Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Institutionen för energi och teknik

Safe Retrieval of Nutrients to Improve Food security

Marcus Hjalmarsson

Examensarbete 2012:10
ISSN 1654-9392
Uppsala 2012

SLU, Sveriges lantbruksuniversitet
SUAS, Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Sciences
Institutionen för energi och teknik
Department of Energy and technology

Swedish titel: Säker växtnäringsåtervinning för ökad livsmedelssäkerhet

English titel: Safe Retrieval of Nutrients to Improve Food Security

Marcus Hjalmarsson

Supervisor: Annika Nordin
Examiner: Björn Vinnerås
Credits: 15 ECTS
Nivå: G2E
Course titel: Självständigt arbete I biologi - Kandidatarbete
Course code: EX0689
Programme/education: Agronomprogrammet - Livsmedel

Place of publication: Uppsala
Year of publication: 2012
Titel of series: Examensarbete 2012:10
ISSN 1654-9392

Keyword: Ammonia, Food Safety, Food Security, Sanitation, Urea

Elektronisk publicering: http://stud.epsilon.slu

Sammanfattning

Säker återföring av näringsämnen genom att gödsla åkrar med mänsklig avföring kan ge ökade skördar och komma att öka livsmedelssäkerheten i jordbruksbygder med näringsfattiga jordar. Säkra och näringsriktiga livsmedel kommer att uppfylla den individuellas näringsbehov, vilket främjar folkhälsan och förmågan till ett aktivt liv. Mänsklig avföring borde ses som en resurs istället för att antas vara avfall. Fekalier och urin är utmärkta gödningsämnen som kommer att sluta näringskretsloppet om det används korrekt. Dock behövs avföringen hygieniseras för att förhindra att sjukdomar sprids genom att reducera antalet gastrointestinala patogener som bakterier, virus och parasiter.

Syftet med den här studien var att i liten skala utvärdera hygieniseringen i en kombinerad våtkompost- och ureabehandling genom att följa inaktiveringen av bakterier och virus vid tre olika starttemperaturer, 45, 40 och 35 °C, och med tre olika mängder av tillsatt urea, 0,5, 0,75 och 1 %, och en referens med 0 % tillsatt urea. Substrat buffrat till pH 9 användes också som en referens. Urea tillsattes till redan komposterat materaial och temperaturen sänktes efter start av behandlingarn 1°C per dag. Den mikrobiella reduktionen av *Enterococcus* spp., termotoleranta coliforma bakterier, *salmonella* och colifager övervakades med plattmetoder.

Urea tillsatsen resulterade i en ökning av pH och ammoniakkvävehalten, som orsakade i olika grad inaktivering i de olika temperatur- och urea behandlingarna. För att få en tillräcklig inaktivering av bakterier inom en veckas behandling måste avföringen behandlas med minst 0,5 % urea vid minst 40 °C starttemperatur.

Abstract

Safe retrieval of nutrients from human excreta to fertilize crops in order to gain adequate yields will increase food security and sustainability in agriculture areas with nutrient-poor fields. Safe and nutritious food will meet the dietary needs of the individual which promotes public health and the ability of an active life. Human excreta should be considered as a resource instead of being assumed as waste. Human faeces and urine is excellent fertilizers which may close the nutrient loop if utilized properly. However, sanitation of the excreta is required to prevent disease transmission by reducing the content of gastrointestinal pathogens such as bacteria, viruses and parasites.

The objective of this study was to in small scale evaluate the sanitation in a combined liquid compost and urea treatment by monitor the inactivation of bacteria and viruses at the combination three start temperatures, 45, 40 and 35 °C, and three additions of urea, 0.5, 0.75 and 1 % and reference, 0 % urea addition. A inoculate buffered to pH 9 was used as an additional reference. Urea was added to already composted material and from the start of the treatment temperature was decreased 1 °C per day. The microbial reduction of *Enterococcus* spp., thermotolerant coliform bacteria, *salmonella* and coliphages was monitored by plate count methods together with pH and TAN.

Urea addition resulted in an increase in pH and TAN and caused inactivation of various degrees among the different temperature and urea addition treatments. To obtain a sufficient inactivation of bacteria within a week of treatment the excreta has to be treated with at least 0.5 % urea and at least 40 °C initial temperature.

Key words: Ammonia treatment, Food Safety, Food Security, Hygiene, Sanitation, Urea

Table of contents

1 INTRODUCTION

World Food Summit in 1996 defines food security as: “Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs, and food preferences for an active and healthy life.” To gain food security three requirements must be fulfilled. Food availability is where the actual physical presence of food in the area of concern. It takes in consideration all kinds of domestic production, imports or food aid, and is therefore conditionally dependent on production of food in the area, trade in the manner that food is brought into the area and also stocks and food which are supplied by the government. Food access describes a household’s capacity to gain adequate amounts of food. The food could be gained from own home production, purchases, exchanges, gifts, borrowing and food aid. Food utilization illustrates the use of the food which is in access. Both in how the food is processed with focus on different parameters, such as production, storage, preparation and hygiene conditions and the ability for individuals to absorb and metabolize nutrients. The dependency of food production is crucial and therefore cannot the trade, government supplies etc. be fulfilled if the production of food declines(WFP, 2009). Lowering the costs of inputs and increasing the production of food per unit space land by obtaining a sustainable and safe fertilizer will increase access to affordable and more nutritious food (WHO, 2006).

Since the world’s population is increasing there will be an increasing demand for food and fibre, furthermore a greater population will generate more waste, such as excreta and greywater. With the focus on the production of food to increase the food availability, the recognition of using human excreta and greywater as soil fertilizing agents has grown (WHO 2006). One of the driving forces behind the increased use of excreta and greywater is the knowledge that they improving soil fertility by containing nutrients and add humus-like substances (Schönning et al.,

2007; WHO, 2006). Urine and faeces are fertilizers of high quality and gives the best fertilizing affect when used together (Jönsson, Richert Stintzing, Vinnerås, & Salomon, 2004). Recovery and recycling of nutrients in the form of human excreta and other organic matter does not just provide complete nutrition for the plants and crops. By using excreta the input cost for production of food can be lowered. This opens the possibility of growing more food per unit space and raise access to a greater amount and more nutritious foods (WHO, 2006). The human to land nutrient loop might be closed if using the excreta as fertilizer for food production (Schönning & Stenström 2004).

However gastrointestinal infections are widespread throughout the world and the enteric pathogens might be found in high concentrations in faeces. The pathogens in the form of bacteria, viruses and parasites results in a wide variety of illnesses like diarrhoea and malnutrition. Pathogens in fresh faeces may be immediately infectious, require time outside a host to become infectious or require an intermediate host or vector prior to becoming infectious (Winblad & Simpson-Hébert, 2004). A main objective for increased use of human excreta in crop production is that it not should end up with worse disease transmission and an increased number of infections (Schönning & Stenström 2004). Excreta associated infections are common in human populations and are related to high concentrations of excreted pathogens (WHO, 2006) and the incidence of infections reflects the hygienic situation among the population. In order to produce safe food, not containing any pathogens or at least an amount which is not harmful for the population, sanitation is necessary. A sustainable sanitation prevents disease and promotes health, protects the environment and conserves water, which opens the possibility to recycle nutrients and organic matter (S. Esrey, 2001). Sanitation is obtained by construct barriers against disease transmission. These barriers could be treatment of the excreta, reducing contact with the excreta or proper handling of foods from fields fertilized with human excreta (Schönning & Stenström 2004).

Treating excreta early inactivate pathogens before further handling and prevents transmission of diseases and reduce the importance of other barriers. Such treatments can be composting inactivating pathogens by heat or ammonia based treatment which gives increased pH and concentration of ammonia (Björn Vinnerås, Holmqvist, Bagge, Albihn, & Jönsson, 2003). In the progress of developing new methods for sustainable sanitation a method of liquid compost with an addition of urea has been constructed as a primary treatment. Heat, which can be obtained in

compost, has been shown to be one of the most effective ways to inactivate pathogens (Schönning & Stenström 2004). Heat together with an additive like urea would give synergies. Higher temperature and also a greater addition of urea would result in more effective inactivation. Still, in order to make the treatment more sustainable it is necessary to optimize the treatment, by e.g. reducing the temperature or reducing the addition of urea, to reduce energy expenditure and transports (Nordin, personal communication).

2 OBJECTIVES

The main objective of this study was to evaluate a liquid compost treatment at three different temperatures combined with three different additions of urea by monitoring the inactivation of bacteria and viruses.

3 BACKGROUND

3.1 Pathogens, their transmission routes and barriers

The pathogens of major concern and one of the reason for require sanitation systems are enteric infection causing bacteria, viruses and parasites. The common gastrointestinal symptoms are diarrhoea, vomiting and stomach cramps, but may also affect other organs and cause severe impacts. A way of decrease the pathogen transmission and identify the actual hazard is to distinguish epidemiological types, persistence in different environments, transmission routes, various treatment techniques and manage control measures (WHO, 2006).

