Ammonia sanitization of blackwater for safe use as fertilizer
Jörgen Fidjeland, Sven-Erik Svensson and Björn Vinnerås

ABSTRACT
Source-separated blackwater from low-flush toilets contains plant-available nutrients and can be used as a fertilizer. The aim of the study was to evaluate the impact on pathogen inactivation when treating blackwater with urea and/or lime. Blackwater was spiked with *Salmonella typhimurium*, *Escherichia coli* O157, *Enterococcus faecalis*, and *Ascaris suum* eggs, and treated with urea and/or lime in concentrations up to 0.1% w/w. The bottles were kept in a storage facility (manure slurry tank) for 102 days while monitoring the pathogen concentrations. The treatment time needed to meet the requirement for *Salmonella* and *E. coli* reduction could be reduced at least six-fold. The enterococci were more persistent, and only the highest treatment doses had a significantly higher inactivation than the controls. The *Ascaris* egg viability was only reduced by around 50%, so higher urea/lime doses and/or longer treatment times are required to fulfill the treatment requirements of 3 log₁₀ reductions of parasite eggs.

Key words | ammonia, blackwater, *E. coli*, inactivation, *Salmonella*, urea

INTRODUCTION
Source-separated wastewater from toilets only, often called blackwater, has relatively high concentrations of plant nutrients such as nitrogen and phosphorus. By using blackwater as a fertilizer in agriculture, these plant nutrients can be recycled together with the water fraction. In this way, the nutrients become a resource instead of being a pollutant when wastewater is discharged into water bodies. However, the blackwater may contain pathogenic microorganisms, which must be inactivated in order to reduce any health risks associated with recycling. New suggested requirements for sanitization of wastewater fractions for agricultural use in Sweden require the treatment to reduce the levels of *Escherichia coli* to <100 per gram wet weight and <1 *Salmonella* spp. per 50 g wet weight (Swedish EPA 2013).

One option for pathogen inactivation is ammonia sanitization, which utilizes the ability of ammonia to inactivate pathogens (Warren 1962; Park & Diez-Gonzalez 2003; Nordin et al. 2009b). The nitrogen in blackwater is mostly in the form of ammonia, which originates mainly from the hydrolysis of urea in urine. The average concentration of ammonia in undiluted urine is 6.9 g NH₄⁺·N L⁻¹ (Jönsson et al. 2005), which is sufficient for inactivation of bacteria and phages in urine (Vinnerås et al. 2008), and even *Ascaris* eggs at temperatures above 20 °C (Nordin et al. 2009a). This intrinsic ammonia is also sufficient for pathogen inactivation in sludge from toilets using low amounts of flush water, such as vacuum toilets or pour flush toilets (Fidjeland et al. 2015).

If the concentration of uncharged ammonia is too low for sanitization it can be increased by adding urea, as urea hydrolyzes into ammonia through the enzyme urease. Addition of urea or lime will also raise the pH, which affects the ammonia equilibrium and transfers a larger proportion of the ammonia into the uncharged form, NH₃, which is the substance responsible for the inactivation of pathogens. Toilet waste with a high dry matter content, such as sewage sludge or pit latrine/fecal sludge, typically requires a higher dose of urea (1–4%) to increase the pH sufficiently. Owing to the high dilution of blackwater, it has a lower buffer capacity, and a lower urea and/or lime dose is hence required to increase the pH.

As ammonia treatment is a scale-independent technology, it can be used for local treatment of blackwater from single houses through to whole communities. Existing farm equipment can be used for field application of treated blackwater, thus simplifying the transition to local
blackwater management. Local management also decreases the need for transport, compared with long journeys to a central sewage treatment plant, often on narrow local roads. Ammonia treatment also enables recycling of all plant nutrients in the wastewater, as compared with the reuse of wastewater treatment plant sludge, which mainly recycles the phosphorus in the wastewater. Furthermore, local treatment reduces the energy used for nitrogen removal at the wastewater treatment plant. This results in environmental savings due to lower emissions and lower usage of virgin resources (Vidal 2014).

The aim of the study was to evaluate the potential for sanitizing blackwater with a low dosage of urea and/or lime by monitoring the inactivation of *E. coli*, *Salmonella* spp., *Enterococcus*, and *Ascaris suum* eggs during storage at ambient temperature in Sweden.

