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Evaluation of Process Performance and Sludge Properties of an up-flow staged Sludge Blanket (USSB) reactor for Treatment of Molasses Wastewater

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ABSTRACT: A combination of an acidification reactor and an up-flow staged sludge bed (USSB) reactor was applied to treatment of molasses wastewater. The USSB, equipped with three gas solid separators, was selected because of superior organic removal by means of good retention of granular sludge. The combined system was continuously operated at mesophilic (35°C) conditions for 600 days. The USSB achieved a high organic removal rate of 37 kgCOD/m^3 day (organic loading rate of 43 kgCOD/m^3 day with 86.2% COD removal). The USSB retained high sludge concentration of approximately 58 gVSS/L based on the reactor volume. The retained granular sludge had good settle-ability with a sludge volume index (SVI) of 4 to 20 ml/gSS. The retained sludge possessed a sufficient level of methanogenic activities for acetate (1.2 gCOD/gVSS day) and for hydrogen (1.7 gCOD/gVSS day). Analysis of microbial community revealed that genus *Methanobacterium* as hydrogen utilizing methanogen and order *Methanosarcinales* as acetate utilizing methanogen were detected in the retained sludge of the USSB reactor. The superior performance of the USSB was attributed to good retention of a large amount of granular sludge with high methanogenic activity.

Key words: USSB, Molasses wastewater, Sludge property, Sludge activity, Microbial community structure

INTRODUCTION

Cane molasses is one of the most valuable raw materials for bio-ethanol production of sustainable fuel. Bio-ethanol production using molasses is increasing year by year in developing tropical countries. However, the distillation process generates a considerable amount of high strength wastewater (molasses wastewater). The discharge of molasses wastewater causes serious pollution in aquatic environments and emission of greenhouse gases such as methane. Baruah et al. (1993) reported serious contamination of river water in India from the distillery effluent. Thus an appropriate wastewater treatment system is required for treatment of molasses wastewater. The anaerobic treatment process such as up-flow anaerobic sludge blanket (UASB) may be a suitable option, because it has both low operational cost and high organic removal efficiency (Satyawali and Balakrishnan, 2008; Safari *et al.*, 2011; Akbarpor Toloti and Mehrdadi, 2011; Arshad *et al.*, 2011; Takahashi *et al.*, 2011; Amani *et al.*, 2011; Arshad and Hashim, 2012; Hatamoto *et al.*, 2012; Selvamurugan *et al.*, 2012; Rahman and Al-Malack, 2012; Adl *et al.*, 2012; Mobarak-Qamsari *et al.*, 2012). The granular sludge based technologies, such as UASB and expanded granular sludge bed (EGSB), have dominated the full-scale application for industrial wastewater treatment in the past decades (Van Lier, 2008). However the molasses wastewater contains a

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large amount of organic compounds, sulfate, and mineral salts (Boopathy and Tilche, 1991; Wilkie *et al.*, 2000). These compounds caused the deterioration of anaerobic digestion process by sulfide inhibition (Koster *et al.*, 1986; Lens *et al.*, 1998) and by cation inhibition (Chen *et al.*, 2008). In addition, a high rate treatment of alcohol (whiskey) distillery wastewater by UASB might cause a sludge washout by a vigorous biogas production (Syutsubo *et al.*, 1998). Therefore, an up-flow staged sludge blanket (USSB) reactor equipped with several gas solid separators (GSS) (Van Lier *et al.*, 1994, Onodera *et al.*, 2011) was used in this test study. In order to optimize the anaerobic process for molasses wastewater treatment, accumulation of basic knowledge on sludge retention together with its physical and microbial properties is necessary. With this background, in this study a combination of an acidification reactor and a USSB reactor was used for treatment of molasses wastewater so as to investigate the process performance. Furthermore, physical and microbial properties of retained sludge in terms of sludge concentration, sludge settle-ability, methanogenic activity, and microbial structure were investigated to gain basic knowledge on stable treatment.

MATERIALS & METHODS

Fig. 1 is a schematic diagram of the treatment system. The acidification reactor with a liquid volume of 13.7 L and the USSB reactor with a liquid volume of 13.4 L were continuously operated at 35 °C. The acidification reactor had an agitator operating at 10 rpm. The USSB had three GSS along the reactor height to efficiently remove biogas produced. The operating conditions for the treatment system are shown in Table 1. The molasses was diluted with tap water to prepare synthetic wastewater with the desired influent COD concentration. Sodium bicarbonate was mixed with the influent wastewater to provide additional alkalinity. An anti-foam reagent (KM-70, Shin-Etsu Silicone, Japan) was also added to the influent. Hydraulic retention time (HRT) of the acidification reactor and the USSB were 24.0 hr and 23.7 hr, respectively. Effluent recirculation in the USSB was carried out for 9 times the influent volume. The acidification reactor was started up without any seed sludge. The USSB was seeded with mesophilic granular sludge (VSS/SS 0.67, SVI 9 ml/gSS) preserved at 4°C for a few years. The total amount of seed sludge was 315 gVSS. The organic loading rate (OLR) was stepwise increased with increasing influent COD concentration at the fixed HRT. On day 262, the volume of the acidification reactor was increased to 22.5 L corresponding to HRT of 40.3 hr to promote acidification.

