Resilient Consortia for Anaerobic Digestion: Insights from "Omics"

Susan K. De Long

Sybil Sharvelle,

L. Paige Wilson, Lucas Loetscher

NREL Anaerobic Digestion Mini-Summit

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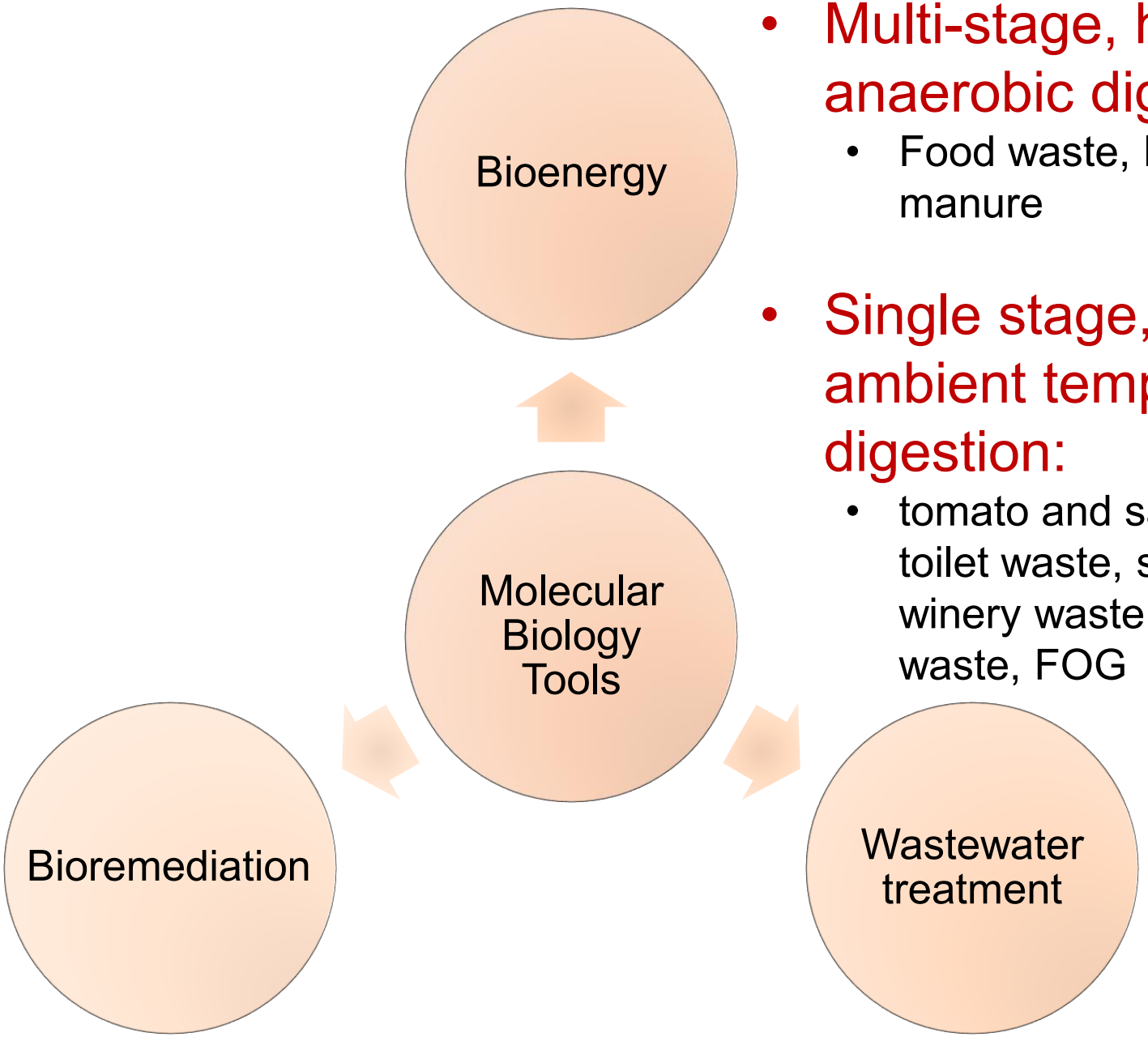
Colorado State University

Research Focus Areas

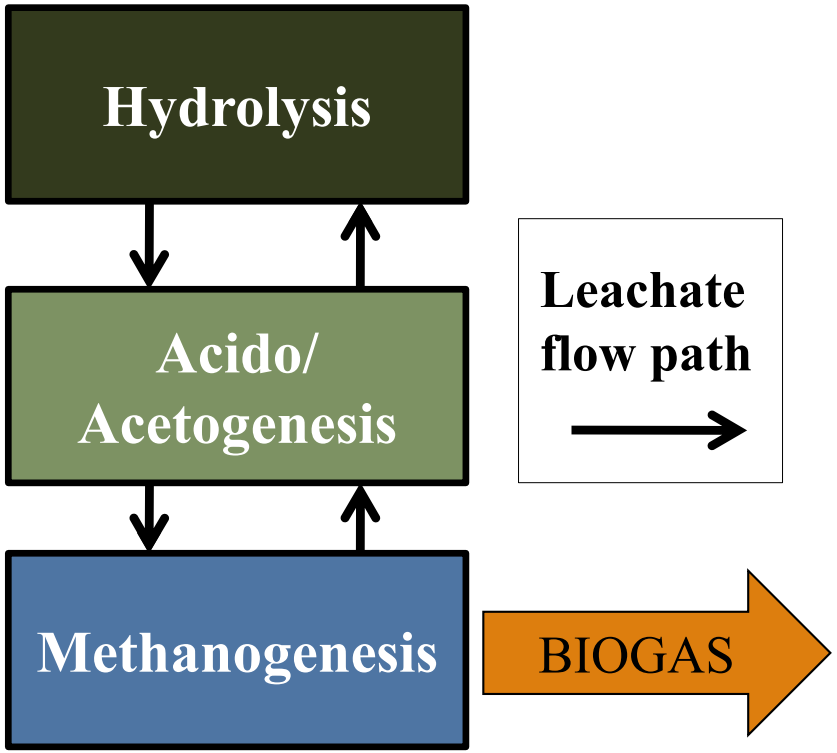


- Multi-stage, high solids anaerobic digestion:
  - Food waste, landscaping waste, manure
- Single stage, low-solids, ambient temperature digestion:
  - tomato and salsa waste, portable toilet waste, septic tank waste, winery waste, beer and cider waste, FOG

