JOURNAL OF PLANT RESEARCH AND INNOVATIONS
Jan-Jun 2025, VOL. 3, ISSUE 1, pp. 14-22
https://doi.org/10.61577/jpri.2025.100003

RESEAPRO JOURNALS

ORIGINAL ARTICLE

OPEN ACCESS

Pretreatment, enzymatic hydrolysis and fermentation of pearl millet (bajra) for bioethanol production

Shritika Bajpai and Snigdha Subhadarshini Sahoo

Department of Biotechnology, Utkal University, Bhubaneswar, India

ABSTRACT

Introduction

The growing need for renewable and sustainable energy sources is intensifying interest in lignocellulosic biomass as a potentially viable feedstock used in bioethanol production. Pearl millet straw, a readily available agricultural residue, represents a valuable substrate due to its high cellulose and hemicellulose content.

Methods

The present study investigated the feasibility of producing bioethanol from pearl millet straw biomass. The reaction of temperature, acid concentration, hydrolysis time, and substrate concentration were examined using Response Surface Methodology (RSM) with an L27 orthogonal array design. Comparative analyses of alkali and acid pretreatments were performed to enhance lignin removal and cellulose accessibility.

Results

The results indicated that alkali pretreatment was more effective than acid pretreatment, achieving approximately 2% higher delignification and improved cellulose exposure (590 mg/g). The optimized parameters for enzymatic hydrolysis, determined through RSM, were pH 5.5, incubation time of 16 h, enzyme concentration of 200 µl, and temperature of 60°C, yielding 605 mg/g of glucose and 465 mg/g of xylose.

Discussion

Characterization studies using SEM and FTIR analyses confirmed significant structural and chemical modifications in pretreated samples, including enhanced surface porosity and lignin removal, validating the efficacy of the pretreatment approach.

Limitations

Further research is required to optimize the fermentation stage and evaluate the overall bioethanol yield under scaled-up operational conditions.

Objectives

This study focus to identify and upgrade the main parameters influencing hydrolysis efficiency and sugar release to establish an effective process for bioethanol production from pearl millet straw.

KEY WORDS

Cellulose; Lignin; Hemicellulose; Xylose; Pennisetum; Glucose; Hydrolysis; Biomass

ARTICLE HISTORY

Received 07 March 2025;
Revised 28 March 2025;
Accepted 07 April 2025

Introduction

The global reliance on fossil fuels has led to growing environmental concerns, resource depletion, and economic instability due to fluctuating petroleum imports. Major energy-consuming nations such as the United States, China, and India face increasing energy demands and carbon emissions, intensifying the need for renewable and sustainable alternatives [1]. In this context, bioenergy derived from lignocellulosic biomass offers a promising solution to reduce dependence on conventional fuels and mitigate climate impacts.

India's energy consumption is expected to rise from 750 million tons of oil equivalent (Mtoe) in 2011 to between 1258 and 1647 Mtoe by 2035 (IEA, 2013). To address this challenge, the Government of India introduced a biofuel policy targeting 20% ethanol blending with petrol by 2017. Bioethanol has been prioritized as a sustainable automotive fuel to curb import dependency and promote rural development through the utilization of agricultural residues [2]. However, progress toward achieving these targets remains slow due to feedstock limitations and high production costs.

Biofuels are generally produced from three categories of biomass feedstocks sugar-based, starch-based, and lignocellulosic materials. Among these, lignocellulosic biomass is most promising as it is abundant, renewable, and non-competitive with food crops. It can be converted into transport fuels, heat, and chemical feedstocks. Despite India's progress in biofuel programs, challenges such as feedstock scarcity, inefficient conversion technologies, and high processing costs remain significant barriers. Two major strategies are often employed to overcome these constraints: utilizing readily available lignocellulosic biomass and modifying biomass composition through molecular or genetic techniques to enhance fermentable sugar yield. Pearl millet (Pennisetum glaucum), commonly known as bajra, is an annual C4 crop widely cultivated in arid and semi-arid areas of India. It ranks fourth among cereal crops in India and sixth globally, following rice, wheat, maize, barley, and sorghum [3]. The crop thrives under low-input conditions, tolerates poor soils, and grows efficiently in regions unsuitable for other grains. While its grains are rich in protein and amino acids, the residual biomass serves as valuable livestock feed

***Correspondence:** Miss. Shritika Bajpai, Department of Biotechnology, Utkal University, Bhubaneswar, India, email: shritikabajpai16@gmail.com

and bioresource material. In India and Africa, pearl millet residues are used as fodder, building material, and domestic fuel, while in the United States, it is grown primarily as forage and bird feed. The huge amount of leftover straw makes pearl millet a potential feedstock for bioethanol production.

In recent years, several ethanol distilleries in Haryana have adopted broken rice and pearl millet grains as feedstocks, with each plant requiring approximately 48,000 tons of grain annually. These developments demonstrate the growing industrial interest in alternative biomass-based ethanol production. The lignocellulosic portion of pearl millet straw composed primarily of cellulose, hemicellulose, and lignin offers a renewable substrate for enzymatic saccharification and fermentation. Previous studies have demonstrated that lignocellulosic materials such as oats, barley, wheat, and pearl millet are suitable for saccharification and fermentation. The efficiency of bioethanol production from such residues depends on the crop's adaptability, yield potential, and ease of conversion. Pearl millet possesses several favorable characteristics, including short crop duration, drought tolerance, and high biomass productivity, making it suitable for bioenergy applications [4]. Optimizing pretreatment and hydrolysis processes is essential to enhance cellulose accessibility and fermentable sugar release for efficient ethanol production.

Despite its potential, large-scale utilization of pearl millet biomass for bioethanol production remains limited. Challenges include the need for cost-effective pretreatment methods, optimization of enzymatic hydrolysis, and improved fermentation efficiency. Moreover, systematic studies on the use of pearl millet residues as a bioenergy source in India are still insufficient.

