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Optimization of Acid-Catalyzed Hydrolysis of Water Hyacinth without Delignification

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Abstract. Water hyacinth (*Eichhornia crassipes*) is a rapidly proliferating invasive aquatic plant causing severe ecological disruptions and economic challenges worldwide. Its uncontrolled spread significantly affects aquatic biodiversity and local livelihoods. Although water hyacinth is rich in cellulose, conventional hydrolysis methods to convert it into valuable bioproducts, such as biofertilizer substrates, often require costly and environmentally harmful pretreatment steps, limiting its broader utilization. This study aimed to optimize acid-catalyzed hydrolysis of water hyacinth into glucose-rich hydrolysate without alkaline pretreatment. Response surface methodology (RSM) was employed to determine optimal conditions using hydrochloric acid (HCl) and sulfuric acid (H_2SO_4). Optimal hydrolysis conditions were found to be 2.36N concentration, 89.33°C, and 76.94 minutes for HCl, and 1.91N concentration, 100.03°C, and 79.66 minutes for H_2SO_4. Model validation showed high R^2 values of 0.82 and 0.95 for HCl and H_2SO_4, respectively. Subsequent biofertilizer fermentation experiments demonstrated that H_2SO_4-derived hydrolysate facilitated superior microbial growth compared to HCl, indicating better glucose bioavailability. Hydrolysates from HCl hydrolysis showed higher bacterial toxicity. These findings highlight the potential of optimized acid-catalyzed hydrolysis as an effective, sustainable strategy for converting invasive water hyacinth into glucose-rich substrates for biofertilizer production. This bioprocess-friendly approach not only mitigates environmental impacts but also enhances resource efficiency, contributing significantly to sustainable agricultural practices.

Keywords: Biofertilizer Production, Glucose Yield, Hydrolysis Optimization, Response Surface Methodology, Water Hyacinth

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INTRODUCTION

Water hyacinth (*Eichhornia crassipes*) is an aquatic plant widely recognized for its invasive nature, leading to significant ecological and socio-economic consequences worldwide (Adornado *et al.*, 2017; Jha, 2024). This plant has drastically reduced macrophyte biodiversity, impacting both ecosystems and local economies (Mengistu *et al.*, 2017). Its rapid growth and ability to proliferate in various environments have resulted in its spread to temperate river systems in the Midwest United States, highlighting its adaptability and the challenges it poses to biodiversity (VonBank *et al.*, 2018). In regions such as the Brahmaputra floodplain in Assam, India, water hyacinth's unchecked growth has led to biomass expansion and altered floral compositions, transforming natural habitats (Lahon *et al.*, 2023).

Efforts to control water hyacinth have gained urgency, with researchers promoting sustainable strategies to limit its spread (Cerveira & Carvalho, 2019). Remote sensing is used to track its rapid proliferation, highlighting the need for timely intervention (Janssens *et al.*, 2022). Global studies emphasize the ecological threat to aquatic biodiversity (Cordeiro *et al.*, 2020). The plant's socio-ecological impacts are evident in locations such as Lake Tana (Ethiopia), Shagashe River (Zimbabwe), and Lake Toba (Indonesia), where it disrupts ecosystems, hinders economic activities, and affects human well-being (Brunerová *et al.*, 2017; Chapungu *et al.*, 2018; Endgaw, 2021).

The proliferation of water hyacinth presents a significant challenge due to its adverse effects on aquatic environments. However, its high cellulose content, ranging from 17.3% to 34.19%, along with substantial hemicellulose (17.66%–49.2%) and lignin content (1.1%–12.22%), has been reported across multiple studies as summarized by (Gaurav *et al.*, 2020), offers a potential opportunity for its utilization in industrial and environmental applications (Arivendan *et al.*, 2022). The challenge lies in efficiently converting this abundant resource into valuable products, such as glucose, which can serve as a precursor for biofuels and other bioproducts. Current hydrolysis methods often require pretreatment steps that can be costly and environmentally taxing, presenting barriers to widespread adoption (Ruan *et al.*, 2016). Figure 1 shows the schematic illustration of the cellulose hydrolysis process.

