

Article

The Efficiency of Nitrogen and Flue Gas as Operating Gases in Explosive Decompression Pretreatment

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Received: 3 July 2018; Accepted: 7 August 2018; Published: 9 August 2018



Abstract: As the pretreatment process is the most expensive and energy-consuming step in the overall second generation bioethanol production process, it is vital that it is studied and optimized in order to be able to develop the most efficient production process. The aim of this paper was to investigate chemical and physical changes in biomass during the process of applying the explosive decompression pretreatment method using two different gases—N₂ and synthetic flue gas. The explosive decompression method is economically and environmentally attractive since no chemicals are used—rather it is pressure that is applied—and water is used to break down the biomass structure. Both pre-treatment methods were used at different temperatures. To be able to compare the effects of the pretreatment, samples from different process steps were gathered together and analysed. The results were used to assess the efficiency of the pretreatment, the chemical and physical changes in the biomass and, finally, the mass balances were compiled for the process during the different process steps of bioethanol production. The results showed that both pre-treatment methods are effective in hemicellulose dissolution, while the cellulose content decreases to a smaller degree. The high glucose and ethanol yields were gained with both explosive pretreatment methods at 175 °C (15.2–16.0 g glucose and 5.6–9.0 g ethanol per 100 g of dry biomass, respectively).

Keywords: lignocellulose; pretreatment; bioethanol; second generation biofuel; mass balance

1. Introduction

The most common way of producing biofuels from lignocellulose is to convert the biomass into sugars and ferment the sugars into fuel, such as bioethanol. However, the low levels of accessibility of cellulose due to its rigid association with lignin and hemicellulose makes the degradation of cellulose difficult for cellulases. In order to increase the overall process efficiency, it is necessary to break down the lignin and hemicellulose seal in order to make cellulose more accessible for further processing [1]. Therefore, pretreatment is an essential step before the step involving hydrolysis of lignocellulosic biomass into sugars [2].

A wide variety of pretreatment methods have been proposed and studied. The most common are physical, chemical and physio-chemical methods, which combine the effect of both. The physical methods usually target biomass particle size reduction and are energy-intensive with low efficiency. The chemical methods, on the other hand, are expensive due to the high cost of the chemicals. Additionally, inhibitors are formed during chemical pretreatment, which may disturb the subsequent hydrolysis and fermentation processes. The combined physio-chemical methods are in most cases thermo-mechano-chemical processes, in which a combination of temperature and pressure is used for pretreatment. In physio-chemical pretreatment process, the biomass is de-structured, and lignin and hemicellulose are separated by changing the operating conditions—usually by means of pressure and/or temperature [3].

The most common physio-chemical method is the steam explosion method (SE). SE is a widely-used method, one in which the structural components of the biomass are broken down by heating it with steam, shearing takes place due to the sudden decompression and expansion of moisture, and then hydrolysis of the glycosidic bonds takes place [4,5]. In this method, chipped biomass is treated with high-pressure saturated steam at a desired temperature, which initiates the process of autohydrolysis [6,7]. The autohydrolysis enhances the hydrolysis of hemicelluloses by means of hydrolysis of acetyl groups, which are included in heterogeneous polysaccharides, and by breaking the linkages between hemicellulose and cellulose [8]. When the pressure is suddenly reduced to atmospheric pressure, the material undergoes an explosive breakdown due to moisture evaporation and gas volume expansion [9].

The SE process can be modified by adding SO_2 , CO_2 , or ammonia to the pretreatment reactor along with the steam in order to increase pressure. In a CO_2 explosion (which is also referred to as supercritical CO_2 -assisted autohydrolysis), supercritical CO_2 under pressure and at high temperature is used to enhance the digestibility of the lignocellulosic biomass [5]. Small CO_2 molecules are capable of penetrating into the biomass cells [1] and, when dissolved, the mixture becomes more acidic due to the formation and dissociation of carbonic acid. By means of the dissolution of the CO_2 , the pH value for the water- CO_2 mixture decreases, making the environment more acidic and thereby facilitating the hydrolysis of the biomass [10].

In addition to SE, a new approach was recently proposed, known as the N_2 explosive decompression (NED) pretreatment of biomass [11,12]. The explosive decompression (ED) method is based on the treatment of biomass at high temperature and with high pressure, which has been generated by the addition of gas. The heating initiates autohydrolysis and at the same time (in the case of NED) nitrogen molecules effectively penetrate into the cells of the biomass. Due to the high temperature, hemicellulose is hydrolysed and this reaction is further catalysed by the presence of organic acids formed during pretreatment. The following explosive depressurisation causes the excess of dissolved nitrogen gas to be released. This opens the cellulose structure of the biomass and increases the accessible surface area for enzymatic hydrolysis [11,12].

Similarly to SE, the NED pretreatment method can also be modified by adding different gases or gas mixtures to replace nitrogen. A comparative study of ED pretreatments with nitrogen and compressed air has been published by Raud et al. [13]. The study showed that explosive decompression pretreatment is more effective when pressurising with nitrogen gas, as this enables higher glucose and ethanol yields to be gained. A study showed that compressed air, although consisting for the most part of nitrogen, was not as effective in the explosive decompression pretreatment process as was nitrogen [13].

While oxygen molecules in compressed air hindered the efficiency of the ED pretreatment, other gases have shown great promise in combination with SE. One of these gases is CO_2 . CO_2 molecules, similarly to nitrogen, can easily penetrate into the biomass structure. CO_2 dissolves in water to produce carbonic acid, and initiates an acid-catalysed process [1], which also increases the dissolution of hemicellulose [5]. Both nitrogen and CO_2 require purification, which increases the cost of the pretreatment process. However, flue gas naturally contains both of these gases and could thereby be used directly without further purification.

