Ammonia sanitisation of sewage sludge using urea
Jørgen Fidjeland, Cecilia Lalander, Håkan Jönsson and Björn Vinnerås

ABSTRACT

The aim of the study was to develop a simple, low-cost treatment for sewage sludge using urea as a sanitising agent. Sewage sludge was spiked with Enterococcus faecalis and Salmonella typhimurium, treated with 0.5, 1, 1.5 and 2% w/w urea at laboratory scale, and the viability was monitored during 4 months of storage at 4, 10 and 22 °C (only 0.5%). A linear relationship was identified between Salmonella spp. inactivation rate and ammonia (NH₃) concentration. Temperature had a positive impact on Salmonella spp. inactivation at higher temperatures, but in the range 4–10 °C temperature influenced this inactivation merely by its impact on the ammonia equilibrium. Enterococcus spp. was more persistent and a lag phase of up to 11 weeks was observed. Higher temperature and ammonia concentration reduced the lag phase duration significantly, and also had a clear effect on the inactivation rate for the treatments with 0.5% urea at 22 °C and 2% urea at 4 and 10 °C. Urea sanitisation of sewage sludge can give a 2 log₁₀ reduction of Enterococcus spp. and more than a 5 log₁₀ reduction of Salmonella spp. within 6 weeks with either 0.5% w/w urea at 22 °C or 2% urea at 10 °C.

Key words | ammonia sanitisation, Enterococcus, inactivation, Salmonella, sewage sludge

INTRODUCTION

Sewage sludge is a residual product from the sewage treatment process and contains a high proportion of the phosphorus found in the wastewater if the process includes phosphorus removal. In addition to phosphorus, contaminants may be present in the sludge, depending on the systems connected to the sewer. In many cases upstream management has reduced heavy metal contamination, but wastewater still reflects the domestic use of metals (Vinnerås et al. 2006). Furthermore, there are risks of organic pollutants and pathogen contamination (Sahlstrom et al. 2004). Most countries have legislation on permissible levels of metal pollution, but fewer have regulated the levels of microbial pollution. In Sweden, hygiene requirements have been proposed for class A use, i.e. for agricultural use. It is suggested that the level of enterococci and thermotolerant coliforms per g dry matter should be <1,000, while no Salmonella spp. should be detected per 25 g of wet sludge (Swedish EPA 2010). In the EU, the use of Escherichia coli or enterococci as indicators for monitoring and destruction of at least 4 log₁₀ Salmonella Senftenberg for validation of sewage sludge treatment are suggested (European Commission 2001). US EPA guidelines for Class A biosolids require <1,000 colony forming units (cfu) of faecal coliforms per g dry solids or <3 most probable number Salmonella spp. per 4 g dry solids, less than 1 plaque forming unit of enteric viruses per g dry solids, and no viable Ascaris eggs per 4 g dry solids (U.S.EPA 2003).

Introducing a hygiene requirement on sludge reuse would require most treatment plants to invest in a pre-pasteurisation step or to upgrade to thermophilic anaerobic digestion. The costs of these treatment alternatives would increase treatment costs by an estimated €1–3 per year and person equivalent (Peter Balmer, personal communication, 2010). One possible treatment alternative used for treatment of other pathogen-contaminated organic materials is addition of ammonia to dewatered sludge prior to airtight storage. Ammonia treatment uses the known antibiotic effect of uncharged ammonia (NH₃) (Warren 1962). This treatment has been implemented in several different sectors and both intrinsic and added ammonia have been utilised for sanitisation of urine (Höglund et al. 2002; Vinnerås et al. 2008), faeces (Vinnerås et al. 2003; Nordin 2010), manure (Ottoson et al. 2008), hatchery waste (Emmoth et al. 2011) and sewage sludge (Mendez et al. 2004; Pecson et al. 2007). The ammonia has high efficiency in deactivating bacteria at all temperatures studied to date, from 4 °C up to 50 °C.
Urea has been suggested as a source of ammonia, as it is a harmless substance to work with. When urea is dissolved in organic matter, the enzyme urease hydrolyses it into carbon dioxide and ammonia, both of which are bactericidal (Park & Diez-Gonzalez 2005). The estimated costs of urea treatment are lower than for thermal treatment alternatives. Assuming a yearly sludge production of 100 kg per person and a 1% urea dose, the cost of urea for treatment is equivalent to 0.5 € per person equivalent. One advantage of using ammonia treatment for sewage sludge is that the process can be used on demand for a selected fraction of the sludge to be used as Class A fertiliser, while the remaining sludge fraction can be stored for a year for Class B, i.e. non-agricultural use. This would minimise the treatment costs in cases where the sludge market is limited.

