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ORIGINAL ARTICLE

Growth potential of faecal bacteria in simulated psychrophilic/mesophilic zones during composting of organic waste

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Abstract

Aim: This study investigated the growth potential of *Salmonella* serotype Typhimurium and faecal indicator organisms in compost materials and the correlation between bacterial growth potential and the physico-chemical composition of the compost substrate and temperature.
Methods and Results: Survival of *Salm.* Typhimurium, *Enterococcus* spp. and total coliforms at 14, 24 and 37°C was determined in material of different degrees of maturity collected from composting plants for household waste and manure. All three micro-organisms showed the potential for growth in the material from active composts (Solvita index 4) but inactivation generally occurred over time in mature compost material (Solvita index 7–8).
Conclusions: *Salm.* Typhimurium had the potential for growth in psychrophilic/mesophilic (P/M) zones of immature compost material and its growth potential correlated negatively with the maturity of the compost and the temperature within the simulated P/M zone.
Significance and Impact of the Study: The risk of pathogen regrowth in P/M zones during organic waste composting further emphasizes the importance of good management practices and of avoiding P/M zones in combination with low compost maturity.

Introduction

To improve the sustainability of the agricultural sector, organic waste products must be used as a soil quality conditioner, because they can serve as a valuable plant nutrient resource. Furthermore, the amount of organic waste disposed in landfills can be decreased. However, recycling of organic waste, e.g. waste from households, food industries, slaughterhouses, manure and sewage sludge, might pose a potential health risk for both human and animals because of pathogenic micro-organisms that may be present in the materials. A wide range of pathogens may be present in organic waste, some of them with zoonotic potential such as *Cryptosporidium parvum*, *Salmonella* spp., *Campylobacter* and *Escherichia coli* O157 (Feachem *et al.* 1983; Sahlström *et al.* 2004; Gerba and Smith 2005; Sidhu and Toze 2009). New transmission routes of pathogens between rural and urban areas can be created through the use of organic waste products such as compost in both rural and urban environments. Transmission of pathogens might occur through aerosols, run-off from arable land to adjacent watercourse, contamination of groundwater, contamination of food or feed as well as because of vector animals such as birds and rodents. Thus, the recycling of organic waste might pose a potential health risk unless properly managed. Sanitation of organic waste before application to soil is recommended because the pathogens may survive for extended periods in the soil environment (Nicholson *et al.* 2005).

A common treatment option for handling of organic waste is composting during which micro-organisms stabilize the material. The temperatures that may be reached during the composting process are lethal to many human and animal pathogens (Feachem *et al.* 1983; Epstein 1997). However, unless a well-insulated vessel is used, and the incoming air is preheated, there will always be a risk for psychrophilic/mesophilic (P/M) zones in the material, because of e.g. pore aeration or surface heat loss (Vinnerås 2007). The inactivation of micro-organisms cannot be guaranteed in these P/M zones, and in some cases survival has been observe associated to the condense water at the top of the reactors (Vinnerås 2007; Niwagaba *et al.* 2009). Therefore, the compost should be turned or mixed so that all of the material reaches a high temperature (>50°C) to minimize the risk for a poorly sanitized end-product (Haug 1993). *Salmonella* spp. has been shown to survive the composting process in low numbers and thereafter, under favourable conditions, repopulate soil amendments and organic wastes during storage (Skanavis and Yanko 1994; Gibbs *et al.* 1997; Ceustermans *et al.* 2007). It is possible for bacterial pathogens such as *Salmonella* spp. and *E. coli* O157 to increase in numbers during the composting process at temperatures between 15 and 45°C. Models to assure a sanitized end-product based on temperature in the process have been developed (Haug 1993; Vinnerås *et al.* 2003). However, these models do not take into account possible pathogen regrowth in P/M zones during the composting process, but rather assumed no change in number of organisms in the P/M zones. To model pathogenic bacterial die-off during composting, the growth/inactivation of these pathogens in the P/M zone needs to be determined.

The objective of the present study was to investigate the growth potential of *Salmonella* spp. and faecal indicator organisms in compost materials, with the focus on the influence of the physico-chemical composition (maturity, pH, moisture content, volatile solids) of the compost substrate and the temperature of the P/M zone.

