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OPTIMIZATION AND KINETICS STUDY OF BIOETHANOL PRODUCTION FROM PALM OIL MILL EFFLUENT UNDER ANAEROBIC PROCESS

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Abstrak: *Various factors has influenced to the products during the anaerobic fermentation process pathway including pH as the main factors. This research study on kinetics of the bioethanol production at different pH conditions (5.5, 6.5-7.5 and 8.5) with artificial palm oil mill effluent with various concentrations of COD (10 g/L, 15 g/L, and 20 g/L) as a substrate. The optimum condition of pH value which provided the maximum bioethanol production under fermentation process from artificial wastewater will be re-implemented and adjusted the pH of palm oil mill effluent (POME) according to the characteristic of COD. Batch reactor will be used in this research study to preserve the microbial activity for converting substrate into bioethanol production within 72 hours. Nitrogen purged for the first 24 hours will be conducted to remove any residual oxygen from the reactor then internal gas in headspace of the reactor circulation system will be replaced as a mixing system. Microorganisms are taken from cow rumen mixed with palm oil mill effluent sludge. The kinetic studies will be determined such as product formation as bioethanol using Modified Gompertz model and substrate consumption using first-order kinetic. Bioethanol and acids yield and production rate will be determined as well. The result of ethanol production from the experiment was shown that the optimum pH condition from initial COD concentration of 10 g/L was in neutral condition and from 15 and 20 g/L were in acidic condition with the concentration of 12.36 g/L, 10.42 g/L, and 20.91 g/L, respectively. According to characteristic of POME wastewater, the concentration of COD was about 15 g/L then pH condition was operated into acidic condition (pH 5.5). From the experiment of the POME wastewater, the ethanol production obtained the maximum in 72 hours of 3.73 g/L. Ethanol yield and production rate from POME were 1.93 g/g and 51.87 mg/L/hr, respectively. Total volatile fatty acids yield and production rate from POME were 2.34 g/g and 62.95 mg/L/hr, respectively.secara rata-rata faktor keamanan sampah terkompaksi melewati standar TPA sementara dan permanen.*

Kata kunci: *pH, kinetic, fermentation, ethanol production, palm oil mill effluent (POME)*

Abstract: *Beberapa factor dapat mempengaruhi produk selama proses anaerobic fermentasi termasuk pH sebagai faktor utama. Penelitian ini mengkaji kinetika produksi bioetanol pada kondisi pH yang berbeda (5,5, 6,5-7,5 dan 8,5) menggunakan limbah pabrik kelapa sawit artifisial dengan berbagai konsentrasi COD (10 g/L, 15 g/L, dan 20 g/L) sebagai substrat. Kondisi optimum nilai pH yang menghasilkan produksi bioetanol maksimum dalam proses fermentasi dari limbah artifisial akan diimplementasikan kembali dan disesuaikan pH limbah pabrik kelapa sawit (POME) sesuai karakteristik COD. Reaktor batch akan digunakan dalam penelitian ini untuk mempertahankan aktivitas mikroba dalam mengubah substrat menjadi bioetanol dalam waktu 72 jam. Nitrogen yang dimurnikan selama 24 jam pertama akan dilakukan untuk menghilangkan sisa oksigen dari reaktor maka gas internal di ruang kepala sistem sirkulasi reaktor akan diganti sebagai sistem pencampuran. Mikroorganisme yang digunakan diambil dari rumen sapi yang dicampur dengan lumpur buangan pabrik kelapa sawit. Studi kinetik akan ditentukan seperti pembentukan produk bioetanol dengan menggunakan model modifikasi Gompertz dan konsumsi substrat menggunakan kinetika orde satu . Nilai Yield dari bioetanol dan asam, serta laju produksi dari bioethanol dan asam juga akan ditentukan. Hasil produksi etanol dari percobaan menunjukkan bahwa kondisi pH optimum dari konsentrasi COD awal 10 g/L berada dalam kondisi netral dan dari 15 dan 20 g/L berada dalam kondisi asam dengan konsentrasi masing-masing 12,36 g/L, 10,42 g/L, dan 20,91 g/L. Menurut karakteristik air limbah POME, konsentrasi COD sekitar 15 g/L kemudian kondisi pH dioperasikan ke dalam kondisi asam (pH 5.5). Dari percobaan limbah cair POME, produksi etanol diperoleh maksimum dalam 72 jam adalah*

3,73 g/L. Hasil etanol dan laju produksi POME adalah 1,93 g/g dan 51,87 mg/L /jam. Total hasil asam lemak volatil dan laju produksi dari POME masing-masing adalah 2,34g/g dan 62,95 mg/L/jam.

