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Characterization of methane, benzene and toluene-oxidizing consortia enriched from landfill and riparian wetland soils

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ABSTRACT

The microbial oxidations of methane (M) and volatile organic compounds (VOCs) were compared with those of M and VOCs alone after enriching soil samples with M and/or VOCs. Landfill cover and riparian wetland soils from which M and VOCs were simultaneously emitted were selected as representative samples. Benzene (B) and toluene (T) were employed as the model VOCs. With the landfill soil consortia, the rate of M oxidation decreased from 4.15–5.56 to 2.26–3.42 μmol g-dry soil^{-1} h^{-1} in the presence of both B and T, but with the wetland soil consortia the rate of M oxidation (3.09 μmol g-dry soil^{-1} h^{-1}) in the mixture of M as well as both B and T was similar to that of M alone (3.04 μmol g-dry soil^{-1} h^{-1}). Compared with the methanotrophic community with M alone, the portion of type II methanotrophs was greater in the landfill consortia; whereas, the proportion in wetland consortia was less in the presence of both B and T. The oxidations of B and T were stimulated by the presence of M with both the landfill and wetland consortia. There were no correlations between the oxidation rate of M and those of B and T with the gene copy numbers of *pmoA* and *tmoA* responsible for the oxidations.

1. Introduction

Methane (M) is the second most abundant greenhouse gas after CO_2, and contributes to approximately 18% of atmospheric radiative forcing [1,2]. M is mainly emitted by the anaerobic degradation of organic compounds via methanogens [3], and volatile organic compounds (VOCs) are simultaneously emitted with M during this process [4,5].

Wetlands, such as swamps, bogs and rich paddies, are regarded as representative M emission sources. The global emissions of M from wetlands have been estimated to be between 92 and 225 Tg CH_4 year^{-1} [6]. As with wetlands, landfills are also major sources of M emission, and contribute to approximately 35% of the anthropogenic M emissions in the US and between 5 and 10% of the global M emissions to the atmosphere [7].

M is mainly eliminated in the troposphere through the reaction with OH radicals (490 ± 85 Tg year^{-1}), and its oxidation by soil microbes, including methanotrophs, has been estimated at approximately 30 ± 15 Tg year^{-1} [8]. Therefore, the oxidation of M by methanotrophs plays an important role in the mitigation of major M emission sources, such as wetlands and landfills [9–11].

Most previous studies have been focused on the performance and methanotroph dynamics of the oxidation of M in wetland and landfill soils. However, there have been few reports on the effects of VOCs on the oxidation of M or vice versa, even though M and VOCs are simultaneously emitted from a variety of environments [10]. Information on the biodegradation of a mixture of M and VOCs would be helpful in understanding the *in situ* microbial degradations of M and VOCs. Therefore, in this study, the microbial oxidation of mixtures of M and VOCs was compared with those of M or VOCs alone. Landfill and wetland soils, which are sources of simultaneous M and VOCs emissions, were selected as representative soil samples. Benzene (B) and toluene (T) were employed as the model VOCs. Each soil sample was enriched with M and/or B and T as the sole carbon and energy sources. The rates of oxidation of M as well as B and T by the resulting enriched consortia (M, B and T, and M, B and T – oxidizing consortia) were compared. The abundances of bacteria associated with the oxidations of M, as well as B and T in the enriched consortia were compared using quantitative real-time PCR (qRT-PCR), employing primer sets for the *pmoA* and *tmoA* functional genes encoding for particulate methane monooxygenase (pMMO) and toluene monooxygenase, respectively [12,13]. To characterize the bacterial communities responsible for the oxidations of M, as well as B and T in the enriched consortia, the communities were qualitatively analyzed, after amplification of the *pmoA* and *tmoA* genes, using a terminal restriction fragment length polymorphism (T-RFLP) method.

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2. Materials and methods

2.1. Soil sampling

Landfills A and B (LCA and LCB, respectively) and riparian wetland (RW) soils were sampled to obtain the M-, and B- and T-oxidizing consortia. The sampling sites are shown in Fig. 1. The LCA and RW soils were sampled at Gongju-si, Chungcheongnam-do, South Korea, and the LCB soil at Gapyeong-gun, Gyeonggi-do, South Korea. The LCA and LCB soils were obtained from domestic waste sanitary landfill cover soils from which landfill gases (LFGs) were continuously emitted. The dominant vegetation at the RW site was reeds (*Phragmites communis*). All the soils were sampled at a depth of 30 cm from the surface, with the large particles sieved through a 2 mm-sieve and then stored at 4 °C.

2.2. Physico-chemical properties and texture of the sampled soils

Each wet soil (2 g) was dried in an oven at 105 °C (VS-1202D3, Vision Scientific Co., Ltd., Gyeonggi-do, Korea) for 24 h to determine the water content (WC). Each dried soil was burned in a furnace at 600 °C (Electric muffle furnace, Dae Han Scientific Co., Seoul, Korea) for 3 h, with the organic matter content (OC) then calculated. The pH value of the soil was measured using a pH meter (Model 420A, Thermo Orion, MA, USA) after mixing the soil with tap water (1:2, w/v).

To analyze the texture of the sampled soil, a pipette method was used [14]. 30% hydrogen peroxide (H_2O_2) (20 ml) was added to 10 g of dried soil in a 250 ml Erlenmeyer flask (at 105 °C for 24 h) to oxidize the organic matter. 10 ml of 5% sodium hexametaphosphate [$(NaPO_3)_n$] in deionized water was then added to the flask and manually mixed for 10 min. The dispersed mixture was transferred to a settling cylinder, with deionized water then added to the 1000 ml mark. A plunger was inserted into the cylinder, with the suspension thoroughly mixed by pulling the plunger upwards in short jerks for 1 min. When the suspension had been thoroughly mixed, the plunger was removed and the start time recorded. A pipette was slowly inserted 10 cm under the suspension, with 25 ml of the suspension removed as a sample. The sampled suspension was then quantified, after drying, using the soil separate classification of the United States Department of Agriculture (USDA). The calculation method employed has previously been described in Kettler et al. [15]. All measurements were performed in triplicate.