Materials and methods

Compost material

Compost samples for the study were collected from two large-scale municipal plants (A, B) for source separated household waste composting mixed with garden waste and straw or woodchip, with three samples from each plant (A1-3, B1-3), plus six samples from one plant for composting of beef cattle manure mixed with peat (C1-6). All three samples from plants, A and B, were collected on single occasions, while the six samples from plant C were collected on two occasions. Because of practical reasons, three samples were collected at each occasion, one in the end of September and one in the end of November. Collection of samples from plant A occurred in the middle of April and from plant B in the middle of May. Before the start of laboratory trials, samples were kept at 4°C for a maximum of 13 days. The three composting plants A–C differed in size, type of composting system and compost material. In addition, one sample of fresh manure (D) was collected from dairy cattle housed indoors. Thus in total, 13 samples were included in the study.

The samples were analysed for pH using an Inolab pH metre (WTW, Germany), and moisture and volatile solids content were determined gravimetrically. All samples were then calibrated to a moisture content of 40–50%, and the degree of maturity was determined by measuring the self-heating capacity of the material based on the Rottegrad scale, which ranges from I (immature) to V (mature) [FCQAO (Federal Compost Quality Assurance Organization) 1994], and by using the Solvita® compost maturity kit (Earthcare Ltd, UK) to measure CO_2 evolution and NH_3 emissions based on a scale ranging from one (immature) to eight (mature). Solvita® test was performed in accordance with the Solvita® test kit manual also described by Changa *et al.* (2003). In addition, the materials were analysed for the presence of naturally occurring *Salmonella* spp., *Enterococcus* spp. and total coliforms. The enumeration of bacteria was performed as described under bacterial enumeration.

Indicator organisms

The bacterial strains used were *Salmonella* serotype Typhimurium, phage type 178, isolated from sewage sludge in Sweden by Sahlström *et al.* (2004), *Enterococcus faecalis* (ATCC 29212) and *Escherichia coli* (ATCC 35218). Bacteria were enriched in overnight cultures in nutrient broth (Oxoid, Malmö, Sweden) (37°C, 18–24 h) in separate flasks. After enrichment, the organisms were mixed and diluted to a concentration of *c.* 10^7–10^8 CFU ml^{-1} in phosphate buffer (M15 pH 7·2, Oxoid) before addition to the compost material. Organisms were added to a final concentration of 10^6–10^7 CFU g^{-1} material.

Experimental design

Each of the 13 different compost and manure samples was inoculated with micro-organisms and thoroughly mixed manually. Each sample was then split into subsamples and incubated in closed containers at three different temperatures (14, 24 and 37°C) simulating different P/M zones in the compost. A total of 15 subsamples from each sample were incubated at each temperature and analysed

in triplicate at the start of incubation (day 0) and thereafter on days 1, 2, 6 and 8.

Bacterial enumeration

The compost material was serially diluted in saline solution (0·86–0·90% NaCl) (Oxoid) to appropriate dilutions before plating of the samples. *Salmonella* Typhimurium was isolated on xylose-lysin-deoxycholate (XLD) agar (Oxoid) containing 0·15% sodium-novobiocin. In brief, 0·1 ml sample was surface spread on XLD agar, and the agar plates were incubated at 37°C for 24 ± 2 h, colonies exhibiting a typical growth with black centres on XLD were registered as *Salm.* Typhimurium. *Enterococcus* spp. was cultured on Slanetz–Bartley (SlaBa) agar (Oxoid), where 0·1 ml was surface spread, and colonies were counted after incubation at 44°C for 48 ± 4 h, without differentiating the added *Ent. faecalis* from *Enterococcus* spp. Total coliform bacteria were analysed by first mixing 1 ml of the diluted compost samples in a layer with violet-red bile (VRB) agar (Oxoid). After solidification, a second layer of VRB was added and the plates incubated at 37°C for 24 ± 2 h. The total coliform bacteria were analysed with a detection limit of 10 CFU g^{-1} compost and *Enterococcus* spp. and *Salm.* Typhimurium with a detection limit of 100 CFU g^{-1} compost.

