Sanitising black water by auto-thermal aerobic digestion (ATAD) combined with ammonia treatment

Annika C. Nordin and Björn Vinnerås

ABSTRACT

The effect of a two-step process on the concentration of pathogens and indicator microorganisms in black water (0.9–1% total solids) was studied. The treatment combined auto-thermal aerobic digestion (ATAD) and ammonia sanitisation. First, the temperature of the black water was increased through ATAD and when a targeted temperature was reached (33, 41 and 45.5 °C studied), urea was added to a 0.5% concentration (total ammonia nitrogen >2.9 g L^{-1}). *Escherichia coli* and *Salmonella* spp. were reduced to non-detectable levels within 3 days following urea addition at temperatures above 40 °C, whereas when urea was added at 33 °C *E. coli* was still present after 8 days. By adding urea at temperatures of 40 °C and above, a 5 $\log_{10}$ reduction in *Enterococcus* spp. and a 3 $\log_{10}$ reduction in *Ascaris suum* eggs was achieved 1 week after the addition. With combined ATAD and ammonia treatment using 0.5% ww urea added at an aerobic digestion temperature >40 °C, black water was sanitised regarding the pathogens studied in 2 weeks of total treatment time.

Key words | ammonia, auto-thermal aerobic digestion, black water, pathogen, urea, sanitisation

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INTRODUCTION

Human excreta contain the majority of household nutrients and, when collected separately, these nutrients can be recirculated to agricultural land as a valuable resource. Such management would decrease environmental pollution from on-site sanitation systems, which are a source of eutrophication of surface waters, and would reduce the use of fossil-based chemical fertilisers (Lundin *et al.* 1999; Winker *et al.* 2009). However, human excreta potentially contain pathogens, which can infect humans and animals if spread in the environment (Albihn & Vinnerås 2007). Human urine can be separated at source and self-sanitises when urea in the urine decomposes into ammonia, if stored in closed vessels (Höglund 2001). Similarly, the intrinsic ammonia in the urine can sanitise the faecal pathogens in systems using low-flush toilets in which urine and faeces are collected together (Fidjeland *et al.* 2013) and source-separated faeces can be sanitised by adding urea to increase the ammonia concentration (Nordin *et al.* 2009b; Vinnerås *et al.* 2003b). If low-flush toilets are used, no change of in-house practice is necessary, which could increase user acceptance, in contrast to source separating, dry sanitation solutions. It is thus likely that a future sanitation reuse scenario involves the use of black water rather than source separated fractions.

An established method to stabilise and sanitise separately collected black water is auto-thermal aerobic digestion (ATAD), where organic material is degraded under aerobic conditions and sanitisation occurs due to the heat generated. For Swedish conditions, a time and temperature combination of 55 °C for 6 hours is currently required (SEPA 2003). However, the energy content of black water is often not sufficiently high to create and maintain the temperatures required for sanitisation and therefore energy-rich organic material, e.g. food waste, manure and industrial waste, has to be added. This addition of external material increases the cost of treatment for the black water, as added material increases the oxygen demand. Treating black water alone would be favourable, as the dependency on suitable locally available complementary material is omitted and transport minimised.

Ammonia has been proven to efficiently inactivate many groups of pathogenic microorganisms and sanitisation has been evaluated for source-separated faeces, sewage sludge and manure (Nordin *et al.* 2009b; Ottoson *et al.* 2008; Pecson *et al.* 2007). Adding ammonia in the form of urea is favourable for working conditions and worker safety, compared with use of aqueous ammonia. Temperature has

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been found to be an important parameter for the inactivation rate. In the case of parasite eggs, an increased temperature is crucial to enable inactivation at the ammonia concentrations that are reasonable to use if the treatment residue is to be used as a fertiliser (Nordin *et al.* 2009a). By combining ATAD with ammonia treatment, the ATAD induced temperature elevation gives higher ammonia sanitisation rates compared with ammonia treatment at ambient temperatures and less urea is needed. By sanitising by a mechanism other than heat treatment, the need for adding complementary energy-rich material to black water is eliminated. However, the efficiency of this combined treatment depends on a number of variables: the temperature reached by the aerobic digestion, the subsequent temperature decline, the pH and ammonia concentration.

Regulations and guidelines regarding hygiene for sewage fractions applied to agricultural land generally set either a concentration limit for the end product and/or require a pre-determined reduction in organisms to validate the treatment process. In Sweden, no such regulation currently exists, but at the request of the government, the Swedish Environmental Protection Agency proposed regulations in 2010 and revised these in 2013 (SEPA 2010, 2013). The proposals for regulation include the assessment of pathogen reduction by threshold levels as well as the set reduction requirements of *Enterococcus*, *Escherichia coli*, *Salmonella*, parasite eggs and viruses. At the time of writing, the proposal has not come into force, and nor has the proposed amendment to the EU Sewage Sludge Directive 86/278/EEC (EC 2000), which also aims to regulate organism reductions and post-treatment concentrations. Due to the lack of European regulation in force, we also refer in the discussion to the EU Animal By-Products (ABP) regulation regarding the use of animal manure on arable land (EC 2009, 2011).