2.3. Enrichment of M- and BT-oxidizing consortia

To prepare the enriched microbial consortia, 8 g (wet-weight) of the soil (LCA, LCB and RW soil) was added to a 600 ml-serum bottle, with 20 ml of nitrate mineral salts (NMS) medium then added. The NMS medium contained $MgSO_4{\cdot}7H_2O$ 1 g l^{-1}, $CaCl_2{\cdot}2H_2O$ 0.295 g l^{-1}, KNO_3 1 g l^{-1}, KH_2PO_4 0.26 g l^{-1}, $Na_2HPO_4{\cdot}2H_2O$ 0.41 g l^{-1} and $CuSO_4{\cdot}5H_2O$ 0.0025 g l^{-1}. Three serum bottles were prepared for each soil sample. Each bottle was sealed with a butyl rubber septum and an aluminum cap, and then mixed by vortexing for 3 min. M was sampled from a gas cylinder containing 99% M (Dong-A gases, Seoul, Korea), using a 50-ml syringe (50-ml KOVAX syringe, Korea Vaccine Co., Ltd., Seoul, Korea), with 30 ml injected into a serum bottle to a final M concentration of 5% (v/v) (1194.7 μmol/bottle at 30 °C). B (HPLC grade, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and T (HPLC grade, J.T. Baker, Phillipsburg, USA) solutions (10 μl each) were injected into a serum bottle using a 10-μl syringe (Hamilton Company, Nevada, USA). The amounts of B and T corresponded to 111.9 and 93.6 μmol/bottle, respectively. M gas (30 ml) and B and T solutions (10 μl each) were injected into a serum bottle in the same manners as described above. The serum bottles, supplemented with M, B and T and M + B and T, were incubated at 30 °C with shaking (180 rpm), with the gases in the headspace of each serum bottle periodically sampled using a 1-ml gas-tight syringe (Hamilton company, Nevada, USA) and analyzed via gas chromatography (GC 6850N, Agilent Technologies Inc., Santa Clara, USA) to measure the M, B and T concentrations. When the M, B, and T concentrations dropped below 200, 20 and 20 ppm, respectively, each serum bottle was opened and left to stand on a clean bench for 1 h to allow the gases inside the bottle to be replaced with air. Each bottle was then re-sealed with a butyl rubber stopper. After M or B and T had been re-injected at the same concentration as described above, the bottle was re-incubated at 30 °C and 180 rpm. The injections of M or B and T were repeated in this way a further 5 times. To prevent exhaustion of the N and P sources in the NMS medium, 1 ml of concentrated N and P solutions were separately added to the bottles every second injection. The concentrated N and P solutions contained KNO_3 20 g l^{-1}, and KH_2PO_4 5.2 g l^{-1} and $Na_2HPO_4{\cdot}12H_2O$ 16.5 g l^{-1}, respectively.

The resulting microbial consortia enriched with LCA soil in the NMS medium supplemented with M, B and T and M + B and T were named LCA-M, LCA-BT and LCA-MBT, respectively. The microbial consortia enriched with LCB, RW and F soils were named in the same manner described above.

The M, and B and T oxidation rates were calculated using the concentrations of M and BT removed vs. time, including the lag period [16]. The M, B and T concentrations were analyzed using gas chromatography (GC 6850N, Agilent Technologies Inc., Santa Clara, USA), equipped with a flame ionization detector and a wax column (30 m × 0.32 mm × 0.25 μm, Supelco, Bellefonte, USA). The detail analytical procedure has been described previously [17]. The analyses of the M, B and T concentrations were performed in triplicate.

2.4. DNA extraction

To analyze the bacterial community in the enriched consortia and raw soils, genomic DNA samples were extracted from 1 ml of each of the consortia and 0.5 g of each raw soil using BIO101 FastDNA SPIN Kits for Soil (MP Biomedicals LLC, Solon, USA). All the genomic DNA samples were stored at −20 °C before use. For the quantitative analysis of the functional genes associated with the oxidations of M, and B and T, genomic DNA samples from *Methylobacter luteus* (NCIMB11914) and *Pseudomonas stutzeri* OX1 [18] were extracted using the same kits, which were used to prepare calibration curves for the gene copy numbers of *pmoA* and *tmoA*, respectively.

2.5. Quantitative analysis of the bacteria responsible for M and BT oxidation

A qRT-PCR method was employed for the quantitative analysis of the bacteria responsible for the oxidations of M, and B and T by targeting the *pmoA* and *tmoA* genes. The *pmoA* gene (ca. 510 bp) is known to encode the α subunit in pMMO, and the *tmoA* gene (ca. 505 bp) that of the toluene monooxygenase gene [12,13]. The fragments of the *pmoA* gene were amplified with the primer set, A189f (5′-GGN GAC TGG GAC TTC TGG-3′) [19], labeled with 6-carboxyfluorescein (6-FAM) and mb661r (5′-CCG GMG CAA CGT CYT TAC C-3′) [20]; whereas, those of the *tmoA* gene were amplified with the primer set, TMOAf (5′-CGA AAC CGG CTT YAC CAA YAT G-3′), labeled with 6-FAM and TMOAr (5′-ACC GGG ATA TTT YTC TTC SAG CCA-3′) [12]. Eight sets of PCR were carried out with 25-μl (total reaction volume) of a mixture containing 1 μl of genomic DNA as a template, 1.25 g bovine serum albumin, 0.4 M of each primer,

