FUDMA Journal of Engineering and Technology
ISSN: 3092-9385
https://sites.google.com/fudutsinma.edu.ng/fjet
Volume 1, Issue 1, 2025, 1-14

Optimization of Alkaline Pretreatment and Fermentation Conditions for Improved Physicochemical Properties and Yield of Bioethanol Produced from Sugarcane Bagasse

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Abstract

Selected processing parameters were optimized to determine their impact on the yield and some physicochemical properties of bioethanol derived from sugar cane (Saccharum officinarum) bagasse. Bioethanol was produced from sugar cane bagasse using alkaline pretreatment method, simultaneous saccharification and fermentation processes. The physicochemical properties determined were: Density, kinematic viscosity, specific gravity, boiling point, pour point, pH value and ethanol concentration. The analytical methods used followed standard procedures. Two-way factor was used for the analysis of variance (ANOVA) to evaluate the effect of these processing parameters on the yield and physicochemical properties. The results revealed that the processing methods (alkaline pretreatment method, simultaneous saccharification and fermentation processes), had significant effect ($p<0.05$) on the yield and physicochemical properties of the bioethanol produced from sugar cane bagasse. The results ranged from 6.5 – 42.2% for the yield; 0.8462 – 0.9541 g/ml for density; 1.98 – 2.43 mm^2/s for kinematic viscosity; 0.8462 – 0.9541 for specific gravity; 88 – 98 °C for boiling point; -8 – 2 °C for pour point; 5.2 – 8.3 for the pH value and 37.2 – 85.06% for the bioethanol concentration. In conclusion, the results suggested that 5% sodium hydroxide concentration and 7days simultaneous saccharification and fermentation process has highest yield and ethanol concentration, and it was deduced that both the sodium hydroxide concentration and fermentation independently and jointly have significant effect on the yield and physicochemical properties.The favourable results obtained for the physicochemical properties of the bioethanol also suggested that it can find use as blend for gasoline.

Keywords: *Bioethanol, sugarcane bagasse, simultaneous saccharification, alkaline pretreatment, fermentation process.*

1.0 Introduction

The world is increasingly turning to biofuels as renewable and sustainable alternative to fossil fuels; among the various types of biofuels, ethanol is a leading contender. Ethanol is a biofuel produced through fermentation of carbohydrates, such as sugar, starches, or cellulose. It is commonly used as a transportation fuel, either as a pure ethanol or blended with gasoline [1]. However, harnessing ethanol from bagasse poses challenges due to its complex lignocellulosic structure. Despite this, research has shown encouraging results, with studies achieving significant ethanol yields from bagasse using various pre-treatment and hydrolysis methods. In the quest for sustainable energy solutions globally, bioethanol from sugarcane bagasse is poised to play a significant role. With further research and development, this promising feedstock can help meet the growing demand for renewable energy [1].

Bioethanol results from fermenting sugars derived from biomass. Biomass utilized for bioethanol production can include sucrose-rich sources like sugarcane and sugar beet, starch-rich crops like corn and wheat, or lignocellulosic materials such as sugarcane bagasse, wood, and straw. Corn and sugarcane serve as the primary feedstock in the US and Brazil, respectively, the world's leading ethanol producers. Sugarcane is a very efficient bioethanol raw material, with considerably lower fossil energy consumption during processing compared to corn. There is however room for further enhancing the bioethanol production process from sugarcane, leading to significant reductions in energy consumption [2].

The transformation of lignocellulosic residues into bioethanol is currently a globally significant area of interest. This procedure involves three key stages: (i) treating the raw material to decreasing lignin levels and enhancing polysaccharide exposure, (ii) enzymatic hydrolysis to convert polysaccharides into glucose and xylose monomers, and (iii) fermenting sugars into ethanol. The main focus in this process revolve around developing cost-effective pre-treatments that enhances enzyme access to cellulose, without generating compounds harmful to fermentation, notably phenolic, and without sacrificing reducing sugar during pre-treatment [3].

Received: 24/06/2025; Revised: 30/06/2025; Accepted: 01/07/2025; Published: 03/07/2025

2.0 Materials and Methods

2.1 Materials

Samples of sugarcane bagasse used for the study were sourced locally from Garatu Market, Bosso local government, Niger State, Nigeria. Other materials used were: Distilled water, Instant dry Yeast (baker's yeast), Aspergillus niger and sodium pellets. Equipment used were cooling system (HTF-319S model), Digital weighing balance (LB-3000 mode, Distillation setup (PSC-47-10963 model), magnetic stirrer (BIMS-005 model), Autoclave (LZ-VA-C24 mode), electric oven dryer (TG-9023A model) and stainless-steel grinder (SK-30-SS model).

2.2 Methods

The method of [4] with some modifications was used in the production of bioethanol from sugar cane bagasse. In the conversion of lignocellulosic biomass such as sugarcane bagasse into bioethanol; four major unit operations are usually employed. These include: pre-treatment, hydrolysis, fermentation and product recovery/ distillation. Pre-treatment was carried out by employing Alkali pre-treatment methods.

2.2.1 Pre-treatment of substrate

Alkali pre-treatment method was used in this study, as it is one of the best methods for pre-treating lignocellulosic biomaterial [4]. 50g of milled sample was placed in 5% concentration of sodium hydroxide and stirred continuously at 50rpm for 30 minutes at 500 ^{o}C using a magnetic stirrer. After pre-treatment, the solution was cooled and filtered using a filter paper and rinsed with distilled water to remove excess sodium hydroxide. The substrate was transferred to a conical flask and distilled water was added to 100ml, the flasks were then covered with cotton wool and wrapped with foil paper to avoid contamination. The mixtures were sterilized in an autoclave at 1210C for 15 minutes, allowed to cool and distilled water was added to make up to the 100ml mark again. This was done for 3% and 1% concentration of sodium hydroxide respectively.

2.2.2 Preparation of Sodium Hydroxide Concentration

5% sodium hydroxide concentration in 100ml of distilled water was prepared by dissolving 5 grams of sodium hydroxide in 50ml of water and stirred properly, once the sodium hydroxide was dissolved completely, distilled water was then added to make up the 100ml mark.