Acknowledgments

Colorado State University Agricultural Experiment Station

Multi-stage Anaerobic Digestion

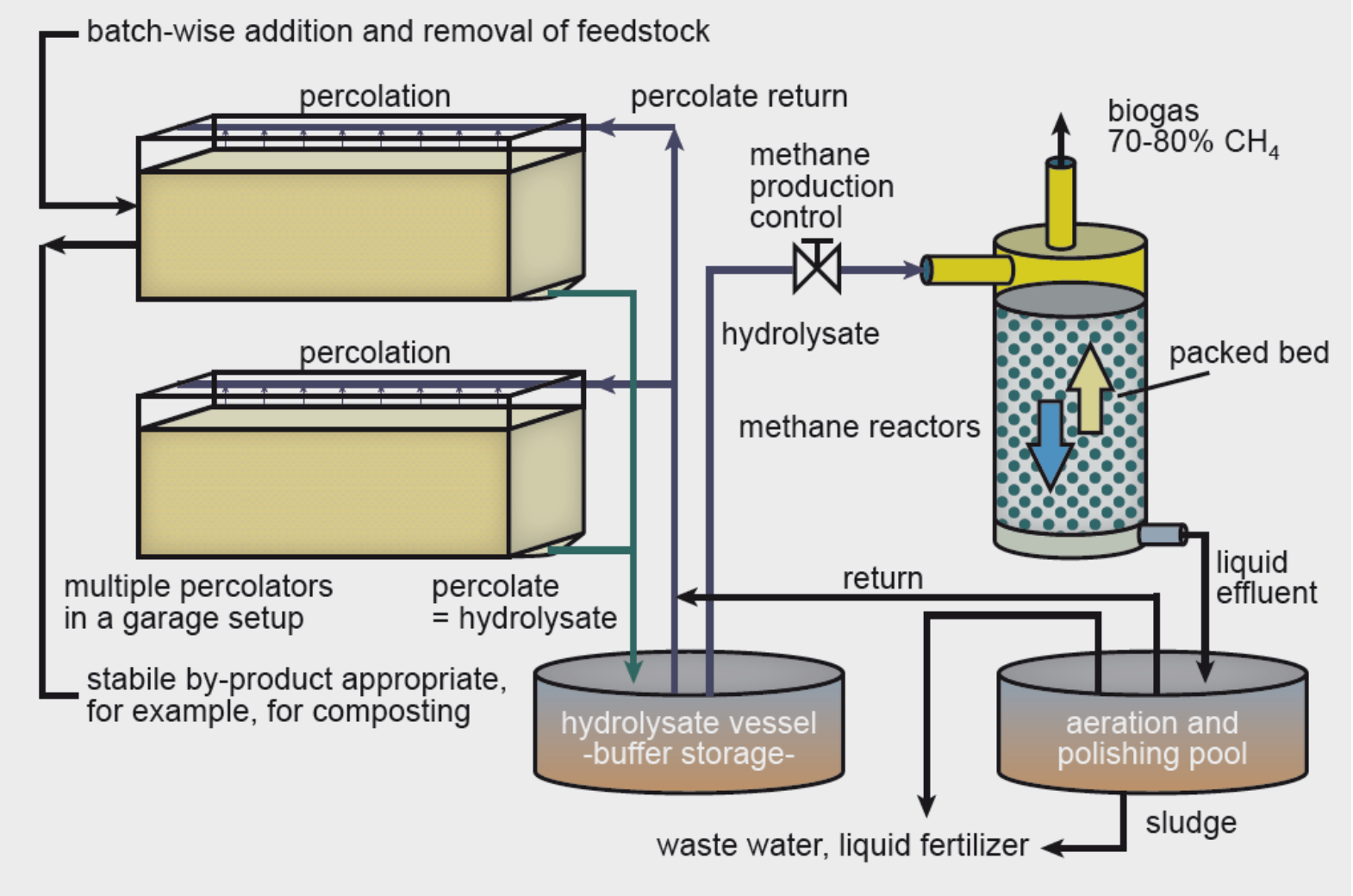


- **Benefits:**
  - Individual stage optimization
  - High solids waste treatment
  - Leachate recycle → reduces fresh water usage

- **Challenges:**
  - Leachate recycle increases ammonia and salinity concentrations

High-solids Multi-stage

- Increasingly popular for application to MSW.
- Feedstock stream can contain >20% total solids (typically 20-40%).



Process flow diagram of GICON Biogas Process
(http://www.gicon.de/uploads/tx_sbdownloader/Biogas_GICON_USA_02.pdf)

SMARTFERM Leachate Beds

San Jose, CA

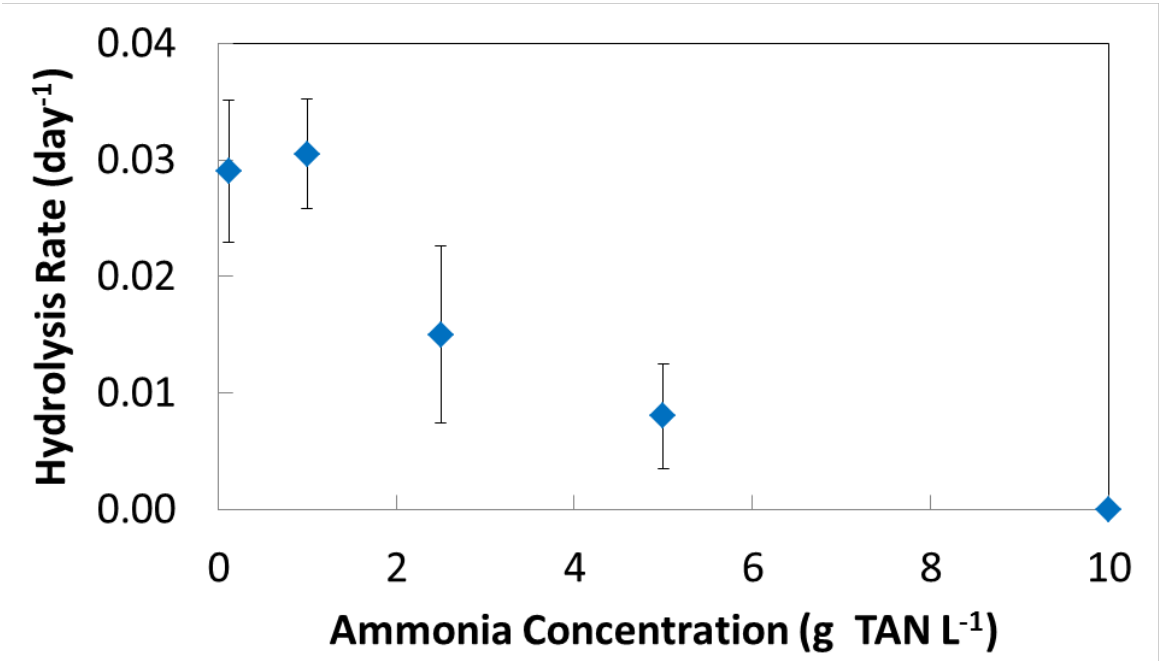
Motivation

- Biogas production limited by inhibitors: salt and ammonia
  - Methanogenesis
  - Hydrolysis
    - Up to 4-10 fold decrease in rates

