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Mestrado em Inovação Química e Regulamentação

Trabalho efetuado sob a orientação de:

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Exploring Agricultural Waste as Raw Material for the Production of Bioflocculants

Declaration of Authorship

I declare that I am the author of this work, which is original. The work cites other authors and works, which are adequately referred in the text and are listed in the bibliography.

(Eyob Mulugeta Kebede)

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Dedication

I dedicate this thesis to my daughter Shalom Eyob

Abstract

Plant derived materials can be applied in water clarification since they reduce the use of chemically based coagulants. This study aims to evaluate the use of powders (particle size ≤ 0.5 mm) of pine needles, spent coffee ground, almond shell and banana tree bark, as well as their extracts, as coagulants for municipal wastewater treatment, in order to remove turbidity. A representative model municipal wastewater was synthesized in the laboratory, presenting a turbidity ranging from 50 to 130 NTU. Jar-tests were performed using this model synthetic wastewater and either a commercial aluminum-based coagulant-flocculant, for comparison purposes, or the prepared materials. As results showed no turbidity removal, banana tree bark powder was physically and physico-chemically treated in order to obtain suspensions of cellulose microfibrils. Both the powder and the microfibrils were chemically modified by reaction with glycidyltrimethylammonium chloride (GTMAC). These materials were tested as coagulant-flocculants to check their potential on turbidity removal. Physico-chemically treated banana tree bark powder with particle size between 25 and 100 μm showed a modest result, with a turbidity removal efficiency of 36.5%. Finally, the GTMAC cationized cellulose microfibrils removed 78.8% of the synthetic wastewater turbidity. When this coagulant was tested in a real wastewater, the result was quite similar, as 79.7% of turbidity was removed.

Keywords: Coagulant, Bio-flocculant, Cellulose, Synthetic Wastewater

Resumo

Os materiais produzidos a partir de matéria vegetal podem ser aplicados na clarificação de águas como forma de reduzir a utilização de coagulantes químicos. O objetivo deste estudo é a utilização de pós (tamanho de partícula ≤ 0,5 mm) obtidos por moagem de agulhas de pinheiro, borra de café, casca de amêndoa e casca de bananeira, bem como os seus extratos, para a remoção de turvação de águas residuais municipais. Com esse objetivo, produziu-se no laboratório uma água residual municipal sintética, com uma turvação entre 50 e 130 NTU, que foi usada como modelo. Foram feitos *jar-tests* utilizando esta água modelo e um coagulante-floculante comercial à base de alumínio, para comparação, ou os materiais vegetais produzidos. Uma vez que não foram obtidos valores significativos de remoção de turvação, o pó de casca de bananeira foi sujeito a um tratamento físico e outro físico-químico para obter suspensões de microfibrilas de celulose. Tanto o pó como as microfibrilas foram modificados quimicamente por reação com cloreto de glicidiltrimetilamónio (GTMAC). Estes materiais foram testados quanto à sua capacidade como coagulantes-floculantes. Com o pó de casca de bananeira sujeito a tratamento físico-químico, com um tamanho de partícula entre 25 e 100 □m, obteve-se uma redução de turvação modesta, na ordem dos 36,5%. Já as microfibrilas de celulose cationisadas com GTMAC removeram 78,8% da turvação da água residual municipal sintética. Quando este material foi aplicado numa água residual real, obteve-se um resultado semelhante, com 79,7% de remoção de turvação.

Table of Contents

List of figures

Lists of tables

List of abbreviations

AD	Alzheimer disease
ADAC	Anionic Derivatives of Dialdehyde Cellulose
CDAC	Cationic Dialdehyde Cellulose
CHPTAC	3-chloro-2-hydroxypropyltrimethylammonium chloride
CMCNa	Sodium Carboxymethylcellulose
DCC	Dicarboxylic Acid Nanocellulose
DOC	Dissolved organic Carbon
FTIR	Fourier-transform infrared spectroscopy
GTMAC	Glycidyltrimethylammonium chloride
NTU	Nephelometric Turbidity Unit
PACl	Polyaluminium chloride
SCG	Spent Coffee Ground
TSS	Total Suspended solid
WHO	World Health Organization

1 Introduction

Coagulation-flocculation is one of the most widely used water treatment process for the purification of raw and wastewaters. Conventional coagulants include alum, ferric chloride and polyaluminium chloride which, despite their effectiveness, present some major drawbacks, such as: high sludge production, low removal of arsenic (Crini and Lichtfouse 2019), and some are very high handling costs (Dkhissi et al. 2018). Other synthetic flocculants like polyacrylamides, polyacrylic and polysulfonic acids, and polyethyleneimine, also lack biodegradability and renewability along with high cost (Sarika, Kalogerakis, and Mantzavinos 2005).

Direct flocculation, which uses medium charge density high molecular weight cationic polymers, thus avoiding the coagulation step, has been proposed as a cost and time saving alternative, but it too suffers from the latter issues(Chong et al. 2009). Therefore, regarding the increasing demand for ecofriendly and sustainable water treatment technologies, natural polymers have been widely investigated for that purpose (Siah, Robinson, and Fong 2014).

Cellulose, the most abundant biopolymer on earth (Vignolini 2019; Yu 2018) is considered to have great potential as raw material for such applications, especially if it is obtained from non-food sources, like agricultural and agro-industrial residues or marine biomass, as it could contribute to their valorization as well as to carbon sequestration, limiting the accumulation of greenhouse gases in the atmosphere, without impacting food crop prices. Moreover, due to its biodegradable nature, the sludge generated in the process is ready for disposal, thus reducing the overall treatment cost (Suopajärvi et al. 2013; R Khiari et al. 2010). Plant cell walls are microfibril-based nanocomposites comprised of a variety of polysaccharides, which include cellulose, hemicelluloses and pectin or lignin, depending on the cell wall type (Cosgrove and Jarvis 2012).

Plant derived materials also have been explored to be used in coagulation-flocculation processes (Siah, Robinson, and Fong 2014; Suopajärvi et al. 2014). From simply preparing solvent extracts from ground plant material (Šćiban, Klašnja, and Stojimirović 2005; Klašnja et al. 2009; Ramavandi 2014) to producing biopolymer derivatives by chemical modification (Shaghaleh, Xu, and Wang 2018; Z. Wang et al. 2019), or from using as obtained wood pulp to disintegrating it in nanofibrils (Fiol et al. 2019), a number of strategies have been pursued to produce bioflocculants.

Although in some cases good efficiencies were observed (Dkhissi et al. 2018), most of the times the bioflocculants were not effective by themselves, but only when conjugated with conventional coagulants acting solely as an aid (Khiari et al., 2010). Moreover, some of the developed synthetic strategies involve steps like dialysis and freeze-drying, and therefore are not easily scalable at a reasonable cost. In fact, to obtain an eco-friendly and sustainable flocculating agent, the processing of the raw material should be kept at a minimum in order to decrease the production costs with energy and chemicals as well as to avoid effluent and waste production. Hence, based on the present knowledge, there seems to be a potential in the use of plant wastes to produce bioflocculants that deserves to be further explored.

Therefore, this project aims to explore plant materials originating from agriculture, agro-industrial, gardening and forestry activities, that otherwise would be worthless residues, as raw materials to produce added-value products intended to be used as flocculants in water treatment. Plant materials of choice come from local activity such as pine needles, carob seed husks, almond shells, branches and leaves of banana tree and coffee grounds. For example, about 100 metric tons per hectare of banana by-products are produced annually in the world and widely available in large amounts throughout the year (Chen et al. 2017) and the composition of extracted cellulose by 2% NaOH were 33.86 ± 1.53 % (Zhang et al. 2013). Besides cellulose, these materials comprise hemicelluloses, pectin, lignin and, eventually, tannins. Processing the plant material in order to obtain wood pulp, cellulose nanofibrils or extracts leads to either leaching or loss of some of these components, which have been recognized to have flocculant (tannins) and cell adhesion (pectin and hemicelluloses) properties.

Therefore, since native cellulose presents a relatively low activity as flocculant, due to the high degree of intra- and inter-chain hydrogen bonding, which is also responsible for its low water solubility, the presence of such components may well turn out to be an added value. In fact, Gershlak et al., (2017) observed higher turbidity removal with a carboxymethyl derivative prepared directly from crushed date palm rachis, when compared to a similar derivative prepared from the same material after extraction and bleaching.

1.1 Objectives

The main objective of this project is to develop a cellulose-based bioflocculant from agricultural waste materials with minimal processing of the raw material.

Specific objectives are:

- identify and select local wastes from agriculture activity to be tested;
- assess the porosity and size distribution of the materials selected;
- produce a synthetic wastewater;
- produce a sustainable bioflocculant;
- assess the coagulation/flocculation ability of the bioflocculant using the synthetic wastewater;
- compare the bioflocculant efficiency with the commercial coagulants using the synthetic wastewater;
- assess the coagulation/flocculation efficiency of the bioflocculant using a real wastewater.

