Sugarcane molasses-based bio-ethanol wastewater treatment by two-phase multi-staged up-flow anaerobic sludge blanket (UASB) combination with up-flow UASB and down-flow hanging sponge

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ABSTRACT

This study was designed to evaluate a treatment system for high strength wastewater (vinasse) from a sugarcane molasses-based bio-ethanol plant in Thailand. A laboratory-scale two-phase treatment system composed of a sulfate reducing (SR) tank and multi-staged up-flow anaerobic sludge blanket (MS-UASB) reactor was used as the pre-treatment unit. Conventional UASB and down-flow hanging sponge (DHS) reactors were used as the post-treatment unit. The treatment system was operated for 300 days under ambient temperature conditions (24.6–29.6 °C). The hydraulic retention time (HRT) in each unit was kept at 25 h for the two-phase system and 23 h for the UASB&DHS. The influent concentration was allowed to reach up to 15,000 mg chemical oxygen demand (COD)/L. COD removal efficiency (based on influent COD) of the two-phase MS-UASB and the UASB&DHS was 54.9 and 18.7%, respectively. Due to the effective removal of sulfate in the SR tank, the MS-UASB achieved a high methane conversion ratio of up to 97%. In DHS, nitrification occurred at the outside portion of the sponge media while denitrification occurred at the inside. Consequently, 27% of the total nitrogen (TN) was removed. An amount of 32% of residual nitrogen (28 mgN/L) was in the form of nitrate, a better nitrogen state for fertilizer.

INTRODUCTION

Subsequent to encouragement from the government of Thailand in recent years to use the bio-ethanol substitute of methyl tert-butyl ether and gasoline, production of bio-ethanol in Thailand reached 84.2 million litres per month in February 2013 (Bank of Thailand 2013). Sugarcane molasses is used as a main feedstock for bio-ethanol production in Thailand. For every 1 L of sugarcane molasses bio-ethanol production, 7–9 L of wastewater is discharged. Vinasse, wastewater from a sugarcane molasses bio-ethanol distillation plant, has a high organic strength (chemical oxygen demand (COD) 65–130 g/L), high nutrients and a dark brown color (Satyawali & Balakrishnan 2008). Moreover, it contains many inhibitors of methanogens, such as potassium (K⁺), sodium (Na⁺), sulfate (SO₄²⁻), it reduced to sulfide) and chloride (Cl⁻). These concentrations vary according to the production process of each distillery (Mohana et al. 2009). The vinasse of our focus contained especially high SO₄²⁻/C₀, around 1,600–5,000 mg/L, and this SO₄²⁻ is considered to be more serious than the other methanogenic inhibitors (Chen et al. 2008). For this reason, there has been limited success in the application of high-rate anaerobic treatment, which is able to efficiently recover methane from vinasse (Wilkie et al. 2000). For instance, the application of a laboratory-scale thermophilic up-flow anaerobic sludge blanket (UASB) reactor for Thai sugarcane molasses-based vinasse treatment achieved only 39–67% COD removal. With 83% of methane recovery based on removed COD, this is equivalent to 0.29 Nm³-CH₄/kg-COD removed (Harada et al. 1996).
However, from the environmental perspective and taking into account renewable energy concerns, high-rate anaerobic treatment followed by aerobic post treatment is required, especially for bio-ethanol plants situated in high cost land areas.

Our proposed treatment system takes advantage of the use of a high rate system to reach both efficient methane recovery and efficient removal of pollutants from vinasse under ambient temperatures in a tropical region. The process performance of the proposed system was investigated during 300 days of operation in the context of treating vinasse from bio-ethanol plants in Thailand at ambient temperatures.

**MATERIALS AND METHODS**

Figure 1 shows a diagram of the laboratory-scale treatment system used. The total treatment system was comprised of the pre-treatment unit (two-phase MS-UASB) and post-treatment unit (UASB&DHS (down-flow hanging sponge)). These units were connected in series.

A two-phase anaerobic reactor was installed as the pre-treatment unit to increase both the organic loading rate (OLR) and methane recovery by sulfate reduction (sulfide production/removal) and by acidification in the first phase (Wei et al. 2007). Therefore, the pre-treatment two-phase unit consisted of a sulfate reducing (SR) tank and multi-staged UASB reactor (MS-UASB, sometimes referred to as an up-flow staged sludge bed (USSB)) (van Lier et al. 1994; Yamada et al. 2006). The MS-UASB, as a staged concept reactor equipped with several gas solid separators (GSSs) along the reactor height reduced high up-flow velocity caused by vigorous biogas production when applied to the high organic concentration of the wastewater, resulting in good sludge retention and high process performance at a high OLR (Kucivilize et al. 2005).