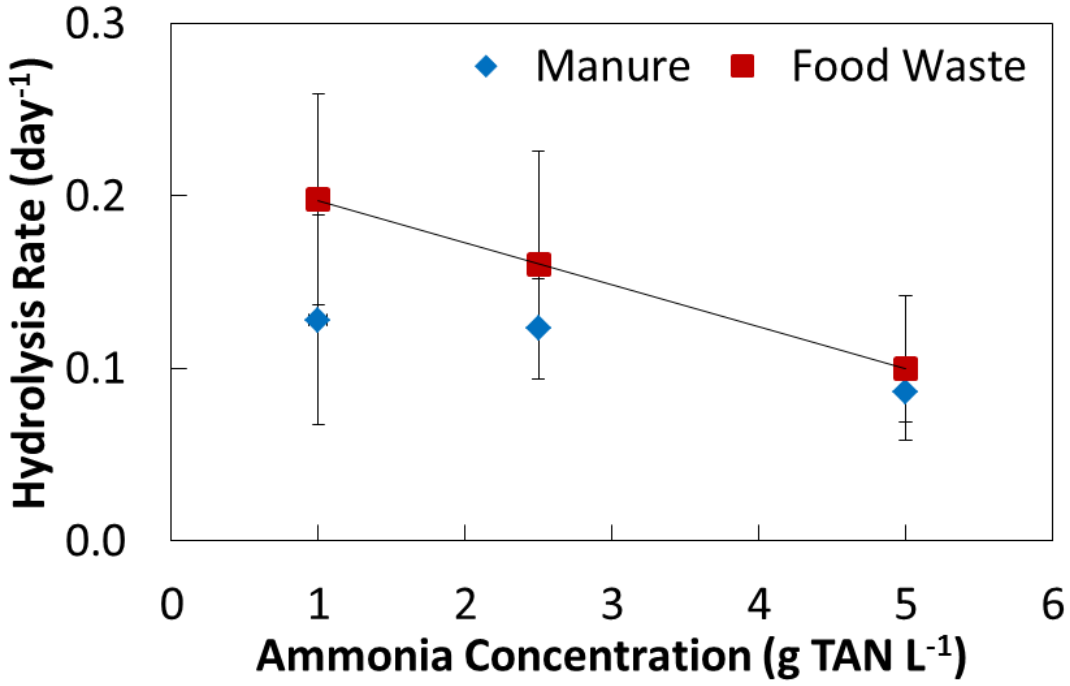
- Salt/ammonia-tolerant microbial inocula needed
- Methods for maintaining desired microbes needed

Ammonia/Salt Tolerant Inoculum

Unacclimated Inoculum



Acclimated Inoculum



Wilson, L.P., Loetscher, L.H., Sharvelle, S.E. and De Long, S.K. (2013) *Bioresour Technol* **146**, 15-22.

Research Objectives

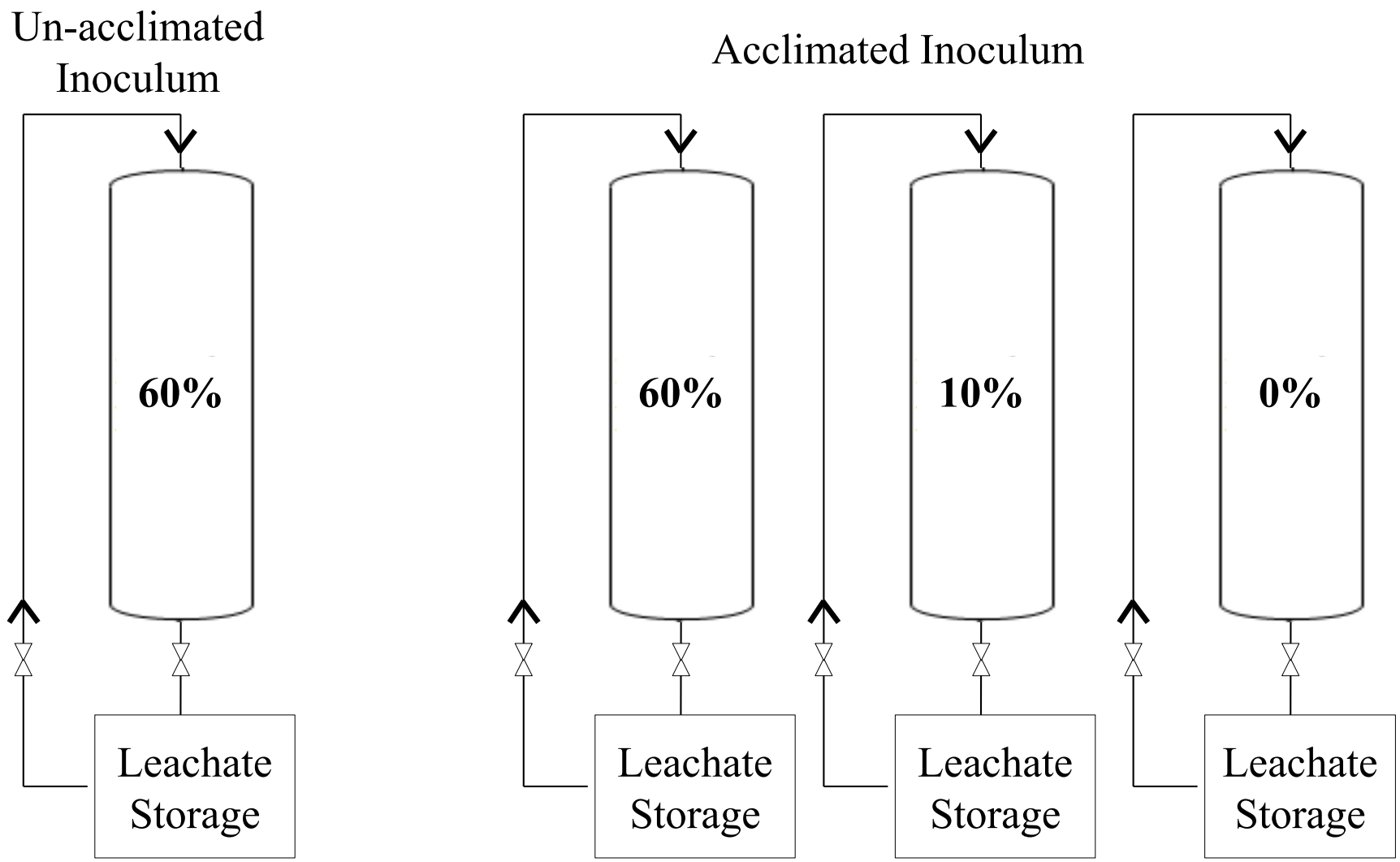
1) Develop leachate-bed seeding methods & operational approaches to control microbial populations
   - at startup/ when inhibitors start increasing
   - over long-term operation
2) Conduct an economic analysis

Research Approach

1) Test ratios (0-60% by mass) of seed (previously digested waste) to fresh waste
   - Elevated ammonia and salinity

