# Inactivation of Salmonella spp. by Low-Frequency Electric Fields in Sewage Sludge

C. Keles[1], G. T. Icemer.[1], and S. Ozen[2]
[1]Akdeniz University, Engineering Faculty, Environmental Engineering, 07058 Campus Antalya, Turkey
[2]Akdeniz University, Engineering Faculty, Electrical & Electronics Engineering, 07058 Campus Antalya, Turkey

***Abstract*— The treatment of the sewage sludge produced in the biological treatment plants have increasingly become a serious problem. For this reason, when the reuse and the disposal of the sludge are taken into consideration, the pathogens that the sludge contains should be seriously reduced. In this study, as an alternative to traditional treatment methods for the purpose of pathogen inactivation in sewage sludge, AC electric field, which is a non-thermal technology, was used. The electric field energy designed for the inactivation of the microorganisms in the sewage sludge affects directly the pores in the cell wall in a physical manner. In order to evaluate the inactivation effect of the electric field, AC electric field (50 Hz, 0,6 -1,2kV cm-1) was applied to the sewage sludge containing Salmonella spp. and *Salmonella typhimurium*. It was observed that Salmonella spp. population reduced 1.4 log in 0.6 kVcm-1, 1.1 log in 1.2 kV cm-1and *S. typhimurium* reduced 0.5 log in pure culture. The results showed that the electric field treatment increased the transmittance of the cell wall and that caused destructions in the cell membranes.**

***Index Term*— AC electric field, pathogen inactivation, sewage sludge, biosolid**

## I. INTRODUCTION

Today, the use of sewage sludge as biosolid material is becoming more and more common. Waste water and sewage sludge contains many enteric pathogens (such as E. coli, Salomonella spp.). Salmonella spp. and particularly the pathogen types cause food contamination because of the contact of food with feces and problems arise for their elimination for humans health and environmental factors. For this reason, it is of great importance that the sludge be purified from its harmful material before being used as a means of treatment. According to the statistical data of 2004 in Turkey, 600,000 tones/year urban sewage sludge and 161 tones year-1 industrial sludge are produced and most of this sludge is released to the environment without any supervision [1].

The inactivation of pathogen microorganisms in the food industry was applied with pasteurization, high temperature pasteurization and UV light techniques, which are common methods. However, in the recent years, the studies have been concentrated on non-thermal technologies which prevent the degeneration of the feeding value of food, such as microwave [2], high pressure [3], pulsed electric field [4], pulsed light [5], oscillating magnetic fields [6], ultrasound [7], pulsed X-Rays [8] and AC electric field [9]. The studies about the use of these new inactivation methods aiming to inactivate pathogens in the waste water have just started. It was reported in a study about the pulsed electric field (PEF) process in the treatment discharges of domestic waste water that there was a new technology which could deal with the combined waste water treatment for the bacterial configuration without any serious disadvantages of ozone and chlorine [10]. In addition, it was reported that flock structure became a micro-flock structure and that they destroyed the cells by applying 70 kW high electric field to the sludge expansion and/or the foam formed by the microorganisms with filaments in the actively working sludge treatment plants [11,12]. In the first study in Turkey in which AC electric field and pathogen inactivation were experimented, 1.4 log Salmonella spp. inactivation was obtained [14].

Because of the high cost of PEF process materials; there are some difficulties in large-scaled plants in terms of application [15]. When AC electric field power is applied to the water via electroporation in low temperature, similar results are obtained to those of the PEF process. In our study, instead of high-cost PEF method, we preferred to use sinusoidal and continuous current techniques for the pathogen inactivation and then we applied pre-testing about the inactivation of pathogens in the sewage sludge. The purpose of this study is to eliminate pathogens in sewage sludge by means of AC electric field process, which is regarded as an environment-friendly and necessary method for obtaining needed data in Turkey.

