Optimization of Bioethanol Yield from Liquid Pineapple Waste using Immobilized *Saccharomyces cerevisiae var. ellipsoideus* in PVA-Alginate-Sulfate Matrix

Sarina Mat Rosid[1], and *Nor Azimah Mohd* Zain[2*]

[1]School of Agricultural Science and Biotechnology, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia
[2]Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, Skudai, 81310 Johor Bahru, Johor, Malaysia

Abstract. The increasing significance of bioethanol as a viable alternative to fossil fuels is driven by the depletion of conventional energy sources and the urgent need to reduce greenhouse gas emissions. Utilizing industrial or food wastes for bioethanol production is highly promising due to their rich sugar content. This study explores the feasibility of producing bioethanol from liquid pineapple waste using immobilized *Saccharomyces cerevisiae* var. *ellipsoideus* ATCC560 within PVA-alginate-sulfate beads. The novelty of this work lies in the post-immobilization treatment of beads with boric acid, sodium alginate, and sodium sulfate, which enhanced bead stability, improved yeast activity, and increased ethanol yield. A statistical approach with a 2-level, 3-factorial design was employed, considering four variables: initial inoculum (3-6 g), temperature (30-50 °C), pH (4.5-5.5), and agitation speed (100-200 rpm). Additionally, Field Emission Scanning Electron Microscope (FESEM) analysis was performed to examine bead morphology. The optimal conditions for bioethanol production were identified, yielding a maximum concentration of 10.0438 g/L at pH 5.5, 30 °C, and 200 rpm using 3 g of beads. FESEM analysis verified the efficiency of this technique by confirming the successful immobilization of S. *cerevisiae* var. *ellipsoideus* within the matrix. This study highlights the potential of immobilized *S. cerevisiae* var. *ellipsoideus* to produce bioethanol from liquid pineapple waste, providing an environmentally friendly and sustainable approach to renewable energy production.

1 introduction

Global energy demand continues to rise, driven by population growth and rapid industrial expansion in developing countries. Petroleum-based sources such as oil, coal, and natural gas remain dominant; however, reliance on these fuels threatens ecological balance, depletes natural reserves, and accelerates climate change. Issues such as greenhouse gas emissions,

* Corresponding author: azimah@utm.my

public health risks, and volatile fuel prices emphasize the urgent need for sustainable alternatives [1].

Bioethanol is particularly promising as a clean and sustainable fuel, produced biologically from sugars, starches, cellulose, and lignocellulosic feedstocks. A key challenge in bioethanol production is the availability of raw materials, leading to growing interest in agricultural residues and crop-based wastes [2]. Agricultural biomass provides an abundant renewable feedstock that can be used directly or blended with conventional fuels. In this study, pineapple waste—a byproduct of fruit processing rich in sucrose, glucose, and fructose—was utilized. Improper disposal of such waste raises water pH and biochemical oxygen demand (BOD), harming aquatic ecosystems and posing environmental and health risks.

Saccharomyces cerevisiae is widely applied in bioethanol production due to its ability to ferment hexoses, particularly glucose, into ethanol under anaerobic conditions. It achieves up to 95% of the theoretical yield and tolerates ethanol concentrations of about 150 g L^{-1} [3]. Furthermore, its resilience to fermentation inhibitors further supports its use, with S. cerevisiae var. ellipsoideus shown as highly effective for maize meal hydrolyzates [4].

Immobilising microbial cells offers significant technical and economic benefits over suspension cultures, including high cell density, easier recovery, reusability, and protection from harsh conditions [5]. This approach improves efficiency in continuous ethanol fermentation. This can be seen in study by Liu et al. [6] that showed ethanol yield up to 95.88% than other tested method. Various immobilisation techniques using yeast, such as gel entrapment, adsorption, and cross-linking, which enhance ethanol yield by protecting cells from toxic substrate and product concentrations. Polyvinyl alcohol (PVA) is widely used for microbial immobilisation due to its non-toxic, stable, and cost-effective properties. Common methods to form the PVA matrix include freezing-thawing, jet cutting, and cross-linking with UV or boric acid. Additionally, PVA–alginate beads can be further stabilised with sodium sulfate to enhance structural integrity and durability [7].

In this study, Design Expert software (DOE) was used to statistically optimise bioethanol production conditions using immobilised *Saccharomyces cerevisiae var.* ellipsoideus in a PVA-alginate-sulfate matrix. Key fermentation factors evaluated included temperature, pH, initial inoculum size, and agitation speed to enhance production efficiency.

2 Materials and Method

2.1 Materials

Saccharomyces cerevisiae var. *ellipsoideus* ATCC560 was obtained as a pellet from the American Type Culture Collection (University Blvd). It was cultured on Potato Dextrose Agar (PDA) from Oxoid. Liquid pineapple waste was sourced from Lee Pineapple Sdn. Bhd. Chemicals including Polyvinyl alcohol (PVA) (60,000 MW), and boric acid were purchased from Merck Germany, while sodium alginate from Fluka Chemie Switzerland, calcium chloride from GCE Laboratory Chemicals, and sodium sulfate were procured from R&M Marketing in Essex, U.K. Figure 1 shows the flowchart for experimental setup.

