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Production of glucose from the acid hydrolysis of anhydrosugars

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Abstract

Two anhydrosugar model compounds (cellobiose and levoglucosan), and a mixture of anhydrosugars from the fast-pyrolysis of birch wood were subjected to acid hydrolysis using sulfuric acid as catalyst. The anhydrosugars mixture or bio-oil aqueous fraction was found to contain mainly levoglucosan with a concentration of 30 g L⁻¹. Hydrolysis temperature, reaction time, and catalyst to substrate molar ratios (c/s), were varied to identify their influence for glucose production. At 120 °C, 60 minutes, and 0.9 c/s ratio; glucose yields of 98.55% and 96.56%, and substrate conversions of 100% and ~92%, were achieved when hydrolysing cellobiose and levoglucosan respectively. An increase in the temperature to 135 °C, resulted in a decrease in both glucose yield and selectivity; whereas substrate conversions around 90% were maintained for both anhydrosugars. During the hydrolysis of the bio-oil fraction, a range of conditions to achieve glucose yields above 90%, was depicted. It was found that c/s ratios between 0.17 and 0.90, and temperatures between 118 °C and 126 °C were suitable to achieve glucose yields around 100% (30 g L⁻¹). Furthermore glucose concentrations ~117% (35 g L⁻¹) and levoglucosan conversions above 90%, were attained at 135 °C, 20 minutes and 0.2 estimated c/s ratio.

Keywords: hydrolysis, glucose, anhydrosugars, levoglucosan, cellobiose

Introduction

Renewable liquid fuels, high-value chemicals, and derived products can be obtained from the thermal processing of lignocellulosic biomass, for example via fast pyrolysis. During fast-pyrolysis, the solid lignocellulosic biomass is thermally converted in the absence of oxygen, into three main fractions namely char, gases, and pyrolysis oil, the latter commonly called bio-oil [1-5].

Bio-oil can contain more than 400 compounds covering a wide range of molecular weights and functionalities [6-8]. The overall bio-oil composition highly depends upon the type of lignocellulosic material used as feedstock, and the pyrolysis processing conditions e.g. temperature and residence time [6, 9]. The major reported components of bio-oils include water, carboxylic acids, ketones, phenols, furans, and anhydrosugars [10, 11].

During fast pyrolysis, the cellulose present in lignocellulosic biomass, degrades into diverse products including anhydrosugars such as 1,6-anhydro- β -D-glucopyranose, referred to as levoglucosan (LG). Levoglucosan is a relevant anhydrosugar, which can be hydrolysed to monomeric glucose, which is a valuable chemical platform that can be fermented to produce bio-fuels such as bio-ethanol and bio-butanol [9, 12-14]. Alternatively, levoglucosan in the bio-oil can be separated for crystallization which opens valorisation routes of the anhydrosugar itself as compared to glucose [15].

The bio-oil fraction normally contains an aqueous and a non-aqueous fraction that can be separated for example by extraction. The aqueous extract of bio-oil is composed by low molecular weight aldehydes such as glycoaldehyde as well as phenolic compounds [16]. Anhydrosugars such as levoglucosan are also normally present in the bio-oil aqueous fraction, and some studies have been directed in optimising levoglucosan extraction [9, 12]. For example Li et al. 2013 [12], used water during the extraction of levoglucosan from bio-oil in order to maximise the amount of levoglucosan obtained. The optimal parameters reported included a water-to-bio-oil ratio of 1.3:1, 25 °C, and 20 min extraction time to yield 12.7 wt.% of levoglucosan. Bennet et al., 2009 [9], studied the extraction of levoglucosan from bio-oil, and its further hydrolysis to produce glucose. The optimal conditions reported for the extraction stage were 41 wt.% of water at 34 °C, which resulted in an aqueous fraction containing about 88 g L⁻¹ of levoglucosan. A glucose yield as high as 216% (based on levoglucosan in the substrate) was reported during the hydrolysis of levoglucosan at 125 °C, 44 minutes reaction time and using 0.5 M sulphuric acid [9]. The extraction of levoglucosan is seen as a necessary step when the levoglucosan is further processed via hydrolysis.

To date, several studies have indicated that levoglucosan yields in bio-oil can be greatly increased if a mild or dilute acid biomass pre-treatment precedes the fast pyrolysis [12, 17-

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3 21]. For example Scott D. S., et al., [17], examined the production of anhydrosugars from
4 cellulose-containing biomass via a series of processes. The first step was a biomass pre-
5 treatment with diluted acid in order to remove alkaline materials, which was followed by the
6 separation of cellulose and hemicellulose fractions. Then the solid hemicellulose-free
7 fraction was subjected to fast pyrolysis at temperatures between 400-650 °C and residence
8 time <10 seconds, and finally the anhydrosugars produced were isolated [17].
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12 Lian et al., [7], pyrolysed acid washed poplar at with an organic phase (containing phenols)
13 and an aqueous phase containing anhydrosugars. The anhydrosugars were separated from
14 phenols by solvent extraction and then subjected to acid hydrolysis using sulphuric acid as
15 catalyst. The HPLC analysis of the phenol-rich fraction, revealed the presence of
16 levoglucosan, sorbitol, cellobiosan, arabinose, galactose, glucose, mannose/xylose,
17 fructose, cellobiose, and some other unknown compounds. After acid hydrolysis at 120 °C,
18 42 minutes and using H₂SO₄ 0.5 M as catalyst, a glucose yield of 220% was achieved. This
19 high glucose yield was attributed to the contribution of unidentified anhydrosugars into the
20 final glucose formation [7].
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26 Acid hydrolysis is one of the most common processes to obtain low-cost fermentable sugars
27 from anhydrosugars. However, it is a complex process as several parameters can be varied
28 including temperature, residence time, acid catalyst type, acid concentration, and catalyst to
29 anhydrosugars ratio. Meaning that many experiments need to be conducted to clearly
30 identify clear trends for glucose yields. So far in the literature there have been a handful of
31 reported studies on acid hydrolysis of anhydrosugar model compounds such as
32 levoglucosan and cellobiose [22-24]. However little has been reported about the acid
33 hydrolysis of bio-oil fractions at different conditions [9, 22].
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38 The feasibility of a bio-refinery concept in which an anhydrosugar-rich liquid from the fast
39 steam pyrolysis of birch-wood is hydrolysed into glucose, with the intention for it to be
40 fermented into bio-ethanol or bio-butanol as a fuel is experimentally addressed in this work.
41 The acid loading and reaction time will affect the economics of the overall process
42 pronouncedly, so in-depth information about this important reaction step is required.
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46 Ultimately this work experimentally investigated the potential of obtaining glucose from the
47 acid hydrolysis of anhydrosugars. Initially levoglucosan and cellobiose were used as
48 anhydrosugar model compounds. The influence of selected hydrolysis conditions over both
49 the conversion of anhydrosugar model compounds and glucose yields was analysed. In a
50 second stage an anhydrosugars mixture from bio-oil was hydrolysed; glucose concentrations
51 and substrate conversions were studied also at different hydrolysis conditions. Overall the
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3 hydrolysis parameters varied included reaction time, temperature and catalyst/substrate
4 molar ratios.
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6 **Materials and methods**

7 **Materials**

8 **Anhydrosugars**

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11 For the anhydrosugar model compounds, levoglucosan (CAS 498-07-7) and β -D-cellobiose
12 (CAS 528-50-7) were purchased from Carbosynth Limited, Berkshire, United Kingdom.
13 Solutions of levoglucosan and cellobiose were prepared at concentrations of 62.3 g L^{-1} , and
14 100 g L^{-1} respectively. The concentration of the levoglucosan solution was adjusted to this
15 value, as it was expected that the real anhydrosugars mixture from bio-oil will contain about
16 60 g L^{-1} of levoglucosan.
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22 **HPLC compounds**

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24 For the HPLC calibration, solutions of levoglucosan, cellobiose, together with solutions of
25 cellobiosan (CAS 35405-71-1, Carbosynth Limited UK), and glucose (CAS 50-99-7, Sigma-
26 Aldrich) were used. For the HPLC mobile phase, the following substances were used: water,
27 acetonitrile (ACN), both HPLC grade from VWR chemicals, and a solution of 100mM
28 ammonium acetate (Sigma-Aldrich). The pH of the ammonium acetate solution was adjusted
29 to pH=5.4 using a concentrated hydrochloric acid (HCl, Sigma-Aldrich). The percentages to
30 prepare the mobile phase were 75vol.% ACN, 15vol.% water, and 10vol.% 100 mM
31 ammonium acetate solution.
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36 **Liquid acid catalyst**

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38 For the homogeneous catalyst, concentrated sulfuric acid (H_2SO_4 >95%, from Fisher
39 Chemicals), it was used to prepare a 0.5 M sulfuric acid solution. This solution was used for
40 all the hydrolysis tests reported in this work. The volumes of acid catalyst and substrates
41 were therefore varied in order to achieve different catalyst/substrate ratios.
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45 **Aqueous fraction of bio-oil**

