

Research Paper

In-toilet disinfection of fresh fecal sludge with ammonia naturally present in excreta

Temitope A. Ogunyoku, Fikreselam Habebo and Kara L. Nelson

ABSTRACT

A simple treatment method, Safe Sludge disinfection, was developed to disinfect pathogens in fresh fecal sludge using the ammonia naturally present in excreta. In the first step, urea is hydrolyzed to ammonia ($\text{NH}_3/\text{NH}_4^+$). In the second step, $\text{Ca}(\text{OH})_2$ is added to raise the pH level such that NH_3 , a known disinfectant, is the dominant form of ammonia; subsequently, the waste is stored until sufficient disinfection is achieved. In a closed system at 23 °C, Safe Sludge disinfection achieved $>9.3 \log_{10}$ and $>4.0 \log_{10}$ decrease of indigenous *Escherichia coli* and seeded MS2 coliphage, respectively, within 10.6 hours, and 2.0 \log_{10} inactivation of seeded *Ascaris suum* eggs within 2 weeks.

Disinfection of feces at high pH with no urine addition was tested for comparison, and similar inactivation levels were achieved for *E. coli* and MS2 bacteriophage. However, for *Ascaris* eggs only 0.38 \log_{10} inactivation was achieved over 2 weeks. For control samples (feces plus urine only), no inactivation of bacteria or virus indicators was observed and inactivation of *Ascaris* eggs was also low (0.42 \log_{10}). To illustrate how the Safe Sludge concept could be incorporated into a waterless household toilet, a conceptual design and prototype was developed, called the pHree Loo.

Key words | ammonia, disinfection, fecal sludge management, helminth eggs, latrine

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INTRODUCTION

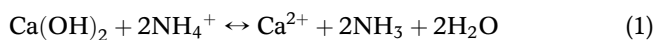
The storage, removal, transport, treatment, and disposal or end-use of fecal sludge continues to be an enormous challenge in developing countries. Residents of urban communities, especially informal settlements, are one of the most vulnerable groups that lack access to sanitation hardware devices and fecal sludge management services (Martine & Marshall 2007). If basic sanitation is accessible, the most common technology used within these communities is shared pit latrines. Pit latrines with a high number of users often have a high sludge accumulation rate. Other factors that affect the sludge accumulation rate of latrines are drainage and disposal of other household waste (Still 2002). Pit emptying services are in high demand within these communities because there is limited space to construct new latrines once the existing pit latrines are full. Many pit emptying approaches are inadequate, exposing workers to the waste,

which may still contain high concentrations of pathogens. Furthermore, the fecal sludge that is removed is often disposed without treatment in close proximity to residents, potentially exposing large numbers of people to pathogens, and degrading ecosystems (Klingel *et al.* 2002; Chowdhry & Koné 2012). If the waste does receive treatment, the most common fecal sludge treatment processes include storage, mesophilic composting, mesophilic anaerobic digestion, aerobic digestion, and planted drying beds; in most cases, these treatment processes have been found to provide incomplete disinfection of the waste (International Reference Centre for Waste Disposal 1985; Gibbs *et al.* 1995; Carrington 2001; Gantzer *et al.* 2001; Strande *et al.* 2014).

The goal of this project was to develop a method for disinfecting fresh excreta within the toilet itself, thereby reducing the risk of pathogen exposure associated with removal and

disposal or end-use of the fecal sludge. We aimed to develop a process that could be incorporated into self-contained waterless toilets (the waste is stored in containers rather than a pit) that are emptied regularly by a service provider. The approach investigated was to utilize the neutral form of ammonia (NH₃) as the disinfectant, which is known to inactivate a wide range of pathogens of concern in human waste, including viruses, bacteria, protozoan cysts, and helminth eggs (Jenkins *et al.* 1998; Mendez *et al.* 2004; Pecson *et al.* 2007; Nordin *et al.* 2009a, 2009b; Decrey *et al.* 2015; Magri *et al.* 2015). Recognizing that ammonia can be produced from the urea naturally present in urine (Fidjeland *et al.* 2013), we investigated the feasibility of producing the ammonia *in situ*. This approach is different from prior work investigating the disinfection of feces from source-separating latrines by addition of urea (Nordin *et al.* 2009b; Magri *et al.* 2013).

In-situ ammonia is mainly produced through biological hydrolysis of urea by urease. Ammonia is a weak base and exists in equilibrium with its conjugate acid (NH₄⁺). Typical fresh excreta has a neutral pH, at which the dominant form is ammonium, NH₄⁺, which is not an effective disinfectant. At pH values above the pKa (9.3 at 25 °C (Stumm & Morgan 1996)), the dominant form is NH₃, the form needed for disinfection. With the addition of an alkalinizing agent such as slaked lime, Ca(OH)₂, to raise the pH of the waste, the equilibrium shifts towards NH₃ (Equation (1)):



The basis of the Safe Sludge approach is to produce NH₄⁺/NH₃ from urine and feces, and convert it to a disinfectant by adding an alkalinizing agent to raise the pH. The first objective of this research was to determine the time necessary for the hydrolysis of urea with different sources of urease (i.e., commercially available urease and feces that naturally contains urease) and pH levels. The second objective was to determine the necessary dose of alkalinizing agent to achieve a stable, high pH level. The third objective was to apply the newly developed Safe Sludge approach to excreta and quantify the inactivation of three classes of model organisms (i.e., *E. coli*, MS2 coliphage, and *A. suum* eggs). Finally, a conceptual design was developed to illustrate how the approach could be incorporated into a household toilet.

