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Biomass Electrochemistry : from cellulose to sorbitol

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6

Cellobiose hydrolysis and decomposition by electrochemical generation of acid and hydroxyl radicals

Abstract

This chapter addresses the hydrolysis of cellobiose to glucose and its further decomposition with electrochemically generated acid (H^+) on a platinum electrode, and with electrochemically generated hydroxyl radical ($OH\bullet$) on boron-doped diamond (BDD). Results are compared with the hydrolysis promoted by conventional acid (sulfuric acid) and $OH\bullet$ (from Fenton's reaction) with support of product analysis by online high-performance liquid chromatography (for soluble products) and online electrochemical mass spectrometry (for CO_2). Cellobiose hydrolysis follows a first-order reaction obeying Arrhenius' law over the temperature range from 25°C to 80°C with different activation energies for the acid- and radical-promoted reaction, i.e. ca. 118 ± 8 kJ mol⁻¹ and ca. 55 ± 1 kJ mol⁻¹, respectively. The high local acidity with electrochemically generated acid (H^+) on the Pt electrode increases the rate of glucose formation, however the active electrode (PtO_x) interacts with glucose and decomposes it further to smaller organic acids. In addition, the molecular oxygen formed during oxygen evolution reaction (OER) lowers the selectivity for glucose by forming side-products. The hydroxyl radical ($OH\bullet$) generated on a BDD electrode first hydrolyzes the cellobiose to glucose, but rapidly attacks the aldehyde on glucose, which is further decomposed to smaller aldoses and finally formaldehyde, which is subsequently oxidized electrochemically to formic acid.

The contents of this chapter have been published: Y. Kwon, S. E. F. Kleijn, K. J. P. Schouten, M. T. M. Koper, *ChemSusChem*, **2012**, 5, 1935-1943.

6.1 Introduction

Cellulose is the most abundant source of biomass, and holds widespread recognized potential as an alternative carbon source to fossil fuels for the sustainable production of fuels and chemicals.^[1-3] However, the utilization of cellulose as a major renewable energy source is restricted by its crystalline structure of β -1-4-glycosidic linkages by the tight packing of cellulose chains in microfibrils.^[2] Although the hydrolysis of cellulose produces glucose, which is a versatile precursor to fuels, plastics, pharmaceuticals, and other value-added chemicals, the efficient hydrolysis of cellulose to glucose with low environmental impact remains a challenge.^[4] An extensive number of works have been devoted to the hydrolysis of cellulose using enzymes, mineral acids, supercritical water, and heterogeneous catalysts.^[5-10] However, the application of electrochemical methods for cellulose hydrolysis has been limited mainly by the low solubility of cellulose in aqueous media.^[2] Baizer and Nobe^[11] introduced many years ago the acid-catalyzed cellulose hydrolysis by electrochemically generated acid (H^+) at the platinum anode in an electrochemical cell. Recently, Li^[12] reported the effect of hydroxyl radical ($OH\bullet$) generated on a Pb/PbO₂ anode for depolymerization of cotton cellulose. In these cases, the electrode provides the active homogeneous catalysts or promoters (i.e. H^+ , $OH\bullet$) for the reaction. However, more detailed studies of reaction kinetics, intermediate species, and the effect of heterogeneous reactions between reactants and electrode have not been reported.

In this chapter, we selected cellobiose, which consists of two glucose molecules linked by a β -1-4-glycosidic bond, as a model of cellulose, and aim at understanding the cellobiose hydrolysis by electrochemically generated acid (H^+) on a Pt electrode, and hydroxyl radical ($OH\bullet$) on boron-doped diamond (BDD) electrode, in comparison with hydrolysis by conventional acid (sulfuric acid) and $OH\bullet$ radical generated from Fenton's reaction. The details of the reaction pathways are discussed with the support of both quantitative and qualitative product distributions, as determined by using a combination of voltammetry with online high-performance liquid chromatography (HPLC) for soluble reaction products and online electrochemical mass spectroscopy (OLEMS) mainly for CO₂. Finally, the potential of the electrochemistry-assisted hydrolysis and decomposition of cellobiose and cellulose are discussed.

6.2 Experimental

6.2.1 Chemical reactions

10 mM cellobiose in sulfuric acid (0.5, 1, 2, 5, and 10 M) was prepared in 5 mL vials for acid cellobiose hydrolysis and reaction temperature was controlled in an oven (25, 60, and 80°C). Reaction products were collected at different reaction times (0, 0.5, 1, 2, 3, and 22 hr) and cooled down immediately to room temperature to prevent further reaction. For radical cellobiose hydrolysis, 2 mM Fe(II) was introduced into a solution of hydrogen peroxide (5, 10, and 20 mM H₂O₂) and experiments were carried out under the same conditions as for the acid-catalyzed hydrolysis (i.e. temperature and sample collection). Residual hydroxyl radical was quenched by adding a stoichiometric excess of Na₂SO₃ after the sample collection. Selectivity to glucose was calculated by the following equation, involving the measured concentrations of glucose and cellobiose:

$$\frac{\text{glucose (mM)}}{2 \times \text{converted cellobiose (mM)}} \times 100\%$$

6.2.2 Electrochemical reactions

All measurements were carried out in a conventional single compartment three-electrode glass cell, which was cleaned by a standard procedure^[13] to remove all traces of organic contaminations. Cellobiose (10 mM) was dissolved into solutions of neutral (0.1 M Na₂SO₄) and acid (0.5 M H₂SO₄). Prior to the experiments oxygen was removed by bubbling argon through the solution for at least 20 minutes. The working electrode for acid cellobiose hydrolysis was a polycrystalline Pt plate. A boron-doped diamond electrode (Windsor Scientific Ltd, 0.2%) was applied for radical cellobiose decomposition. In all experiments, a platinum plate was used as a counter electrode, while a reversible hydrogen electrode (RHE) was employed as a reference electrode. Electrochemical cell potentials were controlled with a potentiostat/galvanostat (μ -Autolab Type III). Reaction temperatures were controlled with a heating bath (Lauda E100, Ecoline). All chemicals used for this work were at least analytical grade.