In summary, the pre-treatment unit consisted of a UASB type SR (1.2 m in height, 10 cm in internal diameter, 8.5 L of liquid volume) and MS-UASB (1.0 m in height, 10 cm in width, 20 cm in length, 12.7 L of liquid volume), with three GSSs installed along the height of the column.

The UASB combination with DHS post treatment unit (Machdar et al. 2000) was proposed in order to further treatments of both residual organic compounds and nitrogen. DHS is an aerobic trickling filter with the polyurethane sponge as media for sludge attachment and is designed to use in combination with UASB. The attractive advantages of DHS are that no aeration is required, construction is simple, a small land area is required, and it has low operation and maintenance costs.

The post-treatment unit, therefore, consisted of a conventional UASB (14 L) and a curtain type DHS, with a sponge-media volume of 4.1 L (placed in a 36 L box, resulting in a void ratio of 98.4%).

These units were operated at an ambient temperature throughout the experiment. Thermometers were installed in each reactor to monitor the temperature. Biogas

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**Figure 1** Schematic diagram of proposed treatment system.
generated from the MS-UASB and conventional UASB was introduced to a column filled with iron-oxide pellets to remove the H$_2$S. The amount of gas production from the reactor was then measured by a wet-test gas meter (Shingawa WS-1A).

To investigate the physical properties of the retained sludge in the MS-UASB, the mixed liquor volatile suspended solids (MLVSS) concentration and the sludge volume index (SVI) were analyzed. Microbial properties were analyzed regarding methane producing activity under anaerobic conditions at 35°C in a 105 mL serum vial following the method of Syutsubo et al. (1998).

The seed sludge used for the MS-UASB and UASB reactors was granular sludge obtained from a brewery wastewater treatment process. The concentration of granular sludge was 64 gVSS/L. The MS-UASB was seeded with 7 L and the UASB with 4.7 L. There was no seeding for the start-up of the SR and DHS reactors.

Raw vinasse obtained from a sugarcane molasses-based bio-ethanol plant in northeastern Thailand and diluted with tap water to the desirable concentration was used as influent into the pre-treatment unit. The characteristics of the raw vinasse used are shown in Table 1. In order to supply appropriate alkalinity (adjust the pH), sodium bicarbonate (NaHCO$_3$) was added to the influent wastewater. Neither supplementation of trace minerals nor adjustment of temperatures was performed on the wastewater. The effluent from the pre-treatment system was used as the influent for the post-treatment unit. In the post-treatment unit, effluent from the UASB was fed by gravity to the DHS.

Operational conditions were separated into a start-up period and a high strength influent acclimation period. Hydraulic retention time (HRT) was kept constant for 10 h for the SR, 15 h for the MS-UASB (calculated without recirculation flow rate), 19.4 h for the UASB and 5.5 h for the DHS, with flow rate control at 20 L/d. The inner recirculation ratio of the MS-UASB and UASB was kept constant at 1:1 (influent: recirculation) to achieve the up-flow velocity of more than 1 m/h. OLR was increased by increasing the influent COD concentration, with dilution factors from 100 to 7.5.

The pH and temperature of both influent and effluent wastewater were measured daily. The following parameters of the wastewater samples were analyzed twice a week following the approach set forth in Standard Methods 18th edition (1992): COD, suspended solids (SS), SO$_4^{2-}$, volatile fatty acids (VFA), total nitrogen (TN), total Kjeldahl nitrogen (TKN), ammonium (NH$_4^+$) and nitrate (NO$_3^-$). Gas composition was analyzed with a gas chromatograph, Shimadzu GC-2014 with a TCD detector.

