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Utilizing Soybean and Corn Residue for Bioethanol Production

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The study utilized soybean and corn residue by-products to produce bioethanol through separate saccharification and fermentation processes. The saccharification process of soybean and corn residue liquid was carried out with α-amylase enzyme, and the fermentation process of the saccharification solution used Saccharomyces cerevisiae yeast. The reducing sugar content and ethanol content of the products were determined by high-performance liquid chromatography analysis. The saccharified product contained the highest reducing sugar content of 90.09 g/L when using 7.5 g/L of α-amylase enzyme for 4 h of saccharification at 65 °C. The fermented product achieved the highest ethanol content of 5.56 vol% with the yeast content used at 6 g/L for 96 h of fermentation at 30 °C. Synthesizing bioethanol from soybean and corn residue by-products not only contributes to reducing the consumption of petroleum resources but also to environmental protection.

1. Introduction

Currently, global fuel demand continues to increase and forecasts of fossil fuel depletion as well as environmental pollution of emissions from fossil fuel use are among the most serious problems (Onyeaka et al., 2022). One of the proposed solutions can be to focus on research on the use of renewable resources and the development of environmentally friendly biofuel production processes. Bioethanol mixed with gasoline in certain proportions has met almost all quality requirements for gasoline products (Devi and Professor, 2020). Furthermore, ethanol has a higher octane number (99) than gasoline (80–100), so there is no detonation when using ethanol instead of gasoline, helping to extend engine life and save fuel. On the other hand, ethanol burns cleaner, and the emissions and pollutants in the exhaust are lower than when using gasoline. This not only helps to resolve the stress of dependence on petroleum resources but also helps to reduce environmental pollution (Junchen and Lin, 2012).

Vietnam is in the process of promoting industrialization and modernization of the country, so it needs a lot of machinery, engines, means of transport, freight transport, and travel, etc. Therefore, in recent years, the demand for fuel in general and gasoline in particular in Vietnam has increased. As a result, the environment is affected by a large amount of polluting emissions. With the goal of ensuring energy security and environmental protection, many countries in the world have used biofuels in general or bioethanol in particular. In Vietnam, E5 gasoline (containing 5% (v) ethanol) has been used to replace RON92 gasoline since 2005, and the roadmap for using E10 biofuel can be applied mandatorily on January 1, 2026. Therefore, using biofuels is a common trend in the world and in Vietnam. (El-Araby, 2024).

Materials containing high levels of carbohydrates, such as food waste, molasses, sugar beet pulp, or biomass such as banana peels, wheat husks, straw, rice husks, etc., are considered suitable raw materials for bioethanol production through hydrolysis and fermentation methods (Onyeaka et al., 2022). Based on statistical research data, it is concluded that bioethanol produced from starch biomass accounts for 60 % of global ethanol production (Larrea et al., 2020). Bioethanol was synthesized from starchy wastes through the fermentation of simple sugars present in the raw materials after enzymatic hydrolysis. Food wastes contain high levels of polysaccharides, but they are difficult to degrade by microorganisms. Therefore, the proposed ethanol production process includes an enzymatic hydrolysis stage to increase the reducing power of the raw materials and then ferment the hydrolysate to produce ethanol. Enzymes such as amylase, glucoamylase, cacbohydrase,

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and cellulase have been used to improve the saccharification of food waste, and the microorganisms used in the fermentation stage are Saccharomyces cerevisiae (Prasoulas et al., 2020). Research analyzing the composition of food waste has concluded that this is a suitable raw material for synthesizing bioethanol through saccharification and fermentation processes (Carmona-Cabello et al., 2020). In another study, from biomass materials such as molasses, sugar beet pulp, or banana peel, bioethanol was produced through the hydrolysis of biomass with acid or alkali and fermentation of the hydrolysate. The ethanol concentrations obtained from molasses, banana stems, and sugar beet pulp reached values of 30.8 g/L, 16 g/L, and 5.5 g/L, respectively (Santos Pinargote and Villalta Pastuzo, 2021). Ethanol is produced from date palm seeds through stages such as treating raw materials with acid, hydrolyzing materials with enzymes, and fermenting the hydrolysate with Saccharomyces cerevisiae yeast for 24 h (Bouaziz et al., 2020).

