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Microbial consortium involving biological methane oxidation in relation to the biodegradation of waste plastics in a solid waste disposal open dump site

Sutharat Muenmee, Wilai Chiemchaisri*, Chart Chiemchaisri

Department of Environmental Engineering, Faculty of Engineering, Kasetsart University, Bangkok 10900, Thailand

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ABSTRACT

The simulated lysimeters of an open waste dump were employed to investigate microbial consortium in relation to the biodegradation of waste plastics (high/low density polyethylene, HDPE/LDPE; polypropylene, PP; polystyrene, PS). A low flow rate of a synthetic biogas was purged continuously to imitate methane oxidation. The plastics were examined periodically for chemical composition and microbial consortium. The PCR-DGGE and FISH techniques revealed bacterial consortium such as heterotrophs, autotrophs and methanotrophs colonizing simultaneously on the waste plastics. Only methanotrophs tended to increase with time throughout the waste bed, particularly for type I/II methanotrophs (*Methylobacter* sp./*Methylocella* sp.). Some microbes were found only at a 15 cm depth such as *Methylococcus capsulatus, Acinetrobacter* spp., and *Flavobacteria* spp. Biodegradation of waste plastics in terms of weight loss, chemical changes and surface deterioration clearly occurred where there was a high density of methanotrophs on the waste plastics. Methanotrophs functioned as the principal decomposer in plastic biodeterioration particularly in the upper zone of the lysimeter. The highest kinetic decay rate was of HDPE (K, $0.128\ y^{-1}$) whereas the lowest K ($0.048\ y^{-1}$) was of LDPE. In summary, the waste plastics were biodegraded in the simulated lysimeters which yielded a good correlation between plastic degradation kinetics and rate of methane oxidation.

Introduction

The total consumption of plastics will reach 297.5 million tons worldwide by 2015, with Asia the world's largest plastics consumer accounting for 30% of global consumption over the last few years (Saisinchai, 2013). Plastic wastes are estimated at approximately 16% of the total weight of municipal solid waste. Polyolefin, a common plastic, is less biodegradable which poses a long-term negative effect to the environment after being disposed as solid waste. Studies of the biodegradation of various types of plastics such as high density polyethylene (HDPE), low density polyethylene (LDPE), polypropylene (PP) and polystyrene (PS) in natural soils revealed that some soil microorganisms including fungi and bacteria showed a specific metabolic capacity to assimilate these plastics as carbon and energy sources for their growth. For example, some fungi (*Penicillium simplicissimum*) degraded the high density polyethylene (HDPE) of a molecular weight of up to 2800 (Yamada-Onodera, 2001). The soil thermophilic bacterium *Brevibaccillus borstelensis* strain 707 assimilates LDPE as a carbon source (Hadad et al., 2005). *Rhodococcus ruber* in a liquid media culture was capable of degrading PE (Gilan et al., 2004). Polypropylene (PP), which is very hydrophobic and of high molecular weight without an active functional group, has been reported for its high persistence in biodeterioration (Arkatkar et al., 2009). However, the consortium of the fungus (*Aspergillus niger*) and the bacteria such as *Pseudomonas* and *Vibrio* species were synergic in the biodegradation of polypropylene (Cacciari et al., 1993).

In many undeveloped and developing countries, an open dump is a common method of municipal solid waste disposal because it is inexpensive in terms of capital investment and operating cost. In waste disposal sites such as landfills and open dumps, the waste plastics have greater potential to be biodetoriated by various types of indigenous microorganisms from the soil. Generally, microorganisms differ from each other in that they have their own optimal growth conditions. The biodegradation of plastics is hypothesized to be accelerated under an open dump environment, which differs

* Corresponding author.
E-mail address: fengwlc@ku.ac.th (W. Chiemchaisri).

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from the anaerobic condition of a landfill. One of the differences is the lower amount of methane generation in the semi-aerobic condition of an open dump. With the available methane and oxygen in between an open dump mass, methanotrophic bacteria are expected to be one of the species present. They are also actively present in the soils where methane is generated such as wetlands, rice fields and landfills including the stabilized organic wastes in the open dump site (Chiemchaisri et al., 2013). They utilize methane as their sole carbon and energy sources via enzyme methane monooxygenase (MMO) that is capable of the co-metabolism of some persistent compounds concurrently (Rockne et al., 1998; Hazen, 2010). Although many co-metabolizing bacterial species have been identified, the more frequently investigated bacteria are the methanotrophs. They have been intensely studied in the application of the biodegradation of some chlorinated solvents, most notably trichloroethylene, to environmentally acceptable concentrations in soils, sediment, and ground water (Hazen, 2010). Although methanotrophs are ubiquitous co-metabolizers in many aliphatic compounds, alkanes (Lee et al., 2012), and aromatic compounds (Rockne et al., 1998), current information on methanotrophic capability in the biodeterioration of waste plastics in a real situation like the waste mass of a dumping site where methane is being produced and atmospheric oxygen is transferring is limited. Thus, the aim of this study is to investigate the potential of waste plastic's biodegradation via methane oxidation in the simulated open dump site of solid wastes using laboratory scale lysimeters. The obtained information is helpful for solid waste management particularly in terms of plastics waste mitigation.

Materials and method

Treatment of plastics

This study was set up by the hypothesis that municipal solid waste generally consisting of biodegradable organic wastes (food wastes and papers) and persistent biodegradable organic waste (plastics) was buried in an open dump site for several months. After the biodegradable wastes were anaerobically decomposed to biogas, the waste plastics remained in large portions in the open dump mass. In order to simulate the practical composition of the waste plastics, all types of commercial plastic samples (HDPE, LDPE, PP and PS) available in local supermarkets in Bangkok, Thailand were prepared by being manually cut into two shapes, square and rectangle, for convenient segregation of plastics after the experiment. The equivalent surface area of each plastic shape was 9 cm^2. Because the disposed plastics in a waste dump site are generally exposed to sunlight, the plastics were pre-treated under synthetic sunlight exposure carried out under a UV-B lamp (30WT8, Tokiwa lamp) which emits radiation of 280–370 nm with a maximum intensity of 313 nm. These shortest wavelengths are similar to those of sunlight (Andrady et al., 2003) which can decrease plastic hydrophobicity (Hadad et al., 2005). The pretreated plastics of 200 h irradiation thereafter were employed in the simulated lysimeters.

