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## ***Introduction to Bioethanol Lab on Cassava for Biology Education Students at UIN Jakarta***

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### ***Abstract***

This study discusses conventional bioethanol production through fermentation as a response to the need for environmentally friendly alternative energy sources. The objectives of this practicum are to understand the basic principles of fermentation, identify the factors that influence it, carry out a simple fermentation process using natural materials (sugar/corn/cassava) and *Saccharomyces cerevisiae* yeast, and analyze the results. The methods used include preparing a sugar solution, inoculating yeast, anaerobic incubation at 30–35 °C for 2–5 days, observing CO<sub>2</sub> formation, filtration, and simple purification through distillation. The study employed an experimental design, with 63 biology education students participating. The results of the practicum indicate that students understand the success rate of bioethanol fermentation, as evidenced by the formation of CO<sub>2</sub> bubbles and the feasibility of ethanol separation by distillation. Students can understand the factors that influence conventional bioethanol production.

**Keywords:** *Biotechnology, Bioethanol, Biology Education*

## **INTRODUCTION**

Biology education in higher education is not limited to mastering theoretical concepts; it also encompasses the development of scientific research skills through practical activities (Barata, 2020; Harahap et al., 2020). Practical work plays a crucial role in building comprehensive conceptual understanding, fostering scientific thinking, and developing students' scientific inclinations (Solihin & Dedah, 2022; Inayah & Harahap, 2024; Harahap & Harahap, 2024). In preparing prospective biology teachers, purposeful practical experiences are crucial for enabling students to implement experiential learning effectively in schools. One

applied topic in biology relevant to current global issues is bioethanol as a renewable energy source. Bioethanol can be produced from local food sources such as cassava, which is abundant in Indonesia. The use of cassava as a raw material for bioethanol production has not only scientific value but also contextual and educational value, connecting the study of biology to environmental issues, energy security, and local resource potential (Fahmi et al., 2021).

In the field of biotechnology, practical work on bioethanol production enables students to directly understand fermentation, the roles of microorganisms, and the factors that influence biochemical reactions (Putri et al., 2025). However, the success rate of bioethanol production experiments varies. This discrepancy can affect students' understanding of the concepts they have learned, particularly when the practical outcomes do not align with the theory presented (Jati & Widayanto, 2022). As prospective teachers, Biology Education students at the State Islamic University of Jakarta are expected not only to conduct laboratory experiments but also to understand the processes, analyze the results, and reflect on the factors that contribute to success and failure. Therefore, introducing laboratory experiments on bioethanol production from cassava requires an in-depth educational study, particularly regarding students' understanding of the success rate of these experiments and the factors that influence it.

Therefore, this study is crucial for determining the extent to which biology education students understand laboratory experiments for bioethanol production from cassava and for identifying the factors influencing their success. The research findings are expected to contribute to improving laboratory design and biotechnology learning strategies, making them more effective, contextually relevant, and beneficial for students, particularly in the development of alternative fuels (Fahmi et al., 2021).

Biofuel is among the options under consideration. Bioethanol is considered the most promising biofuel to replace gasoline, primarily due to its properties (Rachman et al., 2025). This fuel is an oxygenated liquid fuel containing 35% oxygen, produced by the microbial fermentation of monomeric sugars obtained from carbohydrate sources such as corn, soybeans, and sugarcane. Global bioethanol production in 2018 was 110 billion liters and is projected to reach 140 billion liters by 2022, with a compound annual growth rate (CAGR) of 7.6%, attributable to the anticipated economic feasibility of the process (Okolieuwa et al., 2025).

The United States, Brazil, the European Union, China, and Canada are the leading countries in global bioethanol production. The United States uses corn as a feedstock for bioethanol production. It has a production capacity of approximately 57.7 billion liters, while Brazil produces bioethanol from sugarcane and had a total production capacity of approximately 27.6 billion liters in 2016. Bioethanol is considered a potential substitute for

conventional gasoline and can be used directly in vehicles or blended with gasoline, thereby reducing greenhouse gas emissions and gasoline consumption (Kamal & Aditama, 2025). The advantages of bioethanol include a high-octane rating that improves engine efficiency and performance, a low boiling point, a wide ignition range, higher compression ratios and heats of vaporization, comparable energy content, shorter combustion times, and the use of lean combustion (Okolieuwa et al., 2025).

