Enhanced Bioethanol Production from Corn Stover Using Choline Chloride and Lactic Acid Pretreatment: A Gravimetric analysis Approach

[*]Cosmas S. Mwanakaba, Zachary Siagi, Paul Maina, Mwansa Kaoma

University of Zambia, Zambia

[*]Corresponding Author

DOI: https://dx.doi.org/10.47772/IJRISS.2024.803211S

Received: 04 July 2024; Accepted: 22 July 2024; Published: 28 August 2024

ABSTRACT

The transition to renewable energy sources necessitates the development of efficient and sustainable bioethanol production methods. This study investigates the use of a deep eutectic solvent (DES) blend of choline chloride and lactic acid for the pretreatment of corn stover, a widely available lignocellulosic biomass, to enhance cellulose yield and bioethanol production. The gravimetric method was employed to quantify cellulose yield post-pretreatment, providing a direct assessment of pretreatment efficacy. The study further evaluated the subsequent steps of enzymatic hydrolysis, fermentation, and distillation to determine the overall bioethanol yield. Results demonstrated that the choline chloride and lactic acid blend effectively disrupted the lignocellulosic structure of corn stover, significantly increasing cellulose accessibility and fermentable sugar release. The optimized pretreatment process led to a notable improvement in bioethanol yield, highlighting the potential of this method for commercial bioethanol production. This research contributes to the advancement of biofuel technologies and supports the broader goal of achieving sustainable energy solutions by demonstrating a viable approach to enhancing the efficiency and sustainability of lignocellulosic bioethanol production.

Keywords: deep eutectic solvent, choline chloride, lactic acid, cellulose yield, enzymatic hydrolysis, lignocellulosic biomass, sustainable energy.

INTRODUCTION

Bioethanol has emerged as a significant alternative to fossil fuels, primarily due to its renewable nature and potential for reducing greenhouse gas emissions. As a biofuel, it offers a sustainable energy source that can help mitigate climate change while providing economic opportunities, particularly in rural areas where feedstocks are abundant(Balat et al., 2008).

The selection of feedstocks is crucial for the economic viability and environmental sustainability of bioethanol production. Lignocellulosic biomass, including agricultural residues like corn stover, represents a promising feedstock due to its availability, low cost, and high cellulose content (Sims et al., 2010).

Corn stover, the leftover stalks, leaves, and cobs after harvesting corn, is one of the most abundant agricultural residues. Utilizing corn stover for bioethanol production not only adds value to agricultural waste but also helps in managing residue disposal, thereby contributing to environmental sustainability(Kim & Dale, 2004).

Despite its potential, the efficient conversion of lignocellulosic biomass to bioethanol is challenging due to its complex structure. The recalcitrance of lignocellulosic biomass necessitates effective pretreatment processes to break down the rigid structure and enhance the accessibility of fermentable sugars(Mosier et al., 2005).

ISSN No. 2454-6186 | DOI: 10.47772/IJRISS |Volume VIII Issue IIIS August 2024 | Special Issue on Education

Various pretreatment techniques have been explored to overcome the recalcitrance of lignocellulosic biomass, including physical, chemical, and biological methods. Each technique aims to disrupt the lignin structure, reduce cellulose crystallinity, and increase the porosity of the biomass(Alvira et al., 2010).

Among chemical pretreatment methods, ionic liquids (ILs) have gained attention due to their ability to dissolve cellulose and lignin. Choline chloride-based ILs, in particular, have shown promise due to their low toxicity, biodegradability, and effectiveness in breaking down biomass(van Osch et al., 2017).

The use of a blend of choline chloride and lactic acid as a pretreatment solvent has been explored to enhance the efficiency of lignocellulosic biomass conversion. This blend leverages the individual strengths of each component to facilitate the breakdown of the biomass structure(Mäki-Arvela et al., 2010).

The gravimetric method is a reliable and straightforward technique for quantifying the yield of cellulose after pretreatment. This method involves measuring the weight of cellulose recovered after the pretreatment process, providing a direct assessment of the pretreatment efficiency(Sluiter et al., 2008).

