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Saccharification of lignocellulosic biomass for biofuel and biorefinery applications – A renaissance for the concentrated acid hydrolysis?

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Abstract

Hydrolysis of lignocelluloses using concentrated acids achieves near-theoretical sugar yields, and with fewer degradation products than the more commonly employed dilute acid hydrolysis process. In this paper, the dependence of sugar yield and the production of fermentation inhibitors on central process parameters is investigated, and the “severity factor” concept of one single process parameter characterizing the extent of the reaction is applied for the first time to concentrated acid hydrolysis of lignocellulosic biomass. Selected hydrolyzates have been fermented in the laboratory to investigate the effect of analyzed and unknown fermentation inhibitors in the hydrolyzates on fermentation performance. The concentrated acid hydrolysis process appears to be an interesting process for saccharification of lignocellulosic biomass for biofuel and biorefinery applications, with high sugar yields, low levels of fermentation inhibitors, good fermentability and good robustness towards changes in raw material quality.

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1. Introduction

The increased concern for anthropogenic CO₂ emissions and limited availability of petroleum resources in the future has spurred a steadily increasing interest in energy and fuel based on renewable

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resources. Although currently exploited biomass resources don't seem to be sufficient to cover the world's transport energy needs, much less the world's total energy needs [1,2], lignocellulosic biomass is still regarded by many as the only large-scale currently available non-fossil carbon source for energy carriers, chemicals and materials. Thus, there is still a strong motivation for research into industrially and economically viable processes for conversion of biomass to energy, fuels, chemicals, and materials.

Currently investigated technologies for conversion of lignocellulosic biomass to fuels and chemicals are diverse; however the main interest in the research community seems to focus on either gasification followed by hydrocarbon synthesis, or saccharification followed by biochemical or catalytic processing of sugars and/or lignin. The use of simple monosaccharides as process intermediates may enable the production of a diverse range of compounds, either by fermentation [3] or by catalytic hydrodeoxygenation [3,4] and is thus a very interesting approach for a biorefinery producing a diverse range of products, of which the fuel components may not necessarily provide the highest income for the biorefinery [5]. However, successful and effective production of monosaccharides in high yields is still a challenge. The main saccharification methods currently being investigated are acidic or enzymatic hydrolysis of biomass polysaccharides after a pretreatment stage, followed by chemical or biochemical conversion of the sugar intermediates. Another approach is the simultaneous saccharification and fermentation (SSF), or consolidated bioprocessing (CBP) [6] to overcome feedback inhibition of the hydrolytic enzymes employed.

1.1. Saccharification methods

Dilute acid pretreatment and hydrolysis is extensively investigated, and the technology is today at the pilot plant level. However, dilute acid hydrolysis has some drawbacks, namely the acid-catalysed dehydration of the sugar intermediates into furfural-type components, decreasing sugar yields and inhibiting biochemical conversion of the sugars into *e.g.* fuel ethanol [7]. Due to the mild conditions employed during enzymatic hydrolysis, furfural production is essentially negligible, and enzymatic saccharification is therefore an appealing alternative to acid hydrolysis. However, the recalcitrance of lignocellulosic substrates and the cost of currently available enzyme preparations are regarded as the major obstacles to an economically viable lignocellulose-based biorefinery employing enzymatic hydrolysis. Also, finding appropriate pretreatment methods to increase substrate availability is a challenge for successful employment of enzymatic hydrolysis in a lignocellulose-based biorefinery [7]. It is believed, however, that improved pretreatment methods and enzyme preparations may overcome this challenge in the future.

Concentrated acid saccharification is a process which was in industrial use during the early 20th century [8,9], but it is now discontinued due to the large consumption of acid making the process less economically favorable. However, the development of effective acid recovery solutions for the concentrated acid hydrolysis process [10,11,12] has renewed the interest [11,13-21] in this process, which previously was regarded as economically not viable due to the large amounts of acid required. In concentrated acid saccharification, concentrated mineral acids effectively de-crystallize the cellulose, making the reaction system during subsequent hydrolysis more homogeneous and thus less vulnerable to monosaccharide degradation before complete hydrolysis is obtained.

1.2. The severity factor and its applicability to concentrated acid hydrolysis

The severity factor concept has been used as a single-parameter characterization of the extent of reaction in lignocellulose processes like pyrolysis [22], delignification [23,24], or autohydrolysis [25,26], but has seen its most widespread use for characterization of dilute acid hydrolysis [22,27].