Disadvantages include high production costs associated with feedstock, enzyme, detoxification, and ethanol recovery. Bioethanol has a low volumetric energy density, meaning more bioethanol/km (up to 50%) will be consumed compared to conventional gasoline (Kamal & Aditama, 2025). However, to reduce production costs, lignocellulosic biomass is being considered as a feedstock due to its availability and low cost. Unfortunately, processing costs remain high, making this process less economically attractive.

## RESEARCH METHODS

This study employs a descriptive, laboratory-based approach to observe and understand the conventional bioethanol production process via fermentation. This study aimed to ferment a simple carbohydrate solution using microorganisms, specifically yeast. *Saccharomyces cerevisiae* is used to produce bioethanol. This study used a quantitative descriptive approach with an educational laboratory research design. This approach was chosen because the study aimed to describe students' understanding of cassava-based bioethanol practicum activities, the practicum success rate, and the factors influencing that success, without conducting hypothesis testing or inferential statistics. The subjects were students in the Biology Education Study Program, Faculty of Islamic Education and Teacher Training, Syarif Hidayatullah State Islamic University Jakarta, who participated in biotechnology practicums on bioethanol. A total of 63 students participated directly in the entire practicum. The research objectives included students' understanding of the concepts and procedures of cassava-based bioethanol practicums, as well as the practicum success rate, as evidenced by fermentation and bioethanol purification results.

The primary variable in this study was students' understanding of the bioethanol practicum, which encompassed fermentation, the role of *Saccharomyces cerevisiae*, the function of heating materials, and the interpretation of practicum results. The supporting variable was the success rate of the bioethanol practicum, as measured by indicators including CO<sub>2</sub> emissions, alcohol aroma, and ethanol content in the distillate.

Data were collected using three primary techniques: observation, questionnaires, and documentation. (Ahyar et al., 2020). Observation was used to monitor the practicum's

implementation and the success of the bioethanol fermentation process. The observation instrument was a practicum implementation observation sheet, which included the accuracy of work procedures, student activities, and indicators of fermentation success. A questionnaire was administered to assess students' understanding of the bioethanol practicum. The questionnaire was structured on a Likert scale with categories of very understanding, understanding, and not understanding. This included understanding fermentation concepts, the role of yeast, the function of cassava heating, and the analysis of practicum results. Documentation was used to support the research data, including photographs of practicum activities, observation notes, and ethanol content measurements obtained using a refractometer.

The research was conducted through several stages: preparation, practicum implementation, and evaluation. In the preparation phase, researchers prepared the laboratory equipment, research instruments, and necessary materials and tools. The implementation phase included a laboratory activity on producing bioethanol from cassava using two treatments: heated and unheated cassava, followed by a simple fermentation and distillation process. The evaluation phase involved completing a student understanding questionnaire and collecting data from the laboratory results.

The data obtained were analyzed using quantitative descriptive analysis.(Diswantika et al., 2022). The questionnaire and observation data were analyzed as percentages to assess the level of student understanding and the laboratory's success rate. The results of the analysis were presented in tables and narrative descriptions to provide a comprehensive overview of student understanding and the factors influencing the success of the bioethanol laboratory from cassava.

## RESULTS AND DISCUSSION

The fermentation stage is carried out using mashed cassava as the raw material, after which 200 mL of water is added, and the mixture is heated to the boiling point. Once the mixture has reached room temperature, 3 grams of yeast are added. *Saccharomyces cerevisiae*. Then, the container is tightly closed and left for five days. During fermentation, physical changes include gas bubble formation and the development of a distinctive alcoholic aroma. The appearance of bubbles indicates that the yeast has converted sugar into ethanol and carbon dioxide. *Saccharomyces cerevisiae*.