Keywords: *pH, kinetik, fermentasi, produksi etanol, limbah pabrik kelapa sawit (POME)*

INTRODUCTION

Population growth caused the global warming from the daily activity of fossil fuel burning to provide the energy supporting. In this recent year, the level of greenhouse gas has increased noticeably. Due to the decline of current fossil fuel, numerous researches have been seeking for the environmental friendly technologies to produce bioenergy including physical, chemical, and biological process. Three type of organic matter including; sugar, starch, and lignocellulosic material which contains of carbohydrate was found as a source of bioethanol and another product (Somda *et al.*, 2011). Using ethanol as fuel is given 15% greater combustion efficiency compared to gasoline due to the oxygen content in ethanol is about 34.7% (Zabed *et al.*, 2016). Anaerobic digestion is one of the biological processes which are feasible to convert organic matter into bio energy such as bio-methane, bi-hydrogen, bioethanol, or other product from the different pathway as well in the absence of oxygen. This process was considered as the less energy consuming process to produce other energies. This process consists of four main stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Vögeli *et al.*, 2014 and Nugroho *et al.*, 2007). Palm oil mill effluent (POME) is the wastewater from the extraction of crude palm oil contained of various type of organic matter which can be used as a substrate to convert into energy. Bioethanol is produced during the acidonegic stages when other products can be formed as volatile fatty acids including acetic acid, butyric acid, propionic acid, valeric acid, carbon dioxide, and hydrogen gases through two different fermentations are butyric-type fermentation and propionic-type fermentation (Kuruti et al., 2015 and Juang *et al.*, 2011). There are many factors affects to the end-products from the fermentation pathway such as: pH, substrate, temperature, organic loading rate, particularly, pH is one of the important factors significantly influence to the products by the influence on microorganisms activity including consumption of energy and carbon source, substrate degradation, nutrients and many types of materials (Zhen *et al.*, 2004 and Infantes *et al.*, 2012). Under acidic, neutral, and alkaline pH condition have been studied and showed different result of bioethanol concentration.

The objectives of this research aim to identify the effect of pH using artificial wastewater as a substrate to optimize the production of ethanol from POME. Using microorganism taken from cow rumen and batch experiments was conducted. Substrate degradation and bioethanol formation kinetics have been studied along with products yield and production rate.

MATERIALS AND METHODS

Culture Medium

There are two main substrate were used in this research study such as artificial wastewater and palm oil mill effluent (POME). The artificial wastewater derived from various types including starch (8.4 g/L), peptone (3 g/L) and other nutrients. POME is the main substrate was taken from the palm oil industry with the characteristic as shown in the **Table 1**.

Microorganisms

Microbe was obtained from the cow rumen which taken from cow slaughterhouse located in Bandung city and mixed with microbe taken from palm oil mill sludge in Garut, Indonesia. Mixture microbe was grown in batch culture by seeding process using palm oil mill effluent (POME) as a substrate. The seed reactor consisted of 20% (v/v) microbe from cow rumen, 20% (v/v) from palm oil mill sludge, and 60% (v/v) of POME. Microbe was prepared and used as seed microorganism in batch reactor to observe the growth by analyzing volatile suspended solid every two days until the growth reached stationary phase (Khalseh, 2016).

