CHEMICAL ENGINEERING TRANSACTIONS

VOL. 45, 2015

A publication of AIDIC
The Italian Association of Chemical Engineering
www.aidic.it/cet

Guest Editors: Petar Sabev Varbanov, Jiří Jaromír Klemeš, Sharifah Rafidah Wan Alwi, Jun Yow Yong, Xia Liu

ISBN 978-88-95608-36-5; **ISSN** 2283-9216

DOI:10.3303/CET1545265

Screening on Biogas Optimization of Lignocellulose-Based Materials using Enzymatic Hydrolysis Process

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Cow manure is one of the promising sources of biogas. However, due to poor biodegradability of fibrous material that is present in the total solid (TS) content, the optimal biogas yield is not achieved. The main purpose of this study is to examine the effect of enzymatic hydrolysis pre-treatment of lignocellulose rich substrate to enhance the biogas production. Biogas production of cow manure was investigated using two anaerobic reactors (R1 and R2) in batch mode. Reactor R1 (controlled) was operated using cow manure as the sole substrate and reactor R2 consist of cow manure and mixed fungal, as inoculum. Each vessel consists of 3 L substrates, operated at the temperature of 35 °C and pH controlled at 6.9. The stirring was maintained at 150 rpm. Effluent was subjected to several analytical tests which includes chemical oxygen demand (COD), ammonia nitrogen content (NH_3-N), total solid (TS) and volatile solid (VS). Lignin, cellulose (as glucose) and hemicellulose (as xylose) were investigated using van Soest Fiber Analysis System and HPLC, respectively. Result indicated that, raw cow manure constitutes about 11.9 wt% of TS, with VS content of 10.7 wt%. The degradable fractions of cow manure consist of 25.44 % of cellulose with 13.43 % of hemicellulose and 13.36 % lignin. Glucose and xylose indicated in raw cow manure was about 15.39 g/L and 10.92 g/L, respectively. Apparently, the results on the biogas production from reactor R1 at 30 d operation showed this sample produced slightly higher than reactor R1 about 6.91 L compared to reactor R2 (6.5 L). This study indicated that the raw cow manure which is without any pre-treatment could be potentially considered as one of the most effective alternative for generating biogas.

1. Introduction

Renewable Energy (RE) and natural gas collectively meet almost two-thirds of incremental energy world's demand in 2010-2035 (Hoeven, 2011). In Malaysia, RE was announced as the fifth fuel in the energy supply mix and it is being targeted to be a significant contributor to the country's total electricity supply (Hashim and Ho, 2011). Based on Administration (2014) about 4 % of biomass and waste is consumed for energy production in Malaysia and it is predicted to increase in 2035. One of the low cost substrate which is suitable for biofuel production (such as hydrogen and methane) is animal manure (Petronia Carilloa et al., 2014). In agricultural sector for east coast region, especially in Terengganu, cow manure is among the largest waste produced (Ministry of Agricultural & Agro-Based Industry Malaysia, 2015) and needs to be properly managed to reduce the negative impact on the environment (Nasir et al., 2012). Besides, the recovery process can be put to a better use of cattle manure for producing RE and retain the methane rather than being released from it natural decomposition process (Sutaryo et al., 2012). As there is an increasing demand in RE and good supply of cow manure, it being one of the potential sources as a biowaste energy source. However, hydrolysis is the first step in anaerobic digestion (AD) process which is known as rate limiting step when animal slurry with high fiber (cellulose, hemicellulose and lignin) concentration is treated (Sutaryo et al., 2014). Based on Azman (2011), due to the negative effects of persistent molecules such as lignin encrustation, only about 30 % of complex organics can be converted to methane. There are several pre-treatment methods are implemented to improve the biodegradation such as chemical, mechanical, thermal and biological pre-treatment (Baatar et al., 2014). With regards to the low energy input, no chemical requisite and environmental friendly Sutaryo et al. (2014), enzymatic hydrolysis is one of biological pre-treatment. Therefore, this paper investigates the enzymatic hydrolysis in terms of the potential on biogas production and biodegradation of sample.