METHODS

Sample collection

Urine and feces samples were collected separately from adult volunteers (sample collection procedures were approved by the University of California, Berkeley, Committee for Protection of Human Subjects). Urine and feces samples were stored for a maximum of 1 and 3 days, respectively, at 4 °C before experimentation. Several different urine to feces volume ratios (v/v) were used for the experiments; urine volumes were varied while the feces volume was kept constant. A urine to feces ratio of 2.6:1 was used to represent all of the urine and feces being deposited in the same toilet. A urine to feces ratio of 1.3:1 was used to represent about half of the urine but all of the feces being deposited in the same toilet, as some users may urinate in other locations throughout a day. These ratios were determined assuming a person living in a low-income country in a tropical climate with vegetarian diet would produce 400 g (wet mass) of feces and 1.0 L of urine per day (Franceys *et al.* 1992), and accounting for the actual mass and volume of fecal samples collected for the research.

Hydrolysis rates of urea and pH adjustment

Three different sets of experiments at varying pH levels were conducted to determine the hydrolysis rate of urea in pure urine, urine with urease extracted from *Canavalia ensiformis* (jack bean), and urine with feces (urease is naturally present in feces). For the pure urine experiment, individual urine samples were collected and amended. Duplicate 10 mL urine samples were aliquoted into individual vials. The jack bean condition consisted of 10 mL urine samples spiked with 1 mL of 5 mg/mL of urease (Type IX; Sigma-Aldrich; catalog # U4002-100KU; St. Louis, MO). Different quantities of calcium hydroxide (Ca(OH)₂) powder (Thermo Fisher Scientific; Fairlawn, NJ) were added to the pure urine and jack bean samples to obtain the desired pH levels (i.e., 8, 9, 10, 11, 12). For the feces condition, the pH of urine was first adjusted with Ca(OH)₂ to obtain a pH of 12. Feces was then combined with high pH urine to obtain a urine to feces ratio of 2.6:1. Un-amended samples, which represented the control (i.e., feces and urine), did not have

the addition of an urease agent or $\text{Ca}(\text{OH})_2$. For each condition, duplicate 10 mL fecal slurry samples were aliquoted into individual vials. The experiments were conducted in a closed system at room temperature (23°C), and analyzed for total ammonia (total $\text{NH}_3\text{-N} = \text{NH}_3\text{-N} + \text{NH}_4^+\text{-N}$) and pH over the duration of the experiment using an ion selective electrode (Beckman Coulter pH/Temp/mV/ISE Meter PHI 295; Waltham, MA) and a general purpose pH electrode (Thermo Fisher Scientific; Fairlawn, NJ), respectively.

Experiments to measure the pH increase upon addition of alkalinizing agents were conducted similarly to hydrolysis experiments: fecal slurries were first well-mixed, then alkalinizing agent was added, followed by pH measurement. Different alkalinizing agents were tested for increasing the pH of urine-feces slurries: quicklime (CaO), slaked lime ($\text{Ca}(\text{OH})_2$), limestone, ash, and biochar. To determine the amount of $\text{Ca}(\text{OH})_2$ needed to maintain the desired pH, samples were aliquoted into 10 mL tubes for storage for 35 days.

Inactivation of *E. coli*, MS2 coliphage, and *A. suum* eggs

Fecal slurry conditions

Three different fecal slurry conditions were investigated to determine inactivation rates of *E. coli*, MS2 coliphage, and *A. suum* eggs. It was assumed that a person would not have access to the same toilet throughout the day. Therefore, inactivation experiments were conducted with fecal slurries consisting of urine to feces ratio of 1.3:1. Condition 1 (i.e., Safe Sludge disinfection approach) consisted of a slurry with a urine to feces ratio of 1.3:1 (v/v) and the addition of $\text{Ca}(\text{OH})_2$. For Condition 2, the urine was replaced with deionized water (no urine control) and the addition of $\text{Ca}(\text{OH})_2$, and for Condition 3 (urine and feces), no $\text{Ca}(\text{OH})_2$ was added (no lime control). The final volume for all conditions was 200 mL. Samples were spiked with 1.5 mL of a stock solution of 1.0×10^5 eggs/mL *A. suum* (Excelsior Sentinel, Inc., Ithaca, NY) and 3 mL of the 3.04×10^8 PFU/mL MS2 coliphage stock solution (see below) and mixed well.

Preparation of MS2 stock solution

MS2 coliphage (ATCC 15597; Manassas, VA) was propagated using broth enrichment in *E. coli* Famp host (ATCC

no. 700891) using previously described methods (Love *et al.* 2010). After incubation, the broth culture was subjected to three cycles of freeze/thaw followed by chloroform extraction (1:3, vol:vol) and centrifugation ($4,000 \times g$ for 10 min) to remove cell debris. The supernatant was filtered through a $0.22 \mu\text{m}$ filter. The 1.90×10^{10} PFU/mL stock solution of MS2 was stored at -80°C until use. Prior to experiments, the stock was diluted with sterilized sodium chloride (10 mM NaCl) to obtain a spiking solution of 3.04×10^8 PFU/mL.