6.2.3 Fraction Collection and Product Analysis

The reaction products from acid-catalyzed (H_2SO_4) and radical-promoted (Fenton's reaction) cellobiose hydrolysis were collected at different reaction time (i.e. 0, 0.5, 1, 2, 3, and 22 hr) and further reaction was quenched immediately. The reaction products from electrochemical acidic and radical cellobiose hydrolysis were collected with a micro-sized sample collecting tip^[14] close to electrode surface. Collected samples during chemical and electrochemical reactions were analyzed in an HPLC system as already described in Chapter 2~3.^[14,15] The microtiter plate with the collected samples was placed in an auto-sampler holder and 20 μL of sample was injected into the column. The columns used were a single Aminex HPX 87-H (Bio-Rad) column or a series of two columns of an Aminex and a Sugar SH1011 (Shodex). Diluted sulfuric acid (0.5 and 5 mM) was used as eluent. The selected temperature of column oven was adjusted to 85°C. Details of the system configuration are described in Chapter 2~3.^[14,15]

6.2.4 On-line Electrochemical Mass Spectrometry (OLEMS)

OLEMS measurements were performed on an EvoLution mass spectrometer system (European Spectrometry Systems Ltd.).^[16] The system consists of a Prisma QMS200 (Pfeiffer), brought to vacuum with a TMH-071P turbo molecular pump (60 l/s, Pfeiffer) and a Duo 2.5 rotary vane pump (2.5 m^3/h , Pfeiffer). During measurements, the pressure inside the MS was $1\sim 5\times 10^{-9}$ bar. Pretreatment procedures and details were explained in a previous paper.^[17]

6.3 Results and discussion

6.3.1 Hydrolysis of cellobiose in acid

The molecular mechanism of acid-catalyzed hydrolysis of cellulose (cleavage of β -1-4-glycosidic bond) proceeds in three steps: 1) a proton interacts with the glycosidic oxygen linking two glucose units to form the corresponding conjugate acid, 2) C-O bond cleavage and breakdown of the conjugate acid into the cyclic carbonium ion, and 3) glucose and a proton are liberated after the rapid addition of water.^[7,18] From the reaction mechanism, the

hydrolysis conditions (i.e. acid concentration) strongly influence the physical and chemical stability of cellulose. Apart from the acidity of the solution, reaction temperature also plays a key role in accelerating the rate of cellulose hydrolysis. Acidity and temperature are typically applied in two combinations: strong acid hydrolysis at ambient temperature (25~45°C) and dilute acid hydrolysis at moderate temperature (170~240°C).^[7,11] Strong acid breaks down cellulose into oligosaccharides and glucose within a few hours without formation of large amounts of dehydration products, such as 5-hydroxymethylfurfural (HMF), levulinic acid, and formic acid.^[19] Dilute acid hydrolysis is efficient because of the short residence time of the hydrolysis products (i.e. glucose) in the continuous reactor minimizing the formation of degradation products from glucose.^[11]

Cellobiose, a glucose dimer connecting two glucose units by a glycosidic bond, is selected as a model molecule in order to understand the mechanism of cellulose hydrolysis. The effect of acid concentration and reaction temperature were studied for later comparison to the electrochemistry-assisted hydrolysis. We investigated the time-dependent hydrolysis and degradation of cellobiose (10 mM) at several concentrations of sulfuric acid (0.5, 1, 2, 5, and 10 M) and reaction temperatures (25, 60, and 80°C). The reaction temperature was limited to below 100°C considering standard electrochemical reaction conditions in aqueous media. Samples were collected at different reaction times (0, 0.5, 1, 3, and 22 hr), and collected samples were immediately cooled down to room temperature and diluted with water to less than 0.5 M in order to prevent further reactions, after which they were analyzed in an HPLC system.

The amount of remaining cellobiose (%) as a function of reaction time summarized in a logarithmic plot follows first-order reaction kinetic as confirmed by the linearity of the plots for all temperatures, and the slope of the plot indicates the first-order rate constant (k , s^{-1}). Based on the obtained rate constant, Arrhenius plots, $\ln(k, s^{-1})$ vs. $1/T$ (1/K) are drawn in Figure 1a. Even though three data points are not sufficient for an accurate determination of the activation energy, the values obtained from Figure 1, ca. $118 \pm 8 \text{ kJ mol}^{-1}$, is within the error range of the value, $110 \pm 30 \text{ kJ mol}^{-1}$, reported by Mosier.^[20] It is also significantly lower than the value reported for conventional cellulose hydrolysis (ca. 176 kJ mol^{-1}).^[11,21,22] Interestingly, the activation energy for glucose degradation is ca. 138 kJ mol^{-1} ,^[11,21] which means cellobiose hydrolysis is more facile than glucose degradation during

the acid-catalyzed reaction, and therefore we can obtain a higher selectivity toward glucose in dilute acid hydrolysis.

The rates of glucose formation, $\ln(R, \text{mM s}^{-1})$ vs. $1/T$ (1/K), based on the HPLC results are shown in Figure 1b. The values for high concentration of sulfuric acid (i.e. 5 and 10 M) are extracted from the initial glucose formation rate before decomposition of glucose. We note that the rate of glucose formation also follows the type of Arrhenius plots as shown in Figure 1a, since cellobiose converts to glucose with high selectivity. The activation energy obtained from Figure 1b is ca. $110 \pm 7 \text{ kJ mol}^{-1}$, which is consistent with the value from Figure 1a. Especially low concentrations of sulfuric acid and low temperature lead to a very low activity for cellobiose conversion, which indicates that acid-catalyzed cellulose hydrolysis is hardly applicable to electrochemical reactions at room temperature. At elevated temperatures, for example at 80°C , however, even 0.5 M sulfuric acid actively hydrolyzes cellobiose to glucose since the reaction rate increases exponentially as the reaction temperature increases, with high selectivity ($> 98\%$) to glucose.