World-wide, faecal bacteria are believed to be a major cause of gastrointestinal illness. Among bacteria, *Salmonella spp.*, *Campylobacter spp.*, enterohaemorrhagic *Esherichia coli* (EHEC) and *Yersinia spp.* are important in both industrialized and developing countries. In areas with insufficient sanitation *Salmonella typhi* and *paratyphi*, *Vibrio cholera* and *Shigella spp.* are major risks in relation to improper sanitation and contamination of water (Schönning & Stenström 2004; WHO, 2006).

Viruses transmitted by faeces may be an enteropathogenic virus such as astrovirus, calicivirus and rotavirus or non-enteropathogenic viruses such as adenovirus, enterovirus and hepatitis A/E virus. Viruses can in general sustain longer in the environment than non-spore-forming bacteria (Nordin, 2010). Viruses may be zoonotic e.g. hepatitis E which might infect both humans and pigs (B Vinnerås, Clemens, & Winker, 2008).

Parasites like protozoa and helminths are of concern because of their high environmental persistence and low infectious doses. *Cryptosporidium parvum* is a pro-

tozoa which is associated with waterborne enteric outbreaks. *Giardia intestinalis* is another pathogenic protozoa. The helminths *Ascaris* and *Taenia* are regarded as indicator organisms and is an index of hygienic quality (WHO, 2006).

Viruses and parasites can infect at low doses, as low as 1 unit, and persist in the environment for long (Höglund, 2001), while bacteria have an infectious dose at approximately 10^6 units. Even though virus has a lower infectious dose than bacteria, bacteria are able to reproduce outside a host.

The pathogens of concern and the reason for require sanitation systems are usually transmitted through the faecal-oral route (Schönning & Stenström 2004), however some pathogens infect through the skin while others infect when meat from infected animals is consumed. Overall, when using excreta as a fertilizer, the most common transmission route and source of infection for humans is through contaminated food (Nordin, 2010). Approaches to prevent disease transmission from excreta are to make up barriers. (Figure 1) Common barriers are treatment to remove pathogens, restricted use of the excreta on fields, post-harvest processing and food hygiene (Nordin, 2010; WHO, 2006). The most effective barrier before even spreading potential pathogens in the environment is to sanitise the material. Pathogens gets eliminated or reduced in numbers. (SEPA, 2010). Application of excreta on agricultural land will also lead to inactivation. The inactivation is dependent on ambient temperature, moisture and sunshine. Microorganisms in the soil will compete with the introduced pathogens and therefore enhance the inactivation (Schönning & Stenström 2004).

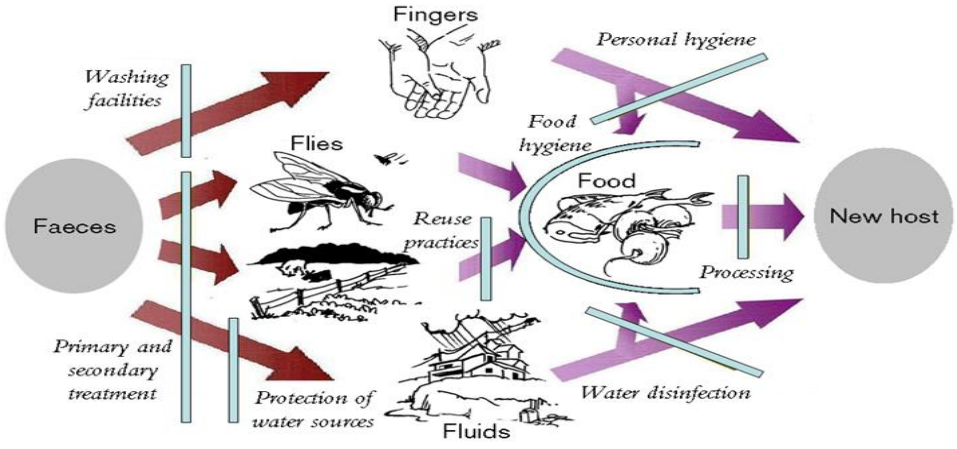

Figure 1. Disease transmission routes and barriers for disease transmission. (Nordin, 2010)

3.2 Mesophilic compost and chemical sanitation to inactivate pathogens

Mesophilic compost does not achieve the same sanitation as thermophilic compost do, mainly because of not reaching elevated temperatures (Jönsson, et al., 2004). In order to achieve successful sanitation, alkaline material or chemicals that elevate the pH can be added.

Urea addition to the compost will sanitise by supplementing the material with ammonia which is toxic to microbes (Björn Vinnerås, et al., 2003) but volatile for the process. It is therefore important to perform this treatment in a closed container to minimize ammonia losses. Ammonia losses could have negative impact on the level of sanitation and on the performance as a fertilizer (Jönsson, et al., 2004). Urea addition is considered an efficient treatment for attaining hygienically safe faecal matter (Björn Vinnerås, et al., 2003).

3.3 Analysing the prevalence of indicator organisms to determine treatment efficiency

Due to limitations in time, analytical methods and the cost of numerous methods for identifying all pathogens in excreta, indicator organisms are used as representatives for pathogens which not always are present in a material. The inactivation of an indicator organism indicates a functioning treatment and decreased risk from pathogens in the material. It is therefore also important that indicator organisms have similar growth rate and inactivation rate as the pathogens in order to evaluate the efficiency of sanitation treatments.

To assess the potential occurrence of pathogenic bacteria in faecal matter the concentration of indicators such as faecal bacteria as Enterococcus spp. can be monitored (Nordin, 2010). Furthermore total thermotolerant coliforms, which is the historically used indicator of faecal contamination, (Höglund, 2001) can be used.

The presence of pathogenic viruses may be indicated by the persistence of bacteriophages. Bacteriophages are viruses that infect bacteria and are thus not harmful to humans. Viruses are smaller, have greater environmental persistence and behave differently than bacteria. Bacteriophages make it possible to predict the presence, survival and possible transport of human enteric viruses in the environment (Höglund, 2001).

3.4 Regulations and legislation

Potential occurring pathogens in faeces must be reduced in order to minimize the risk of further transmission in the environment if using it as a fertilizer. An increased incidence of disease is at risk when using unsanitized faeces (Schönning & Stenström 2004). One fundamental criteria for a sanitation system is to prevent disease (Winblad & Simpson-Hébert, 2004). Regulation of how the sanitation should be performed and how well it protects the surrounding environment and populations are based on epidemiological evidence, risk assessment predictions, guideline values and performance. Sanitation systems can be evaluated with quantitative microbial risk assessment measures. An estimation of a risk is often based on a validated prediction and belief, but may sometimes overestimate the risk because of variations in behaviour, exposure and other outer factors. Risk assessment may also have limitations valuing the risk, for instance, in their way of solely look for indicator organisms instead of the range of present pathogens. Risk assessment should also be based on background rates of possible diseases. The estimations of risks of infection are related to the use of exposure barriers and the validity of treatments (WHO, 2006).

To regulate risks of disease transmission Swedish Environment Protection Agency has brought up a proposal how to treat and use sewage fractions. According to SEPA (2010) should treatment quality be divided up in two sanitation classifications. Classification A signifies sewage fractions which have been treated at a temperature above 50 °C during a certain amount of time. The application possibilities are varied, because of supposed less survival capacity of pathogens. Classification B concerns defined processes which treat the faeces fractions with a temperature below 50 °C during a longer period of time than class A. B-fractions do not have same application possibilities as A-fractions (SEPA, 2010).

The use of animal excreta outside the farm where it is produced is regulated by the European commission regulation No. 208/2006. The European Commission (2006) states that validation of a process must demonstrate that the process achieves the following overall risk reduction: Reduction of 5 log10 of *E. faecalis* and *Salmonella senftenberg*. In a class A fraction the content of *E. faecalis* should be less than 1000 bacteria in 1 g dry matter. Reduction of infectivity titre of thermo resistant viruses such as parvovirus should be at least 3 log10, whenever they are identified as a relevant hazard. The indicator should be consistently present in high concentration in the raw material. SEPA (2010) suggests a quality demand

that salmonella should be absent in 25 g material. Salmonella analyses might give additional information about contamination in a facility which might be correlating to the handling of the material. *E. coli* must have a content less than 1000 bacteria in 1 g dry matter.

Application restrictions for A and B fractions is considered as an additional barrier for further exposure if the sanitation method did not fulfil to sanitize the sample. Nondependent of which class the fraction should not be used on crops destined for fodder or food (SEPA, 2010).

There is room for new and alternative methods to sanitize human excreta. According to 11§ in the regulation proposal from 2002 in SEPA (2010) is it possible to submit an application to be approved regarding alternative sanitising treatments.

3.5 Food safety to realize the food security requirements

Excreta and potential fertilizers from sanitation systems introduce a new and promising handling of nutrient resources. The implementations of fertilizers with faecal origin introduce new transmission routes for infectious diseases. Applications must be adjusted and risks have to be minimized with appropriate measures to obtain successful fertilizing products for agricultural use (Winker, Vinnerås, Muskolus, Arnold, & Clemens, 2009). In Sweden, application restrictions such as not using the excreta on food aimed for raw consumption are recommended in the current proposal for regulation of the use of sewage fractions as fertiliser (SEPA, 2010). Effective sanitation and hygiene programmes may be combined to grow safe crops. People need to understand why better health depends on the implementation of hygienic practices such as hand washing, sanitation systems for excreta and greywater, and safe storage of drinking-water and food (WHO, 2006). The ways in which the food is stored and processed might have a sanitation effect as well. The knowledge in utilize process techniques of food in order to sanitize them in favour for more hygienic conditions will possibly have health benefits for the populations in question (WFP, 2009; WHO, 2006).

