Methane Production and the Microbial Community During Thermophillic Composting of Human Waste

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Abstract

In order to evaluate the viability of thermophillic composting as an alternative method for disposing of human waste, we characterized the microbial community using PhyloChip analysis. Here, the focus is on Methanogens and Methanotrophs, to gain an understanding of the extent and nature of methane release from these piles during the composting process. In this study, DNA was extracted from three replicate human waste thermophillic compost piles, and the PCR amplified 16S rRNA genes of bacteria and archaea were fragmented, labeled and hybridized to PhyloChips which provides relative abundance values for any species or operational taxonomic unit (OTU) that is present in the GreenGenes database. 10,539 taxa were detected, including 31 methanogens and 79 methanotrophs. Methanogen abundance decreased from the initial values over time, though certain thermophillic species increased. Numbers of OTU detected by PhyloChip decreased over time, more so at the center than the edges of the pile, likely due to increased temperatures at center. Methanotroph abundance varied, decreasing more at the center than edges,.

A Need for Better Waste Disposal

- Processed waste is buried and **nutrients** within are lost rather than returned to the soil
- Waste treatment facilities use vast amounts of **water and energy** to move and process waste
- Many countries cannot afford the **maintenance costs** of waste treatment plants, let alone construction costs
- In regions without proper sanitization of waste, water-borne **diseases** proliferate

Open Sewage and Garbage, Port Au Prince, Haiti – Photograph by Pradip Malde

Thermophillic Composting

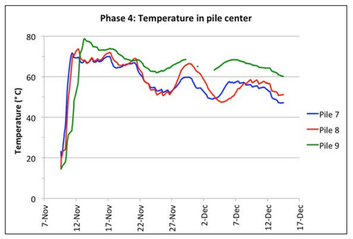
An alternative method for processing human waste.

Given **Proper Conditions**

30:1 Carbon:Nitrogen ratio

55-60% Moisture >5% Oxygen

Bacteria break down the pile material, generating enough heat to raise the temperature at the center to over 70ºC within a few days. Only thermophillic species can survive.

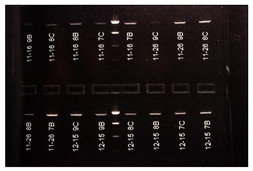


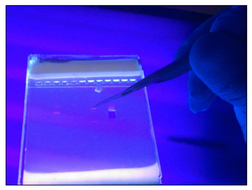
Three replicate compost piles were established in Nicasio, CA with human waste from National Park vault toilets, mixed with wood chips and straw bedding from a stable. Temperature data was measured at the center and edges of the piles, as well as oxygen concentration at the center. Gas samples were taken from inside the pile at the same time as samples of the actual pile material.

This predominately ecologically friendly method has the potential issue of excessive **methane production** from the methanogens (methane producing microbes) in human waste. Methane is a potent greenhouse gas.

Sample Preparation

Samples were taken periodically and stored at -80ºC. 33 were chosen for analysis: Each pile's initial mixture and a center and edge sample from each pile at 5 time points.





Homogenization
Frozen with Liquid Nitrogen. Pulverized in steel grinding jar with TissueLyser ™ machine

DNA Extraction
Zymo™ Fecal Extraction Kit

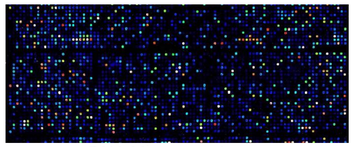
Polymerase Chain Reaction (PCR)
Amplified the 16S rRNA gene.
Archaea: 21F and 1492r primers
Bacteria: 27f and 1492r primers

Gel Extraction
Isolating Archaea 16S rRNA

PhyloChip Analysis

Affymetrix™ PhyloChip

The PhyloChip is a **DNA microarray** developed for microbial ecology by Dr. Gary Andersen and colleagues. This technology provides a way to quickly identify the relative abundances of an entire community of **bacteria and archaea**, based on intensity values of fluorescently stained fragments of the DNA that codes for 16s rRNA.



