Agronomy Research **21**(2), 928–939, 2023
https://doi.org/10.15159/AR.23.060

Comparison of different chemical-free pretreatment methods for the production of sugars, ethanol and methane from lignocellulosic biomass

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Received: February 19th, 2023; Accepted: June 3rd, 2023; Published: July 3rd, 2023

Abstract. Most pretreatment methods for lignocellulosic biomass use strong chemicals, such as sulphuric acid and ammonia, to open up the cellular structure of plant biomass. However, those chemicals are not environmentally friendly and their use leads to safety risks. As a result, different chemical-free pretreatment methods have been developed, which focus on the usage of pressure, high or low temperatures and mild chemicals. Freezing pretreatment and explosive decompression pretreatments, using different operating gases, such as nitrogen and steam, are compared in the context of glucose, ethanol and methane yield in this review. For the methane production, the stillage from bioethanol production is used. The usage of this waste improves the overall valorisation of lignocellulosic biomass. The review also investigates, whether the nitrogen explosive decompression pretreatment is suitable for the treatment of softwoods, hardwoods and herbaceous materials. In the comparison of different chemical-free pretreatment methods, it is concluded that heat and water are the most influential parameters for opening up the lignocellulosic biomass structure. The operating gas and pressure in the pretreatment reactor are less relevant. Steam explosion, nitrogen explosive decompression pretreatment and autohydrolysis pretreatment are the most suitable chemical-free pretreatment methods for lignocellulosic biomass.

Key words: anaerobic digestion, biofuels, fermentation, lignocellulosic inhibitors, steam explosion.

INTRODUCTION

Pretreatment is an important step in the production of biofuels, such as bioethanol and biomethane, from lignocellulosic biomass (LCB). Its main task is to open up the complex lignocellulosic structure in order to improve the enzymatic digestibility of biomass. An ideal pretreatment process should solubilize hemicellulose and/or lignin and decrease the cellulose crystallinity. During pretreatment no inhibitory compounds should be formed, which could impede the subsequent enzymatic hydrolysis and fermentation processes. Additionally, the pretreatment method should be economically feasible, environmentally friendly and technically easy to operate. So far, no pretreatment method, which can fulfill all of these requirements, has been developed. Each of them has at least one limitation of the mentioned aspects (Kumar et al., 2009; Bajpai, 2016; Rocha Meneses et al., 2017; Rooni et al., 2017a).

Pretreatment methods are divided into four main groups: physical, biological, chemical and physio-chemical pretreatment. Methods, such as milling and irradiation, are categorized as physical pretreatment methods. Biological methods use enzymes and microorganisms to open up the LCB structure. In the case of chemical pretreatment, strong chemicals, such as acids, alkalis, organic solvents and ionic liquids, are used. Physio-chemical pretreatment methods combine the advantages of chemical and physical forces in order to open up the biomass structure. Under this group are categorized pretreatments, such as steam explosion, torrefaction and ammonia fiber expansion (AFEX). For example in the case of torrefaction, an inert gas environment and high temperature are used for processing LCB. In the case of AFEX, the biomass is mixed with ammonia, heated and pressurized inside a reactor. After a certain incubation time, the pressure in the reactor is released in an explosive manner. This step additionally opens up the biomass structure. Steam explosion is a similar method to AFEX, but instead of ammonia, hot saturated steam is used as a pretreatment agent. In addition, CO_2 and SO_2 can be utilized to assist the steam explosion process. Other pretreatment methods, which also have an explosive decompression step, use gases, such as flue gas, nitrogen or air, to pressurize the reactor. If the reactor is not pressurized prior heating, the required pressure for the explosive decompression step can be gained from the formed gases during pretreatment. This pretreatment process is classified as autohydrolysis. The autohydrolysis pretreatment is similar to the liquid hot water pretreatment and can be counted to the hydrothermal pretreatment methods. Both use water, as a solubilizing agent, in the pretreatment of LCB (Taherzadeh & Karimi, 2008; Raud et al., 2016b; Zhuang et al., 2016; Amin et al., 2017; Rooni et al., 2017a, 2018, 2019; Cahyanti et al., 2020; Rezania et al., 2020).

