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Anaerobic treatment of municipal wastewater at ambient temperature: Analysis of archaeal community structure and recovery of dissolved methane

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ABSTRACT

Anaerobic treatment is an attractive option for the biological treatment of municipal wastewater. In this study, municipal wastewater was anaerobically treated with a bench-scale upflow anaerobic sludge blanket (UASB) reactor at temperatures from 6–31°C for 18 months to investigate total chemical oxygen demand (COD) removal efficiency, archaeal community structure, and dissolved methane (D-CH_4) recovery efficiency. The COD removal efficiency was more than 50% in summer and below 40% in winter with no evolution of biogas. Analysis of the archaeal community structures of the granular sludge from the UASB using 16S rRNA gene-cloning indicated that after microorganisms had adapted to low temperatures, the archaeal community had a lower diversity and the relative abundance of acetoclastic methanogens decreased together with an increase in hydrogenotrophic methanogens. D-CH_4, which was detected in the UASB effluent throughout the operation, could be collected with a degassing membrane. The ratio of the collection to recovery rates was 60% in summer and 100% in winter. For anaerobic treatment of municipal wastewater at lower temperatures, hydrogenotrophic methanogens play an important role in COD removal and D-CH_4 can be collected to reduce greenhouse gas emissions and avoid wastage of energy resources.

Keywords: Archaeal community structure; Degassing membrane; Dissolved methane; Municipal wastewater; Psychrophilic condition; Upflow anaerobic sludge blanket process

1. Introduction

Anaerobic treatment is an attractive option for the biological treatment of municipal wastewater (Latif et al., 2011; Kayranli and Ugurlu, 2011; Lew et al., 2011; Elefsiniotis et al., 1996). It has numerous advantages, including the generation of useful energy (i.e., biogas), no energy requirement for aeration, and the reduction of the cost of sludge treatment. In temperate regions, the ambient temperature of municipal wastewaters is considerably lower than the optimum value for anaerobic treatment processes (Latif et al., 2011; Dhaked et al., 2010). To operate anaerobic treatment processes under mesophilic conditions (30°C–40°C), a significant input of energy is required to heat the influent wastewaters, resulting in a loss of energy. Thus, the operation of an anaerobic bioreactor for municipal wastewater treatment at ambient temperature in temperate regions offers economic advantages over the operation under mesophilic or thermophilic (45°C–60°C) conditions.

Anaerobic treatment of low-strength wastewaters at ambient or low temperatures has recently been successfully demonstrated (Latif et al., 2011; Kayranli and Ugurlu, 2011; Lew et al., 2011; Urban et al., 2007; Alvarez et al., 2008). However, further studies are required to anaerobically treat low-strength municipal wastewaters at low temperatures. Specifically, chemical oxygen demand (COD) removal rate and methane (CH_4) production rate are low owing to lower microbial activity under psychrophilic (<20°C) conditions (Latif et al., 2011; Dhaked et al., 2010). The economic feasibility of the long-term low-temperature anaerobic treatment relies on sufficient microbial activity to ensure reliable wastewater treatment. However, little is known about psychrophilic methanogenesis. In some systems mesophilic methanogenic communities that adapt to low temperatures contributed to methanogenesis, in contrast, specific psychrophilic methanogenic communities carried out methanogenesis in others. (Dhaked et al., 2010). Greater insights into the relationship between process performance and microbial characteristics in the anaerobic treatment of municipal wastewaters at low temperature are required to achieve stable COD removal and biogas production.

It is also important to consider dissolved methane gas (D-CH_4) in the anaerobic treatment of municipal wastewaters. D-CH_4 in anaerobic treatment effluent is not usually recovered, which results in greenhouse gas emission from the anaerobic treatment process and loss of a potential energy resource (Urban et al., 2007). The loss of D-CH_4 is enhanced at lower temperatures because of the increase in CH_4 solubility at reduced temperatures (Bandara et al., 2011). Thus, it is the case with the anaerobic treatment of municipal wastewaters at low temperatures. In these processes, as much D-CH_4 as possible from the anaerobic treatment effluent should be collected. Several studies have investigated the collection of D-CH_4 from the anaerobic treatment of wastewaters by physical gasification based on gas-liquid equilibrium and mixing with gas or a paddle (Hatamoto et al., 2010; Matsuura et al., 2010; Hartley and Lant, 2006; Pauss et al., 1990). However, the collection efficiency of D-CH_4 was low and/or the recovered CH_4 gas concentration was low using these technologies. Our previous research demonstrated that degasification with a degassing membrane (DM) could effectively collect D-CH_4 without reducing its concentration in the anaerobic treatment of a low-strength synthetic wastewater at low temperature (Bandara et al., 2011). The DM only allows gas molecules to pass through the non-porous layer of the DM (Bandara et al., 2011; Matsunaga et al., 2012). Thus, the DM effectively separates dissolved gases from the liquid. In the present research study, we operated a bench-scale upflow anaerobic sludge blanket (UASB) reactor, of which the liquid outlet was connected to the DM reactor, to treat raw municipal wastewater at ambient temperature (from 6°C to 31°C) over 18 months. The performance of the UASB reactor was monitored. The archaeal community structures of the UASB reactor were analyzed using 16S rRNA gene-cloning techniques. Additionally, the D-CH_4 collection efficiency by degasification was investigated.

