

Comparative Study of Steam- and Nitrogen Explosion Pretreatment Methods

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Abstract The aim of this paper was to investigate chemical and physical changes in biomass during N₂ explosive decompression pretreatment and compare it with steam explosion pretreatment. The methods are economically and environmentally attractive since only pressure and water/steam are used to break down the biomass structure. Two pretreatment methods were used at different temperatures and samples from all process steps were analysed. The results were used to assess the pretreatment effect and the chemical changes in biomass and, finally, the mass balances of the bioethanol process at different process steps. Results show that the highest glucose and ethanol yields were obtained with the steam explosion pretreatment method at 200 °C (24.29 g and 12.72 g per 100 g biomass, respectively). At lower temperatures, the nitrogen explosion treatment produced better yields.

Keywords: • Biomass • Lignocellulose • Bioethanol • Steam explosion pretreatment • Nitrogen explosive decompression pretreatment •

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1 Introduction

Lignocellulosic ethanol has attracted attention as an alternative to fossil fuels. Lignocellulosic biomass is defined as either non-edible residues from food crop production or non-edible whole plant biomass [1]. Different kinds of biomass can be used in bioethanol production, such as by-products (straw, sugar cane bagasse, forest residues), waste materials (organic components of municipal solid waste), and dedicated feedstocks (purpose-grown vegetative grasses, short-rotation wood, etc.) [2-4].

The traditional bioethanol production process consists of three steps – pretreatment, hydrolysis and fermentation. The pretreatment step is used to open the biomass structure and make the cellulose accessible for enzymatic hydrolysis. In the enzymatic hydrolysis, cellulose polymer is degraded into glucose monomers. Hydrolysis is followed by fermentation, where glucose is fermented into ethanol.

Out of the three main components of the lignocellulosic biomass, cellulose, hemicellulose and lignin, only cellulose can be converted into ethanol. However, cellulose is wrapped inside the hemicellulose and lignin matrix, which makes it difficult to access. In order to gain access to cellulose, effective pretreatment is paramount. The goal of the pretreatment is to alter the biomass structure in a way that breaks down the cellulosic structure of the biomass and opens it for further enzymatic hydrolysis [5].

Different methods for biomass pretreatment have been proposed. They can be divided into chemical, physical, and combined pretreatment methods. Physical methods are mainly based on size reduction [6] and not widely used due to high energy input and low efficiency. However, it is often combined with chemical or physio-chemical methods [7]. Chemical methods use acids, bases, solvents, etc. to dissolve some components of the biomass, thereby gaining access to the cellulose. These methods are effective, however, cost of the chemicals raises the total cost of the process and, subsequently, the price of the ethanol produced, which in turn impedes competition with fossil fuels.

Of the combined methods, steam explosion (SE) and its variations – ammonia fiber explosion (AFEX), CO₂ or SO₂ explosions are most common. In SE, biomass is treated with saturated steam at high pressure, [8] where temperatures of 180-220 °C are commonly used, and after a retention time of 2-10 minutes [5], the pressure is suddenly decreased. During the process, hemicellulose is hydrolyzed through the action of steam and this reaction is further catalyzed by the presence of the organic acids formed during treatment [9]. The AFEX process and also CO₂ or SO₂ explosions are variations of steam explosion methods, where ammonia, CO₂ or SO₂ are added to enhance the effect of pretreatment [10]. These methods are very effective at dissolving and removing hemicellulose, however, organic acids and furfural are formed during the pretreatment, which act as inhibitors.

In addition to SE, N₂ explosive decompression (NED) pretreatment of biomass was recently proposed [1, 11]. In NED pretreatment, the biomass is mixed with water and

placed into a pressure reactor. The reactor is then heated and pressure is applied to the biomass mixture by adding N₂ gas. The nitrogen molecules, which are smaller than the water molecules or gas molecules used in other pretreatment methods (CO₂ and SO₂), can more effectively penetrate into the cells of the biomass. The subsequent explosive release of pressure causes the dissolved nitrogen gas to expand. This opens the cellulosic structure of the biomass and increases the accessible surface area for enzymatic hydrolysis. The NED pretreatment method is economically and environmentally attractive since neither catalysts nor chemicals are added in these processes [1, 11]. However, the working principle and the processes that occur during the pretreatment still need further research.

The aim of this paper was to investigate the chemical and physical changes in biomass during the NED pretreatment and compare the results with those of SE pretreatment method. The two pretreatment methods were used at different temperatures and samples from various process steps were analyzed with different methods. Results were used to assess the pretreatment effect and the chemical and physical changes in biomass and finally, a mass balances of the bioethanol process in different process steps was compiled [1].

