Jurnal Crystal: Publikasi Penelitian Kimia dan Terapannya
Vol. 8, No.1, Page. 001 - 006, 2026
e-ISSN 2685-7065

MASS BALANCE ANALYSIS OF GLUCOSE FROM SUGARCANE BAGASSE THROUGH ULTRASONIC ENZYMATIC HYDROLYSIS

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Article History
Received: 07 August 2025; Received in Revision: 10 October 2025; Accepted: 28 October 2025

Abstract

This study explores the conversion of sugarcane bagasse into glucose through an enzymatic ultrasonic hydrolysis process. Initially, the bagasse undergoes delignification using 6% sodium hydroxide to remove lignin and free cellulose for further processing. The delignified bagasse is then hydrolyzed using cellulase enzyme under ultrasonic conditions. The results indicate that glucose production is influenced by factors such as raw material preparation, enzyme activity, temperature, and pH. Mass balance calculations were applied to assess the input and output of materials throughout the delignification and hydrolysis processes. The findings show that 10 grams of delignified sugarcane bagasse yielded 5.955 grams of glucose, demonstrating the potential of sugarcane bagasse as a source of bioenergy through glucose production. These results support the utilization of agricultural waste in bioethanol production and contribute to sustainable energy development.

Keywords: Enzymatic Hydrolysis, Glucose Production, Sugarcane Bagasse

Abstrak

Penelitian ini mengkaji konversi bagasse tebu menjadi glukosa melalui proses hidrolisis enzimatis dengan bantuan ultrasonik. Tahap awal melibatkan delignifikasi menggunakan larutan NaOH 6% untuk menghilangkan lignin sehingga selulosa lebih mudah dihidrolisis. Selanjutnya, bagasse terdilignifikasi diproses menggunakan enzim selulase dalam kondisi ultrasonik. Hasil penelitian menunjukkan bahwa produksi glukosa dipengaruhi oleh persiapan bahan baku, aktivitas enzim, suhu, dan pH. Perhitungan neraca massa menunjukkan bahwa 10gram bagasse terdilignifikasi menghasilkan 5,955gram glukosa. Temuan ini menegaskan potensi pemanfaatan bagasse sebagai sumber bioenergi serta mendukung upaya pengembangan energi terbarukan berbasis limbah pertanian.

Keywords: Hidrolisis Enzimatik, Produksi Glukosa, Ampas Tebu

1. Introduction

Indonesia is one of the major sugar-producing countries, with numerous sugar mills operating throughout the archipelago. In the sugar production process, sugarcane bagasse (SCB), a fibrous residue, accounts for approximately 35–40% of the processed sugarcane, while only about 5% is converted into crystal sugar (Trisakti and br Silitonga, 2015). Alongside molasses and filter cake, bagasse remains one of the most abundant by-products. Unfortunately, these by-products, especially SCB, have not received the same level of attention as the primary product. Considering that SCB is already collected and available on-site in sugar mills, it has significant potential as a biomass feedstock for value-added products (Furlan *et al.*, 2013). Moreover, the increasing global energy demand and the gradual depletion of fossil fuel reserves have intensified interest in renewable energy sources, particularly those derived from biomass (Wong and Sanggari, 2014). Globally, sugarcane industries generate massive volumes of SCB. For instance, during the 2010/2011 harvest season, over 625 million tons of sugarcane were processed, producing

approximately 208 million tons of bagasse (Rocha *et al.*, 2012). While most of this residue is used as boiler fuel in the mills, a substantial portion remains unused due to excess generation beyond the boiler's burning capacity (Amorim *et al.*, 2011). The accumulation of SCB over time poses environmental concerns, as it is flammable and requires significant storage space, leading to pollution and safety risks (Anisya, Fitra Andriana and Islamsyah, 2020). Given these challenges and opportunities, valorizing SCB for bioenergy production, particularly bioethanol via glucose intermediate, emerges as a promising solution.

