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EXPLORING CELLULOSE AS RAW MATERIAL IN THE PRODUCTION OF A COAGULANT/FLOCCULANT FOR WASTEWATER TREATMENT

Faculdade de Ciências e Tecnologia

Erasmus Mundus Mestrado em Qualidade em Laboratórios Analíticos

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Trabalho efetuado sob a orientação de:

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Faculdade de Ciências e Tecnologia

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Declaration of Authorship

I declare I am the author of this work, which is original and unpublished. The sources consulted have been duly cited in the text and included in the list of references.

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(Abdullah Ismail)

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Dedication

I dedicate this thesis to my father, Muhammad Ismail, and my son Ahmed Ismail.

Abstract

Cellulose, the most prevalent natural biopolymer on earth, may provide an efficient, environment-friendly, inexpensive, and chemical-free solution for water and wastewater treatment. Cellulose-based materials are utilized in the food industry, pharmaceutical, paper textile industries, and wastewater treatment due to their low cost, renewability, biodegradability, and nontoxicity. This study aims to explore cellulose as raw material to produce coagulants to remove turbidity from wastewater. All the synthesized coagulants and a commercial coagulant, for reference purposes, were tested in jar test experiments using synthetic wastewater with a turbidity of 92-96 NTU and real urban wastewater with a turbidity of 142-156 NTU. Cellulose extracted from powdered pine needles was modified by reductive amination after the periodate oxidation, showed no turbidity removal during the jar test. Cellulose from filter paper was processed by 2,2,6,6- tetramethyl-4-acetamidopiperidin-1-oxyl (TEMPO) oxidation and examined to assess its coagulation performance, which did not show the desired activity. Finally, three different micro/nanofibrillated cellulose (M/NFCs) derivatives cationized with 3-chloro-2-hydroxypropyltrimethyl ammonium chloride (CHPTAC) and with carboxymethyl trimethyl ammonium chloride hydrazide (Girard's T reagent), as well as anionized by TEMPO oxidation were analyzed. CHPTAC-treated samples showed excellent removal of turbidity, which was close to the performance of polyaluminium chloride commercial-grade (WAC-AB®) coagulant. MFCs (treated with CHPTAC) with a higher degree of substitution (DS 0.106) showed 93.2% turbidity removal for synthetic wastewater and 85.7% for real urban wastewater, while that with a lower degree of substitution (DS 0.06) showed 91.0% and 58.9% removal for turbidity in synthetic and real wastewater, respectively.

Keywords: Cellulose, Coagulant, Pine needles, Micro/nanofibrils, Wastewater, Turbidity removal

Sumário

A cellulose, sendo o polímero natural mais abundante na Terra, pode ser a resposta para o desenvolvimento de soluções amigas do ambiente, a baixo custo e livres de químicos, para tratamento de águas para consumo e de águas residuais. Materiais à base de celulose são já utilizados nas indústrias alimentar, farmacêutica, do papel e têxtil, bem como no tratamento de águas residuais, devido ao seu baixo custo e por serem renováveis, biodegradáveis e não apresentarem toxicidade. Este estudo tem por objetivo explorar a celulose como matéria-prima na produção de coagulantes para remoção de turbidez em águas residuais. Todos os coagulantes sintetizados, bem como um coagulante comercial, usado como referência, foram testados num *jar test* usando uma água residual modelo (turvação de 92-96 NTU) e uma água residual urbana (turvação de142-156 NTU). A celulose extraída de agulhas de pinheiro foi modificada por oxidação com periodato seguida de aminação redutiva, não tendo produzido formação de flocos no *jar test*. A celulose obtida de papel de filtro foi oxidada com (2,2,6,6-tetrametilpiperidin-1-il)oxil (TEMPO), e o seu desempenho como coagulante testado, não se tendo verificado a atividade pretendida. Finalmente, foram testados três derivados de celulose micro/nanofibrilhada (M/NFCs) (cationizadas com cloreto de (3-chloro-2-hidroxipropil) trimetilamónio (CHPTAC) e com reagente de Girard T, e anionizada por oxidação com TEMPO). Os derivados obtidos por reação com CHPTAC demonstraram uma excelente remoção de turvação, próxima da obtida com o coagulante comercial de cloreto de polialunínio (WAC-AB®) O derivado MFCs-CHPTAC com o grau de substituição mais elevado (DS 0.106) removeu 93.2% da turvação na água residual modelo e 85.7% na água residual urbana, enquanto que o derivado menos substituído (DS 0.06) removeu 91.0% e 58.9% da turvação na água modelo e na água residual, respetivamente.

Palavras-chave: Celulose, Coagulante, Agulhas de pinheiro, Micro/nanofibrilhas, Água residual, Remoção de turvação

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List of Abbreviations

PN	Pine Needles
CNF	Cellulose Nanofibers
M/NFCs	Micro/nanofibrillated celluloses
TSS	Total Suspended Solids
TOC	Total Organic Carbon
DS	Degree of substitution
NTU	Nephelometric Turbidity units
TEMPO	2,2,6,6- tetramethyl-4-acetamidopiperidin-1-oxyl
Girard's Reagent T	(carboxymethyl) trimethyl ammonium chloride hydrazide
CHPTAC	3-chloro-2-hydroxypropyltrimethyl ammonium chloride
FTIR	Fourier Transform Infrared Spectroscopy
AGU	Anhydrous Glucose Unit

1. Introduction

From a global perspective, any country's growth compared to other countries can be measured in absolute terms by looking at its rate of industrialization. Over the last few decades, a dramatic increase in industrialization has been observed, and much of it has been concentrated in or near urban areas. This has resulted in high demands on the environment's carrying capacity at a specific location. Water sources, including rivers, lakes, and oceans around these areas, are often highly contaminated due to the release of the contaminants [1]. Consequently, water contamination has become a severe problem in the current context, affecting all living things, including recreation, fishing, household, transportation, and other commercial operations. [2]

To address the issues of water contamination and its treatment, a variety of techniques have been established to treat the wastewater, ranging from traditional and simple methods such as sedimentation and filtration [3] to more complex methods such as ultrafiltration [4], ozonation [5], and reverse osmosis [6], which, however, result in higher process costs. Water treatment techniques should be environmentally friendly, cost-effective, and efficient.

One of the most popular methods for purifying raw water and wastewater in water treatment is the coagulation/flocculation process. It is a widely used simple and cost-effective process capable of removing organic/inorganic chemicals and colloidal particles, depending on the operating conditions, coagulant type, and wastewater properties [7]. It is usual practice to employ inorganic flocculants (salts of multivalent metals like aluminum and iron) because of their low cost, the convenience of use, and availability. Despite this, their use has been curtailed due to several drawbacks that will be covered in more detail below. With growing awareness of the potential harms associated with chemical flocculants and the implementation of more stringent environmental regulations, most countries have begun to strictly regulate their use in drinking water treatment and food processing [8,9].

Biopolymer-based flocculants have generated considerable interest among researchers due to their biodegradability, nontoxicity, and ease of availability from repeatable agricultural resources [9,10,11]. Natural organic flocculants derived from natural polymers or polysaccharides such as starch [12], cellulose [13], chitosan [10], natural gums [14], and mucilage [15], among others, have been studied for their flocculating capabilities in wastewater treatment. Cellulose, the most abundant biopolymer on the planet, is considered to have

enormous potential as a raw material for such applications, mainly if it is derived from nonfood sources such as agricultural and agro-industrial residues or marine biomass, which would reduce atmospheric greenhouse gases in the atmosphere without affecting food crop prices. Additionally, the sludge formed during the process is biodegradable, easy to handle, ready for disposal, and overall reduces the operational cost [16,17]

Although in a majority of cases, bio flocculants were ineffective unless combined with conventional coagulants that acted as sole assistance, even though good efficiencies were seen in a few cases [18] while using plant-derived materials in coagulation/flocculation processes. In addition, some of the developed synthetic techniques include high-cost processes such as dialysis and freeze-drying, making them expensive. To achieve an environment-friendly and sustainable flocculating agent, the processing of the raw material should be maintained to a minimum to reduce energy and chemical expenses and prevent effluent and waste creation. Therefore, based on the current state of knowledge, using plant wastes to make bio flocculants appears promising and warrants additional investigation.

In this context, to develop a bio-flocculant at a lost cost using fewer resources that can remove turbidity from wastewater based on cellulose material, several types of cellulose source materials were investigated, such as pine needles, filter paper, and obtained micro/nanofibrillated celluloses (M/NFCs) derivatives from Jorge et al., [135] and Alves et al., [141], as coagulants in the treatment of turbidity in wastewater. Different chemical modifications were tried on different sources of cellulose, like the reductive amination using cyanoborohydride after the periodate oxidation, along with different purification techniques, i.e., dewaxing, bleaching, and dialysis applied on PN. Pine Trees are abundantly available and easily accessible. Besides cellulose, pine needles contain many hemicelluloses, lignin, and other impurities like wax, tannin, pentosan, ash, etc. [19,20,21].

The 2,2,6,6-tetramethyl-4-acetamidopiperidin-1-oxyl (TEMPO) mediated oxidation reaction was tried on cellulose pulp obtained from filter paper, and the same oxidation reaction was applied to the mixture of (mechanically and enzymatically treated) M/NFCs. M/NFCs treated with (carboxymethyl) trimethyl ammonium chloride hydrazide (Girard's reagent T), and 3-chloro-2-hydroxypropyltrimethyl ammonium chloride (CHPTAC) with different degree of substitution (DS) were also evaluated to assess their behavior in the coagulation/flocculation process.

The plant material has an intricate structure, and it's not easy to access the cellulose sugar units to react with the reagents. Chemical modification of cellulose was challenging due to the high degree of intra and interchain hydrogen bonding, making the cellulose less water soluble.

1.1 Objectives:

The primary purpose of this study is to produce a cellulose-based bio-flocculant from plant material with the minimum usage of chemicals and energy while processing the raw material for wastewater treatment.

The specific objectives of this study are:

- To test pine needles as a plant-based coagulant;
- To chemically modify cellulose in order to obtain charged derivatives that might act as
- coagulant/flocculant in wastewaters;
- To assess pine needles and filter paper as a cellulose source to produce bio-flocculant;
- Develop a synthetic wastewater;
- Assess the coagulation/flocculation ability of the bio-flocculant using model wastewater;
- Assess the coagulation/flocculation ability of the bio-flocculant using the municipal wastewater;
- Compare the bio-flocculant efficiency with commercial coagulants in both wastewaters;

1.2 Cellulose:

Cellulose is the most abundant and renewable polymer material available globally [17, 22]. Cellulose is a structural polymer found not just in plants but also in microbes [23], algae [24], and animals [25]. It is estimated that cellulose accounts for 33 % of all plant material (cotton accounts for 90 % and wood accounts for 40–50 %) [22]. For industrial use, cellulose is primarily derived from wood pulp and cotton. It is generally employed in producing paperboard and paper [26]. In the last few years, natural polymers such as cellulose have gained attention in many applications instead of synthetic polymers because of the growing problems of oil shortage and environmental pollution by synthetic polymers that do not break down [27]. Cellulose possesses other favorable characteristics, such as strength, rigidity, excellent heat stability, and inexpensive cost due to its global availability [28].

1.2.1 Structure & Properties of Cellulose

Cellulose does not have a taste or smell, is hydrophilic with a contact angle of 20°–30°, is insoluble in water and most organic solvents, is chiral, and breaks down in the environment [22]. It can be chemically broken down into glucose units by treating it with concentrated acids at a high temperature for some time [29]. Cellulose comprises a long chain of molecules surrounded by glucose rings. The two anhydroglucose (AGU) rings $(C_6H_{10}O_5)_n$; n = 10,000 to 15,000, where n is dependent on the cellulose source material) are covalently attached to carbon 1 (C1) of one glucose ring. Carbon 4 (C4) of the ring, called glucosidic bond (β 1-4) [30], forms the repeating unit shown in Figure 1. These factors combine to form a linear structure composed of several cellulose chains stacked on top of one another. Thousands of these types of structures are repeated, resulting in a stiff and substantially more robust network; either the intra or interchain hydrogen bonding network makes cellulose a stable biopolymer. A vast number of anhydroglucose rings are connected due to this association. So, cellulose consists of many linearly linked β(1→4) linked D-glucose units [31].