2 Materials and Methods

2.1 Biomass

Barley straw (*Hordeum vulgare*) was used as a biomass in all experiments. The samples were dried to moisture content less than 100 g kg⁻¹ and ground with Cutting Mill SM 100 (Retsch GmbH) and then with Cutting Mill ZM 200 (Retsch GmbH) to a particle-size 3 mm or less.

2.2 NED pretreatment

The instrumentation and working principles of NED pretreatment method are described in detail in previous publications [1, 11, 12]. In NED pretreatment, 650 ml of distilled water was added to 100 g of dried and milled biomass. Samples were mixed thoroughly and heated to temperatures between 100 - 175 ± 3°C and the pressure of 30 bar was applied using compressed nitrogen gas. After reaching the target temperature, the mixture was cooled below the boiling point and pressure was released in an explosive manner. After the explosion, samples were cooled to a temperature below 50°C and used in the enzymatic hydrolysis step.

2.3 Steam explosion pretreatment

SE pretreatment method and instrumentation described by Tutt et al [11] was used in this work. Sample of 900 g pre-dried and milled hay was soaked in 900 g of distilled water. SE was performed in a laboratory scale SE system at the University of Applied Sciences Upper Austria. SE was carried out at temperatures 150 – 200 °C and incubation time of

30 minutes. Pretreated material was then dried at 40 °C to a dry matter content of 95% or more.

2.4 Enzymatic hydrolysis and fermentation

Pretreated biomass was used to prepare suspensions with dry matter content of 10% in citrate buffer (5 mmol L⁻¹, pH 5.0) in case of SE pretreatment. In case of NED pretreatment, distilled water and enzyme was added to the pretreated biomass in order to gain the volume of the mixture 1000 ml. Enzyme mixture was added to biomass suspension at a ratio of 0.3 ml per g of biomass. Enzyme complex Accellerase 1500 from Genencor®; 30 FPU g⁻¹ cellulose) was used in all experiments. Hydrolysis lasted for 72 hours at a temperature of 50°C under constant stirring in rotating shaker/incubator (100 min⁻¹).

After enzymatic hydrolysis the liquid phase of the suspension was separated from solid phase by vacuum filtration. Yeast *Saccharomyces cerevisiae* was added to the liquid phase gained after filtration to start the fermentation process. Fermentation lasted 7 days at the temperature of at a temperature of 30 °C.

2.5 Chemical analysis

The biomass samples were taken for analysis before and after pretreatment and after enzymatic hydrolysis. The solid and liquid parts of the biomass mixture were weighed before and after pretreatment and after enzymatic hydrolysis. The solid biomass gained after pretreatment and enzymatic hydrolysis was dried and milled for analysis.

Dry matter content was analysed with a moisture analyser Ohaus MB 45. The fibre analysis (cellulose, hemicellulose and lignin) was performed using an ANKOM 2000 analyzer.

2.6 Data analysis

All the weights of the liquid and solid phases from all process stages, glucose and ethanol concentrations were measured after hydrolysis and fermentation stages, fiber analysis results of all solid residues were conducted.

The hydrolysis efficiency was calculated based on a formula 1:

$$E_{HY} = \frac{m_{glc}}{m_{cel} \cdot 1,11} \cdot 100\% \quad (1)$$

Where m_{glc} is measured amount of glucose in sample; m_{cel} is measured amount of cellulose in sample and 1.11 is cellulose to glucose conversion factor based on stoichiometric biochemistry of hydrolysis [13]. The fermentation efficiency was calculated based on

$$E_F = \frac{c_{eth}}{c_{glc} \cdot 0.51} \cdot 100\% \quad (2)$$

Where c_{glc} is concentration of glucose in sample; c_{eth} is concentration of ethanol in sample and 0.51 is glucose to ethanol conversion factor based on stoichiometric biochemistry of fermentation [13].

The mass balance was calculated based on the masses of the solid and liquid phases and the calculated masses of the components of biomass and liquid.

Averaged results of at least three parallel measurements are used. Data were analyzed using MS Excel software.

3 Results and discussion

3.1 Biomass characterization

Biomass can be characterised based on its composition - relative proportion of cellulose, hemicellulose, and lignin. Previous research has shown that energy crops for bioethanol production should be selected based on their cellulose content. Bioethanol is produced from glucose, which is formed during cellulose degradation and therefore, the most important property of biomass for bioethanol production is high cellulose content [14]. In addition, a low lignin and hemicellulose content is preferred. The hemicellulose-lignin matrix makes cellulose that is packed into this matrix, inaccessible to enzymes. Furthermore, the degradation products of lignin and hemicellulose inhibit the hydrolysis of cellulose to sugars and therefore, the low lignin content biomasses are preferred [15].

Table 1 shows that barley straw, used in these experiments as a sample biomass, had a relatively high cellulose content of 41.56% while the hemicellulose and lignin contents were lower, 32.9% and 5.35%, respectively. Relatively high cellulose content makes it a suitable biomass for bioethanol production since high ethanol yields can be obtained.