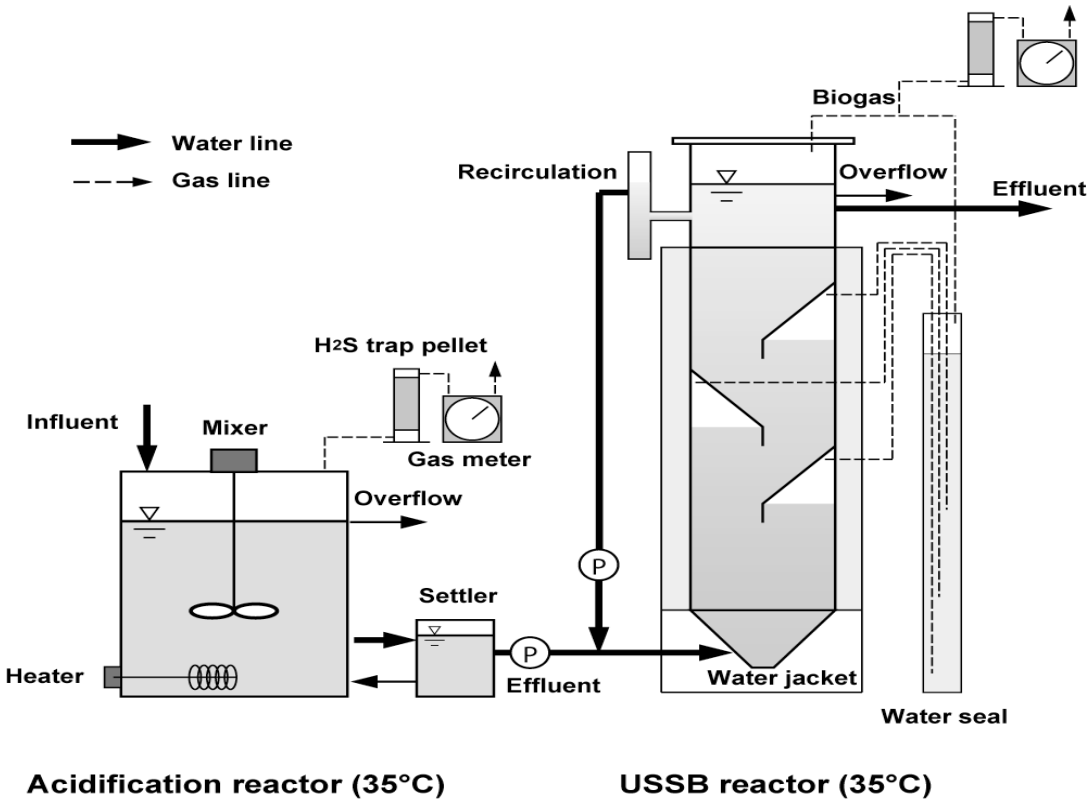

Fig. 1. Systematic diagram of the proposed treatment system

Table 1. Operating conditions for the proposed treatment system

Run	Run 1								
Phase	1	2	3	4	5	6	7	8	9
Day	0-24	25-53	54-80	81-143	144-180	181-190	191-212	214-235	235-279
COD (mg/L)	3,000	5,000	8,000	10,000	15,000	23,000	30,000	40,000	25,000
Sulfate ($mgSO_4$/L)	110	190	300	370	560	850	1,100	1,500	920
$NaHCO_3$ (mg/L)	2,400	4,000	6,400	8,000	9,000	13,800	15,000	16,000	15,000
Recirculation ratio	-	-	-	-	1:9	1:9	1:9	1:9	1:9
Run	Run 2				Run 3				
Phase	10	11	12	13	14	15	16	17	
Day	280-329	330-360	361-401	402-417	418-431	432-458	459-531	532-598	
COD (mg/L)	15,000	22,000	30,000	35,000	23,000	25,000	30,000	43,000	
Sulfate ($mgSO_4$/L)	560	820	1,100	1,300	850	920	1,100	1,600	
$NaHCO_3$ (mg/L)	7,500	11,000	15,000	17,500	11,500	12,500	6,000	6,000	
Recirculation ratio	1:9	1:9	1:9	1:9	1:9	1:9	1:9	1:9	

The water samples obtained from the influent wastewater, the acidification effluent, and the USSB effluent were analyzed twice a week. The total suspended solids (TSS) concentration and the volatile suspended solid (VSS) concentration were measured using a 0.4 μm glass fiber filter (GB-140, ADVANTEC, Japan). The chemical oxygen demand (COD_{Cr}) and sulfate concentration was analyzed using a HACH water quality analyzer (DR-2500, HACH). For COD measurement, a small amount of sulfuric acid was added to the water samples followed by N_2 purge to remove sulfide from the water sample. The volatile fatty acid (VFA) concentration was determined using a flame ionization detector (FID) gas chromatography (GC-2014, Shimadzu, Japan), fitted with a 2.1 m x 3.2 mm (ID) glass column packed with Thermon 3000 (60/80 mesh). Biogas composition was analyzed using a thermal conductivity detector (TCD) gas chromatograph (GC-8A, Shimadzu, Japan), equipped with a 2 m x 3 mm (ID) stainless-steal column with Unibeads-C (60/80 mesh). Other analytical methods were according to Standard methods (APHA, 1998). Granule size distributions were determined by image analysis (Scion Image, USA) of more than 300 granules for each sample taken from port no.1 (20 cm height from the bottom of reactor). The sludge sample was spread in a Petri dish and then, photographed by digital camera. Granular size in both major axis and minor axis was determined by image analysis. Then, the volume and mean diameter of the granules were calculated (Syutsubo *et al.*, 1997).