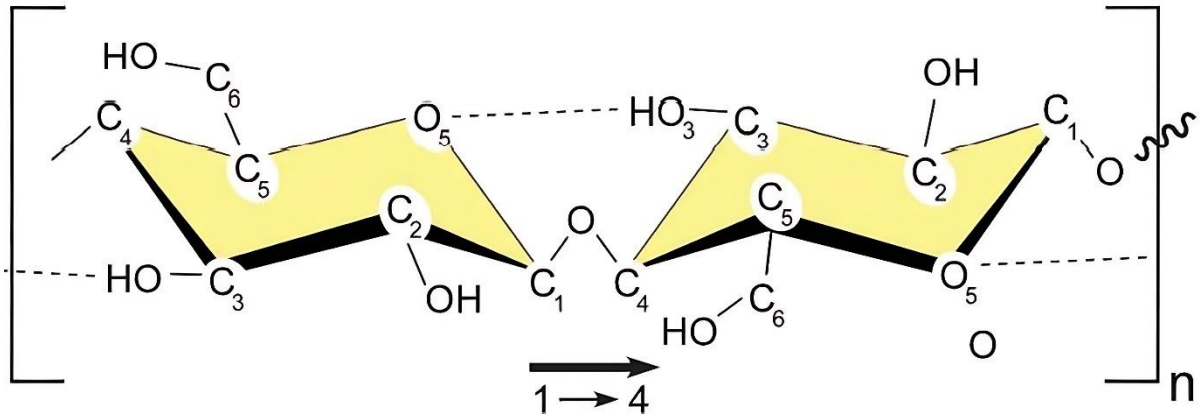

Figure 1.1 Chain of single cellulose repeating unit, 1-4 showing the direction of linkage, and the dotted lines represent intrachain hydrogen bonding [30]

1.3 Processing, Preparation, and Treatment of microfbrillated or nanocellulose

Cellulose chains, made up of several glucose molecules, are linked together by van der Waals and hydrogen bonds to make three-dimensional networks, as shown in Figure 2, called microfbrillated cellulose (MFC). Microfibrils are what make up the plant cell wall. They are tightly bundled together and hard to break apart because of their strong hydrogen bonds [30,32,33]. Several methods have been employed to create cellulose nanofibers, but all of them involve mechanical, chemical, or enzymatic treatments of the fibers to disintegrate them into

nanostructures [34,35]. Some of the common mechanical operations are refining [36], microfluidization [37], homogenization [38], high speed blending [39] and ultrasonication [40].

In addition to these treatments, it is commonly used acid hydrolysis (chemical) treatment to prepare nanocellulose from different plant parts [41]. Still, this process requires high usage of acid. However, Yaxin Zhou [42] reported that nano cellulose generated by an acid-free approach exhibited improved mass recovery ratios, a more significant number of anionic surface groups, and smaller, more uniform dimensions than nano cellulose prepared via acid hydrolysis.

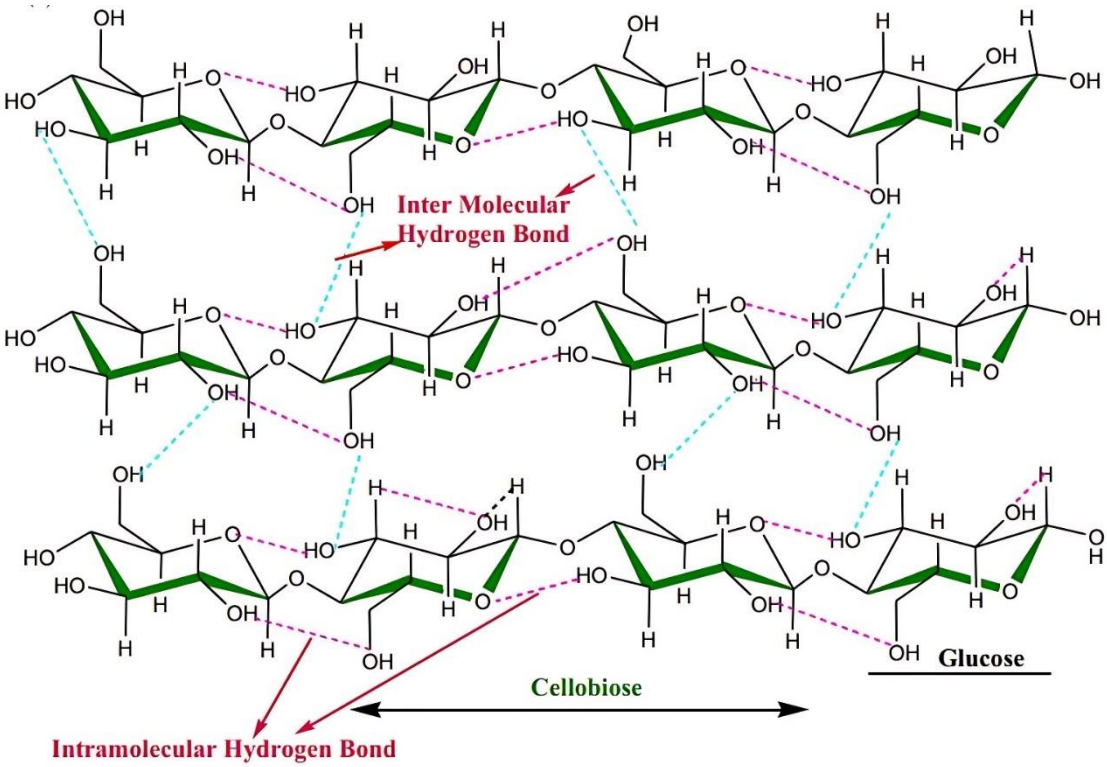

Figure 1.2: Intramolecular and intermolecular hydrogen bonding networks in cellulose structure [43]

1.3.1 Surface functionalization of nanocellulose or microfibril cellulose

Nanocellulose is excellent for various chemical surface modification techniques due to its abundance of hydroxyl groups and high surface-to-volume ratios. The presence of free hydroxyl groups at the surface of nanocellulose is a critical component that plays a significant role in facilitating the attachment of a diverse range of functional groups [44]. Most of the time, these chemical transformations are performed to enhance the processability and performance of

nanocellulose-based materials and, more specifically, to create nanocellulose derivatives that disperse effectively in solvents and matrices [44]. Several functionalization techniques have been studied to graft the functional group or molecule onto the surface of nanocellulose, such as sulfonation, oxidation, carboxylation, esterification, phosphorylation, and amidation [45-48]. The chemical structure of cellulose (Figure 1.3) reveals that the condensation-formed polymer is composed of monomers connected by 1,4 glycosidic oxygen bridges [50]. This natural polymer's repeating unit is a dimer of glucose called cellobiose, which contains three hydroxyl groups that serve as reactive platforms for the chemical changes seen in Figure 1.4.

1.3.2 Cationization of micro or nanocellulose

Chemical modification of cellulose is a crucial step in the cellulose refining process, in which renewable natural cellulose components are used to produce novel chemicals and products. Cationic cellulose is biodegradable, cost-effective, and harmless, so due to these properties, they have been used in various applications [51]. The production of biopolymeric materials requires the introduction of positively charged groups, which can be accomplished by the reaction of the hydroxyl group found in polysaccharides to form micro- and nanocellulose.

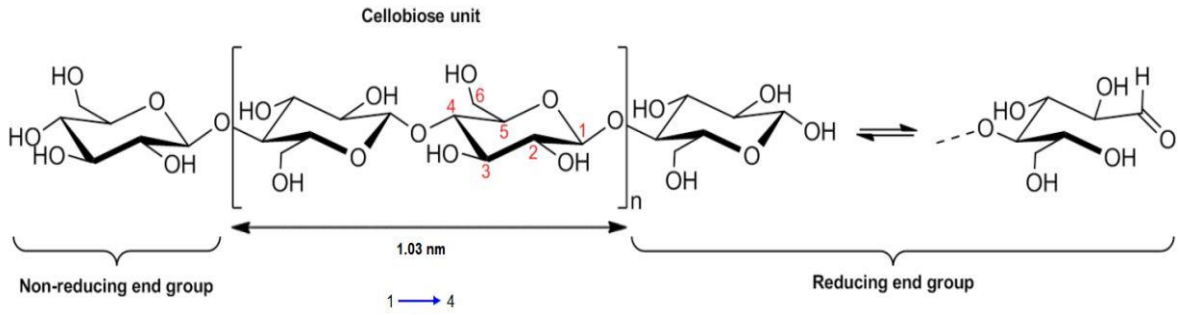

Figure 1.3: Cellobiose repeating unit of cellulose with reducing end at right and non-reducing end at left. The numbering system for carbon atoms in the anhydroglucose unit is shown in red, and the directionality of the β 1-4 connection is shown by the blue arrow [49]

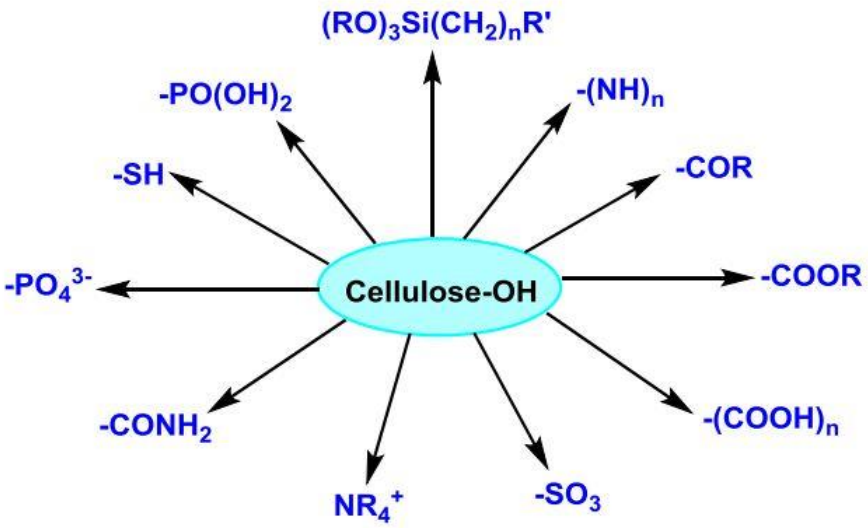

Figure 1.4: The principal surface chemical functionalization of nanocellulose for water treatment applications [48].

1.3.2.1 Periodate Oxidation and Reductive Amination of Cellulose

All OH groups present in cellulose can be modified; cellulose derivative (Dialdehyde Cellulose) is produced using periodate as an oxidizing agent. C2 and C3 are more reactive than primary carbons during the cleavage of two secondary carbons.

Figure 1.5 shows that periodate oxidation involves opening the ring cleavage of cyclic cellulose and decreases crystallinity, resulting in a material with thinner and more flexible fibers. Magnesium and lithium chloride salts were added to the reaction to improve the solubility by disrupting intermolecular hydrogen bonds and promoting sodium periodate's efficiency [52,53].

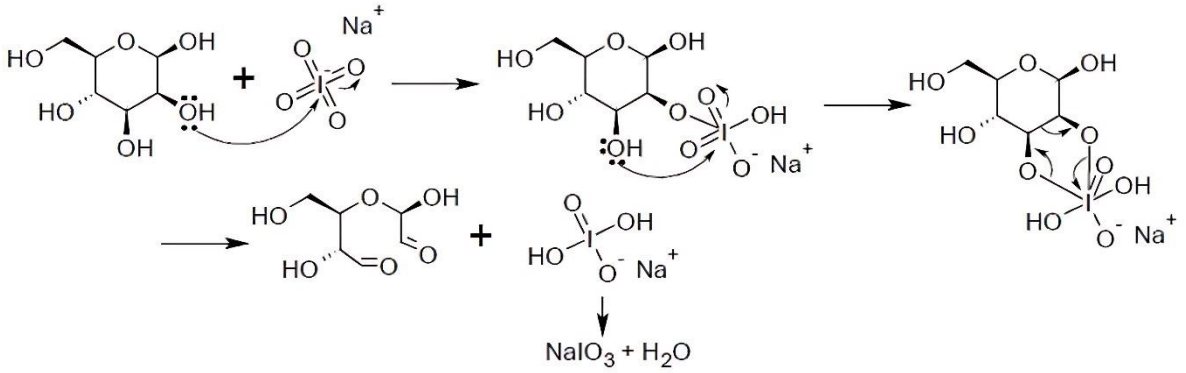

Figure 1.5: Mechanism of periodate cleavage [53]

Figure 1.6 shows the process of reductive animation, a carbonyl and an amino group combine to form an imine, which is then followed by a reduction of the C=N bond created by adding a reducing agent [54].

This is a two-step process; when ammonium chloride was added along with piperidine, nucleophilic addition to the carbonyl group occurs to form an Imine. In the second step, imines were reduced to primary amines by reacting with sodium cyanoborohydride.

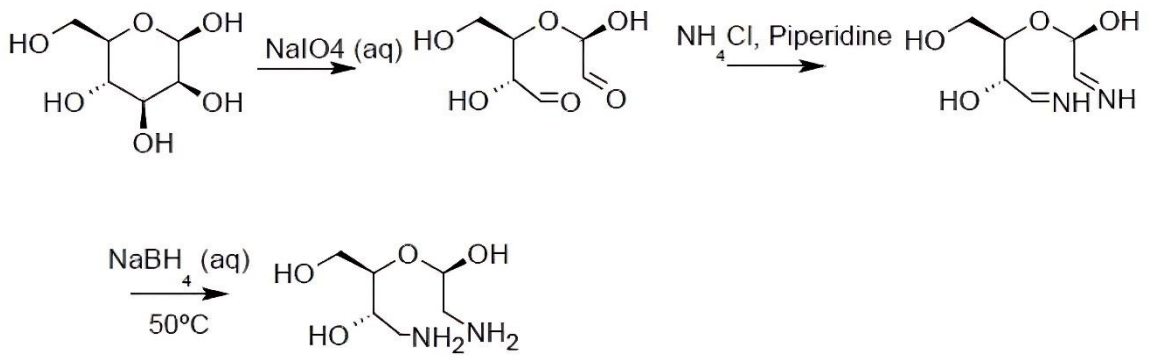

Figure 1.6: Periodate oxidation followed by reductive amination [53]

1.3.2.2 TEMPO Mediated Oxidation

TEMPO mediated oxidation technique is widespread and widely used to functionalize cellulose. During the oxidation of cellulose using NaOCl/NaBr, mediated by TEMPO, the C6 primary hydroxyl alcohol groups are converted preferentially into a C6 aldehyde intermediate, which is then converted into a C6 carboxyl group as demonstrated in Figure 1.7. The NaOCl in this system is the primary oxidant, while the NaBr functions as a co-catalyst in the reaction. In an aqueous media with a pH of 10, the presence of NaBr speeds up the reaction, while the co-catalyst BrO acts as a more powerful oxidizing agent than OCl [55,56].

