ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2012, Vol 19, No 3, 427-430
www.aaem.pl

Sanitization efficacy of anaerobic digestion and aeration of slurry from the aspect of limiting emission of *Salmonella* into the environment

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Paluszak Z, Skowron K, Olszewska H, Skowron K J, Bauza-Kaszewska J, Gryń G. Sanitization efficacy of anaerobic digestion and aeration of slurry from the aspect of limiting emission of *Salmonella* into the environment. Ann Agric Environ Med. 2012; 19(3): 427-430.

Abstract

The aim of this study was to estimate the usefulness of mesophilic anaerobic digestion and aeration for sanitization of slurry from the aspect of limiting transmission of *Salmonella* into the environment. Material for the study was fresh pig slurry. Collected samples were subjected to anaerobic digestion at 35°C and aeration with an initial temperature of 35°C. The efficacy of both methods was examined based on determination of the elimination rate and theoretical time of survival of *Salmonella* Senftenberg W_{775}, *Salmonella* Enteritidis and *Salmonella* Typhimurium introduced into slurry in carriers of type Filter-Sandwich. Samples for the study were collected every 24 hours and the number of bacilli was determined with the MPN (Most Probably Number) method. The study indicated that fermentation is a more effective method for slurry sanitization. A higher rate of elimination and shorter time of survival of all the tested bacteria was observed, compared with the use of aeration. The experiment allowed us to prove the high sanitization efficacy of both examined methods. They ensure the full elimination of the tested serotypes of *Salmonella* in only slightly more than 10 days. The use of fermentation or aeration as a way of slurry treatment for agricultural purposes makes it possible to obtain a fertilizer which is valuable and safe for humans and the environment.

Key words

salmonelloses, survival rate of *Salmonella*, slurry, anaerobic digestion, aeration, sanitization efficacy

INTRODUCTION

Slurry used for agricultural purposes may contain considerable amounts of pathogenic microorganisms. The microbial pathogens most often isolated from slurry are bacteria of the genus *Salmonella* which still remain one of the main causes of food poisonings in humans [1]. According to the data from the Department. of Agriculture, FSIS of 1998 in the USA, bacilli of *Salmonella* are the ethiological factor of from 300 thousand to even 4 million food poisonings annually [2], whereas the mortality can reach up to 30.6% [3].

The above data prove that salmonellosis constitutes a serious health problem; it is therefore necessary to properly handle natural fertilizers which can lead to the contamination of food and drinking water [4, 5].

Soil intensively fertilized with improperly treated slurry may constitute a highly significant link in the transmission of *Salmonella* bacilli to water and field crops [6]. Depending on the conditions, they can survive in soil from 6–71 weeks [7, 8]. Getting into surface and ground waters, they can pose a direct ethiological factor of salmonelloses in humans, particularly if this phenomenon applies to household intakes of drinking water. Both contaminated water and soil favour transmission of *Salmonella* to plants. Research has indicated that *Salmonella* bacilli can be taken up by the plant root system from the soil solution and through cambium, migrate to the edible parts, e.g. lettuce leaves [9]. Introduced to tomato stems by injection, they got to fruits of those plants, even when the application took place at the stage of flowering and survived there for 49 days [10]. Also, water contaminated by microorganisms and used for the watering or sprinkling of crops can be the source of internal or exterior pollution of fruits and vegetables [11, 12]. In such cases, stomata and damage to plant organs constitute an invasive method [10].

Fertilization with slurry can also pose a threat connected with introducing antibiotic resistant bacteria into the environment [6]. Multiple antibiotic resistant (MAR) bacilli of *Salmonella* can pass on this feature to other bacteria present in the soil (e.g. *Proteus* spp., *Pseudomonas* spp.), or in water through the extrachromosomal plasmid or r-plasmid [13, 14].

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Received: 17 February 2012; accepted: 5 June 2012

The above data indicate the necessity of looking for effective methods for the sanitization of slurry intended for agriculture. The promotion of anaerobic digestion or aeration seems to be the optimal solution for big market farms, from the point of view of the biosafety of humans and animals.

