



OPTIMIZATION OF BIOETHANOL PRODUCTION FROM GAMBA GRASS (*Andropogon gayanus*) AND LOVE GRASS (*Eragrostis tremula*) USING ACID HYDROLYSIS

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ABSTRACT

Bioethanol is a widely utilized liquid biofuel and demand for it has been increasing, there is a need to enhance production of it from more affordable and environmentally friendly raw materials. In this study Gamba grass and Love grass both were used as resources for the production of bioethanol using dilute acid hydrolysis. Reducing sugar was determined after hydrolysis with UV spectrophotometer at 540 nm with pH values of 4.0, 4.5, and 5.0 of samples and the results were compared. Optimization of process parameters for comparative production of bioethanol from Gamba grass and Love grass using *Saccharomyces cerevisiae* were carried out using Response surface based on Box-Beinkhen design. The optimum yield of bioethanol from sample A was 69.0% and sample B was 67.0% at the temperature, pH and reaction time of 32.5°C, 5.0, 120 hours respectively. This research shows that Gamba grass has the highest yield of bioethanol when compared with Love grass. The studies revealed suitability of both Gamba and Love grass as potential sources of good quality bioethanol.

Keywords: Bioethanol, Gamba grass, Love grass, *Saccharomyces cerevisiae*, Acid hydrolysis

INTRODUCTION

The non-renewable natural fossil fuels such as coal, petroleum, and natural gas serve as the main sources of energy for the global economy. With the increase in population, transportation, and technological progress, the utilization of fossil fuels also rises, causing a reduction in the availability of these natural resources (Mohammed and Saha, 2022). Additionally, the utilization of fossil fuels is responsible for the generation of greenhouse gases, which bring about changes in the climate and raise air pollution (Mohammed and Saha, 2022). Thus, researchers are actively seeking alternative sources of energy in response to the increasing global energy requirement, global warming, the depletion of fossil resources, and the high cost of petroleum-based fuels. The alternative energy sources ought to be environmentally friendly, productive, renewable, and affordable with no net or little greenhouse gas emissions (Abidin *et al.*, 2023). Brazil, the United States, and Canada are the three leading countries in the production of biofuels (Banerjee *et al.* 2019). Biofuels including bioethanol, biodiesel, and biogas are produced and utilized in order to mitigate the release of greenhouse gases, as well as reduce the dependence on petroleum-derived fuels (Priya *et al.*, 2022).

Among all the various types of biofuels available, bioethanol stands out as an exceedingly encouraging option for automotive fuel since it can be easily produced from renewable sources. Furthermore, bioethanol possesses advantageous characteristics such as biodegradability, non-toxicity, and the potential to effectively mitigate particle emissions originating from compression-ignition engines (Thangavelu *et al.*, 2016).

Gamba grass (*Andropogon gayanus*) originated from the tropical regions of Africa and was introduced to the northern regions of Australia for grazing purposes. The huge tufted perennial grass can reach a height of 4 meters and it is

particularly inclined to areas with lower altitude, in tropical and warmer sub-tropical climates (John, 2016). On the other hand, Love grass (*Eragrostis tremula*) is a broad and extensive genus of grass-family plants that can be found in numerous countries across all continents (Skerman, 2011). Love grass, can be classified as an annual or perennial grass, with erect culms that are often unbranched, frequently used as a source of feed for animals. The grass grows in areas such as road sides, river banks, cultivated regions, and farmland, primarily in soils that are predominantly sandy in nature (Ken, 2014). The aim of this research is to compare the potentials of bioethanol production from gamba grass and love grass using dilute acid hydrolysis.

MATERIALS AND METHODS

Sample Collection

Gamba grass was collected from Badariya area in Birnin Kebbi, Kebbi State, while Love grass was collected behind postgraduate hostel in Usmanu Danfodiyo University Sokoto.

Sample Treatment

The samples were taken to Herbarium unit, Department of Plant Science, Usmanu Danfodiyo University Sokoto for identification and authentication. Gamba grass (*Andropogon gayanus*) was assigned the Voucher No: UDUH/ANS/0295, while love grass (*Eragrostis tremula*) was registered with Voucher No: UDUH/ANS/0330. The samples were cut into tiny pieces. Gamba grass was sun dried for three (3) weeks and Love grass for two (2) weeks. The samples were grounded into fine powder using motor and pestle. The powdered samples were stored at room temperature in an air tight container prior to usage (Tambuwal *et al.*, 2018).

Reactivation of Baker's Yeast (*Saccharomyces cerevisiae*)

The medium was prepared according to manufacturers instruction. The prepared yeast extract agar (28 g) was dissolved in 1 dm³ of distilled water and autoclaved at 120 °C for 15 minute. Then, 1 g of baker yeast was dissolved in 9 cm³ of sterilize distilled water. Thereafter, 1 cm³ of dissolved baker yeast was spread on the prepared yeast extract in the petri dish or media and was incubated for 24 hours (Adrian, 2021).

