

## Development of enzymatic pretreatment of palm oil mill effluent for monomers towards biogas production

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### ARTICLE INFO

### ABSTRACT

#### Article history:

Received 2 July 2016

Received in revised form 1 February 2017

Accepted 4 August 2017

Available online 19 November 2017

In anaerobic digestion for enhancement of biogas production, various types of pre-treatment method have been used with some limitations in terms of sustainable environmental management. Although acid and alkali pretreatment have significant effect on the degradation of biomass, these methods have some negative impacts on environment due to their hazardous nature, while the enzymatic pre-treatment is more environmentally friendly. One of the constrains of using enzyme in pretreatment process for biogas production is high cost which is currently focused to reduce cost through using locally produced enzyme. The hydrolytic enzymes, cellulase and lipase were applied to evaluate the pretreatment and hydrolysis process for POME based biogas production. The results showed that about 66.67% more free fatty acid (FFA) was obtained in this study in compared to untreated sample as the control (POME) by using 15 U/ml lipase at the pH of 4.5. About 3 fold higher reducing sugar was recorded at the loading of 2.4 FPU/ml cellulase enzyme as compared to the control at the pH range 4. Both enzymes were effective in the pretreatment process in conversion of complex substances in the POME into monomers towards biogas production.

#### Keywords:

Biogas, enzyme, pre-treatment, lignocellulose, anaerobic digestion, lipase, cellulase

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## 1. Introduction

With the rapid increase in world population, waste generation and demand for natural resource are increasing with significant correlation as well as reserve of fossil fuel is decreasing with an alarming rate. Biogas from lignocellulosic waste can be an alternative source of renewable energy [1]. Palm oil industry is a prominent agricultural industry which has huge economic value in some

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tropical countries. Malaysia was the second largest palm oil producer in 2006 in the world, with a total of 15.88 million tonnes, which is 1% less behind Indonesia from the total world's supply. Since the palm oil industry is abundant, with 67% of agricultural land covered with palm oil tree, contribution of oil palm biomass is significant. Currently, 86% of biomass residues come from oil palm industry [2].

Malaysia is the second largest producer of palm oil in the world generates about 40 million tons of sludge palm oil (SPO) per year. Only 10% of palm oil fruit is used for the production of oil and the rest 90% biomass can be used to produce renewable energy. This huge waste has a very high free fatty acid (FFA) content which is about 40 to 80% by weight. Every single bunch of the palm fruit generates approximately 21% palm oil, 14-15% of (PF) palm fibers, 6-7% of (PKS) palm kernel shells, 23% of (EFB) empty fruit bunches [3]. Out of all the by-products, POME will be a threat to the environment if directly discharged to the adjacent water body without pre-treatment [4].

Anaerobic digestion may be defined as the decomposition of organic material in the absence of oxygen while simultaneously producing useful biogas [5] consisting of mainly 60% methane (CH<sub>4</sub>), 35% carbon dioxide (CO<sub>2</sub>), and 5% ammonia (NH<sub>3</sub>) and other gases. Anaerobic digestion [2] is the most widely studied technology for organic treatments to alter the waste into renewable energy. Four different stages of reaction involved during anaerobic digestion process: namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis [6].

Many researchers used enzyme as a co-digestion with chemical or physical pre-treatment. As chemical treatment has some negative impact of on the environment, researchers are trying to search alternative way. Enzymatic pre-treatment can be one of the solutions. During anaerobic digestion to produce biogas, various type of pre-treatment gave satisfactory result but enzymatic pre-treatment is comparatively new and environment friendly than other method and which has already gained the global attention. The reason behind pre-treatment is to get more monomers from complex compound so that microbes can easily take the monomers as their food and as well as increase the rate of reaction in anaerobic digestion and produce more biogas [7-9].