SCB is a type of lignocellulosic biomass, primarily contains fibrous components, with cellulose as the major fraction (around 40–45%), followed by hemicellulose (30–35%), and a smaller portion of lignin (20–30%) (Nasution, Lelinasari and Kelana, 2022). Cellulose is a linear homopolymer of glucose ($(C_6H_{10}O_5)_n$), while hemicellulose is a branched heteropolymer of various sugars, and lignin is a complex aromatic polymer that binds the structure tightly (Sarkar *et al.*, 2012). These components form a compact matrix that resists enzymatic attack. Thus, pretreatment is required to break this structure, reduce cellulose crystallinity, increase porosity, and improve hydrolysis efficiency. Among various methods, alkaline pretreatment using NaOH is effective in removing lignin and enhancing cellulose accessibility (Furlan *et al.*, 2012; de Araujo Guilherme *et al.*, 2019). Lignin's presence has been reported to inhibit enzymatic hydrolysis by adsorbing enzymes and reducing their effectiveness (Brienzo *et al.*, 2015).

The conversion of lignocellulosic biomass into glucose involves two key stages: pretreatment (e.g., delignification) and hydrolysis. In the first stage, lignin and hemicellulose are separated to expose the cellulose fibers. In the second stage, the cellulose is broken down into fermentable sugars, including glucose, via enzymatic hydrolysis (Rabelo et al., 2011; Velmurugan and Muthukumar, 2011). Enzymatic hydrolysis using cellulase is environmentally friendly and efficient but requires effective pretreatment to achieve high conversion rates. The glucose obtained can be further fermented into bioethanol or upgraded into other valuable bio-based chemicals (Galadima and Muraza, 2019). The production of glucose from lignocellulose is considered sustainable and cost-effective due to the abundance of agricultural waste like SCB (Cardona, Quintero and Paz, 2010; Restiawaty et al., 2020).

Advancements in pretreatment technologies, including ultrasonic-assisted hydrolysis, have shown promising results in improving cellulose conversion. Combining physical disruption with enzymatic hydrolysis may enhance enzyme-substrate interaction and reduce hydrolysis time (Sritrakul, Nitisinprasert and Keawsompong, 2017). However, pretreatment remains one of the most expensive and least technologically mature steps in the overall biomass-to-bioethanol conversion pathway. Despite this, the biochemical route remains attractive due to its lower environmental impact and potential for high glucose yield, especially when optimized with the appropriate pretreatment conditions (Asgher, Ahmad and Iqbal, 2013; Ajala *et al.*, 2021).

This study aims to evaluate glucose production from sugarcane bagasse through a combination of alkaline pretreatment and enzymatic ultrasonic hydrolysis. A mass balance approach was applied to assess the input and output of materials throughout the process. This research highlights the potential of sugarcane bagasse as a renewable and sustainable source of glucose, contributing to the broader development of bioethanol and bio-based industries.

2.Methodology

2.1. Materials and method

The materials used in this study included sugarcane bagasse (150 g), sodium hydroxide p.a. (Merck, Germany), cellulase enzyme (Nanobio, Indonesia), citric buffer (MitraLab, Indonesia), and distilled water.

Sugarcane bagasse was collected from local sugarcane juice vendors in Surabaya, East Java, Indonesia. The bagasse was sorted to obtain good-quality samples, sun-dried, and subsequently ground with a blender to produce a fine powder. The powdered bagasse was stored in an airtight plastic container to prevent contamination.

Delignification was performed by placing 150 g of powdered bagasse into a beaker glass, followed by the addition of 1500 mL of 6% sodium hydroxide solution (Merck). The mixture was heated at 100 °C for 1 h using a hot plate stirrer. After treatment, the solid residue was separated from the filtrate by filtration, then thoroughly washed with distilled water until a neutral pH was achieved.

The delignified bagasse was finally dried at 105 °C for 2 h to remove moisture and residual alkali, resulting in lignin-reduced sugarcane bagasse suitable for hydrolysis.

For the hydrolysis step, 10 grams of the delignified bagasse were mixed with 100 mL of 0.1 M citric buffer (pH 5) and 5 mL of cellulase enzyme to initiate the enzymatic reaction. The mixture was then subjected to ultrasonic treatment for 30 minutes at 40 °C to enhance enzyme activity and facilitate substrate disruption. After treatment, the hydrolysate was subsequently filtered to separate the solid residue from the liquid fraction, and the glucose content in the filtrate was analyzed using a refractometer for quantitative determination.

2.2. Equipment Used

This subsection presents the equipment utilized during the delignification and hydrolysis processes. The visual documentation and identification of tools aim to provide a clear overview of the experimental setup and support reproducibility of the method.

