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Bioethanol Fermentation from Banana Pseudostems Waste (*Musa balbisiana*) Pretreated with Potassium Hydroxide Microwave

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ABSTRACT

Several nations are committed to developing an alternative energy source to achieve the net zero emissions (NZE) target. A typical alternative is bioethanol, which has been reported to be a renewable energy supporting the achievement of the target. Although banana pseudostem waste is often minimally utilized and discarded by the community, several studies have shown its potential to yield bioethanol due to the high cellulose content. Therefore, this study aimed to synthesize bioethanol from banana pseudostem waste (*Musa balbisiana*) pretreated with potassium hydroxide (KOH) microwave using hydrolysis and fermentation. The effect of yeast concentrations (8%, 10%, and 12%) and fermentation times (6, 7, 8, 12, and 13 days) on the pretreated sample was also analyzed. Fermentation was carried out using enzymatic kinetic modeling with Michaelis Menten's equations to determine the reaction rate. The results showed that the sample with 12% yeast and fermentation time of 13 days produced the highest ethanol content (41.5%). In addition, the appropriate kinetic modeling results were similar to Hanes Woolf's linearization modeling. The 10% yeast concentration led to K_M values of 1.606×10^{-3} g mL^{-1} and V_{max} of 6.837×10^{-4} g mL^{-1} h.

Keywords: bioethanol, fermentation, kinetic modeling, KOH microwave, banana pseudostem waste.

INTRODUCTION

Indonesia was reported to be a major net global exporter of CO_2 in 2016, with emissions totaling 114 million tons. The increased emissions can largely be attributed to the high contributions of the electricity and machinery sectors [Wiloso et al., 2024]. To overcome this condition, several studies have proposed the use of bioethanol compounds, which have similar characteristics to gasoline and can be used as fuel alternatives. In addition, the use of these compounds offers various advantages, including minimal carbon emissions, biodegradability, high octane value, and favorable environmental impact [Morad, 2024]. Indonesia has also emphasized the development of biofuels, such as bioethanol, to meet domestic fuel demands and reduce imports [Wirawan et al., 2024]. Consequently, bioethanol holds significance as one of the pioneer renewable energy sources to achieve the target of net zero emission (NZE). Technological advancements in biomass, such as cellulosic ethanol production offer the potential to reduce carbon footprint further and make a greater contribution to decarbonization. These advancements can help bioethanol play a larger role in achieving long-term NZE goals [Kiehbadroudinezhad et al., 2023].

In line with previous studies, the world's annual production of banana plants is 155.2 million tones, with India being the largest producer, followed by China and Indonesia [Yadav et al., 2020]. Banana pseudostems have been reported to be an underutilized waste, which is often discarded and only used as makeshift bridges over rivers. However, several studies have shown that

this material can be fermented to produce bioethanol products as an alternative energy source [de Souza et al., 2017; Liebl et al., 2019]. Banana pseudostem waste a type of biomass containing lignocellulose, indicating its potential to be processed in bioethanol [Ingale et al., 2014]. This is in line with Kolajo et al. [2023], who reported the use of alkali pretreatment experiments during the processing. Somaprabha et al. [2022] also evaluated the potential of agricultural waste as raw material for bioethanol. The results showed that banana pseudostem waste provided a favorable substrate for microbial activity. In addition, microbial activity served as a biocatalyst to break down the lignocellulosic biomass in the substrate [Badanayak et al., 2023]. The use of banana pseudostem waste offers several sustainability benefits, including reduced waste disposal problems, improved waste management, and the potential for renewable energy production [Li et al., 2016].

According to Sawarkar et al. [2022], a series of physical electric pretreatment processes through ultrasonic microwaves have shown promising progress for pretreating banana pseudostem. The internal heating generated through microwave irradiation directly facilitates the saponification of ester bonds cross-linking hemicelluloses and lignin, thereby improving their removal. In addition, this offers a quicker and more energy-efficient method for pretreating samples [Thi Thuy Van et al., 2022]. In this study, alkali-physical pretreatment, particularly the potassium hydroxide (KOH) microwave method was used on banana pseudostems, followed by hydrolysis and fermentation into bioethanol. Alkali pretreatment combined with microwave assistance can yield higher glucose levels compared to the samples treated without microwave assistance [Samuel Agu et al., 2022]. Alkali played a significant role in disrupting the bonds between lignin and carbohydrates in the plant biomass. This helped to loosen the highly cross-linked structure of lignin, leading to partial depolymerization. Microwave heating accelerated the reaction and its combination with alkali provided an effective pretreatment method by efficiently disrupting biomass structure. The mechanism of action is typically related to delignification, which enhances enzymatic hydrolysis and sugar release [Gabhane et al., 2014].

In line with these results, Kusmiyati et al. [2018] conducted bioethanol fermentation from banana pseudostems using various types of microbes and pH levels. Corrales et al. [2012] also found that the estimated ethanol yield from fermented banana pseudostems after acid pretreatment was 187 liters per ton. On the basis of existing literature, there are no reports on the fermentation of bioethanol with various yeast concentrations and fermentation times *(Musa Balbisiana*) using KOH microwave. Therefore, this study aimed to analyze the effects of yeast concentration percentages and fermentation times on bioethanol production from banana pseudostems pretreated with KOH microwave. This is because concentrations of yeast can enhance ethanol production with increasing fermentation time, allowing microorganisms to decompose glucose into ethanol maximally [Aina Christalia Rinastiti et al., 2022; Roni, Kartika, et al., 2019].

