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Optimised Ammonia Sanitisation of Sewage Sludge

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Abstract

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The usage of sewage sludge as an organic fertiliser offers a way to recycle nutrients and organic matter for a sustainable agriculture. Sewage sludge is rich in plant nutrient like nitrogen, phosphorus, potassium and micronutrients. Concerning the depletion of phosphorus resources, the reuse of sewage sludge can contribute in reducing the production of artificial fertiliser and satisfy agricultural nitrogen and phosphorus demands. Heavy metals and organic contaminants contained in sewage sludge hampered the image of sewage sludge application in agriculture, although reduced since the last decades. Another concern is pathogens in the sludge, posing a risk on human health and the food chain when applied to land. Pathogens in sewage sludge have not been considered as limiting for the use as heavy metals, thus regulations on pathogens putting less restrictions. This has changed over the last years, especially in the EU member states, lowering pathogen limits for sewage sludge (biosolids) when applied to land. Therefore, proper sanitisation of sewage sludge is required and the need for cost-effective, simple and quick treatment methods for sludge stabilisation increased. In this study, an optimum ammonia treatment for sanitising sewage sludge was attempted. Pathogen inactivation was monitored by using indicator and model organisms. The small-scale experiment comprised two phases, an aerobic composting phase (<40°C) and a subsequent anaerobic chemical treatment phase using urea solely or combined with carbonate or ECOX (sodium percarbonate). Urea addition releases ammonia, which has shown to effectively reduce pathogens. This antimicrobial effect is combined with the biological heat development in compost, also reducing pathogens. All tested chemical treatments reached a 5 $\log_{10}$ reduction for total thermotolerant coliforms and an 8 $\log_{10}$ reduction for *Salmonella* spp. within the two weeks experiment, with the best effect using 1.5% urea. Somatic coliphages reached a 1-2 $\log_{10}$ reduction in treatments and control. The treatments had no significant effect on enterococci and only a 1 $\log_{10}$ reduction was achieved. The viability of *Ascaris suum* eggs was reduced in all treatments, but without consistency. No viable eggs were detected with 1.5% urea and 1.5% urea+3% $CaCO_3$, when temperature was maintained over 35°C during four consecutive days. All chemical treatments used in this study increased the pH to around 9. The highest values were reached with 1% urea +2% ECOX. Considering treatment efficiency and cost-effectiveness, the best sanitisation result in this study was achieved with 1.5% urea.

Keywords: ammonia, urea, sanitisation, compost, sewage sludge, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella* Typhimurium, *Ascaris suum,* somatic coliphages

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Contents

Abbreviations

Ascaris	*Ascaris suum*
CFU	Colony-forming units
DM	dry matter
D_R	decimal reduction time
E. coli	*Escherichia coli*
E. faecalis/enterococci	*Enterococcus faecalis*
ECOX	Sodium percarbonate
N-TOT	total Nitrogen
$N\text{-}NH_4^+$	Ammonium Nitrogen
NH_3	Ammonia
PFU	Plaque-forming units
Salmonella Typh.	*Salmonella enterica serovar* Typhimurium
Salmonella	*Salmonella* spp.
TTC	Total Thermotolerant Coliform bacteria
TS	Total Solids
VS	Volatile Solids
WW	wet weight

1. INTRODUCTION

The striving for sustainability has shaped the society though the last decades, but is still in development concerning its concepts for environment and agriculture. One major aspect of the sustainability principle is to establish closed cycles for waste management where nutrient recycling presents an effective way of closing the loop between waste production and agriculture. Between 2003 and 2006 about ten million tons of dry matter sewage sludge were produced within the EU and 37 % of it was recycled in agriculture (EC, 2010b). Regarding the EU Waste Framework Directive (Directive 2008/98/EC) and its waste management hierarchy, the EC clearly states that greater priority is given to recycling compared to energy recovery like incineration, with disposal only as last option. For nutrient recycling, proper treatment of recycled material is needed to provide a safe reuse and to protect the food chain and thus also human health.

Sewage sludge from treatment plants is commonly used as fertiliser but needs an appropriate preparation to exclude harmful substances from ending up in the environment. Simplified and cost-effective treatment methods for sludge stabilisation are needed to extend the reuse as fertiliser instead of producing mineral fertiliser in a world facing future nutrient shortages. Composting is a way of sanitising organic material from pathogens, by increasing temperature with the intrinsic energy content, and to transform it to a suitable fertilizer. Furthermore, the addition of urea with its antimicrobial effect can speed up the sanitisation for sewage sludge composting and increases the fertiliser value concerning nitrogen.

Objective

In this thesis, the optimization of sewage sludge sanitization with ammonia (urea) combined with composting and other supporting chemicals were attempted. The aim of this study was to reduce the time of sanitisation by increased pH, temperature and especially ammonia concentration. All pathogenic indicators and model organism should be notably reduced within a maximum of two weeks. Especially a 3-4 $\log_{10}$ reduction of enterococci should be reached within this period. The amount of chemicals should be in a cost-effective range for large scale-treatment. The end product should be a safe fertiliser with increased nitrogen value.

Hypothesis

One day composting of sewage sludge up to 40°C prior to urea treatment enhances the urea effect by increased temperature. ECOX (Sodium percarbonate) or calcium carbonate combined with urea give an additional increase in pH of sewage sludge within the urea treatment. The combination of composting and urea treatment reduces enterococci, total thermotolerant coliform bacteria, salmonella, bacteriophages and ascaris eggs to the detection limit within two weeks. Urea applied after composting under anaerobic conditions minimizes ammonia losses via volatilization.

Research questions

1. For composting the sewage sludge, 40 °C should be reached before the chemical treatment. How long does 5 kg sewage sludge need to be composted to reach 40°C and how long can this temperature be maintained, when sealing 500 g of the sewage sludge with the chemical amendments in a 1 L vessel?

2. Concerning the sanitisation, are all indicator and model organisms reduced to the detection limit within two weeks or earlier?

3. Are there differences between the chemical treatments for pathogen reduction?

4. Considering cost-effectiveness, which chemical treatment gives the best organism reduction?

5. Sodiumpercarbonate (ECOX) and calcium carbonate ($CaCO_3$) are combined with urea to increase pH. $CaCO_3$ is tested as a cheaper solution ECOX. How do they differ in increasing the pH?

6. Does ECOX have a higher effect on organism reduction, pH increase or temperature development compared to carbonate?

7. Do carbonate or ECOX increase the urea treatment efficiency?

8. Will there be any nitrogen (ammonia) losses from the sewage sludge within the two weeks experiment?

These questions were answered within the experiment and result evaluation.

2. BACKGROUND

2.1. Sewage Sludge

Waste and water treatment plays an essential role for the environment and human health. One issue of major concern is how to deal with the insoluble residues from waste water treatment. This sewage sludge "contains organic compounds, nutrients, heavy metals, organic micro pollutants and pathogens" (EC, 2010b), but the concentration in which they occur in sewage sludge depends on the treatment method and physical-chemical processes the sludge went through (EC, 2014). Wastewater purification processes clean the water and thereby concentrate these unwanted contaminants in the sludge (USEPA, 2003).

Heavy metals accumulating in soils applied sewage sludge casted a negative light on recycling of sludge through the last decades. However, there has been a steady decline of the heavy metal concentration in sewage sludge through the EU since the 1980´s, mainly due to improved practices evolving from new regulations (EC, 2010b). Based on the EU Sludge Directive 86/278/EEC from 1986, the EU set limits on heavy metals in sludge for agricultural use (Article 5.2 (a) and Annex 1B): cadmium 20-40 mg/kg per dry matter, 1000-1750 mg/kg for copper, nickel 300-400 mg/kg, 750-1200 mg/kg lead, zinc 2500-4000 mg/kg, mercury 16-25 mg/kg and no limit set for chromium whereas for example Sweden has 1500 mg/kg, Germany 900 mg/kg and Denmark a limit of 100 mg/kg per dry matter (IEEP, 2009). The member states have implemented the regulations in their national limits, often with stricter limits.

Organic micro-pollutants in sewage sludge, such as AOX (absorbable organic halogen), LASs (linear alkylbenzene sulphonates), NP/NPEs (nonylphenols and nonylphenols ethoxylates), PAHs (polycyclic aromatic hydrocarbons), PCBs (polychlorinated biphenyls), PCDD/Fs (polychlorinated dibenzo-p-dioxins and dibenzo-p-furans) are still contentiously discussed concerning possible effects on environment and appropriate limit values for agricultural use (Smith, 2009). There are no default limit values by the EU Sludge Directive. In the revision process of the Directive, various options and possible limit values are proposed and discussed, for example for PAHs (6 mg/kg dm) and PCBs (0,8mg/kg dm) in sludge (EC, 2010a). Some member states set limits on organic micro-pollutants, yet without consistency: Germany regulates PCBs and PCDD/Fs but not PAHs, while France put a limit on PAHs and PCBs but not PCDD/Fs (EC, 2010a). Other countries, like the UK, U.S. and Canada do not have any limit for organic contaminants in sludge, arguing that they will not pose a risk on human health and environment when applied to soil. Wastewater treatment and sludge treatment, such as anaerobic digestion or composting reduce a major part of organic contaminants, estimated to 20-40%, by microbial biodegradation (Smith, 2009). Additionally, after application compounds are lost via volatilization, fixed due to soil adsorption (persistent contaminants), or further degraded in soil. The EC Joint Research Center stated in 2001: "Organic contaminants in sludge are not expected to pose major health problems to the human population when sludge is re-used for agricultural purposes. In comparison, metal contamination of sludge is much more important with respect to human health"(Prüeß and Erhardt, 2001).

As regards to pathogens in sewage sludge, they are not all removed or inactivated within the waste water cleaning process (Chauret et al., 1999). The majority of microorganisms in

sludge emerge from human feces, giving importance to the general sanitisation standard of a region (EC, 2001a). Hence, the pathogenic contamination of sewage sludge can be expected higher in developing countries with a low sanitation level and higher numbers of infected people excreting the pathogens with their feces. Pathogens are dealt with in more detail in the following chapter 2.2.

When sludge is treated and fulfills requirements for pathogens and other pollutants, it is suitable as fertiliser and in the U.S. termed as "Biosolids" to imply its quality (USEPA, 2003). For this reason pathogenic inactivation plays an important role in sewage sludge treatment and different approaches are currently applied for sewage sludge quality assessment. The USEPA regulation divides biosolids into class A and B to give guidance about land application based on treatment and pathogen and indicator concentrations. Class A biosolids demand a high pathogen reduction for public sale and land application without site restriction: The limit for feacal coliform bacteria in sewage sludge is < 1000 g^{-1} TS and for helminth eggs less than 1 egg in 4 g TS (USEPA, 1994). This class is considered as "pathogen-free" and thus minimizes the risk for human and animal health and the food chain. Class B biosolids are less restricted regarding pathogen inactivation and may still contain a certain amount of pathogenic organisms: Helminth eggs do not have to be reduced and faecal coliform bacteria in the product should be $<2^6$ CFU/g TS or a $2\log_{10}$ reduction during the process has to be achieved. For this reason, class B requires restrictions on usage and land application to decrease the risk to human health. A further pathogen inactivation of class B biosolids can then be provided by exposure to the environment over time, which should be longer then the potential survival of the microorganism in the soil. Based on this, subsequent inactivation after land application, crop harvest and land access for humans and grazing animals is restricted for an appropriate time period when applying Class B biosolids to a field (USEPA, 2003). Recommendations are given by the European Commission (Directive 86/278/EEC) about the usage of treated biosolids in agriculture with supplementary specification in the different member states. Regarding heavy metals the EU set lower limits than the US, while no pathogen limits for biosolids as end product are defined by the European Commission compared to the US regulations. The EC demands treatment of sludge prior to land application, and set restrictions on certain crops. Yet, the EC allows using untreated sludge "if it is injected or worked into the soil" (EC, 2010b). Explicit pathogen limits are left to the member states. In comparison, for manure and class 3 animal byproducts, the EC Animal By-product regulation (2009) requests a 5 $\log_{10}$ reduction for salmonella and *Enterococcus faecalis*, as well as a 3 $\log_{10}$ reduction of *Ascaris* spp. during the treatment process, while the product should be free from salmonella in 25 g wet weight. In Sweden, the SEPA did in 2010 present a proposal for regulation of class A sewage sludge for land application with <1000 *E. coli* and *Enterococcus* g^{-1} TS, and further that no salmonella should be detected in 25 g wet weight sludge.