Experimental set up

Lysimeter study

The waste plastics in municipal solid wastes were mainly composed of HDPE, LDPE and PP at 69.02%, 7.38% and 13.00%, respectively (Pollution Control Department of Thailand, 2005). Therefore, this waste proportion was reproduced in this study. Each type of the pre-treated plastics were mixed to obtain a ratio (%, W/W) of 56: 29:12:3 for HDPE:LDPE:PP:PS. In addition to the plastic wastes, stabilized organic wastes from an actual landfill site were amended in the plastic mixture in order to provide indigenous microorganisms similar to a real environment in an open dump site. The chemical characteristics of the stabilized organic wastes were ammonium (50.31 $\mu g\ g^{-1}$), nitrite (4.52 $\mu g\ g^{-1}$), nitrate (15.84 $\mu g\ g^{-1}$), total organic carbon (TOC, 147.5 mg g^{-1}), TKN (4978 $\mu g\ g^{-1}$) and pH (5.01). Of the pretreated plastics 427 g were mixed with 379 g of the stabilized organic wastes in a ratio of 53:47 based on real waste composition (Chiemchaisri et al., 2010). The chemical characteristics of this waste mixture were ammonium (23.54 $\mu g\ g^{-1}$), nitrite (3.20 $\mu g\ g^{-1}$), nitrate (10.50 $\mu g\ g^{-1}$), and TKN (3938 $\mu g\ g^{-1}$). There were a total of three lysimeters used as the simulated open dump sites, each made of an acrylic cylinder with a diameter of 5 cm and length of 150 cm as described in Chiemchaisri et al. (2013). Prior to filling the lysimeters with the waste mixture, the moisture content was adjusted to 10% for optimum methane oxidation (Visvanathan et al., 1999). The waste bed of the lysimeters had an average bulk density of 0.71 g cm^{-3} with 48% porosity. Following this, a synthetic biogas (60%CH_4:40%CO_2) was purged with a flow rate of 0.56 ml min^{-1} at the bottom of each lysimeter, equivalent to an actual methane loading rate of 26.50 g m^{-3} d^{-1} (Chiemchaisri et al., 2013). During the experimental period, gas samples at depths of 0, 37, 74, 111, and 148 cm of all lysimeters were withdrawn and the gas composition was analyzed using a gas chromatograph (GC6890 Agilent) on a weekly basis. Then, the methane oxidation rate of the lysimeters at different depths and overall were calculated (Chiemchaisri et al., 2013). Every three months, the biogas purging of one of the lysimeters was stopped and the waste matrix was segregated into various depths (0–10 cm; 10–20 cm; 30–40 cm; 65–75 cm; 100–110 cm; 135–145 cm) for determination of methanotrophic activity, chemical analysis (FTIR) and biomolecular analysis (PCR-DGGE, FISH).

Determination of methanotrophic activity in microcosm study

Methanotrophic activity was determined via a batch microcosm study. Reduction of headspace methane in the microcosm was measured over time to evaluate methane oxidation rate (MOR) resulting from methanotrophic activity (Chiemchaisri et al., 2013). A 0.5 g waste sample of each waste bed layer (0–10 cm, 10–20 cm, 30–40 cm, 65–75 cm, 100–110 cm, and 135–145 cm) of the lysimeter was placed in triplicate in a 25 ml serum bottle, then the bottles were sealed with rubber stoppers and aluminum caps. About 2.5 ml of 99.99% CH_4 was injected into each bottle in order to provide 10% CH_4 headspace. All the serum bottles were incubated at room temperature (28–30 °C). The 300 μl of the headspace gas sample was withdrawn every 3 h and analyzed by gas chromatograph (GC, Agilent 6890). The GC was equipped with a thermal conductivity detector (TCD); carrier gas: 99.99% He 49.1 ml/min; a stainless steel column: ID 6.35 mm, 1.8 m length (Alltech CRT I) with the supporting material: activated molecular sieve; injection temperature, 50 °C; oven temperature, 35 °C; detector temperature, 180 °C (Chiemchaisri et al., 2013). Changes in methane, oxygen and carbon dioxide concentrations against time were plotted to determine methane oxidation rate (MOR), oxygen uptake rate (OUR) and carbon dioxide production rate (CPR) using general linear model analysis by Microsoft Excel 2013.

Identification of bacteria via molecular analysis

The waste samples from the lysimeters were separated into two parts: plastics and stabilized wastes. The microbial DNA was extracted from the 0.5 g plastics/stabilized wastes using a soil DNA isolation kit (Farvoprep™). The 16S rRNA was harvested as instructed in the leaflet of the kit. Then, they were amplified using a universal primer (338F and 518R, Table S1) via Toptaq Master Mix Kit, Quiagen by Swift™MaxPro Thermal Cyclers (Esco Healthcare Pte. Ltd). After 10 min of initial denaturation at 95 °C, PCR cycling

was performed as follows: 25 cycles of 95 °C, 10 s; 58 °C, 30 s and 72 °C, 30 s. The PCR products were separated by DGGE technique (Bio Rad DCode™). The PCR products were loaded onto an 8% polyacrylamide gel. A 35%–70% denaturing gradient (100% corresponding to 7 M urea and 40% formamide) was used. Electrophoresis was run in 1× TAE buffer at 60 °C for 7 h at 80 V. After that, gels were stained in an ethidium bromide solution for 15 min, then visualized and photographed under UV light. Small pieces of the selected DGGE bands were punched from the gel. Each piece was then transferred into a 50 μl of sterile water and incubated overnight at 4 °C to allow for diffusion of the DNA. Two microliters of the eluted DNA were used for re-amplification with primer without the GC clamp (Bodelier et al., 2005). Finally, each product band was sequenced by a DNA sequence analyzer (AITbiotechPte Ltd, Singapore). The obtained DNA sequences were compared to the 16S rRNA gene sequences from the database of the National Center for Biotechnology Information (NCBI) using the BLASTn search option. The concentration of the DNA product was calculated using Eq. (1) (LeÓn Ohl et al., 2003). It is noted that the DNA length of the 338F and 518R primers is 180 bp.

$$\text{DNA copies} = 6.02 \times 10^{23}\ (\text{copies mol}^{-1}) \times \text{DNA amount (g)/DNA length (bp)} \times 660\ (\text{g mol}^{-1}\ \text{bp}^{-1}) \quad (1)$$

The fluorescence in situ hybridization (FISH) technique was applied to identify the bacterial types on the wastes (Eller et al., 2001). A 0.5 g of waste was mixed with 20 ml of 0.85% NaCl. 300 μl of solution was fixed with 4% paraformaldehyde at pH 7.2, and kept under 4 °C for 4 h, then washed with a phosphate buffer solution (PBS), and a mixture of ethanol and PBS (1:1) was added. The sample was preserved at −20 °C until hybridizing reaction. The following steps were conducted by the immobilization of a fixative sample onto the gelatin coated slide and dehydration with stepwise concentrations of 50%, 80% and 90% ethanol, respectively. Oligonucleotide probes, Mγ84 + Mγ705 and Mα450, were employed to detect type I and type II methanotrophs, respectively (Wagner et al., 1995). Oligonucleotide probes, NSO 1225, NIT 3 and EUB 338, were used to detect ammonia oxidizer, nitrobacter and domain bacteria, respectively (Table S1). For the hybridization step, a buffer and a probe were overlaid on the sample slides and the slides were incubated under a specific hybridization temperature of each species for 2 h. Then, washing the excess probes by dipping the slides in a washing buffer and sterile water, respectively. After being dried at room temperature, the samples were stained with DAPI (4′,6-diamidino-2-phenylindole) and washed with sterile water in order to observe total microorganisms.

