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INACTIVATION OF *ESCHERICHIA COLI* DURING COMPOSTING PROCESS OF ORGANIC WASTES WITH SEWAGE SLUDGE

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Abstract: The aim of the study was to estimate the effect of the composting process in the container technology Kneer on *E. coli* inactivation. The bacteria placed in the special carriers were introduced into the composted material. The elimination rate of *E. coli* differed depending on both the carriers' location in the biomass and the thermal conditions. The most effective hygienization of the material was noticed in summer – after 48 h in the middle layer, 6 days in the top layer and 10 days in the bottom layer. In spring and autumn, the bacteria survived the longest in the bottom layer – 83 and 45 days, respectively. Apart from the high temperature, the research points out the action of other factors such as competition, antagonism and antibiosis.

INTRODUCTION

The management of organic municipal waste and sewage sludge for fertilization and land reclamation is an essential aspect of shaping the environment. Sewage sludge is a rich source of organic compounds, biogenic elements and microelements [2, 11, 21]. Apart from chemical compounds, sludge can contain a considerable amount of microorganisms which pose a hazard for human health and environmental [7]. Its application as a fertilizer to arable land, for forest cultivation and for land reclamation depends on its previous treatment. One of the methods for obtaining a high-quality and environmentally safe product is the composting process. It allows for obtaining the stable material, being a good soil conditioner and a source of nutrients for plants and microorganisms [10, 15]. Moreover, during the disinfection stage of this process pathogenic bacteria are reduced to low levels. On account of environmental safety, it is crucial that its quality is assessed microbiologically in order to prevent soil and organisms from contamination with pathogenic microorganisms [19].

The aim of the study was to evaluate the hygienic effectiveness of the composting process conducted in closed containers, based on the inactivation of the indicator bacteria *E. coli*. The bacteria are commonly used for assessing the effectiveness of composting organic wastes, as they occur in composted biomass considerably more often than other pathogens and methods of their determination are simpler and safer [3, 8, 12].

MATERIAL AND METHODS

The research was carried out in spring, summer and autumn in a composting plant working in the container technology system Kneer. The material composted consisted of wastes from municipal greens, scraps and sewage sludge mixed in a proportion of 2/3:1/3:1. It was placed in containers, where the intensive composting stage proceeded, lasting about 14 days. Then the material was removed from the containers and formed in a windrow. To provide a suitable biomass aeration, the windrow was turned mechanically every 2 weeks. The compost maturation process lasted for about 4–6 weeks.

In order to perform the microbiological evaluation of the composting process effectiveness, spherical carriers of about 5 cm in diameter were made of pasteurized compost and inoculated with 1 cm^3 of solution of *E. coli*. The concentration of the bacteria in the solution was 10^8–10^9 MPN/cm^3 of a suspension. The carriers were additionally surrounded with compost and placed in special nylon sacks. The carriers with microorganisms were placed in the container in the top, middle and bottom layers of the biomass. Throughout intensive composting the level of *E. coli* inactivation was determined. After 2 weeks that is during the time of windrow forming part of the carriers from the container were transferred to the windrow. In order to estimate the effectiveness of hygienization of the second stage of composting proceeding in the windrow, additional carriers were introduced to it, which were also subjected to microbiological analyses.

The material from each carrier was accurately fragmented and mixed. 1 g weighed portions were taken from each compost carrier and a series of 10-fold dilutions in 0.9% NaCl was prepared. The number of *E. coli* was determined in triplicate using the selective MacConkey medium (MERC105396). The samples were incubated at a temperature of 43°C for 24 h. Next, the material was sieved into the solid selective medium Lactose TTC agar with Tergitol® (MERC107680). The samples were incubated at 37°C for 24 h. In dubious cases the material was transferred on the Standard I nutrient agar (MERC105450). After 24 h of incubation at 37°C the pure colonies obtained were subjected to the IMViC tests. The determination of bacteria number was made based on the method of the most probable number (MPN). The results were analyzed statistically and regression lines were drawn to determine the theoretical survival time of *E. coli* in the tested material.

RESULTS

The results of the research are presented in Tables 1–3. The elimination rate of *E. coli* differed significantly and was dependent both on the location of the carriers and thermal conditions.

In the spring cycle *E. coli* died the most slowly in the bottom part of the biomass. During 14 days of intensive composting their number decreased only by 2 $\log_{10}$. At the same time the full inactivation of *E. coli* occurred in the top parts (Tab. 1).