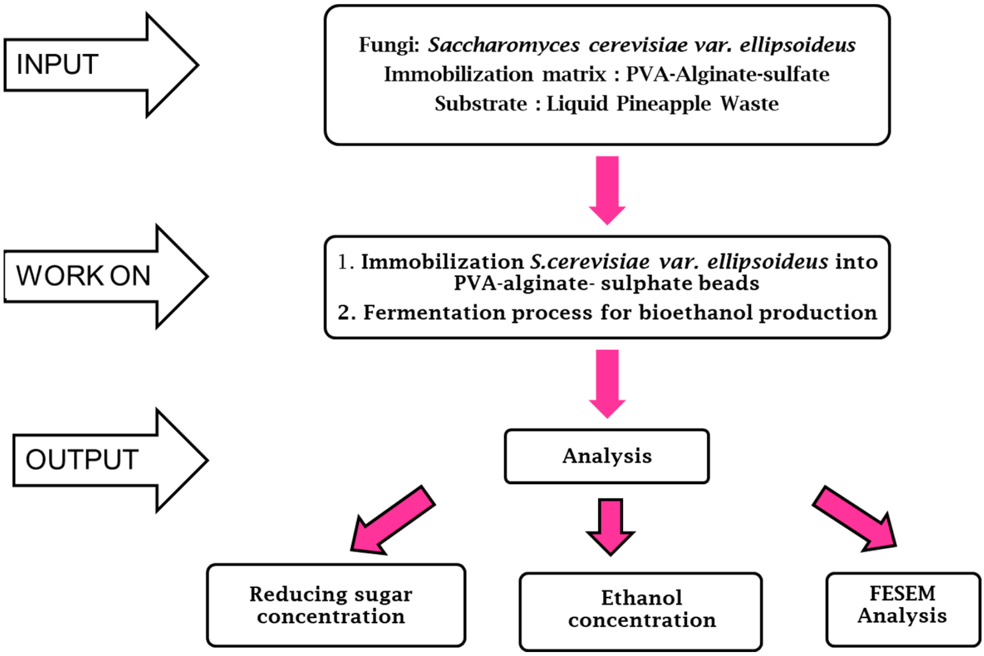

Fig. 1. Flowchart for the experimental setup.

2.2 Pretreatment of liquid pineapple waste

The liquid pineapple waste was boiled for 5 minutes to settle solid particles, then centrifuged at 4000 rpm for 15 minutes at 4 °C using a Sorvall® RC 5C Plus centrifuge. The supernatant was vacuum-filtered and sterilised with a 0.45 µm nylon filter. Before fermentation, the waste was frozen at -20 °C and thawed using a microwave oven to ensure consistency.

2.3 Immobilization of *Saccharomyces cerevisiae var. ellipsoideus*

A 12% (w/v) PVA and 1% (w/v) sodium alginate solution was prepared by mixing at 900 rpm and 80 °C until fully dissolved. After autoclaving, the solution was maintained at 50–70 °C to prevent solidification. Separately, 5.0% boric acid, 2.0% calcium chloride, and 10% boric acid solutions were also autoclaved. For immobilisation, 10 mL of *Saccharomyces cerevisiae* broth culture was mixed with 90 mL of the PVA-alginate solution. This mixture was slowly dropped into a 100 mL solution containing 5.0% boric acid and 2.0% calcium chloride using a 21G x 4 cm syringe needle to form beads. The beads were gently swirled for 30–50 minutes, then stored at 4 °C for 24 hours. Afterward, the beads were washed, treated in 10% boric acid for 30 minutes, followed by sodium sulfate for another 30 minutes, and finally stored in distilled water at 4 °C [7]. All steps were performed under sterile conditions.

2.4 Field emission scanning electron microscopy (FESEM) analysis

The PVA-alginate-sulfate beads were gently dried with tissue and sliced using a surgical knife to expose their cross-section. Mounted on sample stubs, the beads were coated four times with platinum using an Auto-Fine Coater JFC-1600 (Joel, USA). Cross-sectional morphology was examined using a ZEISS SUPRA 35 VP FESEM at 5000X magnification.

2.5 Experimental design for bioethanol production

A two-level, three-factor factorial design was employed to screen parameters influencing ethanol production, focusing on pH, temperature, agitation speed, and initial inoculum beads. Table 1 presents the low and high levels for each factor. Model validation involved randomly set conditions, and analyses were performed in triplicate. Table 2 lists the experimental runs suggested by the design.

Table 1. The low and high level used for screening.

Factors	Unit	Low level	High level
pH		4.5	5.5
Initial inoculums	g	3	6
Temperature	ºC	30	50
Agitation speed	rpm	100	200

Table 2. The Factorial Design for the experiment according to run.

