Inactivation of Dairy Manure-Borne Pathogens by Anaerobic Digestion

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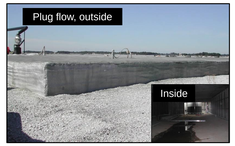
Study Objectives

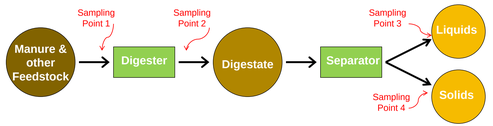
- Measure pathogen inactivation by farm-scale anaerobic digestion of dairy manure
- Evaluate several factors for their effect on pathogen inactivation (pathogen type, farm, time of year)
- Determine proportion of surviving pathogens in digestate liquid and solid fractions after separation
- Determine effect of bedding recovery units (no digestion) on fractionation of pathogens into liquids and solids

On-Farm Digesters



- Seven anaerobic digesters in Wisconsin: six on privately-owned farms; one community digester serving three farms
- All mesophilic digesters, two complete mix and five plug flow
- Primary feedstock was dairy manure
- Separators: five screw press; one blower, one centrifuge
- Plug flow digesters' retention time approximately 21 days

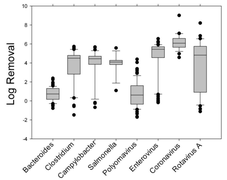
Sampling Plan and Removal Calculation



- Sampled ca. every two weeks for eight months, January – August 2012.
- Four sampling points: 1) Digester manure input; 2) Digestate output; and post-separation 3) solids and 4) liquids
- Log removal = log (microbe concentration at sampling point 1) – log (microbe concentration at sampling point 2)
- Removal for plug flow digesters: digestate concentration offset from manure concentration by two sampling periods to account for retention time

Pathogen and Microbial Indicator Removal

Removal efficiency varied by microbe type

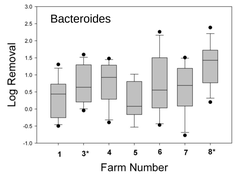


Pathogen	qPCR primer and probe references
Bovine enterovirus (5'NTR)	Ley V, 2002, Appl Environ Microbiol. 68:3455.
Bovine rotavirus -A (VP7)	Chang KO, 1999, J of Virol. 73:9284. (Probe designed by Spencer SK and Nagi Abdou S.)
Bovine coronavirus (M protein)	Spencer SK, Nagi Abdou S. In-house design.
Bovine polyomavirus (VP1)	Wong K, 2011, Appl Microbiol Biotechnol. 90:1521.
S. enterica spp. (invA)	Hoorfar J, 2000, J Clin Microbiol. 38:3429.
C. jejuni (mapA)	Best E, 2003, FEMS Microbiol Lett. 229:237.
Bovine Bacteroides (*16S RNA*)	Mieszkin S, 2010, J Appl Microbiol. 108:974
C. perfringens (cpA)	Gurjar A, 2008, Mol Cell Probes, 22:90

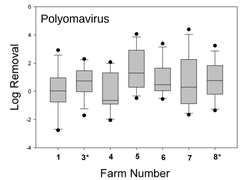
Box plots: horizontal line = median; box = 25th to 75th percentile; whiskers = 10th to 90th percentiles; circles = outlying points

All analyses were performed by qPCR. All concentrations expressed as genomic copies/g.

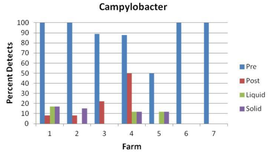
Removal efficiency varied by on-farm digester



* Continuous mix digester

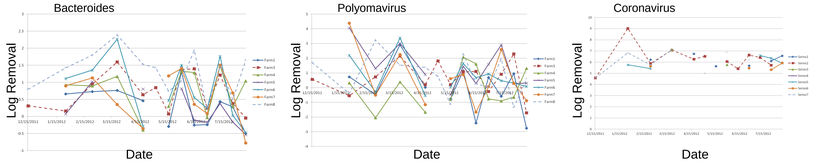


* Continuous mix digester



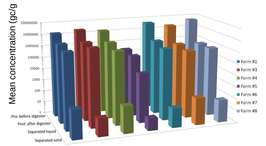
For some microbe targets, like C. jejuni, concentrations were near the qPCR limit of detection, skewing log removal estimates to negative values. Alternatively, removal was evaluated as the change in percent detections.

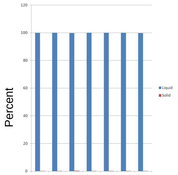
Removal efficiency varied by sampling date



Separation into Liquid and Solids Fractions

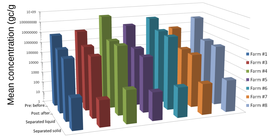
Bovine Bacteroides

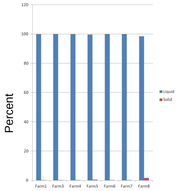




Solids Post-Digestion

Bovine Polyomavirus





Liquids Post-Digestion

Study Limitations

- Sampling frequency was not based on digester retention time therefore the manure and digestate samples are not truly coupled, particularly for the plug-flow digesters.
- Measured inactivation of pathogen/indicator genomes; this is not a measure of infectivity or viability.
- Pathogen concentrations in many samples were near the assay limit of detection, which reduces accuracy of the log removal estimate.

Summary

- Aggregating the data across time, full-scale anaerobic digesters reduced pathogen levels by 90% to 99.9%.
- However, for some microbes, removal efficiency was highly variable with time.
- Removal efficiency also varied by microbe type and digester.
- After digestion and separation, the majority of pathogens were in the liquid.
- Pathogen concentrations in solids were reduced, but still could be problematic when solids are used as bedding.
- Granting an automatic 2-log pathogen removal credit for irrigated manure that is first digested needs further consideration.