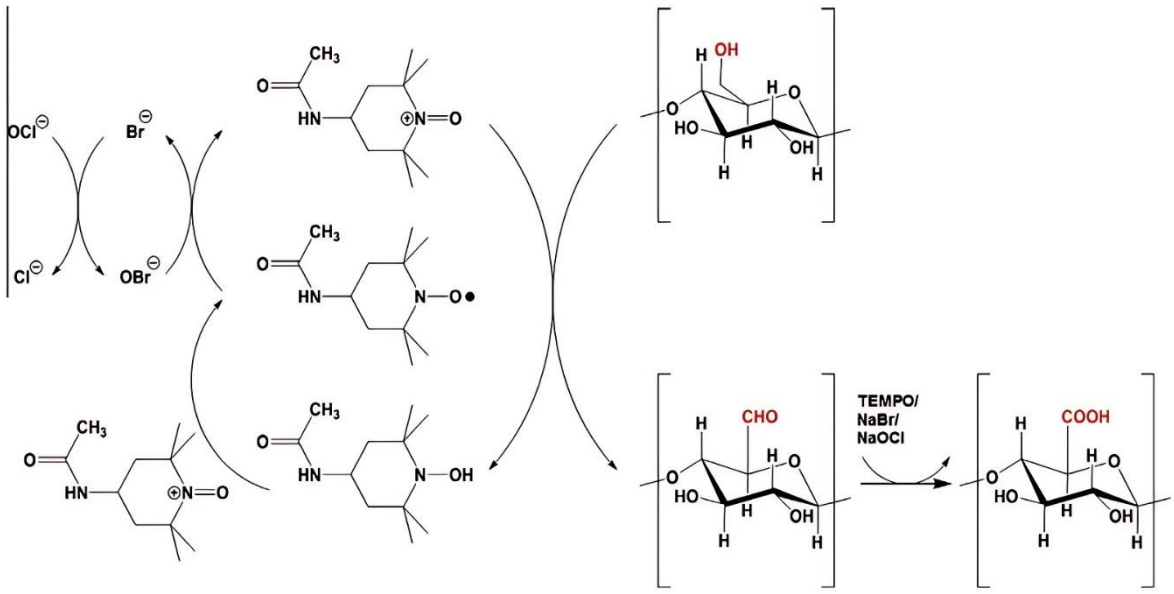

Figure 1.7: Mechanism of TEMPO-mediated oxidation [57]

1.4 Coagulation /Flocculation Process

Coagulation is the process through which a given suspension or solution becomes destabilized. In other words, the purpose of coagulation is to counteract elements that promote the stability of a given system. When particles are destabilized or new particles are created due to the disruption, a process known as flocculation occurs, causing them to clump together and form large(r) agglomerates [7]. Different conventional and innovative methods are utilized to process the raw water into potable water and wastewater into treated effluent before its release into the water bodies [58,59]. Chemical precipitation, lime coagulation, ion exchange, and membrane separation are some physio-chemical methods for treating water and wastewater [60]. Most water and wastewater treatment techniques involve coagulation and flocculation processes, separating the suspended particles in water to generate clear, suspension-free effluent [61]. In water treatment facilities, coagulation procedures are typically followed by flocculation and sedimentation phases. Usually, a coagulant is a positive-charged (often divalent) chemical that can join with negatively charged suspended particles in a solution to form a neutral form of combined molecules [63,64]. Accelerating the rate of particle collision requires a precise mixing of velocity, intensity, and time [65]. The collision of flocs will be produced by an aggregation of particles that results in a rapid rate of sedimentation [66]. During the generation of flocs, multiple mechanisms, including charge neutralization, sweep coagulation, bridging, and patch flocculation, can occur [67,68], as shown in Figure 1.8.

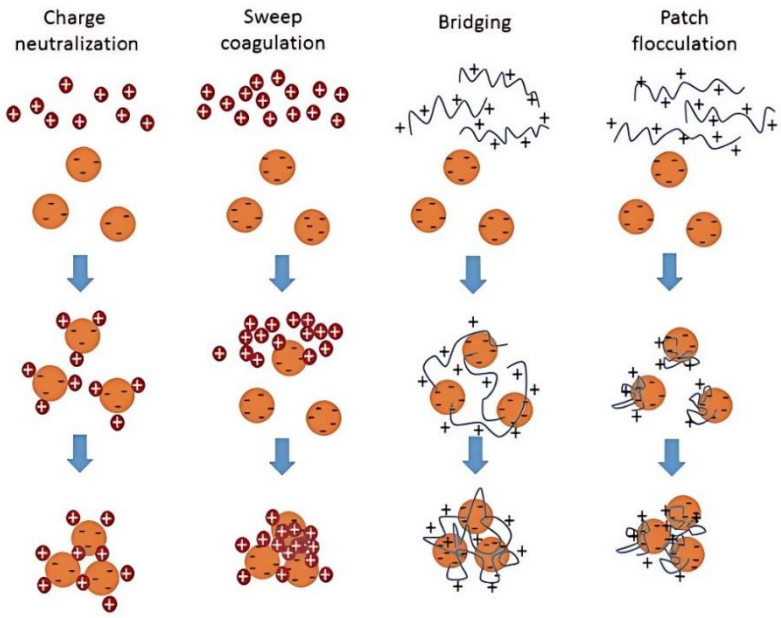

Figure 1.8: Mechanism of Coagulation and Flocculation [67]

Figure 1.8 describes in-detail the mechanism of coagulation and flocculation. In charge neutralization, a positively charged coagulant or flocculant is adsorbed onto the surfaces of negatively charged colloids [62,69,70]. The chemicals coagulants added to the solution permeate into a diffuse double layer around the particles, making them thinner, denser, and smaller in volume, allowing the particles to close to each other [71,07]. Colloid particles can become entangled in the precipitates formed when a metal salt coagulant is introduced to water at a concentration high enough to trigger the precipitation of amorphous metal hydroxide ($M(OH)_3$) [72,73], and this process is called sweep coagulation (Figure 1.8). Bridging (Figure 1.8) happens when a section of a polymer chain absorbs multiple particles, thereby connecting them [72,8]. When the bridging mechanism prevails in the system, the polymers with high molecular weight make macroflocs which improve the flocculation process by adding weight, strengthening the floc, and increasing the settling rate [74,143]. The process of patch coagulation (Figure 8) involves the adsorption of a polymer or coagulant onto the surface of an oppositely charged particle. This results in a non-uniform distribution of the surface charge [75]. Particles having a full negative zeta potential, such as silica, will aggregate in response to polyelectrolytes with a high cationic charge density (>0.15) through patch processes. In contrast, polyelectrolytes with a low cationic charge density (<0.15) will favor bridging [69].

1.4.1 Commercial Coagulants and Flocculants

Coagulants and flocculants used in water and wastewater treatment are divided into organic and inorganic categories. The classification of compounds is shown in Figure 1.9. Both aluminum and iron-based coagulants are among the most frequently utilized types of commercial metal coagulants. Some examples of aluminum coagulants are aluminum sulfate, aluminum chloride, and sodium aluminate, and the most common iron coagulants are ferric sulfate, ferric chloride, and ferric chloride sulfate. Magnesium carbonate and hydrated lime are two other commonly used coagulants in water treatment. [7,76]

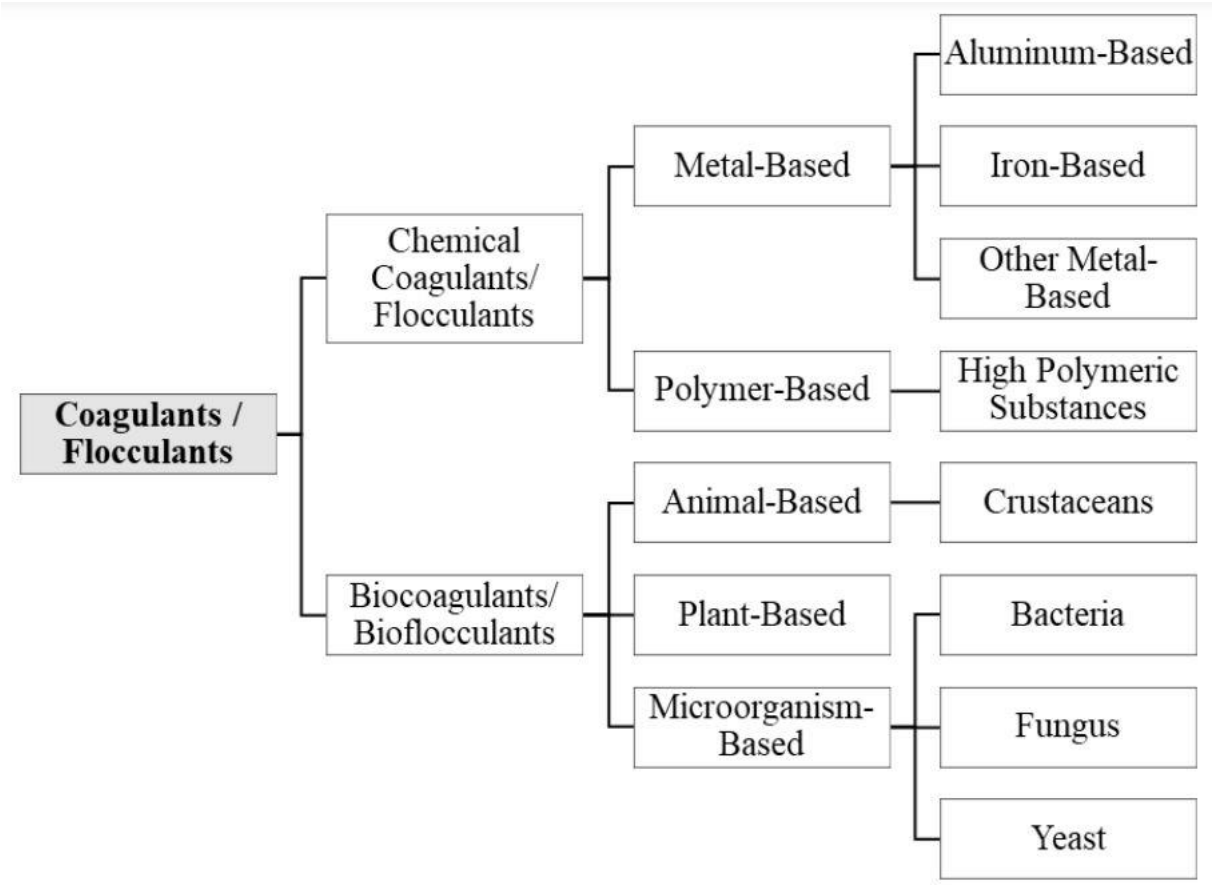

Figure 1.9: Classification of Coagulants / Flocculants [77]

1.4.1.1 Drawbacks of Commercial Coagulants

Although commercial coagulants and flocculants are famous for their effectiveness in water and wastewater treatment, there are human health and environmental pollution concerns are associated with these compounds' usage. Based on the Microtox assay, aluminum-based coagulants exhibit a significant rise in toxicity potential in the concentration range of 100–200 mg/L [78]. Inorganic polymers with a considerable molecular weight exhibit significant

hazardous potential, and synthetic polymers have very limited biodegradability with carcinogenicity potential [79]. Synthetic polymers, iron, aluminum based polymers, and other metals are also nonbiodegradable [77]. When drinking water is consumed, traces of metals that came from using coagulants or flocculants, which easily don't break down, can accumulate in body cells, or metal ions can end up in food chains [80,81]. Recent research highlighted that an unfair amount of aluminum in the human body (particularly the brain) has a high association with neurotoxic disorders [82], Alzheimer's disease [83], and autism[84].

Another significant environmental pollution caused by aluminum-based coagulants is the generation of sludge in higher amounts; according to the report of [85], in the United Kingdom, more than 270,000 tons of iron and aluminum based coagulants were utilized for wastewater treatment purposes in 2014, resulted in the generation of 180,000 tons of dried sludge [86]. When this vast amount of sludge is used without considering the coagulants' toxicity, there is a high possibility of metals leaching into the groundwater and soil. Barakwan et al. also stated in [87] that when aluminum sludge releases directly into the water bodies, it disturbs the aquatic environment, can cause biomagnification and bioaccumulation of aluminum, and releases different toxic metals.

However, Iron-based coagulants generate less sludge. Still, they are also associated with other environmental pollution like corrosion in piping material [88]; iron can also limit bacterial development even though it lowers the pH of the environment by interfering with certain enzyme activities. [89]. A high iron concentration hinders plant development. The excessive intake of iron has detrimental effects on plants, including bronzing and stippling of leaves caused by the production of enzymes to control free radicals, epidermal damage on roots, and a decrease in overall plant growth and survival rate [90,91].

1.4.2 Plant-Based Coagulants and Flocculants

Plant-based coagulants are organic coagulants that are natural, water-soluble, and derived from various parts of the plant and species [92], as shown in figure 1.10. These coagulants fall into two broad classes: ionic and non-ionic polymers of varying molecular weight [93]. Bio-coagulants and bio-flocculants generated from plants have been studied and utilized extensively in freshwater purification [94] and wastewater remediation [95]. Several researchers used various parts of the plants as coagulants and flocculants, such as peels [96], seeds or pods [97,98], fruit wastes such as soya or sago pastes [99], and leaves [100,101]. Moringa oleifera is

the most well-known and extensively researched among the species studied, particularly for its seed proteins [102,103].

