Anaerobic Treatability of Cane Molasses Based Bio-Ethanol Distillery Wastewater Mixed with Cane Sugar Mill Wastewater by Two Phase Multi-Stage Upflow Anaerobic Sludge Blanket Reactor

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Abstract: In a period of 1 year, the volumetric ratio of wastewater from a cane molasses-based bio-ethanol distillery plant (CMBP) and a cane sugar mill (CSM) was monitored and shown to be 1:2. In order to evaluate the validity of mixing the two wastewaters, their biodegradability and methanogenic activity (MA) were measured under a fixed temperature of 35℃ and fixed FM ratio of 0.5 (i.e., 2 g COD/l for test substrate per 4 g VSS/l for test sludge). The results showed that the mixed wastewater was 4 % higher in biodegradability and 1.2 times higher in MA when compared with pure CMBP wastewater. Based on 100% COD input, 46% COD from the mixed wastewater was converted to methane. The MA of the mixed wastewater was 0.0006 g COD-CH_4/gVSS.d. Additionally, a lab scale, 2-phase, multi-stage up-flow anaerobic sludge blanket (MS-UASB) reactor was operated at room temperature (22 to 32°C) and fed with CMBP wastewater and with mixed wastewater to investigate anaerobic treatability. Influent COD concentration and hydraulic retention time (HRT) were fixed at 15 g/l and 36 hours, respectively. The treatment process consisted of a conventional UASB reactor with a liquid volume of 24 l for the first phase of sulfate reduction, in addition to acidification by keeping influent pH at 6.0, followed by an MS-UASB reactor with a liquid volume of 12.7 l. Feeding of the wastewater mixture resulted in a sufficient level of COD removal, with an efficiency of 66.8%, higher than CMBP wastewater treatment by 10.6%.

Keywords: Anaerobic treatment, bio-ethanol distillery wastewater, cane molasses based, multi-stage UASB, 2-phase UASB.

1. Introduction

Bio-ethanol distillery plants are increasing year by year due to demands for renewable energy. In November 2014, the capacity of bio-ethanol production in Thailand was about 3.4 Ml/day. In addition, under Thailand's Alternative Energy Development Plan (AEDP), Thailand's Ministry of Energy set a target for increasing ethanol production volume to 9 million l/day within 10 years (2012-2021). However, the distillation process generates a large amount of high strength wastewater, which has a very high biochemical oxygen demand (BOD) of up to 40,000- 70,000mg/l and a chemical oxygen demand (COD) of up to 100,000- 150,000 mg/l [1]. Thus an appropriate wastewater management and treatment system is very much required for bioethanol plants. A biological treatment process, such as the Upflow Anaerobic Sludge Blanket (UASB) reactor, is the most popular system selected for practical sugarcane molasses-based bio-ethanol distillery plant wastewater (CMBP-WW) treatment, in spite of problems with low treatment efficiency (lower than 60% COD removal) and low methane recovery ratio (lower than 80% based on removed COD) [2]. In addition, attempting to increase treatment efficiency and methane recovery ratio by using multiple stages, i.e. the Multi-stage UASB (MS-UASB) or the Upflow Staged Sludge Bed reactor (USSB) [3][4], has been tested, but it was found that there was a limitation from inhibitants when high strength influent was fed. The expected dilution requirement for successful treatment of CMBP-WW by UASB is more than 10 times to decrease COD concentration to the range of 15,000 mg/l. In real situations, however, it is very difficult to supply sufficient dilution water for system operation.

To solve this problem, this study proposes using cane sugar mill wastewater (CSM-WW) as a dilution water for anaerobic treatment of CMBP-WW, since most cane sugar factories and sugarcane molasses alcohol distilleries are located in the same area or nearby each other. Within this context, discharged CMBP-WW and CSM-WW from factories in Chaiyaphum Province in Northeastern Thailand were collected. Then the validity of the wastewater mixture was evaluated with a mixing ratio according to the volume ratio found of the actual discharge, using serum-vial tests and continuous feeding into a 2-phase MS-UASB reactor.