Dilute Acid Pretreatment of the Samples

Thirty grams (30 g) of each sample was mixed with 250 cm³ of (0.8%) dilute sulphuric acid in a 500 cm³ conical flask and then autoclaved at 121 °C, 15 psi for 30 minute. The mixture was filtered through a Whatman filter paper to separate the solid residue. The residue was washed with distilled water until neutral pH. The sample was oven air dried at 105 °C and stored in tightly sealed plastic bag for further use (Tambuwal et al., 2016).

Acid hydrolysis of the samples

This was carried out according to the method described by Humphrey and Caritas (2007) and Oyeleke and Jibril (2009). Fifty grams (50 g) of dried pretreated Gamba grass was weighed in a 1 dm³ capacity conical flasks and 500 cm³ of (0.4%) dilute sulphuric acid was added to each conical flask. The flasks were covered with aluminum foil and heated for 2 hours at 50 °C on a water bath and then autoclaved for 30 min at 121 °C. The flasks were allowed to cool and filtered through Whatman No.1 filter paper. The pH was adjusted base on design of experiment shown in Table 3 with 5 M sodium hydroxide. The same procedure was repeated for the acid hydrolysis of Love grass.

Determination of Reducing Sugar Content

The reducing sugar content of the hydrolysates was determined by adding 3 cm³ of DNS reagent to 3 cm³ of the sample. The mixture was heated in boiling water for 10 minute to develop the red-brown colour. Then, 1 cm³ of 40% potassium sodium tartrate solution was added to stabilize the colour and cooled to room temperature (Miller, 1959). The absorbance of the samples was measured at 540 nm using UV-VIS spectrophotometer. The reducing sugar content was determined using the following equation.

$$\text{Concentration of reducing sugar} = \frac{\text{Absorbance of sample}}{\text{Absorbance of glucose standard}} \times \text{conc. of standard}$$

Fermentation

The fermentation of the hydrolyzed samples was carried out as described by Rabah et al. (2011). The samples hydrolysates

(100 cm³) was dispensed into 500 cm³ capacity conical flasks. The conical flasks was enclosed with cotton wool, wrapped in aluminum foil, autoclaved for 15 minute at 121 °C, and cooled to room temperature. The flask was then inoculated with *Saccharomyces cerevisiae*. The conical flask was then incubated anaerobically at 30 °C to 35 °C for 24 hours to 120 hours as presented in the experimental design (Table 3). Flasks were removed from each sample every 24 hours incubation period for a period of 5 days. The fermented broth that was produced from each conical flask in each sample was subjected to fractional distillation.

Fractional distillation

Bioethanol derived from the process of fermentation possesses a considerable amount of water; which must be eliminated. The removal of water is achieved using fractional distillation process, by boiling the water and bioethanol mixture. Since bioethanol has a lower boiling point of 78.3 °C in comparison to water's boiling point of 100 °C, the bioethanol turns into the vapour state before the water and was subsequently subjected to condensation and separation (Romano, 2011). After distillation, the resulting mixture was composed of 95.6% bioethanol and 4.4% water.

The fermented broth was poured into a round-bottom flask fitted to a distillation column. The column was supplied with a continuous flow of tap water. A conical flask was attached to the other side of the distillation column to collect the distillate. The round-bottomed flask containing the fermented broth was heated using a heating mantle set at a temperature of 78.3 °C. The resulting liquid that was collected during the distillation process was measured using a measuring cylinder (Oyeleke and Jibril, 2009).

$$\text{Bioethanol Yield (\%)} = \frac{\text{Volume of bioethanol produce}}{\text{Volume of sample used}} \times 100$$

Qualitative test for bioethanol

Two (2) cm³ of acetone was added in a test tube followed by four (4) drops of the fractionated bioethanol, and then two (2) drops of chromic acid was added. The mix was shaken energetically. The change in colour of the mixture forming a blue-green precipitates shortly of adding few drops of chromic acid confirms the presence of bioethanol (Tojo and Fernandez, 2006).

Design of Experiment

The experiment was designed using Response Surface based on Box-Behnken design on MINITAB17 statistical software. The effects of three factors i.e. reaction temperature (30-35 °C), effect of pH (4-5), and retention time (24-120 hours) on the fermentation was investigated (Table 1). The design generated a total of 30 runs for each sample (randomized).

Table1: The Factors for Optimization Fermentation at Low and High Level

Factors	Low level	High level
pH	4	5
Temperature (°C)	30	35
Time (hours)	24	120

RESULTS AND DISCUSSION**Effect of Reducing Sugar of Samples after Hydrolysis**

The effect of various concentrations at different pH of pretreated samples on the reducing sugar concentration was studied and the result is presented in Table 2. The highest reducing sugar concentration was observed on sample A at pH 5 (899 mg/dL) and lowest reducing sugar concentration was observed on sample B at pH 4 (107.1 mg/dL). As seen from

the result, there is high reducing sugar concentration on sample A when compared with sample B. This might be due to large amount of carbohydrate content of sample A, and the reducing sugar yield increased by changing the pH value from 4.0 to 5.0 respectively. Therefore, the higher the pH for all the samples the higher the concentration of reducing sugar. The 107 mg/dL reducing sugar obtained as a lowest yield is almost in agreement with the (111 mg/dL) reducing sugar obtain by

Folake and Ibukun (2022). The highest result from another experiment that used Response Surface Methodology to optimize Bioethanol production from biodegradable municipal solid waste produced a reducing sugar

concentration of 920 mg/dL at pH 5.0 (Nadhim *et al.*, 2018), which is higher than the highest yield obtained in this study (899.5 mg/dL).

