Disinfection kinetics of pathogens in physicochemical sludge treated with ammonia

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Abstract Ammonia is a disinfectant which can diffuse through the membrane of highly resistant structures like helminth ova. Thus, it can be considered an alternative disinfectant of wastewater sludge with high pathogenic content. In this study, the kinetic parameters of the Hom model were used to describe the inactivation with ammonia of faecal coliforms, Salmonella spp. and viable helminth ova. These were obtained in processes considering the addition of ammonia alone as well as for ammonia combined with an increase in temperature. The sludge was sampled from a municipal wastewater treatment plant using an APT (Advanced Primary Treatment) or CEP (Chemical Enhanced Primary) process. With 20% w/w of ammonia, 7 logs of faecal coliforms, 6 logs of Salmonella spp., and 83% of viable helminth ova were reduced in 2 hours contact time. To eliminate 100% of the helminth ova from samples having 88–132 ova/g TS it was needed to combine 20% of ammonia with 50°C. The analysis of parameters $k$, $n$ and $m$ indicate higher resistance to inactivation of helminth ova compared to bacteria and a better performance of the ammonia process than lime stabilization to inactivate microorganisms. In addition, ammonia increased the agricultural value of the biosolids produced.

Keywords Ammonia; disinfection kinetic; faecal coliforms; helminth ova; Salmonella spp.; sludge

Introduction

Currently, proposed regulations limit the content of pathogens in biosolids intended for disposal or reuse. In Mexico, as in other developing countries, sludge contains high levels of pathogens that must be inactivated, particularly helminth ova. Several studies have demonstrated that alkaline stabilization is an alternative for the treatment of sludge with high microbial content (Christy, 2000; Mignotte-Cadiergues et al., 2001). However, its use can increase both the weight and the salinity of treated sludge making necessary to consider other disinfection processes. According to Allievi et al. (1994), ammonia is an effective disinfectant that has been used as bactericide in its non-ionized form (NH$_3$). Furthermore, it has been demonstrated that ammonia can inactivate or even destroy helminth ova like Taenia saginata, Ascaris suum, Ascaris lumbricoides, Trichuris spp., Hymenolepis spp., and Toxocara spp. in sludge from wastewater treatment plants (Bruce, 1984; Reimers et al., 1986; Ghiglietti et al., 1995, 1997; Mendez et al., 2002). As a consequence, ammonia was identified as an alternative for the treatment of sludge containing diverse populations of microorganisms, with the advantage of increasing the fertilizing properties of the biosolids produced for agriculture. To test its capability to disinfect, several studies were performed to study the kinetics of the process. The models used to describe the disinfection reactions are similar to those for chemical reactions. Currently, the most common disinfection model is the one proposed by Chick-Watson expressed as (Pernitsky et al., 1995):

$$
\ln \frac{N}{N_0} = -kC^a t
$$

(1)
Where \( N_0 \) is the initial concentration of microorganisms, \( N \) is the concentration of the remaining microorganisms at time \( t \), \( k \) is the pseudo first-order reaction rate constant, \( C \) is the disinfectant concentration and \( n \) is an empirical coefficient frequently assumed as equal to 1. In 1972, Hom (AWW, 1990) proposed an alternative kinetic model to account for deviations from the Chick-Watson model commonly encountered in practice. The model introduces an empirical constant \((m)\):

\[
\ln \frac{N}{N_0} = -k C^n t^m
\]

Owing to the potential applicability of ammonia to stabilize sludge with high pathogenic content, it was considered necessary to obtain information concerning the inactivation kinetics of faecal coliforms, \textit{Salmonella} spp. and viable helminth ova, which are the microorganisms commonly present in sludge and thus considered in regulations. Furthermore, this study tries to establish the conditions to reduce the total content of helminth ova in sludges with high initial concentrations.

