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Rsm Modeling and Optimization of Acid-Catalyzed Hydrolysis for Sustainable Bioethanol Production from Mixed Waste Biomass

Samyami DP[1], Girisha ST[2], Shruthi HM[3]
[1,2,3]Department of biotechnology, jnana Bharathi campus, Bangalore University.
samyami@bub.ernet.in[1*], stgirisha@gmail.com[2] , shruthichampa49@gmail.com[3]

Abstract:
The generation of ethanol from the acid hydrolysis of Mixed Waste Biomass (MWB) was optimized through the use of response surface methodology (RSM) and central composite design (CCD). Responses were quantified regarding reducing sugars and biomass components, while independent variables included temperature, hydrolysis duration, and concentrations of sulfuric acid and sodium hydroxide. Under particular acid and basic conditions, the highest glucose concentrations were 0.563 ± 0.03 mg mL^{-1} and 0.486 ± 0.07 mg mL^{-1}, respectively. A noteworthy 61% decrease in phenolic compounds was achieved through hydrolysate detoxification using 5% activated charcoal. For ten days, Saccharomyces cerevisiae fermented the detoxified hydrolysate. The ethanol yield peaked on day six of fermentation at 0.61 ± 0.03 g g^{-1}. From S. cerevisiae, an ethanol concentration of 22.56% was obtained. These findings suggest that bioethanol is feasible. The ethanol yield peaked on day six of fermentation at 0.61 ± 0.03 g g^{-1}. From S. cerevisiae, an ethanol concentration of 22.56% was obtained. These findings suggest that producing bioethanol from cellulosic waste commercially is feasible.
Keywords: *Bioethanol, Lignocellulosic biomass, Central composite design, Response surface methodology.*

INTRODUCTION:

The world's energy consumption is growing at an unprecedented pace. It is expected to substantially increase by about 28% of the present level by 2040 (Kumar et al., 2020b). The widespread reliance on fossil fuels is limited by scarcity, high carbon emissions, and quick depletion (Ahmad et al., 2018). Around 8.1 billion people are anticipated to live on Earth as of 2023, and projections indicate that number will increase to 9 billion by 2037 and 10 billion by 2058 (Worldometers 2023). Waste management is a significant issue on a global scale. India has enacted waste management laws to promote sustainable development (Naveen, 2021). Due to global population expansion, industrialization, and geopolitical factors, searches for alternative and carbon-neutral energy sources are now required (Souza et al., 2017; Chandel et al., 2020).

One of the most essential strategic renewable fuels includes biofuel. Because of their relative availability, green credentials, long-term economic viability, and lack of carbon emissions, the second generation of biofuels derived from lignocellulose biomass is the best (Moshood et al., 2021). The medical field is among the several other areas and industries that heavily rely on ethanol (Kratzel et al., 2020). The National Renewable Energy Laboratory (NREL) conducted a techno-economic analysis of lignocellulosic ethanol production and found that the minimum selling price (MSP) of ethanol is USD 2.15 per gallon (Humbird et al., 2011). Various sources, including invasive plant species, energy crops, forestry, and agricultural wastes, generate lignocellulosic biomass. Cellulose (40%–50%), hemicellulose (20%–30%), lignin (10%–25%), and trace amounts of pectin and protein make up the majority of lignocellulose biomass (Bhatia et al., 2020; Nanda et al., 2013). Despite the adverse environmental effects (Kassaye et al., 2017), the use of lignocellulosic biomass (LCB) as a plentiful (200 billion tons/year) source of renewable energy has drawn much attention in this area (Ahmad et al., 2020). According to Sun et al., (2016), pretreatment transforms lignocellulosic biomass from its natural state into a form easier for enzymes to hydrolyze. Hydroxides of potassium, sodium, ammonium, and calcium are used to pretreat biomass in the alkali pretreatment method (Veluchamy et al., 2018). The primary purpose of acid pretreatment of LCB is to eliminate hemicellulose. (Veluchamy & colleagues, 2018). For optimum fermentation, pretreatment and hydrolysis are essential (Vasić et al., 2021). The fermentable sugars generated in the preceding stages are converted into bioethanol by the fermenting microorganisms during the last stage, fermentation. Due to its high productivity and ethanol output, Saccharomyces cerevisiae is the most widely used organism in

fermentation (Sharma et al., 2020). Using the central composite design (CCD) tool of response surface methodology (RSM) for bioethanol production, the effects of optimizing thermochemical pretreatment and enzymatic hydrolysis, followed by co-fermentation using the yeast culture S. cerevisiae (MTCC-36), have been statistically examined. There are notable differences in composition between lignocellulosic biomass that has not been treated and that has undergone chemical pretreatment using various chemical techniques (Manmai et al., 2021). The breakdown of the lignin, hemicellulose, and cellulose components of the lignocellulosic biomass that underwent thermochemical pretreatment has been thoroughly examined (Raina et al., 2020).