Kinetic modeling was carried out with various fermentation times to determine the reaction rate. Therefore, the strengths of the study lied in the application of Michaelis-Menten equation in reaction kinetics modeling. The process comprised the use of microbes, namely *Saccharomyces cerevisiae* which had cellulolytic and solventogenic (ethanol-producing) abilities [Periyasamy et al., 2023]. Due to the non-linear nature of Michaelis-Menten equation, linearization modifications were carried out to better understand kinetic parameters. Several linear forms of the equation that had been developed included Lineweaver-Burk, Eadie-Hofstee, and Hanes-Woolf [Saganuwan, 2021; Tomczak & Węglarz-Tomczak, 2019].

A previous report was conducted regarding KOH microwave pretreatment on banana pseudostems [Anggriani et al., 2023], followed by enzymatic hydrolysis, leading to the production of 1.3 g mL^{-1} sugar [Samara et al., 2024]. Despite the existing literature, fermentation of samples pretreated with KOH microwave has not been explored. Therefore, this study also aimed to analyze the fermentation of banana pseudostems pretreated with KOH microwave as a potential raw material for bioethanol. The kinetic reaction modeling of the fermentation reaction was assessed to provide information for considerations in fermentor design, feed rate, substrate ratio, and fermentation times.

MATERIAL AND METHODS

The study procedures were carried out in various steps, including KOH microwave pretreatment, production of cellulase enzymes, yeast inoculum preparation, enzymatic hydrolysis, fermentation, distillation, and analysis.

Materials

The primary materials used in this study included pseudostems of *Musa balbisiana*, *Aspergillus niger*, and *Saccharomyces cerevisiae*. All chemicals were purchased from Merck Indonesia.

Feedstock preparation

The banana pseudostems were cut into approximately 5 cm sizes and sun-dried for 4 days, then placed in an oven at 80°C for 10 min. The dried banana pseudostems were ground using a grinder to a 35 mesh size, followed by storage and analysis for cellulose, hemicellulose, and lignin composition using the Chesson analysis method.

Chesson method analysis

The modification method [Anggriani et al., 2023] comprised adding 1 g of dried banana pseudostem sample to 150 ml of water, refluxing at 100 °C for 1 hour. In addition, the refluxed solution was filtered and washed using hot water. The residue was weighed (b), followed by the addition of 150 mL of 1 N, and refluxed again.

The solution was neutralized to a neutral pH, and the residue was dried and weighed (c). A total of 10 mL of 72% H_2SO_4 was added and soaked for 4 hours at room temperature, followed by the addition of 150 mL of 1 N H_2SO_4, refluxed for 1 hour at 100°C. The refluxed solution was neutralized to neutral, followed by drying and weighing (d). In addition, the residue (d) was washed and then weighed. Subsequently, the lignin, cellulose, and hemicellulose content (%) in the sample were calculated.

$$Hemicellulose\ content = (b\text{-}c)/a \times 100\% \quad (1)$$

$$Cellulose\ rate = (c\text{-}d)/a \times 100\% \quad (2)$$

$$Lignin = (d\text{-}e)/a \times 100\% \quad (3)$$

KOH microwave pretreatment

A total of 75 g of banana pseudostem samples were soaked in 750 mL of 5% KOH using a 1-liter beaker and maintained at 90 °C for 25 minutes with the lid closed. After this treatment, the samples were rinsed with distilled water until neutral pH was achieved and dried in an oven at 105 °C. Subsequently, banana pseudostems were placed in a microwave with a frequency of 360 Hz for 25 minutes. Rinsing was then carried out with distilled water until neutral pH was achieved, followed by drying in an oven at 105°C until a constant weight was obtained and recorded. The samples were stored and analyzed for cellulose, hemicellulose, and lignin composition.

Production of cellulase enzyme

Procedure modification after Efrinalia et al. [2022]. All equipment used in this study was previously sterilized using an autoclave for 15 minutes at 121°C. *Aspergillus niger* was cultured on PDA media and then incubated at approximately 30 °C for 120 hours. Liquid media were prepared in a volume of 100 mL containing 12.5% sucrose, 0.25% $(NH_4)_2SO_4$, and 0.2% KH_2PO_4. The pH of the liquid media was adjusted to pH 3 using HCl. The tip of an ose wire was sterilized in 96% ethanol and then heated until it turned red. *Aspergillus niger* inoculation was performed using the tip of the ose wire, which was dipped into the liquid media until it became cloudy (forming the *Aspergillus niger* inoculum), then covered with cotton and incubated at approximately 30 °C for 24 hours. Afterwards, 80 mL of growth media was prepared, involving 20 g of pretreated banana pseudostems, 0.03 g of urea nutrient, 0.005 g of $MgSO_4.7H_2O$, and 0.0023 g of KH_2PO_4 adjusted to pH 5.

After 24 hours of incubation, the *Aspergillus Niger* inoculum was added to the growth media and incubated for 96 hours. The result was added to 100 mL of distilled water and stirred on a rotary shaker at 150 rpm for 1 hour. The sample was then filtered and stored, ready for use and analysis.