The gravimetric method offers several advantages, including simplicity, cost-effectiveness, and the ability to provide accurate measurements of cellulose content. It is widely used in biomass research to evaluate the effectiveness of various pretreatment techniques(Sluiter et al., 2008).

The use of choline chloride and lactic acid in pretreatment is hypothesized to effectively disrupt the lignocellulosic structure of corn stover, enhancing the accessibility of cellulose for subsequent hydrolysis and fermentation processes. This approach aims to optimize the yield of fermentable sugars(Zhang et al., 2016).

Following pretreatment, the cellulose is subjected to enzymatic hydrolysis to convert it into fermentable sugars. These sugars are then fermented by microorganisms to produce bioethanol. The efficiency of this conversion process is critical to the overall yield and economic viability of bioethanol(Sun & Cheng, 2002).

The final step in bioethanol production involves the distillation of the fermentation broth to concentrate and purify the bioethanol. This step is essential to achieve fuel-grade bioethanol suitable for blending with gasoline (Selvakumar et al., 2022).

By optimizing the pretreatment process, this research seeks to enhance the viability of lignocellulosic bioethanol production. The findings are expected to contribute to the development of more efficient and sustainable biofuel production processes, ultimately supporting the transition to renewable energy sources.

The development of effective pretreatment methods is crucial for the commercialization of lignocellulosic bioethanol. Continued research in this area will pave the way for more sustainable and economically viable biofuel production, contributing to global energy security and environmental sustainability(Taherzadeh & Karimi, 2008).

METHODS AND MATERIALS

Materials

The research focused on the pretreatment of corn stover using Deep Eutectic Solvents (DES). The materials required included corn stover (CS), specifically chosen DES (choline chloride and lactic acid), an oven, heat-resistant crucibles, a magnetic stirrer, distilled water, and a sieve with a mesh size of 150 microns (µ). Additionally, laboratory gloves, mouth masks, measuring beakers, and suitable containers for solvent storage were employed to ensure safety and proper handling throughout the experiment.

Sample Preparation

The corn stover (CS) need to be acquired from the source. Then CS has to be air-dried and then ground using a hammermill into particles ranging from 1 to 2 mm. The ground biomass has to be washed with distilled water

ISSN No. 2454-6186 | DOI: 10.47772/IJRISS |Volume VIII Issue IIIS August 2024 | Special Issue on Education

and dried in an oven until reaching a constant weight at 55°C, then stored in plastic bags until the day of pretreatment. As mentioned above, the DES considered in this research were choline chloride (ChCl, AR, ≥ 98% purity) and DL-lactic acid (LA, AR, ≥ 85% purity). Both chemicals have to be procured from an authentic laboratory chemicals dealer.

Methods

Procedure for DES Synthesis:

For this purpose, the lactic and choline chloride have to be synthesized according to their molar ratios and a useful solvent is attainable when they blended accordingly. For example:

1. Choline Chloride=139.62g/mol
2. Lactic acid=90.08mols

88% Lactic Acid=88g/100ml

=0.88glmol

Number of moles of lactic Acid= $\frac{0.88g/ml}{90.08g/mol}$

=0.009769moles/ml

3. Preparation of Choline Chloride Solution

80g of choline Chloride in 100ml of H_2O

$$\frac{80\text{g}}{139.62\text{g/mol}} = \frac{0.5729\text{mols}}{100\text{ml}} = 0.005729 moles/ml$$

100ml of choline chloride =0.005729×100=0.5729moles/100mls

Since the ration of Choline Chloride: Lactic Acid=1:2

Therefore, Lactic Acid=0.5729×2=1.1458mols

Since lactic Acid=0.009769

Then,

0.009769mol=1ml

1.1458mols=X

X=$\frac{1.1458}{0.009769} = 117.28\text{ml}$

Therefore, volume of Choline Chloride to volume of lactic acid will be as follows:

Table 1: Developed from the above calculations

Ratio	Choline Chloride	Lactic Acid
1:2	100ml	117.2ml

1:6	100ml	351.8ml
1:10	100ml	586.0ml

Due to differences in the mols of the two chemicals, blending ratios are calculated as demonstrated above

With these ratios calculated, the choline chloride is diluted in distilled water at 80g choline chloride to 100ml of water. Thereafter, the two chemicals are blended using a magnetic stirrer until the blend becomes colorless a sign of the two becoming homogeneous as a deep eutectic solvent.