2. Materials and methods

2.1. Experimental setup and operating conditions

A UASB reactor (height, 80 cm; diameter, 5 cm; working volume, 1.6 L) was operated from January 2010 to June 2011. The reactor was inoculated with 0.7 L of anaerobic granular sludge obtained from the bench-scale UASB reactor operated in our laboratory (Bandara et al., 2011). It had total and volatile solids concentrations of 24 g/L and 18 g/L, respectively. The UASB reactor was fed with domestic wastewater from the Souseigawa municipal wastewater treatment plant in Sapporo, Japan (Okabe et al., 1999). The concentrations (average ± standard deviation) of total COD (T-COD) and the dissolved fraction of COD (D-COD), and pH in the wastewater were 173 ± 38 mg/L, 78 ± 17 mg/L, and 7.2 ± 0.2, respectively. The hydraulic retention time (HRT) was changed in response to changes in the COD removal efficiency (see Figure 1b). The temperature, which was not controlled, varied from 6°C to 31°C. A 20-cm-high filter media (polyester nonwoven fabric sheets; Japan Vilene Co., Ltd., Tokyo, Japan) was installed in the upper part of the UASB reactor on June 22, 2010, to avoid biomass washout. A DM reactor was connected to the effluent of the UASB reactor to collect the residual D-CH_4 in the effluent according to a study described previously. The characteristics of the DM have been described in detail elsewhere (Bandara et al., 2011). D-CH_4 was collected into the lumen of the hollow fibers of the DM using a vacuum pump and the transmembrane pressure was set to 80 kPa using a vacuum gauge. A transmembrane pressure of 97 kPa was also tested after April 25, 2011, to investigate the effect of transmembrane pressure on D-CH_4 collection efficiency.

2.2. DNA extraction and PCR amplification of 16S rRNA genes

Total DNA was extracted from the granular sludge inoculum, termed INO, and a mature granular sludge, termed PSY, in the UASB reactor on day 416 (February 15, 2011) using a Fast DNA spin kit (MP Biomedicals, Irvine, CA) according to the manufacturer’s instructions. To construct archaeal clone libraries, the 16S rRNA gene fragments from the isolated total DNA were amplified using a ONE Shot LA PCR

MIX kit (TaKaRa Bio Inc., Ohtsu, Japan) and a primer set of arc109f (Lueders and Friedrich, 2000) and univ1390r (Zheng et al., 1996). The PCR condition for the archaea was as follows: 5 min of initial denaturation at 94°C, followed by 25 cycles of 30 s at 94°C, 30 s at 50°C, and 1 min at 72°C. The final extension was conducted for 5 min at 72°C. All PCRs were performed using a total volume of 50 μL containing 1 μg of DNA as the template. The PCR products were electrophoresed in a 1% (wt/vol) agarose gel.

2.3. Clone library construction and phylogenetic analysis

The archaeal clone libraries were constructed using previously described methods (Kindaichi et al., 2011). DNA sequencing was performed by Dragon Genomics Center, TaKaRa Bio Inc. (Yokkaichi, Japan). The 16S rRNA gene sequences from the archaeal clone libraries (1,281 bp) were imported and aligned using Integrated Aligners in the ARB software (Ludwig et al., 2004) with the database SSU Ref NR 106 dataset (Pruesse et al., 2007). Sequences with 97% or higher similarity were grouped into operational taxonomic units (OTUs) using the Distance matrix methods with the similarity correction in the ARB software. Phylogenetic trees were constructed using the neighbor-joining (Saitou and Nei, 1987) with jukes-cantor correction model and maximum parsimony (Phylip DNAPARS) approaches using default settings in the ARB software. A bootstrap resampling analysis for 1,000 replicates was conducted to estimate the confidence of tree topologies. The 16S rRNA gene sequence data obtained in this study were deposited in the GenBank/EMBL/DDBJ databases under accession numbers AB123456 to AB123456.

2.4. Sampling and analysis methods

CH_4 concentrations in the headspace of the UASB reactor and inside the DM were measured using a gas chromatography system (Bandara et al., 2011). Dissolved gas concentrations were measured using the headspace method (Bandara et al., 2011). The concentrations of T-COD and D-COD in the influent and effluent of the UASB and DM reactors were measured using the Hach method (Method 8000) (Bandara et al., 2011). The concentration of the particulate fraction of COD (P-COD) was calculated by subtracting the D-COD concentration from the T-COD concentration. The oxidation-reduction potential (ORP) and pH were directly determined using ORP and pH electrodes, respectively.

On the basis of these measurements and liquid flow rates, the rates (mg COD/day) of CH_4 evolved into the UASB headspace ($R(CH_4)_{evo}$), D-CH_4 discharged from the UASB reactor ($R(D\text{-}CH_4)_{UASB}$), D-CH_4 collected with the DM ($R(D\text{-}CH_4)_{col}$), and D-CH_4 discharged from the DM reactor ($R(D\text{-}CH_4)_{dis}$) were calculated. Furthermore, the D-CH_4 collection efficiency was calculated as

$$\text{D-CH}_4\text{ collection efficiency} = \frac{R(D\text{-}CH_4)_{col}}{R(D\text{-}CH_4)_{UASB}}$$

The CH_4 recovery efficiency was calculated as

$$\text{CH}_4\text{ recovery efficiency} = \frac{R(CH_4)_{rec}}{T\text{-}R(CH_4)} = \frac{R(CH_4)_{evo} + R(D\text{-}CH_4)_{col}}{R(CH_4)_{evo} + R(D\text{-}CH_4)_{col} + R(D\text{-}CH_4)_{dis}}$$

Where $R(CH_4)_{rec}$ is the CH_4 recovery rate (mg COD/day) defined as the sum of $R(CH_4)_{evo}$ and $R(D\text{-}CH_4)_{col}$, and $T\text{-}R(CH_4)$ is the total CH_4 production rate (mg COD/day) defined as the sum of $R(CH_4)_{evo}$, $R(D\text{-}CH_4)_{col}$, and $R(D\text{-}CH_4)_{dis}$.

The amounts of suspended solids (SS) and volatile suspended solids (VSS) in the filter media and in the granular sludge (g per reactor) were estimated and used as an indicator of microorganisms (Andrew et al., 2005). Biomass samples were taken from 100 mL of the filter media and 10 mL of the granular sludge. Then, the filtered samples were dried at 105°C and weighed to determine SS. To determine VSS, they were further burned at 550°C to allow volatile substances to evaporate.