Decrystallization and hydrolysis of lignocelluloses using concentrated acids gives a reaction which is described to be homogenous [28,29], and given the basic chemical similarities between dilute acid pretreatment/hydrolysis and concentrated acid decrystallization/hydrolysis, it would be natural to examine the applicability of this concept also for the concentrated acid process.

The generalized severity parameter for acid-catalyzed hydrolysis, CSF_{SA} , is expressed as [27]:

$$CSF_{SA} = \ln(R_{OH}) \quad (1)$$

where

$$R_{OH} = \exp\left(\frac{C - C_{ref}}{\lambda C_{ref}}\right) \times \exp\left(\frac{T - T_{ref}}{\omega}\right) \times t \quad (2)$$

And C is acid concentration, C_{ref} is the reference acid concentration, T is temperature, T_{ref} is the reference temperature λ is a parameter expressing the acid effect in the conversion, t is the reaction time and ω expresses the temperature influence in the conversion and is related to the average activation energy [30].

1.3. Scope and motivation

The purpose of this work has been to gain increased understanding of the concentrated acid hydrolysis process and investigate its applicability towards a sugar-based biorefinery or biofuel production plant.

2. Materials and methods

2.1. Raw materials

Lignocellulosic raw materials were aspen (*Populus tremula*) and Scots pine (*Pinus sylvestris*) chips which were hammer milled to a size between 3mm and 7mm. Biomass composition was determined according to Sluiter *et al* [31,32,33]. Further details on biomass composition are reported by Janga *et al* [20,21].

2.2. Saccharification procedure

Concentrated acid saccharification was performed in two stages. In the decrystallization stage, the biomass was mixed with a predetermined amount of concentrated sulfuric acid and deionized water in 250 ml Erlenmeyer flasks at an acid/wood ratio of 15 to ensure good mixing and complete wetting of the biomass. The flasks were incubated for the required time in a shaking water bath (Stuart Scientific SBS 30) preset at the required reaction temperature and a shaking speed of 200 rev/min. After decrystallization, the slurry was diluted with deionized water to an acid concentration of 20 wt. % in a 250 ml plastic capped Pyrex glass bottle and hydrolyzed at 100 °C in an autoclave (CertoClav CV-EL 12 L GS) open to the atmosphere for 3 hours before cooling and vacuum filtration. The acid/sugar solution was neutralized with solid Ca(OH)_2 to pH 5.0-6.0, and the solution was further filtered to separate the CaSO_4 precipitate from a yellowish sugar solution. The sugar solution was stored at 4°C before monosaccharide and degradation products analysis. Further details on saccharification procedures are reported in [20].

2.3. Chemical analyses

The hydrolyzates were analyzed by HPLC. Monosaccharides were analyzed on a Chrompack Carbohydrates Pb column (Varian, Palo Alto CA, USA) using deionized and degassed water as mobile phase and refractive index (RI) detection. Column temperature was 80 °C, and the flow rate was 0.4 ml/min [34]. Sugar degradation products were analyzed on an Aminex HPX-87H column (Bio-Rad, Hercules CA, USA) using 5 mM sulfuric acid as mobile phase and UV absorbance detection. 5-hydroxymethylfurfural (HMF), furfural, and levulinic acid were detected at 280 nm, while acetic acid and formic acid were detected at 210 nm. The flow rate was 0.6 ml/min, and the column temperature was 65 °C. All samples were filtered through a 0.2 µm syringe filter before injection.

Analyses of sugar and ethanol concentrations during fermentation were performed by HPLC. The concentrations of glucose, xylose, galactose, mannose and arabinose in the samples were determined by using a sugar column (Aminex HPX-87P, Bio-Rad, USA) at 85 °C with deionized water as an eluent at a flow rate of 0.6 ml·min⁻¹. The ethanol concentration in the fermented samples was determined by ion exchange chromatography (Aminex HPX-87H, Bio-Rad, USA) at 65 °C, using 0.005 M sulfuric acid as an eluent at a flow rate of 0.6 ml·min⁻¹. Both sugars and ethanol were detected by a refractive index (RI) detector.

