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# THE ELIMINATION OF *SALMONELLA TYPHIMURIUM* IN SEWAGE SLUDGE BY AEROBIC EXOTHERMIC STABILIZATION AND HYDRATED LIME STABILIZATION

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## Introduction

Over decades, the problems of solid and liquid wastes produced by human activities have been paid increased attention (Strauch, D., Ballarini, G. 1994).
Aerobic stabilization of sewage sludges is a biological oxidation process of organic sludges, which makes use of the anabolic and catabolic activity of aerobic microorganisms capable of producing heat. With an accurately run process (adequate aeration and sludge dry residue) the temperature can rise up to the thermophilic range (45-70°C) in which pathogens are killed and the sludge becomes stabilized, odour free and suitable for agricultural use as a high-quality organic fertilizer (Smith et al., 1975, Novák 1994, Dubinský et al. 2000, Burton et al., 2003).

*Salmonellae* are enterobacteriacae that are widely distributed in the environment and include more than 2,000 serotypes. They are the most predominant pathogenic bacteria in wastewater and they cause typhoid and paratyphoid fever and gastroenteritis. It has been estimated that 0.1% of the population excretes *Salmonella* at any given time. This pathogen produces an endotoxin that causes fever, nausea and diarrhea and may be fatal if not properly treated by antibiotics (Bitton 1994). Species implicated in food contamination are *S. paratyphi* and *S. typhimurium*. These species can grow readily in contaminated foods and cause food poisoning (Cabadaj et al. 1995). Species such as *S. typhimurium* and *S. enteritidis* cause gastroenteritidis, which is characterized by diarrhea and abdominal cramps (Sahlström 2003).

It was the aim of our experimental work to compare the effects of disinfecting municipal sewage sludge using aerobic stabilization or lime hydrate upon the survival of *S. typhimurium*.

## Materials and methods

In our experiments raw (primary) sludges from the mechanical biological municipal sewage treatment plant (STP) at Poprad (Slovak Republic) were used. Six experiments were carried out. Three experiments were carried out during aerobic mesophilic stabilization and three experiments during lime hydrate stabilization. Aerobic stabilization of primary sludges was carried out in a prepared at the Parasitological Institute of the Slovak Academy of Sciences in Kosice, Slovak Republic, according to the scheme of Hotař (1974). The fermentor worked in a discontinuous single filling regime and was placed in a laboratory at a temperature of 19-21°C. The mean hourly volume of air supplied to the fermentor during aerobic stabilization remained constant (Q=0.7 l min-1). The active stabilization volume of the aerobic fermentor was 5,000 ml.

Modified equipment was used to observe the effect of liming. Modification was based upon closing the openings of the pipes for the in-and outlet of air. In order to stabilize the sludge commercially produced lime hydrate (light, airy, white, Kalcit s.r.o., Gombasek, Slovak Republic) was used at an amount of 10g $l^{-1}$ (100 mg $g^{-1}$ dry matter in the sludge). One litre of sludge with no lime hydrate added and kept at 4°C served as a control.

Of the physical-chemical parameters pH and total N were determined according to Plachá et al. (2001) and chemical oxygen demand (COD) according to the Standard Methods for Examination of Water and Wastewater (APHA 1985).

Throughout stabilization temperature values were recorded in 1-h intervals by the programmable registration thermometer. A freeze-dried strain of *S.* typhimurium SK 14/39 (SZÚ, Prague, Czech Republic) was used as the test strain. Prior to stabilization sludge was inoculated with a 24-h broth culture of *S. typhimurium* at a dose of 50 ml per l of sludge. The starting concentrations are given in Table 1.

The time of survival of the investigated microorganism was expressed in $T_{90}$ values. Decimation time ($T_{90}$) as defined by Schlundt (1984) is the time it takes viable counts of a population to decrease by one logarithmic unit ($log_{10}$), which is equivalent to a 90% reduction.

