THE EFFECT OF TEMPERATURE ON THE SURVIVAL OF PATHOGENIC BACTERIA AND *ASCARIS SUUM* IN STORED SEWAGE SLUDGE

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ABSTRACT

The reduction in pathogenic bacteria and *Ascaris suum* were determined during storage of sedimented, dewatered sewage sludge at 7, 13 and 21°C. The kinetics of *Salmonella* Typhimurium reduction differed over time as the initial decrease was temperature dependent while other factors influenced the survival in the later stages of storage. Temperature and reduction rate were highly correlated over time for *Enterococcus* spp., while the coliform bacteria showed an inconsistent reduction. A temperature of 21 °C during 214 days of storage was not enough to inactivate the *Ascaris suum* eggs.
Keywords**:** *Ascaris suum; indicator bacteria; kinetics; Salmonella Typhimurium*

INTRODUCTION

The use of sewage sludge on arable land can give beneficial effects such as provision of plant nutrients, humus build-up and thereby increased water-holding capacity, improved soil structure and increased cation exchange capacity. However, this use could also provide a health risk to humans and animals. Sedimentation and mesophilic digestion of sludge are conventional treatments that cause a limited reduction in pathogens (Carrington, 2001). These methods do not result in a hygienically safe sludge, and pathogens such as *Salmonella* spp. may still be present in the final sludge products (Sahlström et al., 2004). Thus, there may be risks of pathogen spreading and transmission of infectious diseases into the environment and the food web. Long-term storage is a low cost method that may result in a sludge product with a better hygiene standard, and is therefore an interesting option for many small sewage treatment plants. The efficiency of pathogen reduction is, however, to a large extent dependent on prevailing climatic conditions and therefore needs to be tested locally. The aim of this study was to determine how the temperature regime of mid-Sweden affects the inactivation rates of bacteria and nematodes during sewage sludge storage.

MATERIALS AND METHODS

The growth and survival of spiked *Salmonella enterica* subspec *enterica* serovar Typhimurium 178 (resistant to trimethoprim, and previously isolated from sewage sludge by Sahlström *et al*., 2004) and the nematode *Ascaris suum* were monitored in raw sewage sludge at three temperature regimes (7, 13 and 21 °C). In addition, the natural occurrence of *Enterococcus* spp. and coliforms was monitored. Pure culture of *S.* Typhimurium, grown in Tryptic Soy Broth (Difco) for 24 h at 37 °C, was used as inoculum. Unembryonated nematode eggs of *A. suum* from pig faeces was collected and placed in nylon bags (70x70 mm^2, 30µm pore size), approx. 4 $\log_{10}$ eggs/bag, which were then sealed (Johnson et al., 1998). Sedimented and stabilised sewage sludge (29 % total solid, TS) was collected from a commercial sewage treatment plant, analysed and confirmed to be free from *S.* Typhimurium prior to use. Each of six portions of 1000 g

sewage sludge was seeded with the *S.* Typhimurium suspension and mixed thoroughly to obtain a homogeneous mixture with a final concentration of $\log_{10}$ 8.1 most probable number (MPN) g^{-1} TS of sludge. Each sludge portion was thereafter transferred to a glass jar (3000 cm^3) together with nylon bags containing *A. suum* and closed with a glass cover. Two jars were placed in each of three incubators at 7 ± 0.8 °C, 13 ± 0.7 °C and 21 ± 0.4 °C, respectively. Water losses due to evaporation were corrected for during the experiment with sterile distilled water. Aerobic conditions were provided through continuous airing. Sludge samples (approx. 30 g) and two bags with *A. suum* were periodically assayed from each jar, without mixing the sludge, over a period of 214 d.

Quantification of *S.* Typhimurium was carried out by a standard five tube MPN method, using three dilutions with five replicates. The enrichment procedure used was according to the Nordic Committee on Food Analysis (NMKL) 71:5:1999. Serological confirmation was performed against *Salmonella* polyvalent H-antiserum. The concentration of *S.* Typhimurium was calculated from a standard MPN chart (APHA *et al*., 1995). The bags with *A. suum* eggs were microscopically examined and the first 100 eggs were differentiated as either unembryonated or embryonated. The viability was confirmed by further incubation for 21 d in 0.1N -sulphuric acid at 22 °C. Enumeration of the indigenous *Enterococcus* spp. and coliform bacteria (37 °C) was carried out according to NMKL 68:2:1992 and NMKL 44:4:1995, respectively. The pH was measured according to the European Standard (EN) 12176:1998 and the TS of the sludge was determined according to APHA et al. (1992).

The exponential death of microorganisms was expressed in the form $N = N_0 e^{-kt}$ where N is the concentration of microorganisms at time t, N_0 the initial concentration of microorganisms and k the specific reduction rate. Students t-test was used to find significant differences ($p<0.05$) between treatments.