**Methods**

**Sampling and treatment of physicochemical sludge**

Sludge was sampled from a municipal wastewater treatment plant of 35 L/s serving a population of 11,000 persons approximately and located near Mexico City. The wastewater treatment consists of an Advanced Primary Treatment Process (APT) or Chemical Enhanced Primary Treatment (CEPT) which basically is a coagulation-flocculation process. Considering the socioeconomic and public health conditions, sludge has high concentrations of pathogens. This has been reported in several studies (Jimenez \textit{et al.}, 2000; Jimenez \textit{et al.}, 2002). Sludge total solids (TS) averaged 5.4% \((\pm0.3\%)\), pH was 5.3 units, faecal coliforms ranged from 7 to 8.2 log MPN/g TS, \textit{Salmonella} from 5.4 to 6.8 log MPN/g TS, and helminth ova from 88 to 132 ova/g TS. The ammonia stabilization tests were carried out using a hermetically closed 2-litre reactor. The research was divided into three stages. In the first one, ammonia was applied to sludge in doses from 10 to 50% w/w, using a solution of 28–30% v/v of ammonium hydroxide \((\text{NH}_4\text{OH})\) and controlling reactor temperature at 20°C. The samples and a control were homogenized with agitation at 200 rpm for 1 minute, then ammonia was applied and mixed at 300 rpm during 2 hours. At the end of each treatment, samples were taken for microbial analyses. Each test was repeated at least five times. The second experimental stage was performed to evaluate the effect of contact time. In this case, 20% w/w of ammonia was added to the sludge (at 20°C) and microbiological evaluation was carried out after 0.5, 1, 1.5 and 2 h. Mixing conditions were the same as in first stage. In the third stage, the microbial inactivation as a function of the product of the ammonia dose and temperature (dose-temperature-DT product) was studied. In this case, 10% and 20% of ammonia were applied at temperatures of 20, 30, 40 and 50°C. Contact time was 2 hours and mixing conditions were the same as in previous experiments. In all cases, the initial and final concentrations of faecal coliforms, \textit{Salmonella} ssp. and helminth ova were measured, as well as other parameters to characterize the sludges.

**Analysis of samples**

Faecal coliforms and \textit{Salmonella} ssp. were determined according to the \textit{Standard Methods for the Examination of Water and Wastewater} (1995), methods 9221E and 9221B, respectively. Viable helminth ova were quantified by the method described by US EPA (1992). pH, temperature and total solids were evaluated following the procedures 4500 H\(^+\), 2550 B and 2540 B from the \textit{Standard Methods}. 

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Results

First Stage. Kinetic coefficients of Hom model in tests with different ammonia doses

Table 1 shows that ammonia doses of 20% and higher removed more than 7 and 5 logs of faecal coliforms and *Salmonella* spp., respectively, meeting the US EPA limits for class A biosolids with respect to these parameters. However, to inactivate 94% of the 105 viable helminth *ova/g TS* initially present, 50% w/w of ammonia was needed, producing a sludge with 6 viable *ova/g TS* which is higher than class A limits. Evidently, ammonia has a higher disinfection power over bacteria than over helminth ova, as expected since the latter are considered the most resistant microbial structures. Because in theory the conversion of the ammonia was expected to be around 75–96% of the original dose applied, the inactivation is attributed mainly to the molecular form (NH₃) rather than the ionized form (NH₄⁺).

Another important factor that accounts for disinfection was pH. pH increased from an initial acid value (near 5) to 9.7–10.7, depending on the dose of ammonia. However, the higher changes of pH were obtained by applying the first ammonia dose (ΔpH = 4.2 units) while the maximum inactivation of helminth ova was reached with the higher doses at a pH of 10.7. Results suggest that the inactivation of microorganisms, mostly helminth ova, is mainly due to the quantity of ammonia added and not to the pH reached. Allievi *et al*. (1994) and Ghiglietti *et al*. (1997) reported a similar effect in faecal streptococcus and ova of *Ascaris suum*.

