Comparison of the survival of *Salmonella Typhimurium* in the solid fraction from agricultural wastewater treatment plant in summer and winter seasons.

Comparaison de la survie de Salmonella typhimurium dans la fraction solide d'effluents d'élevage issus d'un traitement, en été et en hiver.

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Abstract

Observations of survival of Salmonella typhimurium in the solid fraction from agricultural wastewater treatment plant was carried out in summer and winter seasons. The Salmonella typhimurium microorganisms tested survived for 26 days in summer and 88 days in winter. In the course of the experiment they decreased by one to two orders of magnitude with the exception of fecal coliform microorganisms which exhibited increase by one order on day 5 of the experiment and subsequent decrease to the original count by day 48 of the experiment. The plate counts of indicator microogranisms in winter were in the range from 10^2 to 10^8 CFU/g of the sample. In the course of the experiment they decreased by one to three orders.

Résumé

Les observations sur la survie de *Salmonella typhimurium* dans la fraction solide d'effluents d'élevage issus d'un traitement, ont été réalisées en période estivale et hivernale. Les bactéries *Salmonella typhimurium* testées ont survucé pendant 26 jours en été et 88 jours en hiver.

Les comptages de microorganismes indicateurs en hiver se situaient de 10^2 à 10^8 UFC / g d'échantillon. Au cours de l'essai, ces microorganismes décroissent d'un facteur 1 à 3.

1. Introduction

The occurence of pathogenic and potentially pathogenic bacteria in an ecosystem is associated mainly with animal production. Especially the farms with literless technology, producing excrements in a liquid form, pose a serious threat in view of the absence of biothermic reaction in the liquid medium, the process which is responsible for devitalization of pathogens in the solid animal manure. An important factor is the high initial count of coliform, mesophilic and psychrophilic germs. Of them, for example *salmonellae* have almost ideal conditions in the slurry for their survival.

One of the possibilities how to overcome the problems mentioned are wastewater treatment plants operating at animal farms. They use technology which starts with separation of the solid fraction of slurry on vibrating screens or press belts. The solid fraction of slurry is transported to field heaps or landfills and can be a source of spreading of many pathogens. It is necessary to realize that animal excrements are the biggest source of organic substances that should be returned to soil. This problem was discussed by J u r i š et al. (1993), N o v á k et al. (1994) who arrived to the conclusion that the most suitable way of processing of the solid fraction is its composting by means of biothermic processes that reach thermophilic temperature range which can guarantee devitalization of pathogenic microorganisms. Despite the recommendations mentioned, insufficient attention is paid to manipulation with the solid fraction of slurry. For this reason we investigated the survival of indicator and tested microorganisms (*Salmonella typhimurium*) in the course of storage of the solid fraction. With regard to the fact that the temperature is one of the most important factors affecting the survival of microorganisms, we carried out investigations of above mentioned parameters in summer and winter seasons.

2. Material and methods

The solid fraction of slurry was obtained from wastewater treatment plant operating at pig farm in Košická Polianka and stored in a 50 l container. The temperature of the environment and inside the substrate was recorded with a programmable registration thermometer (Commeter) made by COMET System, Roznov p. R., Czech Republic, in 1 hour intervals.

A lyophilised *Salmonella typhimurium* strain SK 14/39 (State Health Institute, Prague) was used as a test strain. The revitalized culture was inoculated to leather squares (4 x 4 cm) and directly to the solid fraction which was transferred to PVC bottles provided with side openings to ensure contact with the environment. Dried-up leather pieces were incubated in a thermostat for 24 h at 37^{o}C to achieve good adhesion of salmonellae to the carriers. Both types of carriers were transferred to the container with the solid fraction and were examined in time intervals specified in Tab. 3 and 6 by the method according to M ü l l e r (1973). By means of sterile equipment we transferred the carriers to a sterile 0,9% saline physiological solution

and after 30 min of shaking and 30 min of sedimentation we prepared decimal dilutions up to 10^{-10}. The dilutions were inoculated to XLD and SS agar (Imuna) and incubated for 48 h at 37°C.

At the same time, we examined the carriers qualitatively for the presence of salmonellae using nutrient broth No. 2 (Imuna) for non-selective pre-enrichment and selenite (Imuna) and Rappaport and Vassiliadis media (Merck, Darmstadt) for selective cultivation.

After the incubation, we transferred the colonies to XLD and SS agar (Imuna) and incubated for 48 h at 37°C. Suspected colonies were examined serologically and biochemically.

A glass vial with No. 2 broth, inoculated with the revitalised culture of *Salmonella typhimurium*, was used as a control. Each sampling consisted of examination of 3 carriers and 3 control vials.

At the beginning of the experiment, we examined the solid fraction for the presence of *Salmonella* spp. by cultivation on XLD and SS agar following the previous pre-enrichment in the broth and selective cultivation on Rappaport-Vassiliadis medium (Merck).

