Thermophilic composting - a hygienization method of source-separated faecal toilet waste

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SUMMARY

Aims: To evaluate the sanitizing effect of thermophilic composting of faecal material from urine diverting toilets as a function of temperature and exposure-time.

Methods and Results: A composting lab with reactors imitating centralized in-vessel composting systems was used. The elimination of indicator organisms was investigated at temperatures between 50 and 65°C. *Salmonella* serotype Senftenberg 775W and thermotolerant coliforms were rapidly inactivated during less than one day at all tested temperatures. *Enterococcus* spp. showed a tail-shaped die-off and was not totally inactivated during the investigation. *Salmonella* serotype Typhimurium phage 28B showed an initial slower reduction at 50 and 55°C. Phage 28B had the highest T90-values of the tested indicator organisms except at 50°C, when comparing the rapid inactivation phases of the different test organisms.

Conclusions: Aerated in-vessel composting at 55°C for five days gives a satisfying hygienization of faecal material from urine-diverting toilets. Inactivation of phage 28B can be used as an indicator for inactivation of other similar thermoresistant microorganisms, inclusive viruses.

Significance and Impact of Study: Analysis of inactivation of indigenous enterococci in combination with added *Salmonella* serotype Typhimurium phage 28B is an effective tool for evaluating the sanitizing effect when composting faecal material at thermophilic temperatures.

Keywords: Source-separating toilets, thermophilic composting, human faeces, indicator organisms, survival, hygiene

INTRODUCTION

There is an increased interest in the society for local handling and use of human faeces and urine from compost toilet systems with urine diversion. However, it appears that the pathogen die-off and associated health risks from reuse of stored faeces have been inadequately assessed despite the fact that compost toilets have been used for decades in many countries (Jansen and Boisen 2000; Moe *et al*. 2001). Diverted urine contains only low concentrations of pathogens that mostly originate from faecal contamination, and their numbers in urine can be effectively reduced by prolonged storage (Jönsson *et al*. 1997; Höglund *et al*. 1998; Höglund 2001). In contrast, the faecal matter contains high numbers of naturally occurring enteric bacteria, and occasionally disease-causing pathogens like *Salmonella*, *Campylobacter*, *Shigella*, enteric viruses, and parasites. Studies have shown that temperatures high enough to achieve an adequate hygienization are normally not reached during faecal storage in single household compost toilets (Carlander and Westrell 1999; Møller *et al*., in press). Therefore, other treatment methods have to be used to provide a safe end-product that can be disposed off or used for agricultural purposes. In many developing countries, wood ash is added to toilets and the increased pH may lead to sanitation of the faecal material (Franceys *et al*. 1992; Austin 2001; Moe *et al*. 2001). A safer and more controllable method would be to collect faecal material from several toilets and compose it under thermophilic temperatures. Temperatures obtained under thermophilic composting of faecal material, i.e. 55ºC for two weeks, would be expected to inactivate or kill pathogens (Feachem *et al*. 1983). However, other factors are also involved in the inactivation, like changes in pH, presence of metabolic antagonistic compounds produced by the indigenous microflora, accumulation of toxic NH_3 and microbiological competition for nutrients (Golueke 1991; Dumontet *et al*. 1999). Several systems have been developed to compost organic wastes of which windrow or static pile systems are the simplest. However, these systems are constrained by low temperatures in the

outer layers of the material where pathogens may survive or even show re-growth. An isolated reactor system with forced aeration is a more effective way of composting, because it can be assured that the entire material reaches a sanitizing temperature during a certain period of time (Haug 1993).