The present study investigated the effect of a combined two-step treatment process on pathogens (*Salmonella* spp. and *Ascaris suum* eggs) and indicator organisms (*E. coli*, *Enterococcus* spp. and bacteriophages) in black water. The combined process consisted of ATAD followed by ammonia treatment with 0.5% urea concentration in a full-scale ATAD reactor in three separate batch runs. The temperature at which urea was added was varied in three runs: at 33 °C, 41 °C and 45.5 °C. The full-scale experiment was complemented with a laboratory study to extend the *Ascaris suum* egg inactivation data. The overall aim of the study was to evaluate the capacity of the combined treatment to sanitise black water, to enable the production of safe fertiliser products from source separated toilet waste.

MATERIALS AND METHODS

Full-scale study

The aerobic digestion reactor used for the full-scale experiments is located outside Kvicksund, Sweden (59°41′13.24′N, 16°33′83.33′E). The treatment system was brought into use in 1998 and comprises a 92 m^3 collection basin, a 32 m^3 reactor (height 7.5 m, Ø 3 m) (Alfa Laval Agri, Sweden) and a 1,430 m^3 covered post-treatment storage basin (Figure 1). Source-separated black water from on-site sanitation systems is co-treated with manure slurry or latrine waste from holiday cottages at approximately 4% total solids (TS). The normal

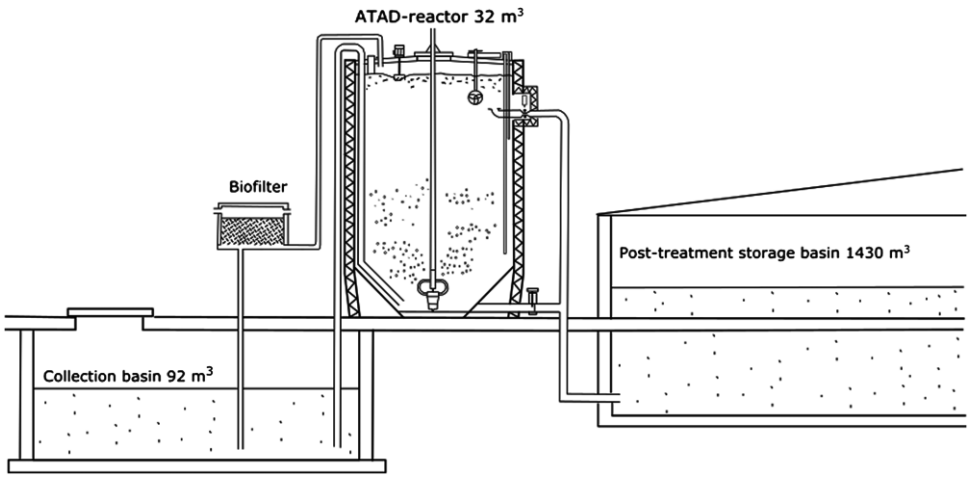

Figure 1 | Schematic overview of the auto-thermal aerobic digestion plant comprising a 92 m^3 collection basin, a 32 m^3 reactor (height 7.5 m, Ø 3 m) (Alfa Laval Agri, Sweden) and a 1,430 m^3 covered post-treatment storage basin.

treatment is semi-continuous thermal sanitisation (55 °C for 12 hours) with an estimated 25 kWh m^{-3} material for aeration and mechanical equipment (Malmén 2005).

For the present study, the reactor was run batch-wise, mainly with source-separated black water from on-site sanitation systems in households, and from a school with 500 pupils, which had some food waste milled into the collection tank (Norin *et al.* 2000). The reactor was completely emptied prior to filling it with 32 m^3 black water. The bags containing *Ascaris suum* eggs were inserted into the reactor from the top with a string, which was held vertically approximately 1 m below the liquid surface. The aim was to increase the temperature through aeration and then add urea, in order to see the effect of temperature on the inactivation. The material was aerated until reaching a temperature of 33, 41 and 45.5 °C in each batch run, respectively (Table 2). After stopping the aeration, 165 kg urea (Yara, Sweden) was added, aiming at a treatment of 0.5% urea weight/weight (ww). The material was mixed by pumping for approximately 12 hours, then the reactor was left unaerated for the sanitisation phase and the material was mixed only before sampling. The three reactor runs with combined treatment were performed during the period 5 December 2011–4 April 2012. During the study period, fresh material was continuously fed into the collection basin (Figure 1).

The *Ascaris suum* eggs added to the reactor were collected from the posterior 2 cm of the uterus of adult worms and washed according to Eriksen (1981), except for the use of sodium hypochlorite. Approximately 10^4 eggs were placed in permeable nylon bags (2×100 cm^2; mesh, 28 microns) that were enclosed in a polyethylene bag (mesh 0.7 mm) connected to the string. At sampling, one nylon bag was detached and kept in 0.05 M sulphuric acid during transport to the laboratory.

