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Some Challenges in Bioethanol Production from Bagasse: Insights from a Preliminary Test Run

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ABSTRACT
This study presents bioethanol production process from bagasse using Simultaneous Saccharification and Fermentation (SSF), utilizing alkaline pretreatment methods and enzymatic hydrolysis techniques, showcasing a practical and cost-effective approach suitable for regions with limited laboratory facilities. To confirm ethanol production, Nuclear Magnetic Resonance (NMR), Fourier Transform Infrared Spectroscopy (FTIR), and Gas Chromatography (GC) analyses were employed to determine the feasibility of the process and specific areas requiring optimization. Proton nuclear magnetic resonance (^{1}H-NMR) analysis confirmed signals for methyl (-CH_3) protons and a broad peak for methylene (-CH_2) protons near a hydroxyl group from the fermented broth, providing insight into the bioethanol produced. Additionally, carbon-13 nuclear magnetic resonance (^{13}C-NMR) analysis corroborated these findings by detecting carbon signals corresponding to methyl and methylene carbons, further validating the bioethanol structure. Complementary FTIR spectroscopy identified characteristic peaks for hydroxyl (O-H stretching) and C-H bending vibrations, which are typical of alcohols, further validating the presence of ethanol. GC analysis revealed an ethanol concentration of 89.2 mg/L, achieved through saccharification at 35˚C for 90 h and fermentation at 37˚C for 96 h, and the setup maintained optimal conditions within 35-37˚C for microbial activity using standard laboratory incubation equipment. Notably, the analyses were performed on the fermented broth without prior distillation, providing direct insights into ethanol presence and concentration. While the ethanol yield was relatively low due to high water content, the methodology is promising for effective bioethanol production. Overall, this study demonstrates the potential of converting bagasse into bioethanol, contributing to renewable energy development and agricultural waste utilization, and highlights areas for refinement to improve yields in future research.

Keywords: Bioethanol, Bagasse Utilization, NMR, FTIR, GC

1. Introduction

Bangladesh, a country with a rich agricultural heritage, faces the dual challenges of rapid population growth and urbanization. With a population of around 170 million, it stands as the eighth most populous nation globally, where agriculture remains essential for the livelihoods of many, especially in rural communities. However, the increasing population is putting immense pressure on food supply and energy consumption, which are rising sharply. Historically, Bangladesh has relied heavily on conventional energy sources, include coal, natural gas, and crude oil to support its development. This dependence on nonrenewable energy has raised significant sustainability concerns and highlighted the risk of resource depletion. To address environmental issues and the diminishing supply of these conventional sources, Bangladesh must shift its focus to renewable energy solutions [1].

Sources of renewable energy like solar, wind, biomass, and hydro energy provide a sustainable alternative, especially for countries like Bangladesh. These abundant and environmentally friendly resources can enhance energy security in rural areas with limited access to the national grid [2]. The energy transition not only benefits the environment but also empowers rural communities, boosting productivity and quality of life. Agriculture is a key sector in Bangladesh, employing about 40% of the workforce [3].

Sugarcane is one of the most significant crops in Bangladesh. Bangladesh Sugarcane Research Institute reporting an average production of 3.92 million metric tons (MMT) in the 2018-19 seasons. Of this, 1.18 MMT (30.11%) was used by sugar mills, 2.11 MMT (53.83%) for goor (jaggery) production, and 0.63 MMT (16.06%) for seeds and chewing. When the sugarcane stalks are crushed for juice extraction, around 30-35% of their weight typically converts into bagasse, resulting in an output of approximately 0.987-1.15 MMT annually [4]. Bagasse, a byproduct of sugarcane processing, was chosen because of its abundance and untapped potential in Bangladesh. Despite being largely discarded as agricultural waste, bagasse holds significant untapped potential. It is valuable for energy generation, paper production, and biomass feedstock. Its high availability and suitability for bioethanol production makes it an excellent resource for renewable energy generation. Furthermore, bagasse supports Bangladesh's energy transition goals by providing a sustainable alternative to fossil fuels, highlighting its need for further development [5].

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2. Biomass Feedstock

Bioethanol production largely depends on regional factors, climate, and the physical characteristics of the available feed stocks [6]. Biofuels are broadly classified into primary and secondary types, and secondary biofuels are further categorized into the first, second, and third generations [7]. Primary biofuels, such as firewood, wood chips, and animal dung, are raw, unprocessed, and directly obtained from nature. Secondary biofuels such as bioethanol, biodiesel, and biogas are processed through thermochemical processes such as pyrolysis, gasification, and hydrothermal liquefaction (HTL), or biochemical methods such as anaerobic digestion, fermentation, and transesterification [8].