Chemical analysis of waste samples

The general parameters of the waste samples such as ammonia, nitrite, nitrate, TKN, total organic content and moisture were analyzed (Anderson and Ingram, 1993) for describing other microbial activities such as heterotrophic/nitrifying activity. In order to reveal the methanotrophs associated with the biodegradation of plastics, the formation or disappearance of functional groups in the plastic polymers during the oxidation process can be evaluated by a Fourier transform infrared (FTIR) spectrometer (Bruker Alpha-E, Germany). FTIR spectroscopy is a tool for identifying various functional groups present in an organic compound (Arkatkar et al., 2010). The spectra were recorded as an average of 32 scans at a resolution of 4 cm^{-1} in the frequency range of 600–4000 cm^{-1}. Moreover, changes in the surface morphology of the virgin plastics, UV treated-plastics, the 210 day incubated plastics (15 and 140 cm depths) were examined using a Scanning Electron Microscope (SEM, Hitachi SU8020) in various magnifications. It was noted that the biomass on the incubated plastics was removed by sonication (ULTRAsonik™) for 15 min and air dried prior to the SEM test in order to observe the physical changes of the plastic surface after bacterial colonization. In terms of weight loss, plastic samples were washed, dried and weighed using a 0.0001 g accurate digital balance (Precisa 240A, Switzerland). Average % weight loss was calculated from multiple samples (Roy et al., 2008). Triplicate tests were maintained for all the samples. Microsoft Excel version 2013 was used for statistical analysis. Data was subjected to analysis of variance (ANOVA). Student's *t*-test was analyzed in order to compare the data of the waste samples at depths of 15 cm and 140 cm. The results were expressed as mean ± standard deviation.

Results and discussion

Methane oxidation in lysimeters

The CH_4 and CO_2 concentrations in the lysimeters showed similar trends of increasing with waste depths, while O_2 concentration displayed a reverse trend to those of CH_4 and CO_2 (Fig. S1). In the first month, the highest oxygen available was 10–13% at 2 cm depth while the other depths were 3–7% which indicates aerobic microbial activity occurring throughout the waste beds. Afterwards, the remarkable disappearance of CH_4 between the 2–39 cm waste depths was observed throughout the 357 days of the experiment which suggests the high activities of methanotrophs in the lysimeters. Inversely, high CH_4 concentrations (45–55%) in the depths of 40–150 cm indicate low methanotrophic activities. The CH_4/CO_2 ratios (1.3–1.5) did not change much between 40 and 150 cm (Fig. 1) whereas that of the 0–40 cm depths tended to decrease with time from 1.3 to 0.8. This appearance confirms the high conversion of CH_4 to CO_2 by methanotrophs in 0–40 cm waste depths. In addition, methane oxidation of the lysimeters was very consistent throughout the experimental period of 357 days. This occurrence differs from several previous reports in which the trend of methane oxidation frequently decreased with time (Visvanathan et al., 1999; Tanthachoon et al., 2008). Besides, methane oxidation in landfill cover soils (Visvanathan et al., 1999; Tanthachoon et al., 2008) and the final cover of the stabilized organic waste (Chiemchaisri et al., 2013) showed high MOR at 10–30 cm depths. This is because the waste mass used in this study had a low water holding capacity (21.26 ± 3.28%, w/w) which was clearly less than conventional cover soils (46%, w/w) (Einola et al., 2007). Although this waste had porosity close to the cover soils (0.4–0.6), the low water holding capacity helps better diffusion of the atmospheric air throughout the waste bed. Water logging caused from methane oxidation is a general problem of continuous methane oxidation in the cover soil/compost-based cover because it limits the oxygen availability (Tanthachoon et al., 2008). As shown in Fig. 1c, the highest moisture content of 14–15% appeared at the 15 cm depth during 210–357 days; subsequently, O_2 depletion and low MOR occurred at the lower part towards the lysimeters. MOR increased about 10% when the O_2 concentration in the gas mixture increased from 3% to 20%, while a decrease in O_2 concentration from 3% to 1% could reduce MOR to more than 50% (Stein and Hettiaratchi, 2001). In conclusion, the simulated lysimeters with a high portion of plastics and stabilized wastes gave high methane oxidation at 0–40 cm depths by giving the maximum MOR of 18.7 mol m^{-3} d^{-1} (Fig. S1d) as compared to 0.64 mol m^{-3} d^{-1} at 65–80 cm of the compost-based cover soil (Chiemchaisri et al., 2013).

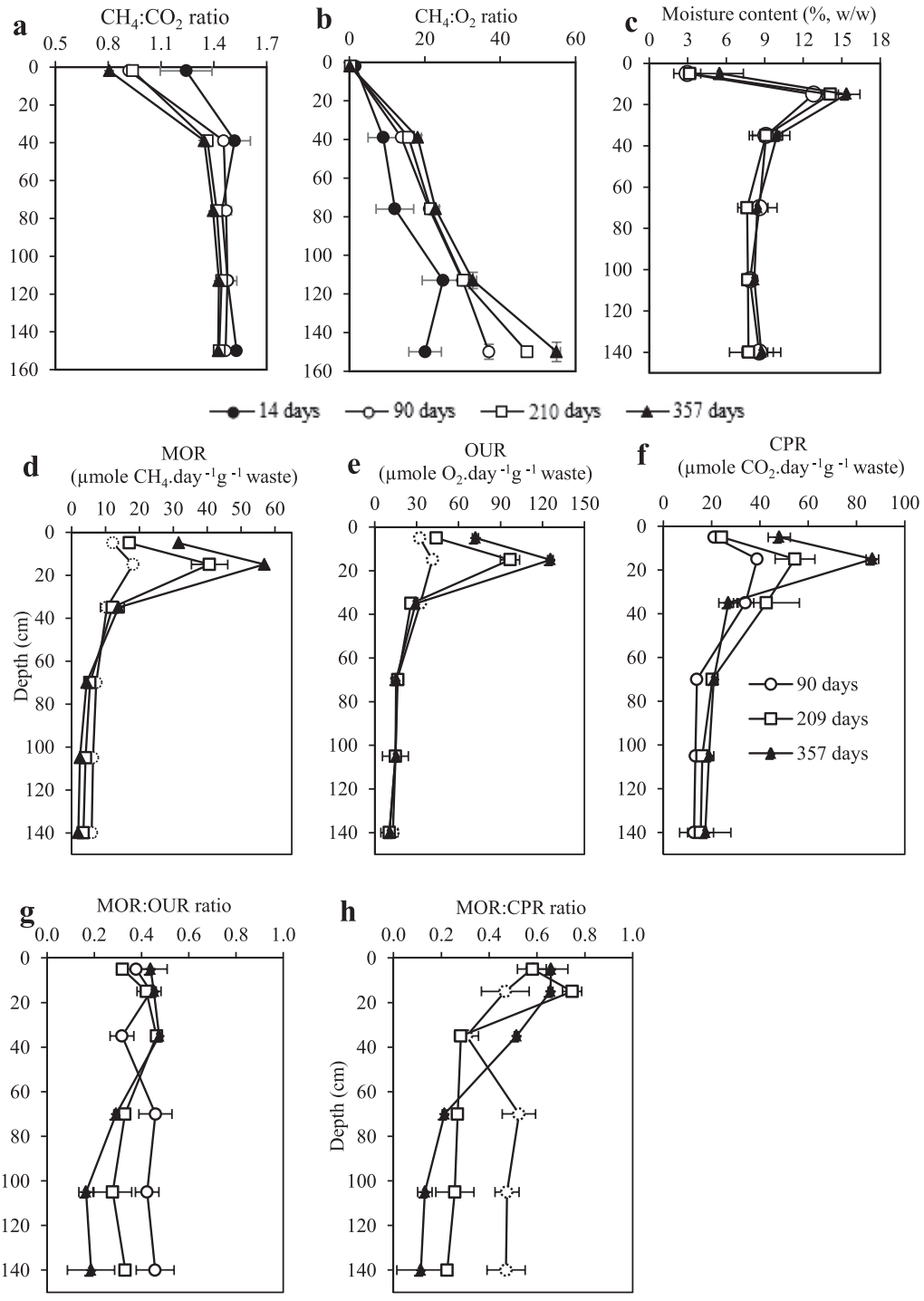

Fig. 1. Changes of ratio CH_4:CO_2 (a), CH_4:O_2 (b) and changes of moisture content (c), MOR (d), OUR (e), CPR (f), MOR:OUR (g), MOR:CPR (h) with time; mean ± SD of triplicate sample incubations; ANOVA, $P < 0.05$.

