Waste Management xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Response of mixed methanotrophic consortia to different methane to oxygen ratios

K. Chidambarampadmavathy [a,c], O.P. Karthikeyan [a,b,c,d], R. Huerlimann [a,b,c], G.E. Maes [a,b,c,f], K. Heimann [a,b,c,e,*]

[a] College of Science and Engineering, James Cook University, Townsville 4811, Queensland, Australia
[b] Comparative Genomics Centre, James Cook University, Townsville 4811, Queensland, Australia
[c] Centre for Sustainable Tropical Fisheries and Aquaculture, James Cook University, Townsville 4811, Queensland, Australia
[d] Sino-Forest Applied Research Centre for Pearl River Delta Environment (ARCPE), Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong Special Administrative Region
[e] Centre for Bio-discovery and Molecular Development of Therapeutics, James Cook University, Townsville 4811, Queensland, Australia
[f] Center for Human Genetics, Genomics Core, KU Leuven, Herestraat 49, 3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 28 January 2016
Revised 31 August 2016
Accepted 4 November 2016
Available online xxxx

Keywords:
Landfill cover soil
Compost soil
Methane
Biopolymer
Methylosarcina
Chryseobacterium

ABSTRACT

Methane (CH_4) and oxygen (air) concentrations affect the CH_4 oxidation capacity (MOC) and mixed methanotrophic community structures in compost (fresh) and landfill (age old) top cover soils. A change in the mixed methanotrophic community structure in response has implications for landfill CH_4 bio-filter remediation and possible bio-product outcomes (i.e., fatty acid methyl esters (FAME) content and profiles and polyhydroxybutyrate (PHB) contents). Therefore the study aimed to evaluate the effect of variable CH_4 to oxygen ratios (10–50% CH_4 in air) on mixed methanotrophic community structures enriched from landfill top cover (LB) and compost soils (CB) and to quantify flow on impacts on MOC, total FAME contents and profiles, and PHB accumulation. A stable consortium developed achieving average MOCs of 3.0 ± 0.12, 4.1 ± 0.26, 6.9 ± 0.7, 7.6 ± 1.3 and 9.2 ± 1.2 mg CH_4 g^{-1} $DW_{biomass}$ h^{-1} in LB and 2.9 ± 0.04, 5.05 ± 0.32, 6.7 ± 0.31, 7.9 ± 0.61 and 8.6 ± 0.48 mg CH_4 g^{-1} $DW_{biomass}$ h^{-1} in CB for a 20 day cultivation period at 10, 20, 30, 40 and 50% CH_4, respectively. CB at 10% CH_4 had a maximal FAME content of 40.5 ± 0.8 mg FAME g^{-1} $DW_{biomass}$, while maximal PHB contents (25 mg g^{-1} $DW_{biomass}$) was observed at 40% CH_4 in LB. Despite variable CH_4/O_2 ratios, the mixed methanotrophic community structures in both LB and CB were relatively stable, dominated by *Methylosarcina* sp., and *Chryseobacterium*, suggesting that a resilient consortium had formed which can now be tested in bio-filter operations for CH_4 mitigations in landfills.

1. Introduction

Contributing to ~20% of the worldwide greenhouse gas (GHG) emissions, methane (CH_4) is considered a potent GHG together with carbon dioxide (CO_2) (IPCC, 2007, 2013). The atmospheric concentration of CH_4 has increased considerably from a pre-industrial level of ~715 to ~1840 ppb in 2015 (Butler and Montzka, 2016; EEA, 2016; IPCC, 2013). Both natural and anthropogenic sources contribute to CH_4 emissions, while anthropogenic activities alone contribute to 50–65% of total CH_4 emissions, causing a sharp rise in GHG emissions (GMI, 2010; Karthikeyan et al., 2015a). Landfill is the third largest anthropogenic source of CH_4, contributing ~600 million metric tons of CO_2 equivalents ($MMTCO_{2eq}$) as of 2010, which is projected to increase by 10% by 2020 (GMI, 2010). Biogenic CH_4 emissions from landfills occur due to microbial anaerobic degradation of organic matter and varies at different time intervals with respect to landfill age (Scheutz et al., 2009). Landfill soil covers naturally act as sinks for CH_4 (Henneberger et al., 2012; Scheutz et al., 2009; Staley and Barlaz, 2009). In addition, various types of compost/mulch materials are being used as landfill cover soil for enhancing CH_4 oxidation (Humer and Lechner, 1999).

