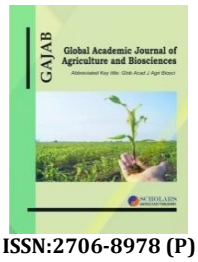
Global Academic Journal of Agriculture and Bio sciences

Available online at https://www.gajrc.com
**DOI:** https://doi.org/10.36348/gajab.2026.v08i04.003

OPEN ACCESS



**ISSN:2706-8978 (P)**
**ISSN: 2707-2568 (O)**

**Original Research Article**

# Enhanced Bioethanol Production from Alkaline-Pretreated Rice Bran Using Locally Isolated *Zymomonas mobilis*

Saidu Isah[1]*, Aminu Yakubu Zubairu[1], Bala Nasir[2], Effiong, E.C[3]
[1]Department of Applied Chemistry, Umaru Ali Shinkafi Polytechnic Sokoto, Nigeria
[2]Department of Applied Physics, Umaru Ali Shinkafi Polytechnic Sokoto, Nigeria
[3]Department of Biological Sciences, Edwin Clark University, Kiagbodo, Delta State, Nigeria

***Corresponding Author**
**Saidu Isah**
Department of Applied Chemistry, Umaru Ali Shinkafi Polytechnic Sokoto, Nigeria

**Article History**
Received: 13.05.2026
Accepted: 01.07.2026
Published: 25.08.2026

**Abstract:** Bioethanol has emerged as an important alternative renewable energy source both an economic and industrial resource providing significant benefits for a several sectors in the world today. The substrate was procured from a rice mill at Argungu, Kebbi State, while the feedstock of the inoculum was isolated from sewage from a local juice vendor, deteriorated fruits, and hostel. The isolate was identified using both biochemical and 16S molecular approaches. Alkaline hydrolysis of the rice bran was conducted using analytical grade reagents, while proximate analysis was performed on the raw rice bran, treated, and conditioned samples. Response surface methodology was applied for optimization using Design Expert. The molecular identity of the bacteria was established to be *Zymomonas mobilis* with a 99% identity with already existing flora in GenBank. Ethanol tolerance and productivity of the isolate. The study identified that the inoculum was thermostable and partitioned excellently with proportionate increase in the ethanol concentration. Proximate composition of the rice bran showed a significant increase in nutrient in pretreated and conditioned samples than the raw substrate. The study was fitted into a quadratic model with an ANOVA of 0.0043 and F-value of 4.44 with a significant iteration showing that inoculum size and temperature having a significant effect on the production of bioethanol production of 22.85 mg/L. The academia and industry must synergize to develop a more robust and efficient technology to advance bioenergy production and commercialization.

**Keywords**: Bioethanol, Optimization, Pretreated Rice Bran, Alkaline Pretreatment, Renewable Energy, Biofuel Production, *Zymomonas Mobilis*.



## 1.0 INTRODUCTION

Biofuels such as bioethanol have been identified as promising alternatives for addressing the world's energy crisis. This has been driven by the depletion of fossil fuels, international policies, and economic challenges today (El-Araby, 2024). Biofuels are biologically sourced and synthesized alternatives to conventional fuels or energy (Malik *et al.,* 2024). The use of food crops as biofuel feedstocks has raised concerns over food security due to competition with the human food supply (Kamani *et al.* 2019). Generations of biofuels have ranged from the use of food and food products in the first to the application of agroresidues (Nisar *et al.,* 2024) from lignocellulosic materials in the second; cellulose, hemicellulose, and lignocellulose occupy a niche in

applied biotechnology and have been harnessed in the production of valuable products (Ojo, 2023). Cellulose is a vital biomaterial in living organisms, especially plants, making it the most abundant biopolymer on Earth, including in algae. It has been linked to pigment production in plants (Drishya *et al.,* 2025).

Cellulose is an insoluble polymer that forms a crucial scaffold network in plant cell walls and, together with other components, forms a complex polymer called lignocellulose through the condensation and cross-linking of sugar moieties (Barhoum *et al.,* 2020). It is a linear polymer composed of D-glucose monomers linked by β(1 → 4) glycosidic bonds (Guo *et al.,* 2025). It is composed of microfibrils, which contribute to its toughness. Lignin is a complex polymer with an aromatic character (Padhiyar *et al.,* 2026). These structurally complex polymers are usually subjected to pretreatments that induce bond-breaking reactions, such as acid, alkaline, fermentative, and enzymatic treatments, ultrasonication, and plasma approaches (Di Fraia *et al.,* 2024), eventually enabling the materials to serve as substrates and feedstocks. The valorization of agro-residues relies on enzymatic hydrolysis of the biomass and glucose fermentation (Joshi *et al.,* 2024). Lignin, a vital component in plants, contributes to cell dry weight. Lignin and cellulose account for over 85% of the plant's dry weight (Riaz *et al.,* 2023). Cellulose, although insoluble, partitions well with water and other solvents due to the presence of numerous hydroxyl groups; cellulose tends to pack in regions of vesicles within the polymeric structure (Acharya *et al.,* 2021). Cellulosic materials on earth are abundant; they exist in various forms and in diverse organic products like jute, cotton, husk, bagasse, fibers, and plant shavings (Vale *et al.,* 2023)

*Zymomonas mobilis* is a Gram-negative, rod-shaped, non-coliform, sugar-fermenting bacterium that ferments simple, bioavailable sugars such as glucose, fructose, and sucrose to yield ethanol and carbon dioxide under anaerobic conditions (Pérez-García *et al.,* 2022). It depends on simple, bioavailable sugars for growth, and there are reports of its industrial use in bioethanol production. Ecological proxies of Z. *mobilis* have been widely reported to affect the production rates of its variants or subspecies (Boismier *et al.,* 2025). It is microaerophilic and a facultative anaerobe; it can metabolize substrates via anaplerotic and non-ATP-yielding systems. There is scientific evidence that it is a nitrate oxidizer, with an optimal metabolic temperature of 35 °C at pH 7.5, and that some subspecies have been isolated from deteriorating fruits, food and feed products, and alcohol-rich waste (Koyande *et al.,* 2021).

Rice bran is a nutrient-rich fibrous byproduct obtained from the processing and milling of rice (Tan *et al.,* 2023). It has emerged as a highly versatile and valuable by-product with medical, industrial, commercial, and therapeutic uses, as it is a non-conventional (Tufail *et al.,* 2024). It is rich in organic and inorganic nutrients; vitamins and minerals have been reported to occur in this residue. It has been widely reported to have beneficial effects due to the high concentrations of soluble and insoluble fiber, which improve gut health and act as anti-inflammatory agents (Gul *et al.,* 2026), although it has also been reported to reduce blood sugar levels. Its shelf life is generally poor, and due to its high enzymatic content, it exhibits high levels of rancidity.

## 2.0 MATERIALS AND METHODS

### 2.1 Sample Collection

The rice bran was obtained from a rice mill at Argungu, Kebbi State, Nigeria. It was pulverized at Argungu market after drying for 3 days. The processed and pulverized substrates were aseptically transported to the laboratory in sterile plastic bags.

#### 2.1.1 Pretreatment of Samples

Alkaline exposure of substrate was conducted on ten grams (10g) of the pulverized sample of the rice bran, which was weighed into separate beakers and suspended with 3.0 % hydrogen peroxide and 1.5% analytical grade dilute sodium hydroxide, and left to stand for five hours (5 hr) and autoclaved at 121ºC for 15 min. The materials were drained of the reagent. Hot water conditioning was performed by exposure of substrates to hot water for three hours.

### 2.2 Proximate Analysis of Agro-Waste

The rice bran materials were aseptically assembled at the Austino Laboratories, at Alakahia, Rivers State. The materials were pulverized after being weighed and dried.

#### 2.2.1 Ash Content:

Two grams (2g) of the rice bran was weighed and charred at the lowest possible temperature, the ash was cooled in a desiccator and re-weighed. Physical removal of moisture to ash at $550^0$C in oven. Mathematically,

**Ash value (%)** = $\frac{W_2 \times 100}{W_1}$

Where: $w_1$ = weight of sample (g) $w_2$ = weight of ash (g)

#### 2.2.2 Crude Fiber

This parameter was determined by AOAC, (1990). The rice bran sample was reacted with 50ml sulphuric acid. The content was reacted with water and strained through a muslin filter. The slurry was reacted with sodium hydroxide for half-an-hour, and

washed off. The net weight was calculated from the container's weight.

**Crude fiber** = Weight of fiber × 100
Weight of sample

### 2.2.3 Crude Protein

This method determined adopting the Macro-Kjeldahl method. Two grams of the de-watered samples, was heated with 20mL sulphuric and assisted with catalyst under controlled condition. The volume was made up to 100% and was back-titrated with 0.2N sulphuric acid. The actual concentration was determined using a factor 6.25 (AOAC, 2000).

Mathematically Calculated as:

**Crude Protein (%)** $=\frac{V_2-V_1-1.4\ x\ 6.25}{W}$ ; **Total Nitrogen** (%) $=\frac{V_2-V_1-N\ x\ 1.4}{W}$

Where: wt (g) of the test protein = W; Volume (ml) of $H_2SO_4$ = $V_1$; Volume (ml) of $H_2SO_4$ for test solution = $V_2$; Normality of $H_2SO_4$ = N

### 2.2.4 Total Available Carbohydrate (TAC):

About 10g of rice bran was digested with perchloric acid, and the soluble sugars produced by starch hydrolysis were determined colorimetrically at 630nm using the Anthrone reagent. (AOAC, 2000). The result was expressed as glucose
Hence mathematically;
**TAC (%)** = (25 × b) (a × w)
Where w = weight of sample; a = Absorbance of standard; b = Absorbance of sample

This figure was confirmed by determining the percentage difference in the sum of other proximate parameters (Ash, Moisture, Protein, Fibre, and Fat).

### 2.2.5 Moisture Content

The controlled-heat approach was adopted to determine the parameter. Here, the pre-weighed rice bran was determined by arithmetically calculating the difference and multiplying by a 100%. Hence, the sample was heated to 105°C for 15 minutes. The crucible was allowed to cool in a desiccator, weighed, and the weight recorded (AOAC, 2000).

**Mathematical Calculations**
Crucible + sample weight (before drying) - Crucible weight = Sample wet weight
Crucible + sample weight (after drying) - Crucible weight = Sample dry weight
Percentage Moisture Content = (water loss/Dry weight) × 100
Crucible + sample weight (after ashing) - Crucible weight = Sample ash weight %Ash = (Ash weight/Dry weight) × 100

### 2.2.6 Crude Fat

The lipid composition of the rice bran was determined by the Soxhlet reflux flask system. 40:60 chloroform-sulphuric acid solution. The oil was extracted by a reflux system. The substrate colour was titrated and the colour removed. The separation of the solution and oil phases was determined. The oil content was determined by the weight gained by the container used (AOAC, 2000).
Crude fat= Weight of fat × 100

Crude fat= $\frac{W2-W1}{w1} \times 100$
Where $W_1$ = weight of sample and flask used (g); $W_2$= weight of sample and flask plus fat (g)

### 2.3 Isolation of the Bacterial Flora

The effluent from the Umaru Ali Shinkafi Polytechnic hostel, a local fruit juice producer in Sokoto, and decomposing fruit from Ramen-Kura market were collected aseptically, and then 10 mL of each was dissolved in 90 mL of de-ionised water. Pure cultures were plated on the nutrient agar slopes. Characteristic colonies were purified by serially sub-culturing the isolate until an axenic culture was attained (Effiong *et al.,* 2026).