The inactivation of faecal coliforms, *Salmonella* spp. and helminth ova at different disinfectant concentrations (D) was described by the modified Hom model. The model was applied considering a constant contact time of 2 h. The resulting equation is

\[
\ln N = -k^* D^n
\]

where

- \( D \): ammonia dose, g/L
- \( k^* \): is a constant associating \( k \) and the time \( t^m \)

In Table 2, \( k^* \) values higher than 1 are shown for bacteria, that suggest that both faecal coliforms and *Salmonella* spp. are not very resistant to ammonia. It is perceptible that a significant inactivation of bacteria is reached even with lower doses (4.7 g/L or 10%). *Salmonella* spp. is the group of bacteria with the highest \( k^* \) value (8.033), which means that faecal coliforms may be used as indicator of this group of bacteria. Also, densities of *Salmonella* were between 1 and 2 log lower than faecal coliforms in raw sludge. In contrast, the \( k^* \) value for helminth ova is clearly lower compared to those for bacteria. This is because: (a) helminth ova concentrations are much lower and consequently not expressed in a logarithmic form; (b) these much lower concentrations need similar ammonia doses to

<table>
<thead>
<tr>
<th>Ammonia dose (average)</th>
<th>pH</th>
<th>% NH₃ converted*</th>
<th>Molecular NH₃ (g/L)</th>
<th>Faecal coliforms Log (N/No)</th>
<th><em>Salmonella</em> spp. Log (N/No)</th>
<th>Viable helminth ova No-N/No × 10²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (0.0)</td>
<td>5.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>10 (4.7)</td>
<td>9.7</td>
<td>75.1</td>
<td>3.5</td>
<td>-3.9</td>
<td>-3.9</td>
<td>68.6</td>
</tr>
<tr>
<td>20 (9.4)</td>
<td>10.3</td>
<td>91.6</td>
<td>8.6</td>
<td>-6.9</td>
<td>-5.7</td>
<td>83.2</td>
</tr>
<tr>
<td>30 (14.1)</td>
<td>10.5</td>
<td>93.9</td>
<td>13.2</td>
<td>-6.9</td>
<td>-5.7</td>
<td>89.9</td>
</tr>
<tr>
<td>40 (18.8)</td>
<td>10.6</td>
<td>95.8</td>
<td>18.0</td>
<td>-6.9</td>
<td>-5.7</td>
<td>91.4</td>
</tr>
<tr>
<td>50 (23.5)</td>
<td>10.7</td>
<td>96.8</td>
<td>22.7</td>
<td>-7.3</td>
<td>-5.7</td>
<td>94.3</td>
</tr>
</tbody>
</table>

* calculated with \( \% \) ammonia = \[ \frac{1}{1 + 1.82 \times 10^{0.9pH}} \] × 100
faecal coliforms and *Salmonella* spp. to be inactivated; and (c) helminth ova are much more resistant.

Comparing these results with those reported by Mendez (2003) for lime stabilization, ammonia was a better disinfectant to reduce bacteria and helminth ova in sludge. For instance, with quicklime a 20% w/w dose and a contact time of 2 h to reduce 7 and 5 logs of faecal coliforms and *Salmonella*, respectively and 90% of the helminth ova (with an initial concentration of 90 ova/g TS). Also the $k^*$ values for lime stabilization of 4.826, 6.551, and 0.755 are much lower than those found for ammonia (Table 2) demonstrating that ammonia is a better disinfectant. Based on this, ammonia is considered an interesting and better option to stabilize sludges with high pathogenic content than lime.