Table 1. Characteristic of palm oil mill effluent (POME) wastewater

Parameter	Result
pH	4.35
tCOD (mg/L)	13,000-15,000
sCOD (mg/L)	4,900-6,500
BOD (mg/L)	6,000-8,000
TVA (mg/L)	3,000 – 3,500
VSS (mg/L)	99-315
TSS (mg/L)	110-350
oil and grease (mg/L)	150-200
Ethanol (mg/L)	1,500-2,000
TN (mg/L-N)	100-200

Batch Fermentation

Bioreactor (**Figure 1)** used in this study is a batch reactor with working volume of five liters contained of four liters substrate and one liter of microbe. Nitrogen purging 1L/min was involved during the first 24 hours to remove the oxygen content in the headspace of the reactor. After nitrogen flushing, internal biogas circulation was replaced as a mixing system from the top to the bottom of the reactor. The Reactor contained of 80% (v/v) substrate from wastewater and 20% (v/v) mixture culture of microbe.

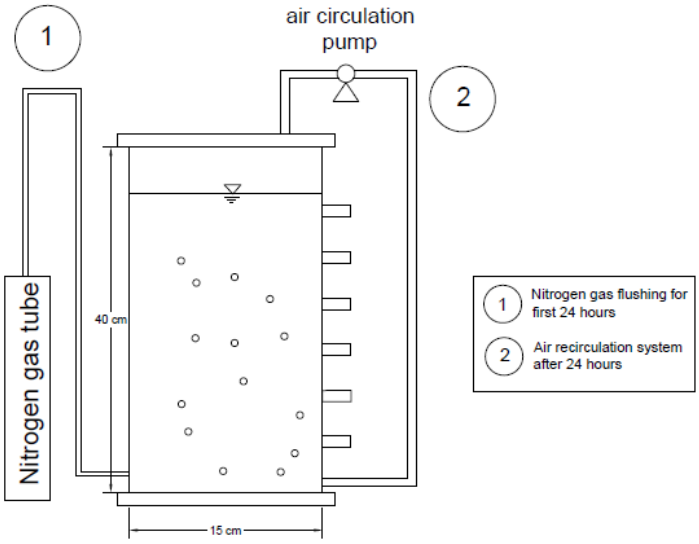

Figure 1. Batch bioreactor for ethanol fermentation

Analytical Methods

Samples were taken from the reactor every 6 hours and analyzed for ethanol, tVFAs, pH, VSS, and sCOD for 72 hours of the experiment. Ethanol and total volatile fatty acids (tVFAs) were analyzed using High Performance Liquid Chromatography (HPLC) (Handajani *et al.*, 2016). Sample was prepared by filtering the previous sample using acrodisc nylon syringe Millipore filter size of 0.22µm. HPLC used the type of interface Hitachi D-7000 HSM which is equipped with UV-Vis detector (Hitachi L-7400) and a pump (Hitachi L-7100). Analytical column was C 18 for ethanol analysis and Aminex HPX-87H (300 mm × 7.8 mm) for tVFAs analysis. The mobile phase of ethanol consisted of eluent, acetate, and methanol and for tVFAs with 5 mm

H_2SO_4, methanol, and aqueous. The operation condition was in temperature of 55°C, flow rate of 1 mL/min, injection volume of 1 mL. Detection of acetate, propionate, butyrate, and valerate used UV-Vis at a wavelength of 210 nm. The concentrations for calibration curve were 50, 75, 100, and 200 nM. pH, VSS, and sCOD were analyzed using pH meter, SM 2540E and SM 5220C method according to the standard methods (Clesceri, 1998).

Calculation

Modified Gompertz Model (**Equation 1**) was able to describe the product (ethanol) formation as the fermentation proceeded (Tussanee *et al.*, 2015) and therefor selected for this study.

$$P = P_m.\exp\left\{-\exp\left[\frac{r_{p,m}.\exp(1)}{P_m}\right].(t_L - t) + 1\right\} \quad (1)$$

Ethanol production yield (**Equation 2**) and tVFAs yield (**Equation 3**) were determined as the maximum amount of each product divided by the amount of substrate utilized as SCOD (Bengtsson *et al.*, 2008). Ethanol production rate (**Equation 4**) and tVFAs production rate (**Equation 5**) were determined as the maximum of each product divided by processing time (Tussanee *et al.*, 2015).