2.4. DOE planning; model fitting

A response surface methodology (RSM) technique using MODDE 8.0.2 software (Umetrics AB, Umeå, Sweden) was used to systematically investigate the effects of temperature, acid concentration and time on sugar yields and degradation [20]. A two-level three-factor Central Composite Face (CCF) design of experiment consisting of 17 experiments with 8 runs at the corner or cube points, 6 axial points, and 3 repeats at the center point was employed. The upper and lower limits of the experimental conditions were selected based on literature data [35-38]. A quadratic function was fitted to the experimental data using multiple linear regression in the MODDE 8.0.2 software.

2.5. Severity factor model fitting

As a first approximation, a simple quadratic function was chosen as suitable for fitting the yield to calculated severity factor; such a function shows the expected qualitative behavior of yield as a function of reaction severity (an increase to maximum, followed by a decrease to zero yield):

$$Y = a + b \cdot R_{OH} + c \cdot R_{OH}^2 \quad (3)$$

The parameters λ and ω in Eqn (2), and a , b , and c in Eqn (3) were determined simultaneously using the non-linear regression function `nlinfit` in MATLAB™. The parameters λ and ω were iterated in an inner loop, and a , b , and c were determined by linear regression for each set of (λ , ω) values in an outer loop.

The reference acid concentration and temperature were chosen at the middle of the data sets as $C_{ref} = 11.9$ mol/L and $T_{ref} = 315.7$ K, respectively, as the reference conditions have been reported have an insignificant influence on the severity analysis and the fitted optimal values of the model parameters [24]. The optimized parameters were then used to calculate the concentrated acid severity factor (CSF_{SA}) for a given set of conditions at the decrystallization stage.

2.6. Fermentation of hydrolyzates

The dilute sugar solutions resulting from the two-stage concentrated sulfuric acid hydrolysis process were concentrated approximately fivefold to increase the concentrations of all reaction products and simulate hydrolysis at lower acid/wood ratios than what was achievable using ordinary laboratory glassware. The neutralized hydrolyzates were evaporated under vacuum (pump negative pressure of 0.8 bar) in a rotating vacuum rotavapor (Heidolph VV 2000) set at 120 rpm and the water bath temperature kept at 60 °C. About 120 ml of a dilute sugar solution was evaporated under vacuum to approximately 20 ±2.5 ml. After concentration, the hydrolyzates were analyzed again by HPLC. Since furfural was lost during the vacuum concentration process, it was replenished after concentrating the hydrolyzates, by a concentration factor equivalent to the concentration factor of the other components.

Anaerobic ethanol fermentation by *Saccharomyces cerevisiae* ATCC 96581 was carried out in 15 ml fermentation tubes incubated at 30 °C without shaking. The tubes had a septum lid which was pierced with a syringe needle during sampling without opening the tube. The volumes were 1.5 ml of the medium and 0.2 ml of the inoculum, and the balance was hydrolyzate to make a total working volume of 15 ml. All hydrolyzates were filter-sterilized through a 0.2 µm filter before inoculation, and fermentation was initiated by adding the inoculum to the tube already filled with the hydrolyzate and the medium. Before incubation a 1 ml sample was taken, and after incubation, a series of 1 ml aliquots were sampled at different time intervals until no more glucose was left. Before sampling the tubes were shaken and the sampled aliquots were centrifuged and later stored at -4 °C for sugar and ethanol analysis. A reference fermentation was run in parallel by charging only the medium, inoculum, glucose and RO-water added to reach the required volume.

3. Results and discussions

3.1. Optimal reaction parameters and inhibitors yields

The initial Design of Experiment (DOE) study indicated that the most significant process variables investigated were acid concentration and temperature. Optimal decrystallization conditions for pinewood maximum yield of about 64 g sugars/100 g dry wood appears in the temperature range of 28 to 40 °C and acid concentration between 70 and 74 wt.%, while optimal decrystallization conditions for aspenwood giving a maximum yield of about 56 g sugars/100 g dry wood occurred in the ranges of 30 to 40 °C and 65 to 69 wt.% sulfuric acid (Figure 1).

As expected from the low content of pentosans in pinewood, the production of furfural was low for the pinewood hydrolyzates (<0.9 g/100g wood), however significant amounts of furfural were detected in aspenwood hydrolyzates (≤2.3 g/100g wood). 5-hydroxymethyl furfural (HMF) yields were lower than 0.3 g/100g for all samples analysed. Generally, the yield of furfural correlated well with the xylose yield, while the yield of HMF correlated well with the yield of hexoses, as expected from the reaction mechanisms for the formation of furfural and HMF from pentoses and hexoses respectively. Further details on inhibitor yields can be found in [21].