The recent study aims to estimate the potential of pearl millet (Pennisetum glaucum) biomass as a sustainable feedstock for bioethanol production. It focuses on optimizing acid and alkaline pretreatment methods to enhance delignification and improve cellulose accessibility for enzymatic hydrolysis. The research further identifies optimal hydrolysis conditions to maximize fermentable sugar yield and examines structural changes in biomass using SEM and FTIR analyses [5]. Ultimately, the study assesses the feasibility of utilizing pretreated pearl millet biomass for efficient and sustainable bioethanol production in semi-arid regions of India (Figure 1).

Figure 1. A close canopy of pearl millet.

Materials and Methods

Optimization of pretreatment of Pearl millet using RSM

The L27 orthogonal array (OA) of the RSM design in Minitab 2.0 was applied to design the pretreatment experiments. The pretreatment for both acid and alkali was regulated separately. A total of 27 experiments were considered using four parameters: substrate concentration (g), acid/alkali concentration (% v/v), temperature (°C), and time (min). The responses taken for these parameters included cellulose (mg/g), hemicellulose (mg/g), and lignin (%).

Optimization of pre-treatment technique using RSM method

Pearl millet straw was washed, shade-dried for 1–2 days, and oven-dried at 60°C to eliminate residual moisture. The dried substances were finely ground into a powder and subjected to acid (H_2SO_4) and alkali (NaOH) pretreatments. A total of 54 observations were conducted using the Response Surface Methodology (RSM), varying substrate concentration (3–9 g/100 mL), temperature (70–120°C), incubation time (10–60 min), and reagent concentration (0.5–2.0%). Cellulose content was estimated using the anthrone reagent method with spectrophotometric analysis, and samples with the highest cellulose and lowest lignin content were selected for further pretreatment [6].

Optimization of enzymatic hydrolysis of pretreated pearl millet using RSM Design

Box-Behnken designs (Minitab 2.0) were employed to conduct enzymatic hydrolysis of the alkali-pretreated pearl millet straw. Four parameters that were considered are-temperature (°C), incubation time (h), enzymatic concentration(µl) and pH. A total of 16 observations were regulated using the aforementioned parameters in which glucose and xylose were considered as the response [7]. 27 variable runs were regulated to produce fermentable sugar in 2 sets which included sulfuric acid (H2SO4) and sodium hydroxide (NaOH) and for enzymatic hydrolysis 16 variable runs were conducted for to produce glucose and xylose.

Optimization of enzymatic hydrolysis of pre-treated pearl millet

A total of 200 g of pearl millet straw was pretreated with NaOH and subjected to enzymatic hydrolysis optimization by the use of the Response Surface Methodology (RSM) [8]. Sixteen experiments were conducted using 5 g of substrate in 100 mL citrate buffer with pH ranging from 5.5 to 7.5. Enzyme concentration varied from 100 to 250 µL, incubation time from 12 to 24 hours, and temperature from 45°C to 60°C. Glucose and xylose contents were estimated using anthrone and DNS reagents, respectively, with spectrophotometric analysis. Samples yielding the highest glucose and xylose concentrations were selected for further NaOH pretreatment and enzymatic hydrolysis.

Fermentation process

For the fermentation process, media were prepared under the optimized enzymatic hydrolysis conditions, and inoculation using Saccharomyces cerevisiae and a mixed culture of S. cerevisiae and Pichia pastoris was carried out. A total of 100 mL

https://doi.org/10.61577/jpri.2025.100003

of hydrolysate was mixed in a 250 mL conical flask with 0.5 g of S. cerevisiae. The flask was covered with aluminum foil to maintain anaerobic conditions and incubated at 30°C and 100 rpm for 48 hours. Fermentation was conducted at a constant temperature of 30°C, with the pH maintained between 5.0 and 5.5 using 1 M NaOH, which represents the optimum range for yeast activity. Freshly prepared yeast culture (25 mL; 1:4 v/v) was added to each flask, and 0.05 g of P. pastoris was added to the second flask to study the co-fermentation effect. Both samples were incubated at 200 rpm for three days, with daily pH adjustments to maintain the optimal range. Following fermentation, the mixtures were subjected to fractional distillation, and ethanol was collected at 78.4°C. The obtained ethanol samples were further examinate using Gas Chromatography (GC) and Mass Spectrometry (MS) to determine ethanol purity and yield.

Analytical method

For the solid fractions of pre-treated samples, cellulose, hemicellulose, and acid-insoluble lignin (AIL) were examined, while the liquid part obtained during AIL processing was used for determining acid-soluble lignin (ASL).

Analysis of cellulose content

For the optimisation of cellulose, 1 g of sample was mixed with 3 g of the Nitric Acid reagent and placed in a water bath at 100 °C for 30 min. The sample in the tubes was then cooled and centrifuged at 5000 rpm for 15–20 min. After this, the residue obtained was washed with distilled water, and 10 mL of 67% sulphuric acid was added to the washed residue, which was then incubated for 1 hr. From this solution, 1 mL of the sample was taken, and the volume was made up to 100 mL using distilled water. From the 100 mL solution, 1 mL was taken, and 10 mL of anthrone reagent was added [9]. The resulting solution was then placed in a water bath at 100 °C for 10 min, cooled to room temperature, and the absorbance was measured at 630 nm.

Lignin content analysis

The Acid Soluble Lignin (ASL) and Acid Insoluble Lignin (AIL) contents of pearl millet biomass were analyzed by following the NREL protocol (Sluiter, 2008). In glass pressure tubes, 300 mg of dried biomass was placed, and 3 mL of 72% (w/w) H_2SO_4 was added. The mixture was then incubated at 30°C for 2 hours, with periodic mixing every 30 minutes to ensure hydrolysis was completed. Afterward, the total volume was brought to 87 mL using double-distilled water, and the samples were autoclaved at 121°C for 1 hour. When cooled to room temperature, the hydrolyzed samples were filtered under vacuum through a filtering crucible. The residue was then washed thoroughly with not less than 50 mL of hot deionized water to remove remaining acid.