The usage of hazardous chemicals, such as acids and alkalis, for pretreatment has many disadvantages. For example, it requires trained personnel for handling hazardous chemicals correctly and safely. The pretreatment equipment needs to be corrosion resistant in order to withstand aggressive chemicals. Additionally, it is necessary to recover chemicals in order to decrease the amount of spent chemicals. This complicates the production process and increases the equipment costs. Furthermore, the chemicals need to be detoxified after usage, which increases the production costs even more.

In order to avoid these disadvantages, research is currently focusing more on the development of pretreatment methods, which use physical forces and harmless chemicals (Rooni et al., 2017a).

An example for a mild/chemical-free pretreatment method is the freezing pretreatment method by Rooni et al. (2017b). This method uses the volume effect of water, when it either freezes or thaws, for disrupting the cellular structure of biomass. In this method, wet biomass is exposed to several freezing-thawing cycles. And with each cycle, the biomass structure opens up more and more. Consequently, it is easier for enzymes to degrade the biomass. Another example for a chemical-free pretreatment method, is the nitrogen explosive decompression pretreatment (NED) by Raud et al. (2016a). This method uses high temperature and pressure for disrupting the biomass cellular structure. The method uses nitrogen as an inert pressurizing gas and is conducted in a similar way as AFEX and steam explosion.

Chemical-free pretreatment methods are milder compared to the methods, which use strong acids and alkalis. Due to their mildness, the sugar yields of chemical-free-pretreated biomass are often lower compared to the yields of other pretreatment methods. In order to increase the sugar yields, two-step pretreatment approaches have been investigated. With the two-step pretreatment approach, it is possible to gain higher sugar yields, however the process also consumes more energy compared to a one-step pretreatment. As a results, it can be argued whether the two-step pretreatment is economically feasible (Sjulander & Kikas, 2022).

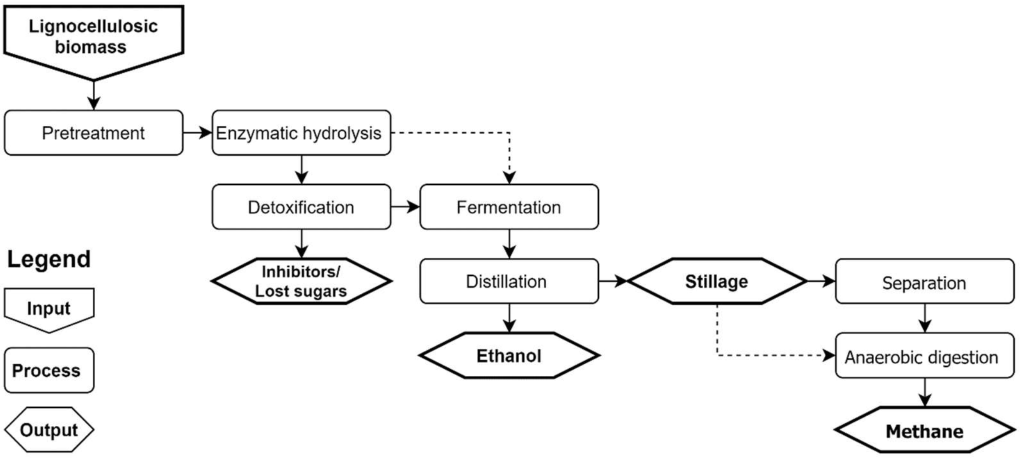

Figure 1. Biomass conversion process of LCB to ethanol and methane (dotted arrows indicate an alternative process path).

A comparison between different chemical-free pretreatment methods will be discussed in the next section. Fig. 1 gives an overview on how the LCB was processed to ethanol and methane, after pretreating it with different chemical-free methods.

