SLU

Nitrogen based sanitation of source separated faecal matter

Annika Nordin

Institutionen för mikrobiologi
Sveriges lantbruksuniversitet

Examensarbete 2006:3

ISSN 1101-8151

Uppsala

ISRN SLU-MIKRO-EX–06/3–SE

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Annika Nordin

Master thesis for the Programme in Natural Resources
Performed at the Department of Biometry and Engineering, SLU and the National Veterinary Institute (SVA)

Supervisors: **Björn Vinnerås, SLU**
Ingela Berggren, SVA

Department of Microbiology
Swedish University of Agricultural Sciences

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SAMMANFATTNING

För att uppnå de av FN uppsatta milleniummålen, att halvera det antal människor som saknar fullgod sanitet, måste alternativ till vattenburen sanitet utvecklas och implementeras. Fekalier och urin måste därvid ses som en resurs och en väg att sluta näringskretsloppet och därmed minimera miljöpåverkan. För att säkerställa mänsklig hälsa när fekalier och urin används för jordbruksproduktion krävs behandling som förhindrar spridning av sjukdomsalstrande mikroorganismer. Kvävebaserade behandlingar med ammoniumhydroxid och urea och har visat sig effektiva att reducera bakteriella patogener, nematodägg och protozoer i fekalier och gödsel. Det är oladdad ammoniak, NH_3, som påverkar mikrobiell inaktivering. Då ammoniak är en svag bas påverkas jämvikten mellan oladdad och laddad ammoniak av pH. Därmed kan andelen ammoniak som förekommer som NH_3 styras med tillsatser av alkaliska substanser, såsom kalk. Ett pH över 8 är nödvändigt för att uppnå betydande koncentration av NH_3.

Syftet med denna studie var att undersöka potentialen hos kvävebaserade behandlingar för reduktion av bakteriella och parasitiska patogener i källsorterade fekalier. Fekalier från ett torrsorterande system behandlades vid 14°C med ammoniak (1%), urea (0,5, 1 och 2%) och/eller kalk och lagring. *Enterococcus faecalis, Salmonella* Typhimurium*, Escherichia coli* O157:H7 and *Ascaris suum* ägg tillsattes före behandling. Den bakteriella reduktionen studerades med plattmetoder och överlevnaden hos ascaris ägg observerades i mikroskop. Innehållet av ammoniak destillerades och titrerades för koncentrationsbestämmning och andelen NH_3 beräknades utifrån pH.

Alla behandlingar förutom lagring resulterade i en ökning av pH från det initiala 7,14. Behandling med 1% ammoniak med samma molara tillsats av total ammoniak som behandling med 2% urea gav ett högre pH jämfört med 2% urea, 10,2 respektive 9,2. Behandling med urea gav ett pH som var stabilare över tid jämfört med pH från behandlingar med kalk. I alla behandlingarna minskade pH över tid vilket således påverkade koncentrationen av NH_3. Dag 21 var det bara behandlingarna med 1% ammoniak och 2% urea som höll ett tillräckligt högt pH för att ge någon betydande andel NH_3. Dock följde den bakteriella reduktionen en exponentiell avdödning även i de behandlingar som efter dag 21 höll låg $NH_{3(aq)}$ koncentration. Inga levande *A. suum* ägg kunde observeras efter 41 dagar i de studerade behandlingarna: urea 0,5% och 2% och lagring. Dag 12 kunde man se en trend med lägre överlevnad med ökad ureatillsats, dock inte statistiskt signifikant. Av de studerade bakterierna visade sig *E. faecalis* minst känslig för de olika behandlingarna. *E. coli* var generellt känsligare för behandlingarna än *S.* Typhimurium, dock var resultaten för dessa två patogena bakterier mer lika vad gäller känslighet och reduktionstid jämfört med *E. faecalis*. Behandling med 1% ammoniak eller 2% urea visade sig vara mest effektiv för bakteriell avdödning med decimalreduktionstid 0,13-5 dagar för 1% ammoniak och 0,2-41 dagar för 2% urea. Regressionsanalys av reduktionskoefficienter *k* mot koncentrationen NH_3 visade ett linjärt samband för alla studerade bakterier. Bara *E. faecalis* gav ett signifikant samband mellan *k* och pH, dock svagare än för NH_3. Det linjära sambandet gav en förändring av *k* med 0,022 enheter per mM NH_3 för *E. coli* och 0,014 for *S*. Typhimurium. *E. faecalis* som var mindre känslig för behandlingarna hade en förändring av koefficienten med 0,00054 enheter per mM NH_3. *E. faecalis* visade sig mycket mer tålig än de patogena bakterierna som

studerades och en högre tröskelkoncentration av oladdad ammoniak verkar krävas för effektiv reduktion. Eftersom reduktionstiden för *E. faecalis* i vissa fall överskred reduktionstiden för de patogena bakterierna mångfalt verkar inte *E. faecalis* vara ett passande val av indikatororganism för reduktion av bakteriella patogener med denna metod.

Slutsatsen är att behandling med 1% ammoniak, 2% urea eller 1% urea med kalktillsats verkar vara goda behandlingsalternativ för källsorterade fekalier då dessa behandlingar gav en 6 $\log_{10}$ reduktion av de patogena bakterierna inom tre veckor. *A.suum* verkar även ha påverkats av andra faktorer än NH_3 koncentration då även lagring gav en snabb reduktion av överlevnaden.

ABSTRACT

To fulfil the UN millennium goals for sustainable development, there is an urgent need for alternatives to conventional water based sanitation. Faeces and urine contain valuable plant nutrients and should be considered as resources rather than wastes. Collection with efficient water usage enables faeces and urine to be reused and environmental pollution may be better prevented. When using human excreta as plant fertilisers, it is important to prevent disease transmission by reducing the content of gastrointestinal pathogens. Nitrogen based treatment of faeces and manure with ammonium hydroxide and urea has been shown to be an efficient method for inactivating bacterial pathogens, nematode eggs and protozoan cysts. The substance responsible for microbial inactivation is uncharged ammonia, $NH_{3\,(aq)}$. As ammonia is a weak base, the ammonia equilibrium can be controlled by additions of alkaline agents such as lime. A pH above 8 is needed to produce substantial amounts of ammonia in the form of NH_3.

The objective of this study was to examine the potential of nitrogen based treatment for reduction of human bacterial and parasitic pathogens in faeces collected separately from a dry sanitation system. The faeces samples were inoculated with *Enterococcus faecalis, Salmonella* Typhimurium*, Escherichia coli* O157:H7 and *Ascaris suum* eggs prior to treatment. Treatments were performed at 14°C and consisted of ammonia (1% w/w), urea (0.5, 1 and 2% w/w) and/or slaked lime and storage. Inactivation of bacteria was monitored by plate count methods and viability of ascaris eggs by microscopy. Ammonia content was determined by distillation and titration and NH_3 concentration calculations based on pH.

Addition of ammonia, urea and/or lime resulted in an increase in pH from the initial 7.14, whereas the pH in the storage treatment decreased. The 1% ammonia treatment with equimolar addition of total ammonia as 2% urea resulted in a higher pH (10.2) than 2% urea (9.2). Addition of urea gave a more stable pH over time compared to addition of lime, although pH declined with time in all treatments, thus affecting NH_3 concentration. On day 21, 1% ammonia and 2% urea were the only treatments with sufficiently high pH to produce a substantial amount of NH_3, although reductions in bacteria fitted well to an exponential function even after day 21. No viable *A. suum* eggs were observed after 41 days in the 0.5% urea, 2% urea and storage treatments. On day 12, no significant differences in viability could be observed between the different treatments. However a tendency for reduced *A. suum* viability according to the urea gradient could be observed. *E. faecalis* was less sensitive to the treatments than any of the pathogenic bacteria studied and *E. coli* was more sensitive than *S.* Typhimurium, although the differences were small. The 1% ammonia and 2% urea treatments were the most efficient at reducing bacteria, resulting in a decimal reduction time of 0.13-5 days for 1% ammonia treatment and 0.2-41 days for 2% urea treatment. Regression analysis of the coefficients *k* for the bacterial reduction function and the concentrations of NH_3 in the treatments revealed a significant linear correlation for all bacteria studied. However, pH was only significantly correlated to *k* for *E. faecalis*, though weaker than to NH_3. The relationship between NH_3 concentration and reduction coefficient gave a change in *k* of 0.022 units per mM NH_3 for *E. coli* and 0.014 units for *S.* Typhimurium. The pathogenic bacteria were more sensitive to NH_3 concentration than *E. faecalis*, with a change in *k* of 0.00054 units per mM

NH_3. As *E. faecalis* seemed to have a higher threshold concentration for inactivation by ammonia based treatments and its reduction time exceeded that of the pathogenic bacteria studied, *E. faecalis* might not be a suitable indicator organism for this method.

In conclusion, treatment with 1% ammonia, 2% urea or 1% urea with lime addition was sufficient to give a 6 $\log_{10}$ reduction of the pathogenic bacteria studied, within 3 weeks of treatment. *A. suum* had a rapid inactivation but seemed to be affected by other environmental parameters in addition to ammonia concentration.

Keywords: ammonia, *Ascaris suum,* chemical disinfection, *Enterococcus faecalis, Escherichia coli* H7:O157, faeces, *Salmonella* Typhimurium, sanitation, urea.

ACKNOWLEDGEMENT

The project was financially supported by the Department of Biometry and Engineering, Swedish University of Agricultural Sciences (SLU), and the Swedish National Veterinary Institute (SVA).

I would like to gratefully thank my supervisors, Ph.D. Björn Vinnerås and Agr.D Ingela Berggren, without whose support this study would not have been initiated or completed.

Last but not least: Katarina, thank you for hospitable lodging on your comfy sofa. To the mates at home, for making me feel talented with computers, which I never felt elsewhere.

CONTENTS

INTRODUCTION

On a global scale, 2.6 billion people do not have access to adequate sanitation. Poor water quality, sanitation and hygiene account for some 1.7 million deaths a year worldwide, mainly through infectious diarrhoea (Ashbolt 2004). One of the UN millennium development goals is to halve the number of people lacking basic sanitation by the year 2015. To fulfil this goal, approximately 100 000 toilets per day need to be constructed. According to Agenda 21, environmentally sound waste management must go beyond mere safe disposal and reuse and recycling must be maximised. Furthermore, improved water use efficiency is encouraged as an action to fulfil protection of the quality and supply of freshwater resources (UN www).

Considering these visions, there is an urgent need for sustainable sanitation systems. Flush produce large wastewater flows and more than 90% of wastewater and excreta worldwide are either only poorly treated or not treated at all before being discharged to water recipients (Fall *et al*. 2003). Nutrients accumulated in wastewater result in eutrophication and contamination of major water bodies. This contamination may place demands on purification and artificial infiltration to ensure the availability and quality of drinking water. The utilisation of nutrients and carbon from wastewater to arable land is often prevented by contaminants from other waste flows such as heavy metals from stormwater and chemicals of industrial origin (Jönsson *et al*. 2004).

To accomplish the above objectives of sustainable development, human faeces and urine must be considered as resources to restore nutrients to arable land so that soil fertility can be preserved and food security assured (WSSCC www, Esrey *et al.* 1998). Human excreta reflect the nutrients consumed and offer a full-value fertiliser in terms of plant macro- and micronutrients (Jönsson *et al*. 2004, Kirchman & Pettersson 1995). Implementation of robust, affordable sanitation systems that enable clean, safe nutrients to be retrieved could provide great potential for the global population to increase crop productivity.

When human excreta are used as fertilisers, the health criteria must be upheld and it is important to prevent disease-causing microorganisms being circulated along with the nutrients. Urine is a fraction with a low microbial content, with any microbes present mainly originating from faecal contamination (Höglund 2001). Faeces carry the potential risk of containing substantial amounts of enteric pathogens when individuals are infected. As the pathogen load in human excreta is mainly concentrated in the faecal fraction, which constitutes only 10% of the total mass, the excreta are most often better handled and treated when urine and faeces are collected separately (Jönsson *et al*. 2004, Schönning & Stenström 2004).

Early treatment to inactivate pathogens in faeces minimises the risks involved during further handling of the material and its use as a fertiliser. Several treatments can be used to sanitise biologically contaminated matter, resulting in various degrees of sanitation and fertiliser value of the biowaste. It has long been recognised that ammonia (NH_3) is capable of inhibiting and killing microorganisms, and its importance in the inactivation of food-borne pathogens has been investigated (Jenkins *et al*. 1998, Himathongkham & Riemann 1999, Park & Diez-Gonzales 2003, Vinnerås 2004). Addition of ammonia nitrogen also enhances the fertiliser value of the treated product. As urea is widely used as a nitrogen fertiliser and easily handled,

it can be used to increase the concentration of free ammonia in solution (Park & Diez-Gonzales 2003, Vinnerås 2004).