Enumeration of microorganisms

For inactivation experiments, duplicate subsamples were collected before and after the hydrolysis period, and at six additional time points throughout the storage period. Separate subsamples were collected and analyzed for pH and total ammonia as described above. For analysis of *E. coli* and total coliforms, 50 μL subsamples of fecal slurry were pipetted into 50 mL sterilized centrifuge vials containing 40 mL of 1 mM phosphate buffered saline (PBS). Centrifuge tubes were vortexed for 20 s, and the well-mixed samples were serially diluted in 1 mM PBS to reach concentrations within the detection range of each method. Quanti-Tray[®]/2000 (IDEXX) was used to determine the most probable number of *E. coli* and total coliforms following the manufacturer's instructions. For analysis of MS2 coliphage, a method developed for the recovery of enteroviruses from sludge was employed (Monpoeho *et al.* 2001). Fecal slurry subsamples (1 mL) were added to 9 mL of 10% beef extract (Sigma-Aldrich; St. Louis, MO) solution. The pH of subsamples was adjusted to ~ 7 with 0.1 N H_2SO_4 to prevent further disinfection. Samples were then vigorously mixed on a shaker table at room temperature at 400 rpm for 30 min. Samples were sonicated on ice for 5 min and then centrifuged at $5,000 g$ for 1 hour at 4°C . The supernatant was transferred into a new sterilized 15 mL centrifuge vial. Serial dilutions ranging from 10^0 to 10^{-4} were prepared from the supernatant. 100 μL samples were then assayed using the double agar layer assay (Adams 1959), with *E. coli* Famp host (ATCC 700891).

For analysis of *Ascaris* eggs, 2 mL subsamples were added to a 50-mL sterilized centrifuge tube that contained 16 mL of PBS with 0.1% Tween 80 (Thermo Fisher

Scientific; Fairlawn, NJ). To prevent further disinfection samples were neutralized with 0.1 N H₂SO₄. Samples were vigorously hand-shaken for 1 min and then vortexed for 20 s. Samples were passed through 48 and 100 mesh sieves while rinsing with a stream of 0.15 M NaCl. The recovered liquid sample was passed through a 400 mesh sieve, which retained the eggs. The retained material was collected in a 50 mL sterilized centrifuge vial. Samples were allowed to settle for 2 h, and the supernatant was removed to a volume of ~3 mL. Approximately 7 mL of 0.1 N sulfuric acid (H₂SO₄) was added to each 50 mL centrifuge vial, capped loosely and incubated for 4 weeks at 28 °C.

After incubation, the supernatant was aspirated from each tube and 10 mL of 15% bleach solution was added to remove the outer shell layer of the eggs and facilitate viewing of the internal egg structures (Bowman *et al.* 2003). After 20 min, 40 mL of DI water was added, and the samples were centrifuged at 800 g for 3 min. The supernatant was removed, and the samples were resuspended in 10 mL of DI water. Duplicate 1.6 mL well-mixed aliquot was pipetted onto a walled glass microscope slide, and eggs were viewed using brightfield microscopy at 100× magnification (Olympus BH-2 microscope). The *A. suum* eggs with larvae were considered viable, and eggs without larvae were considered inactivated. At each time point, the percent reduction in viable eggs was calculated as the concentration of larvated eggs divided by the total initial concentration of larvated eggs, multiplied by 100.

RESULTS AND DISCUSSION

Hydrolysis of urea

Very little hydrolysis of urea occurred in pure urine samples, with a maximum total ammonia concentration of 0.98 g/L after 33 days' incubation. Urine samples with urease addition produced a much greater quantity of total ammonia over a shorter period compared to the pure urine samples (Figure 1(a)). The pH of the urine amended with urease was unstable in samples initially adjusted to ~10 and ~11 (the pH dropped over time), but was stable with storage at pH 12 (Figure 1(b)). However, hydrolysis of urea was negligible at pH 12. Hydrolysis also appears to have been

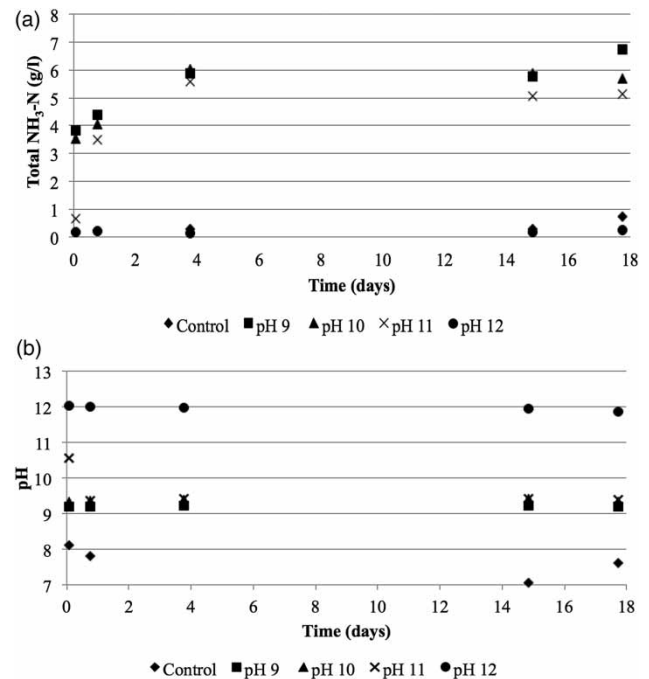


Figure 1 | Stored urine samples at 23 °C spiked with urease from *Canavalia ensiformis* and adjusted to different initial pH values (control sample had no urease amendment and no pH adjustment): (a) total ammonia concentration over time and (b) pH over time.

