

The Effect of COD:N Ratios and pH Control to Biogas Production from Vinasse

S. Sumardiono, I. Syaichurrozi*, Budiyo, S. B. Sasongko

Department of Chemical Engineering, University of Diponegoro Postal Code 50239,
Semarang, Indonesia

ABSTRACT

Aims: Vinasse has potential to be processed into biogas because of its high COD. Various COD:N ratios of substrates and pH control were investigated to know their effect to biogas production from vinasse.

Study Design: This experiment used anaerobic digester-laboratory scale under room temperature and batch system. Urea was added as nitrogen source to make COD:N ratios = 400:7, 500:7, 600:7, and 700:7. Initial pH for all variables was adjusted 7.0.

Place and Duration of Study: Wastetreatment Laboratory, Department of Chemical Engineering, University of Diponegoro, Indonesia, between August 2012 and January 2013.

Methodology: Vinasse used obtained from the alcohol industry that produced alcohol from molasses. Polyethylene bottles which had volume 5 liters was used as digester laboratory scale. Vinasse 1 liter put into digester. Urea was added to make various of COD:N ratio.

Initial pH of all variables was adjusted 7.0 by adding 10 N NaOH solution. Rumen fluid (10%v/v vinasse) was added as methanogenic bacteria provider. Biogas formed was measured every once in two days to know biogas production daily. pH of substrates in the digesters was measured by using pH meter every once in two days to know pH profile daily. COD of substrates was measured by using COD meter. Solution of NaOH 2 N was used to control neutral pH in substrate (7.0 ± 0.2) during the fermentation process.

Results: Biogas production at COD:N ratios of control variable (1436:7), 400:7, 500:7, 600:7, 700:7 were 3.673, 4.909, 6.079, 6.096, 5.630 mL/g COD respectively. pH profiles for all variables were decreasing from beginning until ending of fermentation. With controlled pH, pH of substrates was maintained at neutral condition, so methanogenic bacteria could grow well in the digesters. Consequently biogas formed was larger than uncontrolled pH.

Conclusions: COD:N ratios of 400:7, 500:7, 600:7, 700:7 produced biogas which was larger than control variable. Total biogas production at COD:N = 600:7 was the most of all variables which was 6.096 mL/g COD. Biogas production at pH control was greater than at pH non-control.

Keywords: biogas, COD:N ratio, pH control, vinnase

1. INTRODUCTION

Molasses is the residue remaining of the sugar crystallization process that contains 52% of fermentable sugars [1]. Because of its contents, molasses can be used as substrate for fermentation process. In the chemical industry, molasses the most widely is used to produce alcohol in the alcohol industry [2].

Alcohol is produced by fermentation yeast from molasses. Alcohol formed must be separated from fermentation broth by distillation. The bottom product of distillation process is

* Tel.: +62 85 641679534; fax: +62 24 7460055.

E-mail address: iqbalnyaichurrozi@gmail.com.

26 residue known as vinasse. Vinasse is typical of liquid waste that has the relatively high
27 values of chemical oxygen demand, more than 100,000 mg/L [3-4]. As a consequence,
28 vinasse can contaminate the environment if it be discharged directly into the environment
29 [2,5]. The production of 1-liter alcohol generates 8 - 15 liters of vinasse [2-3,6-7].
30

31 Vinasse must be treated first before it is released into the environment because of its COD
32 contents. Aerobic treatment has some shortages there are extensive land requirement,
33 capital high cost, and operating high cost. On the other hand, Anaerobic treatment can
34 produce energy and be non-polluting [8]. In anaerobic treatment, various type of biomass are
35 decomposed to biogas by microbial activities. This treatment uses digester to destroy COD
36 of biomass into biogas at the anaerobic condition [9]. Biogas is sustainable energy source
37 that contains 50-75% methane gas, 30-50% carbon dioxide, small amounts of other gases
38 (CO, N₂, H₂, H₂S, O₂), depending on the substrate [10-12]. So, anaerobic treatment provides
39 method for disposal of wastes and produce biogas as renewable energy.
40

41 Vinnase has potential to be processed into biogas. The high COD of vinasse can be
42 destroyed by methanogenic bacteria in the digester. Some authors studied treatment of
43 vinasse in the anaerobic digestion. Espinoza-Escalantea et al. [13] studied the effect of pH
44 to biogas production. At pH 6.5, biogas production was greater than pH 4.5 and 5.5. Buitron
45 and Carjaval [14] studied effect of temperature of anaerobic digestion to biogas production.
46 At 35°C, biogas production was greater than 25°C. Siles et al. [15] reported that ozonation
47 pre-treatment had the yield coefficient of methane 13.6% and the production rate of methane
48 41.6% was greater than at without ozonation pre-treatment. However, this process required
49 expensive installation costs.
50

