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Growth Kinetics Studies of *Saccharomyces Cerevisiae* for Bioethanol Production of Oil Palm Empty Fruit Bunches

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ABSTRACT

This study aims to analyze the growth kinetics of *saccharomyces cerevisiae* in the bioethanol production process based on oil palm empty fruit bunch waste. Growth kinetics parameters are an important basis for fermentor design, especially the maximum growth rate constant to determine the minimum biomass residence time. The minimum biomass residence time is a critical point for fermentor operation in processing empty oil palm fruit bunches into bioethanol. This research was the laboratory experimental research method with a quantitative approach. The result in this research shows the growth kinetics parameters of *saccharomyces cerevisiae* have been successfully determined to produce the highest bioethanol at 5% KOH variation. Kinetic analysis established a substrate saturation coefficient (K_S) of 28.195 g/L under the S < 10 K criterion, alongside a maximum specific growth rate (μ_M) of 0.00073 hour^{-1}. Furthermore, the process demonstrated a biomass yield ($Y_{x/S}$) of 0.053 g/g and a product yield ($Y_{P/S}$) of 2.96 g/g, culminating in a final bioethanol productivity of 0.41 g/L·h. Therefore, these growth kinetics parameters can be used in designing a fermentor system for processing empty oil palm fruit bunches into bioethanol.

KEYWORDS: *Bioethanol, Growth kinetics, Palm empty fruit bunches,Saccharomyces cerevisiae.*

NOMENCLATURE

$Y_{X/S}$	The biomass yield
$Y_{P/S}$	The product yield
μ	Specific growth rate of microorganisms
μ_m	Maximum specific growth rate
K_s	Half saturation constant for the reaction substrate hydrolysis
S	Substrate concentration
X	Dry weight of cells
t	time

1. INTRODUCTION

Empty oil palm fruit bunches are the main pollutant component found in various palm oil biomass wastes in the palm oil industry. The biodegradation of empty oil palm fruit bunches into bioethanol occurs through various pathways involving s*accharomyces cerevisiae* and several stages, namely delignification, hydrolysis, and fermentation [1-3] have delignified oil palm fronds with oil bunch ash and delignified empty oil palm bunches [1] as well as delignified oil palm fibre [4] and use NaOH for delignification of empty oil palm bunches [5], while the hydrolysis process uses H_2SO_4 [6-8] and HCl [9-11].

The fermentation process takes place under anaerobic condition [12-16] has reported a kinetic model based on the reaction mechanism, namely the hydrolysis process stage, the process of utilizing the hydrolyzed substrate, and product formation. The model was constructed based on the initial assumption that the microbial distribution in the system is relatively uniform and mixed. Ahmad et al (2022) [7] reported that the fermentation kinetics of bioethanol from oil palm fronds follows first order, while [17] have determined kinetic parameters by observing the anaerobic biodegradation process without involving the hydrolysis process. Kinetic parameters are an important basis in fermentor design, especially the maximum growth rate constant (μ_m) to determine the minimum biomass residence time. The minimum biomass residence time is the critical point for fermentor operation in processing empty palm oil fruit bunch waste. Safe design uses a safety factor of 5 times the minimum biomass residence time [18].

The chosen fermentor system is a stirred suspended growth fermentor because it is more advantageous compared to the attached growth fermentor system. The advantages of a suspended growth fermentor include: it does not require a supporting medium for the growth of microorganisms and is

not easily clogged [19]. In addition, the chosen fermentor has a simple design, is cheap and easy to operate and has high biomass stability. The fermentor design that meets these requirements is a stirred fermentor. This paper attempts to examine the growth kinetic parameters of s*accharomyces cerevisiae* to produce bioethanol from empty oil palm fruit bunches.

This study aims to comprehensively evaluate the growth kinetics of *saccharomyces cerevisiae* during bioethanol production from Oil Palm Empty Fruit Bunches (OPEFB). The research focuses on determining the key kinetic parameters, including the half-saturation constant, maximum specific growth rate, biomass yield, product yield and overall productivity, to accurately represent the fermentation process using an integrated kinetic model. The resulting kinetic parameters establish a reliable quantitative basis for predicting fermentation performance, optimizing bioprocess operating conditions, and improving the efficiency of bioethanol production.

2. METHOD

This study employed a quantitative laboratory experimental approach using a controlled experimental design to investigate the influence of alkaline pretreatment on bioethanol production from oil palm empty fruit bunches (OPEFB). The independent variable was the KOH concentration (5%, 6%, and 7%), while the response variables included glucose concentration, biomass formation, and bioethanol production. Experimental conditions, including pH incubation temperature, inoculum concentration, and fermentation duration, were maintained constant to ensure that the observed responses were solely attributable to variations in KOH concentration. Fermentation was conducted in a closed batch system using *saccharomyces cerevisiae*, without nutrient supplementation or medium withdrawal during cultivation. The temporal profiles of substrate consumption, microbial growth, and ethanol formation were subsequently analyzed through growth kinetic modeling to quantify the specific growth behavior, substrate utilization, and product formation rates under different pretreatment conditions.

