

1 **Evaluation of the pathogen reduction from plug flow and**
2 **continuous feed anaerobic digesters.**
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13 **ABSTRACT.**

14 *Reduction of pathogens from the treatment of animal manure in anaerobic digesters (AD) has implications*
15 *for policies related to land application of the post AD materials and bio-security implications for “community “*
16 *digesters. Samples of pre-AD liquid, post AD liquid, and post AD solid were assayed bi-weekly, on two*
17 *consecutive days, for six sampling events from a continuous feed and plug flow AD. Samples were quantitatively*
18 *assayed for generic E-coli and enterococci and qualitatively assayed for Mycobacterium avium paratuberculosis*
19 *(MAP), and bovine enterovirus, and Salmonella spp. E. coli, enterococci, and enterovirus were selected because*
20 *of their dependable occurrence in bovine feces, their similarity to potential biosecurity agents and their wide*
21 *thermotolerance range. Mycobacterium avium paratuberculosis and Salmonella were selected due to their*
22 *importance as biosecurity agents. Anaerobic digestion resulted in declines of 98.8 % and 99.9 % (at the two*
23 *sites) for generic E. coli and 84.5 % and 95.8 % for enterococci. Four samples of composted solid manure from*
24 *the plug flow digester indicated a reduction of 100 % for generic E-coli and 99.9 % in enterococci. Bovine*
25 *enterovirus and MAP were isolated on numerous occasions from both pre- and post-digestion samples and*
26 *composted material. Salmonella spp. were found in only two samples, both post-digestion. While substantial*
27 *quantitative reductions occurred for E. coli and enterococci, the low level of survival of these indicator*
28 *organisms along with the frequent survival of enterovirus and MAP indicates that anaerobic digestion, even*
29 *followed by composting, would not remove all biosecurity hazard.*

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31 *Keywords. Anaerobic digester, pathogens, bio-security*
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33 Manure is recognized as a means of transmitting some domestic disease agents of biosecurity
34 concern in cattle, including *Mycobacterium paratuberculosis* (Wells 2000), salmonella (Veiling, 2002;
35 Radke, 2002; Warnick, 2001), protozoa and viruses (Guan, 2003). Manure also represents a mode of
36 transfer of zoonotic agents to crops grown for animal or human consumption (Beuchat, 1997; Natvig,
37 2002; Solomon, 2002; Guan, 2003). Manure contamination of crops in international commerce has
38 been implicated as a means of transmission of *E. coli* 0157:H7 (Davis, 2003).

39 While there are numerous claims of the impact that anaerobic digesters (AD) can have on
40 pathogen reduction (<http://www.epa.gov/agstar/library> and Sobsey et al. 2000), limited detailed studies
41 are available that demonstrate efficacy. Most of these studies result in uncertain pathogen reduction
42 estimates and are based on limited lab studies or pilot field studies with few pathogens (Sobsey et al
43 2000). Reductions range from 1- 2 log₁₀ for mesophilic AD to >4 log₁₀ for thermophilic AD. Gamroth
44 and Krahn (2003) did report a 98 % reduction from 60 million to 1.2 million with a continuous flow
45 AD in Oregon.

46 Currently there are a number of AD systems being proposed in Washington State, with only one
47 currently being operational (McConnell, 2004 and Sayre, 2004). One system being proposed is a
48 community digester that would collect manure from many dairies at a centralized facility
49 (<http://www.quilcedapower.com/>). In planning these systems, there has not been a consideration of the
50 implications of pathogens associated with the post AD liquid or solid material. The potential pathogen
51 reduction due to AD treatment is an important consideration since it has been proposed that the post
52 AD solid would be: 1) used in the horticulture and row crop vegetable and fruit industry, 2) recycled
53 back to dairies as bedding material, and the liquid used for fertilizer and irrigation. Movement of post
54 AD liquid or solid has the potential in each case to transfer pathogens amongst agricultural industries
55 or herd-to-herd transmission in the case of the community AD system.

56 **METHODS**

57 Two operating anaerobic digesters in Oregon were the source of pre- and post AD samples. The
58 sampling period was bi-weekly, on two consecutive days, for six sampling events. The samples were

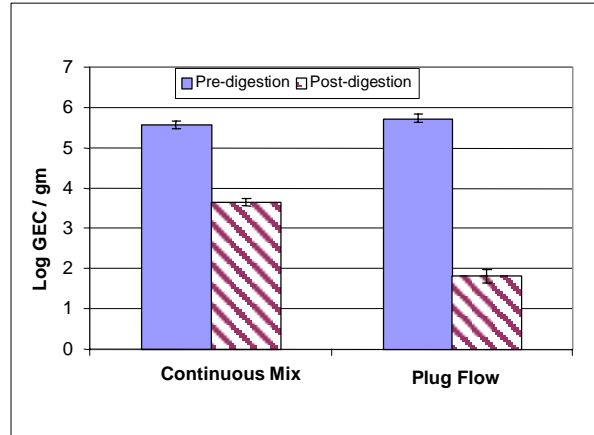
59 obtained from: manure prior to the AD system, and solids and liquids post AD. The design of the two
60 digesters was different, with one being a plug-flow and the other, a continuous feed.

