



Glucose Synthesis from Imperata Cylindrica by Acid Hydrolysis Process

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ABSTRACT

Imperata cylindrica, commonly known as alang-alang, is a fast-growing, abundant, and underutilised grass often considered a weed. However, its high cellulose content makes it a promising lignocellulosic biomass for renewable glucose production. This study explores the synthesis of glucose from *Imperata cylindrica* through acid hydrolysis, to utilize this low-cost biomass as a sustainable raw material for bio-based products. The hydrolysis process was investigated using two main variables: hydrolysis time (1, 1.5, 2, 2.5, and 3 hours) and acid concentration (1%, 1.5%, 2%, 2.5%, and 3%). The resulting hydrolysates containing glucose were analysed using a refractometer and UV-Vis spectrophotometer, and further optimised using Response Surface Methodology (RSM). The highest glucose content obtained by refractometer was 6.5%, while RSM predicted a maximum of 6.49%, and UV-Vis analysis showed 6.34%. All optimal results were achieved under the condition of 2.5 hours of hydrolysis time and a 3% acid concentration. These findings highlight the potential of *Imperata cylindrica* as a viable and sustainable feedstock for glucose production through acid hydrolysis, contributing to the development of alternative bio-resources.

Contribution to Sustainable Development Goals (SDGs):

SDG 6: Clean Water and Sanitation

SDG 11: Sustainable Cities and Communities

SDG 13: Climate Action

SDG 15: Life on Land

1. INTRODUCTION

1.1. Research Background

Indonesia's energy demand continues to grow in line with population and economic development, while non-renewable energy sources are rapidly depleting. As a result, alternative energy such as bioethanol is gaining attention. Bioethanol is commonly produced through the fermentation of glucose sourced from carbohydrates like starch, sugar, or cellulose. However, due to the high cost and food-based competition of starch and sugar, cellulose from non-food biomass has become a promising alternative[3].

Imperata cylindrica (alang-alang), an invasive and fast-growing weed, is one such biomass. It is widely distributed across Indonesia and contains a high amount of cellulose, making it a potential raw material for glucose production. Chemical composition studies show alang-alang contains over 40% α -cellulose and nearly 60% holocellulose. Despite its abundance it remains underutilized[1].

Glucose can be derived from cellulose using acid or enzymatic hydrolysis [9]. This study uses acid hydrolysis with sulfuric acid, chosen for its faster reaction time and lower cost. Previous research has shown that both acid ratio and hydrolysis time significantly affect glucose content, but optimal conditions have not been clearly defined.



1.2. Literature Review

Imperata cylindrica, commonly known as cogon grass, is a grass species belonging to the Poaceae family and is recognized as one of the most widespread and easily encountered weeds. Globally, there are nine known *Imperata* species, which are predominantly distributed across eastern and southern Africa, Australia, Micronesia, India, Melanesia, as well as East and Southeast Asia. This plant can grow to a height ranging from 30 to 200 cm, with leaf widths of approximately 2 cm. Chemically, cogon grass is composed of 40.22% cellulose, 18.40% hemicellulose, 18.12% lignin, 3.6% silica, and 5.42% ash. It thrives extensively in open areas, both on cultivated and uncultivated land[6].

1.2.1. Delignification

Delignification is a process aimed at reducing lignin content by breaking down lignocellulose into its main components: lignin, cellulose, and hemicellulose. This process can be carried out through mechanical, chemical, or biological methods [4]. This process serves to reduce the lignin content, lower the crystallinity of cellulose, and enhance the material's porosity. Among various methods, chemical delignification using sodium hydroxide (NaOH) is considered more advantageous in terms of both technical efficiency and cost-effectiveness. NaOH is particularly effective at breaking down lignin and generates relatively low environmental impact compared to other chemical agents[7].