Thermotolerant coliforms, *Escherichia coli* and enterococci are often used as indicator bacteria to assess the hygienic quality of treated organic waste (Bendixen *et al*. 1995; Redlinger *et al*. 2001; Christensen, Carlsbæk and Kron 2002). *Salmonella* is a relatively common excreted pathogen, and test systems in different countries therefore often demand absence of *Salmonella* in treated faecal polluted material. S*almonella* serotype Senftenberg 775W is often added to the faecal material and used as a test organism, e.g. in Germany (Hösel *et al*. 1995), because of its high thermotolerance compared with other *Salmonella* serotypes (Henry *et al*. 1969; Soldierer and Strauch 1991). Various kinds of bacteriophages have been suggested as viral indicators to evaluate the sanitation effect of different treatment methods (Havelaar *et al*. 1991). *Salmonella* serotype Typhimurium phage 28B (Lilleengen 1948) has a documented resistance towards high temperatures, changes in pH and high NH_3-levels (Eller *et al*. 1996; Carlander and Westrell 1999; Holmqvist and Stenström 2001; Vinnerås *et al*. 2003). This phage does not occur naturally in the environment or in faecal material and must therefore be added if level of hygenisation is to be measured.

Several studies have investigated microbiological aspects of composted sewage sludge under more or less controlled conditions (Pereira-Neto *et al*. 1986; Shuval *et al*. 1991; Dumontet *et al*. 1999). However, few if any studies seem to have investigated the survival of microorganisms under controlled thermophilic composting of source-separated faecal material. Thus, the objective of the present study was to evaluate the inactivation of faecal indicator microorganisms during centralized composting of faecal material from urine

diverting toilets at different temperature and exposure-time experiments in a lab-scale model system, which simulates the conditions in a full-scale composting plant.

MATERIALS AND METHODS

The model composting system

Human faecal material was treated in a model system consisting of six computer-controlled 9-l compost reactors with controlled aeration and temperature regulation. Each reactor had a lid at the top for sampling. Process-air was recirculated by a diaphragm air pump, and heating or cooling the recirculated air controlled the composting temperature. Fresh air was provided by another diaphragm air pump. The temperature of the compost was measured using a Pt_{100} temperature probe placed in the middle of the reactor. A PC with process-control and data acquisition software (Genesis™ for Windows) controlled the system and logged data with one-minute intervals. For a more detailed description of the composting system see Møller and Reeh (2003).

Experimental set-up and use of semi-permeable chambers

Faecal material of an age of up to two months was collected from a non-flushing toilet with urine diversion situated in a Danish eco-village. Although the users had added toilet paper and some sawdust, the material was wet suggesting that urine diversion was not optimal. Additional sawdust was, therefore, added before the composting process was initiated. Approx. 3.5 kg of the faecal mixture was put into each of the composting reactors. The reactors were aerated (150 ml min^{-1}) to initiate the composting process, and the material was allowed to self-heat to approx. 40°C before the temperature was regulated ±2°C to 50, 55, 60

and 65°C, respectively, in the different batch experiments. When the desired temperature was reached in the compost reactors, a number of semi-permeable chambers (Excelsior Sentinel, Inc., NY, USA) were placed into each reactor at a level of 10 cm from the top close to temperature probes. It should be noted, that due to the small time intervals between sampling at 65°C and the subsequent opening and closing of the reactors, the process temperature was difficult to maintain at 65°C during the entire experimental period.

The chambers were cylindrical with a volume of about 2.5 ml and contained in both ends membranes with a pore size 0.45 μm, which allow passage of gasses and ions but not bacteria. For preparation of the chambers, faecal material was homogenised by hand and inoculated with *Salmonella* serotype Typhimurium phage 28B to a final concentration of approximately $8x10^8$ plaque forming units (PFU) g^{-1}. A test analysis was done to determine if the phages were evenly distributed in the material. The chambers were filled up with approx. one g of the inoculated faecal material. A volume of 100 μl of *Salmonella* serotype Senftenberg 775W culture was added to each chamber to obtain a final concentration of approximately $3x10^8$ colony forming units (CFU) g^{-1} just after which the chambers were sealed.

Two chambers were taken out from each reactor at defined time intervals and stored cool at 4-5°C prior to analysis. Most samples were analysed the same day and all samples were analysed within 24 hours. The faecal matter in the chambers was analysed quantitatively for indigenous thermotolerant coliforms and *Enterococcus* spp. and the added test strains of phage 28B and *S*. Senftenberg 775W as described below

Bacteriological and phage analyses

Preparation of test organisms. For each experiment, *S*. Senftenberg 775W was grown in Luria broth (Difco, Maryland, USA) for 10 h at 37°C with constant shaking to obtain a concentration of approximately $3x10^9$ CFU ml^{-1} prior to use.

