	1	0	0	0	6.1	23.7	16.9	34.2
20	0	1	0	0	0	6.1	14.8	6.0	30.7
21	0	1	0	1	0	7.2	15.2	0.0	5.4
22	0	1	-1	0	1	7.5	0.1	53.0	43.8
23	0	-1	1	0	0	7.1	9.4	0.0	5.1
24	1	-1	1	-1	1	8.1	20.1	0.0	12.8
25	1	-1	-1	1	-1	7.2	9.6	14.3	20.5
26	1	-1	-1	-1	-1	7.7	50.5	0.0	5.2
27	1	1	0	-1	0	8.0	15.8	0.0	9.7
28	0	0	1	1	0	6.8	12.3	14.9	94.9
29	-1	1	-1	-1	-1	6.0	5.0	8.6	22.5
30	-1	-1	-1	0	0	5.2	7.8	42.5	39.2
31	1	0	-1	-1	1	7.8	13.4	0.0	11.0

Table 4.1 Experimental variables and their responses.

4.2.1 Analysis of variance (ANOVA)

The analysis of variance (ANOVA) method was chiefly developed by R.A. Fisher (R. A. Fisher, 1925; S. R. A. Fisher et al., 1960), who introduced the term 'analysis of variance' in the world of statistics.

> **Analysis of variance (ANOVA):** A statistical technique for analysing measurements depending on several kinds of effects operating simultaneously, in order to decide which effects are important and to estimate those effects (Bolton, 1997).

Another report defines ANOVA as a graphical analysis of the experimental data in order to obtain the connection between the process variables and the responses (Ghafari, Aziz, Isa, & Zinatizadeh, 2009). Among the features of ANOVA used in this study is the ability to determine how well the polynomial model fits the experimental design by analysing the coefficient of determination, R^2. Besides, the statistical significance can also be evaluated by analysing Fisher's *F*-test in the software. Apart from that, the *P*-value, or probability, was used to analyse the model terms with 95% confidence intervals.

Table 4.2 illustrates the ANOVA for the response surface quadratic model. In this table, the data shows that all the models were significant at the 5% confidence level since *P* values were less than 0.05 where values greater than 0.1 would indicate that the model terms are not significant. The statistical analysis gives several comparative measures for the model selection. Based on high R^2 statistics, the quadratic model was selected as the best for all variables. The desirable R^2 values are the higher values (close to 1) and a reasonable agreement with the adjusted R^2 values is also necessary (Nordin, Venkatesh, Sharif, Elting, & Abdullah, 2004).

Response	*P*	R^2	Adj. R^2	AP
pH	<0.0001	0.9860	0.9580	17.966
COD	0.0425	0.8732	0.5915	8.109
TSS	0.0004	0.9716	0.9006	13.122
Turbidity	0.0003	0.9543	0.8629	12.494

Table 4.2 ANOVA results for response parameters.

Where *P* is the probability of error, R^2 is the coefficient of determination, Adj. R^2 is the adjusted coefficient of determination and AP is adequate precision. The adequate precision (AP) feature is a comparison of the predicted values from the design point of the actual values from experiments. Furthermore, ratios greater than 4 indicate adequate model discrimination (John & John, 1971). According to Table 4.2, the AP values fit this criterion. In this case, the high R^2 values represent a satisfactory application of the quadratic model to the experimental data. This is also evident in Figures A1 to A4 which is a clear plot of the predicted values versus experimental data. In each plot, the experiment data are close to the $y = x$ line which shows the satisfactory performance of the model.

4.2.2 Model verification

In order to verify the model selected, 5 additional runs were conducted to obtain the values of the four response variables using the numerical optimization method in the Design Expert software based on the models selected. Following that, a set of solutions was generated by the software to determine the optimised conditions of the process. This set of solutions for the optimised conditions were repeated 5 times to obtain the average results. The optimised conditions were determined from preliminary experiments and D-optimal model calculation as presented in sections 3.2.2. The

experimental data obtained were compared with the predicted values from the model as tabulated in Table 4.3.

Run	Input variables					Responding variables							
	Initial pH	Coagulation mixing rate (rpm)	Flocculation mixing rate (rpm)	Coagulant dosage (g/L)	Flocculant dosage (g/L)	COD removal (%)		Final pH		Turbidity removal (%)		TSS removal (%)	
						EV	PV	EV	PV	EV	PV	EV	PV
1	5	300	20	1.0	0.1	0.07	0.15	5.8	5.9	83.4	84.9	82.6	80.8
2	5	300	20	1.0	0.1	1.06	0.15	5.9	5.9	56.9	84.9	68.0	80.8
3	5	300	20	1.0	0.1	0.16	0.15	5.9	5.9	73.8	84.9	67.5	80.8
4	5	300	20	1.0	0.1	0.12	0.15	5.3	5.9	54.9	84.9	94.9	80.8
5	5	300	20	1.0	0.1	1.12	0.15	5.5	5.9	89.0	84.9	53.6	80.8
Average						0.51	0.15	5.7	5.9	71.6	84.9	73.3	80.8

Table 4.3 Optimum values of process variables.

Where **EV** is the experimental values and **PV** is the predicted values.

Based on the 5 runs conducted, the EV values are close to the PV values with a difference of 12.4% error for four responding variables. The error is within an acceptable range taking into consideration the varying initial conditions of the POME sample as explained in section 2.2. The average results for the responding variables optimum values of the process variables were: COD removal value, **0.51%**; final pH, **5.7**; turbidity removal, **71.6%** and TSS removal value was **73.3%** at the optimum conditions.

4.2.3 Effect of jering seeds coagulant dosage

Figures C1 to C4 are graphical representations of the effects of jering seeds coagulant dosage on the TSS removal, turbidity removal, final pH and COD removal. From these plots, four different trends can be observed. The TSS removals are shown to increase gradually within the coagulant dosage range of 0.1g/L to 1.0g/L reaching its peak at 94.9% removal when the coagulant dosage was 1.0g/L (Figure C1). Turbidity removals followed an identical trend to that of TSS removal. The turbidity removal values were shown to increase gradually from 17.0% to 89.0% with increasing coagulant dosages (Figure C2). Besides, the final pH values and COD removal values showed a slight increase proportional to the increase in coagulant dosage within a final pH range of 5.5 to 6.5 and a maximum COD removal of 4.4% (Figures C3 and C4). Hence, we notice that there is a significant effect of jering seeds coagulant dosage on the removals of TSS and turbidity but a less significant effect on the final pH and COD removal.

4.2.4 Effect of okra mucilage flocculant dosage

Figures C5 to C8 are graphical representations of the effects of okra mucilage flocculant dosage on the TSS removal, turbidity removal, final pH and COD removal. The TSS removals is shown to increase gradually within the flocculant dosage range of 0.0g/L to 0.1g/L. The maximum TSS removal achieved was 89% when the flocculant dosage was 0.1g/L (Figure C5). Similar to the TSS removal, the turbidity removal values were shown to increase gradually from 17.0% to 83.4% with increasing flocculant dosages (Figure C6). However, the final pH values were found to be decreasing with increasing flocculant dosages within a final pH range of 5.0 and 7.2. Besides, COD removal values showed a steady increase proportional to the increase in flocculant dosage with the highest peak at 4.4%

(Figures C7 and C8). Hence, we conclude that the flocculant dosage has an effect on the removals of TSS and turbidity as well as the final pH but a weaker effect on the COD removal.

4.2.5 Effect of initial pH

Figures C9 to C12 are graphical representations of the effects of initial pH on the TSS removal, turbidity removal, final pH and COD removal. Unlike the two variables discussed previously, the TSS removals is shown to decrease gradually within the initial pH range of 5 to 8 with its lowest removal at 14.3% when the initial pH was 7.5 (Figure C9). Turbidity removals followed an identical trend to that of TSS removal. The turbidity removal values were shown to decrease consistently from 83.4% to 14.3% with increasing initial pH values (Figure C10). On the other hand, the final pH values were found to increase proportionally within a range of 5.8 to 7.5 with the increasing initial pH values (Figure C11). The COD removal values showed a decrease with increasing initial pH values and maximum COD removal of 4.4% at a pH of 5.5 (Figure C12). In conclusion, the coagulation and flocculation process using jering seeds as the coagulant works best in acidic conditions within a pH range of 5 to 6.

4.2.6 Effect of flocculation mixing rate

Figures C13 to C16 are graphical representations of the effects of flocculation mixing rate on the TSS removal, turbidity removal, final pH and COD removal. The flocculation mixing rate is carried out during the flocculation phase of the experiment. The trend for TSS removal showed a decreasing trend line with increasing speeds with the lowest removal at 10.7% when the speed was at 45rpm (Figure C13). Turbidity removals followed an identical trend to that of TSS removal. The turbidity removal values were shown to decrease from 83.4% to 16.9% with increasing speeds within a range of 20rpm to

90rpm (Figure C14). Besides, the final pH values were found to decrease slightly within a range of 6.6 to 5.1 with increasing speeds, however, this may be due to the varying initial pH conditions (Figure C15). The COD removal values showed a sharp decrease with the highest removal of 1.06% at a speed of 20rpm (Figure C16). Based on the trends observed, the slower the flocculation mixing rate, the better the removal efficiencies. This is because flocculation processes require a limiting mixing rate with low velocity gradients for maximum floc formations. The flocculation mixing rate was found to have little to no effect on the final pH values.

4.2.7 Effect of coagulation mixing rate

Figures C17 to C20 are graphical representations of the effects of coagulation mixing rate on the TSS removal, turbidity removal, final pH and COD removal. The coagulation mixing rate is carried out during the coagulation phase of the experiment. The trends observed in this case are the opposite of the trends discussed previously for the effect of flocculation mixing rates. TSS removal showed an increasing trend line with increasing speeds with the highest removal at 94.9% when the speed was at 250rpm (Figure C17). Turbidity removal also showed increasing values within a range of 7.6% to 83.4% at increasing speeds within a range of 200rpm to 300rpm (Figure C18). Besides, the final pH values were found to increase slightly within a range of 5.3 to 6.8 with increasing speeds, however, this may be due to the varying initial pH conditions (Figure C19). The COD removal values showed a slight increase with the highest removal of 1.06% at a speed of 300rpm (Figure C20). The coagulation mixing rate has little to no effect on the final pH values.

4.3 Response surface methodology (RSM) results

The graphical representations of the model from Equation [7] based on the effects of the experimental variables on the responses are demonstrated through 3D surface graphs and contour plots which were obtained using the Design-Expert software and are presented in Figures A5 to A16. These figures are a clear representation of the responses of different combinations of the five experimental variables and the interactions between them (D. Wu et al., 2002). To have a better understanding of the relationship between variables and the responses, the 3D surface graphs will be analysed in pairs through comparison.

4.3.1 Effect of initial pH and coagulation mixing rate

Based on the response surface plots in Figures A5, Figure A8, Figure A11 and Figure A14, a correlation between the initial pH (variable A) and coagulation mixing rate (variable B) with the four responding variables can be observed. From the graphs, a conclusion can be made that as the initial pH increases, the final pH increases rapidly as evident in the steep curvature on the surface graph in Figure A11. This is a desirable outcome because it proves that the pH is not greatly altered during the treatment process but instead, the pH is more dependent on the initial pH that is fixed prior to the treatment process. Apart from that, COD, TSS, and turbidity removals also increased with the increase in initial pH. Though not as rapid as the final pH, the curvatures on Figures A5, A8 and A14 were still visible and significant.

The coagulation mixing rate does not affect the responding variables as much as the initial pH. This is evident through the planar surfaces on the surface graphs in all four graphs. The planar surface represents the minor changes or effects the coagulation mixing rate has on the COD, TSS and turbidity removals as well as

the final pH. This is a desirable outcome because the coagulation proves does not depend as much on the velocity gradient but more dependent on the coagulant dosage which is important especially for the coagulation of fine particles.

4.3.2 Effect of flocculation mixing rate and coagulant dosage

Based on the response surface plots in Figures A6, Figure A9, Figure A12 and Figure A15, a correlation between the flocculation mixing rate (variable B) and coagulant dosage (variable C) with the four responding variables can be observed. A trend can be observed in the effect of the flocculation mixing rates on all the responding variables. From the curvature on the surface graphs towards higher values of COD, TSS, and turbidity removal values, a conclusion can be drawn that the removals significantly improved when the flocculation mixing rates were at lower values. This is because lower velocity gradients would prevent the continuous breakdown of flocs which is not a desirable outcome. Only the final pH was not affected by the changes in the flocculation mixing rate as evident in the flat, planar surface graph with no curvature.

Compared to the flocculation mixing rate, the coagulant dosage had an even stronger impact on the responding variables as noticed in the higher curvatures of the graphs with increasing coagulant dosages in Figures A6, A9 and A15. However, the coagulant dosage does not have an effect on the final pH as evident in the flat, planar surface graph with no curvature.

4.3.3 Effect of coagulant dosage and flocculant dosage

Based on the response surface plots in Figures A7, Figure A10, Figure A13 and Figure A16, a correlation between the coagulant dosage (variable D) and flocculant dosage (variable E) with the four responding variables can be observed. A comparison between these two variables shows greatly pronounced curvatures in Figures A10

and A16 where TSS and turbidity removals were shown to increase with increasing coagulant and flocculant dosages. On the other hand, Figure A13 shows that the flocculant dosage and coagulant dosage had little interaction with the initial pH.

4.4 Analytical tests

The characteristics of jering seeds were identified to further verify their coagulative properties. The elemental characteristics were determined using FTIR spectroscopy and EDX whereas the surface properties of jering seeds were determined using SEM morphology, BET and Zeta potential test.

4.4.1 Fourier Transform Infrared Spectroscopy (FTIR)

In this study, FTIR spectroscopy is used for the characterisation of jering seeds by determining the functional groups that might be involved in the coagulation and flocculation mechanism, mainly protein content. The FTIR spectroscopy was carried out using the Perkin Elmer RX1 FT-IR Imaging System. The sample pellet was prepared using the potassium bromide (KBr) pellet method which is the most commonly utilised measurement method.