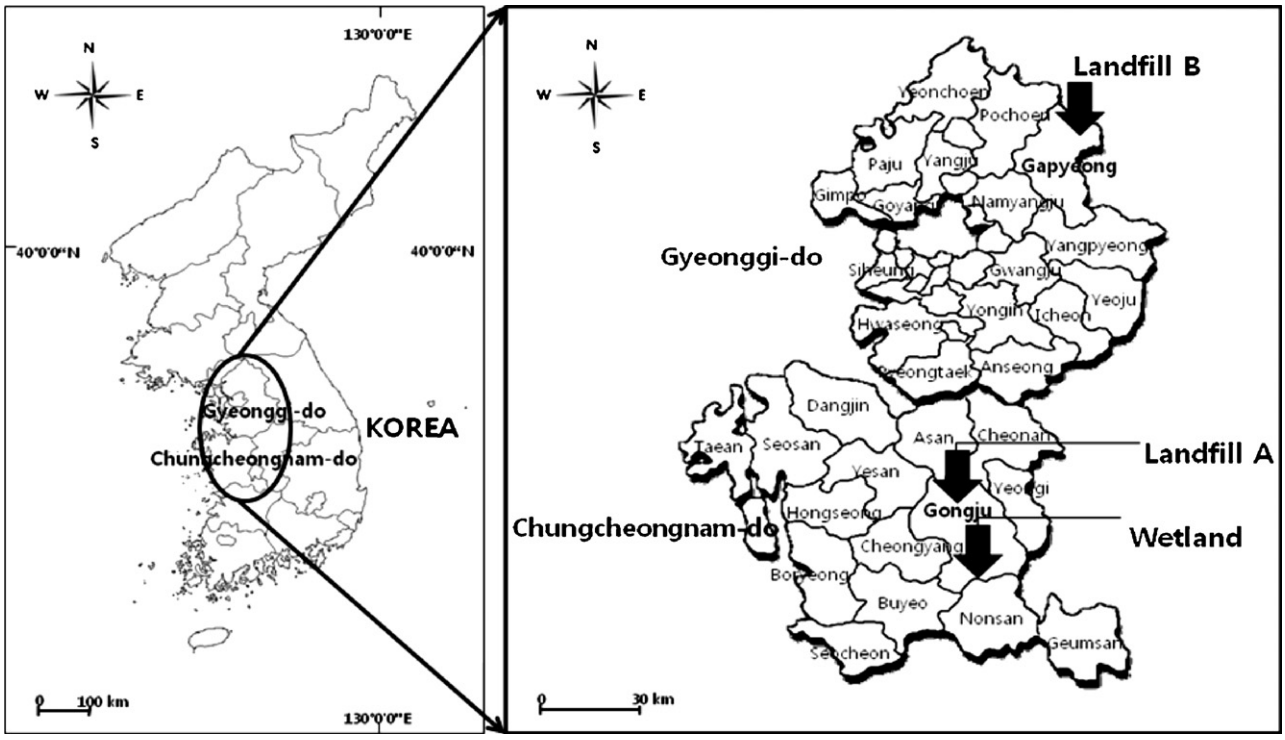

Fig. 1. Map of the soil sampling sites.

1× Ex Taq buffer, 1 U Takara Ex Taq DNA polymerase (TaKaRa Bio Inc., Shiga, Japan) and 200 M dNTP. The reactions were performed in a GeneAmp® PCR system, Model 2700 (Applied Biosystems Inc., Foster City, USA). Initial denaturation was performed at 95 °C for 5 min, followed by 28 cycles of denaturation at 95 °C for 30 s, primer annealing at 60 °C for 30 s and elongation at 72 °C for 30 s, with a final elongation step at 72 °C for 5 min [21]. The size of each PCR product was checked on 1% agarose gel, with each amplicon (each 200-μl) then excised. Excised gels were purified with a QIAquick Gel extraction Kit (Qiagen, Valencia, USA), as specified by the manufacturer. Purified DNAs were used as standard samples for the qRT-PCR. DNA concentrations of standard samples were measured using a micro-volume nucleic acid spectrophotometer (ASP-2680, ACTGene Inc., NJ, USA) at 260 nm. The standard samples, the genomic DNA samples of *M. luteus* and *P. stutzeri* OX1, were diluted 10^{12}-fold, with qRT-PCR then performed. qRT-PCR (Applied Biosystems 7300 real-time PCR system, Applied Biosystems, CA, USA) was performed using 25 μl samples on a MicroAmp Optical 96-well reaction plate (Applied Biosystems, Foster City, USA), with MicroAmp Optical Caps (Applied Biosystems, Foster City, USA). The purified DNA (3 μl) was added as a template to 22 μl of the PCR master mix. The PCR reaction mixture consisted of 12.5 μl of Power SYBR Green PCR master mix (Applied Biosystems, Warrington, UK), 0.2 μl of 25 μM primers (each) and 9.1 μl of distilled water. The PCR conditions were 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min and 77 °C for 30 s. The temperature of the signal detector was 77 °C. After qRT-PCR, a standard curve (*pmoA* or *tmoA* gene copy numbers vs. C_t) was constructed to determine the gene copy numbers. The detection levels of *pmoA* and *tmoA* were 9 and 2 gene copy numbers, respectively.

To quantitatively analyze the abundances of the *pmoA* and *tmoA* gene copy numbers in the enrichment cultures, genomic DNAs were diluted 10^2-fold, with qRT-PCR then performed. At the same time as performing the qRT-PCR, standard samples were also subjected to qRT-PCR. The *pmoA* and *tmoA* gene copy numbers of the template were calculated from standard curves. All analyses were performed in triplicate.

