BIOETHANOL PRODUCTION FROM WOODY BIOMASS AFTER PRETREATMENT AND ENZYMATIC HYDROLYSIS OF CELLULOSE

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Abstract: *The aim of this study was the production of bioethanol from wood by steam-explosion pretreatment, enzymatic hydrolysis and fermentation of glucose. Separate hydrolysis and fermentation was applied for cellulose hydrolysis, followed by fermentation with S. cerevisiae. Using H_2SO_4 – impregnated of wood chips in steam pretreatment resulted in the high ethanol concentration. In this work, the steam-explosion pretreatment conditions for wood chips were optimized to obtain higher ethanol yield. A commercial enzymes mixture was used for enzymatic hydrolysis of wood.*

Keywords: bioethanol, steam-explosion, cellulose, SHF

INTRODUCTION

Lignocelluloses or woody biomass can be used for bioethanol production. Bioethanol produced from woody biomass is a renewable source and can be used at transport fuel, in pure form or mixed with gasoline [1, 2, 3]. Currently, bioethanol is produced from sugarcane and cereals, but these resources are used as food [4, 5]. Woody biomass has a great potential feedstock not compete with food production, and is available in large quantities. Finding alternative biofuel residue must satisfy the requirements of the Kyoto Protocol [6]. Wood consists mainly of cellulose, hemicellulose and lignin. Cellulose and hemicellulose can be converted to bioethanol. Bioethanol is produced from woody biomass by four stages: pretreatment, acid or enzymatic hydrolysis, fermentation of the sugar solution and distillation/ recovery of the ethanol [7, 8, 9]. The enzymatic hydrolysis and fermentation can be conducted simultaneously (SSF) or separate hydrolysis and fermentation (SHF). Cellulose is a crystalline polymer of glucose. Hemicellulose is a mixture of pentoses (glucose, galactose and mannose) and pentoses (xylose and arabinose) [10]. Cellulose can be hydrolyzed to glucose by cellulose enzymes. *Trichoderma reesei* are cellulase enzymes used for enzymatic hydrolysis. The production of ethanol from woody biomass has been intense studies with regard to pretreatment [11, 12], enzymatic hydrolysis [13, 14] and fermentation [15, 16]. Pentose and hexoses can be recovered by steam pretreatment and enzymatic hydrolysis and glucose can be fermented to bioethanol using baker's yeast. Baker's yeast *Saccharomyces cerevisiae* is the most commonly used for fermentation of glucose to bioethanol.

The aim of this work is the production of bioethanol from woody biomass. The pretreatment method used to separate cellulose from hemicellulose and lignin is steam-explosion using H_2SO_4 as catalyst. Steam-explosion pretreatment of wood using H_2SO_4 as catalyst is a good methods for hemicellulose solubility, allowing easier attack of enzymes to cellulose. Cellulose obtained by steam explosion is enzymatic hydrolysis by separate hydrolysis and fermentation (SHF) to glucose. Baker's yeast, *Saccharomyces cerevisiae* is used for glucose fermentation to bioethanol.

MATERIAL AND METHOD

The experimental procedure used to convert wood chips to ethanol is show schematically in Figure 1.