### 2.4 Determination of Reducing Sugar

Reducing sugar was determined using the method DNS, exactly 5 ml of the sample was centrifuged (Sorvall GLC–4, Germany) at 30,000 × g for 5 min. Exactly 1 ml of the supernatant was mixed with 1 ml of 3.5–dinitrosalicylic acid (DNS) in a test tube and heated in a water bath for 10 minutes. It was allowed to cool to ambient temperature. The volume of the solution was adjusted to 12 mL by adding distilled water. A blank was prepared by mixing 1 mL of distilled water with 1 mL of DNS. The samples' optical density was read in a spectrophotometer (Spectrum Lab 735, Germany) at 540 nm. The resultant reducing sugar concentration was estimated using a Glucose standard curve (Lam *et al.,* 2021).

### 2.5 Preparation of 0.5 Mcfarland Standard

The standard was prepared by weighing 1.175% w/v of $BaCl_2.2H_20$ and dissolving in 100 mL of solution. Then, 1 mL of $H_2SO_4$ was measured with a pipette and added to 99 mL of distilled water. Then 0.05ml of a 1.175% w/v $BaCl_2{\cdot}2H_2O$ solution was measured with a pipette and dispensed into a sterile test tube, then mixed with 9.95ml of 1% sulphuric acid solution to obtain 10ml of barium sulphate. This was then properly mixed and distributed into test tubes identical to those used to prepare inoculum suspensions of the test organisms. The tube is stored in a well- sealed container in the dark at room temperature until required. Exactly 200 ml of must was transferred to 500 ml conical flasks and inoculated with 1 $\times 10^8$ CFU/ml (Asfere *et al*., 2018)

### 2.6 Determination of pH and Temperature

The samples were monitored for changes in pH and temperature using the method described by AOAC (1984). Buffers (pH 4 and pH 7) were used to calibrate the pH meter (PHS–25, UK), after which Samples were withdrawn at 4-day intervals to measure pH and temperature.

### 2.7 Effect of pH on Bioethanol Production

The effect of pH on bioethanol production was determined by adjusting the pH to different levels using a standard buffer (6.0-10.0) adjusted with 1N HCl or 1N NaOH. The pH stability of the bioethanol was determined by incubating the must in buffers with different pH values in a water bath at 40 °C for 10 min. The reducing sugar release by enzymatic hydrolysis of soluble starch was determined by the addition of 1ml Dinitrosalicic acid (DNSA) and $KMnO_4$ reagent, then incubated in boiling water for 100° C for 5 minutes, and bioethanol using the $KMnO_4$ standard at 550nm (Omonije, 2023)

### 2.8 Effect of Temperature on Bioethanol Production

Optimal activity of the bacterial strain is critical for industrial-scale production of valuable chemicals. The incubation temperature directly affects the cell's enzymatic activity; thus, overall metabolism is disrupted by any fluctuation in incubation temperature. The varied conditions (20-60 $^oC$) with Ringer's tablet for buffer at pH 7. They were incubated in a water bath at different temperatures for 10 minutes (Agbaji *et al.,* 2023).

### 2.9 Optimization Studies for Bioethanol Production

Response Surface Methodology was applied to optimize bioethanol production. Four factors were selected for the optimization: substrate, pH, inoculum size, and temperature. The ranges of these factors and the chosen level of response surface analysis, based on the Central composite design, are presented in Table 3. The ranges were chosen automatically (Agbaji *et al.,* 2021). About 100 ml of the broth, sufficient for the 30 runs of the experiment, was placed in 250 ml-capacity conical flasks and sterilized at 121 °C for 15 minutes in the Autoclave. After sterilization and cooling, 5-15% of the inoculum were transferred into the broth cultures of the bacterium, separately into the contents of the different flasks. The flasks and their contents were then incubated at ambient temperature (25$^oC$ –45 °C) for 7 days. At the end of the incubation period, biomass, bioethanol, and reducing sugar concentrations in the broth for the experimental runs were determined. The results of the study suggest that the principles of Matrix were applied using Design Expert software® to solve the equations.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{1,2} X_1 X_2 + \beta_{1,3} X_1 X_3 + \beta_{2,3} X_2 X_3 + \beta_{1,1} X_1^2 + \beta_{2,2} X_2^2 + \beta_{3,3} X_3^2$$

Y is the predicted response; $X_1$, $X_2$, and $X_3$ represent the values for the three factors (pH, temperature, and inocula volume); $\beta_0$ is the value of fitted response at the centre point of the design; $\beta_1$, $\beta_2$, and $\beta_3$ are the linear coefficients; $\beta_{1,2}$, $\beta_{1,3}$, and $\beta_{2,3}$ are the interaction coefficients; and $\beta_{1,1}$, $\beta_{2,2}$, and $\beta_{3,3}$ are the quadratic coefficients.

### 2.10 DNA Extraction

DNA extraction method as described by Effiong *et al.,* (2026) was adopted. Five milliliters of an overnight broth culture of bacteria isolates in Luria-Bertani (LB) medium was spun at 1400rpm for 30min; the cells were suspended in 500 µL of normal saline and heated at 950 °C for 20min. Then, the heated bacteria were suspended, quickly cooled on ice, and centrifuged at 1400 rpm for 3min. The supernatant containing the DNA was transferred to a 1.5 ml microcentrifuge tube and stored at -200 °C. A NanoDrop 1000 spectrophotometer was used to quantify the extracted genomic DNA.

#### 2.10.1 DNA Amplification

For bacterial amplification, the 16S rRNA primer was used, and for fungi, the ITS primer was used. The 16S rRNA was amplified on an ABI 9700 thermal cycler with a 25-microliter reaction volume for 35 cycles. The PCR mix included the X2 Dream Taq Master mix (InQaba Biotechnical Ltd, South Africa), Taq polymerase, DNTPs, MgCl, primers at a concentration of 0.4 M, and the extracted DNA template. The PCR steps were as follows: initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 30 seconds, repeated for 35 cycles, followed by a final extension at 72°C for 5 minutes. The products were loaded onto a 1% agarose gel and run at 120 V for 15 minutes, then visualized on a UV transilluminator.

#### 2.10.2 Sequencing

Sanger-Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by (Inqaba Biotechnological, Pretoria South Africa).

#### 2.10.3 Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit. Similar sequences were downloaded from the online NCBI database using BLASTN. These sequences were aligned using ClustalW. The evolutionary relatedness was then deduced using the most likely relatedness tool in MEGA 6.0 (Saitou and Nei, 1987). A bootstrap of 500 replicates was employed, and the evolutionary history of the taxa analyzed was examined. The

evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor, 1969).

## 3.0 RESULTS INTERPRETATION

### 3.1 Proximate Composition of Rice Bran

The results presented in Table 1 show the nutrient concentrations and their variation after pretreatment. The ash content of the rice bran was 7.83%; after pretreatment and conditioning, it was 5.93% and 5.24%, respectively. The moisture content was 7.7%; after pretreatment and conditioning, it was 36.61% and 33.7%, respectively. There was a significant difference between the initial and untreated rice bran, but no significant difference after pretreatment and conditioning.

**Table 1: Proximate and Mineral Composition of Rice bran before and after pretreatment**

| Parameters(%w/w) | Untreated Substrate | After Pretreatment | After conditioning |
|---|---|---|---|
| Moisture | 7.7 ± 0.35[a] | 36.61 ± 2.91[b] | 33.7 ± 1.84[b] |
| Ash | 7.83 ± 0.35[b] | 5.93 ± 0.26[a] | 5.24 ± 0.49[a] |
| Protein | 7.51 ± 0.15[b] | 3.61 ± 0.34[a] | 3.73 ± 0.35[a] |
| Lipid | 1.68 ± 0.14[a] | 0.697 ± 0.299[a] | 0.797 ± 0.17[a] |
| Fibre | 0.97 ± 0.032[a] | 0.70 ± 0.092[b] | 0.433 ± 0.62[c] |
| Carbohydrate | 88.04 ± 4.84[b] | 89.8 ± 1.98[b] | 49.23 ± 1.48[a] |
| Lignin | 46.73 ± 4.84[b] | 34.73 ± 3.49[b] | 15.67 ± 1.95[a] |
| Hemicellulose | 21.3 ± 2.09[a] | 10.33 ± 0.56[a] | 9.0 ± 0.23[b] |
| Potassium | 31.22 ± 4.43[b] | 25.44 ± 0.56[a] | 20.01 ± 0.634[a] |
| Nitrogen | 37.1 | 40.3 | 12.9 |
| Magnesium | 112.8 ± 6.5[a] | 159.21 ± 10.1[b] | 139.13 ± 0.47[b] |
| Calcium | 128.31 ± 22.31[a] | 141.15 ± 27.4[a] | 85.43 ± 0.296[b] |

### 3.2 Biochemical Characterization of Bacterial Isolates

The biochemical attributes of the isolates obtained from the sewage within the polytechnic community are reported in Table 2. The characteristics revealed the presence of rod-shaped and coccoid organisms. The Gram reaction varied between positive and negative isolates. Some of the bacterial isolates showed variable results for catalase, oxidase, indole, methyl red, and Voges-Proskauer. Bacterial isolates included *Bacillus* sp., *Zymomonas* sp., *Serratia* sp., *Citrobacter* sp., *Proteus* sp., and *Pseudomonas* sp.

**Table 2: Biochemical identity of bacterial isolates obtained from Sewage**

| Shape | | GMA1 | GMA2 | GMA3 | GMA4 | GMA6 |
|---|---|---|---|---|---|---|
| | | Rod | Rod | Rod | Cocci | Rod |
| Gram reaction | | + | - | + | + | - |
| Catalase | | - | + | + | - | - |
| Oxidase | | - | + | - | + | + |
| Citrate | | | | | | |
| | Slant | A | A | B | B | B |
| | Butt | A | B | A | A | A |
| | $H_2S$ | - | - | + | - | - |
| | Gas | - | - | - | - | + |
| Urease | | - | - | | - | + |
| Fructose | | - | AG | AG | AG | AG |
| Lactose | | - | - | - | - | - |
| Maltose | | A/G | G | AG | AG | AG |
| Manitol | | + | + | + | + | + |
| Sucrose | | + | + | | + | - |
| Starch hydrolysis | | + | | + | + | - |
| Tentative Identity | | *Bacillus* sp. | *Zymomonas* sp. | *Serratia* sp. | *Citrobacter* sp | *Pseudomonas* sp. |

### 3.3 Calibration and Screening for Bioethanol Production

The result presented in Fig3 and Fig.4 shows the calibration curve for the determination of the concentration of bioethanol and ascertain the concentration of the reducing sugars during the production the line of best fit shows the y=0.2442x-0.0002 was observed for the reducing sugars while Fig6 shows the effect of variation in the concentration of the bioethanol produced with varying reducing sugars and pH of the fermentation must using the *Z. mobilis*; similar responses can be seen Fig 7 and 8.

