Methanotrophic community composition based on pmoA genes in dissolved methane recovery and biological oxidation closed downflow hanging sponge reactors

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Abstract

Dissolved methane in the effluent of anaerobic wastewater treatment processes is unrecovered and released into the atmosphere as methane, a greenhouse gas. To prevent methane emissions from effluent, a post-treatment system consisting of two closed downflow hanging sponge (DHS) reactors for the recovery and biological oxidation of dissolved methane was developed. More than 99% of the dissolved methane was completely eliminated using this system under ambient temperatures for 1 year. In this study, the methanotrophic community composition of the two closed DHS reactors was investigated. The performance of the closed DHS reactor was evaluated at different heights of the reactor in summer and winter. The clone libraries and T-RFLP analyses based on the *pmoA* gene revealed that type I and type II methanotrophs were present in the closed DHS reactors. Furthermore, type I methanotrophs showed wide diversity and contained uncultured phylogenetic clusters of methanotrophs (FWs and LWs), while type II methanotrophs were dominated by *Methylocystis*- and *Methylosarcina*-related clusters. The relative abundance of type II methanotrophs increased during winter. The type I methanotroph population dynamically changed with height of the reactor. These results demonstrate the important role of methanotrophs in removal of dissolved methane from upflow anaerobic sludge blanket effluent treating sewage.

Keywords:

Anaerobic sewage treatment

Closed downflow hanging sponge reactor

Dissolved methane

Methanotrophs

T-RFLP analysis

1. Introduction

Anaerobic wastewater treatment has been widely applied as an eco-friendly technology in terms of improved energy conservation and reduced carbon dioxide (CO_2) emissions. However, the technology has some disadvantages [1] and [2]. One of the disadvantages is that the anaerobic wastewater treatment process discharges unrecovered methane as dissolved methane in the effluent. Methane is a greenhouse gas with a 25-fold greater effect on global warming than CO_2 [3]. In the case of low-strength wastewaters such as municipal sewages with low methane production potential, the ratio of dissolved methane to the recovered methane gas significantly increases with decreasing wastewater strength [4] and [5]. Therefore, new technologies enabling economic recovery or treatment of dissolved methane are needed to reduce greenhouse gas emissions and improve anaerobic wastewater treatment technology.

In recent years, some new technologies to prevent dissolved methane emission have been reported [6], [7] and [8]. We have also developed a post-treatment system consisting of two closed downflow hanging sponge (DHS) reactors for the recovery and oxidation of dissolved methane from sewage effluents after anaerobic treatment processes [9]. The first stage closed DHS reactor is mainly involved in dissolved methane recovery as burnable gas containing over 30% methane; unrecovered residual dissolved methane is almost completely removed by methane-oxidizing bacteria, or methanotrophs, in the second stage closed DHS reactor. The two continuously closed DHS reactor system efficiently removed organic material and also allowed dissolved methane to be recovered as a useful burnable gas, preventing the methane from being emitted into the atmosphere.

Methanotrophs are divided into two main groups—type I (*Gamma-proteobacteria*) and type II (*Alpha-proteobacteria*)—based on differences in several biochemical characteristics. The distribution of methanotrophs in many environments has been well studied, as have the competitive and selective factors for type I versus type II methanotrophs [10]. Methanotrophs show great diversity and many uncultured clusters, based on *pmoA* gene (which encodes the α-subunit of particulate methane monooxygenase (pMMO)) sequences, exist in the environment [11]. The two closed DHS reactors contain a gradient of methane, oxygen and other constituents with height of the reactor. The reactors were operated under ambient temperature for 1 year to assess the effects of temperature and concentrations of key constituents on the methanotrophic community. There is considerable interest in identifying and understanding the ecology of methanotrophs playing a key role in preventing methane

emissions from the two closed DHS reactors. The results of this study will provide insight into understanding dissolved methane removal and methanotrophic ecology.

In this study, we analyzed the water quality and gas compositions with height of the closed DHS reactors in summer and winter. The methanotrophic community structure of the retained sludge responsible for oxidation of dissolved methane was sequenced based on *pmoA* genes. The diversity and distribution of methanotrophs was investigated by terminal restriction fragment length polymorphism (T-RFLP) analysis of samples from different heights in the closed DHS reactors during both seasons.

2. Material and methods

2.1 Two closed DHS reactors post-treatment system

The two closed DHS reactors for dissolved methane recovery and biological oxidation were installed in series with an upflow anaerobic sludge blanket (UASB) reactor treating actual municipal sewage (Fig. 1). Further details regarding the DHS reactors have been described previously [9]. The first and second closed DHS reactors had the same square columnar structure and size. The volume of sponge containing microbes was 35.2 L, accounting for 44% of the volume of the reactor. The reactors had differences in air supply direction and rate. In the first closed DHS reactor, air was supplied from the bottom of the reactor to recover dissolved methane by physical gasification at 250 $L \cdot m^{-3} \cdot day^{-1}$ based on the reactor volume. The off-gas with methane was recovered from the top of the reactor. In the second closed DHS reactor, air was fed from the top at a rate of 2500 $L \cdot m^{-3} \cdot day^{-1}$ to oxidize the unrecovered dissolved methane and complete the wastewater polish-up. The exhaust gas was released from the bottom to the atmosphere. The system was operated under ambient temperature conditions, and the daily average temperature ranged from 10°C to 28°C.