**Figure 1.1.** The results of cassava fermentation are heated



**Figure 1.2.** The results of the cassava fermentation treatment were not heated

On the fifth day, the bubbles began to decrease, and the alcohol odor became more intense, indicating that fermentation was nearing completion. The fermentation liquid appeared cloudy with sediment at the bottom of the container and a strong alcoholic aroma. This indicated that fermentation was progressing well and ethanol had formed. The fermented liquid was then filtered and distilled using a simple distillation apparatus.

A simple distillation apparatus consists of an Erlenmeyer flask as the container for the fermented liquid, a tripod and an alcohol burner as the heat source, and a lid made from a piece of Swallow-brand rubber sandal, shaped into a circle to cover the mouth of the Erlenmeyer flask. Two holes are made in the lid to install a mercury thermometer and a plastic tube to channel the steam produced by heating. The tube is taped to ensure a tight seal and prevent steam leakage. The end of the tube is directed into a laboratory sink filled with water to facilitate cooling and condensation of the ethanol vapor back to liquid. A small beaker is used to collect the distillate. During distillation, the temperature is monitored with a mercury thermometer to ensure that the heating is not too high. Steam appears when the temperature reaches 70–80°C, indicating that ethanol has begun to evaporate.

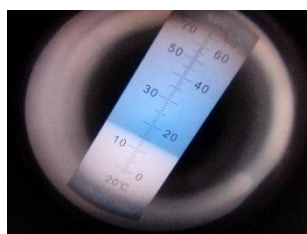


**Figure 2.1:** The results of the cassava fermentation distillation treatment are heated.

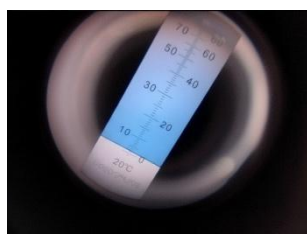


**Figure 2.2:** The results of the distillation of fermented cassava with unheated treatment

After distillation, measure the alcohol content of the distillate using a refractometer. The distilled liquid is dripped onto the refractometer's prism or glass surface, and the ethanol content is measured using an optical scale. This instrument operates on the principle of light refraction, which depends on the ethanol concentration in the solution.



**Figure 3.1.** The results of measuring bioethanol from cassava in the heated treatment



**Figure 3.2.** Results of bioethanol measurements from cassava in the unheated treatment

Based on Figures 3.1 and 3.2, the measured bioethanol content in cassava treated with heating was higher than in cassava not heated. This is because the heating process hydrolyzes cassava starch into simple sugars, which can be fermented by the yeast *Saccharomyces cerevisiae* into ethanol. In the treatment without heating, starch-to-sugar conversion is suboptimal, resulting in low fermentation activity and lower bioethanol content. Therefore, heating is an important step in increasing fermentation efficiency and bioethanol production from cassava.

**Table 1. Results of observations of the alcohol content of cassava treated with heating**

Activity	Observation aspects	Observation result
Initial activity	Source of carbohydrates	Granulated sugar
	Solution concentration	20% (50 grams of sugar in 200 mL of distilled water)
	Amount of yeast	5gram <i>Saccharomyces cerevisiae</i>
	Initial temperature of the solution	28 °C
During Fermentation Incubation (30–35 °C)	Day 1	The CO <sub>2</sub> bubbles are few, small, and slow. The dominant aroma is sweet and yeasty.
	Day 2	CO <sub>2</sub> bubble formation is evident, quite active, medium-sized, and consistent. An alcohol aroma is beginning to be detected.
	Day 3	CO <sub>2</sub> bubble activity decreases, slows down, is sparse, and small. The alcohol aroma is strong and dominant.
	Day 4	There are very few or no CO <sub>2</sub> bubbles (fermentation is complete). The alcohol aroma remains strong.
	Total fermentation time	7 days
After Fermentation (Filtering)	Liquid color	Yellowish turbidity
	Sediment	Brownish white at the bottom of the pumpkin (presumably dead yeast and remaining ingredients)
	Filtered fluid volume	230 mL (slightly reduced from the initial volume)
After Distillation (Purification)	Characteristics of distillate	The condensed steam (distillate) has a sharp, distinctive alcoholic aroma.
Final Analysis	Fermentation success	Successfully observed CO gas production <sup>2</sup> and a strong alcoholic aroma
	Purification success	Quite successful in separating the alcohol-flavored components.
	Alcohol level	10%
	Efficiency conclusion	Demonstrates practical basic principles for an educational laboratory scale.

