

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

S. Sumardiono¹, I. Syaichurrozi^{2*}, Budiyo³, S. B. Sasongko⁴

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 substrate 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. Solution of NaOH 10 N was added initial pH for all variables were 7.0. 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. pH of substrates in the digester 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 (7.0±0.2) during the fermentation process.

Results: Biogas production at COD:N ratios of control (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 neutral condition so methanogenic bacteria could grow well in the digester. Consequently biogas formed was larger than uncontrolled pH.

Conclusions: COD:N ratios of 400:7, 500:7, 600:7, 700:7 produced biogas 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 to produce alcohol in the alcohol industry [2].

* Tel.: +62 85 641679534; fax: +62 24 7460055.
E-mail address: iqbalsyaichurrozi@gmail.com.

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

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

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

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

2. MATERIALS AND METHODS

2.1 Wastewater and Inoculum

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

Table 1. Vinasse properties

Parameters	Values
COD	299,250±1.060
TS	27.94±0.085
pH	3.25±0.212
N	1,458±0.000

Information: COD, chemical oxygen demand (mg/L); TS, total solid (%); pH, power of hydrogen; N, nitrogen content (mg/L).

2.2 Experimental set up

Anaerobic digesters of experimental laboratory were made from polyethylene bottles which had a volume of 5 liters. These bottles were plugged with rubber plug and was equipped with valve for biogas measurement. Anaerobic digesters were operated in batch system and at room temperature. Biogas formed was measured by liquid displacement method as also has been used by the other authors[16-18].

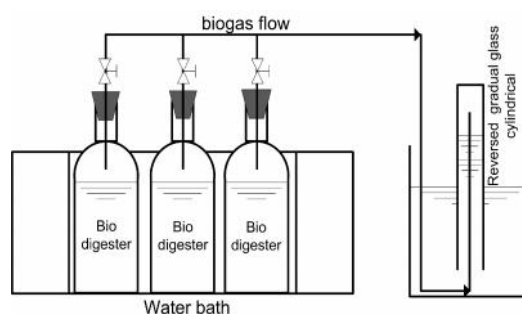


Figure 1. Anaerobic digestion of experimental laboratory

2.3 Experimental design

2.3.1 Study of COD:N ratios

Anaerobic digestions of experimental laboratory using 5-liter volumes were operated in batch system. 1-liter vinnases were put in the digester. Rumen fluid as methanogenic bacteria provider that was added into the digester as much as 10% v/v vinasses. From Table 1, could be known that ratio COD:Nvinasse was 1436:7. Whereas ratio COD:N is optimum to produce biogas at range 350:7 – 1000:7 [9]. Furthermore, urea as nitrogen source was added into the digester to make COD:N ratios of 400:7, 500:7, 600:7, 700:7. Initial pH for all variables was adjusted 7.0 by using NaOH solution 10 N. The variables in this study can be seen in Table 2 (a).

Table 2. Variation of pH treatment and COD:N ratios

	pH treatment	COD:N substrate	Initial pH
(a)	Non-controlled neutral	1436:7 (Control-without urea addition)	7.0
		400:7	7.0
		500:7	7.0
		600:7	7.0
		700:7	7.0
(b)	Controlled neutral	1436:7 (Control-without urea addition)	7.0
		400:7	7.0
		500:7	7.0
		600:7	7.0
		700:7	7.0

2.3.2 pH controlled to biogas production

In this study, pH initial for all variables was adjusted 7.0. Anaerobic digesters (volume 5 L) were operated in batch system. Volume of 1-liter vinasses and 10% v/v vinasses 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 2N that was studied to know its effect to biogas production (Table 2.(b)).

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 substrates in the digester were 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 for 30 days or 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 need to do.

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 [9] statement. 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.

