Growth of faecal coliforms and *Salmonella spp.* in physicochemical sludge treated with acetic acid

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**Abstract** The use of advanced primary treatment (APT) to remove helminth ova from wastewater has raised the issue of treating the generated sludge to allow its reuse or disposal. Several studies have been performed in Mexico in order to treat the sludge with the main goal of destroying helminth ova and bacteria, one of them analysing the acid treatment. Previous research has demonstrated the feasibility of applying such a process using acetic acid to disinfect the sludge, but the potential for bacterial growth was still to be proved. The results of a growth study of faecal coliforms and *Salmonella spp.* in sludge treated with acetic acid are presented in this paper. Physicochemical sludge generated in a semi-rural area of Mexico City was treated using acetic acid in 6 different doses ranging from 3,700 to 22,000 ppm (w/w) and the concentrations of faecal coliforms, *Salmonella spp.*, and total and volatile solids, were monitored after 30 minutes, and 8, 21 and 35 days. Average initial concentrations in sludge were $1.1 \times 10^8$ MPN/g TS and $1.5 \times 10^5$ MPN/g TS for faecal coliforms and *Salmonella spp.*, while pH, total and volatile solids were 5.4, 5.0% and 73% respectively. Apparently, some acidified samples presented anaerobic activity, observed as a change in sludge coloration, as well as bacterial growth. pH of the treated samples with less than 18,400 ppm raised from the initial value of approximately 4.0 up to 5.9 units, while samples with 18,400 and 22,000 ppm maintained the pH close to 4. Total and volatile solids did not present important changes except in the untreated sample where they were reduced by 0.3 and 3.4% respectively. Samples treated with more than 14,700 ppm of acetic acid did not present any increase in bacterial density. Additionally, concentrations of faecal coliforms and *Salmonella spp.* in untreated sludge were reduced throughout the time, in contrast to samples treated with doses lower than 14,700 ppm that showed some growth, which suggests that the use of acetic acid in doses lower than 15,000 ppm stimulates in some way the growth of these bacteria.

**Keywords** Acetic acid; acid treatment; faecal coliforms; physicochemical sludge; *Salmonella*

**Introduction**

The use of advanced primary treatment (APT: coagulation-flocculation-sedimentation) in Mexico for removal of helminth ova from wastewater has demonstrated that the standard for restricted irrigation (1 helminth ova/L) is achievable by applying this technology coupled with filtration and disinfection (Jiménez *et al.*, 1998). As a result of the APT, sludge contains most of the helminth ova removed from wastewater and also high levels of faecal coliforms and *Salmonella spp.* (Jiménez *et al.*, 2000). Since these microorganisms must be destroyed or inactivated to a certain degree before sludge is reused or disposed, stabilisation needs to be carried on. For this purpose, sludge stabilisation is generally accomplished by applying conventional treatment processes such as aerobic and anaerobic digestion, composting or alkaline treatment. Removal of bacteria on those processes varies widely and especially destruction of parasites, like helminth ova, is not always accomplished except on those treatments that increase the temperature of the sludge. However, certain non-conventional processes have demonstrated their ability to reduce bacterial and parasitic levels in sludge. Among these, acid treatment has not been deeply studied but has demonstrated that it may produce biosolids that meet the Class B requirements according to the United States Environmental Protection Agency (US EPA). Previous studies performed with acetic, peracetic, perchloric and sulphuric acids have demonstrated that 3.2 to 6.9 log
units were removed for faecal coliforms and efficiencies from 69 to 90% may be achieved regarding helminth ova reduction in physicochemical (APT) sludge (Barrios et al., 2000). Additionally, it has been determined that acetic acid performed better than the rest of the acids based on microbial reduction (faecal coliforms and helminth ova), cost, pH of the treated sludge and risk of application, all of them under laboratory conditions. Even though several experiments were carried out, microbial stability of the sludge needed to be demonstrated to guarantee that acid treatment (with acetic acid) is an alternative to produce biosolids that may be land applied. This study presents the monitoring of faecal coliforms and Salmonella spp. levels in sludge treated with acetic acid in different doses over a period of 35 days to evaluate the stability of the acid biosolids.

**Methods**

Sludge used on this study was sampled from an APT plant treating municipal wastewater from a semi-rural area in Mexico City. The plant treats a flow of 35 L/s by applying 66 mg/L of aluminium sulphate and 1 mg/L of polymer. The study was performed twice with sludge sampled on different dates. Analyses performed included pH, total solids (TS), volatile solids (VS), faecal coliforms, and Salmonella spp. according to the techniques described by the Standard Methods (1995). Residual acetic acid was measured on certain samples using the method proposed by Orozco (1981). Initial concentrations of helminth ova were determined with the technique described by the US EPA (1992). After sludge was sampled from the APT plant, it was analysed for all the parameters except for residual acetic acid, assuming that baseline concentrations were negligible and that initial concentrations were equal to the amount of acetic acid added. Characterisation of raw sludge is shown in Table 1.