Nowadays, in Vietnam and many countries around the world, people are increasingly interested in choosing to use food products processed from plant seeds such as soybeans and corn kernels because they can bring many health benefits to people. In the process of processing these products, a quantity of by-products such as soybean residue and corn residue is always formed. Thus, every year Vietnam and some countries in the world can provide a large quantity of soybean residue and corn residue by-products. Based on the composition of soybeans and corn kernels, soybean residue and corn residue are predicted to contain high levels of carbohydrates, so they can be considered a promising source of raw materials for the production of biofuels through transformation processes such as saccharification and fermentation.

This study aimed to evaluate the feasibility of using a mixture of soybean and corn residue as raw materials in bioethanol production. The proposed research content includes stages such as hydrothermal treatment of raw materials, saccharification of soybean and corn residue pretreated with α-amylase enzyme, and fermentation of saccharified corn and soybean residue with Yeast Saccharomyces cerevisiae, a yeast commonly used in wine and bread fermentation. Parameters affecting the saccharification and fermentation processes are also investigated to evaluate the efficiency of the process.

2. Experiment

2.1 Materials

The mixture of soybean and corn residue (MSCR) is collected from food processing facilities such as fresh tofu, fresh soy milk, corn milk, cooking oil, etc.**,** and has impurities such as plastic packaging, paper packaging, wet tissues, or other mechanical impurities removed. The MSCR is mixed with water in a volume ratio of 1:3 and crushed, ground to form a liquid. The MSCR liquid is stored in a dark can at a temperature of about 15 – 20 °C to be used as raw material for the saccharification process (Hafid et al., 2017).

2.2 Preliminary processing of raw materials and saccharification

500 mL of MSCR liquid was put into a 1 L reactor. The reactor had an outer thermostatic jacket. The raw material mixture was heated to 100 °C and held for 60 min to pre-treat the raw material. After pre-treatment by the hydrothermal method, the MSCR solution was cooled to 65 °C, ready for the enzymatic saccharification stage (Hafid et al., 2017).

The process of saccharification of MSCR liquid uses α-amylase enzyme with enzyme activity ≥10000 U/g. The amount of loading enzyme was investigated at values (g/L) 5, 7.5, and 10. The enzyme was activated before being put into the process by mixing with water at a ratio of 1:10 (w/v), stirring well, and heating at 50 °C for 15 min (Larrea et al., 2020).

Prepare 3 heat-resistant Erlenmeyer flasks with a capacity of 250 mL. Add 100 mL of pre-treated soybean-corn pulp solution to each flask. Add the calculated volume of activated α-amylase enzyme solution to the reaction flask, mix the mixture well, and place the reaction flask in the incubator. Set the temperature for the incubator at 65 °C. The pH value of the saccharification reaction mixture is controlled and adjusted at 4.2 - 4.5 by adding 10 % (w/v) $(NH_4)_2HPO_4$ solution to the sample (Vučurović et al., 2025). After periods (h) 1, 2, 3, and 4, 20 mL of saccharification product samples were taken and centrifuged at 10,000 rpm for 15 min, and vacuum filtered. The obtained filtrate liquid was analyzed the ethanol content by high-performance liquid chromatography (HPLC) analysis to evaluate the efficiency of the process.

2.3 The fermentation process

The fermentation process of soybean-corn saccharification liquid uses dry yeast, Saccharomyces cerevisiae. The amount of yeast used is investigated at the values (g/L) 3, 6, 9. Prepare 3 heat-resistant Erlenmeyer flasks with a capacity of 250 mL. The Erlenmeyer flasks have lids to ensure anaerobic conditions during the fermentation process (Moura et al., 2024). All materials used for the fermentation process must be sterilized in a steam autoclave at 150 °C for 30 min. Add 100 mL of MSCR liquid to each flask and then add the calculated amount of yeast to the reaction flask. Mix the mixture of MSCR and yeast to activate the yeast for 30 min at 30

°C. Place the fermentation reaction flasks in a thermo-incubator, set the temperature for the incubation flask at 30 °C. After time intervals (h) 24, 48, 72, and 96, 20 mL samples were taken from the fermentation reactors, centrifuged at 10,000 rpm for 15 min, and vacuum filtered. (Hafid et al., 2017). The obtained filtrate liquid was analyzed the ethanol content by high-performance liquid chromatography (HPLC) analysis to evaluate the efficiency of the process.

