Anionic bio-flocculants from sugarcane for purification of sucrose: An application of circular bioeconomy

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ABSTRACT

In sugar production, polyacrylamide-based anionic flocculants are added for juice treatment, the main objective being to remove impurities that affect the quality of the sugar. However, if they remain in the final product, those polymers can present carcinogenic and neurotoxic actions besides contaminating the soils where the waste is discharged. To overcome this problem, the present study proposes, for the first time, natural flocculants based on cellulose obtained from sugarcane bagasse (residue from sugarcane processing) as substitutes for the flocculants based on polyacrylamide, normally used in sugar cane juice purification. Additionally, cellulose-based flocculants obtained from Acacia wood, developed in a previous study, have also been tested for sugar juice treatment. Acacia wood and sugarcane bagasse were first treated with a choline chloride/levulinic acid solution in a molar ratio of 1:2, at 160 °C, for 4 h. Subsequently, the cellulose-rich samples were modified by a two-stage process (oxidation with sodium periodate followed by reaction with sodium metabisulfite), and polyelectrolytes with different characteristics were produced. The final products obtained were characterized, and their performance in the treatment of sugarcane juice, at different concentrations (10, 50, 100, 250, and 500 mg kg^{-1}), was evaluated and compared to the synthetic commercial flocculant (Flonex, based on polyacrylamide) usually used by the sugarcane industry in Brazil. The substitution of petrol-based flocculants by natural-based ones, obtained from sugarcane residues, is presented for the first time in this study, with very relevant performance of the new flocculants. Overall, it was possible to produce anionic flocculants, modifying the cellulose obtained from different raw materials, which showed good results in the purification of sucrose, when compared with the commercial polyacrylamide normally used. It is also important to stress that, for the first time, a residue from sugarcane industry could be used with success in the purification of the sugar juice itself, which constitutes a major novelty.

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1. Introduction

Sugarcane grows worldwide, and its production is mainly used for the sugar industry, currently corresponding to 80% of the sugar produced, contributing significantly to the world economy.

During sugar production, a critical step is the juice treatment, being the main objective to remove impurities, such as polyphenols including flavonoids and phenolic acids (gallic and chlorogenic acids, ferulic acid, etc.) [1,2], that negatively affect the sucrose quality for the production of the final product (crystal sugar). Synthetic anionic flocculants are currently added as clarification aids during this step, consisting of polyacrylamides. If they remain in the final product, these compounds, or their hydrolysis products, can exhibit carcinogenic and neurotoxic actions [3]. Thus, it is essential to consider the concentrations used since high concentrations can result in the retention of polyacrylamide molecules in the final crystal sugar [3]. On the other hand, when carried by the flocs to the effluents, they may contaminate sugarcane crops, due to the use of the effluents as biofertilizers. Thus, replacing synthetic polyelectrolytes with sustainable and non-toxic options is important for the sugarcane industry, and natural polyelectrolytes may be promising alternatives in the treatment of sugarcane juice.

Moreover, during the processing of sugarcane, large amounts of waste, such as sugarcane bagasse (SCB), molasses, and sludge or filter cake, are produced, and the valorisation of these resources is relevant and currently under research [4].

In general, to produce 100 kg of sugarcane, 1 ton of cane is crushed, generating 300 kg of bagasse (with 50% moisture), 40 kg of molasses, and 30 kg of decanter sludge. The molasses are used for ethanol production and the decanter sludge for hydrocarbon or chemical production [4]. On the other hand, the SCB is currently used as a fuel for the boilers in the sugar factory or as a raw material for the manufacture of lignocellulosic products. Various types of building blocks and certain chemicals [5–7] have been obtained from SCB treatment. SCB has a complex structure, and it is primarily composed of cellulose (40–50%), hemicelluloses (25–35%), lignin (15–35%), ash and waxes [8]. The composition of SCB makes it an ideal additive to be applied and utilized as bio-flocculants for purification of sucrose, instead of polyacrylamides.

Cellulose is itself a fascinating polymeric product and possesses several attributes, but also some inherent issues. Among the drawbacks, the poor solubility in common solvents should be highlighted [9]. To overcome such limitations, the controlled physical and/or chemical modification of the cellulose structure is often a strategy which is adopted [10,11].

Polyelectrolytes for application in flocculation should be water-soluble, and so, in order to use cellulose as a flocculation agent, it is crucial to obtain a final water-soluble cellulose derivative with the charged groups spread effectively among the polymeric backbone. However, lignocellulosic biomass has a high degree of heterogeneity, which leads to different interactions among the different bio-macromolecules [12], and therefore, fractionation and isolation of the different components are necessary, important steps before any modification [13]. The effectiveness of the fractionation step has proven to play a key role in the anionisation of the cellulose backbone [14,15].

Regarding the cellulose modification, to obtain an ionic polymer with a better solubility, the use of a two-stage strategy has proven more favourable [16]. The first stage is the selective oxidation with periodate, which partially destroys the crystalline cellulose structure. Periodic acid and its salts, periodates, are known as regioselective oxidation agents capable of converting vicinal diols, such as carbohydrates, to dialdehyde structures [14]. In this case, the diol cleavage of cellulose by periodate, under acidic conditions, occurs in the C2–C3 bond, resulting in the formation of two aldehyde groups, at the OH–C2 and OH–C3 positions, and leading to dialdehyde cellulose (DAC). In this reaction, a high modification degree can be achieved, since two aldehyde groups are introduced per anhydroglucose (AGU) unit, which allows obtaining highly modified end products [13]. On the other hand, it is observed that, in most cases, the degree of polymerization (DP) decreases during this oxidation procedure [17]. Several reaction parameters may influence the properties of obtained DAC, such as the concentration of periodate (i.e., higher concentrations of periodate improve the formation of aldehyde groups and allows to obtain cellulose with higher aldehyde contents), temperature and the reaction time [13]. Anionic water-soluble cellulose-based polymers can be obtained in the second reaction stage, after the first oxidation step, through the sulfonation of DAC [14,15]. The anionic flocculants were obtained by the DAC modification using sodium metabisulfite, which leads to a sulphur trioxide bond resulting in negatively charged groups being introduced into the cellulose backbone. This type of modification allows the introduction of more than one anionic group per AGU. Products obtained in such a way are thus characterized by a high degree of substitution, high ionic character and consequently high charge density and water solubility at room temperature.