Determination of the methane oxidation rate of waste plastics via microcosm study

As shown in Fig. 1d–h, the maximum MOR via microcosm study was found at a depth of 15 cm. It increased with time by giving the MOR of 18.15, 40.70 and 56.86 µmol CH_4 d^{-1} g^{-1} at 90, 210, and 357 days, respectively. This supports the CH_4 disappearance in between depths 0–40 cm (Fig. S1) was the result of methanotrophic activities. Trends of oxygen uptake rate (OUR) correlated well to that of MOR. Nevertheless, the ratios of MOR to OUR (Fig. 1g) throughout the depths were clearly different between the upper part (0–75 cm) and the lower part (75–150 cm) of the lysimeters. A common ratio of MOR to OUR in a stoichiometric equation of methane oxidation is 1–2 (mol/mol) equally 0.5. This ratio of all depths was below 0.5 indicating the utilization of oxygen by other microorganisms. In the upper part, at the 15 cm depth the MOR to OUR ratio did not change during the 357 days of the experimental period. This indicates the dominance of methanotrophic activity in

this depth. Regarding the other depths, the MOR:OUR ratio fluctuated with operational time. For example, at the depths of 5 cm and 35 cm, the lowest ratio was found at 210 days and 90 days, respectively. This evidence suggests O_2 assimilation by other aerobic types of microorganisms in waste bed which might vary in species and growing conditions. Likewise, since a common MOR:CPR ratio of methane oxidation is 1:1, the MOR:CPR ratio of all depths was lower than 1.0 which suggests a consortium of aerobic microorganisms in the waste mass (Fig. 1h). The decreasing MOR:CPR ratio in the lower parts of the lysimeters (40–150 cm depths) with time indicates that more activities of other microbes predominate those of methanotrophs. Finally, this decreasing MOR:CPR ratio from 0.5 to 0.1 with time corresponded well to the decreasing MOR:OUR ratio.

Morphology of biodegraded plastics

Visually, there was a slight change in the color of the plastics (from white to light yellow) after 200 h of UV-exposure, while the plastic surfaces rarely changed. After incubation in the lysimeters, there was the appearance of biofilms on the plastic surfaces. Test of the 210 days waste plastics showed the highest biomass content (5.50 ± 1.12 mg g^{-1}) was of the HDPE at the 15 cm depth where the MOR was highly active. The biomass of each depth was significantly different (ANOVA, $P = 0.005$). The biomass at other waste depths such as at 5, 35, 70, 105 and 140 cm were 3.80 ± 1.13, 3.15 ± 1.10, 2.87 ± 0.85, 2.55 ± 1.05 and 2.28 ± 1.20 mg g^{-1}, respectively. Density of attached microbes at the plastics at 15 cm depth was significantly higher than those at the 140 cm depth ($P = 0.012$). The SEM images revealed various shapes of microorganisms colonized on the HDPE surface (data doesn't show) including cocci and rods. A relatively higher population of cocci than the rods were found in the 15 cm depth. Inversely, the rods were dominant in the 140 cm depth. Chiemchaisri et al. (2013) reports that the cocci methanotrophs favored growing on plastic waste, whereas both rods and cocci methanotrophs were found in higher densities in organic waste. The results of SEM for LDPE, PP and PS showed similar trends to that of HDPE in both depths. They indicate that the different CH_4:O_2 ratios had more influence on the growth of methanotrophs in the lysimeters, whereas for the plastic type it was not an important factor. It is reported that at limited oxygen conditions such as coastal marine sediments, the bacilli were found to be dominant on LDPE surfaces buried in sandy and silty soils (Harrison et al., 2014). After extraction of the biofilms from the plastic surfaces, only the HDPE surface showed more irregularity, cracks and cavities relative to the initial one. The HDPE surface at the 15 cm depth was more cracked compared to that of the 140 cm depth. This suggests that the higher density of microorganisms in the 15 cm depth were more capable of the biodegradation of HDPE than those in the 140 cm depth. Because the factors of microbial population and species are involved in the decomposition of the plastics (Esmaeili et al., 2013; Harrison et al., 2014), the microbial species were identified via the molecular technique as described in the next section.

Bacterial consortium in the simulated lysimeters

Bacterial consortium in the plastic and stabilized organic wastes

The types of bacterial consortium in the wastes by PCR-DGGE are shown in Fig. 2, and their related characteristics are summarized in Table S2. The microbial communities in all samples consisted of heterotrophs, methanotrophs, and chemolithoautotrophs in which most of them were gram-negative. In stabilized organic wastes, the initial composition was of 12 bacterial genera such as heterotrophs (*Shingobacterium* sp., *Flavobacterium* spp., *Pseudoxanthomonas* sp., *Burkholderia* sp. and *Xanthobacter* sp.), methanotrophs (methanotrophs type I:*Methylobacter* sp., *Methylococcus* sp., and methanotrophs type II: *Methylocella* sp., *Methylocystis* sp.) and chemilithoautotroph (*Nitrobacter hamburgensis*, *Nitrobacter wingogradskyi* and *Nitrosomonas* sp.). A total of $2.9–7.4 \times 10^{10}$copies g^{-1} dry waste was found. The populous genera were *Burholderia* sp. (6.3×10^{10}copies g^{-1} dry wastes) and *Methylocystis* sp. (7.4×10^{10}copies g^{-1} dry wastes). In the 210 days wastes including the plastics (HDPE, LDPE, PP, PS) and the stabilized organic wastes (OW), almost microbial genera were similar in all the samples of 15 and 140 cm depths. However, some methanotrophic genera of type II methanotrophs disappeared after 210 days incubation due to the waste pH changing to the neutral range such as *Methylocystis* sp. and *Methylococcus* sp. These methanotroph genera normally grow in weak acidic conditions. Because the stabilized organic waste used as seeding had a pH of 5.1–5.2 which was in their pH optimum growth range (pH 5.0–6.0), the initial organic waste was populated with these methanotrophs. In the landfill, type II methanotrophs (*Methylocystis* sp.) were more abundant in the acid cover soil compared to the other type II methanotrophs (Kong et al., 2013). Regarding the types of methanotrophs residing in the lysimeters (Fig. 2), type I included *Methylococcus capsulatus* and *Methylobacter* sp. was of higher concentration (13.43×10^{10} DNA copies) than type II (6.93×10^{10} DNA copies) in the upper part. Only *M. capsulatus* was absent in the lower part suggesting that the low O_2 could suppress the growth of this methanotroph. This is because type II methanotrophs favor an environment of higher methane level while type I methanotrophs are dominant where there are limited CH_4 and high O_2 levels (Hanson and Hanson, 1996). This implies that *M. capsulatus* corresponded to high MOR in the upper part of the lysimeter.