The crucible containing the acid-insoluble residue was dried at 105°C until a constant weight was obtained, cooled in a desiccator, and weighed [10]. Following this, the crucible and residue were placed in a muffle furnace at 575 ± 25°C for 24 ± 6 hours to determine ash content, allowing accurate calculation of the lignin fractions.

FTIR and SEM content analysis

FTIR spectra and SEM analysis were undertaken to inspect the structural variations in the physical and chemical characteristics of the untreated and pre-treated lignocellulosic biomass (HNG and DG). For the FTIR study, spectra of the untreated and pre-treated lignocellulosic biomass in the KBr phase were recorded using the Perkin FTIR spectrophotometer, with a nominal resolution of 2 cm^{-1} while averaging 44 scans. SEM analysis was carried out to examine the structural characterisation of the native, alkali pre-treated, and ultrasonic-assisted alkali pre-treated pearl millet varieties. The detectors used for SEM were the Secondary Electron, Semiconductor Back-scattered Electron, and Cathodoluminescence detector, respectively. The samples were mounted in very thin layers over the gold-coated copper sample holder and then examined for the morphological changes (Figure 2).

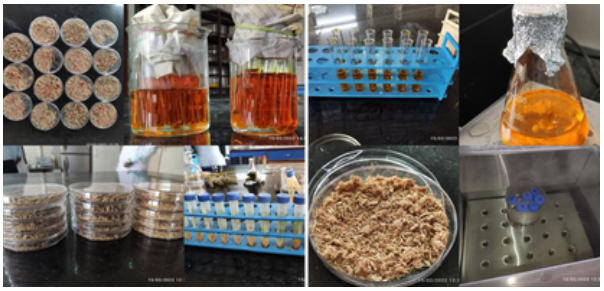

Figure 2. Pretreatment methodology.

Results And Discussion

Pre-treatment of pearl millet using acid hydrolysis

In total, 27 pearl millet straw samples were pretreated with different concentration of sulphuric acid. The RSM method was followed. The pre-treatment values were presented in (Table 1) and (Figure 3). The response table SN ratios show that acid concentration has highest effect on the pretreatment on pearl millet straw than substrate concentration (g), temperature (0C), incubation time (min) represents the important effects plot for the signal to noise ratios of the effect of different parameter (Incubation time (IT), temperature, substrate concentration (SC), acid concentration) on pearl millet straw pretreated with H2SO4. In the figure it is clearly observed that the signal to noise ratio for IT (min), temp. (0C), and SC (g) is relatively less as compare to the SN ratio of Acid concentration (%). Therefore, further observation of acid concentration will be required to obtain the optimum acid concentration value.

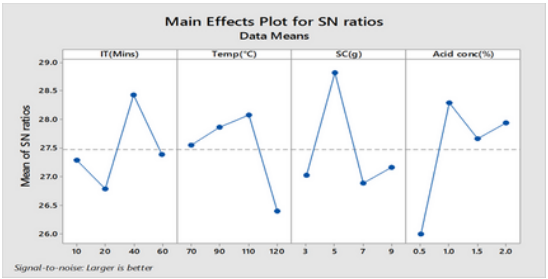

Figure 3. Graphical representation of SN ratio of different parameters of pretreated pearl millet.

Table 3. Surface to noise ratio of pre-treatment of pearl millet.

Level	IT(Mins)	Temp(0C)	SC(g)	Acid conc. (%)
1	27.29	27.55	27.03	26
2	26.79	27.87	28.82	28.29
3	28.43	28.08	26.89	27.67
4	27.39	26.4	27.17	27.94
Delta	1.64	1.68	1.92	2.29
Rank	4	3	2	1

https://doi.org/10.61577/jpri.2025.100003

Calculation of standard deviation and mean

Figure 4 represents the important effect plot for Standard deviation (StDevs) of the effect of different parameters (Incubation time (IT), temperature, substrate concentration (SC), Acid concentration) on pearl millet straw pretreated with H2SO4. In the figure it is clearly viewed that the StDevs of temperature, SC (g), acid concentration is comparatively less than StDevs of IT. As a result, further processing of IT will be required for optimum result (Figure 4 and Figure 5) represents the main effect plot from means of the result of various parameters (Incubation time (IT), temperature, substrate concentration (SC), Acid concentration) on pearl millet pretreated with H2SO4. In the figure it is clearly observed that the mean of means for IT, SC, temperature of lesser mean of mean as compared to acid concentration. Therefore, further optimization of acid concentration is required to obtain the optimum value. The optimum value IT is 60min, temp. is 900C, SC is 5g. Acid concentration has two optimum values 1% and 2%. For prime result further optimization is required for acid concentration.