Regarding the land application, sewage sludge represents a valuable nutrient resource (Table 1), containing a high proportion nitrogen, phosphorus, potassium (Arthurson, 2008), as well as micronutrients. Organic matter contained in the sludge improves soil physical properties like bulk density, porosity and water holding capacity (Singh and Agrawal, 2008). When used as organic fertiliser, sewage sludge can reduce the need for mineral fertiliser and therefore may play an important role in countering nutrient depletion. This recycling route returns the nutrients, deprived via crop production, back to the soil and thus closes part of the nutrient loop (Lepeuple et al., 2004).

Table 1 Chemical composition of digested sewage sludge (Fytili and Zabaniotou, 2008)

Parameters	Digested sludge	
	Range	Typical
Total dry solids (TS), %	6.0-12.0	10.0
Volatile solids (% of TS)	30-60	40
Nitrogen (N, % of TS)	1.6-6.0	3.0
Phosphorus (P_2O_5, % of TS)	1.5-4	2.5
Potassium (K_2O, % of TS)	0.0-3.0	1.0

Sewage sludge mainly undergoes three different options, disposal on landfills, incineration or agricultural recycling (De Brouwere, 2006). Economically, agricultural reuse of sewage sludge presents the cheapest solution. Moreover, for environmental reasons recycling of sewage sludge is the most sustainable way when adequately implemented.

2.2. Pathogen Risk and Indicators

Pathogenic microorganisms in biosolids

Assessing the microbial risk from land application of sewage sludge, a variety of human pathogenic microorganisms has to be considered, belonging to four main groups: bacteria, viruses, protozoa and helminthes (Arthurson, 2008). Davis et al (1999) compiled data presented by the European Commission (2001b) about typical concentration of microorganisms in untreated sewage sludge from various sources. Bacteria samplings counted approximately 10^6 *E. coli*, 10^2- 10^3 *Salmonella* spp. and virus monitoring showed 10^2- 10^4 enteric viruses per gram wet weight sludge. The protozoa *Giardia* spp. and the helminth *Ascaris* spp. could both be detected in a range of 10^2–10^3 oocysts/ovae per gram, whereas other helminths as *Toxacara* showed less occurrence with 10-10^2 and *Taenia* only 5 ovae per g wet weight untreated sludge (EC, 2001b).

While bacteria and viruses die within one to three months, parasite eggs can survive for years in soil or wastewater posing risk of transmission (USEPA, 2003, Sidhu and Toze, 2009). Parasites have a high persistence in the environment, even at low temperatures. Bacteria and virus survival in soil amended with biosolids depends on pH, moisture and temperature, for bacteria nutrient availability is an additional factor (Pepper et al., 2006). Bacteria have the possibility of regrowth in a suitable environment, whereas protozoa, helminthes and enteric viruses “are obligatory parasites and hence unable to multiply in biosolids” (Sidhu and Toze, 2009).

Pathogen exposure can occur in various ways and poses a risk to humans and environment. Direct contact with sewage sludge or treated soil via ingestion, dust inhalation or handling is one way of infection. Indirect routes are the consumption of crops or milk from grazing animals as well as vector transmission through other animals. Runoff from fields with sewage sludge amendment also presents a risk. (USEPA, 2003)

Microorganisms in sludge applied to land may additionally contaminate groundwater, especially viruses is at risk to be transported within the soil due to their small size (Chauret et al., 1999, Pepper et al., 2006). Viruses can adsorb to biosolids and soil particles which limit

transport, but this sorption is regulated by soil pH. At neutral pH soil sorption is low, because viruses are negatively charged as well as most soil particles. In contrast, at acidic soil pH viruses are positively charged and thus sorption is increased (Pepper et al., 2006). However, studies by Chetochine et al. (2006) indicated that only a minor part of viruses is expected to be leached out, because viruses are strongly adsorbed to biosolids, affecting the pH–dependent potential for transport (Pepper et al., 2006). The virus adsoption to biosolids might originate from virus-binding proteins, produced by bacteria in the activated sludge during wastewater treatment (Sano et al., 2004).

Besides exposure, also the infectious dose determines the potential infection risk. Parasites and viruses can infect in very low doses and show an overall high environmental resistance compared to bacteria with an infectious dose often around 10^6 (Santamaría and Toranzos, 2003).

Especially zoonotic microorganisms are of major importance in risk management of sewage sludge, as they are able to infect different animals as well as humans, meaning the pathogen can be transmitted from one organism to another (Arthurson, 2008). This feature increases the pathways exposing humans via animals and food chain.

The risk potential of pathogens determines public concern. Bacteria such as *E. coli* O157:H7, *Listeria*, *Helicobacter pylori* and viruses like coxsackievirus, echovirus, hepatitis A, rotavirus and norovirus as well as the parasites *Cryptosporidium, Cyclospora, Toxoplasma, Microsporidia* and *Giardia* are considered to be particularly critical (Sidhu and Toze, 2009) . The protozoan *Cryptosporidium* is an important waterborne disease often found in wastewater treatment plants and also in surface waters. It has been responsible for several outbreaks and poses a high risk on human health (Dumontet et al., 2001). As a food-borne disease, the bacteria *Salmonella* spp. and *Campylobacter* spp. are responsible for causing gastroenteritis in many cases throughout the world.

Indicator and Model Organisms

For monitoring the environmental hygiene, indicator and model organisms are used to determine pathogen reduction, as it is not practicable to examine all pathogens that potentially can be present. (Nordin, 2010)

Faecal indicators are organisms naturally occurring in faecal material in sufficiently high numbers, so that their presence and reduction can be used to draw conclusions about potential pathogens. These organisms should be easy to cultivate and, of particular importance, they should exhibit a similar behavior to the pathogen of concern (Nordin, 2007). “It is preferable, but not always possible, to use model and indicator organisms that are non- or only weakly pathogenic” (Nordin, 2007). One indicator is not representative for the prediction of all microorganisms. The wider the range of indicators being analyzed, the more accurate is the related risk determination for the substance of interest (e.g. biosolids).

Model organisms, are used to deduce conclusions about another organism with similar behaviour, e.g. the pathogen of concern. These organisms, pathogenic or non-pathogenic, can be initially present or added to serve as a model. The usage of a model organism can be more convenient concerning treatment and costs. Likewise indicators, model organisms should be representative for the inactivation of a certain pathogen. It can be an asset to use

model or indicators, showing a marginally higher resistance to avoid underestimations. Both terms, model and indicator organism, are closely related. An organism can be an indicator and a model organism at the same time, for instance *E. coli* can be a faecal indicator but may be used as a model organism for salmonella (Nordin, 2010).

Traditionally used bacterial indicators are faecal streptococci, thermotolerant coliform bacteria and faecal coliform bacteria, with further addition of *Clostridium perfringens* and enterococci (*E. faecalis*) (Sidhu and Toze, 2009). *E. coli*, for instance is representative for vegetative bacteria as *Shigella, Vibrio* or *Listeria* (Lepeuple et al., 2004).

Virus monitoring have been technically difficult, which is why bacteriophages are used instead as indicators and models. Bacteriophages are defined as viruses which infect bacteria. They show a similar structure, morphology and size as enteric viruses and provide an easier and safer handling (Sidhu and Toze, 2009). Examples of bacteriophages used as indicators are somatic coliphages, F-specific RNA phages and phage infecting *Bacteroides fragilis* (Lepeuple et al., 2004). All three groups show advantages and disadvantages. Coliphages show a "wide host range, presence in natural aquatic environments and ability to multiply in the environment" (Sidhu and Toze, 2009), compared to f-specific RNA phages which don´t multiply in the environment. Moreover, differences in heat resistance between the phages were reported, somatic coliphages being less resistant than f-specific RNA phages and phage infecting *Bacteroides fragilis (Sidhu and Toze, 2009)*. Laboratory experiments also showed possible higher heat resistance for poliovirus, rotavirus and coxsackievirus than bacteriophages, suggesting some weaknesses as indicator organisms for all viruses (Sidhu and Toze, 2009). Another possibility for virus detection are molecular methods for virus monitoring such as PCR-assays (Polymerase Chain Reaction), based on the viral nucleic acid (Metcalf et al., 1995).

Parasite reduction is usually determined by using the eggs of the helminth ascaris, as model organism. For safety reasons and due to its similarity, the pig-infecting *Ascaris suum* eggs are utilized as alternative to the human-pathogenic *Ascaris lumbricoides.* These eggs show a high resistance in environment and thus present an overall good indicator for general parasitic viability.

2.3. Treatment Methods

Methods, like anaerobic digestion, chemical treatment including lime or urea application, pasteurization and composting can be used for stabilising sewage sludge. Stabilisation means reduction of pathogens, odor, water and organic matter content of the residues (Arthurson, 2008). The main common demand on a stabilisation process is a significant pathogen reduction. Besides, it is advantageous when the material constitution is changed concerning organic matter, water content and odor to prevent drawing attention of other organisms (USEPA, 2003).

Concerning the inactivation of pathogens, pH and temperature are important factors determining the rate of reduction. Elevated temperatures lead to cell death, partly because of enzyme denaturation (Haug, 1993). The time/temperature ratio is an important variable which can be used to calculate pathogen reduction, especially concerning aerobic and anaerobic digestion (Dumontet et al., 1999). A high pH has proofed to decrease pathogens (Doyle, 1967).

Composting of Sewage Sludge

Composting of sewage sludge is a common biologic stabilisation technique. The compost process itself does not lead to a sanitisation of the material, but the heat production due to microbial activity can be used. Within an exothermic reaction, mesophilic microbes use the easily available organic matter as energy source (Dumontet et al., 1999). Oxygen is essential for these microbes giving the heat production, thus enough oxygen and free air space within a compost mixture is of importance. This can be provided by mixing the material, aerating or adding bulking agents for a higher porosity.

The compost process consists of three typical temperature phases, namely the mesophilic, thermophilic and lastly the cooling phase (Haug, 1993, Hassen et al., 2001). The self-heating can lead to a temperature rise up to 80°C, but the maximum beneficial temperature for a diverse and stable microbial community lies at 60°C (Dumontet et al., 1999). Small scale composting requires insulation, for instance in suitable vessels, while large scale composts need to have a sufficient height for the pile to conserve the heat inside(Vinnerås, 2007).

Depending on time and temperature, USEPA classifies composted sludge as class A or B biosolids (USEPA, 2003). For class A biosolids, the USEPA (2003) recommends a temperature rise to either 40°C for five days or exceeding 55°C for four hours during the five day period. The temperature kept over a certain time period is an easy way to estimate pathogen inactivation.

Beside these factors, also microbial competition and inhibiting substances can lead to pathogen control or destruction when composting organic material (Haug, 1993). In composts, antagonistic organisms are an additional advantage for pathogen inactivation compared to other biological treatments as anaerobic digestion (Dumontet et al., 1999). The success of composting as sanitisation method has been examined in various experiments: faecal coliform bacteria and *E. coli* can reach an up to 6, enterococci up to 4 $\log_{10}$ reductions (Lepeuple et al., 2004). However, sufficient high temperatures (>50°C) are necessary for this result. In a small-scale compost experiment with feces and maximum temperatures between 33 to 56°C, enterococci could not be significantly reduced within 6 days (Vinnerås, 2007). Vinnerås (2007) pointed out, that maintaining a high temperature over a certain period, as well as the composition of a material play a direct role for pathogenic survival. One factor describing the composition of a material is the carbon/nitrogen (C/N) ratio. The C/N ratio can determine about the degradation time, hence microbial activity. Additionally, a low C/N ratio can increase ammonia emissions. As most beneficial C/N ratio for compost start-up, a value between 25 and 30 is recommended in various studies (Huang et al., 2004).

The end product of the sewage sludge compost demonstrates a change in structure and composition, thus it “turns into a substrate unsuitable for the growth and survival of most pathogens” (Dumontet et al., 1999). Yet, if not totally sanitised, the compost bears still the risk of regrowth for certain pathogens. For minimizing this risk, temperature monitoring can ensure that a certain temperature was remained over the required time for sufficient pathogen inactivation. The mature compost has a long tradition as soil conditioner, supplying nutrients and organic matter for increased humus and nutrient content of the soil (Haug, 1993).

One major problem associated with composting is nitrogen losses due to ammonia volatilization. Ammonia emissions from agriculture are contributing considerably to environmental acidification and eutrophication. Within composting, the process of ammonia volatilization is driven by elevated temperature and alkaline pH, shifting the NH_3/NH^{4+} equilibrium towards ammonia (chapter 2.4) (Pagans et al., 2006). Ammonia emissions during composting are also responsible for undesired odor development. Nowadays, there are easy ways to minimize emissions, either by pH and temperature control, by closing or covering the compost system or exhausting outgoing air. By using a heat exchanger for outgoing air, ventilation air can be pre-heated for better temperature development within the compost, and at the same time emissions can be treated and ammonia captured by condensation (Vinnerås, 2002).