Cellulase enzyme analysis

This study used a modified method [Novia et al., 2022] to analyze the activities of endo-β-glucanase, exo-β-glucanase, and protein content. Analysis of endo-β-glucanase activity involved homogenizing 1 mL of cellulase enzyme and 1% CMC in 3 mL of DNS reagent. The absorbance was then measured using a spectrophotometer at $\lambda = 540$ nm. For the analysis of exo-β-glucanase activity, 1 mL of cellulase enzyme and a 6×1 cm

Whatman no. 1 filter paper soaked in 1 mL of sodium buffer were dissolved in 3 mL of DNS reagent. The absorbance was subsequently measured using a spectrophotometer at λ = 540 nm. Protein analysis was conducted by dissolving 6 mL of cellulase enzyme in 4 mL of Bradford solution, followed by measuring the absorbance using a spectrophotometer at λ = 540 nm.

Yeast inoculum preparation

Procedure modification after Novia et al. [2023]. The equipment was sterilized using an autoclave at 120 °C for 20 minutes and then set aside to cool. YPD medium was prepared, involving 10 g of yeast extract, 20 g of peptone, and 20 g of glucose. Saccharomyces cerevisiae was inoculated with one loopful of yeast into 500 mL shake-flasks containing 150 mL of YPD medium. The inoculum was then incubated at 30 °C for 24 hours.

Enzymatic hydrolysis

Procedure modification after Efrinalia et al. [2022]. This equipment was sterilized in an autoclave at 121 °C for 60 minutes. 20 g of pretreated banana pseudostems were transferred to a 1000 ml Erlenmeyer flask, then distilled water was added at a ratio of 1:10 (w/v) to adjust to pH 5 with the addition of sodium buffer solution. Next, 10 mL of cellulase enzyme was added to the Erlenmeyer flask, securely sealed, placed on a hotplate, stirred at 200 rpm, and maintained at 50 °C for 24 hours, then cooled to 30 °C.

Reducing sugar analysis

Procedure modification after Samara et al. [2024]. Firstly, 1 mL of the hydrolysis sample was carefully transferred into a reaction tube and added with 3 mL of DNS reagent. The solution was heated in boiling water (100 °C) for 10 minutes, then diluted. After cooling, the absorbance value was measured using a spectrophotometer at λ 540 nm.

Fermentation

Procedure modification after Novia et al. [2023]. Fermentation equipment was sterilized using an autoclave at 120 °C for 20 minutes and then cooled. The pretreated banana pseudostem samples, which had been hydrolyzed, were placed in an Erlenmeyer flask equipped with a CO_2 gas outlet. 8% (v/v) of *Saccharomyces cerevisiae* yeast inoculum was added, stirred at 120 rpm, maintained at 30 °C, and fermented at varying durations, i.e. 6, 7, 8, 12, and 13 days. This procedure was repeated with the yeast concentrations of 10% and 12% (v/v). After fermentation, the product samples were distilled, and their glucose content was analyzed using a DNS solution and a UV-Vis spectrophotometer. The distilled samples were also analyzed for ethanol content using an alcohol refractometer.

Kinetic modeling

This method was conducted by plotting graphs of the glucose substrate and ethanol product against time. Slope and intercept values were determined, enabling the calculation of V_{max} and K_M values.

RESULT AND DISCUSSION

Composition of raw materials before and after pretreatment

The initial weight of banana pseudostem waste (*Musa Balbisiana*) obtained was 102.3 kg, which was subsequently prepared to yield 1.2 kg of powdered banana pseudostems after grinding. This preparation process effectively removed 98.8% of the water content from the banana stem. The removal of water content was necessary to facilitate the delignification process and subsequent steps. Water content removal was crucial, as it could impede and reduce the effectiveness of the bioethanol production process. Second-generation bioethanol production from banana pseudostem waste emphasized the necessity of removing water from biomass to enhance the efficiency of the process. This was achieved through following mechanical and chemical pretreatments, which served to improve the bioethanol yield [Chandrasiri et al., 2022].

The composition results were presented in Table 1, explaining that hemicellulose and cellulose increased, while lignin content decreased after pretreatment. This pretreatment was a delignification process to break down and remove lignin from lignocellulosic biomass, to enhance the availability and utility of the cellulose component [Deivy Andhika Permata et al., 2021].

Table 1. Analysis results of banana pseudostem sample composition

Type of treatment	Composition analysis results (%)		
	Hemicellulose	Cellulose	Lignin
Before KOH pretreatment microwave	30.84	58.58	5.16
After KOH pretreatment microwave	43.67	74.76	3.98

Table 2. Comparison of banana stem composition analysis results in previous studies