Table 2: Determination of Reducing Sugar of the Samples after Hydrolysis

pH Value	Sample A (mg/dL)	Sample B (mg/dL)
pH 4.0	613.3 ± 3.97	107.1 ± 1.65
pH 4.5	681.9 ± 3.99	193.2 ± 1.36
pH 5.0	899.5 ± 2.34	367.8 ± 0.86

Values are Arithmetic Mean ± Standard Deviation of three replicate determinations

Optimization Process

In the optimization process, the Response surface based on Box-Behnken design on MINITAB17 statistical software was able to function as an optimal design for the desired response based on the model obtained and input criteria. The optimization of bioethanol yield was conducted based on three fermentation variables i.e. pH, reaction temperature, and

reaction time, which are in three different levels of experimental runs. Table 3 show the experimental conditions and percentage of bioethanol yield from various experimental runs. The percentage of bioethanol yield obtained from sample A and sample B ranges from 52% to 69%. Highest yield of 69% was obtained from sample A at a pH of 5, temperature of 32.5 °C, and time of 120 hours.

Table 3: Optimization Process for Bioethanol Production from Gamba and Love Grass and the Yield Obtained.

StdOrder	RunOrder	PtType	Blocks	pH	Temperature	Time	Yield (%)	
							A	B
1	30	2	1	4.0	30.0	72	58	58
2	2	2	1	5.0	30.0	72	59	58
3	25	2	1	4.0	35.0	72	57	56
4	4	2	1	5.0	35.0	72	60	58
5	29	2	1	4.0	32.5	24	54	52
6	18	2	1	5.0	32.5	24	55	52
7	21	2	1	4.0	32.5	120	67	67
8	24	2	1	5.0	32.5	120	69	67
9	6	2	1	4.5	30.0	24	52	53
10	28	2	1	4.5	35.0	24	53	53
11	23	2	1	4.5	30.0	120	66	64
12	16	2	1	4.5	35.0	120	63	60
13	9	0	1	4.5	32.5	72	60	58
14	27	0	1	4.5	32.5	72	59	58
15	5	0	1	4.5	32.5	72	61	60
16	20	2	1	4.0	30.0	72	59	55
17	17	2	1	5.0	30.0	72	58	59
18	11	2	1	4.0	35.0	72	58	55
19	22	2	1	5.0	35.0	72	60	60
20	8	2	1	4.0	32.5	24	54	53
21	14	2	1	5.0	32.5	24	53	55
22	7	2	1	4.0	32.5	120	67	65
23	3	2	1	5.0	32.5	120	68	67
24	26	2	1	4.5	30.0	24	54	52
25	15	2	1	4.5	35.0	24	54	52
26	13	2	1	4.5	30.0	120	65	66
27	19	2	1	4.5	35.0	120	66	66
28	12	0	1	4.5	32.5	72	58	59
29	10	0	1	4.5	32.5	72	57	56
30	1	0	1	4.5	32.5	72	59	59

Analysis of Variance

The analysis of variance (ANOVA) shown in Table 4 for sample A and Table 5 for sample B, was carried out to fit the response variable and to investigate the variable that is significant. The "P" value less than 0.05 showed that the specific term was statistically significant. The results of analysis of variance revealed that all the linear interactions terms of the process variables are statistically insignificant except reaction time on sample A and sample B. The analysis

of variance results also indicate that apart from pH*pH on sample A and sample B interaction, all other square interaction terms of the process variables are statistically significant. It also indicates that all the interactions of 2-way terms are statistically insignificant in both sample A and sample B. The correlation coefficient (R^2) of the analysis of sample A is 96.12% and for sample B is 90.97% which shows the variables fit the model.

Table 4: Analysis of Variance for Sample A

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	666.325	74.036	55.10	0.000
Linear	3	640.625	213.542	158.91	0.000
Ph	1	3.063	3.063	2.28	0.147
Temperature	1	0.000	0.000	0.00	1.000
Time	1	637.563	637.563	474.47	0.000
Square	3	20.950	6.983	5.20	0.008
pH*pH	1	2.885	2.885	2.15	0.158
Temperature*Temperature	1	7.385	7.385	5.50	0.030
Time*Time	1	9.346	9.346	6.96	0.016
2-Way Interaction	3	4.750	1.583	1.18	0.343
pH*Temperature	1	3.125	3.125	2.33	0.143
pH*Time	1	0.500	0.500	0.37	0.549
Temperature*Time	1	1.125	1.125	0.84	0.371
Error	20	26.875	1.344		
Lack-of-Fit	3	3.875	1.292	0.95	0.437
Pure Error	17	23.000	1.353		
Total	29	693.200			

Key: Adj SS= adjusted sum of squares, DF= degree of freedom, F-Value =F-statistic values, Adj MS= adjusted mean squares.