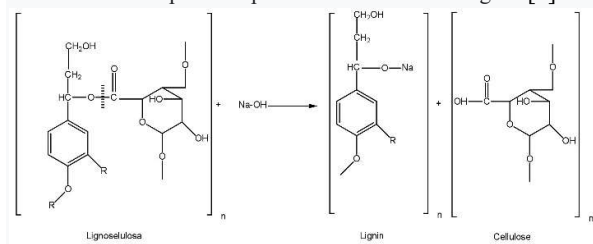


Fig. 1. Delignification of Lignocellulose with NaOH

1.2.2. Hydrolysis

Hydrolysis is a chemical process that utilizes water to break chemical bonds within a compound. In cellulose hydrolysis, two common methods are acid hydrolysis and enzymatic hydrolysis. Acid hydrolysis involves the use of acid as a catalyst to convert polysaccharides into monosaccharides. In this process, the acid acts as a catalyst that facilitates the breakdown of carbohydrates into simple sugars[10]. Acid hydrolysis typically employs strong acids such as sulfuric or hydrochloric acid under high temperature conditions, while enzymatic hydrolysis uses cellulase enzymes under milder and more environmentally friendly conditions. This process plays a critical role in biorefinery applications, as the resulting glucose can serve as a key feedstock for fermentation in the production of bioethanol and other value-added biochemicals.

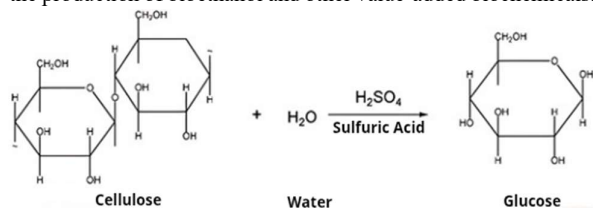


Fig. 2. Hydrolysis of Cellulose

1.3. Research Objective

This study aims to synthesize glucose from *Imperata cylindrica* using acid hydrolysis, with variations in hydrolysis time and acid-to-water ratio. Optimization is carried out using Response Surface Methodology (RSM) to determine the most effective conditions for maximizing glucose production.

2. MATERIALS AND METHODS

2.2.1. Materials and Equipment

The main material used in this research is alang-alang (*Imperata cylindrica*), specifically the stem part. The supporting materials used include technical grade sulfuric acid (H₂SO₄) at 98%, technical grade sodium hydroxide (NaOH), and distilled water (aquadest). The equipment used in this research, as shown in Figure 2, includes a chopper to reduce the size of the alang-alang, a 100-mesh sieve, a magnetic stirrer used during the delignification process with NaOH, and a reflux apparatus set for the hydrolysis process.

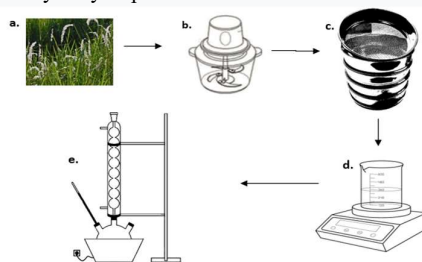


Fig. 3. Process sequence and equipment for hydrolysis

Description:

a. Alang-alang (*Imperata cylindrica*)

b. Chopper

c. Sieve

d. Magnetic Stirrer

Hydrolysis Apparatus Set

2.2.2. Procedure

The dried alang-alang (*Imperata cylindrica*) was first chopped and then sieved using a 100-mesh screen to ensure uniform particle size. Delignification was performed using a 2% (w/v) sodium hydroxide (NaOH) solution with a solid-to-liquid ratio of 1:20, at 121°C for one hour, to remove lignin which can hinder the hydrolysis process. After delignification, the mixture was filtered and the residue was dried. Hydrolysis was then carried out using sulfuric acid (H₂SO₄) solutions at concentrations of 1%, 1.5%, 2%, 2.5%, and 3%, with a solid-to-liquid ratio of 1:15. Each sample was treated at 100°C for varying durations: 1 hour, 1.5 hours, 2 hours, 2.5 hours, and 3 hours. After hydrolysis, the mixture was filtered and the filtrate was collected for glucose analysis, which was conducted using a Brix refractometer.

3. RESULT AND DISCUSSION

3.1. Analysis of glucose content from hydrolysis of alang alang with sulfuric acid

Analysis of glucose content was carried out using a glucose refractometer. The results are presented in Table 1.