Raw material	Pretreatment method	% increase in cellulose	% loss of lignin	References
Banana pseudosteam (unknown)	KOH microwave (6% KOH, 95 °C, 50 min)	43.26	60.68	[Anggriani et al., 2023]
Banana pseudostems (*Musa balbisiana*)	KOH microwave (5% KOH, 90 °C, 25 min)	27.62	22.87	This study
Banana pseudosteam (unknown)	KOH microwave (5% KOH, 80 °C, 25 min)	11.87	8.70	[Samara et al., 2024]
Banana pseudostems (*Musa balbisiana*)	NaOH (10% NaOH, 150 °C, 30 min)	20.80	48.72	[Erlinawati et al., 2023]
Parthenium hysterophorus	KOH (conc. 0.5%)	12.29	20.33	[Kumar et al., 2023]
Banana pseudostems (from local area in Duque de Caxias)	NaOH (conc. 25%)	15.68	9.61	[Shimizu et al., 2018]
	H_2O_2 (conc. 8%)	13.53	10.09	
	H_2SO_4 (conc. 25%)	5.44	(just increased lignin about 52.3%)	

Table 2 illustrated that the KOH microwave pretreatment method in this study could remove 22.87% of lignin while increasing cellulose by 27.62%, and hemicellulose by 41.60% compared to the raw composition in banana pseudostem waste (*Musa balbisiana*) before pretreatment. The study conducted by Anggriani et al. [2023] showed a greater increase in cellulose and a substantial reduction in lignin compared to these findings. Consequently, this concluded the KOH microwave pretreatment method, by increasing its concentration, raising the temperature, and extending the duration. This led to increased cellulose and reduced lignin in banana pseudostems.

Compared to other studies, KOH was utilized by following microwave pretreatment on banana pseudostems that increased the release of lignin from the covalent bonds of lignocellulose. As depicted in Figure 1, the filtrate water was dark black, indicating that the lignin bonds had been successfully released from the cellulose. However, the banana pseudostems treated with the microwave also exhibited the filtrated color indicating the presence of lignin in the samples. The use of a microwave in this process maximized the efficiency due to its magnetron circuit component, which could emit electromagnetic waves, thus degrading the lignocellulosic bonds that remained in the

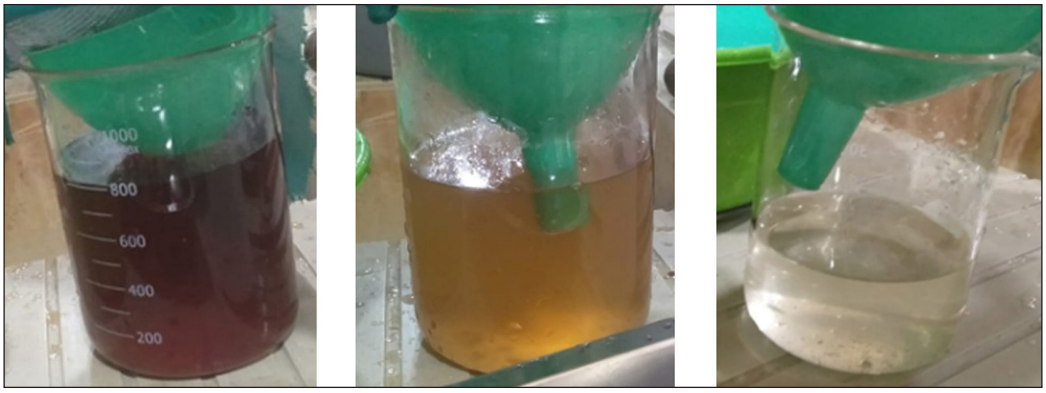

Figure 1. Filtrate colors of samples: (a) untreated; (b) pretreated KOH, (c) pretreated KOH microwave

banana pseudostems. Microwave radiation could reduce the production of inhibitors, facilitating the depolymerization of cellulose into easily fermentable monomers [Sawarkar et al., 2022].

Characteristics of cellulase enzyme

As presented in Table 3, endo-β-glucanase and exo-β-glucanase had an activity of 81.43 U·mL^{-1} and 211.43 U·mL^{-1} respectively. In the study by Malik & Javed [2024], the maximum enzyme activity of endoglucanase was found to be 0.56 U mL^{-1} using carboxymethyl cellulose substrate, this enzyme was produced by *Bacillus tequilensis*. Sarangthem et al. [2023] investigated the cellulase enzyme activity produced from *Bacillus velezensis* with substrates of rusk straw resulting from CMCase activity of 48 U·mL^{-1}. This was due to straw, which had higher lignin content as the straw plant parts function as a structure that provided strength and resilience to rice plants, this strength held the lignocellulosic matrix together [Erfani Jazi et al., 2019]. Conversely, banana pseudostems had a softer structure with lower lignin contained. Compared to Malik & Javed [2024], carboxymethyl cellulose substrate was chemically modified, making it difficult for cellulase enzymes to access, thereby reducing cellulose breakdown efficiency compared to natural substrates in banana pseudostems. It was concluded that the cellulase enzymes exhibited higher activity, since *Aspergillus niger* was inoculated into the sample media of banana pseudostems which had amount of cellulose composition positively being more accessible. Moreover, its structure was more easily degraded compared to rice straw and carboxymethyl cellulose. This study also had higher activity of cellulase enzymes compared to the findigs of Samara et al. [2024] who obtained 77.55 U mL^{-1} of endo-β-glucanase and 0.05 U·mL^{-1} of exo-β-glucanase. Although both studies used the same method on banana pseudostems with KOH microwave, the different results were caused by sterilization maintenance practices conducted to ensure that environmental conditions, such as pH, temperature, and nutrients, remained optimal for the growth and production of enzymes by microorganisms. Sterilization was also important in the enzyme purification stage to prevent contamination and maintain the purity of the produced cellulase enzyme.

