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Kinetics Study of Bioethanol Production from Cassava Peels Waste using *Saccharomyces diastaticus*

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Abstract

In addition to affecting its supply, the usage of fossil fuels pollutes the environment. A potential remedy for the diminishing supply of fossil fuels is bioethanol. It is possible to make bioethanol from substances that include carbs. In this study, cassava peel waste is the primary component used to make bioethanol. Prior to fermentation, lignin must be reduced as it has the potential to obstruct the production of ethanol. In this study, an alkaline solution in the form of NaOH was used as the chemical pretreatment. *Saccharomyces diastaticus* is used in this study to aid in the fermentation of bioethanol from leftover cassava peels. Saccharification and fermentation were used concurrently during the fermentation process because they are easy, quick, and affordable. The objectives of this study are to characterize cassava peel flour, investigate kinetics parameters such as *Saccharomyces diastaticus* growth kinetics, product formation kinetics, and substrate usage kinetics, and determine the impact of substrate variation on ethanol produced. The percentages of substrate that were used were 10%, 15%, and 20% (weight/volume). At 15% (weight/volume) substrate concentration, or 6.172 g/L, the maximum bioethanol concentration was reached. With a maximal specific growth rate of 0.0442 h^{-1}, a product formation coefficient of 0.503, an unfollowed product formation coefficient of 0.005, and a substrate utilization coefficient of 0.005 h^{-1}, the kinetic parameters of yeast growth, substrate consumption, and ethanol production were displayed.

Keywords: Bioethanol, cassava peel waste, kinetic parameters, *Saccharomyces diastaticus*, SSF.

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1. Introduction

Future fuel consumption in Indonesia will be influenced by patterns of daily energy consumption that involve the use of fossil fuels. It is highly probable that Indonesia will eventually have to import coal, natural gas, and petroleum if the country's fossil energy consumption habits remain unchanged. In addition, using fossil fuels pollutes the environment. The earth's atmosphere is becoming hotter due to environmental pollution, which includes greenhouse gases like CO_2, CH_4, and NO_2, global warming, and CO_2 emissions. These gases can form a layer in the atmosphere and retain heat that would otherwise escape the planet [1,2]. Bioethanol is one of the environmentally acceptable alternative fuels that are therefore required. Broadly speaking, bioethanol is ethanol that is made through fermentation using raw materials derived from renewable biomass sources, including organic waste, forests, agricultural plants, and other plants that are high in carbs. This carbohydrate is used to produce bioethanol from cassava peels (*Manihot esculenta crantz* or *Manihot utilissima*). Salatiga produces about 4,793 tons of cassava year with a 142hectare land area under cultivation and 337.51 quintals per hectare of yield, according to Statistics Indonesia data. 36.5% starch is found in cassava peels, according to the analysis's findings. Microorganisms can use cassava peel as an energy source because of its high starch content [2]. Lignin, another substance found in cassava peel waste, inhibits the production of ethanol. Alkaline solutions must be used to treat cassava peel waste in order to lower or completely remove the lignin level. It is possible to pretreat lignocellulosic material physically, chemically, or biologically. Acid, alkali, and cellulose solvent reagents are used to treat chemical pretreatments that can be performed [3]. The delignification method is a raw material pretreatment that makes the application of hemicellulose easier. The lignin content is decreased using this method. There are several ways to do delignification first steps, including chemical (solvent and SO_2 gas) and physical (milling, heating, radiation, or heating with dry air). Following the substrates concentration and length of immersion, a chemical and physical delignification treatment using NaOH was employed

