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ORIGINAL ARTICLE

Use of the Filter-Sandwich carriers in continuous effectiveness monitoring of slurry treatment methods as an element improving biosafety in agriculture

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Abstract

Slurry, due to high microbiological contamination, requires hygienization before spreading. The agricultural usage of treated slurry has to guarantee biosafety. Therefore, constant monitoring of the slurry treatment process should be conducted. The use of Filter-Sandwich carriers seems to be a prospective solution. The aim of the research was to test whether Filter-Sandwich carriers influence the survivability of microorganisms during the slurry hygienization process and hence, whether they are safe for the environment. Raw cattle and swine slurry with different dry matter content was the research material. *Salmonella* Senftenberg W_{775} rods were introduced directly into the slurry and into the carriers placed in the liquid excrements stored at 4 and 20 °C, and underwent anaerobic digestion at 35 °C. The number of tested bacteria obtained from the slurry and carriers was determined using the MPN method with proper microbiological media. The values of physicochemical parameters of the raw and treated slurry were determined, both for the carriers and for slurry only. Biosafety control was also conducted for the carriers in slurry containers. The differences in the theoretical survivability between *Salmonella* Senftenberg W_{775} re-isolated from the slurry and the carriers, and in the values of the selected physicochemical parameters obtained at the end of the process, were not statistically significant. The re-contamination of the sterile slurry caused by the bacteria in the carrier was not observed after placement of the carrier with inoculated material. The conducted research proves the usefulness of Filter-Sandwich carriers for continuous hygienization monitoring of the slurry treatment process. This refers not only to the semi-technical scale, but also to the full-scale process.

Key words

Filter-Sandwich carrier, survivability of the *Salmonella* Senftenberg W_{775}, slurry storage, mesophilic anaerobic digestion

INTRODUCTION

Slurry is a valuable natural fertilizer commonly used in agriculture [1]. As it may be contaminated by many microbial and parasitological agents, its application should be preceded by effective hygienization [2].

The pathogens most often isolated from slurry include: *Salmonella* spp., *Escherichia coli* (enteropathogenic strains resistant to antibiotics), *Campylobacter* spp., and others [3, 4]. Many viruses and parasite eggs can also be found in liquid livestock excrements [4, 5]. The presence of high quantities of these pathogens may pose a significant sanitary and hygienic threat.

The above data indicates that proper hygienization of slurry before its agricultural use is crucial for environmental biosafety. The methods applied for slurry treatment require procedures for evaluation of their efficiency. The major difficulty in the treatment efficiency monitoring is providing slurry homogeneity in the whole container, and obtaining the representative sample for analyses.

The use of bacteriological Filter-Sandwich carriers according to Rapp [6], made of polycarbonate with nitrocellulose filtering membranes, seem to be a good solution. Placing the microorganisms in these carriers guarantees the influence of the outside environment factors on the bacteria inside, and at the same time preventing the surrounding slurry from microbiological contamination. This makes it possible to continuously monitor any method of slurry treatment.

The aim of this study was to estimate whether the Rapp Filter-Sandwich carriers have an effect on the survivability of the microorganisms in hygienized slurry. This was conducted on the basis of comparison of the inactivation rates for *Salmonella* Senftenberg W_{775} rods, introduced directly into the slurry and the carriers. Two methods of slurry treatment were examined.

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MATERIALS AND METHODS

FILTER-SANDWICH carrier construction. The Filter-Sandwich carrier according to Rapp is made of a tube from polycarbonate with screws at both ends. The carrier is closed with nitrocellulose membranes of 0.2 µm diameter pores at each end. The membranes are assembled on the perforated polycarbonate discs. Between the interior disc and the filter, a silicone seal is placed. The whole tube is screwed with special nuts (Photo 1).

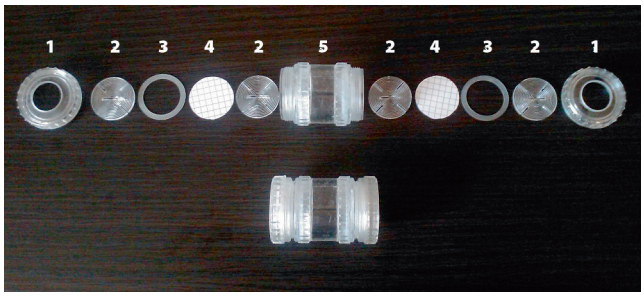

Photo 1. FILTER-SANDWICH carrier construction: 1 – nuts; 2 – perforated disc; 3 – silicone seals; 4 – nitrocellulose filters; 5 – carrier capsule].

Tested samples. The material used for the research was cattle and swine slurry samples taken according to the sample collecting instruction on the basis of PN-B-12098:1997 norm. On the basis of the obtained samples, using centrifugation and mixing of the fractions in the appropriate proportions, the slurry with reduced (1–2% – thin slurry), average (5–6% – medium slurry) and increased (14–15% – thick slurry) dry matter content were prepared.

Physicochemical tests. Within the framework of the experiment, both raw slurry and that obtained from the reactor and carriers underwent the basic physicochemical analysis after finishing a particular treatment method. Temperature, pH, REDOX potential, dry matter and organic dry matter content were measured. The measurements were conducted according to the recommendations by Eaton et al. [7].