**Second stage. Kinetic coefficients for Hom model obtained in tests using different contact times**

Based on a statistical analysis, the dose of 20% of ammonia was selected for performing the tests during this experimental stage. From experiments with different contact times it is observed, in Table 3, that less time is needed to inactivate *Salmonella* spp. and faecal coliforms (1 h) than to inactivate helminth ova (2 h). Even though results seem to indicate that 90 minutes are enough to perform the process, it is recommended, as for lime (US EPA, 1994), to use at least 2 hours for ammonia disinfection. Also, these experiments show that due to the very high initial content of helminth ova (98 ova/g TS, for this stage) compared with concentrations reported in sludges from the United States (2–13 ova/g TS; Lue – Hing *et al*., 1992) it was not possible to destroy them completely and produce Class A biosolids.

The inactivation of microorganisms for this stage was described using a first order model. For this, the Hom model was modified replacing the $k$ parameter and the ammonia dose ($D$) by $k^{**}$.

$$
\ln \frac{N}{N_0} = -k^{**} t^n
$$

(4)

The values of the kinetic parameters obtained applying Eq. (4) are shown in Table 4. These results confirm that bacteria inactivation is faster than helminth ova, since $k^{**}$ for bacteria was of 5.859 and 6.953 for faecal coliforms and *Salmonella*, respectively, while for helminth ova it was of only 0.211.

**Table 2** Hom modified model kinetics parameters obtained during the first experimental stage

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>$k^*$</th>
<th>$n$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faecal coliforms</td>
<td>7.585</td>
<td>0.2669</td>
<td>0.90</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>8.033</td>
<td>0.1940</td>
<td>0.84</td>
</tr>
<tr>
<td>Helminth ova</td>
<td>0.741</td>
<td>0.4370</td>
<td>0.85</td>
</tr>
</tbody>
</table>

**Table 3** Summary of results of the second experimental stage

<table>
<thead>
<tr>
<th>Contact time (min)</th>
<th>Ammonia dose % w/w (g/L)</th>
<th>pH (average)</th>
<th>% NH$_3$ converted *</th>
<th>Molecular NH$_3$ (g/L)</th>
<th>Faecal coliforms Log (N/No)</th>
<th><em>Salmonella</em> spp. Log (N/No)</th>
<th>Viable helminth ova (No-N)/No $\times 10^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 (0)</td>
<td>5.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>30</td>
<td>20 (9.4)</td>
<td>10.3</td>
<td>91.6</td>
<td>8.6</td>
<td>-4.8</td>
<td>-3.3</td>
<td>76.9</td>
</tr>
<tr>
<td>60</td>
<td>20 (9.4)</td>
<td>10.3</td>
<td>91.6</td>
<td>8.6</td>
<td>-5.6</td>
<td>-4.5</td>
<td>88.1</td>
</tr>
<tr>
<td>90</td>
<td>20 (9.4)</td>
<td>10.3</td>
<td>91.6</td>
<td>8.6</td>
<td>-5.8</td>
<td>-4.5</td>
<td>93.8</td>
</tr>
<tr>
<td>120</td>
<td>20 (9.4)</td>
<td>10.3</td>
<td>91.6</td>
<td>8.6</td>
<td>-6.4</td>
<td>-4.5</td>
<td>96.0</td>
</tr>
</tbody>
</table>

* calculated with $\%$ ammonia = \[ \frac{1}{1 + 1.82 \times 10^{-9} \cdot pH} \times 100 \]
To obtain the $k$ value of the Hom model it is necessary to use the following equations:

Stage 1 (Constant contact time and variable ammonia doses). From Eq. (3)

$$k = \frac{k^*}{t^m}$$  \hspace{1cm} (5)

The value of the coefficient $m$ is presented in the second stage (Table 4).

Stage 2 (Constant ammonia dose and variable contact time). From Eq. (4)

$$k = \frac{k^{**}}{D^n}$$  \hspace{1cm} (6)

During the first stage (Table 2), the value of the coefficient $n$ was calculated. Thus, with the results, global values for $k$ were calculated for each microorganism and each experimental stage. Table 5 displays these values for faecal coliforms, Salmonella spp., and helminth ova. Although data were obtained in different experimentation stages, they are similar, which expresses that these parameters are independent of the contact time and the ammonia dose.