PRETREATMENT OF BARLEY STRAW WITH DIFFERENT CHEMICAL-FREE PRETREATMENT METHODS

In this section, different chemical-free pretreatment methods are compared. The comparison is done based on received glucose and ethanol yields (Table 1). Each tested method can be considered as a physio-chemical pretreatment. However, their operating parameters are mild compared to acidic/alkaline pretreatment methods. The usage of harsh chemicals is avoided, instead different gases and water are used for breaking down the biomass. Explosive decompression pretreatment methods, such as steam explosion, NED and autohydrolysis, are examples of chemical-free pretreatment methods. The freezing pretreatment method by Rooni et al. (2017b) is another example. For pressurizing the pretreatment reactor, gases, such as flue gas, nitrogen, saturated steam and air, are used (Raud et al., 2016b; Rooni et al., 2017b; Raud et al., 2018, 2019).

Since the mentioned pretreatment methods were tested on the same material, barley straw (*Hordeum vulgare*), a comparison between the methods is possible. Barley straw had a cellulose content of ≈ 46%, hemicellulose content of ≈ 33% and lignin content of

≈ 5%. The highest glucose yield of 292 g per 1 kg of dry biomass (g kg^{-1}) was achieved using a two-step NED pretreatment at the temperatures of 175 and 200 °C and pressure of 10 bar. The highest ethanol yield of 127 g kg^{-1} was produced with steam explosion at 200 °C. The untreated barley straw released a low amount of glucose (12 g kg^{-1}), which also resulted in a low amount of ethanol (22 g kg^{-1}). These results show that pretreatment is necessary for opening up the complex structure of LCB prior hydrolysis and fermentation. However, the presented chemical-free pretreatment methods still need further improvement, since the theoretical glucose and ethanol yield of 511 and 261 g kg^{-1} are not yet achieved.

Table 1. Glucose and ethanol yields from barley straw using different chemical-free pretreatment methods (Raud et al., 2016a, 2016b; Rooni et al., 2017b; Raud et al., 2018, 2019; Sjulander & Kikas, 2022)

Pretreatment	Thermal system	Parameters °C	bar	Glucose g kg^{-1} of dry biomass	Ethanol
Untreated				12	22[c]
Freezing in bales outside	Winter	-9.2 to 12.8	n.a.	13	22
Freezing in swathes outside	Winter	-9.2 to 12.8	n.a.	45	30
Freezing (four freezing cycles)	Freezer	-18	n.a.[b]	89	54
Flue gas with bubbling (liquid fraction)	CHJ[a]	175	30	160	56
Autohydrolysis	CHJ	150	1	158	82
Compressed air	CHJ	175	30	192	89
Steam (liquid fraction)	Steam	180	n.a.	154	90
Nitrogen (liquid fraction)	CHJ	175	30	152	90
Flue gas without bubbling (liquid fraction)	CHJ	175	30	158	94
Two-step pretreatment nitrogen (liquid fraction)	CHJ	175 & 200	10	292	100
Steam (liquid fraction)	Steam	200	n.a.	243	127
Theoretical yield				511	261

[a] – CHJ = ceramic heating jacket; [b] – n.a. = not available; [c] – sorted from the lowest to the highest yield.

Some of the results in Table 1 are marked with the term 'liquid fraction'. This means that the hydrolysate was filtrated after the enzymatic hydrolysis Fig. 1. For the ones without marking, no filtration was performed. The filtration step improved the fermentation process, since inhibitors were partly retained in the solid biomass. However, the sugar yields also decreased due to the filtration, since some of the sugars were also retained in the solid biomass. For example, NED-pretreated biomass (175 °C, 30 bar) without filtration achieved a glucose and ethanol yield of 250 and 22 g kg^{-1}, respectively. The experiment was repeated using the same pretreatment parameters adding a filtration step after the hydrolysis. As a result, the glucose yield decreased from 250 to 152 g kg^{-1}, but at the same time the ethanol yield increased from 22 to 90 g kg^{-1}. (Raud et al., 2016a, 2019).

