Research Paper

Inactivation of *Ascaris* eggs and *Salmonella* spp. in fecal sludge by treatment with urea and ammonia solution

J. Fidjeland, A. Nordin and B. Vinnerås

ABSTRACT

Ammonia sanitisation is a promising treatment alternative for inactivation of pathogens in fecal sludge intended for agricultural use. Inactivation of *Ascaris* eggs and *Salmonella* spp. was studied in fecal sludge at ≥28 °C treated with low doses of urea, and in fecal sludge at ≤17 °C treated with high doses of ammonia solution. The effect of ammonia and carbonate on *Ascaris* inactivation in buffer was also studied. *Ascaris* eggs and *Salmonella* spp. were inactivated in fecal sludge treated with 0.4% urea or more at ≥28 °C. With lower doses of urea, the pH of the fecal sludge decreased during the experiment, resulting in low NH_3 concentrations and subsequently no inactivation of *Ascaris* eggs. *Ascaris* was successfully inactivated at 5 °C, but the NH_3 concentrations required were 10-fold higher than at high temperatures and the storage time required was longer. The buffer study showed that carbonate (CO_3^{2-}) had a statistically significant impact on *Ascaris* inactivation, but the effect was low compared with that of NH_3. Thus for inactivation of *Salmonella* spp. with urea at low temperatures, CO_3^{2-} is probably a more important factor than NH_3.

Key words | ammonia, *Ascaris*, carbonate, inactivation, *Salmonella*

J. Fidjeland
A. Nordin
B. Vinnerås (corresponding author)
Department of Energy and Technology,
Swedish University of Agricultural Sciences,
Uppsala,
Sweden
E-mail: *bjorn.vinneras@slu.se*

INTRODUCTION

Human excreta often contain high concentrations of pathogens and represent a health risk, as they are often dumped into water bodies used as freshwater sources (Yajima & Koottatep 2010). In many places, it is common to use human excreta as fertiliser, but as the excreta are often not sufficiently treated, this practice is associated with an increased prevalence of diseases and infections (Corrales *et al.* 2006; Do Thuy *et al.* 2007). As human excreta contain nitrogen, phosphorus and micronutrients, their use as fertiliser should not be discouraged, but there is a need to communicate the risk of using insufficiently treated excreta and to develop treatment options to reduce the pathogen concentrations to sufficiently low levels.

The biocidal property of ammonia is exploited in ammonia sanitisation, which is a simple treatment as it only requires sealed storage to avoid ammonia volatilisation, sufficient storage time to ensure pathogen inactivation and a source of ammonia. The source of ammonia can be urine (Yang *et al.* 2003; Jensen *et al.* 2009; Fidjeland *et al.* 2013), but in fecal sludge from toilets using flushwater (sometimes called blackwater), the ammonia from urine is not sufficient due to the dilution effect. More ammonia can be added in the form of urea (Nordin *et al.* 2009a), which is a common nitrogen fertiliser. Urea is degraded into carbonate and ammonia upon contact with the enzyme urease, which is present in fecal matter. Another potential source of ammonia is ammonia solution, which elevates the pH more than the equivalent nitrogen dose of urea, but does not contain carbonate. However, while urea is harmless to handle, ammonia solution is strongly alkaline and hazardous to work with. As ammonia is not consumed during treatment, addition of urea or ammonia solution both increases the fertiliser value of the fecal sludge and enhances pathogen inactivation.

doi: 10.2166/washdev.2016.017

In addition to transport costs, the main costs for ammonia treatment are the addition of urea or ammonia solution and storage facilities. It is therefore of interest to reduce both the amount of ammonia added and the storage time required for sanitisation. However, the lower threshold for pathogen inactivation by ammonia in fecal sludge is not well studied, especially at the higher range of ambient temperatures. At low temperatures (<14 °C), reduction of *Ascaris* egg viability by ammonia treatment has been found to take a very long time, with 1 year or more required for a 3 $\log_{10}$ reduction in *Ascaris* egg viability in urine, or urine mixed with feces (Nordin *et al.* 2009a; Fidjeland *et al.* 2013).

Several studies on ammonia inactivation of *Ascaris* and *Salmonella* spp. at ambient temperatures have used urea as an ammonia source (Nordin *et al.* 2009a; Fidjeland *et al.* 2013; Nordin *et al.* 2013). As one urea molecule degrades into one carbonate molecule and two ammonia molecules, it is not clear whether the inactivation is caused by ammonia alone, or a combination of the two. Carbonate, CO_3^{2-}, has previously been shown to have an inactivating effect on *Salmonella* Typhimurium (Park & Diez-Gonzalez 2003), but its inactivating effect on *Ascaris* eggs has not previously been tested.

The main aim of this work was to evaluate the potential to sanitise fecal sludge: (1) with low additions of urea at high temperature, specifically by studying the inactivation of *Ascaris* eggs and *Salmonella* spp.; and (2) at low temperatures using high doses of ammonia solution for inactivation of *Ascaris* eggs. An additional aim was to investigate the inactivation effect of CO_3^{2-} on *Ascaris* eggs in comparison with the inactivation by NH_3.

