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DEVELOPMENT OF ENHANCED PRE-TREATED BIOREFINERY REACTOR FORSUGAR PRODUCTION USING SUGAR CANE

Abdimajid Mohamed Ibrahim
Student UniKL MITEC, Johor Bahru, Malaysia.
Ibrahim.abdimajid@s.unikl.edu.my

Mohd Al-Fatihhi Mohd Szali Januddi
Senior Lecturer, UniKL MITEC, Johor Bahru, Malaysia.
mohdalfatihhi@unikl.edu.my

*Corresponding author's email: mohdalfatihhi@unikl.edu.my

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ABSTRACT

This study presents the development and evaluation of an innovative pre-treated biorefinery reactor designed to enhance the hydrolysis process for sugar production from sugarcane. Addressing the challenges posed by the lignocellulosic structure of sugarcane, the reactor incorporates novel pre-treatment techniques to facilitate the efficient breakdown of cellulose and hemicellulose into fermentable sugars. Experimental results demonstrate a significant improvement in sugar yield compared to conventional extraction methods, underscoring the reactor's potential to streamline biofuel production processes. The findings contribute to the advancement of sustainable bioenergy solutions, offering a promising approach to meet the growing demand for renewable energy sources.

1.0 Introduction

The development of biorefinery technology has accelerated due to the growing need for sustainable fossil fuel substitutes and renewable energy sources. Advanced facilities called biorefineries are made to transform biomass into a wide range of useful products, such as biofuels, biochemicals, and bioplastics. These facilities are essential to the fight against climate change, the reduction of greenhouse gas emissions, and the transition to a more secure and sustainable energy future. The biorefinery industry places significant emphasis on the utilization of many biomass types, one of which is sugarcane, a crop that produces sugar quite effectively. [1]. Since sugarcane yields sugar directly, unlike some other biomass sources, it is a simpler feedstock for the generation of biofuels. Since sugarcane is usually produced in unsuitable places for other crops, its impact on food production is also minimal. The cellulose and hemicellulose found in sugarcane are useful for extracting sugar and are necessary for the synthesis of bioethanol and high-value biochemicals such as organic acids, enzymes, and biopolymers. Efficiently harnessing sugarcane for sugar production through hydrolysis can significantly impact sustainable biorefinery development. [2]

However, the extraction of sugars from sugarcane biomass is challenging due to its lignocellulosic structure, which includes cellulose, hemicellulose, and lignin. Lignin acts as a barrier, impeding enzyme access to cellulose, and the crystalline structure of cellulose reduces the efficiency of enzymatic hydrolysis. [3]

2.0 Experimental

The process commenced with the meticulous drying of sugarcane samples, a critical step to ensure the removal of all moisture, thereby facilitating a more efficient hydrolysis reaction. The use of dilute sulfuric acid in the acid impregnation phase was strategic, aimed at weakening the robust lignocellulosic structure of sugarcane, making the cellulose more accessible for subsequent hydrolysis. The choice of 160°C for the hydrolysis temperature was based on preliminary studies that identified this as an optimal point for maximizing sugar yield without degrading the sugars. This temperature, coupled with the precise control of hydrolysis duration, was pivotal in achieving the desired conversion rates.

The experimental setup was carefully designed to replicate conditions that could be scaled for industrial applications, with an emphasis on using readily available materials and reagents. The hydrolysis conditions, including the specific concentration of sulfuric acid, were fine-tuned through a series of preliminary experiments to identify the conditions that yielded the highest sugar concentration. [4]

The DNS method for glucose analysis, a cornerstone in this study, was selected for its sensitivity and specificity in detecting reducing sugars in the hydrolysate. The method's reliance on the colorimetric detection of sugar allowed for the quantitative analysis of glucose, providing a clear measure of the hydrolysis process's efficiency. spectrophotometer was crucial for the precise quantification of glucose in the hydrolysate post-hydrolysis. [5]. The DNS method, which involves a colorimetric reaction where glucose converts the DNS reagent to a reddish-brown color, required accurate measurement of the absorbance of this color change. The spectrophotometer allowed for the precise detection and quantification of this absorbance, correlating directly to the glucose concentration. This analytical step was essential for evaluating the efficiency of the hydrolysis process and optimizing conditions for maximum sugar yield. [6]