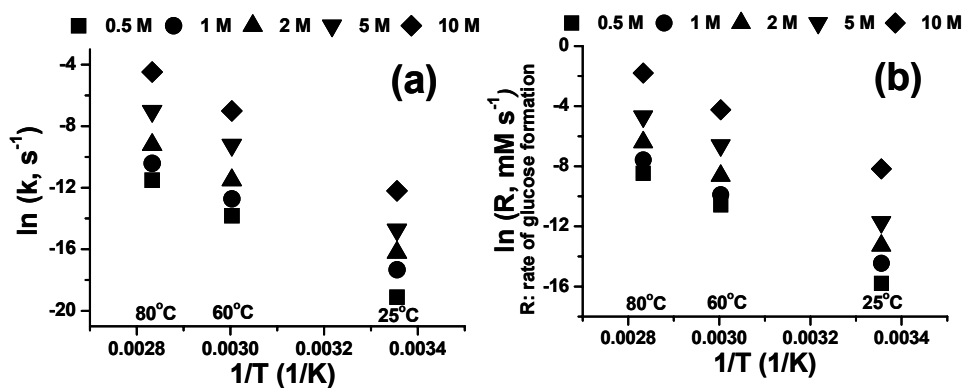
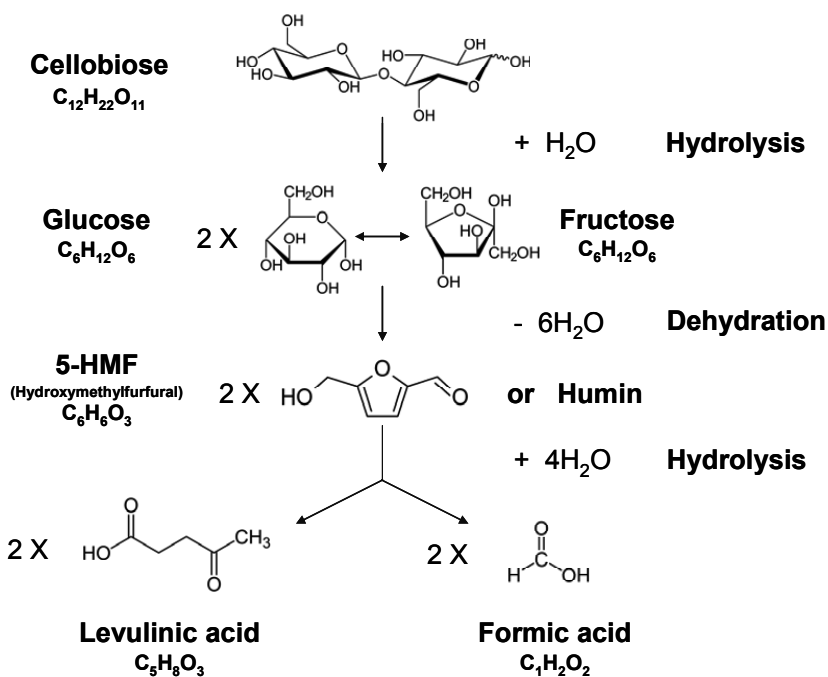


Figure 1. (a) Arrhenius plots for the acid-catalyzed cellobiose hydrolysis and (b) the rates of glucose formation at diverse concentrations of sulfuric acid (■ 0.5 M, ● 1 M, ▲ 2 M, ▼ 5 M, and ◆ 10 M) at different temperatures (25, 60, and 80°C).

Although the condition of 10 M sulfuric acid at 80°C shows extremely high cellobiose conversion and glucose formation rate in a short reaction time (2.6 mM cellobiose converted to glucose within 30 s corresponding to a rate of glucose formation of ca. 10 mM/min, and the conversion of cellobiose complete within 0.5 hr), the subsequent degradation of glucose lowers the yield and selectivity. The results from the HPLC analysis

Cellobiose hydrolysis and decomposition by electrochemical generation of acid and hydroxyl radicals

reveal that glucose is ultimately decomposed to equal amounts of levulinic acid and formic acid. However 5-hydroxymethylfurfural (HMF), a key intermediate species, was not observed. In general, the pathway for glucose decomposition is believed to be initiated by fructose isomerization from glucose, which is subsequently dehydrated to give HMF or humin (a carbonaceous hydrocarbon)-type side products due to condensation reactions of intermediates during fructose dehydration.^[23] Next, HMF is further rehydrated to give levulinic acid and formic acid.^[24] Since we observed the formation of humin at 60°C and 80°C from 5 and 10 M sulfuric acid, we are convinced that the two reaction steps of glucose isomerization to fructose and fructose dehydration to HMF must have taken place. Therefore, as illustrated in Scheme 1, a cellobiose molecule is hydrolyzed to two molecules of glucose, then glucose isomerizes to fructose, followed by dehydration to HMF and humin, and finally HMF is hydrolyzed to levulinic acid and formic acid in strong acid and at high temperature.



Scheme 1. Reaction pathway for acid-catalyzed cellobiose hydrolysis to glucose and its decomposition.

As a general conclusion, cellobiose is hydrolyzed by acid with a lower activation energy (118 ± 8 kJ mol⁻¹) than conventional cellulose hydrolysis (ca. 176 kJ mol⁻¹ [11,21,22]). Cellobiose hydrolysis is strongly dependent on reaction temperature and concentration of acid. Higher concentration of acid and higher temperature accelerate the rate of cellobiose conversion, however glucose is not stable under these conditions, as it may isomerize to fructose, followed by dehydration to 5-HMF or humin, and finally hydrolyzes to levulinic acid and formic acid.

6.3.2 Cellobiose hydrolysis by electrochemically generated acid

The easiest way to generate acid (H⁺) in an electrochemical cell is by the oxygen evolution reaction (OER) at an anode. The main potential advantage of using an electrochemical method for cellobiose hydrolysis is that we can control the local acidity during oxygen evolution by applying a controlled potential or current. Based on the experimental results obtained from chemical (H₂SO₄) cellobiose hydrolysis in the previous section, we selected a relatively low concentration of sulfuric acid (0.5 M) as electrolyte with high reaction temperature (80°C), in order to examine whether the electrochemically generated acid can accelerate the rate of cellobiose hydrolysis, in a background electrolyte in which the further degradation of glucose may be limited.