2) Compare performance for acclimated and unacclimated seed

Methods – Reactor Start-up/ Condition Change



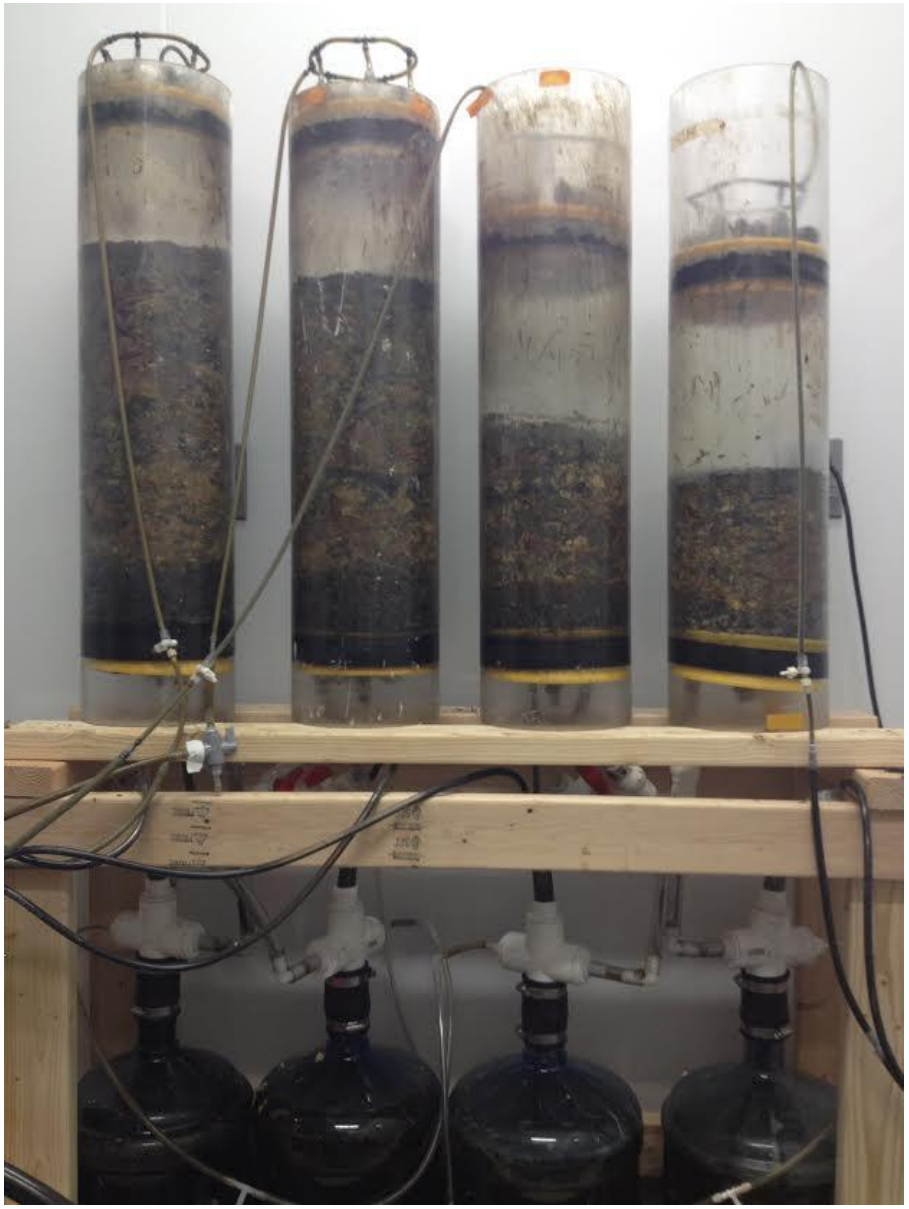
- Ammonia concentration: 3.5 g TAN/L
- Salinity concentration: 6 g Na^+/L
- Fed combination of food and yard waste
- Each batch lasted 16 days

Feedstock

- Food waste collected in dining centers
- Food waste mixed with water, ground, and dewatered.
- Food waste pulp was mixed with
  - yard (grass and leaves) waste (10% w/w)
  - wood chips (7% w/w) as bulking agent.

Methods

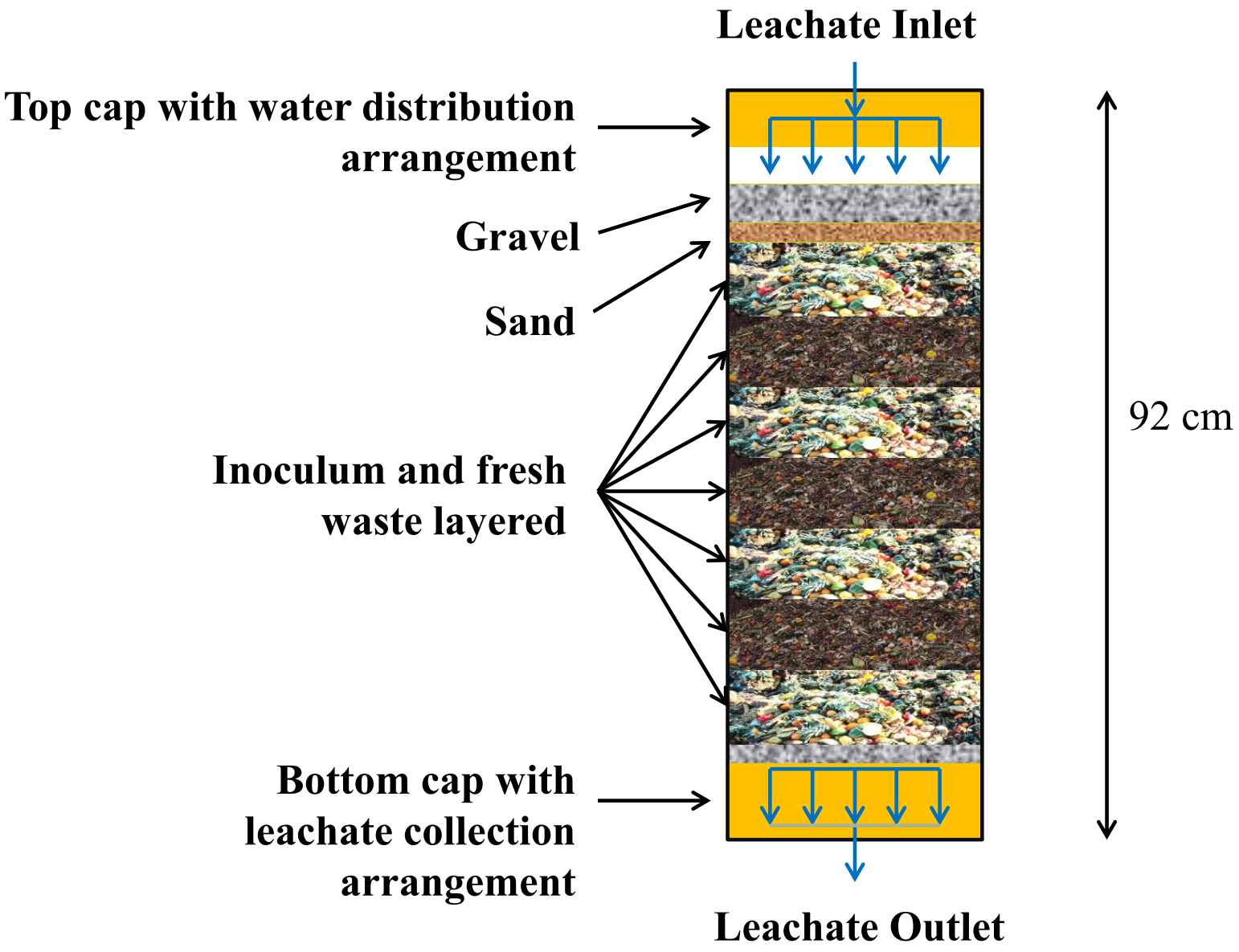
Leach bed reactors



- 10-25L waste

Leachate storage tanks

Feedstock Addition

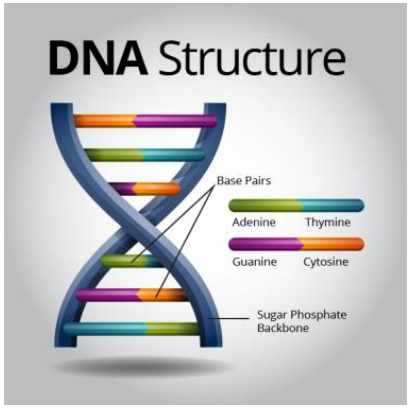


Analytical Methods

- Reactor performance monitoring:
  - Volatile Solids- by layer
  - Methane
  - Dissolved chemical oxygen demand (DCOD)
  - Volatile fatty acids (VFAs)
  - pH
- Tracking microorganisms
  - qPCR to quantify total bacteria: leachate and inoculum
  - 16S rRNA gene-targeted TRFLP
  - Next generation sequencing of 16S rRNA genes