The specific activity of cellulase enzymes in the previous study was considered appropriate when it fell within the range of 100 to 1000 U mg^{-1}, protein also typically ranging from 500 to 1000 U mg^{-1}. Purification studies of cellulase enzymes from *Aspergillus niger* using sawdust resulted in enzyme activity of 500–1500 U·mg^{-1} protein depending on the level of purification [Emmanuel & Queen, 2017]. Optimization studies of cellulase enzyme production from *Aspergillus flavus* yielded enzyme activity of 1000–1500 U mg^{-1} protein [Arun Sasi, 2012]. The conversion of enzyme activity units, which were valued at 1118 U mg^{-1} of enzyme activity. It was concluded that cellulase enzymes were produced effectively for hydrolyzing cellulose into glucose.

The analysis results depicted in Table 3 also indicated that there was 0.18904 mg mL^{-1} of protein contained in the cellulase enzyme, compared to Samara et al. [2024], where protein contented 0.086 mg·mL^{-1} of cellulase enzymes. Therefore, this study indicated higher enzyme purity, which could support the efficiency of cellulose hydrolysis into glucose. On the basis of the analysis, this study achieved higher activity, which was due to the different types of banana pseudostem substrates and the precision of sterilization practices during the findings.

Reducing sugar content after hydrolysis

According to the result shown in Table 4, the amount of reducing sugar produced from the enzymatic hydrolysis process for 24 hours was 5.47390 mg·mL^{-1}. This was high compared to the study by Samara et al. [2024], which obtained a sugar reduction content of 1.3 mg·mL^{-1} for the

Table 3. Results of cellulase enzyme activity analysis

Cellulase enzyme	Absorbance 540 nm	Concentration		Activity (U mL^{-1})
		ppm	(mg mL^{-1})	
Endo-β-glucanase	0.11461	879.40000	0.87940	81.42593
Exo-β-glucanase	0.29759	2283.40000	2.28340	211.42593
Protein	0.02261	189.04000	0.18904	-

Table 4. Comparison of reducing sugar content in previous studies

Raw material	Pretreatment	Hydrolysis	Reducing sugar content	References
Banana pseudostems (*Musa balbisiana*)	KOH microwave (5% KOH, 90 °C, 25 min)	Enzyme-to-substrate ratios 1:2 (Cellulase enzyme from Aspergillus Niger) (50 °C, 200 rpm, 45 h)	5.47 mg mL^{-1}	This study
Banana pseudosteam (unknown)	KOH microwave (5% KOH, 80 °C, 25 min)	Enzyme-to-substrate ratios 1:1 (Cellulase enzyme from Aspergillus Niger) (50 °C, 200 rpm, 45 h)	1.3 mg mL^{-1}	[Samara et al., 2024]
Rice husks	H_2O_2 aqueous ammonia pretreatment (3% H_2O_2, 85 °C, 6 h)	Enzyme-to-substrate ratios 25% to 50% (v/w) (Cellulase enzyme from Aspergillus Niger) (50 °C, 200 rpm, 45 h)	2.21 mg mL^{-1}	[Efrinalia et al., 2022]
Banana pseudosteam (unknown)	NaOH (NaOH, 2N, 90 min)	Enzyme-to-solution ratios 1:10 (SQzyme CS 20,000 u/g) (50 °C, 150 rpm, 72 h)	5.0 mg mL^{-1}	[Kusmiyati & Sukmaningtyas, 2018]

same samples of banana pseudostems pretreated with a KOH microwave. This was due to the different enzyme-to-substrate ratios used. Samara et al. [2024] used a 1:1, while this study used a 1:2. Efrinalia et al. [2022] also revealed that the highest reducing sugar concentration was 2.21 mg·mL^{-1} with enzyme-to-substrate ratios ranging from 25% to 50% (v/w). It was concluded that the enzyme-to-substrate ratios provided proper nutritional sufficiency for cellulase enzyme activity in banana pseudostem substrates. This ratio effectively minimized the appearance of contaminants that could interfere with enzyme activity during the hydrolysis process. Kusmiyati & Sukmaningtyas [2018] found a sugar content of 5.0 mg·mL^{-1} after 24 hours of enzymatic hydrolysis, their study used cellulase enzymes with higher activity on banana pseudostem samples. The conclusion was that enzyme activity and the enzyme-to-substrate ratios affected the reduction of sugar in enzymatic hydrolysis activity.