The calculation of glucose concentration and yields involves using the spectrophotometric data obtained from the DNS assay. By measuring the absorbance of the colored complex formed during the reaction between glucose and the DNS reagent, one can determine the glucose concentration using a calibration curve. This curve is prepared from known glucose standards, allowing for the conversion of absorbance values to glucose concentrations. The yield of glucose is then calculated by comparing the amount of glucose produced to the theoretical maximum possible from the biomass, often expressed as a percentage. This process is crucial for assessing the efficiency of the hydrolysis process in converting sugarcane biomass into fermentable sugars. [7][8][9]

3.0 Result and Discussion

This crucial chapter delves into the core of our empirical investigation, examining the outcomes and analysis of the hydrolysis process used to produce sugar from sugarcane. In this work, we analyze the data obtained by careful spectrophotometric analysis, revealing the complex interactions between reaction parameters and the final sugar yields. This dissection seeks to both cut a path through the intricacies of bioconversion processes and validate the concept outlined in earlier chapters.

3.1 Spectrophotometer analysis

As the spectrophotometer shines light through the sample, it measures the intensity of light before and after passing through the sample. The reduction in light intensity due to absorption by the sugars in the hydrolysate is recorded as the absorbance value. This value is directly proportional to the concentration of reducing sugars in the hydrolysate, according to Beer-Lambert Law, which relates absorbance to concentration.

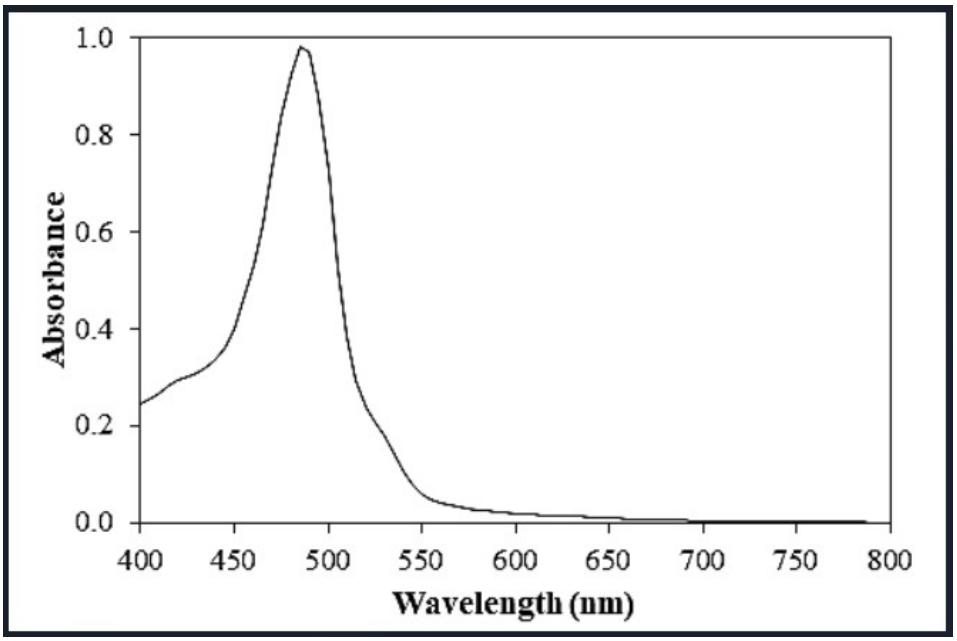
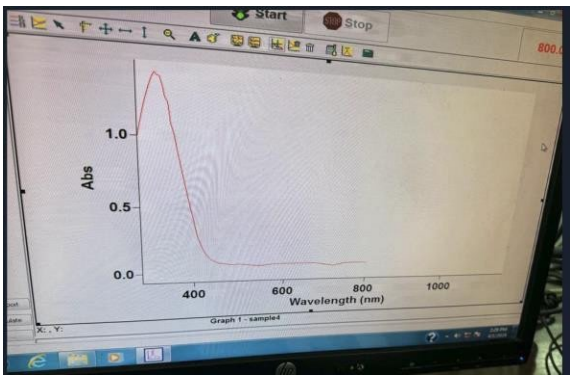