Table 5: Analysis of Variance for Sample B

A	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	621.758	69.084	22.39	0.000
Linear	3	596.625	198.875	64.46	0.000
pH	1	7.563	7.563	2.45	0.133
Temperature	1	1.000	1.000	0.32	0.575
Time	1	588.063	588.063	190.59	0.000
Square	3	22.883	7.628	2.47	0.091
pH*pH	1	0.321	0.321	0.10	0.751
Temperature*Temperature	1	8.051	8.051	5.26	0.036
Time*Time	1	9.782	9.782	5.49	0.026
2-Way Interaction	3	2.250	0.750	0.24	0.865
pH*Temperature	1	1.125	1.125	0.36	0.553
pH*Time	1	0.000	0.000	0.00	1.000
Temperature*Time	1	1.125	1.125	0.36	0.553
Error	20	61.708	3.085		
Lack-of-Fit	3	8.375	2.792	0.89	0.466
Pure Error	17	53.333	3.137		
Total	29	683.467			

Key: Adj SS= adjusted sum of squares, DF= degree of freedom, F-Value =F-statistic values, Adj MS= adjusted mean squares.

Regression Analysis

The analysis showed that reaction time for both sample, reaction temperature*reaction temperature and reaction time*reaction time for both sample were significantly affect the bioethanol yield. Reaction temperature, pH, pH*pH, pH*reaction time, reaction temperature*reaction time for both samples were found to be statistically insignificant as shown in Table 6 for sample A and Table 7 for sample B. The model developed was successful in capturing the correlation between the fermentation conditions variables to the bioethanol yield. The result of regression analysis suggests

that bioethanol yield was only significantly affected by reaction time and reaction temperature. Significant interaction terms were found to exist between the main factor reaction time. After removing the insignificant terms from the model the new regression model (equation 3 for sample A and 4 for sample B) with significant terms is better than the previous model with terms (adjusted $R^2 = 94.25\%$ compared to 93.38% for sample A and adjusted $R^2 = 88.20\%$ compared to 83.82% for sample B).

Table 6: Results of Regression Analysis of Sample A Showing the Estimated Coefficients of the Model and their Significance

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF	Significance
Constant		59.000	0.473	124.67	0.000		S
pH	0.875	0.437	0.290	1.51	0.147	1.00	NS
Temperature	0.000	0.000	0.290	0.00	1.000	1.00	NS

Time	12.625	6.313	0.290	21.78	0.000	1.00	S
pH*pH	1.250	0.625	0.427	1.47	0.158	1.01	NS
Temperature*Temperature	-2.000	-1.000	0.427	-2.34	0.030	1.01	S
Time*Time	2.250	1.125	0.427	2.64	0.016	1.01	S
pH*Temperature	1.250	0.625	0.410	1.52	0.143	1.00	NS
pH*Time	0.500	0.250	0.410	0.61	0.549	1.00	NS
Temperature*Time	-0.750	-0.375	0.410	-0.91	0.371	1.00	NS

Key: NS= statistically not significant, and S=statistically significant, SE Coeff= Standard error for coefficients, Coeff= regression equation coefficient, T-value = t-statistics value, P-Value = probability value.

Table 7: Results of Regression Analysis of Sample B showing the Estimated Coefficients of the Model and their Significance

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF	Significance
Constant		58.333	0.717	81.35	0.000		S
pH	1.375	0.688	0.439	1.57	0.133	1.00	NS
Temperature	-0.500	-0.250	0.439	-0.57	0.575	1.00	NS
Time	12.125	6.063	0.439	13.81	0.000	1.00	S
pH*Ph	0.417	0.208	0.646	0.32	0.751	1.01	NS
Temperature*Temperature	-2.111	-1.127	0.646	-1.70	0.036	1.01	S
Time*Time	2.217	1.108	0.646	1.77	0.026	1.01	S
pH*Temperature	0.750	0.375	0.621	0.60	0.553	1.00	NS
pH*Time	-0.000	-0.000	0.621	-0.00	1.000	1.00	NS
Temperature*Time	-0.750	-0.375	0.621	-0.60	0.553	1.00	NS

Key: NS= statistically not significant, and S=statistically significant, SE Coeff= Standard error for coefficients, Coeff= regression equation coefficient, T-value = t-statistics value, P-Value = probability value.