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47 The aqueous fraction was extracted from a bio-oil prepared by Nova Pangaea Technologies,
48 in the United Kingdom. Birch-wood chips from UK were used as feedstock for the fast
49 pyrolysis. Initially hemicelluloses and alkali materials were removed from the biomass by a
50 dilute sulphuric acid based hemicellulose hydrolysis process (H_2SO_4 : biomass ~0.02:1.0), at
51 $170 \text{ }^\circ\text{C}$, and 15 min. The use of dilute acid (H_2SO_4 <2 wt.%) as pre-treatment to alter or
52 break the structure of lignocellulosic biomass is a widely used technique. It is mainly used to
53 remove hemicelluloses in the form of sugars and oligomers with limited effects on cellulose
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3 and lignin compounds. This pre-treatment involves low acid consumption and increases the
4 material's porosity [25-29]. Commercial processes using sulfuric acid as pre-treatment for
5 biomass include BlueFire Renewables (USA), and Abengoa Bioenergy (Spain) [25, 30].
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8 The hydrolysed hemicellulose fraction was segregated, and the pre-treated stream was
9 subjected to a fast steam pyrolysis process. A continuous pyrolysis reaction with a capacity
10 of 15-25 kg/h was used to process the pre-treated biomass. For the pyrolysis about 4.5 kg of
11 superheated steam were added per kg of dry biomass feed, the pyrolysis temperature was
12 around 380-410 °C, the pressure ~1 atm and a short biomass residence time in the order of
13 seconds were used as process conditions. Under these conditions about 75wt.% of liquid
14 fraction (wet basis), 20wt.% solid fraction (char), and 5wt.% of gases (dry basis) were
15 produced. The aqueous fraction from the condensed pyrolysis liquid was segregated from
16 non-aqueous fraction, as it was known to contain anhydrosugars. The aqueous fraction
17 containing anhydrosugars was characterised using HPLC and GC-MS, in order to identify its
18 major components. The aqueous bio-oil fraction was stored at 4 °C with no light exposure
19 until used for the hydrolysis tests.
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26 **Methods**

27 **Experimental: Acid hydrolysis**

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29 The anhydrosugar model compounds (cellobiose and levoglucosan) and the aqueous
30 fraction from the bio-oil were subjected to acid hydrolysis. A schematic of the 15 mL
31 autoclave reaction system used for the hydrolysis experiments is shown in Figure 1. The
32 reagents were loaded at atmospheric pressure, and due to the closed nature of the system
33 the pressure slightly increased to autogenous pressure of water about 2 bar to 6 bar, when
34 using temperatures of 135 °C and 150 °C respectively. The influence of an inert vs an air
35 atmosphere on the glucose yield, was not studied as it was not within the scope of this
36 research. During the hydrolysis tests the parameters selected to be varied included
37 temperature, reaction time, and catalyst to substrate molar ratio.
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44 A typical hydrolysis experiment was carried out as a batch experiment (Figure 1); certain
45 volume of substrate solution with a fixed concentration (cellobiose, levoglucosan, or
46 pyrolysis-oil), was loaded in a glass liner together with a magnetic stirrer and the calculated
47 volume of catalyst (0.5 M H₂SO₄). The total volume for all the hydrolysis experiments was
48 kept constant at 10 mL and only the volumes of both substrate solution and sulphuric acid
49 were varied in order to achieve different catalyst/substrate molar ratios. For all the
50 experiments stirring was set at 600 RPM and a heating rate of about 2.5 °C min⁻¹ was used
51 until reaching the set temperature (±3 °C). The reaction time began to be measured once the
52 temperature reached the set value.
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The independent variables for acid hydrolysis were X_1 , X_2 , and X_3 , representing the temperature in °C, reaction time in minutes, and catalyst/substrate molar ratio respectively. Each variable was coded at three levels (-1, 0, 1); these coded values were obtained according to Eq. (1):

$$CV = \frac{(AV - M)}{HR} \quad (1)$$

where CV is the coded value, AV is the actual value, M is the mean and HR is the half of range. Values were considered for each test, and a matrix of coded factor levels was obtained as shown in Table 1. The initial fixed conditions were temperatures of 80 °C, 100 °C, and 120 °C, reaction times of 20, 40, and 60 minutes, and catalyst to substrate molar ratios of 0.2, 0.6, and 0.9. All these values were selected based on hydrolysis conditions reported in the literature. Additional experiments were carried out at 135 °C and 150 °C, in order to study the decomposition routes of the anhydrosugars at higher temperatures.

After each hydrolysis test, the resulting samples were analysed using HPLC and the concentrations of the different compounds were calculated using their corresponding calibration curves. The conversion of substrate (cellobiose or levoglucosan), represented how much out of the initial 100% of substrate was converted during the reaction, and it can be expressed by Eq. (2):

$$C_s = 100 - \left[\frac{(Y_{is} - Y_{fs})}{Y_i} \times 100 \right] \quad (2)$$

where C_s is the conversion of substrate in percent (%); Y_{is} are the initial moles of the substrate at $t=0$, and Y_{fs} are the final moles of substrate after acid hydrolysis.

The glucose yields were calculated considering the actual and theoretical glucose amounts. The actual glucose was calculated by Eq. (3):

$$A_G = Y_{fG} \times M_G \quad (3)$$

where A_G indicates the actual amount of glucose in grams (g), Y_{fG} represent the final moles of glucose after hydrolysis, and M_G is the molecular weight of glucose (180.16 g/mol).

The theoretical amount of glucose was then calculated using Eq. (4):

$$T_G = Y_{is} \times M_G \quad (4)$$

where T_G is the theoretical maximum amount of glucose in grams (g), and Y_{is} are the initial moles of substrate. The 1:1 and 1:2 stoichiometry (moles) were used to calculate the theoretical amount of glucose from the hydrolysis of levoglucosan and cellobiose respectively.

The overall glucose yield was therefore obtained combining the theoretical and actual glucose amounts, and using the following expression (Eq. (5)):

$$G_Y = \left(\frac{A_G}{T_G} \right) \times 100 \quad (5)$$

where G_Y , is the glucose yield in percent (%); A_G is the actual glucose (g), and T_G is the theoretical amount of glucose in grams (g).

Finally, the selectivity was calculated considering the conversion of the substrate to glucose, using the following Eq. (6), and reported by Deng et al [31]:

$$S = \left(\frac{Y_{fG}}{Y_{is} - Y_{fs}} \right) \times 100 \quad (6)$$

where Y_{fG} are the moles of glucose in the final product, Y_{is} and Y_{fs} are the initial and final moles of substrate. When calculating the selectivity of glucose from the substrate cellobiose, the denominator was multiplied by 2, considering the reaction stoichiometry.

Characterisation of bio-oil aqueous fraction

In the present research the bio-oil from fast pyrolysis was extracted or separated into water soluble and water-insoluble fractions. Similar procedures have been reported in the literature by Yu et al, 2016 [32]; Lian et al, 2010 [7], and Bennett et al, 2009 [9].

The water soluble or aqueous fraction of bio-oil, was characterised using diverse techniques in order to identify its major compounds.

Moisture content

Initially the water content of the bio-oil was quantified using a Mettler Toledo V20 Volumetric Karl-Fisher Titrator as per American Society for Testing and Materials (ASTM) E203-96. It was determined that the pyrolysis oil had a water content higher than 90.0%. This water content, together with the water contained in the liquid acid catalyst (0.5 M H_2SO_4), contributed to the hydrolysis process.

GC-MS

A Varian 450-GC gas chromatograph, coupled to a Varian 220-MS, IT mass spectrometer (GC-MS), was used for the analysis of the chemical compounds contained in the aqueous fraction of the pyrolysis oil and in some hydrolysates. The system was equipped with a capillary column Elite-1701, L 30 m x I. D. 0.25 mm, d_f 0.25 μm . The identification of the compounds was based on the existing library for different types of bio-oil.

HPLC

All the samples from acid hydrolysis tests were analysed by high-performance liquid chromatography (HPLC), using a 1200 Infinity Series from Agilent Technologies equipped with an auto sampler, gradient pump, and UV/ RI detection systems. The separation of sugars was performed using a 2.6 μm 150 Amide-HILIC HPLC column (250 x 2.1 mm with guard 10 x 3.0 mm). The column was set at 30 °C with a flowrate 0.1 mL min⁻¹, and an injection volume of 5 μL . The mobile phase used was 75/10/15 (ACN/100 mM ammonium acetate pH 5.4/H₂O); 1 L of the solution was premixed in order to avoid variations in the RI signal when using the mixing pump.

The HPLC column was calibrated using prepared stock solutions of levoglucosan, cellobiose, cellobiosan, and glucose at 5 different concentrations. Linear calibration curves (average $R^2=0.997$) were obtained for each compound and the elution times for the different compounds were identified. Each sample was analysed in duplicate and the average was used to report the concentration of each compound. During HPLC analysis, the typical relative standard error was ± 0.00026 for multiple injections from the same sample.