Future trends for sewage sludge usage in Europe prognosticated by Kelessidis and Stasinakis (2012) is an overall decrease of landfilling, while incineration will increase in some countries (e.g. Austria, Portugal, Spain, Belgium, Denmark). Regarding agricultural reuse, a positive development is in progress: Sweden, the Czech Republic, Lithuania, Poland and partly Spain and Italy appear to extend composting as sewage sludge treatment (Kelessidis and Stasinakis, 2012).

2.4. Sanitisation with Ammonia in form of Urea

Urea treatments have been studied in various experiments and turned out to be a cheap and effective additive for sanitising sewage sludge, or other organic substances (Vinnerås, 2002, Nordin, 2010, Fidjeland et al., 2013). When urea is added to sewage sludge, the enzyme urease transforms it by hydrolysis to ammonia and carbon dioxide (equation 1) both products showing disinfectant properties towards microorganisms. (Fidjeland et al., 2013, Vinnerås, 2002).

$$CO(NH_2)_2 + 2\,H_2O \rightarrow\ H_2CO_3 +\ NH_3 \quad \textbf{(1)}$$

Ammonia prevails in two forms, the unionized form NH_3 and the ammonium ion NH_4^+, being in equilibrium (equation 2). The ionized form is an important nutrient for plants and microorganisms, while NH_3 has an antibiotic effect (Vinnerås, 2002, Nordin, 2010).

$$NH_3(aq) + H_2O(l) \leftrightarrow\ {NH_4}^+(aq) +\ OH^-(aq) \quad \textbf{(2)}$$

An increase in temperature and pH leads to a shifting towards the side of uncharged ammonia (NH_3). In contrast, at pH-values lower than 8, the ammonia fraction will be very low and mainly the NH_4^+ form will be prevailing, thus only a minor sanitisation effect can be expected at total nitrogen (NH_3+NH_4^+) concentrations relevant for fertilier (Vinnerås, 2002, Nordin, 2010). The temperature and pH dependence of NH_3 is expressed with equation 4 and 5. The pk_a-value (acid dissociation constant) is regulated by temperature (4) and is used for calculating the ammonia concentration together with pH in equation 5.

$$[NH_3] = [NH_{tot}]\ \cdot fNH_3 \quad \textbf{(3)}$$

$$pk_a = \frac{2728{,}92}{T(^{\circ}\mathrm{C}) + 273} + 0{,}090181 \quad \textbf{(4)}$$

$$fNH_3 = \frac{1}{10^{(pk_a - pH)} + 1} \quad \textbf{(5)}$$

Urea treatment requires one hour of incubation time to degrade urea to ammonia, before it becomes antibiotic effective (Vinnerås et al., 2003). Vinnerås et al (2003) showed that 6% urea, added to faecal matter increased pH to 9.2 within only one hour at room temperature (20 °C), which lead to a high sanitisation effect in a short time: *E. coli* and *Salmonella* spp. reached the detection limit (<1 $\log_{10}$) within 5 days of this treatment, while *Enterococcus* spp. needed 21 days for inactivation (detection limit: <2 $\log_{10}$). As virus-indicator of this study, *Salmonella* Typhimurium 28B phage required 50 days for inactivation, showing a high ammonia-resistance. Bacteria and phages were significantly reduced compared to the control without treatment, showing an accelerated sanitisation effect with urea. Concerning ascaris eggs 50 days were necessary to achieve 0% viability (Vinnerås et al., 2003). Fidjeland et al (2013) documented within experiments on sewage sludge, a 5 $\log_{10}$ reduction of *Salmonella* spp. after 1 month with 1.5% urea at 10°C or 0.5% urea at 22°C. In contrast, enterococci were reduced about 2 $\log_{10}$ with either 2% urea at 10°C or 0.5% urea at 22°C within less than six weeks.

Ammonia, as shown, has the potential to speed up the sanitisation and as long as it is contained, it also protects the material from potential regrowth (Nordin, 2010).The addition of urea to improve sanitisation also increases the sewage sludge quality as fertiliser with the ammonia-nitrogen. Regarding the positive environmental effect of nutrient recycling and the economic expense for urea treatment, the sanitisation costs can be compensated by the usage as fertiliser (Vinnerås, 2007).

Ammonia sanitisation is even more effective at higher temperatures, proved for example by Nordin (2010) with experiments on human excreta. With 0.5-2% urea at 34°C , all concentrations lead to a 2 $\log_{10}$ reduction of *Ascaris suum* eggs within <5 days. To reach the same reduction at 24°C with 1-2% urea, 30 -60 days were needed. A salmonella reduction of 6 $\log_{10}$ was reached in 10 days with only 0.5% urea and 34 °C, compared to lower temperature like 24°C, when the reduction time is doubled to 20 days (Nordin, 2010). In this as in previous studies, enterococci showed low resistance to higher temperatures, but higher ammonia-resistance. With 0.5-2% urea added to feces at 34 °C, 4-2 days were needed to reach 1 $\log_{10}$ reduction for enterococci.

Nordin (2010) concluded, that an “addition of 1% urea to feces at temperatures from 14 to 34°C is sufficient to produce a safe fertiliser for unrestricted use (6 $\log_{10}$ pathogen reduction) within 2 months of treatment” and 2% urea at 34°C with 1 month of treatment.

To get a better effect from the urea amendments, the sewage sludge in this study was composted beforehand to increase temperature. In addition, urea was tested in combination with ECOX or calcium carbonate to increase pH. Both, temperature and pH elevation should improve the effect of urea, by increasing the ammonia (NH_3) fraction for pathogen reduction

3. MATERIAL AND METHODS

3.1. Experimental design

Anaerobically digested, dewatered sewage sludge used in the experiments was collected from the municipal wastewater treatment plant in Uppsala, Uppsala Vatten och Avfall AB.

The experiment consisted of two different parts: one initial compost phase (aerobic) for one day, followed by a chemical treatment phase (day 1-14) after adding chemicals and sealing (anaerobic) of compost vessels (Figure 2).

A pre-test was performed to assess the experimental design and to specify the concentrations of urea, urea+$CaCO_3$ and urea+ECOX needed for desired pH elevation to around 9. Six different treatment combinations and one control were tested in the pre-test (Table 2). The concentrations of urea, urea+$CaCO_3$ and urea+ECOX in the main experiment (trial 1-3) were selected based on combinations showing the best pathogen inactivation in the pre-test (Table 2).

Table 2 Treatment combinations for the pre-test and the modifications for the experiment (trial 1-3)

Pre-Test	Experiment
Control	Control
0.5% Urea + 0.5% ECOX	1% Urea + 2% ECOX
0.5% Urea + 2% ECOX	0.5% Urea + 2% ECOX
0.5% Urea + 1% $CaCO_3$	Replicate (alternating)
0.5% Urea	1% Urea
1.5% Urea	1.5% Urea
0.5% Urea + 3% $CaCO_3$	1.5% Urea + 3% $CaCO_3$

Five different treatments and one control were selected for the experiment and three composting trials were performed in six composting vessels giving opportunity to in each trail replicate one of the treatments. To avoid variations in temperature between the composts at start-up, as experienced in the pre-test, approximately 5 kg of fresh sludge was composted in a styrofoam container with a volume of about 500 cm^3, before divided upon the different treatments for the chemical treatment phase. Total thermotolerant coliform bacteria, enterococci, somatic coliphages and f-RNA phages were analyzed in the pre-test. *Salmonella* Typhimurium and *Ascaris suum* eggs were used as additional model organisms for the hygiene monitoring in the main experiment. The f-RNA phages were not analyzed as they in the pre-test appeared to be at the detection limit.

3.2. Procedure

In the pre-test 350 g of fresh sewage sludge was composted in seven 1 liter Dewar vessels until the material reached 40°C, when the chemicals were added. At the start of composting, the sewage sludge was loosened up, by mixing it thoroughly with a spoon and hands, before transferring it to the vessel. A theromocouple fixed in the middle of the sludge constantly provided data to a logger. The lid was closed in a way which still provided oxygen supply.

After one day, when the compost reached about 40°C, samples were taken and the material received its chemical treatment. Compost vessels, containing sewage sludge not reaching temperature ≥40°C were heated up in a 44°C oven with its content. The chemical additives were weighed into stomacher bags, filled up with the heated sludge, hand-shaked and then retransferred to the vessels. Parafilm PM-996 (BEMIS, USA) (later a Stomacher bag) was used to seal the lid and to stop aeration.

Salmonella cultivation for the main experiment began one day before compost start-up, by adding one colony *Salmonella enterica serovar* Typhimurium (Sahlström et al., 2006) to 5 ml nutrient broth and pre-incubating at 37°C for a couple of hours. The mixture was then transferred to a 500 ml-Erlenmeyer flask and filled up with approximately 50 ml nutrient broth. After over-night incubation at 37°C, the bacterial solution was applied with a pipette to the sewage sludge in the styrofoam-container, before starting up the compost. First, 1/5 of the sludge was added to the container and inoculated with 10 ml of salmonella. In succession, the remaining sludge was added with additional 40 ml salmonella solution. For homogenization, the whole material was mixed by hand.

After the inoculation the sewage sludge was shaped into a pile with seven *Ascaris suum* egg containing nylon bags being buried in the middle of the compost. After reaching an elevated temperature of over 40°C, the sludge was mixed and distributed to the preheated Dewar vessels (500 g sewage sludge per vessel), which were warmed up without lid in a 44°C oven to provide the same start temperature for each treatment. The chemical amendments (Table 2) were prepared in stomacher bags, filled with sludge, hand-shaked and transferred to the vessels. All the composts received one *Ascaris suum* egg bag from the styrofoam container, buried in the middle. After linking the vessels to a thermocouple, the lids were sealed.

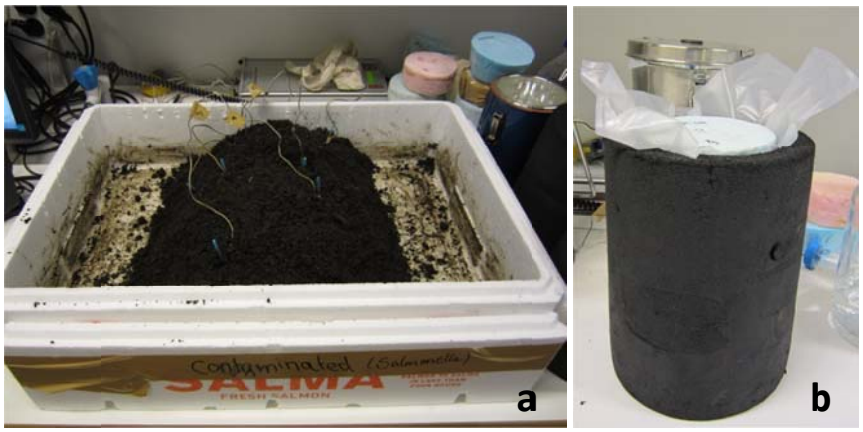

Figure 1a) 5 kg of sewage sludge compost in a styrofoam container with open lid with thermocouples fixed in the middle; b) a 1 l Dewar vessel sealed with lid and plastic bag showing the set-up during the chemical treatment phase

3.3. Sampling

For pre-test and main experiment, a similar sampling procedure was performed. Thermocouples with data logger (Intab, Sweden) and the Easy View software measured the compost temperature every minute over two weeks.

At each sampling day (day 0, 1, 2, 7, 10, 14), the pH and hygiene for total thermotolerant coliform bacteria, salmonella, enterococci, f-RNA phages and somatic coliphages was determined, while VS, TS, Nitrogen and Ammonium were only measured at the compost start-up and end (day 0/1 and 14) (Figure 2). For the second and third trial, one additional sample day (day 4) was included for indicator bacteria, as salmonella and total thermotolerant coliform bacteria were rapidly reduced within the first week. At each sampling, all material in the Dewar vessel was intermixed from bottom to top using a metal spoon, to get a representative sample. Then, the vessels were sealed immediately. At the start of the composting and after reaching 40°C (day 1), three replicate samples were taken from the styrofoam container, after mixing the entire material, to get a representative average value for the 5 kg sewage sludge. For all other sampling days, only one sample per Dewar vessel and analysis were withdrawn. For sampling days including pH and hygiene, sludge was withdrawn from the vessel and distributed for hygiene (10 g) and pH measurement (7 g). For full sampling days at start-up (day 0/1) and end (day 14) approximately 50 g sludge were taken out and distributed to the measurements, for N-TOT/ammonia 10 g and for TS/VS approximately 10 g. The samples were kept in a stomacher bag for further dilution in the bag, described in chapter 3.5. *Ascaris suum* egg bag sampling was only done at the last sampling day 14.

