COMPARATIVE PERFORMANCE OF NATURAL COAGULANTS FOR THE TREATMENT OF FISH FARM WASTEWATER

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by

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LIST OF SYMBOLS

	Symbol	Unit
A	Coagulant dosage	mg/l
B	pH of wastewater	-
b	Constant for RSM equation	-
C	Mixing time	min
D	Settling time	min
N	Total number of experiments required	-
n	Number of factors	-
n_c	Number of replicates at the center point	-
Y_1	Turbidity value for chitosan	NTU
Y_2	Turbidity value for *M. oleifera*	NTU

LIST OF ABBREVIATIONS

CCD	Central Composite Design
CCF	Central composite design face-centered
COD	Chemical Oxygen Demand
DOE	Design of Experiment
HCl	Hydrochloric Acid
H_2O	Water
MLVSS	Mixed Liquor Volatile Suspended Solid
NaOH	Sodium Hydroxide
NTU	Nephelometric Turbidity Units
OFAT	One Factor At A Time
RSM	Response Surface Methodology
rpm	Rotation per minute
TSS	Total Suspended Solids
TVS	Total Volatile Solids

PERBANDINGAN PRESTASI BAHAN GUMPAL SEMULA JADI UNTUK RAWATAN AIR SISA PENTERNAKAN IKAN

ABSTRAK

Proses pembekuan-pemberbukuan menggunakan chitosan dan ekstrak daripada benih *Moringa oleifera* sebagai bahan gumpal semula jadi telah dilaporkan untuk rawatan air sisa ternakan ikan dalam kajian ini. Objektif utama yang perlu dicapai adalah untuk menentukan bahan gumpal yang terbaik untuk penyingkiran tertinggi pepejal terampai melalui proses pembekuan-pemberbukuan. Oleh itu, rawatan pengoptimuman untuk mencapai tujuan ini telah dilakukan dengan menggunakan ujian balang dan menggunakan Kaedah Gerak Balas Permukaan (KGBP) untuk menganalisa keputusan.2^4 sepenuh faktorial Reka Bentuk Komposit Pusat (RBKP) telah dipilih untuk menerangkan kesan dan interaksi empat faktor: dos bahan gumpal, pH, masa pencampuran dan masa tenggelam. 84% daripada penyingkiran kekeruhan dapat diperhatikan melalui sampel air sisa yang dirawat dengan chitosan pada keadaan yang optimum iaitu 100 mg / l dos bahan gumpal, pH 6, 15 minit masa pencampuran dan 10 minit masa tenggelam manakala 47% penyingkiran kekeruhan pada keadaan optimum 400 mg / l dos bahan gumpal, pH 10, 15 minit masa pencampuran dan 10 minit masa tenggelam dapat dilihat untuk ekstrak benih *M. oleifera*. Sampel air sisa yang dirawat menggunakan keadaan pengoptimuman yang dipilih telah dianalisis untuk kadar ammonia, Permintaan Oksigen Kimia (POK), jumlah pepejal terampai (TSS), jumlah pepejal meruap (TVS) dan kepekatan fosfat. Chitosan menunjukkan kecekapan penyingkiran yang lebih baik daripada *M. oleifera* dengan 64% penyingkiran POK, 70% ke atas TSS, 64% ke atas VSS dan 80% penyingkiran pada fosfat manakala *M. oleifera* mempunyai prestasi yang sedikit baik dalam kecekapan penyingkiran ammonia pada 32%.

COMPARATIVE PERFORMANCE OF NATURAL COAGULANTS FOR THE TREATMENT OF FISH FARM WASTEWATER

ABSTRACT

A coagulation – flocculation process using chitosan and *Moringa oleifera* seeds extract as natural coagulants is reported for fish farm wastewater treatment in this study. The main objective to be achieved is to determine the best coagulant for highest removal of suspended solid through the coagulation - flocculation process. Therefore, optimization treatment to serve this purpose was performed using jar tests and applying a response surface methodology (RSM) to the results. A 2^4 full-factorial central composite design (CCD) was chosen to explain the effect and interaction of four factors: coagulant dosage, pH, mixing time and settling time. The CCD is successfully demonstrated to efficiently determine the optimized parameters, where 84% of turbidity removal was observed for wastewater samples treated with chitosan at optimized condition of 100 mg/l coagulant dosage, pH 6, 15 minutes mixing time and 10 minutes settling time and 47% of turbidity removal at optimized condition of 400 mg/l coagulant dosage, pH 10, 15 minutes mixing time and 10 minutes settling time for *M. oleifera* seeds extract. The wastewater samples treated using the selected optimized condition were further analyzed on their final values of ammonia, Chemical Oxygen Demand (COD), total suspended solids (TSS),total volatile solids (TVS) and phosphate concentrations. Chitosan demonstrated a better removal efficiency than *M. oleifera* with 64% removal on COD, 70% on TSS, 64% on VSS and 80% removal on phosphate while *M. oleifera* has a slightly better performance in ammonia removal efficiency at 32%. Hence, chitosan is proven to be a better choice of coagulant than *M.oleifera*.