1.2 History of coagulants/flocculants

Coagulation and flocculation could be achieved using either natural coagulants or chemical-based coagulants. The former have been acknowledged for their application in traditional water purification which was evidences from various ancient records (Bratby John 2006). However, with the invasion of chemical coagulants, traditional water clarification methods using natural coagulants are no longer used, except in rural and developing countries which have limited accessibility to those chemicals. In the 19^{th} century, the French and British chemists and engineers achieved the optimum conditions to use alum ($Al_2(SO_4)_3$) as a coagulant for public water supply, which was globally disseminated due to its high efficiency (Jahn 2001). However, Kroehler (2014) referred that China was the earliest alum user for water clarification in the world. In 1880, in the United States, ferric salts were the mainly used coagulant in water treatment (Choy et al. 2014). The introduction of polymerized coagulants in water purification process occurred due to problems of slow-settling flocs in low-temperature coagulation using alum. From the different polymerized coagulants found in the market polyaluminium chloride (PACl) has growing due to its less sludge production, insensitive on change of temperature, pH and lower consumption of alkalinity (Cao et al. 2016). Common Organic synthetic polymer flocculants such as polyacrylamide are also available in the market; offering a wider selection of chemical coagulants to provide diverse choices of the individual water treatment plants. This marked the beginning of a paradigm shift towards the dependence of chemical coagulants in treating turbid water (Choy et al. 2014).

1.3 Drawbacks of chemical coagulants

Despite the superiority of chemical coagulants in treating turbid water, they are still lacking in terms of green chemistry. In the 1960s, detrimental effects of chemical coagulants on the human health were published (Heydenrych et al. 2011). In alum treated water, residual aluminum has been the center of debate as it is linked to serious health issues such as the development of dementia and Alzheimer's disease (AD) (McLachlan, 1995; J.R. Walton, 2013; Redford, 2001). The results from several epidemiological studies and clinical observations have suggested the association between the concentration of aluminum in drinking water and AD (Flaten 2001). Thus, to minimize this risk over prolonged water consumption, threshold aluminum concentration values in treated water have been reported to be 0.05 to 0.2 mg/L (EPA 2018). Similarly, WHO guideline suggested the concentration of residual aluminum as 0.2 mg/L (WHO 2011). As for iron salts, excessive iron residual in the treated water will lead to highly visible rust or blood colored stains due to the hydrolysis of iron salts (Gebbie 2005).

Another major drawback of hydrolyzing metal coagulant is the generation of huge hydrous oxide sludge which is non-biodegradable due to the nature of the coagulant. The raw alum sludge is 99% water and it is hard to dewater (Choy et al. 2014). A review by Dassanayake et al., (2015) shows that the amount of alum sludge production in Portugal is 66,000 ton yr^{-1} and sludge disposal cost in Netherlands was £30–£40 million per year. Moreover, treatment of highly turbid water requires several proteolytic additives, which degrade proteins into smaller polypeptides or amino acids (Pham et al. 2014), along with alum making it an expensive process (Pavankumar et al. 2014). Owing to the downfalls of chemical coagulants, there is a need to consider other potential alternatives for water clarification to minimize the environmental damages and to safeguard the wellbeing of human population.

1.4 Plant-based coagulants

Fruits are consumed fresh, dried, or processed with diverse demands in the food industries. A significant amount of fruit waste such as peels and seeds of the total fruit weight are commonly non-edible. These large proportions of waste are generally discarded into the environment as they lack in commercial values. Nearly 1000 million tons of Agricultural Waste is produced in a year in the world and they have environmental pollution potential leaching into soil and water sources if not correctly disposed (Agamuthu 2009). Historically back to the 1100, *P. armeniaca*

had been used as primary coagulant in China and Egypt (Jahn 2001). A report by Yongabi et al., (2011) using carica papaya seeds (deshelled, pulverized and sprinkled onto samples) showed 90% of turbidity removal of storm water with an initial turbidity of 119 NTU. In another work by Sowmeyan et al., (2011), 85% turbidity removal was obtained from raw surface water using *Citrus sinensis* peel/skin (washed with formaldehyde, acid-alkaline and pulverized). Generally, more than 80% of turbidity reduction for plant based coagulants were achieved (Saleem and Bachmann 2019) using different model wastewater, like Abidin et al., (2011) reported a 98% turbidity removal with a coagulant dosage of 120 mg/L from a kaolin suspension with initial turbidities from 100 NTU to 8000 NTU at pH 1–3 and pH 11–12, using Jatropha curcas, presscake as a coagulant, which was obtained by solid–liquid extraction with hexane.

1.5 Potentials of natural coagulants

1.5.1 Tannins based coagulant

Currently, tannin-based coagulants are used in coagulation/flocculation processes for water purification. Tannins are high molecular weight polycyclic aromatic compounds which are called large polyphenol compounds. Tannins are widely distributed in the plant kingdom obtained from natural materials, for example, the sodium hydroxide extracted from pine needle contains higher concentrations of tannin (47.02 %) (Thakur and Choubey 2014; Graciela et al. 2014). Tannin-derived coagulant, using Mannich base reaction and tannin extracts from *Acacia mearnsii*, *Schinopsis balansae*, and *Pinus pinaster*, has been widely used in the potable and wastewater treatment industries and commercialized as flocculant (Bratby John 2006; Beltrán-Heredia, Sánchez-Martín, and Gómez-Muñoz 2010). The features highlighted could be viewed favorably as a selling point to develop and promote tannins as a commercialized plant-based natural coagulant in line with the urgency towards sustainable water treatment. As potential investors, stakeholders and suppliers discover the social-economic and environmental values in developing tannins, the financial aspect and market awareness are resolved easily, contributing to successful commercialization.

1.5.2 Cellulose based coagulant

Mechanical or chemical treatment of cellulosic materials are readily available, they are cost-effective raw materials and have an excellent capacity for water purification (D. Wang 2019). In line with this, several studies have been carried out on the modifications and applications of cellulose for water treatments, such as anionic sodium carboxymethylcellulose (CMCNa), anionic dicarboxylic acid nanocellulose (DCC), cationic dialdehyde cellulose (CDAC) and anionic derivatives of dialdehyde cellulose (ADAC). CMCNa, obtained by chemical

modification of cellulose from date palm rachis, was tested as flocculant to treat surface water and removed 95% of turbidity (Ramzi Khiari et al. 2010). DCC was synthesized by the periodate and chlorite oxidation of cellulose from bleached birch (*Betula verrucosa and B. pendula*) chemical wood pulp, and showed a turbidity reduction of 40–80% and a COD removal of 40–60% in coagulation/flocculation of municipal wastewater (Suopajärvi et al., 2013). CDAC was synthesized from bleached birch (*Betula verrucosa*) commercial chemical wood pulp and showed a maximum flocculation efficiency of 85% treating ground calcium carbonate filler suspension as model water (Sirviö et al. 2011). Liimatainen et al., (2012) ADAC was modified from bleached birch (*Betula verrucosa and pendula*) chemical wood pulp combined with alum (250 mg/L ADAC:62.5 mg/L alum) and removed 72 % of colloidal material efficiently from suspension containing kaolin with initial turbidity of 90 NTU.

1.6 Benefits of plant-based natural coagulants

In contrast to chemical coagulants, plant-based natural coagulants are safe, eco-friendly and generally toxic free (Kumar and Gunasundari 2018). Alternative to chemical coagulants, natural coagulants are low cost and generate five times lower sludge volume with a higher nutritional value (Ugya and Imam, 2016). Local availability, low cost, milder treatment conditions (Maurya and Daverey 2018), high efficiency, and minimal sludge handling cost make natural coagulants as logical and a sustainable choice for turbidity removal (Bouaouine et al. 2018). **Figure 1** summarizes the benefits of using natural coagulants as an alternative to chemical coagulants in water clarification process (Choy et al. 2014)

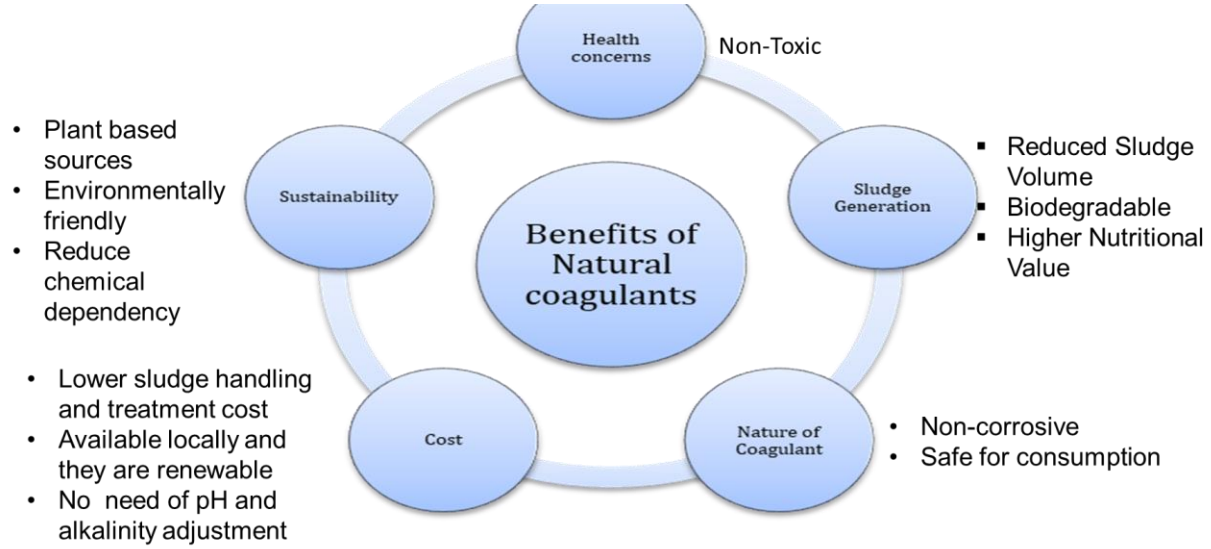

Figure 1: Advantages of natural coagulants as an alternative to chemical coagulants in water clarification process (Choy et al., 2014)

1.7 Microfibril or nanocellulose: preparation, functionalization and processing

1.7.1 Preparation of microfibril or nanocellulose

In the biosphere, cellulose is one of the most important natural and abundant renewable polymers (Suopajärvi et al. 2013). It represents about 1.5 x 10^{12} tons of total annual biomass production and is considered an inexhaustible source of raw material capable of meeting the increasing demand for environmentally friendly and biocompatible products (Klemm et al. 2005). It also contributes 95% of the crystallinities and strength of the plant bodies (Brinchi et al. 2013). As it is shown in **Figure 2**, due to hydrogen bonding and van der Waals forces, it is difficult to isolate these natural cellulose fibrils from their sources. Thus, to remove the non-cellulosic materials and to prepare cellulose nanofibers and nanocrystals from lignocellulosic biomass physical/mechanical or chemical treatments, different techniques should be employed. Among the physical/mechanical pretreatment processes ultrasonication, high-pressure homogenization, grinding/crushing and microfluidization are commonly employed (Wang, 2019; Xie et al., 2018).