Equation 1: Regression equation of sample A with insignificant terms

Yield Sample A = $-1.1 - 38.6 \text{ pH} + 8.38 \text{ Temperature} + 0.116 \text{ Time} + 2.50 \text{ pH*pH}$
 $- 0.1600 \text{ Temperature*Temperature} + 0.000488 \text{ Time*Time} + 0.500 \text{ pH*Temperature}$
 $+ 0.0104 \text{ pH*Time} - 0.00312 \text{ Temperature*Time}$

Equation 2: Regression equation of sample B with insignificant terms

Yield Sample B = $-95 - 15.9 \text{ pH} + 10.91 \text{ Temperature} + 0.152 \text{ Time} + 0.83 \text{ pH*pH}$
 $- 0.187 \text{ Temperature*Temperature} + 0.000524 \text{ Time*Time} + 0.300 \text{ pH*Temperature}$
 $+ 0.0000 \text{ pH*Time} - 0.00312 \text{ Temperature*Time}$

Equation 3: Regression equation of sample A without insignificant terms

Yield Sample A = $-70.3 - 21.6 \text{ pH} + 10.40 \text{ Temperature} + 0.0612 \text{ Time} + 2.50 \text{ pH*pH}$
 $- 0.1600 \text{ Temperature*Temperature} + 0.000488 \text{ Time*Time}$

Equation 4: Regression equation of sample B without insignificant terms

Yield Sample B = $-131 - 6.1 \text{ pH} + 12.03 \text{ Temperature} + 0.0508 \text{ Time} + 0.83 \text{ pH*pH}$
 $- 0.1867 \text{ Temperature*Temperature} + 0.000524 \text{ Time*Time}$

Effects of Fermentation Variables on Bioethanol Yield

The effects of reaction time, pH, reaction temperature and their interactions were studied to check the effect of each variable toward bioethanol production. The contour plots were used to analyse the interaction as shows in Figure 1 to 6 for both samples A and B.

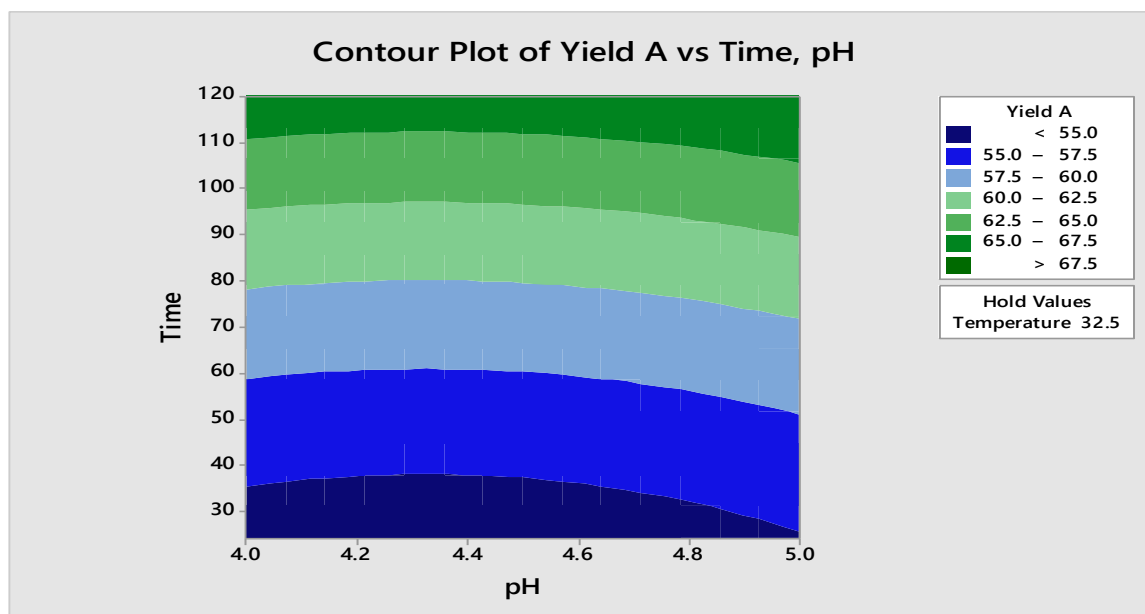


Figure 1: Effects of Time and pH of Sample A (Gamba grass) on the yield of bioethanol