The aim of this study was to assess the sanitization effectiveness of mesophilic anaerobic digestion and aeration with an initial temperature of 35 °C, based on the kinetics of inactivation of bacilli of the genus *Salmonella*.

MATERIAL AND METHODS

Material for the study was fresh pig slurry collected from a pig farm in the Kujawsko-Pomorskie province.

The experiment was carried out on the semi-technical scale in 3 replications using equipment consisting of a laboratory bioreactor BIOMER 10, allowing both aeration of the batch and providing anaerobic conditions.

Each time, 10 dm^3 of slurry was subjected respectively to mesophilic anaerobic digestion (35 °C) and aeration with an initial temperature of 35 °C. The intensity of aeration ensured maintaining the amount of oxygen dissolved in slurry on a level of 1 $mg \times dm^{-3}$.

Bacilli of *Salmonella* Senftenberg W_{775}, *Salmonella* Enteritidis and *Salmonella* Typhimurium were used for the study. The bacteria were initially multiplied in nutrient broth (24-hour incubation at 37 °C) obtaining initial suspensions with concentrations from 1.78×10^{10} – 2.40×10^{10} $cfu \times cm^{-3}$. Using suspensions of such high concentrations allowed observation of the sanitization effectiveness of the selected methods under conditions of the extreme level of slurry contamination. Carriers of the Filter-Sandwich type, containing 10 cm^3 of slurry, each inoculated with the tested microorganisms, were placed in the slurry subjected to anaerobic digestion aeration. Both in the case of anaerobic digestion and aeration, samples from the bioreactor were collected after 1 hour after inoculation (zero sample), and then at 1-day intervals for the period of 5 days. In the process of re-isolation of bacilli of the genus *Salmonella* 1% buffered peptone water was used for initial multiplication (24-hour incubation at 37 °C). Selective multiplication was carried out on the liquid medium according to Rappaport (24-hour incubation at 43 °C). The BPLS agar was used for growth on the solid medium (24-hour incubation at 37 °C). The number of *Salmonella* was determined based on the MPN method in the 3-tube design. The final identification was conducted using the kit of diagnostic sera according to the scheme of Kauffmann-White.

The results obtained were subjected to statistical analysis involving the determination of regression line equations and calculation of the elimination rate and the theoretical time of survival. Additionally, the significance of differences between the elimination rate and the theoretical time of survival was estimated for both methods by means of Tukey's test using the application SAS 9.2 PL.

RESULTS

The initial microbiological analyses of fresh pig slurry prior to its contamination did not indicate the presence of *Salmonella*.

In slurry subjected to mesophilic anaerobic digestion, a gradual decrease in the population count of all the tested bacilli of the genus *Salmonella* was observed. The initial concentration of the tested microorganisms determined an hour after the inoculation of liquid faeces amounted to 3.02×10^8 $MPN \times cm^{-3}$ and 3.89×10^8 $MPN \times cm^{-3}$, respectively, in the case of *Salmonella* Senftenberg W_{775} and *Salmonella* Typhimurium (Tab. 1); whereas the initial number of *Salmonella* Enteritidis was the highest and amounted to 1.48×10^9 $MPN \times cm^{-3}$ (Tab. 1). After 24 hours of the anaerobic digestion proceeding at 35 °C the population of *Salmonella* Senftenberg W_{775} and *Salmonella* Enteritidis decreased by about 1 log, and that of *Salmonella* Typhimurium by as much as 2 log. On the second day of the experiment, the bacteria of *Salmonella* Senftenberg $W_{775,}$ which were isolated in an amount of 5.89×10^6 $MPN \times cm^{-3}$, occurred most numerously in slurry (Tab. 1). The concentration of *Salmonella* Enteritidis and *Salmonella* Typhimurium after 48 hours of the study remained at the level of 9.30×10^1 $MPN \times cm^{-3}$ and 8.91×10^3 $MPN \times cm^{-3}$, respectively (Tab. 1). After 4 days of anaerobic digestion, the bacteria *Salmonella* Typhimurium were completely eliminated from the tested slurry, whereas the count of populations of the bacteria *Salmonella* Enteritidis and *Salmonella* Senftenberg W_{775} on the fifth day of the anaerobic process was still 0.20×10^1 $MPN \times cm^{-3}$ and 8.71×10^3 $MPN \times cm^{-3}$, respectively (Tab. 1).

