Land Application Sustainability Studies with Class B Biosolids, and Novel Approaches for Obtaining Class A Biosolids

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BACKGROUND: FOR LAND APPLICATION

• Class A and Class B biosolids well-defined on the basis of treatment

• Land application of Class A and Class B regulated by EPA Part 503 Regulations

• Benefits of biosolids as a useful soil amendment well documented by peer reviewed research

• No peer reviewed scientific evidence of harm or disease from land application
BACKGROUND: AGAINST LAND APPLICATION

- Environmental activists loathe land application
- Residential complaints caused by odors
- Historically odors associated with disease
- Psychosomatic problems?
- National debate on the safety of land application, e.g., Kern County Case
LAND APPLICATION OF BIOSOLIDS

- 7.2 million dry tons used or disposed of annually in U.S.
- 55% land applied
- Approximately 0.1% of available agricultural land utilized for land application of biosolids
POTENTIAL HAZARDS IN BIOSOLIDS

- Metals
- Organics: EPA priority pollutants
- Pharmaceuticals
- Nitrates
- PATHOGENS
# PATHOGENS IN BIOSOLIDS

<table>
<thead>
<tr>
<th></th>
<th>CLASS A BIOSOLIDS</th>
<th>CLASS B BIOSOLIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>&lt;3 per 4 g</td>
<td>≈10–400 per g</td>
</tr>
<tr>
<td>Fecal coliform</td>
<td>&lt;1000 per g</td>
<td>&lt;2 × 10^6 per g</td>
</tr>
<tr>
<td>Enteric viruses</td>
<td>&lt;1 per 4 g</td>
<td>≈1 per g</td>
</tr>
<tr>
<td>Helminth ova</td>
<td>&lt;1 per 4 g</td>
<td>Rarely found in U.S.</td>
</tr>
</tbody>
</table>
OTHER BIOLOGICAL CONCERNS

- *Staphylococcus aureus*
- Antibiotic Resistant Bacteria
- Endotoxin
- Prions
LAND APPLIED PATHOGENS AS A HAZARD

DIRECT EXPOSURE

● From biosolid amended soil at the land application site
● From stored biosolids

INDIRECT EXPOSURE

● Groundwater beneath (or surface water close to) land application site subsequently used for potable water
● Bioaerosols that reach communities some distance away from the land application site
SUSTAINABILITY STUDIES:
REGROWTH OF PATHOGENS

- Human viruses never regrow in biosolids or soil: no host
- Bacterial regrowth can occur
Objective:

Evaluate regrowth of *Salmonella* and fecal coliforms in Class B biosolids and biosolid amended soil
FIELD STUDIES

- Biosolid drying beds at Avra Valley WWTP in Tucson
- Survival studied as a function of:
  - moisture content
  - desiccation rate
  - effect of rainfall events
- Study regrowth after rainfall events
Field Experiment Set up
RESULTS—Ina Road

Class A regulation levels were achieved during weeks 3–7
Regrowth occurred during week 8 following rainfall
CONCLUSIONS: Class A Regrowth

- Regrowth in Class A biosolids only occurred under saturated conditions
- No regrowth in Class A biosolid-amended soil, under any moisture regime
- Care must be taken during storage of Class A biosolids prior to land application i.e., cover biosolids and protect from rainfall and bird or animal feces
SUSTAINABILITY STUDIES: INDIRECT PATHOGEN EXPOSURE VIA TRANSPORT TO GROUNDWATER

- Transport of phage from land applied biosolids less than from phage in pure culture (2-4% of total phage) due to phage adsorbed within biosolids

- Contamination of groundwater from phage or virus unlikely

- Human virus concentration in Class B biosolids <1 per g

- U of A annual monitoring of groundwater from wells near land applied biosolids negative (1985–Present)
SUSTAINABILITY STUDIES: INDIRECT PATHOGEN EXPOSURE VIA BIOAEROSOLS

● How many microorganisms are aerosolized?
● What is the duration of exposure?
● What is the nature of the bioaerosol plume?
● Does method of application influence rate of aerosolization?
● What is the fate and transport of aerosolized microorganisms?
Background
National Study within the United States

- Bioaerosol samples already taken
- Seminar presentations
Loading
Spraying of 2% Solids
Spraying of 7% Solids
OCCUPATIONAL RISK

- *Salmonella* annual risk of infection: 0.0001% to 0.013%
- Viral annual risk of infection: 0.78% to 2.1%
COMMUNITY RISK:
Risk of infection to residents during land application

- Risk varies with distance from application site
- How far are microbes transported?
- How much inactivation?
ANNUAL RISK OF INFECTION:

B) Loading operations

- *Salmonella* risk at 30.5 meters:
  \[ \sim 10^{-4} \approx 1.3 \]