Methanogenic activity of the sludge samples was evaluated by serum vial test at 35°C in duplicate. The sludge sample was taken at 0.2 m from the bottom of the reactor. The harvested sludge was washed with phosphate buffer and then homogenized. The medium containing 25 mM of phosphate buffer (pH 7.0), 400 mg/L $MgCl_2 \cdot 9H_2O$, 150 mg/L $CaCl_2 \cdot 2H_2O$, and 500 mg/L NH_4Cl. A $Na_2S \cdot 9H_2O$ solution was used as a reducing agent at final concentration of 250 mg/L. The substrates in terms of acetate, H_2/CO_2 (80%:20%, v/v), propionate and sucrose were used for acetoclastic methanogen, hydrogentrophic methanogen, acetogenic bacteria, and sucrose-degrading acid-forming bacteria, respectively. A control (without the addition of the substrate) was also prepared. The headspace in the hydrogen-fed vials was filled with H_2/CO_2 gas at 1.4 atm (142 kPa). For the liquid substrate-fed vials, COD strength was set at 2,000 mgCOD/L for acetate, 1,000 mgCOD/L for propionate, and 1,500 mgCOD/L for sucrose. The prepared vial bottles were shaken reciprocally at 120 rpm. Methane production rate was determined by measuring gas amount and composition in the vial bottles at regular intervals.

Sulfate reducing activity was determined using substrates in terms of H_2/CO_2, acetate, and propionate. The initial SO_4 concentration was set at 300 $mgSO_4$/L. The activity was calculated by the reduction rate of the SO_4 as COD based on the sludge weight.

The microbial community structure of retained sludge was analyzed by denaturing gradient gel electrophoresis (DGGE) targeting the 16S rRNA gene. Genomic DNA was extracted from the sludge sample by Isoil beads beating kit (Nippon Gene, Japan). Polymerase chain reaction (PCR) was performed using a specific primer set (PARCH 341F-GC, PARCH 519R), designed to amplify the 16S rRNA genes of the Archaea domain (Ovreås *et al.*, 1997). DGGE analysis was carried out using a D Code™ system (Bio-Rad Laboratories, USA). Electrophoresis was made with a gradient gel (40% to 55% of denaturant) at 60°C for 3.5 hr. Major bands containing DNA were excised, and nucleotide

sequences were determined using a 3100 genetic analyzer (Applied Biosystems Japan, Japan). The nucleotide sequences obtained were compared with the data stored in NCBI GenBank using BLAST.

RESULTS & DISCUSSION

Fig. 2 shows the total COD concentration in the system and the methane production in the USSB during the whole operational period. The treatment system was started up at an influent concentration of 3,000 mgCOD/L. The influent concentration was stepwise increased at a constant HRT of 23.7 hr in the USSB. The complex organic compounds such as sucrose in the influent wastewater were effectively acidified in the acidification reactor. The USSB was successfully operated at high OLR of 30 kgCOD/m^3 day in phase 7. The effluent COD concentration was less than 5,000 mgCOD/L and the COD removal efficiency was 75 to 87%.

In phase 8, at 40 kgCOD/m^3 day, VFA accumulated in the USSB effluent due to overload. The accumulated VFA concentration was 9,660 mgCOD/L, and consisted of acetate (6,750 mgCOD/L), propionate (1,740 mgCOD/L), and n-butyrate (520 mgCOD/L). Sanchez Riera *et al.* (1985) reported that accumulation of both acetate and propionate occurs in the effluent of the UASB reactor for treatment of molasses wastewater under overloading conditions (at more than 22 kgCOD/m^3 day). To recover the process performance of the USSB,