2.1 Materials

The palm oil biomass used was empty palm fruit bunches. The process variables used were pretreatment with varying KOH concentrations of 5%, 6%, and 7%, followed by a delignification process using 3% H_2O_2 and followed by a hydrolysis process using 2 M H_2SO_4. The fermentor operating conditions were at pH 4.5 ± 0.2 with room temperature. The parameters observed included glucose concentration, biomass concentration as dry cell weight, and bioethanol concentration. Fermentation lasted for 72 hours with a batch process [8],[20].

2.2 Determination of Microorganism Growth Rate

According to [21], the decomposition of complex organic compounds occurs through the hydrolysis process. Only dissolved compounds resulting from the hydrolysis reaction can act as growth regulators for microorganisms, as expressed in the Monod equation. [22] have developed a kinetic model based on the hydrolysis process, the transfer of substrates resulting from the hydrolysis reaction into cells, and the utilization of substrates for cell growth and product formation.

Developing a kinetic model of anaerobic processes requires a number of complex and intricate kinetic equations and parameters. To simplify the kinetic model, several assumptions are made, including:

1. There are no microorganisms in the feed stream.
2. The system is perfectly mixed. The fermenter has a relatively uniform distribution of biomass throughout the system due to the appropriate stirring rate and the resulting turbulence effect on the system.
3. The death rate of microorganisms is negligible compared to the growth rate.
4. The process takes place without oxygen.

Based on the above assumptions, a kinetics model of microorganism growth and process productivity in an anaerobic bioreactor system is constructed through three stages of biodegradation, namely the hydrolysis stage, the substrate transfer stage into the cell, the substrate utilization stage for cell growth and product formation [23]. Mathematically, the relationship between the specific growth rate and substrate concentration is expressed by the Monod equation [22],[24]:

$$\mu = \frac{\mu_m S}{K_s + S} \quad (1)$$

With μ = specific growth rate of microorganisms (hours^{-1}), μ_m= maximum specific growth rate (hours^{-1}), K_s= half saturation constant for the reaction substratehydrolysis (g/L), S= substrate concentration (g/L).
Meanwhile,

$$\mu = \frac{1}{X}\frac{dX}{dT1} \quad (2)$$

With μ= specific growth rate of microorganisms (hours^{-1}), X= dry weight of cells (g/L), t= time (hours).
Equation (1) is linearized into equation (3):

$$\frac{1}{\mu} = \frac{K_s}{\mu_m}\frac{1}{S} + \frac{1}{\mu_m} \quad (3)$$

Equation (3) if 1/μ is plotted with 1/S, the slope is obtained = Ks/μ_m and the intercept = $1/\mu_m$, so that the kinetic parameters of microorganism growth are obtained: K_S and μ_m [25]. Then, the cell yield against the substrate is calculated from the following equation:

$$\frac{dS}{dt} = \frac{1}{Y_{X/S}}\frac{dX}{dt} \quad (4)$$

Equation (4) when differentiated with respect to -dS/dt and dX/dt yields a slope = $1/Y_{X/S}$ [25]. The yield of product with respect to substrate is calculated from the following equation:

$$\frac{dS}{dt} = \frac{1}{Y_{P/S}}\frac{dP}{dt} \quad (5)$$

Equation (5) when differentiated with respect to -dS/dt and dP/dt yields a slope = $1/Y_{P/S}$ [25]. The productivity of the fermentation process is calculated based on the relationship between fermentation time and the concentration of the product obtained [25].

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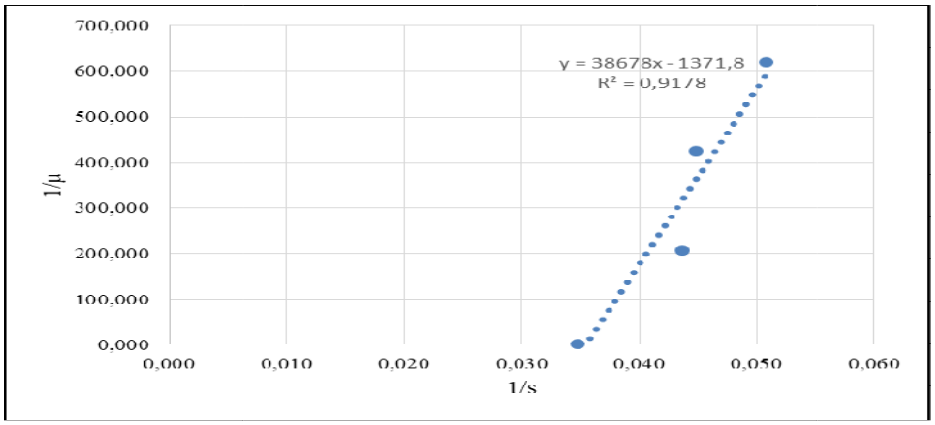
3. RESULT AND DISCUSSION

3.1. Microorganism Growth Kinetics

The growth rate of microorganisms is quantitatively influenced by the concentration of substrate and nutrients in the media. If the substrate concentration is high, the growth rate of microorganisms will also be high. The growth rate is expressed as the specific growth rate, which is the rate of biomass formation per unit cell mass. The determination of the kinetic parameters Ks and μ_m is shown in Figure 1. It can be seen in Figure 1(a), (b) and (c) the graphical determination of the half-saturation constant (Ks) and maximum specific growth rate (μm) for *saccharomyces cerevisiae* during bioethanol production from empty oil palm fruit bunches, specifically at varying concentrations of KOH (5%, 6%, and 7%). In Figure 1(a) shows the linearized Monod plot for the fermentation process using OPEFB pretreated with 5% KOH concentration, the intercept of the linearized plot is 1/μm = 1371.8.This translates to a maximum specific growth rate (μm) of 0.00073 hour^{-1}.