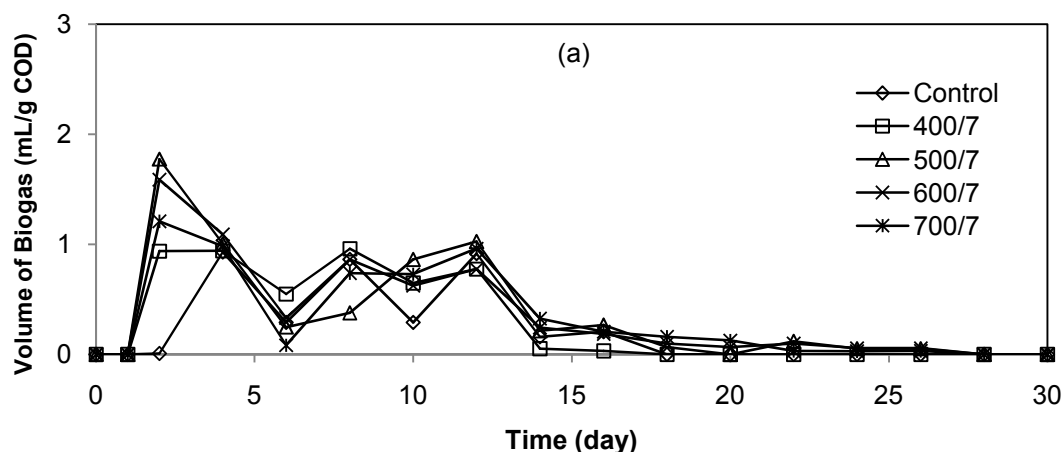
Control variable, 400:7, 500:7, 600:7, 700:7 had total biogas production 3.673, 4.909, 6.079, 6.096, 5.630 ml/g COD respectively. Among the five variable in various COD:N ratios, COD:N ratio = 600:7 had biogas production was the most and control variable had biogas production was the least of the all variables (Fig. 2. (b))

Urea is compound that unstable and easily decomposed being ammonia, CO₂ and H₂O. So, urea in the digester was decomposed into ammonia. Ammonia can be used by microbe as a nitrogen source [21]. However, the ammonia may be toxic for microbe if the amount of ammonia is too much [22]. Ammonia concentrations below 200 mg/L in the digester are good for anaerobic microbes [23]. Methanogen bacteria was the least tolerant and the most easily killed to ammonia inhibition among the four anaerobic microorganisms in four step biogas production there were hydrolysis, acidogenic, acetogenic, methanogenic [24].

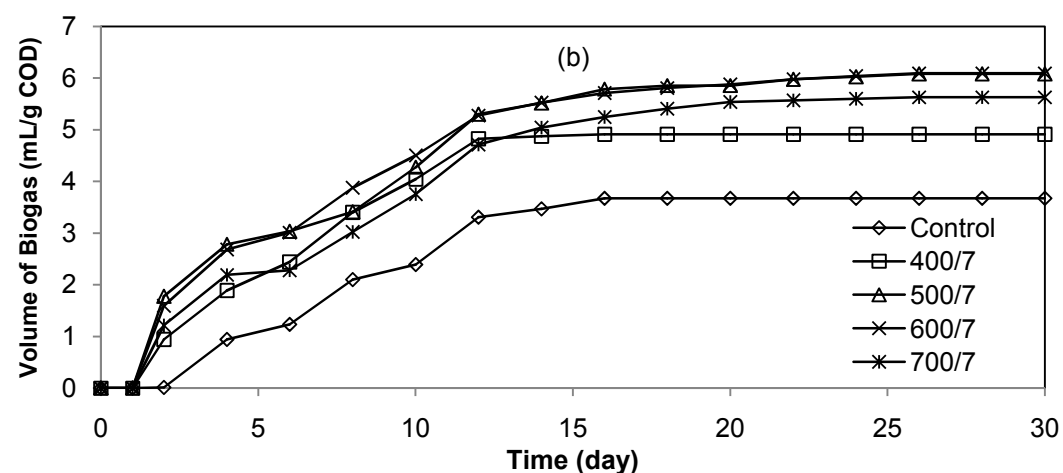
Chen et al.[25] reported that COD:N ratio of 70 has been suggested for the stable performance of anaerobic digestion. That report could approach with the result of this experiment in which range of COD:N ratios 71.4 – 85.7 (500:7 – 600:7). In control variable, availability of nitrogen source was too few (Table 1.). Hence, microbe in the digester could not build cell structures well and the rate biogas production was low (Fig 2. (a)).