Lipases are also one of the most important classes of industrial enzymes which is capable of hydrolyse triglycerides into diglycerides, monoglycerides, glycerol and fatty acids and vast range of reaction in nature [10, 11]. Cellulase enzymes can capable of bioconversion of cellulosic wastes into the simplest carbohydrate monomer, glucose [12]. Therefore, the hydrolytic enzymes, cellulase and lipase based on the major constituents of POME were selected to evaluate the pretreatment and hydrolysis process for production of monomers towards biogas production.

## **2. Materials and Methods**

### **2.1 Sample Collection**

Palm Kernel Cake (PKC) was collected in clean autoclave bags, from West Oil Mill, Sime Darby Sdn. Bhd. in Carey Island, Banting, Selangor, Malaysia. It was ground to 1.0 mm and dried at 60°C has been left in an oven for 72 h to reduce the moisture content to about 3.0%. All chemicals and consumables used in this research were of analytical grade. The raw material palm oil mill effluent (POME) was collected from an oil palm industry named Seri Ulu Langat Palm Oil Mill Sdn. Bhd., Dengkil, Selangor, Malaysia. The liquid sample was collected in a plastic container and stored at 4°C.

### **2.2 Sub Culturing, Inoculum Preparation and SSB**

The microbial strain, *Candida cylindracea* (ATCC 14830) used in this study was purchased from the American Type Tissue Culture, USA. *C. cylindracea* was grown on PDA plates at 28°C for four

days in an incubator (Incucell, Germany) and sub-cultured every two weeks. Each plate was washed with 10.0 ml sterile distilled water and the suspension was used to prepare the inoculum in the appropriate medium. Locally fabricated PKC Lipase through the solid state fermentation (SSB) was produced according to method suggested by [13, 14].

*Trichoderma reesei* RUT C-30 (ATCC 56765) was cultured on the PDA plate as inocula source and incubated at 30°C for 8-10 days until the good sporulation was observed. After maturation each plate was washed with 25 ml of sterile water and then filtered the spore suspension with Whatman.1 filter paper. Cellulase enzyme was produced and assayed through the method described by [15]

### 2.3. Lipase loading on POME for FFA content

#### 2.3.1 Lipase activity, pH, time and agitation on FFA content of POME

Summary of Lipase loading to the POME to get FFA at different process condition has been shown in Table 1. In all operation to produce monomer (Free Fatty Acids) from POME, commonly 20 ml diluted sterilized POME with 1.25% TSS loading in 100 ml shake flask was used to put on shaker under room temperature (28±2°C). pH range of POME was recorded 4.5 for all operation.

**Table 1**

Summary of Lipase loading to the POME in different process condition

Experiment	Lipase activity (U/ml)	(TSS), %	Rotation (rpm)	pH	Time (Hours)
Effect of Lipase activity	3, 6, 9, 15, 21, 27, 33, 39, 45	1.25	150	4.5	24
Effect of pH	3, 6, 9, 15	1.25	150	4.5, 5, 6, 7	24
Effect of Time	15	1.25	150	4.5	1, 3, 6, 12, 24, 36
Effect of agitation	3, 15	1.25	150, 120	4.5	24

To evaluate lipase activity, the experiments were carried out with different doses of PKC lipase, while the control sample was left without enzyme loading. Free fatty acids (FFAs) content was measured titrating against sodium hydroxide (0.1N) at the presence of phenolphthalein indicator suggested by Vêras *et al.* [16]. To evaluate the optimum time to get highest level of FFA after 1, 3, 6, 12, 24, 36 and 48 hours of operation, FFA content was measured.

### 2.4 Cellulase Loading on POME

#### 2.4.1 Cellulase activity, pH, time and agitation on reducing sugar of POME

Summary of cellulase loading to the POME to get reducing sugar at different process condition has been shown in Table 2. The experiments were conducted with 20 ml diluted sterilized POME with 1.25% TSS loading in 100 ml shake flask on the shaker under room temperature (28±2°C). Reducing sugar according to the method suggested by Rashid *et al.* [15]. A set of sterilized control sample was put as same process.