Table 1. Composition of biomass used in the experiments

Component	Content (%)
Hemicellulose	32.9
Cellulose	41.6
Lignin	5.4

3.2 Effect of pretreatment to biomass

Two different methods– NED and SE were used for biomass pretreatment. Biomass was pretreated at different temperatures, at constant pressure, and pretreatment duration. After the pretreatment, fiber analysis was performed to investigate the change in the biomass due to pretreatment. Since no chemicals are used in either of pretreatments, the methods require high temperatures and/or high pressures to effectively dissolve hemicellulose and expose cellulose fibres to enzyme molecules in the following hydrolysis process [11]. In addition, the heating step in both methods is followed by a swift release of pressure causing the steam or N₂ gas to expand within the lignocellulosic matrix. During the explosion, the plant biomass particles are shattered into small pieces, the fibres of the plant biomass are separated, and the ordered structure of the plant biomass is significantly disrupted [16]. The results of fiber analysis from pretreated biomass are shown in table 2. The results show that both pretreatment methods decrease the concentration of hemicellulose in the biomass (to not detectable level at 190 °C with SE pretreatment or to 7.3% at 175 °C with NED pretreatment).

Table 2. The composition of biomass after pretreatment with NED or SE pretreatment method at different temperatures

Temperature (°C)	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Ash (%)
Steam explosion pretreatment				
150	26.7	42.1	8.7	3.59
160	14.0	38.4	8.7	4.23
170	11.2	35.3	10.8	3.81
180	0.8	33.0	12.8	4.74
190	ND*	38.4	14.1	4.55
200	ND*	39.5	13.9	4.01
N₂ explosive pretreatment				
100	28.3	44.2	2.3	4.00
125	27.1	44.1	3.3	3.50
150	24.1	40.8	4.6	3.89
175	7.3	36.8	8.9	4.27

Both pretreatment methods decrease the hemicellulose content in biomass by dissolving the hemicellulose at high temperature, which increases substantially the availability of the cellulosic fraction of the material [15]. This also enables to improve the hydrolysis yields of cellulosic fraction in the further steps of process since the cellulose is more accessible to enzymes [15].

At the same time the cellulose proportion varies between 42.1% and 33.0% with SE pretreatment and decreases only slightly to 36.8% with NED pretreatment. This shows

that there is a loss in cellulose content however, compared to dissolution of hemicellulose the cellulose losses are negligible

3.3 Hydrolysis and fermentation of the pretreated biomass

The pretreated biomass was used in a traditional three-step bioethanol production process, where enzymatic hydrolysis and fermentation followed pretreatment. After these steps, the glucose and ethanol concentrations were measured and hydrolysis and fermentation efficiencies were calculated (figure 1) to assess the effect of pretreatment.

From the figure 1 it can be seen that the higher hydrolysis efficiency was gained using NED method at temperatures up to 175 °C where 52.6% hydrolysis efficiency was gained. At lower temperatures, at 100 °C, the hydrolysis efficiency was 26.0% but increased steadily to maximum values at 175°C, where glucose yield of 15.2 g of glucose from 100 g of biomass was gained. Higher temperatures were not tested with NED pretreatment method because we already demonstrated that the glucose yield reaches a plateau or decreases at temperatures higher than 175 °C [1, 11].

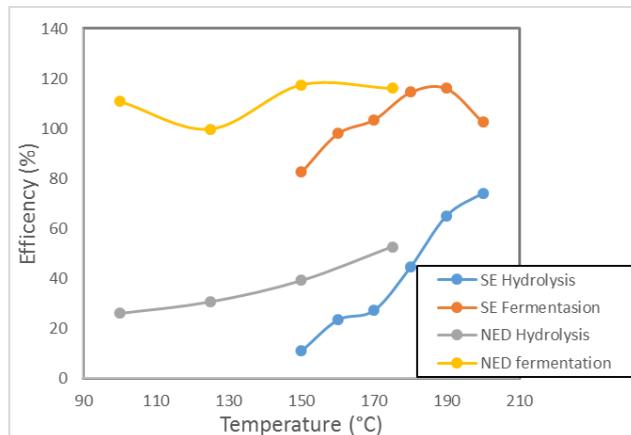


Figure 1. The hydrolysis and fermentation efficiencies when steam explosion pretreatment (SE) and explosive decomposition pretreatment (NED) was used at different temperatures.