51 Previous research did not study the effect of COD:N ratios of substrate to biogas production
52 from vinasse yet. According to Speece [9], wastewater containing COD will be destroyed
53 and produced biogas optimally if substrate contained COD:N ratios in the range 350:7 –
54 1000:7. While Lutoslawski et al. [4] reported that biodegradation at pH control caused the
55 rate of degradation by microorganisms was greater than at pH non control. So, the objective
56 of this experiment was study the effect of COD:N ratios of substrate and pH control to biogas
57 production from vinasse.
58

59 2. MATERIALS AND METHODS

60 2.1 Wastewater and Inoculum

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63 The wastewater used was vinasse obtained from an alcohol production. The alcohol industry
64 located in Solo, Central Java, Indonesia, that produces alcohol from molasses. Table 1 lists
65 the vinasse properties that was used as biogas feedstock. The rumen fluid was used as
66 inoculum. In this research, rumen fluid that was in fresh condition was obtained from
67 slaughterhouse in Semarang, Central Java, Indonesia.
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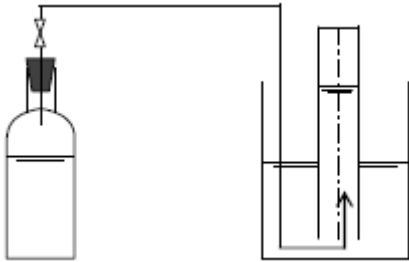
70 **Table 1. Vinasse characteristics**

Parameters	Values
COD	299,250±1,060.660
Carbohydrate	268,647±0.000
Protein	9,117±0.000
TS	27.94±0.085
pH	3.25±0.212
N	1,458±0.000
COD:N	1436:7

71 Remarks: COD, chemical oxygen demand (mg/L); TS, total solid (%); pH, power of hydrogen; N,
72 nitrogen content (mg/L); Carbohydrate (mg/L); Protein (mg/L).
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74 **2.2 Experimental set up**
75

76 Anaerobic digesters of experimental laboratory were made from polyethylene bottles which
77 had a volume of 5 liters. These bottles were plugged with rubber plug and were equipped
78 with valve for biogas measurement. Anaerobic digesters were operated in batch system and
79 at room temperature. Biogas formed was measured by liquid displacement method as also
80 has been used by the other authors [16-18]. The schematic diagram of experimental set up
81 as shown in Fig. 1. This study were performed in ten variables (Table 2.), each variable was
82 repeated two times using two digesters having the same contents, so this study needed 20
83 digesters.
84



85
86 **Figure 1. Anaerobic digestion of experimental laboratory**
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88 **2.3 Experimental design**
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90 **2.3.1 Study of COD:N ratios**
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92 Anaerobic digestions of experimental laboratory using 5-liter volumes were operated in batch
93 system. 1-liter vinnase was put in the digester. Rumen fluid as methanogenic bacteria
94 provider that was added into the digester as much as 10% v/v vinasses. From Table 1, could
95 be known that ratio COD:N vinnase was 1436:7. Whereas ratio COD:N is optimum to
96 produce biogas at range 350:7 – 1000:7 [9]. Furthermore, urea as nitrogen source was
97 added into the digester to make COD:N ratios of 400:7, 500:7, 600:7, 700:7. Initial pH for all
98 variables was adjusted 7.0 by using NaOH solution 10 N. The variables in this study can be
99 seen in Table 2 (a).
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Table 2. Variation of pH treatment and COD:N ratios

Condition of pH		COD:N substrate
(a) Non-controlled		1436:7 (Control-without urea addition)
		400:7
		500:7
		600:7
		700:7
(b) Controlled		1436:7 (Control-without urea addition)
		400:7
		500:7
		600:7
		700:7

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103 **2.3.2 pH controlled to biogas production**
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In this study, pH initial for all variables was adjusted 7.0 by using NaOH 10 N before rumen fluid was added. Anaerobic digesters (volume 5 L) were operated in batch system. Volume of 1-liter vinasse and 10% v/v vinasse of rumen fluid were put into digesters. Furthermore, urea as nitrogen was added into the digester to make COD:N ratios of 400:7, 500:7, 600:7, 700:7. In this study, pH was controlled constant (pH 7.0±0.2) during biogas formed by using NaOH solution 2 N that was studied to know its effect to biogas production (Table 2.(b)). NaOH 10 N was used by authors to adjust initial pH because characteristic of vinasse was very acid. Using NaOH with high concentration (10 N) can avoid the addition of initial total volume in the digester. Whereas, during fermentation NaOH 2 N was used to control pH at range 7.0±0.2 because the high concentration of NaOH (more than 2 N) can kill bacteria in the digesters.