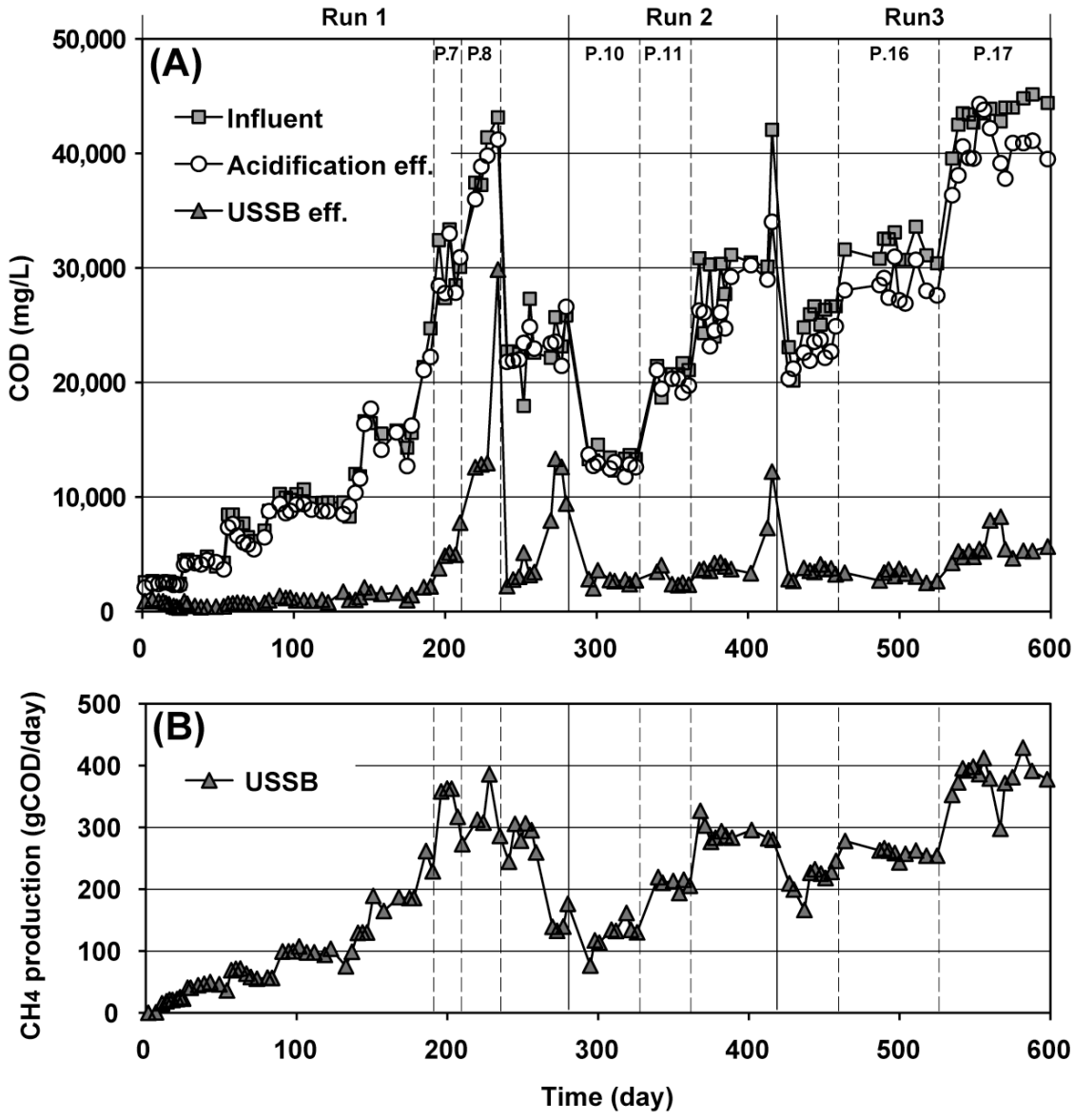

Fig. 2. COD in the treatment system (A) and methane production in the USSB (B)

OLR was temporarily reduced to 15 kgCOD/m^3 day in phase 10. After recovery of COD removal, OLR was again increased in phase 11.

In phase 16, the concentration of sodium bicarbonate added to the influent was reduced from 15,000 to 6,000 mg/L to reduce sodium inhibition of methanogenic bacteria. As a result, pH of the acidification effluent decreased from 6.3 in phase 15 to 5.1 in phase 16. The relatively low pH (5.0-5.6) affected the bacterial activity with respect to acidification and sulfate reduction in the acidification reactor. Although the total VFA to soluble COD ratio in phase 16 was almost equal to that in phase 15, the ratio of acetate in total VFA was relatively low in phase 16. Consequently, the ratio of n-butyrate increased from about 40% in phase 15 to about 70% in phase 16 based on total VFA concentration as COD. In addition, sulfate reduction efficiency in the acidification reactor was significantly suppressed from 82% to 15%. Due to incomplete reduction of sulfate in the acidification reactor, sulfate reduction followed by sulfide production took place mainly in the USSB. However, the process performance of the USSB was retained due to the maintenance of relatively high pH (7.8-8.1) by effluent recirculation and effective discharge of H_2S by biogas produced from the USSB in phase 16.

In phase 17, the USSB achieved high process performance at OLR of 43 kgCOD/m^3 day. This high process performance may be attributed to good retention of sludge as described below and a reduction of sodium inhibition. The COD removal efficiency was 86.2% for total COD and 90.4% for soluble COD. The effluent COD level was 5,500 mg/L for total COD and 3,500 mg/L for soluble COD on average. The high COD removal of the USSB was caused by high methane gas production of 380 gCOD/day on average. Composition of the biogas after removal of hydrogen sulfide was 62.9% methane, 36.5% carbon dioxide, 0.4% nitrogen, and 0.2% hydrogen. The methane recovery rate based on the removed COD was 80.0% in the USSB. The biogas produced was mainly removed from the bottom GSS (GSS 1) and the middle GSS (GSS 2) and the percentage amount of biogas produced was 34% and 38% based on the total amount.

The result clearly shows that the USSB achieved a higher COD removal rate of 37 kgCOD/m^3 day (COD removal efficiency of 86.2% at OLR of 43 kgCOD/m^3 day) for treatment of molasses wastewater at 35°C when compared with previous studies. So far, several types of the anaerobic systems have been used to treat molasses wastewater (Satyawali and Balakrishnan, 2008). The remarkable experiments show that the COD removal rate was 18 kgCOD/m^3 day (COD removal efficiency of 75% at OLR of 24 kgCOD/m^3 day) in an UASB for treatment of stillage from sugar cane molasses at 40°C (Sanchez *et al.*, 1985) and 14 kg COD/m^3 day (COD removal efficiency was over 70% at OLR of 20 kgCOD/m^3 day) in a hybrid anaerobic baffled reactor (HABR) for treatment of molasses wastewater at 37°C (Boopathy and Tiche, 1991). The COD removal rate of the USSB is more than two times higher than that of other high-rate anaerobic reactors. This advantage contributes to smaller reactor equipment (small footprint) and high energy-yield (low energy consumption) in full-scale applications.