Microbial community analysis

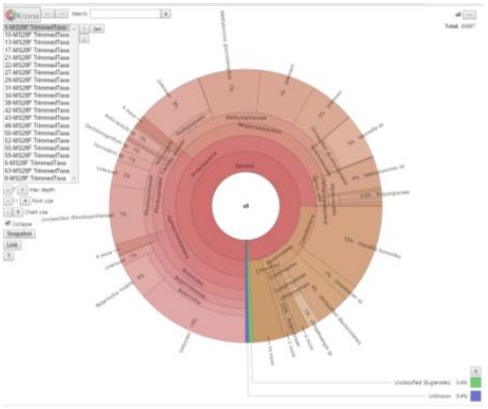
DNA extraction



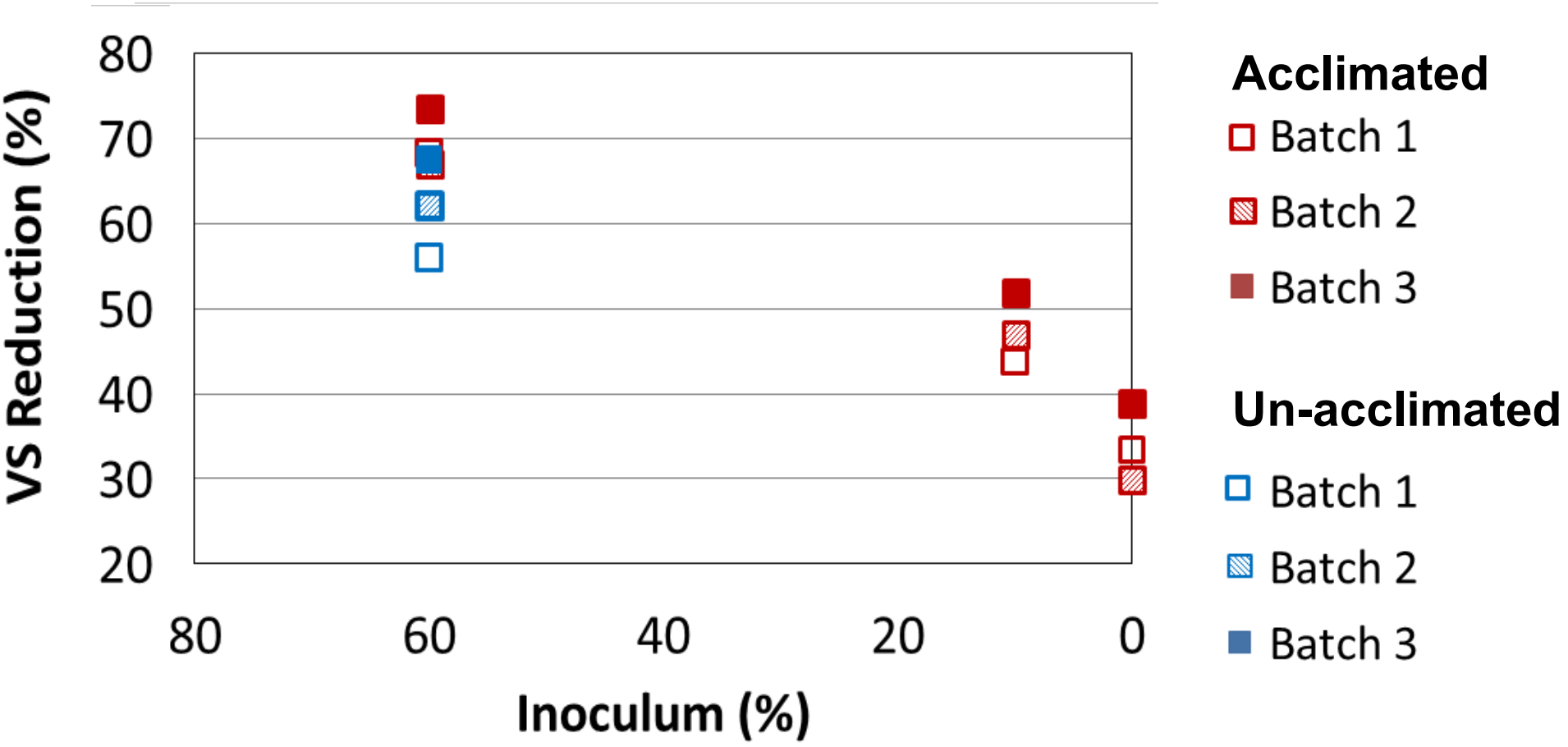
Sequencing

Bioinformatics

Microbes identification

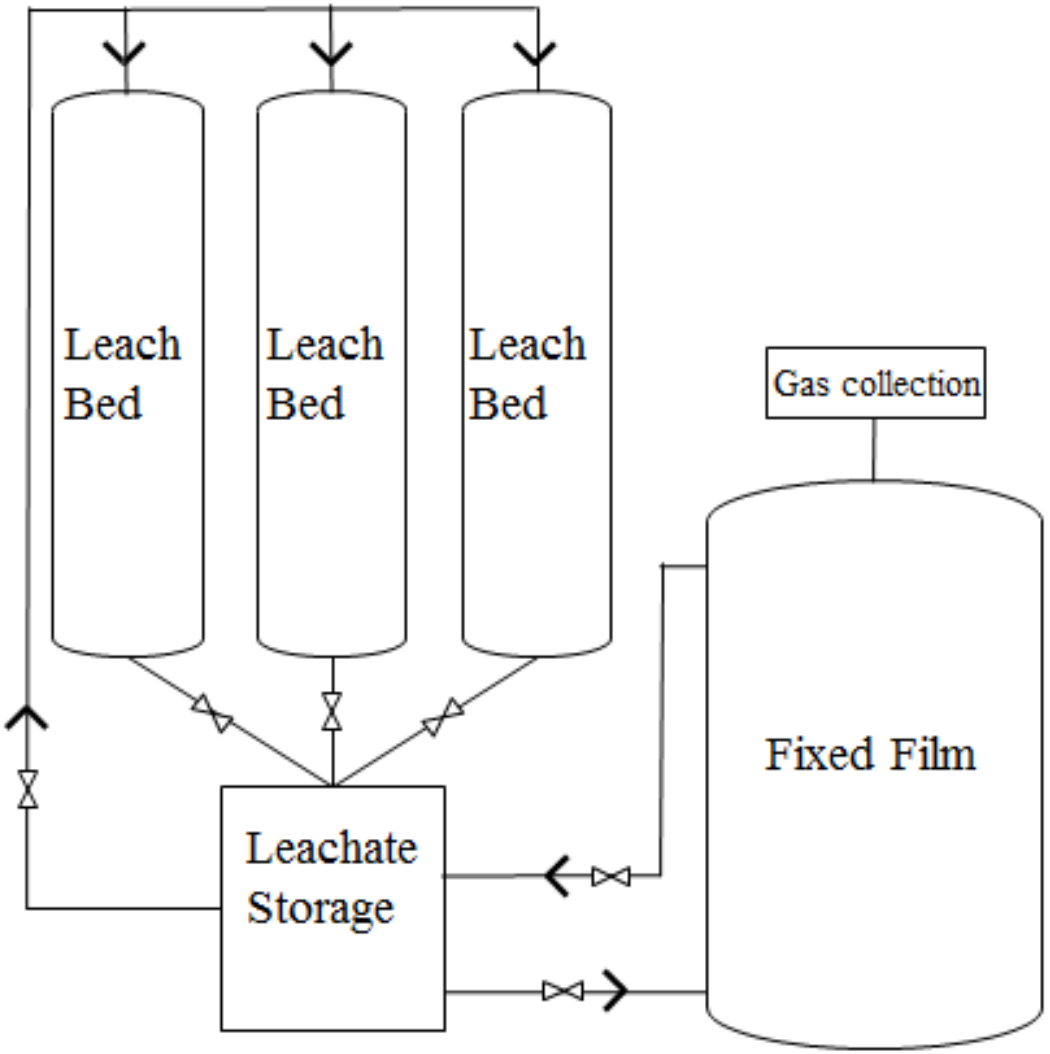


