

SURVIVAL OF SALMONELLA TYPHIMURIUM AND SALMONELLA SEFTENBERG IN SOLID FRACTION OF SLURRY FROM PIG FARM WASTEWATER TREATMENT PLANT AMENDED WITH ZEOLITE

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Abstract

This study deals with influence of 1 vol% and 2 vol% zeolites to survival of *Salmonella typhimurium*, *S. seftenberg* and indicator bacteria (psychrophilic, mesophilic, coliform and faecal coliform) in the solid fraction of pig slurry from agricultural wastewater treatment plant. PVC bottles with openings served as test carriers. In solid fraction with 1 vol% zeolites *S. typhimurium* survived for 9 days and *S. seftenberg* for 12 days. In solid fraction with 2 vol% zeolites *S. typhimurium* survived for 6 days and *S. seftenberg* for 9 days. In the control samples (solid fraction without zeolites) *S. typhimurium* survived 28 days and *S. seftenberg* 40 days. The present study demonstrated that addition of 1 vol% and 2 vol% zeolites have good disinfecting effect.

Key words: wastewater treatment plant, solid fraction, slurry, survival, *Salmonella typhimurium*, *Salmonella seftenberg*, zeolites

INTRODUCTION

The zeolites are a popular group of minerals for collectors and an important group of minerals for industrial and other purposes. Zeolites have many useful purposes. They can perform ion exchange, filtering, odor removal, chemical sieve and gas absorption tasks. Zeolites added to livestock feed have been shown to absorb toxins that are damaging and even fatal to the growth of the animals, while the basic structure of the zeolite is biologically neutral. Most municipal water supplies are processed through zeolites before public consumption.

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. Equilibrium relationships and kinetics of NH_4^+ adsorption and desorption by clinoptilolite have been investigated (Bernal *et al.*, 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.

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. 300 kg of the solid fraction was treated by

natural zeolite (40 – 56% clinoptilolite) mined at Nižný Hrabovec, Slovakia (main fractions: 76.9 % - 0.125-0.250 mm, 10.8 % - 0.25-0.5 mm, cation exchange capacity 0.77 mol/L, pre-dried at 105 °C) which was added at 1 and 2 vol%. The mixtures were stored in a closed concrete dungyard. 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).

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). The presumptive salmonella colonies were examined biochemically and serologically.

From indicator microorganisms we investigated the dynamics of psychrophilic, mesophilic, faecal coliform and coliform bacteria.

The physical-chemical parameters - pH, dry matter content, ammonia nitrogen, total nitrogen and volatile solids were determined according to Plachá *et al.* (2001).

The time of survival of the observed microorganisms was expressed as the decimation time value (T_{90}).

RESULTS AND DISCUSSION

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.

The decimation time value (T_{90}) for *S. typhimurium* in the PVC bottles was 1.23, 1.37 and 3.32 days in the 1% and 2% zeolite and the control, respectively, and in the glass ampoules 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* in the PVC bottles was 1.82, 1.70 and 8.99 days in the 1% and 2% zeolite and the control, 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.

The counts of indicator bacteria in the solid fraction ranged from 10^1 to 10^9 in the heap containing 1 vol% zeolites, from 10^2 to 10^9 in the heap containing 2% zeolites and the same counts of indicator bacteria were in the control heap. In the heap with 1 vol% zeolites coliforms bacteria were detected until d 15, with 2% zeolites until d 12 and faecal coliforms until d 18 (1 vol% zeolites) and until d 6 (2 vol% zeolites). The numbers of mesophilic bacteria decreased by six orders of magnitude (1 vol% zeolites) and by seven orders of magnitude (2 vol% zeolites). The number of psychrophilic bacteria decreased by five order of magnitude (1 vol% zeolites) and by seven order of magnitude (2 vol% zeolites). In control heap the mesophilic bacteria decreased by six order of magnitude, psychrophilic bacteria by seven, coliforms by five and faecal coliforms by four order of magnitude. The values of decimation time T_{90} ranged from 2,71 d (coliforms) to 13,29 d (psychrophilic bacteria) (1 vol% of zelites) and from 1,62 d (faecal coliforms) to 10,39 d (psychrophilic bacteria) (2 vol% of zeolites). In the control heaps the values of decimation time T_{90} ranged from 8,74 d (psychrophilic bacteria) to 17,69 d (faecal coliforms).

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. For all other values a decreasing tendency was observed (Figures 1, 2 and 3).

