

**MICROBIAL STABILIZATION OF PIG SLURRY SOLIDS
AMENDED WITH NATURAL ZEOLITE AND/OR SAWDUST**

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Summary

The experiment was conducted to investigate the effect of natural zeolite clinoptilolite on microbial stabilization of the solid fraction (SF) of pig slurry amended with zeolite and /or sawdust during 6-week storage with turning after 1 and 3 weeks. The solid fraction was obtained by mechanical separation on vibrating sieves in the first stage of aerobic pig slurry treatment. The temperatures recorded in the substrates S1-S4 (S1=control; S2=SF+2% zeolite; S3=SF+2% sawdust; S4=SF+2% zeolite+2% sawdust) showed a significant ($P<0.001$) positive effect of zeolite-amendment during the first 3 weeks of storage. Plate counts of psychrophilic, mesophilic and coliform bacteria were also affected by zeolite and corresponded to the course of temperature. At the end of the experiment, fecal coliform counts were by about one order lower in the amended substrates (S2-S3) in comparison with the control (S1).

Key words: zeolite clinoptilolite, pig slurry solids, decomposition, bacterial plate counts

Introduction

The development of intensive pig farming is influenced by processing and disposal of pig excrements. The treatment of the liquid fraction of pig slurry has been resolved by the activated sludge process but the solid fraction, obtained in the first mechanical stage of treatment, may present a problem. During the mechanical separation, considerable portion of bacteria, viruses,

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protozoa and different parasitic stages may pass to the solid fraction and survive there for different periods. Therefore additional treatment of this material, preferably by mixing with some bulking agents and subsequent composting, is recommended before application to agricultural land (Juriš *et al.* 1991). During the storage of animal manure some organic substances are decomposed and the product obtained is less odorous and safer also from the hygiene point of view (Killham 1994). Depending on the conditions of storage (temperature, aeration, humidity, pH, content of nutrients, type and structure of the material, and others) organic matter is decomposed at various rates in several temperature stages in which specific microorganisms play a dominant role. Piggery solid wastes are nutrient rich fibrous material readily amenable to aerobic composting (Bhamidimarri and Pandey 1996). However, the basic conditions for aerobic processes, particularly optimum moisture, carbon content and sufficient aeration are not always met in practice. This may result in insufficient decomposition of organic matter and poor sanitation of the product. Moreover, a considerable portion of nutrients may be lost to the atmosphere and soil and pollute the environment.

Zeolites are minerals with unique structure that allows them to entrap or release various substances as a consequence of cation-exchange reactions and adsorption. The framework structure of clinoptilolite, the most abundant and frequently used natural zeolite, consists of interlinked four- and five-tetrahedral rings creating a layer. Between these layers are open eight- and 10-tetrahedral ring channels. These rings form the ion sieving channels of clinoptilolite and are also responsible for its extensive internal surface (Breck 1974). The high affinity of zeolites for NH_4^+ and the possibility of releasing it over time is of special interest for minimizing environmental pollution during animal waste management (Kithome *et al.* 1998) and when used for bedding (Pintarič, 1999). Application of zeolites to soil can improve some chemical and physical properties of the soil system, such as pH, cation exchange capacity, water-holding capacity and the use of nutrients.

The present study focused on the processes of microbial decomposition in the solid fraction of pig slurry amended with clinoptilolite and sawdust during its storage.

Material and methods

The experiment was carried out on the solid fraction (SF) of slurry from a pig fattening farm obtained in the first stage of its aerobic treatment by separation on vibrating sieves. Substrates of the following composition were

prepared by manual mixing and stored in opened plastic bags for 6 weeks with collection of the released liquid: a) S1 = 25 kg unamended solid fraction; b) S2 = 25 kg solid fraction amended with 2% zeolite; c) S3 = 25 kg solid fraction + sawdust 1:1 in volume (2% in weight); d) S4 = 25 kg solid fraction + 2% zeolite + sawdust 1:1 in volume (2% in weight). After 1 and 3 weeks of storage the substrates were thoroughly mixed and returned to the bags. Samples were taken at the beginning and after 1, 3, and 6 weeks of storage, prior to mixing.