3. Results and discussion

3.1. Performance of the UASB reactor

The bench-scale UASB reactor was operated at ambient temperature from January 2010 to June 2011. Changes in T-COD and D-COD concentrations in the influent, T-COD concentration in the effluent, and T-COD removal efficiency are observed (Figure 1a). The influent T-COD and D-COD concentrations were in the ranges 70–310 mg/L and 50–160 mg/L, respectively. Based on the COD results, the municipal wastewater used in this study could be classified as a low-strength domestic wastewater (Tchobanoglous et al., 2003). The average (± standard deviation) of the influent P-COD/T-COD ratio remained relatively constant (0.56 ± 0.05) throughout the operation.

Temperature, HRT, and pH in the UASB reactor are shown in Figure 1b. Temperature varied from 6°C to 31°C during the operation. HRT was adjusted in the range of 2–8 h in response to changes in the COD removal efficiency. The pH in the UASB reactor became lower than that in the influent after July 2010.

T-COD was not removed during the first winter (Figure 1a). Between July and October 2010, the T-COD removal efficiency was in the range 50%–71%. The temperature ranged from 20°C to 31°C during this period (Figure 1b), indicating that temperature was a critical factor for effective COD removal. Although disintegration of the granules was observed during the operation, the average amounts of VSS in the granular bed increased from 78 ± 15 to 150 ± 40 g per reactor by installation of the filter media on June 22, 2010 because of prevention of biomass washout. This is a common problem with the anaerobic treatment of low-strength wastewaters for short HRT (Latif et al., 2011). Enhancement of the retention and growth of biomass by the installation of the filter media may have contributed to the improvement in T-COD removal efficiency (Figure 1a) and increase in $R(CH_4)_{evo}$ (see Figure 1c) between July and October 2010. The T-COD removal efficiency started to decrease at the beginning of Nov 2010 accompanying the temperature drop. This might be attributed to the low methanogenic activity at low temperatures (Dhaked et al., 2010). Volatile fatty acids (mainly acetic acid) were detected in winter, but not in summer, indicating that acidogenic activity was not inhibited compared to methanogenic activity at low temperatures. Subsequently, the T-COD removal efficiency gradually increased from

10% to around 60% in April 2011 owing to the gradual increase in temperature. The COD removal efficiency was lower (<71%) in this study than those in UASB reactors treating high-strength wastewaters under mesophilic conditions (Latif et al., 2011). This might be because of the higher P-COD fraction of the municipal wastewater used in this study and the operation at ambient temperature (Figures 1a and 1b) (Alvarez et al., 2008). Table 1 summarizes the operating parameters and T-COD removal efficiencies of UASB reactors treating low-strength wastewaters under psychrophilic conditions reported in the previous studies. These results indicate that the treatment of low-strength wastewaters at low temperatures results in low COD removal efficiencies. Therefore, an aerobic post-treatment is needed to achieve the appropriate T-COD removal efficiency (Khan et al., 2011).

The biogas production rate and $R(CH_4)_{evo}$ in the UASB reactor are presented in Figure 1c. From January to May 2010, no biogas was evolved into the UASB headspace because of the low T-COD removal efficiency (Figure 1a). It should be noted that a barely detectable amount of D-CH_4 was found in the UASB reactor and D-CH_4 was discharged from the DM reactor during this period (see Figure 3a). Biogas was evolved in summer. The CH_4 concentrations in the UASB headspace and in the biogas collected with the DM were 50% ± 11% and 52% ± 8%, respectively, from July to October, which are comparable to or lower than those when treating high-strength wastewaters (Latif et al., 2011; Bandara et al., 2011). Biogas evolution ceased again in the second winter season. Thereafter, biogas evolution occurred as temperature increased from May 2011.

3.2. Archaeal community structure

To investigate the microbial community structure in the UASB reactor, archaeal clone libraries of INO and PSY were constructed. No chimeric sequences were detected in the clone libraries. We obtained 69 and 72 clones from the INO- and PSY-clone libraries, respectively. At least 5 archaeal groups at the order level including 15 different OTUs on the basis of more than 97% sequence similarity within an OTU were identified (Figure 2). It should be noted that there were no differences in the topology of the three trees generated using the neighbor-joining, the maximum-parsimony, and the maximum-likelihood methods. In the granular sludge inoculum, nine different OTUs were identified. *Methanosaeta*- and *Methanobacterium*-like clones comprised 57% (39 out of 69 clones) and 28% (19 out of 69 clones) of the INO-clone library, respectively. However, by day 416, a shift in the archaeal community structure was evident. The number of OTUs in the PSY-clone library had decreased to 6, indicating that the archaeal community had a lower diversity after microorganisms had adapted to low temperatures. Although high levels of *Methanosaeta*-like acetoclastic methanogens were maintained in the PSY-clone library, the relative abundance of *Methanosaeta concilii*-like clones diminished and the relative abundance of *Methanosaeta harundinacea*-like clones increased in the granular sludge inoculum. The most frequently detected clones in the PSY-clone library were affiliated with *Methanolinea tarda* (21 out of 72 clones; detection frequency, 29%). In addition, the clones affiliated with *Methanobacterium beijingense* comprised 7% (5 out of 72 clones) of the PSY-clone library. These two OTUs, which were absent in the INO-clone library, utilize H_2 and formate for growth and methane production (Imachi et al., 2008; Ma et al., 2005).