CHAPTER 1

INTRODUCTION

1.1 Research Background

In the last decades, development of aquacultures as monocultures has manifested tremendous growth, from keeping fish in ponds for easier harvesting to high technological fish farms extensively using feed, hormones and antibiotics. In 2015 alone, global aquaculture production has reached 106 million tonnes, where 76.6 million tonnes of it comes from aquatic animals while the rest is from aquatic plants, with average growth percentage of 6.6% since 1995 (FAO, 2017). This positive trend is projected to continue as the aquaculture sector plays a huge role in contributing to food security and poverty alleviation of the poor.

However, it is well known that aquaculture activities are the major contributor to the increasing level of organic waste and toxic compounds in the aquaculture industry. Wastewater discharged from aquaculture contains phosphorus, dissolved organic carbon and nitrogenous compounds such as ammonia, nitrite and nitrate. This contaminants will cause environmental deterioration at high concentrations in the receiving water body if not being treated effectively. Ammonium and nitrite especially, could be harmful to aquatic life while nitrate is known to cause ‘blue baby syndrome’. Nutrients such as nitrogen and phosphorus could stimulate the growth of algae and other photosynthetic aquatic life, leading to excessive eutrophication and excessive loss of oxygen resources (Nora’aini et al., 2005). Hence, it is of vital importance to incorporate effective wastewater treatment to treat the effluents from aquaculture industries.

1.2 Conventional Wastewater Treatment

To achieve high quality of receiving waters in aquaculture systems, various conventional methods have being implemented such as settling systems, centrifugal systems, mechanical filters and biological processes. In Malaysia particularly, one of the most commonly approached method is by activated sludge, which consists of flocs of bacteria suspended and mixed with wastewater in an aerated tank. The bacteria will degrade organics and remove nutrients from wastewater to produce a high-quality effluent. However, this conventional method can only achieve partial effectiveness, with the disadvantage of producing sludge that contains a lot of moisture which needs to be dewatered. Apart from that, as this process rely solely on microorganisms to break down the pollutant, frequent monitoring is required to ensure a conducive environment for their development as the microbes are very sensitive to the surrounding temperature and pH (Latif et al.,2003).

1.3 Coagulation and Flocculation

To counter the problems arise from using the conventional wastewater treatment method, coagulation and flocculation method is used as a simple and cost-effective alternative. This is a type of physico-chemical process in which compounds such as coagulants are added to wastewater in order to destabilize the colloidal materials, causing the small particles to agglomerate into larger settleable flocs. It is a simple and efficient method for wastewater treatment, and has been extensively used for the treatment of various types of wastewater such as dairy wastewater, domestic wastewater, tannery wastewater, textile wastewater and aquaculture wastewater (Freitas et al.,2018; Ugwu et al.,2017; Ayoub et al., 2011; Kushwaha et al., 2010; Ebeling et al., 2003).

1.4 Problem Statement

In view of the complex nature of wastewater effluent, careful treatment strategies need to be implemented for better quality of treated effluent. The chemical coagulation process is commonly employed in this regard, with inorganic coagulants such as aluminum, iron, and magnesium salts being widely used. However, the coagulation process is invariably associated with the generation of a secondary waste stream in the form of sludge, and low sludge volume is crucial to the process. The usage of chemical coagulants, in particular, could lead to production of sludge with high toxicity because of its aluminum content, not to mention the presence of residual aluminum concentration in the treated water will result in the reaction of aluminum with alkalinity present in the water leading to the big drop in the pH of wastewater. The exorbitantly expensive cost of imported chemicals in some developing countries is also one of the drawbacks for its application.

As an effort to introduce a more environmental-friendly approach for the treatment of wastewater, the focus of this research is to evaluate the effectiveness of natural coagulants (chitosan and *M. oleifera*) which is believed to be a cheaper and better alternative for conventional coagulants considering their biodegradable nature and similarity of potential as aluminum coagulants with an enhanced economic profile. This study will emphasize on treating wastewater obtained from fish farm with further analysis on its water quality using the optimized condition of each natural coagulants by Response Surface Methodology (RSM).