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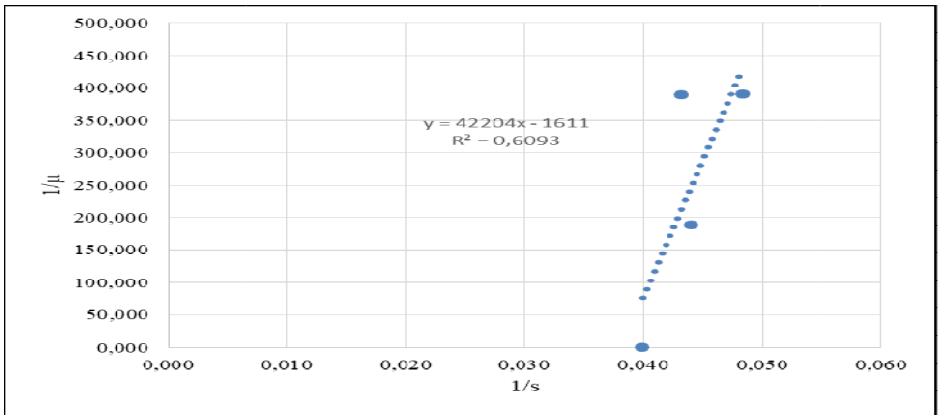

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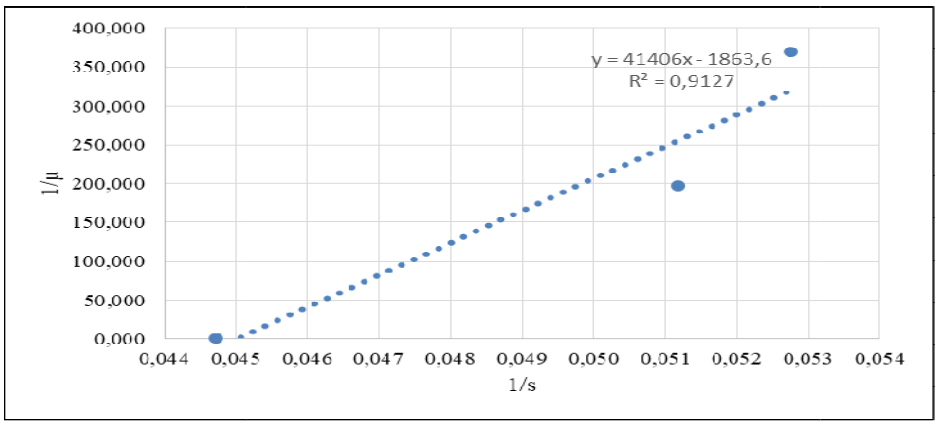

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Figure 1: Determining the kinetic parameters Ks and μ_m : (a) KOH 5%; (b) KOH 6%; (c) KOH 7%

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The µm value represents the highest rate at the yeast can grow under optimal substrate conditions. The slope of the plot for 5% KOH is Ks/µm = 38678. Using the previously calculated µm, the half-saturation constant (Ks) is determined to be 28.195 g/L. Ks indicates the substrate concentration at the specific growth rate is half of the maximum specific growth rate. A higher Ks value suggests a lower affinity of the microorganism for the substrate, meaning more substrate is needed to achieve half of the maximum growth rate.

Figure 1(b) at a 6% KOH concentration, the intercept is 1/µm = 1611, resulting in a maximum specific growth rate (µm) of 0.00062 hour^{-1}. This value is slightly lower than that observed at 5% KOH. The slope for 6% KOH is Ks/µm = 42204, which yields a Ks value of 26.197 g/L. Compared to the 5% KOH condition, the Ks value is slightly lower, suggesting a potentially improved substrate affinity despite the reduced maximum growth rate.

Figure 1(c) for the 7% KOH concentration, the intercept is 1/µm = 1863.6, leading to a µm of 0.0005 hour^{-1}. This represents the lowest maximum specific growth rate among the three KOH concentrations tested. The slope for 7% KOH is Ks/µm = 41406, which gives a Ks value of 22.218 g/L. This is the lowest Ks value among the three concentrations, indicating the highest affinity of the microorganism for the substrate under these conditions, even though the maximum growth rate is the lowest.

3.2. Cell Acquisition of Substrate

The determination of the cell gain coefficient is shown in Figure 2. It can be seen in Figure 2 the graphical determination of the cell recovery coefficient (Yx/s) for *saccharomyces cerevisiae* at varying KOH concentrations (5%, 6%, and 7%) during bioethanol production. The Yx/s value represents the biomass yield per unit of substrate consumed, indicating the efficiency with which the microorganism converts the substrate into its own biomass. These values are derived from the slopes of the linearized plots, where the slope is equal to 1/Yx/s.