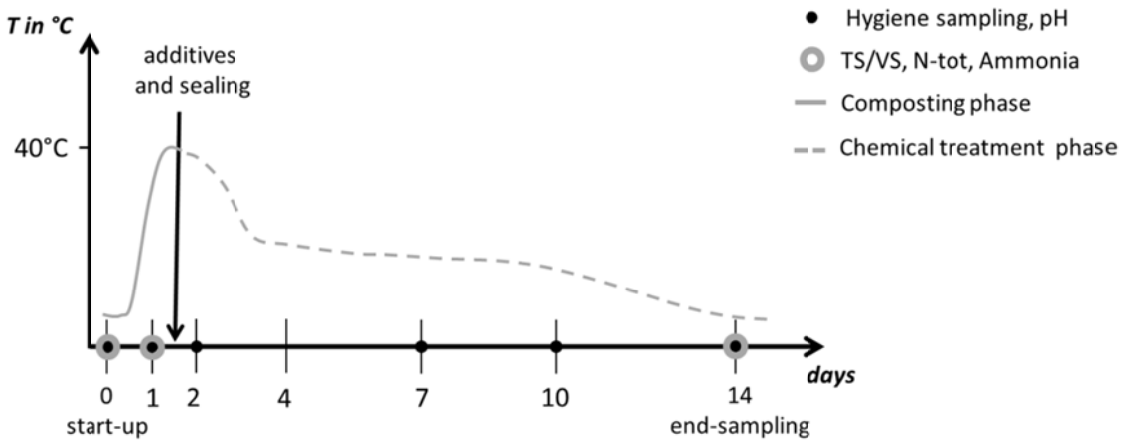

Figure 2 Experimental principle for the pre-test with chemicals added after one day composting to elevated temperature of 40°C (composting and chemical treatment phase), showing the time for microbial sampling at day 0, 1, 2, 7, 10 and 14 and TS/VS, NH_3/NH^{4+} sampling days 0, 1 and 14

3.4. Analysis

Total Solids (TS) and Volatile Solids (VS)

Approximately 10 g of sludge were weighed into an empty beaker with recorded weight. The material was dried at 105°C for 14 hours to determine the Total Solids (dry matter) content. Afterwards, the dried and weighed samples were combusted for four hours at 550°C. The weight of the ash corresponds to the inorganic fraction of the material. The weight loss of the dried matter compared to the ash complies with the volatile solids or organic matter content.

$$Total\ Solids\ (\%) = \frac{dry\ weight}{wet\ weight} \cdot 100 \quad \textbf{(6)}$$

$$Volatile\ Solids\ (\%) = \frac{dry\ weight - ash\ weight}{dry\ weight} \quad \textbf{(7)}$$

pH

The pH was measured in a 5-fold dilution of 7 g sewage sludge with deionized water. The sludge was mixed in Stomacher®400 (BA6041, UK) or VWR® Blender bags (129-0729, Leuven) (by hand-shaking and squeezing to get a homogenized dilution, which was poured into a centrifuge tube. The closed tubes rested one hour for settling of the particles before determining the pH with pH-electrode PHC 2051 (Radiometer, Copenhagen) and meter PHM 210 (Radiometer, Copenhagen) at room temperature.

Total Nitrogen and Ammonium

The Total Nitrogen (TOT-N) and Total Ammonium Nitrogen (NH^{4+}-N) concentration in the sludge was determined with the Spectroquant® Test Kit (Merck, Darmstadt). The measuring range for the cell tests lies between 10-150 mg/l N and within 4–80 mg/l NH^{4+}-N for the TOT-N (total Nitrogen Cell Test, Cat. No 1.14763) and the NH^{4+}-N kit (Ammonium Cell Test, Cat. No 1.14559), respectively. A 100-fold dilution with deionized water was sufficient for treated and untreated sludge samples to be within the measuring range. For the ammonium kit, samples were filtered (0.45 µm) before adding the reagents. As the digested sewage sludge sample for TOT-N showed turbidity and no complete homogenization, it was also filtered (0.45 µm) to avoid false high readings at the photometric measurement. For TOT-N digestion, a thermo-reactor TR 420 (Merck, Darmstadt) was used. The photometric measurement was performed with the Spectroquant®NOVA 60 photometer (Merck, Darmstadt) in accordance to the Nitrogen and Ammonia test kits.

3.5. Microorganisms

Bacterial sampling

Ten (10) g of sludge was squeezed and intermixed thoroughly with buffered NaCl peptone solution with tween in proportions 1:9 in a stomacher bag and further 10-fold serial diluted depending on the expected pathogen concentration.

For enterococci, 0.1 ml of the diluted sample was applied to Slanetzy Bartley (SlaBa) agar plates, spread with spatulas and incubated for 48 hours at 44°C for enumeration. The detection limit is 100 CFU/g when 0.1 ml is applied, but was increased to 10 CFU/g by using 0.2 ml on five SlaBa agar plates.

Total thermo tolerant coliform bacteria analyzes was prepared by adding 1 ml sample on a petri dish and pouring two times 8 ml warm Violet red bile agar (VRG) over: once directly over the sample, the second time after the agar solidified. The counting was carried out after incubation for 24 ± 3 hours at 42-44°C. The detection limit of this analysis was 10 CFU/g sludge.

The salmonella enumeration was performed with 0.1 ml diluted sample being applied on Xylose-Lysine-Desoxycholate (XLD) agar plates with 24±3 hours incubation at 37±1°C. Reaching low concentrations, 0.2 ml sample was applied on 5 XLD plates respectively giving a detection limit of 10 CFU/g. When plating on XLD reached the plating method detection limit, salmonella enrichment was performed. Then, 10 g or 50 g sludge was diluted 10-fold with buffered Peptone-water and mixed within a stomacher bag. The bags were incubated for 18 hours at 37°C. After incubation and mixing, three drops were withdrawn with a micro-loop and applied on a Modified Semi-Solid Rappaport-Vassiliadis (MSRV) agar plate. One *Salmonella* Typh. colony diluted in 5 ml peptone-water was used as a positive control. After incubating for at least 16 hours at 42°C, the plates were examined whether salmonella was detected or not. In indistinct cases, when only a white slight sign of salmonella was observed, a micro loop was used to pick up some sample from the MSRV agar to spread on XLD (37°C, 24 hours) for further confirmation. The detection limit was 1 CFU per 10 g and 50 g sewage sludge.

For sampling of bacteriophages, the corresponding host bacteria solution has to be incubated beforehand: One colony of either *Salmonella* Typh. WG49 (ATCC 700730), the host bacteria of FRNA-phages or *Escherichia coli* 13706 for somatic coliphages, was transferred to an Erlenmeyer flask filled up to 10-20% of its volume with nutrient broth (SVA B311040, Sweden) and incubated at 37 °C for three hours while shaking. The diluted samples were filtered (0.45 µm) first to exclude bacteria from the sample, while the phages are small enough to pass. In a heat block at 46°C, 2 ml melted softagar were provided in a test tube and mixed with 1 ml sample and 1 ml host bacteria solution. This mixture was rapidly applied on blood agar base (BAB) agar plates and then incubated for 17 to 24 hours at 37 °C for counting plaques in the agar. The resulting detection limit was 10 PFU/g sludge. To verify that the method worked, the phiX 174 phages control strain (ATCC 13706-B1) was used.

All bacteria and phages plate counts were calculated back, depending on the dilution of the original sample. This way the results can be presented in CFU or PFU/g sewage sludge. For fitting a regression line to the data points from repeated trials having different start concentrations the data were normalized. Detection limits were included for the regression line, when it improved the inactivation rate, which was indicated by a steeper line.

Ascaris suum

A solution of $2 \cdot 10^5$ *Ascaris suum* eggs/ml (Excelsior Sentinel, USA) was diluted with 0.1 N sulfuric acid and approximately 10^4 eggs injected to a nylon bag (2·25 cm^2; mesh, 35 µm). The injection hole was closed with melt glue and each bag was transferred into sulfuric acid. The bags were stored in this acid under aerated conditions in the fridge until use. The nylon bags keep the eggs inside but are permeable for fluids, thus allow to be affected by the tested treatments in the experiment. One ascaris egg bag per compost vessel was used for monitoring reduction in egg viability. Before applying the bag, it was taken from the sulfuric acid, rinsed in tap water and shortly drained with paper. The same procedure was performed after removing the bags at the end of the two week-trial. The bags from the different treatments and one untreated reference bag were stored in 0.1 N sulfuric acid in petri dishes for 28-35 days at room temperature. The reference bag was used to determine the initial viability of eggs used in the experiment. The egg bags were smoothly rubbed to gather eggs in one corner to withdraw them with a syringe by stitching into the nylon bag.

Withdrawn solution with eggs was applied to an object slide and topped with cover glass for observation under the microscope. The incubated ascaris eggs withdrawn from the bags were counted and differentiated into four categories: destroyed eggs, eggs, eggs developed into pre-larvae stages and larvated eggs. When larvae were detected 100 eggs were counted whereas 1000 eggs were counted for bags without larvae to increase the certainty when all eggs were unviable and 200 eggs were counted for the initial viability.

As equation 8 shows, all stages before larval development were considered as non-viable egg. Eggs with started development were still included as non-viable due to their unfinished development compared to others being able to reach the larval stage. The viability within the experiment was then divided by the initial viability, as the reduction has to be related to the initial viability.

$$Viability\ (\%) = \frac{larvated\ eggs}{larvated\ eggs + developed\ eggs + unviable\ eggs} \cdot 100 \quad \textbf{(8)}$$

3.6. Statistical analysis

For analyzing the organism data, linear regression was used in Minitab 16. Each treatment was compared with the control for the different studied organisms respectively. Significant differences were determined at α=0.05. Residual plots (Residuals versus fitted values) assessed the model by the randomness of the error.

4. RESULTS

4.1. Total Solids (TS) and Volatile Solids (VS)

The total solids and volatile solids slightly varied over the three trials, as each time a new batch of sludge was used. The TS content at compost start-up (day 0) was between 29.6-33.2% and the volatile solids between 49.8-55.2% of TS. When comparing the data from day 0 with day 1, the VS was reduced in all samples, at most about 2%, indicating degradation during the compost phase. Chemicals were added on day 1, which is why TS and VS were determined again on day 2. Comparing day 2 with day 14 (Appendix, Table 10), an additional VS decrease of around 5% could be observed in some treatments and trials. Yet, some vessels only recorded minor VS-losses and all losses between trials and treatments randomly varied within the experiment. The control and 0.5% urea+2% ECOX treatment showed no or only a small decrease in VS. TS changed only slightly within the chemical treatment phase (comparing day 2 and 14) and alterations in TS were mainly occurring within the composting phase with an increase of 1-2% in all trials (comparing day 0 and 1), as the material became drier.

Table 3 Total Solids (TS) and Volatile Solids (VS) for day 0 and 1, as means for trial 1-3 ± the standard deviation

Trial	Day 0*		Day 1	
	TS %	VS %	TS %	VS %
1	29.9±0.3	54.4±0,8	31.7±0.1	50.6±0.3
2	31.8±0.5	51.4±1.6	32.8±1.0	49.8±1.7
3	32.8±0.4	52.7±1.5	35.0±0.0	49.1±0.0

***salmonella enriched sludge**

4.2. Temperature development

The first temperature development when composting the sewage sludge in the Styrofoam box in all trials corresponds to the mesophilic phase within a compost process. Shaped into a pile, the material reached over 40°C in trial 2 and 3 (Figure 3) and without pile-shape only 35°C in trial 1. For reasons of repeatability the sewage sludge in each trial was heated up in an oven (42 to 44°C), before adding the chemicals.

The temperature development within a treatment varied between the trials. The first day of composting was similar for each trial, as the material was in the Styrofoam container. During compost phase (day 0-1), trial 2 and 3 reached 40°C, compared to trial 1 with only 35°C. In trial 3 the temperature even exceeded 40°C. Therefore temperature-induced pathogen reduction led to higher pathogen numbers on day 2 in trial 1, compared to 2 and 3. As the vessels were sealed on day 1, temperature dropped in most cases from ≥40°C to 30-35°C during day 2, excepted three treatments (1.5% urea, 1.5% urea + 3% $CaCO_3$, Control) in trial 2 (Figure 3d-f) and the 1% urea treatment in trial 1c (Figure 3c). For these treatments the temperature curve shows a more gradual decrease, approaching the other trial curves after seven days in case of 1.5% urea and after ten days for the other treatments. Each treatment and trial in the chemical treatment phase (day 1-14) shows small temperature peaks, increasing up to 3°C higher than before. These peaks were created by opening of the vessels and mixing of the material, with the related oxygen supply. All treatments ended up at 25±1°C after 14 days.

