

**BIOCOAGULANT FROM BIOMASS FOR  
WATER TREATMENT**

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**BIOCOAGULANT FROM BIOMASS FOR WATER TREATMENT**

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**A project report submitted in partial fulfilment of the  
requirements for the award of Bachelor of Civil  
Engineering with Honours**

**Lee Kong Chian Faculty of Engineering and Science  
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**April 2024**

**DECLARATION**

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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**APPROVAL FOR SUBMISSION**

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Approved by,

Signature



Supervisor

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: 18 April 2024

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## ABSTRACT

The escalating demand for sustainable and environmentally friendly water treatment solutions has sparked interest in exploring alternative coagulants derived from natural sources. This study investigates the efficacy of biomass extracted from *Annona* seeds as a biocoagulant for treating turbidity in wastewater. The effectiveness of the *Annona* seed biomass is compared against alum and ferric chloride through various operating conditions such as turbidity removal efficiency and dosage requirements. Additionally, the study examines the influence of operating conditions such as pH, mixing intensity, and stirring duration on the coagulation process. *Annona* seed will be grinded into powder, sieved through a 2mm wire mesh, and left to dry overnight before mixing with distilled water. The concentration of all three coagulants will be modified to 1000mg/L to treat wastewater with a dosage of 30mL. Moreover, the kaolin wastewater will have a concentration of 100mg/L, and a working volume of 300mL is used throughout the study. The initial and final turbidity of wastewater will be measured in the testing, whereas the turbidity reduction efficiency will be calculated using the given formula. The research shows that all the coagulants struggle to fully perform when the wastewater is under high pH. When the pH value of wastewater reaches 11, the turbidity reduction of alum, *Annona* seed, and ferric chloride dropped to 11.09%, 13.01%, and 7.89%, respectively. This is because the coagulants are less effective at neutralizing the charges on particles at higher pH, resulting in reduced aggregation and poorer coagulation efficiency. Additionally, the turbidity reduction rate of all three coagulants performs better when the mixing time during fast-mixing processes is longer. The turbidity reduction rate of alum, *Annona* seed, and ferric chloride increases to 66.17%, 59.35%, and 51.55% when the high-speed mixing duration is at 40 minutes. For wastewater treatment under different mixing speeds, the turbidity reduction rate increases when the speed of the initial mixing process increases. The turbidity reduction rate of alum, *Annona*, and ferric chloride elevated to 50.55%, 45.77%, and 21.47%, respectively, at high-speed mixing of 250rpm for 15 minutes followed by slow-mixing speed of 40rpm for 30 minutes.

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## LIST OF SYMBOLS / ABBREVIATIONS

CO <sub>2</sub>	carbon dioxide
H <sub>2</sub> CO <sub>3</sub>	carbonic acid
HCO <sub>3</sub> <sup>-</sup>	bicarbonate ion
CO <sub>3</sub> <sup>2-</sup>	carbonate ion
-COOH	carboxylic acid group
OH <sup>-</sup>	hydroxyl group
H <sup>+</sup>	hydrogen ion
Al	aluminium
PAM	polyacrylamide
SDG	Sustainable Development Goal
COD	chemical oxygen demand
BOD	biochemical oxygen demand
PAS	potassium aluminium sulphate
SS	suspended solid
PFS	poly ferric sulphate
PAC	poly aluminium chloride
WTS	water treatment sludge
NTU	Nephelometric Turbidity Units
SEM	scanning electron microscope

## CHAPTER 1

### INTRODUCTION

#### 1.1 General Introduction

Water is an invaluable resource for every living organism as it is also essential for sustaining life and promoting development. However, the growing global population and rapid industrialization have created an alarming issue on freshwater sources. For instance, potential water shortage and pollution in water are the few alarming issues that are jeopardizing water resources. Dealing with the obstacles related to water treatment and purification has emerged as a critical issue, including both environmental preservation and public health. According to the United Nations (2022), over 85% of wetlands have vanished in the past 300 years and if this issue was not solved immediately, the supply of drinking water for sanitation or consumption may end.

Wastewater treatment serves multifaceted roles crucial for both public health and environmental preservation. Primarily, it acts as a frontline defence against waterborne diseases by removing harmful pathogens, bacteria, and viruses from wastewater, thus safeguarding public health. Moreover, by eliminating pollutants like nutrients, organic matter, heavy metals, and chemicals, treatment processes prevent contamination of natural water bodies, reducing adverse impacts on aquatic ecosystems and biodiversity. Furthermore, reclaimed wastewater has the potential to be repurposed for a range of non-drinking applications like irrigation and industrial use. This approach helps preserve freshwater reservoirs and promotes sustainable water management strategies. Furthermore, wastewater treatment ensures compliance with regulatory standards, mitigating legal and environmental risks while fostering environmental responsibility and stewardship. Consequently, wastewater treatment plays a pivotal role in promoting clean and healthy environments, supporting economic growth, and advancing sustainable development efforts globally.

Traditional water treatment methods often rely on the use of chemical coagulants to remove suspended particles and impurities from water. However,

conventional coagulants, such as aluminium sulphate and ferric chloride, have drawbacks such as excessive costs, potential health hazards, and the creation of toxic sludge. Biocoagulants that are derived from biomass play a key role in overcoming these issues in a more sustainable approach that minimizes the adverse impact on the environment. Biomass consists of a variety of organic materials, providing an abundant and renewable resource that can be harnessed for environmentally friendly water treatment solutions.

In older times, traditional societies have historically used natural materials like seeds, nuts, and plant extracts to purify water, proving that the concept of utilizing biocoagulants is the perfect way to solve the issue related to water resources. In this modernized era, the current technology and scientific understanding have become more advanced with the help of scientists and researchers. Thus, specific biocoagulant compounds can be identified and extracted easily from various biomass sources, optimizing their effectiveness and applicability for large-scale water treatment processes.

According to Usman, et al (2023), plant-derived coagulants are recognized as a cost-efficient and eco-friendly method for the coagulation and flocculation procedures used in water treatment. The seed of *Annona* fruit otherwise known as *Annona diversifolia* is one of the plant-based biomasses that can be extracted into biocoagulants deemed as a promising potential for water treatment applications. However, the chemistry and mechanisms behind the biomass coagulation properties are complicated along with their efficiency in removing distinct type of contaminants from water matrices are proved to be complex. Other than that, multiple challenges are associated with their implementation, and potential areas for further research and improvement cannot be overlooked.

## **1.2 Importance of the Study**

The significance of obtaining biocoagulant from biomass for water treatment study lies in its capacity to transform water purification methods and tackle numerous critical environmental and social issues. The importance of the study include:

- i. This water treatment approach provides a sustainable and environmentally friendly substitute for conventional chemical coagulants. It aims to decrease the environmental footprint of water treatment procedures and lessen dependence on finite resources.
- ii. Traditional chemical coagulants often generate toxic by-products that can pollute water bodies and harm ecosystems. Biocoagulants have the potential to minimize water pollution by creating cleaner waterways and healthier aquatic environments.
- iii. Implementing biocoagulants can lead to cost savings in water treatment operations. Many biomass sources are locally available and can be obtained at lower costs compared to chemical coagulants, making the treatment process more economically viable, particularly in resource-constrained regions.
- iv. Biocoagulants produce fewer harmful by-products which can help reduce potential health risks associated with consuming chemically treated water. This study, therefore, contributes to safeguarding public health and well-being.
- v. The study of biocoagulants from biomass fosters research and innovation in the field of water treatment. This can lead to further discoveries and improvements in the efficiency and effectiveness of biocoagulants, benefiting the water treatment industry.
- vi. Biocoagulants are important in improving wastewater treatment processes. By reducing half of the proportion of untreated wastewater and enhancing recycling and safe reuse practices, this study supports more efficient wastewater management and resource conservation.

The potential benefits of employing biocoagulants in water treatment also align with the Sustainable Development Goals (SDG). According to SDG, enhancing water quality must be done without creating pollution, eradicating dumping, and minimizing the release of hazardous chemicals and materials, cutting the proportion of untreated wastewater in half, and promoting global recycling and safe reuse practices (THE GLOBAL GOALS, 2022).

### 1.3 Problem Statement

Despite multiple benefits in utilizing biomass biocoagulants to improve water treatment, it also has disadvantages. Due to the field of study in using biocoagulant from biomass for water treatment is new, limited data and knowledge on the various processes are hard to obtain due to the lack of extensive research and understanding of the specific biocoagulant compounds. Optimal extraction processes, coagulation properties, and effectiveness of these biocoagulants in treating several types of water contaminants are equally important in conducting this study.

Furthermore, the variability in the composition and performance of biocoagulants from biomass is also one of the limitations of conducting the research. This is because the composition and effectiveness of biocoagulant may depend on factors such as growing conditions, geographical location, seed maturity, and plant species. As a result, this variability can lead to inconsistent coagulation performance, making it difficult to establish standardized treatment procedures or methods. Comprehensive studies must be conducted to ensure consistent performance and ideal results. In addition, the dosage and efficiency of implementing biocoagulants such as *Annona* seed as a substitute for chemical coagulants is still unknown. This is because sludge production will increase if too many *Annona* seeds are extracted for coagulation.

Moreover, the scalability and cost-effectiveness may pose challenges in large-scale implementation. While biocoagulants from biomass have the potential to be more sustainable and economically feasible than chemical coagulants, there may be initial costs involved in setting up extraction and processing facilities, and transportation, especially in remote or resource-constrained areas. Other than that, the contaminant removal efficiency of biocoagulants from biomass is predetermined due to insufficient research or tests having been conducted by past researchers. As mentioned before, *Annona* seeds proved to have certain coagulation properties, their effectiveness in eliminating numerous types of contaminants might be limited

compared to chemical coagulants such as ferric chloride and aluminum sulphate.

#### **1.4 Aim and Objective**

This report aims to explore the potential and effectiveness of biocoagulants from biomass as a sustainable and eco-friendly alternative for water treatment processes. The study aims to investigate the extraction methods and coagulation properties to understand their applicability in purifying water and removing contaminants. The objective of this report include:

- i. To examine the turbidity reduction rate of coagulants under different pH levels in wastewater.
- ii. To assess the efficiency of coagulants in reducing turbidity of wastewater based on different initial mixing duration.
- iii. To analyze the effectiveness of coagulants under different initial mixing intensities.

#### **1.5 Scope and Limitation of the Study**

The primary scope of the study is proving the biocoagulants from biomass are suitable for future water treatment. Further research will be carried out to learn more about the chemical and physical processes involved in the coagulation properties of biocoagulants from biomass and its mechanisms in removing contaminants from water. Efficiency in removing distinct types of contaminants such as suspended solids, turbidity, heavy metals, organic matter, and microorganisms will be assessed in this study. Apart from that, the scope of the study will compare the amount or level of chemical residues between chemical coagulants and biocoagulants from biomass as to which of them is safer for both environmental discharge and human consumption.

However, there are several limitations on using biocoagulants from biomass as the method in curbing water contaminants that must be considered. The requirements prior to the treatment process must be outlined. For example, the extraction of biocoagulants from biomass might require specific ways to extract the coagulating agents whether it is needed to be grounded or soaked. Moreover, potential side effects are yet to be discovered in utilizing

biocoagulants from biomass as a substitute for chemical coagulants due to the research of coagulation properties is still ongoing.

Furthermore, the environmental impact of using biocoagulants from biomass for water treatment must be assessed. For instance, the sustainability of sourcing the various biomass and impact of any waste generated during the extraction processes. In addition, the storage and shelf life of using natural coagulants such as *Annona* seeds may decrease over time which can affect the coagulation performance. Hence, proper storage conditions and limitations in shelf life can cause the practical application to be used in a larger scale water treatment become more challenging.

### **1.6 Contribution of the Study**

The contribution of this study is to determine whether the biocoagulants from biomass can prove to be a better coagulant compared to synthetic coagulants in terms of its effectiveness, eco-friendly, and efficiency. This study provides in-depth research and understanding of promoting *Annona* seeds as a natural coagulant that can be used in the current water treatment to society.

### **1.7 Outline of the Report**

A total of five chapters is included in this report which are introduction, literature review, methodology and work plan, results, and discussion along with conclusions and recommendations. References and appendices will be included after the main chapter. Readers are encouraged to read and study the report following its sequence.

Introduction is the first chapter of the report which consists of background information of biocoagulants from biomass and the role of clean water in our world. Moreover, the importance of the study, problem statements, aim and objective, scope and limitations of the study, and contribution of the study are included in this chapter.

The report is followed by Chapter 2 which will dwelled into the literature review. Various past research on water treatment will be reviewed in this chapter. For instance, the usage of plant-based coagulant, natural coagulant, and *Annona* seeds that have been utilized in the water treatment.

Certain methodology of the water treatment that involve organic materials in water treatment will be discussed as well such as microalgae cultivation and biomass filtration. However, the main variables that will be discussed is the effect of pH in terms of influencing the turbidity in water.

Chapter 3 will further discuss the methodology and work plan of the Annona seeds from biomass to be used as a coagulant for water treatment. Two other chemical coagulants which are ferric chloride and potassium aluminium sulphate (PAS) otherwise known as alum will be introduced in the experiment. The purpose is to compare the result between natural coagulant and chemical coagulant in tackling turbidity in water. In the experiment, jar testing will be conducted to determine the turbidity reduction in water where pH of the turbid water will play a significant role.

In Chapter 4, the results that had been obtained from the turbidity reduction testing will be compared and discussed. Three distinct types of experiment results will be obtained from three distinct types of coagulants which are the testing of different pH water, different mixing speed, and different mixing time. All the results will be presented in a precise tabulation and graphical method which will be presented in this chapter. The discussion will be compared and studied from the resources of past research.

Lastly, Chapter 5 will include the conclusion and recommendations regarding the experiment. The conclusion will summarize the aim of the study of the topic and outline the objectives accordingly. Several recommendations will be given and emphasised for improving the studies in the future.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Introduction

Although coagulation does not eliminate all bacteria and viruses from water, this process still holds the utmost importance in the water treatment process. The effectiveness of coagulants is crucial in the initial phase of water treatment procedures because they play a vital role in the removal of various particles, including dissolved organic carbon, which can create difficulties for disinfection (Safe Drinking Water Foundation, n.d.). Coagulation is a water treatment method that involves the manipulation of the electric charges of suspended particles in water to eliminate solids. Coagulation introduces minuscule, strongly charged molecules to water, causing the disruption of charges on suspended particles, colloids, or oily substances (Bradley, 2022). Conventional physical methods used to treat colloidal wastewater, like sedimentation, prove insufficient due to the presence of particles ranging from  $0.1\mu\text{m}$  to  $1\text{nm}$  within colloidal systems (Barrera-Díaz, Balderas-Hernández, and Bilyeu, 2018). Figure 2.1 below shows one of the few tests that are required to be carried out in the coagulation process for water treatment purposes.

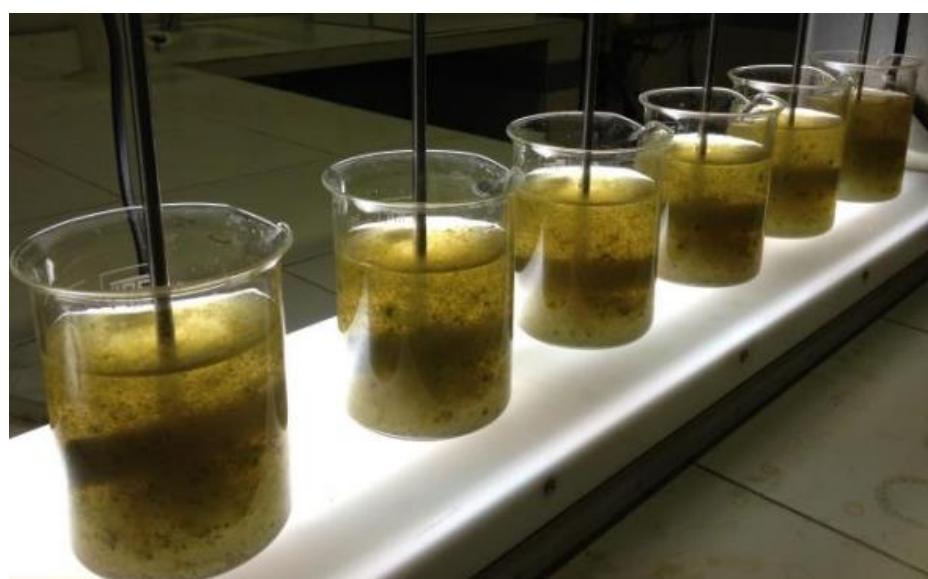


Figure 2.1: Jar Testing for Coagulation in a Water Treatment Facility.

In this time of age, chemical and synthetic coagulants are often used in the treatment of water due to its high efficiency, effectiveness, and large-scale capability. Aluminium sulphate stands as the most common chemical employed in wastewater treatment for coagulation purposes. Other frequently utilized coagulants include sodium aluminate, ferric sulphate, and ferric chloride. Other than that, synthetic coagulants could exhibit high charge densities on comparatively bigger molecules which is dependent on their production process, certain synthetic variations might function as flocculants (Greenwood and Greaves, 2022). The chemical coagulation process utilized in treatments represents one of the pivotal steps aimed at mitigating or diminishing turbidity, color, and microorganisms in water. However, numerous side effects and drawbacks can be found in utilizing chemical coagulants in current wastewater treatment.

On the other hand, the high performance of biocoagulants in water treatment can successfully remove pollutants like SS, COD, BOD, algae, and color, which stands in stark contrast to the performance of conventional metal-based coagulants (Kurniawan, 2020). Biocoagulants might even carry superior levels of pollutant removal efficiency compared to conventional flocculants. Most countries engaged in biocoagulant research are in tropical and developing regions. This phenomenon can be attributed to the abundant and diverse potential resources, particularly plants and crustaceans, that can be harnessed as biocoagulants due to the tropical climate conditions (Kurniawan et al., 2020).

The evolution of biocoagulants has reached a new height due to the introduction of biomass that can be extracted to be utilized as a biocoagulant in water treatment. The investigation of biocoagulants based on their capacity to enhance the efficiency of eliminating contaminants from water. These substances rely on natural polymers such as cellulose, mucilage, and collagen, deriving from sources like animals, plants, microorganisms, and bacteria (Aguilera Flores et al., 2022). Most of the biocoagulant that are extracted from biomass are plant-based such as banana pith, Jatropha curcas, Moringa oleifera seeds, and Annona diversifolia seeds.

## 2.2 Microalgae Cultivation

Algae are known as primarily aquatic and organisms with nuclei to carry out photosynthesis. They are categorized into macroalgae and microalgae. Algae are recognized as a sustainable energy resource because of their ability to grow quickly and yield a substantial biomass. Microalgae cultivation can be conducted using two methods, which are the suspended and attached growth systems that are suitable for both open and closed cultivation systems. In the attached growth system, a carrier medium serves as a substrate for microalgae adhesion, whereas the suspended growth system operates without requiring any medium (Economou et al., 2015). However, there are multiple methods to harvest microalgae with the help of biocoagulants.

### 2.2.1 Charge Neutralization

In the suspended growth system, microalgae harvesting is primarily accomplished through coagulation-flocculation and sedimentation techniques (Oladoja et al., 2020). Coagulation refers to the process of disrupting the negative charge of colloidal particles such as microalgae, whereas flocculation involves aggregating these neutralized particles into structures known as flocs (Gutiérrez et al., 2015). According to Bhalkaran and Wilson (2016), the process of harvesting microalgae through charge neutralization takes place when positively charged biocoagulants undergo hydrolysis upon dissolving in water and then interact with microalgae. Figure 2.2 below illustrates the harvesting mechanism of microalgae through charge neutralization.

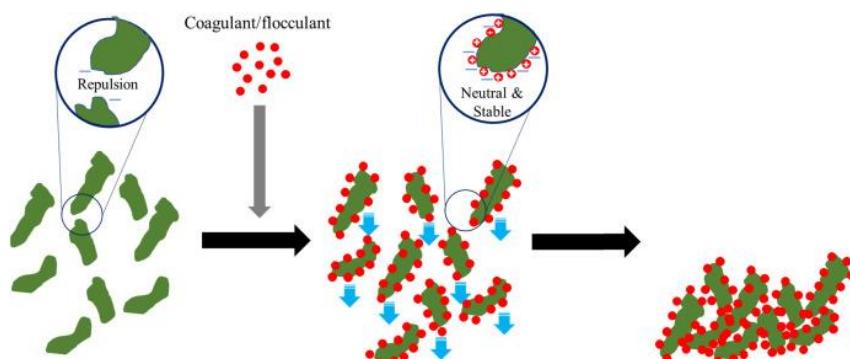


Figure 2.2: Microalgae Harvesting Mechanism through Charge Neutralization  
(Kurniawan et al., 2020).

