

A comparative study between ionic liquid and acidic pretreatment of waste cotton textiles with simultaneous saccharification and fermentation

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Abstract

Millions of tons of waste cotton textiles are discarded every year while they could have been used for the production of bioethanol as an alternative for fossil fuel. In this study, the potential of cotton as raw material for ethanol production using ionic liquid (IL) pretreatment and acidic pretreatment is examined and compared, along with non-pretreated cotton samples. The study also aims to determine the optimal temperature of ionic liquid pretreatment, for which experiments under three temperatures (95°C, 150°C, and 175°C) were carried out. Subsequently, the pretreated cotton samples were converted to bioethanol via simultaneous saccharification and fermentation (SSF). Ethanol yields resulting from different pretreatment methods (also non-pretreatment) were compared. Findings suggest that ionic liquid pretreatment under 175°C gives the highest ethanol yield, proving it to be a competitive alternative to traditional acid pretreatment widely applied in industry, as well as demonstrating the prospective usage of waste cotton textiles as biomass for the production of renewable biofuel.

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Introduction

With the advent of fast fashion, more garments are bought and discarded than ever; 57% of them end up in landfills, including 35% of materials that end up as waste in the supply chain (Objective, 2018). Cotton with a yearly production of over 25 million tons (OECD-FAO Agricultural Outlook 2019–2028, 2019) stands for about 30% of the global market of textile fibers (Krifia and Stevens, 2016). The industry pays less attention to waste than to other ecological issues, meaning that tons and tons of waste textiles are ending up in landfills. To give an idea, the US Environmental Protection Agency reported that in 2018, only 3 million out of 17 tons of waste textiles were recycled in the US (National Overview: Facts and Figures on Materials, Wastes and Recycling, 2021).

Cotton is made out of fibers that consist mainly of cellulose. According to Liu (2018), there can be as much as 96.5% cellulose in cotton. The biopolymer, after some pretreatment, can undergo a hydrolysis reaction to generate glucose, a component that is essential in the

production of ethanol. It would be beneficial both in terms of a circular economy as well as solving the energy crisis and controlling pollution by fossil fuel if waste cotton in the textile industry can be further utilized to produce bioethanol.

Cotton belongs to the category of lignocellulosic biomass, which is the foundation of second-generation bioethanol. A lot of studies have already been carried out, converting a wide range of lignocellulosic biomasses to ethanol, including rice straw (Binod *et al.*, 2010), sugar cane, rice hull, corn stover and many others (Aditya *et al.*, 2016). However, very limited research is done on cotton, especially waste cotton textiles. In a study by Jeihanipour and Taherzadeh (2009), acid, as well as alkali-pretreated cotton waste from blue jeans were used to produce bioethanol, with cotton linter as reference. High ethanol yield was obtained under conditions of proper pretreatment, demonstrating the great potential of cotton in the production of bioethanol.

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Ionic liquids (IL) are a broad range of “liquid salts” that essentially have melting points below 100°C. They can consist of organic and/or inorganic ions and can be comprised of multiple anions or cations - making them complex and diverse at the same time (Shamshina *et al.*, 2018). One of their interesting features is high thermal stability which can reach up to 300°C, depending on the combination of cation and anions (Zhao and Anderson, 2012). They are able to dissolve high amounts of cellulose under moderate conditions and they are considered to be environmentally friendly because of their low volatility, resulting in minimal emissions to the ambient air. Another remarkable property of ionic liquids is that in cases of dissolving cellulose, cellulose precipitates immediately after the addition of an anti-solvent such as deionized water, allowing for easy cellulose recovery. These characteristics of ionic liquids make them an ideal solvent for dissolving and pretreating cellulose (Dadi *et al.*, 2006).

Indeed, ionic liquid pretreatment is a promising non-conventional pretreatment method for lignocellulosic biomasses that emerged more recently than conventional acid or alkali pretreatments. It is reported to significantly increase ethanol yields (Alayoubi *et al.*, 2020) and has advantages over the traditional methods in terms of eco-friendliness, 100% cellulose recovery, and its application produces no toxic, hazardous, or degradation compounds (Brandt-Talbot *et al.*, 2017).