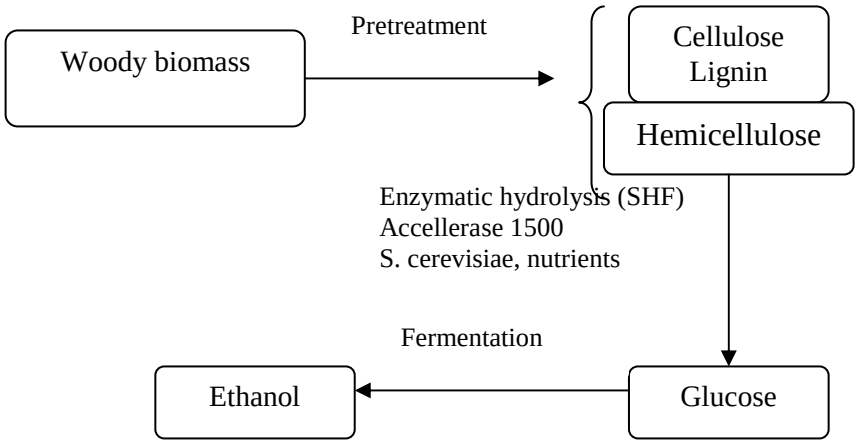

Fig.1. Schematic presentation of the procedure to convert wood to ethanol

The raw material used in these experiments was wood chips was obtained from the forest around the city Cluj-Napoca in Romania. The wood chip was dried before to use. The fresh wood was stored in plastic bags at 4°C. The fir wood was cutter-milled to pass through either a 2- or 0.2-mm pore size sieve. The moisture content of raw material was determined by the weight loss after drying (105°C, 12 h), and to be 11%. Determination of ash in fir has expressed as the percentage of residue remaining after dry oxidation of raw material at 590°C. Extractives were analyzed by extracted by using one step extraction process which includes ethanol an extractive solvent. The holocellulose (mixture of cellulose and hemicellulose) and lignin content was determined as the $NaClO_2$ delignified methods.

The woody biomass was extracted in a Soxhlet extractor with ethanol for 6 h, and dried at 40°C for 24 h in vacuo. Hollocellulose content was determined as the $NaClO_2$ – delignified residue: 1 g sample was repeatedly (2 times) treated with 0.8 g $NaClO_2$ in acetic acid (60 ml) at 70°C for 1 h. The holocellulose product was filtered, washed with water, and dried at 105°C for 24 h, and weight. α-cellulose content was determined as the residue insoluble in 20% NaOH: 10 ml 20% NaOH

solution was added to 0.5 g sample of the obtained hollocellulose. The mixture was stirrer at 20°C for 50 min. The residue was filtrated and weight. Lignin was determined as the amount of residue insoluble in 75% sulfuric acid solution.

Steam-explosion pretreatment held in a 1L Parr reactor loaded with 40 g wood chips and 280 ml water containing sulfuric acid as catalyst (2% H_2SO_4, pH 2). The molar ration wood: solvent was 1:7 (w/w). The mixture is heated to 180 -210°C, 50-60 bar pressures for a residence time of 4-15 minutes, followed by rapid decompression, the products was recovered by filtration. The exploded material was washed tree times with water. The pretreated material was separated by filtration into solid residue and a liquid.

Enzymatic hydrolysis was performed on solid material recovered after pretreatment. A commercial cellulase *Accellerase1500* (Genencor, Finland) was used in this experiments. The activity of Accellerse1500 enzyme complex is expressed in carboxymethycellulose (CMC U) activity units. One CMC U unit of activity liberates 1 µmol of reducing sugars (expressed as glucose equivalents) in one minute under specific assay conditions of 50°C (122°F) and pH 4.8. Beta-glucosidase is reported in pNPG units. One pNPG unit denotes 1 µmol of Nitrophenol liberated from *para*-nitrophenyl-B-D-glucopyranoside in 10 minutes at 50°C (122°F) and pH 4.8 [17].

The enzymatic hydrolysis of the pretreated material was carried out in 0.1 M Na-acetate buffer (pH 4.8) for 72h.

Fermentation of the liquid was performed after enzymatic hydrolysis of cellulose. The pH of the liquid was adjusted to 5.5 with NaOH (0.8 M). Fermentation was performed according to Soderstrom [8] with small modifications.

The ethanol concentration was determined by gas chromatograph (Agilent technologies, 6890N GC) coupled with mass spectrometer (Agilent technologies, 5973N MSD). Headspace using a FID detector and a column HPS-5MS of 30m length×0.25mm I.D.×0.25µm HP-3 MS film thickness. The carrier gas was helium at constant flow rate of 1.2 mL min^{-1}.