1.5 Research Objectives

The objectives of this research are;

i. To develop regression models and optimize the coagulation and flocculation process using *M. oleifera* and chitosan utilizing Response Surface Methodology (RSM).

ii. To determine the quality of the treated wastewater in terms of turbidity, ammonia and phosphate content, Chemical Oxygen Demand (COD), Total Suspended Solid (TSS) and Total Volatile Solid (TVS) removal.

iii. To evaluate and compare the performance of *M. oleifera* with chitosan as natural coagulants for the treatment of fish farm wastewater.

CHAPTER 2

LITERATURE REVIEW

2.1 Fish Farm Wastewater in Malaysia

Fish farming is the principal form of aquaculture, which involves raising fish commercially in tanks or enclosures, usually for food. Aquaculture is the breeding, rearing, and harvesting of fish, shellfish, plants, algae and other organisms in all types of water environments. Generally, there are two kinds of aquaculture: extensive aquaculture based on local photosynthetical production and intensive aquaculture, in which the fishes are fed with external food supply.

In 2008 alone, freshwater aquaculture had contributed 39.5% or 95,917 tonnes to the entire fish production in Malaysia (FAO, 2017). Despite the huge economic benefits of the industry, its negative impact on the environment needed to be controlled due to its large volume of water demand and the effluent volume that is discharged into water. Aquaculture wastewaters exert adverse environmental impacts when the effluents from these systems are discharged into receiving waters , as they will reduce the dissolved oxygen level, contribute to the buildup of bottom sediments and impair water quality by stimulating excessive phytoplankton production due to high nutrient loading (Ghaly et al., 2005). Therefore, an appropriate wastewater treatment process was required for sustaining aquaculture development in Malaysia. The quality of effluent from treatment plants is regulated by the Environmental Quality Act 1974 and its regulations such as the Environmental Quality (Sewage) Regulations 2009 and Environmental Quality (Industrial Effluent) Regulations 2009.

Two different standards have been established (Standard A and Standard B) for the quality of effluent discharged from treatment plants to receiving waters, as shown in Table 2.1. Standard A is for effluent that is discharged upstream of a water supply intake while Standard B is for effluent that is discharged downstream.

Table 2.1 Parameter limits of effluent standard A and B (Act, 1974)

		Standard	
Parameter	Units	A	B
Temperature	˚C	40	40
pH Value	-	6.0-9.0	5.5-9.0
BOD5 at 20C	mg/l	20	50
COD	mg/l	50	100
Suspended Solids	mg/l	50	100
Mercury	mg/l	0.005	0.05
Cadmium	mg/l	0.01	0.02
Chromium, Hexavalent	mg/l	0.05	0.05
Arsenic	mg/l	0.05	0.1
Cyanide	mg/l	0.05	0.1
Lead	mg/l	0.1	0.5
Chromium, Trivalent	mg/l	0.2	1
Copper	mg/l	0.2	1
Manganese	mg/l	0.2	1
Nickel	mg/l	0.2	1
Tin	mg/l	0.2	1
Zinc	mg/l	1	1
Boron	mg/l	1	4
Iron (Fe)	mg/l	1	5
Phenol	mg/l	0.001	1
Free Chlorine	mg/l	1	2
Sulphide	mg/l	0.5	0.5
Oil and Grease	mg/l	Not Detectable	10

The three main methods of water and wastewater treatment are physical, chemical and biological treatment, with the physico-chemical process being the primary treatment for water and wastewater.

2.2 Physico-chemical Methods of Treatment

Physico-chemical treatment of wastewater focuses primarily on the separation of colloidal particles (size < 1 μm) which is achieved through the addition of chemicals (called coagulants and flocculants).One of the methods in physico-chemical treatment is coagulation and flocculation which is then followed by sedimentation.

2.2.1 Coagulation

Coagulation is by far the most widely used process to remove the substances producing turbidity in water. It is the process whereby destabilization of a given colloidal suspension or solution is taking place. Primary function of coagulation is mainly to overcome the factors that promote the stability of a given system, which is caused by the nature of these particles having electrostatic surface charges of the same sign (usually negative). These charges create electrostatic repulsive forces between the particles, constraining them from approaching each other which in turn prevents the process of aggregation and subsequent settling to happen. Coagulation is achieved with the use of appropriate chemicals, the so-called 'coagulant agents' which are usually aluminium or iron salts.

The importance of size is illustrated in Table 2.2, which shows the relative settling times of spheres of different sizes. It can be seen that with decreasing size, the time required for particles to settle increases, which can be up to several years for certain solutions ingredients. For some particle sizes, the only way for settling to happen proceeded with subsequent separation is for them to come into contacts and form larger particles (flocs), which ease the settling process. However, this procedure is hindered due to negative charges this material carries, which prolong its settling time. In order to accelerate the process, destabilization is required, denoting the importance of coagulation.