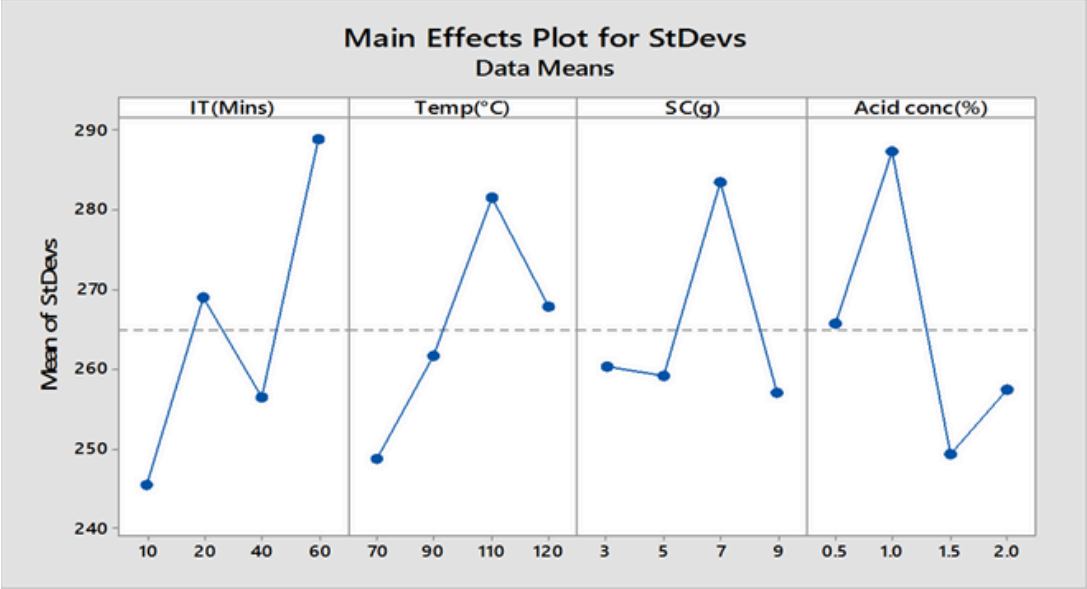

Figure 4. Standard deviation of different parameters of pretreated pearl millet with acid.

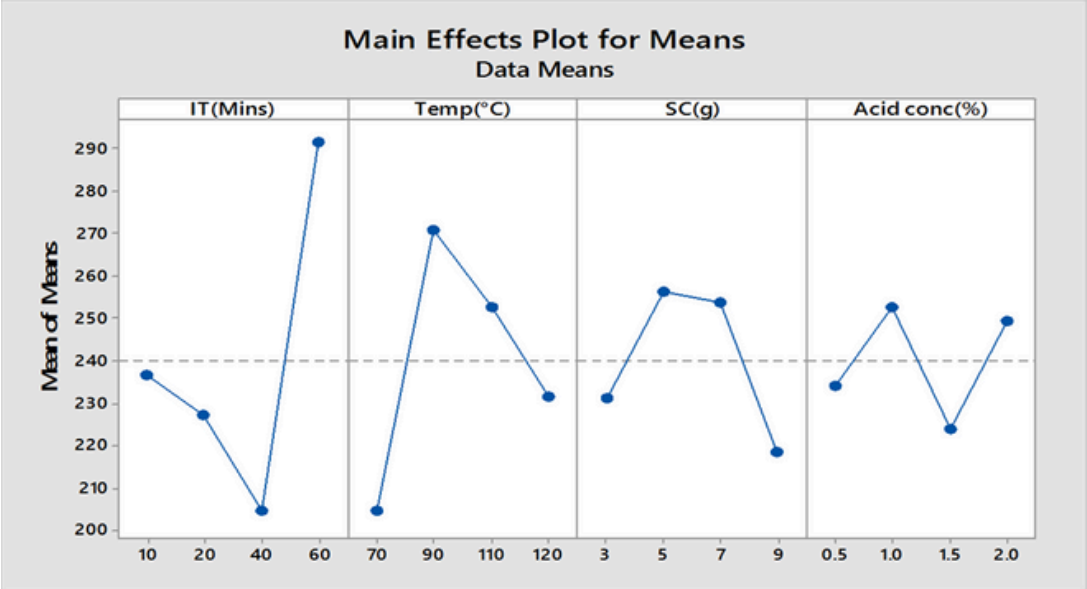

Figure 5. Mean effects plot of different parameters of pretreated pearl millet.

Predicted response model and normal probability

To determine whether the model satisfies the assumptions of the analysis of variance (ANOVA), which is shown in (Table 2). The probability (P-values) was used as a device to check the significance of each co-efficient which shows the interaction strength of each parameter. The smaller the p values are the bigger the significance of the corresponding co- efficient. In the ANOVA table it shows that the R-Squared value is 94.1%, which is quite significant.

https://doi.org/10.61577/jpri.2025.100003

Table 2. Analysis of variance (ANOVA) for SN ratio.

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
IT(mins)	3	5.67	5.67	1.8899	2.64	0.223
Temp(0C)	3	6.721	6.721	2.2404	3.13	0.183
SC(g)	3	9.741	9.741	3.247	4.54	0.123
Acid conc.(%)	3	12.396	12.396	4.1322	5.77	0.092
Residual Error	3	2.148	2.148	0.7158		
Total	15	36.676				

Pre-treatment of pearl millet using alkali hydrolysis (NaOH)

In total, 27 pearl millet straw samples were pretreated with different concentration of sodium hydroxide (NaOH). The RSM method was followed. The pre-treatment values were presented in (Table 3). The main effects plot for SN ratios of the parameters Incubation Time (IT), temperature (°C), substrate concentration (g), and alkali concentration (%) for pearl millet pretreated with alkali NaOH is represented in (Figure 6). In the figure it is clearly observed that SN ratio for IT and SC comparatively less than SN ratio of temperature and alkali concentration. Therefore, further optimization of temperature of alkali concentration will be required to obtain the optimum value of both. Response (Table 3) for Signal to Noise Ratios Larger is better. The response table SN ratios shows that acid temperature has highest effect on the pretreatment on pearl millet. SC has comparatively less effect on pretreatment than temperature. IT has milder effect on while alkali concentration has the least effect on pretreatment.

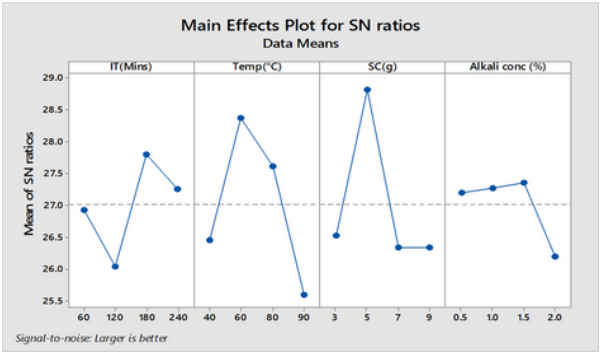

Figure 6. Signal-to-noise ratio of pre-treatment of pearl millet with alkali.