**MATERIALS AND METHODS**

Blackwater was collected, spiked with microorganisms and placed in 10 2-L polyethylene bottles. The blackwater had been collected from households with a source-separated wastewater system with low-flush toilets (2/4 L per flush) connected to a closed holding tank. The microorganisms added were *Salmonella typhimurium* phage type 178, which was isolated from sewage sludge by Sahlström et al. (2004), a non-virulent and non-toxin producing strain of *E. coli* O157 (EHEC) (CCUG 44857), and *Enterococcus faecalis* (ATCC 29212). The bacteria were grown in nutrient broth overnight to a concentration of \( \sim 10^7 \) for *Enterococcus faecalis* and \( \sim 10^8 \) for *Salmonella typhimurium* and *E. coli* O157. In addition, *Ascaris suum* was used as a model for parasites. *Ascaris suum* eggs were collected from the intestines of slaughtered pigs, washed as described by Eriksen et al. (1996) except for the use of sodium hypochlorite, and placed in permeable nylon bags (mesh 35 μm) with approximately \( 10^4 \) eggs per bag. Three bags were added to each bottle for three of the treatments and the two controls.

The samples were treated with different urea doses up to 0.1% and/or lime doses up to 0.05% (Table 1). In total, 10 bottles were prepared: three of which were kept close to the surface of an open manure slurry tank half-filled with 500 m³ blackwater; the other seven were kept at the bottom, approximately 2 m below the surface. The temperature in the tank was estimated to be 10–20 °C.

Levels of viable microorganisms were analyzed on days 0, 28, 68, and 102. All samples for a treatment and placement combination were extracted from the same bottle. After mixing the contents of the bottle, three subsamples were extracted and stored at 5 °C, and analysed microbiologically within 4 h. Bacteria levels were evaluated by the plate spreading technique; *Salmonella* spp. was cultivated on xylose lysine desoxycholate agar (Oxoid) containing 0.15% novobiocin incubated at 37 °C for 24 h, and *Enterococcus* spp. on Slanetz–Bartley agar incubated at 44 °C for 48 h. Enterohemorrhagic *E. coli* were cultivated on Chromocult agar incubated at 37 °C for 24 h, and thermotolerant coliforms were enumerated by cultivation on violet red bile agar incubated at 44 °C for 24 h. At sampling, one bag with *Ascaris* eggs was collected from the five bottles containing eggs, rinsed in physical saline solution (0.9% NaCl), and then incubated in 0.1 M HCl at 20 °C for 21 days. After incubation, 100 eggs were examined by

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<th>Treatment</th>
<th>Decimal-reduction time, ( T_{90} ) (days)</th>
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<tr>
<td></td>
<td><em>Salmonella</em></td>
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<td>Control</td>
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<td>Control</td>
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<td>0.1%</td>
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<td>0.025%</td>
<td>6.7</td>
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<td>0.0125%</td>
<td>&lt;6.3</td>
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microscope and the eggs that had developed to motile larvae were counted. A nylon bag with *Ascaris* eggs was incubated without being exposed to blackwater in order to determine the initial viability (viability at day 0).

The inactivation data were analysed statistically using linear regression after logarithmizing (log10) the concentration of bacteria and the viability of the *Ascaris* eggs. The decimal-reduction time (*T*<sub>90</sub>), which is the time required for 90% inactivation, was calculated as the negative inverse of the slope of the regression lines. In cases where the bacteria concentration was below the detection limit after 28 days, *T*<sub>90</sub> was estimated based on the detection limit. The detection limit was also used for figures where the concentration of bacteria was below the detection limit. Student's *t*-test was used for evaluating the significance of the difference between regression line slopes. The statistical analysis was done using R v. 3.1.0 (R Development Core Team 2014).