Table 2.2 Effect of decreasing size of spheres (Hiestand, 1964)

Diameter of particle mm	Order of size	Total surface area°	Time required to settle Ψ
10	Gravel	0.487 sq. in.	0.3 sec
1	Coarse sand	4.87 sq. in.	3 sec
0.1	Fine sand	48.7 sq. in.	38 sec
0.01	Silt	3.38 sq. ft.	33 min
0.001	Bacteria	33.8 sq. ft.	55 hr
0.0001	Colloidal particles	3.8 sq. ft.	230 days
0.00001	Colloidal particles	0.7 acre	6.3 yr.
0.000001	Colloidal particles	7.0 acres	63 years Minimum

°Area for particles of indicated size produced from a particle 10 mm in diameter with a specific gravity of 2.65

Ψ Calculations based on sphere with a specific gravity of 2.65 to settle 1 ft.

2.2.1.1 Mechanism of Coagulation

Based on Table 2.2, it should be noted that finely divided and colloidal particles have very small settling velocities that is deemed impractical for ordinary sedimentation. Hence, it is necessary to implement procedures to agglomerate these particles into small aggregates, which in turn makes them into larger aggregates with practical settling velocities .Formation of larger particles from those smaller ones, which could be obtained through flocculation process, is also required for their removal by filtration (Sengupta and Hashim, 1996).

Although both “coagulation” and “flocculation” terms are widely used to describe the turbidity removal process, there is definitely a clear distinction between these two terms. “Coagulation” comes from the Latin word coagulare, which means to drive together while “flocculation” is derived from the Latin word floculare, referring to the formation of flocs and bridges.

2.2.1.2 Fundamentals of Coagulation

It is best to describe the fundamentals of coagulation by introducing three mechanisms that influence the destabilization of colloidal particles, which are double layer compression, adsorption and charge neutralization and sweep flocculation (Faust and Aly, 1998).

i) Double layer compression

In a solution, the primary charge of the colloids attracts ions of the opposite charges, also known as counterions. These ions, which are held by electrostatic and van der Waals forces, will form a compact layer (also known as Stern layer) around the primary charge as shown in Figure 2.1.The counterions attached to the surface will in turn attract their own counterions (the co-ions of the primary charge), forming the diffuse layer. However, only a part of the diffused layer will move along with the colloid by shearing at the shear plane (Sincero and Sincero, 2002). The potential at this surface of shear is generally called as “zeta potential” and is measured in wastewater treatment operations by means of a zeta-potential meter. This measurement gives a good approximation of the surface charge of the colloidal particle, which in the case of colloids in the water, a zeta potential between −5 and −40 mV is usually acquired due to the presence of its charged groups (Parsons and Jefferson, 2006).

As for the double layer compression, it involves adding salts (such as aluminium or iron) to the system, which increases the ionic concentration. Under this condition, the double layer and the repulsion energy curves are compressed until there is no longer an energy barrier, which causes the particles to agglomerate rapidly. The thickness of the double layer will depend upon the concentrations of ions in the solution. Higher level of ions means more positively-charged ions available for charge neutralization, hence thinner double layer.

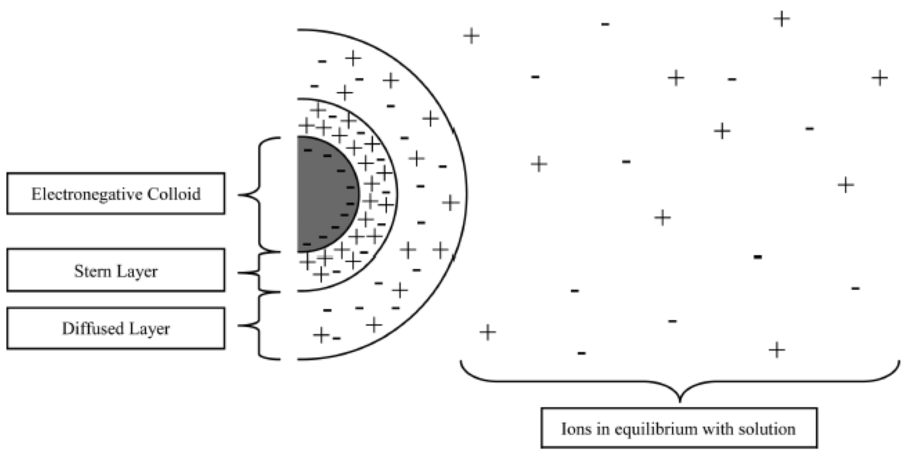

Figure 2.1 Double layer around a negatively-charged colloid (Tchobanoglous et al., 2003)

ii) Adsorption and charge neutralization

Inorganic coagulants (such as alum and ferric chloride) often work through charge neutralization. When any metal-based coagulants are added to water, it will dissociate and form metal ions, such as Fe^{3+} and Al^{3+} , if ferrous and aluminium salts are used respectively. These positively-charged ions, along with OH^- ions inside the water will react to form various monomers and polymeric hydrolyzed species, with its concentration highly dependent on the metal concentration and pH of the solution (Kushwaha et al., 2010).

