High yields of sugars via the non-enzymatic hydrolysis of cellulose

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Abstract

Given the cost of cellulosics (quasi-homogeneous residual feeds range in North America, between $US 60–80/tonne, dry basis, FOB conversion plant) their fractionation and subsequent use of the intermediate fractions is a strategy that makes economic sense. Furthermore, it permits the isolation of cellulose with low contents of lignin and hemicellulose. Once the cellulose is isolated, its use as a chemically pulped fibre and the conversion of the fines into glucose becomes possible. Our group has been working on the chemical depolymerisation of the cellulose (both the fines and the fibres as well) using highly ionic solutions. The method implies recovery of both anions and cations by state of the art technologies. This paper presents the fractionation + ionic decrystallization and depolymerisation approach, provides and discusses its energy balance and compares it with the enzymatic route for hydrolysis in applications to < 40 MML Biofuels/y plants which correspond to < 100 000 t/y of input lignocellulosics, dry basis.
Keywords: cellulose, hydrolysis, depolymerization, electrodialysis, biofuels.

1 Introduction

In North America, residual forest and agricultural biomass cost actually 60-80$ US per dry tonne FOB. Such biomass could be considered chemically as quasi-homogeneous since although it may contain the same macromolecules and metabolites (extractives, hemicelluloses, cellulose and lignin), the concentration

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of each may vary as a function of season, due to weathering, etc. Energy crops or "non-conventional" cultures could also be included in this category since they usually are a "mixture" of different tissues although at this point, this biomass is slightly more expensive, reaching close to 100$ US per dry tonne FOB. Conversion of biomass to biofuels and "green chemicals" can be achieved through two general approaches which are categorized as "thermo" or "bio" pathways [1, 2]. Conversion of lignocellulosic biomass could also be achieved through a combination of both [3].

The "bio" approach relies on biological conversion of biomass at one point or the other during the process. This approach is somehow at this point limited by three technological challenges which are 1) the isolation of the cellulose and 2) its hydrolysis to glucose. The last technological aspect (3) that has to be considered is the fermentation of cellulosic sugars which may require additional nutriment to be efficient. The first challenge has been overcome for years by the pulp and paper industry although there is actually a need to develop new techniques leading to the production of pulp which will overall lead to cheaper and less water- and chemicals-consuming processes. Many approaches have been considered to isolate cellulose among which different steam treatments and solvent-related process have been thoroughly investigated [4]. Although isolation of cellulose from the biomass matrix could be performed under different conditions, another key to the economic viability of a biorefinery process is the isolation and utilisation of the other macromolecular fractions of the biomass as lignin and hemicelluloses. Although in many cases, the preliminary conversion process will use thermal and chemical energy, some reports have been made in literature on biological pre-treatment to isolate cellulose [4].

The second key technological challenge that needs to be overcome is the hydrolysis of glucose which is a crucial aspect of the production of cellulosic ethanol. Cellulose is composed of a crystalline and an amorphous phase. In most cases of the case, the amorphous phase is the more vulnerable to hydrolysis, chemical and biological as well. The latter usually relays on a mixture of 3 type of enzymes, endoglucanases, exoglucanases (cellobiohydrolases), and β-glucosidases [5]. The major problems delaying commercialisation of enzyme-based technologies are related on the cost of enzymes. Cellulose is composed of glucose units linked together by acetal bonds and the latter are weaken by acid catalyst. Therefore, utilisation of acid should in theory be an option, although the major problem in this case is the penetration of the acid in the cellulose crystalline and amorphous structure. Such a concept is not applicable since the cellulose macromolecules are oriented so that the polar functional groups are all linked together via hydrogen bonding making the outside section of cellulose highly hydrophobic. Penetration of water would ease the conversion of cellulose to glucose since it would expose the acetal bonds to any type of Lewis acid. Specific compounds can be used to swell cellulose which means that the swelling molecules can "move" between the microfibrils, reach the cellulosic chains and break its hydrogen bonded structure making a hydrogel. Cellulose is therefore less shelled against an attack since the acetal groups are exposed. The compounds that usually allow such specific interactions are usually ionic. This

brings the issue of removing them before fermentation of the cellulosic sugars to avoid inhibition. Another key aspect is to efficiently remove and recover the ions from the mixture.

