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From Trash to Treasure: The Prospect of Producing Bioethanol from Wastepaper through Pretreatment with Sulphuric Acid

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Abstract

Bioethanol is a prospective alternative to nonrenewable energy sources and this study aimed to produce bioethanol from waste paper using a pre-treatment technique. Freshly fermented palm wine was analyzed using standard microbiological techniques to identify the microorganisms used in the study. The pre-treatment process utilized sulphuric acid and sodium hydroxide at varying concentrations (5%, 10%, 25%, 40% and 50%). The substrates had glucose concentrations ranging from 0.2 to 0.9 ppm and the peak yield was recorded at 10% sulphuric acid pretreatment. Bioethanol was produced through fractional distillation and sugar fermentation with *Saccharomyces cerevisiae*. The viability of bioethanol production using waste paper has been demonstrated as a sustainable method of waste management and a potential solution to energy shortages, particularly in developing countries.

Keywords: Bioethanol, Pre-treatment, *Saccharomyces cerevisiae,* Sulphuric acid, Waste paper

Introduction

In recent years, the global focus on sustainable energy sources has intensified in response to the mounting challenges of climate change and the exhaustion of non-renewable resources (Ramos *et al*., 2022). Bioethanol production has emerged as a promising solution to mitigate these challenges by offering a renewable alternative to fossil fuels (Oyebanji and Kirikkaleli, 2022). Derived from organic matter such as biomass and agricultural residues, bioethanol presents multifaceted benefits including reduced greenhouse gas emissions, economic growth and enhanced energy security (Thiyagarajan *et al*., 2023; de Mello *et al*., 2023). The utilization of diverse feedstocks for bioethanol production, including cellulosic biomass, sugarcane and maize, underscores its flexibility and potential as a substitute for traditional fossil sources (Abdulsalam *et al*., 2022).

Despite the advancements in bioethanol production, challenges persist, particularly concerning feedstock availability and sustainability. Conventional feedstocks face constraints related to land use competition, water consumption and potential impacts on food security (Abdulsalam *et al*., 2022). Therefore, there is a critical need to explore alternative feedstocks that are abundant, cost-effective and environmentally sustainable. Wastepaper emerges as a promising yet underutilized substrate for bioethanol production, offering a dual solution to the pressing issues of waste management and renewable energy generation.

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Annually, millions of tons of wastepaper are discarded worldwide as part of municipal solid waste, constituting a significant environmental burden (Nanda and Berruti, 2021). Repurposing wastepaper as a feedstock for biofuel production alleviates this burden and reduces reliance on finite fossil fuels, thereby contributing to efforts aimed at mitigating climate change (Osman *et al*., 2024). Moreover, the utilization of wastepaper for bioethanol production encourages a circular economy to reduce landfill waste (Igbokwe *et al*., 2022).

Against this backdrop, this research investigates the feasibility of producing bioethanol from wastepaper through pretreatment with sulphuric acid (H_2SO_4). Sulphuric acid pretreatment has demonstrated effectiveness in breaking down the complex lignocellulosic structure of wastepaper, thereby releasing fermentable sugars suitable for bioethanol fermentation (Verma *et al*., 2022). The objectives of this study include evaluating the effectiveness of sulphuric acid pretreatment, assessing the potential of bioethanol derived from wastepaper as a sustainable energy source and exploring the economic and environmental implications of utilizing wastepaper for bioethanol production. By integrating empirical evidence with theoretical insights, this study contributes to the broader discourse on renewable energy and waste management, laying the groundwork for transformative solutions in both domains.

Materials and Methods

Study Area

Skyline University Nigeria, Kano State, positioned at coordinates 11°59′7″ N and 8°31′59″ E. The university is centrally located within Kano State of Nigeria.

Collection of Sample

Waste papers were sourced from various locations within Skyline University Nigeria classrooms, including used old notebooks/ papers, assignment/ practical report papers, cardboard, posters and snack wrappers. Fifty (50) g of the sample underwent a combination of alkali and acidic pre-treatment to optimize production using a similar protocol. To perform acid pre-treatment, 50 g of paper sample was treated with sulphuric acid at various concentrations of 5%, 10%, 25%, 40% and 50% (v/v) and allowed to stand for 6 hours while alkali pre-treatment was *via* sodium hydroxide respectively (Egbe *et al*., 2022). Analytical-grade chemicals and reagents were utilized in this study, including sulphuric acid (H_2SO_4), sodium hydroxide (NaOH), basal salt medium and potato dextrose agar (PDA).