2. Experimental

2.1 Discharged volume and wastewater characteristics

Wastewater was received from a CMBP and CSM in Chaiyaphum Province in Northeastern Thailand. Data regarding the discharged volume of CMBP-WW and CSM-WW for each month during a yearlong span from 2010 to 2011 was obtained from the factories in Northeastern Thailand. Discharged volume of CMBP-WW and CSM-WW depended on seasonal production, as shown in Table 1. The discharged volume per year of CMBP-WW and CSM-WW was 272,680 m^3 and 570,318 m^3, respectively. It can be summarized that the ratio discharge volume of CMBP-WW and CSM-WW was 1:2. Consequently, the mixed wastewater (Mixed-WW) refers to the raw wastewater of CMBP-WW and

Table 1. Discharged volume of wastewater from the CMBP and CSM in a yearlong span from 2010 to 2011 (unit in m^3)

Year	2010					2011						1 year
Month	Jul	Aug	Sep	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	
CMBP	34,364	30,231	23,461	16,093	0	26,585	26,519	27,428	26,144	30,112	22,865	272,680
CSM	40,129	40,066	41,767	43,062	40,278	52,675	47,362	62,981	51,382	67,198	56,244	570,318

CSM-WW mixed at the volumetric ratio of 1:2 before dilution using tap water into the desired influent concentration for the experiment. The characteristics of each raw unmixed wastewater and the raw mixed wastewater were analyzed, as shown in Table 2.

2.2 Methanogenic Activity Test and Biodegradability Test

Methanogenic activity of retained sludge and anaerobic biodegradability of wastewater were determined using the serum vial-bottle technique [5]. The test temperature was controlled at 35°C. The F/M ratio was set at the same ratio concentration in each test vial, specifically at 2,000 mg COD/l for test substrates and 4,000 mg VSS/l for test sludge. The test sludge was sludge retained from the MS-UASB on day 15, during the start-up period. The MS-UASB reactor was fed with CMBP-WW at an organic loading rate of 10 kg COD/m^3.d with the VSS/SS ratio at 0.94.

For measurement of methanogenic activity, specific substrates H_2/CO_2 and acetate were used. For biodegradability evaluation, sucrose (the control substrate), CMBP-WW, CSM-WW and Mixed-WW (a mixture of CMBP-WW and CSM-WW at the volume ratio of 1:2) were used as the carbon source. Trace elements were added at the following concentrations (in mg/l): 2.0 $FeCl_2 \cdot 4H_2O$, 0.17 $CoCl_2 \cdot 6H_2O$, 0.07 $ZnCl_2$, 0.06 H_3BO_3, 0.50 $MnCl_2 \cdot 2H_2O$, 0.04 $NiCl_2 \cdot 6H_2O$, 0.027 $CuCl_2 \cdot 2H_2O$, 0.025 $NaMoO_4 \cdot 2H_2O$ and 5.0 EDTA. The concentration of the medium in each test vial (in mg/l) was 1.0 $MgCl_2 \cdot 6H_2O$, 0.375 $CaCl_2 \cdot 2H_2O$, 1.25 NH_4Cl, 2.18 K_2HPO_4 and 1.70 KH_2PO_4.

2.3 2-phase MS-UASB set-up

Figure 1 illustrates a schematic diagram of the multiphase system, which consisted of a conventional UASB type reactor called a sulfate reducing (SR) reactor and a multi-stage UASB (MS-UASB) reactor. Influent was mixed and pH adjusted every day, and it was stored in the equalization tank (EQ) tank. Influent was fed from the EQ tank to the SR reactor, and the effluent from the SR reactor was fed into the MS-UASB by peristaltic pumps. The SR reactor and the MS-UASB reactor were made from polyvinylchloride (PVC). The SR reactor had a total volume of 30 l, was 20 cm in length, 15 cm in width and 100 cm in height and had a liquid volume of 24 l. The MS-UASB reactor had a total volume of 16 l, a length of 20 cm, width of 10 cm and height of 80 cm and a liquid volume of 12.7 l. Three gas solid separators (GSSs) were installed along the reactor height. Thermometers were installed in each reactor to monitor the temperature. Biogas released from the SR-reactor and the MS-UASB reactor was collected and hydrogen sulfide (H_2S) removed using ferric pellets before it was directed into the wet test gas meter (Shingawa, WS-1A).