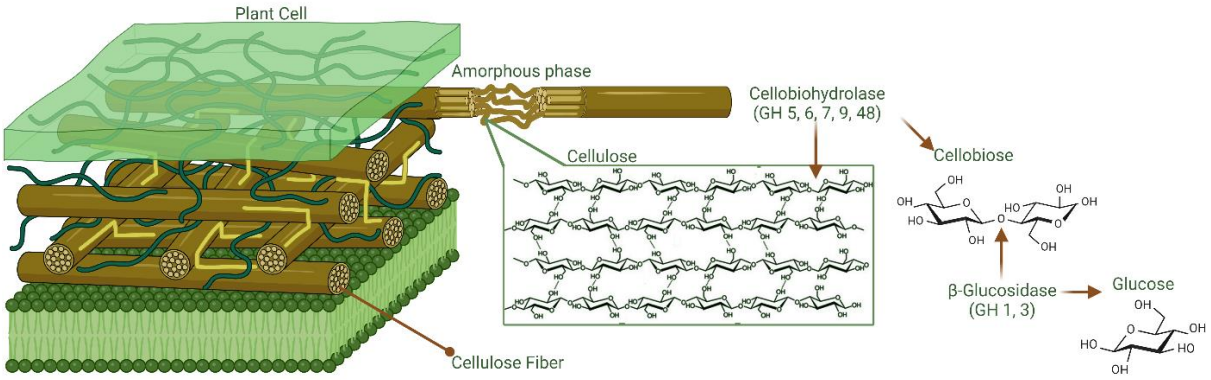

Fig. 1: Schematic of cellulose hydrolysis process

This research proposes the use of acid-catalyzed hydrolysis as a promising approach to convert water hyacinth cellulose into glucose without alkaline pretreatment. Generally, alkaline pretreatment is often necessary in cellulose hydrolysis processes to remove lignin and hemicellulose, thereby increasing cellulose accessibility for subsequent hydrolysis with acid catalysts (Hidayatulloh *et al.*, 2021; Martínez *et al*., 2015). In the case of water hyacinth hydrolysis, the alkali pretreatment used in the previous study (Wulandari *et al.*, 2023) resulted in a yield of 8.67 - 17.17%, which did not demonstrate significant effects from the alkali pretreatment. Considering this, when the lignin content is not high, that is 7% (Ratnani *et al*., 2021), and the hydrolysate will be used as a substrate in a subsequent bioprocess, eliminating alkaline pretreatment will be beneficial because by doing so, we avoid using excessive chemicals. By focusing on optimizing the hydrolysis conditions, specifically temperature, catalyst types, concentrations, and time, this study aims to develop an acid hydrolysis method that uses fewer chemicals so that the hydrolysate will be bioprocess-friendly, i.e., more consumable as a substrate by the microorganism in the subsequent fermentation. Several studies on chemical hydrolysis have primarily focused on energy-efficient and cost-effective methods (H. Wang *et al*., 2020; Zhang *et al*., 2012), while research on minimizing chemical addition and testing the hydrolysate as the substrate in the bioprocess application has not yet been conducted. This approach could lead to more sustainable and economically viable solutions for managing water hyacinth invasions and utilizing it as a renewable resource.

Chemical hydrolysis using acids such as HCl and H_2SO_4 has been identified as a highly effective method for breaking down cellulose in water hyacinth into glucose, a crucial step in producing bioethanol and other bioproducts (Dahiya *et al*., 2021). Acids, particularly HCl and H_2SO_4, can penetrate the cellulose structure more effectively than alkaline solutions, which primarily target lignin removal. Acid hydrolysis can directly cleave the glycosidic bonds in cellulose, leading to faster solubilization of carbohydrates and better glucose yield (Cheng *et al*., 2014). In comparison, alkaline pretreatments are primarily used to remove lignin and hemicellulose, but are less effective at breaking down cellulose itself. Acid hydrolysis provides a rich source of fermentable sugars, essential for bioproduct production.

Numerous studies have explored the hydrolysis of water hyacinth, yet challenges remain in achieving efficient conversion without pretreatment steps (Bronzato *et al*., 2018). While acid hydrolysis is effective, variations in experimental conditions have led to inconsistent results, indicating the need for optimized protocols tailored to specific catalysts and conditions (Z. Wang *et al*., 2019). Furthermore, existing research often overlooks the potential for milder, more sustainable hydrolysis conditions that can maintain high glucose yields (Cheng *et al*., 2014).

There is a noticeable gap in studies focusing on optimizing hydrolysis conditions that are more environmentally friendly and cost-effective. The absence of alkaline pretreatment and the potential for lower temperature and catalyst concentration conditions present an opportunity for innovation in the field (Tadesse *et al*., 2022). This research aims to address these gaps by systematically investigating the optimal hydrolysis parameters.

Studies have shown that optimizing these parameters can lead to significant improvements in glucose yield, paving the way for the efficient conversion of water hyacinth into biofuels and other valuable products (Ruan *et al*., 2016). One of the optimization methods that is widely used is response surface methodology (RSM). By leveraging the capabilities of RSM, this research aims to refine the hydrolysis process that helps to determine the optimal hydrolysis conditions, temperature, catalyst concentration (using HCl and H_2SO_4), and time for maximizing glucose yield and contributing to the sustainable utilization of water hyacinth.

RSM has been effectively employed in various studies to optimize the conditions for hydrolysis, providing a systematic approach to evaluating the effects of multiple variables and their interactions (Bronzato *et al*., 2018). The central composite design (CCD) within RSM allows for the identification of optimal conditions by assessing the impact of factors such as temperature, catalyst concentration, and hydrolysis time (Das *et al*., 2016). Previous research has highlighted the importance of these factors in maximizing glucose yield while minimizing degradation to undesired byproducts (Dhankhar *et al*., 2014).

This research introduces a novel approach to acid-catalyzed hydrolysis by focusing on more bioprocess-friendly conditions that do not require alkaline pretreatment. This research supports bioprocess and bioproduct applications essential for the green industry era and advancing sustainable development through resource efficiency and eco-friendly technologies. By utilizing RSM, this study seeks to enhance the efficiency and sustainability of the hydrolysis process, contributing to innovative solutions for managing water hyacinth invasions. The study involves laboratory-scale experiments to identify optimal conditions for hydrolyzing water hyacinth. The independent variables include temperature, HCl, and H_2SO_4 concentrations, and hydrolysis time, while the dependent variable is glucose yield. Statistical analyses, including ANOVA and optimization algorithms, will be employed to validate and refine the predictive model. The findings will provide insights into the effective conversion of water hyacinth into glucose, facilitating its use as a renewable resource.

MATERIALS AND METHODS

Design of the Experiment Based on RSM

The experimental design was structured using response surface methodology (RSM) with a central composite design (CCD) to optimize the hydrolysis of water hyacinth. The design varied three factors: concentration of the catalyst (X_A), temperature (X_B), and reaction time (X_C). These factors were tested at five levels: -1.68, -1, 0, 1, and 1.68, corresponding to actual values for concentration from 0.32 N to 3.68 N, temperature from 59.77°C to 110.23°C, and time from 46.36 minutes to 113.63 minutes. This approach facilitated a comprehensive analysis of each variable's effect on the glucose yield and its interactions. The factors and levels used for hydrolysis are shown in Table 1.

Experiment Design

The experiments were structured according to the design matrix provided in Table 2, detailing 20 runs for each catalyst (HCl and H_2SO_4). Each combination of coded factors X_A, X_B, and X_C corresponded to specific real-world conditions of acid concentration,

temperature, and time. This matrix allowed the systematic exploration of the experimental space to identify optimal conditions for maximizing glucose yield from water hyacinth hydrolysis.

Materials

The materials used in this study included water hyacinth, which served as the raw material due to its high cellulose content conducive for hydrolysis into glucose (Arivendan *et al*., 2022). For the hydrolysis reaction, HCl and H_2SO_4 were employed as catalysts. Additional materials used in the experimental setup included deionized water, Fehling's solution A and B, NaOH, methylene blue, and anhydrous glucose, which were used for the glucose analysis post-hydrolysis.