### 3.3 Molecular Characterization of Bacterial Isolates

Figure 1 shows the amplified regions of the bacterial isolates, particularly the 16S rRNA. Lane L represents the 100bp molecular ladder. Two frequently isolated bacterial isolates were amplified and identified. The gel for the bacterial isolates ran at 1500 bp. The ladder on the far right is used to determine gene sizes.

### 3.4 Phylogenetic Construct

The result presented in Figure 2 shows the relatedness of the isolates; the bootstrap values indicate their relatedness to the already identified and deposited identity in GenBank. Isolate was observed to have 100% with isolate GA102017 with *Zymomonas mobilis* as presented in Fig 2.

### 3.5 Optimisation Response Surface Methodology

The results presented in Table 4 show the real-time data obtained from the design of experiments using variables that promote bioethanol production. The thirty runs for the experimentation, and the information around the center points and the other points.

The contour plots for optimal bioethanol production, as presented in Figure 9, suggest an interaction between temperature and inoculum size. The bioethanol concentrations produced within the range suggest that extreme conditions did not favour bioethanol production. An increase from the center point suggests a marginal increase of about 24% v/w in bioethanol within the range of 30-32%, and in inoculum within the range of 10-12%. However, there was a significant decline at 13-15% and at a temperature of 33-35 °C.

Figure 8 shows the contour plot, which indicates that at the optimal point, increasing the substrate size could increase the amount of bioethanol produced. The study using these two parameters revealed that an increase in the inoculum size led to a slight increase in bioethanol production.

The result shown in Figure 10 illustrates the interaction between temperature and pH during bioethanol production. A pH of 7.5 to 8.1 was found to significantly affect the synthesis of bioethanol by *Zymomonas mobilis*. Changes in temperature did not significantly affect bioethanol production at an actual value of 4.5% and an inoculum size of 10%. The result suggested that changes in the parameter did not have a significant effect on bioethanol production; the decline in the optimal points decreased from 24% to 23% v/w.

The scatter plot of the points from the minimal, optimal, and maximal points along a regression line or goodness-of-fit line. The proximity to unity (1.0). The plots of the predicted and actual values side-by-side are presented in Figure 13.

### 3.6 Reducing Sugar

Reducing sugar=-945.45146+106.74583* Substrate load-61.79583* pH+43.06917* Inoculum size+43.17250* Temperature+3.22500* Substrate load * pH+2.41500 * Substrate load * Inoculum size-3.52500* Substrate load * Temperature+0.80500* pH * Inoculum size-0.63500* pH * Temperature-1.57700* Inoculum size * Temperature-5.42917* Substrate load$^2$+3.62083* pH$^2$-0.33917* Inoculum size$^2$-0.039167* Temperature$^2$

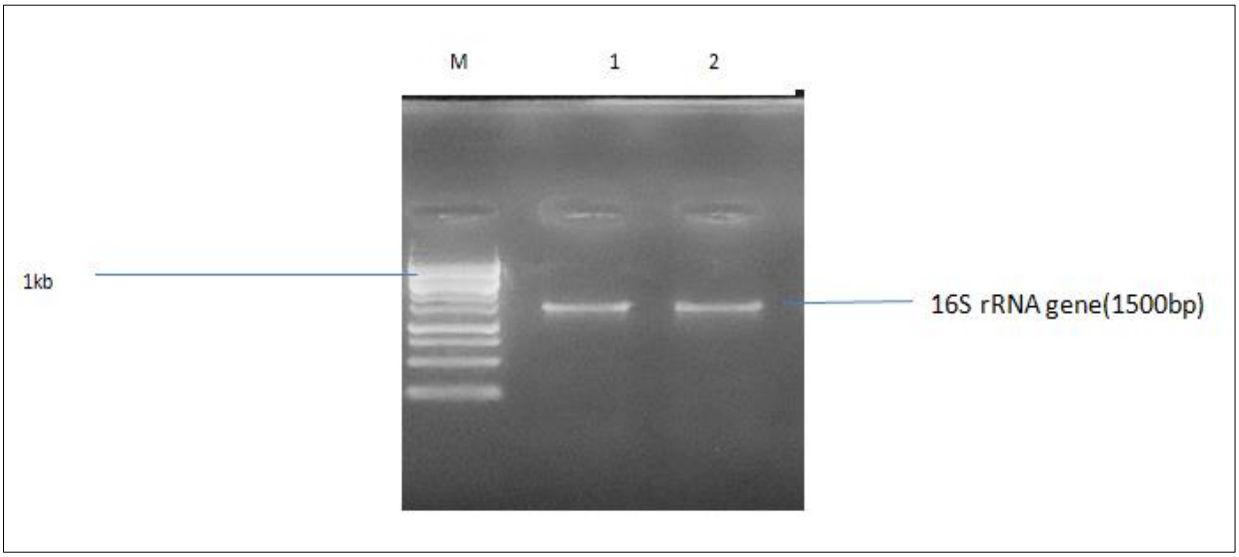



**Fig. 1: Agarose gel electrophoresis showing 16SrRNA gene bands: Lanes 1 and 2 represent the amplified gene from the isolates, M represents the 1kb ladder**

**Table 3: Ethanol tolerance screening from Congo dye stain of the zones of inhibition**

| Isolate Code | 10.0% | 6.0% | 2.0% | 1.0% |
|---|---|---|---|---|
| A1 | 9.20 | 14.2 | 18.7 | 16.3 |
| A2 | - | 12.4 | 10.6 | 12.6 |
| A3 | 12 | 25 | 32.1 | 18.1 |
| A4 | - | 8 | 6.3 | 4.2 |