### 2.2.2 Sweep Coagulation

Another frequently employed method for harvesting microalgae from the culture medium involves pH adjustment using metal hydroxides. With this approach, microalgae can be separated from the culture medium through processes like sweep coagulation. This phenomenon occurs when the pH of the system or pond is alkaline and employs metallic coagulants or flocculants, such as chitosan (Kurniawan et al., 2020). Sweep coagulation is the process whereby microalgae are precipitated simultaneously due to the formation of solid-state hydroxides or deprotonated chitosan after hydrolysis in an aqueous solution or because of the interaction between metals and hydroxide ions under basic pH conditions. Figure 2.3 depicted below illustrates the mechanism of microalgae harvesting using coprecipitation with metal hydroxides.

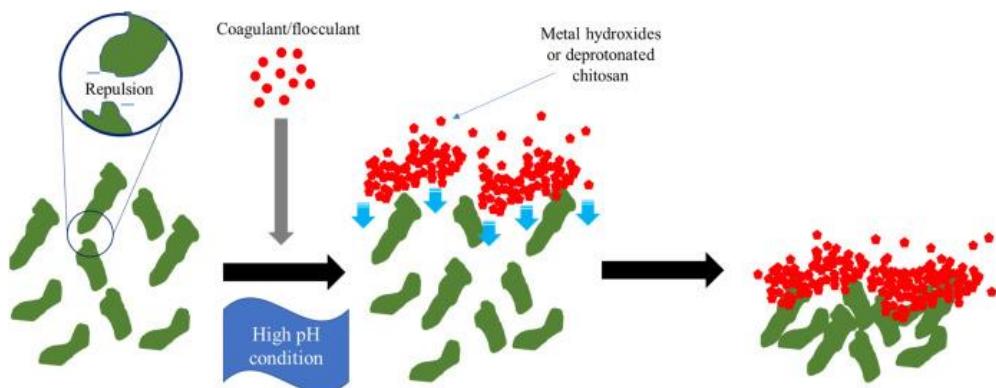


Figure 2.3: Microalgae Harvesting Mechanism through Sweep Coagulation (Kurniawan et al., 2020).

### 2.2.3 Auto Flocculation

Auto flocculation is triggered by adjusting the pH of the system, which happens when lime or similar alkaline compounds are introduced (Lananan et al., 2016). The negatively charged surface of microalgae becomes unstable under high pH conditions, modifying its isoelectric point (Li, Hu, & Zhu, 2021). Additionally, destabilization arises from the creation of calcium phosphate on the surface or cell of microalgae, prompting the organisms to

aggregate into flocs and subsequently settle. Figure 2.4 below will clarify the mechanism of harvesting microalgae with auto flocculation.

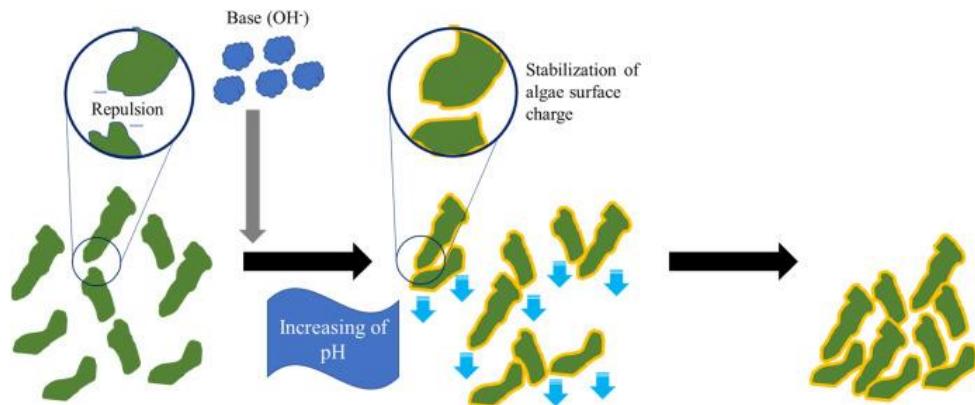


Figure 2.4: Microalga Harvesting Mechanism through Auto Flocculation (Kurniawan et al., 2020).

#### 2.2.4 Bridging

The most popular mechanism in microalga harvesting using biocoagulants is known as bridging, especially when polymer-based and starch-based compounds are used. In this process, biocoagulants serve as connectors between microalgae, leading to the formation of flocs with greater density than water (Kurniawan et al., 2020). This occurrence triggers the settling and subsequent separation of microalgae from the culture medium. Figure 2.5 shows the mechanism of microalga harvesting by using a bridging method.

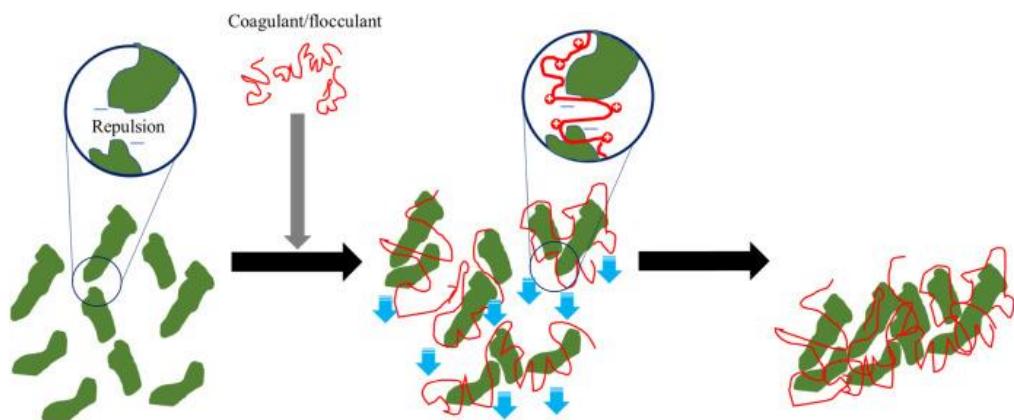


Figure 2.5: Microalga Harvesting Mechanism through Bridging (Kurniawan et al., 2020).

### 2.2.5 Adsorption-patch Flocculation

According to Gregory and Barany (2011), the application of biocoagulants results in their absorption into the pores of microalgae, causing alterations in the zeta potential at specific microalgal sites. The variation in zeta potential between the region of the microalgae where these biocoagulants are absorbed and other sections of the microalgae that maintain a negative charge due to potential differences leads to a phenomenon known as patch flocculation. This interconnected chain of organisms, formed by those microalgae that have adsorbed these substances, creates a denser community compared to the surrounding water (Hatta et al., 2021). Consequently, this promotes the settling of the biomass. This adsorption-patch flocculation mechanism is most prominent when harvesting using substances like chitosan or other coagulants and flocculants derived from faeces and fats from animals or leaves and seeds from plants with low molecular weight.

Because of their low molecular weight, these substances are unable to perform charge neutralization and instead tend to attach or adsorb to specific microalgal sites, acting as positive functional groups. This facilitates their interconnection with other microalgal cells (Czemierska et al., 2015). When the pH exceeds a value of 9, as observed with chitosan, it triggers distinct flocculation mechanisms due to the partial deprotonation of chitosan. This results in the formation of an interconnecting network that eventually sweeps microalgae along as coprecipitates. Figure 2.6 below illustrates the mechanism of microalgae harvesting using the adsorption-patch flocculation method.

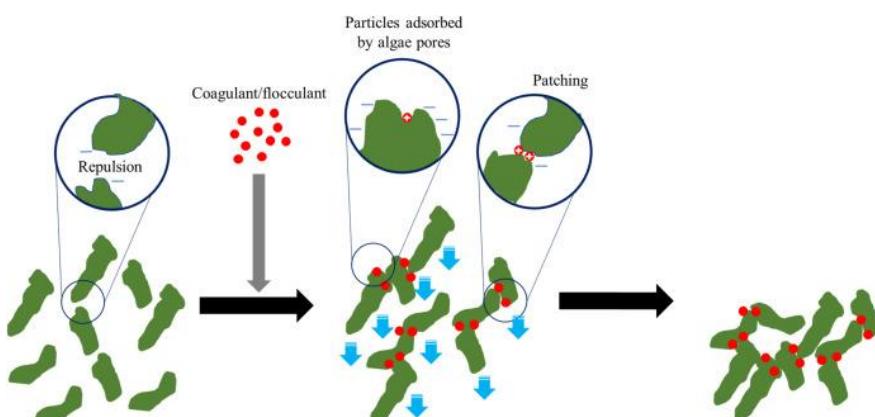


Figure 2.6: Microalgae Harvesting Mechanism through Adsorption-patch Flocculation (Kurniawan et al., 2020).

### 2.3 Effect of pH

Harvesting plays a pivotal role in the production of microalgal biomass for several important reasons. Among the various techniques employed to harvest and separate water from microalgal biomass, the most cost-effective approach has been the recovery from the aqueous medium through coagulation-flocculation, with its effectiveness significantly influenced by pH levels (Cassini et al., 2017). For example, cationic starch demonstrated superior recovery of microalgal biomass across a wider pH range. Conversely, the use of powdered seeds from *Moringa oleifera* and gum extracted from *Hibiscus esculentus* was effective in biomass removal but limited to an acidic pH range. Following the sedimentation of the microalgal biomass, the effluents exhibited impressive removal rates of phosphorus, nitrogen, biochemical oxygen demand, and chemical oxygen demand (Cassini et al., 2017). However, this effect was observed only when aluminium sulphate, cationic starch, and modified tannin were employed as coagulants.

Natural organic coagulants, which can function within a wide pH range have the potential to replace aluminium sulphate. This is a commonly used reference coagulant in microalgal biomass recovery. In addition, natural organic coagulants will not compromise the efficiency of microalgal biomass harvesting and the quality of the final effluent. The coagulation-flocculation procedure encourages the clustering of microalgae by introducing coagulants such as electrolytes. Electrolytes can take the form of metal salts, naturally occurring or artificially produced polymers, and by adjusting the pH value. This results in the creation of flocks that help in the settling of microalgal biomass.

According to Cassini et al. (2017), research has confirmed that microalgae typically employ two inorganic carbon species, namely  $\text{CO}_2$  and  $\text{HCO}_3^-$ . The assimilation of  $\text{HCO}_3^-$  is facilitated by the enzyme carbonic anhydrase, which is responsible for extracting  $\text{H}^+$  ions from the water medium, resulting in an elevation of pH levels. At elevated pH values, where above pH 9, a huge portion of the inorganic carbon exists in the form of carbonate ( $\text{CO}_3^{2-}$ ), which microalgae also utilize in conditions characterized by high

alkalinity. Additionally, this process contributes to the removal of  $H^+$  ions from the water, consequently leading to an increase in pH levels. Equation 2.1 illustrates the equilibrium between  $CO_2$  and  $CO_3^{2-}$ .



Throughout the night, respiration and various metabolic activities replenish the  $CO_2$  levels in the medium, resulting in a decline in pH values and concluding the pH cycle. The introduction or removal of  $CO_2$  does not have a direct impact on alkalinity, but it does exert influence on pH levels, contributing to significant daily pH fluctuations. Given these pH fluctuations during the daily cycle of microalgal growth, the selection of the coagulant type emerges as a critical factor. Therefore, it is advisable to opt for an optimized microalgal biomass recovery process via coagulation-flocculation based on the pH range of the selected medium.

#### 2.4 **Annona Diversifolia**

*Annona diversifolia*, commonly known as "ilama" or "papausa," is a tropical fruit-bearing tree that belongs to the Annonaceae family. It also includes other well-known fruits like cherimoya (*Annona cherimola*) and sugar apple (*Annona squamosa*). Despite the various nutrient content, its seed also contain biomass which is a natural coagulant can be extracted and supplied for biodiesel feedstock and even water treatment. Water treatment is highly essential especially when the treatment process focus in an eco-friendly approach. Additionally, it also has the tendency to reduce the use of chemicals and the generation of harmful sludges in water and wastewater treatment facilities.

The seeds of *Feronia limonia*, *Carica papaya*, *Prunus armeniaca*, *Persea americana*, *Mangifera indica*, and the peels of *Citrus sinensis* have the potential to reduce water turbidity by as much as 80%. Additionally, extracts derived from *Annona diversifolia* seeds have been found to contain polysaccharides capable of reducing both chemical oxygen demand and turbidity in wastewater (Usman et al., 2023). These seed extracts exhibit

functional carboxyl and hydroxyl groups, which are strongly associated with their ability to aggregate suspended particles. The pH and turbidity of cloudy water are assessed using a portable pH meter (HACH Sension 1) and a turbidity meter (HACH 2100Q), respectively.

In the coagulation process, pH can impact the electrical potential gradient on the surface of particles. When *Annona diversifolia* seed extract coagulant is added during coagulation and flocculation, it destabilizes the negative surface charge of particles in the water, enhancing the London–Van der Waals force of attraction between particles, thereby promoting aggregation (Usman et al., 2023). At higher pH levels, the concentration of  $H^+$  ions decrease. This results in negatively charged functional groups present in *Annona* seed such as hydroxyl  $OH^-$  and carboxyl groups where the negatively charged kaolin surface generates a repulsive force (Usman et al., 2023). This repulsion allows the coagulant molecules to extend and form loops and tails, facilitating bridging mechanisms and the formation of large, open-structure flocs. Figure 2.7 illustrates the schematic diagram depicting the adsorption and bridging mechanisms.

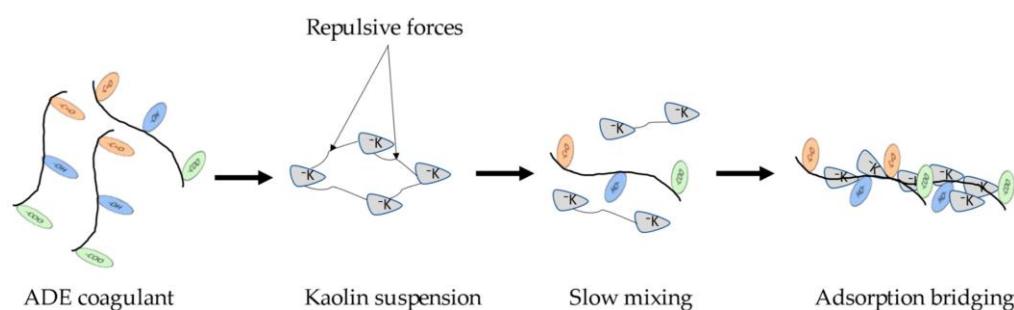


Figure 2.7: Diagram Illustrating the Mechanisms of Coagulant Adsorption and Bridging. (Usman et al., 2023).

## 2.5 Dewatered Sludge

In the water treatment process, the treatment of water results in the generation of significant quantities of 'alum sludge', a by-product traditionally viewed as waste and disposed of in landfills (Nguyen et al., 2022). However, challenges such as reduced access to landfill sites, increasing disposal expenses, and the implementation for zero-waste initiatives will demand an alternative approach to sludge management and disposal. Henceforth, it is vital to study the typical

characteristics of WTS, current disposal practices, and sustainable resource management options, including sludge reuse, recycling, and recovery strategies. Additionally, other aspects such as social, economic, and environmental advantages and obstacles associated with implementing sustainable resource management practices concerning WTS within the framework of the Circular Economy cannot be overlooked. Figure 2.8 below shows the diagram of the water treatment process using water treatment sludge.

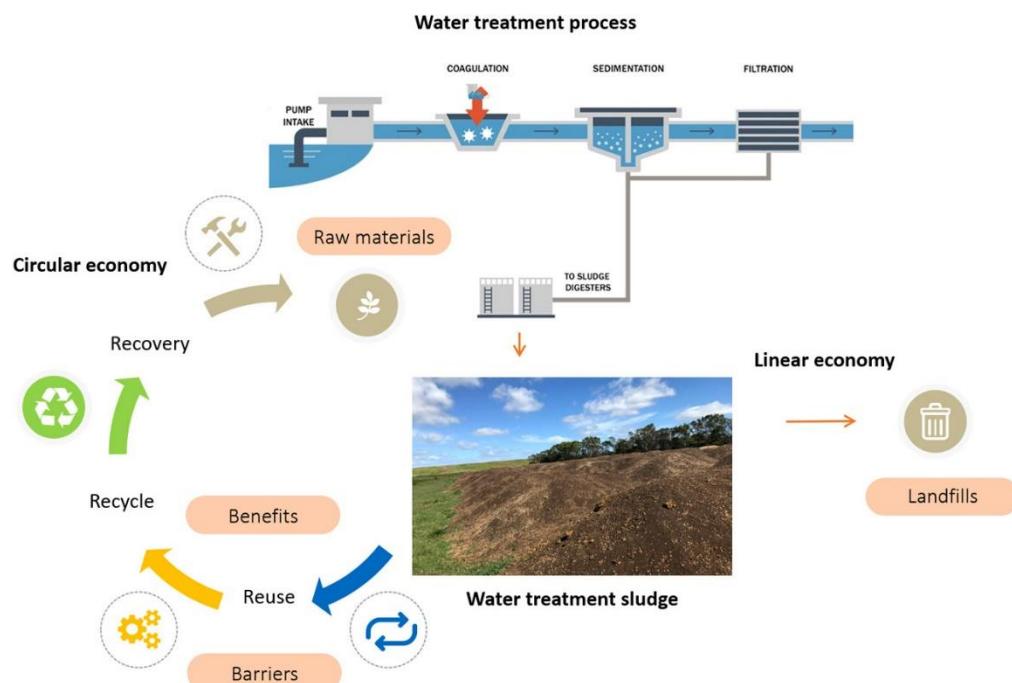


Figure 2.8: Schematic Diagram of the Water Treatment Process Using Water Treatment Sludge.

Sludge, a residual product of the wastewater treatment process, is commonly repurposed as fertilizer or WTS to purify wastewater. However, utilizing sludge for fertilization carries certain drawbacks that pose risks of environmental contamination or pollution, and potential harm to living organisms. This is because sludge may contain heavy metals, microbes, impurities, and chemicals, which can adversely affect the environment and living organisms if not managed properly. The sludge obtained from water treatment plants primarily comprises hydrolysis byproducts of utilized

coagulants, remains of other chemicals employed during treatment such as polymers, organic compounds including humic colloids, microorganisms, and plankton, along with various other substances that have been extracted from the water such as insoluble or poorly soluble metal salts (Kuldeyev et al., 2023). WTS is typically alkaline and contains aluminium or metal oxides, which carry a positive charge. This suggests that WTS can act as an effective coagulant. In fact, several countries had already begun implanting WTS as their primary method in providing clean water to their inhabitants.

According to Zainol et al. (2022), the presence of ionic charges of distinct O-H bond in WTS helps in forming various soluble species like  $\text{Al(OH)}^{2+}$  or  $\text{Al(OH)}^{3+}$ . These species serve as effective coagulants by exhibiting strong adsorption onto the surfaces of negatively charged colloids. However, there is a major step that needs to be included before carrying out turbidity reduction testing using WTS which is the acidification of sludge. Acidification was employed to reduce the high aluminium content in the WTS. This involved a chemical reaction between the aluminium hydroxide present in the WTS and sulfuric acid, resulting in the formation of aluminium sulphate. This aluminium sulphate can then serve as an effective coagulant in wastewater treatment plants. Other than that, the addition of WTS, specifically alum sludge containing insoluble aluminium hydroxides, to the raw effluent during the pre-treatment stage results in a COD removal efficiency of 20% and SS removal efficiency of 15% (Kuldeyev et al., 2023). Furthermore, when alum sludge is employed in wastewater treatment, it achieves a COD removal efficiency of 74% and reduces turbidity by 89%.

While this method can enhance the efficiency of removing organic compounds and chemicals from wastewater, it also poses certain risks. These include the potential for increased sludge accumulation in both the sewer system and wastewater treatment plants along with other potential interference with wastewater and sludge treatment processes. The prevailing approach for WTS disposal involves its storage on sludge drying beds. However, this method is subject to various limitations, including compliance with increasingly stringent environmental regulations governing waste management and constraints related to limited storage space availability. Consequently, in

recent years, there has been a growing interest among researchers in addressing sludge management issues in a manner consistent with the principles of sustainable development.