Performance Results at Start-up



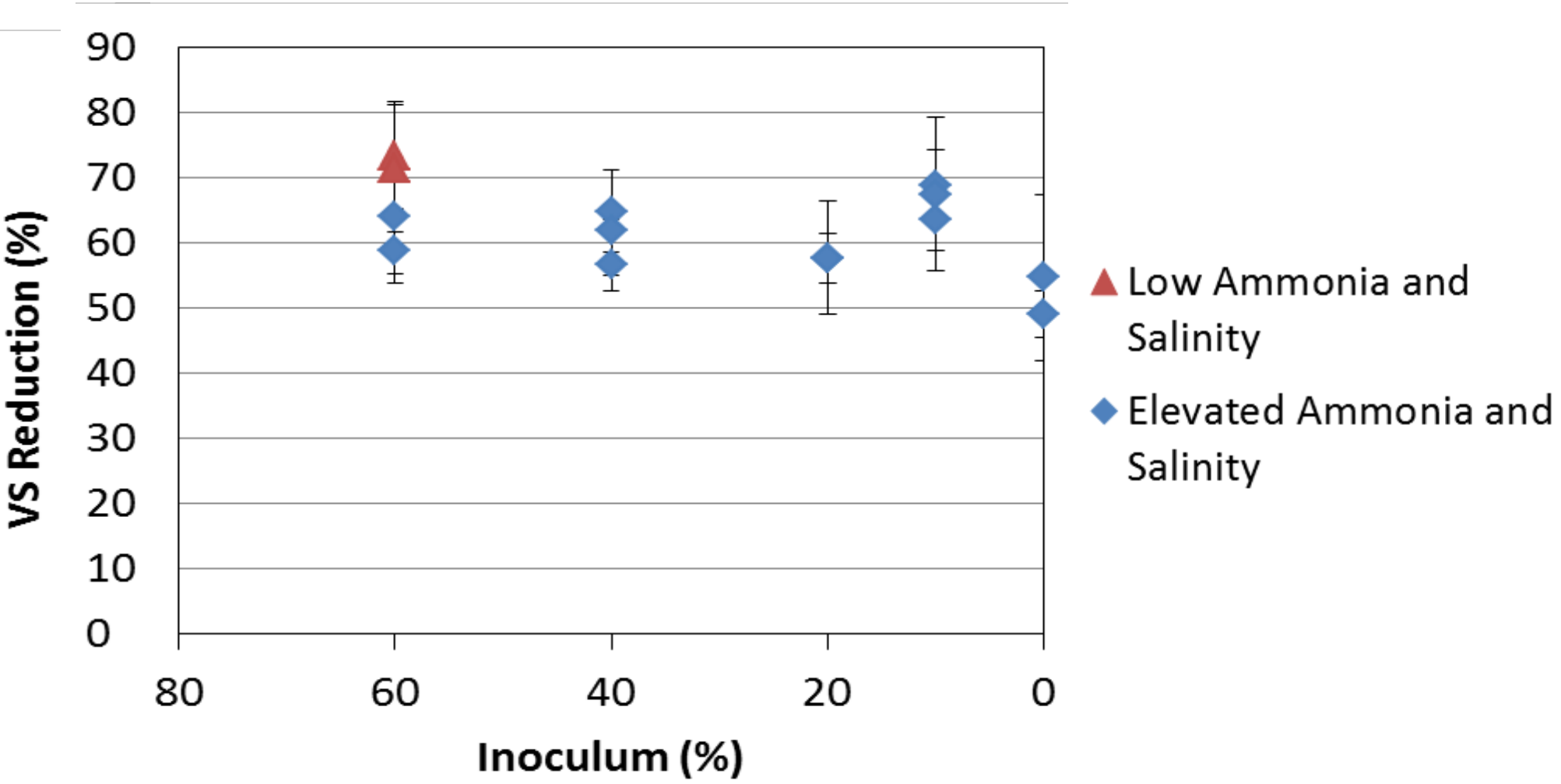
But, 60% inoculum by mass is not viable over long-term.

Methods – Long Term Operation



- Operated system for 190 days.
- Inoculum percentages (0-60%) tested in series.