Phage 28B was propagated in Nutrient broth (Oxoid, Hampshire, UK) against its host strain, *Salmonella* serotype Typhimurium type five, to a concentration of approx. 10^{10} PFU ml^{-1}. After the bacterial host was killed by addition of chloroform (10 ml l^{-1}), the solution was centrifuged for 30 min at 4300 × *g* and filtered through 0.45 μm membrane filters to remove cell debris. Initial analyses of the faecal material showed that there was no indigenous *Salmonella*, or phages present that lyzed the host strain. The phage 28B did not lyze *S*. Senftenberg 775W.

Enumeration of bacteriological and phage parameters. The faecal material was taken out sterile from the chambers and weighed. Peptone water (1%, Difco) was added to obtain a concentration of 1:10. The mixture was homogenized in a stomacher for 30 s, and subsequently serially diluted before enumeration of *S*. Senftenberg 775W, thermotolerant coliforms, *Enterococcus* spp. and phage 28B.

A semi-quantitative analyse for *S*. Senftenberg 775W was done by transferring one ml from respective sample dilutions to nine ml buffered peptone water (Oxoid) and incubated at 37°C for 16 h. From this resuscitation step, 0.1 ml was transferred to nine ml tubes of Rappaport-Vassiliadis soy peptone broth (Oxoid) and incubated at 42.0°C for 24 h. A sterile loop was used to streak enriched samples onto Brilliant green Lactose Saccharose Phenol red agar (Oxoid) and incubated at 37°C for 24 h. Representative suspected colonies were confirmed as *Salmonella* by agglutination with polyvalent Salmonella-O-antiserum (DS 266 1988). The detection limit for *S*. Senftenberg 775W was 10 bacteria g^{-1}.

For analysis of thermotolerant coliforms and *Enterococcus* spp., 0.1 ml from the sample dilutions was spread onto agar plates. When the expected amount of bacteria was less than 10^2 CFU g^{-1}, 1 ml from the –1 dilution was spread onto two plates to increase the detection limit to 10 CFU g^{-1}. Thermotolerant coliforms was counted as typical yellow colonies on membrane lauryl sulphate agar (Difco) after incubation at 44°C for 21±3 h (ISO/DIS 9308-1 mod. 1998). Numbers of *Enterococcus* spp. was determined as typical red-red brown colonies on Slanetz & Bartley agar (Difco) following incubation at 44°C for 48 ± 4 h (DS 2401 1999). It was observed for some samples that pin-point sized red-red brown colonies appeared among the normal two to three mm sized colonies. It was assumed that the pin-point sized colonies may represent stressed bacterial cells showing impared growth, and agar plates with such colonies were therefore incubated for another two days at 44°C to see if the pin point colonies would grow into typical sized *Enterococcus* spp. colonies. After incubation of totally four days the colonies were therefore counted again. Often the numbers of colonies with typical shape and size of enterococci had significantly increased from day two to day four. To the result presentation, both the colonies counted after two days and the new colonies counted after four days were included.

Phage 28B was enumerated by a double-agar layer method (Adams, 1959). The host strain *S*. Typhimurium type five was grown in Nutrient broth at 37°C for four h. From the 10-fold diluted samples, one ml was taken and mixed with one ml broth culture of the host strain and three ml of soft agar (a mixture of 70% Blood agar base (Oxoid) and 30% Nutrient broth). The mixture was spread on a well-dried Blood agar base plate which was incubated at 37°C for 18 h. Clear zones (plaques) were counted as PFU. When a high bacterial background flora was expected (mainly at the lower dilutions), the samples were filtered through 0.45 μm pore size filters before mixed with the soft agar. The detection limit for phage 28B was 10^2 PFU g^{-1}.

For all experiments, enumeration of the target microorganisms was done until two subsequent samples were found not to contain the target microorganism.

