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Kinetics Studies Impact of Initial pH and Addition of Yeast *Saccharomyces cerevisiae* on Biogas Production from Tofu Wastewater in Indonesia

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ABSTRACT

The purpose of this work was to study the effect of initial pH and yeast *Saccharomyces cerevisiae* on biogas production from tofu wastewater (TW). The initial pH was varied in ranging of 5 – 9 in substrate without yeast (T5-T9) and with yeast (TY5-TY9). The results showed that optimum initial pH was 8. The maximum biogas was resulted in T8 (275 mL) and TY8 (421 mL). Yeast addition increased total biogas compared with no yeast addition. Kinetic of biogas production was modeled through modified Gompertz and Cone models. The predicted biogas in Cone model was more precise than that in modified Gompertz. The difference between measured and predicted biogas in Cone and modified Gompertz models was 0.193 – 2.809 and 0.316 – 3.115 % respectively. The presence of yeast increased the kinetic constant of ym (biogas potential, mL) and λ (lag period, days), and decreased k_{hyd} (hydrolysis rate, /day).

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1. INTRODUCTION

Indonesia is one of the developing countries in the world. As a developing country, Indonesia has tremendous amount of tofu Small and Medium Enterprises (SMEs) as much as 84,000 units. Tofu is a popular food in Indonesia, because of its associated health benefits and acceptable price [1]. Those units are wide spread all over the districts in Indonesia and contribute to produce wastewater up to about 2.56 million meter cubic per year.

Tofu is traditional oriental food produced from soybean as raw materials through some steps, i.e. soybean grinding, cooking (boiling), filtration, protein coagulation, preservation, and packaging [2]. In Indonesia, the 80 kg tofu is produced from 60 kg soybean and 2,700 kg water. During the tofu production process, the tofu industries generate byproducts of 70 kg soybean curd and 2,610 kg wastewater (TW). The soybean curd is utilized as nutritious feed for livestock as derivative food. In the other hand, TW has not been treated completely, so that it directly enters the environment and produces bad odor, Green House Gasses (GHG) emission and pollution in water and soil.

The bad impacts of TW in the environment are caused by its huge amount and high organic contents [3]. According to Intergovernmental Panel on Climate Change (IPCC) emission reduction calculation in AM 0013 method, if the TW is discharged directly into the rivers without treating before, total baseline of GHG emission is 46,494,000 kg CH_4/year or 976,374 ton CO_2/year. Meanwhile, if the TW is treated as much as 80%, the potential emission reduction will approach 744,469 ton CO_2/year. The Indonesian government has goals which are voluntarily commitment to reduce carbon emissions by 26 percent in 2020 and to reduce fossil fuels need by 5 percent in 2025. Therefore, the best way in treating TW is using anaerobic digestion (AD), so that its organic contents are transformed into biogas. Hence, the biogas is utilized as alternative energy to substitute by 5 percent of fossil fuels need in Indonesia. Especially, biogas can be used to fulfill energy required by society for rural development.

In this work, we focused on biogas enhancement from TW. Microbial strains (some bacteria and fungi)

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can increase total biogas by stimulating the activity of particular enzymes [3]. Yeast *Saccharomyces cerevisiae* was selected as microbial agent in this study. This yeast is contained in *Ragi*. The *Ragi* can be found in traditional markets in Indonesia. Generally, *Ragi* is made in home industries, which is then sold with or without trade mark. *Ragi* is usually used by home industries to produce ethanol because it contains mixed cultures with dominant of *Saccharomyces cerevisiae* strain. In ethanol production, the yeast can transform carbohydrates to glucose and subsequent to ethanol, acetic and butyric acid. We guessed that the presence of yeast can help the anaerobic-bacterial activity to degrade organic materials. Hence, it can increase biogas production from TW.

Besides that, the initial pH was also investigated. The initial pH is a fundamental factor for microbial activity [6]. By yeast addition, there are some microbes in the substrate, not only anaerobic bacteria which are derived from rumen fluid but also the yeast *Saccharomyces cerevisiae*. Therefore, the optimum initial pH must be found. In this study, the initial pH was varied in ranging from 5 to 9.

Furthermore, the kinetic model of modified Gompertz and Cone model were chosen to simulate the actual data from experiment. Also, we compared between the two models to find which one is better in predicting of biogas production from TW. Many authors have investigated the accuracy and precision between modified Gompertz and first order kinetic model [4-7]. They found that the modified Gompertz could predict biogas potential with low error prediction. Whereas, the first order kinetic was just suitable to be used in biogas from rich-carbohydrate substrates, such as vinasse [4]. In this work, we used TW as feedstock of biogas. The TW contained high protein/nitrogen content (Table 1). Hence, the first order kinetic was not recommended for this study.

The other kinetic models which can be used to predict biogas rate were Monod, Andrew, and Logistic. Among them, the Logistic was usually used in predicting of biogas production. Many authors have compared the modified Gompertz and Logistic model in fitting (R^2) between experimental data and predicted data. According to some literatures, modified Gompertz model and Logistic model had the fitting error value (R^2) of 0.9895-0.999 and 0.9775-0.999 respectively [8-10]. Hence, modified Gompertz model was more accurate in predicting of biogas production than Logistic model. Therefore, in this research, we chose modified Gompertz model.

Pitt et al. [11] was the first authors who proposed Cone model. Furthermore, the model most recently was used by Zhen et al. [12] for simulating the anaerobic co-digestion waste activated sludge and *Egeria densa*. The existence of Cone model was still low, because the literatures which discussed about the model were limited. The information about the model on biogas modeling from tofu wastewater has not been reported by other authors yet.

This research is new and original because it has not been conducted and reported by other authors yet. Achmad et al. [13] studied the effect of rumen liquid and *S. cerevisiae* dose on the quantity of biogas generation from fresh market garbage. Ekpeni et al. [14] investigated the potential of yeast as biomass substrate for biogas production. Whereas, Colussi et al. [15] studied the influence of fermentative yeast (*S. cerevisiae*) on biogas production from solid potatoes using two-stage anaerobic digestion. From the informations above, we concluded that the study of *S. cerevisiae* addition and initial pH on biogas production from particular substrate which was tofu wastewater from Indonesian country has not been reported yet.