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

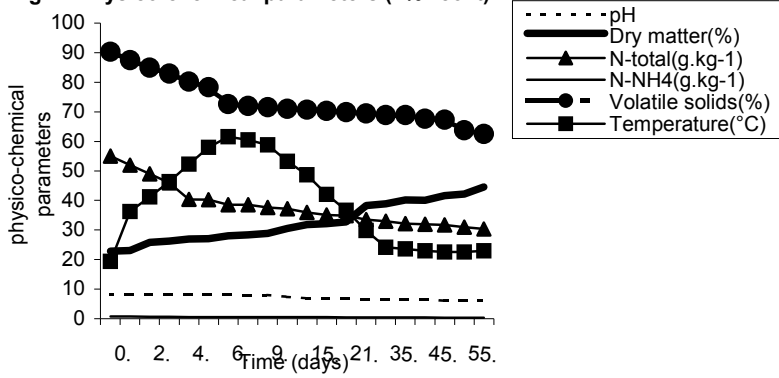


Fig. 2. Physico-chemical parameters (2% zeolite)

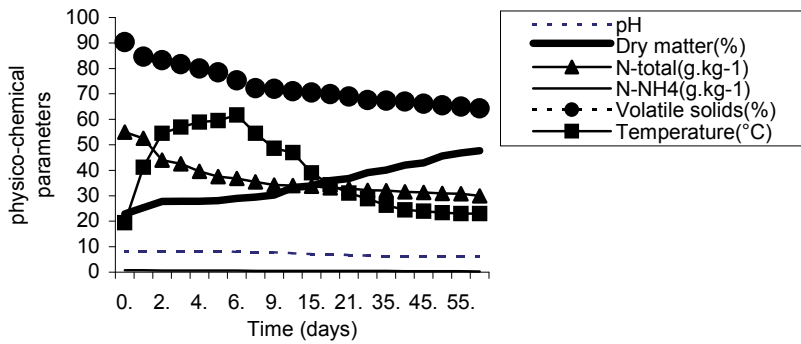
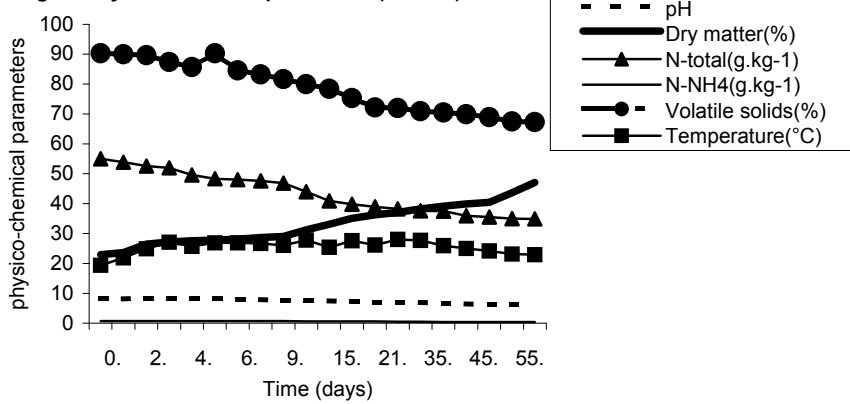


Fig. 3. Physico-chemical parameters(control)



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 samples (40 d vs. 45 d). 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 that *S. typhimurium* survive in the waste-water sludge compost at about 60°C a minimum of 5 days.

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.

The elimination of Salmonella from the solid fraction was found to correspond with the decrease in numbers of the indicator microorganisms, faecal coliforms and coliforms. In the present experiment, the fact that Salmonella die-off was faster than that of indicator microorganisms suggests that salmonella was the most heat-sensitive genus.

The survival of salmonellae is also affected by the dry matter content. Our results are comparable with observations by Riche *et al.* (1995) according to whom a significant correlation exists between the decrease of the Salmonella population and the decrease in moisture content. In our experiment the zeolite-containing solid fraction was more easily dewatered than the normal solid fraction.

These results may suggest a direct inhibition of the bacteria but it is unclear whether the zeolite compound alone or in combination with other factors, such as moisture, is responsible for this. Zeolites have been used as drying agents and in this study lower moisture levels were observed with clinoptilolites. This may reflect a decreased water activity that decreases Salmonella viability. Other possible indirect effects by zeolites include adsorption of products of microbial metabolism, such as ammonium or p-cresol (Shurson *et al.*, 1984).

Our results proved that the decrease of the pH value has a devitalization effect on microorganisms. This results confirmed the observations of Plachá *et al.* (2001), Cabadaj *et al.* (1995), Zeleňáková *et al.* (1996) and Vasil' (2001) according to whom the decrease of pH has a devitalizing 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 studies suggest that zeolites retard bacterial survival only under certain specific conditions. Detailed investigations of the mechanisms of microbial attachment to zeolites and of the effects of zeolites on bacterial viability are needed.

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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