Methanosaeta-like acetoclastic methanogens were dominant in the granular sludge inoculum, such that these species are believed to be competitive in established methanogenic communities (Zhang et al., 2012; McKeown et al., 2009a; Satoh et al., 2007; O'Reilly et al., 2009). In contrast, several studies have documented that methanogenesis predominantly proceeds through the hydrogenotrophic route in anaerobic bioreactors under psychrophilic conditions (O'Reilly et al., 2009, 2010; McKeown et al., 2009b; Connaughton et al., 2006; McHugh et al., 2004). Under psychrophilic conditions, improved thermodynamics of methane formation from H_2/CO_2, coupled with the enhanced solubility and therefore accessibility of H_2/CO_2 in the reactor liquor, are thought to account for this phenomenon (Lettinga et al., 2001). The observed granule disintegration during the operation might be attributed to a decrease in the relative abundance of the filamentous *Methanosaeta*, which are suggested to play an important role in the formation and maintenance of granular sludge in an anaerobic bioreactor (McHugh et al., 2005).

OTU PSY-e7 (11 out of 72) and PSY-e1 (1 out of 72), which were not detected in the INO-clone library, were affiliated with the deep-sea hydrothermal-vent euryarchaeotal group 6 (DHVEG-6) and the terrestrial miscellaneous group (TMEG) based on the SILVA taxonomy (Pruesse et al., 2007), respectively. Members of these groups have primarily been detected in habitats such as ocean water, coastal water, polar seawater, deep-sea hydrothermal vents, hypersaline microbial mat, and methanogenic granular sludge (Nunoura et al., 2008; Takai and Horikoshi, 1999; Robertson et al., 2009; Yashiro et al., 2011). The ecophysiology and functions of the groups remained unknown.

3.3. Degasification with the DM

Figure 3a shows D-CH_4 concentrations discharged from the UASB and DM reactors. D-CH_4 concentrations discharged from the DM reactor were clearly lower than those from the UASB reactor, indicating the residual D-CH_4 in the effluent of the UASB reactor was successfully collected by the DM. The average D-CH_4 concentrations discharged from the UASB and DM reactors were 51 ± 12 mg COD/L and 22 ± 4 mg COD/L from July to October 2010, and 48 ± 9 mg COD/L and 16 ± 3 mg COD/L from December 2010 to March 2011, respectively. The average biogas flux through the DM was 55 ± 10 mL/m^2/day during the operating period. This indicates that membrane fouling of the DM was insignificant for 18 months. An increase in transmembrane pressure on April 25, 2011, resulted in a further decrease in the concentration of D-CH_4 discharged from the DM reactor.

$\mathrm{R(CH_4)_{evo}}$, $\mathrm{R(D\text{-}CH_4)_{col}}$, and $\mathrm{R(D\text{-}CH_4)_{dis}}$ were calculated in order to investigate the mass balance of CH_4 in this system. Figure 3b shows $\mathrm{R(CH_4)_{evo}}$, $\mathrm{R(D\text{-}CH_4)_{col}}$, and $\mathrm{R(D\text{-}CH_4)_{dis}}$, and the CH_4 recovery efficiency. From January to

May 2010, no CH_4 was evolved from the UASB reactor. From July to October 2010, $R(CH_4)_{evo}$, $R(D\text{-}CH_4)_{col}$, and $R(D\text{-}CH_4)_{dis}$ were 210 ± 110 mg COD/day, 280 ± 100 mg COD/day, and 330 ± 140 mg COD/day, respectively. Their average rates were 0 mg COD/day, 125 ± 20 mg COD/day, and 180 ± 35 mg COD/day, respectively, from mid-December 2010 to March 2011. An absence of CH_4 evolution was attributed to the decrease in organic matter degradation rate at lower temperatures (Figures 1a and 1b). The average CH_4 recovery efficiency was 59% ± 9% from July to October 2010 and 41% ± 6% from mid-December 2010 to March 2011. The average ratio of $R(D\text{-}CH_4)_{col}$ to $R(CH_4)_{rec}$ was 60% ± 12% from July to October 2010, but it was 100% during winter because no biogas was evolved into the UASB headspace. These values were greater than those in the UASB and DM reactors treating high-strength wastewater (<35%) (Bandara et al., 2011). Thus, we concluded that degasification technology is advantageous, especially when low-strength wastewater is treated under ambient conditions in comparison to mesophilic conditions (Khan et al., 2011). However, a reduction in energy for degasification should be investigated in future studies.

$T\text{-}R(CH_4)$ decreased with decreasing temperature (Figure 3b). This is attributed to the decrease in the organic matter degradation rate associated with a drop in temperature (Figures 1a and 1b) (Latif et al., 2011; Dhaked et al., 2010). As shown in Figure 3b, at low temperatures (<10°C), $R(CH_4)_{evo}$ was almost zero and $R(D\text{-}CH_4)_{dis}$ was 180 ± 35 mg COD/day from mid-December 2010 to March 2011. D-CH_4 concentrations in the DM reactor were 29 ± 9 mg COD/L from July to October

2010 and 32 ± 9 mg COD/L from mid-December 2010 to March 2011, because the solubility of D-CH_4 increased with decreasing temperature. Consequently, the D-CH_4 collection efficiency increased relatively from 57% ± 7% to 66% ± 8% with a decrease in temperature. The remaining D-CH_4 concentrations in the effluent from the DM reactor were similar to those from the treatment of high-strength synthetic wastewaters (Bandara et al., 2011) because the same type of DM and the same transmembrane pressure were applied. Increasing the transmembrane pressure (after April 25, 2011) improved the D-CH_4 collection efficiency (Figure 3b). Thus, transmembrane pressure was a critical operating parameter for the DM.

4. Conclusions

In this study, municipal wastewater was anaerobically treated with a bench-scale UASB reactor at ambient temperature (from 6°C to 31°C) for 18 months. The data presented here indicated that low-strength municipal wastewaters could be treated anaerobically at ambient temperature, although COD removal efficiency is low in winter. Hydrogenotrophic methanogens were dominant under psychrophilic conditions. A DM is useful for collecting D-CH_4 especially under psychrophilic conditions. From an economic point of view, a further reduction in the energy required for degasification is needed.