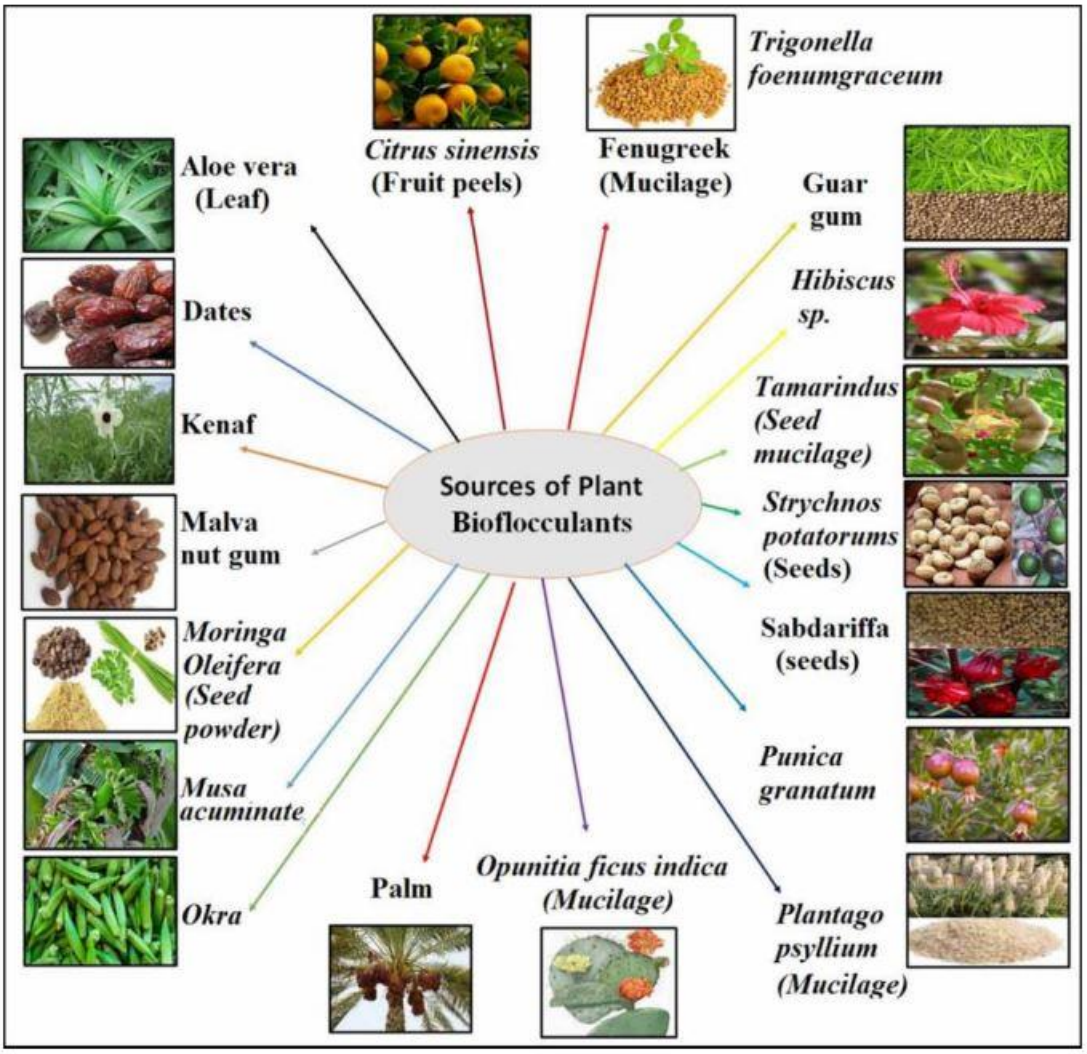

Figure 1.10: Sources of plant-based flocculants [104]

1.4.2.1 Performance and Potential of Plant-based Coagulants

The sustainable and greener approach to using plant-based coagulants has gained considerable attention as a feasible alternative to synthetic chemical reagents used for the coagulation/ flocculation process. Several researchers concluded the excellent performance of plant-based coagulants, which means they have a great potential to replace traditional chemical coagulants [105]. A study by Yongabi et al. [105] utilizing *Carica papaya* seeds that were deshelled, pulverized, and dusted demonstrated a turbidity reduction rate of 90% in stormwater with initial turbidity of 119 NTU. *Citrus sinensis* peel and skin (washed with formaldehyde, acid-alkaline,

and pulverized) were used in another experiment conducted by Sowmeyan [106], and they were successful in removing 60% of the turbidity from raw surface water and 90% of turbidity removal while using *Moringa oleifera* with initial turbidity of 32 mg/L. In most cases, plant-based coagulants successfully achieved a turbidity reduction of greater than 80% on synthetic turbid waters with initial turbidity around 50-500 NTU [93], displayed in table 1.1.

Table 1.1: List of plant materials investigated to remove turbidity from several types of water.

Wastewater type	Natural Coagulant	Type of Extraction	Initial Turbidity (NTU)	Removal (%)	Reference
Wastewater	*Casava peels*	Casava Peels Starch	194±14.43	60.19	[107]
Tap Water (Kaolin)	*M. oleifera*	M. Oliefera Seeds	105	93.33	[108]
Synthetic Turbid Water (Kaolin)	*Pistachio green*	Pistachio green hull	300	88	[109]
Wastewater	*Cactus*	Cactus Pads	50	98.33	[110]
Synthetic Turbid Water (Kaolin)	*Corn and Potato*	Conventional Starches	165±5	50	[111]
Raw Surface Water	*Plantago ovata*	Plantago seeds extracted by FeCl3	76	95.6	[112]
Synthetic Turbid Water	*Pine cone*	Pine Cones	71±4	77	[113]
Palm Oil Mill Effluent	*Chitosan*	Chitosan Powder	>1000	98.4	[114]
Synthetic Turbid	*Jatropha curcas*	J Curcas Seeds	500	99.4	[115]

1.4.2.2 Advantages of Plant-Based Coagulants

There are several benefits to use plant-based coagulants instead of chemical-based; some of the significant advantages in using bio-coagulants/flocculants are their natural abundance, safe to use, cost-effective [116], non-corrosive, non-toxic [117], biodegradable [118]. In the presence of microorganisms, bacteria, and fungi, plant-based coagulants are biodegradable or readily broken down [120] and make less sludge with a high nutritional value, so it is easy to handle or treat with less cost [119]. In addition to being biodegradable and nutrient-rich, the sludge produced by the coagulation process can be further processed into biofertilizers. Compared to chemical coagulants, plant-based coagulants result in five times less sludge generation [77]. While comparing the cost of operation between chemical and plant-based coagulation/flocculation processes, it is impossible to compare the operation cost of their end-to-end procedure due to less information. Saleem and Bachmann [93] quoted one of the extensive economic evaluations based on the work of Jahn and Samia [121], stating that USD 2,370 was saved by replacing alum with *Moringa oleifira* as the coagulant aid to treat 1.74 million m^3 of water in Nagpur, India. In response to the effects of climate change, the depletion of natural resources, and environmental destruction, using plant-based coagulants in water and wastewater treatment as replacements for chemical coagulants is a significant effort to manage environmental challenges. This effort is in line with an initiative to achieve the Sustainable Development Goals, and possible plant-based coagulants include agriculture biomass, such as plant wastes (vegetables and fruits). An estimated 3.28 billion tons of potential agricultural waste are created annually, according to data from many countries, including the United States of America, India, China, Brazil, Argentina, Europe, and Canada. [122]. The advantages of Plant-Based coagulants are summarized in Figure 1.11.

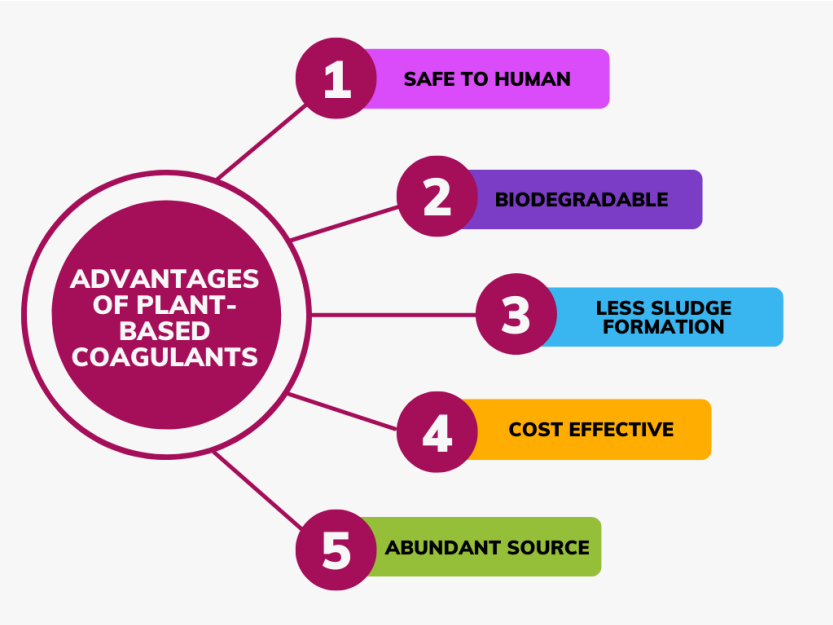

Figure 1.11: Advantages of Plant-Based Coagulants

1.5 Pine Needle as a source of cellulose

Pine needles are a natural bioresource that can be found easily and is abundant in Portugal [123]. Pine needles can be collected all year round; usually, one ton of needles can be collected from two acres of pine [21,124]. Some benefits of pine needles are that they increase, regrow, are relatively strong, and are flexible [124]. Different parts of pine trees contain considerable amounts of cellulose. Like Shoaling et al. [21]., reported, pine needles to consist of approximately 30% cellulose. According to Fradinho et al [123]., pine bark contains around 48% of cellulose, while Avinash kumar et al [20]., reported pine cones contain 24% of hemicellulose and 37% of alpha-cellulose. They all stated that pine extracts also contain 23% to 33% of lignin with other substrates.

Numerous studies have been conducted to investigate the use of pine sawdust, pine cones, and pine needles as bio-flocculants and bio-adsorbents in the removal of heavy metals, nutrients, and contaminants from water [113,125,126,127,128]. However, pine needles extract has not been investigated extensively for use in a coagulation/flocculation process to remove turbidity. Some researchers utilized pine bark [125] and pine cone extract [113] to remove turbidity from wastewater and reported a positive result.

Pine bark and pine sawdust are extensively used in many applications, such as herbal products, antioxidant support, health aging, immunity, brain function, pharmaceuticals, chemicals, food

additives, and plant cultivation [129-131]. In contrast, dried pine needles falling to the ground are often discarded and not utilized to create any particular product. Dwivedi et al. [132] reported that a bunch of fallen pine needles could cause forest fires as they are an excellent biomass fuel source. Hamilton et al. [133] also stated that forest fires caused by pine needles that have dried out are a significant source of greenhouse gas emissions in the atmosphere. Furthermore, when fallen pine needles make a thick layer on the ground, they block the sunshine, stopping grass's growth, the primary food of cattle feed [132].

2. Experimental Part

2.1 Reagents and equipment

$MgCl.H_2O$, LiCl, and KOH were obtained from Scharlau Chemie S.A; sodium periodate and piperidine from Merck, HCl, and NH_3 from PanReac AppliChem. $NaIO_4$, TEMPO (2,2,6,6-Tetramethylpiperidine 1 oxyl), toluene, and ethylene from Sigma-Aldrich. Ethylene glycol and Sodium cyanoborohydride from Fluka Analytical. Commercial coagulant (WAC-AB®) was obtained from Aguas do Algarve, Portugal.

Powdered samples were obtained using a grinder (MF-10 basic, Model IKA-WERKE). Fourier-transform infrared (FTIR) spectra were acquired in a Bruker Tensor 27 (UK). Total organic carbon (TOC) was measured using a TOC-500, Shimadzu (Japan), turbidity was measured using a HACH (USA) 2100N turbidity meter, and pH was measured in a Crison Basic 20+ pH meter (Spain). The conductivity of the samples was measured by Crison GLP 32 (Spain) conductivity meter, and zeta potential was measured using a ZetaSizer Nano-ZS Zen-3600 Malvern Inc. (UK). Dialysis was performed using D tube snake skin (Ref 68035) by Thermo Scientific.

2.2 Preparation of cellulose-based coagulant/flocculants

2.2.1 Cationization of cellulose extracted from pine needles

Pine needles are abundant and readily accessible in the Gambelas campus of the University of Algarve. The fallen pine needles shown in Figure 2.12 were collected from the campus. They were rinsed with tap water to eliminate dust and then air-dried for three days. Then, PN was ground into a powder using a 0.5 mm mesh sieve with a grinding machine. Several methods were evaluated to extract cellulose from PN. The methods used are presented following.