in this investigation. Since caustic soda is comparatively less expensive than other chemical reagents and efficiently boosts the hydrolysis output, it was selected [4]. The two primary steps in the production of bioethanol from biomass are generally hydrolysis and fermentation. The majority of earlier techniques involved doing the hydrolysis and fermentation processes independently, or separated hydrolysis and fermentation (SHF), and the most recent technique combined saccharification and fermentation. One method for producing ethanol from lignocellulose is simultaneous saccharification and fermentation, or SSF. Reduced end-product inhibition of the enzymatic hydrolysis and lower investment costs are the main advantages of carrying out the enzymatic hydrolysis concurrently with the fermentation as opposed to doing it in a separate step after the hydrolysis. On the other hand, the main disadvantages are the inability to recycle the fermenting organism and the enzymes and the requirement to establish ideal conditions (such as pH and temperature) for both enzymatic hydrolysis and fermentation [5]. In order to meet the first criterion, the temperature is often maintained below 37°C. On the other hand, because it is challenging to recycle the yeast, it is advantageous to operate at low yeast concentration and high solid loading. The amount of bioethanol produced during fermentation is influenced by the duration of the fermentation process. Up until the maximum amount of bioethanol is reached, the amount of bioethanol generated increases with the length of the fermentation process [6]. In order to fill in the previously described knowledge gap, the following general aims requirements were met in this work: (1) To measure the amount of lignin, cellulose, and hemicellulose in cassava peel flour; (2) To find out how much of a yield is produced when bioethanol is produced using cassava peel and *Saccharomyces diastaticus*; (3) To determine how much of a yield is produced when fermentation time is taken into account; and (4) To determine the kinetics parameters of bioethanol fermentation using cassava peel raw materials using *Saccharomyces diastaticus*.

2. Materials and methods

2.1 Biomass and pretreatment

Waste made from cassava peels was gathered at Salatiga, Central Java, Indonesia. Before being used, the leftover cassava peel waste is cleaned, dried in the sun, ground into flour, and dried once more for 12 hours at 55°C in an oven. Using the Chesson Datta method, the content of hemicellulose, cellulose, and lignin was ascertained [7]. A 5M NaOH solution was applied to the cassava peel flour and left it at 100°C for 90 minutes. After that, the biomass was dried at 50°C and cleaned with distilled water for later usage [8].

2.2 Preparation cultures

Using a sterile tube, cultures that develop on slanted agar media with scratch grooves are extracted. After that, it is slantedly scratched onto MEA (Malt Extract Agar) media to inoculate it. It was then incubated for 24 hours at 30 °C [9].

2.3 Growth pattern of Saccharomyces diastaticus

10% (v/v) of the culture was cultured in Malt Extract Broth (MEB) medium to ascertain the growth pattern of *Saccharomyces diastaticus*. The optical density (OD) was then measured every hour for 24 hours using a spectrophotometer set to 660 nm wavelength (λ). After that, the OD value was transformed to show how many cells are growing in MEB medium [10].

2.4 Simultaneous saccharification and fermentation

Following treatment (per treatment), the 250 ml Erlenmeyer flask containing cassava peel flour was autoclaved for 15 minutes at 121°C. Next, a 10% (v/v) inoculum and the saccharification enzyme were introduced. For 60 hours, the fermentation process was run at 30°C with 120 rpm of stirring speed. The amount of ethanol and glucose that were created was then analyzed.

2.5 Analysis of bioethanol content

The examination of bioethanol content was performed using Shimadzu LT-04-044 Chromatography GC-17A.

2.6 Kinetic parameters

The net specific growth rate, which can be expressed as follows, is a measure of the microbiological growth rate:

$$\mu=\frac{1}{X}\frac{dX}{dt}$$

where μ is the specific microbial growth, t is the time (hours), and X is the biomass concentration (g/L). The maximum specific microbiological growth rate (μ_m) and saturation constant (K_s) are found using the Monod equation [11].

$$\mu=\frac{\mu_m S}{K_S+S}$$

$$\frac{1}{\mu}=\frac{K_S}{\mu}\frac{1}{S}+\frac{1}{\mu m}$$

where, S is substrat concentration (gram/liter). The slope value of the line equation, referred to as μ_m, was obtained for all three substrate concentrations [11].

Plotting -dS/dt against the time parameter allows for the estimation of the substrate utilization coefficient (k_3) in the first-order equation using linear regression analysis (first-order model) [12]. The following formula is applied:

$$-\frac{dS}{dt}=k_3 S$$

The Luedeking-Piret mixed association equation can be used to find the product formation coefficient (k_1) and the non-associated product formation coefficient (k_2) [13]. This is the formula that was applied.

$$\frac{dP}{dt}=k_1\frac{dX}{dt}+k_2 X$$

P stands for product concentration in grams per liter.