The polymeric hydrolyzed species possess high positive charges, which are then adsorbed to the surface of the negatively-charged colloids. This results in a reduction of the zeta potential, which brings to the destabilization of the colloidal particles. The destabilized particles, with the hydrolyzed species adsorbed onto it, will aggregate via interparticulate Van Der Waals forces.

iii) Sweep Flocculation

Sweep flocculation happens when a very high dosage of coagulant (usually aluminium or iron salts) is added into the water, making the water saturated and causes the coagulant to precipitate out as hydrous metal oxides. Most of the colloids and some dissolved solids are swept from the bulk of the water as they become enmeshed in the settling hydrous oxide flocs (Duan and Gregory, 2003).

2.2.1.3 Chemistry of Metal Coagulants

There are several chemicals that have been conventionally and commercially used as coagulant in the water and wastewater treatment. Aluminium and iron salts are widely used coagulants to treat water and wastewater, where each of the coagulant will affect the destabilization degree of the colloidal particles differently. Generally, it is known that when aluminium and iron salts are added to water, they will react with water or with alkalinity present in the water, yielding insoluble materials which are aluminium hydroxide ($Al(OH)_3$) and ferric hydroxide ($Fe(OH)_3$) respectively. In the reaction, an acid is produced which reduces the pH of the solution and consumes alkalinity in approximately the ratio of 1 mg/l of alkalinity for each 2 mg/l of commercial alum (Sengupta and Hashim, 1996).

When aluminium salt is added to the water, it will almost instantly enter into a series of hydrolytic reactions with water to form a series of multivalent charged hydrous oxide species. These compounds are pH dependent, which means they may range from positively-charged at lower pH values and negatively-charged at higher pH values. The reactions can be represented as follows:

$$[Al(H_2O)_6]^{3+} + H_2O \rightleftharpoons [\,Al(H_2\,O)_5OH]^{2+} + H_3O^+ \quad (2.1)$$

$$[Al(H_2O)_5OH]^{2+} + H_2O \rightleftharpoons [\,Al(H_2\,O)_4(OH)_2]^{+} + H_3O^+ \quad (2.2)$$

These reactions can proceed until the neutral species $[Al(H_2O)_3(OH)_3]$ or a negatively-charged species $[Al(H_2O)_2(OH)_4]^-$ is formed (HDR Engineering, 1997).

Similarly, hydrolysis of iron can be seen as follows:

$$[Fe(H_2O)_6]^{3+} + H_2O \rightleftharpoons [\,Fe(H_2\,O)_5OH]^{2+} + H_3O^+ \quad (2.3)$$

which yields ferric hydroxo and polynuclear complexes (HDR Engineering, 1997).

From the study of these reactions, it can be seen that both hydroxide and hydrogen ions are involved. This shows the significance of pH value in coagulation, as pH of water is of vital importance in establishing the average charge of the hydrolysis products and consequently determine the coagulation effectiveness. When pH of the water increases, the density of positive charges decreases, which leads to weaker charge neutralization capability (Cao et al., 2010).

However, the usage of these metal or inorganic coagulants has imposed a problem of its own, mainly on the side health effects that may occur on human being and the expensive cost, which may be a downside to several developing countries struggling to afford them. Hence, researchers have developed an interest in studying and evaluating the performances of natural coagulants, which could be a great eco-friendly alternative to the current problems.

2.2.1.4 Natural Coagulants

Natural or organic coagulants have been known to have several advantages in the treatment of wastewater. Among them are the production of biodegradable sludge, availability in local region and low in cost besides virtually toxin-free. Natural coagulant could be plant-based and non-plant based. The use of plant materials as natural coagulants in the reduction of turbidity in wastewaters has long been proposed since the ancient time, and has been recently making a comeback in the emerging economies. Such plant-based coagulant that has been extensively studied is *Moringa oleifera*, which is a multipurpose tree with considerable potential and its cultivation being actively promoted in many developing countries (Shan et al., 2017). On the other hand, the application of natural coagulant derived from non-plant based could be seen in the treatment of wastewater using chitosan, which is a partially deacetylated polymer obtained from the alkaline deacetylation of chitin, a biopolymer extracted from shellfish sources.

