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Original Research

Validation of Biodegradable Waste Composting Process Based on the Inactivation of *Salmonella senftenberg* W775

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Abstract

Due to frequent occurrence of pathogenic organisms in organic wastes, there is a need for a microbiological evaluation of the utilization process in the case of using them for agricultural purposes. The effectiveness of organic waste composting process using Kneer container technology was evaluated on the basis of *Salmonella senftenberg* W775 inactivation. The study was conducted during the spring, summer and autumn, placing specially prepared carriers in three layers of the composted biomass. In the top and middle layers the total elimination of the *Salmonella* rods was obtained. The inactivation rate of the bacteria in the bottom layer was slower.

Keywords: *Salmonella senftenberg* W 775, composting, sewage sludge

Introduction

The management of organic municipal waste for fertilization and land reclamation is an essential aspect of the biosafety of the human environment. Biowaste, due to the large organic matter content, makes an excellent medium for microbial growth [1-3]. Apart from autochthonous microorganisms, organic waste mixed with sewage sludge might contain some allochthonous microorganisms, including diverse pathogens infectious for several species of animals as well as for humans [4, 5]. Basic data concerning the occurrence of *Salmonella* in raw and treated sludge has been given by several authors [6-8]. Their count in raw sludge normally amounts to 10^2 cfu/g, although the density can rise even to 10^7 cfu/g [9, 10]. A significant aspect in spreading *Salmonella* is their resistance to unfavourable environmental factors and the ability to survive in different ecological niches for a longer period of time [11, 12]. In water the bacteria can survive for several months, and a decrease in temperature extends their survival time. In soil, they can survive from 12 to 28 weeks. If ambient temperature rises to about 30°C, this time is shortened to 4 weeks. In dry environment *Salmonella* rods can survive from 1 to 7 days, showing higher resistance than *E. coli* [13]. In a number of European states this microorganism is used as an indicator organism for the evaluation of the sanitary quality of sewage sludge and compost.

The Regulation of the Minister of the Environment in Poland on municipal sewage sludge allows it to be applied in agriculture and for land reclamation, when no bacteria of the genus *Salmonella* is detected in 100 g of sludge [14]. It implies the necessity of the validation of the technologies applied to organic waste treatment, particularly the effectiveness of a composting process.

The aim of the present study was the microbiological evaluation of the effectiveness of Kneer container technology, which is used for composting of municipal green waste with the addition of sewage sludge. The inactivation rate of *Salmonella senftenberg* W 775 was the basis for hygienic assessment of this process.

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Material and Methods

The experiment was carried out at a sewage sludge composting plant using Kneer container technology. Kneer container technology consists of composting organic waste in closed containers and then in a windrow. The containers are connected to a system aerating the biomass. Water during the process circulates in a closed cycle. The nascent gases are transferred through a biological filter and released to the atmosphere.

A properly prepared material consisting of waste from municipal green areas, cuts and sewage sludge mixed in the proportion 2/3:1/3:1 was placed in containers. A stage of intensive composting lasted for about 14 days. Afterwards, the composted material was removed from the containers and heaped up into the compost windrow. To provide the biomass proper aeration, the windrow was turned mechanically every two weeks. The process of the compost maturation lasted about 4–6 weeks.

The effectiveness of compost hygienization process was controlled during spring, summer and autumn on the basis of the inactivation rate of *Salmonella senftenberg* W775 introduced into the biomass. *Salmonella senftenberg* W 775 was used in microbiological tests due to its high thermoresistance. These bacteria are rarely present in sewage sludge, so there is low risk of infection of the carriers used with native strains.

In order to perform a microbiological evaluation of the effectiveness of composting waste from municipal green areas and sewage sludge, spherical carriers of about 5 cm in diameter were prepared from pasteurized compost. The carriers were inoculated with a suspension of *Salmonella senftenberg* W 775 (1 ml each). The concentration of bacteria in the suspension ranged from 10^8 to 10^9 cfu/ml. The carriers were additionally surrounded with compost and placed in special nylon sacks.