OBJECTIVES

The main objective of this study was to evaluate addition of urea, ammonia and lime compared to storage as secondary treatments for inactivation of bacteria and parasites in source separated faeces.

BACKGROUND

Faecal disease transmission

Enteric infections can be caused by viruses, bacteria and parasites such as protozoa and helminths. High concentrations of enteric pathogens are excreted in the faeces when individuals are infected (Acha & Szyfres 2003). All microorganisms that infect the intestinal tract are shed in the faeces, while organisms shed into the bile, such as hepatitis A and typhoid bacilli, also appear in the faeces (Mims *et al.* 2001). Viruses and parasites are unable to multiply outside their hosts and thereby do not increase in numbers after excretion but these groups of organisms are normally more stable in the environment compared to non spore-forming gastrointestinal bacteria (Schönning & Stenström 2004).

Pathogenic organisms cause disease to varying degrees. To susceptible people such as children and elderly or immuno-compromised individuals an infection may be lethal, although it does not cause clinical symptoms in healthy adults. The most common symptoms of enteric infections are diarrhoea with abdominal pain, often accompanied by fever, and as the disease becomes severe it can develop into dysentery. Even if the infection is self-limiting, the diarrhoea may result in dehydration and malnutrition if prolonged. Helminths may also result in nutrient depletion if the burden is great and may cause abdominal pain due to mechanical obstruction. Even if not clinically symptomatic, infections may increase the susceptibility to other disease-causing agents and create a cumulative burden. As many of the symptoms are non-specific, the disease often passes without detection (Feachem *et al*. 1983, Acha & Szyfres 2003).

The pathogenic microorganisms in the faeces are mainly spread through the faecal-oral route. Where open defecation or inadequate sanitation is practised, transmission through runoff water and mechanical and biological vectors exposes individuals to faecal pathogens mainly through food and water (Figure 1).

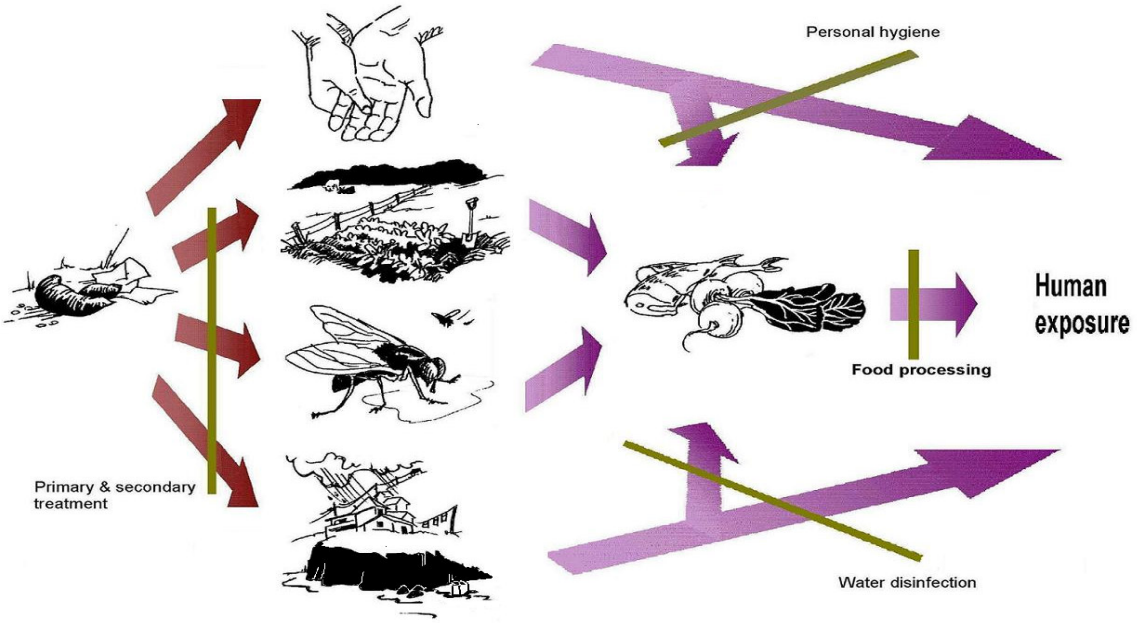

***Figure 1**. Transmission routes for pathogenic organisms from human excreta and restriction barriers for prevention of disease transmission. Illustration Annika Nordin.*

The helminths differ from the other groups of pathogenic organisms by having a more complex life cycle that may require an intermediate host. They do not infect just by the faecal-oral route but also via skin penetration by free-living larvae or by consumption of infected intermediate hosts. Animal exposure to untreated faeces enables helminths that may require an intermediate host to complete their life cycle. Furthermore, as many of the pathogenic bacteria and some parasites and viruses are zoonotic, transmission to animals might result in disease or the animal functioning as a reservoir and transmitter of the pathogen.

By establishing barriers in the transmission route, the spread of pathogenic organisms can be minimised. Adequate collection of faeces and treatment performed at an early stage reduces the need for later precautions as regards food handling and water status (Figure 1). Personal hygiene is of great importance for restricting disease transmission and should always be considered, as it never can be substituted for by other preventive actions.

Prevalence of faecal pathogens

The prevalence of enteric diseases depends on environmental factors such as climate, sanitation status and control programmes. Globalisation of trade and travel enhances the distribution of pathogenic organisms. For many of the gastrointestinal pathogens, infection and disease are more prevalent in children of a young age, probably as a result of poor hygiene and immature immune system (Bitton 1999)

Bacteria have traditionally been considered to be the major agent causing gastrointestinal illness but today viruses are considered to cause the majority of gastrointestinal infections in industrialised regions (Svensson 2000). Epidemic viral diarrhoea is caused primarily by the genus Caliciviridae, especially the group of noroviruses, which contribute significantly to

viral gastroenteritis in adults, particularly in Europe and Australia (Vasickova *et al*. 2005). Caliciviruses are often transmitted by faecally contaminated food or water and tremendous antigenic diversity and short-lived immunity to infection permit repeated episodes throughout life (Ashbolt 2004). Rotaviruses are by far the largest cause of diarrhoea in children and largely contribute to child mortality in developing countries (Bitton 1999). Hepatitis A is currently recognised as one of the most important food-borne pathogens in Europe with regard to number of outbreaks and people affected (Vasickova *et al.* 2005).

Most species of pathogenic bacteria causing enteritis and diarrhoea in man, such as *Salmonella*, *Campylobacter* and enterohaemorrhagic *E. coli*, are distributed worldwide, in both industrialised and developing countries. In developing countries where sanitation is poor, *Salmonella typhi*, *Vibrio cholerae* and *Shigella* are common causes of diarrhoea (Schönning & Stenström 2004).

Parasites are of greater concern in developing countries than in the industrialised regions. Protozoa are responsible for the majority of enteric diseases, with *Entamoeba histolytica* being an important cause of morbidity and mortality (DPD www). *Cyclospora cayetanesis* is suggested to be the cause of many cases of "traveller's diarrhoea" (DPD www). Helminth infections are a major concern, especially in rural regions with poor sanitation practices. On a worldwide basis, *Ascaris lumbricoides* is the most common helminth infection, infecting more than 25% of all humans (OSU www). Another helminthosis of importance is *Scistosomiasis*, which is endemic in 74 developing countries and considered very important to public health because of its debilitating effect on people throughout large areas of the world (Acha & Szyfres 2003).

Environmental persistence of faecal pathogens

Viral pathogens

Numerous viruses can be found in the human gut, but only a few are frequently recognised as common food-borne pathogens spread by the faecal-oral route. The common viral pathogen genera causing gastroenteritis and their stability in the environment are listed in Table 1 (Bitton 1999, Ashbolt 2004, Schönning & Stenström 2004).

Most of the viruses are not zoonotic but recent studies suggest that a reservoir for hepatitis E may exist in domestic animals, including swine. Hepatitis A infects other non-human primates even though the infection seldom is spread by this means (PHA Canada www). For most viruses the infective dose (ID) is unknown with the exception of the coxsackie and hepatitis A viruses, with an ID of less than 20 and 10-100 viral particles, respectively.

Table 1. *Characteristics of enteric viruses (University of Florida www, PHA Canada www, virology-online www). All viral agents mentioned have an icosahedral structure and have linear genomes (University of Florida www).*

Viral genera and groups	Genome	Stability in environment and physical inactivation
Epidemic viral disease		
HEV-like viruses Hepatitis E	ssRNA	Survives in water and sewage for long periods. Inactivated by heat (56°C for 30 min, 70° C for 4 min) and radiation.
Caliciviruses Noroviruses	ssRNA	Stability unknown, found in contaminated water supplies and lakes. Survives 60°C for 30 min. Resistant to pH 5-10, ether, acid.
Endemic viral disease		
Adenoviruses	dsDNA	Stable for some time in the environment. Inactivated by heat (56°C for 30 min); infectivity sensitive to ionizing radiation; stable to lipid solvents.
Enteroviruses Coxsackie A&B	ssRNA	Survives in stool for weeks at room temperature. Relatively stable: pH 2.3-9.4 for 1 day. Inactivated by heat (56-60°C for 30 min).
Echoviruses		Survives at room temperature up to 3 weeks; stable for many weeks in liquid environments, water, body fluids and sewage**.** Inactivated by heat (50°C for 2 hrs); stable at acidic pH 3–5.
Polioviruses		No data available
Enteroviruses (types 68-71)		No data available
Hepatoviruses Hepatitis A	ssRNA	Survives in water and sewage for long periods, at 4°C infectivity is reduced 0.5 $\log_{10}$ after 6 weeks survival. Partially resistant to heat, still infectious after 10-12 hrs at 60°C, stable under extremes of pH (pH 1).
Reoviruses Rotaviruses	dsRNA	Rather durable and resistant to disinfection processes.
Astroviruses 3 serogroups	ssRNA	No data available

Pathogenic viruses are of concern when applying faeces on agricultural land due to the low infective dose, potential stability in the environment and ease of transport by water due to their small size. In effluents with temperatures below 5°C in particular, the persistence increases and survival in groundwater is assumed to be longer than that in surface water (Schönning & Stenström 2004). The general survival time in faeces, nightsoil and sludge at a temperature of 20-30°C is less than 100 days. For enteroviruses, the survival time is usually

less than 20 days (Feachem *et al.* 1983). Inactivation studies on enteroviruses and rotaviruses have shown that inactivation is more rapid under aerobic than anaerobic conditions and at higher temperatures (Turner & Burton 1997).

Bacteria

Table 2 lists important bacterial pathogens and some properties affecting their survival in the environment and disease transmission. The infective dose (ID) does not give a precise number and attention must be given to the fact that the doses are determined in healthy adults. However, ID provides the potential to compare the risk of exposure between the pathogen organisms.

Table 2. *Characteristics of pathogenic bacteria transmitted via faeces (PHA Canada www, IFST www, arrowscientific www, textbook of bacteriology www)*

Bacterium	Principal reservoir	ID[a]	Survival in environment and physical inactivation
Campylobacter jejuni; coli	Domestic animals, poultry, rodents, birds	≤500	Faeces 9 days, water 2-5 days. Inactivated by 48°C. Sensitive to drying, environmental stresses.
Escherichia coli 1) enterohaemorrhagic enteropathogenic	Cattle	$10\text{-}10^{10}$	Faeces and soil: small reduction over 2 months. Dust 4 -27 days. Survives well at low temperatures, heat-sensitive.
2) enteroinvasive, enterotoxigenic	Humans	$10\text{-}10^{10}$	Survives well in contaminated faeces, food, soil or water, dust 4 -27 days, faeces and soil 84 days.
Salmonella 1) *enteridis;* Typhimurium	Humans, domestic and wild animals, poultry	$10^2\text{-}10^3$	Survives for long periods in the environment.
2) *typhi; paratyphi*	Humans	$10^3\text{-}10^5$	Faeces 62 days, ashes 130 days, dust 30 days. Some strains can survive in the environment for years
Shigella flexneri; dysenteriae; boydii; sonnei	Humans	10-200	Faeces 11 days, flies 12 days, water 2-3 days.
Vibrio cholerae	Humans, waters associated with zooplankton	$10^6\text{-}10^{11}$	Faeces 50 days, soil 16 days, dust 3-16 days. Survives well in waters. Very sensitive to cold temperatures and acids. Simple growth requirements.

a) ID-Infective dose

Most of the pathogenic enteric bacteria are gramnegative and facultative anaerobes or aerobes. *Campylobacter spp.* are microaerophilic and thus directly affected by the oxygen level. Gramnegative bacteria seem to be more persistent in nature and to overcome the immune defence of their hosts due to their more complex cell structure (Mims *et al*. 2001). As the pathogenic bacteria infect humans, they are mesophiles with an optimum of growth between 30 and 40°C. Despite a lower temperature pathogenic bacteria may multiply outside the host and thus increase the risk of exposure.

