Decentralised Black water Treatment with Combined Auto-thermal Aerobic Digestion and Ammonia – a Pilot Study Optimising Treatment Capacity

A. Nordin, D. Göttert and B. Vinnerås

Department of Energy and Technology, Swedish University of Agricultural Sciences, P.O. Box 70 30, SE-750 07, Uppsala.
(E-mail: *annika.c.nordin@slu.se*)

Abstract
Part heating of black water was combined with addition of 1% w/w urea and pathogen and indicator organism followed over 21 days. After initial mixing the 160 m^3 black water was left undisturbed. First after 14 days could the urea be confirmed fully degraded into ammonia (5.1 g N L^{-1}) whereas the pH stabilised around 9.2 after 1 week. The initial temperature of 17 ºC declined by 6 ºC during the study. *E. coli* and *Salmonella* spp. that are sensitive to ammonia was inactivated during the first days of the study despite that urea was only partly hydrolysed. Also f-RNA bacteriophages were undetected at day 14. Somatic coliphages, *Enterococcus* spp. and *Ascaris* eggs showed significant but slow inactivation. The treatment showed efficient for Salmonella which is a target pathogen in a Swedish context but for parasite egg inactivation a higher temperature is needed. The treatment would benefit from a more frequent stirring to speed up the hydrolysis of urea and thus the treatment efficiency.

Keywords
ammonia; auto-thermal aerobic digestion; black water; pathogen; urea; sanitisation

BACKGROUND

Collection and recycling of nutrients from human excreta to agricultural land would decrease environmental pollution from on-site sanitation systems and would reduce the use of fossil-based chemical fertilisers (Lundin et al., 1999; Winker et al., 2009). 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. Black water potentially contain pathogens and thus sanitising treatment is a requirement for a safe nutrient reuse.

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 (SEPA) proposed regulations in 2010 and revised these in 2013 (SEPA, 2013; SEPA, 2010). The proposals for regulation include assessment of the pathogen reduction by threshold levels as well as set reduction requirements of *Enterococcus*, *E. 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. In the meanwhile, source separated black water from households can be certified according to a voluntary certification, SPCR 178, which regulate traceability and product quality including hygienic quality (SP, 2012).

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., 2009; Ottoson et al., 2008; Pecson et al., 2007). Adding ammonia in the form of urea is favourable for working conditions and workers safety, compared with use of aqueous ammonia.

To sanitise black water with low dry matter a combination of auto-thermal aerobic digestion (ATAD) to 40°C and subsequent ammonia treatment by adding 0.5% urea to the ATAD reactors was developed (patent WO 2012/115589 A1, (Nordin & Vinneras, 2015) and is since 2012 applied in the Hölö plant, located south of Stockholm, Sweden (Fig. 1). Ammonia sanitsation is enhanced by temperature, especially the inactivation of parasite eggs (Fidjeland et al., 2015). 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 was eliminated. However, the total retention time for the treatment in the combined ATAD and ammonia reactors are two weeks which becomes a bottle neck for the system capacity. The present study investigated optimisation of the treatment capacity by heating only part (60 m^3 of 160 m^3 treated) of incoming black water and treat at a higher urea addition (1% w/w) in one of the collection wells (Fig. 1: C) instead of in the reactors (Fig.1: R). The main focus was the sanitising effect on pathogens (*Salmonella* spp. and *Ascaris suum* eggs) and indicator organisms (*Escherichia coli*, *Enterococcus* spp. and bacteriophages), with the aim to enable the production of safe fertiliser products from source separated toilet waste.