In the present study, the objective was to obtain anionic cellulose-based polyelectrolytes, using as raw material sugarcane bagasse. The first stage was to isolate cellulose from the bagasse using an eco-friendly procedure based on the use of a mixture of choline chloride and levulinic acid. This procedure was compared with the traditional methodology based on the treatment of the bagasse in a basic medium using a NaOH solution [18]. Afterwards, the cellulose was modified (anionised) using the two-stage procedure previously applied by the research group for other raw materials [14,15]. Finally, the efficiency of the new bio-based polyelectrolytes developed was validated for the treatment of sugarcane juice in the sugar production process, as an alternative to the polyacrylamide polymers normally used nowadays, which constitutes a complete novelty and a step forward towards a circular bio economy. It can be stated that the new polymers developed will be fully biodegradable. Still, it is foreseen to perform, in the future, a complete toxicological assessment of these new natural-based polyelectrolytes.

2. Materials and methods

2.1. Materials

The sugarcane bagasse (Saccharum officinarum) was supplied by the Hugot Laboratory of Sugar Technology at the University of

São Paulo and had, on average, 38.78% of cellulose, 23.81% of hemicellulose, 24.02% of lignin, determined according to the procedure which will be described later. The average particle size was 0.84 mm after milling (knife mill, supplied by Thomas Scientific, USA).

The Acacia wood (Acacia dealbata) used in this work was collected and harvested in Midões (Tábua), Portugal, and the main components are given in Table 1. The raw material consisted of waste branches, which were finely milled in the same laboratory mill (knife mill, Thomas Scientific, USA) and classified in a mechanical sieve shaker (Thomas Scientific, USA). The sawdust sample with a particle size between 0.25 and 0.84 mm was selected for the subsequent pre-treatment.

Levulinic acid (98 wt% purity) and choline chloride (99 wt% purity) were purchased from Acros Organics, while sulfuric acid (72 wt%) was purchased from Chem-Lab NV. The 10% sodium hydroxide solution was prepared with distilled water and 1–2 mm sodium hydroxide beads purchased from LabKem. Calcium carbonate (>99%), lithium chloride (99 wt%) and sodium metabisulfite (>98%) were purchased from Sigma-Aldrich. The acetate buffer solution with pH = 4.5 was prepared with anhydrous sodium acetate (99 wt%) and glacial acetic acid (99 wt%) purchased from Chem-Lab and VWR Chemicals, respectively. Sodium periodate (99 wt%) was purchased from Fisher scientific, propanol-2 (99.94 wt% with 0.012 wt% water) was purchased from José Manuel Gomes dos Santos, Lda and ethylene glycol from ITW Reagents.

2.2. Biomass fractionation and cellulose purification

The acacia wood and the sugarcane bagasse fractionation was performed using a deep eutectic solvent system (choline chloride (ChCl):levulinic acid (Lev) in a 1:2 molar ratio). First, 0.75 g of sugarcane bagasse or acacia chips (dry basis, previously dried in an oven at 105 °C) were introduced into the fractionation vessel (a cylindrical metallic reactor capable of supporting high pressures), and 10 mL of eutectic solvent (ChCl:Lev) were added. Next, the reactor was placed in the oven at 160 °C for 4 h.

After fractionation was completed, the cylindrical reactor was carefully opened, and the cellulose-rich fraction was meticulously washed with distilled water, in order to drag the lignin-rich material into the liquid fraction. Afterwards, both the cellulose-rich fraction and the lignin-rich fraction were vacuum filtered. The cellulose-rich material, initially washed with water to remove the insoluble lignin, was then washed during vacuum filtration with a 10 wt% NaOH aqueous solution to remove any residual lignin that remain on the fibres. Finally, the cellulose-rich fraction was further washed extensively with distilled water to remove any remaining chemicals and neutralize the fibres. The final washing with water was conducted till the used cleaning water exhibited neutral pH, which usually corresponded to 5 to 6 washing cycles. On average, 100 g of cellulose fibres required 200 mL of water for the washing process. It must be stressed that the washing water can be later purified and reused. After filtration, the two materials, one rich in lignin and the other in cellulose were dried in an oven at 50-60 °C for 24 h, for further characterization.

Another method for sugarcane bagasse fractionation evaluated in the present study was the experimental procedure described by Aguiar and Menezes (2000) [17]: 100 g of washed and milled sugarcane bagasse (dry basis) was treated with 2000 mL of 4% sodium hydroxide solution in an autoclave at 121 °C for 30 min. The material recovered by filtration was then washed with running water until pH neutrality and dried at 65 °C until constant mass.

2.2.1. Determination of lignin, cellulose, and hemicellulose content

The lignin content was estimated using the standard LAP-004 protocol from the National Renewable Energy Laboratory LAP-004 (NREL/TP-510-42618) [19]. In brief, ca. 300 mg of the reaction extract was weighed and hydrolysed in 3 mL of 72% sulfuric acid solution (12 mol L^{-1}) for 1 h at 30 °C, with stirring. Then, the hydrolysed sample was diluted till a 4% sulfuric acid solution was obtained, which was then placed in the autoclave to react for 1 h at 121 °C. Subsequently, the samples were vacuum filtered using filter crucibles (40 mm diameter and pore size 0.6 μm), to determine the acid-insoluble lignin by gravimetry. The acid-soluble lignin was determined using aliquots of the filtrate, which were adequately diluted, and by measuring the absorbance of the solutions at 205 nm in a UV-VIS spectrometer (JASCO V650 spectrophotometer, JASCO, Germany).

