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Enhancement of biodegradation of plastic wastes via methane oxidation in semi-aerobic landfill

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ABSTRACT

The biodegradation of waste plastics (high/low density polyethylene, HDPE/LDPE; polypropylene, PP; polystyrene, PS) in the simulated lysimeters of semi-aerobic landfill was investigated under different range of aeration rates (1–2 ml min^{-1}) concurrently with a constant rate of a synthetic landfill gas. Methane oxidation was found throughout waste bed of a 1 ml min^{-1} aerated lysimeter while methane was absolutely oxidized at the bottom of 2 ml min^{-1} aerated lysimeter. After 3 months of experiment, aeration of 1 ml min^{-1} gave higher significant percentage losses of weight (15%–20%HDPE and 5%–9%PP) than those of 2 ml min^{-1} (11%–12%, HDPE and 1%–2%, PP) where high methane oxidation rate (MOR) appeared. Species variety of methanotrophs, heterotrophs, and autotrophs was revealed using PCR-DGGE technique. Only heterotrophs (*Burkholderia* sp.), *nitrifying bacteria* (*Nitrosomonas* sp. *AL212*, *Nitrobacter winogradkyi*), Type I methanotrophs (*Methylobactor* sp. and *Methylococcus capsulatus*), Type II methanotrophs (*Methylocystic* sp., *Methylocella* sp.) were found correlatively to plastics degradation in the lysimeters in different conditions. Many low molecular weight of hydrocarbon compounds such as alkane, alkene, alcohol, acid and epoxide were detected as biodegraded products. In conclusion, biodegradation of plastic wastes in semi-aerobic landfill could be accelerated by supplying an optimum aeration in proportion to methane available in the waste bed.

1. Introduction

Approximately 265 million tonnes of plastic materials were produced in 2010, and plastic consumption of developing countries has been higher than the world average due to rapid urbanization and economic development (UNEPIETC, 2015). The amount does matter in waste landfill site, plastic waste is estimated at approximately 13% of the municipal solid waste generated in 2013 (EPA, 2015). Waste plastics pose enormous burden on the environment, because of their resistance to degradation. They tend to accumulate in nature due to lack of functional groups as well as water insolubility, low chemical reactivity and bioavailability for microorganisms. Waste plastics buried in soil cause water clogging phenomena and devastate soil for agricultural cultivation. Polyethylene (PE) films have been reported to undergo both abiotic (photo degradation, oxidative degradation) and biotic (biodegradation, bio-oxidation) degradation (Albertsson et al., 1995). Nevertheless, many researches have been carried out to improve degradability of waste polyethylene by introducing polar groups such as carbonyl groups to the polyethylene backbone chain and thus facilitate the microbes to metabolize the unwieldy plastics (Albertsson et al., 1995; Kavitha et al., 2014). Generally, conversion of a long chain polymer compound into CO_2 and water is a complex process. The various different types of microorganisms are co-operated, in which one leads to breakdown a polymer into smaller constituents, another utilizes a monomer and excretes simple waste compounds as a by-product and one uses the excreted waste. A few microbes are capable of decomposition of polyethylene isolated from soil, sea water, and compost. These include *Pseudomonas* sp., *Bacillus* sp., *Staphylococcus* sp., *Micrococcus* sp., *Streptococcus* sp., *Acinetobacter* sp., *Rhodococcus* sp., *Flavobacterium* sp., *Chelatococcus* sp. (Gilan et al., 2004; Hadad et al., 2005; Koutny et al., 2009; Jeon and Kim, 2013). Plastic polymer is broken into monomers by heterotrophic microorganisms of which plastic degradation pathway is a result of various physical and biochemical reactions (Albertsson et al., 1995; Roy et al., 2008; Kyaw et al., 2012).