3.2. Application of the severity factor concept

The severity factor concept seemed to explain satisfactorily the differences in reaction conditions required for optimal yield of the two different raw materials (Figure 2). It can be determined from the model equation and also seen from Figure 2 that the glucose yield reached a maximum at CSF_{SA} of approximately around 4.4 for pine, while for aspen the maximum conversion of hexosans to glucose

appeared at CSF_{SA} of approximately 3.6. The difference in severity requirements illustrates the highly recalcitrant nature of softwoods as compared to hardwoods. It has previously been pointed out that softwoods are less easily treated as compared to hardwoods [39], and the findings reported in this paper thus correspond well with the literature.

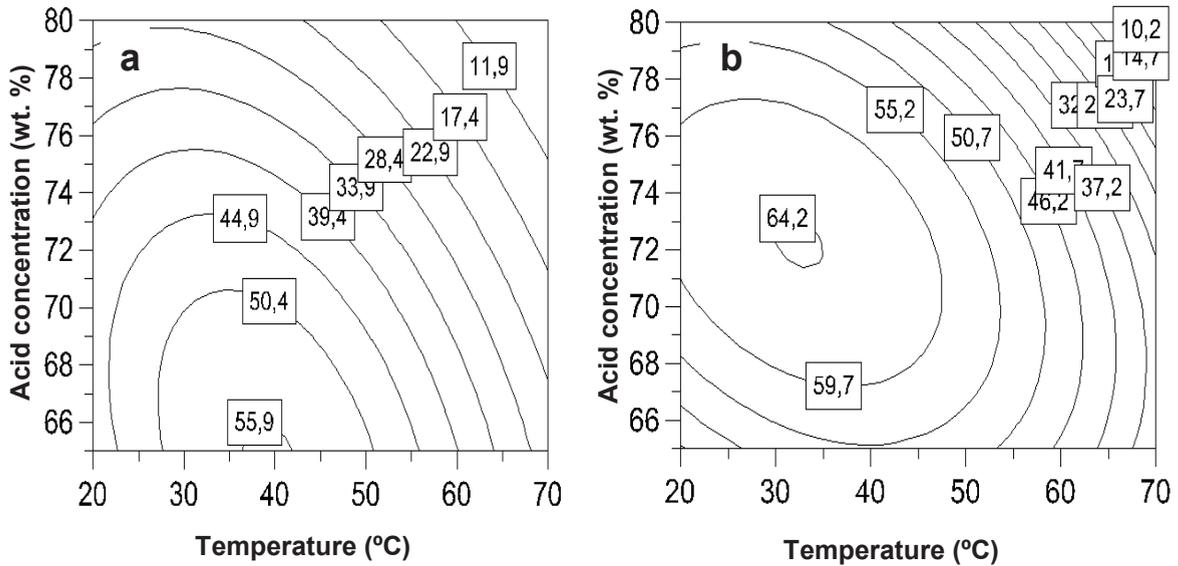


Figure 1. Constant yield contour plots showing the effect of temperature and acid concentration on the total sugar yield at decrystallization time of 60 minutes (a) aspen (b) pine

