DISCUSSION

Of: **Preventing Growth of Pathogens in Pasteurized Digester Solids,** A. Ward, H.D. Stensel, J.F. Ferguson, G. Ma, S. Hummel, **71,** 176 (1999).

R. Iranpour, O. Moghaddam, M. Stenstrom, D. Miller, S. Yaghmaei

This paper seems to be a significant advance for the beneficial reuse of wastewater solids because it provides insight to past observations of pathogen behavior and demonstrates that anaerobic mesophilic digestion after pasteurization fosters the growth of an anaerobic culture that inhibits growth of pathogenic bacteria in the solids. It seems likely that this self-disinfecting effect also should occur in anaerobic digestion processes (Huyard et al., 1998; Streeter et al., 1997; and Wilson and Dichtl, 1998) in which a primary phase of thermophilic digestion that lasts for 2 to 5 days at 55 ℃ is followed by 10 or more days of mesophilic digestion. If so, the result in this paper opens the possibility of adapting diverse digestion processes to produce biosolids that meet Class A standards for killing pathogenic bacteria (U.S. EPA, 1995).

Because the results are so striking, the following questions are provided to promote additional studies and are offered out of a desire to see the excellent findings of this study replicated and extended. First, when during the run with the secondary mesophilic digesters was the spiking test in Figure 1 conducted? Was it after 8 weeks of not detecting pathogens in the digester that was being fed pasteurized solids, as seems to be implied on p. 179? Did this observation period begin when the digesters began operating or after an initial acclimation or stabilization period? Second, is the *Salmonella* analysis from *Standard Methods* (APHA et al., 1998) or some other established reference, such as Neidhardt (1987)?

Third, is there any prospect that the authors, or some of them, will extend this study by analyzing this effect in more detail? The following questions and considerations seem reasonable. (a) Is the rise in pH observed in several of the tests attributable primarily to ammonia production from protein decomposition that more than neutralizes the volatile fatty acid production that is observed, or is something else involved? (b) Although using centrate as the soluble substrate was obviously both convenient and sure to be appropriate for the bacterial population, it might be informative to identify at least the primary organic components and nutrients of the soluble substrate. Is there any prospect of doing this? (c) It seems that identifying the anaerobic bacteria responsible for killing the pathogens would be quite difficult, but do the authors see any hope of doing so? (d) On the other hand, it seems that it might be informative to filter or centrifuge solids that kill *Salmonella* and separate the filtrate or centrate by liquid chromatography or a resin separation column so that the fractions could be tested for effectiveness in killing pathogens. This would be a step toward characterizing the chemical mechanism by which the killing occurs. Do the authors have any access to facilities that would make this possible and do they consider this a reasonable undertaking? (e) Coventry et al. (1997) describe a bacterial inhibition effect caused by substances produced by other bacteria. These substances are called bacteriocins. Is it possible that these substances cause the observed *Salmonella* inhibition?

Fourth, what about temperature-phased anaerobic digestion (TPAD) and other processes involving thermophilic digestion that are used now to produce biosolids that are dried and sold for soil application and sometimes claim to meet the Class A standard, although it is unclear that this is true for anything except coliform bacteria. Has anyone analyzed the pathogen content of such material after it has been stored for several weeks or months or the pathogen content of soil to which it has been applied?

Fifth, in the approximately 2 years since this paper was submitted, have the authors made any effort to extend this work to TPAD or related processes? Last, because Seattle is not far from Vancouver, British Columbia, Canada, have the authors looked for self-disinfecting effects in digested solids from the thermophilic systems described in, for example, Volpe et al. (1993). This paper describes not only the design for the Annacis Island plant, but some results from the Lions' Gate plant.

Significant as this paper is likely to be for the wastewater treatment industry, it would have a much broader significance if the mechanism by which the pathogens are killed in self-disinfecting biosolids turns out to be both new and adaptable to other contexts. At this point it would not be productive to speculate further about what else might be done with the mechanism, but we hope that not only the authors but the readers of this journal recognize the potential significance of additional investigation of the result in this important paper.

Acknowledgments

Authors. R. Iranpour and O. Moghaddam are Principal Investigator and Energy Manager of the Bureau of Sanitation, City of Los Angeles, California; M. Stenstrom, D. Miller, and S. Yaghmaei are professors and researchers at the University of California, Los Angeles; University of Southern California, Los Angeles; and Sharif (Tehran, Iran) University, respectively. Correspondence should be addressed to R. Iranpour, 229 21st Street, Santa Monica, CA 90402.

References

American Public Health Association; American Water Works Association; and Water Environment Federation (1998) *Standard Methods for the Examination of Water and Wastewater*. 20th Ed., Washington, D.C.