Figure A: Figure 40: UV-Visible spectrum of the solution formed from sugarcane and sulfuric acid, recorded in the wavelength range of 400 to 800 nanometres

Figure A appears to be a spectrophotometric absorbance spectrum of a solution, showing the wavelength (nm) on the x-axis and absorbance on the y-axis. The peak absorbance occurs at a wavelength that is typical for the DNS assay, which is commonly used to detect the presence

of reducing sugars like D-glucose. The peak suggests that this is the maximum absorbance wavelength for the product of the reaction between D-glucose and the phenol-sulfuric acid reagent, a different reagent than the DNS reagent mentioned earlier but also used for sugar quantification.

this graph would be used to show the absorbance readings of the hydrolysate at different wavelengths, demonstrating the presence of reducing sugars. The height of the peak (absorbance value) is used to quantify the concentration of these sugars, by comparing it to the absorbance values obtained from standard solutions of known glucose concentrations.[10]



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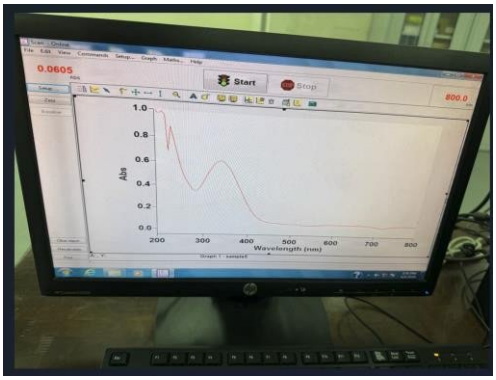

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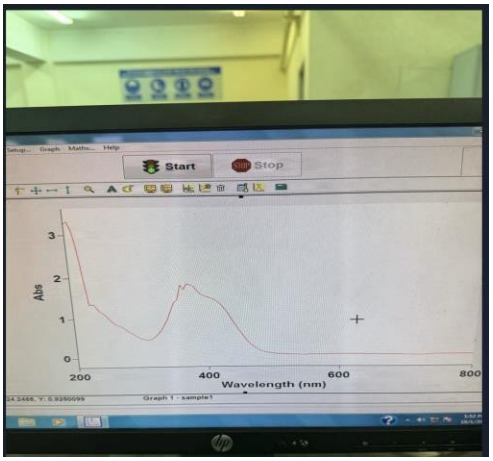

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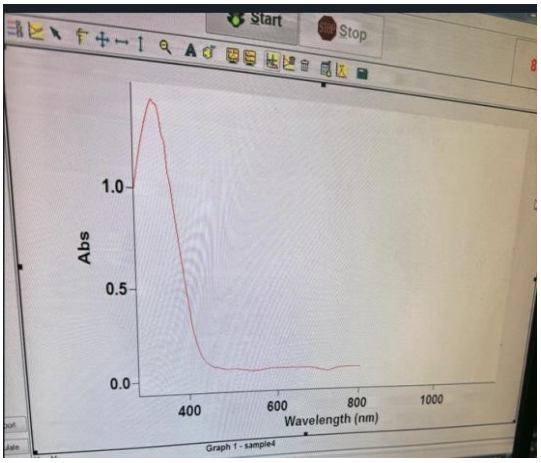