Preparing of the suspensions. The *Salmonella* Senftenberg W_{775} rods were obtained from The National *Salmonella* Institute in Gdańsk, Poland. After 24 hour growth on standard nutritious agar I, the proper volume of the suspension was prepared using 0.9% saline solution. The obtained density was 4.50×10^{9} CFU×cm^{-3}.

Slurry inoculation. The slurry was inoculated with *Salmonella* Senftenberg W_{775} rods suspension in an amount of 25 cm^3 per 1 dm^3. Next, 30 dm^3 of cattle and swine slurry were placed in separate reactors and 10 cm^3 in the Filter-Sandwich carriers.

Course of treatment procedures. The experiment testing the hygienization efficiency of storage and the mesophilic anaerobic digestion of slurry was conducted on a semi-technical scale.

Storage was performed in 2 variants of temperature: 4 and 20 °C. At each temperature, liquid excrements were inoculated with bacterial suspension and stirred twice a day for 30 minutes. Then, 12 Filter-Sandwich carriers with the inoculated swine and cattle slurry were placed in the reactor chambers. Samples used for tests (from the slurry and from the carriers), were collected after 1 hour (control sample), after 1 day, and then once a week for 70 days, regardless of the slurry type and storage temperature.

In order to assess the mesophilic anaerobic digestion, the cattle and swine slurry samples (30 dm^3 each) were placed in separate reactor chambers and heated to 35°C. Samples were inoculated with *Salmonella* Senftenberg W_{775} rods, and 8 Filter-Sandwich carriers with inoculated slurry of a proper type were introduced. During the experiment, slurry was stirred every 2 hours for 5 minutes with a mechanical stirrer. The produced biogas was passing via a one-way draining valve. The samples were collected after 1 hour (control sample), 1 day, 2 days, and then at 2-day intervals until the 12th day after the inoculation.

Determination of quantities of re-isolated *Salmonella* Senftenberg W_{775} rods. The quantitive assessment of bacteria in the samples was conducted on the basis of counting the most probable number (MPN) of microorganisms in a 3-sample arrangement, using Mc Crady's tables.

In the process of isolating bacilli of the *Salmonella* genus, 1% buffered peptonic water was used for initial multiplication (incubation for 24 hours at 37 °C). Selective enrichment was conducted on the liquid medium according to Rappaport (incubation for 24 hours at 42 °C). BPLS agar was used as the solid growing medium (incubation for 24 hours at 37 °C).

Identification of the reisolated *Salmonella* rods serotypes was conducted according to the shortened version of antigen *Salmonella's* constitution by White- Kauffman-Le Minor (data from 1 January 2007) [8].

Carrier biosafety control. In order to test the biosafety of the Filter-Sandwich method, carriers inoculated with suspension of *Salmonella* Senftenberg W_{775} rods were added to 1 dm^3 of cattle and swine sterilized slurry samples. The samples were stored or underwent methane fermentation, and were then tested for the presence of the bacteria from carriers in the autoclaved slurry.

Statistical analysis of results. The results of physicochemical tests were statistically analyzed with the programme SAS 9.2 PL. The normality of distribution was checked and multi-factor variance analysis conducted using the GLM model. The significance of differences was determined for $p\leq0.05$ and $p\leq0.01$ between tested factors for the slurry from reactor and carriers using Tukey's multiple comparison test.

On the basis of the *Salmonella* Senftenberg W_{775} rods quantity changes, the regression lines equations were defined. According to these equations, theoretical survival time, the time of decimal elimination (DRT) and elimination rate were determined. Afterwards, each of the treatment methods was tested for significance of differences between parameters describing inactivation kinetics of the tested rods, both from the slurry and carriers, using Tukey's test (programme SAS 9.2.PL).

RESULTS

The results of physicochemical analyses are presented in Figures 1–5. Values of parameters determined both for slurry obtained directly from the reactor and from Filter-Sandwich

carriers were similar, and observed differences were not statistically significant at the end of the study. This tendency was maintained for each of the examined slurry treatment methods, regardless of its dry matter content, and allowed the observation that the parameters inside the carrier and in the external environment were almost the same.