Mitscherlich and Marth (1983) reviewed microbial survival in various environments including faeces (human, poultry and cow). Their results indicate that bacterial (*S.* Typhimurium*, S.* Dysenteriae*, S.* Flexneri) survival time is longer in pulpy samples compared to liquid and that survival time is shorter at higher temperatures for several salmonella species analysed in poultry.

Parasites

The parasitic microorganisms conclude helminths and protozoa. Protozoa are a heterogenic group of single-celled, eukaryote organisms within the Protista kingdom. Protozoa that might be spread through faeces are parasites of the intestinal tract. They are excreted through the faeces most commonly in cyst form, the infectious state, where the cells is very resistant to environmental factors. Table 3 lists some of the parasitic protozoan that may be spread by faeces.

Table 3. *Characteristics of protozoan parasites potentially spread through faeces (Esrey et al. 1998, Ashbolt 2004, Schönning & Stenström 2004, DPD www)*

Protozoa	Survival in environment
Giardia duodenalis	Sensitive to desiccation, sunlight, high and low temperature. Survives several months in cold water. Resistant to a wide range of pH and osmotic pressure. Die-off rates at 1 and 23°C, are 0.015 and 0.28 $\log_{10}$ units per day, respectively
Entamoeba histolytica	Sensitive to desiccation, in moist environment inactivation mainly dependent on temperature and time. Survival in environment from days to weeks.
Cryptosporidium parvum	Highly resistant to disinfectants, high temperatures and freezing. Reported sensitive to dehydration. Lime treatment for water softening can partially inactivate the cysts. Ammonia at alkaline pH has proven efficient for inactivation. Viable 178 days in faeces of 4°C. Survives 18 months in cool wet or damp environment. Die-off rate in natural waters is 0.005-0.037 $\log_{10}$ units per day.
Cyclospora cayetanesis	Survival 2 months at 4°C, likely to survive longer at lower temperatures

Whereas many of the protozoan cysts are infective upon excretion or shortly thereafter, the cysts of *Cyclospora cayetanesis* become infective after excretion sporulation, which occurs after days to weeks at temperatures between 22-32°C. Many of the protozoa mentioned are zoonotic, even though humans in most cases are the main reservoir of the organisms (Bitton 1999, Acha & Szyfres 2003, Ashbolt 2004). The minimum infective dose is as low as 10 cysts for several of the protozoa (Bitton 1999).

Table 4 presents helminths of concern when using faeces for agricultural purposes. Helminths with a direct life cycle, without an intermediate host, are more likely to infect humans as the life cycle is less extended. The infectious state for the direct life cycle can be eggs or larvae, where the eggs infect by the oral route and the larvae by skin penetration.

For all the helminths with an indirect life cycle (except *Schistosoma* spp.), human exposure is due to ingestion of the infected intermediate host. The risk is thus mainly due to inadequate food processing or accidental ingestion through contaminated food.

Helminth larvae and eggs excreted with the faeces can be infective immediately or may need an incubation time to become infective. The incubation time is affected by environmental conditions such as temperature and moisture (DPD www, Acha & Szyfres 2003).

Table 4. *Helminths of concern when using faeces as a fertiliser (Gaspard et al. 1995; 1997, Bitton 1999, Schwartzbrod & Banas 2003, Schönning & Stenström 2004, DPD www)*

Helminths	
Direct life cycle	
Ascaris lumbricoides NE	egg[b]→human
Trichuris spp. NE	egg[b]→human
[a]*Hymenolepis nana* CE	egg[c]→human
Hookworms: *Necator americanus, Ancylostoma duodenale* NE	egg[b]→larvae[b]→human
Indirect life cycle	
Taenia solium/saginata CE	egg[c]→pig/cattle→human
Paragonimus westermani TR	egg[b]→larvae[c]→snail→crustacean→human
Clonorchis sinensis TR	egg[c]→snail→fish→human
[a]*Hymenolepis nana* CE	egg[c]→arthropod→human
Schistosoma mansoni TR	egg[c]→larvae[c]→snail→larvae→human
S. japonicum TR	egg[c]→snail→larvae→human

a) Have both direct and indirect life cycle
b) Incubation time for egg or larvae to become infective
c) Directly infective eggs or larvae

Hygienisation of faeces

One commonly used method for sanitation of faeces is storage for a specific time. Time is a factor that reduces pathogenic organisms, in faeces as well as other environments. The main influence is due to alteration of environmental factors such as moisture, temperature, carbon content and nutrient availability. However, any shift in the external environment, *e.g*. seasonal shifts in temperature and humidity, can result in an increased number of pathogenic bacteria. As many of the environmental parameters affecting pathogen reduction are difficult to control, storage is an uncertain method to ensure sufficient sanitation.

Composting is a treatment promoted for sanitation of collected solid toilet waste but the level of sanitation is uncertain, since achieving constant and high temperatures during the composting demands skilful management. Some studies have identified insulation and low energy content as key factors for unsuccessful small-scale composting of faeces (Björklund 2004, Karlsson & Larsson 2000). Temperatures above 50°C have been reached when composting of faeces was performed in insulated boxes (Vinnerås *et al.* 2003). Even when high temperatures are reached in parts of the compost, there may be uneven heat distribution in the material, allowing re-growth of bacteria. When faecal composting treatments do not reach temperatures high enough for sanitation, they are comparable to faecal storage as regards pathogen reduction.

Anaerobic digestion is a treatment that also allows biogas production but the process requires external heating to ensure sufficient microbial inactivation.

Liming can be used for sanitation and stabilisation of sewage sludge by altering the pH into the alkaline range. As pH affects ionisation of other substances, *e.g*. by increasing the amount of uncharged ammonia in solution, pH often works synergistically with other inactivating factors (Mendez *et al.* 2002).

Alkaline treatment for microbial inactivation

Most organisms thrive in a neutral pH of 6-8, and may be inactivated by more acidic or alkaline environments (Prescott *et al*. 1996). The pH affects the activity of microbial enzymes and the ionisation of chemicals and thus plays a role in the transport of nutrients and toxic chemicals into the cell (Bitton 1999).

Lime has been suggested for stabilisation and sanitation of sewage sludge. Treatment of contaminated manure with slaked lime for inactivation of viruses and bacteria, except mycobacteria, has been recommended by Swedish governmental institutions concerning foodproduction and animal health (Jordbruksverket 1997).

Addition of lime increases the pH. The lime can be added in various forms: limestone ($CaCO_3$), quick lime (CaO) and slaked lime ($Ca(OH)_2$) are varieties of lime with different alkaline properties. Potassium hydroxide (KOH) also functions as an alkaline agent, while at the same time enriching the material with potassium. Alkaline inactivation of microorganisms seems to be synergistic with temperature.

Wood ash can be used as an alkaline agent. It is commonly used in dry toilets as a continuous amendment to cover the fresh faeces and create a physical barrier to flies and other disease spreading vectors. The ash addition also lowers the moisture content and thus inactivates faecal pathogens (Esrey *et al*. 1998, Schönning & Stenström 2004). Calcium is the most abundant element in wood ash and gives the ash properties that are similar to agricultural lime ($CaCO_3$). The liming ability of wood ash can be expressed as calcium carbonate equivalents (CCE), that for most ash forms range between 25 and 60%, depending on wood type and combustion process (Risse www).

Many studies on reduction of food-borne pathogens by alkaline treatment have been performed on sewage sludge. For example, the pH sensitivity for several organisms inserted into sewage sludge has been studied after treatment with lime and ash (Boost & Poon 1998). *E. coli* and *S.* Typhimurium appeared more resistant to the alkaline treatment than *Salmonella* Typhi, *Shigella sonnei*, *Vibrio parahaemolytica* and *Campylobacter jejuni*. *E. coli* and *S.* Typhimurium had a maximum pH tolerance at 10.5, whereas the latter group of bacteria had a maximum pH tolerance at 9.5, when organisms were detected after 24h.

Other studies with alkaline treatment of sludge also indicate that salmonella are tolerant to high pH. A pH above 12 for 2-3 months seems to be necessary to affect salmonella and nematode eggs (Gaspard *et al*. 1995, Gantzer *et al.* 2001). Among pathogens of epidemiological relevance, *Ascaris* eggs seem to be the most resistant to liming (Gaspard *et al*. 1995, Capizzi-Banas *et al.* 2004).

Ammonia treatment for microbial inactivation

Unlike other common disinfectants, ammonia is a natural product that occurs in the environment as a product of degradation of urea, proteins and other nitrogen-containing compounds (Jenkins *et al*. 1998). For human urine, storage has been proven to be a sufficient treatment to inactivate pathogens due to degradation of urea and other nitrous material (Höglund 2001). Aqueous and gaseous ammonia has been used to control microbial growth in stored fruits, hay and grain, and has proven more effective against fungal than against bacterial spoilage of food (Inchem www).

Ammonia is considered very toxic for all types of organisms but the mechanism of inactivation is not yet fully understood. It is the uncharged ammonia, NH_3, that is responsible for the microbial inactivation. One hypothesis is that NH_3 causes a rapid alkalinisation of the bacterial cytoplasm, as it easily penetrates the cell membrane by simple diffusion and reduces the proton concentration as NH_4^+ is formed (Park & Diez-Gonzales 2003). For virus inactivation, there are studies indicating that the inactivation is achieved by rupture of the RNA chain (Burge *et al.* 1983).

When urea is used as an ammonia-forming additive, the degradation of urea, catalysed by urease in the faeces, results in hydroxide ions and ammonia:

$$CO(NH_2)_2 + 3\ H_2O \rightarrow 2\ NH_4^+ + OH^- + HCO_3^- \qquad \text{equation 1}$$

Furthermore, an analytic reaction between the strongest acid (NH_4^+) and the strongest base (OH^-) takes place, leading to the production of new protolytes. The equilibrium between the final main protolytes, HCO_3^- and NH_3, determines the pH and the speciation of the reactants and products.

The amount of the uncharged species (NH_3) is dependent on the acidic properties of charged ammonium:

$NH_4^+ + H_2O \leftrightarrow NH_3 + H_3O^+$ equation 2

The acid constant (K_a) for the ammonia/ammonium equilibrium can be calculated for any temperature between 0 and 50 °C using equation 3, where T is the temperature in Kelvin:

$K_a = [NH_3] * [H_3O^+] / [NH_4+] = 10^{-(2729,92/T+0,09018)}$ equation 3

$[NH_3] + [NH_4^+] = [NH_{tot}]$ equation 4

From equation 3 and 4 the following can be derived.

$[NH_3] = K_a * [NH_{tot}] / ([H_3O^+] + K_a)$ equation 5

Equation 5 gives the speciation into NH_3 from pH, total ammonia and the acid constant. The concentration of non-ionised ammonia is lowered with increasing ionic strengths.

At temperatures from 4°C, the fraction of NH_3 at equilibrium shows that a pH above 8 is needed to get ammonia in the uncharged form and that at pH 11, almost all ammonia is in the uncharged form (Figure 2).

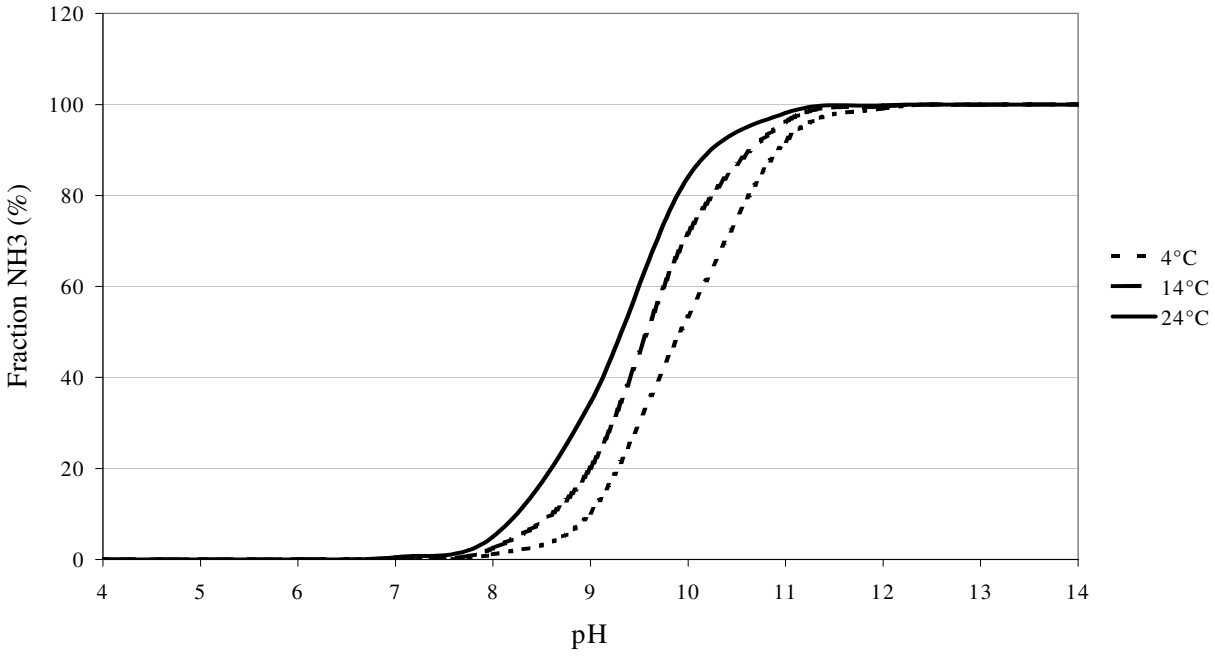

Figure 2. *The percentage of uncharged species in the total dissolved ammonia depending on pH at temperatures 4, 14 and 24 °C.*

When treating with ammonia or ammonia-forming substances, closed containers must be used to reduce losses of volatile ammonia. If this is fulfilled the ammonia will work as a disinfectant until the material is spread, as the ammonia is not consumed during the treatment, compared to e.g. oxidising agents.