## 2.6 Biomass Filtration

The animal waste generated by the livestock industry can undergo anaerobic digestion, a biological process that transforms it into methane-rich biogas and nutrient-rich digestate (Cui et al., 2022). These products can then be used on-site as a renewable energy source, organic fertilizer, and water treatment, respectively. This is due to the nutrient-rich biogas slurry from animal faeces containing substances like ammonia, phosphate, and humus that can enhance plant growth (Akhiar et al., 2017). Apart from the increase in nutrient content, membrane processes can efficiently capture and concentrate residual contaminants like heavy metals and antibiotics present in biogas slurry, making it unsuitable for agricultural use but useful for water treatment (Li et al., 2020).

Extensive evaluations have been carried out on both inorganic and organic materials to enhance the rapid filtration of wastewater or to establish a foundation for biofilm formation in wastewater biofiltration processes. Organic materials that are used for biocoagulant containing mulch filters have the potential to significantly reduce the costs associated with wastewater treatment (Hunter and Deshusses, 2020). Furthermore, dry corn stalks have been found to remove 66% of SS and 46% of COD during the rapid filtration of biogas slurry (Du et al., 2019). The remaining corn stalks, which still contain SS and organic materials after filtration, can be composted directly. This approach eliminates the need for regular backflushing, a common requirement for inorganic filters, thereby reducing waste discharge and maximizing nutrient recovery from biogas slurry.

In China, multiple researchers had conducted research and experiments on comparing inorganic and organic coagulants for water treatment to test out their efficiency. PAS represented an inorganic coagulant, while chitosan represented an organic coagulant. It is widely known that both coagulants are highly effective in eliminating SS, colloids, and organic

substances. Both coagulants contribute to the improvement of wastewater treatment and its potential for reuse. To enhance the coagulation process, PAM, an efficient polymer flocculant, was combined with chitosan.

The effectiveness of PAS and chitosan in pre-treating biogas slurry was evaluated under various conditions, including dosage, temperature, and coagulation duration. The objective was to determine the optimal operational parameters for removing organic substances and suspended particles, as quantified by COD and SS levels. Subsequently, the coagulation process was integrated with cornstalk filtration. This involved mixing the coagulants with cornstalks and then introducing them into the column filter based on their respective treatment capacities, which were determined from earlier filtration and coagulation-flocculation experiments. Figure 2.9 will illustrate the mechanism of the water treatment process using corn stalks.

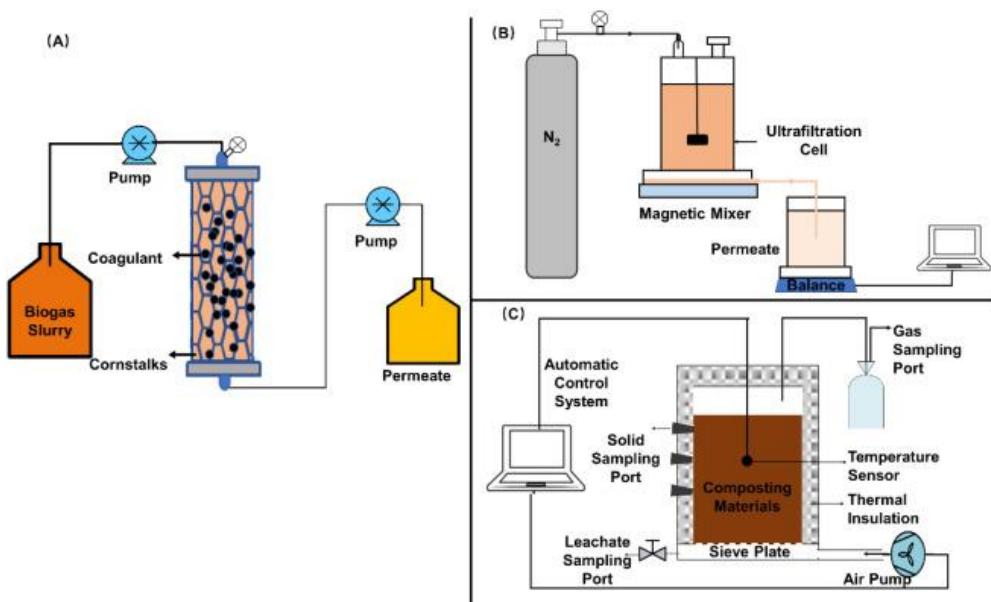


Figure 2.9: Schematic Diagram of (A) Cornstalk and Coagulant Hybrid Filter, (B) Ultrafiltration System, and (C) Aerobic Composting Reactor (Cui et al., 2022).

The utilization of cornstalk filtration resulted in an approximately 20% decrease in SS, leading to a reduction in the turbidity of biogas slurry. This decrease primarily occurred due to the physical trapping of SS by the cornstalk, as there was limited biofilm formation to initiate biodegradation during the

rapid filtration process, which extended for approximately 3 hours. Consequently, organic matter, as indicated by COD, and nutrients present in the biogas slurry were also captured to varying extents, ranging from 10% to 33%. The porous surface of the cornstalk possesses the ability to absorb soluble substances, thereby contributing to the removal of COD and nutrients.

In comparison to cornstalk filtration, both the PAS and chitosan coagulation processes exhibited significantly greater effectiveness in removing SS and nutrients. It is well-established that PAS achieves the coagulation of suspended particles and nutrient removal by forming floc sediment through processes like charge neutralization, sweep, and bridge formation, as previously documented (Kumar et al., 2009). Additionally, chitosan, being a biopolymer, has the capacity to directly adsorb organic molecules in solid form, complementing the traditional coagulation pathways in the liquid state, as discussed by Desbrières and Guibal (2018). Notably, PAM, with its high cation density and long chains, facilitates the aggregation of flocs, thereby assisting chitosan in the removal of SS, COD, BOD, turbidity, and organic substances (Sun et al., 2020).

In the context of removing heavy metals and antibiotics from biogas slurry during rapid filtration, the combination of PAS and cornstalk filtration demonstrated remarkable effectiveness in eliminating all identified heavy metals, except for aluminium. When PAS was introduced into the cornstalk media, it initiated a coagulation process, resulting in the formation of flocs as suspended particles and dissolved substances passed through the filter. Consequently, the removal of heavy metals can be attributed to their entrapment by these flocs. This entrapment process enhances their physical retention within the interconnected cornstalk media. Notably, heavy metals exhibit a strong affinity for colloidal and organic complexes commonly encountered in waste streams like biogas slurry and leachate (Won et al., 2019). Furthermore, owing to its microporous network structure and expansive surface area containing various functional groups like alcohols, aldehydes, ketones, carboxylic acids, phenols, and ethers, cornstalk serves as an absorbent capable of sequestering heavy metals from wastewater (Salman et al., 2015).

## 2.7 Natural Coagulant

Natural coagulants, also known as biocoagulants, are substances sourced from natural materials. The utilization of natural coagulants involves three primary stages: molecule bridging, adsorption, and charge neutralization. In addition to these stages, natural coagulants offer significant advantages in wastewater treatment.

Chemical water treatment, on the other hand, comes with various drawbacks, such as excessive costs, toxicity to both humans and the environment, corrosive properties, potential carcinogenicity, alteration of pH levels in treated water, the production of non-biodegradable hazardous sludge, and increased disposal expenses (Koul, 2022). Therefore, it has become imperative to explore alternative measures to mitigate the adverse impacts of these chemical coagulants on the ecosystem. Consequently, there has been a recent shift in the water treatment paradigm, prompting industries to raise awareness among water operators regarding the adoption and implementation of sustainable practices in their operations.

One effective approach involves replacing chemicals with natural substances in water treatment procedures, resulting in decreased environmental impacts concerning production, consumption, and the management of secondary waste. Natural coagulants, which consist of polyelectrolytes composed of anionic, cationic, or neutral polymers, play a crucial role in facilitating this transition (Koul, 2022). They provide a combination of safety and cost-effectiveness, demonstrating remarkable capability in preserving the pH levels of treated water. In contrast to chemical coagulants, natural coagulants avoid introducing extra metal content into the treatment process. Furthermore, they generate minimal sludge volume, which can significantly reduce disposal expenses. Consequently, they represent a sustainable substitute for chemical coagulants.

The industrial acceptance of natural coagulants depends on their ability to produce results comparable to those of chemical coagulants, making them a viable alternative. However, reluctance to embrace natural coagulants in industries stems from the absence of real-world or pilot-scale applications in drinking water treatment, along with the absence of official approval and

regulatory guidelines. The adoption of water treatment utilizing natural coagulants, especially in rural areas, holds promise for enhancing health, sanitation, and overall living standards for all individuals, as illustrated in Figure 2.10. The technical dimension of sustainability encompasses treatment effectiveness, long-term durability, material accessibility, and compatibility with other techniques.

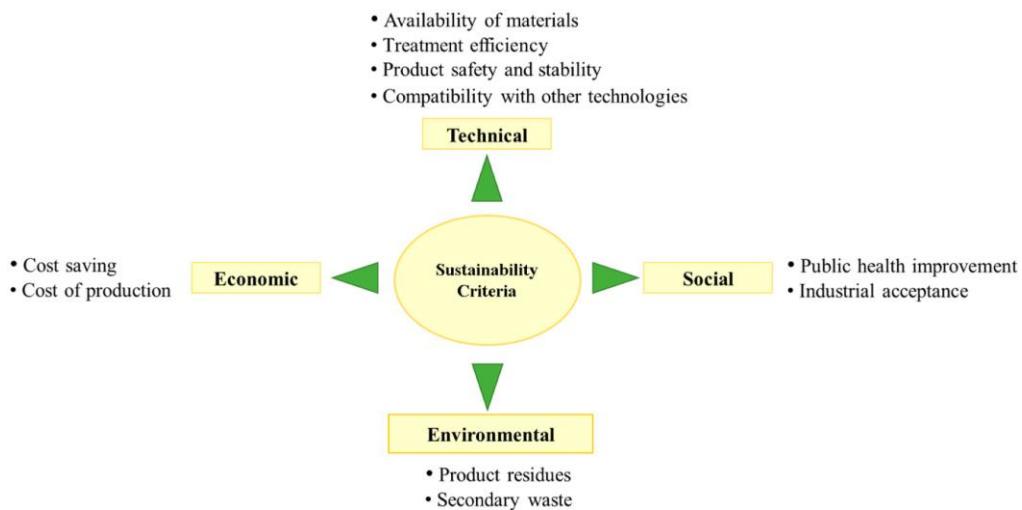


Figure 2.10: Sustainability Standards for Natural Coagulants (Koul, 2022).

However, apprehensions regarding the possible harmful effects of organic coagulants on both human health and the environment necessitate additional research into their environmental impact. Therefore, a meticulous selection process and the fine-tuning of dosages for efficient natural coagulants in water treatment could offer a viable alternative to chemical coagulants. However, their vulnerability to degradation by microbial or other environmental factors presents obstacles to their extended storage and commercial feasibility (Koul, 2022). In terms of environmental sustainability, the emphasis lies on the use of biodegradable, plant-derived coagulants that are environmentally friendly and can produce biodegradable sludge. This sludge can find applications in various fields, including agriculture, landfill management, and civil engineering projects.

## 2.8 Plant-based Coagulant

Plant-derived coagulants are more readily accessible compared to those derived from animals or microorganisms. There is a substantial body of evidence displaying the successful utilization of various plant-based resources, including materials like *Jatropha curcas* L., and *Moringa oleifera*, in the treatment of contaminated water. These plant-derived coagulants, characterized by macromolecules such as proteins, polysaccharides, and specific functional groups, play a significant role in facilitating processes like adsorption, polymer bridging, and charge neutralization.

Nonetheless, the effectiveness of natural coagulants can be further improved by optimizing the extraction and purification procedures employed for these coagulants (Kurniawan et al., 2020). Consequently, employing a well-designed extraction process utilizing plant-based materials can yield superior performance in the coagulation process, resulting in enhanced efficiency in waste removal. Several plant-based coagulants are commonly employed in water treatment such as nirmali seeds serve as a source of anionic polyelectrolytes, benefiting from their carboxylic acid and hydroxyl groups, which enhance coagulation efficiency. Furthermore, plants such as *Acacia*, *Catenae*, and *Schinopsis* contain natural tannins, enabling them to effectively remove contaminants from water.

Certain cactus species, such as *Opuntia latifaria* L., are utilized as natural coagulants owing to their composition, which includes compounds like d-galactose, d-rhamanosei, d-xylose, l-arabinose, and galacturonic acid. These constituents play a crucial role in connecting contaminants in water during the coagulation process. The way natural coagulants are applied during the coagulation process can also impact the efficiency of turbidity reduction. For example, when *Tamarindus indica* L. seeds were mixed in water, they exhibited greater effectiveness compared to water extracts obtained from ground seeds. Additionally, beyond their effectiveness in reducing turbidity, certain fruit by-products exhibit coagulation properties, while others show antimicrobial activity attributed to compounds like saponins, phenols, and flavonoids. Moreover, colloids found in the leaves of *H. undatus* Haw. bear resemblance to those in the seeds of *M. oleifera* Lam. and exhibit a cationic

character. Colloid adsorption and charge neutralization mechanisms are regarded as viable processes for coagulation, resulting in the formation of flocs.

The extraction process of plant-based natural coagulants follows a sequence of three stages: primary, secondary, and tertiary extraction. Initially, seeds are gathered and traditionally dried, after which manual or mechanical methods are employed to crush and finely grind them into powder. During this phase, a settling tank is employed to allow water to settle, separating denser solids at the bottom of the tank while lighter solids float to the surface. These settled solids are retained, while the remaining liquid proceeds to the more intensive secondary water treatment phase. In some cases, tanks may feature mechanical scrapers that continuously gather sludge from the base of the tank, transporting it to a hopper for further processing. While this primary extraction method is common in rural areas, it has a drawback: the coagulating agent constitutes only a small portion of the seed powder, resulting in an organic load in the treated water.

To overcome this limitation, secondary and tertiary stages are implemented. In the secondary stage, active coagulants are extracted using various solvent-based methods. Among these methods, water extraction is the most widely adopted due to its abundance and cost-effectiveness. The tertiary stage is a less commonly utilized approach, employed in academic research due to its higher associated expenses. Its primary aim is to further improve water quality to meet domestic, industrial, and specific requirements for safe water discharge. Tertiary water treatment also places emphasis on the elimination of microbes and pathogens, ensuring that the treated water is safe for consumption, especially in municipal water treatment settings. A visual representation of the natural coagulant extraction process is presented in Figure 2.11.

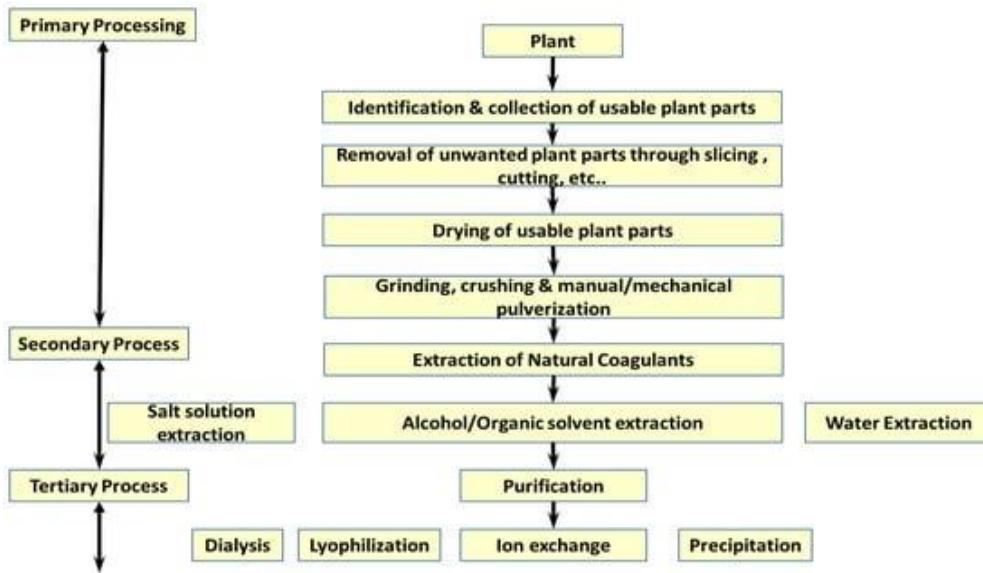


Figure 2.11: Diagram Illustrating the Extraction Process of Natural Coagulants (Koul, 2022).

## 2.9 Mixing Conditions

Despite pH in water and dosage of coagulant play significant roles in determining the performance of coagulant to treat water, other variables such as the alteration of mixing time or speed may also affect the outcome of the water treatment. When it comes to mixing coagulants with wastewater to effectively remove turbidity in water, there are several procedures and techniques based on past research. Effective turbidity removal can be obtained through either brief mixing periods, approximately 10 seconds, or extended mixing durations spanning 60 to 90 seconds (Daryabeigi et al., 2010). While certain researchers recommended prolonged mixing periods lasting up to 10 minutes, there are also other researchers who prefer instantaneous mixing techniques. The average distribution of floc size and the effectiveness of the coagulation-flocculation process are influenced by mixing conditions. Mixing speed is employed in two distinct phases, namely fast and slow, each defined by its specific speed in revolutions per minute (rpm) and duration in minutes or seconds.

According to Ahmed et al. (2022), the characteristics of the water under treatment, including pH and suspended solids (SS) concentration, impact the optimal mixing speed and duration required to achieve effective aggregation. Fast mixing serves as a foundational stage in water and

wastewater treatment, playing a crucial role in the success of the coagulation-flocculation process. Its primary objective is to uniformly distribute the coagulant throughout the suspension, thereby stimulating effective flocculation. Fast mixing assumes greater importance, particularly in highly turbid wastewater scenarios, where the elevated particle concentration leads to a heightened collision rate. This increased collision rate enhances both adsorption and floc formation rates, underscoring the significance of fast mixing in such instances. The duration of the fast-mixing phase significantly influences floc formation during the subsequent slow mixing phase (Ahmed et al., 2022). Fast mixing periods that fall shorter than the optimal duration will result in higher residuals with higher turbidity and larger flocs.

From past studies that conducted by Yu et al. (2011), a fast mix duration of 10 seconds at a speed of 200 rpm, followed by slow mixing at 50 rpm for 10 minutes proved insufficient for complete adsorption when treating a 50 mg/L kaolin clay suspension with alum as a coagulant. Notably, a stable flocculation index (FI) plateau was rapidly reached with shorter fast mixing durations, around 5 and 10 seconds compared to 30 seconds at 400 rpm, resulting in higher FI values and lower residual turbidity when alum was added to a 50 mg/dm<sup>3</sup> kaolin clay suspension. Moreover, several studies have demonstrated that higher fast mixing speeds lead to more efficient turbidity removal.

## 2.10 Summary

Biocoagulant from biomass, specifically *Annona* seed is a promising substitute for chemical coagulant because of its eco-friendly aspects where the operating cost also can be reduced in the water treatment. Besides that, *Annona* plant is available abundantly across the world. However, due to the lack of past research and experiment, many aspects in terms of its efficiency, side effects, and drawbacks remain unknown to the public. The major challenges encountered using *Annona* seeds is the suitability of the treatment process and the long-term stability in water treatment. Apart from that, there are several operating conditions and aspects needed to be considered before carrying out the testing on evaluating the turbidity reduction efficiency of coagulants.

Hence, many viable solutions have been proposed by various researchers, but all these only increase the complexity of the utilization of *Annona* seed in water treatment.

## CHAPTER 3

### METHODOLOGY AND WORK PLAN

#### 3.1 Introduction

In this chapter, all the procedures and processes in using the biomass from *Annona* seeds as a biocoagulant in water treatment will be laid out and explained precisely. Apart from using *Annona* seeds, two types of chemical coagulants which are ferric chloride and alum will also be used in water treatment to compare the results and efficiency of the water treatment. The obtained results and effects of the water treatment with the utilisation of *Annona* seeds will be analysed and assessed.