Based on the analysis of regression equation (Fig. 1) it was estimated that the bacilli of *Salmonella* Typhimurium were characterized by the shortest theoretical survival time during the mesophilic anaerobic digestion of slurry, amounting to 3.91 days at the elimination rate of 2.23 log $MPN \times day^{-1}$, whereas *Salmonella* Senftenberg W_{775} – by the longest, equal to 9.52 days at the elimination rate of 0.89 log $MPN \times day^{-1}$ (Tab. 2). The bacteria *Salmonella* Enteritidis, in turn, were able to survive for 4.52 days and the daily elimination rate determined for them amounted to 1.90 log MPN.

In slurry subjected to aeration at 35 °C a gradual reduction in the population of all the tested microorganisms was

Table 1. Number of tested *Salmonella* and standard deviation [MPN xdm^3] at individual determination times for methane fermentation and aeration

TIME [days]	0	1	2	3	4	5
BACTERIA						
MESOPHILIC METHANE FERMENTATION						
***S.* Senftenberg W_{775}**	3.02×10^8	2.75×10^7	5.89×10^6	9.33×10^5	7.76×10^4	8.71×10^3
STD	0	1.48×10^6	6.62×10^5	8.13×10^5	4.68×10^3	5.34×10^2
***S.* Enteritidis**	1.48×10^9	3.63×10^8	9.30×10^1	5.30×10^1	1.60×10^1	0.20×10^1
STD	7.87×10^8	4.23×10^7	6.12×10^1	6.90×10^1	0.70×10^1	0.50×10^0
***S.* Typhimurium**	3.89×10^8	9.55×10^6	8.91×10^3	6.90×10^1	nd.[a]	nd.
STD	2.79×10^7	7.86×10^5	1.44×10^3	2.9×10^1	___	___
MESOPHILIC FINE-BUBBLE AERATION						
***S.* Senftenberg W_{775}**	2.34×10^8	1.05×10^7	3.63×10^6	9.55×10^5	9.55×10^4	9.77×10^3
STD	2.93×10^7	2.56×10^6	6.16×10^5	2.15×10^4	5.49×10^3	1.94×10^2
***S.* Enteritidis**	2.69×10^8	4.37×10^7	6.76×10^5	4.90×10^4	7.94×10^3	9.33×10^2
STD	2.93×10^7	2.56×10^6	6.16×10^4	2.15×10^3	5.49×10^2	1.94×10^1
***S.* Typhimurium**	5.37×10^8	9.55×10^6	1.45×10^6	6.03×10^4	4.68×10^3	6.46×10^2
STD	1.8×10^7	1.42×10^6	2.08×10^5	4.50×10^3	2.33×10^2	4.23×10^1

a – not detected

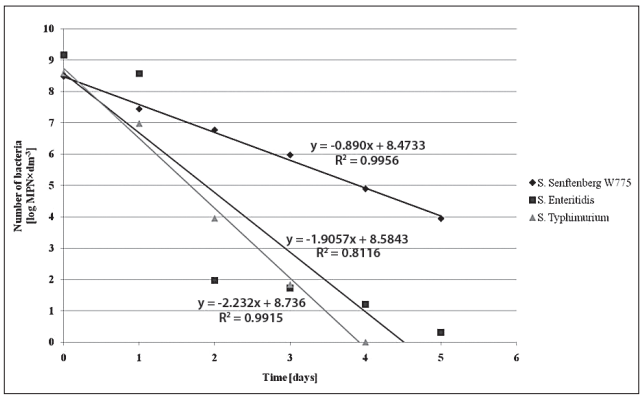

Figure 1. Regression lines equations describing the dynamics of *Salmonella* population during mesophilic anaerobic digestion

