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Original Research

Evaluation of Meat Waste Composting Process Based on Fecal Streptococci Survival

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Abstract

Animal by-products of category 3 after proper processing may be a valuable fertilizer for agricultural purposes. However, they can contain numerous bacterial and viral pathogens and, in cases of improper handling, may pose a health risk for people and animals. This study is aimed at monitoring the number of fecal streptococci introduced into carriers of different types imitating fragments of meat and bone wastes during composting process in a drum bio-reactor. Fecal streptococci are indicator microorganisms, and are known by their thermoresistant characteristics, so it was assumed that their elimination will also diminish the pathogenic microorganisms present in wastes. Three research cycles were carried out in a drum-type bio-reactor, and a different course of temperature was noted in each of them. In cycle 1, in which the temperature exceeded 60ºC, fecal streptococci died the fastest, 139.0-154.4 hours later (depending on carrier type). In cycles 2 and 3, maximum temperatures were similar (57.2ºC and 58.8ºC, respectively), but secondary multiplication of the streptococci in the 102nd hour of the processes was observed. In cycle 2 at this time their number was similar to the level of initial suspension. The type and size of the carriers were of no major importance to streptococci survival in the bio-reactor. Yet in each of the cycles analyzed, effective reduction was accomplished and the product obtained can be considered to be environmentally safe.

Keywords: group D - streptococci, enterococci, composting, animal by-products

Introduction

Animal by-products of category 3 make a valuable source of various elements and are easily biodegradable, which make it possible to use them as a fertilizer [1, 2]. Bacteriological contamination of meat wastes poses a risk for public and animal health, and environmental sustainability. Therefore, processing under controlled conditions is required. Some viral animal pathogens are classical swine fever virus (CSFV), foot-and-mouth disease virus (FMDV), and swine vesicular disease virus (SVDV). There are also pathogens that infect human beings, such as *Campylobacter, Salmonella, Listeria, Yersinia* and verocytotoxygenic *E. coli* species (including O:157) [3-5]. One of the methods which can be allowed for their sanitation is composting, and a major factor responsible for pathogen reduction, is the temperature generated during the process. A temperature range of 60-72ºC has been reported to kill effectively the pathogens [6]. Since it is impossible to test each treated material for each of the pathogenic agents which may occur, alternative strategies are needed to ensure the safety of the finished product. A representative group of microorganisms, called indicator microorganisms, can therefore be used for this purpose. Of many microorganisms applied for validation of organic waste composting processes, *Salmonella senftenberg* W_{775} (H_2S-), *Escherichia coli, Campylobacter* sp., and enterococci should be noted [7-10]. Their detection indirectly testifies to the potential ability of the presence of enteric pathogens in this environment.

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The analysis of enterococci survival in the environment shows their high resistance to stress when compared to fecal coliforms [11, 12]. This is also confirmed by the results of many experiments on the influence of composting processes on the inactivation rate of fecal streptococci. In bovine slurry warmed up to 55ºC after 24 hours their reduction by 4-5 log was observed [13]. According to Bachman [14] and Selbitz [15] they survive more than 30 minutes in 60ºC. The values Dº calculated for slurry and household wastes warmed up to 70ºC in anaerobic conditions ranging from 8.8-9.8 min., and a decrease in their number during one hour was 6.1-6.8 log [16]. Thus, this group of bacteria is frequently suggested to be used as indicators of the fecal contamination of different habitats as well as potential human and animal pathogens [17]. In this study, group D - streptococci were used for evaluation of the meat waste composting process. When this group of bacteria occurs in wastes subjected to insufficient sanitization and is used for fertilizing, there might be a risk of gene transmission of antibiotic-resistance to soil bacteria, such as *Proteus* spp., *Pseudomonas* spp. and *Staphylococcus aureus* [18-20].

The aim of this study was to evaluate the safety of meat waste composting based on inactivation of fecal streptococci. Several types of carriers were tested, to which bacteria were introduced and placed in the composted biomass. This allowed monitoring of the process and, consequently, the elimination of pathogen transmission into the compost produced.

Material and Methods

Bio-Reactor and Composting Ingredients

A drum bio-reactor loaded with 60% of batches of meat waste (stomach contents, scraps, fats, blood) and 40% of sawdust was used in the experiment. In three sites of the reactor (front, centre, back) were shallow (30 cm in depth) perforated recesses closed from the outside, in which small meat and bone carriers were placed. Additionally, a metal perforated cylindrical capsule of 50x15 cm with big meat and bone carriers was placed inside the bio-reactor (Fig. 1). Time of composting was different in each cycle and amounted to: 84 hours in cycle 1, 126 hours in cycle 2 and 210 hours in cycle 3, depending on temperature.