MATERIALS AND METHODS:

Proximate Composition Profiling of Mixed Waste Biomass:

The Bangalore-based Agricultural Produce Market Committee (APMC) provided the mixed waste biomass (MWB), comprising agricultural waste such as rice, paddy, sugar cane, maize cob, and wheat. Furthermore, over sixteen types of vegetable waste, including tomato, potato, and beet root peels, were gathered from different parts of Bangalore. Additionally, fruit wastes, including banana, amla, avocado, and other fruit wastes, were collected from fruit juice centres across the city. The discarded materials were rinsed with water. After that, they were dried at 60 °C for over two days. After drying, the substrate was ground into a fine powder with particles ranging in size from 2 to 5 mm, which were then sieved. Miller's (1959) DNS approach was used to quantify different compositional reducing sugars. The ash and moisture contents were evaluated following the established AOAC, 2023. Lignin, cellulose, and hemicellulose contents were quantified according to Lin et al., (2010).

Pretreatment with sulfuric acid and sodium hydroxide, followed by hydrolysate optimisation by CCD

MWB pretreatment was aided by sulfuric acid and NaOH. The sample to acid ratio used in the hydrolysis was 1:10. The reaction took place in 100 mL conical flasks wrapped in aluminium foil. During 20 experimental runs, three parameters, acid content, hydrolysis temperature, and duration, were methodically investigated using CCD. Every experiment was carried out in triplicate under these specific conditions, and the flask contents were maintained in a shaking incubator at 100 rpm. To preserve volume uniformity, the liquid was filtered and neutralised with NaOH. Following neutralisation, the mixture was filtered and detoxified. To maximise the reducing sugar yield necessary for bioethanol fermentation, the MWB hydrolysis was carried out using CCD with Design Expert software (version 13) (Myers et al., 2004). Table 1 describes the experimental setup for the three dependent variables: time (X_3, min), hydrolysis temperature (X_2, °C), and acid concentration (X_1, %). Table 2 shows the 20 experimental design runs. With the use of RSM, the link between input variables and responses [reducing sugars (Y)] is demonstrated by the generic quadratic equation "Y" (1) as follows:

$$Y = \beta_o + \beta_A X_1 + \beta_B X_2 + \beta_C X_3 + \beta_{AA} X_1^2 + \beta_{BB} X_2^2 + \beta_{CC} X_3^3 + \beta_{AB} X_1 X_2 + \beta_{AC} X_1 X_3 + \beta_{BC} X_2 X_3 + e \quad (1)$$

Were,
Y = predicted response
$ß_0$ = constant coefficient
$ß_A$, $ß_B$, and $ß_C$ = linear coefficients
$ß_{AA}$, $ß_{BB}$, and $ß_{CC}$ = quadratic coefficients
$ß_{AB}$, $ß_{AC}$, and $ß_{BC}$ = cross product coefficients
X_1, X_2, and X_3 = input variables
e = residual error between the observed Y and the predicted ($\hat{Y}$)

Table 1: Variable values that have been coded for the central composite design of MWB pretreatment

Variable	Actual value of coded level			
	Coded symbol	Low level	Centre point	High level
Acid concentration (%)	X_1	1	3	5
Hydrolysis temperature (°C)	X_2	100	120	130
Hydrolysis time (min)	X_3	30	60	90

Table 2: MWB pre-treatment responses using a central composite design matrix comprising three independent variables

Std	Run	A: Acid concentration	B: Hydrolysis Time	C: Hydrolysis Temperature
		%(W/V)	Min	°C
9	1	1	60	122.5
13	2	2	60	118.296
2	3	3	30	120
8	4	3	90	125
3	5	1	90	120
11	6	2	9.54622	122.5
7	7	1	90	125
6	8	3	30	125
14	9	2	60	126.704
1	10	1	30	120
19	11	2	60	122.5
20	12	2	60	122.5
17	13	2	60	122.5
12	14	2	110.454	122.5
5	15	1	30	125
10	16	3.68179	60	122.5
16	17	2	60	122.5
18	18	2	60	122.5
15	19	2	60	122.5
4	20	3	90	120