3% sodium hydroxide concentration in 100ml of distilled water was prepared by dissolving 3 grams of sodium hydroxide in 50ml of water and stirred properly, once the sodium hydroxide was dissolved completely, distilled water was then added to make up the 100ml mark.

1% sodium hydroxide concentration in 100ml of distilled water was prepared by dissolving 1 gram of sodium hydroxide in 50ml of water and stirred properly, once the sodium hydroxide was dissolved completely, distilled water was then added to make up the 100ml mark.

2.2.3 Simultaneous saccharification and fermentation (SSF) of sugarcane bagasse

Simultaneous saccharification and fermentation was conducted in a 500ml conical flask with a working volume of 100ml. 10ml of aspergillus niger and 1.5g of instant dry yeast (Saccharomyces cerevisiae) were added to the flask which was corked properly, sealed with aluminium foil paper and incubated at room temperature for 7 days, 5 days and 3 days.

2.2.4 Fractional distillation

The fermented broth was poured into a round-bottom flask connected to a distillation column enclosed by continuous flowing water. A conical flask was attached to the other end of the distillation column to collect the distilled liquid. The round-bottom flasks with the fermented broth were heated using a heating mantle set to 78°C (standard ethanol boiling point).

2.2.5 Determination of quantity of ethanol produced

The distilled ethanol was measured using a measuring cylinder. The quantity of ethanol produced in g/l was calculated by multiplying the volume of distilled ethanol by the density of ethanol (0.79 g/ml). The ethanol yield in g/l is equivalent to the yield of 100 g of dried substrate.

2.2.6 Determination of bioethanol yields

The initial mass of the dried sugarcane bagasse was weighed and recorded as Mbagasse, the volume of bioethanol produced after distillation was measured and recorded as Vethanol, the concentration of ethanol in the distillated bioethanol was measured using an alcoholmeter and recorded as Cethanol. Bioethanol yield was determined using the formula in equation 1.,

Received: 24/06/2025; Revised: 30/06/2025; Accepted: 01/07/2025; Published: 03/07/2025

$$\text{Bioethanol yield} = \frac{Methanol}{Mbagasse} \times 100\% \quad (1)$$

$$M_{ethanol} = V_{ethanol} \times \varrho_{ethanol} \times C_{ethanol}$$

where: ϱethanol is the density of ethanol (0.79/ml).

2.2.7 Characterization of produced bioethanol

The physicochemical properties of the produced bioethanol determined based on standard methods are: density, kinematic viscosity, specific gravity, boiling point, pour point and pH value. These properties were compared with those of standard ethanol.The ethanol concentration of the produced bioethanol was also determined.

2.2.8 Experimental design

The experimental design for the study was done with the aid of the design expert 13 software. The data obtained from the study was subjected to statistical analysis using Analysis of Variance (ANOVA).

3.0 Results and Discussion

3.1 Results

The result of this study is presented in Table 1.

Table 1: Bioethanol yield from sugarcane bagasse and its physicochemical properties

Run	NaOH Conc. (%)	Fermentation Duration (days)	Bioethanol Yield (%)	Density (g/ml)	Kinematic viscosity (mm^2/s)	Specific gravity	Boiling point ^0C	Pour point ^0C	pH Value	Ethanol Conc. (%)
1	0	3	6.5	0.9541	2.25	0.9541	94	-3	5.6	37.2
2	1	3	18.7	0.9135	2.35	0.9135	95	-2	6.4	58.77
3	3	3	28.1	0.8942	2.46	0.8942	97	0	7.2	67.12
4	5	3	33	0.8901	2.62	0.8901	98	2	8.3	68.81
5	0	5	9.3	0.9432	2.11	0.9432	90	-5	5.2	43.89
6	1	5	27.5	0.9051	2.23	0.9051	92	-4	5.7	62.49
7	3	5	33.3	0.8853	2.38	0.8853	93	-2	6.8	70.74
8	5	5	37.4	0.8622	2.54	0.8622	95	1	7.7	79.5
9	0	7	11.2	0.9354	1.98	0.9354	88	-8	4.4	48.15
10	1	7	30.8	0.916	2.05	0.916	90	-7	5.3	57.64
11	3	7	37.3	0.875	2.16	0.875	91	-4	6.4	74.76
12	5	7	42.2	0.8462	2.43	0.875	93	-1		7.3

3.2 Discussion

3.2.1 Bioethanol yield from sugarcane bagasse

The bioethanol yield from sugarcane bagasse showed positive correlation with both increasing NaOH concentration and fermentation duration as shown in Table 1. The highest yield of 42.2% was achieved under the most extreme conditions tested: 5% NaOH and 7 days of fermentation. This trend agrees with previous studies on lignocellulosic biomass pretreatment and fermentation. For instance, [5] reported that alkaline pretreatment enhances cellulose accessibility by breaking lignin-carbohydrate complexes and partially dissolving hemicellulose, thereby improving enzymatic hydrolysis efficiency.

The effect of NaOH concentration and fermentation duration on the bioethanol yield was tested for significance using analysis of variance, ANOVA (Table 2). In the analysis, P-values ≤ 0.05 indicate that the model terms are significant. The overall model for bioethanol yield showed high significance with a P-value of 0.0010, suggesting that the chosen factors have a Substantial impact on the yield. This aligns with previous studies that have demonstrated the importance of pretreatment conditions and fermentation time on bioethanol production from lignocellulosic biomass [6].

Table 2: Analysis of variance (ANOVA) for bioethanol yield

Source	Sum of squares	Df	Mean square	F-value	P-value	
Model	1496.44	5	299.29	20.83	0.0010	significant
A-NaOH concentration	1183.24	1	1183.24	82.36	0.0001	
B-Fermentation duration	156.14	1	156.14	10.87	0.0165	

Received: 24/06/2025; Revised: 30/06/2025; Accepted: 01/07/2025; Published: 03/07/2025

Source	Sum of squares	Df	Mean square	F-value	P-value
AB	1.43	1	1.43	0.0997	0.7629
A^2	176.81	1	176.81	12.31	0.0127
B^2	2.16	1	2.16	0.1503	0.7116
Residual	86.20	6	14.37		
Cor Total	1582.64	11			
Std. Dev.	3.79				
Mean	26.27				
C.V. %	14.43				
R^2	0.9455				
Adjusted R^2	0.9001				
Predicted R^2	0.8075				
Adeq Precision	12.9744				

Linear terms for both NaOH concentration (A) and fermentation duration (B) were found to be highly significant, with P-values of 0.0001 and 0.0165 respectively. This indicates that both factors independently exert a strong influence on the bioethanol yield. The interaction term (AB) between NaOH concentration and fermentation duration was not significant (P-value = 0.7629). This suggests that the effects of these two factors on bioethanol yield are largely independent of each other. The quadratic term for NaOH concentration (A^2) was significant (P-value = 0.0127), indicating a non-linear relationship between NaOH concentration and yield.