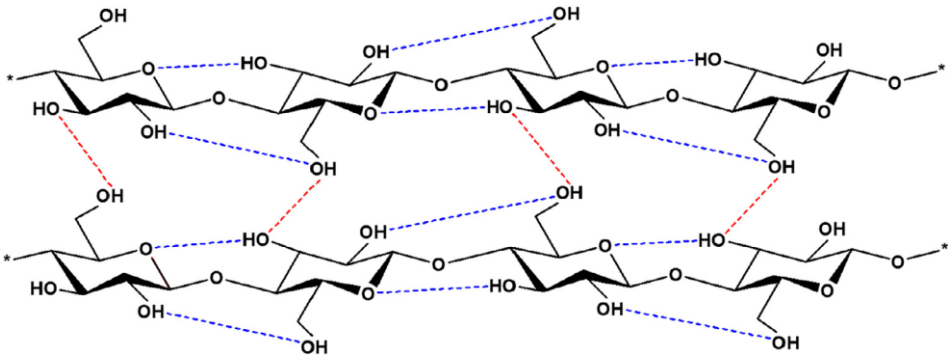

Figure 2: Intramolecular (----) and intermolecular (----) hydrogen bonding networks in cellulose structure (Xie et al. 2018)

In addition to these pretreatments, chemical treatment such as acid hydrolysis is used to prepare micro or nanocellulose from different plant part (Tang et al. 2017). However, as Zhou et al., (2018) reported nano cellulose prepared through acid-free preparation procedure displayed superior mass recovery ratios, higher number of surface anionic groups, and smaller and homogeneous dimensions than which prepared with acid hydrolysis. The different types, sources and their average sizes of nanocellulose are presented in

Table 1.

Table 1: Types and sources of nanocellulose (Wang, 2019)

Type	Origin	Average size
Cellulose nanocrystals	Wood, cotton, hemp, flax, straw, tunicin, avicel	Width: 5–70 nm Length: 100 nm to several micrometers
Cellulose nanofibrils	Wood, cotton, hemp, flax, straw, tunicin, tubers, algae, bacteria	Width: 5–60 nm Length: several micrometers
Cellulose nanowhiskers	Wood, cotton, hemp, flax	Width: 2–60 nm Length: 100–500 nm
Bacterial nanocellulose	Sugar, alcohol	Width: 5–70 nm Length: several micrometers

1.7.2 Surface functionalization of microfibril or nanocellulose

Chemical pretreatments such as carboxylation, sulfonation, oxidation, phosphorylation, esterification, etherification, hydrolysis and amidation are techniques used to functionalize the surface of nanocellulose (D. Wang 2019). **Figure 3** shows the functional groups or molecules that can be grafted onto the nanocellulose surface through different techniques.

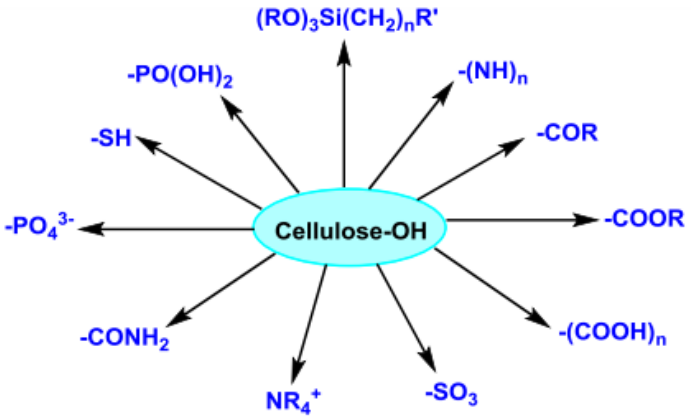

Figure 3: The major surface chemical functionalization of nanocellulose for application in water purification (D. Wang 2019)

The chemical structure of cellulose (Figure 4) shows that the polymer, formed by condensation, consists of monomers joined together by β-1,4 glycosidic oxygen bridges (George and

Sabapathi 2015). The repeating unit of this natural polymer is a dimer of glucose, known as cellobiose, which, possess three hydroxyl groups, which provide reactive platforms for chemical modifications listed in **Figure 3.**

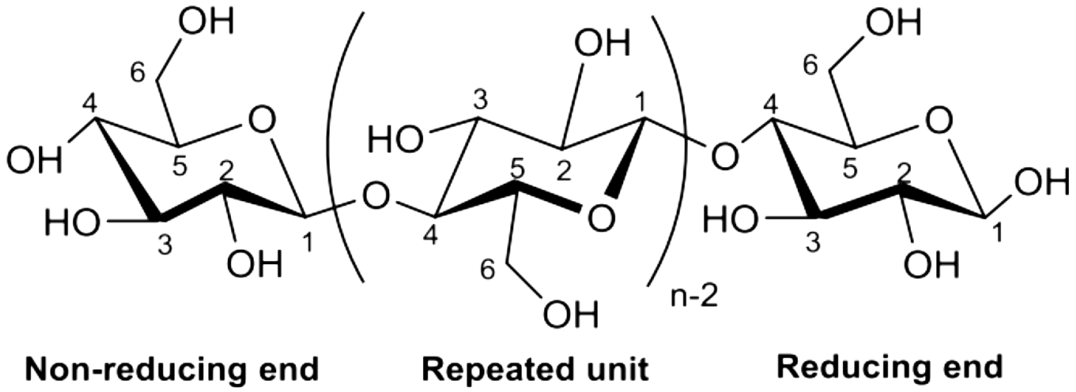

Figure 4: The chemical structure of cellulose (D. Wang 2019)

Aside from abundant hydroxyl groups, the surface of cellulose nanocrystals may contain other types of functional groups that are directly related to its preparation and processing conditions. With the introduction of common functional groups, as it is shown in **Figure 3**, on the cellulose surface the product exhibit different charge properties. As an example, nanocellulose functionalized by sulfate or carboxylate groups on the surface is negatively charged over a wide range of pH conditions (above its pKa), while the amino groups are positively charged below the pKa values of the weak base. In addition, modifying the cellulose nanocrystals with quaternary ammonium groups such as Glycidyltrimethylammonium chloride (GTMAC) can provide their surface with permanent cationic charges (Tang et al. 2017).

1.7.2.1 Cationizations of micro or nanocellulose

Cationic celluloses have found diverse application areas because of their properties such as biodegradability, low cost and low toxicity (Yang, Asoh, and Uyama 2019). Micro/nanocellulose functionalization involves the introduction of positively charged groups by reacting the hydroxyl group of polysaccharides to produce biopolymeric materials. As reported by Sirviö et al., (2011) a water-soluble cationic dialdehyde cellulose (CDAC) was effectively synthesized from birch cellulose pulp using cationic Girard's reagent T ((2-hydrazinyl-2-oxoethyl)-trimethylazanium chloride). Similarly, sulfuric acid hydrolyzed cotton cellulose is treated with epoxypropyl trimethyl ammonium chloride (EPTMAC) to make cationic cellulose. After modification, the characterization had done by using zeta-potential, conductometric titration and polarized light microscopy. The zeta-potential result shows a slight

decrease in the magnitude from -39 ± 3 mV before treatment to +30 ± 5 mV after treatment which confirmed the surface cationization of the cellulose with EPTMAC and the formation of stable suspension before and after functionalization (Hasani et al. 2008). Another study by Zaman et al., (2012) showed that the surface of commercially available cellulosic nanocrystal was effectively cationized using GTMAC based on wet and semi-dry method. For this work the cationization had been confirmed by Fourier Transform Infrared Spectroscopy (FTIR), which reviled new band at 1479 cm^{-1} attributed to CH_2 bending mode and methyl groups of the cationic substituent, and zeta-potential measurements which showed charge reversal from −57 ± 1.2 mV for the un-modified to +63 ± 1.65 mV the modified at 36% water content of the reaction system. **Figure 5** shows the reactions during the cationic modification of cellulose using GTMAC/H_2O/NaOH System (Courtenay et al. 2018). Generally, cationization of cellulosic material using different reagents such as with ammonium or amino functional groups had proven to have immense value for a wide range of applications due to their properties, which include biodegradability, low cost and low toxicity (Yang, Asoh, and Uyama 2019).