MATERIALS AND METHODS

Fecal sludge preparation

Fecal sludge was prepared by mixing tapwater with feces and urine collected from volunteers. The feces and urine constituted 0.65 and 1.6% (w/w), respectively, of the mix, equivalent to 30 L flushwater, 0.5 L urine and 200 g feces per person and day. Feces were kept frozen until use. At preparation, feces and urine were measured separately into 8- and 50-mL tubes and mixed with ammonia solution or urea to reach 2–10% ammonia solution and 0.05–1.5% urea (Table 1). Different treatment concentrations were used at the different temperatures, thereby covering the different knowledge gaps in treatment efficiency of ammonia and carbonate at the temperatures tested. In the treatments with urea, urease enzymes were added (~500 U/g urea). Finally, the tubes were filled with tapwater to the desired volume, aiming at minimised headspace. The tubes were sealed with an O-ring in the lid to prevent ammonia losses. After preparation, the tubes with urea addition were shaken at 50 rpm for 3 hours in 37 °C to hydrolyse the urea, which was confirmed by ammonia measurement. The tubes were then stored cold (5 °C) until the addition of pathogens.

Ascaris egg bags were prepared by adding ~10,000 *Ascaris suum* eggs from pig feces (Excelsior Entinel, Inc. (New York, USA)) to permeable nylon bags (mesh: 28 μm). The bags were kept in 0.1 N H_2SO_4 until use. The bags were rinsed thoroughly in deionised water before being added to the tubes with fecal sludge. *Salmonella enterica* subspecies 1 serovar Typhimurium phage type 178, which was first isolated from sewage sludge by Sahlstrom *et al.* (2004), was cultivated overnight in nutrient broth to a concentration of 10^8 per mL.

After the addition of one *Ascaris* bag per tube and 1% *Salmonella* Typhimurium culture, the tubes were kept at the respective treatment temperature (4, 11, 17, 23, 28 or 32 °C) until sampling.

Buffer preparation

Buffers with ammonia and/or carbonate were prepared by adding ammonium chloride and sodium carbonate to saline solution (0.85% NaCl). At 32 °C, buffers were prepared with 588 mM NH_4Cl or 160 mM Na_2CO_3, or a combination of both. At 11 °C, buffers with 975 mM NH_4Cl or 5100 mM Na_2CO_3 or a combination of both were prepared. The pH was adjusted to 9 with NaOH and HCl. Ammonia and carbonate-free pH controls were made from M15 phosphate buffer (SVA, Sweden) adjusted to pH 7.2, 9 and 12 with NaOH and HCl. One *Ascaris* bag was added to each tube and they were incubated at 32 and 11 °C.

Microbial analysis

At sampling, which was destructive, i.e. each tube was only sampled once, the bags with *Ascaris* eggs were removed and

Table 1 | Treatment time required for 4 $\log_{10}$ reduction of *Ascaris* egg viability and 7 $\log_{10}$ reduction of *Salmonella* spp., number of samples (#), ammonia activity (NH_3, mM) and carbonate activity (CO_3^{2-}, mM) and pH for the different treatments

Temp.	Treatment	4 log red. *Ascaris* (days)	#	7 log red. *Salmonella* (days)	#	NH_3 (mM)	CO_3^{2-} (mM)	pH
32 °C	10% ammonia	3	2	<0.21	2	915	4	11.2
32 °C	0.4% urea	20	7	2.1	4	50 (61–41)	2	9.0 (9.2–8.9)
32 °C	0.15% urea	NR (24)	6	112	12	13 (23–7)	0.5	8.6 (8.9–8.3)
32 °C	0.05% urea	NR (30)	6	NR (30)	12	2 (4–2)	0.1	7.9 (8.3–7.9)
32 °C	no treatment	NR (51)	7	NR (51)	13	1 (2–1)	0.02	7.7 (8.6–7.7)
32 °C	CO_3^{2-} control	67[a,b]	7	–	–	0	7	9.0
32 °C	NH_3 control	19[a]	7	–	–	73	0	9.0
32 °C	$NH_3 + CO_3^2$ control	15[a]	7	–	–	62	6	9.0
32 °C	Control	NR[a] (40)	3	–	–	0	0	9.0
28 °C	1.5% urea	17	3	1.4	3	196	8	9.3
28 °C	0.75% urea	28	4	2.8	4	92	4	9.2
28 °C	0.4% urea	45	7	4.2	5	39	2	9.0
28 °C	0.15% urea	NR (48)	7	399	11	12 (19–8)	0.5	8.6 (8.9–8.4)
28 °C	0.05% urea	NR (102)	6	NR (85)	11	1 (2–1)	0.0	7.9 (8.1–7.9)
28 °C	no treatment	NR (152)	5	NR (85)	10	0.4 (2–0.5)	0.01	7.7 (8.5–7.7)
23 °C	10% ammonia	<9	2	<0.7	2	1,205	5	11.3
23 °C	1.5% urea	52	3	1.4	3	171	9	9.3
23 °C	no treatment	NR (152)	3	NR (152)	11	0.7 (3–0.2)	0.03	8.1 (8.8–7.5)
17 °C	3% ammonia	73	4	0.7	2	355	3	10.7
17 °C	2% ammonia	82	3	1.4	2	225	3	10.6
17 °C	1.5% urea	130	5	1.4	3	149	8	9.3
17 °C	0.75% urea	216	4	2.8	4	62	4	9.2
17 °C	no treatment	NR (204)	3	NR (108)	10	1 (2–0.3)	0.04	8.3 (8.8–7.3)
11 °C	CO_3^{2-} control	NR[a] (220)	7	–	–	0	6	9.0
11 °C	NH_3 control	159[a]	6	–	–	550	0	9.0
11 °C	$NH_3 + CO_3^2$ control	200[a]	6	–	–	304	1	9.0
11 °C	Control	NR[a] (220)						
10 °C	10% ammonia	100	2	2.1	2	1,107	4	11.3
10 °C	5% ammonia	131	3	4.2	4	570	3	11.0
10 °C	1.5% urea	NR (108)	2	3.5	4	94 (99–92)	7	9.3 (9.4–9.3)
10 °C	no treatment	NR (204)	3	28	6	0.3 (1–1)	0.03	8.2 (8.8–7.4)
4 °C	10% ammonia	149	3	2.1	2	1,064	4	11.3
4 °C	5% ammonia	188	4	7	5	620	3	11.0
4 °C	1.5% urea	NR (204)	3	3.5	4	71 (69–69)	7	9.4 (9.3–9.4)
4 °C	no treatment	NR (204)	3	28	6	0.4 (1–1)	0.05	8.6 (8.8–7.4)