The model demonstrated good fit characteristics, with a high R^2 value of 0.9455, indicating that 94.55% of the variability in bioethanol yield could be explained by the model. The adjusted R^2 (0.9001) and predicted R^2 (0.8075) were in reasonable agreement, suggesting good predictive capability. The adequate precision ratio of 12.9744, being greater than 4, indicates adequate signal-to-noise ratio. The coefficient of variation (C.V.) was 14.43%, which is acceptable for biological processes that often exhibit inherent variability [7]. This level of variation is comparable to those reported in similar bioethanol production studies [8]. The standard deviation of 3.79 relative to a mean yield of 26.27% suggests reasonably consistent experimental results. Fig 1 shows the 3D plot of NaOH concentration and fermentation duration on bioethanol yield.

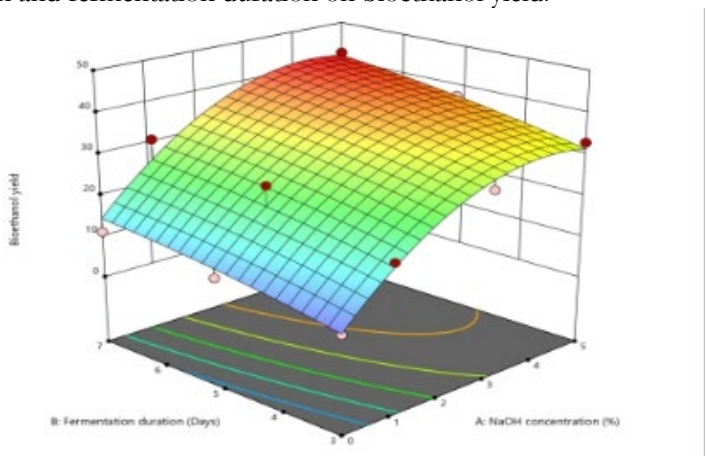


Fig. 1: 3D plot of NaOH concentration and fermentation duration on bioethanol yield

3.2.2 Density of bioethanol from sugarcane bagasse

The density of the produced bioethanol showed a consistent decrease with both increasing NaOH concentration and fermentation duration. The lowest density (0.8462 g/ml) was observed under conditions of 5% NaOH and 7 days of fermentation as seen in Table 1. The decreasing trend in density can be primarily attributed to the increasing ethanol content in the fermentation broth. Ethanol has a lower density (0.789 g/ml at 20°C) compared to water (1.000 g/ml at 4°C), so. The more efficient pretreatment (higher NaOH concentration) likely led to greater sugar availability for fermentation, resulting in higher ethanol production and consequently lower density.

Table 3 shows the effect of NaOH concentration and fermentation duration on the density of the bioethanol produced. The overall model for density demonstrated exceptionally high significance with a P-value of 0.0002, this means that the selected factors have a substantial impact on the density of the bioethanol.

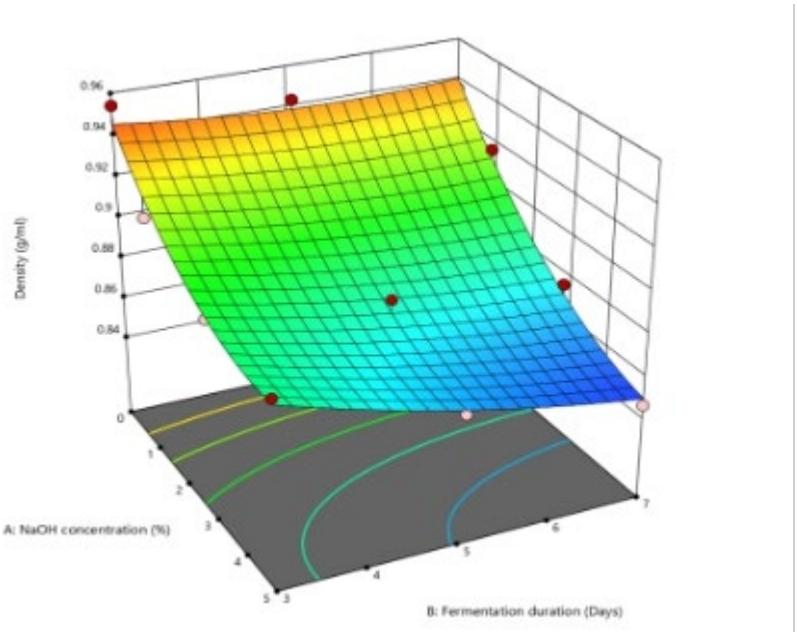
Received: 24/06/2025; Revised: 30/06/2025; Accepted: 01/07/2025; Published: 03/07/2025

Table 3: Analysis of Variance (ANOVA) for density

Source	Sum of squares	df	Mean square	F-value	P-value	
Model	0.0114	5	0.0023	34.33	0.0002	significant
A-NaOH concentration	0.0098	1	0.0098	147.99	< 0.0001	
B-Fermentation duration	0.0009	1	0.0009	13.66	0.0101	
AB	0.0003	1	0.0003	4.73	0.0725	
A^2	0.0005	1	0.0005	8.03	0.0298	
B^2	0.0000	1	0.0000	0.6807	0.4409	
Residual	0.0004	6	0.0001			
Cor Total	0.0118	11				
Std. Dev.	0.0081					
Mean	0.9017					
C.V. %	0.9028					
R^2	0.9662					
Adjusted R^2	0.9381					
Predicted R^2	0.8558					
Adeq Precision	16.6629					

Both linear terms, NaOH concentration (A) and fermentation duration (B), were found to be highly significant, with P-values of < 0.0001 and 0.0101 respectively. This indicates that both factors independently exert a strong influence on the density of the bioethanol. The significance of fermentation duration aligns with findings by [9], who noted that longer fermentation times lead to higher ethanol concentrations, which in turn affects the density of the final product.