Table 3. Surface to noise ratio of pre-treatment of pearl millet.

Level	IT(Mins)	Temp(0C)	SC(g)	Alkali conc. (%)
1	26.93	26.45	26.53	27.19
2	26.04	28.36	28.81	27.27
3	27.8	27.61	26.34	27.35
4	27.26	25.59	26.34	26.2
Delta	1.76	2.77	2.47	1.15
Rank	3	1	2	4

Calculation of standard deviation and mean

Figure 7 represents the main effects plot for Standard Deviation (StDevs) showing the effect of different parameters Incubation Time (IT), temperature, substrate concentration (SC), and acid concentration on pearl millet straw pretreated with NaOH. In the figure it is clearly viewed that the StDevs of temperature, SC (g), acid concentration is comparatively less than StDevs of IT. As a result, further processing of IT will be required for optimum results (Figure 8). represents the main effect plot from means of the result of various parameters (Incubation time (IT), temperature, substrate concentration (SC), Acid concentration) on pearl millet straw pretreated with NaOH. In the figure it is clearly observed that the mean of means for IT and SC, half lesser mean of mean as compared to temperature and alkali concentration. Therefore, further optimization of temperature and alkali concentration is required to obtain the optimum value. The optimum value IT is 250min, SC is 3g temperature and alkali concentration has optimum values of 600C and 2% respectively.

Figure 7. Standard deviation of different parameters of pretreated pearl millet with alkali.

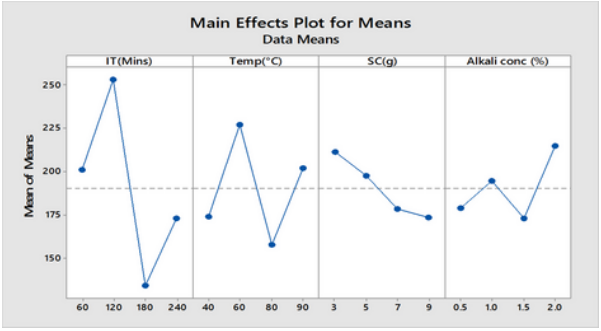

Figure 8. Mean effectsplot of different parameters of pretreated pearlmillet with alkali.

Predicted response model and normal probability (ANOVA)

To examine whether the model satisfies the assumptions of the analysis of variance (ANOVA), the results shown in (Table 4) were considered. The probability (P-values) was used as a device for checking the significance of each coefficient, which indicates the interaction strength of each parameter. The smaller the P-values, the greater the significance of the

https://doi.org/10.61577/jpri.2025.100003

corresponding coefficient. In the ANOVA table, it is shown that the R-Squared value is 85.4%, which is quite significant.

Table 4. Analysis of Variance (ANOVA) for SN ratio.

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
IT(mins)	3	6.552	6.552	2.184	0.84	0.556
Temp(0C)	3	18.11	18.11	6.037	2.31	0.254
SC(g)	3	17.494	17.494	5.831	2.23	0.263
Alkali conc. (%)	3	3.499	3.499	1.166	0.45	0.737
Residual Error	3	7.83	7.83	2.61		
Total	15	53.485				

RSM method of enzymatic hydrolysis

A total of sixteen experiments were conducted to optimize glucose and xylose production using Response Surface Methodology (RSM). Twenty-seven variable runs were performed for pretreatment with sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH), followed by sixteen runs for enzymatic hydrolysis. Pearl millet straw samples were prepared under 32 different conditions, each containing varied concentrations of crushed straw in 100 mL of distilled water. The pretreatment parameters included temperatures of 70°C, 90°C, 110°C, and 120°C; acid and alkali concentrations of 0.5%, 1.0%, 1.5%, and 2.0%; and incubation times of 10, 20, 40, and 60 minutes. After incubation, samples were filtered through muslin cloth and shade-dried [11,12]. The pretreated biomass was analyzed for cellulose, hemicellulose, and lignin content to assess delignification efficiency. Enzymatic hydrolysis was then performed to convert polysaccharides into fermentable sugars. The response surface plots revealed that glucose production was significantly influenced by the interaction in between incubation time and enzyme concentration, forming a concave surface. In contrast, other parameter combinations such as temperature with incubation time, enzyme concentration, or pH showed relatively flat response surfaces, indicating weak interactions during enzymatic hydrolysis (Figure. 9).

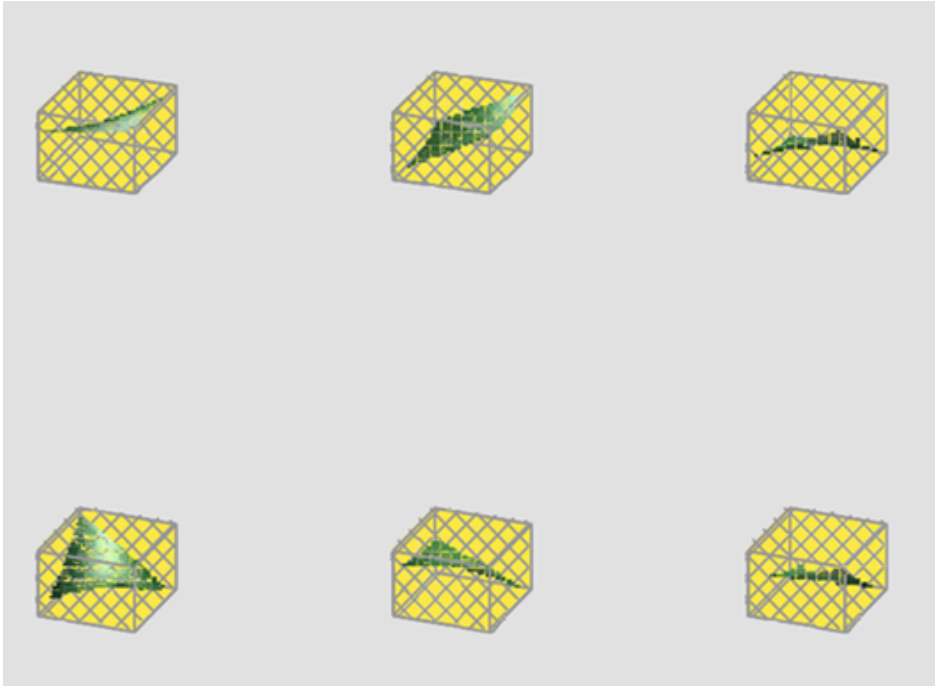

Figure 9. Response Surface Regression method for 4 different parameters of glucose production.