Jering seeds extract were mixed together with KBr powder, finely pulverised and placed into a pellet-forming die to form 13mm-diameter pellets for FTIR scanning. Table 4.4 shows the FTIR results between the wavelength of 400-4000cm^{-1} for jering seeds compared with the more established biocoagulant, *moringa oleifera* seeds. To understand the mechanism of each functional group, a description of each functional group present in the material is listed down.

Material	Wavenumber(cm^{-1})	Bond present
Jering seeds	3283.85	O-H bond in carboxylic acids
	2927.97	C-H (asymmetric stretching)
	2854.66	C-H (symmetric stretching)
	1642.46	C=O
	1239.60	C-N
	1053.15	C-C, C-O, C-N
M. oleifera seeds	3344.20	O-H bond in carboxylic acids
	2925.04	C-H (asymmetric stretching)
	2854.36	C-H (symmetric stretching)
	2373.06	C≡C, C≡N
	1654.52	C=O
	1236.45	C-N
	1058.66	C-C, C-O, C-N

Table 4.4 Comparison of FTIR spectroscopy results.

- O-H bond in carboxylic acids

Region: 3650-3200cm^{-1}

An elongated U shape at this region represents the stretching of OH bonds present in proteins, fatty acids, carbohydrates and lignin units. Due to the high content of protein present in a seed, there is also a contribution in this region from N-H stretching of the amide bond.

- C-H (asymmetric stretching)

Region: 2960-2850cm^{-1}

Sharp peaks that occur in a small range that corresponding to asymmetric and symmetric stretching of the C-H bond of the CH_2 group. Due to the high intensity, it is possible to assign them to the lipid component of the seed, which is present in a high proportion similar to that of protein.

- C=O bond

Region: 1750-1630cm^{-1}

A strong peak which can be attributed to C=O stretching. Due to the heterogeneous nature of a seed, the carbonyl group may be bonded to different neighbourhoods as part of the fatty acids of the lipid portion or amides of the protein portion.

- C-C and C-N bond

Region: 1000-1200cm^{-1}

A sharp peak which corresponds to either the C-N bond or cyclic C-C bond stretching. This bond is found in the ring structure of protein molecules.

4.4.2 Scanning Electron Microscopy (SEM)

The scanning electron microscope is a powerful instrument which is utilised in the observation and characterisation of heterogeneous organic and inorganic materials and surfaces (Hayat & others, 1974). In this study, SEM is conducted on jering seeds to determine their surface properties and correlate the finding to the efficiency in the coagulation and flocculation mechanisms.

Among the advantages of using SEM imaging compared to the conventional light microscope is greater magnification, an increase in depth of field and higher resolution (Weber Jr, Pirbazari, & Melson, 1978). However, the most notable contribution of SEM to this study is its three-dimensional imaging which is an invaluable contribution to the study of the topography and structural properties of jering seeds.

Jering seeds extracts were scattered on adhesive carbon disks, which were then mounted on aluminium microstubs and examined by varying the magnification and electron voltage, the

physical surface morphology of jering seeds were obtained as seen in Figures 4.1 and 4.2 which reveal bean-shaped, heterogeneous microporous structures, and rough surface properties on jering seeds.

Primarily, there are two types of particle shapes; spherical and fibrous (Haider & Levenspiel, 1989). For jering seeds, the particle shapes fall under the spherical category with a bean-shaped microstructure. Through these images, we have a clear perception of the finely structured details especially the porous surface of the sample which is an accurate representation of high permeability and therefore, evident for coagulating and flocculating properties (X. Zhang et al., 2015).

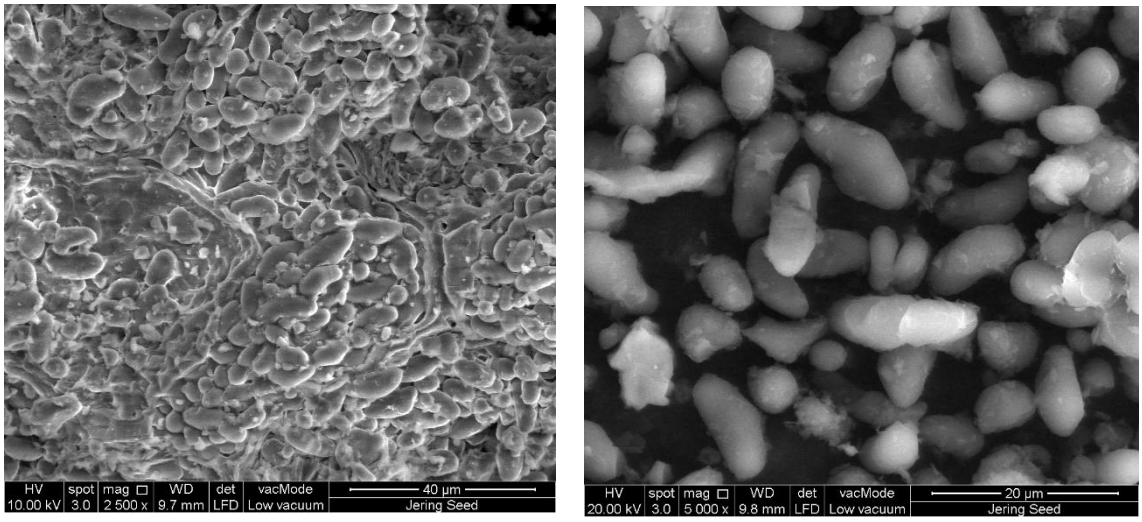


Figure 4.1 Surface morphology of jering seeds.

A study on the porosity and structure of alum coagulation by Gorczyca (2000) reported that porosity is the basis for floc permeability. A microporous structure on jering seeds was observed and proved to improve floc permeability and aggregation which meets the requirements of a coagulating agent.

4.4.3 Energy Dispersive X-ray Spectroscopy (EDX)

Obtained simultaneously with SEM, EDX is used for the elemental analysis as well as the chemical characterization of the sample. The working principle behind this is that each element has a unique atomic structure, thus EDX allows the elemental composition of jering seeds to be measured. Table 4.5 shows the comparison between the elemental composition of jering seeds and *moringa oleifera* seeds as obtained from the EDX spectroscopy.

From the EDX results, it is evident that carbon and oxygen in jering seeds are the highest amounts in weight and atomic percentage similar to that of *moringa oleifera* seeds.

Jering seeds			***Moringa oleifera* seeds**		
Element	Wt.%	At.%	Element	Wt.%	At.%
C	52.80	60.33	C	57.77	68.44
O	45.39	38.93	O	30.57	27.19
K	1.38	0.59	K	2.57	0.94
Ca	0.42	0.15	Ca	7.07	2.51

Table 4.5 EDX Results for jering seeds and *moringa oleifera* seeds.

A study conducted by Bello et al. (2015) reported that high amounts of carbon improve flocs formation. This is because the carbon element is a binding agent in the formation of microflocs. Therefore, it can be deduced that jering seeds have the elemental characteristics required for the coagulation and flocculation process.

4.4.4 Brunauer-Emmett-Teller (BET) Analysis

The BET analysis is mainly used to explain the physical adsorption of gas molecules on a solid surface and by doing so, provides an analysis technique for the measurement of the specific surface area of a material (den Berg, 1985). In this study, the BET analysis was carried out to analyse the surface area and adsorption properties of jering seeds. Using the ASAP 2020 V4.02 equipment and exposed

the sample in nitrogen gas for 3 hours to allow for adsorption, a comparison between the surface area for jering seeds and *moringa oleifera* seeds were determined as tabulated in Table 4.6.

Area	**Jering seeds**	***Moringa oleifera* seeds**
Single point surface area	0.1765 m^2/g	0.0293 m^2/g
BET Surface area	0.1575 ± 0.03 m^2/g	-

Table 4.6 BET results on jering seeds and *moringa oleifera* seeds.

The BET theory is an extension of the Langmuir theory and the surface area of the molecules is a function of the volume of gas adsorbed. In order to understand the surface area values of jering seeds, a correlation can be made between the surface area and the SEM imaging on the jering seeds. The more irregular and porous the structures would lead to a significantly higher BET surface area (Mohammed & Chong, 2014). In addition, the higher surface area values of the jering seeds in comparison to *moringa oleifera* seeds verifies the effective adsorption properties of jering seeds.

4.4.5 Zeta potential test

Zeta potential is a controlling parameter of coagulation and flocculation. The particles in a colloidal suspension such as anaerobic POME usually carry an electrical charge (Colloidal Dynamics, 1999). Zeta potential is a measure of the magnitude of the electrostatic or charge repulsion/attraction between particles, and is one of the fundamental parameters known to affect stability (Malvern Instruments Ltd., 2014). Using the Malvern Zetasizer Nano ZS90 equipment, a zeta potential analysis on the jering seeds powder was carried out three times to obtain an average reading. Table 4.7 shows a comparison between the zeta potential values of jering seeds, okra mucilage and fenugreek seeds.

Reading	Zeta potential values (mV)		
	Fenugreek seeds (Anastasakis et al., 2009)	**Jering seeds**	**Okra mucilage**
1	-25.2	-22.1	106.1
2	-25.7	-20.5	103.5
3	-24.5	-19.9	103.7
Average	-25.1	-20.9	104.8

Table 4.7 Zeta potential values for coagulants.

A study conducted by Li et al. (2006) on the characterisation of flocs reported that higher zeta potential values signify higher charges, hence, results in faster and denser floc formation which explains the highly positive values observed in okra mucilage. The negative charges of the jering seeds is a desirable outcome because the lower surface charge indicates that a low coagulant dosage is required for coagulation to take place. Therefore, the potential of jering seeds as a coagulant and okra mucilage as a flocculant can be verified.

4.5 Cost evaluation

Among the main advantages of using plant-based polymers as coagulants in anaerobic POME treatment is that they are cost-effective. Though the effectiveness of plant-based coagulants in wastewater treatment is undoubtedly successful, from a commercialization point of view, it is still primarily an issue of whether the scale-up system of the lab-scale study could sustain and maintain a similar treatment performance at a reduced cost compared to the existing systems which use conventional coagulants. Studying and comparing the full cost difference between both systems would be a challenging task due to the different processing stages as well as the fluctuating prices in the world palm oil market. However, a general cost analysis can be conducted on the existing information.

For example, in Malaysia, the purchase price for 20 pieces of jering seeds cost approximately RM4 on average. This would then result in 0.3kg of pure jering seeds extract (Kaur et al., 2009). Therefore, the purchase price to produce 1kg of jering seed extract (67 seeds) would cost approximately RM13.40. In comparison, the cultivation cost to produce 1kg of *moringa oleifera* extract (3400 seeds) is approximately RM7.80 (Katayon et al., 2006).

However, it is important to note that different countries have different cost-effectiveness. For example, in Malawi, the purchase price for moringa oleifera costs only MK 75 (=RM0.41) per 1000 m^3 wastewater treated compared to the cost of alum which costs MK 501 (=RM2.86) per 1000 m^3 wastewater treated (Sutherland et al., 1994). Though the cost evaluation was done based on *moringa oleifera* seeds, a study by Yin (2010) reported that it can be used as a baseline in the costing for other plant-based coagulants in general. In conclusion, the cost evaluation evidently shows that the utilization of plant-based polymers for the treatment of industrial wastewater is a low-cost option.

CHAPTER 5: GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

Plant-based polymers as coagulant and flocculant in the coagulation and flocculation method for the treatment of POME is highly effective for the removal of harmful pollutants in colloidal forms. The goal of this research was to verify the use of jering seeds as a novel biocoagulant in the treatment of anaerobic POME.

In this study, two types of plant-based polymers: jering seeds and okra mucilage were studied, where jering seeds were studied as a novel biocoagulant and was selected based on its polymeric properties and protein solubility.

Using the response surface methodology (RSM), an experimental design was utilised to determine the effects of initial pH, coagulation mixing rate (rpm), flocculation mixing rate (rpm), flocculant dosage (g/L) and coagulation dosage (g/L) to obtain the optimum values of four responding variables: final pH, COD removal (%), TSS removal (%) and turbidity removal (%). The compatibility of the model was verified effectively using the analysis of variance (ANOVA) feature, 3D surface graphs, and contour plots as well as verification of the experimental data by comparing predicted values with actual values. This produced the optimised coagulant dosage of 1.0g/L, flocculant dosage of 0.1g/L, pH of 5, flocculation mixing rate of 20rpm and coagulation mixing rate of 300rpm which resulted in a maximum reduction in total suspended solids (TSS) and turbidity (82.6% and 83.4% respectively).

Five different types of analytical tests: FTIR, SEM, EDX, BET and Zeta Potential Test were conducted to understand the elemental and surface characteristics of jering seeds. Results from these tests further confirmed the effectiveness of jering seeds as a coagulant in the treatment of anaerobic POME. Some of the notable

findings from these tests were the functional groups detected in the FTIR test that proved the existence of protein bonds in jering seeds. Furthermore, EDX results showed high amounts of carbon in the elemental composition of jering seeds which is essential in flocs formation. BET and SEM results, on the other hand, showed microporous and rough surface structures as well as a high surface area of the jering seeds.

Several measures can be taken to further improve the effectiveness of using jering seeds in POME treatment. Firstly, the adsorption of pollutants onto the polymers could be improvised through adsorption equilibrium and kinetics modelling in order to fit the optimised adsorption criteria during the flocculation process, hence, improve flocs formation.

Furthermore, extraction methods could be improved by applying sonication extraction method due to its high reproducibility by increasing the extraction efficiency and also reduce extraction time (Melecchi et al., 2006). Optimisation studies could also be conducted on the sonication extraction method by varying the extraction time, sample amount, sample volume and sample particle size (Mierzwa, Sun, & Yang, 1997).

Besides, the chemical characteristics of POME vary throughout the year depending on mill operations and seasonal cropping (Latif et al., 2011). In order to obtain a more consistent reading, the experimental study should be conducted on a single batch of POME and analysed according to the specific characteristics of that particular batch of POME. More importantly, periodic research could be done on the effluent to better understand the varying output properties and ways to standardize the POME characteristics.