3. Results and Discussions

3.2 Characteristics of cassava peel flour

Approximate characterisation is used to determine the water, ash, protein, fat, and carbohydrate contents of cassava peel flour, as shown in Table 1. Proximate tests were used to assess the nutritional value of different cassava peels. Three separate locations Otukpo City, Apa, and Ushongo are the sources of cassava peels. The nutrients found in cassava peels were found to have moisture contents between 9.20 and 16.50%, ash contents between 1.28 and 4.05%, protein contents between 2.06 and 4.55%, lipid contents between 0.40 and 1.33%, and carbohydrate contents between 69.35 and 77.28%. A portion of the results reported regarding nutrient content do not align with current theories. The obtained protein and water contents were lower than those of earlier investigations. The results showed higher levels of fat and ash concentration than in earlier research. Various cassava cultivars, peeling techniques, and soil composition can all contribute to this [14].

3.2 Pretreatment of cassava peel waste

The stable complex lignin is what gives biomass lignocellulose its structure. The molecular structure of lignin is radically different due to its aromatic polysaccharide system, which is made up of units of phenyl propane. Pretreatment is required for lignin, a molecule that slows down the fermentation process because it is sticky. The flour made from cassava peels contains lignin, hemicellulose, and cellulose, which is also referred to as lignocellulosic biomass. A large network of intra- and intermolecular hydrogen bonds found in cellulose allow it to produce microfibrils with a high glucose bond strength. Another type of carbohydrate that gives the cell wall structural strength is hemicellulose, which forms microfibrils from cellulose fibers and binds them together with lignin. The complex molecule known as the lignin carbohydrate complex is created when lignin forms covalent connections with hemicellulose. Hemicellulose fibers are linked to cellulose microfibrils, which are covered in lignin molecules. As a result, it is challenging to disassemble the intricate structure that has developed [15].

When testing the content of cassava peel flour after delignification, the Chesson-Datta method revealed hemicellulose of 42.6%, cellulose of 54.7%, and lignin of 1.7%. However, the Chesson-Datta analysis for the content of cassava peel flour showed hemicellulose of 36.0%, cellulose of 44.0%, and lignin of 15.6%. According to the tests, following delignification, there was a drop in the amount of lignin and an increase in the amount of cellulose and hemicellulose. The chemical composition of plant materials can be examined using the Chesson-Datta method, which is particularly useful when examining the impact of treatments like sodium hydroxide on cereal straw. The process entails evaluating how treatment has affected the structural polysaccharide and other component composition of the plant material. The outcomes are typically displayed in a table that compares the chemical makeup of the plant material before and after treatment, along with the elements that were recovered. Studies on how microorganisms in the stomachs of ruminant animals break down plant material frequently employ this technique [7].

Previous research (Pooja & Padmaja, 2015) yielded hemicellulose content of 23.40%, cellulose content of 14.17%, and lignin content of 10.88% in cassava peel flour that was not subjected to the delignification process [16]. According to another study, 32.36% hemicellulose, 9.71% cellulose, and 16.89% lignin were found in cassava peel flour that had not undergone delignification process [17]. According to the two earlier investigations, the predominant composition in cassava peel flour is hemicellulose, with cellulose and lignin coming in second. Table 2 results for the flour composition of cassava peel differ from those of earlier research. Whereas hemicellulose predominated in earlier investigations, cellulose made up the majority of the composition in the results obtained from cassava peel flour. Various cassava varieties, peeling techniques, and soil composition can all contribute to variations in the results [14].

