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Application of Chitosan and Its Derivatives as Bioflocculants for Iron and Turbidity Removal from Filter Backwash Water

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Abstract: The present study aimed to investigate the potential application of chitosan (CS) and its two derivatives, dialdehyde chitosan (DCT) and carboxymethyl chitosan (CMC), as new flocculants in the purification of filter backwash water. The main objective of the work was to remove the disadvantages and inconveniences associated with using synthetic flocculants, mainly based on polyacrylamide, by using biodegradable and non-toxic biopolymer flocculants. The chitosan derivatives were obtained in the process of the chemical modification of this polysaccharide. CS was oxidated with periodate in acetic acid solution to obtain DCT with a degree of substitution (DS) of 44.29%. For the CMC synthesis, after the alkaline activation of chitosan, the solution was treated with monochloroacetic acid (MCA) at 60 °C for 3 h, leading to carboxymethylated chitosan formation with a DS of 49.55%. Characterizations of the flocculants using Fourier transform infrared (FT-IR) spectroscopy, ^{13}C nuclear magnetic resonance (^{13}C NMR) spectroscopy, and an elementary analysis confirmed the successful modification of the chitosan. The morphology and thermal stability of the samples were investigated using scanning electron microscopy (SEM) and a thermogravimetric analysis (TGA), respectively. Moreover, the biopolymer materials were studied in a series of flocculation jar tests for filter backwash water samples collected at a water treatment plant (WTP) in Kutno, Poland. CMC turned out to be a particularly effective agent in an optimal amount of 0.2 mg/L, achieving nearly 99% iron removal and 98.26% turbidity removal efficiency rates, which indicates that chitosan–based materials can be ecological alternatives for polyacrylamide flocculants, effectively removing the impurities of iron ions.

Keywords: flocculant; filter backwash water; chitosan; carboxymethyl chitosan; dialdehyde chitosan

1. Introduction

Coagulation is one of the most used methods for treating water contaminated with colloidal substances, including heavy metals [1].

In this phenomenon, chemical compounds called coagulants, which are usually aluminum or iron salts, are added to the water in a controlled dose, which causes the formation of agglomerates (flocs). Coagulation takes place in two steps. In the first step, after adding the coagulant, chemical and physical reactions take place, destabilizing the colloidal particles [2].

In the second phase, called flocculation, the molecules combine into larger agglomerates due to particle collisions. To remove a post-flocculation suspension from water, sedimentation is used in water treatment systems. Polymeric compounds (flocculants) capable of forming chemical bonds between colloid molecules are added to the solution to speed up this process. The polyelectrolyte's action mechanism consists of its adsorption on a destabilized colloid particle, which leads to the creation of larger and denser agglomerates. The effectiveness of the coagulation or flocculation depends largely on the selection of the doses of coagulant and flocculant and the technological parameters, mainly the mixing rate and the processing time [1–3].

An important factor significantly impacting the flocculation process is the pH of the solution, the optimization of which has been the subject of many detailed studies [4–9]. The pH influences the surface charges of contaminants, the solubility of impurities, colloid stabilization, as well as the agglomeration of flocs. Since the effectiveness of water purification depends on the type of flocculant, it should be remembered that its chemical structure may also change with pH (this is the case, for example, with chitosan, which dissolves in acidic environments, changing from a neutral to a cationic form). Moreover, the optimal pH range depends on the contamination type.

Saritha et al., testing various coagulants or flocculants depending on pH [4], found that the best results in removing color and haze were observed for alum (aluminum sulfate) at pH 7, for sago (a type of starch) at pH 6–7, and for chitin at pH 6–8. These authors also determined the optimal coagulant dose range (0.1–0.4 g/L), mixing speed, and time (a rapid step of 100 rpm for 10 min and slow step of 20 rpm for 20 min).

Naceradska et al. [5] observed that a neutral environment is most favorable for removing inorganic contaminants, while an acidic pH is better for removing organic matter from drinking water.

Studies on polyferric silicate sulphate (PFSS) and polyferric sulphate (PFS), used in the purification of Yellow River water, showed that both compounds act most efficiently in turbidity removal at pH 5.5 [6]. Additionally, polyferric chloride (PFC) showed the highest removal efficiency at a pH range of 5.50–5.75 [7].

Statistical and mathematical methods are applied to avoid repeated and long-lasting investigations when changing individual parameters influencing flocculation efficiency [8,9]. This approach was used in the study of an innovative hybrid copolymer, ferric chloride-(polyvinylpyrrolidone-grafted-polyacrylamide), proposed for turbidity and COD (chemical oxygen demand) removal in municipal wastewater [8]. The optimal pH (close to 7) and dose range (130–1590 mg/L) were found for this copolymer, which appeared to be a good alternative for conventional flocculants.

Another paper describes the study of wastewater over a wide range of pH values (from acidic to alkaline) [9]. To omit pH adjustment stages, the authors proposed selecting an alkaline pH. They concluded that by applying statistical analyses, it is possible to improve the real industrial process.

Polyacrylamide compounds are the most used polyelectrolytes for water treatment. However, due to the possibility of water contamination with toxic acrylamide monomers and oligomers, attempts are being made to find more ecological flocculants, of which biopolymers are becoming increasingly popular [10–13].

Among the natural polymers, the most attention is paid to polysaccharides such as starch [14], cellulose [15,16], chitosan, and their derivatives [17–25]. Moreover, alginates [26,27], lignin [28], cyclodextrin [29], xanthan, pectin, pullulan, and gelatine flocculants are also proposed [11]. They are biodegradable, easily available, and cheap, which justifies their use in technological processes in sewage treatment plants. However, they still show limited flocculation efficiency (lower than inorganic compounds or synthetic polymers). Therefore, better materials based on natural sources are sought.

Analyzing the latest literature on the use of chitosan in the water and wastewater treatment process [17–22], it can be stated that it is a biopolymer that contributes to the effective removal of various pollutants such as metal ions, anions (nitrates, phosphates), organic compounds (including hydrocarbons, dyes, humic substances, oil, and grease), as well as microorganisms (e.g., algae), which is mainly due to its chemical structure determining its valuable physicochemical properties.

Chitosan is a partially deacetylated derivative of chitin obtained from crustacean shells. It is a copolymer of glucosamine and N-acetylglucosamine (Figure 1) characterized by its biodegradability, non-toxicity, ease of modification, good absorption capacity, high affinity for metals and dyes, chemical stability, chelation capacity, and antibacterial properties. It is applicable in many industries, from medicine through pharmacy and material sciences to environmental protection [30–34].
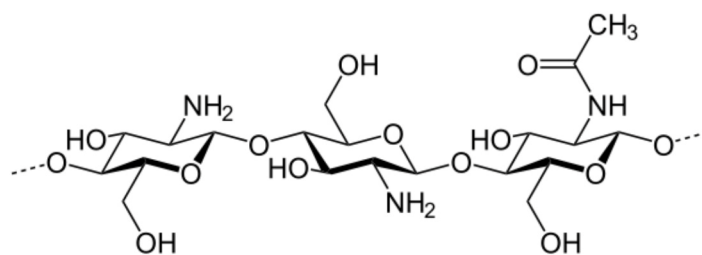
Figure 1. Chemical structure of chitosan copolymer of D-glucosamine and N-acetyl-D-glucosamine.

Despite its many advantages, chitosan is not soluble in aqueous solutions (except for acidic solutions, where due to the protonation of amino groups it takes the form of a polycation), limiting its direct application in numerous areas. The solution to this problem is to modify the chemical structure of the chitosan. The applied modification methods include thermal treatment or the use of electric and magnetic fields, which cause changes in the structure of this biopolymer. Most often, however, chemical modification is used, leading to the introduction of new functional groups or their transformation, intramolecular cyclization or the opening of glucoside rings, oxidation, or reduction [35–37]. Numerous studies were conducted to improve the solubility of chitosan and facilitate its application for water and wastewater treatments, mainly via grafting [38–40], the cross-linking of polymer chains [41], and etherification [41,42].

The carboxymethylation of chitosan leads to the formation of its water-soluble, amphiprotic ether derivative with -COOH and -NH$_2$ groups in the molecules [43]. The presence of these active sites in the polysaccharide backbone enhances their adsorption properties [44,45], which determines their potential application in the process of aqueous colloidal solution purification.

The new concepts for the design of innovative flocculation materials include, among others, the ultrasonic-initiated graft copolymerization of chitosan with acrylamide in the presence of kaolin, which is particularly efficient in the removal of acid blue 83 dye [22]; the preparation of chitosan-containing ferromagnetic nanoparticles, which have proven effective in removing nitrate, fluoride, and phosphate anions from aqueous solutions [23]; the synthesis of a flocculant based on carboxylated chitosan using a photochemical method [24]; the preparation of a hybrid adsorbent composed of chitosan doped with low-cost fly ash (exhibiting 98–100% removal efficiencies for Zn and Pb cations) in the alkali-activated process [34]; and the grafting of 3-chloro-2-hydroxypropyl trimethylammonium chloride onto carboxymethyl chitosan, which is effective in the removal of turbidity and phosphates [46].

It is worth noting that chitosan not only effectively removes organic and inorganic contaminants but also pathogens due to its bactericidal effect. An example is the removal of the pathogen *Edwardsiella ictalurid* while simultaneously maintaining the nitrifying bacteria in aquaculture wastewater [47].

Moreover, chitosan-based flocculants are used to remove dangerous algae appearing seasonally in some lakes and rivers, causing eutrophication, which is a serious threat to the lives of fish and other organisms living in these environments, and also is harmful to humans [48,49]

Recently, chitosan has been proposed for the harvesting of microalgae (*Chlorella* sp.) from biomass to obtain green fuels replacing petroleum [50].

It should also be added that in the process of biosynthesis, valuable polysaccharides of various compositions and promising flocculation properties can be obtained. The biopolymers produced by microorganisms, secreted outside cells, apart from exopolysaccharides, additionally consist of proteins, glycoproteins, humic acids, lipids, and nucleic acids. However, this is a complicated, multi-stage process involving organic precursors and enzymes, which results in high production costs [11,51].

The selected papers cited above show that despite the intensive research on biopolymer flocculants, a universal material that meets the requirements of modern water treatment

technologies has not yet been found. In particular, high efficiency in removing various pollutants (suspended solids, colloids, or dissolved impurities, even in trace amounts or in the form of very fine particles) at a relatively low dose is needed, regardless of the conditions (i.e., over a wide range of pH and temperature values). Moreover, for application on an industrial scale, a simple method of obtaining and using agents that are safe for people and do not burden the environment, a high rate (i.e., short time) of water purification, and an affordable price is expected. Obtaining the desired flocculation properties must be based on the possibly comprehensive characteristics of the designed materials. Hence, further research is necessary to determine the relationship between the structure and properties.

