The Use of Immobilized Methanotrophic Bacteria for Biodegradation of Landfill Methane Emissions

B.L. Adams[1], F. Besnard[1], L. Talmadge[1], J. Bogner[2], J.D. Oliver[1], H. Hilger[1]*

***Corresponding Author:** 9201 University City Blvd., Charlotte NC 28223 hhilger@uncc.edu

[1] University of North Carolina at Charlotte
Charlotte, NC

[2] Landfills +, Inc
Wheaton, IL

Q-210

UNC CHARLOTTE

ABSTRACT

Methanotrophic bacteria oxidize methane to carbon dioxide and water, and they are abundant in landfills where they biodegrade methane passing through soil or compost covers. Although highly engineered bio-based systems exist to trap and collect methane generated after landfill closure, no such system exists for open, active landfill cells. Data suggest that significant amounts of methane can be produced soon after waste placement, and large amounts of methane may enter the atmosphere before final capping and gas collection system installation.

This paper will report on investigations to assess the methane oxidation capabilities of immobilized methanotrophic bacteria in a "biotarp" that could be used to control emissions from open landfill cells. A population of methanotrophic bacteria enriched from landfill cover soil was immobilized by adsorption onto various combinations of commercial materials with varying physical properties. Batch studies were conducted under a 10% methane-in-air headspace, and gas chromatography was used to measure the relative performance of the bacteria on different material supports. The results showed that methanotrophs adsorbed to synthetic geotextile with high density and water absorbency yielded the best methane uptake rates. Methane oxidation rates by the adsorbed cells were assessed at varying temperatures, under 12-hour starvation cycles, and after agitation in water to simulate hard rainfall. A continuous flow bioreactor designed to simulate field gas flux rates was used to examine biotarp prototypes based on the results from batch experiments. Methanotroph-embedded biotarp samples tested in continuous flow landfill gas simulation chambers (methane flux was 22.5 $g/m^2/day$) retained good methane oxidation activity when tested in batch after three weeks of incubation. Field trials of the methane oxidation capacity of immobilized methanotrophs are underway using flux chambers atop recently placed waste at a local landfill. Data will be presented on the methane biodegradation activity of the immobilized cells under batch, continuous flow, and field conditions.

INTRODUCTION

Methanotrophs

- Gram negative, aerobic bacteria
- Oxidize methane to carbon dioxide and water
  $CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$
- Found in many different environments, including landfill cover soil
- Also capable of co-metabolizing other hydrocarbon compounds

Methane

- A potent greenhouse gas
- Produced by natural (wetlands, termites, oceans) and anthropogenic (fossil fuels, domestic ruminates, waste decomposition, rice cultivation, and biomass burning) source
- Naturally removed by reactions with hydroxyl radicals in the troposphere and by methane oxidizing bacteria in the soil

Landfills

- Account for 37% of annual US methane emissions
- Biotic methane production begins soon after waste placement
- Observed to emit methane fluxes as high as several hundred $g/m^2/d$ on open, active landfill cells
- Final landfill capping systems efficiently prevent fugitive methane emissions
- No methane mitigation strategies used on open, active cells

Methanotroph Embedded Biotarp

- A methanotroph embedded alternative daily cover
- Applied on open landfill cells at night to prevent wind blow litter and animal scavenging as well as to mitigate early methane emissions
- Removed at the beginning of the working day and stored until reapplied at the end of the day

Objectives

- To find an appropriate support on which to adsorb cells for incorporation into a biotarp
- To evaluate activity of adsorbed cells under potential environmental stresses
- To evaluate methane oxidation of a biotarp prototype in a simulated landfill bioreactor
- To evaluate methane oxidation of a biotarp prototype under field conditions

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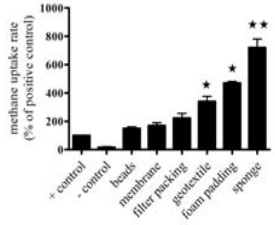

Fig. 1. Adsorption of a mixed population of methanotrophic cells to various types of supports. Error bars represent the standard deviation of two replicates. ★ indicates $p<0.05$ and ★★ indicates $p<0.01$.