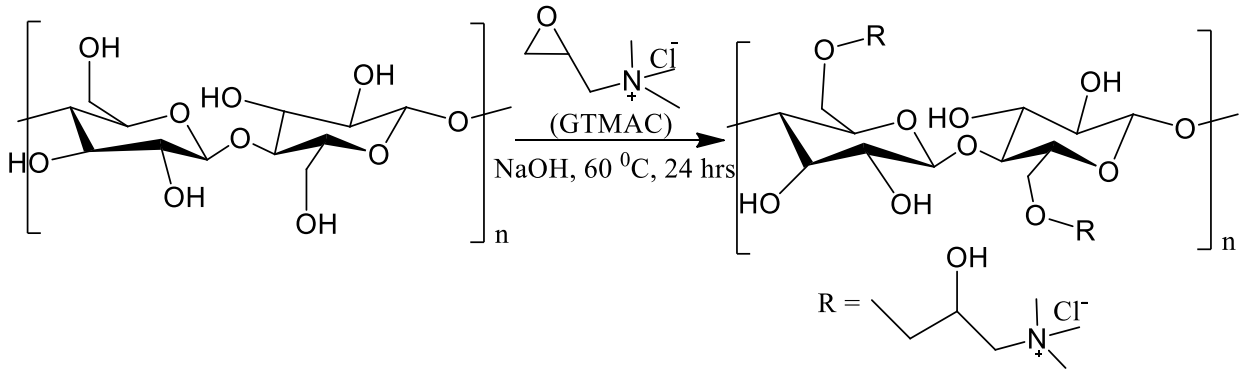

Figure 5: Reactions during the cationic modification of cellulose nanocrystals using GTMAC/H_2O/NaOH System (Courtenay et al. 2018)

2 Experimental part

This chapter includes the materials used for the experiments and the different methods applied in each phase of the work. **Figure 6** shows the summery of the overall work done.

2.1 Analytical methods, reagents and equipment

Corn Starch, $MnCl_2 \cdot 4H_2O$, $Na_2MoO_4 \cdot 2H_2O$, and $CaCl_2.2H_2O$ were obtained: from Riedel Haen; $CuCl_2 \cdot 2H_2O$ and GTMAC (purity≥99.0%) from Sigma-Aldrich; $MgSO_4.7H_2O$ and $K_2H_2PO_4$ from Merck; NH_4Cl from J.M Gomes dos Santos; $ZnCl_2$ from Vaz Pereira; yeast extract from Fluka 9182; $Co(NO_3)_2 \cdot 6H_2O$ from Fisher; meat extract from Scharlau chemie S.A, Spain; Bacto Peptone from Becton Dickinson, USA; and $NaHCO_3$ and NaOH from Pronalab, Portugal.

All samples were analysed for dissolved organic carbon (DOC) (TOC-5000, Shimadzu, Japan), after filtration through a 0.45 µm acrodisk filter, on the same day that the experiments were conducted. The Fourier-transform infrared spectra (FTIR) were recorded using a Bruker, Tensor 27, FTIR spectrophotometer (UK). Samples turbidity was measured in a HACH 2100N (USA) turbidimeter, conductivity was measured with a Crison GLP 32 (Spain) conductivity meter, and pH was measured in a Crison Basic 20+ (Spain) pH meter. The zeta potentials of the samples were also measured. Analyses were performed in a ZetaSizer Nano ZS90 (Malvern Inc., UK) by electrophoresis mobility measurements at 25 ºC using a disposable polycarbonate capillary cell (DTS1061, Malvern Inc., UK).

2.2 Synthetic wastewater preparation

A model water, representative of a municipal wastewater was prepared. It is composed of the following components (**Table 2**) and trace amount of heavy metals, such as $MnCl_2 \cdot 4H_2O$ (2.49 mg/L), $ZnCl_2$ (0.11 mg/L), $Na_2MoO_4 \cdot 2H_2O$ (0.39 mg/L), $CuCl_2 \cdot 2H_2O$ (0.054 mg/L) and $Co(NO_3)_2 \cdot 6H_2O$ (0.049 mg/L). All the chemicals used were analytical grade. All the components were dissolved in 15 L of chlorine free tap water (left overnight in an open vessel to allow for chlorine release) and left for about four days in an oven at 30 ^{0}C, until its turbidity reached 50 – 130 NTU.

Table 2: Components of the synthetic wastewater

Component	Amount in mg/L	C	N	P	CO_3^{2-}	Cl^-	SO_4^{2-}
Bactopeptone	115	122	36				
Corn-starch	670	196					
Dry-Meat extract	15	6	0.018				
Yeast extracts	28	11.2	2.8	0.5			
Oleic acid	10	7.65					
NH_4Cl	84		22			55	
$CaCl_2.2H_2O$	118					57.0	
$MgSO_4.7H_2O$	88						34.4
KH_2PO_4	21.75			5			
$NaHCO_3$	255.5				146		
NaOH	35						
Total		239.85	42.818	5.5	146	112	34.4

2.3 Preparation of a coagulant from pine needles

Fallen pine needles were collected from the University of Algarve, Gambelas campus, washed with tap water to remove dust particles, air dried in the hood for two weeks, and ground to ≤ 0.5 mm particles size using a grinder equipped with a cutting-grinding head (MF-10 basic, Model-IKA-WERKE). All the extraction procedure was based on the previous work reported by (de Hoyos-Martínez et al. 2019) unless otherwise stated.

2.3.1 Tannin extraction by sonication

Pine needle powder (50, 75,100, 200 and 500 mg) was mixed with 50 ml of distilled water in five 50 ml volumetric flasks. The suspension was then thoroughly mixed and sonicated for about 15 minutes (GT Sonic VGT-1620QTD). The obtained stock solutions from each of these flasks were preserved at -4°C until used for the jar-test.

2.3.2 Tannin extraction by sonication followed by heating

Pine needle powder (50, 75,100, 200 and 500 mg) was mixed with 50 ml of distilled water each in five 50 ml volumetric flask. The suspension was then thoroughly mixed and sonicated for about 15 minutes (GT Sonic VGT-1620QTD) followed by heating at 80 ^{o}C for 15 minutes. The obtained stock solutions from each of these methods were preserved at -4°C until used for the jar-test.

2.3.3 Tannin extraction by 1M NaCl

Pine needle powder (5 g) was mixed with 500 ml of 1M NaCl. Then the suspension was thoroughly mixed using a magnetic stirrer for 30 min followed by filtration of the solution, under vacuum, through a filter paper (Whatman® grade 41). The collected supernatant was expected to have the active component used as coagulant (Camacho et al. 2017). The obtained stock solution from this method was preserved at -4°C until used for the jar-test.

2.3.4 Tannin extraction by 1% NaOH and 10% NaOH

In each of three different 100 ml beakers, 2 g of pine needle powder and 50 ml of 1% NaOH aqueous solution were added. The suspension was mixed using a magnetic stirrer and heated at 80 ^{o}C, 90 ^{o}C, and 100 ^{o}C for 10 min to extract the active component (tannins), followed by separation of the solution through centrifugation at 2000 rpm for 10 min. The procedure was repeated for 20 min and 30 min heating to optimize the extraction time and temperature. After optimization of time and temperature using 1% NaOH, 2 g of pine needle were extracted with 10% NaOH for 30 minutes at 90 ^{o}C. The obtained stock solutions from each of these methods were preserved at -4°C until used for the jar-test.

2.4 Preparation of a coagulant from almond shell

A sample of almonds was obtained from a local supermarket and the shell removed from the seed in the laboratory. The shell was ground to ≤ 0.5 mm particles size using a grinder equipped with a cutting-grinding head (MF-10 basic, Model-IKA-WERKE).

2.5 Preparation of a coagulant from spent coffee grounds (SCG)

Spent coffee grounds (SCG) were collected from a cafeteria at the University of Algarve, Gambelas campus, which was branded by Nova Delta-Comércio e Indústria de Cafés, S.A. (Campo Maior, Portugal). The collected SCG was dried at room temperature in the hood and tested as coagulant using jar-test.

2.6 Preparation of a coagulant from banana tree bark

The banana tree bark was collected from a backyard near the campus and washed with tap water to remove dust particles, air dried in the hood for three weeks, and ground to ≤ 0.5 mm particles

size using a grinder equipped with a cutting-grinding head (MF-10 basic, Model-IKA-WERKE).

2.6.1 Physico-chemical treatment of banana tree bark

The procedure was based on the work published by Pillon and Picolli, (2004) with some modifications. Banana tree bark powder (7.14 g) was added to a 500 ml conical flask which contained 3.41g of NaOH dissolved in 200 ml of distilled water and autoclaved for about 45 minutes at 85 °C. The mixture was cooled to room temperature and ground for about 30 minutes using a domestic blender. After 3 hours heating at 95°C, grinding was repeated for 60 minutes. The homogenized mass was subsequently introduced in a conical flask and autoclaved for about 45 min at a temperature of 85°C again. The obtained mixture was divided in two parts. One part was neutralized using 2 M HCl and dialyzed (cellulose dialysis membrane, molecular weight cut-off 12,400 from Sigma–Aldrich) against distilled water for 2 days changing the water twice per day. After dialysis the mixture was divided in two parts and homogenized using an Ultra-Turrax (model AKI-25T) with two different conditions, one part homogenized for 1 minute and the other for 2 minutes. The second part from the first division was transferred into centrifuge tubes and centrifuged at 10000 rpm for 10 min. The supernatant was discarded. The precipitated product was washed with distilled water three times to neutralize and centrifuged under the same conditions until getting a neutral product. Finally, the size of the products was analyzed using a Mastersizer (Malvern Instruments, UK). Then the jar-test was conducted using this material.