The carbohydrate content of the extracted cellulose-rich fractions was accessed by HPLC (High Pressure Liquid Chromatography, Knauer, Germany, model K301). Samples were first autoclaved, and a 20 mL sample of the hydrolysate, resulting from the procedure described above to determine the lignin content, were transferred to a 50 mL Erlenmeyer flask, and slowly neutralized with calcium carbonate until reaching a final pH of 5–6. The pH of the solution was monitored with a pH meter (inoLab, WTW of Weilheim, Germany). Then, the solution was filtered with a 0.22 μm nylon filter directly into an Eppendorf tube, and this sample was used for further HPLC analysis. The pump and detector used were Smartline 1000 and Smartline RI S2300 (refractive index detector), respectively. An Agilent Hi-Plex Ca, 300 × 7.7 mm column from Agilent Technologies was used. Ultra-pure water without any added buffer or other compounds was used as the mobile phase. The pH was set to 5.9. The stationary phase was a Hi-Plex Ca - strong cation exchange resin, consisting of sulfonated crosslinked styrene-divinylbenzene copolymer in the calcium form, with a particle size of 8

Table 1
Composition of the Acacia wood and Sugarcane bagasse.

Biomass	Composition (wt%)		
	Lignin	Cellulose	Hemicellulose
Acacia wood	22.20	47.41	18.79
Sugarcane bagasse	24.02	38.78	23.81

μm. For microporous resins, the crosslink content controls the pore size and hence the molecular weight range of the materials which can be analysed, determining the size exclusion properties of the resin. The lower the crosslink content, the higher the molecular weight that can be analysed. The 8% crosslinked resin has a low exclusion limit and is suitable for oligosaccharides with a DP (degree of polymerization) lower than 5. The flow rate was set to 0.6 mL min^{-1} and the run time was 60 min. The chemical composition and purity of the cellulose and extracted hemicellulose samples were accessed based on the monosaccharide composition obtained by HPLC (i.e., cellulose is determined based on the glucose content and hemicellulose based on the xylose content). The calibration standard was prepared in our lab, and included the following compounds: glucose, cellobiose, xylose, arabinose, formic and acetic acids and levulinic acid.

2.3. Synthesis and characterization of anionic cellulose-based polyelectrolytes

The anionisation of the cellulosic material was achieved using a dual-step procedure, in which the cellulose is firstly oxidized to DAC followed by anionisation with sodium metabisulfite. Fig. 1 summarizes, schematically, the two reactions that occur (oxidation followed by anionization) and the structure of the final product (anionic polyelectrolyte) obtained.

The procedure used was based on the work developed by Grenda et al. (2020) [14]. The first step focuses on the oxidation of the cellulose material, which avoids the use of alkali treatments. Briefly, a highly oxidized cellulose is produced by reacting the extracted cellulose with NaIO$_4$. To do so, it is prepared a cellulose dispersion in distilled water (4 g of cellulose dry basis in 100 mL of water), stirring overnight using a magnetic stirrer. Then, the suspension was placed in a round flask and diluted with 200 mL of distilled water. Precise quantities of NaIO$_4$, 7.2 g, and LiCl, 8.2 g (per 4 g of cellulose, dry basis) were added to the aqueous dispersion to initiate the reaction. According to Sirviö et al. (2011) [20], LiCl can act as a catalyst and improve the oxidation efficiency. The improvement obtained is attributed to the ability of the lithium ions to disrupt the hydrogen bonds between the cellulose chains which facilitates the interaction between the chemical reagents and the cellulose chains, as described by Sirviö et al. (2011) [21]. After the reaction period, the product was filtered and washed several times with distilled water to remove iodine compounds from the obtained DAC. Washing was performed several times (3–6 times, depending on the degree of substitution of the DACs) till the conductivity of the washing water and that of the collected water were the same, in order to guarantee a full removal of the iodine compounds. The aldehyde content of the oxidized cellulose was determined based on the oxime reaction between the aldehyde groups with a hydroxylamine salt (NH$_2$OH HCl). The non-dried periodate-oxidized cellulose (approximately 0.1 g) was placed in a 250 mL beaker to which 1.39 g of NH$_2$OH HCl and 100 mL of a sodium acetate buffer solution (0.1 M and pH = 4.5) were added. The mixture was then put under stirring (300 rpm) and left to react for 48 h. Then, the product was vacuum filtered and washed with distilled water and dried at 60 °C in an oven. Based on the reaction described, it is known that 1 mol of aldehyde reacts with 1 mol of NH$_2$OH HCl resulting in 1 mol of the oxime derivative. Thus, the degree of substitution by aldehyde groups in DAC and the aldehyde content can be calculated directly from the measurement of the nitrogen content in the oxime sample, determined using the elemental analysis technique, and using equation (1) and equation (2), respectively, which relate the nitrogen content (N%) with the degree of substitution by aldehyde groups in the cellulose chain (DS$_{DAC}$).

$$DS_{DAC} = \frac{\frac{N\%}{100} \times \frac{1}{14 \times 2}}{\frac{N\%}{100} \times \frac{1}{14 \times 2} + \left(1 - \frac{N\%}{100} \times \frac{190}{14 \times 2}\right)/162} \times 2 \tag{1}$$

$$Aldeyde\ content = \frac{DS_{DAC} \times 10}{MW_{DAC}} \times 1000 \tag{2}$$

where 14 (g mol^{-1}) represents the molar mass of the nitrogen atom, 190 (g/mol) is the molar mass of the oxime unit, 162 (g/mol) is the molar mass of the AGU unit, N% corresponds to the percentage of nitrogen present in the oxime sample, and MW$_{DAC}$ stands for the molecular weight of 10 mol of DAC units (g mol^{-1}).

The anionisation followed the procedure developed in the work by Grenda et al. (2020) [14]. In the present study the undried DAC was weighed in a 200 mL beaker and distilled water was added in order to obtain a ratio of 1.5 g of dried DAC to 60 mL of distilled water. Subsequently, sodium metabisulfite was added to the beaker in the proportion of 14 mmol of Na$_2$S$_2$O$_5$/g of dried DAC. Finally, the reaction mixture was placed under stirring (500 rpm) for 24 h at room temperature.