## II. MATERIAL AND METHODS

### 2.1. Microorganisms

In the experiment, ATTC#14028 *Salmonella typhimurium* lyophilized culture and raw sludge of Hurma

Corresponding author: G. T. Icemer (gicemer@akdeniz.edu.tr).
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Biological Wastewater Treatment Plant located in the west part of Antalya were used. Lyophilized culture was developed by inoculating tryptic soy broth (Merck) in 37 °C and 24 h and kept up to 4°C experiment. In the pre-experiment period, the hearth infusion agar (Merck) was inoculated. The cultures in stationer phases were used for positive and negative controls and for the spike procedure. On the other hand, the raw sludge was brought to the laboratory from the treatment plant in a cold chain.

2.2 AC Electric Field Treatments

In this study, in order to obtain an AC electric field of 50 Hz frequency, the experiment design was prepared as shown in Figure 1. The parallel plates had a separation distance of "d" on the ground plane surface in order to obtain a homogenous electric field.

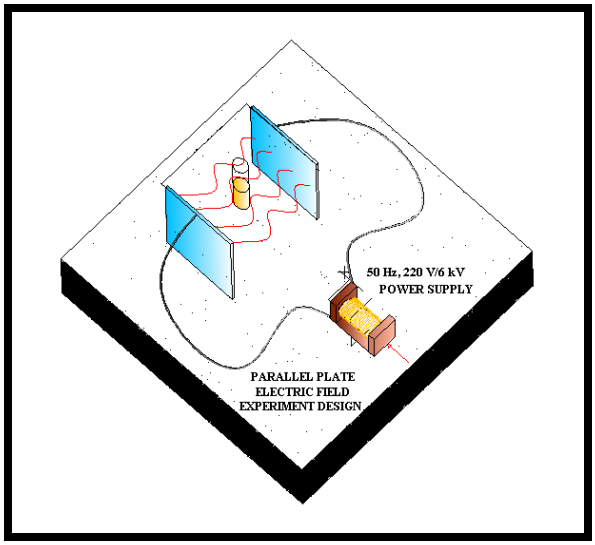



Fig. 1. Experimental setup.

6000 V (6 kV) 50-Hz-frequency sinusoidal voltage was applied to the parallel plates. This voltage was provided by a transformer with one-phase 220V/6000V (30mA) output characteristics. Parallel plates are made of two 40x20 cm coppers covered by 2mm-thick zinc. In order to keep the homogenous structure in the electric field distribution the sharp ends of the plates were rounded. The plates were determined on wooden pedestals and they were placed in parallel to each other. The interval could be adjusted according to the intensity of the electric field applied. For avoiding the degenerative effects in the electric field distribution the supply cables were plugged in the center points of the plates.

The electric field between the plates changes according to the distance between plates and the voltage applied:

$$E = \frac{V}{d} \qquad (1)$$

where, V (kV) is the voltage applied to the plates, and d (m) is the distance between the plates.

2.3 Experiments

The sludge samples were taken from the sludge concentration unit and they were dried immediately with the dry matter analysis method at 105°C. The distance between the plaques in the study was determined as 5 cm and the tubes containing *S typhimurium* pure culture, raw sewage sludge (unspiked) and spiked sludge (approximately $10^7$ CFU $ml^{-1}$ stationer phase *S. typhimurium*). The only 10 cm distance was applied only for the raw sludge. The time intervals were determined as 10, 20, 40 and 80 minutes and then the electric field was applied. As a result of these processes, some samples were taken from the tubes. Multiple tube fermentation method [16] was used for *Salmonella* spp. species and *S. typhimurium*. This method is for the detection and enumeration of Salmonella in treated biosolids by enrichment, selection and characterization. All the experiments were triplicate. The performance of the analysis was tested in accordance with the initial and ongoing precision and recovery (IPR and OPR) acceptance criteria. (IPR: %16,8 -OPR: %1,48).

In the stabilization experiment with the slaked lime ($Ca(OH)_2$) under normal conditions, more caustic lime was added at a rate of 40% of the dry matter content of the sludge. They were then heated at 65°C and sludge samples were taken in every two minutes. As a result, the analysis of *Salmonella* spp. was made.