significantly inhibited at pH 10.6, as ammonia production was much lower in the second-highest pH sample until sampling point 2 (0.8 day), by which time the pH had decreased to 9.4. In a 35 day experiment with urine amended with feces, the pH was maintained at a stable value above 12.5, but urea hydrolysis was negligible (data not shown). Previous research has shown that urease is most effective at a pH range of 6.8–7.6 (Muck 1982). These experiments demonstrated that hydrolysis of urea needs to occur before pH adjustment, and that the pH of urine-feces slurries should be above ~pH 12.5 to remain stable for several weeks.

To compare the effectiveness of urease naturally present in feces to urease from jack bean, experiments were conducted to determine the total ammonia that could be produced from sludge slurries at different urine to feces ratios and pure urine with urease from jack beans within a 24 hour period at pH 7.2. As expected, sludge slurries with a higher ratio of urine produced a larger quantity of NH₃ (Table 1). Urease from feces produced a similar amount of NH₃ as the urease from jack bean. Given that urease from

Table 1 | Total ammonia production after 24 hours from urine at three different urine to feces ratios and in pure urine spiked with urease from *Canavalia ensiformis*

Urine:Feces	Total NH ₃ -N (g/l) ± SD ^a
1.3:1	6.66 ± 0.47
2.6:1	8.28 ± 0.58
5.2:1	9.01 ± 0.16
Pure urine	8.59 ± 0.013

^a(n = 2, SD = standard deviation).

feces was just as effective as the commercial urease, it was decided to use feces as the urease source for the Safe Sludge disinfection approach.

Experiments were conducted to determine the optimal hydrolysis period for urea. Fecal slurry samples with a urine to feces ratio (v/v) of 1.3:1 were sampled at five different time points and total ammonia was measured (Table 2). The greatest increase in ammonia concentration occurred in the first 4 hours; thus, for applications in which the shortest hydrolysis time is desired, 4 hours is recommended. Longer hydrolysis periods (e.g., 24 hours or several days) could result in greater ammonia production.

pH adjustment

Different alkalinizing agents (i.e., quicklime, slaked lime, limestone, ash, and biochar) were tested for increasing the pH of urine-feces slurries (results not shown). Ca(OH)₂ was chosen because it was the only alkalinizing agent that maintained a stable pH above 12. Quicklime (CaO) was determined to be unsafe because the exothermic hydrolysis reaction created high temperatures, which were unsafe for human contact and damaged the container materials. Finally, we determined the dosage of Ca(OH)₂ needed to

Table 2 | Total ammonia concentration of urine to feces ratio 1.3:1 samples that underwent hydrolysis over a 24 hour period

Time (hours)	Total NH ₃ -N (g/l) ± SD ^a
0.1	0.91 ± 1.28
1	2.89 ± 0.24
2	3.29 ± 0.09
4	5.13 ± 0.08
24	6.15 ± 0.30

^a(n = 2, SD = standard deviation).

Table 3 | Percentage of lime required to maintain a stable pH of 12.5 for samples with different urine to feces ratios

Urine:Feces ratio	Dry matter % ± SD	Lime in slurry % ± SD ^a
1.3:1	13.7 ± 0.647	3.35 ± 0.356
2.6:1	9.97 ± 1.10	2.63 ± 0.403

^a(n = 2, SD = standard deviation).

reach a stable pH of 12.5 for the fecal slurries with varying urine to feces ratios. A larger amount of Ca(OH)₂ was needed to maintain a stable pH for slurries that had a higher percentage of dry matter (Table 3). It is hypothesized that slurries with a higher percentage of dry matter form more weak acids due to the increased microbial activity from the feces.

Based on these results, the Safe Sludge disinfection approach was defined to have a 4 hour minimum contact period for urine and feces to allow for hydrolysis, followed by the addition of sufficient Ca(OH)₂ to increase pH to above 12.5.

Inactivation *E. coli* and MS2 coliphage

Over the 4 hour hydrolysis period, 4.90–5.20 g/L of total NH₃-N was produced by the Safe Sludge and no lime conditions (Figure 2(a) and 2(c)), and the concentration remained fairly stable throughout the 30 hour experiment (data after 12 hours not shown). The no urine (high pH, low ammonia) condition contained a minimal amount of ammonia (i.e., 0.50–0.57 g/L total NH₃-N) (Figure 2(b)). The ammonia concentrations remained relatively constant for the slurries throughout the duration of the experiments.