There is wide variation between the resistance of various microbial pathogens to chemical inactivation. Generally spore-forming bacteria are more resistant to disinfectants than are vegetative bacteria, as has been proven by inactivation with urea (Vinnerås 2004), where *Clostridia* spp. seem to be resistant to ammonia. Although resistant to various sanitation methods, spore-forming bacteria such as *Clostridia* spp. and *Bacillus* spp. are not considered gastrointestinal pathogens.

Resistance to disinfectants also varies among vegetative bacteria and among strains belonging to the same species. Physical interference from particulate matter is also reported to affect disinfectant inactivation, and clumping or aggregation of microorganisms generally reduces the disinfectant efficiency. Laboratory-grown pathogenic bacteria are generally more sensitive to disinfectants than those that occur in natural environments (Bitton 1999).

When Park & Diez-Gonzales (2003) evaluated the threshold inhibitory concentration for free ammonia with respect to *E. coli* O157:H7 and *S.* Typhimurium, 5mM was the concentration where reduction could be observed in pure broth cultivation. To achieve reduction in cattle manure they reported a concentration of 30 mM NH_3(aq). In the same study *E. coli* was reportedly more resistant than *S.* Typhimurium to urea treatment. When urea was added to cattle manure and the manure incubated at room temperature, resulting in a pH above 8.5 and NH_3(aq) concentration of 40, 125 and 245 mM, respectively, *S.* Typhimurium*, E. coli* O157:H7 and total coliforms were reduced more than 7 $\log_{10}$ in 7 days for all urea treatments. When urea addition resulted in pH 9 and NH_3(aq) concentration of 245 mM, a total reduction (8 $\log_{10}$) was achieved in four days for both bacteria. Furthermore, Park and Diez-Gonzales (2003) concluded that carbonate produced by urea degradation was responsible for the bacterial reduction.

Faecal material (DM 10%) was treated with 6% urea at 20°C, resulting in 8000 ppm (470 mM) of NH_3(aq) (Vinnerås *et al.* 2004). The decimal reduction time (Dr) was less than 0.7 days for both *Salmonella* and *E. coli* and less than 3 days for *Enterococcus* spp. No viable *A. suum* eggs were found after 50 days, corresponding to <0.1% viability. For *S.* Typhimurium phage 28 B, the Dr time was 7.5 days. *Clostridia* spp. did not show any reduction within 50 days.

A study of survival of *A. suum* eggs in wastewater sludge treated with ammonium hydroxide, (NH_4OH 0.5-4% ww), at 22°C resulted in no viability after 40 days for 1.5% NH_4OH and after 21 days for concentrations above 1.5% NH_4OH (Ghiglietti *et al.* 1997). The percentage of viable eggs decreased progressively with incubation time and ammonia concentrations. The 1.5% treatment resulted in 146 mM $NH_{3(aq)}$.

In a study by Jenkins *et al*. (1998), wild-type cryptosporidium oocysts were treated at 24°C with ammonium solution at concentrations of 7-148 mM $NH_{3(aq)}$ with exposure time 10

minutes to 24 hours. Exposure to 148 mM for 24 hours reduced the viable oocysts to 20% and 5.8 days were estimated to be required for 99.999% inactivation.

Reovirus has been reported to be relatively resistant to inactivation by ammonia, explained by the double stranded RNA. The single stranded viruses polio, coxsackie, and echovirus 11 were reported to be more sensitive to ammonia treatments (Burge *et al.* 1983).

Indicator organisms

An indicator organism is an organism used to indicate the potential presence of other, usually pathogenic, organisms. Indicator organisms are usually associated with the other organisms, but are usually more easily sampled and measured. When pathogen-containing material such as faeces and sludge are treated, the hygienic characteristics of the biosolids and process efficiency can be indirectly evaluated. The ideal faecal indicator organism should be (Feachem *et al.* 1983, Bitton 1999):

- A normal member of the intestinal flora of healthy people.
- Exclusively intestinal in its habitat and hence exclusively faecal in origin when found in the environment.
- Absent from non-human animals (a requirement not met by any of the bacteria currently used).
- Present in higher numbers than the faecal pathogens.
- Unable to grow outside the intestine, with a die-off rate slightly less than that of faecal pathogens.
- Resistant to natural antagonistic factors and to waste and wastewater treatment processes to a degree equal to or greater than faecal pathogens.
- Easy to detect and count by affordable methods
- Non-pathogenic.

Model organisms used in this study

Enterococcus faecalis

E. faecalis, which fulfils many of the criteria for a indicator organism listed above, was used in this study to evaluate its possibilities to functioning as indicator organism for the pathogenic model organisms studied . *E. faecalis* is a gram positive coccus, occurring in pairs or short chains mostly found in the intestines of warm-blooded animals. It is less prone to re-growth and generally survives somewhat longer than faecal coliforms and has been suggested as an indicator for the presence of viruses, particularly in biosolids and seawater (Feachem *et al*. 1983, Bitton 1999). *E. faecalis* is often found in numbers of 10^5-10^8 cells per gram of fresh faeces (Feachem *et al*. 1983).

E. faecalis grows in the temperature range 0 to 44°C, with optimum temperature at 37°C (Prescott *et al.* 1996). It is extremely hardy and halotolerant and can survive for weeks on

environmental surfaces; in soil up to 77 days, on soiled linen up to 90 days (PHA Canada www). *Enterococcus* spp. appear to be more resistant to lime treatment than salmonella and faecal coliforms (Allievi *et al.* 1994) and can grow at pH 9.6 (Prescott *et al.* 1996). Treatment at pH 10.5 with KOH or NH_4OH reveals the impact of ammonia on *Enterococcus* spp. The KOH treatment did not affect viability but for the ammonia treatment a 4-log reduction was achieved in 2 weeks. Treatment with H_2PO_4 resulting in pH 4 gave a faster reduction than pH 8.5-10.5 (Allievi *et al.* 1994). In the same study, the viability of *Enterococcus* spp. was found to be similar when stored in a temperature range from 10 to 20°C, whereas the reduction was very limited at 5°C.

Escherichia coli O157:H7

E. coli is one of the coliforms commonly used as faecal indicator organism in drinking water. Currently, there are four recognised classes of enterovirulent *E. coli* that cause gastroenteritis in humans. *E. coli* serotype O157:H7 is an unusual variety of *E. coli* that has been recognised since 1982 as a human pathogen causing food-borne disease with worldwide distribution (U.S Food & Drug administration www). Recent studies have indicated long survival times in soil for *E. coli* O157:H7 (Berggren *et al.* 2005). This finding, together with the zoonotic character, indicates the epidemiological importance of the organism. *E. coli* is of the genera facultative anaerobic, gram-negative, oxidase negative rods belonging to the family *Enterobacteriace*. *E. coli* is acid tolerant and a maximum pH tolerance at 10.5 is reported (Boost & Poon 1998).

Salmonella Typhimurium

As *Salmonella* spp. can resist dehydration and freezing for a long time and have proven to survive (4-14 months) and grow in the environment, salmonella is of epidemiological importance (Mitscherlich & Marth 1983). *Salmonella* is of the same genus as *Escherichia*, facultative anaerobic gram-negative rods that are oxidase negative. *S.* Typhimurium is acid tolerant (pH 4-8) and *Salmonella* spp. multiply in the temperature range 8-45°C (Acha & Szyfres 2003). Studies on lime treated sludge indicate that salmonella is one of the more resistant bacteria to alkaline treatment and that *S.* Typhimurium might be more resistant than *S. typhi*, with a maximum pH tolerance limit at pH 10.5 (Boost & Poon 1998). Allievi *et al*. (1994) report contradictory findings where various species of *Salmonella* spp. inoculated into lime treated sludge were no longer viable after 6 days, even in sludge with pH 9.

Ascaris suum

A. suum is a nematode mainly infecting swine and shows only slight morphological and physiological differences to *A. lumbricoides,* which infects man (Acha & Szyfres, 2003). *A. suum* has been used as an indicator organism for *Ascaris* spp. and as a model for other intestinal parasites. Eggs of the nematodes *Ascaris* and *Taenia* are very persistent in the

environment and to several sanitation methods and are therefore regarded as a good standard for parasitic survival (Feachem *et al*. 1983, Johnson *et al.* 1997). Gaspard *et al.* (1995) found 26±14% viable nematode eggs (*Ascaris*, *Toxocara*, *Trichuris* and *Capillaria*) in 6-year old lagoon sediment, showing the great persistence of these nematode eggs. The survival time for *Ascaris* spp. in faeces, sludge and soil at 20-30°C is estimated to be several months (Feachem *et al.* 1983).

The outstanding resistant properties of *A. suum* eggs are due to an outer layer made up of dense sticky material secreted by the uterine cells (Schwartsbrod 2001, Capizzi-Banas *et al.* 2004). An *in vitro* test of *A. suum* eggs exposed to various sewage treatment processes revealed that eggs inserted in embryonated form showed a greater viability than eggs inserted un-embryonated (Johnson *et al*. 1997). Embryonation occurs at 15 to 20 days under ideal conditions of humidity, temperature, shade and oxygen, but under adverse conditions the process can take much longer (Acha & Szyfres 2003). Anaerobic conditions prevent gas exchange necessary for egg metabolism and prolong the maturation of *Ascaris* eggs (Gaspard *et al.* 1995). An evaluation of methods with eggs in permeable bags showed a higher viability of *A. suum* eggs contained in bags compared to free eggs, indicating that reduction in egg viability might be underestimated when treated within bags (Eriksen *et al*. 1995).

Functions for bacterial inactivation

Ideally, inactivation of pathogens with disinfectants should follow first-order kinetics (Bitton 1999). The inactivation is assumed not to be affected by the concentration of microorganisms and thus the rate of inactivation should be constant over time. The number of organisms N_t at time t can be expressed as the logarithmic function $N_t = N_0 * e^{-kt}$, where N_0 is the number of organisms at time 0 and k is the decay constant.

MATERIALS AND METHODS

Experimental set-up

Approximately 300 g of faeces were weighed into 500 ml screw-capped plastic bottles (Figure 3). Bacteria suspended in horse serum broth were added to reach 10^7-10^8 cfu g^{-1} faeces (ww) for each bacterium studied. *A. suum* were inserted to the faeces in nylon bags (60 x 60 mm), permeable to surrounding fluid, containing approximately10^4 eggs each (Figure 3).

Figure 3. *Nylon bags containing eggs of A. suum during incubation in sulphuric acid (left) and the plastic bottles in which the infected faeces were treated (right).*

After the addition of model organisms, the faeces were treated with urea and/or lime and ammonia, with each treatment made in duplicate. Urea was added at 0.5, 1 and 2% according to wet weight. The ammonia treatment was designed to give the same molar addition of total ammonia as 2% urea (Table 5). The lime, $CO(OH)_2$, was added to give a final pH of 9.2, *i.e.* the same as in the 2% urea treatment. This was done both for the lime treatment and the urea-lime treatment. The final dry matter content was adjusted to 20% in all treatments with ionised water.