This paper will discuss the conversion of residual quasi-homogeneous biomass to ethanol. Cellulose has been isolated from the lignocellulosic matrix using the Feedstock Impregnation Rapid and Sequential Steam Treatment (FIRSST) process. The cellulose-rich pulp produced from this process is then hydrolysed to glucose using a non-enzymatic approach involving ionic aqueous solutions. The broth is then purified using a sequential approach with one of the steps being electrodialysis and the remaining sugars fermented to ethanol using industrial grade yeasts. The energy and mass balance of the whole process will be evaluated to see where such a process could be position in comparison to other biological techniques leading to the production of cellulosic ethanol.

2 Experimental

2.1 Feedstock impregnation rapid and sequential steam treatment (FIRSST)

Two steps FIRSST process. Scheme of the one step FIRSST process is depicted in Figure 1 below. After extraction of the secondary metabolites the biomass was impregnated with water without any catalyst and was then pressed at 6.8 atm (100 psi) to remove the excess water and leave a saturated fiber. After pressing, the biomass is transferred into the 4.5 litres steam gun where about 200 g (dry basis), of chips were cooked at temperatures from 190 to 220 °C for 2-5 minutes. Delignification was performed on the pulp obtained from the first steam treatment using a solution of NaOH (2-10%wt of the lignocellulosic material). The wet fibrous solids filtered (as per Figure 1) were then washed again with

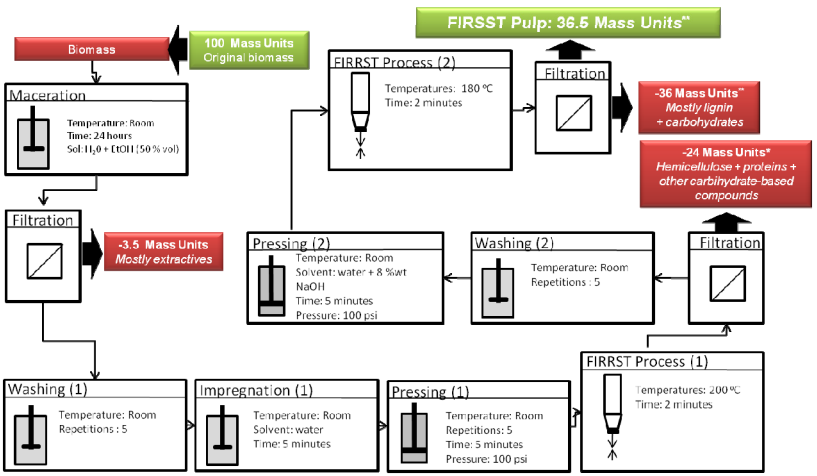

Figure 1: Two steps FIRSST process.

water 5 times using a water/biomass weight ratio of 5/1. The wet biomass was then impregnated with the alkali solution at 6.8 atm (100 psi) for 5 minutes. Delignification was performed at a cooking temperature in the 170-190 ^{o}C range for 2–5 minutes with concentration of NaOH ranging from 2 to 10%wt.

2.1.1 Analysis of the fibres

The testing methods used to evaluate the fibres produced both using FIRSST pulp and kraft pulp (in terms of comparison) is presented in *Table 1* below.

Table 1: Identification of the test and the standard techniques used for characterization of both FIRSST and kraft pulp.

Test	Standard technique identification
Freeness Determination	ATPPC C.1
Laboratory Screening of Pulp (Pulmac-Type Instrument)	ATPPC C.12
Fibre classification (Bauer-McNett)	ATPPC C.5U
Fibre Length by Automated Optical Analyzer Using Polarized Light	ATPPC B.4P
Forming Handsheets for Physical Tests with Pulp	ATPPC C.4
Forming Handsheets for Optical Tests with Pulp (British Sheet Machine Method)	ATPPC C.5
Grammage	ATPPC D.3
Brightness	ATPPC E.1
Colour Measurement with a Diffuse/Zero Geometry Tristimulus Reflectometer	ATPPC E.5
Opacity	ATPPC E.2
Thickness and Apparent Density	ATPPC D.4
Internal Tear Resistance	ATPPC D.9
Bursting Strength	ATPPC D.8
Length of rupture	ATPPC D.34
TEA	ATPPC D.34

2.2 Hydrolysis of the cellulose

The method used for cellulose hydrolysis is described in the patent #80685-2 [8]. This method includes an acid pretreatment followed by addition of a source of hydroxide ions. The mixture is then heated to obtain a glucose-rich solution, which is filtered before glucose purification.