The reactor content was sampled through a tap connected to a pipe system pumping the black water from the bottom to the top of the reactor. Before each sample was collected, the black water in the reactor was stirred for 1 hour, the material was pumped round through the pipes for 5 min and before collecting the sample, the tap was flushed with 1 L material, which was discarded. Triplicate 1-L samples of the black water were taken at three times: (1) after the reactor was filled, prior to the start of aeration; (2) when the material had reached the target temperatures, but before adding urea; and (3) at the end of the combined treatment. These samples were analysed for TS, volatile solids (VS), total ammonia nitrogen (TAN), pH and detection/count for *E. coli*, *Enterococcus* spp., somatic coliphages, f-specific phages, *Salmonella* spp. and *Ascaris suum* eggs. Triplicate 45-mL samples were taken every 24 hours during the aeration phase for analysis of pH and ammonia and every 48 hours after urea addition for analysis of pH, ammonia, bacteria and phage counts. All samples were cooled and transported overnight to the laboratory, where the temperature of the black water on arrival was confirmed to be ≤10 °C, and the analyses were performed.

Laboratory study

The viability of *Ascaris suum* eggs retrieved by sieving swine faeces (Excelsior Sentinel, Inc., USA) were studied in composted black water. Similarly to the full scale study, 0.5% urea (w/w) was added at a start temperature of 35, 40 and 45 °C. To simulate the temperature drop occurring in the full-scale plant, the temperature was decreased by 1 °C per day using a heat block (Grant Instruments Ltd, UK). The black water was collected from a treatment plant in Södertälje municipality (Telge Nät, Sweden) where it had been aerated for 7 days, giving a temperature of 43.6 °C. After transport to the laboratory, 0.75 g urea was added to 150 g black water and blended for 30 minutes in an enclosed container. The black water was aliquoted into 7-mL polypropylene tubes with O-ring lined screw caps (Sarstedt AG & Co, Sweden). Approximately 10,000 *Ascaris suum* eggs in nylon bags were inserted into each tube. The tubes were inserted into the heat block, along with tubes containing *Ascaris suum* egg bags in buffered NaCl (0.9% w/v) peptone water with the surfactant Tween (pH 7) as temperature control.

Analyses

The pH was analysed with a glass electrode PHC 2,051 (Radiometer Analytic, France) connected to a PHM 210 meter (MeterLab, Denmark) in undiluted samples that were allowed to adjust to room temperature (23 °C). Black water filtered through 45 μm mesh (Filtropur, Sarstedt AG & Co, Sweden) was analysed for TAN by Spectroquant® reagent kit 114,544 (Merck, USA) based on the indophenol blue method, at 660 nm on a Genesys 20 spectrophotometer (Thermo Scientific, USA). Then, TS and VS were analysed according to EN 12880:2000. To fulfil the requirements of a minimum dry matter amount of 0.5 g per analysis and a representative measure, approximately 200 g wet material was used.

Salmonella spp. was analysed by the Modified Semisolid Rappaport Vassiliadis (MSRV) method (Annex D of EN ISO 6579:2002). In brief, 25 mL black water was diluted 10-fold

and pre-enriched in Buffered Peptone Water (BPW) (18 h at 37 ± 1 °C). Three drops (0.1 mL) of the BPW were placed on the surface of MSRV plates supplemented with 1.0% Novobiocin (SVA, Sweden) for selective enrichment at 41.5 ± 0.5 °C. The MSRV plates were examined for *Salmonella* spp. after 24 ± 3 h and, if negative, after an additional 24 hours. Suspect *Salmonella* spp. growth was plated on Xylose Lysine Deoxycholate agar (XLD) with 1.5% Novobiocin and on Brilliant Green agar (BGA) (SVA, Sweden). Further confirmation of presumptive *Salmonella* was performed with triple sugar iron agar and urease broth. Isolated *Salmonella* spp. colonies were plated on blue-agar plates (37 ± 1 °C, 24 ± 3 h) and serotyped according to Kaufmann and White (Popoff & Le Minor 2001) at the National Veterinary Institute (SVA), Uppsala, Sweden.

Buffered NaCl peptone water with surfactant Tween (pH 7) (SVA, Sweden) was used for the dilution series when enumerating the other bacteria and phages. Total thermotolerant coliform analysis specifying the fraction of *E. coli* was performed by pour plate methods with a double layer of violet red bile agar (SVA, Sweden) incubated at 44 ± 0.5 °C for 24 ± 3 h. Typical colonies were confirmed by growth and gas production in lactose-tryptone-lauryl sulphate broth (44 ± 0.5 °C; 24 ± 3 h), and *E. coli* was determined by Kovacs indole regent. In addition, lauryl sulphate tryptose broth with 100 mg L^{-1} 4-methylumbelliferyl-β-D-glucuronide (MUG) (Fluorocult) (44 ± 1 °C; 24 ± 3 h) was used and tubes testing positive for *E. coli* were confirmed by Kovacs indole reagent. *Enterococcus* spp. was detected by the most probable number (MPN) method in enterococcosel broth using 1 mL for three dilutions and five replicates and by plating 1 mL on Slanetz-Bartley (SlaBa) agar (Oxoid) (44 ± 0.2 °C, 48 ± 2 h) and confirmed as catalase negative. F-specific RNA phages and somatic coliphages were detected by standards ISO10705–1:1995 and 10705–2:2000, the double-layer agar method with *E. coli* (ATCC 13706) and *S. typhimurium* WG 49 (ATCC 700730) were used as bacterial host strains for enumeration of the respective bacteriophages, cultured in nutrient broth (Oxoid; Sollentuna, Sweden).