After the reaction period, the solution was mixed with isopropanol to precipitate the soluble material and then centrifuged, being
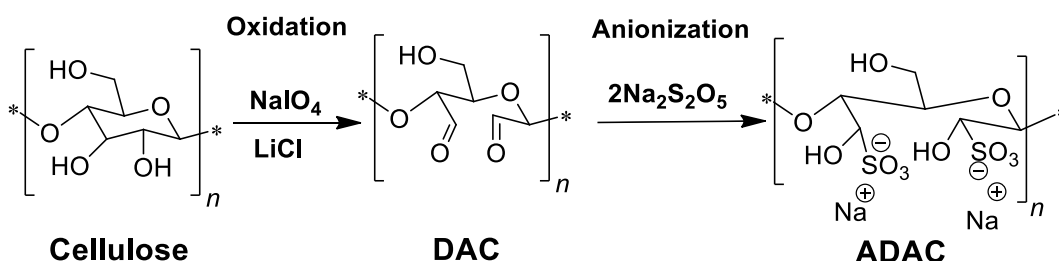

Fig. 1. Two-step reaction procedure used to produce anionic lignocellulose-based polyelectrolytes (ADAC).

the precipitated material washed afterwards four times with a water/isopropanol mixture (1:9, v/v) until the conductivity of the liquid after cleaning is close to that of the washing aqueous solution used in the process after cleaning, the anionic precipitate (ADAC) was placed in the oven at 60 °C to dry. The final ADAC was characterized by elemental analysis (EA 1108 CHNS, from Fisons, UK) in order to determine the degree of substitution by anionic groups (DS_{ADAC}) (equations (3)–(5)) and the anionicity index (equations (3) and (6)), through the measurement of the sulphur content.

$$Amount\ of\ anionic\ units\ (Aa) = \frac{\frac{S\%}{100} \times 368.1}{32.06 \times 2} \tag{3}$$

$$Mole\ fraction\ of\ anionic\ units = \frac{mol_{anionic\ units}}{mol_{total}} = \frac{\frac{Aa}{368.1}}{\frac{Aa}{368.1} + \frac{1-Aa}{162}} \tag{4}$$

$$DS = \frac{2 \times mol_{anionic\ units}}{mol_{total}} = \frac{2 \times \frac{Aa}{368.1}}{\frac{Aa}{368.1} + \frac{1-Aa}{162}} \tag{5}$$

$$Anionicity\ index = \frac{Aa}{368.1} \times 2 \times 1000 \tag{6}$$

where Aa represents the mass (g) of anionic units in 1 g of sample, 162 (g mol^{-1}) corresponds to the molar mass of the AGU unit and 368.1 (g mol^{-1}) corresponds to the molar mass of the anionic unit. It was assumed that only anhydroglucose and anionic units are present in the final product (no cellulosic dialdehyde exists in the final product).

The zeta potential values of each polyelectrolyte were also measured by electrophoretic light scattering (ELS) in a Zetasizer NanoZS from Malvern Instruments, UK. For that a 0.5% (wt%) solution of the polyelectrolyte was prepared in deionized water.

2.4. Evaluation of performance in the treatment of sugarcane juice

2.4.1. Cane plants and juice extraction

Healthy canes were disintegrated in forage and then pressed at 250 kgf cm^{-2} during 1 min to obtain the raw sugarcane juice (SCJ). SCJ was filtered through a 0.074 mm sieve to remove inorganic impurities and fibres and after that it was stored at −18 °C until the purification tests were performed. SCJ was characterized and presented 24.8 ± 0.01 °Brix (total soluble solids; wt%), turbidity of 1287 ± 0.01 NTU (Nephelometric Turbidity Unit), pH 5.20 ± 0.01, conductivity of 1.96 ± 0.01 mS cm^{-1}, 1.32 ± 0.1% g of reducing sugars per 100 g of juice, and pol%juice of 16.7 ± 0.4% (apparent sucrose content per 100 g of juice). The procedures for the analysis of the cane juice samples followed the official methods from Icumsa (2011) [22] and Consecana (2015) [23].

2.4.2. Treatment of sugarcane juice by cellulose-based polyelectrolytes

The extracted sugarcane juice was subjected to a treatment similar to that applied in cane mills for the purification of sugarcane juice using the commercial polyelectrolyte (Flonex at 6 mg kg^{-1}). In this study, the commercial polyelectrolyte was replaced by different doses of cellulose-based polyelectrolytes obtained from Acacia wood or from sugarcane bagasse, as shown in Fig. 2. First, the
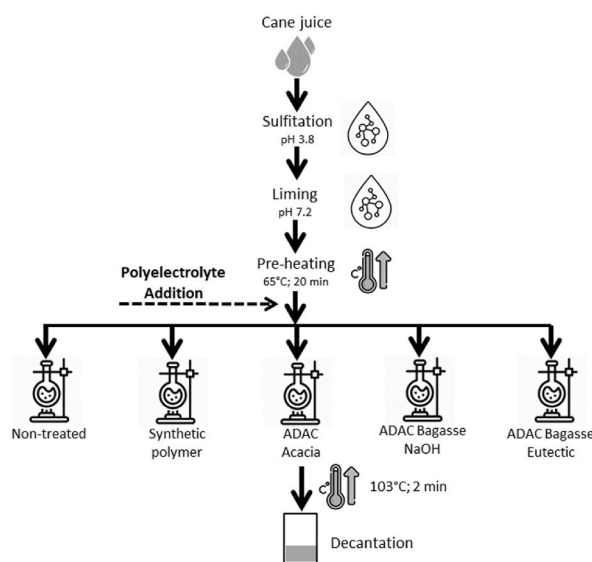
Fig. 2. Schematic representation of the steps in the treatment of simulated juice at a laboratory scale.

sugarcane juice was subjected to clarification by sulfitation using sulfurous acid (analytical grade) to adjust the pH to 3.8. Then, the juice pH was neutralized by a calcium hydroxide suspension (milk of lime) to pH 7.2. For protein denaturation and promotion of phospholipid coagulation, the juice was preheated to 65 °C for 20 min, simulating the operating conditions of a sugar industry. The temperature was then raised to 103 °C for 2 min, to remove microbubbles dispersed in the cane juice. Then, different polyelectrolytes, at different dosages, were added to the pre-treated juice to analyse the sedimentation and purification efficiency and the treated juice was collected.