MWB hydrolysate saccharification

The amount of reducing and non-reducing sugars was estimated by analyzing the MWB hydrolysate produced during chemical pretreatment. The following formula was used to estimate the saccharification of MWB (Begum & Alimon, 2011):

$$\text{Saccharification yield (mg mL}^{-1}) = \frac{\text{Reducing sugars in hydrolysate}}{\text{Reducing sugars in MWB}}$$

Detoxification of MWB hydrolysate

Detoxifying the hydrolysate before fermentation is considered necessary. To achieve this, 2.5% activated charcoal was used to detoxify the MWB hydrolysate (Mussatto & Roberto, 2005). After agitating the activated charcoal for an hour at 30 °C at 200 rpm, it was subsequently separated using filter paper. Centrifugation was then carried out for 20 minutes at 2000 rpm. NaOH pellets were used to neutralise the resultant supernatant. As Gonzalez et al. (2003) described, the Folin-Ciocalteu technique was used to measure the hydrolysate's total phenolic content.

Bioethanol production with MWB hydrolysate

The microbe Saccharomyces cerevisiae was chosen in this investigation and cultured on YDP agar supplemented with extract (10 g/L), peptone (20 g/L), glucose (20 g/L), and agar (15 g/L). YDP broth was sterilised in an autoclave for 15 minutes at 121 °C and 15 psi, inoculated into YDP broth and incubated for 24 hours at 35 °C at 150 rpm in a shaker to prepare the inoculum. Further, it was diluted with NaOH, preserved at pH 5.6, and kept in an aseptic refrigerator at 4 °C. The yeast biomass was harvested by centrifugation at 7000 rpm for 10 min at 4°C (Bautista et al., 2019).

Statistical analysis

Every optimization experiment undertaken in CCD was carried out in triplicate. ANOVA and regression for the response surface quadratic model were employed to analyse CCD data using Design Expert Software (version 13.0.5.0, Stat-Ease). After the Duncan multiple range test (version 13.0.5.0 Software, Stat-Ease), the experimental data from fermentation studies were analyzed using a one-way ANOVA.

RESULTS AND DISCUSSION:

A study by Miezah et al. (2015) indicates that Ghanaian households produce about 12,700 tons of municipal solid waste (MSW) daily, constituting 70–80% of urban waste, with 8400 tons being biodegradable (50–70%). Bioenergy, primarily derived from lignocellulosic biomass such as rice straw, corn stover, wheat straw, and MSW, has garnered significant interest due to its capacity to mitigate global greenhouse gas (GHG) emissions (Jiang et al. 2020; Liu et al. 2022). The creation of sustainable products may arise from the utilization of renewable resources as eco-friendly alternatives to fossil fuels, which are detrimental to the environment (Kaparaju et al., 2009; Deshmukh and Pathan, 2023). This study involved the management of MWB after sodium hydroxide and sulfuric acid pretreatment, followed by fermentation with yeast isolates to synthesize ethanol. The compositional analysis in Table 3 indicates that MWB holds significant potential for microbial development. The rate of municipal solid waste (MSW) generation is increasing more rapidly than population growth (Hoornweg and Bhada-Tata, 2012). Due to its high fermentable sugar content, municipal waste biomass (MWB) has become a promising substrate for ethanologens (Das et al., 2021). Prior studies indicated that the cellulose and hemicellulose in MWB were hydrolyzed through chemical and enzymatic methods by various bacteria to enhance ethanol production. The hemicellulose percentages in various waste fractions were notably lower, with pretreated yard waste exhibiting the highest lignin content, as Miezah et al. (2017) in the present study sample of biodegradable municipal solid waste contain Hemicellulose (0.282 ± 0.15 mg mL−1), lignin (0.279 ± 0.45 mg mL−1), cellulose (0.34 ± 0.34 mg mL−1), reducing sugars (0.377 ± 0.12 mg mL−1), and moisture contents (0.118 ± 0.24 mg mL−1).

Table 3: Structural composition of mixed waste biomass

Parameter	Quantity
Moisture content (%)	11.85 ±0.24
Reducing sugar content (g/L-1)	37.78 ±0.12
Hemicellulose content (%)	28.28 ±0.15
Lignin content (%)	27.97 ±0.45
Cellulose content (%)	34.06 ±0.34
Ash content (%)	3.87 ±0.0046

Dilute sulfuric acid and sodium hydroxide pretreatment optimized by central composite design.

