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Supercritical water treatment for cello-oligosaccharide production from microcrystalline cellulose

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ABSTRACT

Microcrystalline cellulose was treated in supercritical water at 380 °C and at a pressure of 250 bar for 0.2, 0.4, and 0.6 s. The yield of the ambient-water-insoluble precipitate and its average molar mass decreased with an extended treatment time. The highest yield of 42 wt % for DP2-9 cello-oligosaccharides was achieved after the 0.4 s treatment. The reaction products included also 11 wt % ambient-water-insoluble precipitate with a DP_w of 16, and 6.1 wt % monomeric sugars, and 37 wt % unidentified degradation products. Oligo- and monosaccharide-derived dehydration and retro-aldol fragmentation products were analyzed via a combination of HPAEC-PAD–MS, ESI-MS/MS, and GC–MS techniques. The total amount of degradation products increased with treatment time, and fragmented (glucosyl$_n$-erythrose, glucosyl$_n$-glycolaldehyde), and dehydrated (glucosyl$_n$-levoglucosan) were identified as the main oligomeric degradation products from the cello-oligosaccharides.

1. Introduction

Cellulose, being globally and abundantly available, is probably the most important biopolymer that is currently used in a wide variety of applications such as paper products, textile fibers and for the production of biofuels and platform chemicals.[1] Cellulose is also the natural source for the manufacture of cello-oligosaccharides. In general, oligosaccharides are water-soluble saccharide polymers, typically consisting of two to ten monosaccharide units, although the definition varies. An example of the possible applications for oligosaccharides is their use as prebiotics that are added in increasing quantities to foods with the focus on beverages and milk products.[2–4] By definition, prebiotics are food ingredients that are not digested by humans and have zero metabolizable energy value. Instead they provide a source of carbon for the intestinal microflora stimulating the beneficial bacteria in the colon.[2] Not all oligosaccharides are, however, recognized or used as prebiotics and this applies also to cello-oligosaccharides.[2] Yet there is a reason to believe that cello-oligosaccharides are potential prebiotics because the human digestion system lacks the enzyme that is required to hydrolyze the β-glucosidic bond in the cello-oligosaccharides.[3] Fermentation activity in the human intestine was reported for cellobiose indicating that it acts as energy source for certain bacteria.[5]

An obvious reason for the lack of extensive research on cello-oligosaccharides is their limited availability and high price, which is caused by cellulose's recalcitrant nature compared with other polysaccharides like starch. Cellulose is a linear polysaccharide consisting of β(1 → 4) linked D-anhydroglucopyranose units (AGU) in which every second AGU is rotated 180° in the plane, adjacent units forming a cellobiose. Cellulose exists as a polymer with the degree of polymerization (DP) up to 10,000 AGUs.[1] In the naturally existing cellulose I allomorph, the cellulose chains are aligned parallel, forming sheets which are stacked on top of each other, thus forming ordered crystalline domains interrupted by less ordered domains.[1] In the crystalline domains, a rigid intra and interchain hydrogen bond network is formed in the cellulose sheets whereas these sheets are held together by Van der Waals—forces which were in fact reported to be the pivotal factor for cellulose recalcitrance.[6] Also hydrophobic interaction, which is caused by the affinity of water molecules to each other, is important regarding the insolubility of cellulose in aqueous systems.[7] As a result, in order to produce water-soluble cello-oligosaccharides, the cellulose must be depolymerized in a controlled manner which will render it water-soluble. The challenge is to hydrolyze cellulose as oligomers instead of obtaining mere monomers, which

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is typically not possible via acid-catalyzed hydrolysis at near-ambient temperatures. Therefore, a separate dissolution step is typically needed to create a homogeneous environment for the hydrolysis of cellulose to oligosaccharides.

In the early 1990s it was discovered that crystalline cellulose can be converted to water-soluble species via a noncatalytic subcritical or supercritical water treatment.[8] This process has the advantage that the hydrolyzed cellulose is dissolved not as a monomer but rather as oligomers or short polymers whose DP depends on the treatment temperature.[9] No actual swelling and dissolution of the cellulose crystallites take place in subcritical water at least below 300 °C,[10] while at temperatures higher than 300 °C and at a pressure of 25 MPa, a crystalline-to-amorphous transformation was reported.[11] This transformation results in a rapid destruction of the crystallites in near critical and supercritical water.[12–14] When the temperature reaches the critical point (374 °C and 22.1 MPa) at a pressure of 25 MPa, the internal energy of the system increases almost stepwise and the physical properties of water are drastically changed. These factors were attributed to a stepwise acceleration of cellulose dissolution.[12,14] Overall, the rate of cellulose dissolution increases faster than the corresponding rate of degradation of sugar, which enables the recovery of the dissolved compounds in high yields.[12]

Although supercritical water treatment was shown to dissolve and hydrolyze cellulose in one stage without a dedicated cellulose solvent, several degradation reactions concomitantly occur during the supercritical water treatment. These formed reaction products may have an effect on the use of the produced oligosaccharides in applications where a high purity is required. In subcritical water the proton concentration is higher than in ambient water catalyzing hydrolytic depolymerization and dehydration; in near- and supercritical water dehydration and fractionation via retro-aldol reactions become more prominent favoring reaction products such as levoglucosan, 5-hydroxymethylfurfural, erythrose, methylglyoxal, glycolaldehyde, and dihydroxyacetone.[12,15,16] These degradation reactions are not restricted to monosaccharides but take place also at the reducing end groups of polysaccharides.[17]

In this study we investigated the dissolution and depolymerization of microcrystalline cellulose to water-soluble cello-oligosaccharides and the formation of degradation products thereof. The yields and molar mass distributions were determined from ambient-water-soluble oligosaccharides and cellulose precipitate which was insoluble in ambient water. The formed monomeric and oligomeric degradation products were investigated by HPAEC-PAD–MS, ESI-MS/MS, and GC–MS techniques.