Preparation of the synthetic wastewater using kaolin powder is required instead of obtaining the wastewater from alternate sources such as water from river or sewer. Apart from that, determining the appropriate ratio of kaolin powder to water before creating the turbid water is vital to ensure the initial turbidity of water is consistent. Before conducting the experiment, calculation of the required stock solution for the three types of coagulants, acidic solution, and alkaline solution are required. The last step before Jar testing can begin is to measure the initial turbidity and pH of water using turbidity meter and pH meter, respectively. Throughout the study, the experiment will be conducted in the Chemical Engineering Laboratory where the sample beaker will be placed on a magnetic stirrer.

There are three types of coagulants used in this study which are *Annona* seeds, ferric chloride, and alum. Each coagulant will be inserted into three beakers filled with the same volume and turbidity of water to test their performance on reducing the turbidity in the synthetic wastewater. In this study, the Jar testing will be carried out under three distinct types of variables. The performance of coagulant will be observed under different pH value, amount of time taken, and mixing speed. After the Jar testing under three different variables is conducted, the final turbidity of water is recorded to calculate the turbidity reduction from three different coagulants.

After all the testing is completed, the data obtained from the results will be tabulated in tables and graphs. The results will be precisely discussed and analysed where the conclusion will determine whether the objectives of this study are fulfilled. Figure 3.1 illustrates the workflow of the entire study.

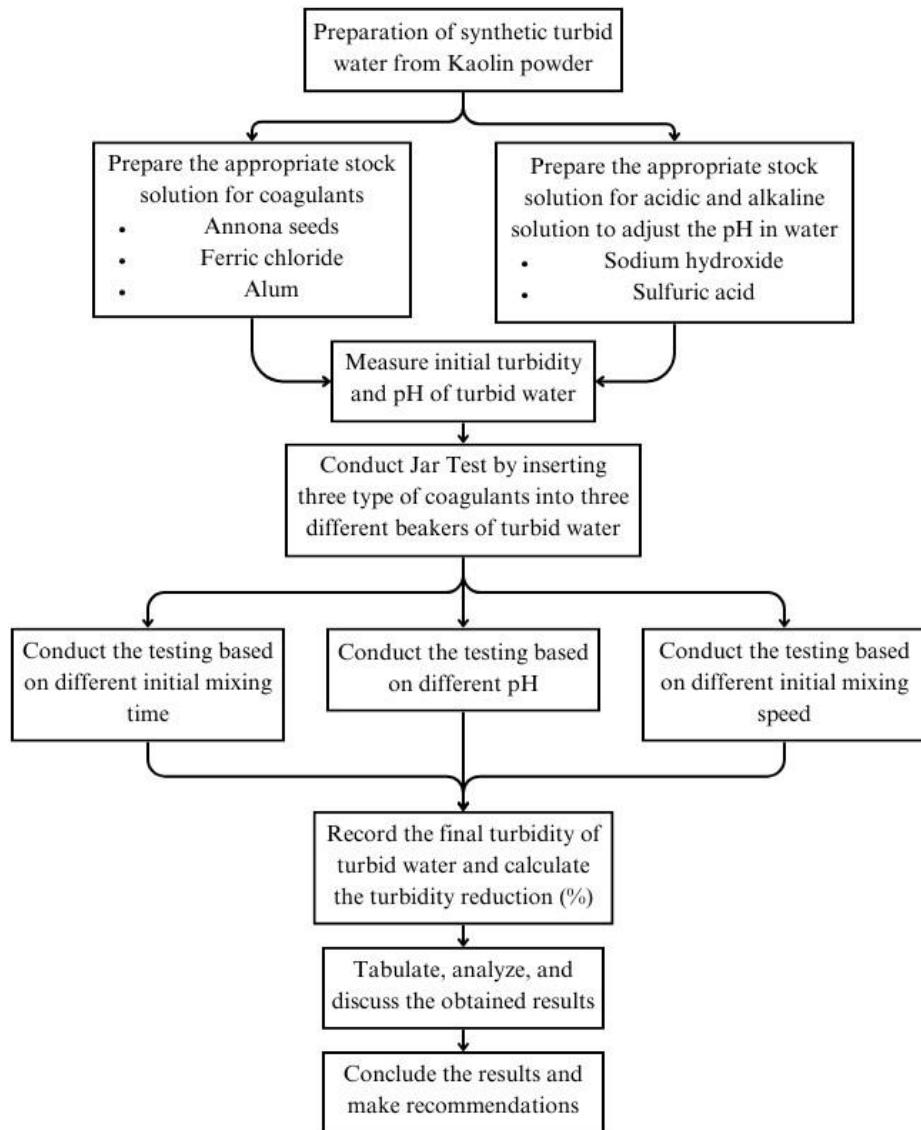


Figure 3.1: Work Flowchart.

### 3.2 Annona Seed

The Annona seeds is the main material that will be used in testing out the efficiency of utilising biocoagulants from biomass for water treatment. The Annona plant, often simply referred to as "Annona", is a diverse genus of flowering plants that belongs to the family Annonaceae. The Annona genus

includes a wide variety of species, each with unique characteristics and uses. Figure 3.2 shows the *Annona* plant and its seed that will be used for extraction. *Annona* seeds possess antimicrobial, anti-inflammatory, and antioxidant properties (Vimala et al., 2021). Hence, they are deemed as a highly potential source of biomass for extracting biocoagulants to be used in water treatment. Although *Annona* seeds are not known to be common in suburban areas, it has various rich nutrients such as proteins and polysaccharides that can act as coagulants.



Figure 3.2: Fruit and Seed of *Annona Squamosa* L.

In this study, the *Annona* seeds are cleaned and dried to avoid unwanted contamination by mold or dust. After all the seeds are dried, the seeds will be crushed with mortar and pestle before grinding it into powder with a grinding machine. The powder will then be left overnight at room temperature. After sufficient time has passed, the powder will be grinded again to ensure the biomass within the kennel of the seed extract are grinded thoroughly without sticking into a big clump after drying overnight. Figure 3.3 below shows the *Annona* seed that had been grinded.



Figure 3.3: Powder of Annona Seeds after Grinding.

After the powder had been grinded for the second time, the Annona seed powder extract underwent sieving using a 2mm wire mesh pan to eliminate any stones and debris present in the sieve. Throughout this study, the solution of the biocoagulant is created by combining Annona seed extracts with distilled water at a ratio of 1000mg per 1L for 10 minutes. Mixing speed of the biocoagulant in the magnetic stirrer is 200rpm. The solution of biocoagulant Annona seed extract is light brownish in color with the seed extract powder settling at the bottom. Figure 3.4 below shows the Annona seed extract solution with a concentration of 1000mg/L.

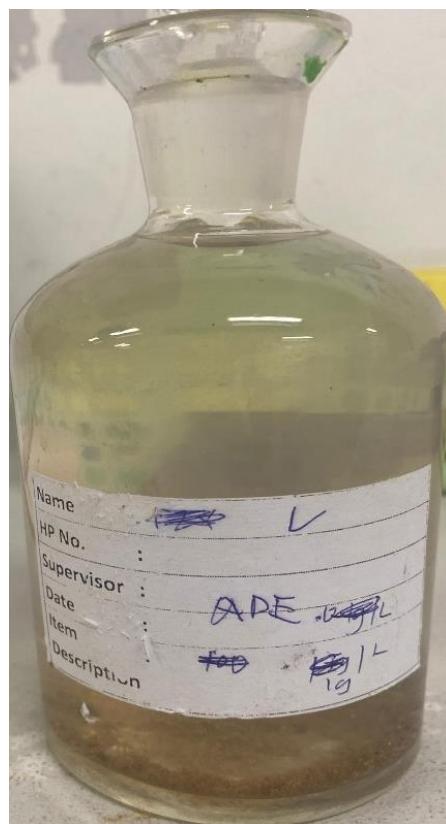


Figure 3.4: Annona Seed Extract Solution.

### 3.3 Chemical Coagulant

Traditional coagulant otherwise known as chemical coagulant is an important material used in treating wastewater to produce viable drinking water. The vital role of chemical coagulants is to destabilize and aggregate suspended particles in water, making them easier to remove through processes such as filtration and sedimentation. The sedimentation process can be accelerated by inserting specific chemicals into the water. Chemical coagulants facilitate the aggregation of sand, silt, and clay particles, causing them to unite and create larger clumps, thereby facilitating their settling to the bottom of the container (Centre for Affordable Water and Safe Technology, n.d.).

Aluminum and iron salts are the prevailing coagulants traditionally employed in water treatment. The substances employed in coagulation and flocculation processes can be categorized into two main groups: organic coagulants and mineral coagulants (inorganic). Organic coagulants consist of cationic, anionic polyelectrolytes, and nonionic polymers (Sohrabi et al., 2018). Among the most used coagulant materials for reducing water turbidity,

both from mineral and synthetic sources, are iron salts and aluminum compounds. These include ferrous sulfate, ferric sulfate, ferric chloride, alum, PFS, and PAC. Other than *Annona* seeds will be used as coagulants, ferric chloride, and alum will be introduced as another two alternate coagulants in this study. Figure 3.5 and Figure 3.6 show the two types of chemical coagulants used in this study.



Figure 3.5: Ferric Chloride.

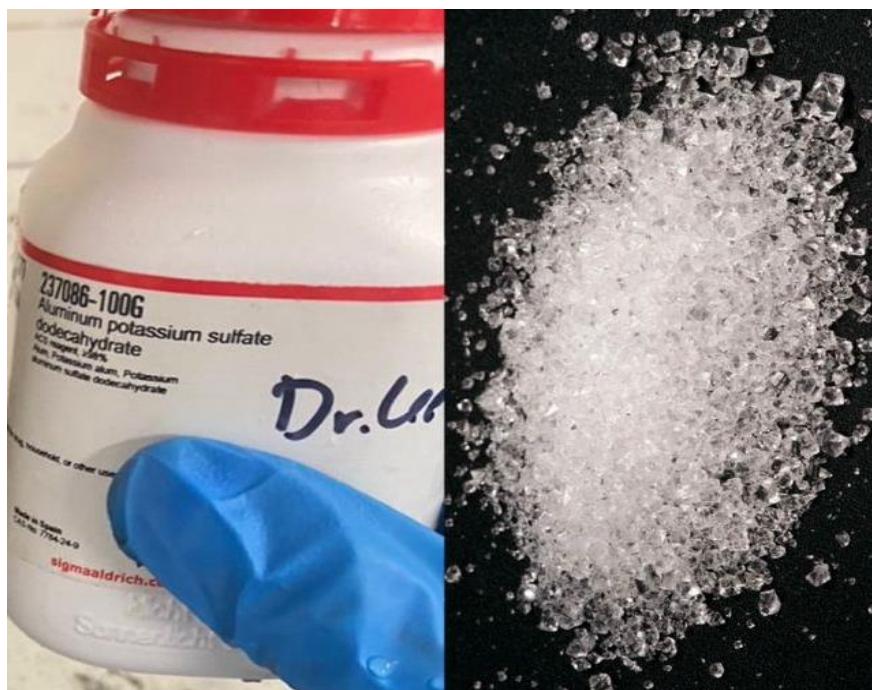


Figure 3.6: Alum.

The three distinct types of coagulants will be used throughout this study and their performance in treating the turbidity of water will be compared. For this study, the solution of coagulant is prepared by stirring the chemical coagulant powder with distilled water at a ratio of 1000mg per 1L. The solution is mixed in a magnetic stirrer at a speed of 200rpm for 10 minutes. Thus, the concentration of both chemical coagulants will be the same which is 1000mg/L. The solution of coagulant ferric chloride is yellowish whereas alum is transparent and colourless. Figure 3.7 and Figure 3.8 shows the chemical coagulant solution with a concentration of 1000mg/L of ferric chloride and alum.

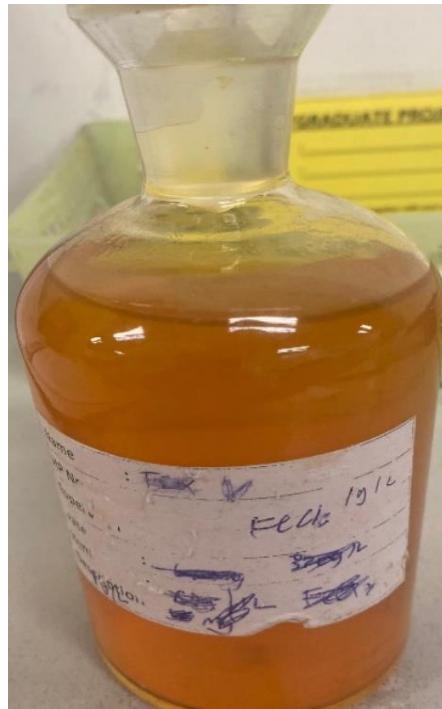


Figure 3.7: Ferric Chloride Solution.

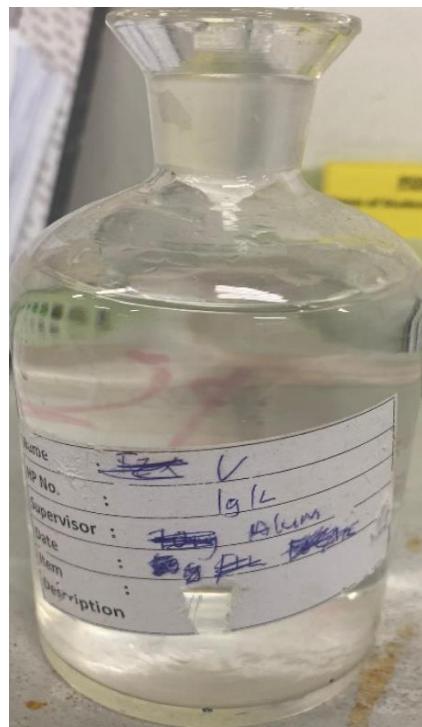


Figure 3.8: Alum Solution.

### 3.4 Synthetic Wastewater

Kaolin is a type of powder derived from soft white clay which is often used in manufacturing various products such as rubber, ceramic, paper, and paint.

When kaolin is combined with water within the 20 to 35 percent range, it gains plasticity, maintaining its shape once pressure is no longer applied (Britannica, 2024). With higher water concentrations, kaolin transforms into a slurry, a watery suspension which makes the water turbid. Hence, if the appropriate ratio of kaolin powder is mixed with distilled water, an ideal synthetic wastewater can be created. Figure 3.4 below shows the kaolin powder that will be used to create the turbid water.



Figure 3.9: Kaolin Powder.

The purpose of using kaolin powder as a substitute to other sources of wastewater is to maintain the consistency in measuring the initial turbidity of water. In other words, it is far easier to control the range of turbidity in water by using kaolin powder rather than other water sources that contain unknown chemicals and microorganisms that might affect the accuracy of the result. The main reason for using kaolin to create synthetic wastewater in this study is due to its colloidal properties which are identical in real wastewater. Hence, it can enable the study of processes such as coagulation, flocculation, and sedimentation. Furthermore, kaolin particles typically have a well-defined size distribution, ranging from submicron to several micrometres in diameter. This characteristic makes kaolin suitable for simulating the particle size distribution commonly found in natural wastewater sources. For this study, the synthetic

turbid water is created by mixing kaolin with distilled water at a ratio of 100mg per 1L. The mixing is done with a magnetic stirrer for 10 minutes at the speed of 200rpm. After mixing has completed, the mixture will be left to settle for 30 minutes to stabilize the flocs. The solution of the synthetic wastewater is cloudy and white in colour. Figure 3.10 below shows the synthetic wastewater solution with a concentration of 100mg/L.

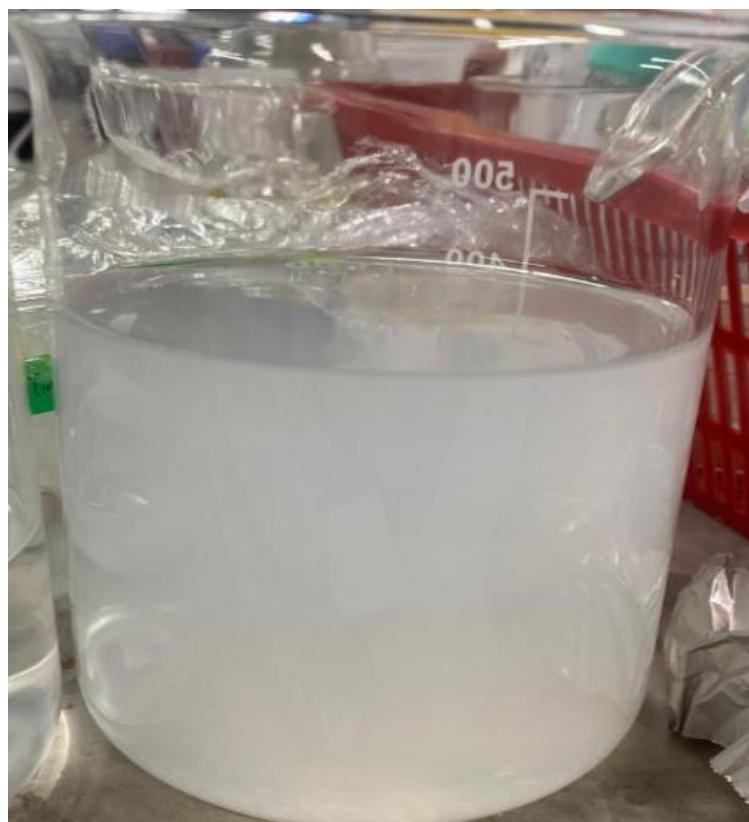


Figure 3.10: Synthetic Wastewater Solution.

### 3.5 Dosage of Coagulant

Preparation of the stock solution is the most important part of this study as it is essential to determine the ideal dosage that is required to be inserted into the wastewater without jeopardizing its result. To obtain the appropriate dosage, a mathematical formula of the dilution factor equation is introduced. Dilution is characterized by a decrease in the pH of a substance, which can exist in the form of a gas, vapor, or solution (BYJUS, n.d.). It involves reducing the concentration of a solute within a solution, typically achieved by mixing it with a solvent. To dilute a solution, additional solvent is inserted without

introducing more solute. Ensure thorough mixing of the resulting solution to achieve uniformity across all its components. Equation 3.1 below shows the dilution factor formula that is used in this study to obtain the stock solution of the coagulants.

$$C_1V_1 = C_2V_2 \quad (3.1)$$

where

$C_1$  represents the concentration of wastewater (mg/L)

$V_1$  represents the volume of wastewater (L)

$C_2$  represents the concentration of coagulant (mg/L)

$V_2$  represents the volume of coagulant needed in this experiment (L)

Prior to preparing the stock solution for each coagulant, accurate calculations needed to be made based on the concentration of the coagulant. The pH and all the coagulant dosage were modified individually for each beaker according to the experimental designs. For this experiment, the concentration of Annona seeds, ferric chloride, and alum will be determined as 1000mg/L. Throughout the study, the working beaker volume of the wastewater is 0.3L with the constant concentration of 100mg/L. Equation 3.2 below depicts the calculation of the stock solution.

$$(100\text{mg/L})(0.3\text{L}) = (1000\text{mg/L}) V_2 \quad (3.2)$$

$$V_2 = 0.03\text{L} = 30\text{mL}$$

Based on the calculation of the stock solution shown above, the stock solution of the coagulant that will be used in treating the wastewater is concluded to be 30mL. The dosage with the volume of 30mL is the fixed variable which is used across the entire study.

### 3.6 Scanning Electron Microscope (SEM)

SEM is an advanced analytical technique renowned for its ability to examine various materials at high magnifications, yielding high-resolution images. It is

commonly employed to analyse the morphology, topology, and fine surface structure of solid materials (Khaled, 2019). SEM is employed to scan the surfaces of a specimen at magnifications ranging from 1 $\mu$ m to 1nm, contingent upon the hardware utilized to generate electron beams, which involves a variety of lenses and vacuum systems (Singh and Singh, 2022). Additionally, SEM are often equipped with energy-dispersive spectrometers to enable elemental analysis of specimens at the surface.

In this case, SEM was utilized to analyse the morphology of *Annona* seed extracts. During SEM analysis, the *Annona* seeds extracts are subjected to electron beams emitted from an electron gun, which accelerate as they traverse the SEM column. The principle behind SEM involves the detection of high-energy electrons emitted from the surface of the sample upon exposure to the tightly focused electron beam from the electron gun. This electron beam is precisely focused onto a small area of the sample surface using the SEM objective lens. Optimal imaging conditions are achieved by adjusting variables such as the aperture size, accelerating voltage, and working distance between the *Annona* seed extracts and the electron gun.