The nylon bags containing *Ascaris suum* eggs were washed with 0.1 N sulphuric acid to remove particles on the bag, and eggs were withdrawn from the bag and checked for any development during the period in the reactor/heat block. The remaining eggs in the bag were incubated at 28 °C in 0.1 N sulphuric acid for 30 days to allow larval development (Arene 1986). Viability counts were performed under the microscope. Unfertilised eggs, identified by their incomplete egg shells, were excluded from further counting. Eggs developing to the larval stage were considered viable, while pre-larval stages were not. Initial viability of the *Ascaris* eggs was 75% (95% confidence interval (CI_{95}) 71–77%) for the full-scale studies and 89% (CI_{95} 88–91%) for the laboratory study.

Ambient temperature data were obtained from a weather data station in Kvicksund (59 °50′N, 16 °31′67″E; 18 m above sea level), measured with 1-wire type equipment (www.temperatur.nu). Concentration of ammonia (NH_3) was calculated from TAN concentration, pH and temperature according to Emerson *et al.* (1975). Regression analysis and single factor Anova followed by post-hoc analysis with Tukey's Honestly Significant Difference (HSD) test (at family rate 5) were performed in Minitab 16 (Minitab Inc., USA), with $\alpha \leq 0.05$ unless otherwise stated. Confidence intervals for proportions of viable *Ascaris* eggs were derived using the Wilson procedure (Vassarstats.net), and the US Environmental Protection Agency's MPN software programme (Avineon, USA) was used to derive the MPN per mL from the positive tubes.

RESULTS AND DISCUSSION

Black water and temperature development

Some of the characteristics of the black water differed between runs, but there were no consistent differences for a specific batch of black water (Table 1). The black water with some food waste had TS of 0.89–0.98% (Table 1), which was higher than in black water from low flush systems evaluated by Norin (1996) and Palm & Malmén (2003) (TS 0.75 and 0.4%, respectively). The content of VS, which constituted 73–76% of TS (Table 1), was similar to the Swedish design value of 74% for black water including toilet paper (Jönsson *et al.* 2005), and comparable to the 71% reported by Norin (1996). The temperature increase in the reactor during the aeration phase (4–11 °C at start) was slightly slower during the first day, but was approximated to be linear, with 5.7–7.1 °C per day of aeration. Temperatures of 33–45.5 °C were reached during the aerobic digestion, and aeration stopped without reaching its potential maximum (Figure 2). The discrepancy between actual and targeted temperatures (35, 40 and 45 °C) was due to operational limitations, i.e. that aeration was stopped during the daytime. No tests were performed to determine the temperature potential of the material. However, the operator of the system reported that such energy-poor material does not reach sufficiently high temperatures for

Table 1 | Characterisation of the incoming black water for the three reactor runs with the values given as mean ± S.D. ($n = 3$) and significance between runs marked with an asterisk

Parameter	Unit	Run 1	Run 2	Run 3
TS*	% ww	0.92 ± 0.01	0.98 ± 0.02	0.89 ± 0.04
VS*	% of TS	73 ± 0.01	77 ± 0.01	76 ± 0.01
pH*	Unitless	6.8 ± 0.08	6.7 ± 0.02	6.5 ± 0.06
TAN	g L^{-1}	0.67 ± 0.03	0.74 ± 0.06	0.62 ± 0.09
Salmonella spp.	Species	*S.* Stanley	*S.* Bareilly	*S.* Bareilly
Enterococcus spp.	Log_{10} cfu mL^{-1}		5.5 ± 0.03	5.6 ± 0.21
	Log_{10} MPN mL^{-1}	4.1 ± 0.12	3.7 ± 0.24	
TTCs	Log_{10} cfu mL^{-1}		3.9 ± 0.10	5.9 ± 0.14
	Log_{10} MPN mL^{-1}	4.2 ± 0.24		
Coliphages	Log_{10} pfu mL^{-1}	5.1 ± 0.05	5.2 ± 0.05	4.8 ± 0.34
f-RNA phages*	Log_{10} pfu mL^{-1}	4.4 ± 0.16	3.5 ± 0.05	3.1 ± 0.1

validated sanitisation, i.e. >55 °C for 6 h. The temperature decline after stopping aeration and adding urea was linear ($p < 0.001$) (Figure 2, Table 2).

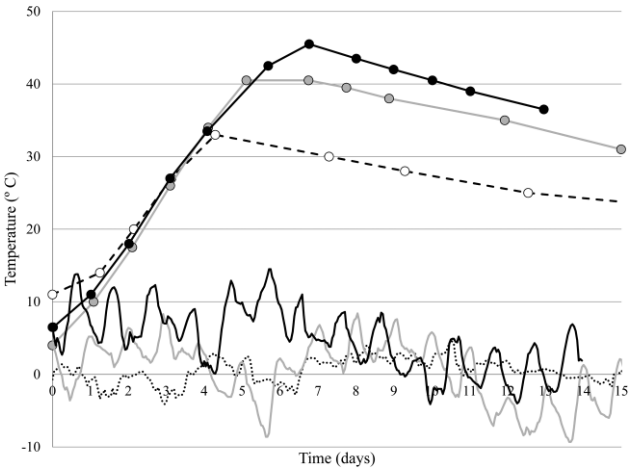

Figure 2 | Temperature during the combined treatment showing the aerobic digestion phase (temperature increase) and ammonia sanitisation phase (temperature decrease) for the runs reaching 33 (white/broken line), 41 (grey) and 45.5 °C (black), together with the ambient temperature for the periods (same colour coding).