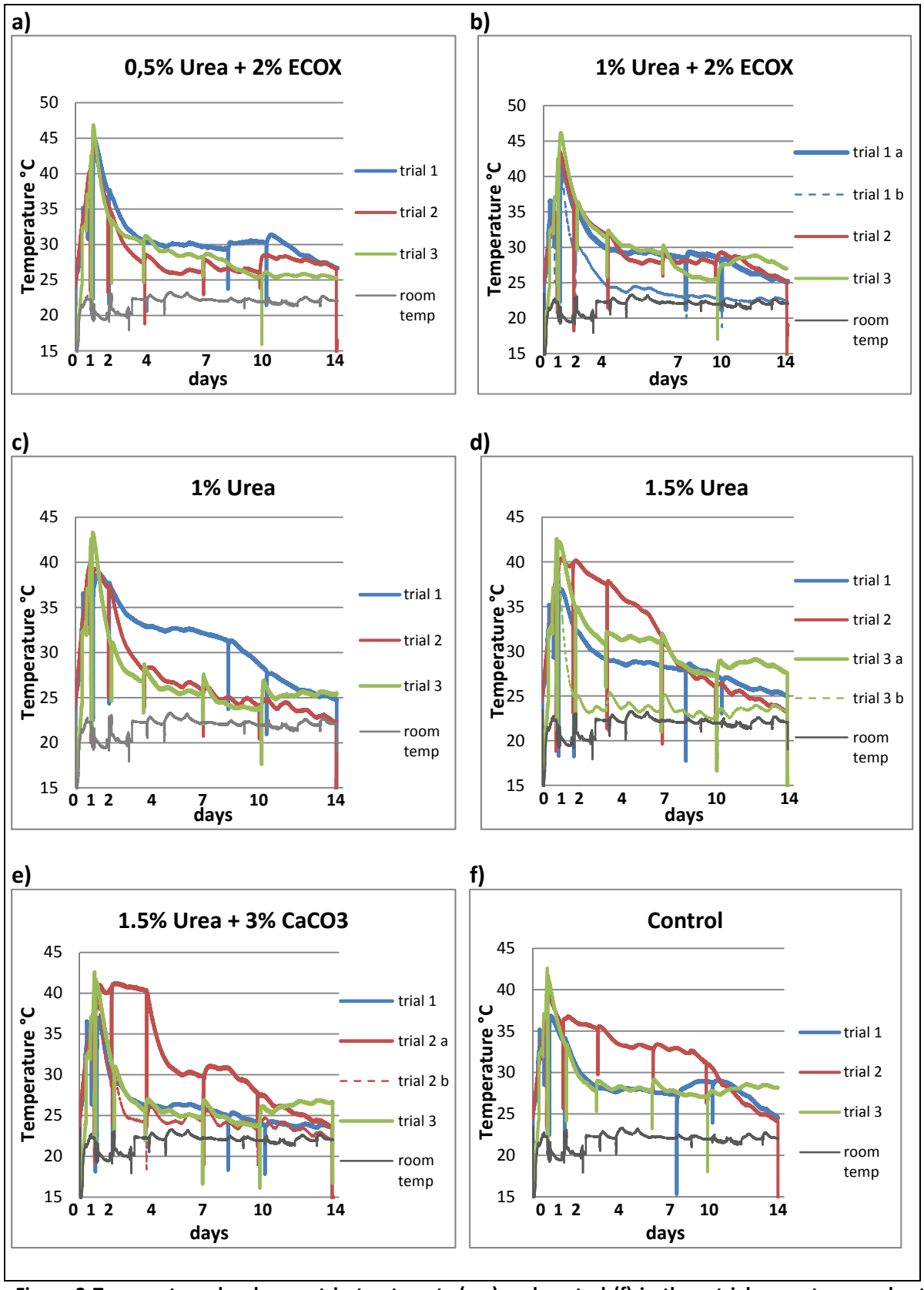

Figure 3 Temperature development in treatments (a-e) and control (f) in three trials over two weeks, the room temperature of the first trial was used as representative for all trials; sudden drops of the temperature curve are due to sampling (day 1, 2, 4, 7, 10, 14) and removing of the temperature logger for a few minutes

4.3. pH development

The pH (Figure 4a-c) showed an immediate rise at sampling day 2, one day after chemicals were added. The treatments reached a pH of 8.7 to 9.2 in all trials, compared to the 1 pH unit lower control (pH 8.1). There are variations within the treatments and trials over time and the control depicts a considerably different pH development. The control shows a slight increase until day 7, but then a decrease of 2.5 pH units in trial 1 and 2 (Figure 4f) and one unit in trial 3 (Figure 4c). Overall, 1% urea+2% ECOX and also the 1.5% urea treatment showed the highest pH-values. However, the difference between the highest and lowest pH reached with chemicals, including all treatments, varied only within 0.3 pH-units. All chemical amendments showed an increase of pH up to 9±0.2 and stayed within that range until the end of the two weeks trial period.

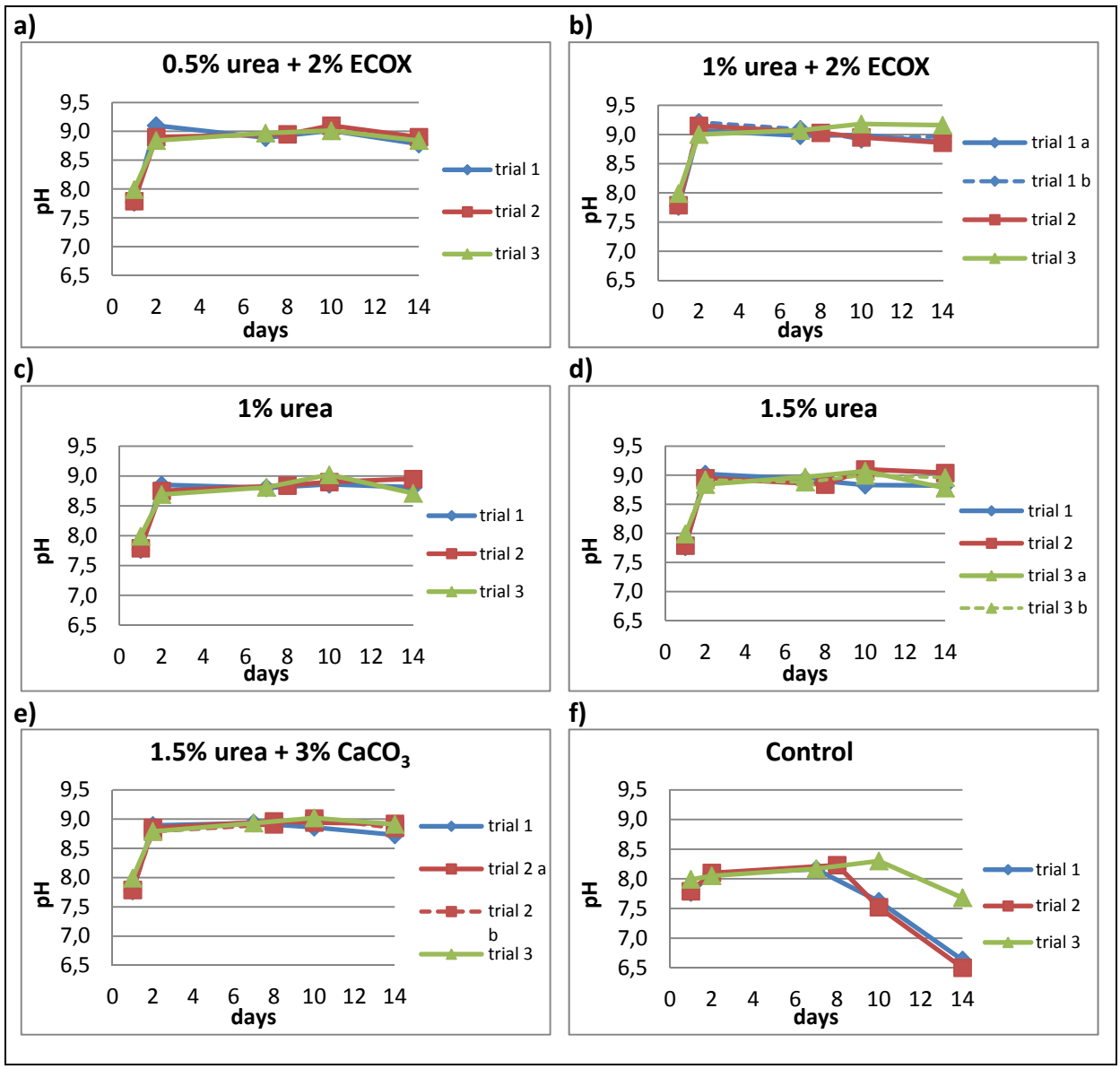

Figure 4 pH development for 5 treatments and control within three trials over 14 days; data points correspond to the sampling days (1, 2, 7, 10 and 14)

4.4. Total Nitrogen (N-TOT) and Ammonium (NH^{4+}-N)

Urea consists of 47% nitrogen by weight. The nitrogen added with the urea to the sewage sludge do for 1.5% urea corresponds to 7 g N/kg sludge; 1% urea to 4.7 g/kg and 0.5% urea to 2.35 g N/kg sludge. According to the urea additions and initial nitrogen concentration of the used sewage sludge, the total nitrogen of the amended sludge (2.4-3.3 g/kg) is expected to rise up to 9.4-10.3 g/kg in case of 1.5% urea, up to 7.1-8 with 1% urea and to 4.8-5.7 g/kg with 0.5% urea. However, for measurements at day 2, only few values (highlighted in bold, Table 4) are within or close (+/-1 g/kg) the expected range. The increase in total nitrogen, due to the treatment additives can be seen in Table 4 (day 2). Mostly, the values are lower than the calculated addition of nitrogen indicating losses. Comparing total Nitrogen measurements of day 2 and final sampling day 14, almost all treatments show a slight decrease in total Nitrogen over the 2 weeks experiment, with bigger losses than 1 g/kg, Table 4, highlighted with *) in some trials. Total Nitrogen content is shown in Figure 5 with an increase of nitrogen from day 1 to 2 due to urea treatment. The NH_4^+-N ranged from 1.2-1.4 g/kg and increased at day 2 after ammonia amendment on day 1 (Table 4). The NH_4^+-N decreased from day 2 to 14 in all chemical treatments.

Table 4 Total Nitrogen (N-TOT) and Ammonium-Nitrogen (NH^{4+}-N) in g/kg sewage sludge at day 2 and 14 for each treatment and trial; for day 2 and N-TOT values in bold are close(+/-1 g/kg) to the expected calculated value; * points out values with higher N-losses than 1 g/kg from day 2 to 14

Treatment	Trial	Day 2		Day 14	
		N-TOT	NH^{4+}	N-TOT	NH^{4+}
Control	1	1.7	1.2	2.1	1.7
	2	2.8	1.5	4.6	3.7
	3	3.5	1.7	3.2	1.8
1% Urea	1	3.5	1.7	3.2	1.8
	2	5.0	3.9	4.4	3.2
	3	**6.8**	4.7	4.8*	4.1
1.5% Urea	1	6.0	5.8	5.5	5.1
	2	7.2	5.8	6.0*	5.5
	3a	7.6	6.8	8.3	6.3
	3b	7.4	6.3	5.8*	4.5
0.5% Urea + 2% ECOX	1	3.3	2.5	2.9	2.6
	2	**4.2**	2.8	4.5	3.6
	3	**6.7**	4.1	6.7	4.7
1% Urea + 2% ECOX	1a	4.5	3.1	4.1	3.3
	1b	4.3	3.7	3.9	3.3
	2	6	4.3	6.1	5.5
	3	**6.8**	6.5	6.5	5.3
1.5% Urea + 3% $CaCO_3$	1	6.9	5.6	6.1	4.9
	2a	6.1	4.8	2.5*	0.8
	2b	5.8	5.2	4.4*	3.8
	3	7.9	7.1	8.3	5.8