2. Material and Methods

2.1 Sample Preparation

Fresh cow manure was collected from a cattle farm in Kuala Terengganu, Malaysia. All samples were transported immediately to the research laboratory University Malaysia Terengganu. The sample was mixed with distilled water with 1:1 (w/v) ratio. The mixture was minced and homogenized by blender (HR2001/70) for 1 min and was kept in 4 °C to prevent the biological decomposition or ready to be analyzed (Liao et al., 2006). The homogenized sample was used to characterize the sample properties. A consortium of mixed fungi culture used in this pretreatment process consists mainly of *Aspergillus sp*. This consortium may pose the ability of producing cellulolytic enzyme such as xylanase, cellulose and CMCase. These three enzymes were responsible for hydrolysis of cellulosic material in the substrate. The preparation of a consortium mixed fungi culture was prepared according to Ang et al. (2013).

2.2 Bioreactor Operation

Reactor R1 (controlled) was operated using cow manure as the sole substrate and reactor R2 consist of cow manure and mixed fungal, as inoculum. Reactors R1 and R2 were operated in batch mode for 30 d. The reactor consists of 5 L vessel (Sartorius, Melsungen, Germany), which has a cylindrical geometry having a diameter of 16 cm and a height of 25 cm. Mixing each vessel was done by mechanical stirring (Rushton impeller), built into the vessel. Other equipment attached to the fermenters were an electrical heating jacket for temperature control, an OxyFerm FDA DO sensor, an EasyFerm plus K8 325 pH sensor (both Hamilton, Bonaduz, Switzerland) and a Pt-100 temperature sensor (Sartorius, Melsungen, Germany). Process temperature was maintained at 35 ± 3 °C by using heating jacket. The pH 6.9 was controlled automatically by adding 2M HCl and 2M NaOH as appropriate and stirring was maintained at a speed of about 150 rpm. In order to ensure the anaerobic environment, the reactors were sealed and purged with nitrogen gas before the operation.

2.3 Analytical Methods

The sample were subjected to several analytical tests such as pH, total solid (TS), volatile solid (VS), chemical oxygen demand (COD), ammonia nitrogen content (NH_3-N) and also ultimate analyses were according to the Standard Methods (APHA, 2012) Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) of the manure were analyzed using van Soest Fiber Analysis System (Liao et al., 2006). Total cellulosic material (cellulose, hemicellulose and lignin) was estimated using NDF value while ADF was use to estimate the concentration of lignin and cellulose. The percentage of NDF-ADF indicated the value of hemicellulose. The content of mono-sugar in the hydrolysed cow manure was determined using High Performance Liquid Chromatograph (HPLC) (Rombke et al., 2007). The biogas production was measured daily by the volumetric water displacement method from the reactor to 1 L measuring cylinder.

3. Results and Discussion

3.1 Characteristic of Substrates

The fresh cow manure were analysed and compared to the literature data with respect to total solid (TS), volatile solid (VS), chemical oxygen demand (COD), ammonia nitrogen content as well as pH. The lignocellulose material was measured and the summarized value was tabulated in Table 1. The initial TS and VS content of cow manure was about 11.9 %, 10.7 %, respectively. The lignocellulose fractions consist of 25.44 % of cellulose with 13.43 % of hemicellulose and 13.36 % lignin.

Table 1: Characteristic of cow manure

Parameters	(Zhang et al., 2013)	(Yen and Brune, 2007)	(Chen et al., 2003)	This Study
Ammonia nitrogen, mg/L	N.D	159,000	N.D	2,175
VS/TS ratio	0.81	N.D	N.D	0.90
Carbon, % dry basis	26.7 ± 1.8	N.D	N.D	42.03 ± 1.3

Table 2: Characteristic of cow manure (continued)

Parameters	(Zhang et al., 2013)	(Yen and Brune, 2007)	(Chen et al., 2003)	This Study
Nitrogen, % dry basis	5.1 ± 0.6	N.D	N.D	2.93 ± 0.2
C/N Ratio	5.2	4.8	N.D	14.3
Cellulose, % dry matter	N.D	N.D	21.9	25.44
Hemicellulose, % dry matter	N.D	53.7	17.4	13.43
Lignin, % dry matter	N.D	N.D	12.2	13.36