4 Materials and Methods

4.1 Experiment

To evaluate different start temperatures in combination with different urea additions compost material from a full scale reactor were used in a laboratory scale experiment simulating the temperature decline in the reactor. Three urea treatments (0.5, 0.75 and 1 % urea), along with two references (0 % urea and pH 9) were studied at starting temperatures 35, 40 and 45 °C, with a daily 1°C falling temperature (Figure 2).

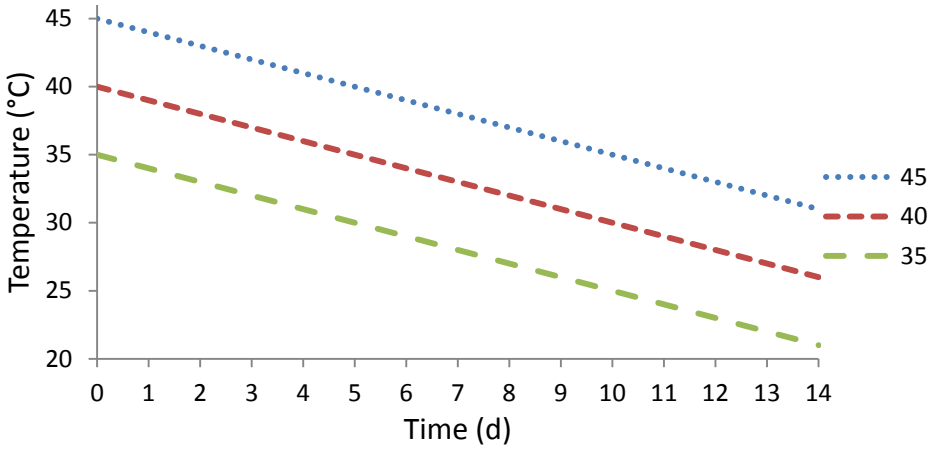

Figure 2. Temperature decline over time with starting temperatures 35, 40 and 45 °C.

4.2 Compost material

The material used in the present study were collected from a compost rector located in Kvicksund, at the border of the municipalities of Eskilstuna and Västerås (lat

59° 41´ 13.24” N, long 16° 33´ 83.33”E), Sweden. The treatment system was taken in use in 1998 and is constituted of a 92 m3 collection basin, a 32 m3 reactor (heigth 7.5 m, Ø 3m; Alfa Laval Agri) and a 1 430 m3 covered post-treatment storage basin. The normal use of the system is to compost source-separated toilet-waste, form on-site sanitation systems and from a nearby school where also some kitchen waste grinded and mixed into the toilet waste, together with manure slurry or latrine buckets from summer houses to reach TS of approximately 4%. The normal treatment is semi continuous with thermal sanitisation (55°C for 12 hours) with estimated energy need for aeration and mechanical equipment of 25 kWh per cubic meter material (Malmén, 1999). Process air is condensed in a peat bio filter which absorbs ammonia from the outgoing air. For the present study the reactor were run batch wise mainly with source-separated toilet-waste from septi tanks from on-site sanitation systems and some mixed waste from the school. The toilet waste was composted to a temperature of 45°C. 3 L material was stored at 4°C from the 29th of March 2012 until the start of the experiment the 11th and the 16th of April 2012.

4.3 Addition of salmonella

According to an ongoing full scale project managed by Annika Nordin (personal communication) the composted material from Kvicksund did contain very low concentrations of salmonella. To monitor the inactivation of salmonella, *Salmonella enterica* subspecies 1 serovar Typhimurium, phage type 178 isolated from sewage sludge. S. Typhimurium was cultivated in 100 ml of nutrient broth (SVA311040) for 18-20 hours to reach a final concentration of 10^8 cfu g-1 suspension. One ml of room temperate enriched strain of S. Typhimurium was added to 1000 g of room tempered compost material so that the initial concentration of S. Typhimurium in the material before treatment was approximately 10^5 cfu/g compost material (ww).

4.4 Treatments

The temperatures were set in two heat blocks in which the tubes were held covered with aluminium foil for a darker environment (Grant Instruments Ltd, England). One heat block was started at 45 °C and the other one at 35 °C and temperatures were lowered one degree a day, to simulate the temperature decline in the full scale reactor. After 5 days of the temperature in the heat block with starting tem-

perature 45 °C had declined to 40 °C and the second set up was started. The temperature in the heat blocks at to levels in a water filled tube was measured with a PC logger 3100 (Intab. Sweden) every minute and recorded as the mean value for each 10 minutes. The temperature logging started after about 12 hours of treatment. In further calculations is the initial temperature the same as the one after half a day of treatment. A mean value of the two temperature measurements is used for further calculations and presentations. The 35 and 45 °C treatments were in progress for 14 days, the 40 °C treatment for 9 days.

The salmonella inoculated material was divided into five aliquots, 200 g each. Three of them were added with 1 g, 1.5 g respectively 2 g of dry urea each (VWR, Sweden) to a final amount of 0.5 %, 0.75 % and 1 % according to the wet weight of the material. The urea was added approximately 3 hours after *S.* Typhimurium was added. One aliquot was used as urea free reference to examine the effect from temperature alone on inactivating potential organisms and the last aliquot used for an ammonia free pH 9.0 reference. To study the same microorganisms the ammonia free pH 9 phosphate buffer (SVA302407) was inoculated 1:100 to a final volume of 500 ml with the last aliquot of the salmonella enriched compost material. The ammonia free pH 9.0 reference examined whether pH 9 alone affects the organisms. The urea treated and the reference aliquots were distributed upon screw-capped polypropylene 57*16.5 mm tubes (Sarstedt AG & Co, Sweden). Of the urea treated material and the references nine tubes were destined for each temperature. The described procedure were conducted at 11th of April for treatments studied at 35 and 45 °C and on the 16th for treatments at 40 °C treatment, but with six tubes per treatment combination due to lack of space in the heat block.

4.5 Microbial analyses

The compost material was analysed before urea addition to obtain the initial concentrations. The treatments at 40 and 45 °C and with a urea addition of 0.75 and 1 % were analysed more frequently (once or twice a day) in the beginning of the experiment due to the predicted fast inactivation of organisms and for less intense treatments and more persistent microorganisms with a lower frequency. The inactivation of the microbes was monitored by growth on each respectively designed medium after specific incubation time and temperature (Table 1). To be able to count the colonies in high concentration, dilution series were made with buffered NaCl-peptone solution with tween, pH 7.0 (SVA301161). The normal procedure is

to add 0.1 ml to SlaBa and XLD (detection limit 10 cfu/ml) and 1 ml to VRG and BAB/softagar (detection limit 1 cfu/pfu /ml). To lower the detection to1 cfu/ml for SlaBa and XLD plates when reaching low concentrations 5 plates were added 0.2 ml material.

Table 1. Medium used for enumeration of the studied microorganisms together with incubation temperature and time.

Microbe	Medium used	Incubation temp and time
Enterococcus spp.	Slanetzy Bartley (SlaBa) Agar (SVA342050)	44 °C 48 h
Total Thermotolerant Coliforms	Violet Red Bile (VRG) Agar (SVA342460)	44 °C 24 h
Salmonella Typhimurium	Xylose-Lysine-Desoxycholate (XLD) Agar with Novobiocin (SVA342521)	37 °C 24 h
Coliphages	*E.coli* ATCC 13706, Soft Agar (SVA331085), Blood Agar Base (BAB) Agar (SVA331020)	37 °C 24 h

Typical colonies growing on Violet Red Bile (VRG) Agar were confimed by growth and gas production in Lactose-Tryptone-Lauryl Sulphate broth (LTLSB) (SVA321290) (44 °C 24 h) and *E. Coli* by adding Kovacs reagent (SVA381730) to the tubes with growth and gas. Typhimurium confirmation was performed in Triple Sugar Iron (TSI) (SVA342420) Agar and Urea Agar Base (SVA321660).

4.6 Chemical analyses

The pH and ammonia (NH_4^- and NH_3) content of the compost material were measured before the treatments started and during the experiment when microbial analyses were performed. The pH was measured directly in the material using a pH meter (PHM 210, MeterLab, Denmark). The ammonia measurement was performed with a photometric method using a test kit: Ammonium Test (Merck, Germany) The absorbance was measured at 660 nm on the spectrophotometer (Genesys 20, Thermo Scientific, USA), and the concentration was calculated from a linear equation obtained from a standard calibration curve.