The carriers with microorganisms were introduced into the material composted in a container in the top, medium and bottom layer of the biomass. At the stage of intensive composting, the number of *Salmonella* rods was determined in the carriers. After about 2 weeks, when the biomass was removed from the containers and formed into a windrow, part of the carriers was transferred from the containers into the windrow. For examination the hygienization effectiveness of the 2nd stage of the composting process in the windrow, additional carriers were introduced inside containing a high concentration of the tested bacteria (10^8 cfu/ml). They were removed at several days' intervals and subjected to microbiological analyses.

1g weighed portions were taken from each compost carrier and placed in tubes containing 9ml of 0.9% NaCl. Next, a series of tenfold dilutions were prepared. From each dilution 1ml was transferred to 3 tubes with 1% peptonic water and incubated at 37°C for 24 h. Then 0.1ml of the suspension was transferred from each tube to tubes containing the liquid medium acc. to Rappaport [15]. The samples were incubated at 43°C for 24h. Next, the material was transferred on the solid selective medium Brilliant-Green Phenol-Red Lactose Agar (BPLA acc. to Kauffmann) (37°C, 24h) [16]. All the tests were made in three replications. On the basis of the positive readings on the solid medium BPL, a three-digit characteristic number was assigned, and then the most probable number of bacteria in the samples was determined using the McCrady tables. The bacteria concentration in the suspension inoculating the carriers before their introduction into the compost was determined in the same way. A series of tenfold dilutions of 1ml suspension of *Salmonella* was prepared, and then incubated on liquid and solid media according to the above-mentioned procedures. The control carriers were protected with the sterile compost and stored under laboratory conditions. Additionally, a serological test was applied using the polyvalent serum Hm in order to confirm the identification.

The moisture content of the composted biomass, its pH value and temperature were also monitored during the study. The moisture content measurements were made in two replications, at intervals of several days, while pH measurements were made in three replications at intervals of several days, using the pH-meter MP 120 Mettleer Toledo. During the phase of intensive composting in the containers, the temperature was monitored by two sensors placed in the bottom and top parts of the containers. During the compost maturation in the windrow, the temperature was measured manually using a thermometer in the upper, medium and bottom layers of the biomass.

The results obtained were verified and analyzed using the program Statistica Microsoft software. Regression lines were drawn, on the basis of which a theoretical survival time of *Salmonella* in the composted material was calculated.

Results and Discussion

The results of the microbiological tests have been presented in Tables 1–3.

The hygienization efficiency of the composting process was different between seasons. In the spring cycle, the number of *Salmonella senftenberg* W 775 in the carriers placed in the container decreased substantially after 19 days of composting from 1.15 x 10^9 cfu/g to 4.00 x 10^2 cfu/g in the top layer, to 3.00 x 10^2 cfu/g in the medium layer, and 9.00 x 10^2 cfu/g were isolated in the bottom layer. The complete elimination of the *Salmonella* rods in the carriers transferred from the container to the windrow was obtained after 37 days of composting (Table 1).

Particularly unfavourable for *Salmonella* appeared the conditions in the container and windrow during the composting process in the summer cycle. As early as after 7 days, the bacteria in the carriers introduced into the container were reduced by 7 log in the top layer, and by 4 log in the bottom layer. At the same time, the microorganisms in the medium layer were totally eliminated. After 15 days of intensive composting there were no tested bacteria in the biomass (Table 1).

Salmonella senftenberg W 775 in the carriers taken from the container in the autumn cycle acted differently.

Table 1. The average number of three replications of *Salmonella senftenberg* W 775 in the carriers in the containers and in the windrow [cfu/g].