**Table 2**

Summary of Cellulase loading to the POME in different process condition

Experiment	Cellulase activity (FPU/ml)	(TSS), %	Rotation (rpm)	pH	Time (Hours)	Temperature (°C)
Effect of cellulase activity	0.16, 0.48, 0.8, 1.44, 2.4	1.25	150	4.5	24	28±2
Effect of Sterilization	0.16, 0.48, 0.8	1.25	150	4.5	24	28±2
Effect of pH	0.16, 0.48, 0.8	1.25	150	4.5, 5, 6,	24	28±2

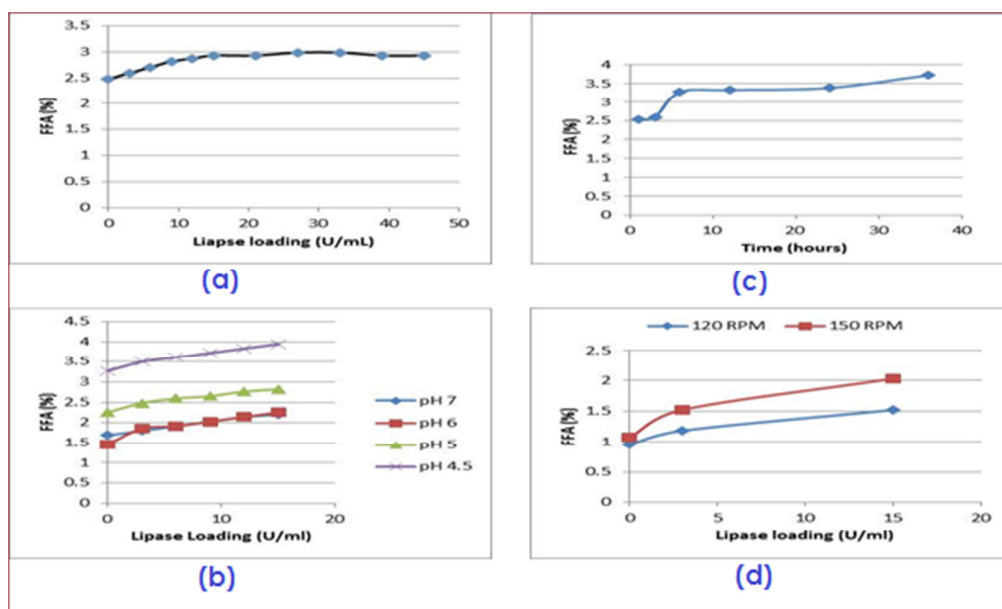
Effect of Time 0.48 1.25 150 4.5 1, 3, 6, 12, 24, 36, 48 28±2

### 3. Results and Discussion

#### 3.1 Lipase Loading on POME

To evaluate the lipase activity to produce monomer (FFA), different dose of lipase was added to the POME with various process conditions (Figure 1). A sharp increase of FFA was noticed from 3 U/ml until 15 U/ml Lipase loading. Further increase of enzyme loading has no significant effect on increase of FFA. Lipase activity on POME has been shown in Figure 1(a).

Among the pH levels, higher FFA was observed at pH level 4.5. A study by Elgharbawy et al. [14]) reported optimal activity of PKC lipase at pH 6-8 and temperature of 25 -45°C while pH lower than 4 the activity decreases to 50%. At high temperature (60°C), no activity was found. NaOH was added to increase pH level from 4.5 to 7, which itself neutralize the FFA. Lower FFA was recorded at the pH range 7 than at the pH range 4.5. Effect of pH to produce FFA from POME has been represented in Figure 1(b).



**Fig. 1.** Effect of lipase loading on POME pretreatment with various conditions: (a) enzyme with fixed conditions; (b) pHs; (c) contact time; (d) agitation rates

Contact time of the enzyme has the impact in producing FFA from POME. A sharp increase of FFA was observed from 1 to 6 hours was observed. Effect of time to produce FFA with 15 U/ml enzyme dose has been shown in Figure 1(c). After 6 hour a gradual increase was reported due to lower of enzyme activity after reactions.