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Figure Captions

Figure 1a. Concentrations of influent T-COD and D-COD and effluent T-COD of the UASB reactor, and the T-COD removal efficiency of the UASB reactor. The dotted line indicates the day when the filter media was installed.

Figure 1b. Variation in temperature, HRT, and pH in the UASB reactor. The dotted line indicates the day when the filter media was installed.

Figure 1c. Variation in biogas production and methane gas evolution ($R(CH_4)_{evo}$) rates into the headspace of the UASB reactor. The dotted line indicates the day when the filter media was installed.

Figure 2. Phylogenetic tree of clones obtained from the UASB reactors. The tree was generated by the maximum likelihood method. The numbers in parentheses indicate the frequencies of the identical clones analyzed. The scale bar represents the number of nucleotide changes per sequence position. The symbols at each branch point show the bootstrap values obtained from 1,000 resampling based on the neighbor-joining method (left circle) and the maximum-parsimony method (right circle). Accession numbers are also indicated. Designations at the order level are bracketed on the right. The *Thermotoga* spp. sequences were used as an outgroup to rooting the tree. INO and PSY represent the granular sludge inoculum and the sludge collected from the reactor after day 416 of the operation, respectively.

Figure 3a. Variation in the D-CH_4 concentration discharged from the UASB and DM reactors. The dotted lines indicate the days when the filter media was installed (June 2010) and transmembrane pressure was increased to 97 kPa (April 2011).

Figure 3b. CH_4 evolution rate in the UASB reactor, D-CH_4 collection and discharge rates in the DM reactor, and CH_4 recovery efficiency. The dotted lines indicate the days when the filter media was installed (June 2010) and transmembrane pressure was increased to 97 kPa (April 2011).

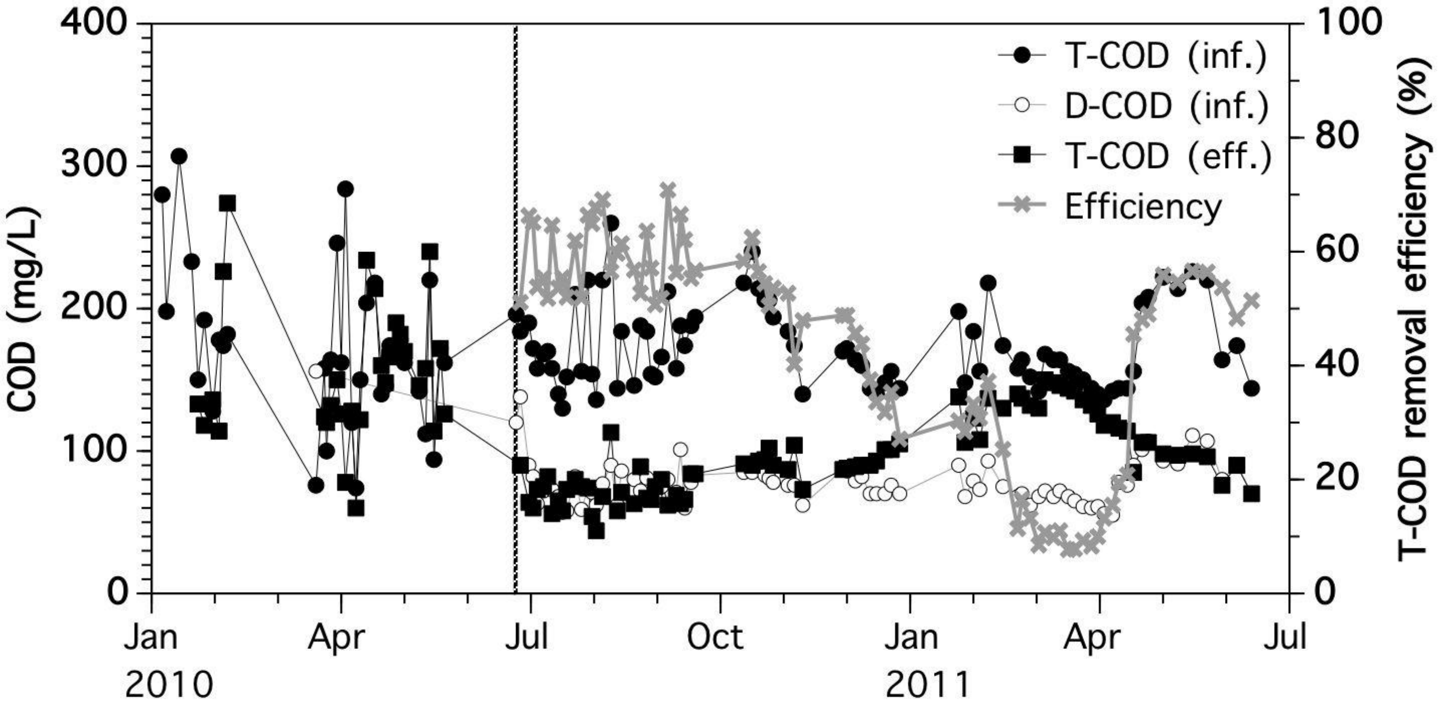

Figure 1a
Wasala et al.

