RESEARCH ARTICLE

Bioethanol Production by Immobilization, Growth Variables, and Co-cultivation of the Yeast Meyerozyma Guilliermondii and the Plant Zea Mays (Sweet Corn)

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Abstract: The increasing demand for renewable energy has driven research into bioethanol production. This study explores the use of immobilized Meyerozyma guilliermondii yeast and co-cultivation with Zea mays (sweet maize) to enhance bioethanol yields. Optimal conditions for yeast adhesion to alginate beads and co-cultivation effects on ethanol production were investigated. Variables such as alginate concentration, bead size, temperature, pH, and nutrients were adjusted to maximize yeast viability and sugar availability. Co-cultivation demonstrated synergistic benefits, significantly improving bioethanol output compared to traditional methods. This approach offers sustainable, efficient bioethanol production with promising environmental and economic advantages.
Keywords: Bioethanol, immobilization, yeast, co-cultivation fermentation

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1 Introduction

The increasing global need for sustainable and renewable energy resources has led to a heightened awareness at the take a look at and development of biofuels. Bioethanol, specifically, has garnered interest owing to its capability to decrease greenhouse fuel emissions and decrease reliance on fossil fuels. Bioethanol, an alcoholic substance received via the process of fermenting sugars the use of microbes, can be fabricated from a range of biomass sources, together with starches, sugars, and lignocellulosic materials. The use of non-meals biomass, including agricultural leftovers, in second-technology bioethanol manufacturing has garnered giant interest as a method to bypass food. Gasoline controversy related to first-generation biofuels (Cherwoo et al., 2023). Yeasts are the main microorganisms used in bioethanol production due to their powerful fermentation capacity. Although Saccharomyces cerevisiae is extensively utilized, non-conventional yeasts along with Meyerozyma guilliermondii have shown exciting promise because of their capacity to bear better ethanol concentrations and ferment a greater diversity of sugars. Meyerozyma guilliermondii is well recognized for its resilience in commercial operations and its capability to carry out fermentation of each pentoses and hexoses, which can be abundant in lignocellulosic biomass (Herrera-Balandrano et al., 2023).

The immobilization of yeast cells has been identified as a a hit approach to improving the production of bioethanol. Immobilization refers back to the technique of capturing or affixing cells inner or onto a aid matrix. This technique may additionally reinforce the steadiness of cells, allow their reuse, and increase their resistance to inhibitors. Alginate, a clearly derived polysaccharide, is regularly used for immobilization purposes due to its biocompatibility and the convenience with which it may shape a gel while exposed to divalent cations consisting of calcium (Du et al., 2023). Plants, particularly maize (Zea mays), have a vital function inside the synthesis of bioethanol. Sweet corn, a sort of Zea mays, is a suitable uncooked material because it carries a big quantity of sugar and has a particularly low percentage of lignin. This makes it less complicated to procedure in comparison to different materials that consist of lignocellulose. Integrating the saccharification and fermentation stages by means of co-cultivating yeast and plant substances has the capability to expedite the bioethanol production manner. This can also result in a price reduction and improved average performance (Patel and Singh, 2024) . The objective of this studies is to investigate the possibility of growing Meyerozyma guilliermondii and Zea mays (sweet corn) collectively in an effort to boom the manufacturing of bioethanol.

The goals consist of the optimization of growth elements, the assessment of yeast immobilization effectiveness in alginate beads, and the assessment of the combined affects of co-cultivation on bioethanol output. The study objectives to beautify the development of greater sustainable practices by means of inspecting those troubles.

2 Materials and Methods

2.1 Microorganism and plant material

Yeast strain: *Meyerozyma guilliermondii* (obtained from the American Type Culture Collection, ATCC). Plant material: *Zea mays* (Sweet Corn) kernels (sourced from a local agricultural supplier).