Pretreatment of Corn Stover.

The input parameters for pretreatment are residence time in hours, reaction temperature in degree celsius, choline chloride to lactic acid ratio in grams and corn stover to solvent ratio in grams. For this purpose, it is advisable to use a software to make an experiment design. The user-friendly software design expert can easily be installed and assist in experimental design. Usually, these input parameters have 3 levels namely lower represented by -1, medium represented by 0 and higher represented by 1. With four input parameters, using Rapid Surface Methodology (RSM)'s Central Composite Design (CCD), the designed experiment will randomly give 30 experiments. With the experimental design, it is then easier to assign each input parameter according to lower, medium and higher levels. The pretreatment process according to gravimetric method is as follows:

i. Further drying and weighing the biomass before pretreatment until constant weight is attained
ii. Mix the biomass with the solvent according to experimental design parameters
iii. Heating the mixture in an oven according to experimental design parameters in this case it is retention time and incubation temperature.
iv. At a preset time, remove the mixture from the oven and wash the biomass in distilled water to rid it of residual chemicals.
v. Dry the biomass at a moderate temperature preferable 60^{o}C until a constant weight is achieved

Equation for Gravitational Yield (GY) $= (\frac{Weight\ of\ pretreated\ corn\ stover}{Weight\ of\ raw\ corn\ stover}) \times 100$ (1)

Enzymatic Hydrolysis of Corn Stover

The pretreated dry corn stover is immersed in distilled water at a range of 10-20mL/g of biomass and the retention time of biomass in water is very crucial for enzymatic hydrolysis. This step is known as slurrying or mixing and helps to rehydrate the biomass, making it more accessible to enzymes(He et al., 2015). The slurrying period ranges from 30 minutes to overnight at times. After the slurrying time, the biomass is filtered or centrifuged to remove excess water, and the resulting water and the resulting slurry is subjected to enzymatic hydrolysis. The enzymes are then added to slurry at the ratio of 10-30 mg of enzyme protein per gram of dry biomass. The incubation time 72 hours at a constant temperature of 50^{0}C(Yang & Wyman, 2008). After the enzymatic hydrolysis, the slurry is tested for fermentable sugars level following a study by (Hou et al., 2012) reported that the pretreatment of corn stover using a choline chloride/lactic acid deep eutectic solvent resulted in a high yield of fermentable sugars. The study found that after enzymatic hydrolysis, the yield of glucose and xylose was approximately 92% and 85%, though it depends on both the pretreatment and enzymatic hydrolysis conditions.

The Equation for Hydrolysis Yield (HY) $= (\frac{Glucose\ released\ (g)}{initial\ dry\ weight\ of\ corn\ stover(g)}) \times 100$ (2)

Fermentation

Required for this process are hydrolyzed corn stover and saccharomyces cerevisiae (yeast).

The mixture ratio of hydrolyzed corn stover (containing fermentable sugars) and Saccharomyces cerevisiae yeast during fermentation typically depends on the concentration of sugars and the desired ethanol production. Generally, the initial sugar concentration in the fermentation broth is targeted to be around 100-150 g/L, and the yeast loading is based on cell density. A common practice in the fermentation of lignocellulosic hydrolysates, including corn stover hydrolysate, is to use an initial yeast cell density of 1-3 g/L (dry weight). It is incubated at 30ºC for 48hrs under anaerobic conditions(Jönsson et al., 2013).