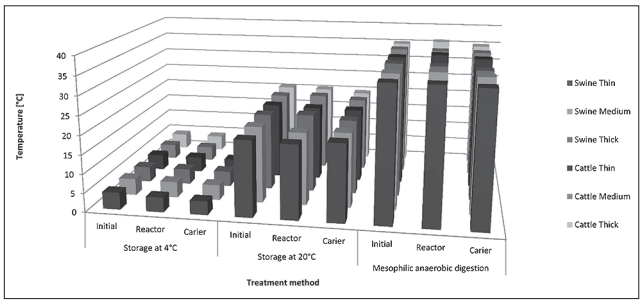

Figure 1. Changes in temperature during slurry treatment

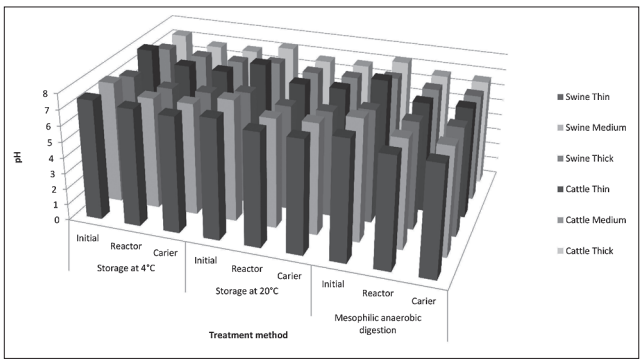

Figure 2. Changes in pH value during slurry treatment

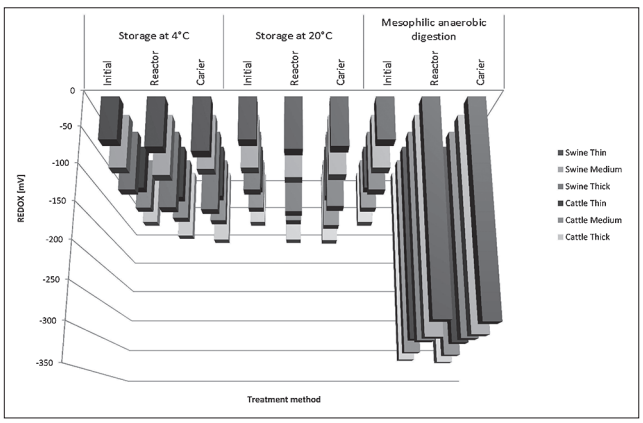

Figure 3. Changes in REDOX potential during slurry treatment

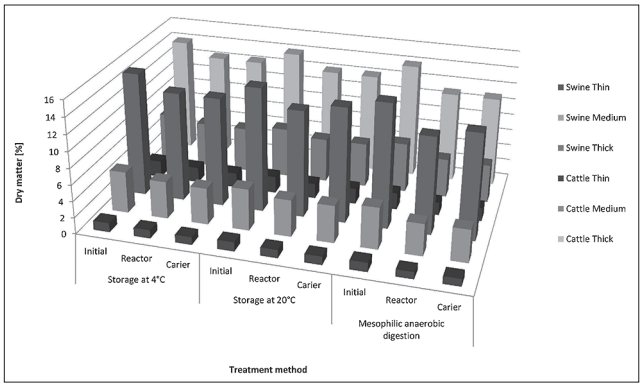

Figure 4. Changes in dry matter content during slurry treatment

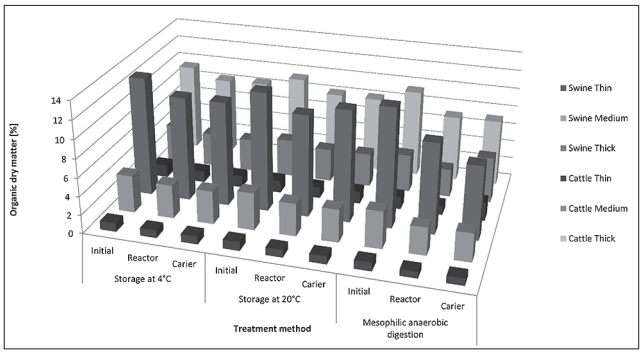

Figure 5. Changes in organic dry matter content during slurry treatment

The isolated microorganisms growing on BPLS agar represented the serotype *Salmonella* Senftenberg W_{775}. This was proved by the positive result of tests with the sera HM; OE_4; O19; Hf,g; Hs and Ht and negative with the O15 and Hf, as well as a negative result of the test for autoaglutination [8].

Control tests, aimed at checking the proof against leaks of the carrier, conducted for both hygienization methods, did not indicate the presence of *Salmonella* Senftenberg W_{775} rods in samples of the autoclaved cattle and swine slurry.

During slurry storage, regardless of the temperature, dry matter content and type of the liquid excrements, a gradual decrease in the *Salmonella* Senftenberg W_{775} rod quantity was observed, regardless of the inoculation method (Tab. 1–2).

Table 1. Changes in number of *Salmonella* Senftenberg W_{775} in particular sampling terms during storage at 4 °C.