2.4. Electron Microscopy

The main aspect of the pathogen inactivation with electric field is the breakage of the cell walls because of the "electroporation [17] occurring in the pores of the cell membranes which differs from the surface treatment of membranes by plasma [18]. While the plasma is behaving as a sputtering, the electric field application to the cell and the transmembrane potential of the cell forms a driving force to the opposite sides (+) (-) of the cell between the opposite charges and thus the thicknesses of the membranes are reduced and the some breakages occur in the cell walls. During our study, in order to determine the effect of electrical field on the cell walls the electron microscopy images from samples were taken. Prior to transmission electron microscopic (TEM) analysis, the dehydrated cells of the samples, polymerized at 60°C for 48 hours. The polymerized

samples sectioned by ultra microtome, stained with 2% of uranyl acetate and Reynolds's lead citrate. The sections were than observed with a transmission electron microscope (TEM, Leiss-Leo 1430-TEM) at 8 kV.

## III. Results and Discussion

*S. typhimurium* in the sewage sludge was made considerably inactive with AC electric field method under non-thermal conditions. Some of the physical characteristics of the sewage sludge and the inactivation rates of pathogens in the ambient temperature in raw sludge, spiked sludge and in pure culture are shown in Table 1. The results of the pathogen elimination are presented in Figure 2. According to the results of the experiment it was observed that there was no effect of electric field on the pH, but there was only an increase of 1.8°C.

During the inactivation 50 Hz frequency and 0.6-1.2 kV $cm^{-1}$ different electric field intensities were applied. The inactivation of *Salmonella* spp. increased to 94% in the 40th minute at 0.6 kV/cm. The number of bacteria subjected to 1.2 kV cm-1 was 85% in the 40th minute whereas it was constant in the 80th minute with 92% of reduction rates.

Table I
Some of the physical characteristics of the sewage sludge and the rates of inactivation with AC electric field.

| | Temperature (°C) | pH | Dry Weight ( %) | Inactivation (0.6 kVcm-1) (%) | Inactivation (1.2 kVcm-1) (%) | Inactivation (Slime and 65°C) (%) |
|---|---|---|---|---|---|---|
| *S. typhimuruium* | 21 | 7.7 | - | - | 66 | - |
| Unspiked sludge | 21 | 7.7 | 0.8 | 96.2 | 92 | 99.9 |
| Spiked sludge | 21 | 7.6 | 0.8 | - | 84 | - |