2.6.2 Cationization of physico-chemically treated banana tree bark

The procedure reported in this section is an adaptation of a work published by Dionísio et al., (2016) with some modifications. The cationic cellulose-based flocculant was synthesized by a reaction between cellulose and glycidyl trimethylammonium chloride (GTMAC). In a round-bottom flask 5 ml of KOH (0.504 g) aqueous solution was prepared and heated under magnetic stirring in a pre-heated oil bath at 60 °C, to which 500 mg of physico-chemically treated banana tree bark was added and 3.62 ml of GTMAC (4.9 g) were added dropwise to the reaction solution, continuously stirred. After the addition of the reagent, the flask was closed with a condenser and kept on stirring for 24 h to react until completion. The temperature was kept constant during the reaction with a temperature controller connected to the oil bath. After the reaction, the mixture was diluted with 10 ml of milli-Q water, left to cool down to room temperature and neutralized with 2 M HCl. Then the suspension was dialyzed (cellulose dialysis

membrane, molecular weight cut-off 12,400 from Sigma–Aldrich) against distilled water for 3 days to remove residues of any unreacted reagents and by-products. The distilled water was replaced every 6 h in the first day and once a day in the following 2 days. For recording FTIR spectra of unmodified and GTMAC modified banana bark, samples were ground with KBr in a mortar and compressed into discs using a Hydraulic Pellet Press (Specac Ltd). For each spectrum, a 32-scan interferogram was collected in transmittance mode with a 4 cm^{-1} resolution in the 4000–400 cm^{-1} region.

2.7 Jar-test experiments

The coagulation/flocculation/sedimentation experiments were carried out using a Jar-test equipment (Flocumatic, Selecta, Spain) with four paddles for 1-L-capacity beakers. Experiments were performed using 800 ml of the model synthetic wastewater having turbidities within the range 50-130 NTU, and a commercial coagulant and the natural coagulants were tested at different concentrations. The operational conditions used for each wastewater sample were as follows: the beakers were filled up with wastewater, placed in the jar tester, different concentrations of coagulants added to each of the beakers and the rapid mixing started at 200 rpm for 2 min. Then, the mixing was reduced to 20 rpm for 20 min, after which was stopped, and sedimentation started for 20 min. After this, 75 ml samples were collected at approximately 5 cm from the water surface for residual turbidity measurement, and DOC whenever considered necessary. For the best coagulant/flocculant results obtained with the synthetic wastewater, jar-test experiments were made using a real wastewater from a carob processing factory. This wastewater was chosen because of its characteristics for turbidity and organic matter, similar to the synthetic wastewater prepared. The commercial coagulant (WAC-AB®) tested, kindly supplied by Águas do Algarve S.A., was used with synthetic and real wastewaters for comparison purposes.

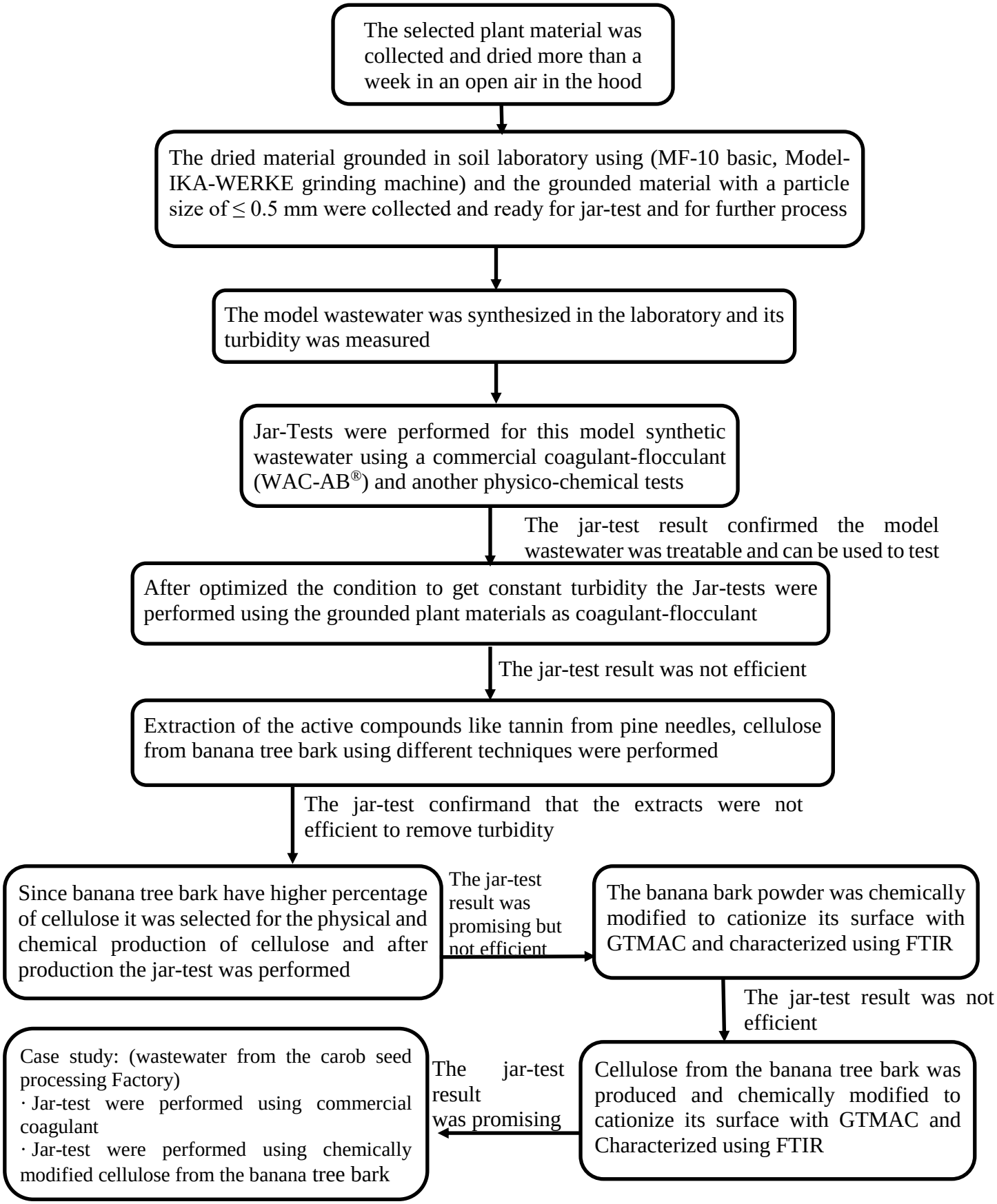

Figure 6: flow diagram of the overall work

3 Results and Discussion

This chapter includes the results obtained in the present work and their discussion, namely: the production and characterization of the model wastewater, the characterization of the materials used as bio-flocculants, the performance results of both untreated and treated bio-flocculants from different plant types.

3.1 Production and characterization of a model municipal wastewater

A representative model municipal wastewater was produced in the laboratory based on (Lv et al. 2017; Metcalf and Eddy 1991). **Table 3** shows the physico-chemical characteristics of the synthetic wastewater produced.

Table 3: Physico-chemical characteristics of the synthetic wastewater

Characteristics	Values
Turbidity	50-130 NTU
TSS	270±22.6 mg/L
pH	7.37± 0.1
Conductivity	951.2 ± 46.2 µS/cm^{-1}
DOC	0.957±0.022 mg/L

TSS: Total suspended solid; DOC: Dissolved organic carbon

Several researchers have used synthetic wastewater as model water for their works such as Priyatharishini et al.,(2019) who made a wastewater by dissolving 10 g of ground cat food into 1 liter of tap water, which has a composition of 30% crude protein, 10% crude fat, 5% crude fiber and 12% moisture, in order to test banana peel as a natural coagulant for the treatment of household wastewater. This wastewater had the following characteristic: TSS (216 mg/L), COD_5 (1500 mg/L), BOD (300 mg/L), NH_3-N (15 mg/L), NO_3-N (27 mg/L), and P (42 mg/L). Similarly Joshi et al., (2019) and Fitria et al.,(2014) prepared domestic synthetic wastewater by the following recipes: dextrin 150 mg/L, NH_4Cl 130 mg/L, yeast extract 120 mg/L, glucose 100 mg/L, soluble starch 100 mg/L, Na_2CO_3 150 mg/L, commercial detergent 10 mg/L, $NaH_2PO_4 \cdot 2H_2O$ 100 mg/L, and K_2SO_4 8.3 mg/L in hot water followed by addition of a Kaolin suspension at 10,000 mg/L. The former used the wastewater to study *Bacillus licheniformis* NJ3 as a bioflocculant and on the latter to study the impact of sludge floc size and water composition on dewaterability.

3.2 Turbidity removal efficiency of ground materials and a commercial coagulant

The ground materials with particle size of ≤ 0.5 mm of pine needle, almond shell, spent ground coffee, carob seed husk, and banana tree bark were used as coagulants/ flocculants, and turbidity removal was evaluated. Similarly, in order to check the treatability of the model wastewater, a commercial coagulant (WAC-AB®) was also used. **Figure 7** shows that the turbidity removal efficiency of the model water without coagulant addition (0 mg/L) was ca. 50% in all tests. This might be due to the wastewater having suspended particles that are sufficiently heavy to sediment naturally by their own without the help of coagulant/flocculants. For pine needles, removals were from 47.3 to 38.8% with the increase of coagulant dose. The same happens with the other materials except for the commercial coagulant, in which case the removal increased with coagulant dose. For the commercial coagulant, 99.2% removal was obtained with the addition of 100 mg/L of coagulant. Thus, this result proved that our model water was treatable and could mimic the wastewater. However, the powders were not efficient as coagulants/flocculants, probably because they are devoid of charge and the hydroxyl groups from the plant material components are not available to interact with the colloids in the model water. Previous studies indicate that the negatively charged functional groups of COO^- and OH^- in the polyelectrolyte of the seeds of *Strychnos potatorum* have been found to be responsible for the reported coagulation activities (Choy et al. 2014).

