Salt-assisted organic-acid-catalyzed depolymerization of cellulose

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Dicarboxylic acids (*e.g.* oxalic and maleic acid) are able to depolymerize cellulose, producing oligomers and glucose. However, to reach efficient organic-acid-catalyzed performances with crystalline celluloses, high temperatures (>160 °C) are needed. These energetically-demanding conditions lead to undesired sugar degradation as well. Herein it is shown that organic acid-catalyzed cellulose depolymerization can proceed efficiently in water under mild reaction conditions (100–125 °C) by the addition of inexpensive NaCl (30 wt%). The application of some pressure in the reactor (10–30 bar) also influences and improves the depolymerization outcome. It is believed that the salt solutions act in a mechanism similar to ionic liquids and disrupt the hydrogen-bond matrix among cellulose fibers. Depolymerization proceeds efficiently with amorphous cellulose, α-cellulose, as well as with microcrystalline cellulose (Avicel®). Importantly, catalysis can be easily controlled by temperature, catalyst loading and salt concentrations, as well as by the applied pressure in the reactor, and thus sugar degradation can be diminished. Furthermore, experiments conducted using concentrated seawater as solvent and maleic acid as catalyst showed positive results in the hydrolysis of Avicel®.

1. Introduction

There is presently a great interest in developing strategies for the processing of lignocellulosic materials, with the goal of generating chemicals and fuels from renewable feedstocks that could replace fossil resources.[1] In this area one crucial step that needs to be addressed is efficient cellulose depolymerization, since that would enable the production of fermentable sugars and oligomers at low cost and high yields.[2] However, because of its inherent stable structure, especially crystalline cellulose is a challenging material from a chemical-processing point of view.[3]

Several alternatives for cellulose depolymerization have been put forward, like, inorganic acid treatment (*e.g.* H_2SO_4, HCl, H_3PO_4) at high temperatures (>150 °C), or biocatalysis (cellulases).[1,2] In this area, ionic liquids have emerged as alternative solvents to overcome the challenge of catalyst accessibility in crystalline celluloses.[4–6] It is believed that ions from the ionic liquid interact with the extensive hydrogen bonding of cellulose fibers, breaking the inter- and intramolecular net and therefore facilitating its dissolution. Another approach for cellulose processing is the addition of some inorganic salts (*e.g.* LiCl, $ZnCl_2$, $CaCl_2$) during strong inorganic acid catalysis in aqueous media. This leads to the swelling or even dissolution of the cellulose slurry, thus increasing the cellulose hydrolysis by mineral acids. The action of the salt is believed to be analogous to that of ionic liquids, and the penetration of ions among cellulose fibers disrupts the hydrogen bonding network.[1a,7]

Apart from mineral acids and enzyme catalysts, some organic dicarboxylic acids (*e.g.* oxalic, maleic, fumaric) have been studied for cellulose depolymerization as well.[8,9] Dicarboxylic acids present lower pK_a values than monocarboxylic organic acids, which may explain their performance as catalysts for polysaccharide depolymerization.[8g,9a] Remarkably, it has also been suggested that the mechanism of these dicarboxylic acids could be analogous to that of glycosidases (*e.g.* cellulases). These enzymes harbour two carboxylic groups within the active site, usually either aspartic or glutamic amino acidic residues, hydrolyzing the glycosidic linkage in a selective, concerted way (Scheme 1).[9]

However, apart from the two amino acidic residues, the remaining protein scaffold of cellulases is also crucial for an adequate micro-environment to afford polysaccharide hydrolysis at room temperature. In contrast, to efficiently process crystalline celluloses with simple dicarboxylic acids, very high temperatures (>160 °C) must be applied.[8,9] These conditions also lead, however, to unselective sugar degradation, making the process less attractive.[8c] At milder conditions (*e.g.* 90–130 °C), organic acids are unable to process packed celluloses to oligomers or fermentable sugars in an efficient way, and only more amorphous regions are somewhat prone to depolymerization.[8,9]