First of all, a blank experiment was carried out without electrochemical reaction and without the electrode in solution, and the concentrations of cellobiose and its reaction products were obtained by continuous fraction collection and subsequent analysis in an HPLC system, as shown in Figure 2a. Note that the concentration of cellobiose decreases linearly, and was hydrolyzed and converted mainly to glucose with only a small amount of side-product. The rate of glucose formation starts at 1.87 mM/hr and decreases to 1.27 mM/hr after one hour, but it is higher than the decomposition rate from chemical cellobiose hydrolysis (0.94 mM/hr) under the same conditions in 0.5 M H₂SO₄ at 80°C (see previous section), most likely due to the convection of solution introduced by the continuous sample collection.^[14] For the generation of acid at the anode by water electrolysis, a platinum electrode was applied as working electrode using different current densities, i.e. 10, 30, 60, and 120 mA cm⁻² as shown in Figure 2b. As expected, a higher applied current causes a faster conversion of cellobiose to glucose at the initial stage of the reaction (< 30 min) since

an applied current of 120 mA cm^{-2} shows the highest concentration of glucose in Figure 2b. However, after 30 min, the rate of glucose formation decreases as the applied current increases. Interestingly, only a current of 10 mA cm^{-2} shows an overall enhanced rate for glucose formation (1.73 mM/hr on average) in comparison with the blank (1.57 mM/hr on average), whilst higher applied currents cause lower rates of glucose formation, on average 1.56 , 1.54 , and 1.3 mM/hr for 30 , 60 , and 120 mA cm^{-2} , respectively. The reason for this lower glucose yield is that the glucose hydrolyzed from cellobiose degrades to by-products, i.e. glyceric acid and formic acid on the activated Pt surface by electro-oxidation.

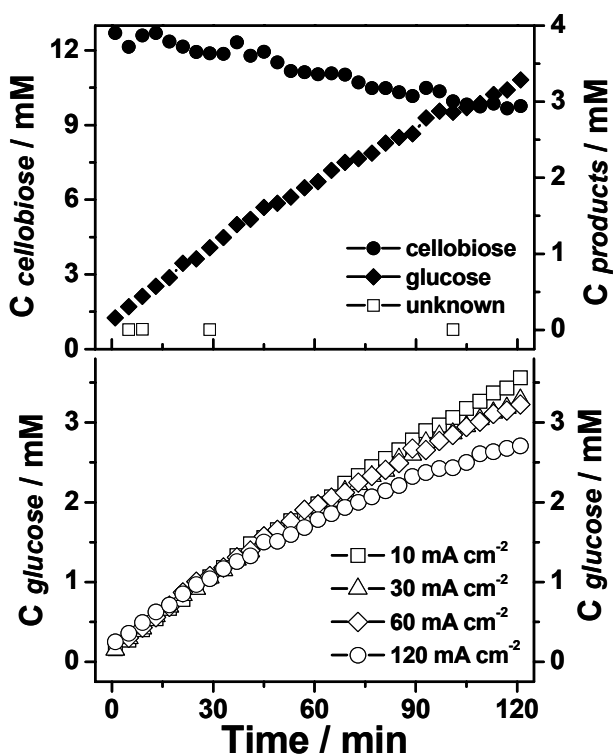


Figure 2. Cellobiose conversion to glucose in $0.5 \text{ M H}_2\text{SO}_4$ at 80°C in (a) blank and (b) with support of electrochemically generated acid by applying different current densities (10 , 30 , 60 , and 120 mA cm^{-2}) on Pt electrode.

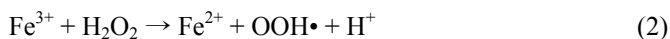
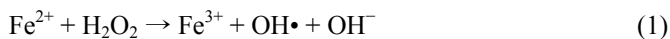
A similar effect was observed during glycerol oxidation,^[15] in which glycerol oxidizes dominantly to glyceraldehyde and glyceric acid on a clean Pt surface, and further oxidizes

to glycolic acid and formic acid on a PtO_x surface (> 1.2 V vs. RHE). The applied currents on the Pt electrode cause the corresponding potential to range from 1.6 to 1.9 V (vs. RHE), at which potentials the Pt electrode is oxidized to PtO_x , leading to C-C bond breaking, i.e. glucose to form smaller organic molecules. In a related experiment, Baizer and Nobe^[11] separated the electrochemically generated acid from the platinum anode and introduced it to a high-pressure autoclave, obtaining 98% glucose selectivity with 29% of cellulose conversion at 160°C under optimized operating conditions. In our experiment, the concentration of unknown side-product increases at higher applied currents, which also produces a large amount of molecular oxygen. This “unknown” side product was also seen in an oxygen bubbled blank experiment, indicating oxygen produced during water electrolysis decreases the glucose selectivity.

As a brief conclusion, electrochemically generated acid can be applied to hydrolyze cellobiose to glucose during oxygen evolution reaction on a Pt anode. However, the interaction between reactants (cellobiose or glucose) and the activated Pt electrode decomposes glucose to smaller organic molecules in case of high applied potential or current. In addition, the oxygen produced during water electrolysis produces side-products and lowers the selectivity to glucose. Therefore, for a higher yield of glucose, an active catalyst for the oxygen evolution reaction is needed which is not-active towards glucose. Also separation of evolved oxygen will be important. Besides that, in order to achieve total cellobiose conversion in solution with our electrochemical method, one will need a large electrode area within a limited volume of solution.

6.3.3 Cellobiose decomposition by Fenton’s reaction

Hydrogen peroxide has been used for the pretreatment of cellulose to solubilize the cellulosic matrix and reduce the cellulose crystallinity thus improving enzyme digestibility.^[25,26] The advanced oxidation process (AOP) (i.e. wet air oxidation) utilizes hydrogen peroxide or hydroxyl radical ($\text{OH}\bullet$) as an active intermediate species to oxidize cellulose at high temperature (150-350°C) with high pressure of O_2 (5-20 MPa).^[27] However, only a few reports have studied the effect of the hydroxyl radical on cellulose hydrolysis and degradation.^[12,28,29] In an attempt to achieve cellobiose hydrolysis, we employed hydroxyl radical ($\text{OH}\bullet$) from Fenton’s reaction as follows^[30]:



In reaction (1) the iron(II) introduced into the solution is oxidized by hydrogen peroxide to the ion iron(III), a hydroxyl radical, and a hydroxyl anion. In reaction (2) the iron(III) is subsequently reduced back to iron(II) by hydrogen peroxide, forming a peroxide radical and a proton. Therefore the iron(II) is regenerated during the cycle and only hydrogen peroxide is consumed in the overall reaction. As the typical ratios of Fe(II) and H₂O₂, we chose 1:5-10, and the time dependent hydrolysis of 10 mM cellobiose in water was investigated with 2 mM Fe(II) and 5, 10, and 20 mM of H₂O₂ at three different temperatures, i.e. 25, 60, and 80°C. Samples were collected at different reaction times (0, 0.5, 1, 2, 3, and 22 hr), and residual H₂O₂ was immediately quenched by using a stoichiometric excess of Na₂SO₃ in order to prevent further reactions, and subsequently analyzed in an HPLC system.