Figure 2.12: Pine Needles Collection at University of Algarve, Gambelas Campus

Method I:

Seven grams of PN were dissolved in 200 ml of water, and 3 grams of NaOH using a 500 ml round bottom flask. This solution was autoclaved for 45 min at 85 °C. Then, the suspension was mixed using a stirrer and heated at 95 °C for 3 hours. Again, the suspension went to an autoclave using the same conditions after it cooled down, transferred to centrifuge tubes. The suspension was centrifuged at 1000 rpm for 20 min, the supernatant was discarded and the centrifugation process was repeated until a clear solution was obtained with pH 7. All remaining solids were transferred to a petri dish and air-dried for two days. After weighing, the remaining solids were transferred into a flask containing 0.2 M LiCl and 0.2M $MgCl{\cdot}H_2O$ dissolved in water. Using a mole ratio of 0.606, 0.8 grams of Sodium periodate ($NaIO_4$) for 1 gram of cellulose was added to the flask. The mixture was heated for 6 hours at 55 °C following the procedure described in the reference [52]. 400 µL of Ethylene glycol was added to react with excess periodate and continued stirring for one hour at room temperature. After completing the reaction, the sample mixture was centrifuged and washed. One hour of additional stirring was performed to neutralize the excess $NaIO_4$.

After that, for the amination, the sample mixture was moistened using ethanol, then ammonium chloride and piperidine in the equivalent ratio (1:1.2) were added to the reaction mixture; the mixture was kept at 75 °C in a hot bath for 5 hours; the reaction mixture was protected from light and moisture throughout the experiment. Sodium cyanoborohydride was added to the

reaction flask in a weight ratio of 1:1 ($NaBH_3CN$/Cellulose) dissolved in ethanol and heated at 50 °C for 4 hours; after 4 hours, water was added and continued for further stirring of one hour to release the amine from boron complex. A small sample was taken after each step for FTIR analysis.

Method II:

The previous method was repeated with some modifications. 16.7 g of PN powder was weighed and followed the same procedure. After the centrifugation, it was observed that a good amount of solid was dissolved, so the further procedure was continued with all of the liquid suspension. This time the sample mixture was dialyzed using a cellulose membrane after the oxidation and amination reaction for 3 days until we got rid of all the chemicals used during extraction.

Method III:

Following the procedure of Shaoliang et al. [21], fifteen grams of PN sample was extracted using a mixture of toluene/ethanol (2:1, V: V) in Soxhlet apparatus at 90°C for 5 hours until we got the clear solvent in the extraction part. After that sample was transferred to a conical flask, and 100-120 ml of commercial bleach (3.5% active chlorine) was added and stirred and room temperature for two hours. After every two hours, the sample was centrifuged and the supernatant was discarded; the bleaching procedure was repeated five times by adding more bleach until the sample became white as shown in figure 2.13. After getting the white product, chemical treatment of the sample was done by adding 100 ml of 6 wt % KOH to the sample mixture and stirring for 2 hours. Then the sample was treated with 1 M HCl at 80C for two hours. After the series of treatments, the sample was washed several times with distilled water using centrifugation until the residues were neutralized. The sample further proceeded with the same method as method I for the oxidation. Then the amination reaction was performed by using ammonium hydroxide solution (1 gram of cellulose was added to 50 ml of 37% NH_3). The sample mixture was stirred at 80°C for 3 hours under a light-protected environment. After 3 hours, sodium cyanoborohydride in a weight ratio of 1:1 ($NaBH_3CN$/Cellulose) dissolved in

ethanol was added to the reaction flask and stirred for 18 hours at 100°C. Then, the purification of the sample was done using a rotavapor and dialysis tube.

Figure 2.13: PN sample after dewaxing (left) and bleaching (right)

2.2.2 Anionization of cellulose from filter paper

The filter paper from the laboratory was shredded and immersed in water for a week. The suspension was stirred for a few hours and homogenized. following the method of [134], one gram of cellulose suspended in 100 ml of water containing (0.016g,0.1mmol) 2,2,6,6-tetramethylpiperidine 1-oxyl and the sodium bromide (0.1g,1mmol). The oxidation reaction was initiated by adding the necessary amount of bleach (1.3-5.0 mmol NaClO per gram of cellulose) at room temperature by stirring at a speed of 500 revolutions per minute (rpm). The pH of NaClO was adjusted to 10 before the addition to the solution by using 0.2 M HCl. The pH was maintained at 10 by adding 0.5 M NaOH until the pH stat showed no more NaOH was consumed. The sample was rinsed and neutralized to pH 7.

2.2.3 Cationization of microfibrillated Cellulose

Two micro/nanofibrillated celluloses (M/NFCs) samples (in which one was mechanically treated and the other was enzymatic) were obtained from [141] were mixed, and proceeded with the same procedure as PN for the cationization. Periodate oxidation and reductive amination reaction were applied and the sample was purified using rotavapor and dialysis tubing. After the purification, a clear solution was obtained. FTIR spectrum was recorded after the oxidation and amination reaction.

2.2.4 Cationic and anionic micro/nanofibrillated cellulose derivatives

The characteristics of cationic micro fibrillated cellulose derivatives obtained by reaction of cellulose microfibrils with 3-chloro-2-hydroxypropyl trimethylammonium chloride (CHPTAC) and Girard's T reagent, as well as of the anionic nano fibrillated cellulose derivative modified by TEMPO oxidation are shown in table 2.2.

Table 2.2: Characterization of cationic and anionic micro/nanofibrillated cellulose derivatives

Sample	Degree of Substitution
Anionic CNF (TEMPO treated)	0.23
Cationic CHPTAC-treated M/NFC (less substituted)	0.06
Cationic CHPTAC-treated M/NFC (more substituted)	0.106
Cationic GTA	0.04
Cationic GTB	0.16
Cationic GTC	0.36

All micro/nanofibrillated cellulose obtained from the research group of Universidade de Coimbra [135, 141] were tested as coagulants on synthetic wastewater with initial turbidity of 92-96 NTU and real wastewater with initial turbidity of 142-156 NTU.

2.3 Assessment of Cellulose-based coagulant/flocculants

2.3.1 Synthetic Wastewater Preparation

A model wastewater characteristic of domestic wastewater was prepared for the jar test experiments. Commercial semi-skimmed milk purchased from a neighborhood grocery store was used to prepare model wastewater to achieve the desired turbidity. The sample had the following nutrient values per 100 ml: fat/lipids 1.60 g, carbohydrates 4.80 g, protein 3.20 g, and calcium 120 mg. Two mili litre of milk was added to 1 litre of chlorine-free tap water and the

turbidity, total suspended solids (TSS), pH, conductivity, and total organic carbon (TOC) were measured.

2.3.2 Real Wastewater

Real wastewater is collected from the domestic effluent treatment facility in Vilamoura, located in the Algarve at (37° 5' 52.0656", -8° 8' 34.5042"). The same physio-chemical parameters as synthetic water were measured before proceeding with the jar test.

2.3.3 Coagulation/flocculation experiments

The experiments of the coagulation/ flocculation process were performed using a Jar-Test flocculator equipment by JP-Selecta Spain consisting of four paddles (Figure 2.14) that accommodate beakers up to 1000 mL with a speed of 15 rpm (min) to 200 rpm (max). Commercial and natural coagulants were tested on synthetic and real urban wastewater at different concentrations, described in table 2.3. Experiments were made using four beakers with 800 mL each containing wastewater; coagulants with different concentrations were added to three beakers, and one beaker was used as a control. Rapid mixing started for 2 min at 200 rpm, then reduced the mixing speed to 20 rpm for 20 min. Rapid mixing was performed to disperse the coagulant material by rapid agitation into the wastewater. Slow agitation for a more extended period helps the tiny particles to join together and make flocs settle. After 20 minutes of slow agitation, the agitation was turned off, and wait another 20 minutes to settle down all the flocculation material properly. After this, carefully took 50 ml of the sample (approx. 5 cm from the surface of the beaker) in a small beaker from all of the jar test beakers to check the turbidity after the coagulation/flocculation/sedimentation process. All natural and commercial coagulants were tested for turbidity removal on both synthetic and real wastewater.

Table 2.3: Concentration/volume used during the coagulation/flocculation experiment

Sample	Concentration / Volume used in Experiment
WAC-AB (Commercial Coagulant)	0.5, 5 and 25 mg/L
Pine needle extract	10, 17.5, and 22.5 mg/ml (obtained from method I) 22, 35, and 55 ml (obtained from method II) 5, 10, and 16 mg/ml (obtained from method III)
M/NFCs (Cationized followed by mechanical & enzymatic treatment)	10, 25, and 40 ml
Filter paper extract	10, 25, and 40 ml
GTA, GTB and GTC	10, 25 and 40 ml
M/NFCs (more substituted)	24, 49, and 74 mg/L
M/NFCs (less substituted)	153, 214 and 276 mg/L

The experiments were repeated for both M/NFCs samples (CHPTAC treated) that showed flocs formation, and three replicates were measured using both wastewaters. Three replicates were also measured for commercial coagulants on both wastewaters for comparison.

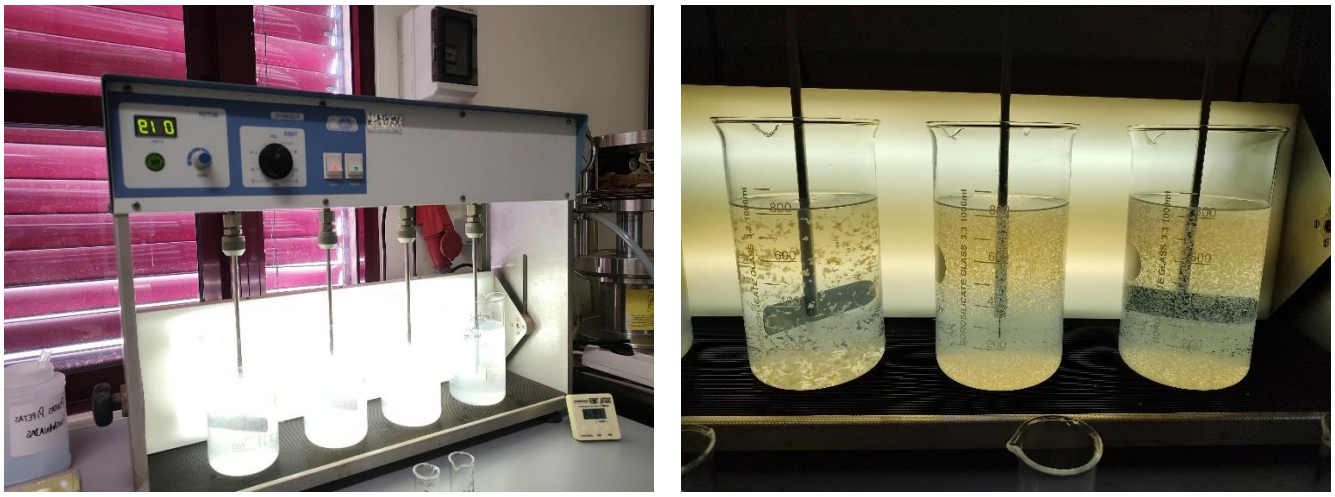

Figure 2.14: Coagulation/flocculation process using jar-test flocculator

3 Results and Discussion

This chapter contains the results obtained and the discussion of the current work, including the characterization of synthetic wastewater, the characterization of the materials used as bio coagulants/flocculants, the process efficiency and the output results of all the coagulants tested on synthetic and real wastewaters.

3.1 Characterization of synthetic and real wastewater

Representative synthetic wastewater with a target turbidity of 80-100 NTU was prepared in the laboratory. Table 3.4 shows the parameter measures of the synthetic wastewater produced.

Table 3.4: Physio-chemical parameters of synthetic wastewater

Characteristics	Values
Turbidity	90-96 NTU
TSS	35.6±12.2 mg/L
pH	7.05±0.2
Conductivity	56.5±14.5 µs/cm
TOC	108.5±2.7 mg/L

Synthetic wastewater is used by many researchers as a model urban wastewater of a real wastewater. Vieira, A. M. S. et al. [136] who prepared synthetic wastewater using milk powder with different concentrations (0.1–2.2 g of milk powder/L of tap water) and tested *Moringa oleifera* seed as a natural adsorbent. The wastewater characteristics were turbidity (856 NTU), color (4006 µH), COD (1299 mgO2/L), and pH (7.2). Altaher, H et al. [137] prepared synthetic wastewaters with the following recipes: 10 g of kaolin in a litre of distilled water, kaolin clay contains traces of different metals with the major components of SiO_2 and Al_2O_3, achieved turbidity of 100 NTU. Kebede [138] used a recipe that contains the composition of bactopeptone, corn-starch, dry meat extract, yeast extract, oleic acid, some salts, and metals. This model wastewater reached a turbidity of 50-130 NTU.

By creating synthetic wastewater from a substantially smaller volume of milk and obtaining the necessary turbidity, the use of many chemicals was restricted.

Real wastewater collected from an effluent treatment plant was also measured for physio-chemical characteristics. Natural and commercial coagulants were tried on this wastewater and tested for turbidity removal. Table 3.5 shows the measured values of physio-chemical parameters of the collected real wastewater.

Table 3.5: Physio-chemical parameters of the collected real wastewater

Characteristics	Values
Turbidity	142-156 NTU
TSS	42.2±11.5 mg/L
pH	6.92±0.3
Conductivity	1250±55 µs/cm
TOC	115±2.5 mg/L

3.2 Cellulose from pine needles

Grounded pine needles powder (0.5mm) were used as a coagulant / flocculant for the evaluation of turbidity removal from wastewater. Before tried on a jar test PN powder was processed by different extraction methods and chemical modifications. Sample was first hydrolyzed by NaOH hydrolysis, sonification was performed to separate and suspend the fibers, and then

chemical modifications were tried to charge the PN product. Purification was done by dialysis to remove the impurities and access amount of reagents. Bleaching of the PN sample also tried to get rid of its dark color. The amount of sample left after all treatment and purification was very less. However, the treated PN sample was found inefficient in the coagulation / flocculation experiments, this is most likely due to the fact that functionalization of cellulose were not occurred as seen by FTIR analysis, also may be the hydroxyl groups that are present in the plant material components were not available to interact with the colloids that were present in wastewater.