Analysis of variance (ANOVA)

To know if the model satisfies the assumptions of variance (ANOVA), as shown in Table 5, the probability (P) value was used to determine the significance of interaction for each parameter. The smaller P shows a larger significance of the interaction. In this case, pH is quite less than 0.05 value, which indicates pH value is significant, and Incubation (h)* enzyme conc. (µl) term is also quite near to 0.05 value. The regression equation in uncoated units for glucose production is shown in the following equation:

Glucose(mg/g) = -3815+ 47 Temperature(°C)+ 107 Incubation(h)+ 7.64 Enzyme conc(µl) + 483 pH+0.089 Temperature(°C)*Temperature(°C)- 0.35 Incubation(h)*Incubation(h) + 0.00489Enzyme conc(µl)*Enzyme conc(µl)+ 20.0 pH*pH+ 0.92 Temperature(°C)*Incubation(h)- 0.007Temperature(°C)*Enzyme conc(µl)- 9.67 Temperature(°C)*pH- 0.289 Incubation(h)*Enzyme conc(µl) - 15.2 Incubation(h)*pH- 0.550Enzyme conc(µl)*pH

https://doi.org/10.61577/jpri.2025.100003

Table 5. Response surface regression: Glucose (mg/g) versus temperature, Incubation (h), Enzyme concentration, pH.

Source	DF	Adj. SS	Adj. MS	F- Value	P- Value
Model	14	440375	31455	1.93	0.556
Linear	4	296208	74052	4.55	0.018
Temperature (0C)	1	56033	56033	3.44	0.088
Incubation (h)	1	20833	20833	1.28	1.28
Enzyme conc. (µl)	1	3333	3333	0.2	0.659
pH	1	216008	216008	13.23	0.003
Square	4	8567	2142	0.13	0.968
Temperature (0C)* temperature (0C)	1	133	133	0.01	0.929
Incubation (h)* Incubation (h)	1	833	833	0.05	0.825
Enzyme conc.(µl)* enzyme conc.(µl)	1	4033	4033	0.25	0.628
pH* pH	1	2133	2133	0.13	0.724
2- Way Interaction	6	135600	22600	1.39	0.295
Temperature(0C)* Incubation (h)	1	6806	6806	0.42	0.53
Temperature (0C)* enzyme conc. (µl)	1	56	56	0	0.954
Temperature (0C)* pH	1	21025	21025	1.29	0.278
Incubation (h)* enzyme conc. (µl)	1	67600	67600	4.16	0.064
Incubation (h)* pH	1	33306	33306	2.05	0.178
Enzyme conc.(µl)* pH	3	6806	6806	0.42	0.53
Error	12	195192	16266		
Lack – of – fit	10	152075	15208	0.71	0.713
Pure Error	2	43117	21558		
Total	**26**	**6355**			

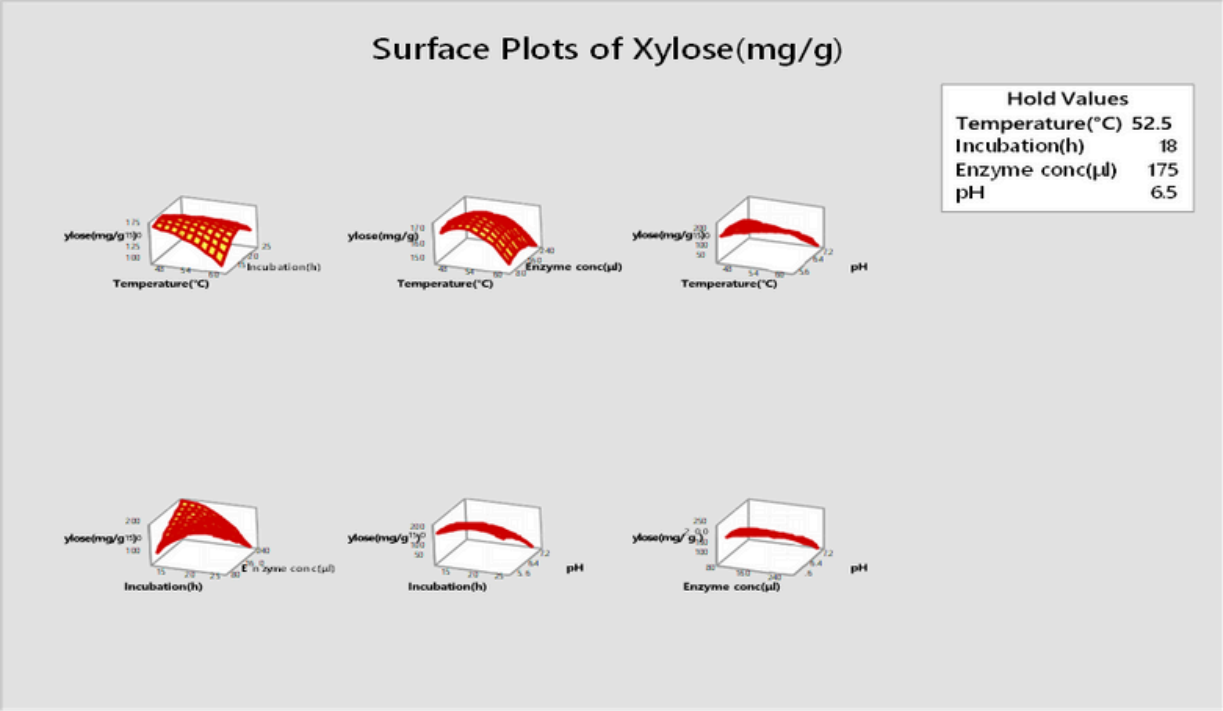

Figure 10. Response surface regression method of 4 different parameter of xylose production.