Urea as ammonia additive

Adding urea to the full-scale reactor (165 kg to 32 m^3 black water) increased the initial TAN concentration (0.54–0.58 g L^{-1}) by five- to six-fold, to exceed the theoretical concentration of 2.9 g L^{-1} after, at most, 3 days from urea addition. Throughout the study period, the TAN concentration continued to increase, to a peak of 3.1–3.8 g L^{-1}, which represented urea addition of 0.54–0.68% (w/w), i.e. 170–220 kg added to the 32 m^3 black water. The highest TAN concentration after urea addition was measured in the material digested at a temperature reaching 41 °C. In the complementary laboratory study, the TAN concentration increased more slowly than in the full-scale study and by day 6 had reached 2.6–2.8 g L^{-1} for all start temperatures. From the initial TAN concentrations (1.0 ± 0.04 g L^{-1}) and the urea addition (2.3 g N L^{-1}), a higher TAN concentration was expected.

The reason for the TAN concentrations in the reactor being higher than expected from the targeted urea additions may have been the result of inaccurate estimates of urea

Table 2 | Temperature data for the three combined treatment runs. When not a single value, these are given as means (range in brackets) or as a specific temperature at a specific time

	Unit	Run 1	Run 2	Run 3
Period	Date	5 Dec–4 Jan	21 Feb–7 March	22 March–4 April
Ambient temperature	°C	1.2 (−8.4–10.4)	0.3 (−9.4–8.6)	4.8 (−4.9–14.9)
Start: reactor/ambient	°C	11/0.1	4/3.9	6.5/13.7
Temp. increase	°C day^{-1}	+5.73	+7.14	+5.70
Aeration max: reactor/ambient	°C	33.0/2.9	41.0/1.9	45.5/8.7
Aeration phase	Days	4.3	5.1	6.8
Temperature decline	°C day^{-1}	−0.65	−1.14	−1.46

addition/reactor contents, or of lysed microorganisms and easily degraded organic material releasing TAN during the treatment. In the laboratory study, the final nitrogen recovery from urea was, at most, 78%. The slower hydrolysis in the laboratory study may be due to the black water having a less active microflora than the black water used in reactor runs, as indicated by the *Enterococcus* spp. and total thermotolerant coliform bacteria concentrations of 1.7 $\log_{10}$ cfu mL^{-1} and 1.0 $\log_{10}$ cfu mL^{-1}, respectively.

pH

The pH for the triplicate samples from each of the reactor runs showed little deviation (CV $\leq$1.1%). The pH differed between the runs at the start (Table 1) and at the end of the aerobic digestion. During the aeration period, the pH increased by 0.6–1.0 pH units, from the initial 6.5–6.8 to 7.3–7.5, with a linear trend of a 0.10–0.14 pH-unit increase per day. The addition of urea resulted in the pH reaching almost 9.0 for the 33 and 45.5 °C digested material, whereas for the 41 °C digested material, a pH of 9.2 was reached on day 2, but by day 3 it was pH 9 ± 0.1, as for the other digestion temperatures. In the complementary laboratory study (pH 7.8 at the start), the pH reached 9 ± 0.1 on day 2–3 after urea addition, and maintained this pH throughout the study. The black water did not buffer against the pH change from the urea additions, as previously observed for more solid material such as faeces (Nordin *et al.* 2009b) and sewage sludge (Nordin *et al.* 2015). A complementary laboratory study showed that a pH of 9 can be achieved with a 0.3–0.4% urea addition to anaerobically digested black water (data not shown), indicating that a lower urea dose may be used to attain the same pH increase.

Formation of NH_3

The formation of NH_3, as a function of TAN, pH and temperature, increased with the temperature and the decomposition of the added urea. Before the addition of urea, the NH_3 concentration ranged from 0.1–2.6 mM (Figure 3(a)). The NH_3 concentration was expected to be higher with higher digestion temperature, as the urea addition was targeted to be the same for all treatments. However, due to the slightly higher pH and TAN concentrations in the 41 °C run, the NH_3 formed exceeded the concentration in the 45.5 °C run, with a maximum concentration of 166 and 134 mM, respectively (Figure 3(b)).

In the complementary study of *Ascaris suum* eggs in the laboratory, the concentration of NH_3 increased after the addition of urea due to increased TAN and pH. After 5 days, the NH_3 concentration reached its maximum value (105–116 mM), a concentration which remained quite stable for the start temperature of 45 °C (due to the continuously increasing TAN), whereas with 40 and 35 °C start temperatures, the concentrations declined to 87–88 mM by day 9 (Figure 4).