After forming a windrow, a biomass hygienization did not proceed evenly. After 9 days the number of the tested bacteria in the top part decreased from $1.48 \cdot 10^9$ MPN/g to $4.67 \cdot 10^2$ MPN/g and the total elimination occurred in the middle part. In the bottom part the bacteria number was still high and amounted to $2.65 \cdot 10^8$ MPN/g (Tab. 2).

In the summer cycle the reduction of *E. coli* proceeded very quickly. After 48 h their presence was not detected in the middle part of the composted material (Tab. 1). The

Table 1. The average number of *E. coli* in the carriers placed in the containers and the windrow [MPN/g]

Cycles	Layers of biomass	Time of sampling [days]							
		in the container					in the windrow (after transfer the carriers from the container)		
		0		5	9	14	19	23	34
Spring	top	$1.30 \cdot 10^9$		$4.67 \cdot 10^2$	$4.67 \cdot 10^2$	nd	nd	nd	nd
	middle			$4.98 \cdot 10^7$	$2.65 \cdot 10^3$	nd	nd	nd	nd
	bottom			$7.33 \cdot 10^8$	$2.65 \cdot 10^8$	$3.83 \cdot 10^7$	$3.08 \cdot 10^7$	$2.65 \cdot 10^6$	$2.83 \cdot 10^5$
	control			$2.15 \cdot 10^9$	$7.33 \cdot 10^8$	$3.32 \cdot 10^8$	$6.17 \cdot 10^7$	$9.67 \cdot 10^7$	$2.83 \cdot 10^7$
		0	1	2	4	7	–*	–*	–*
Summer	top	$1.48 \cdot 10^9$	$3.83 \cdot 10^2$	$2.34 \cdot 10^2$	$2.34 \cdot 10^2$	nd	–	–	–
	middle		$1.14 \cdot 10^2$	nd	nd	nd	–	–	–
	bottom		$4.98 \cdot 10^8$	$4.75 \cdot 10^7$	$5.50 \cdot 10^7$	$3.83 \cdot 10^2$	–	–	–
	control		$6.17 \cdot 10^8$	$1.30 \cdot 10^8$	$1.30 \cdot 10^8$	$2.65 \cdot 10^7$	–	–	–
		0		4	8	13	18	22	–
Autumn	top	$3.83 \cdot 10^9$		$1.30 \cdot 10^6$	$3.83 \cdot 10^3$	$2.34 \cdot 10^2$	nd	nd	–
	middle			$5.50 \cdot 10^5$	$3.07 \cdot 10^1$	nd	nd	nd	–
	bottom			$5.50 \cdot 10^8$	$6.17 \cdot 10^6$	$3.83 \cdot 10^6$	$6.17 \cdot 10^5$	$3.17 \cdot 10^5$	–
	control			$6.17 \cdot 10^8$	$6.48 \cdot 10^8$	$3.83 \cdot 10^8$	$7.83 \cdot 10^7$	$7.83 \cdot 10^7$	–

nd – tested bacteria not detected

* – experiment was not continued due to lack of bacteria presence in the carriers

Table 2. The average number of *E. coli* in additional carriers with a high concentration of bacteria placed in the windrow [MPN/g]

Cycles	Layers of biomass	Time of sampling [days]				
		0	5	9	20	23
Spring	top	$1.48 \cdot 10^9$	$1.48 \cdot 10^9$	$4.67 \cdot 10^2$	nd	nd
	middle		$1.80 \cdot 10^2$	nd	nd	nd
	bottom		$2.83 \cdot 10^8$	$2.65 \cdot 10^8$	$2.83 \cdot 10^8$	$4.98 \cdot 10^8$
	control		$2.83 \cdot 10^9$	$2.65 \cdot 10^9$	$5.17 \cdot 10^8$	$1.13 \cdot 10^8$
Summer		0	2	–*	–*	–*
	top	$3.17 \cdot 10^8$	nd	–	–	–
	middle		nd	–	–	–
	bottom		nd	–	–	–
	control		$2.83 \cdot 10^8$	–	–	–
Autumn		0	5	9	16	–
	top	$2.65 \cdot 10^9$	$3.83 \cdot 10^4$	$1.40 \cdot 10^1$	nd	–
	middle		$3.17 \cdot 10^5$	nd	nd	–
	bottom		$3.83 \cdot 10^6$	$3.83 \cdot 10^6$	$3.32 \cdot 10^6$	–
	control		3.83x10^6	$4.72 \cdot 10^6$	$3.83 \cdot 10^6$	–

nd – tested bacteria not detected

* – experiment was not continued due to lack of bacteria presence in the carriers