### 3.7 Turbidity Testing

There are three types of testing using *Annona* seeds, ferric chloride, and alum that will be used to reduce the turbidity of wastewater in the experiment. The purpose is to compare and investigate the different effects of each type of coagulant in water treatment. Each type of coagulants has different impacts on parameters of their respective performance, such as pH, turbidity, mixing time, and mixing speed. All these analyses were conducted with chemical coagulants and *Annona* seed extracts to compare the performance and result of the water treatment. The model of turbidity meter used in this study is Waterproof TN 100 Waterproof Turbidimeter which has a turbidity measurement range of 0NTU to 1000NTU. Before conducting each testing, the initial turbidity of wastewater must be taken after completing the calibration of the turbidity meter. Initial turbidity of the wastewater will be taken three times to ensure the accuracy of the data. Figure 3.11 below shows the model of the turbidity meter used in the study.



Figure 3.11: Waterproof TN 100 Waterproof Turbidimeter.

The turbidity removal efficiency of both biocoagulant and chemical coagulants depends on numerous factors, including the specific coagulant used, the characteristics of the water being treated, dosage of coagulants, and the treatment conditions. The method to calculate the turbidity reduction is based on APHA Method 2130B, with NTU utilized for turbidity measurement. Turbidity reduction percentage was calculated using Equation 3.3 provided below.

$$\text{Turbidity Reduction} = \frac{\text{Initial Turbidity} - \text{Final Turbidity}}{\text{Initial Turbidity}} \times 100 \quad (3.3)$$

The section below will further delve into the explanation on preparing the three distinct types of testing on each performance of different type of coagulants.

### 3.7.1 PH

The pH values of biocoagulants and chemical coagulants can vary depending on the specific product and its intended application. Both types of coagulants are used in water treatment and wastewater treatment processes to destabilise suspended particles and facilitate their removal. The purpose of the testing is to compare the turbidity reduction efficiency of the three distinct types of coagulant in tackling the wastewater under different pH values. Prior to the testing, two distinct types of chemical solutions which are an acidic and

alkaline solution need to be prepared in advance. 10g of sulfuric acid is slowly mixed in a magnetic stirrer with 1L of distilled water and mixed at the speed of 200rpm for 10 minutes to create an acidic solution with 10g/L concentration. On the other hand, 10g of sodium hydroxide is slowly mixed in a magnetic stirrer with 1L of distilled water and stirred for 10 minutes to create an alkaline solution with 10g/L concentration. Figure 3.12 and Figure 3.13 illustrate the acidic and alkaline chemical that will be used in the study.

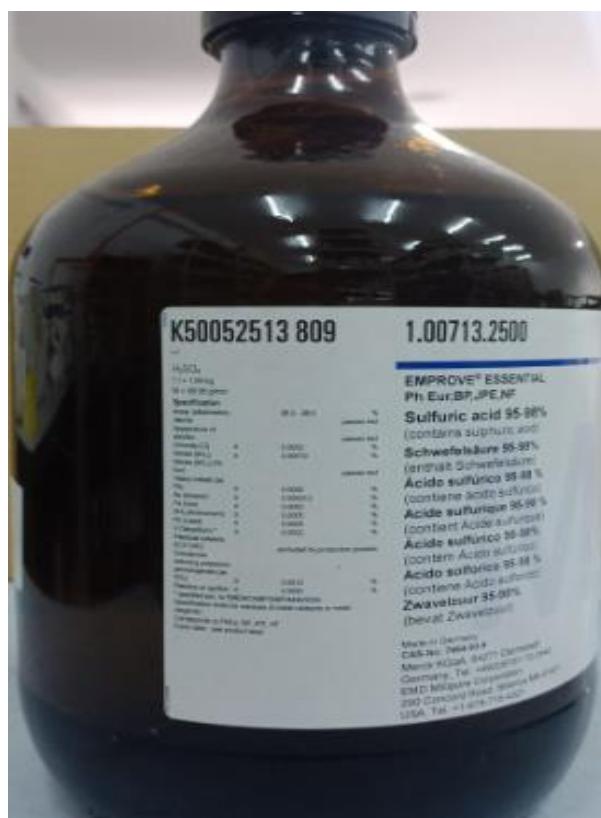


Figure 3.12: Sulfuric Acid

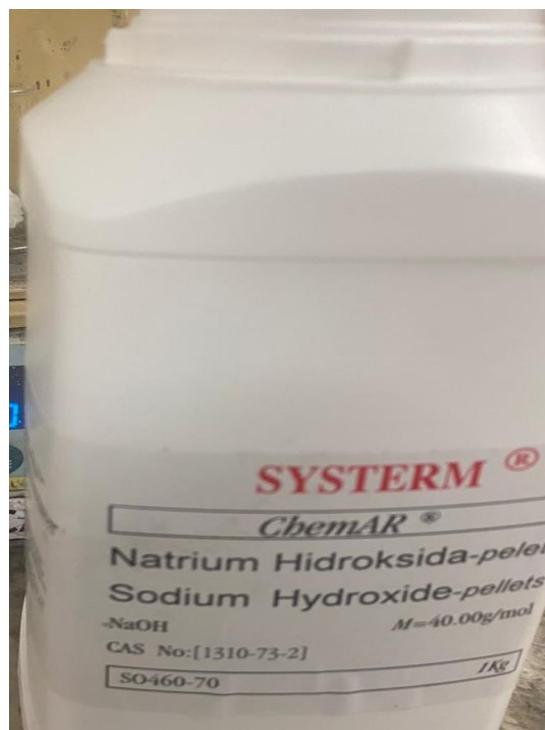


Figure 3.13: Sodium Hydroxide.

For this study, sodium hydroxide and sulfuric acid are the alkaline and acidic solution that are used for pH adjustment in the wastewater. The pH levels required for this experiment were 3, 5, 7, 9, and 11. A total of 3 sets of beakers with the same working volume will be prepared for each of the pH levels of wastewater. Therefore, it is essential to measure the pH of the wastewater before conducting the pH adjustment. The model for the pH meter that are used in the entirety of the study is HT-1202 PH meter. Before testing or adjusting the pH of the wastewater, calibration of the pH meter is required to guarantee the precision of the data and result. Other than that, the testing will involve 10 minutes of high-speed mixing of 150rpm followed by 30 minutes of low speed mixing of 40rpm for three of the coagulant in each of their respective beaker at a constant temperature of 28°C. Lastly, the final turbidity after coagulation of the wastewater will be recorded. Figure 3.14 shows one of the sample readings of the pH meter.



Figure 3.14: HT-1202 PH Meter.

### 3.7.2 Mixing Time

Mixing time between coagulant and wastewater at a constant speed is also one of the variables that can test the performance of the coagulant. Chemical coagulants, such as alum or ferric chloride are often known for their fast coagulation and flocculation kinetics. Suspended particles can be destabilised quickly in water and promote the formation of larger flocs, which settle or are easier to remove through filtration. Depending on the specific application and water quality, chemical coagulants can achieve the desired treatment results within a brief period, often within minutes to hours. The speed of chemical coagulation can determine whether the coagulant is suitable for applications requiring rapid treatment. In some cases, coagulants may require slightly longer contact times to achieve ideal or jeopardize treatment results. Hence, it is important to test on the turbidity reduction efficiency relative to their mixing time.

In this research, the testing will involve two minutes of high-speed mixing of 150rpm followed by low speed mixing of 40rpm for three of the coagulants in each of their respective beakers. Apart from that, the pH value of the wastewater will be adjusted to 7 and maintained at a constant temperature of 28°C for the mixing of the three of the working beakers. However, the procedure where the initial high-speed mixing takes place, the mixing time will be ranging from 5 minutes to 40 minutes with a 5-minute interval. The final turbidity of the wastewater will be measured and recorded. Hence, this will produce 8 different results for each of the three different coagulants which total up to a sum of 24 data to be obtained.

### **3.7.3 Mixing Speed**

Despite the relationship of the turbidity in wastewater are dependent on the pH and coagulant dosage, mixing speed during coagulation may have the tendency to affect turbidity efficiency. Testing the turbidity reduction efficiency of the three different coagulants based on different mixing speed can also determine the performance of the coagulant. The procedure of this testing will have some similarities with previous two testing such as constant temperature, pH, mixing speed during low speed, settling time, and overall mixing time. However, the only different factor in this testing is the initial mixing speed where the high-speed mixing takes place. In this testing, it will involve 10 minutes of high-speed mixing followed by 30 minutes of low speed mixing of 40rpm. The initial mixing speed will be ranging from 0rpm to 300rpm with a speed interval of 50rpm. In other words, the data of the final turbidity acquired through measurement of turbidity will produce 7 results for three of the respective coagulants.

## CHAPTER 4

### RESULTS AND DISCUSSION

#### 4.1 SEM Analysis

SEM images of the *Annona* seed extract were captured at multiple magnifications to achieve optimal resolution of fine particles and to encompass more particles within the images. The size and morphology of the particles significantly impact the characteristics of the *Annona* seed extracts, which may affect performance in treating wastewater. Smaller particles tend to bind more tightly and exhibit greater agglomeration compared to larger particles (Gurak et al., 2014). The captured images of SEM for *Annona* seed extract in 10 $\mu$ m magnification of 1,000 $\times$ , 5 $\mu$ m magnification of 4,000 $\times$ , and 2 $\mu$ m magnification of 8,000 $\times$  are present in Figure 4.1, Figure 4.2, and Figure 4.3 below respectively.

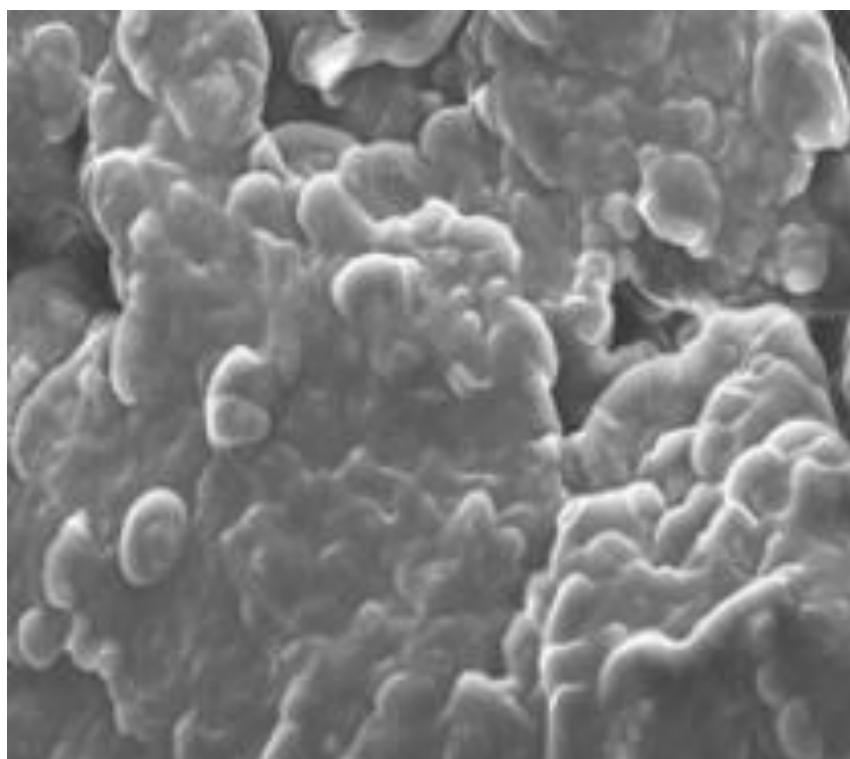


Figure 4.1: SEM Image of *Annona* Seed Extract in 10 $\mu$ m Magnification of 1,000 $\times$ .

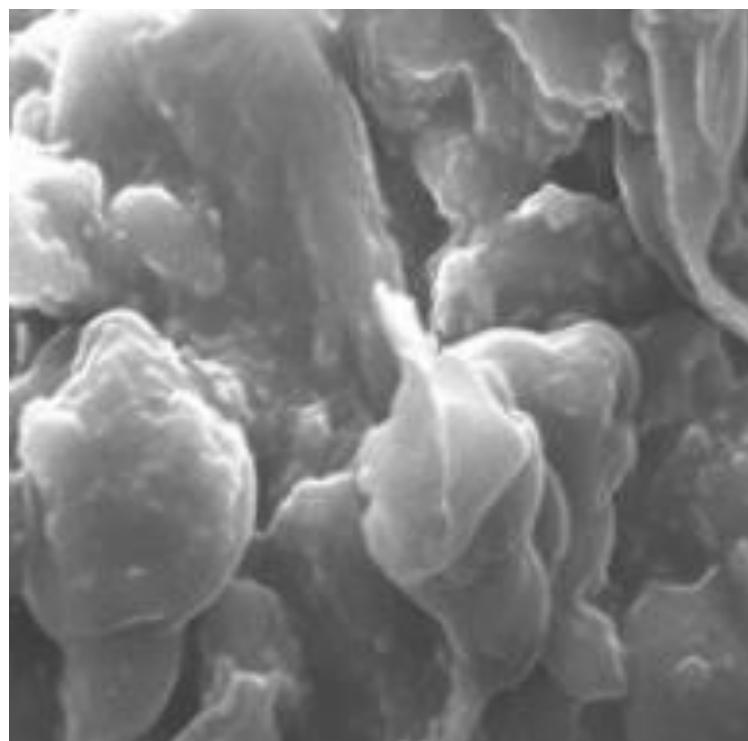


Figure 4.2: SEM Image of Annona Seed Extract in  $5\mu\text{m}$  Magnification of 4,000 $\times$ .

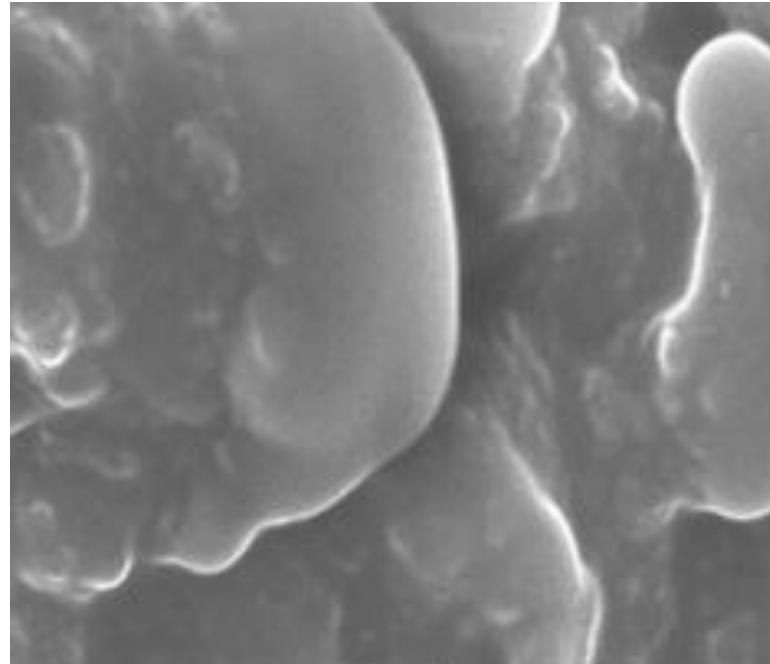


Figure 4.3: SEM Image of Annona Seed Extract in  $2\mu\text{m}$  Magnification of 8,000 $\times$ .

In Figure 4.1, tiny oil droplets visible on the surface of the *Annona* seed extract. Meanwhile, in Figure 4.2, it shows multiple openings in the vegetable matrix surface which allow the efficient retrieval of denser particles during coagulation. During the investigation of *Annona* seed in this study, it was found to be nonuniform and irregular in shape. The image of *Annona* seed extract under different magnification shows that it contains multiple clumps of rough surface structure with irregular shape distribution. This represents the presence of biomass particles within the seed. Consequently, the *Annona* seed exhibits an amorphous structural configuration which can help in capturing particles. The examination of surface morphology also indicated that both materials exhibited irregular and porous structures, which would enable the adsorption of metal ions across various regions of the materials. SEM micrographs revealed the presence of pores with varying sizes and shapes on the external surface of *Annona* seed extracts, confirming the adsorption of metals on the surface of the adsorbent during coagulation.

#### 4.2 PH Testing

In this testing, three types of coagulants which are *Annona* seed extract, ferric chloride, and alum will be used in reducing the turbidity of kaolin wastewater. Three separate beakers of the same concentration of kaolin water with similar initial turbidity of wastewater were prepared in each of the pH tested. All three of the beakers will have the same working volume of 300mL. Each of the beakers will have the measurement of initial turbidity and pH value taken three times where the average turbidity will be calculated to ensure the precision of the results. Table 4.1 below shows the tabulation of average initial turbidity under different average pH values measured before testing. In Table 4.2, the final turbidity of wastewater after the mixing of each coagulant with kaolin wastewater and the turbidity reduction percentage was measured and calculated. The parameters that will remain constant throughout this testing will be listed in Table 4.3 below.

Table 4.1: Initial Turbidity of Wastewater under Different pH.

<b>1<sup>st</sup> Testing</b>		
<b>Measurement</b>	<b>Initial Turbidity (NTU)</b>	<b>pH</b>
First	142	3.1
Second	139	3.07
Third	137	3.04
Average	139.33	3.07
<b>2<sup>nd</sup> Testing</b>		
<b>Measurement</b>	<b>Initial Turbidity (NTU)</b>	<b>pH</b>
First	133	4.94
Second	129	5.05
Third	126	4.97
Average	129.33	4.99
<b>3<sup>rd</sup> Testing</b>		
<b>Measurement</b>	<b>Initial Turbidity (NTU)</b>	<b>pH</b>
First	128	7.09
Second	126	7.11
Third	120	7.05
Average	124.67	7.08
<b>4<sup>th</sup> Testing</b>		
<b>Measurement</b>	<b>Initial Turbidity (NTU)</b>	<b>pH</b>
First	148	8.97
Second	147	9.09
Third	143	9.04
Average	146	9.03
<b>5<sup>th</sup> Testing</b>		
<b>Measurement</b>	<b>Initial Turbidity (NTU)</b>	<b>pH</b>
First	159	11
Second	156	10.97
Third	154	10.98
Average	156.33	10.98

Table 4.2: Final Turbidity and Turbidity Reduction Rate under Different pH.

<b>pH = 3.07; Initial Turbidity = 139.33NTU</b>		
<b>Coagulant</b>	<b>Final Turbidity (NTU)</b>	<b>Turbidity Reduction (%)</b>
Alum	55.9	59.88
Annona	58.4	57.85
Ferric Chloride	76.8	44.88
<b>pH = 4.99; Initial Turbidity = 129.33NTU</b>		
<b>Coagulant</b>	<b>Final Turbidity (NTU)</b>	<b>Turbidity Reduction (%)</b>
Alum	69.2	46.49
Annona	72.5	43.94
Ferric Chloride	79.4	38.61
<b>pH = 7.08; Initial Turbidity = 124.67NTU</b>		
<b>Coagulant</b>	<b>Final Turbidity (NTU)</b>	<b>Turbidity Reduction (%)</b>
Alum	69.7	44.09
Annona	77.7	37.67
Ferric Chloride	91.9	26.28
<b>pH = 9.03; 146NTU</b>		
<b>Coagulant</b>	<b>Final Turbidity (NTU)</b>	<b>Turbidity Reduction (%)</b>
Alum	127	13.01
Annona	123	15.75
Ferric Chloride	130	10.96
<b>pH = 10.98; Initial Turbidity = 156.33NTU</b>		
<b>Coagulant</b>	<b>Final Turbidity (NTU)</b>	<b>Turbidity Reduction (%)</b>
Alum	139	11.09
Annona	136	13.01
Ferric Chloride	144	7.89

Table 4.3: Constant Variable of pH Testing.