2.2 Analytical methods

Chemical oxygen demand (COD), ammonium nitrogen, nitrate, pH, dissolved methane, oxygen gas and methane gas concentrations were measured as previously described [12] to evaluate the methanotrophic conditions with height of the closed DHS reactors during summer and winter. Ammonium and nitrate were analyzed by high-performance liquid chromatography (HPLC; Shimadzu LC 20-ADsp). Gas samples were collected from the top, middle (Port 2 in the first closed DHS reactor or Port 5 in the second closed DHS reactor) and bottom of each closed DHS reactor. The gas composition was analyzed using a gas

chromatograph equipped with thermal conductivity detector (GC-TCD; Shimadzu GC-8A).

2.3 Sludge sampling and DNA extraction

Sludge samples were squeezed and collected from the upper, middle, and lower parts of sponges from each reactor on day 110 during summer and day 261 in winter. The collected sludge samples were washed with phosphate buffer. DNA from the washed sludges was extracted using a Fast DNA spin kit for soil (MP Biomedicals, Irvine, CA, USA) according to the manufacturer's instructions.

2.4 Phylogenetic analysis based on *pmoA* genes

Extracted DNA was used for amplification of *pmoA* gene fragments with the primer pair A189f/mb661 [13]. PCR amplification was performed using the ONE Shot LA PCR MIX (TAKARA BIO, Otsu, Japan). PCR amplification of *pmoA* genes was conducted by subjecting the samples to 5 min of initial denaturation at 94°C, followed by 25 cycles of 30 s at 94°C, 30 s at 56°C and 1 min at 72°C. The final extension step was at 72°C for 4 min. The PCR products were electrophoresed on a 1% (w/v) agarose gel, after which they were purified with a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany).

The PCR products were cloned using the TOPO XL PCR Cloning Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Cloned *pmoA* genes were randomly picked from each clone library and subjected to sequencing from both ends at the Dragon Genomics Center (Takara Bio, Yokkaichi, Japan). Chimeric sequences were identified and removed using Bellerophon [14]. The *pmoA* gene sequences were classified using the FastGroupII program [15]. Fragments with 98% sequence identity were considered unique operational taxonomic units [16]. A phylogenetic tree was constructed using the *pmoA* database, which includes 6628 reference sequences corresponding to 53 low-level taxa [17], with the ARB program package [18]. Sequences from samples were imported into the ARB program and the phylogenetic tree of translated PmoA amino acid sequences was reconstructed based on the neighbor-joining method implemented in the ARB program. Bootstrap resampling analysis for 1000 replicates was conducted to estimate the confidence of tree topologies. The *pmoA* gene sequences obtained in this study were deposited in the GenBank/EMBL/DDBJ databases under accession numbers LC201876 to LC201919.

2.5 T-RFLP analysis of *pmoA* genes

Terminal restriction fragment length polymorphism (T-RFLP) analyses were used to analyze the diversity of methanotrophic communities. The fragments of *pmoA* genes were amplified using primer pair A189f/mb661r with the A189f primer labeled with Beckman-dye D4. The PCR products were digested at 37°C for 2 h by the restriction endonuclease *Msp*I. All the digested fragments were analyzed on the CEG-2000XL (Beckman Coulter, Fullerton, CA) as previously described [19].

3. Results

3.1 System performances

The two closed DHS reactors were operated at a sewage treatment plant without temperature control (10°C–28°C) for approximately 1 year. A detailed description of the system's performance is presented elsewhere [9]. Water quality in the wastewater and gas compositions were measured every day for one week prior to taking sludge samples to evaluate changes in the methanotrophic communities with height of the reactor in summer and winter (Table 1). The average temperatures were 26.7°C in summer and 12.2°C in winter.

The first closed DHS reactor was operated at a low air flow rate of 250 L m^{-3} d^{-1} for dissolved methane recovery. Methane and oxygen, except for those in the inlets, were sampled directly using a gas-tight syringe from the reactor sampling port or exhaust gas. About 40% of the methane gas concentration was recovered as burnable gas on the top of the first closed DHS reactor at 4 m. In the middle port, at 3 m the methane concentrations reached 32% during summer and 30% in winter (Fig. 2(A)). Oxygen concentrations were below detection limits (< 0.1%) at both sampling points, even though air was supplied to the reactor (Fig. 2(B)). In the first DHS reactor, the sulfate concentration increased from 5.1–5.9 mg SO_4^{2-} L^{-1} in the influent to 18–19 mg SO_4^{2-} L^{-1} in the effluent. About 5.5 g SO_4^{2-} day^{-1} of sulfate was produced in the first DHS reactor, which corresponded to about 60% of the oxygen in the supplied air being consumed (Table 1).

In the second closed DHS reactor, air was supplied from the top of the column at a low air flow rate of 2500 L m^{-3} d^{-1} and the exhaust gas was emitted from the bottom of the column. Air and dissolved methane from the first closed DHS effluent were mixed in the top of the column. The mixing methane concentration at the inlet point could not be directly measured; therefore, the methane concentrations at that point were theoretical values in which influent dissolved methane was assumed not to undergo degradation. The calculated values were 1.4% during summer and 2.1% in winter (Fig. 2(A)). Methane gas concentrations

rapidly decreased in the top of the second DHS reactor; the methane concentrations in the middle of the reactor (at 1 m) were 150 ppm during summer and 2570 ppm in winter. The final methane gas concentrations in the bottom of the reactor (at 0 m) were below the detection limit (<100 ppm) during summer and 270 ppm in the winter. Seasonal fluctuations in the oxygen concentrations tended to be similar to those for the methane concentrations. The final off-gas contained oxygen at 2.3% in summer and 8.6% during winter.