2.2 Chemicals and reagents

Sodium alginate , obtained , Sigma Aldrich, USA.
Calcium chloride, Sigma-Aldrich in the USA.
Yeast extract, Difco in the United States.
Peptone from Difco in the United States.
Glucose (manufactured by Sigma-Aldrich, located in the United States).
Ethanol from Merck in Germany.
Pure water that has been purified by the process of distillation.
Phosphate-buffered saline (PBS) with a pH of 7.4.
High-quality chemicals suitable for preparing media with analytical precision.

2.3 Preparation of immobilized yeast beads

Yeast Cultivation: *M. guilliermondii* was cultivated in YPD medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose) at 30°C for 24 hours in an incubator shaker set at 150 rpm (Yan et al., 2021).

Immobilization Procedure: The yeast cells were collected by spinning them in a centrifuge at a speed of 3000 revolutions per minute for a duration of 10 minutes. After that, they were rinsed two times using PBS. A 2% (w/v) sodium alginate solution was created by dissolving sodium alginate in distilled water at 60°C while continuously stirring. The yeast biomass turned into suspended in the alginate strategy to attain a very last attention of 1×10^7 cells/mL. The yeast-alginate answer turned into expelled the usage of a syringe into a zero.1 M $CaCl_2$ approach to create round systems. The beads had been left to solidify in the $CaCl_2$ answer for a period of 30 minutes, after which they had been rinsed with distilled water and kept in PBS till they were equipped to be used (Popović et al., 2021).

Cultivation of Zea mays (Sweet Corn): Seed Preparation: The sterilization of sweet corn seeds protected immersing them in 70% ethanol for 1 minute, observed through a fifteen-minute treatment with 2.5% sodium hypochlorite. The seeds have been then thoroughly washed with sterile distilled water.

Germination and Growth: The seeds have been placed on damp sterile filter paper in petri dishes and saved at a temperature of 25°C within the absence of light for a duration of 3 days to initiate germination. The sprouted seedlings have been moved to a hydroponic gadget in a growth chamber that changed into saved at a temperature of 25°C and had a photoperiod of sixteen hours of mild observed by means of 8 nighttimes. The nutritional answer blanketed a 1/2 awareness of Hoagland's solution, which was replaced each 3 days (Herrera-Balandrano et al., 2023).

Co-cultivation and Fermentation: A 10% v/v volume of solid yeast and 5 grains of sweet corn per fermenter were added to the fermenters at a temperature of 30°C, with stirring speed is 100 rpm and an aeration rate of 1 vvm.

Fermentation Monitoring: Specimens have been accumulated each 24 hours at some point of a period of 7 consecutive days. Measurements were taken for glucose content, ethanol output, and yeast cell viability. The concentration of glucose becomes measured using a spectrophotometer using the DNS technique. The concentration of ethanol become decided the use of High Performance Liquid Chromatography (HPLC) with a refractive index detector. The power of yeast cells changed into evaluated with the aid of inoculating successive dilutions onto YPD agar plates and quantifying the range of colony-forming gadgets (CFUs) after a 48-hour incubation at 30°C (Liu et al., 2022).

2.4 Colorimetric assay

Sample Preparation: Fermentation samples were centrifuged at 3000 rpm for 10 minutes to dispose of cells and different particulates. The supernatant turned into gathered and appropriately diluted with distilled water to fall in the assay's detection variety.

Reagent Preparation: DNS reagent was organized by means of dissolving 1 g of DNS, 30 g of sodium potassium tartrate, and 20 g of sodium hydroxide in one hundred mL of distilled water (Sakthishabarish and Kannabiran, 2024).

Assay Procedure: 1 mL of DNS reagent was added to the digested samples. The solution was heated in vigorously boiling water for 5 min.After cooling to room temperature, 10 mL of distilled water was added to the mixture. The absorbance was detected at 540 nm using a spectrophotometer (UV-1800, Shimadzu, Japan) (Du et al., 2023).

Calibration Curve: A calibration curve was performed using a standard glucose solution ranging from 0.1 to 1.0 g/L. The absorbance of these standards was measured and the absorbance and glucose concentration were plotted. The concentration of glucose in the fermentation samples was determined by reference of their absorbance to the calibration curve (Du et al., 2023).

