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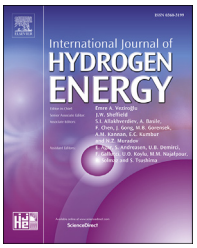

Direct hydrolysis of palm oil mill effluent by xylanase enzyme to enhance biogas production using two-steps thermophilic fermentation under non-sterile condition

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ABSTRACT

Palm oil mill effluent (POME) is well known as the potential raw material for biogas production. However, the high hemicellulose and cellulose content (10–14% w/w in dry basis) of the palm fibre in POME are less accessible for biodegradable to sugars, therefore, limiting the carbon source for microorganisms for the biogas production. This study attempted to enhance the hydrolysis step of the biomass by enzymatic pretreatment of POME. The optimum temperature and hydrolysis time using the commercial xylanase (5 unit/mL) were at 50 °C for 12 h. Two-steps thermophilic process consisting of POME pretreatment by various concentrations of xylanase (5–20 unit/mL) under the optimum condition followed by biogas production at 60 °C for 45 days was carried out. In all cases, the substrate to inoculum volatile solids (VS) ratio and volume ratio were 1:1 and 4:1, respectively. The maximum biomethane (CH_4) yield of 914 mL CH_4/g VS was obtained from the fermentation of POME hydrolysed by 15 unit/mL of xylanase. However, it was not significantly different from those of the other three concentrations of xylanase (845, 870 and 851 mL CH_4/g VS, respectively). They were about 2.5–3 folds higher than that of the control (POME without enzymatic hydrolysis) (297 mL CH_4/g VS). Microbial community analysis revealed the presence of *Clostridium* sp. and *Methanocaldococcus* sp. as the dominant strains.

Introduction

Palm oil mill effluent (POME) is one of the polluting sources among various agro-industrial wastewaters in Thailand. It contains high organic matter (chemical oxygen demand of 75–96 g COD/L), high solids (35–42 g/L total solids and 8.5–12 g/L suspended solids) and has high temperature (70–80 °C) when discharged from the oil extraction process [1,2]. Based on its characteristics, POME can easily cause the environmental problem if not properly treated. Anaerobic open pond is commonly used to treat POME and this practice

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is responsible for the greenhouse gas (CH_4 and CO_2) emission. Hence, closed anaerobic system that produces biogas as an alternative fuel source could solve the problem.

The specific organic load from palm oil mills in Thailand was calculated to be 19.8 kg BOD/t FFB (fresh fruit bunch) [3]. High organic load of POME made it suitable for biogas production, which has been implemented extensively in most of the palm oil mills. In Thailand, the quantity of POME was varied in the range of 0.5–1.2 m^3/t FFB or average 0.87 m^3/t FFB [4]. In 2006, under Energy & Eco Efficiency in Agro-Industry (E3 Agro) Project, it was reported that the specific wastewater generation was 0.56 m^3 POME/t FFB [3]. One tonne of POME can produce up to 20 m^3 biogas [5]. The biogas is used as fuel for boilers to generate steam and electricity for milling processes. The surplus electricity was sold to the Provincial Electricity Authority under the Very Small Power Plant (VSPP) scheme supported under National Alternative Energy Promotion Plan [6]. Another alternative form of energy under this plan is hydrogen. This encouraged research and development on biohydrogen production from POME under dark fermentation and photo-fermentation by our group [7–16]. The effluent from hydrogen reactor was used as substrate for biogas (biomethane) production which formed the two-stage process. Thermophilic and mesophilic fermentation process for production of biohythane (hydrogen + methane) from POME was recently developed [17,18].

POME solid is lignocellulosic material, mainly consisting of cellulose, hemicellulose and lignin. Improvement of biogas production efficiency from POME could be achieved by various methods including co-digestion of substrates [19–25], pretreatment of POME before fermentation [26–31]. Pretreatment could disrupt the recalcitrant structure of lignocellulosic material, increase reducing sugar and improve biogas production [32]. The pretreatment methods include heat, chemical, biological treatment prior to biogas production. The enzymatic pretreatment was reported to increase 24% and 15% CH_4 yield of sugar beet pulp and spent hops hydrolysate fermentation, respectively [33]. Using plant biomass hydrolysate could increase the biogas yield up to 20% [34].