2.4 Seed sludge for 2-phase MS-UASB

The SR reactor and MS-UASB reactor were seeded with granular sludge from the UASB reactor of a brewery in Khon Kaen Province. The seed sludge had a concentration of 64 g/l for MLSS and 61 g/l for MLVSS. The total amount of sludge added to the SR reactor was 9.6 l, which corresponds to 614.4 g for MLSS and 580.8 g for MLVSS, and the total amount of sludge added to the MS-UASB reactor was 4.8 l, corresponding to 307.2 g for MLSS and 290.4 g for MLVSS.

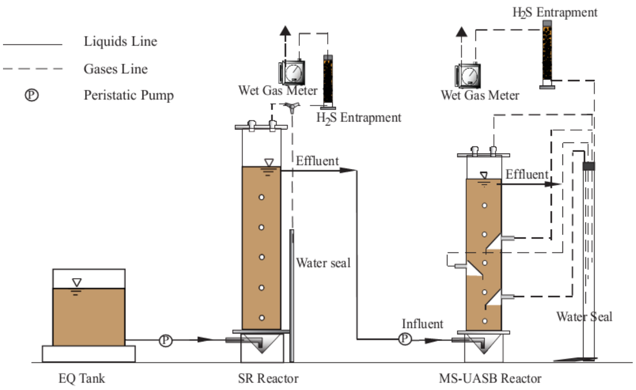

Figure 1. Experimental set-up of the wastewater treatment system.

2.5 Start-up for 2-phase MS-UASB

Start-up of the wastewater treatment system was done by feeding of diluted CMBP-WW to each reactor separately during days 0-25. After day 25 (days 26-40), the 2-phase MS-UASB was connected as shown in Figure 1. The CMBP-WW was diluted using tap water to the desired different concentrations for each reactor, as shown in Table 3. Flow rate was fixed for 40 days during the start-up period at 24 l/d for both reactors. Using an addition of $NaHCO_3$ into the influent at the concentration of 1,250 mg/l for the SR and 1,500 mg/l for the MS-UASB during days 0-25, the pH was controlled to create appropriate conditions for sulfate reducing bacteria at the SR reactor (around 6.0) and for methane producing bacteria at the MS-UASB (around 7.0). After day 25, pH was controlled by an addition of $NaHCO_3$ in the EQ tank at the concentration of 1,250 mg/l.

2.6 System operation during the experimental period for 2-phase MS-UASB

To find out the difference in performance between the treatment systems, one using diluted CMBP-WW (single wastewater) and the other Mixed-WW (composed of CMBP-

Table 2. Characteristics of raw CMBP, CSM and mixed wastewater.

Parameters	CMBP-WW	CSM-WW	Mixed-WW
pH	4.2±0.19	3.8±0.09	4.0±0.16
COD (mg/l)	300,000±60,000	15,900±920	158,000±0
SS (mg/l)	62,000±20,000	825±21.2	5,800±2,000
VSS (mg/l)	6,300±3,300	90±10	1,380±360
SO_4^{2-} (mg/l)	7,940±135	490±2	3,830±1,110

Table 3. Operating conditions for the start-up period.

Parameter	SR reactor	MS-UASB reactor
Feed wastewater	CMBP-WW	CMBP-WW
Influent COD (days 0-25)	6,800±1,420	3,890±1,090
Influent COD (days 26-40)	10,500±1,300	7,250±790
pH	5.9±0.3	7.0±0.1
Flow rate (l/d)	24	24
Hydraulic retention time (HRT) (h)	24	12

WW and CSM-WW at the mixing ratio of 1:2), the 2-phase MS-UASB was operated under room temperature with a matching influent COD concentration of 15,000 mg/l and constant flow rate of 24 l/d. During the test, CMBP-WW was fed continuously during days 41-85. Then after day 85, influent was changed to Mixed-WW and influent COD concentration was decreased to 8,000 mg/l to accumulate anaerobic sludge before increasing influent COD concentration to its former condition. Finally, Mixed-WW was fed at the same influent COD concentration of 15,000 mg/l and at a flow rate of 24 l/d during days 105-145. Test temperature during days 41-85 and days 105-145 averaged 28.6±1.8°C (in the range of 24.0-32.0°C) and 25.8±1.6°C (in the range of 22.1-28.9°C), respectively. The temperature in the reactor was around 2℃ lower than the room temperature. $NaHCO_3$ was added into the influent at the EQ tank at the concentration of 1,250 mg/l to control pH in the SR reactor during the experiment, with no further addition of $NaHCO_3$ prior to the MS-UASB inlet. The operational conditions of the single wastewater and mixed wastewater at the same influent COD concentrations and flow rates are given in Table 4.