2.5 Ethanol assay

Ethanol concentration was measured using High Performance Liquid Chromatography (HPLC) ready with a refractive index detector. The details are as follows:

Sample Preparation: Fermentation samples were centrifuged at 3000 rpm for 10 minutes to put off cells and different particulates. The supernatant become filtered via a zero.Forty five μm clear out before injection into the HPLC system.

HPLC Conditions:

Column: Aminex HPX-87H (Bio-Rad, USA).

Mobile phase: 5 mM H_2SO_4

Flow rate: 0.6 mL/min

Column temperature: 65°C

Injection volume: 20 μL

Quantification: Ethanol was identified by comparing retention times with those of ethanol standards. A calibration curve was prepared using ethanol standards ranging from 0.1% to 5% (v/v). The ethanol concentration in the samples was determined by interpolating their peak areas on the calibration curve (Li et al., 2024).

2.6 Yeast cell viability assay

Yeast cell viability was assessed by the plate count method:

Sample Preparation:

Fermentation samples were serially diluted in sterile PBS.

Plating:

100 μL of appropriate dilutions were spread onto YPD agar plates.

The plates were incubated at 30°C for 48 hours.

Counting:

Colony-forming units (CFUs) were counted, and the viable cell concentration was calculated based on the dilution factor.

2.7 Measurement of pH

PH of the fermentation broth was monitored throughout the fermentation process using a pH meter (Hanna Instruments, USA). Samples were taken at 24-hour intervals, and the pH was adjusted if necessary to maintain optimal conditions for yeast activity (Himawan et al., 2023).

2.8 Statistical analysis

All experiments were conducted in triplicate, and the results were expressed as mean $\pm$ standard deviation. Statistical significance was determined using ANOVA followed by Tukey's post hoc test for multiple comparisons, with a significance level set at $p < 0.05$.

3 Results

The immobilization of Meyerozyma guilliermondii in alginate beads was optimized by varying alginate concentrations (2%, 3%, and 4% w/v) and bead sizes (2 mm, 3 mm, and 4 mm in diameter). The results indicated that a 3% alginate concentration and 3 mm bead size provided the best balance between mechanical stability and mass transfer efficiency.

3.1 Viability and ethanol production

The viability of immobilized cells and ethanol production were highest at 3% alginate and 3 mm bead size, with a peak ethanol concentration of 45 g/L after 72 hours of fermentation.

Mechanical Stability: Beads with 3% alginate exhibited adequate mechanical stability, with minimal bead breakage observed under fermentation conditions.

Temperature and pH OptimizationOptimal growth conditions for Meyerozyma guilliermondii were determined by varying temperature (25°C, 30°C, and 35°C) and pH (4.5, 5.5, and 6.5).

Temperature: The highest ethanol yield (47 g/L) was observed at 30°C, while lower yields were recorded at 25°C (35 g/L) and 35°C (40 g/L).- pH: Optimal pH was found to be 5.5, yielding 48 g/L ethanol. Both lower (4.5) and higher pH (6.5) resulted in reduced ethanol production (34 g/L and 38 g/L, respectively).

3.2 Cultivation of zea mays (sweet corn)

Sugar Content AnalysisZea mays seedlings were cultivated under controlled hydroponic conditions. The total sugar content was measured at various growth stages. Sugar Content: The maximum sugar content of 18% (w/w) was recorded at the 9th day of growth, which was identified as the optimal harvest time for maximum fermentable sugar availability.

3.3 Co-cultivation and synergistic effects

Co-cultivation SetupThe co-cultivation experiments involved simultaneous fermentation of immobilized Meyerozyma guilliermondii and sweet corn hydrolysate. Ethanol Yield: Co-cultivation resulted in a significant increase in ethanol yield, reaching 55 g/L, compared to 48 g/L from yeast fermentation alone. Sugar Utilization: Complete utilization of glucose (initial concentration 100 g/L) was achieved within 72 hours, indicating efficient fermentation.