In this work, the possibility of using chitosan-based materials as flocculants for a filter backwash water treatment was investigated. Carboxymethyl chitosan (CMC), obtained from the etherification reaction of low molecular weight chitosan, was proposed for a new flocculation material, whose properties were compared with dialdehyde chitosan (DCT) and unmodified chitosan. The structure and properties of all compared biopolymers were characterized by spectroscopy (FTIR, NMR), scanning electron microscopy (SEM), an elemental analysis, and thermogravimetry. Furthermore, the prepared materials were used as flocculants in the purification process of filter backwash water (FBW) contaminated mainly with iron compounds. The use of an ecological biopolymer—carboxymethyl chitosan—as a flocculant in the purification of filter backwash water is a scientific novelty.

2. Materials and Methods

2.1. Materials

The chitosan (100–300 cps) (low molecular weight, 90% deacetylated; CAS 9012-76-4) was purchased from CHEMAT (Gdańsk, Poland). The acetic acid, propane-2-ol, and methanol of reagent grade were obtained from Chempur (PiekaryŚląskie, Poland). The acetone of reagent grade was from Avantor Performance Materials Poland S.A. (Gliwice, Poland). The sodium periodate and chloroacetic acid were purchased from Sigma-Aldrich (Munich, Germany). The synthesis and characteristics of the bio-based flocculants were assessed at the Faculty of Chemistry, Nicolaus Copernicus University (NCU) in Toruń, Poland.

The commercial flocculant Superfloc A100PWG, produced by Kemira Company (Helsinki, Finland), was used for comparison.

2.2. Water Samples

The research involved the use of filter backwash water (FBW), rich in contaminating iron compounds remaining after the water treatment process at the water treatment plant (WTP) in Kutno, Poland. In this process, water is aerated and ventilated, leading to its saturation with oxygen and a simultaneous increase in the pH value. This catalyzes the oxidation of iron compounds dissolved in water to form Fe^{3+}, which precipitates and can be separated on sand filters.

The precipitated sludge is then washed out of the sand grains, and together with the washing water, goes through the treatment process based on the phenomenon of coagulation and flocculation. FBW is a suspension of colloidal compounds of iron hydroxides with a surface charge that hinders their effective coagulation. For this reason, a coagulant is added to the water. In the case of the WTP in Kutno, it is an aluminum coagulant in the form of hydrolyzed polyaluminum chloride, which neutralizes the impurities' charge. Coagulation causes oxidation of the outer layer of the colloid, and neutralization of the charge is possible due to the use of rapid mixing. In practice, the mixing speed is most often 250 rpm, which enables coagulation to take place within a few seconds. Due to the large load carried with the purified washing water (the amount of iron compounds determined at the level of approximately 30 mg/L), a polymer flocculant is added to the water to accelerate the agglomeration process, reducing at the same time the dose of the aluminum coagulant used and causing the formation of larger and more compact agglomerates that are easy to remove.

For measurement, one liter samples of filter backwash water (FBW) were taken each time at the water treatment plant (WTP) in Kutno, Poland.

2.3. Coagulants and Flocculants—Preparation for Application

The effectiveness of the polysaccharide-based flocculants was evaluated with the presence of PAX XL10 hydrolyzed polyaluminum chloride in the amount of 1 mg Al^{3+}/L as a primary coagulant.

2.4. Synthesis of Chitosan Derivatives (DCT and CMC)

The dialdehyde derivative of chitosan (DCT) was obtained via oxidation with sodium periodate ($NaIO_4$) in an acetic acid solution, according to procedures described in our previous research [52].

For the CMC synthesis, three grams of chitosan was dispersed in propane-2-ol and mixed with an appropriate quantity of 30% aqueous sodium hydroxide (NaOH) solution, then blended for one hour. Further, monochloroacetic acid (MCA) was added in certain portions (3.60 g, 38.09 mmol), and the solution was mixed using a magnetic stirrer at 60 °C for 3 h. The post-reaction solution was decanted and the remaining precipitate was rinsed with 80% methanol, then with anhydrous methanol twice, and vacuum-dried at 40 °C. The obtained product was carboxymethylated chitosan. A simplified scheme of the CMC preparation process is shown in Figure 2 below.
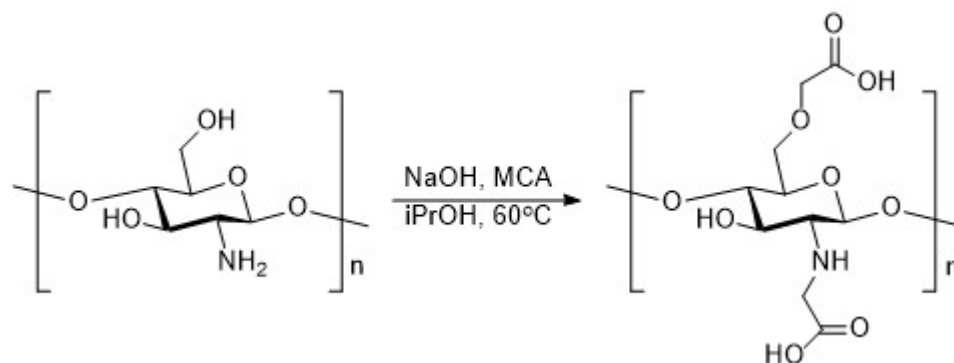

Figure 2. Scheme of CMC synthesis from chitosan.

The chemical structure of the synthesized chitosan derivatives was confirmed by FTIR and ^{13}C-NMR spectroscopy.

2.5. Degree of Substitution (DS)

The degree of substitution of the chitosan derivatives was calculated using the titration method. For the carboxymethyl chitosan, the DS was calculated using Equation (1), and for dialdehyde chitosan, Equation (2) was used, as presented below:

$$DS_{CMC} = 161A/(m_{CMC}/59A) \tag{1}$$

where $A = V_{NaOH} \times C_{NaOH,}$

$$DS_{DCT} = (A-B)/(m_{DCT}/161) \tag{2}$$

where $A = V_{NaOH} \times C_{NaOH}$, $B = V_{HCl} \times C_{HCl}$; V_{NaOH} and C_{NaOH} are the volume and molarity of the aqueous NaOH solution, respectively; V_{HCl} and C_{HCl} are the volume and molarity of aqueous HCl, respectively; m_{CMC} is the mass of CMC (g); m_{DCT} is the mass of the DCT sample (g); 161 and 59 are the molecular weights of the anhydroglucosamine unit (AGU) in the polymer backbone and the carboxymethyl group, respectively.

A sample of DCT (0.1 g) and NaOH solution (5 mL, 0.25 M) was placed in a 200 mL volumetric flask and heated in a 60 °C water bath until dissolved. After cool-

ing, the HCl solution (7.5 mL, 0.25 M) and distilled water (15 mL) were added. Afterward, the phenolphthalein solution was added as a pH indicator and the sample was titrated with the NaOH solution (0.25 M).

The CMC (0.1 g) was dissolved in distilled water (heated in 60 °C water bath) with the addition of HCl solution (0.1 M) to reach pH < 2. Then, the sample was titrated with NaOH solution (0.1 M) using phenolphthalein as an indicator.

2.6. Characterization of Chitosan and Its Derivatives

An elemental analysis (i.e., the determination of the percentage shares of carbon, hydrogen, and nitrogen) was carried out using a Vario MACRO CHN ELEMENTAR microanalyzer (Analysensysteme GmbH Hanau/Frankfurt, Germany).

The FTIR spectra of the polysaccharides were captured on a Perkin Elmer Spectrum Two^{TM} FT-IR Spectrometer (Perkin Elmer, Waltham, MA, USA) with an ATR tool equipped with a diamond crystal. The scanning range was 400–4000 cm^{-1}.

The ^{13}C-NMR spectra in a solid state were recorded on a Bruker Avance III 700 MHz spectrometer. A data analysis was carried out using TopSpin 3.6.2 software.

The morphologies of chitosan, DCT, and CMC were studied with the help of a scanning electron microscope (LEO Electron Microscopy Ltd., Cambridge, UK, model 1430 VP) without sputtering.

The thermal properties of the polysaccharides were investigated using a TA Instruments Discovery SDT 650 Thermoanalyzer (TA Instruments, New Castle, DE, USA). The samples were heated at a constant rate of 10 °C/min up to 600 °C under a nitrogen atmosphere.

2.7. Jar Tests

A jar test is a standard method to simulate a full-scale water treatment process. The use of this method allows for the determination of which coagulant or flocculant works best and in what dose it should be used.

The jar test procedure consists of:

— Placing the samples of the tested water in equal amounts (1 L of the sample) in the beakers;
— Dosing each beaker with a different dose of the appropriate chemical compound, i.e., the flocculant;
— Setting the appropriate mixing speed, which should reflect the conditions prevailing in a given water treatment system (usually these values oscillate around 250 rpm for fast mixing and 25–30 rpm for slow mixing);
— Subjecting the water in the beakers to the sedimentation process at a time corresponding to the actual process in the WTP;
— Taking a sample from each beaker for the analysis, the results of which allow for determination of the optimal parameters of the water purification process [53].

In our studies, jar tests were employed to evaluate the flocculation properties of chitosan-based bioflocculants. The studies were conducted using a JLT6 VELP Scientifica flocculation tester (VELP Scientifica, Bohemia, NY, USA). The experiment simulated the standard procedures engaged at the water treatment plant (WTP) in Kutno. For each sample, the total iron concentration and turbidity were measured. The iron content in each water sample was determined using the colorimetric method with 1,10-phenanthroline, and the nephelometric method was used for the turbidity assessment.

The flocculation jar tests were carried out at the water treatment plant (WTP) in Kutno, Water Supply and Sewage Enterprise LLC (Kutno, Poland).

2.8. Determination of Total Iron Concentration and Turbidity of Water Samples

The total iron concentration (IR) caused high turbidity (TR) in the water samples, so these parameters were used in the flocculation test to calculate the removal efficiency according to Equations (3) and (4) below:

$$TR = [(T_A - T_T)/T_A] \times 100\% \tag{3}$$

$$IR = [(Fe_A - Fe_T)/Fe_A] \times 100\% \tag{4}$$

where TR is the turbidity removal efficiency; T_A and T_T are the average turbidity values of raw FBW and treated water, respectively; IR is the iron removal efficiency; Fe_A is the average iron concentration of FBW; and Fe_T is the iron concentration after treatment. Each trial was repeated at least three times. The same batch was used for the compared results.