**RESULTS AND DISCUSSION**

**E. coli**

*E. coli* O157 was inactivated very quickly and detectable only in the two controls after 28 days. Therefore, the number of thermotolerant coliforms was monitored instead (Figure 1). The initial population of coliforms was probably mainly *E. coli* O157, and later other more persistent coliforms. The decimal-reduction time (*T*<sub>90</sub>) was <4.8 days for the treatment with 0.1% urea, and <7.3 days for all other treatments, except the one with 0.0125% urea and 0.0125% lime (Table 1); the actual *T*<sub>90</sub> could not be determined for either as the concentration was below the detection limit. The decimal-reduction times (*T*<sub>90</sub>) for the controls were 12 and 29 days for the surface and bottom samples, respectively. In all treatments, the inactivation of coliforms was faster than the controls stored in the same location, despite low doses of chemicals in some of the treatments.

As the *E. coli* O157 was more sensitive than the thermotolerant coliforms, the estimated decimal-reduction times are thus probably an underestimate for the inactivation of thermotolerant coliforms, but a conservative estimate of *E. coli* O157 inactivation. The treatment with 0.05% urea and the control, both stored at the surface, and the treatments with 0.05% urea and 0.025% urea stored in the bottom of the tank, all had a coliform concentration below the detection limit on day 68, but viable colonies were detected at day 102. This is probably caused by the growth of other species more persistent than *E. coli*, such as *Klebsiella* or *Citrobacter*, which are also detected by the enumeration of thermotolerant coliforms (Hachich et al. 2012).

**Salmonella**

The blackwater contained no *Salmonella* before spiking. After 28 days of treatment, the number of *Salmonella* spp. was below the detection limit for the treatments with 0.1% urea (<10 cfu mL<sup>−1</sup>), 0.05% urea (<100 cfu mL<sup>−1</sup>), and 0.05% lime (<100 cfu mL<sup>−1</sup>). On day 68, the concentration was also below the detection limit for the treatments with 0.025% urea only and with 0.025% urea and 0.025% lime...
(Figure 2). For Salmonella, there was a significant ($P < 0.005$) difference between the treated samples ($T_{90} \leq 6.7$ days) and the controls (surface $T_{90} = 16$; bottom $T_{90} = 19$).

**Enterococcus**

Enterococcus spp. were more persistent than the Gram-negative bacteria, and possible to detect throughout the experiment, except for the urea treatments kept at the surface of the slurry tank. The decimal-reduction times decreased with higher doses of urea and/or lime (Figure 3). However, only in the treatments with 0.1% urea, 0.025% urea (surface sample), or 0.05% lime, were enterococci inactivated significantly faster than the respective controls (stored in the same location in the tank) ($p < 0.05$).

**Ascaris**

Ascaris suum eggs were more resistant to treatment than bacteria, and in the treatment with the higher urea dose (0.1%), only 43% inactivation was observed compared with the initial viability (Figure 4). This is probably due to the low ambient temperature, as Ascaris egg inactivation has been shown to be very slow at temperatures below 20 °C (Nordin et al. 2009a).

**Treatment aspects**

All studied microorganisms were inactivated faster in the bottles stored at the surface compared with the bottles with the same treatment stored at the bottom of the tank.

This is probably due to algal growth in the bottles stored at the surface, which can cause an increase in pH (Araki et al. 2001). Furthermore, the temperature was probably higher in the bottles stored at the surface due to direct sunlight. Comparing the pathogen inactivation observed in the slurry tank (not reported) with the bottles stored at the surface, indicates that the pH increase at the surface is not sufficient to increase the pH in the whole tank. Therefore, the bottom samples are more representative of the average treatment effect in the tank.

As the pathogen inactivation is due to ammonia and not urea, the hydrolysis of urea is needed in order to achieve pathogen inactivation. As the pathogen inactivation in treatments with urea was faster than the treatments without, hydrolysis of urea did occur in the treatments, but the proportion of urea that was hydrolyzed was not measured. In treatment systems with higher flush water volumes, the hydrolysis of urea may be a limiting factor, as the hydrolysis is dependent on the urease enzyme, which mainly comes from the faecal fraction.