Inactivation trends for *E. coli* and total coliforms were indistinguishable, thus, only data for *E. coli* are reported. No significant inactivation of *E. coli* occurred during the 4 hour hydrolysis period. Within 10.6 hours (including the hydrolysis period), >9.3 log₁₀ decrease of *E. coli* was achieved for the Safe Sludge condition (Figure 2(a)). The inactivation of *E. coli* was similar in the no urine condition (Figure 2(b)), although it appears there was a slightly longer lag period before rapid inactivation began (the Safe Sludge condition achieved >6.0 log₁₀ removal of bacteria within 1.3 hours after the hydrolysis period

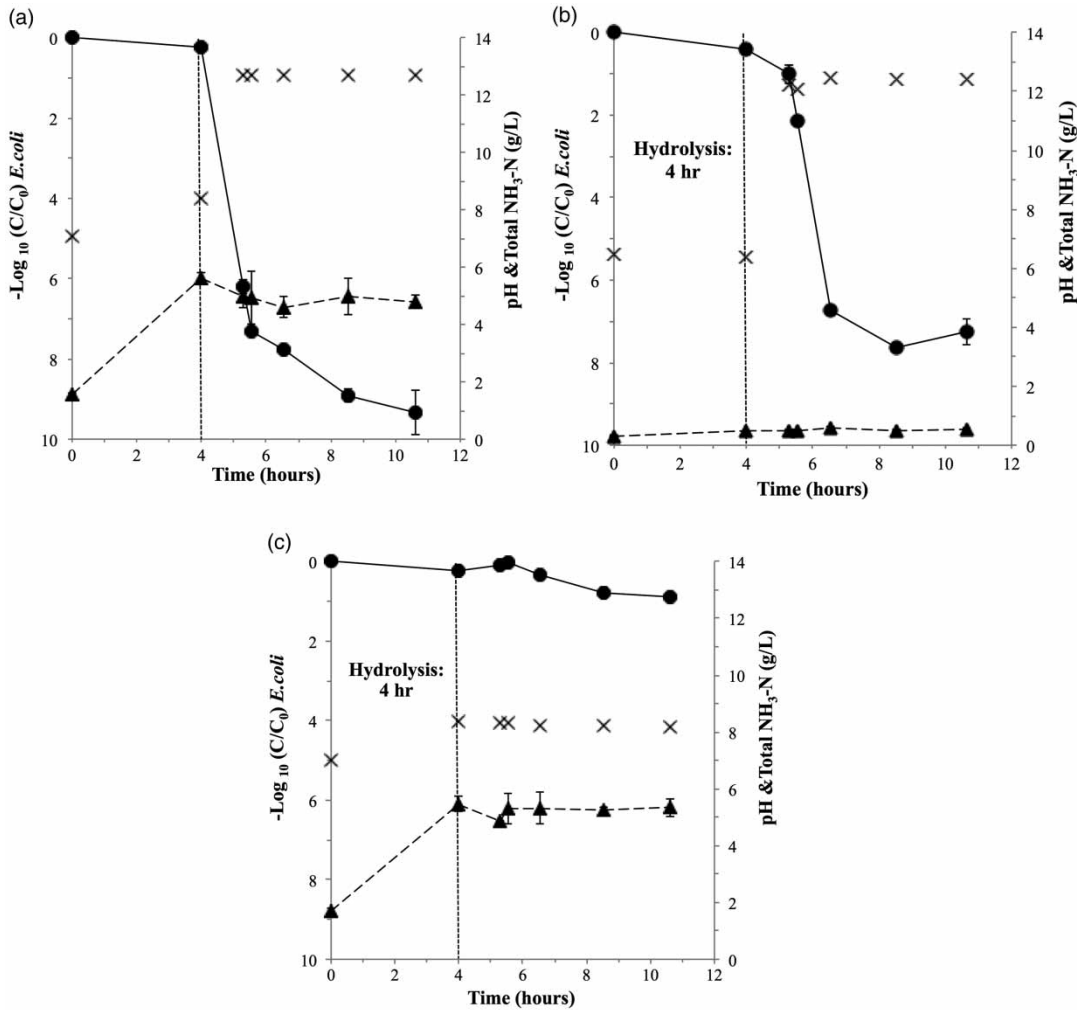


Figure 2 | Inactivation of *E. coli* during the hydrolysis period (the first 4 hours) and after lime addition (at 4 hours). Total ammonia g/L (\blacktriangle), pH (\times), and log inactivation of *E. coli* (\bullet), over time in the: (a) Safe Sludge condition, (b) no urine (high pH) condition, (c) no lime (high total ammonia, neutral pH) condition. Closed system and stored at 23 °C.

whereas the high pH condition achieved only $\sim 1.0 \log_{10}$ removal within the same time frame) and the final concentration was about 2 log higher. The no lime condition achieved $< 1 \log_{10}$ removal of *E. coli* over the 10.6 hour experiment (Figure 2(c)). Note that the log inactivation may be slightly underestimated for the Safe Sludge and no lime condition, because the initial concentrations were above the detection limit (insufficient dilution). Thus, the initial concentrations were set to the detection limit of 2.50×10^9 CFU/100 mL. This value was close to the initial concentration in the no urine samples, which was 2.28×10^9 CFU/100 mL.