The model's statistical interpretation for biomass optimization was used to analyze and summarize a number of parameters (Table 4). Hayder et al., (2018) state that response surface methodology (RSM) was used to optimize the parameters of the bioethanol manufacturing process for municipal solid waste (MSW). A 24-complete factorial central composite design (CCD) required thirty distinct experimental cycles to practically reach the maximal bioethanol output of 332.9 mg/L. Chen et al. (2021) reported optimizing acidic pretreatment, while Yildirim et al., (2021) used a central composite design to maximize sugar recovery from cotton and sunflower straws using diluted acid pretreatment. Additionally, Zakaria & Pa (2023) recommend pre-treating Bambusa Wray at 4% H_2SO_4 + 5% NaOH due to its high glucose production. For the release of reducing sugars (Y1) in this investigation, the quadratic regression (Eq. 2) was as follows:

$$Y^1 = 0.26 - 0.039\ X_1 + 0.062\ X_2 - 0.069\ X_3 + 0.007\ X_1^2 - 0.032\ X_2^2 + 0.0054\ X_3^2 - 0.0032\ X_1 X_2 - 0.0052\ X_1 X_3 - 0.062\ X_2 X_3 + 0.00037 \quad (2)$$

Table 4: Central composite design matrix with three independent variables, sulfuric acid hydrolyses mixed waste biomasses to reduce their contents of sugars, cellulose, hemicellulose, and lignin.

		Factor 1	Factor 2	Factor 3	Respon se 1	Respon se 2	Respon se 3	Response 4	Respon se 5
Std	Run	A: Acid concentration	B: Hydrolysis Time	C: Hydrolysis Temperature	Reducing sugar for H_2SO_4	Reducing sugar for NaOH	Cellulose	Hemicellulose	Lignin

					pretreated	Pretreated			
		%(W/V)	Min	°C	g/L^{-1}	g/L^{-1}	%	%	%
3	1	1	90	120	38.78	21.32	34.98	19.09	27.7
10	2	3.68179	60	122.5	34.99	22.01	39.83	21.38	34.23
13	3	2	60	118.296	36.21	23.89	42.6	23.36	38.74
16	4	2	60	122.5	36.09	35.31	32.56	18.98	29.45
20	5	2	60	122.5	38.22	32.11	34.98	25.11	26.93
7	6	1	90	125	43.22	39.12	29.43	32.56	40.21
14	7	2	60	126.704	48.54	30.78	23.78	19.21	34.76
9	8	0.318207	60	122.5	33.9	32.01	28.92	28.99	31.08
8	9	3	90	125	42.11	28.12	36.06	18.94	43.91
11	10	2	95.5462	122.5	36.54	46.98	31.11	25.98	34.87
2	11	3	30	120	33.54	34.45	42.34	29.21	37.11
6	12	3	30	125	37.25	24.03	34.13	24.02	32.38
12	13	2	110.454	122.5	26.01	29.48	38.07	31.45	22.08
17	14	2	60	122.5	32.11	19.43	46.03	23.11	32.34
4	15	3	90	120	39.65	22.38	21.32	33.98	37.38
18	16	2	60	122.5	23.33	36.54	36.05	16.72	36.43
15	17	2	60	122.5	21.76	29.09	39.03	20.09	32.14
5	18	1	30	125	39.01	18.9	35.22	32.54	29.3
19	19	2	60	122.5	36.65	23.56	41.76	23.56	20.44
1	20	1	30	120	22.98	26.31	37.28	28.96	32.71

Minus signs expressed the factors' antagonistic relationships, whereas plus signs represented the synergistic association. The statistical model's Y = f (X1, X2) factors and the answer Y had a quadratic connection with X12, X22, and a linear interaction with X1, X2. By comparing the factor coefficient, the equation in the current study assists in determining the relative effects of the hydrolysis parameters, including the acid concentration (X1), hydrolysis temperature (X2), and hydrolysis time (X3), on reducing sugars (Y1). By pre-treating MWB, one individual factor, such as acid concentration ($-0.039\ X_1 + 0.062\ X_2$), raised the amount of reducing sugars. It demonstrated a synergistic association because it had no interaction effects with the other components examined in the experiment. For negative signs, the same principle holds.

The B3 slope results from the linear interaction between X1 and X2 on Y1 and Y2. X2 is more positive, and X1's influence on reaction is more positive if B3 is positive (the interaction effect is positive). One interpretation of this is a synergistic relationship. On the other hand, X1's effect on Y gets more antagonistic, that is, more negative, the more negative X2. Additionally, these effects depend on either high or low amounts of X1 or X2. In this study, reducing sugars in the saccharification process were positively impacted by the linear relationship between two parameters (X1X2, X2X3, and X1X3), as previously mentioned.