155
156
157
158
159
160
161

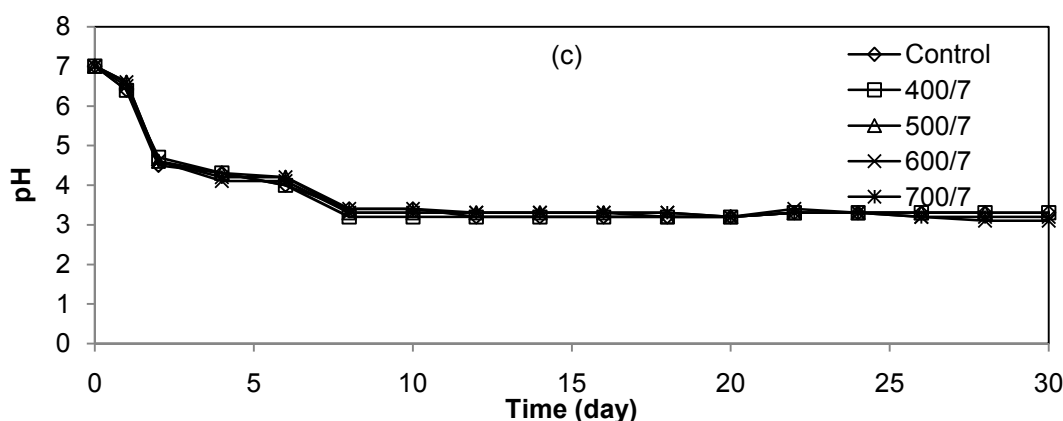
Mata-Alvarez et al.[26] reported that carbohydrate-rich substrates were good producers of VFAs and protein-rich substrates were good buffering capacity due to production of ammonia. So, if ratio between carbohydrate (C) and protein (N) are appropriate, biogas will be produced optimally. The value of C (carbon) can be obtained from COD where $C = \text{COD value} \times (12/32)$ [27].



162



163



164
165

Figure 2. Biogas production daily (a), biogas production cumulative (b), pH profile (c) at various of COD:N

Elbeshbishy and Nakhla [28] 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 NH₄-N during protein degradation, as ammonia which was a base combines with carbon dioxide and water to form ammonium bicarbonate, a natural pH buffer. However, in this experiment, pH decreased until the end (Fig 2.(c)). This phenomena was caused accumulation of VFAs production in the digester was very large. Condition of pH in the substrate was very acid and methanogenic bacteria was death. 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 4.). The comparison total biogas between pH control and pH non-control can be seen in Fig. 3. 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, H₂CO₃ 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 (Na₂CO₃) 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 Lutoslawskiet al. [4], pH neutralization could be did by addition H₂SO₄ 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.

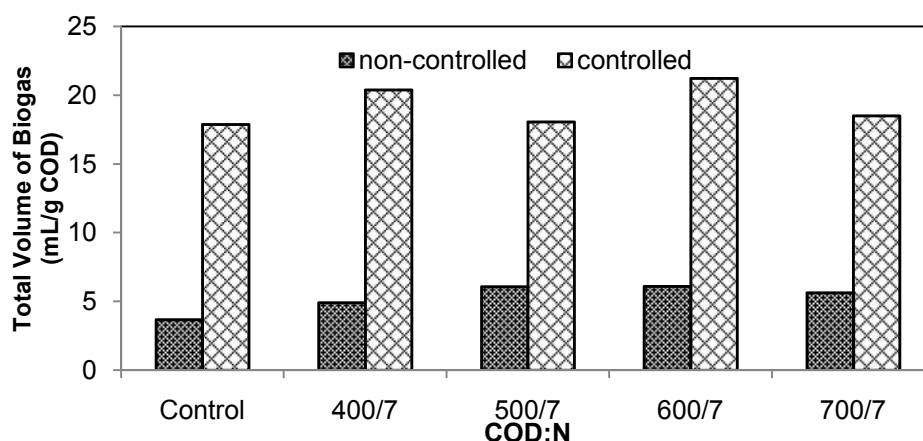


Figure 3. Comparison between pH controlled and pH non-controlled to total biogas production

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.