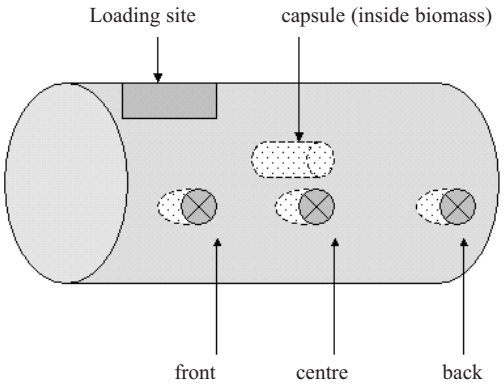

Fig. 1. The bio-reactor scheme.

Inoculum Preparation

Streptococci were grown on Nutrient Broth medium (Merck, nr 7882) at 37ºC for 18 hours. Suspensions with a density of $10^{6\text{-}10}$ cfu/ml were used in the experiment (DU-65 Spectrophotometer Beckman).

Carrier Preparation

Bone carriers included fragments of shafts and distal parts of thighbones of pigs. Bone marrow was removed and polycarbonate bags containing bacteria suspension were placed inside. Pores with a diameter of 0.01 μm in the polycarbonate foil allowed contact between microorganisms and composting material. Meat carriers were particles of pork closed in cubes made of metal netting measuring 3 by 3 cm and 5 by 5 cm, which protected them from deformation during composting. Bacteria suspension was injected inside the particles. Large meat carriers and bone heads, due to small recesses in the bio-reactor, were placed only in a metal perforated capsule inside the reactor and analyzed only at the final stage of the process. In theory, they were supposed to protect the bacterial suspension, the best from thermal conditions in the bio-reactor.

Microbiological Analysis

In each of the cycles analyzed samples were taken at different times. This resulted from diverse temperature conditions in particular cycles. It has been assumed that an increase temperature will accelerate the elimination rate of the microorganisms. Thus samples were taken more frequently in such cases. The carriers were removed from the bio-reactor at several-hour intervals and the number of streptococci were determined based on the most probable method (MPN) in a 3-test tube set. One-gram meat sample or 1 ml of the suspension was diluted 10-fold in 0.9% NaCl. One millilitre of suspension was taken from each of them and added to 9 ml of the broth bouillon with glucose and azide enriching medium for selective growth of fecal streptococci (Azid Dextrose Broth, Merck, nr 1590). After 48 hours of incubation at 37ºC, a loopfull of culture showing growth was streaked to agar containing esculine and azide for confirmation (Kanamycin Esculine Azide Agar, Merck, nr 5222). The serological test for re-confirmation of preconfirmed colony was also made (Phadebac Strep D Test, Boule Diagnostics AB, Huddinge, Sweden). This test is based on co-agglutination reaction. Characteristically growing colonies of streptococci surrounded by dark zone were selected and placed on the slide. Then a reagent (antibodies) was added and after 1 min the reaction of agglutination was observed.

Compost Analysis

The following analyses of final product were made: pH value – with the electrometric method in water, dry matter content (DM), organic matter content, the heavy metal (Hg, Cd, Cr, Cu, Ni, Pb, Zn) content (atomic absorption

Table 1. The number of group D-streptococci in cycle 1.

Carrier	Sampling location	Time [h]				
		0	24	36	48	84
Meat carrier 3x3 cm	front	$1.4x10^{10}$	$1.4x10^{10}$	$1.4x10^{10}$	$1.4x10^{8}$	$2.5x10^{3}$
	centre	$1.4x10^{10}$	$1.4x10^{10}$	$1.4x10^{10}$	$1.4x10^{8}$	$2.5x10^{3}$
	back	$1.4x10^{10}$	$1.4x10^{10}$	$4.5x10^{9}$	$1.4x10^{6}$	$2.5x10^{3}$
Bone shaft	front	$1.4x10^{10}$	$1.4x10^{10}$	$4.5x10^{9}$	$1.4x10^{10}$	$2.5x10^{3}$
	centre	$1.4x10^{10}$	$1.4x10^{10}$	$1.4x10^{10}$	$1.5x10^{9}$	$2.5x10^{3}$
	back	$1.4x10^{10}$	$1.4x10^{10}$	$1.4x10^{10}$	$1.4x10^{10}$	$2.5x10^{3}$
Meat carrier 5x5 cm	capsule	$1.4x10^{10}$	n.e.	n.e.	n.e.	$2.5x10^{3}$
Bone head	capsule	$1.4x10^{10}$	n.e.	n.e.	n.e.	$2.5x10^{3}$

n.e. – not examined

Table 2. The number of group-D streptococci in cycle 2.