APPENDIX A: Optimisation results

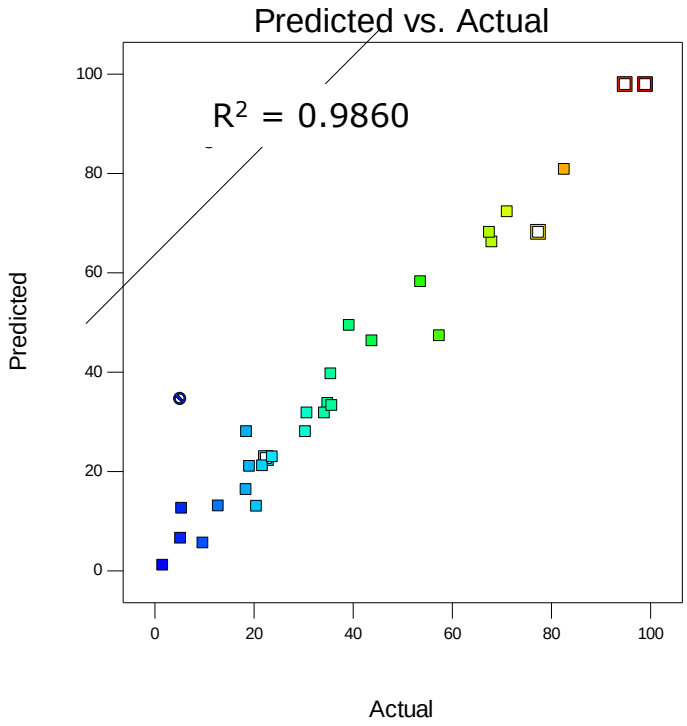


Figure A1: Predicted vs. Actual values plot for final pH.

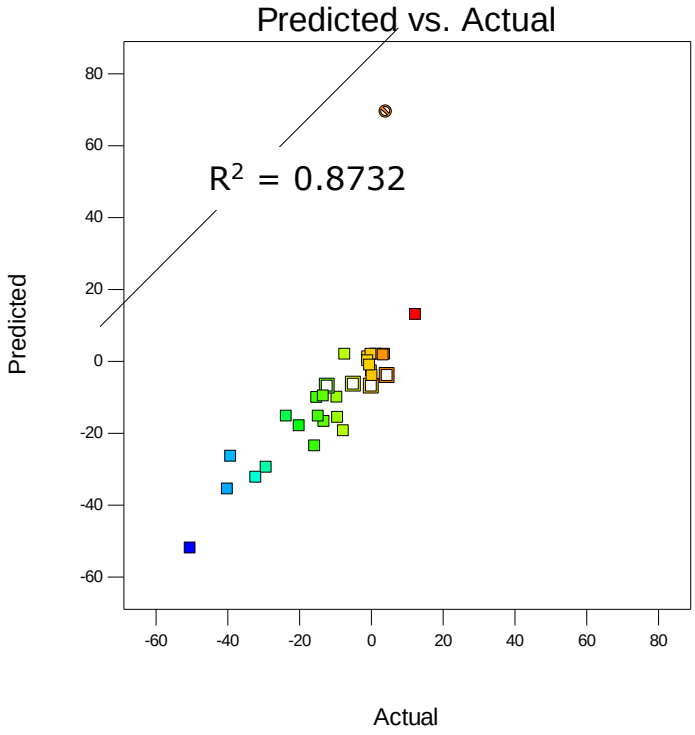


Figure A2: Predicted vs. Actual values plot for COD removal.

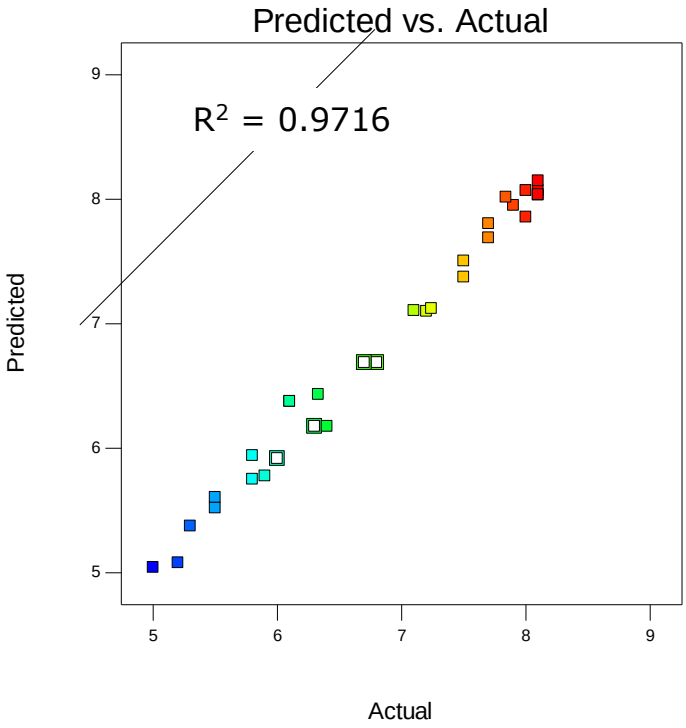


Figure A3: Predicted vs. Actual values plot for TSS removal.

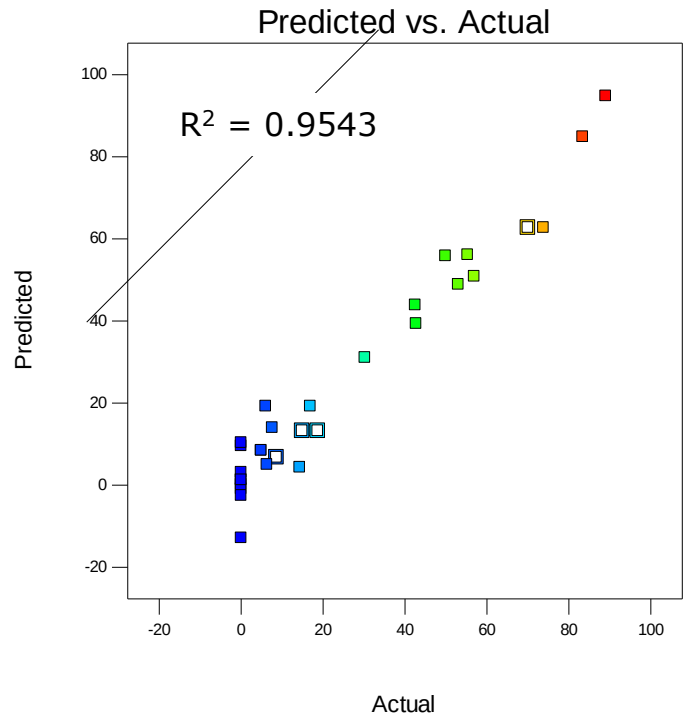


Figure A4: Predicted vs. Actual values plot for turbidity removal.

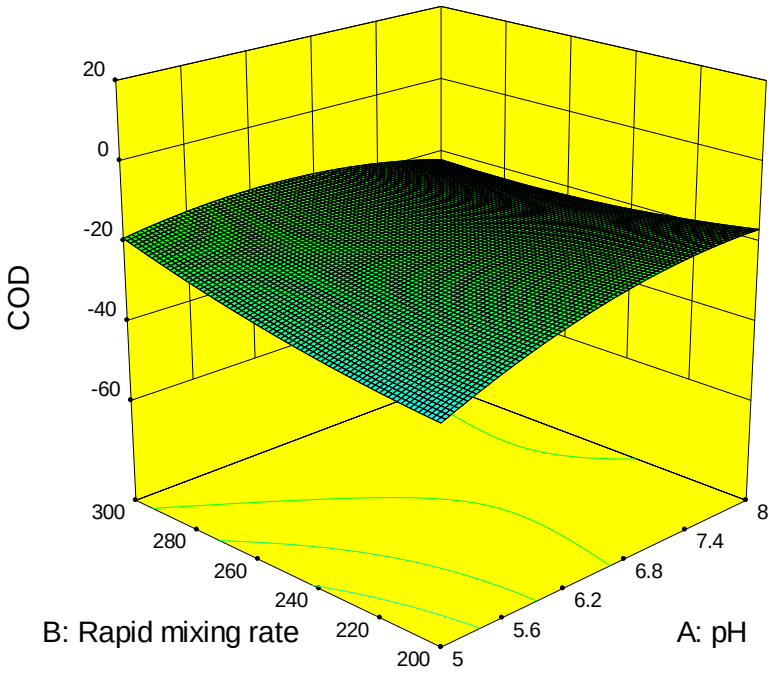


Figure A5: Effects of coagulation mixing rate and initial pH on chemical oxygen demand (COD) removal; 3D surface graph and contour plot.

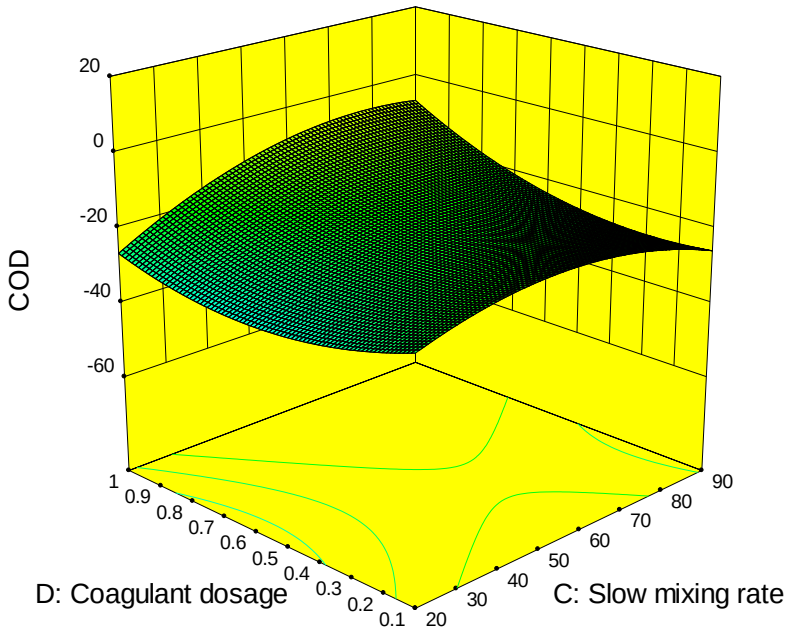


Figure A6: Effects of coagulant dosage and flocculation mixing rate on chemical oxygen demand (COD) removal; 3D surface graph and contour plot.

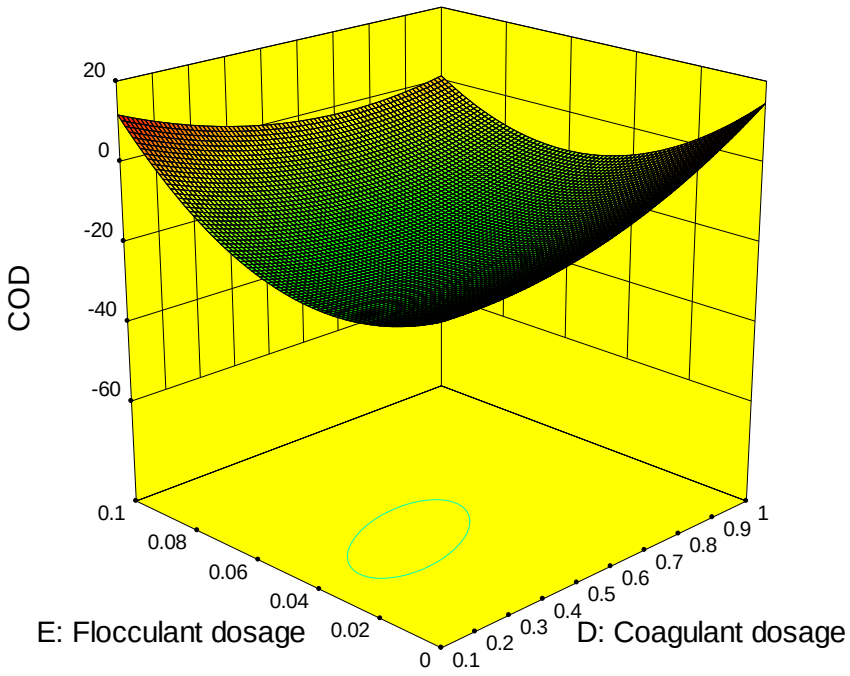


Figure A7: Effects of flocculant dosage and coagulant dosage on chemical oxygen demand (COD) removal; 3D surface graph and contour plot.

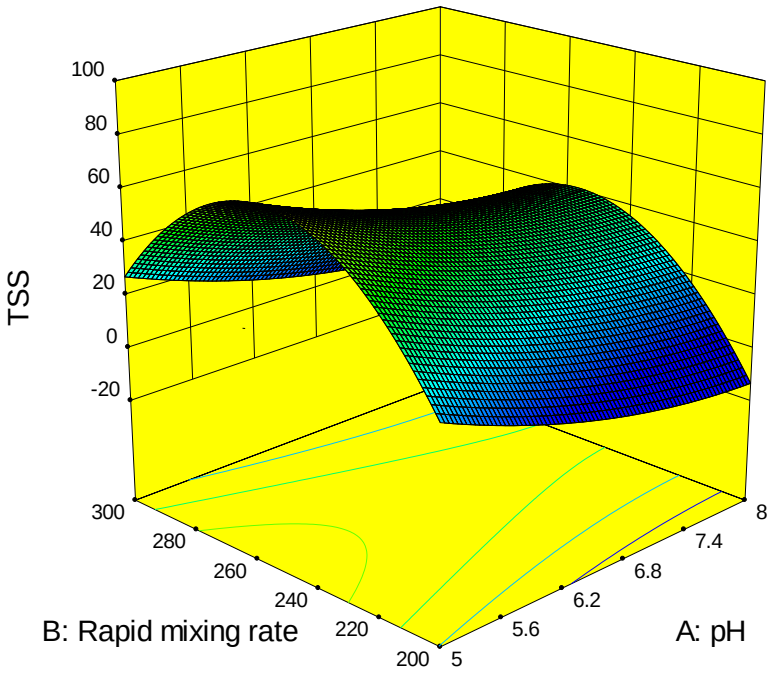


Figure A8: Effects of coagulation mixing rate and initial pH on total suspended solids (TSS) removal; 3D surface graph and contour plot.