Similarly to NED, with SE the lowest glucose yield was gained at lower temperatures at 150 °C. However as the pretreatment temperature was increased, the hydrolysis efficiency increased steeply up to 66.7% and exceeded the highest efficiency gained with NED method, which was 52.7%. In comparison, in temperature range of 150-175° C, where both methods were applied the NED method enabled to gain 72-53% more glucose than SE pretreatment method. However, at higher temperature the SE method was more efficient. Fermentation efficiency was higher when NED pretreatment was used and it varied between 99% to 117%. With SE pretreatment, the fermentation efficiency increased from 82% at 150 °C to 116% at 190 °C and then decreased to 102%. In the

temperature range of 150-175 °C, NED method enabled to gain higher fermentation efficiencies.

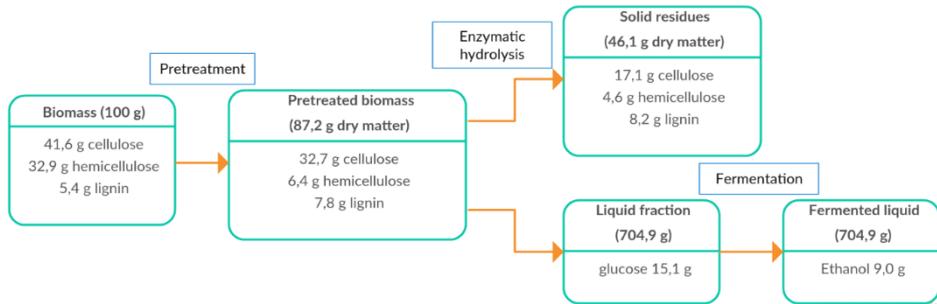


Figure 2. Mass balance of bioethanol production with NED pretreatment at 175 °C

The fermentation efficiencies exceeding 100% indicate that during fermentation more sugars were available than measured after enzymatic hydrolysis. After enzymatic hydrolysis, the liquid fraction was separated from solid fraction and glucose concentration was measured. However, the liquid fraction also contained dissolved sugar oligomers that remained undetected when glucose was measured but were hydrolyzed into glucose during fermentation, which increased the concentration of available sugars and enabled to gain fermentation efficiencies over 100%.

3.4 Mass balance of processes

Mass balance was calculated based on the weight of liquid and solid phases from each process stage, fiber content of those solid residues, and glucose and ethanol concentrations, measured after hydrolysis and fermentation stages, respectively. Mass flow charts were prepared from ethanol production processes using different pretreatments at 175 °C and at 180 °C with NED and SE pretreatment methods, respectively.

Bioethanol production process started with 100 g on biomass, which contained 41.6% of cellulose. After pretreatment, the dry matter content of biomass decreased to 87.2 g and 83.2 g with NED and SE pretreatment methods, respectively. Main effect on both pretreatment methods is the dissolution of hemicellulose, which decreased in biomass 80.5 % and 97.3% in case of NED and SE pretreatments, respectively. At the same time, decrease for cellulose is negligible and lignin mass increases slightly.

After the pretreatment and the enzymatic hydrolysis, solid and liquid phases were separated by filtration. The weight of solid residue was 46.1 g in case of NED pretreatment and 34.5 g with SE pretreatment, with the polymeric cellulose part 17.1 g and 12.7 g, respectively. These results show that SE is more effective in hemicellulose dissolution. Additionally, the method enables to gain more effective cellulose usage since less cellulose remains intact after hydrolysis, which results in higher glucose yield.

In hydrolysis and fermentation steps both pretreatment methods under similar conditions result in similar glucose and ethanol yields. Although, NED method enabled to gain more of liquid fraction, almost equal amount of glucose was produced with both pretreatments. However, SE pretreatment was also used at 200 °C and there 24.3 g of glucose was produced in comparison to 15.4 g at 180 °C. Furthermore, equal amounts of ethanol (9 g) were measured in the fermentation broth with both methods. Similarly to hydrolysis step, more ethanol was produced with SE method at 200 °C where 36% more ethanol was gained from the same amount of initial biomass.

4 Conclusions

Two pretreatment methods were used in three-step bioethanol production process to investigate the effect of pretreatment to chemical and physical changes in biomass. Biomass was pretreated at different temperatures, samples from various process stages were analyzed and finally, mass balances of the bioethanol process in different process steps were compiled.

The results show that both pretreatment methods are effective in hemicellulose removal, while decrease in cellulose content is negligible. After pretreatment, hydrolysis efficiencies exceeded 58% and 74% for NED and SE pretreatments, respectively. Fermentation efficiencies reached over 100%, which shows that fermentation inhibiting compounds did not form during pretreatment. Mass analysis of the process shows that at similar pretreatment temperatures the methods gave similar results in terms of glucose and ethanol yields. However, SE is more effective in hemicellulose removal and after fermentation, less cellulose remained unused in the solid residue. Higher temperature enables to gain higher yields and efficiencies and thereby, the highest glucose and ethanol yields, 24.3 g and 12.7 g from 100 g of biomass, respectively, were gained with SE pretreatment at 200 °C.

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