Both urea and lime proved to be efficient for treating blackwater, and Salmonella spp. and E. coli were inactivated much faster in all treatments than in the controls. Treatment with 0.1% urea could reduce the storage time by a factor of at least 6 to achieve the same E. coli reduction, and could thus fulfill the suggested Swedish requirements of $<100$ E. coli per gram wet weight and $<1$ Salmonella per 50 g wet weight (Swedish EPA 2013). However, the inactivation of Ascaris suum eggs was slow, and the suggested guidelines also include a $3 \log_{10}$ inactivation of parasites for verification of new methods, similar to the EU animal by-product regulation (European Union 2011). Assuming linear extrapolation of the Ascaris egg inactivation, the
resulting treatment time required for 90% reduction of *Ascaris* viability is ~1 year, which is not practical for reaching a 3 log₁₀ reduction. However, ammonia inactivation of *Ascaris* eggs typically has an initial lag phase, with low inactivation followed by a more rapid inactivation (Fidjeland et al. 2013). Furthermore, it has been shown in the case of urine storage tanks that the concentration of both pathogens and ammonia is higher close to the bottom of the tank (Högblund et al. 2000). A similar effect is expected to expose sedimented *Ascaris* eggs to an NH₃ concentration above average in blackwater storage tanks, thereby increasing the effect of the urea dose. A limitation of this study using bottles and nylon bags is that these conditions and effects are not simulated; full-scale studies are needed to evaluate this. Still, it is most likely that either higher chemical doses or longer storage times are required than those studied here in order to achieve a 3 log₁₀ reduction of *Ascaris* eggs. However, if the final regulations allow it, other hygiene barriers, such as crop selection, could be implemented instead, as *Ascaris lumbricoides* infections are very rare in Sweden. In areas where *Ascaris* eggs are normally found in wastewater, the ambient temperature is usually higher, giving a much higher die-off of *Ascaris* eggs (Nordin et al. 2009a). Heating and/or higher doses of urea or lime than those used in this study are required to decrease the storage time needed for *Ascaris* egg inactivation in Sweden.

The treatment with 0.05% lime has about the same inactivating effect as 0.1% urea. Both nitrogen and lime are regularly spread on soil in Swedish agriculture, and adding them to the blackwater as a treatment is beneficial as the chemicals are not consumed in the process. However, the alkaline effect of lime is somewhat reduced during storage due to its reaction with CO₂ in the air, which reduces the pH and the alkaline effect of lime. Adding lime to the storage tank causes precipitation of calcium phosphates, which even binds to other suspended material during sedimentation. This makes the phosphorus concentration uneven, and mixing is required before use of the blackwater as fertilizer.

As ammonia is highly volatile, ammonia loss during treatment needs to be avoided either by putting a roof on the storage tank or, as in this case, by having a scum layer on top of the slurry, which reduces (but does not eliminate) ammonia loss. The treatment assures sanitization, and, as long as the ammonia remains in the blackwater, there is no risk of regrowth during storage (Vinnerås et al. 2008; Nordin et al. 2009b), which is a risk after other treatments, for example, autothermal aerobic digestion or anaerobic digestion (Sidhu et al. 2001; Elving et al. 2010).

Ammonia treatment offers an efficient, low-tech treatment alternative for the safe recycling of plant nutrients from separately collected blackwater. As the treatment is very simple and mainly consists of storage, the investment in infrastructure is limited, especially in this case where a disused but fully functional slurry tank was used for the treatment. Addition of urea improves the fertilizing value of the treated product, as the ammonia is not consumed in the process. Thereby, the cost of the added urea can be allocated to the increased value of the fertilizer product.

**CONCLUSIONS**

This study shows that there is potential to use low concentrations of urea and/or lime treatment for the sanitization of blackwater prior to agricultural use as a fertilizer. Sanitization of blackwater with urea or lime can reduce the required treatment time for *E. coli* inactivation by a factor of up to 6 (treatment with 0.1% urea). There was a high effect on the inactivation of *Salmonella* spp. and *E. coli* from all treatments studied, except the lowest dose of 0.0125% urea and 0.0125% lime, which had no impact on *E. coli* inactivation compared with the control. The enterococci were more persistent, and only the treatments with the highest doses, 0.1% urea or 0.05% lime, had an effect on enterococci inactivation in the storage tank. The inactivation of *Ascaris* eggs was slow and higher storage temperature, higher doses of urea and/or lime are probably required for faster inactivation of *Ascaris* eggs.

**REFERENCES**


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