DNA Microarray Image

Procedure

DNase cuts 16S rRNA genes- Purified DNA is fragmented to 50-200 b.p.

Hybridized to PhyloChip - Fragments bind to probe for specific sequence

Stained in Fluidics Station - Primary and secondary antibodies bind to probes

Chip Scanner - Measures fluorescence of each probe

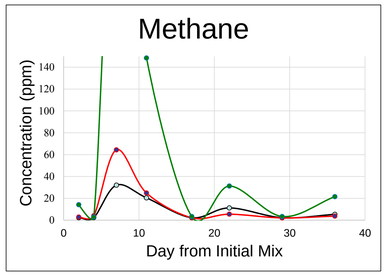
Results

10,539 Taxa Detected by PhyloChip
31 Methanogens
79 Methanotrophs

A Bray-Curtis similarity matrix showed grouping of samples by pile position and time point

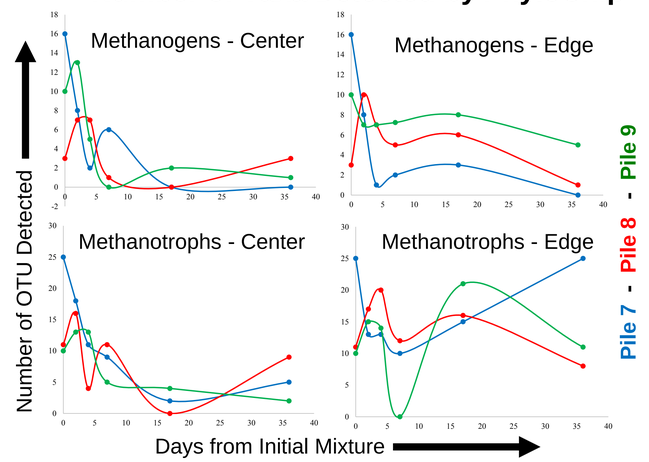
Many methanotrophs identified were thermophillic species, such as *Methanothermobacter thermautotrophicus*.

Overall methanogen abundance decreased in all piles, to a greater extent in the center samples.

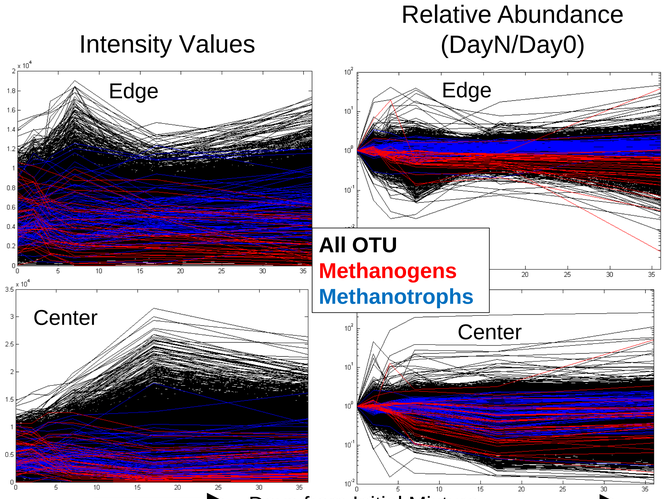


Methane concentrations followed similar patterns for all three piles, with a large peak around 7 days and smaller peak at 22 days. Pile 9 had consistently higher concentrations.

Number of Taxa Detected by PhyloChip



Methanogen diversity decreases at center of pile. Methanotroph diversity dropped in center but varied at edges



Methanogen abundance clearly decreases in almost all taxa, especially in center samples. Methanotroph abundance is seen to generally increase in edge samples, and varies in center samples

Discussion

This study shows that thermophillic composting is a viable method for processing human waste, particularly for regions which have no systems in place. The decrease in methanogen presence and abundance suggests that this method may help to minimize methane emissions. Methanotroph abundance increasing relative to methanogen abundance indicated a potential for methane cycling within the pile. The piles produced lower amounts of methane than previous phases of this project, increased aeration may have inhibited methanogenesis.

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