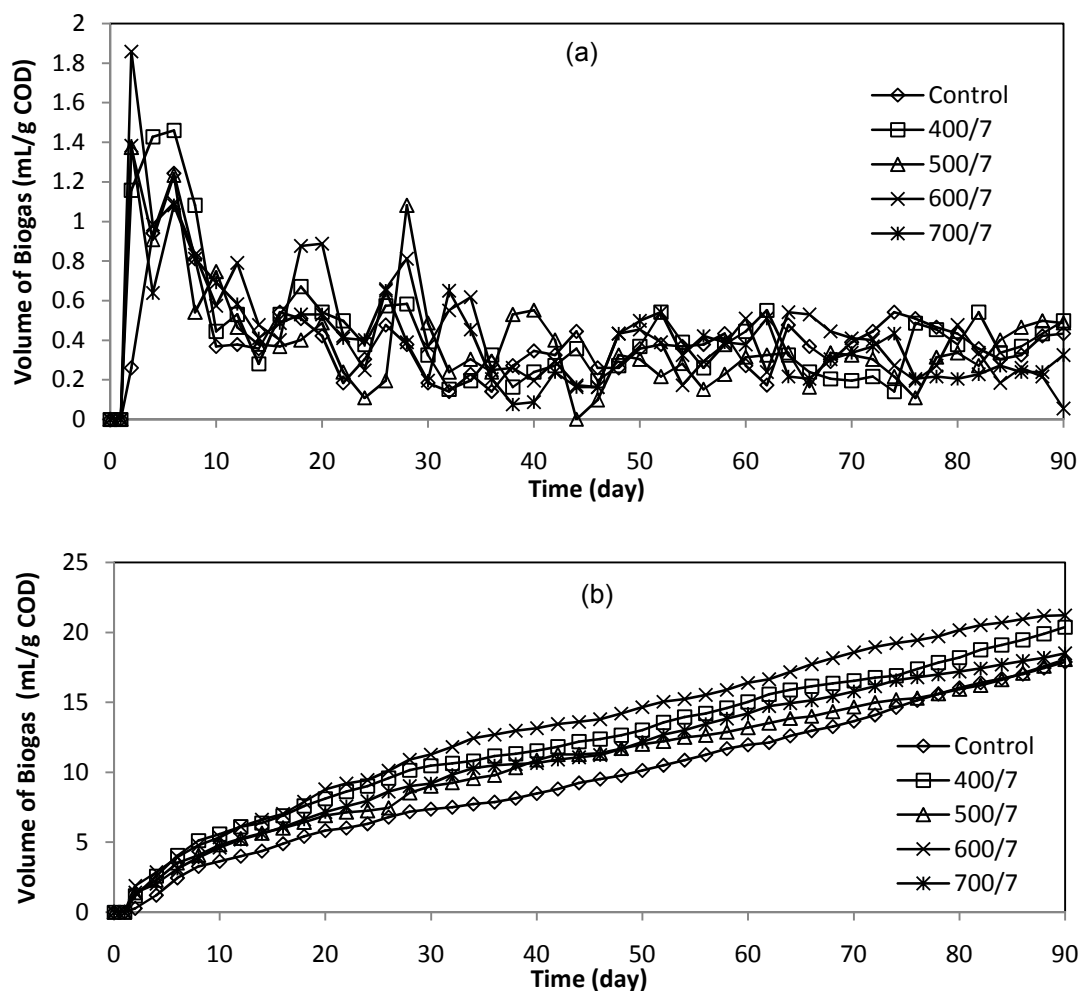


Figure 4. Biogas production daily (a), biogas production cumulative (b)

Organic contents in the substrate will be destroyed to be biogas. Running out biogas production was caused two problems. 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 conditions of substrates are 7.0 ± 0.2 , so pH is not a limiting parameter and microorganisms in the digesters can grow well (Fig. 4.). From Fig. 4. 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.

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 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.