2.6. Analysis of bacterial communities responsible for M and BT oxidation using a T-RFLP method

To compare the bacterial communities in the enriched consortia responsible for the oxidations of M, and B and T, the *pmoA* and *tmoA* genes fragments were amplified using the primer sets A189f/mb661r and TMOAf/TMOAr, respectively. The PCR conditions and mixture were the same as described for the qRT-PCR method. Each purified *pmoA*-gene amplicon and *tmoA*-gene amplicon (approximately 200 ng) were digested at 37 °C for 4 h, with 5 U of restriction endonuclease *Msp* I (5′-C↓CGG-3′, BEAMS Biotechnology, Seongnam, Korea) for the *pmoA* gene amplicon, and *Hha* I (5′-GCG↓C-3′, BEAMS Biotechnology, Seongnam, Korea) for the *tmoA* gene amplicon. An analysis of the terminal restriction fragment (T-RF) size composition of each sample was performed using electrophoresis in denaturing polyacrylamide gel (6 M urea and 5% polyacrylamide), employing an ABI 377 DNA auto sequencer (GMI Inc., Ramsey, USA). The selected T-RFs (ca. 510 or 505 bp) were quantified by their peak areas using the GENESCAN analytical software (Applied Biosystems Inc., Foster, USA), with the minimum peak heights threshold set at 50 relative fluorescent units (RFU). The relative abundance (%) of a population was assessed as the percentage of the individual peak area to that of the total peak area.

The similarities among the T-RF profiles of the M or BT-oxidizing bacteria in the enriched consortia were analyzed using the PC-ORD software version 4.0 [22] to calculate the cluster similarities from the composite data set of the relative abundance values for each peak, using the un-weighted pair group method, via the arithmetic mean (UPGMA) and Sorenson coefficient values. An analysis of variance (ANOVA) test was performed using the SPSS 14.0K (SPSS Inc., Chicago, USA) program.

Table 1
Physico-chemical properties of the sampled soils.

Soil sampling site	pH	Moisture content (%)	Organic matter content (%)	Soil texture
Landfill A cover soil (LCA)	7.23 ± 0.18	10.02 ± 0.09	2.40 ± 0.05	Sandy loam (sand 65.0%, silt 23.3%, clay 11.8%)
Landfill B cover soil (LCB)	6.68 ± 0.20	10.13 ± 0.07	3.80 ± 0.02	Sandy loam (sand 77.1%, silt 14.0%, clay 8.9%)
Riparian wetland soil (RW)	7.40 ± 0.14	28.24 ± 0.47	1.00 ± 0.05	Sandy loam (sand 74.6%, silt 16.2%, clay 9.2%)

3. Results and discussion

3.1. Physico-chemical properties of the soil samples

Table 1 shows the physico-chemical properties of the soils used in this study. The pH values of the LCA, LCB and RW soils were neutral (6.68–7.40). The water contents of the LCA and LCB soils ranged from 10.02 to 10.13%; whereas, that of the RW soil was 28.24%. The organic matter contents were in the order: LCB (3.80%) > LCA (2.40%) > RW (1.00%). The texture of all the soil samples was sandy loam.

3.2. Comparison of M and BT oxidation

Fig. 2 shows the oxidations of M, B and T with the LCA-M (Fig. 2a), LCA-BT (Fig. 2b) and LCA-MBT consortia (Fig. 2c). A relatively short lag period of 7.3 h was observed when initiating the oxidation of M with the LCA-M, but a relatively long lag period of 13.6 h was required for the oxidation of M with the LCA-MBT. Unlike the oxidation of M, the oxidation patterns of B and T with LCA-MBT were similar to those with the LCA-BT. Interestingly, with the LCA-MBT, M was oxidized after B and T had been completely removed. The oxidations of M and/or BT with the LCB-M (Fig. 3a), LCB-BT (Fig. 3b) and LCB-MBT (Fig. 3c) showed similar properties to those with the LCA consortia (Fig. 2). With the RW-M and RW-MBT consortia, M was removed after lag periods of 19.5 and 22.5 h, respectively (Fig. 4a and c). B and T were slowly removed with the RW-BT consortium compared with those with the LCA-BT and LCB-BT consortia. However, the oxidation of B and T with the RW-MBT was relatively quick, within 13.5 h.

Based on the results shown in Figs. 2–4, the oxidation rates of M, B and T with the consortia were calculated, and are represented in Table 2. When landfill cover soils were used as the inoculum sources, the oxidation rates of M in the enriched consortia with MBT were lower than those in the enriched consortia with M alone: the oxidation rates of M with the LCA-MBT and the LCB-MBT were 3.42 ± 0.02 and 2.26 ± 0.21 μmol g-dry soil^{-1} h^{-1}, respectively; whereas, those with the LCA-M and LCB-M were 5.56 ± 0.03 and 4.15 ± 0.06 μmol g-dry soil^{-1} h^{-1}, respectively. The reductions in the oxidation rates of M with the mixtures of M, B and T were caused by delayed oxidation, as M was removed after the complete removals of B and T, as shown in Figs. 2c and 3c. Unlike the LCA and LCB consortia, the oxidation rate of M with the RW-MBT consortium (3.09 μmol g-dry soil^{-1} h^{-1}) was not significantly different from that with the RW-M consortium (3.04 μmol g-dry soil^{-1} h^{-1}). Conversely, irrespective of the inoculum source, the oxidation rates of B and T with the consortia enriched with mixtures of M and BT were higher than those with the consortia enriched with B and T alone.

Many investigations have measured the maximum oxidation rates of M from landfill cover soils. Kightley et al. [23] reported that 1 μmol g-dry soil^{-1} h^{-1} was the maximum oxidation rate for 5% (v/v) M; whereas, Börjesson et al. [24] measured 1.18–1.58 μmol g-dry soil^{-1} h^{-1} for the same system. Bender and Conrad [25] and Börjesson [26] reported maximum oxidation rates for 5% M of 6×10^{-4} and 10.8 μmol g-dry soil^{-1} h^{-1}, respectively. Although the specific oxidation rates of M with the LCA-M and LCB-M consortia were lower than those found by Börjesson [26], they were higher than those found in previous studies.