The aim of this study is to determine the optimal temperature for pretreating waste cotton textile with ionic liquid as well as to compare the effect with the conventional acid pretreatment using phosphoric acid in terms of the bioethanol yield obtained after SSF using cellulase and yeast. Cotton linter (100% pure cotton) is used as a reference, as cotton textiles have gone through a lot of processing such as bleaching and scouring which might have changed the chemical composition of cotton.

Materials and methods

Mechanical pretreatment

Starting from a white T-shirt as a source for our waste cotton (and cotton linter as reference), the size of the samples should be reduced as much as possible. Scissors and tweezers were used to cut and tear apart the cotton fibers.

Ionic liquid pretreatment

The mechanically pretreated cotton was subjected to ionic liquid treatment according to the standard procedure (Li *et al.*, 2010). The ionic liquid used was 1-ethyl-3-methylimidazoliumchloride (Sigma Aldrich), and the biomasses were pretreated for 2h at respectively 95°C, 150°C, and 175°C in separate oil baths. A small amount of sodium azide (in the range of 0.01 g) was added and the samples of pretreated cotton were kept in the refrigerator for further use.

Acid pretreatment

In parallel to the ionic pretreatment, the mechanically pretreated cotton underwent acid pretreatment based on the protocol of Zhang *et al.* (2006). The phosphoric acid used was 85% purchased from Sigma Aldrich and the cotton samples were pretreated for 100 minutes.

SSF

Microorganism preparation

To activate the yeast, 8 g of sugar and 800 mg of the yeast (*Saccharomyces cerevisiae*) were added in 100 mL lukewarm (+/- 43.3°C) water. After the yeast was activated, it was added to the inoculum medium YEPD (yeast extract peptone dextrose) which was prepared based on the paper of Dymond (2013).

Enzymatic hydrolysis

The saccharification is performed with a commercial enzyme extract, cellulase from *Trichoderma reesei* (Sigma Aldrich). Based on the protocol of Doran-Peterson *et al.* (2009), the total calculated volume of commercial enzyme preparation to add to the fermentation was 0.25 mL. The needed enzymes were filter-sterilized using a 0.22- μ m filter.

SSF

During the actual SSF the yeast, obtained after centrifuging the microorganism preparation at 10 000 x g for 10 min and removing the supernatants, was added together with 125 mL of the diluent prepared based on Doran-Peterson *et al.* (2009), the filtered enzymes, and the pretreated cotton in one Erlenmeyer flask of 250 mL. After adjusting the pH to obtain a value of around 5 according to the protocol of Doran-Peterson *et al.* (2009), the fermentation airlock was

added for CO₂ ventilation and the bioreactor was incubated at 37°C at 150 rpm in a rotary shaker. Samples were taken every 24 hrs (for a total of 72 hours) for the detection.

Detection

Detection of ethanol concentration was carried out with a commercial enzymatic UV-based detection kit (Dialab, Austria). The lower detection limit is 10 mg/dL and the range of linearity is up to 350 mg/dL when measured at 376 nm. First, a calibration curve was set up by measuring the optical densities (OD) of standard solutions with known ethanol concentrations, then ethanol concentrations of samples obtained from SSF were determined based on the calibration curve.

Results and discussion

Calibration curve for ethanol detection

Figure 1 represents OD values against ethanol concentration (in per mille). The measured OD values