2.4 Analytical method

The reducing sugar content of the saccharification product and the ethanol content contained in the fermentation product were determined by high-performance liquid chromatography (HPLC) analysis. The glucose and ethanol standard curves were constructed, and the standard curves were used to quantify the reducing sugar concentration and ethanol concentration contained in the samples by comparing the peak areas of the samples with the peak areas of the standard solutions (Debebe et al., 2018).

In this study, the HPLC analysis was performed using an automatic sample injector (Agilent, USA). The reverse-phase analytical column (C18, 150 × 2.1 mm, 1.7 µm; Waters, Ireland) and a photodiode array (PDA) detector were used. In the case of determining the reducing sugar content in the saccharification product, the mobile phase used was deionized water. The flow rate was 0.7 mL/min. The sample injection volume was 20 µL. The detection wavelength was 230 nm. The analysis was maintained at 40 ºC. (Fetyan et al., 2022). In the case of determining the ethanol content in the fermentation product, the mobile phase used was a mixture of water/ethanol ratio at 40/60 (v/v). The injection volume was 20 µL. The flow rate was 0.5 mL/min. The detection wavelength was 235 nm. The elution was carried out in isocratic mode at a flow rate of 0.7 mL/min. The analytical column compartment was maintained at 40 ºC (Albaseer and Dören, 2022).

3. Results and discussion

3.1 Results of the saccharification process

The D-Glucose standard curve equation was constructed as shown in Eq(1):

$$y = 195332x - 947498 \quad (1)$$

With $R^2 = 0.9923$. The process of saccharifying MSCR liquid with the α-amylase enzyme was carried out at 65 °C in a stable pH range of 4.2 - 4.5. The affecting parameters of saccharification were investigated, including the amount of α-amylase enzyme loading (5, 7.5, and 10 g/L) and saccharification time (1, 2, 3, and 4 h). By comparing the pick area of the surveyed samples with that of the standard glucose solution, the reducing sugar content of the samples was determined. The results are presented in Figure 1.

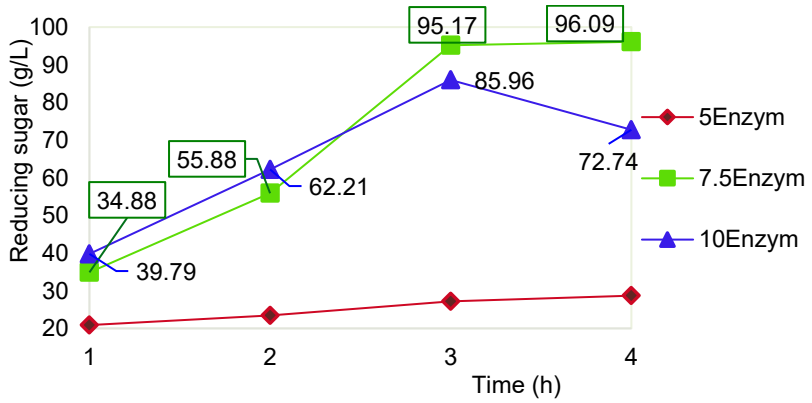

Figure 1: Effect of α-amylase enzyme amount and time on saccharification efficiency

The data presented in Figure 3.1 show that, during the survey period (1 - 4 h), the glucose content of the saccharification product gradually increased when the amount of loading α-amylase enzyme increased from 5 g/L to 7.5 g/L, but the reducing sugar content of the product was decreased when the amount of enzyme used continued to increase to 10 g/L even though the saccharification time was extended to 4 h.

This may be because when the reaction used a low amount of biocatalyst, 5 g/L, the catalyst may not be enough to activate the reaction, or in other words, the process has an excess amount of substrate that has not participated in the reaction, so the amount of sugar released was low. When the amount of enzyme was increased to 7.5 (g/L), the process efficiency increased significantly, and this may be the most suitable amount of enzyme used for the substrate concentration, so the reaction rate increased.

This can be consistent with the theory of enzyme affinity for the resulting product. In a biocatalytic reaction, when the amount of biocatalyst and substrate creates a strong enough affinity between them, it will promote the reaction and ensure that the product can be released from the enzyme immediately after being formed so that the enzyme can be ready to participate in new reaction cycles. When the reaction uses a large amount of biocatalyst, the catalytic activity is strong, so at the early stages of the reaction, the reaction efficiency increases. However, in the later reaction times, the amount of product formed is large, and in the step where the product is released from the enzyme, there is still a small amount that creates a strong affinity with the enzyme, sticks to the enzyme and inhibits the enzyme, causing the enzyme to lose activity in new reaction cycles (Triwahyuni et al., 2015).