Cycles	Layers of biomass	Times of sampling (days)							
		in the container				in the windrow (after transferring the carriers from the container)			
		0		7	19	23	30	37	-*
spring	top	$1.15x10^9$		$2.83x10^6$	$4.00x10^2$	$1.67x10^2$	nd	nd	-
	medium			$7.83x10^6$	$3.00x10^2$	nd	nd	nd	-
	bottom			$1.92x10^7$	$9.00x10^2$	$4.67x10^2$	$3.00x10^2$	nd	-
	control			$3.67x10^7$	$4.50x10^6$	$9.67x10^6$	$6.00x10^5$	$4.33x10^4$	-
		0		7	15	-*	-*	-	-
summer	top	$3.67x10^8$		$3.07x10^1$	nd	-	-	-	-
	medium			nd	nd	-	-	-	-
	bottom			$1.80x10^4$	nd	-	-	-	-
	control			$3.17x10^8$	$5.50x10^8$	-	-	-	-
		0	2	6	14	20	27	31	38
autumn	top	$2.83x10^9$	$1.00x10^6$	nd	nd	nd	nd	nd	nd
	medium		$1.30x10^5$	$1.28x10^4$	$2.83x10^4$	$2.83x10^4$	$2.65x10^4$	$5.17x10^3$	nd
	bottom		$3.17x10^8$	$1.48x10^8$	$1.23x10^8$	$3.83x10^7$	$7.33x10^7$	$3.08x10^7$	$2.58x10^6$
	control		$5.50x10^9$	$2.32x10^9$	$7.33x10^8$	$8.00x10^7$	$5.50x10^7$	$5.50x10^7$	$9.67x10^6$

nd – no occurrence detected of the tested bacteria

* – experiment discontinued due to no bacteria being detected in the carriers

After 6 days of the thermophilic stage of the process, the total inactivation of the bacteria was observed only in the top layer of the biomass. In the medium layer the *Salmonella* rods survived the composting process in the container and were eliminated after transferring the carriers into the windrow – after 38 days (Table 1). The elimination of the bacteria proceeded for the longest time in the carriers from the bottom level. The theoretical time of their survival calculated on the basis of regression lines was 148 days, with a very low daily decrease in number – 0.06 $\log_{10}$ (Table 3).

The hygienization efficiency of the 2nd stage of the composting process in the windrow was also determined, on the basis of a change in the number of bacteria introduced at a high concentration in additional carriers. The results obtained are presented in Tables 2, 3.

In the spring cycle, *Salmonella* survived remarkably longest in the bottom layer of the windrow. After 18 days, the bacteria number was still high – 1.07×10^6 cfu/g, while at the same time in the medium layer the presence of the bacteria was no longer detected (Table 2).

In the summer cycle, the inactivation of *Salmonella* proceeded very quickly. Although after 6 days from the moment of placing the carriers in the biomass a significant decrease in the bacteria number did not occur, as early as after 13 days, no tested microorganisms were isolated in the top and medium layers (Table 2). In the bottom part, however, after 20 days the bacteria number decreased to 3.17×10^3 cfu/g, and a daily decrease in number amounted to 0.32 $\log_{10}$ (Tables 2, 3).

In the autumn cycle *Salmonella* was eliminated faster in the higher parts of the biomass and its presence was not detected in the material tested after 24 days. At the same time, the bacteria number in the bottom level decreased only to 7.15×10^5 cfu/g (Table 2).

Salmonella senftenberg W 775 was used for the evaluation of the sanitary cleanliness of the composted biomass as it is extremely resistant to high temperatures and it rarely infects people. Moreover, the bacteria are relatively easy to identify [5, 9, 17].

Many authors confirm the hygienization effectiveness of a composting process for the inactivation of pathogenic organisms, including *Salmonella* [5, 17-20]. However, Russ and Yanko [21] point out that to provide the full elimination of pathogens in compost, the process of utilization should be performed thoroughly, and any changes in technological parameters such as temperature, moisture or pH value, have a strong impact on the final result of the process.

The research indicates that the most essential hygienization factor is the temperature generated during the composting process, which should reach the required level and

Table 2. The average number of three replications of *Salmonella senftenberg* W 775 in additional carriers with a high bacteria concentration placed in the windrow (cfu/g).