**Table 2. Results of observations of the alcohol content of cassava treated without heating**

Activity	Observation Aspects	Observation result
Initial activity	Source of carbohydrates	Grated cassava (not heated)
Initial activity	Solution concentration	Concentration around 20% (50 g grated cassava in 200 mL water)
Initial activity	Amount of yeast	5 gram <i>Saccharomyces cerevisiae</i>
Initial activity	Initial temperature of the solution	28 °C
During Incubation (30–35 °C)	Day 1	Few CO <sub>2</sub> bubbles; mild sweet aroma; slightly cloudy solution
During Incubation (30–35 °C)	Day 2	Bubbles begin to appear; the aroma of alcohol is very faint; a thin foam appears.
During Incubation (30–35 °C)	Day 3	Gas activity is reduced; alcohol aroma is weak; color remains cloudy.
During Incubation (30–35 °C)	Day 4	Bubbles subside; fine sediment appears
During Incubation (30–35 °C)	Day 5 – Day 7	Activity has nearly ceased; fermentation is essentially complete.
Total fermentation time	-	7 days (significant activity only days 1–3)
After Fermentation (Filtering)	Liquid color	Turbid yellowish-light brown
After Fermentation (Filtering)	Sediment	Yellowish white sediment (yeast + cassava fiber)
After Fermentation (Filtering)	Filtered fluid volume	~225 mL (slightly reduced)
After Distillation (Purification)	Characteristics of distillate	The aroma of alcohol is very weak; the amount of distillate is small.
Final Analysis	Fermentation success	Fermentation occurs but is inefficient; starch is not degraded.
Final Analysis	Purification success	Distillation was successful, but the alcohol yield was low.
Final Analysis	Alcohol content (final)	1% (v/v)
Final Analysis	Efficiency conclusion	Ethanol production is low due to the absence of heating; a saccharification stage is required.
Final Analysis	Scientific notes	Cassava starch must be converted to sugar to enable optimal yeast fermentation.



Bioethanol production is a biotechnological process that uses microorganisms, such as *Saccharomyces cerevisiae*, to ferment carbohydrates into ethanol. In this experiment, two treatments were used: cassava heated and cassava not heated prior to fermentation. This difference significantly affected the alcohol content produced (Putri et al., 2025). Cassava is heated, and the heating process breaks down starch (*amylum*) into simple sugars, such as glucose and maltose (Kamal & Aditama, 2025). These simple sugars then serve as substrates that can be directly fermented by the yeast *Saccharomyces cerevisiae* into ethanol and carbon dioxide (CO<sub>2</sub>) (Ridwan et al., 2023). This is evident in the observation results, which indicate numerous gas bubbles and a strong alcoholic aroma during fermentation (Mustafa Hauwa M. et al., 2019). The measured alcohol content was 10%, indicating that sugar conversion to ethanol was optimal.

High fermentation activity indicates that the yeast is working efficiently under favorable conditions, namely an incubation temperature of 30–35°C and the availability of a sugar source (Dinata & Kartawiria, 2021). The appearance of bubbles indicates the fermentation process, namely, the conversion of sugar into ethanol and carbon dioxide by yeast. In contrast, in unprocessed cassava, the alcohol content produced is only around 1%. This is because the starch in the cassava has not been converted into sugar.