Sample Preparation

The preparation of the water hyacinth involved cleaning and separating the leaves

Table 1. Factors and levels used for hydrolysis

Factor	Unit	Notation	Level				
			-1.68	**-1**	**0**	**1**	**1.68**
Concentration	N	X_A	0.32	1	2	3	3.68
Temperature	^oC	X_B	59.77	70	85	100	110.23
Time	minutes	X_C	46.36	60	80	100	113.63

Table 2. Design matrix of the experiment

HCl	H_2SO_4	Coded Factor			Uncoded Factor		
		X_A	X_B	X_C	X_A	X_B	X_C
A1	B1	-1	-1	-1	1	70	60
A2	B2	1	-1	-1	3	70	60
A3	B3	-1	1	-1	1	100	60
A4	B4	1	1	-1	3	100	60
A5	B5	-1	-1	1	1	70	100
A6	B6	1	-1	1	3	70	100
A7	B7	-1	1	1	1	100	100
A8	B8	1	1	1	3	100	100
A9	B9	-1,68	0	0	0.32	85	80
A10	B10	1,68	0	0	3.68	85	80
A11	B11	0	-1,68	0	2	59.77	80
A12	B12	0	1,68	0	2	110.23	80
A13	B13	0	0	-1,68	2	85	46.36
A14	B14	0	0	1,68	2	85	113.63
A15	B15	0	0	0	2	85	80
A16	B16	0	0	0	2	85	80
A17	B17	0	0	0	2	85	80
A18	B18	0	0	0	2	85	80
A19	B19	0	0	0	2	85	80
A20	A20	0	0	0	2	85	80

and stems, which were then cut into 1-3 cm segments. The prepared biomass was oven-dried at 80°C for 30 minutes to reduce moisture content, enhancing the efficiency of the hydrolysis process. Subsequently, the dried biomass was ground and sieved to achieve a uniform particle size, ensuring consistent exposure of reactant during the hydrolysis.

Experimental Setup

The experimental setup for the hydrolysis included a hotplate stirrer, boiling flasks with flat bottoms, cooling condensers, and digital thermometers to monitor the reaction temperature accurately. Hydrolysis was performed by dissolving the water hyacinth in a dilute acid solution, maintaining a substrate to solvent ratio of 1:20 (w/v). The system was equipped with a reflux setup to maintain the reaction conditions and a magnetic stirrer to ensure homogenous mixing throughout the process.

Parameters

The primary parameters measured during the hydrolysis process included the concentration of HCl and H_2SO_4, temperature, and reaction time. These parameters were varied within specified ranges to identify the optimum conditions that maximize glucose yield. The concentration of the catalyst ranged from 1 N to 3 N, temperatures from 70°C to 100°C, and hydrolysis time from 60 to 100 minutes, aligning with the conditions used in previous studies to allow a comprehensive analysis of their impacts on glucose yield (Arivendan *et al*., 2022).

Statistical Analysis

Statistical analysis was employed to evaluate the significance of the hydrolysis parameters on the glucose yield using the RSM. ANOVA was utilized to determine the fit of the regression models and the significance of each parameter and its interactions. The results were interpreted to refine the model, ensuring optimal predictions of glucose yields from the hydrolysis process. The software used for the statistical analysis was Minitab 19, which facilitated the regression analysis and optimization of the hydrolysis conditions.

Fermentation Experiment Setup

Fermentation was conducted using hydrolysates from acid-catalyzed water hyacinth hydrolysis as substrates, neutralized with NaOH. Following hydrolysis, the fibrous water hyacinth (initially chopped to 1–3 cm) was fully broken down into a slurry-like consistency, indicating substantial degradation of the biomass structure. Effective microorganisms 4 (EM4) served as the fermentation starter. Each fermentation batch had a volume of 2000 ml, and the process was monitored over two weeks by measuring microbial growth (OD600). The glucose in the hydrolysates was the primary carbon source. Comparisons were made between substrates hydrolyzed with HCl, H_2SO_4, and untreated control.

RESULT AND DISCUSSION

The experimental results obtained from the optimization of hydrolysis conditions using HCl and H_2SO_4 for water hyacinth are summarized in Table 3, which provides the glucose yields for each experiment set according to the RSM.

Effectiveness of hydrolysis without alkaline pretreatment

This study explores acid-catalyzed

hydrolysis as an efficient method for converting water hyacinth cellulose into glucose without the need for alkaline pretreatment. Traditionally, alkaline pretreatment is used to remove lignin and hemicellulose, increasing cellulose accessibility for subsequent acid hydrolysis (Martínez *et al.*, 2015). However, water hyacinth has a relatively low lignin content, and previous research by (Wulandari *et a*l., 2023) showed that alkaline pretreatment produced glucose yields of 8.67% to 17.17% based on raw biomass, with limited improvement compared to acid hydrolysis alone. Another study (Jongmeesuk *et al.*, 2014) that used alkaline pretreatment also showed a relatively similar glucose yield of 15.63%. Given these findings, eliminating alkaline pretreatment offers the advantage of reducing chemical use, streamlining the process, and making the hydrolysate more suitable for bioprocessing in fermentation. In this study, a maximum yield of 19.25% was obtained with HCl and 17.50% with H_2SO_4 based on raw biomass, which is a higher yield compared to several of the previously mentioned studies.

Optimizing hydrolysis conditions such as temperature, catalyst concentration, and reaction time has proven effective in breaking down cellulose without the need for lignin removal. A study by Choudhary *et al.* (2016) confirmed that sulfuric acid (H_2SO_4) hydrolysis, even without alkaline pretreatment, yields high glucose concentrations, supporting the view that under optimized conditions, significant results can be achieved without additional pretreatment complexity. Choudhary found that 4% H_2SO_4 at optimal temperatures produced glucose yields comparable to those involving alkaline pretreatment, showing the viability of this simplified approach.