Untreated sawdust and natural zeolite (40-56 % clinoptilolite), mined at Nižná 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⁻¹; pre-dried at 105°C), were used in the experiment.

Temperatures in the core of the substrates were recorded in 1 h intervals using a programmable Commeter System (Rožnov pod Radh., CR) with appropriate probes.

Microbiological examination, carried out according to Slovak standards, included determination of plate counts of psychrophilic (STN 83 0531-5), mesophilic (STN 83 0531-4), coliform (STN 83 0531-3) and fecal coliform bacteria (STN 83 0531-6). Decimal dilutions for cultivation were prepared in physiological saline solution.

All determinations were carried out at least in duplicate. Results were analysed statistically by means of paired Student's t-test.

Results of chemical examination of substrate samples and the released liquid were presented by Vargová et al. (1999) and Sasáková et al. (1999).

Results and discussion

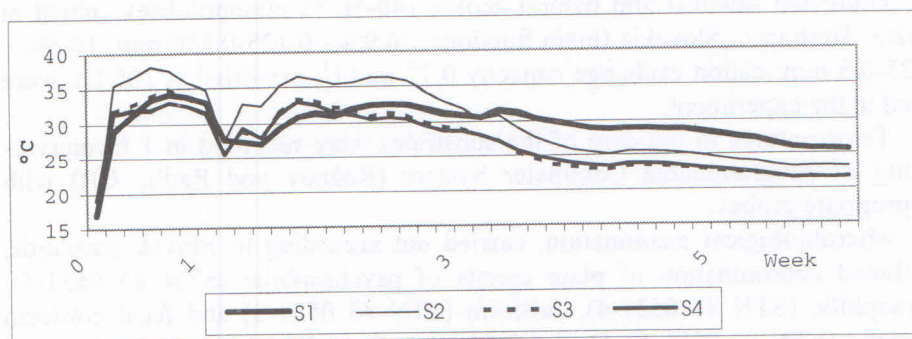
Many factors determine the microbial community during stabilization of organic materials. Pig manure itself contains a high density of bacteria with different functional groups and is biologically active (O'Neill and Phillips 1991). The nutrient and organic matter in pig manure can also provide the necessary environmental conditions for continuous proliferation of bacterial populations (Tam et al. 1996).

Temperature in the substrate is one of the most important indicators of decomposition processes. In our experiment a positive influence of zeolite amendment on the development of temperature was observed already on day 4 of the experiment when the temperature in the core of substrate 2 reached 38.1°C in comparison with 34.2°C in the control substrate S1 (Fig.1).

This positive influence persisted up to 3rd week of storage and was statistically significant (P<0.001). Negative significant differences (P<0.001)

were observed between substrates 2 and 4 and 3 and 4. During weeks 3-6 the highest temperature developed in substrate 1 and differed significantly ($P < 0.001$) from that in substrates 2, 3 and 4.

Fig. 1: - TEMPERATURES IN THE CORE OF SUBSTRATES S1-S4 DURING 6-WEEK STORAGE ($T_a = 19-21.5^\circ\text{C}$)



Natural zeolite can potentially capture ammoniacal nitrogen from mixed or liquid wastes, changing liquid-phase N to solid-phase N, thereby improving the C/N ratio (Dwairi, 1998). It is possible that the effect of zeolite depends on this. Retention of water and different loss of nutrients through the released liquid may also be involved (Sasáková et al. 1999). Our experiments show that the thermophilic phase can hardly be reached if pig slurry solids are stored without addition of the sufficient amount of bulking agent and frequent turning.