2.3.3 COD removal

Chemical Oxygen Demand (COD) was converted by bacteria to biogas at anaerobic condition in the digester [9]. Influent COD and effluent COD of substrates were measured by COD meter. COD of influent substrates and COD of effluent substrates were used to calculated COD removal (%).

$$\text{COD removal (\%)} = [(\text{Influent COD} - \text{Effluent COD}) / \text{Influent COD}] \times 100\%$$

2.4 Experimental procedures

Biogas formed was measured every once in two days to know biogas production by using water displacement method (Fig. 1). pH substrate in the digesters was measured by using pH meter every once in two days to know pH profiles daily. COD of substrates was measured by using COD meter. At the non-controlled pH neutral, fermentation process was done in the digester until biogas ran out to produce. While at the controlled pH neutral, fermentation process was done for 90 days.

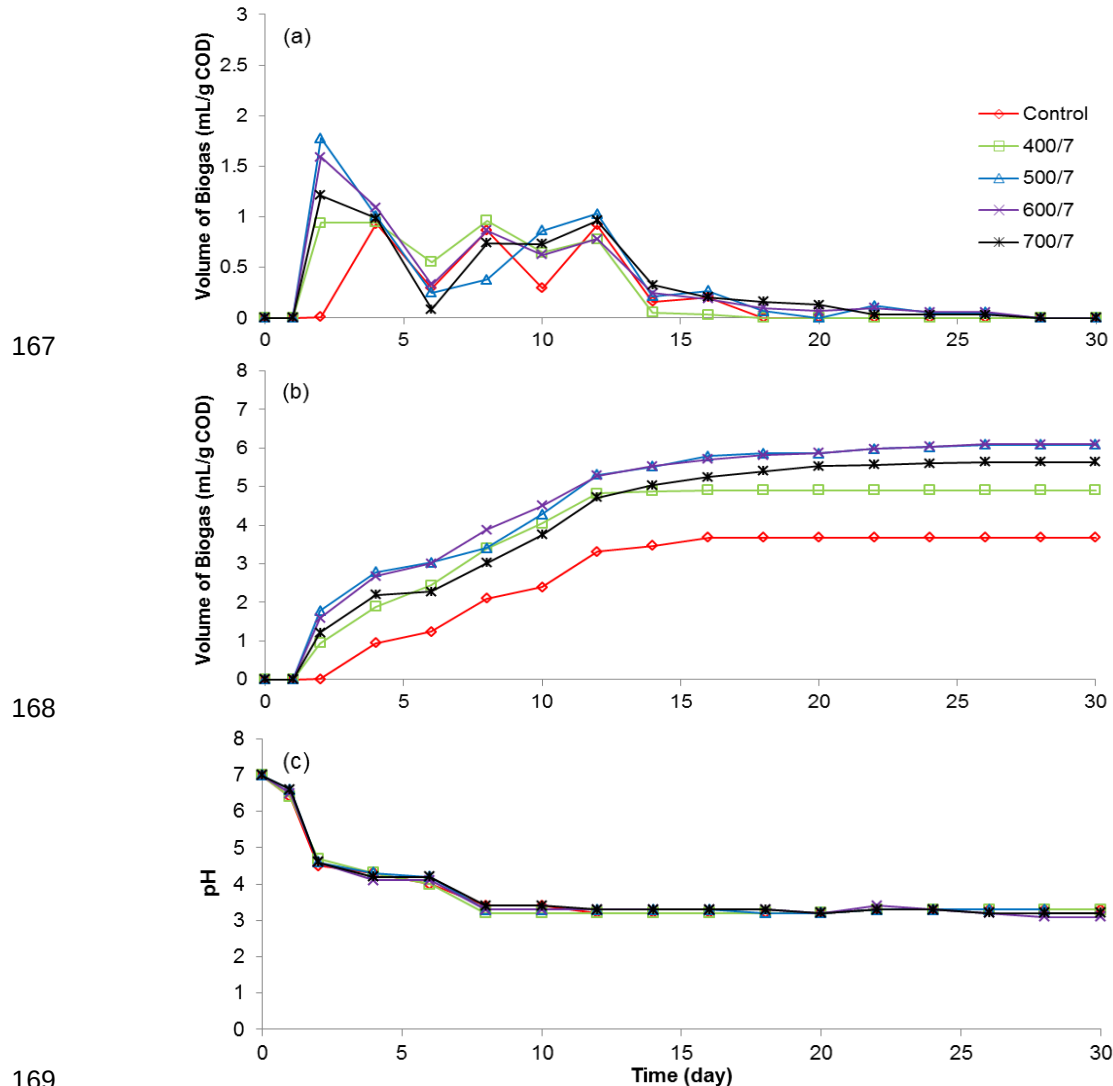
3. RESULTS AND DISCUSSION

3.1 Effect of COD:N ratio to Biogas Production

COD is amount of oxygen needed for waste material in the water can be oxidized through a chemical reaction. COD:N ratio of substrate is necessary parameter to produce biogas optimally that is in the range 350:7 – 1000:7 [9]. If more or less than the range, microbial growth in the digester will be hampered. So, adjustment of COD:N of substrate was needed to be done. In this experiment, vinnase obtained had COD:N = 1436:7 consequently nitrogen source must be added into the substrate. Proteins, amino acids and urea are nitrogen source needed by microbe to build cell structures [19-20]. For this experiment, urea [(NH₂)₂CO] was added as nitrogen source.