2. METHODS

2. 1. Wastewater, Inoculums, and Yeast The wastewater used was tofu wastewater (TW) obtained from a tofu industry. The industry located in Serang City, Banten Province-Indonesia, which produced tofu from soybean. The TW contained 576 mg/L COD, 13.5 mg/L nitrogen total, COD/N of 298.7/7, pH level of 4.2. The rumen fluid was used as inoculums. The rumen fluid in fresh condition was obtained from slaughterhouse in Serang, Banten Province, Indonesia. Rumen fluid contained *Clostridium sp.*, *Clostridium sporogenes*, *Clostridium butyricum* and rich methanogenic bacteria. *Ragi* was used as yeast *Saccharomyces cerevisiae* provider. *Ragi* was easy to be found in traditional markets and usually used in ethanol production industries. The shape of *Ragi* was flat round with diameter of 4 – 6 cm and thickness of 0.5 cm. The *Ragi* was crushed into powder mode.

2. 2. Experimental Set Up Anaerobic digesters were made from polyethylene bottles having volume of 600 mL. The bottles were plugged with rubber plug and were equipped with valve for biogas measurement. Biogas formed was measured by liquid displacement method as also has been used by the other authors [16-18]. In this method, each digester was connected to gas collector that was reserved cylindrical glass. The connection was done using connecting tube. Each gas collector was immersed in through of water to ensure complete sealing. Biogas formed from digesters was collected by the downward displacement of water.

2. 3. Experimental Design Anaerobic digestion of experimental laboratory using 600-mL volumes was operated in batch system, at room temperature and pressure of 1 atm. 250-mL substrate was put into digesters. Rumen fluid as methanogenic bacteria

provider was added into the digesters as much as 10% v/v substrate. In this work, we compared the effect of initial pH and yeast *Saccharomyces cerevisiae* addition to biogas production. The pH of substrates was adjusted 5, 6, 7, 8 and 9 by using NaOH solution 5 N. *Ragi* as much as 1 gram was added into variables of TY5-TY9. All variables in this study can be seen in Table 1.

2. 4. Experimental Procedures Fermentation was done until no longer produced biogas at room temperature and pressure of 1 atm. Biogas formed was measured once in two days to know biogas production by using water displacement method. The pH of substrate was measured by using pH meter once in two days. The organic materials removal was calculated based on total solid (TS) removal. In the end of fermentation, the final TS of all variables were measured.

2. 5. Kinetic Model of Biogas Production Biogas production kinetic was modeled through modified Gompertz model [16, 19, 20] and Cone model [12]. Kinetic of biogas production in batch condition was assumed that had correspondence to specific growth rate of methanogenic bacteria in digesters. Kinetic constant of ym, λ, U, k_{hyd}, n was determined by using non-linear regression with help of polymath software [16, 19, 20]. The equations of modified Gompertz model (1) and cone model (2) were shown below:

$$y(t) = ym.\exp\left\{-\exp\left[\frac{U.e}{ym}(\lambda - t) + 1\right]\right\}, t \geq 0 \quad (1)$$

$$y(t) = \frac{ym}{1 + (k_{hyd}.t)^{-n}}, t > 0 \quad (2)$$

where:

y(t) = the cumulative biogas at digestion time t days (mL)
ym = the biogas production potential (mL)
U = the maximum biogas production rate (mL/day)
λ = lag phase period or minimum time to produce biogas (days)
t = cumulative time for biogas production (days)
e = mathematical constant (2.718282)
k_{hyd} = hydrolysis rate constant (/day)
n = shape factor

3. RESULTS AND DISCUSSION

3. 1. Biogas Production

3. 1. 1. The Effect of Initial pH The variables of T5, T6, T7, T8, T9 produced 179, 183, 237, 275, 263 mL total biogas respectively. The optimum variable was T8 which had initial pH of 8. From Figure 1, biogas amount at 1st day of fermentation from T8 was higher than T5, T6, T7, T9. That means anaerobic bacteria were easy to adapt in the substrate. Initial pH of 8 was comfortable for bacterial activity. Speece [21] also stated that pH level up to 8.2 can produce biogas optimally.

The substrate pH of T5, T6, T7, T8, T9 was changing during fermentation, from 5 to 5.66, 6 to 5.87, 7 to 6.11, 8 to 6.31, 9 to 6.32 respectively. For all variables, the decreasing of substrate pH was occurred from 1-day until 8-day fermentation. Furthermore, above the 8-day fermentation, the substrate pH increased. The decreasing of pH was caused by accumulation of Volatile Fatty Acids (VFAs) produced from decomposition of carbohydrate contents.

TABLE 1. Biogas production and digester performances at various initial pH and yeast addition

Variables	TW (mL)	Rumen fluid (mL)	*Ragi*;Yeast (gram)	pH		TS			Total Biogas (mL)
				Initial	Final	Initial (%g/g)	Final (%g/g)	Removal (%)	
T5	250	25	-	5	5.66	1.355	0.903	33.337	179
T6	250	25	-	6	5.87	1.355	0.898	33.716	183
T7	250	25	-	7	6.11	1.355	1.175	13.265	237
T8	250	25	-	8	6.31	1.355	1.121	17.292	275
T9	250	25	-	9	6.32	1.355	1.094	19.251	263
TY5	250	25	1	5	5.43	1.355	0.707	47.792	220
TY6	250	25	1	6	5.46	1.355	0.658	51.448	333
TY7	250	25	1	7	5.63	1.355	1.184	12.628	370
TY8	250	25	1	8	5.74	1.355	0.467	65.517	421
TY9	250	25	1	9	5.71	1.355	0.452	66.669	374

Remarks: TW, tofu wastewater; TS, total solid

In the other hand, decomposition of nitrogen contents produced ammonia (NH_3) or ammonium (NH_4^+). The accumulation of these could increase the pH level. Carbohydrate was more easily to be degraded than protein [7]. Hence, in anaerobic digestion, pH of substrate was always decreasing in first time of digestion, then that was increasing gradually. In this work, decreasing of pH was not sharply. That was due to the COD/N ratio in TW. The TW contained low COD and high N content (COD/N = 298.7/7).