For treatments with decreasing pH (>0.2 units decrease), initial and final values of pH and NH_3 activity are given in brackets. For treatments with no reduction, i.e. less than 0.3 $\log_{10}$ reduction (NR), the study duration is given in brackets (days).

[a]Time for reduction of 3$\log_{10}$.

[b]Extrapolated value, ~0.5 $\log_{10}$ reductions observed.

rinsed in 0.1 N sulphuric acid prior to incubation in 0.1 N sulphuric acid for 30–40 days at 28 °C (Cruz Espinoza *et al.* 2012). The *Ascaris* eggs were then extracted from the bag using a syringe and needle and counted under the microscope. Eggs that had developed to larvae were counted as viable, while non-developed eggs were assumed to be dead. For viability above 50%, 200 eggs were counted, for viabilities between 50 and 5%, 500 eggs were counted and for viabilities below 5%, 1,000 eggs were counted.

For salmonella enumeration, a direct plate count was performed due to the high initial concentration, using xylose lysine desoxycholate (XLD) agar for detection as described in NMKL 71:5.1999 (www.nmkl.org/index.php/en/om-nmkl). At sampling, 1 mL fecal sludge was serially diluted in buffered (pH 7) NaCl peptone water with Tween 80, plated on XLD agar containing 0.15% sodium-novobiocin (Oxoid AB, Sweden) and incubated at 37 °C for 24 h.

Physiochemical measurements

The pH was measured directly in the 50-/8-mL tubes after reaching room temperature, using a Radiometer pH electrode at room temperature (Meterlab pH meter 210, Copenhagen, Denmark). The total ammonia nitrogen (TAN) concentration was measured spectrophotometrically using ammonia kit reagents (Merck, art.nr. 1.00683.0001). The storage temperature was monitored using Tinytag® loggers (Intab, Sweden).

The activity of uncharged ammonia (NH_3) and carbonate $\left(CO_3^{2-}\right)$ was estimated using the Pitzer approach. The estimation was performed using PHREEQC Interactive v. 3.1.1. For estimation of NH_3 and CO_3^{2-} activity in the fecal sludge, the concentration of total ammonia and total carbonate was used. The total carbonate concentration for the urea-treated fecal sludge was assumed to be half the measured total ammonia concentration, based on the stoichiometric relationship from degradation of urea. For untreated fecal sludge and sludge treated with ammonia solution, the total carbonate concentration was assumed to be half the measured total ammonia concentration in the untreated samples. The buffer composition that was used in the Pitzer approach included NaCl from the physical saline solution, added NH_4Cl, Na_2HCO_3 and HCl and NaOH used for pH adjustment.

Statistics

The decrease in *Ascaris* egg viability over time was fitted to the lag phase formula (Equation (1)), where the inactivation rate constant, k, is the inverse of the decimal reduction time (t_{90}), and n is a parameter determining the lag phase (Fidjeland *et al.* 2015):

$$N = N_0\left[1 - \left(1 - 10^{-k\cdot t}\right)^{10^n}\right] \quad (1)$$

A log-linear regression (Equation (2)) was used for *Salmonella* spp. inactivation, and also for *Ascaris* egg inactivation in cases with no lag phase or only two data points:

$$N = N_0 \cdot 10^{-k\cdot t} \quad (2)$$

The t-test was used to compare the *Ascaris* egg inactivation slope (k) and/or lag phase for the buffers with ammonia and/or carbonate and the pH controls at pH 7, 9 and 12. All statistical analyses were performed with the statistical software R v. 2.14.0 (R Development Core Team 2014), and the nls-package was used for the non-linear analysis.

RESULTS AND DISCUSSION

Fecal sludge

The pH development

In several treatments with urea and the untreated controls, there was a decrease in pH over time at 28 and 32 °C, while at 23 °C and below only the controls were affected (Figure 1). The decrease in pH had a strong impact on NH_3 activity in the treatments (Table 1). The pH decrease was slower at lower temperatures (Figure 1), while at 4 °C the pH of the untreated control never went below 8.5. The reason for the pH decrease is not clear, but it may be due to degradation of organic matter, producing fatty acids and carbonate. None of the treatments with ammonia solution experienced any significant pH decrease ($p > 0.1$).