2.7 Analytical parameters for anaerobic treatability by 2-phase MS-UASB

Influent from the EQ tank and effluent from the SR reactor and the MS-UASB reactor were sampled twice a week for analysis. The parameters of COD, SS, VSS and sulfate were analyzed following Standard methods (1998) [6]. Sulfate content was determined using a HACH water quality analyzer (HACH, SulfaVer® 4). Biogas compositions were analyzed using a thermal conductivity detector (TCD) gas chromatograph (GC-2014, Shimadzu, Unibeads-C 60/80 mesh). Concentrations of volatile fatty acids (VFA) were determined using a flame ionization detector (FID) gas chromatograph (GC-14B, Shimadzu, Thermon 3000 60/80 mesh).

3. Results and Discussion

3.1 Methanogenic activity and biodegradability

Methanogenic activity (MA) and biodegradability tests were done to evaluate the validity of the wastewater mixture before anaerobic treatability test by the 2-phase MS-UASB reactor. Table 5 summarizes the MA in each test substrate. MA from the CSM-WW test substrate showed itself to be 2.3 times higher than that of the CMBP-WW. Consequently, MA from the Mixed-WW test substrate was measured to be 1.2 times higher when compared to MA from the CMBP-WW test substrate. The test sludge was retained sludge on day 15 during the start-up period, resulting in the low MA value.

An anaerobic biodegradability test was carried out after 24 days of the test period. The COD fraction was calculated into percentages based on 100% input COD of test substrate, and the test substrates (sucrose, CMBP-WW, CSM-WW and Mixed-WW) were compared, as shown in Figure 2. The biodegradable COD fraction converted to methane from CSM-WW was high at 77% based on input COD, even higher than from the sucrose test substrate. Conversely, the biodegradable COD fraction from the CMBP-WW was low at 42% based on input COD. The biodegradable COD fraction from the Mixed-WW was 46% based on input COD, an increase of 4% compared to the CMBP-WW, elucidating the validity of the mixed wastewater.

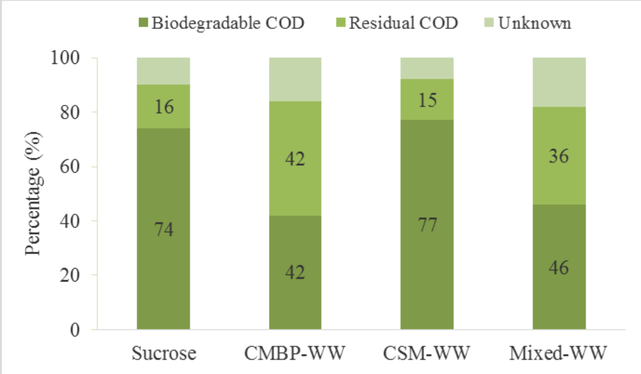

Figure 2. Anaerobic biodegradability of each test substrate.

3.2 Anaerobic treatability by 2-phase MS-UASB

Process performance of the 2-phase MS-UASB under matching operating conditions and with feeding as described in Table 4 was compared with the different wastewaters (CMBP-WW and Mixed-WW). Figure 3 shows the total COD concentration in the influent and effluent, the room temperature and the water temperature in each reactor during the CMBP-WW feeding period (days 41-85, Figure 3 (a)) and during the Mixed-WW feeding period (days 105-145, Figure 3 (c)). Figure 3 (b) and (d) show the COD removal in percentage ratio at each reactor and the COD removal from the overall system during the CMBP-WW feeding period and Mixed-WW feeding period, respectively. Average organic loading rate (OLR) of the SR and the MS-UASB were 15.4 and 16.6 gCOD/l.d, respectively, during the CMBP-WW feeding period and 15.4 and 24.1 gCOD/l.d during the Mixed-WW feeding period. The higher OLR of the MS-UASB during the Mixed-WW feeding period was due to the lower COD removal at the SR reactor. The changes of test temperature affected the fluctuation of COD removal in the system. The COD removal efficiency of the overall system during feeding with Mixed-WW (66.8±15.7 %) was 10.6 % higher than during feeding with CMBP-WW (56.2±7.32 %).