This study focused on bioethanol production from sugarcane bagasse, which is a second-generation biofuel derived from lignocellulosic biomass. Bagasse is mainly composed of cellulose (35-40%), hemicellulose (25-30%), and lignin (20-25%) [9]. This substantial amount of cellulose and hemicellulose can be converted into fermentable sugars through advanced technologies such as enzymatic hydrolysis and fermentation [10]. Utilizing bagasse not only offers a pathway to produce renewable energy but also serves as a cleaner alternative to fossil fuels. This study focuses on three main steps: pretreatment, saccharification, and fermentation, to convert bagasse into bioethanol effectively.

3. Methodology

The experimental setup was designed based on insights from previous research. Established methods, adapted with specific measurements for bagasse, nutrients, and other variables, were employed. These methods widely validated in similar studies, are summarized in the theoretical workflow shown in Tab 1.

3.1 Theoretical Approach

Bioethanol production can be achieved through two primary methods: SSF and Separate Hydrolysis and Fermentation (SHF). In SSF, enzymatic hydrolysis and fermentation are carried out in a single reactor. According to F. Alfani (2000), using a single bioreactor in SSF lowers investment and operational costs. Data on glucose and ethanol production in SSF demonstrated that glucose conversion to ethanol approaches theoretical values [11]. A.Wingren (2003) compared SSF with SHF, concluding that the SHF process has a higher initial investment and reduced overall ethanol yield for softwood. Wingren's study indicated that SHF has a higher risk of contamination than SSF [12]. L. Zhang (2011) compared SSF with SHF and Partial SSF using sweet potato as raw material. The study concluded that SSF was more advantageous than SHF and Partial SSF, yielding higher ethanol production [13]. After considering all these factors, SSF is considered to be employed due to its advantages over SHF, including higher ethanol yields, cost-effectiveness, ease of implementation, and a lower risk of contamination [12].

In the SSF process, the pretreatment of lignocellulosic biomass is a critical step, particularly when using acid or alkaline methods. Lignocellulosic biomass consists of three primary components: cellulose, hemicellulose, and lignin [14]. Lignin serves as a protective barrier around cellulose and hemicellulose, making them less susceptible to enzymatic breakdown. Acid pretreatment degrades hemicellulose into simpler sugars, such as xylose and arabinose, while disrupting the lignin structure [15]. In contrast, alkaline pretreatment, specifically using sodium hydroxide (NaOH), primarily targets lignin removal, thus enhancing cellulose accessibility while preserving a significant portion of hemicellulose content [8].

Alkaline pretreatment (NaOH) was used in this experiment. Through this process, the pore size of the biomass is increased and the crystallinity of cellulose decreased, making the cellulose more accessible. By using an appropriate concentration of NaOH, it is possible to reduce or eliminate lignin, exposing cellulose and hemicellulose (including xylan) fibers, which facilitates more efficient enzymatic hydrolysis.