3.2 Chemical oxygen demand (COD) concentrations and biogas production

The COD concentration for both bioreactors showed the reduction over a period of 30 d of digestion due to the depletion in substrate availability (Figure 1). Reactor R1 demonstrated that COD percentage reduction peak was 4 % higher than reactor R2. The fluctuation in reactor R2 indicates the unstable nature of the microorganism due to the actual sample might contains a complex organic material. However, the COD percentage reduction efficiency in reactor R2 was 50 % higher compared to study conducted by Saidu et al. (2013). It might be because of the supplementation of a consortium mixed fungi in this study compared to that study which only use only POME as inoculum. The cumulative biogas production for reactors R1 and R2 were 6.91 L and 6.5 L respectively (Figure 1). In the first 10 d of the anaerobic digestion, more than 50 % of the total biogas produced in reactor R1 meanwhile reactor R2 showed the slow progress where it took about 12 d to produce 50 % of biogas. The insignificant difference in total biogas production between both reactors R1 and R2 showed the mixed fungi has no effect on the biogas production.

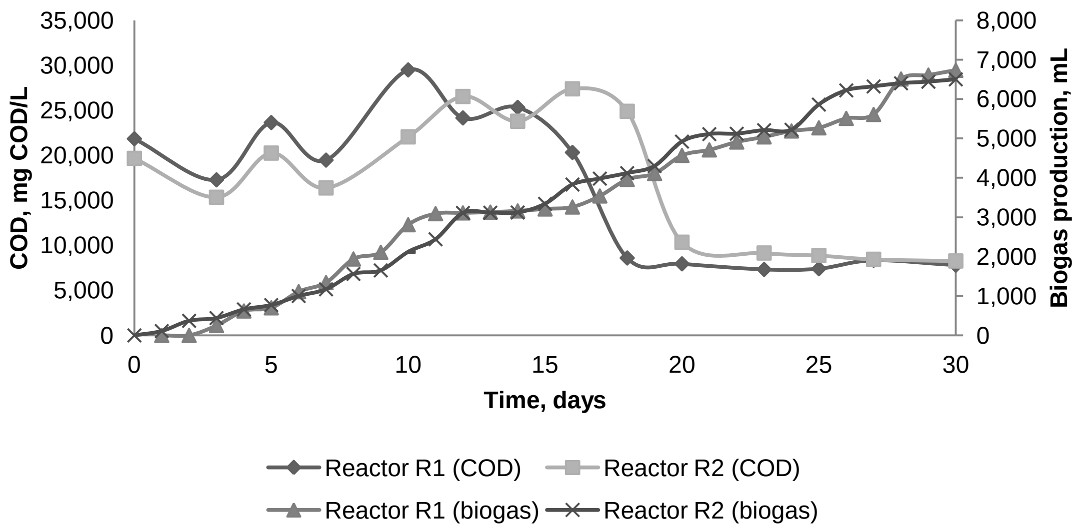

Figure 1: COsD concentration and biogas production for anaerobic digestion of cow manure