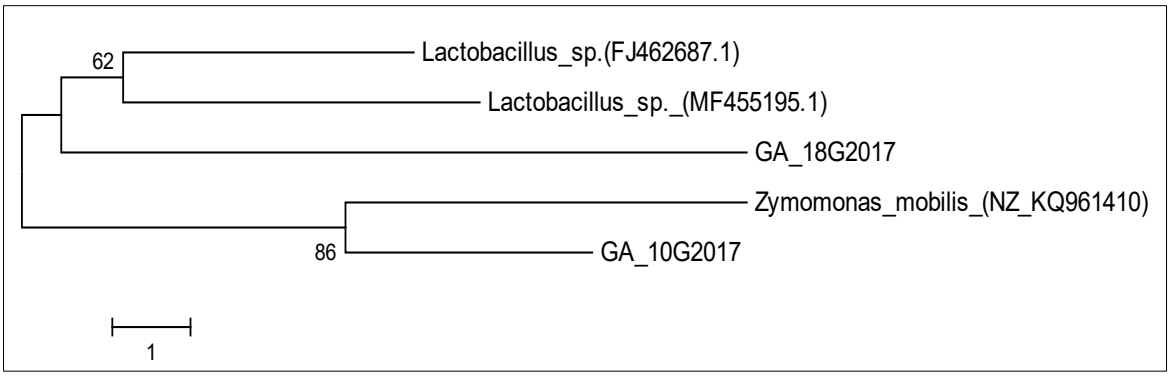



**Fig. 2: Phylogenetic construct of bacterial isolates employed in the production of bioethanol**

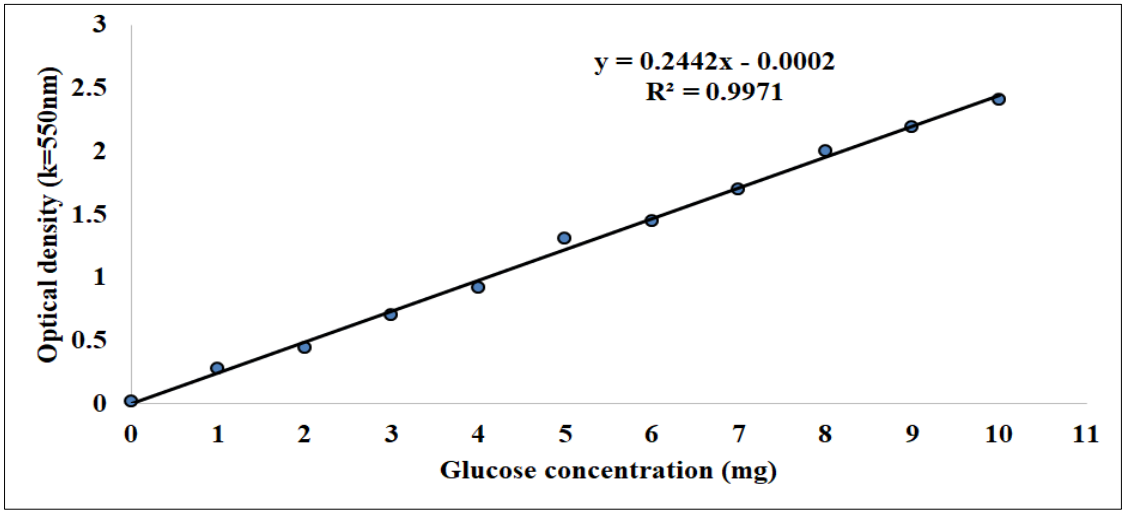



**Fig. 3: Calibration curve for reducing sugar estimation using Di-Nitrosalicyclic acid (DNSA)**

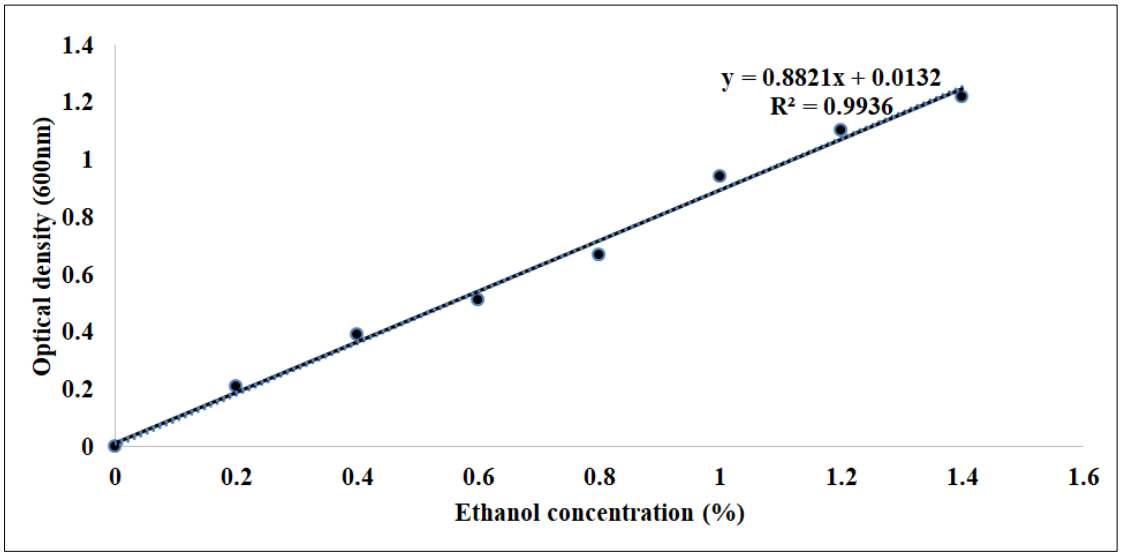



**Fig. 4: Calibration curve for ethanol estimation using potassium dichromate (vii)**

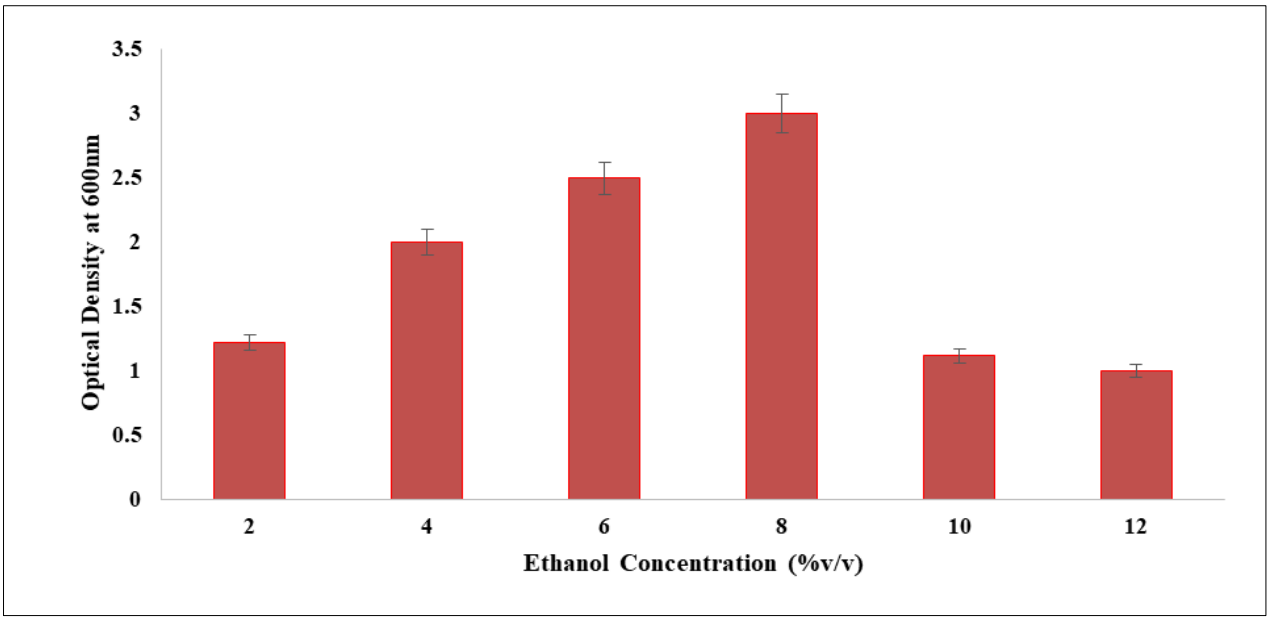



**Fig. 5: Ethanol tolerance for *Zymomonas* sp. Isolated from soil**

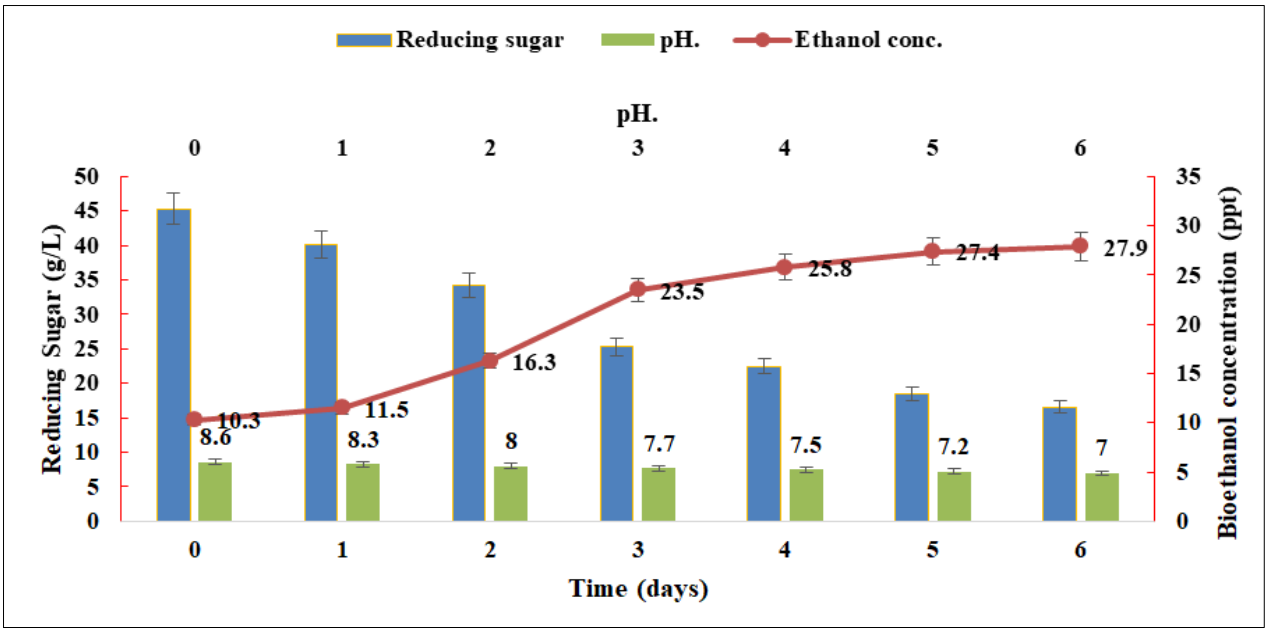



**Fig. 6: Effect of reducing sugar, pH on bioethanol production by *Zymomonas* sp using pretreated composite substrate**

**Table 4: Response plot for Actual and predicted ethanol production by *Zymomonas* sp**

| Std | run | Factor 1 Substrate % | Factor 2 pH | Factor 3 Inoculum Conc (%) | Factor 4 Temperature (%) | Actual Bioethanol | Predicted Bioethanol |
|---|---|---|---|---|---|---|---|
| 1 | 7 | 4 | 7.5 | 10 | 30 | 20.21 | 17.98 |
| 2 | 16 | 5 | 7.5 | 10 | 30 | 19.9 | 22.85 |
| 3 | 9 | 4 | 8.5 | 10 | 30 | 25.1 | 17.32 |
| 4 | 11 | 5 | 8.5 | 10 | 30 | 18.1 | 17.75 |
| 5 | 4 | 4 | 7.5 | 15 | 30 | 13.2 | 17.86 |
| 6 | 3 | 5 | 7.5 | 15 | 30 | 18.84 | 9.78 |
| 7 | 8 | 4 | 8.5 | 15 | 30 | 4.4 | 22.92 |
| 8 | 6 | 5 | 8.5 | 15 | 30 | 8.3 | 19.59 |
| 9 | 5 | 4 | 7.5 | 10 | 35 | 18.3 | 15.72 |
| 10 | 1 | 5 | 7.5 | 10 | 35 | 21.5 | 18.66 |
| 11 | 18 | 4 | 8.5 | 10 | 35 | 11.8 | 23.55 |

| Std | run | Factor 1 Substrate % | Factor 2 pH | Factor 3 Inoculum Conc (%) | Factor 4 Temperature (%) | Actual Bioethanol | Predicted Bioethanol |
|---|---|---|---|---|---|---|---|
| 12 | 10 | 5 | 8.5 | 10 | 35 | 19.23 | 22.85 |
| 13 | 15 | 4 | 7.5 | 15 | 35 | 21.2 | 16.27 |
| 14 | 12 | 5 | 7.5 | 15 | 35 | 17 | 22.12 |
| 15 | 17 | 4 | 8.5 | 15 | 35 | 14.3 | 21.19 |
| 16 | 14 | 5 | 8.5 | 15 | 35 | 17.5 | 14.05 |
| *17* | *2* | *4.5* | *8* | *12.5* | *32.5* | *28.37* | *14.80* |
| *18* | *19* | *4.5* | *8* | *12.5* | *32.5* | *29.8* | *22.85* |
| *19* | *13* | *4.5* | *8* | *12.5* | *32.5* | *27.5* | *22.85* |
| 20 | 20 | 4.5 | 8 | 12.5 | 32.5 | 15.78 | 13.81 |
| 21 | 30 | 3.5 | 8 | 12.5 | 32.5 | 21.11 | 22.58 |
| 22 | 24 | 5.5 | 8 | 12.5 | 32.5 | 16.67 | 24.01 |
| 23 | 27 | 4.5 | 7 | 12.5 | 32.5 | 24.5 | 17.88 |
| 24 | 28 | 4.5 | 9 | 12.5 | 32.5 | 8.4 | 23.47 |
| 25 | 29 | 4.5 | 8 | 7.5 | 32.5 | 14.45 | 23.47 |
| 26 | 21 | 4.5 | 8 | 17.5 | 32.5 | 20.3 | 20.49 |
| 27 | 22 | 4.5 | 8 | 12.5 | 27.5 | 25.6 | 9.89 |
| 28 | 23 | 4.5 | 8 | 12.5 | 37.5 | 23.5 | 18.42 |
| 29 | 26 | 4.5 | 8 | 12.5 | 32.5 | 19.33 | 17.38 |
| 30 | 25 | 4.5 | 8 | 12.5 | 32.5 | 17.54 | 19.7 |

| **ANOVA for Response Surface Quadratic model** | | | | | | |
|---|---|---|---|---|---|---|
| **Analysis of variance table [Partial sum of squares - Type III]** | | | | | | |
| | **Sum of** | | **Mean** | **F** | **p-value** | |
| **Source** | **Squares** | **df** | **Square** | **Value** | **Prob > F** | |
| Block | 40.34 | 1 | 40.34 | | | |
| Model | 2318.38 | 14 | 165.60 | 4.44 | 0.0043 | significant |
| *A-Substrate load* | *71.25* | *1* | *71.25* | *1.91* | *0.1887* | |
| *B-pH* | *17.50* | *1* | *17.50* | *0.47* | *0.5047* | |
| *C-Inoculum size* | *328.31* | *1* | *328.31* | *8.80* | *0.0102* | |
| *D-Temperature* | *246.22* | *1* | *246.22* | *6.60* | *0.0223* | |
| *AB* | *10.40* | *1* | *10.40* | *0.28* | *0.6059* | |
| *AC* | *145.81* | *1* | *145.81* | *3.91* | *0.0681* | |
| *AD* | *310.64* | *1* | *310.64* | *8.32* | *0.0120* | |
| *BC* | *16.20* | *1* | *16.20* | *0.43* | *0.5207* | |
| *BD* | *10.08* | *1* | *10.08* | *0.27* | *0.6114* | |
| *CD* | *1554.33* | *1* | *1554.33* | *41.64* | *< 0.0001* | |
| $A^2$ | *50.53* | *1* | *50.53* | *1.35* | *0.2641* | |
| $B^2$ | *22.48* | *1* | *22.48* | *0.60* | *0.4507* | |
| $C^2$ | *123.25* | *1* | *123.25* | *3.30* | *0.0907* | |
| $D^2$ | *1.64* | *1* | *1.64* | *0.044* | *0.8368* | |
| Residual | 522.56 | 14 | 37.33 | | | |
| *Lack of Fit* | *512.45* | *10* | *25.24* | *20.28* | *0.53* | *Not significant* |
| *Pure Error* | *10.11* | *4* | *2.53* | | | |
| Cor Total | 2881.28 | 29 | | | | |