Cycles	Layers of biomass	Times of sampling in the windrow (days)				
		0		4	11	18
spring	top	3.83×10^8		4.82×10^5	2.45×10^4	1.64×10^2
	medium			3.15×10^4	1.34×10^2	nd
	bottom			1.20×10^7	1.97×10^7	1.07×10^6
	control			5.50×10^8	1.12×10^8	3.83×10^6
summer		0	6	13	20	27
	top	2.83×10^8	3.08×10^8	nd	nd	nd
	medium		6.65×10^7	nd	nd	nd
	bottom		1.07×10^8	6.67×10^5	3.17×10^3	nd
	control		2.83×10^8	3.83×10^8	3.83×10^8	3.17×10^7
autumn		0	6	13	17	24
	top	9.67×10^8	4.32×10^6	7.83×10^4	1.40×10^3	nd
	medium		1.48×10^6	1.30×10^6	8.00×10^6	nd
	bottom		2.65×10^7	9.00×10^6	5.50×10^6	7.15×10^5
	control		7.83×10^7	4.83×10^7	9.67×10^7	6.17×10^7

nd – no occurrence detected of the tested bacteria

remain at it for sufficient time, in all parts of the biomass [19, 22].

The results of the present study also indicated the high efficiency of composting in the applied Kneer container technology. Full hygienization was observed in all layers of the composted biomass both in the spring and summer cycles. The elimination of *Salmonella senftenberg* W 775 during the composting process allows one to conclude that other pathogenic microorganisms with less resistance to high temperatures also will become inactivated [23]. A particularly high rate of *Salmonella* elimination was observed in the summer cycle, where as early as after 2 weeks the bacteria tested were not detected in the carriers. In this cycle, the high temperature generated during the stage of intensive composting plays a vital role. It exceeded 50°C, remaining in this level for several days. In the literature, many authors emphasize that temperature not only influences proper mineralization and stabilization of organic matter, but also plays a crucial role in the process of elimination of pathogenic organisms [18, 19]. Yanko [24] reports that the action of a temperature of 55°C for 3 days is sufficient for the necessary elimination of pathogenic bacteria. A significant effect of temperature was also observed in the present study in the spring cycle. At first, at the stage of intensive composting in a container, a properly high temperature was not obtained. Accordingly, *Salmonella* was still detected in spite of a substantial decrease in number. Only after heaping up the windrow and also better biomass aeration the temperature at higher parts of the material rose to 53°C and ranged from 47°C to 52°C for several days, which resulted in the total elimination of the bacteria tested.

Although the temperature in the autumn cycle did not exceed 50°C, a satisfying state of hygienization was achieved. One might suppose that there were some other mechanisms eliminating pathogenic bacteria here, such as competition for food, antagonistic effect of mezophilic organisms inhabiting compost, unfavourable oxygen conditions and action of antibiotics generated by actinomycetes present in the composted material. Sidhu [25] claims that at the cool stage of composting the variability of microorganisms increases, and thus competition for nutrients is exacerbated. At the same time, the activity of autochthonous microorganisms rises and it increases the effect of antagonistic action. Weaker competitors, that is to say pathogenic microorganisms, may be eliminated in such conditions. Active anaerobic bacteria which appear in poorly aerated places in the biomass might also contribute to the inactivation of *Salmonella* [26].

The analyses conducted indicated the presence of a hazard zone at the bottom layer of the composted material. In all the experimental cycles *Salmonella* survived for the longest time in the carriers taken from this part of the biomass. A particularly slow elimination rate was observed in autumn – after a month's proceeding the number of bacteria decreased only to 7.33×10^7 cfu/g, and the theoretical time of full inactivation amounted to 148 days. One may suppose that the lack of proper aera-

Table 3. Regression line equations presenting the dynamics of *Salmonella senftenberg* W 775 inactivation in the composted material.