In the pretreatment with flue gas (80% nitrogen and 20% CO_2) at 175 °C and 30 bar, the reactor was pressurized in two different ways: into the headspace of the reactor or through a gas sparger at the bottom of the reactor (marked in Table 1. as 'bubbling'). The glucose yields for the two gas insertion methods were nearly the same with 158 and 160 g kg^{-1}. Yet, the ethanol results were different. The pretreatment method with gas sparger achieved an ethanol yield of 56 g kg^{-1} and the pretreatment method without gas sparger an ethanol yield of 94 g kg^{-1}. The smaller amount of ethanol for the pretreatment with gas sparger indicates an inhibition of the fermentation process (Raud et al., 2018). Carbonic acid might be the cause for the inhibition, since it lowers the pH of the hydrolysate. Consequently, the yeast was not able to survive in this acidic environment. Carbonic acid is formed when CO_2 is dissolved in water. In the case where the gas sparger was used, more CO_2 was dissolved compared to the case where the CO_2 was inserted into the headspace of the reactor. The dissolving process of CO_2 worked better with the gas sparger, because the CO_2 permeated the biomass-water mixture directly (Boyd, 2015).

Compressed air was also tested as an operating gas. The highest glucose and ethanol yield with this gas were 192 and 89 g kg^{-1}, respectively. The pretreatment parameters were 175 °C and 30 bar. The glucose yield was higher compared to the other pretreatment methods with nitrogen, steam or flue gas at the same parameters. This can be explained by the fact that the hydrolysate was not filtrated after the enzymatic hydrolysis, which means no sugars got lost. This is why, the glucose yield is higher compared to the other pretreatment methods (Galbe et al., 2011; Raud et al., 2016b).

So far, the results of different explosive decompression pretreatment methods have been discussed. In order to confirm the positive effect of the used gases on the pretreatment of LCB, the glucose and ethanol yield without any added gas are listed in Table 1. as well (marked as 'autohydrolysis') (Raud et al., 2016b). For the autohydrolysis pretreatment (150 °C and one bar), the glucose (158 g kg^{-1}) and ethanol yield (82 g kg^{-1}) were in the same range as compared to the pretreatment methods with pressurizing gases. Additionally, the biomass was not filtrated after the enzymatic hydrolysis, which means no sugars got lost. The results of the autohydrolysis pretreatment show that the addition of flue gas, steam, compressed air and nitrogen has no extreme benefits to the pretreatment efficiency. The addition of flue gas even turned out to be disadvantageous, since it inhibited the fermentation process after pretreatment.

The least efficient pretreatment method for barley straw was the freezing method. After four cycles of freezing (-18 °C in freezer) and thawing (room temperature) of the biomass, a glucose and ethanol yield of 89 and 54 g kg^{-1} was achieved. A natural freezing method was tested as well, which means, the straw was stored outside throughout the winter. When the straw was stacked in bales, the freezing and thawing of the biomass had a low effect on the glucose and ethanol yields. When the straw was left out on the field in swathes, the glucose and ethanol yield increased to 45 and 30 g kg^{-1}. The swathes yielded higher results, because the straw was less densely packed as compared to the baled straw. This means that the biomass had a chance to freeze and thaw over the winter. The baled straw stayed mostly unfrozen, since the temperature inside the bales never dropped below zero degrees. Only, the surface layer of the bale actually froze and thawed. In the paper by Rooni et al. (2017b), where the freezing method is described, the author suggests to use the method as a pre-pretreatment method but not as a pretreatment method, because the gained glucose and ethanol yields were too low. Yet, the method is cheap and can be easily applied on biomass (Rooni et al., 2017b).

To sum up, it is proven that pretreatment is a necessary step in the production of biofuels from LCB. A comparison between the results, presented in Table 1. of untreated and treated barley straw, demonstrates it clearly. However, not all of the tested pretreatment methods are efficient. The freezing method is the least effective, therefore not recommended as a standalone pretreatment method. In the case of explosive decompression pretreatment with different gases, none of the gases showed to be extremely beneficial for higher sugar yields compared to the autohydrolysis pretreatment. The pretreatment results with air, flue gas, steam and nitrogen are too similar to the autohydrolysis results. It seems that the driving forces for improved sugar yields are heat and water, since those are the parameters that all pretreatment methods have in common.