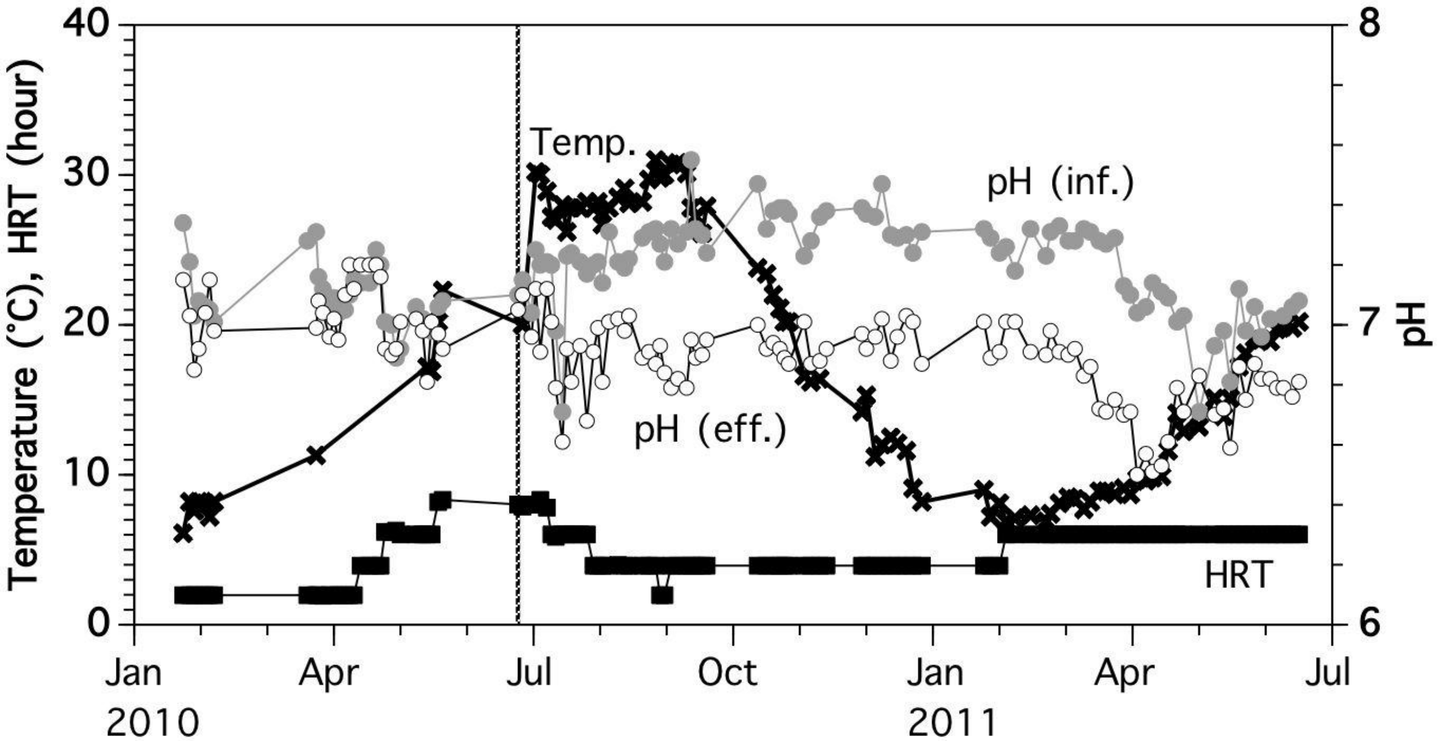

Figure 1b
Wasala et al.

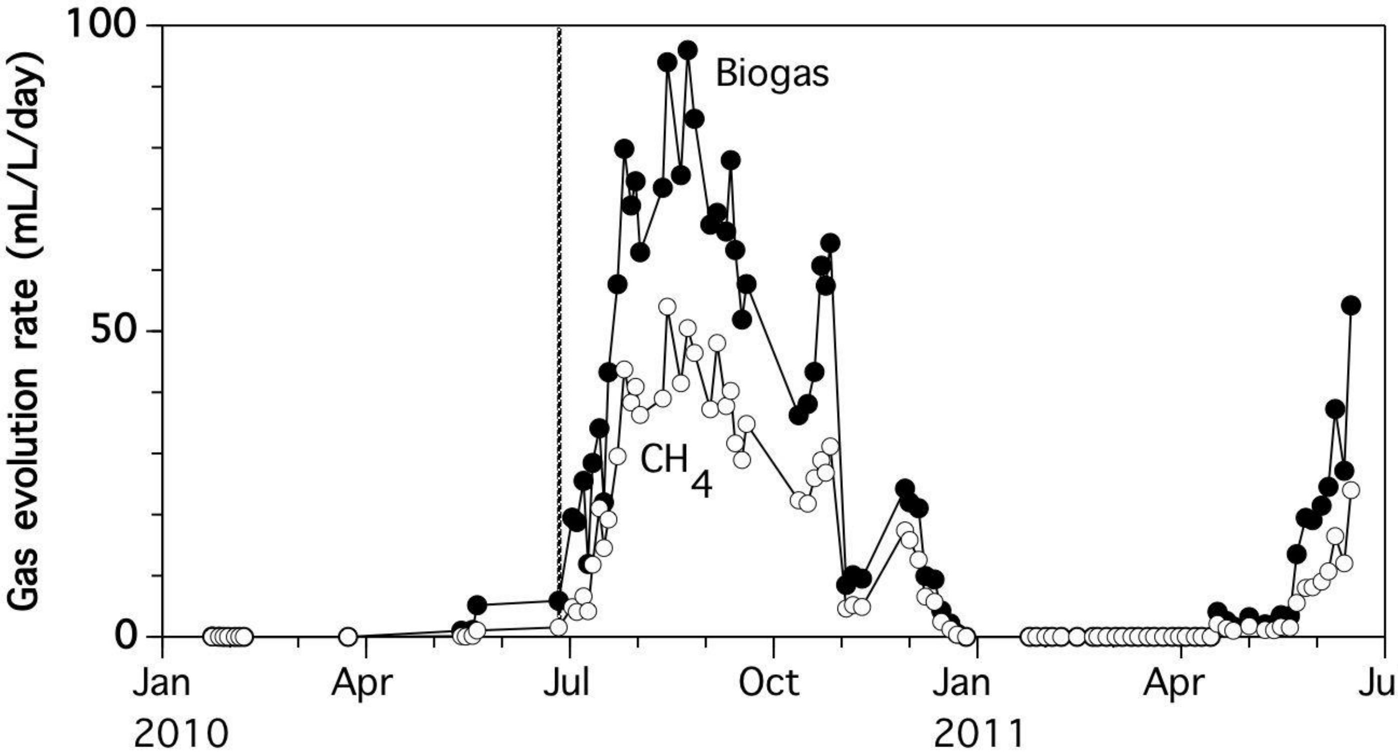

Figure 1c
Wasala et al.