## Results

(Table 1- 4). Statistical evaluation

A significant negative correlation was observed between the survival of *S. typhimurium* and the pH in experiment 2 (r = -0.9794) and a tendency to negative correlations was seen in experiments 1 and 3 in which sludges were stabilized by liming (r = -0.8925 and -

0.8714, respectively). In all three experiments with aerobic stabilization of sludges significant negative correlations were also observed between the temperature and *S. typhimurium* survival (r=-0.9174, r = -0.9248 and r = -0.9857, respectively). As to the relation between total N and *S. typhimurium* survival a significantly positive correlation was stated in the control in experiments 1 and 2 (r = 0.9226 and r = 0.9076, respectively) and a tendency towards positive correlation in experiment 3 (r = 0.8333). With aerobic stabilization a negative correlation occurred in experiment 2 (r = -0.9101) and a positive one in experiment (r = 0.9557) whereas experiment 1 revealed a positive tendency (r = 0.8222). If lime hydrate was used for stabilization, correlation between total N levels and the survival of *S. typhimurium* appeared to be significantly positive in all three experiments (r = 0.9220, r = 0.9942 and r = 0.9999, respectively).

## Discussion

Our study confirmed that of the methods investigated, liming of communal sludges has a higher hygienic effect than aerobic mesophilic stabilization. We could confirm that S.*typhimurium* was inactivated by high temperature as well as high pH values.

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Table 1 Detection time of S. typhimurium in sewage sludge during aerobic stabilization, liming and in the control sample

| Time (h) | Aerobic stabilization ($log_{10}$ cfu $ml^{-1}$) | | | Liming ($log_{10}$ cfu $ml^{-1}$) | | | Control sample ($log_{10}$ cfu $ml^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | Exp.2 | Exp.3 | Exp.1 | Exp.2 | Exp.3 | Exp.1 | Exp.2 | Exp.3 |
| 0 | 8.06 | 8.51 | 8.27 | 8.06 | 8.51 | 8.27 | 8.06 | 8.51 | 8.27 |
| 0.5 | 7.99 | 7.93 | 7.72 | 4.95 | 3.99 | 5.93 | 7.99 | 8.01 | 7.85 |
| 1 | 6.59 | 6.75 | 5.99 | 2.38 | 2.75 | 3.64 | 7.33 | 7.19 | 6.94 |
| 6 | 5.47 | 6.08 | 5.06 | — | — | — | 6.93 | 6.99 | 6.77 |
| 12 | 4.63 | 5.93 | 4.33 | — | — | — | 6.71 | 6.79 | 6.61 |
| 24 | 3.65 | 4.80 | 3.95 | — | — | — | 6.51 | 5.66 | 6.56 |
| 48 | + | + | — | — | — | — | 6.88 | 5.83 | 5.36 |
| 72 | — | — | — | — | — | — | 5.33 | 5.93 | 5.64 |
| 96 | — | — | — | — | — | — | 5.64 | 5.51 | 5.83 |
| 120 | — | — | — | — | — | — | 4.53 | 5.34 | 5.86 |
| $T_{90}$ (h) | 6.28 | 8.37 | 5.76 | 0.18 | 0.17 | 0.22 | 44.88 | 54.93 | 70.46 |