NITROGEN EXPLOSIVE DECOMPRESSION PRETREATMENT

This section discusses the results of NED-pretreated biomass and elaborates, how the type of biomass influences the efficiency of the pretreatment method.

In the paper by Raud et al. (2019), it was investigated whether NED pretreatment or steam explosion is more efficient in the pretreatment of barley straw. It has been determined that NED is more efficient than steam explosion up to a temperature of 175ºC. From 180ºC and higher, steam explosion was more efficient than NED. The compositional changes of hemicellulose, cellulose and lignin during pretreatment have been investigated as well. Both pretreatment methods are capable to dissolve hemicellulose completely. The dissolving process of hemicellulose is strongly connected to the pretreatment temperature. The cellulose content decreases slightly for both pretreatment methods, but stays overall the same. Surprisingly, the lignin content increases with rising pretreatment temperature for both pretreatment methods. This can be an indicator for the formation of pseudo-lignin. Pseudo-lignin is formed, when the pretreatment conditions are harsh. Sugars, such as glucose and xylose, start to degrade and agglomerate to a humin-like substance and appear in the form of droplets on the biomass surface. Pseudo-lignin can be falsely detected as lignin, when fiber analysis are performed from pretreated biomass (Aarum et al., 2018; Shinde et al., 2018; Raud et al., 2019). Its formation should be avoided, since it leads to the loss of sugars, which areneeded for fermentation. In addition,it can deactivate enzymes during hydrolysis (Hu et al., 2012).

Table 2. Fiber analysis results of raw materials, which were later NED-pretreated (Raud et al., 2016a; Sjulander, 2019; Rooni et al., 2021; Sjulander & Kikas, 2022)

Biomass	Hemicellulose, %	Cellulose, %	Lignin, %
Ash	17	42	27
Aspen	17	49	19
Birch	21	43	20
Grey alder	16	38	24
Willow	13	51	20
Barley straw	33	46	5
Pine	13	40	24

Table 2 provides an overview about the cellulose, hemicellulose and lignin content of different raw plant materials, which were later pretreated with NED. From the tested materials, barley straw had the highest hemicellulose content (33%). The other materials had a significantly lower hemicellulose content, ranging from 13 to 21%. The cellulose content for the different materials ranged from 38 to 51%. Barley straw, with 5%, had the lowest lignin content. For the other tested materials, the lignin content ranged from 19 to 27% (Raud et al., 2016a; Sjulander, 2019; Rooni et al., 2021; Sjulander & Kikas, 2022). The fiber analysis results help to understand the NED-results in Table 3 and explain why certain materials are more resilient than others.

Table 3 is a comparison between different NED-pretreated materials and illustrates the differences in product yields, depending on the type of biomass. The highest glucose yield, 250 g kg^{-1}, was achieved from barley straw and the lowest yield, 31 g kg^{-1}, from pine. The glucose yields of the tested hardwoods range from 50 to 126 g kg^{-1}. The hardwoods also released xylose after the enzymatic hydrolysis, which is not surprising, since the hemicellulose of hardwoods is mainly built up from xylans. Barley straw should also release xylose after the hydrolysis, because its hemicellulose also contains xylans (Sun et al., 2011). However, xylose results are not available for this biomass. Pine released just a low amount of xylose. This is due to the fact that the hemicellulose of pine is mainly built up from mannans and the major released sugar would be mannose (Rowell, 2012).

Table 3. Comparsion between different NED-pretreated materials in regard to glucose, xylose and acetic acid yield after the enzymatic hydrolysis and ethanol yield after the fermentation (Raud et al., 2016a; Sjulander, 2019)

Biomass	Parameters		Glucose	Xylose	Acetic acid	Ethanol
	°C	bar	g kg^{-1} of dry biomass			
Pine	175	10	31[b]	12	3	10
Grey alder	175	30	50	90	34	0
Ash	200	30	64	88	30	4
Birch	200	30	87	90	43	0
Willow	200	30	105	79	33	0
Aspen	200	30	126	53	44	0
Barley straw	175	30	250	n.a.[a]	n.a.	22

[a] – n.a. = not available; [b] – sorted from the lowest to the highest yield.