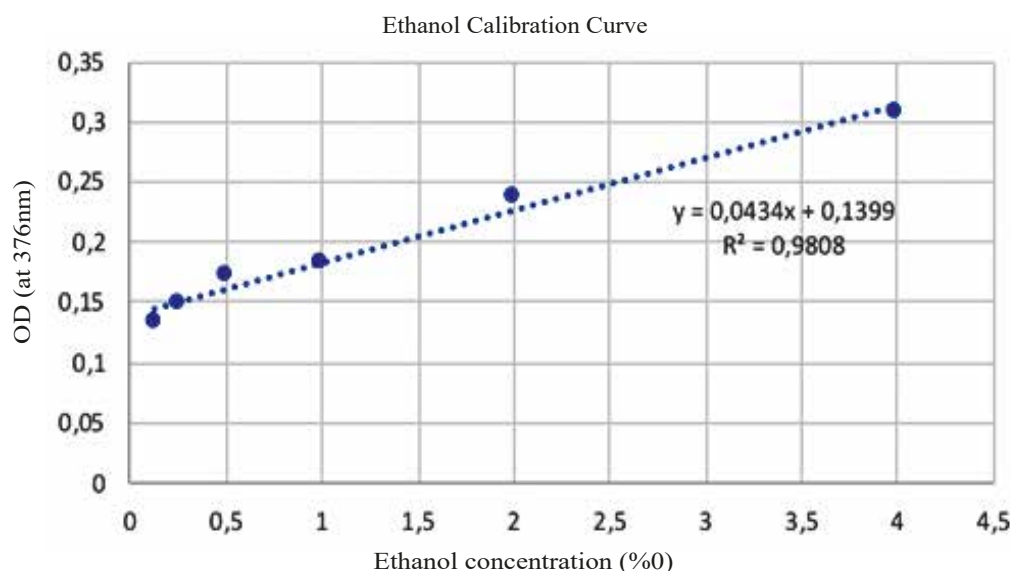


Fig. 1. Ethanol calibration curve determined from dilutions of ethanol absolute (99.4%)

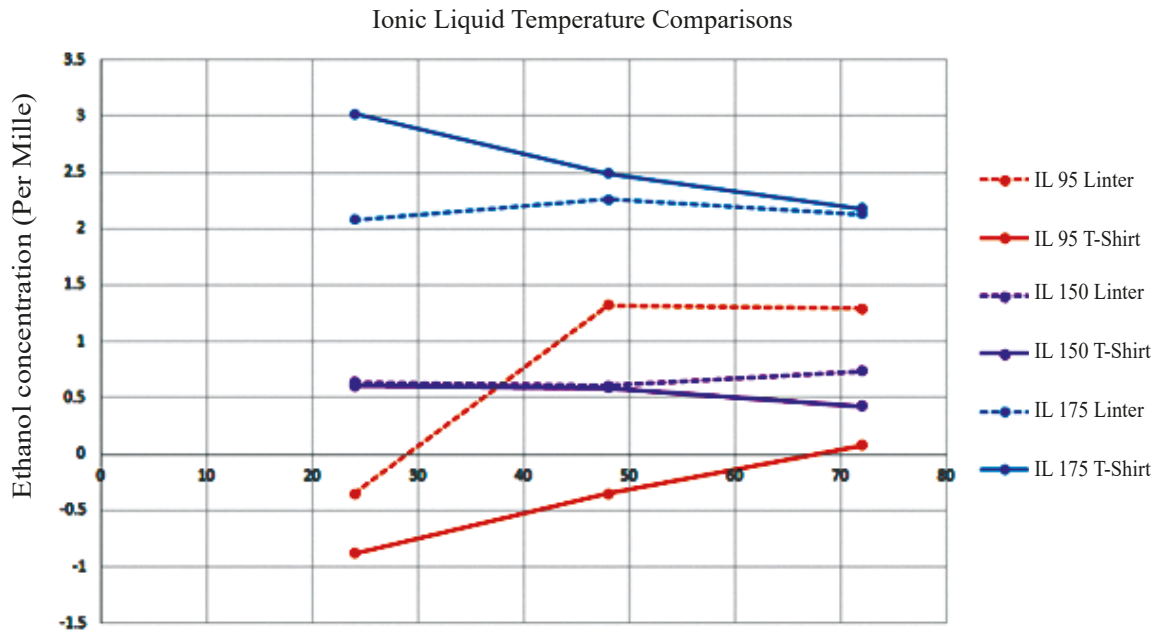
correspond to the different dilutions made from ethanol stock solution (99.4%). This graph has been used to determine the concentration of ethanol obtained from all the SSF experiments.