In this work, initial pH of 8 generated the largest total biogas (275 mL). Lay et al. [22] reported the same results, that initial pH of 7.5-8 was suitable for biogas production from tofu wastewater. The acidogenic and acetogenic bacteria produced VFAs (acetate, propionate, i-butyrate, n-butyrate), then methanogenic bacteria utilized VFAs to produce biogas. At initial pH of 4-7, biogas formed was just a little and the VFAs were still in large amount after 48-day fermentation. Whereas, at initial pH of 7.5-8, VFAs were just a little because these were converted into biogas. Thus, the total biogas was bigger than that at pH below of 7.5 [4]. Based on that, the initial pH of 8 was the most suitable for anaerobic bacteria, especially methanogenic bacteria in biogas generation from TW.

3. 1. 2. The Effect of Yeast Addition Total biogas from variables of TY5, TY6, TY7, TY8, TY9 was 220, 333, 370, 421, 374 mL respectively. By addition of yeast (TY5-TY9), total biogas was increased as much as 22.91, 81.97, 56.12, 53.09, 42.21 % respectively compared T5-T9 (without yeast addition). Yeast *Saccharomyces cerevisiae* converted glucose into ethanol, acetic acid and butyric acid [23]. The reaction can be seen in Equations (3) and (4). In Equation (3), 4 mol of glucose were converted to 2 mol of acetic acid and 3 mol of butyric acid. Whereas in Equation (4), 1 mol of glucose was converted to 1 mol ethanol and 1 mol of acetic acid.

$$4C_6H_{12}O_6 \rightarrow 2CH_3COOH + 3CH_3(CH_2)_2COOH + 8H_2 + 8CO_2 \quad (3)$$

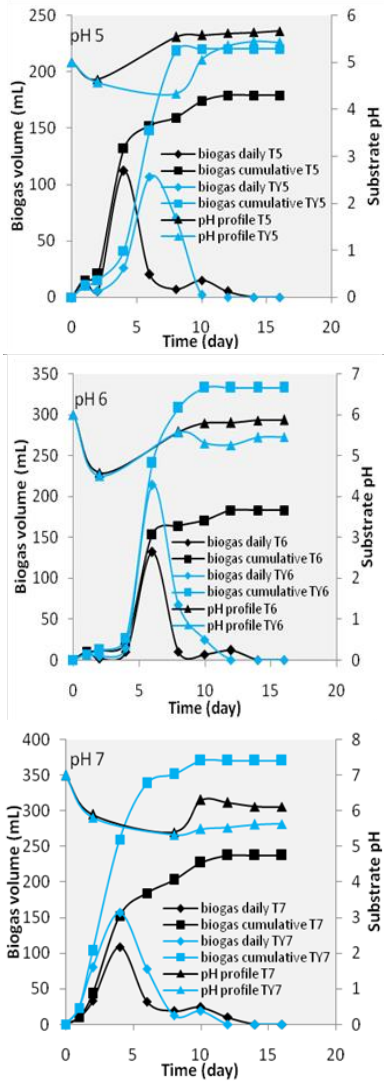
$$C_6H_{12}O_6 + H_2O \rightarrow C_2H_5OH + CH_3COOH + 2H_2 + 2CO_2 \quad (4)$$

Meanwhile, in biogas production processing, ethanol, acetic acid and butyric acid were resulted from acidogenesis phase. The acetic acid formed could be converted into methane and carbon dioxide by methanogenic bacteria directly. However, the methanogenic bacteria could not convert ethanol and butyric acid into methane. Hence, the methanogenic bacteria needed help of acetogenic bacteria to change ethanol and butyric acid into acetic acid and hydrogen. Then, acetic acid was converted to be methane [24]. In TY5-TY9, the yeast would convert glucose into ethanol, acetic and butyric acid. These compounds were intermediate products of biogas. Thus, total biogas formed was more than that in substrates without yeast addition (T5-T9).

Figure 1 showed the results of the batch test used to investigate the effect of initial pH on biogas production with yeast addition. When the pH was 5, the total biogas was 220 mL. Whereas, when the pH was above 5, the quantity of biogas produced substantially increased. The most biogas production (421 mL) was reached at initial pH of 8. In addition, when the pH was 9, the total biogas was lower than that when pH of 8. Lin et al. [23] stated that yeast produced ethanol, acetic and butyric acid with composition depended by initial pH. The best initial pH for ethanol production was 5, the composition of ethanol, acetic acid and butyric acid was 65.54, 1.63, 0.02 %, respectively. Furthermore, at initial pH of 6, the composition of them was 48.80, 9.00, 17.05 %, respectively. We concluded that, the more alkaline of pH, the less of ethanol and the more acetic and butyric acid formed. Thus, in this work, initial pH of 5 – 7 produced ethanol in high concentration. The high amount of ethanol was in the system, methanogenic bacteria were death. Whereas, initial pH of 8 – 9 produced ethanol in concentration which was still tolerance for methanogenic bacteria, and high acetic and butyric acid which were used as raw materials to produce biogas.

The substrate pH of TY5, TY6, TY7, TY8, TY9 was changing during fermentation, from 5, 6, 7, 8, 9 to 5.43, 5.46, 5.63, 5.74, 5.71, respectively. By presence of yeast, the final pH was lower than that at no yeast addition (Figure 1 and Table 1). The ethanol, acetic and butyric acid, which were produced by yeast activity, were accumulated in the system. Thus, the substrates of TY5-TY9 were more acidic than substrates of T5-T9.