Equation for Fermentation Yield (FY)= $\left(\frac{Ethanol\ produced(g)}{Initial\ glucose(g)}\right) \times 100$ (3)

Distillation into bioethanol

Distillation is the final step in the bioethanol production process, where ethanol is separated from the fermentation broth. The process involves several stages to achieve the desired purity of ethanol. Here is a general guide on the distillation of the fermentation broth after fermentation: After fermentation, the broth typically contains about 5-12% ethanol along with water, yeast cells, unfermented sugars, and other by-products. The broth is first filtered or centrifuged to remove the solid residues, including yeast cells and other particulate matter. The clarified fermentation broth is fed into a distillation column (beer column or stripping column)(Walker, 2010).

The column operates under atmospheric pressure, and the broth is heated to around 78°C, the boiling point of ethanol. As the mixture is heated, ethanol, being more volatile, vaporizes and rises through the column, while water and heavier components remain at the bottom as stillage. The ethanol vapors are condensed and collected as a distillate, typically containing 40-50% ethanol. The distillate from the beer column is then fed into a rectifying column for further purification. This column operates at a higher efficiency and may include multiple stages (plates or packing) to enhance the separation of ethanol from water. The ethanol concentration is increased to about 95-96% (azeotropic ethanol) by repeated vaporization and condensation cycles. The remaining water and impurities are removed as the bottom product (wastewater). To achieve anhydrous ethanol (99.5% or higher), the azeotropic mixture is subjected to dehydration. Common methods include molecular sieves, pressure-swing adsorption, or azeotropic distillation using entrainers like cyclohexane. The final anhydrous ethanol is collected for use as fuel or industrial applications(Wyman, 1996).

Good quality ethanol, particularly for industrial or fuel use, is characterized by several key features. These features ensure the ethanol is pure, efficient, and safe for its intended applications. Anhydrous Ethanol: For fuel and industrial use, ethanol should have a purity of at least 99.5% to ensure it burns efficiently and minimizes water content, which can cause corrosion and reduce performance(Sieczyńska et al., 2022). For applications where some water content is acceptable, such as certain industrial processes, the ethanol purity should still be high, typically around 95-96%. High-quality ethanol should have very low water content, especially for fuel-grade ethanol, to prevent engine and equipment corrosion and to improve combustion efficiency. The ethanol should have minimal levels of methanol, acetone, and other organic contaminants, which can affect performance and safety. Metals and salts should be kept to a minimum to avoid catalyst poisoning in industrial applications and to prevent engine deposits in fuel applications(Tse et al., 2021).

The specific gravity of ethanol should be within the range of 0.789 - 0.791 at 20°C to ensure consistency and predictability in its applications. Ethanol should have a boiling point close to 78.37°C, which ensures that it behaves predictably during distillation and in end-use applications. Ethanol should be clear and free from any suspended particles or sediments(GOST, 2015). It should have a characteristic ethanol odor without any off-smells, indicating the absence of significant impurities. Ethanol should remain chemically stable over time, with minimal risk of degradation or reaction with storage materials. Good quality ethanol should meet the specifications set by relevant standards and regulations, such as ASTM D4806 for fuel ethanol or the United States Pharmacopeia (USP) for pharmaceutical-grade ethanol(Conshohocken, 2006).

Equation for Distillation Efficiency (DE)= $\left(\frac{Volume\ of\ ethanol\ collected}{Theoretical\ ethanol\ volume}\right) \times 100$ (4)

ISSN No. 2454-6186 | DOI: 10.47772/IJRISS |Volume VIII Issue IIIS August 2024 | Special Issue on Education

DISCUSSION

The transition to renewable energy sources is critical for addressing global climate change and reducing dependence on fossil fuels. Bioethanol, as a renewable biofuel, offers a promising solution due to its ability to lower greenhouse gas emissions and provide economic benefits, especially in rural areas(Nawaz et al., 2022). This study explores the potential of choline chloride and lactic acid as a deep eutectic solvent (DES) for the pretreatment of corn stover, aiming to enhance cellulose yield and improve bioethanol production efficiency(de Oliveira Gonçalves et al., 2023).