The hydrolysis yield is calculated by comparison with the ASTM method No E1758-01R07 [9]. Glucose concentration of the filtrate was measured by HPLC, using an Agilent Chromatograph equipped with an RoA-Organic acid (8%) column (Phenomenex) and a refractive index detector. The column was eluted with 5 mM sulphuric acid at a flow rate of 0.6 ml min^{-1} and maintained at 60°C.

The injection volume was 30 µl. Sulphate concentration was measured by a colorimetric method [10] and ammonium or sodium concentration, by IC. The apparatus used was a Dionex ICS-3000 ion chromatograph loaded with an IonPac CS12A (2x250mm) and detection was made with electric conductivity.

Three identical cellulosic hydrolyses were performed with 46 g of wet cellulose (64% humidity) each. These tests were done in 2 L erlenmeyer and hydrolysed in an autoclave. The solution was filtered in a Buchner with Glass fiber Fisher Brand filter. The three filtrates were then mixed for the purification step which, in these tests, was made by using electrodialysis only.

2.3 Purification of the cellulosic hydrolysate

A 3 L mixture of the hydrolysis broth (composed of ~300 g/L sodium sulphate, ~300 g/L sulphuric acid and between 10-20 g/L glucose) was purified using an electrodialysis system.

Testing was done at the *Energy technology Laboratory (LTE)* in Shawinigan, QC. The electrodialysis system used was a CS-O batch system from Asahi Glass Co. The cathode and the anode were of iridium oxide and 4 membranes pairs of 180 cm^2 each were used. The experimental conditions were: 30 ^oC, 150-200 L/h and 20 A fixed (i = 111 mA/cm^2). The pH, temperature, conductivity, voltage and intensity were measure automatically at time intervals. Each volume of the three compartments was also measured.

2.4 Fermentation of cellulosic sugars

Inocula were prepared using a medium containing 0.07% w/w gluco-amylase, 16 mM ammonium sulphate, 0.01 g/L Lactrol, 20 g/l yeast (Ethanol Red, Fermentis), 60 ml corn mash (32% solids) and 40 ml water was used. The medium was incubated in an Erlenmeyer flask at 32^oC, 150 RPM for 4 hours.

4.6 ml (2 g/l of yeast) of the pre-fermentation medium were then added to 200 ml of the purified lignocellulosic sugars. 8 mM ammonium sulphate and 0.01 g/l Lactrol were also added to the fermentation medium. The corn mash used in the pre-fermentation medium provided the necessary trace nutriments. The medium was incubated in 250 ml Erlenmeyer flasks coupled with a fermentation lock at 34.5^oC and 150 RPM during 44 hours.

Monitoring of the fermentation was made by HPLC, using an Agilent Chromatograph equipped with a RoA-Organic acid (8%) column (Phenomenex) and a refractive index detector.

3 Results and discussion

3.1 Feedstock impregnation rapid and sequential steam treatment (FIRSST)

The FIRSST process was shown effective for the isolation of the macromolecular structures from different types of lignocellulosic biomass including hardwood (willow), softwood (balsam and fir) and energy crops (hemp

and triticale). Production of pulp via the FIRSST process was of 30%wt (dry mass) for willow [6], 40%wt for softwoods [7], 37%wt for hemp and 34%wt for triticale. Pulp produces contained about 3–6% of lignin and the residual fibre was mostly composed of C_6 sugars. Evaluation of the fibres was made both on chemical and mechanical aspects to verify how the severity of the combined steam treatments could be comparable to a classical kraft pulping process. Comparative results are shown in *Table 2* below.

Table 2: Mechanical properties of FIRSST pulp and kraft pulp for a species of hardwood (*Salix viminalis*) and a mixed species of softwood (*Abies balsamea* and *Picea mariana*).