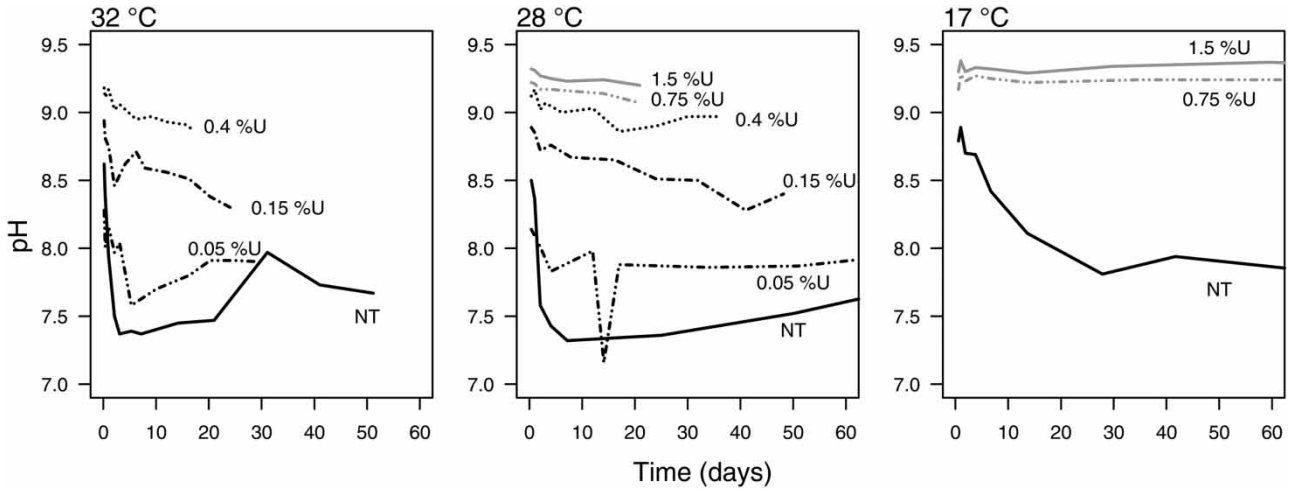

Figure 1 | Development of pH at 32, 28 and 17 °C for different additions of urea: 1.5% (grey solid line), 0.75% (grey dashed line), 0.4% (dotted line), 0.15% (dotted dashed line), 0.05% (dashed line), untreated (solid line).

A decrease in pH over time has also been reported by Nordin *et al.* (2009b), who studied urea treatment of feces. The observed decrease in pH of urea-treated feces varied from 0.1 to 1.5 pH units over 60 days, and a greater decrease was observed for feces with a low pH prior to urea addition. In general, the decrease in pH is probably likely to be potentially larger for wastewater fractions with a high dry matter (DM) content.

Ascaris inactivation

Ascaris eggs were inactivated rapidly compared with the untreated controls in all treatments with 0.4% urea or more at 28 and 32 °C (Figure 2). The inactivation was slower at lower temperatures and no inactivation was observed in treatments with 1.5% urea at 4 and 10 °C, while 100–200 days were required for a 4 $\log_{10}$ inactivation in the treatments with 5 and 10% ammonia solution (Table 1). This shows that *Ascaris* egg inactivation is possible also at low temperature, but the doses and treatment times required are much larger than at higher temperatures.

No inactivation of *Ascaris* occurred at 28 °C with 12 mM NH_3, while 39 mM NH_3 resulted in a 4 $\log_{10}$ reduction within 45 days (Table 1). This is in agreement with previous reports that *Ascaris* eggs are not inactivated at 20 mM at 24 °C in urine, while 40 mM NH_3 inactivates *Ascaris* eggs at 34 °C (Nordin *et al.* 2009a). As treatment with 0.15% urea did not result in a stable pH, the NH_3 activity was decreasing over time. For ammonia inactivation of *Ascaris* eggs at high temperature, the lowest possible operating NH_3 activity is therefore probably decided by the pH stability.

Salmonella inactivation

The *Salmonella* spp. was inactivated far more rapidly than *Ascaris* eggs in all treatments except for the untreated fecal sludge and the treatments with 0.15 and 0.05% urea, where no or very slow inactivation occurred in both *Ascaris* eggs and *Salmonella* spp. (Table 1). The *Salmonella* spp. inactivation was less temperature-sensitive than the *Ascaris* egg inactivation. With 50 mM NH_3 at 32 °C, the inactivation was only 1.6-fold faster than in the treatment with 71 mM at 5 °C, despite the large temperature difference (Figure 3). This agrees well with earlier findings (Vinnerås *et al.* 2008; Nordin *et al.* 2009b).