When using sewage sludge as a fertiliser, one main restriction on application of sludge with low heavy metal content is the dose of phosphorus permitted for application. The allowed application dose on most soils in Sweden today is 22 kg phosphorus per year, but, to decrease the cost of spreading, a 5-year dose of phosphorus is allowed and consequently the permissible application dose is 110 kg in one application every 5 years. This represents application of approximately 11 Mg sludge per hectare, as the phosphorus concentration is around 1%. When a phosphorus fertiliser is applied, a nitrogen fertiliser is also required for optimal plant growth (Winker et al. 2009). When applying nitrogen fertiliser, the application rate of nitrogen is limited by the 1-year dose. In the EU, the maximum permissible amount is currently 170 kg total nitrogen per hectare and season. This limitation, in conjunction with intrinsic nitrogen and a sludge application rate of 11 Mg per hectare, results in a possible maximum addition of around 1% urea for sludge treatment.

The objective of this study was to evaluate the efficiency of using urea for ammonia sanitisation of sewage sludge regarding inactivation of enterococci and Salmonella spp., and to study how the inactivation depends on urea dose and temperature.

**METHODS**

**Experimental setup**

Anaerobically digested (37 °C), dewatered sewage sludge with a dry-matter content of 31% was obtained from a municipal wastewater treatment plant with some biological phosphorus removal in combination with phosphorus precipitation using aluminium (Al³⁺). The initial bacterial concentrations in the sludge were 10⁶ cfu/g Salmonella spp. and 10⁵ cfu/g Enterococcus spp. The sludge was disintegrated into small pieces and sprayed with a mix of urea dissolved in bacterial inoculate solution to final concentrations of 10⁶ cfu/g Salmonella spp. and 10⁶ cfu/g Enterococcus spp. The final urea concentration corresponded to 0.5, 1, 1.5 and 2% w/w in the sludge, and the bacterial solutions corresponded to 2.5% w/w. The bacterial inoculate solution consisted of Enterococcus faecalis (ATCC 29212) and Salmonella typhimurium phage type 178 isolated from sewage sludge (Sahlstrom et al. 2004), both grown in nutrient broth. After mixing, the sludge was distributed to Stomacher bags (thickness: 30 μm) with 9.5-10 g sludge in each bag. The bags were welded and kept at 4, 10 or 22 °C (only 0.5% urea) to simulate storage under different ambient temperature conditions. Independent single samples from each treatment and temperature combination were analysed on 13 occasions with exponentially increasing time intervals, the last sampling performed on day 134. Each bag was used for one sampling only, as the bag was destroyed during sampling.

**Bacterial sampling**

At sampling, the bag was opened and the sample was diluted in 90 mL phosphate-buffered saline solution (pH 7). After homogenisation, 1 mL of the diluted sludge was diluted further in phosphate-buffered saline solution (pH 7), and 0.1 mL of the diluted samples was spread on agar plates for bacterial enumeration. Enterococcus spp., including added Ent. faealis and Ent. spp. originating from the sludge, were grown on Slanetz-Bartley agar (Oxoid AB, Sweden) and incubated at 44 °C for 48 hours. Salmonella spp. was grown on xylose lysine desoxycholate agar (Oxoid AB, Sweden) containing 0.15% sodium-novobiocin and incubated at 37 °C for 24 hours.

**Physiochemical analysis**

The pH was measured with a Radiometer pH electrode (Meterlab pH meter 210, Copenhagen, Denmark) at room temperature after 1:5 dilution of the sample in deionised water. The content of total ammonia nitrogen (TAN) was measured spectrophotometrically using ammonia kit reagents (Merck, art.nr. 1.00683.0001) after three-fold dilution in
deionised water and filtration (0.45 μm). The concentration of uncharged ammonia, given as NH₃ concentration in water (mM), was calculated using the dissociation constant pKa = 0.09018 + 2729.92/T (Clement & Merlin 1995).