Figure 1. *The treatment plant sanitising black water with a combination of auto-thermal aerobic digestion and ammonia treatment, showing the two 32 m^3 ATAD reactors (R) (built in), the two below surface, insulated 200 m^3 collection basins (C) and the covered, above ground 1500 m^3 covered post-treatment storage basin (S) (www.telge.se/vatten-avlopp/kretsloppsanlaggning).*

METHODS

The study took place from the middle of March 2016 until the end of May 2016 studying two separate batch runs. Source-separated black water was collected from sealed septic tanks from on-site sanitation systems, part of them using vacuum toilets. Both reactors was used to aerate and heat 2*30 m^3 black water to ≥40 ºC which took 10 days. The heated black water was mixed for 1 hour with 100 m^3 raw black water and 1600 kg urea (1% w/w) (Yara, Sweden) and retained in one of the collection basins. Temperature was monitored with Tiny tag Aquatic 2 probes (Intab, Sweden) at three depths, surface (0 m) middle (1.6) and bottom (3.2 m deep). *Ascaris suum* eggs from sieved swine feces (Excelsior Sentinel, Inc, USA), where added to the blackwater in permeable nylon bags

(mesh 28 microns) (Bigman AB, Sefar) which were held in 1 L outer containers (0.7 mm mesh), at the same depths as the temperature probes.

At start and end the black water was analysed for total solids (105 °C, 24 h) and volatile solids (550 °C, 4 h). During the study triplicate samples of 100 mL was taken at each depth and at sampling of ascaris eggs, the egg containing nylon bags was removed and kept in 0.1 N sulphuric acid during the cold transport to the laboratory. At the laboratory the ascaris egg bags were incubated at 28 °C in 0.1 N sulphuric acid for 30 days to allow larval development (Arene, 1986). Viability counts was 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%). The pH was analysed with a glass electrode PHC 2011 (Radiometer Analytic, France) connected to a PHM 210 meter (MeterLab, Denmark) in undiluted samples allowed to adjust to room temperature (23 ºC). For total nitrogen analysis samples were digested with oxidizing agents (Spectroquant® 114963; Merck, Germany) in a thermoreactor (TR 420 and TR 320; Merck, Germany) followed by analysis of nitrate formed (Spectroquant® 109713; Merck, Germany). Total ammonia nitrogen analyses (Spectroquant® 100683; Merck, Germany) were performed on filtered black water (Filtropur 45 µm, Sarstedt AG & Co, Sweden). Colorimetric readings were performed in a Spectroquant® NOVA 60 (Merck, Germany).

Salmonella spp. was analysed by pre-enriching 50 mL black water 10-fold diluted in Buffered Peptone Water (BPW) (18 h at 37±1 ºC) followed by selective enrichment on Modified Semi solid Rappaport Vassiliadis (MSRV) agar supplemented with 1.0% Novobiocin (41.5±0.5 °C, 24+24 h). Suspect *Salmonella* spp. growth was further investigated by Xylose Lysine Deoxycholate agar (XLD) with 1.5% Novobiocin, triple sugar iron agar tubes and urease broth.

For enumeration 10-fold dilution series of samples with buffered NaCl peptone water with surfactant Tween (pH 7) (SVA, Sweden) was performed. Chromocult Coliform agar (Merck, Germany), at low dilutions added *E. coli*/Coliform Selective-Supplement, was used for total coliform analysis specifying the fraction of *E. coli*. *Enterococcus* spp. was detected on Slanetz-Bartley (SlaBa) agar (Oxoid) (48±2 h at 41.5±0.5 °C). F-specific RNA phages and somatic coliphages were detected by standards ISO10705-1:1995 and 10705-2:2000, using the double-layer agar method (17-24 h at 37±1°C,) with *Salmonella* Typhimirium WG49 (ATCC® 700730™) and *E. coli* 13706 (ATCC® 13706™) used as bacterial host strains for the respective bacteriophages. Samples for phage analysis were filtered (0.45µm) to reduce the potential of bacterial growth. Phage ϕx174 (ATCC® 13706-B1™) and f-RNA phage MS2 (ATCC® 15597-B1™) was used as positive phage controls. 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., US), with $\alpha \leq 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Characterisation of black water