Many bacterial genera grew following incubation in the lysimters such as *Acinetobacter* sp., *Chitiphaga* sp., *Hyphomicrobium* sp., and *Methylococcus capsulatus*, particularly in the 15 cm depth. This evidence suggests that new environmental conditions such as neutral pH (6.8–7.0) and high available O_2 in the lysimeters promoted other methanotrophic genera including other microorganisms. Higher extracellular polysaccharides (EPS) production by methanotrophs was found in the upper part than in the lower part (Table 1). Subsequently, the heterotrophs which assimilate the carbon source from the EPS had been growing (Giroldo et al., 2005). In addition, they could alternatively utilize organic matters from the debris of microbial cells such as methanotrophs and other chemilithotrophs. Using the PCR-DGGE technique, the results agree with the SEM that more microbial genera and densities were in the upper part of the lysimeters (Table 1). The microorganisms had been absent in the lower part including *Acinetobacter* sp., *Flavobacterium* sp., *Chitiphaga* sp. (Fig. 2). It is reported that these three genera were capable of decomposition in various types of hydrocarbon such as plastics (Gnanavel et al., 2013), diesel-oil, *n*-hexadecane (Kang et al., 2011), gelatin, cellulose and chitin (Chung et al., 2012). These microorganisms will be discussed in relation to plastic decomposition in section 3.5.

For the nitrifying bacteria, *Nitrobacter* sp. and *Nitrosomonas* sp. were found in the same amounts throughout the lysimeter depths (Table 1). This indicates that different O_2 and CH_4 ratios did not affect the growth of these autotrophic species. The nitrifying bacteria could help the growth of methanotrophs by providing nitrate as a nitrogen source. The chemical analysis of nitrogen species in the wastes showed that there was not much difference in nitrogen speciation between the upper and lower parts of the lysimeter (data not shown). Some reports state that nitrifying bacteria can degrade some persistent matters such as halogenated aliphatic and crude oil (John and Okpokwasili, 2012).

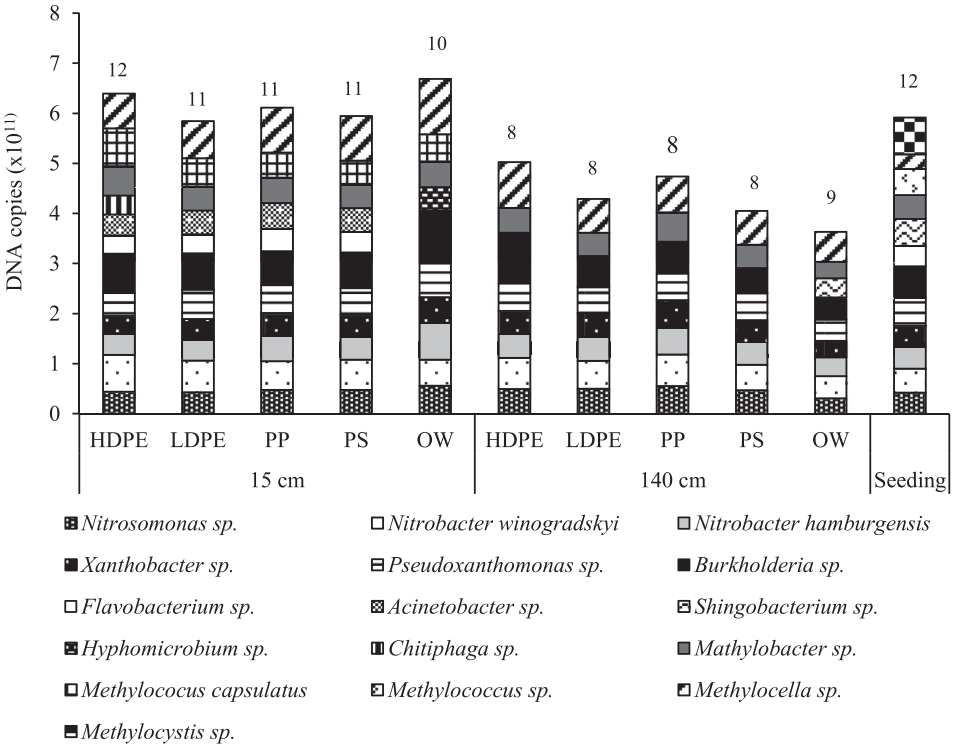

Fig. 2. Bacterial consortium of various waste plastics and organic wastes after 210 days incubation in comparison between the seeding, the 15 and 140 cm depths.

Chemical composition of initial and UV treated waste plastics

A wide variety of compounds were detected by FTIR for the initial plastics and the UV-irradiated plastics. All types of the initial plastic spectrum are shown in Fig. 3. All spectra of initial plastics had slight differences. HDPE consisted mainly of alkane (−CH, 2800–3000 cm^{-1}), alkene (=CH, 690–720 cm^{-1}), and methylene ($-CH_2$, 1400–1500 cm^{-1}) as functional groups. LDPE contained similar functional groups as HDPE with slightly higher intensities of all compounds. PP had a high portion of alkane groups (−CH, 2850–3000 cm^{-1}), and methyl groups (CH_3, 1375 cm^{-1}) with a low portion of alkene groups (=CH, 650–1000 cm^{-1}). Finally, PS contained similar compounds as PP and additional aromatic compounds. After 200 h of UV irradiation, new peaks of all the plastics appeared in the ranges of 1665–1760 cm^{-1}which were a carbonyl functional group (C=O), the common products of abiotic oxidation (Arkatkar et al., 2009). This appearance indicates the surface hydrophilicity of plastics (Roy et al., 2008). The synthetic polymers especially polyolefin, made up of only carbon and hydrogen atoms, are generally less susceptible to microbial attack. Their inertness is probably due to a total lack of carbon-to-oxygen bonds such as C=O, C−OR, C−OH which are the normal appropriate sites of microbial attacking enzymes (Motta et al., 2009). In addition, increasing carbonyl compounds give much greater opportunity for microbial decomposition because the substrate is more water soluble (Raaman et al., 2012).