The turbidity of the sample was determined on a Hach 2100Q Portable Turbidimeter (Hach, Ames, IA, USA). Here, 10 mL of a sample was taken from each beaker with a measuring pipette and placed directly in a glass cuvette for each measurement. Before the examination, a calibration was performed using standard formazine solutions (TABLCAL Formazin Standard) with specified turbidity rates of 10, 20, 100, and 800 NTU. The analysis was carried out according to the Hach procedures approved by the United States Environmental Protection Agency (USEPA) [54,55].

The total iron concentration was determined on a Hach DR1900 spectrophotometer (HACH LANGE GmbH, Berlin, Germany) in accordance with the USEPA FerroVer® method. The procedure includes the following steps:

- Selecting the measurement method and setting the wavelength to 510 nm;
- Collecting 10 mL from the water samples in a measurement cell;
- Adding the reagent (FerroVer Iron Reagent Powder Pillow) to the sample cell;
- Mixing the solution followed by standing for 3 min;
- Performing a blank measurement without the addition of a reagent (i.e., the total iron concentration in mg/L);
- After the required time, the cell is placed in the cuvette holder and the reading starts
- The device performs several measurements of the placed sample and displays the finished result.

The reagent is a mixture of 1,10-phenatroline derivative and a reducing agent, which converts the iron ions to Fe^{2+}. Fe^{2+} forms an orange complex with 1,10-phenantroline, absorbing light at 510 nm. The full composition of the iron determination reagent is sodium thiosulfate, disodium bisulfate (IV), sodium hyposulfite, and 1,10-phenanthroline-p-toluosulfonacid salt.

3. Results

3.1. Synthesis of Chitosan Derivatives

The dialdehyde derivative of chitosan (DCT) was obtained by employing methods described in our previous work [52]. The reaction leads to the formation of two aldehyde groups in the anhydroglucose unit (Figure 3).
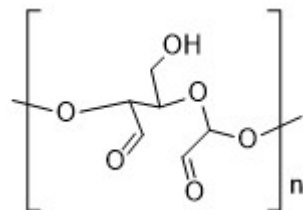

Figure 3. Chemical structure of the DCT unit.

Carboxymethyl chitosan (CMC) was synthesized via the etherification of chitosan with chloroacetic acid. The aqueous NaOH solution causes the complete dissolution of chitosan, and simultaneously its activation facilitates carboxymethylation. As a result of the penetration of the alkali into the polysaccharide structure, internal hydrogen bonds are broken, which leads to an increase in the number of available hydroxyl groups (-OH) [56]. Polymer activation enables the attachment of carboxymethyl (-CH₂COOH) groups on both amino and hydroxyl groups of chitosan, resulting in O-carboxymethyl chitosan (O-CMC)

(Figure 4), N-carboxymethyl chitosan (N-CMC) (Figure 5), or N,O-carboxymethyl chitosan (N,O-CMC) (Figure 6). Further spectroscopic characterization confirmed the possibility of the formation of the structures shown in Figures 4–6.
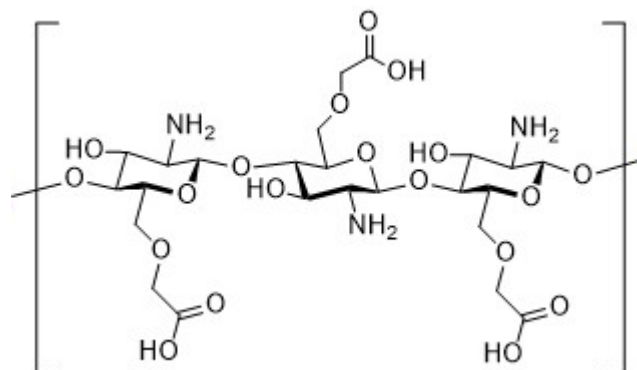

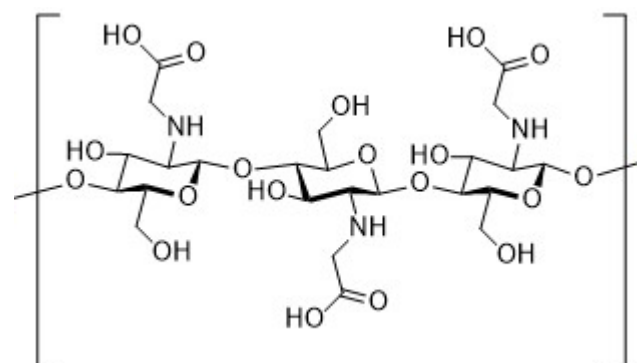**Figure 4.** Chemical structure of O-carboxymethyl chitosan.

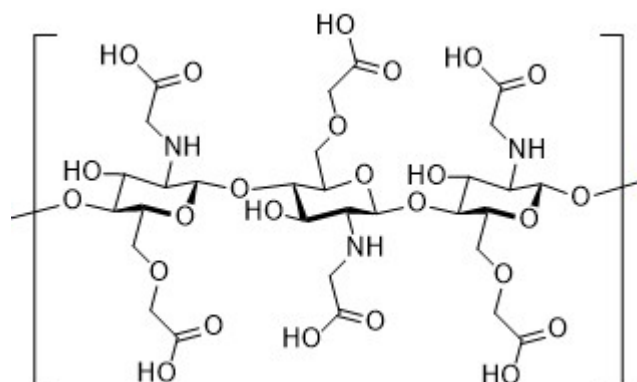**Figure 5.** Chemical structure of N-carboxymethyl chitosan.

Figure 6. Chemical structure of N,O-carboxymethyl chitosan.

3.2. Degree of Substitution and the Elemental Analysis

The degree of substitution can be defined as the average number of substituted functional groups per one anhydroglucose unit (AGU) of polysaccharides. For these assays, we assumed that chitosan consisted only of glucosamine rings. Table 1 shows the percentage shares of the constituent elements of native chitosan and its two derivatives. Based on these data, the empirical formulas of the obtained products were determined. A significant increase in the oxygen content, and at the same time a decrease in the nitrogen content in the CMC in relation to the starting chitosan, is proof of the effective modification

and introduction of carboxymethyl substituents in the place of hydroxyl and amino groups. In the case of DCT, an increase in O and a drop in the N amount can also be observed, resulting in glucosamine ring opening.

Table 1. Percentages of chitosan, DCT, and CMC constituent elements and degrees of chitosan substitution in DCT and CMC.

Sample	Theoretical Values [%]				Elemental Analysis [%]				Empirical Formula	DS [%]
	C	H	N	O	C	H	N	O		
Chitosan	44.72	6.83	8.70	39.75	40.57	5.21	7.07	47.15	$C_{6.6}H_{10.2}NO_{5.8}$	-
DCT	45.00	5.00	0.00	50.00	29.52	3.75	3.86	62.80	$C_{8.8}H_{13.4}NO_{14}$	44.29
CMC	42.86	5.36	4.17	47.62	35.40	5.99	5.12	53.49	$C_8H_{16.2}NO_9$	49.55

The determined degrees of substitution were about 44% and 50% for DCT and CMC, respectively.

3.3. FTIR Spectra

ATR-FTIR spectra of chitosan, CMC, and DCT are shown in Figure 7. In the recorded spectrum of chitosan, characteristic peaks can be observed for the stretching vibrations of the glycosidic bond (C-O-C), with the maximum at 1052 cm^{-1}. Furthermore, there are absorption bands for C-H stretching vibrations at 2837 cm^{-1} and broad bands corresponding to OH groups (3200–3500 cm^{-1}). For the dialdehyde derivative, the presence of a sharp peak at 1630 cm^{-1} confirms the oxidation of polysaccharides [52,57]. In addition, the intensity of the CH bands at 2837 cm^{-1} as well as the band in the range of 1000–1100 cm^{-1} attributed to a glycosidic bond (C-O-C) decreases. Moreover, new strong bands can be observed in the fingerprint region at wavenumbers of 785–723 cm^{-1}, confirming the glucoside ring's opening. The band attributed to the carbonyl group vibrations is very weak and almost imperceptible, which could be related to the formation of hemiacetals or imine groups with not fully oxidized polymer molecules. This observation is similar to a previously described study [52].
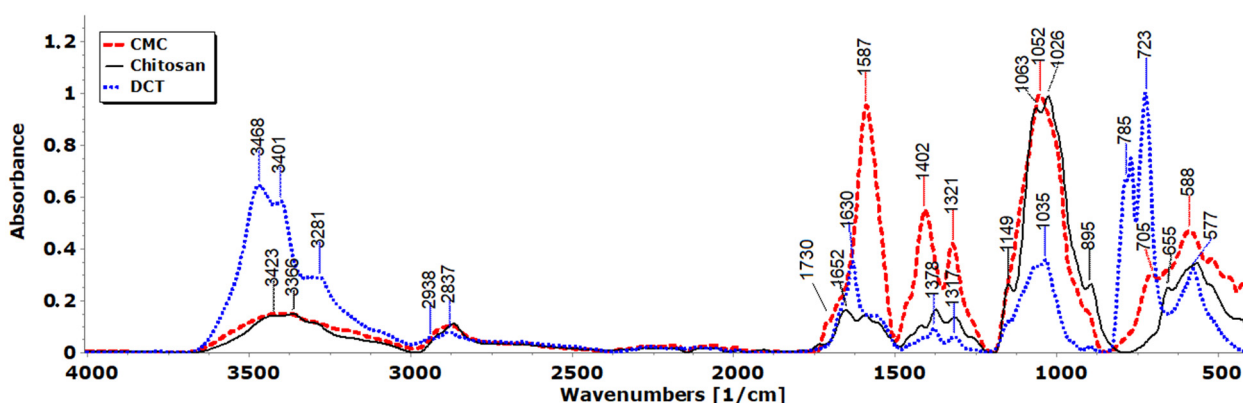

Figure 7. FTIR spectra of chitosan, DCT, and CMC.

For the CMC spectrum, the most characteristic feature is the absorption band at about 1730 cm^{-1} of the -COOH group [58,59]. However, for the synthesized carboxymethyl chitosan, this band is shifted to 1587 cm^{-1}, which is typical for sodium salt of CMC, when –COOH becomes –COO$^-$Na$^+$ [43]. Another characteristic band occurs at 1402 cm^{-1} due to the N-carboxymethylation of chitosan. The results indicate both N-carboxymethylation and O-carboxymethylation of the chitosan (N,O-CMC) [60].