The results from the NED pretreatment method have been published. However, the modification of this method so that it uses flue gas has not been reported. Flue gas is usually composed of CO_2 and N_2 in a certain proportion depending upon the fuel type and combustion parameters. The use of dry flue gas has been reported where it contains 13.3% CO_2 and 78.7% N_2 gas from wood pellet combustion [14]. However, with different solid fuels the CO_2 content can range between 6–12% [15]. By adding flue gas to the biomass mixture, enhanced pretreatment could be achieved due to the acid-catalysed process that is initiated by the dissolution of CO_2 . The utilisation of flue gas also has additional advantages. Firstly, this is a waste product which is generated by any burning process and is considered as being one of the greenhouse gases. Thanks to this, it requires minimisation. Secondly,

flue gas is released into the atmosphere at a high temperature, a temperature that could be used to heat the pretreatment mixture and thereby further reduce pretreatment costs.

To be able to study the explosive decompression pretreatment method, the biomass was pretreated with two different gases—nitrogen and synthetic flue gas to compare the efficiency of the ED pretreatment with these gases. The NED was used as a reference pretreatment method in order to be able to compare the results. In the modified version, the synthetic flue gas which contained the CO₂ and nitrogen mixture was used to generate a high level of pressure. The traditional three-step bioethanol production process was used after pretreatment, with samples from various process stages being analysed to investigate the effect of the pretreatment in regards to chemical and physical changes in the biomass. In addition, the mass balance of the process was prepared and analysed.

2. Results and Discussion

2.1. Biomass

Biomass can be characterised according to its relative proportion of cellulose, hemicellulose and lignin. Previous research has shown that the selection of energy crops for bioethanol production should be based upon their cellulose content. The most important property of biomass is high cellulose content, since bioethanol is produced from glucose that is formed during cellulose hydrolysis [16]. However, since cellulose is packed into a hemicellulose-lignin matrix, which makes it inaccessible to enzymes, it is additionally preferable to have a low lignin and hemicellulose content. Furthermore, the degradation products that are contained in lignin and hemicellulose inhibit the hydrolysis of cellulose to sugars and, therefore, the low lignin content biomasses are preferred [17,18].

Table 1 shows the results of the fibre analysis of barley straw that is used in these. It can be seen that this biomass was a suitable biomass for bioethanol production due to a relatively high cellulose content of 41.6%. At the same time, the hemicellulose and lignin contents were lower, at 32.9% and 5.4% respectively. From these results it can be assumed that this type of biomass is suitable for use in the bioethanol production process.

Table 1. The composition of biomass used in the experiments.

Component	Content (%)
Hemicellulose	32.9
Cellulose	41.6
Lignin	5.4
Ash	3.8
Moisture	5.4

2.2. The Physical Effect of Carrying out Pretreatment on Biomass

Explosive decompression pretreatment with nitrogen and flue gas using different gas insertion methods and temperatures were used in this study. During the pretreatment process, the wet biomass is placed inside the reactor, gas is added and, simultaneously, the reactor is heated until the final pressure and temperature are gained. At an elevated pressure the dissolution of the gas is enhanced and the dissolved gas penetrates into the biomass cells. Additionally, autohydrolysis is initiated by heating the biomass which leads to the hydrolysis of acetyl groups, and the release of acetic acid, which catalyses the hydrolysis of polysaccharides, mainly in terms of hemicelluloses [6,7,19,20]. The CO₂ in the flue gas mixture dissolves and produces carbonic acid, which enhances the acid-catalysed process [1] and increases the dissolution of hemicellulose. The addition of gas and heating is followed by explosive decompression which causes the dissolved gas to expand within the lignocellulosic matrix. During the explosion, the plant biomass is shattered into small pieces and the plant biomass fibres are separated [16].

This kind of destruction of the biomass during the pretreatment process can be seen in SEM pictures (see Figure 1). The SEM pictures show the biomass structure before pretreatment, where the biomass surface is smooth; after the pretreatment process, where the biomass is fragmented into smaller pieces; and when it exhibits rough surfaces.

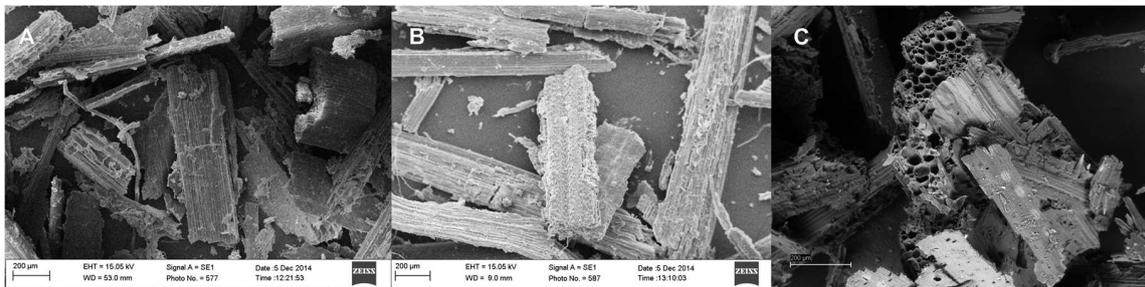


Figure 1. SEM pictures of different biomass samples: (A) non-treated, (B) NED pretreated, and (C) flue gas pretreated.

In order to analyse the effects of different pretreatments on the main components in the biomass, a fibre analysis was also conducted after the pretreatment processes. To be able to do this, 100 g of biomass was used in a pretreatment process at different temperatures. After the pretreatment, biomass was collected and a fibre analysis was carried out.