Similar results compared to the acid-catalyzed cellobiose hydrolysis were observed with the radical reaction. The amount of remaining cellobiose (%) plotted logarithmically as a function of time represents a first-order reaction as confirmed by the linearity of the plots for all temperatures, and the slope of the plot gives the first-order rate constant (k , s⁻¹). From the obtained rate constant, Arrhenius plots of $\ln(k, \text{s}^{-1})$ vs. $1/T$ (1/K) are shown in Figure 3a. High reaction temperature and high concentration of hydrogen peroxide accelerate the cellobiose conversion, with glucose as initial product, indicating that the hydroxyl radical generated during Fenton's reaction cleaves the β -1-4-glycosidic bond of cellobiose to form glucose. Interestingly, the activation energy derived from the slope of the Arrhenius plots in Figure 3a is ca. $55 \pm 1 \text{ kJ mol}^{-1}$, about twice lower than that of acid-catalyzed reaction (ca. $118 \pm 8 \text{ kJ mol}^{-1}$), indicating that the application of hydroxyl radicals for cellulose hydrolysis can be a very promising technique with enhanced cellobiose conversion compared to acid catalysis. However, the glucose is also fractionized by radicals to smaller molecules as observed in the product spectra showing glyceric acid, glycolic acid, acetic acid, and formic acid. In contrast to the acid-catalyzed hydrolysis, in which the proton is not consumed, the hydroxyl radical is continuously consumed and needs to be generated by the cycles of reaction (1) and (2), which is limited by the residual amount of H₂O₂. In addition, the stability of H₂O₂ is dependent on the pH of solution and it decomposes easily to O₂ and H₂O at pH>1.^[31]

Concerning the neutral condition for our experiment, H_2O_2 is not stable and therefore decomposes, which causes a lower conversion of cellobiose during long-term reaction. Therefore, after consumption of all H_2O_2 in the solution, cellobiose conversion does not proceed anymore. In addition, the rate of hydroxyl radical formation, which determines the rate of cellobiose conversion, is mainly dependent on the concentration of H_2O_2 and temperature. Figure 3b clearly shows that a higher concentration of H_2O_2 and a higher reaction temperature generate more hydroxyl radicals, which results in higher conversion of cellobiose to glucose with Arrhenius-type temperature dependence. The activation energy obtained from Figure 3b is ca. $54 \pm 2 \text{ kJ mol}^{-1}$, which value is consistent with that from Figure 3a. Room temperature is almost inactive for cellobiose conversion, however at high temperature (at 80°C), the conversion ratio of cellobiose increases to 28, 45, and 70% with 5, 10, and 20 mM H_2O_2 , respectively. Interestingly, a higher conversion of cellobiose does not lead to a higher selectivity for glucose as shown in Figure 3c, since the hydroxyl radical further decomposes glucose into smaller fractions. Therefore, in general, the selectivity (%) of glucose is less than 30% under all reaction conditions studied, and decreases as the rate of hydroxyl radical formation increases.

As an intermediate conclusion, the hydroxyl radical generated by Fenton's reaction initially hydrolyzes cellobiose to glucose with the activation energy of ca. $55 \pm 1 \text{ kJ mol}^{-1}$, but it further decomposes glucose to smaller organic molecules. The rate of cellobiose conversion is dependent on the rate of hydroxyl radical formation mainly determined by the amount of H_2O_2 and reaction temperature, and the selectivity of glucose is less than 30% in general, and inversely proportional to the rate of reaction.

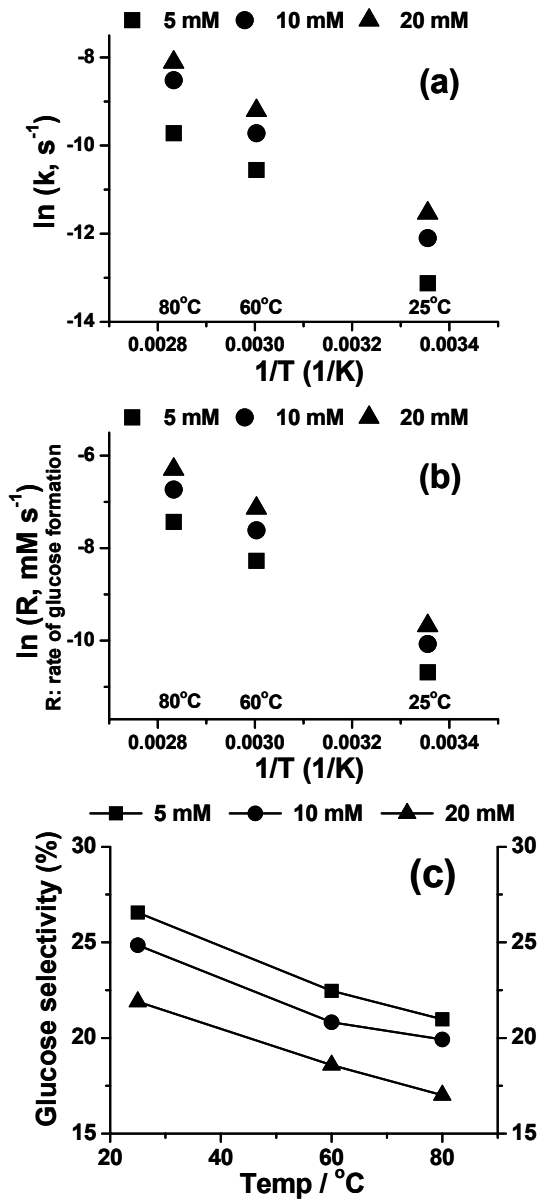


Figure 3. (a) Arrhenius plots for the first-order rate constants in radical-enhanced cellobiose hydrolysis, (b) the rates of glucose formation, and (c) glucose selectivity (%) at different concentrations of hydroxyl radical generated by Fenton's reaction (2 mM Fe(II) with 5, 10, and 20 mM H_2O_2) at 25, 60, and 80°C.