Despite the beneficial effects of dicarboxylic acids, when compared with mineral acids, the combination of organic acids with inorganic salts in aqueous media has not been studied for cellulose depolymerization. A potential synergy between non-hazardous and readily available salts like NaCl with (bio-based) dicarboxylic acids could be relevant for the

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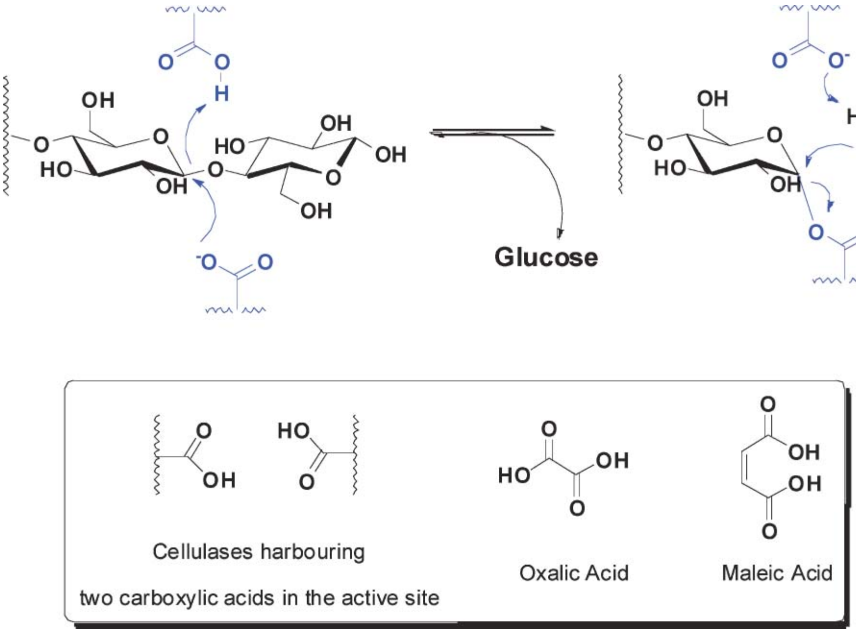

Scheme 1 Postulated mechanism for the hydrolysis of a glycosidic bond, catalyzed by glycosidases harbouring two carboxylic acid residues in the active site. It has been suggested that dicarboxylic acids might mimic these biocatalysts.[9]

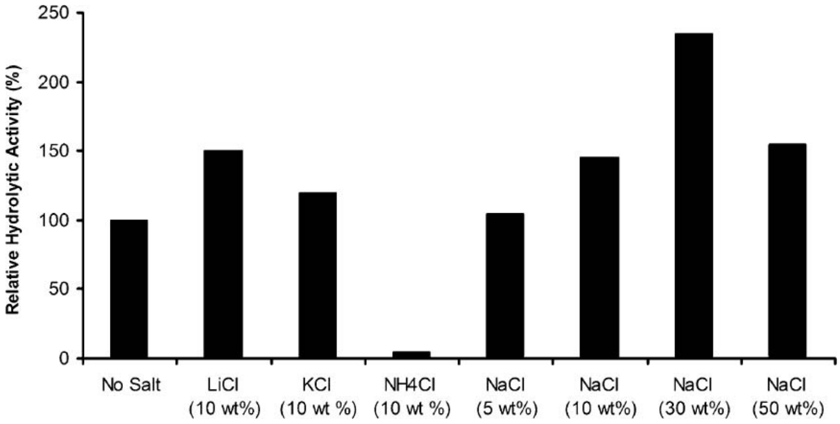

Fig. 1 Relative activities in amorphous-cellulose depolymerization catalyzed by oxalic acid (0.1 M) alone, or in combination with different salts. Conditions: salt (5–50 wt%), 105 °C (oil bath). Amorphous cellulose (20 g L^{-1}, slurry). Activity measured as amount of reducing-end sugars in water (PAHBAH). 100% activity is defined as the hydrolysis catalyzed by oxalic acid (0.1 M) without addition of salts.

cellulose-processing arena, especially if mild reactions conditions, less energy demand, and therefore less by-product formation, could be achieved. Herein we wish to report on our results in this area.