Initial water turbidity: 50 NTU

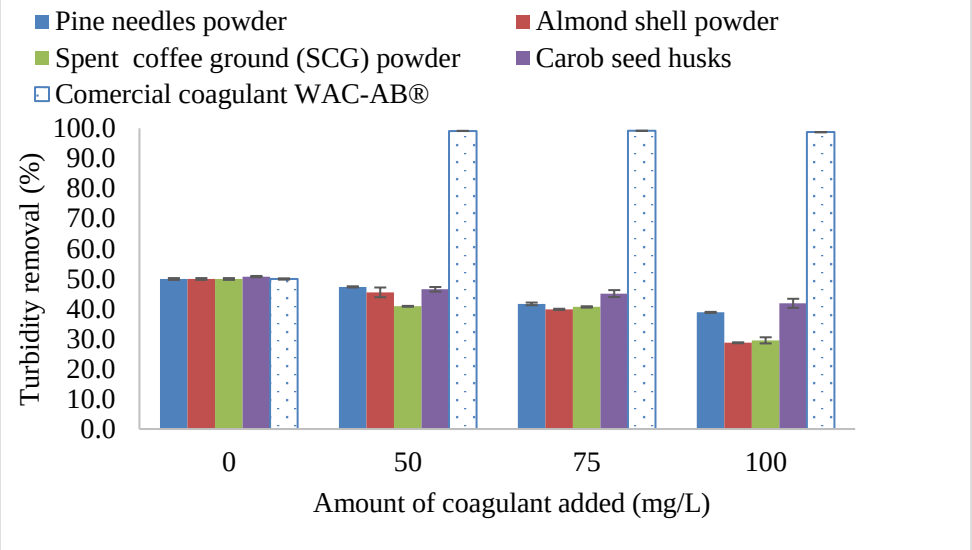

Figure 7: Turbidity removal efficiency of ground materials and a commercial coagulant

3.3 Turbidity removal efficiency of pine needles powder extracts

Results of turbidity removal by tannins extracted from pine needles are shown in **Figure 8**. The turbidity removal efficiency from the model water without coagulant addition (0 mg/L) ranged from 28.6% to 52% for all the tested materials, depending on the initial turbidity of the wastewater, which, in this case, ranged from 70 to 122 NTU. The highest turbidity removal of 80.1% was obtained by the addition of 100 mg/l of coagulant extracted by a hot 10% NaOH aqueous solution. However, using the same tannin extraction method but neutralizing with 2 M HCl the turbidity removal was only 26.5%. Based on this result, we may assume that the highest turbidity removal might not be due to coagulation/flocculation, but to precipitation because of the high pH (11.56). In addition, the percentage removal decreased when the concentration of the extract increases for the other extracts using different extraction methods, and the turbidity removal without the addition of any coagulant was even a little bit higher than with the lowest coagulant dose added (50 mg/L). This result might be due to the colorful nature of the extract or suspended materials that would add turbidity to the tested water.

Initial water turbidity:70-122

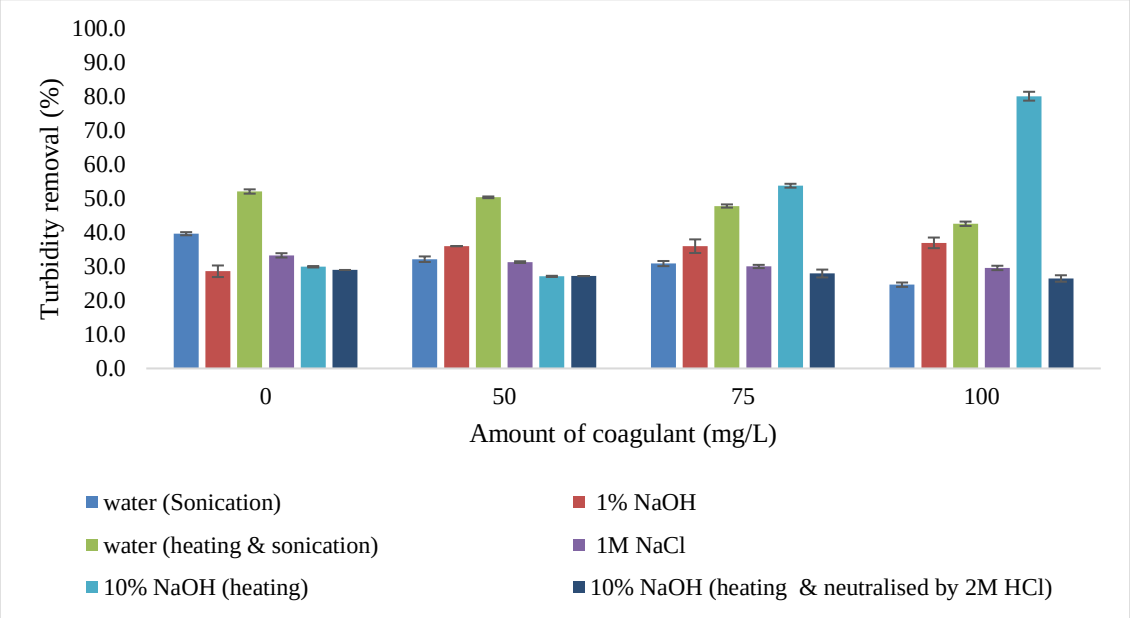

Figure 8: Turbidity removal efficiency of pine needles powder extracts

3.4 Physically and physico-chemically treated banana tree bark powder and its removal efficiency

The results in **Table 4** show that the particle size distribution of the banana tree bark powder treated with water was approximately 150 µm, while all the other processing methods, that use 0.02 M NaOH, gave the same particle size distribution, in the range 25-100 µm. Based on these results it seems that NaOH is essential to produce cellulose microfibrils. These results are comparable with the patent published by Engelen et al., (2015) using potato wastes they prepared cellulose with particle size distribution in the range of 25-110 µm. Azanaw et al., (2019) extracted cellulose fibers from *Yucca elephantine* plant using water and 3% NaOH. These authors found approximately 242 µm and 135 µm for the diameter of cellulose fibrils, respectively.

Table 4: Size distributions of physically and physico-chemically treated banana tree bark powder

Code	Production treatment method	Average particle size
B1	Autoclaved (water) + Mixer (3 hrs)	~ 150 µm
B2	Autoclaved (0.02 M NaOH) + Mixer (3 hrs) + Neutralized (2 M HCl)	~ 25 – 100 µm
B3	0.02 M NaOH(aq) + Mixer (3 hrs) + Neutralized (2 M HCl) +Dialysis (2 days) + Homogenized (Ultra Turrax, 1 min)	~ 25 – 100 µm
B4	0.02 M NaOH(aq) + Mixer (3 hrs) + Neutralized (2 M HCl) + Dialysis (2 days) + Homogenized (Ultra Turrax, 2 min)	~ 25 – 100 µm
B5	0.02 M NaOH(aq) + Mixer (3 hrs) + Centrifuged + Washed (distilled water)	~ 25 – 100 µm

The cellulose microfibrils obtained from each method were tested as coagulant using the model wastewater in a jar-test. The highest turbidity removal was 36% obtained using the coagulant produced by method B5 (**Table 4**), while the lowest turbidity removal was 12% when using the coagulant produced by method B2 (**Table 5**). The low values of turbidity removal might be due to the lack of charge in the cellulose microfibrils. Therefore, it was decided to chemically

modify the microfibrils surface by introducing charged groups. Method B5 was selected to produce the microfibrils for surface modification because relative to the others it could be cost effective.

Table 5: Turbidity removals using 7.26 mg/l of banana tree bark produce with different physical and physico-chemical methods

Code	Production treatment method	Turbidity removal (%)
0	No treatment applied	12
B1	Autoclaved (water) + Mixer (3 hrs)	16
B2	Autoclaved (0.02 M NaOH) + Mixer (3 hrs) + Neutralized (2 M HCl)	12
B3	0.02 M NaOH(aq) + Mixer (3 hrs) + Neutralized (2 M HCl) + Dialysis (2 days) + Homogenized (Ultra Turrax, 1 min)	24
B4	0.02 M NaOH(aq) + Mixer (3 hrs) + Neutralized (2 M HCl) + Dialysis (2 days) + Homogenized (Ultra Turrax, 2 min)	25.5
B5	0.02 M NaOH(aq) + Mixer (3 hrs) + Centrifuged + Washed (distilled water)	36.5

3.5 Chemical modification of the untreated banana tree bark powder

The FTIR spectra of GTMAC modified and unmodified banana tree bark powder are shown in **Figure 9**. Since the quaternary ammonium groups do not display any characteristic IR absorption bands, an evidence for the formation of the functionalized derivative would come from the broadening of the band at 1088 cm^{-1} (ether C-O symmetric stretching) and the new bands at 1479 and 914 cm^{-1} (C-H scissoring in methyl groups of trimethylammonium and ether C-O asymmetric stretching, respectively) (Braz et al. 2018). However, these changes were not observed in the FTIR spectra of the synthesized sample, which means the reaction did not occur at all or occurred only to a small extent. The only change which was observed was the disappearance of the bands at 1669 cm^{-1} and 1252 cm^{-1} from the unmodified banana tree after the treatment. These bands may be due to the presence of lignin (the former due to C=O stretching and the latter to C-O and C-C stretching combined with C=C-H in-plane bending) (Fan, Dai, and Huang 2012). Their disappearance may be related to lignin degradation in the basic reaction medium, during the reaction course. The general characteristic of the modified material was to be insoluble in water.