The start sample of the first run deviated by having higher TS than for round two and both end samples (Tab. 1) and was observed darker and more turbid. For this sample the volatile solids constituted a larger proportion of the TS. Considering this sample an outlier due to unrepresentative

sampling, the TS of the black water, 0.26-37% (Tab. 1), was lower than reported in black water from low flush systems evaluated by Norin et al. (1996) and Palm and Malmén (2003) (TS 0.75 and 0.4%, respectively) and also the VS was constituting less of the TS than the Swedish design value of 74% (Jönsson et al., 2005), and to the 71% reported by Norin et al. (1996). Considering the design values the TS would correspond to a flush volume of 17-18 L per person and day whereas the total nitrogen concentration of 0.7-0.8 g L^{-1} corresponds to 14-15 L per person and day. Apart from the TS and VS the composition of the black water did not differ between the two rounds other than for the enumerated microorganisms which were consistently higher in the second round (Tab. 1).

The part of the black water that was composted to 40 °C had concentrations of *Enterococcus* spp. reduced with 0.2-0.6 $\log_{10}$ and coliphages with 0.8-1.2 $\log_{10}$ but after mixing with the raw black water the final concentration in the mix did not differ from the initial concentrations in the raw black water (Tab. 1). The aerobic digestion of the black water for 10 days led to a pH increase (from 7.8-7.9 to 8.5-8.9) and subsequently a higher pH in the mix compared to the raw black water.

Table 1. Characterisation of the incoming black water given as mean ± S.D with significance between runs marked with an asterisk

Parameter	Unit	Run 1	Run 2
TS_{start*}	% ww	0.69±0.15	0.37±0.040
TS_{end}	% ww	0.35±0.070	0.26±0.037
VS_{start*}	% of TS	82±2.7	55±5.4
VS_{end}	% of TS	53±25	52±6.60
pH	-	7.8±0.13	7.9±0.025
TAN	g L^{-1}	0.52±.010	0.51±.012
Tot-N	g L^{-1}	0.69±0.066	0.82±.040
Salmonella spp.	per 50 g wet weight	Positive	Positive
Enterococcus spp.*	$\log_{10}$ cfu mL^{-1}	3.9±0.012	4.5±0.046
*E. coli**	$\log_{10}$ cfu mL^{-1}	2.6±0.14	3.0±0.00
coliphages*	$\log_{10}$ pfu mL^{-1}	3.8±0.030	4.4±0.061
f-RNA phages	$\log_{10}$ pfu mL^{-1}	-	2.0±0.058

Treatment parameters

After the addition of urea and undisturbed containment in the collection basin a stratification over the depths was expected but not observed except for the temperature. For some of the investigated parameters and sampling occasions there were significant difference between the depths but not following any consistent trend, thus the data was used and shown without differentiating upon depths.

Adding urea to the black water increased the total nitrogen (tot-N) concentration more than 6-fold and was for most measurements exceeding the 5.3-5.4 g N L^{-1} expected from the addition and intrinsic nitrogen. The addition of urea to the black water is expected to cause an increase in the pH both through urea itself, which is alkaline, and through the degradation of the urea into ammonia and carbonate, both of which cause microbial inactivation (Diez-Gonzalez et al., 2000; Fidjeland, 2015). The degradation of urea is dependent on a variety of factors, including the temperature, the

initial pH, the TS, and the amount of urea added to the treated material. The degradation of urea into total ammonia nitrogen (TAN) showed linear trend and first at day 14-15 was the theoretical concentration of 5.1 g TAN L^{-1} reached indicating full hydrolysis of urea (Fig. 2a). The addition of urea instantly increased the pH and after day 4 the pH stabilized around 9.2. The pre-heating of 60 m^3 black water to 40 ºC increased the temperature in the final mix to 17.6 ºC. The temperature showed a constant decline and had at day 21 decreased to 11-12 ºC. The temperature were highest at the middle and approximately 0.5 and 1.5 ºC lower for the surface and the bottom, respectively. This temperature difference was established at day 5 and remained throughout the study (Fig. 2b).