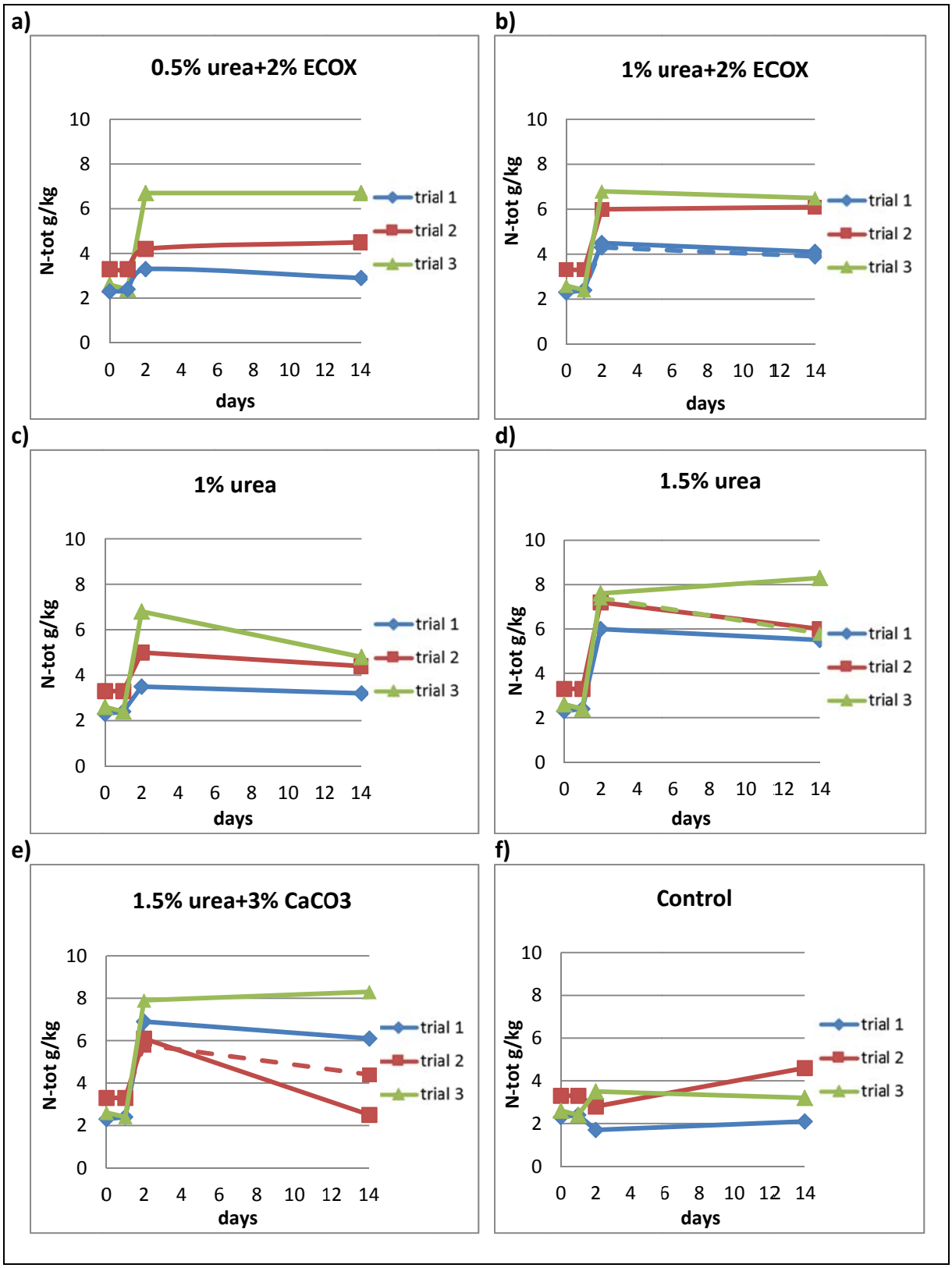

Figure 5 Total Nitrogen content in the treatments (a-e) and control (f) in three trials over two weeks, sampling (day 0, 1, 2, 14)

4.5. Microorganism monitoring

Bacteria and Phage

The fresh sewage sludge from the waste water treatment plant contained in average: Total thermotolerant coliform bacteria $4.3 \cdot 10^6$ CFU/g sludge, *Enterococcus* spp. $4.1 \cdot 10^4$ CFU/g and somatic coliphages $1.9 \cdot 10^3$ PFU/g. Using *Salmonella* spp. as a model bacteria, the sludge was enriched to an average concentration of $1.7 \cdot 10^7$ CFU/g. The treatment effects on reduction differed between the organisms studied. Salmonella and total thermotolerant coliform bacteria (TTC) showed a rapid pathogen inactivation compared to the control, whereas somatic coliphages and enterococci were reduced in a similar range as the control (Figure 6a-e).

For total thermotolerant coliform bacteria (Figure 6a), a 5 $\log_{10}$ reduction was reached in all treatments within 7-10 days, while the control only had a 2 $\log_{10}$ reduction within 14 days (D_R=8.3 days) (Table 5). All treatments showed similar good reduction, but the treatments 1.5% urea and 1.5% urea+3% $CaCO_3$ have a slightly shorter decimal reduction time (D_R=1.5 days). In contrast, for the 1% urea treatment, the time for one log reduction was longest with D_R=1.8 day pointing towards a slower reduction of TTC. However, all treatments reached the detection limit of 1 $\log_{10}$ CFU/g sewage sludge in the 2 weeks-experiment in all trials.

The untreated control displays a time and heat related *Salmonella* spp. reduction of 2-3 $\log_{10}$ CFU/g (Figure 6b). In contrast, all treatments achieved an 8 $\log_{10}$ reduction within 7-8 days ($D_R \leq 1$ day) by reaching the detection limit of 0.1 CFU/g except for 1% urea, accomplishing the same result on sampling day 14 (D_R=1.6 days). Comparing the decimal reduction time, the time needed by the 1% urea treatment for pathogen reduction was double that needed by 1.5% urea or 1% urea+2% ECOX (Table 5). The detection limit of 0.02 CFU/g was not reached in all treatments for trial 3 (for 1% urea; 1.5% urea and its replicate).

All sampling data for *Enterococcus* spp. reduction (Figure 6c) are in a range of 2-5 $\log_{10}$ CFU/g. About 1 $\log_{10}$ reduction was reached in control and all treatments (D_R=10.9-17.4 days) except for 0.5% urea+2% ECOX (D_R=32.4). This treatment (0.5% urea+2% ECOX) stagnates at concentrations between 4 and 5 $\log_{10}$ CFU/g or even appears to show an increase in number towards the end of the two weeks study. The lowest concentrations of enterococci occurred with 1.5% urea alone (D_R=10.9) or combined with 3% $CaCO_3$ (D_R=14.5) and with 1% urea+2% ECOX (D_R=15.2), seen in Figure 6c and Table 5. Overall the chemical additives don´t seem to have an effect on the reduction, but mainly the temperature and time, as the control was reduced in a similar range (D_R=16.6) and even faster than the 1% urea treatment (D_R=17.4).

Treatments and control showed a similar reduction of somatic coliphages to around 1-2 $\log_{10}$ PFU/g (D_R=8.5-13.7). There were huge variations within the same treatment during the three trials. Altogether, the detection limit (10 PFU/g) was not reached consistently, but 0.5% urea+2% ECOX (D_R=8.5) and 1.5% urea (D_R=9.4) reached in trial 2 and 3 concentrations close to the detection limit (Figure 6d). The control showed the same decimal reduction time (D_R=10.4) as 1% urea+2% ECOX, which was 2-3 days shorter than the D_R of 1% urea and 1.5% urea+3% $CaCO_3$ (Table 5).

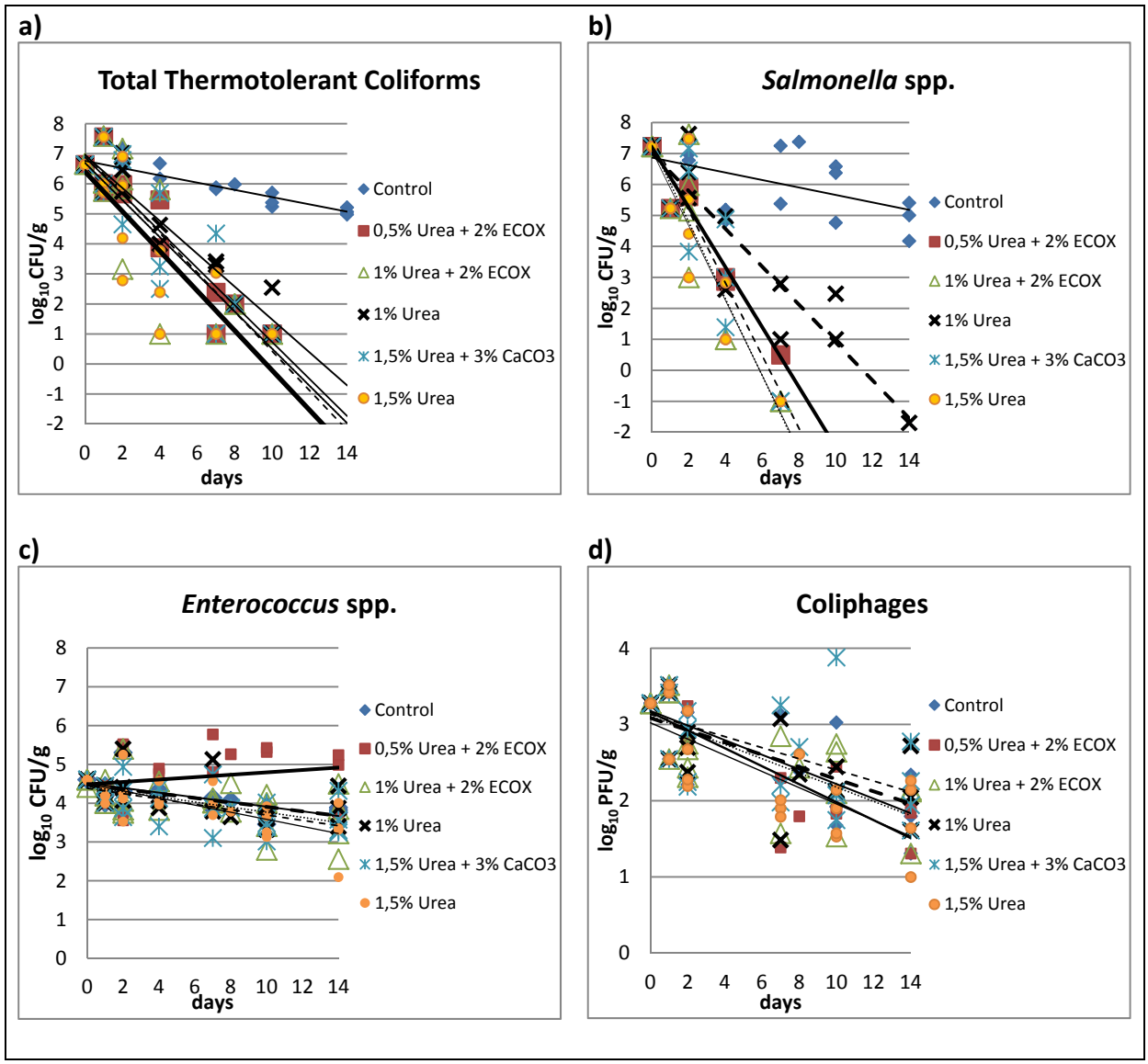

Figure 6 Concentrations (log_{10}CFU/g (a-c) or PFU/g (d)) of a) total thermotolerant coliform bacteria (detection limit 1 log_{10}CFU/g) and detection limit of -1 log10 CFU/g or -1,7 log10 CFU/g reached for b) *Salmonella* spp.; c) *Enterococcus* spp. and d) somatic coliphages; linear trend lines for treatment and control are indicated; normalized data are used

Table 5 Decimal reduction time (D_R) for the pathogens during each treatment, coefficient of determination (R^2) for each regression line in Figure 6

Sample type	Treatment	D_R value	R^2
TTC	Control	8.3	0.70
	1% Urea	1.8	0.91
	1.5% Urea	1.5	0.69
	0.5% Urea+2% ECOX	1.6	0.93
	1% Urea+2% ECOX	1.6	0.76
	1.5% Urea+3% $CaCO_3$	1.5	0.77
Salmonella spp.	Control	8.3	0.30
	1% Urea	1.6	0.87
	1.5% Urea	0.8	0.87
	0.5% Urea+2% ECOX	1.0	0.97
	1% Urea+2% ECOX	0.8	0.87
	1.5% Urea+3% $CaCO_3$	0.9	0.88
Enterococcus spp.	Control	16.6	0.53
	1% Urea	17.4	0.28
	1.5% Urea	10.9	0.52
	0.5% Urea+2% ECOX	32.4	0.05
	1% Urea+2% ECOX	15.2	0.31
	1.5% Urea+3% $CaCO_3$	14.5	0.39
Coliphages	Control	10.4	0.57
	1% Urea	12.5	0.48
	1.5% Urea	9.4	0.63
	0.5% Urea+2% ECOX	8.5	0.73
	1% Urea+2% ECOX	10.4	0.55
	1.5% Urea+3% $CaCO_3$	13.7	0.33

Table 6 Results from regression analysis for indicator organisms and treatment.
Significance of treatment compared to the control (*) at α=0.05 (**) at α=0.01, (***) at α=0.001

Treatment	TTC	Salmonella	Enterococci	Coliphages
1% Urea	***	***	-	-
1.5% Urea	***	***	-	-
0.5% Urea+2% ECOX	***	***	*	-
1% Urea+2% ECOX	***	***	-	-
1.5% Urea+3% $CaCO_3$	***	***	-	-

Regression analysis was performed for each treatment and organisms (Table 6). All treatments showed a significant reduction for TTC and salmonella compared to the control. Coliphages and enterococci had concentrations not significantly different from the control, with the one exception for enterococci in the 0.5% urea+2% ECOX treatment being significantly different compared to the control. However, the increase over time was not significant (p-value 0.32). All other treatments and the control are significantly reduced over time. For TTC, the regression line explained the reduction rate to 69-93%. The regression line of salmonella had a similar R^2 of 87-97% for the treatments but only 30% for the control. The regression of coliphages and enterococci showed a lower R^2 than TTC and salmonella. The lower R^2 indicates that the linear regression model of coliphages and enterococci fit less to the data than the model of salmonella and TTC.