Results and discussion

Acid hydrolysis of cellobiose

Cellobiose is not commonly identified as a component of pyrolysis oil, however it was selected as it can be formed as intermediate during the acid hydrolysis of cellobiosan; the latter is a common compound present in the bio-oil composition together with levoglucosan [24, 32, 33]. During the hydrolysis of cellobiosan, two molecules of glucose can be formed via two different routes as shown in Figure 2 [24, 34]. The upper path occurs via the hydrolysis of β -(1 \rightarrow 4) glycosidic bonds to form one molecule of glucose and one molecule of levoglucosan, then levoglucosan might further hydrolyse into glucose [35]. The second glucose formation route from cellobiosan is via the hydrolysis of 1,6-anhydro bond, resulting in the formation of the disaccharide cellobiose. Cellobiose can further hydrolyse yielding two molecules of glucose via rupture of the O-glycosidic bond (Figure 2).

For the hydrolysis of cellobiose different temperatures (80, 100, and 120 °C), reaction times (20, 40, and 60 min), and catalyst to substrate molar ratios (0.2, 0.6, and 0.9) were varied in order to identify their influence in both the glucose yield and substrate conversion (Table 1). The volumes of the cellobiose stock solution (100 g L⁻¹), and catalyst (H₂SO₄, 0.5 M) were adjusted to achieve different catalyst/substrate molar ratios.

The effects of varying the hydrolysis parameters were studied via monitoring both cellobiose conversion and glucose yields, as shown in Figure 3. Figure 3 a, b, and c, depicts the

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aforementioned trends at 20, 40 and 60 min reaction times respectively. From Figure 3 it can be seen that at a hydrolysis temperature of 80 °C, low substrate conversions and low glucose yields were achieved. For example at 80 °C (Figure 3) just between 20 and 30% of the initial cellobiose was converted, resulting in relatively low glucose yields between 2 and 15% at different reaction times and different catalysts to cellobiose ratios. At a hydrolysis temperature of 80 °C, by increasing the reaction time from 40 minutes (Figure 3b) up to 60 minutes (Figure 3c), slightly increased the glucose yields from 7% up to 17%, for a catalyst/substrate ratio of 0.6 whereas the glucose yield was maintained around 14% for 0.9 catalyst/substrate. In addition, the selectivity towards glucose increased from 0.4 up to 0.9 when increasing the catalyst/substrate ratio from 0.6 to 0.9. Nevertheless, both the low conversion of cellobiose and relatively low glucose yields (~15%), were attributed to the mild hydrolysis temperature used.

At 120 °C and a catalyst to substrate ratio of 0.2, the glucose yield increased from 60% to 77% as the hydrolysis time was increased from 20 min (Figure 3a) up to 60 min (Figure 3c); whereas at a ratio of 0.6, the glucose yield remained somewhat constant around 87% regardless the reaction time. Full conversion of cellobiose at a ratio of 0.6 was seen after approximately 40 minutes at 120 °C. A very similar yield observed after 40 and 60 minutes indicates that only marginal glucose degradation occurs at this reaction condition. At 120 °C the further increase of the catalyst to substrate ratio from 0.6 up to 0.9, resulted in a 100% conversion of cellobiose after 40 min (Figure 3b) and 60 min (Figure 3c) of hydrolysis. This is in accordance with reported results where the increase in the H₂SO₄ concentration increased the substrate conversion and thus the glucose yields [24]. For cellobiose hydrolysis, the highest glucose yield of 98% was achieved after 60 min (Figure 3c), H₂SO₄/cellobiose ratio of 0.9, and a reaction temperature of 120 °C, with a 100% cellobiose conversion.

In order to study the effect of further increasing the temperature during the hydrolysis of cellobiose, selected experiments were carried out at 135 °C, 40 and 60 min reaction time, and 0.6 and 0.9 catalyst/substrate ratio. It was observed that all the experiments carried out at 135 °C resulted in a 100% conversion of cellobiose, but not necessarily 100% yield of glucose. For example at a catalyst/substrate of 0.6 and 135 °C, the glucose yield decreased from 92% down to 89.5%, as the reaction time increased from 40min up to 60min; showing that glucose degradation starts becoming significant at this temperature. Furthermore, the presence of levoglucosan was observed and its concentration was noted to increase from 2.4 g L⁻¹ up to 3.4 g L⁻¹ at the aforementioned hydrolysis conditions. It is postulated that the increase in the temperature from 120 °C up to 135 °C, promoted the dehydration of glucose into levoglucosan as shown in Figure 5 [36, 37]. The dehydration of glucose into levoglucosan can occur via two pathways. The first one is the formation of a key

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3 intermediate that becomes stable due to the solvation energy due to the high temperature
4 and high density of hot water. In the second route two water molecules close to glucose
5 transfer hydrogen atoms into the hydroxyl groups of glucose, eliminating a water molecule
6 from glucose. Then a bi-radical is formed, which can finally leads to the formation of
7 levoglucosan [38].
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10 The glucose selectivity after 60 minutes of reaction time is shown in Figure 4. From Figure 4
11 it is observed a positive influence of the temperature on the glucose selectivity (dashed
12 lines). For example, for a catalyst/substrate ratio of 0.2, the glucose selectivity gradually
13 increases from 14% to 36%, and up to 87%, as the temperature increases from 80 °C to 100
14 °C and up to 120 °C respectively (Figure 4). At 120 °C, the glucose selectivity increased
15 from 87.6% to 98.5% when the catalyst to substrate ratio was increased from 0.6 up to 0.9
16 (Figure 4). Interestingly, the selectivity was reduced down to 86% when the temperature was
17 increased up to 135 °C for a catalyst/substrate of 0.9. This behaviour is therefore linked to
18 the potential glucose dehydration reactions taking place at hydrolysis temperatures higher
19 than 120 °C (Figure 5).
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26 The results from the experiments carried out at different temperatures were used to create a
27 matrix and obtain a 3D surface area and contour plot for glucose yields from the hydrolysis
28 of cellobiose (Figure 6). From Figure 6, it can be seen that glucose yields above 90% can be
29 attained during the hydrolysis of cellobiose, at temperatures between 120-135 °C, and using
30 catalyst/substrate ratios between 0.6 and 0.9.
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34 In order to study the influence of increasing the temperature beyond 135 °C, cellobiose
35 hydrolysis tests were carried out at 150 °C, 10 min, and at catalyst/substrate ratios of 0.6
36 and 0.9. Under these conditions it was observed that after 10 min, the glucose yield was
37 reduced from 83% down to 79% as the H₂SO₄/cellobiose ratio increased from 0.6 up to 0.9.
38 Contrastingly glucose yields higher than 87% were attained when hydrolysing cellobiose at
39 120 °C and 135 °C, 40 minutes of reaction time, at both 0.6 and 0.9 catalyst/substrate ratios.
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43 For the hydrolysis experiments carried out at 150 °C, there was a visual presence of solids
44 in the collected sample, which has been related with the further degradation of glucose at
45 high temperatures. For example the isomerization of glucose into fructose, followed by the
46 dehydration of fructose into 5-hydroxymethyl furfural (5-HMF), as shown in Figure 5 [39].
47 The resulting 5-HMF (Figure 5) is an unstable molecule which tends to condense into a
48 black insoluble carbonaceous heterogeneous materials, often referred to as "humins" [40-
49 47]. Alternatively the subsequent addition of water (hydration reaction) to the C₂-C₃ bond of
50 the furan ring in the 5-HMF structure, might yield to both levulinic acid and formic acid in a
51 1:1 mol ratio [44]. van Zandvoort et al., 2013 [44], reported humins yields up to 36% when
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3 hydrolysing glucose at 113 °C and 247 °C, and after 6h of reaction, and found a strong
4 relationship between the humins yield and temperature rather than acid concentration and
5 humins yield. Generally, the presence of humins in hydrolysis product indicates that 150 °C
6 temperature is too harsh for high yield glucose production [44, 45].
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9 **Acid hydrolysis of levoglucosan**