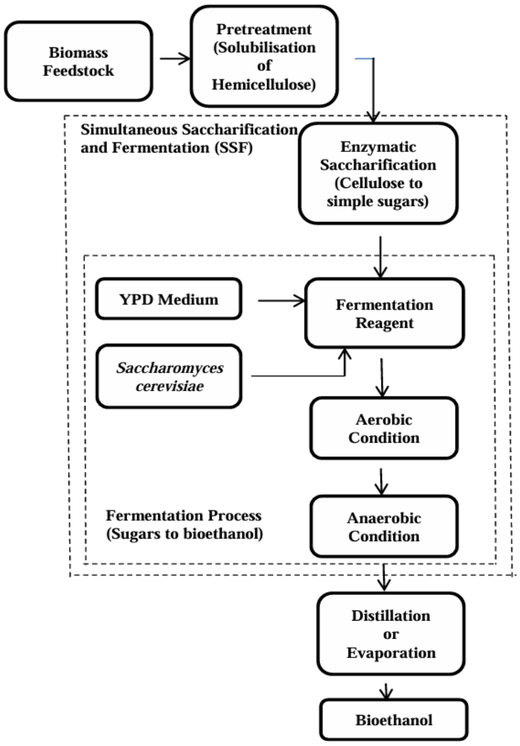


Fig. 1: Workflow of the SSF process

On the enzymatic side, cellulose enzymes are generally categorized into three main types based on their action [16]. Endoglucanases (EG or Endo-1, 4-β-D-glucanases) cleave internal bonds within the cellulose chain, breaking the long chains into shorter polysaccharides and creating new chain ends that allow for further enzymatic action. Exoglucanases (CBH or Exo-1, 4-β-D-glucanases) then act on these newly exposed chain ends, releasing cellobiose or glucose units. β-glucosidases (BGL or Cellobiase) complete the process by hydrolyzing cellobiose and small oligosaccharides generated by the action of endoglucanases and exoglucanases into glucose. This step is crucial for completing the hydrolysis of cellulose and producing fermentable sugars [15].

Optimal cellulase activity for most fungal-derived cellulases and β-glucosidases occurs between 45°C and 55°C, within a pH range of 4 to 5. In this study, neutral cellulase, which typically contain a mix of cellulases including endoglucanase, were used for enzymatic hydrolysis to break down cellulose into fermentable sugars. These sugars were then simultaneously fermented into bioethanol in the same reactor, optimizing the overall process efficiency [17].

During the fermentation process, yeast (or other microorganisms such as fungi or bacteria) converts fermentable sugars into bioethanol (ethyl alcohol) under anaerobic conditions. In bioethanol production from lignocellulosic materials involves mixed sugar fermentation, and several inhibitory compounds can affect fermentation efficiency. These compounds such as low molecular weight organic acids, furfural, hydroxymethylfurfural (HMF), and various inorganic substances are released during hydrolysis phases [18]. Several strategies can be implemented to enhance fermentation include: controlling reaction conditions, such as temperature, time, and pH, adding detoxification agents, and supplementing nutrients. These measures can help mitigate the detrimental effects of inhibitory compounds found in bagasse, thus supporting yeast growth and activity [19].

A variety of yeast type are used in the SSF process, including *Zymomonas mobilis*, *Trichoderma reesei*, *Kluveromyces spp.*, *kluyveromyces marxianus*, *Mucor indicus*, *Pichia stipitis*, *Schizosaccharomyces pombe*, *Rhizopus spp.*, *Liriodendron tulipifera*, *Saccharomyces pastorianus*, *Saccharomyces cerevisiae* (Baker's yeast), among others [20]. However, numerous studies have shown that *Saccharomyces cerevisiae* is the most prevalent yeast in fermentation processes, capable of producing nearly 90% of the theoretical bioethanol from glucose through fermentation [21].

3.2 Experimental Procedure

The experimental procedure was divided into several stages, including pretreatment, enzymatic saccharification, fermentation, and bioethanol yield analysis. All the experiments were conducted in a controlled laboratory environment with a specific set of conditions for each process. Table 1 presents the various experimental stages, including pH and retention time, while Fig. 2 shows the experimental setups.

3.2.1 Pretreatment of Biomass Feedstock

The raw material (bagasse) was collected and stored at room temperature. To prepare for further processing, bagasse was chopped and crushed to achieve a particle size smaller than 2 mm. A 2.5% (w/v) NaOH solution was prepared by mixing 15 g of NaOH with 600 mL of distilled water. Subsequently, 30 g of bagasse was added to the NaOH solution. This mixture was allowed to soak for 1 h and then sterilized at 120°C for 4 h to ensure decontamination and improve the chemical and physical properties of the bagasse. After sterilization, the bagasse mixture was washed multiple times with distilled water until the pH reached 6.6 to 7.5. The washed sample was then filtered and placed in an oven at 110°C for drying. Once fully dried, the samples were stored in airtight envelopes to maintain their condition for future analysis.

3.2.2 Enzymatic Saccharification

For enzymatic saccharification, a sodium citrate buffer solution was prepared by dissolving 3.71 g of sodium citrate in 250 mL of distilled water. This buffer solution was then combined with 10 g of the oven-dried sample. The next step involved adding the enzyme to this mixture to ensure an enzyme-to-bagasse ratio of 1:2. The mixture was placed in an orbital shaker at 35°C and maintained at a shaking speed of 150 rpm for 90 h. This setup aimed to optimize the conditions for enzymatic activity and maximize saccharification efficiency.