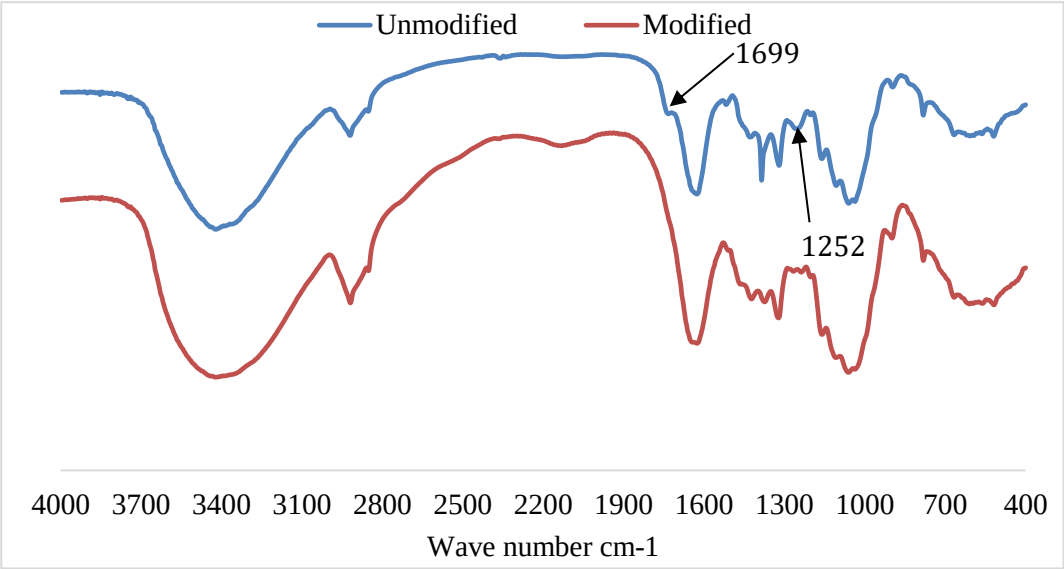

Figure 9: FTIR spectra of GTMAC modified and unmodified banana tree bark powder

The highest turbidity removal efficiency from the jar-test experiment was 26% with the addition of 500 mg/L of the new coagulant, while the lowest was 22.8% with the addition of 50 mg/L of coagulant (Figure 9). The low turbidity removal might be due to the very small number of cations on the surface of the coagulant. As stated above, this may due to the fact that cationization of the cellulose surface by GTMAC did not occur or occurred to a small extent. During cationization, GTMAC is consumed in a competition between two reactions: cationization of cellulose, and GTMAC hydrolysis. Zaman et al., (2012) reported that higher percentage of water affected the cationization of nanocellulose using GTMAC and concluded that the water content of the reaction system is critical for the cationization process in addition to the side reaction which is hydrolysis of the GTMAC in the presence of higher concentration of NaOH/KOH.

Initial turbidity: 115 NTU

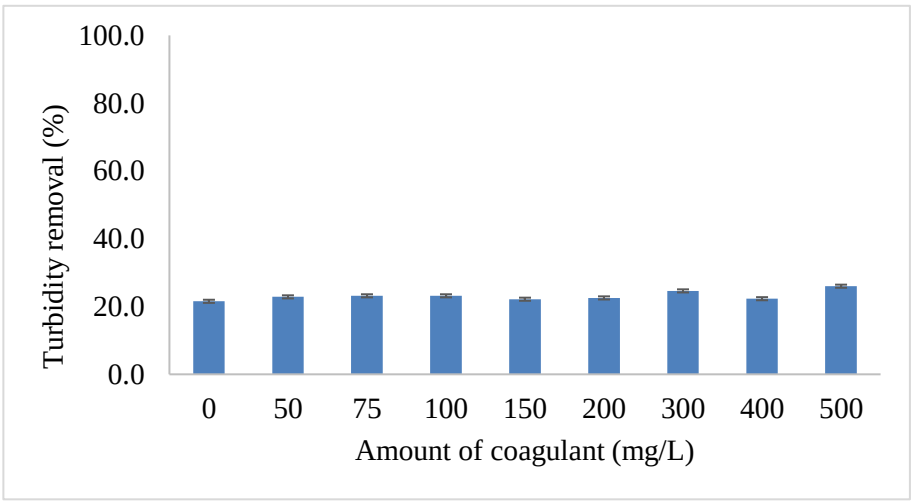

Figure 10: Turbidity removal of banana tree bark powder modified with GTMAC

3.6 Chemical modification of the physico-chemically treated banana bark

Based on the experiment reported on 3.5, the amount of water and concentration of kOH was reduced and the cellulose from the banana tree bark powder was produced by the method previously reported on 2.6.1. The FTIR spectra (Figure 10) show that the cellulose was effectively cationized by GTMAC since the band at 1479 cm^{-1} is now well visible. Moreover, the band at 897 cm^{-1} in the unmodified material broadened upon modification and the band at 1103 cm^{-1} also intensified. In addition, the change of zeta potential (ζ-potential) from -12.7 mV for the unmodified cellulose to +19.8 mV for the GTMAC modified cellulose reveals that the material's surface was effectively cationized. Similar to this result, Morantes et al., (2019) reported that cellulose nanocrystals were modified using 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC) and the zeta potential changed from -33 mV to +33 mV for unmodified and modified cellulose nanocrystals, respectively.

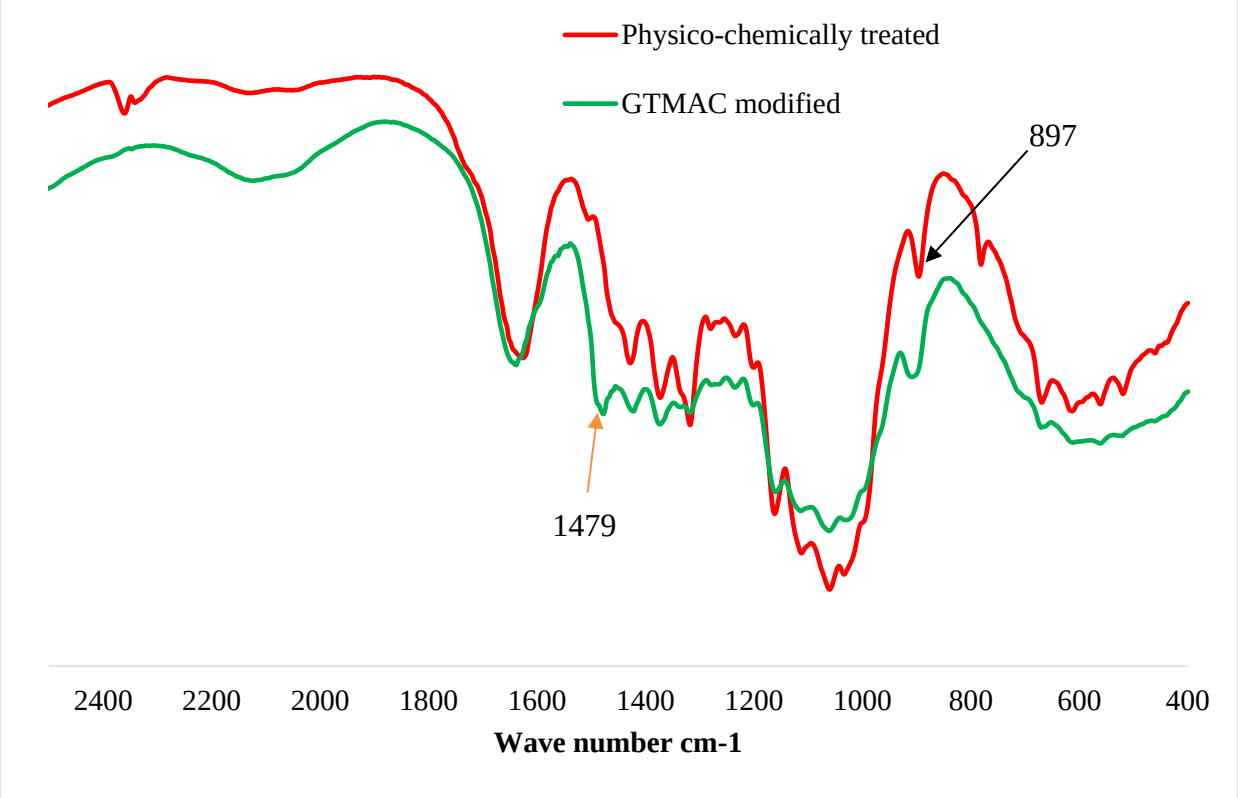

Figure 11: FTIR spectra of the physio-chemically treated banana tree bark powder pre and post-GTMAC modification

The efficiency to remove turbidity was evaluated using this new coagulant and results are in **Figure 12**. The highest turbidity removal obtained was 78.8% with 15 mL of coagulant suspension at pH 6.95 and the lowest 22.6 % without the addition of coagulant. DOC values

were 1592, 1591, 1923, 2244, 2901 and 13880 mg/L for 0, 5, 10, and 15 mL coagulant dose, respectively. These results show that when increasing the dose, DOC also increases, which may be due to the high carbon contents of the coagulant. On the other hand, the zeta potential measured for each sample shows the following results: 18.4, -20.1, -21.8, -21.3, and -20.8 mV for 0, 5, 10, and 15 mL coagulant dose, respectively. This shows that the coagulation flocculation mechanism might be bridging not a neutralization since the coagulant has a positive zeta potential (+19.8 mV) therefore, if it were neutralization the value of the tested samples should be neutral or positive, however, the reverse happened. Similar to this result was found by Joshi et al., (2019), where the zeta potential value of kaolin suspension was found to be −13.6 mV, indicative of negatively charged nature of kaolin particles. Whereas flocculated kaolin suspension showed zeta potential value of −27.7 mV, representing negative charge impartment by bioflocculant produced by *Bacillus licheniformis* NJ3. If the net charge of suspension (kaolin + bioflocculant produced by *Bacillus licheniformis* NJ3) had changed from negative to a positive value, the mechanism would be charge neutralization. But negative charge impartment might suggest a bridging mechanism of flocculation instead of charge neutralization.