Sampling terms [days]	STORAGE AT 4 °C					
	Cattle slurry					
	Thin		Medium		Thick	
	Reactor	Carrier	Reactor	Carrier	Reactor	Carrier
0	4.47×10^8	9.55×10^8	7.59×10^8	9.55×10^8	7.59×10^8	4.47×10^8
1	3.16×10^8	7.24×10^8	4.90×10^8	5.75×10^8	5.75×10^8	3.63×10^8
7	2.14×10^8	5.01×10^8	2.69×10^8	3.24×10^8	2.14×10^8	1.29×10^8
14	1.45×10^8	3.47×10^8	1.45×10^8	1.82×10^8	8.13×10^7	5.50×10^7
21	8.13×10^7	1.70×10^8	7.94×10^7	1.00×10^8	3.63×10^7	3.16×10^7
28	4.27×10^7	7.76×10^7	4.07×10^7	5.25×10^7	2.14×10^7	1.70×10^7
35	2.14×10^7	3.47×10^7	2.40×10^7	2.82×10^7	1.32×10^7	9.55×10^6
42	8.32×10^6	1.51×10^7	1.29×10^7	1.55×10^7	8.13×10^6	6.17×10^6
49	3.31×10^6	6.17×10^6	6.92×10^6	8.71×10^6	5.37×10^6	4.07×10^6
56	1.17×10^6	2.24×10^6	3.80×10^6	4.47×10^6	3.89×10^6	2.75×10^6
63	3.89×10^5	7.41×10^5	2.04×10^6	2.40×10^6	2.69×10^6	1.91×10^6
70	1.15×10^5	2.24×10^5	1.10×10^6	1.29×10^6	1.95×10^6	1.38×10^6
	Swine slurry					
0	7.59×10^8	7.59×10^8	9.55×10^8	9.55×10^8	7.59×10^8	9.55×10^8
1	4.47×10^8	4.90×10^8	3.89×10^8	4.68×10^8	5.01×10^8	6.17×10^8
7	2.63×10^8	3.02×10^8	1.48×10^8	1.82×10^8	1.00×10^8	1.35×10^8
14	1.58×10^8	1.35×10^8	5.75×10^7	6.92×10^7	2.04×10^7	2.14×10^7
21	6.17×10^7	5.01×10^7	2.19×10^7	2.57×10^7	7.94×10^6	8.71×10^6
28	2.09×10^7	1.58×10^7	8.13×10^6	9.55×10^6	3.24×10^6	4.07×10^6
35	6.03×10^6	4.37×10^6	3.09×10^6	3.55×10^6	1.48×10^6	2.04×10^6
42	1.51×10^6	1.17×10^6	1.12×10^6	1.29×10^6	7.76×10^5	1.10×10^6
49	3.02×10^5	2.88×10^5	4.17×10^5	4.79×10^5	4.47×10^5	6.03×10^5
56	5.62×10^4	6.03×10^4	1.58×10^5	1.82×10^5	3.02×10^5	3.39×10^5
63	1.00×10^4	1.17×10^4	5.89×10^4	6.76×10^4	1.62×10^5	1.95×10^5
70	1.58×10^3	2.19×10^3	2.19×10^4	2.51×10^4	1.04×10^5	1.23×10^5

Table 2. Changes in number of *Salmonella* Senftenberg W_{775} in particular sampling terms during storage at 20 °C.

Sampling terms [days]	STORAGE AT 20 °C					
	Cattle slurry					
	Thin		Medium		Thick	
	Reactor	Carrier	Reactor	Carrier	Reactor	Carrier
0	9.55×10^{8}	9.55×10^{8}	9.55×10^{8}	9.55×10^{8}	9.55×10^{8}	7.59×10^{8}
1	4.90×10^{8}	5.50×10^{8}	5.62×10^{8}	3.39×10^{8}	7.24×10^{8}	5.01×10^{8}
7	2.45×10^{8}	2.75×10^{8}	1.38×10^{8}	8.91×10^{7}	3.16×10^{7}	3.24×10^{7}
14	1.15×10^{8}	1.29×10^{8}	3.47×10^{7}	2.40×10^{7}	2.63×10^{6}	2.75×10^{6}
21	2.34×10^{7}	3.09×10^{7}	8.51×10^{6}	6.31×10^{6}	6.61×10^{5}	5.01×10^{5}
28	4.47×10^{6}	6.03×10^{6}	2.09×10^{6}	1.58×10^{6}	1.95×10^{5}	1.82×10^{5}
35	8.32×10^{5}	1.07×10^{6}	5.13×10^{5}	4.27×10^{5}	6.76×10^{4}	7.59×10^{4}
42	1.41×10^{5}	1.70×10^{5}	1.23×10^{5}	1.12×10^{5}	3.02×10^{4}	3.39×10^{4}
49	2.00×10^{4}	2.63×10^{4}	3.16×10^{4}	3.09×10^{4}	1.55×10^{4}	1.58×10^{4}
56	2.75×10^{3}	3.89×10^{3}	8.13×10^{3}	8.51×10^{3}	8.75×10^{3}	9.33×10^{3}
63	3.24×10^{2}	4.47×10^{2}	2.04×10^{3}	2.24×10^{3}	5.37×10^{3}	5.75×10^{3}
70	3.02×10^{1}	3.31×10^{1}	4.90×10^{2}	5.62×10^{2}	3.55×10^{3}	3.72×10^{3}
	Swine slurry					
0	9.55×10^{8}	7.59×10^{8}	9.55×10^{8}	9.55×10^{8}	4.47×10^{8}	7.59×10^{8}
1	7.59×10^{8}	5.01×10^{8}	1.91×10^{8}	3.55×10^{8}	2.82×10^{8}	5.01×10^{8}
7	4.07×10^{8}	2.45×10^{8}	3.31×10^{7}	6.03×10^{7}	8.91×10^{6}	1.53×10^{7}
14	7.59×10^{7}	3.31×10^{7}	5.62×10^{6}	1.05×10^{7}	5.37×10^{5}	7.24×10^{5}
21	5.89×10^{6}	3.16×10^{6}	9.33×10^{5}	1.70×10^{6}	7.94×10^{4}	9.77×10^{4}
28	3.55×10^{5}	2.63×10^{5}	1.66×10^{5}	2.69×10^{5}	1.95×10^{4}	2.24×10^{4}
35	2.00×10^{4}	2.00×10^{4}	2.95×10^{4}	4.37×10^{4}	5.25×10^{3}	5.89×10^{3}
42	1.07×10^{3}	1.32×10^{3}	5.37×10^{3}	7.41×10^{3}	1.62×10^{3}	2.04×10^{3}
49	5.12×10^{1}	8.13×10^{1}	9.33×10^{2}	1.20×10^{3}	6.17×10^{2}	8.13×10^{2}
56	0.24×10^{1}	0.46×10^{1}	1.66×10^{2}	2.00×10^{2}	2.82×10^{2}	4.07×10^{2}
63	n.d.*	n.d.	3.02×10^{1}	3.39×10^{1}	1.58×10^{2}	2.09×10^{2}
70	n.d.	n.d.	0.55×10^{1}	0.56×10^{1}	9.20×10^{1}	1.29×10^{2}