i) *Moringa oleifera* seeds

M. oleifera, a pantropical plant, is one of approximately thirteen species belonging to the monogeneric Moringaceae family. The property of *M. oleifera* that permits its ability to function as a coagulant is due to the presence of cationic protein called 'lectin', which is an active ingredient specifically found in its seed. The efficiency of *M. oleifera* as a natural coagulant in the treatment of several types of wastewaters has been shown in numerous researches (Ugwu et al., 2017; Shan et al., 2017; Ruelas-Leyva et al., 2017; Hendrawati et al., 2016; Dehghani and Alizadeh, 2016). Ugwu et al. (2017) has studied the effectiveness of the seeds in the treatment of domestic wastewater, comparing it with the usage of alum. The results obtained has been fairly impressive, with almost all parameters (pH, BOD, nutrients, hardness and coli form) showed values within WHO tolerable level with less sludge production compared to alum. *M. oleifera* seed flour has also demonstrate capability in removing heavy metals in river water, agricultural wastewater, and mixed wastewater, with Pb removal of up to 95% and Mn removal of up to 90% (Ruelas-Leyva et al., 2017). Mateus et al. (2017) has studied the usage of the coagulants in the treatment of dairy wastewater treatment with the aid of membrane filtration. The results obtained are high removal efficiencies of organic matter, color, and turbidity with permeate generated within this treatment meeting all the standards required for reuse.

ii) **Chitosan**

The development of chitosan-based materials as useful coagulants and flocculants is an expanding field in the area of water and wastewater treatment. Their coagulation and flocculation properties, which is based from the protonation of the chitosan amine group can be used to remove particulate inorganic or organic suspensions, and also dissolved organic substances. Khodapanah et al. (2013) has studied the efficiency of chitosan in surface water clarity using different initial pH and comparing it with other natural and inorganic coagulants. The results have shown that chitosan is on par with the other well-known coagulants, with good turbidity removal and tolerable pH value of discharged water (according to WHO (2011) guideline value). Bergamasco et al. (2011) has studied the potential application of chitosan as natural coagulant in CF-UF hybrid processes for treating drinking water with relatively high turbidity and the obtained result showed higher quality of permeate released after the ultrafiltration. Chitosan can also be combined with other coagulants to achieve higher efficiency of COD, suspended solids and heavy metal removal (Zeng et al., 2008).

2.2.2 Flocculation

The second stage of physico-chemical treatment is flocculation, which is the formation of settleable particles from destabilized colloidal-sized particles. Flocculation occurs by chemical bridging or physical enmeshment mechanism, with its primary force being electrostatic or inter-ionic. This process is obtained by gentle and prolonged mixing, which convert the submicroscopic coagulated particles into discrete, visible suspended particles called are pinflocs .These flocs will continue to build with additional collisions and form macroflocs, which are large enough to settle rapidly under the influence of gravity. Coagulant aids may also be added to help bridge, bind and strengthen the flocs, thus increase its settling rate.

2.2.2.1 Floc Formation Mechanism

To understand the mechanism of floc formation, it is important to know the physical and chemical behavior of the colloids initially present and of the colloids formed from the external addition of aluminium or iron salts.

The negatively-charged colloids present in the turbid water induce the formation of electrical double layers, and the stability of the colloidal system is established by the repulsive force existed in the interaction between the double layer (Faust and Aly, 1998). Upon addition of coagulants to the water, the electrostatic attraction between the negatively-charged particles and the positively-charged hydrolysis products enhances the adsorption of coagulant species onto the surface of the turbidity particles, which will be “coated” with coagulant. The result is the reduced electrical charges on the particles, which may vary from slightly negative to neutral to slightly positive depending on the coagulant dose and pH. The suspension is considered to be destabilized, which ease the flocculation process where particles agglomerate to settleable sizes. This further enhanced by slow mixing or slight turbulence which promotes particle collision.

In addition, physical and mechanical process may be taking place concurrently (enmeshment). In the process of this formation, and during its settling, the floc may physically enmesh the turbidity particles and simply act like a “sweep” as it settles.

On the other hand, there is a slightly modified mechanism for destabilization of hydrophilic colloids, which are colloids that contain polar groups such as hydroxyl, carboxyl or phosphate groups that are negatively – charged . For instance, natural colour in water is an example for hydrophilic colloids. These colloids are able to combine chemically rather than by electrostatic force with the positively-charged coagulant hydrolysis products, forming insoluble products that is electrically neutral or destabilized (HDR Engineering, 1997)

There are three major mechanisms of flocculation (HDR Engineering, 1997):

- *Perikinetic*, which is the aggregation of particles resulting from Brownian diffusion. The driving force for this type of particle movement is the thermal energy of the fluid, which causes continuous bombardment of particles with the surrounding fluid molecules. This is predominant for sub-micron particles.
- *Orthokinetic*, which is the aggregation of particles by induced velocity gradients in the fluid. This is the predominant mechanism in water treatment, where suspended

particles will follow the streamlines with different velocity which eventually leads to interparticle contacts. The velocity gradient is directly related to the energy dissipated into the water (via mixing).