The equipment used in the delignification process consisted of a beaker glass, a thermometer, and a hot plate stirrer. The beaker glass was employed as the reaction vessel to contain the mixture of sugarcane bagasse and NaOH solution. The thermometer was used to monitor and control the reaction temperature throughout the process. Meanwhile, the hot plate stirrer provided both heating and continuous stirring, ensuring a uniform temperature distribution and homogeneous mixing between the sample and the chemical solution.

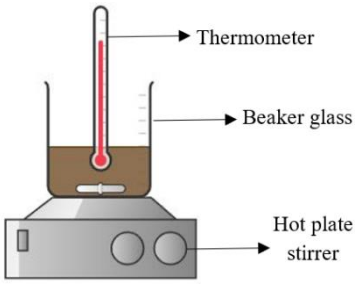

Figure 1. Delignification Equipment

The equipment used in the hydrolysis process consisted of a beaker glass and an ultrasonic bath device. The beaker glass was used as a container for the enzymatic hydrolysis reaction, while the ultrasonic bath device provided ultrasonic waves to enhance the hydrolysis process by improving enzyme penetration and substrate disruption.

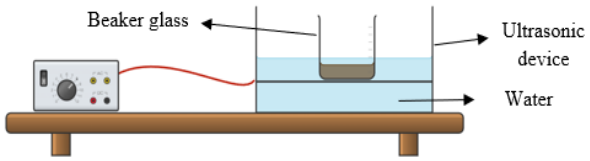

Figure 2. Hydrolysis Equipment

3. Results and Discussion

Mass balance, also referred to as material balance, is a fundamental principle derived from the law of conservation of mass, which states that the total mass entering a system must be equal to the total mass leaving it (Saputro *et al.*, 2021). In this context, no accumulation occurs within the system, and the transformation of materials does not alter the overall mass quantity (Heriyadi and Kusnandar, 2014). This principle was applied in the analysis of glucose production from sugarcane bagasse (SCB), as summarized in Table 1.

Based on the mass balance shown in Table 1, the hydrolysis of 10 grams of delignified SCB resulted in 5.955 grams of hydrolysis filtrate. The glucose yield is influenced by several factors, including substrate quality, enzyme concentration, hydrolysis temperature, and reaction time. The pretreatment step, particularly delignification with 6% NaOH, plays a vital role in enhancing glucose yield by removing lignin, reducing cellulose crystallinity, and increasing porosity, thereby improving enzyme accessibility during the hydrolysis process.

The enzymatic hydrolysis was conducted under ultrasonic conditions (40 kHz, 60 W) for 30 minutes, resulting in 5.955 grams of glucose, alongside 25 grams of dregs and 96.709 grams of residual solution. This corresponded to a glucose content of 5.8%. This finding is consistent with previous studies, which have shown that cellulase enzymes effectively degrade cellulose into glucose (Gomez Del Pulgar and Saadeddin, 2014). The hydrolysis reaction scheme is illustrated in Figure 3, while the reaction mechanism is shown in Figure 4. The breakdown of cellulose into glucose involves a consortium of enzymes: endoglucanases that cleave internal β-1,4-glucosidic linkages, exoglucanases that release cellobiose from cellulose chain ends, cellobiohydrolases, and β-glucosidases that convert cellobiose into glucose (Lakhundi, Siddiqui and Khan, 2015). These enzymes act synergistically to disrupt the solid–liquid interface and hydrolyze cellulose polymers into fermentable sugars. Ultrasonication further enhances this process by improving mass transfer and enzyme–substrate interactions. Through acoustic cavitation, ultrasonic waves generate microbubbles that collapse violently, producing localized high temperatures and pressures. This phenomenon increases the substrate surface area, enhances porosity, and ultimately facilitates more efficient hydrolysis (Córdova *et al.*, 2022).

Table 1. Mass Balance of Sugarcane Bagasse Hydrolysis by Enzymatic–Ultrasonic Process

No	Process	Input		Output	
1	Delignification	Sugarcane bagasse	150 g	Lignin free sugarcane bagasse	131.577 g
		Solution of sodium hydroxide	1575.241 g	Residue	1593.664 g
	Total		**1725.241 g**		**1725.241 g**
2	Hydrolysis	Lignin free sugarcane bagasse	10 g	Dregs	15 g
		Citric Buffer	101.546 g	Residual solution	96.709 g
		cellulase enzyme	6.118 g	Glucose	5.955 g
	Total		**117.664 g**		**117.664 g**