Control samples. Approximately 20 g of the original faecal material with phage 28B inoculated was put into 100 ml sterile bottles after which *S*. Senftenberg 775W was added and mixed with the material to a final concentration of $3x10^8$ CFU g^{-1}. The bottles were incubated at five and 20°C during the trial, as control samples to check for either die-off or growth of thermotolerant coliforms, *Enterococcus* spp. and *S*. Senftenberg 775W or die-off of phage 28B.

RESULTS

Reduction in numbers of bacteria and phage

The reduction in numbers of bacteria studied and the phage is shown in Fig. 1 and 2. In general, numbers of *S*. Senftenberg 775W and thermotolerant coliforms were reduced to <10 CFU g^{-1} (detection limit) within six h for all temperatures studied. In one of two parallel samples exposed at 50°C, the number of *S*. Senftenberg 775W was reduced below the detection limit of 10 CFU g^{-1} within six h, while both parallel samples were found negative for *S*. Senftenberg 775W after 22 h (Fig. 1A). At 50 and 55°C, the thermotolerant coliforms were reduced to below the detection limit within six and four h respectively (Fig. 1B). At 60°C, numbers of *S*. Senftenberg 775W and thermotolerant coliforms were reduced to below the detection limit within two h, and at 65°C within one h.

Enterococcus spp. had at all temperatures a rapid initial decline after which only minimal reductions in numbers occurred (Fig. 2A). Numbers of *Enterococcus* spp. were not reduced

below the detection limit during the duration of the experiment (238, 143, 66 and 43 h at 50, 55, 60 and 65°C, respectively). The reduction of phage 28B at 50 and 55°C showed that PFU could not be seen after five and three days, respectively (Fig. 2B). At 60 and 65°C, numbers of phage 28B were reduced below detection limit within less than two days.

The same material as added in the chambers were used as control material and incubated in glass bottles at five and 20°C. Reduction or growth of target organisms were seen neither at five nor at 20°C incubation for *S*. Senftenberg 775W, thermotolerant coliforms or *Enterococcus* spp. over the investigation period (five days for *S*. Senftenberg 775W, 11 days for the indicator bacteria). No reduction was seen for phage 28B (investigation period 11 days).

Regression analysis and T90-values

Linear regression analysis was done on mean values from duplicate experiments of log-transformed data of numbers of surviving microorganisms as a function of exposure time at the different temperatures investigated. Based on the regression analysis, T90-values (the time required to reduce the population by 90%) were calculated (Tables 1 and 2). The ability to survive thermophile conditions was - in decreasing order - phage 28B > *Enterococcus* spp. > thermotolerant coliforms > *S*. Senftenberg 775W, except at 50°C where *Enterococcus* spp. survived better than phage 28B. *S*. Senftenberg 775W and thermotolerant coliforms had very similar degradation kinetics. The regression analysis of the die-off of *Enterococcus* spp. was divided into two phases, as there was a rapid initial decline, followed by a slower reduction in numbers during the second phase. At 50 and 55°C, the reduction during the first phase was only 3.2 $\log_{10}$ CFU g^{-1}. At the shift from phase one to phase two, the numbers of *Enterococcus* spp. were nearly the same at all temperatures, with a mean value of 3.3 $\log_{10}$ CFU g^{-1}

(CV=0.64%, n=4). The regression analysis of reduction of phage 28B PFU was divided into two phases for 50 and 55°C, as there was a very slow initial reduction at these temperatures, followed by a faster reduction. At 60 and 65°C, a one phase regression analysis was performed.

The T90-values for each organism (Table 1 and 2) as a function of temperature are shown in Fig 3. For *Enterococcus* spp. the values from the first rapid die-off phase was used instead of the higher T90-values found during the second degradation phase. Based on Fig. 3, T90-values (or 4-$\log_{10}$ reduction times equalling four times the T90-value) for other temperature regimes not tested in our experiments can be calculated. The slope of the curve indicates that phage 28B was by far the most thermotolerant of the organisms tested. Extrapolating the curve to 70°C, a 4-$\log_{10}$-reduction time of approx. 10 h for phage 28B can be calculated.