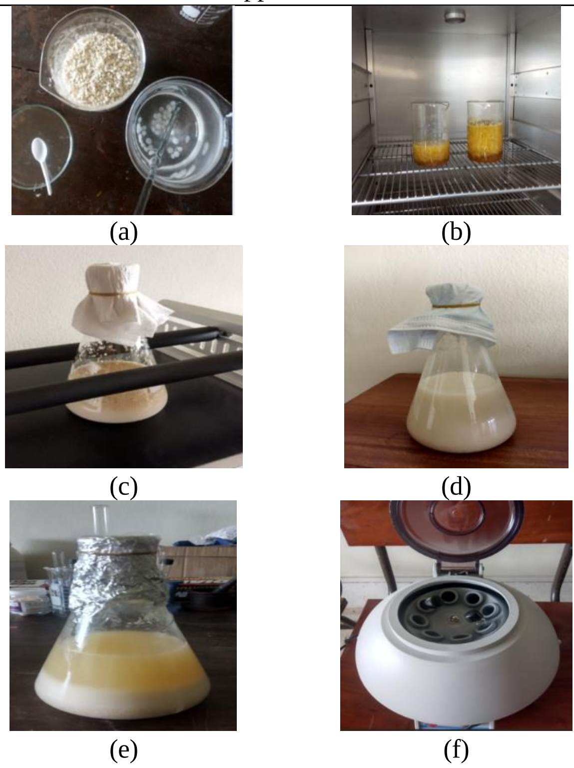


Fig. 2: Experimental procedure: (a) bagasse pretreatment, (b) sterilization, (c) enzymatic saccharification, (d) aerobic conditions, (e) anaerobic conditions, and (f) centrifugation

3.2.3 Fermentation

For the fermentation process, Yeast, Peptone, and Dextrose (YPD) media were prepared by mixing 2 g of glucose, 2 g of peptone, and 1 g of yeast extract in 100 mL of distilled water to culture *Saccharomyces cerevisiae*. This solution was incubated at 37°C for 24 h to allow for yeast growth. The fermentation medium was prepared by adding yeast, $CaCl_2.2H_2O$, $(NH_4)_2SO_4$, $MgSO_4.7H_2O$, and KH_2PO_4, along with 60 mL of distilled water, and thoroughly stirred. The saccharified slurry was subsequently added to the fermentation and YPD media. The mixture was kept under aerobic conditions for 24 h before being shifted to anaerobic conditions for 72 h at 35°C to complete the fermentation process.

3.2.4 Sample Collection

After completion of the fermentation process, the fermented broth was collected for centrifugation at 3000 RPM for 10 minutes. After centrifugation, clear supernatants were carefully collected and prepared for bioethanol estimation.

From Table 1 it is notable that each stage has specific pH, temperature, and retention time requirements to facilitate different biochemical processes. In this process, the retention time represents the cumulative duration for each stage as the materials progress through the entire procedure, ensuring optimal conditions for each phase. The pretreatment stage starts at near-neutral pH of 7.45 with high temperatures of 120˚C and 110˚C, requiring 13 h to break down complex structures effectively. Following that, the saccharification stage takes place with a lower pH of 5.63 at 35˚C, over a prolonged period of 90 h, which allows enzyme activity to convert complex sugars into simpler forms. The YPD medium stage is maintained at a pH of 6.81 and 35˚C for 24 h, providing nutrients to foster microbial growth. Moving to the fermentation stage, the temperature increases slightly to 37˚C, and the retention time extends to 96 h, enabling microorganisms to convert sugars into bioethanol. Finally, the final slurry reaches a pH of 4.92 at temperatures of 35˚C and 37˚C.

Table 1: Temperature and pH measurements at various stages of the fermentation

Stage	pH	Temperature (˚C)	Retention Time (h)
Pretreatment	7.45	120, 110	13
Saccharification	5.63	35	90
YPD Medium	6.81	35	24
Fermentation Medium	-	37	96
Final Slurry	4.92	35, 37	-

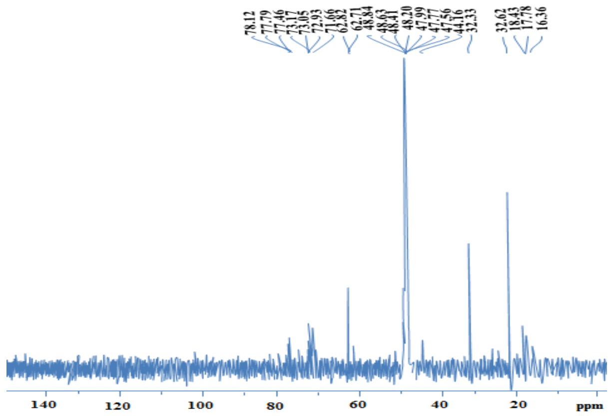
The temperature during all stages, except pretreatment, was maintained using standard laboratory incubation equipment, ensuring an aqueous medium and oxygen controlled environment necessary for *S. cerevisiae* to thrive. The observed drop in pH in the final slurry is likely due to acidic byproducts generated during fermentation. As the microorganisms ferment sugars into bioethanol, they also produce organic acids, contributing to the lowered pH [22].