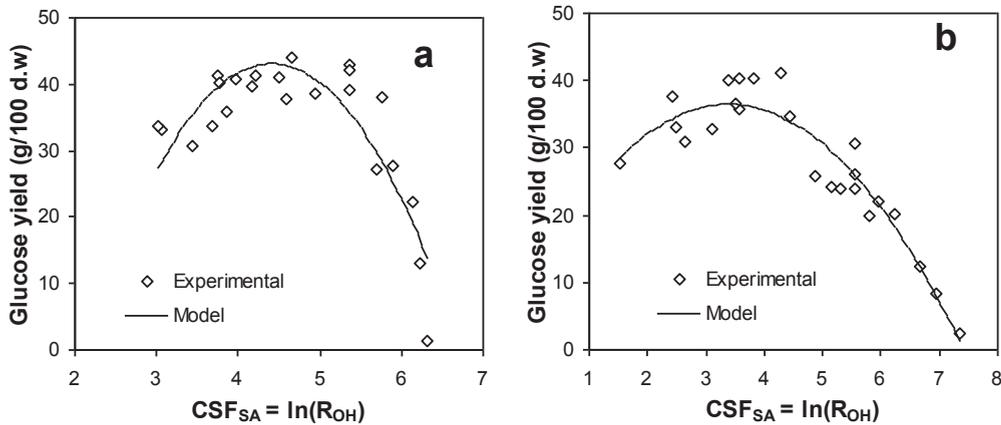


Figure 2. Effect of decrystallization severity on glucose yield (a) Pine ($\lambda=0.21$; $\omega=20.26$; Optimal severity ≈ 4.5 ; $R^2=0.76$) (b) aspen ($\lambda=0.12$; $\omega=15.82$; Optimal severity ≈ 3.5 ; $R^2=0.90$)

3.3. Fermentability of hydrolyzates

Fermentation experiments indicated that furfural was the most important inhibitor to fermentation performance. No adverse effect of fermentation inhibitors were observed at furfural levels below approximately 1 g/L, and for all hydrolyzates all glucose fermented totally to ethanol [21]. Final ethanol production seemed to depend only on the hexose concentration of the samples.

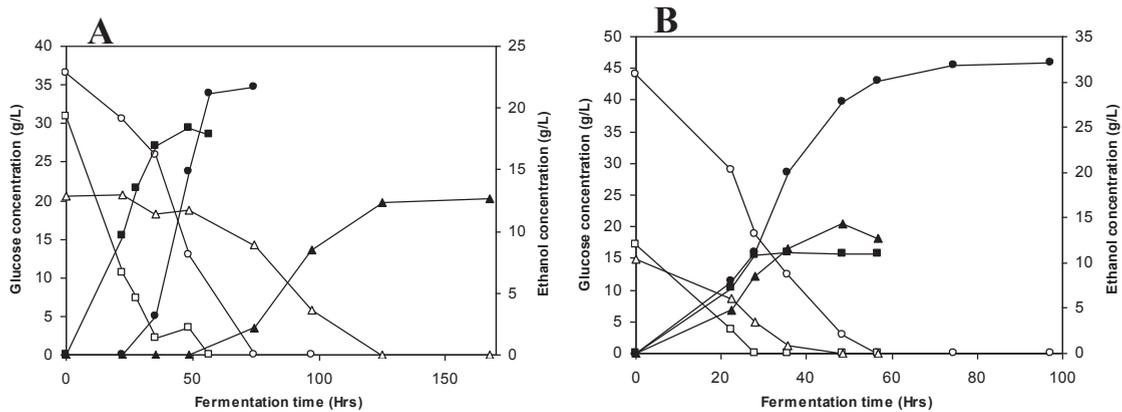


Figure 3. Fermentation profiles of concentrated hydrolyzates. Glucose consumption (open symbols), ethanol production (filled symbols). ○: Moderate severity, △: Mild severity, □: High severity. (A) Aspen (B) Pine.

Due to the low stability of xylose under acidic conditions, the highest yield of xylose was observed for the low severity treated samples while the lowest xylose yield was observed for the most severely treated samples. Since xylose and furfural yields correlated in this study, this was also observed for furfural yield. Only two samples (aspen at low and medium severity treatment), had a furfural concentration in excess of 1 g/L and showed a detectable lag phase during fermentation. For all the other samples, having a furfural concentration below 1 g/L, no lag phase was observed during fermentation.

4. Conclusions

The good sugar yields and low content of fermentation inhibitors indicates that two-stage concentrated acid hydrolysis may be a good alternative for saccharification of biomass, especially softwood. This may open up the possibility for utilizing low-value softwood residues like logging waste for a sugar-based biorefinery/biofuel production plant, considering the maturity and the relative simplicity of the concentrated acid hydrolysis process. Given the reaction conditions investigated, fermentation inhibitor levels in softwood hydrolyzates did not increase to levels affecting anaerobic fermentation by *Saccharomyces cerevisiae* ATCC 96581. Concentrated acid decrystallization and hydrolysis may also be a viable alternative for saccharification of xylan-rich lignocellulosic biomass like hardwoods. However, some furfural production must be expected, possibly necessitating a detoxification treatment before fermentation.

The application of the severity factor concept to the concentrated acid process was able to indicate the expected differences in recalcitrance between hardwood and softwood, however further studies are

needed to make the severity factor a universal tool for evaluating the extent of reaction during concentrated acid decrystallization and hydrolysis of lignocellulosic biomass.

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