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DISINFECTING EFFECTS OF ZEOLITES AND SURVIVAL OF SALMONELLA TYPHIMURIUM AND SALMONELLA SEFTENBERG IN THE SOLID FRACTION OF SLURRY FROM A PIGGERY WASTEWATER TREATMENT PLANT

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ABSTRACT

The influence of the addition of 1% and 2% zeolite upon the survival of *Salmonella typhimurium* and *S. seftenberg* was observed in the solid fraction of slurry. In PVC bottles the decimation time value (T_{90}) for *S. typhimurium* was 1.23, 1.37 and 3.32 days in the heaps containing 1% and 2% zeolite and in the control heap, respectively whereas in the control ampoules the respective values reached 1.53, 3.27 and 19.83 days for 1% and 2% zeolite and the controls. The T_{90} value for *S. seftenberg* was 1.82, 1.70 and 8.99 days in the 1% and 2% zeolite-amended heaps and in the control one, respectively whereas in the control ampoules the respective values reached 2.76, 1.78 and 25.26 days for 1% and 2% zeolite and the controls. The addition of zeolites to the solid fraction was observed to have a good devitalizing effect.

INTRODUCTION

The potential for environmental contamination through the improper handling of agricultural wastes has been extensively studied. Environmental contamination includes bacterial pollution through application of manures and sludges to soil which can affect the indigenous soil populations and thereby change the decomposition and nutrient recycling rate. A more immediate public health concern, however, is the potential for disease transmission.

Wastes from agricultural production can be processed by several biological, physical and chemical technologies. From the ecological viewpoint those methods are important which enable nutrients to return into the circulation of natural substances. The microbiological and hygienic demand is that waste processing should eliminate pathogenic microorganisms.

Natural zeolite compounds or clinoptilolites have the potential of being extensively used in agricultural applications and wastewater treatment (Bernal et al., 1993; Pond, 1993). In our experiments the survival of *Salmonella typhimurium* and *S. seftenberg* was observed in the zeolite-treated solid fraction of slurry.

MATERIALS AND METHODS

The solid fraction of slurry was obtained from a pig farm with a capacity of 23 000 fattening pigs situated at Košická Polianka, Slovak Republic. Slurry was treated by an aerobic process in a WTP with mechanical, chemical and biological treatment stages.
The solid fraction was stored in three heaps, each of a volume of 2 m^3. One and 2 vol%

of zeolite were added to the first and second heap of solid fraction; the third heap served as a control. The experiments were carried out between April and June. During the experiment temperature in the solid fraction was recorded in 1 hour intervals by means of a programmable registration thermometer (Commeter - COMET System, Rožnov p. R., Czech Republic). The natural zeolite (40-56% clinoptilolite) used in our experiment was obtained from Nižný Hrabovec, Slovak Republic.

At the beginning of our experiment the original solid fraction was examined for the presence of *Salmonella* spp. using the method of Philipp *et al*. (1990).

A 20 g sample of the solid fraction of slurry was transferred to each PVC bottle to which 0.5 ml aliquots of a broth culture of *S. typhimurium* (Sk 14/39) and *S. seftenberg* (Sk 87/58) were applied. The PVC bottles were inserted directly into the heaps of the solid fraction. Sterile 20 cm long glass ampoules, 1 cm diameter, were used as controls. These ampoules were applied directly into the solid fraction, too.

The carriers were examined by the method of Müller (1973). Each carrier was subjected to quantitative and qualitative examination for the presence of salmonellae.

The presumptive salmonella colonies were examined biochemically and serologically.

The physical-chemical parameters - pH, dry matter content, ammonia nitrogen and total nitrogen - were determined according to Plachá et al. (1997) and total phosphorus according to the Standard Methods for Examination of Water and Wastewater (APHA 1985).

Statistical analyses

The time of survival of the observed microorganisms was expressed as the T_{90} value. The decimation time (T_{90}) as defined by Schlundt (1984) is the time viable counts of a population need to decrease by one logarithmic unit (log_{10}) which is equivalent to a 90% reduction.

RESULTS

The carriers and control ampoules were examined in the time intervals presented in Table 1 and 2. The values obtained by quantitative examination and presented in the tables are the arithmetic means of three parallel examinations. Qualitative tests were considered negative in those cases in which all three parallel examinations for the presence of *S. typhimurium* and *S. seftenberg* were negative.