It can be seen in Figure 2(a), for the 5% KOH concentration, the slope of the plot is 1/Yx/s = 28.724. Hence, Ittranslates to a biomass yield (Yx/s) of 0.035 g/g. The results for the 6% KOH concentration are presented in Figure 2(b). The slope of the linearized plot decreased to 1/Yx/s=18.799indicating improved substrate conversion efficiency. Consequently, the biomass yield coefficient (Yx/s) increased to 0.053 g/g, demonstrating more efficient biomass production per unit of substrate consumed.This indicates an improved efficiency in converting substrate to biomass at 6% KOH compared to 5% KOH. Finally, at 7% KOH (Figure 2c), the slope is further reduced to 1/Yx/s = 7.3384, yielding the highest Yx/s value of 0.136 g/g.

Therefore, Figure 2 demonstrates a clear trend, as the KOH concentration increases from 5% to 7%, the cell recovery coefficient (Yx/s) significantly increases. This suggests that higher KOH pretreatment concentrations lead to a more efficient conversion of the substrate into *Saccharomyces cerevisiae* biomass. This enhanced biomass yield at higher KOH concentrations is a positive outcome, as increased biomass can potentially lead to higher bioethanol production. The study notes that a higher KOH concentration results in an increased biomass yield, product yield, and productivity.

3.3. Product Obtaining Against Substrate

The determination of the cell gain coefficient is shown in Figure 3. Figure 3 shows the product yield coefficient (Yp/s) for *saccharomyces cerevisiae* during bioethanol production from empty oil palm fruit bunches, specifically at varying concentrations of KOH (5%, 6%, and 7%).Figure 3(a) for the 5% KOH concentration, the slope of the plot is 1/Yp/s = 0.3378. This value indicates a product yield (Yp/s) of 2.960 g/g. This means that for every gram of substrate consumed, 2.960 grams of bioethanol are produced under these conditions.Figure 3(b) at 6% KOH concentration, the slope of the plot is 1/Yp/s = 0.2413. This results in a product yield (Yp/s) of 4.144 g/g. This value is significantly higher than that observed at 5% KOH, suggesting an improved efficiency in converting substrate to bioethanol at this concentration.Figure 3(c) for the 7% KOH concentration, the slope is further reduced to 1/Yp/s = 0.2142. This yields the highest product yield (Yp/s) of 4.668 g/g among the three concentrations tested. This indicates that the 7% KOH pretreatment leads to the most efficient conversion of substrate into bioethanol.

The KOH concentration increases from 5% to 7%, the product yield coefficient (Yp/s) significantly increases. This suggests that higher KOH pretreatment concentrations are more effective in enhancing the conversion of substrate into bioethanol by *saccharomyces cerevisiae*. The product yield (Yp/s) in this research, at 2.96 g/g, was relatively higher than some other studies. This improvement in product yield at higher KOH concentrations is a crucial finding for optimizing bioethanol production from oil palm empty fruit bunches.

3.4 Fermentation Process Productivity

The productivity of the fermentation process is calculated based on the relationship between fermentation time and product concentration as shown in Figure 4. It can be seen in Figure 4, the fermentation process productivity for *saccharomyces cerevisiae* at varying KOH concentrations (5%, 6%, and 7%) during bioethanol production. Figure 4 demonstrates that increasing the KOH concentration from 5% to 6% leads to a notable increase in bioethanol productivity. Although the productivity slightly decreases at 7% KOH compared to 6% KOH, both 6% and 7% KOH concentrations show significantly higher productivity than 5% KOH. This suggests that optimal KOH concentrations, around 6-7%, are crucial for maximizing the rate of bioethanol production from empty oil palm fruit bunches. The study generally indicates that higher KOH concentrations lead to increased biomass yield, product yield, and productivity.

For the 5% KOH concentration (Figure 4a), the slope of the plot is 0.4142. This directly translates to a productivity of 0.4142 g/L-hour. This indicates that at this KOH concentration, bioethanol is produced at a rate of 0.4142 grams per liter per hour. At a 6% KOH concentration (Figure 4b), the slope of the plot is 0.5457, resulting in a productivity of 0.5457 g/L-hour. This value is higher than that observed at 5% KOH, suggesting an improved rate of bioethanol production at this concentration. For the 7% KOH concentration (Figure 4c), the slope of the plot is 0.5326, which yields a productivity of 0.5326 g/L-hour. Therefore, it is slightly lower than the productivity at 6% KOH but it is still significantly higher than that at 5% KOH.