Ethanol concentrations from experiments

In Figure 2, the results (ethanol concentration in function of time) of the ionic liquid pretreatments are shown. The difference between the three temperatures (95°C, 150°C, and 175°C) used during the experiment can be seen on the graph. This set of experiments was designed to find the optimal temperature for the ionic liquid pretreatment based on the obtained ethanol yield. For both cotton linter and T-shirt, ethanol yields obtained at 175°C are evidently higher than those obtained at 95°C and 150°C.

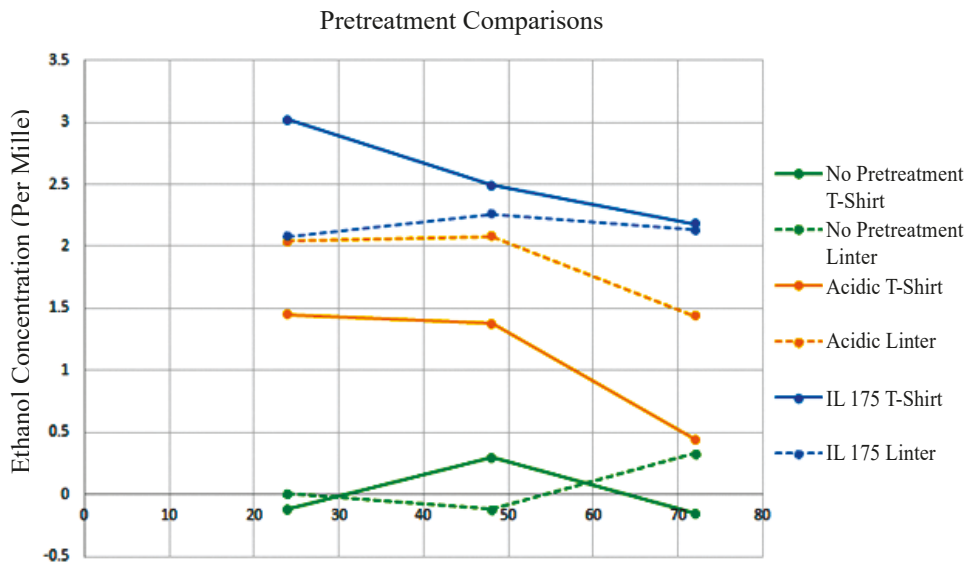
In addition, no obvious trend can be observed between the different experiments with regard to the number of hours after which the samples were taken. In the case of 95°C and 150°C, the pretreated cotton linters have higher ethanol concentrations than the pretreated cotton T-shirts. In the case of the pretreatment at 175°C, this is not the case, there the cotton T-shirt has higher ethanol concentrations than the cotton linters. This suggests that no conclusion can be drawn for differences between waste cotton T-shirts and cotton linters in terms of ethanol production from these experiments.

Figure 3 compares the ethanol concentrations (obtained from different pretreatment methods experimented) in function of the hours of SSF (72 hours in total in each case - ethanol concentrations detected every 24 hours). As can be seen and expected, very little to no ethanol (the values of the



Legends in full form: IL 95 Linter - Ionic Liquid Pretreatment at 95°C using Cotton Linters, IL 95 T-Shirt - Ionic Liquid Pretreatment at 95°C using Cotton T-Shirt, IL 150 Linter/T-Shirt - Ionic Liquid Pretreatment at 150°C using Cotton Linter/Cotton T-Shirt, and IL 175 Linter/T-shirt - Ionic Liquid Pretreatment at 175°C using Cotton Linter/Cotton T-Shirt.

Fig. 2. Ethanol concentrations in function of time obtained from ionic liquid pretreatments carried out at three different temperatures: 95°C, 150°C and 175°C.



Legends in full form: No Pretreatment T-Shirt/Linter - Non-Pretreated T-Shirt/Linter (Control), Acidic T-Shirt/Linter - Acid Pretreated T-shirt/Linter and IL 175 T-shirt/Linter - Ionic Liquid Pretreated at 175°C (the optimal temperature) T-Shirt/Linter.