Table 1. Survival of S. typhimurium and S. seftenberg in the solid fraction of slurry

Time (days)	*S. typhimurium* (cfu.ml^{-1}) 1% zeolite	2% zeolite	control	*S.seftenberg* (cfu.ml^{-1}) 1% zeolite	2% zeolite	control
0	$1.54x10^{5}$				$1.54x10^{5}$	
1	$1.61x10^{4}$	$4.00x10^{3}$	$4.40x10^{5}$	$2.12x10^{5}$	$2.25x10^{4}$	$5.78x10^{5}$
2	$2.28x10^{3}$	$4.30x10^{2}$	$8.67x10^{5}$	$9.10x10^{4}$	$1.16x10^{3}$	$2.21x10^{4}$
3	$1.98x10^{2}$	$9.87x10^{1}$	$6.43x10^{4}$	$2.32x10^{4}$	$3.21x10^{3}$	$1.95x10^{4}$
4	$8.76x10^{1}$	$5.32x10^{1}$	$5.98x10^{3}$	$5.34x10^{3}$	$8.98x10^{2}$	$1.92x10^{4}$
5	$1.21x10^{1}$	$2.12x10^{1}$	$5.67x10^{3}$	$8.54x10^{2}$	$6.54x10^{1}$	$1.56x10^{4}$
6	+	+	$1.78x10^{3}$	$1.12x10^{2}$	$2.12x10^{1}$	$1.34x10^{4}$
7	+		$9.76x10^{2}$	$7.54x10^{1}$	+	$1.23x10^{4}$
9			$8.76x10^{2}$	+		$9.56x10^{3}$
12			$1.43x10^{2}$			$6.76x10^{3}$
15			$8.67x10^{1}$			$1.12x10^{3}$
18			$1.23x10^{1}$			$8.67x10^{2}$
21			+			$5.43x10^{2}$
28			+			$8.54x10^{1}$
35			+			+
40			+			+
45						+
50						
60						
Slope	-0.8108	-0.73067	-0.30125	-0.55092	-0.59886	-0.11114
T_{90}	1.23335	1.368607	3.319502	1.815146	1.669839	8.997661

Tab. 2. Control ampoules

Time (Days)	*S. typhimurium* (cfu.ml^{-1}) 1% zeolite	2% zeolite	control	*S.seftenberg* (cfu.ml^{-1}) 1% zeolite	2% zeolite	control
0.		$1.54x10^{5}$			$1.54x10^{5}$	
1.	$2.34x10^{4}$	$3.56x10^{3}$	$1.23x10^{5}$	$7.86x10^{5}$	$6.76x10^{5}$	$1.34x10^{5}$
2.	$4.56x10^{4}$	$1.32x10^{2}$	$1.15x10^{5}$	$8.40x10^{4}$	$6.78x10^{4}$	$1.12x10^{5}$
3	$9.87x10^{3}$	$9.87x10^{1}$	$9.87x10^{4}$	$5.65x10^{4}$	$2.23x10^{4}$	$1.02x10^{5}$
4	$8.76x10^{2}$	$5.65x10^{1}$	$5.43x10^{4}$	$1.13x10^{4}$	$8.76x10^{3}$	$9.67x10^{4}$
5.	$2.19x10^{2}$	$3.21x10^{1}$	$4.56x10^{4}$	$6.98x10^{3}$	$4.12x10^{2}$	$6.78x10^{4}$
6	$8.98x10^{1}$	$1.23x10^{1}$	$3.92x10^{4}$	$8.76x10^{2}$	$2.34x10^{2}$	$6.12x10^{4}$
7.	$4.31x10^{1}$	+	$2.13x10^{4}$	$5.34x10^{2}$	$5.65x10^{1}$	$5.98x10^{4}$
9	+	—	$2.11x10^{4}$	$3.43x10^{2}$	$1.23x10^{1}$	$4.12x10^{4}$
12.	—	—	$1.98x10^{4}$	$1.12x10^{2}$	—	$3.12x10^{4}$
15	—	—	$9.98x10^{3}$	—	—	$2.98x10^{4}$
18	—	—	$8.76x10^{3}$	—	—	$2.54x10^{4}$
21.	—	—	$8.54x10^{3}$	—	—	$2.23x10^{4}$
28.	—	—	$9.76x10^{2}$	—	—	$1.34x10^{4}$
35	—	—	$7.43x10^{2}$	—	—	$9.87x10^{3}$
40.	—	—	$6.54x10^{2}$	—	—	$4.56x10^{3}$
45	—	—	$5.87x10^{2}$	—	—	$1.12x10^{3}$
50	—	—	$1.98x10^{2}$	—	—	$9.78x10^{2}$
60	—	—	$9.98x10^{1}$	—	—	$6.78x10^{2}$
slope	-0.65381	-0.3057	-0.05042	-0.36262	-0.5612	-0.03959
T_{90}	1.529496	3.271181	19.8334	2.757708	1.781896	25.2589

For physical-chemical examinations samples were taken at time intervals specified in Figures 1, 2 and 3.

During the experiment *S. seftenberg* survived longer periods in the PVC bottles and in the control ampoules than did *S. typhimurium*. For both bacteria maximum survival was confirmed in the control ampoules (Tables 1 and 2).

The decimation time value (T_{90}) for *S. typhimurium* was 1.23, 1.37 and 3.32 days in the 1% and 2% zeolite and the control bottles, respectively, and 1.53, 3.27 and 19.83 days in the 1% and 2% zeolite and the control ampoules, respectively. The T_{90} value for *S. seftenberg* was 1.82, 1.70 and 8.99 days in the 1% and 2% zeolite and the control bottles, respectively, whereas in the glass ampoules the respective value reached 2.76, 1.78 and 25.26 days in the 1% and 2% zeolite-containing and the control ones (Tables 1 and 2).