Std	A	B	C	D
1	4.5	30	100	3
2	4.5	30	100	3
3	4.5	30	100	3
4	5.5	30	100	6
5	5.5	30	100	6
6	5.5	30	100	6
7	4.5	50	100	6
8	4.5	50	100	6
9	4.5	50	100	6
10	5.5	50	100	3
11	5.5	50	100	3
12	5.5	50	100	3
13	4.5	30	200	6
14	4.5	30	200	6
15	4.5	30	200	6
16	5.5	30	200	3
17	5.5	30	200	3
18	5.5	30	200	3
19	4.5	50	200	3
20	4.5	50	200	3
21	4.5	50	200	3
22	5.5	50	200	6
23	5.5	50	200	6
24	5.5	50	200	6
25	5	40	150	4.5
26	5	40	150	4.5
27	5	40	150	4.5
28	5	40	150	4.5
29	5	40	150	4.5

*(A = pH, B = Temperature, C = Agitation Speed and D = Initial Inoculum).

2.6 Bioethanol production

Batch fermentation was carried out in 250 mL Erlenmeyer flasks, each containing 100 mL of treated liquid pineapple waste. The process was tested at temperatures ranging from 30 °C to 50 °C, pH 4.5 to 5.5, and agitation speeds of 100–200 rpm. Each flask received 3 g of inoculum and 6 g of immobilised *Saccharomyces cerevisiae* var. *ellipsoideus* [8]. All experiments were conducted in triplicate. A control using untreated waste without yeast was included. Fermentation samples (5 mL) were taken every 12 hours and centrifuged at 4000 rpm for 15 minutes.

2.7 Determination of bioethanol and sugar concentration

Bioethanol concentration was determined using High-Performance Liquid Chromatography (HPLC) with a Rezex ROA-organic acid column (300 mm × 7.8 mm ID) and a refractive index (RI) detector. The eluent used was 0.005 N sulfuric acid, delivered at a flow rate of 0.6 mL/min, with the column maintained at 79.5 °C. A 10 µL aliquot of the filtered sample was injected, and peak heights or areas were compared with a standard ethanol solution to determine concentration.

Sugar concentration, including glucose, fructose, and sucrose, was measured based on the Official Method of Analysis (1984). HPLC analysis was conducted using a Rezex RCM-Monosaccharide column (300 mm × 7.8 mm ID) with an RI detector. The mobile phase was distilled water at 0.6 mL/min, with a column temperature of 75 °C. Prior to injection, samples were filtered through a 0.2 µm membrane filter. Calibration was done using standard sugar solutions.

2.8 Determination of reducing sugar concentration

To determine reducing sugar content, the 3,5-dinitrosalicylic acid (DNS) method was used, which detects free carbonyl groups by oxidizing aldehydes to carboxylic acids. A blank solution (1.0 mL distilled water) and standard glucose solutions (0.25–1.5 g/L) were prepared in labeled test tubes. Each tube received 1.0 mL DNS reagent and 2 mL distilled water, followed by heating in a boiling water bath for 5 minutes. After cooling, the mixtures were diluted to 10 mL with distilled water, mixed thoroughly, and their absorbance was measured at 540 nm. Reducing sugar concentrations were calculated using a standard glucose curve [9].

3 Results and Discussion

3.1 Morphology of the immobilized *Saccharomyces cerevisiae var. ellipsoideus* beads

FESEM analysis was used to examine cross-sections of PVA-alginate-sulfate beads. Figure 2(A) shows control beads without *Sacharomyces cerevisiae*, while Figure 2(B) displays the internal structure with immobilised *Sacharomyces cerevisiae* var. *ellipsoideus*, confirming successful entrapment within the matrix.

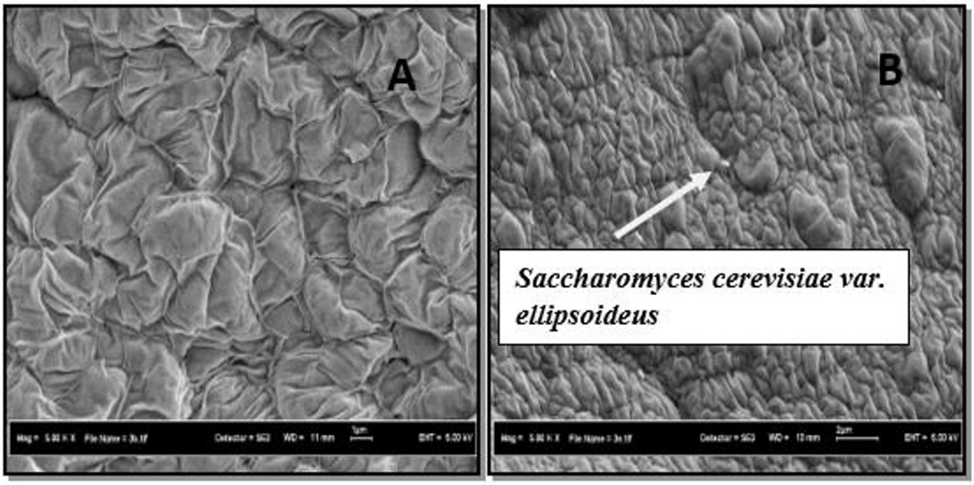

Fig. 2. FESEM images of PVA-Alginate-Sulfate beads' cross section with 5000X magnification for empty bead (A) and immobilized *Saccharomyces cerevisiae var. ellipsoideus* bead (B)