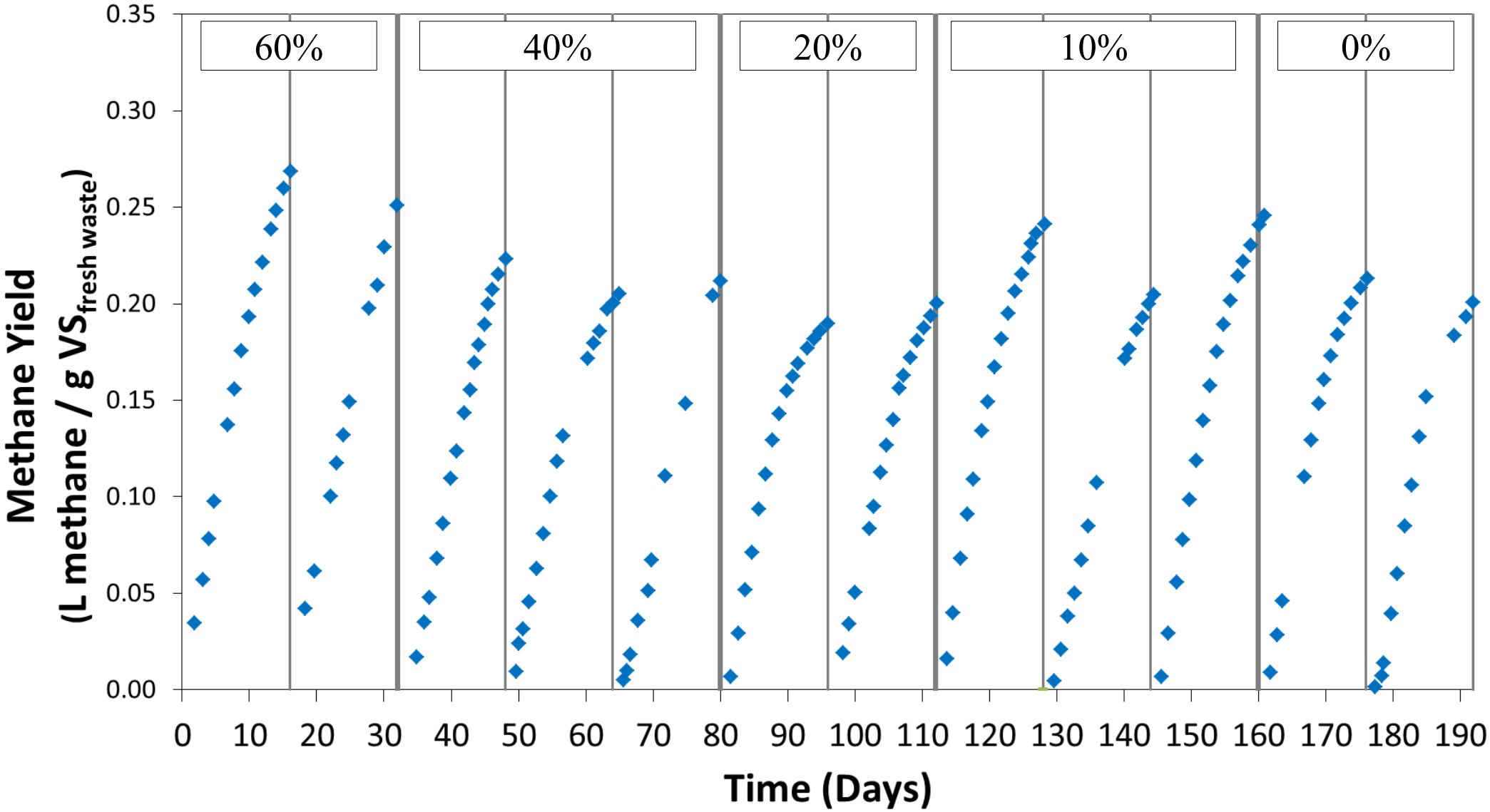
Performance Results Over Time



- Elevated ammonia and salinity inhibited VS reduction.
- Minimal decrease in VS reduction with decreased inoculum.

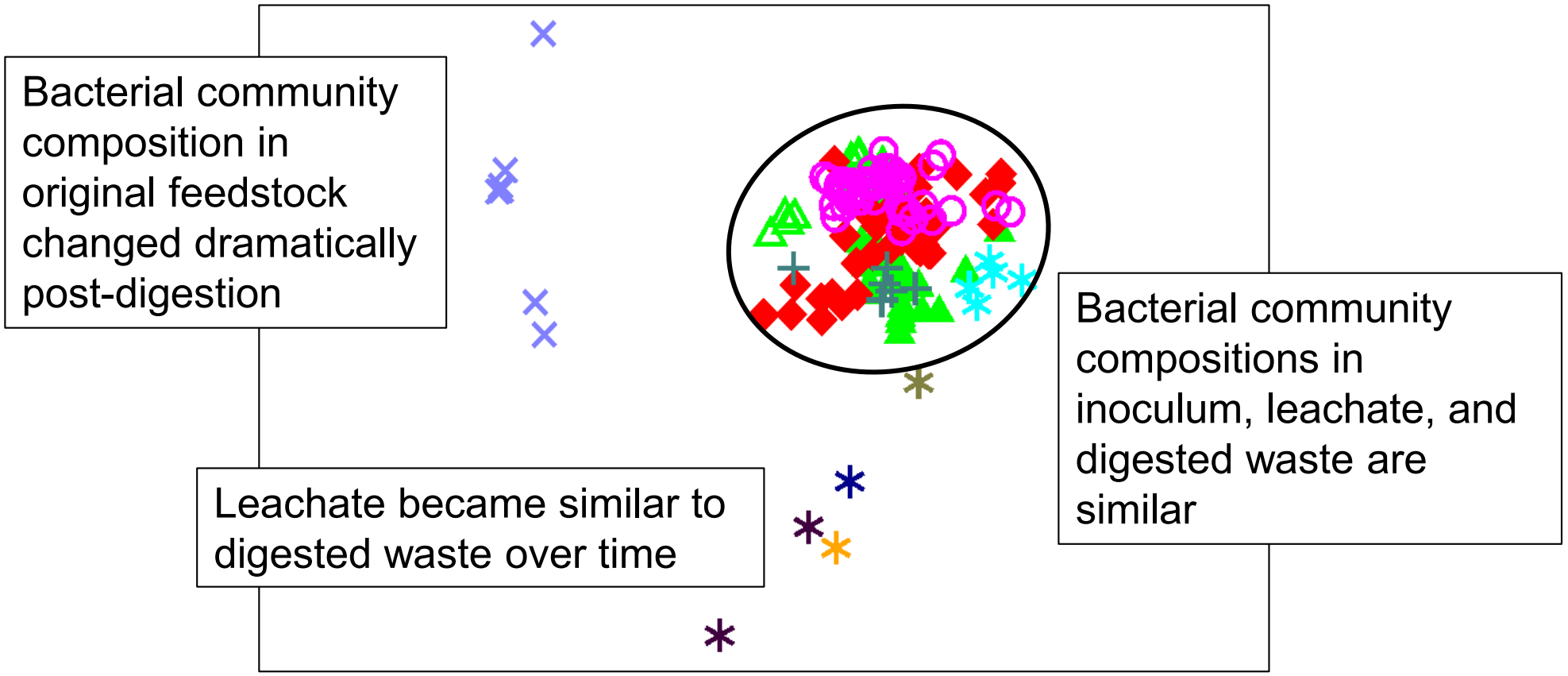
⇨ Organisms built up over time in the leachate.

Performance Results over Time



- Decrease in methane generation until day 112.
- Increase in methane generation while operating at 10% inoculum.