Cycles	Location carriers	Layers of biomass	Regression equations	r^2(%)	Survival of bacteria (days)
spring	container	top	y = – 0.31x + 8.62	79.21	28
		medium	y = – 0.40x + 9.27	81.00	23
		bottom	y = – 0.25x + 8.48	90.25	34
		control	y = – 0.12x + 9.16	94.09	76
	windrow	top		88.36	22
		medium	y = – 0.36x + 7.89 y = – 0.45x + 7.35	88.36	16
		bottom	y = – 0.13x + 8.16 y = – 0.09x + 8.44	70.56	64
		control		72.25	94
summer	container	top	-	-	7*
		medium	–	–	nd (after 7days)
		bottom	–	–	7*
		control	y = – 0.07x + 8.70	77.44	124
	windrow	top	-	-	6*
		medium	–	–	6*
		bottom	y = – 0.32x + 9.35	92.16	29
		control	y = – 0.04x + 8.62	60.84	216
autumn	container	top	-	-	2*
		medium	y = – 0.14x + 6.80	56.25	49
		bottom	y = – 0.06x + 8.86	56.25	148
		control	y = – 0.07x + 9.60	81.00	137
	windrow	top	y = – 0.36x + 8.99	98.01	25
		medium	y = – 0.30x + 9.12	67.24	30
		bottom	y = – 0.13x + 8.59	82.81	66
		control	y = – 0.04x + 8.49	39.69	212

*- the day of the process when the bacteria were last isolated

tion and biomass mixing may have been the reason for too weak warming in the bottom part of the composted organic wastes, both in the container and in the windrow. It is likely that obtaining too low temperature resulted from direct contact of the composted biomass with the base of the container and a cooling effect of the air pumped in order to aerate the biomass. Tateda [27] came to similar conclusions on the basis of his study.

The phenomenon of various inactivation rates of pathogens and differentiated distribution of temperature in particular layers of the composted material is supposed by the research of Paluszak and Bauza-Kaszewska [28]. Experiments conducted by Tateda [27] during composting the material in closed facilities also indicated that the highest temperatures were noted in the top layer of the biomass, while the lowest were observed in the bottom level.

Insufficient amounts of heat generated during the composting process or a decrease in temperature during biomass maturing may be the cause of not only the failure in *Salmonella* inactivation, but also their regrowth, particularly when a composted material is rich in nutrients [29]. This phenomenon is frequently described in the literature [30, 31]. It has been noted that another increase in the bacteria number depends not only on temperature, but also on moisture content, availability of nutrients, or the presence of autochthonous organisms in compost [25]. In the present study, in spite of the fact that the carriers introduced to the composted material contained a high concentration of bacteria, and also low temperatures were noted during the process, the above-mentioned phenomenon was not observed.

Moisture and pH value of the material monitored during composting did not show any changes significant for the inactivation of the tested indicator bacteria. In all the research cycles, the moisture values ranged from 53% do 60%, while the average pH values ranged from 7.8 to 8.5.

The study confirms the necessity of microbiological control of biowaste utilization process, particularly sewage sludge, being the main source of pathogenic microorganisms. These actions allow us to evaluate and avoid the risk of environmental contamination in the case of using obtained products for fertilization and land reclamation.

Conclusions

1. The applied research model based on the inactivation of the indicator bacteria *Salmonella senftenberg* W 775 enables the validation of organic waste composting methods and predicting the hygienic quality of the utilized product.
2. The research proved the high hygienization efficiency of the container composting technology with the Kneer system. It applies particularly to the top and medium layers of the composted material.
3. It was indicated that the bottom layers of the composted biomass make the hazard zone, where the reduc-

tion of the introduced pathogen elimination rate can be observed. This phenomenon resulted from poor aeration and improper material mixing.

4. Temperature appeared not to be the only factor playing a vital role in the elimination of allochthonous pathogenic microorganisms during the composting process. Biotic actions are also of substantial importance.

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