* n.d. – not detected

The number of *Salmonella Senftenberg* W_{775} rods introduced directly into slurry stored at 4°C decreased during 70 days of storage by 2.59–5.68 log (Tab. 1). In contrast, at 20°C the decrease amounted to 5.43–8.24 log, and in the case of liquid swine excrements with a low content of dry matter, the tested bacteria were not isolated earier than the 63rd day of the study (Tab. 2). Decrease in the number of *Salmonella Senftenberg* W_{775} was larger in swine slurry than in cattle slurry. The decreasing of dry matter content in excrements created favourable conditions for intensity of elimination of the tested bacteria (Tab. 1–2).

Similar tendencies were found in the tested bacteria introduced into Filter-Sandwich carriers (Tab. 1–2).

The times of theoretical survival and decimal elimination calculated, based on regression line equations (Fig. 6–9), were longer at 4 °C than at 20°C, regardless of *Salmonella Senftenberg* W_{775} introduction method. Also, they survived longer in cattle slurry than in swine slurry (Tab. 3).

The theoretical survival time of *Salmonella Senftenberg* W_{775} rods introduced directly into slurry remained within the range of 61.91–237.83 days, whereas in Filter-Sandwich carriers it amounted to 62.98–238.79 days, depending on the experiment variant (Tab.3). Observed differences in theoretical survival time, between volume of reactor and carriers inside, did not exceed 2.5 days, and were not statistically significant (Tab. 3).

The DRT value for the *Salmonella Senftenberg* W_{775} populations introduced into slurry were within the range 6.51–27.78 days, and for those placed in the carriers it amounted to 6.80–28.49 days, depending on the storage temperature, the type of liquid excrements and dry matter content (Tab. 3). Observed differences were not statistically significant (Tab. 3).

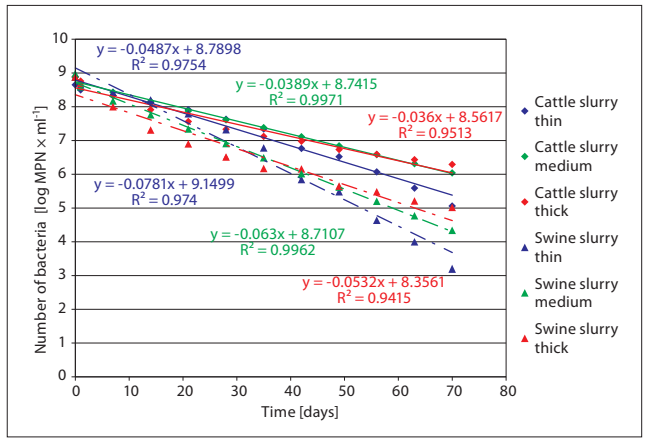

Figure 6. Regression lines illustrating changes of *Salmonella* Senftenberg W_{775} number in reactor during slurry storage at 4 °C

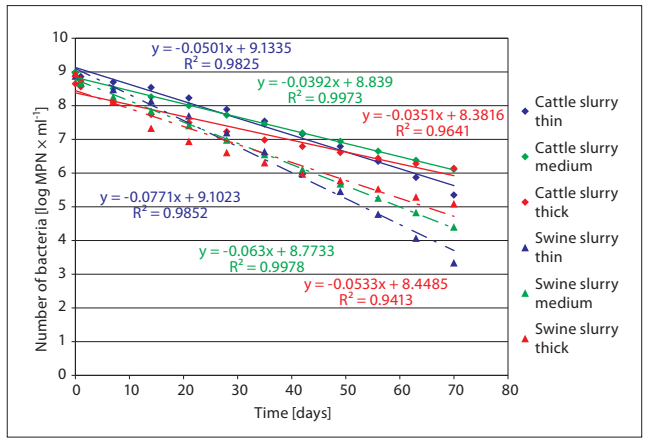

Figure 7. Regression lines illustrating changes of *Salmonella* Senftenberg W_{775} number in carrier during slurry storage at 4 °C

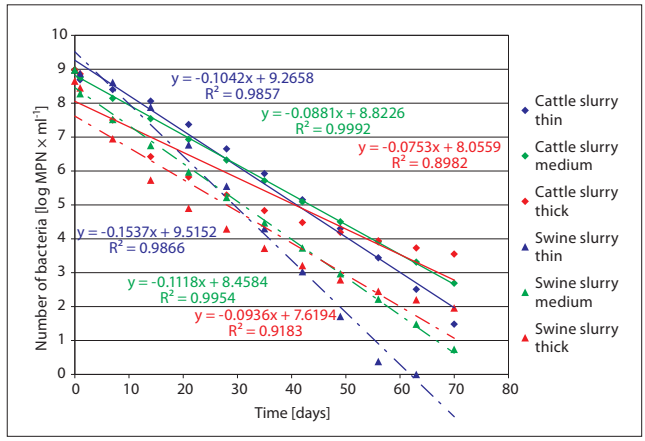

Figure 8. Regression lines illustrating changes of *Salmonella* Senftenberg W_{775} number in reactor during slurry storage at 20 °C