2. Results and discussion

Three commercially available cellulose types (amorphous PASC cellulose, α-cellulose, and microcrystalline Avicel®) were tested. To measure the depolymerization, two approaches were followed: colorimetric PAHBAH method for determination of reducing-end sugars (oligomers),[10] and a commercially available glucose kit for the glucose measurement as final product in cellulose depolymerization (see experimental section). Initially, oxalic acid was tested as prototypical bifunctional organic acid (Scheme 1).

At 105 °C in pure water, oxalic acid (0.1 M) showed activity in the hydrolysis of amorphous PASC cellulose. Therefore, we firstly studied the influence of the addition of different inorganic salts (5–50 wt%) in the oxalic-acid-catalyzed hydrolysis (Fig. 1).

Remarkably in all cases the addition of different alkaline chlorides (LiCl, KCl, NaCl, 10 wt%) leads to a more efficient processing of amorphous cellulose catalyzed by oxalic acid. Notably, it was observed that NaCl is practically as effective as LiCl as ionic additive for cellulose hydrolysis. This is somewhat surprising, since NaCl has been reported to be not effective as additive for cellulose depolymerization when inorganic acids (such as HCl) are used as catalysts.[7b] Studying the influence of the amount of NaCl in the range from 5 to 50 wt% the maximum effect was noted at 30 wt% NaCl, where more than 2 fold improvement was achieved (Fig. 1). Considering availabilities and costs of both LiCl and NaCl, it is obvious that the combination of organic dicarboxylic acids with NaCl may provide a promising tool for processing cellulosic residues under mild reaction conditions. From a practical perspective, as previously shown in the literature for analogous salt-based protocols, salt removal should be efficiently afforded by commercially applied technologies, like electrodialysis.[7b]

Following the promising results with amorphous PASC cellulose, the next step was to study the oxalic-acid catalyzed hydrolysis of more crystalline α-cellulose at 105 °C in water, with or without addition of NaCl (30 wt%) (Fig. 2).

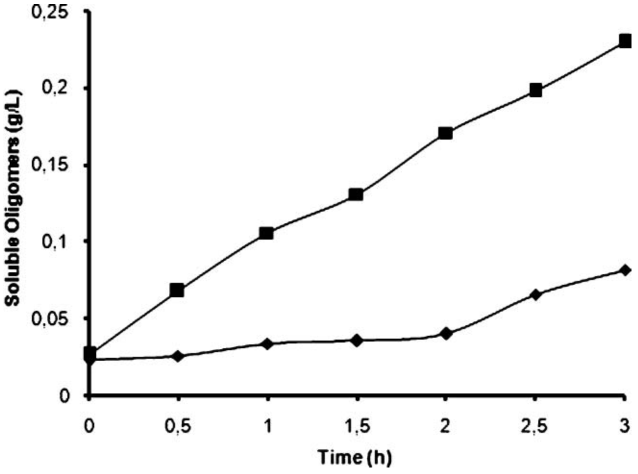

Fig. 2 Comparison of depolymerization of α-cellulose (20 g L^{-1}) at 105 °C (oil bath) with oxalic acid as catalyst in water. (◆) Only oxalic acid (0.1 M); (■) Oxalic acid (0.1 M) and NaCl (30 wt%). Hydrolysis measured as the increase of reducing sugars (PAHBAH).

As shown in Fig. 2, at 105 °C oxalic acid alone is not effective for the hydrolysis of α-cellulose, since this derivative presents a more crystalline structure than the amorphous cellulose. This is consistent with literature reports, where higher temperatures (>160 °C) have been applied for processing crystalline celluloses with organic acids.[8,9] However, it is interesting to note that after 2 h reaction time some slight α-cellulose hydrolysis started. Presumably this may be due to production of some (more or less soluble) oligomers from the less crystalline regions, which are further hydrolyzed by oxalic acid in the aqueous solution, increasing the yield of reducing-end sugars. In any case, a major improvement is observed when NaCl is added, since a constant, efficient α-cellulose depolymerization is achieved. It is supposed that the two components act in a concerted manner: while the salt ions are breaking hydrogen bonds among cellulose fibers, oxalic acid affords depolymerization under mild reaction conditions. Due to these mild reaction conditions no significant glucose degradation is observed, in agreement with previous reports from literature at these mild temperatures (<130 °C).[8,9]