Statistical analysis

The data were analysed statistically using non-linear regression in R v. 2.14.0 (R Development Core Team 2011). The inactivation rate constant, k, and the lag phase, l, were estimated by fitting the data to a model for shouldered inactivation (Equation (1)) (Harm 1980), where k is the inverse of the decimal reduction time (T90), N₀ and N are the concentration of bacteria at start and after time t, respectively, and n is a parameter determining lag phase duration (Equation (2)). The standard error (SE) of the lag phase (σ_l) was estimated based on standard errors of n (σ_n) and k (σ_k) and the covariance of n and k (Equation (3)). The t-test was applied to assess if the parameter n and hence the lag-phase was significantly different from 0. For the treatments with an estimated lag period <0, the value of n was set to 0, simplifying Equation (1) to exponential decay. When no colonies were detected, the detection limit was used for graphics and regression. Detection limit data which decreased estimated die-off were not included in the regression.

\[
N = N_0 \left[ 1 - \left( 1 - 10^{-k_1} \right)^{10^t} \right] \quad (1)
\]

Lag phase: \[ l = \frac{n}{k} \quad (2) \]

\[
\sigma_l = l \sqrt{\frac{(\sigma_n)^2}{n} + \frac{(\sigma_k)^2}{k} - \frac{2\text{cov}(n,k)}{nk}} \quad (3)
\]

RESULTS

Ammonia and pH

The urea was hydrolysed quickly, and after 1 week the measured TAN concentration was in the range 75–115% of the theoretical TAN, i.e. intrinsic TAN + TAN from urea. A decrease in TAN recovery was observed after 2–4 months in most cases (Table 1). This was believed to be due to leakage through the seal of the sample bag and was thus not thought to be representative of urea treatment of sewage sludge. The pH value decreased by 0.4 units during storage for the sludge treated with 2% urea, an effect that was also believed to be related to ammonia leakage. The pH of the sewage sludge was lower than reported levels for faeces treated with similar doses of urea (Nordin et al. 2009b). This is believed to be due to the higher dry matter (DM) content in sewage sludge and anaerobic digestion resulting in increased carbonate concentration.

Bacterial inactivation

Salmonella spp. was inactivated faster than Enterococcus spp. and was not detected by plate spreading after 134 days in any treatment except for 0.5% w/w urea at 4°C (Figure 1). Only the treatment at 22°C reached an enterococci concentration below the detection limit. Different Enterococcus species could be differentiated on the Slanetz-Bartley agar plates by the appearance of the colonies, and the proportion of species other than Ent. faecalis was in general higher for the samples with low concentration of enterococci.

Reduction rates and lag phases for bacterial inactivation

It was possible to model bacterial inactivation by the lag phase model with a good fit (Equation (3)). Of the 16

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>TAN-concentration, and TAN recovery rate of urea-treated sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 59</td>
</tr>
<tr>
<td>No treatment</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>0.5% urea</td>
<td>8.2</td>
<td>8.2</td>
</tr>
<tr>
<td>1% urea</td>
<td>8.5</td>
<td>8.6</td>
</tr>
<tr>
<td>1.5% urea</td>
<td>8.7</td>
<td>8.6</td>
</tr>
<tr>
<td>2% urea</td>
<td>9.0</td>
<td>8.7</td>
</tr>
</tbody>
</table>
bacterial decays, nine had a significant lag phase \((p < 0.05)\), four had a negative lag phase and were thus simplified to first order kinetics, and three had a positive lag phase which was not statistically significant. Two of these three had a \(p\)-value of \(\approx 0.3\) and were included in the results, while one had a very high \(p\)-value \(\approx 0.98\) and was rejected. The lag phase lasted up to 11 and 3 weeks for Enterococcus spp. and Salmonella spp., respectively, and in some treatments it was double the decimal reduction time (Table 2).

In general, the lag phase decreased with increasing temperature and urea dose, although there were several exceptions. Similarly, the time required for 90% die-off decreased in most cases with increasing temperature and urea dose (Table 2).

**DISCUSSION**

**Impact of ammonia and pH**

Ammonia concentration had a rather small impact on the inactivation rate of Enterococcus spp., with the exception for the treatments with 2% urea (Figure 2). This suggests that a threshold concentration of ammonia exists for enterococci inactivation at around 50 mM NH3. The inactivation at 22 °C is faster despite the lower ammonia concentration, but due to the lack of an untreated control it is not clear if the inactivation is caused by ammonia. However, another study reported an inactivation of enterococci by sludge storage at 21 °C which was slower than the inactivation observed in this study, indicating that ammonia is the inactivating factor (Berggren et al. 2004). It is not possible to separate the impact of pH from the impact of ammonia in this study, as they are correlated, but previous studies have shown faster inactivation of Enterococcus spp. at higher pH for similar ammonia concentrations (Ottoson et al. 2008). However, studies have shown no inactivation of E. faecalis in ammonia-free controls at pH 9 for a period of 6 months (Nordin et al. 2013), and that E. faecium have been shown to be able to grow at pH 9.9 (Berge et al. 2006).