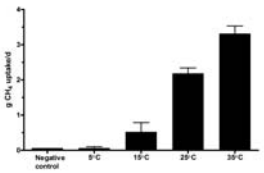

Fig. 2 . Methane uptake rate by methanotrophic cells absorbed to a synthetic geotextile at various temperatures. Error bars represent the standard deviation of three replicates.

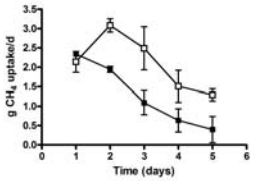

Fig. 3 . Methane uptake rates by methanotrophic cells absorbed to a synthetic geotextile with a constant methane atmosphere (□) or methane deprived for 12 hours every 24 hours (■). Error bars represent the standard deviation of two replicates.

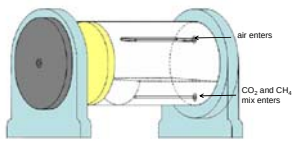

Fig. 4. Schematic of a continuous flow bioreactor to simulate the flow of landfill gases.

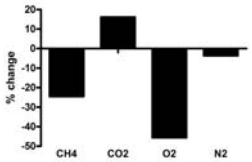

Fig. 5 . Overall percent change in each major gas analyzed in the continuous flow bioreactor. Negative values indicate consumption and positive values indicate production.

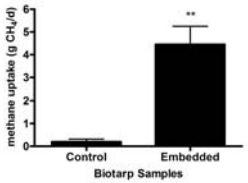

Fig. 6. Methane uptake of biotarp subsamples removed to batch after incubation in the bioreactor. Error bars represent the standard deviation of three replicates. ★★ indicates $p<0.01$

Fig. 7. Flux chamber inserted into the landfill surface to allow changes in emitted landfill gas to be monitored.

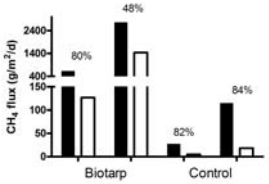

Fig. 8. Methane fluxes for two methanotroph-embedded biotarp samples and two controls placed on intermediate landfill cover. ■ represents bare soil methane flux and □ represents methane flux with the biotarp or control tarp in place. Values indicate the percent reduction in methane flux for each sample.

MATERIALS AND METHODS

Cultures and Conditions

- Isolated a mixed methanotroph population from landfill cover soil
- Maintained at room temperature in Whittenbury's Nitrate Mineral Salts (NMS) media in custom gas-tight bottles with a 10% methane in air headspace

Adsorption

- Six types of supports tested for their ability to maintain a robust population of methane oxidizers: natural sponge, a 0.95 cm x 2 cm x 4 cm sample of a highly wettable PP nonwoven geotextile, a 2.5 x 3 x 4 cm piece of synthetic foam padding with a 1.2 lb/ft^3 density, a small sub-section of injection molded polypropylene plastic tower packing material, a 90 mm diameter circle of polycarbonate membrane with a 0.22µm pore and glass beads with a 200-300 mm diameter
- An overnight culture of mixed methanotrophs was added to a support material in a gas-tight bottle under a 10% methane:air headspace and incubated at room temperature.
- Methane uptake analyzed by gas chromatography after 24 hours

Temperature Stress

- An overnight culture of the mixed methanotrophs added to the geotextile in a gas-tight bottle under a 10% methane:air headspace and incubated at 5°C, 15°C, 25°C, and 35°C
- Methane uptake analyzed by gas chromatography after 24 hours

Methane Starvation

- An overnight culture of the mixed methanotrophs added to the geotextile in a gas-tight bottle under a 10% methane:air headspace and incubated at room temperature
- Samples alternately exposed and deprived of methane in 12-hour cycles, while controls were continually exposed to methane
- Methane uptake analyzed by gas chromatography

Continuous Flow Bioreactor Design

- 45cm long clear PVC pipe (ID 20cm) sealed at one end
- Tee-union was inserted 1.3 cm from the top and used to attaching ¼ " perforated stainless steel tubing (8cm x 20cm "U" shape)
- ¼" Swagelok bulkhead fitting inserted 1.3cm from the bottom and connected to 20cm length of ¼" perforated stainless steel tubing covered by gravel
- Gas flow volumes introduced through flow regulators
- Circle of commercial furnace air filter fabric positioned across the cross-section of the cylinder to ensure that the gas sampled from the outlet port was well mixed
- Threaded bolts and a gasket used to ensure a gas tight-seal
- Gas samples collected through a septum mounted in a Swagelok bulkhead fitting through the outlet cover
- Biotarp samples sandwiched between two plexi-glass frames (16.8cm x 25.5cm ¼" with a central section removed)
- A 10cm diameter semi-circle sealed the bottom portion of the platform