The fibre analysis was conducted with all of the pretreated biomass samples and the results are shown in Figure 2. It can be seen that in the case of all of the pretreatment samples, the hemicellulose content in pretreated biomass decreases from an initial 32.9 g to around 6.3–6.9 g at the pretreatment temperature of 175 °C. A moderate decrease in hemicellulose content can be seen up to a temperature of 150 °C, but the level of decrease increases sharply when the temperature is elevated further, up to 175 °C. The decrease in the hemicellulose component can be attributed to the formation of acetic acid during autohydrolysis and carbonic acid formation due to CO₂ dissolution, which initiates the acid-catalysed process. This is similar to processes that occur during a dilute acid treatment, where the van der Waals forces, hydrogen bonds, and covalent bonds that hold together the biomass components are disrupted and broken [21]. Similar pretreatment conditions as those of the current study are also reviewed during dilute acid pretreatment, involving low acid concentration, and high temperatures (100–240 °C) at a pressure that is higher than 10 bars [22,23]. In addition, it has been shown that steam explosion pretreatment disrupts the structure of plant cell walls and removes hemicellulose; however, the method requires high temperatures to effectively dissolve hemicellulose. Effectiveness of steam explosion increases rapidly at temperatures over 180 °C and at 200 °C; hemicellulose content biomass was decreased to 15.27%, compared to the 29.15% in the untreated sample [24]. The main effect of this type of treatment is the solubilisation and hydrolysis of polysaccharides, especially hemicellulose in the biomass, which can be removed with levels of efficiency that reach up to 100% [21]. However, this method only disrupts the lignin structure but is not effective in lignin removal [23].

The results presented in Figure 2 show that in the case of all of the pretreatment methods the mass of remaining cellulose does not change significantly up to 150 °C, and the cellulose mass varies between 37.1 g and 40.2 g. When compared to hemicellulose dissolution, the cellulose dissolution was noticed only to a small degree. The cellulose content in biomass varied between 38.5–32.1%, 40.2–33.8%, and 39.0–37.1% after pretreatment with SE, flue gas and flue gas bubbling pretreatments, respectively. As the pretreatment temperature was increased, the cellulose mass decreased, which shows that there is a loss in cellulose due to dissolution. However, when compared to the dissolution of hemicellulose, the cellulose losses are relatively small even at higher temperatures. Similar results for glucan recovery of between 70.5–89.3% after pretreatment have been reported when barley straw was pretreated with sodium hydroxide in the twin-screw reactor [24]

The mass of lignin in pretreated biomass was smaller after pretreatment at lower temperatures, and the lignin mass increased as the pretreatment temperature increased. The lignin mass in pretreated biomass was at 2.0–7.2 g when pretreating with different gases at 100 °C; however, when a pretreatment temperature of 175 °C was used, the lignin content was seen to be between 7.8–11.9 g. The lignin content in the initial biomass was 5.4 g in 100 g of biomass. Therefore, it can be said that at lower temperatures, some of the lignin is removed. However, at higher pretreatment temperatures, different processes such as depolymerisation and condensation reactions occurred, which altered the complex native lignin structure and resulted in more lignin being measured [25]. Higher lignin content was found in the case of flue gas pretreatment, with between 6.0–9.0 g of lignin remaining at different temperatures. Whilst the greatest amount of lignin was removed when N₂ gas was used for pretreatment, between 2.0–7.8 g of lignin remained at different pretreatment temperatures. As the lignin is usually removed using alkaline pretreatment methods, the preservation of lignin in biomass is expected [26]. However, in this work, flue gas was used in the pretreatment process, which moved the acid-base balance in the biomass mixture towards acidic, favouring the dissolution of hemicellulose. Similar results have been gained by Tutt et al. (2014), where after steam explosion pretreatment the lignin remained in insoluble form [24].

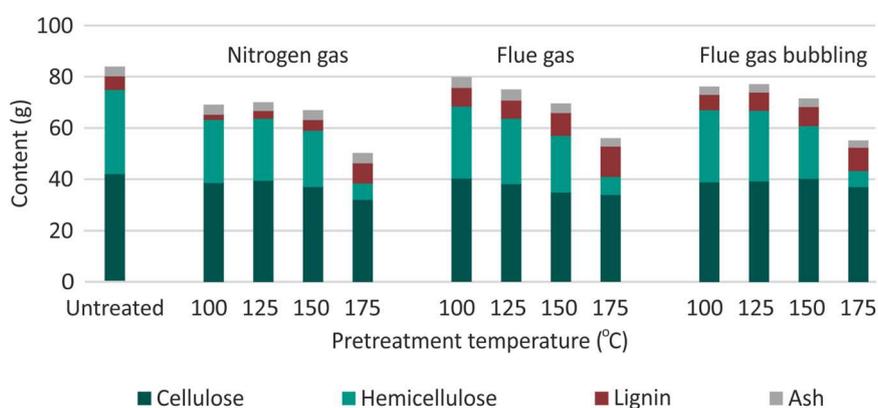


Figure 2. The content of the main components in the solid phase of the biomass/water mixture after pretreatment with different gases at different temperatures when 100 g of initial biomass was used.

2.3. Bioethanol Production

The pretreated biomass was used in a conventional three-step bioethanol production process, where pretreated biomass was used in enzymatic hydrolysis and fermentation steps. To assess the effect of the pretreatment process, glucose and ethanol concentrations were measured in terms of ethanol and glucose yields, but hydrolysis and fermentation efficiencies were also calculated after each process step in order to assess the effect of the pretreatment process.