From Fig. 2., COD:N = 400:7 – 700:7 had biogas production was greater than control variable (COD:N = 1436:7). This result corresponded with speece's [9] statement that substrate with COD:N ratio more than 1000:7 will produce a little biogas volume. Biogas produced at COD:N ratios of 500:7 and 600:7 were in nearly equal numbers. On other hand, at COD:N ratio 400:7 biogas produced was less than 500:7, 600:7, 700:7. Control variable, 400:7, 500:7, 600:7, 700:7 had total biogas production 3.673, 4.909, 6.079, 6.096, 5.630 ml/g COD respectively. Among the five variable in various COD:N ratios, COD:N ratio = 600:7 had biogas production which was the most and control variable had biogas production which was the least of the all variables (Fig. 2. (b))

158 Urea is compound that unstable and easily decomposed being ammonia, CO₂ and H₂O. So,
 159 urea in the digester was decomposed into ammonia. Ammonia can be used by microbe as a
 160 nitrogen source [21]. However, the ammonia may be toxic for microbe if the amount of
 161 ammonia is too much [22]. Ammonia concentrations below 200 mg/L in the digester are
 162 good for anaerobic microbes [23]. Methanogen bacteria was the least tolerant and the most
 163 easily killed to ammonia inhibition among the four anaerobic microorganisms in four step
 164 biogas production there were hydrolysis, acidogenic, acetogenic, methanogenic [24].
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 170
 171 **Figure 2. Biogas production daily (a), biogas production cumulative (b), pH profile (c)**
 172 **at various ratio of COD:N**
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174 Chen et al. [25] reported that COD:N ratio of 70 has been suggested for the stable
 175 performance of anaerobic digestion. That report could approach with the result of this
 176 experiment in which range of COD:N ratios 71.4 – 85.7 (500:7 – 600:7). In control variable,
 177 availability of nitrogen source was too few (Table 1.). Hence, microbe in the digester could
 178 not build cell structures well and the rate biogas production was low (Fig 2. (a)).

Mata-Alvarez et al. [26] reported that carbohydrate-rich substrates were good producers of VFAs and protein-rich substrates were good buffering capacity due to production of ammonia. So, if ratio between carbohydrate (C) and protein (N) are appropriate, biogas will be produced optimally. The value of C (carbon) can be obtained from COD where $C = \text{COD value} \times (12/32)$ [27]. Vinasse used in this experiment contained large amounts of carbohydrate and protein in small amount (Table 1.). During the fermentation process, VFAs was formed in large quantities and pH substrates dropped directly (Fig. 2.(c)) Elbeshbishy and Nakhla [28] also reported that decreasing in the pH could be due to the rapid VFAs production at the beginning, while the increasing in the pH from 3rd day to 10th day could be due to generation of $\text{NH}_4\text{-N}$ during protein degradation, as ammonia which was a base combines with carbon dioxide and water to form ammonium bicarbonate, a natural pH buffer. In this experiment, pH decreased from beginning until ending of fermentation (Fig 2.(c)). This phenomena was caused by accumulation of VFAs production in the digester which very large. Condition of pH in the substrates which very acid caused methanogenic bacteria to die. Ammonia production from degradation urea and protein could not increase the pH because VFAs was produced rapidly.

3.2 Effect of pH control to Biogas Production

With pH control, biogas formed was greater than pH non-control. Biogas was produced until 90th day and might still be produced until organic matter in the substrate discharged (Fig 3.). The comparison total biogas between pH control and pH non-control can be seen in Fig. 4. The influence changes in pH was very sensitive to fermentation processing by bacteria activity. So, pH control was important for application in biogas production [4,9]. The drop of pH was caused acidogenic bacteria produced acetate, hydrogen gas, carbon dioxide, and few other VFA such as propionic and butyric acid. A low pH value inhibited the activity of microorganisms involved in the biogas production especially methanogenic bacteria [9,29].

The neutral condition characterize was optimal anaerobic biotechnology pH conditions. Low pH condition might be caused by two sources of acidity, H_2CO_3 and VFA, which were generated in microbial reactions. These acids required alkalinity for neutralization so that the microbial activity was not hindered by pH depression [9]. According to Ayu and Aryanti [30], to allow the methanogenic bacteria to grow, digester should be properly fed and buffered to rising alkalinity. Sodium carbonate (Na_2CO_3) could increase alkalinity or buffering capacity of fermenting slurry to control pH substrate. Viswanath et al. [31] mentioned that there was a perfect link of the acidogenic and methanogenic phases when the pH was remained at 7.0 and there was no drastic increase in acidity or alkalinity. According to Lutoslawski et al. [4], pH neutralization could be done by addition of H_2SO_4 and NaOH to maintain constant pH. Biodegradation at pH control caused the final number of microbial cell total in the digester was more than process with no pH control. That caused pH control contributed largely to rate of degradation by microorganisms.