RESULTS AND DISCUSSION

The initial reduction rate in *S.* Typhimurium increased with temperature, as the accumulated reduction during the first 56 days of storage was 1.2 ± 1.0, 1.8 ± 0.3 and 3.8 ± 0.5 $\log_{10}$ MPN g^{-1} TS of sludge at 7, 13 and 21 °C, respectively (Fig. 1). This observation is in accordance with earlier reports (Ahmed and Sorensen, 1995; Himathongkham et al., 1999; Bujoczek et al., 2001). Ignoring the initial loss of *S.* Typhimurium, the k obtained for each temperature showed that 7 °C (0.0050 h^{-1}) was different from 13 °C (0.0038 h^{-1}) and 21 °C (0.0028 h^{-1}), but that there was no significant difference between 13 and 21 °C. Thus, we found the reduction rate to decline over time at 13 and 21°C, but not at 7 °C (Fig. 1). Hussong et al. (1985)

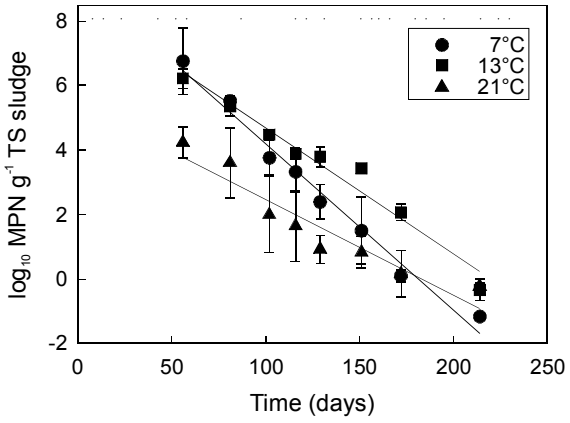

Figure 1. *Effects of different temperatures on the survival of Salmonella Typhimurium in sewage sludge. Dotted line is number of S. Typhimurium at start of experiment. Bars denote standard deviation.*

reported a similar death rate constants when studying the reduction in *S.* Typhimurium and *S.* Newport composted sewage sludge at 36 °C and their results also support our observed decline in reduction rate over time. Similarly, Wang et al. (1996) found a first order reduction in *E. coli* stored at 5 °C in cow manure but a decline in the reduction rate over time during storage at 22 and 37 °C. Based on the above, we suggest that the initial decrease in *S.* Typhimurium was to a high degree temperature dependent, but that other factors such as changes in the indigenous microbial population (Sidhu et al., 2001) may have a large influence on pathogen survival in the later stages of sewage sludge storage. After 214 days the survival of *S.* Typhimurium was similar regardless of storage temperature (Fig. 1).

We found a clear relationship between temperature and reduction rate of *Enterococcus* spp. (Fig. 2). The *k* obtained for each temperature showed that 21 °C (0.0035 h^{-1}) was different to 7 °C (0.0010 h^{-1}), but that 13 °C (0.0015 h^{-1}) was not different to the other temperatures. There was a lag period of about 60 days in the response of the coliform bacteria to the storage conditions (Fig. 3). The death phase was modeled and the *k* obtained for each temperature showed that 21 °C (0.017 h^{-1}) was different from 7 °C (0.0024 h^{-1}) and 13 °C (0.0020 h^{-1}), while the two latter temperatures did not differ in their effect on *k*. However, the reduction in the coliform bacteria was not stable over time during the later phase of storage (Fig. 3). Thus, the investigated organisms responded differently when incubated over time at different temperatures. Our results indicate that the coliform bacteria in particular may not be a usable indicator of the hygiene properties during storage of sewage sludge. A shift within the bacterial population of this group probably occurs during sludge storage, which makes an evaluation of the sanitary stability difficult (Christensen et al., 2002).

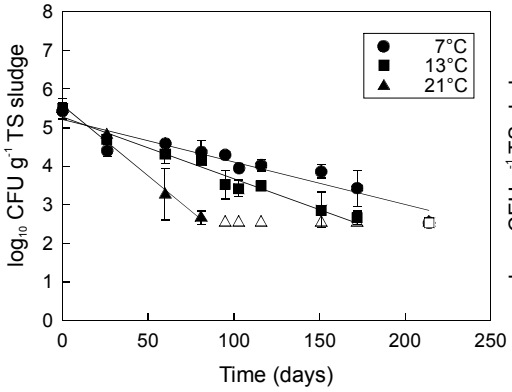

Figure 2. *Effects of different temperatures on the survival of Enterococcus spp. in sewage sludge. Black symbols represent data included in the analysis. Bars denote standard deviation.*