From Table 1 it is clear that sludge did not significantly change its quality between samples except for total solids concentration and Salmonella spp. density. In the first case (TS), this parameter is affected by how the treatment process is operated as sludge wasting, coagulant dosage and water quality vary over time. However, it changes less than 1% between samples which is considered within the operating range for the plant, based on previous data (4.3–6.7%). Regarding concentration of Salmonella spp. it depends more on health issues rather than operation procedures at the treatment plant and thus it cannot be controlled. On this aspect, it should be noticed that, based on the microbial content of the sludge, population from the served area presents high levels of infection with Salmonella and helminths. As mentioned before, the area that generates the wastewater is a semi-rural area that to some extent is based on farming activities that may contribute some pathogens and parasites. However, considering the parameters analysed, it was assumed that samples taken for this study were representative of the quality of the sludge generated at the treatment plant.

Acid treatment of the sludge was performed by adding different amounts of acetic acid solution (50% v/v) to APT sludge and mixed in a jar-test apparatus at 320 rpm for 30 minutes. The first experiment was carried on with 1-litre samples but at the end of the experimental period (35 days) total solids concentration indicated that evaporation had dewatered the sludge and thus for the second experiment samples with 2 litres of sludge

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample A</th>
<th>Sample B</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.39</td>
<td>5.43</td>
</tr>
<tr>
<td>Total solids, %</td>
<td>5.3</td>
<td>4.7</td>
</tr>
<tr>
<td>Volatile solids, % of TS</td>
<td>73.1</td>
<td>72.3</td>
</tr>
<tr>
<td>Faecal coliforms, MPN/g TS</td>
<td>$1.10 \times 10^8$</td>
<td>$2.40 \times 10^8$</td>
</tr>
<tr>
<td>Salmonella spp., MPN/g TS</td>
<td>$1.50 \times 10^5$</td>
<td>$2.30 \times 10^6$</td>
</tr>
<tr>
<td>Helminth ova, ova/g TS</td>
<td>105.2</td>
<td>94.0</td>
</tr>
</tbody>
</table>
were used. After the mixing period, samples were taken for analyses and the sludge was kept at room temperature for 35 days in the laboratory. All samples were open to the atmosphere during the storage period and the parameters listed on Table 1 analysed after 8, 21 and 35 days except for helminth ova.

Results and discussion

**pH, total solids, volatile solids and residual acetic acid**

pH is in certain ways related to the destruction or inactivation of bacteria and thus certain organic acids (including acetic) are used as food preservatives to avoid potential contamination. The toxicity of weak organic acids is related to the concentration of the undissociated acid that diffuses through the cell membrane and may dissociate intracellularly (Taherzadeh et al., 1997). This means that at pH values below the pKa for a specific acid the toxicity is greater.

Figure 1 shows that the initial pH in all of the treated samples was lower than the pKa of acetic acid (4.8) meaning that toxicity would be greater than at pH values higher than that. In raw sludge, pH slowly increased from 5.4 up to 6.9 units after 35 days, allowing for fermentation to take place. This was confirmed by the reduction in volatile solids from 72.7 to 69.3% in contrast with the treated samples that did not show the same behaviour. Also, total solids decreased slightly during the first 21 days until 35 days when average solids increased in all samples due to evaporation in the first experiment, giving a large standard deviation (up to 2.8%).

In treated sludge, total and volatile solids did not show a clear trend but their concentration was to some extent reduced especially at doses lower than 15,000 ppm.

pH data also shows that for those samples acidified with less than 15,000 ppm, an increase in this parameter was measured, reaching 5 units after 35 days except for a dose of 14,700 which raised the pH to 4.7. In this regard, samples with 18,400 and 22,000 ppm maintained their pH close to 4 units (4.1 and 4.0 respectively) reducing the possibility of bacterial regrowth. Nonetheless, it was expected that if the pH had been monitored on these two samples, in the long term it would have started to rise gradually, increasing the chance for regrowth.

![Figure 1](https://iwaponline.com/wst/article-pdf/44/10/85/424258/85.pdf)

**Figure 1** pH, total solids, volatile solids and residual acetic acid in physicochemical sludge treated with acetic acid
Residual acetic acid was measured at 21 and 35 days because it was assumed that at the beginning of the experiment the baseline concentration was negligible as the sludge did not present any signs of fermentation. As a result, initial concentration was estimated as the concentration of acetic acid added to each sample. In raw sludge, acetic acid concentration was 0.6 g/L at 21 and 35 days, amount apparently generated during anaerobic fermentation. In samples of acidified sludge with 3,700, 7,300 and 11,000 ppm the acetic acid concentration decreased gradually over time while the pH increased, indicating the consumption of acetic acid by bacteria as part of their metabolism. On the other hand, when 14,700, 18,400 and 22,000 ppm of acetic acid were applied to sludge, acid concentrations, between 30 minutes and 21 days of contact time, were reduced by less than 0.4 g/L or maintained, and they increased slightly between 21 and 35 days, especially with the higher dose. These differences may indicate the production of acetic acid by acetogenic bacteria under anaerobic conditions and the subsequent accumulation as it cannot be consumed by methanogenic bacteria under those acidic conditions.