Finally, the microcrystalline Avicel® was tested under the new reaction conditions. Avicel® is a highly crystalline form of cellulose produced by hydrolysis of wood in refluxing acid. In this process, any amorphous cellulosic material is hydrolyzed, and the remnant, which is highly resistant to acid hydrolysis, is filtered, washed, and dried to yield the Avicel®.[1c] For an efficient Avicel® hydrolysis, either high temperatures (>160 °C),[4e] or its previous dissolution in ionic-liquids followed by solid-acid hydrolysis, have been proposed.[4e] For the present study the challenging substrate Avicel® was subjected to the same hydrolytic conditions as α-cellulose, that is oxalic acid (0.1 M) in water, at 105 °C, with or without addition of NaCl (30 wt%). Results are shown in Fig. 3.

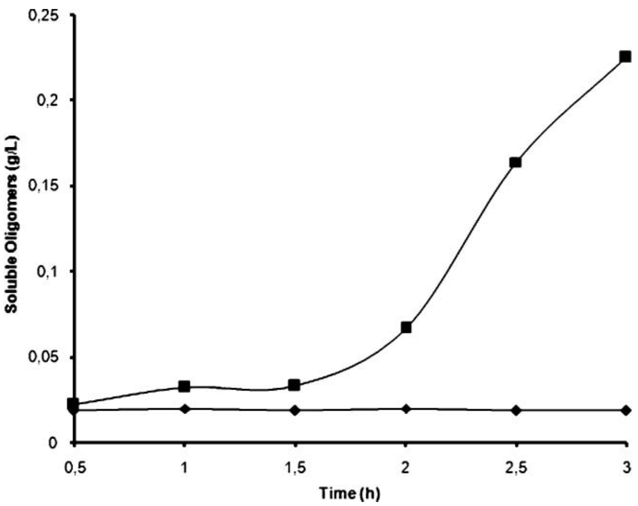

Fig. 3 Comparison of depolymerization of microcrystalline cellulose (Avicel®) at 105 °C (oil bath) catalyzed by oxalic acid in water. (◆) Only oxalic acid (0.1 M); (■) Oxalic acid (0.1 M) and NaCl (30 wt%). Hydrolysis measured as the increase of reducing sugars (PAHBAH).

As seen from Fig. 3, the synergy of NaCl and oxalic acid also enables Avicel® depolymerization under very mild reaction conditions in water. When the hydrolysis of Avicel® was carried out at a slightly higher temperature of 125 °C (oil bath), it was already possible to detect not only oligomers, but also some glucose in the reaction system (Fig. 4). Starting from cellulose, glucose is the final product in the depolymerization (cellulose to oligomers to finally glucose). Thus, results suggest an appreciable hydrolytic rate.

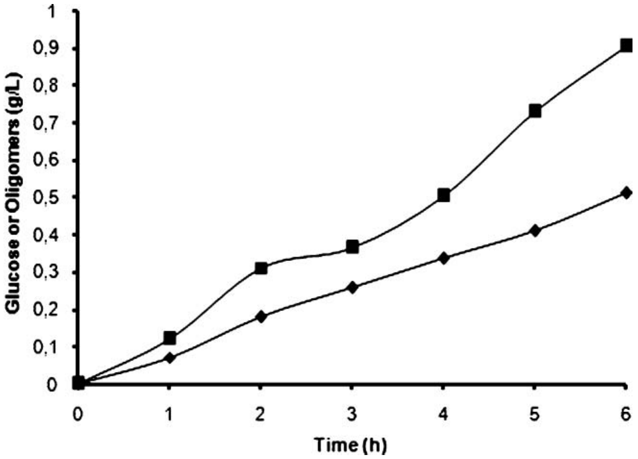

Fig. 4 Formation of soluble oligomers (reducing-end sugars) (■), and glucose (◆) in the hydrolysis of Avicel® (20 g L^{-1}) at 125 °C under reflux conditions, using a combination of oxalic acid (0.1 M) and NaCl (30 wt%). Hydrolysis measured as the increase of reducing sugars by PAHBAH method (oligomers), and glucose quantification by an enzymatic Glucose (HK) Assay Kit (GAHK20). 125 °C measured in oil bath, a temperature of 106 °C is measured within the reactor.