3.4. ^{13}C NMR Data Analysis

Figure 8 presents the ^{13}C NMR spectra of chitosan, CMC, and DCT. In the unmodified chitosan's spectrum (Figure 8a), signals at 105.3, 82.5, 75.3, 60.8, and 56.9 ppm are attributed to the C-1, C-4, C-3,5, C-6, and C-2, respectfully, in the chitosan chain's [61]. The most characteristic signal in the DCT spectrum occurs at 177.5 ppm and comes from carbonyl groups, confirming the chitosan's oxidation (Figure 8b). Moreover, two signals at 22.4 and 31.0 ppm can be attributed to the carbons from CH_2 groups after glucopyranose ring opening during oxidation. For the CMC spectrum (Figure 8c), signals at 105.7, 83.0, 74.5, and 61.2 ppm are attributed to carbons in the glucopyranose ring. Chemical shifts at 177.5 ppm and 57.6 ppm are assigned to –CH_2COOH groups, verifying the carboxymethylation of chitosan [62].

3.5. SEM Images

The morphological structures for the derivatives of chitosan are shown in Figure 9. The SEM images at different magnifications (200×, 1000×, 10,000×) present changes in the architecture of the investigated compounds.

The native chitosan forms irregular aggregates of varying sizes. They are organized in flake-shaped structures with lengths of 50 to 300 μm (Figure 9a).

The chitosan oxidation causes fragmentation of the particles. The images of the DCT revealed the emergence of irregular granular groupings and spindle structures (Figure 9b). The change in the spatial arrangement of the dialdehyde chitosan particles increased their surface area, improving the adsorption properties of the DCT.

Partial fragmentation can also be seen in the morphology of carboxymethylated derivative of chitosan (Figure 9c). The particles take an irregular form similar to native chitosan, with sizes varying from 50 μm to 200 μm. The introduction of carboxymethyl groups increases the surface roughness of the particles.

The observed morphology changes may improve the flocculation properties of the tested samples. This is mainly due to an increase in the adsorption surface, which is rich in reactive hydroxyl and carboxymethyl groups capable of binding metal ions present in water.
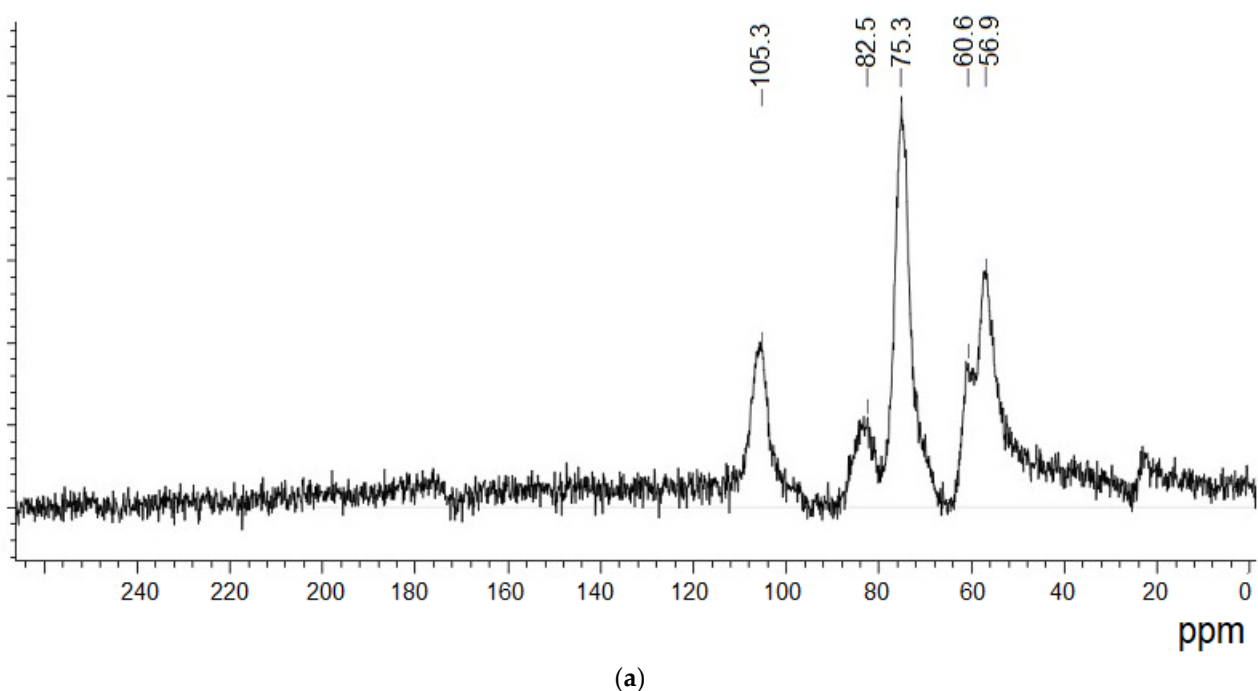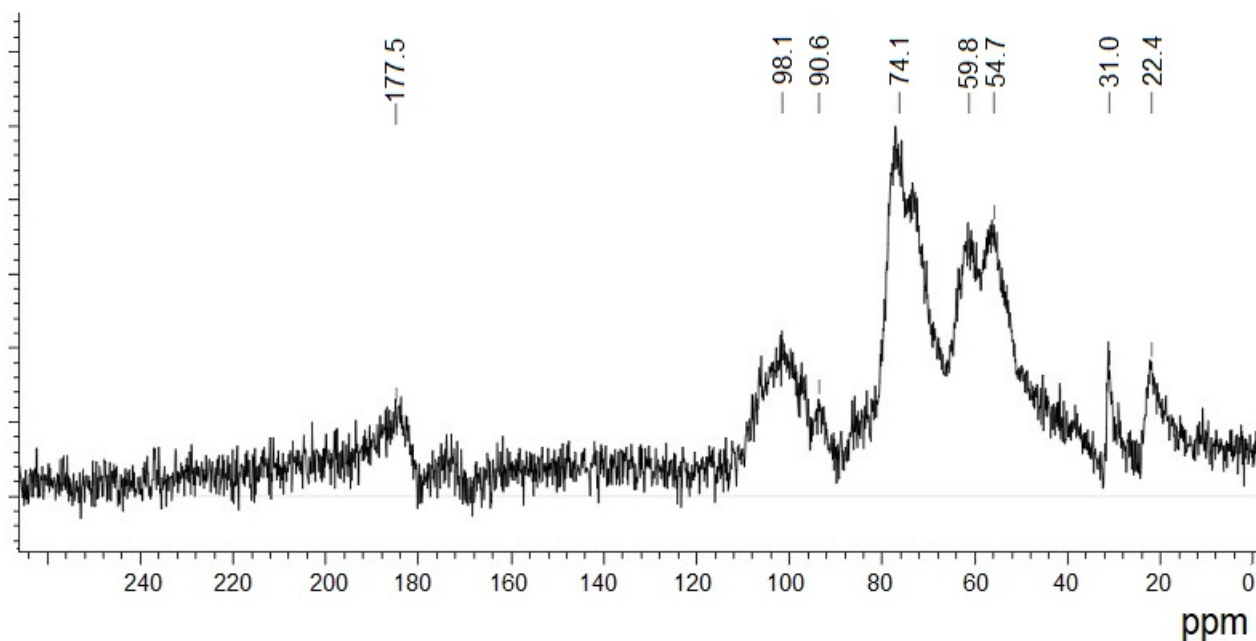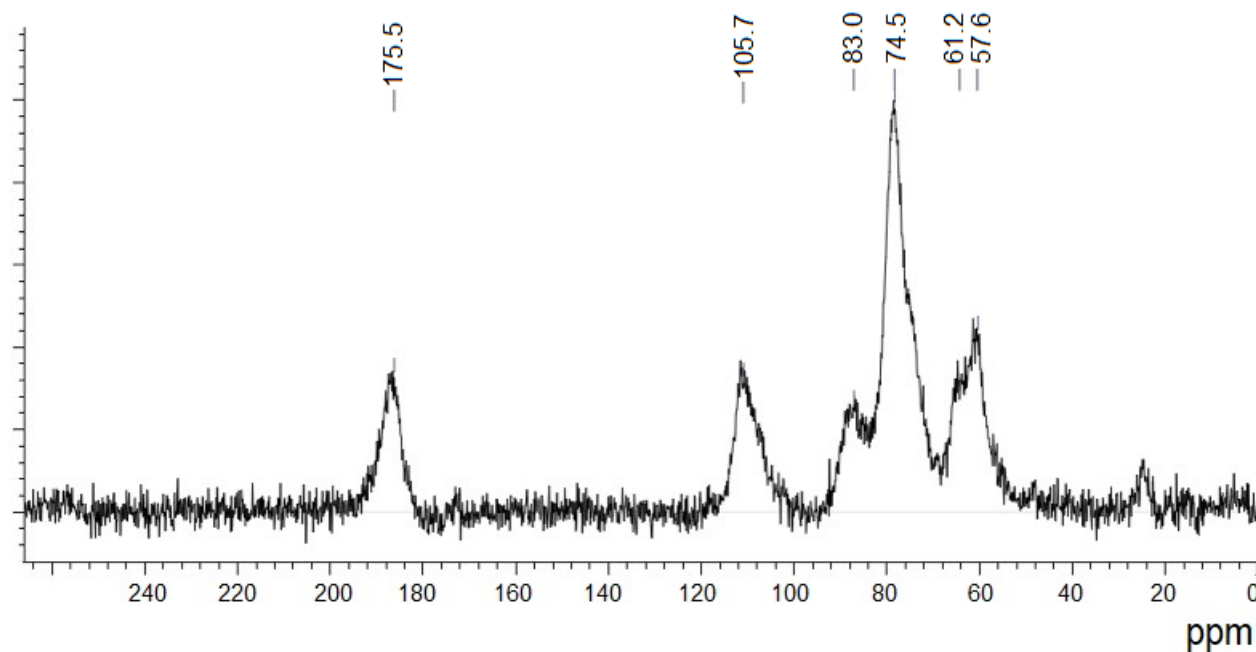

(a)

Figure 8. *Cont.*

(b)

(c)

Figure 8. ^{13}C-NMR spectra of chitosan (**a**), DCT (**b**), and CMC (**c**).

3.6. Thermal Analysis

The thermogravimetric technique was used to determine the thermal stability of chitosan and its derivatives. This method also provides valuable information about the structures of the obtained chitosan derivatives and confirms successful chitosan modification.