For Salmonella spp., the effect of increased urea doses on the inactivation rate was pronounced. The inactivation rate \((k)\) increased linearly with increasing ammonia concentration (Figure 2). However, the concentrations of ammonia are highly correlated with the concentrations of carbonate \((\text{CO}_3^{2-})\) \((r = 0.97)\) for the range of pH values studied here, and, as carbonate has also been shown to inactivate Salmonella spp. (Park & Diez-Gonzalez 2003), the increased inactivation is probably caused by the increase in the concentration of the two substances.

For both Salmonella spp. and Enterococcus spp., the duration of the lag phase decreased with increased ammonia concentration in most cases (Figure 3). The rapid hydrolysis of urea excludes slow ammonia generation as the cause of the lag phase. A longer lag phase was observed for Enterococcus spp. than for Salmonella spp., possibly due to the more complex cell membrane with lower permeability of the Gram-positive bacteria.
Impact of temperature

There was no observed temperature effect on the inactivation rate of *Enterococcus* spp. except for the treatment at 22 °C, which was faster than treatments at 4–10 °C with similar ammonia concentrations (Figure 2). However, the duration of the lag phase decreased with increasing temperature (Figure 3). This confirms other studies which have reported low temperature dependency of *Enterococcus* spp. inactivation rates (Vinnerås et al. 2013) and a shorter lag phase with treatment at 14 °C compared with 4 °C where the linear die-off phase was not reached (Ottoson et al. 2008).

In the present study, the inactivation rate for *Enterococcus* spp. decreased for the treatments with the highest urea dose at concentrations of 3–4 log_{10} cfu/g, while no such decrease was observed for the treatment with 0.5% urea at 22 °C. This decrease in inactivation may have been due to a sub-population of enterococci being more persistent towards ammonia than *Ent. faecalis*. Some *Enterococcus* species have been shown to be more persistent than *Ent. faecalis*; for example, Son ThiThThanh et al. (2011) observed *Ent. gallinarum* growing in stored urine in which *Ent. faecium* and *Ent. faecalis* were inactivated. There was no evidence of such regrowth during treatment in the present study, but the tailed inactivation observed at 10 °C which was not evident at 22 °C suggests that the intrinsic *Enterococcus* species were more susceptible to ammonia at 22 °C than at lower temperatures. Additional studies are required to evaluate the inactivation effect of ammonia and regrowth potential of *Enterococcus* species other than *Ent. faecalis*.

For *Salmonella* spp., a clear increase in k-value was observed at 22 °C compared with 4–10 °C, demonstrating the temperature dependency of *Salmonella* spp. inactivation (Figure 2). The increase in k-value observed at 10 °C compared with 4 °C for a given urea dose can be explained by

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**Table 2**  Estimated lag phase (days) ± SE, post-lag phase decimal reduction time t_{90} (days) ± SE and concentration of uncharged ammonia, NH3 [mM] ± SD (%)

<table>
<thead>
<tr>
<th>Urea Concentration</th>
<th>Temperature °C</th>
<th>Lag phase Enterococcus</th>
<th>Lag phase Salmonella</th>
<th>t_{90}</th>
<th>t_{90}</th>
<th>NH3 [mM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% urea</td>
<td>4°C</td>
<td>35.2 ± 7.0</td>
<td>37.4 ± 5.3</td>
<td>68 ± 4</td>
<td>14 ± 4</td>
<td>5 ± 30</td>
</tr>
<tr>
<td>0.5% urea</td>
<td>10°C</td>
<td>36.5 ± 11.4</td>
<td>20.9 ± 6.0</td>
<td>55 ± 7</td>
<td>16 ± 3</td>
<td>8 ± 27</td>
</tr>
<tr>
<td>0.5% urea</td>
<td>22°C</td>
<td>18.8 ± 5.5</td>
<td>3.1 ± 13.4</td>
<td>–</td>
<td>2 ± 2</td>
<td>19 ± 31</td>
</tr>
<tr>
<td>1% urea</td>
<td>4°C</td>
<td>28.0 ± 12.9</td>
<td>12.6 ± 8.4</td>
<td>81 ± 6</td>
<td>20 ± 3</td>
<td>18 ± 26</td>
</tr>
<tr>
<td>1% urea</td>
<td>10°C</td>
<td>40.5 ± 6.2</td>
<td>6.8 ± 7.4</td>
<td>–</td>
<td>23 ± 2</td>
<td>28 ± 23</td>
</tr>
<tr>
<td>1.5% urea</td>
<td>4°C</td>
<td>34.6 ± 8.5</td>
<td>6.2 ± 9.9</td>
<td>35 ± 6</td>
<td>3 ± 2</td>
<td>32 ± 30</td>
</tr>
<tr>
<td>1.5% urea</td>
<td>10°C</td>
<td>41.2 ± 6.3</td>
<td>5.2 ± 3.6</td>
<td>–</td>
<td>4 ± 1</td>
<td>49 ± 27</td>
</tr>
<tr>
<td>2% urea</td>
<td>4°C</td>
<td>23.4 ± 7.5</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>49 ± 40</td>
</tr>
<tr>
<td>2% urea</td>
<td>10°C</td>
<td>17.7 ± 10.8</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>75 ± 37</td>
</tr>
</tbody>
</table>