DISCUSSION

The objective for this study was to evaluate the sanitizing effect of thermophilic composting of faecal material from urine-diverting toilets. It was found that at temperatures between 50 and 65°C, the studied microorganisms were reduced to below detection limit after two to five days, with exception of *Enterococcus* spp. At 50 and 55°C, the tested organisms could be divided into two groups according to their survival, where *S*. Senftenberg 775W and thermotolerant coliforms had T90-values about one hour, while *Enterococcus* and phage 28B were reduced at least 10 times slower. At 60 and 65°C, phage 28B were still more slowly reduced, while *Enterococcus* spp., *S*. Senftenberg 775W and thermotolerant coliforms had a more similar survival. Bendixen *et al*. (1995) suggested the group of faecal streptococci (enterococci) as a suitable indicator at temperatures below 60°C, also for survival of viruses

and parasites. His results are in good agreement with the results of our investigation. Above 60°C, enterococci are more heat sensitive than i.e. thermoresistant viruses (Bendixen *et al*. 1995). Therefore, other more heat-resistant organisms has to be used when the composting temperature reaches 60°C or above. In our investigation, phage 28B was shown to be a promising indicator organism for composting at higher temperatures, due to its high thermoresistance. This was also shown by E. Emmoth, A. Holmqvist and L. Sahlström (personal communication), who found phage 28B even more thermoresistant than porcine parvovirus. Among human enteric viruses, the most heat-resistant appears to be the hepatitis A virus (HAV). In a study of Murphy *et al*. (1993), HAV was inactivated by 3.6 $\log_{10}$-units during heat-treatment at 60°C for six hours in comparison with phage 28B that needed 21 h at 60°C for a 4-$\log_{10}$-inactivation in the present study. As phage 28B also is resistant to high pH and high ammonia rates (Vinnerås *et al*. 2003), it makes it even more suitable as an indicator for the sanitizing effect of composting, as the composting process often raises the pH and ammonia is released. Other bacteriophages has been tested as indicator organisms for enteric viruses and other pathogenic microorganisms, but none of them was found to show heat-resistance that matched the most heat-resistant human enteric viruses, like HAV (Burge *et al*. 1981; Mariam and Cliver 2000).

As the different test organisms behaved in different ways in our investigation, it also showed the value of using more than one test organism to evaluate a treatment system. As shown in our investigation, phage 28B had an initial prolonged survival at 50 and 55°C, followed by a faster die-off to a level under detection limit. In contrast, *Enterococcus* spp. had a more rapid initial die-off, followed by a slower degradation phase that still ended above the detection limit. Using only phage 28B as an indicator organism would not have shown the tail of survivors among the enterococci. *S*. Senftenberg 775W and thermotolerant coliforms showed a too rapid die-off to indicate survival of more thermoresistant organisms. But the

results show that thermotolerant coliforms could be a good indicator for *Salmonella*. Parasites like helminths or protozoan were not included in this study. However, other investigations have shown that they are less resistance to heat (Van Praagh *et al*. 1993; Plym-Forshell 1995; Whitmore and Robertson 1995) than phage 28B, used in this study. Like bacteriophages, parasites do not have the ability to re-grow outside their host. Altogether, phage 28B seems to be a useful and conservative indicator for survival of helminths and protozoans, like *Ascaris*, *Giardia* and *Cryptosporidium*.

Several authors have suggested that a 4-$\log_{10}$-reduction of pathogenic microorganisms or indicator organisms should be achieved before a treated waste product is hygienically safe (Bendixen *et al*. 1995; Carrington 2001). An extrapolation made from the T90-calculations for phage 28B (Fig. 3) predict that at 70°C more than 10 h treatment time will be needed to achieve a 4-$\log_{10}$-reduction. This means that, according to Danish legislation for composting sewage sludge (Danish EPA 1996, Danish EPA 1998), a sanitation period of one h at 70°C would not be enough to reduce thermo-resistant viruses to appropriate levels. In contrast, a treatment period of less than five days at 55°C gave a reduction of phage 28B PFU with more than six $\log_{10}$ PFU g^{-1} (Fig. 2B) and numbers of *Enterococcus* spp. with four $\log_{10}$ CFU g^{-1} (Fig 2A). This indicates that composting at 55°C under controlled conditions at five days could be preferable to achieve sanitised compost compared to sanitation at 70°C for one h, even when considering very thermoresistant viruses. There are some other advantages with composting at a lower temperature. S. Smårs showed that the degradation of the organic material when composting municipal household waste is more effective at 55°C than at 67°C (personal communication). This suggests that if the hygienic demands could be satisfied at 55°C, this should be both an economical and practical advantage for compost facilities. Also the risk for repopulation/re-growth of pathogenic bacteria is considered to be lower when composting at 55°C rather that at 70°C. Millner *et al*. (1987) showed that no suppression of