6.3.4 Electrochemical cellobiose decomposition on BDD

Boron-doped diamond (BDD) seems a suitable anode to produce large amounts of OH• to hydrolyze cellobiose to glucose as shown in the previous section with hydroxyl radicals generated from Fenton's reaction. BDD shows a higher O₂ evolution overvoltage than conventional anodes such as PbO₂, doped SnO₂, IrO₂, and Pt. The OH• formation follows from the reaction^[32]:



This formation of a hydroxyl radical has been confirmed by electron paramagnetic resonance spectroscopy.^[27,33] The hydroxyl radical is considered as a non-selective, very powerful oxidant.^[34] Here we will demonstrate its reactivity toward cellobiose hydrolysis to glucose and the further decomposition of glucose. As the hydroxyl radical from Fenton's reaction is highly active for cellobiose hydrolysis and accelerates the glucose decomposition at high reaction temperature, which lowers the selectivity toward glucose, we only show the electrochemical results obtained at room temperature. Also note that the number of protons generated by reaction (3) will be too small to lead to significant pH changes, so that we disregard any acid-catalyzed decomposition induced by reaction (3).

Shown in Figure 4 are the voltammograms of cellobiose decomposition in 0.1 M Na₂SO₄ (Figure 4A) and in 0.5 M H₂SO₄ (Figure 4B) alongside the concentration profiles of the reaction products, both in the absence (dashed line) and in the presence (solid line) of cellobiose (10 mM) in the solution. First of all, the blank voltammograms (dashed line) seem to have a higher overpotential for oxidation than the voltammogram with cellobiose in solution. However, we note that the potential for the formation of hydroxyl radical (blank, dashed line) on the BDD electrode is identical with the onset potential of the oxidation current with cellobiose (solid line) in the solution (see insets in Figure 4A-a, and 4B-a), since hydroxyl radical initiates the cellobiose hydrolysis and further oxidizes its fractions. Also note that the onset potential of radical formation depends on the acidity of the solution. The oxidation current on BDD with cellobiose in neutral solution begins from 2.15 V, and the current increases significantly from 2.7 V. In acidic condition, 2.05 V is the onset potential for the oxidation with cellobiose, and the current increases exponentially. Comparing the current density at 3 V clearly shows that the current in acid

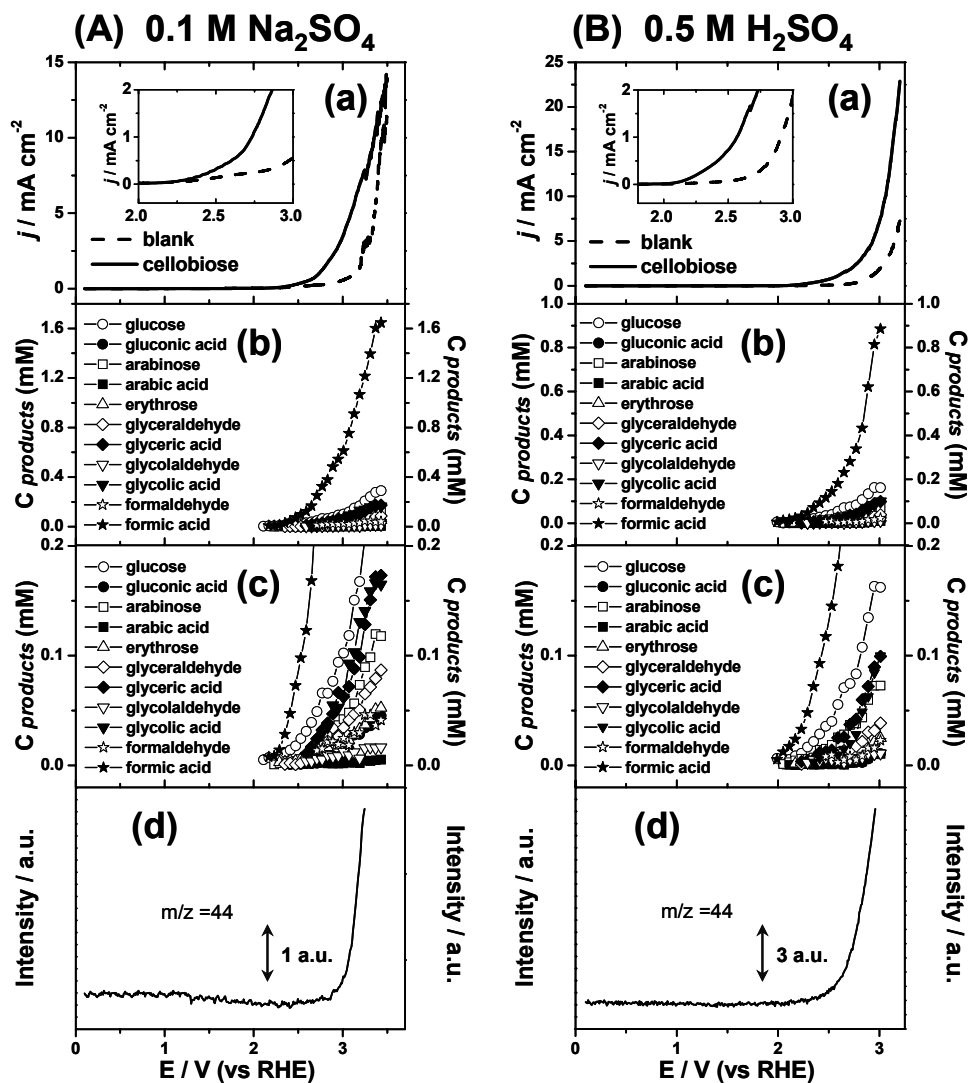


Figure 4. Cellobiose (10 mM) decomposition on boron-doped diamond (BDD) under (A) neutral (0.1 M Na₂SO₄) and (B) acidic (0.5 M H₂SO₄) conditions: a) current density measured with (solid line) and without (dash line) cellobiose in the solution during linear sweep voltammetry with a scan rate of 1 mV s⁻¹; b) concentration profiles of reaction products collected with the fraction collection system; c) enlargement of b) with different scale; and d) ion current profiles for $m/z=44$ (CO₂) obtained with OLEMS measured during voltammetry.

solution is almost twice of that in neutral solution, which indicates that the oxidation or decomposition of cellobiose and its by-products by hydroxyl radicals on BDD is enhanced under acidic conditions.