Table 5. *Treatments performed and organisms studied in the treatments*

Treatment	Urea	Ammonia	Lime	*S.* Typhimurium	*E.faecalis*	*A.suum*	*E.coli*
0.5% urea	0.5%			x	x	x	x
1% urea	1%			x	x		
2% urea	2%	680±17 mM[a]		x	x	x	x
Ammonia		690±13 mM		x	x		x
Urea-lime			pH 9.2[b]	x	x		
Lime			pH 9.2[b]	x	x		
Storage				x	x	x	x

a) Calculated from the amount of urea added.
b) Added to a final pH of 9.2

In a first experiment, the reduction in *S*. Typhimurium, *E. faecalis* and *A. suum* was monitored over 86 days. In a following complementary experiment, the reduction in *S.* Typhimurium in the ammonia treatment was monitored for 42 days. In addition, *E. coli* O157 was studied in some of the treatments in the 42-day experiment. All treatments in the main study were set up in duplicate, including the complementary treatment for *S*. Typhimurium. The reduction in *E.coli* O157 was studied in single treatments. The bottles were incubated at 14°C during the treatments and shaken on every sampling or measuring occasion.

Faecal material

The faecal material was collected from a single household of 5 persons, consisting of both children and adults, using a urine-diverting, dry toilet. The material consisted of faeces and some toilet paper and had been collected continuously during approximately three months. The moisture content of the material was determined by drying at 105°C for 20 hours.

Organisms

Bacteria

The strains of bacteria used were *S.* Typhimurium (CCUG 3169), *E. faecalis* (ATCC 29212) and a non verotoxin producing strain of *E. coli* O157:H7 (CCUG 42744), all obtained from the strain collection at SVA. Enrichment was performed in horse serum broth (SVA art no. 311060) (37°C, 18-24 h). After enrichment a serial dilution with 0.9% physiological saline solution was performed and samples spread on selective agar plates for enumeration and confirmation of viability. *S.* Typhimurium was detected on xylose lysin desoxycholate agar with 0.15% natrium-novobiocin (37°C, 18-24 h), *E. faecalis* on Slanetz-Bartley agar (44°C, 36-48 h) and *E. coli* O157:H7 on sorbitol MacConkey agar with cefixine and kalimtellarite (37°C, 18-24 h) (SVA production).

At sampling, two 10 gram samples of faecal material from each duplicate were collected and separately diluted up to 100 ml in phosphate buffer M 15 pH 7.2 (SVA), to neutralise the effect of ammonia. After this a tenfold serial dilution was performed in 0.9% physiological saline solution. The serial dilutions were plated on the selective agar media and incubated according to temperature and time mentioned above, to give 30-300 cfu per plate, i.e. suitable for concentration count (Bitton 1999).

Ascaris suum

A. suum eggs were collected from the ovaries of mature helminths obtained from slaughtered pigs and inserted into 60 x 60 mm permeable nylon bags.

At sampling, the bags were removed and incubated for 21 days at 22°C in 0.1 N sulphuric acid (Figure 3) before a viability count was performed for approximately 100-300 eggs per bag. Eggs containing larvae were counted as viable. The initial viability of the *A. suum* eggs was 75%.

Chemical analyses

Buffer capacity and pH

The pH was measured without dilution, at water content of approximately 80%, using an inoLab 720 pH meter (Wissenschaftlich-Technische Werkstätten, Hamburg, Germany.)

The buffer capacity of the faeces was calculated from the amount of lime added and the pH increase achieved in the lime treatment.

Ammonia measurement

The total ammonia in solution was analysed with a distillation-titration method. For the distillation, 0.3–2 g of the samples was dissolved in 250 ml deionised water with 25 ml borate buffer added to increase the pH. The distillate containing the ammonia from the samples was collected in Erlenmeyer flasks with 50 ml boric acid (2%). The distillate was titrated with sulphuric acid 0.02 M and a Misch indicator 5 (pH 4.4) was used for detection of the titre point. Concentrations of total ammonia in the samples were calculated from the titrate volume. There was no interference from the material on concentrations of ammonia.

Statistical analyses

Microsoft Excel 2000 was used for descriptive statistics calculations. Bacterial numbers, cfu g^{-1}, were plotted against time and linear regression for a function following the first order kinetics was tested. The least square method was used to estimate the suitability of the regression and from the functions the decimal reduction time (Dr) for the different treatments was derived. Means of unadjusted variables were compared using Excel Student's t-test. Confidence levels are reported in the results. Regression analysis was performed between decay coefficient k and disinfectant concentration, $[NH_3]$ or $[H_3O^+]$, using SigmaStat (SPSS; Chicago, IL, USA).

RESULTS

Buffer capacity and pH

The pH of the faecal material in the 86-day experiment was 7.14 ±0.11 before treatment. The initial pH of the 42-day experiment was 0.2 units lower and the difference was consistent for all the treatments throughout the study period. As the pH trends for both experiments were similar, only the pH from the 86-day experiment is presented in Figure 4. Tables are presented in Appendix 1.

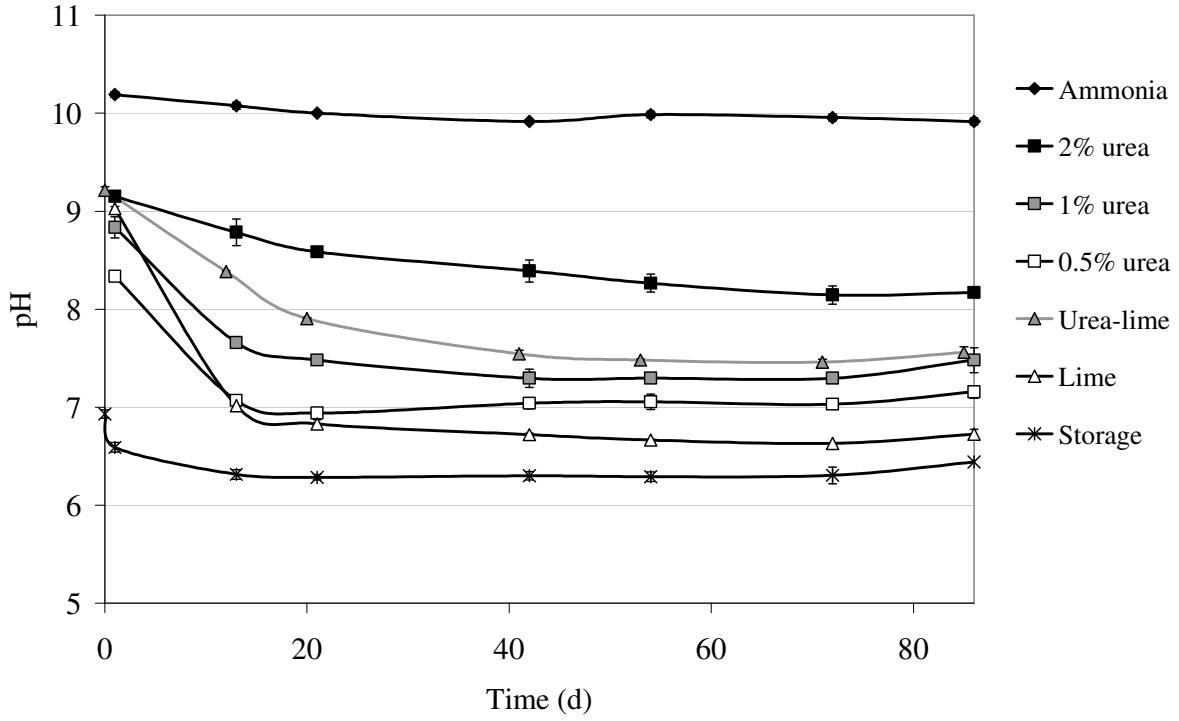

Figure 4. *The pH of the different treatments during the 86 days of study.*

The alkaline agents increased the pH by main proteolytic reactions. All additions resulted in a pH significantly higher ($p<0.01$) than that of storage and the difference was persistent during the study. Only the 1% urea, 0.5% urea and lime treatments showed less significant values ($p<0.05$) and only at the end of the study. Treatment with 1% ammonia gave the highest initial pH (10.2), while 2% urea, which supplied the same amount of total ammonia, increased the pH to 9.2. For the lime and urea-lime treatments, $Ca(OH)_2$ was added to give the same pH as for 2% urea (9.2). However, the pH attained in the lime treatment was 0.2 units lower than that of the 2% urea and urea-lime treatment (Table 5).

During the study period, the pH decreased somewhat in all the treatments, more rapidly in the beginning. Between days 72 and 86, the pH increased slightly for all treatments except ammonia and 2% urea.

On day 1, the pH in the storage treatment after addition of bacterial solution was 6.6 and it thereafter fluctuated some and finally decreased by 0.2 pH units during the 86 days of study. The ammonia treatment gave the most stable pH during the study, with a total drop of only 0.3 units. The largest decrease in pH was seen in the lime and urea-lime treatments, with a decrease of 2.3 and 1.6 pH units, respectively. On day 13, the pH in the lime and 0.5% urea treatments had reached pH values almost as low as or lower pre-treatment, 7.14±0.11.

The buffer capacity was 96.3 cmole kg^{-1} dry faeces.

Ammonia measurement

The concentrations of ammonia nitrogen measured day 86, showed values that roughly followed the additions of urea and ammonia (Table 7). The ammonia treatment had a lower concentration of total ammonia than 2% urea, 11.1 and 13.6 g respectively.

Table 7. *Concentrations of total ammonia in solution from ammonia distillation-titration method compared to concentrations from ammonia addition*

	NH_{tot}-N ($g\ kg^{-1}$)	
Treatment	From distillation	From addition
Ammonia	11.1 ±0.50	9.5 ±0.23
2% urea	13.6 ±0.18	9.6 ±0.19
1% urea	10.3 ±0.12	4.7 ±0.00
0.5% urea	7.3 ±0.48	2.3 ±0.00
Urea-lime	10.4 ±0.02	4.6 ±0.00
Lime	5.4 ±0.06	-
Storage	4.1 ±0.36	-

The urea-lime and the 1% urea treatments, both with an addition of 1% urea, gave figures of total ammonia in the same range, 10.3-10.4 g kg^{-1}. Lime and storage treatment where no ammonia was added gave concentrations ranging from 4.1-5.4 g kg^{-1} faeces, where the concentration of total ammonia was greater in the lime treatment than the storage. A standard was used to calculate the uncertainty within the method, which was at most 5%.

Bacterial inactivation

Data from bacterial counts are presented in the Appendix, Tables A2 to A5.

Inactivation of Enterococcus faecalis

The start concentration, day 0, of *E.faecalis*, was calculated from the inoculum, enumerated prior to addition. The enumeration of bacteria was performed with double dilution series and the standard deviation was ±20.4%. The bacterial enumeration was affected by outer performing the 10, but the results were linear for later measurements and further used for the reduction kinetics (Figure 5).

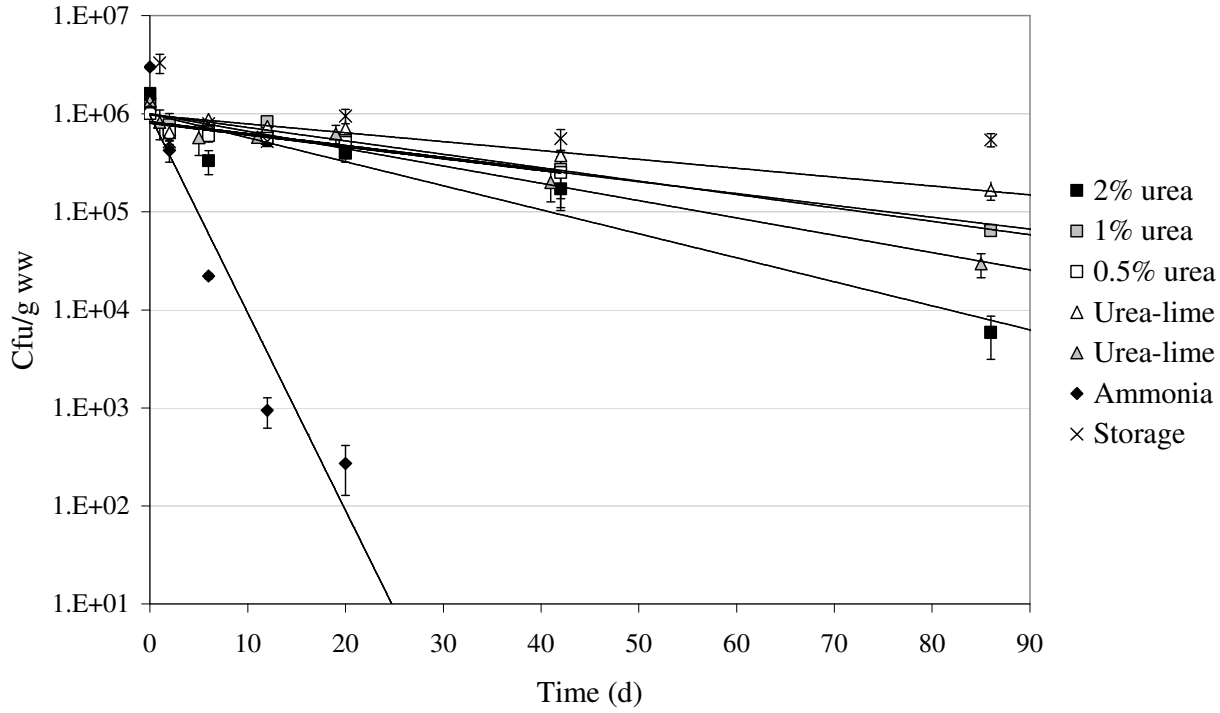

Figure 5. *Bacterial inactivation of E. faecalis in faeces by different nitrogen treatments and storage at 14 °C. The starting concentration was calculated from inoculum, prior to addition.*

E. faecalis proved more resistant to treatments compared to other model organisms and ammonia was the only treatment where no viable organisms were detectable after 86 days of study (detection limit 100 cfu g^{-1}). The reduction in bacterial concentration between sampling occasions was significant ($p<0.01$) in the ammonia treatment.