Enzymatic hydrolysis of various lignocellulosic materials was reported but mostly employed cellulases [36–38]. Among lignocellulosic components in POME, hemicelluloses are probably the most accessible substrate for microorganisms. In the previous study [35], commercial xylanase and the mixed enzymes of *Aspergillus niger* ATCC6275 (with equal xylanase activity) had demonstrated to enhance oil recovery from POME by enhancing the release of oil entrapped within the residual fibre in POME and allowed the oil and solid particles to float to the upper layer under static condition. This phenomenon is commonly seen as layer of acid oil and solid particles on the surface of the first pond of the wastewater treatment system in palm oil mills. The comparable performance of commercial xylanase and the fungal mixed enzymes may indicate the strong hydrolytic reaction of xylanase and/or synergistic interaction of xylanase and cellulase. Due to the presence of dissolved hemicellulose in POME, during oil extraction process, addition of xylanase to directly hydrolyse the hemicellulose to sugars was anticipated to accelerate the hydrolytic reaction that could lead to enhance biogas production.

The objectives of this study are to determine the optimum temperature and hydrolysis time for the pretreatment of POME by xylanase and the effect of enzyme concentrations on biogas production from POME.

Materials and methods

Seed sludge and inoculum preparation for biogas production

Seed sludge was collected from covered lagoon of biogas production system at the receiving pond at Southern Palm (1978) Co., Ltd. in Surat Thani Province, Southern Thailand. An inoculum for biogas production was prepared by mixing the seed sludge with POME in the volume ratio of 1:1 and acclimatized by adding POME (in the same ratio) every day for 5 days at 60 °C incubation. After the biogas production decreased and sedimentation appeared in the reactor, the clear supernatant was decanted. POME was then added into the seed sludge in the same volume ratio, giving the total volume of 2.5 L in a 4 L reactor. The fermentation was conducted for 4 days prior to use as the inoculum. The prepared inoculum contained 119.60 g/L total solid (TS), 39.98 g/L volatile solids (VS) and 3.98 g/L volatile suspended solids (VSS).

Optimum temperature and hydrolysis time for enzymatic pretreatment of POME

POME was taken from Southern Palm (1978) Co., Ltd. in Southern Thailand. Commercial xylanase from *Trichoderma viride* (3.34 unit/mg) (Sigma–Aldrich) was used as source of enzyme in this study.

In order to optimize the operational temperature and time for the enzymatic pretreatment of POME to achieve the highest reducing sugars, the experiments were conducted in a 500-mL-Erlenmeyer flasks containing 100 mL of non-sterilized POME. It was added with xylanase at the final concentration of 5 unit/mL, while without xylanase was used as a control. All of them were incubated at 30, 40, 50 and 60 °C for 30 h. Samples were taken every 6 h for measuring pH and reducing sugars (glucose and xylose) concentration.

Two-steps thermophilic process for biogas production from POME

Two-steps thermophilic process were consisted of (1) enzymatic pretreatment of POME with the various xylanase concentrations of 0, 5, 10, 15 and 20 unit/mL under the optimum temperature and hydrolysis time and (2) biogas production from the POME hydrolysate. In the second step, the inoculum (20% v/v) was added into each POME hydrolysate samples (total volume of 40 mL) in 120 mL glass serum bottles for biogas production. Next, the gas mixture of N_2:CO_2 (80%/20% v/v) was flushed into the tested serum bottles for 2–3 min to guarantee anaerobic condition, then, the bottles were closed with butyl stoppers and incubated at 60 °C for 45 days. Biogas production was determined by using the water replacement method [21] every 24 h in the first 7 days and every 72 h thereafter.

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