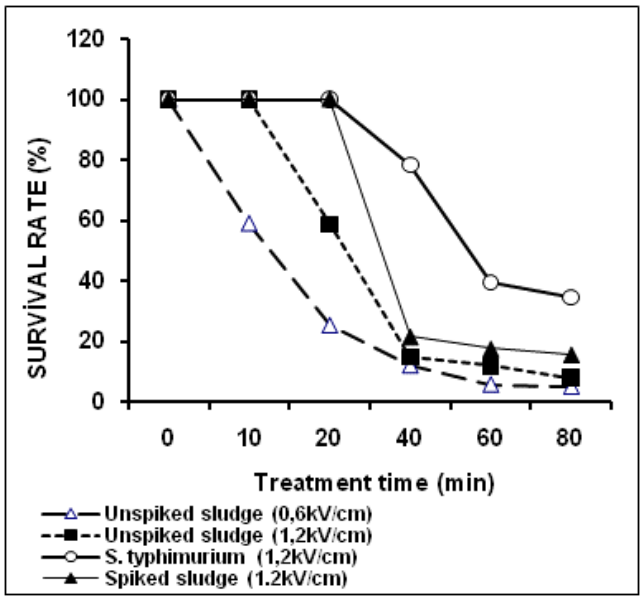


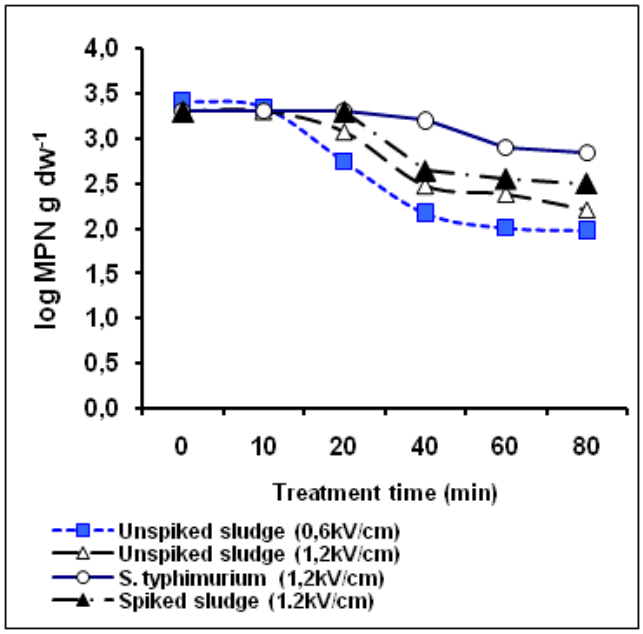

Fig. 2. Effect of electric field strength on the inactivation Salmonella typhimurium and Salmonella spp. at 50 kHz.

On the other hand, in the experiment of the inactivation with pure culture of and 1.2 kV cm-1 electric field power, there was a 0.5 log reduction. The results of inactivation of *S. typhimurium* might be due to the differences of the electric charges in the cell membranes of the other gram (-) Salmonella types and their bacteria. In this respect, Garci et al [19] reported that gram (-) Salmonella serotype sentenberg was more resistant at low pH degrees than other grams (+) by carrying out PEF application. On the other hand, Zhang et al.[20] reported that the cells in the logarithmic phases were much more inactive (30%) than the cells in the stationary phases. Korolczuk et al. [21] reported that there was a significant reduction in the continuous system as a result of the treatment with Salmonella enteritis PEF at 30 kV cm-1. Although the systems of electric fields were different and the values of the powers were lower, we obtained similar results with the ones of the above researchers in terms of inactivation values without calorific effects. However, inactivation occurred 40-60 minutes later in this study while the inactivation occurred in millisecond periods in PEF and AC electric field with µs methods.

Uemura et al [9] who obtained a 5 log *E. coli* reduction by applying 400 times of frequency (20 kHz) and 14 kV $cm^{-1}$ AC electric field, succeeded in obtaining a much higher inactivation than their experiments in which they had used electric field with 74°C pasteurization. Noval et al [22] studied the magnetic field impact on *Saccaromyses cerevisia*, *E. coli*, *Staphylococcus aureus* at laboratory temperatures (24-26°C) and put forward the idea that the inactivation rate was low because the magnetic field impact killed the cells of the bacteria and because the rest of the living bacteria went on growing. The raw waste water in the conventional waste water treatment plants contains $7x10^3$ MPN 100 $ml^{-1}$[23] *Salmonella* spp and $10^7$-$10^8$ CFU 100 $ml^{-1}$ indicator bacteria. However, these bacteria reduce at a rate of 90% in the treatment process [24]. The cause of the low inactivation rate in our study was thought to be the high concentration of bacteria. However, although some researchers [25] determined that the high concentration bacteria affected the rate of inactivation, Geveke and Brunkhorst [26] and Zhang

et al [27] put forward that the initial higher concentrations of bacteria did not have an impact on the inactivation rate. The number of Salmonella spp., whose surviving periods vary between 30-60 days in sludge and waste water, in unwatered sludge is 105 g $dw^{-1}$ [28]. In this study applied in the sewage sludge, our inactivation rate maintained in 1.1-1.43 log MPN g $dw^{-1}$ interval because of testing electric field without using any heat. The difference between the results of these studies can vary according to the PEF and AC electric field applications and the types of the microorganisms.

An elimination experiment was conducted under thermal conditions (65°C) with slaked lime and the inactivation efficiencies were compared between the AC electric field and the caustic lime (Figure 3). In order to determine both the impacts of temperature and the lime treatment, 3 log reduction was observed in *Salmonella* spp, which was subjected to caustic lime at 65°C. When the results of the electric field and slaked lime were compared, it was determined that the lime and pasteurization applications in bacteria were more easily and efficiently inactivated. However, the increase in the use of energy and sludge in this study poses a disadvantage.