Similar to *E. coli*, no significant inactivation of MS2 occurred during the 4 hour hydrolysis period. Under all conditions, the inactivation rate of MS2 was slightly slower than *E. coli*. Overall, the Safe Sludge condition was slightly more effective (i.e., $4.22 \log_{10}$ decrease) than the no urine condition (i.e., $3.72 \log_{10}$ decrease) in the inactivation of MS2 coliphage (Figure 3(a) and 3(b)) at 5.53 hours (including the 4 hour hydrolysis period). Both conditions were below the detection limit (> 4.40 and $> 4.32 \log_{10}$ removal for the Safe Sludge and no urine conditions, respectively) by 6.53 hours. Interestingly, the no urine condition required less $\text{Ca}(\text{OH})_2$ (i.e., 0.87% v/v) to

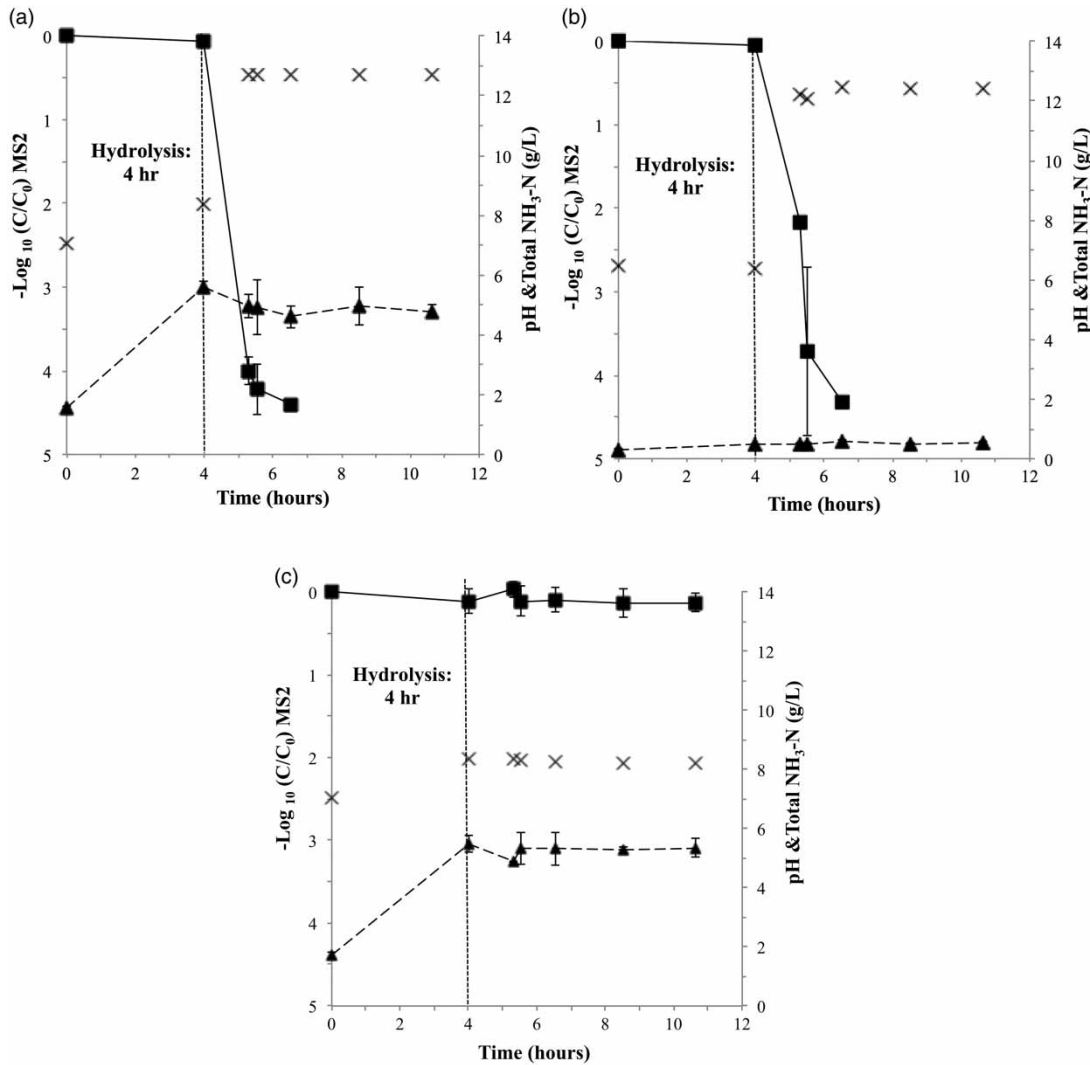


Figure 3 | Inactivation of MS2 during the hydrolysis period (the first 4 hours) and after lime addition (at 4 hours). Total ammonia g/L (\blacktriangle), pH (\times), and log inactivation of MS2 coliphage (\blacksquare): (a) Safe Sludge condition, (b) no urine (high pH) condition, (c) no lime (high total ammonia, neutral pH) condition; last time point for (a) and (b) represent the detection limit. Closed system and stored at 23 °C.

achieve a stable pH of 12.5, indicating that urine had a significant buffering capacity (resisting the pH increase). In the no lime condition, there was no significant reduction in the MS2 coliphage concentration after 28 hours (Figure 3 (c); data at 28 hours not shown).

Inactivation of *A. suum* eggs

The average total ammonia concentration that was produced by the Safe Sludge Disinfection condition was 6.60 g/L (Figure 4(a)), and the concentration was fairly

stable over the 2 week experiment. Similar to the *E. coli* experiment, the no urine condition produced a minimal amount of total NH₃-N (i.e., 0.64 g/L) (Figure 4(b)). Interestingly, ammonia continued to be produced (at a much slower rate) after the initial hydrolysis period in the no lime condition, presumably because urea hydrolysis was not arrested by high pH, with an average total ammonia concentration of 9.10 g/L (Figure 4(c)). This phenomenon was not observed in the *E. coli*/MS2 experiments, which only lasted 30 hours compared to 2 weeks.