3. 2. Kinetic Analysis Two types of models including modified Gompertz and Cone models were subsequently employed to simulate the principal kinetic patterns of biogas production obtained from experimental test. The kinetic parameters, such as ym, λ, U, k_{hyd}, n were estimated based on the best fit of the studied models and the results were summarized in Table 2. For all studied models, the predicted maximum biogas potential (ym) increased with increased the initial pH from 5 until 8. Furthermore, at initial pH of 9, the ym decreased. The difference between the measured biogas and predicted biogas observed in modified Gompertz model was 0.316 – 3.115 % and in Cone model was 0.193 – 2.809 % (Table 2). Clearly, between the proposed models, Cone model better fitted the actual evolution of biogas production, which was also strongly supported by its high correlation coefficient (R^2 of 0.978 – 0.999) and the low Root Mean Square Deviation (RMSD of 1.379 – 5.384).



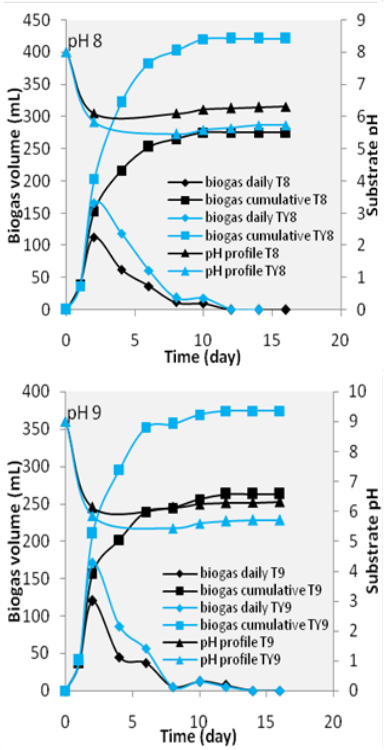


Figure 1. The pH profile, biogas production daily and cumulative during fermentation at variation of initial pH in substrates without and with yeast addition

Meanwhile, modified Gompertz model had R^2 of 0.977 – 0.999 and RMSD of 1.748 – 6.293. To further verify the above observations, the predicted values of biogas from modified Gompertz and Cone model were plotted against the actual values, as presented in Figure 2.

TABLE 2. Estimated parameters of modified Gompertz and Cone model

	Without yeast addition					With yeast addition				
	pH 5	pH 6	pH 7	pH 8	pH 9	pH 5	pH 6	pH 7	pH 8	pH 9
Modified Gompertz Model										
λ (days)	1.561	3.672	1.005	0.354	0.335	3.446	3.895	0.916	0.589	0.522
U (mL/day)	54.865	71.449	46.753	79.108	77.988	64.796	121.829	91.368	103.317	110.455
R^2	0.987	0.991	0.991	0.987	0.977	0.994	0.999	0.999	0.984	0.983
RMSD	2.561	2.439	2.718	3.528	4.448	2.292	1.748	1.245	6.293	5.626
Measured biogas (mL)	179	183	237	275	263	220	333	370	421	374
ym (mL)	173.424	177.738	232.435	270.364	254.441	222.754	331.948	368.796	414.469	366.87
Diff. (%)	3.115	2.875	1.926	1.686	3.254	1.252	0.316	0.325	1.551	1.906
Cone Model										
K_{hyd} (/day)	0.315	0.202	0.284	0.501	0.523	0.191	0.187	0.344	0.424	0.506
n	3.591	8.769	2.428	2.171	2.144	5.829	7.941	2.669	2.688	2.462
R^2	0.985	0.990	0.994	0.991	0.978	0.993	0.998	0.999	0.984	0.987
RMSD	2.562	2.508	2.174	2.419	3.468	2.502	2.08	1.379	5.384	4.024
Measured biogas (mL)	179	183	237	275	263	220	333	370	421	374
ym (mL)	175.982	177.859	243.022	278.318	262.292	223.777	331.651	377.887	414.36	373.279
Diff. (%)	1.686	2.809	2.541	1.206	0.269	1.717	0.405	2.132	1.577	0.193

Remarks: ym, the biogas production potential; U, the maximum biogas production rate; λ, lag phase period or minimum time to produce biogas; k_{hyd}, hydrolysis rate constant; n, shape factor; R^2, correlation coefficient; RMSD, Root Mean Square Deviation; Diff, difference between measured and predicted biogas

Most of works undertaken in the past have often compared the first order kinetic and modified Gompertz model to model the experimental data. Kafle et al. [6] and Budiyono et al. [4] reported that modified Gompertz model can predict the biogas with lower diff (%) than first order kinetic. Furthermore, Zhen et al. [12] found that the Cone model had the best fitness for realistic simulation of the measured biogas, compared with first order kinetic and modified Gompertz model. The finding drawn from this study supported that the Cone model was as the most suitable method for the prediction of biogas production. However, many authors did not use this model to simulate the biogas kinetic, because of its low familiarity. Thus, with this work, we proposed the Cone model as potential model in biogas modeling because of its high precision and credibility.

Furthermore, the correlation between λ, k_{hyd}, and total biogas was shown in Figure 3. The value of λ indicated the time that was required for methanogenic bacteria to adapt in the substrates [25]. By without and with yeast addition, the λ value decreased with increasing of pH from 5 to 9. The lower the λ value, the more comfortable the substrate for the methanogenic bacteria. During fermentation process, there were two kinds of organic acids in the substrate, which were not dissociated acids and dissociated acids [26]. The composition of them was depended on pH value. The more acidic condition of substrate, the more the not dissociated acids formed. Thus, not dissociated acids were dominant in substrates with initial pH of below 7. That hampered the methanogenic-bacterial activity because not dissociated acids were penetrated into cell and denatured the protein of bacteria [26]. Whereas, according to Brannen and Davidson [27], the inhibitory mechanism of bacterial activity by organic acids was related to acid-base equilibrium. Acid-base equilibrium in cell of bacteria was in neutral pH condition. Organic acids penetrated into the cell, disturbed acid-base equilibrium so that bacteria experienced cell-lysis. Hence, that could spoil protein, nucleic acid and phospholipid in cell bacteria. At initial pH of above 7, methanogenic bacteria thrived and produced biogas in large amount. Lay et al. [22] also reported the same results, substrate with initial pH of 7.5-8 produced more biogas than that with initial of 4-7. The λ value of T5-T9 and TY5-TY9 was 0.335-3.672 and 0.522-3.895 days respectively. The presence of yeast increased the total amount of organic acids and ethanol. The abundant of organic acids and ethanol can disturb the anaerobic-bacterial activity, so that the bacteria needed the longer adaptation time (λ).