2.4.3. Total soluble solids (°Brix) of the sugarcane juice

Total soluble solids were measured for each independent juice obtained from sugarcane juice with or without treatment. Brix values were measured using a refractometer (RFM712 refractometer, Bellingham + Stanley Ltd., Tunbridge Wells, UK) expressed as °Brix (wt %) according to CONSECANA (2015) [23].

2.4.4. Conductivity of the sugarcane juice

Conductivity was measured for each independent juice obtained from samples before and after the treatment with the addition of the polyelectrolytes. The values were measured using a conductivity meter HI8820 N (Hanna Instruments Co., Portugal) and expressed as mS cm^{-1} according to the ASTM Method D1125-95 (1999).

2.4.5. Apparent sucrose content (pol%juice) of sugarcane juice

The analysis of pol%juice was done through the saccharimetric reading of the clarified samples, using a clarifying mixture based on aluminium, in a polarimeter model ADS420 (Bellingham + Stanley Ltd., Tunbridge Wells, United Kingdom), according to CONSEC-ANA (2015) [23]. The conversion of saccharimetric reading using the aluminium-based clarifying mixture (L_{Al}) to the equivalent reading in lead acetate (L_{Pb}) was done using equation (7). The clarifying step is necessary to remove the interference of solids on the optical measurement inside the polarimeter. The pol%juice (S) was expressed by equivalent reading in lead acetate (LPb) and initial total soluble solids (°Brix0) as wt% (or g of apparent sucrose per 100 g of cane juice, (equation (8)).

$$L_{Pb} = (1.00621 \times L_{Al}) + 0.05117 \tag{7}$$

$$S = L_{Pb} \times (0.2605 - (0.0009882 \times °Brix_0) \tag{8}$$

The purification index of sucrose in the sugarcane juice treated is given by equation (9):

$$Purification\ index\ of\ sucrose\ (\%) = \frac{(S_{final} - S_{initial})}{S_{initial}} \ x\ 100 \tag{9}$$

where S_{final} and $S_{initial}$ represent final (after the treatments by polyelectrolytes) and initial pol%juice (SCJ without treatment), respectively.

2.4.6. Turbidity measurements of sugarcane juice

The SCJ's turbidity was measured to determine the amount of cloudiness, and it was expressed as Nephelometric Turbidity Units (NTU). The turbidity of the samples was measured using a bench-top turbidimeter (Tecnopon TB1000, Brazil), properly calibrated against a curve at 0 to 1000 NTU [24]. The turbidity removal efficiency of the sugarcane juice treated is given by equation (10):

$$Removal\ turbidity\ efficiency\ (\%) = \frac{(residual\ turbidity - initial\ turbidity)}{initial\ turbidity} \ x\ 100 \tag{10}$$

2.4.7. Statistical treatment of the data

Data are presented as average ± standard deviation (minimum 3 measurements). Differences between average values of individual

Table 2
Characterization of the ADACs produced.

	ADAC Acacia-1	ADAC Bagasse -NaOH	ADAC Bagasse-3
Initial raw material			
Lignin (wt%)	2.83 (±0.18)	2.34 (±0.24)	0.84 (±0.02)
Cellulose (wt%)	86.88 (±0.71)	71.63 (±0.24)	84.71 (±1.11)
Hemicellulose (wt%)	2.75 (±0.01)	15.17 (±0.39)	4.67 (±0.03)
Aldehyde's introduction			
DS_{DAC}	1.06	1.28	0.94
Aldehydes (mmol g^{-1})	6.58	7.98	5.88
DS after anionisation reaction			
DS_{ADAC}	0.79	1.85	0.62
Anionicity index (mmol g^{-1})	3.25	5.25	2.74
Zeta potential (mV)	−22.4 (±1)	−36.8 (±2)	−29.3 (±2)

*At least three replicates were conducted for each test.

groups were assessed by one-way analysis of variance and Tukey's multiple range tests using the Excel package software. Differences were considered significant at $p \leq 0.05$.

3. Results and discussion

3.1. Synthesis and characterization of anionic of cellulose-based polyelectrolytes

Three anionic polyelectrolytes, containing different amounts of anionic modification, were successfully synthesized. They differ in the biomass origin and the fractionation treatment. Sugarcane bagasse was treated, in one of the tests, with the solvent mixture (ChCl: Lev in a 1:2 molar ratio for 4 h at 160 °C) and, in another test, with a 4% NaOH solution for 30 min at 121 °C [17], leading, after the modification, to ADAC Bagasse-3 and ADAC Bagasse-NaOH, respectively. Acacia wood was treated with ChCl:Lev in a 1:2 molar ratio for 4 h at 160 °C, leading, after modification, to ADAC Acacia-1. The characterization of the synthesized anionic polyelectrolytes is presented in Table 2.

Looking at Tables 2 and it is possible to conclude that it was always possible to introduce anionic groups in the cellulose backbone obtained from the extraction procedure applied to the sugarcane bagasse.

In addition, the results presented in Table 2 show that the extraction procedure used to produce ADAC Bagasse-3, using the eutectic solvent, led to a purer cellulose-rich fraction with a lower percentage of lignin and hemicellulose than the NaOH treatment of the bagasse. Even so, the final product does not show a higher degree of substitution than when using the other extracted materials. This is because both the origin of the raw material and the presence of hemicellulose in the raw material can play an important role in the final product characteristics. In fact, the presence of hemicellulose in the fractionated material seems to lead to higher DS in the final ADAC. The material used to produce ADAC Bagasse-NaOH presents the highest hemicellulose content, which seems to facilitate the chemical modification due to its branched structure and smaller DP, which allows a better interaction of the modification chemicals with the OH groups, due to the higher aqueous solubility compared to pure cellulose, as described by Bajpai (2018) [25]. Thus, both a higher aldehyde modification and final degree of substitution were obtained, even if, probably, the average molecular weight of the final materials can be smaller, compared to the other two samples.