Fungal Isolation

The study utilized *Saccharomyces cerevisiae*, which was isolated from fresh palm wine *via* the pour plate method on PDA (Nwinyi and Hassan, 2021).

Purification and Identification of the Fungal Isolate

To identify the fungi, a series of physiological tests and standard morphological were carried out, including examining their morphology, vegetative reproduction, surface characteristics and ascospore formation. Pure isolate was obtained through multiple sub-cultures and kept in a PDA slant for further study (Nwinyi and Hassan, 2021).

Wastepaper Hydrolysate

A 250 ml Erlenmeyer flask was filled with 50 g of pre-treated samples and was left to stand at room temperature for 5-7 days to facilitate additional processing. This will ensure the release of required constituents for biofuel production. The incubation period is crucial as it allows for the complete breakdown of the paper substrate and ensures the maximum yield of fermentable sugars (Robak and Balcerek, 2020).

Assessment of Reducing Sugar Concentration (DNS)

A 0.5 ml sample combined with 2.5 ml of distilled water and 3 ml of DNS was used to evaluate the pre-treatment process's efficacy on the substrate. The solution was heated in a water bath for 5 minutes and allowed to cool before the addition of 1 ml of 40% Rochelle's salt. The spectrophotometer was used to determine the absorbance of the resulting solution at 540 nm.

Production of Bioethanol

Saccharomyces cerevisiae was used in this study to produce bioethanol by converting sugar into ethanol through fractional distillation at 78 °C. Using an inoculating loop, the fermentation process began by inoculating slant-stored yeast cells into a hydrolyzed sugar-filled Erlenmeyer flask. The flask and substrate mixture were then positioned in a shaker and rotated at 800 rpm for an hour daily over three days. The mixture was filtered, the resulting filtrate was used for distillation by heating to 78 °C of ethanol boiling point and the residue was discarded (Jacobus *et al*., 2021).

Results and Discussion

Measurement of Concentration of Reducing Sugars

The capability of the pretreatment process to degrade the lignocellulosic matrix of the substrate affected the quantity of sugar released. Enzymes produced by microorganisms were then able to efficiently degrade cellulose, resulting in the production of glucose.

The concentration of released sugar (measured in ppm) and absorbance (measured at 540 nm) was observed to vary across the different acid and alkali concentrations used for pre-treating the substrate. The sugar yield ranged from 0.2 to 0.9 ppm, with the highest yield obtained at 10% H_2SO_4 pre-treatment. The concentration was deemed moderate to prevent the creation of substances that could hinder the effectiveness of enzymes. The results indicated that acid pre-treatment was more effective in breaking down the lignocellulosic structure of the paper than alkali pre-treatment. In contrast, the use of alkali pre-treatment resulted in decreased levels of sugar concentration since it lacked the required strength to fully decompose the structure, causing the cellulose to become less receptive to enzyme action.

Alcohol Yield

The ethanol yield was determined after the distillation process and the results showed that the pre-treatment using 10% acid concentration produced the highest percentage of ethanol.

Filamentous fungi play an important role in the breakdown of cellulose chains by secreting cellulase, which includes carboxymethyl-cellulase. This process releases glucose and cellooligosaccharides of various lengths (Pang *et al*., 2021). Pre-treatment is essential for improving productivity by converting cellulose and hemicellulose into more easily processable forms, which necessitates the deletion of lignin and the disruption of the cellulose's crystalline structure, as illustrated in figures 1 and 2. Various substrates with distinct characteristics were obtained using different sodium hydroxide and sulfuric acid concentrations in pre-treatment, as demonstrated in table 1. The choice of pre-treatment method and the concentration used can significantly affect the amount of sugar released from the substrate (Jojima *et al*., 2021). The pre-treatment method that resulted in the highest sugar yield (0.9 ppm) was the use of a mild concentration (10%) of sulfuric acid, which prevented the formation of inhibitory compounds that could hinder enzyme activity (Park *et al*., 2020).