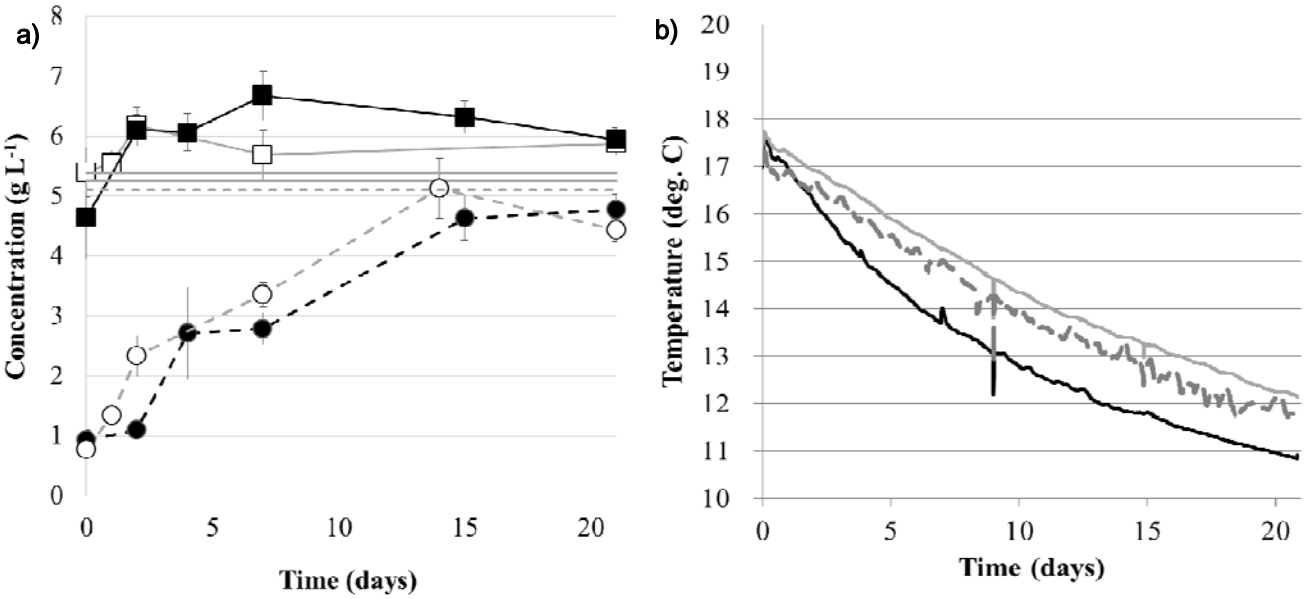

Figure 2a) Total Nitrogen (■/□) and total ammonia nitrogen (○/●) concentrations for run 1(black) and run 2 (white) showing the average± 95% CI (n=9). The shaded band show the theoretical concentration of total nitrogen (dark grey) and total ammonia nitrogen (light grey band) expected from the addition of urea and the average initial concentrations. **b**) The temperature during the 21 days at the bottom (black line), middle (grey line) and surface (broken line).

Microorganism inactivation

In both runs *Salmonella* spp. was isolated from the black water though, since not all start samples was positive, probably in concentrations close to the detection limit of 1 per 50 mL. In run 1 salmonella was no longer detected at day 4, and for set 2 at day 2. The difference in time to reach detection limit can be a result of different start concentrations and may thus not be a true difference since initial enumeration not was performed. A study by Vinnerås et al. (2008) tested the inactivation of *Salmonella* Typhimurium in undiluted urine, which had a similar ammonia concentration to the blackwater in this study. At 14°C they had a decimal reduction time of <1.2 ± 5 days. This range is similar to the 2-4 day reduction time needed in this study. For coliform bacteria, including *E. coli*, both sets reached the detection limit (1 cfu mL^{-1}) within one week. *E. coli,* which initially constituted about 50-55% (2.6-3.0 log_{10}) of the total coliform bacteria, was inactivated faster that the coliforms in general as observed also by Fidjeland et al. (2015). The voluntary certification that the plant complies with require, similar to SEPA 2010 proposal, an end product concentration of <1000 *E. coli* g^{-1} TS (SP, 2012) which could be confirmed. The latest proposal to a regulation from SEPA has a lower limit for end concertation of <100 *E. coli* g^{-1} wet weight.