**Table 3. Level of Student Understanding of Cassava Bioethanol Practical**

No	Student Understanding Aspects	Very Understand (%)	Understand (%)	Not really understand (%)
1	Bioethanol fermentation concept	38	46	16
2	Role <i>Saccharomyces cerevisiae</i>	41	44	15
3	Heating function (starch saccharification)	35	48	17
4	The relationship between CO <sub>2</sub> formation and fermentation	43	42	15
5	Interpretation of alcohol content from distillation results	32	49	19

Based on the data in Table 3, the majority of students fall into the understanding and very understanding categories across all aspects of the cassava bioethanol practicum. The highest level of understanding is in the relationship between CO<sub>2</sub> formation and the fermentation process, with 85% of students in the understanding and very understanding

categories, indicating students' success in linking biochemical concepts to practical phenomena. Interpretation of the alcohol content in distillation results is less well understood than other aspects, indicating a need to strengthen the analysis of practicum data.

**Table 4. Success Rate of Students' Bioethanol Practicals**

Success Category	Criteria	Number of Students (%)
Succeed	Active CO <sub>2</sub> is formed, and the ethanol content is $\geq 8\%$	68
Quite Successful	CO <sub>2</sub> is formed, but the ethanol content is low (2–7%)	21
Less Successful	Very little/no CO <sub>2</sub> formed, ethanol $\leq 1\%$	11

Based on Table 4, the majority of students (68%) were in the successful category, as evidenced by the formation of CO<sub>2</sub> and an ethanol content  $\geq 8\%$ , indicating an optimal fermentation process. Twenty-one percent of students were in the moderately successful category, in which CO<sub>2</sub> formation had occurred. However, ethanol content remained relatively low, indicating a problem with the fermentation conditions or the distillation stage. Meanwhile, 11% of students were in the less successful category, with very little or no CO<sub>2</sub> formation and an ethanol content of  $\leq 1\%$ , reflecting suboptimal understanding and practical skills.

**Table 5. Student Difficulties in Bioethanol Practicals**

No	Type of Difficulty	Percentage of Students (%)
1	Determining the heating temperature of cassava	29
2	Understanding the heating function of materials	26
3	Maintaining anaerobic fermentation conditions	18
4	Simple distillation process	17
5	Reading refractometer results	10

Based on the table of student difficulty types, the most significant obstacles were encountered in determining the cassava heating temperature (29%) and understanding the function of heating materials (26%), both of which are directly related to the starch saccharification stage. Difficulties in maintaining anaerobic conditions during fermentation (18%) and in performing simple distillation processes (17%) indicate that students still require reinforcement of their procedural skills during the practicum. The difficulty in interpreting refractometer results was relatively low (10%), indicating that most students were able to use the measuring instrument effectively.

**Table 6. Student Responses to the Cassava Bioethanol Practical**

Statement	Agree (%)	Disagree (%)
The lab helps to understand the concept of fermentation.	87	13
Practicums improve understanding of biotechnology	84	16
Practical work relevant to alternative energy issues	89	11
Practicums train laboratory skills	91	9
Practical work is important for prospective biology teachers	93	7

Based on Table 6, the majority of students agreed that the bioethanol practicum made a positive contribution to learning. The highest levels of agreement were observed for the statements that the practicum is important for prospective biology teachers (93%) and that it trains laboratory skills (91%), demonstrating the practicum's relevance to students' professional competencies. Furthermore, the high level of agreement regarding the relevance to alternative energy issues (89%) and understanding of fermentation concepts (87%) confirmed that the practicum was contextual and meaningful.

The cassava-based bioethanol experiment in this study confirmed the basic principle of fermentation biotechnology, as proposed by (Adetunji et al., 2015) The success of ethanol fermentation is primarily determined by the availability of simple sugars as the primary substrate for *Saccharomyces cerevisiae*. The results showed that heating cassava increased ethanol content by up to 10%, whereas heating was not required for a yield of about 1%. This finding aligns with the research of (Efeovbokhan et al., 2019), which states that starch gelatinization and saccharification are crucial steps in bioethanol production from starchy materials such as cassava.