61 Specific organisms selected for evaluation were: Salmonella, Generic *E. coli* (including
62 0157:H7), enterococci, salmonella, *mycobacterium paratuberculosis* (Johnes), and enterovirus.
63 Generic *E. coli* was selected because high concentrations are dependably present in bovine fecal waste,
64 and, because of its relatively low thermotolerance, survival of this organism in residues would indicate
65 that a wide variety of biosecurity agents could likely survive. Enterococci were selected because they
66 are dependably present in bovine fecal waste, and, because of their relatively high thermotolerance,
67 survival of these organisms in residues would indicate that thermotolerant biosecurity agents could
68 likely survive. Salmonella and *Mycobacterium paratuberculosis* were selected because they are
69 themselves important biosecurity agents, because they occur frequently enough in dairy herds that a
70 good chance exists of finding them (at least in pre-digestion samples), and because they are
71 environmentally resistant to a lesser (Salmonella) or greater (*Mycobacterium*) degree. Enteroviruses
72 were selected because they occur ubiquitously in cattle populations at a high prevalence (Ley et al,
73 2002) and they have a similar level of environmental resistance as certain viruses with biosecurity
74 implications.

75 **RESULTS AND CONCLUSIONS**

76 A summary of results from pre- and post AD samples collected from the two AD systems are
77 shown in Figures 1-3 and Table 1. The data indicated reductions in pathogen concentration were >
78 98% (generic *E. coli*, enterococci, and enterovirus) in most cases. While the detection of
79 *Mycobacterium paratuberculosis* was reduced in post digested samples, however, greater than 50% of
80 samples had detectable levels. The overall data suggest that AD treatment of dairy manure would not
81 remove all biosecurity hazard.

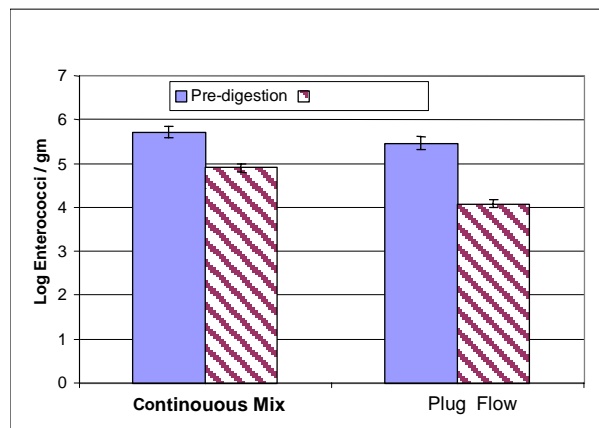
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Figure 1. Generic E.Coli concentration in anaerobic digester samples.

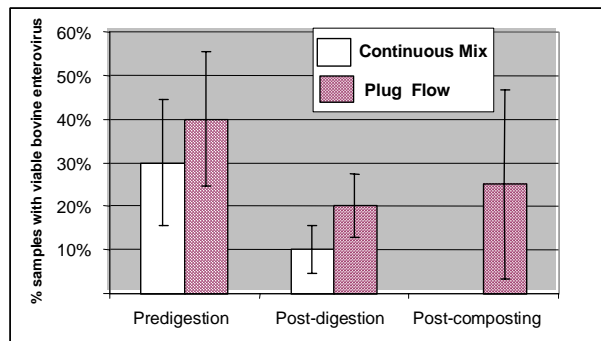
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Figure 2. Enterococci concentration in anaerobic digester samples.

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Figure 3. Enterovirus concentration in anaerobic digester samples.

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Table 1. Summary of anaerobic digester samples for *Mycobacterium paratuberculosis*.

		Pre-digestion	Post-digestion	Post-composted solids
Continuous Mix	Number of samples	10	30	NA
	% Samples with <i>Mycobacterium paratuberculosis</i>	80	40	NA
Plug Flow	Number of samples	10	30	4
	% Samples with <i>Mycobacterium paratuberculosis</i>	90	63.3	0

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