To study the formation of gaseous products, in particular CO_2 , an online electrochemical mass spectrometry experiment was carried out.^[16] Figure 4 A-d and B-d shows the MS ion current for $m/z=44$, which implies CO_2 formation, clearly demonstrating that formic acid is further oxidized to CO_2 from ca. 2.8 V in neutral and from ca. 2.5 V in acidic condition, respectively. The potential for CO_2 formation indicates that formic acid is relatively stable at low potentials (< ca. 2.5 V), but it is oxidized to CO_2 at high concentration of hydroxyl radical at high potentials. The electro-oxidation of formaldehyde (10 mM) and formic acid (10 mM) on a BDD electrode were studied in separate experiments, the results of which are shown in Figure 5. Formaldehyde clearly shows a higher oxidation current and a lower onset potential than formic acid, especially in neutral condition, suggesting that the CH_2O detached from aldose is easily oxidized to formic acid, but the oxidation of formic acid is sluggish. Interestingly, from the OLEMS experiment, we did not observe the formation of O_2 , $m/z=32$, during cellobiose decomposition even at higher potentials (> 3 V), which implies that the oxygen evolution reaction is sluggish on a BDD electrode and the blank currents in Figure 4 A-a and B-a are mainly due to the water oxidation to hydroxyl radical following the one electron transfer reaction (3).

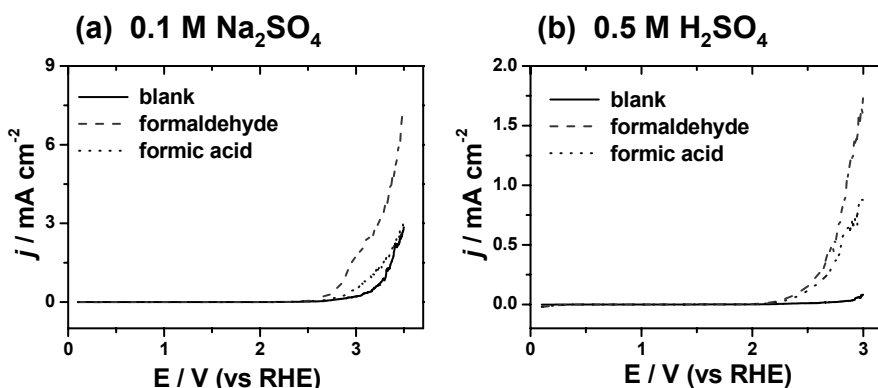


Figure 5. Formaldehyde (10 mM) and formic acid (10 mM) oxidation on boron-doped diamond (BDD) in (a) neutral (0.1 M Na₂SO₄) and (b) acidic (0.5 M H₂SO₄) conditions with a scan rate of 1 mV s⁻¹.

Turning our attention to the detailed reaction pathway, Figure 6 shows the onset potentials for the observed reaction products in 0.1 M Na₂SO₄ and in 0.5 M H₂SO₄ from panel (b) in Figure 4. First of all, we can clearly see how the sequence of product formation depends on the applied potential. For the formation of aldoses under neutral conditions, Figure 6a shows that glucose (C₆H₁₂O₆) hydrolyzed from cellobiose appears as the first product from ca. 2.11 V, followed by arabinose (C₅H₁₀O₅, 2.23 V), erythrose (C₄H₈O₄, 2.29 V), glyceraldehyde (C₃H₆O₃, 2.35 V), glycol-aldehyde (C₂H₄O₂, 2.59 V), and formaldehyde (CH₂O, 2.71 V), indicating that the hydroxyl radical selectively attacks the aldehyde on the aldose and breaks the nearby C-C bond, thereby producing a smaller aldose and formic acid (as formed by oxidation of CH₂O). This is evidenced by the observation of formic acid from 2.17 V, the potential in between the formation of glucose and arabinose, with the CH₂O detached from aldose immediately oxidized to formic acid. We also observed several organic acids oxidized from their corresponding aldoses, which enhances the total current density compared to the blank. The formation of organic acids follows the same potential dependence as the aldoses, i.e. gluconic acid was observed from 2.41 V, followed by arabic acid (2.47 V), glyceric acid (2.59 V), and glycolic acid (2.65 V).

The onset potentials of the observed aldoses and their corresponding organic acids in acidic solution (Figure 6b) are shifted to lower potentials compared to neutral solution (Figure 6a), however they also follow the general trend described above. The first reaction products from cellobiose decomposition by hydroxyl radical are glucose and formic acid observed from 1.99 V, with other aldoses observed at consecutively higher potentials, i.e. arabinose (2.05 V), erythrose (2.17 V), glyceraldehyde (2.23 V), glycolaldehyde (2.29 V), and formaldehyde (2.47 V), and the organic acids, i.e. gluconic acid (2.05 V), arabic acid (2.11 V), glyceric acid (2.23 V), and glycolic acid (2.35 V). Therefore we conclude that acidic conditions are preferred to generate hydroxyl radicals on BDD,^[35] which actively hydrolyze cellobiose to glucose, and then further decompose and oxidize glucose to smaller aldoses and their corresponding organic acids at lower potentials than in neutral condition.