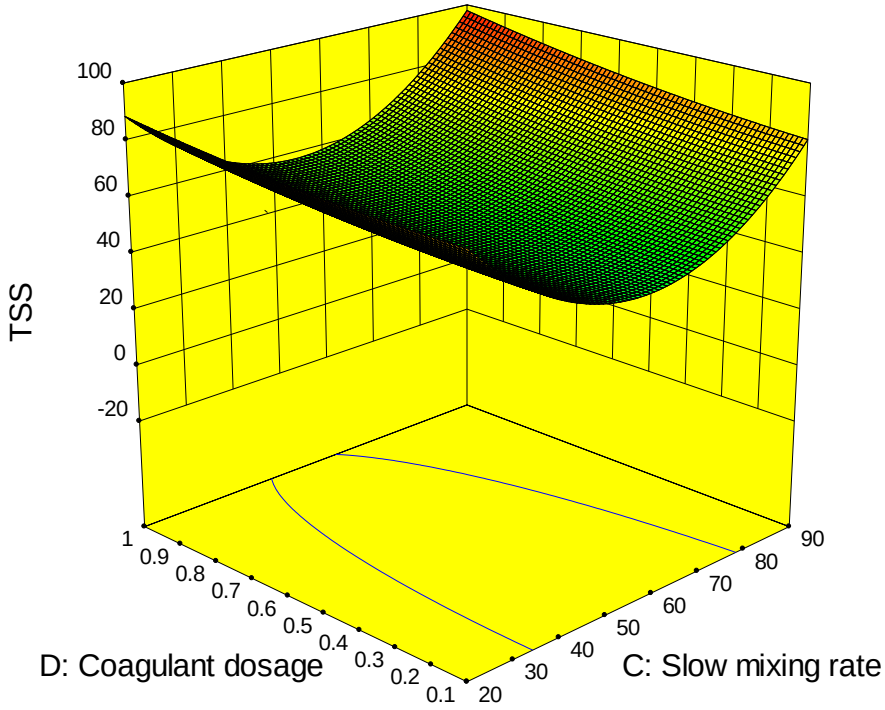


Figure A9: Effects of coagulant dosage and flocculation mixing rate on total suspended solids (TSS); 3D surface graph and contour plot.

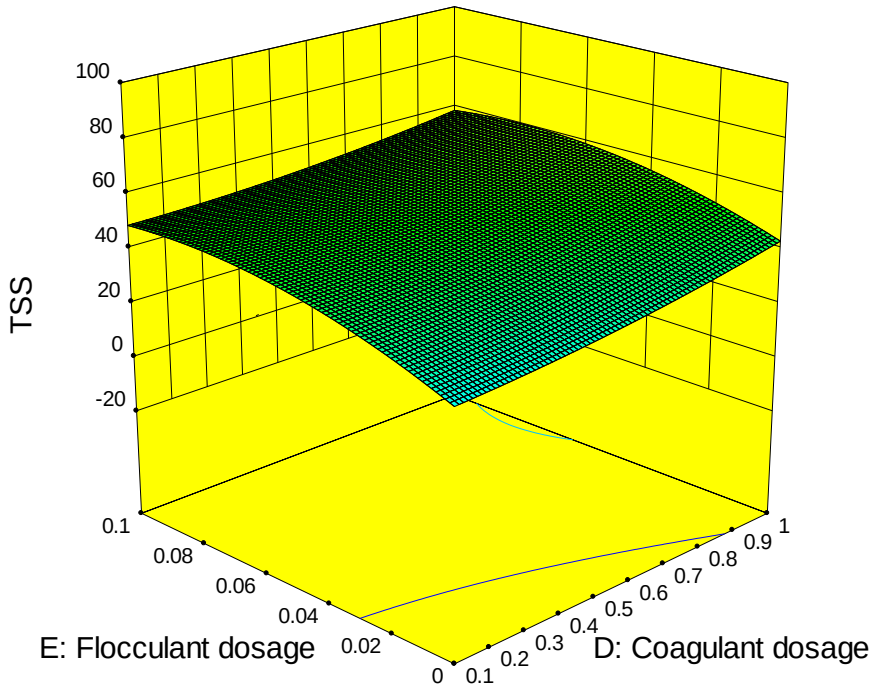


Figure A10: Effects of flocculant dosage and coagulant dosage on total suspended solids (TSS); 3D surface graph and contour plot.

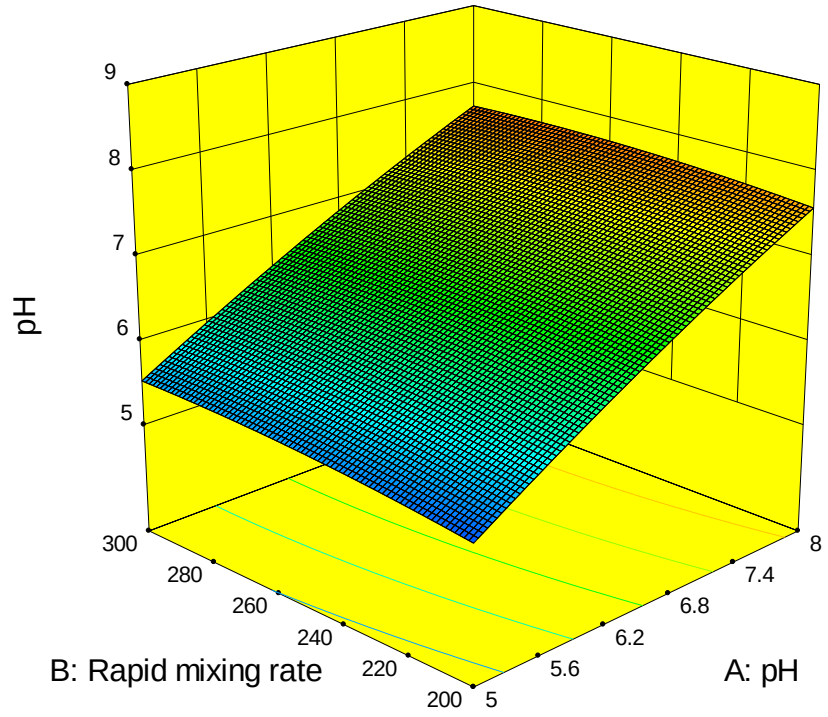


Figure A11: Effects of coagulation mixing rate and initial pH on final pH; 3D surface graph and contour plot.

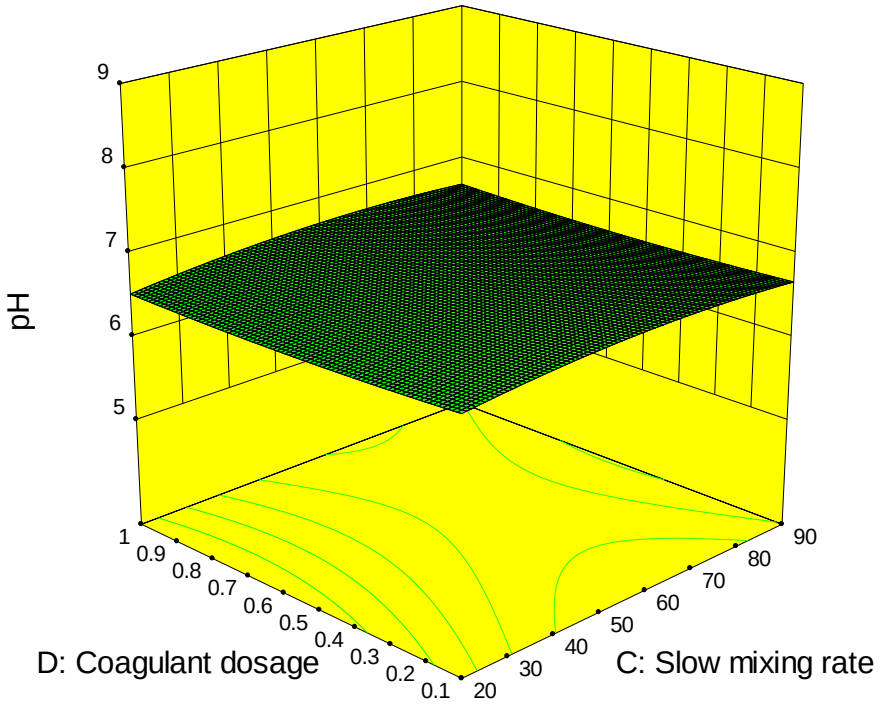


Figure A12: Effects of coagulant dosage and flocculation mixing rate on final pH; 3D surface graph and contour plot.

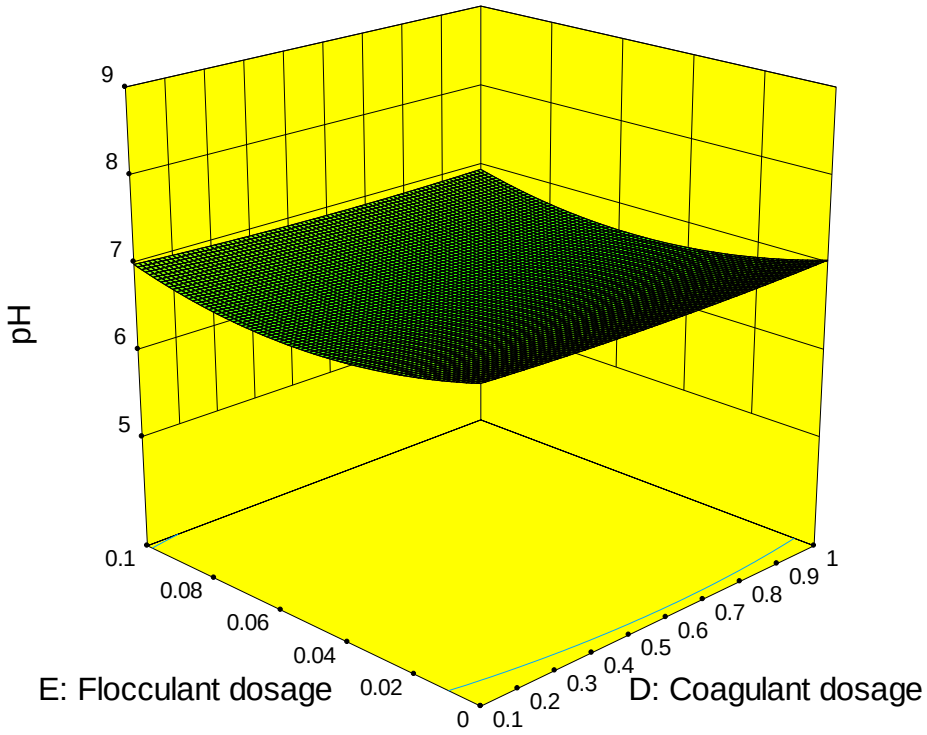


Figure A13: Effects of flocculant dosage and coagulant dosage on final pH; 3D surface graph and contour plot.

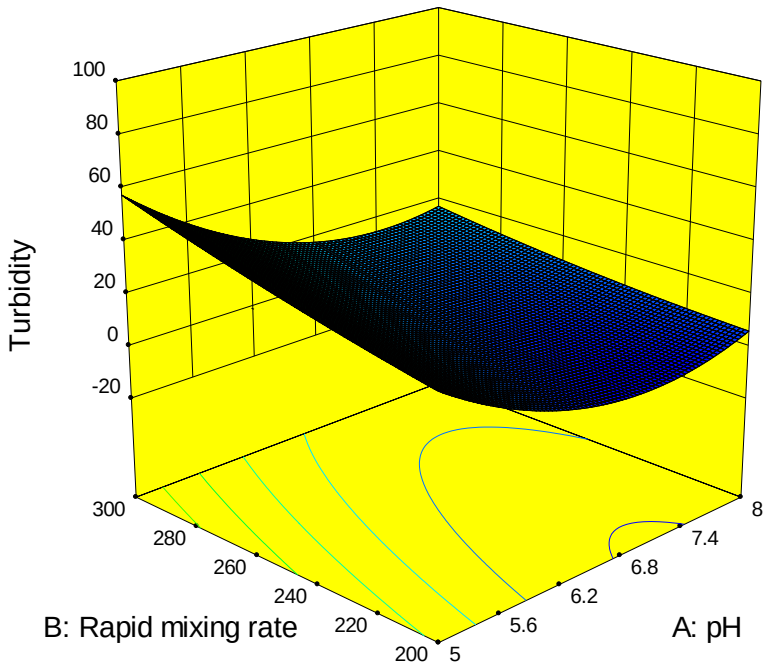


Figure A14: Effects of coagulation mixing rate and initial pH on turbidity removal; 3D surface graph and contour plot.

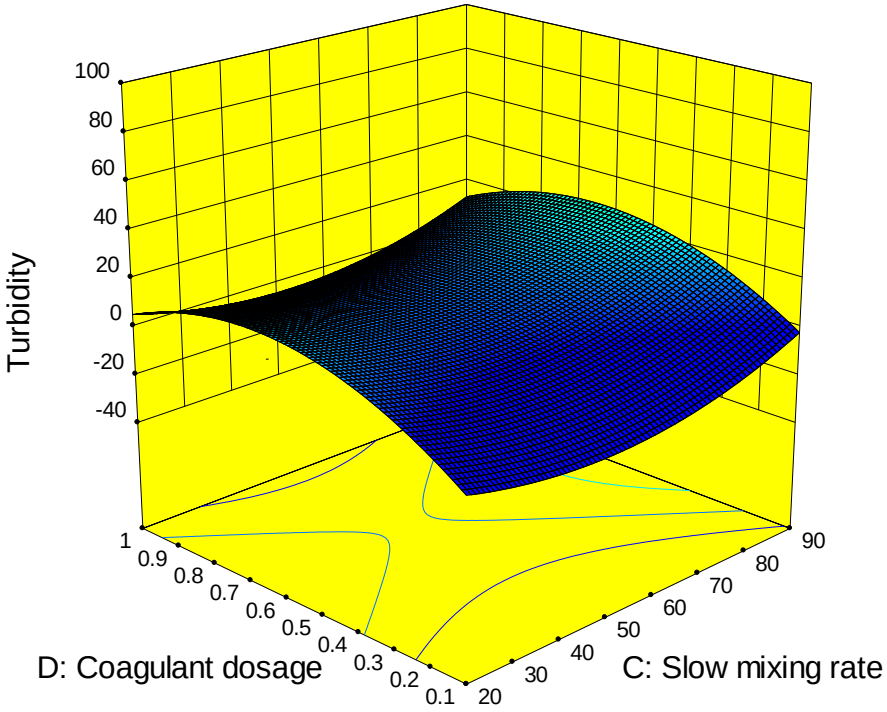


Figure A15: Effects of coagulant dosage and flocculation mixing rate on turbidity removal; 3D surface graph and contour plot.