Table 3. Design matrix and response value for glucose yield

HCl	Response (Yield) %	H_2SO_4	Response (Yield) %
A1	7.52	B1	5.60
A2	12.75	B2	9.80
A3	12.60	B3	14.53
A4	14.18	B4	12.25
A5	12.08	B5	7.18
A6	11.37	B6	8.93
A7	16.10	B7	12.60
A8	11.20	B8	12.78
A9	6.83	B9	7.88
A10	17.15	B10	10.50
A11	9.45	B11	7.35
A12	14.53	B12	17.50
A13	13.65	B13	12.60
A14	13.82	B14	14.70
A15	16.10	B15	15.05
A16	17.15	B16	15.58
A17	19.25	B17	16.10
A18	18.55	B18	15.93
A19	15.92	B19	15.23
A20	15.75	A20	17.33

The effectiveness of acid hydrolysis, particularly with H_2SO_4, is further demonstrated in the SEM images (Figure 2), which reveal distinct morphological changes in the cellulose structure. The untreated cellulose appears smooth and dense, with minimal disruption to its surface, highlighting the natural barriers lignin and hemicellulose pose in limiting access to cellulose. In contrast, HCl-treated fibers display roughened surfaces, with cracks and fissures that suggest partial disruption of the cellulose structure. Hydrochloric acid is effective for moderate depolymerization, loosening the fibers without fully fragmenting them. This level of disruption improves accessibility but is less aggressive than H_2SO_4.

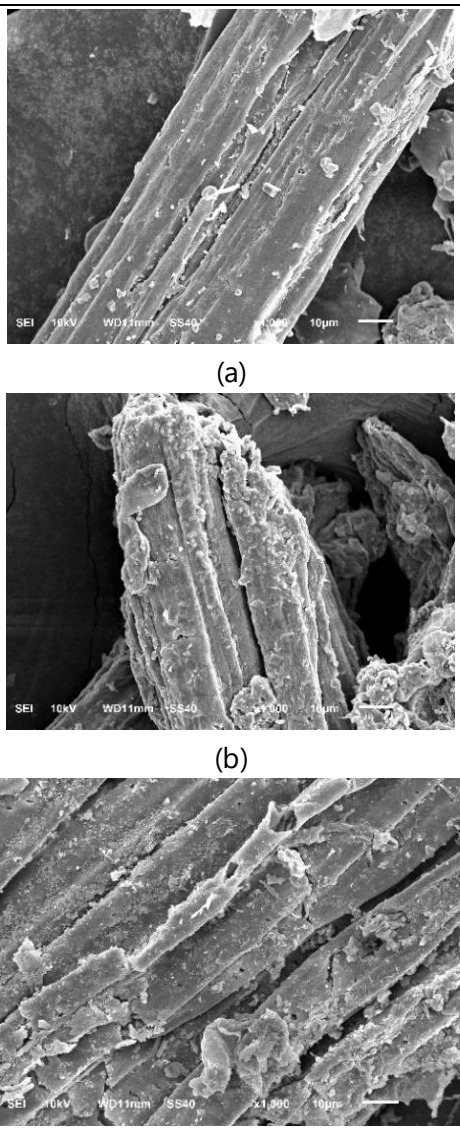

Fig. 2: SEM images for (a) before hydrolysis, (b) hydrolyzed by HCl, and (c) hydrolyzed by H_2SO_4

The chemical properties and degradation mechanisms can explain the difference in aggressiveness between HCl and H_2SO_4 in cellulose hydrolysis. HCl, with a lower acid strength (*p*Ka = -8) compared to H_2SO_4 (*p*Ka = -3), causes moderate depolymerization by cleaving glycosidic bonds without esterifying the fiber surface, resulting in looser fibers with rough surfaces and partial cracks (Wang *et al.*, 2020). This improves cellulose accessibility but does not achieve complete fragmentation as with H_2SO_4. In contrast, H_2SO_4 not only cleaves glycosidic bonds but also esterifies cellulose hydroxyl groups with sulfate, creating negative surface charges that accelerate fiber dissociation into crystalline nanoparticles (CNCs) with more intensive fragmentation (Ioelovich, 2023). Comparative studies show that H_2SO_4 hydrolysis produces CNCs with better colloidal stability in water but reduces thermal stability due to sulfate groups. In comparison, HCl yields fibers with higher thermal stability despite lower colloidal stability (Wang *et al.*, 2020). These results align with the SEM findings in the manuscript, where H_2SO_4 causes extensive porosity and fragmentation, whereas HCl only damages the surface structure without complete disintegration. This difference is also reflected in applications: H_2SO_4 is more effective for high glucose production in biorefineries, while HCl is suitable for applications requiring partial fiber integrity, such as composite materials (Ashfaq, 2023). Thus, the choice of catalyst depends on the end goal of the process and the trade-off between hydrolysis efficiency and desired product properties.

The sulfuric acid-treated fibers, however, show extensive fragmentation and porosity, indicating a more complete breakdown of the cellulose-lignin matrix. This aggressive hydrolysis exposes a larger surface area, maximizing glucose yield but potentially complicating material handling due to over-fragmentation. Comparing the two acids, H_2SO_4 proves more effective at degrading cellulose, offering superior glucose production, although HCl may be easier to handle in industrial applications. The SEM analysis, along with the optimization of hydrolysis conditions, highlights the importance of tailoring the process to the specific goals of yield, efficiency, and downstream processing.

Table 4. Glucose yield for X_A, X_B, and X_C

Source	Notation	Sum of Squares	Mean Square	Coefficient	Standard Error	*p*	R^2	R^2 (adj)
HCl								
Constant				0.17122	0.00815	0.000		
Concentration	X_A	0.002521	0.002521	0.01359	0.00541	0.031		
Temperature	X_B	0.002617	0.002617	0.01384	0.00541	0.028		
Time	X_C	0.000116	0.000116	0.00292	0.00541	0.601		
Concentration* Concentration	$X_A * X_A$	0.004819	0.004819	-0.01829	0.00526	0.006		
Temperature* Temperature	$X_B * X_B$	0.004819	0.004819	-0.01829	0.00526	0.006		
Time*Time	$X_C * X_C$	0.002116	0.002116	-0.01212	0.00526	0.044	0,82	0,65
Concentration* Temperature	$X_A * X_B$	0.000768	0.000768	-0.00980	0.00706	0.196		
Concentration* Time	$X_A * X_C$	0.001928	0.001928	-0.01553	0.00706	0.053		
Temperature* Time	$X_B * X_C$	0.000088	0.000088	-0.00333	0.00706	0.648		
Lack of Fit		0.002899	0.000580			0.154		
Error		0.003993	0.000399					
Total		0.021939						
H_2SO_4								
Constant				0.15904	0.00455	0.000		
Concentration	X_A	0.00050	0.00050	0.00605	0.00302	0.073		
Temperature	X_B	0.01042	0.01042	0.02762	0.00302	0.000		
Time	X_C	0.00006	0.00006	0.00208	0.00302	0.507		
Concentration* Concentration	$X_A * X_A$	0.00961	0.00961	-0.02583	0.00294	0.000		
Temperature* Temperature	$X_B * X_B$	0.00298	0.00298	-0.01439	0.00294	0.001		
Time*Time	$X_C * X_C$	0.00146	0.00146	-0.01006	0.00294	0.007	0.95	0.91
Concentration* Temperature	$X_A * X_B$	0.00081	0.00081	-0.01006	0.00395	0.029		
Concentration* Time	$X_A * X_C$	0.00000	0.00000	0.00001	0.00395	0.998		
Temperature* Time	$X_B * X_C$	0.00006	0.00006	-0.00264	0.00395	0.519		
Lack of Fit		0.00091	0.00018			0.148		
Error		0.00125	0.00013					
Total		0.02534						