The sulfate concentration of the acidification effluent was about 1,600 mgSO_4/L (530 mgS/L) in phase 17. It was completely reduced to sulfide in the USSB. As a result, approximately 3% of the total COD removal was caused by sulfate reduction. The USSB discharged approximately 70% of the sulfide produced as a hydrogen sulfide included in biogas. This phenomenon indicates that sulfide produced was effectively discharged from the liquid through three GSS by biogas production, despite no additional chemical-agents and equipment. The removal-characteristic of sulfide in the USSB was evaluated in the previous study (Onodera *et al.*, 2011). The removal of hydrogen sulfide was important to maintain the process performance, because the sulfur level of the USSB influent was high enough to inhibit the methanogenic activity. (Koster *et al.*, 1986; Karhadkar *et al.*, 1990; Onodera *et al.*, 2011). Composition of organic compounds removed in the USSB was determined on day 570. Fig. 3 shows soluble COD and VFA concentration along the reactor height. The USSB was fed with a mixture of the acidification effluent and the USSB effluent recirculation. The soluble COD was about 6,770 mg/L at the inlet (height 0 m). It was reduced from 5,570 mg/L to 4,570 mg/L at 30 cm height from the inlet. The final effluent COD was approximately 3,370 mg/L.

The influent VFA concentration was 3,220 mg/L. The influent VFA consisted of acetate (520 mgCOD/L), propionate (230 mgCOD/L), i-butyrate (90 mgCOD/L), and n-butyrate (2,380 mgCOD/L). Degradation of n-butyrate occurred in the lower portion of the USSB reactor. The ratio of n-butyrate to total VFA decreased with increasing height in the reactor. On the other hand, the ratio of acetate to total VFA increased when going from the lower to the middle portion of the reactor. The acetate was only 370 mgCOD/L in the upper portion of the reactor. These results suggest that the USSB has high capability for organic removal despite relatively high OLR conditions of 43 kgCOD/m^3 day. Additionally, these results indicate that both acetogenic reaction and methane production occurred at a sufficient level in the sludge bed. It is known that the anaerobic

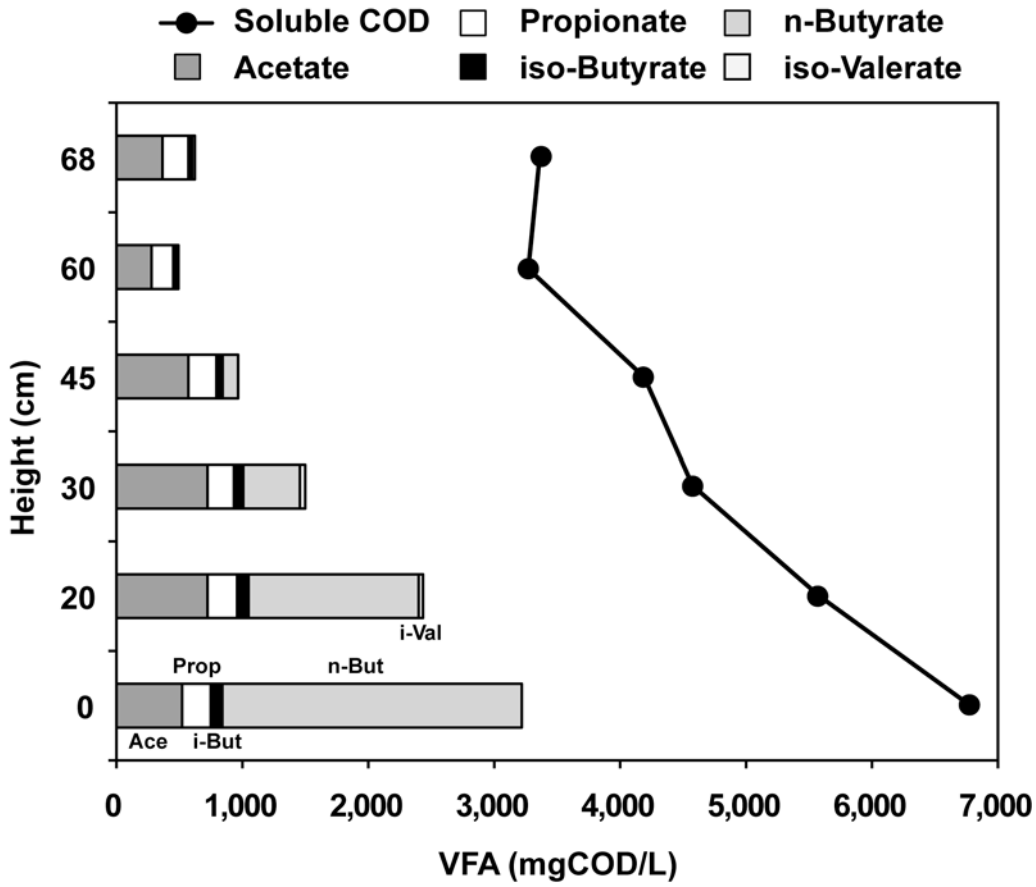

Fig. 3. Removal characteristic of organic compounds in the USSB on day 570

degradation of propionate and butyrate, which is an endergonic reaction under standard conditions, can occur if the hydrogen partial pressure is kept low enough (Schink, 1997). Therefore, low VFA level in the USSB might be attributed to maintaining a low partial pressure of hydrogen in the upper portion of the USSB which is provided by the high activity of hydrogen scavengers such as methanogens and sufficient removal of biogas (hydrogen) through each GSS.