4. Result Analysis

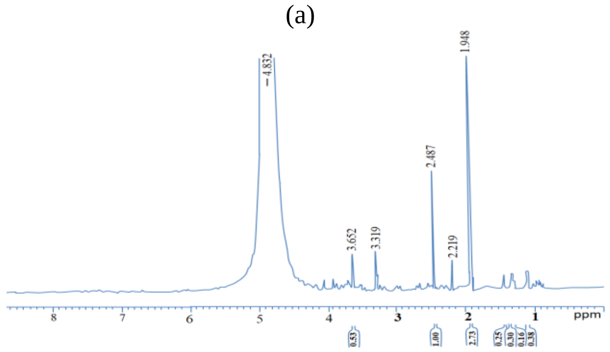
To confirm the presence of bioethanol in the fermented broth samples, FTIR and NMR techniques were performed. Each technique validated the presence of bioethanol through characteristic peaks in the spectra, providing a qualitative assessment before concentration levels were determined using GC analysis.

The Bruker Ascend 400 MHz NMR spectrometer was used for ^{1}H-NMR, featuring an UltraShield™ Plus 9.4 Tesla magnet and a 5mm BBFO (Broad Band Fluorine Observe) probe optimized for X-nuclei direct observation. After sample injection, data acquisition and analysis were performed using Bruker's dedicated NMR software. Fig. 3 shows ^{1}H-NMR and ^{13}C NMR spectra characterizing of the proton signals that align with the structure of bioethanol. (i) Sharp Peak at 2 ppm: The methyl group (-CH_3) protons adjacent to electronegative atoms (such as oxygen) in ethanol typically showed up around 2 ppm. The deshielding caused by oxygen or a carbonyl group (like in ethanol's -CH_2-CH_3 structure) leads to a shift in the proton signal, which is characterized ethanol. (ii) Broad Peak at 5 ppm: The broadening of the peak around 5 ppm indicates the presence of methylene (-CH_2) protons that are adjacent to the hydroxyl (-OH) group. This is a hallmark of alcohols, and the broadening is often caused by hydrogen bonding or exchange, which is especially prominent in alcohols like ethanol. The use of methanol-d4 (MeOD) as a solvent further supports this, as it enhances the behavior of exchangeable proton, confirming the presence of the hydroxyl group.

FTIR spectroscopy is essential for identifying chemical compounds and functional groups. The Shimadzu IRTracer-100 FTIR spectrophotometer was used, covering a spectral range of 4000 to 400 cm^{-1} with a resolution of 0.5 to 16 cm^{-1} with a KBr (potassium bromide) window. After injecting the sample into the cell, it was analyzed using software. Fig. 4 shows the FTIR spectrum, highlighting two significant regions, which characterized the functional groups associated with ethanol. The first peak was observed between 1500 and 1750 cm^{-1} with a transmittance (%T) range of 90 to 88, corresponds C=O and C-H bending vibrations. And second stronger peak appeared between 3000 and 3500 cm^{-1}, with %T ranging from 90 to 68, indicating O-H stretching, which characterized of alcohol presence. These observed peaks confirmed the existence of hydroxyl groups, consistent with the functional groups in ethanol, thereby supporting the identification of bioethanol in the collected broth.