Observing in Figure 3.1, the case of saccharification reaction using an enzyme amount increased to 10 g/L creates very strong enzyme activity, so at the initial time (1 and 2 h), compared to the case of reaction using 7.5 g/L enzyme, the saccharification reaction speed was higher, meaning that more glucose was formed. But in the following time periods (3 and 4 h), a large amount of glucose product could create a greater affinity with the enzyme, so a certain amount of product was not released but remained on the enzyme, inhibiting the enzyme and reducing the enzyme activity in the 3 and 4 h reaction cycles. On the other hand, when the saccharification reaction used a high amount of enzyme, at the initial reaction time (1 and 2 h), the amount of sugar released increased, and some of these products may promote the formation of products that inhibit enzyme activity, such as organic acids, furfural, etc. They may negatively affect the recovery of enzyme activity in the 3 and 4 h reaction cycles. Therefore, the overall efficiency of the saccharification process was reduced. This has also been explained similarly in the study of bioethanol production from bagasse (Raghavi et al., 2016).

In the study to determine the optimal conditions for starch hydrolysis with thermostable α-amylase enzyme, the variation of the process and the obtained results tended to be and were explained similarly (Kolusheva and Marinova, 2007). A similar result was found in the study of ethanol production from municipal solid waste in India and Nepal, where the process of hydrolysis of raw materials with acid at a concentration of 7.5 vol% at a temperature of 135 °C obtained a hydrolysate containing a sugar content of 32.636 g/100 g of dry material (Prasoulas et al., 2020).

Thus, in the investigated cases, the product of the MSCR liquid saccharification process using α-amylase enzyme achieved the highest reducing sugar concentration of 96.09 g/L with the amount of enzyme used 10 g/L, temperature 65 °C, and saccharification time 4 h. The saccharification of MSCR liquid was performed using enzymes under the optimal conditions just determined. The raw product was centrifuged at 10,000 rpm for 15 min and vacuum filtered. The resulting filtrate was stored in a dark can at 4-5 °C and used as material for the fermentation process to produce bioethanol.

3.2 Results of the fermentation process

The ethanol standard curve equation was constructed as shown in Eq(2):

$$y = 471301x - 36680 \quad (2)$$

With $R^2 = 0.9924$. The fermentation process of soybean-corn pulp by yeast was carried out at 30 °C with a stable pH of 4.5. The affecting parameters of the fermentation efficiency were investigated, including the amount of yeast used (3, 6, 9 g/L) and saccharification time (24, 48, 72, and 96 h). The pick area of the investigated samples was compared with the pick area of the standard ethanol solution, and the ethanol content of the samples was determined.

The results in Figure 2 show that, during the entire period of investigation (24 - 96 h), the ethanol content of the fermented product gradually increased as the amount of yeast used increased from 3 g/L to 6 g/L. However, when the amount of yeast used continued to increase to 9 g/L, the ethanol content of the product decreased despite the fermentation time being extended to 96 h.

This may be because when the fermentation reaction uses a low level of biocatalyst, 3 g/L, the catalyst activity is low, and a certain amount of substrate may not be catalyzed to stimulate the reaction, so the amount of ethanol produced is low. In this case, the highest ethanol content is only 2.07 vol%. When the amount of yeast used is increased to 6 g/L, the process efficiency has increased significantly. The ethanol content in the product reaches a concentration of 5.56 vol% after 96 h of fermentation, so this may be the most suitable amount of yeast used for the substrate concentration, so the reaction rate has increased significantly.

The decrease in ethanol concentration in the product in the case of fermentation reaction using a high amount of yeast (9 g/L) may be due to biochemical changes occurring in the cells that are detrimental to the reaction. When the reaction uses a lot of yeast, the catalytic activity is enhanced, so at the initial reaction times, 24 and 48 h, the ethanol product is formed more than in the case of fermentation using 6 g/L yeast. But at later times, 72 and 96 h, a large amount of ethanol product in the large amount of ethanol formed tends to be consumed for the reaction to form substances that inhibit the recovery of yeast in the next reaction cycles, reducing the activity of the yeast, so the reaction rate in the time periods of 72 and 96 h was reduced (Hafid et al., 2017). This has

also been explained similarly in the study of bioethanol production from sugarcane bagasse. The authors have proposed that there must be a stage of adding chemicals capable of treating the enzyme inhibitors so that the fermentation process of sugarcane bagasse hydrolysate yields a product with a high ethanol content (Raghavi et al., 2016).