Fig. 4 shows the sludge concentration over time in the USSB during the experimental period. The average sludge concentration increased from 23.6 gVSS/L (day 0) to 57.1 gVSS/L (day 394). Between day 233 and day 326, sludge concentration decreased significantly due to withdrawing of the floating agglomerated sludge from the top of the reactor. The high sludge concentration was attributed to the fact that the reactor was mostly occupied by granular sludge and the relatively moderate biogas flux in the reactor provided by the GSS. The superior retention of the sludge in the reactor can lead directly to high potential for organic removal. The MLSS increased with increasing operational days, corresponding to the increasing VSS/TSS ratio. Inorganic particles were found in the retained sludge especially toward the end of the experimental period. The accumulation of inorganic particles caused no operational trouble of the system during the experimental period.

The high sludge concentration in the USSB might be a result of certain physical sludge properties. The retained sludge was formed in a granular shape. On day 401, 90% of the granule sludge based on sludge volume had a mean diameter of more than 2.0 mm at port 1 (20 cm height). The granular sludge had high sludge settle-ability of approximately 4 to 14 ml/gSS of SVI at port 1 (20 cm height) and 8 to 20 ml/gSS at port 2 (30 cm height). The relatively low VSS/TSS ratio of 0.42 was observed in the retained sludge on day 326. The low value of VSS/TSS ratio might be due to accumulation of inorganic particles such as $CaCO_3$ (identified by X-ray diffraction, data not shown) in the retained sludge. The $CaCO_3$ accumulation could be caused by the fact that the influent wastewater contained amounts of calcium in the molasses of 7.5 mgCa/gCOD and bicarbonate in the range of 150 to 500 mg$NaHCO_3$/gCOD. The USSB maintained relatively high pH of approximately 8.0 by effluent recirculation. Note that operational trouble of the USSB did not

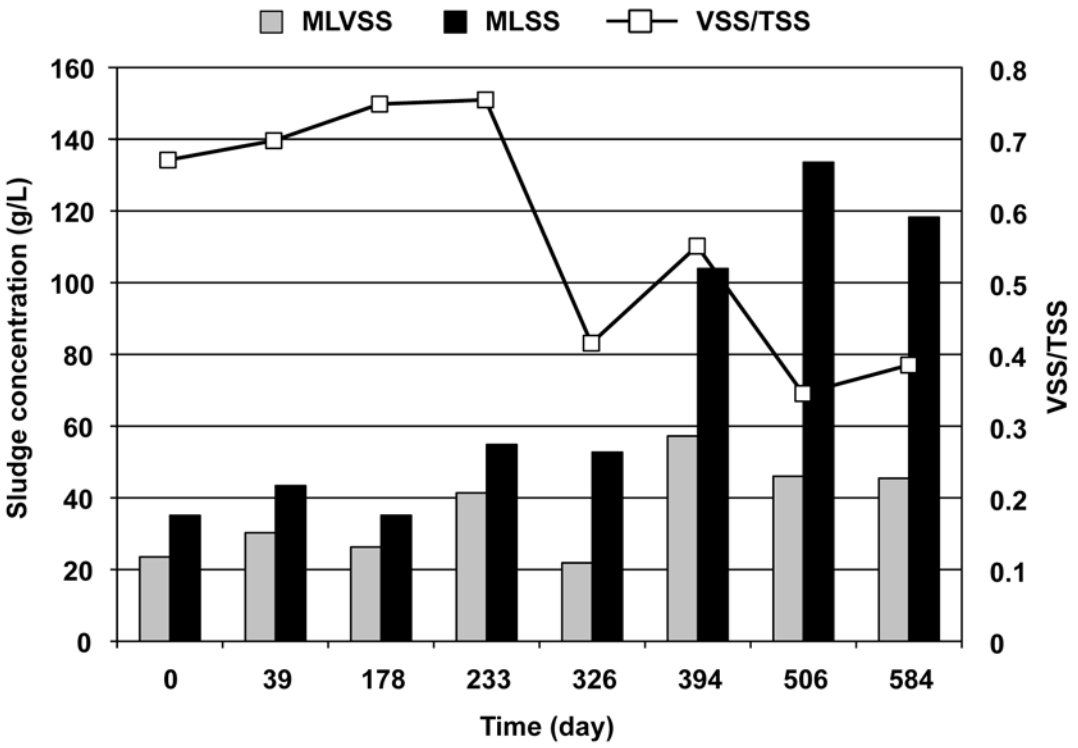

Fig.4. Evolution of retained sludge concentration in the USSB

occur because of the $CaCO_3$ accumulation in the retained sludge during the operational period.

Fig. 5 shows the methanogenic activity of retained sludge in the USSB with regards to the representative substrates over time. Methanogenic activity of the seed granular sludge was very low. The methanogenic activity of the retained sludge gradually increased with increasing levels of OLR until day 178. The high sludge activity was observed at 0.88 gCOD/gVSS day in the acetate substrate, 2.6 gCOD/gVSS day in the hydrogen substrate, and 0.48 gCOD/gVSS day in the propionate substrate on day 178. On day 584, the methanogenic activity was 1.2 gCOD/gVSS day in the acetate substrate and 1.7 gCOD/gVSS day in the hydrogen substrate. This result indicates that the methanogenic activity in the acetate substrate was high under high OLR condition. On day 584, methanogenic activity in the n-butyrate substrate was also determined because n-butyrate concentration was relatively high at the lower portion of the USSB on day 570 (Fig. 3). It confirmed that the methane producing activity in the n-butyrate substrate was high enough at 1.1 gCOD/gVSS day. Fig. 6 shows the sulfate reducing activity of the retained sludge in the USSB. The sulfate reducing activity of the seed sludge was very low. The sulfate reducing activity reached to 0.31 gCOD/gVSS day for H_2/CO_2 and 0.07 gCOD/gVSS day for propionate on day 584. The activity for acetate was very low. In the USSB, sulfate was absent even at port 1 (0.2 m height). This means that the sulfate reduction followed by sulfide production occurred quickly at the lower portion of the USSB. The sludge activity test reveals that both metanogenic activity and sulfate-reducing activity of the retained sludge in hydrogen substrate was high enough. As described above, maintaining a low partial pressure of hydrogen is important for degrading intermediate VFA such as butyrate and propionate. Therefore, the high activity of the retained sludge in the hydrogen substrate can offer a sufficient VFA degradation potential in the USSB even under a high OLR of 43 $kgCOD/m^3$ day.