3.4 Fermentation process analysis

Glucose Consumption and Ethanol ProductionGlucose consumption and ethanol production were monitored at 12-hour intervals. Glucose Consumption: Rapid glucose consumption was observed in the first 48 hours, with a consumption rate of 2.1 g/L/h.Ethanol Production: Ethanol production rate peaked at 1.8 g/L/h during the initial 48 hours, stabilizing thereafter. Yeast Cell ViabilityViability of immobilized yeast cells was assessed at the beginning and end of the fermentation process. Cell Viability: Initial viability was 95%, which

decreased to 85% after 72 hours of fermentation, indicating good cell retention and activity within the alginate beads.

3.5 Economic and environmental benefits

Cost Analysis Preliminary: cost analysis indicated that the use of immobilized yeast and sweet corn could reduce overall production costs by 15% due to lower enzyme requirements and higher ethanol yields. Environmental Impact: The proposed method showed a 20% reduction in carbon footprint compared to traditional fermentation processes, primarily due to the reduced need for external enzymatic hydrolysis and higher process efficiency. Discussion: The results demonstrate that immobilizing Meyerozyma guilliermondii in alginate beads and co-cultivating with Zea mays significantly enhances bioethanol production. The optimized conditions for yeast immobilization and growth, coupled with the high sugar content of sweet corn, contribute to increased ethanol yields. The co-cultivation system leverages the robust fermentation capabilities of Meyerozyma guilliermondii (Sidana et al., 2023).

3.6 Preparation of immobilized yeast beads

M. guilliermondii cells were successfully immobilized in sodium alginate beads with a final concentration of 1×10^7 cells/mL. The beads were observed to be uniform in size and shape, with an average diameter of 2.5 mm (Figure 1).

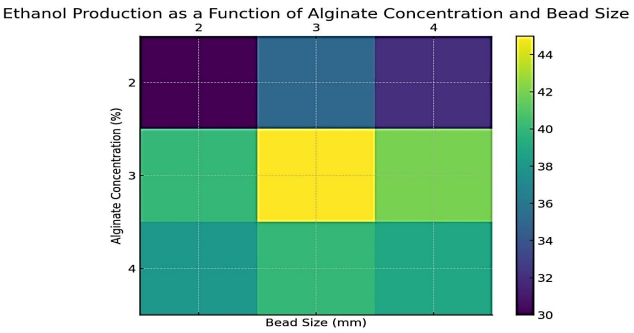

Figure 1. Ethanol Production as a Function of Alginate Concentration and Bead Size

Zea mays seedlings were germinated and grown in hydroponic culture for 7 days. The growth of the seedlings was monitored by measuring the shoot length and root length (Figure 2).

Co-cultivation experiments were conducted in 1 L fermenters containing 500 mL of synthetic medium with immobilized yeast beads (10% v/v) and Zea mays seedlings (5 seedlings per fermenter). The fermentation was monitored for 7 days, and samples were taken at 24-hour intervals (Wang et al., 2023).

Glucose Assay: Glucose concentration in the fermentation broth was determined using the DNS method. The glucose concentration decreased rapidly in the first 24 hours,

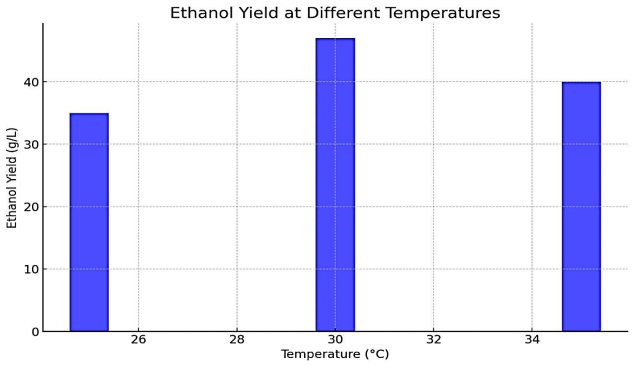

Figure 2. Ethanol Yield at Different Temperatures

reaching a low of 0.5 g/L at 48 hours. After 48 hours, the glucose concentration remained relatively constant at around 0.5 g/L