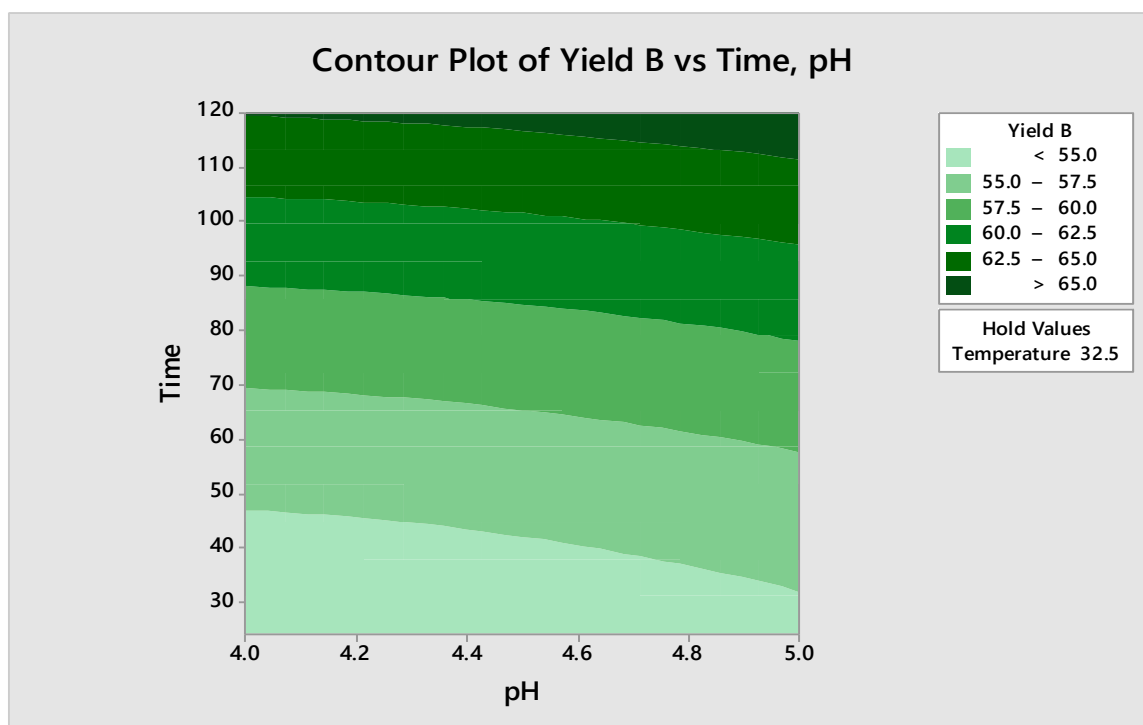


Figure 2: Effects of Time and pH of Sample B (Love grass) on the yield of bioethanol

Effect of Different Time on Bioethanol Production

Different time duration were examined to optimize the required time for maximum production of bioethanol. Five days, three days and one day were used to precede fermentation of sample A and B as presented in Figure 1 to 4. The production was increased with the increase in incubation time. Bioethanol production after one day was 55% as the highest yield for sample A and 52% was the lowest yield for both samples. The bioethanol production was recorded after three days and it was found that 61% as the highest yield for sample A and 55% lowest yield respectively for sample B. The bioethanol production was also recorded

after five days and sample A was found as the highest yield of 69% while sample B has the yield of 67% respectively. On the effect of incubation time, 120 hours was found to be the optimum incubation time. Sonali and Banwari (2007) has obtained maximum yield of bioethanol at 120 hours of incubation time in a similar study. Furthermore, Yingjie *et al.* (2019) observed that the maximum amount of bioethanol produced was after 120 hours, confirming that the optimum bioethanol yield was produced during the fermentation time of 5 days (120 hours), which declined with the highest fermentation time of 6 days (144 hours).

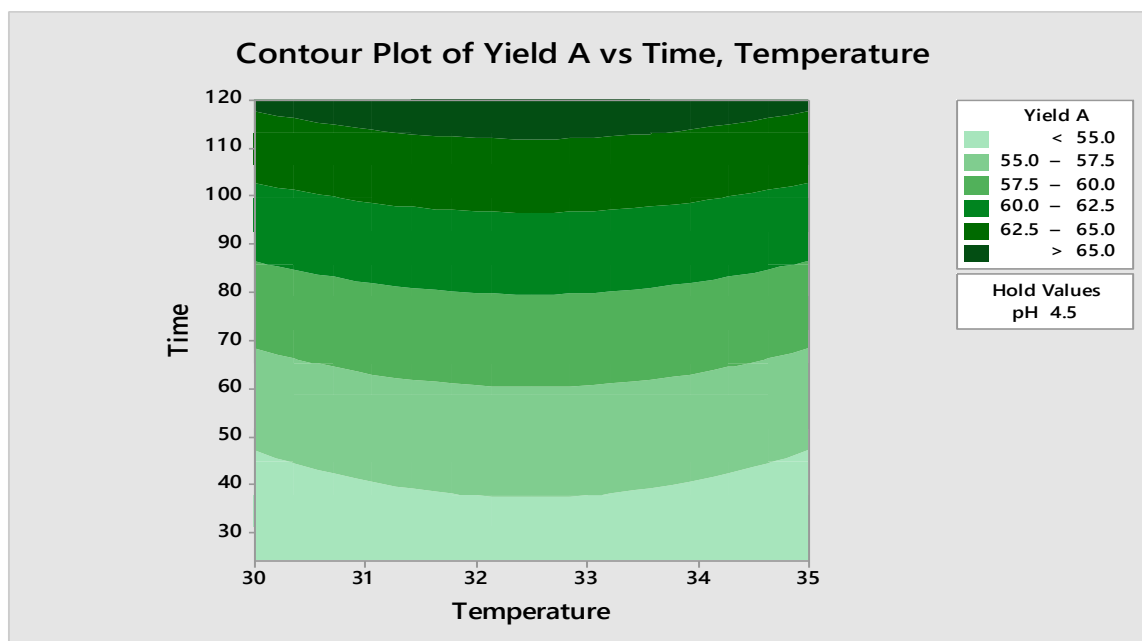


Figure 3: Effects of time and temperature of Sample A (Gamba grass) on the yield of bioethanol