Conversely, Rahalkar et al. [13] measured the oxidation rates of M in the littoral and profundal sediments from Lake Constance, Germany. At depths between 1.6 and 3.5 cm in the littoral sedi-

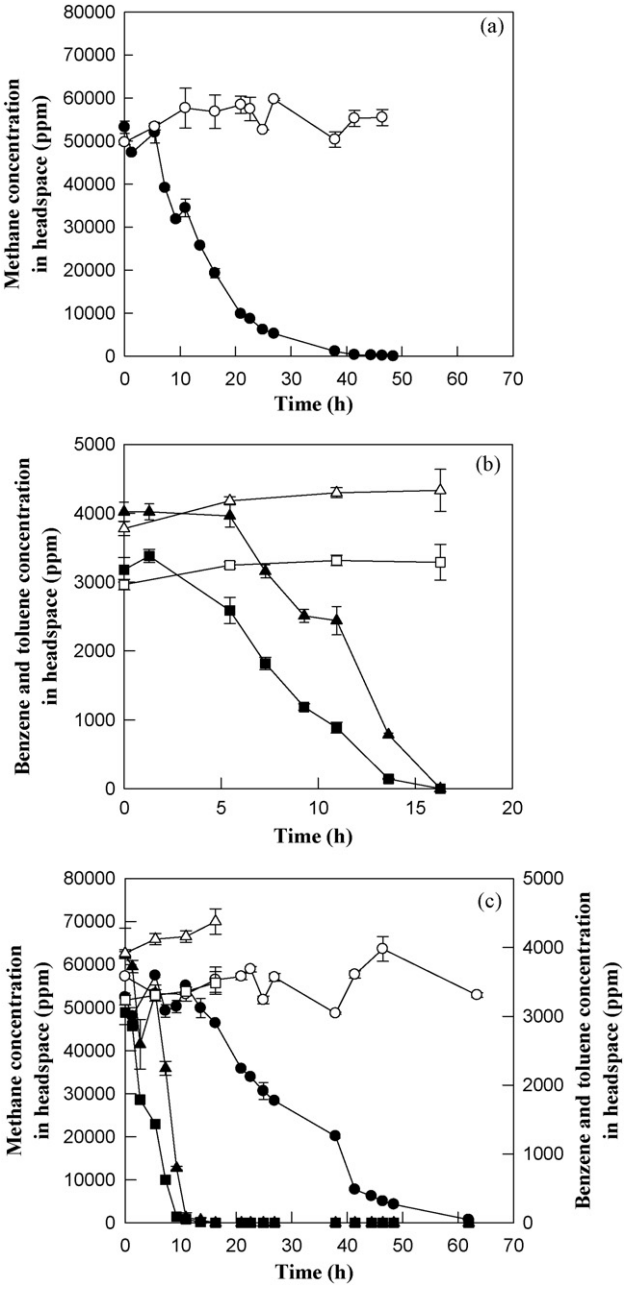

Fig. 2. The degradation patterns of methane (M), benzene (B) and toluene (T) by the LCA-M (a), LCA-BT (b) and LCA-MBT consortia (c). Open symbols, abiotic control; closed symbols, the consortium; circles, triangles and squares indicate M, B and T, respectively.

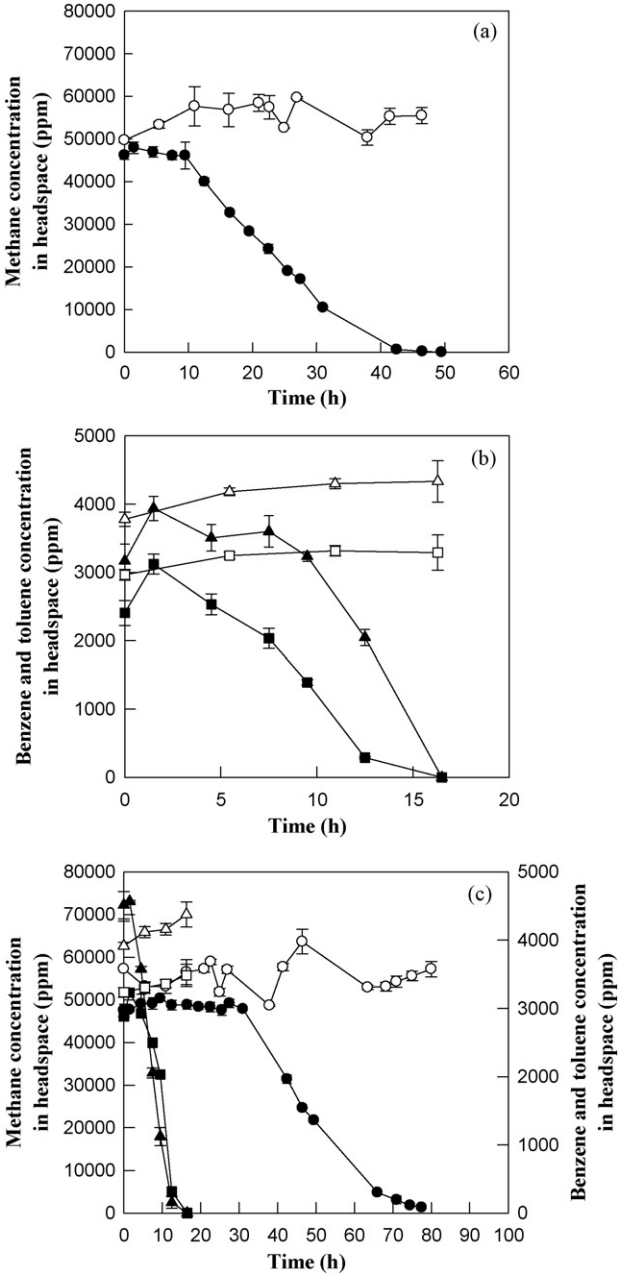

Fig. 3. The degradation patterns of methane (M), benzene (B) and toluene (T) by the LCB-M (a), LCB-BT (b) and LCB-MBT consortia (c). Open symbols, abiotic control; closed symbols, the consortium; circles, triangles and squares indicate M, B and T, respectively.