The similarity in results between this study and the work of Takei et al. [7] may be attributed to the comparable methodology employed. In their study, sodium sulfate concentrations ranging from 0.1 to 1.5 M were tested, with 0.5 M yielding optimal stability, low toxicity, and good biological activity. This result consistent with the concentration used in our research. However, a key distinction in our method is the post-immobilisation treatment of beads with 10% boric acid, which was not applied by them. Boric acid enhances the mechanical and chemical strength of the beads, improves biological activity, and increases product yield. Additionally, the incorporation of sodium alginate reduces molecular agglomeration and the tendency of PVA molecules to attract one another [8]. Sodium sulfate treatment further prevents bead dissolution in water by forming thiosulfate bonds with PVA ions and exhibits lower toxicity to yeast cells. The gelation process inside the beads completes after 24 hours of incubation. This improved method supports the successful immobilisation of *Saccharomyces cerevisiae* var. *ellipsoideus*, as confirmed by FESEM imaging in Figure 2.

3.2 Level factorial design data analysis

By using Design-Expert software, there were 29 runs generated including three replicates and centre point. Table 3 presents the concentrations of bioethanol, glucose, fructose, sucrose, and reducing sugars. The highest bioethanol yield was 16.6412 g/L (run 27), while the lowest was 0.2528 g/L (run 7).

Table 3. The factorial design for the experiment according to run.

Std	A	B	C	D	X1	X2	X3	X4	X5
1	4.5	30	100	3	4.125	1.331	12.393	2.020	0.226
2	4.5	30	100	3	4.192	1.353	12.408	2.010	0.220
3	4.5	30	100	3	4.122	1.450	12.537	2.112	0.225
4	5.5	30	100	6	2.953	3.090	2.537	2.282	0.057
5	5.5	30	100	6	2.806	3.248	2.351	2.341	0.057
6	5.5	30	100	6	2.934	3.112	2.361	2.205	0.057
7	4.5	50	100	6	0.253	70.756	51.495	2.413	0.375

Std	A	B	C	D	X1	X2	X3	X4	X5
8	4.5	50	100	6	0.277	70.247	51.443	2.408	0.377
9	4.5	50	100	6	0.284	70.382	51.440	2.402	0.383
10	5.5	50	100	3	6.326	24.603	31.922	5.387	2.349
11	5.5	50	100	3	6.558	24.621	31.862	5.315	2.340
12	5.5	50	100	3	6.496	24.468	31.811	5.328	2.344
13	4.5	30	200	6	3.726	0.330	0.747	0.534	0.353
14	4.5	30	200	6	3.638	0.328	0.713	0.554	0.352
15	4.5	30	200	6	3.514	0.313	0.700	0.538	0.352
16	5.5	30	200	3	10.030	3.850	3.763	2.459	0.056
17	5.5	30	200	3	10.020	3.758	3.504	2.601	0.048
18	5.5	30	200	3	10.085	3.965	3.034	2.564	0.047
19	4.5	50	200	3	0.458	22.030	23.935	8.008	0.772
20	4.5	50	200	3	0.440	22.067	24.220	8.075	0.777
21	4.5	50	200	3	0.457	22.079	23.608	8.004	0.775
22	5.5	50	200	6	1.501	39.643	22.303	3.152	1.470
23	5.5	50	200	6	1.509	39.317	22.127	3.001	1.475
24	5.5	50	200	6	1.5007	39.172	22.469	3.154	1.479
25	5	40	150	4.5	16.613	2.269	2.561	8.591	0.604
26	5	40	150	4.5	16.540	2.433	2.232	8.300	0.608
27	5	40	150	4.5	16.641	2.170	2.672	8.489	0.613
28	5	40	150	4.5	16.532	2.378	2.451	8.511	0.604
29	5	40	150	4.5	16.632	2.250	2.354	8.231	0.614

*(A = pH, B = Temperature, C = Agitation Speed and D = Initial Inoculum)
*X1 = Ethanol Concentration g/L, X2= Glucose Concentration g/L, X3= Fructose Concentration g/L, X4 = Sucrose Concentration g/L, X5 = Reducing sugar g/L.

The results in Table 3 were analysed using Analysis of Variance (ANOVA), along with diagnostic and model graphs suited to the experimental design. ANOVA assesses variability within the experiment to identify significant factors. The model was found to be statistically significant, with a *p*-value (Prob > F) less than 0.05, indicating strong model reliability. The ANOVA also highlighted the significance of individual factors. Main effects with *p*-values less than 0.01 demonstrated high significance. This confirms that all tested variables—pH (A), temperature (B), agitation speed (C), and initial inoculum of immobilised beads (D)—had significant factorial impacts on the measured responses. Table 4 summarises the ANOVA results for ethanol concentration, as well as glucose, fructose, sucrose, and reducing sugar concentrations, supporting the statistical validity of the model and the importance of each factor in influencing bioethanol production outcomes.