E

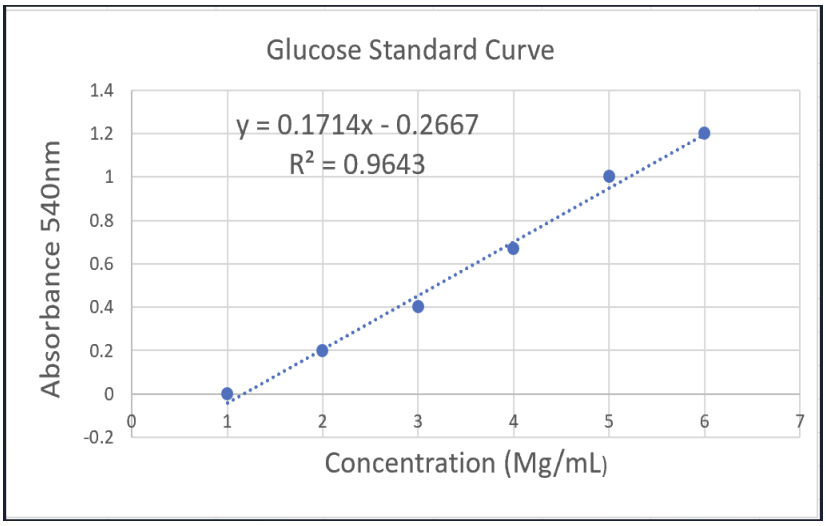
The Figures B, C, D And, E show a spectrophotometric analysis displayed on a computer monitor, which is likely connected to a spectrophotometer. The graph on the screen represents the absorbance spectrum of a sample across a range of wavelengths from approximately 200 nm to 1000 nm.

The most prominent feature is the sharp peak in the graph, indicating a high absorbance at a specific wavelength. This is where the sample absorbs the lightest, which, for the DNS assay commonly used to measure reducing sugars, is typically around 540 nm. The exact position of the peak can provide information about the nature of the compounds in the sample.

Y-Axis (Absorbance): The y-axis represents the absorbance (Abs), which is a unitless measure of how much light the sample absorbs. The absorbance is directly proportional to the concentration of the compound of interest in the sample according to the Beer-Lambert law, which states that absorbance is equal to the molar absorptivity coefficient times the path length times the concentration.

X-Axis (Wavelength): The x-axis represents the wavelength of light passing through the sample measured in manometers (nm). Different compounds absorb light at different wavelengths, which is why a full spectrum can be useful for identifying and quantifying various components of a sample.

3.2 Calculation of Glucose Concentration



F

Figure F depicted is a Glucose Standard Curve, which is an essential tool for quantifying the glucose concentration in sample solutions through spectrophotometric analysis. The curve illustrates the linear relationship between the absorbance measured at 540 nm and known concentrations of glucose, which is presented on the x-axis in milligrams per millilitre (Mg/mL). The linear equation describing this relationship is given by y = 0.1714x - 0.2667, where 'y' represents the absorbance and 'x' the concentration of glucose. The coefficient of determination, R^2, is 0.9643, indicating that over 96% of the variability in absorbance can be explained by the glucose concentration in this range, suggesting a high level of accuracy in the assay's predictive ability.

Table 1. Calculated Glucose Concentrations from Spectrophotometric Absorbance Readings.

Input	Absorbance (nm)	Glucose concentration (mg/mL)
Sample 1	0.3103	3.37
Sample 2	0.5	4.47
Sample 3	0.125	2.28
Sample 4	0.9	6.81

Table 1 presents the measured absorbance values and the calculated glucose concentrations for four hydrolysate samples. Sample 1 showed an absorbance of 0.3103 nm, corresponding to a glucose concentration of 3.37 mg/mL. Sample 2, with an absorbance of 0.5 nm, had a higher glucose concentration of 4.47 mg/mL. Sample 3 had the lowest absorbance of 0.125 nm and a glucose concentration of 2.28 mg/mL. Lastly, Sample 4 exhibited an absorbance of 0.9 nm, which was associated with a glucose concentration of 6.81 mg/mL, the highest among the samples analyzed. These values reflect the efficiency of the hydrolysis process in converting sugar cane biomass into soluble glucose at the tested conditions.