Table 1

GC column temperature program

Step	Ramp. °C/min	Temperature, °C	Holding time, min
1	10	35	3
2	0	150	1
3	3	230	18

RESULTS AND DISCUSSION

The chemical composition of woody biomass, the raw material used in this study, is presented in Table 2. Determination of the initial composition of wood was made by the gravimetric method presented in material and methods. The chemical composition was determined as the $NaClO_2$–delignified residue; the holocellulose and lignin were calculated using their respective weight.

Chemical composition of woody biomass used in this study was determined and is show in Table 2.

Table 2

Composition of wood (percent of dry material)

Carbohydrates	
Cellulose	43 ± 0.5
Hemicellulose	21 ± 0.1
Lignin	
Acid-insoluble	24.2 ± 0.1
Acid-soluble	1.8 ± 0.2
Ash	1.16 ± 0.1
Extractives	1.11 ± 0.1
Others	7.73

Steam-explosion pretreatment carried out in a lab scale apparatus (1 L steam Parr reactor) to ensure a good process control and effective recovery of pretreatment fractions. In general, the recovery yield of pretreated material was when pretreatment was carried out in absence of acid as catalyst. In this condition, our experiments were carried out in presence at sulfuric acid as catalyst. The factors that affecting steam-explosion pretreatment are: temperature, residence time, pressure and severity factor.

In this study, steam pretreated of wood with sulfuric acid has been investigated by varying the temperature (180 -200°C) and the residence time (4-15 min.). The pressure (60 bars) was maintained constant in all experiments. The severity factor ($Log(R_0)$) and combined severity factor (CSF) was calculed according to Stenberg definition [15].

$$Log(R_0) = Log\left(t \exp\left(\frac{T - T_{ref}}{14{,}75} \right) \right) \quad (1)$$

where:

t temperature (°C);

T_{ref} 100 °C

14,75 Stenberg coeficient.

The severity factor shows the severity correlation between temperatures, time and is possible to choose the duration and the temperature of the pretreatment. The combined severity factor shows the good qualitative measure of how harsh a certain set of pretreatment conditions will be [15].

Table 3

Experimental design for steam-explosion pretreatment of wood chips

Experiment number	Temperature (°C)	Residence time (min.)	Log(R_0)	CSF (Log(R_0)-pH)
1.	180	15	3.52	1.52
2.	190	5	3.34	1.34
3.	190	10	3.64	1.64
4.	190	15	3.82	1.82
5	200	5	3.93	1.93

Cellulose was separated from hemicellulose by steam-explosion pretreatment due to the hard solubility of cellulose comparative with hemicellulose that is very slowly soluble in water and solvents. Temperature and steam cause the breaking of the chemical bonds between the macromolecules that constitutes the wood, hemicellulose appear as monomeric mixture sugars in liquid fraction and cellulose remain in solid fraction.

During the pretreatment acid acetic, furfural and HMF were formed. Acetic acid, furfural and HMF are inhibitors for enzymatic hydrolysis.