The interaction term (AB) between NaOH concentration and fermentation duration was not significant (P-value = 0.0725), although it was close to the significance threshold. This suggests that while there might be a slight interaction effect, the impacts of these two factors on density are largely independent of each other. The quadratic term for NaOH concentration (A^2) was significant (P-value = 0.0298), indicating a non-linear relationship between NaOH concentration and density. The model exhibited excellent fit characteristics, with a very high R^2 value of 0.9662, indicating that 96.62% of the variability in density could be explained by the model. The adjusted R^2 (0.9381) and predicted R^2 (0.8558) were in good agreement, suggesting strong predictive capability. The adequate precision ratio of 16.6629, being substantially greater than 4, indicates an excellent signal-to-noise ratio. The coefficient of variation (C.V.) was remarkably low at 0.9028%, which indicates exceptional precision and reliability of the experiments, the standard deviation of 0.0081 relative to a mean density of 0.9017 g/ml suggests highly consistent and precise experimental results. Fig. 2 shows the 3D plot of NaOH concentration and fermentation duration on density.



Received: 24/06/2025; Revised: 30/06/2025; Accepted: 01/07/2025; Published: 03/07/2025

Fig. 2: 3D plot of NaOH concentration and fermentation duration on density

3.2.3 Kinematic viscosity of bioethanol from sugarcane bagasse

The kinematic viscosity results show a slight increase with higher NaOH concentrations but a decrease with longer fermentation durations from Table 1, the lowest viscosity (1.98 mm^2/s) was observed at 0% NaOH and 7 days of fermentation. This complex behaviour might be due to the interplay between ethanol concentration, residual sugars, and other byproducts of the fermentation process. The increase in viscosity with higher NaOH concentrations could be attributed to the presence of residual sodium ions or other soluble compounds released during the more aggressive pretreatment. These dissolved solids can increase the viscosity of the solution [10].

Table 4 shows the influence of NaOH concentration and fermentation duration on the kinematic viscosity of the produced bioethanol. The overall model for kinematic viscosity demonstrated exceptionally high significance with a P-value of < 0.0001, which indicates that the selected factors have a substantial impact on the kinematic viscosity of the bioethanol.

Table 4: : Analysis of variance (ANOVA) for kinematic viscosity

Source	**Sum of squares**	**df**	**Mean square**	**F-value**	**P-value**	
Model	0.4332	5	0.0866	85.51	< 0.0001	significant
A-NaOH concentration	0.2871	1	0.2871	283.33	< 0.0001	
B-Fermentation duration	0.1340	1	0.1340	132.25	< 0.0001	
AB	0.0019	1	0.0019	1.85	0.2229	
A^2	0.0008	1	0.0008	0.8178	0.4007	
B^2	0.0020	1	0.0020	1.99	0.2080	
Residual	0.0061	6	0.0010			
Cor Total	0.4393	11				
Std. Dev.	0.0318					
Mean	2.30					
C.V. %	1.39					
R^2	0.9862					
Adjusted R^2	0.9746					
Predicted R^2	0.9368					
Adeq Precision	29.4952					

Both linear terms, NaOH concentration (A) and fermentation duration (B), were found to be extremely significant, with P-values of < 0.0001 for both factors. This indicates that both variables independently exert a strong influence on the kinematic viscosity of the bioethanol. The interaction term (AB) between NaOH concentration and fermentation duration was not significant (P-value = 0.2229). This suggests that the effects of these two factors on kinematic viscosity are largely independent of each other, which could simplify process optimization strategies. The quadratic terms for both NaOH concentration (A^2) and fermentation duration (B^2) were also not significant (P-values of 0.4007 and 0.2080 respectively), indicating that the relationship between these factors and kinematic viscosity is primarily linear within the studied range.

The model exhibited excellent fit characteristics, with an extremely high R^2 value of 0.9862, indicating that 98.62% of the variability in kinematic viscosity could be explained by the model. The adjusted R^2 (0.9746) and predicted R^2 (0.9368) were in very good agreement, suggesting strong predictive capability. The adequate precision ratio of 29.4952, being substantially greater than 4, indicates an excellent signal-to-noise ratio, the coefficient of variation (C.V.) was remarkably low at 1.39%, which indicates exceptional precision and reliability of the experiments. The standard deviation of 0.0318 mm^2/s relative to a mean kinematic viscosity of 2.30 mm^2/s suggests highly consistent and precise experimental results. Fig. 3 shows the 3D plot of NaOH concentration and fermentation duration on kinematic viscosity.

Received: 24/06/2025; Revised: 30/06/2025; Accepted: 01/07/2025; Published: 03/07/2025

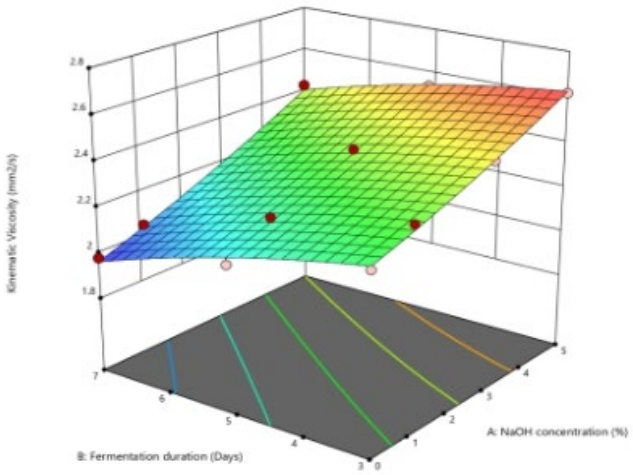


Fig. 3: 3D plot of NaOH concentration and fermentation duration on kinematic viscosity

3.2.4 Specific gravity of bioethanol from sugarcane bagasse

The specific gravity results follow the density trends; it shows a decrease with both increasing NaOH concentration and fermentation duration. The lowest specific gravity (0.8462) was observed under conditions of 5% NaOH and 7 days of fermentation as shown in Table 1, it matches the density result as expected. Specific gravity is the ratio of the density of a substance to the density of a reference substance (usually water at 4°C). It provides a dimensionless measure that is often used in the fermentation industry. The decreasing trend in specific gravity indicates an increase in ethanol concentration, as ethanol has a lower specific gravity (0.789 at 20°C) than water [11].