In this investigation, the best theoretical and practical values (g L−1) were 48.54 ± 0.06 and 42.11 ± 0.03 at 3% H2SO4 concentration at 126°C for 60–90 minutes of hydrolysis and 46.98 ± 0.04 at 2% NaOH concentration at 122°C for 100 minutes of hydrolysis, respectively. To break down cellulose and hemicellulose into simple sugars, Jennings & Schell (2011) also reported using the same pretreatment. Yildirim et al. (2021) reported that the ideal pretreatment conditions for maximum sugar yield were temperature at 121.7°C, acid concentration at 2.28% (v/v), and time at 36.82 minutes for cotton straw, but Yildirim et al. (2021) reported temperature at 87.03°C, acid concentration at 3.68% (v/v), and time at 36.82 minutes for sunflower straw.

This work used ANOVA (Table 5) employing RSM to investigate the model's reliability. The model's importance was demonstrated by its F value of 3.08 and p value of 0.0474. According to Table 5, the R2 and Adj R2 coefficients were 0.6154 and 0.3308, respectively. The value for "Adeq Precision" was

determined to be 6.587. A statistical metric known as R-squared (R2) indicates the percentage of a dependent variable's variance that can be accounted for by an independent variable or variables in a regression model. R-squared values are often expressed as percentages between 0% and 100% and range from 0 to 1. R-squared values that fall into the weak/low, moderate, and considerable effect size categories are $0.3 < r < 0.5$, $0.5 < r < 0.7$, and $r > 0.7$. Regression analysis was used to get the R2 values using Design Expert Software (version 13.0.6, Stat-Ease). The better the regression model fits the data, the higher the R2 value. Response Y1 in the current study has an R2 value of 0.2657.

The response surface graph exhibited the contours on the base, representing projections of the surface plot to show trends in reducing sugar concentration as sulfuric acid hydrolysates and sodium hydroxide hydrolysates concentration and hydrolysis time vary. Increasing the acid concentration (% w/v) initially increases the reducing sugar yield. However, beyond a certain point, further increases in acid/alkaline concentration may result in diminishing returns or a plateau in sugar yield. Similarly, increasing hydrolysis time leads to a rise in reducing sugar yield, but beyond a specific duration, the yield stabilizes or may decrease, likely due to degradation of sugars. Figures 1A and 2A showed an increase in reducing sugar contents because of an increase in the acid concentration, as well as an increase in the temperature of hydrolysis. Figure 1B and Figure 2B depict a decrease in reducing sugars with an increase in both acid concentration plus time. The results elucidate that an increase in temperature exhibited a sharp elevation in contents of reducing sugars, whereas an increased time showed a slight elevation (Figure 1C and Figure 2C).

Table 5 Single-factor ANOVA ($p < 0.05$) of fitted quadratic regression model for reducing sugars and total carbohydrates examined by sulfuric acid-treated hydrolysates

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	368.69	9	40.97	3.08	0.0474	significant
A-Acid concentration	1.41	1	1.41	0.1061	0.7513	
B-Hydrolysis Time	65.02	1	65.02	4.88	0.0516	
C-Hydrolysis Temperature	125.36	1	125.36	9.41	0.0119	
AB	1.16	1	1.16	0.0867	0.7744	
AC	8.61	1	8.61	0.6466	0.4400	
BC	5.85	1	5.85	0.4392	0.5225	
A^2	10.83	1	10.83	0.8132	0.3884	
B^2	6.53	1	6.53	0.4901	0.4998	
C^2	191.32	1	191.32	14.37	0.0035	
Residual	133.17	10	13.32			
Lack of Fit	33.22	5	6.64	0.3323	0.8740	not significant
Pure Error	99.95	5	19.99			
Cor Total	501.86	19				