The breakdown of lignin, which covers cellulose in lignocellulosic structures, is known as delignification. Delignification research, which involved the use of alkali and microwave heating techniques, the cellulose content increased from 14.73% to 48.34% prior to delignification [18]. The decreased content of other components, including hemicellulose, is one of the causes of the increase in cellulose content. Because cellulose has a higher degree of polymerization and is partially shielded by a crystalline structure, it is less prone to deterioration than hemicellulose. Short sugar chains formed by the hemicellulose content can impede the fermentation process. Hemicellulose has an acid-soluble structure, a low degree of polymerization, and it dissolves readily in alkali. Studies employing techniques like microwave and alkali heating revealed a 13.09% to 49.32% reduction in hemicellulose concentration compared to pre-delignification levels. Lignin shields cellulose from moisture and is a stiff material. At high temperatures, lignin can transform into methanol, acetic acid, and formic acid. Alkaline solutions can be used to oxidize lignin. By lowering the lignin proportion, delignification seeks to degrade lignin and make cellulose more soluble for the subsequent procedure. Studies that employed techniques like microwave cooking and alkaline heating revealed a 3.65% drop in lignin content to 32.73% from pre-delignification levels. The results of delignification contrast from those of earlier studies, which showed that hemicellulose content increased from 16% to 28.6% following delignification a rise of 78.75% from before to delignification. Numerous reasons, including restricted hemicellulose breakdown, loss of lignin covering hemicellulose, and chemical interactions during delignification, can contribute to the increase in hemicellulose during delignification of cassava peels [19].

The results of the fourier-transform infrared spectroscopy (FTIR) analysis, which are shown in Figure 1 and indicate that the peak absorption in cassava skin and delignified cassava skin have essentially the same wave number, corroborate the findings of the Chesson Datta analytical test. This suggests that the majority of each material's constituent functional groups are identical. Nevertheless, minor variations in the absorption peaks generated by each material are still discernible; these variations are visible in a few tiny absorption peaks at particular wave numbers. The primary characteristic of cellulose is the stretching of the O-H functional group, which results in a large absorption peak at wave numbers 3200 cm^{-1} and 3400 cm^{-1}. An absorption peak at 2890 cm^{-1} was observed using the lignin removal method, showing both symmetrical and asymmetrical stretching of the C-H functional groups. The aromatic ring structure of lignin is represented by the absorption peaks at 1609 cm^{-1} and 1611 cm^{-1}, which correspond to C=C functional groups and -CH2

bonds, respectively. The aromatic ring structure of lignin exhibits a diminishing absorption peak in the 1318 cm^{-1} wave number region of delignified cassava peels [20].

This suggests that cassava peels lignin content may be decreased through the delignification process. The wave number range of 1148 cm^{-1} and 1146 cm^{-1} contained the absorption peak of cellulose containing the CH_2 functional group. The stretching of β-glycosidic bonds, specifically the stretching of the C-O-C functional group and the lowest C-H vibration, which indicate an increase in the crystallinity value of cellulose, is indicated by the absorption peaks of cassava peel products both before and after delignification. These peaks showed an increase in absorption intensity in the area of 1015 cm^{-1} and 572 cm^{-1}. The delignified cassava peel and its derivatives underwent fourier-transform infrared spectroscopy (FTIR) analysis. The findings indicate that the lignin and amorphous areas in the lignocellulosic biomass underwent successful degradation, owing to a decrease in the absorption intensity of the aromatic rings in the lignin at multiple wave numbers [21].

3.3 Growth microbial curve of Saccharomyces diastaticus

Saccharomyces diastaticus was cultivated, and the development stage was measured every hour by observing the optical density (OD) value. This allowed researchers to calculate the length of incubation required to produce bioethanol. At a wavelength of 600 nm, spectrophotometry is the technique employed [22]. The growth curve of *Saccharomyces diastaticus* was derived from the research findings and is shown in Figure 2. At different dates, the *Saccharomyces diastaticus* growth curve was obtained. Owing to adaptation (lag phase), *Saccharomyces diastaticus* did not grow much between hours 0 and 4. After that, *Saccharomyces diastaticus* grew exponentially from the fourth to the eighteenth hour. Moreover, *Saccharomyces diastaticus* did not significantly develop or die during the 18^{th} and 21^{st} hours (stationary phase). Death caused *Saccharomyces diastaticus* to decline in population after the twenty-first hour. According to current understanding, the growth of microbes occurs in four separate phases: the lag phase, the stationary phase, the exponential or logarithmic phase, and the death phase. Microbes adjust to their new surroundings and are ready to proliferate during the lag phase. They are not reproductive, but they do grow larger and have an active metabolism. The creation of RNA, growth factors, and other substances required for cell division characterizes this phase. When the proper conditions are met, microbes can reproduce at their maximal rate during the exponential or logarithmic phase of active growth. This stage of growth is exponential. A constant cell population results from the stationary phase, where the rate of cell growth and death are equal. Poisonous chemical buildup restricts growth. The last stage, known as the death phase, is when the population as a whole decline because more cells are dying than are being created [23].