Effect of yeast concentrations and fermentation time on the alcohol content produced

The banana pseudostems that were hydrolyzed were further fermented with varying yeast concentrations of 8%, 10%, and 12% for 6, 7, 8, 12, and 13 days of fermentation duration. The results of the ethanol content analysis were illustrated in Figure 2. The banana pseudostems treated with KOH-microwave pretreatment in variation 12% yeast concentrations during 13 days of fermentation times led to a higher ethanol content compared to the samples with 8% and 10% yeast concentrations. The study by Roni, Hastarina, et al. [2019] also revealed the highest ethanol content with the maximum yeast weight (12 g) and the longest fermentation duration (9 days). Pratiwi & Ni Made [2023] also indicated that the longer the fermentation time produced, the higher the ethanol, due to microbial activity converting glucose into ethanol. Therefore, the higher the concentration of yeast

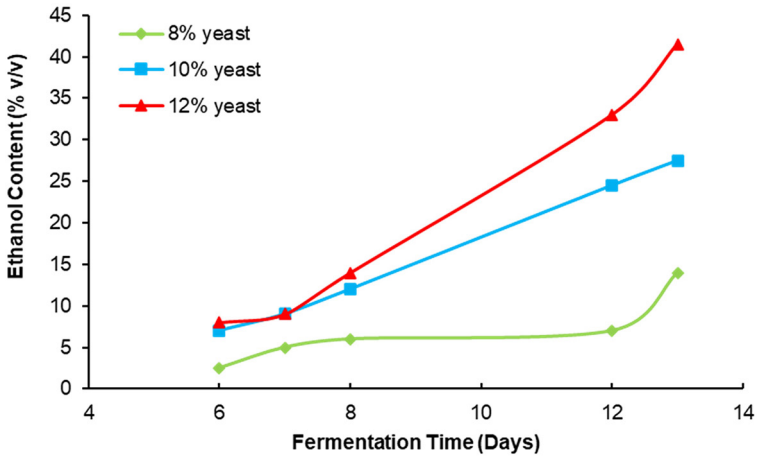

Figure 2. Fermentation times vs ethanol content

and the longer the fermentation time, the higher the ethanol production could be. Table 4 presented the results of ethanol content analysis from banana pseudostems compared to the previous study.

According to Table 5, it was shown that this study produced higher ethanol content compared to other findings, which used banana pseudostems as lignocellulosic biomass mostly. Overall, the combination of appropriate pretreatment, hydrolysis, and fermentation methods led to higher ethanol yielded from lignocellulosic biomass. Optimizing each process stage through the selection of suitable methods was important for improving the efficiency of biomass conversion into ethanol.

Kinetic modeling

The fermentation process applied enzymatic kinetics, as it involved *Saccharomyces cerevisiae*, which produced enzymes that catalyzed glucose into ethanol [Felix et al., 2014; Nnaemeka et al., 2021], using alcohol dehydrogenase (ADH). The fermentation process was described as applying enzymatic kinetics, where the enzymes were responsible for the interconversion of acetaldehyde and ethanol [Abu Rayyan et al., 2019; de Smidt et al., 2012].

$$v = \frac{Vmax \cdot [S]}{K_M + [S]} \quad (4)$$

The Michaelis Menten equation explained the enzyme catalysis rate as a function of substrate concentration [Srinivasan, 2022]. This study plotted graphs using the linearization of the Michaelis-Menten, which consisted of Lineweaver and Burk, Eadie Hofstee, and Hanes Woolf. The graph were shown in Figures 3, 4, and 5. After plotting, the definitions of the K_M and V_{max} values were obtained [Riyadi et al., 2024], as presented in Table 6.

Vmax indicated the maximum enzymatic reaction rate, while K_M showed the Michaelis-Menten constant. This revealed that the substrate concentration was at the optimum level, while the substrate was converting appropriately into products [Abdullah et al., 2024]. Variations of 10% yeast concentrations in the Hanes-Woolf model plot gave the coefficient of determination closest to 1 compared to the Lineweaver-Burk and Eadie-Hofstee plots. This sample provided an R^2 value of 0.9978, indicating that this linear regression model could accurately explain the variation in the data. In other words, 99% of the data variability was explained by the Hanes-Woolf linear regression model, which provided better regression values compared to other linearization equations. The Hanes-Woolf plot minimized the influence of experimental errors at low substrate values, where 1/[S] in other linearization equations tended to amplify the errors. Aledo's [2021] study found that Viscozyme-L data extracted from the literature using the Hanes-Woolf method was

Table 5. Comparison of ethanol content in previous studies

Raw Material	Pretreatment	Hydrolysis	Fermentation	Ethanol content % (v/v)	References
Banana pseudostems (*Musa balbisiana*)	KOH microwave (5% KOH, 90 °C, 25 min)	*Cellulase* enzymes (50% (v/w), 24 h)	*Saccharomyces* yeast (12% (v/v), 13 days)	41.50	This study
Banana pulp, peels and pseudostem (*Musa spp. Cavendishii*)	H_2SO_4 (conc. 2%, 120°C, 15 min)	*Cellulase-hemicellulase* enzymes (6 wt% Cellic CTec2, 2 wt% *Aspergillus niger* H2125 , 45°C, 24 h, pH 5.5, 120 rpm.	*Saccharomyces* yeast-*Pachysolen tannophilus* (6 g/L, 30°C, 120 rpm)	40.0	[Uchôa et al., 2021]
Banana pseudostems (unknown)	NaOH (NaOH 2 N, 90 min)	Cellulase enzymes (SQzyme CS) (1 g, 24 h)	*Saccharomyces* yeast (10% (v/v), 5 days)	0.43	[Kusmiyati & Sukmaningtyas, 2018]
Banana pseudostems (unknown)	Acid-alkali (H_2SO_4-NaOH) (2% (w/v), 121 °C, 30 min)	SSF *Aspergillus niger*, *Trichoderma reesei*, *Zymomonas mobilis* (1:1:2, pH 5)		0.85	[Kusmiyati et al., 2018]
Sorghum seeds	-	*Alpha amylase, gluco amylase*	*Saccharomyce cerevisiae*, *Rhizopus oryzae*, *Acetobacter xylinum*, *Mucor* sp., *Aspergilus niger* (Conc 2.5%, 42 h)	11	[Roni, Kartika, et al., 2019]

