

Bioethanol from lignocellulosic materials

Iuliana Leustean

„Dunarea de Jos” University Galati, Food Science and Engineering Faculty
Domneasca Street, no. 111

Abstract

Lignocellulosic biomass can be utilised to produce ethanol. There are two processes involved in the conversion: hydrolysis of cellulose in the cellulosic biomass to produce reducing sugars, and fermentation of the sugars to bioethanol. For this, pretreatments for lignocellulosic materials are required. The goal of any pretreatment is to alter or remove the structural and compositional impediments to hydrolysis and to increase the yields of fermentable sugar. In this work is made a review of the physical or chemical pretreatment methods that could be used to produce bioethanol from cellulosic materials.

Keywords: lignocellulose, pretreatments, cellulase, hydrolysis, bioethanol.

1. Introduction

This study examines the hydrolysis of waste lignocelluloses for the purpose of investigating feedstocks for ethanol production while providing an alternative solid waste management strategy. Lignocellulosic materials from agriculture, forest management and urban waste are the largest sources by hexose (C-6) and pentose (C-5), sugars with a potential for the production of biofuels, chemicals and other economic by-products.

Directive was accepted that requests member states to establish legislation about utilization of fuels from renewable resources. Biofuels are considered to be most promising in the short-term as their market maturity.[13]

Ethanol is an attractive alternative fuel because it is a renewable bio-based resource and it is oxygenated thereby provides the potential to reduce particulate emissions in compression- ignition engines.

Lignocellulose composed of cellulose (40–50%), hemicelluloses (25–35%) and lignin (15–20%) is extremely resistant to enzymatic digestion. Pretreatments are

usually necessary to disrupt the plant cell wall (lignin) in order to improve enzymatic digestibility. [3,4-13]

2. Materials and Method

Various processes are employed for lignocellulosic conversion a general process includes size reduction and pretreatment, hydrolysis, fermentation and separation. Size reduction and pretreatment are required to alter the biomass structure and increase the accessible surface area of cellulose, so that hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yield. Hydrolysis includes the processing steps that convert the carbohydrate polymers into monomeric sugars. During the fermentation process, the monomeric sugars are converted to ethanol and then ethanol is recovered from the fermentation broth, usually by distillation.[8,12]

The purpose of the pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials.[12] The crystallinity of cellulose, accessible surface area, protection of cellulose by lignin, the heterogeneous character of biomass

particles, and cellulose sheathing by hemicellulose all contribute to the recalcitrance of lignocellulosic biomass to hydrolysis. Rigorous process economic analysis is necessary to determine the best pretreatment process options for a particular feedstock and product opportunity. Pretreatment methods are either physical or chemical. Physical pretreatment methods include comminution (mechanical reduction in biomass particulate size), steam explosion, and hydrothermolysis. The comminution consists of dry, wet, and vibratory ball milling. Acids or bases promote hydrolysis and improve the yield of glucose recovery from cellulose by removing hemicelluloses or lignin during pretreatment. Cellulose solvents are another type of chemical additive. Solvents that dissolve cellulose resulted in 90% conversion of cellulose to glucose and showed enzyme hydrolysis could be greatly enhanced when the biomass structure is disrupted before hydrolysis. Alkaline H₂O₂, ozone, organosolv (uses Lewis acids, FeCl₃, Al₂SO₄ in aqueous alcohols), glycerol, dioxane, phenol, or ethylene glycol are among solvents known to disrupt cellulose structure and promote hydrolysis. Concentrated mineral acids (H₂SO₄, HCl), ammonia-based solvents (NH₃, hydrazine), metal complexes (ferric sodium tartrate, cadoxen, and cuoxan), and wet oxidation also reduces cellulose crystallinity and disrupt the association of lignin with cellulose, as well as dissolve hemicellulose.[8,12]

The first step in bioconversion of lignocellulosics to bioethanol is size reduction and pretreatment. The goal of any pretreatment technology is to alter or remove structural and compositional impediments hydrolysis in order to improve the rate of enzyme hydrolysis and increase yields of fermentable sugars from cellulose or hemicelluloses. Pretreatment is an important tool for practical cellulose conversion processes. Pretreatment is required to alter the structure of cellulosic biomass to make more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars and to

cellulose producing microorganisms. A successful pretreatment must meet the following requirements: improve formation of sugars or the ability to subsequently formsugars in hydrolysis, avoid degradation or loss of carbohydrate, avoid formation of byproducts inhibitory to subsequent hydrolysis and fermentation processes.[3]

Physical pretreatment

1. Mechanical comminution

Waste cellulosic materials are comminuted by a combination of chipping, grinding and milling to reduce cellulose crystallinity. The size of the materials is usually 10–30 mm after chipping and 0.2–2 mm after milling or grinding. Vibratory ball milling has been found to be effective in breaking down the cellulose crystallinity.