Although landfill is a common method as a final disposal of

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municipal solid waste, nevertheless it generates a great amount of greenhouse gas (CH_4) and various pollutants in leachate. Thus, a semi-aerobic landfill is a new concept of solid waste disposal which accelerate waste decomposition with low emission of methane. Air is supplied into a landfill through a large leachate collection pipe by heat convection between higher waste temperature and lower atmospheric temperature. In semi-aerobic landfill condition, the observed oxygen content in waste bed varied between 3% and 8% (v/v) (Qifei et al., 2008). This enhances activities of aerobic bacteria that give increasing rate of solid waste decomposition and improve leachate quality (Matsuto et al., 2015). Although many microorganisms in the waste play an important role in degrading persistent wastes, one of the more interesting microbes is methanotrophs, which play protection role in global warming and natural resource contamination. They utilize methane as their sole carbon and energy source. Over 300 persistent biodegraded compounds such as aliphatics, alkanes, aromatics and chlorinated hydrocarbon are co-metabolized by enzyme methane monooxygenase (MMO) from methanotrophs (Hanson and Hanson, 1996; Hazen, 2010; Semrau, 2011). It is reported that some types of methanotrophs such as *Methylobacter* sp., *Methylococcus* sp. and *Methylocella* sp. are the principle decomposer of plastic degradation in simulated open dump site where methane is available in waste beds (Muenmee et al., 2015). Because both methane and oxygen are available in a waste bed of a semi-aerobic landfill, there is supposed to be a lot of methanotrophs reside in this type of landfill. By the fact that oxygen is a key factor of methane oxidation (Chiemchaisri et al., 2001), thus this study aims to investigate biodegradation of plastic waste in semi-aerobic landfill condition under different aeration rates. Microbial consortium in relation to plastic degradation and methane oxidation was investigated. The obtained information is useful to improve design of semi-aerobic landfill in order to accelerate plastic waste degradation.

2. Materials and methods

2.1. Set-up of lysimeters of semi-aerobic landfill

Four types of plastics HDPE (high density polyethylene), LDPE (low density polyethylene), PP (polypropylene) and PS (polystyrene) were employed in experiment. The same procedure of preparation and pre-treatment of plastics was done as described in previous report (Muenmee et al., 2015). They were manually cut into two shapes, square and rectangle, for convenient segregation of plastics after experiment. Surface area of every plastic is equivalent to 9 cm^2. After pre-treatment by 200 h UV exposure; HDPE, LDPE, PP and PS were mixed to obtain the ratio of 56: 29: 12: 3 (%W/W) which is a ratio commonly found in waste composition in waste disposal site (Pollution Control Department of Thailand, 2005). The stabilized organic wastes were taken from a practical landfill site and mixed with the mixed plastics in order to simulate disposal wastes in a landfill. The chemical characteristics of the stabilized organic wastes were ammonium (42.11 ± 8.5 μg g^{-1}), nitrite (5.02 ± 1.4 μg g^{-1}), nitrate (18.43 ± 4.2 μg g^{-1}), total organic carbon (TOC, 137.3 ± 12.1 mg g^{-1}), TKN (4812.5 ± 82.5 μg g^{-1}). These stabilized organic wastes also provided natural microorganisms as seeding of the simulated lysimeters. 427 g of the mixed plastics were mixed with 379 g of the stabilized organic wastes to obtain 53:47 of a common ratio based on real condition of landfill (Chiemchaisri et al., 2010). The chemical characteristics of this mixture were ammonium (20.8 ± 2.7 μg g^{-1}), nitrite (4.3 ± 1.5 μg g^{-1}), nitrate (12.6 ± 3.3 μg g^{-1}), TKN (4138 ± 29.4 μg g^{-1}). The moisture content of the waste mixture was adjusted to 10% (w/w) which is an optimum for methane oxidation (Visvanathan et al., 1999), then the waste mixture was filled into the simulated lysimeters. The waste bed of the lysimeters had an average bulk density of 0.71 g cm^3 with 48% porosity. There were 3 lysimeters made of acrylic cylinders with a diameter of 5 cm and a length of 150 cm each. In order to stimulate semi-aerobic condition, aeration was supplied at the bottom of the lysimeters. The lowest of average O_2 content was set at 1 ml min⁻1 (lysimeter No.1, L1) because it was given the initial oxygen available in the waste bed about 8–10%. It was noted that the lowest detected oxygen in the waste bed was about 1.3% during experiment which was still in semi-aerobic condition. Concurrently, lysimeter No. 2, L2, aeration was set at 2 ml min^{-1} in order to increase methane oxidation rate. A synthetic landfill gas (60%CH_4:40%CO_2) was purged into both lysimeters at a flow rate of 0.56 ml min^{-1} equivalent to an actual methane loading rate of 26.50 g m^3 d^{-1} (Chiemchaisri et al., 2013). For lysimeter No. 3 (L3), only 1 ml min^{-1} aeration was applied to serve as control (no purged synthetic landfill gas). During experimental period, the gas samples at 0, 37, 74, 111, and 148 cm depths of all lysimeters were withdrawn and analyzed for composition via gas chromatograph (GC6890 Agilent) on weekly basis. After 3 months, the waste samples of all lysimeters from different layers of waste bed (0–10 cm; 10–15 cm; 30–40 cm; 65–75 cm; 100–110 cm; 140–150 cm) were analyzed for determination of methanotrophic activity, molecular analysis (PCR-DGGE) and plastic decomposition (weight loss and GC/MS).