$$Y_{etha}, \text{ (g/g)} = \frac{\text{Maximum ethanol concentration (g/l)}}{\text{Total utilised substrate as COD (g/l)}} \quad (2)$$

$$Y_{tVFAs}, (\text{ g/g}) = \frac{\text{Maximum acids concentration (g/l)}}{\text{Total utilised substrate as COD (g/l)}} \quad (3)$$

$$r_{p,etha} \text{ (g/L/hr)} = \frac{\text{Maximum ethanol concentration (g/L)}}{\text{Fermentation time (h)}} \quad (4)$$

$$r_{p,tVFAs} \text{ (g/L/hr)} = \frac{\text{Maximum acids concentration (g/L)}}{\text{Fermentation time (h)}} \quad (5)$$

RESULT AND DISCUSSION

Artificial Wastewater

Artificial wastewater was used and diluted into three different initial COD concentration (10, 15, 20 g/L) and operated by different pH conditions (5.5, 6.5-7.5, 8.5) to preserve the concentration of ethanol from the fermentation process. The result of ethanol production was shown as in the **Figure 2**.

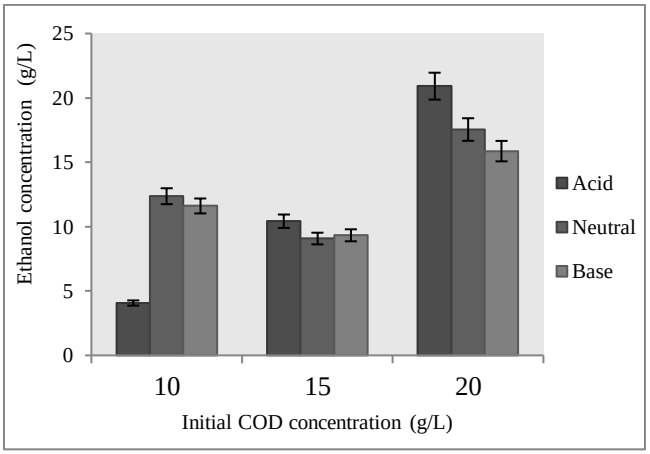

Figure 2. Maximum ethanol productions from artificial wastewater at different pH

When the initial COD concentration started from 10 g/L, the optimum ethanol production obtained in the neutral condition with the concentration of 12.36 g/L and in basic and acidic condition were 11.6 and 4.06 g/L, respectively. With the initial COD concentration of 15 g/L, the maximum ethanol production produced in the acidic condition with the concentration of 10.41 g/L, while in basic and neutral condition produced ethanol of 9.32 and 9.07 g/L, respectively. Otherwise, using artificial wastewater with COD concentration of 20 g/L, the ethanol production reached the maximum concentration of 20.91 g/L while in neutral and basic condition got 17.54 and 15.86 g/L, respectively.

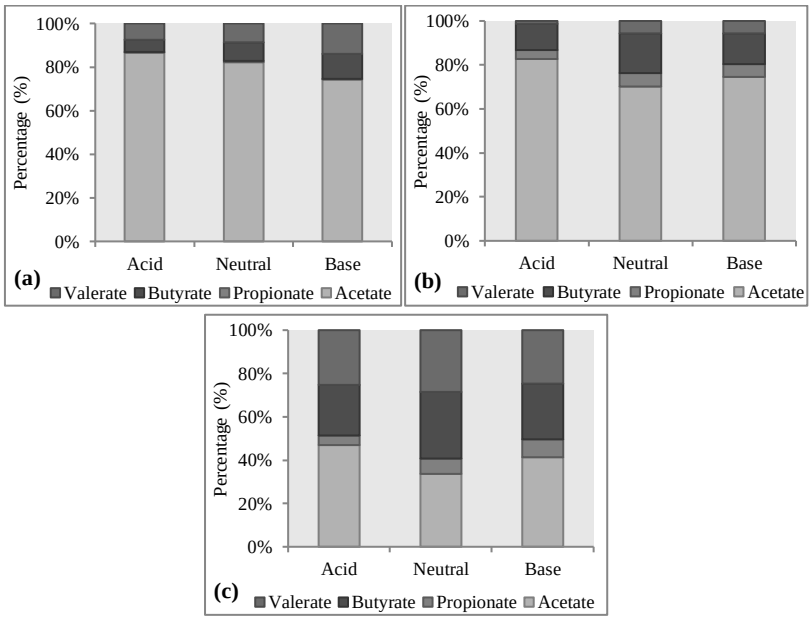

Figure 3. Volatile fatty acids type proportion (Initial COD concentration of (a) 10 g/L, (b) 15 g/L, and (c) 20 g/L)