To explore the scope of potential diacidic catalysts, other organic acids like maleic acid, succinic acid and citric acid were investigated as well. Both succinic and citric acid displayed very low hydrolysis rates under the studied reaction conditions (data not shown). Herein the differences in pK_a among the different dicarboxylic acids (higher values for citric or succinic than for maleic or oxalic) should be considered as a possible explanation of the poor results.[8g,9a] Conversely, maleic acid was active in the hydrolysis of Avicel® both in combination with NaCl, as well as with $CaCl_2$. As observed, rates of maleic acid-catalyzed hydrolysis were only slightly lower to that of oxalic acid when the same reaction conditions (temperature and catalyst, salt and cellulose loading) were applied (Fig. 5).

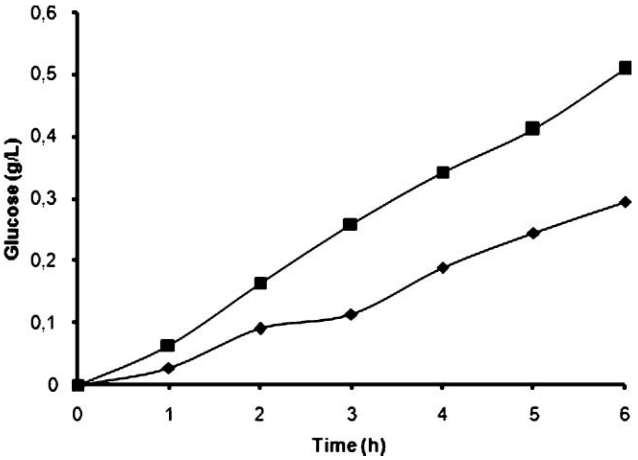

Fig. 5 Formation of glucose in the hydrolysis of Avicel® (20 g L^{-1}) at 125 °C under reflux conditions, using maleic acid (0.1 M) as catalyst, and different inorganic salts. (30 wt%). (■) $CaCl_2$; (◆) NaCl. Hydrolysis measured as glucose quantification by an enzymatic Glucose (HK) Assay Kit (GAHK20). 125 °C measured in oil bath, a temperature of 106 °C is measured within the reactor.

Importantly, due to precipitation in the form of calcium oxalate, oxalic acid could not be used in combination with $CaCl_2$ as salt additive. The use of maleic acid might thus open other research lines, by combining that acid with different salts from NaCl. In that respect, preliminary experiments by directly using seawater (NaCl *ca.* 4 wt%) as reaction media for the salt-assisted organic acid-catalyzed concept were conducted. Due to the fact that some Ca^{2+} is also present in seawater (together with Mg^{2+}, SO_4^{2-}, *etc.*), experiments with oxalic acid did not provide satisfactory results. Therefore, maleic acid (0.1 M) was used as catalyst for the depolymerization of a slurry of Avicel® (Fig. 6).