2. Pyrolysis

Pyrolysis has also been used for pretreatment of cellulosic materials. When the materials are treated at temperatures greater than 300⁰ C, cellulose rapidly decomposes to produce gaseous products and residual char.

Chemical pretreatment

1. Acid hydrolysis

Concentrated acids such as H₂SO₄ and HCl have been used to treat cellulosic materials. Although they are powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive and hazardous and require reactors that are resistant to corrosion. In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible. Dilute acid hydrolysis has been successfully developed

as a pretreatment of lignocellulosic materials. The dilute sulfuric acid pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis [8]. At moderate temperature, direct saccharification suffered from low yields because of sugar decomposition. High temperature in dilute acid treatment is favorable for cellulose hydrolysis. The pretreatment usually involves mineral acids

(e.g., HCl and H₂SO₄) at a concentration from 0.3 to 2% (w/w). The temperature for treatment ranged from 120 to 180°C, and the length of treatment ranged from less than 1 min to 2 h or longer. The objective of dilute acid treatments is to separate hemicellulose from lignin and cellulose. [10,11]

Higher temperature dilute acid pretreatment has been shown to increase cellulose digestibility of pretreated residues. [2] Dilute sulfuric acid has some important limitations including corrosion that mandates expensive materials of construction. The acid must be neutralized before the sugars proceed to fermentation. [8]

2. Alkaline hydrolysis

Some bases can also be used for pretreatment of lignocellulosic materials and the effect of alkaline pretreatment depends on the lignin content of the materials [3,8-10]. The mechanism of alkaline hydrolysis is believed to be saponification of intermolecular ester bonds cross linking xylan hemicelluloses and other components, for example, lignin and other hemicellulose. The porosity of the lignocellulosic materials increases with the removal of the cross links. Dilute NaOH treatment of lignocellulosic materials caused swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure.

Alkaline treatment causes lignocellulosic materials to swell; increased swelling leads to higher susceptibility of cellulose to saccharification. In the presence of alkaline chemicals (e.g., NaOH or NH₃), cellulose, hemicellulose, and lignin bonds can be disrupted, which permits cellulose to swell beyond normal water-swollen stages. Consequently, the pore size, intraparticle porosity, and capillary size are increased. There is also a phase change in the cellulose crystalline structure. Sodium hydroxide is the most commonly used chemical in the treatment of lignocellulose. It is a strong swelling agent for cellulose.

The amount of NaOH used for treatment ranges from 2 to 20%, and the temperature for the treatment ranges from ambient to 120°C. Under mild conditions (low concentration and low temperature) substrate components remain unchanged. Under harsher conditions, most of the lignin and hemicellulose are solubilized. [10,11]

Alkali pretreatment reduces the lignin and hemicelluloses content in biomass, increases the surface area, allowing penetration of water molecules to the inner layers, and breaks the bonds between hemicellulose and lignincarbohydrate. Dilute NaOH is usually used for alkali pretreatment. Considering economic and environmental aspects, dilute NaOH treatment would be much more suitable than the concentrated NaOH pretreatment.

Combination of dilute NaOH treatment and other treatments seems more efficient.[3]

Alkali pretreatment may be carried out at ambient conditions, but pretreatment time is measured in terms of hours or days rather than minutes or seconds. Unlike acid-catalyzed pretreatments, a limitation occurs because some of the alkali is converted to irrecoverable salts or incorporated as salts into the biomass by the pretreatment reactions. The process of lime pretreatment involves slurring the lime with water, spraying it onto the biomass material, and storing the material in a pile for a period of hours to weeks. The particle size of the biomass is typically 10 mm or less. Elevated temperatures reduce contact time.

[8]

3. Organosolv process

In the organosolv process, an organic or aqueous organic solvent mixture with inorganic acid catalysts (HCl or H₂SO₄) is used to break the internal lignin and hemicellulose bonds. The organic solvents used in the process include methanol, ethanol, acetone, ethylene glycol, triethylene glycol and tetrahydrofurfuryl alcohol. Solvents used in the process need to be drained from the reactor, evaporated, condensed and recycled to reduce the cost.