With pH control, biogas production daily and biogas production cumulative were more than pH non-control (Fig 4.). Methanogens prefer nearly neutral pH conditions with generally accepted optimum range of approximately 6.5-8.2 and biogas production rate will decline at pH condition either higher or lower [9]. So if pH condition was maintained, methanogens would grow well and produce biogas in large. Total biogas of control, 400:7, 500:7, 600:7, 700:7 were 17.875, 20.385, 18.060, 21.229, 18.504 mL/g COD respectively.

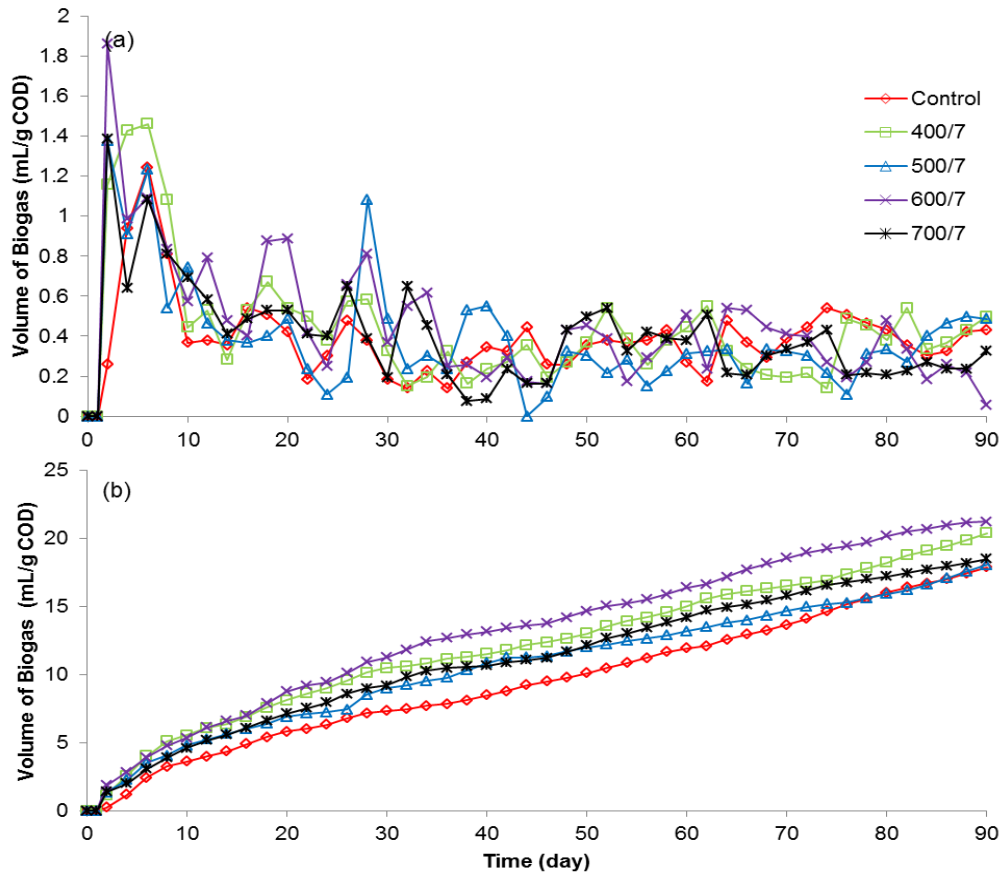


Figure 3. Biogas production daily (a), biogas production cumulative (b)

Organic contents in the substrate will be destroyed to be biogas. Running out biogas production was caused two things. First, methanogenic bacteria in the digester cannot grow well and finally death. This phenomena can occur if condition inside the digester not support the growth of methanogenic bacteria such as temperature, pressure, nutrition, and pH condition. At non-controlled neutral pH, pH of all variables were decreasing until pH 3.0 (Fig. 2(c)). This condition caused methanogenic bacteria death. Second, organic contents or COD of substrate is exhausted. This phenomena can occur if condition of digester is not a limiting parameter. Organic contents or COD are destroyed by microorganisms into biogas. At controlled neutral pH, pH condition of substrates was 7.0 ± 0.2 , so pH was not a limiting parameter and microorganisms in the digesters could grow well (Fig. 3.).

From Fig. 3. can be seen that biogas production did not stop yet after 90 days observation. Biogas might be still produced until organic contents were completely discharged. Budiyo et al. [17] reported that after 90 days cattle manure in the anaerobic digester still produced biogas. This case was caused organic contents of cattle manure did not exhausted yet and microorganisms still destroyed organic contents actively. Lopes et al. [32] state that organic content of substrate did not completely after 360 days observation yet.