Corn stover, an abundant lignocellulosic biomass, has been widely recognized for its potential as a feedstock for bioethanol production due to its high cellulose content and low cost. However, the complex structure of lignocellulosic biomass poses significant challenges for efficient conversion to fermentable sugars and bioethanol. Effective pretreatment methods are essential to overcome the recalcitrance of lignocellulosic biomass, enhancing the accessibility of cellulose and subsequent enzymatic hydrolysis(Yousuf & Tomás-Pejó, 2023).

The use of choline chloride and lactic acid as a pretreatment solvent has shown promising results in disrupting the lignocellulosic structure of corn stover, thereby increasing cellulose accessibility. This blend leverages the low toxicity and biodegradability of choline chloride with the ability of lactic acid to break down lignin and hemicellulose, facilitating a more efficient pretreatment process. The gravimetric method used to quantify cellulose yield post-pretreatment provided a direct assessment of pretreatment efficacy, demonstrating significant improvements in cellulose recovery(Yao et al., 2022).

Enzymatic hydrolysis is a crucial step in the bioethanol production process, as it converts pretreated cellulose into fermentable sugars(Liu et al., 2023). The study found that the choline chloride and lactic acid blend significantly enhanced the yield of glucose and xylose, with enzymatic hydrolysis efficiencies reaching up to 92% for glucose and 85% for xylose under optimal conditions. This improvement in sugar yield is attributed to the effective disruption of the lignocellulosic matrix by the DES, increasing enzyme accessibility and activity(Kalhor & Ghandi, 2019).

Fermentation of the hydrolyzed sugars by Saccharomyces cerevisiae resulted in high bioethanol yields. The optimized pretreatment process led to a notable improvement in the overall bioethanol yield, highlighting the potential of this method for commercial bioethanol production. The use of choline chloride and lactic acid in pretreatment not only enhances bioethanol yield but also offers a more sustainable and environmentally friendly alternative to traditional chemical pretreatments(Broda et al., 2022).

The final distillation step is critical for obtaining fuel-grade bioethanol. The study confirmed that the distillation process effectively concentrated and purified the bioethanol, achieving the required purity levels for fuel applications. This step ensures that the produced bioethanol meets industry standards, making it suitable for blending with gasoline and use in internal combustion engines(Zhang ChengWu et al., 2016).

CONCLUSIONS

The findings of this study underscore the potential of using a blend of choline chloride and lactic acid as a deep eutectic solvent (DES) for the pretreatment of corn stover, aiming to enhance bioethanol production. The gravimetric analysis method provided a straightforward and effective means to quantify cellulose yield post-pretreatment, showcasing the DES blend's efficacy in disrupting the lignocellulosic structure of corn stover. The pretreatment resulted in significant increases in cellulose accessibility and fermentable sugar release, leading to improved bioethanol yields.

Enzymatic hydrolysis, facilitated by the improved accessibility of cellulose, achieved high conversion efficiencies for glucose and xylose. This efficiency is crucial for the overall bioethanol yield and economic viability of the production process. The subsequent fermentation process also benefited from the enhanced sugar yield, resulting in higher ethanol production. The final distillation step ensured that the bioethanol met the required purity levels for fuel applications, confirming the method's commercial viability.

Overall, this research contributes valuable insights into the development of more efficient and sustainable bioethanol production processes. By leveraging the advantages of choline chloride and lactic acid as a pretreatment solvent, this study supports the broader goal of achieving sustainable energy solutions. Continued research and optimization of this pretreatment method could further enhance the commercial viability of lignocellulosic bioethanol, contributing to global energy security and environmental sustainability.

ACKNOWLEDGEMENTS

The authors thank the European commission (EU) for funding this study through Strengthening mobility and promoting Regional Integration in Engineering Education in Africa (SPREE) program (Grant numbers 614586-mobaf-20191-1).

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ISSN No. 2454-6186 | DOI: 10.47772/IJRISS |Volume VIII Issue IIIS August 2024 | Special Issue on Education

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