For the treatments where viable organisms were still detected on day 86, only 2% urea gave more than a 2 $\log_{10}$ reduction (2.4). The $\log_{10}$ reduction was 1.7 for lime-urea and 1.6 for 0.5% urea, whereas 1% urea gave a 1.3 $\log_{10}$ reduction during the 86 days of study. Lime and storage gave less than 1 $\log_{10}$ reduction, 0.9 and 0.4, respectively.

All the treatments except ammonia showed initial fluctuations as shown by significant ($p<0.05$-0.001) increases in bacterial concentration in the 2% urea, 1% urea, lime and storage treatments. Lime and 0.5% urea gave no significant reduction in bacterial concentration between sampling occasions until day 20. However, there was still an overall trend for decreasing bacterial concentrations and after day 20 significant reductions were observed for all treatments except storage, where there was a significant reduction ($p<0.01$) from day 6. However no significant reduction was measured in storage between the two last occasions, days 42 and 86.

Inactivation of Salmonella Typhimurium

The start concentration, day 0, of *S.* Typhimurium, was calculated from the inoculum, enumerated prior to addition. The enumeration of bacteria was performed with a single

dilution series and the standard deviation for the start concentration was calculated from the bacterial additions in the duplicates of treatments. As *S*. Typhimurium was not detectable in the ammonia treatment at the first measurement (day 2), a second experiment was performed though with pH values 0.2 units lower. For the second trial, the standard deviation for the bacterial concentration in solution was ±16% (Figure 6).

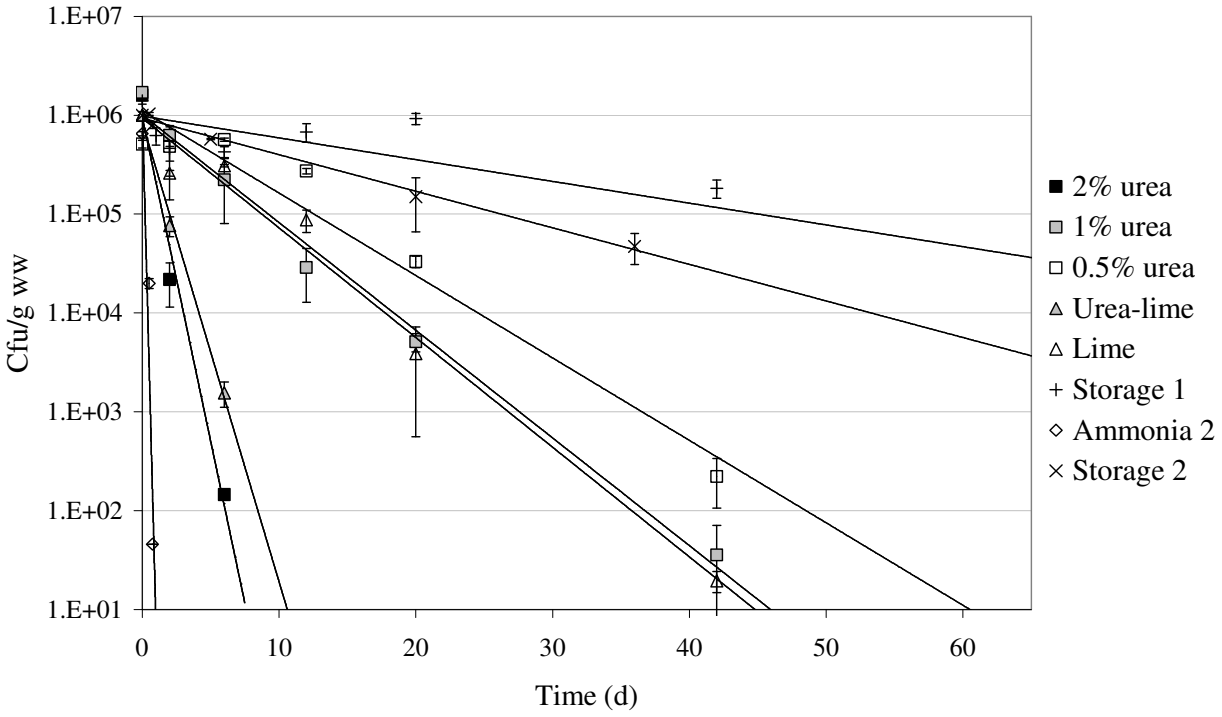

Figure 6. *Bacterial inactivation of Salmonella Typhimurium in faeces by different nitrogen treatments and storage 14 ℃. The starting concentration was calculated from bacterial inoculum.*

Ammonia and 2% urea were the most effective treatments for reduction of *Salmonella* Typhimurium, where no viable organisms were detectable after 5 and 12 days, respectively. After the 86 days of study, no viable organisms were detectable in any of the treatments except storage. The storage treatment gave a 2.2 $\log_{10}$ reduction during the whole period.

The 1% urea treatment gave a significant reduction in bacterial concentration ($p<0.05$) between measuring occasions, whereas 0.5% urea gave a significant reduction only after day 6. The lime treatment gave a significant ($p<0.01$) increase in bacterial concentration from days 2 to day 6, after which there was a significant reduction in bacterial concentration. The storage treatment showed significant fluctuations ($p<0.05$) until day 20 and then a significant ($p<0.001$) reduction during the remaining days of study. In the 36-day study, the storage treatment showed a significant reduction ($p<0.05$) from day 0.5 until day 20.

Where the significance of the reduction could not be determined, *e.g*. for the urea-lime and ammonia treatments where the data were insufficient to perform a t-test, the bacterial concentrations still showed a trend for a constant decrease.

Inactivation of Escherichia coli O157:H7

The start concentration, day 0, of *E.* coli, was calculated from the inoculum, enumerated prior to addition. The enumeration of bacteria was performed with double dilution series and the standard deviation was ±46% (Figure 7).

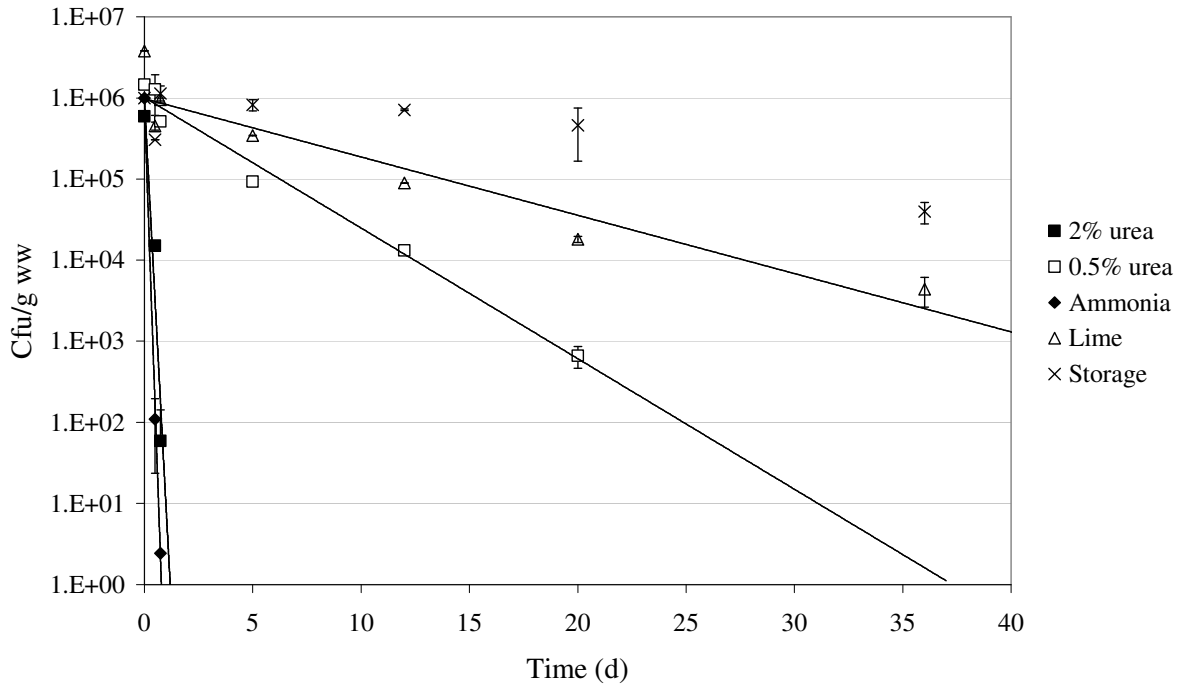

Figure 7. *Inactivation of Escherichia coli O157 in faeces by different nitrogen treatments and storage at 14℃. The starting concentration is calculated from bacterial inoculum.*

The ammonia and 2% urea treatments gave the fastest reduction of *E.coli* and no viable organisms were detectable on day 0.75 and 5, respectively (Figure 7). With 0.5% urea, no viable organisms were detectable by day 36. After 36 days of treatment, the lime and the storage treatments had bacterial concentrations corresponding to 2.9 and 1.4 $\log_{10}$ reduction, respectively.

The 0.5% urea treatment showed a significant ($p<0.05$) increase in bacterial concentration between 0.5 and 0.75 days, after which a significant ($p<0.01$) reduction was observed between sampling occasions. The storage treatment gave a significant ($p<0.001$) increase in bacterial concentration between 0.5 and 0.75 days and not until day 36 was the concentration significantly ($p<0.05$) lower compared to 0.5 days, although there was a trend for decreasing bacterial concentrations during the study. Where the significance of the reduction could not be determined, *e.g.* for the ammonia and 2% urea treatments where the data were insufficient to perform a t-test, the bacterial concentrations still showed a trend for a constant decrease.

Viable count of *Ascaris suum* eggs

The *A. suum* eggs were studied in treatments with 0.5% and 2% urea and in storage. By day 12 the viability had decreased from the initial 75% to between 40 and 70% for the treatments (Table 8). For all these treatments no viable eggs were observed after 41 days, corresponding to <0.04% viability. The viability of *A. suum* eggs did not differ significantly between treatments, even though the figures showed a tendency for lower viability for the urea treatments.

Table 8. *Viability of Ascaris suum eggs after12 and 41 days treatment with urea or storage at 14°C*

Treatment	Day 12		Day 41	
	No. eggs counted	Viability (%)	No. eggs counted	Viability (%)
2% urea	468	45±3.1	518	<0.04
0.5% urea	379	47±10	631	<0.04
Storage	409	62±10	376	<0.04

DISCUSSION

Buffer capacity and pH

The initial drop in pH observed for all treatments (Figure 4) may have been caused by increased bacterial activity, as high numbers of organisms were added to the faecal matter by the bacterial inoculum. The increased moisture content in combination with repeated mixing of the material may further have enhanced the bacterial activity. Bacterial activity produces acids and carbon dioxide, and further carbon dioxide from the air can dissolve in the solution and thus neutralise hydroxide ions by forming hydrogen carbonate. The ammonia and 2% urea treatments, where bacterial reduction was achieved much faster than for the other treatments, showed a more stable pH. The impact of bacterial activity on pH was demonstrated by a study of bacterial reduction in sewage sludge, where the decline in pH was smaller at 5°C than at 28°C as a result of bacterial activity (Allievi *et al.* 1994).

While exceeding the buffer range of the faecal material, the pH values achieved by the alkaline treatments were probably much more sensitive to the acidic effects than pH in the storage treatment (Figure 4). The ammonia treatment, which had a high and stable pH, was probably less sensitive to neutralisation as a result of high concentrations of hydroxide ions compared to neutralising agents.

Comparing the 2% urea and lime treatments, which both had an initial pH of around 9.2, the decrease in pH was less for the 2% urea treatment (Figure 4). This implies that the alkaline pH achieved from urea treatment was less sensitive to neutralisation as the urea produces a more complex system with several equilibriums. The alkalinity achieved in lime and 0.5% urea treatment was neutralised by day 13, as the pH of these both treatments was then stabilised at around 7.0.