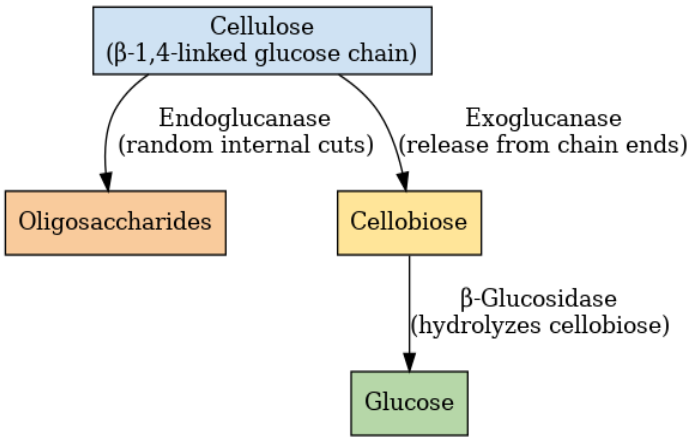

Figure 3. Schematic Diagram of Cellulose Enzymatic Degradation to Glucose by Cellulase

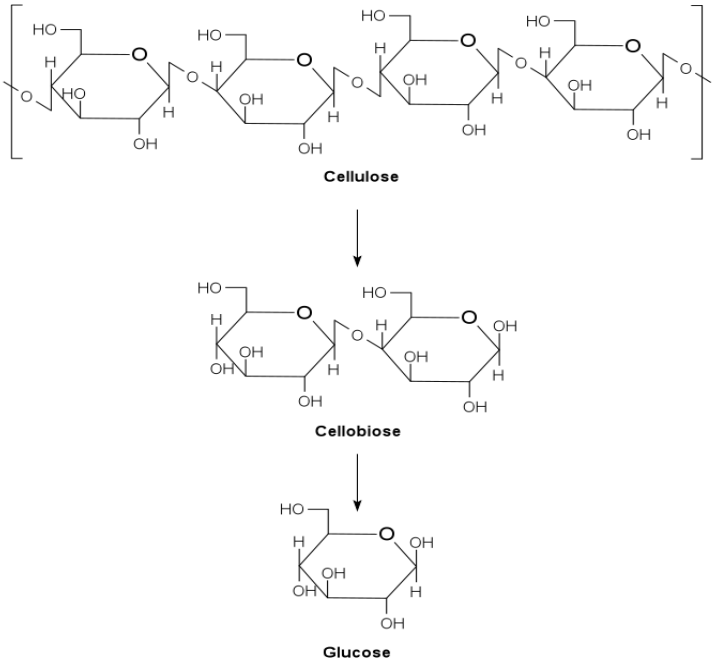

Figure 4. Enzymatic Hydrolysis of Cellulose to Glucose

Compared to conventional methods, the integration of enzymatic hydrolysis and ultrasonication offers time efficiency and energy savings. For instance, Wang et al. (2022) achieved a glucose yield of 91.2% from SCB using 10% NaOH and ethylene glycol pretreatment at 130 °C for 60 minutes, followed by 72 hours of enzymatic hydrolysis. Another study reached a yield of 91.6% after pretreatment at 180 °C for 30 minutes and 72-hour enzymatic hydrolysis (Córdova et al., 2022). While these yields are higher, they required significantly longer processing times. In contrast, the current study achieved a 5.8% glucose yield in just 30 minutes of hydrolysis, highlighting the potential of combining ultrasonication with enzymatic treatment as a rapid and effective method for SCB conversion.

The 5.955 g glucose yield from 10 g of delignified SCB demonstrates the viability of SCB as a renewable feedstock for bio-based chemical production. This process valorizes agricultural waste and supports sustainable bioenergy initiatives. Future studies should focus on optimizing hydrolysis conditions, enzyme dosage, and pretreatment parameters to increase yield and scalability. In conclusion, SCB shows promising potential as a raw material for glucose production through a combination of alkaline pretreatment and ultrasonic enzymatic hydrolysis. This pathway contributes to the development of sustainable biomass utilization strategies and circular economy initiatives.

4. Conclusion

This study demonstrated that sugarcane bagasse (SCB) can be effectively converted into glucose through alkaline pretreatment followed by ultrasonic-assisted enzymatic hydrolysis. The process yielded 5.955 g of glucose from 10 g of delignified SCB in just 30 minutes, indicating its potential for rapid biomass valorization. To enhance efficiency and scalability, further research is recommended to optimize enzyme loading, reaction time, and pretreatment conditions.

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