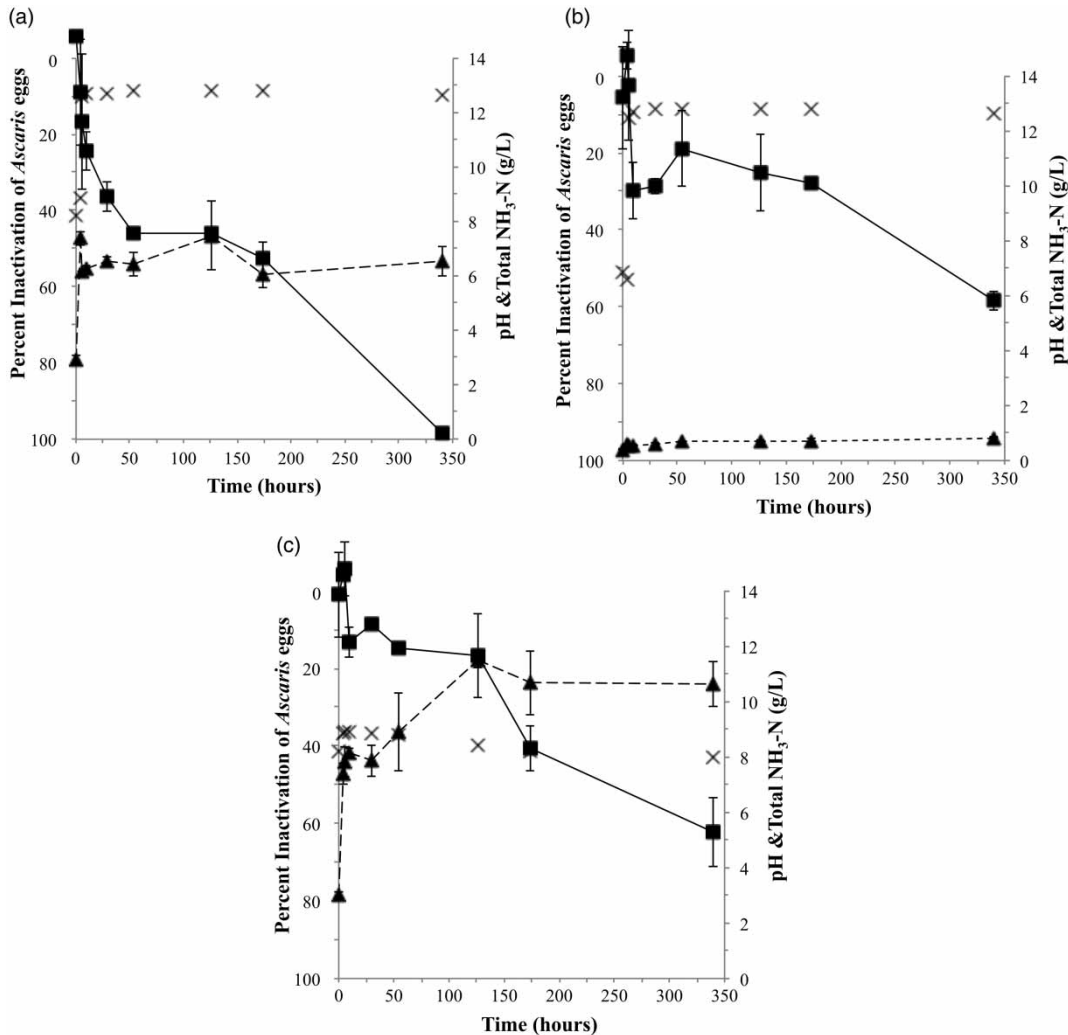


Figure 4 | Inactivation of *Ascaris suum* eggs during the hydrolysis period (the first 4 hours) and after lime addition (at 4 hours). Total ammonia g/L (▲), pH (×), and percent inactivation of *Ascaris* eggs (■): (a) Safe Sludge condition, (b) no urine (high pH) condition, (c) no lime (high total ammonia, neutral pH) condition. Closed system and stored at 23 °C.

At the end of the 2 week period, 99% ($2 \log_{10}$) inactivation of *A. suum* eggs was observed for the Safe Sludge condition (Figure 4(a); note the use of linear scale rather than log scale). For the no urine and no lime conditions, only 58% ($0.38 \log_{10}$) and 62% ($0.42 \log_{10}$), respectively, of the *A. suum* eggs were inactivated over the 2 week period (Figure 4(b) and 4(c)). The higher inactivation in the Safe Sludge condition can be attributed to the presence of unionized ammonia, since the Safe Sludge and no urine conditions had similar pH, but the Safe Sludge condition contained ~10 times more ammonia than the no urine condition. As is typical for ammonia inactivation of *Ascaris*

eggs, the Safe Sludge inactivation curve had a shoulder or lag period (evident when graphed on a log scale) (Fidjeland *et al.* 2015). The largest difference in the inactivation curves was observed at the last time point; good agreement between the experimental replicates provides confidence in these values. The time required for 99% inactivation of *Ascaris* eggs in the present research (14 days, pH 12.5, 24 °C, 6.6 g/L total $\text{NH}_3\text{-N}$) was in between that found for indigenous *Ascaris* eggs in sewage sludge by Pecson *et al.* (2007) for 5 g/L of added $\text{NH}_3\text{-N}$ and pH 12 at 20 °C (25 days) and 30 °C (4.8 days). Similar to our results, Pecson *et al.* found a large difference between samples

with high and low ammonia: the time required to achieve 99% ($2 \log_{10}$) inactivation at 20 °C and pH 12 was nine times lower when 5 g/L of $\text{NH}_3\text{-N}$ was added compared to sewage sludge with no added ammonia. Thus, these previous findings are consistent with our current results that the contribution of high pH to inactivation of *Ascaris* eggs is small compared to the contribution of unionized ammonia.