As the pH affects the concentration of uncharged ammonia, a stable pH is necessary in order to predict the microbial inactivation. The results of the present study show that lime could be

used to increase the pH and thus the NH_3 concentration. However, the alkalinity created by lime is more sensitive to neutralising influenses than that created by urea, as the alkalinity is achieved by simpler mechanisms and not buffered against pH changes.

The peak pH was reached on day 1, indicating a rapid degradation of urea at 14°C. Other studies performed at room temperature also report a fast degradation of urea, as rapidly as 75 minutes (Park & Diez-Gonzales 2003, Vinnerås 2004).

The high buffering capacity, resembling the capacity in peat soils, is probably due to recalcitrant carbonic material consisting of a variety of weak acids and organic complexes that produce acidity through hydrolysis (McBride 1994). In a material with lower dry matter content, the buffer capacity per kg wet weight would be less and the same percentage of urea would probably result in higher pH and a higher amount of NH_3.

Concentration of ammonia in solution

The concentration of total soluble ammonia in the storage treatment was lower than in the lime treatment, 290 and 390 mM respectively. This may have been due to assimilation of nitrogen into microorganisms, as the survival was higher in the storage treatment than in the lime treatment. For a comparison of data the intrinsic ammonia was estimated to correspond to the concentration from distillation of lime (390 ±5 mM), as treatment factors were assumed to resemble those in the lime treatment more than those in the storage treatment when having alkaline additions (Table 9).

Table 9. *Concentrations of soluble ammonia in the treatments and calculations of NH_3 concentrations on days 1 and 42. Calculations were based on addition of ammonia-forming substances plus intrinsic ammonia from distillation. For concentrations on day 86 and treatments without ammonia addition, the calculations were based on distillation concentrations*

	NH_{tot} (mM)			pH			NH_3 (mM)		
	Distillation	Addition	Add+intr	d 1	d 42	d 86	d 1	d 42	d 86
Ammonia	800 ±36	680 ±17	1070	10.2	9.9	9.9	850	720	540
2% urea	970 ±13	690 ±13	1080	9.2	8.4	8.2	280	63	35
1% urea	730 ±9	340 ±0.0	730	8.8	7.3	7.5	107	3.6	5.5
0.5% urea	520 ±34	170 ±0.0	560	8.3	7.0	7.2	29	1.5	1.9
Urea-lime	740 ±2	330 ±0.0	720	9.2[a]	7.5[a]	7.6	210	6.7	6.7
Lime	390 ±5	-		9.0	6.7	6.7	80	0.51	0.52
Storage[1]	290 ±25	-		6.6	6.3	6.4	0.30	0.15	0.20

a) For urea-lime treatment, the pH is from days 0 and 41

For ammonia and 2% urea, the distillation gave concentrations of total ammonia nitrogen lower than expected from the additions and the intrinsic concentration in the faeces. This may be explained by losses of volatile ammonia during the repeated samplings. These losses could be assumed to be greater for the ammonia treatment with higher pH. The 0.5% urea, 1% urea and urea-lime treatments held concentrations that corresponded to the added amounts plus

calculation of intrinsic ammonia even slightly higher for urea-lime than expected from additions (Table 9).

Calculation of free ammonia was based on three pH values; the highest achieved in the study (day 1), that on day 42 when the pH had stabilised after the initial decline, and that at the end of the study (day 86). Furthermore the two first calculations were based on additions and intrinsic concentration, to avoid overestimation of the reducing effects as the distillation measurement was performed on day 86, with mostly lower concentrations than expected (Table 9). For the lime and storage treatments, concentrations of total ammonia from distillation were used.

The treatment with ammonia gave much higher pH and thus much higher concentration of NH_3 than the 2% urea treatment (Table 9). Thus the treatments were not comparable for evaluation of effects from other substances originating from urea, *e.g*. carbonate.

When comparing the concentration of NH_3 estimated from pH on days 0 and 42 (Figure 8), the influence of pH was obvious as the concentrations decreased by one or two orders of magnitude when the pH reached neutral, 6.3-7.3. In the storage and lime treatments, where no ammonia was added, the difference in pH gave a great response in NH_3 concentration, 0.29 and 82 mM respectively. Park and Diez-Gonzales (2003) reported a threshold limit of 30 mM free ammonia to achieve bacterial reduction in cattle manure and this concentration was initially (day 1) achieved by all the treatments except storage and 0.5% urea, which held an initial NH_3 concentration of 0.30 and 29 mM, respectively. By day 42, only the ammonia and 2% urea treatments held concentrations above 30 mM NH_3.

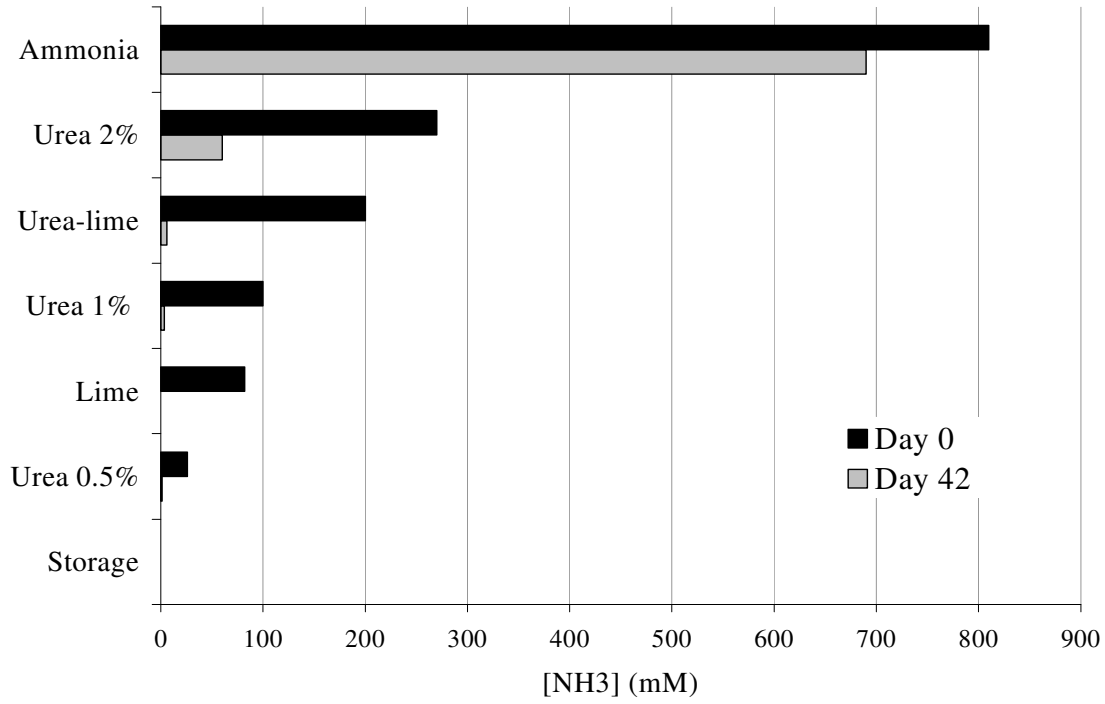

***Figure 8**. NH_3 concentrations in the treated faeces at days 0 and 42. The concentrations are calculated values based on nitrogen additions and pH.*

Bacterial reduction

Reduction kinetics

Even though ammonia concentrations declined during the study period, most treatments showed a reduction in bacteria following first order kinetics. Table 10 shows the decay coefficients *k* and the r^2 values. The r^2 values were around or above 0.90 except for the reduction of *E. faecalis* in storage treatment, where no significant reduction was observed. *E. faecalis* proved quite resistant to all treatments, although storage was the only treatment where the reduction not could be explained by an exponential function (Table 10).

Table 10. *Decay coefficients (k) and r2 values for the bacterial reduction, Nt(t) = N0·e-kt*

	E. faecalis		*S.* Typhimurium		*E. coli*	
Treatment[1]	k	r^2	k	r_2	K	r_2
Ammonia	-0.4638	0.91	-11.92	0.89	-18.239	1.00
2% urea	-0.0564	0.93	-1.5089	0.98	-11.576	0.91
Urea-lime	-0.0407	0.96	-1.0628	0.99	np	np
1% urea	-0.0315	0.97	-0.2509	0.99	np	np
0.5% urea	-0.0278	0.92	-0.192	0.95	-0.3709	0.98
Lime	-0.0208	0.90	-0.2554	0.98	-0.1656	0.90
Storage	-	0.15	-0.0507	0.87		
			-0.0852	0.99	-0.0722	0.69

np) For *E. coli*, treatments with 1% urea and urea-lime were not performed.

Ammonia effects on bacterial reduction

Regression analysis gave a linear relationship between the decay coefficient *k* and concentration of free ammonia that was significant (<0.05) for all the bacteria studied, even though fewer observations were available for *E. coli* and thus the test was weaker (Table 11). The linear correlation gives a model with change in *k* per mM uncharged ammonia, revealing *E. coli* as most sensitive to the treatments with the largest effect on the reduction rate per mM uncharged ammonia (Table 11).

Table 11. *NH_3 dependent change in decay coefficients derived from regression analysis*

	Change in k mM^{-1} NH_3	p	r^2	Observations
E. faecalis	0.000539	<0.001	0.93	7
S. Typhimurium	0.0140	<0.001	0.94	8
E. coli	0.0224	<0.05	0.90	5

When Park and Diez-Gonzales (2003) investigated the inactivation of *E. coli* and *S.* Typhimurium by ammonia in broth solutions at room temperature the bacteria decreased at a rate of 0.14 log and 0.03 log cell reduction per mM free ammonia, respectively, when studied during 6 days. In the present study no such relation between cell reduction and ammonia was obtained during the first 6 days, neither for the whole study and calculations revealed less reduction per mM than reported by Park & Diez-Gonzales (2003). This may depend on the

different media in which the reduction was studied, as particulate matter may reduce disinfectant efficiency.

When testing the correlation between reduction coefficient *k* and pH, only *E. faecalis* had a significant ($p<0.05$) but weak linear relationship ($r^2=0.69$). *S.* Typhimurium and *E. coli* did not show a significant ($p>0.05$) relation between pH and reduction, with R^2 values of 0.37 and 0.58, respectively. As *E. faecalis* was resistant to the ammonia based treatment, other parameters can be assumed to also be involved in the inactivation process.

Of the organisms studied, *E. coli* was the bacterium that was most sensitive overall to the treatments, even though other studies (Himathongkham & Riemann 1999, Park & Diez-Gonzales 2003) found *E. coli* O157:H7 to be more resistant than *S.* Typhimurium to ammonia treatment.

Efficiency of the treatments

Ammonia was the most efficient treatment in reducing the bacteria studied and in the case of *E. faecalis* the ammonia treatment showed a much faster reduction than the other treatments, resulting in a Dr time one or two orders of magnitude less (Table 12). For the other bacteria studied, the difference in reduction time was less between treatments and affected by the whole urea gradient. Note however that *E.coli* was not as thoroughly studied.

Table 12. *Decimal reduction time (Dr) and time to achieve 6 log_{10} reduction for the bacteria in the various treatments. Treatments arranged according to ammonia gradient*

Treatment[a]	Dr (days)/ 6 log_{10} reduction					
	E. faecalis		*S.* Typhimurium		*E. coli*	
Ammonia	5	30	0.2	1.2	0.13	0.78
2% urea	41	246	1.5	9	0.2	1.2
Urea-lime	57	342	2.2	13.2	-	
1% urea	73	438	9.2	55	-	
Lime	111	666	9.0	54	14	84
0.5% urea	83	498	12	72	6.2	37
Storage 1			45	270	32	192
2			27	162		

a) Highest concentration from ammonia addition +intrinsic distillation values calculated with pH on day 0. Lowest concentration from distillation values and pH on day 86.

Urea-lime treatment with the same level of urea addition as 1% urea had a faster reduction than 1% urea for the bacteria studied, *E. faecalis* and *S.* Typhimurium. This indicates that lime addition can be used to get a more rapid reduction from the same ammonia addition as the NH_3 concentration increased (Figure 8).

S. Typhimurium was equally sensitive to the 1% urea and urea-lime treatments, with a Dr time around 9 days, implying effect from both ammonia and pH at those levels. Both *E. faecalis* and *E. coli* were more sensitive to 0.5% urea treatment than to lime treatment, even though there was initially a higher pH and ammonia concentration in the lime treatment.

However, the 0.5% urea maintained a more stable pH, resulting in higher ammonia concentration in the latter part of the study (from day 42), and ammonia seemed to be the factor affecting the reduction even at that low concentration. Lime was the only treatment where *E. coli* proved to be more resistant than *S*. Typhimurium, indicating that *E. coli* is more resistant to pH than *S*. Typhimurium but more sensitive towards ammonia.