Salmonella occured in compost taken from 70°C compost-pile zones despite the presence and growth of many types of microorganisms. With higher numbers and types of microorganisms in compost kept at 55°C, growth of *Salmonella* was suppressed 100-10.000-fold. Also other authors have noticed the important role of the indigenous microflora and its diversity in suppressing regrowth of unwanted bacteria (i.e. Hussong *et al*. 1985; Soares *et al*. 1995). Altogether, a compost process at 55°C rather than 70°C, seems to give a safer compost end product.

It is important to be aware of the potential big differences between a controlled, aerated and isolated in-vessel system and an open windrow or static pile system. One disadvantage for open treatment systems is the possibility of re-growth of indicator and pathogenic bacteria in the cooler outer layer between the turnings. Therefore the time for adequate reduction of these bacteria may be several times longer than in reactor composting, although the inner layer may reach temperatures of 55 to 70°C in the open treatment systems. In the investigation of Shuval *et al*. (1991), where sewage sludge was composted in windrow piles, 83 days was needed to eliminate *Salmonella*. Faecal streptococci were reduced four $\log_{10}$ after 100 days, although the mean temperature was above 55°C for this period. As shown in our investigation, we found that composting of faecal material in a controlled environment needs a much more reduced treatment time to become hygienizated. Though our experiments were performed in laboratory reactors, we think the results can be used as a guideline also for large scale composting, if an isolated and aerated reactor is used, and where thermophilic temperatures are achieved in the entire material. Hence, the temperature has to be monitored frequently and at different positions in the material.

Today's sewage system produces large amounts of sludge, rich of valuable plant nutrients. In a sustainable society these nutrients have to be recycled. But contaminated with several toxic compounds like metals and organic substances, the sludge is not suitable as fertilizer in

a long-term perspective. The main part of the plant nutrients in the sewage is originating from human excreta (Vinnerås and Jönsson 2002). A source-separating toilet system is needed to keep nutrient rich fractions apart from the polluting fractions. With an adequate treatment of the faecal fraction, like thermophilic composting at 55°C for at least five days (enclosed aerated and isolated systems), a nutrient rich and hygienically safe product may be obtained. The finished compost could then be used for applications like organic farming or other agricultural practices while minimizing microbial food safety hazards caused by contamination.

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Table 1

Test organism and temperature	T90-values (hours)
Salmonella serotype Senftenberg 775W	
50°C	0.99
55°C	0.82
60°C	0.26
65°C	0.12
Thermotolerant coliforms	
50°C	1.2
55°C	0.75
60°C	0.36
65°C	0.16

Table 2

Test organism and temperature	Phase 1			Phase 2
	Reduction ($\log_{10}$ CFU or PFU g^{-1})	Length (h)	T90-values (h)	T90-values (h)
Enterococcus spp.				
50°C	3.2	60	19	360
55°C	3.2	23	7.0	120
60°C	4.7	3.1	0.65	74
65°C	4.5	1.9	0.42	85
Phage 28B				
50°C	0.8	38	-*	12
55°C	0.5	17	-*	8.5
60°C†	-	-	-	5.3
65°C†	-	-	-	3.8

* One $\log_{10}$ reduction was not achieved during the first phase.
† For phage 28B at 60 and 65°C there was no initial phase with slower reduction.