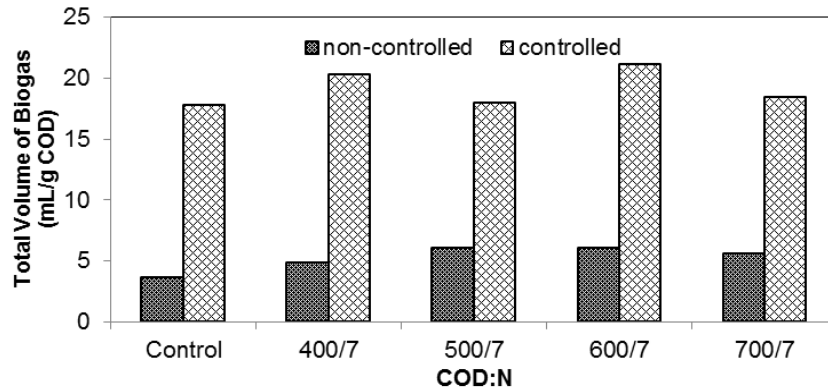


Figure 4. Comparison between pH controlled and pH non-controlled to total biogas production

Speece [9] stated that 1 gram COD can convert into 395 mL biogas at room temperature. In this study, maximum biogas formed was 21.229 mL/g COD. This result was very far from the speece's estimation. This result also was lower than other authors (Table 3.). This difference in results might be related to differences in amount of organic material in the substrates. According to Soeprijanto et al. [33], biogas formed very influenced by the amount of organic materials in the initial substrates. Besides that, organic material which too large in the substrates caused overloading in the digesters [17]. From Table 3., vinasse used in this study had high COD content and this value was larger than other studies. Case of overloading caused bacteria destroyed organic material slowly, so the rate of biogas formation to be slow and biogas formed at the end fermentation was little.

Table 3. Comparison of result between this study and other studies

Influent COD (g/L)	Volume (mL/ g COD)	COD:N	Reff
64	43.36 ^a	-	[13]
66.10	284 ^a	-	[15]
5	110 ^a	500:5	[33]
35 - 45	252 ^b	-	[34]
61.2	330 ^a	-	[35]
299.25	21.23 ^b	600:7	This study

Remarks: a = CH₄ volume (mL/g COD), b = biogas volume (mL/g COD)

3.3 COD Removal

Organic materials (COD) of substrates will be converted to biogas by activities of bacteria. These activities consist of four levels which are hydrolysis, acidogenesis, acetogenesis and methanogenesis. COD amount that is degraded by bacteria in the digester is COD removal [9]. In this study, COD substrate for all variables decreased at the end of fermentation process. Value of COD removal for all variables can be seen in Table 4.

At non-controlled pH condition, COD removal of 600:7 was the most of all variables which was 3.21±0.49%. Then followed by 500:7, 700:7, 400:7 and control variable (Table 4). From Fig. 4., the sequence of variables that generate biogas from the most to the less total biogas was 600:7, 500:7, 700:7, 400:7 and control variable. From this result, can be concluded that the more COD was removed, the more biogas was produced.

COD removal at controlled pH condition was bigger than at non-controlled pH condition. Lutoslawski et al [4] stated that degradation rate of organic contents would do fast, if pH condition of substrate was adjusted neutral. COD:N of 600:7 had COD removal value which was the most of all variables. From Fig. 4., the most of total biogas production was 600:7. This result similar with statement of Speece [9] and Soeprijanto et al. [33] that biogas formed would be comparable to the number of missing COD.

Table 4. COD removal for all variables

Variable		Influent COD	Effluent COD	COD removal
COD:N	pH condition	(gram)	(gram)	(%)
Control	Non-controlled	300.85±1.06	297.02±0.38	1.27±0.43
400:7		300.85±1.06	296.06±0.38	1.59±0.43
500:7		300.85±1.06	292.27±0.00	2.85±0.39
600:7		300.85±1.06	291.19±0.76	3.21±0.49
700:7		300.85±1.06	294.17±0.00	2.22±0.39
Control	Controlled	300.85±1.06	264.80±1.13	11.98±0.56
400:7		300.85±1.06	262.29±1.13	12.82±0.56
500:7		300.85±1.06	264.65±2.25	12.03±0.94
600:7		300.85±1.06	261.58±0.00	13.05±0.35
700:7		300.85±1.06	262.91±1.13	12.61±0.56

4. CONCLUSIONS

Vinnase obtained had COD:N = 1436:7. Consequently urea addition was necessary to do. COD:N ratio of 600:7 had total biogas production which was the most of all COD:N variables which was 6.096 mL/g COD. Control variable (without urea addition) had total biogas production was the least of all variables which was 3.673 mL/g COD. Biogas production with controlled pH was greater than that with uncontrolled pH. At controlled pH, total biogas maximum which was produced at COD:N ratio of 600:7 was 21.229 mL/g COD. From this study, the optimal condition was substrate which had COD:N ratio of 600:7 either at uncontrolled or controlled pH. There was proportional relationship between COD was removed and biogas was produced. The more in COD removal value caused the more in biogas production too. At non-controlled pH, COD:N ratio of 600:7 had COD removal 3.21±0.49%. Whereas at controlled pH, COD:N ratio of 600:7 had COD removal 13.05±0.35%.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

'Author 1' and 'Author 2' designed the study, wrote the protocol, and wrote the first draft of the manuscript. 'Author 1', 'Author 3' and 'Author 4' managed the analyses of the study. 'Author 2' managed the literature searches. All of authors read and approved the final manuscript.