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11 As shown in Table 1 the conditions for acid hydrolysis of levoglucosan were the same as
12 those described for the hydrolysis of cellobiose. However as demonstrated by Figure 2, the
13 theoretical quantity of glucose was calculated using a 1:1 molar stoichiometry (levoglucosan
14 into glucose).
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17 The conversion of levoglucosan and glucose yields at 80 °C, 100 °C, 120 °C;
18 H₂SO₄/levoglucosan molar ratios of 0.2, 0.6, 0.9; and reaction times of 20 min, 40 min and
19 60 min, are shown in Figure 7. Similar to the trends observed for cellobiose hydrolysis
20 (Figure 3), at 80 °C both levoglucosan conversions and glucose yields <11% were obtained
21 at 20 min (Figure 7a) and 40 min (Figure 7b), whereas a slight increase in the glucose yield
22 up to 14% is observed after 60 min (Figure 7c). At 80 °C, 0.6 ratio, and 60 minutes,
23 levoglucosan conversion was just 10% (Figure 7), whereas the conversion of cellobiose
24 under similar hydrolysis conditions was ~33% (Figure 3). Which shows that at this relatively
25 low temperature of 80 °C, the conversion of cellobiose occurs at a faster rate when
26 compared with the hydrolysis of levoglucosan.
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30 When the levoglucosan hydrolysis temperature was increased to 100 °C, glucose yields as
31 high as 85%, and ~90% levoglucosan conversion were attained, after 60 minutes of reaction
32 time and 0.6 catalyst/substrate molar ratio (Figure 7c). At 100 °C, and 20 minutes of reaction
33 time (Figure 7a), an increase in the catalyst/substrate ratio from 0.2 to 0.6 increased the
34 glucose yield from 21% up to 32%. Experiments at a catalyst/substrate ratio of 0.2 revealed
35 that an increase in the temperature from 80 °C up to 100 °C, had a similar effect than
36 increasing the catalyst/substrate ratio from 0.2 up to 0.9 (Figure 7). This means that either
37 the increase in the sulfuric acid at a given temperature, or the increase in the temperature at
38 a given sulfuric acid concentration, can have similar effects towards both levoglucosan
39 conversion rate and glucose yield [24].
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43 However it was observed that the further increase in the temperature from 100°C up to 120
44 °C (Figure 7), had a major influence on both levoglucosan conversion and glucose yields.
45 For example at 120 °C, levoglucosan conversions around 99% and glucose yields 90-100%
46 were achieved for all the hydrolysis conditions tested. The levoglucosan conversion trends
47 observed by increasing the temperature up to 120 °C, are in agreement with previous results
48 reported by Bennett et al, 2009, and Helle et al, 2007 [9, 24].
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3 Levoglucosan conversions and glucose concentrations at different reaction times (20, 40, 60
4 minutes) and temperatures (80 °C, 100 °C, 110 °C, and 120 °C), are shown in Figure 8.
5 From Figure 8 values for levoglucosan conversion and glucose concentration at 110 °C were
6 calculated using the first-order kinetic equations reported by Helle et al, 2007 [24], for the
7 hydrolysis of levoglucosan. Equations (6) and (7), allowed us to estimate the concentrations
8 for levoglucosan and glucose at 110 °C, respectively
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$$\frac{A}{A_0} = e^{-k_1 t} \quad (6)$$

$$\frac{D}{A_0} = (1 - e^{-k_1 t}) \quad (7)$$

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18 Where A and D , are the concentrations of levoglucosan and glucose, respectively, and k_1 is
19 the first order rate constant for the hydrolysis of levoglucosan (0.00135 s^{-1}); A_0 is the initial
20 concentration of levoglucosan.
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23 Figure 8 aims to show the proximity between our experimental data and that calculated
24 using a kinetic expression reported somewhere else [24]. From Figure 8, a major increase in
25 the glucose concentration and levoglucosan conversion was observed for temperatures
26 above 100 °C, even at the low catalyst/substrate ratio of 0.2. The calculated data at 110 °C
27 was obtained using a kinetic constant (k_1) for H_2SO_4 , 500 mM. For our experimental values
28 at 120 °C we used the same acid concentration and a catalyst/substrate ratio of 0.2. The
29 calculated kinetic values were slightly above our experimental ones, which indicated that a
30 good estimation can be obtained based on the kinetics, however experimental data is also
31 necessary to verify these estimated trends.
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37 Similar to the cellobiose hydrolysis analysis, the influence of further increasing the
38 levoglucosan hydrolysis temperature was studied. Hydrolysis experiments were carried out
39 at 135 °C, reaction times of 10 min, 40 min and 60 min, at catalyst/substrate molar ratios of
40 0.6 and 0.9. The results were integrated with the previous ones in order to create a matrix
41 and to obtain a 3D surface area and a contour plot as shown in Figure 9.
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45 In general, glucose yields following cellobiose hydrolysis (Figure 3) were lower than those
46 obtained from the levoglucosan hydrolysis (Figure 7). For example, at a hydrolysis
47 temperature of 120 °C, average glucose yields of 83% and 93% were obtained for the
48 hydrolysis of cellobiose and levoglucosan respectively. Furthermore, at 120 °C values for
49 glucose selectivity of 91% and 98% were calculated for the hydrolysis of cellobiose and
50 levoglucosan respectively. This means that glucose selectivity is favoured for hydrolysing
51 levoglucosan rather than cellobiose under similar conditions. This variation can be related to
52 the slower rate of reaction during the hydrolysis of cellobiose, associated to the kinetics of
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3 this particular reaction [24]. This can also explain the different patterns showed in the
4 contour plots for the hydrolysis of cellobiosan (Figure 6b) and levoglucosan (Figure 9b).
5 Whereas for hydrolysis of cellobiosan, glucose yields higher than 80% are concentrated at
6 temperatures around 120 °C and 135 °C and catalyst/substrate ratios of 0.45 and 0.9 (Figure
7 6); for levoglucosan this area is greater from temperatures between 110 °C up to 135 °C,
8 and catalyst/substrate ratios of 0.2 up to 0.9 (Figure 9).
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10 For the hydrolysis levoglucosan at 150 °C, 10 minutes at catalyst/substrate molar ratios of
11 0.6 and 0.9, glucose yields around 67% were obtained, with conversions of substrate around
12 80%. It is worth to mention that 5-HMF was also identified in the hydrolysates (GC-MS),
13 which might indicate the further degradation of this particular compound into humins. This
14 was observed physically as also solids were observed in the collected hydrolysate samples.
15 During the hydrolysis tests at 135 °C and 150 °C, the pressure in the autoclave system went
16 up to 2 bar and 6 bar respectively, which was due to the nature of the closed system and
17 higher reaction temperatures, but it might have implications when thinking about scaling up
18 the process at these conditions as the reaction system should be capable to cope with these
19 conditions.
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27 **Acid hydrolysis of aqueous bio-oil fraction**

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29 The extracted aqueous fraction from the bio-oil was analysed by GC-MS and HPLC. The
30 GC-MS chromatogram of this fraction is shown in Figure 10. From Figure 10, it is observed
31 that the major compound identified by GC-MS it was levoglucosan, as the area of this
32 compound represented about 75% among all the peaks identified. Other major compounds
33 identified by GC-MS included furfural, guaiacol, 2-methoxy-4-methylphenol, 5-hydroxymethyl
34 furfural (5-HMF), syringol, 1,2,4-trimethoxy benzene, syringaldehyde, and possibly the last
35 peak corresponds to 1,6-anhydro- β -D-glucofuranose. This last compound is a furanose
36 isomer of levoglucosan and it has been has been proven to be present in similar pyrolysis
37 products [48, 49]. The presence of this particular compound can also be attributed to the
38 dehydration of glucose as shown in Figure 5. However, a conclusive assignment could not
39 be achieved due to the unavailability of the pure 1,6-anhydro- β -D-glucofuranose.
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46 From HPLC analysis of the aqueous fraction, it was determined that the initial concentrations
47 of levoglucosan and cellobiosan were 31 g L⁻¹ (3.1 w/v.%) and 2.1 g L⁻¹ (0.021 w/v.%),
48 respectively. Also other unknown compounds, possibly anhydrosugars and acids, might be
49 present in the aqueous fraction as several unidentified peaks were observed by both GC-MS
50 and HPLC analysis (Figure 10). Previous studies [12, 18, 50, 51], have identified monomeric
51 and oligomeric (anhydro)-sugars such as cellobiosan and levoglucosan as bio-oil component
52 in pyrolysis oil from the fast pyrolysis of different biomass, being levoglucosan the most
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3 abundant anhydrosugar. For example Dobele et al., 2003 [18], reported about 15 wt.% from
4 the analytical pyrolysis of birch wood sawdust; Li et al., 2013 [50], reported about 16 wt.% of
5 levoglucosan in the organic fraction of pyrolysis liquids from red oak; and Oudenhoven and
6 collaborators, 2015 [51], reported ~35 wt.% of levoglucosan in pyrolysis liquids condensed at
7 80 °C from acid leached biomasses. Also Lian et al., 2010 [7] reported that the aqueous
8 phase of bio-oil from the pyrolysis of acid washed poplar contained 19 g L⁻¹ of levoglucosan
9 and 15 g L⁻¹ of cellobiosan. Overall, the variability in the bio-oil composition and therefore the
10 composition of the aqueous fraction, depends upon the reactor's configuration, feedstock,
11 and pyrolysis process conditions.
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16 During the hydrolysis of the bio-oil aqueous fraction, the catalyst to substrate ratios were
17 estimated considering the initial concentration of levoglucosan of 31 g L⁻¹ obtained by HPLC.
18 Levoglucosan was used as reference compound to estimate the catalyst to substrate molar
19 ratios, as it was the anhydrosugar present in the bio-oil in a higher concentration (Figure 10).
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23 When calculating glucose yields (%) it is necessary to estimate the theoretical glucose
24 based on the initial moles of substrate (Section 0), however the aqueous fraction of bio-oil is
25 a mixture of diverse compounds some of which may contribute to glucose formation, thus is
26 not possible to report an accurate glucose yield value. Therefore unlike the hydrolysis of
27 cellobiose and levoglucosan, in this section the glucose produced was reported as
28 concentration (g L⁻¹) instead of a percentage.
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32 Glucose concentrations therefore will give a better idea about the amount of glucose
33 produced, as well as the potential contribution from other components in the aqueous
34 fraction towards glucose. Figure 11 depicts glucose and levoglucosan concentrations from
35 the acid hydrolysis of the bio-oil aqueous fraction at different reaction times of 20 minutes
36 (Figure 11a), 40 minutes (Figure 11b), and 60 minutes (Figure 11c).
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40 From Figure 11 it is observed that 80 °C was not high enough to allow the conversion of
41 potential substrates present in the aqueous fraction, even at a high catalyst to substrate ratio
42 of 0.9 and reaction time of 60 minutes (Figure 11c). As the temperature was increased up to
43 100 °C, slightly improvement towards substrate conversion and glucose concentration were
44 observed. For example, at 60 minutes (Figure 11c) the concentration of glucose was
45 positively influenced as it increased from 9 g L⁻¹ up to 17 g L⁻¹ as the catalyst/substrate ratio
46 increased from 0.2 up to 0.9.
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51 From Figure 11, the positive effect of further increasing the hydrolysis temperature from 100
52 °C up to 120 °C and 135 °C, is clearly observed in both glucose concentrations and
53 substrate conversion. Average glucose concentrations around 35.5 g L⁻¹ were attained at a
54 catalyst/substrate ratio of 0.2 and hydrolysis temperature of 135 °C. At catalyst/substrate
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3 ratios of 0.6 and 0.9, glucose concentrations of about 32 g L^{-1} were attained for different
4 reaction times at both $120 \text{ }^{\circ}\text{C}$ and $135 \text{ }^{\circ}\text{C}$, whereas levoglucosan was nearly depleted after
5 just 20 minutes of reaction time. Yu and Zhang, 2003 [14], hydrolysed a pyrolysate from dry
6 waste cotton. They found that after 20 minutes at $120 \text{ }^{\circ}\text{C}$, and using $0.3 \text{ M H}_2\text{SO}_4$ per litre of
7 pyrolysate, over 100% of the levoglucosan in the pyrolysate was converted into glucose. The
8 excess of glucose produced was therefore attributed to the contribution of other compounds
9 such as cellobiosan in the pyrolysate. For our pyrolysate we observed that at 20 minutes
10 (Figure 11a), the glucose concentrations and substrate conversions could be further
11 improved by increasing the hydrolysis temperature from $120 \text{ }^{\circ}\text{C}$ up to $135 \text{ }^{\circ}\text{C}$, particularly at
12 a low catalyst/substrate ratio of 0.2.