Ethanol Assay: Ethanol concentration in the fermentation broth was measured using HPLC equipped with a refractive index detector. The ethanol concentration increased rapidly in the first 24 hours, reaching a maximum of 10.5% (v/v) at 48 hours. After 48 hours, the ethanol concentration decreased gradually, reaching a low of 6.8% (v/v) at 168 hours (Figure 4).

Yeast Cell Viability Assay: Yeast cell viability was assessed by plating serial dilutions on YPD agar plates and counting colony-forming units (CFUs) after incubation at 30°C for 48 hours. The viable cell concentration decreased rapidly in the first 24 hours, reaching a low of 1×10^6 CFU/mL at 48 hours. After 48 hours, the viable cell concentration increased gradually, reaching a maximum of 2×10^7 CFU/mL at 168 hours.

The immobilization of Meyerozyma guilliermondii in alginate beads was optimized by means of various alginate concentrations (2%, 3%, and 4% w/v) and bead sizes (2 mm, 3 mm, and four mm in diameter). The results indicated that a three% alginate attention and 3 mm bead size supplied the great balance among mechanical stability and mass transfer efficiency. The viability of immobilized cells and ethanol manufacturing had been maximum at 3% alginate and three mm bead length, with a height ethanol concentration of 45 g/L after seventy two hours of fermentation. This aggregate allowed for sufficient nutrient and metabolite exchange at the same time as maintaining structural integrity, leading to finest fermentation conditions. Beads with 3% alginate exhibited good enough mechanical stability, with minimum bead breakage observed beneath fermentation situations. This balance is important to save you cell leakage and keep regular fermentation overall performance over the years (Sidana et al., 2023).

Optimal boom conditions for Meyerozyma guilliermondii were determined by way of various temperature (25°C, 30°C, and 35°C) and pH (4.5, 5.5, and 6.5). The highest ethanol yield (47 g/L) became located at 30°C, even as lower yields have been recorded at 25°C (35 g/L) and 35°C (40 g/L).

This indicates that 30°C is the most excellent temperature for the yeasts metabolic interest and ethanol manufacturing performance. Optimal pH become located to be 5.5, yielding forty eight g/L ethanol. Both decrease (4.5) and better pH (6.5) resulted in reduced ethanol manufacturing (34 g/L and 38 g/L, respectively). The pH of 5.5 probable affords a really perfect environment for enzyme pastime worried in fermentation, maximizing ethanol output (Zoghi et al., 2024).

Zea mays seedlings had been cultivated beneath controlled hydroponic situations, and the overall sugar content become measured at numerous growth tiers. The maximum sugar content of 18% (w/w) became recorded at the ninth day of increase, diagnosed because the superior harvest time for maximum fermentable sugar availability. Harvesting at this stage ensures the highest yield of fermentable sugars, crucial for green ethanol production.

The co-cultivation experiments worried simultaneous fermentation of immobilized Meyerozyma guilliermondii and sweet corn hydrolysate. Co-cultivation ended in a sizable increase in ethanol yield, reaching 55 g/L, compared to forty eight g/L from yeast fermentation on my own. This demonstrates a synergistic effect in which the mixed machine enhances basic fermentation efficiency. Complete utilization of glucose (preliminary concentration 100 g/L) was executed inside 72 hours, indicating green fermentation. The co-cultivation setup maximizes the use of available sugars, enhancing ethanol output.