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REFERENCES

- 328 1. Baikow VE. *Manufacture and Refining of Raw Cane Sugar*. 1982. Elsevier Scientific
329 Publishing Company, Amsterdam-Oxford-New York.
- 330 2. Garcia IG, Venceslada JLB, Jimenez Pena PR, Gomez ER. Biodegradation of Phenol
331 Compounds in Vinasse Using *Aspergillus Terreus* and *Geotrichum Candidum*. *Wat. Res.*
332 1997;31(8):2005-2011.
- 333 3. Robles-Gonzalez V, Lopez-Lopez E, Martinez-Jeronimo F, Ortega-Clemente A, Ruiz-
334 Ordaz N, Galindez-Mayer J, Rinderknecht-Seijas N, Poggi-Varaldo HM. Combined
335 treatment of mezcal vinasses by ozonation and aerobic biological post-treatment.
336 *Proceedings of 14th International Biotechnology Symposium*. Rimini, Italy, 14–18,
337 September 2010.
- 338 4. Lutoslawski K, Ryznar-Luty A, Cibis E, Krzywonos M, Miskiewicz T. Biodegradation of
339 beet molasses vinasse by a mixed culture of microorganisms: Effect of aeration
340 conditions and pH control. *Journal of Environmental Sciences*. 2011;23(11):1823–30.
- 341 5. Zayas T, Romero V, Salgado L, Meraz M, Morales U. Applicability of
342 coagulation/flocculation and electrochemical processes to the purification of biologically
343 treated vinasse effluent. *Separation and Purification Technology*. 2007;57:270–276.
- 344 6. Jimenez AM, Borja R, Martin A, Raposo F. Mathematical modelling of aerobic
345 degradation of vinasses with *Penicillium decumbens*. *Process Biochem*. 2005;40:2805-
346 11.
- 347 7. Preeti CS, Aniruddha BP. Enhancement in biodegradability of distillery wastewater using
348 enzymatic pretreatment. *J. Environ. Manage*. 2006;78(1):77–85.
- 349 8. Tang Y-Q, Fujimura Y, Shigematsu T, Morimura S, Kida K. Anaerobic treatment
350 performance and microbial population of thermophilic upflow anaerobic filter reactor
351 treating awamori distillery wastewater. *J Biosci Bioeng*. 2007;104(4):281–7.
- 352 9. Speece RE. *Anaerobic Technology for Industrial Wastewaters*. 1996. USA: Archae
353 Press.
- 354 10. Sasse L. *Biogas Plants*. Eschborn. 1988. Germany: Deutsche Gesellschaft für
355 Technische Zusammenarbeit (GTZ) GmbH.
- 356 11. Juanga JP, Visvanathan C, Tränkler J. Optimization of Anaerobic Digestion of Municipal
357 Solid Waste in Combined Process and Sequential Staging. *J Waste Manage Res*. 2007;
358 25:30–38.
- 359 12. Karellas SB. Development of an investment decision tool for biogas production from
360 agricultural waste. *Jurnal Renewable and Sustainable Energy Reviews* 2010;14:1273-
361 82.
- 362 13. Espinoza-Escalante FM, Pelayo-Ortiz C, Navarro-Corona J, Gonzalez-Garcia Y, Bories
363 A, Gutierrez-Pulido H. Anaerobic digestion of the vinasses from the fermentation of
364 Agave tequilana Weber to tequila: The effect of pH, temperature and hydraulic retention
365 time on the production of hydrogen and methane. *Journal Biomass and Bioenergy*.
366 2009;33:14–20.
- 367 14. Buitron G, Carjaval C. Biohydrogen Production from Tequila Vinasse in an Anaerobic
368 Sequencing Batch Reactor: Effect of Initial Substrate Concentration, Temperature, and
369 Hydraulic Retention Time. *Bioresource Technology* 2010;101:9071 – 9077.
- 370 15. Siles JA, García-García I, Martín A, Martín MA. Integrated ozonation and
371 biomethanization treatments of vinasse derived from ethanol manufacturing. *Journal of*
372 *Hazardous Materials*. 2011;188:247–253.
- 373 16. Yetilmezsoy K, Sakar S. Development of empirical models for performance evaluation of
374 UASB reactors treating poultry manure wastewater under different operational
375 conditions. *J. Hazardous Materials*. 2008;153: 532–543.