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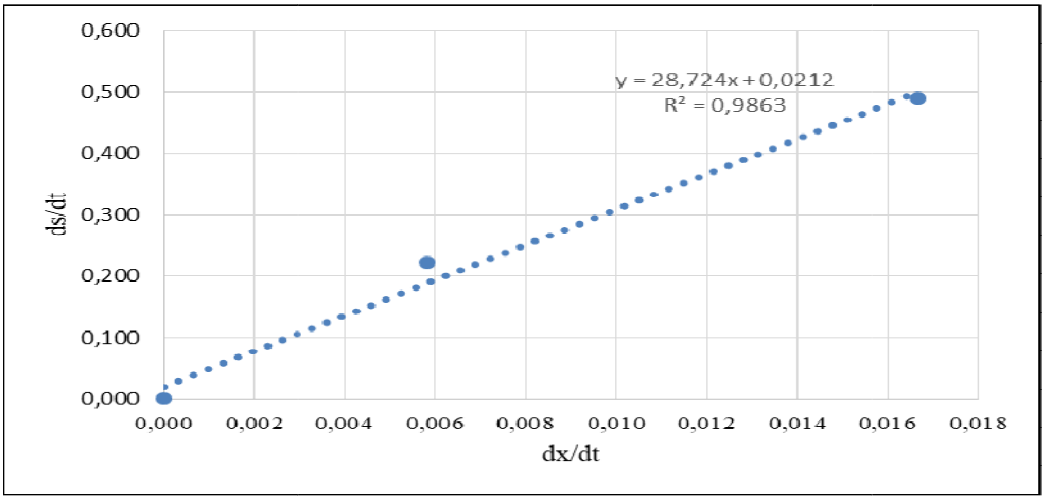

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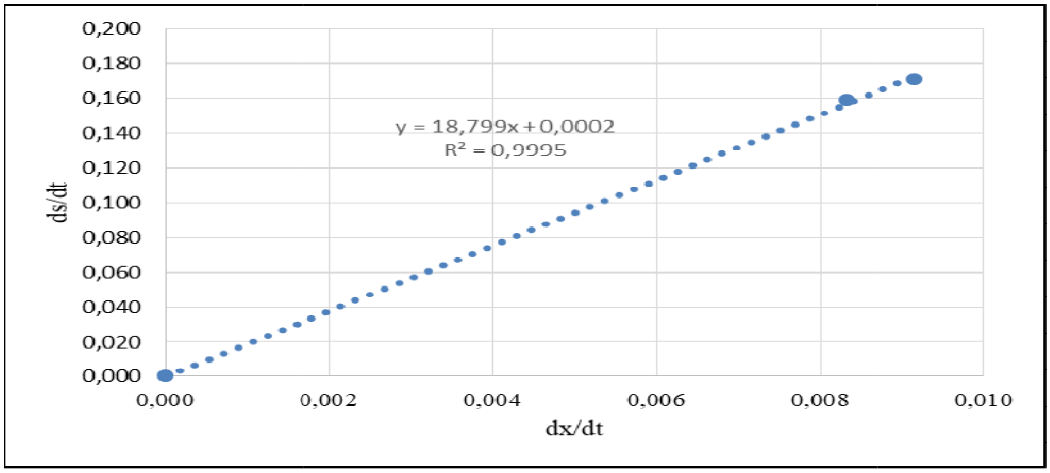

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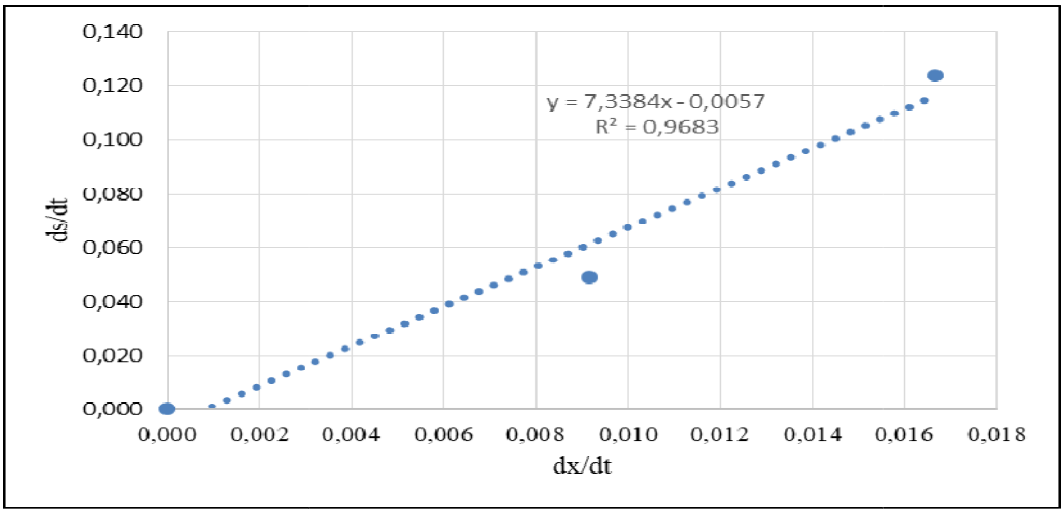

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Figure 2: Determining the $Y_{X/S}$ cell recovery coefficient: (a) 5% KOH; (b) 6% KOH; (c) 7% KOH