3.2 Methanotrophic communities based on *pmoA* genes

Methane oxidation actively occurred in the second DHS reactor based on evidence of reactor performance and methane oxidation activity [12]. The methanotrophic community based on *pmoA* gene cloning was constructed from port 4 to port 6 of the second closed DHS sludge samples in summer and winter (Fig. 3). A wide variety of methanotrophs, including type I and II methanotrophs, were detected in the DHS reactor. Type I methanotrophs were mainly composed of six groups. *Methylosarcina* and *Methylomicrobium* groups were dominant in the summer, whereas *Methyloparacoccus* and Uncultured methanotrophs FWs cluster were detected during winter. An uncultured methanotrophs LWs cluster was detected in both seasons. Type II methanotrophs were dominated by the *Methylocystis* and *Methylosinus* groups in both seasons. The proportions of type II methanotrophs increased from 18% during winter to 44% in summer.

3.3 T-RFLP analysis

To determine changes in the methanotrophic community with height of the reactor, sludge samples taken from ports 1 to 6 of the two closed DHS reactors in summer and winter were analyzed by *pmoA* gene based T-RFLP (Fig. 4, Fig. S1). T-RFLP analysis with *Msp*I permitted assignment of a putative taxonomic affiliation to the dominant T-RFs in the profile through comparison with *pmoA* sequences in the NCBI database (Fig. 4). The T-RFLP profiles of sludge samples in the two-closed DHS reactors changed dramatically with height of the reactor and season. Enzymatic digestion of the *pmoA* amplicon with *Msp*I produced four detectable T-RFs (77, 244, 351 and 443 bp) and an undigested fragment (515 bp). In the first DHS reactor, the 443 bp fragment of the *Methylomonas* group dominated during summer and the population did not change significantly with reactor height. In winter, the methanotrophic community in the reactor was dominated by the 224 bp T-RF of *Methylosystis*/*Methylosinus*. In the second DHS reactor, the composition of the

methanotrophic population changed dynamically with height Five T-RFs (77, 241, 351, 443 and 515 bp T-RFs) were detected in the top of the second DHS reactor (port 4). The 77 bp T-RF of the methanotrophic community represented several diverse groups, including *Methyloparacoccus* and uncultured methanotrophs (FWs and LWs). In summer, the abundance of this population gradually increased from port 4 (14%) to port 6 (57%). Conversely, the relative abundance of the 351 bp T-RF of *Methylomicrobium* gradually decreased from 22% to 5% toward the lower portion of port 6. The trends in winter were the same as those during summer, but the 515 bp T-RF of *Methylobacter*/*Methylomicrobium* and the 244 bp T-RF of *Methylosystis*/*Methylosinus* did not increase significantly during winter.

4. Discussion

Psychrophilic species capable of methane oxidation have been reported as type I and type II methanotrophs [20]. However, there is little information available regarding the effects of temperature on methanotrophic community compositions in the environment. Mohanty et al. [21] reported the effects of temperature on composition of the methanotrophic community in rice fields and forest soil. The relative abundance of the type II methanotrophic population in the rice field decreased as temperature decreased from 25°C to 10°C, while the methanotrophic population in the forest soil showed very little change. Column tests using landfill-cover soil revealed that the relative abundance of type II methanotrophs was higher at 20°C than at 4°C and 12°C [22]. Our results showed for the first time that the relative abundance of the type II methanotrophic population increased during winter at temperatures of about 12°C. In particular, the type I and type II methanotrophic populations were affected by temperature under extremely low oxygen conditions in the first DHS reactor, suggesting that type II methanotrophs contributed more to dissolved methane removal in sewage in winter than in summer.

The second DHS reactor was maintained under aerobic conditions for methanotrophs in both seasons because air was supplied at a rate 10-times higher than in the first DHS reactor (Fig. 2). The relative abundance of type I and type II methanotrophs is affected by the availability of oxygen and methane. Type I methanotrophs dominated under low methane and high oxygen concentrations, whereas type II methanotrophs favored high methane and low oxygen concentrations [23]. Methane concentration had a greater effect on type I and type II methanotroph communities than oxygen concentration [24]. The data from

the second reactor indicated that the oxygen concentration ranged from 20% to 5% and the methane concentration ranged from 2% to 0.01%, corresponding to high oxygen and low methane conditions as previously reported [23] and [24]. Type I methanotrophs outcompeted the type II methanotrophs in the second DHS reactor (Figs. 3 and 4). The ratio of type I to type II the methanotrophs did not change substantially with position in the second DHS reactor, despite substantial differences in methane and oxygen concentrations. These results suggest that the DHS reactor conditions were not favorable for type II methanotrophs, because methane and oxygen were used simultaneously throughout the height of the reactor.