However, at 4, 10 and 17 °C, treatments with ammonia solution had similar or slower inactivation than urea-treated samples, despite much higher NH_3 activity (Table 1). As urea is also split into carbonate during hydrolysis, this indicates that the carbonate effect is important for *Salmonella* spp. inactivation. It should also be noted that for urea-treated samples, the relative activity of CO_3^{2-} compared with NH_3 activity is higher at lower temperatures, due to different impacts of temperature on the equilibriums. Still, it is possible that ammonia is relatively more important at high

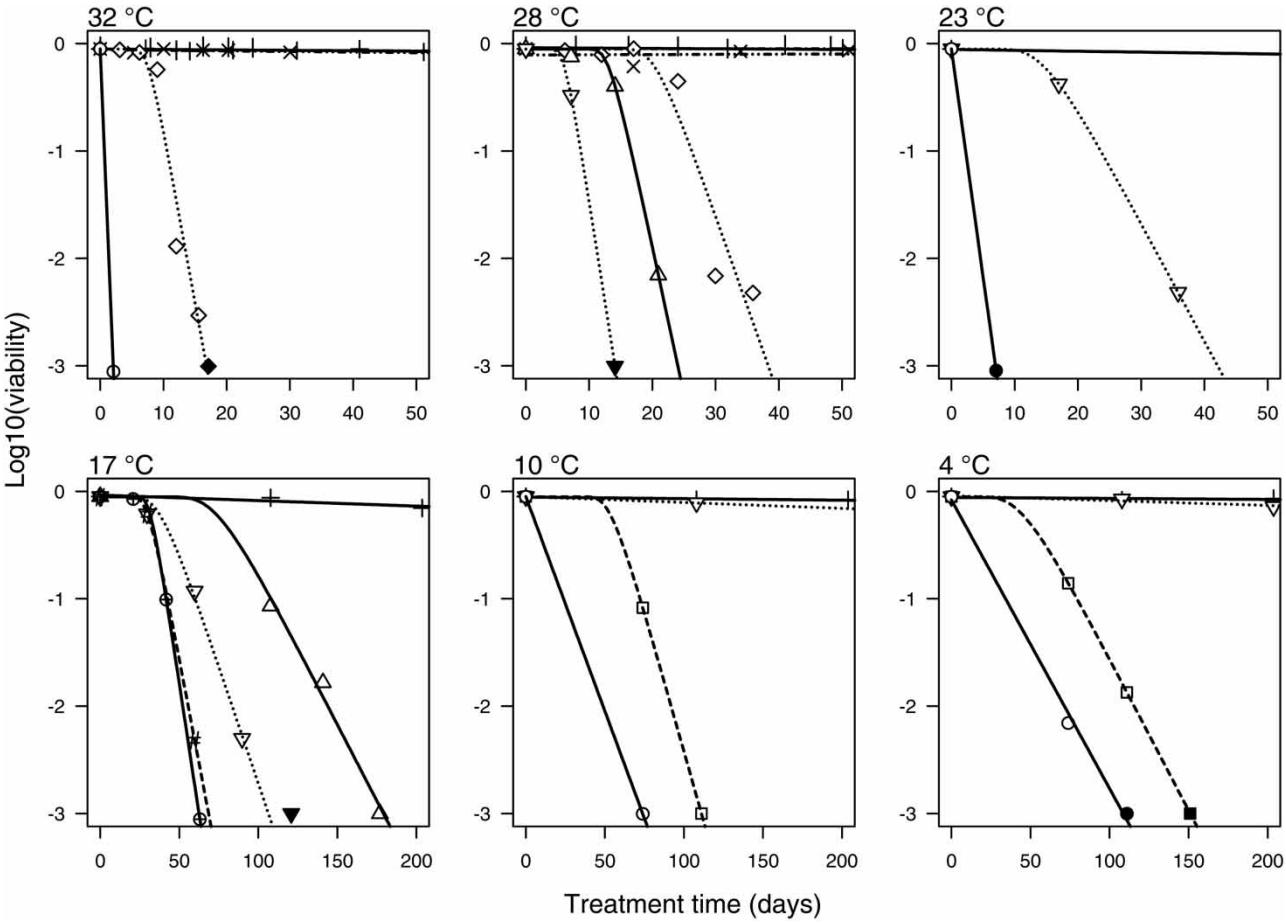

Figure 2 | *Ascaris* egg viability (log) reduction with fitted regression lines during the treatment time (days) at different temperatures. Filled points indicate that no viable eggs were detected. Treatments: 10% ammonia (○, solid line), 5% ammonia (□, dashed line), 3% ammonia (⊕, solid line), 2% ammonia (#, dashed line), 1.5% urea (∇, dotted line), 0.75% urea (Δ, dotted dashed line), 0.4% urea (◇, dotted), 0.15% urea (|, dotted dashed line), 0.05% urea (x, dotted dashed line), no treatment (+, solid line).

temperatures, while carbonate is more important at low temperatures. This is supported in a study by Park & Diez-Gonzalez (2003), which found that the inactivation was similar in solutions with similar concentrations of NH_3 and CO_3^{2-} at 37 °C. As the CO_3^{2-} concentration was a factor of 3–12 lower than the NH_3 concentration for urea-treated samples at high temperatures (≥28 °C, data not shown, Table 1 shows activity), the NH_3 effect is probably more important at these temperatures.

Ascaris egg inactivation in buffer

At 32 °C the carbonate buffer had significantly greater inactivation effect on *Ascaris* egg viability compared with the pH 9 control ($p = 0.01$), but the inactivation was slow compared to the buffer with ammonia only (Figure 4). At 32 °C, the inactivation was significantly faster in the buffer with both ammonia and carbonate compared to the buffer with ammonia alone ($p < 0.001$), despite slightly lower NH_3 activity in the buffer with both NH_3 and CO_3^{2-}. However, the resulting difference in inactivation time was not very great (Table 1). The ratio between the carbonate and ammonia activity in the buffers at 32 °C was approximately 1:10, which is in the higher range of the CO_3^{2-}:NH_3 ratio in the urea-treated samples (1:11–1:30) (Table 1). As the impact of CO_3^{2-} compared with NH_3 was low, the *Ascaris* inactivation observed in urea-treated fecal sludge at this temperature is therefore probably mainly due to NH_3. For treatment of substrates with a higher concentration of carbonate, e.g. digestate, the inactivating effect of carbonate may be significant.