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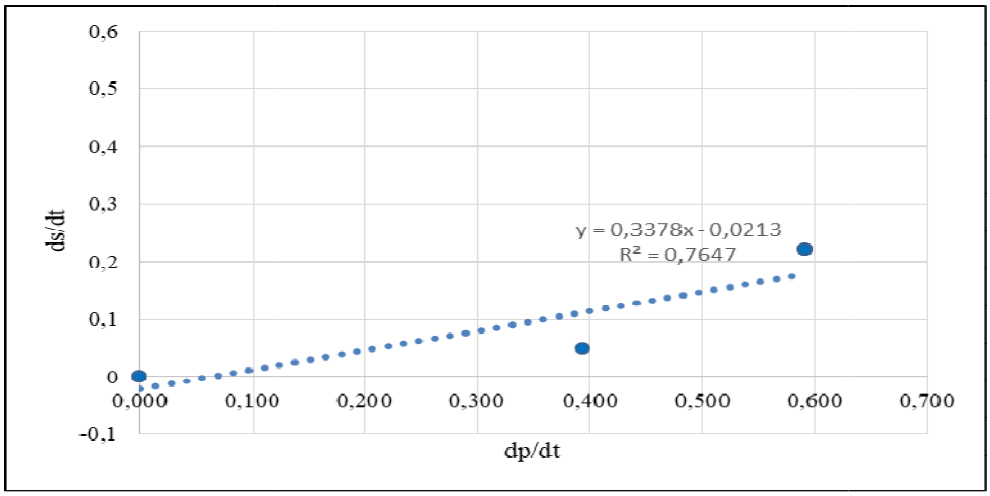

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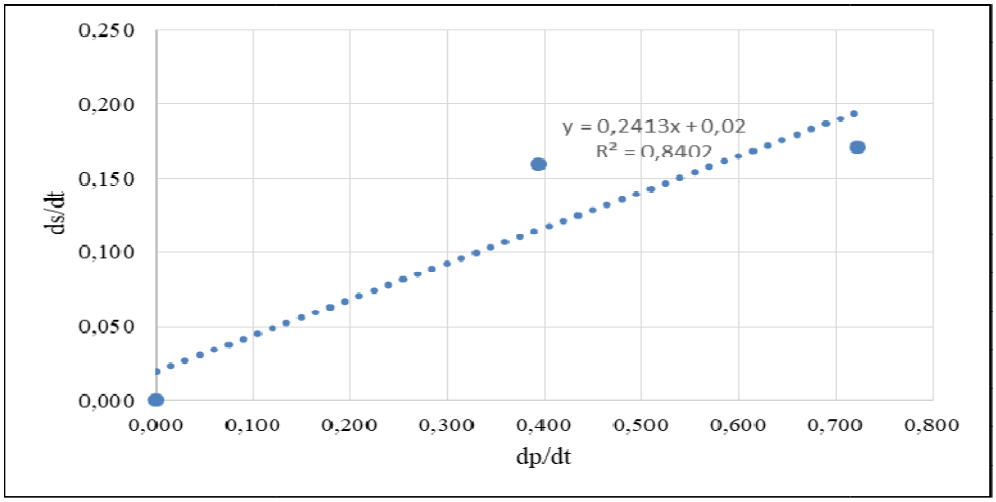

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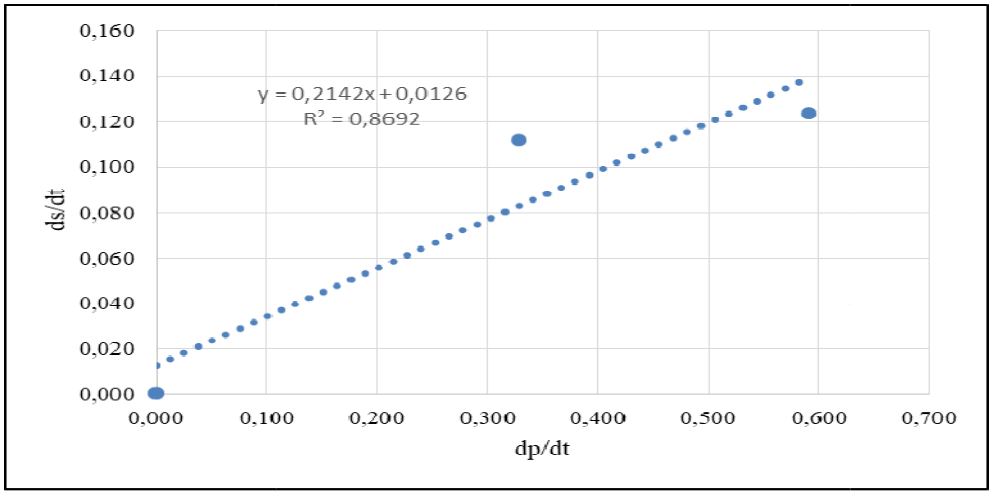

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Figure 3: Determining the product yield coefficient $Y_{P/S}$: (a) KOH 5%; (b) KOH 6%; (c) KOH7%