Note*: $p < 0.05$

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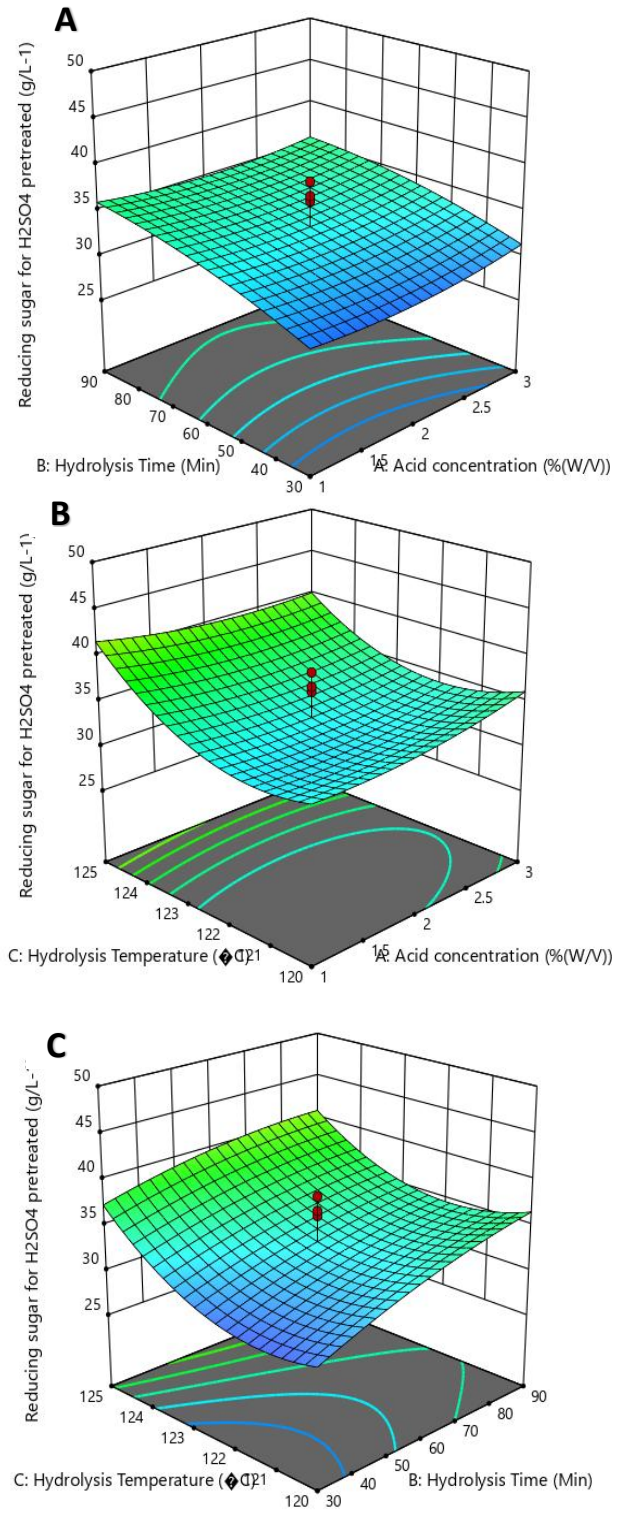

Figure 1: Response surface plot for reducing sugars (mg mL^{-1}) in MWB from various treatments of sulfuric acid concentration with different combination of hydrolysis time and Acid concentration(A), temperature and acid concentration (B), and