4.7 Statistical analyses

In order to examine whether values were significantly different or similar to each other regression tests in Microsoft Excel was used. A p-value lower than 5 % prove significance. Microbial concentrations were plotted against time in a first order kinetic function. The equation of the function was used to determine the D-value. To do the inactivation of the microbes visual for the reader in the diagrams the concentrations were normalized for each microbe and treatment.

5 RESULTS

5.1 Initial material

Some characteristics of the composted material from the reactor had changed during the 13 and 18 days of storage at 4 °C. The pH and TAN concentration was higher after storage. The dry matter content was lower than before storage. The volatile matter was not significantly different after the time of storage. The concentrations in the microbial analyses were neither changed after storage. (Table 2)

Table 2. Characteristics of the initial material before storage and before starting the treatments. The salmonella is added the same day as the treatment starts.

	From compost	35/45	40
Storage (d)	1	13	18
pH	7.49	7.78	7.60
TAN (g/L)	0.58	0.76	0.73
Dry matter (%)	0.86	0.69	0.71
Volatile matter (%)	75.1	70.4	78.0
Enterococcus (cfu/g)	$1.4*10^3$	$1.7*10^3$	$1.7*10^3$
Thermotolerant coliforms (cfu/g)	$9.3*10^4$	$2.1*10^5$	$4.6*10^4$
Salmonella (cfu/g)		$5.5*10^5$	$8.0*10^5$
Coliphages (pfu/g)	$1.2*10^5$	$8.9*10^3$	$1.4*10^4$

5.2 Temperature

The temperatures in the heat blocks where decreasing gradually, with slightly more than 1 degree a day. The temperature in the 35 °C levelled out at about 5 °C above room temperature at day 7 since that was lower than the threshold temperature for the heat block (Figure 3).

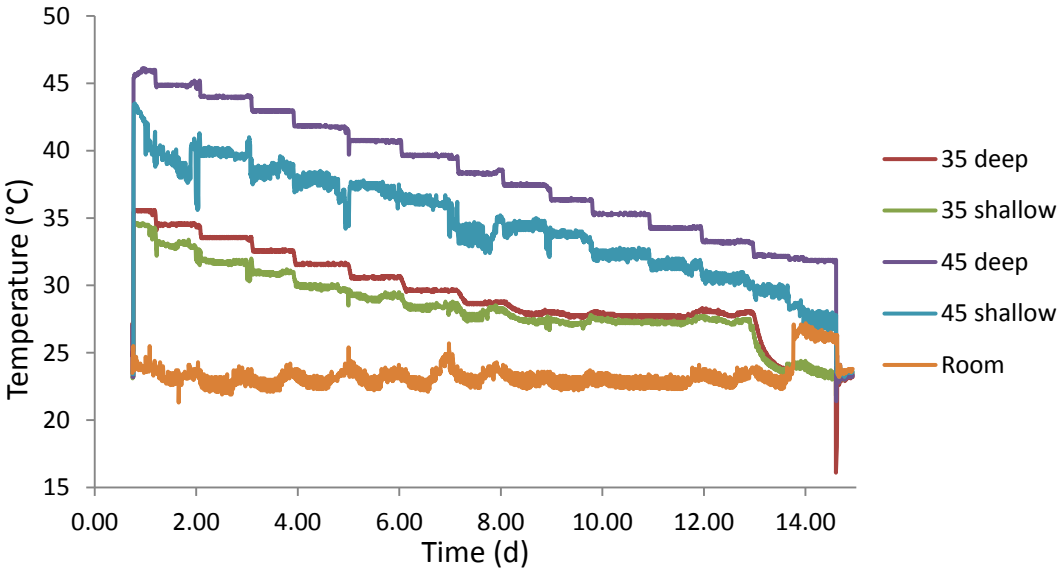

Figure 3. The temperature in the two heat blocks during 14 (35 and 45 °C) respectively 9 (40 °C) days of treatment.

5.3 Ammonia

The theoretical concentrations of ammonia when adding 0.5 % and 0.75 % were estimated to 2.93 g/l and 4.10 g/l, respectively. Addition of 1 % urea should give 5.24-5.26 g/l according to the two starting concentrations.

In the treatments without urea (0 %) at the three temperatures ammonia concentrations varied from 0.56 to 0.80 g/l with no significant trend of increase or decrease in concentration. When urea was added the ammonia concentration increased over time to reach the theoretical concentrations at day 3-5, and during further incubation it exceeded the theoretical concentration. (Figure 4) Treatments with same urea addition did during the incubation at different temperatures reach similar ammonia concentrations.

For the pH 9 reference treatments the compost material was diluted 1:100 with buffer and ammonia concentrations measured during the treatment accordingly low ranging from 0 to 0.1 g/L.

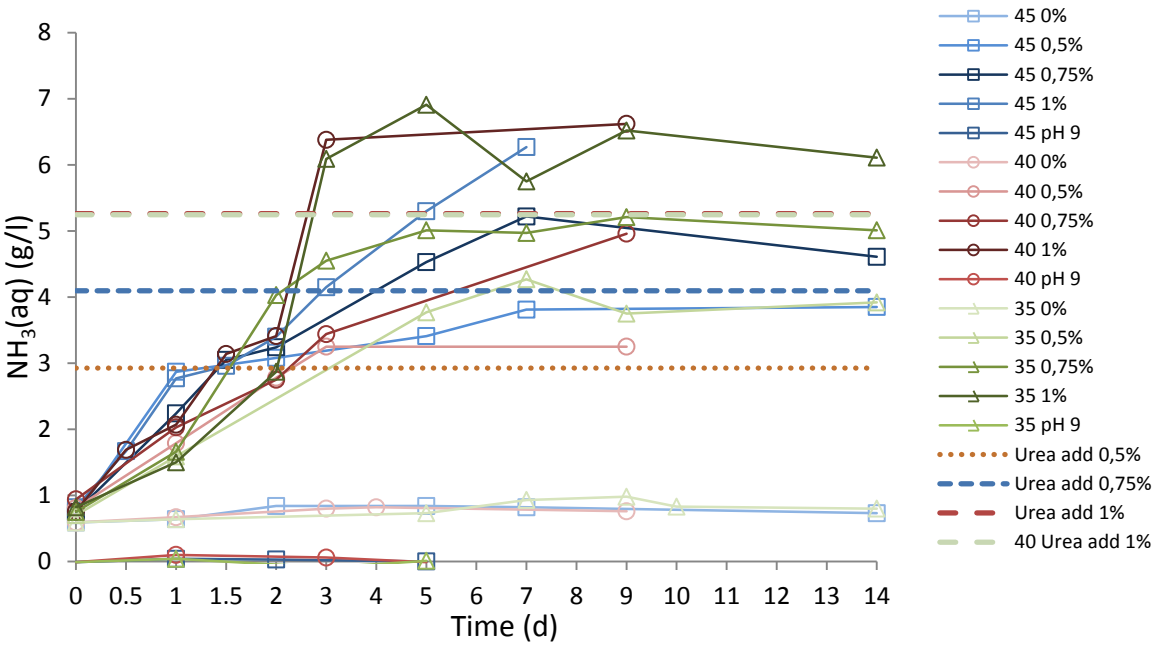

Figure 4. Total ammonia expressed in g/l in different urea treatments during all heat treatments.

5.4 pH

The pH in treatments with urea addition increased from 8.13 to at the most 9.20. The pH increased fast during the first 2 days. These treatments with urea were not decreasing or increasing drastically throughout the next following 7 and 13 days of treatment at 40 respectively 35/45 °C. (Figure 5) The pH 9 reference sample did at all temperatures decrease to a final pH of about 8.50, while the no-urea-treatment decreased the first day and ranged between pH 7.40 and 7.90 throughout the next coming 13 respectively 8 days of treatment.

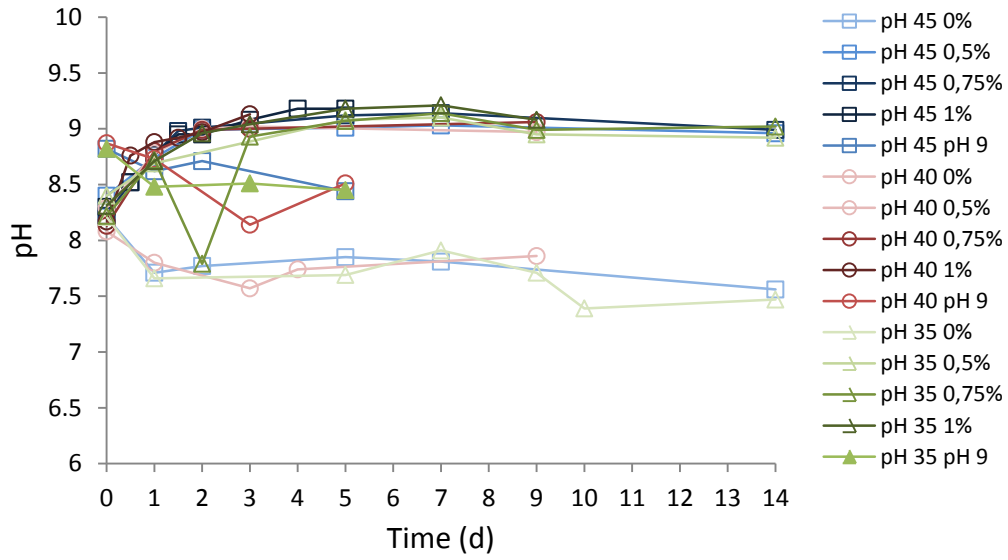

Figure 5. pH during time of the treatments.