Table 1. Glucose Content

Acid Concentration (%)	Hydrolysis Time (h)	Glucose Content (%)
1	1	1.1
1.5	1	2.8
2	1	3
2.5	1	4.9
3	1	5
1	1.5	1.9
1.5	1.5	3.5
2	1.5	3.9
2.5	1.5	5.2
3	1.5	5.5
1	2	2.5
1.5	2	3.9
2	2	4.5
2.5	2	5.5
3	2	6.2
1	2.5	2.7
1.5	2.5	4.2
2	2.5	4.9
2.5	2.5	5.7
3	2.5	6.5
1	3	3
1.5	3	4.5
2	3	4.1
2.5	3	5.2
3	3	6.5

Based on the data table above, the glucose content increases with increasing hydrolysis time and acid concentration. The highest glucose content was at 2.5 hours hydrolysis time and 3% acid concentration. Furthermore, the hydrolysis time of 3 hours and acid concentration of 3% did not increase glucose. This can occur because glucose is degraded due to the length of hydrolysis time and the increase in acid concentration.

3.2. Effect of hydrolysis time and acid concentration

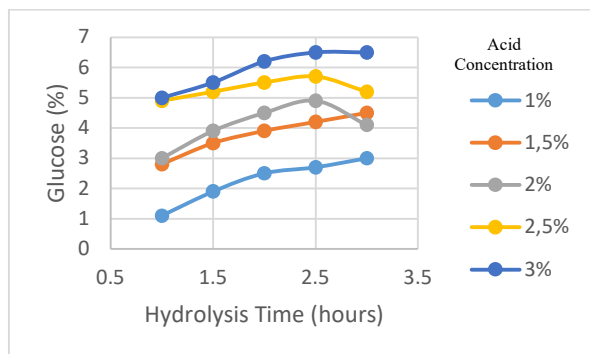


Fig. 4. Graph of the Relationship Between Hydrolysis Time (h) and Glucose Content (%)

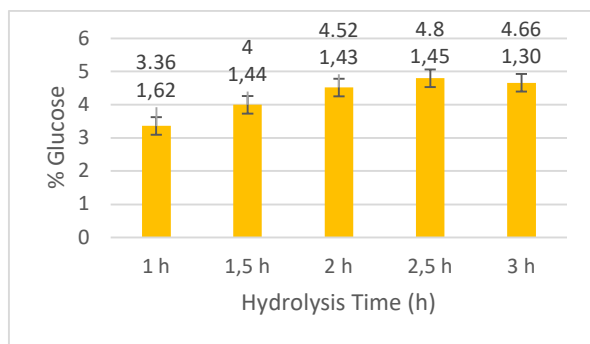


Fig. 5. Error Bar Graph of Hydrolysis Time with Glucose Content

Figures 3 and 4 show the effect of hydrolysis time and acid concentration on glucose content. Glucose content increased up to 6.5% at a hydrolysis time of 2.5 hours, but then remained

unchanged or even decreased. Figure 4 shows the same trend and the smallest deviation at 2.5% acid concentration, which means that this condition is more stable. Prolonging the hydrolysis time enhances the interaction between acid molecules and cellulose structures, thereby facilitating the depolymerization of cellulose into glucose monomers. While an extended reaction time generally increases glucose concentration, excessively long hydrolysis durations may induce glucose degradation, forming undesired by-products such as formic acid and hydroxymethylfurfural (HMF)[5].

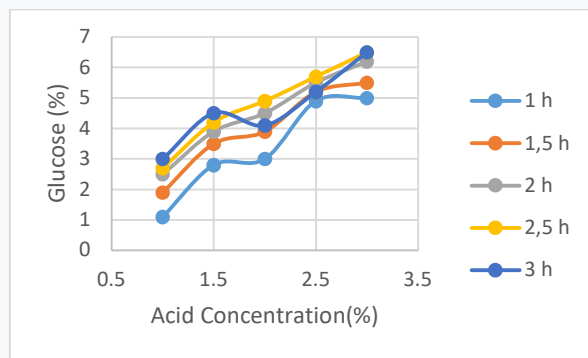


Fig. 6. Graph of the Relationship Between Acid Concentration (%) and Glucose Content (%)