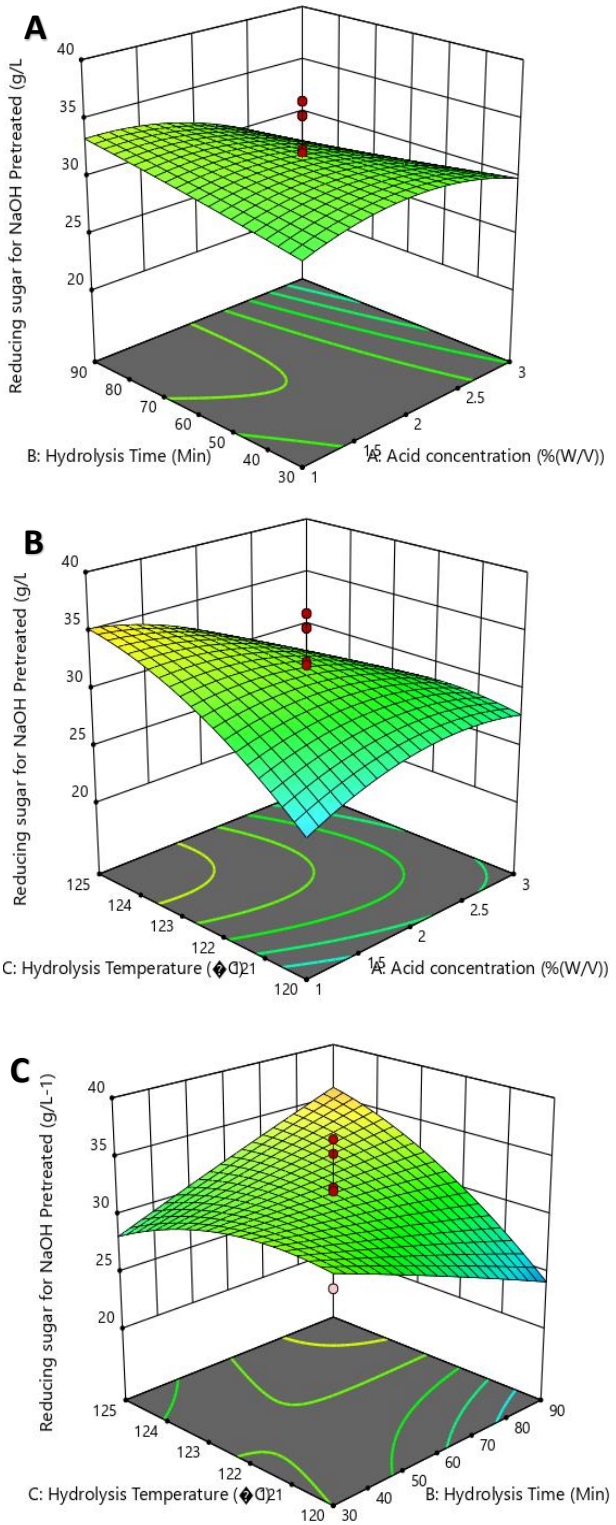

Figure 2: Response surface plot for reducing sugars (mg mL^{-1}) in MWB from various treatments of sodium hydroxide concentration with different combination of hydrolysis time and Acid concentration(A), temperature and acid concentration (B), and temperature and time(C)

Saccharification and detoxification of MWB hydrolysate

According to Zhang et al., (2021) and Guo et al., (2022), biodetoxification by inoculating Paecilomyces variotii to remove inhibitors produced by citric acid pretreatment, and the ethanol concentration reached 70.2 g/L using semi-SSF at 25% of solid content, and ethanol production reached 0.280 g/g initial corn fibre. And also, Juneja et al., (2021) used the combination of liquid hot water pretreatment with wet disk milling to pretreat corn fibre, resulting in a final ethanol concentration of 21.54 g/L at 20% of solid content. However, in the present study, the percentage of saccharification yield of reducing sugars estimated after hydrolysis was 12.76%. The 56% reduction in phenol content was estimated after detoxification using 5 % activated charcoal. The number of phenolic compounds (mg mL−1) in MWB hydrolysate before detoxification was 0.38 ± 0.27, and the amount lessened to 2.32 ± 2.14 after detoxification.

Production of ethanol from MWB hydrolysate

Teoh et al., 2014 reported that spent mushroom sawdust waste (SMSW) from Pleurotus spp. Followed by fermentation of the sugar solution obtained from SMSW with baker's yeast, Saccharomyces cerevisiae, yielded up to 4 g ethanol/100 g lignocellulose, which equals 40 L of biofuel per ton of dried SMSW. In this study, S. cerevisiae manifested 0.61 ± 0.03 g g^{-1} ethanol yield at day 6. Regarding percentage, 22.56 % of ethanol was obtained from S. cerevisiae, respectively. The reduction in reducing sugar contents was noticed day-wise, on Days 8 to 10, because of ethanol bioproduction (Figures 3 & 4). Stability in yeast growth after days 6 and 7 envisaged that the organisms could be promising candidates, as they can tolerate ethanol (Figure 5).

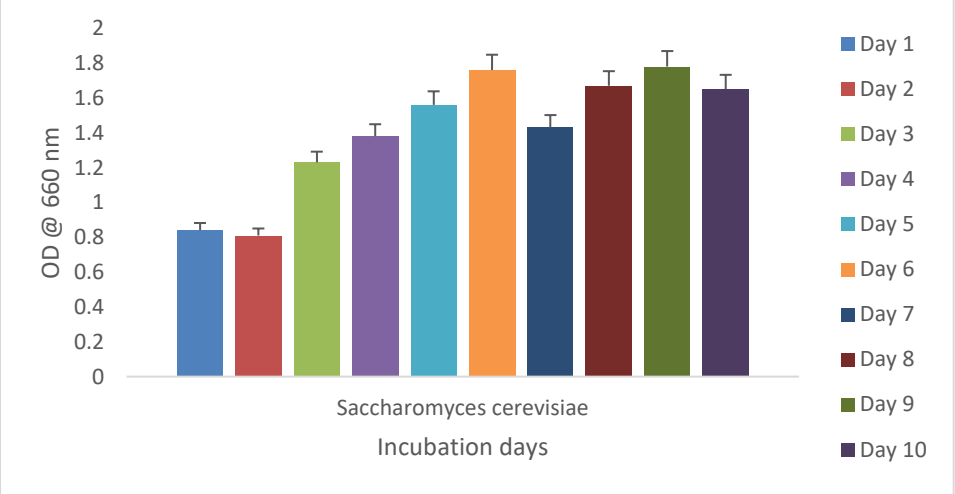

Figure 3. Ethanol production (mg mL−1) by S. cerevisiae isolate using MWB hydrolysate