Carrier	Sampling location	Time [h]			
		0	72	102	126
Meat carrier 3x3 cm	front	$2.5x10^{7}$	$2.5x10^{8}$	$1.5x10^{9}$	$0.4x10^{2}$
	centre	$2.5x10^{7}$	$7.5x10^{8}$	$2.5x10^{8}$	$0.4x10^{2}$
	back	$2.5x10^{7}$	$2.5x10^{9}$	$2.5x10^{8}$	$0.4x10^{2}$
Bone shaft	front	$4.5x10^{9}$	$2.5x10^{8}$	$2.5x10^{10}$	n.d.
	centre	$4.5x10^{9}$	$7.5x10^{8}$	$0.4x10^{6}$	n.d.
	back	$4.5x10^{9}$	$9.5x10^{5}$	$0.7x10^{9}$	n.d.
Meat carrier 5x5 cm	capsule	$2.5x10^{6}$	n.e.	n.e.	n.d.
Bone head	capsule	$1.4x10^{6}$	n.e.	n.e.	n.d.

spectrometer, Philips PU 9100X) and C:N ratio (Elementar Vario Max CN). The biomass was not analyzed chemically before composting.

Statistical Analysis

In order to compare the inactivation rate of streptococci in different cycles, the results for bacteria count were reported in $\log_{10} \cdot g^{-1}$ of bacterial reduction per hour. Simple linear regression analysis was applied to determine the regression equation:

$$log_{10}(N) = ax + b$$

...where N is the number of bacteria at time t, x is the time in hours, a is the slope of the curve - $\log_{10}$ of bacterial reduction per hour and b is the intercept - $\log_{10}$ of bacterial number at time 0.

To calculate theoretical survival time of bacteria, b was divided by a. Statistical analysis was carried out using Statistica Microsoft Software. The temperatures in all the cycles were also compared using Tukey'a HSD test (Statistica 8.0).

Results

Three cycles of composting runs at different time were analyzed. The temperature was monitored at three locations of the bioreactor near the recesses in the carriers. During the first cycle of composting the temperature inside the bio-reactor reached a maximum value of 62.9ºC (Fig. 2). The temperature values at three locations were close. The bacteria were present in all carriers even after 84 hours (Table 1). Their number did not change for the first 36 hours in meat carrier and 48 hours in bone carrier. Fecal streptococci were isolated from the samples placed in the capsule for 84 hours both in small (meat carrier 3x3cm, bone shaft), and big carriers (meat carrier 5x5cm, bone head) (Table 1).

In the second cycle, the maximum temperature was 57.2ºC (Fig. 3). The analyses of samples in terms of the bacteria indicated that the cycle did not proceed effectively (Table 2). In meat carriers a secondary bacteria multiplication occurred after 72 hours. It might have been due to low temperature, but in the last term analyzed (126 hour) a very fast reduction occurred in number of the microorganisms by 6 log. For bone carriers, a remarkable fluctuation in the bacteria count was observed, as increasing after 102 hours of the cycle up to the initial level. The final sample from the last time did not contain the tested microorganisms either in small and big meat carriers or in the head and shaft of the thigh bone (Table 2).

At the third cycle, the temperature reached a maximum value of 58.8ºC at the back of the reactor (Fig. 4). Microbiological analysis of the samples revealed streptococci in both carriers after 112 hours, no streptococci were isolated in the samples from the capsule after 210 hours (Table 3).

The statistical analysis and regression line equations indicate that cycle 1 proceeded most effectively with respect to the elimination rate of streptococci. Daily decrease in the bacteria population ranged from 0.07-0.09 log irrespective of the bio-reactor location and the carrier type (Table 4). In the second cycle it was impossible to carry out this analysis because the measurements were single replicated.

Table 3. The number of group D-streptococci in cycle 3.