Test	**H-FIRSST**	**H-Kraft**	**S-FIRSST**	**S-Kraft**
ATPPC C.1 ± 1 mL	409	454	664	721
ATPPC C.12 ± 0.01%	12.7	0.16	5.58	0.11
ATPPC B.4P ± 0.01 mm	0.39	0.41	2.08	2.56
ATPPC D.3 ± 0.1 g/m^2	60.9	60.1	59.6	61.0
ATPPC E.1 ± 0.1%	24.7	33.3	24.8	27.6
ATPPC E.5				
L* ± 0.01	64.22	71.45	67.26	69.36
a* ± 0.01	2.82	2.18	4.41	4.12
b* ± 0.01	13.26	12.42	18.31	17.43
ATPPC E.2 ± 0.1%	99.6	99.5	98.5	99.3
ATPPC D.4 ± 0.01 cm^3/g	2.17	1.79	2.24	2.36
ATPPC D.9 ± 0.01 mN*m^2/g	4.09	3.14	8.04	25.9
ATPPC D.8 ± 0.01 kPa*m^2/g	1.35	1.56	3.05	2.28
ATPPC D.34 ± 0.01 km	2.78	4.24	5.03	3.87
ATPPC D.34 ± 0.1 J/m^2	15.6	19.6	37.8	22.9

Results shown in Table 2 show that for hardwood and softwood, two steps steam treatment allowed the isolation of a high quality fibre. These fibres showed less resistance to mechanical stress but showed overall better optical properties. Overall, the FIRSST process allowed the production of fibres that could be converted either to pulp or hydrolysed to glucose depending on market potential.

3.2 Hydrolysis of the cellulose

The cellulosic hydrolysis yields obtained were 96, 83 and 88%, and the average glucose concentration was 10 g/L. These yields can be maximized by changing

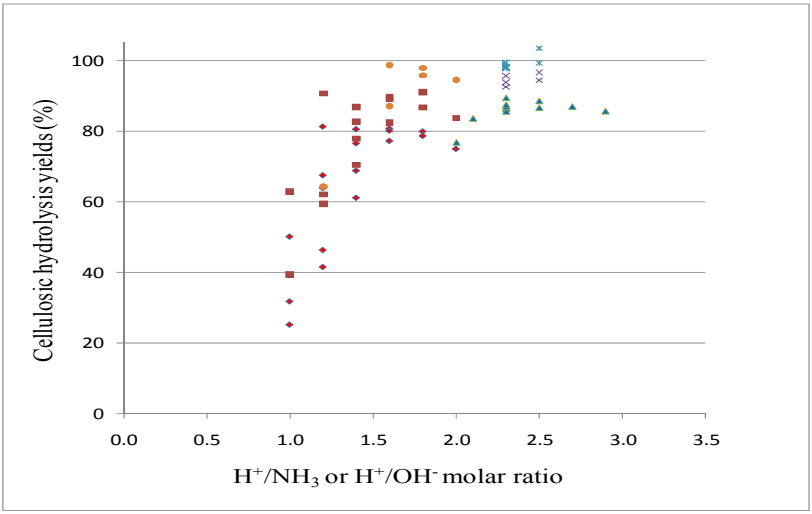

Figure 2: Cellulosic hydrolysis yield versus H^+/OH^- or H^+/NH_3 molar ratio.

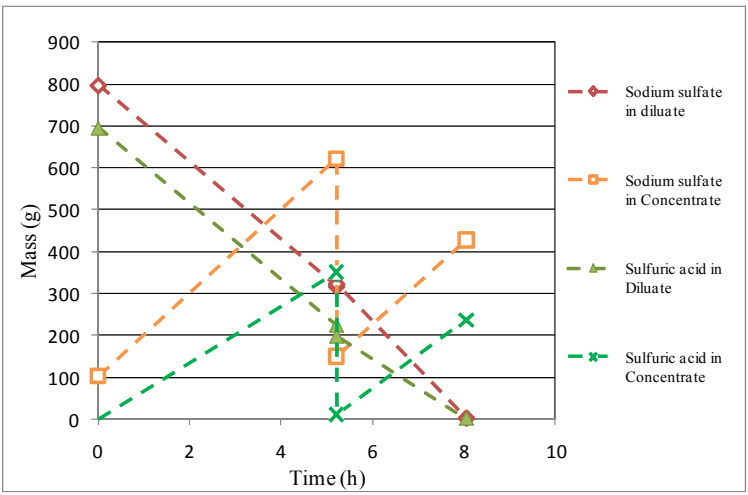

Figure 3: Composition of the diluate and of the concentrate vs. time for the electrodialysis purification.

some parameters, proven by laboratory tests realised to optimise cellulosic hydrolysis.