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18 When increasing the catalyst/substrate ratio from 0.6 up to 0.9 and at $135 \text{ }^{\circ}\text{C}$, resulted in
19 glucose concentration reductions at reaction times longer than 20 minutes. For example at
20 40 minutes (Figure 11b), glucose concentration was reduced from 32.5 g L^{-1} down to 28.56 g
21 L^{-1} ; similarly at 60 minutes (Figure 11c), the concentration of glucose was reduced from 30 g
22 L^{-1} down to 27.58 g L^{-1} . This might indicate that at this particular temperature of $135 \text{ }^{\circ}\text{C}$ and
23 catalyst/substrate ratio of 0.9, glucose might not be stable and dehydrate into levoglucosan
24 or 5- HMF, as depicted in Figure 5.

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29 Additional hydrolysis experiments were carried out at $135 \text{ }^{\circ}\text{C}$, at a shorter reaction time of 10
30 minutes, and catalyst/substrate ratios of 0.2, 0.6, and 0.9. These results were compared with
31 those obtained at 20 minutes and at $135 \text{ }^{\circ}\text{C}$, in order to study the conversion of levoglucosan
32 and glucose concentrations (Figure 12). It was found that after 10 minutes and a catalyst to
33 substrate ratio of 0.2, a glucose concentration of about 33.0 g L^{-1} could be attained, whereas
34 the levoglucosan concentration was reduced from 31.0 g L^{-1} down to 3.0 g L^{-1} . When the
35 reaction time was increased to 20 minutes, the final glucose concentration reached 35 g L^{-1} ,
36 whereas levoglucosan concentration was about 2.5 g L^{-1} . This might be due to more
37 compounds contained in the bio-oil aqueous phase continue converting after 10 minutes into
38 glucose as the hydrolysis progresses, thus contributing to this slight increase. From Figure
39 12, at 10 minutes of reaction time the increase in the catalyst/substrate from 0.6 to 0.9,
40 reduced the glucose concentration from 32 g L^{-1} down to 26 g L^{-1} ; whereas at 20 minutes the
41 concentration of glucose was maintained around 31 g L^{-1} as the catalyst to substrate ratio
42 increased from 0.6 to 0.9.

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51 Figure 13 shows a 3D surface map and contour plot created using the experimental data
52 obtained from the hydrolysis of the aqueous fraction of bio-oil. In Figure 13 glucose
53 concentration is shown as function of both temperature and catalyst/substrate ratios for the
54 hydrolysis conditions studied. At catalyst/substrate ratios between 0.2-0.6, and temperatures

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3 between 120-135 °C, resulted in glucose concentrations higher than 30.0 g L⁻¹. Choi et al.,
4 [28], reported that during the hydrolysis of starch higher glucose yields were achieved at low
5 acid concentrations around 2% and 132 °C; at this temperature the acid concentration is
6 critical as the decomposition rate of glucose is increased. A similar trend is observed in
7 Figure 13b, as it seems the red area representing glucose concentrations > 35 g L⁻¹,
8 becomes more reduced as the temperature increases beyond 125 °C, but also it
9 concentrates in lower catalyst/substrate ratios between 0.24 and 0.43. Glucose
10 concentrations were reduced at catalyst/substrate ratios higher than 0.43 and at
11 temperatures beyond 125 °C. This might be due to some of the glucose product is further
12 dehydrating into other products, when hydrolysing this particular bio-oil aqueous fraction.

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14 Overall it was observed that during the hydrolysis of the aqueous fraction at 120 °C, lower
15 catalyst/substrate ratios required longer reactions times in order to achieve glucose
16 concentrations similar to those attained at higher ratios and shorter reaction times. For
17 example to attain 32.5 g L⁻¹ of glucose at 120 °C, catalyst/substrate ratios of 0.2 and 0.6 can
18 be used, but the reaction times required are 60 min and 20 min respectively. At 120 °C,
19 catalyst/substrate ratio of 0.9, and different reaction times (20, 40, 60min), the glucose
20 concentration in the hydrolysate was maintained constant ~31 g L⁻¹. However the
21 levoglucosan concentration increased in the product from 2 g L⁻¹ up to 5.8 g L⁻¹ as the
22 reaction time was increased from 20 min up to 40 min, which might indicate that the potential
23 glucose produced at longer reaction times, dehydrated into levoglucosan due to the higher
24 amount of acid. A similar undesirable effect was observed at higher temperature of 135 °C
25 and when using a catalyst/substrate ratio of 0.9. For instance 33.4 g L⁻¹ of glucose was
26 attained in the hydrolysate at 10 min, 135 °C and with a catalyst/substrate of 0.2; whereas
27 the increase in the catalyst/substrate ratio to 0.6 and up to 0.9 under the same conditions,
28 resulted in reductions in the glucose concentration down to 32 g L⁻¹ and 26 g L⁻¹
29 respectively.

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31 Finally from Figure 13, the optimum range to attain glucose yields above 30 g L⁻¹ were at
32 catalyst/substrate ratios between 0.16-0.90, and temperatures between 118-135 °C.
33 Ultimately, we report that following the acid hydrolysis of an aqueous fraction from bio-oil,
34 the glucose produced comes not only from levoglucosan but also from other potential
35 substrates present in this particular fraction [14, 24]. It will be therefore interesting to create a
36 similar mixture using other glucose contributors and undertake hydrolysis experiments in
37 order to identify and verify this particular trend.
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Conclusions

This research demonstrates the feasibility of producing glucose from the acid hydrolysis of anhydrosugar model compounds as well as anhydrosugars contained in the aqueous fraction of bio-oil from the fast pyrolysis of birch-wood.

Acid hydrolysis of cellobiose and levoglucosan can achieve substrate conversions close to 100% and glucose yields as high as 96% within various ranges of hydrolysis conditions including temperature, reaction time and catalyst to substrate ratio.

The aqueous fraction from bio-oil, containing mainly levoglucosan can be hydrolysed at 135 °C, 20 mins reaction time and with a levoglucosan to H₂SO₄ molar ratio of 0.2, for the production of 35.3 g L⁻¹ of glucose (117% yield). At these conditions a conversion of levoglucosan was 92%.

Hydrolysing the bio-oil aqueous fraction at 135 °C, with a reaction time as short as 10 min, and catalyst/substrate ratios of 0.2, can result in conversions of levoglucosan of 90% and a glucose concentrations of 32.4 g L⁻¹. This short residence time can promote a more continuous operation when scaling up acid hydrolysis.

For the hydrolysis of the aqueous fraction at 120 °C, the highest glucose concentration of 32.5 g L⁻¹ can be achieved at 20min, and a catalyst/substrate of 0.6, or at 60 minutes and a catalyst/substrate of 0.2.