The TGA and DTG curves recorded for the tested compounds are shown in Figure 10. The initial weight losses are associated with the evaporation of physically adsorbed water molecules, and in all samples these range from 6% to 12% at 100 °C. The main degradation stage for native chitosan began at about 242 °C and achieved a maximal thermal decomposition rate at 269 °C. The weight loss of this step was approximately 71% (Figure 10a).
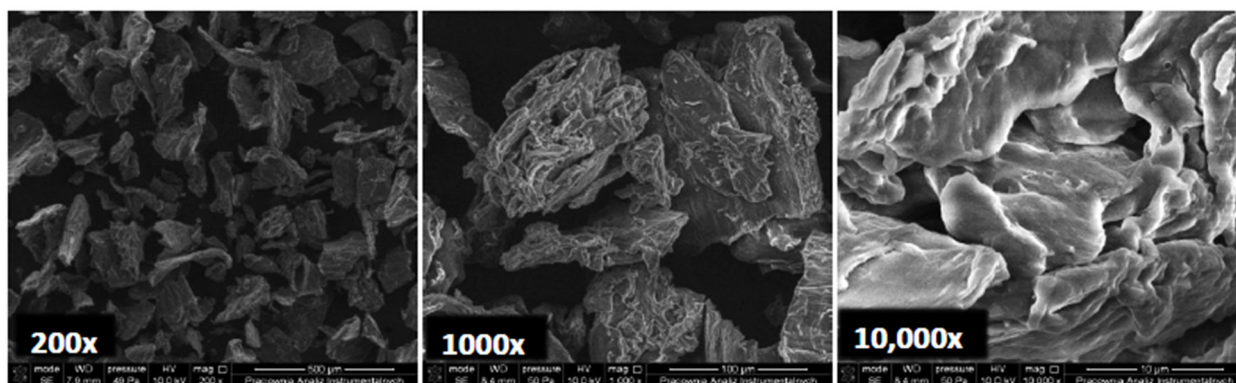
(a)
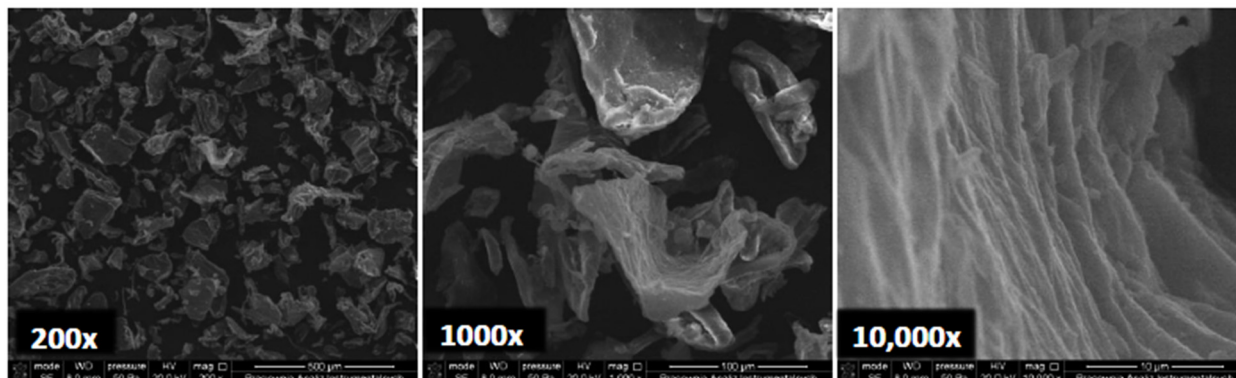
(b)
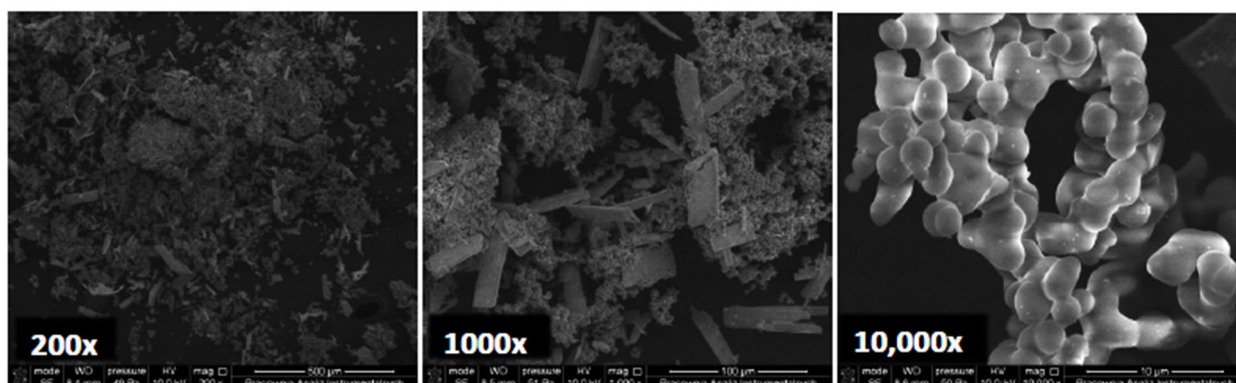
(c)

Figure 9. SEM images of chitosan (**a**), dialdehyde chitosan (**b**), and carboxymethyl chitosan (**c**) at different magnifications.

The course of DCT's thermal degradation differs significantly from unmodified chitosan. Systematic weight loss during sample heating up to about 300 °C can be observed. Initially (i.e., at lower temperatures), this is associated with the evaporation of water and then with the gradual decomposition of macromolecules. The main decomposition peak occurs at 313 °C (at this temperature, the rate reaches the highest value among the 3 tested samples of 2.7%/°C).
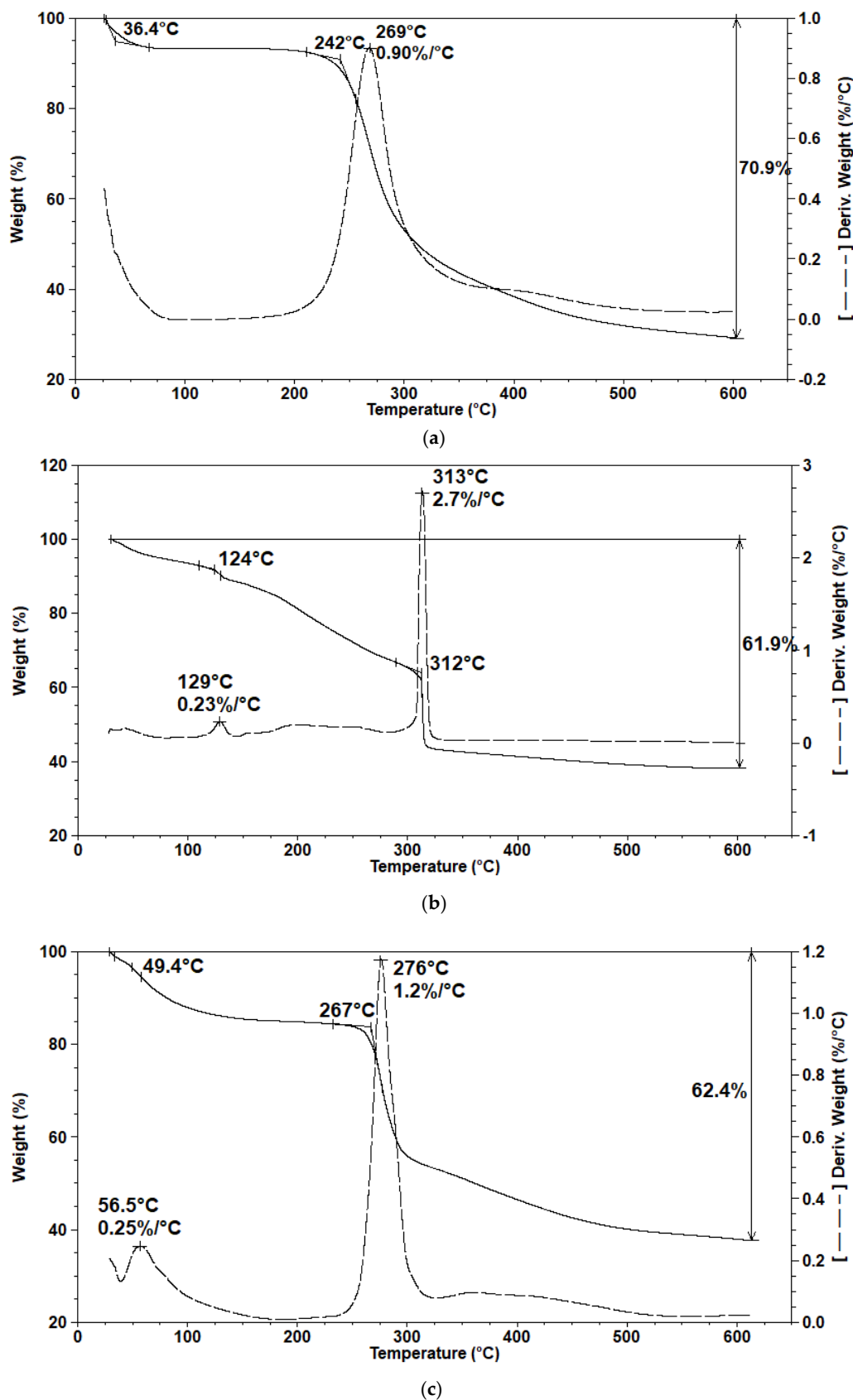
Figure 10. TGA-DTG curves for (**a**) chitosan, (**b**) DCT, and (**c**) CMC.

In the CMC sample, the main thermal transformation occurs at 276 °C, with a 1.2%/°C degradation rate. The shape of TG is also different (as in DCT) than that of chitosan alone,

which clearly proves the successful chemical modification of chitosan. Notably, the carbon residue measured at 600 °C is about 10% higher in both modified samples than in pure chitosan. This may be due to the ability to form stable cross-linked structures formed during the heating of DCT and CMC due to the presence of carbonyl groups, which are absent in the initial chitosan.