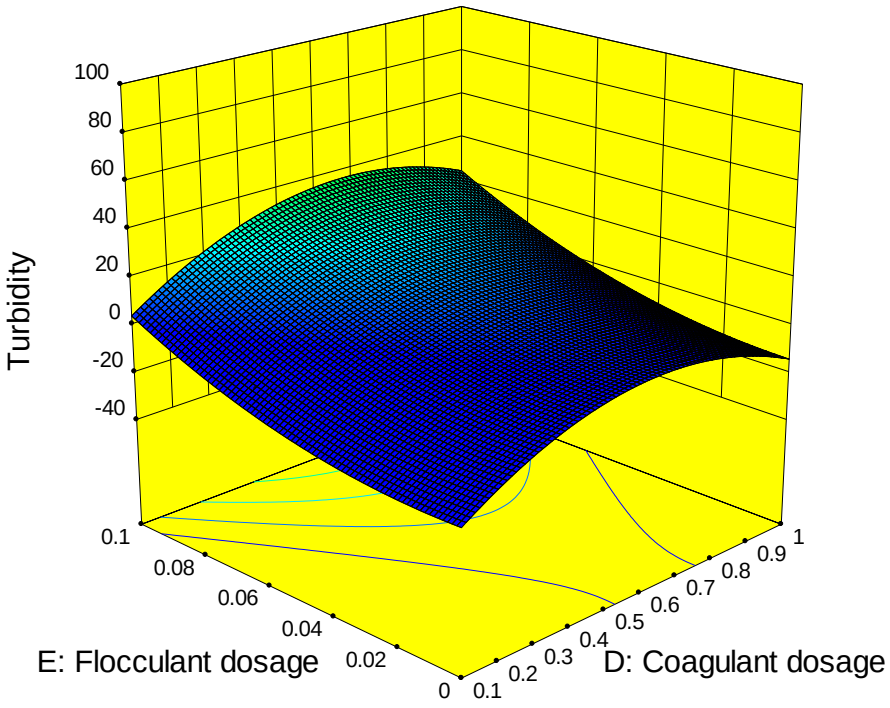


Figure A16: Effects of flocculant dosage and coagulant dosage on turbidity removal; 3D surface graph and contour plot.

APPENDIX B: SEM Images

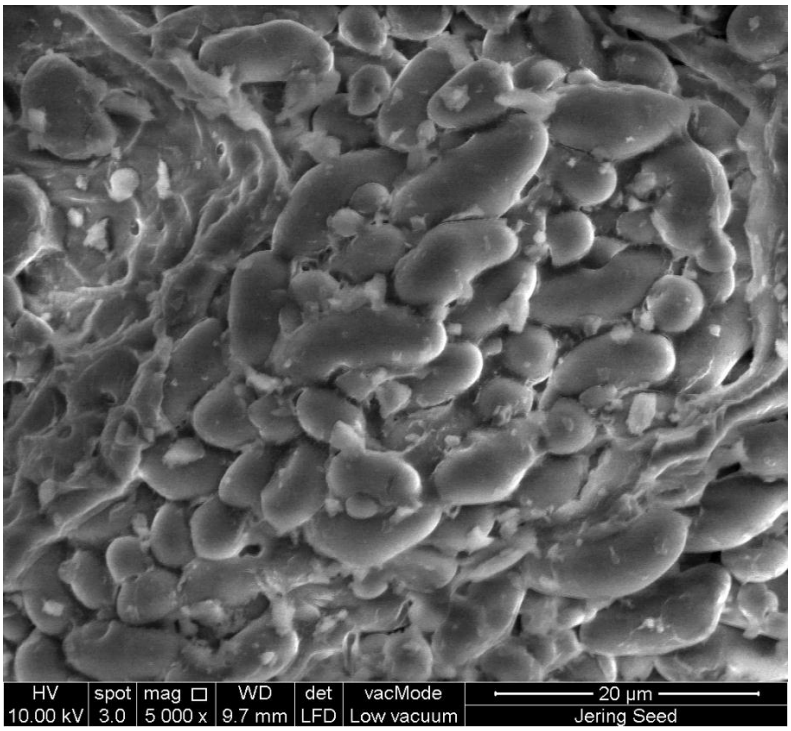


Figure B1: SEM image of jering seed powder at 10kV and 5000x magnification.

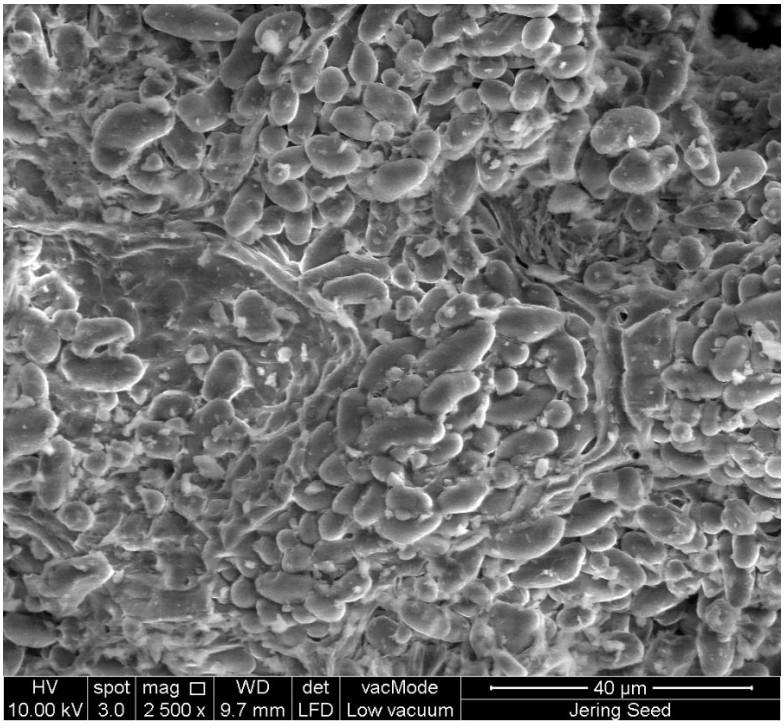


Figure B2: SEM image of jering seed powder at 10kV and 2500x magnification.

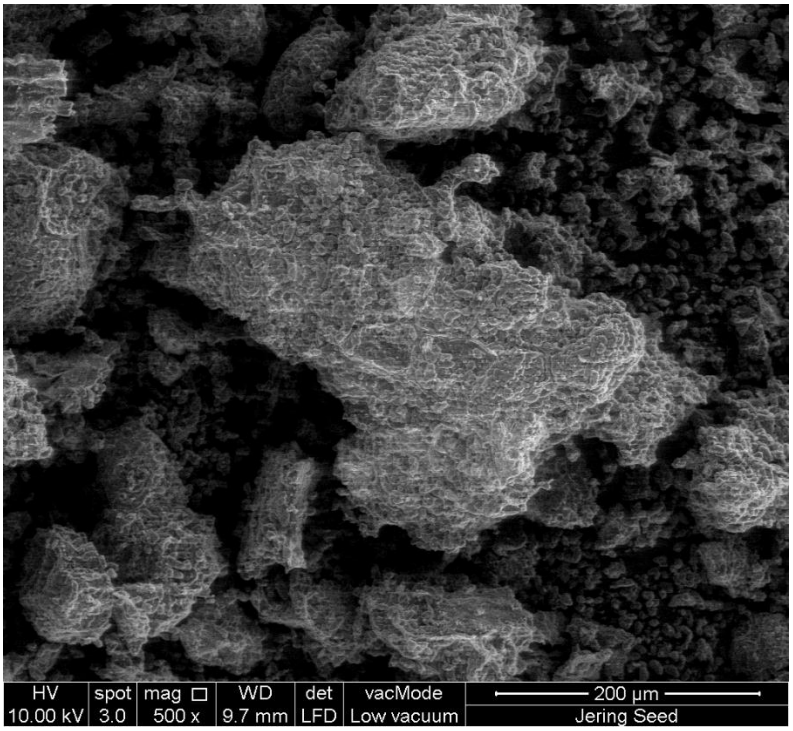


Figure B3: SEM image of jering seed powder at 10kV and 500x magnification.

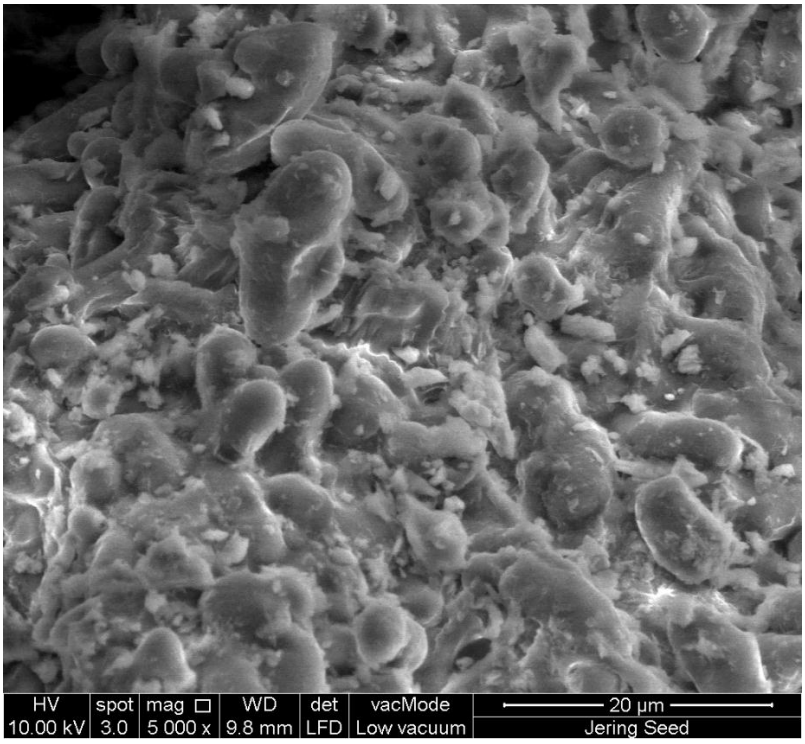


Figure B4: SEM image of jering seed powder at 10kV and 5000x magnification.

APPENDIX C: Jar test results

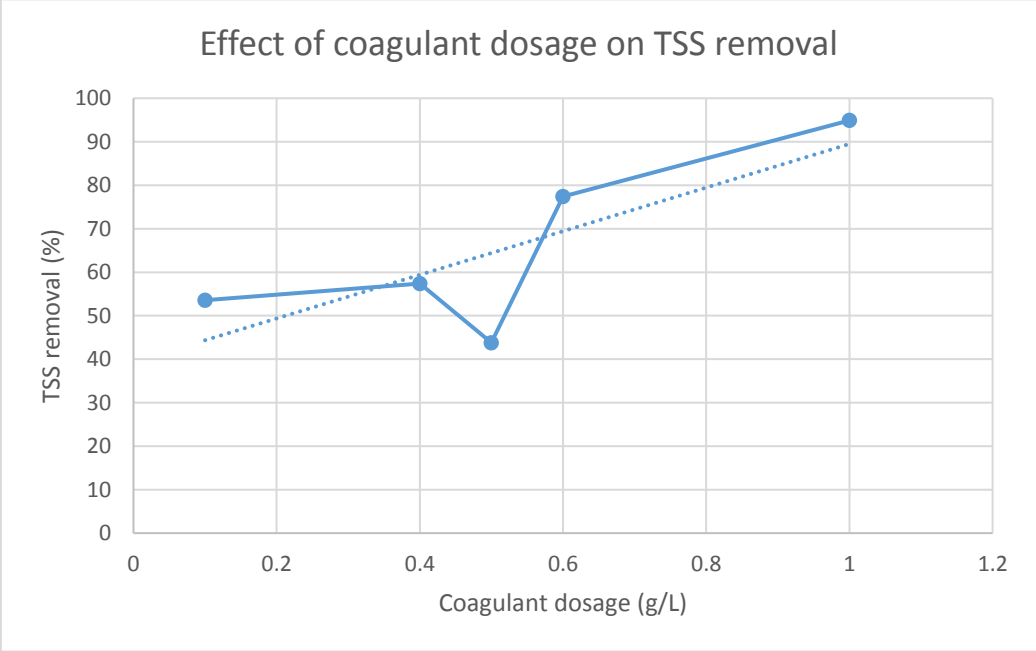


Figure C1: Effect of coagulant dosage (g/L) on TSS removal (%).