Table 4. ANOVA table for all responses.

Response Model	Ethanol	Glucose	Fructose	Sucrose	Reducing Sugar
F-test	7996.13	1.007E +005	27119.51	2313.57	1.352E+005
Prob> F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Significant interaction Effect	A, B, C, D, AB, AC, AD	A, B, C, D, AB, AC, AD	A, B, C, D, AB, AC, AD	B, C, D, AB, AC, AD	A, B, C, D, AB, AC, AD

The experimental data were further validated using the R-squared (R^2) value, which estimates the proportion of total variation in the data explained by the model. An R^2 value close to +1 indicates a good fit, confirming that the model accurately represents the experimental results and is suitable for prediction purposes [10]. Additionally, Adequate Precision (Adeq. Precision) measures the signal-to-noise ratio, where values greater than 4 are considered desirable, indicating sufficient model discrimination. Table 5 presents the R^2 and Adequate Precision values for all responses, supporting the model's reliability and accuracy.

Table 5. R-squared value for all responses.

	R-squared	Adj R-squared	Pred R-squared	Adeq precision
Ethanol	0.9996	0.9995	0.9998	459.456
Glucose	1.0000	1.0000	0.9999	939.077
Fructose	0.9999	0.9999	0.9998	494.446
Sucrose	0.9986	0.9982	0.9987	168.269
Reducing Sugar	1.0000	1.0000	1.0000	1078.762

The empirical models for ethanol, glucose, fructose, sucrose, and reducing sugar concentrations are crucial for model validation. These models, expressed in terms of actual variables, are presented in Table 6 for reference and further analysis.

Table 6. The empirical model for all the responses.

Responses	Equations
Ethanol	- 24.63495 + 7.68640 * pH – 0.40907 * temp - 0.058676* agitation + 8.43565 * initial inoculum + 0.051742* pH * temp + 0.012661* pH * agitation – 1.90054 * pH * initial inoculum
Glucose	-84.87397 + 4.26504 * pH + 6.08259 * temp -1.70931 * agitation + 32.82304 * initial inoculum - 0.84714 * pH * temp + 0.32489 * pH * agitation - 5.54038 * pH * initial inoculum
Fructose	-38.36597 + 3.52817 * pH + 3.11913 * temp - 0.88745 * agitation + 22.43363 * initial inoculum - 0.34750 * pH * temp + 0.15358 * pH * agitation - 4.39959 * pH * initial inoculum
Sucrose	-26.52090 + 5.37626 * pH + 0.67066 * temp + 0.15759 * agitation - 4.65571 * initial inoculum - 0.10545 * pH * temp - 0.030448 * pH * agitation + 0.77057 * pH * initial inoculum
Reducing Sugar	+4.55264 - 1.08675 * pH - 0.33818 * temp + 0.034191 * agitation + 0.40162 * initial inoculum + 0.078355 * pH * temp - 7.01359E-003 * pH * agitation - 0.099184 * pH * initial inoculum

3.3 Diagnostics of the results obtained using two level factorial designs

Figure 3 (A–E) presents the normal plots of residuals for ethanol, glucose, fructose, sucrose, and reducing sugar concentrations. The residual points closely follow a straight line, indicating that the errors are normally distributed [11]. This supports the validity and reliability of the model used in predicting the experimental outcomes.

 https://doi.org/10.1051/bioconf/202518901021

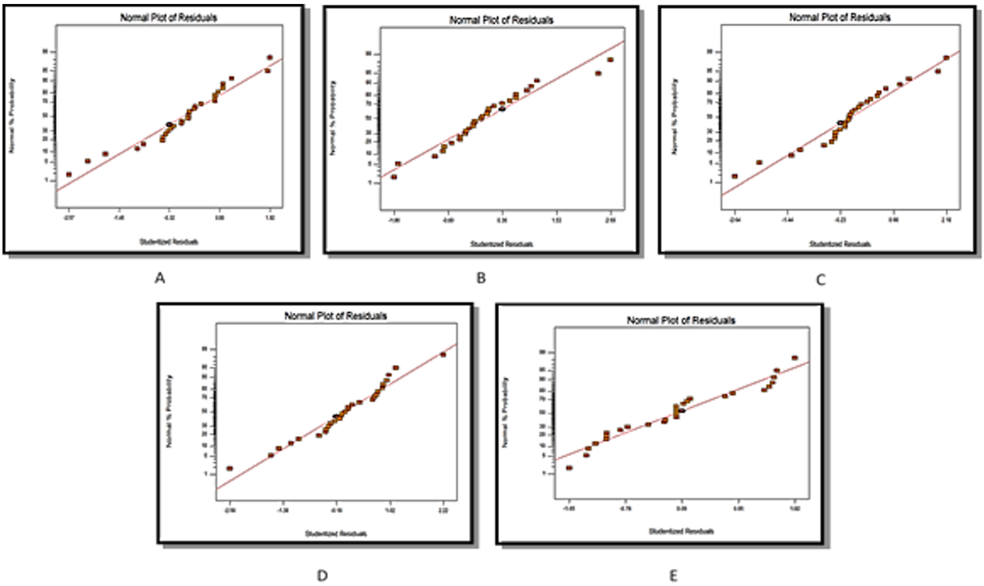

Fig. 3. Normal plot of residuals for all responses A) ethanol concentration, B) glucose concentration, C) fructose concentration, D) sucrose concentration, and E) reducing sugar concentration.