The type I methanotrophic community, except the 443 bp T-RF group, changed dramatically at every point in the second DHS reactor (Fig. 4). *Methylomonas* species, including the 443 bp T-RF group, were detected in a denitrification reactor with oxygen concentrations of <1 mg O_2/L [25]. Araki et al. [26] demonstrated that a DHS sponge material maintained aerobic conditions down to a depth of 0.75 cm from the surface, beyond which the environment became anoxic. Methanotrophs corresponding to the 443 bp T-RF fragment might be active in methane removal inside the sponge material in the second DHS reactor. The 351 bp T-RF was detected at the top of the reactor and the relative abundance of this T-RF gradually decreased toward the lowest port in the second DHS reactor at port 6. It was reported that *Methylomicrobium album*, belonging to the 351 bp T-RF group, could grow under low methane concentrations of 100 ppm [27], which is in contrast to the results obtained in this study (Fig.4). The 351 bp T-RF group could not be detected in the first DHS reactor under low oxygen concentrations (Fig. 4). The results show that this group responded most strongly to oxygen concentration. Thus, it is likely that members of the 351 bp T-RF group were not affected by methane concentration, but were mainly affected by oxygen concentration in the closed DHS reactors.

Conversely, the abundance of the 77 bp T-RF gradually increased toward the lowest port during both seasons; a population ratio of 36–57% was reached in the lower portion of the second closed DHS reactor at port 6, which was characterized by very low methane concentrations. Sewage contains ammonium at about 30 mg N/L, and nitrate was produced to a concentration of 3–7 mg N/L by ammonium and nitrite oxidizers in the second DHS reactor (Table 1). Isolates of some species in the 77 bp T-RF group were known to be able to use ammonia and nitrate as nitrogen sources [28], [29], [30] and [31]. The 77 bp T-RF group was the dominant methanotroph detected in activated sludge treating sewage [32]. Hatamoto et al. [8] investigated the methanotrophic community structure in a closed DHS reactor treating

dissolved methane and ammonium. The 77 bp T-RF group of methanotrophs was dominant in the presence of low methane concentrations and nitrate in the bottom of the reactor. An increase in the relative abundance of this group in the bottom of the reactor might be related to nitrate concentrations, as demonstrated by our water quality profile data, the T-RFLP profiles in this study and the results of the abovementioned reports. Interestingly, sequenced clones of the 77 bp T-RF group were assigned to uncultured LWs and FWs clusters (Fig. 3). The clones in the uncultured LWs cluster were detected in both seasons, whereas the clones in the uncultured FWs cluster were mainly present during winter. These data are important to understanding the ecology of uncultured methanotrophs within the 77 bp T-RF group. Thus, this group includes uncultured methanotrophs that might play a key role in oxidizing low methane concentrations in the DHS reactor treating sewage.

5. Conclusions

This study showed that influent dissolved methane was quickly removed in the top of the methane oxidation DHS reactor by methanotrophs during summer and winter. Type I and type II methanotrophs were found in the closed DHS reactors, and the relative abundance of type II methanotrophs increased during winter when compared with summer. The type I methanotrophic population changed dramatically with reactor height based on the concentrations of methane and oxygen. These findings indicate the importance of the methanotrophic population for stabilization of methane removal in the methane oxidation DHS reactor.

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Figure captions

Fig. 1 Two-stage closed downflow hanging sponge (DHS) reactor for dissolved methane recovery and biological dissolved methane oxidation.

Fig. 2 Profiles of methane (A) and oxygen (B) concentrations in the gas phase with height of the two closed downflow hanging sponge (DHS) reactors. Methane concentrations at the inlet point were theoretically calculated from the influent dissolved methane and inlet air. Oxygen concentrations at the inlet point are those of supplied air.

Fig. 3 Phylogenetic tree of the derived amino acid sequences (146 amino acids) of *pmoA* genes from summer and winter from the second closed downflow hanging sponge (DHS) reactor. The sludge samples were taken from three parts of the reactor (upper, middle, and lower) and the extracted DNA was mixed and used for cloning. The measured terminal-restriction fragment (T-RF) length of phylotypes digested with *Msp*I is indicated. The scale bar represents the number of nucleotide changes per sequence position. The symbols at each branch point show the bootstrap values obtained with 1000 resamplings. Ten ammonia oxidizing gene (*amoA*) sequences were used as the outgroup, but were removed for presentation.

Fig. 4 Terminal restriction fragment length polymorphism (T-RFLP) analyses of *pmoA* gene populations present in the two closed downflow hanging sponge (DHS) reactors in summer (A) and winter (B). Data show the relative contribution of each terminal restriction fragment (T-RF) to the total signal intensity.

Table 1 Overview of reactor performance during summer and winter.

	Summer							Winter						
	Temp.	pH	COD [a]	Sulfate	Ammonia	Nitrate	Dissolved CH_4	Temp.	pH	COD [a]	Sulfate	Ammonia	Nitrate	Dissolved CH_4
	°C	–	mg COD·L^{-1}	mg SO_4^{2-}·L^{-1}	mg N·L^{-1}	mg N·L^{-1}	mg CH_4·L^{-1}	°C	–	mg COD·L^{-1}	mg SO_4^{2-}·L^{-1}	mg N·L^{-1}	mg N·L^{-1}	mg CH_4·L^{-1}
UASB [b] effluent	26.7 ± 0.2	6.6	122 ± 22	5.9 ± 0.6	27 ± 2	N.D. [d]	17.6 ± 0.8	12.2 ± 0.6	6.8	128 ± 25	5.1 ± 0.3	28 ± 2	N.D. [d]	19.8 ± 0.8
First DHS [c] effluent		6.5	106 ± 12	19 ± 1	27 ± 2	N.D. [d]	5.1 ± 0.7		6.7	125 ± 14	18 ± 2	28 ± 2	N.D. [d]	7.3 ± 0.4
Second DHS [c] effluent		6.1	44 ± 5	30 ± 3	18 ± 3	7.0 ± 1.2	N.D. [e]		6.4	68 ± 10	32 ± 3	24 ± 2	3.5 ± 0.6	0.05 ± 0.02