Ascaris egg inactivation

In general, the recommended lowest sanitisation temperature is 50–55 °C (WHO 2006), although lower temperatures may still cause inactivation (Elving *et al.* 2014). Of the organisms studied here, *Ascaris* spp. eggs are most sensitive to heat, with temperatures of 45 °C considered to be lethal and with total inactivation at such temperatures estimated to take 5 days (Bruce *et al.* 1990; Koné *et al.* 2007). At 50 °C, inactivation takes place within hours (Aitken *et al.* 2005). In an alkaline solution of pH 9, Pecson & Nelson (2005) found

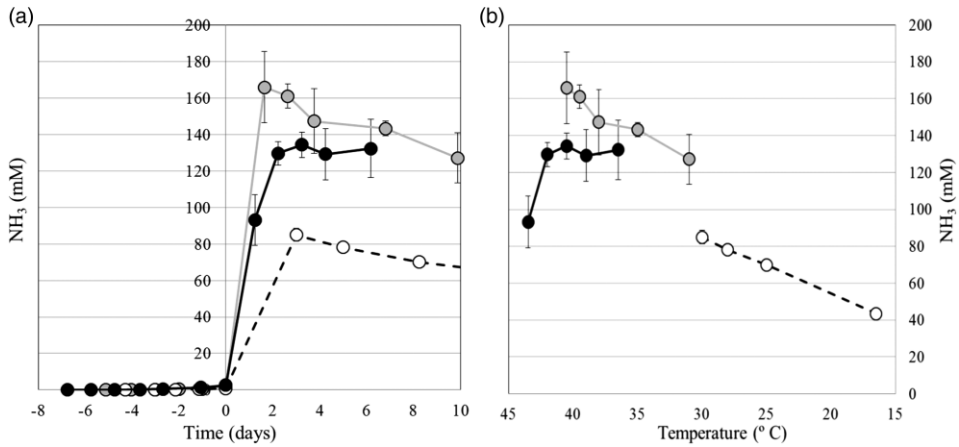

Figure 3 | (a) Ammonia concentrations during the aerobic digestion phase (negative time) and after the addition of 0.5% urea ww (day 0) to black water and (b) during the urea sanitisation phase, plotted against temperature in the reactor at the time of sampling, digested at temperatures reaching 33 (white/broken line), 41 (grey) and 45.5 °C (black). Error bars show the 95% confidence interval of the mean ($n = 3$), which for some means was too small to be visible.

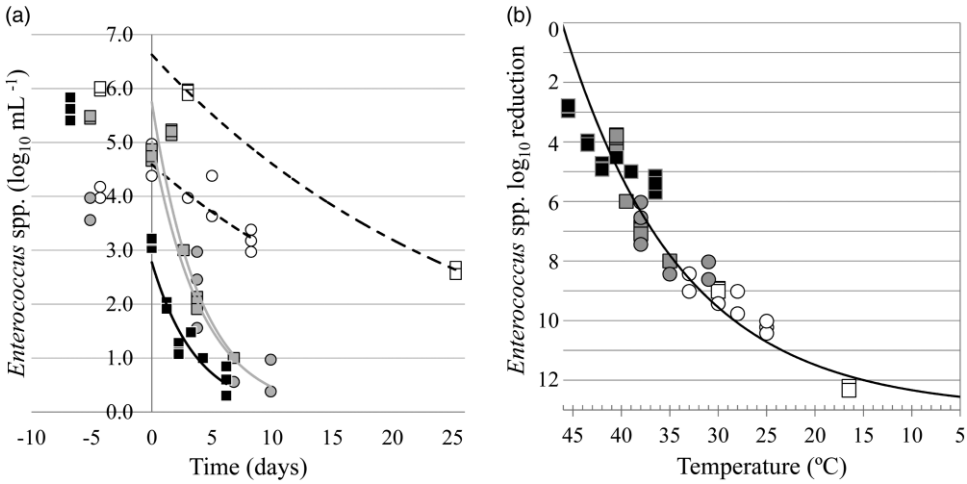

Figure 4 | (a) Concentrations of *Enterococcus* spp. during the aerobic digestion phase (negative time) and after the addition of 0.5% urea ww (at day 0) for black water digested at temperatures reaching 33 (white/broken line), 41 (grey) and 45.5 °C (black), enumerated on SlaBa agar (squares) and by the MPN method in Enterococcosel agar (circles) with the trends of inactivation depicted with exponential trend lines. (b) Summarised inactivation trend for *Enterococcus* spp. after adjustment to fit concentrations for the two detection media at overlapping temperatures after 0.5% ww urea treatment (starting at temperature 45.5 °C) and the fitted exponential trend line.

that 47 °C, but not 45–46 °C, gave 100% (2 $\log_{10}$) *Ascaris* egg inactivation in 24 hours, indicating the effect small differences in treatment temperature can have for egg inactivation. In the present full-scale study, the aerobic digestion phase with temperature increased to 41 °C gave a 35% (CI_{95} 22–48) reduction in egg viability, whereas for the two other aerobic digestion temperatures (33 and 45.5 °C) these samples were lost or destroyed in the reactor. The ammonia-free temperature controls in the laboratory study gave an 82% (CI_{95} 79–82%) reduction in egg viability in 7 days with a start temperature of 45 °C, whereas for start temperatures of 40 and 35 °C, the egg viability was stable (CI_{95} 94–101%) for the 11 days studied. Considering the declining temperature (1 °C day^{-1}), these results are consistent with the literature cited above.