The results from the enzymatic hydrolysis step are shown in Figure 3, and it can be seen that the glucose concentration in the biomass mixture after hydrolysis, and hydrolysis levels of efficiency depend upon the pretreatment temperature; higher results were gained at elevated temperatures. The pretreatment process, where it involved nitrogen gas, was more effective at lower temperatures, while at temperatures of 175 °C nearly equal results were gained with all of the pretreatment methods. Compared to NED pretreatment, pretreatment with flue gas and flue gas bubbling yielded 37.4% and 16.3% lower hydrolysis efficiencies at 100 °C, respectively. As the pretreatment temperature was increased, hydrolysis efficiency also increased along with glucose concentration in the biomass mixture, finally equalising at a temperature of 175 °C for all methods. The hydrolysis efficiency levels for different pretreatment gases at temperatures 150 °C and 175 °C reached 38.4–41.2% and 45.3–46.7%, respectively. The final glucose concentration in the biomass mixture after hydrolysis was between 17.7–19.0 g L⁻¹ and 20.9–21.5 g L⁻¹ at temperatures of 150 °C and 175 °C respectively. Higher temperatures were not tested since it has already been demonstrated that the glucose yield

reaches a plateau or decreases at pretreatment temperatures that are higher than 175 °C with the NED pretreatment [11,12]. Similar results were gained with steam explosion pretreatment where 30.8% and 46.7% hydrolysis efficiency were gained at 150 °C and 170 °C, respectively. The application of higher temperatures with steam explosion yielded higher results and up to 86.3% hydrolysis efficiency [12]; however, it has been shown that pretreatment temperatures result in the formation compounds that inhibit the fermentation process [11], which lead to lower final ethanol yields.

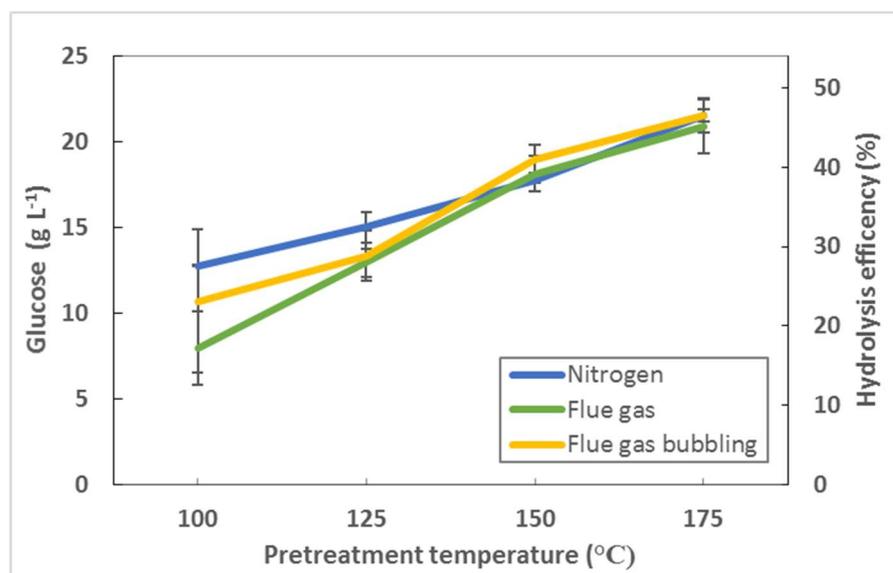


Figure 3. Hydrolysis efficiency and glucose concentration in the hydrolysis mixture after biomass pretreatment with different gases and at different temperatures.

After the hydrolysis stage, the liquid fraction of the mixture was used in the fermentation process. The fermentation efficiency level (Figure 4a) varied between 99.6–117.5% depending upon the pretreatment temperature; however, it was at its highest with the NED pretreatment. This permitted ethanol concentrations to be gained from the fermentation broth which ranged from 7.6 g at 125 °C to 12.8 g L⁻¹ when the biomass was pretreated at 175 °C. When flue gas and flue gas bubbling pretreatments were used, fermentation efficiencies were gained of between 99.6–116.1% and 51.2–83.2%, respectively. The highest ethanol concentration in the fermentation broth was gained with the NED pretreatment at 175 °C. Flue gas pretreatment enabled the collection of similar ethanol concentration in the fermentation broth (only 1.4%); however, flue gas bubbling pretreatments yielded results that were 41.1% lower than with the NED pretreatment. The lower results that were gained with the flue gas pretreatments can be explained by the acidification formation of by-products during the pretreatment process, which later inhibits the fermentation process [26]. The pH of the mixture is lowered by the dissolution of CO₂ during the pretreatment process and the formation of carbonic acid. Additionally, fermentation-inhibiting compounds, which are usually formed during the dilute acid pretreatment process [27], could also be formed during the pretreatment process using flue gas. The results show that the dissolution of CO₂ during the flue gas bubbling pretreatment was more effective than the standard flue gas pretreatment, and this yielded lower ethanol concentrations and fermentation efficiencies.

In the case of the flue gas bubbling pretreatment, fermentation efficiency was at its highest at 125 °C, but decreased when the pretreatment temperature was increased. On the other hand, fermentation efficiencies increased with flue gas and NED pretreatment when temperature was increased from 125 °C to 150 °C. The ethanol concentrations in the fermentation broth, however, increased with the increase in the pretreatment temperature and no decrease was detected. This can be attributed to the increasing glucose concentration when the pretreatment temperature was increased.

Since more glucose was available when the biomass was pretreated at a higher temperature, higher ethanol concentrations were also achieved.