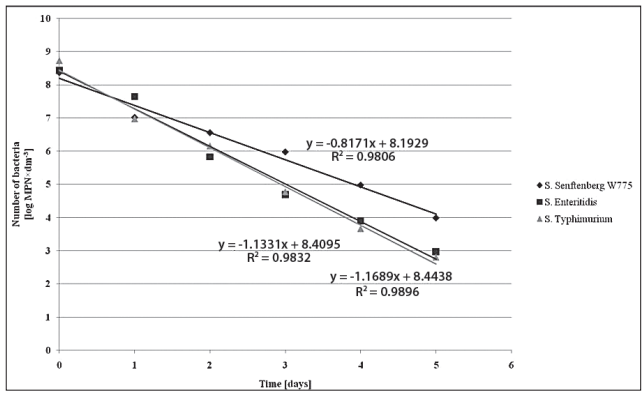

Figure 2. Regression lines equations describing the dynamics of *Salmonella* population during the mesophilic aeration process

Table 2. Results of statistical analysis of microbiological data obtained for both examined methods

BACTERIA	METHOD	ELIMINATION RATE [logMPN×day^{-1}]	R^2	THEORETICAL TIME OF SURVIVAL [days]
Salmonella Senftenberg W_{775}	Fermentation	0.89	0.99	9.52
	Aeration	0.82	0.98	10.03
Salmonella Enteritidis	Fermentation	1.91^{A}	0.81	4.50^{A}
	Aeration	1.13^{B}	0.98	7.42^{B}
Salmonella Typhimurium	Fermentation	2.23^{A}	0.99	3.91^{A}
	Aeration	1.17^{B}	0.99	7.22^{B}

A, B, – highly statistically significant differences ($p \leq 0.01$) determined for the given bacteria between tested methods

also observed. Decreases in the number of the tested bacteria found between successive dates of determination were more balanced, particularly in the case of bacilli *Salmonella* Enteritidis (Tab. 1). The initial concentration of microorganisms was on a level of 10^8 MPN×cm^{-3} (Tab. 1). After 24 hours of aeration, the number of *Salmonella* Senftenberg W_{775} and *Salmonella* Enteritidis decreased by 1 log and amounted to 1.05×10^7 MPN×cm^{-3} and 4.37×107 MPN×cm^{-3}, respectively. The concentration of bacilli of *Salmonella* Typhimurium decreased by 2 log, and their number was 9.55×10^6 MPN×cm^{-3} (Tab. 1). The population of *Salmonella* Enteritidis and *Salmonella* Typhimurium on the second day of aeration was definitely higher than that in slurry subjected to anaerobic digestion and was on a level of 10^5 and 10^6 MPN×cm^{-3}, respectively (Tab. 1). In contrast with fermentation of slurry, in liquid faeces subjected to aeration, each of the tested bacteria was isolated on all the dates of sample collection (Tab. 1). The highest final concentration, amounting to 9.77×10^3 MPN×cm$^{-3,}$ was recorded in the case of *Salmonella* Senftenberg $W_{775,}$ the same as during fermentation, whereas the lowest (6.46×10^2 MPN×cm^{-3}) was found for *Salmonella* Typhimurium (Tab. 1).

The analysis of regression equations (Fig. 2) made it possible to determine that in aerated pig slurry the bacteria *Salmonella* Typhimurium were theoretically able to survive for 7.22 days, *Salmonella* Enteritidis for 7.43 days, and *Salmonella* Senftenberg W_{775} for 10.03 days, at the elimination rate amounting to 1.17, 1.13, and 0.82 log MPN×day^{-1}, respectively (Tab. 2).

The times of survival and the daily elimination rate of bacilli of *Salmonella* Typhimurium and *Salmonella* Enteritidis in the process of slurry aeration were highly statistically significantly longer than those determined for those microorganisms in slurry subjected to mesophilic anaerobic digestion (Tab. 2). Only in the case of bacteria *Salmonella* Senftenberg W_{775} no statistically significant differences were observed (Tab. 2).

On the basis of the conducted study it was found that of the tested microorganisms, *Salmonella* Senftenberg W_{775} bacilli were the most resistant to the sanitization activity of both tested processes, whereas *Salmonella* Typhimurium proved to be the most sensitive (Tab. 1 and 2).