Fig. 3. Ethanol concentrations in function of time obtained from different pretreatment methods

concentrations lie very close to 0) can be detected from non-pretreated cotton sourced from both T-shirt and linters. For acidic pretreatment, higher ethanol concentration can be detected, which drops over the course of 72 hours of SSF. Interestingly, cotton linters

examined, IL pretreatment at 175°C proved to obtain the highest ethanol yield. This difference could be attributed to the different lignocellulosic make-up of switchgrass and cotton, as well as the different ionic liquids used (imidazolium chloride in this study). However, this finding

Table I. Summary of results from different pretreatment experiments

Cotton Type	Findings	Ionic Liquid Pretreatment			Acidic	No
		95°C	150°C	175°C	Pretreatment	Pretreatment
Linters (Pure)	Highest ethanol yield (%0)	1.30	0.75	2.30	-	-
	Time to reach highest ethanol yield (hrs)	48	72	48	48	72
T-Shirt (Waste)	Highest ethanol yield (%0)	0.10	0.60	3.00	1.45	0.80
	Time to reach highest ethanol yield (hrs)	72	24	24	24	48

showed higher ethanol concentration than cotton from waste T-shirts in this case. Furthermore, the highest ethanol concentration is seen to be obtained from ionic liquid pretreatment at 175°C. The key results are summarized in Table 1 for both cotton linters and T-shirt.

Discussion and future perspectives

A key question to be answered in this paper is to determine the optimal temperature for ionic liquid pretreatment for cotton, and three temperatures were examined in this study (i.e. 95°C, 150°C, and 175°C). The choice of this temperature range was based on a reference study done by Li *et al.* (2010) on switchgrass, where they found the optimal temperature to be 160°C using imidazolium acetate. Findings in this study differ from the reference study. Out of the three temperatures

can still be explained by previous literature (Raj *et al.*, 2018), as enhanced porosity and increased surface area were seen with ionic liquid pretreatment at increasing temperatures.

The aim of this study is also to compare acid pretreatment and ionic liquid pretreatment for cotton in terms of ethanol yield. Results show that ionic liquid pretreatment at 175°C gave the highest concentration compared to the rest, even slightly higher than the most popular acid pretreatment. The results obtained in these experiments are, in fact, compatible with the research done by other scientists and their claims about ionic liquids. As stated by Solowski *et al.* (2020), ionic liquid allows for almost 100% saccharification under the right conditions (by decreasing the crystallinity of the cellulose and increasing porosity and surface area) which could eventually out-perform acidic pretreatment, if it was not too expensive. Acidic pretreatments' failure to compete

with ionic liquid could be due to the formation of degradation and inhibitory products which affect the subsequent SSF. This seems reasonable when comparing the ionic liquid pretreatment at 175 °C that led to higher yields than acid pretreatment.

Over the course of the 72 hours of SSF, ethanol concentrations for both IL pretreatment at 175°C and acidic pretreatment are seen to be decreasing, this could be due to the evaporation of ethanol. Saturation point seemed to be reached by the first 24 hours and production of ethanol might have stopped then, leading to a net loss of ethanol concentration by evaporation. In the case of IL pretreatments at 95°C and 150°C, the ethanol concentrations are seen to be increasing over the course of 72 hours. This could be due to slower ethanol production by the yeast due to the lower quality of pretreatment. It could be that the crystalline structure of the cellulose in these cases was not sufficiently disrupted leading to difficult access of the enzymes and yeast to the cellulose structure, hence taking more time to reach saturation. The net ethanol concentration (despite evaporation) is thus seen to be increasing. More thorough research has to be done to confirm this hypothesis.

As mentioned earlier, for the detection of ethanol, a Dialab kit was utilized. The kit requires the addition of two solutions into the ethanol samples before recording the OD values. The solutions could be the reason why the ethanol calibration curve shows a non-zero intercept. Another important thing to note is that the detection limit of the kit and the linearity range was slightly exceeded, which can give somewhat anomalous values of ethanol concentration.