The samples of solid fraction (50 g) intended for determination of dynamics of indicator microorganisms and chemical examinations were taken as average samples in time intervals specified in Tab 1 and 3. The examinations of dynamics of indicator microorganisms were carried out according to CSN 83 0531 and included determinations of numbers of psychrophilic, mesophilic, fecal coliform and coliform microorganisms and fecal streptococci. Samples were diluted in 0,9% saline solution up to concentrations 10^{-6}. The quantitative determinations of psychrophilic and mesophilic microorganisms were carried out on nutrient agar No. 2 (Imuna), of fecal coliform and coliform on Endo agar (Imuna) and of fecal streptococci on the selective agar for isolation of fecal streptococci (Imuna).

The examination of physical-chemical parameters, pH, dry matter, ammonia nitrogen and total nitrogen were carried out according to CSN 83 0540 and those of total phosphorus according to the standard methods APHA (1985).

3. Results

At the beginning of the experiment carried out in the summer season (june-august), the numbers of indicator microorganisms in the solid fraction of pig slurry ranged from 10^3 to 10^7 CFU/g sample. The most marked decrease by two orders was observed in psychrophilic microorganisms while the coliform and mesophilic germs decreased by one order only. Fecal coliform microorganisms and fecal streptococci exhibited no changes except for an increase by one order on days 5 and 26 and subsequent decrease to the original value on day 48 for the fecal coliforms. Fecal

streptococci exhibited the same values throughout the experiment with the exception of day 26 when a decrease by one order was observed followed by subsequent increase to the original value (Tab 1).

days	mesophilous CFU/1g	coliforms CFU/1g	psychrophilous CFU/1g	faecal coliforms CFU/1g	faecal streptococci CFU/1g
0.	3,38E+07	6,92E+05	6,67E+07	9,35E+04	2,37E+04
5.	1,64E+07	6,20E+06	4,88E+05	9,76E+05	1,93E+04
26.	1,57E+06	4,52E+06	2,18E+07	8,51E+05	4,63E+03
48.	3,12E+06	6,11E+04	2,71E+05	8,53E+04	5,00E+04
60.	2,01E+06	3,02E+04	3,51E+05	7,02E+04	3,01E+04

Table 1
Dynamics of indicator microorganisms in solid fraction - summer.

The *Salmonella typhimurium* microorganisms tested in this summer season were recovered the longest from the PVC carrier, namely for 26 days, when a change of pH from alkaline to acidic value (from 8,08 to 6,5 – Tab 2) was also recorded. The number of *Salmonella typhimurium* microorganisms in the control glass vial remained the same.

days	pH	dry mater (%)	N-total (g/kg)	$N\text{-}NH_4$ (g/kg)	organic matter(%)	inorganic matter(%)	P-total (g/kg)
0.	8,08	21,37	39,56	0,78	93,2	6,8	1 223,50
5.	7,28	20,02	42,99	0,84	92,74	7,26	1 218,60
26.	6,5	20,66	20,2	0,54	91,8	8,2	1 192,30
48.	5,75	14,96	28,21	0,48	90,88	9,12	934,2
60.	5,8	13,81	31,2	0,52	88,02	10,01	980,9

Table 2
Physico-chemical parameters - summer

The physical-chemical examinations showed marked decrease in pH from the initial value of 8,08 to the final value of 5,80. The values of $N\text{-}NH_4$ showed a decrease with the exception of day 5 of the experiment when a slight increase with regard to initial value was detected. Other values exhibited slight decrease with the exception of inorganic substances the values of which were on slight increase (Tab 2).
The external temperature ranged from 10 to 33^{o}C and that inside the solid fraction from 17 to 26^{o}C.
The numbers of indicator microorganisms in the solid fraction in winter season (january-june) ranged from 10^2 to 10^8 CFU /g sample. During the storage the numbers of psychrophilic and mesophilic microorganisms decreased by one order. The numbers of fecal streptococci and fecal coliforms decreased by two orders and the numbers of coliform microorganisms by one order on day 54 of the experiment, however, the latter increased to the original number by day 120 (Tab 3).

days	mesophilous CFU/1g	coliforms CFU/1g	psychrophilous CFU/1g	faecal coliforms CFU/1g	faecal streptococci CFU/1g
0.	3,01E+07	3,48E+05	2,59E+07	5,32E+05	1,38E+05
4.	5,37E+07	1,58E+05	2,80E+08	7,04E+04	1,80E+05
40.	4,21E+06	2,31E+05	1,87E+08	3,51E+04	1,49E+04
54.	1,73E+05	5,03E+04	7,12E+07	1,29E+04	3,72E+04
76.	6,50E+06	2,55E+04	3,44E+07	1,69E+04	1,50E+04
85.	9,17E+06	3,90E+04	9,64E+06	1,23E+04	2,93E+04
120.	6,32E+05	5,05E+05	9,00E+06	3,00E+02	7,30E+03
137.	3,03E+06	5,19E+05	6,41E+06	5,15E+03	6,85E+03