Microbial community structure of the retained sludge in the acidificaiton reactor and the USSB was investigated by a 16S rDNA-targeted DGGE analysis with respect to Archaea domain. The sludge samples were harvested from the acidificaiton reactor on day 233 and the USSB reactor on days 0, 40, 178, and 233. The DGGE profiles of the retained sludge for both reactors are shown in Fig. 7. The microbial community structure was different between the acidification sludge and the USSB sludge. The presence of genus relatives of *Methanocorpusculum* (band A, similarity 98%) and genus *Methanofollis* (band C, similarity 98%), which methanogens use hydrogen and formic acid, was confirmed in the sludge of the acidification reactor on day 233. The genus *Methanofollis* (band C) are able to tolerate high salinity conditions (Lai and Chen, 2001). The results obtained indicate that those

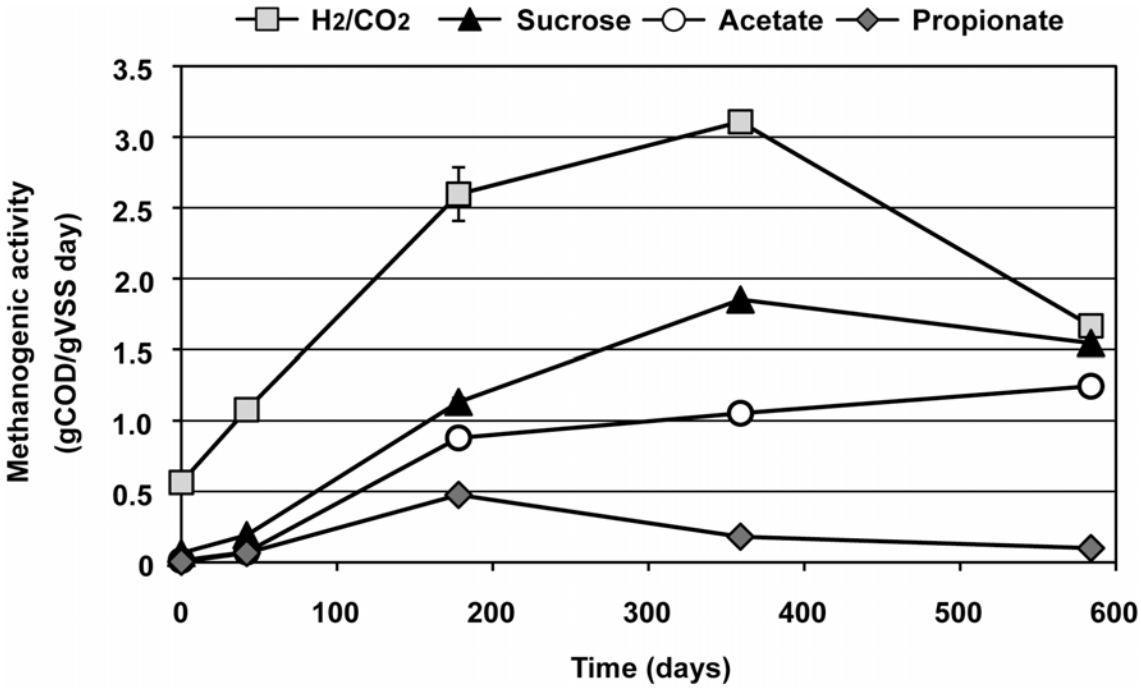

Fig. 5. Methanogenic activity of the retained sludge over time in the USSB

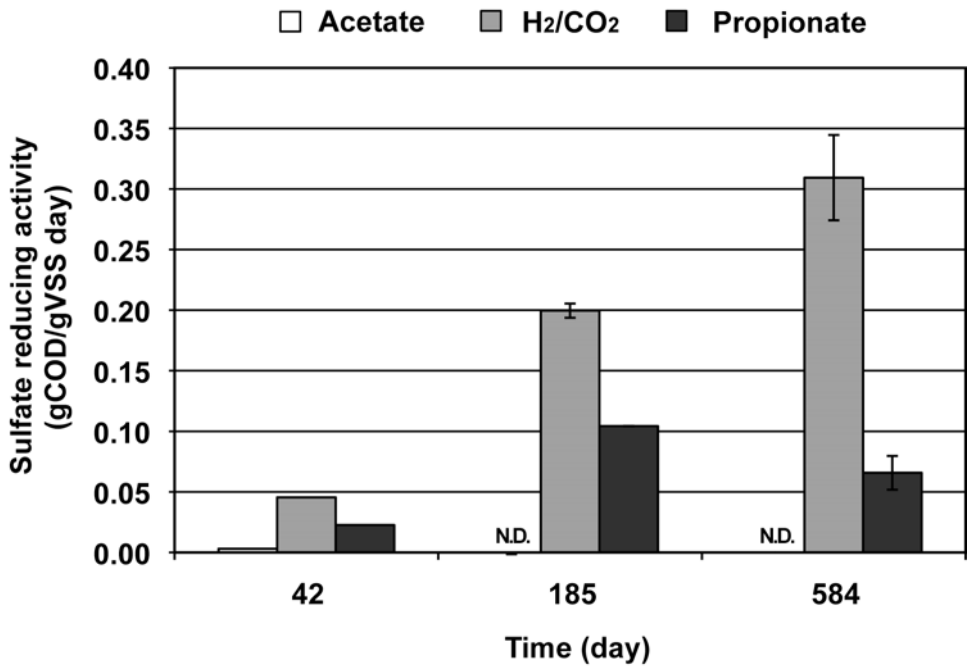

Fig. 6. Sulfate reducing activity of the retained sludge in the USSB

methanogens produced methane gas even from the acidification reactor. The low methane gas production in the acidification reactor was confirmed between phases 6 and 15. On the other hand, relatives of genus *Methanobacterium* (band B) as hydrogen utilizing methanogen and order *Methanosarcinales* (band D) as acetate utilizing methanogen were prevalent in the USSB. Moreover, many kinds of hydrogen utilizing methanogens belonging to order *Methanomicrobiales* (bands A, C, and E) were present in the sludge of the USSB. The wide variety of DGGE bands of hydrogen utilizing microorganisms is consistent with the high activity for hydrogen utilizing methane production. This may be provided by relatively high hydrogen pressure resulting from acidification conditions at the lower portion of the USSB. It was observed that