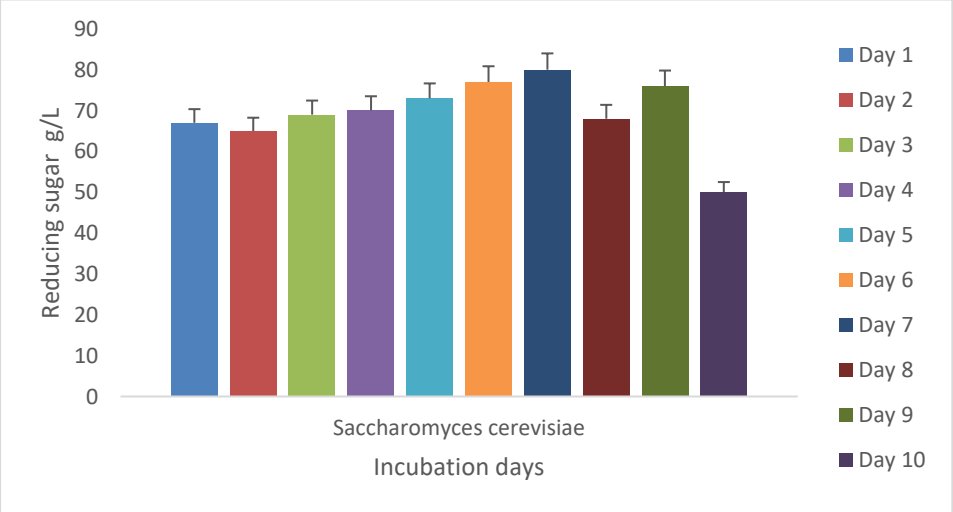

Figure 4. Periodic reduction in reducing sugars (g/L) by S. cerevisiae

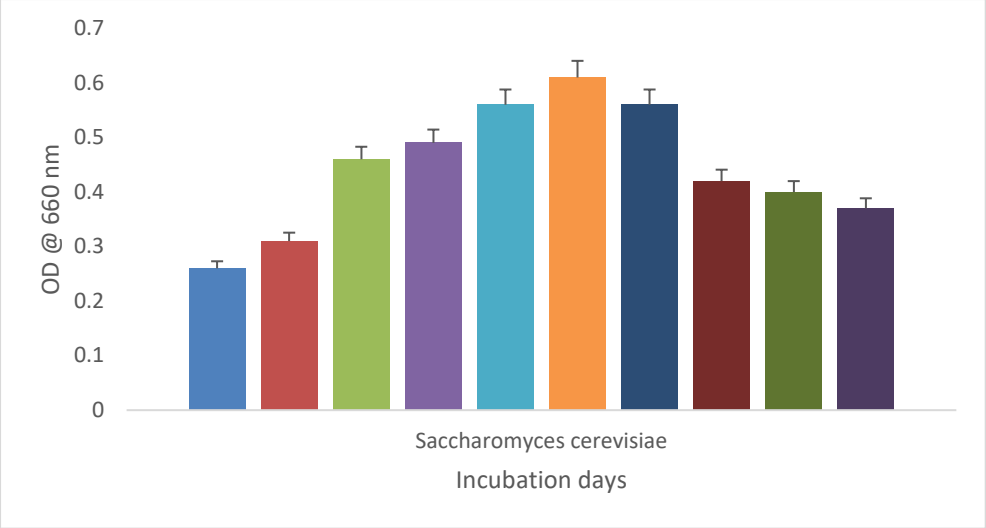

Figure 5. Growth of S. cerevisiae in MWB hydrolysate

CONCLUSION:

This study demonstrates the successful application of Response Surface Methodology (RSM) using a Central Composite Design (CCD) for optimising acid and alkali hydrolysis parameters to maximise fermentable sugar yield from Mixed Waste Biomass (MWB). Among the experimental conditions, optimal sugar release was observed at 3% sulfuric acid concentration, 126 °C temperature, and 60–90 minutes of hydrolysis. The detoxification step employs 5% activated charcoal, significantly reduces phenolic inhibitors, and enhances fermentability. Subsequent fermentation with Saccharomyces cerevisiae yielded an ethanol concentration of 0.61 ± 0.03 g/g, equivalent to 22.56% ethanol content, with peak production observed on day six. These results affirm the viability of MWB as a cost-effective, sustainable feedstock for second-generation bioethanol production, supporting waste valorization and renewable energy goals.

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