Statistical Analysis of Glucose Yield

RSM was employed to identify the optimal conditions for hydrolyzing water hyacinth into glucose using HCl and H_2SO_4 as catalysts. The regression analysis and ANOVA results presented in Table 4 provide a comprehensive understanding of the effects of varying hydrolysis temperature (X_B), catalyst concentration (X_A), and hydrolysis time (X_C) on glucose yield.

The regression equations derived for both catalysts are presented as Eq. (1) for HCl and Eq. (2) for H_2SO_4.

$$Yield_{HCl} = -1.111 + 0.2044\,X_A + 0.01693\,X_B + 0.00749\,X_C - 0.01829\,X_A^2 - 0.000081\,X_B^2 - 0.000030\,X_C^2 - 0.000653\,X_A X_B - \textit{0.000776}\,X_A X_C - 0.000011\,X_B X_C \quad (1)$$

$$Yield_{H2SO4} = -0.918 + 0.1663\,X_A + 0.01476\,X_B + 0.00487\,X_C - 0.02583\,X_A^2 - 0.000064\,X_B^2 - 0.000025\,X_C^2 - 0.000671\,X_A X_B + \textit{0.000001}\,X_A X_C - 0.000009\,X_B X_C \quad (2)$$

The coefficients signify the impact of each variable and its interactions on glucose yield. Key observations from the ANOVA include. The p-values indicate which factors significantly affect glucose yield. For HCl, the significant terms include the concentration of HCl (X_A), temperature (X_B), the square of concentration (X_A^2), and the interaction between concentration and temperature (X_AX_B). For H_2SO_4, significant factors include temperature (X_B) and its square (X_B^2), with interactions between concentration and temperature also playing a notable role. Although time is generally a critical factor in biomass hydrolysis processes, particularly for pretreatment, the lack of a significant effect of time X_c on glucose yield in this study can be explained by the optimized reaction conditions and the specific characteristics of the acid-catalyst system used. The ANOVA analysis (Table 4) shows that the *p-value* for time (0.601 for HCl; 0.507 for H_2SO_4) is not significant ($\alpha = 0.05$), indicating that variations in time (46–114 minutes) do not statistically affect glucose yield under optimized concentration and temperature conditions. This may be due to several reasons. First, the high reaction rates facilitated by strong acid concentrations (HCl 2.36N; H_2SO_4 1.91N) and optimal temperatures (89–100°C), which accelerate cellulose depolymerization, reaching reaction saturation within a relatively short time (80 minutes). Similar studies using 43% HCl at room temperature show that hydrolysis reactions reach maximum efficiency within 24 hours (Leenders *et al*., 2025), but under high acid concentrations and elevated temperatures, the main reaction stages can be completed within minutes (Chang *et al*., 2018). The Second is dominance of concentration and temperature factors: the quadratic interactions of concentration (X_A^2) and temperature (X_B^2) are more dominant in the regression model, suggesting that the non-linear effects of these parameters "overshadow" the contribution of time. In systems with strong catalysts, increasing concentration and temperature can compensate for the need for longer reaction times (Świątek *et al*., 2020). The third is glucose degradation at prolonged times: under strong acid conditions, the produced glucose can degrade into byproducts (e.g., HMF or levulinic acid) if the reaction time is extended. Data in Table 3 show that HCl glucose yield decreases from 19.25% (A17) to 15.75% (A20) as time increases, although not statistically significant. A similar phenomenon is reported in concentrated acid hydrolysis, where excessive reaction time reduces glucose yield due to degradation reactions (Chang *et al*., 2018; Świątek *et al.*, 2020). Thus, the insignificance of time in this study reflects that other parameters (concentration, temperature) have been adjusted to achieve maximum efficiency within a narrow time range, while prolonged time may be counterproductive.

The coefficient of determination (R^2) for the models using HCl and H_2SO_4 is 0.82 and 0.85, respectively. These values suggest that

the models can predict the optimal glucose yield with high accuracy, as R^2 values closer to 1.0 indicate a better fit of the model to the data. The lack of fit being non-significant further validates the suitability of the model for predicting the outcomes under the given experimental conditions.

The regression coefficients describe the direction and magnitude of the influence exerted by each factor on the glucose yield. Positive coefficients indicate an enhancing effect on yield, while negative coefficients suggest inhibitory effects. For instance, the negative coefficients for the square terms (X_A^2 and X_B^2) imply that beyond a certain level, increasing the concentration or temperature may reduce the yield, highlighting the importance of optimizing these parameters.

The interaction terms in the regression equation, such as X_AX_B, demonstrate how the effects of one factor depend on the level of another. For example, the negative interaction term between concentration and temperature for HCl suggests that the combined increase of these factors may lead to a decrease in glucose yield, emphasizing the need for careful control of experimental conditions during hydrolysis.

Statistical analysis underscores the complexity of the hydrolysis process and the significant role played by each parameter and its interactions. This detailed understanding allows for precise adjustment of conditions to maximize glucose yield from water hyacinth, supporting the development of more efficient bioconversion processes.