The results reported for the hydrolysis of this particular bio-oil fraction, can serve as a basis for selecting acid hydrolysis conditions for a larger scale operation. With that being said, further work is required on the effect and presence of inhibitors in the bio-oil, particularly when fermentation is considered as a next process stage.

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Table 1. Coded factors matrix for the required experiments

Experiment	Temperature (°C)	Reaction time (min)	Catalyst/substrate molar ratio
	X_1	X_2	X_3
1	-1	-1	-1
2	-1	-1	0
3	-1	-1	1
4	0	-1	-1
5	0	-1	0
6	0	-1	1
7	1	-1	-1
8	1	-1	0
9	1	-1	1
10	-1	0	-1
11	-1	0	0
12	-1	0	1
13	0	0	-1
14	0	0	0
15	0	0	1
16	1	0	-1
17	1	0	0
18	1	0	1
19	-1	1	-1
20	-1	1	0
21	-1	1	1
22	0	1	-1
23	0	1	0
24	0	1	1
25	1	1	-1
26	1	1	0
27	1	1	1

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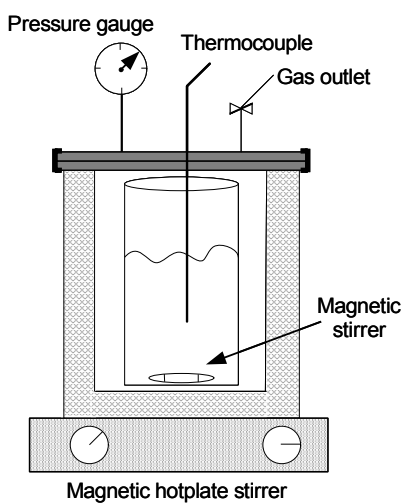


Figure 1. Schematic diagram of the autoclave reaction system used for acid hydrolysis

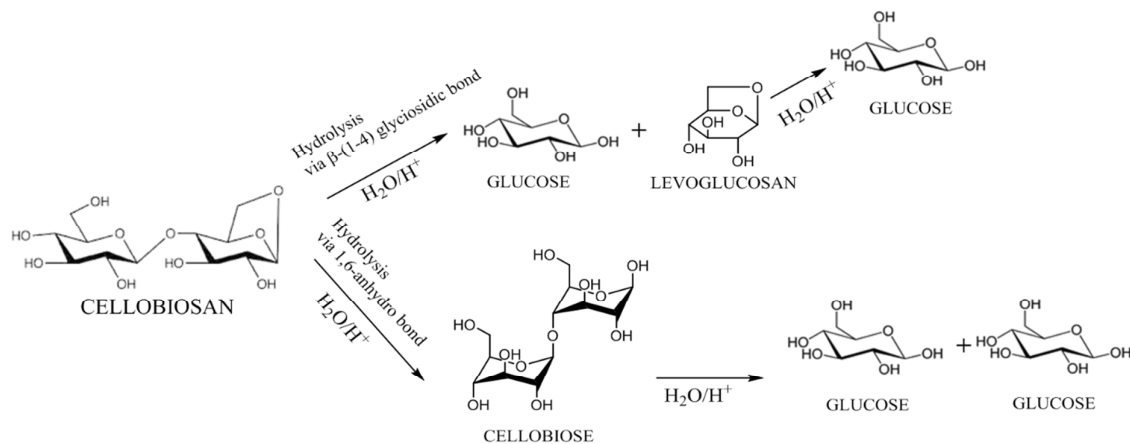


Figure 2. Hydrolysis of cellobiosan into glucose (adapted from Helle et al, 2007 [24])

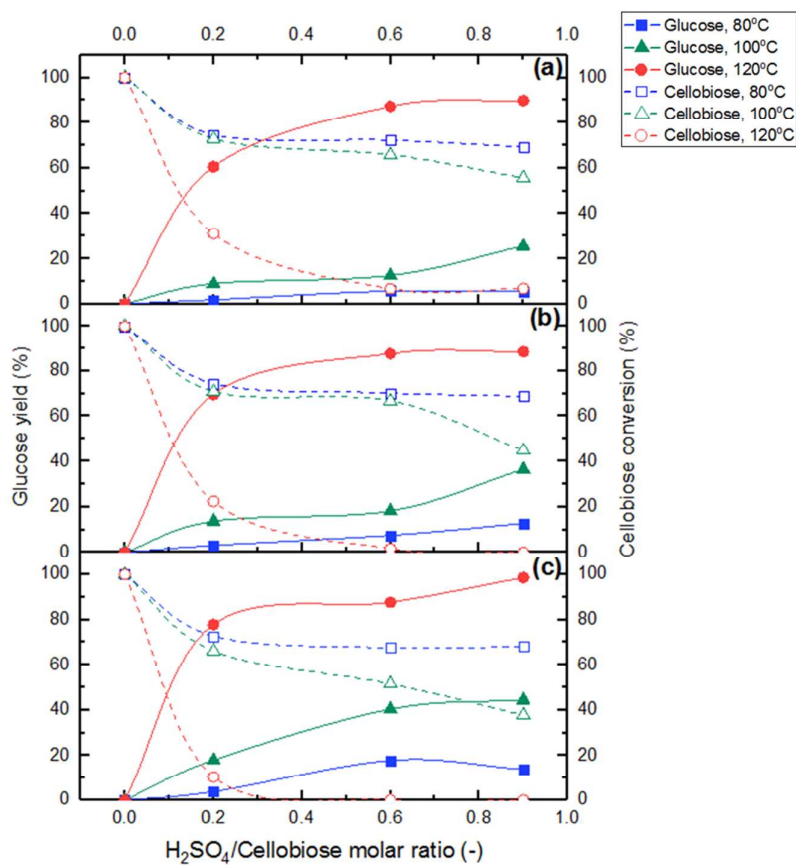


Figure 3. Cellobiose conversion (%) and glucose yields (%) at different reaction times: (a) 20 min; (b) 40 min; and (c) 60 min

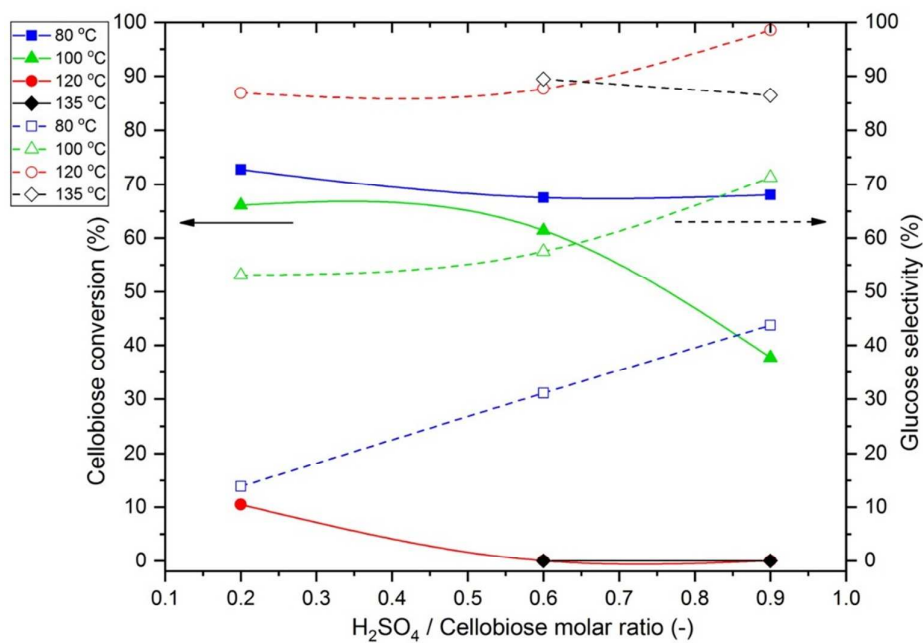


Figure 4. Cellobiose conversion (%) and Glucose selectivity (%) at 60 minutes reaction time, different temperatures and catalyst/cellobiose ratios