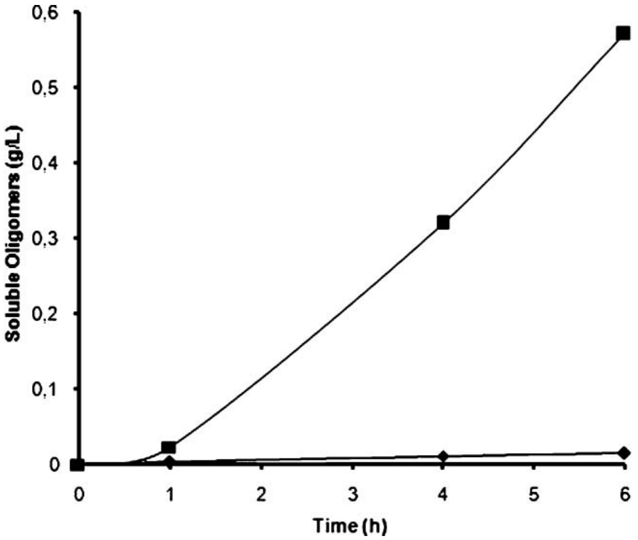

Fig. 6 Maleic-acid-catalyzed Avicel® depolymerization using seawater as reaction medium. Conditions: Avicel® (20 g L^{-1}), maleic acid (0.1 M), reflux, 125 °C. (◆): Reaction performed directly in seawater; (■): Reaction performed in concentrated (*ca.* 5×) seawater. 125 °C measured in oil bath, a temperature of 106 °C is measured within the reactor.

By directly using seawater a low activity in Avicel® hydrolysis was observed, as expected by our previous results on salt screening (see above). Thus seawater was concentrated *ca.* 5-fold to *ca.* 20 wt% NaCl (by evaporating water). Under the new conditions hydrolytic activity was observed (Fig. 6), and thus maleic acid depolymerizes Avicel® in concentrated seawater. It is important to state, however, that seawater is composed not only of NaCl, but also of many other inorganic ions. Therefore it is possible that those other ions can also play a role in the disruption of hydrogen bonding net in crystalline cellulose fibers, and thus a direct comparison of data obtained in seawater experiments with the other values reported in this paper (water and NaCl) might not be appropriate. From a practical perspective the use of (concentrated) seawater as reaction media might open novel leads in biomass processing (*e.g.* for marine algae technology).

Experiments conducted in Fig. 4–6 were performed in open vessels under refluxing conditions. A further improvement in cellulose formation was observed when the reactions were conducted in closed reactors, where some pressure is created at those temperatures. This was further studied by performing experiments of Avicel® hydrolysis with oxalic acid (0.1 M) at different temperatures (85–125 °C). As observed in Fig. 7, after 2 h reaction a higher amount of glucose was observed than compared with reflux systems (Fig. 4–6).

The pressure in the closed reactor amounted to about 6–8 bar under these conditions. In order to investigate the pressure influence without further increasing the temperature, 30 bar of CO_2 pressure was applied in a 10 mL stainless steel high pressure reactor. Relevant results of the pressure influence are summarized in Table 1.

From data reported in Table 1, it can be confirmed that it is only the combination of an organic acid together with an inorganic salt that leads to an efficient Avicel® depolymerization under mild reaction conditions. Furthermore, the application of pressure in the reaction enhances significantly the glucose production, in particular when applied through additional overpressure of CO_2. Thus, under 30 bar of CO_2 and at 125 °C, the simple oxalic acid/NaCl system provides yields in glucose of ~ 2.8–3.9 g L^{-1}, corresponding to *ca.* 15–20% of total glucose from Avicel® in 6 h. Overall, the judicious combination of organic acids, inorganic salts and applied pressures might open relevant options in the processing of bio-based materials, once the herein reported concepts are optimized and properly integrated in an adequate process set-up.

Table 1 Glucose concentration of Avicel® processing experiments carried out at different pressure conditions: reflux (ambient pressure), closed reactor, and standard 10 mL stainless steel high pressure reactor, applying 30 bar of CO_2 pressure. Different inorganic salts were added (30 wt%), as well as different organic acids (0.1 M). Heating bath temperature: 125 °C. Data are at 6 h reaction.

Entry	Salt (30 wt%)	Process set-up (Pressure, bar)	Catalyst (0.1 M)	Glucose/g L^{-1}[a]
1	NaCl	Reflux	Oxalic	0.50
2	NaCl	Reflux	Maleic	0.30
3	$CaCl_2$	Reflux	Maleic	0.50
4	NaCl	Closed reactor	Oxalic	1.90
5	NaCl	Closed reactor	Maleic	1.50
6	$CaCl_2$	Closed reactor	Maleic	1.60
7	No Salt	High-pressure reactor, CO_2 (30 bar)[b]	Oxalic	0.20
8	NaCl	High-pressure reactor, CO_2 (30 bar)[b]	Oxalic	2.80–3.90[c]
9	NaCl	High-pressure reactor, CO_2 (30 bar)[b]	No Catalyst	0.00

[a] Full conversion in glucose expected at *ca.* 22 g L^{-1}. [b] Pressure of 20 bar at room temperature, at 125 °C, *ca.* 30 bar pressure is reached. [c] Value range from several independent experiments.