(a)



(b)

Fig. 3: NMR spectra of the broth derived from bagasse (a) ^{13}C NMR and (b) ^{1}H-NMR

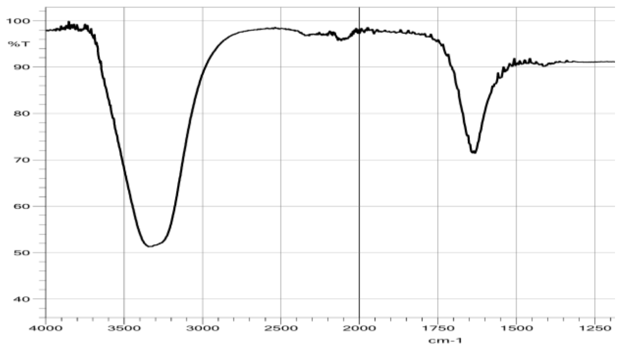
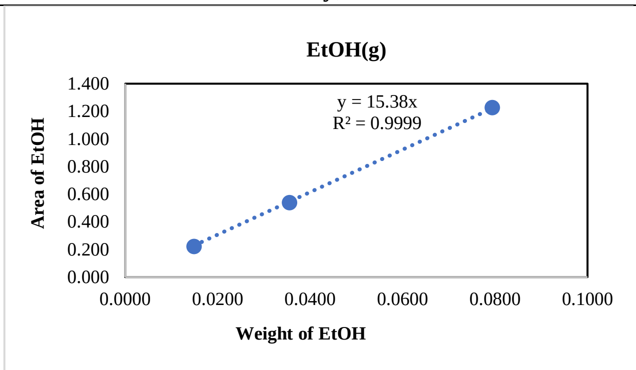
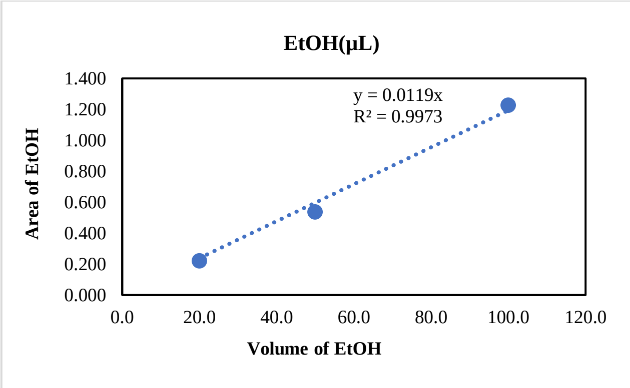


Fig. 4: FTIR Spectra of the broth derived from bagasse

To quantify the concentration of ethanol (EtOH) and methanol (MeOH) in the collected broth, calibration curves were constructed using standard solutions with known concentrations. Ethanol concentration in the collected broth was determined using both volume-based and weight-based calibration curves. The use of both methods provides a robust measurement by cross-validation of the results, which is shown in Fig. 5.



(a) Weight based calibration curve of EtOH



(b) Volume based calibration curve of EtOH

Fig. 5: Calibration curves for ethanol based on GC data

The DANI Master GC was used to conduct GC analysis. The sample injection volume was 3 μL, and the injector temperature was maintained at 220°C. Nitrogen was used as the carrier gas for the analysis. Calibration curve was constructed using three standard ethanol solutions with known volumes and corresponding GC peak areas. The calculated ethanol concentration from the curve revealed that the collected broth contained 0.0892 g of EtOH. This value was obtained by comparing the GC peak areas of the sample with those on the respective calibration curves. Ethanol is the primary product of this fermentation process, while methanol is also present at the sample and it was below quantification limit.

5. Discussion

The objective of this research work was to assess the challenges of bioethanol production from bagasse using the SSF process as a test run, assessing the overall process performance and estimating the ethanol yield. However, the results revealed a relatively low ethanol concentration of 89.2 mg/L without prior distillation, which is lower than the yield reported in similar studies on bagasse based bioethanol production. For instance, S. Johri (2016) obtained 9.15 g/L of ethanol using *Pachysolen Tannophilus* MTCC 1077 [23], and Huang (2015) reported a yield of 18.79 g/L by using *S. cerevisiae* ZM1-5 [24]. These higher yields underline the potential for improvement in this study, particularly by optimizing experimental conditions such as microorganism selection, bagasse pretreatment and nutrient supplementation.

The relatively lower yield in this work highlights the unique challenges associated with the used experimental conditions used. It also demonstrates the opportunity for further refinement to unlock the full potential of bagasse as a feedstock for bioethanol production. Some challenges include:

- The properties of bagasse are influenced by the sugarcane variety. The bagasse used in this study was collected from a local market, where sugarcane is primarily used for juice extraction. This may have impacted the yield.
- The yeast and enzyme employed in this study may not have been the optimal choices for maximizing yield.
- The process can be further optimized by fine-tuning parameters, such as pH, temperature, nutrients availability, and oxygen level, to create the most favorable environment for yeast growth and ethanol production.
- The presence of fermentation inhibitors such as furfural, acetic acid, and phenolic compounds offers a chance to reduce the fermentation efficiency and significantly affect microorganism activity.
- Incomplete hydrolysis of cellulose and hemicellulose, limited sugar release, and suboptimal yeast activity likely contributed to the lower yield indicated room for improvement.