5.5 Ammonia and pH

The treatment with 0.75 % urea at 35 °C pH had one measurement with low pH and deviated from the overall pH trend (Figure 5 pH, Figure 6), where the increase in pH is related to the increase in ammonia.

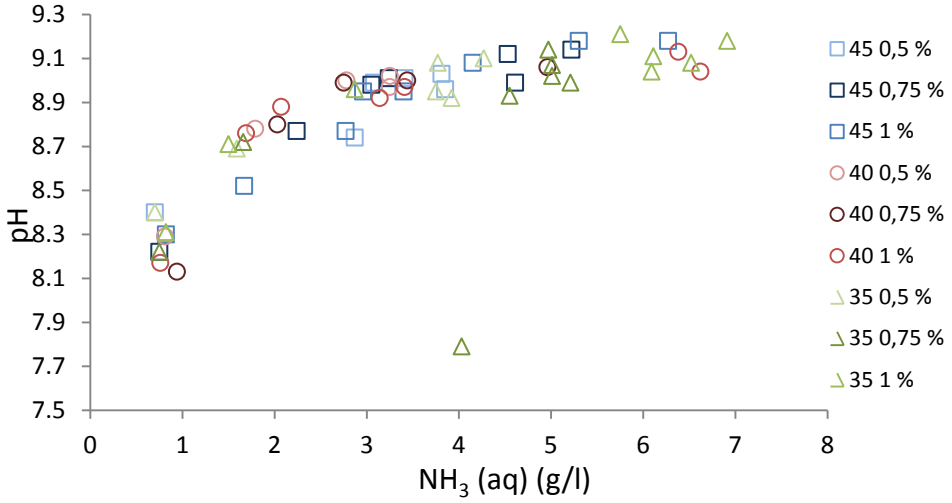

Figure 6. The relation between pH and ammonia in the different heat treatments and urea additions (Green 1 %, Blue 0.75 %, Red 0.5 %)

5.6 Microbial inactivation

5.6.1 Inactivation of *Enterococcus spp.*

The post-treatment concentrations of *Enterocoocus spp.* were reduced below the detection limit (1 cfu/ml) in all urea treatments at 45 °C whereas in 0 % urea treatment at 40 °C and all treatments at 35 °C enterococci were still detectable at the end of the study (9 and 14 days respectively). Figure 7 presents the inactivation with normalized concentrations and table 3 presents D-values for enterococci inactivation. Enterococci were the most resistant of the 3 bacterial indicator organisms.

*Table 3.*D-values for *Enterococcus spp.* in each temperature-urea addition treatment combo.

	0%	0.50%	0.75%	1%
35 °C	9.37	2.68	2.80	3.28
40 °C	6.34	1.40	1.04	1.31
45 °C	1.24	0.95	0.45	1.08

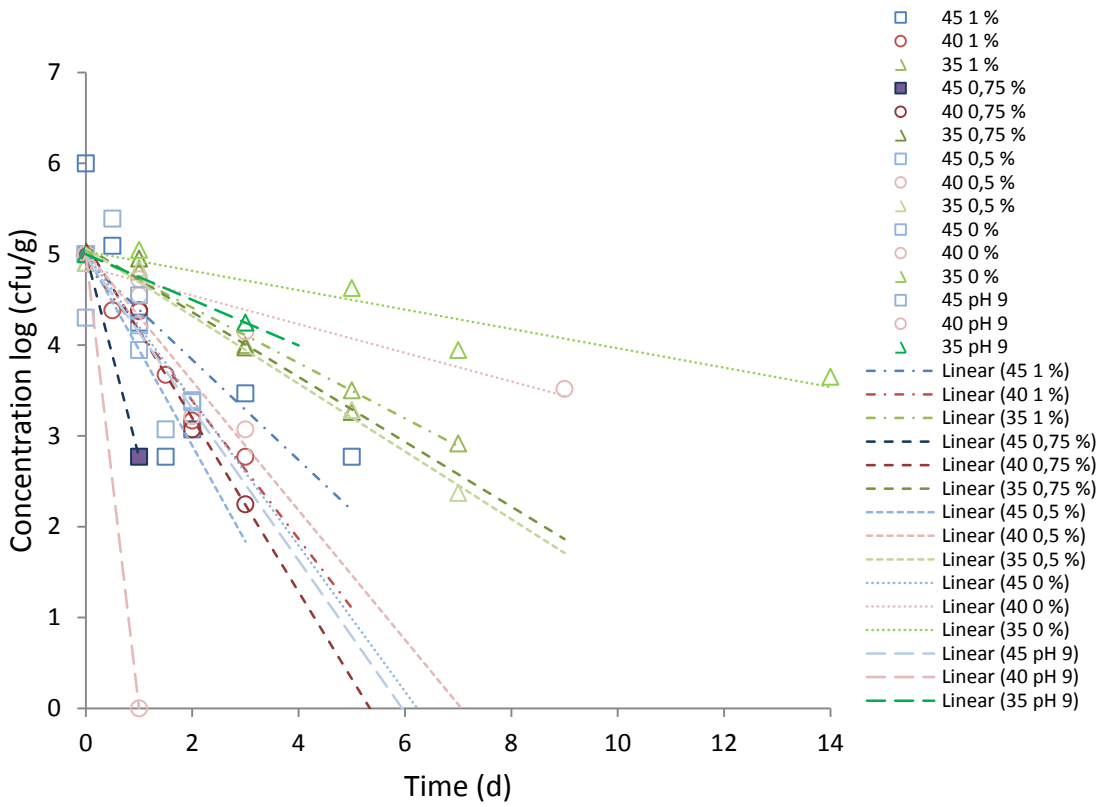

*Figure 7.*Inactivation of *Enterococcus spp.* over time in different treatments. Blue: 45°C, Red: 40°C, Green: 35 °C

5.6.2 Inactivation of Total Thermotolerant Coliforms

The inactivation rate of total thermotolerant coliforms increases with temperature and urea addition. The two highest temperatures had similar inactivation rates. (Figure 8, Table 4) 0.75 % urea addition at 45 °C obtained the fastest rate.

At all temperatures were rates of inactivation increasing with urea addition except at 1 % urea addition. In the treatment without urea (0 %) addition was the count never reduced below detection limit (1cfu/ml). Treatments with urea addition were all reduced below detection limit after at the most 5 days of treatment.

The 0.5 and 0.75 % values are marked as less than 0.20 day / 1 log10 reduction; because just initial and final concentration of <1 cfu/ml was obtained.

*Table 4.*D-values for Thermotolerant coliforms in each temperature-urea addition treatment combo.

	0%	0.50%	0.75%	1%
35 °C	7.34	0.98	0.42	0.74
40 °C	0.99	≤0.20	≤0.20	0.38
45 °C	1.34	≤0.20	≤0.20	0.15

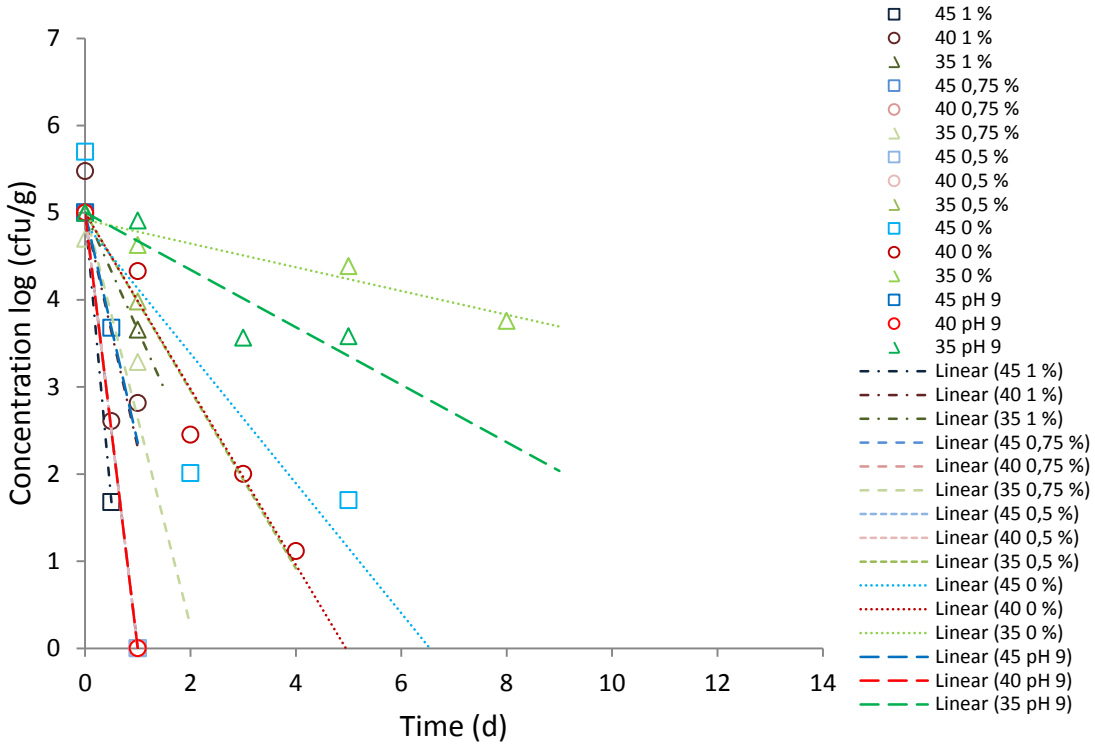

Figure 8. Inactivation of Thermotolerant Coliforms over time in different treatments. Blue: 45°C, Red: 40°C, Green: 35 °C

5.6.3 Inactivation of salmonella

Salmonella was reduced to the detection limit 1cfu/ml in all treatments with urea addition. The fastest rates was obtained in 1 % 40 °C and 0.75 %, 45 °C and was estimated to less than 0.25 day for 1 log10 reduction (Table 5).