Statistical analysis

Student's *t*-test was performed to compare the bacterial count on day 0 with the bacterial count after 8 days. Possible correlations between the $\log_{10}$ changes (growth/inactivation) in *Salm.* Typhimurium numbers after 8 days and $\log_{10}$ changes in indicator numbers, moisture content, volatile solids and pH were analysed using Pearson product moment correlation. Correlation between changes in *Salm.* Typhimurium and temperature, Solvita index and Rottegrad index were also analysed using Spearman rank correlation. Statistical analyses were performed in SigmaStat 3.0 (SPSS Inc., Chicago, IL, USA).

Results

The composition of the compost samples regarding pH, moisture content, volatile solids, age and maturity of samples is presented in Table 1. The initial pH in the samples of household waste compost (A1-3 and B1-3) varied from 5·3 to 8·5, while that in samples of cattle manure compost (C1-6) and fresh dairy cattle manure (D1) varied from 5·6 to 8·1. Moisture content of household waste compost varied between 34 and 62% and volatile solids between 58 and 84%, in cattle manure compost and fresh manure the moisture content varied between 79 and 85% and volatile solids between 81 and 94% of the dry matter. The maturity of household waste compost varied between Rottegrad index II-IV and Solvita index 3–8. All samples collected from the manure compost plant (C1-6) had a Rottegrad index above IV and Solvita index above 5, while the fresh manure had a Solvita index of 4 (Rottegrad index not measured because of excessive moisture content of the sample).

Inactivation and growth of *Salm.* Typhimurium in simulated P/M zones

The average change in bacterial count for *Salm.* Typhimurium in household waste compost (samples A1-3, B1-3) at incubation temperatures 14, 24 and 37°C is shown in Fig. 1. During the study, growth of *Salm.* Typhimurium was observed in sample A1 when incubated at 14 and

Table 1 Comparison of compost material (pH, moisture content, volatile solids, age and maturity) collected from systems for household waste compost (A1-3 and B1-3), beef cattle manure compost (C1-6) and fresh dairy cattle manure (D1)

Sample no.	pH	Moisture content (%)	Volatile solids (%)	Age (weeks)	Maturity	
					Rottegrad	Solvita®
A1	5·3	54	82	<1	III	4
A2	5·5	55	84	1–1·5	IV	4
A3	8·5	34	61	6–9	III	5
B1	5·2	56	58	2	IV	6
B2	5·6	62	80	8	IV	8
B3	7·7	34	75	15	II	3
C1	8·1	81	92	*	IV	5
C2	5·7	85	87	*	V	7
C3	7·1	83	82	*	V	7
C4	7·0	79	88	*	V	8
C5	7·2	81	86	*	V	8
C6	5·6	81	81	*	V	8
D1	7·9	84	94	0	–	4

*age of sample unknown.