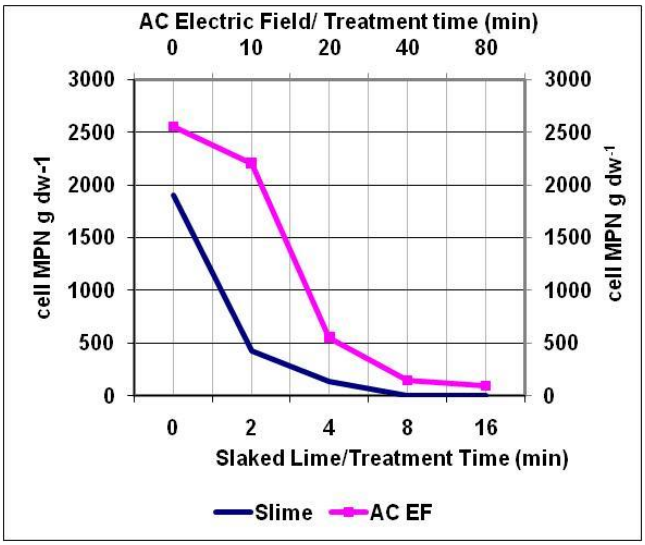




Fig. 3. Effect of AC electric field strength at 50 kHz. and slime treatment on *Salmonella* spp.

When the TEM images were examined (Fig. 4), it was found that the cells subjected to 0.6 kV cm-1 electric field were seriously destroyed. There became a deformation in membranes and the materials in the cytoplasm shrank because of the intracellular pressure and then they were spread out of the cell.

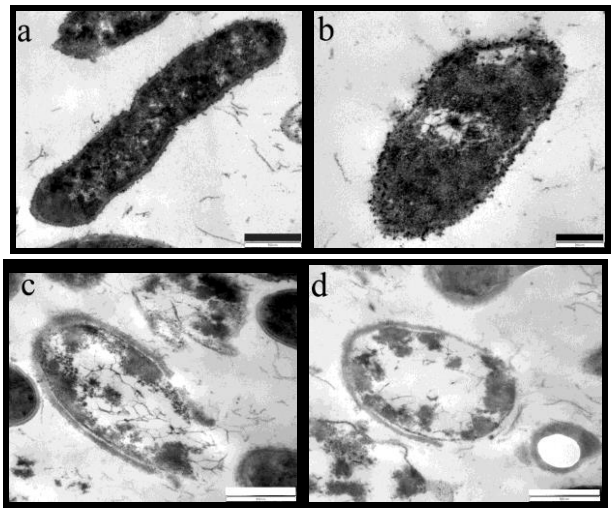



Fig. 4. The TEM images of untreated and 50 Hz AC electric field treated *Salmonella typhimurium* bacteria. Untreated *S. typhimurium* (a), treated for (b) 40 min, (c) 60 min, (d) 80 min. (scale bar: (a, c ,d) 500nm, (b) 200 nm).

## IV. Conclusion

In this study whose purpose was to pretest the inactivation of pathogens in sewage sludge under non-thermal conditions in a batch system, the pathogens were inactivated remarkably. As a result of the electric field application of 50 kHz frequency and 0.6-1.2 kV cm-1;

- The inactivation rate of Salmonella spp. in the raw sludge in 0.6 kV cm-1 electric field reduced at a rate of 1.4 log (92%) in the 80th minute.

- There was a reduction in S. typhimuruium 0.5 log (66%), spiked sludge Salmonella spp. 0.8 log (84%), unspiked (raw) sludge 1.1 log(92%) in 1.2 kV cm-1 electric power.
- As a result of the heat and slaked lime application, there was an inactivation 99.5% of Salmonella spp. When the results of the electric field and slaked lime treatment were compared, the effects of the pasteurization and the lime application were found to be more efficient and quicker. However, the increase in the volumes of the energy and sludge used in this process poses a disadvantage. The daily electricity cost of AC electric field in the laboratory scale was calculated as $16, 00.
- TEM images demonstrate that the inner areas of the AC-low frequency electric field treated cells were seriously disrupted. The membranes show deformation and materials within the cytoplasm seem to be shrunken and unevenly dispersed.

While PEF inactivation has the advantage of quicker and more efficient inactivation, AC electric field may be used in the elimination of waste water and sewage sludge because of its uncomplicated equipment demands. It may be an environment-friendly method as it does not release any chemicals to nature.

## ACKNOWLEDGMENT

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