Initial turbidity: 113 NTU

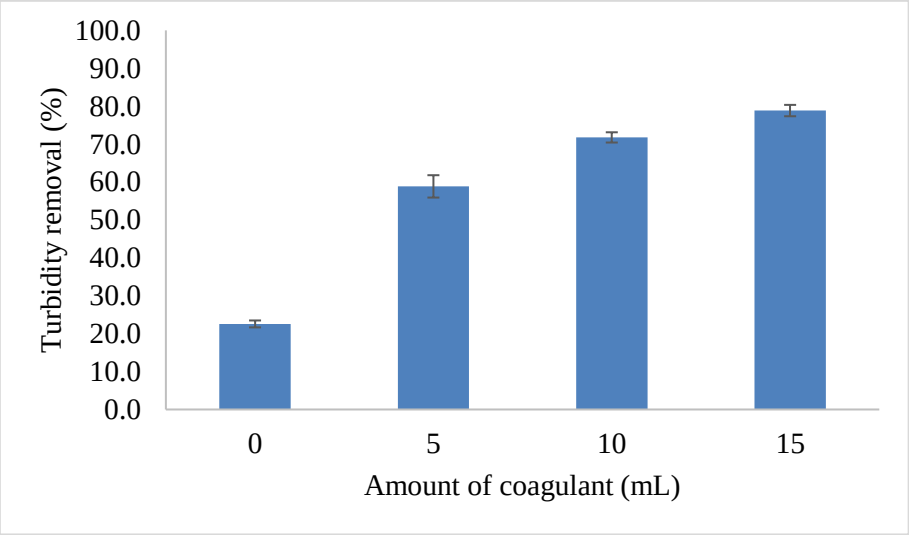

Figure 12: Turbidity removal of physico-chemically treated banana tree bark powder post-GTMAC modification

3.7 Case study: Wastewater from a carob processing factory

A wastewater of a carob processing factory from Algarve, Portugal, was used as case study. This wastewater presented the following characteristics: turbidity ranged from 92-98 NTU, pH 2-2.5 and COD from 300-350 mg/l. A commercial coagulant (WAC-AB®) and the optimized

natural coagulant (GTMAC modified physico-chemically treated banana tree bark powder) were tested for turbidity removal. The water pH was brought to near 7 by the addition of calcium carbonate prior to treatment.

3.7.1 Results from the commercial coagulant

Figure 13 shows that the highest turbidity removal of 83.6 % using 50 mg/l of the coagulant and a lowest removal of 8.3 % without using the coagulant were observed. There is no significant difference turbidity removal between replicates of all coagulant dose except at a coagulant dose 100 mg/L.

Initial turbidity 92 NTU

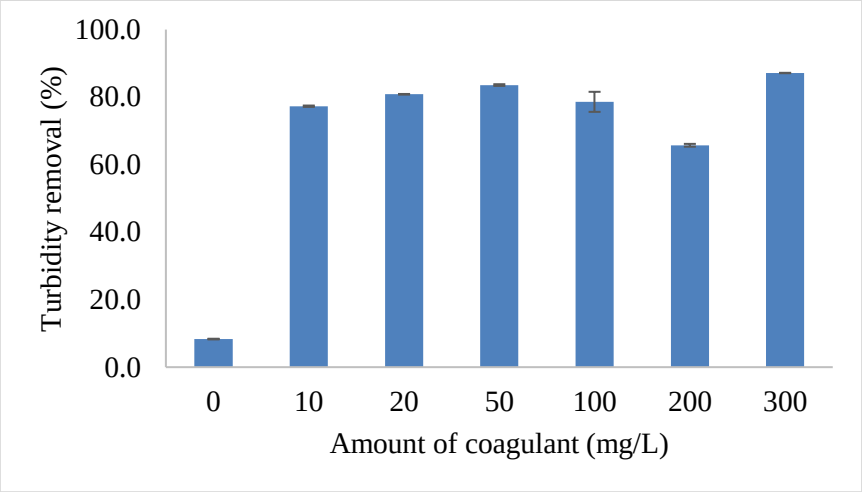

Figure 13: Turbidity removal from real wastewater (RWW) using commercial coagulant

3.8 Results from GTMAC modified physico-chemically treated banana tree bark power

Figure 14 shows that the highest turbidity removal of 79.8 % when using 15 mL of coagulant dose at 20-minute settling time was obtained, while when the settling time increases the turbidity removal increased to 86.5% at 80 minutes. This trend was observed for all tested coagulant doses. On the other hand, with the smallest coagulant dose, 71 % removal of turbidity was found at 20 minutes settling and when the time increased to 80 minutes a 76 % removal was achieved, which is almost near to the highest value obtained at the highest coagulant dose with the lowest settling time. This result might be the flocs which formed after coagulation/flocculation were soft and reluctant to undergo sedimentation. After this time (80

minutes), the highest removal rate exhibited could be ascribed to a density increase of the initially formed flocs. However, DOC values for the tested water increased with coagulant dose: 1219.7, 8206, 8579, and 8869 mg/L for 0, 5, 10 and 15 mL of added coagulant. This result shows that DOC increases when the coagulant dose increases, which might be due to the high carbon content of the bioflocculant. Generally, this is one of the shortcomings of natural coagulants (Okuda et al. 2001).

Initial turbidity: 94 NTU

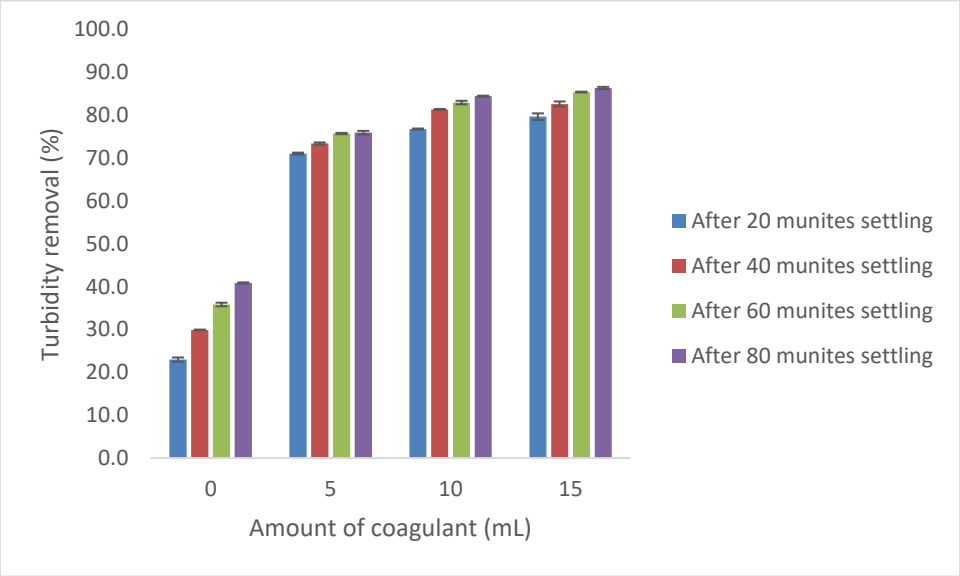

Figure 14: Turbidity removal from real wastewater using GTMAC modified banana tree bark at different time of sedimentation

4. Conclusions and future work

4.1 Conclusions

The conclusions of these work are:

- The selected plant materials in the form of powder did not work as coagulant/flocculant for the removal of turbid municipal wastewater (50-130 NTU);
- Physico-chemically treated banana tree bark powder with particle size between 25 and 100 µm showed a turbidity removal of 36.5%;
- The cellulose from the banana tree bark was successfully modified by GTMAC as confirmed by the FTIR spectra and zeta potential;
- Banana tree bark modified by GTMAC showed good coagulation/flocculation performance with the synthetic model wastewater (78.8% of turbidity removal) and real wastewater (79.7 % of turbidity removal).

In summary, it can be referred that the cellulose based coagulant, which are safe for the environment, produced represent a promising alternative for the treatment of municipal wastewaters, substituting the usual aluminum based and synthetic polyelectrolytes.

4.2 Future work

The present work is not definitive, and it leaves open many doors for future improvements. Regarding the synthesis of cellulose based coagulant, the proposals are:

- Characterize the starting material;
- Improve cellulose production process in order to increase the reaction yield;
- Improve the separation of the modified cellulose from the undissolved part to avoid contaminations of the product;
- Vary the molar ratio of GTMAC to anhydroglucose unit to understand its influence in the reaction;
- Verify the residual content of reagents in the final product;
- Improve the reproducibility
- Estimation of the costs of producing functionalized nanocelluloses.

Regarding the turbidity removal tests the proposals are:

- Test model wastewaters with other turbidities;

- Check the influence of the degree of substitution of the modified cellulose on turbidity removal efficiency;
- Study more deeply the influence of pH and cellulose from different plant source on turbidity removal.

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