3.3 Effect on solubilisation and composition of cow manure after pre-treatment process

The enzymatic hydrolysis pre-treatment by of a consortium mixed fungi consists mainly of *Aspergillus sp*. was conducted to improve the hydrolytic process and to increase the solubilisation of the organic matter of cow manure. Figure 2 illustrated the composition of cow manure before and after pre-treatment in terms of cellulose, hemicellulose, lignin and water soluble carbon fraction. The water soluble carbon fraction of raw cow manure was about 47 % and other 53 % represented the lignocellulose compounds (cellulose, hemicellulose and lignin) This fiber value was similar to the range suggested by Chen et al. (2003) as showed in the characteristic of raw cow manure in Table 1. After pre-treatment, the water soluble carbon increased slightly in reactor R1 from 47.77 % to 50.98 %. In this condition, the fiber composition was also altered. Lignin composition in reactor R1 reduced from 13.36 % to 12.44 % while reactor R2 marked a greater reduction after pre-treatment where the fraction was from 13.36 % to 8.33 %. While, the initial cellulose content in raw cow manure was 25.44 % demonstrated degradation with 11.33 % in reactor R1 and 19.05 % in reactor R2 after pre-treatment. Surprisingly, the hemicellulose content in both reactors R1 and R2 after pre-treatment showed an increment with 25.29 % and 27.84 % from initial hemicellulose content (13.43 %). HPLC analysis revealed the enzymatic degradation by uncultured mixed fungi could produce fermented sugars such as glucose and xylose. The glucose produced after pre-treatment process in reactors (R1 and R2) was about 16.75 g/L and 21.26 g/L respectively. On the other hand, about 15.62 g/L and 9.28 g/L xylose was detected in reactors (R1 and R2). The value of sugar recovery achieved by this process is in line with the delignification process. However, hemicellulose fractions are increased and lower concentration of sugar recovery in reactor R2. It may happen due to incomplete hydrolysis of xylan which requires higher concentration and different types of enzymes (Binod et al., 2011).