Studies on lignocellulosic biomass frequently encounter challenges such as incomplete fermentation, lignin inhibition, and inefficient pretreatment methods [25]. Therefore, while the yield in this study was lower than expected, this is not unusual for initial experiments, especially with complex feedstock like bagasse.

6. Conclusion

The results, validated through NMR, FTIR, and GC analyses, demonstrated the successful conversion of bagasse into bioethanol, with a measured ethanol concentration of 89.2 mg/L. This yield achieved with saccharification at 35˚C for 90 h and fermentation at 37˚C for 96 h, was relatively low compared to yield reported in other study. For example, S. Johri (2016) used saccharification at 50˚C for 24 h and fermentation at 30-34˚C for 72 h, and Huang (2015) saccharification at 50˚C for 96 h and fermentation at 50˚C for 96 h.

These findings highlight the complexities involved in bioethanol production from bagasse under the conditions tested. Several factors, including previously mentioned limitations such as the high water content in the broth, absence of distillation, and suboptimal process parameters, likely contributed to the reduced ethanol concentration. Additionally, the relatively lower saccharification and fermentation temperature used in this study compared to others may have interrupted the efficiency of microbial activity and enzyme action, further reducing the yield. In the context of bioethanol production, small adjustments in these parameters could have a substantial effect on yield, making it a pivotal area for optimization in future studies. Such improvements would contribute significantly to renewable energy development and the sustainable utilization of lignocellulosic biomass.

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References

[1] Uddin, M. N., Rahman, M. A., Mofijur, M., Taweekun, J., Techato, K., Rasul, M. G., Renewable energy in Bangladesh: Status and prospects, *Energy Procedia*, vol. 160, pp. 655–661, 2019.

[2] McKendry, P., Energy production from biomass (part 1): Overview of biomass, *Bioresour. Technol.*, vol. 83, no. 1, pp. 37–46, 2002.

[3] Mondal, M. H., Crop agriculture of Bangladesh: challenges and opportunities., Bangladesh J. Agril. Res. 35(2): pp. 235-245," 2010.

[4] Reza, M. S., Riazi, M. H., Khan, M. M. H., Productivity and Profitability of Sugarcane Production in Northern Bangladesh, *Indian J. Commer. Manag. Stud.*, vol. 7, no. 1, pp. 38–46, 2016.

[5] Tabriz, S. S., Kader, M. A., Rokonuzzaman, M., Hossen, M. S., Awal, M. A., Prospects and challenges of conservation agriculture in Bangladesh for sustainable sugarcane cultivation, *Environ. Dev. Sustain.*, vol. 23, no. 11, pp. 15667–15694, 2021.

[6] Sebayang, A. H., Masjuki, H. H., Ong, H. C., Dharma, S., Silitonga, A. S., Mahlia, T. M. I., Aditiya, H. B., A perspective on bioethanol production from biomass as alternative fuel for spark ignition engine, *RSC Adv.*, vol. 6, no. 18, pp. 14964–14992, 2016.

[7] Jain, S., Kumar, S., A comprehensive review of bioethanol production from diverse feedstocks: Current advancements and economic perspectives, *Energy*, p. 131130, 2024.

[8] Limayem, A., Ricke, S. C., Lignocellulosic biomass for bioethanol production: current perspectives, potential issues and future prospects, *Prog. energy Combust. Sci.*, vol. 38, no. 4, pp. 449–467, 2012.

[9] Bezerra, T. L., Ragauskas, A. J., A review of sugarcane bagasse for second-generation bioethanol and biopower production, *Biofuels, Bioprod. Biorefining*, vol. 10, no. 5, pp. 634–647, 2016.

[10] Silva, G. M., Giordano, R. L. C., Cruz, A. J. G., Ramachandriya, K. D., Banat, I. M., Wilkins, M. R., Ethanol production from sugarcane bagasse using SSF process and thermotolerant yeast, *Trans. ASABE*, vol. 58, no. 2, pp. 193–200, 2015.