### RESULTS AND DISCUSSION

Figure 2(a) shows the time course of influent and effluent COD concentrations of each reactor. During the start-up period (days 0–180) the OLR increased to sufficient levels within 53 days and was kept constant for 127 days to evaluate performance (25.2 kgCOD/m$^3$.d in the SR, 12.7 kgCOD/m$^3$.d in the MS-UASB, 5.8 kgCOD/m$^3$.d in the UASB and 13.8 kgCOD/m$^3$. sponge-d in the DHS). The time course of operated temperatures is shown in Figure 2(b). The stability of treatment efficiency was temperature dependent. The fluctuation of temperatures caused by seasonal weather changes, with a decreasing tendency effect after day 90, resulted in a higher VFA concentration in the MS-UASB effluent and a slightly lower treatment efficiency. Total COD removal efficiency based on total influent COD at the inlet during days 53–180 averaged 23.0% at the SR, 37.7% at the MS-UASB, 10.6% at the UASB and 5.8% at the DHS, or 77.1% for the whole system.

Due to the installation of the GSSs along the reactor height, the MS-UASB was advantageous in reducing hydrogen partial pressure by exhausting biogas at the generating point (Yamada et al. 2006). This advantage resulted in sustaining a low concentration of VFA (such as propionate) in the effluent, as shown in Figure 2(c). The effluent contained small amounts of VFAs, on average 85 mgCOD/L of acetate, 164 mgCOD/L of propionate and 55 mgCOD/L of butyric acid.

During the stable state of maximum OLR in the start-up period (days 53–80), the SR almost completely reduced

![Table 1: Characteristics of raw vinasse used](https://iwaponline.com/wst/article-pdf/69/6/1174/472307/1174.pdf)
the 298 mgSO$_4^{2-}$/L contained in the wastewater (COD/SO$_4^{2-}$ at 40.5), as shown in Figure 2(d). Therefore, a suitable condition for methane production was secured in the MS-UASB. It was also assured by the superior value of methane conversion in the MS-UASB, which had a ratio of 96% compared to 83% in the SR (Figure 3). The remarkable advantages of sulfide removal by a two-phase process using a staged reactor concept for treatment of molasses wastewater were also affirmed by Onodera et al. (2011). The phenomenon of sulfide removal in our proposed system is elucidated in the following manner: under low pH conditions (6.0–6.3) in the SR reactor, sulfate was reduced to sulfide, and then stripping of hydrogen sulfide from the wastewater occurred by biogas production. The remaining sulfur (sulfide) was effectively removed as biogas under neutral pH conditions via the GSSs installed in the MS-UASB compartmentalized reactor. In this experiment, influent pH was kept at approximately 6.0 by the addition of NaHCO$_3$. According to the results, the SR effluent pH was an average of 7.2 and the MS-UASB effluent pH was an average of 7.6. Regarding addition of an alkalinity source such as NaHCO$_3$ in a real plant, this would increase operational cost. Therefore, measures such as effluent recirculation, CO$_2$ scrubbing or a gas recycle system are considered reasonable measures for alkalinity supplementation in
the context of cost minimization (Speece 1996; van Lier 2008; Syutsubo et al. 2013).

The SR reactor was started up without seeding sludge. At the sludge bed, granulation of dark yellow, brown, fluffy sludge with a SVI of 20 mL/gVSS was found. After 300 days of operation, the sludge concentration in the SR increased to 15.6 gVSS/L-reactor.

To investigate the sludge characteristics of the MS-UASB, MLVSS and methane producing activity of the retained sludge were measured on day 282 (Table 2). According to the results, a highly retained sludge concentration in the MS-UASB was confirmed with an MLVSS concentration of 61.3 gVSS/L-reactor. Also, retained sludge possessed good settleability (SVI 12.2 mL/gVSS). The multi-number GSSs in the MS-UASB caused sufficient retention of the granular sludge by decreasing biogas flux in the column. In addition, levels of methane producing activity of the retained sludge were 0.37 gCOD/gVSS-d for acetate and 0.32 gCOD/gVSS-d for H₂/CO₂. Consequently, good retention of the granular sludge with sufficient levels of methane producing activity is cause for the superior performance of the MS-UASB in vinasse treatment.

The effluent from the MS-UASB was fed to the UASB in the post treatment unit for further organic treatment and methane recovery. With a low BOD/COD ratio of 0.11 in the UASB influent, COD removal efficiency and the methane conversion ratio of the UASB were only 29% and 55%, respectively. Without aeration, dissolved oxygen (DO) in the effluent of aerobic DHS increased to 4.0 mg/L under average water temperatures of 26.0 °C. Consequently, organic compounds were further removed, with 22% COD in the received wastewater (effluent from the UASB), which had a lower BOD/COD ratio of 0.07.