Table 5. D-values for salmonella in each temperature-urea addition treatment combo.

	0%	0.50%	0.75%	1%
35 °C	4.64	1.32	0.75	0.81
40 °C	2.08	≤0.50	0.37	≤0.25
45 °C	1.20	≤0.33	≤0.25	0.33

The inactivation of salmonella at 35 °C was slower than at the higher temperatures. The pH 9 reference treatment (35 °C) had a lower rate than the 0 % urea treatment at 35 °C (Figure 9). The salmonella was reduced below detection limit in all treatments at 45 °C.

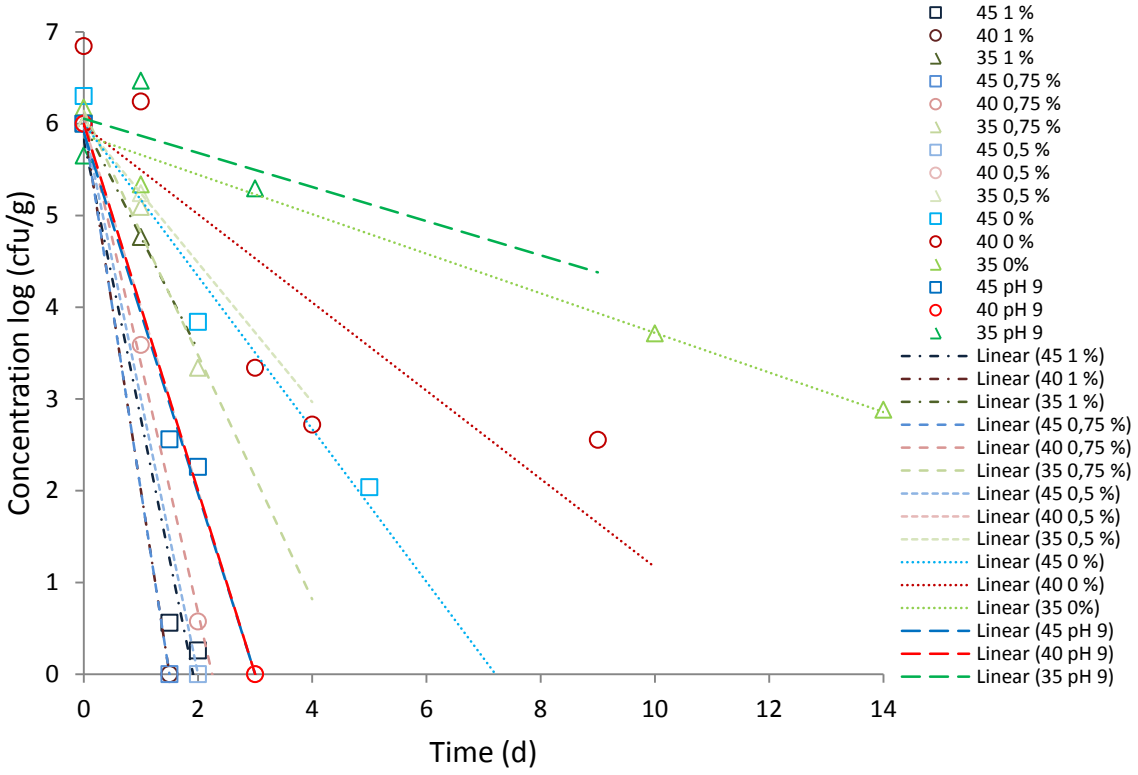

Figure 9. Inactivation of salmonella over time in different treatments. Blue: 45°C, Red: 40°C, Green: 35 °C

5.6.4 Inactivation of Coliphages

It was only in the diluted pH 9 treatments where the phages were approximately 100 times lower at start where they were decreased to the detection limit. Phages in pH 9 treatments were inactivated in 35 and 45 °C, but increased extensively in the 40 °C. (Figure 10) Concentrations were also increasing at 35 °C with 0 and 0.5 % urea addition. The fastest inactivation rates, 12 and 24 days for a 1 log10 reduction, were obtained at 45 °C with 1 and 0.75 % urea addition, respectively. (Table 6)

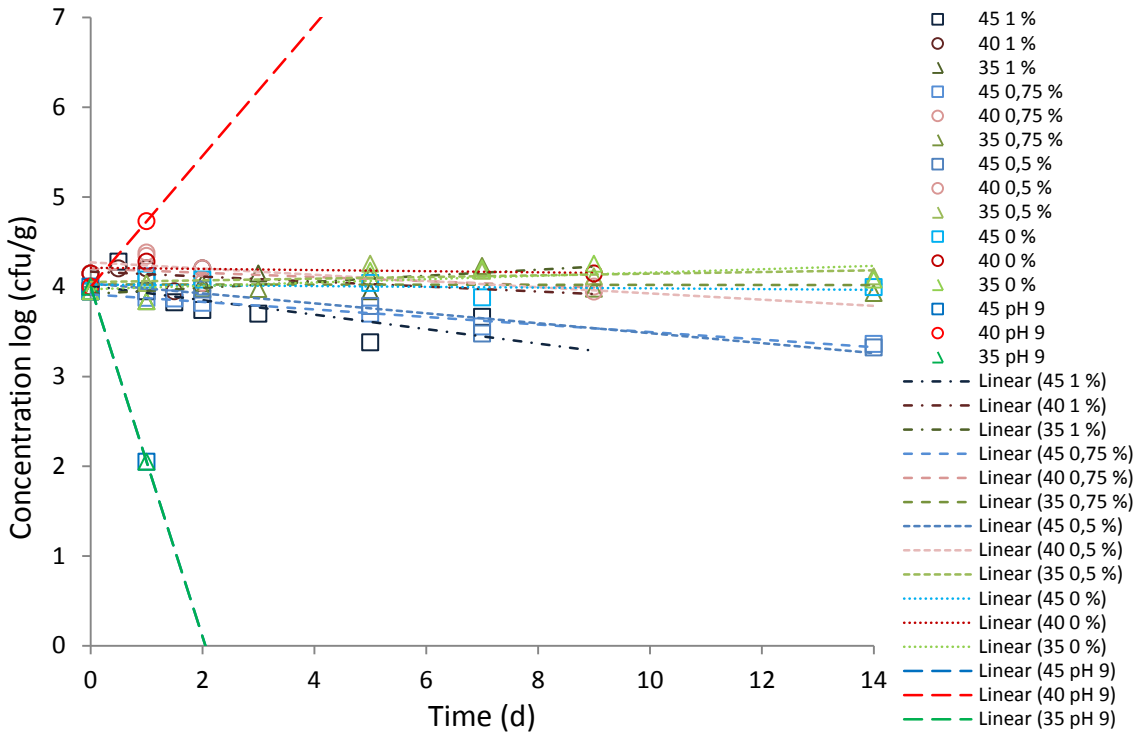

Figure 10. Inactivation of coliphages over time in different treatment. Blue: 45°C, Red: 40°C, Green:35 °C

Table 6. D-values for *the coliphages* in each temperature-urea addition treatment combo.

	0%	0,50%	0,75%	1%
35	No reduction	No reduction	2000	30
40	156	29	43	36
45	233	18	24	12

6 DISCUSSION

6.1 Initial material

The material changed during storage. The pH and ammonia concentration increased. This might be because there were still biological and biochemical degradation in the material. The material was stored at 4 °C. There is an ammonification during composting, which will increase NH_3-concentration (Schönning & Stenström 2004). This ammonification would probably not be discontinued forthright; then, ammonia will continue to increase during storage. The ammonia formation from the breakdown of nitrogen rich substances and volatile fatty acids affects pH. The dry matter content was slightly lower than before storage due to breakdown of organic material or difficulties taking a representative sample when not totally homogeneous. The material which was used in the present laboratory study had been composted in the full scale reactor at 45 °C which had an impact on the first initial content of microbes and start concentrations in material composted to only 35 °C may be higher, a difference that was not encountered for in the lab-scale set up. The initial microbial analyses were indicating no change in the concentrations of microbes during the storage. The variation in ammonia and pH during storage is small compared to the increase of pH and ammonia when urea is added. The addition of salmonella to an initial concentration of 5-6log10 is much higher than what could be found in a naturally contaminated toilet waste but make it possible to follow the detection kinetics. It might be a difference in resistance between naturally occurring salmonella and the added strain.