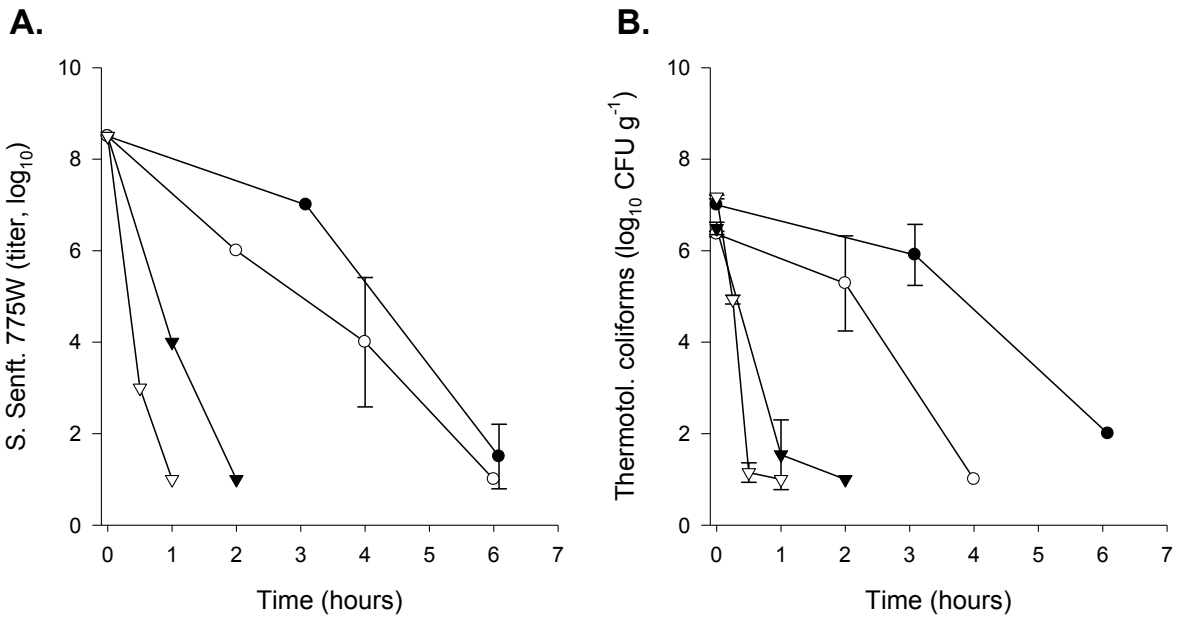
A.
B.
S. Senft. 775W (titer, log10)
Thermotol. coliforms (log10 CFU g-1)
Time (hours)
Time (hours)
0
1
2
3
4
5
6
7
8
10

Fig. 1

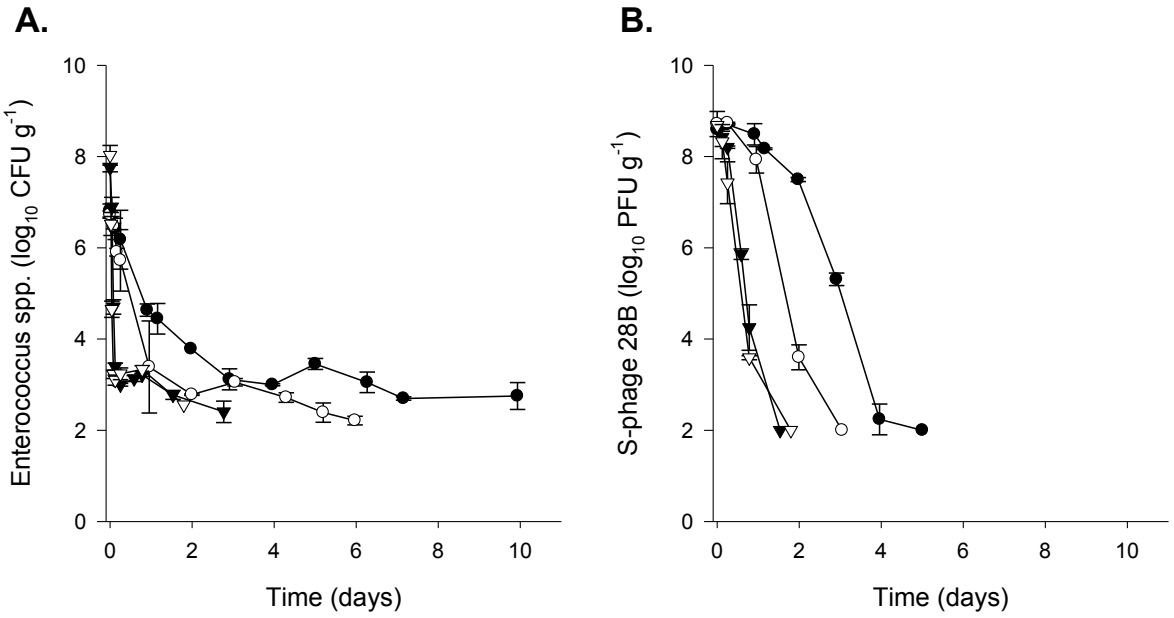
A.
B.
Enterococcus spp. (log10 CFU g-1)
S-phage 28B (log10 PFU g-1)
Time (days)
Time (days)
0
2
4
6
8
10