+ , detected only qualitatively
—, non detected

Table 2 Physico-chemical parameters in sewage sludge – aerobic stabilization

| Time (h) | pH | | | N-total (g $l^{-1}$) | | | Temperature (°C) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Exp. 1 | Exp.2 | Exp.3 | Exp.1 | Exp.2 | Exp.3 | Exp.1 | Exp.2 | Exp.3 |
| 0 | 5.51 | 5.98 | 5.65 | 6.07 | 6.95 | 7.01 | 20.15 | 21.55 | 23.31 |
| 0.5 | 5.22 | 6.01 | 5.69 | 5.97 | 6.91 | 6.98 | 23.91 | 25.21 | 27.91 |
| 1 | 5.21 | 6.05 | 6.01 | 5.95 | 6.89 | 6.55 | 29.81 | 30.12 | 33.90 |
| 6 | 5.51 | 6.14 | 6.54 | 5.91 | 6.15 | 6.32 | 30.12 | 31.14 | 34.52 |
| 12 | 5.12 | 6.18 | 6.49 | 5.89 | 5.79 | 5.89 | 31.86 | 32.31 | 35.12 |
| 24 | 5.01 | 6.15 | 6.45 | 5.47 | 5.25 | 5.25 | 33.31 | 35.23 | 35.71 |
| 48 | 5.13 | 6.11 | 6.39 | 5.25 | 4.72 | 4.81 | 30.52 | 38.71 | 45.00 |
| 72 | 5.18 | 6.10 | 6.31 | 4.79 | 4.10 | 4.79 | 29.12 | 33.44 | 38.81 |
| 96 | 5.28 | 6.10 | 6.29 | 4.77 | 3.99 | 4.28 | 28.54 | 30.12 | 36.12 |
| 120 | 5.29 | 5.99 | 6.01 | 4.03 | 3.68 | 4.05 | 26.65 | 27.16 | 29.12 |

Table 3 Physico-chemical parameters in sewage sludge – hydrated lime stabilization

| Time (h) | pH | | | N-total (g $l^{-1}$) | | | Temperature (°C) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Exp. 1 | Exp.2 | Exp.3 | Exp.1 | Exp.2 | Exp.3 | Exp.1 | Exp.2 | Exp.3 |
| 0 | 5.51 | 5.98 | 5.65 | 6.07 | 6.95 | 7.01 | 20.15 | 21.55 | 23.31 |
| 0.5 | 12.10 | 12.00 | 12.22 | 5.99 | 6.11 | 6.85 | 20.21 | 21.60 | 23.21 |
| 1 | 12.11 | 12.22 | 12.26 | 5.65 | 6.01 | 6.70 | 20.20 | 21.68 | 23.24 |
| 6 | 12.10 | 12.21 | 12.27 | 5.33 | 5.99 | 6.52 | 20.22 | 21.88 | 23.53 |
| 12 | 12.13 | 12.23 | 12.26 | 5.21 | 5.56 | 6.49 | 20.23 | 22.00 | 23.85 |
| 24 | 12.12 | 12.22 | 12.28 | 4.19 | 5.32 | 6.35 | 20.19 | 22.24 | 24.02 |
| 48 | 12.24 | 12.24 | 12.31 | 4.13 | 4.95 | 6.02 | 20.29 | 22.65 | 24.10 |
| 72 | 12.25 | 12.24 | 12.32 | 4.02 | 4.52 | 5.96 | 20.88 | 22.85 | 24.19 |
| 96 | 12.25 | 12.25 | 12.31 | 3.98 | 4.21 | 5.32 | 21.18 | 22.80 | 24.22 |
| 120 | 12.27 | 12.26 | 12.31 | 3.91 | 4.18 | 5.01 | 21.17 | 22.98 | 24.29 |

Table 4 Statistical evaluation between survival of *S. typhimurium* and physico-chemical parameters

| Physico-chemical parameters | Aerobic stabilization | | | Liming | | | Control sample | | |
|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | Exp.2 | Exp.3 | Exp.1 | Exp.2 | Exp.3 | Exp.1 | Exp.2 | Exp.3 |
| pH | 0.8211 | 0.5387 | 0.3358 | -0.8925 | -0.9794 | -0.8714 | 0.1786 | -0.2485 | -0.6756 |
| N-total | 0.8222 | -0.9101 | 0.9577 | 0.9220 | 0.9942 | 0.9999 | 0.9226 | 0.9076 | 0.8333 |
| Temperature | -0.9174 | -0.9248 | -0.9857 | -0.8107 | -0.6656 | -0.9905 | 0.3870 | 0.5906 | -0.8355 |