Additionally, during the second stage the effect of long contact times was also analyzed. It was found that times higher than 24 h did not significantly increase the efficiencies reached in 2 h, either for bacteria or helminth ova, and that, concerning bacteria, no regrowth was observed. Figure 1 illustrates the application of the Hom model to helminth ova data from this stage.

### Table 4  Hom modified model kinetics parameters obtained during the second experimental stage

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>$k^*$</th>
<th>$m$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faecal coliforms</td>
<td>5.859</td>
<td>0.1893</td>
<td>0.84</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>3.953</td>
<td>0.2128</td>
<td>0.92</td>
</tr>
<tr>
<td>Helminth ova</td>
<td>0.211</td>
<td>0.5741</td>
<td>0.94</td>
</tr>
</tbody>
</table>

### Table 5  Values of $k$ (global parameter) of the Hom model

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>First stage</th>
<th>Second stage</th>
<th>Mean Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faecal coliforms</td>
<td>3.17</td>
<td>3.28</td>
<td>3.21</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>2.88</td>
<td>2.62</td>
<td>2.70</td>
</tr>
<tr>
<td>Helminth ova</td>
<td>0.0474</td>
<td>0.0823</td>
<td>0.0552</td>
</tr>
</tbody>
</table>

Figure 1  Application of Hom model to helminth ova data from Stage 2
Third Stage. Kinetic coefficients at different temperatures

Because temperature also increases inactivation, a third stage was carried out to evaluate the combined effect of varying temperature and ammonia dose. For all the microorganisms studied, inactivation increased dramatically when temperature was raised above 40°C. Table 6 shows that when 10% ammonia was applied, the best conditions were obtained at 50°C, reducing 7.6 logs of faecal coliforms, 4.9 logs of *Salmonella* spp., and up to 76.5% of helminth ova. However, with the combination of ammonia (20%) and temperature 50°C, 100% of the helminth ova are destroyed. This effect cannot be achieved with ammonia alone, even for the highest concentration (8.6 g/L or 20% at 20°C), showing that inactivation is highly increased by raising the temperature. According to Booth (1999), temperature increases the permeability of the cellular wall, allowing the penetration of external agents. In these conditions, molecular ammonia can diffuse through the bacterial membrane and even in highly resistant structures like helminth ova. Also, it is known that temperature accelerates chemical reactions. Allievi *et al.* (1994) mentioned that in a process where ammonia was added to anaerobic sludge to achieve a pH value of 10, environmental temperature (28°C) can produce the inactivation of 1 unit log of faecal streptococcus in comparison with a treatment using the same dose but at 5°C, where there was not a significant removal. Moreover, Ghiglietti *et al.* (1995) reported that in biological sludge treated at 30°C in presence of ammonia (added in a dose to accomplish a pH of 11), more than 90% of ova of *Ascaris suum* and *Ascaris lumbricoides* were inactivated.

Veschetti *et al.* (2003) related the inactivation of bacteria (*E. coli* and faecal streptococcus) with the product of the disinfectant concentration and the contact time (CT product). Results showed that microorganisms’ concentration was linearly reduced when the CT product value was increased. Similarly, in this case, from 27 tests a relationship was found between the product of the ammonia dose (D) and the temperature (T) with the microbial inactivation. Actually, the totality of data could be represented by the first-order Hom model, where time is associated with $k$ in $k^*$ and the $m^*$ parameter is introduced to account for the Chick–Watson model deviations, which are frequently encountered in practice. The Hom model was represented by

$$\log \frac{N}{N_0} = -k^* (DT)^{m^*}$$

(7)

The $k^*$ values obtained are presented in Table 7 and suggest that the inactivation is carried out gradually with the increase of the product DT (ammonia dose × temperature) and that helminth ova display bigger resistance to both variables compared to bacteria. It is important to point out that the $k^*$ value obtained in this stage cannot be compared with the $k^*$ and $k^{**}$ obtained in the previous ones, since it involves the combined effect of two variables.