3.2. Evaluation of the performance of cellulose-based polyelectrolytes in sugarcane juice treatment

The data from the sugarcane juice (SCJ) purification trials with cellulose-based polyelectrolytes was compared with data referring to untreated juice (raw juice) and juice treated with synthetic polyacrylamide-based polymer.

As a whole, the different polyelectrolytes, in the respective doses used, did not affect significantly the SCJ pH value, soluble solids content and conductivity. However, some statistical differences were observed by Tukey's test (at $p < 0.05$), which can be explained since the standard deviations calculated for the mean values of the treatments were large enough to denote the differences between the means of the different treatments (indicated by the different letters).

The most crucial parameter for sugar production is the apparent sucrose content, which indicates whether sucrose degradation occurred during the process. In the present study, we can conclude that, regardless of the polyelectrolyte and the doses used, there was efficient purification of the juice, resulting in increased sucrose purity. The reduction in soluble solids content can confirm this, representing a reduction of 1–3% points. The treatments with cellulose-based polyelectrolytes (regardless of the source of cellulose) were able to remove, initially, some soluble solids in the sugarcane juice, such as proteins, peptides, and phospholipids, etc, which is confirmed by the reduced soluble solids levels after the treatment.

In the production of crystal sugar, especially white sugar, an essential variable in the industrial process is the content of reducing sugars (for glucose and fructose in sugarcane see Xiao et al. (2017) [26] and Misra et al. (2022) [27]. Since commercial crystal sugar contains at least 99.3% of sucrose, the total glucose and fructose contents (i.e., reducing sugars) should be as low as possible. According to Ogando et al. (2022) [28], reducing sugars content in sugarcane juice was around 1.5 g L^{-1}. A possible increase in the reducing sugar content may be a consequence of two physiological factors: first, the sugarcane is still at an early physiological state or immature or in an advanced state of maturity or ageing; this increase may be associated with the action of hydrolytic enzymatic extracts from the sugarcane itself or to microorganisms presence that degrade sucrose as a carbon source for its development or production of protective metabolites, such as organic acids, exopolysaccharides (levan, dextran, etc.) [26,27]. According to the data obtained for the sugarcane juice used in the trials, the reducing sugar content was 1.32 ± 0.10 wt%, which is within the expected range of reducing sugars, i.e., 0.50 to 1.50 wt%. Furthermore, the sugarcane juice conventionally treated with the polyacrylamide-based synthetic polyelectrolyte also showed reducing sugar values within the expected range, i.e., 1.40 ± 0.10 wt%. The same is observed when using the cellulose-based polyelectrolytes, independently of the cellulose source, with lower values of reducing sugars obtained when using the ADAC polyelectrolytes. Additionally, in general, higher dosages of ADAC lead to lower values of reducing sugars, demonstrating that the bio-polyelectrolytes were capable of not altering the physiological environment of the sugarcane juice and thus contributed to avoid the sucrose degradation increasing the reducing sugar content.

The synthetic polyelectrolyte used in this work was a commercial polyacrylamide-based polymer characterized as an extended-chain anionic flocculant. Its mechanism of action-interaction has been characterized as bridging [29]. The reducing sugar content in the cane juice treated with polyacrylamide-based polymer showed a slight increase (5.7%) with a nominal value of 1.40 ± 0.10 wt%, compared to the raw SCJ. Polyacrylamide-based polymers contain a large number of carboxylic groups (-COOH) that, depending on the pH, can dissociate with the release of protons in the medium (H^+), reducing the pH of the medium and leading to acid hydrolysis of sucrose and release of glucose and fructose in the juice. Dawber et al., in 1966 demonstrated the mechanism and kinetics of sucrose

acid hydrolysis [30].

On the other hand, another route of sucrose hydrolysis associated with temperature increase has been reported by Richards and Shafizadeh in 1978 [31]. Nolasco and Massauger [32] demonstrated the thermal degradation of sucrose at temperatures of 110, 120, 130, and 140 °C. In conventional sugarcane juice treatment, the juice is heated with superheated steam at 103–107 °C. Simulated test protocols, with similar operating conditions to those employed in sugar mills, were applied in this study during the treatments with synthetic or cellulose-based polyelectrolytes. For the heat treatment, the juices in the trials were subjected to heating in an oil bath in two steps: preheating at 65 °C for 20 min followed by removal of microbubbles of air (flashing) at 103 °C for 2 min. Thus, it is very likely that thermal degradation of sucrose to glucose and fructose occurs initially. Subsequently, the monosaccharides undergo thermal degradation to low molecular weight compounds, or copolymerization to high molecular weight compounds, as reported by Aguiar et al. (2015) [33] and Rockland (1960) [34]. Conversely, cellulose-based anionic polyelectrolytes were shown to have little or no influence on sucrose hydrolysis, in the assays performed. For the three cellulose-based polyelectrolytes used, at different doses, the levels of reducing sugars were lower than those found for the raw juice, or the juice treated with the synthetic polyelectrolyte, on the order of 79.6% for ADAC Acacia-1; 78.0% for ADAC Bagasse-NaOH; and 60.6% for ADAC Bagasse-3, considering the best results for reducing sugars content when using the three ADACs at 100 mg of ADAC per kg of sugarcane juice.

It is important to note that analyzing the performance of the bio-based polyelectrolytes, even with a low dosage, 10 mg kg^{-1} (similar to the dosage of the synthetic polyelectrolyte), in the treatment of sugarcane juice, the obtained results showed an improved efficiency compared to the synthetic option, regardless of the variable analysed (see Table 4). In the case of the ADACs, concentrations lower than 10 mg kg^{-1} (not shown here), led to low total solids removal, in spite of the reasonable results for the other purification parameters.