Figure 4 shows the residuals versus predicted values for ethanol, glucose, fructose, sucrose, and reducing sugar concentrations. The residuals are randomly scattered with no clear trend or unusual structure across all responses (A to E), indicating constant variance and confirming the adequacy and reliability of the model proposed through ANOVA.

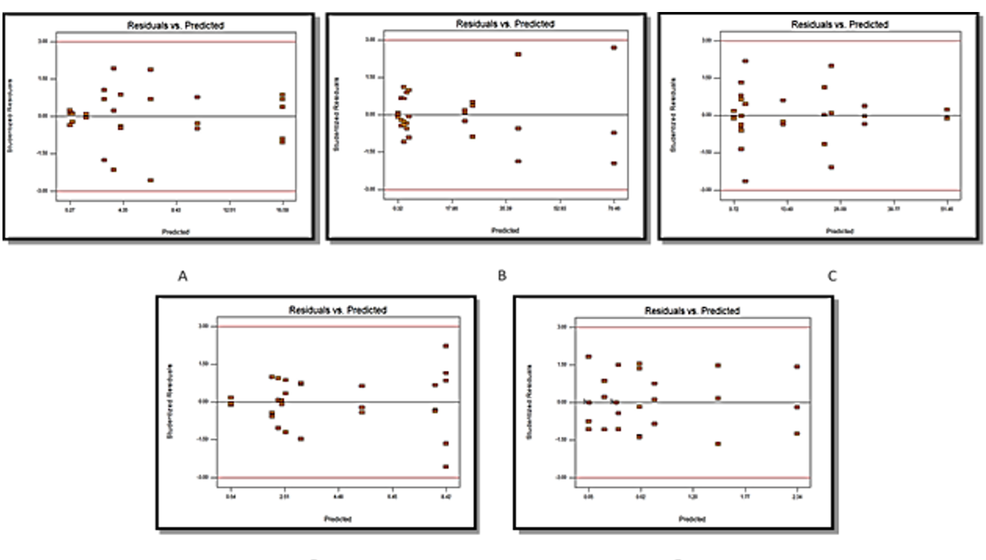

Fig. 4. The diagnostics graph of residuals versus the predicted value, for A) ethanol, B) glucose, C) fructose, D) sucrose, and E) reducing sugar concentration.

Figure 5 displays the outlier T graph, where all data points fall within the baseline range, indicating the absence of outliers. This clear distribution further confirms the model's consistency and accuracy. Additionally, the graph supports the adequacy of the ANOVA-proposed model, reinforcing its reliability for predicting experimental responses.

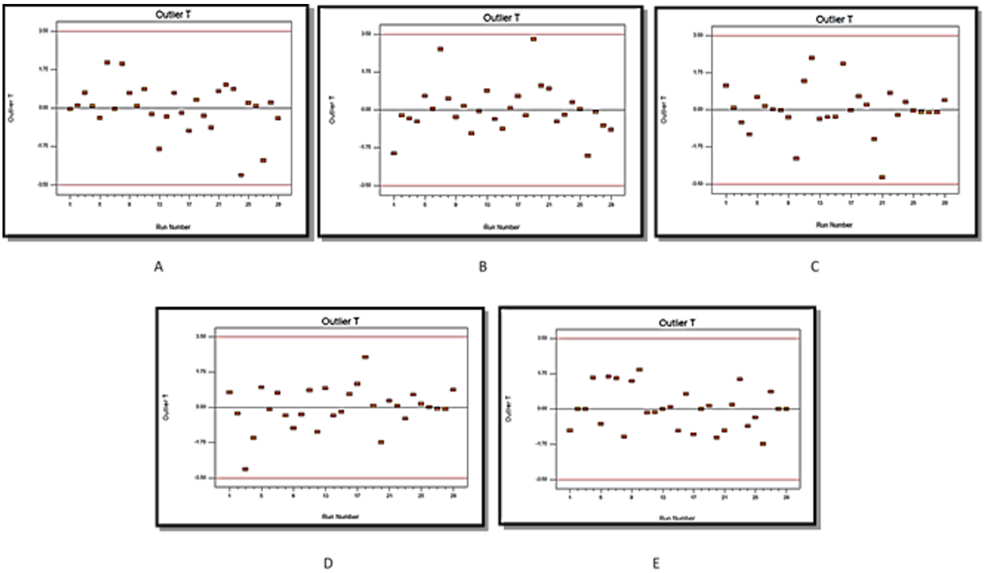

Fig. 5. The diagnostics graph of outlier T graph for A) ethanol, B) glucose, C) fructose, D) sucrose, and E) reducing sugar concentration.