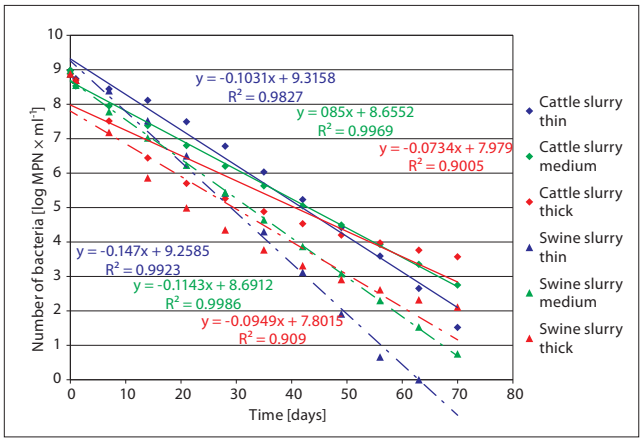

Figure 9. Regression lines illustrating changes of *Salmonella* Senftenberg W_{775} number in carrier during slurry storage at 20 °C

Table 3. Statistical parameters describing the inactivation kinetic of *Salmonella* Senftenberg W_{775} introduced into the reactor and the Filter-Sandwich carrier

Method	Slurry type	Slurry density	Place	Theoretical survival time [days]*	Elimination rate [log×day^{-1}]*	DRT [days]*
Storage at 4 °C	Swine	Thin	Reactor	117.16	0.08	12.80
			Carrier	118.06	0.08	12.97
		Medium	Reactor	138.27	0.06	15.87
			Carrier	139.26	0.06	15,87
		Thick	Reactor	157.07	0.05	18.80
			Carrier	158.51	0.05	18.76
	Cattle	Thin	Reactor	180.49	0.05	20.53
			Carrier	182.31	0.05	19.96
		Medium	Reactor	224.72	0.04	25.71
			Carrier	225.48	0.04	25.51
		Thick	Reactor	237.83	0.04	27.78
			Carrier	238.79	0.04	28.49
Storage at 20 °C	Swine	Thin	Reactor	61.91	0.15	6.51
			Carrier	62.98	0.15	6.80
		Medium	Reactor	75.66	0.11	8.94
			Carrier	76.04	0.11	8.75
		Thick	Reactor	81.40	0.09	10.68
			Carrier	82.21	0.09	10.54
	Cattle	Thin	Reactor	88.92	0.10	9.60
			Carrier	90.36	0.10	9.70
		Medium	Reactor	100.14	0.09	11.35
			Carrier	101.83	0.09	11.76
		Thick	Reactor	106.98	0.08	13.28
			Carrier	108.71	0.07	13.62
Mesophilic anaerobic digestion	Swine	Thin	Reactor	18.42	0.51	1.94
			Carrier	18.91	0.49	2.03
		Medium	Reactor	19.92	0.45	2.24
			Carrier	20.59	0.44	2.30
		Thick	Reactor	21.57	0.39	2.55
			Carrier	22.05	0.37	2.71
	Cattle	Thin	Reactor	22.14	0.42	2.37
			Carrier	23.11	0.40	2.50
		Medium	Reactor	22.93	0.39	2.59
			Carrier	23.93	0.36	2.77
		Thick	Reactor	24.83	0.34	2.93
			Carrier	25.64	0.33	3.04

* – statistically significant differences ($p \leq 0.05$) between values of parameters for reactor volume and carriers are not shown

In slurry subjected to anaerobic digestion conducted at 35 °C, a gradual elimination of the tested bacteria was observed, regardless of the method for introduction of *Salmonella Senftenberg* W_{775} and of the type and density of liquid excrements (Tab. 4).

The observed declines in the number of population of the tested rods, regardless of the way of introducing the inoculum, were higher in swine slurry than in cattle slurry, and increased together with a decrease in the dry matter content in the liquid excrements (Tab. 4). The number of *Salmonella Senftenberg* W_{775} rods introduced directly into the slurry during 12 days of mesophilic anaerobic digestion decreased by 3.97–6.02 log, and the number of those placed in the carriers by 3.92–5.79 log (Tab. 4).

Table 4. Changes in number of *Salmonella* Senftenberg W_{775} in particular sampling terms during mesophilic anaerobic digestion at 35 °C.