The FTIR spectra, shown in figure 3.15, were recorded while different methods for cellulose extraction from pine needles samples and different procedures were applied for its cationization. The peaks observed at 2855 and 2924 cm^{-1} were due to the stretching vibration of C-H [140]. O-H band, which were recorded in all spectrum vibrates around 3300-3500 cm^{-1} probably due to the OH of cellulose and other organic materials along with adsorbed / crystallization water. Two prominent peaks at 1637 and 1734 cm^{-1} were attributed to the ester linkage of a carboxylic group or hemicellulose acetyl groups or lignin which indicates that hemicellulose and lignin were still in the sample after treatment [139,147]. These peaks did not disappear after the chemical treatment, which means there was no removal of impurities, a very low yield of pure cellulose from the PN were obtained. Also, there were no bands of amines, which was a clear indication of an unsuccessful reaction. Another notable peak appeared at 893 cm^{-1}, which might be an amorphous cellulose vibration (glucose ring stretch).

It also possible that undesired oxidative cleavage of glycosidic bonds could occur during the periodate reaction due to the acidic/ oxidative hydrolysis, which leads to the loss of yields [151]. A study by Bo Sun et al. [146] also highlighted the effect of temperature during this reaction; a high temperature may lead to more production of iodine with more decomposition of sodium periodate, which will end up with a low aldehyde content [146].

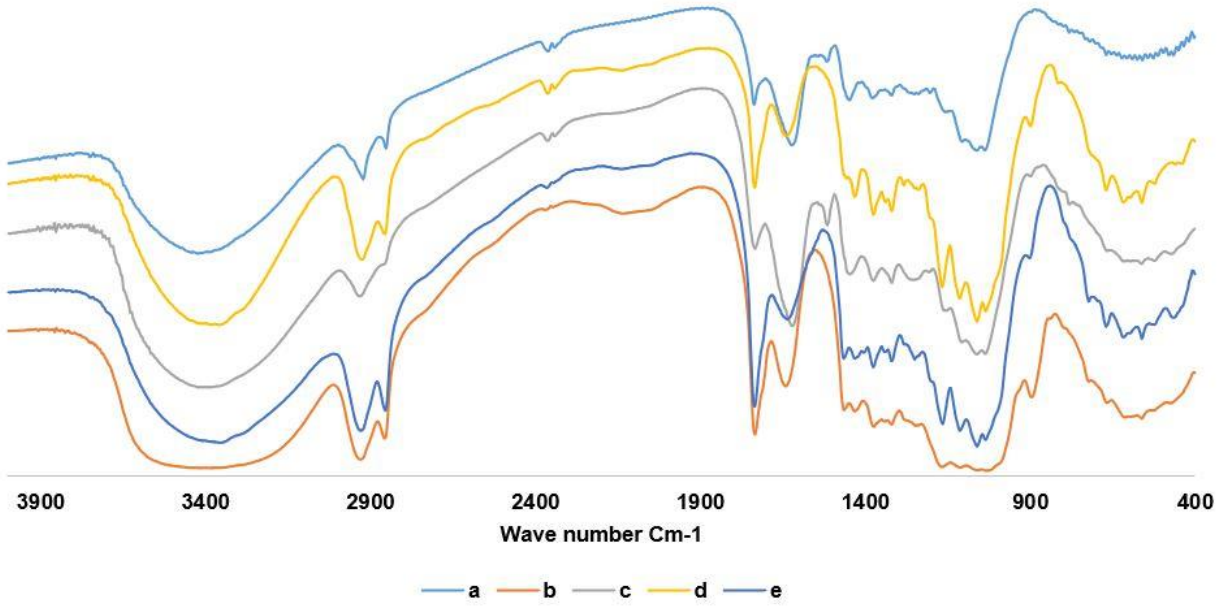

Figure 3.15: FTIR Spectra of (PN) sample recorded at different stages during cellulose extraction. (a) Raw PN sample, (b) after periodate oxidation, (c) after dewaxing, (d) after bleaching, and (e) after the reductive amination reaction.

In addition, as shown in figures 3.16A and B, the size and surface charge of the fibers prepared from pine needles showed an average value of -26.3 mV which means the net charge of the sample was negative. Therefore, hydroxyl groups and the remaining of lignin may still exist.

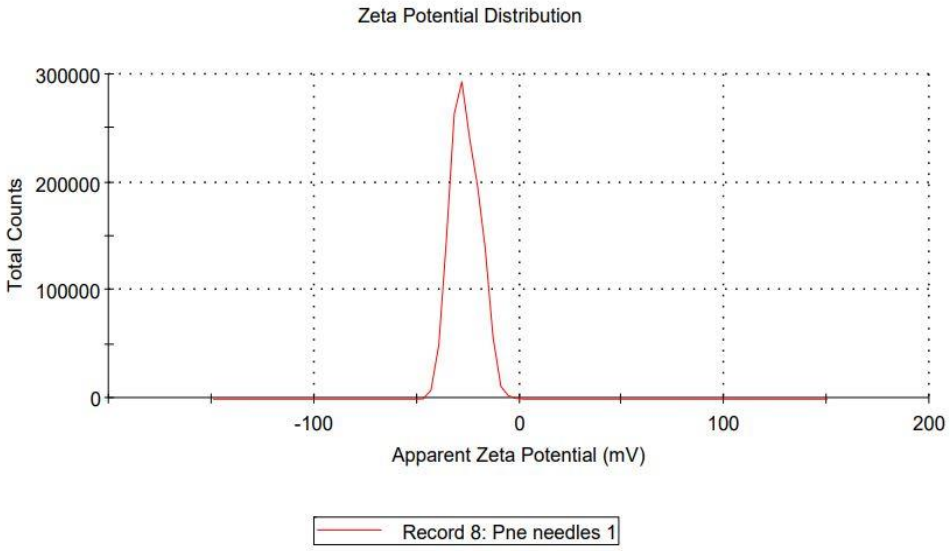

Figure 3.16A: Particle size distribution of the suspension of fibers obtained from pine needles after hydrolysis and sonification.

We may also have tannins attached to the cellulose and other impurities to hinder the interaction of hydroxyl groups. The large particle size distribution of the PN suspension shows that fibers may aggregate in aqueous suspension during hydrolysis [147]

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	183,7	Peak 1:	252,2	98,1	149,6
PdI:	0,392	Peak 2:	5099	1,9	556,0
Intercept:	0,961	Peak 3:	0,000	0,0	0,000
Result quality	Good				

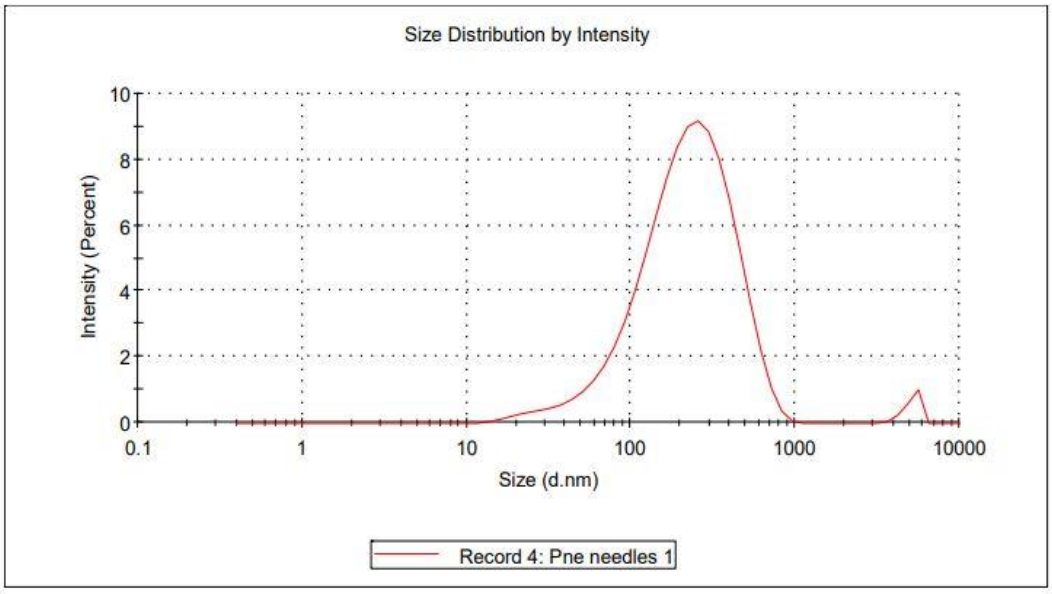

Figure 3.16B: Zeta potential distribution of PN

The final product of the pine needle after all of the extraction treatments and chemical modification was tested for coagulation/flocculation and no flocs formation was observed, even the PN product was increasing the turbidity of the suspension as seen in figure 3.17. However, a study reported pine needles' efficiency in the removal of turbidity using a large amount of coagulant [138], which might produce a severe sludging problem. Hussain et al. [113] tried pine cones and Banchon et al. used pine sawdust [127] and got satisfactory results. Besides the chemical treatment, another reason may be the change in morphological variability in pine needle species and allometry between the tree composition and specific species. [148] Local environmental conditions [149], stand management, and growing conditions [150].

Figure 3.17: Pine needle suspension during the coagulation/flocculation process

3.3 Cellulose from filter paper

There was no flocs formation observed during the coagulation/flocculation experiments tried by TEMPO treated filter paper shown in figure 19. The coagulant added sedimented at the bottom of the jar without making any interaction with the wastewater. This result shows that the TEMPO reaction had not successful, or the source of cellulose did not have the proper content of microfibers or readily available bonds to interact with the reagent and make the reaction successful. Okahisa et al [142] reported that NaClO played an essential role in the TEMPO reaction for the delignification of the cellulose material, which resulted in a change in the morphology and structure of cellulose. If NaClO used in the reaction was not pure may lead to the cellulose fibers to significantly lower carboxyl content, which leads to lower physical and mechanical performance [143].

Several researchers used the TEMPO oxidation reaction to produce nanocellulose and usually obtained ready microfibers cellulose, cellulose with high yield, or kraft pulps to proceed with the reaction. Such as, Rajalaxmi et al [144] used fully commercial bleached softwood Kraft pulp, to produce micro/nanofibrillated celluloses (M/NFCs). Taipale et al. [145] used bleached softwood kraft pulp (elemental chlorine free) and bleached hardwood kraft pulp both obtained from finish mill .Bo Sun et al. [146] also used bleached kraft eucalyptus pulp to modify the cellulose to nanocellulose and later made CNF beads with further reactions and used them as adsorbent for water treatment.

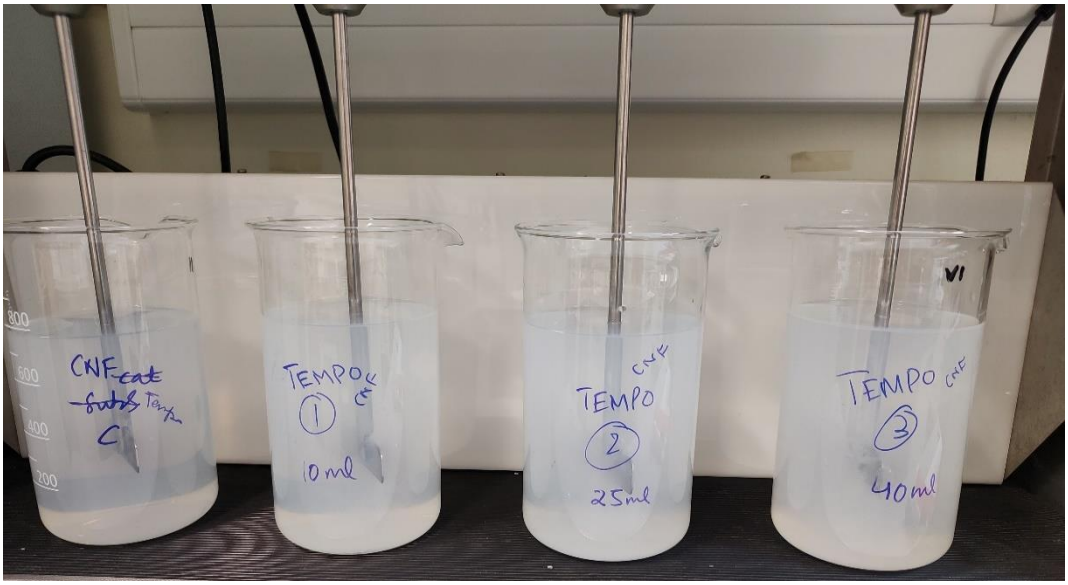

Figure 3.18: Coagulation/flocculation test using TEMPO-treated cellulose from filter paper

3.4 Mechanically and enzymatically produced M/NFCs

The FTIR spectra in figure 20 were recorded while trying the cationization of a combined sample of M/NFCs using periodate oxidation followed by an amination reaction. The carbonyl group which was supposed to appear around 1740 cm^{-1} [146] did not appear. The N-H bending vibration band normally shows absorption at 1564 cm^{-1} which did not appear and after the amination reaction a broad peak appears near 1400 cm^{-1}. It may possible that excessive use of ammonia with sodium cyanoborohydride destroyed the fibers. The absorbance between 1060 to 1161 cm^{-1} in raw M/NFCs sample are assigned to C-O stretching [146], The bands appear at 1630 cm^{-1} in (b) and 1623 cm^{-1}in (c) probably due to the adsorbed moisture by M/NFCs sample