3.4 Factors that influence the bioethanol concentration

The factors significantly influencing ethanol concentration are illustrated in Figure 6 (A–C), each representing interaction effects between key parameters. Figure 6(A) displays the interaction between pH and temperature. At both 30 °C and 50 °C, ethanol concentration increases with higher pH values. This observation aligns with findings by Orij et al. [12], who reported that cell replication activity decreases linearly with declining intracellular pH. The enhanced ethanol production at higher pH may be attributed to the absence of weak acids and a higher proton gradient across the plasma membrane, facilitating better fermentation conditions [13]. Figure 6(B) illustrates the interaction between pH and agitation speed. The graph suggests a slight positive relationship, although changes in agitation speed (100 rpm and 200 rpm) did not significantly affect ethanol output. Liu and Shen [14] noted that excessively high agitation speeds may not enhance ethanol production due to the metabolic limitations of yeast cells, which may become stressed under such conditions.

Figure 6(C) shows the interaction between pH and initial inoculum size. At 3 g of immobilised beads, ethanol concentration increases with rising pH. However, at 6 g, no significant difference is observed across the pH range. This may be due to the relationship between fermentation pH and cell growth, particularly in sugar hydrolysates containing undissociated weak acids at lower pH (4.5–5.5) [15]. Elevated fermentation pH (≥6.5), however, can result in reduced ethanol yields due to bacterial contamination, which competes with yeast for available sugars, thereby compromising fermentation efficiency.

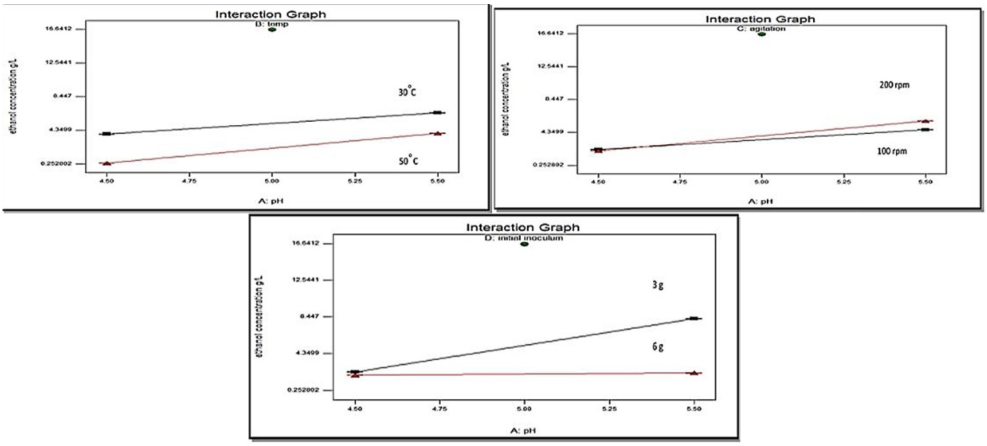

Fig. 6. Interaction graphs A) pH and temperature, B) pH and agitation speed, and C) pH and initial inoculums that influenced ethanol concentration.

3.5 Optimal value by numerical optimization for each variable

Figure 7 presents the optimal values for each variable, as determined through numerical optimisation using Design-Expert software. Based on the 2-level factorial design, the ideal fermentation conditions were identified as pH 5.50, temperature 30 °C, agitation speed 200 rpm, and an initial inoculum of 3 g immobilised beads. Under these optimised conditions, the maximum predicted ethanol concentration was 10.043 g/L, indicating efficient bioethanol production potential.

Fig. 7. Optimal operating conditions suggested by Numerical Optimization of Design-Expert Software.

3.6 Confirmation run

An experiment was conducted to validate the adequacy of the model developed from the screening phase. The confirmation results are shown in Table 7. The validation experiment was performed using the same conditions identified earlier: pH 5.50, temperature 30 °C, agitation speed 200 rpm, and 3 g of immobilised yeast inoculum. The experimental outcomes closely matched the model's predicted values, with an error margin of less than 5%, indicating that the developed factorial model is reliable and accurately reflects actual fermentation performance.

Table 7. Confirmation runs results.

Responses	Predicted Values	Actual values	% Error
Ethanol (g/L)	10.0438	9.7025	3.40
Glucose (g/L)	3.87319	3.23742	3.50
Fructose (g/L)	3.44796	3.67142	2.22
Sucrose (g/L)	2.54238	2.50154	1.63
Reducing Sugar	0.0514669	0.05021	2.44

4 Conclusion

Bioethanol production was carried out using the direct utilisation of liquid pineapple waste by immobilised *Saccharomyces cerevisiae* var. *ellipsoideus* in a modified PVA-alginate-sulfate matrix under batch fermentation conditions. The successful immobilisation of the yeast cells was confirmed through Field Emission Scanning Electron Microscopy (FESEM) micrographs, which revealed their entrapment within the matrix structure. Optimisation of the fermentation parameters was performed using a two-level, three-factor factorial design. The results identified the optimal conditions for maximum bioethanol yield: a temperature of 30 °C, agitation speed of 200 rpm, pH 5.5, and an initial inoculum size of 3.0 g of immobilised beads. This study demonstrates the potential of converting agricultural residues into biofuels, providing a sustainable waste management solution while supporting renewable energy initiatives. In the context of Malaysia and other ASEAN countries, these findings provide guidance for integrating bioethanol production into national and regional energy policies, reducing fossil fuel dependence, lowering greenhouse gas emissions, and promoting circular economy practices aligned with sustainable development goals.