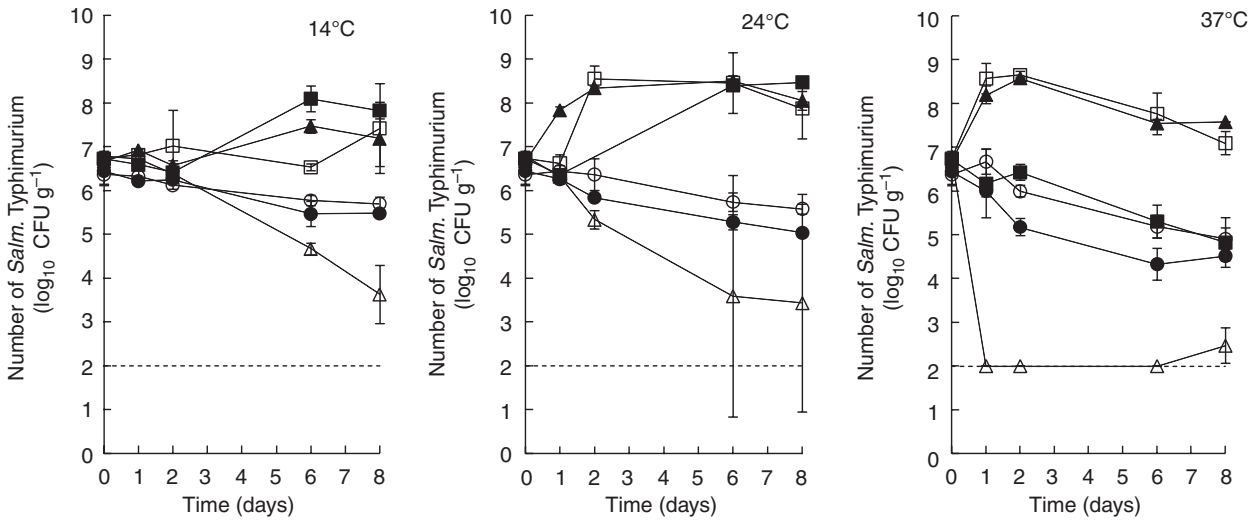

Figure 1 Growth/inactivation of *Salmonella* Typhimurium (CFU g^{-1} wet weight) in composted household waste samples A1 (■), A2 (▲), A3 (●), B1 (□), B2 (Δ) and B3 (○) incubated at 14, 24 and 37°C. Results of CFU counts shown as mean ± SD (n = 3). Detection limit for the analysis marked with a broken line.

24°C and in samples A2 and B1 at all three incubation temperatures. At 24°C growth of *Salm.* Typhimurium occurred in samples A1, A2 and B1 between day 2 and 6, and thereafter the bacterial count remained constant throughout the incubation period. The growth of *Salm.* Typhimurium in samples A2 and B1 at 37°C peaked during the first couple of days of incubation and after that a reduction in bacterial count occurred, but not to below the initial bacterial count in the samples. In samples collected from manure compost (C1-6), a reduction in the bacterial count in comparison with the initial count occurred in all samples and temperatures (Fig. 2). In fresh manure (D1), growth of *Salm.* Typhimurium occurred during the first two days of incubation at 14°C and during the first day at 24 and 37°C. After this initial increase in bacterial count, a reduction occurred at 37°C, while the bacterial count at 14 and 24°C remained constant throughout the incubation period (Fig. 2).

Correlation between bacterial count and sample parameters

Student's *t*-test comparing bacterial count at the start of the study with the bacterial count after 8 days of incubation showed significant changes in bacterial count of *Salm.* Typhimurium, *Enterococcus* spp. and total coliforms in several samples from both household waste compost and manure compost and in fresh manure (Table 2).

Results from statistical analysis of the correlation between organisms and the parameters temperature, Solvita index, Rottegrad index, pH, moisture content and volatile solids can be seen in Table 3. A significant positive correlation was found between bacterial counts of *Salm.* Typhimurium and those of total coliforms and *Enterococcus* spp. Furthermore, changes in *Salm.* Typhimurium and total coliform numbers showed a significant negative correlation to the Solvita® and incubation temperature of the samples, while *Enterococcus* spp. numbers showed a significant negative correlation only to the incubation temperature (Table 3).

Discussion

The results show that during the composting process there is a possibility that, instead of inactivation, growth of pathogens can occur in P/M zones of the compost. Significant growth of *Salm.* Typhimurium occurred in household waste composts at all three incubation temperatures tested (14, 24 and 37°C) and in fresh manure at 24°C (Table 2). However, no significant growth of *Salm.* Typhimurium could be seen in manure compost, where the samples generally had a higher degree of maturity (Table 1). The *Salm.* Typhimurium inoculated into the household waste compost rapidly increased to more than 8 $\log_{10}$ CFU g^{-1} after 2 days in several of the samples, which correspond to an increase in bacterial count of more than 1·5 $\log_{10}$ CFU g^{-1} compost.