3.4 The effect of substrate concentration on bioethanol yield

Table 3 displays the yields obtained at different substrate concentration variations based on a 60-hour fermentation. Yield and substrate concentration obtained from *Saccharomyces diastaticus* mediated bioethanol fermentation of cassava peel. $Y_{X/S}$ and $Y_{P/S}$ were measured during fermentation at a 10% substrate concentration and 0.331, respectively. $Y_{X/S}$ and $Y_{P/S}$ during fermentation with 15% substrate concentration were 0.605 and 0.491, respectively. $Y_{X/S}$ and $Y_{P/S}$ were measured during fermentation at a 20% substrate concentration and 0.309, respectively. The current idea states that the yield increases with the starting sugar concentration. On the other hand, average specific growth rate and average biomass yield significantly decreased with increasing starting sugar content. There was an increase in average specific substrate uptake, average specific ethanol productivity, and average ethanol yield [24]. Based on the study findings, 15% of the substrate was the ideal concentration, with $Y_{X/S}$ and $Y_{P/S}$ of 0.605 and 0.491, respectively. The yield results that were obtained showed data variations. This happens as a result of the high concentration of substrate in the fermentation medium, which, when increased over its optimal level, can have a strong inhibitory effect on yeast development and their capacity to create ethanol. In the last stages of fermentation, there may not be enough nutrients and excessive sugar concentrations can be highly hazardous to yeast [25].

3.5 The effect of fermentation time on bioethanol yield

Table 4 presents the yields achieved at different time variations based on the fermentation that has been carried out. The current theory states that the relationship between yield and fermentation time is direct. Accordingly, the percentage of yield produced increases with the length of the fermentation period [26]. The amounts of bioethanol generated during *Ulva sp.* fermentation provide additional evidence for this. In their study, the extended growth and development of the yeast resulted in an increase in the amount of ethanol obtained as the fermentation process lasted seven days, going from 0% to 7.55% [27]. The research findings showed that the 24-hour fermentation duration was the longest. The study's results exhibit swings, with the percentage yield gained increasing and decreasing with time. This is brought on by the medium's decreased nutritional content, which inhibits the growth of microbes. Because there are fewer microbes growing, the percentage yield produced likewise drops [28].

3.6 Kinetic paramaters on cassava peels fermentation

The processes that take place in the substrate, cell, and production variables during fermentation are identified using fermentation kinetics. The *Saccharomyces diastaticus* growth model, substrate use, and product production are all modeled within these kinetics. The biomass concentration (X), substrate concentration (reducing sugar, in this case), and product concentration (ethanol, P) at each substrate concentration 10% (w/v), 15% (w/v), and 20% (w/v) are displayed in Table 5. The maximum specific bacterial growth rate (μ_m) of 0.032/hour and the saturation constant (K_s) of 17.18 g/L were found in the research she conducted on producing ethanol from pineapple peel waste [29]. In a study using cassava peel flour to produce bioethanol, it was recorded that the highest possible specific bacterial growth rate (μ_m) was 1.037/hour. According to him, a number of variables, such as the type of substrate used, the yeast used, and the fermentation conditions, affect the variation in the maximum specific bacterial growth rate (μ_m) [30].