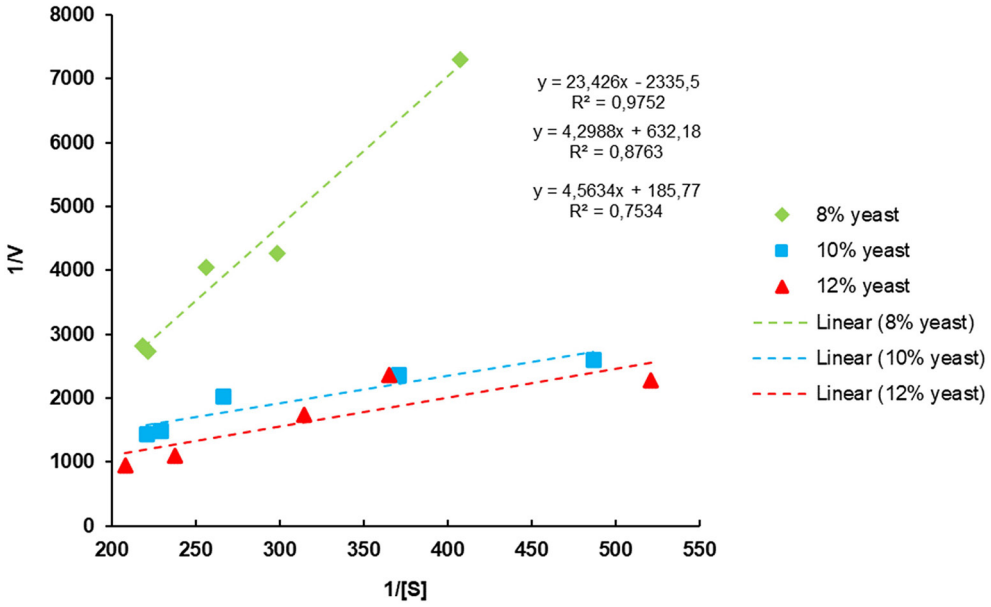

Figure 3. Lineweaver and Burk Plot of 1/[S] versus 1/V

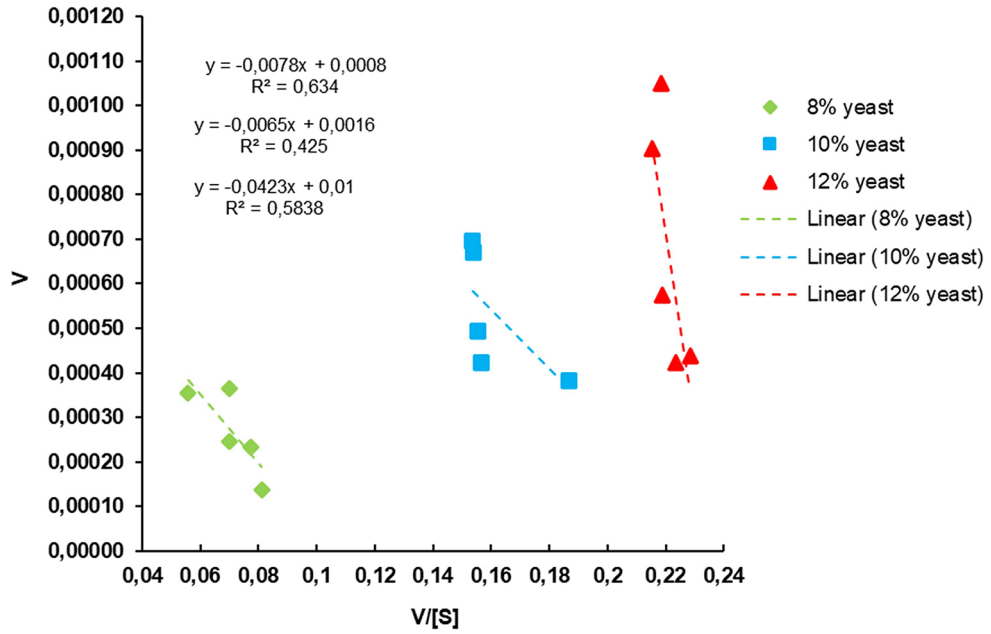

Figure 4. Eadie Hofstee Plot of V/[S] versus V

able to produce the estimates of K_M and V_{max} that were almost identical to those obtained through direct nonlinear regression of the Michaelis-Menten equation. Aledo's [2021] study also revealed a negative K_M value, which demonstrated that the analysis yielded physiologically invalid kinetic parameters. Knizikevičius's study [2018] indicated that the Hanes-Woolf plot provided kinetic constant values with the lowest mean absolute percentage errors compared to the true values determined from the saturation curves. Meanwhile, the Eadie-Hofstee plot underestimated the experimental error variance, detecting outlier data points, but yielding less accurate constants. The Lineweaver-Burk plot gave the poorest agreement with saturation curve analysis due to emphasizing low-pressure data.