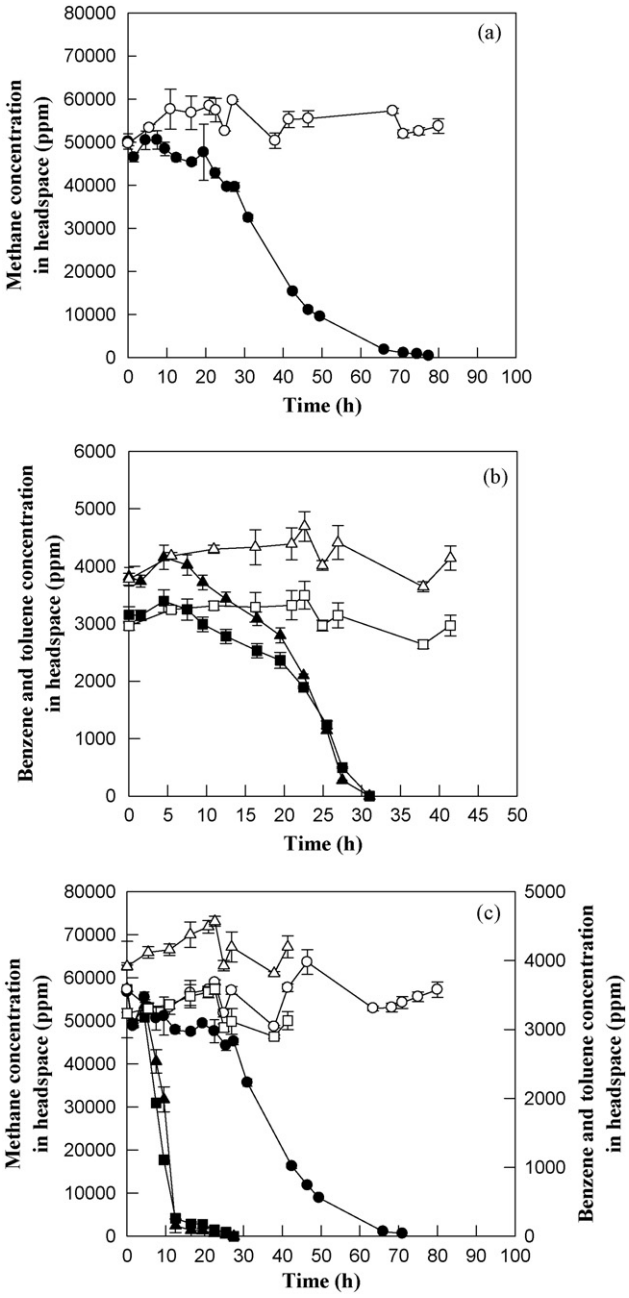

Fig. 4. The degradation patterns of methane (M), benzene (B) and toluene (T) by the RW-M (a), RW-BT (b) and RW-MBT consortia (c). Open symbols, abiotic control; closed symbols, the consortium; circles, triangles and squares indicate M, B and T, respectively.

ment, the oxidation rate of M was highest, at 0.18 μmol l^{-1} h^{-1}, but at depths between 0.5 and 2.3 cm in the profundal sediment, the highest rate was 0.28 μmol l^{-1} h^{-1} [13]. However, Auman et al. [27] reported the highest oxidation rate of M to be 346 μmol l^{-1} h^{-1} at depths between 0 and 0.5 cm in sediments from Lake Washington, USA. Zelenkina et al. [28] reported uptake rates between 0.2 and 7.7 nmol cm^{-1} h^{-1} M with sediment from Lake Baikal.

The inhibition effects of BT on the oxidation of M have been observed in previous studies: Scheutz and Kjeldsen [10] reported that VOCs strongly influenced the activity of methanotrophs. In this study, the oxidation rates of M with the LCA and LCB consortia were decreased and delayed by the coexistence of B and T, except for RW (Figs. 2 and 3, Table 2). The inhibition of B and T on the oxidation of M may have been caused by the toxicities of catechol and phenol as representative byproducts of B and T. Moreover, a delay in the initiation of the oxidation of M after the complete oxidations of B and T may have caused the higher solubilities of B and T over that of M.

The oxidation rates of B and T were enhanced by the coexistence of M (Figs. 2–4, Table 2). Methanotrophic bacteria are known to co-oxidize various aliphatic and aromatic hydrocarbons, including several halogenated hydrocarbons [3]. Recently, numerous VOCs were verified as being co-oxidized by methanotrophs in soils [29]. Methanotrophs are considered important organisms, which transform substrates to susceptible compounds easily utilized by heterotrophs [30]. Considering this information, the methanotrophs in the LCA-MBT, LCB-MBT and RW-MBT consortia might participate in the oxidation reactions of B and T, resulting in their enhanced oxidation rates.

Table 2
Methane (M), benzene (B) and toluene (T) oxidation rates by the enriched consortia.

Inoculum source	Enriched consortium	Oxidation rate (μmol g-dry soil^{-1} h^{-1})		
		M	B	T
Landfill A cover soil	LCA-M	5.56 ± 0.03^{a}		
	LCA-BT		0.92 ± 0.00^{a}	0.91 ± 0.00^{a}
	LCA-MBT	3.42 ± 0.02^{b}	1.82 ± 0.03^{b}	1.36 ± 0.01^{b}
Landfill B cover soil	LCB-M	4.15 ± 0.06^{c}		
	LCB-BT		0.94 ± 0.00^{a}	0.92 ± 0.00^{a}
	LCB-MBT	2.26 ± 0.21^{d}	1.20 ± 0.03^{c}	0.93 ± 0.00^{a}
Riparian wetland soil	RW-M	3.04 ± 0.00^{e}		
	RW-BT		0.66 ± 0.00^{d}	0.50 ± 0.00^{c}
	RW-MBT	3.09 ± 0.00^{e}	1.49 ± 0.50^{e}	1.21 ± 0.05^{d}

Different letters (a–e) indicate statistical significance ($P < 0.05$).