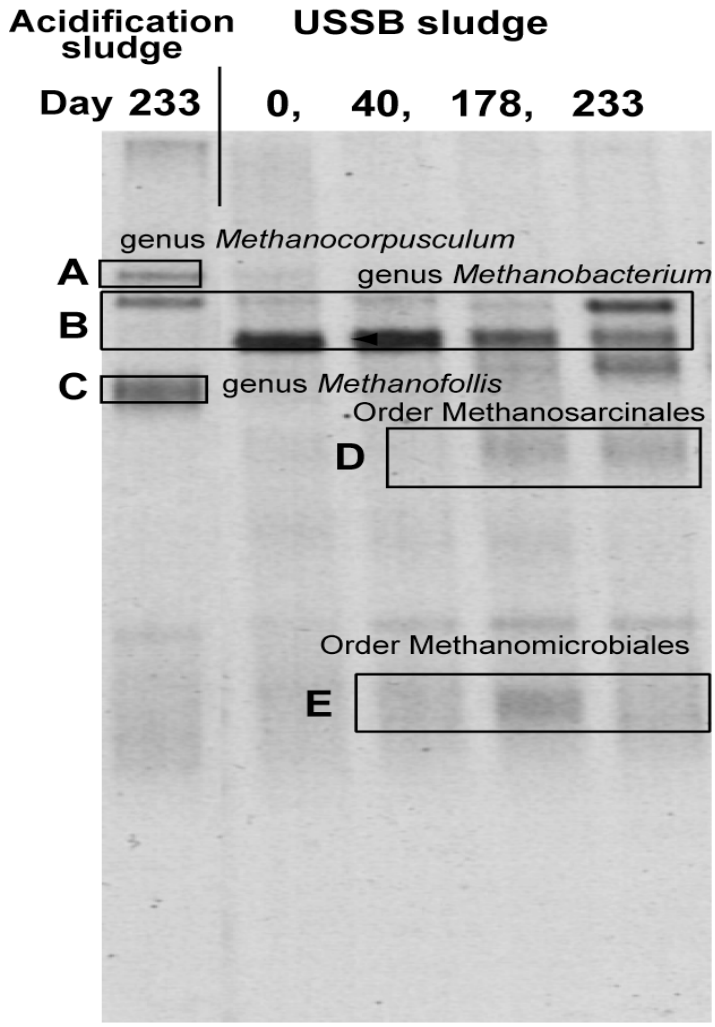

Fig. 7. DGGE proûles of the retained sludge with respect to 16S rDNA of Archaea domain

hydrogen partial pressure at the lower portion of the USSB reactor (0.3 m height) was about 1,300 Pa on day 234. In addition, relates of genus *Clostridium* and gens *Lactococcus*, which are sugar-degrading acid-forming bacteria, were frequently detected in the sludge of the USSB reactor on day 233 (Onodera *et al*., 2011).

CONCLUSION

The system consisting of the acidification reactor and the USSB reactor was successfully operated to treat molasses wastewater at mesophilic (35°C) conditions. The USSB achieved a high organic removal rate of 37 kgCOD/m^3 day (at OLR of 43 kgCOD/m^3 day with 86.2% COD removal). Evaluation of the sludge properties revealed that the USSB retained high sludge concentration of 58 gVSS/L based on the reactor volume. The high sludge concentration was attributed to sufficient sludge settle-ability, with an SVI of 4 to 20 ml/gSS. The retained sludge had relatively high ash content with VSS/TSS ratio of 0.34 toward the end of the experiment. Moreover, the retained sludge accumulated various methanogens such as genus *Methanobacterium* and order *Methanosarcinales* during the operation and had high methanogenic activity in amounts of 1.2 gCOD/gVSS day in acetate substrate and 1.7 gCOD/gVSS day in hydrogen substrate on OLR of 43 kgCOD/m^3 day. The results obtained indicate that superior performance of the USSB for molasses wastewater was attributed to good retention of the granular sludge with high activity provided by the USSB reactor equipped with three GSS.

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