The hemicellulose in plants is often acetylated, as a result acetic acid is formed during pretreatment, when the acetyl-groups cleave off (Jönsson & Martín, 2016). For hardwoods, the acetic acid yields range from 30 to 44 g kg^{-1}, which indicates a much higher acetylation compared to pine wood with 3 g kg^{-1}.

The gained sugars from the NED-pretreated materials were fermented to ethanol after the enzymatic hydrolysis. As can be seen in Table 3, little or no ethanol was produced, which shows that the fermentation process was inhibited. The inhibition can be due to a low pH in the hydrolysate or the presence of inhibitors, such as acetic acid, hydroxymethylfurfural, furfural and aromatic compounds (Sjulander & Kikas, 2020).

Table 1 shows results, where ethanol was produced from pretreated barley straw. However, Table shows results, where the ethanol production from the same material was inhibited. The pretreatment conditions where the same for both results. It seems contradicting, yet can be explained by the detoxification of the material. Raud et al. (2019) filtrated the pretreated barley straw after the hydrolysis (see Fig. 1). As a result, the hydrolysate became detoxified and the fermentation process was not impeded anymore. A negative side effect of filtration is that some sugars were lost, which explains why the glucose yields from barley straw in Table 3 are higher compared to Table 1. In order to avoid the formation of inhibitors and the loss of sugars, the NED pretreatment method should be optimized for each specific material in order to reduce the need for detoxification (Sjulander & Kikas, 2020). Yet, this can be difficult to achieve, when the production process aims for high sugar yields. As can be seen in Table 3, the highest sugar yields were achieved with NED pretreatment at high temperatures. At lower temperatures, e.g. 125 and 150 °C, the sugar yields (data not shown) were significantly lower (Raud et al., 2016a). In addition, the experiments have shown that increasing the pretreatment temperature also increases the formation of inhibitors. Therefore it can be argued, whether the NED pretreatment method should be optimized or the pretreated biomass should be detoxified.

From the NED-pretreated materials, the highest sugar yields were gained from barley straw, the second highest from hardwoods and the least amount from pine. This indicates that the recalcitrance of materials for NED pretreatment goes as follows: softwoods > hardwoods > herbaceous materials. Lignin plays a vital role in the recalcitrance of materials, since it gives plant fibers strength and protects them from environmental decay. As a result, it is not surprising that the materials with a higher lignin content released less sugars compared to the ones with a lower lignin content (see Table 2).

To sum up, NED pretreatment can efficiently solubilize hemicellulose from LCB. However, the risk is that the solubilized hemicellulose is turned into pseudo-lignin during pretreatment, in case the pretreatment temperature is too high. The lignin content of a material indicates how recalcitrant a material is, and allows to predict the success of the NED pretreatment method on a particular material. During NED pretreatment, inhibitors can be formed, which in turn can impede the subsequent enzymatic hydrolysis and fermentation. The type of inhibitor and its quantity depends on the material and the pretreatment conditions. As a results, the NED pretreatment method should be adjusted to each specific material, so that the formation of inhibitors can be avoided or kept low. If an adjustment is not possible, the pretreated material can also be detoxified.

VALORIZATION OF BIOETHANOL PRODUCTION WASTE

Bioethanol production is an energy-intensive process with several steps. Depending on the type of biomass, more or less energy is required for converting the biomass into bioethanol or other valuable products. In order to make the production profitable, each side stream or residue should be utilized or recycled. For example, organic residues from bioethanol production can be converted into biomethane, using anaerobic digestion, or burned in boilers for the production of heat and electricity. Process water can be recycled in order to save water. Residues from the enzymatic hydrolysis of different materials can be used as animal feed. (Klemeš, 2012; Rocha Meneses et al., 2017; Chatzifragkou & Charalampopoulos, 2018; Solarte-Toro et al., 2018).