The impact of NaOH concentration and fermentation duration on the specific gravity of the produced bioethanol was assessed using analysis of variance (ANOVA), as presented in Table 5. The overall model for specific gravity demonstrated exceptionally high significance with a P-value of 0.0002, this means that the selected factors have a substantial influence on the specific gravity of the bioethanol.

Table 5: Analysis of variance (ANOVA) for specific gravity

Source	Sum of squares	Df	Mean square	F-value	P-value	
Model	0.0114	5	0.0023	34.33	0.0002	significant
A-NaOH concentration	0.0098	1	0.0098	147.99	< 0.0001	
B-Fermentation duration	0.0009	1	0.0009	13.66	0.0101	
AB	0.0003	1	0.0003	4.73	0.0725	
A^2	0.0005	1	0.0005	8.03	0.0298	
B^2	0.0000	1	0.0000	0.6807	0.4409	
Residual	0.0004	6	0.0001			
Cor Total	0.0118	11				
Std. Dev.	0.0081					
Mean	0.9017					
C.V. %	0.9028					
R^2	0.9662					
Adjusted R^2	0.9381					
Predicted R^2	0.8558					
Adeq Precision	16.6629					

Both linear terms, NaOH concentration (A) and fermentation duration (B), were found to be highly significant, with P-values of < 0.0001 and 0.0101 respectively. This indicates that both factors independently exert a strong influence on the specific gravity of the bioethanol. The interaction term (AB) between NaOH concentration and fermentation duration was not significant (P-value = 0.0725), although it was close to the significance threshold. This suggests that while there might be a slight interaction effect, the impacts of these two factors on specific gravity are largely independent of each other. The quadratic term for NaOH concentration (A^2) was significant (P-value = 0.0298), indicating a non-linear relationship between NaOH concentration and specific gravity.

Received: 24/06/2025; Revised: 30/06/2025; Accepted: 01/07/2025; Published: 03/07/2025

The model exhibited excellent fit characteristics, with a very high R^2 value of 0.9662, indicating that 96.62% of the variability in specific gravity could be explained by the model. The adjusted R^2 (0.9381) and predicted R^2 (0.8558) were in good agreement, suggesting strong predictive capability. The adequate precision ratio of 16.6629, being substantially greater than 4, indicates an excellent signal-to-noise ratio. The coefficient of variation (C.V.) was remarkably low at 0.9028%, which indicates exceptional precision and reliability of the experiments. The standard deviation of 0.0081 relative to a mean specific gravity of 0.9017 suggests highly consistent and precise experimental results. Fig. 4 shows the 3D plot of NaOH and fermentation duration on specific gravity.

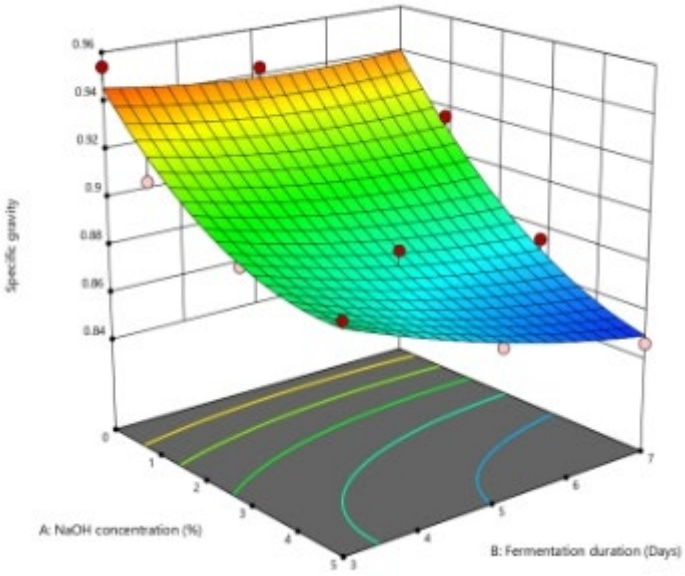


Fig. 4: 3D plot of NaOH concentration and fermentation duration on specific gravity

3.2.5 Boiling point of bioethanol from sugarcane bagasse

The boiling point results show a general decrease with longer fermentation durations but a slight increase with higher NaOH concentrations (Table 1). The lowest boiling point (88°C) was observed at 0% NaOH and 7 days of fermentation, the decrease in boiling point with longer fermentation times is consistent with an increase in ethanol concentration (Table 1). Pure ethanol has a boiling point of 78.4°C, significantly lower than that of water (100°C). As the proportion of ethanol in the mixture increases, the boiling point of the solution decreases, approaching that of pure ethanol [12].

Table 6 shows the effect of NaOH concentration and fermentation duration on the boiling point of the produced bioethanol produced. The overall model for boiling point demonstrated exceptionally high significance with a P-value of < 0.0001, indicating that the selected factors have a substantial impact on the boiling point of the bioethanol. This finding is consistent with previous studies that emphasizes the influence of pretreatment conditions and fermentation parameters on the thermal properties of bioethanol [13].

Table 6: : Analysis of variance (ANOVA) for boiling point

Source	Sum of squares	df	Mean square	F-value	P-value	
Model	96.80	5	19.36	96.72	< 0.0001	significant
A-NaOH concentration	34.53	1	34.53	172.49	< 0.0001	
B-Fermentation duration	58.94	1	58.94	294.48	< 0.0001	
AB	0.0763	1	0.0763	0.3811	0.5597	
A^2	0.3499	1	0.3499	1.75	0.2343	
B^2	1.50	1	1.50	7.49	0.0338	
Residual	1.20	6	0.2002			
Cor Total	98.00	11				
Std. Dev.	0.4474					
Mean	93.00					
C.V. %	0.4811					
R^2	0.9877					

Received: 24/06/2025; Revised: 30/06/2025; Accepted: 01/07/2025; Published: 03/07/2025

Source	Sum of squares	df	Mean square	F-value	P-value
Adjusted R^2	0.9775				
Predicted R^2	0.9609				
Adeq Precision	31.2705				

Both linear terms, NaOH concentration (A) and fermentation duration (B), were found to be extremely significant, with P-values of < 0.0001 for both factors. This indicates that both variables independently exert a strong influence on the boiling point of the bioethanol. The interaction term (AB) between NaOH concentration and fermentation duration was not significant (P-value = 0.5597). This suggests that the effects of these two factors on boiling point are largely independent of each other, which could simplify process optimization strategies. The quadratic term for NaOH concentration (A^2) was not significant (P-value = 0.2343), but the quadratic term for fermentation duration (B^2) was significant (P-value = 0.0338). This indicates a non-linear relationship between fermentation duration and boiling point, this suggests that the effect of fermentation time on boiling point may not be constant throughout the process.