3.7. Iron and Turbidity Removal Evaluation

A flocculation test procedure, called the jar test, determines how much flocculants need to be added to a contaminated medium to induce floc formation. The flocculation capability of bio-based materials was specified in a series of jar tests. The experiments were carried out to simulate practical standards. The WTP in Kutno uses the technology of a continuous sand filter, in which the filter bed rinsing process takes place in parallel with filtration. Continuously generated backwash water constitutes nearly 15% of the total treated water. To minimize water losses, the FBW undergoes a purification process, in which after the sedimentation on the lamellar settling tank, it is returned to the beginning of the water treatment system, where it mixes with oxygenated raw water directed to the filters. The purification process starts with the addition of an electrolyte in the form of polyaluminum chloride (PAC) with an amount of 1 $mgAl^{3+}$/L, causing the destabilization of colloidal particles suspended in FBW. Next, a polymeric flocculant is dosed to form well-defined flocs that settle out from the water. The jar test experiments were designed to reflect this real scale conditions, as illustrated in Figure 11.
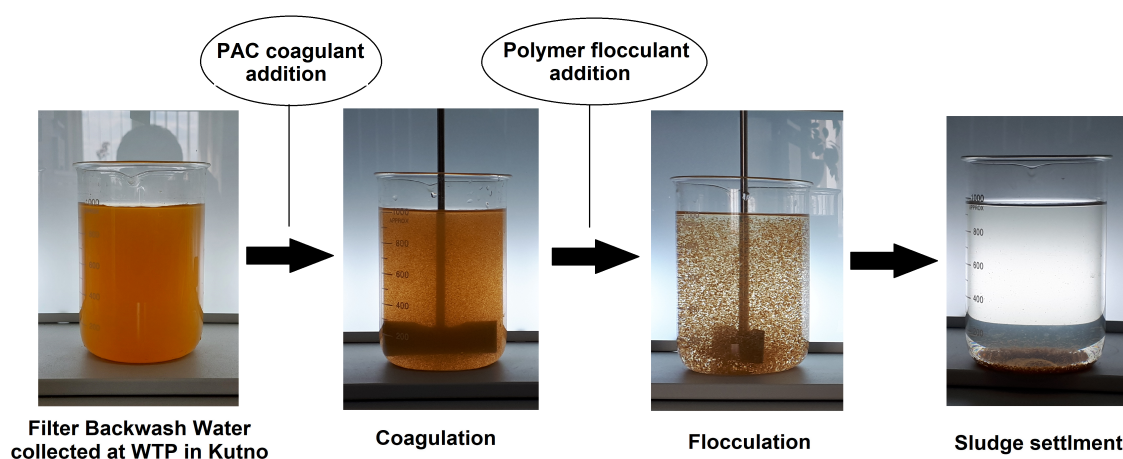

Figure 11. Illustration of the jar test.

The water samples (FBW) were taken directly from the collector pipe that discharges filter backwash water at the WTP in Kutno, Poland. Before the tests, basic FBW parameters were determined (Table 2), and the average iron concentration of 29.59 mg/L and turbidity of 260.33 NTU were used to calculate the flocculation efficiency.

Table 2. The average values of raw water and the FBW's basic parameters at the WTP in Kutno.

Water Sample	Total Iron Concentration [mg Fe/L]	Turbidity [NTU]	Temperature [°C]	pH
Raw ground water	5.41	6.48	9.80	6.94
Filter Backwash Water	29.59	260.33	13.40	7.56

In our research, we compared the flocculation capability of bioflocculants in amounts from 0.1 to 2.0 mg/L. The tests were carried out with a specific timing and stirring rate regime, as well as with the sedimentation time based on the processing conditions of the technological procedures at the WTP in Kutno, Poland. The primary coagulant polyalu-

minum chloride (PAX XL10) at a dose of 1 mg Al^{3+}/L was added to the glass beakers with 1 L of filter backwash water. Subsequently, rapid mixing (250 rpm) was initiated and an appropriate flocculant dose (ranging from 0.1 to 2.0 mg/L) was added, with continuing stirring for 1 min. Then, slow mixing (25 rpm) was carried out for 15 min to accelerate the flocs' agglomeration. Finally, the suspension was subjected to a 0.5 h sedimentation process. For each polysaccharide sample, the test was performed in triplicate. The results of the flocculation tests are presented in Figures 12 and 13.
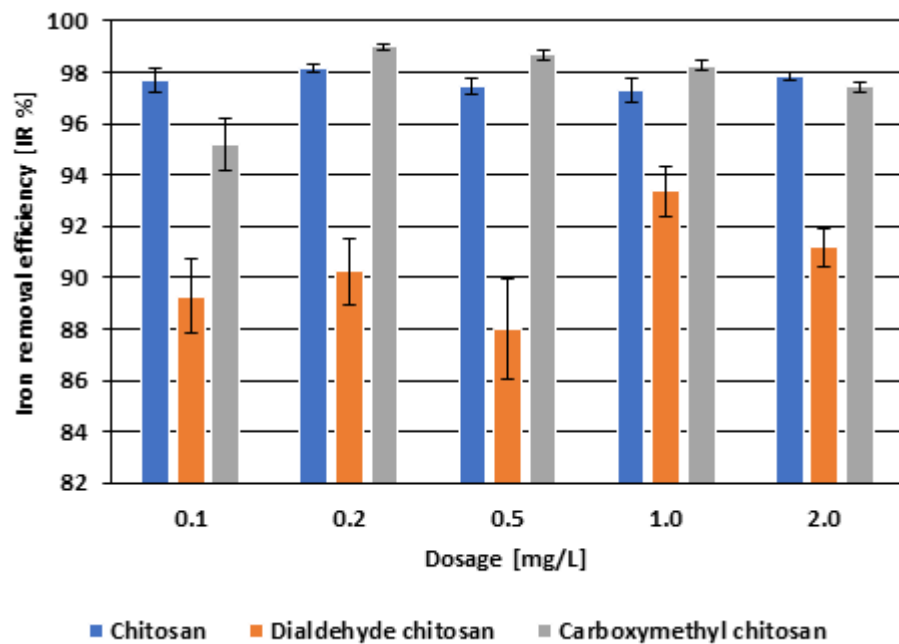

Figure 12. Iron removal efficiency for filter backwash water according to the flocculant dosage.
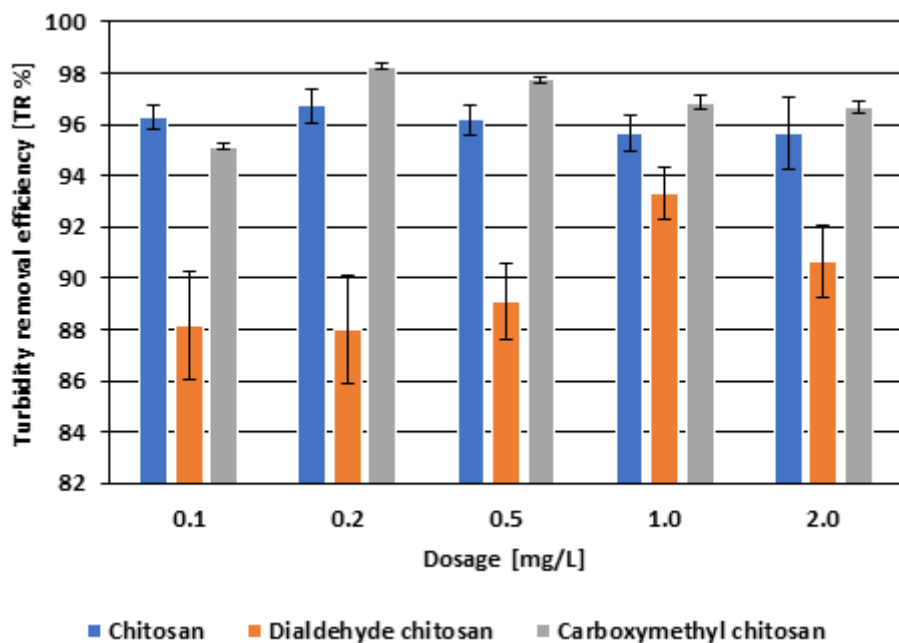

Figure 13. Turbidity removal efficiency for filter backwash water treatment according to the flocculant dosage.

The data in Table 3 present the iron and turbidity removal efficiency results for bio-based flocculants in their optimal doses. The tested polymer samples showed over 90% reductions in water parameters. The most effective flocculation agent was CMC in an optimal amount of 0.2 mg/L (Table 3).

Table 3. Iron concentration and turbidity removal efficiency values for the tested flocculants in optimal doses.

Sample	Optimal Dose [mg/L]	Removal Efficiency [%]	
		Iron Concentration	Turbidity
Chitosan	0.2	98.11 ± 0.17	96.76 ± 0.65
DCT	1.0	93.19 ± 0.98	93.51 ± 1.01
CMC	0.2	98.97 ± 0.13	98.26 ± 0.10
Superfloc A100PWG *	0.5	92.17 ± 0,37	93.89 ± 0.41

* Description in: *Materials* 2022, *15*(3), 1056; https://doi.org/10.3390/ma15031056 [52].

Table 3 compares the results for these novel polysaccharide flocculants and the selected synthetic polymer Superfloc A100PWG (which is a commercial polyacrylamide-based flocculant), which is currently used at the WTP in Kutno. Considering the data presented in that work for polyacrylamide, it can be concluded that chitosan and CMC show better flocculation properties than the polyacrylamide flocculants used in practice. In the case of DCT, only the turbidity removal efficiency was slightly worse compared to the commercial Superfloc A100PWG.

4. Discussion

The proposed conditions for modifying chitosan allowed for high degrees of substitution with the introduced functional groups, at over 44% for DCT and about 50% for CMC. The applied modification method is uncomplicated and repeatable.

Chitosan materials modified in this way show high efficiency in removing impurities in the form of iron ions from water solutions (specifically filter backwash water with a high content of iron ions).

The performed chemical modification of chitosan changes the morphology and porosity of the samples, which undoubtedly affects the flocculation process.

Moreover, the course of thermal degradation of DCT and CMC differs from this process in unmodified chitosan, confirming the successful functionalization of the biopolymer. In the initial stage of heating of the chitosan derivatives, the adsorbed low-molecular compounds (e.g., moisture, solvent residues, or other impurities) are evaporated, which is accompanied by relatively small weight losses (a few %). Moreover, the hydrogen bonds are destroyed. When the macromolecules obtain the appropriate energy value, i.e., after exceeding a certain temperature, the covalent bonds are broken, the side groups are abstracted, and the polymer chains undergo chain scission. The fragmentation of macromolecules leads to a large weight loss, although the thermal decomposition is not complete at 600 °C, at which a thermally stable residue is observed. This is typical for polysaccharides [63]. Interestingly, the main stage of decomposition of DCT and CMC derivatives occurs at a higher temperature than in chitosan itself. This can be explained by the thermal crosslinking taking place due to the functional groups (COOH, CHO) involved in this process. The result is macromolecules linked by transversal covalent bonds. They are very resistant to high temperatures, which is visible in the form of approximately 10% more carbonaceous residue at 600 °C.

The bioflocculant prepared using the proposed method was used to purify the filter backwash water (FBW), which is water containing a suspension of ironhydroxides at a relatively high concentration, washed out of the filter bed.

The results for the determination of the turbidity and iron ion content indicated a possible mechanism of colloid flocculation in the filter backwash water. In the literature, two mechanisms of flocculation with the use of biopolymers are most often given: charge

neutralization and bridging [64–66]. The first mentioned mechanism may occur if the flocculant has a different surface charge than the colloidal particle. Then, the charge density on the molecule is reduced by polymer adsorption. In other words, it destabilizes the colloidal impurities by electrostatic attraction. This mechanism is particularly effective for low molecular weight polymers [64–66]. In the case of bridging, the polymers can be adsorbed by van der Waals forces, hydrogen bonding, or a reaction with functional groups in the polymer molecule. According to the obtained results, bridging is the most likely mechanism. Both chitosan and CMC contain numerous reactive groups if $-NH_2$, $-OH$, and $-COOH$ in their structure, capable of binding iron ions. Another factor causing effective flocculation is the ability of chitosan macromolecules to adopt privileged conformations that allow them to surround impurity particles.