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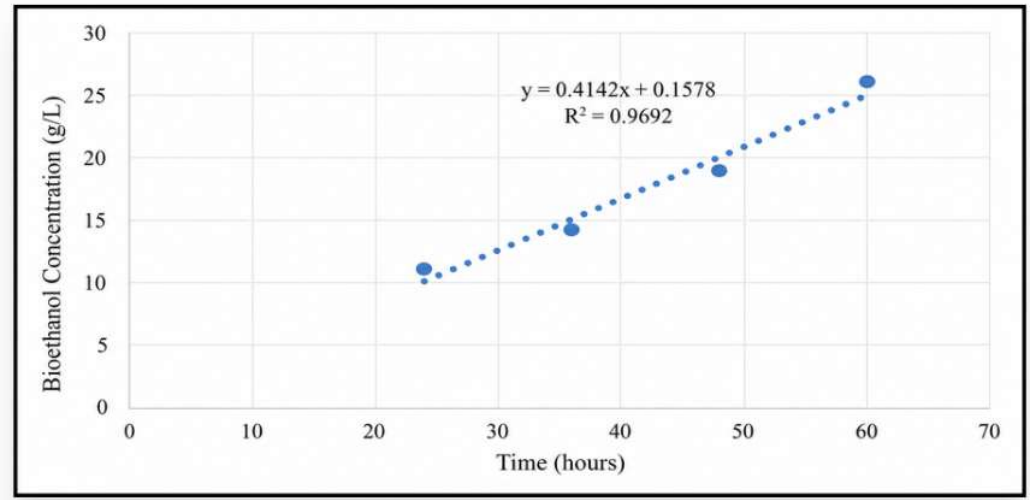

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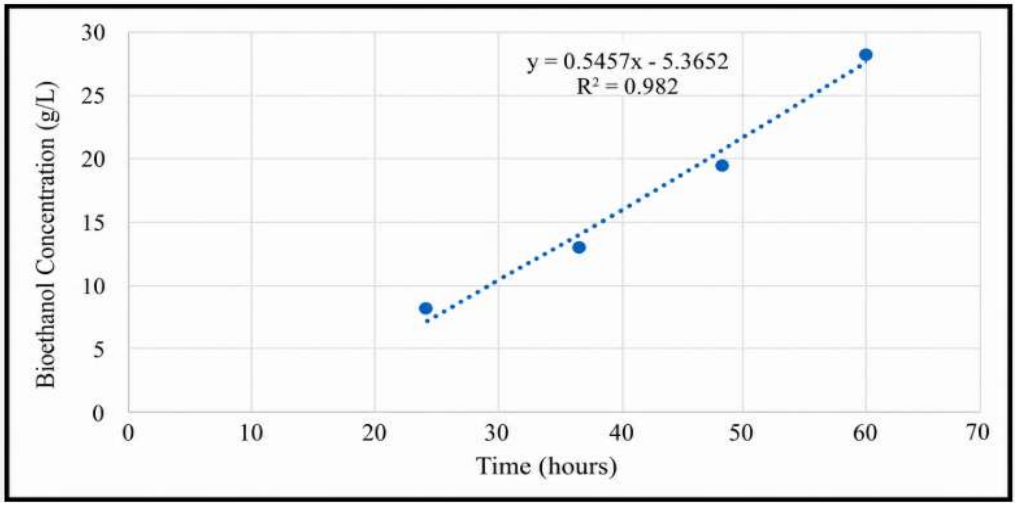

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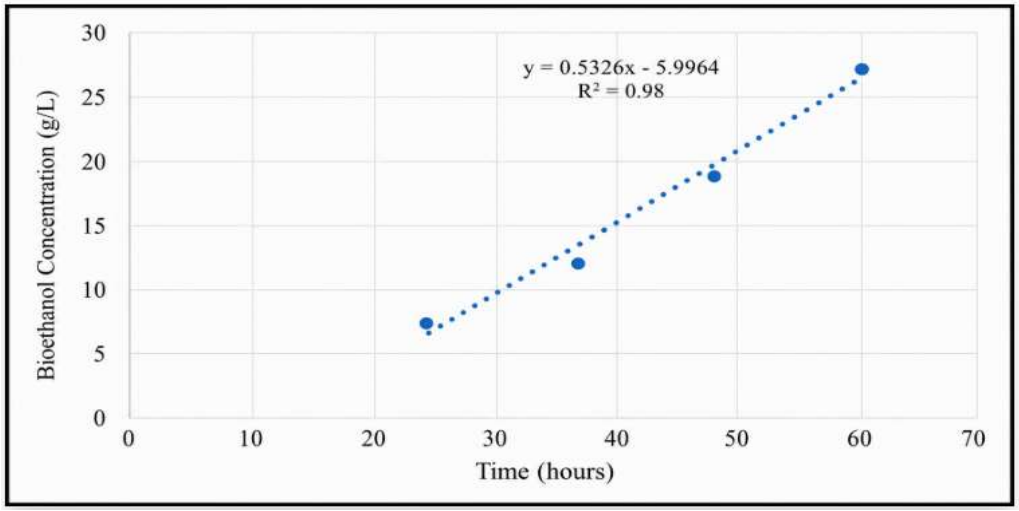

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Figure 4: Fermentation process productivity: (a) KOH5%; (b) KOH6%; (c) KOH7%

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3.5. Kinetic Parameters

The calculation results obtained the following kinetic and productivity parameters as shown in Table 1. Table 1 showsthe Ks value in this study was influenced by the initial treatment using KOH. The research results showed that the higher the KOH concentration, the lower the Ks value obtained, as well as the maximum microorganism growth rate value, while biomass yield, product yield, and productivity increased. The same thing was also found in research conducted by [26]. The highest Ks value obtained in this study was 28.195 g/L, significantly different from that obtained by [27] which was 4.032 g/L and lower than that obtained by [17],[28]. The difference in Ks values was caused by the different substrates used and the different substrate concentrations given. High substrate concentrations will result in high Ks values. The Ks value in this study was lower than the Ks value obtained by [16] and greater than the Ks value obtained by [14] which was 0.75 g/L.

