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UASB performance and microbial adaptation during a transition from mesophilic to thermophilic treatment of palm oil mill effluent

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ABSTRACT

The treatment of palm oil mill effluent (POME) by an upflow anaerobic sludge bed (UASB) at organic loading rates (OLR) between 2.2 and 9.5 g COD l^{-1} day^{-1} was achieved by acclimatizing the mesophilic (37 °C) microbial seed to the thermophilic temperature (57 °C) by a series of stepwise temperature shifts. The UASB produced up to 13.2 l biogas d^{-1} with methane content on an average of 76%. The COD removal efficiency ranged between 76 and 86%. Microbial diversity of granules from the UASB reactor was also investigated. The PCR-based DGGE analysis showed that the bacterial population profiles significantly changed with the temperature transition from mesophilic to thermophilic conditions. In addition, the results suggested that even though the thermophilic temperature of 57 °C was suitable for a number of hydrolytic, acidogenic and acetogenic bacteria, it may not be suitable for some *Methanosaeta* species acclimatized from 37 °C. Specifically, the bands associated with *Methanosaeta thermophila* PT and *Methanosaeta harundinacea* can be detected during the four consecutive operation phases of 37 °C, 42 °C, 47 °C and 52 °C, but their corresponding bands were found to fade out at 57 °C. The DGGE analysis predicted that the temperature transition can result in significant methanogenic biomass washout at 57 °C.

1. Introduction

A palm oil milling process generates significant amounts of oily wastewater. Palm oil mill effluent (POME) is regarded as a highly polluting wastewater. It has high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) and contains a wide-range of biological substances from complex biopolymers such as proteins, starches and hemicelluloses to simple sugars and amino-acids. POME may also contain dissolved oil and fatty acids, glycerin, crude oil solids and short fibers as well as soluble materials that are harmful to the environment. POME is usually discharged at temperatures around 80–90 °C. The high discharge temperatures mean that both mesophilic and thermophilic treatments are possible (Quarmby and Forster, 1995; Mustapha et al., 2003; Najafpour et al., 2006; Zhang et al., 2008).

It has been shown that operating temperature is a major factor that greatly influences digester performance (Yu et al., 2002; Choorit and Wisarnwan, 2007; Poh and Chong, 2009). Thermophilic operation of anaerobic reactors has been reported to provide some advantages over mesophilic operation in areas such as higher rates of substrate degradation and biogas production. However, it has also been reported that mesophilic reactors can be preferable because of greater process stability (Quarmby and Forster, 1995; Ugoji, 1997; Mustapha et al., 2003; Poh and Chong, 2009).

The upflow anaerobic sludge bed (UASB) process enables anaerobic degradation of organic matter in wastewater and subsequent solid–liquid–gas separation to occur in a single reactor (Lettinga et al., 1983). A UASB reactor is able to treat wastewater with a high suspended solid content. This type of reactor also provides high production of methane (Beccari et al., 1996). There are a few studies on the effects of operational temperature on UASB performances (Dinsdale et al., 1997; Yu et al., 2002; Ryan

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et al., 2010) and it has been known that the key microbial populations which significantly accelerate anaerobic granule development are acetogenic bacteria and *Methanosaeta* sp. (El-Mamouni et al., 1997). However, there appear to be few studies reported in the literature of the detailed mechanism for the microbial adaptation process from mesophilic to thermophilic conditions under UASB operations and the effects of temperature on the diversity of acetogenic bacteria and methanogenic archaea.

In this study, results are given of studies on microbial adaptation during the transition from mesophilic (37 °C) to thermophilic conditions (57 °C) of a 5-L UASB treating POME. Polymerase chain reaction (PCR)-based denaturing gradient gel electrophoresis (DGGE) was employed to determine the diversity of bacteria and methanogenic population in granules at different temperatures of 37 °C, 42 °C, 47 °C, 52 °C and 57 °C. The effects of the operational temperatures on the reactor performance and the granular microbial consortium are discussed.

2. Materials and methods

2.1. Sludge suspension

The sludge used as a source of the biological inoculum was an anaerobic sludge obtained from Ngaung-Khaem water quality control plant (Bangkok, Thailand). This is a domestic wastewater treatment plant operated under mesophilic conditions. The sludge was screened with a sieve of 500 μm (mesh no. 35) to remove material that would not be easily biodegraded. The sludge had a volatile suspension solid (VSS) concentration of approximately 20.6 g VSS l^{-1}.