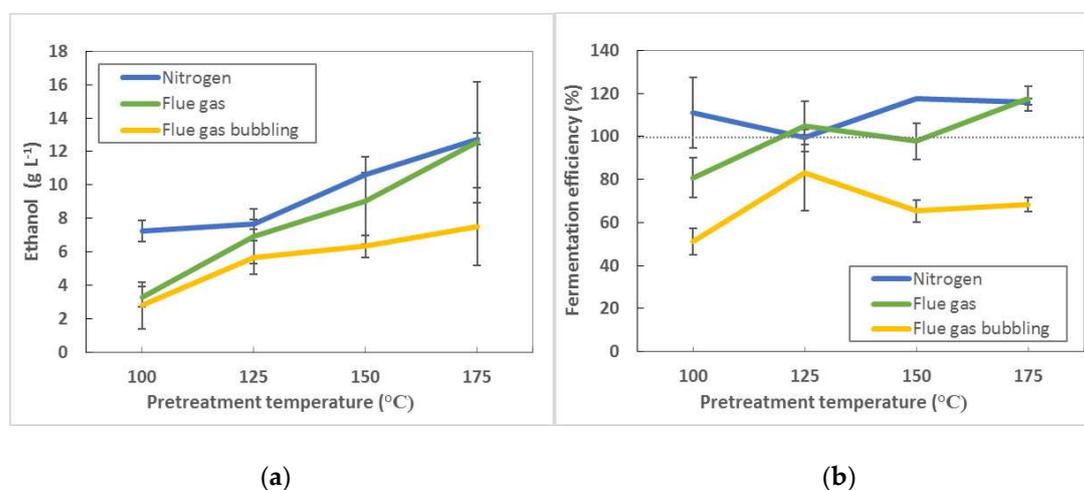


Figure 4. Ethanol concentration in fermentation broth (a) and fermentation efficiency (b) which was gained following biomass pretreatment with different gases and at different temperatures.

2.4. Mass Balance Studies

After the hydrolysis and fermentation stages, various analyses were carried out in order to collect data for the mass balance studies. The weight of the liquid and solid fractions were measured from all of the process stages; a fibre analysis was carried out for all biomasses and solid residues; and glucose and ethanol concentrations were measured in all liquid fractions. Based on the data that was collected, the mass balance was calculated and mass flow charts were prepared for ethanol production processes using different pretreatments at 175 °C (Figure 5).

As can be seen in Figure 5, there is a slight loss of mass during pretreatment. A total of 100 g of biomass was used in the case of all pretreatments, but only between 87.2–92.6 g of dry biomass, depending upon the pretreatment method being used, was recovered after pretreatment had been completed. The loss in dry mass during the pretreatment stage is caused by the dissolution of biomass components and also partially due to the evaporation of moisture and dissolved compounds during explosive decompression.

As can be seen in the figures, the main effect of the pretreatment is the dissolution of hemicellulose, which decreases from 32.9 g to 6.4 g, 7.0 g or 6.3 g, respectively when NED, flue gas or flue gas bubbling pretreatments were used. The amount of cellulose decreases to a smaller degree and most of the cellulose remains in the solid phase. Similarly, no significant loss in lignin mass was detected, which inversely increased slightly. Domínguez et al. reported similar results for hemicellulose dissolution in a study, where the fast growing biomass of *P. tomentosa* was pretreated using autohydrolysis. In that study, a total of 98% of glucan, the main component of cellulose, and 80% of Klason lignin were recovered in the solid phase, and xylan, as a main component of hemicellulose, was almost totally solubilised at 230 °C [28]. The slightly lower hemicellulose removal rate in this study was due to lower temperatures being used in the pretreatment process.

A total of 17.1–17.8 g of cellulose still remained in the solid residue following enzymatic hydrolysis. This shows that either the pretreatment step or the hydrolysis step could be more effective. The cellulose can remain in the solid residue following hydrolysis if the pretreatment does not break down the biomass structure enough to be able to open up access to cellulose fibrils. In this case, the enzyme cannot reach the cellulose and will not be hydrolysed, which in turn means that it remains in the residual biomass. Additionally, hydrolysis efficiency can be low due to the low enzyme activity or enzyme unsuitability for this biomass or hydrolysis conditions. Similar results were reported by

Akanksha et al., where native sorghum was used with an acid pretreatment and of 37.9 g of cellulose in the initial biomass (comprised of 100 g of sorghum), a total of 18.4 g remained in the residual biomass after the process of enzymatic hydrolysis [29].

Although almost similar glucose yields were recorded with different pretreatment methods, the ethanol yields were different and the highest results were gained with the flue gas and NED pretreatment methods. The flue gas and NED pretreatments allowed gains of 9.4 g and 9.0 g respectively of ethanol from 100 g of biomass, while the flue gas bubbling pretreatment yielded results that were 37.8% lower. The lower ethanol yields that were gained from the flue gas bubbling pretreatment method were probably caused by the formation of by-products during the pretreatment process, which later inhibited the fermentation process. As more CO₂ was dissolved during the pretreatment process by bubbling the CO₂ through the biomass, the lowest results were achieved via this method.