https://doi.org/10.61577/jpri.2025.100003

Figure 10 represents the surface plots xylose (mg/g) during enzymatic hydrolysis. They show interaction of each parameters during enzymatic hydrolysis for xylose production. The surface which shows interaction between temperature and incubation time, temperature and enzyme concentration and incubation time and enzyme concentration have a concave graph. This show there is significant interaction temperature and incubation time, temperature and enzyme concentration and incubation time and enzyme concentration. Rest other surface plots which shows interaction between temperature and pH, incubation time and pH and enzyme concentration and pH have flat surface plots which shows weak interaction between parameters during enzymatic hydrolysis.

Table 6 shows analysis of variance (ANOVA) for the production of xylose during enzymatic hydrolysis. The smaller P shows larger significance of interaction. In this case pH is equal to 0.05 value which indicates pH value is quite significant.

The regression equation in uncoaded units for xylose production is shown in following equation:

Xylose(mg/g) = -3382+ 43.5 Temperature(°C)+ 45.9 Incubation(h)+ 3.89 Enzyme conc(µl)+ 585 pH- 0.196 Temperature(°C)*Temperature(°C)- 0.914 Incubation(h)*Incubation(h)- 0.00085 Enzyme conc(µl)*Enzyme conc(µl)- 20.4 pH*pH + 0.528 Temperature(°C)*Incubation(h)+ 0.0022 Temperature(°C)*Enzyme conc(µl)-
5.17 Temperature(°C)*pH- 0.1111 Incubation(h)*Enzyme conc(µl)- 3.54 Incubation(h)*pH- 0.267 Enzyme conc(µl)*pH

Table 6. Response surface regression: xylose (mg/g) versus Temperature, Incubation (h), enzyme concentration, pH.

Source	DF	Adj. SS	Adj. MS	F- Value	P- Value
Model	14	76021	5430	1.43	0.271
Linear	4	47563	11890.6	3.13	0.056
Temperature (0C)	1	469	468.7	0.12	0.732
Incubation (h)	1	1408	1408.3	0.37	0.554
Enzyme conc. (µl)	1	52	52.1	0.01	0.909
pH	1	45633	45633.3	12	0.005
Square	4	6783	1695.8	0.45	0.774
Temperature (0C)* temperature (0C)	1	650	650.2	0.17	0.687
Incubation (h)* Incubation (h)	1	5779	5778.7	1.52	0.241
Enzyme conc.(µl)* enzyme conc.(µl)	1	122	122.5	0.03	0.861
pH* pH	1	2223	2223.1	0.58	0.459
2- Way Interaction	6	21675	3612.5	0.95	0.496
Temperature(0C)* Incubation (h)	1	2256	2256.3	0.59	0.456
Temperature (0C)* enzyme conc. (µl)	1	6	6.2	0	0.968
Temperature (0C)* pH	1	6006	6006.3	1.58	0.233
Incubation (h)* enzyme conc. (µl)	1	10000	10000	2.63	0.131
Incubation (h)* pH	1	1806	1806.2	0.47	0.504
Enzyme conc.(µl)* pH	3	1600	1600	0.42	0.529
Error	12	45648	3804		
Lack – of – fit	10	35831	3583.1	0.73	0.702
Pure Error	2	9817	4908.3		
Total	**26**	**121669**			

Assessment of pretreatment using FTIR

The structural alterations in lignocellulosic biomass before and after pretreatment were analyzed using FTIR spectroscopy. Comparisons between untreated, alkali-treated, and alkali combined with ultrasonication-treated samples of Pennisetum sp. revealed notable vibrational changes. Prominent absorption bands near 3334 and 2917 cm^{-1} indicated disruption of cellulosic hydrogen bonds in AAU-treated samples compared to untreated biomass. Peaks at 1611, 1320, and 1037 cm^{-1} suggested lignin removal, evident in AA and AAU-treated samples but absent in untreated ones. A shift from 1033 cm^{-1} (AA-treated DG) to 1250 cm^{-1} (AAU-treated DG) reflected C=O absorption from acetyl group cleavage, while such shifts were not observed in HNG. Additional peaks at 1091 and 900 cm^{-1} (AAU DG) and 1086 and 905 cm^{-1} (AAU HNG) confirmed

https://doi.org/10.61577/jpri.2025.100003

structural modifications and improved cellulose accessibility after ultrasonication-assisted alkali pretreatment. (Figure 11 & Figure 12) [13,14].

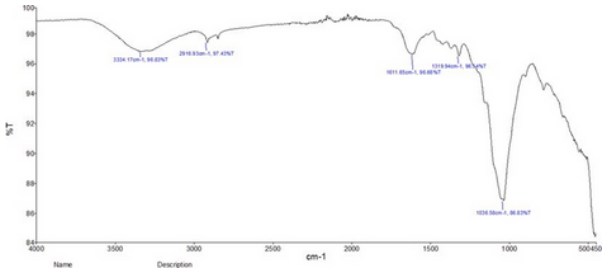

Figure 11. FTIR analysis of crude pearl millet (S1 Pearl millet).