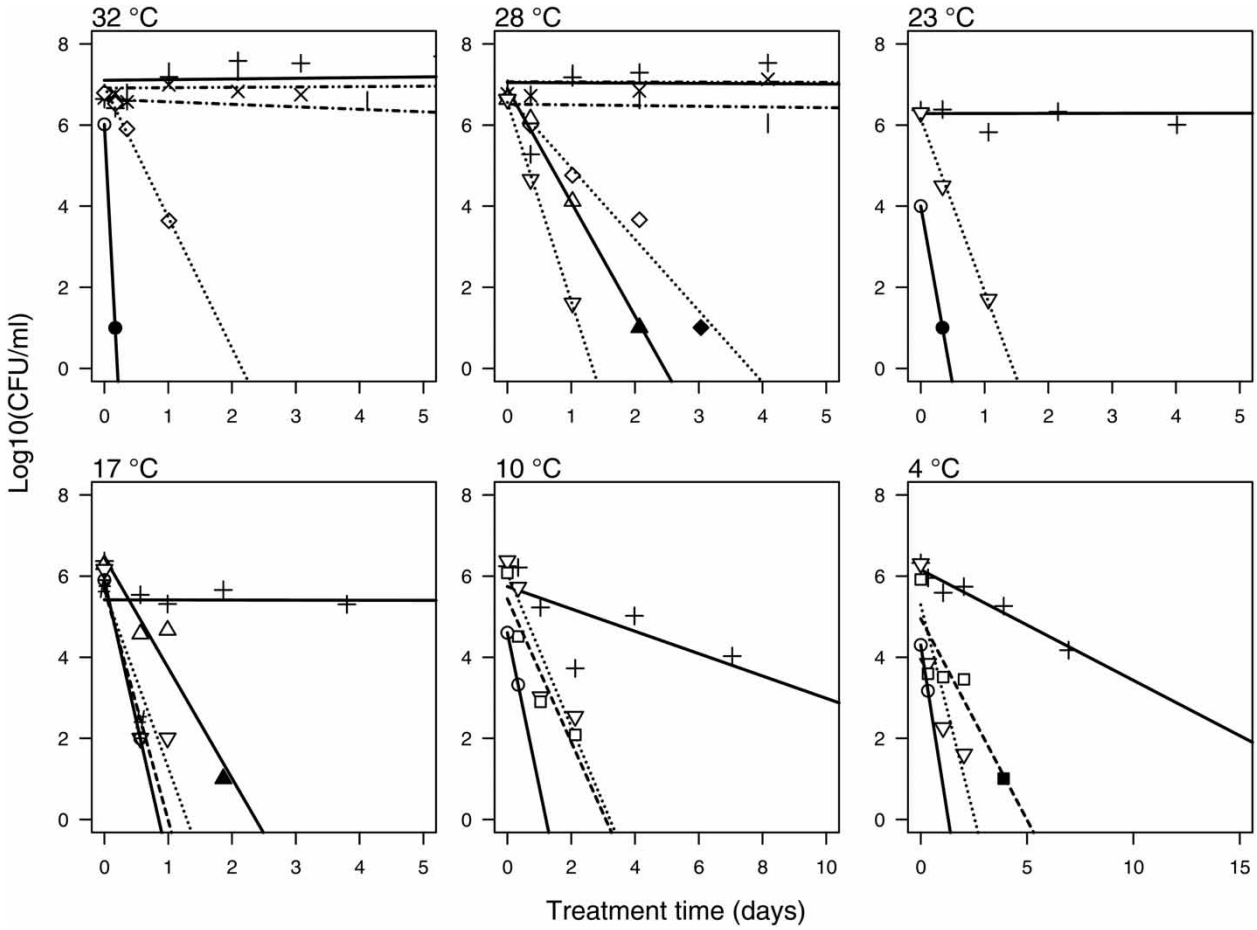

Figure 3 | *Salmonella* spp. inactivation and fitted regression during the treatment (days) at different temperatures. Filled points indicate concentration below detection limit (1 or 2 $\log_{10}$ (cfu)). Treatments: 10% ammonia (○, solid line), 5% ammonia (□, dashed line), 3% ammonia (⊕, solid line), 2% ammonia (#, dashed line), 1.5% urea (∇, dotted line), 0.75% urea (Δ, dotted dashed line), 0.4% urea (◇, dotted line), 0.15% urea (|, dotted dashed line), 0.05% urea (x, dotted dashed line), no treatment (+, solid line).