Table 1. Proximate analysis of cassava peels

Compound	(%)
Water	7.37
Ash	4.49
Proteins	1.41
Fat	10.31
Carbohydrate	76.42
Water	7.37
Ash	4.49
Proteins	1.41
Fat	10.31

Table 2. Chesson Datta analysis of cassava peel before and after pretreatment

Composition	Before Pretreatment (%)	After Pretreatment (%)
Hemicellulose	36.0	42.6
Cellulose	44.0	54.7
Lignin	15.6	1.7

Table 3. Yield of bioethanol

Substrate Concentration (%)	$Y_{X/S}$	$Y_{P/S}$
10%	0.440	0.331
15%	0.551	0.491
20%	0.423	0.320

Table 4. Effect of time in bioethanol production

Time(hour)	Substrate (%)	Reducing Sugar(g/L)	Bioethanol(g/L)	Biomass(g/L)	$Y_{X/S}$	$Y_{P/S}$
0	10%	10.2727	0.0010	0.8000	-	-
12		7.8485	0.4110	1.2000	0.1650	0.1691
24		6.6364	1.4416	2.7333	0.5317	0.3962
36		5.1212	1.7280	3.0667	0.4400	0.3352
48		3.6061	2.3710	4.0000	0.4800	0.3555
60		2.0909	2.7120	4.4000	0.4400	0.3313
0	15%	15.2840	0.0034	0.8000	-	-
12		13.2710	0.7280	1.9333	0.5630	0.3600
24		11.3540	3.0753	3.2000	0.6107	0.7816
36		5.3820	3.6660	4.4000	0.3636	0.3699
48		4.1280	4.7120	6.6667	0.5259	0.4221
60		2.7120	6.1720	7.7333	0.5515	0.4907
0	20%	20.0030	0.0020	0.5333	-	-
12		14.2710	0.6440	1.5380	0.1745	0.1120
24		12.3710	2.4870	4.1820	0.4804	0.3256
36		8.2710	2.8504	5.1220	0.3921	0.2428
48		4.8710	3.1240	7.1120	0.4362	0.2063
60		3.1230	5.4120	7.6667	0.4226	0.3205

Table 5. Values of kinetics parameters

Substrate Concentration	μ_m (hour^{-1})	K_S (g/L)	k_1 (gP/gX)	k_2 (gP/gX.h)	k_3 (hour^{-1})	R^2
10%	0.0364	11.07	0,636	0,001	0,003	0.82
15%	0.0442	13.08	0,503	0,005	0,005	0.89
20%	0.1371	14.11	0,257	0,006	0,006	0.85