Table 1: The yield of reducing sugar about acid and alkali concentration

Concentrations (ppm)	Absorbance (540 nm)	Concentrations of acid and alkaline
0.0	0.0	Vacant
0.2	0.17	5% H_2SO_4
0.9	0.44	10% H_2SO_4
0.2	0.35	25% H_2SO_4
0.3	0.29	40% H_2SO_4
0.3	0.20	50% H_2SO_4
0.2	0.15	5% NaOH
0.2	0.16	10% NaOH
0.2	0.14	25% NaOH
0.2	0.12	40% NaOH
0.2	0.11	50% NaOH

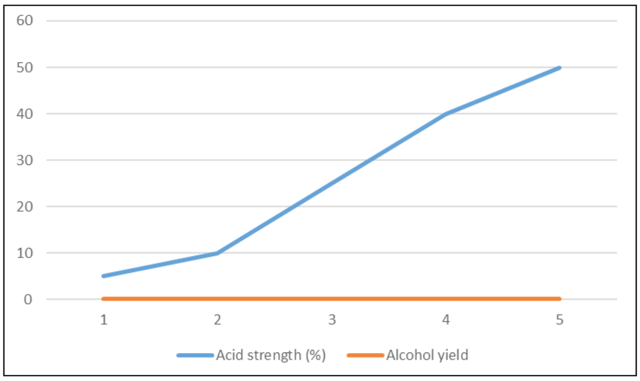

Figure 1: The data presented show the alcohol yield resulting from acid pre-treatment of glucose, using sulphuric acid at different concentrations of 5%, 10%, 25%, 40% and 50%. The glucose yield was also recorded.

However, the use of different pre-treatment methods and concentrations can also affect the efficiency of cellulose breakdown. The study discovered that acid pretreatment

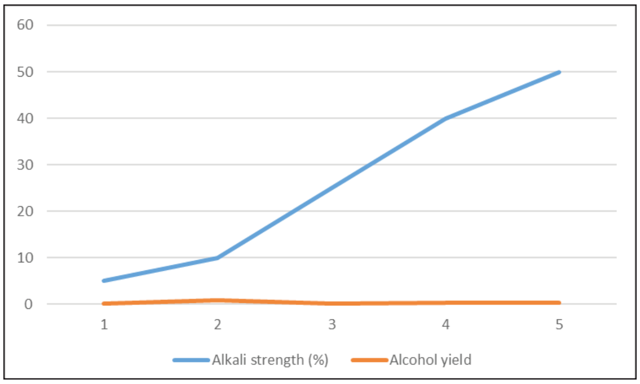

Figure 2: The data presented show the alcohol yield resulting from alkali pre-treatment of glucose, using sodium hydroxide at different concentrations of 5%, 10%, 25%, 40% and 50%. The glucose yield was also recorded.

was more effective than alkali pretreatment at breaking down the paper's lignocellulosic structure. Alkaline pre-treatment led to low sugar concentrations because it was unable to completely degrade the cellulose structure and reduced its receptivity to enzyme activity (Park *et al*., 2020). This information can be useful for optimizing the pre-treatment process in biofuel production from lignocellulosic materials (Saini *et al*., 2022).

Conclusion

The efficient production of bioethanol from paper substrate requires pre-treatment to break down its lignocellulosic structure. The use of 10% sulphuric acid is effective in releasing glucose which glucose is broken down by yeast through either the Embden-Meyerhof-Parnasor glycolytic pathway producing pyruvate to produce bioethanol. Pyruvate decarboxylase then facilitates decarboxylation, leading to the formation of aldehyde and CO_2. Acetaldehyde, an electron acceptor during fermentation with *Saccharomyces cerevisiae*, oxidizes the NADH and generates ethanol, as evidenced by bubble release. Diluted acid is widely used for the effective release of glucose, while alkali is less efficient but can prevent the formation of inhibitory products. The degradation of the paper substrate during hydrolysis releases soluble oligomers and monomeric sugars that can be utilized by fungi for the production of bioethanol. However, this study has demonstrated that biofuel production can be achieved with minimal capital investment, offering a cost-effective and environmentally friendly solution to waste paper disposal. By converting waste paper into bioethanol, this method not only addresses the issue of waste accumulation but also provides a practical way to produce energy. Ultimately, this approach has the potential to mitigate pollution caused by improper waste management practices while offering a sustainable alternative to fossil fuels.

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