- 376 17. Budiyono, Widiasta IN, Johari S, Sunarso. Increasing Biogas Production Rate from Cattle
377 Manure Using Rumen Fluid as Inoculums. *International Journal of Basic & Applied*
378 *Sciences IJBAS-IJENS* 2010;10(1):68-75.
- 379 18. Budiyono, Widiasta IN, Johari S, Sunarso. The Kinetic of Biogas Production rate from
380 Cattle Manure in Batch Mode. *International Journal of Chemical and Biological*
381 *Engineering*. 2010;3(1):40-4.
- 382 19. Angelidaki I, Ahring BK. Thermophilic anaerobic digestion of livestock waste: the effect
383 of ammonia. *Appl Microbiol Biotechnol*. 1993;38(4):560–4.
- 384 20. Fang HHP, Chui HK, Li YY, Chen T. Performance and granule characteristics of UASB
385 process treating wastewater with hydrolyzed proteins. *Water Sci Technol*
386 1994;30(8):55–63.
- 387 21. Sung S, Liu T. Ammonia inhibition on thermophilic anaerobic digestion. *Chemo-sphere*.
388 2003;53(1):43–52.
- 389 22. Soubes M, Muxõ L, Fernandez A, Tarlera S, Quirolo M. Inhibition of methanogenesis
390 from acetate by Cr+3 and ammonia. *Biotechnol. Lett*. 1994;16:195–200.
- 391 23. Liu T, Sung S. Ammonia inhibition on thermophilic aceticlastic methanogens. *Water Sci.*
392 *Technol*. 2002;45:113–120.
- 393 24. Kayhanian M. Performance of a high-solids anaerobic digestion process under various
394 ammonia concentrations. *J. Chem. Tech. Biotechnol*. 1994;59:349–352.
- 395 25. Chen Y, Cheng JJ, Creamer KS. Inhibition of anaerobic digestion process: a review.
396 *Bioresour. Technol*. 2008;99:4044–4064.
- 397 26. Mata-Alvarez J, Mace S, Llabres P. Anaerobic digestion of organic wastes. An overview
398 of research achievements and perspectives. *Bioresour. Technol*. 2000;74:3–16.
- 399 27. Phang SM, Miah MS, Yeoh BG, Hashim MA. Spirulina cultivation in digested sago
400 starch factory wastewater. *Journal of Applied Phycology* 2000;12:395–400.
- 401 28. Elbeshbishy E, Nakhla G. Batch anaerobic co-digestion of proteins and carbohydrates.
402 *Bioresource Technology*. 2012;116:170–178.
- 403 29. Vicenta M, Pacheco G, Alamis MLA, Anglo P G, Tan BV, Silverio CM. A Study of Some
404 Factors Affecting Biogas Production from Pineapple Peelings, In Bidin R., Chong C. N.
405 and Wang C. W. (eds), *Proceeding of the Second ASEAN Workshop on Biogas*
406 *Technology Applied in the Management and Utilization of Food Waste Materials* (pp.
407 189-202), Kuala Terengganu, Malaysia, 1984. Quoted in Anunputtikul W, Rodtong S,
408 *Biogas Production from Cassava Tubers, Thailand, 2004*.
- 409 30. Ayu AO, Aryanti VD. Biogas Production Using Anaerobic Biodigester From Cassava
410 Starch Effluent With Ruminant Bacteria As Biocatalyst. 2010. Master Thesis.
411 Diponegoro University, Indonesia.
- 412 31. Viswanath P, Devi SS, Nand K. Anaerobic Digestion of Fruit and Vegetable Processing
413 Waste for Biogas Production, *Bioresource Technology*. 1992;40:43-48. Quoted in
414 Anunputtikul W, Rodtong S. *Biogas Production from Cassava Tubers, Thailand, 2004*.
- 415 32. Lopes WS, Leite VD, Prasad S. Influence of inoculum on performance of anaerobic
416 reactors for treating municipal solid waste. *Bioresour. Technol*. 2004;94:261-266.
- 417 33. Soeprijanto, Ismail T, Lastuti MD, Niken B. Treatment of Vinasse From Alcohol Industry
418 Wastewater Into Biogas Using Upflow Anaerobic Sludge Blanket (UASB) Bioreactor.
419 *Jurnal Purifikasi*, 2010;11:11 – 20.
- 420 34. Saputra, Eka B, Guna, Hadi R. Enhancment Effort of Biogas Production from Liquid
421 waste Water of Ethanol Industry. 2009. Master Thesis. ITS, Surabaya.
- 422 35. Lalov IG, Krysteva MA, Phelouzat JL. Improvement of Biogas Production from Vinasse
423 Via Covalently Immobilized Methanogens. *Bioresource Technology*. 2001;79:83-85.