6.2 Temperature

The set up aimed to achieve a homogeny temperature in the tubes with urea treated compost and the temperature should decline 1°C a day. The temperature difference

between the bottom and the surface of the tubes were not constant even if the heat block followed the same temperature schedule. For instance is the temperature difference greater in the 45 °C heat block than in the 35 °C heat block between day one and two than in any other time period (Figure 3).

In this small scale experiment there are no real environmental effects to consider, which might be an issue in a full scale liquid compost reactor. Depending on the outside environment will the temperature decline in various rates. Performance target of an inactivation method should ensure the inactivation of real pathogens in addition to the indicator organisms. The outcome of a lower temperature can be an incomplete inactivation (WHO, 2006). A difference between this study and a big scale reactor is the daily decrease in temperature. Concerning the 35 °C treatment is the treatment more intense and causing more inactivation since it is not decreasing below room temperature.

The differences in temperature between top and bottom of the tube was as high as approximately 5 °C which could have an effect on the result, by treating unevenly, since there was no stirring of the material. The uneven temperature might have been avoided with a more isolated environment, i.e. by having the tubes in an incubation cupboard.

6.3 Ammonia

The concentrations of ammonia increased after additions of urea, and exceeded the theoretical concentrations which indicate another ammonia nitrogen source which may be inactivated bacteria. The treatments with no urea (0 %) addition did not increase in ammonia during the treatment which shows that temperature does not affect the total ammonia concentrations. However temperature has impact on the fraction of ammonia in form of NH_3 in the solution. The free ammonia is one explanation for the fast inactivation of bacteria.

The pH is increasing during the urea treatment. Ammonia and carbonate (HCO_3^-) is formed during the degradation of urea. The equilibrium between ammonia and carbonate determines the pH. The formation is in need for hydrogen which is taken from the surrounding solution. A lowering concentration of hydrogen results in an increasing pH. Carbonate also buffers for a high pH. The low pH in the 0.75 %, 35 °C was probably due to an error. If comparing the plots in figure 5 should the

pH in mind be somewhere between 8.8 and 8.9 instead of 7.8 as indicated in figure 6.

6.4 Microbial inactivation

6.4.1 Enterococcus spp.

The initial concentration of *Enterococcus spp.* was about 3 log10. According to EC (2006) the treatment should have an overall risk reduction of 5 log10 of *Enterococci*. This is however not succeeded when having an initial value much less than the expectant reduction. Nevertheless, there are indications of continuous reduction, which is shown when normalizing all concentration in figure 7. Regarding the inactivation kinetics it was considered to follow first order kinetics. Even though the inactivation is faster within this treatment than with e.g. termophilic compost, there is still questionable if the inactivation should be exponential or not, in such early state of inactivation. The inactivation factors temperature and thus NH_3 concentration are decreasing over time therefore is the hypothesis about a strict first order reduction not accepted completely. When combining the inactivation for the three starting temperatures the inactivation tends to be log-exponential (Figure 11). In thermophilic compost, where the inactivation is slower, is the bacteria reduced in a first order kinetic until about 10-12 days before it begins to level out to an endless exponential inactivation (Shuval, Gunnerson, & Julius, 1981).

Time (d)

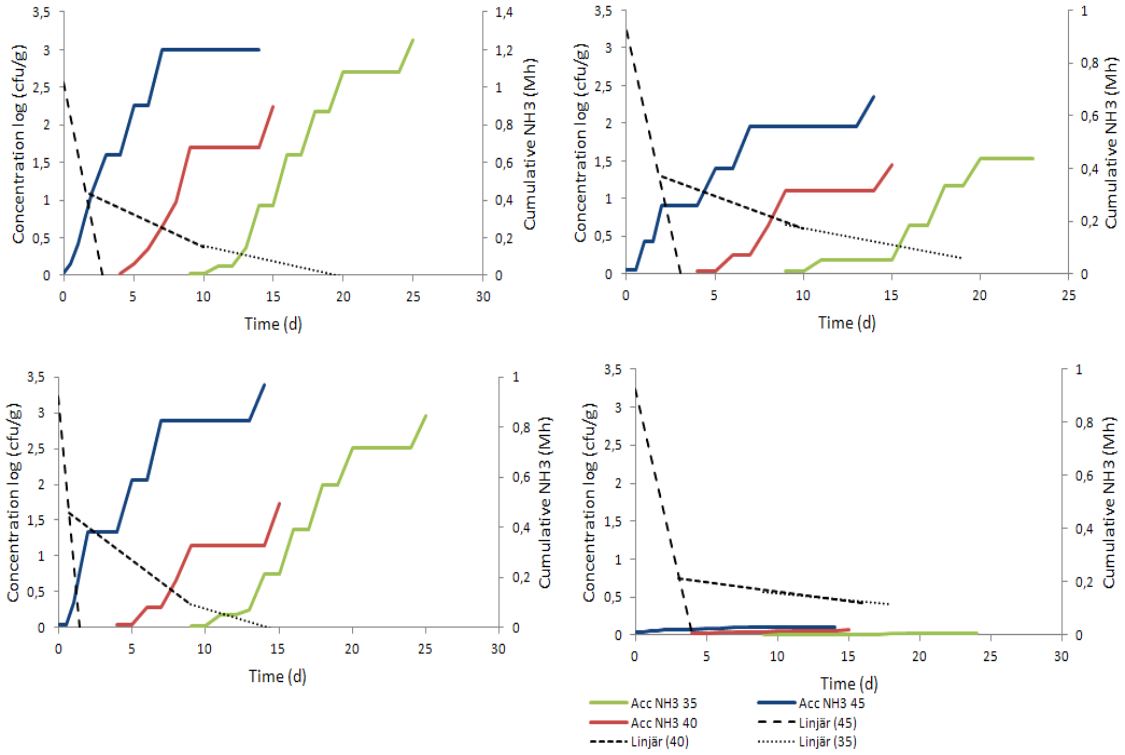

Figure 11. Inactivation of *Enterococcus* spp. over time with reduction rate taken from the different temperature treatments showed with the dotted lines. The colored and solid lines demonstrate the cumulative ammonia concentration. Diagram upper left: 1 % Urea addition, bottom left: 0.75 %, upper right: 0.5 %, bottom right: 0 %.

By examine the D-values of the inactivation (table 3), in other words the time for 1 log10 inactivation, the inactivation rate was as high as 0.45 day per 1 log10, which was obtained in the 45 °C treatment with 0.75 % urea addition. The rate in the 1 % treatment should presumably be higher. A reason for obtaining a slower rate might be because of lower pH than in the 0.75 % treatment at day 1 and 2. The inactivation for the 1 % treatment might have been faster. More analyses should have been performed since there is just one value which separates the two and make the 0.75 % treatment slightly faster.

The inactivation time for a 5 log10 reduction, if keeping a steady rate between 0.45 and 1.40, would be between 2.25 and 7 days. This time period of reduction in this study is achieved by treating the material with a starting temperature of at least 40 °C and with an addition of urea of at least 0.5 %, or treating at minimum 45 °C if treating the excreta without urea. These parameters should be fulfilled in the full-scale reactor in order to inactivate bacteria within a week of time. Entero-

cocci seem to be more resistant than salmonella and the thermotolerant coliforms. That confirms previous findings that *Enterococcus spp.* has longer survival in relation to ammonia than *Salmonella* spp. and other bacterial pathogens when studied in faeces (Björn Vinnerås, et al., 2003). Enterococci are therefore setting the limit for bacterial inactivation.

6.4.2 Total thermotolerant coliforms

Total thermotolerant coliform count determines the presence and concentration of *Enterobacteriaceae* of faecal origin, faecal coliforms and is to a large extent comprised of *E. coli*. Other pathogens such as salmonella usually occur in lower densities, faecal coliforms may therefore be considered as indicators for pathogens such as salmonella and enteropathogenic *E. coli* (Nordin, 2010). The coliforms seem to be more sensitive to temperature than Enterococci, if comparing the D-values of the two (table 4) in same urea treatments, though the differences in inactivation between the urea additions are comparable.

The thermotolerant coliforms started at a concentration of about 5 log10 and were, if following the inactivation rate, reduced to 1 log10 in between 0.75 and 36.7 days. The fastest rate for inactivation was at 45 °C with an addition of 1 % urea. However the inactivation was showed to be faster in 0.75 % treatment in 35 and 40 °C treatments than in 1 %, but only when including the last concentration. *E. coli* should be less than 1000 in 1 g dry matter for the treatment to be approved.

6.4.3 Salmonella

Sanitisation of human excreta before use to fertilize crops is crucial in being an additional barrier for *Salmonella spp*. It is important to detain all possibilities to transmit in the environment through humans and animals in order to first of all decrease the incidence of salmonellosis in humans.