DISCUSSION

In 2009, 8,964 cases of salmonelloses were recorded in Poland, of which as many as 3,464 referred to rural inhabitants [15]. It can be concluded that 23.3 persons per each 100 thousand people living in the country had salmonelloses [15]. In 2009, 45 cases of extra-intestinal salmonellosis were found among people living in agricultural regions [15].

From the conducted study it follows that aeration and anaerobic digestion of liquid animal faeces allow the effective elimination of *Salmonella* bacilli from slurry.

Mesophilic anaerobic digestion proved to be a more effective method for slurry sanitization. The theoretical time of survival of *Salmonella* in the present experiment ranged from 3.91-9.52 days, and did not differ from the literature data. Kumar *et al.* [16] indicated that bacilli of *E. coli* and *Salmonella* Typhi survive in slurry in the process of fermentation at room temperature for 20 days, and during mesophilic fermentation for 10 days, thus longer than in the presented experiment. Bendixen [17], in an experiment carried out on a technical scale, observed that in the process of mesophilic fermentation the number of bacteria in the biomass decreased quickly by 1-2 log. According to the study by Martens *et al.* [18], in the process of anaerobic digestion at 31°C *Salmonella* bacilli were isolated from cattle slurry for 10 hours. Such a short time of survival was probably caused by a lower initial concentration used for the experiment, compared with the presented study. Olsen [19], in turn, found that the bacteria *Salmonella* Typhimurium in pig slurry subjected to fermentation at 35°C at the time of hydraulic retention ranging from 0.8-4.2 days were eliminated within the range 0.8-1.3 log. It should be assumed that decimal reduction time (DRT) during fermentation amounts to 1-2 days for *E. coli* and 2-4 days in the case of the bacteria *Salmonella* Typhimurium [20, 21]. This is also confirmed by

the studies by Olsen and Larsen [22], according to whom the bacilli of *Salmonella* Typhimurium underwent 90% reduction during 2 days in pig slurry after continuous fermentation at 35°C, and during 2.5 days in cattle slurry under the same conditions. Similarly, the decimal reduction time of *Salmonella* Dublin was less than 1 day in pig faeces and 2.2 days in cattle faeces. Paluszak *et al.* [23] report that the theoretical time of survival of *Salmonella* Enteritidis in cattle slurry subjected to mesophilic anaerobic digestion (at 35°C) on a semi-technical scale was 12.94 days, and their daily elimination rate amounted to 0.59 log MPN. A decrease in the number of the tested bacteria by 2 log MPN took place as early as 24 hours [23].

In turn, a longer time of survival of *Salmonella* in aerated slurry obtained in the presented study indicated a lower effectiveness of this method. This was not confirmed in the studies of other authors. Hanajima *et al.* [24] observed that significant changes in the population of intestinal bacteria present in slurry occur as early as after 1 day of aeration, as a result of generating ammonia, growth of pH and competition with developing aerobic microflora. Paluszak [25] showed that the complete sanitization of pig slurry at 40°C and at pH=8.5 occurs after 2 days, and according to Wassen [26], such an effect is obtained under the same conditions as early as after 10 hours.

On the basis of the obtained results it should be concluded that the propagation and application of modern methods for sanitization of liquid animal faeces, to a considerable degree, can improve the bio-safety of humans living in the areas of industrial animal farms, and significantly decrease the risk of spreading salmonelloses through water, soil and agricultural crops.

CONCLUSIONS

1. The presented study proved that both anaerobic digestion and aeration effectively eliminate *Salmonella* in slurry.
2. In slurry subjected to anaerobic digestion, pathogenic microorganisms are eliminated more effectively in comparison with aerated slurry.
3. Differences were observed in the elimination rates of individual serotypes of *Salmonella* in slurry subjected to both methods of sanitization.
4. Application of modern methods of slurry treatment on a wide scale can considerably decrease the degree of transmission of *Salmonella* to the environment.

Acknowledgement

The research was financed from Key Project No. POIG.01.01.02.-00-016/08 'Model agroenergy complexes as an example of distributed cogeneration based on local renewable energy sources'.

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