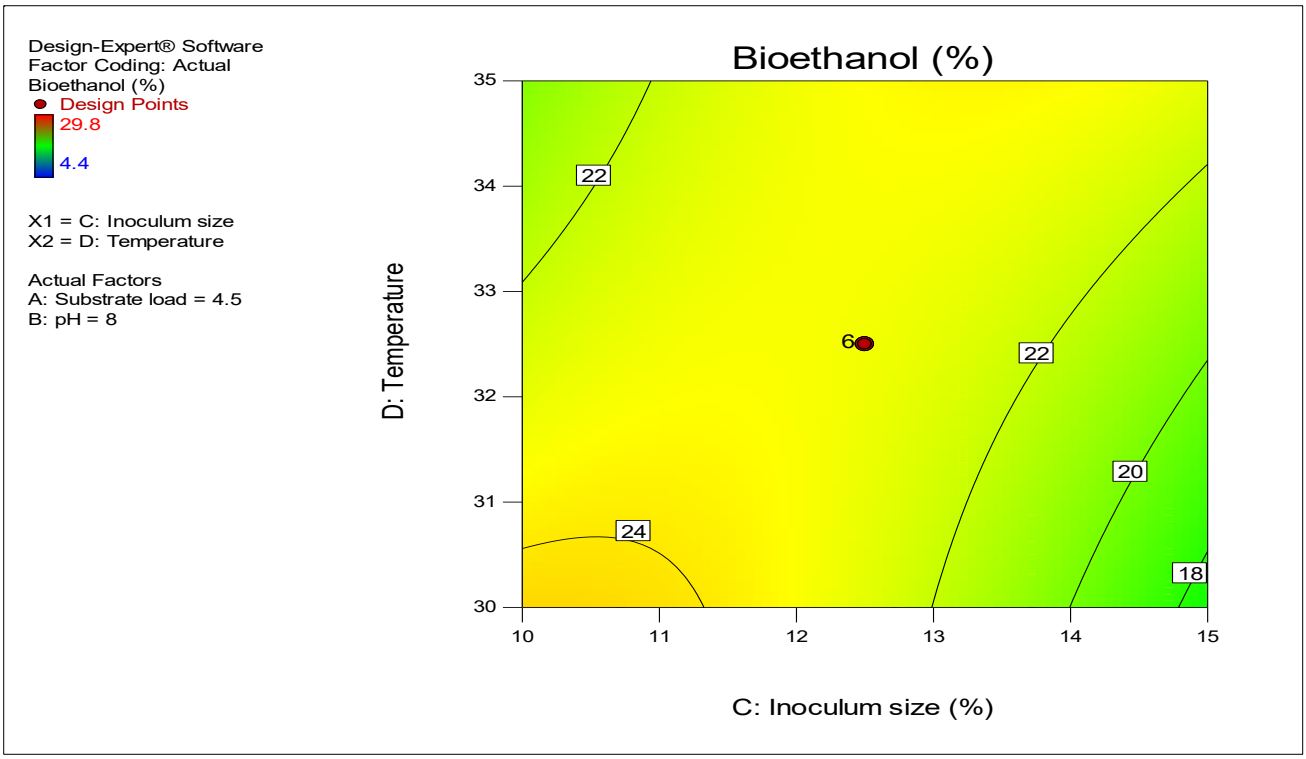



**Fig. 7: Contour-plot of the iteration effect of Inoculum size (*Z. mobilis*) and Temperature on Bioethanol production**

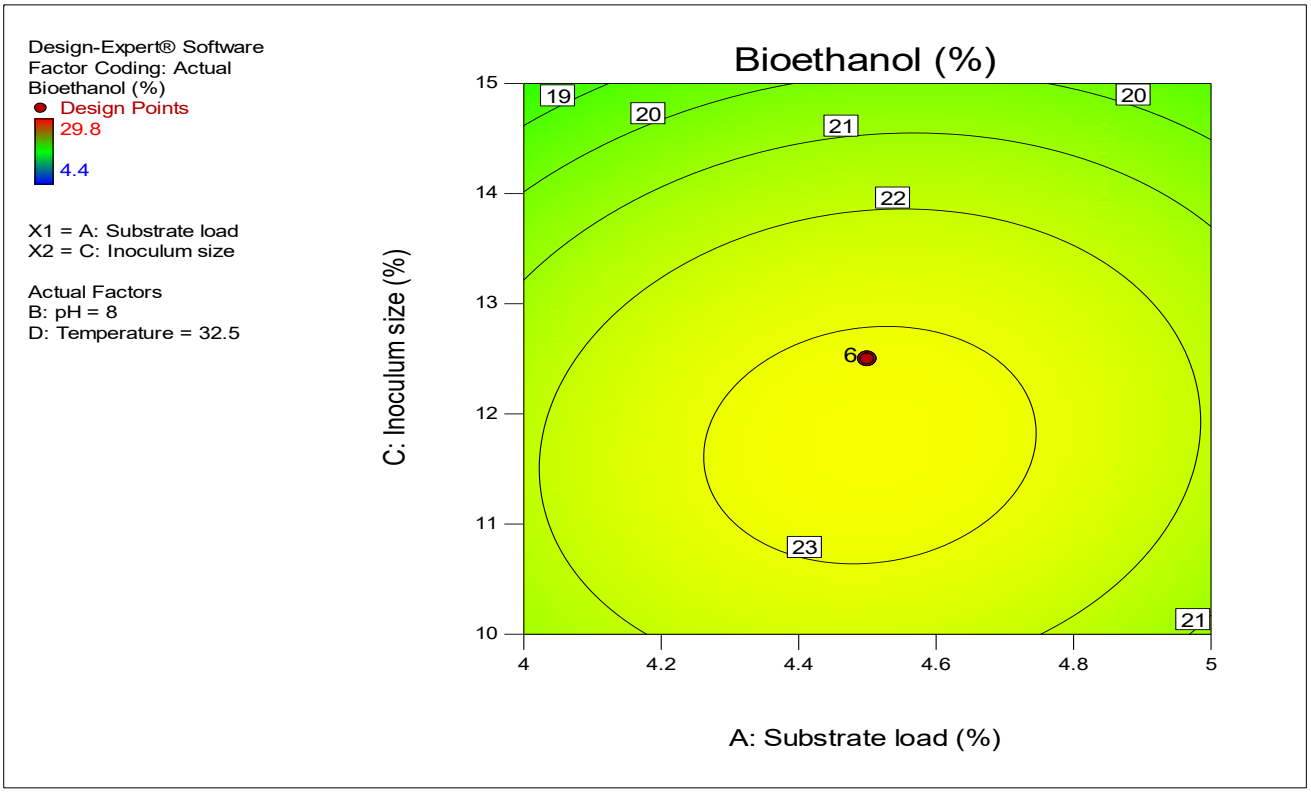



**Fig. 8: Contour-plot of the iteration effect of Inoculum size (*Z. mobilis*) and substrate load (Rice Bran) on Bioethanol production**