The results of volatile fatty acids were found acetate was the major product compared to propionate, butyrate, and valerate as shown in the Figure 3 above. From the lower COD concentration **(Figure 3a & 3b)**, the percentage of acetate obtained of more than 70% of the total VFAs. From the initial COD concentration of 20 g/L, acetate, butyrate and valerate were found by different pH condition in a comparable amount **(Figure 3c).**

Palm Oil Mill Effluent

Characterizing of POME wastewater found that COD was about 15,000 mg/L, according to the optimum condition of artificial wastewater, the initial pH operation was adjusted to 5.5 and preserved the product formation during the fermentation process. Ethanol concentration using POME as a substrate was shown as in the **Figure 4**.

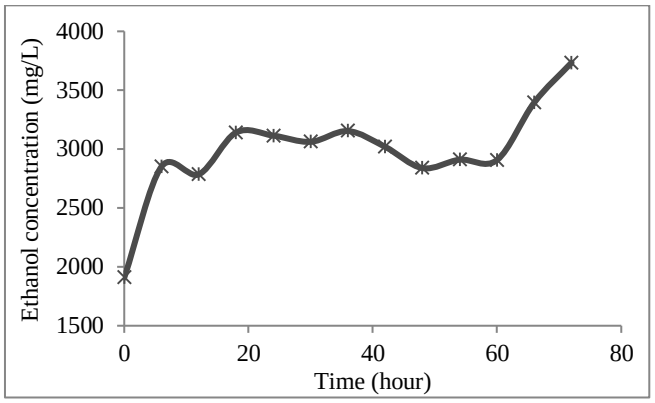

Figure 4. Ethanol production from POME

Ethanol started from the initial concentration of about 1,900 mg/L and increased rapidly to 3,140 mg/L at 18 hours then fluctuated until 36 hours with the concentration of 3,153 mg/L. Ethanol concentration dropped down after 36 hours to about 2,907 mg/L at 60 hours then rose steeply to the maximum concentration of ethanol of 3,734 mg/L. This trend seems the ethanol concentration still increase with the longer time of the fermentation process.

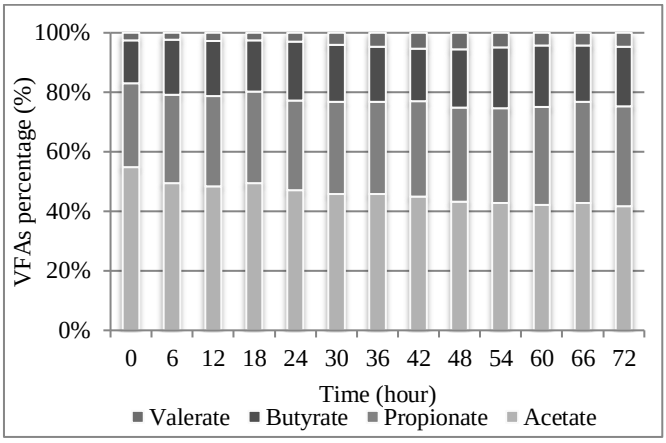

Figure 5. Volatile fatty acids compositions concentration from POME

Production of VFAs from POME (**Figure 5**) has shown that acetate was the major product with the initial concentration of about 2,500 mg/L and dropped down at the first 6 hours to 2,000 mg/L then started to increase slightly resulting of pH drop. Acetate decreased slowly which could cause accumulation of ethanol. Abubackar et al., (2016) found that the interesting observation and conclusion from the experiment, that was used for the next studies, is that during the initial period of the experiment with no pH regulation and thus pH increase, biomass build-up took place and acids were produced; while maintaining a low, constant pH during the remaining part of the study allowed to completely convert the accumulated acetic acid to alcohols. Since the accumulation of acetate and propionate accounted of more than 70% among the VFAs products,

this process was considered as propionic type fermentation with no significantly gas production (Juang *et al.*, 2011).