Sampling terms [days]	MESOPHILIC ANAEROBIC DIGESTION (35 °C)					
	Cattle slurry					
	Thin		Medium		Thick	
	Reactor	Carrier	Reactor	Carrier	Reactor	Carrier
0	9.55×10^8	9.55×10^8	7.59×10^8	7.59×10^8	7.59×10^8	7.59×10^8
1	7.24×10^8	6.03×10^8	4.27×10^8	2.14×10^8	4.68×10^8	3.98×10^8
2	5.37×10^8	4.27×10^8	8.32×10^7	5.13×10^7	3.72×10^7	3.09×10^7
4	7.94×10^7	6.46×10^7	1.66×10^7	1.05×10^7	2.82×10^6	3.09×10^6
6	1.00×10^7	9.12×10^6	3.16×10^6	2.57×10^6	8.91×10^5	9.77×10^5
8	1.12×10^6	1.23×10^6	5.50×10^5	5.01×10^5	3.16×10^5	3.80×10^5
10	1.20×10^5	1.62×10^5	1.02×10^5	1.07×10^5	1.38×10^5	1.82×10^5
12	1.20×10^4	2.04×10^4	1.82×10^4	2.69×10^4	8.13×10^4	9.12×10^4
	Swine slurry					
0	9.55×10^8	9.55×10^8	9.55×10^8	9.55×10^8	4.47×10^8	4.47×10^8
1	6.17×10^8	5.62×10^8	4.68×10^8	5.37×10^8	3.63×10^8	1.86×10^8
2	4.47×10^8	3.02×10^8	6.17×10^7	7.76×10^7	2.69×10^7	9.77×10^6
4	7.59×10^7	3.47×10^7	8.91×10^6	1.55×10^7	3.55×10^6	1.12×10^6
6	5.25×10^6	3.24×10^6	1.29×10^6	2.63×10^6	7.24×10^5	3.16×10^5
8	3.16×10^5	2.95×10^5	2.40×10^5	2.57×10^5	1.48×10^5	1.00×10^5
10	1.74×10^4	2.24×10^4	2.81×10^4	4.07×10^4	3.80×10^4	3.55×10^4
12	9.12×10^2	1.55×10^3	3.89×10^3	6.17×10^3	1.20×10^4	1.48×10^4

In the process of mesophilic fermentation, the theoretical time of survival and decimal elimination calculated on the basis of regression lines equations (fig. 10–11) for *Salmonella Senftenberg* W_{775} rods, regardless of the way of their introduction, were longer in cattle excrements and increased along with an increase in the proportion of dry matter (Tab. 3).

Theoretical survival times of *Salmonella Senftenberg* W_{775} remained within the range 18.42–24.84 days for bacteria introduced directly into slurry, and 18.91–25.64 days for those placed in the carriers (Tab. 3).

The time needed to decrease the quantity of *Salmonella* Senftenberg W_{775} rods introduced into the reactor by 90% was 1.94–2.93 days, and for those placed in carriers 2.03- 3.04 days, depending on the type of slurry and the content of dry matter (Tab. 3).

The differences in theoretical survivability, elimination rate and times of decimal reduction of *Salmonella Senftenberg* W_{775} introduced directly into slurry and placed in carriers were small and statistically non-significant (Tab. 3).

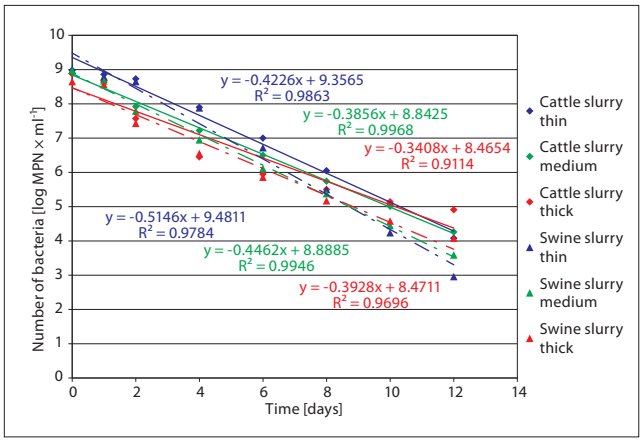

Figure 10. Regression lines illustrating changes of *Salmonella* Senftenberg W_{775} number in reactor during slurry anaerobic digestion at 35 °C

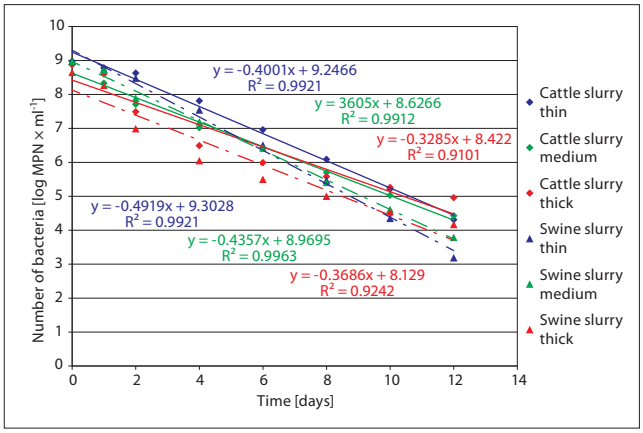

Figure 11. Regression lines illustrating changes of *Salmonella* Senftenberg W_{775} number in carrier during slurry anaerobic digestion at 35 °C

DISCUSSION

The agricultural use of improperly treated slurry may cause the spread of pathogenic factors and the antibiotic resistance among the environmental microflora [9]. Therefore, the proper monitoring of hygienization processes has a high priority. Control of the end product is frequently insufficient and the continuous monitoring creates numerous technical difficulties.

Assessment of the hygienization effectiveness of any slurry treatment method with Filter-Sandwich carriers prevents recontamination of the slurry and guarantees proper efficiency control. This makes it possible to correct the parameters of the ongoing process. However, there are doubts about whether the carriers have a protective effect on indicator microorganisms by increasing their survivability.