Application of the safe sludge disinfection approach to pHree Loo (pathogen-free toilet)

Based on the laboratory experiments, Safe Sludge disinfection appears to be a promising low-cost and simple approach for inactivating fresh excreta. Consistent with prior research, the combination of high NH_3 and high pH was necessary to achieve significant inactivation of *Ascaris* eggs; the effect of pH is indirect because it influences the speciation of $\text{NH}_4^+/\text{NH}_3$ (Fidjeland *et al.* 2015). *E. coli* and MS2 inactivation was much faster than inactivation of *Ascaris* eggs, and high pH was effective by itself. At lower pH values, other researchers have documented higher inactivation in the presence of NH_3 (Nordin *et al.* 2009b; Decrey *et al.* 2015). The log reduction necessary to reduce pathogen levels to an acceptable risk level will depend on which pathogens are present and their initial concentrations in excreta, and the characteristics of exposure that occur (e.g., the mass of waste accidentally ingested by sanitation workers or children that play near fecal sludge disposal sites). For any scenario, however, a $2 \log_{10}$ reduction in viable *Ascaris* eggs, $4 \log_{10}$ reduction in MS2, and $9 \log_{10}$ reduction in *E. coli* would be an enormous improvement over no treatment conditions.

To illustrate the potential for the Safe Sludge disinfection approach, a conceptual toilet was designed and built. The pHree Loo ('pathogen-free toilet') was designed to be a stand-alone household toilet that requires no water, sewerage connection, or power supply (Figure 5). The hypothetical use scenario is for a family of five. The first compartment of the toilet consists of a cylindrical tube that provides mixing of the urine and feces via an auger that is turned with a crank by the user, and a storage time of ~4 hours (the dimensions were determined assuming high use in the morning). After traveling to the end of the

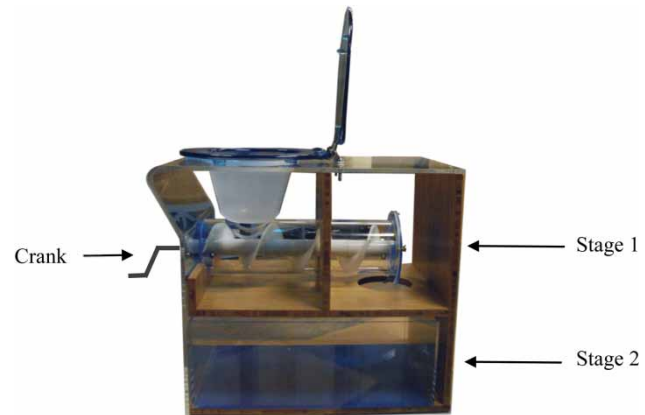


Figure 5 | The pHree Loo. Stage 1: hydrolysis of urea, mixing, and 4 hour detention time. The hand crank moves the auger and provides mixing. Stage 2: pH increase with Ca(OH)_2 , conversion of total ammonia to NH_3 , and pathogen inactivation.

first compartment, the waste empties into the bottom chamber, which is fitted with a collection bin containing the Ca(OH)_2 solution. The only requirement by the user is turning of the crank to provide mixing of the waste after each use. The conceptual service model consists of a sanitation service provider that exchanges the collection bin with a fresh bin containing Ca(OH)_2 solution on a weekly basis. The pHree Loo prototype was built to illustrate the potential design, but was not tested with real human waste because the materials were not selected to enable easy cleaning and disinfecting. The conceptual prototype was useful for identifying priorities for future designs: testing to ensure adequate mixing occurs during Stage 1 and Stage 2, a mechanism for sealing the bottom chamber to prevent loss of NH_3 , odor control, and a toilet bowl design that is easy to clean without significant amounts of water, and prevents users from seeing the deposited waste.

CONCLUSIONS

The public health risks associated with fecal sludge management practices can be significantly reduced with the application of the Safe Sludge disinfection approach, which disinfects waste at the point of collection and makes all subsequent steps safer. If excreta management ends after collection and disposal, the pathogen exposure of sanitation workers and individuals that come into contact

with the disposed waste will be significantly reduced. Ideally, however, the fecal sludge will receive additional treatment to further stabilize it, recover resources, and enable beneficial end use (Strande *et al.* 2014). By combining Safe Sludge disinfection with composting, anaerobic digestion, or drying beds, the overall treatment train will be more likely to achieve adequate pathogen inactivation for safe end-use to occur; however, further research is needed to determine the effects that the 'Safe Sludge' has on these treatment processes (e.g., high pH and high calcium concentration). In terms of the pHree Loo, the volatilization rate of ammonia and the effects it has on inactivation rates in an open system needs to be investigated. Finally, it will be essential to pursue a user-centered design process for developing actual prototypes to ensure eventual adoption by target users.

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