[146]. The sample after all the reactions was clear, and it shows no activity in the coagulation/ flocculation experiment

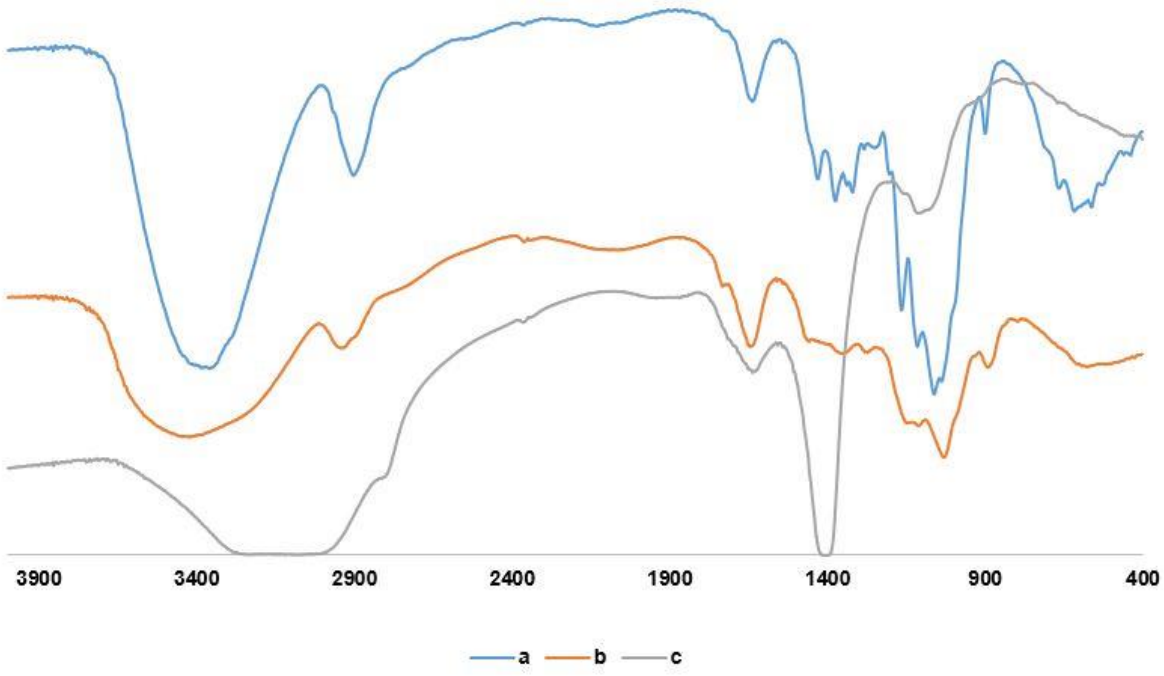

Figure 3.19: FTIR spectra of cellulose nano/microfibers sample recorded at different stages during cationization (a) Raw M/NFCs (mechanically and enzymatically produced), (b) after periodate oxidation, (c) after amination

3.5 Micro/nanofibrillated celluloses (M/NFCs)

Cationic M/NFCs more (DS=0.106) and less (DS=0.06) substituted samples showed satisfactory results for removing turbidity from both, model and real urban wastewater at different concentrations in coagulation/flocculation experiments shown in figure 3.22 and the turbidity removal efficiencies graph are in figures 3.20 (a and b) and 3.21 (a and b).

Figure 3.20 (a and b) shows the turbidity removal efficiencies using more and less substituted cationic samples on synthetic wastewater. The highest removal of turbidity (93.2%) from synthetic wastewater was observed using a more substituted coagulant at the concentration of 49 mg/L which was the medium concentration tried. In comparison, the highest turbidity removal (91.0%) from less substituted coagulant on synthetic wastewater was observed at its highest concentration of coagulant tried i.e., 276 mg/L. This reflects that the degree of substitution is one of the important factors in the structure of fibers, from both samples the

highest removal efficiency was >90% but the concentration used from the less substituted sample was five times higher than the more substituted. Kinga also concluded in [152] that polymers with a high degree of substitution showed better performance in flocs formation and removal of impurities as compared to the ones which have less.

At the highest concentration tried for the more substituted coagulant, 74 mg/L, less turbidity removal was observed than using 49 mg/L of this coagulant, which means that the dosage exceeded its optimum level. Overdosing with coagulant induces charge reversal and particle stabilization. If the coagulant's concentration exceeds the optimum dosage, it be can result in less turbidity reduction [153].

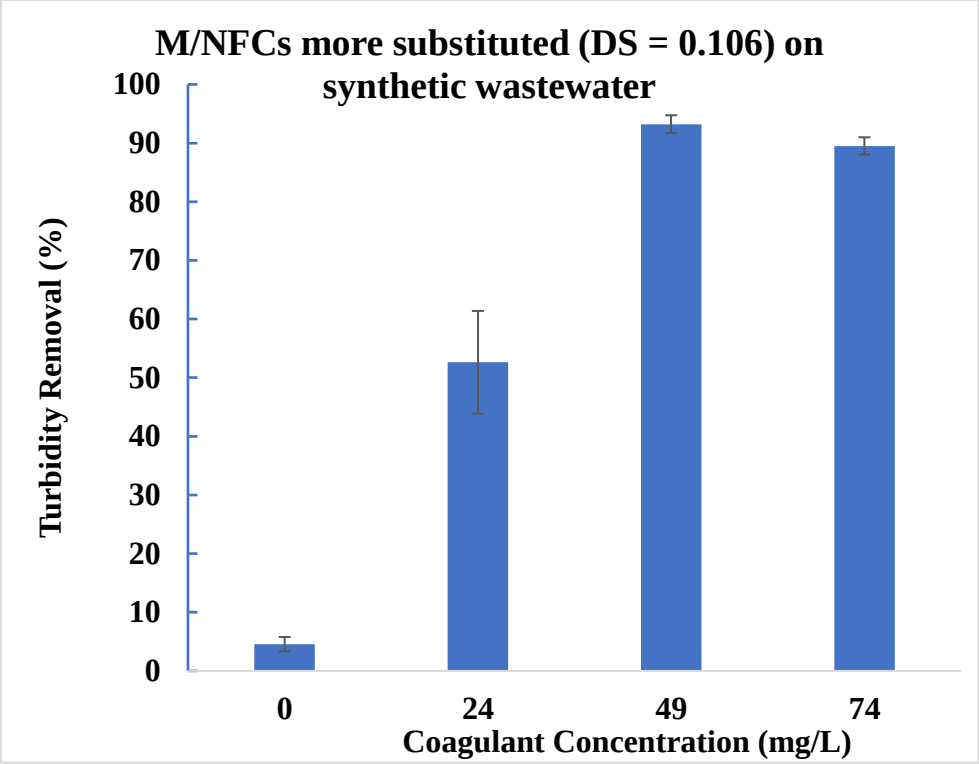

Figure: 3.20a Turbidity removal from synthetic wastewater using more suspended cationic coagulants

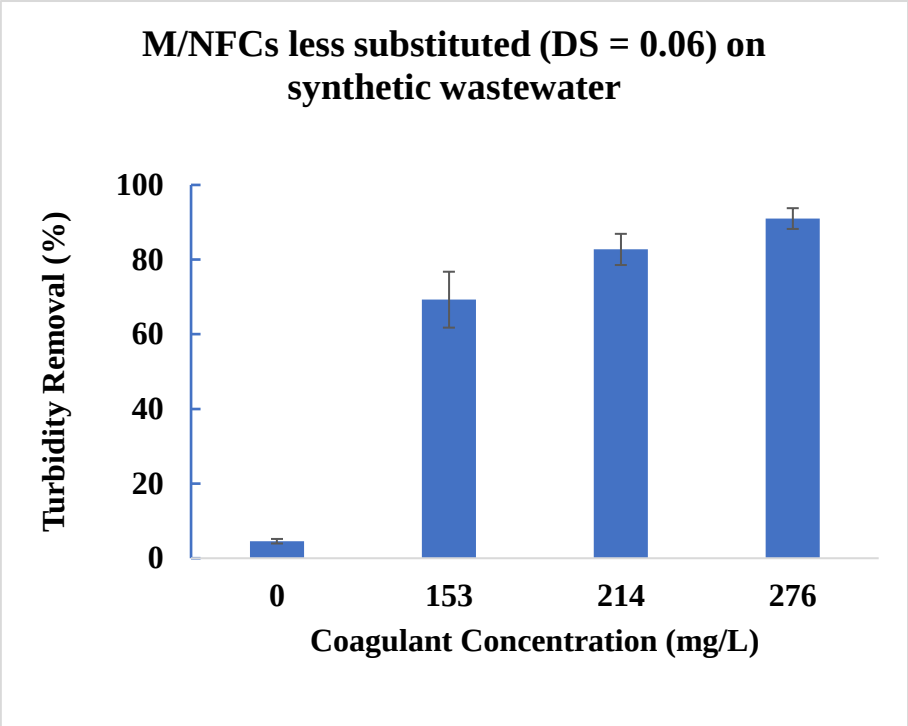

Figure 3.20b: Turbidity removal from real wastewater using more suspended cationic coagulants

Figure 3.21 (a and b) shows the turbidity removal efficiencies using more and less substituted cationic samples on real urban wastewater. The highest removal of turbidity (85.7%) from real wastewater was observed using the more substituted coagulant at the highest coagulant concentration tried i.e., 74 mg/L. In comparison, the highest turbidity removal of 58.0% using the less substituted coagulant was measured at the highest tested coagulant concentration of 276 mg/L. Less turbidity reduction was observed on real wastewater as compared to synthetic wastewater which can be due to the nature of real wastewater as it can have much more components than the synthetic wastewater. Considering the degree of substitution, the trend was the same as more substituted coagulant showed better performance, at maximum removal, using four times less coagulant dosage as compared to the less substituted coagulant.

Besides good turbidity removal, there was also a 25-30% reduction in the concentration of total organic carbon (TOC) recorded using both cationic samples on synthetic wastewater which indicates that natural coagulants also have potential to reduce TOC.

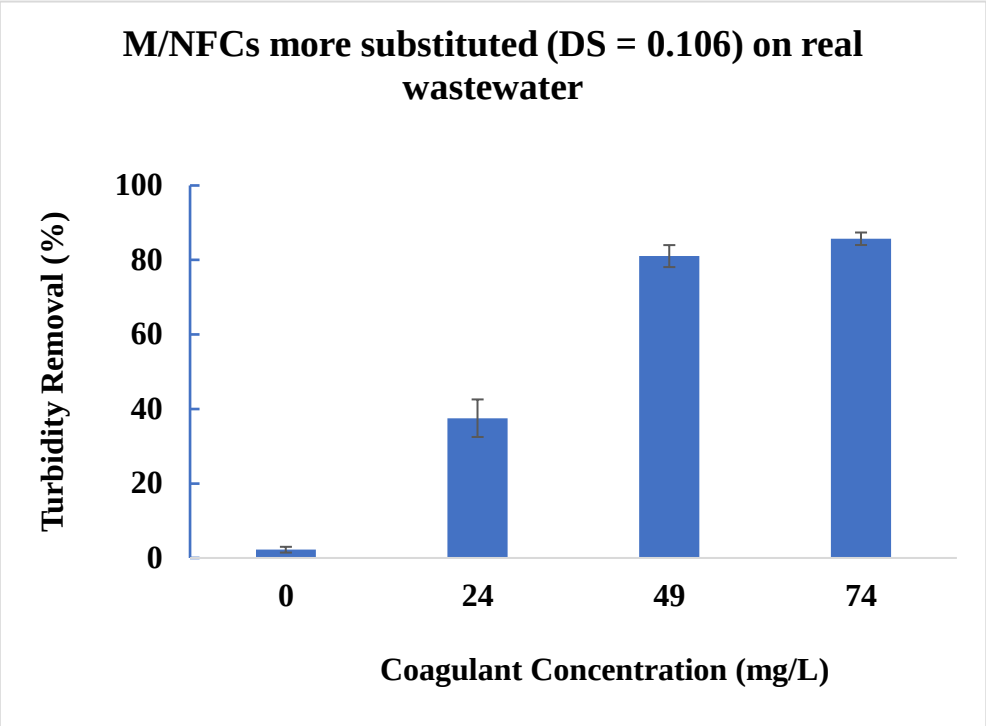

Figure 3.21a: Turbidity removal from real urban wastewater using more suspended cationic coagulants