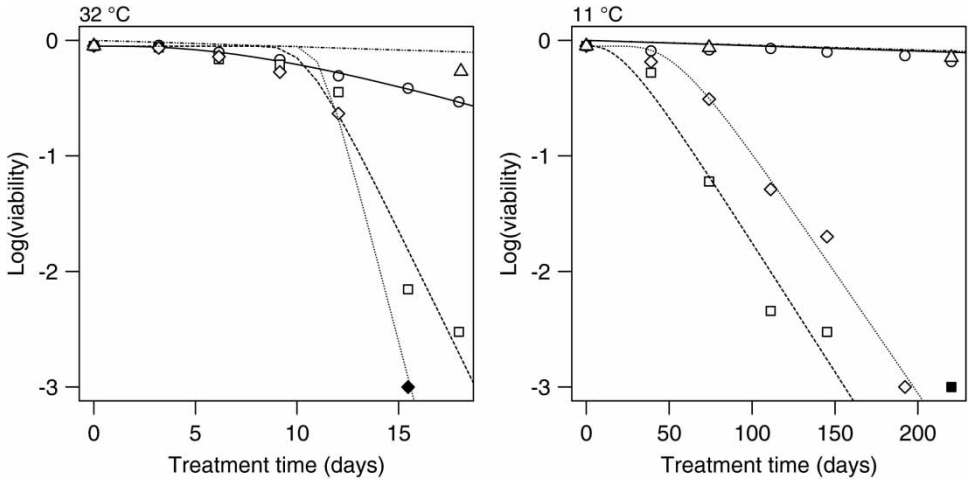

Figure 4 | Reduction of *Ascaris* egg viability and fitted regression lines in buffers, with carbonate only (○, solid line), ammonia only (□, dashed line), carbonate and ammonia (◇, dotted line) and control pH 9 (Δ, long dashed line). Filled points indicate that no viable eggs were observed.

Further studies are required to quantify the effect of carbonate in relation to pH and ammonia concentrations.

At 11 °C, no *Ascaris* egg inactivation was observed in the buffer with carbonate only during the 220 days of the study (Figure 4). The inactivation in the combined buffer with both ammonia and carbonate was slower than in the buffer with ammonia only. This was due to the lower NH_3 activity in the combined buffer due to the ionic strength. At 11 °C, the ratio between CO_3^{2-} activity in the carbonate buffer and NH_3 activity in the ammonia buffer was 1:90. The fecal sludge treated with ammonia solution had a CO_3^{2-}:NH_3 ratio of 1:120–1:340. As no inactivation occurred in the buffer with carbonate, it is therefore reasonable to assume that the effect of carbonate is negligible when using ammonia solution for *Ascaris* inactivation at low temperatures. Urea-treated sludge may have higher relative carbonate activity at low temperatures, but urea is less likely to be used for *Ascaris* inactivation in fecal sludge at low temperatures, as the dose required would be extremely high.

Less than 0.3 $\log_{10}$ reduction in *Ascaris* egg viability occurred in the ammonia- and carbonate-free controls at pH 7, 9 and 12 during the experiment, and there were no differences between the controls at different pH ($p > 0.3$). This shows that the observed inactivation in fecal sludge treated with ammonia and urea was not caused by pH directly.

Practical implementations

According to US EPA standards for class A biosolids, there should be no viable helminth eggs and <3 MPN *Salmonella* spp. per 4 g dry matter. The USEPA target is used for relating the treatment goal to an internationally accepted product quality. Typical concentrations in sludge in low- and mid-income countries are in the range of 70–3,000 *Ascaris* egg/g DM and 10^5–10^7 CFU *Salmonella* spp./g DM (Mendez *et al.* 2004; Jimenez *et al.* 2006). A 4 $\log_{10}$ reduction in *Ascaris* egg viability and a 7 $\log_{10}$ reduction of *Salmonella* spp. should therefore be sufficient to meet the USEPA targets in most cases. As *Salmonella* spp. inactivation is so rapid compared with inactivation of *Ascaris* eggs, it is sufficient to consider the *Ascaris* egg inactivation when deciding on the treatment design for pathogen inactivation.

For urea-treated fecal sludge, it is sufficient to consider the activity of NH_3 and the temperature when deciding on treatment time for *Ascaris* egg inactivation, as the effect of CO_3^{2-} is negligible. For the fecal sludge studied here (0.2% DM), at least 0.4% urea was required to maintain a sufficiently stable pH to achieve inactivation of pathogens. However, this is probably strongly dependent on the flush-water volume, and less diluted fecal sludge with a higher dry matter content would probably require a larger urea dose to maintain a sufficiently stable pH.

It is crucial to ensure that the urea is hydrolysed, as the pathogens will otherwise not be inactivated. The hydrolysis of urea may take several days or even weeks to degrade to ammonia for fecal sludge with low dry matter content, especially at low temperatures (unpublished data), but this may be compensated for by adding urea early so that the hydrolysis can take place during the time the tank is filled with fecal sludge. Furthermore, during emptying of the tank, a fraction of the dry matter may be left to enhance the urea hydrolysis of the next batch, as the urease is mainly associated with the dry matter.

Instead of using a septic tank with infiltration of the effluent into the ground, which is often found to contaminate groundwater (Scandura & Sobsey 1997), two sealed collection tanks can be installed. One can be used for filling while the other is used for storage during the time required for pathogen inactivation. The urea dose can then be adjusted so the storage duration needed for pathogen inactivation is shorter than the filling time. Another option is to transport the untreated fecal sludge to mobile storage tanks located on farms. In this case, the urea dose can be adjusted so the pathogen inactivation is completed by the time the fertiliser is scheduled to be applied.