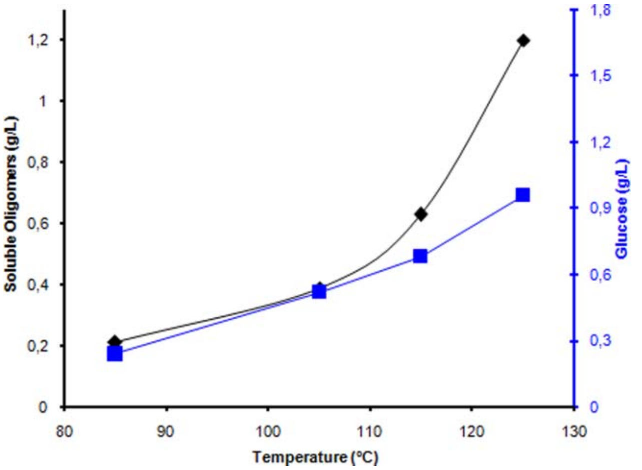

Fig. 7 Concentration of soluble oligomers (reducing-end oligomers) (◆), and glucose (■) at 2 h reaction at different temperatures (85–125 °C) (oil bath) in water. Conditions: Avicel® 20 g L^{-1}, oxalic acid (0.1 M), NaCl (30 wt%), closed reactors. Hydrolysis measured as the increase of reducing sugars by PAHBAH method (oligomers), and glucose quantification by an enzymatic Glucose (HK) Assay Kit (GAHK20).

3. Experimental

3.1. Chemicals

Amorphous cellulose (phosphoric-acid-swollen-cellulose, PASC), α-cellulose, and Avicel® (PH-101), NaCl, $CaCl_2$, oxalic acid, maleic acid, succinic acid, and citric acid were purchased from *Sigma-Aldrich*. Glucose (HK) Assay Kit (GAHK20) and reagents for PAHBAH method were obtained from *Sigma-Aldrich* as well. Seawater was collected in Playa de las Canteras (Gran Canaria, Canary Islands, Spain), and stored at 4 °C until its use.

3.2. Standard reaction conditions

Slurries of cellulose (20 g L^{-1}) were suspended in water, in which different amounts of dicarboxylic acids and salts were aggregated. Refluxing systems, closed reactors, or 10 mL stainless steel high-pressure reactors were used. Reactions were magnetically stirred at different temperatures.

3.3. Reducing-end sugars determination

A colorimetric method, PAHBAH, was used as reported in literature.[10] A calibration curve was recorded taking glucose as standard substrate. All samples were diluted until absorbance values were within the linear region of the curve. Equation for linear region: Absorbance = 0.66[oligomers] + 0.028(r^2 = 0.997).

3.4. Glucose determination

As stated before, a commercially available enzymatic glucose kit (GAHK20) was used. A calibration curve was recorded taking glucose as standard substrate. All samples were diluted until absorbance values were within the linear region of the curve. Equation for linear region: Absorbance = 4.36[Glucose] + 0.14(r^2 = 0.996).

4. Conclusions

The application of aqueous solutions of simple organic acids and inorganic salts might open a new approach in the field of cellulose depolymerization, in which environmental concerns can be tackled, while simultaneously keeping process costs (energy, substrates, catalysts, *etc.*) at an acceptable level. Mechanistically, salts are believed to act as ionic liquids do, disrupting the massive hydrogen bond network of cellulose fibers, and therefore allowing the hydrolysis catalyzed by oxalic or maleic acids. Furthermore, preliminary results suggest that (more or less concentrated) seawater may be another useful reaction media for cellulose depolymerization. It is obvious that more research is needed in the area to provide a clear picture on the synergistic action of the components of the catalytic system, and to explore the practical potentialities that this approach may have.

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