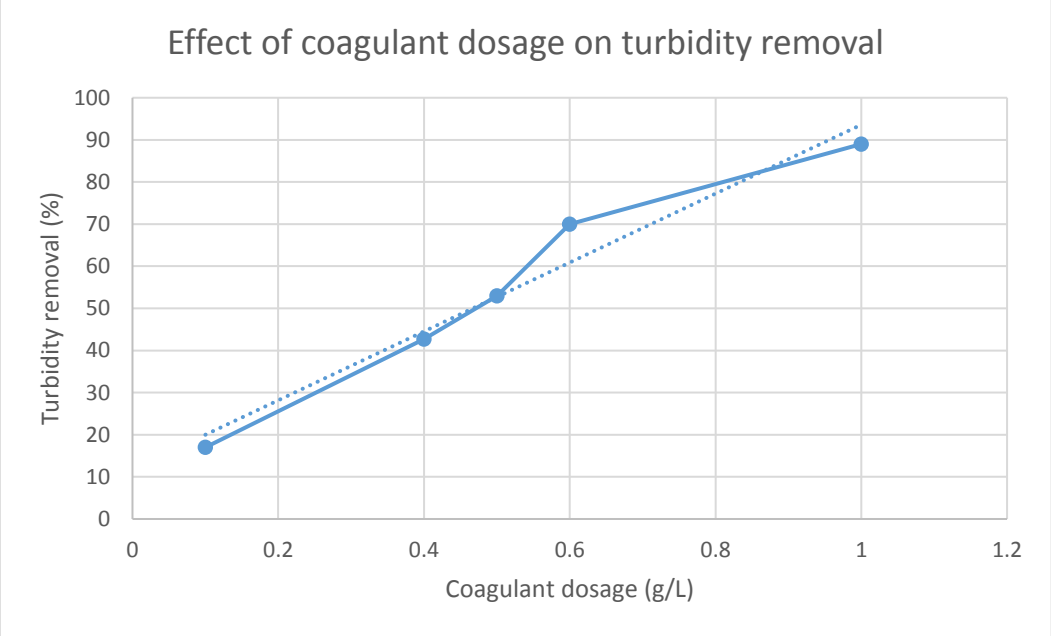


Figure C2: Effect of coagulant dosage (g/L) on turbidity removal (%).

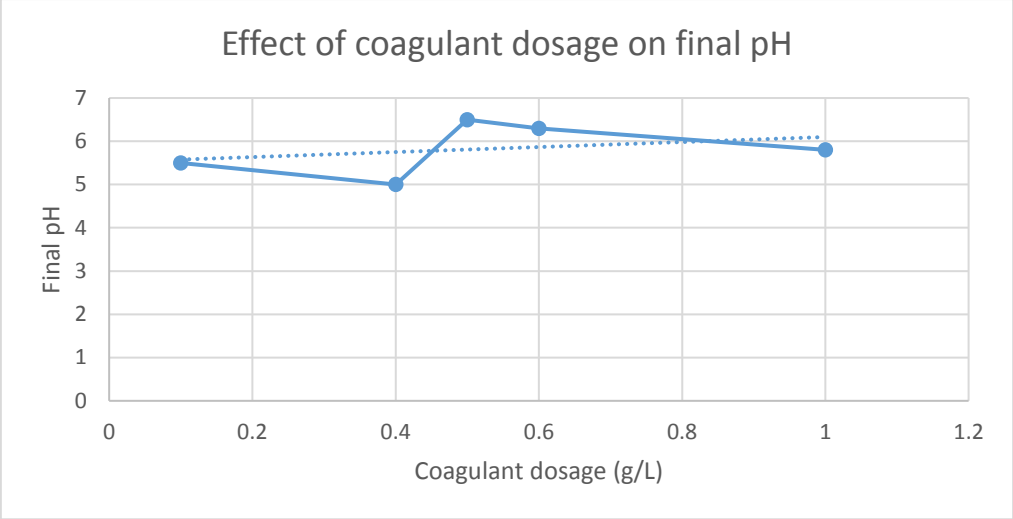


Figure C3: Effect of coagulant dosage (g/L) on final pH.

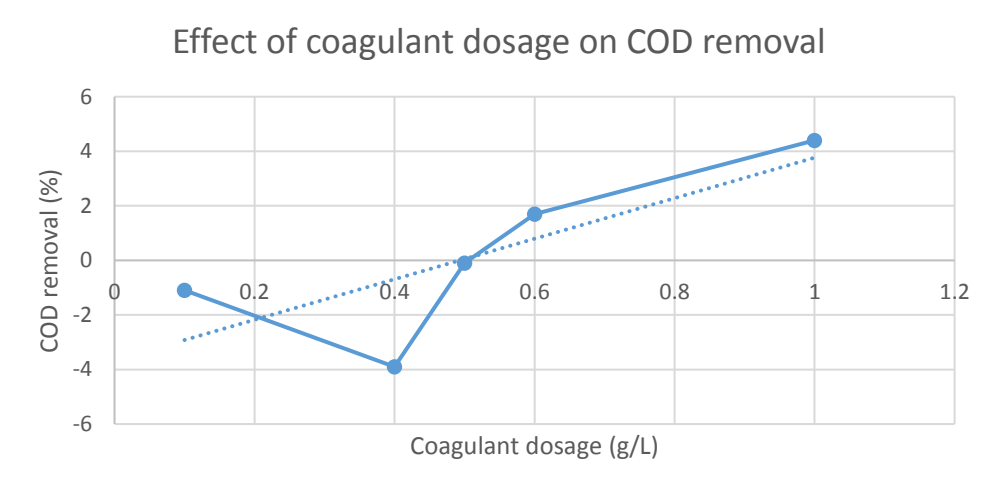


Figure C4: Effect of coagulant dosage (g/L) on COD removal (%).

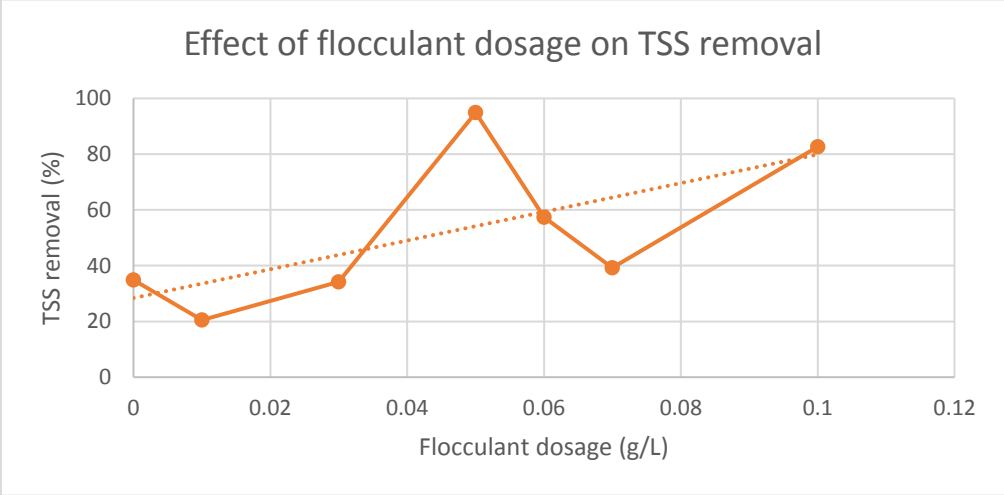


Figure C5: Effect of flocculant dosage (g/L) on TSS removal (%).

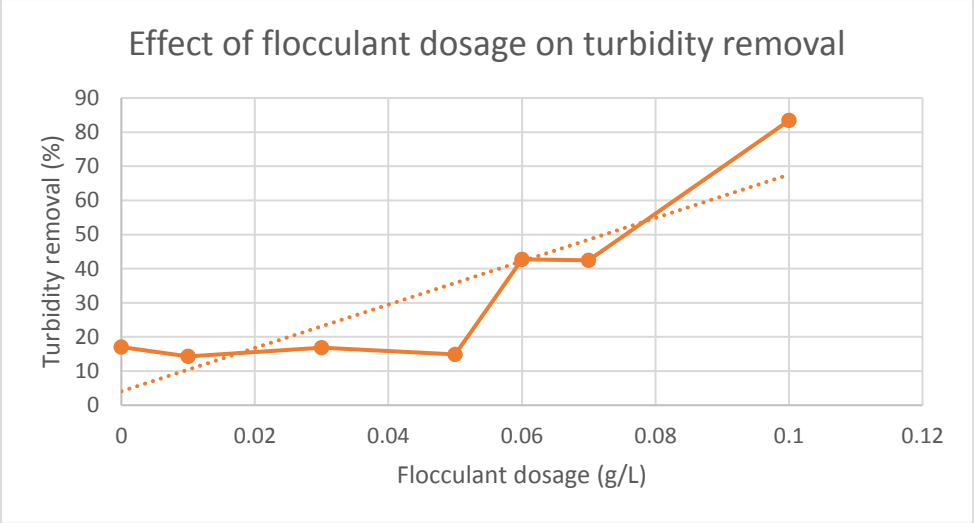


Figure C6: Effect of flocculant dosage (g/L) on turbidity removal (%).

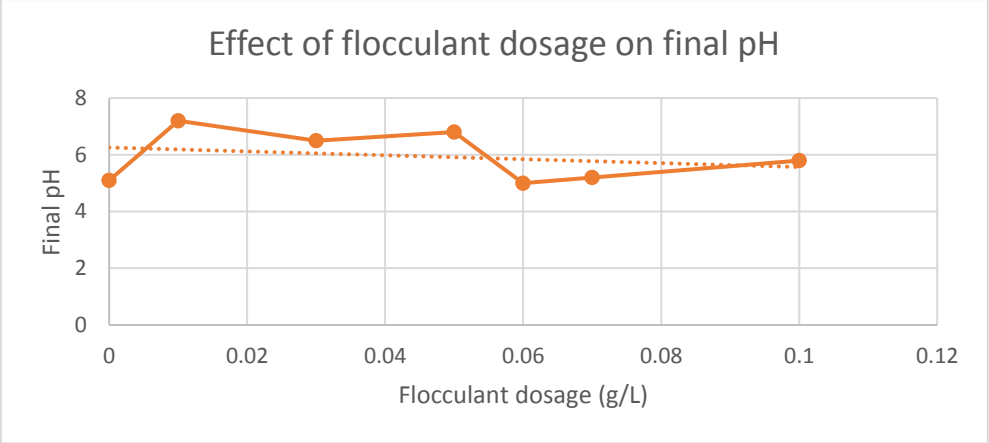


Figure C7: Effect of flocculant dosage (g/L) on final pH.

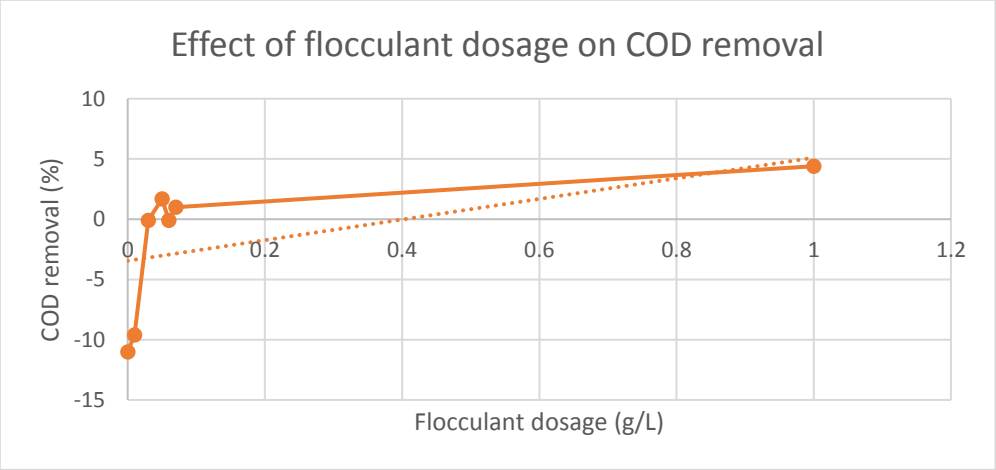


Figure C8: Effect of flocculant dosage (g/L) on COD removal (%).

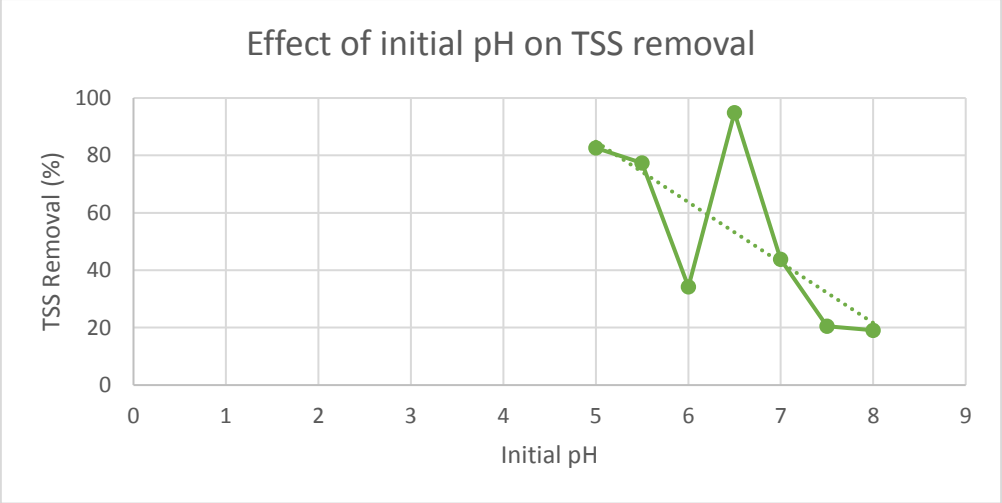


Figure C9: Effect of initial pH on TSS removal (%).

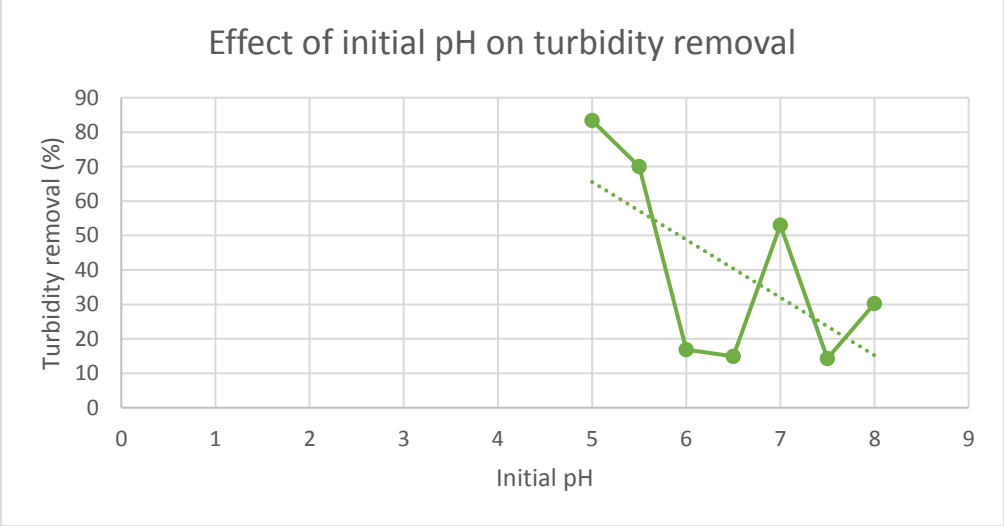


Figure C10: Effect of initial pH on turbidity removal (%).