According to Strauch and Ballarini (1994) only the thermophilic range of 55°C is sufficient to destroy pathogens. Novák (1994) stated that temperature in the composting substrate should reach min. 45°C and remain at this level for at least 5 days to ensure sanitation of the material. If the presence of pathogens is suspected, temperature min. of 55°C should be maintained for at least 21 days. Counts of total and fecal coliforms and moulds in the composted material can serve as basic hygiene and epizootologic criteria indicating devitalization of pathogens.

According to Farrell (1992) the recommended density of fecal coliforms indicating sanitization is 1000 CFU.g^{-1} solids because *Salmonellae* are considered to be absent from all samples containing amounts of fecal coliforms lower than that. Strauch (1987) recommended a limit of $5 \times 10^2 \text{ CFU}$ for fecal coliforms and $5 \times 10^3 \text{ CFU}$ for fecal streptococci per 1 g of fresh composted material (FW - fresh weight). In the study published by Tiquia and Tam

(1998) the elimination of *Salmonella* spp. from spent-litter piles corresponded with the progressive decrease in the numbers of fecal coliforms and fecal streptococci.

The majority of papers on the use of zeolites in the treatment of animal wastes focuses on the chemical aspects, particularly on adsorption and release of ammonia nitrogen. Ricke et al. (1995) investigated the influence of different types of clinoptilolite on survival of *S. typhimurium* (a primary poultry isolate, resistant to nalidixic acid and novobiocin) in various artificial microcosms (soil, water) in relation to the frequent amendment of poultry litter with zeolites and its subsequent application to soil. They observed direct inhibition of bacteria by small mesh clinoptilolite. According to these authors a direct inhibition of bacteria may be considered but it is unclear whether the zeolite compound alone or in combination with other factors, such as moisture, is responsible for this. Other possible indirect effects by zeolites include the adsorption of products of microbial metabolism, such as ammonium or cresol (Shurson 1984).

Results of our microbiological examination are presented in Table 1 and Fig. 2. Plate counts of psychrophilic populations showed similar trend in all the substrates without significant differences. The addition of either zeolite or sawdust induced similar changes in the density of mesophiles, however, their numbers were lower in the presence of zeolite. In the substrate 4 the effect of both sawdust and zeolite resulted in a more pronounced decrease after 3 weeks and increase almost to the highest level after 6 weeks. Plate counts of mesophiles in the control (S1) showed an opposite trend. The biggest difference in total coliforms between substrates was observed after 3 weeks of storage and decreased to a minimum after 6 weeks. Fecal coliforms counts were lower in all amended substrates in comparison with unamended substrate after 3 and 6 weeks of storage. The final level in amended substrates was by about one order lower in comparison with the control, however, it did not decrease down to the limit indicating sufficient sanitation.

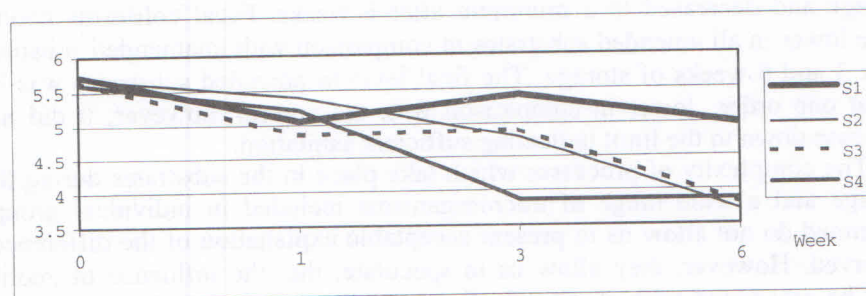
The complexity of processes which take place in the substrates during the storage and a wide range of microorganisms included in individual groups examined do not allow us to present acceptable explanation of the differences observed. However, they allow us to speculate, that the influence of zeolite may be associated with changes in the dry matter content and retention and gradual release of ammonia. Considerable retention of ammonia has been reported also for sawdust (Bhamidimarri and Pandey 1996). The additive effect of zeolite and sawdust on microorganisms at some stages of storage supports our assumption. Due to frequent application of zeolite to litter and, together with excrements, to cropland the effect of zeolite on microbial decomposition deserves further attention.