Examination of Biotarp Prototypes in Continuous Flow Chambers

- A four layered geotextile biotarp prototype was soaked for 10 minutes in 300 mL overnight mixed methanotrophs
- dI water negative control geotextile prepared in a similar manner
- Inlet and exit methane concentrations and flows monitored for mass balance analysis:

$$\% \text{ removal} = \frac{Q_{in}C_{in} - Q_{out}C_{out}}{Q_{in}C_{in}} \times 100$$

Where Q_{in} = flow in, mL gas/min
C_{in} = inflow methane concentration, mL CH_4/100 mL gas
Q_{out} = exit flow, mL gas/min
C_{out} = exit flow methane concentration, mL CH_4/100 mL gas

- Samples were monitored for one week, after which 4cm x 4cm subsamples were placed in gas-tight bottles in a 10% methane in air headspace
- Methane uptake was monitored in batch for 24-hours and methane uptake was analyzed by gas chromatography

Field Testing and Flux Chambers

- Field tests conducted at the Allied Waste landfill in Cabarrus County, NC
- Flux chambers utilized to measure gas emissions from the landfill surface
- Chambers had metal collar bases inserted and secured in intermediate cover surface
- Stainless steel lid, fitted with a gas-tight sampling port, attached to the collar and water used to fill the joint between the two in order to prevent gas leakage
- Biotarp prototype soaked in 1 L of an overnight mixed methanotroph population for 15 minutes
- Control samples prepared with dI water only
- Gas flux measured with and without biotarps in place by withdrawing samples from the sealed chamber at three minute intervals for 15 minutes using a 60 mL syringe fitted with a gas-tight stop cock and needle
- Gas samples were stored and transported to the lab in 60 mL gas-tight, evacuated collection vials and analyzed by gas chromatography
- Methane flux at each chamber site calculated using the change in methane concentration over time (dC/dt) and the volume:area ratio of the chamber

RESULTS AND DISCUSSION

- Highest methane uptake observed by cells adsorbed to supports with a high moisture holding content – synthetic geotextile and natural sponge (Fig. 1)
- Geotextile further examined due good feasibility as a biotarp component but natural sponge eliminated for poor durability
- Optimal methane oxidation rate by cells adsorbed to geotextile occurred between 25°C and 35°C, with negligible uptake occurring at 5°C (Fig. 2)
- Methane deprivation reduced oxidation over time, but did not eliminate it. Controls showed a less pronounced decline (Fig. 3)
- Storage of the biotarp during the working day may reduce the methane mitigation potential of the biotarp
- Methane oxidation of approximately 25% uptake/d was observed under continuous flow conditions (Fig. 5)
- Biotarp samples tested in batch after continuous flow incubation had a significantly higher methane oxidation rate than in the chambers(Fig. 6), suggesting retention time under continuous flow limited performance
- Under field conditions, baseline fluxes were found to be highly variability between sampling sites, ranging from 2700 to 27 g $CH_4/m^2/d$
- Application of the biotarp to the landfill surface was found to reduce methane fluxes to varying degrees, depending on the bare soil flux (Fig. 8)

CONCLUSIONS

- A methanotroph embedded biotarp was constructed and methane mitigation was observed under batch, continuous flow, and field conditions
- Methanotrophs can be adsorbed to a synthetic geotextile support
- Low temperatures and methane deprivation reduce methane oxidation rates by adsorbed cells
- Methanotrophs adsorbed to a layered geotextile biotarp prototype oxidized methane, although maximal oxidation was not achieved under simulated landfill conditions
- Methanotrophs adsorbed to a layered geotextile biotarp were able to reduce methane emissions from intermediate landfill cover soil
- Future works will include the use of hybrid biotarp designs with compost and expanded clay layers in future trials
- Water and nutrient holding capacity of these materials may improve biotarp performance