Table 1 shows that the μ_{max} and K_S parameters are highly dependent on the type of microorganism and the substrate used. If a particular microorganism species is cultured on several types of substrates under the same conditions, then the observed μ_{max} and K_S values will be highly dependent on the type of substrate. Conversely, if several types of pure cultures are cultured on the same substrate media. In general, substrates that are difficult to degrade will have low μ_{max} values and high K_S. Conversely, substrates that are easily degraded will have high μ_{max} values and low K_S. The substrate saturation constant (Ks) obtained in this study was 28.195 g/L, while the substrate concentration was 34.07 g/L. According to [25], the substrate saturation constant with the criteria S < 10 Ks means that the specific growth rate is a function of the substrate concentration. A large Ks value indicates that the affinity between cells and the substrate is small, this is because the substrate used has a complex composition so that initial treatment is needed that can remove organic and inorganic inhibitors while initial treatment with delignification and hydrolysis methods can only remove inorganic inhibitors. *Saccharomyces cervisiae* to empty palm fruit bunch substrates needs to be adapted because the adaptation period shortens the lag phase in bioethanol fermentation and aims to condition *saccharomyces cervisiae* to survive in empty palm fruit bunch substrates so that the growth of *saccharomyces cervisiae* is not hampered.

The results of the kinetic parameters in Table 1 are compared with those of previous researchers. The comparison of kinetic parameters is shown in Table 2.

Table 1: Kinetic parameters and productivity of bioethanol fermentation

KOH Variables	Ks g/L	μ_m hour^{-1}	$Y_{X/S}$ g/g	$Y_{P/S}$ g/g	Productivity g/L-hour
5%	28.195	0.00073	0.053	2.960	0.4142
6%	26.197	0.00062	0.059	4.144	0.5457
7%	22.218	0.0005	0.136	4.668	0.5326

Table 2: Comparison of kinetic parameters from various research results

Types of Bioreactors	Raw material	μ_m hour^{-1}	$Y_{X/S}$ g/g	$Y_{P/S}$ g/g	Productivity g/L.hour	Data source
BIOPAN	POME	0.762	0.059	-	-	[29]
BUFAN	Synthetic	0.16	0.08	-	-	[17]
HABR	Molasses	0.296	1.227	-	-	[28]
BIOPAN	POME	0.187	0.395	-	-	[16]
AEROBIC FERMENTOR	Molasses	0.07	0.36	0.17	0.17	[26]
ANAEROBIC FERMENTOR	Empty Palm Fruit Bunches	0.00073	0.053	2.96	0.41	This research

Information:
BUFAN = anaerobic fluidized bed bioreactor; HABR = hybrid anaerobic baffled reactor;
BIOPAN = anaerobic baffled bioreactor; POME = palm oil mill effluent

Table 2 shows that the maximum specific growth rate (μ_m) in this study was obtained at 0.0007 hour^{-1}, relatively lower than that obtained by [17],[28],[26] and lower than that obtained by [16], which was 0.187 hour^{-1}. The maximum specific growth rate (μ_m) in this study was much lower than that obtained by other researchers due to the different substrates used and the different substrate concentrations given. High substrate concentrations will cause high growth rates.

The biomass yield ($Y_{X/S}$) in this research was 0.053 g/g, not much different from that obtained by [29], which was 0.059 g/g and much lower than that obtained by other researchers [15],[26],[28]. This biomass yield was also relatively lower than the kinetic data obtained by [17], which was 0.08 g/g. Therefore, the product yield ($Y_{P/S}$) in this research was 2.96 g/g, which is relatively more than high from that obtained by [26], which was 0.17 g/g. The productivity of this research was 0.41 g/Lhours, which is relatively different from that obtained by [26], which was 0.17 g/g.

4. CONCLUSION

The behavior of bioethanol fermentation from empty oil palm fruit bunches can be described by a kinetic model that is compiled based on kinetic parameters. This study successfully determined the principal kinetic parameters governing the

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performance of the investigated bioprocess, providing a quantitative basis for evaluating its operational efficiency. The estimated substrate saturation coefficient (Ks) = 28.195 g/L and the substrate saturation constant with the criteria S < 10 Ks and the maximum specific growth rate (μ_m) = 0.00073 h-1. The biomass yield ($Y_{X/S}$) was obtained at 0.053 g/g and the product yield ($Y_{P/S}$) was obtained at 2.96 g/g. Therefore, the productivity was obtained at 0.41 g/L.h. Overall, the kinetic parameters obtained in this study establish a reliable framework for describing the bioprocess behavior and provide valuable input for predictive kinetic modeling, process optimization, and the scale-up of bioreactor operations.

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