Fig. 2

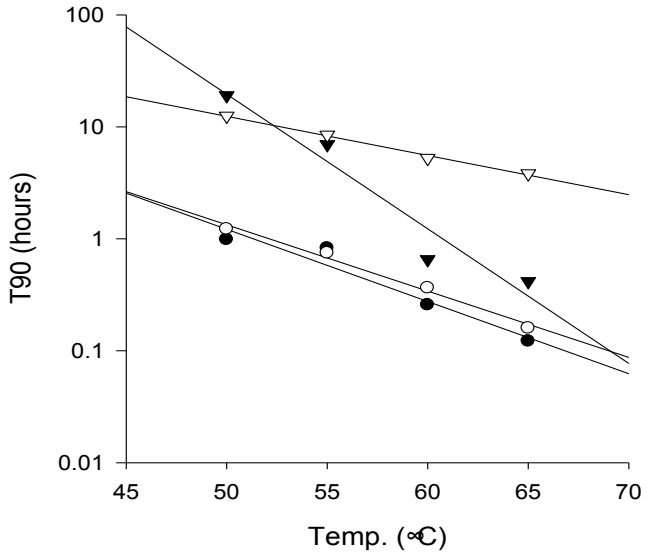

Fig. 3

Table 1 Calculated T90-values for *Salmonella* serotype Senftenberg 775W and thermotolerant coliforms when composting faecal material at different temperatures

Table 2 Calculated T90-values for *Enterococcus* spp. and *Salmonella* serotype Typhimurium phage 28B when composting faecal material at different temperatures. For *Enterococcus* spp. at all temperatures and phage 28B at 50 and 55°C, a two phase regression analysis were made

Fig. 1 Reduction of *Salmonella* serotype Senftenberg 775W (A) and thermotolerant coliforms (B) over time when composting faecal material at 50 (●), 55 (○), 60 (▼) and (▽) 65°C. Analysis of *S*. Senftenberg 775W was performed semi-quantitatively, and is expressed as log titer values. Detection limit was 10 bacteria g^{-1} for *S*. Senftenberg 775W. Detection limit for thermotolerant coliforms was 10 CFU g^{-1} except for 50°C, where it was 10^2 CFU g^{-1}. Each point represents the average of duplicate trials, and the bars at each point indicate the standard deviation of results obtained at each sampling time

Fig. 2 Reduction of *Enterococcus* spp. (A) and *Salmonella* serotype Typhimurium phage 28B (B) over time when composting faecal material at 50 (●), 55 (○), 60 (▼) and (▽) 65°C. Detection limit was 10 CFU g^{-1} for *Enterococcus* spp. and 10^2 PFU g^{-1} for phage 28B. Each point represents the average of duplicate trials, and the bars at each point indicate the standard deviation of results obtained at each sampling time

Fig. 3 Log_{10}-transformed T90-values as function of composting temperature for *Salmonella* serotype Senftenberg 775W (●), thermotolerant coliforms (○), *Enterococcus* spp. (▼) and *Salmonella* serotype Typhimurium phage 28B (▽). The curves were fitted with linear regression