The effect of rotation speeds are shown in Figure 1(d). Result showed that higher FFA at the 150 of rotation was observed than the 120 of rotation. High agitation speed may be favorable to the good mixing in the substrate and help in reactions for the conversion process.

#### 3.2 Cellulase Loading on POME

A sharp increase of reducing sugar was observed in the POME pretreatment from various cellulase loading with different process conditions (Figure 2). The increased trend was noticed with

increase of activity of cellulase until the activity of 2.4 FPU/ml. Effect of cellulase activity was shown in Figure 2(a).

Results of pH effect on reducing sugar in the POME are shown in the Figure 2(b). With 3 different pH ranges (pH 4, pH 5, and pH 6), higher reducing sugar was noticed at the pH 5. A 2.8 fold higher reducing sugar than control sample was recorded with 0.8 FPU/ml cellulase loading at pH 5 where 2.4 and 2.3 fold higher reducing sugar was recorded at pH 4 and 6 respectively.

Effect of time to produce reducing sugar is shown in Figure 2(c). For commercial product economical viability is a major factor. To evaluate economical viability effect of time in producing reducing sugar was noticed from 1 hour to 48 hours with same dose of cellulase. Until 10 hours no significant increase was noticed. After 10 hours, gradual increase was observed until 48 hours of pretreatment process. An increased 86.9% yield was measured after 48 hours.

Effect of agitation rates on reducing sugar is shown in the Figure 2 (d). No significant difference was observed between 150 rpm and 120 rpm of rotation speeds.

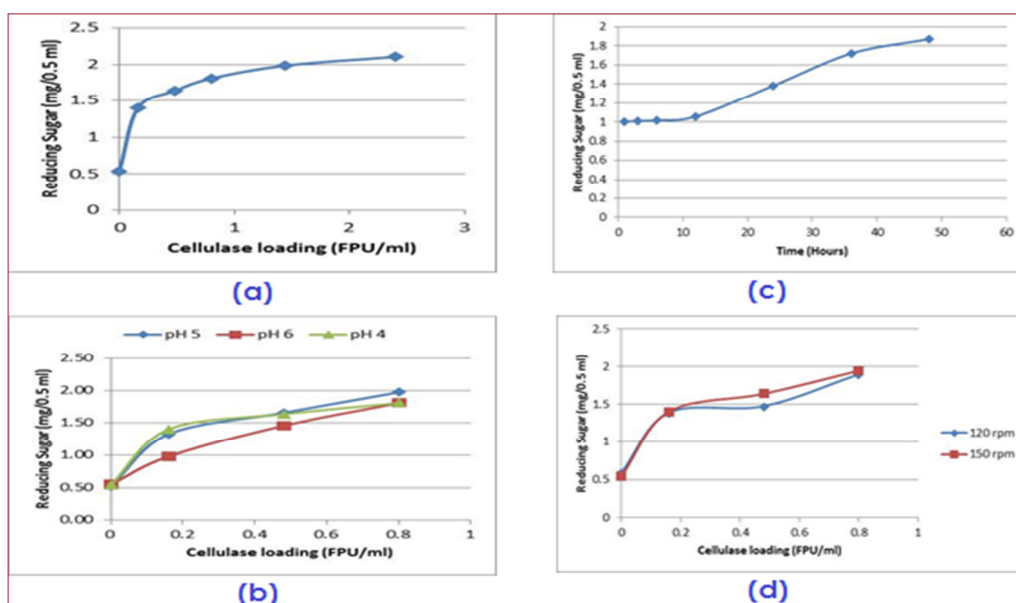


Fig. 2. Effect of cellulase loading on reducing sugar in the POME pretreatment: (a): enzyme loads; (b) pHs; (c) reaction time; (d) agitation rates

#### 4. Conclusion

The results obtained in this study shows that the cellulase and lipase enzymes have significant effect on the POME to produce monomers towards enhanced biogas production in anaerobic digestion. The findings provide a potential solution to high yield biogas from POME for oil palm industry with further optimization and development of the processes.

#### Acknowledgement

Authors are grateful to the Department of Biotechnology Engineering for providing the lab facilities.

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