Table 2. Result of modified Gompertz model

Initial COD (g/L)	pH	P_m (g/L)	$r_{p,m}$ (mg/L/hr)	t_l (hr)
10	**6.5-7.5**	12.36	171.70	24
15	**5.5**	10.41	144.70	12
20	**5.5**	20.91	290.44	12
POME	**5.5**	3.73	51.87	1

The products formation result obtained from the Modified Gompertz model for the optimum condition using artificial and POME given as shown in the **Table 2**.

Table 3. Ethanol and total volatile fatty acids yield and production rate

Initial COD (g/L)	pH	Ethanol yields (g/g)	Ethanol production rate (mg/L/hr)	Acids yields (g/g)	Acids production rate (mg/L/hr)
10	**5.5**	2.90	56.45	3.90	75.85
	6.5-7.5	7.34	171.70	3.27	76.59
	8.5	12.44	161.20	6.19	80.24
15	**5.5**	4.74	144.71	1.09	33.37
	6.5-7.5	8.51	126.02	1.65	24.44
	8.5	4.00	129.53	0.95	30.67
20	**5.5**	3.11	290.44	0.38	35.42
	6.5-7.5	2.77	243.65	0.25	22.24
	8.5	1.89	220.34	0.22	26.23
POME	**5.5**	1.93	51.87	2.34	62.95

From the artificial wastewater (10, 15, and 20 g/L of COD), ethanol yields (g/g) from the optimum condition given the result 7.34, 4.74, and 3.11 and the ethanol production rate (mg/L/hr) were 171.7, 144.71, and 290.44, respectively. For the volatile fatty acids product, in the optimum condition VFAs yields (g/g) were 3.27, 1.09, and 0.38, respectively. The VFAs production rates (mg/L/hr) were 76.59, 33.37, and 35.42, respectively. The performance of starch fermentation for ethanol production from the previous studies has shown different result of product yield and production rate. Liu & Lien, (2016) found that using potato and cassava startch without any pretreatment process using *A. awamori*, *R. juponiaus/Z. Mobilis* obtained the yield of 0.5 and 0.47 g/g, and the production rate of 1.18 and 0.85 g/L/hr, respectively.Using POME as a substrate, the ethanol yield was 1.93 g/g and production rate was 51.87 mg/L/hr while the VFAs yield was 2.34 g/g and VFAs production rate was 62.95 mg/L/hr, relatively higher than ethanol did (**Table 3**).

Table 4. First order reaction of the sCOD degradation from artificial wastewater and POME

Initial COD (g/L)	pH	Rate constant k_h	R^2
10	5.5	0.0072	0.61
	6.5-7.5	0.01	0.8
	8.5	0.0066	0.62
15	5.5	0.0184	0.9
	6.5-7.5	0.018	0.91
	8.5	0.016	0.95
20	5.5	0.02	0.97
	6.5-7.5	0.021	0.97
	8.5	0.023	0.92
POME	5.5	0.0051	0.765

Substrate degradation as soluble COD during the fermentation time was plotted using inverse of concentration versus time giving the straight line where the slop is the rate constant of first-order kinetic. The correlation coefficients (R^2) of each pH operation with different initial concentration have been shown in the **Table 4**. A plot of logarithm of substrate concentration as sCOD versus time (hour) is a straight line with slope ($-k_h$) which can be implied the reaction rate of the reactant in the batch reactor.

CONCLUSION

Through the operation of pH on artificial wastewater, the ethanol productions were obtained maximum in neutral condition with 10 g/L of initial COD concentration, and acidic condition from 15 g/L and 20 g/L with the concentration of 12.36 g/L, 10.41 g/L, and 20.91 g/L, respectively. POME was adjust the pH into acidic condition (pH 5.5), the appropriated condition, since the concentration of COD was about 15 g/L. The maximum ethanol production from POME obtained 3.73 g/L from the fermentation process of 72 hours. Ethanol yield and production rate from POME were 1.93 g/g and 51.87 mg/L/hr, respectively and total volatile fatty acids yield and production rate from POME were 2.34 g/g and 62.95 mg/L/hr, respectively.

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