For *Ascaris* egg inactivation after urea addition, the results from the laboratory study confirmed and refined the few results from the full-scale study. For the 33 °C full-scale run, the viability (of 100 eggs) was reduced by 57% (CI_{95} 40–70%) and 76% (CI_{95} 68–82%) after 5 and 8 days from urea addition, respectively. Taking into consideration the low precision in viability estimates, these were similar to values obtained in the laboratory study (Figure 5(b)). In the full-scale study with a start temperature of 33 °C, the

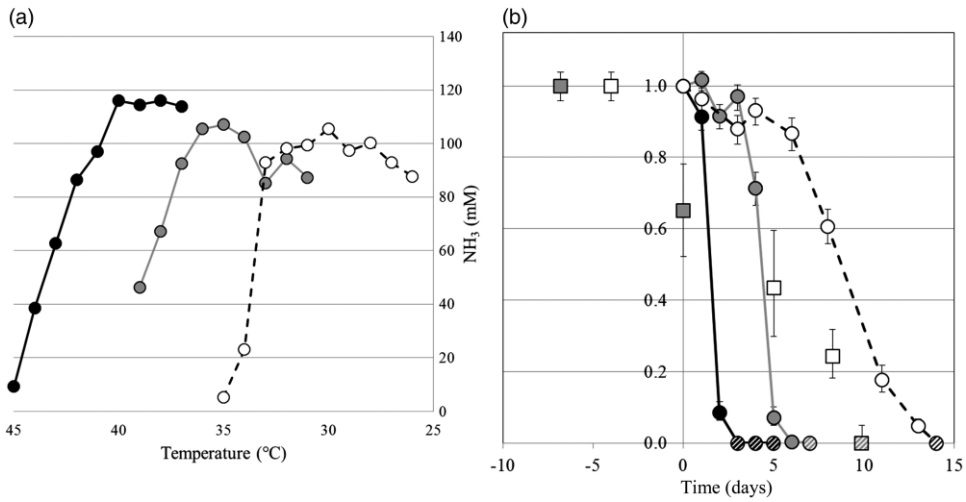

Figure 5 | (a) Ammonia concentrations in black water after the addition of 0.5% urea ww plotted against temperature, which started at 35 (white), 40 (grey) and 45 °C (black) and was set to decline by 1 °C day^{-1}. (b) *Ascaris suum* egg viability for the full-scale (squares) and laboratory study (circles) during composting (negative time) and after the addition of 0.5% urea (day 0) at temperatures 45.5/45 (black), 41/40 (grey) and 33/35 °C (white). Striped symbols indicate samples with no viable eggs (from 1,000).

point of total inactivation was not reached, but in the laboratory study, it was determined (1,000 eggs) to be 14 days from urea addition. With ammonia treatment at 41 °C, in the full-scale run, no viable eggs could be found (from 100 eggs) at the first sampling after 10 days of urea treatment. In the laboratory, with a higher sampling frequency, total inactivation (of 1,000 eggs) was achieved in 6 days (Figure 5(b)).

Studies of *Ascaris* egg inactivation in urine with similar pH and ammonia concentrations (100 mM NH_3) at 34 °C constant temperature have reported a 99% viability reduction in <7 days (Nordin *et al.* 2009a), a finding confirmed in the present study. At 45 °C, in the laboratory study, the *Ascaris* eggs were inactivated (1,000 eggs) within 3 days of urea addition, despite the fact that the urea was not fully hydrolysed and the NH_3 concentration was at most 86 mM during the period of the inactivation (Figure 5(a)). Both the EC ABP Regulation (EC 2009) and the revised Swedish proposal (SEPA 2013) suggest that treatments could be validated by a 3 $\log_{10}$ reduction in *Ascaris* eggs, aiming at a product free of viable helminth eggs, which is in accordance with US EPA Part 503 (1994) regarding sludge management and WHO Guidelines for the reuse of human excreta (2006). Such a degree of inactivation was observed in the laboratory study at all temperatures, within 2 weeks. Since the laboratory study only assessed the decrease in *Ascaris* egg viability after addition of urea, and did not take into account any reduction occurring during the composting phase (as observed in the full-scale study), the estimated time for a 3 $\log_{10}$ reduction could thus be considered conservative.

Bacterial inactivation

For all three treatment runs, *Salmonella* spp. were isolated from the start material and identified as *Salmonella enterica* subspecies 1 serovar Stanley (*S.* Stanley) (first run) and serovar Bareilly (*S.* Bareilly) (second and third runs). Enumeration indicated concentrations of 2–3.5 cfu per 10 mL of black water. *Salmonella* spp. was still detected after the aerobic digestion phase at all temperatures, but decreased to below the detection limit (1 cfu per 25 g wet material) at the first sampling after urea addition (2–3 days). In the raw black water, *E. coli* constituted approximately 88% of thermotolerant coliform bacteria (TTCs) with a concentration of 4–6 $\log_{10}$ mL^{-1}. The aerobic digestion phase reduced the TTCs by 0.4–1 $\log_{10}$, with a higher reduction after aerobic digestion at 45.5 °C compared with 31–41 °C. After the aerobic digestion phase, *E. coli* constituted 100% of TTCs. The small reduction in *Salmonella* spp and *E. coli* during the aerobic digestion phase shows their tolerance to temperatures up to 50 °C (Vinnerås *et al.* 2003a). At 41 and 45 °C, TTCs were reduced to below the detection limit (1 cfu mL^{-1}) at the first or second sampling after urea addition (2–3 days of ammonia treatment), whereas they were still detectable in low concentrations after 5 days of ammonia treatment starting at 33 °C.