Parameters	Value
Initial Mixing Speed (rpm)	150
Slow Mixing Speed (rpm)	40
Initial Mixing Time (mins)	15
Low Speed Mixing Time (mins)	30
Dosage (mL)	30
Coagulant Concentration (mg/L)	1000
Kaolin Water Concentration (mg/L)	100
Working Volume (mL)	300
Temperature (°C)	28

In this study, it was observed that the efficacy of turbidity removal exhibited a slight enhancement at lower pH levels and a drastic drop can be observed on the turbidity reduction percentage of the three coagulants. From Table 4.2, it is revealed that the turbidity removal efficiency of alum, Annona seed extract, and ferric chloride coagulant are administered at a dosage equivalent to 1000mg/L of raw seeds, was 59.88%, 57.85%, and 44.88% at pH of 3. For wastewater with a pH of 7.08, three of the coagulants are still able to reduce the turbidity of water by at least up to 26%. When the wastewater is adjusted to the pH of 11, the coagulant fails to coagulate and flocculate with most of the SS and other particles in the wastewater. Despite the function of the coagulant in treating the wastewater, the turbid removal efficiency of alum, Annona seed, and ferric chloride had plummeted to 11.09%, 13.01%, and 7.89%, respectively.

Furthermore, by comparing the results, it can be observed that alum yields the highest percentage in reducing turbidity in wastewater, followed by Annona seeds which have similar efficiency with alum. However, ferric chloride shows the worst performance in treating wastewater compared to the other two coagulants. To further understand the results obtained from the experiment, Figure 4.4, Figure 4.5, and Figure 4.6 will illustrate the graph for turbidity removal percentage of alum, Annona, and ferric chloride under

different pH of wastewater. On the other hand, Figure 4.7 will show the combination of the turbidity reduction percentage under different pH.

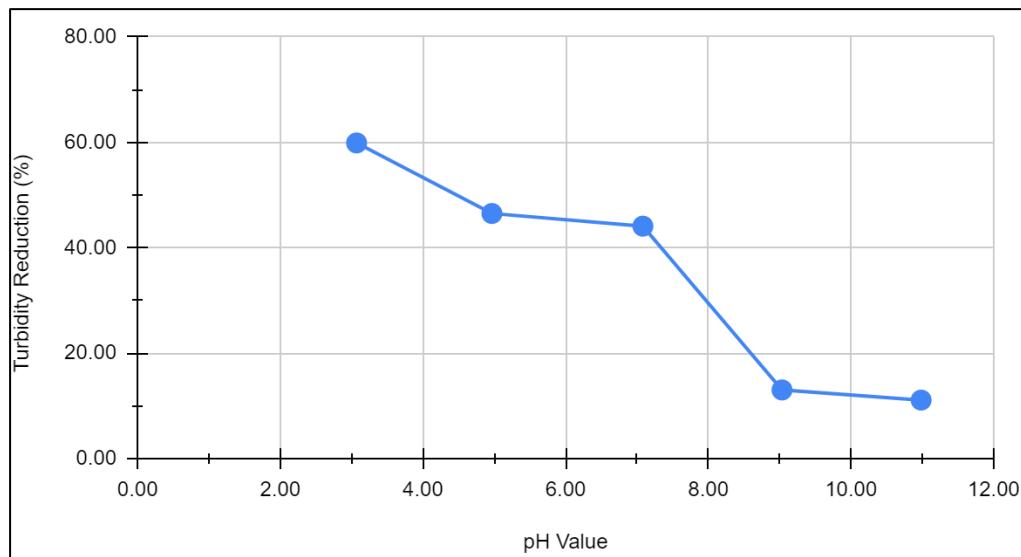


Figure 4.4: Turbidity Reduction Rate of Different pH Using Alum.

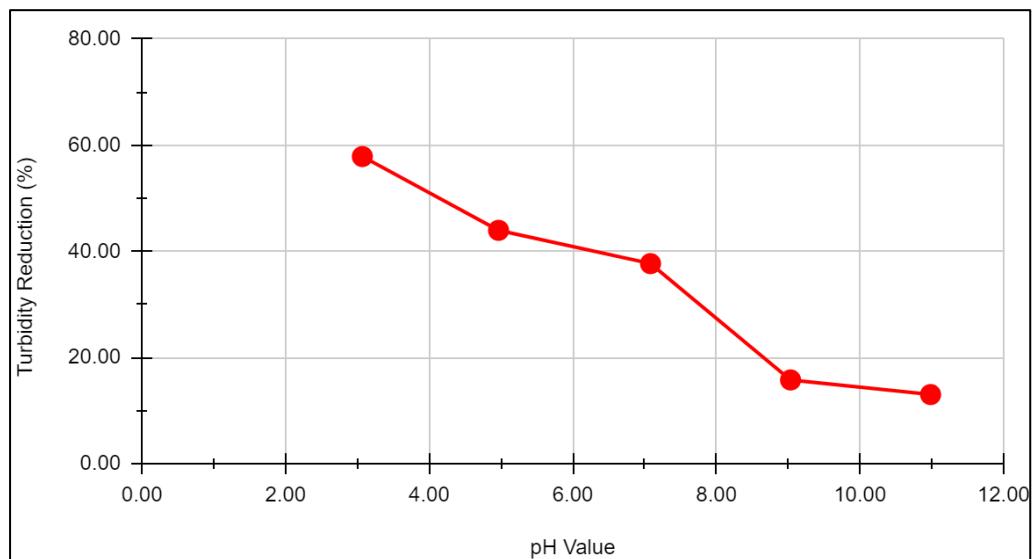


Figure 4.5: Turbidity Reduction Rate of Different pH Using Annona Seed.

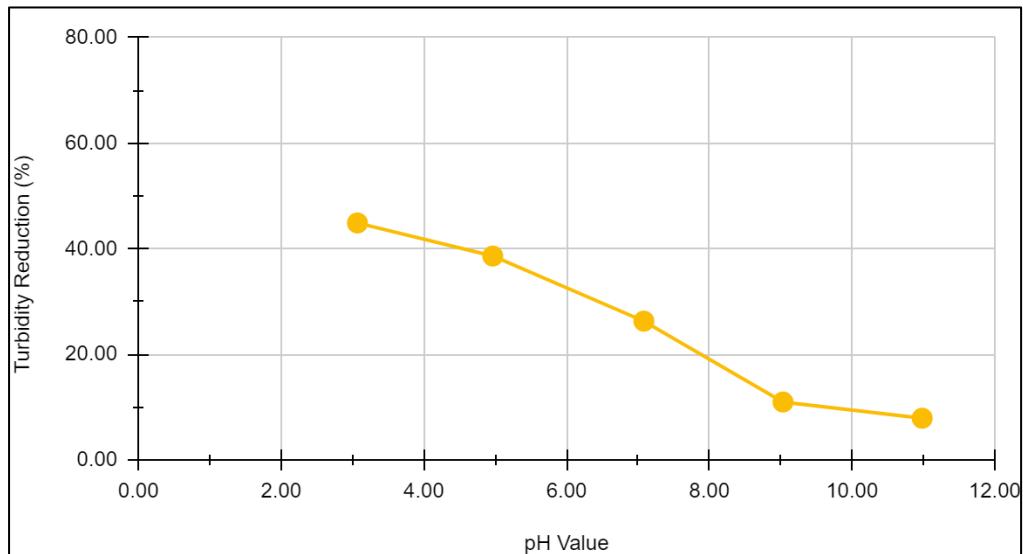


Figure 4.6: Turbidity Reduction Rate of Different pH Using Ferric Chloride.

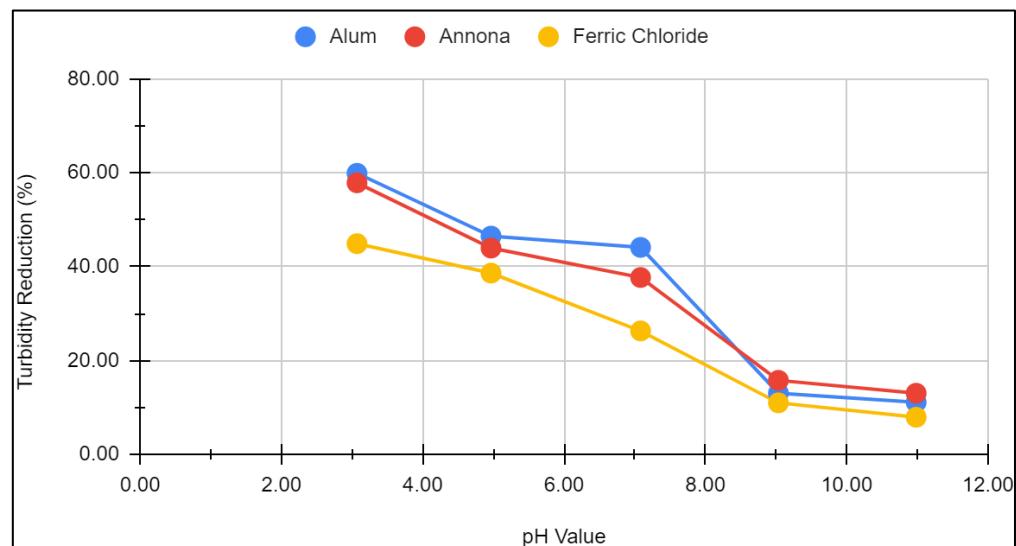


Figure 4.7: Turbidity Reduction Rate of Different pH for Alum, Annona Seed, and Ferric Chloride.

From all the graphs in the figure above, it has been proven that all the coagulants reduce the turbidity in water efficiently under lower pH compared to higher pH. The graphical trend of pH level in wastewater is inversely proportional to the turbidity reduction performance of coagulants where all three coagulants fail to achieve even 20% under pH 11. At acidic pH levels, natural organic matter like humic and algal substances can be effectively removed by charge neutralization (Naceradska, Pivokonska, and Pivokonsky, 2019). Algal cells are best coagulated at slightly acidic to neutral pH levels

because of their interaction with coagulant hydroxide precipitates. When mixtures of impurities are coagulated together, their interactions can affect the required dose of coagulant and the optimal pH range.

#### 4.3 Mixing Time

For turbidity reduction testing on different mixing times, the mixing time during initial high-speed mixing that will be evaluated is ranging from 5 minutes to 40 minutes with 5-minutes interval at a constant speed of 150rpm. The initial turbidity and pH are recorded before mixing, whereas the final turbidity of the three coagulant mixing will be documented after a slow-mixing process of 30 minutes is completed. Table 4.4 below shows the measurement of final turbidity whereas Table 4.5 shows the calculation of turbidity reduction percentage of all three coagulants. The parameters that will remain constant throughout this testing will be listed in Table 4.6 below.

Table 4.4: Final Turbidity of Different Initial Mixing Time.

Initial Mixing Time (mins)	Alum	Annona	Ferric Chloride
	Initial Turbidity (NTU)		
	169	167	165
Final Turbidity (NTU)			
5	158	154	164
10	149	149	161
15	140	138	156
20	134	129	152
25	128	133	153
30	131	123	155
35	124	129	146
40	127	126	147

Table 4.5: Turbidity Reduction Rate of Different Initial Mixing Time.

<b>Initial Mixing Time (mins)</b>	Alum	Annona	Ferric Chloride
	<b>Turbidity Reduction (%)</b>		
5	17.75	18.82	2.45
10	32.28	26.05	9.82
15	46.80	41.98	22.09
20	56.49	55.00	31.91
25	66.17	49.21	29.46
30	61.33	63.69	24.55
35	72.62	55.00	46.64
40	67.78	59.35	44.19

Table 4.6: Constant Variables of Different Initial Mixing Time Test.

<b>Parameters</b>	<b>Value</b>
Initial Mixing Speed (rpm)	150
Slow Mixing Speed (rpm)	40
Low Speed Mixing Time (mins)	30
pH	7.12
Temperature (°C)	28
Dosage (mL)	30
Coagulant Concentration (mg/L)	1000
Kaolin Water Concentration (mg/L)	100
Working Volume (mL)	300

Certain variables mentioned in Table 4.6 such as initial mixing speed, slow mixing speed, low speed mixing time, pH, and temperature will remain constant throughout this testing to ensure a more accurate result. It can be observed that the turbidity reduction rate for alum, Annona, and ferric chloride are poor when the duration of initial mixing time at 5 minutes is 17.75%, 18.82%, and 2.45%. The turbidity reduction percentage of the coagulants that undergo mixing time of 40 minutes improved to 67.78%, 59.35%, and 44.19% for alum, Annona seed, and ferric chloride, respectively. From the research, it is proven that longer initial mixing time can enhance the performance of the

coagulation-flocculation process of the wastewater. However, the mixing time of coagulants in achieving their peak performance differs from one another. Alum reached the highest turbidity reduction percentage is 72.62% when the wastewater is mixed for 25 minutes, Annona seed yield the best performance during mixing time of 30 minutes at reduction rate of 63.69%, and ferric chloride had a 46.64% of reduction percentage when mixing time is at 35 minutes.

The duration of initial mixing time during coagulation can significantly impact the turbidity reduction achieved by both the biocoagulant and coagulant. A longer initial mixing time allows for more thorough dispersion of the coagulant throughout the water, ensuring better interaction between the coagulant and the suspended particles. This increased interaction enhances the effectiveness of the coagulation process, resulting in greater turbidity reduction. Conversely, a shorter initial mixing time may not allow for adequate dispersion of the coagulant, leading to incomplete particle destabilization and lower turbidity reduction efficiency (Zainol et al. 2022). Therefore, the duration of the initial mixing time is important in evaluating the overall performance of the coagulant in reducing turbidity. Figure 4.8, Figure 4.9, and Figure 4.10 allow for clearer illustration on the results of turbidity reduction rate under different initial mixing duration. Figure 4.11 demonstrates a combined graphical result of alum, Annona seed, and ferric chloride.

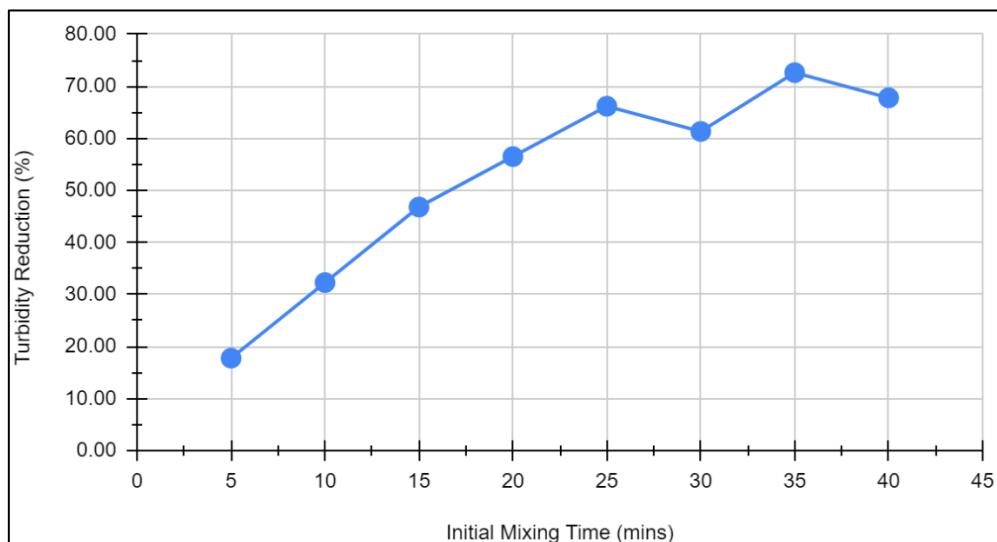


Figure 4.8: Turbidity Reduction Rate of Different Initial Mixing Time Using Alum.

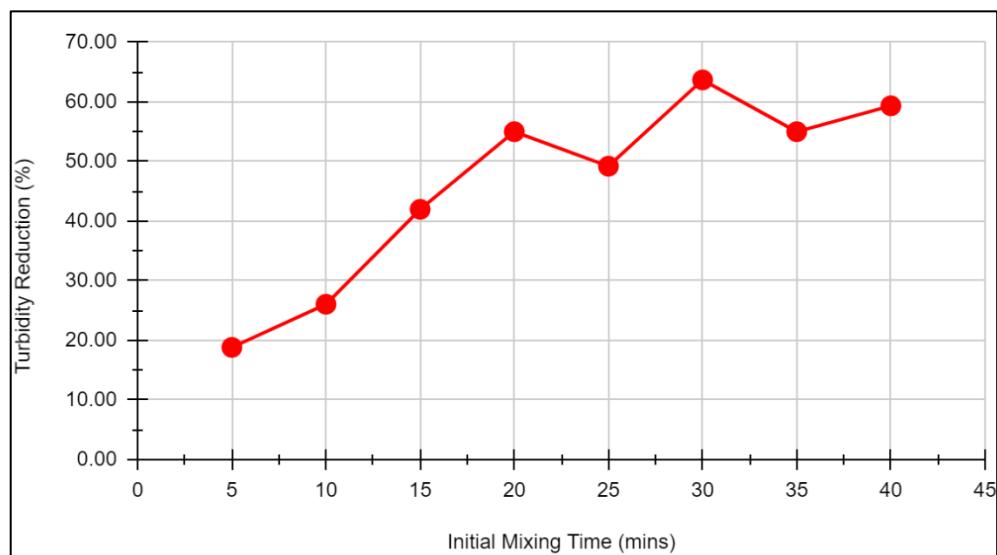


Figure 4.9: Turbidity Reduction Rate of Different Initial Mixing Time Using Annona Seed.

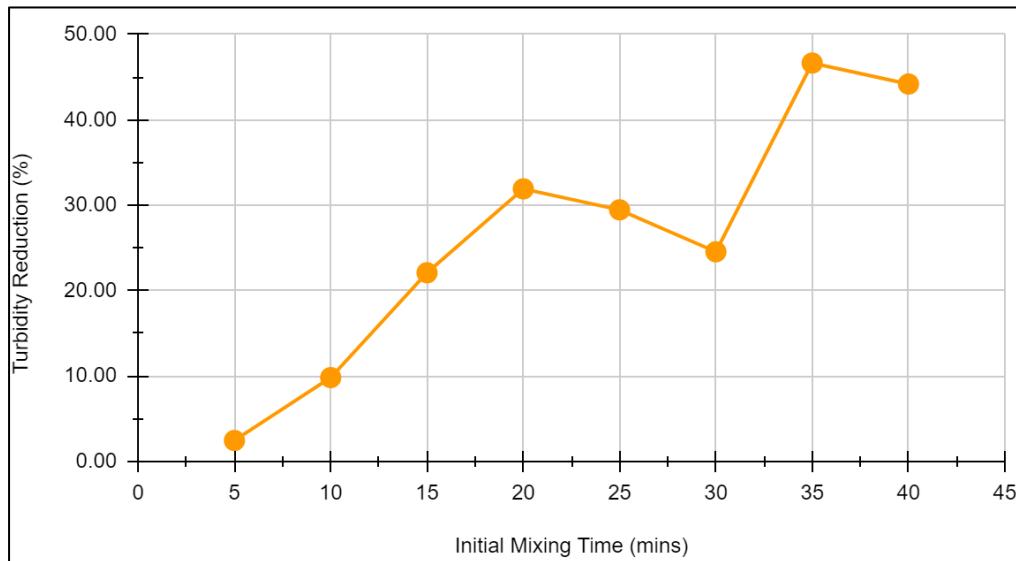


Figure 4.10: Turbidity Reduction Rate of Different Initial Mixing Time Using Ferric Chloride.

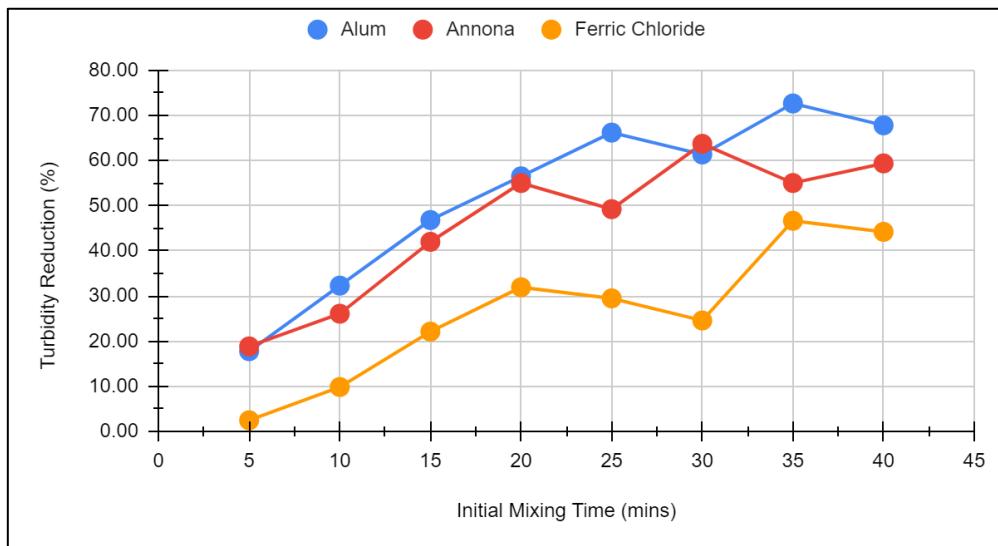


Figure 4.11: Turbidity Reduction Rate of Different Initial Mixing Time Using Alum, Annona Seed, and Ferric Chloride.

