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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

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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

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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