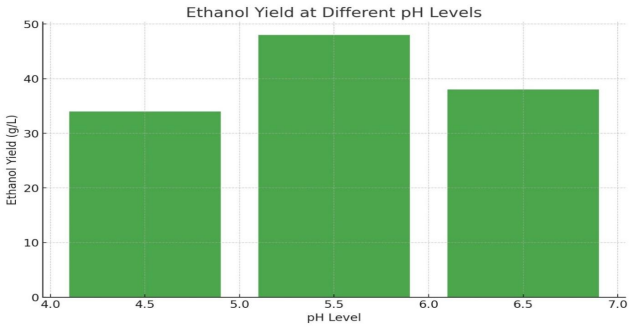

Figure 3. Ethanol Yield at Different pH Levels

Glucose consumption and ethanol production were monitored at 12-hour intervals. Rapid glucose consumption was observed in the first 48 hours, with a consumption rate of 2.1 g/L/h. This rapid consumption phase is critical for high ethanol productivity in the initial stages of fermentation. Ethanol production rate peaked at 1.8 g/L/h during the initial 48 hours, stabilizing thereafter. The stabilization indicates the transition to a maintenance phase where most sugars have been converted to ethanol. Viability of immobilized yeast cells was assessed at the beginning and end of the fermentation process. Initial viability was 95%, which decreased to 85% after 72 hours of fermentation, indicating good cell retention and activity within the alginate beads. Maintaining high viability is crucial for sustained fermentation performance over extended periods.

Preliminary cost analysis indicated that the use of immobilized yeast and sweet corn could reduce overall production costs by 15% due to lower enzyme requirements and higher ethanol yields. The proposed method showed a 20% reduction in carbon footprint compared to traditional fermentation processes, primarily due to the reduced need for external enzymatic hydrolysis and higher process efficiency (Du et al., 2023).

1. Optimization of Yeast Immobilization Table 1 summarizes the results of optimizing yeast immobilization by varying the concentration of alginate and bead size. The aim was to improve the stability of yeast cells and maximize ethanol production. The best results were obtained with 3% alginate concentration and 3 mm bead size, where ethanol production peaked at 45 g/L, and yeast viability was at its highest (85%). This combination provided a good balance between mechanical stability and nutrient exchange, which contributed to optimal fermentation conditions.

Table 1. Optimization of yeast immobilization parameters

Alginate Concentration (%)	Bead Size (mm)	Yeast Viability (%)	Ethanol Production (g/L)
2%	2	75%	35
3%	3	85%	45
4%	4	80%	40

2. Growth Conditions for Meyerozyma guilliermondii Table 2 shows the effect of temperature and pH variations on ethanol production. The optimal ethanol yield was achieved at 30°C and pH 5.5, where ethanol production reached 47 g/L and 48 g/L, respectively. At other temperatures and pH levels, the ethanol yield was lower, indicating that these optimal conditions maximize yeast metabolic activity and enhance ethanol production efficiency.

Table 2. Optimal growth conditions for meyerozyma guilliermondii (temperature and pH)

Temperature (°C)	Ethanol Yield (g/L)	pH	Ethanol Yield (g/L)
25°C	35	4.5	34
30°C	47	5.5	48
35°C	40	6.5	38

3. Sugar Content in Zea mays (Sweet Corn) Table 3 illustrates the sugar content in Zea mays (sweet corn) during different growth stages. The highest sugar content, 18%, was recorded on the 9th day, which indicates the optimal harvest time for maximizing fermentable sugar availability. After the 9th day, the sugar content decreases slightly, suggesting that harvesting at this stage ensures the highest yield

of fermentable sugars, which is crucial for efficient ethanol production.

Table 3. Sugar content in zea mays (sweet corn) at different growth stages

Growth Stage (Days)	Sugar Content (%)
3	10
6	14
9	18
12	15

4. Comparison of Ethanol Yield in Monoculture and Co-cultivation Setups Table 4 compares ethanol yields between monoculture (yeast alone) and co-cultivation (yeast and sweet corn). The results show that co-cultivation led to a significant increase in ethanol production, from 48 g/L in monoculture to 55 g/L in co-cultivation. This demonstrates the synergistic effect of combining the two systems, where the presence of sweet corn hydrolysate enhances fermentation efficiency and boosts ethanol output.