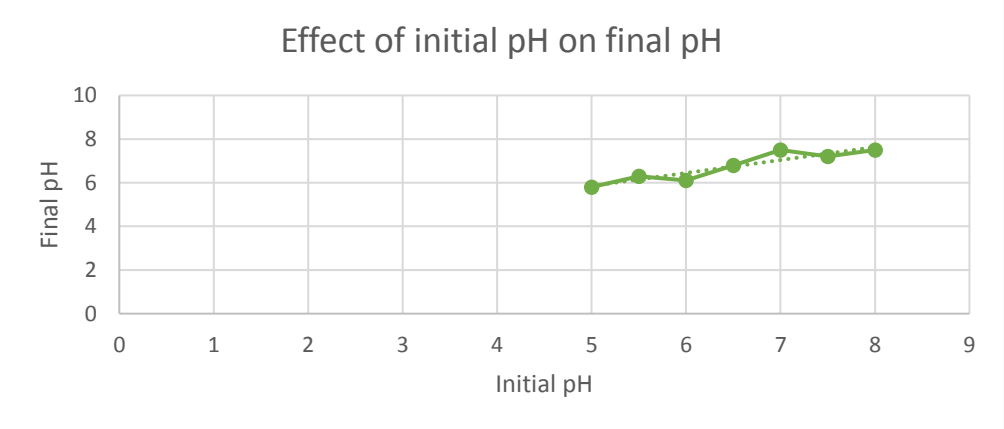


Figure C11: Effect of initial pH on final pH.

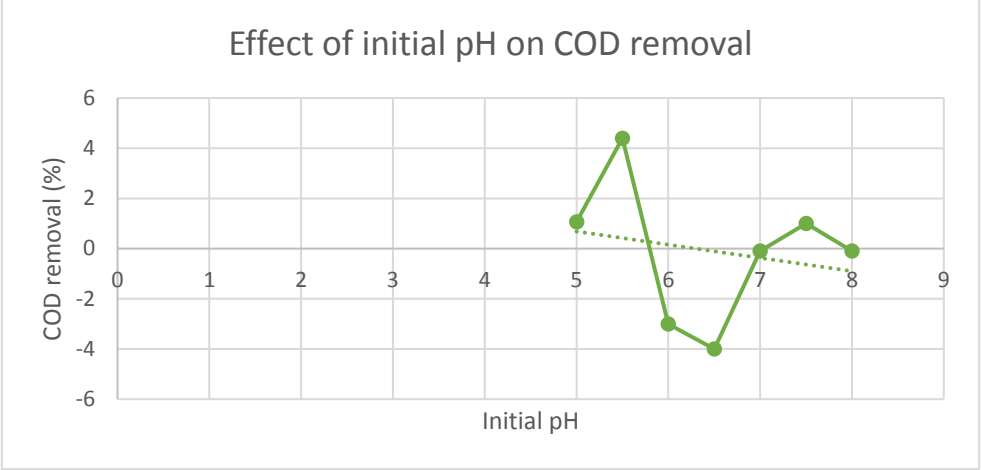


Figure C12: Effect of initial pH on COD removal (%).

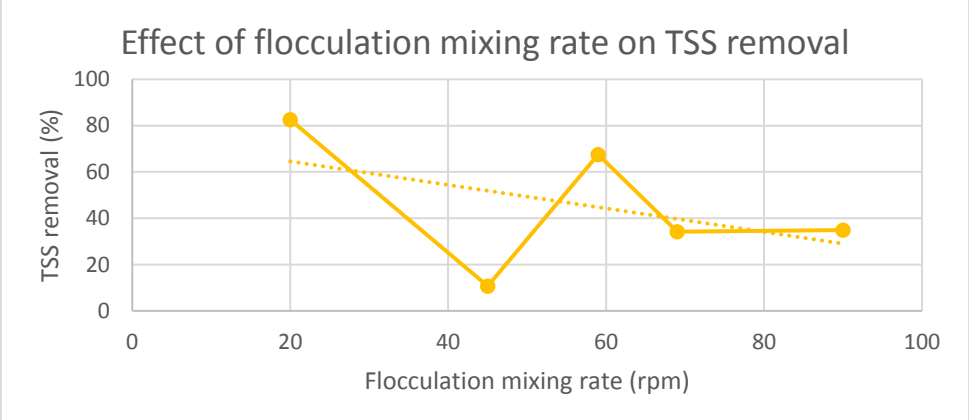


Figure C13: Effect of flocculation mixing rate (rpm) on TSS removal (%).

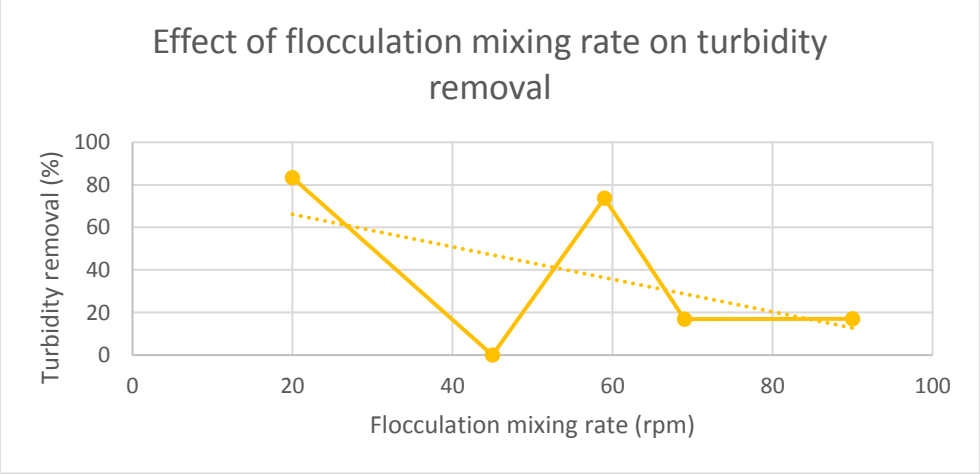


Figure C14: Effect of flocculation mixing rate (rpm) on turbidity removal (%).

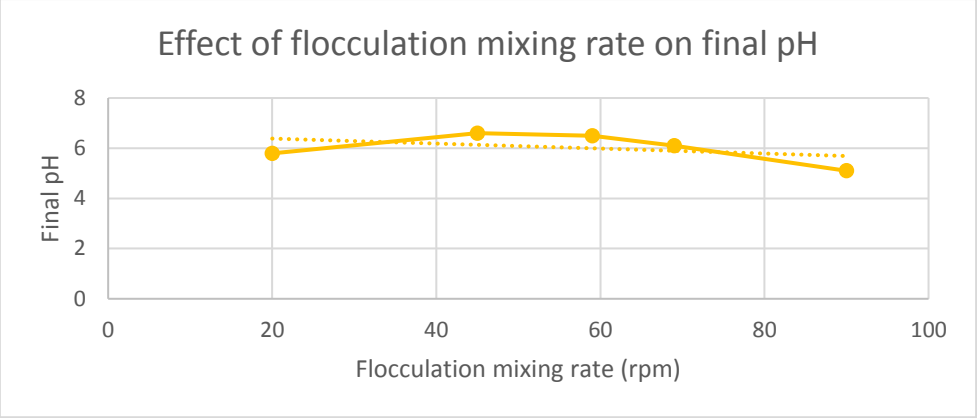


Figure C15: Effect of flocculation mixing rate (rpm) on final pH.

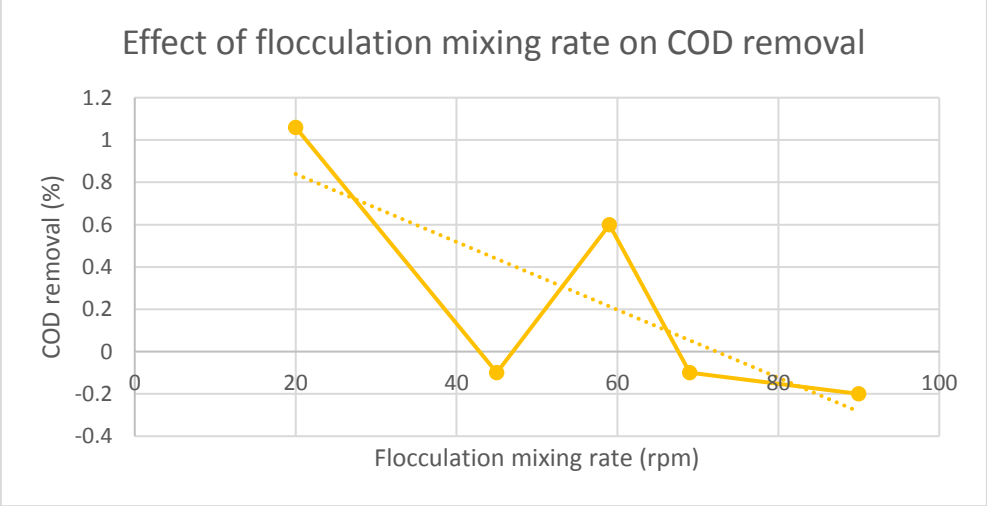


Figure C16: Effect of flocculation mixing rate (rpm) on COD removal (%).

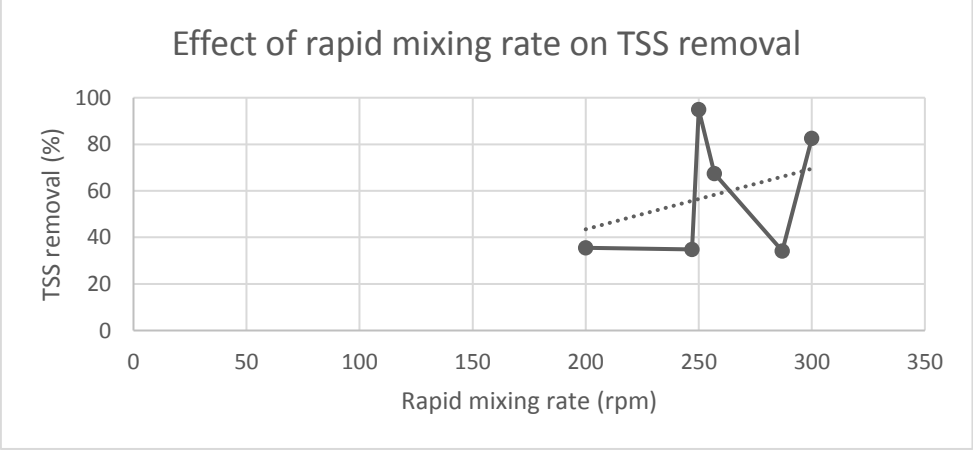


Figure C17: Effect of coagulation mixing rate (rpm) on TSS removal (%).

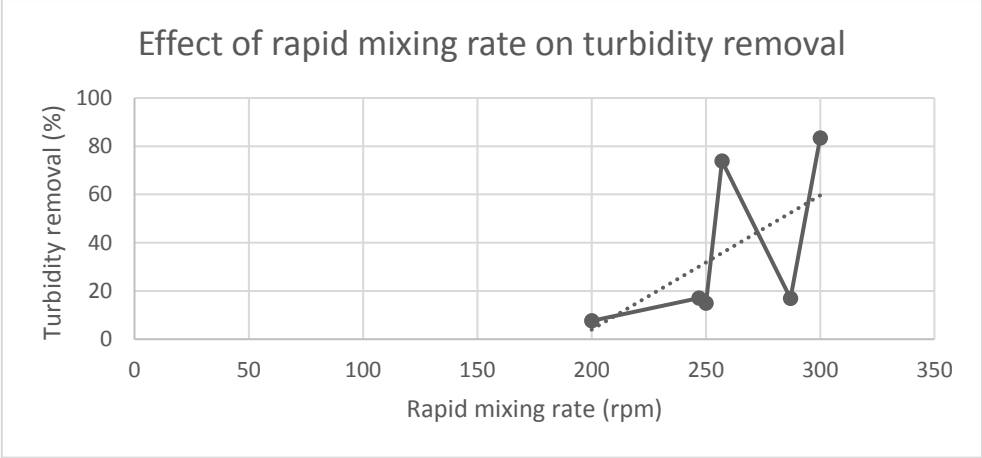


Figure C18: Effect of coagulation mixing rate (rpm) on turbidity removal (%).

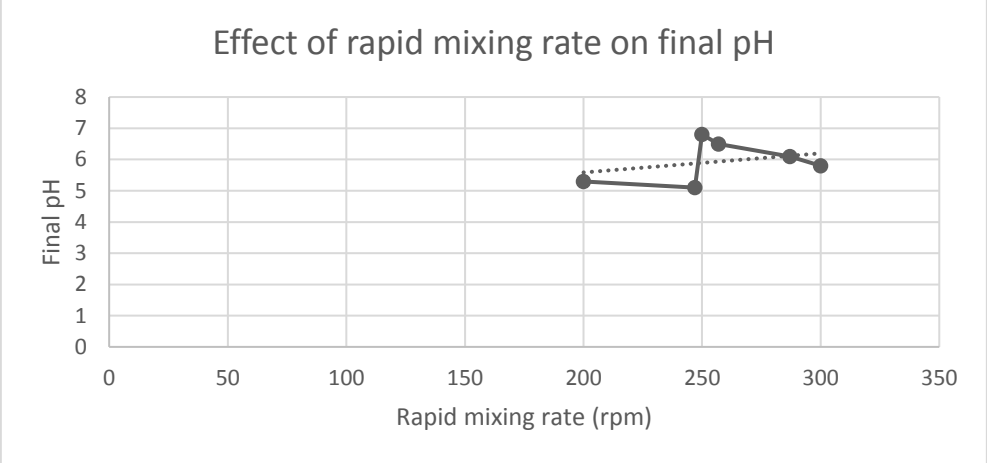


Figure C19: Effect of coagulation mixing rate (rpm) on final pH.

