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Effect of biomass concentration on methane oxidation activity using mature compost and graphite granules as substrata

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ABSTRACT

Reported methane oxidation activity (MOA) varies widely for common landfill cover materials. Variation is expected due to differences in surface area, the composition of the substratum and culturing conditions. MOA per methanotrophic cell has been calculated in the study of natural systems such as lake sediments to examine the inherent conditions for methanotrophic activity. In this study, biomass normalised MOA (i.e., MOA per methanotophic cell) was measured on stabilised compost, a commonly used cover in landfills, and on graphite granules, an inert substratum widely used in microbial electrosynthesis studies. After initially enriching methanotrophs on both substrata, biomass normalised MOA was quantified under excess oxygen and limiting methane conditions in 160 ml serum vials on both substrata and blends of the substrata. Biomass concentration was measured using the bicinchoninic acid assay for microbial protein. The biomass normalised MOA was consistent across all compost-to-graphite granules blends, but varied with time, reflecting the growth phase of the microorganisms. The biomass normalised MOA ranged from 0.069 ± 0.006 μmol CH_4/mg dry biomass/h during active growth, to 0.024 ± 0.001 μmol CH_4/mg dry biomass/h for established biofilms regardless of the substrata employed, indicating the substrata were equally effective in terms of inherent composition. The correlation of MOA with biomass is consistent with studies on methanotrophic activity in natural systems, but biomass normalised MOA varies by over 5 orders of magnitude between studies. This is partially due to different methods being used to quantify biomass, such as *pmoA* gene quantification and the culture dependent Most Probable Number method, but also indicates that long term exposure of materials to a supply of methane in an aerobic environment, as can occur in natural systems, leads to the enrichment and adaptation of types suitable for those conditions.

1. Introduction

The waste sector accounts for 2.4% of total greenhouse gas (GHG) emissions in Australia and 80% of these emissions are from landfills (DCCEE, 2012). Despite the existence of biogas collection systems in landfills, a portion of biogas still diffuses through landfill slopes and covers, particularly in active cells where gas extraction does not commence until a viable methane concentration is reached, or in old landfills where extraction of combustible biogas is no longer viable due to deteriorating capping systems. Landfill covers can be designed to enhance the growth of methanotrophic bacteria to mitigate GHG emissions (Barlaz et al., 2004). So called biocovers have been intensively studied with the aim of optimizing their efficiency in mitigating methane emissions generated at landfills, particularly with the regard to various types of biocover material (He et al., 2008; Pedersen et al., 2011), landfill gas flux (Stern et al., 2007; Bogner et al., 2010) and environmental conditions such as pH, temperature and moisture content of the biocover (Mor et al., 2006; Stern et al., 2007; He et al., 2008; Wang et al., 2011). All of these factors will affect the required thickness of a biocover.

A range of materials, including soils (sand, loam and clay), or organic material such as biochar, compost from municipal solid waste, agricultural wastes (straw, sugar cane mulch and shredded wood) and shredded garden waste, have been investigated as substrata or co-substrata for enhanced methanotrophic activity within landfill covers (Huber-Humer et al., 2011; Reddy et al., 2014; Scheutz et al., 2009).

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Table 1
Variation of MOA on different landfill cover materials.

Landfill cover materials	Configurations	MOA (μmol CH_4/g substratum/h)	References
Mature compost	Batch	0.70–1.49	Einola et al. (2007)
Manure compost	Batch	0.51–3.70	Wang et al. (2011)
Loamy sand	Batch	4.5–7.38	Scheutz and Kjeldsen (2004)
Sandy loam	Batch	1.15–1.33	Park et al. (2005)
Mature compost	Column	0.99–1.60	Nikiema et al. (2005)
Soil	Column	0.58–0.68	Reddy et al. (2014)
Soil + 20% biochar	Column	0.86–4.86	Reddy et al. (2014)
Coal	Column	0.13–1.39[a]	Limbri et al. (2014)

[a] Assumes that the bulk density of the coal was 1.22 kg/l.

There are wide variations in the MOA reported for each these landfill cover materials, as summarised in Table 1 (Huber-Humer et al., 2011; Limbri et al., 2014; Scheutz et al., 2009). A range of MOA values is expected because the composition and colonisable surface area of broadly defined materials such as compost and soil will vary from one study to another. Furthermore, biofilm establishment may not be repeatable on the same material for a variety of reasons including gas and moisture channelling due to non-uniform packing; the blockage of pore space by biofilm; or different growth phases of microorganisms. The variability in reported rates therefore makes it difficult for the practitioner to select the most suitable biocover material, or to anticipate if the biocover will improve with time, or to decide whether additional moisture or nutrient might enhance performance.