3.3 Purification of the cellulosic hydrolysate

Purification of the mixed hydrolysate by electrodialysis leaded to a solution composed of 4.3 g/L sodium sulphate and 1.9 g/L sulphuric acid with glucose. The separation concentrates the glucose solution by a factor of 4.7. With the use of AMV and CMV Selemion membranes (Asahi Glass), it is possible to keep around 95% of the glucose in the diluate. Presence of glucose did not seem to foul the membranes over the time duration of the preliminary process design steps.

Figure 3 presents the composition in sulphuric acid and sodium sulphate of the different compartments versus time:

Due to the voltage increase caused by high ionic concentration in the concentrate (223 g/L sodium sulphate and 126 g/L sulphuric acid)-which means a depletion of the ionic content in the diluate-, the solution in the compartment was replaced by a 50 g/L sodium sulphate solution after 5.7 h of purification.

The energy demand was calculated to 0.57 kWh/kg of separated ions, corresponding to 26 kWh/kg of glucose recovered. Another purification by electrodialysis was realised with a solution containing ammonium sulphate instead of sodium sulphate. This purification had an energy demand of 0.52 kWh/kg of separated ions, corresponding to 24 kWh/kg of glucose. 90% current efficiency was calculated for the test with sodium sulphate and 92% with ammonium sulphate. Since the electrical demand is too high translating into an excessive energy cost per kg of glucose, separation of the ions will have to be done by a combination of less energy intensive steps followed by a final electrodialysis step.

3.4 Fermentation of cellulosic sugars

2 ml of the fermentation medium were withdrawn after 2, 22 and 44 hours in order to be analysed for ethanol and glucose content, as well and acetic and lactic acid content. The results shown are the mean of duplicates. The progress of the alcoholic fermentation is shown in Figure 4.

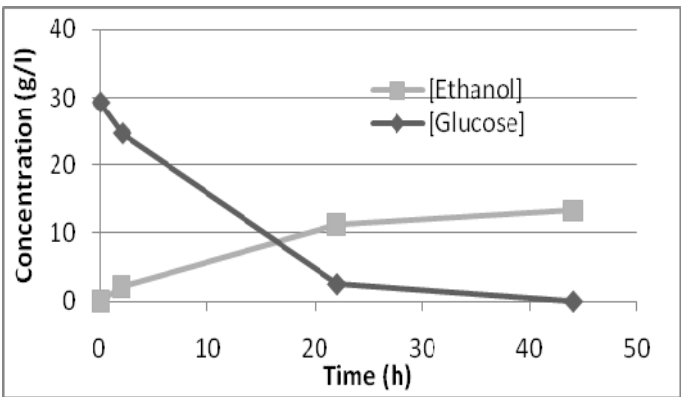

Figure 4: Alcoholic fermentation progress of Ethanol Red *Saccharomyces cerevisiae* yeasts in lignocellulosic sugars medium.

Yields of .46 g/g, an efficiency of 90.1% and a consumption of 100% of the glucose were achieved after 44 hours. No methanol was produced during the fermentation (data not shown). Inhibitors such as acetic acid and lactic acid were also quantified. Table 3 shows the inhibitor composition of the medium through the fermentation.

Table 3: Major fermentation inhibitor concentration through an alcoholic fermentation by Ethanol Red Saccharomyces cerevisiae yeasts.

Time (h)	[lactic acid] mg/l	[acetic acid] mg/l
0	< 100	< 100
2	< 100	< 100
22	< 100	501.0
44	< 100	503.6

Table 3 shows an increase in acetic acid concentration after 22 hours as a by-product of the yeast growth. An acetic acid concentration of .05% (w/v) is considered to have no influence on the yeast growth [11]. The results show that no major inhibitor is in a sufficient concentration to inhibit the yeast fermentation throughout the whole process.

4 Conclusion

The Feedstock Impregnation Rapid and Sequential Steam Treatment (FIRRST) was shown to be suitable not only for the isolation of the cellulosic matrix but also to produce a high quality pulp that could have a potential for paper production. Results have shown that cellulose can be hydrolysed at 90%+ using a strong ionic solution and that this solution could be purified using membrane technologies. Following treatments, the residual glucose solution can easily be fermented with common yeasts to produce cellulosic ethanol. Key for the economics of this approach will be to optimise the energy consumption for the separation process and recovery of ions.

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