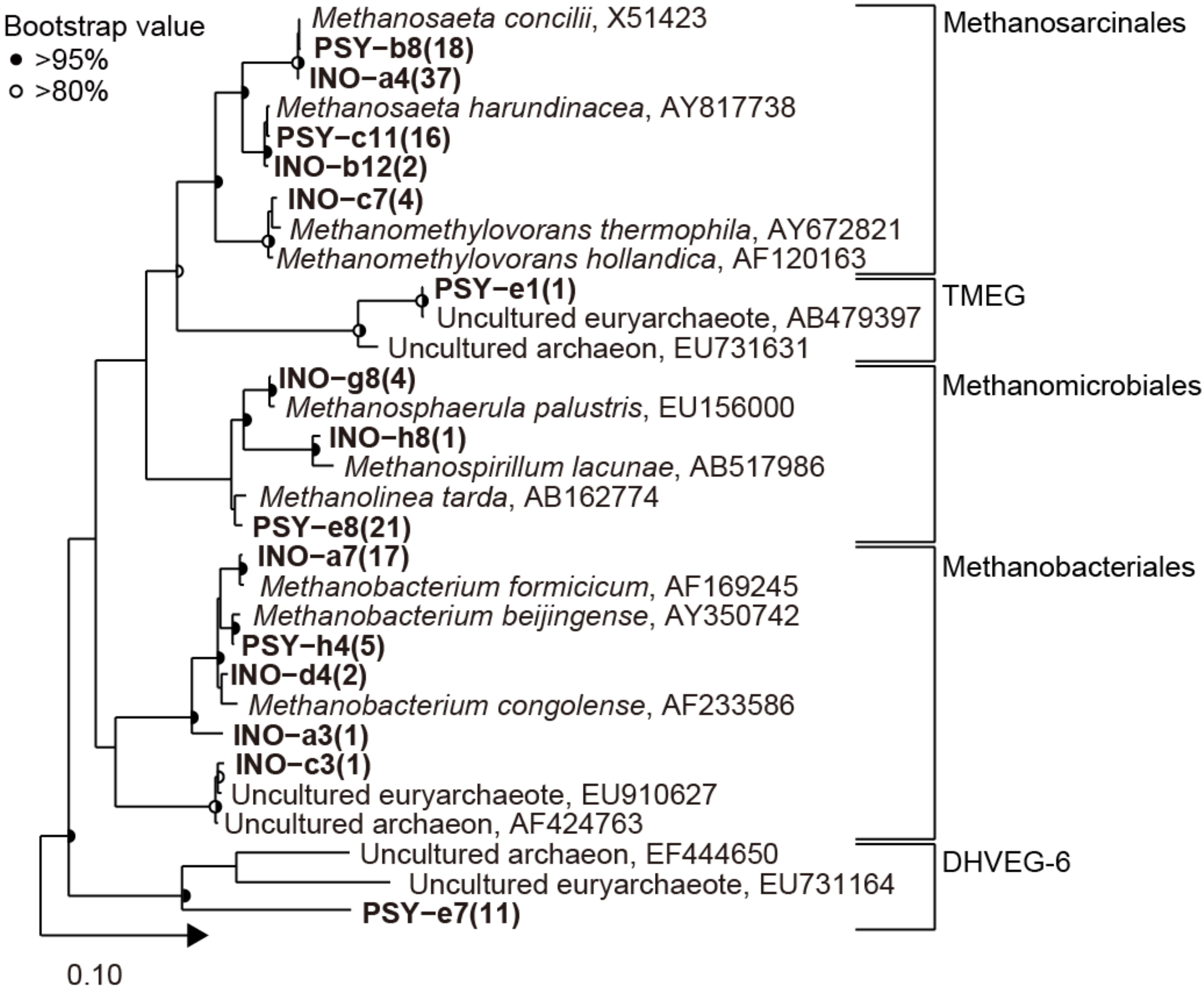

Figure 2
Wasala et al.