The most important factors limiting the bacteria survivability during any of the slurry hygienization methods are: physical and chemical parameters of the material, presence of antagonistic microflora, and the concentration of the indicator microorganisms and their features [10]. The conducted physicochemical tests showed no statistically important differences in the temperatures, pH, REDOX potential and dry matter or organic dry matter contents recorded at the end of the process. This indicates that the use of the carriers does not isolate tested organisms form the environment and factors limiting their survivability.

In the conducted experiment, the indicator organisms were *Salmonella* Senftenberg W_{775}. These rods are characterized by thermo-resistance, and their specific antigenic structure makes their identification simple [11, 12].

The conducted research proved the influence of storage temperature, slurry type and dry matter content on the survivability of *Salmonella* Senftenberg W_{775} rods. It is worth noting that the observed tendencies were analogical for bacteria introduced directly into the reactor and for those placed in carriers. This is consistent with the findings of other authors.

In the presented study, lower storage temperature stabilized the *Salmonella* Senftenberg W_{775} rods survivability. Budzińska [13] also reported that for cattle slurry stored at 4 °C *Salmonella* Senftenberg rods survived for 42 days and at 20 °C – 36 days [13]. These times were visibly shorter than those in the presented study. In the research by Mitscherlich and Marth [14] these bacteria were isolated from the slurry for 18–63 days, depending on the temperature of storage. Olszewska et al. [15] observed the visible impact of temperature on the *Salmonella* Typhimurium rods survivability. Use of higher temperatures increases the storage hygienic efficiency. Ahmed and Sorensen [16] proved that *Salmonella* Typhimurium can survive for 7 days at 49 °C, at least 20 days at 38 °C, and more than 62 days at 22 °C. Himathongkhama et al [17] observed the variety of decimal elimination time (DRT), depending on the temperature of liquid excrement storage.

Many authors [4, 18, 19, 20] have observed the influence of the type of slurry on the bacteria survivability, and that *Salmonella* rods can survive for 110 days in stored swine excrement [19] and for 286 days in cattle excrement [20]. According to the research by Strauch [4], *Salmonella* survive for the longest time in cattle slurry, shorter in swine slurry, and they are eliminated at the fastest rate in moist poultry brood and in calf slurry [4]. This tendency is supported by Burton and Turner [18], who proved that *Salmonella* rods can survive in cattle slurry for 200–300 days, and in swine slurry for only 90–120 days. Moreover, in the present study, *Salmonella* Senftenberg W_{775} rods survived longer in cattle slurry, both for the reactor volume and for the Filter-Sandwich carrier.

One of the major factors determining bacteria survivability, apart from temperature and slurry type, was the dry matter content. Both the presented study and the research by Mitscherlich and Marth [14] proved that *Salmonella* Typhimurium rods survivability depends on the dry mass content. In most cases, the higher dry mass content caused an increase in survival time. According to Jones [21], an increase in the solid particle amount from 10 to 50 g×dm^3 increases the *Salmonella* rods survival by 40%. This tendency was true for both the bacteria from the reactor and for those in the carriers.

Another very important factor limiting the survivability of the microorganisms is the slurry pH. The decrease in liquid excrement pH, according to Jones [22], indicates the production of acidic substances toxic for the intestine parasites [22]. The pH of slurry from the reactor and for the carriers was very similar at the end of the experiment.

Numerous factors have an impact on the bacteria survivability during anaerobic digestion. These are: time of hydraulic retention, concentration of the volatile fatty acids, pH, temperature, slurry type, dry mass content and the fermentation type [23].

Tappouni [24] proved that an increase in the volatile fatty acid concentration caused a decrease in the pH and has an influence on the bacteria numbers reduction. Moreover, development of the methane bacteria being able to produce antibiotics shortens the survival time of the pathogens [12].

On the other hand, the research by Kumar et al. [25] indicated that the *Salmonella* Typhi survivability during psychrophilic fermentation was dependent on the dry matter content. Fertilizer consisting of 5–10% dry matter was characterized by bacteria surviving for 20 days, whereas in that with 15% of dry matter they survived for 25 days in the same thermal conditions [25]. A similar tendency was observed in both reactor volume and for the carriers.

The temperature impact is visible in research by Kumar at al. [25]. They claimed that *Salmonella* Typhi rods survive at 18–20 °C anaerobic digestion for 20 days, and increasing the temperature to 35 °C shortened their survival time to 10 days. According to the literature data, *Salmonella* Enteritidis and *Salmonella* Senftenberg rods can be retrieved from slurry at 31 °C for less than 9 days [18]. In contrast, in the presented study *Salmonella* Senftenberg W_{775} theoretical survivability based on regression line equation ranged from 18.42–25.64 days at 35 °C.

The conducted research proves the usefulness of Filter-Sandwich carriers for the continuous hygienization monitoring of the slurry treatment process, not only on the semi-technical scale but also in the full-scale process.

CONCLUSION

Filter-Sandwich carriers proved to be useful in the continuous assessment of the efficiency of slurry hygienization.

The determined differences of the physico-chemical slurry parameters of the reactor slurry and for the carriers observed at the end of the process were insignificant. The results proved that the carrier construction provides the influence of the outside environment on the tested organisms inside.

No protective effect of the Filter-Sandwich carriers was proved, which could increase the survivability of the indicator bacteria placed in carriers compared to those from the reactor.

Microorganisms placed in the carriers did not cause recontamination of the treated slurry, which ensures the biosafety of their use in monitoring.

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