Due to the limited time scope of this study, the results are still preliminary and the experiments could be more fine-tuned for further improvement. Certain variables were not taken into account during the experiments, for example, the evaporation of ethanol. Since ethanol is a rather volatile compound, this could have influenced the accuracy of the final ethanol concentrations. Another factor to consider is the amount of yeast used. The activated yeast started from the same amount of dry yeast, but since there was liquid content in the activated yeast that was added to the bioreactors, the weight was slightly inconsistent from batch to batch. Because the yeast has been activated with glucose/sucrose before being added to the bioreactor, additional ethanol could be present in our samples introduced by the wet yeast leading to slightly misleading ethanol concentrations. There is also no clear trend that can be understood in the ethanol concentration fluctuations between cotton linters and waste

cotton from T-shirts, it is beyond the scope of this paper to provide explanations for that.

Another limitation was that the ionic liquid pretreatment was only performed at three different temperatures. The results only suggest the best condition between the three chosen temperatures instead of an accurate approximation of the real optimal temperature. A wider range of temperatures should be studied (including temperatures above 175°C) to determine the real optimal temperature for the pretreatment of IL of cotton. Also, more duplicates of each experimental setup should be performed in order to obtain more reliable results.

For further improvement, more samples could be taken in shorter time intervals for the determination of ethanol production over time, to determine the shortest amount of time needed to get the highest ethanol yield. Additionally, purification is still required to obtain concentrated products without remainders of fermentation medium. These two aspects do not fall into the scope of this study but could be crucial for future commercialization.

The findings of this experiment offer new insights into bioethanol production, both in terms of raw material and pretreatment method. This study proved the potential of ionic liquids in efficient pretreatment of lignocellulosic biomass by significantly increasing the yield of bioethanol, which is in accordance with previous studies (Alayoubi *et al.*, 2020; Dadi *et al.*, 2006). Results even suggest that ethanol yields obtained from ionic liquid pretreated biomass (under appropriate temperature) surpass those from acid pretreated biomass, providing a more eco-friendly alternative to acid pretreatment, which is widely used in industry. A summary of the differences between these two pretreatment methods is shown in Table 2. Moreover, this study also demonstrated the possibility of using waste textile for bioethanol production, a rather novel area on which little research has been done. With further research on the industrialization and commercialization of waste cotton textile as raw material for ethanol, it could be a valuable contribution to some of our most concerning issues, such as creating a circular economy to avoid resource depletion and reduce waste, as well as solving the energy crisis caused by over-dependence on fossil fuels.

Table II. Summary of the key differences between the novel IL pretreatment and conventional acid pretreatment

Acidic Pretreatment	Ionic Liquid Pretreatment
Commercially under development	Currently under research
Hazardous and corrosive to equipment	Eco-friendly and non-corrosive
High ethanol yield	Higher ethanol yield (at high temperatures)
Solubilizes hemicelluloses, however, condensed lignin remains on the surface of crystalline cellulose - hindering enzyme accessibility	Efficient in biomass dissolution and recovery of cellulose upon addition of anti-solvent
Degradation products can form which are inhibitory to enzymatic activity	No such degradation or by-products formed

Conclusion

In this study, bioethanol was produced starting from waste cotton textiles and cotton linter as a reference, treated with acid and ionic liquid pretreatment prior to performing simultaneous saccharification and fermentation. For both pretreatments, significantly higher yields were obtained compared to cases without pretreatment, proving pretreatment to be an essential step for ethanol production from lignocellulosic biomass. For acidic pretreatment, an ethanol yield of 1.45% was obtained for waste cotton T-shirt and 2.08% for cotton linter, while for ionic liquid pretreatment an optimal ethanol yield of 3.02% was obtained for waste cotton T-shirt and 2.26% for cotton linter at 175°C. Lower temperatures (95°C and 150°C) of ionic liquid pretreatment resulted in lower ethanol yields compared to the best case of 175°C.

Based on the obtained yields, it is safe to conclude that ionic liquid pretreatment is a competitive alternative for traditional acid pretreatment for cotton, with comparable or even higher bioethanol yields under optimal conditions. The study also proves the potential of waste cotton textiles to be used in the bioethanol industry, with high conversion rates and large biomass availability.

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