Variations of 10% yeast concentrations in the Hanes-Woolf plot resulted Vmax value of 6.836×10^{-4} g mL^{-1} h^{-1}, indicating the maximum reaction rate value, where the reaction rate curve did not change when more substrate was

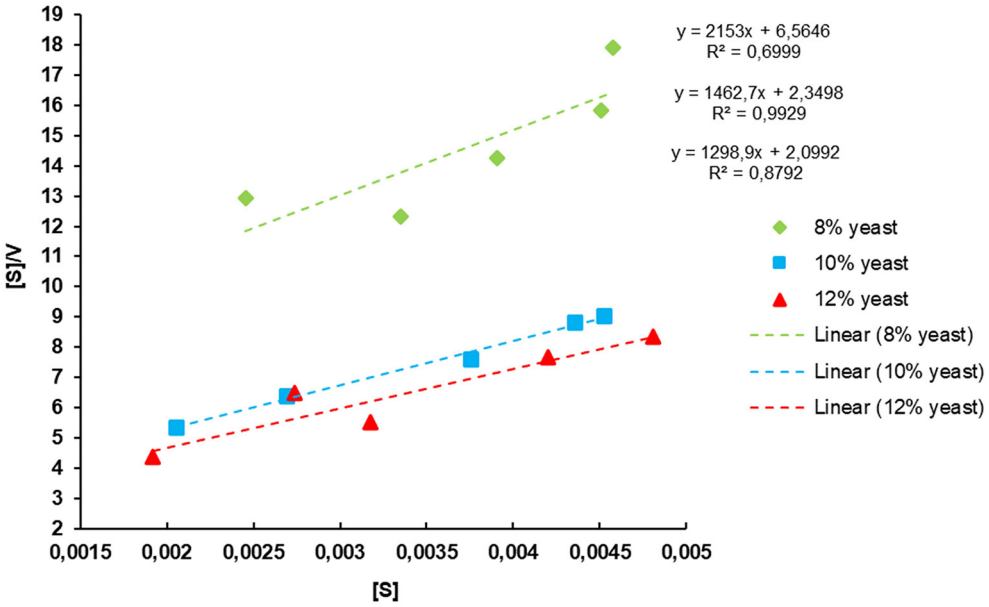

Figure 5. Hanes Woolf plot of [S] versus [S]/V

Table 6. Result K_M and V_{max}

Linearization equation	Yeast concetrantions (% v/v)	K_M (g mL^{-1})	V_{max} (g mL^{-1} h^{-1})	R^2
Lineweaver and Burk	8	3.039×10^{-5}	4.282×10^{-4}	0.9752
	10	4.799×10^{-3}	1.582×10^{-3}	0.8763
	12	2.456×10^{-2}	5.383×10^{-3}	0.7534
Eadie-Hofstee	8	7.800×10^{-3}	8.000×10^{-5}	0.6340
	10	6.500×10^{-3}	1.600×10^{-4}	0.4250
	12	4.230×10^{-2}	1.000×10^{-3}	0.5840
Hanes-Woolf	8	3.049×10^{-3}	46.444×10^{-5}	0.6999
	10	1.606×10^{-3}	6.837×10^{-4}	0.9929
	12	702.308×10^{-5}	334.56×10^{-5}	0.8792

continuously added. Furthermore, a K_M value of 1.606×10^{-3} g mL^{-1} indicated that the substrate tended to form enzyme-substrate complexes, which subsequently form final products. Oktoh et al. [2024] study, which modeled the kinetics of bioethanol fermentation from sweet sorghum stalk juice, revealed a fermentation kinetics V_{max} value of 35×10^{-5} g mL^{-1} h^{-1}, however, with an R^2 of 0.77. A higher V_{max} value was found, compared to Oktoh et al. [2024], the regression obtained was closer to 1, thus explaining the data variation accurately. Oktoh et al. [2024] also found a K_M value of 1.256×10^{-2} g mL^{-1}, compared to this study, which resulted in a smaller K_M value. However, according to Tomczak & Węglarz-Tomczak [2019], the smaller KM indicated higher substrate affinities for binding with the enzyme. It was concluded that an appropriate kinetic modeling was the Hanes-Woolf linearization modeling at variation 10% yeast concentrations, which resulted in K_M value of 1.606×10^{-3} g mL^{-1}, V_{max} value of 6.837×10^{-4} g mL^{-1} h^{-1}, and 0.9929 of regression.

CONCLUSIONS

In conclusion, banana pseudostem waste (*Musa Balbisiana*) pretreated with KOH microwave had good potential in producing bioethanol as an alternative fuel source. This was evident by the analysis of the influences of varying percentages of yeast concentrations and fermentation duration. Furthermore, banana pseudostems (*Musa B/albisiana*) with 12% yeast concentrations and 13 days of duration produced higher ethanol contents compared to other variations.

The appropriate kinetic modeling for this fermentation reaction pretreated with KOH microwave was the linearization modeling of Hanes-Woolf, which showed K_M value of 1.606×10^{-3} g mL^{-1} and V_{max} value of 6.837×10^{-4} g mL^{-1} h^{-1} with regression of 0.9929.

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