The residual plots (Figure 7 and Figure 8) from the regression analysis for coliphages and enterococci show a random pattern with symmetric character. A random error for linear models produces residuals which are normally distributed, showing a symmetric but random pattern in the residual plot. The residual plots for salmonella and *E. coli* both show a tendency towards non-symmetry. This means the error is not totally random and it is possible to predict residuals, thus the model does not reflect the measured values sufficiently. The datasets for both *E. coli* and *Salmonella* spp. due to wrong estimates of dilutions to sample were smaller, consequently leading to less data points for fitting the models. In contrast, phages and enterococci did not contain lacking data points and hence have a slightly higher certainty. Another explanation for the residual plots of *E. coli* and salmonella can be the usage of a linear model to describe the pathogen reduction. The non-symmetrical pattern in the plot can indicate that the log linear models for *E. coli* and salmonella might not be valid and don´t sufficiently describe the pathogen reduction. (Minitab Inc, 2014)

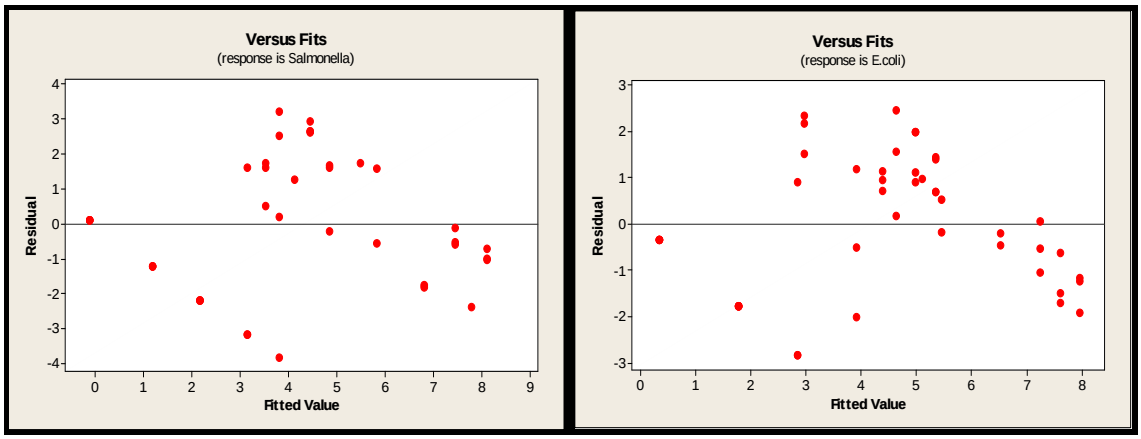

Figure 7 Residual plots: 1.5% urea+3% $CaCO_3$ (as a representative example for all treatments) for *Salmonella* spp. (left) and total thermotolerant coliform bacteria (*E. coli*) (right)

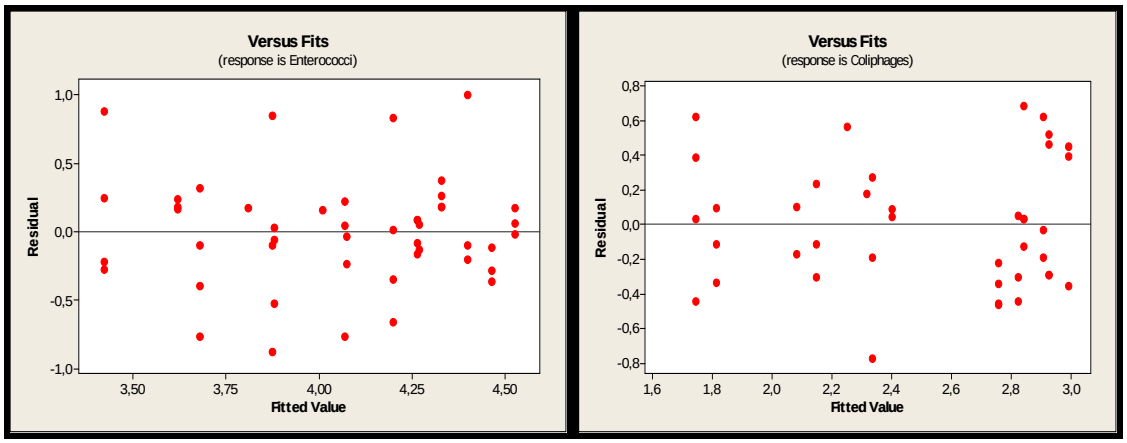

Figure 8 Residual plots: 1.5% urea+3% $CaCO_3$ (as example for all treatments) for enterococci (left) and somatic coliphages (right)

Ascaris suum

The viability of *Ascaris suum* eggs in the experiment showed a high variability within a treatment over the three trials (Table 7). Due to high variation in viability within the same treatment between trial 1-3 and because of the different temperature development between the trials of the same treatment single values or ranges are presented instead of summarising statistics. Comparing the control with the initial viability of 86%, there is a 15-44% reduction due to the compost process itself. Each chemical treatment gave an additional reduction of ascaris egg viability compared to the control. For the 1% urea treatment, all three trials had similar results with 37-45% viable eggs, which is a slightly lower viability than the control. The higher urea content of 1.5%, resulted in a total inactivation with no viable eggs in trial 2 and only 3% viability for trial 3a. However, trial 3a is completely contradictive to 3b with 48% viable eggs, that is more in the range of the first trial. Excluding trial 1b replicate (72% viability eggs), the 1% urea+2% ECOX treatment shows the tendency of reaching a lower viability than 0.5% urea+2% ECOX: 6-36% (1% urea) instead of 29-51% viable ascaris eggs (0.5% urea). The urea-carbonate amendment (1.5% urea+3% $CaCO_3$) shows around 50% viable eggs in all trials, except for 2a. This is questionable, especially as trial 2a shows a total inactivation of ascaris eggs (no viable eggs were detected, $\leq$0.1%). The variations between trials within the same treatment can be attributed to temperature variations. Both samples without viable eggs ($\leq$0.1%) were within treatments involving 1.5% urea. Furthermore, the comparison of 1% urea with or without ECOX tends towards a better effect with the combined treatment.

Table 7 Viable *Ascaris suum* eggs (%) for treatments and control; trial 1-3 with replicates a and b; Viability calculated as part of initial viability before the experiment (initial viability=86%), 100 eggs counted when larvae were detected, 1000 eggs without larvae and 200 eggs for the initial viability

Treatment	Viability (%)		
	Trial 1	**Trial 2**	**Trial 3**
Control	71	42	56
1% Urea	37	45	41
1.5% Urea	44	≤0.1	3 (a) 48 (b)
0.5% Urea + 2% ECOX	41	29	51
1% Urea + 2% ECOX	36 (a) 72 (b)	13	6
1.5% Urea + 3% $CaCO_3$	50	≤0.1 (a) 51 (b)	53

5. DISCUSSION

5.1. Total Solids (TS) and Volatile Solids (VS)

Measurements at beginning and end of the trial showed a decrease in VS due to ammonia volatilization and composting (Table 3, Table 10). The decrease in VS during the composting phase can be explained by the compost process itself, with decomposing organic matter, and ammonia volatilization due to high temperatures. It seemed like, the more VS decreased in the chemical treatment phase, the higher the ammonia losses. Low changes in VS over time indicated that less ammonia was lost by volatilization. To differentiate ammonia volatilization from decomposition of organic matter within the VS decrease, the VS of treatments could be compared to the control. The control showed a VS decrease of 1-2% within the chemical treatment phase. In contrast to the control, the treatments showed a 1-5% decrease in VS. Some treatments showed similar losses to the control, indicating no or only minor ammonia losses, as 1-2% can be attributed to decomposition. Therefore, higher VS losses than the control suggest ammonia loss.

TS (at the beginning 30-33%) changed during the compost phase for most of the treatments, with an increase of 1-2% TS (Table 3). The highest temperatures were reached within this phase, thus water was evaporated from the moist sewage sludge as observed when opening the vessels on sampling days when the lid was covered with water droplets. The high standard deviation in some cases can be attributed to heterogeneous material, as for each replicate a new sample was taken. Variations between the trials can be explained by using a new batch of sludge for each trial, having slightly different TS/VS starting values.

5.2. Temperature

The temperature development (Figure 3) showed huge variations between trials of the same treatment, causing some difficulties in comparing the effects of chemicals on pathogen reduction. Temperature development in the chemical treatment phase showed small peaks due to mixing of the material in the vessels, performed on each sampling day. The oxygen supplied by mixing increased microbial activity causing the temperature peaks.

For composting of sewage sludge, 55°C is often recommended to be maintained over a certain period. In this study, 45 °C was not exceeded and the heating within the short compost phase was not considered as main treatment factor. The chemical treatments were added after reaching 40°C, to provide no disturbance for heat development during composting. In a previous study at SLU, urea amendments, especially combined with ECOX, seemed to have a negative impact on the first heat development at compost start-up (Annika Nordin, personal communication, 02/2014). Having the chemicals added at compost start do also risk larger losses of ammonia, due to the high compost temperatures.

5.3. pH

The control shows the beginning of a typical pH development. Composting, typically starts with a first pH drop due to production of organic acids, carbon dioxide and nitrification, followed by an increase in pH and stabilisation at the end phase. Within the two weeks experiment, the pH drop could partly be captured, but the period was too short to show the later increase and stabilisation of the pH. The pH was notably increased by urea alone and combined with carbonate or ECOX compared to the control (Figure 4). Vinnerås et al. (2003) documented an increase up to 9.2 one hour after application of 6% urea to faecal matter. In this study less urea was used, but a pH of 9.2 was reached with 1% urea+2% ECOX. After addition, 1% urea+2% ECOX developed the highest pH increase in all trials and stayed the highest until the end of the experiment, except for trial 2 which was higher than the other treatments only until day 8. ECOX+urea seem to have a slightly better impact on raising the pH than carbonate combined with urea. However, the sole 1.5% urea treatment raised the pH in a similar range as combined with carbonate, suggesting that the combination with carbonate is not necessary for the 1.5% urea treatment. 1% urea and 2% ECOX could reach 0.1-0.3 pH units higher than 1.5% urea over the whole trial period. Considering cost-effectiveness, this marginally higher pH increase with 1% urea+2% ECOX might not be necessary.

5.4. Ammonia

The nitrogen content measured in the sludge after amendment did not correspond to the nitrogen added with urea to the sewage sludge. It can be assumed that ammonia was lost after application during day 1 and 2, when the sludge had the highest temperature and pH, thus accelerating ammonia volatilization. Afterwards, ammonia was lost over the period of the chemical treatment phase, indicated by lower nitrogen amounts on day 14 compared to day 2 (Figure 5). The gas could possibly be lost when opening the lid for sampling or because of insufficient sealing of the lid with parafilm. The amount of ammonia being lost, randomly varied between the chemical treatments and trials.

The control showed the lowest nitrogen losses over the two weeks experiment (Figure 5). This can be explained by generally lower temperature development in the anaerobic treatment phase and significantly lower pH between 8.2 at the beginning and down to 6.5 at the end. Both, lower temperature and lower pH decreased the risk of ammonia volatilization in the control. Moreover, the control did not receive any urea addition, hence less ammonia was contained in the material, which could be lost.