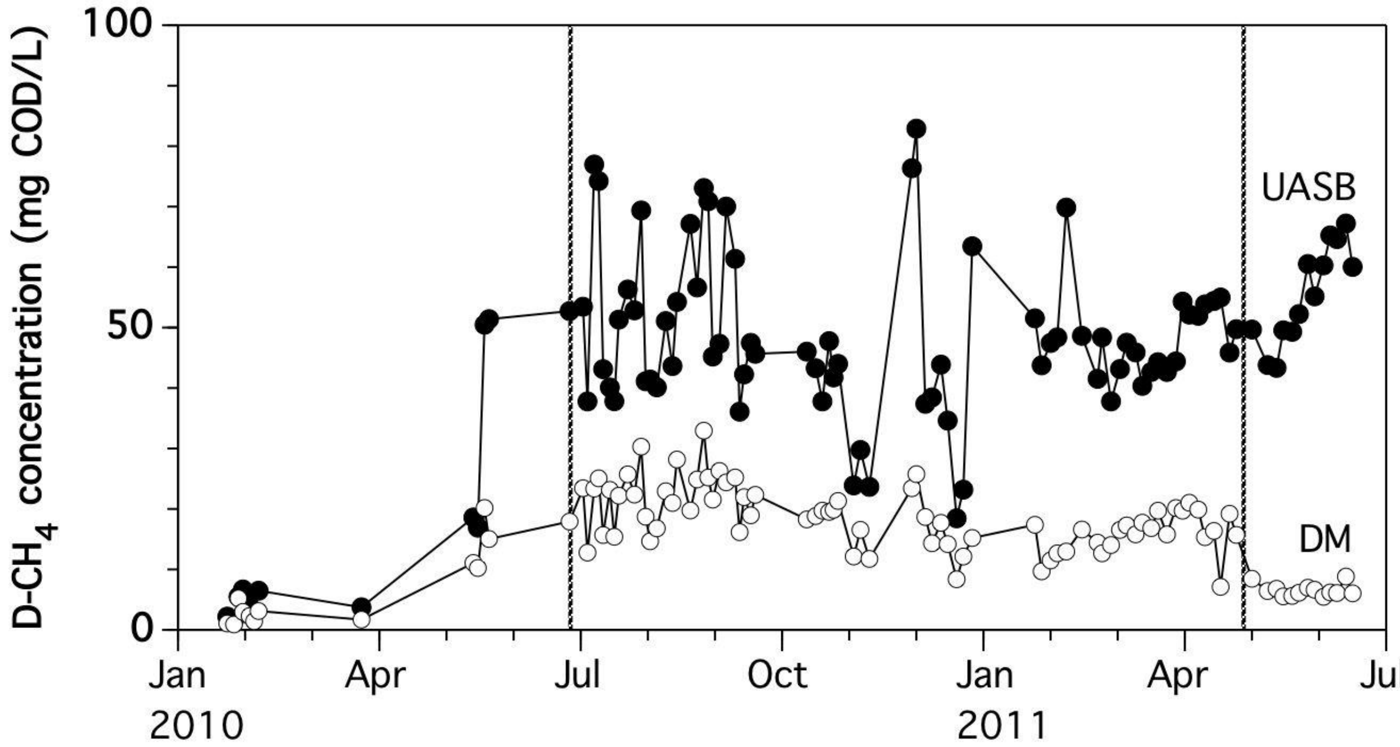

Figure 3a
Wasala et al.

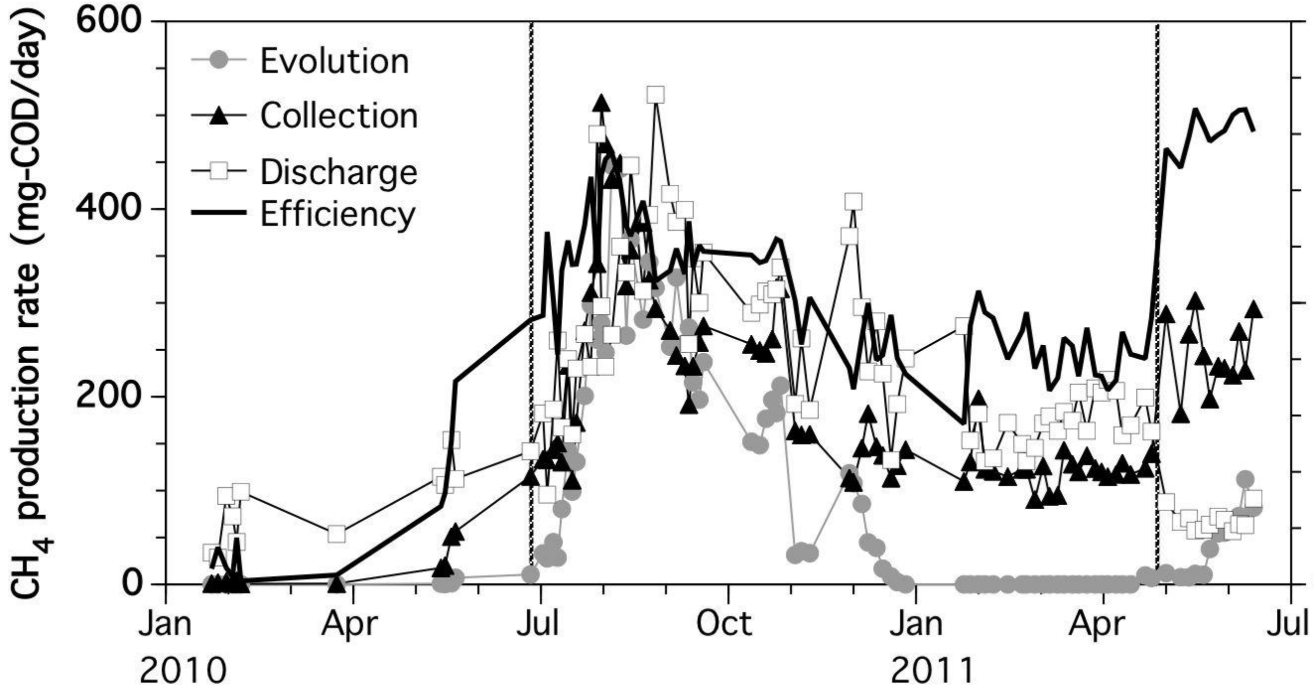

Figure 3b
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Table 1. A summary of the operating parameters and T-COD removal efficiencies of UASB reactors treating low-strength wastewaters under psychrophilic conditions.

COD concentration (mg/L)[a]	Organic loading rate (mg COD/L/day)	Temperature (°C)	HRT (h)	T-COD removal efficiency (%)	Reference
70–310	370–3740	6–31	2–8	8–71	This study
690 ± 133	191 ± 74	10	84 ± 20	51 ± 16	Luostarinen and Rintala, 2005
1500–2200	1500–2500	20	18–12	50–80	Sayed et al., 1984
115–595	-	13–25	4.7	64–72	Uemura and Harada, 2000
1247 ± 220	2560	15.5	8	23	Sawajneh et al., 2010

[a] COD concentrations are indicated as the lowest value–the highest value or as average ± standard deviation.