Biodegradation of polyethylene (HDPE, LDPE)

The waste plastics after 210 days of biogas exposure in the lysimeters, in case of polyethylene (HDPE and LDPE), are shown in Fig. 3a–b. The carbonyl peaks (1710–1750 cm^{-1}) of HDPE slightly decreased, whereas that of LDPE greatly decreased. Many reports stated that the carbonyl groups initially increased when polyethylene was exposed to abiotic environments such as UV light with a long exposure time (Hadad et al., 2005), high temperature (Arkatkar et al., 2009; Jeyakumar et al., 2013), hot nitric acid (Yamada-Onodera et al., 2001) etc. Most of the abiotic exposed plastics often decreased in water soluble compounds after they were continually biodegraded by various species of microorganisms such as *Brevibacillus borstelensis* (Hadad et al., 2005), *Aspergillusniger* (Raaman et al., 2012), and *Rodococcus* sp. (Mor and Sivan, 2008), etc. Inversely, the carbonyl compounds such as ketone or aldehyde are the products of plastics by biodegradation of many soil microorganisms (Esmaeili et al., 2013). As seen in HDPE, the carbonyl compounds could still be detected in the 210 days as a result of continuous indigenous microbial activities. In general, the greatest difficulty of microorganisms in the digestion of plastics is the initial breakaway of the long-chain structure (Esmaeili et al., 2013). In addition to the carbonyl groups, the alkane group (C−H, 2850–3000 cm^{-1}), the alkene peak (=CH) and methylene peak (CH_2) were observed in both depths, and slightly higher intensity in 140 cm. The remaining of these compounds appeared because lower oxidation in 140 cm depth. Conversely, the oxygenated (−OH, 3400 cm^{-1}, C−O, 1060 cm^{-1}) groups of HDPE appeared particularly in the 15 cm depth. The appearance of the hydroxyl group (−OH) is commonly classified as that of carboxylic acids and alcoholic compounds (Motta et al., 2009). This indicates the transformation of hydrocarbon compounds (−CH) to oxygenated hydrocarbon compounds (−OH, C−O) by oxidation reaction which resulted in lower double bond peaks in the 15 cm depth. Although most biodegradation was slow, the microorganisms in the lysimeters were able to oxidize HDPE into more water-soluble compounds. As described in section 3.4.1, there were several microorganisms involved in hydrocarbon biodegradation such as *Acinetobacter* sp., *Flavobacterium* sp., *Chitiphaga* sp. including methanotrophs which were dominant in the upper part of the lysimeters. As illustrated in Fig. S2, weight loss percentages of each depth were significantly different (ANOVA, $P < 0.05$), and the weight loss percentages of HDPE (a) and LDPE (b) at the 15 cm depth were the highest

Table 1
Changes of EPS and bacterial type during the experiment by FISH technique (using ANOVA).

Depth (cm)	90 days						210 days						357 days					
	EPS (mgg^{-1})	M1 $P = 0.003$	M2	NB	AOB	Others	EPS (mgg^{-1})	M1	M2	NB	AOB	Other	EPS (mgg^{-1})	M1	M2	NB	AOB	Others
		($\times 10^7$ cells g^{-1})						($\times 10^7$ cells g^{-1})						($\times 10^7$ cells g^{-1})				
5	8.8 ± 1.6	2.2 ± 0.5	1.3 ± 0.3	2.5 ± 0.9	2.9 ± 0.9	4.8 ± 0.9	10.1 ± 1.7	2.9 ± 1.3	1.2 ± 0.4	2.7 ± 1.4	3.1 ± 1.1	1.3 ± 0.8	12.4 ± 1.9	3.8 ± 0.9	2.6 ± 0.9	3.5 ± 1.5	3.2 ± 1.1	1.0 ± 0.3
15	8.0 ± 1.6	3.5 ± 0.3	1.8 ± 0.8	2.0 ± 0.9	2.1 ± 0.6	4.6 ± 0.5	9.6 ± 1.6	4.8 ± 1.9	2.2 ± 0.5	3.0 ± 0.8	2.9 ± 1.4	1.2 ± 0.6	11.6 ± 1.8	4.9 ± 1.0	3.3 ± 1.3	3.7 ± 0.8	3.9 ± 0.7	0.8 ± 0.8
35	5.6 ± 1.7	2.4 ± 1.2	2.0 ± 0.9	1.7 ± 0.9	1.9 ± 0.8	5.0 ± 0.6	6.2 ± 1.6	1.8 ± 0.7	2.2 ± 0.6	1.9 ± 0.9	2.2 ± 0.9	2.5 ± 0.9	8.4 ± 1.9	2.9 ± 0.6	2.6 ± 0.5	2.1 ± 0.5	2.7 ± 0.9	1.6 ± 0.5
70	3.2 ± 0.8	2.1 ± 0.7	2.2 ± 0.8	1.4 ± 0.9	1.6 ± 0.4	4.7 ± 1.0	4.1 ± 1.5	1.1 ± 0.3	2.2 ± 1.2	1.3 ± 0.4	1.5 ± 0.4	3.2 ± 0.5	7.8 ± 2.2	2.1 ± 0.5	2.4 ± 1.2	1.3 ± 0.3	1.5 ± 0.5	1.7 ± 0.5
105	3.0 ± 0.8	1.7 ± 0.9	2.3 ± 1.1	1.1 ± 0.9	1.3 ± 0.6	5.3 ± 1.0	3.7 ± 1.0	0.8 ± 0.4	2.3 ± 0.8	1.1 ± 0.5	1.2 ± 0.3	3.9 ± 0.2	7.5 ± 2.0	1.3 ± 0.9	2.2 ± 1.0	1.1 ± 0.5	1.2 ± 0.6	2.5 ± 0.5
140	2.7 ± 0.7	1.4 ± 0.3	2.1 ± 0.4	0.9 ± 0.9	1.1 ± 0.7	5.3 ± 0.5	3.3 ± 0.8	0.7 ± 0.3	2.2 ± 1.1	1.0 ± 0.7	1.0 ± 0.5	4.0 ± 0.2	7.1 ± 2.0	0.9 ± 0.9	2.2 ± 0.7	0.8 ± 0.4	1.1 ± 0.5	3.1 ± 0.8
P value	0.001	0.003	0.047	0.005	0.003	0.075	0.001	0.001	0.009	0.001	0.001	0.002	0.003	0.001	0.044	0.001	0.001	0.001

Note: M1: Type I methanotrophs; M2: Type II methanotrophs; NB: nitrobacter; AOB: ammonia oxidizing bacteria.

compared to those of the lower depths. In correlation with the methane oxidation rate (MOR, Fig. 1) and bacterial consortium (Fig. 2) including plastic weight loss (Fig. S2), this implies that the methanotrophs were responsible for the decomposition of the plastic samples. However, *Acinetobacter* sp., *Flavobacterium* sp., *Chitiphaga* sp. were detected on the plastic samples in the upper part of the lysimeters and absent in the lower part, which corresponded to the lowest weight loss of the plastics. Thus, these heterotrophs might be involved in HDPE/LDPE biodegradation in this situation.

Biodegradation of polypropylene and polystyrene

In the case of polypropylene (PP), the carbonyl intensities in both depths decreased in opposite to that of the −CH group as compared to beginning initial PP (Fig. 3c). This occurrence was similar to the polyethylene cases (HDPE/LDPE). The appearance/disappearance of carbonyl groups also indicates microbial biodegradation of PP (Arkatkar et al., 2010). It is reported that the carbonyl of the chemically pretreated PP had decreased while that of unpretreated PP increased under incubation with *Bacillus flexus*, *Bacillus subtilis* and *Pseudomonas azotoformans* (Arkatkar et al., 2010). In contrast with the polyethylene, the OH peak did not appear at both depths. This indicates that the hydrolysis reaction rarely occurred in PP due to its hydrophobic property. Biodegradation of the C−O peaks at both depths was not observed due to the lack of change in the peak areas in comparison to the initial PP. Nevertheless, the increasing intensities of the −CH peaks indicated the polymer chain cleavage by microbial activities subsequently increasing the alkane intensity of PP in the 15 cm depth. In addition, the CH_3 intensity decreased, which indicates that oxidation took place at the primary position of the polymer chain, to be further decomposed into ketone and esters (Ambika et al., 2009; Jeyakumar et al., 2013). The lower CH_3 intensity of the 15 cm depth suggests more microbial activities in PP decomposition. This is good relative to the increasing −CH peaks. The intensity of CH_3 tends to decrease as a function of time when PP is incubated with some microorganisms such as *Phanerochaetechryso sporium* NCIM 1170 and *Engyodontium album* MTP019 (Jeyakumar et al., 2013).