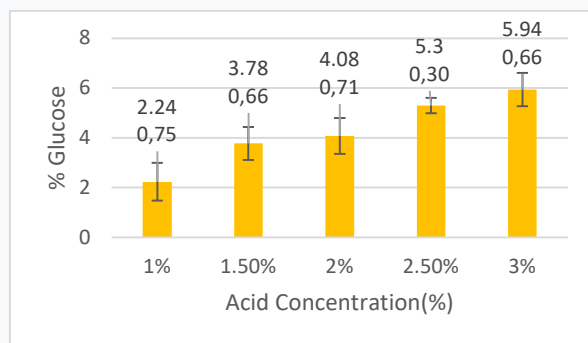


Fig. 7. Error Bar Graph of Acid Concentration (%) with Glucose Content

Figure 4 and 5 illustrate the relationship between acid concentration and glucose yield, showing a positive correlation between the two variables. Higher acid concentrations enhance the breakdown of cellulose into glucose due to increased availability of H^+ ions from sulfuric acid (H_2SO_4), which catalyze the cleavage of glycosidic bonds. A more notable increase in glucose yield was observed at acid concentrations of 2.5% and 3%, reaching 5.3% (± 0.30) and 5.94% (± 0.66), respectively. At 2.5%, the smaller standard deviation suggests more consistent results across replicates, indicating a more efficient and stable hydrolysis process within this concentration range.

3.3. Glucose Optimization Using Response Surface Methodology (RSM)

The optimization of glucose yield was carried out using Response Surface Methodology (RSM) with a Central Composite Design (CCD). Two key factors, acid concentration and hydrolysis time, were selected to evaluate their individual and interactive effects. Table 2 presents the 13 experimental runs generated by the CCD

approach, which served as the basis for model development and determination of optimal conditions.

Table 2. 13 experimental data with the CCD method by combining 2 factors

Acid Concentration (%)	Hydrolysis Time (h)	Glucose Content (%)
1	2.5	4.9
1	3	5
1.5	2.5	5.2
1.5	3	5.5
2	2	4.5
2	2.5	5.5
2	3	6.2
2.5	2	4.9
2.5	2.5	5.7
2.5	3	6.5
3	2	4.1
3	2.5	5.2
3	3	6.5

The desired optimal condition is the maximum glucose response. From the 13 data, the regression equation is obtained as follows.

$$y = 2,89 - 0,28X_1 + 0,69X_2 - 0,433X_1^2 - 0,229X_2^2 + 0,924X_1 * X_2$$

Description :

y = glucose content (%)

X₁ = Hydrolysis time (hours)

X₂ = Acid Concentration (%)

3.3.1. Optimization results from 13 experimental data

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,202759	95,50%	92,28%	82,69%

Parameters

Response	Goal	Lower	Target	Upper	Weight	Importance
Glucose (%)	Maximum	4,1	6,5		1	1

Solution

Solution	Hydrolysis Time (h)	Acid Concentration (%)	Glucose (%) Fit	Composite Desirability
1	2,87879	3	6,49043	0,996014

Multiple Response Prediction

Variable	Setting
Hydrolysis Time (h)	2,87879
Acid Concentration (%)	3

Response	Fit	SE Fit	95% CI	95% PI
Glucose (%)	6,490	0,149	(6,138; 6,843)	(5,895; 7,086)

In Table, the optimization results using RSM resulted in an optimum glucose level of 6.49% at a hydrolysis time of 2.878 hours and an acid concentration of 3% with a desirability value of 0.996 indicating that this solution is very close to actual conditions. This strengthens the reliability of the model in accurately predicting the response[2]. Validation of the results was done by comparing the predicted values of the model against the experimental data using the Confidence Interval (CI) and Prediction Interval (PI) at the 95% confidence level. The CI falls within the range of 6.138 to 6.843, while the PI ranges from 5.895 to 7.086. The CI indicates the level of precision of the estimate produced by the model. At the same time, the PI includes variations in the observation data and model error, thus providing a wider range of predictions.

3.3.2. Analysis using ANOVA method

Table 2. Anova Analysis

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	6,1029	1,2206	26,69	0,000
Linear	2	2,3614	1,1807	28,72	0,012
X ₁	1	0,3436	0,3436	8,36	0,023
X ₂	1	2,3212	2,3212	56,46	0,011
Square	2	0,3841	0,1920	4,67	0,051
X ₁ *X ₁	1	0,3836	0,3836	9,33	0,018
X ₂ *X ₂	1	0,0080	0,0080	0,19	0,672
2-way interaction	1	0,4408	0,4408	10,72	0,014
X ₁ *X ₂	1	0,4408	0,4408	10,72	0,014
Error	7	0,2877	0,0411		
Total	12	6,3907			

Description :

X₁ = Hydrolysis Time (h)

X₂ = Acid Concentration (%)

Based on the analysis of variance (ANOVA), both the linear effects and the two-way interaction significantly contributed to the model. The linear terms of hydrolysis time (X₁) and acid concentration (X₂) were statistically significant, with P-values of 0.023 and 0.011, respectively ($P < 0.05$), indicating that variations in either factor directly influenced the response[8]. Furthermore, the interaction between X₁ and X₂ also showed a significant effect (P -value = 0.014), suggesting that the impact of one factor on the response depends on the level of the other. These results confirm that both linear effects and the interaction between hydrolysis time and acid concentration play a critical role in accurately modeling the system response.