Almost all regulations on land application of sewage sludge and other sewage fractions require the absence of *Salmonella*. spp. in the end product (US EPA 1994; EC 2000; WHO 2006; SEPA 2010, 2013). The present full-scale study proved that *Salmonella* spp. were absent in 3 × 25 g material (wet weight), which can be assumed to be representative results due to the homogeneity and mixing of the material. The limit for *E. coli* or TTCs (which mainly comprise *E. coli*) is generally set to $<10^3$ g^{-1} TS (US EPA 1994; SEPA 2010), or process efficiency is validated with a 5–6 $\log_{10}$ reduction (EC 2000, 2009; SEPA 2013). Nordin (2010) observed similar inactivation rates for *Salmonella* spp. and *E. coli* (O157:H7) by ammonia sanitisation in laboratory studies. In the present study, the two bacteria were not detected 2–3 days after urea addition at 41 and 45.5 °C. Due to the high proportion of *E. coli* in relation to *Salmonella* spp. in black water, *E. coli* can be used as an indicator organism to estimate the *Salmonella* spp. inactivation effect of combined aerobic digestion and urea treatment.

The two methods for enumerating *Enterococcus* spp., when used in parallel, gave deviating concentration counts, with approximately 2 $\log_{10}$ lower concentrations for the enterococcosel MPN method, but fairly similar inactivation patterns (Figure 4(a)). *Enterococcus* spp. tolerates high pH (Berghe *et al.* 2006) and salt concentrations, but appears to be more sensitive to heat than *Salmonella.* spp. and *E. coli* (Nordin 2010). The start concentrations for the two enumeration methods, of approximately 4 $\log_{10}$ cfu mL^{-1} and 6 $\log_{10}$ cfu mL^{-1}, respectively, were reduced by 2 $\log_{10}$ at aerobic digestion to 45.5 °C, whereas they were not affected by digestion at 41 °C and below.

In the present study, the inactivation of *Enterococcus* spp. at 33 °C was slow and as the reactor contents became cooler, the rate of inactivation decreased over time (Figure 4(a)). With a temperature of 41 and 45.5 °C, an ammonia sanitisation phase of approximately 1 week gave a 5 $\log_{10}$ reduction in *Enterococcus* spp. (Figure 4(a)). However, the effect of the higher temperature of 45.5 °C was counteracted by the higher NH_3 concentrations in the 41 °C treatment (Figure 4). If a 5 $\log_{10}$ reduction in *Enterococcus* is used as a measure of sanitisation for the combined aerobic digestion and ammonia treatment, as required for validation of treatments for animal manure (EC 2009), a corresponding

3 $\log_{10}$ reduction of *Ascaris* eggs can be expected. Moreover, due to the higher sensitivity of *E. coli* and other *Enterobacteriaceae* to ammonia, great margins for the inactivation of, for example, *Salmonella* spp. can be achieved.

Bacteriophage inactivation

Both somatic coliphages and f-RNA phages were present in the untreated black water at concentrations of 3–5 $\log_{10}$ mL^{-1}. The coliphages survived the combined aerobic digestion and urea treatment at all start temperatures, but the f-RNA phages were sensitive to aerobic digestion up to 41 °C and above, which reduced them to below the detection limit (1 cfu mL^{-1}). Aerobic digestion at temperatures up to 33 °C did not affect the f-RNA phage concentrations, while they were undetected at the first sampling 3 days after urea addition at 33 °C. When naturally occurring phages have been analysed, f-RNA phages were considerably more sensitive to ammonia treatment than somatic coliphages (Nordin *et al.* 2015), in accordance with to the results in this study. This does however, contradict laboratory studies in which the two phage groups have been represented by single phages MS2 and Phi-X, where it has been found that the inactivation of the two phages was in the same range, or even that the f-RNA phage MS2 was more persistent (Vinnerås *et al.* 2008). In comparison with animal viruses, the MS2 and Phi-X phage inactivation by ammonia was found to be slower (Fidjeland 2015). To confirm that this relation to viruses is also valid for naturally occurring bacteriophages, further studies are needed.

CONCLUSIONS

This study showed that temperatures up to 45.5 °C could be reached when aerobically digesting black water with TS content <1%. On reaching 41 °C and above during the aerobic digestion phase, combined ATAD and ammonia treatment (0.5% ww urea addition) gave a 5 $\log_{10}$ reduction in *Enterococcus* spp. with 2 weeks of combined treatment. This occurred simultaneously with a high reduction in *E. coli* and *Salmonella.* spp., and a 3 $\log_{10}$ reduction in *Ascaris suum* egg viability.

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