Carrier	Sampling location	Time [h]				
		0	60	100	112	210
Meat carrier 3x3 cm	front	$1.5x10^7$	$2.5x10^7$	$1.5x10^6$	$4.5 x10^4$	n.d.
	centre	$1.5x10^7$	$4.5x10^6$	$2.5x10^5$	$7.5x10^5$	n.d.
	back	$1.5x10^7$	$4.5x10^7$	$2.5x10^6$	$2.5x10^5$	n.d.
Bone shaft	front	$2.5x10^8$	$2.5x10^6$	$9.5x10^4$	$4.5x10^2$	n.d.
	centre	$2.5x10^8$	$9.5x10^5$	$2.5x10^6$	$2.5x10^6$	n.d.
	back	$2.5x10^8$	$7.5x10^4$	$4.5x10^5$	$4.5x10^3$	n.d.
Meat carrier 5x5 cm	capsule	$1.4x10^{10}$	n.e.	n.e.	n.e.	n.d.
Bone head	capsule	$1.4x10^{10}$	n.e.	n.e.	n.e.	n.d.

n.e. – not examined
n.d. – not detected

Table 4. Regression line equations describing the inactivation rate of streptococci in particular cycles.

The part of bio-reactor	cycle 1			
	Meat carrier (3x3)	R^2*	Bone shaft	R^2
front	y = - 0.08x + 11.65 x = 145.6	80.89	y = - 0.07x + 11.75 x = 167.8	68.98
centre	y = - 0.08x + 11.65 x = 145.6	80.89	y = - 0.08x + 11.76 x = 147.0	75.34
back	y = - 0.09x + 11.33 x = 125.8	84.43	y = - 0.08x + 11.86 x = 148.3	67.35
x_{mean}**	139.0		154.4	
cycle 3				
front	y = - 0.02x + 6.82 x = 341.0	85.37	y = - 0.02x + 6.70 x = 335.0	95.23
centre	y = - 0.02x + 6.58 x = 329.0	85.28	y = - 0.02x + 7.33 x = 366.5	84.98
back	y = - 0.02x + 7.05 x = 352.0	81.66	y = - 0.02x + 6.43 x = 321.0	92.48
x_{mean}	340.7		340.8	

* R^2 – determination of a decrease in bacteria number depending on time [%].
** x_{mean} – mean bacteria survival [h] in front, centre and back of bio-reactor.

The same situation occurred with big meat carriers and bone heads placed in the capsule. The bacteria number was analyzed only once, at the end of the process after emptying the bio-reactor. The least effective course of the process was observed in cycle 3. Irrespective of place in the bio-reactor and carrier type, a daily decrease in streptococci number was 0.02 log. In cycle 3 the theoretical survival time of the microorganisms was too long to provide sanitation of the composted waste at the intended time. It seems that the inactivation rate of the bacteria depended on the temperature at which the process occurred. The elimination of streptococci proceeded the most effectively during cycle 1, when the highest temperature was noted, although no statistically significant differences were found between the temperatures at each cycle (data not shown).

The results from the chemical analysis of the final product of composting were presented in Table 5. The composting process resulted in obtaining a homogenous material with dark colour and earthy smell. Its physico-chemical parameters and heavy metal content are within the standards. The biomass was not analyzed chemically before composting.

Discussion

Composting is one of the most useful methods of waste treatment, besides pasteurization, stabilization in thermophilic or mesophilic anaerobic conditions and liming. The final product is characterized by its agricultural benefits. The present work involved microbiological monitoring

Table 5. The chemical parameters of the composting final product.

Compost parameters	Values						
pH	7.63						
Dry matter (%)	68.29						
Organic matter (DM%)	46.9						
Heavy metals	Cd	Cr	Cu	Ni	Pb	Zn	Hg
(mg/kg DM)	0.26	6.71	8.26	1.79	6.03	64.90	0.016
C:N	30:1						

of the waste treatment process by composting in a drum bioreactor, on the grounds of survival of fecal streptococci.

Different courses of temperatures were observed at each cycle, but no statistically significant differences at $P<0.05$ between them were found. The mean theoretical survival time of the bacteria was the shortest in cycle 1. In cycles 2 and 3 a secondary growth of bacteria during composting was observed.