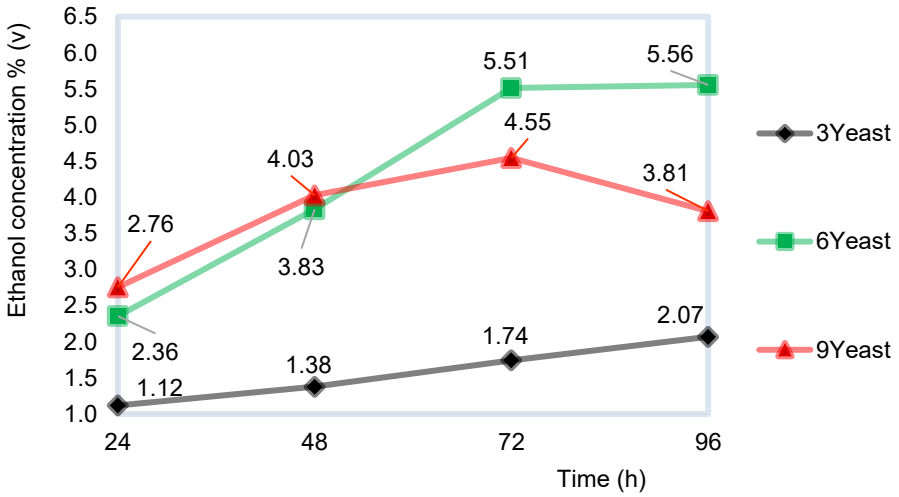

Figure 2: Effect of Yeast amount and time on fermentation efficiency

Thus, in the surveyed cases, the MSCR saccharification solution fermentation product (reducing sugar content reached at 96 g/L) using dry yeast Saccharomyces cerevisiae achieved the highest ethanol content of 5.56 vol% with the amount of yeast used 6 g/L, temperature 30 °C during fermentation time 96 h.

This result can be compared with the results of other studies on bioethanol production from high carbohydrate sources such as food waste and biomass. In the study on bioethanol production from food waste by hydrolysis and fermentation methods. The food waste hydrolysate (glucose content 33.7 g/L - 42.13 g/L) was fermented with mixed yeast strains F. oxysporum and S. cerevisiae, with the addition of glucoamylase enzyme to the culture medium, resulting in a product containing bioethanol content of 30.3 g/L (Prasoulas et al., 2020). A similar result was found in the study of bioethanol production from food waste containing high carbohydrate content (117 g/L) through raw material pretreatment, acid-enzymatic hydrolysis, and fermentation separately. The hydrolysate of food waste treated and hydrolyzed by an acid-enzymatic combination contained reducing sugar content at 103.4 g/L. Fermentation of the hydrolysate (diluted to a concentration of 25 g/L) yielded an ethanol product with a content of about 40 g/L in the first 24 h of fermentation, with a sugar consumption during fermentation of 88.68 % (Hafid et al., 2017). In the study of bioethanol production from starchy materials such as cassava, potato, sweet potato, the product of saccharification of 10 wt% cassava, potato, or sweet potato starch solution with glucoamylase enzyme for 2 h at 60 °C obtained reducing sugar content in the range of (66 - 90.9 g/L) and the product of fermentation of saccharification solutions with Saccharomyces cerevisiae yeast contained an average ethanol content of 10 vol% in a fermentation time of 24-72 h (Larrea et al., 2020).

4. Conclusion

The study utilized a mixture of soybean and corn residue as raw materials for bioethanol production. The effects of enzyme content and time on the efficiency of the saccharification process were investigated. The saccharification product reached the highest reducing sugar content of 96.09 g/L with the amount of α-amylase enzyme used at 7.5 g/L in a saccharification time of 4 h and a temperature of 65 °C. Experiments were conducted to investigate the effects of yeast content and time on the efficiency of the fermentation process of the saccharification product of a mixture of soybean and corn residue liquid and the fermentation product contained the highest ethanol content at 5.56 vol% with the yeast content used at 6 g/L in a fermentation time of 96 h at a temperature of 30 °C. The results of this study may have practical significance in exploiting renewable resources, reducing dependence on fossil resources, and opening up a direction for clean energy development in Vietnam.

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