2.2. Analyses of plastic waste samples

2.2.1. Methane oxidation rate

Methanotrophic activity was determined via batch microcosm study as described in (Chiemchaisri et al., 2013). Briefly, 0.5 g of the waste sample was placed in a 25 ml serum bottle in triplicate in sealed condition. A pure CH_4 gas was provided at 10% CH_4 head space. All the serum bottles were incubated at room temperature (28–30 °C). Head space gas was withdrawn every 3 h and analyzed by gas chromatograph (GC, Agilent-6890). Changes of CH_4, O_2 and CO_2 concentrations against time were plotted to determine methane oxidation rate (MOR) oxygen uptake rate (OUR) and carbon dioxide production rate (CPR).

2.2.2. Biomolecular analysis

A 0.5 g of plastic waste was extracted for microbial DNA using a soil DNA isolation kit (Farvoprep™) according to the manufacturer's protocol. The concentration of the DNA product was measured via a Qubit 2.0 fluorometer (Invitrogen Corporation) and was calculated by Eq. (1) (LeOn Ohl et al., 2003). The procedure of DNA amplifying was similar description as our previous report (Muenmee et al., 2015) using a universal primer (338F and 518R) via Toptaq Master Mix Kit, Quiagen by Swift™ MaxPro thermal cyclers (Esco Healthcare Pte. Ltd). The condition of thermal cycle operation was set as 95 °C for 10 min, followed by 25 cycles of 95 °C for 10 s, 58C for 30 s and 72C for 10 s; and a final extension step of 72 °C for 10 min. Then, the PCR product was verified via electrophoresis in 1% agarose, and separated by DGGE technique (Bio Rad DCode™). The concentration of PCR product was determined by a Qubit 2.0 fluorometer (Invitrogen Corporation). Then, the PCR product was loaded onto an 8% polyacrylamide gel. The gel was made by a denaturing gradient from 35 to 70% (where 100% denaturant contains 7 M urea and 40% formamide). The electrophoresis was run using TAE buffer (20 mM Tris, 10 mM acetate, 0.5 mM EDTA; pH 7.4) for 7 h at 80 V at 60 °C. After electrophoresis, the gel was stained with ethidium bromide (0.5 mg l^{-1}) for 30 min and destained in water for 15 min. The, gel image was captured, and DNA band profiles were analyzed to determine the intensity of each band by using VisionWorks™LS Analysis Software (Ultra-Violet Products Ltd.). The percentage of the intensity for each band based on the