- Viral risk at 30.5 meters:
  \[ \sim 10^{-4} \approx 3.8 \]
EXPOSURE VIA BIOAEROSOLS: Conclusions

- Occupation risk greater than community risk
- Community risk is insignificant
SUSTAINABILITY STUDIES: 
THE STAPHYLOCOCCUS STORY: 
FACTS

*Staphylococcus aureus*

- Gram positive coccus
- Commonly found within nose of healthy people
- Can result in minor or major skin infections
- To date, no scientific data or epidemiological study has been published linking *S. aureus* to land application of biosolids
THE **STAPHYLOCOCCUS** STORY: ALLEGATIONS

- *S. aureus* is found in biosolids
- *S. aureus* from biosolids results in public health affects
- *S. aureus* from land applied biosolids has resulted in deaths
S. AUREUS RESEARCH AT THE UNIVERSITY OF ARIZONA

- *S. aureus* found in 3 of 5 sewage samples (60% incidence)
- *S. aureus* never detected in 23 biosolid samples (8 Class A and 15 Class B) (0% incidence)
- *S. aureus* never detected in bioaerosol samples (0% incidence)
This study provides scientific evidence for the absence of *S. aureus* in land applied biosolids.

It shows that biosolids are not a source of *S. aureus* human exposure.

SUSTAINABILITY STUDIES:

OTHER BIOLOGICAL CONCERNS:

ENDOTOXIN
## POTENTIAL FOR AEROSOLIZED ENDOTOXIN DURING LAND APPLICATION OF BIOSOLIDS

<table>
<thead>
<tr>
<th>Sample Type</th>
<th># of Samples Collected</th>
<th>Distance from Site (m)</th>
<th>Avg EU m*</th>
<th>Median EU m*</th>
<th>Minimum EU m*</th>
<th>Maximum EU m*</th>
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</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Background</td>
<td>12</td>
<td>NA</td>
<td>2.6</td>
<td>2.49</td>
<td>2.33</td>
<td>3.84</td>
</tr>
<tr>
<td><strong>Biosolids Operations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Loading</td>
<td>39</td>
<td>2–50</td>
<td>343.7</td>
<td>91.5</td>
<td>5.6</td>
<td>1807.6</td>
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<tr>
<td>Slinging</td>
<td>24</td>
<td>10–200</td>
<td>33.5</td>
<td>6.3</td>
<td>4.9</td>
<td>14.29</td>
</tr>
<tr>
<td>Biosolids Pile</td>
<td>6</td>
<td>2</td>
<td>103</td>
<td>85.4</td>
<td>48.9</td>
<td>207.1</td>
</tr>
<tr>
<td>Total Operation</td>
<td>33</td>
<td>10–200</td>
<td>133.9</td>
<td>55.6</td>
<td>5.6</td>
<td>623.6</td>
</tr>
<tr>
<td><strong>Wastewater Treatment Plant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aeration Basin</td>
<td>6</td>
<td>2</td>
<td>627.3</td>
<td>639</td>
<td>294.4</td>
<td>891.1</td>
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<tr>
<td><strong>Non Biosolids Field</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Tractor</td>
<td>6</td>
<td>2</td>
<td>469.8</td>
<td>490.9</td>
<td>284.4</td>
<td>659.1</td>
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</table>
AEROSOLIZED ENDOTOXIN DURING LAND APPLICATION

• Aerosolized endotoxin levels during land application of biosolids similar to other agricultural activities not involving biosolids and soil and biosolids both contribute to aerosolized endotoxin

• Greatest exposure to endotoxin occurs at wastewater treatment plant
Antibiotic Resistant Bacteria

- Land applied biosolids did not influence ARB concentrations
- Soil is the original source of antibiotics
- Exposures more common from handling foods
- Ready to eat lettuce—greatest relative concentrations of ARB
- Hospitals more important
SUSTAINABILITY STUDIES: SURVIVAL OF INFECTIOUS PRIONS DURING WASTEWATER TREATMENT
An infectious prion (PrP\textsuperscript{sc}) causes a normal prion (PrP\textsuperscript{c}) to convert to an infectious prion.

Disease occurs when PrP\textsuperscript{sc} concentration threshold is reached.

Previous studies have suggested that prions are highly stable.

http://www.le.ac.uk/biology/research/phyto/prions.jpg
Infectious prion proteins (PrP$^{\text{Sc}}$) cause Transmissible Spongiform Encephalopathy (TSE)

Most common to humans is Creutzfeldt-Jakob disease (CJD)
TSEs IN ANIMALS

- Most common TSE in animals is called scrapie which affects sheep and goats.