In the present study, the size and type of the carriers did not influence the elimination rate of streptococci with the exception of cycle 2, where a longer survival time of streptococci in a meat carrier was observed. The time needed for warming the inside of the bones to a temperature of 56ºC, while the surrounding temperature is 60ºC, is reported to be 40 hours [21]. Thus, a slower elimination rate of streptococci in bone carriers was expected, but this was not the case in the present study. The largest meat samples of 5x5 cm and bone carriers made of thigh bone heads, removed from the capsule on the last day of composting did not contain any streptococci, except cycle 1. However, a 7 log - decrease in number occurred inside, which, as Böhm reported [22], was enough to make the final product environmentally safe. The temperature generated during the process is the most important of all factors decisive in the proper conduct of sanitization process. Its maintenance at a proper level results in the elimination of pathogens in the waste. Most authors have reported that composting waste for several days at temperatures above 50ºC causes elimination of pathogenic microorganisms. According to Epstein, a reduction in the number of *Escherichia coli, Salmonella enteritidis* and fecal streptococci occurred within the range of 55-65ºC [23]. The author reported that after 14 days of composting the final material did not contain any pathogens. Joshua conducted a composting process in a completely closed system, in cycles from 5 to 11 days [24]. In his experiment he used a closed reactor with a forced aeration system. During the first 48 hours, the temperature was 65ºC, later it ranged from 46 to 50ºC. The final product was good at hygienic quality. Generating a high temperature during the process is strictly connected with biomass richness in easily degradable carbohydrates. The time of high temperature action is inversely proportional to the temperature value. Böhm reported that a temperature of 55°C should remain for at least two weeks in a pile system or 65°C for a week in closed bio-reactors [25].

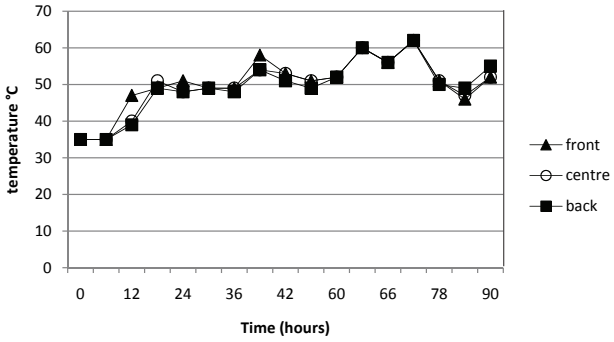

Fig. 2. Time course of temperature in the bioreactor during cycle1.

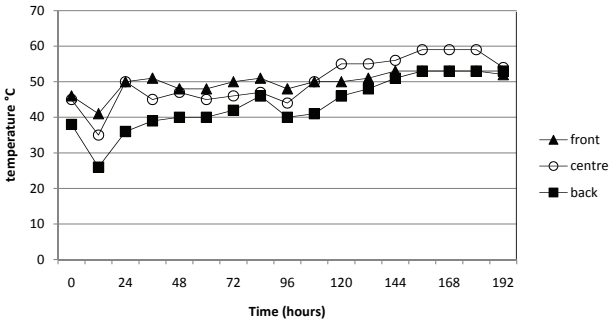

Fig. 3. Time course of temperature in the bioreactor during cycle 2.

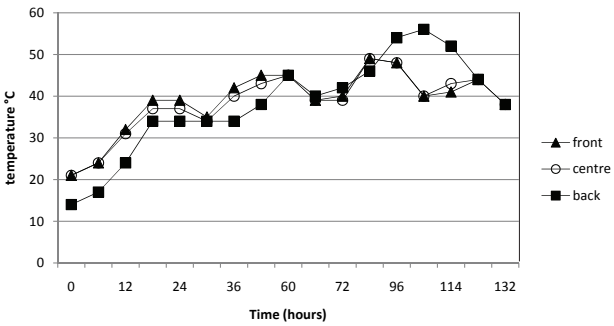

Fig. 4. Time course of temperature in the bioreactor during cycle 3.

Fecal streptococci are far more resistant to unfavourable conditions that occur during waste hygienization than other indicator bacteria (*Salmonella senftenberg* W_{775}, *Escherichia coli*). For instance, Shaban observed a long survival time of fecal streptococci during sewage sludge composting in various systems, while the rods of *Salmonella* and *Escherichia coli* died considerably faster. In a pile with forced aeration they were killed after 9 days, whereas enterococci were isolated until day 73 of the process [26]. In turn, Cekmecelioglu et al. [27] studied the survival rate of different microorganisms in a forced-aerated in-vessel system. The feedstock consisted of food waste, cow manure and bulking materials. Maximum temperatures of composting during the 12-day trials ranged from 54.7 to 56.6ºC and were remained for 3.3 days. This enabled the number of *Salmonella* and *E. coli* O157:H7 to be reduced by 92.3%, and the fecal streptococci only by 27.1%. Jepsen in his experiments obtained a reduction by 4 log. He claimed that providing high temperature values in all parts of a pile was necessary for the total sanitation of the wastes [28]. In the present study, a slower elimination of streptococci ranging from 0.02 – 0.09 log/day was observed, which was equivalent to a theoretical survival time of bacteria within 139 – 340 hours. Therefore, monitoring fecal streptococci over composting can be used to suggest the absence or presence of pathogens at different conditions of composting process.

Acknowledgments

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