This confirms our earlier observations on the mechanism of flocculation using polysaccharide-based flocculants. The presence of hydroxyls capable of creating hydrogen bonds also enhances the aggregation process [52]. However, these bonds exhibit weak interactions with colloid particle surfaces. Dialdehyde chitosan, containing mainly $-OH$ and CHO groups in its structure, achieved 93% removal efficiency at a higher dosage (1.0 mg/L).

The best removal efficiency of iron ions and turbidity being found for CMC indicates that carboxyl groups most effectively interact with flocs in an aqueous solution, causing their agglomeration. However, slight differences in the determined parameters allow us to conclude that all the tested biopolymers meet the expectations in the treatment of post-wash water. In all studied flocculation systems, the flocs were large, dense, and easy to separate in the sedimentation and filtration processes.

The results obtained are closely related to the search for ecological solutions for water and wastewater treatments, not only in the water supply and sewage enterprise (WPT, Kutno, Poland). The technology used in the WTP primarily concerns the removal of high iron concentrations, which in the washing water oscillate around 30 mg/L. In the designed jar test experiment, the polyaluminum chloride was added in a much smaller amount of 1 mgAl^{3+}/L to initiate coagulation. Such a reduction in the amount of aluminum compound is an undoubted ecological benefit. Then, the polysaccharide flocculant was dosed into the water, which enabled the formation of compact flocs and facilitated its sedimentation. Compared to the commercial polyacrylamide flocculant currently used in the FBW purification process, the designed chitosan-based flocculants showed a higher removal efficiency for the tested compounds. This can bring measurable economic effects to the WTP and other similar enterprises, and more importantly biodegradable chitosan-based flocculants do not burden the natural environment and are safe for humans.

5. Conclusions

Chitosan and its two derivatives oxidized, dialdehyde chitosan (DCT) and etherified carboxymethyl chitosan (CMC), were obtained and their chemical structures were confirmed using spectroscopic methods (FTIR, ^{13}C-NMR) and an elemental analysis. Significant changes in the structure of the modified chitosan were also proven by a thermogravimetric analysis, which at the same time showed differences in the pyrolysis process of the tested materials. SEM microscopy allowed us to observe the various morphologies of the samples, which had no significant effect on the flocculation efficiency in this case.

The process of obtaining DCT and CMC is well developed, well understood, and uncomplicated.

Both chitosan and CMC can be used at a very low dose of 0.2 mg/L, while the optimal amount for DCT is somewhat higher at 1 mg/L. The parameters characterizing the flocculation efficiency (removal of iron ions and turbidity) in all tested samples exceed 90%, and in the case of CMC they are close to 100%. Thus, it has been proven that CMC is the most effective agent in the filter backwash water purification process among the three compared biopolymers. All proposed materials allow for FBW treatment with a lower environmental impact (due to the elimination of synthetic polymers and reduction in the

PAC amount added to the wastewater) than common coagulant or flocculant systems. The sludge remaining after the flocculation process can be safely disposed of in landfills.

The proposed flocculation materials and the efficient treatment of these polluted waters allow them to be returned to the technological circulation and contribute to a reduction in water consumption, which is another important achievement from the point of view of water resource protection.

Although these studies strictly corresponded to the requirements of the technological process in the Kutno plant (WTP), the proposed environmentally friendly flocculants obtained from renewable raw materials may find wider application in related fields of industrial or municipal sewage treatment, as well as for obtaining clean, compliant water for the food and beverage industry. The very important advantages of chitosan and its derivatives are the lack of toxicity and safety for humans and high susceptibility to biodegradation, which helps to maintain a clean environment.

In summary, we can conclude that the CMC chitosan derivative is a good ecological alternative to the flocculants currently used in practice. Further planned work with these chitosan-based flocculants will allow us to determine the possibility of using them to remove other pollutants from water.

References

1. El-taweel, R.M.; Mohamed, N.; Alrefaey, K.A.; Husien, S.; Abdel-Aziz, A.B.; Salim, A.I.; Mostafa, N.G.; Said, L.A.; Fahim, I.S.; Radwan, A.G. A review of coagulation explaining its definition, mechanism, coagulant types, and optimization models; RSM, and ANN. *Curr. Opin. Green Sustain. Chem.* **2023**, *6*, 100358. [CrossRef]
2. Bray, R.T.; Fitobór, K. Sizes of iron hydroxide particles formed during ferric coagulation processes. *Des. Water* **2017**, *64*, 419–424. [CrossRef]
3. Cui, H.; Huang, X.; Yu, Z.; Chen, P.; Cao, X. Application progress of enhanced coagulation in water treatment. *RSC Adv.* **2020**, *10*, 20231–20244. [CrossRef] [PubMed]
4. Saritha, V.; Srinivas, N.; Srikanth Vuppala, N.V. Analysis and optimization of coagulation and flocculation process. *Appl. Water Sci.* **2017**, *7*, 451–460. [CrossRef]
5. Naceradska, J.; Pivokonska, L.; Pivokonsky, M. On the importance of pH value in coagulation. *J. Water Supply Res. Technol. Aqua* **2019**, *68*, 222–230. [CrossRef]
6. Cao, B.; Gao, B.; Xu, C.; Fu, Y.; Liu, X. Effects of pH on coagulation behavior and floc properties in Yellow River water treatment using ferric based coagulants. *Chin. Sci. Bull.* **2010**, *55*, 1382–1387. [CrossRef]
7. Cao, B.; Gao, B.; Liu, X.; Wang, M.; Yang, Z.; Yue, Q. The impact of pH on floc structure characteristic of polyferric chloride in a low DOC and high alkalinity surface water treatment. *Water Res.* **2011**, *45*, 6181–6188. [CrossRef] [PubMed]
8. Dawood, A.S.; Li, Y. Modeling and Optimization of New Flocculant Dosage and pH for Flocculation: Removal of Pollutants from Wastewater. *Water* **2013**, *5*, 342–355. [CrossRef]
9. Martín, M.A.; González, I.; Berrios, M.; Siles, J.A.; Martín, A. Optimization of coagulation–flocculation process for wastewater derived from sauce manufacturing using factorial design of experiments. *Chem. Eng. J.* **2011**, *172*, 771–782. [CrossRef]
10. Crini, G. Recent developments in polysaccharide-based materials used as adsorbents in wastewater treatment. *Progr. Polym. Sci.* **2005**, *30*, 38–70. [CrossRef]
11. Salehizadeh, H.; Yan, N.; Farnood, R. Recent advances in polysaccharide bio-based flocculants. *Biotechnol. Adv.* **2018**, *62*, 92–119. [CrossRef]
12. Jiang, X.; Li, Y.; Tang, X.; Jiang, J.; He, Q.; Xiong, Z.; Zheng, H. Biopolymer-based flocculants: A review of recent technologies. *Environ. Sci. Pollut. Res.* **2021**, *28*, 46934–46963. [CrossRef] [PubMed]
13. Mansour, Y.H.; Othmani, B.; Rebah, F.B.; Mnif, W.; Saoudi, M.; Khadhraoui, M. Could plant-based flocculants substitute the conventional synthetic chemicals in the sludge dewatering process? *Water* **2023**, *15*, 2602. [CrossRef]
14. Kolya, H.; Sasmal, D.; Tripathy, T. Novel biodegradable flocculating agents based on grafted starch family for the industrial effluent treatment. *Polym. Environ.* **2017**, *25*, 408–418. [CrossRef]

15. Grenda, K.; Arnold, J.; Gamelas, A.F.; Rasteiro, M.G. Environmental friendly cellulose-based polyelectrolytes in water treatment. *Water Sci. Technol.* **2017**, *76*, 1490–1499.

16. Barrero-Fernández, A.; Aguado, R.; Moral, A.; Brindley, C.; Ballesteros, M. Applications of cellulose-based agents for flocculation processes: A bibliometric analysis. *Cellulose* **2021**, *28*, 9857–9871. [CrossRef]

17. Pontius, F.W. Chitosan as a drinking water treatment coagulant. *Am. J. Civil Eng.* **2016**, *4*, 205–2015. [CrossRef]

18. Al-Manhel, A.J.; Al-Hilphy, A.R.S.; Niamah, A.K. Extraction of chitosan, characterisation and its use for water purification. *J. Saudi Soc. Agricultur. Sci.* **2018**, *17*, 186–190. [CrossRef]

19. Feng, L.; Li, X.; Lu, W.; Liu, Z.; Xu, C.; Chen, Y.; Zheng, H. Preparation of a graft modified flocculant based on chitosan by ultrasonic initiation and its synergistic effect with kaolin for the improvement of acid blue 83 (AB 83) removal. *Int. J. Biol. Macromol.* **2020**, *150*, 617–630. [CrossRef]

20. Mohammadi, E.; Daraei, H.; Ghanbari, R.; Athar, S.D.; Zandsalimi, Y.; Ziaee, A.; Maleki, A.; Yetilmezsoy, K. Synthesis of carboxylated chitosan modified with ferromagnetic nanoparticles for adsorptive removal of fluoride, nitrate, and phosphate anions from aqueous solutions. *J. Mol. Liquids.* **2019**, *273*, 116–124. [CrossRef]

21. Sun, Y.; Chen, A.; Pan, S.; Sun, W.; Zhu, C.; Shah, K.J.; Zheng, H. Novel chitosan-based flocculants for chromium and nickle removal in wastewater via integrated chelation and flocculation. *J. Environ. Management* **2019**, *248*, 109241. [CrossRef] [PubMed]

22. Renault, F.; Sancey, B.; Badot, P.-M.; Crini, G. Chitosan for coagulation/flocculation processes—An eco-friendly approach. *Eur. Polym. J.* **2009**, *45*, 1337–1348. [CrossRef]

23. Lan, L.E.; Reina, F.D.; De Seta, G.E.; Meichtry, J.M.; Litter, M.I. Comparison between different technologies (zerovalent iron, coagulation-flocculation adsorption) for arsenic treatment at high concentration. *Water* **2023**, *15*, 1481. [CrossRef]

24. Yu, Y.; Sun, Y.; Zhou, J.; Chen, A.; Shah, K.J. preparation and characterization of high-efficiency magnetic heavy metal capture flocculation. *Water* **2021**, *13*, 1732. [CrossRef]