In addition to methane emission mitigations, the deposition of fossil fuel-derived non-biodegradable plastics into landfills also causes landfill management issues. Despite all recycling efforts for non-biodegradable plastics, approximately 20% (w/v) are deposited in landfills (Staley and Barlaz, 2009), posing a threat to

* Corresponding author at: College of Science and Engineering, James Cook University, Townsville 4811, Queensland, Australia.
E-mail address: kirsten.heimann@jcu.edu.au (K. Heimann).

http://dx.doi.org/10.1016/j.wasman.2016.11.007
0956-053X/

adjacent environments, impeding environmentally sustainable landfill management practices. Therefore, reducing deposition of non-biodegradable plastics is of equal importance to developing GHG mitigation strategies. As a cradle-to-grave/cradle approach, the emanated CH_4 from landfill could potentially be re-routed for bio-plastic production using methanotrophs (Karthikeyan et al., 2015a; Rostkowski et al., 2012). Methanotrophs, a group of Gram-negative bacteria utilize landfill CH_4 as a carbon source and can store the CH_4-carbon in form of polyhydroxybutyrate (PHB), a key ingredient for the production of biodegradable plastics (Hanson and Hanson, 1996; López et al., 2014; Pieja et al., 2011a). To date, two major groups of methanotrophs have been identified: type-I methanotrophs belonging to the gamma proteobacteria (includes sub types-Ia and -Ib, family Methylococcaceae; 14 genera), and type-II methanotrophs belonging to the alpha proteobacteria group (family Methylocystaceae; 4 genera) (Bowman, 2014; Chidambarampadmavathy et al., 2015b; Hanson and Hanson, 1996; Semrau, 2011). Compared to type-I methanotrophs, type-II are capable of fixing atmospheric nitrogen, utilize CO_2, produce C18 as dominant fatty acids and are capable of storing carbon as PHB. Type-II methanotrophs are therefore beneficial for industrial bio-product development (Pieja et al., 2011a; Rostkowski et al., 2012; Strong et al., 2015; Wendlandt et al., 2010). Other than these, some methanotrophs are also grouped under Verrucomicrobia and NC10 phylum, with the latter reported to perform CH_4 oxidation coupled with denitrification process (Op den Camp et al., 2009; van Teeseling et al., 2014; Wu et al., 2011).

Studies on CH_4 oxidation capacity (MOC) and production of PHB have been previously carried out with pure cultures of methanotrophs, but research utilizing mixed microbial communities are rare (Helm et al., 2006; Karthikeyan et al., 2015b; López et al., 2014). Furthermore, complex interactions occur within the mixed microbial communities, whereby the co-habiting bacteria consume toxic and over-produced metabolites of methanotrophs such as methanol and formaldehyde, thereby potentially improving methanotrophic activity and PHB accumulation (Ho et al., 2014; Hršak and Begonja, 2000; Wendlandt et al., 2010). Provision of methanol and formaldehyde to heterotrophs could prove beneficial for methanotrophs in terms of supply with vitamins in exchange. Such a scenario is possible given the evidence that heterotrophs provide cobalamin to methanotrophs (Ho et al., 2014). To achieve these beneficial outcomes, sufficient supply of oxygen (O_2) is vital for supporting CH_4 oxidation and heterotrophic growth.

Theoretically, methanotrophs require 2 mol of O_2 to oxidize 1 mol of CH_4. Generally type-I methanotrophs dominate in niches where CH_4 levels are low and O_2 levels are high, with the opposite being the case for type-II methanotrophs (López et al., 2014; Wei et al., 2015). Low O_2 concentrations favor type-II, as the nitrogenase complex responsible for N_2-fixation is sensitive to O_2 (Whittenbury and Dalton, 1981). Another reason may be the formation of exopolysaccharides/extracellular polymeric substance (EPS) by type I methanotrophs, which, in mixed natural samples, may limit supply of O_2, thereby favoring the growth of type-II (Whittenbury and Dalton, 1981). Therefore, it is critically important to consider CH_4 to air (hereafter CH_4/O_2) ratios, as the responses of methanotrophs may vary, potentially affecting MOC in-turn, depending on the type enriched. Understanding CH_4/O_2 ratio-induced microbial community shifts is also important for developing/customizing bio-filters that work economically well for CH_4 mitigation in the varying CH_4/O_2 ratio fluxes, as experienced in landfills.