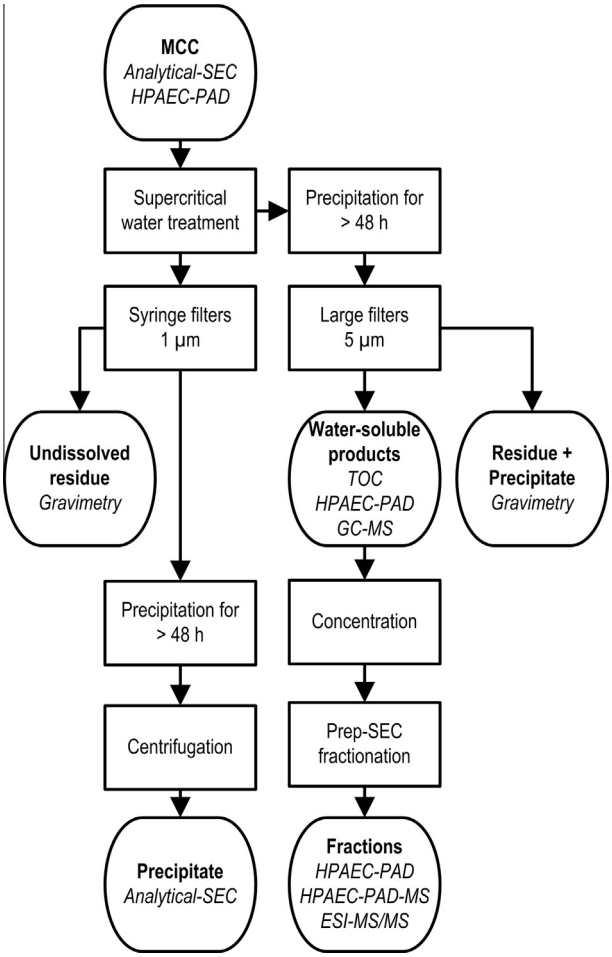
2. Experimental

Commercial microcrystalline cellulose (MCC) powder was purchased from Merck and used as raw material. The mass average molar mass of the MCC was 32.9 kg mol^{-1} and polydispersity index 2.03.[10] The carbohydrate composition of the MCC after total hydrolysis was 97.8% glucose, 1.0% xylose, and 1.2% mannose, analyzed by HPAEC-PAD with a CarboPac PA20 column after the hydrolysis to monosugars as described earlier.[18] Water–MCC suspension was prepared by mixing the MCC powder thoroughly with deionized water. Nitrogen purging was applied in order to remove oxygen from the suspension. The prepared cellulose suspension was treated with a bench-scale tubular flow reactor system described earlier.[13] Three experiments were conducted at the temperature of 380 °C employing treatment times of 0.20 s, 0.40 s, and 0.60 s. The pressure was held at 25.0 MPa in all experiments. The concentration of the cellulose suspension was 0.50 wt % in the feeding tank and 0.20 wt % in the beginning of the supercritical water treatment after dilution by supercritical heating water.

The overall sampling and analysis procedure is illustrated in Scheme 1. In order to avoid the earlier reported problem with the re-deposition of precipitated cellulose, the time required for the removal of the undissolved residue was reduced to approximately 15 s.[13] The solution from the reactor's outlet was taken into a 10 mL syringe and immediately pressed through a syringe filter (Acrodisk, PN4523T). In total 50 mL of reaction product solution was filtered in this way. The syringe filters were then dried at 105 °C, and the amount of undissolved cellulose residue was determined gravimetrically. The filtered solution was stored in a cold room to allow the precipitation of cellulose take place. The formed precipitate was separated by centrifugation for analytical size exclusion chromatography.

Besides the samples collected with the syringe filters, several liters of reaction solution were collected and stored in a cold room for more than 48 h. During that time nearly all of the dissolved cellulose chains precipitated. The solid fraction containing the undissolved residue and the formed precipitate was removed using filters (Whatman Polycap heavy duty 5.0/10.0 μm). The filters were dried overnight at 105 °C and the total amount of undissolved residue and precipitate was determined gravimetrically. The amount of cellulose precipitate was obtained by subtracting the amount of undissolved residue from the total amount of residue and precipitate.

A part of the 0.4 s treated, filtrated sample was fractionated by preparative-scale size exclusion chromatography (Prep-SEC). First the sample was concentrated to 1:20 of its volume by a rotary evaporator (55 °C and 80 mbar) to compensate for the dilution in



Scheme 1. Experimental procedure. Conducted analyses indicated by italics.