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THE EFFECT OF TWO PRE-TREATMENT METHODS ON BIOGAS PRODUCTION POTENTIAL FROM COW MANURE

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Abstract: Hydrolysis process is considered as a rate limiting step in anaerobic digestion (AD) of lignocellulosic material in cow manure (CM). Due to the negative effects of lignin in lignocellulosic material, only about 30 % of this material can be converted to biogas. To accelerate the hydrolysis process and increase the biogas production, pretreatment of CM is needed. Two different pretreatment methods, biological and physical, were evaluated. Four sets of anaerobic digestion assays were carried out, with a working volume of 500 mL at 35 ± 2 °C and 120 rpm. The first and second sets of digestion were classified under physical pretreatment which contained dry grinded (DG) and fresh blended (FB) pretreated samples. The third set of fermentation involved biological pretreatment with the addition of *A. Fumigatus* SK1, while the last set contained *Trichoderma* sp. These four pretreated samples were compared to the untreated CM. Several analyses were subjected to determine the biomethane potential (BMP) of CM. The highest BMP was observed in DG pretreatment ($P<0.05$) which was 0.27, followed by 0.17, 0.02 and 0.01 LCH_4-STP g VS^{-1}. These results were obtained after the samples were pretreated with FB and added with pretreated *Trichoderma* sp. and *A. Fumigatus* SK1, respectively. These results collectively suggested that DG pretreatment could be a promising pretreatment method for the higher methane production in the anaerobic mono-digestion process.

Keywords: Anaerobic mono-digestion, biomethane potential, biological, physical pre-treatment

Introduction

In Malaysia, about 4 % biomass and waste were consumed for energy production in 2014 and the number is predicted to increase further in 2035 . In the agricultural sector especially in Terengganu, Cow Manure (CM) is listed as one of the largest biomass produced (Veterinar, 2015) with a percentage of approximately 60 % (329 kt/yr), followed by poultry 23 % (125kt/yr) and the other 17 % (91 kt/yr) were from buffalo, goat and sheep (Ghani *et al.*, 2013). Up until 2017, no proper manure disposal procedure had been proposed and executed which triggered the problems of odor, water pollution as well as continuing discharge of greenhouse gases (Sutaryo, 2012; Vancov *et al.*, 2015). Therefore, a more sensible alternative to turn CM into value-added products such as bioenergy become an urgent demand for the long term. On the bright side, CM is a good candidate to be used in Anaerobic Digestion (AD) because of its high buffering capacity which can regulate the optimum pH in the reactor. Despite having high moisture and high organic biodegradable material, CM also contained a high level of nutrients which is a good requirement for optimal bacteria growth (Kaoutar Aboudi *et al.*, 2015; Li *et al.*, 2015). CM is considered a lignocellulosic biomass. Lignocellulosic biomass consists of lignin, cellulose and hemicellulose (Wi *et al.*, 2015). In lignocellulosic biomass, about 63-78 % are made of holocellulose (Angelidaki & Ahring, 2000). Holocellulose (cellulose and hemicellulose) is packed and supported by lignin, which composed of phenylpropanoid units, act as a barrier for hydrolysis as well as resistance against enzyme and microbial attack (Hendriks & Zeeman, 2009) which leads to low cellulose and hemicellulose degradation (Chaikitkaew *et al.*, 2015). Only about 30 % of this lignocellulosic material can be converted to biogas because of the negative effects on lignin (Li *et al.*, 2015; Zhou & Li, 2016). Thus, pre-treatment is needed to increase the biogas production. The aim of pretreatment is to increase the solubilistion of substrates, making it more accessible for enzyme and microbial attack on biomass. As a result, the hydrolysis rates, bio-digestibility of wastes and the accessibility of enzymes improved while it decreased the association of lignin with the degradable part of biofibers (Leung & Wang, 2016, Farrukh Raza Amin *et al.*, 2017). Cell walls in biomass feedstock differ in structure and chemical composition. Thus, one pretreatment method will not necessarily fit all applications. Therefore, developing pretreatment technology that is effective over a wide range of biomass materials is important (Wi *et al.*, 2015). A variety of methods has been applied for pre-treating lignocellulosic biomass, but only a few seem promising for pre-treating CM. After undergoing this physical pretreatment, the substrates showed promising results such as improved anaerobic biodegradability by increment up to 20% of methane or biogas potential (Angelidaki & Ahring, 2000; Pereira, 2009), reduced digestion time by 23-59%, increased 5 to 25% total hydrolysis yield (Hendriks & Zeeman, 2009) and improved VS destruction as the effect of mechanical shear by 16-110% (Muller *et al*., 2007). In addition, biological pretreatments of lignocellulosic waste showed positive effects after pretreatment. *Aspergillus* sp. and *Trichoderma* sp. are capable of degrading lignin, cellulose and hemicellulose up to 75 to 80% thus increasing 30 to 101% by increment in methane yield (Muthangya *et al*., 2009). At present, a lot of research have implemented pretreatment methods on co-digestion in AD process (Kaparaju *et al*., 2002; Castrillón *et al*., 2011; Yufang Wei *et al*., 2015) and others. However, limited attention has been paid on mono-digestion process of pretreated CM. The aim of this work is to investigate the effectiveness of enzymatic hydrolysis process after undergoing different pretreatment processes. The focus of the study was on the influence of lignin removal on reducing sugar and ways to improve the biogas production.