The k_{hyd} indicated the hydrolysis rate of organic materials. The initial pH increased from 5 to 9, not only caused decreasing in λ value, but also caused increasing in k_{hyd} value (Figure 3). We concluded that the comfortable substrate was good for bacterial activity, so that the bacteria were easy to adapt. Hence, hydrolysis phase was carried out well. The k_{hyd} value in TY5-TY9 (0.187-0.506 /day) was less than that in T5-T9 (0.202-0.523 /day). The rumen fluid contained hydrolysis bacteria (*Clostridium sp.*), acidogenic bacteria (*Clostridium sporogenes*), acetogenic bacteria (*Clostridium butyricum*) and rich methanogenic bacteria. In variables of T5-T9, *Clostridium sp.* converted complex organics (carbohydrate, protein, fat) into simple organics (sugar, amino acid, LVFA). Whereas, according to Christy et al. [3], the yeast *Saccharomyces cerevisiae* have been genetically engineered to carry out simultaneous saccharification and fermentation (SSF) to produce extracellular endoglucanase and glucosidase which are able to ferment polysaccharide/carbohydrate to 6-carbon and 5-carbon sugars (glucose). Thus, in variables TY5-TY9, yeast hydrolyzed carbohydrates and produced glucose. Furthermore, glucose formed was converted into organic acids and ethanol. The accumulation of these compounds disturbed the hydrolysis stage which was carrying out by microbes including in the substrates. Thus, the k_{hyd} was low.

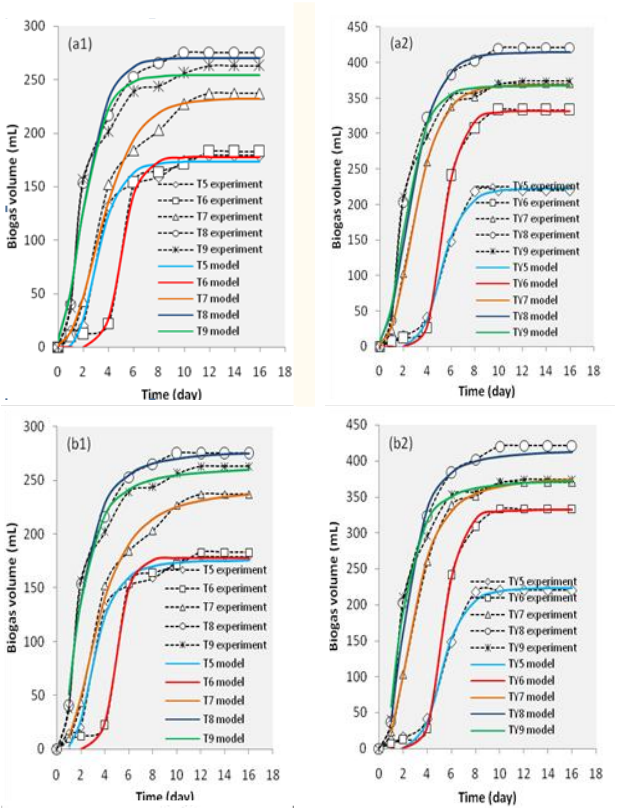


Figure 2. Ploting between measured value and predicted value obtained from modified Gompertz model (a1 = without yeast addition, a2 = with yeast addition) and Cone model (b1 = without yeast addition, b2 = with yeast addition)

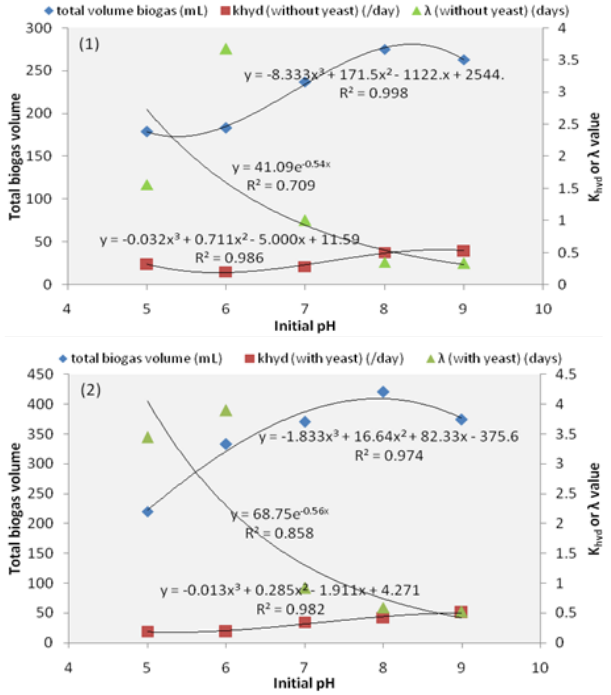


Figure 3. The effect of initial pH on biogas production, λ value, k_{hyd} in substrate (1) without yeast and (2) with yeast addition

3. 3. Total Solid Removal The total solid (TS) removal was analyzed to know the effect of initial pH and yeast addition on organic material removal. The initial and final TS were shown in Table 1. In addition, Figure 4 presented the TS removal for all variables. The more the final TS value, the less the TS removal value. The TS removal in TY5-TY9 (12.628-66.669 %) was more than that in T5-T9 (13.265-33.716 %). The yeast helped the anaerobic bacteria to degrade organic materials into biogas. Both without and with yeast addition, the TS removal increased when the initial pH was increased from pH 5 to 6. Furthermore, at pH 7, the TS removal value was the least. When the initial pH was increased from pH 7 to 9, the TS removal increased. The biggest of TS removal was at pH of 9.