The potential growth of pathogens in the compost materials ought to influence the way these materials are to be handled. Account must be taken of the potential growth of pathogens when calculating the amount of turnings of compost material needed during the composting process if P/M zones are present within the organic matter. Depending on the amount of the material

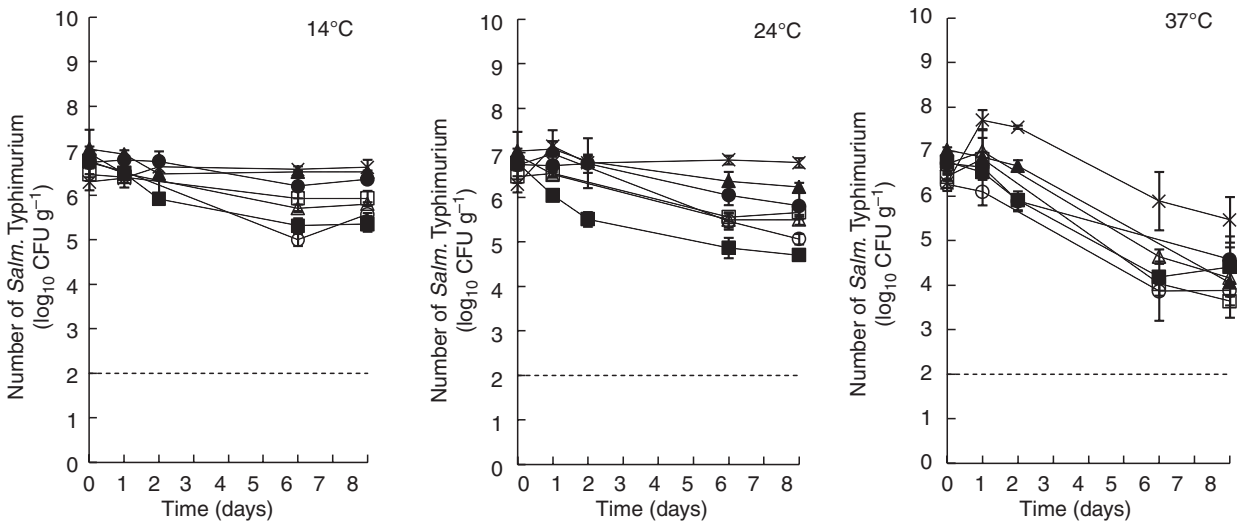

Figure 2 Growth/inactivation of *Salmonella* Typhimurium (CFU g^{-1} wet weight) in composted beef cattle manure samples C1 (■), C2 (▲), C3 (●), C4 (□), C5 (Δ) and C6 (○) and fresh dairy cattle manure D (×) incubated at 14, 24 and 37°C. Results of CFU counts shown as mean ± SD (n = 3). Detection limit for the analysis marked with a broken line.

Table 2 Growth (bold) and inactivation of bacteria when comparing the bacterial count at start to the numbers after 8 days of incubation expressed as log_{10} CFU g^{-1}. See the legend of Table 1 for description of samples A, B, C and D

	Salmonella Typhimurium			*Enterococcus* spp.			Total coliforms		
Sample no.	14°C	24°C	37°C	14°C	24°C	37°C	14°C	24°C	37°C
A1	**1·1****	**1·8*****	−1·9**	**2·3*****	**1·2***	−1·6*	**1·7*****	**2·2*****	−1·1*
A2	**0·5**	**1·4****	**0·9****	**1·8*****	**1·4****	−0·4	**1·7*****	**1·7*****	**0·6**
A3	−1·0*	−1·4**	−1·9***	−0·2	−1·6	−3·0***	−0·8**	−1·0***	−1·8***
B1	**0·7**	**1·1***	**0·4**	**1·7****	**1·9***	−0·3	**0·3**	**1·0**	−0·6
B2	−3·2**	−3·4	−4·3***	−2·2	−2·0	−3·0**	−2·6***	−4·3	−4·1*
B3	−0·7*	−0·8**	−1·5	**0·4**	**0·3**	−0·5*	**0·2**	−0·1	−1·3**
C1	−1·4***	−2·1***	−2·4***	**0·4**	−0·3	−1·1	−1·2***	−2·0***	−2·5***
C2	−0·5***	−0·8***	−3·0**	**0·8*****	**0·5****	−1·0	−0·6**	−0·8**	−3·1**
C3	−0·4**	−0·9**	−2·2**	**0·8*****	**0·2**	−0·7	−0·2	−1·0**	−2·1
C4	−0·5*	−0·8**	−2·8***	−0·5	−0·6**	−0·8	−0·5**	−0·9***	−5·5***
C5	−1·1*	−1·4**	−2·6***	−0·6*	−1·0**	−1·3**	−0·8***	−1·5***	−3·2***
C6	−1·2***	−1·7***	−2·4***	−0·4*	−0·6*	−0·5	−1·0***	−1·4**	−2·5**
D1	**0·3**	**0·5***	−0·8	−0·1	−0·6	−2·0*	**0·3**	**0·2**	−0·8