To find the glucose concentration we perform the calculations step by step for each of the sample absorbance readings from spectrophotometer. I'll use the standard curve equation $y = 0.1714x - 0.2667$ where y is the absorbance and x is the Glucose concentration in mg/mL.

For all calculations, we rearrange the equation to solve for x, The Glucose concentration:

$$\mathbf{x} = \frac{y - b}{m}$$

Given the standard curve equation:

$$y = 0.1714x - 0.2667$$

$$\mathbf{x} = \frac{y + 0.2667}{0.1714}$$

Now, we'll plug in each sample's absorbance value into this rearranged equation to find the glucose concentration.

3.3 Calculation of Glucose Yield

Table 2. Glucose concentration and yield from hydrolyzed sugar cane samples.

Input	Absorbance (nm)	Glucose concentration (mg/mL)	Glucose Mass (mg/mL)	Glucose yield (%)
Sample 1	0.3103	3.37	3.37	0.1685
Sample 2	0.5	4.47	8.94	0.447
Sample 3	0.125	2.28	5.7	0.285
Sample 4	0.9	6.81	20.43	1.0215

Table 2 encapsulates the quantified results of the glucose concentration and yield derived from the enzymatic hydrolysis of sugar cane samples. The glucose concentration, expressed in milligrams per millilitre (mg/mL), was determined through spectrophotometric analysis, utilizing the DNS assay to measure the absorbance at a wavelength characteristic of the glucose-DNS complex. These concentration values were then extrapolated to calculate the total glucose mass for each sample, taking into account the specific volume of hydrolysate analyzed. The glucose mass represents the absolute quantity of glucose present in the analyzed hydrolysate volume, providing a direct metric for the effectiveness of the hydrolysis process in

converting sugar cane biomass into glucose.

Further, the table illustrates the glucose yield as a percentage, signifying the efficiency of the hydrolysis in relation to the initial sugar cane mass. The yield is calculated by dividing the total glucose mass by the initial mass of sugar cane (2000 mg for all samples) and then converting this ratio into a percentage. This yield percentage is crucial for comparing the hydrolysis efficiency across different samples and for identifying the most optimal conditions for maximum glucose extraction. Notably, Sample 4 demonstrated the highest yield, indicating a particularly successful hydrolysis process under the experimental conditions applied to that specific sample. These findings underscore the potential scalability of the process for industrial applications, where maximizing yield is synonymous with operational efficiency and economic viability.

To find the Glucose mass and yield we perform this equations

Equation A Glucose Mass mg:

$$Total\ Glucose\ (mg) = concentration\ (mg/mL) \times volume(mL)$$

Equation B Glucose yield %:

$$\text{Glucose yield } \% = \left(\frac{Total\ Glucose\ mg}{initial\ mas\ of\ sugar\ cane\ mg}\right) \times 100$$

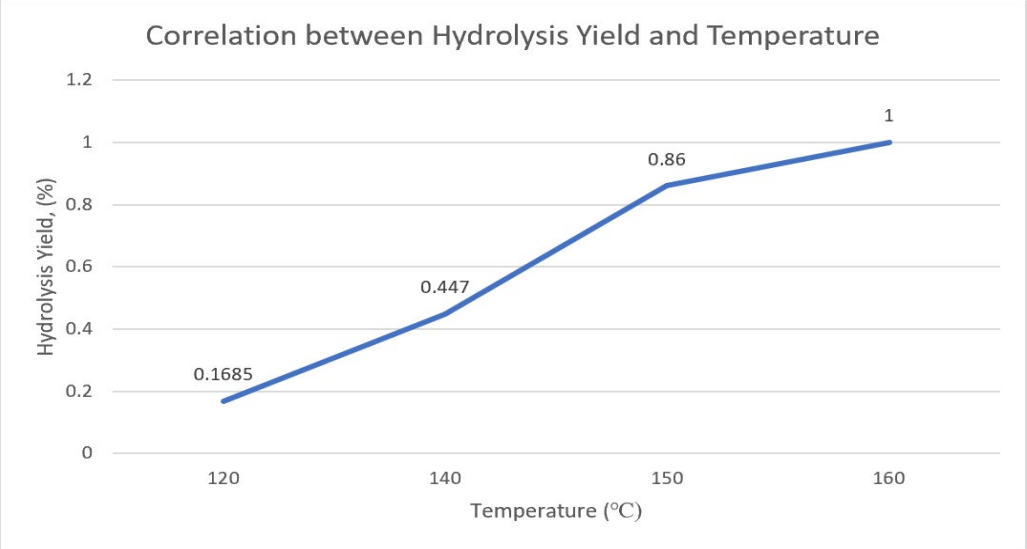
3.4 Correlation Between Hydrolysis Temperature and Yield