Coventry, M.J.; Gordon, J.B.; Wilcock, A.; Harmark, K.; Davidson, B.E.; Hickey, M.W.; Hillier, A.J.; and Wan, J. (1997) Detection of Bacteriocins of Lactic Acid Bacteria Isolated from Foods and Comparison with Pediocin and Nisin. *J. Appl. Microbiol.*, **83**, 248.

Huyard, A.; Ferran, B.; Audic, J.M.; Dieterien, J.; Adamick, J.; and Noel, T. (1998) A Challenge for the Two Phase Anaerobic Digestion: To Produce Class A Biosolids and Meet PFRP Equivalency. *Proc. Water Environ. Fed. 71st Annu. Conf. Exposition*, Orlando, Fla.

Neidhardt, F.D. (1987) *Salmonella typhimurium*. Am. Soc. Microbiol., Washington, D.C.

Streeter, M.D.; Dague, R.R.; and Main, R.E. (1997) Evaluation of a Field Application, Temperature-Phased Anaerobic Digestion. *Proc. Water Environ. Fed. 70th Annu. Conf. Exposition,* Chicago, Ill.

U.S. Environmental Protection Agency (1995) A Guide to Biosolids Risk Assessments for the EPA Part 503 Rule. EPA-832/B-93-005.

Volpe, G.; Rabinowitz, R.; Peddie, C.C.; and Krugel, S. (1993) Class A (High Grade) Sludge Process Design for the Greater Vancouver Regional District (GVRD) Annacis Island Wastewater Treatment Plant. *Proc. Water Environ. Fed. 66th Annu. Conf. Exhibition,* Anaheim, Calif.

Wilson, T.E., and Dichtl, N.A. (1998) Two-Phase Anaerobic Digestion: An Assessment. *Proc. Water Environ. Fed. 12th Annu. Residuals Biosolids Manage. Conf.*, Washington, D.C., 195.

Closure

Amanda Ward, H. David Stensel, John F. Ferguson, Gregory Ma, Stan Hummel

We greatly appreciate the discussion by Iranpour et al. that pointed out an issue of importance to us, which is the need to understand the mechanism of *Salmonella* kill in mesophillic anaerobic digestion. Although our work was able to show that *Salmonella* regrowth can be prevented in pasteurized solids, determining the mechanism was beyond the scope of our available resources and time. Determining the mechanism would be an exciting venture with possibly far-reaching implications about pathogen kill, but as of yet we have not had the opportunity to further pursue this interest. A number of other specific questions were raised about the research, which we will answer here. With respect for space allowed for discussion articles, we would also be happy to provide additional specific information if contacted directly.

The *Salmonella*-spiking test shown in Figure 1 was done after the anaerobic digesters had been operating for more than 8 weeks without detecting pathogens. Both digesters were operating at normal pH when the test began (7.7 in the pasteurized digester, 7.5 in the control digester). The method used to quantify the *Salmonella* population with time was described in detail in the paper. Ammonia concentrations were not measured during incubation following *Salmonella* inoculation; so we are not able to define the exact cause of 0.2 to 0.5 pH increases in some of the bottles. Although there were no data on the centrate itself, ammonia, alkalinity, soluble chemical oxygen demand (SCOD), and volatile fatty acid (VFA) analyses were done. Table 1 summarizes the results of those analyses. A number of interesting research methodologies can be undertaken to study the *Salmonella* die-off mechanism. The idea to study liquid fractions to determine possible chemical factors is good and would be recommended as part of a research effort. Work with specific bacteria would also be helpful.

Table 1—Ammonia, alkalinity, SCOD, and VFA concentrations in Tacoma Central autothermal reactor sludge.

	Number of Samples	VFA Value
NH_3–N, mg/L	13	500 ± 200
Alkalinity, as $CaCO_3$, g/L	59	3.0 ± 0.9
SCOD, g/L	110	8.8 ± 1.2
Acetate as COD, mg/L	24	2000 ± 500
Propionate as COD, mg/L	24	300 ± 200
Butyrate as COD, mg/L	24	500 ± 100
Iso-butyrate as COD, mg/L	24	300 ± 100
pH	194	6.6 ± 0.3

In 1996, the Great Vancouver Regional District inoculated Class A thermophilic biosolids, topsoil, and compost with *Salmonella*. Samples were incubated at 35 °C. They claimed that *Salmonella* grew in short-term bursts (no data are given) in thermophilic and mesophilic biosolids after 2 days and died off after 2 months. *Salmonella* died off immediately in top soils and composts (Krugel et al., 1996).

References

Krugel, S.; Mlianna, L.; and Palsenbourg, R. (1996) Sludge and Scum Screening at the Greater Vancouver Regional District's Annacis Isonal and Lulu Island Wastewater Treatment Plants. *Proc. Water Environ. Fed. 10th Annu. Residuals Biosolids Manage. Conf.*, Washington, D.C.