Biochemically, heating cassava plays a role in changing the starch structure (starch) into a form that is more easily hydrolyzed into glucose and maltose (Oyeleke et al., 2011). Explained that without the saccharification stage, starch cannot be optimally utilized by yeast because *S. cerevisiae* lacks the amylase enzyme. This explains the low fermentation activity in the unheated treatment, characterized by minimal CO<sub>2</sub> production and a weak alcoholic aroma, as evidenced by the observed results and the low ethanol content. Intense fermentation activity during the heating treatment was demonstrated by the active formation of CO<sub>2</sub> bubbles on days 2 and 3 of incubation. This phenomenon is consistent with the theory of alcoholic fermentation proposed by Purnama et al., (2023) This states that the exponential phase of yeast growth is characterized by high CO<sub>2</sub> production and increasing ethanol levels. At the optimal incubation temperature (30–35°C), the yeast works optimally, resulting in efficient fermentation.

From a biology education perspective, these results reinforce Uno's (2011) view that lab-based learning can bridge abstract concepts with real-world experiences (Arnita Sari, 2019). The data in Table 3 shows that more than 80% of students were in the "understand" or "very understanding" categories regarding the concept of fermentation and the relationship between CO<sub>2</sub> formation and biochemical processes. This indicates that the bioethanol lab is not only procedural but also conceptual (Hidayah et al., 2025).

The student success rate, which reached 68% in the "successful" category (Table 4), indicates that this lab model is effective as a learning medium for biotechnology. Contextual laboratory experiments can improve conceptual understanding, scientific skills, and positive attitudes toward science. Connecting the experiments to alternative energy issues also strengthens the relevance of learning to global challenges. However, data on student difficulties (Table 5) indicate that the saccharification stage remains a significant obstacle, particularly in determining the appropriate heating temperature. This supports the findings of (2024), who stated that failures in bioethanol experiments at the higher education level are often caused by students' lack of understanding of process variable control. Therefore, reinforcing pre-practical concepts, using supporting e-LKM or demonstration videos are crucial pedagogical needs.

The positive student responses to the experiments (Table 6) demonstrate that the cassava-based bioethanol learning approach has high educational and motivational value. According to Restudila et al., (2025) Active learning through experiments can increase student engagement and long-term concept retention. The high level of agreement among prospective biology teachers that the experiments are important demonstrates their relevance to developing students' professional competencies as future educators. The novelty of this research lies in the integration of a bioethanol practicum using local ingredients (cassava) with a comprehensive evaluation of cognitive aspects, practical skills, and student responses within the context of pre-service biology teacher education. (Mahmudatun Nisa, 2017). Unlike previous research that focused on the technical aspects of bioethanol production, this study positions the practicum as a contextual biotechnology learning medium aligned with renewable energy issues and strengthening scientific literacy. (Fadillah et al., 2021). Thus, this research makes a new contribution to the development of a simple, practical, and educational biotechnology practicum model for higher education institutions. (S Solihin, A Fitriyah, 2022).

## CONCLUSION

Based on the results and discussion, it can be concluded that the bioethanol production process from cassava is strongly influenced by the initial processing stage of the raw material,

particularly the heating process. Cassava that was heated prior to fermentation produced a higher ethanol content (10%) compared to unheated cassava (1%). Heating plays a crucial role in hydrolyzing starch into simple sugars that can be more readily fermented by *Saccharomyces cerevisiae*, thereby increasing fermentation efficiency and ethanol yield. These findings emphasize that the success of bioethanol production largely depends on the availability of easily degradable sugar substrates and optimal fermentation conditions.

From an educational perspective, particularly in biology education, the implementation of cassava-based bioethanol practicum has been shown to enhance students' conceptual understanding of fermentation, biotechnology, and alternative energy. Through practicum activities, students not only gain theoretical knowledge but also connect abstract biochemical processes to real laboratory experiences. In addition, this practicum develops students' science process and laboratory skills, including observation, measurement, data analysis, and drawing scientific conclusions. Cassava-based bioethanol practicum using local materials has high pedagogical value as a contextual learning medium. This activity effectively bridges biotechnology concepts with current issues in renewable energy. It prepares students—especially prospective biology teachers—to implement meaningful, applicable, and contextually relevant practicum-based learning aligned with societal needs and advances in science and technology.

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