Table 4. Operating conditions of 2-phase MS-UASB reactor during test period

Parameter	CMBP-WW (days 41-85)		Mixed-WW (days 105-145)	
	SR	MS-UASB	SR	MS-UASB
Room temperature (℃)	28.6±1.8 (24.0 - 32.0)		25.8±1.6 (22.1 - 28.9)	
pH	5.9±0.2	6.9±0.3	5.6±0.2	6.0±0.5
Hydraulic Retention Time (h)	24	12	24	12
Flow rate (l/d)	24	24	24	24
Influent COD (mg/l)	15,300±1,570	8,330±780	15,400±2,280	12,400±1,880
Organic Loading Rate (gCOD/l.d)	15.4	16.6	15.4	24.1
$NaHCO_3$ (mg/l)	1,250	-	1,250	-

Table 5. Methanogenic activity of test sludge for each test substrate (unit in gCOD-CH_4/gVSS.d)

Test Substrate	H_2/CO_2	Acetate	Sucrose	CMBP-WW	CSM-WW	Mixed-WW
MA	0.0016	0.0026	0.0013	0.0006	0.0014	0.0007

The reason for the higher COD removal of the 2-phase MS-UASB when feeding with Mixed-WW compared to CMBP-WW at the same COD concentration was assumed to be the lowering of anaerobic inhibitors in the wastewater by the mixture of CSM-WW. There are various kinds of anaerobic inhibitors in CMBP-WW to regulate the anaerobic biodegradability, such as potassium (K^+), sodium (Na^+), sulfate (SO_4^{2-} when reduced to sulfide) and chloride (Cl^-) [7]. The anaerobic inhibitor in CMBP-WW that was considered in this study was sulfate, because the 2-phase MS-UASB system has the advantage of sulfate removal at the SR reactor.

The average sulfate concentration in the SR influent during the CMBP-WW feeding and Mixed-WW feeding periods were 2,490±1,310 $mgSO_4$/l (830±435 mgS/l) and 2,320±778 $mgSO_4$/l (774±260 mgS/l), respectively. The effluent sulfate concentration from the SR reactor was 92.3±15 $mgSO_4$/l (31±5 mgS/l) when feeding with CMBP-WW. This value was higher than when feeding with Mixed-WW (on average 21.3±15.2 $mgSO_4$/l [7±5 mgS/l]). The sulfate removal efficiency at the SR reactor was 84.2±10.4% during the CMBP-WW feeding period and was 98.7±1.7% during the Mixed-WW feeding period. As a result of controlling the pH at the SR reactor to be lower than 6.0, lower COD removal was observed (by suppression of methane production) in the SR reactor while sulfate reduction progressed. Considering the COD/SO_4^{2-} ratio, the theoretical minimum COD/SO_4^{2-} ratio to achieve complete sulfate reduction would be 0.67 [8]. The influent COD/SO_4^{2-} ratio during feeding with single wastewater and mixed wastewater exceeded 6 throughout the experiment. Results show that sulfate was almost completely reduced by the SR reactor in both types of wastewater.

The differentiation of methanogenic activity (MA) of the retained sludge in the MS-UASB reactor during the test period was also assessed using the retained sludge of the MS-UASB reactor. Sludge samples were harvested for testing on days 66 (CMBP-WW feeding period) and 145 (Mixed-WW feeding period). Test substrates were H_2/CO_2, acetate, sucrose, CMBP-WW, CSM-WW and Mixed-WW. The results are summarized in Table 6. MA is given in terms of COD equivalent rate (gCOD-CH_4/gVSS.d). From the results, MA of the retained sludge during feeding with Mixed-WW (day 145) was higher than that of the retained sludge during feeding with CMBP-WW (day 66) in all tested substrates. When comparing the H_2/CO_2 and acetate as test substrates, the MA of the retained sludge during feeding with Mixed-WW was 3.2 times and 11 times higher than the MA of retained sludge during feeding with CMBP-WW, respectively. The results emphasize the benefits in anaerobic treatability when mixing CMBP-WW with CSM-WW, as compared to feeding with CMBP-WW as a single wastewater.