The model exhibited excellent fit characteristics, with an extremely high R^2 value of 0.9877, indicating that 98.77% of the variability in boiling point could be explained by the model. The adjusted R^2 (0.9775) and predicted R^2 (0.9609) were in very good agreement, suggesting strong predictive capability. The adequate precision ratio of 31.2705, being substantially greater than 4, indicates an excellent signal-to-noise ratio, the coefficient of variation (C.V.) was remarkably low at 0.4811%, which indicates exceptional precision and reliability of the experiments. The standard deviation of 0.4474°C relative to a mean boiling point of 93.00°C suggests highly consistent and precise experimental results. Fig.5 shows the 3D plot of NaOH concentration and fermentation duration on boiling point.

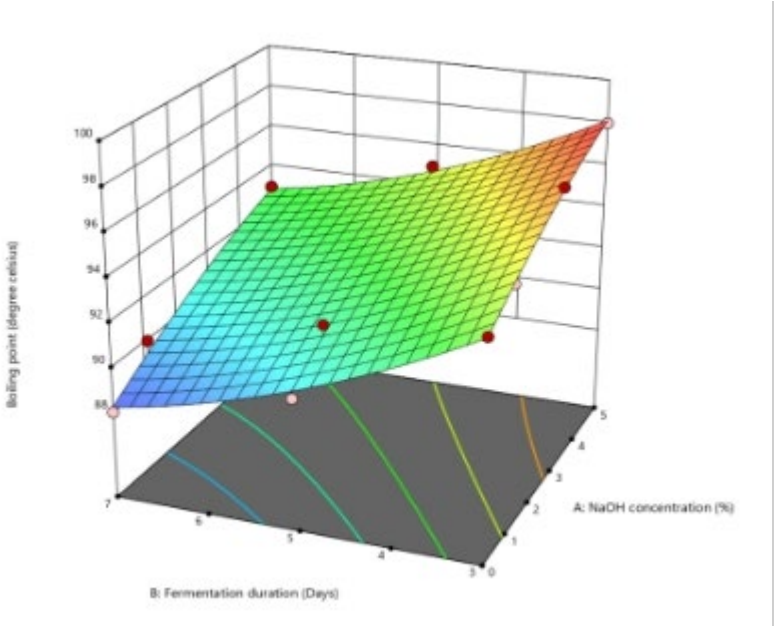


Fig. 5: 3D plot of NaOH concentration and fermentation duration on boiling point

3.2.6 Pour point of bioethanol from sugarcane bagasse

The pour point results as shown in Table 1 shows a general decrease (becoming more negative) with longer fermentation durations but an increase with higher NaOH concentrations. The lowest pour point (-8°C) was observed at 0% NaOH and 7 days of fermentation. The decrease in pour point with longer fermentation times is consistent with an increase in ethanol concentration. Ethanol has excellent antifreeze properties, with a freezing point of -114°C, significantly lower than that of water (0°C). As the ethanol concentration in the mixture increases, the pour point decreases, improving the cold flow properties of the fuel [14].

The effect of NaOH concentration and fermentation duration on the pour point of bioethanol from sugarcane bagasse was tested for significance using analysis of variance (ANOVA), as presented in Table 7. In the analysis, P-values ≤ 0.05 indicate that the model terms are significant. The overall model for pour point demonstrated exceptionally high significance with a P-value of < 0.0001, indicating that the selected factors have a substantial impact on the pour point of the bioethanol.

Received: 24/06/2025; Revised: 30/06/2025; Accepted: 01/07/2025; Published: 03/07/2025

Table 7: Analysis of variance (ANOVA) for pour point

Source	Sum of squares	df	Mean square	F-value	P-value	
Model	102.07	5	20.41	693.26	< 0.0001	significant
A-NaOH concentration	63.88	1	63.88	2169.14	< 0.0001	
B-Fermentation duration	33.77	1	33.77	1146.93	< 0.0001	
AB	1.32	1	1.32	44.97	0.0005	
A^2	0.1687	1	0.1687	5.73	0.0538	
B^2	0.3750	1	0.3750	12.73	0.0118	
Residual	0.1767	6	0.0294			
Cor Total	102.25	11				
Std. Dev.	0.1716					
Mean	-2.75					
C.V. %	6.24					
R^2	0.9983					
Adjusted R^2	0.9968					
Predicted R^2	0.9931					
Adeq Precision	83.6753					

Both linear terms, NaOH concentration (A) and fermentation duration (B), were found to be extremely significant, with P-values of < 0.0001 for both factors. This indicates that both variables independently exert a strong influence on the pour point of the bioethanol. The interaction term (AB) between NaOH concentration and fermentation duration was also found to be significant (P-value = 0.0005). This suggests a synergistic effect between these two factors on the pour point, indicating that the impact of NaOH concentration on pour point depends on the fermentation duration, and vice versa. This interaction effect highlights the complexity of the bioethanol production process and the importance of considering multiple factors simultaneously when optimizing fuel properties[15].

The quadratic term for NaOH concentration (A^2) was not significant (P-value = 0.0538), but it was very close to the significance threshold. This suggests that there might be a slight non-linear relationship between NaOH concentration and pour point, On the other hand, the quadratic term for fermentation duration (B^2) was significant (P-value = 0.0118), indicating a clear non-linear relationship between fermentation time and pour point. This non-linearity is consistent with the findings of [16], who observed that the impact of fermentation time on ethanol production and fuel properties is not always linear due to the complex dynamics of microbial metabolism.