Acknowledgments

This work was supported by Ministry of Higher Education (MOHE) and Universiti Teknologi Malaysia for financial in Geran Universiti Penyelidikan (GUP) under vote 02J29.

References

1. Wang, J., & Azam, W. (2024). Natural resource scarcity , fossil fuel energy consumption , and total greenhouse gas emissions in top emitting countries. Geoscience Frontiers, 15(2), 101757. https://doi.org/10.1016/j.gsf.2023.101757
2. Saleem, M. (2022). Possibility of utilizing agriculture biomass as a renewable and sustainable future energy source. Heliyon, 8, e08905. https://doi.org/10.1016/j.heliyon.2022.e08905

3. Caspeta, L., Castillo, T., & Nielsen, J. (2015). Modifying Yeast Tolerance to inhibitory Conditions of ethanol Production Processes. Frontiers in Bioengineering and Biotechnology, 3(184r), 1–15. https://doi.org/10.3389/fbioe.2015.00184
4. Rakin, M., Mojovic, L., Nikolic, S., Vukasinovic, M., & Nedovic, V. (2009). Bioethanol production by immobilized Sacharomyces cerevisiae var . ellipsoideus cells. African Journal of Biotechnology, 8(3), 464–471.
5. Takei, T., Ikeda, K., Ijima, H., & Kawakami, K. (2011). Fabrication of poly (vinyl alcohol) hydrogel beads crosslinked using sodium sulfate for microorganism immobilization. Process Biochemistry, 46(2), 566–571. https://doi.org/10.1016/j.procbio.2010.10.011
6. Liu, Q., Zhao, N., Zou, Y., Ying, H., Liu, D., Chen, Y. (2020). Feasibility study on long-term continuous ethanol production from cassava supernatant by immobilized yeast cells in packed bed reactor. Journal of Microbiology and Biotechnology, 30(8): 1227-1234. https://doi.org/10.4014/jmb.1908.08017
7. Zain, N. A. M., Suhaimi, M. S., & Idris, A. (2011). Development and modification of PVA – alginate as a suitable immobilization matrix. Process Biochemistry, 46(11), 2122–2129. https://doi.org/10.1016/j.procbio.2011.08.010
8. Zain, N. A. M., Suhaimi, M. S., & Idris, A. (2010). Hydrolysis of liquid pineapple waste by invertase immobilized in PVA – alginate matrix. Biochemical Engineering Journal, 50(3), 83–89. https://doi.org/10.1016/j.bej.2010.02.009
9. Miller, G. L. (1959). Use of DinitrosaIicyIic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry, 31(3), 426–428. https://doi.org/10.1021/ac60147a030
10. Chicco, D., Warrens, M. J., & Jurman, G. (2021). The coefficient of determination R-squared is more informative than SMAPE, MAE, MAPE, MSE and RMSE in regression analysis evaluation. PeerJ Computer Science, 7, 1–24. https://doi.org/10.7717/peerj-cs.623
11. Gottipati, R., & Mishra, S. (2010). Process optimization of adsorption of Cr (VI) on activated carbons prepared from plant precursors by a two-level full factorial design. Chemical Engineering Journal, 160(1), 99–107. https://doi.org/10.1016/j.cej.2010.03.015
12. Orij, R., Urbanus, M. L., Vizeacoumar, F. J., Giaever, G., Boone, C., Nislow, C., Brul, S., & Smits, G. J. (2012). Genome-wide analysis of intracellular pH reveals quantitative control of cell division rate by pHc in Saccharomyces cerevisiae. Genome Biology, 13, 1–15. https://doi.org/10.1186/gb-2012-13-9-r80
13. Yang, Q., Yang, Y., Tang, Y., Wang, X., Chen, Y., Shen, W., Zhan, Y., Gao, J., Wu, B., He, M., Chen, S., & Yang, S. (2020). Development and characterization of acidic-pH-tolerant mutants of Zymomonas mobilis through adaptation and next-generation sequencing-based genome resequencing and RNA-Seq. Biotechnology for Biofuels, 13(144), 1–17. https://doi.org/10.1186/s13068-020-01781-1
14. Liu, R., & Shen, F. (2008). Impacts of main factors on bioethanol fermentation from stalk juice of sweet sorghum by immobilized Saccharomyces cerevisiae (CICC 1308). Bioresource Technology, 99, 847–854. https://doi.org/10.1016/j.biortech.2007.01.009
15. Felipe, M. G. A., Vitolo, M., Mancilha, I. M., & Silva, S. S. (1997). Fermentation of sugar cane bagasse hemicellulosic hydrolysate for xylitol production: Effect of pH. Biomass and Bioenergy, 13(97), 11–14. https://doi.org/10.1016/S0961-9534(97)00032-9