Removal of solvents from the system is necessary because the solvents may be inhibitory to the growth of organisms, enzymatic hydrolysis, and fermentation.

[12]

3. Results and Discussion

Lignocellulosic materials used in this study are waste cellulosic materials, such as: office paper, newspaper and cardboard.

The tests was analised after and before milling at a vibratory ball milling.

The enzyme used in this study, Celulaza Onozuka R 10 (by *Trichoderma viride*), 1 U/mg, was a commercial broth obtained from SC. Nordic Invest SRL, Cluj-Napoca.

The buffer used was 50 mM citrate, pH 4,8.

The reducing sugar reagent was the DNS reagent containing 1416 mL of deionized water, 10,6 g of 3,5-dinitrosalicylic acid, 19.8 g of NaOH, 306 g of Rochelle salts (Na-K tartrate), 7,6 mL of phenol, and 8,3 g of sodium metabisulfite. Glucose was used as the reducing sugar stock standard and was diluted to produce a standard curve. All

chemicals were obtained from “Elena Doamna” Food Industry College, Galati or from SC. Nordic Invest SRL, Cluj-Napoca. Glucose concentrations were analyzed using a dinitrosalicylic acid (DNS) assay.

Five pretreatments were examined prior to enzymatic hydrolysis: untreated waste cellulose materials, milled waste materials, organosolvents treatments, pyrolysis, and chemical treatments in wet and dry air.

The lignocellulosic materials pretreated were dried, grinded and stored in dry atmosphere until it was used in enzymatic hydrolysis.

The assays were performed in duplicate.

The hydrolysis assay procedure was performed in 250 ml flasks that contained 0,1 g cellulosic material, 2 ml cellulose, suspended in 20 ml final volume with distillated water; the pH value was adjusted at 4,8. Te sample flasks were incubated in a thermostat, at 45°C, for 1 hour.

The enzymatic reaction was stopped by assays keeping, for 10 minutes, in boil water. The mixture was centrifuged at 5000 rpm for 10 minutes, to separate the hydrolyzate; the samples were analyzed for reducing sugar content in the supernatant by the dinitrosalicylic acid (DNS) method, used pure glucose to the standard curve.

1. Mechanical comminution

Waste materials was comminuted by a combination of chipping, grinding and milling, at a size of the materials by 10–30 mm after chipping and 0.2–2 mm after milling or grinding, at a vibratory ball milling. These materials were hydrolyzed, and the reducing sugar concentration was:

To enhance cellulose extraction from the lignocellulosic materials, grinding pretreatments were investigated.

Table 1: Milling cellulosic materials

Cellulosic material	Office paper	Newspaper	Cardboard
Reducing sugar, mg/ml	0,074	0,037	0,037

The reducing sugar concentration after the enzymatic hydrolysis is low, but higher than the untreated material, assayed in the same conditions. This would suggest the cellulose is not as readily extractable from the lignocellulosic material, thereby limiting enzyme access to the cellulose molecules. As a result, milling pretreatment alone had little effect on improving the glucose yield from the enzymatic hydrolysis of waste cellulosic materials.

2. Pyrolysis

The ground cellulosic material are treated at temperatures of 300⁰ C, when the cellulose was rapidly decompose to produce gaseous products and residual char, in a calcination oven. After treatment and enzymatic hydrolysis the reducing sugar concentration was:

Table 2: Pyrolysed cellulosic materials

Cellulosic material	Office paper	Newspaper	Cardboard
Reducing sugar, mg/ml	0,14	0,11	0,11

The pyrolysis pretreatment of ground material improved the conversion of cellulose to glucose yield from enzymatic hydrolysis.

3.Organic solvents pretreatments

The organic solvent used in the process was acetone and methanol. For this 1 g of cellulosic material was suspended in 10 ml distilled water with 2 ml organic solvent. The samples were kept at room temperature for 24 hours. The samples were washed with distilled water to remove the solvent, and then it was enzymatically hydrolysed. In table 3 it is shown the reducing sugar content:

Table 3: Organic solvents pretreatment

Cellulosic material	Organic solvent	
	methanol	acetone
	Reducing sugar, mg/ml	
Office paper	0,037	0,037
Newspaper	0,074	0,11
Cardboard	0,037	0,074

After organic solvents pretreatment, the reactivity of cellulosic materials is the same with acid or alkali pretreatment in mild conditions (low concentration and low teperature).