ACKNOWLEDGMENTS

The authors thank University of Diponegoro and Indonesian Ministry of Higher Education for financial support.

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.

REFERENCES

1. Baikow VE. Manufacture and Refining of Raw Cane Sugar. 1982. Elsevier Scientific Publishing Company, Amsterdam-Oxford-New York.
2. Garcia IG, Venceslada JLB, Jimenez Pena PR, Gomez ER. Biodegradation of Phenol Compounds in Vinasse Using *Aspergillus Terreus* and *Geotrichum Candidum*. *Wat. Res.* 1997;31(8):2005-2011.
3. Robles-Gonzalez V, Lopez-Lopez E, Martinez-Jeronimo F, Ortega-Clemente A, Ruiz-Ordaz N, Galindez-Mayer J, Rinderknecht-Seijas N, Poggi-Varaldo HM. Combined treatment of mezcalvinasses by ozonation and aerobic biological post-treatment. *Proceedings of 14th International Biotechnology Symposium*. Rimini, Italy, 14–18, September 2010.
4. Lutoslawski K, Ryznar-Luty A, Cibis E, Krzywonos M, Miskiewicz T. Biodegradation of beet molasses vinasse by a mixed culture of microorganisms: Effect of aeration conditions and pH control. *Journal of Environmental Sciences*. 2011;23(11):1823–30.
5. Zayas T, Romero V, Salgado L, Meraz M, Morales U. Applicability of coagulation/flocculation and electrochemical processes to the purification of biologically treated vinasse effluent. *Separation and Purification Technology*. 2007;57:270–276.

- 279 6. Jimenez AM, Borja R, Martin A, Raposo F. Mathematical modelling of aerobic
280 degradation of vinasses with *Penicillium decumbens*. *Process Biochem.* 2005;40:2805-
281 11.
- 282 7. Preeti CS, Aniruddha BP. Enhancement in biodegradability of distillery wastewater using
283 enzymatic pretreatment. *J. Environ. Manage.* 2006;78(1):77–85.
- 284 8. Tang Y-Q, Fujimura Y, Shigematsu T, Morimura S, Kida K. Anaerobic treatment
285 performance and microbial population of thermophilic upflow anaerobic filter reactor
286 treating awamori distillery wastewater. *J Biosci Bioeng.* 2007;104(4):281–7.
- 287 9. Speece RE. *Anaerobic Technology for Industrial Wastewaters.* 1996. USA: Archae
288 Press.
- 289 10. Sasse L. *Biogas Plants.* Eschborn. 1988. Germany: Deutsche
290 Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH.
- 291 11. Juanga JP, Visvanathan C, Tränkler J. Optimization of Anaerobic Digestion of Municipal
292 Solid Waste in Combined Process and Sequential Staging. *J Waste Manage Res.*
293 2007;25:30–38.
- 294 12. Karellas SB. Development of an investment decision tool for biogas production from
295 agricultural waste. *Jurnal Renewable and Sustainable Energy Reviews* 2010;14:1273-
296 82.
- 297 13. Espinoza-Escalante FM, Pelayo-Ortiz C, Navarro-Corona J, Gonzalez-Garcia Y, Bories
298 A, Gutierrez-Pulido H. Anaerobic digestion of the vinasses from the fermentation of
299 Agave tequilana Weber to tequila: The effect of pH, temperature and hydraulic retention
300 time on the production of hydrogen and methane. *Journal Biomass and Bioenergy.*
301 2009;33:14–20.
- 302 14. Buitron G, Carjaval C. Biohydrogen Production from Tequila Vinasse in an Anaerobic
303 Sequencing Batch Reactor: Effect of Initial Substrate Concentration, Temperature, and
304 Hydraulic Retention Time. *Bioresource Technology* 2010;101:9071 – 9077.
- 305 15. Siles JA, García-García I, Martín A, Martín MA. Integrated ozonation and
306 biomethanization treatments of vinasse derived from ethanol manufacturing. *Journal of*
307 *Hazardous Materials.* 2011;188:247–253.
- 308 16. Yetilmezsoy K, Sakar S. Development of empirical models for performance evaluation of
309 UASB reactors treating poultry manure wastewater under different operational
310 conditions. *J. Hazardous Materials.* 2008;153: 532–543.
- 311 17. Budiyo, Widiya IN, Johari S, Sunarso. Increasing Biogas Production Rate from Cattle
312 Manure Using Rumen Fluid as Inoculums. *International Journal of Basic & Applied*
313 *Sciences IJBAS-IJENS* 2010;10(1):68-75.
- 314 18. Budiyo, Widiya IN, Johari S, Sunarso. The Kinetic of Biogas Production rate from
315 Cattle Manure in Batch Mode. *International Journal of Chemical and Biological*
316 *Engineering.* 2010;3(1):40-4.
- 317 19. Angelidaki I, Ahring BK. Thermophilic anaerobic digestion of livestock waste: the effect of
318 ammonia. *Appl Microbiol Biotechnol.* 1993;38(4):560–4.
- 319 20. Fang HHP, Chui HK, Li YY, Chen T. Performance and granule characteristics of UASB
320 process treating wastewater with hydrolyzed proteins. *Water Sci Technol* 1994;30(8):55–
321 63.
- 322 21. Sung S, Liu T. Ammonia inhibition on thermophilic anaerobic digestion. *Chemo-sphere.*
323 2003;53(1):43–52.
- 324 22. Soubes M, Muxõ L, Fernandez A, Tarlera S, Quirolo M. Inhibition of methanogenesis
325 from acetate by Cr+3 and ammonia. *Biotechnol. Lett.* 1994;16:195–200.
- 326 23. Liu T, Sung S. Ammonia inhibition on thermophilic aceticlastic methanogens. *Water Sci.*
327 *Technol.* 2002;45:113–120.