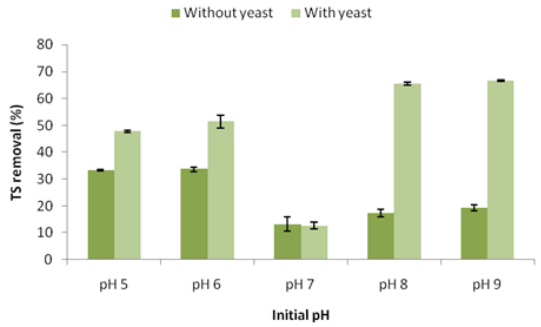


Figure 4. The effect of initial pH and yeast addition on TS removal

Yeast *Saccharomyces cerevisiae* grew optimally at pH of 5. In that condition, the yeast produced ethanol, acetic acid and butyric acid with composition of 65.54, 1.63, 0.02 % respectively. When the pH was above 7, the ethanol production decreased and the acetic and butyric acid increased. Although TS removal at initial pH 5-6 was higher than that at pH 7, the biogas production at pH 7 was more than that at pH 5-6. That might be caused by ethanol production in large amount. Hence, that disturbed methanogenic bacteria so that the biogas production was just little at pH 5-6. When pH of 7, the *Saccharomyces cerevisiae* still grew but its growth rate was lower. The yeast also produced high acetic and butyric acid. The methanogenic bacteria converted these compounds into biogas. Thus, the biogas production was higher than that at pH 5-6, although the TS removal value was low. The pH of 8 was the best condition, because *Saccharomyces cerevisiae* produced compounds with high acetic and butyric acid which was higher than that at pH 7. Anaerobic bacteria, especially methanogenic bacteria, grew well in substrate of tofu wastewater at initial pH of 8. Therefore, the biogas formed was the highest of all variables. The methanogenic-bacterial activity was hampered at pH above 8. However, the hydrolysis bacteria contained in rumen fluid still can live at the condition. Hence, total biogas at pH 9 was lower than that at pH 8, although the TS removal was higher than at pH 8. We concluded that at pH 5-6, the organic materials removal was dominant by yeast *Saccharomyces cerevisiae*. At pH 8-9, the organic materials removal was dominant by hydrolysis bacteria (*Clostridium sp.*). Whereas at pH 7, both of microbes, *Saccharomyces cerevisiae* and *Clostridium sp.* could grow well but not optimally, so that the TS removal was low although the biogas formed was high enough.

3. 4. Prediction of Scheme of Biogas Production Process In this section, we tried to predict the scheme of biogas processing during fermentation in anaerobic digesters. The rumen fluid used contained *Clostridium sp.*, *Clostridium sporogenes*, *Clostridium butyricum* and rich methanogenic bacteria. Meanwhile, *Ragi* contained yeast *Saccharomyces cerevisiae*. The anaerobic bacteria and yeast were in the system simultaneously to produce biogas. Biogas was generated through four phases i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis phase. The predicted scheme of biogas production can be seen in Figure 5.

In hydrolysis phase:

In this phase, the insoluble materials such as polysaccharides, lipids, proteins were converted into soluble materials such as simple sugars (glucoses), long-chain fatty acids, amino acids [26] by *Clostridium sp.* involved in rumen fluid. Meanwhile, yeast *Saccharomyces cerevisiae* also converted polysaccharides to glucose with help of amylase

enzyme. However, the yeast could not produce protease and lipase enzyme so that lipids and proteins could not be changed to long-chain fatty acids and amino acids.

In acidogenesis phase:

In this phase, glucoses were converted into acetic acid, butyric acid, propionic acid, hydrogen, carbon dioxide, methanol and ethanol. The amino acids were converted into acetic acid, butyric acid, propionic acid, carbon dioxide, ethanol, methanol, ammonia/ammonium. The long-chain fatty acids were converted to acetic acid, hydrogen and carbon dioxide [26]. The *Clostridium sporogenes* played a role at this phase. Meanwhile the yeast *Saccharomyces cerevisiae* converted glucose to acetic acid, butyric acid and ethanol (Equations (3)-(4)) [23].

In acetogenesis phase:

Methanogenic bacteria could not use the compounds that contained more than two of carbon atom to produce biogas. Hence, compounds which were produced from acidogenesis phase, such us propionic acid, butyric acid and ethanol, had to be converted into acetic acid, carbon dioxide and hydrogen (Equations (5)–(7)) [28]. This process was done by *Clostridium butyricum* that was found in rumen fluid.

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 \quad (5)$$

Propionic acid Acetic acid

$$CH_3CH_2CH_2COOH \rightarrow 2CH_3COOH + 2H_2 \quad (6)$$

Butyric acid Acetic acid

$$CH_3CH_2OH + H_2O \rightarrow CH_3COOH + 2H_2 \quad (7)$$

Ethanol Acetic acid

In methanogenesis phase:

Methanogenic bacteria, that were contained in rumen fluid in high amount, converted biogas through 3 (three) reaction types. The reaction types were hydrogenotrophic methanogenesis, acetoclastic methanogenesis and methyltrophic methanogenesis [28, 29]. In hydrogenotrophic methanogenesis reaction (Equation (8)), the bacteria changed carbon dioxide and hydrogen into methane and water. Furthermore, in acetoclastic methanogenesis (Equation (9)), the bacteria changed acetic acid into methane and carbon dioxide. Moreover, in methyltrophic methanogenesis (Equation (10)), the bacteria converted methanol into methane, carbon dioxide and water.