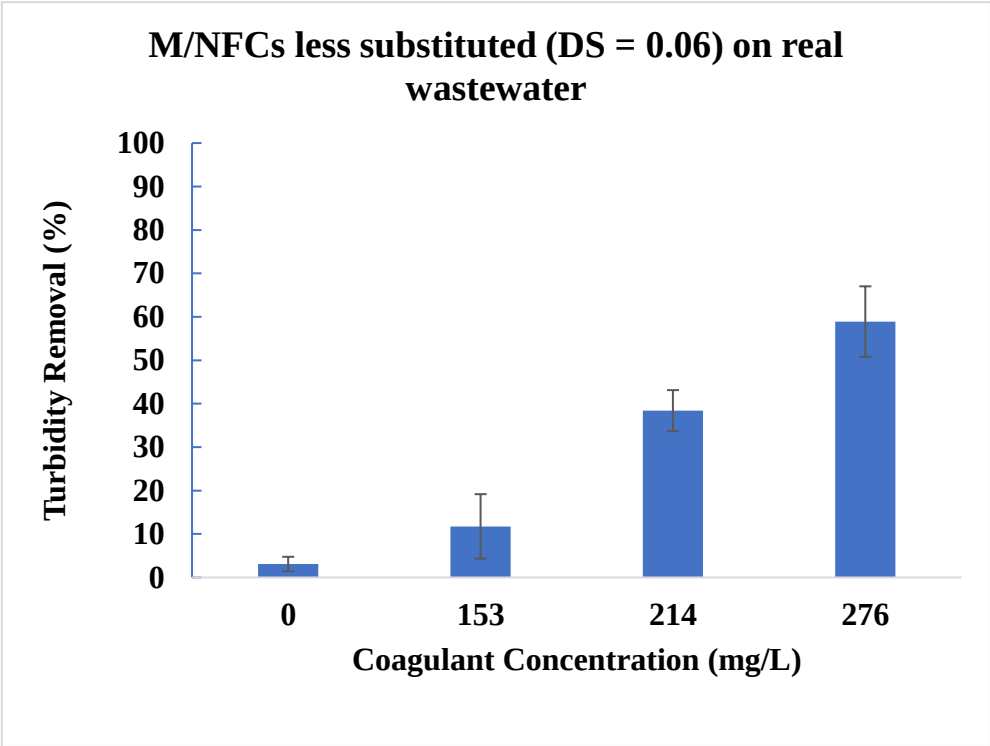

Figure 3.21b: Turbidity removal from real urban wastewater less suspended cationic coagulants

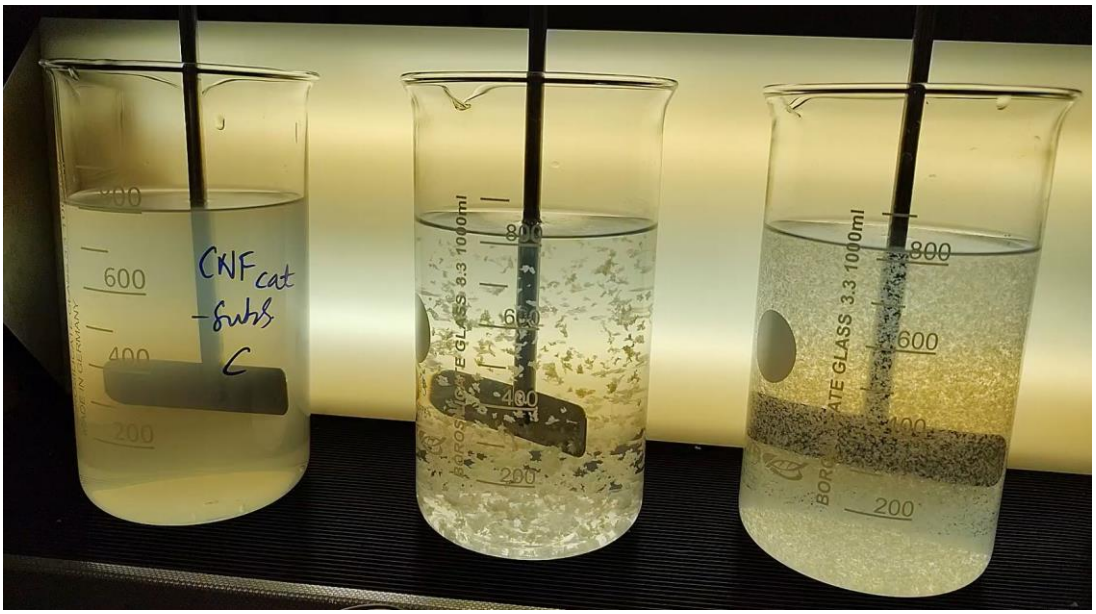

Figure 3.22: Flocs formation during coagulation/flocculation test using cationic sample

3.6 Comparison of the M/NFCs and Commercial Coagulants

Polyaluminum chloride commercial coagulant (WAC-AB®) obtained from Aguas do Algarve company was tested on synthetic and real wastewaters. Figure 3.23a shows the lowest turbidity removal of 4.7% with a coagulant dosage of 0.5 mg/L, and the highest removal of turbidity (98.4%) was observed at 25 mg/L using synthetic wastewater which had an initial turbidity of 92-96 NTU. The commercial coagulant was also tested for turbidity removal efficiency on real wastewater with initial turbidity of 142-156 NTU. The highest turbidity removal for real wastewater was recorded at 98.4% at a concentration of 25 mg/L. In comparison, the lowest turbidity removal of 15.9% was recorded at the concentration of 0.5 mg/ L shown in figure 3.23b, with a little difference between the replicates. The turbidity removal can be seen in figure 3.24.

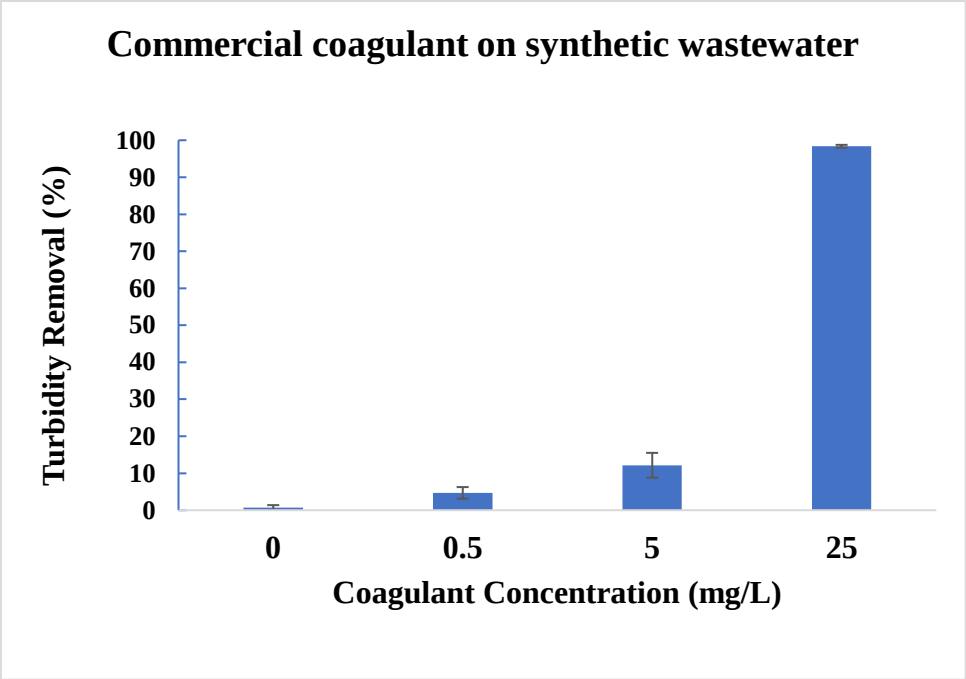

Figure 3.23a: Removal efficiency of turbidity from synthetic wastewater using commercial coagulant

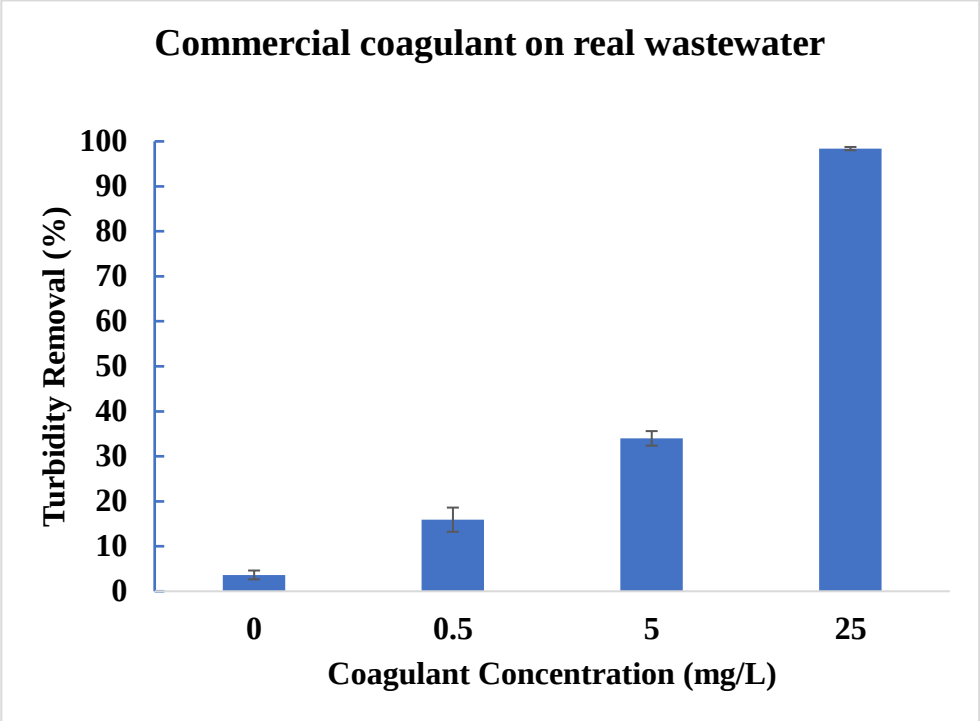

Figure 3.23b: Removal efficiency of turbidity from real wastewater using commercial coagulant

Figure 3.24: Turbidity removal after coagulation/flocculation test using commercial coagulant

Results from micro/nanofibrillated celluloses demonstrate that they are promising alternatives to commercial coagulants. The maximum turbidity removal from M/NFCs more loaded cation on synthetic wastewater was 93% which was very close to the maximum removal of 98% while using the commercial coagulant on synthetic wastewater. The concentration of commercial coagulant used at their maximum removal efficiencies was 25 mg/L, and from more substituted samples was 49 mg/L and both recorded removal efficiencies were > 90%, so it is possible to have the same removal using cellulose-based coagulants which are harmless, abundant, and sustainable. In contrast, commercial coagulants generate hazardous sludge. M/NFCs with less substitution also showed good turbidity removal of 91% on synthetic wastewater, but the concentration used was 276 mg/L, much higher than the commercial coagulant.

4. Conclusion and Future Work

This section includes the conclusion of the research and the proposed future improvements for similar work.

4.1 Conclusive Remarks

The present work explores the performance of cellulose derivatives from different sources tried as a coagulant/flocculant to remove turbidity from wastewater. Extracted and chemically modified products from pine needles, filter paper, and cellulose micro/nanofibers were tried to check their efficiency in removing turbidity. From all the synthesized products, the M/NFCs cationized with CHPTAC (high degree of substitution) performed well as a coagulant and showed a removal efficiency of 92.2% on synthetic wastewater and 85.7% on real wastewater. CHPTAC (low degree of substitution) also showed satisfactory results, with a removal efficiency of 91.0% on the model and 58.9% on real wastewater.

In this assay, a representative model of turbid water with a turbidity of 92-96 NTU was produced without using chemicals. While in the exploration, it was attempted to synthesize the abundantly available plant material (powdered pine needles of 0.5 mm) by adding amine groups. Periodate oxidation followed by reductive amination was performed to functionalize the cellulose from pine needles and then tried as a coagulant. Before the oxidation reaction, different methods were also applied to pine needles, like dewaxing and bleaching. After each reaction, dialysis was performed for purification, but no functional group appeared on the structure, which was confirmed by the FTIR analysis. However, no turbidity removal was observed using synthetic or real urban wastewaters.

Another source of cellulose (filter paper) and a mixture of M/NFCs (mechanical + Enzymatic) treated was examined to assess its coagulation performance processed by TEMPO oxidation and results also showed no flocculation activity. Turbidity removal performance of CHPTAC were >90% on synthetic wastewater which is near to the performance of commercial coagulant and concluded to the point that coagulants obtained from cellulose source, which is harmless for the environment and abundantly available, can be a cost-effective replacement for hazardous synthetic polymers in treating municipal wastewater.

4.2 Recommendation for Future Work

Taking everything into consideration, a significant amount of additional work and improvements must be investigated in producing bio-flocculants from cellulose. New approaches need to be tested to get more effective results in wastewater treatment by coagulation/flocculation. These may include further purification operations and the addition of salts to boost the polymer's solubility, which may aid in floc formation and sediment rate.

While the reaction is being used to get dialdehyde cellulose, it is essential to investigate the possibility of using alternative reagents that oxidize the carbon C_2 and C_3 found in cellulose.

Other equivalents of periodate, ammonia, and TEMPO should be tried to understand the reaction outcome better. The various reaction steps should also be optimized for temperature and time to get a greater yield and a higher substitution degree.

Regarding the safety and efficacy of using CNF, their biodegradability is one of the attractive characteristics that can promote the implementation of green products in water treatment as alternatives to chemicals that don't disintegrate. Therefore, adequate research must be conducted into natural extract's stability and shelf life, most of which are proteins or polysaccharides. In addition to this, the influence of storage conditions and the effective extract formulation that will subsequently be available for usage in commercial settings should be thoroughly investigated.

Finally, laboratory research should be scaled up on pilot plant feasibility employing effective plant extracts to increase the widespread use of bio-based coagulants. In addition, researchers should also continue their search to look for new coagulants and flocculants derived from abundantly available waste plant sources. This will help eliminate chemical reagents in water treatment, paving the way for a sustainable environment and green technology.

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