2.2. Wastewater feed

The POME wastewater was obtained from Suksomboon Palm Oil Co., Ltd. (Chonburi province, Thailand). The raw POME was collected from the wastewater collection tank of the factory. The POME was then filtered through two-layer cheesecloth to remove dirt, plant cell debris, fibers and other solids whose size was in the order of millimeters. The POME was then allowed to stand overnight to allow precipitation. The supernatant portion of the POME was then separated. It was then pre-treated to remove oil and grease by adding aluminum sulfate to a final concentration of 25 ppm. The suspension was rapidly mixed at 150 rpm for 5 min and then slowly mixed at 30 rpm for 30 min. The wastewater was then allowed to settle for 6 h and the supernatant was separated and stored in a cold room at 4 °C. The characteristics of the POME before and after the pre-treatment are shown in Table 1.

In preparing the substrate for UASB operation, the pre-treated POME was prepared at five different concentrations by diluting with tap water at the POME: water ratios of 1:10, 2:10, 3:10, 4:10 and 5:10. These five POME samples were treated at operating temperatures of 37, 42, 47, 52 and 57 °C. $NaHCO_3$ was added to the samples to obtain an alkalinity concentration of 500 mg l^{-1} and 6 N NaOH was added to obtain a pH of 7.

Table 1
Characteristics of POME.

Parameter	Raw POME	Settled POME	Chemically-treated POME
pH	4.4–4.6	4.4–4.6	4.3–4.5
Alkalinity (mg $CaCO_3$ l^{-1})	50–150	50–150	50–150
TVFA (mg CH_3COOH l^{-1})	300–500	300–500	300–500
COD $_{(soluble)}$ (mg l^{-1})	55,000–60,000	53,000–55,000	52,000–54,000
COD $_{(total)}$ (mg l^{-1})	80,000–95,000	68,000–83,000	60,000–64,000
TS (mg l^{-1})	58,000–62,000	40,000–50,000	39,000–40,000
SS (mg l^{-1})	30,000–34,000	16,000–21,000	10,000–15,000
Oil and grease (mg l^{-1})	4600–5100	4500–5000	1500–2500

2.3. Reactor system

A cylindrical shape UASB reactor was used with a working volume of 5.3 l. It was 14 cm in diameter, 40 cm in height and had 4 sampling ports located at 4, 14, 24, and 37 cm from the bottom, respectively.

2.4. Start up and acclimation

Sludge with an initial volatile suspended solid (VSS) concentration of 12 g VSS l^{-1} was inoculated into the reactor. The POME substrate prepared by diluting POME with tap water at a ratio of 1:10 (pre-treated POME: tap water) was fed to the reactor with a hydraulic retention time (HRT) of 2.4 days and an upflow velocity of 0.3 m h^{-1}. The effluent from the reactor was recirculated to the reactor with a ratio of influent: effluent of 1:50 in order to maintain an upflow velocity of 0.3 m h^{-1}. The reactor temperature was maintained by circulating hot water through the reactor jacket. The starting temperature was 37 °C. After the COD removal in a reactor was greater than 80% for at least 3 times the HRT, the temperature of the reactor was increased by a step of 5 °C–42 °C. Simultaneously, the OLR of the reactor was increased by feeding the 2:10 diluted substrate. The process was then repeated for temperatures of 47 °C, 52 °C and 57 °C and feeds of 3:10, 4:10 and 5:10, respectively. Therefore, there were five steps of simultaneous temperature and OLR increase. The average OLR in each step was 2.23, 3.95, 5.76, 7.77 and 9.47 g COD l^{-1} day^{-1}, respectively.

2.5. Analytical methods

The influent and effluent samples from the UASB reactor were collected every 3 days for analyses of COD, alkalinity, volatile fatty acids (VFA) and VSS according to the Standard Methods (APHA, 1998). Gas composition was determined by a gas chromatograph (GC) equipped with a thermal conductivity detector (GC-2014; SHIMADZU, Japan), a stainless steel packed column and a helium carrier gas with a flow rate of 50 ml min^{-1}. The amount of biogas generated was recorded using liquid displacement gas meters and the pH value was measured every day. In order to measure the VFA in the sample drawn from the reactor, it was first necessary to stop any changes in the VFA concentration that could occur after withdrawal of the sample from the reactor. 100 ml of the sample from the reactor were placed in a 500-ml distillation flask with 100 ml distilled water, 5 ml concentrated sulfuric acid, and several glass beads. The flask was placed on a hot plate and connected by an adapter tube to a condenser. The samples were then distilled at a rate of approximately 5 ml min^{-1}, and 150 ml of the distillate were collected. The distillate was then titrated by 0.1 N sodium hydroxide until the phenolphthalein endpoint was reached.

2.6. Analysis of microbial community structure

After the COD removal reached approximately 80% after each temperature and OLR increase, granular samples were collected from the sludge bed at a fixed position of the UASB. All samples were collected in duplicate. Total genomic DNA was extracted from the microbial samples according to the method of Zhou et al. (1996). The bacterial 16S rRNA genes were amplified by Polymerase