4. Acid / alkali hydrolysis

The pretreatment involves mineral acids H₂SO₄ or alkali NaOH in concentration of 2% (w/w). The temperature for the treatment was 120⁰C, and the duration of the treatment ranged from 15 min to 1.5 hours. For this the ground cellulosic material, was mixed with H₂SO₄ 2% or NaOH 2%, 1:3 solid/liquid raport.

The samples were kept in an autoclave at 120⁰C for 15, 30, 60 and 90 minutes. The samples were washed with distilled water

up to a neutral pH, and then they were enzymatically hydrolysed.

It was observed that the chemical treatment, in wet atmosphere, at 120⁰C temperature, increased the reducing sugar yield. The reducing sugar concentration after enzymatic hydrolysis of the samples, was increased, compared with the reducing sugar concentration at low temperature pretreatments.

Table 4: Chemical pretreatment at 120⁰C, in wet atmosphere (1 h)

Pretreatment	Cellulosic material	Reducing sugar, mg/ml			
		Time, minutes			
		15	30	60	90
H ₂ SO ₄ , 2%	Office paper	0,37	0,37	0,48	0,19
	News paper	0,11	0,26	0,3	0,11
	Cardboard	0,3	0,33	0,33	0,3
NaOH, 2%	Office paper	0,11	0,37	0,48	0,3
	News paper	0,11	0,37	0,3	0,11
	Cardboard	0,037	0,037	0,037	0,037

It was observed that the treatment had maximal efficiency after a one hour pretreatment. For office paper the reducing sugar yield was increased thirteen times, for newspaper paper eight times and for cardboard nine times. The acid pretreatment, with H₂SO₄ 2%, in wet atmosphere, was analysed by graphic representation: saccharification percentage vs. incubation time. The samples of cellulose materials (office newspaper paper and cardboard) were treated according to the above mentioned method. The samples was enzymatically hydrolysed, for: 24, 48, 72, 96 hours.

Was calculated the saccharification percentage a follows:

$$\text{Saccharification \%} = \frac{\text{reducing sugar, } \frac{\text{mg}}{\text{ml}} - 0,9}{\text{initial substrat, mg/ml}} \cdot 100$$

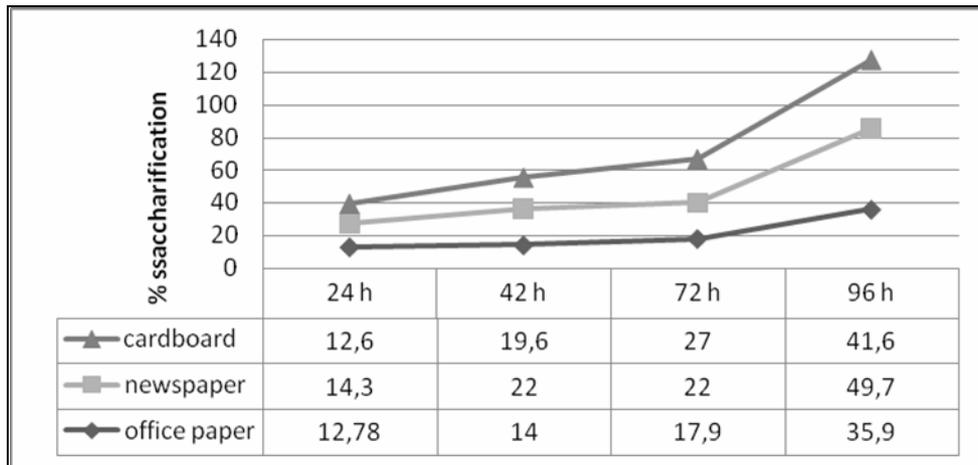


Figure 1: Saccharification percent vs. incubation time

Enhanced the reactivity of waste cellulosic materials after five different test conditions is shown below.