Materials and Methods

Fungi and Pre-treatment Sample Preparation

The fungi used in the biological pretreatment, *Trichoderma* sp. and *A. Fumigatus* SK1. *Trichoderma* sp., were samples from the Setiu Wetlands, Setiu, Terengganu, whereas, *A. Fumigatus* SK1 was locally isolated from UiTM Kuala Pilah and previously identified using 18rNA characterization (Umor *et al*., 2016). After 7 days of incubation, the spores were collected and put into a conical flask, diluted and used as spore suspension of 10^8 spores/g of CM, as described by Ang *et al*., 2013. In the physical pretreatment studies, the homogenized CM was divided into two fractions: one to be freshly used and the second fraction dried at 65 °C for 24 hours and ground to pass a 1 mm.

Biomethane Potential Assay

Four sets of experiments were employed for the biomethane potential evaluation. The first set of fermentation contained *A.Fumigatus* SK1 and the second set had *Trichoderma* sp. Meanwhile, the third set contained an addition of freshly blended CM and the last set contained the addition of dry grinded CM.

All four sets of experiments were compared with the untreated CM. The experiments were performed in duplicates. The anaerobic digestion assay was carried out using 1000 mL Oxitop® bottles with a working volume of 500 mL, at the temperature of 35 ± 2°C. The corresponding substrate-to-inoculum ratio (S/I) was 0.5, on a VS basis. After filling the bottles, the samples were flushed with N_2 gas for 5 minutes, tightly sealed, incubated and shaken at 130 rpm. The pressure produced was monitored using an Oxitop® control 6, WTW. The highest pressure in each pretreatment process was used to calculate the BMP after pretreatment based on Eq. 1 (Pereira, 2009):

$$\mathrm{BMP} = \frac{\left[\left(\frac{(P_S \pm P_{atm})V_S}{RT}\right) \times \frac{\%CH_{4,s}}{100}\right] \mp \left[\left(\frac{(P_{bl} \pm P_{atm})V_{bl}}{RT}\right) \times \frac{\%CH_{4,bl}}{100}\right]}{So} \text{ X } 22.4 \quad (1)$$

Theoretical Methane Yield and Biodegradability

The theoretical methane yield was calculated based on Buswell equation (Eq. 2) (Angelidaki and Sanders, 2004). The analysis of gas was reported on cumulative methane basis at standard temperature and pressure (Eq. 3) (STP, 1 atm, 273.15 K)

$$C_aH_bO_cN_dS_e + [A]H_2O \rightarrow [B]CO_2 + [C]CH_4 + [d]NH_3 + [e]H_2S \quad (2)$$

$$\mathrm{TMY}_{ele} = \frac{(\frac{n}{2} + \frac{a}{8} - \frac{b}{4})22.4}{12n + a + 16b} \text{ (STP } LCH_4\text{/ g VS)} \quad (3)$$

The biodegradability of substrate was calculated based on theoretical methane yield and experimental methane yield as in (Eq. 4) (Li *et al*., 2013; Zhang *et al*., 2013).

$$\mathrm{Bd}\ (\%) = \frac{\mathrm{TMY}}{\mathrm{BMP}} \text{x } 100 \quad (4)$$

Where Bd is the percentage biodegradability, TMY is the theoretical methane yield and BMP is biomethane potential.

Statistical Analysis

To determine the degree of significance among biological and physical pretreatment methods, the data set was subjected to a one-way analysis using ANOVA, adopting SPSS version 22. Probabilities of $P < 0.05$ are considered as significant.

Analytical Methods

Characterization of the CM and inoculum including pH, Total Solids (TS) and Volatile Solids (VS) was performed based on standard methods (APHA, 2012). Samples were dried in the (UFB 400, Memmert) oven, overnight at 65°C. Grinding was performed in a (A11 Basic, IKA) grinder equipped with a 1 mm sieving device, and blending was performed in a (MX-337, Panasonic) commercial laboratory blender. The total fiber content was determined by the gravimetric methods (Goering & Soest, 1970).

Results and Discussion

Biomethane Potential and Anaerobic Biodegradability of Different Pre-Treatment

To obtain the value of BMP, the highest pressure in each pretreatment was applied to the equation (1). The results illustrated the highest BMP after DG pretreatment with 0.27 followed by FB, *Trichoderma* sp., and *A.Fumigatus* SK1 with 0.17, 0.02 and 0.01 LCH_4-STP g VS^{-1}, respectively. In addition, a previous study showed that the BMP range of CM was 0.11 – 0.24 LCH_4-STP g VS^{-1} (Lehtomaki *et al.*, 2005). Thus, this study highlighted that the DG pretreatment of CM can give the highest BMP in anaerobic mono-digestion process. Meanwhile, the effectiveness of the pretreatment can be evaluated based on BMP and the biodegradability of the samples (Chaikitkaew *et al.*, 2015). The biodegradability of the samples can be determined based on elemental compositions of the organic substrates using Buswell's equation as described previously by Angelidaki and Sanders, (2004). Based on