Polystyrene's (PS) chemical structure shows an aromatic ring which is normally difficult to biodegrade (Singh and Sharma, 2008). As shown in Fig. 3d, the intensities of the C=O peak slightly increased after 200 h of exposure to UV-radiation. However, the C=O peak at the 15 cm depth PS continually increased after 210 days incubation in concurrence with aromatic peak appearance (C−CH, 1600 cm^{-1}) in the PS of both depths. The intensities of the C=O peak did not decrease with time which differed from the polyethylene. This is because it might be bonded with the aromatic ring, which is not a simple chemical structure for biodeterioration. Furthermore, the polystyrene-related peaks showed wavelengths of 1600, 1510, 759 and 694 cm^{-1} in both PS of the 15 cm and 140 cm depths with higher intensities after 210 days of incubation. These peak appearances suggest that some of the bonds in polystyrene have possibly been weakened (Pushpadass et al., 2010). Clear changes were observed for peaks at 1000–1700 cm^{-1} and 3400 cm^{-1} which indicates depolymerization and biodegradation into monomers.

Interrelation of biodegradation of waste plastics and methanotrophic activities

The overall percentage change in weight (a loss) is a common indicator of plastic degradation as shown in Fig. S2. It illustrates that after 357 days of incubation with an indigenous microbial consortium of the open dump lysimeters, the highest weight losses of HDPE, LDPE, PP and PS were 15%, 4.96%, 6.7% and 5.29%,

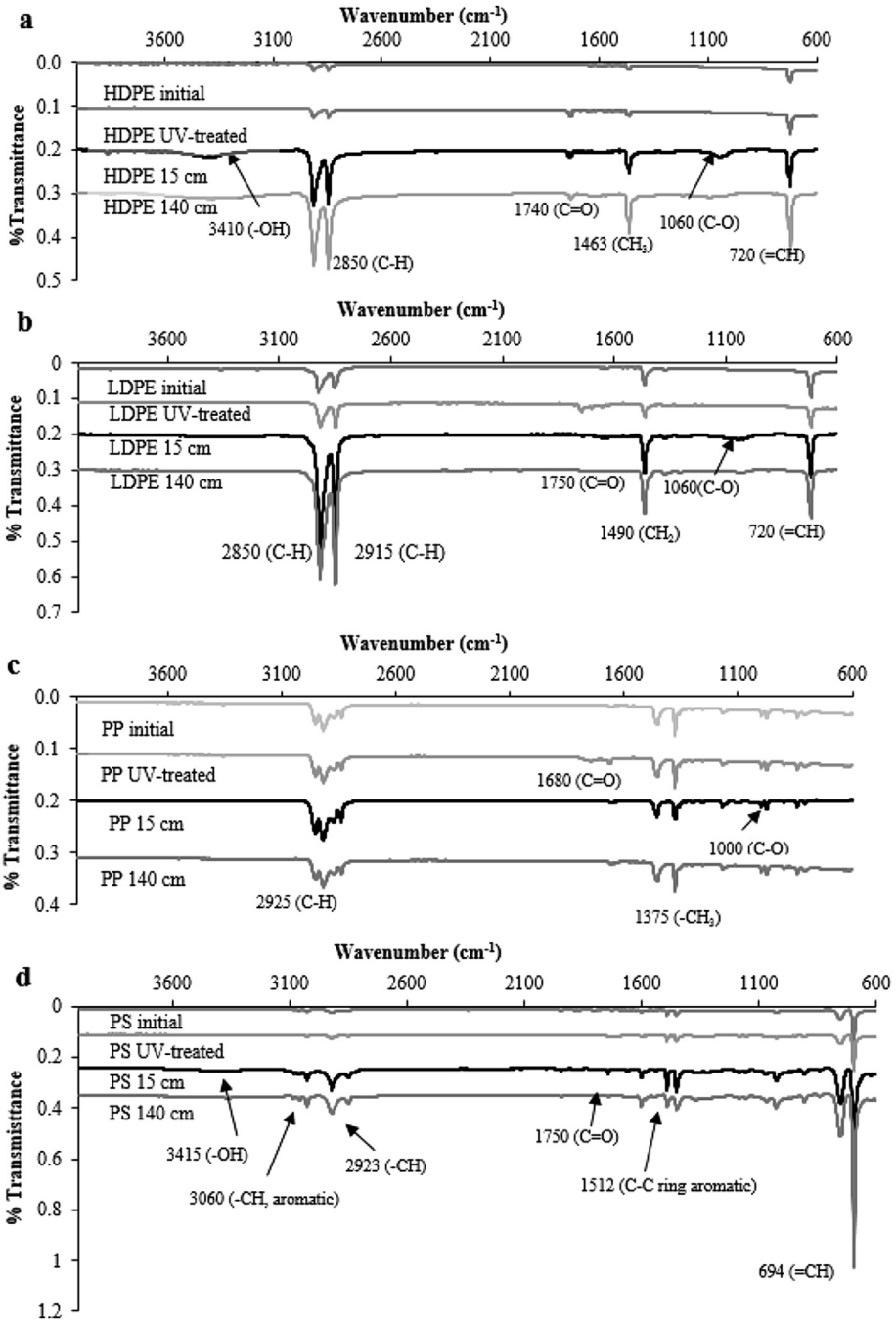

Fig. 3. Intensities of functional groups of HDPE (a) LDPE (b), PP (c), PS (d) in various samples: initial, UV exposed, incubated samples at 15/140 cm depths.

respectively at the 15 cm depth where the highest bacteria population was found. HDPE is the highest biodegraded plastic, followed by PP, PS and LDPE. The greater HDPE biodegradation can be explained as there is greater cross-linking in the void volume of HDPE, although HDPE is of higher density as compared with LDPE. This provides much more carbon content, subsequently enhancing sites for microbial reaction (Alok et al., 2008). The type of additive or plasticizer is one of the most important factors affecting the degradation rate of polymers for the microbial consortium environment. The LDPE containing Mn/Fe + $CaCO_3$ was the CO_2 mineralized to about 16%, whilst none of the $CaCO_3$ additive was the CO_2 mineralized to about 7% after 13 months in the soil (Husarova et al., 2010). In the case of PP, the weight loss of the UV-treated PP (6.7%) at 15 cm waste depth in this study was higher than the UV-treated PP that was incubated with *Bacillus flexus* (2.5%) in the previous study (Arkatkar et al., 2010). This indicates that the condition of the open dump lysimeters was appropriate for plastic degradation. The weight loss of the UV-treated PP in the lysimeter in this study was lower than the thermal pretreated one, but higher than the un-pretreated PP that was incubated with soil consortia for 12 months (Arkatkar et al., 2009, 2010). In addition, bacteria species highly influence plastic biodegradation. *Bacillus flexus* oxidizes the PP that was not pretreated more than *Bacillus subtilis*, *Bacillus stutzeri* and *Pseudomonas azotoforman* were individually. It can clearly be seen that the plastic degradation rate under aerobic conditions (the upper zone) was higher than the semi-aerobic

conditions (the lower zone) in the lysimeters. The results suggest that aerobic conditions are appropriate for the biodegradation of plastics in this situation.