$$2H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (8)$$

Methane

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (9)$$

Acetic acid Methane

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O \quad (10)$$

Methanol Methane

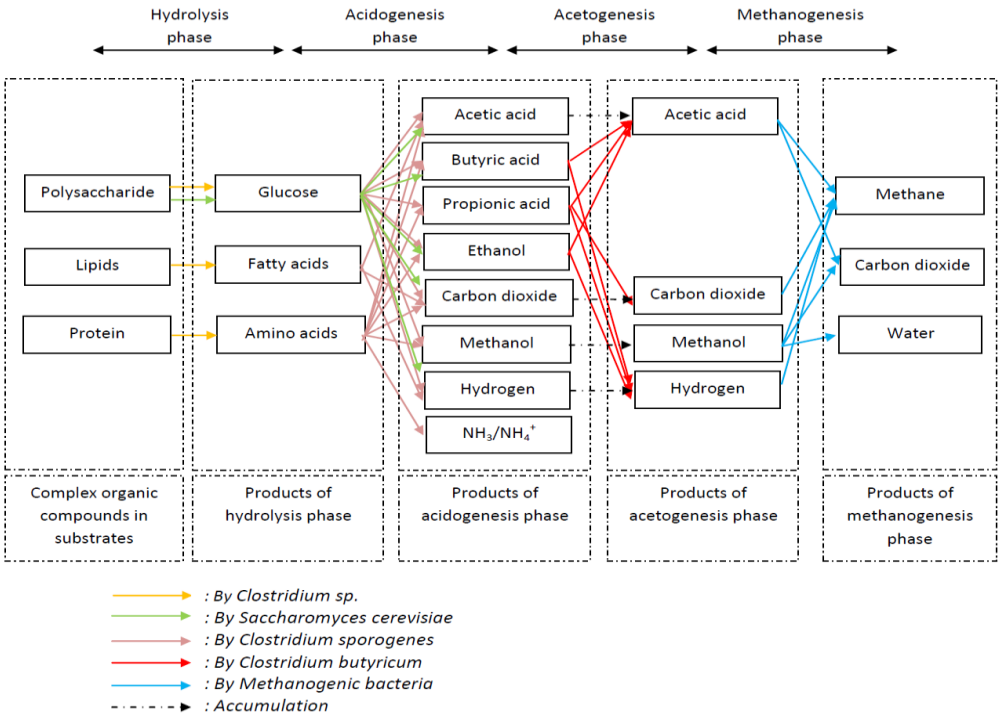


Figure 5. Biogas production from activity of anaerobic bacteria from rumen fluid and yeast *Saccharomyces cerevisiae* simultaneously

4. CONCLUSION

The total biogas formed in T5-T9 and TY5-TY9 was 179, 183, 237, 275, 263 mL and 220, 333, 370, 421, 374 mL, respectively. The presence of yeast could increase the biogas production. The best initial pH was 8. The Cone model could predict the biogas potential with higher accuracy than modified Gompertz model. The difference between measured and predicted biogas in modified Gompertz and Cone model was 0.316 – 3.115 % and 0.193 – 2.809 %, respectively. The presence of microbial agent (*Saccharomyces cerevisiae*) not only increased the ym and λ value but also decreased the k_{hyd}.