Microorganisms in Waste and Leachate

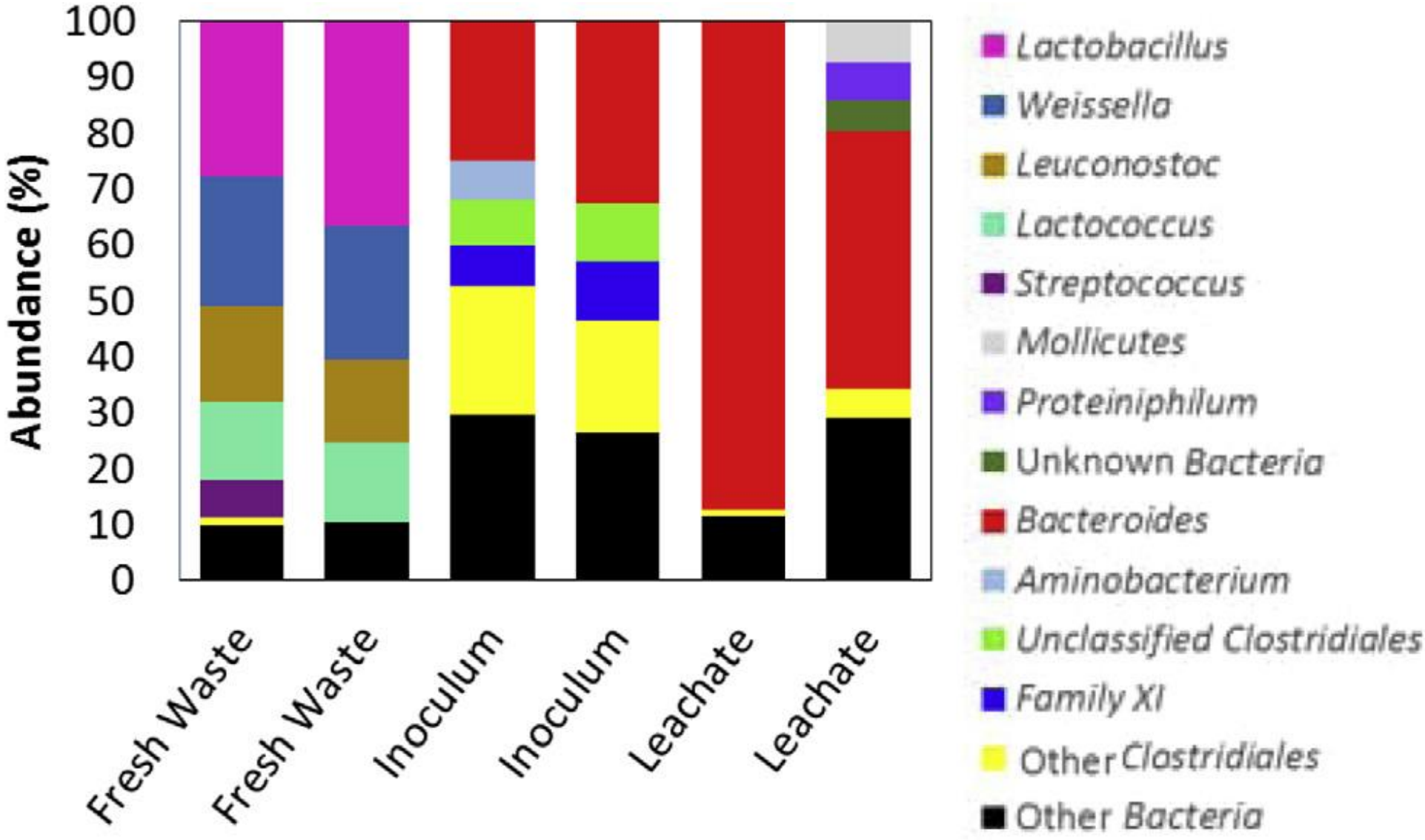


× Fresh waste + Original inoculum

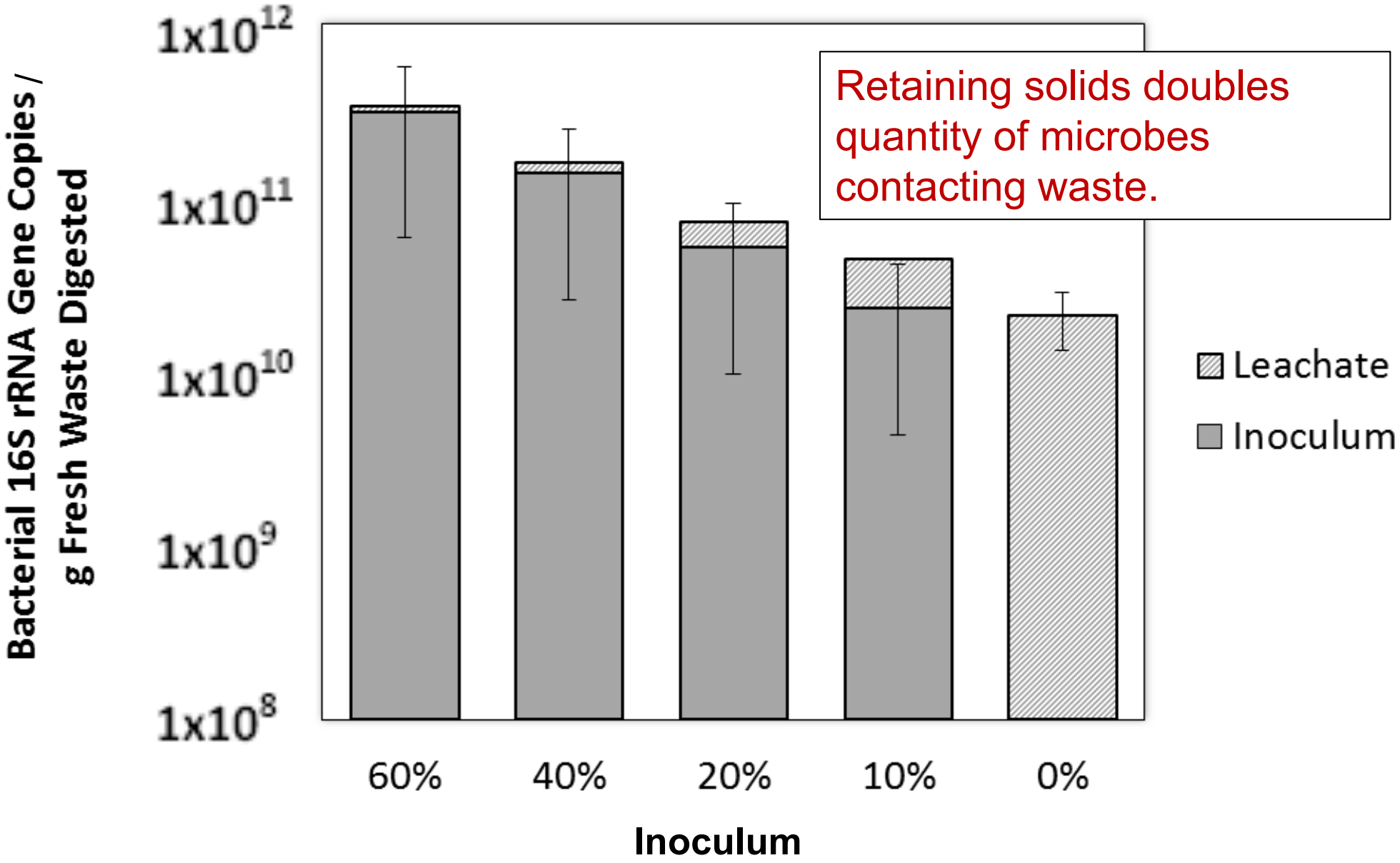
Leachate over time:
- Day 16 (60%, batch 1)
- Day 32 (60%, batch 2)
- Day 48 (40%, batch 1)
- Day 80 (20%, batch 1)
- Day 112 (10%, batch 1)
- Day 192 (0%, batch 2)

Waste after a 5-day digestion period operated with leachate from day 192:
- LI: Inoculum layer
- LI: Fresh waste layer
- MI: Fresh waste mixed with inoculum
- NI: Fresh waste (without inoculum)

Microbial Phylotypes in Waste, Leachate and Inoculum



Microorganisms in Waste and Leachate



Conclusions

- High percentages of inoculum are beneficial at start-up/ when salinity and ammonia are increasing.
- Low percentages of inoculum (0-10%) are sufficient for optimal performance afterwards.
- Key hydrolyzers were present in leachate after ~100 days of operation.
- Combining leachate-based and solids-based inoculation maximizes performance of hydrolysis reactors by providing *Clostridia* and *Bacteroides*

Questions?