- *Differential settling,* which is caused by the different settling velocities of particles.

Coagulation and flocculation occurs in successive steps, with coagulation happens first to allow particle collision and then followed by flocculation to promote growth of flocs. After both of the processes happen successfully, sedimentation will occur prior to filtration. If coagulation is incomplete, flocculation step will be unsuccessful, which hindered the progress of the following steps.

2.3 Factors Affecting Coagulation and Flocculation

Efficiency of coagulation−flocculation treatment is highly dependent on the process parameters of the process. Optimization of significant parameters, such as coagulant or flocculant dosage, initial pH, settling time, mixing time and temperature are necessary to ensure a more effective coagulation−flocculation performance.

2.3.1 Coagulant or Flocculant Dosage

Optimal coagulant dosage is established as a significant and critical factor for effective coagulation−flocculation performance. This optimal dosage varies with the molecular weight, ionic character and ionic degree of the coagulant. Inadequate or excess dosing of coagulants or flocculants may result in poor coagulation-flocculation performance. Overdosing of coagulants can give charge reversal and restabilisation of colloids or lead to insignificant changes in the quality of water treated (Bazrafshan et al., 2015). Overdosing of coagulants can be identified when the applied dosage reaches an inflection point known as critical coagulation concentration, which when the dosage is applied beyond this point, insignificant effect may be observed. This is because sufficient ionic strength derived from high concentration of counterions will allow for double layer compression. On the other hand, particle restabilisation may occur when bridge formation between adjacent particles is prevented due to lack of adsorption sites as most of the sites are already occupied by polymeric species.

Besides that, coagulant dosage also highly influences the mechanism of particle destabilization. Smaller dosage of metal salts tend to destabilize particles through effect of ionic strength, which cause double layer compression by charged counterions. Sweep-floc coagulation, on the other hand, is predominant when high coagulant dosage is applied.

To indicate optimum coagulant or flocculant dosage needed for wastewater treatment, the use of zeta potential is proposed. Zero zeta potential means that the coagulant or flocculant dosage is at the optimum level ,while particle restabilisation will occur when the flocs potential shift from negative to positive potential (Wei et al., 2015).

2.3.2 Initial pH of Wastewater

Most coagulants and flocculants require pH adjustment for effective treatment. Generally, optimum pH in the coagulation process is specific to the type of coagulant used in the treatment, with specific pH range catering to specific type of wastewater and coagulant used in order to achieve high coagulation efficiency. The destabilization of the colloidal particles are made possible through addition of chemicals such as acid or alkali, which promotes electrostatic attraction as the interparticle force between the particles is eliminated due to reduced surface charges (Teh et al., 2016).

Most inorganic coagulants perform in pH ranging from pH 3 to 9, where around this range the formation of the most effective hydrolysis species of the coagulant is induced. The predominant charges during coagulation tend to be positive at lower pH and negative at higher pH. As for natural coagulant from majority of plant seeds, the coagulation performance is usually effective at lower pH as the particles are prone to coalesce into flocs at this pH range owing to the presence of positively loosed charged particles to bind with negatively-charged colloids (Renault et al., 2009).

2.3.3 Settling Time

The efficiency of the solid−liquid separation process is highly influenced by the characteristics of flocs, as the flocs act as a medium to transport suspended particles to the bottom of wastewater through settling and separation from the treated effluent. Production of small flocs is not ideal due to their fragility, lower settling velocity, and difficulty to separate them from the effluent. The capability of flocs to settle usually depends on the type of coagulant or/and flocculant used in treatment and also, the type of wastewater.

In general, addition of flocculants are much more effective in the rapid settling of flocs when being compared to coagulants, as it aids the process of clumping and formation of flocs from the destabilized particles .The size of flocs highly influenced its settling capability. The predominant mechanisms for this process are the adsorption of polymers and polymer bridging which assist the formation of macroflocs for rapid settling.

