Source Tracking of Microbial Aerosols Emitted at Class B Biosolids Land Application Sites

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Introduction

Land application of biosolids (processed domestic wastewater sludge) is the preferred recycling practice in the US. Class B biosolids contain pathogen concentrations that are greater than the soils to which they are applied. Concerns have been expressed over possible wind aerosolization and off site transport of biosolids derived pathogens.

The objectives of this research were to develop, test, and apply BST methods to trace the wind aerosolization of biosolids from land applied soils. The methods were selected based on microbial population information obtained from cultural and phylogenetic analysis. They include culturing and sequencing *Clostridium bifermentans* from aerosol samples, direct PCR amplification and sequencing of aerosols for an unidentified *Chloroflexi* bacterium that is commonly present in biosolids clone libraries, and direct PCR amplification of Euryarchaeota coupled with terminal restriction fragment length polymorphism (t-RFLP) to distinguish biosolids specific terminal fragments. Each method was confirmed by testing for presence in bulk biosolids and absence in soils. Each method was then applied to a group of aerosol samples collected under high wind conditions (wind speed > 5 m/s) at biosolids land application sites and also to aerosol samples taken at distances up to 170 m downwind of disking operations to determine the occurrence and extent of biosolids wind aerosolization and transport.

Hypotheses

1. Bioaerosols, derived from biosolids, can be specifically and sensitively tracked on and off site through PCR-based analytical methods.
2. High wind (average speed > 5 m/s) can aerosolize microorganisms from soils to which biosolids have been applied.
3. Bioaerosols, derived from disk incorporating biosolids, can be transported up to 170 m from their source.

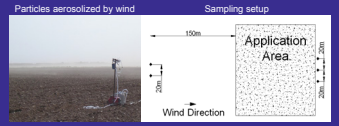
Materials and Methods

Sampling

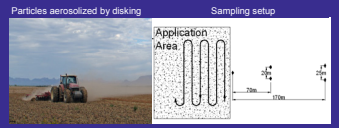
Samples Collected:
- Air (liquid impinger)
- Biosolids (Bulk)
- Soil (Bulk)

Supporting Data:
- Wind Speed and Direction
- PM_{10}
- Soil Moisture and Composition

Wind Aerosolization



Off Site Transport



BST Methods

- BST methods are first applied to a large group of bulk biosolids and soil samples to test for their ability to discriminate between microorganisms originating from biosolids and from soil.
- BST methods are then applied to a variety of aerosol samples to
  - Determine the sensitivity of the methods.
  - Investigate aerosolization of microorganisms from applied biosolids during high wind events.
  - Investigate off site transport of bioaerosols.

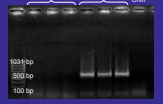
Sulfite Reducing *Clostridia*
- Culturing on TSC agar

- Black colonies selected
- PCR with Universal Primers
- Sequencing
- Phylogenetic Analysis

Chloroflexi

Method Development
- Clone libraries indicate *Chloroflexi* are enriched in biosolids

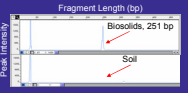
Method Application
- Extraction
- PCR with Chloroflexi Primers



- Sequencing

Euryarchaeota (methanogens)

Method Development
- Cloning of DNA from Biosolids and Soil to identify relevant Euryarchaeota
- Analyze clones for fragments that are unique to biosolids

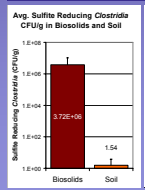


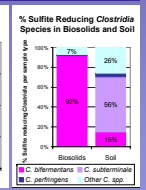
Method Application
- Extraction and PCR with Methanobacteriales and Archaea Primers
- Restriction Digestion with SfcI
- T-RFLP analysis

Results

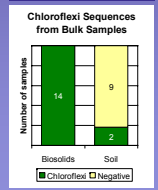
BST Methods

Sulfite Reducing *Clostridia*

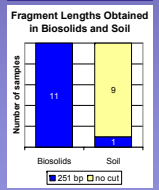




Chloroflexi

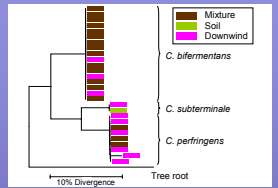


Euryarchaeota

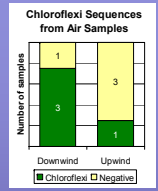


Wind Aerosolization

Sulfite Reducing *Clostridia*



Chloroflexi



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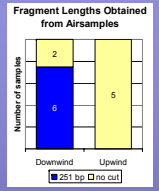
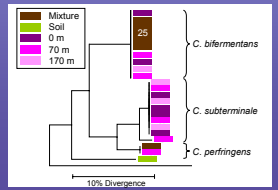
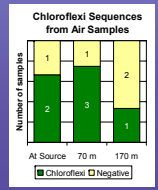


Off Site Transport

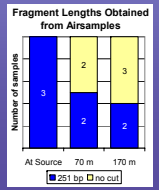
Sulfite Reducing *Clostridia*



Chloroflexi



Euryarchaeota



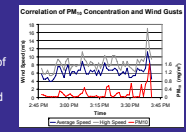
Discussion

BST Methods

The average rate of correct classification (ARCC) for the BST methods was 95.1% for sulfite reducing *Clostridia*, 87.5% for Chloroflexi, and 91.7% for Euryarchaeota. The ARCC increases to 100% if all three methods are applied to the same sample.

Wind Aerosolization

All three BST methods were positive for bioaerosols emitted from biosolids in samples collected during high winds. One of 15 upwind samples was positive. The PM_{10} data supports these findings. PM_{10} generally increases with increased wind speed above 5 m/s.



Off Site Transport

All three source tracking methods were positive for bioaerosols emitted from biosolids in samples collected during transport experiments after biosolids application (biosolids disking) for all three stations (at the source, 70m, and 170m).

Supporting Data

Soils were classified sandy-loam with soil moisture < 5%.

Conclusion

BST Methods
- PCR-based BST methods can be used to discriminate between samples originating from biosolids and soil with an ARCC of 87.5 to 100%. These methods are sensitive enough to detect microorganisms in aerosol samples

Wind Aerosolization
- Average wind speeds exceeding 5 m/s can aerosolize microorganisms from biosolids that have been incorporated into soils by disking. Wind aerosolization was tested under dry soil conditions and limited to sandy loams.

Off Site Transport
- BST methods consistently detected bioaerosols originating from biosolids disking at distances of 170 m from the source. Atmospheric conditions during disking were moderately to slightly unstable.

Acknowledgements

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