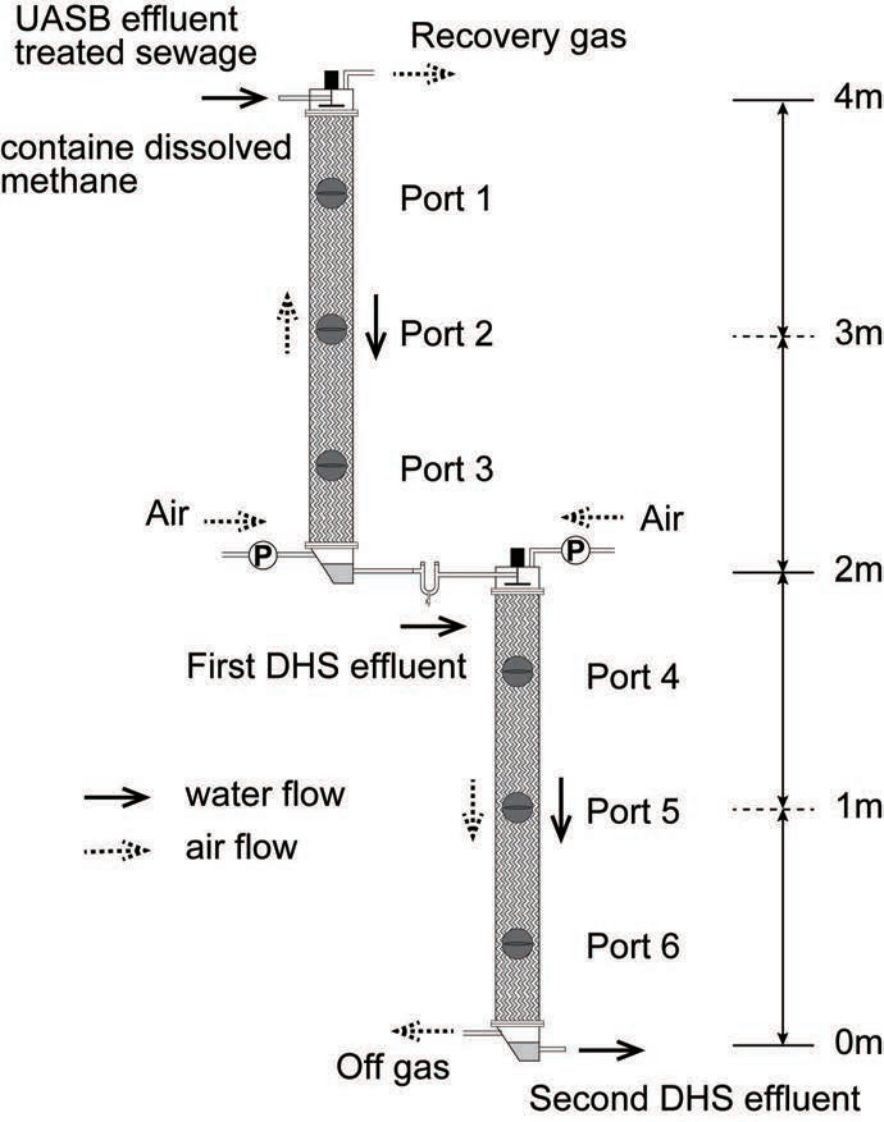
a Chemical Oxygen Demand

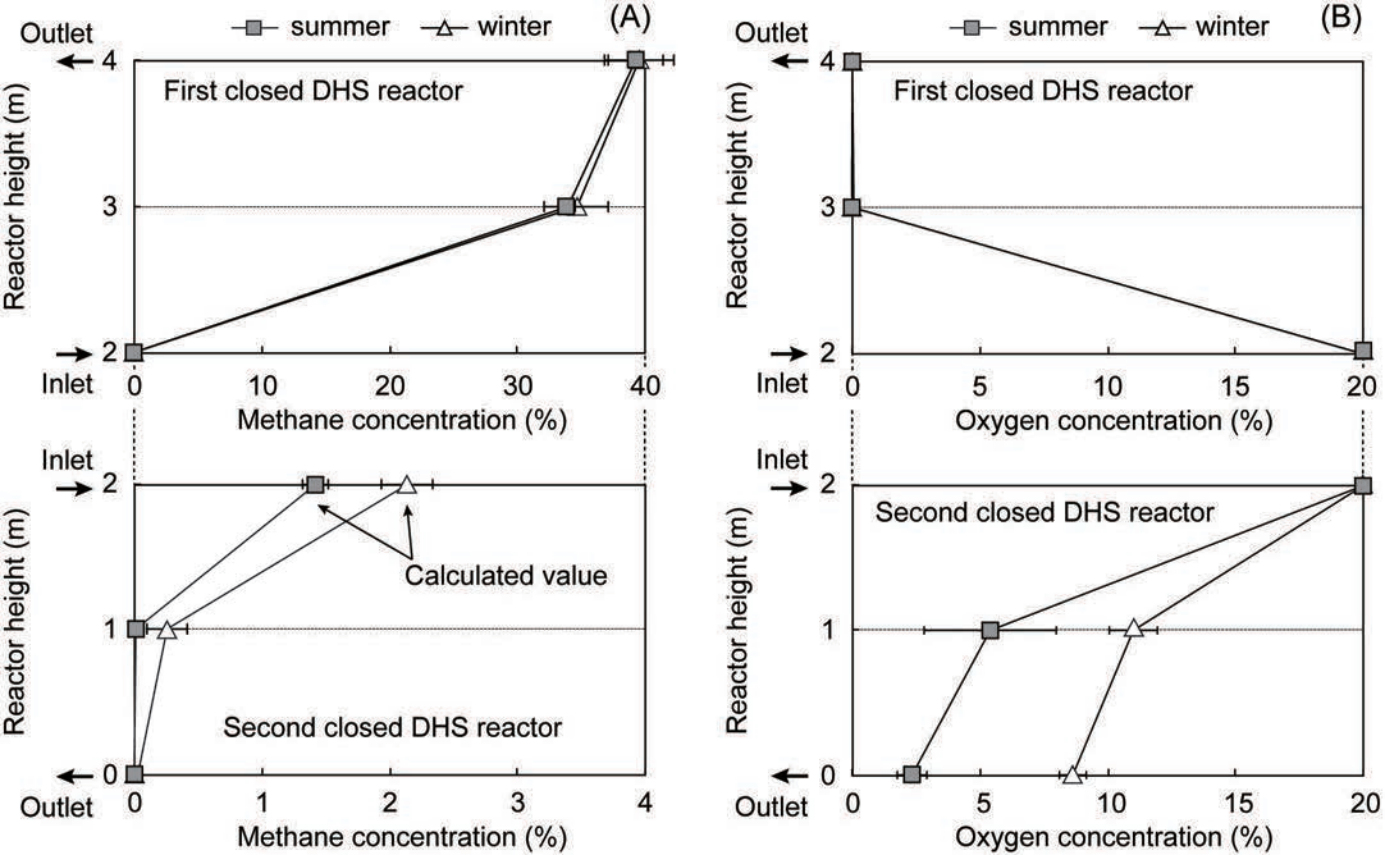
b Upflow Anaerobic Sludge Blanket

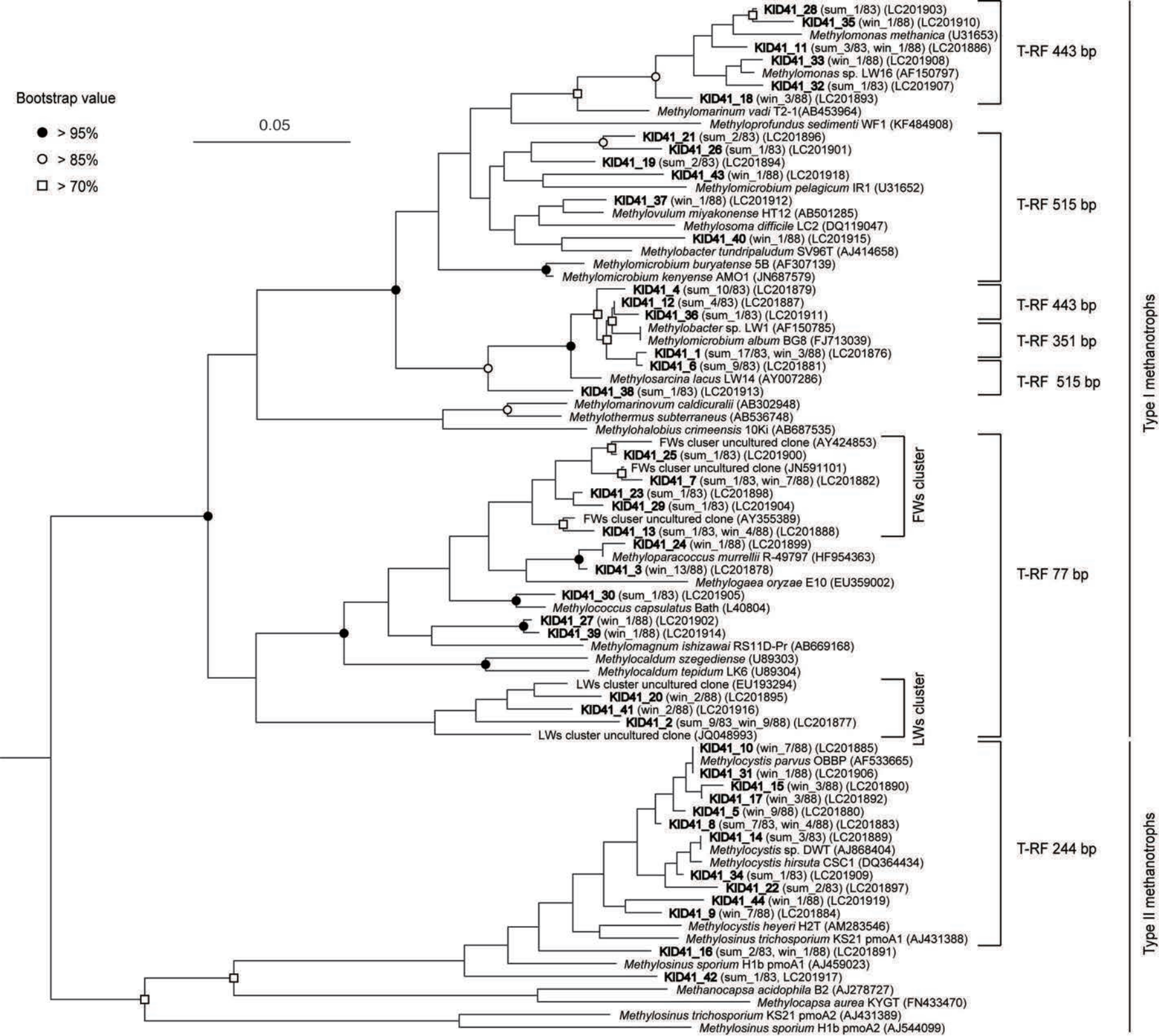
c Downflow Hanging Sponge

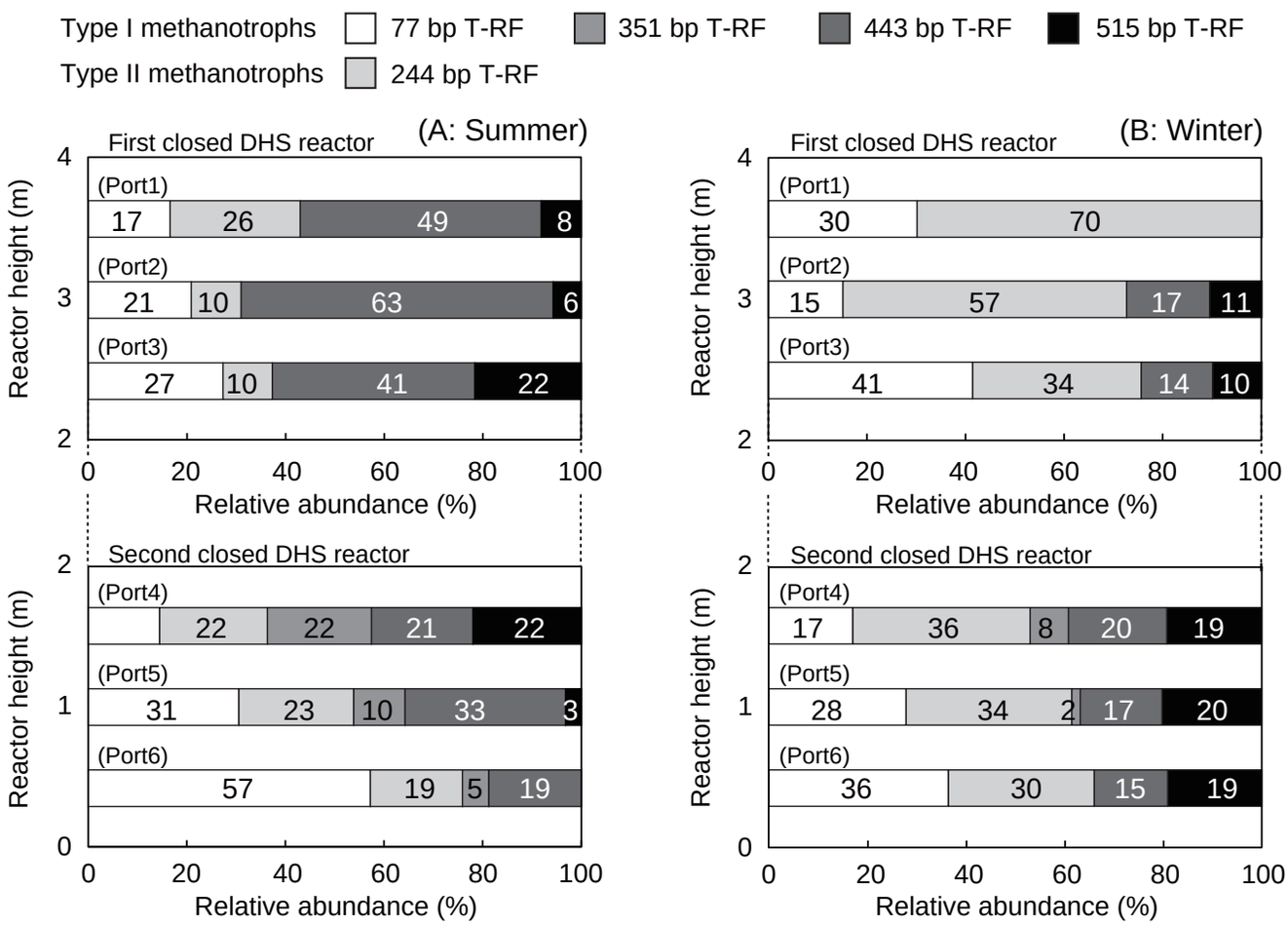
d high-performance liquid chromatography detection limit is 0.05 mg N·L^{-1}

e gas chromatography detection limit is 100 ppm corresponding to 0.02 mgCH_4·L^{-1}


UASB effluent
treated sewage
Recovery gas
containe dissolved
methane
Port 1
Port 2
Port 3
Air
Air
P
P
First DHS effluent
Port 4
water flow
air flow
Port 5
Port 6
Off gas
Second DHS effluent
4m
3m
2m
1m
0m


(A)
summer
winter
Outlet
4
First closed DHS reactor
Reactor height (m)
3
2
Inlet
0
10
20
30
40
Methane concentration (%)
Inlet
2
Calculated value
1
Second closed DHS reactor
0
Outlet
0
1
2
3
4
Methane concentration (%)
(B)
summer
winter
Outlet
4
First closed DHS reactor
Reactor height (m)
3
2
Inlet
0
5
10
15
20
Oxygen concentration (%)
Inlet
2
Second closed DHS reactor
Reactor height (m)
1
0
Outlet
0
5
10
15
20
Oxygen concentration (%)