The $m^*$ value < 1 for faecal coliforms from Table 7 suggests that actual disinfection kinetics are deviating from the Chick–Watson model with a tendency already defined by

<table>
<thead>
<tr>
<th>Parameter</th>
<th>20°C</th>
<th>30°C</th>
<th>40°C</th>
<th>50°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.5</td>
<td>9.7</td>
<td>10.3</td>
<td>9.7</td>
</tr>
<tr>
<td>Faecal coliforms*</td>
<td>0</td>
<td>-4.4</td>
<td>-6.6</td>
<td>-4.8</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>0</td>
<td>-3.3</td>
<td>-4.9</td>
<td>-3.5</td>
</tr>
<tr>
<td>Viable helminth ova b</td>
<td>0</td>
<td>-0.7</td>
<td>-1.7</td>
<td>-0.8</td>
</tr>
</tbody>
</table>

a Log (N/No); b Ln (N/No)
Hom as “tailing”. This tendency means a very fast bacteria inactivation, even with lower DT product values, thus having a less dependency of this product. For *Salmonella* spp, an ammonia dose of 20% w/w (8.6 g/L) at 20°C was enough to inactivate all of them, and thus the effect of the DT product could not be evaluated. Concerning helminth ova, *m* \* was close to 1, so there is no deviation from the lineal model and the results adjusted, once again, to the Hom model.

The kinetic parameters determined may be used to predict the inactivation of the main microorganisms present in sludge and to design and operate a process using ammonia. In addition, the coefficients presented in Table 7 may be used in Eq. (7) to predict microbial removal when temperature is added to the process to increase efficiency. Also, it should be mentioned that the same variables are dependent on the CaO dose in the alkaline stabilization process, frequently used to hygienize sludge, and they can be used to improve lime stabilization.

**Conclusions**

Based on the results, ammonia can be considered an effective alternative disinfectant for sludges, especially to inactivate helminth ova. In contrast with lime stabilization, this process does not increase both, the weight and the salinity of the treated sludge. Quite the opposite, ammonia enhances the fertilization capacity of the biosolids making them more useful on agricultural lands. For these purposes it is recommended to apply 20% w/w of ammonia during at least 2 hours. Results suggest that ammonia can inactivate microorganisms in relatively short contact times, anyway it is recommended to use at least 2 hours that is equal to the time needed when using lime stabilization (2 h) but much shorter than when sludges are composted (> 20 days). This comparison is relevant since these two processes are often considered as the cheapest options to treat sludges with high pathogenic content.

The parameters obtained for the Hom kinetic model (*k*, *n* and *m*) using ammonia indicate higher resistance to inactivation of helminth ova than bacteria. But also, these values obtained when compared to those for lime stabilization for the same type of sludges indicate that ammonia is a better disinfectant than lime.

The kinetic parameters determined in this research are useful to design, operate and control a process using ammonia. Nonetheless, they were obtained from experimental tests carried out in physicochemical sludge, which, in addition to having a complex composition, had very important helminth ova content. Different types of sludges, particularly those from biological processes or from developed countries with less pathogenic content might need less ammonia.

Since the reduction of the microorganisms increases with higher values of dose and temperature (DT product), the heat generated in an alkaline stabilization may be used to improve the process and to reduce costs.

**References**


**Table 7** Hom model kinetic parameters obtained during the third experimental stage

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th><em>k</em></th>
<th><em>m</em></th>
<th><em>R</em>^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faecal coliforms</td>
<td>1.524</td>
<td>0.2765</td>
<td>0.93</td>
</tr>
<tr>
<td>Helminth ova</td>
<td>0.00924</td>
<td>1.011</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Note: helminth ova inactivation was calculated with Ln (N/No)