From all the graphs stated in the figure above, it has been proven that the turbidity reduction rate in all three coagulants gets higher as the initial mixing duration increases. The graphical trend of high-speed mixing time of coagulant with wastewater is directly proportional to the turbidity reduction efficiency. All three coagulants achieve below 20% when the given initial mixing time is only 5 minutes. When coagulants are added to water during the treatment process, they need to mix thoroughly with the water to effectively

interact with the suspended particles causing turbidity. The initial mixing time refers to the duration during which the coagulant is rapidly mixed with the water after addition.

A longer initial mixing time allows more time for the coagulant to disperse evenly throughout the water and react with the suspended particles. This prolonged contact time facilitates the process of particle destabilization and aggregation, leading to the formation of larger flocs. These larger flocs are more easily removed through sedimentation or filtration, resulting in greater turbidity reduction. However, if the initial mixing time is too short, the coagulant may not have enough time to disperse adequately, leading to uneven distribution and incomplete interaction with the suspended particles. This can result in ineffective particle destabilization and smaller floc formation, leading to lower turbidity reduction efficiency.

#### **4.4 Mixing Speed**

For turbidity reduction testing on different mixing speeds, the mixing speed during initial mixing that will be assessed is ranging from 0rpm to 300rpm with 50rpm interval at a constant mixing time of 15 minutes. The initial turbidity and pH are recorded before mixing, whereas the final turbidity of the three coagulant mixing will be documented after a slow-mixing process of 30 minutes is completed. Table 4.7 below shows the measurement of initial turbidity and final turbidity of the wastewater whereas Table 4.8 shows the calculation value of turbidity reduction percentage of all three coagulants. The parameters that will remain constant throughout this testing will be listed in Table 4.9 below.

Table 4.7: Final Turbidity on the Testing of Different Initial Mixing Speed.

<b>Initial Mixing Speed (rpm)</b>	<b>Initial Turbidity (NTU)</b>	Alum	Annona	Ferric Chloride
		<b>Final Turbidity (NTU)</b>		
0	154	131	128	141
50	142	101	118	123
100	156	103	120	131
150	172	91.5	99.8	134
200	148	80.9	79.7	109
250	163	80.6	88.4	128
300	168	96.5	98.8	135

Table 4.8: Turbidity Reduction Rate on the Testing of Different Initial Mixing Speed.

<b>Initial Mixing Speed (rpm)</b>	Alum	Annona	Ferric Chloride
	<b>Turbidity Reduction (%)</b>		
0	14.94	16.88	8.44
50	28.87	16.90	13.38
100	33.97	23.08	16.03
150	46.80	41.98	22.09
200	45.34	46.15	26.35
250	50.55	45.77	21.47
300	42.56	41.19	19.64

Table 4.9: Constant Variables of Different Initial Mixing Speed Test.

Parameters	Value
Slow Mixing Speed (rpm)	40
Initial Mixing Time (mins)	15
Low Speed Mixing Time (mins)	30
pH	7.04
Temperature (°C)	28
Dosage (mL)	30
Coagulant Concentration (mg/L)	1000
Kaolin Water Concentration (mg/L)	100
Working Volume (mL)	300

It can be noted that the effectiveness of alum, Annona seed, and ferric chloride in reducing turbidity is notably low when there is no initial mixing (0rpm), with reductions of 14.94%, 16.88%, and 8.44%, respectively. The turbidity reduction efficiency of Annona seed and ferric chloride peaks at an initial mixing speed of 200rpm, with improvements to 46.15% and 26.35%, respectively, while alum achieves its highest reduction rate of 50.55% at an initial mixing speed of 250rpm. This research demonstrates that higher initial mixing speeds significantly enhance the performance of the coagulation-flocculation process in wastewater treatment. However, it is evident that the mixing time required for each coagulant to reach peak performance varies. When the coagulant is initially mixed with wastewater at a speed of 300rpm, there is a slight decrease in turbidity reduction rates to 42.56%, 41.19%, and 19.64% for alum, Annona seed, and ferric chloride, respectively.

The initial mixing speed during the coagulation process plays a crucial role in determining the effectiveness of turbidity reduction achieved by both biocoagulants and coagulants. When mixing speeds are higher between wastewater and coagulant, larger flocs are formed, whereas lower mixing speeds result in smaller flocs. According to Ahmed et al. (2022), the efficiencies of turbidity and SS removal are not only highly dependent on the initial mixing time but also on the initial mixing speeds. Faster mixing speed in the initial phase significantly influences the formation of flocs during the

slow mixing phase. Fast mixing for a duration shorter than the optimum leads to higher residual turbidity and larger flocs. Figure 4.12, Figure 4.13, and Figure 4.14 allow for clearer illustration on the results of turbidity reduction rate under different initial mixing speeds. Figure 4.15 demonstrates a combined graphical result of alum, Annona seed, and ferric chloride.

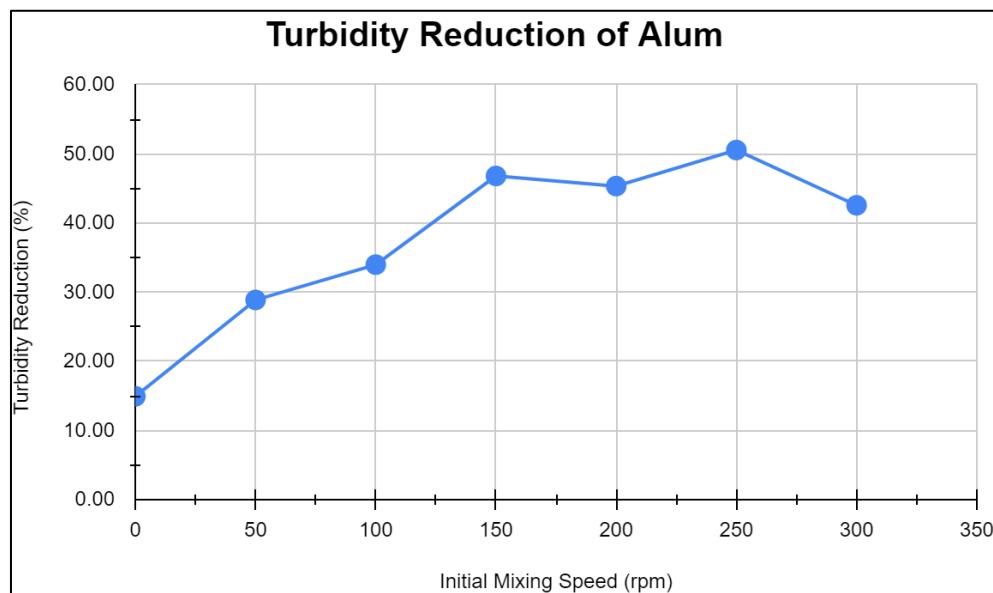


Figure 4.12: Turbidity Reduction Rate of Different Initial Mixing Speed Using Alum.

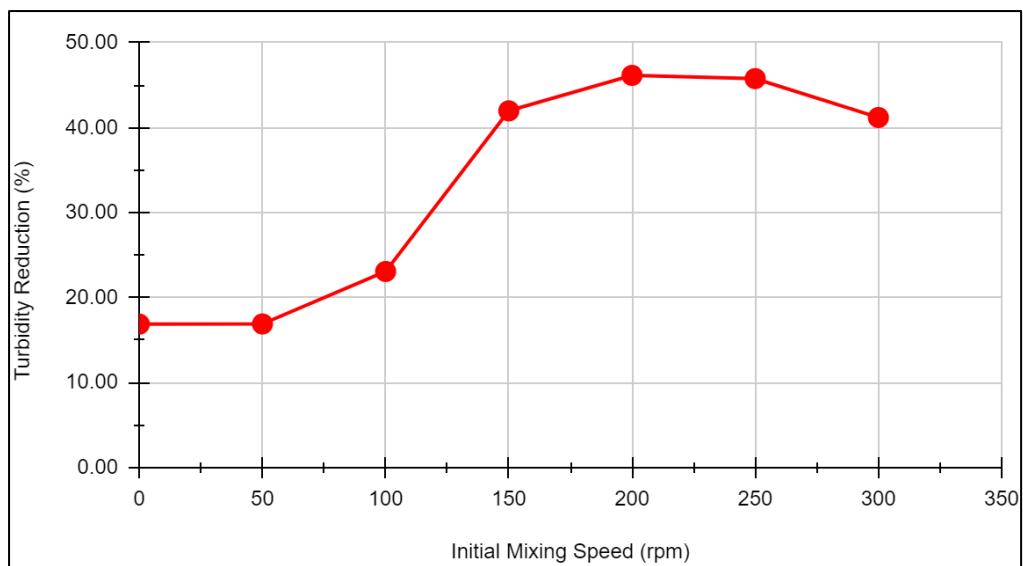


Figure 4.13: Turbidity Reduction Rate of Different Initial Mixing Speed Using Annona Seed.

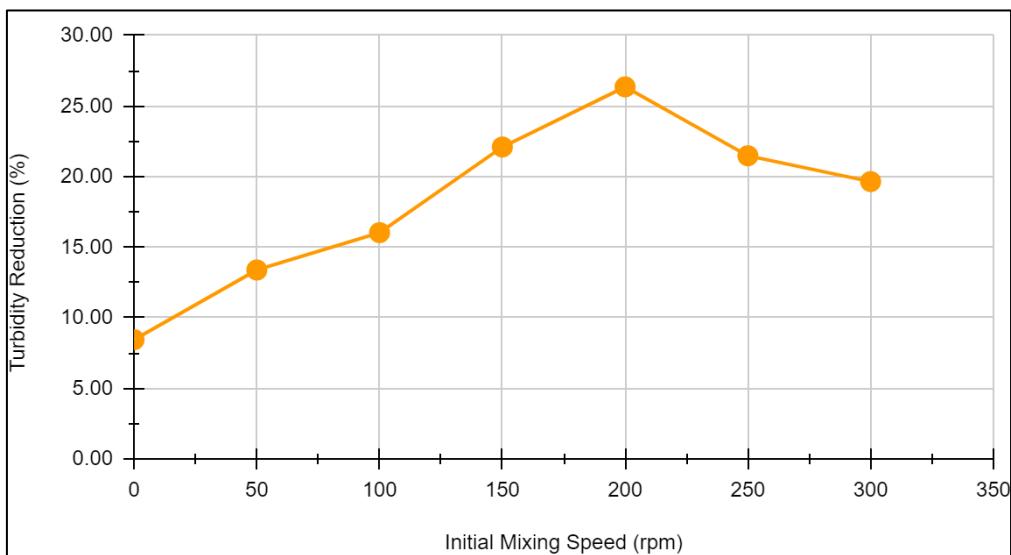


Figure 4.14: Turbidity Reduction Rate of Different Initial Mixing Speed Using Ferric Chloride.

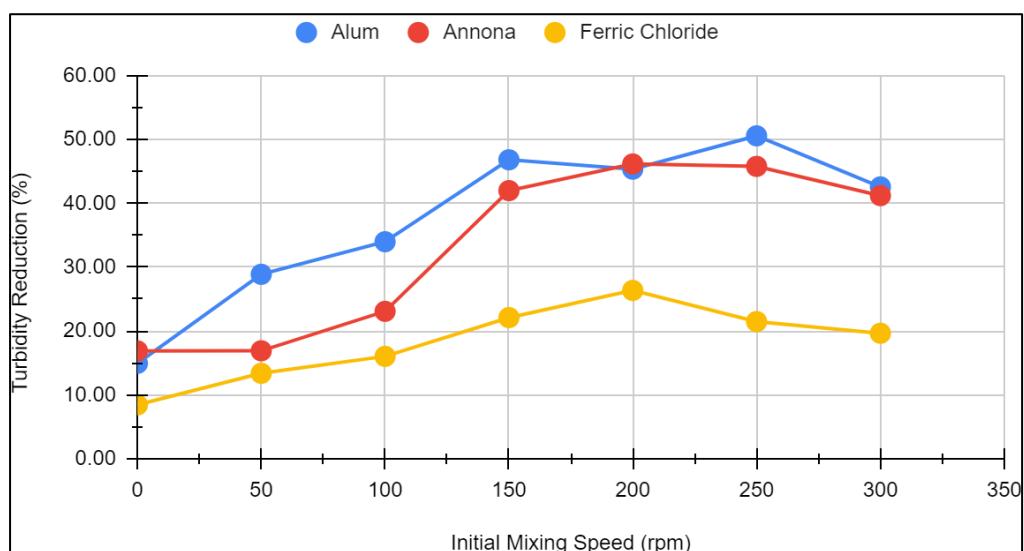


Figure 4.15: Turbidity Reduction Rate of Different Initial Mixing Speed Using Alum, Annona Seed, and Ferric Chloride.

From the graphs presented in all the figures above, it is evident that the turbidity reduction rate for all three coagulants increases as the initial mixing speed rises. The graphical trend indicates a direct proportionality between the initial mixing speed of the coagulant with wastewater and the turbidity reduction efficiency. When mixing with wastewater does not undergo any high-speed mixing, all three coagulants achieve turbidity reduction rates below 20%. However, when coagulants are added to water during the

treatment process, higher mixing speeds enable effective interaction between the coagulant particles and the suspended particles, thereby reducing turbidity. Therefore, the speed of the initial mixing process during coagulation plays a significant role in determining the effectiveness of turbidity reduction by the coagulant.

Higher mixing speeds result in more efficient dispersion of the coagulant throughout the wastewater. This improved dispersion enhances the interaction between the coagulant and the suspended particles, promoting the formation of larger and denser flocs. Higher initial mixing speeds between wastewater and coagulant can accelerate the collision frequency between the coagulant particles and the suspended particles in the wastewater. According to Sohrabi et al. (2018), this higher collision frequency enhances the aggregation of suspended particles into larger flocs, allowing their subsequent sedimentation or filtration. On the other hand, if the initial mixing speed is too low, the dispersion of the coagulant may be inadequate, leading to incomplete or inefficient interaction with the suspended particles (Yu et al., 2011). This can result in insufficient floc formation and reduced turbidity reduction efficiency of coagulant to wastewater. Therefore, optimizing the speed of the initial mixing process is crucial to ensure effective coagulation and turbidity reduction in wastewater treatment processes.

#### **4.5 Colour and Texture**

Coagulation can affect the color of wastewater by allowing the removal of colored impurities through the formation of flocs. When coagulants are added to wastewater, they neutralize the negative charges on suspended particles and dissolved substances, causing them to clump together and form larger aggregates called flocs (Getahun, Befekadu, and Alemayehu, 2024). These flocs can trap and encapsulate colored molecules present in the wastewater, including organic compounds, dyes, and other pigments. As a result, the flocs settle more readily during sedimentation or are easier to remove during filtration processes, effectively reducing the concentration of colored impurities in the water. Figure 4.16, Figure 4.17, and Figure 4.18 show one of

the visual results of the turbidity removal in wastewater by alum, Annona seed, and ferric chloride, respectively.



Figure 4.16: Texture of Wastewater Before Coagulation (left) vs After Coagulation (right) by Alum.



Figure 4.17: Texture of Wastewater Before Coagulation (left) vs After Coagulation (right) by Annona Seed.

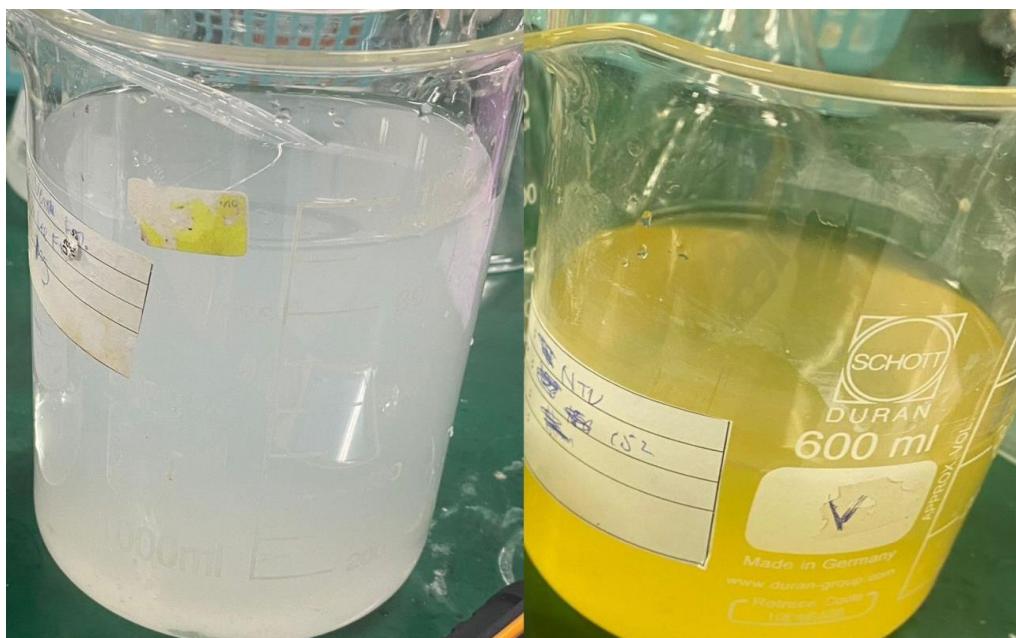


Figure 4.18: Texture of Wastewater Before Coagulation (left) vs After Coagulation (right) by Ferric Chloride.

Coagulation can promote the adsorption of colored molecules onto the surfaces of the flocs, further enhancing their removal from the wastewater. This adsorption mechanism helps to physically remove colored substances from the water, resulting in clearer and less colored effluent. Overall, coagulation plays a vital role in reducing the color of wastewater by promoting the aggregation and removal of colored impurities, improving the aesthetic quality and environmental acceptability of the treated water. The process responsible for removing color in this scenario is primarily coagulation and flocculation, rather than improved softening or other factors (Malakootian and Fatehizadeh, 2010). From Figure 4.16 and Figure 4.17, it proves that alum and Annona seeds had made the texture of the water clearer by efficiently decreasing turbidity in water. This is due to the successful coagulation-flocculation process between wastewater and the coagulants where the captured flocculants had settled to the bottom of the beaker.

On the other hand, wastewater treated by ferric chloride shows the worst visual result in color removal compared to the other two coagulants as the treated wastewater was left with dark yellowish color as shown in Figure 4.18 above. The utilization of ferric chloride shows quite promising results in removing turbidity of kaolin water based on all three different testing that

were done in this study. However, the predetermined concentration of ferric chloride coagulant used throughout this study was higher than the ratio of working volume of wastewater. Hence, this makes the concentration of ferric chloride to be slightly unfit for the working volume of wastewater.

#### **4.6 Summary**

In short, *Annona* seed is a viable option for water treatment as its turbidity reduction rate proved to be superior to the performance of ferric chloride. Although alum has given the best efficiency in treating turbid water, the results of *Annona* seeds from all three testing can be equally matched or deemed identical with alum. Further studies can be conducted if given the right tools or apparatus that can aid in further improving the study such as in removing the fat content in *Annona* seeds or magnetic stirrer that has temperature adjustment. From the testing of pH, it is proven that coagulants can work better under lower pH rather than higher pH. In the testing of different initial mixing times, the higher the duration of initial mixing, the lower the turbidity in water due to sufficient time for the coagulation-flocculation process to take place. Lastly, for the testing of different initial mixing speeds, higher mixing speed can enhance the turbidity removal efficiency of coagulant by allowing thorough mixing between the residues of wastewater and coagulant to react with one another. Therefore, *Annona* seeds have solidified as an ideal biocoagulant to replace conventional chemical coagulant for future wastewater treatment.