The model exhibited excellent fit characteristics, with an extremely high R^2 value of 0.9983, indicating that 99.83% of the variability in pour point could be explained by the model. This exceptionally high R^2 value suggests that the model captures almost all of the variation in the data, providing a robust tool for predicting pour point based on NaOH concentration and fermentation duration. The adjusted R^2 (0.9968) and predicted R^2 (0.9931) were in very close agreement, suggesting strong predictive capability and minimal overfitting. The adequate precision ratio of 83.6753, being substantially greater than the desired value of 4, indicates an excellent signal-to-noise ratio. The coefficient of variation (C.V.) was relatively low at 6.24%, which indicates good precision and reliability of the experiments.

The standard deviation of 0.1716°C relative to a mean pour point of -2.75°C suggests highly consistent and precise experimental results. This level of precision is crucial for accurately characterizing the cold flow properties of bioethanol, which are critical for its use as a fuel, especially in colder climates [17]. Fig. 6 shows the 3D plot of NaOH concentration and fermentation duration on pour point.

Received: 24/06/2025; Revised: 30/06/2025; Accepted: 01/07/2025; Published: 03/07/2025

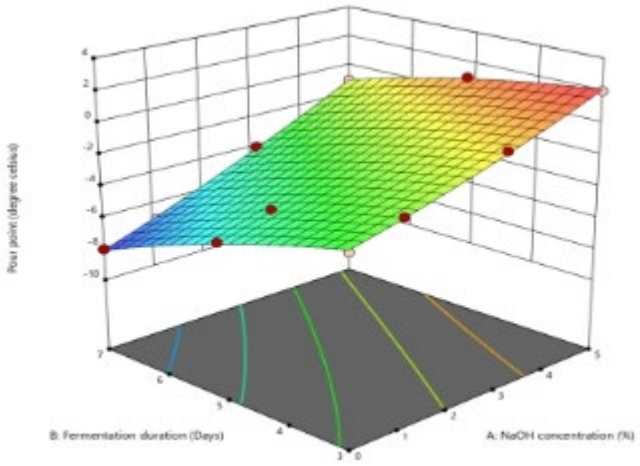


Fig. 6: 3D plot of NaOH concentration and fermentation duration on pour point

3.2.7 pH value of bioethanol from sugarcane bagasse

The pH results show an increase with higher NaOH concentrations but a slight decrease with longer fermentation durations (Table 1). The highest pH (8.3) was observed at 5% NaOH and 3 days of fermentation. The increase in pH with higher NaOH concentrations is expected due to the alkaline nature of the pretreatment. NaOH is a strong base, and residual hydroxide ions contribute to the elevated pH.

The effect of NaOH concentration and fermentation duration on the pH value of bioethanol from sugarcane bagasse was tested for significance using analysis of variance (ANOVA), as presented in Table 8. The overall model for pH value demonstrated exceptionally high significance with a P-value of < 0.0001, this means that the selected factors have a substantial impact on the pH of the bioethanol. This finding aligns with previous studies that have emphasized the influence of pretreatment conditions and fermentation parameters on the chemical properties of bioethanol [18].

Table 8: : Analysis of variance (ANOVA) for pH value

Source	Sum of squares	df	Mean square	F-value	P-value	
Model	14.57	5	2.91	179.58	< 0.0001	significant
A-NaOH concentration	12.43	1	12.43	766.09	< 0.0001	
B-Fermentation duration	2.02	1	2.02	124.28	< 0.0001	
AB	0.0178	1	0.0178	1.10	0.3351	
A^2	0.0516	1	0.0516	3.18	0.1248	
B^2	0.0004	1	0.0004	0.0257	0.8780	
Residual	0.0974	6	0.0162			
Cor Total	14.67	11				
Std. Dev.	0.1274					
Mean	6.36					
C.V. %	2.00					
R^2	0.9934					
Adjusted R^2	0.9878					
Predicted R^2	0.9655					
Adeq Precision	40.6733					

Both linear terms, NaOH concentration (A) and fermentation duration (B), were found to be extremely significant, with P-values of < 0.0001 for both factors. This indicates that both variables independently exert a strong influence on the pH value of the bioethanol. The high significance of NaOH concentration is expected, given its direct impact on the alkalinity of the solution. This is consistent with the work of [19], who reported that the severity of alkaline pretreatment significantly affects the pH and other properties of the resulting bioethanol [20].

The significance of fermentation duration on pH is also noteworthy. This could be attributed to the production of organic acids during fermentation, which can lower the pH over time. This finding is in line with

the research of [21], who observed changes in pH throughout the fermentation process due to microbial metabolic activities. The interaction term (AB) between NaOH concentration and fermentation duration was not significant (P-value = 0.3351). This suggests that the effects of these two factors on pH are largely independent of each other. In practical terms, this means that changes in NaOH concentration will affect pH similarly regardless of fermentation duration, and vice versa.

The quadratic terms for both NaOH concentration (A^2) and fermentation duration (B^2) were not significant (P-values of 0.1248 and 0.8780 respectively). This indicates that the relationship between these factors and pH is primarily linear within the studied range, the model exhibited excellent fit characteristics, with an extremely high R^2 value of 0.9934, indicating that 99.34% of the variability in pH could be explained by the model. This exceptionally high R^2 value suggests that the model captures almost all of the variation in the data. The adjusted R^2 (0.9878) and predicted R^2 (0.9655) were in very close agreement, suggesting strong predictive capability and minimal overfitting.

The adequate precision ratio of 40.6733, being substantially greater than the desired value of 4, indicates an excellent signal-to-noise ratio, the coefficient of variation (C.V.) was remarkably low at 2.00%, which indicates exceptional precision and reliability of the experiments, the standard deviation of 0.1274 relative to a mean pH value of 6.36 suggests highly consistent and precise experimental results. Fig. 7 shows the 3D plot of NaOH concentration and fermentation duration on pHvalue.