3.3.3. Contour plot and surface plot

Contour Plot of Glucose (%) vs Acid Concentration (%); Hydrolysis Time

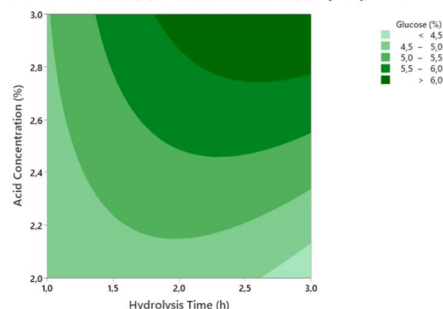


Fig. 8. Contour Plot

The contour plot shows that increasing both hydrolysis time and acid: water ratio together causes a significant increase in the glucose content produced. The dark green region on the upper right indicates the optimum region, which is the combination of hydrolysis time and acid: water ratio that can produce glucose levels of more than 6%. In contrast, the light green color in the lower left indicates glucose levels below 4.5%, which occurs under low time and concentration conditions.

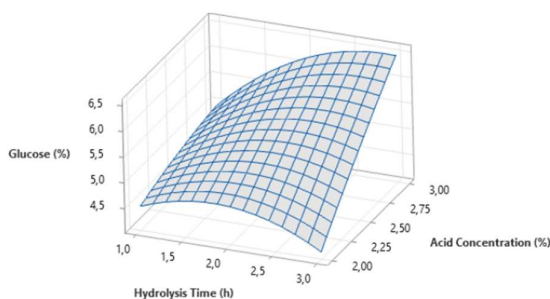


Fig. 9. Surface Plot

From the graph, it can be observed that the percentage of glucose increases as the Acid: Water ratio and hydrolysis time increase. At a higher Acid:Water ratio (close to 3.0), as well as longer hydrolysis time (about 3 hours), a higher glucose yield of 6.49% was obtained.

3.4. Glucose Content Results Using UV-VIS Spectrophotometer

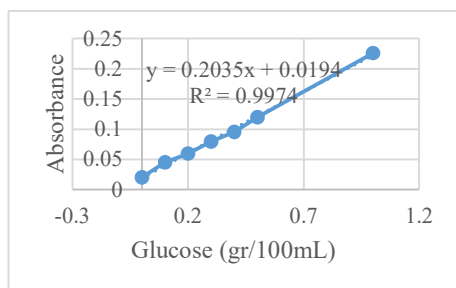


Fig. 10. Calibration Curve of Standard Sugar Solution

The glucose standard calibration curve at a wavelength of 740 nm is shown in Figure 1. This curve illustrates the relationship between glucose concentration (gr/100 mL) and absorbance values measured using a UV-Vis spectrophotometer. The results of linear regression analysis showed that the relationship between concentration and absorbance was linear with a regression equation. Based on the measurement results, the absorbance of the sample was recorded as 0.1484. This absorbance value was then entered into the linear regression equation of the standard solution, and obtained a glucose concentration in the sample of 6.34% or 6.34 grams per 100 mL.

4. CONCLUSION

Based on the this research, several test results were obtained. Refractometer analysis at 25 data points indicated that the highest glucose content, 6.5%, was achieved using a hydrolysis duration of 2.5 hours and an acid concentration of 3%. Further optimization using Response Surface Methodology (RSM) and based on 13 experimental data points, confirmed the same optimal condition (2,88-hour hydrolysis with 3% acid concentration), yielding a predicted glucose content of 6.49%. Additionally, spectrophotometric analysis showed a maximum glucose content of 6.34% under the same condition. For future research, it is recommended to explore the use of acid catalysts and alternative raw materials to enhance glucose content.

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