In the case of methane production from bioethanol production waste, Rocha-Meneses et al. (2019c) investigated the methane potential from distillation residues (stillage). In her experiments, barley straw was pretreated with explosive decompression pretreatment, using flue gas or nitrogen, at 150 °C and 30 bar. Then, the pretreated material was enzymatically hydrolyzed, fermented and distilled. Afterwards, the stillage was anaerobically digested. Table 4 shows the methane yields from barley straw stillage, which was pretreated with different pretreatment methods. The lowest methane yield of 157 g kg^{-1} was gained when barley straw was pretreated with flue gas and the gas was inserted into the headspace of the reactor (marked in Table 4 as ‘without bubbling'). Higher methane yields were gained when nitrogen was inserted into the headspace of the reactor or flue gas was inserted through a gas sparger at the bottom of the reactor (marked in Table 4 as 'with bubbling'). The highest methane yields were achieved when the stillage was separated into a solid and liquid fraction and each fraction was separately anaerobically digested (see Fig. 1). In this case, pretreatment with nitrogen achieved a total methane yield of 365 g kg^{-1}. The reason why the solid-liquid separation improved the methane yields, is not known. However, Drosg et al. (2013) and Town et al. (2014) observed in their experiments as well that the methane yield increases when the bioethanol production stillage was separated into a solid and liquid fractions before anaerobic digestion.

Table 4. Methane yields from bioethanol production stillage, using barley straw as a feedstock and different explosive decompression pretreatment methods (Rocha-Meneses et al., 2019a, 2019b, 2019c)

Pretreatment	Methane g kg^{-1} of dry biomass
Untreated	146
Flue gas without bubbling	157
Nitrogen	188
Flue gas with bubbling	192
Flue gas with bubbling (solid & liquid fraction)[a]	292
Nitrogen (solid & liquid fraction)	366

[a] – separation of stillage into a solid and liquid fraction.

The results in Table 4 demonstrate that the valorization of stillage can be beneficial for the overall energy output of a bioethanol production plant (Rocha-Meneses et al., 2019c, 2019b, 2019a).

CONCLUSIONS

In the comparison of different chemical-free pretreatment methods on barley straw, it can be concluded that the pretreatment temperature and water, as a solubilizing agent, are the most important parameters for an efficient pretreatment of LCB. The pretreatment pressure and the pressurizing gas are less relevant for the success of the pretreatment. However, they can be beneficial. For the fermentation, the type of operating gas is important. For example, flue gas has shown to be inhibiting to the fermentation process, since it lowers the pH of the hydrolysate and creates an unfavourable environment for the yeast, but this only occurred when the flue gas was inserted through a gas sparger at the bottom of the reactor.

Depending on the pretreated biomass (herbaceous materials, softwoods or hardwoods), the sugar yields were different, although the NED pretreatment with the same parameters was applied on all of them. The difference can be explained by the compositional and structural diversity of LCB. The lignin content can be used as an indicator, how recalcitrant a material is. Biomass with a higher lignin content, such as softwoods, is more difficult to hydrolyze than biomass with a lower lignin content, such as straw. During NED pretreatment, inhibitors can be formed that impede the subsequent enzymatic hydrolysis and fermentation. The NED pretreatment parameters should be adjusted according to the processed material in order to avoid the formation of inhibitors. The pretreated material can also be detoxified, if adjustment is not possible.

Bioethanol production waste, such as stillage, can be converted to methane by anaerobic digestion. This allows to process waste in an environmentally friendly and economical way. Additionally, the produced methane can cover partly the energy demand of a bioethanol production plant. The separation of stillage into a liquid and solid fraction and the separate anaerobic digestion of both fractions improves the methane yield in comparison to the stillage alone. The biochemical and physical reason for the increased methane yield is yet unknown.

Overall, it is concluded in this review that autohydrolysis, steam explosion and NED pretreatment are the most suitable chemical-free pretreatment methods for LCB.

ACKNOWLEDGEMENTS. We gratefully acknowledge the financial support from the base-funded project P200195 by the Estonian University of Life Sciences.

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