[11] Alfani, F., Gallifuoco, A., Saporosi, A., Spera, A., Cantarella, M., Comparison of SHF and SSF processes for the bioconversion of steam-exploded wheat straw, *J. Ind. Microbiol. Biotechnol.*, vol. 25, no. 4, pp. 184–192, 2000.

[12] Wingren, A., Galbe, M., Zacchi, G., Techno-economic evaluation of producing ethanol from softwood: Comparison of SSF and SHF and identification of bottlenecks, *Biotechnol. Prog.*, vol. 19, no. 4, pp. 1109–1117, 2003.

[13] Zhang, L., Zhao, H., Gan, M., Jin, Y., Gao, X., Chen, Q., Guan, J., Wang, Z., Application of simultaneous saccharification and fermentation (SSF) from viscosity reducing of raw sweet potato for bioethanol production at laboratory, pilot and industrial scales, *Bioresour. Technol.*, vol. 102, no. 6, pp. 4573–4579, 2011.

[14] Huang, J., Khan, M. T., Perecin, D., Coelho, S. T., Zhang, M., Sugarcane for bioethanol production: Potential of bagasse in Chinese perspective, *Renew. Sustain. Energy Rev.*, vol. 133, p. 110296, 2020.

[15] Dien, B. S., Cotta, M. A., Jeffries, T., Bacteria engineered for fuel ethanol production: current status, *Appl. Microbiol. Biotechnol.*, vol. 63, pp. 258–266, 2003.

[16] Tengborg, C., Galbe, M., Zacchi, G., Influence of enzyme loading and physical parameters on the enzymatic hydrolysis of steam-pretreated softwood, *Biotechnol. Prog.*, vol. 17, no. 1, pp. 110–117, 2001.

[17] Miskat, M. I., Ahmed, A., Chowdhury, H., Chowdhury, T., Chowdhury, P., Sait, S. M., Park, Y-K., Assessing the theoretical prospects of bioethanol production as a biofuel from agricultural residues in Bangladesh: a review. Sustainability, 12(20), *Sustainability*, vol. 12, no. 20, p. 8583, 2020.

[18] Hahn-Hägerdal, B., Galbe, M., Gorwa-Grauslund, M. F., Lidén, G., Zacchi, G., Bio-ethanol - the fuel of tomorrow from the residues of today, *Trends Biotechnol.*, vol. 24, no. 12, pp. 549–556, 2006.

[19] Wong, Y. C., Sanggari, V., Bioethanol production from sugarcane bagasse using fermentation process, *Orient. J. Chem.*, vol. 30, no. 2, pp. 507–513, 2014.

[20] Bharathiraja, B., Jayamuthunagai, J., Praveenkumar, R., VinothArulraj, J., Vinoshmuthukumar, P., Saravanaraj, A., Bioethanol production from lignocellulosic materials--an overview, *SciTech J.*, vol. 1, no. 7, pp. 28–36, 2014.

[21] Afifi, M. M. I., Massoud, O. N., El-Akasher, Y. S., Bioethanol production by simultaneous saccharification and fermentation using pretreated rice straw, *Sciences (New. York).*, vol. 5, no. 03, pp. 769–776, 2015.

[22] Huang, Y., Wang, Y., Shang, N., Li, P., Microbial fermentation processes of lactic acid: challenges, solutions, and future prospects, *Foods*, vol. 12, no. 12, p. 2311, 2023.

[23] Bhatia, L., Johri, S., Optimization of simultaneous saccharification and fermentation parameters for sustainable ethanol production from sugarcane bagasse by Pachysolen tannophilus MTCC 1077, *Sugar Tech*, vol. 18, no. 5, pp. 457–467, 2016.

[24] Huang, Y., Qin, X., Luo, X., Nong, Q., Yang, Q., Zhang, Z., Gao, Y., Lv, F., Chen, Y., Yu, Z., Liu, J., Feng, J., Efficient enzymatic hydrolysis and simultaneous saccharification and fermentation of sugarcane bagasse pulp for ethanol production by cellulase from Penicillium oxalicum EU2106 and thermotolerant Saccharomyces cerevisiae ZM1-5, *Biomass and Bioenergy*, vol. 77, pp. 53–63, 2015.

[25] Pinaki, D., Lhakpa, W., Joginder, S., Simultaneous saccharification and fermentation (SSF), an efficient process for bio-ethanol production: an overview, *Biosci Biotechnol Res Asia*, vol. 12, no. 1, pp. 87–100, 2015.