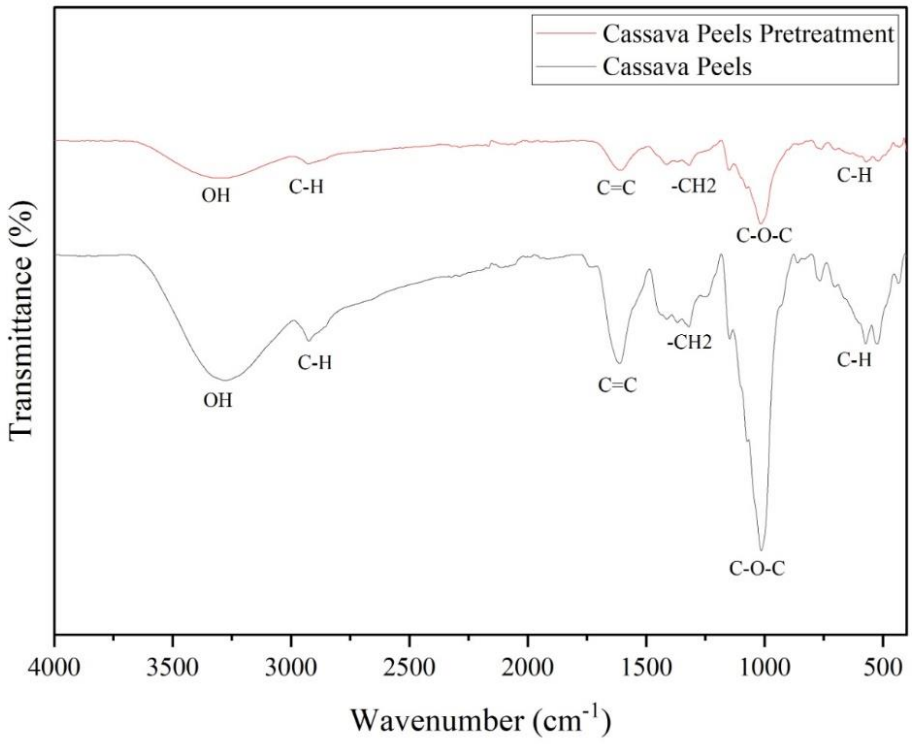


Figure 1. FTIR result of lignocellulose before and after pretreatment

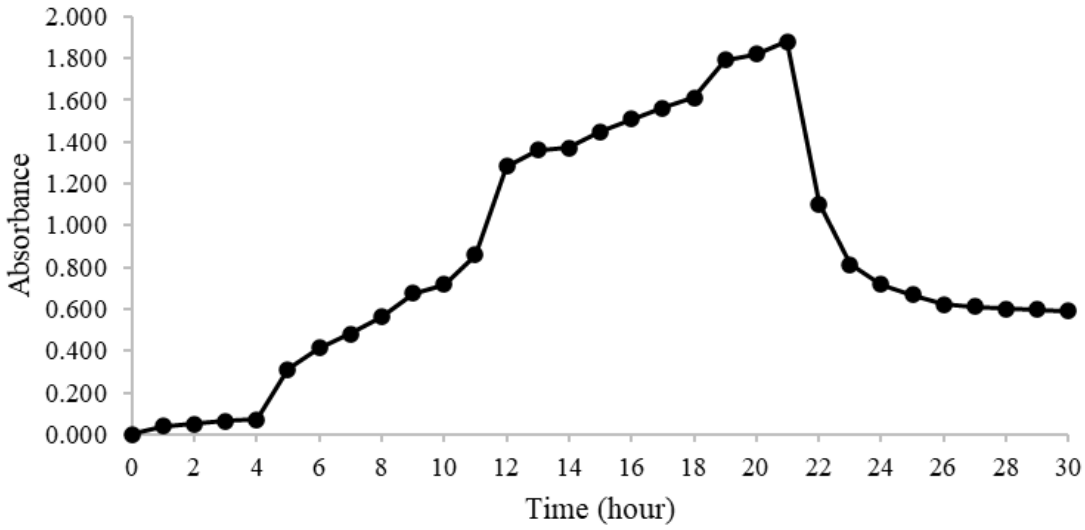
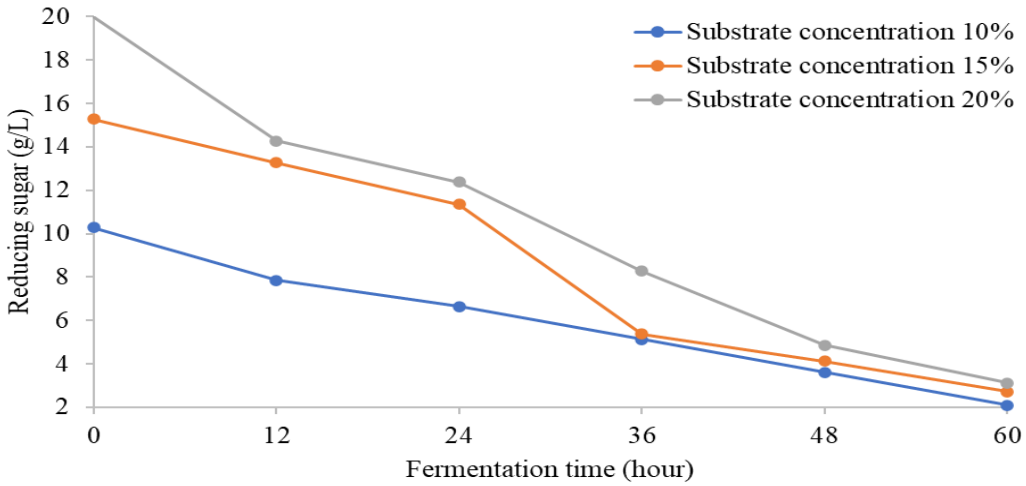
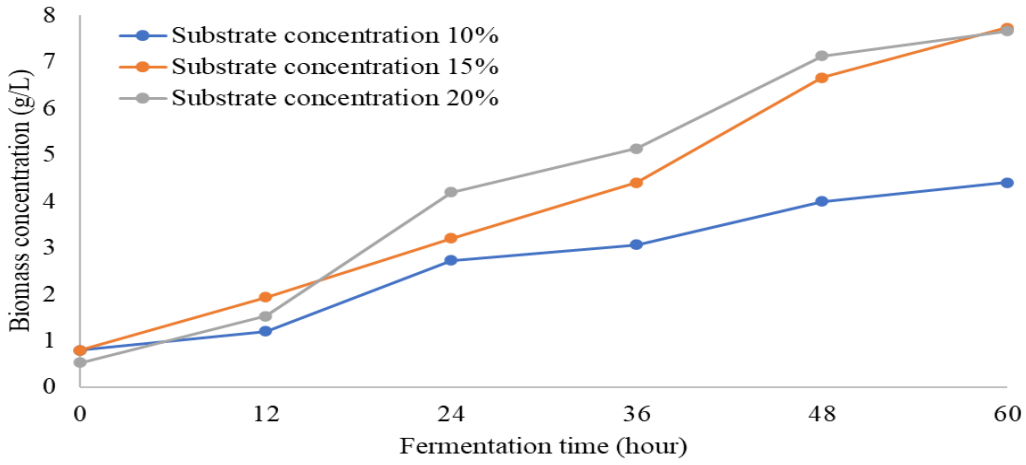