4. Conclusions

A mixing volume ratio between cane sugar mill wastewater (CSM-WW) and cane molasses-based bio-ethanol distillery plant wastewater (CMBP-WW) was found and considered to match the actual discharged volume from each factory at 2:1. With this mixing volume ratio, methanogenic activity (MA) from mixed wastewater (Mixed-WW) as the test substrate was 1.2 times higher and anaerobic biodegradability increased 4% when compared with CMBP-WW as the test substrate under the same FM ratio testing conditions.

The 2-phase multi-stage UASB (MS-UASB) performed COD removal 10.6% more efficiently and sulfate removal was 14.5% higher when feeding with Mixed-WW compared with feeding with CMBP-WW.

The higher methanogenic activity (MA) of retained sludge in the MS-UASB during feeding with Mixed-WW also added to the benefits of anaerobic treatability using Mixed-WW, with MA 3.15 times higher when using H_2/CO_2 as a test substrate and 11 times higher when using acetate as a test substrate.

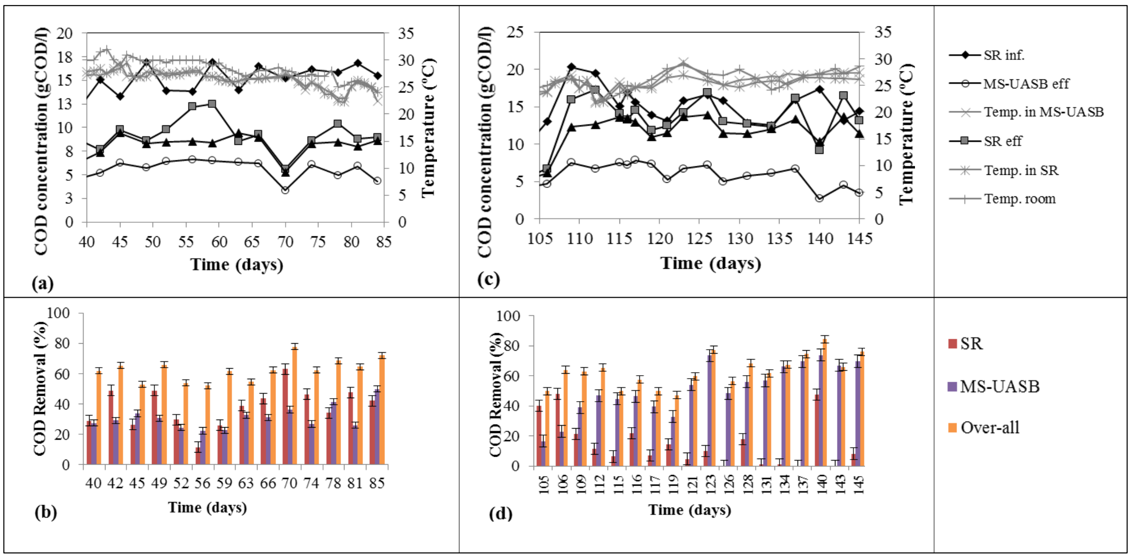

Figure 3. (a) COD and temperature in the treatment system while feeding single wastewater (CMBP-WW), (b) COD removal from the system while feeding single wastewater (CMBP-WW), (c) COD and temperature in the treatment system while feeding mixed wastewater and (d) COD removal from the system while feeding mixed wastewater.

Table 6. Methanogenic activity of retained sludge in 2-phase MS-UASB for each test substrate (unit in gCOD-CH_4/gVSS.d).

Test sludge	Test substrate					
	H_2/CO_2	Acetate	Sucrose	CMBP-WW	CSM-WW	Mixed-WW
CMBP-WW feeding(day 66)	0.030	0.035	0.009	0.024	0.006	0.023
Mixed-WW feeding (day 145)	0.093	0.38	0.25	0.15	0.23	0.24

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