A valid treatment should reduce salmonella 5 log10 and should in the end product be absent in 25 g sample wet weight (EC, 2006; SEPA, 2010). The initial concentration of salmonella for the treatment performed in this study was about 6 log10. Inactivation took place in all treatments, but was best performed in the treatment with added urea. A 1 log10 reduction is made in less than 1 day of treatment at all temperatures when adding at least 0.5 % urea.

6.4.4 Coliphages

The viruses, the bacteriophages, were more resistant to treatment than bacteria. The highest inactivation rate was obtained in the 45 °C treatment with 1 % urea addition. Bacteriophages differ in such way from animal viruses in that they infect bacteria instead of animals. They tend to be more resistant to treatment than many animal viruses (Nordin, personal communication). The body temperature reaches about 40 °C when fever in an attempt to inactivate viruses. Fever and temperature are certainly not the only defence the body has against viruses of any kind. However a temperature at 45 °C had none or little effect on bacteriophages. To obtain a faster inactivation, without increasing the temperature, probably a greater amount of urea has to be added.

6.4.5 Inactivation in general

Including detection limit concentration in the regression of inactivation kinetics gives censored D-values and the inactivation rates could in some treatments have been higher. The final concentration was included in the inactivation plot, which in some cases had to be done to get an overall inactivation. All these inactivation rates are obtained when starting with this experiment initial concentrations of microbes. However if the initial concentrations were in different ratios, e.g. Enterococci in greater amount than salmonella, would perhaps the inactivation rates be differently due to longer lag-phases before inactivation proceeded. The reason might be because the inactivation may be correlated with the concentration of other microbes.

The regulations about which amount of microbes that have to be inactivated do not state any time span for the treatments. Time is an important factor to consider, especially when e.g. temperature are decreasing with time. There is a risk for regrowth of microbes if e.g. the temperature increases again and if the ammonia sanitation e.g. not is processed in a closed container. A loss of ammonia would perhaps give the microorganisms the possibility to grow again.

6.5 Reach food security with sanitized excreta

Sanitized excreta are a good option to commercial fertilizers. Constructing a use for excreta instead of wasting all good nutrients has to be a goal for all governments worldwide. The excreta should be used as a resource rather than be assumed as a waste. The very idea that excreta are waste with no useful purpose is a mod-

ern misconception. Nature has no waste; all the products of living things are used as raw materials by others. Recycling of nutrients prevents direct pollution caused by sewage being discharged or seeping into water resources and ecosystems. The excreta benefits soil and crops and reduce the need for chemical fertilizer (Winblad & Simpson-Hébert, 2004). By sanitizing the excreta with this liquid compost/urea treatment there is according to the results a possibility for using the excreta on fields aimed for food production. The sanitizing, which is obtained by combining urea and heat for a certain period of time, is performed in a closed container which minimizes the loss of nitrogen in the form of ammonia. A fertilizer with a sufficient content of nitrogen is one essential feature for being a good nutrient source for plants, as well as it is hygienically safe in the way that the disease transmission is stopped. By increasing the parameters, i.e. heat or urea, the time to obtain sanitized excreta is reduced.

Nutrients recovered from human excreta may enhance the productivity of any kind of crop growing industry when there is no possibility of gaining commercial fertilizers. However, excreta should be handled and treated according to the guidelines (Schönning & Stenström 2004) before use in cultivation (Jönsson, et al., 2004).

Farmers in developed countries are dependent on the consumer's opinion on either processing (food industry) or eating (end user) a crop which have been fertilized with excreta. The demand from SEPA (2010) to increase the quality assurance of sewage fractions ought to increase the acceptance from the food industry, to process and sell products derived from a sustainable agriculture. The consumers, the industry, the government should come to a conclusion together whether they are willing to process and eat excreta fertilized products.

Farmers in developing countries can by using their excreta increase their average yield with lower cost than with commercial fertilizer. Producing more food per unit space can result in preventing malnutrition by letting people get the opportunity to have better diets, by improving macro- and micronutrient intakes. Yet if not proper sanitation guidelines are introduced their health is threatened. Because there is always possible pathogens excreted, barriers must be continuous without failure or breakdown (S. A. Esrey, 2001). The health benefits must be further maintained, by proper handling of sanitized excreta and utilization of the food the farmers and households get from the field fertilized with excreta.

7 Conclusions

In this small-scale treatment actions such as temperature and urea addition which determine inactivation parameters, were quite easy to monitor and regulate. Decreasing temperature does not make the inactivation less sufficient. Urea addition gives an addition of nitrogen which can form ammonia and thus increase the pH to obtain the possibility for sufficient inactivation.

To obtain sufficient inactivation of bacteria within a week the excreta has to treated at least 40 °C, and with 0.5 % urea addition. In order to obtain a faster inactivation the excreta should be treated with more urea or at a warmer temperature.

References

EC. (2006). Comission regulation (EC) No 208/2006 ammending Annexes VI and VIII to Regulation (EC) No 1774/2002 of the European parliment of the Council as regards processing standards for biogas and composting plants and requirements for manure. In E. C. Regulations (Ed.), (Vol. EC No. 208/2006).

Esrey, S. (2001). Towards a recycling society: ecological sanitationâ€"closing the loop to food security. *Water Sci. Technol., 43*, 177.

Esrey, S. A. (2001). Towards a recycling society: ecological sanitation - closing the loop to food security *WATER SCIENCE AND TECHNOLOGY, 43*(4), 177-187.

Höglund, C. (2001). *Evaluation of microbial health risks associated with the reuse of source-separated human urine.* Doctoral Doctoral thesis, Royal Institute of Technology, Stockholm. Retrieved from http://urn.kb.se/resolve?urn=urn:nbn:se:kth:diva-3090

Jönsson, H., Richert Stintzing, A., Vinnerås, B., & Salomon, E. (2004). Guidelines on the use of urine and faeces in crop production. In Stockholm Environment Institute & E. Programme (Eds.), *EcoSanRes publications series, 2004:2* (pp. 35). Stockholm: Stockholm Environment Institute.

Malmén, L. (1999). Kretsloppsanpassning av hushållens avlopp och organiska köksavfall på Vätö. In J. institutet (Ed.), *Kretslopp och avfall* (Vol. 20). Uppsala: Jordbrukstekniska institutet.

Nordin, A. (2010). *Ammonia sanitisation of human excreta: treatment technology for production of fertiliser*. PhD, SLU, Uppsala.

Schönning , C., & Stenström , T. A. (2004). Guidelines on the safe use of urine and faeces in ecological sanitation systems. In Stockholm Environment Institute & E. Programme (Eds.), *EcoSanRes publications series, 2004:1* (pp. 38). Stockholm: Stockholm Environment Institute.

Schönning, C., Westrell, T., Stenström, T. A., Arnbjerg-Nielsen, K., Hasling, A. B., Hoibye, L., & Carlsen, A. (2007). Microbial risk assessment of local handling and use of human faeces. *J Water Health, 5*(1), 117-128.

SEPA. (2010). Redovisning av regeringsuppdrag 21 Uppdatering av "Aktionsplan för återföring av fosfor ur avlopp"

Bilaga 1: Förslag till förordning Dnr 525-205-09 (Vol. Dnr 525-205-09).

WFP. (2009). Emergency Food Security Assessment Handbook 2nd Edition (Vol. 2). Rome: World Food Programme, Food Security Analysis Service.

WHO. (2006). Volume 4: The use of excreta and greywater in agriculture *Guidelines for the safe use of wastewater, excreta and greywater* (Vol. 4). Geneva: World Health Organization.

Winblad, U., & Simpson-Hébert, M. (Eds.). (2004). *Ecological sanitation – revised and enlarged edition.*: SEI, Stockholm.

Winker, M., Vinnerås, B., Muskolus, A., Arnold, U., & Clemens, J. (2009). Fertiliser products from new sanitation systems: Their potential values and risks. *Bioresource Technology, 100*(18), 4090-4096.

Vinnerås, B., Clemens, J., & Winker, M. (2008, 12th December). *Non-metallic contaminants in domestic waste, wastewater and manures: constraints to agricultural use?* Paper presented at the The International Fertiliser Society Annual Meeting, Cambridge, United kingdom.

Vinnerås, B., Holmqvist, A., Bagge, E., Albihn, A., & Jönsson, H. (2003). The potential for disinfection of separated faecal matter by urea and by peracetic acid for hygienic nutrient recycling. *Bioresource Technology, 89*(2), 155-161.

Personal Communication

Nordin, Annika, post-doc, Swedish University of Agricultural Sciences, Uppsala. April – May 2012

Acknowledgement

This study was financially supported by the Department of Energy and Technology, Swedish University of Agricultural Sciences (SLU). Laboratory facilities were provided by The National Veterinary Institute.

I would like to thank my supervisor PhD Annika Nordin for her knowledge and valuable inputs about the performance of the study. Thanks to Alexander Johansson, research assistant, who helped me combine, which seemed to be a never ending stream, of results.

I appreciate I got the possibility to join the research team and the work group at the Department of Energy and Technology and somehow someday make a change.