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6. REFERENCES

1. Faisal, M., Mulana, F., Gani, A. and Daimon, H., "Physical and chemical properties of wastewater discharged from tofu industries in banda aceh city, indonesia", ***Research Journal of Pharmaceutical, Biological and Chemical Sciences***, Vol. 6, No. 4, (2015), 1053-1058.
2. Kim, D.-H., Lee, D.-Y. and Kim, M.-S., "Enhanced biohydrogen production from tofu residue by acid/base pretreatment and sewage sludge addition", ***international Journal of Hydrogen Energy***, Vol. 36, No. 21, (2011), 13922-13927.
3. Christy, P.M., Gopinath, L. and Divya, D., "A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms", ***Renewable and Sustainable Energy Reviews***, Vol. 34, No., (2014), 167-173.
4. Budiyono, I. and Sumardiono, S., "Kinetic model of biogas yield production from vinasse at various initial ph: Comparison between modified gompertz model and first order kinetic model", ***Research Journal of Applied Sciences, Engineering and Technology***, Vol. 7, No. 13, (2014), 2798-2805.
5. Kafle, G.K. and Kim, S.H., "Anaerobic treatment of apple waste with swine manure for biogas production: Batch and continuous operation", ***Applied Energy***, Vol. 103, (2013), 61-72.
6. Kafle, G.K., Kim, S.H. and Sung, K.I., "Ensiling of fish industry waste for biogas production: A lab scale evaluation of biochemical methane potential (bmp) and kinetics", ***Bioresource Technology***, Vol. 127, No., (2013), 326-336.
7. Zhang, W., Wei, Q., Wu, S., Qi, D., Li, W., Zuo, Z. and Dong, R., "Batch anaerobic co-digestion of pig manure with dewatered sewage sludge under mesophilic conditions", ***Applied Energy***, Vol. 128, (2014), 175-183.
8. Latinwo, G. and Agarry, S., "Modelling the kinetics of biogas generation from mesophilic anaerobic co-digestion of sewage sludge with municipal organic waste", ***Chem Process Engineering Research***, Vol. 31, (2015), 2224-7467.
9. Latinwo, G.K. and Agarry, S.E., "Modelling the kinetics of biogas production from mesophilic anaerobic co-digestion of cow dung with plantain peels", ***International Journal of Renewable Energy Development***, Vol. 4, No. 1, (2015), 55-63.
10. Ghatak, M.D. and Mahanta, P., "Comparison of kinetic models for biogas production rate from saw dust", ***Carbon***, Vol. 63, (2014), 35-43.
11. Pitt, R., Cross, T., Pell, A., Schofield, P. and Doane, P., "Use of in vitro gas production models in ruminal kinetics", ***Mathematical Biosciences***, Vol. 159, No. 2, (1999), 145-163.
12. Zhen, G., Lu, X., Kobayashi, T., Li, Y.-Y., Xu, K. and Zhao, Y., "Mesophilic anaerobic co-digestion of waste activated sludge and egeria densa: Performance assessment and kinetic analysis", ***Applied Energy***, Vol. 148, (2015), 78-86.
13. Achmad, K., Gani, S., Sunarto, S. and Hamidah, I., "The use of rumen liquid and saccharomyces cerevisiae as activators in biogas production from fresh market garbage", ***Lucrari Stiintifice-Universitatea de Stiinţe Agricole si Medicina Veterinara, Seria Zootehnie***, Vol. 56, (2011), 117-120.
14. Ekpeni, L.E., Nkem-Ekpeni, F.F., Benyounis, K.Y., Aboderheeba, A.K., Stokes, J. and Olabi, A.-G., "Yeast: A potential biomass substrate for the production of cleaner energy (biogas)", ***Energy Procedia***, Vol. 61, (2014), 1718-1731.
15. Colussi, I., Cortesi, A., Gallo, V., Rubesa Fernandez, A. and Vitanza, R., "Methane production from solid potatoes by a procedure simulating a bench-scale sequencing batch reactor anaerobic process", ***Chemical and Biochemical Engineering Quarterly***, Vol. 28, No. 1, (2014), 135-141.
16. Syaichurrozi, I. and Sumardiono, S., "Predicting kinetic model of biogas production and biodegradability organic materials: Biogas production from vinasse at variation of cod/n ratio", ***Bioresource Technology***, Vol. 149, (2013), 390-397.
17. Yusuf, M., Debora, A. and Ogheneruona, D., "Ambient temperature kinetic assessment of biogas production from co-digestion of horse and cow dung", ***Research in Agricultural Engineering***, Vol. 57, No. 3, (2011), 97-104.
18. Yusuf, M.O. and Ify, N.L., "The effect of waste paper on the kinetics of biogas yield from the co-digestion of cow dung and water hyacinth", ***Biomass and Bioenergy***, Vol. 35, No. 3, (2011), 1345-1351.
19. Budiyono, B. and Sumardiono, S., "Effect of total solid content to biogas production rate from vinasse", ***International Journal of Engineering***, Vol. 27, No. 2, (2014), 177-184.
20. Sumardiono, S. and Mardiani, D.T., "Microwave pretreatment of fresh water hyacinth (eichhornia crassipes) in batch anaerobic digestion tank (research note)", ***International Journal of Engineering-Transactions C: Aspects***, Vol. 28, No. 6, (2015), 832-840.
21. Speece, R., "Anaerobic technology for industrial wastewaters, USA, Archae Press, (1996).
22. Lay, C.-H., Sen, B., Huang, S.-C., Chen, C.-C. and Lin, C.-Y., "Sustainable bioenergy production from tofu-processing wastewater by anaerobic hydrogen fermentation for onsite energy recovery", ***Renewable Energy***, Vol. 58, (2013), 60-67.
23. Lin, Y., Zhang, W., Li, C., Sakakibara, K., Tanaka, S. and Kong, H., "Factors affecting ethanol fermentation using saccharomyces cerevisiae by4742", ***Biomass and Bioenergy***, Vol. 47, (2012), 395-401.
24. Esposito, G., Frunzo, L., Panico, A. and Pirozzi, F., "Modelling the effect of the olr and ofmsw particle size on the performances of an anaerobic co-digestion reactor", ***Process Biochemistry***, Vol. 46, No. 2, (2011), 557-565.
25. Zwietering, M., Jongenburger, I., Rombouts, F. and Van't Riet, K., "Modeling of the bacterial growth curve", ***Applied and Environmental Microbiology***, Vol. 56, No. 6, (1990), 1875-1881.
26. Deublein, D. and Steinhauser, A., "Biogas from waste and renewable resources: An introduction, John Wiley & Sons, (2011).

27. Brannen, A.L. and Davidson, P.M., "Antimicrobial in foods, New York, Marcel Dekker, (1993).
28. Werner, U., Stöhr, U. and Hees, N., "Biogas plants in animal husbandry", ***Deutsches Zentrum fur Entwicklungstechnologien-GATE***, (1989).
29. Gerardi, M.H., "The microbiology of anaerobic digesters, John Wiley & Sons, (2003).

Kinetics Studies Impact of Initial pH and Addition of Yeast *Saccharomyces cerevisiae* on Biogas Production from Tofu Wastewater in Indonesia

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چکیده

هدف از این کار، مطالعه اثر pH اولیه و مخمر ساکارومایسس سرویزیه در تولید بیوگاز از فاضلاب توفو (TW) بود. pH اولیه در سوبسترای بدون مخمر (T5-T9) و با مخمر (TY5-TY9) در محدوده ۵ تا ۹ تغییر کرد. نتایج نشان داد که pH اولیه بهینه ۸ بود. حداکثر بیوگاز در T8 (۲۷۵ میلی لیتر) و TY8 (٤٢١ میلی لیتر) نتیجه داد. اضافه کردن مخمر در مقایسه با اضافه نکردن مخمر، بیوگاز کل را افزایش داد. سینتیک تولید بیوگاز از طریق مدل های Gompertz و Cone مدل شد. بیوگاز پیش بینی شده در مدل Cone دقیق تر از Gompertz اصلاح شده بود. تفاوت بین بیوگاز پیش بینی شده و اصلاح شده در مدل های Gompertz اصلاح شده و Cone به ترتیب ۰/۱۹۳ تا ۲/۸۰۹ و ۰/۳۱٦ تا ۳/۱۱۵ درصد بود. حضور مخمر ثابت سینتیک ym (پتانسیل بیوگاز، میلی لیتر) و λ (دوره تاخیر، روز) را افزایش و k_{hyd} (سرعت هیدرولیز، /روز) را کاهش داد.

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