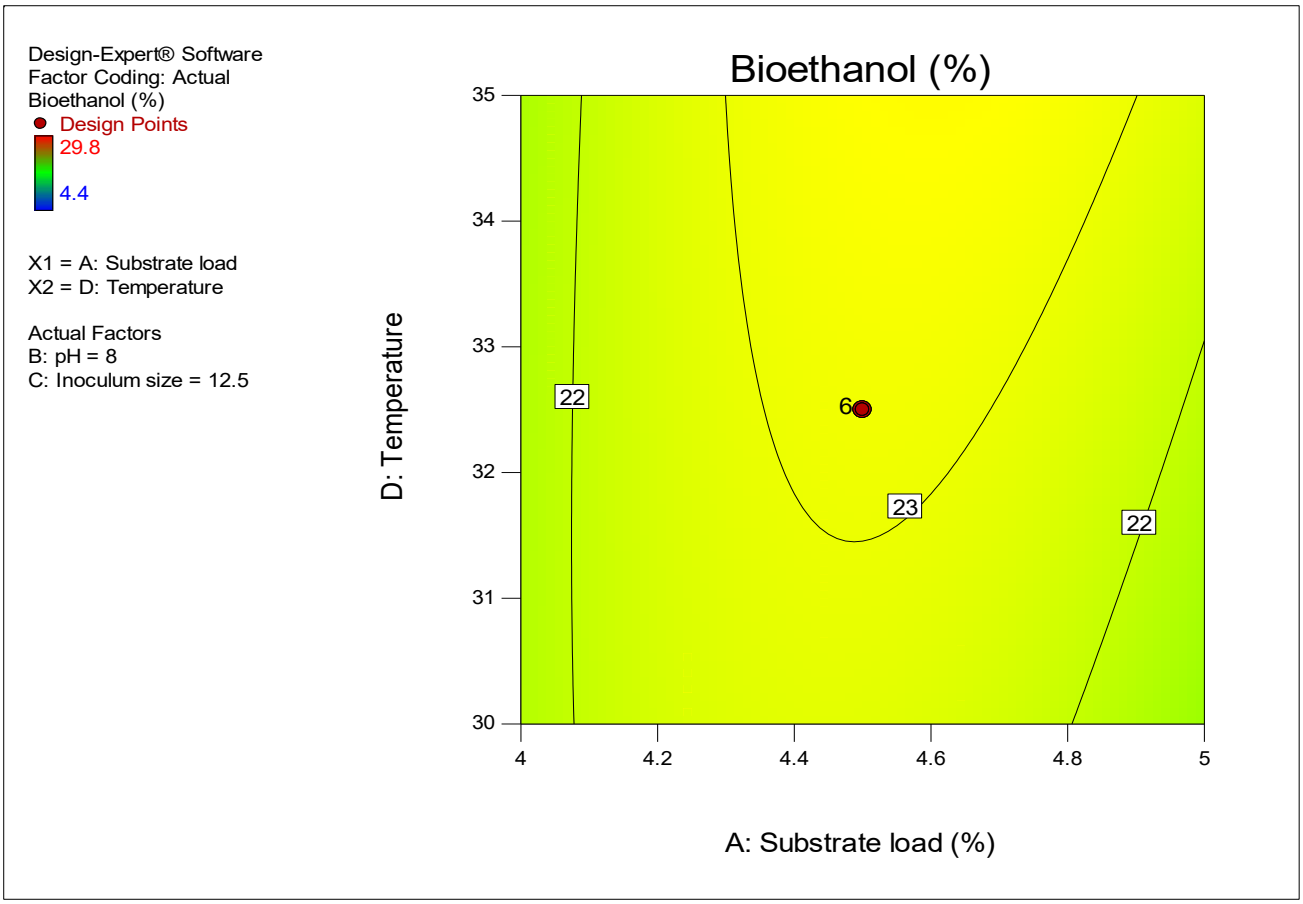



**Fig. 9: Contour-plot of the iteration effect of Temperature and substrate load (Rice Bran) on Bioethanol production**

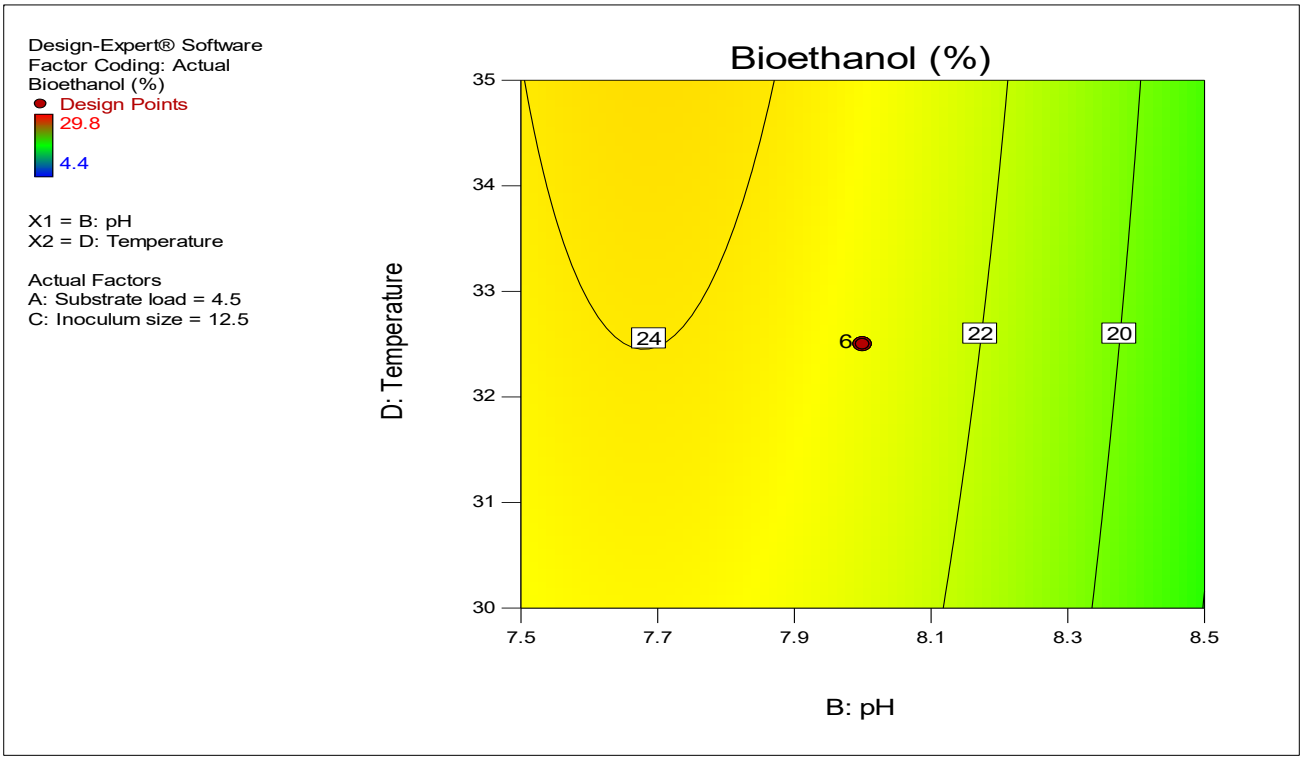



**Fig. 10: Contour-plot of the iteration effect of Temperature and pH on Bioethanol production**

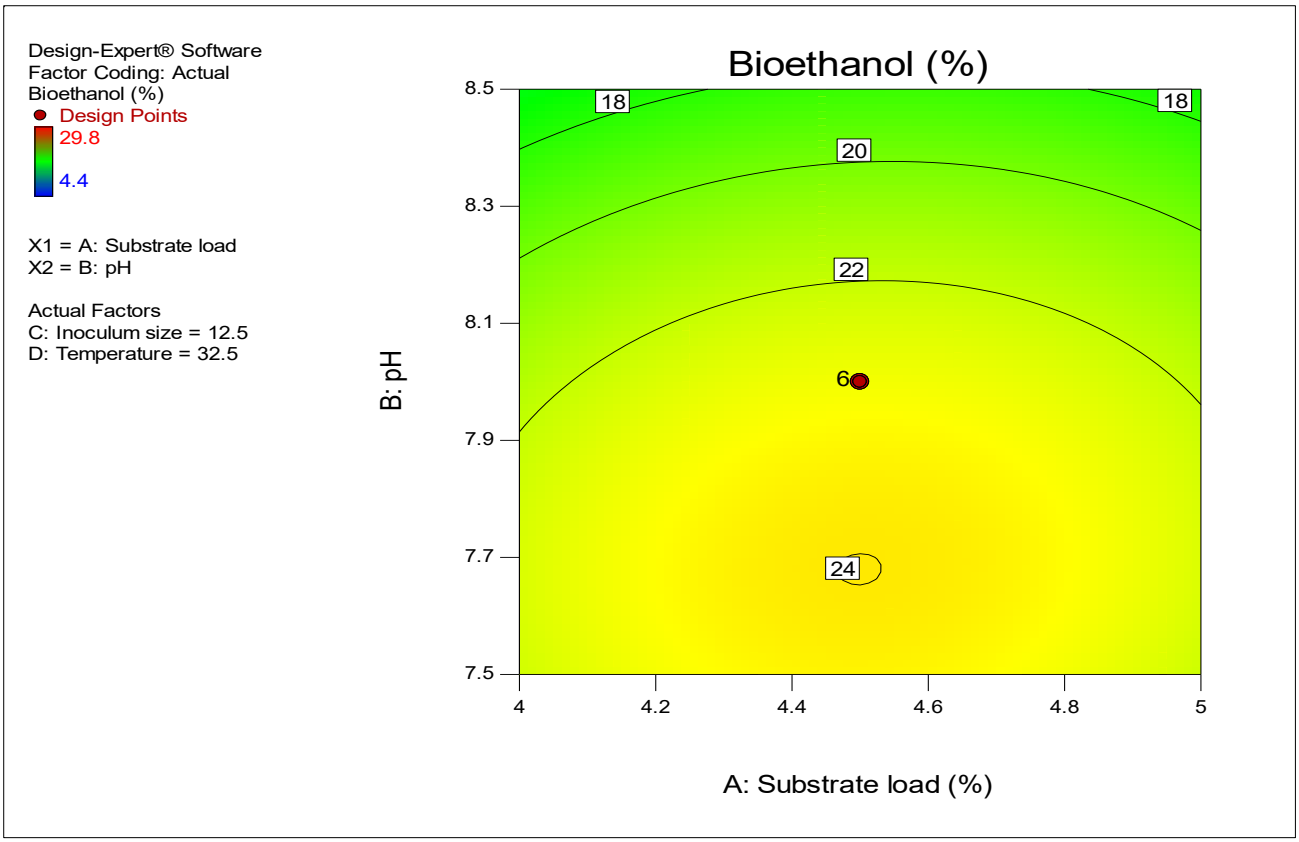
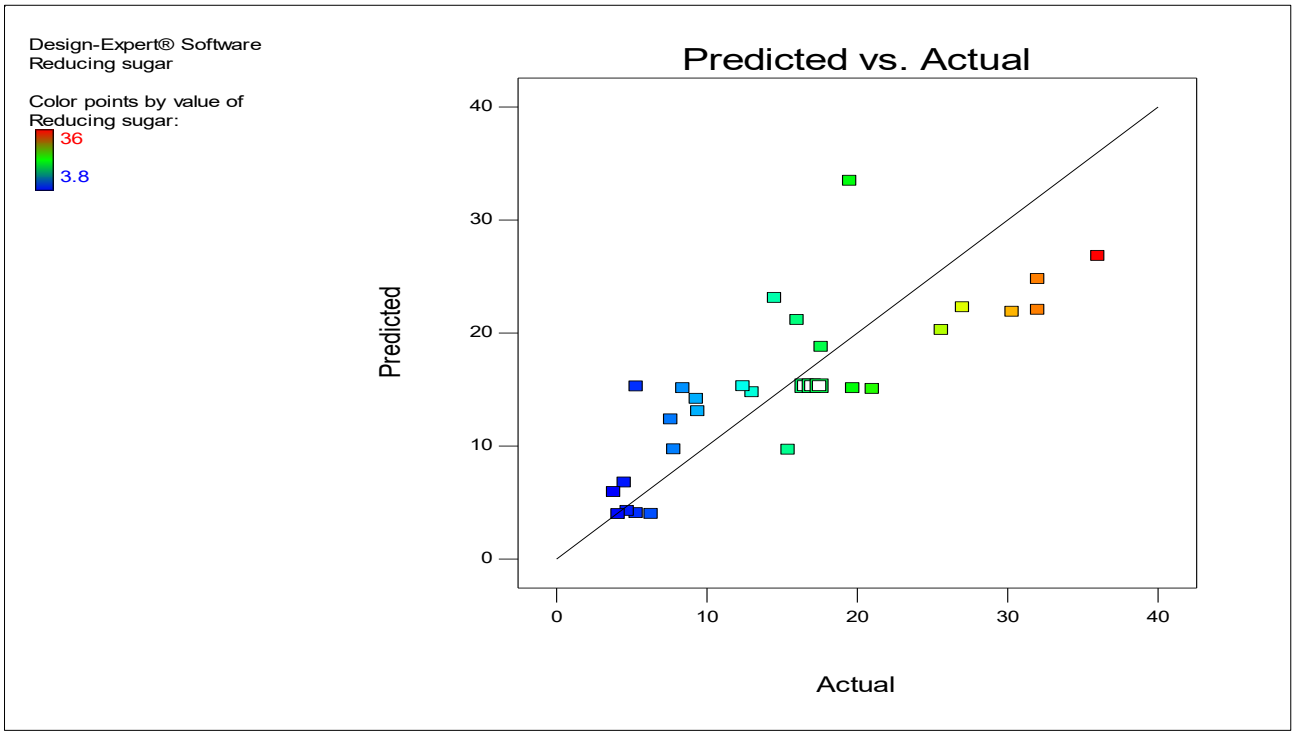