3.3. Abundance of pmoA and tmoA genes

Fig. 5 shows the abundances of the *pmoA* and *tmoA* genes, which are functional genes for the oxidations of M, and B and T, respectively [12,13]. The *pmoA* gene is encoded in the α subunit of pMMO [31], and essentially requires Cu to function. When the gene copy numbers in the raw soils were compared, those for *pmoA* were in the order: LCB ($4.15 \pm 0.04 \times 10^{6}$) > LCA ($1.02 \pm 0.15 \times 10^{5}$) > RW ($1.40 \pm 0.14 \times 10^{4}$) consortia (Fig. 5a), and those for *tmoA* were in the same order: LCB ($3.35 \pm 0.05 \times 10^{4}$) > LCA ($4.05 \pm 2.15 \times 10^{3}$) > RW ($2.37 \pm 1.96 \times 10^{3}$) (Fig. 5b). Compared with the copy numbers in the raw soils, those for *pmoA* and *tmoA* were increased in all the consortia after enrichment of the culture. In the case of the landfill cover soils, the gene copy numbers for *pmoA* in the consortia enriched with M alone (LCA-M and LCB-M consortia) were similar to those in the consortia enriched with M, and B and T (LCA-MBT and LCB-MBT consortia). However, the gene copy number for *pmoA* in the RW-MBT ($1.98 \pm 0.01 \times 10^{8}$) was higher than that in the RW-M ($2.64 \pm 0.26 \times 10^{7}$). Conversely, the gene copy number for *tmoA* in the LCA-BT ($2.60 \pm 0.00 \times 10^{7}$) was higher than that in the LCA-MBT ($6.16 \pm 0.38 \times 10^{6}$). Similar to that found with the LCA consortia, the gene copy number in the LCB-BT ($9.84 \pm 0.19 \times 10^{6}$) was also higher than that in the LCB-MBT ($1.65 \pm 0.22 \times 10^{5}$). With the RW enriched consortia; however, there were no significant differences between the gene copy numbers in the RW-BT and RW-MBT consortia.

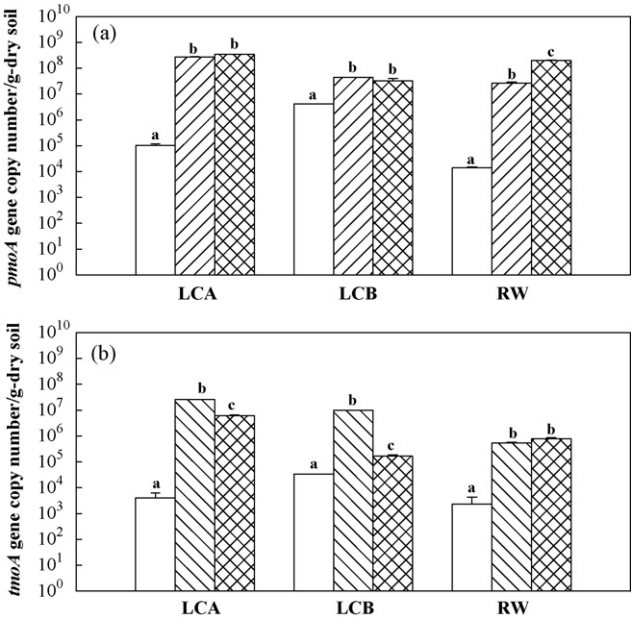

Fig. 5. Abundance of methane-oxidizing bacteria containing the *pmoA* gene (a) and BT-oxidizing bacteria containing the *tmoA* gene (b). Different letters in each sample indicate statistical significance ($P < 0.05$).

The *pmoA* and *tmoA* gene copy numbers were not correlated with the oxidation rates of M, and B and T, as shown in Table 2 (data not shown), indicating that the *pmoA* gene copy numbers were not affected by the presence of BT; whereas, the oxidation rate of M was significantly inhibited by the presence of B and T with the LCA-MBT and LCB-MBT consortia (Table 2). It would seem that the oxidation rates of M with the consortia decreased because another MMO (e.g. sMMO, *pmoB*, and *pmoC* encoded in the β and γ subunit of pMMO) seemed to be inhibited by the presence of B and T, and/or their metabolites [32]. Detailed studies, however, will be required to more clearly understand the mechanism.

3.4. Characterization of bacterial communities responsible for M and BT oxidation

To compare structures of the M and BT-oxidizing bacterial communities in the enrichment cultures, T-RFLP analyses of the *pmoA* (Fig. 6a) and *tmoA* genes (Fig. 6b), respectively, were conducted. With the LCA-M consortium, *Methylobacter* (type I methanotroph) was found to be the most dominant species, followed by *Methylomonas* (type I) and *Methylomicrobium* (type I). As with the LCA-M, *Methylobacter* was the most dominant species in the LCA-MBT consortium, but type II methanotrophs, such as *Methylocystis*/*Methylosinus* (type II), were the second most dominant, but their proportions were decreased compared to those in the LCA-M. The most dominant methanotroph was *Methylocystis*/*Methylosinus*, followed by *Methylococcus*/*Methylocadum* and *Methylobacter* in both the LCB-M and LCB-MBT consortia. Compared with those in the LCB-M, the proportion of *Methylocystis*/*Methylosinus* in the LCB-MBT was increased, but those of *Methylococcus*/*Methylocadum* and *Methylobacter* were decreased. *Methylocystis*/*Methylosinus* was the most abundant, followed by *Methylobacter,* in the RW-M consortium. Interestingly, the abundance of *Methylococcus*/*Methylocadum* in the RW-MBT consortium was markedly decreased, but that of *Methylobacter* was increased. The similarities in the microbial communities relating to the oxidations of M between LCA-M and LCA-MBT, LCB-M and LCB-MBT, and RW-M and RE-MBT were 97.3, 85.0 and 44.9%, respectively.