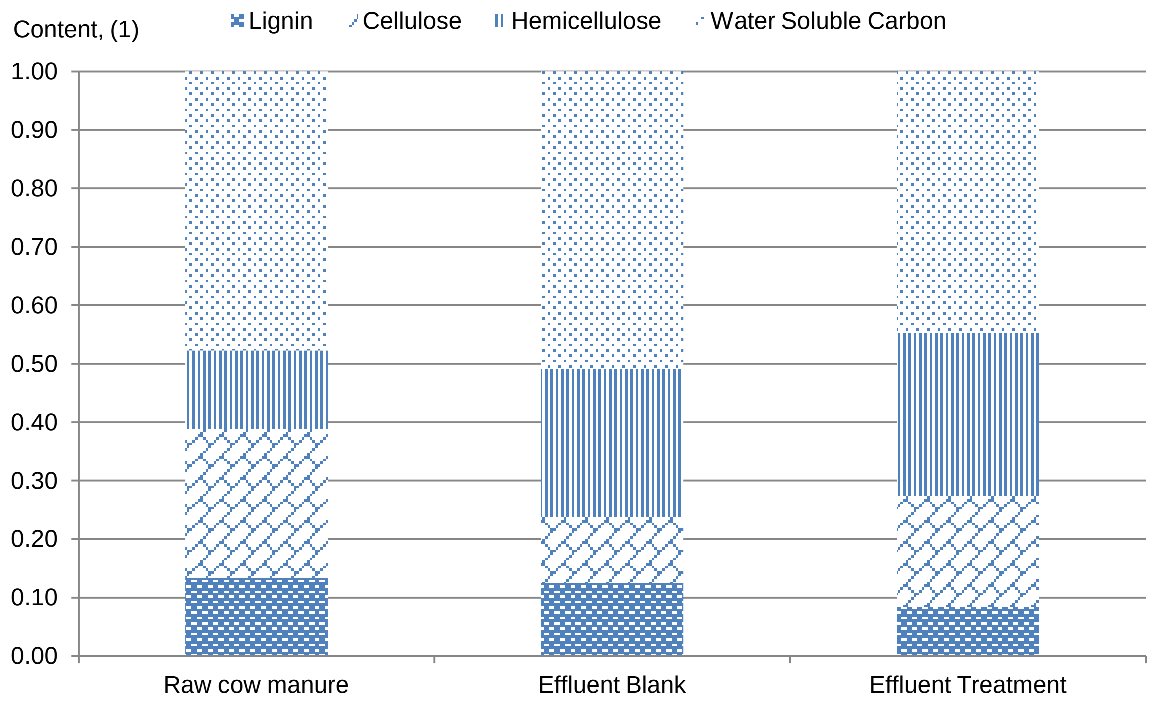

Figure 2: Fiber and soluble fraction of cow manure before and after pretreatment

3.4 Effect of pH through 30 days digestion operation

Figure 3 showed the pH profile in both reactors of 30 d bioreactor operation. Initially, the pH values for both reactors were setup at 6.9. However, both reactors showed a fluctuation trend at the beginning of experiment and it is due to the adaptation of the environment. After 10 d of digestion, R2 showed gradual decreased to 6.8 at the end of the experiment. It might be due to the optimum pH of a consortium mixed fungi consist mainly of *Aspergillus sp*. in acidic condition as reported by (Ang et al., 2013). However, the pH value for R1 illustrated the fluctuation but still in range of optimum pH in AD as reported by previous studies of 6.8 - 8.0 (Yohaness, 2010); 6.65- 7.81 (Bakar and Ismail, 2012) and 6.8 – 7.4 (Zhang et al., 2013). Although the pH value is a fixed parameter in this study, the variation from the initial values might be because of the activity or interaction of microorganism inside the reactor. The pH is one of the essential parameter used to test the substrate’s acidity which is important to control the growth of microbes during anaerobic digestion (Yohaness, 2010).

4. Conclusion

In this study, the result shows there is no significant effect on biogas production. The biodegradation of the sample in reactor R2 shows the highest lignin removal of 5.03 % compared to 0.92 % in reactor R1. On other hand, the increase in hemicellulose content in reactor R2 demonstrates the incomplete degradation of hemicellulose. This result is supported by low concentration of xylose produced. Therefore, there is limitation of anaerobic digestion of cow manure with addition of cultured mixed fungi solely.

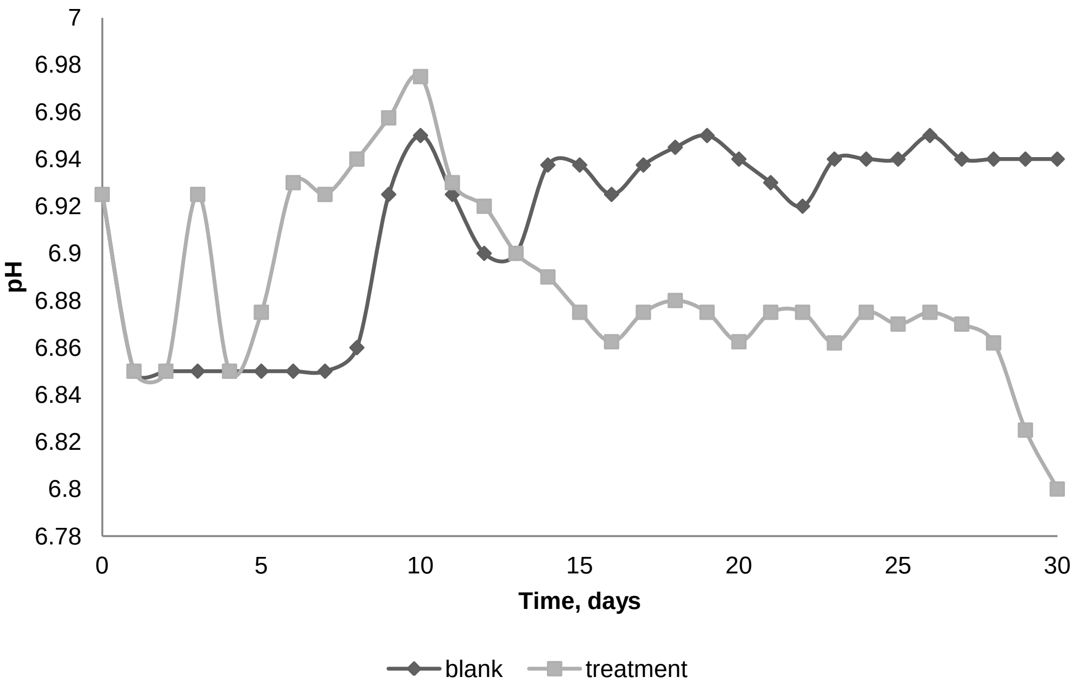

Figure 3: pH profile of 30 d bioreactor operation

Acknowledgement

This work was supported financially by the Research Acculturation Grant Scheme (RAGS) Vote No 57083 from Ministry of Education Malaysia.

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