Effects of Factors on Glucose Yield

The RSM was utilized to elucidate the interactions between catalyst concentration, temperature, and time in the hydrolysis of water hyacinth using HCl and H_2SO_4. The associated response surface plots vividly demonstrate how these variables interact to optimize glucose yield, as shown in Figure 3.

The highest glucose yields for HCl were achieved at a concentration of 2N and a temperature of 85°C, aligning with previous studies that suggest moderate temperatures combined with optimal acid concentrations maximize efficiency (Moodley & Gueguim Kana, 2018). For H_2SO_4, the optimal yield appeared at a higher temperature of 110.23°C at the same concentration, supporting findings by (Fileto-Pèrez *et al*., 2013) that higher temperatures can facilitate more effective cellulose breakdown.

The interaction between time and temperature also highlights the importance of adequate processing time. For HCl, the optimal yield at 80 minutes and 85°C confirms the need for sufficient reaction time to achieve significant hydrolysis, a finding consistent with (Xia *et al*., 2013). Similarly, for H_2SO_4, optimal results were obtained at 80 minutes and 110.23°C, underscoring the necessity of balancing higher temperatures with precise times to prevent degradation of glucose, as noted by Zhang *et al*. (2012).

The effects of time and concentration reveal that for HCl, peak efficiency was at 76.94 minutes and 2.36N. This supports the theory from (Sun *et al*., 2014) that higher concentrations increase hydrolysis efficiency up to a certain threshold. For H_2SO_4, the optimal conditions were slightly different at 79.66 minutes and 1.91N, suggesting a more delicate balance for sulfuric acid, likely due to its stronger nature, as discussed in (Tantayotai *et al*., 2019).

The results of this study illustrate that glucose yields for HCl ranged from 6.83% to 19.25%, and for H_2SO_4 from 5.60% to 17.50%. These findings are in line with the broader literature, which notes that the effectiveness of hydrolysis depends significantly on the

specific conditions and type of acid used (Eka-Sari *et al*., 2014). The optimal conditions discovered here validate the robustness of using RSM to optimize hydrolysis processes and align well with previously established parameters in the literature.

Overall, this detailed understanding of how temperature, concentration, and time interplay offers substantial implications for improving the commercial viability of biofuel production from water hyacinth. By refining these parameters, the process becomes more sustainable and efficient, promoting better utilization of this invasive species for bioenergy applications.

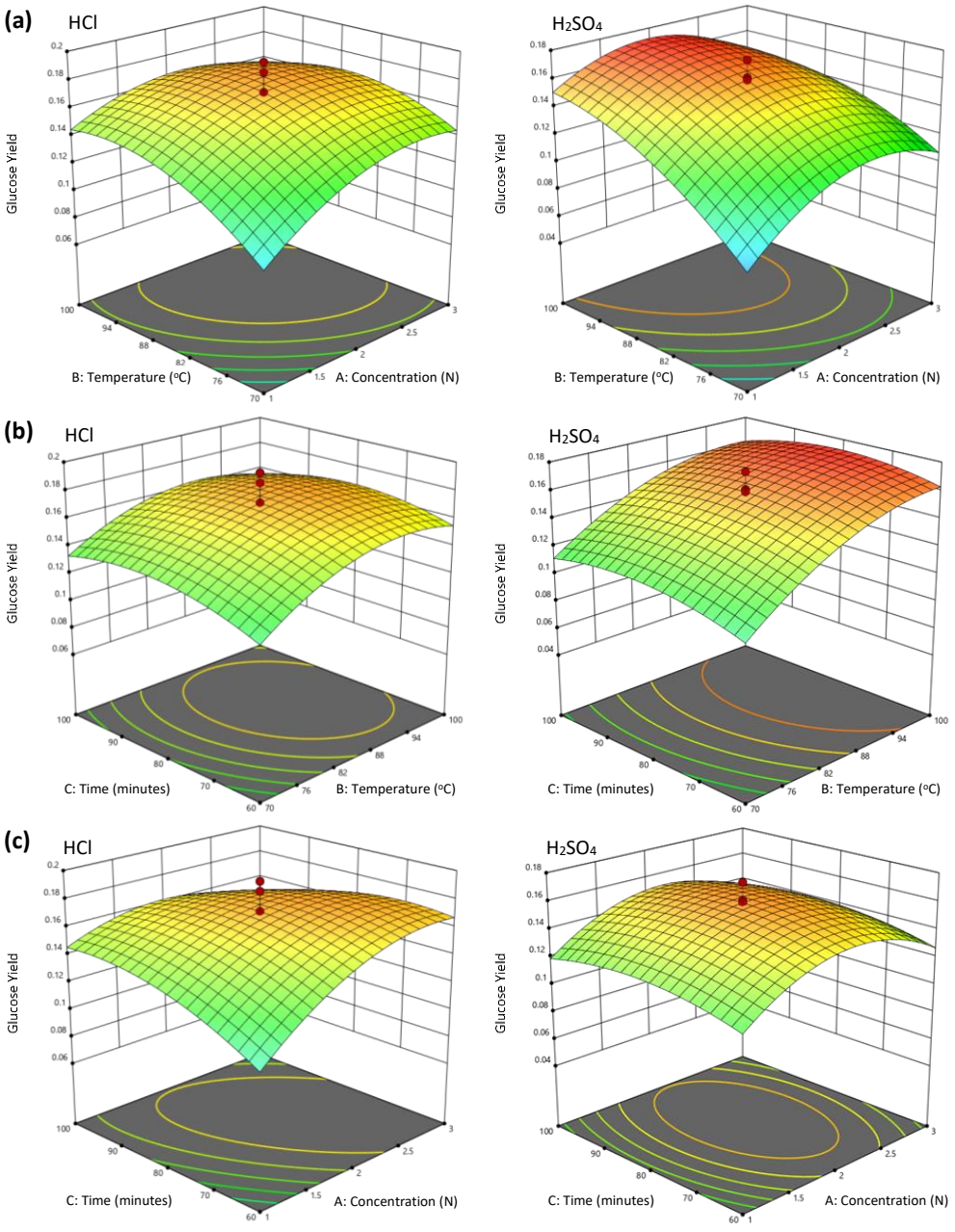

Fig. 3: Response surface plot for glucose yield of HCl and H_2SO_4 catalysts: the plots for (a) concentration and temperature, (b) time and temperature, and (c) time and concentration illustrate

Optimization of Glucose Yield

Optimization studies focused on identifying which catalyst, between HCl and H_2SO_4, produces the highest glucose yield under controlled conditions of concentration, temperature, and time. The resulting data were graphically represented (in Figure 4) to show how each factor influenced the hydrolysis process clearly.