As discussed above, the maximum biodegradation rate in terms of weight (Fig. S2) appeared at the 15 cm depth, while the minimum biodegradation ate was found at the 140 cm depth. Because the appearance of heterotrophs such as *Acinetrobacter*, *Flavobacterium,* occurred in only the 15 cm depth, this suggests that they participated in plastic deterioration. This evidence could be explained by the interrelation of bacterial consortium. One possibility is the heterotrophs are the principal microbes in plastic decomposition while methanotrophs function as a supporter in the case of the upper zone of the lysimeters. Most of the relationships between heterotrophs and methanotrophs provide beneficial relations in the interaction among population. The bacterial consortium can be adaptable to various environments and increase in the compound degradation rate compared to the individual population (Hršak and Begonja, 2000; Hesselsoe et al., 2005). The methanotrophs, as the only member was able to oxidize methane and produced the oxidation product as methanol/formate and extracellular polysaccharide (EPS). These can serve as carbon sources or growth factors for the heterotrophs (Hršak and Begonja, 2000; Chiemchaisri et al., 2001). For example, the mixed communities of heterotrophs and methanotrophs were more efficient in the transformation of the linear alkylbenzene sulfonates (LAS) than any of the individual populations (Hršak and Bengonja, 2000). Therefore in the lysimeter, the methanotrophs indirectly stimulate heterotroph growth and increase the heterotroph population. Although heterotrophs act as competition for O_2 consumption, the probable beneficial effect of the heterotrophs is the reduction of oxygen tension, thus creating more favorable conditions (suitable CH_4:O_2 ratio) for methanotrophs to grow. High O_2 tension can simulate EPS production in methanotrophs as a mechanism for reduction of O_2 tension (Chiemchaisri et al., 2001). In the lysimeters, the highest EPS content from methanotrophs was at the 5 cm depth due to the effect of high oxygen tension. If these heterotrophs utilized EPS as the carbon source, a high population of the plastic degrading heterotrophs (*Acinetrobacter* sp. and *Flavobacterium* sp.) must appear in the upper zone of the lysimeter (5–15 cm depths) with high biodegradation of plastics throughout the upper zone. Nevertheless, as shown in Table 1, the population of heterotrophs (within the other bacteria) showed a decreasing trend with time. The rate of their disappearance was high particularly in the upper zone of the lysimeter with the lowest population at the 15 cm depth. Because the lower biodegradation of HPDE/LDPE was found in the 5 cm depth as opposed to the 15 cm depth, this indicates that these heterotrophs were not the principal microbes functioning in plastic degradation (particularly in the 15 cm depth). This is because the maximum biodegradation rate of plastics was found at this depth where the maximum MOR was also found (Fig. S2e). As a result, it can be concluded that the methanotrophs mainly functioned as the principal decomposer in plastic biodeterioration, particularly in the upper zone of the lysimeter. Methane monooxygenase (MMO) of methanotrophs is a high potential enzyme in the decomposition of the C–H compounds via co-metabolism. The sMMO shows a wide range of substrate specificity (C1–C10), including alkenes, aromatic, alicyclic and hetrocyclic compounds whereas pMMO mediates the oxidation of a small group of alkanes (C1–C5) (Kotani et al., 2007). These enzymes commonly attack unactivated C–H bonds (Goldman, 2009). Likewise, a plastic normally contains a lot of C–C and C–H bonds. Alternatively, a small alkane and an alkene from the other bacterial activities could be the substrates of MMO which can be future oxidized to become alcohol and ketone and fatty acids respectively. Because *Methylococcus capsulatus* was found only in the 15 cm depth, this microbe might respond in the high biodegradation of waste plastics. MMO from *Methylococcuscapsulatus* can degrade xenobiotic compounds such as TCE, styrene, C1–C8 n-alkanes, cyclohexane, pyridine and methyl bromide (Oremland et al., 1994; Lontoh et al., 2000). Many studies report that multiple factors affect polymer degradation such as chemical structure, chemical composition, and additives (Tokiwa et al., 2009). In biological environments, one important factor is the presence of a microbial consortium with the appropriate metabolic capabilities and beneficial interaction population. The results from this study show that the kinetic degradation rate in terms of the weight loss of any plastic was the first-order reaction. The plotting of decay rates (K) of each plastic type and methane oxidation rate in various depths is illustrated in Fig. S2e. The correlation of the degradation kinetic and MOR of any plastic is an exponential relationship. This result indicates the degradation rate of waste plastics increased as the function of methanotrophic activities. The equations are obtained from the relationships shown in Eqs. (2)–(5) for HDPE, LDPE, PP and PS, respectively. The plastics which lead to a high possibility of decomposition via methane oxidation under open dump conditions are HDPE, PP, PS and LDPE, respectively.

$$Y = 0.0314 \ln(X) + 0.0141, \quad (R^2, 0.97) \tag{2}$$

$$Y = 0.0165 \ln(X) + 0.0125, \quad (R^2, 0.97) \tag{3}$$

$$Y = 0.0112 \ln(X) + 0.0216, \quad (R^2, 0.94) \tag{4}$$

$$Y = 0.0140 \ln(X) + 0.0003, \quad (R^2, 0.90) \tag{5}$$

where X and Y refer to the MOR (μmole CH_4 d^{-1} g^{-1} waste) and degradation rate (y^{-1}), respectively.

Conclusion

The maximum MOR appeared at the 15 cm waste depth whereas the minimum MOR was found at the 140 cm waste depth. The types of methanotrophs and their activities in the lysimeters depended on CH_4:O_2 ratio. At the upper zone (15 cm depth), type I including *Methylococcus capsulatus* and *Methylobacter* sp. were of a higher population than type II (*Methylocella* sp.). Many species of heterotrophs found in the upper and lower zones of the lysimeter were *Shingobacterium* sp., *Pseudoxanthomonas* sp., *Burkholderia* sp. and *Xanthobacter* sp. Only *Acinetrobacter*, *Flavobacterium*, and *Chitiphaga* sp. resided in the 15 cm depth. For nitrifying bacteria, *Nitrobactor* spp. and *Nitrosomonas* sp. lived in the lysimeters of similar densities throughout the lysimeters. The results of FTIR indicate that the microbial consortiums in the lysimeters were able to oxidize waste plastics into more water-soluble compounds such as carbonyl groups which contain compounds and shorter chains compounds. The plastic degradation rate in aerobic conditions was higher than in semi-aerobic conditions. The weight losses of HDPE, LDPE, PP and PS were 15%, 4.96%, 6.7% and 5.29%, respectively, at the 15 cm depth where the highest bacteria population was found. Methanotrophs mainly functioned as the principal decomposer in plastic biodeterioration, particularly in the upper zone of the lysimeter. The correlation of the degradation kinetic and MOR of any plastic is an exponential relationship.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ibiod.2015.03.015.

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