**Fig. 11: Contour-plot of the iteration effect of pH and Substrate load (Rice Bran) on Bioethanol production**

**Final Equation in Terms of Actual Factors**

Reducing sugar=-945.45146+106.74583* Substrate load-61.79583* pH+43.06917* Inoculum size+43.17250* Temperature+3.22500* Substrate load * pH+2.41500 * Substrate load * Inoculum size-3.52500* Substrate load * Temperature+0.80500* pH * Inoculum size-0.63500* pH * Temperature-1.57700* Inoculum size * Temperature-5.42917* Substrate load$^2$+3.62083* pH$^2$-0.33917* Inoculum size$^2$-0.039167* Temperature$^2$



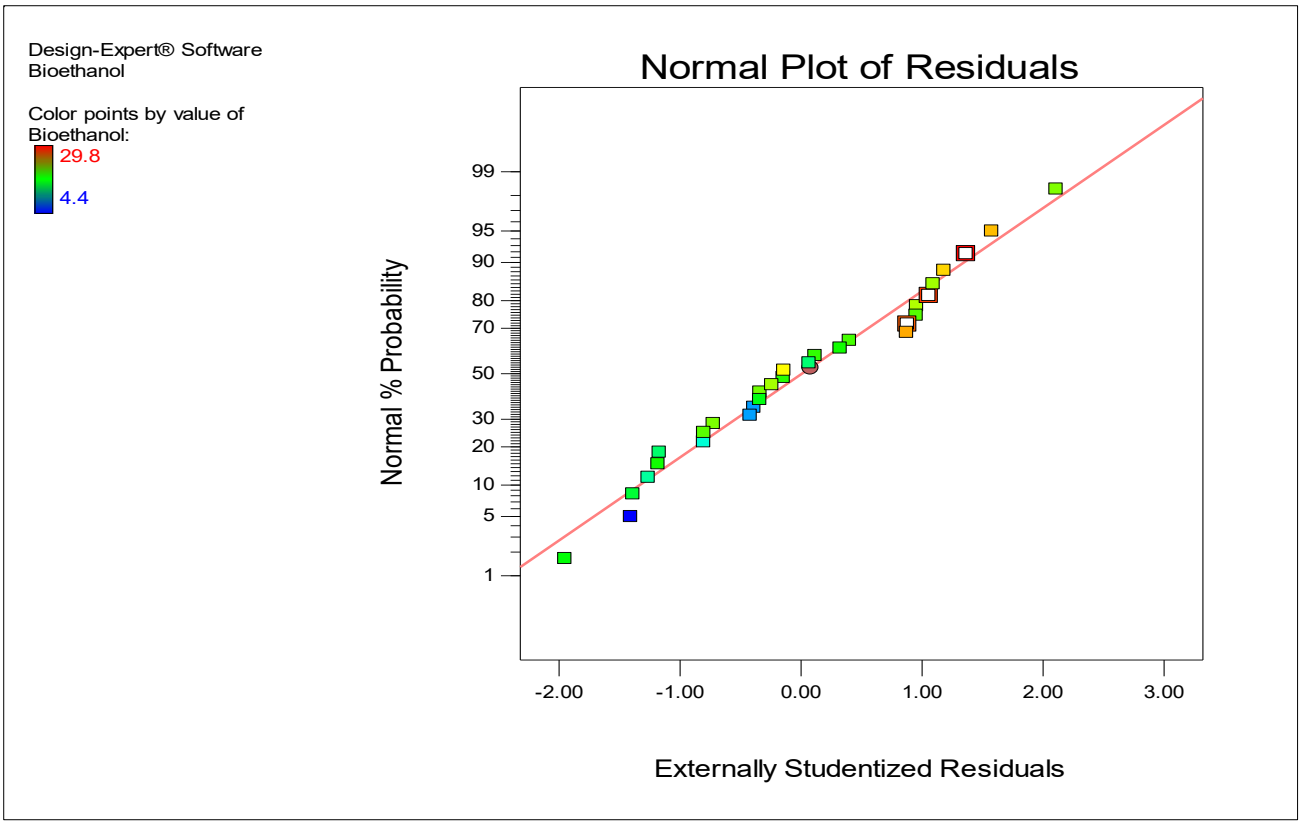



**Fig. 13: Line of regression of the studentized and experiments**

## 4.0 DISCUSSION

The carbohydrate content of the rice bran was 78.04 ± 4.84b, 82.8 ± 1.98b, and .23 ± 1.48a. Fiber content was 0.97 ± 0.032a, 0.70 ± 0.092b, 0.433 ± 0.62c, and 11.01 ± 0.34b for raw, pre-treated, and steam-conditioned samples, as reported in Table 1. The lipid content was 1.21 ± 0.058a, 0.92 ± 0.03a, and 0.91 ± 0.05b. The protein content for raw, treated, and steam-conditioned samples was 0.45 ± 0.18a, 0.07 ± 0.04a, and 0.157 ± 0.06a, respectively. The results suggest that lipid content was unaffected by treatment but was significantly affected by steam conditioning at p-value < 0.05. A similar result was observed for moisture, where the pretreatment data were significant. The results reported for rice bran indicated moisture contents of 11.7 ± 0.56a, 9.93 ± 0.60b, and 7.63 ± 0.24c for the raw, treated, and steam-conditioned samples, as presented in Table 1. Ash content was 22.56 ± 1.07a and 20.63 ± 0.41a. These findings align with the position of Fussy and Papenbrock (2022), who argued that most substrates could be used to avoid the cost of using organic nutrients. This is because these wastes are components of farm-sourced waste and can be obtained in commercial quantities. Furthermore, Dwivedi *et al.,* (2022) suggested that simple carbohydrates with potential for biotechnological milestones were repurposed from these waste materials. Mullick *et al.,* (2026) argue that 9-40% contain lignocellulosic materials and carry useful materials for energy generation.

Biomining for high-throughput strains has continually dominated current issues. Dumpsites are archives of high-throughput biotechnological innovations. Anand *et al*., (2023) report that most microbes in sewage can produce useful bioactive compounds, such as bioethanol. pH is an important factor in biosynthetic and physiological processes. Yeast cells have been used at an optimal activity pH of 3.0-5.5, as reported by Fatima *et al*., (2024), for effective bioethanol production. In their findings, an increase in pH to 5.0 was associated with an astronomical increase in production. Clearly, pH 6.0 to 8.0 enhanced production with rice bran, showing a steady increase from 2.0% v/w to 9.8% v/w. The bioethanol increased from 4.2% to 11.8% v/w. pH 8.5 to 9.0 was observed to decrease bioethanol production. The effect on bioethanol production using yam peel was observed to increase from pH 6.5 to 7.0, with a 5.1-11.2% v/w increase, and to decrease with increasing pH from 7.5 to 9.5. Furthermore, the decline in ethanol production was more pronounced at pH 8.0-8.5. There was an exponential increase from 6.0 to 11.5% v/w at pH 6.0-7.0 using *Z. mobilis*, and a *Paenibacillus* sp. co-culture system was reported at pH 5.5, corroborating the results obtained in this study. This is because the pH of a must undergoing fermentation controls the viability of the process and the interaction of selected strains. Extreme pH could lead to strain loss and lower yield. The findings of Saleh *et al*., (2022), in their work with Pichia sp. (a fungus), revealed that a pH of 6.0 enhanced

bioethanol production. Another study using Theobroma cacao opined that the highest concentration was 8.0% v/w at a pH of 8.0. They further felt that liming, as a pH-adjustment tool, affected the media.

Optimal temperature controls the interaction between the fermentation must and the inoculum size. Extreme temperatures can reduce the production and yield of valuable products. Temperatures between 20-45 °C were observed. An *et al*., (2010) found that the metabolic activity of the raw material could favor fermentation. Panahi *et al*., (2022) reported that ¼ of the bioethanol yield can be lost due to fermentation temperature. A temperature range of 25-30 °C was observed to have a significant effect on bioethanol production. During the study, a temperature of 35 °C was observed to enhance bioethanol production by *Zymomonas mobilis*, yielding 23.1% v/w ethanol. A temperature range of 40-45 °C was observed to reduce bioethanol production using agro residues as the substrate. Temperatures of 35-45 °C did not favor the production of bioethanol. Isolates were intolerant to extreme temperatures.

Inoculum size is of extreme and critical importance for the production of bioethanol. The study conducted by Tenkolu *et al*., (2024) reported that a 10% inoculum size was useful for high-yield bioethanol production. This was not agreed by Behera *et al*., (2021), who opined that 10% of a viable inoculum could be seeded in production. Although *Zymomonas mobilis* activity was optimal on day 3 of the study. Experiments using other substrates in previous studies reported 9.1% within the same duration.

Optimization of process parameters using multivariate methods, as revealed by the Box-Behnken approach in the design of the bioethanol system, proved productive. This technology represents a holistic approach to production. In another study, Chinedu *et al*., (2026) applied a multivariate approach to three variables and found that pH below 3.0 and above 8.0 did not yield bioethanol. In this study, a similar approach was employed with a four-factor quadratic design to model the prediction. The output suggests feasible synthesis under controlled conditions, with the proximity to unity (1.0). The plots of the predicted and actual values are presented side by side. The contour plots for optimal bioethanol production, as presented in Figures 9-10, suggest an interaction between temperature and inoculum size. The concentration of bioethanol produced within the range suggests that extreme conditions did not favor bioethanol production. An increase from the center point suggests a marginal increase of about 24% v/w of bioethanol within the range 30-32%, with inoculum within 10-12%. However, there was a significant decline at 13-15% and at temperatures of 33-35°C. Quantification of bioethanol using single cultures and co-culture conditions, and the substrates. It was observed that there was a significant difference in the substrate. The co-culture samples had 16% v/w, while the pure cultures had lower concentrations.