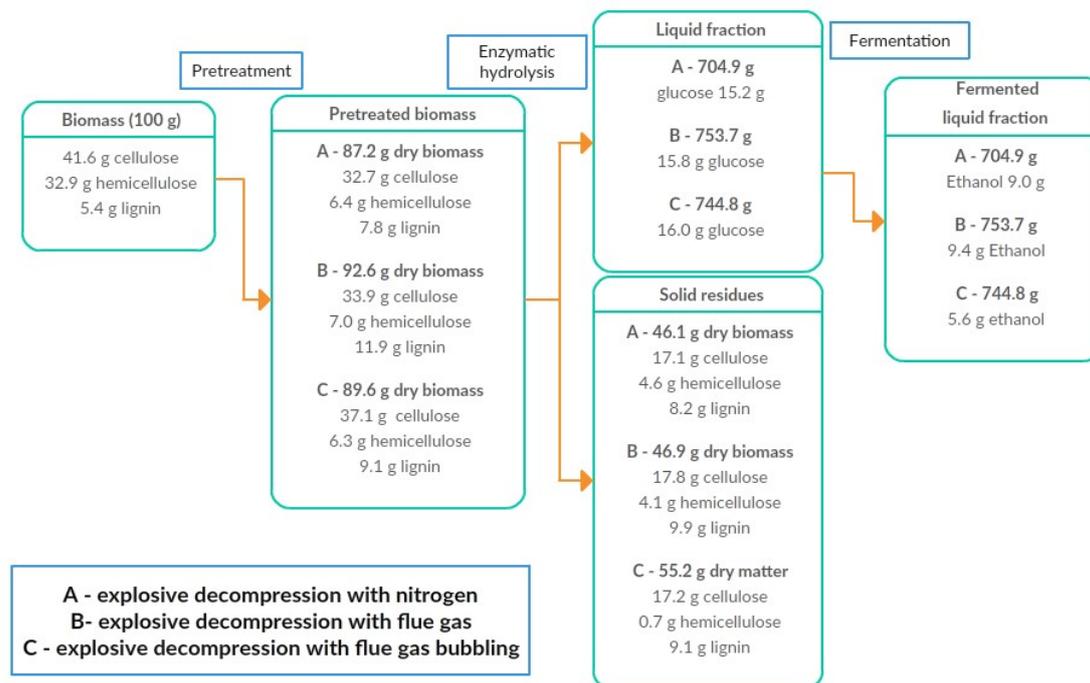


Figure 5. The mass balance for the bioethanol process using different pretreatment methods at 175 °C and 30 bar: A—NED pretreatment; B—flue gas pretreatment; C—flue gas bubbling pretreatment.

3. Materials and Methods

3.1. Biomass

Barley straw (*Hordeum vulgare*) was used as a biomass in all of the experiments. The samples were dried to a moisture content less than 100 g kg⁻¹ and were ground using a Cutting Mill SM 100 comfort (Retsch GmbH, Haan, Germany) to a particle-size of 3 mm or less.

3.2. Explosive Decompression Pretreatment

The instrumentation for, and working principles of, the ED pretreatment method are described in detail in previous publications [11–13]. Distilled water at 700 ml was added to 100 g of dried and milled biomass and was mixed thoroughly. The biomass slurry was heated to different temperatures between (100–175) ± 3 °C. A compressed nitrogen gas or synthetic flue gas mixture was added to the reactor in order to achieve a pressure of 30 bar. The synthetic flue gas imitated the composition of flue gases from a boiler room, with the mixture's composition at N₂ (20%) and CO₂ (80%) (AGA Eesti AS, Tartu, Estonia). The nitrogen and flue gas were added to the reactor through its top and

into the headspace. Additional experiments were conducted with flue gas when the gas was added using a tube with a spiral perforated tip, which extended down to the bottom of the reactor. In this case, the gas bubbled through the biomass during the heating process to enhance the dissolution and dissociation of CO₂. After reaching the target temperature, the mixture was cooled below the boiling point and pressure was released in an explosive manner. Following the explosion, samples were cooled to a temperature below 50 °C and were used in the enzymatic hydrolysis step or were stored for later analysis.

3.3. Enzymatic Hydrolysis and Fermentation

Enzymatic hydrolysis was used to convert cellulose to glucose. Distilled water and enzyme were added to the pretreated biomass in order to gain the final volume of the mixture at 1000 ml. Enzyme mixture (30 FPU g⁻¹ cellulose; Accellerase 1500 Genencor[®], Palo Alto, CA, USA) was added to the biomass suspension at a ratio of 0.3 ml for each gram of biomass. Hydrolysis lasted for 72 h at a temperature of 50 °C under constant stirring in rotating shaker/incubator (250 min⁻¹) (Unimax 1010, Heidolph Instruments GmbH & Co.KG, Schwabach, Germany).

Following enzymatic hydrolysis, vacuum filtration was used to separate the liquid part of the suspension from the solid part. A total of 1 g of *Saccharomyces cerevisiae* yeast was added to the 200 ml liquid phase to start the fermentation process. Fermentation lasted for 7 days at room temperature (22 ± 1°C). The solid fraction was weighed and dried for later analysis.

3.4. Biomass Analysis

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

Dry matter content was analysed with a Kern MLS-D moisture analyser (KERN & SOHN GmbH Balingen, Germany), and ash content was measured according to NREL Technical Report NREL/TP-510-42622 [30]. The fibre analysis (cellulose, hemicellulose and lignin) was carried out using ANKOM 2000 analyser (ANKOM Technology, Macedon NY, USA). Acid- and neutral detergent solutions (ANKOM Technology), and 72% H₂SO₄ were used to determine acid- and neutral detergent fibre contents, and acid insoluble lignin content, respectively.

The glucose and ethanol yields were determined using an electrochemical analyser Analox GL6 (Analox Instruments Ltd., Stourbridge, UK), with determination following both the hydrolysis and fermentation steps.

Pictures of the biomass were taken using a scanning electron microscope (SEM) at the University of Tartu's Institute of Geology. SEM imaging of uncoated samples was carried out using a ZEISS EVO MA15 SEM (Carl Zeiss Microscopy GmbH, Jena, Germany) in variable pressure mode. The images were captured in backscattered electron (BSE) and variable pressure secondary electron (VPSE) modes.