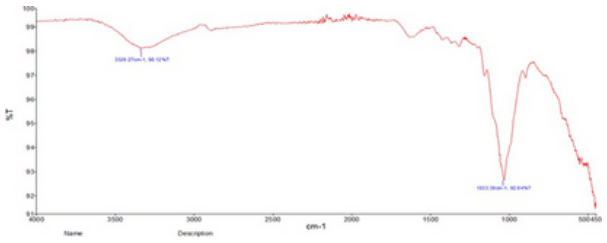

Figure 12. FTIR analysis of pre-treated pearl millet.

Conclusion

Pearl millet straw, which is an agricultural waste product, has the potential to be an economical feedstock for bioethanol production. However, a series of steps such as pretreatment, enzymatic hydrolysis and fermentation are required for the production of bioethanol from pearl millet. In the present study, a comparative study was conducted for alkali and acid pretreatment using the RSM L27 orthogonal array. It was evident from the study that compared to acid pretreatment, alkali pretreatment was an effective technique for enhanced delignification and cellulose exposure (590mg/g). Further, the RSM method was used for determining the optimised parameter for the hydrolysis of cellulose and hemicellulose that were exposed after the alkali pretreatment. The optimal parameters for enzymatic hydrolysis were determined to be pH 5.5, incubation time 16h, enzyme concentration 200µl and temperature 600C which resulted in 605 mg/g of glucose and 465 mg/g of xylose. The pretreated and enzyme-hydrolysed samples were further subjected to co-culture fermentation at 10 g and 90 g substrate concentration (separately) using S. Cerevisiae and Pichia sp. in batch cultures. The fermentation experiments resulted in enhanced ethanol production as revealed by the gas chromatography analysis. It is evident from this study that pearl millet straw has tremendous potential to be an economical feedstock for a biorefinery-based bioethanol concept.

References

1. Mignogna D, Szabó M, Ceci P, Avino P. Biomass energy and biofuels: perspective, potentials, and challenges in the energy transition. Sustain. 2024;16(16):7036. https://doi.org/10.3390/su16167036
2. Mutabaruka M, Kaur M, Singla S, Sharma P, Kaur G, Singh G. Biofuels Policy as the Indian Strategy to Achieve the 2030 Sustainable Development Goal 7: Targets, Progress, and Barriers. 2024. https://doi.org/10.2174/0126662558309359241004102906
3. Cheah WY, Sankaran R, Show PL, Tg Ibrahim TN, Chew KW, Culaba A, et al. Pretreatment methods for lignocellulosic biofuels production: current advances, challenges and future prospects. Biofuel Res J. 2020;7(1):1115-1127. https://doi.org/10.18331/brj2020.7.1.4
4. Packiam M, Karthikeyan S. A Catalytic Hydrothermal Pretreatment process for sugar recovery from pearl millet biomass for Biofuels Production. Madras Agric J. 2020.
5. Blessie JJ, Kennady ZJ, Karthikeyan S, Ramesh D. Pearl millet (Pennisetum glaucum) as a sustainable feedstock for bioethanol production by catalytic downflow liquid contact reactor. Crop Res. 2021;56(5):270-275. https://doi.org/10.31830/2454-1761.2021.043
6. Kumar PS, Kumari PK, Uma A, Umakanth AV. Evaluation of alkali and acid pretreatments on brown mid rib sorghum varieties for maximum cellulose yield. J Sci Res. 2021;65(1). https://doi.org/10.37398/jsr.2020.640316
7. Punia P, Singh L. Optimization of alkali pre-treatment of sweet sorghum [Sorghum bicolor (L.) Moench] residue to improve enzymatic hydrolysis for fermentable sugars. Waste Manag Bull. 2024;2(1):131-141. https://doi.org/10.1016/j.wmb.2023.12.007
8. Zhang H, Wu J. Optimization of Sodium Hydroxide Pretreatment and Enzymatic Hydrolysis of Wheat Straw Powder by Cellulases and Xylanases From Aspergillus Niger HQ-1 Using Response Surface Methodology. 2021. https://doi.org/10.21203/RS.3.RS-374151/V1
9. Ogura I, Sugiyama M, Tai R, Mano H, Matsuzawa T. Optimization of microplate-based phenol-sulfuric acid method and application to the multi-sample measurements of cellulose nanofibers. Anal Biochem. 2023; 681:115329. https://doi.org/10.1016/j.ab.2023.115329
10. Rasheed HA, Adeleke AA, Nzerem P, Olosho AI, Ogedengbe TS, Jesuloluwa S. Isolation, characterization and response surface method optimization of cellulose from hybridized agricultural wastes. Sci Rep. 2024;14(1):14310. https://doi.org/10.1038/s41598-024-65229-4
11. Aziz NK, Mohd Sukri SS, Wan Yaacob WS. The optimization of sodium hydroxide (NaOH) pretreatment for reducing sugar production from rice husk using Response Surface Methodology (RSM). ESTEEM Acad J. 2024;20:36-44. https://doi.org/10.24191/esteem.v20imarch.572.g485
12. Pereira LM, Milan TM, Tapia-Blácido DR. Using Response Surface Methodology (RSM) to optimize 2G bioethanol production: A review. Biomass and Bioenergy. 2021; 151:106166. https://doi.org/10.1016/j.biombioe.2021.106166
13. Lavudi S, Oberoi HS, Mangamoori LN. Ethanol production from sweet sorghum bagasse through process optimization using response surface methodology. 3 Biotech. 2017;7(4):233. https://doi.org/10.1007/s13205-017-0863-x
14. Mohapatra S, Mishra C, Merritt BB, Pattathil S, Thatoi H. Evaluating the Role of Ultrasonication-Assisted Alkali Pretreatment and Enzymatic Hydrolysis on Cellwall Polysaccharides of Pennisetum Grass Varieties as Potential Biofuel Feedstock. ChemistrySelect. 2019;4(3):1042-1054. https://doi.org/10.1002/SLCT.201802187

https://doi.org/10.61577/jpri.2025.100003