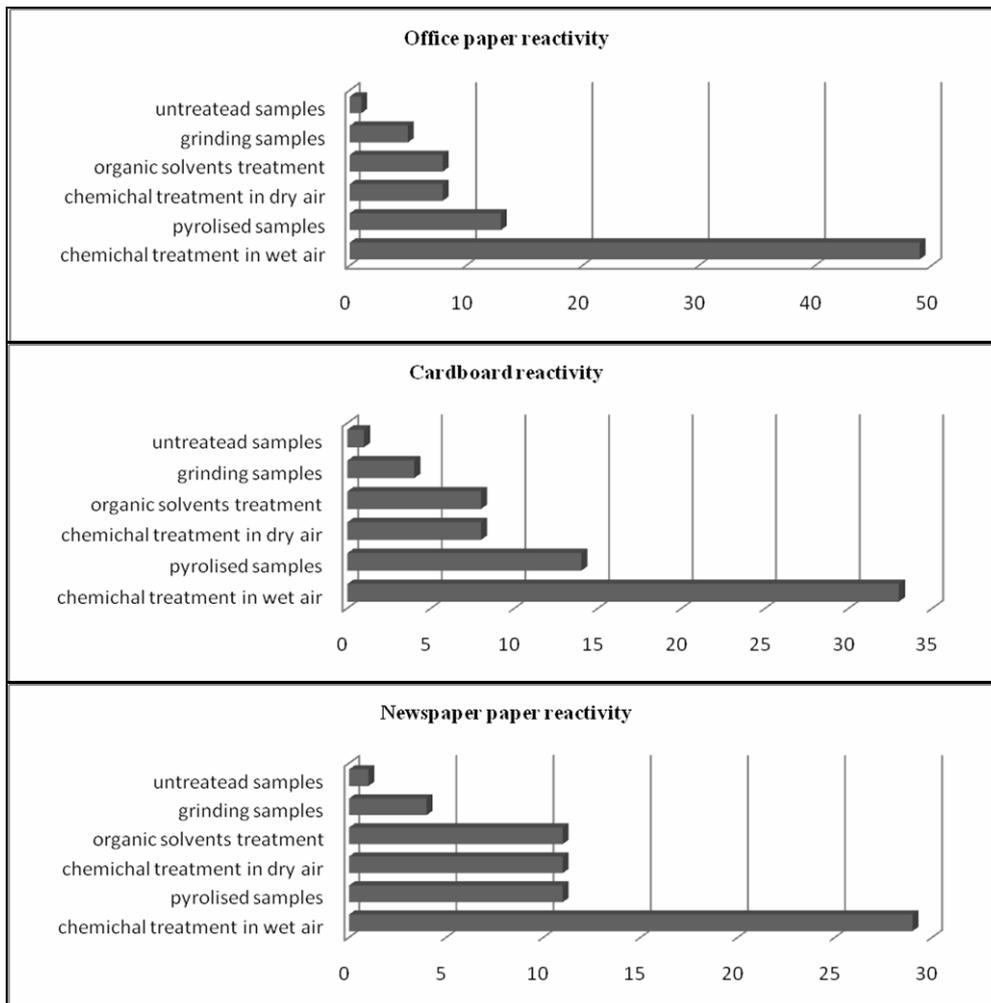


Figure 2: Enhanced the reactivity of waste cellulosic materials after five different test conditions

4. Conclusion

The highest conversion percentage to glucose resulting from the enzymatic hydrolysis of waste cellulose materials was achieved using the ground, H₂SO₄ or NaOH 2% pretreated materials.

High yield could be achieved through the enzymatic hydrolysis of waste cellulose materials at 120°C with a reaction time of 24 hours. The hydrolysis of untreated waste cellulose materials yielded only a slight increase in enzymatic conversion obtained in 24 hours.

On the other hand, physical pretreatment proved to be effective in enhancing the conversion of waste cellulose materials to glucose.

When the waste cellulose materials was pyrolysis-treated or organic solvent treated before being enzymatically hydrolysed, the reducing sugar content increase to double its amount. Moreover, when the ground substrate was treated with mineral acids H₂SO₄ or alkali NaOH in a concentration of 2%(w/w) treatment at a temperature of 120°C, in wet atmosphere, the glucose conversion percentage increased about 13 times.

The results of this study would suggest that the main barrier for the enzyme to access the cellulose in waste cellulose materials is the recalcitrance of untreated materials.

This study demonstrated that a significant glucose yield could be achieved through the enzymatic hydrolysis of waste cellulosic materials at 120°C, in wet atmosphere, with H₂SO₄ or alkali NaOH in a 2% (w/w) concentration by and a reaction time of 24 hours. It was observed that the saccharification percentage increases with the incubation time.

On the other hand, the physical pretreatment proved to be effective in enhancing the conversion of cellulose into glucose. This study has demonstrated that it could be feasible to employ waste cellulosic materials as a possible feedstock for glucose recovery, which could subsequently be employed in the production of bioethanol.

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