2.3.4 Mixing Time

Mixing, which is achieved via stirring, is a crucial step in water and wastewater treatment via coagulation−flocculation process. There are two types of mixing: fast and slow. The main purpose of fast mixing is to ensure uniform dispersion of coagulant into effluent for efficient treatment while slow mixing is to encourage the flocs to form and grow to a size which will easily settle. Fast and slow mixing time has an effect on the size and strength of formed flocs. Relatively shorter fast mixing time allowed for larger floc growth, but the flocs will be less compact or have less shear resistant, whereas extended fast mixing time produces stronger flocs but smaller in size. This phenomenon could be explained by the presence of lower collision efficiency of small flocs and floc breakage due to extended high shear rate (Wei et al., 2015). As for slow mixing, extended slow mixing will lead to an increase in floc size (Ayoub et al., 2011). Mixing time has an important role in ensuring successful collision efficiency by allowing ample time for aggregates to restructure into a more compact and shear resistant flocs.

2.3.5 Temperature of Wastewater

Temperature affects the coagulation and flocculation performance by influencing the particle transport and collision rate by varying density and viscosity of the suspension at different temperatures. The effectiveness of each type of coagulant of flocculant varies with temperature. At lower temperatures, the performance of metal coagulants such as alum is less effective due to decreased hydrolysis and precipitation kinetics compared to that of readily hydrolyzed coagulants such as polyaluminum chloride. Low temperature hinders the aggregation rate of flocs, producing irregular and less compact besides slowing down perikinetic collision (Xiao et al., 2009). Similarly, there is poor formation and settling of flocs due to the convection currents generated from high heat and an increase of kinetic energy at very high temperature (Shak and Wu, 2014).

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Materials and Chemicals

Throughout the empirical investigation on coagulation and flocculation treatment on fish farm wastewater, different types of materials and chemicals were employed. Those components involved in the experimental studies are listed in Table 3.1 with some additional information.

Table 3.1 List of materials and chemicals used

Materials/Chemicals	Chemical Formula	Purpose
Moringa oleifera Seeds	-	Natural Coagulant
Chitosan Powder (Medium Molecular Weight)	-	Natural Coagulant
Hydrochloric Acid	HCl	pH Adjuster
Sodium Hydroxide	$NaOH$	pH Adjuster

3.2 Equipment and Instrumentations

All the equipments used in this research study are listed in the Table 3.2. These equipments helped the experiment being conducted efficiently and smoothly.

Table 3.2: List of equipments used in this experiment

Equipment	Model	Usage
Jar Test Equipment	-	To conduct jar test experiment
Photometer	LOVIBOND Maxidirect MD600	To determine the ammonia concentration and COD of water samples
Turbidity meter	HANNA HI 93703	To determine the turbidity of water samples
Spectrophotometer	HACH DR2800	To determine the nitrate and phosphate concentration of water samples
pH Meter	HANNA HI 2020-01	To adjust/determine the pH of water samples

3.3 Design of Experiment (DOE)

Design of Experiment (DOE) is systematic planning and studies to determine the relationship between the experimental variable and the effect of the response. There are several techniques of DOE, including best guess approach (trial and error), One Factor at a Time (OFAT) and Response Surface Methodology (RSM).

In this experiment, a standard Response Surface Methodology (RSM) design using central composite design (CCD) was employed to study the parameters involved in the coagulation and flocculation process:

i. X_1 , Coagulant dosage(mg/l)

ii. X_2, pH of wastewater

iii. X_3, Mixing time(min)

iv. X_4, Settling time(min)

A central composite design face-centered (CCD) were used throughout the two sets of experiments (chitosan and *M. oleifera*). Table 3.3 summarizes the levels for each factor involved in the design strategy.

Table 3.3: Range of variations of the parameter used in CCD

Parameters (Factors)		Coded Variables Level		
		Low (-1)	Center (0)	High (+1)
X_1: Coagulant dosage (mg/l)	*M. oleifera*	400	800	1200
	Chitosan	100	450	800
X_2: pH of wastewater		4	7	10
X_3: Mixing time (min)		5	10	15
X_4: Settling time (min)		10	20	30

Based on the four variables studied, there were 8 axial or star points (α=+1) located at the center and both extreme levels of the experimental models and 6 central, replicates of the central point. There are 30 experiments for chitosan and 30 experiments for *M. oleifera*, as calculated in Equation 3.1:

$$N = 2^n + 2n + n_c \quad (3.1)$$

where N is the total number of experiments required, n is the number of factors and n_c is the number of replicates at the center point.

Experiments were initiated as a preliminary study for determining a narrower range of coagulant dosage prior to designing the experimental runs. Accordingly, coagulant dosages from 10 mg/l were tried and the increments continued until appreciable reductions in turbidity were observed. As a result, the study ranges were chosen as coagulant dosage 100-800 mg/l for chitosan and 400 – 1200 mg/l for *M. oleifera*. There is one response of each experiments which is turbidity (NTU). The response is used to develop an empirical model which