Tableau 3
Dynamics of indicator microorganisms in solid fraction - winter

The *Salmonella typhimurium* microorganisms tested were recovered qualitatively on day 85 from both carriers. Quantitative determination allowed us to recover them up to day 4 on the leather carrier and up to day 54 on the PVC carrier. The number of microorganisms in the glass vial remained the same.

days	pH	dry matter (%)	N-total (g/kg)	N-NH4 (g/kg)	organic matter(%)	anorganic matter(%)	P-total (g/kg)
0.	8,52	16,06	3,49	1,37	91,07	8,93	1 680.22
4.	8,14	15,95	3,67	0,99	89,33	10,67	2 088,22
40.	8,26	13,09	4,54	1,12	89,47	10,53	1 274,66
54.	8,6	15,484	4,97	0,7	91,66	8,34	1 157,89
76.	8,31	15,55	4,11	0,84	89,44	10,56	1 202,11
85.	8,1	15,138	4,41	0,91	87,71	12,29	1 062,67
120.	7,01	15,089	4,75	0,94	87,91	12,09	2 012,42
137.	7,28	16,68	4,49	0,49	88,88	11,12	1 876,32

Tableau 4
Physico-chemical parameters - winter

The physical-chemical examination showed 3-fold decrease in ammonia nitrogen in comparison with the initial value. The values of total nitrogen, inorganic substances and total phosphorus showed slightly increasing trend. The value of pH decreased from 8,52 to 7,28 and the values of organic substances also exhibited slight decrease. The dry matter content decreased up to day 40 after which it increased to the original value (Tab 4).

The external temperature in this season ranged from -11 to 48^{o}C and that in the solid fraction from –1 to 30^{o}C.

4. Discussion

The results obtained indicate that the temperature has a pronounced effect on the survival of *Salmonella typhimurium* germs. Our results point to the differences in

the survival of salmonellae in the summer (26 days) and winter (85 days) periods which is in an agreement with the data of many authors (D e a n, 1981; S t r a u ch, 1991; A h m e d, 1995 and others) that the temperature is a significant factor of survival of salmonellae. M ü l l e r (1973) stated that the viability of various salmonella species in slurry ranges from 4 to 97 days in summer and up to 87 days in winter which is comparable with results obtained in our study.

In addition to the temperature the dry matter content also affects significantly the survival of salmonellae. M i t c h e r l i c h and M a r t h (1984) compared the survival of *Salmonella typhimurium* in three types of manure with different content of dry matter and arrived to the conclusion that the *S.typhimurium* germs survived for 84 days at 10^{o}C in the manure with dry matter content of 17,3% which is the value comparable with the dry matter content in the solid fraction used in our experiment (Tab 4). The results of authors mentioned above resemble our observations of survival of *S.typhimurium* for 85 days.

S t r a u c h (1991) stated that salmonellae survive the longest at dry matter content above 5% and temperature below 10^{o}C which is in agreement with the results of our experiment in which, in the summer season at higher content of dry matter (Tab 2) and higher temperature, *S.typhimurium* germs survived for shorter period of time (26 days) in comparison with the winter season when the temperatures and the dry matter content were lower (Tab 4) and *S.typhimurium* germs survived longer (85 days).

M í k o v á (1997) stated that the minimum pH for the growth of salmonella is 3.8, optimum 7 and maximum 9. The pH values of the solid fraction used in our experiment were in the range mentioned above therefore this substrate represented a suitable medium for the survival of salmonellae.

The numbers of indicator microorganisms recorded in winter and summer periods are comparable with values reported by O n d r a š o v i c o v á et al. (1994) during the storage of slurry with the difference that the results of the author mentioned showed considerable decrease in the number of coliform and fecal coliform microorganisms during 6-weeks of storage while our experiment showed considerable decrease only in psychrophilic microorganisms in summer and fecal streptococci in winter.

High contamination with indicator microorganisms was recorded by V e n g l o v s k ý et al. (1994) and P l a c h á et al. (1997) who detected higher numbers of coliform and fecal coliform bacteria in the solid fraction of wastewaters than in the inflow to the wastewater treatment plant.
Our results as well as results of other authors - V e n g l o v s k ý et al. (1994); O n d r a š o v i c o v á et al. (1994); P a c a j o v á et V e n g l o v s k ý (1997) indicate that the solid fraction of pig slurry can be contaminated with high numbers of pathogenic microorganisms and therefore increased attention should be paid to its handling and processing.

N i e w o l a k (1994) stated that microorganisms (*Escherichia, Salmonella*)

introduced to the soil by application of contaminated pig slurry can penetrate to depths of 160-180cm. H e n r y et al. (1995) isolated salmonellae from pasture after 2 months and from soil after 8 months following the application of contaminated pig slurry. For this reason the proper treatment of contaminated slurry before its application on land should be ensured. The most suitable way of treatment of the solid fraction of pig slurry is the composting. N i e w o l a k (1997), N o v á k (1994) and P l a c h ý (1995) stated that the composting results in significant decrease in the number of pathogenic bacteria, fungi and eggs of parasites and in production of high-quality manure with high portion of humic substances.

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