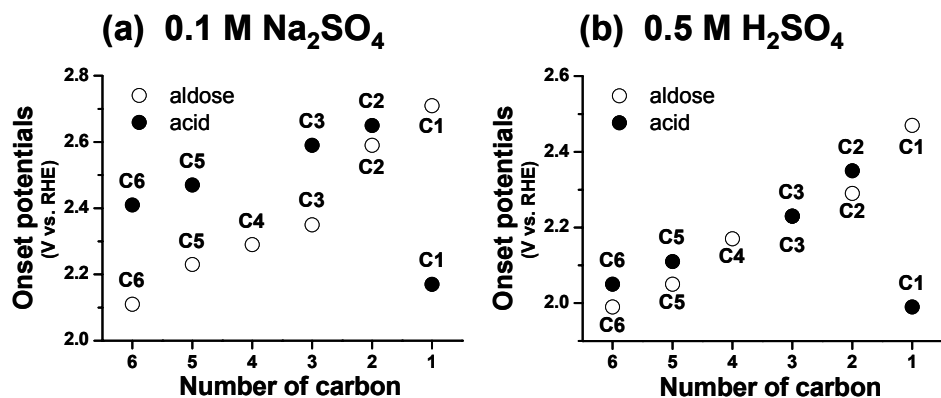
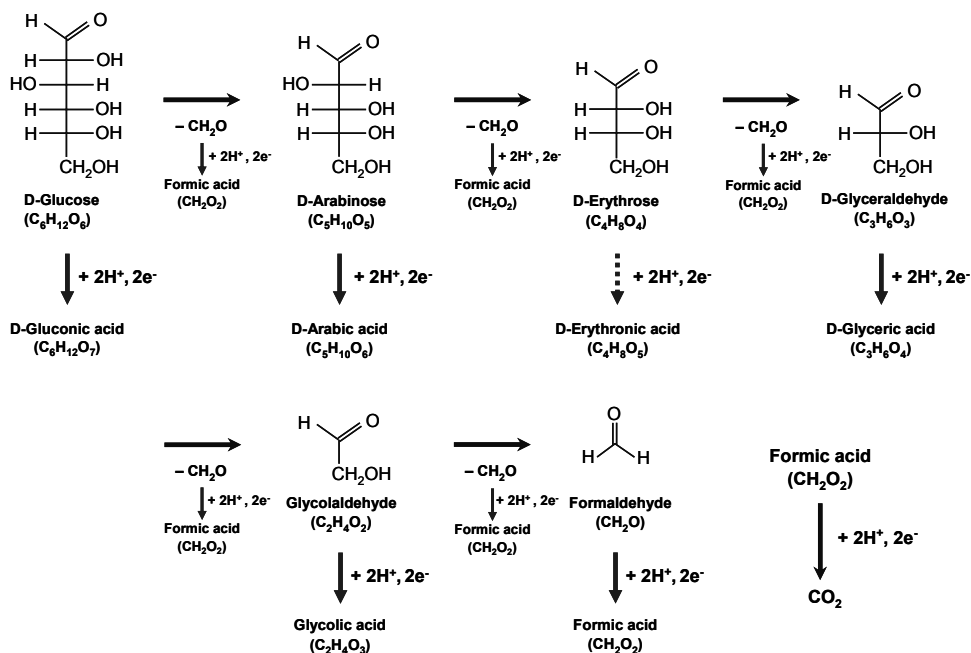


Figure 6. The onset potentials for observed reaction products as a function of the number of carbon atoms of the aldoses and their corresponding acids on a BDD electrode in (a) neutral (0.1 M Na₂SO₄) and (b) acidic (0.5 M H₂SO₄) solution.



Scheme 2. Reaction pathway for radical cellobiose decomposition on BDD electrode.

On the basis of the results shown in Figure 4–6, we suggest a general reaction pathway for the cellobiose hydrolysis to glucose and its further decomposition by the hydroxyl radicals generated on a BDD electrode as shown in Scheme 2. First of all, the hydroxyl radical generated on a BDD surface depolymerizes cellobiose to glucose. A possible pathway for the cleavage of cotton cellulose was proposed recently by Li et al.,^[12] suggesting that the hydroxyl radical extracts the hydrogen atom from the C4 of D-anhydroglucopyranose unit forming a carbon radical, which is subsequently oxidized to form a superoxide radical of cellulose by addition of molecular oxygen. This is followed by the cleavage of the glycosidic bond to form glucose after the leaving of superoxide anion radical. However, this suggestion does not fit our case since there is no molecular oxygen in our experiment, as confirmed by the OLEMS measurements. After the hydrolysis of cellobiose by the hydroxyl radical, glucose is subsequently decomposed to arabinose, erythrose, glyceraldehyde, glycol-aldehyde, and formaldehyde, which indicates that the hydroxyl radical selectively attacks the C-C bond of the aldehyde to break it to smaller aldoses. After the C-C bond cleavage, CH₂O is detached from the aldose molecule, which is immediately oxidized to formic acid. We note that, however, the formation of CO₂ from formic acid oxidation is a bit sluggish on the BDD electrode (see Figures 4A-d, 4B-d, and 5), even at high potential. In addition, we did not observe stereoisomers, but only D-aldose during hydrolysis and decomposition, indicating that the hydroxyl radical does not change the configuration of sugar molecules.

6.4 Conclusion

This work has described the potential of cellobiose hydrolysis and decomposition by electrochemical methods. We investigated the cellobiose hydrolysis to glucose and its further decomposition during the acid-catalyzed reaction by comparison of sulfuric acid vs. electrochemically generated acid, and the radical reaction by hydroxyl radicals from Fenton's reaction vs. hydroxyl radicals from water oxidation on a BDD electrode. The reaction products were determined by (online) HPLC for dissolved reaction products and by OLEMS for CO₂. The experiments with homogeneous chemical reactions provided the key reaction parameters for cellobiose conversion to glucose, i.e. concentration of acid and radical, and reaction temperature. The hydrolysis of cellobiose follows a first-order reaction with Arrhenius-type temperature dependence for the various concentrations of sulfuric acid

and hydroxyl radicals with different activation energies of ca. $118 \pm 8 \text{ kJ mol}^{-1}$ and ca. $55 \pm 1 \text{ kJ mol}^{-1}$, respectively. The main advantage of electrochemical approach would be to steer the reaction rate by controlling the amount of acid or radical formation at the electrode surface. The electrochemically generated acid during the oxygen evolution reaction (OER) only marginally increases the rate of glucose formation, but lowers the selectivity towards glucose since the electrode (PtO_x) interacts with glucose and decomposes it further to smaller organic acids. In addition, the oxygen formed during OER lowers the selectivity of glucose, since oxygen causes the formation of side-products. Therefore, an electrode with high activity for OER and low activity for glucose decomposition is necessary for such an application. Hydroxyl radicals generated on a BDD electrode first hydrolyze the cellobiose to glucose, and then attacks the aldehyde group on the glucose species, which is then further fractionized to smaller aldoses and formic acid. The application of hydroxyl radicals may be a promising technique with lower activation energy than that of the acid-catalyzed reaction, provided the hydrolyzed glucose is immediately separated from the active radical. In combination with the ability of potential-controlled electrolysis, as recently demonstrated for the oxidation of glycerol,^[15,36] it may be possible to use this method to achieve higher selectivity towards certain interesting end products.

6.5 References

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