Figure 2. FTIR result of lignocellulose before and after pretreatment



(a)



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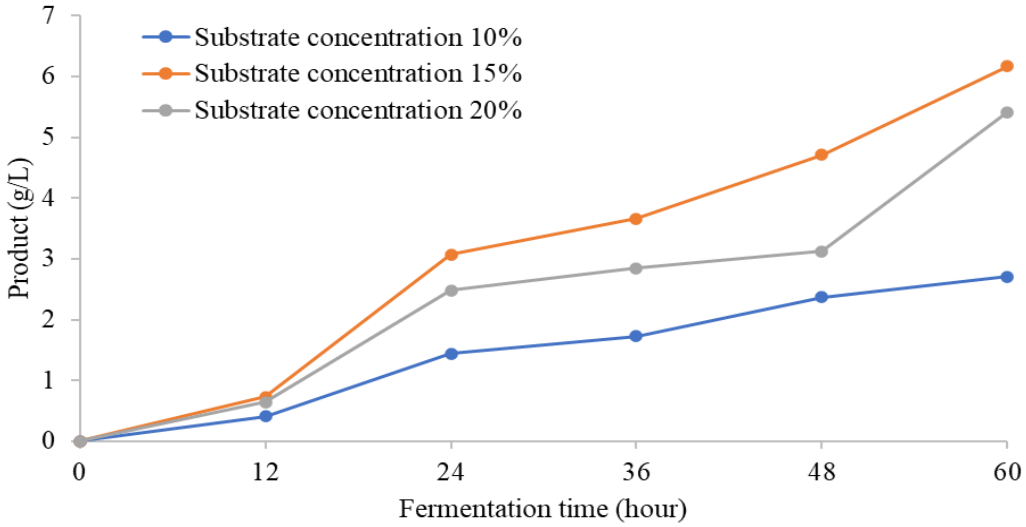

(c)

Figure 3. *S.diastaticus* growth modeling (a), substrate utilization modeling (b), and product formation modeling (c)

4. Conclusions

This study uses different strains of *Saccharomyces diastaticus* to quantify the quantity of ethanol produced from cassava peel waste. The study's cassava waste is composed of 44% cellulose, 36% hemicellulose, and 15.6% lignin. The maximum yield, 6.172 g/L of ethanol, was achieved with a 15% (w/v) variation. The greatest specific growth rate (μ_{max}), as determined by the kinetics parameter, was 0.0442 h^{-1}. Based on existing research, it is possible to turn waste cassava peels into ecologically acceptable bioethanol, which can help support the development of alternative energy sources.

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