The physical and chemical examination revealed a considerable increase of temperature in the solid fraction amended with 1% and 2% zeolite. On day 6 the temperature rapidly increased from 19.4°C to 61.5°C and 61.7°C, respectively. During this time inactivation of both salmonella strains was observed (Tables 1 and 2). For pH values an increasing tendency could be stated whereas all other values were observed to decrease (Figures 1, 2 and 3).

Fig. 1. Physico-chemical parameters (1% zeolit)

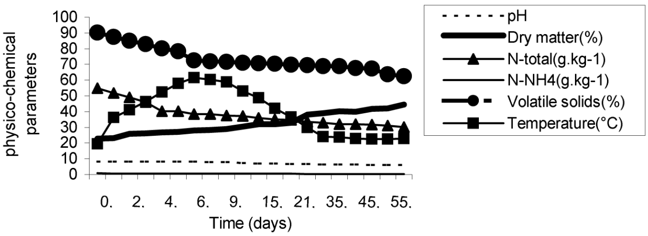


Fig. 2. Physico-chemical parameters (2% zeolite)

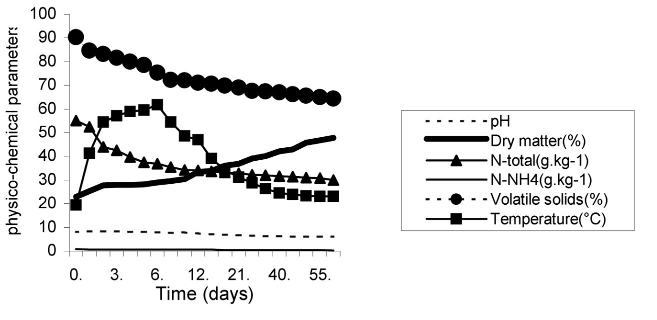
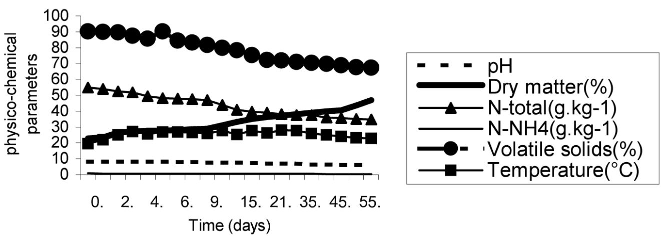


Fig. 3. Physico-chemical parameters(control)



DISCUSSION

The zeolite clinoptilolite is a natural silicate mineral with internal channelling, a large surface area and high cation exchange capacity. It has an affinity for NH_4^+ ions and has been successfully used to remove these ions from wastewaters, to retain them in soils and to reduce NH_3 emissions from animal housing and during the composting of nitrogen-rich wastes. Equilibrum relationships and kinetics of NH_4^+ adsorption and desorption by clinoptilolite have been investigated (Bernal and Lopez-Real 1993) but no information is available in the literature on the influence of zeolites upon the survival of bacteria. From this point of view we investigated the survival of salmonellae in the solid fraction of slurry after the addition of zeolite.

Our results indicate that the survival of *S. typhimurium* and *S. seftenberg* is considerably affected by temperature. They also point at differences in the survival of both salmonella strains in the solid fraction treated with 1% zeolite (7 d vs. 9 d) and 2% zeolite (6 d vs. 7 d) and in the control ampoules (40 d vs. 45 d) (Table 1). In the zeolite-treated solid fraction inactivation of *S. typhimurium* and *S. seftenberg* was extensively influenced by the rapid increase of temperature from 19.4°C to 60°C. These results are comparable with the findings of Droffner and Brinton (1995) who observed *Salmonella typhimurium* to survive in the waste-water sludge compost at about 60°C a minimum of 5 days (Table 1, Fig. 1 and 2).

In the present study *S. seftenberg* was undetectable after 15 and 12 days (1% and 2% zeolite, respectively) when the temperature decreased from about 60°C to about 40°C. This is comparable to the results quoted by Droffner and Brinton (1995) according to whom salmonellae became undetectable after temperature in the compost decreased from 62°C to about 40°C.

Our results confirmed the observations of Plachá *et al.* (2001), Cabadaj *et al*. (1995) and Vasiľ (2001) according to whom the decrease of pH has a devitalizaing effect on micro-organisms. According to Strauch (1987) the decrease of the pH value during storage is influenced by the natural bacterial flora producing fatty acids which are toxic to salmonellae. In contrast to natural bacterial flora the latter are not able to secure nutrients and this probably causes their extinction.

This study makes it obvious that the addition of zeolites to the solid fraction had a sufficient devitalizing effect. From this point of view zeolites can be recommended as an efficient fertilizer and soil conditioner.

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