The *Enterococcus* spp. inactivation (Fig. 3c) was slow with around a 2 log_{10} reduction over the 21

days and did not meet the SPCR 178 requirement of <1000 cfu g^{-1} TS (SP, 2012) also proposed by SEPA in 2010. However, the fraction of sub species consisting of *Enterococcus faecalis* was inactivated faster and decreased from 70 to 5% as observed also by Fidjeland et al. (2013a). The use of *Enterococcus* spp. as an indicator for ammonia sanitisation may lead to overestimation of the risk since they are more persistent than gram negative bacteria (e.g. *Salmonella* spp. and *E. coli*), especially at low temperatures (Fidjeland et al., 2015b; Vinnerås et al., 2008; Allievi et al., 1994). The SEPA 2013 proposal have omitted to regulate *Enterococcus* spp. for that reason (SEPA, 2013).

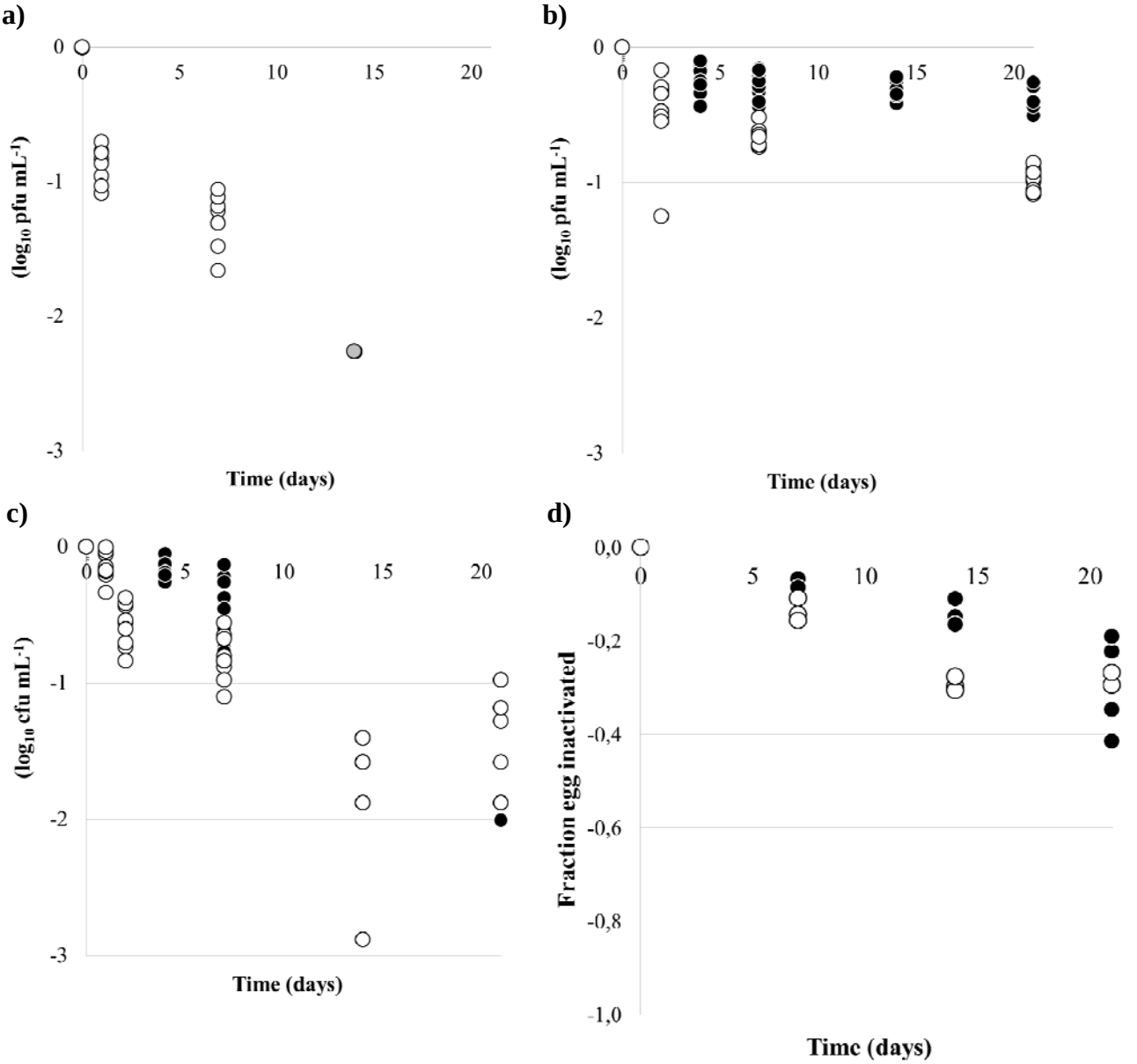

Figure 3a). Log_{10} inactivation of **a)** f-RNA phages, **b)** somatic coliphages, **c)** *Enterococcus* spp., and **d)** fraction inactivated *Ascaris suum* eggs, over the 21 days of study for run 1(black) and run 2 (white) showing data for all depths. Grey symbols show when the detection limit were met.

The 2013 SEPA proposal (SwedishEPA, 2013; SP, 2012) suggest a 3 log_{10} reduction of viruses for validation of alternate treatments. F-RNA bacteriophages, which are more sensitive to inactivation than somatic coliphages, has been found a good indicator for estimating enterovirus inactivation

(Vinnerås et al., 2008; Cramer et al., 1983). F-RNA bacteriophages (only studied in set 2 due to methodological problems), were inactivated to below the detection limit (1 pfu mL^{-1}) by day 14 (Fig. 3a). Estimating inactivation to be linear, a 3 $\log_{10}$ reduction would be met in 21 days in contrast to the more persistent somatic coliphages which were reduced less than 1 $\log_{10}$ in 21 days (Fig. 3b).