Bootstrap value
> 95%
> 85%
> 70%
0.05
KID41_28 (sum_1/83) (LC201903)
KID41_35 (win_1/88) (LC201910)
Methylomonas methanica (U31653)
KID41_11 (sum_3/83, win_1/88) (LC201886)
KID41_33 (win_1/88) (LC201908)
Methylomonas sp. LW16 (AF150797)
KID41_32 (sum_1/83) (LC201907)
KID41_18 (win_3/88) (LC201893)
Methylomarinum vadi T2-1(AB453964)
Methyloprofundus sedimenti WF1 (KF484908)
KID41_21 (sum_2/83) (LC201896)
KID41_26 (sum_1/83) (LC201901)
KID41_19 (sum_2/83) (LC201894)
KID41_43 (win_1/88) (LC201918)
Methylomicrobium pelagicum IR1 (U31652)
KID41_37 (win_1/88) (LC201912)
Methylovulum miyakonense HT12 (AB501285)
Methylosoma difficile LC2 (DQ119047)
KID41_40 (win_1/88) (LC201915)
Methylobacter tundripaludum SV96T (AJ414658)
Methylomicrobium buryatense 5B (AF307139)
Methylomicrobium kenyense AMO1 (JN687579)
KID41_4 (sum_10/83) (LC201879)
KID41_12 (sum_4/83) (LC201887)
KID41_36 (sum_1/83) (LC201911)
Methylobacter sp. LW1 (AF150785)
Methylomicrobium album BG8 (FJ713039)
KID41_1 (sum_17/83, win_3/88) (LC201876)
KID41_6 (sum_9/83) (LC201881)
Methylosarcina lacus LW14 (AY007286)
KID41_38 (sum_1/83) (LC201913)
Methylomarinovum caldicuralii (AB302948)
Methylothermus subterraneus (AB536748)
Methylohalobius crimeensis 10Ki (AB687535)
FWs cluser uncultured clone (AY424853)
KID41_25 (sum_1/83) (LC201900)
FWs cluser uncultured clone (JN591101)
KID41_7 (sum_1/83, win_7/88) (LC201882)
KID41_23 (sum_1/83) (LC201898)
KID41_29 (sum_1/83) (LC201904)
FWs cluser uncultured clone (AY355389)
KID41_13 (sum_1/83, win_4/88) (LC201888)
KID41_24 (win_1/88) (LC201899)
Methyloparacoccus murrellii R-49797 (HF954363)
KID41_3 (win_13/88) (LC201878)
Methylogaea oryzae E10 (EU359002)
KID41_30 (sum_1/83) (LC201905)
Methylococcus capsulatus Bath (L40804)
KID41_27 (win_1/88) (LC201902)
KID41_39 (win_1/88) (LC201914)
Methylomagnum ishizawai RS11D-Pr (AB669168)
Methylocaldum szegediense (U89303)
Methylocaldum tepidum LK6 (U89304)
LWs cluster uncultured clone (EU193294)
KID41_20 (win_2/88) (LC201895)
KID41_41 (win_2/88) (LC201916)
KID41_2 (sum_9/83_win_9/88) (LC201877)
LWs cluster uncultured clone (JQ048993)
KID41_10 (win_7/88) (LC201885)
Methylocystis parvus OBBP (AF533665)
KID41_31 (win_1/88) (LC201906)
KID41_15 (win_3/88) (LC201890)
KID41_17 (win_3/88) (LC201892)
KID41_5 (win_9/88) (LC201880)
KID41_8 (sum_7/83, win_4/88) (LC201883)
KID41_14 (sum_3/83) (LC201889)
Methylocystis sp. DWT (AJ868404)
Methylocystis hirsuta CSC1 (DQ364434)
KID41_34 (sum_1/83) (LC201909)
KID41_22 (sum_2/83) (LC201897)
KID41_44 (win_1/88) (LC201919)
KID41_9 (win_7/88) (LC201884)
Methylocystis heyeri H2T (AM283546)
Methylosinus trichosporium KS21 pmoA1 (AJ431388)
KID41_16 (sum_2/83, win_1/88) (LC201891)
Methylosinus sporium H1b pmoA1 (AJ459023)
KID41_42 (sum_1/83, LC201917)
Methanocapsa acidophila B2 (AJ278727)
Methylocapsa aurea KYGT (FN433470)
Methylosinus trichosporium KS21 pmoA2 (AJ431389)
Methylosinus sporium H1b pmoA2 (AJ544099)
T-RF 443 bp
T-RF 515 bp
T-RF 443 bp
T-RF 351 bp
T-RF 515 bp
FWs cluster
LWs cluster
T-RF 77 bp
T-RF 244 bp
Type I methanotrophs
Type II methanotrophs


Type I methanotrophs
77 bp T-RF
351 bp T-RF
443 bp T-RF
515 bp T-RF
Type II methanotrophs
244 bp T-RF
(A: Summer)
(B: Winter)
First closed DHS reactor
Second closed DHS reactor
Reactor height (m)
Relative abundance (%)
(Port1)
(Port2)
(Port3)
(Port4)
(Port5)
(Port6)
17 26 49 8
21 10 63 6
27 10 41 22
22 22 21 22
31 23 10 33 3
57 19 5 19
30 70
15 57 17 11
41 34 14 10
17 36 8 20 19
28 34 2 17 20
36 30 15 19