Table 3. Regression line equations showing the dynamics of *E.coli* inactivation in the composted material

Cycles	Location of the carriers	Layers of biomass	Regression equations	r^2 [%]	Survival of bacteria [days]
Spring	container	top	y = -0.60x + 7.36	62.41	12
		middle	y = -0.67x + 9.69	77.44	14
		bottom	y = -0.11x + 9.14	92.16	83
		control	y = -0.06x + 9.19	53.29	153
	windrow	top	y = -0.54x + 9.82	73.96	18
		middle	–	–	5*
		bottom	y = -0.06x + 9.35	53.29	156
		control	y = -0.05x + 9.83	70.56	197
Summer	container	top	y = -0.95x + 5.67	51.84	6
		middle	–	–	nd (after 2 d)
		bottom	y = -0.90x + 9.37	79.21	10
		control	y = -0.27x + 8.80	72.25	33
	windrow	top	–	–	nd (after 2 d)
		middle	–	–	nd (after 2 d)
		bottom	–	–	nd (after 2 d)
Autumn	container	top	y = -0.52x + 8.60	72.25	17
		middle	y = -0.77x + 8.79	70.56	11
		bottom	y = -0.21x + 9.45	79.21	45
		control	y = -0.07x + 9.28	77.44	133
	windrow	top	y = -0.59x + 8.03	82.81	14
		middle	–	–	5*
		bottom	y = -0.16x + 8.43	57.76	53
		control	y = -0.16x + 8.35	47.61	52

nd – tested bacteria not detected

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

survival calculated on the basis of regression lines in the top part was 6 days, and it was only 4 days longer in the bottom part (Tab. 3). In spite of the high concentration of *E. coli* ($3.17 \cdot 10^8$ MPN/g) in additional carriers introduced into the composting windrow the first analysis carried out two days after its forming did not show the bacteria presence at any level (Tab. 2).

In the autumn cycle, as well as in spring one, *E. coli* died the most quickly in the top parts of the composted material. In the middle part of the biomass, they were not detected after 13 days. At the same time the concentration of the bacteria in the top part was $2.34 \cdot 10^2$ MPN/g, and in the bottom part – $3.83 \cdot 10^6$ MPN/g (Tab. 1). *E. coli* were not found in the windrow after 9 days in the middle part and after two weeks in the top part. In the bottom part of the composted biomass the theoretical time of their survival amounted to 53 days (Tab. 3).This reflects a considerable difference between a daily reduction rate of the tested bacteria in the top part – 0.59 $\log_{10}$ and in the bottom part – 0.16 $\log_{10}$ (Tab. 3).

DISCUSSION

The properly conducted composting process is an effective method of pathogen elimination in the biomass [4, 13]. In the experiment by Savage [14] the author reported a reduction in coliform concentration from 10^7 to 10^3 cfu/g during 35 days of the composting process. Similar results were obtained by Hassen [9]. After the stage of hygienization he observed a decrease in *E. coli* concentration from $2 \cdot 10^7$ to $3.1 \cdot 10^3$ cfu/g d.m.

The present studies, conducted during the spring and summer, also confirmed a high efficiency of the composting process in indicator bacteria inactivation, but only in the top and middle layers of the biomass. In both layers the survival rates of *E. coli* ranged from 12 days in spring to 17 days in autumn (Tab. 3). The highest effectiveness of the composted biomass hygienization was noticed in summer. After 24 h a reduction in indicator bacteria concentration in the top layers of the biomass amounted to 7 $\log_{10}$. The elimination rate of *E. coli* was particularly rapid in additional carriers introduced into the compost windrow. The total inactivation of the microorganisms occurred within 48 h (Tab. 2). This was mainly due to the rise in temperature from 39°C to 66°C in the composted material (Fig. 1). Numerous authors point out the decisive effect of high temperature on pathogen elimination [6, 17, 18]. A high temperature should be obtained in all parts of the biomass [4].