5.5 Treatment effects and organism inactivation

Five treatments were tested for the best possible effect on sanitising sewage sludge (Figure 6). Five microorganisms were used as indicator or model organisms for determining the sanitisation in the experiment. It could be shown, that total thermotolerant coliform bacteria (TTC) and salmonella were reduced to the detection limit within each treatment which was a reduction that was significantly different from the control (Table 6). Yet, the 1.5% urea solely and combined with 3% $CaCO_3$ tended to reach the quickest reduction for TTC (D_R=1.5) compared to the other treatments

(D_R=1.6-1.8) (Table 5). The additional carbonate amendment would therefore not be necessary. For salmonella, the shortest D_R and therefore the best effect was reached with 1.5% urea and 1% urea+2% ECOX (D_R=0.8) and also 1.5% urea+3% $CaCO_3$ (D_R=0.9). The reduction time also shows that the addition of ECOX and carbonate is not necessary since 1.5% urea solely applied gave the same result. Though, the combination with 2% ECOX, halved the D_R of 1% urea solely to 0.8 days and indicates an improved effect on salmonella inactivation (Table 5). For salmonella enrichment, methods giving different detection limits were used: Day 7 with 1 CFU per 10 g (trial 1 and 2) and on day 14 with 1 CFU per 50 g ww sewage sludge (trial 3). Using 50 g sample for enrichment, salmonella had a lower detection limit. Although the 1.5% urea treatment showed an overall fast salmonella reduction trend, the detection limit of 1 CFU per 50 g was not reached in trial 3 on day 14. In contrast, 1 CFU per 50 g could be reached with 1.5% urea+3% $CaCO_3$ and 0.5%/1%+2% ECOX. The data point at day 14 for salmonella with 1.5% urea (trial 3) was excluded from the regression line, as it deviates from the previous inactivation trend and when comparing to trial 1 and 2, or other treatments. Explanations for the exceptional data point for 1.5% urea could be contaminated MSRV or XLD plates. Such contamination might be caused within handling. Several factors can play a role for a different outcome, but 1.5% urea, solely or combined, showed over all three trials and compared to the other treatments the best effect on salmonella and TTC.

Enterococci and phages in treatments had a reduction which however was not significant compared to the control (Table 6). A 3 log_{10} reduction for enterococci was not reached in the experiment and phages were not reduced to the detection limit. The 1.5% urea treatment seemed to have the best effect on enterococci (D_R=10.9), however only of minor extend compared to control and other treatments, as they all showed similar reduction (Figure 6). In the control, other factors than those from the chemical amendments was effecting the inactivation. In previous studies of urea sanitisation, enterococci were able to grow at low ammonia concentrations at 24°C and showed higher sensitivity to temperature than ammonia (Nordin, 2010). This could explain the slightly positive increase (approx. 0.5 log_{10} CFU/g) of enterococci with 0.5% urea+2% ECOX (Figure 6). Trial 3 with 0.5% urea+2% ECOX, reduced *Enterococcus* spp. 1 log_{10}, whereas trial 1 and 2 show a slight increase instead of reduction. However, the control (D_R=16.6) did not show any increase in Enterococcus like 0.5% urea+2% ECOX (D_R=32.4) and was reduced similar to the other treatments. Somatic coliphages on the other hand, showed the best effect with 0.5% urea+2% ECOX (D_R=8.5) and 1.5% urea (D_R=9.4). However, similar to enterococci, control (D_R=10.4) and treatments differed only to a minor extend from the best D_R.

Ascaris spp. had the largest egg inactivation with 1.5% urea solely and combined with $CaCO_3$, especially in trial 2, where no viable eggs could be detected at day 14 (Table 7). When considering the temperature in these treatments, trial 2 reached the highest temperatures which were maintained until day 4: 40°C with 1.5% urea+3% $CaCO_3$ and approximately 37°C with 1.5% urea (Figure 3). Trial 1 and 3 for 1.5% urea+3% $CaCO_3$ still showed 50% viability and a temperature decrease from day 1 with <40°C to 27°C on day 4. Higher temperatures over a longer period might have played an additional role for egg viability reduction to 0%. The temperature effect can also be seen with only 3% viable ascaris eggs in trial 3a with 1.5% urea. Temperature development of

trial 3a (1.5% urea) maintained 32°C until day 7, which was much higher than trial 1 and 3b with approximately 40% viable eggs. Thus, a different temperature development caused considerably different viability rates within the same treatment. The 1% urea+2% ECOX treatment showed rather similar temperature development throughout the trials, except for trial 1b. An average viability of 18% (36%, 13%, 6%) was achieved indicating 1% urea+2% ECOX reached lower viability rates than 1% urea alone, which resulted in an average of 41% viable eggs. Therefore an increase of urea to 1.5% combined with 2% ECOX might be an interesting combination for further testing. Considering cost-effectiveness, however, this would be questionable, as ECOX presents higher costs for the treatment, and if urea solely (≥1.5%) can reach the same reduction, it will be more favorable regarding the cost.

5.5. Sewage sludge as fertiliser

No treatment could completely fulfill the proposed Swedish demands on class A biosolids (2010) concerning enterococci (<1000 g^{-1} TS). Only 1.5% urea solely or with $CaCO_3$ and 1% urea+2% ECOX showed the potential of reaching lower concentrations of enterococci (10^2) in trial 2, due to higher temperatures being maintained for a longer period than in trial 1 and 3. For total thermotolerant coliform bacteria, the Swedish demands of 2010, <$10^3 g^{-1}$ TS, could be fulfilled with all chemical treatments. For Class A sewage sludge, no salmonella should be detected in 25 g ww sludge. In trial 1 all chemical treatments reached <1 CFU per 10 g (as it was not tested for 50 g) and class A could depending on the salmonella concentration be expected, but not verified. In trial 2, a salmonella concentration of <1 CFU/50 g was reached in all tested treatments fulfilling the Swedish recommendation, while in trial 3, 1% urea did not reach class A, whereas all ECOX related treatments and 1.5%+$CaCO_3$ did. 1.5% urea solely reached salmonella concentrations between 1 CFU/10g and 1 CFU/50 g ww sludge and it can be assumed to fulfill Swedish obligations. Bacteriophages are not included in class A recommendations. However, considering the relatively high resistance of somatic coliphages in this experiment, bacteriophages can be a valuable addition to pathogen recommendations to draw conclusions about the occurrence of enteric virus. Including phages as virus indication can improve the risk determination of sewage sludge and other substances.

5.7. Recommendations and Shortcomings

The three trials show high variations for the same treatment, leading to difficulties when comparing the effectiveness of amendments. Also within trials, i.e. same sludge used, large differences between replicated treatments were observed, especially for the temperature development and *Ascaris suum* egg viability. Problems like this show the high dependence on similar conditions within an experiment. Enterococci showed the highest resistance to the treatments. Recent experiments on urea effect during different temperatures demonstrated a 3 $\log_{10}$ reduction of enterococci within one week at 41.5°C with 0.5% urea or at 38°C with 1.5% urea (Fredrik Wirell, personal communication, 08/2014). Thus to reach sufficient enterococci reduction, the temperature hast to be maintained longer time at a high level (>38°C).

The experiment and its treatment could be improved in several ways:

1. Sealing of the 1 L vessels was improvised for each vessel separately by adding layers of parafilm or a plastic bag in between opening and lid. Insufficient sealing might be the reason for temperature variations, when oxygen could still get into the vessels. By providing a proper sealing, similar for each vessel the experiment could be improved.

2. Another aspect is the length of the composting phase at beginning. One additional day could be given for the compost process to maintain a high temperature over a longer period, which could increase the reduction of enterococci and somatic coliphages.

3. In this small-scale experiment with only 5 kg being composted at start, the fast temperature drop on day 2 might not be a problem for a large-scale trial. A bigger volume of sewage sludge (tons) has a higher potential for heat conservation after composting and hence, might maintain a higher temperature for longer time within the chemical treatment phase.

6. CONCLUSION

All chemical treatments resulted in significant reduction of total thermotolerant coliform bacteria and *Salmonella* spp. reaching detection limits within two weeks, though with the best effect using 1.5% urea without other chemicals. Somatic coliphages reached a 1-2 $\log_{10}$ reduction in both treatments and control. Nor for enterococci, did the treatments have any significant effect compared to the control and only a 1 $\log_{10}$ reduction was achieved over two weeks. The hypothesis of reaching the detection limit for all indicator and model organisms within the two weeks experiment could not be confirmed for any treatment and only TTC and salmonella were significantly reduced by all chemical treatments. The viability of *Ascaris suum* eggs was reduced in all treatments, yet without consistency. No viable eggs were detected with 1.5% urea and 1.5% urea+3% $CaCO_3$, when temperature was maintained over 35°C during four days. The nitrogen applied in form of urea, was partly lost within the experiment, but only to a minor degree in most cases ($\leq$10%, maximum 30%). All chemical treatments increased the pH up to 9 ± 0.2 and the highest values were reached with 1% urea +2% ECOX. Regarding the hypothesis of an additional benefit for pH development with $CaCO_3$ or ECOX, ECOX showed the best result. Carbonate did not give an additional pH increase to the urea treatment. Considering treatment efficiency and cost-effectiveness, the best sanitisation result in this study was achieved with 1.5% urea.

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8. APPENDIX

Table 8 Results from regression analysis for indicator organisms and treatment. Significance of treatment compared to the control, p-values

Treatment	TTC	Salmonella	Enterococci	Coliphages
1% Urea	0.001	0.001	0.880	0.987
1.5% Urea	0.001	0.001	0.109	0.125
0.5% Urea+2% ECOX	0.001	0.001	0.010	0.320
1% Urea+2% ECOX	0.001	0.001	0.373	0.711
1.5% Urea+3% $CaCO_3$	0.001	0.001	0.123	0.589

Table 9 Total Solids (TS) and Volatile Solids (VS) for day 0 and 1, trial 1-3

Trial	Day 0 (Salmonella enriched sludge)		Day 1	
	TS %	**VS %**	**TS %**	**VS %**
1	29,93±0,30	54,43±0,77	31,69±0,14	50,61±0,34
2	31,83±0,48	51,35±1,57	32,80±0,97	49,81±1,67
3	32,85±0,36	52,67±1,48	34,95±0,00	49,06±0,00

Table 10: Total Solids (%) and Volatile solids (%) on day 2 and 14 for each trial and treatment with standard deviation (±)

	Day 2						Day 14					
	TS (%)			VS (%)			TS (%)			VS (%)		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Control	31,9±0,3	32,3±0	33,3±0,1	50,3±0,8	49,3±1,9	50,7±0,4	31,2±0,4	30,9±1,3	33,8±0,04	48,2±1,2	48,9±3,1	52,0±1,1
1% Urea	31,4±0,3	31,3±0,7	33,9±0,6	51,7±1,9	53,3±0,6	48,8±3,6	31,2±1,7	29,5±1,5	32,7±0,6	46,1±2,9	51,9±2,9	52,3±1,6
1,5% Urea	31,6±0,4	31,0±0,2	(a)32,8±0,2 (b)33,9±1,2	49,4±1,6	53,7±0,8	(a)51,8±0,4 (b)49,6±4	30,3±0,03	29,9±0,4	(a)33,2±1,3 (b)28±4,5	49,1±0,2	51,7±0,8	(a)51,0±0,9 (b)47,9±8,9
0,5% Urea+2%ECOX	32,1±0,2	31,8±0,1	34,0±1,2	47,9±0,7	50,6±0,4	48,2±2,5	30,2±0,2	31,5±1,5	29,2±3,7	47,2±0,2	48,9±2,9	49,1±1,3
1% Urea+2% ECOX	(a)31,7±0,1 (b)31,9±0	30,7±0,9	33,6±0,3	(a)48,6±0,1 (b)48,8±0,1	52,0±0,6	49,7±0,02	(a)30,7±0,8 (b)30±0,1	30,0±0,8	33,4±0,7	(a)46,1±2 (b)48,3±0,02	50,7±0,5	51,1±1,7
1,5% Urea+3% $CaCO_3$	33,5±0,2	(a)33,3±0,3 (b)34,2±0,7	34,9±0,2	46,1±0,6	(a)48,7±0,4 (b)47,6±0,4	47,7±0,3	33,9±2,5	(a)32,7±0,3 (b)31,1±0,3	33,5±0,2	41,4±3,9	(a)48,9±1,2 (b)49,1±1,6	49,0±0,3

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