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Conversion of Sugarcane Bagasse for production of Ethanol using Saccharomyces cerevisiae

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ABSTRACT

ORIGINAL RESEARCH ARTICLE

The purpose of this study is to use a fermentation procedure to manufacture bioethanol from sugarcane bagasse and to ascertain how temperature and pH affect the output of bioethanol. Utilizing enzymes like glucoamylase and alpha-amylase, the cellulose in sugarcane bagasse was broken down. In the experiment, yeast, specifically Saccharomyces cerevisiae, was also utilized for fermentation. To investigate the impacts of pH on ethanol yield at 37^0 C, five samples were prepared at different pH values. In addition, five samples were prepared while maintaining a constant pH of 4.5 to investigate the effects of temperature on ethanol output. After subjecting the samples to High-Performance Liquid Chromatography, the quantities of ethanol were ascertained (HPLC). The findings demonstrated that pH 4.5 and the maximum ethanol concentration were reached.

INTRODUCTION

Bioethanol, a type of renewable fuel, is gaining increasing attention as an alternative to traditional fossil fuels due to its potential to mitigate environmental impacts and reduce dependence on non-renewable resources. Produced through the fermentation of biomass, particularly sugars and starches derived from crops or organic waste, bioethanol offers a promising avenue for sustainable energy production (Misono and Yamaguchi, 1990).

The production of bioethanol involves several key steps, beginning with the selection and preparation of biomass feedstocks. Common feedstocks include sugarcane, corn, wheat, and cellulosic materials such as agricultural residues, forestry waste, and dedicated energy crops like switchgrass. These feedstocks are processed to extract fermentable sugars, which serve as the primary precursor for ethanol production. Once the biomass is obtained, it undergoes pretreatment to break down complex carbohydrates into simpler sugars, making them more accessible to fermentation microorganisms (McMeckin, *et al.*, 2002; This step may involve mechanical, chemical, or enzymatic treatments to optimize sugar release. Subsequently, the pretreated biomass is subjected to enzymatic hydrolysis, where enzymes break down polysaccharides into fermentable sugars such as glucose and xylose.

The next crucial stage is fermentation, where microorganisms such as yeast or bacteria metabolize the sugars present in the biomass feedstock to produce ethanol and carbon dioxide (Torija, et al., 2003). Yeasts are commonly used due to their high ethanol

tolerance and efficiency in converting sugars to ethanol under anaerobic conditions. Fermentation conditions such as temperature, pH, and nutrient availability are carefully controlled to maximize ethanol yield and minimize the formation of unwanted by-products (Lucero, *et al.*, 2000; Narendranath, *et al.*, 2001)

Following fermentation, the resulting ethanol mixture undergoes purification to remove impurities and water, typically through distillation, dehydration, and rectification. This purification step is essential for achieving the desired ethanol concentration suitable for blending with gasoline or other fuel applications (Narendranath and Power, 2005).

Bioethanol production offers several environmental and economic benefits. Firstly, it contributes to reducing greenhouse gas emissions compared to fossil fuels, as the carbon dioxide released during ethanol combustion is offset by the carbon dioxide absorbed during biomass growth. Additionally, bioethanol production can create opportunities for rural development, providing income for farmers and fostering regional economic growth (Pramanik K., 2003).

However, bioethanol production also poses certain challenges and considerations. Competition with food crops for land and resources raises concerns about food security and land use sustainability (Nigam, J. N., 1999; Togarepi, *et al.*, 2012). Furthermore, the energy balance of bioethanol production, including the energy inputs required for cultivation, processing, and transportation, must be carefully evaluated to ensure overall environmental sustainability (Yadav, et al., 1997).

In conclusion, bioethanol production represents a promising pathway towards sustainable energy production, offering the potential to reduce greenhouse gas emissions, promote rural development, and decrease reliance on finite fossil fuel resources. Continued research and technological advancements are essential to address challenges and optimize the efficiency and sustainability of bioethanol production processes.

MATERIAL & METHODS

The local market of Ganganagar, Meerut U.P. provided the sugarcane bagasse. After harvesting sugarcane bagasse, about 1 kg was removed and sun-dried for two weeks to extract all of the juice and remaining moisture. To make sure the bagasse was completely dry, it was further dried for two hours at 60 °C in an oven. The local milling machine was used to grind the dried bagasse. After grinding, 200g of powdered bagasse was found.