3.5. Data Analysis

The hydrolysis efficiency was calculated based on Equation (1):

$$E_{HY} = \frac{m_{glc}}{m_{cel} \cdot 1.11} \cdot 100\% \quad (1)$$

where m_{glc} is the measured amount of glucose in the sample; m_{cel} is the measured amount of cellulose in the sample and 1.11 is the cellulose-to-glucose conversion factor based on the stoichiometric biochemistry of hydrolysis [31]. Fermentation efficiency was calculated based on Equation (2):

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

where c_{glc} is the concentration of glucose in the sample; c_{eth} is the concentration of ethanol in the sample and 0.51 is glucose-to-ethanol conversion factor based on the stoichiometric biochemistry of the fermentation process [31].

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.

The averaged results of at least three parallel measurements were used and standard deviations were calculated. Data were analysed using MS Excel software (2013).

4. Conclusions

The explosive decompression pretreatment method was studied and used with two different gases—nitrogen and synthetic flue gas. The latter was added into the process in two different ways. In order to investigate the effects of pretreatment upon chemical and physical changes in the biomass, a traditional three-step bioethanol production process was used with pretreatment, hydrolysis and fermentation.

The results show that explosive decompression pretreatment is a suitable method for biomass destruction since all usages of this pretreatment method were effective in the dissolution of hemicellulose, especially at higher temperatures. A small loss of cellulose due to dissolution was noticed after pretreatment; however, when compared to the extent of hemicellulose dissolution, the cellulose losses were rather small. At lower temperatures, higher hydrolysis efficiencies were gained when using the NED pretreatment, while at higher temperatures similar results were gained with all pretreatment methods, and there the hydrolysis efficiency levels reached as high as 46.7%.

Fermentation efficiencies were higher and, at times, exceeded 100% when using the NED and flue gas pretreatment methods. On the other hand, when the flue gas bubbling pretreatment method was used, ethanol concentrations were achieved that were as much as 41% lower depending upon the pretreatment temperature. The flue gas pretreatment method yielded lower ethanol concentrations and fermentation efficiencies due to the enhanced dissolution of CO₂ during pretreatment. This is most likely caused by the acidification of the biomass and the formation of fermentation inhibitors. It is necessary to investigate further to discover what kinds of compounds are being formed here and the options which may be available when it comes to removing these compounds.

Author Contributions: Conceptualization, M.R.; Methodology, M.R., V.R.; Software, M.R.; Validation, M.R.; Formal Analysis, M.R.; Investigation, M.R., V.R.; Resources, M.R., V.R. and T.K.; Data Curation, M.R.; Writing—Original Draft Preparation, M.R.; Writing—Review & Editing, M.R., V.R. and T.K.; Visualization, M.R. and T.K.; Supervision, T.K.; Project Administration, M.R.; Funding Acquisition, M.R.

Funding: This research was funded by European Regional Development Fund via the Mobilitas Pluss (project MOBERA2) of the Estonian Research Council.



Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Mood, S.H.; Golfeshan, A.H.; Tabatabaei, M.; Jouzani, G.S.; Najafi, G.H.; Gholami, M.; Ardjmand, M. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. *Renew. Sustain. Energy Rev.* **2013**, *27*, 77–93. [CrossRef]
2. Bhutto, A.W.; Qureshi, K.; Harijan, K.; Abro, R.; Abbas, T.; Bazmi, A.A.; Karim, S.; Yu, G. Insight into progress in pre-treatment of lignocellulosic biomass. *Energy* **2017**, *122*, 724–745. [CrossRef]
3. Shirkavand, E.; Baroutian, S.; Gapes, D.J.; Young, B.R. Combination of fungal and physicochemical processes for lignocellulosic biomass pretreatment—A review. *Renew. Sustain. Energy Rev.* **2016**, *54*, 217–234. [CrossRef]
4. Gírio, F.M.; Fonseca, C.; Carvalheiro, F.; Duarte, L.C.; Marques, S.; Bogel-Lukasik, R. Hemicelluloses for fuel ethanol: A review. *Bioresour. Technol.* **2010**, *101*, 4775–4800. [CrossRef] [PubMed]
5. Agbor, V.B.; Cicek, N.; Sparling, R.; Berlin, A.; Levin, D.B. Biomass pretreatment: Fundamentals toward application. *Biotechnol. Adv.* **2011**, *29*, 675–685. [CrossRef] [PubMed]
6. Samuel, R.; Cao, S.; Das, B.K.; Hu, F.; Pu, Y.; Ragauskas, A.J. Investigation of the fate of poplar lignin during autohydrolysis pretreatment to understand the biomass recalcitrance. *RSC Adv.* **2013**, *3*, 5305–5309. [CrossRef]
7. Oliveira, F.M.V.; Pinheiro, I.O.; Souto-Maior, A.M.; Martin, C.; Gonçalves, A.R.; Rocha, G.J.M. Industrial-scale steam explosion pretreatment of sugarcane straw for enzymatic hydrolysis of cellulose for production of second generation ethanol and value-added products. *Bioresour. Technol.* **2013**, *130*, 168–173. [CrossRef] [PubMed]
8. Verardi, A.; Blasi, A.; Marino, T.; Molino, A.; Calabrò, V. Effect of steam-pretreatment combined with hydrogen peroxide on lignocellulosic agricultural wastes for bioethanol production: Analysis of derived sugars and other by-products. *J. Energy Chem.* **2018**, *27*, 535–543. [CrossRef]
9. Balat, M. Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review. *Energy Convers. Manag.* **2011**, *52*, 858–875. [CrossRef]
10. Magalhaes da Silva, S.P.; Morais, A.R.C.; Bogel-Lukasik, R. The CO₂-assisted autohydrolysis of wheat straw. *Green Chem.* **2014**, *16*, 238–246. [CrossRef]
11. Raud, M.; Olt, J.; Kikas, T. N₂ explosive decompression pretreatment of biomass for lignocellulosic ethanol production. *Biomass Bioenergy* **2016**, *90*, 1–6. [CrossRef]
12. Tutt, M.; Raud, M.; Kahr, H.; Pointner, M.; Olt, J.; Kikas, T. Nitrogen explosion pretreatment of lignocellulosic material for bioethanol production. *Energy Sources Recovery Util. Environ. Eff.* **2016**, *38*, 1785–1789. [CrossRef]
13. Raud, M.; Rooni, V.; Kikas, T. Explosive decompression pretreatment: Nitrogen vs. compressed air. *Agron. Res.* **2016**, *14*, 569–578.
14. Hansen, M.T.; Jain, A.R.; Hayes, S.; Bateman, P. *English Handbook for Wood Pellet Combustion*; National Energy Foundation: Milton Keynes, UK, 2015.
15. Zevenhoven, R.; Kilpinen, P. Control of Pollutants in Flue Gases and Fuel Gases. Available online: [https://www.adeq.state.ar.us/downloads/commission/p/closed%20permit%20dockets%202006-2017/08-006-p%20aep%20service%20corp.%20&%20swepco-sierra%20club%20&%20audubon\(consolidated\)/2009-04-24_swepco-mu-ex.10.pdf](https://www.adeq.state.ar.us/downloads/commission/p/closed%20permit%20dockets%202006-2017/08-006-p%20aep%20service%20corp.%20&%20swepco-sierra%20club%20&%20audubon(consolidated)/2009-04-24_swepco-mu-ex.10.pdf) (accessed on 31 May 2018).
16. Raud, M.; Mitt, M.; Oja, T.; Olt, J.; Orupöld, K.; Kikas, T. The utilisation potential of urban greening waste: Tartu case study. *Urban For. Urban Green.* **2017**, *21*, 96–101. [CrossRef]
17. Raud, M.; Tutt, M.; Olt, J.; Kikas, T. Dependence of the hydrolysis efficiency on the lignin content in lignocellulosic material. *Int J. Hydrog. Energy* **2016**, *41*, 16338–16343. [CrossRef]
18. Kikas, T.; Tutt, M.; Raud, M.; Alaru, M.; Lauk, R.; Olt, J. Basis of energy crop selection for biofuel production: Cellulose vs. lignin. *Int. J. Green Energy* **2016**, *13*, 49–54. [CrossRef]
19. Naik, S.N.; Goud, V.V.; Rout, P.K.; Dalai, A.K. Production of first and second generation biofuels: A comprehensive review. *Renew. Sustain. Energy Rev.* **2010**, *14*, 578–597. [CrossRef]
20. Chiaramonti, D.; Prussi, M.; Ferrero, S.; Oriani, L.; Ottonello, P.; Torre, P.; Cherchi, F. Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method. *Biomass Bioenergy* **2012**, *46*, 25–35. [CrossRef]
21. Amin, F.R.; Khalid, H.; Zhang, H.; Rahman, S.U.; Zhang, R.; Liu, G.; Chen, C. Pretreatment methods of lignocellulosic biomass for anaerobic digestion. *AMB Express* **2017**, *7*, 72. [CrossRef] [PubMed]

22. Chen, H.; Liu, J.; Chang, X.; Chen, D.; Xue, Y.; Liu, P.; Lin, H.; Han, S. A review on the pretreatment of lignocellulose for high-value chemicals. *Fuel Process. Technol.* **2017**, *160*, 196–206. [[CrossRef](#)]
23. Taherzadeh, M.J.; Karimi, K. Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. *Int. J. Mol. Sci.* **2008**, *9*, 1621–1651. [[CrossRef](#)] [[PubMed](#)]
24. Han, M.; Kang, K.E.; Kim, Y.; Choi, G.-W. High efficiency bioethanol production from barley straw using a continuous pretreatment reactor. *Process Biochem.* **2013**, *48*, 488–495. [[CrossRef](#)]
25. Leskinen, T.; Kelley, S.S.; Argyropoulos, D.S. E-beam irradiation & steam explosion as biomass pretreatment, and the complex role of lignin in substrate recalcitrance. *Biomass Bioenerg.* **2017**, *103*, 21–28.
26. Tutt, M.; Kikas, T.; Olt, J. Influence of different pretreatment methods on bioethanol production from wheat straw. *Agron. Res.* **2012**, *10*, 269–276.
27. Sarris, D.; Papanikolaou, S. Biotechnological production of ethanol: Biochemistry, processes and technologies. *Eng. Life Sci.* **2016**, *16*, 307–329. [[CrossRef](#)]
28. Domínguez, E.; Romani, A.; Domingues, L.; Garrote, G. Evaluation of strategies for second generation bioethanol production from fast growing biomass Paulownia within a biorefinery scheme. *Appl. Energy* **2017**, *187*, 777–789. [[CrossRef](#)]
29. Akanksha, K.; Sukumaran, R.K.; Pandey, A.; Rao, S.S.; Binod, P. Material balance studies for the conversion of sorghum stover to bioethanol. *Biomass Bioenergy* **2016**, *85*, 48–52. [[CrossRef](#)]
30. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. Determination of Ash in Biomass. Available online: <https://www.nrel.gov/docs/gen/fy08/42622.pdf> (accessed on 30 January 2018).
31. Lu, J.; Li, X.; Zhao, J.; Qu, Y. Enzymatic Saccharification and Ethanol Fermentation of Reed Pretreated with Liquid Hot Water. *J. Biomed. Biotechnol.* **2012**, *2012*, 9. [[CrossRef](#)] [[PubMed](#)]



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