## CHAPTER 5

### CONCLUSIONS AND RECOMMENDATIONS

#### 5.1 Conclusion

Concisely, the turbidity tests were conducted to study the overall performance and effectiveness of the different coagulants. Multiple manipulative variables were predetermined before conducting the testing to observe and compare the results whether the variables will bring a significant impact on the turbidity reduction efficiency by each of the coagulants. This study introduced the utilization and benefits of nature-based solutions, specifically focusing on the extraction of a natural coagulant from *Annona* seed. Three experiments were carried out to establish the ideal pH, initial mixing speed, and initial mixing time of the coagulant for reducing turbidity in kaolin wastewater. Ferric chloride and alum coagulants were also employed in kaolin wastewater samples for comparative analysis. Independent variables were optimized in each of the three testing to identify the optimal conditions for achieving the most effective treatment outcomes.

The performance of alum, *Annona* seeds, and ferric chloride in reducing the turbidity in water had been compared with which alum produced the best results, followed by *Annona* seeds, and then ferric chloride. To produce a balanced and fair results, all three types of coagulant are customized with the same concentration which is 1000mg/L whereas the working volume of kaolin wastewater was determined to be 300mL with the concentration of 100mg/L throughout the experiment. Hence, the optimal dosage of all three coagulants required for effective water treatment will be the same which is 30mL. The theory that coagulants can perform well under lower pH level had been proven through this study. When alum, *Annona* seed, and ferric chloride treat the wastewater under the pH level of 3.07, their turbidity reduction efficiency is at 59.88%, 57.85%, and 44.88%, respectively. However, when treating the turbid water with a pH level of 10.98, all three coagulants were only able to achieve turbidity reduction rate of below 15%.

In turbidity reduction testing of coagulants under different initial mixing time, alum produces the best result when mixed for 35 minutes where its turbidity reduction rate is at 72.62%. Annona seeds can reduce turbidity in water up to 63.69% when the mixing duration is at 30 minutes. However, ferric chloride produced the worst result compared to the other two coagulants where it can only reduce 46.64% of turbidity in water for 30 minutes. For turbidity reduction testing under different initial mixing speed, alum and Annona seeds show the identical results where the intensity of mixing at 250rpm, both coagulants can reduce turbid water above 45%. Unfortunately for ferric chloride, its best turbidity reduction rate is only at 26.35% occurring at the mixing speed of 200rpm.

## 5.2 Recommendations

There are several operating conditions that can be further studied in determining whether Annona seeds can be suitable to be used in future wastewater treatment industries such as different temperature, dosage, and concentration of coagulant or wastewater. Different operating conditions of wastewater can affect the performance of both biocoagulant and coagulant due to their influence on the chemical reactions involved in the coagulation process. Wastewater tends to contain elevated levels of organic compounds, such as humic acids or proteins. This can interfere with the coagulation-flocculation process by competing for binding sites on coagulant molecules. Therefore, this will result in the reduction of the effectiveness of coagulants and require higher doses to achieve desired treatment outcomes.

In addition, the optimization of the extraction process of Annona seeds may impact its workability in water treatment. Different seed extraction methods need to be explored and investigated to maximize the yield of the biomass content which function as active coagulant agents to treat turbid water. This could involve varying parameters such as solvent type, extraction temperature, and extraction time to determine the most efficient method. In terms of the biomass properties within the Annona seeds, precise characterization of coagulant content can be done through techniques like chromatography, spectroscopy, and microscopy. The purpose is to analyze the

chemical composition and structural properties of the coagulant, meanwhile identify and quantify the active compounds present in *Annona* seed extracts responsible for coagulation activity.

Other than that, exploring alternate removal types with *Annona* seeds can prove fruitful as most wastewater contains several types of waste content. Henceforth, studies can be conducted on the removal of odor, color, organic matter removal, heavy metal removal, and presence of organic content within the wastewater using *Annona* seeds. An extra procedure must be added during the *Annona* seed extraction process which is the removal of the fat content within the seed. This is because the fat in the seed may interfere with the coagulation process by forming emulsions or coatings on particles, reducing the effectiveness of coagulation agents. Removing fat ensures better contact between the coagulant and the suspended particles, leading to improved coagulation efficiency.

## REFERENCES

- Aguilera Flores, M.M., Medellín Castillo, N.A., Ávila Vázquez, V., González García, R., Cardona Benavides, A., and Carranza Álvarez, C. (2022). Evaluation of a biocoagulant from devilfish invasive species for the removal of contaminants in ceramic industry wastewater. *Scientific Reports*, 12(1). doi: <https://doi.org/10.1038/s41598-022-14242-6> [Accessed 21 Aug. 2023].
- Ahmed, S., Ayoub, G., Al-Hindi, M. and Azizi, F. (2022). *The Effect of Fast Mixing Conditions on the Coagulation-Flocculation of Highly Turbid Suspensions Using Magnesium Hydroxide Coagulant*. [online] American University of Beirut. Available at: <http://uest.ntua.gr/win4life/proceedings/papers/ahmedetal.pdf> [Accessed 3 Apr. 2024].
- Akhiar, A., Battimelli, A., Torrijos, M. and Carrere, H. (2017). Comprehensive characterization of the liquid fraction of digestates from full-scale anaerobic co-digestion. *Waste Management*, [online] 59, pp.118–128. doi: <https://doi.org/10.1016/j.wasman.2016.11.005> [Accessed 21 Aug. 2023].
- Barrera-Díaz, C.E., Balderas-Hernández, P. and Bilyeu, B. (2018). *Chapter 3 - Electrocoagulation: Fundamentals and Prospectives*. [online] ScienceDirect. Available at: <https://www.sciencedirect.com/topics/engineering/chemical-coagulation#:~:text=Traditional%20chemical%20coagulation%20uses%20aluminum,ferric%20chloride%20sulfate%20%5B4%5D>. [Accessed 1 Apr. 2024].
- Bhalkaran, S. and Wilson, L. (2016). Investigation of Self-Assembly Processes for Chitosan-Based Coagulant-Flocculant Systems: A Mini-Review. *International Journal of Molecular Sciences*, [online] 17(10), p.1662. doi: <https://doi.org/10.3390/ijms17101662> [Accessed 28 Aug. 2023].
- Bradley, E. (2022). *Wastewater Coagulation*. [online] www.doer.com. Available at: <https://www.doer.com/water-treatment/resources/wastewater-coagulation#:~:text=Coagulation%20is%20the%20chemical%20water> [Accessed 16 Aug. 2023].

Britannica (2020). Kaolin | clay. In: *Encyclopædia Britannica*. [online] Available at: <https://www.britannica.com/science/kaolin> [Accessed 28 Mar. 2024].

BYJUS. (n.d.). *Dilution Factor Equation - Overview of Dilution, Dilution Factor Equation along with FAQs*. [online] Available at: <https://byjus.com/chemistry/dilution-factor-equation/> [Accessed 28 Mar. 2024].

Cassini, S.T., Francisco, S.A., Antunes, P.W.P., Oss, R.N. and Keller, R. (2017). Harvesting Microalgal Biomass grown in Anaerobic Sewage Treatment Effluent by the Coagulation-Flocculation Method: Effect of pH. *Brazilian Archives of Biology and Technology*, [online] 60(0). doi: <https://doi.org/10.1590/1678-4324-2017160174> [Accessed 28 Aug. 2023].

Centre For Affordable Water and Safe Technology (n.d.). *Chemical Coagulants / Multi-barrier Approach / Sedimentation*. [online] CAWST. Available at: <https://www.hwts.info/products-technologies/25154a7c/chemical-coagulants/technical-information> [Accessed 1 Apr. 2024].

Cui, W., Li, S., Xie, M., Chen, Q., Li, G. and Luo, W. (2022). Performance of coagulant-aided biomass filtration to protect ultrafiltration from membrane fouling in biogas slurry concentration. *Environmental Technology & Innovation*, [online] 28, p.102659. doi: <https://doi.org/10.1016/j.eti.2022.102659> [Accessed 28 Aug. 2023].

Czemierska, M., Szcześ, A. and Jarosz-Wilkołazka, A. (2015). Purification of wastewater by natural flocculants. *BioTechnologia*, [online] 4, pp.272–278. doi: <https://doi.org/10.5114/bta.2015.57731> [Accessed 18 Aug. 2023].

Daryabeigi, A., Baghvand, A., Zand, A., Mehrdadi, N. and Karbassi, A. (2010). Optimizing Coagulation Process for Low to High Turbidity Waters Using Aluminum and Iron Salts. *American Journal of Environmental Sciences*, [online] 6(5), pp.442–448. Available at: <https://thescipub.com/pdf/ajessp.2010.442.448.pdf> [Accessed 3 Apr. 2024].

Desbrières, J. and Guibal, E. (2017). Chitosan for wastewater treatment. *Polymer International*, [online] 67(1), pp.7–14. doi: <https://doi.org/10.1002/pi.5464> [Accessed 18 Aug. 2023].

Du, L., Zhang, Z., Li, G., Sun, Q. and Zhang, B. (2019). Composting of Cornstalks Used as Filtering Materials for the Pretreatment of Anaerobically Digested Centrate. *Compost Science & Utilization*, [online] 27(2), pp.81–87. doi: <https://doi.org/10.1080/1065657x.2019.1571460> [Accessed 18 Aug. 2023].

Economou, C.N., Marinakis, N., Moustaka-Gouni, M., Kehayias, G., Aggelis, G. and Vayenas, D.V. (2015). Lipid production by the filamentous cyanobacterium *Limnothrix* sp. growing in synthetic wastewater in suspended- and attached-growth photobioreactor systems. *Annals of Microbiology*, 65(4), pp.1941–1948. doi: <https://doi.org/10.1007/s13213-014-1032-7> [Accessed 24 Aug. 2023].

Greenwood, J. and Greaves, J. (2022). *How are coagulants and flocculants used in water and wastewater treatment?* [online] [www.wcs-group.co.uk](http://www.wcs-group.co.uk). Available at: <https://www.wcs-group.co.uk/wcs-blog/coagulants-flocculants-wastewater-treatment#Which-coagulant> [Accessed 16 Aug. 2023].

Gregory, J. and Barany, S. (2011). Adsorption and flocculation by polymers and polymer mixtures. *Advances in Colloid and Interface Science*, [online] 169(1), pp.1–12. doi: <https://doi.org/10.1016/j.cis.2011.06.004> [Accessed 18 Aug. 2023].

Gurak P.D., De Bona G.S., Tessaro I.C., and Marczak L.D.F. (2014). Jaboticaba pomace powder obtained as a coproduct of juice extraction: a comparative study of powder obtained from peel and whole fruit. *Food Res. Int.*, 62(2014): 786-792.

Gutiérrez, R., Passos, F., Ferrer, I., Uggetti, E. and García, J. (2015). Harvesting microalgae from wastewater treatment systems with natural flocculants: Effect on biomass settling and biogas production. *Algal Research*, 9, pp.204–211. doi: <https://doi.org/10.1016/j.algal.2015.03.010> [Accessed 18 Aug. 2023].

Hunter, B. and Deshusses, M.A. (2020). Resources recovery from high-strength human waste anaerobic digestate using simple nitrification and denitrification filters. *Science of The Total Environment*, [online] 712, p.135509. doi: <https://doi.org/10.1016/j.scitotenv.2019.135509> [Accessed 18 Aug. 2023].

Khaled, P. (2019). Characterization Techniques of Two-Dimensional Nanomaterials. [online] Available at: <https://www.sciencedirect.com/topics/materials-science/scanning-electronmicroscopy>. [Accessed on 11 April 2024].

Koul, B., Bhat, N., Abubakar, M., Mishra, M., Arukha, A.P. and Yadav, D. (2022). Application of Natural Coagulants in Water Treatment: A Sustainable Alternative to Chemicals. *Water*, [online] 14(22), p.3751. doi: <https://doi.org/10.3390/w14223751> [Accessed 18 Aug. 2023].

Kuldeyev, E., Ospanov, K., Andraka, D. and Merkýreva, S. (2023). Experimental Study on the Application of Sludge from Water Treatment Plant as a Reagent for Phosphate Removal from Wastewater. *Water*, [online] 15(15), pp.2691–2691. doi: <https://doi.org/10.3390/w15152691>. [Accessed 3 Apr. 2024].

Kumar, P., Prasad, B. and Chand, S. (2009). Treatment of desizing wastewater by catalytic thermal treatment and coagulation. *Journal of Hazardous Materials*, 163(1), pp.433–440. doi: <https://doi.org/10.1016/j.jhazmat.2008.06.114> [Accessed 18 Aug. 2023].

Kurniawan, S., Abdullah, S., Imron, M., Said, N., Ismail, N., Hasan, H., Othman, A. and Purwanti, I. (2020). Challenges and Opportunities of Biocoagulant/Biofloculant Application for Drinking Water and Wastewater Treatment and Its Potential for Sludge Recovery. *International Journal of Environmental Research and Public Health*, [online] 17(24), p.9312. doi: <https://doi.org/10.3390/ijerph17249312>. [Accessed 18 Aug. 2023].

Lananan, F., Mohd Yunos, F.H., Mohd Nasir, N., Abu Bakar, N.S., Lam, S.S. and Jusoh, A. (2016). Optimization of biomass harvesting of microalgae, Chlorella sp. utilizing auto-flocculating microalgae, Ankistrodesmus sp. as bio-flocculant. *International Biodegradation & Biodegradation*, [online] 113, pp.391–396. doi: <https://doi.org/10.1016/j.ibiod.2016.04.022> [Accessed 15 Aug. 2023].

Li, T., Hu, J. and Zhu, L. (2021). Self-Flocculation as an Efficient Method to Harvest Microalgae: A Mini-Review. *Water*, [online] 13(18), p.2585. doi: <https://doi.org/10.3390/w13182585> [Accessed 29 Aug. 2023].

Li, Y., Xu, Z.C., Xie, M., Zhang, B., Li, G. and Luo, W. (2020). Resource recovery from digested manure centrate: Comparison between conventional and aquaporin thin film composite forward osmosis membranes. *Journal of Membrane Science*, [online] 593, pp.117436–117436. doi: <https://doi.org/10.1016/j.memsci.2019.117436> [Accessed 18 Aug. 2023].

Malakootian, M. and Fatehizadeh, A. (2010). Color Removal from Water by Coagulation/Caustic Soda and Lime. *Iranian Journal of Environmental Health Science & Engineering*, [online] 7(3), pp.267–272. Available at: [https://www.researchgate.net/publication/228821249\\_Color\\_removal\\_fom\\_water\\_by\\_coagulationcaustic\\_soda\\_and\\_lime](https://www.researchgate.net/publication/228821249_Color_removal_fom_water_by_coagulationcaustic_soda_and_lime) [Accessed 13 Apr. 2024].

Mohamed Hatta, N.S., Lau, S.W., Takeo, M., Chua, H.B., Baranwal, P., Mubarak, N.M. and Khalid, M. (2021). Novel cationic chitosan-like bioflocculant from *Citrobacter youngae* GTC 01314 for the treatment of kaolin suspension and activated sludge. *Journal of Environmental Chemical Engineering*, [online] 9(4), p.105297. doi: <https://doi.org/10.1016/j.jece.2021.105297> [Accessed 30 Aug. 2023].

Naceradska, J., Pivokonska, L. and Pivokonsky, M. (2019). On the importance of pH value in coagulation. *Journal of Water Supply: Research and Technology-Aqua*, [online] 68(3), pp.222–230. doi: <https://doi.org/10.2166/aqua.2019.155>. [Accessed 1 Apr. 2024].

Nguyen, M.D., Thomas, M., Surapaneni, A., Moon, E.M. and Milne, N.A. (2022). Beneficial reuse of water treatment sludge in the context of circular economy. *Environmental Technology & Innovation*, [online] 28(2352-1864), p.102651. doi: <https://doi.org/10.1016/j.eti.2022.102651>. [Accessed 1 Apr. 2024].

Oladoja, N.A., Ali, J., Lei, W., Yudong, N. and Pan, G. (2020). Coagulant derived from waste biogenic material for sustainable algae biomass harvesting. *Algal Research*, 50, p.101982. doi: <https://doi.org/10.1016/j.algal.2020.101982> [Accessed 16 Aug. 2023].

Safe Drinking Water Foundation (n.d.). *Conventional Water Treatment: Coagulation and Filtration*. [online] Safe Drinking Water Foundation. Available at: <https://www.safewater.org/fact-sheets-1/2017/1/23/conventional-water-treatment#:~:text=It%20is%20however%20an%20important> [Accessed 16 Aug. 2023].

Sohrabi, Y., Rahimi, S., Nafez, A.H., Mirzaei, N., Bagheri, A., Ghadiri, S.K., Rezaei, S. and Chargineh, S.S. (2018). Chemical Coagulation Efficiency in Removal of Water Turbidity. *International Journal of Pharmaceutical Research*, [online] 10(03). doi: <https://doi.org/10.31838/ijpr/2018.10.03.071>. [Accessed 1 Apr. 2024].

Salman, M., Athar, M. and Farooq, U. (2015). Biosorption of heavy metals from aqueous solutions using Indigenous and modified lignocellulosic materials. *Reviews in Environmental Science and Bio/Technology*, [online] 14(2), pp.211–228. doi: <https://doi.org/10.1007/s11157-015-9362-x> [Accessed 18 Aug. 2023].

Singh, M.K. and Singh, A. (2022). *Chapter 17 - Scanning electron microscope*. [online] ScienceDirect. Available at: <https://www.sciencedirect.com/science/article/abs/pii/B9780128239865000087> [Accessed 4 Apr. 2024].

Sun, Q., Zhao, S., Yan, Y., Jia, W. and Yang, W. (2020). Parameter optimization and mechanism research of enhanced coagulation treatment for Yuquan River water. *Water Science & Technology: Water Supply*, [online] 20(3), pp.1072–1082. doi: <https://doi.org/10.2166/ws.2020.030> [Accessed 18 Aug. 2023].

THE GLOBAL GOALS (2022). Goal 6: Clean water and sanitation. [online] The Global Goals. Available at: <https://www.globalgoals.org/goals/6-clean-water-and-sanitation/> [Accessed 24 Jul. 2023].

Usman, I.M.T., Lee, F.W., Ho, Y.C., Khaw, H.P., Chong, Q.W., Kee, Y.M., Lim, J.W. and Show, P.L. (2023). Evaluation of *Annona diversifolia* Seed Extract as A Natural Coagulant for Water Treatment. *Sustainability*, [online] 15(7), p.6324. doi: <https://doi.org/10.3390/su15076324>. [Accessed 18 Aug. 2023].

United Nations (2022). *Goal 6 / Department of Economic and Social Affairs*. [online] [sdgs.un.org](https://sdgs.un.org). Available at: <https://sdgs.un.org/goals/goal6> [Accessed 24 Jul. 2023].

Vimala, J., Lidiya, R., Aiswariya, K.S. and Paulson, M. (2021). Green synthesis of silver nanoparticles using *Annona squamosa* L. seed extract: characterization, photocatalytic and biological activity assay. *ResearchGate*, [online] 44(9), pp.1–11. Available at: [https://www.researchgate.net/publication/350682341\\_Green\\_synthesis\\_of\\_silver\\_nanoparticles\\_using\\_Annona\\_squamosa\\_L\\_seed\\_extract\\_characterization\\_photocatalytic\\_and\\_biological\\_activity\\_assay](https://www.researchgate.net/publication/350682341_Green_synthesis_of_silver_nanoparticles_using_Annona_squamosa_L_seed_extract_characterization_photocatalytic_and_biological_activity_assay) [Accessed 11 Sep. 2023].

Won, J., Wirth, X. and Burns, S.E. (2019). An experimental study of cotransport of heavy metals with kaolinite colloids. *Journal of Hazardous Materials*, [online] 373, pp.476–482. doi: <https://doi.org/10.1016/j.jhazmat.2019.03.110> [Accessed 18 Aug. 2023].

Yu, W., Gregory, J., Campos, L., and Li, G. (2011). The role of mixing conditions on floc growth, breakage and regrowth, *Chemical Engineering Journal*, 171, pp.425-430.

Zainol, N.A., Khalilullah, P.A.B., Ghani, A.A., Rashid, N.A. and Makhtar, S.M.Z. (2022). Turbidity Removal from Kaolin Synthetic Wastewater via Coagulation Process Using Sludge from Water Treatment Plant. *International Journal of Integrated Engineering*, [online] 14(9), pp.222–231. doi: <https://doi.org/10.30880/ijie.2022.14.09.028>. [Accessed 4 Apr. 2024].