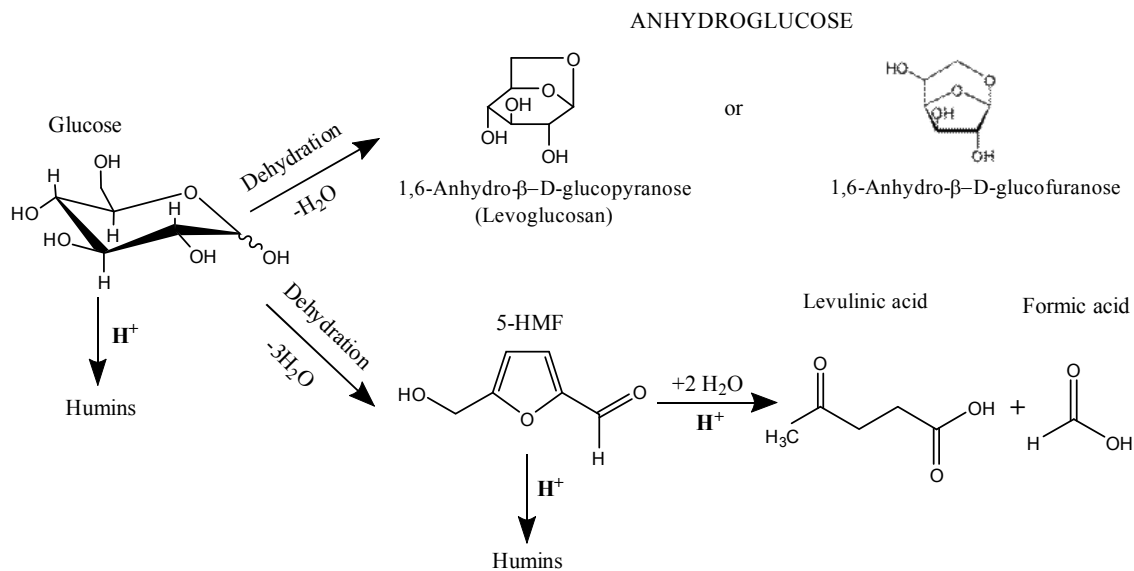


Figure 5. Dehydration reactions of glucose

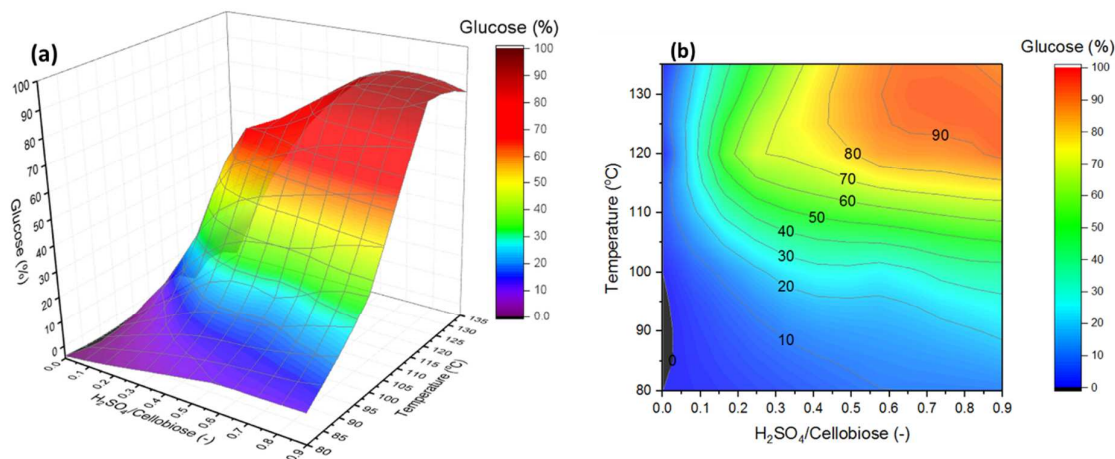


Figure 6. Glucose yields (%) from the hydrolysis of cellobiose at different temperatures and catalyst to substrate ratios: (a) 3D surface area; (b) contour plot

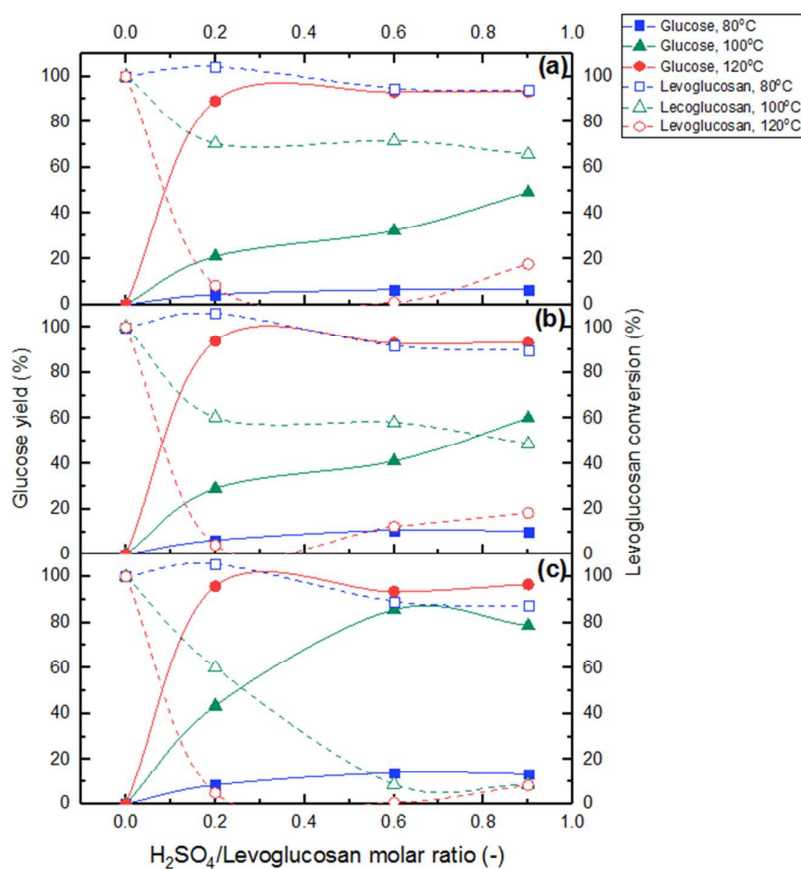


Figure 7. Levoglucosan conversion (%) and glucose yields (%) at different reaction times: (a) 20 min; (b) 40 min; and (c) 60 min

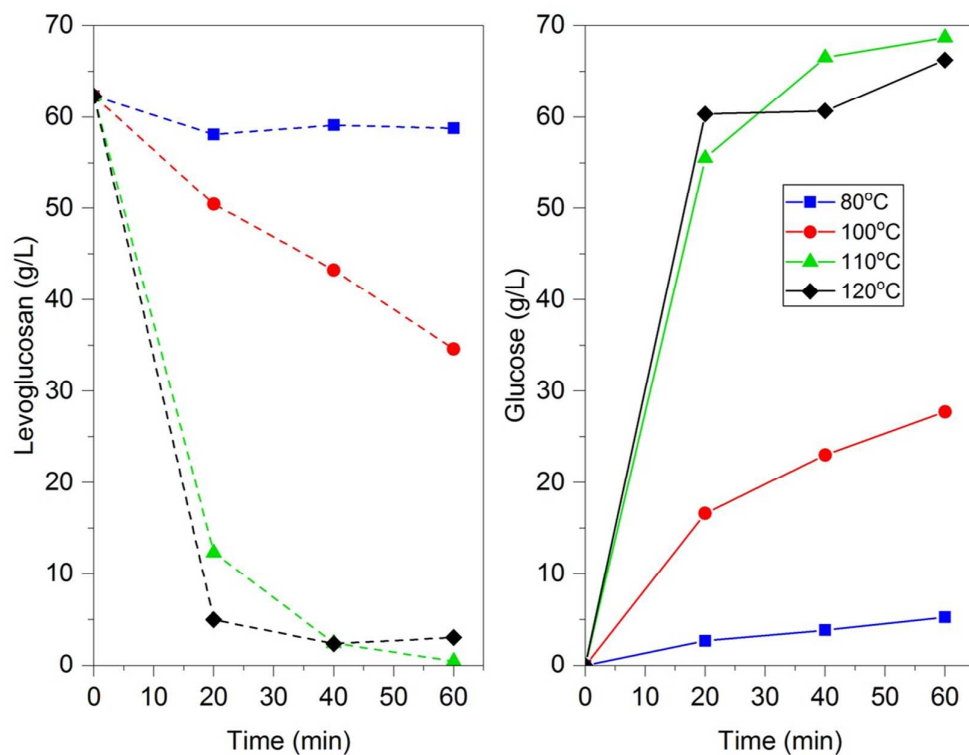


Figure 8. Levoglucosan conversions (%) and glucose yields (%) at catalyst/substrate molar ratio 0.2, and temperatures of ■80°C; ●100°C; ▲110°C (calculated); ◆120°C.