**Faecal coliforms and Salmonella spp.**

The mechanism of disinfection with acetic acid is not well understood but some studies suggest that organic acids may act either as a carbon source and energy, or as inhibitory agents, depending on their concentration and its ability to enter the cell and the capacity of the organism to metabolise it (Nunn, 1987; Cherrington et al., 1991). Regarding sludge treatment, the information reported in the literature is scarce and only a few studies have used acetic acid to reduce microbial content in sludge, and especially focusing on helminth ova (Kiff and Lewis-Jones, 1984).

On this study, the application of acetic acid in doses from 3,700, to 22,000 ppm reduced faecal coliforms from 3.2 to 5.0 log (average for 2 experiments) 30 minutes after the acid addition (Figure 1). However, after the initial reduction, faecal coliforms recovered and started to grow on acidified sludge with doses of up to 11,000 ppm in contrast with the rest of the samples that maintained or reduced their concentration. This recovery was faster when the acid dose was lower. As can be seen, in the case of the sludge treated with 11,000 ppm there is a noted delay in the faecal coliforms growth and it was not until the period between days 21 and 35 that they started to reproduce. Some studies have reported that certain varieties of *Escherichia coli*, included in the faecal coliform group, do not always grow in acid conditions with acetic acid at a pH below 4.4 while some species of *Salmonella* may grow at a pH of 4 (Lin et al., 1995; Ryu et al., 1999). Nevertheless, under certain circumstances both of these bacteria may develop some acid tolerance. Presumably, faecal coliforms adapted to the acidified sludge which, together with the pH recovery, accounted for this adaptation allowing them to grow under less stressful conditions.

On the other hand, concentration of faecal coliforms in raw sludge (0 ppm of acetic acid) decreased over time due to the fermentation that took place on the sludge. These conditions reduced more than 4 log in raw sludge indicating that if sludge is anaerobically digested it would comply with the Class B limits for land application. However, mesophilic anaerobic digestion does not reduce helminth ova viability according to Pike et al. (1988).

It is important to mention that since the sludge comes from alum coagulation and contains a large amount of sulphate, hydrogen sulphide was generated in samples containing raw sludge and 3,700 and 7,300 ppm, turning sludge from a light into a dark brown colour. Also, gas production increased as pH recovered, noted as tiny bubbles released to the surface of the sludge in those same samples but also in the sample with 11,000 ppm. All these indicates that microbial activity continued in the sludge when less than 14,700 ppm of acetic acid were applied, suggesting that doses in the order of 15,000 ppm should be used if regrowth is to be avoided.
Regarding *Salmonella* evolution, Figure 3 shows that their concentration in raw sludge was reduced by more than 3 log just by the fermentation process. Again, some acidified samples presented an initial inhibition but after some time they started to grow or did not further reduce their concentration as noted in the results for samples with 3,700 to 11,000 ppm. By observing the data for a dose of 11,000 ppm and also the pH for that same sample, it is clear that *Salmonella* recovered from an initial pH lower than 4 units (3.8 after 30 minutes) and grew at pHs of 4.2 and 5.2 at 21 and 35 days. These results differ somewhat with the work by Foster and Hall (1991) which indicates that internal cell systems help to maintain the internal pH of bacterial cells close to a neutral value but at a pH below 4, these systems begin to fail allowing internal H⁺ concentration to raise to lethal levels. As in the case of faecal coliforms, *Salmonella spp.* developed some acid tolerance and recovered from the initial acid shock, supported by a reduction in acetic acid concentration. In contrast, samples with doses equal or higher than 14,700 ppm did not present any *Salmonella* regrowth even after 35 days of treatment which again suggest the use of doses higher than 15,000 ppm for stabilisation.
Conclusions

Based on the results, it is possible to apply the acid treatment for stabilisation of physicochemical sludge, reducing the concentrations of faecal coliforms and Salmonella spp. However, doses higher than 15,000 ppm of acetic acid must be applied to reduce the possibility of bacterial regrowth, since lower doses promote their acid tolerance and increase their concentration in the short term.

Considering the evolution of volatile solids (i.e. no reduction), it is possible to demonstrate that faecal coliforms and Salmonella spp. mainly used the acetic acid as a source of carbon and energy before using other organic compounds. Additionally, both types of bacteria were stimulated by the acetic acid addition increasing their concentration after their initial reduction. Also, it is clear that sludge treatment with acetic acid limits the normal development of the microbial community and slows down their establishment because of the pH reduction, but this reduction will not necessarily guarantee the stability of the product. For this reason, an excess amount of acetic acid is needed to totally inhibit faecal coliforms and Salmonella spp. the recommended dose being higher than 15,000 ppm.

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References