## 5. CONCLUSION AND RECOMMENDATION

Bioethanol production has advanced with the application of substrates that offer numerous ecological benefits, and such applications have been reported in recent times. Pretreatment of agroresidues such as rice bran has been shown to improve proximate composition after conditioning. Isolation and characterization of *Z. mobilis* were conducted using a molecular approach, analyzing potential sewage sources. Bacteriological screening demonstrated tolerance to varied concentrations of synthetic ethanol. The study further identified a direct proportionality between the increase in bioethanol and the variables tested. Iteration using operational and fermentation parameters was observed to fit the model for bioethanol production as a polynomial function, with the optimal conditions for bioethanol production being a substrate load of 4.5%, pH 8.0, inoculum size of 12.5%, and a temperature of 32.5°C. These findings further buttress the need for academia to develop proper reactors for the commercialization of industrially and economically viable products.

**Conflict of Interest**: The authors declare no competing interests regarding this study.

**Acknowledgment**: The authors explicitly acknowledge and express their profound gratitude to the Tertiary Education Trust Fund (TETFund), Nigeria, for providing the institutional research grant and financial sponsorship that made this study possible.

## REFERENCES


- Acharya, S., Liyanage, S., Parajuli, P., Rumi, S. S., Shamshina, J. L., & Abidi, N. (2021). Utilization of cellulose to its full potential: a review on cellulose dissolution, regeneration, and applications. *Polymers*, *13*(24), 4344.
- Agbaji, J. E., Nwaichi, E. O., & Abu, G. O. (2021). Attenuation of petroleum hydrocarbon fractions using rhizobacterial isolates possessing alkB, C23O, and nahR genes for degradation of n-alkane and aromatics. *Journal of Environmental Science and Health, Part A*, *56*(6), 635-645.a
- An, F., Wu, J., Feng, Y., Pan, G., Ma, Y., Jiang, J., ... & Zhao, M. (2023). A systematic review on the

flavor of soy-based fermented foods: Core fermentation microbiome, multisensory flavor substances, key enzymes, and metabolic pathways. *Comprehensive reviews in food science and food safety*, *22*(4), 2773-2801.

- Anand, U., Dey, S., Parial, D., Federici, S., Ducoli, S., Bolan, N. S., ... & Bontempi, E. (2023). Algae and bacteria consortia for wastewater decontamination and transformation into biodiesel, bioethanol, biohydrogen, biofertilizers and animal feed: a review. *Environmental Chemistry Letters*, *21*(3), 1585-1609.
- Asfere, Y., Kebede, A., & Muthuswamy, M. (2018). In-Vitro Antimicrobial Activities and Phytochemical Screening of Calotropis Procera (Ait) and Vernonia Amygdalina (Del.) Extracts Against Some Medically Important Pathogenic Bacteria. *American Journal of Bioscience and Bioengineering*, *6*(6), 42-55.
- Barhoum, A., Jeevanandam, J., Rastogi, A., Samyn, P., Boluk, Y., Dufresne, A., ... & Bechelany, M. (2020). Plant celluloses, hemicelluloses, lignins, and volatile oils for the synthesis of nanoparticles and nanostructured materials. *Nanoscale*, *12*(45), 22845-22890.
- Behera, B., Das, T. K., Raj, R., Ghosh, S., Raza, M. B., & Sen, S. (2021). Microbial consortia for sustaining productivity of non-legume crops: prospects and challenges. *Agricultural Research*, *10*(1), 1-14.
- Boismier, E. C., Aboulnaga, E. A., & TerAvest, M. A. (2025). Zymomonas mobilis: bringing an ancient human tool into the genomic era. *Current Opinion in Biotechnology*, *92*, 103257.
- Chinedu, N. B., Orhuebor, E. N., Ben, M. G., Joshua, O. O., Nnanna, A. C., & Oghenebrume, D. N. (2026). Comparative modelling and statistical optimization of bioethanol production from yam, plantain, and potato peels using fermentation performance indicators. *World News of Natural Sciences*, *65*, 228-244.
- Di Fraia, A., Di Fraia, S., & Massarotti, N. (2024). Role of advanced oxidation processes in lignocellulose pretreatment towards biorefinery applications: a review on emerging trends and economic considerations. *Green Chemistry*, *26*(15), 8461-8496.
- Drishya, P. K., Reddy, M. V., Mohanakrishna, G., Sarkar, O., Isha, Rohit, M. V., ... & Chang, Y. C. (2025). Advances in microbial and plant-based biopolymers: synthesis and applications in next-generation materials. *Macromol*, *5*(2), 21.
- Dwivedi, S., Tanveer, A., Yadav, S., Anand, G., & Yadav, D. (2022). Agro-wastes for cost effective production of industrially important microbial enzymes: an overview. *Microbial biotechnology: role in ecological sustainability and research*, 435-460.
- Effiong, E. C., & Asionye, E. I. (2025). Evaluation of Quality proxies of roof-harvested rainwater obtained from tertiary institutions in Orlu Zone, Imo State. *Evaluation*, *8*(1), 132-145.
- El-Araby, R. (2024). Biofuel production: exploring renewable energy solutions for a greener future. *Biotechnology for Biofuels and Bioproducts*, *17*(1), 129.
- Fatima, N., Malik, A. Y., Shah, M. A., & Khan, H. (2024). The effect of glucose, temperature and ph on bioethanol production by Saccharomyces Cerevisiae. *Journal of Population Therapeutics and Clinical Pharmacology*, *31*(1), 656-667.
- Fussy, A., & Papenbrock, J. (2022). An overview of soil and soilless cultivation techniques—chances, challenges and the neglected question of sustainability. *Plants*, *11*(9), 1153.
- Gul, M. Z., Das, P. P., Babu, K. R., Juarez-Colunga, S., Weber, A. M., Yoosuf, B., ... & Ghazi, I. A. (2026). Rice bran: a comprehensive review of phytochemicals and bioactive components with therapeutic potential and health benefits. *Nutrition Reviews*, *84*(7), 1453-1472.
- Guo, S. H., Zhu, Y., & Zhao, J. (2025). Enhancing Students' Understanding of Saccharides by 3D Printed Glucose Models. *Journal of Chemical Education*, *102*(2), 573-582.
- Joshi, A., Yadav, P., Yadav, C., Kanthaliya, B., Verma, K. K., & Arora, J. (2024). Valorization strategies for agriculture residue: an overview. *Transforming Agriculture Residues for Sustainable Development: From Waste to Wealth*, 21-43.
- Kamani, M. H., Eş, I., Lorenzo, J. M., Remize, F., Roselló-Soto, E., Barba, F. J., ... & Khaneghah, A. M. (2019). Advances in plant materials, food by-products, and algae conversion into biofuels: use of environmentally friendly technologies. *Green chemistry*, *21*(12), 3213-3231.
- Koyande, A. K. (2021). *Biorefinery of Chlorella sp. using integrated multiphasic systems for biofuel, feed and wound healing application*. University of Nottingham (United Kingdom).
- Lam, H. H., Nguyen, T. M. T., Do, T. A. S., Dinh, T. H., & Dang-Bao, T. (2021, December). Quantification of total sugars and reducing sugars of dragon fruit-derived sugar-samples by UV-Vis spectrophotometric method. In *IOP Conference Series: Earth and Environmental Science* (Vol. 947, No. 1, p. 012041). IOP Publishing.
- Malik, K., Capareda, S. C., Kamboj, B. R., Malik, S., Singh, K., Arya, S., & Bishnoi, D. K. (2024). Biofuels production: A review on sustainable alternatives to traditional fuels and energy sources. *Fuels*, *5*(2), 157-175.
- Mullick, K., Adari, R., Srinivasan, V., & Radha, P. (2026). Waste to Wealth: In Silico Biorefineries

Harnessing Microbial Intelligence for Lignocellulosic-Based Circular Bioeconomy. In *Industrial Microbiology and Biotechnology: Trends in Microbial Biotechnology* (pp. 727-761). Singapore: Springer Nature Singapore.

- Nisar, A., Hashum, K., Bashir, M., Mubeen, N., Younus, S., Mehmood, S., ... & Haroon, M. (2024). Advancing sustainable biofuel production from agricultural residues: a comprehensive mini-review. *Sustainable Chemical Engineering*, 115-128.
- Ojo, A. O. (2023). An overview of lignocellulose and its biotechnological importance in high-value product production. *Fermentation*, *9*(11), 990.
- Omonije, O. O. (2023). Optimization Of Microwave-Alkaline Pre-Treatments Conditions and Co-Immobilization of Xylanase, Cellulase and Fungi Species for the Production of Bioethanol from Some Agrowastes (Doctoral Dissertation).
- Padhiyar, S., Parikh, J., Kumar, D. S., Yousuf, M., Sachuthananthan, B., Nagabhooshanam, N., ... & Saravanan, A. (2026). Effect of lignin biopolymer, microfiber, and rubber matrix on the mechanical, fatigue, dielectric, and electromagnetic shielding properties of composites. *Polymer Bulletin*, *83*(3), 127.
- Panahi, H. K. S., Dehhaghi, M., Guillemin, G. J., Gupta, V. K., Lam, S. S., Aghbashlo, M., & Tabatabaei, M. (2022). Bioethanol production from food wastes rich in carbohydrates. *Current Opinion in Food Science*, *43*, 71-81.
- Pérez-García, L. A., Del Rio-Arellano, C. N., Rincón, D. F. L., & De La Fuente-Salcido, N. M. (2022). Physiology of ethanol production by Zymomonas mobilis. In *Bioethanol* (pp. 21-42). Apple Academic Press.
- Riaz, M. W., Yousaf, M. I., Hussain, Q., Yasir, M., Sajjad, M., & Shah, L. (2023). Role of lignin in wheat plant for the enhancement of resistance against lodging and biotic and abiotic stresses. *Stresses*, *3*(2), 434-453.
- Saleh, A. K., Abdel-Fattah, Y. R., Soliman, N. A., Ibrahim, M. M., El-Sayed, M. H., Abd El-Aziz, Z. K., & El-Zawawy, W. K. (2022). Box-Behnken design for the optimization of bioethanol production from rice straw and sugarcane bagasse by newly isolated Pichia occidentalis strain AS. 2. *Energy & Environment*, *33*(8), 1613-1635.
- Tan, B. L., Norhaizan, M. E., & Chan, L. C. (2023). Rice bran: From waste to nutritious food ingredients. *Nutrients*, *15*(11), 2503.
- Tenkolu, G. A., Kuffi, K. D., & Gindaba, G. T. (2024). Optimization of fermentation condition in bioethanol production from waste potato and product characterization. *Biomass Conversion and Biorefinery*, *14*(4), 5205-5223.
- Tufail, T., Ain, H. B. U., Chen, J., Virk, M. S., Ahmed, Z., Ashraf, J., ... & Xu, B. (2024). Contemporary views of the extraction, health benefits, and industrial integration of rice bran oil: A prominent ingredient for holistic human health. *Foods*, *13*(9), 1305.
- Vale, A. C., Leite, L., Pais, V., Bessa, J., Cunha, F., & Fangueiro, R. (2024). Extraction of natural-based raw materials towards the production of sustainable man-made organic fibres. *Polymers*, *16*(24), 3602.