In contrast, the MOA per methanotrophic cell would reflect the microbial genera present in the system, the degree to which the organisms have adapted to local conditions and the availability of nutrients and the presence of inhibitory factors. Inhibitory agents may be associated with the supporting substratum or the culturing media. As an example, a number of studies have demonstrated the inhibitory effect of various metabolites on MOA including organic acids and ethanol, nitrogen species (NH_4^+, NO_3^-, and NO_2^-) and H_2S (Duan et al., 2013; Long et al., 2013; Wieczorek et al., 2011; Wilshusen et al., 2004). Nutrient deficiency could also occur with an inert substratum such as crushed rock, in which case essential nutrients and trace elements must be supplied with the growth media.

The use of biomass normalised activity assays is well established in environmental biotechnology. Biomass normalised assays are used in anaerobic digestion to examine the effect of inhibitory substances on digester performance (Soto et al., 1993; Angelidaki et al., 2009) and in a number of studies on methanotrophic activity in natural environments including temperate lakes (Sundh et al., 2005), lake sediments (Rahalkar et al., 2009), on the surfaces of plants that generate methane as a by-product from photosynthesis (Yoshida et al., 2014) and in soils over naturally occurring subterranean sources of methane (Bender and Conrad, 1992). Correlations between MOA and the amount of methanotophic biomass was noted in all of these studies, although the biomass normalised rate varied between studies. The aim of this study was to measure biomass normalised MOA on selected substrata and to compare the rates with those reported in the studies listed above.

Biomass normalised MOA was measured on two substrata in this study: stabilised compost which is an established landfill cover material, and graphite granules, a proven electrode applied widely in microbial electrosynthesis studies (Erable et al., 2009; Freguia et al., 2007; Rabaey and Verstraete, 2005). Although graphite granules are too expensive to be a viable landfill cover material, they are an effective inert biofilm substratum with a well characterised shape and size range and they can be readily picked out of blends with compost, as required in this study, as opposed to fine grained materials such as sand.

2. Materials and methods

2.1. Substrata

Woodchip based mature compost (10 months old) was used as a representative biocover material. The fraction passing through an 8 mm sieve was used in the experiments. The sub-8 mm fraction had a moisture content of 55.5% and volatile solid content of 35.4% of total solids (TS). A property of interest for material acting as a substratum is surface area. The compost particles had a wide size distribution (Table 2) and varied in shape. The graphite granules were typically spherical with a diameter of between 2 and 6 mm (El Carb 100, Graphite Sales, Inc., USA). The nominal surface area of 4 mm diameter graphite granules is 0.7 m^2/kg (Freguia et al., 2007). The total surface area according to mercury porosimetry was three orders of magnitude higher (Freguia et al., 2007), but comprised of pores less than 1 μm in diameter and inaccessible to bacteria. The compost was likely to have had a greater surface area per unit mass, given the irregular shape and texture of compost particles and the fact that spheres, characteristic of the granules, represent the minimum surface area to volume ratio.

2.2. Colonisation of methanotrophs on substrata

The experiments consisted of a colonisation process, followed by MOA measurements of colonised compost and graphite granule samples. Colonisation was achieved by feeding 100 ml/min of 2.5% (v/v) CH_4 and 2.5% (v/v) CO_2 in N_2 to 5 kg of stabilised compost packed to a bed height of 22 cm in a Perspex column with an inner diameter of 24 cm. The packed bed was supported by a 10 cm gas distribution layer consisting of 5 mm river pebbles on a standard wire mesh above a plenum. The total active bed volume was approximately 10 L. Air was fed together with the dilute CH_4:CO_2 feed. Two litres of nutrient solution was circulated at a flow rate of 1.9 L/day using a peristaltic pump to maintain moisture levels of approximately 55% in the bed. The solution was sprayed over the surface of the bed using a nozzle (POPE, Microjet Mist Spray) and allowed to drain through the layers to the plenum and the storage vessel.

The nutrient solution consisted of a nitrate mineral salts medium (NMS) and 0.5 ml trace elements solution per litre of NMS (Whittenbury et al., 1970):

Table 2
Characteristics of compost and graphite granules.

Parameters	Compost	Graphite granules
Dry bulk density (kg/m^3)	223	1081
Moisture content (%)	55.5	0.8
Volatile solids (%, dry solids)	35.4	100
Packed bed porosity (%)	37	45
Size range	0.1–8 mm particles	2–6 mm particles