25. Xu, Y.; Gan, K.; Liang, S.; Liu, H.; Wang, Q. Investigation and optimization of chitosan performance in flocculating kaolin suspensions using a real-time suspending solid concentration measuring method. *Water* **2021**, *13*, 513. [CrossRef]

26. Tian, Z.; Zhang, L.; Sang, X.; Shi, G.; Ni, C. Preparation and flocculation performance study of a novel amphoteric alginate flocculant. *J. Phys. Chem. Solids* **2020**, *141*, 109408. [CrossRef]

27. Zhao, X.; Wang, X.; Song, G.; Lou, T. Microwave assisted copolymerization of sodium alginate and dimethyl diallyl ammonium chloride as flocculant for dye removal. *Int. J. Biol. Macromol.* **2020**, *156*, 585–590. [CrossRef]

28. Wang, B.; Wang, S.-F.; Lam, S.S.; Sonne, C.; Yuan, T.-Q.; Song, G.-Y.; Sun, R.-C. A review on production of lignin-based flocculants: Sustainable feedstock and low carbon footprint applications. *Renewable Sustainable Energy Rev.* **2020**, *134*, 110384. [CrossRef]

29. Patra, P.; Patra, A.S.; Mukherjee, A.K.; Pal, S. Development of a highly efficient selective flocculant based on functionalized β-cyclodextrin toward beneficiation of low-quality iron ore. *Polym. Adv. Technol.* **2021**, *32*, 2169–2175. [CrossRef]

30. Baran, T.; Menteş, A.; Arslan, H. Synthesis and characterization of water soluble O-carboxymethyl chitosan Schiff bases and Cu(II) complexes. *Int. J. Biol. Macromol.* **2015**, *72*, 94–103. [CrossRef] [PubMed]

31. Hejazi, R.; Amiji, M. Chitosan-based gastrointestinal delivery systems. *J. Control Release* **2003**, *89*, 151–165. [CrossRef]

32. Jain, A.; Gulbake, A.; Shilpi, S.; Jain, A.; Hurkat, P.; Jain, S.K. A new horizon in modifications of chitosan: Syntheses and applications. *Crit. Rev. Ther. Drug* **2013**, *30*, 91–181. [CrossRef] [PubMed]

33. Lv, K.L.; Du, Y.L.; Wang, C.M. Synthesis of carboxymethylated chitosan and its adsorption properties for cadmium (II), lead (II) and copper (II) from aqueous solutions. *Water Sci. Technol.* **2009**, *60*, 467–474. [CrossRef]

34. Mondal, S.K.; Wu, C.; Nwadire, F.C.; Rownaghi, A.; Kumar, A.; Adewuyi, Y.; Okoronkwo, M.U. Examining the effect of chitosan biopolymer on alkali-activated inorganic material for aqueous Pb (II) and Zn (II) sorption. *Langmuir* **2022**, *38*, 903–913. [CrossRef] [PubMed]

35. Ostrowska-Czubenko, J.; Piróg, M.; Gierszewska, M. Modification of chitosan—A concise overview. *Wiad.Chem.* **2016**, *70*, 9–10.

36. Kelar, K. *Modyfikacja Polimerów*; Wydawnictwo Politechniki Poznańskiej: Poznań, Poland, 1991.

37. Braun, D.; Cherdron, H.; Rehahn, M.; Ritter, H.; Voit, B. *Polymer Synthesis: Theory and Practice. Fundamentals, Methods, Experiments*; Springer: Berlin/Heidelberg, Germany, 2001.

38. Wang, B.; Zhang, Y.; Miao, C. Preparation of cationic chitosan-polyacrylamide flocculant and its properties in wastewater treatment. *J. Ocean. Univ. China* **2011**, *10*, 42–46. [CrossRef]

39. Yang, Z.; Shang, Y.; Lu, Y.; Chen, Y.; Huang, X.; Chen, A.; Jiang, Y.; Gu, W.; Qian, X.; Yang, H.; et al. Flocculation properties of biodegradable amphoteric chitosan-based flocculants. *Chem. Eng. J.* **2011**, *172*, 287–295. [CrossRef]

40. Tao, L.; Xuejun, W.; Guojun, S.; Guangpeng, C. Synthesis and flocculation performance of a chitosan-acrylamide-fulvic acid ternary copolymer. *Carbohydr. Polym.* **2017**, *170*, 182–189.

41. You, L.; Lu, F.; Li, D.; Qiao, Z.; Yin, Y. Preparation and flocculation properties of cationic starch/chitosan crosslinking-copolymer. *J. Hazard. Mater.* **2009**, *172*, 38–45. [CrossRef]

42. Wei, T.; Wu, L.; Yu, F.; Lv, Y.; Chen, L.; Shi, Y.; Dai, B. pH-responsive chitosan-based flocculant for precise dye flocculation control and the recycling of textile dyeing effluents. *RSC Adv.* **2018**, *8*, 39334–39340. [CrossRef]

43. Chen, X.G.; Park, H.J. Chemical characteristics of O-carboxymethyl chitosans related to the preparation conditions. *Carbohydr. Polym.* **2003**, *53*, 355–359. [CrossRef]

44. Musarurwa, H.; Tavengwa, N.T. Application of carboxymethyl polysaccharides as bio-sorbents for the sequestration of heavy metals in aquatic environments. *Carbohydr. Polym.* **2020**, *237*, 116142. [CrossRef] [PubMed]

45. Mucha, M. *Chitozan Wszechstronny Polimer ze Źródeł Odnawialnych*, 1st ed.; WNT: Warszawa, Poland, 2010; pp. 91–96.
46. Agbovi, H.K.; Wilson, L.D. Design of amphoteric chitosan flocculants for phosphate and turbidity removal in wastewater. *Carbohydr. Polym.* **2018**, *189*, 360–370. [CrossRef] [PubMed]
47. Chung, Y.C. Improvement of aquaculture wastewater using chitosan of different degrees of deacetylation. *Environ. Technol.* **2006**, *27*, 1199–1208. [CrossRef]
48. Dong, C.; Chen, W.; Liu, C. Flocculation of algal cells by amphoteric chitosan-based flocculant. *Bioresour. Technol.* **2014**, *170*, 239–247. [CrossRef] [PubMed]
49. Pei, H.-Y.; Ma, C.-X.; Wen-Rong Hu, W.-R.; Sun, F. The behaviors of Microcystis aeruginosa cells and extracellular microcystins during chitosan flocculation and flocs storage processes. *Bioresour. Technol.* **2014**, *151*, 314–322. [CrossRef]
50. Hadiyanto, H.; Widayat, W.; Christwardana, M.; Monica Pratiwi, M.E. The flocculation process of Chlorella sp. using chitosan as a bio-flocculant: Optimization of operating conditions by response surface methodology. *Curr. Res. Green Sustain. Chem.* **2022**, *5*, 100291. [CrossRef]
51. Salehizadeh, H.; Yan, N. Recent advances in extracellular biopolymer flocculants. *Biotechnol. Adv.* **2014**, *32*, 1506–1522. [CrossRef]
52. Maćczak, P.; Kaczmarek, H.; Ziegler-Borowska, M.; Węgrzynowska-Drzymalska, K.; Burkowska-But, A. The use of chitosan and starch-based flocculants for filter backwash water treatment. *Materials* **2022**, *15*, 1056. [CrossRef]
53. Zane Satterfield, P.E. Jar Testing; Tech Brief, Publisher by The National Environmental Services Center, Spring. 2005, Volume 5. Issue 1. Available online: https://www.nesc.wvu.edu/files/d/3cf372e5-ba40-450c-a3ad-cd774f4c3345/jar-testing.pdf (accessed on 22 June 2023).
54. Hach Company. *Hach Method 8008. USEPA FerroVer® Method*; DOC316.53.01053; Hach Company: Ames, IA, USA, 2014.
55. Method 180.1: Determination of Turbidity by Nephelometry. Available online: https://www.epa.gov/sites/production/files/2015-08/documents/method_180-1_1993.pdf (accessed on 26 March 2023).
56. Heinze, T.; Koschella, A. Carboxymethyl ethers of cellulose and starch—Review. *Macromol. Symp.* **2005**, *223*, 13–40. [CrossRef]
57. Węgrzynowska-Drzymalska, K.; Gebicka, P.; Młynarczyk, D.T.; Chełminiak-Dudkiewicz, D.; Kaczmarek, H.; Gośliński, T.; Ziegler-Borowska, M. Crosslinking of chitosan with dialdehyde chitosan as a new approach for biomedical applications. *Materials* **2020**, *13*, 3413. [CrossRef]
58. Zhang, L.; Guo, J.; Zhou, J.; Yang, G.; Du, Y. Blend membranes from carboxymethylated chitosan/alginate in aqueous solution. *J. App. Polym. Sci.* **2000**, *77*, 610–616. [CrossRef]
59. Zhao, X.; Kato, K.; Fukumoto, Y.; Nakamae, K. Synthesis of bioadhesive hydrogels from chitin derivatives. *Int. J. Adhes. Adhes.* **2001**, *21*, 227–232. [CrossRef]
60. Mourya, V.K.; Inamdar, N.N.; Tiwari, A. Carboxymethyl chitosan and its applications. *Adv. Mater. Lett.* **2010**, *1*, 11–33. [CrossRef]
61. Sun, L.; Du, Y.; Fan, L.; Chen, X.; Yang, J. Preparation, characterization and antimicrobial activity of quaternized carboxymethyl chitosan and application as pulp-cap. *Polymer* **2006**, *47*, 1796–1804. [CrossRef]
62. Zheng, M.; Han, B.; Yang, Y.; Liu, W. Syntheis, characterization and biological safety of O-carboxymethyl chitosan used to treat Sarcoma 180 tumor. *Carbohydr. Polym.* **2011**, *86*, 231–238. [CrossRef]
63. Almeida, V.; Frollini, E.; Castellan, A.; Coma, V. Chitosan, sisal cellulose, and biocomposite chitosan/sisal cellulose films prepared from thiourea/NaOH aqueous solution. *Carbohydr. Polym.* **2010**, *80*, 655–664. [CrossRef]
64. Dao, V.H.; Cameron, N.R.; Saito, K. Synthesis, properties and performances of organic polymers employed in flocculation applications. *Polym. Chem.* **2016**, *7*, 11–25. [CrossRef]
65. Bolto, B.; Gregory, J. Organic polyelectrolytes in water treatment. *Water Res.* **2007**, *41*, 2301–2324. [CrossRef] [PubMed]
66. Maćczak, P.; Kaczmarek, H.; Ziegler-Borowska, M. Recent achievements in polymer-based flocculants for water treatment. *Materials* **2020**, *13*, 3951. [CrossRef] [PubMed]