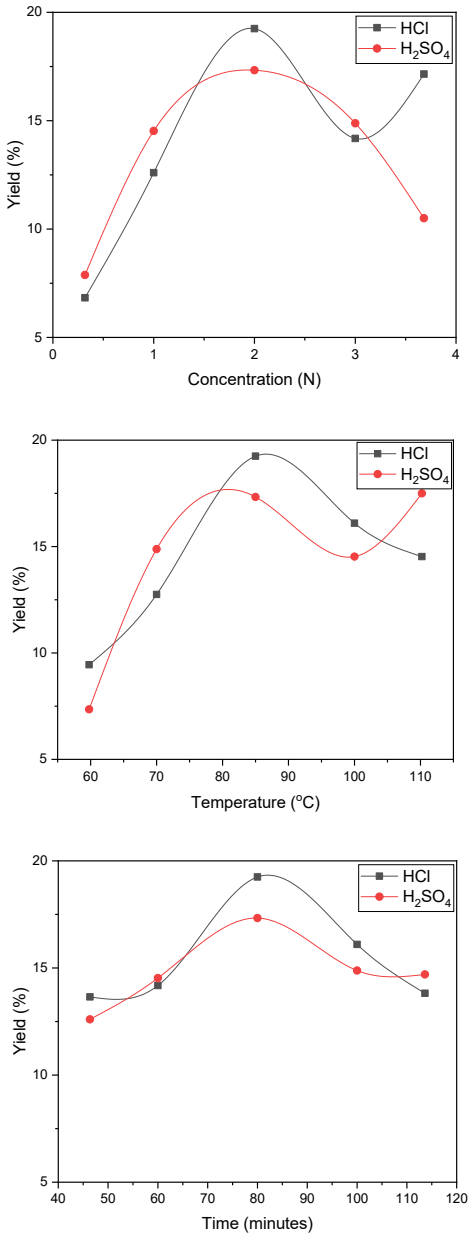

Fig. 4: Optimization plot for the optimum (a) Concentration, (b) Temperature, and (c) Time

The plots demonstrate the optimal conditions for maximizing glucose yield with each catalyst. For both HCl and H_2SO_4, the glucose yield peaked at a concentration of 2N. This peak is consistent with optimal yields observed in previous studies, where a balance between acid strength and material breakdown was achieved without excessive degradation of glucose.

The optimal temperature for achieving maximum glucose yield with HCl was approximately 85°C, while for H_2SO_4, it was higher, around 110°C. This difference underscores the varying thermal stability and reaction kinetics associated with each acid, corroborating literature that suggests H_2SO_4 may require higher temperatures to effectively break down cellulose (Ashfaq, 2023; Ioelovich, 2023).

A reaction time of around 80 minutes was found to be optimal for both catalysts, aligning with findings that longer exposure times allow for more complete cellulose hydrolysis, thus increasing glucose yield (Ashfaq, 2023).

The composite optimization values, D, calculated were 0.86 for HCl and 0.98 for H_2SO_4, indicating a slightly higher efficiency for H_2SO_4 under the conditions tested. Comparative analysis with prior research highlights the importance of carefully balancing hydrolysis conditions. Studies have emphasized that while higher temperatures can accelerate hydrolysis reactions, they must be carefully controlled to prevent glucose degradation (Ashfaq, 2023). Similarly, the concentration of the hydrolyzing agent plays a crucial role, with optimal levels needing to be identified to maximize yield while avoiding cost inefficiencies (Sun *et al.*, 2014; Zhen-lei *et al.*, 2019). The relationship between reaction time and yield further supports the necessity of optimizing hydrolysis duration to enhance

overall process efficiency (Adewuyi & Deshmane, 2015).

This study's findings offer valuable insights into the optimal hydrolysis conditions for converting water hyacinth into glucose, using either HCl or H_2SO_4. The research not only supports existing literature on the subject but also provides practical guidelines for scaling up biofuel production processes, promoting more efficient and sustainable industrial applications.

Experimental Verification

Experimental verification was conducted to validate the predictive accuracy of the optimized hydrolysis conditions using HCl and H_2SO_4 as catalysts. This verification utilized two main approaches: regression analysis for predicted versus actual yields, shown in Figure 5, and experimental re-testing under optimal conditions shown in Table 5.

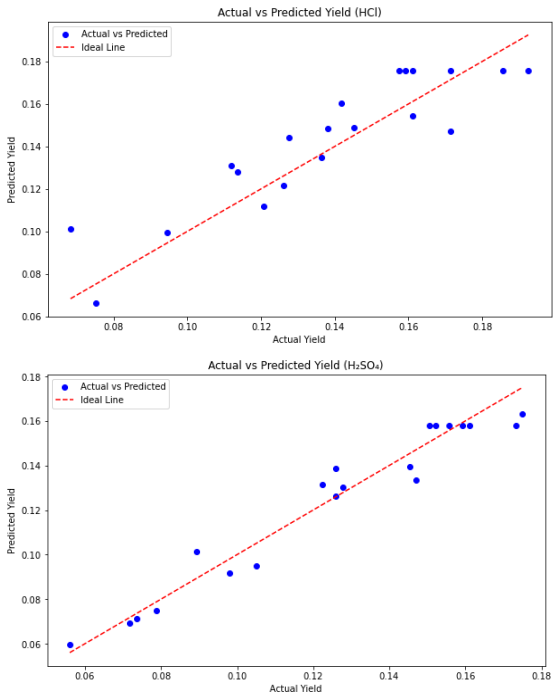

Fig. 5: Plot for predicted versus actual glucose yield from hydrolyzed with (a) HCl (b) H_2SO_4

Table 5. Experiment verification

Sample	Yield		
	Predicted	Verification	Relative Deviation (%)
HCL	17.55	17.37	1.02
H_2SO_4	17.24	17.20	0.23

The regression analysis revealed, that for HCl, the regression plot shows an R^2 value of 0.80, indicating good predictive accuracy of the model, which captures a substantial portion of the variability in glucose yields. Although the fit is strong, there is still some room for refinement to account for unexplained variability. The regression plot of H_2SO_4 displays an even higher R^2 value of 0.95. This exceptional level of fit suggests that the model predictions are extremely close to the actual outcomes, demonstrating that the model effectively captures the dynamics of the hydrolysis process under the influence of sulfuric acid. Following the regression analysis, the experimental re-testing was conducted as shown in Table 5.