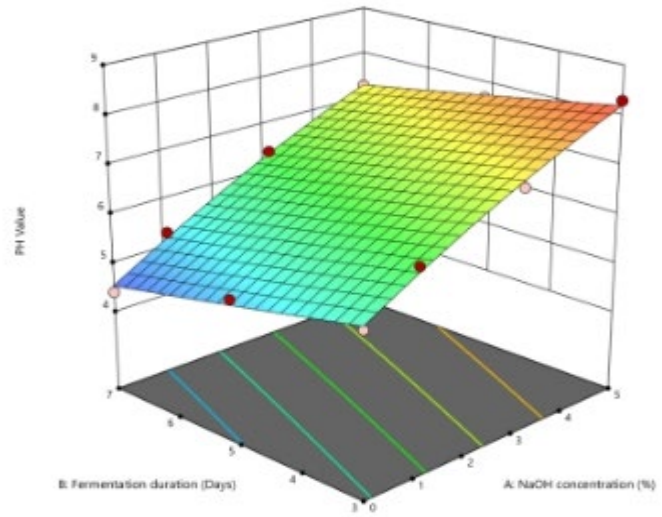


Fig. 7: 3D plot of NaOH concentration and fermentation duration on pH value

3.2.8 Ethanol concentration of bioethanol from sugarcane bagasse

The ethanol concentration results show a clear increase with both higher NaOH concentrations and longer fermentation durations, reaching a maximum of 85.06% at 5% NaOH and 7 days of fermentation as shown in Table 1. This trend corroborates the bioethanol yield results and is consistent with improved cellulose accessibility due to alkaline pretreatment and more complete fermentation over time.

The increase in ethanol concentration with longer fermentation times is expected because, longer fermentation allows yeasts to consume a higher percentage of available sugars, which leads to higher ethanol yields as stated by [22] in their study on yeast, in sustainable bioethanol production, additionally, initial fermentation products or biomass-derived compounds can inhibit yeast activity. Longer fermentation times may allow yeasts to adapt and overcome this inhibition [21].

Table 9 shows the influence of NaOH concentration and fermentation duration on the ethanol concentration of the bioethanol. The overall model for ethanol concentration demonstrated high significance with a P-value of 0.0006, this means that the selected factors impact the ethanol concentration.

Table 9: : Analysis of variance (ANOVA) for ethanol concentration

Source	Sum of squares	Df	Mean square	F-value	P-value	
Model	2201.92	5	440.38	25.44	0.0006	significant
A-NaOH concentration	1885.80	1	1885.80	108.95	< 0.0001	
B-Fermentation duration	155.37	1	155.37	8.98	0.0241	

Received: 24/06/2025; Revised: 30/06/2025; Accepted: 01/07/2025; Published: 03/07/2025

Source	Sum of squares	Df	Mean square	F-value	P-value
AB	25.07	1	25.07	1.45	0.2742
A^2	165.42	1	165.42	9.56	0.0213
B^2	10.31	1	10.31	0.5956	0.4695
Residual	103.86	6	17.31		
Cor Total	2305.78	11			
Std. Dev.	4.16				
Mean	62.84				
C.V. %	6.62				
R^2	0.9550				
Adjusted R^2	0.9174				
Predicted R^2	0.8143				
Adeq Precision	14.1201				

Both linear terms, NaOH concentration (A) and fermentation duration (B), were found to be highly significant, with P-values of < 0.0001 and 0.0241 respectively. This indicates that both factors independently exert a strong influence on the ethanol concentration. The interaction term (AB) between NaOH concentration and fermentation duration was not significant (P-value = 0.2742). This suggests that the effects of these two factors on ethanol concentration are largely independent of each other, which could simplify process optimization strategies. The quadratic term for NaOH concentration (A^2) was significant (P-value = 0.0213), indicating a non-linear relationship between NaOH concentration and ethanol concentration.

The model exhibited excellent fit characteristics, with a high R^2 value of 0.9550, indicating that 95.50% of the variability in ethanol concentration could be explained by the model. The adjusted R^2 (0.9174) and predicted R^2 (0.8143) were in good agreement, suggesting strong predictive capability. The adequate precision ratio of 14.1201, being substantially greater than 4, indicates an excellent signal-to-noise ratio. The coefficient of variation (C.V.) was 6.62%, which is quite low and indicates good precision and reliability of the experiments. The standard deviation of 4.16 relative to a mean ethanol concentration of 62.84% suggests highly consistent experimental results. Fig.8 shows the 3D plot of NaOH concentration and fermentation duration on ethanol concentration.

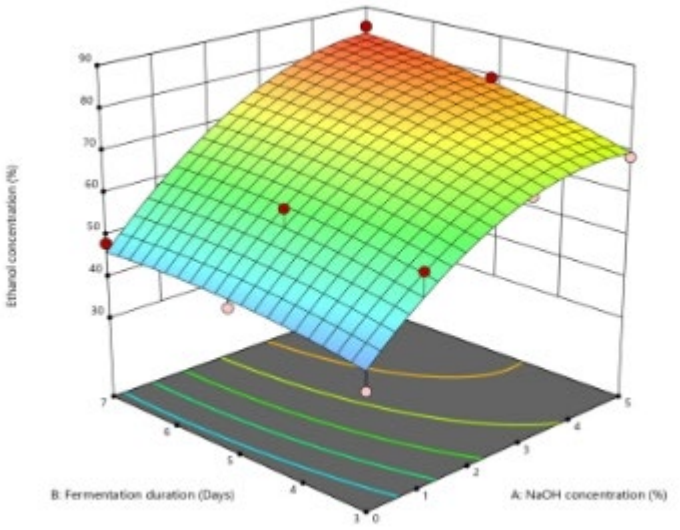


Fig. 8: 3D plot of NaOH concentration and fermentation duration on ethanol concentration

4.0 Conclusion

The yield of bioethanol was significantly affected (P≤ 0.05) by the processing parameters and conditions (pretreated with 1, 3, and 5% sodium hydroxide concentrations and simultaneous saccharification and fermentation for 3, 5, and 7 days). Higher sodium hydroxide concentration and longer fermentation days gave the highest yield of bioethanol (42.2%). So, both the sodium hydroxide concentration and fermentation have significant impact on the yield of bioethanol. Also, sll the physicochemical properties of the bioethanol were all positively influenced (P≤ 0.05) by the processing parameters.

Received: 24/06/2025; Revised: 30/06/2025; Accepted: 01/07/2025; Published: 03/07/2025

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Received: 24/06/2025; Revised: 30/06/2025; Accepted: 01/07/2025; Published: 03/07/2025