The T-RFLP results for the *tmoA* gene are shown in Fig. 6b. Compared with the LCA-BT, the relative abundance of the 107 bp T-RF was significantly increased in the LCA-MBT consortium, but those of the 172 and 122 bp T-RFs were obviously decreased. In the LCB-BT, two T-RFs, 114 and 107 bp, were the dominant fragments, with

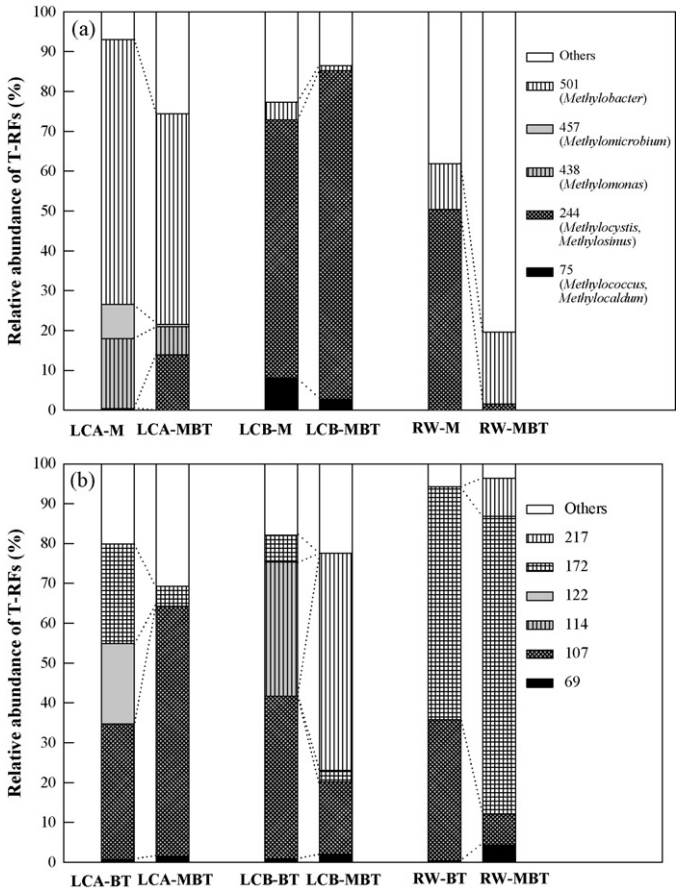

Fig. 6. Relative abundance of T-RFs for *pmo*A (a) and *tmoA* (b) functional genes. *In silico* analysis, [a]*Methylobacter* (Type I); [b]*Methylomicrobium* (Type I); [c]*Methylomonas* (Type I); [d]*Methylocystis/Methylosinus* (Type II); [e]*Methylococcus*/*Methylocaldum* (Type X) [11].

the 172 bp T-RF being the next most dominant fragment. However, the 114 and 172 bp T-RFs were markedly decreased to below 1%, and the 107 bp T-RF was also decreased to 20%. In the LCB-MBT consortium, two fragments, the 217 and 107 bp T-RFs, with relative abundances of 54 and 18%, respectively, were insignificant fragments in the LCB-BT consortium. The abundances of the 172 and 107 bp T-RFs were relatively high in the RW-BT consortium, whereas, in the RW-MBT, the relative abundance of the 172 bp T-RF was increased; whereas, that of the 107 bp T-RF was decreased. Besides, the abundance of the 217 bp T-RF, which was a trivial fragment in the RW-BT consortium, was increased to 10% in the RW-MBT consortium. Regardless of inoculum source, the relative abundance of the 69 bp T-RF in the consortium enriched with the mixture of M + B and T was higher than that in the consortium enriched with B and T alone. The similarities in the microbial communities relating to the oxidations of B between the LCA-BT and LCA-MBT, LCB-BT and LCB-MBT, and RW-BT and RE-MBT were 59.2, 80.8 and 92.7%, respectively.

Kallistova et al. [33] reported that type I methanotrophs were more dominant than type II in a municipal landfill cover soil from Russia. Conversely, Cébron et al. [34] reported that type II methanotrophs dominated over type I in a peat landfill soil from Ireland; their results were similar to those found for the LCB consortia in this study (Fig. 6). Furthermore, Wang et al. [35] reported that landfill cover soil from China was dominated by the genus *Methylosarcina* (type I); whereas, Héry et al. (2008) reported that *Methylobacter* (type I) and *Methylocystis* (type II) dominated in a microcosms test using landfill cover soil from the UK. The results from Héry et al. [36] showed a similar pattern with the bacterial structure in the LCA consortia (Fig. 6), where the genera *Methylobacter* (type I) and *Methylocystis* (type II) predominated. DeJournett et al. [37] reported that both types I and II methanotrophs predominated, in similar portions, in a wetland near a lake in the USA. Similar to their results, type II methanotrophs, such as *Methylocystis* and *Methylosinus,* and type I methanotrophs, such as *Methylobacter*, were dominant in the RW consortia (RW-M and RW-MBT).

4. Conclusions

M and VOCs are simultaneously emitted from various sources; therefore, it is important to investigate the effects of each compound on their removals, as well as the associated microbial community structures responsible for their degradations. In this study, consortia from landfill cover and wetland soils were used as inoculum sources. M alone, VOCs alone (BT), and a mixture of M and VOCs (BT) were supplied as substrates, with the effects of B and T on the abundance of functional genes, as well as the community structure associated with the oxidation of M its oxidation rate then characterized. Consequently, the oxidations of M as well as of B and T were found to be affected by the counter compounds. The results obtained in this study offer basic information for understanding the interactions between M and VOCs, which can be subsequently used as basic data for gaining a better understanding of the mechanisms involved in the biological removals of M and VOCs, and as reference data of the *in situ* interactions between M and VOCs.

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