Significant results of student's t-test given as *($P < 0·05$), ** ($P < 0·01$) and *** ($P < 0·001$).

estimated to be within P/M zones, Haug (1993) devised an equation to calculate the number of turnings needed to achieve a certain reduction target based on the assumption that neither growth nor inactivation occurs in P/M zones. However, the present study demonstrated regrowth potential in immature compost material, so this has to be taken into account when modelling pathogen reduction in the composting process. The 1·8 log_{10} growth of *Salm.* Typhimurium in P/M zones that we recorded in immature compost would lead to an increase in salmonella numbers during the first week if the P/M zones represented >1·5% of the total compost, resulting in a lower pathogen reduction than expected in the end-product. This can explain the occurrence of *Salmonella* spp. in composted material despite high temperatures having been measured in central parts of the compost for extended periods of time.

The risk for growth of *Salmonella* spp. in organic waste under favourable conditions has previously been shown to depend on moisture content, temperature and bio-available nutrient content (Russ and Yanko 1981; Hussong *et al.* 1985; Burge *et al.* 1987; Gibbs *et al.* 1997; Sidhu *et al.* 1999) but also on competing microbiota (Hussong *et al.* 1985; Sidhu *et al.* 2001; Pietronave *et al.*

Table 3 Results from correlation analyses of changes in micro-organism numbers and treatment temperature, maturity (measured as Solvita index, Rottegrad index), pH, moisture content (MC) and volatile solids (VS) of manure and household waste compost

Organism	Total coliforms	*Enterococcus* spp.	Temperature	Solvita	Rottegrad	pH	MC	VS
Salmonella Typhimurium								
R	0·81	0·79	−0·40	−0·53	−0·29	−0·27	−0·22	0·15
P-value	**$5{\cdot}1 \times 10^{-10}$**	**$1{\cdot}7 \times 10^{-9}$**	**0·011**	**<0·0001**	0·086	0·10	0·17	0·36
n	39	39	39	39	36	39	39	39
Total coliforms								
R		0·69	−0·48	−0·58	−0·36	−0·013	−0·20	0·03
P-value		**$1{\cdot}4 \times 10^{-6}$**	**0·0023**	**<0·0001**	**0·029**	0·94	0·23	0·86
n		39	39	39	36	39	39	39
Enterococcus spp.								
R			−0·50	−0·27	−0·087	−0·26	−0·12	0·12
P-value			**0·0012**	0·090	0·61	0·11	0·47	0·46
n			39	39	36	39	39	39

R, correlation coefficient; *P*, *P*-value (bold when significant correlation, α = 95%); *n*, number of samples.

2004). For example, Russ and Yanko (1981) showed that for growth potential to exist in organic waste, the moisture content must be 20% or greater. Furthermore, the high content of bio-available nutrients in immature composts has been shown to support growth of pathogens such as *Salmonella* spp. (Hussong *et al.* 1985; Sidhu *et al.* 2001). As the compost mature the concentration of bio-available nutrients decreases (Inbar *et al.* 1990; Sidhu *et al.* 2001). None of the samples tested in the study had a moisture content below 34% and the maturity varied between samples (Table 1) and hence also the content of bio-available nutrients. Within the present study, no significant correlation could be found between the separate parameters pH, moisture content and volatile solids. However, the previously mentioned parameters are reflected as part of the compost maturity that has been shown to significantly influence the bacterial count. Further, the results from this study indicate that except maturity the temperature of the compost is an important factor affecting the growth potential of *Salm.* Typhimurium in organic waste. The correlation between growth or inactivation of *Salm.* Typhimurium in the samples and the maturity of the samples shows that growth of pathogens is less likely in mature compost materials than in raw or immature compost materials. This is supported by other reports by showing that bio-available nutrients present in immature compost supports growth of pathogens (Hussong *et al.* 1985; Inbar *et al.* 1990; Sidhu *et al.* 2001). Although growth of *Salm.* Typhimurium occurred in a few of the more mature compost samples, the effect was transient, with peaks around day 1 and 2 followed by inactivation. Furthermore, despite the low maturity of household waste compost sample B3, no growth of *Salm.* Typhimurium could be seen and instead a reduction in the bacterial count occurred in this sample. There was no obvious reason for the lack of growth in this sample. Even though significant correlations were found between bacterial counts in the samples and the parameters incubation temperature and Solvita index, these correlations were not strong (R^2 values between 0·16 and 0·34). Thus, the results indicate that in addition to the relevance of compost maturity and temperature other factors influence the growth potential of *Salm.* Typhimurium in organic waste.