Enzymatic hydrolysis of sugarcane bagasse

10g of sugarcane **bagasse**was weighed. The weighed sample was placed into conical flask and 200ml of distilled water was added to the sample. 0.5 ml of NaOH was added to adjust the pH to 4.5. then 0.2 microliters of enzyme alpha- amylase diluted with phosphate buffer was added. The mixture was heated until 50^0C.The mixture was cooled down to 40^0C. Then, 0.2 microliters of secondary enzyme, glucoamylase was added. The mixture was maintained at 50^0C as the glucoamylase hydrolyzed the dextrin to fermentable glucose. The mixture was cooled down to 32^0C and 10 ml of *Saccharomyces cerevisiae*was added to the sample before transferred to conical flask.

Fermentation of sugarcane

Saccharomyces cerevisiae fermented the simple sugar to ethanol and carbon dioxide. To determine the effects of pH on ethanol yield, the temperature was kept constant at 37^0C while the pH was varied from 3, 3.5, 4, 4.5, and 5. To determine the effect of temperature on ethanol yield, the pH was kept constant at 4.5. The fermentation process continued for 48 hours (Yah, et al., 2010).

Distillation of ethanol

After 48 hours, the sample was filtered using Whatman Filter Paper to separate the ethanol from the residue. The bioethanol was distilled. The sample was heated at 80^0C to get the bioethanol.

Determine bioethanol yield

Bioethanol produced was analyzed by high-performance liquid

chromatography(HPLC). The HPLC analysis parameters were determined using the following conditions: column, C18 RP (53 x 7mm); injector temperature was 30°C, 20 µL of the sample was injected into the HPLC system. The mobile phase was phosphoric acid and the flow rate was 1.5mL/min; and detection was set at a wavelength of 210 nm (Phisalaphong, *et al.*, 2006).

RESULTS & DISCUSSION

Calibration curve

The standard was prepared at different concentrations to such as 25%, 50%, 75%, and 100%. The calibration equation of the ethanol standard was determined to be y = 367.94x-2853 (R^2 = 0.9515)

Table 1: Standard calculation

Concentration (%)	Volume of ethanol Standard (ml)	Volume of mobile phase (ml)	Peak Area
25	0.5	1.5	5241
50	1.0	1.0	15233
75	1.5	0.5	28750
100	2.0	0	31352

Effects of pH on ethanol concentration

The sample was fermented at different pH values from 3, 3.5, 4, 4.5, and 5 while the temperature was kept constant at 37^{0}C to obtain the maximum yield of bioethanol Table 2.

Table 2: The Effects of pH on Ethanol Concentration (%) in Water

pH	Ethanol concentration in water (%)
3.0	10.2
3.5	11.1
4.0	11.3
4.5	13.4
5.0	8.2

Based on the results obtained, pH 4.5 showed the highest ethanol content. The lowest ethanol concentration was achieved at pH 5.0. The maximum ethanol concentration in water at pH 4.5 reflects enzyme function in an environment [1] while the lower ethanol concentration in water at pH reflects lesser yeast activity.

Effects of temperature on ethanol concentration

Temperature is one of the major factors that determine ethanol production. Table 3 shows the ethanol concentration obtained at different temperatures. Based on the result obtained, no ethanol concentration in water was observed at 25 and 30^{0}C.

Table 3: Effects of temperature on ethanol concentration in water (%)

Temperature (^{0}C)	Ethanol concentration in water with water (%)
25	3.4
30	3.8
35	12.7
40	11.3
45	11.1

However, as the temperature increases beyond 30^0C it shows an increase in the production of ethanol. At 35^0C ethanol concentration in water was maximum and turned out to be 13.7% followed by 40^0C where 12.3% ethanol was obtained.

CONCLUSION

This study shows that pH 4.5 showed the highest ethanol content which is 14.8 %, followed by pH 4.0 which is 11.9 %, then pH 3.5 at 11.6 %, and pH 3.0 at 10.7 %. The lowest ethanol concentration was achieved at pH 5.0. The study also shows that at 35^0C ethanol concentration in water was maximum and turned out to be 13.7% followed by 40^0C where 12.3% ethanol. The Conclusion is that pH 4.5 and 35^0C are the optimum conditions for ethanol production.

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