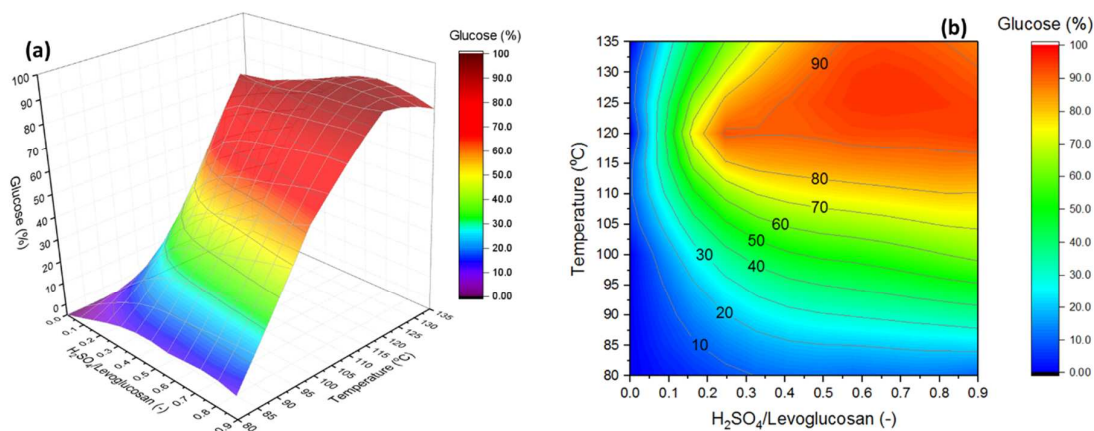


Figure 9. Glucose yields (%) from the acid hydrolysis of levoglucosan at different temperatures and catalyst to substrate ratios: (a) 3D surface area; (b) contour plot

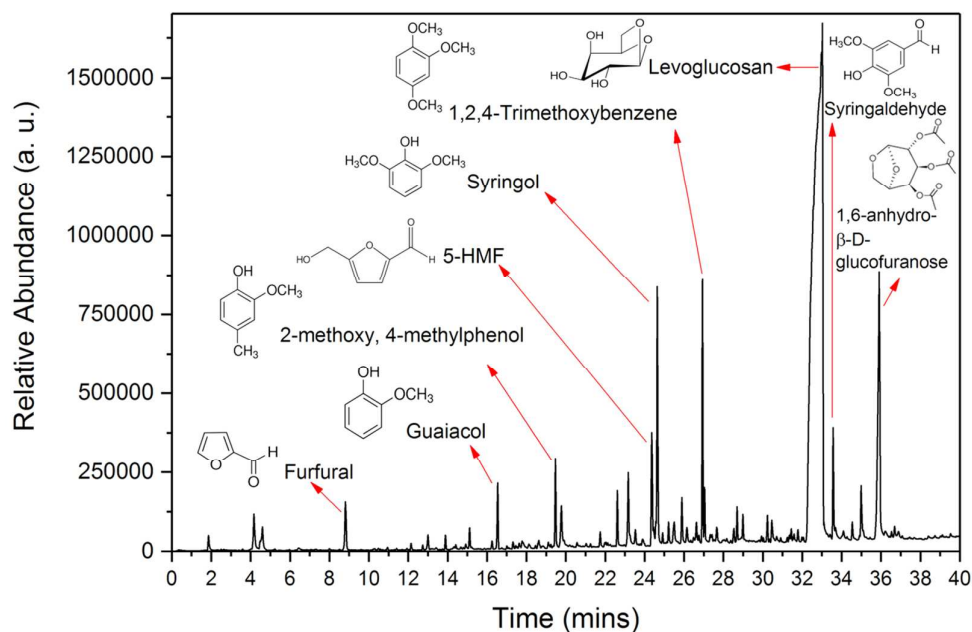


Figure 10. GC-MS chromatogram of the aqueous fraction of bio-oil

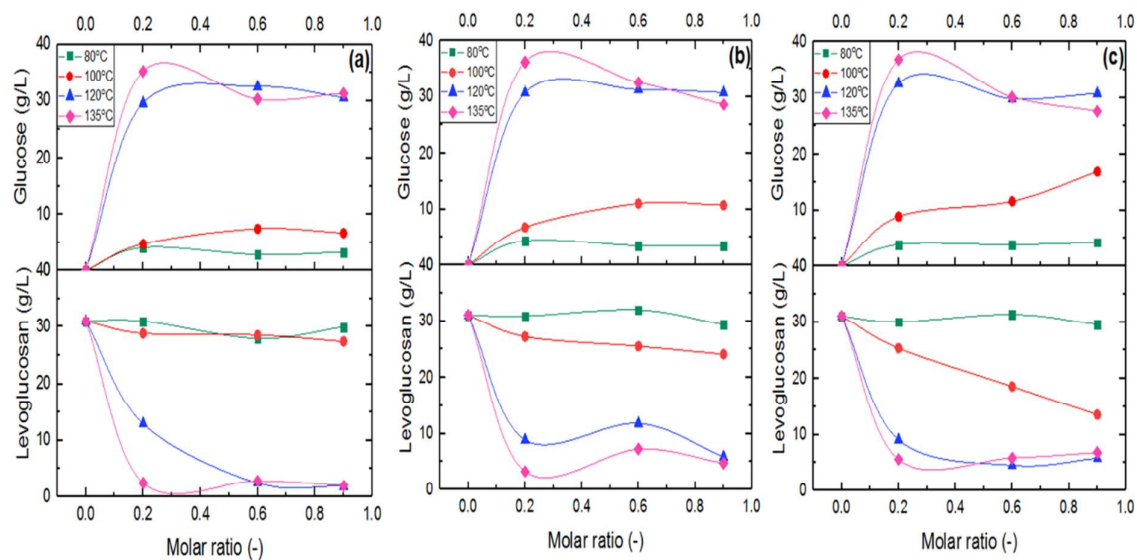


Figure 11. Concentrations of Glucose and levoglucosan (g L^{-1}) at different temperatures, molar ratios and reaction times of (a) 20 min; (b) 40 min; (c) 60 min

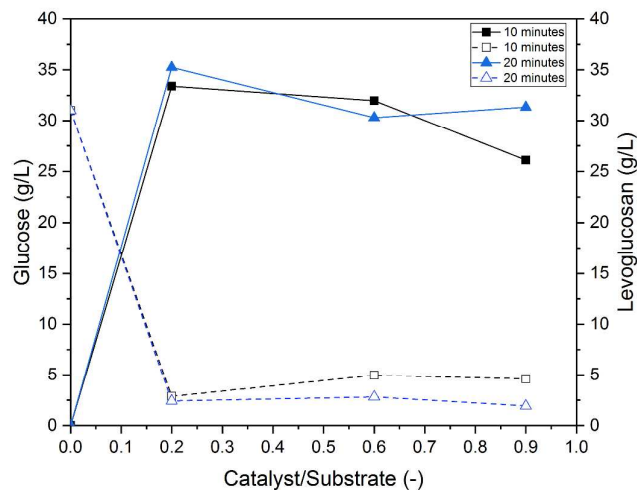


Figure 12. Concentrations of levoglucosan and glucose from the hydrolysis of pyrolysis oil at 135 °C, 10 and 20 minutes reaction time and 0.2, 0.6, and 0.9 catalyst/substrate ratios.

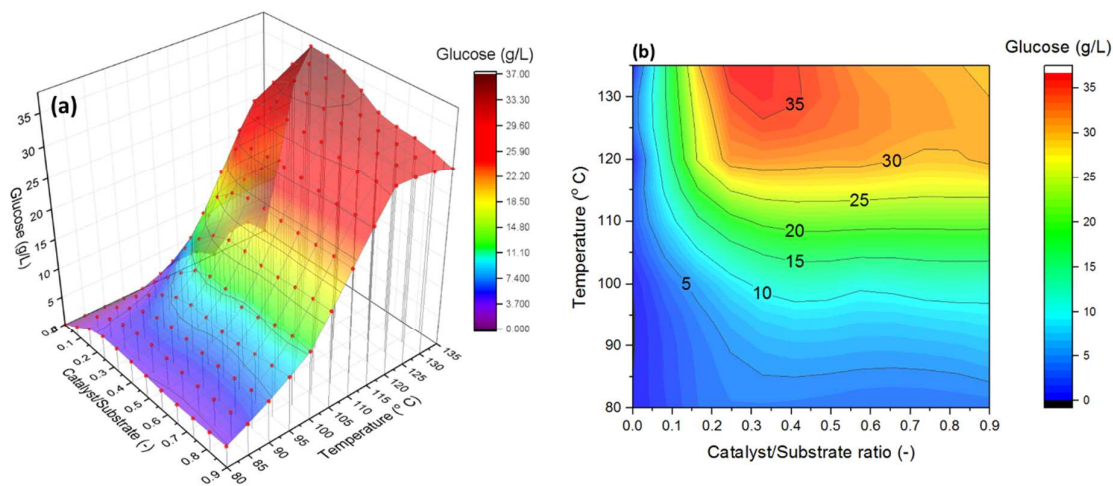
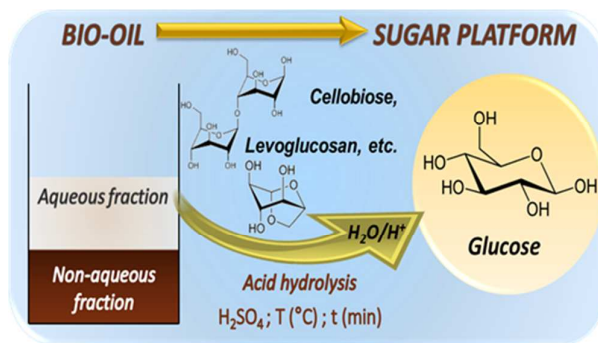


Figure 13. Glucose concentration from the acid hydrolysis of pyrolysis oil: (a) 3D surface area; (b) contour plot

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Synopsis

This paper envisages a conversion pathway from a biomass renewable feedstock into glucose which is a high-value sugar platform.