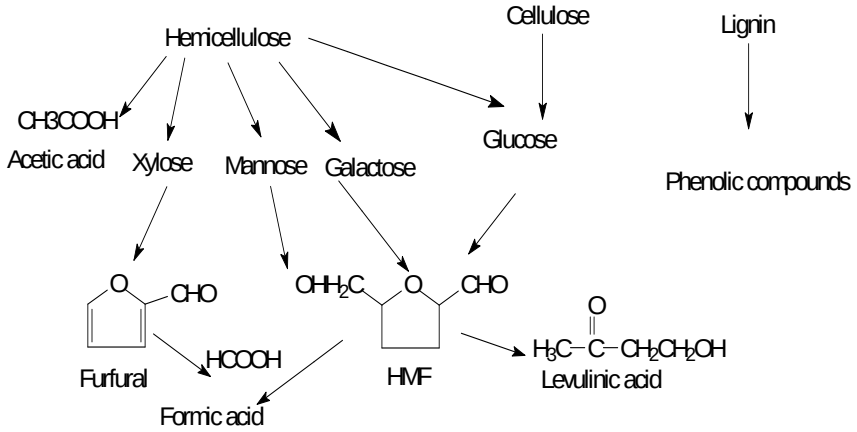

Fig. 2. Inhibitor structures formed during the pretreatment process

Separate hydrolysis and fermentation (SHF) of cellulose (solid fraction) was performed in all experiments. The commercial enzyme used in this study was Accellerase1500 who contains enzymes with exoglucanase, endoglucanase, hemicellulase and beta-glucosidase activity [17].

In this process, cellulose is enzymatic hydrolyzed to glucose; the glucose eliberate is then fermented into ethanol by yeast. Lignin is recovered as solid fraction after enzymatic hydrolysis of cellulose. The enzymatic hydrolysis yield was calculated assuming that no lignin was degraded during the pretreatment. Enzymatic hydrolysis gave the highest yields for pretreatment at a combined severity factor of CSF = 1.64. The fermentation depends of hydrolysis of cellulose. The enzymatic hydrolysates obtained after enzymatic hydrolysis was fermented with *Saccharomyces cerevisiae* according to Soderstrom method [8].

The theoretical ethanol production was calculated based on conversion of the entire glucose in the process including glucose from cellulose.

$$\text{Theoretical ethanol production} = TSC^{*} \times 0.51 \quad (2)$$

TSC^{*} = Total sugar from cellulose (after pre-treatment and enzymatic hydrolysis) (g/100 g raw material)

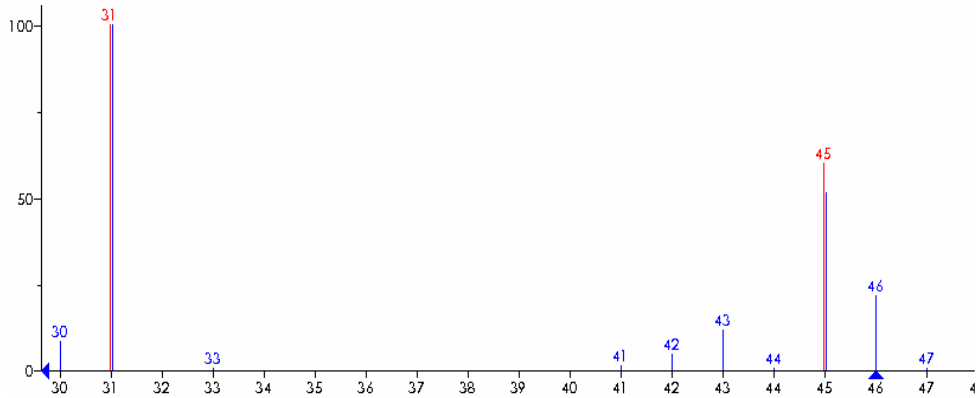

Fig. 3. Mass spectra of bioethanol (mass spectrum of ethanol from MS data overlap with mass spectrum obtained)

The ethanol concentration after 72 h was 25,5 g/l obtained for experiment number 3, which correspond to ethanol yield pf 75% based on the amount of cellulose in the raw material. For other experiments were obtained much lower concentrations of ethanol.

CONCLUSIONS

Ethanol production process used energy from renewable energy sources; no net carbon dioxide is added to the atmosphere, making ethanol an environmentally beneficial energy source. Ethanol from woody biomass can replace gasoline and is the only liquid transportation fuel that does not contribute to the green house gas effect. Bioethanol was obtained from woody biomass by steam-explosion pretreatment, enzymatic hydrolysis followed by the fermentation of the obtained sugar.

Steam-explosion is a successful method pretreatment due to the following characteristics: improve formation of cellulose and hemicellulose, the ability to subsequently form sugars by hydrolysis; avoid degradation or loss of carbohydrate; avoid formation of by products inhibitory to subsequent hydrolysis and fermentation processes and be cost effective.

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