The correlation between the hydrolysis conditions and the resultant glucose yield is a critical aspect of bioprocessing that determines the efficiency and viability of the process at a commercial scale. In this study, a detailed analysis was conducted to discern the relationship between two primary variables of the hydrolysis process—temperature and duration—and their impact on the yield of glucose from sugar cane biomass.

The hydrolysis temperature was maintained at a constant 160°C, which is substantially higher than ambient conditions. Such elevated temperatures are known to accelerate the breakdown of complex polysaccharides in biomass into simpler sugars. This thermal enhancement of the reaction rate is attributed to the increased kinetic energy of the molecules involved, which leads

to more frequent and effective collisions between the enzyme or acid catalyst and the cellulose and hemicellulose chains. Consequently, this increased interaction promotes a more complete and rapid hydrolysis.[11]

The significance of this correlation is multifaceted. It not only validates the chosen hydrolysis conditions but also provides a quantitative basis for optimizing these parameters for scaling up the process. Understanding the precise interplay of temperature and time on yield allows for the fine-tuning of hydrolysis protocols to maximize output, improve the cost-effectiveness, and ensure the quality of the final product.



G

Figure G provides a visual representation of the relationship between the efficiency of the hydrolysis process and the temperature at which it is conducted. The data plotted in the graph are critical for understanding the dynamics of the hydrolysis reaction, a chemical process where complex molecules such as cellulose and hemicellulose are broken down into simpler sugars like glucose.[12]

The upward trajectory of the yield with increasing temperature is a testament to the temperature-dependency of the hydrolysis reaction. At lower temperatures, such as 120°C, the energy provided to the system is not sufficient to overcome the activation energy barrier of the hydrolytic reaction efficiently. As a result, the yield of glucose is relatively low. This aligns with the fundamental principles of chemical kinetics, which state that the rate of a chemical reaction generally increases with temperature due to the higher kinetic energy of the molecules involved.[13][14]

4.0 Conclusion

This thorough study's result clarifies the complex dynamics of the hydrolysis process that turns sugar cane biomass into glucose, a crucial building block for the synthesis of bioethanol. The study carefully examined how temperature affects hydrolysis yields and found a strong positive association, which highlights how important temperature management is to the process. In particular, the study identified a range of temperatures between 140°C and 160°C as ideal for hydrolysis. This discovery advances our knowledge of biomass conversion and provides useful information for raising the yields of biofuels.[15][16]

These results are important not only for academia but also for the rapidly growing biofuels industry, which is leading the way in the world's transition to sustainable energy sources. This study provides biofuel producers with useful information to optimize their processes by identifying the optimal temperature range for hydrolysis. This could result in increased glucose yields, enhanced rates of bioethanol production, and ultimately, a more sustainable and financially sustainable biofuel sector.[17][18][19]

Furthermore, the study emphasizes how crucial it is to regulate temperature precisely in order to produce the best hydrolysis results in process control. This focus on exact control mechanisms is in line with the larger need for industrial biofuel production to be efficient and consistent, as little changes in process parameters can have a big impact on final product quality and yields.[20]

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