During the 21 days a small but significant inactivation of ascaris egg viability was observed (Fig. 3d). The inactivation of ascaris eggs for alkaline and ammonia-based treatment have in repeated studies show to be comprised of a lag phase followed by first order inactivation (Fidjeland et al., 2013b; Nordin et al., 2013; Nordin et al., 2009a; Ghiglietti et al., 1997 pecson) and can be modelled according to Harm. Fidjeland et al (2015) presented a lag-model for ascaris inactivation in relation to ammonia, pH, temperature and TS that can be used to estimate the time for egg inactivation. Using the model presented by Fidjeland et al using an average temperature of 14 deg C a 3 $\log_{10}$ reduction of egg viability was estimated to take 164 days whereas applying the Harm model the same inactivation was estimated to take 200 days. In order to decrease the inactivation time of the ascaris eggs by the studied treatment either a greater concentration of urea needs to be added and/or the temperature of the blackwater has to be increased. However, the SEPA 2010 plant regulations do not require an inactivation of parasite eggs since not prevalent in Sweden.

For the formation of NH_3 the temperature and pH was at its most beneficial combination day 5 to 7 with the fraction present as NH3 30-35% of total ammonia nitrogen. However, the slow hydrolysis counteracted the high temperature and pH and the highest actual concentration NH_3 was during day 14-21, reaching 80-110 mM, when urea was fully degraded. Despite that more frequent stirring not is necessary for preventing any stratification over the depths, the hydrolysis of urea into ammonia would most likely take place faster with a frequent mixin, at least initially, and then the temperature from the pre-heating would be used more efficiently for pathogen inactivation.

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