In this context, the present study aimed to evaluate the impact of variable CH_4/O_2 ratios during the enrichment of mixed methanotrophic consortia from landfill top cover (aged) and compost soil (fresh) and on MOC, FAME and PHB content. Specifically, we examined the community composition of methanotrophs and associated heterotrophs using 16s rRNA gene sequencing, generating community structure and biochemical profile results that are directly applicable to continuous landfill bio-filter operations and for evaluating bio-product development potential.

2. Material and methods

2.1. Inoculum-enrichment conditions

Inoculum-enrichment was carried out using a top cover soil taken from a 7 year-old landfill (aged) and a 6-week-old compost soil (fresh land cover soil) collected from McCahills landscaping supplies, Townsville, Australia (latitude 19°15′0″S/longitude 146°48′0″E). Both soil types shared similar characteristics, predominantly containing a mixture of bio-solids and woodchips. The soils were equally rich in organic content (20% and 25.8% TOC), but compost soil had high concentrations of total nitrogen ($10{,}600 \pm 280$ mg $kg_{dry\ weight}^{-1}$) and copper (166 ± 0.8 mg $kg_{dry\ weight}^{-1}$). Microbial communities of the collected soils were continuously enriched in nitrate mineral salts medium (NMS) for 20 days at 20% (v/v) CH_4 concentration in the reactor headspace. The inoculi obtained were transferred into fresh NMS medium every 20 days for four sequential transfers to obtain obligate mixed methanotrophic consortia. Mother cultures obtained after enrichment were labeled as LB and CB to designate the soil type from which they were established; LB: landfill top cover soil-derived biomass and CB: compost soil-derived biomass. The physiochemical characteristics of seed soil and enrichment conditions of the consortia are detailed in (Chidambarampadmavathy et al., 2015a; Karthikeyan et al., 2016).

2.2. Influence of CH_4/O_2 ratio

To study the effect of CH_4/O_2 ratios, 20×500 ml mini-bench top reactors (Schott-Duran® gas-wash bottle, VWR International, QLD, Australia) were used for establishing microbial cultures. Of the 20 reactors, 15 reactors were used to set up triplicate cultures to study the effects of five CH_4 to air ratio (10:90, 20:80, 30:70, 40:60 and 50:50%) in a working volume of 200 ml NMS, inoculated with 5 mL of each mother culture, yielding a biomass concentration of 0.090 ± 0.015 and 0.110 ± 0.009 (OD600) with a protein content of 37.93 ± 0.06 μg mL^{-1} and 40.69 ± 0.08 (Lowry method, TP0300, Sigma-Aldrich, NSW, Australia) for LB and CB, respectively. The other five reactors served as CH_4 dissolution controls. The bench top reactors head space (~300 mL) was purged with 10:90, 20:80, 30:70, 40:60 and 50:50% of CH_4 to O_2 (i.e., 0.20–1.6 CH_4/O_2 ratios) every 24 h for 20 days.

Biomass growth (OD_{600}) was monitored spectrophotometrically (Enspire-2300, PerkinElmer, USA) every two days and biomass protein was measured every five days using the Lowry method (TP0300, Sigma-Aldrich, Castle Hill, NSW, Australia). Dry-weight (DW) was measured every 5th (5, 10, 15 and 20) day by harvesting 10 mL of culture sample and sample loss was compensated for by adding fresh NMS medium. Head space CH_4 samples were collected in vacuumed screw cap GC vials using an air tight syringe (Hamilton; 100 μL, Model 1710 RN, Grace Davison Discovery Science, Vic., Australia) and analysed by gas-chromatography equipped with thermal conductivity and flame ionization detectors (GC-TCD-FID; Varian-CP 3800, Vic., Australia). Methane removal efficiencies were calculated based on $[CH_{4in} - CH_{4out}/CH_{4in}] * 100$). MOC, fatty acid methyl esters (FAME) and PHB contents of the biomass was measured at 5, 10, 15 and 20 day as detailed in Section 2.4. All results are expressed in mg $g^{-1}DW_{biomass}$.

Please cite this article in press as: Chidambarampadmavathy, K., et al. Response of mixed methanotrophic consortia to different methane to oxygen ratios. Waste Management (2016), http://dx.doi.org/10.1016/j.wasman.2016.11.007