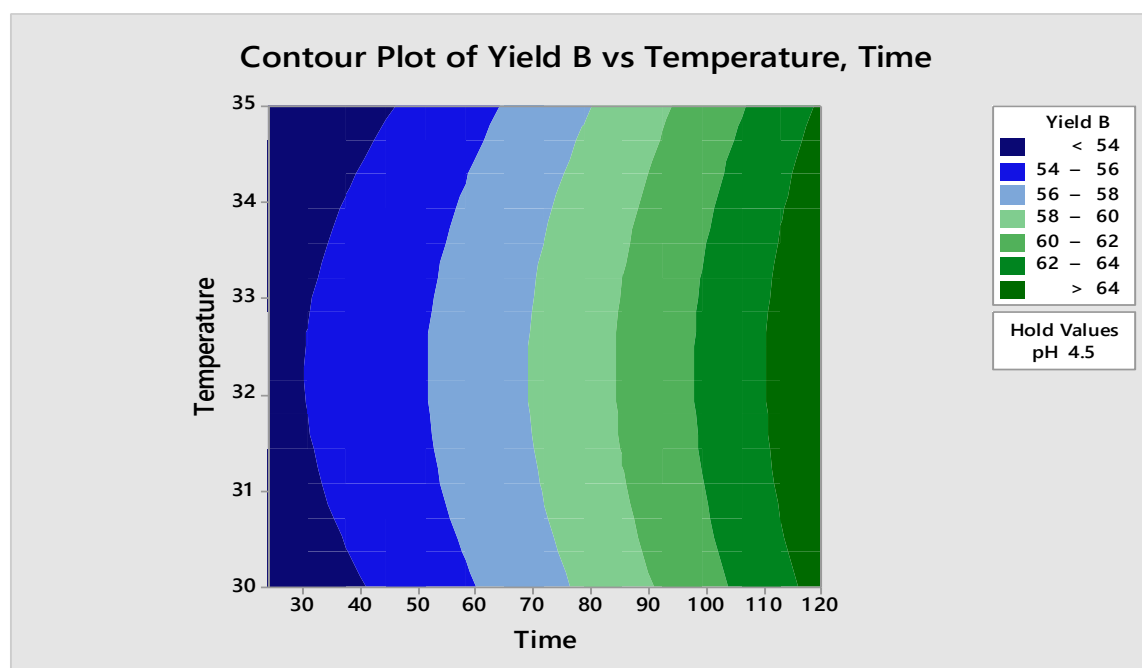


Figure 4: Effects of time and temperature of Sample B (Love grass) on the yield of bioethanol

Effect of pH

Samples were fermented in different pH ranges from 4.0 to 5.0, to attain the highest yield of bioethanol. The Figure 1,2 and 5,6 shows the bioethanol yield from fermentation of sample A and sample B at different reaction temperature from 30 °C to 35 °C. The samples were fermented at pH 4.0, 4.5 and 5.0. It has been found that the optimal yield was attained at pH 5.0 for both the samples. Therefore, rising pH causes the production of bioethanol to rise until pH 5.0 and the lowest

yield was observed at pH 4.0. This aligned with an experimental study on the effect of pH on the production of bioethanol, which indicated that the highest yield was obtained at a pH of 5.0 and the lowest at 7.0 (Kemka *et al.*, 2013). The pH range for increase can range from 4 to 6 when behaving as yeast (Phu *et al.*, 2022). The range in which it generated the most yield of bioethanol within this research was pH 5.0.