- Chronic Wasting Disease (CWD) - affecting deer and elk.
  - Affecting deer population in U.S.: Colorado, Wyoming, Nebraska, New Mexico, South Dakota, Wisconsin.
PRION SURVIVAL IN BIOSOLIDS

● Previous study reported that prions survived wastewater treatment

● Assay used was Western Blot technology which did not distinguish between infectious and normal prions

● We developed a new assay for infectious prions
REDUCTION OF INFECTIOUS PRIONS AT MESOPHILIC (37°C) AND THERMOPHILIC (60°C) TEMPERATURES

A) In PBS

Mesophilic: \( \log_{10} \frac{N}{N_0} \) (15 days) = -1.13
Thermophilic: \( \log_{10} \frac{N}{N_0} \) (10 days) = -1.80

B) Class B biosolids

Mesophilic: \( \log_{10} \frac{N}{N_0} \) (15 days) = -2.43
Thermophilic: \( \log_{10} \frac{N}{N_0} \) (10 days) = -3.41
EVALUATION OF ANAEROBIC DIGESTION ON INFECTIOUS PRIONS

- Anaerobic digestion of primary undigested sludge with activated sludge (mesophilic 35°C or thermophilic 50°C)
- Incubation for 21 days in miniature anaerobic digesters spiked with infectious prions
- Miniature anaerobic digesters initially tested for efficacy of digestion by monitoring volatile fatty acids
Sealed test tubes utilized as miniature anaerobic digesters
Fate of infectious prions during wastewater treatment

Fate of Infectious Prions During Anaerobic Digestion

- Mesophilic
- Sterile Mesophilic
- Thermophilic

TCID50/mL vs. Weeks
INFLUENCE OF LIME TREATMENT (Class A Treatment Process) ON INFECTIOUS PRION INACTIVATION

- Class B biosolids treated with lime and incubated at 52°C at pH 12.9
- Within 2 hours of lime treatment, $2.9 \log_{10}$ inactivation
CONCLUSIONS

● Data indicate that infectious prions do not survive wastewater treatment and that land application of biosolids is not a viable route of human prion exposure
● Anaerobic digestion at 35 °C or 50 °C resulted in a significant reduction in infectious prion numbers
● Lime treatment was particularly effective in reducing numbers
● Microorganisms involved in prion inactivation
Novel Approaches for Obtaining Class A Biosolids: Top Choice Organic
**Top Choice Organic** - Class A Pelletized Biosolids (Mannco Environmental Services, Inc.)

- Anaerobically digested, dewatered then heat dried
- Nutrient content 5-3-0
- Pellets, 1 to 4 mm diameter
- No odor
Objectives

- Determine the efficacy of the Class A biosolids for cotton (*Gossypium hirsutum* L.) production relative to traditional inorganic fertilizer

- To evaluate the effect of land application of Class A biosolids on soil quality enhancement (carbon sequestration)
Sandy loam soil plots were planted on 27 May 2011
Clay soil plots were planted on 6 June 2011
Approach–Yield Processing: 2011

- Post harvesting soil core samples collected for soil microbial and chemical properties
- Total yields ginned for lint turnout percentages and seedcotton weight:lint ratios
Results – Cotton Bolls Count & Yield: 2011

FIGURE 2. Total cotton harvest (before ginning), data based on yield harvested from a 3m long interior row of each plot.
SUMMARY

- Top Choice an attractive organic fertilizer

- In 2012, boll data suggests Top Choice will significantly enhance yields relative to inorganic controls

- Need soil analysis post-harvest to estimate C sequestration
NOVEL APPROACHES FOR OBTAINING CLASS A BIOSOLIDS: The MagnaGro Process
MagnaGro Process

- Involves addition of Sodium Metam to Class B biosolids and increasing pH to 1 for 24 hours
- Sodium Metam decomposition product volatilizes within 24 hrs
- Helminthes and virus are killed
● Soil fumigant used extensively in the U.S.
● Once in soil it decomposes to gaseous product (methylisothiocyanate) with nematocidal, fungicidal, herbicidal and insecticidal activity
● Used on potatoes, carrots, beets and ornamentals
MagnaGro Process™ Testing

• Two tests in Arizona:
  – Green Valley Wastewater Treatment Facility
  – Ina Road WTF

• In both tests, biosolids spiked with:
  – Enteric Virus (100,000 PFU/4 grams solids)
  – Helminth (Ascaris) Eggs (1,000 eggs/4 grams Solids)
  – Enough Fecal Coliforms were already present
# Microbial Inactivation Due to Sodium Metam

## Table of Organisms and Reductions

<table>
<thead>
<tr>
<th>Organism</th>
<th>$\log_{10}$ Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal coliforms</td>
<td>5.3</td>
</tr>
<tr>
<td><em>Ascaris</em> eggs (Helminthes)</td>
<td>2.7</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>5.2</td>
</tr>
</tbody>
</table>
PROPOSED UA RESEARCH

Evaluate MagnaGro process in a field study with Asarco Mine, MagnaGro and Pima County Wastewater