Tab. 1. - BACTERIAL COUNTS (Log_{10} CFUg⁻¹ DW) - RESULTS OF TWO REPLICATE SERIES

Psychrophilic bacteria				
Weeks	S1	S2	S3	S4
0	7.47-7.67	7.21-7.83	7.21-7.75	7.30-7.60
1	6.59-6.71	6.70-6.94	6.61-6.87	6.91-7.09
3	3.80-4.10	5.65-5.91	6.20-6.30	5.83-5.99
6	6.24-6.44	6.00-6.14	6.79-7.05	6.65-6.95
Mesophilic bacteria				
Weeks	S1	S2	S3	S4
0	7.10-7.24	6.95-7.29	7.80-7.34	6.91-7.19
1	5.71-5.91	6.17-6.37	6.59-6.71	6.35-6.47
3	6.50-6.76	5.85-5.95	6.31-6.53	5.40-5.98
6	5.61-5.91	5.77-5.97	6.17-6.55	6.16-6.34
Total coliforms				
Weeks	S1	S2	S3	S4
0	6.25-6.45	6.08-6.54	6.12-6.42	5.99-6.49
1	5.76-5.98	5.70-6.06	5.41-5.81	5.87-6.07
3	6.27-6.67	5.80-5.96	6.10-6.24	5.40-5.56
6	4.92-5.14	4.97-5.14	5.08-5.12	5.01-5.25

DW - dry weight; S1 = solid fraction (SF); S2 = SF + zeolite; S3 = SF + sawdust;
S4 = SF + sawdust + zeolite

Fig. 2. - PLATE COUNTS OF FECAL COLIFORMS (Log_{10} CFUg⁻¹ DW) IN SUBSTRATES S1-S4



Conclusion

- The results of our experiment allow us to draw the following conclusions:
- natural zeolite clinoptilolite, added to pig slurry solids in a powder form can affect the microbial decomposition processes reflected in the

temperature. Under conditions of our experiment the effect of 2% zeolite-amendment was positive.

- Plate counts of selected groups of bacteria in the observed substrates were affected by zeolite and sawdust and corresponded to the temperature development. Amendment with zeolite and/or sawdust resulted in lower plate counts of fecal coliforms at the end of the experiment.

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MIKROBNA STABILIZACIJA KRUTE FAZE SVINJSKOG TEKUĆEG GNOJA S DODANIM ZEOLITOM I/ILI PILOVINOM

Sažetak

Istraživan je utjecaj dodatka prirodnog zeolita klinoptilolita i/ili pilovine na mikrobnu stabilizaciju krute faze svinjskog tekućeg gnoja (SF) kroz šest tjedana uskladištenja uz obrtanje nakon prvog i trećeg tjedna. Kruta faza je dobivena mehaničkom separacijom na vibracionim sitima pri prvom stupnju aerobne obrade svinjskog tekućeg gnoja. Temperature izmjerene u supstratima kojima su dodani zeolit i/ili pilovina S1 - S4 (S1 = kontrola; S2 = SF+2% zeolita; S3 = SF+2% pilovine; S4 = SF+2% zeolita+2% pilovine) su pokazale značajan ($P < 0.001$) pozitivan utjecaj kroz prva tri tjedna skladištenja na broj psihrofilnih, mezofilnih i koliformnih bakterija čiji je broj bio u skladu s razvojem temperature. Na kraju pokusa broj fekalnih koliforma bio je za oko jednu potenciju niži u tretiranim supstratima (S2 - S3) u usporedbi s kontrolom (S1). Iz pokusa se može zaključiti da dodani prirodni zeolit i/ili pilovina dodani u krutu fazu svinjskog tekućeg gnoja utječu na mikrobnu procese razgradnje koji se očituju u promjeni temperatura pa je stoga i broj selektivnih vrsta mikroorganizama, naročito fekalnih koliforma, niži na kraju pokusa.

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