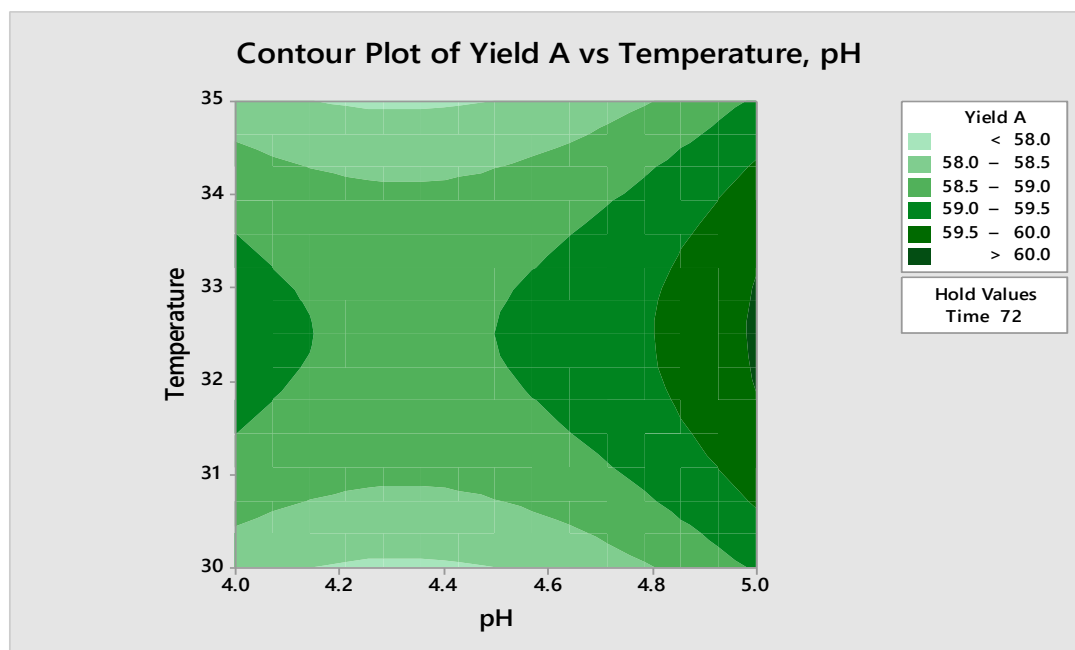


Figure 5: Effects of temperature and pH of Sample A (Gamba grass) on the yield of bioethanol

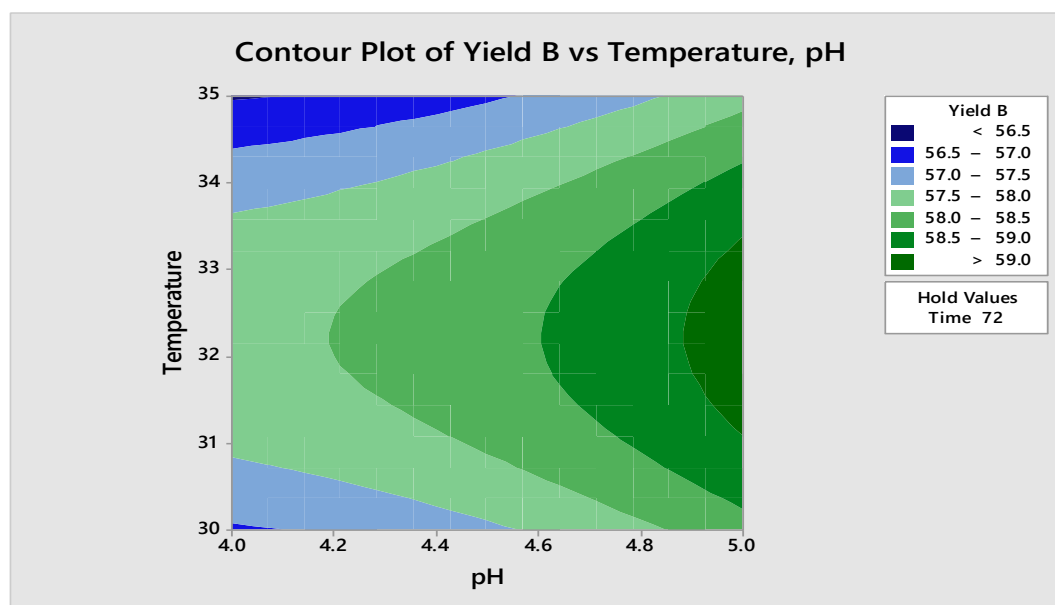


Figure 6: Effects of temperature and pH of Sample B (Love grass) on the yield of bioethanol

Effect of Fermentation Temperature

The Figure 3 to 6 shows the bioethanol yield from fermentation of sample A and sample B at different reaction temperature from 30 °C to 35 °C. The samples were fermented for 120 hours, 72 hours and 24 hours. It was discovered that the optimum yield was attained in 32.5 °C for both the samples with bioethanol yield of 69%, for sample A and 67% for sample B. Bioethanol yield was observed to increase with increasing temperature until 32.5 °C. Moreover, by further increasing fermentation temperature the yield declines. Therefore, as shown in Figure 3 and 4, for both samples, temperature is an extremely delicate parameter for *Saccharomyces cerevisiae* because it produced maximum yield at 32.5 °C. However, at higher temperatures, the production of bioethanol decreased because of the denaturation of *Saccharomyces cerevisiae* cells. The organism could not withstand the high temperature, due to this factor in summer; the production yield of bioethanol is

relatively low in distilleries. Kirk and Aswad (2013) investigated the effect of fermentation temperature and discovered that for yeast fermentation, 32 °C can produce the highest results. Similarly, Slavikova and Nadketrova (2003) observed that yeast generally grows best at temperatures between 30 °C and 37 °C. This temperature range corresponds to the range at which the maximum amounts of bioethanol were produced in the current research.

CONCLUSION

This research was designed to make use of waste grass for bioethanol production as fuel. Response Surface Methodology was successfully used to optimize the fermentation with the help of *saccharomyces cerevisiae*. The optimum yield of bioethanol from both samples was obtained at temperature 32.5°C, pH 5.0, and reaction time 120 hours. The study revealed suitability of both Gamba and Love grass as potential sources of good quality bioethanol.

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