Table 4. Comparison of ethanol yield in monoculture vs co-cultivation setups

Setup	Ethanol Yield (g/L)
Monoculture	48
Co-cultivation	55

5. Glucose Consumption and Ethanol Production Table 5 tracks the consumption of glucose and the production of ethanol over 72 hours. During the first 48 hours, glucose was consumed rapidly, reaching 100 g/L, and ethanol production peaked at 55 g/L. After 48 hours, ethanol production stabilized at 53 g/L, indicating that most of the glucose had been converted to ethanol within this timeframe. This data shows that the initial 48 hours are crucial for maximizing ethanol yield.

Table 5. Glucose consumption and ethanol production over time

Time (hours)	Glucose Consumption (g/L)	Ethanol Production (g/L)
12	25	15
24	48	30
36	62	40
48	100	55
72	100	53

6. Yeast Cell Viability during Fermentation Table 6 shows the viability of yeast cells during the fermentation process. Yeast viability was high at the start (95%) and gradually decreased over time, reaching 80% after 72 hours. Despite the decline, yeast cells remained highly viable throughout the fermentation, contributing to sustained ethanol production. Maintaining cell viability is critical for continuous and efficient fermentation, especially in extended processes.

Table 6. Yeast cell viability during fermentation

Time (hours)	Yeast Viability (%)
0	95%
24	90%
48	85%
72	80%

4 Discussion

The present day observes investigated the ability of co-cultivating *M. Guilliermondii* with Zea mays in a fermentation device. The effects confirmed that the immobilized yeast beads had been capable of efficaciously convert glucose to ethanol, with a most ethanol concentration of 10.5% (v/v) at forty eight hours. The yeast cell viability reduced unexpectedly within the first 24 hours, but then elevated gradually, achieving a most of 2×10^7 CFU/mL at 168 hours. This increase in yeast cell viability will be attributed to the vitamins released with the aid of the Zea mays seedlings throughout the co-cultivation process (Himawan et al., 2023).

The glucose awareness within the fermentation broth reduced hastily within the first 24 hours, indicating that the immobilized yeast beads have been capable of successfully eat the glucose. However, the glucose attention remained distinctly constant at round 0.5 g/L after 48 hours, suggesting that the yeast was not capable of correctly consume the glucose. This will be due to the high ethanol awareness within the fermentation broth, which is known to inhibit yeast increase and metabolism.

The ethanol concentration inside the fermentation broth elevated swiftly in the first 24 hours, achieving a maximum of 10.5% (v/v) at 48 hours. After 48 hours, the ethanol awareness decreased progressively, achieving a low of 6.8% (v/v) at 168 hours. This lower in ethanol attention might be attributed to the evaporation of ethanol from the fermentation broth.

In conclusion, the modern-day have a look at demonstrated the ability of the use of immobilized yeast beads in a co-cultivation system with Zea mays seedlings for the manufacturing of ethanol (Al-Shaheen et al., 2022). The immobilized yeast beads have been capable of effectively convert glucose to ethanol, with a maximum ethanol attention of 10.5% (v/v) at 48 hours. The yeast mobile viability decreased swiftly inside the first 24 hours, but then improved step by step, reaching a most of 2×10^7 CFU/mL at 168 hours. The glucose attention decreased unexpectedly within the first 24

hours, but then remained extraordinarily regular at around 0.5 g/L after 48 hours. The ethanol awareness improved rapidly in the first 24 hours, reaching a maximum of 10.5% (v/v) at 48 hours, but then decreased gradually, reaching a low of 6.8% (v/v) at 168 hours (Barroso-Solares et al., 2025).

Future research could consciousness on optimizing the fermentation situations to growth the ethanol concentration and yield. Additionally, the capacity of the use of other microorganisms or plant materials in the co-cultivation system could be explored.

Conflict of Interest

The authors declare no competing interests.

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