- 328 24. Kayhanian M. Performance of a high-solids anaerobic digestion process under various
329 ammonia concentrations. *J. Chem. Tech. Biotechnol.* 1994;59:349–352.
- 330 25. Chen Y, Cheng JJ, Creamer KS. Inhibition of anaerobic digestion process: a review.
331 *Bioresour. Technol.* 2008;99:4044–4064.
- 332 26. Mata-Alvarez J, Mace S, Llabres P. Anaerobic digestion of organic wastes. An overview
333 of research achievements and perspectives. *Bioresour. Technol.* 2000;74:3–16.
- 334 27. Phang SM, Miah MS, Yeoh BG, Hashim MA. Spirulinacultivation in digested sago starch
335 factory wastewater. *Journal of Applied Phycology* 2000;12:395–400.
- 336 28. Elbeshbishy E, Nakhla G. Batch anaerobic co-digestion of proteins and carbohydrates.
337 *Bioresource Technology.* 2012;116:170–178.
- 338 29. Vicenta M, Pacheco G, Alamis MLA, Anglo P G, Tan BV, Silverio CM. A Study of Some
339 Factors Affecting Biogas Production from Pineapple Peelings, In Bidin R., Chong C. N.
340 and Wang C. W. (eds), *Proceeding of the Second ASEAN Workshop on Biogas*
341 *Technology Applied in the Management and Utilization of Food Waste Materials* (pp.
342 189-202), Kuala Terengganu, Malaysia, 1984. Quoted in Anunputtikul W, Rodtong S,
343 *Biogas Production from Cassava Tubers, Thailand, 2004.*
- 344 30. AyuAO, Aryanti VD. Biogas Production Using Anaerobic Biodigester From Cassava
345 Starch Effluent With Ruminant Bacteria As Biocatalyst. 2010. Master Thesis.
346 Diponegoro University, Indonesia
- 347 31. Viswanath P, Devi SS, Nand K. Anaerobic Digestion of Fruit and Vegetable Processing
348 Waste for Biogas Production, *Bioresource Technology.* 1992;40:43-48. Quoted in
349 Anunputtikul W, Rodtong S. *Biogas Production from Cassava Tubers, Thailand, 2004.*
- 350 32. Lopes WS, Leite VD, Prasad S. Influence of inoculum on performance of anaerobic
351 reactors for treating municipal solid waste. *Bioresour. Technol.* 2004;94: 261-266.
352