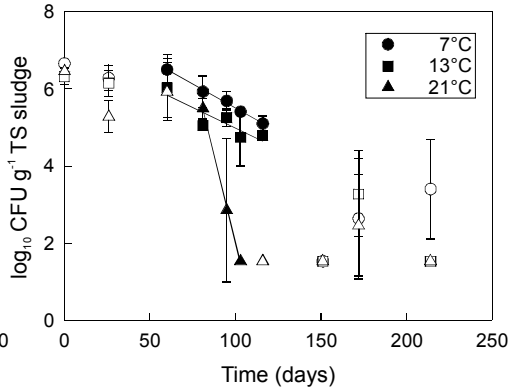

Figure 3. *Effects of different temperatures on the survival of coliform bacteria in sewage sludge. Black symbols represent data included in the analysis. Bars denote standard deviation.*

The viability (developed and viable larvae) of the *A. suum* eggs in sewage sludge stored at 21 °C was between 81 and 99% through-out the experiment. Temperatures of 7 and 13 °C during the 214 d of storage had no effect on the eggs (no cell division and no damaged interior). Our results show that higher temperatures and/or longer storage period are required in order to inactivate the nematode eggs. O´Donnell et al. (1984) used a sludge storage period of 16 months at 25 °C before the viability was affected. Gantzer et al. (2001) showed that the temperature had to be over 45 °C or the pH not less than 11.5 in order to produce sanitized sludge.

During storage, the pH was reduced in all treatments and the final reduction was 2.0, 2.7, and 1.5 pH units in 7, 13 and 21 °C, respectively. However, according to Ahmed and Sorensen (1995) and Foster (1995) the reduction in *S.* Typhimurium does not appear to be directly related to pH.

CONCLUSIONS

The relation between pathogen reduction and temperature differs over time between the pathogens studied. In addition to temperature, other factors also have to be considered when evaluating long-term storage of sewage sludge as a hygienisation method.

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REFERENCES

Ahmed, A.U., Sorensen, D.L. 1995. Kinetics of pathogen destruction during storage of dewatered biosolids. Wat. Environ. Res., 67: 143-150.

APHA, AWWA and WEF. 1992. Standard Methods for the Examination of Water and Wastewater, 18th ed. American Public Health Association, Washington, DC.

APHA, AWWA and WPCF. 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association, Washington, DC.

Bujoczek, G., Reiners, R.S., Olaszkiewicz, J.A. 2001. Abiotic factors affecting inactivation of pathogens in sludge. Wat. Sci. Tech., 44: 79-84.

Carrington, E.G. Evaluation of sludge treatments for pathogen reduction -Final report. European Commission. Report No 5026/1; 2001.

Christensen, K.K., Carlsbaek, M., Kron, E. 2002. Strategies for evaluating the sanitary quality of composting. J. Appl. Microbiol., 92: 1143-1158.

European Committee for Standardization, Central Secretariat: rue de Strassart 36, B-1050 Brussels.

Foster, J.W. 1995. Low pH adaption and the acid tolerance response of Salmonella typhimurium. Critical Rev. Microbial., 21: 215-237.

Gantzer, C., Gaspard, P., Galvez, L., Huyard, A., Dumouthier, N., Schwartzbrod, J. 2001. Monitoring of bacterial and parasitological contamination during various treatment of sludge. Wat. Res., 35: 3763-3770.

Himathongkham, S., Bahari, S., Reimann, H., Cliver, D. 1999. Survival of Escherichia coli O157:H7 and Salmonella typimurium in cow manure and manure slurry. FEMS Microbiol. Letters, 178: 251-257.

Hussong, D., Burge, W.D., Enkiri, N.K. 1985. Occurrence, growth, and suppression of Salmonellae in composted sewage sludge. Appl. Environ. Microbiol., 50: 887-893.

Johnson, P.W., Dixon, R., Ross, A.D. 1998. An in-vitro test for assessing the viability of Ascaris suum eggs exposed to various sewage treatment processes. Int. J. Parasitology., 28: 627-633.

Nordic Committee on Food Analysis, Veterinareinaerinstitutet, Department of Food Hygiene, Postbox 8156, n-0033 Oslo, Norway.

O´Donnell, C.J., Meyer, B., Jones, V.J., Benton, T., Kaneshiro, E.S., Nichols, J.S., Schaefer, F.W. 1984. Survival of parasite eggs upon storage in sludge. Appl. Environ. Microbiol., 48: 618-625.

Sahlström, L., Aspan, A., Bagge, E., Danielsson-Tham, M-L., Albihn, A. 2004. Bacterial pathogen incidences in sludge from Swedish sewage treatment plants. Wat. Res., 38: 1989-1994.

Sidhu, J., Gibbs, R.A., Ho, G.E., Unkovich, I. 2001. The role of indigenous microorganisms in suppression of Salmonella regrowth in composted biosolids. Wat. Res., 35: 913-920.

Wang, G., Zhao, T., Doyle, M.P. 1996. Fate of Enterohemorrhagic Escherichia coli O157:H7 in bovine feces. Appl. Environ. Microbiol., 62: 2567-2570.