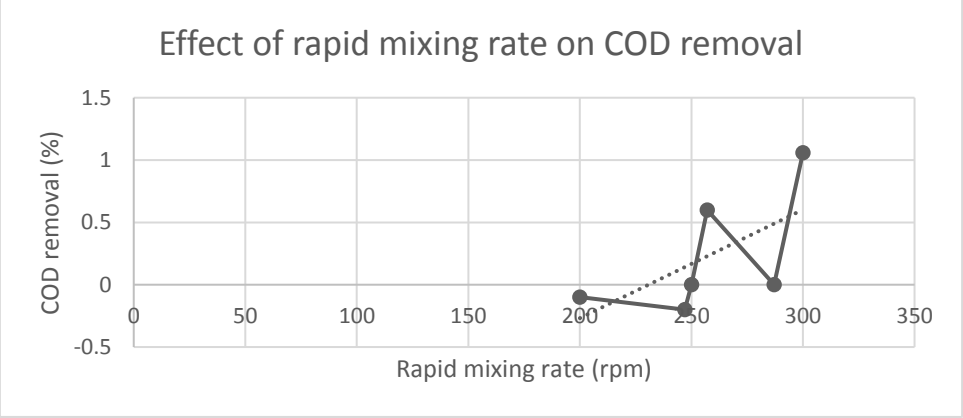


Figure C20: Effect of coagulation mixing rate (rpm) on COD removal (%).

Run	pH	Rapid mixing rate (rpm)	Slow mixing rate (rpm)	Coagulant dosage (g/L)	Coagulant dosage (mL)	Flocculant dosage (g/L)	Flocculant dosage (mL)	COD (mg/L)	TSS (%)	Final pH	Turbidity (%)
Blank	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0**	**-**	**-**	**-**
BLANK	**7.4**	**300**	**90**	**0**	**0**	**0**	**0**	**1753**	**53.3**	**7.9**	**0**
1	5	300	90	1	33	0	0	1782	68.0	5.9	56.9
2	5.5	257	59	0.6	20	0.1	50	1755	77.4	6.3	70
3	5	300	20	1	33	0.1	50	1783	82.6	5.8	83.4
4	8	300	20	1	33	0	0	2020	18.4	7.9	-6.3
5	5	200	20	0.4	13	0.06	30	3018	57.4	5	42.7
6	8	300	90	1	33	0.1	50	1762	35.7	7.7	55.3
7	5	300	90	0.1	3	0.1	50	1773	53.6	5.5	89
8	8	247	55	0.6	20	0	0	1768	18.5	8	4.9
9	5	200	90	0.1	3	0	0	2595	34.9	5.8	17
10	8	247	55	0.6	20	0	0	1980	30.4	8.1	4.9
11	8	200	90	1	33	0	0	1763	23.7	8.1	0
12	6.5	250	90	1	33	0.05	25	0	124.8	6.7	18.7
13	5.5	257	59	0.6	20	0.1	50	1830	67.5	6.4	73.8
14	6	200	45	0.1	1	0.1	50	1794	1.6	6.33	0
15	8	200	20	1	33	0.1	50	1775	19.1	7.5	30.2
16	5	200	90	1	33	0.1	50	3060	71.1	5.5	49.9
17	5	200	20	1	33	0	0	2702	35.5	5.3	7.6
18	8	300	90	0.1	3	0	0	1730	21.7	8.1	0
19	6	287	69	0.4	13	0.03	15	2403	34.2	6.1	16.9
20	6	287	69	0.4	13	0.03	15	2152	30.7	6.1	6
21	7	300	48	1	10	0.06	30	2377	5.4	7.24	0
22	7	300	20	0.5	17	0.1	50	1755	43.8	7.5	53
23	7	205	90	0.4	13	0.03	15	2309	5.1	7.1	0
24	8	200	90	0.1	3	0.1	50	2295	12.8	8.1	0
25	7.5	200	20	1	33	0.01	5	2028	20.5	7.2	14.3
26	8	200	20	0.1	3	0	0	3707	5.2	7.7	0
27	8	300	51	0.1	1	0.06	30	2394	9.7	8	0
28	6.5	250	90	1	33	0.05	25	2089	94.9	6.8	14.9
29	5	300	20	0.1	3	0	0	1846	22.5	6	8.63
30	5	200	20	0.4	13	0.07	35	1987	39.2	5.2	42.5
31	8	257	20	0.1	3	0.1	50	2327	11	7.84	0

Table C1: Jar test results from preliminary experiments.

APPENDIX D: FTIR results

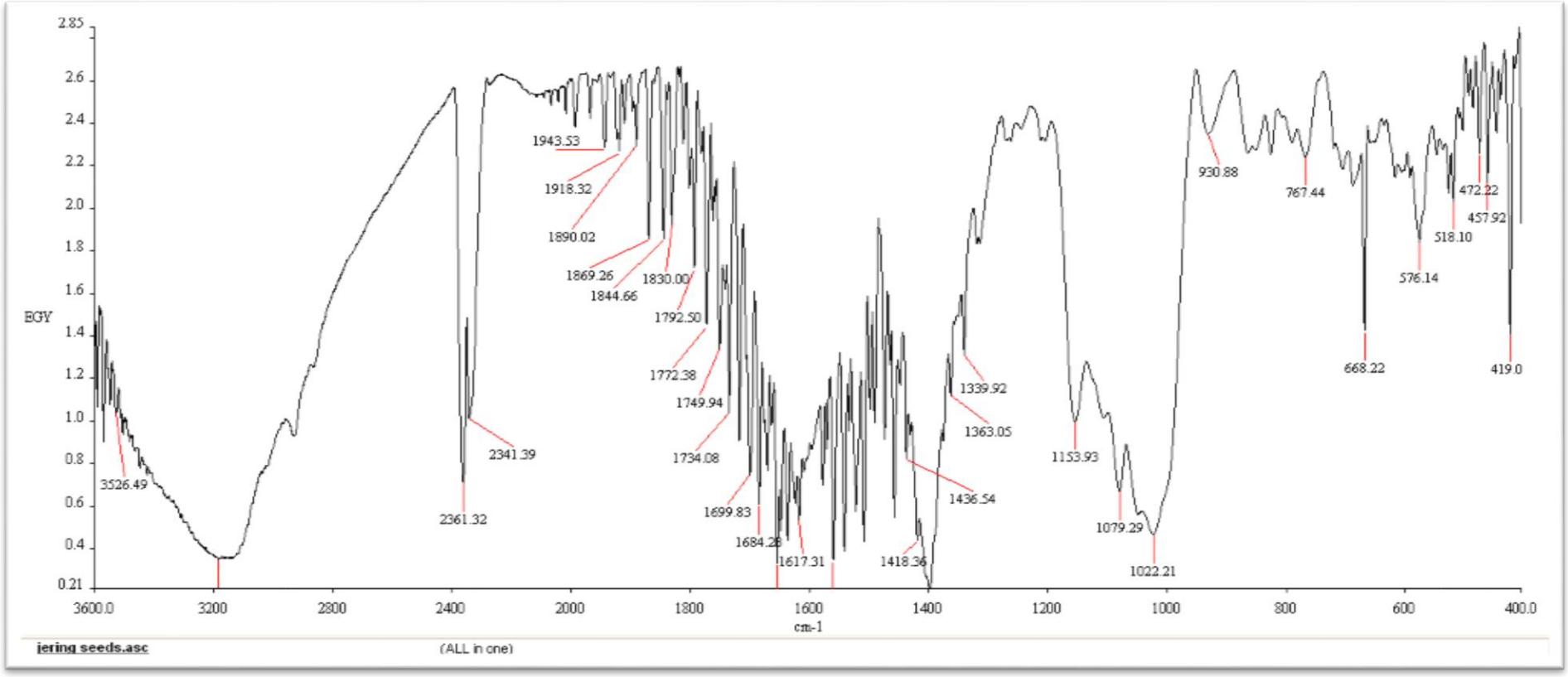


Figure D1: FTIR results for jering seeds.

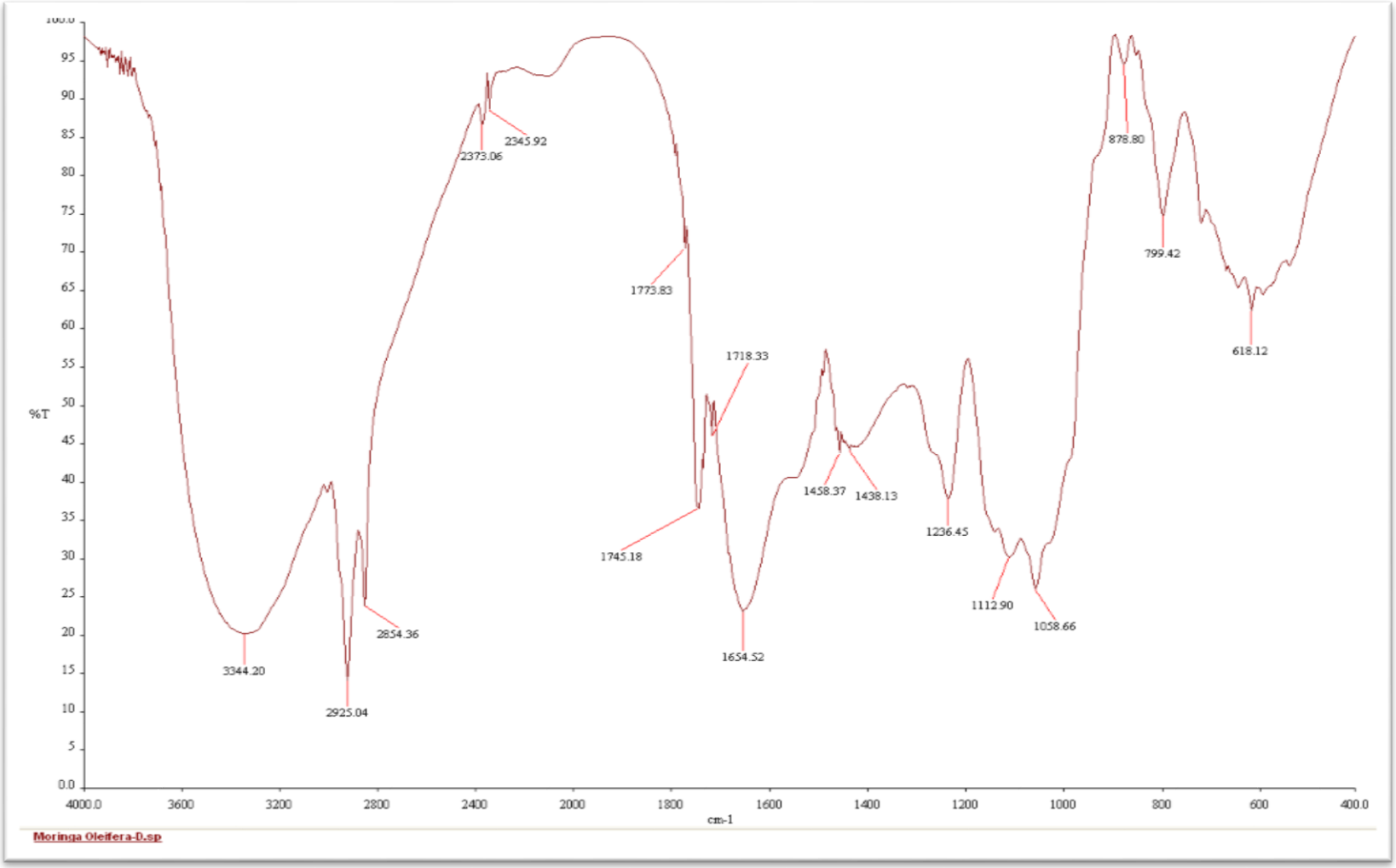


Figure D2: FTIR results for *moringa oleifera* seeds.

APPENDIX E: Zeta potential test results

Zeta Potential Report

v2.3

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Sample Details

Sample Name: Jering -1 trial avg
SOP Name: 030516-trial.sop
General Notes: Average result created from record number(s): 1 2 3

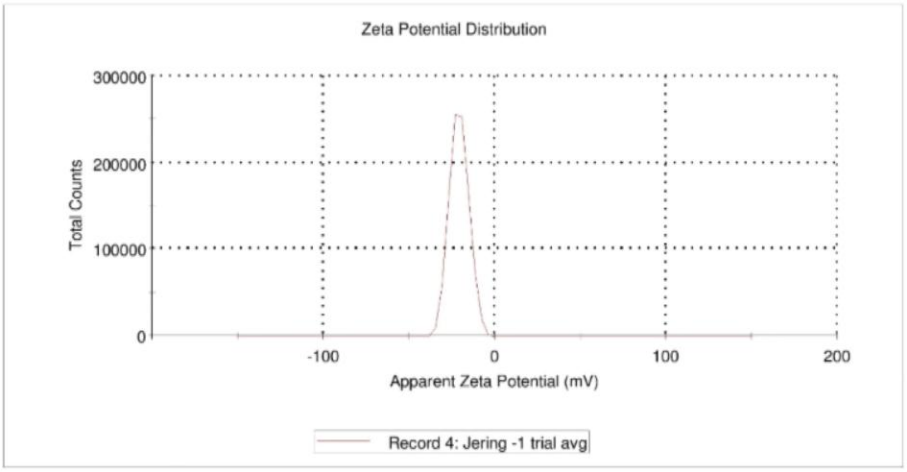
File Name: Jering trial 030516.dts
Record Number: 4
Date and Time: Tuesday, May 03, 2016 5:35:50 PM
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0
Count Rate (kcps): 187.0
Cell Description: Clear disposable zeta cell
Zeta Runs: 13
Measurement Position (mm): 2.00
Attenuator: 6

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-20.9	**Peak 1:**	-20.9	100.0	5.53
Zeta Deviation (mV):	5.38	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.104	**Peak 3:**	0.00	0.0	0.00
Result quality :	Good				



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Zetasizer Ver. 7.03
Serial Number : MAL1038260

File name: Jering trial 030516.d
Record Number: 4

Figure E1: Zeta potential results for jering seeds.

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