Bacterial indicator organisms, such as total coliforms and *Enterococcus* spp., are generally monitored to check the quality of the compost end-product. The analysis by Pearson product moment correlation test of the data obtained in the present study showed a significant correlation between *Salm.* Typhimurium and *Enterococcus* spp. or total coliforms. However, when using indicator organisms to predict the inactivation of pathogens, care is needed when interpreting the data and when selecting the indicator organism (Shuval *et al.* 1991; Sidhu *et al.* 1999; Ugwuanyi *et al.* 1999). For example, *Enterococcus* spp. has been shown to survive thermal composting long time after organisms such as *Salmonella* spp. has been inactivated, and thus the use of *Enterococcus* spp. as an indicator has been questioned because of the irregular heat resistance behaviour of the organism (Shuval *et al.* 1991; Craven *et al.* 1997; Vinnerås 2007). The significant correlations between *Enterococcus* spp., total coliforms and *Salm.* Typhimurium in the present study indicated that both *Enterococcus* spp. and total coliforms well represents the growth potential for pathogens such as *Salmonella* spp. in raw material and composted organic waste and thus could be seen as adequate indicator organisms for this purpose. The *Salm.* Typhimurium strain used in the present study is of clinical importance and has been shown to be present in organic waste (Sahlström *et al.* 2004). It has also been used in earlier studies (Ottoson *et al.* 2008; Vinnerås *et al.* 2008). Although the use of

Salmonella to predict the growth and inactivation of other important pathogens such as *Campylobacter* has its limitations. However, under environmental stress *Campylobacter* has been reported to enter a viable but nonculturable (VBNC) state, which currently used methods are not able to detect and hence the data on survival of *Campylobacter* is uncertain (Sidhu and Toze 2009).

To avoid the problems posed by the possible regrowth of pathogens within P/M zones, these should preferably be avoided. In general, it can be said that heat losses are greater in a windrow composting system than in a reactor process. When composting at large-scale the windrow can be high enough to allow heat to be conserved by using the outer layer as insulation (Vinnerås 2007) although parts of the insulation layer might be a P/M zone. Thus, assuming that there is a regrowth of micro-organisms in P/M zones, as has been demonstrated in the present study, the use of an insulation layer of 5% of the total mass of compost material may result in an increase in S*almonella* numbers during the first week of composting. In this case, the amount of turnings or mixings must be sufficient to achieve a sanitized end-product, taking into account the risk of growth in the P/M zones. Measuring the heat distribution in the windrow/reactor makes it possible to calculate the required number of turnings. USEPA (1999) recommends that windrow compost should reach at least 55°C for at least 15 consecutive days. During this period, there should be a minimum of five turnings of the windrow. However, when there are legislative hygienic demands on the quality of the end-product, for example in accordance with European law (Commission regulation (EC) No 208/2006) or national regulations, composting should preferably take place in a reactor, in e.g. countries with Nordic climate where the temperatures during the winter period goes well below 0°C a sufficient insulation of the reactor is preferable. To avoid heat losses in a reactor process good insulation of the reactor and preheating of ventilation air may be required. In addition to minimizing the P/M zones in the compost, the reactor process also reduces the risk of vector-borne contamination, which makes this process preferable for hazardous materials such as animal by-products and sewage sludge.

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