CONCLUSIONS

Ascaris eggs and *Salmonella* spp. in fecal sludge can be inactivated by relatively low urea doses (0.4% urea) and short treatment times at high temperatures (>28 °C). Doses of urea lower than 0.4% do not inactivate pathogens due to decreased pH and hence decreased NH_3 and CO_3^{2-} activity. *Ascaris* eggs

can be inactivated at low temperatures (<17 °C) but this requires high ammonia concentrations. *Salmonella* spp. inactivation is less temperature-dependent than *Ascaris* egg inactivation and can be achieved with 1.5% urea even at 4 °C.

Ascaris eggs seem to be mainly inactivated by ammonia, while carbonate seems to be an important factor for *Salmonella* spp. inactivation at low temperatures. *Ascaris* eggs are far more persistent than *Salmonella* spp. and *Ascaris* egg inactivation should, in most cases, be the design criterion for treatment. Therefore, only temperature and ammonia activity need to be monitored in most cases. Both the time required for initial hydrolysis of urea and the pH decrease may be important for treatment design.

REFERENCES

Corrales, L. F., Izurieta, R. & Moe, C. L. 2006 Association between intestinal parasitic infections and type of sanitation system in rural El Salvador. *Trop. Med. Int. Health.* **11** (12), 1821–1831.

Cruz Espinoza, L. M., Yeh, D., Vinnerås, B., Rajaram, L., Whiteford, L., Corvin, J. & Izurieta, R. 2012 Inactivation of *Ascaris* suum by ammonia in feces simulating the parameters of the solar toilet. *J. Appl. Sci. Environ. Sanit.* **7** (3), 173–182.

Do Thuy, T., Molbak, K., Phung Dac, C. & Dalsgaard, A. 2007 Helminth infections among people using wastewater and human excreta in peri-urban agriculture and aquaculture in Hanoi, Vietnam. *Trop. Med. Int. Health.* **12** (Suppl 2), 82–90.

Fidjeland, J., Magri, M. E., Jönsson, H., Albihn, A. & Vinnerås, B. 2013 The potential for self-sanitisation of fecal sludge by intrinsic ammonia. *Water Res.* **47** (16), 6014–6023.

Fidjeland, J., Nordin, A., Pecson, B. M., Nelson, K. L. & Vinnerås, B. 2015 Modeling the inactivation of *Ascaris* eggs as a function of ammonia concentration and temperature. *Water Res.* **83**, 153–160.

Jensen, P. K. M., Phuc, P. D., Konradsen, F., Klank, L. T. & Dalsgaard, A. 2009 Survival of *Ascaris* eggs and hygienic quality of human excreta in Vietnamese composting latrines. *Environ. Health*. **8** (57).

Jimenez, B., Austin, A., Cloete, E. & Phasha, C. 2006 Using Ecosan sludge for crop production. *Water Sci. Technol.* **54** (5), 169–177.

Mendez, J. M., Jimenez, B. & Maya, C. 2004 Disinfection kinetics of pathogens in physicochemical sludge treated with ammonia. *Water Sci. Technol.* **50** (9), 67–74.

Nordin, A., Nyberg, K. & Vinnerås, B. 2009a Inactivation of *Ascaris* eggs in source-separated urine and feces by ammonia at ambient temperatures. *Appl. Environ. Microbiol.* **75** (3), 662–667.

Nordin, A., Ottoson, J. R. & Vinnerås, B. 2009b Sanitation of feces from source-separating dry toilets using urea. *J. Appl. Microbiol.* **107** (5), 1579–1587.

Nordin, A., Niwagaba, C., Jonsson, H. & Vinneras, B. 2013 Pathogen and indicator inactivation in source-separated human urine heated by The Sun. *J. Water Sanit. Hyg. Dev.* **3** (2), 181–188.

Park, G. W. & Diez-Gonzalez, F. 2003 Utilization of carbonate and ammonia-based treatments to eliminate *Escherichia coli* O157:H7 and *Salmonella Typhimurium* DT104 from cattle manure. *J. Appl. Microbiol.* **94** (4), 675–685.

R Development Core Team 2014 *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Sahlstrom, L., Aspan, A., Bagge, E., Danielsson-Tham, M. L. & Albihn, A. 2004 Bacterial pathogen incidences in sludge from Swedish sewage treatment plants. *Water Res.* **38** (8), 1989–1994.

Scandura, J. E. & Sobsey, M. D. 1997 Viral and bacterial contamination of groundwater from on-site sewage treatment systems. *Water Sci. Technol.* **35** (11–12), 141–146.

Vinnerås, B., Nordin, A., Niwagaba, C. & Nyberg, K. 2008 Inactivation of bacteria and viruses in human urine depending on temperature and dilution rate. *Water Res.* **42** (15), 4067–4074.

Yajima, A. & Koottatep, T. 2010 Assessment of *E. coli* and *Salmonella* spp. infection risks associated with different fecal sludge disposal practices in Thailand. *J. Water Health* **8** (2), 355–364.

Yang, L., Li, X., Wu, Q., Xu, H., Nong, C., Nong, L., Li, L. & Lin, J. 2003 Observation on the inactivation effect on *Ascaris* suum eggs by urine from toilets. *Chinese J. Parasitic Disease Control* **16** (5), 299–301.

First received 26 January 2016; accepted in revised form 29 May 2016. Available online 30 June 2016