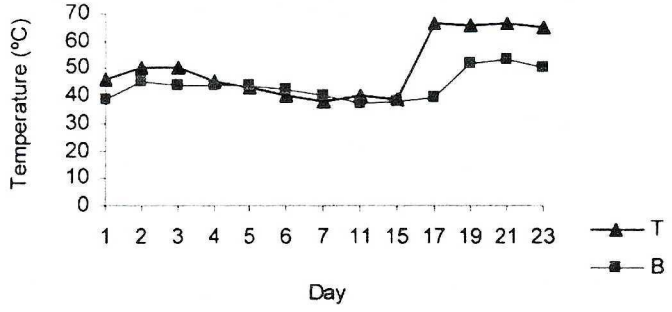

Fig. 1. Temperature distribution in composted biomass in the container and in the windrow in the top (T) and bottom (B) layers – summer cycle

Tateda [20] reported that during the composting process in a closed reactor the highest temperature occurred in the top part, and the lowest in the bottom part of the material. Similar observations were made in the studies conducted in spring and autumn after building a compost windrow. In the top layer of material the temperature was remarkably higher than in the bottom (Figs 2 and 3). It might have resulted from improper material aeration. Such temperature distribution caused a notably slower course of *E. coli* elimination in the bottom part of the biomass. Epstein [5] also found out that a number of fecal coliforms was high in the lower parts of the compost pile, which he ascribed to the excessively low temperature generated in the biomass.

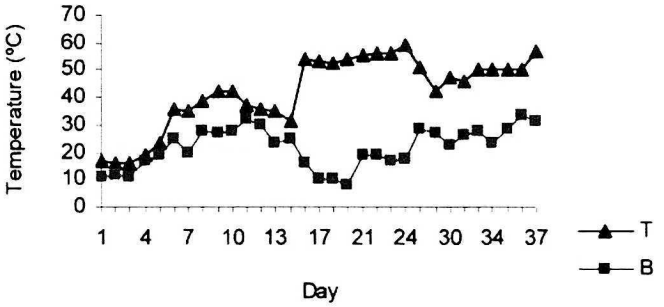

Fig. 2. Temperature distribution in composted biomass in the container and in the windrow in the top (T) and bottom (B) layers – spring cycle

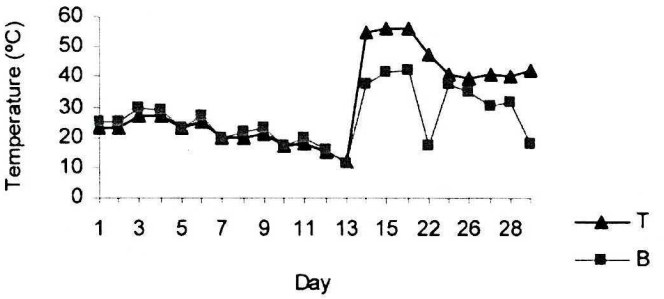

Fig. 3. Temperature distribution in composted biomass in the container and in the windrow, in the top (T) and bottom (B) layers – autumn cycle

In the spring and autumn cycles, a decrease in the bacteria number was observed in the top and middle parts of the biomass in the container, though the temperature obtained was lower than required. This indicates the presence of other important factors such as competition, antagonism or antibiosis. Competition among microorganisms for available carbon and nitrogen compounds can be an essential mechanism destroying pathogens in the compost [1, 9]. Being in competition for food with organisms which naturally occur in the compost, pathogens are definitely weaker rivals. The antagonistic effect of autochthonic microorganisms is strongest during initial composting stages and decreases slowly during compost maturation [16]. Such interactions might have caused *E. coli* inactivation in the biomass intensive composting in the container in the present study.

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INAKTYWACJA PAŁECZEK *ESCHERICHIA COLI* PODCZAS KOMPOSTOWANIA ODPADÓW ORGANICZNYCH WRAZ Z OSADEM ŚCIEKOWYM

Badano wpływ procesu kompostowania w kontenerowej technologii Kneer na inaktywacje pałeczek *E. coli*. Bakterie, w specjalnych nośnikach, wprowadzano do warstwy górnej, środkowej i dolnej kompostowanego materiału. Doświadczenia prowadzono w cyklu wiosennym, letnim i jesiennym. Tempo eliminacji pałeczek coli było zróżnicowane i zależało zarówno od usytuowania nośników w biomasie, jak i warunków cieplnych. Higienizacja materiału nastąpiła najszybciej w cyklu letnim – po 48 godz. w warstwie środkowej, 6 dniach w górnej i 10 dniach w dolnej. Wiosną i jesienią, bakterie najdłużej przeżywały w warstwie dolnej – odpowiednio 83 i 45 dni. Badania wskazują również na działanie, oprócz wysokiej temperatury, innych czynników, takich jak konkurencja, antagonizm czy antybioza.