*Regression of data from day 0 to day 45 (10 °C) and 57 (4 °C).

*No data; agar plate overgrown with non-Salmonella colonies on day 2.

*Non-significant lag phase (p > 0.3).*

**Figure 2**  Relationship between ammonia ([NH3] [mM]) and inactivation rate constant (k ± SE) [days⁻¹] for *Salmonella* spp. (a) and *Enterococcus* spp. (b). Closed symbols — 4 °C, open symbols — 10 °C, shaded symbol — 22 °C.
the effect on the ammonia equilibrium, with higher temperatures shifting the equilibrium towards higher concentrations of uncharged ammonia. In other studies on inactivation in pig slurry (Bolton et al. 2013) and urine (Vinnerås et al. 2008), a significant shift in the inactivation rates with a temperature increase from 14 to 24/25°C has been reported, similar to the variation presented in Figure 2. However, in the same studies, faster inactivation was observed at 14°C than at 4°C at similar ammonia concentrations. One possible reason why the temperature dependency of Salmonella spp. inactivation was not observed at 4–10°C in this study could be the limited mobility of ammonia in materials with high DM content (Vinnerås et al. 2009), as the lower moisture content limits the impact of the temperature-driven Brownian motion of the ammonia in the liquid phase. Another reason for the difference could be that increased DM provides a more protective environment, as zones with microenvironments may be more frequent.

Treatment aspects

The temperature dependency of bacteria inactivation at temperatures above 10°C increases the advantages of performing urea treatment at higher temperatures, especially in regions with low ambient temperature. One option is to perform the ammonia treatment directly after dewatering, when the sludge has a higher temperature. The urea should not be added to the sludge prior to dewatering, as more than 20% of the urea has been shown to end up in the reject water in that case (Sylwan 2010). Another option is to apply urea treatment after heating of the sludge by composting. No aeration should be used after application of urea, due to the ammonia evaporation. Furthermore, ammonia inactivates the microorganisms responsible for the composting process and hence stops heat production from composting.

While the suggested sludge treatment regulations in Sweden contain bacterial reduction requirements only, the sludge treatment regulations in other countries often contain requirements on virus and helminth egg viability. While ammonia has been shown to inactivate both viruses and helminth eggs, especially helminth eggs are shown to be more persistent than bacteria, and thus higher temperatures, higher urea doses and/or longer storage times are required to inactivate helminth eggs compared with bacteria, depending on the initial concentration of helminth eggs in the sludge. More studies are needed on the survival of these microorganisms in urea treated sludge.

CONCLUSIONS

Urea treatment of sludge was demonstrated to be a viable option for sanitisation of sludge regarding bacteria reduction requirements. Salmonella spp. was reduced by 5 log<sub>10</sub> after one month with 1.5% w/w urea at 10°C or 0.5% urea at 22°C. Enterococcus spp. was inactivated more slowly, with a 2 log<sub>10</sub> reduction observed after less than 6 weeks with 2% w/w urea at 10°C or 0.5% urea at 22°C. However, other species of Enterococcus and Salmonella that are possibly present in sludge may be more persistent than the Ent. faecalis and S. typhimurium used in this study.

Inactivation rate of Salmonella spp. showed a linear relationship with increasing ammonia concentration, but no temperature effect was observed in the range 4–10°C. The inactivation rate of enterococci did not increase with increasing ammonia concentration or with temperature elevation from 4 to 10°C except in the treatment with 2% urea. However, the observed lag phase decreased drastically with increasing urea dose and temperature.
ACKNOWLEDGEMENTS

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