If we consider the primary variable of this study, that is, the reduction in the turbidity of the sugarcane juice (Table 4), the efficiency of the bio-based polyelectrolytes varied in the range of 99.1%–99.8% for ADAC Acacia-1, between 99.1% and 99.7% for ADAC Bagasse-NaOH, and from 98.9% to 99.8% for ADAC Bagasse-3, which demonstrates very high efficiencies of the three bio-polyelectrolytes in the reduction of the turbidity. Fig. 3 presents an example of a microscope image (optical microscope Olympus BH2, Olympus Optical Co., Japan, coupled with a digital camera Olympus ColorView III) of a juice treated with ADAC Bagasse 3, confirming that flocculation occurred during the treatment. Large flocs are visible in the microscope image. In addition, it was observed that the efficiency to reduce turbidity changed only slightly with the cellulose-based polyelectrolyte dosage, the efficiencies observed being always higher than for the synthetic polyelectrolyte. For example, for ADAC Bagasse-3, the best turbidity reduction (99.8%) was with 250 mg kg^{-1} (Table 4), while it was observed an efficiency of 98.9% for the lowest dosage. So, it can be concluded that even ADACs concentration as low as 10 mg kg^{-1} present good efficiency in the SCJ treatment.

Rationalizing the mechanism of turbidity reduction, it is interesting to note that all the three bio-based polyelectrolytes presented similar efficiencies, even if having different DS and anionicity indexes, revealing that the charge of the polyelectrolyte is not the only factor to take into account in the turbidity reduction mechanism. Not only electrostatic interactions are involved in the flocculation mechanism, but also other interactions, such as hydrophobic interactions, which can play a key role in the flocculation process. An important factor influencing the polyelectrolyte characteristics and performance is that all initial raw materials used in the modification (cellulose fibres) presented a certain amount of lignin, which can positively impact the interaction between the bio-based polyelectrolytes and the particles in suspension in the sugarcane juice, such as proteins, peptides, and phospholipids, which are clearly amphiphilic molecules. The presence of lignin can induce hydrophobic interactions with this type of molecules and influence positively the performance of the bio-based flocculants [35,36].

The results obtained for reducing the sugarcane juice turbidity by using the cellulose-based polyelectrolytes, show a good correlation to the increase of apparent sucrose contents (Tables 3 and 4). In Tables 4 and it is possible to note that the reduction of sugarcane juice turbidity confirmed the removal of insoluble or soluble impurities by the joint action of polyelectrolyte addition and heating.
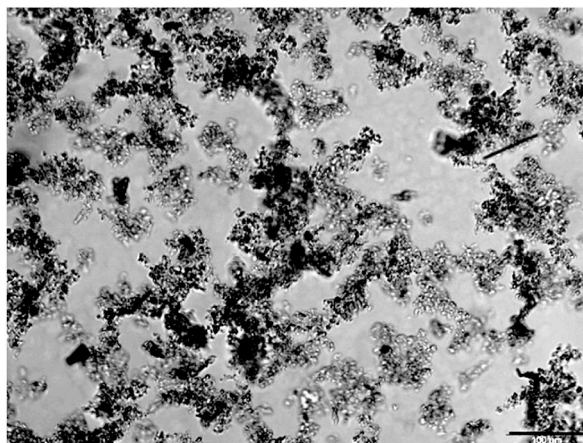

Fig. 3. Microscope image of SCJ flocs obtained after treatment with ADAC Bagasse 3.

Table 3
Results of sugarcane juice treatment using different polyelectrolytes.

	Polyelectrolyte concentration (mg kg^{-1})	pH	Total soluble solids (%)	Conductivity (mS cm^{-1})	Turbidity (NTU)	Apparent sucrose (wt%)	*Reducing sugars (wt%)*
Raw sugarcane juice (without the treatment)		5.2 ± 0.01 e	24.8 ± 0.01 a	1.96 ± 0.01 e	1287 ± 0.01 a	16.7 ± 0.4 c	1.32 ± 0.1 a
Synthetic polymer	6	7.1 ± 0.02 d	24.2 ± 0.01 b	2.11 ± 0.02 d	18.0 ± 0.80 b	19.4 ± 0.2 c	1.40 ± 0.1 a
ADAC Acacia 1	10	6.8 ± 0.03 c	23.7 ± 0.0 c	2.11 ± 0.01 d	8.5 ± 1.0 bc	21.7 ± 0.4 a	0.85 ± 0.1 b
	50	7.2 ± 0.04 a	22.0 ± 0.0 f	2.11 ± 0.01 d	12.2 ± 0.7 bc	19.5 ± 0.4 b	1.1 ± 0.0 b
	100	7.0 ± 0.01 b	23.2 ± 0.06 d	2.30 ± 0.02 c	2.4 ± 1.07 c	22.8 ± 0.8 a	0.27 ± 0.1 d
	250	7.0 ± 0.04 bc	22.4 ± 0.0 e	2.70 ± 0.03 a	5.4 ± 0.25 c	19.6 ± 0.5 b	0.62 ± 0.1 c
	500	7.3 ± 0.05 a	21.9 ± 0.0 f	2.60 ± 0.04 b	8.3 ± 0.25 bc	19.1 ± 0.4 b	0.66 ± 0.1 c
Standard deviation		0.02	0.04	0.02	4.14	0.46	0.07
MSD at 5%		0.05	0.12	0.06	11.53	1.27	0.19
CV (%)		0.27	0.19	0.89	2.16	2.35	7.81
ADAC Bagasse NaOH	10	6.9 ± 0.02 c	23.7 ± 0.0 c	2.11 ± 0.03 d	4.7 ± 0.13 c	19.8 ± 0.2 b	1.2 ± 0.0 ab
	50	7.2 ± 0.01 a	22.1 ± 0.06 e	2.11 ± 0.01 cd	10.5 ± 0.6 bc	18.9 ± 0.2 bc	1.1 ± 0.0 b
	100	6.8 ± 0.02 d	21.8 ± 0.0 f	2.30 ± 0.02 b	4.1 ± 0.66 c	21.3 ± 0.7 a	0.29 ± 0.1 d
	250	7.1 ± 0.01 b	22.3 ± 0.0 d	2.60 ± 0.02 a	5.9 ± 0.06 c	19.7 ± 0.4 b	0.61 ± 0.1 c
	500	7.0 ± 0.04 c	21.7 ± 0.06 g	2.60 ± 0.02 a	11.6 ± 0.46 bc	18.2 ± 0.8 cd	0.77 ± 0.1 c
Standard deviation		0.03	0.03	0.02	4.14	0.46	0.08
MSD at 5%		0.09	0.09	0.06	11.53	1.27	0.21
CV (%)		0.47	0.13	0.89	2.16	2.35	8.01
ADAC Bagasse 3	10	6.8 ± 0.05 d	23.7 ± 0.0 c	2.11 ± 0.02 e	14.5 ± 0.93 b	18.7 ± 0.1 ab	1.3 ± 0.0 a
	50	7.2 ± 0.02 b	22.0 ± 0.0 e	2.11 ± 0.01 d	14.1 ± 0.26 b	18.7 ± 0.2 b	1.1 ± 0.0 b
	100	7.1 ± 0.01 c	21.0 ± 0.0 g	2.30 ± 0.03 c	8.0 ± 0.52 bc	19.1 ± 0.4 ab	0.52 ± 0.1 c
	250	7.4 ± 0.04 a	22.4 ± 0.06 d	2.70 ± 0.03 a	2.4 ± 0.17 c	19.7 ± 0.5 a	0.64 ± 0.1 c
	500	7.1 ± 0.03 bc	21.2 ± 0.06 f	2.60 ± 0.01 b	7.0 ± 0.26 bc	18.3 ± 0.5 b	0.68 ± 0.1 c
Standard deviation		0.03	0.04	0.01	4.11	0.37	0.06
MSD at 5%		0.08	0.11	0.04	11.46	1.03	0.16
CV (%)		0.43	0.17	0.60	2.13	2.01	5.82