The results from this phase of verification include the predicted yield for HCl, which was 17.55%, and the actual experimental yield was closely matched at 17.37%, yielding a relative deviation of 1.02%. This close match confirms the model's effectiveness and its applicability in practical scenarios. Similarly, the predicted yield for H_2SO_4 was 17.24%, with an actual yield of 17.20%, resulting in an even smaller relative deviation of 0.23%. This negligible deviation strongly validates the model's high precision for predicting outcomes with sulfuric acid.

This structured approach to verification involves first assessing the regression model's accuracy with Figure 5 and then confirming the model's reliability with actual experimental data in Table 5. effectively demonstrates the robustness and applicability of the optimized hydrolysis

conditions. Both the regression analysis and the experimental verification underscore the models' accuracy in predicting glucose yields, reinforcing the confidence in using these models for scaling up the hydrolysis process in industrial applications.

Fermentation Verification

Fermentation experiments were conducted using the hydrolyzed results of HCl and H_2SO_4 as substrates for biofertilizer fermentation, utilizing Effective Microorganisms 4 (EM4) as the fermentation starter. The growth rate of microorganisms on different substrates was monitored to evaluate the effectiveness of the hydrolysis process in providing a viable carbon source for biofertilizer production.

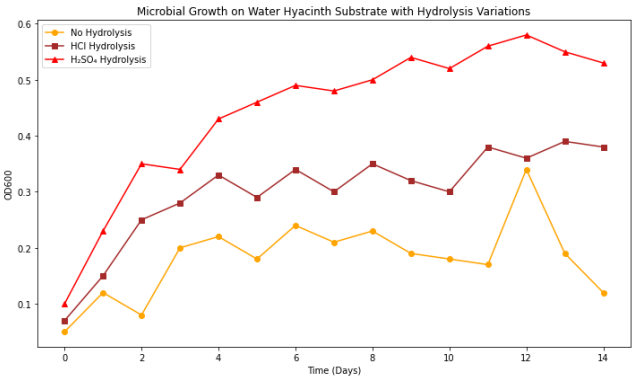

Fig. 6: Verification of biofertilizer fermentation

The observed growth rates, as illustrated in Figure 6, show a marked difference between substrates without hydrolysis and those treated with HCl and H_2SO_4. Notably, the growth trajectory using the substrate hydrolyzed with H_2SO_4 exhibited a higher growth rate, indicating a superior availability of carbon (glucose), which is essential for microbial activity and growth.

The glucose derived from hydrolysis served as a crucial carbon source, significantly impacting the growth and metabolic activities of the biofertilizer bacteria. This is evident from the higher optical density (OD600) values in substrates treated with H_2SO_4 compared to those treated with HCl and the control (substrate without hydrolysis).

The substrate hydrolyzed with H_2SO_4 supported more robust microbial growth, suggesting that the hydrolysis process was more efficient in breaking down the water hyacinth into usable glucose. This efficiency is critical, as biofertilizer bacteria, including plant growth-promoting bacteria (PGPB), rely heavily on readily available glucose to trigger metabolic processes that benefit plant growth (Madhaiyan *et al.*, 2009; Ng *et al.*, 2024).

Glucose availability influences key metabolic pathways in biofertilizer bacteria, such as carbon source utilization and catabolite repression (Yao *et al.*, 2011). The efficient use of glucose facilitates better nutrient uptake from the host plant, enhancing overall plant development (Ng *et al.*, 2024).

Adequate glucose supply not only enhances the immediate microbial activity but also influences the broader soil microbial community, leading to improved nutrient transformation and plant growth promotion (Li *et al.*, 2023). This dynamic condition underscores the importance of optimizing hydrolysis states to maximize glucose yield, thereby supporting more effective and sustainable biofertilizer applications.

In conclusion, the fermentation verification highlighted the significant role of glucose as a substrate derived from hydrolyzed water hyacinth, particularly when using H_2SO_4. The results demonstrate that the optimized hydrolysis conditions effectively support microbial growth, which is pivotal for developing biofertilizer products aimed at

enhancing agricultural productivity and sustainability.

CONCLUSION

The hydrolysis conducted without prior alkaline pretreatment resulted in a good glucose yield, demonstrating that the process can proceed effectively under optimized acid-catalyzed conditions. The research confirmed that both catalysts could hydrolyze water hyacinth efficiently, provided the hydrolysis was carried out under optimal conditions, thus avoiding the additional complexity and cost of pretreatment. Optimum conditions were identified for each catalyst: for HCl, a concentration of 2.36N, a temperature of 89.33°C, and a hydrolysis time of 76.94 minutes; for H_2SO_4, a concentration of 1.91N, a temperature of 100.03°C, and a hydrolysis time of 79.66 minutes.

Notably, H_2SO_4 outperformed HCl, producing a slightly higher glucose yield under optimal conditions and demonstrating better performance in fermentation trials. Fermentation experiments revealed that the glucose derived from H_2SO_4 hydrolysis provided a more effective carbon source, supporting higher microbial growth rates than HCl, further underscoring H2SO4's superior performance. This finding highlights the important implications of hydrolysis optimization to prepare an appropriate feedstock for the subsequent bioprocess.

Overall, the study underscores that water hyacinth, an invasive aquatic plant, is a valuable resource for further processing to produce useful products. By refining hydrolysis conditions, this research contributes to the development of cost-effective and environmentally friendly technologies for biomass conversion, offering a promising approach to managing water hyacinth infestations while producing valuable bioproducts. Future work will focus on further improving the efficiency of these processes and exploring the scalability of the technology in industrial applications.

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