Table 1, DG pretreatment remarked the highest biodegradability with 38%, followed by 24% after FB pretreatment, 15% after CM was pre-treated with *Trichoderma* sp. and 9% after CM was pre-treated with *A.Fumigatus* SK1. The results on the biodegradability of CM after each pretreatment were in accordance with the BMP achieved. It was due to more substrate available to be digested by anaerobic microorganisms (Muthangya *et al.*, 2009). These results were supported by a previous finding, which concluded that increasing bio-degradability has contributed to increasing biomethane yield production (Meng *et al.*, 2015).

Table 1: Biomethane Potential and Biodegradability of CM with Different Types of Pre-treatment

Type of Pre-Treatment	BMP (LCH_4–STP g VS^{-1})	TMY (LCH_4–STP g VS^{-1})	B_d (%)
CM + *A.Fumigatus* SK1	0.01	0.10	9
CM + *Trichoderma* sp.	0.02	0.33	15
CM + Fresh Blended	0.17	0.04	24
CM + Dry Grinded Ground	0.27	0.10	38

BMP = Bio methane potential, TMY = Theoretical Methane Yield, B_d =Biodegradability,

Composition of Pre-Treated Sample and Reducing Sugar Production

Anaerobic degradation of organic components such as carbohydrates, proteins and lipids in CM will produce methane gas (Hendriks & Zeeman, 2009). The effectiveness of pretreatment process can be determined by hydrolysis process which converts polymer (carbohydrate) to fermentable sugar (Alvira *et al.*, 2010). The percentage of methane to be produced was influenced by the composition of fiber, cellulose, hemicellulose, protein, fat, starch and sugar content (Aslanzadeh *et al.*, 2011). A previous study done by Li *et al.* (2013) found a good correlation between lignin content and methane potential where the lignin content was one of the significant parameters affecting the methane production potential.

Figure 1 shows lignin removal and reducing sugar produced after several pretreatment methods.

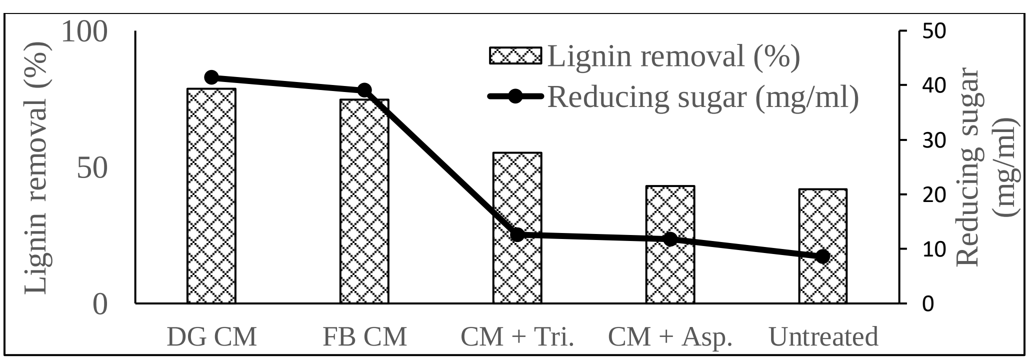

Figure 1: Lignin and Reducing Sugar Produced After Different Pretreatments

The DG pre-treatment showed the highest total lignin removal with 78.7%, followed by 74.8% after FB pretreatment. About 55.3% and 41.9% lignin can be removed after pre-treated with *Trichoderma* sp., and *A.Fumigatus* SK1, respectively and the percentage of lignin removal was higher than the untreated CM

(P<0.05). The amount of reducing sugar produced was in accordance with the percentage of lignin removal after each pretreatment. After 30 days of digestion, DG and FB pretreatment produced about 41.4 mg/ml glucose and 39.1 mg/ml glucose, respectively. Meanwhile, CM treated with *Trichoderma* sp. and CM treated with *A. Fumigatus* SK1 produced about 12.6 and 11.8 mg/ml glucose respectively. Statistical analysis showed that reducing sugar after a different pretreatment was higher compared to the untreated CM (P<0.05). These results illustrated that there was significant interaction between reducing sugar produced and lignin removal (P<0.05). As reported by (Alvira *et al*., 2010) , lignin removal can contribute to higher sugar production. This is due to the pre-treatment process that opened up the protective sheathing and hydrophobic nature of the lignin, which retards cellulose accessibility to enzyme and microbial attacks in further degradation during the following digestion process (Chaikitkaew *et al*., 2015). The result demonstrated that pretreatment of CM may accelerate the hydrolysis process.

Conclusion

In conclusion, this study found that there was a good correlation between lignin content and reducing sugar of CM to the BMP. From the four pre-treatments conducted, DG showed the highest lignin removal and the highest reducing sugar produced, which eventually contributed to the highest BMP recorded. Based on the results of the study, it can be suggested that DG pre-treatment is the best method for mono-digestion of CM through AD process.

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