After day 180, influent concentration was gradually increased to assess maximum OLR. Accordingly, the influent COD for stable operation was determined as 15,000 mg/L. Consequently, the maximum OLR of each reactor was on average 38.2 kgCOD/m³-d at the SR, 19.1 kgCOD/m³-d at the MS-UASB, 20.8 kgCOD/m³-d at the UASB and 7.4 kgCOD/m³ sponge-d at the DHS (OLR was calculated based on the inlet COD of each reactor). At the maximum OLR, COD removal (based on influent COD) of the two-phase MS-UASB and the UASB&DHS was 54.9% and 18.7%, respectively. Proportions of 22.4, 32.5, 13.5 and 5.2% of input COD were removed at the SR, MS-UASB, UASB and DHS, respectively.

The DHS was originally used as post treatment for sewage after the UASB reactor, with an OLR of 2.35 kgCOD/m³ sponge-d (Tandukar et al. 2006). Tawfik et al. (2011) recommended an optimal OLR for DHS, as applied for grey wastewater treatment under 3.6 kgCOD/m³ sponge-d. In this study, the BOD/COD ratio of the UASB effluent (DHS influent) was only 0.06. Therefore, a comparatively high OLR of up to 7.4 kgCOD/m³ sponge-d was allocated in the DHS.

Although the proposed system showed good performance with a comparatively high OLR and high methane recovery, there was the limitation of a maximum influent concentration of only 15 g COD/L, under a short HRT of 47.9 h.

Table 2 | Characteristics of retained sludge in the MS-UASB, day 282

<table>
<thead>
<tr>
<th>MLVSS (gVSS/L-reactor)</th>
<th>SVI (mL/gVSS)</th>
<th>Methane producing activity at 35 °C (gCOD/gVSS-d)</th>
<th>H₂/CO₂</th>
<th>Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>61.3</td>
<td>12.2</td>
<td>0.32</td>
<td>0.37</td>
<td></td>
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</tbody>
</table>

Figure 3 | Relationship between COD removal rate and methane production rate in the (a) SR, (b) MS-UASB.
for the whole system. The limitation may have been caused by a variety of factors: e.g. temperature fluctuation; cation inhibition, especially by the potassium ion (Onodera et al. 2013); or phenolic compounds present in vinasse (Harada et al. 1996).

In the DHS, TKN (mainly NH$_4^+$) was oxidized to NO$_3^-$ form in the aerobic condition. The occurrence of denitrification (nitrogen removal) was also confirmed. The oxygen concentration in the DHS sponge media varied according to the zone (portion). The outside portion of the sponge was the aerobic zone, where nitrification occurred. In contrast, the inside of the sponge included the anoxic zone, where denitrification occurred (Chuang et al. 2007). Concentrations of TN, TKN, NH$_4^+$ and NO$_3^-$ in the DHS were analyzed and are shown in Figure 4. With a high BOD:N ratio of 100:31 in the DHS, 27% of TN, 45% of TKN and 85% of NH$_4^+$ were removed. Also, in the effluent of the DHS, 28 mg/L-N of NO$_3^-$ was detected. Reuse of wastewater treated by our proposed system as a fertilizer and/or as irrigation water for sugarcane was also investigated. Reuse for these purposes would result in low risk of greenhouse gas emissions from the field soil and would have a beneficial influence on sugarcane growth (Syutsubo et al. 2013).

**CONCLUSIONS**

For 300 days of continuous operation at ambient temperatures in Thailand, a two-phase MS-UASB in combination with UASB&DHS achieved the maximum available OLR of 38.2 kgCOD/m$^3$-d in the SR, 19.1 kgCOD/m$^3$-d in the MS-UASB, 20.8 kgCOD/m$^3$-d in the UASB and 7.4 kgCOD/m$^3$ sponge-d in the DHS, calculated based on COD concentration at the inlet of each reactor, with removal of 74% COD by the whole system. The characteristics of wastewater treatment (organic and nitrogen removal) at each unit were revealed as follows. The SR reduced SO$_4^{2-}$ completely, the MS-UASB converted COD to methane with 96% efficiency, and the UASB&DHS removed residual organic compounds of influent COD by 18.7%. In addition, the DHS performed nitrogen removal (27% TN removal, 45% TKN removal) and oxidized the Kjeldahl nitrogen into nitrate, which is better for liquid fertilizer.

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**REFERENCES**