For *S.* Typhimurium and *E. coli*, the storage treatment also fitted an exponential reduction, indicating that those pathogenic bacteria are more sensitive to environmental factors than *E. faecalis* and that storage as a treatment does reduce bacterial numbers over time. However, for storage to produce a 6 $\log_{10}$ reduction treatment for 300 and 200 days are needed for *S.* Typhimurium and *E. coli*, respectively. The results from the repeated trial for *S*. Typhimurium indicated that the reduction in storage treatment is affected by many parameters that will be difficult to control and that the bacterial reduction may be unpredictable (Table 15).

Of the bacteria studied, *E. faecalis* was most resistant to the treatments and, using a 6 $\log_{10}$ reduction as a value for hygienisation, one month of ammonia treatment was needed. Thus *E. faecalis* may not be a good indicator of pathogen removal, although if *E. faecalis* is not found, it is likely that also pathogenic bacteria are absent.

Viable count of Ascaris

For the *Ascaris suum* no viable eggs were found (<0.03%) after 41 days in all treatments, including the storage. There was a tendency for lower viability with the urea treatments after 12 days, although this was not significant. It was not possible to correlate the inactivation rate to differences in concentration, as too few dates of sampling were available. The low concentration of free ammonia in the storage, 0.22mM, was assumed to be too low to affect the viability (Ghiglietti *et al.* 1997). This inactivation in the storage treatment was rapid compared to inactivation in animal faeces, where *A. suum* and other nematodes have been recorded as highly persistent (Helle *et al.* 1989, Caballero-Hernandez *et al*. 2004). However, the results were consistent with Vinnerås *et al.* (2003), who achieved very low viability (0.005%) in 50 days of storage, using comparable to storage method to this study. Eggs from the same batch, as in this experiment, were used in treatment of blackwater. In those trials with a DM of less than 1%, the viability of the *A. suum* was high (over 50%) throughout the 100 days of storage at a temperature similar to the temperature in this experiment (Vinnerås 2005). This might imply that some characteristics in the faecal material or some environmental parameters enhanced the inactivation of *A. suum* eggs.

How to sanitise faecal matter for safe fertiliser production

A person infected with *e.g.* Salmonella may excrete the pathogenic bacteria in concentrations of 10^6 g^{-1} faeces (Feachem *et al.* 1983). As dilution will take place, by healthy users and over time by the recovered person, a 6$\log_{10}$ reduction can be set as a standard for suitable hygienisation (Table 12).

If faeces are to be used as soil conditioner, the treatment time must be adjusted to seasonal use and to the available potential to store the faecal matter. The concentration of the treatment amendment can thus be adjusted according to time limitations. Ammonia, 2% urea and urea-lime appear to be treatments capable of achieving a rapid reduction, as these treatments could sanitise faeces within one month considering the faecal pathogens studied (Table 12). For treatment with 1% urea, two months were needed to achieve sufficient reduction of the pathogenic bacteria studied. *A. suum* is considered very persistent in the environment and is resistant to sanitation methods, thus affecting the total treatment time needed. As the nitrogen based treatment proved to be efficient, it gives good possibilities to shorten treatment time of faeces compared to other methods, *e.g*. liming.

As ammonia buffers at a higher pH than urea, it has a higher reduction efficiency per gram nitrogen added and will thus be cheaper to use than urea if nitrogen costs are equal. As the most common form of ammonia is a solution, it may be very suitable for a mechanised system where the mixing may be performed within closed containers and the emissions minimised.

In a rural small-scale context, urea may be the most available form of ammonia nitrogen. As the urea needs some time to degrade into ammonia, the emissions during initial blending will be minimised and urea may thus be suitable for blending by hand. Closed containers can be constructed by simple means even in rural areas in developing countries. Compared with liming, urea treatment is more user-friendly since it is manufactured in granular form and stabilise at lower pH.

When considering fertiliser value, the additions of ammonia nitrogen can be adjusted to meet the fertiliser requirements and the NH_3 concentration can be increased by other alkaline additions such as lime, which also counteracts soil acidification.

A limitation may be the initial mixing of urea and faeces to get a homogeneous ammonia concentration. Treatment at higher water content may enhance the mixing efficiency and distribution of ammonia in the faecal material, but demands larger storage capacity.

CONCLUSIONS

A pH higher than neutral is necessary to achieve uncharged ammonia. At this temperature, 14°C, a pH of at least 9 is preferable but also a stable pH is important for giving a constant concentration of free ammonia. Ammonia and urea treatments gave a more stable pH than lime addition. Closed treatment is also of importance to get treatment at constant $NH_{3(aq)}$ concentration. However, the volatile losses in this study cannot be considered representative, as small volumes were treated and the containers were opened on every sampling occasion.

Even though losses of ammonia occurred, this study showed that treatments with 1% ammonia and 2% urea, which gave the same addition of total ammonia, resulted in a fast reduction of the bacterial pathogens studied. The different chemical constitution and buffer interval resulted in a higher pH in the ammonia treatment than the 2% urea treatment and thus higher concentrations of NH_3. Addition of lime to the 1% urea treatment gave a faster reduction than urea alone by increasing the pH and concentration of NH_3. A 6 $\log_{10}$ reduction

of the pathogenic bacteria studied was achieved within three weeks in the ammonia, 2% urea and urea-lime treatments, with a good margin of error.

Comparing the patterns of bacterial reduction in the study, it seems as though *E. faecalis* has a higher threshold concentration at which it is affected by ammonia. If *E. faecalis* is to be used as a indicator of sufficient hygienisation, its presence only will not be a good parameter, as *E. faecalis* will exceed the hygienisation time of the pathogens studied by as much as several months.

For the *Ascaris suum* eggs, there seemed to be a tendency for urea treatments to give lower viability. However, after 41 days of storage no viable eggs were to be found either, indicating that other factors affected the viability.

When human faeces are to be used as a soil conditioner, the seasonal use and storage possibilities will set the limits for the treatment time and the concentrations of ammonia nitrogen used should be adjusted to the available treatment time. As nitrogen based sanitation gives a product with enhanced fertiliser value, the costs for fertilisers can be allocated to the treatment instead, with the same total expense.

FURTHER STUDIES

For the future adoption of the nitrogen based sanitation methods, it would be of interest to study other model organisms and correlate their reduction to endemic and emerging pathogenic microorganisms. It would also be of interest to include viral models that are persistent in the environment. As a reduction in *A. suum* occurred in storage, it would be of interest both to determine the factors other than NH_3 that affect viability and to study how NH_3 affects the egg cell.

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APPENDIX

Table A1. The pH values for the treatments during 86 days of study. The standard deviation was consistently small; the greatest deviation was ±0.13 pH units

Time (d)	Ammonia	2% urea	1% urea	0.5% urea	Urea-lime	Lime	Storage
-	7.0	7.2	7.2	7.2	7.2	7.2	6.9
0					9.2		
1	10.2	9.2	8.8	8.3		9.0	6.6
12					8.4		
13	10.1	8.8	7.7	7.1		7.0	6.3
20					7.9		
21	10.0	8.6	7.5	6.9		6.8	6.3
41					7.6		
42	9.9	8.4	7.3	7.0		6.7	6.3
53					7.5		
54	10.0	8.3	7.3	7.1		6.7	6.3
71					7.5		
72	10.0	8.2	7.3	7.0		6.6	6.3
85					7.6		
86	9.9	8.2	7.5	7.2		6.7	6.4

Table A2. Concentrations of *E. faecalis* (cfu g^{-1} faeces, wet weight ± s.d.%). Start concentration was calculated from bacterial inoculum. All treatments were performed at 14°C

Day	Ammonia	2% Urea	1% Urea	0.5% Urea	Urea-lime	Lime	Storage
0	7.4×10^{7}(±5)	8.5×10^{7}(±11)	7.9×10^{7}(±5)	6.9×10^{7}(±9)	8.0×10^{7}(±5)	8.0×10^{7}(±1)	7.0×10^{7}(±8)
1					4.8×10^{7}(±34)		3.6×10^{7}(±22)
2	1.0×10^{7}(±24)	3.5×10^{7}(±30)	5.1×10^{7}(±31)	4.7×10^{7}(±33)		3.8×10^{7}(±24)	2.9×10^{7}(±17)
5					3.4×10^{7}(±34)		
6	5.4×10^{5}(±6)	1.8×10^{7}(±27)	4.3×10^{7}(±10)	4.1×10^{7}(±14)		5.2×10^{7}(±3)	4.4×10^{7}(±8)
11					3.4×10^{7}(±12)		
12	2.3×10^{4}(±34)	3.0×10^{7}(±17)	5.4×10^{7}(±3)	3.9×10^{7}(±17)		4.4×10^{7}(±17)	2.9×10^{7}(±10)
19					3.7×10^{7}(±21)		
20	6.6×10^{3}(±53)	2.1×10^{7}(±18)	3.7×10^{7}(±9)	3.5×10^{7}(±17)		4.2×10^{7}(±21)	5.3×10^{7}(±17)
41					1.2×10^{7}(±37)		
42	ND	9.1×10^{6}(±36)	1.8×10^{7}(±50)	1.7×10^{7}(±59)		2.2×10^{7}(±39	3.1×10^{7}(±24)
85					1.7×10^{6}(±28)		
86		3.1×10^{5}(±47)	4.2×10^{6}(±10)			9.8×10^{6}(±21)	3.0×10^{7}(±15)

ND) not detected (detection limit 10^{2} cfu g^{-1} faeces)

Table A3. Concentrations of *Salmonella* Typhimurium (cfu g^{-1} faeces, wet weight ± s.d.%) from the 86-day study. Start concentration was calculated from bacterial inoculum. All treatments were performed at 14°C

Day	2% urea	1% urea	0.5% urea	Urea-lime	Lime	Storage
0	1.1×10^{7}(±12)	1.1×10^{7}(±5)	9.7×10^{6}(±9)	1.1×10^{7}(±5)	1.1×10^{7}(±1)	9.8×10^{6}(±8)
1				8.6×10^{5}±23		4.4×10^{6}(±20)
2	1.5×10^{5}(±47)	4.1×10^{6}(±22)	9.2×10^{6}(±29)		2.9×10^{6}(±7)	3.3×10^{6}(±71)
5				1.8×10^{4}(±29)		
6	$<1\times10^{3}$	1.5×10^{6}(±64)	1.1×10^{7}(±4)		3.5×10^{6}(±2)	3.0×10^{6}(±13)
11				1×10^{2}		
12	ND	1.9×10^{5}(±55)	5.2×10^{6}(±6)		9.8×10^{5}(±26)	4.8×10^{6}(±21)
20		3.3×10^{4}(±21)	6.3×10^{5}(±13)	ND	4.4×10^{4}(±86)	6.5×10^{6}(±13)
42		2.3×10^{2}(±98)	4.2×10^{3}(±52)		2.2×10^{2}(±24)	1.3×10^{6}(±21)
86		ND	ND		ND	5.8×10^{4}(±28)

ND) not detected (detection limit 10^{2} cfu g^{-1} faeces)

Table A4. Concentrations of *Salmonella* Typhimurium (cfu g^{-1} faeces, wet weight ± s.d.%) from the 36-day study. Start concentration was calculated from bacterial inoculum. All treatments were performed at 14°C

Day	Ammonia	Storage
0	1.4×10^{8}(±16)	1.4×10^{8}(±16)
0.5	4.4×10^{6}(±11)	1.4×10^{8}(±3)
0.75	$<1\times10^{4}$	1.1×10^{8}(±8)
5	ND	7.9×10^{7}(±2)
20		2.1×10^{7}(±56)
36		6.5×10^{6}(±35)

ND) not detected (detection limit 10^{2} cfu g^{-1} faeces)

Table A5. Concentrations of *E. coli* (cfu g^{-1} faeces, wet weight ± s.d.%). Start concentration was calculated from bacterial inoculum. All treatments were performed at 14°C

Day	Ammonia	2% urea	Urea 0.5%	Lime	Storage
0	4.1×10^{7}(±46)	5.0×10^{7}(±46)	4.3×10^{7}(±46)	4.1×10^{7}(±46)	4.0×10^{7}(±46)
0.5	4.5×10^{3}(±79)	1.3×10^{6}(±13)	3.8×10^{6}(±52)	4.9×10^{6}(±42)	1.2×10^{7}(±24)
0.75	ND	1×10^{4}	1.5×10^{7}(±3)	1.0×10^{7}(±15)	4.5×10^{7}(±23)
5		ND	2.8×10^{6}(±12)	3.7×10^{6}(±3)	3.3×10^{7}(±16)
12			3.9×10^{5}(±3)	9.7×10^{5}(±3)	2.9×10^{7}(±2)
20			2.0×10^{4}(±30)	2.0×10^{5}(±32)	1.8×10^{7}(±64)
36			ND	4.8×10^{4}(±152)	1.6×10^{6}(±29)

ND) not detected (detection limit 10^{2} cfu g^{-1} faeces)