Different letters in the same column indicate significant statistical differences at $p < 0.05$ by Tukey test. Minimum Significant Difference (MSD) at 5% by Tukey test. Coefficient of variation (CV) expressed in percentage. NTU, nephelometric turbidity units. Apparent sucrose was determined by polarimetric analysis and expressed as g of sucrose per 100 g of cane juice.

4. Conclusions

In this work, anionic polyelectrolytes were successfully synthesized from Acacia wood and sugarcane bagasse, in a two-step process, and applied in sugarcane juice purification. The tests were performed for different polyelectrolyte concentrations (10, 50, 100, 250 and 500 mg kg^{-1}) and compared with the synthetic polyacrylamide-based polymer, normally used in these processes, at the most commonly used concentration (6 mg kg^{-1}). All flocculants could purify the juice regardless of the dose. However, in general, with the increase, above a certain level, of the concentration of flocculant added, the performance in the juice treatment decreases, and the concentration of the cellulose-based polyelectrolyte that presented the best results, regardless of the variable analysed, was 100 mg kg^{-1}, even if the differences in efficiency for the lower concentrations used were small. Overall, the results obtained with the bio-based polyelectrolytes were better than the ones obtained with the synthetic polyelectrolyte. Also, it was possible to conclude that the flocculation mechanism is not only based in electrostatic interactions, but also other interactions, such as hydrophobic interactions, can play here an important role. It is also important to note that the bio-based polyelectrolytes are eco-friendly and are expected not to have any kind of negative impact on nature or on the purified sugars, considering the high biodegradability. Additionally, no or very low toxicity is expected to be associated to these biopolymers, especially considering the washing procedures conducted during the modification process, even if further studies will have to be conducted, to assess this parameter in a more comprehensive way, including a full toxicological assessment of the new polymers, which is foreseen to be performed in the near future.

Lastly, it should be stressed that the results presented demonstrate the viability of using sugarcane bagasse, a residue from sugar

Table 4
Results of sugarcane juice treatment using different polyelectrolytes, turbidity removal.

	Polymer concentration (mg kg^{-1})	Total soluble solids removal (%)	Purification index of sucrose (%)	Turbidity removal efficiency (%)
Juice treated by synthetic polymer	6	7.1 ± 0.02 d	24.2 ± 0.01 b	98.6 ± 0.01 d
ADAC Acacia 1	10	4.4 ± 0.21d	29.9 ± 0.2b	99.3 ± 0.07b
	50	11.3 ± 0.17a	16.8 ± 0.3c	99.1 ± 0.03c
	100	6.5 ± 0.39c	36.1 ± 1.4a	99.8 ± 0.09a
	250	9.6 ± 0.21b	17.1 ± 0.4c	99.6 ± 0.02b
	500	11.6 ± 0.21a	14.0 ± 0.7d	99.4 ± 0.02b
ADAC Bagasse NaOH	10	4.5 ± 0.05c	18.6 ± 0.4b	99.6 ± 0.02a
	50	10.9 ± 0.19b	13.2 ± 0.1c	99.2 ± 0.04b
	100	12.0 ± 0.21a	27.5 ± 0.6a	99.7 ± 0.06a
	250	10.0 ± 0.21b	17.7 ± 0.6b	99.6 ± 0.00a
	500	12.5 ± 0.03a	8.6 ± 1.8d	99.1 ± 0.04b
ADAC Bagasse 3	10	4.5 ± 0.11e	12.0 ± 0.2c	98.9 ± 0.03b
	50	11.3 ± 0.21c	12.0 ± 0.1c	98.9 ± 0.03b
	100	15.2 ± 0.20a	14.4 ± 0.7b	99.3 ± 0.04a
	250	9.4 ± 0.22d	17.5 ± 0.0a	99.8 ± 0.01a
	500	14.3 ± 0.22b	9.7 ± 0.5d	99.5 ± 0.02a

Different letters in the same column indicate significant statistical differences at $p < 0.05$ by Tukey test.

production, to produce a polyelectrolyte that can be used in sugarcane juice treatment, even if a full economic evaluation is still required, which must also take into account the possibility of recycling/reusing the excess chemicals from the different steps, including solvents. This strategy is fully aligned with the principles of a circular bioeconomy.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available …

Declaration of competing interest

The authors have no special declaration of interest to declare.

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