Ammonia Based Sanitation Technology

Safe Plant Nutrient Recovery from Source Separated Human Excreta

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Licentiate Thesis

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Rapport 6
Report 6

Uppsala 2007
ISSN 1652-3261

Tryck: SLU Service/Repro, Uppsala 2007

Abstract

Water-borne sanitation of toilet waste is not a viable option for the estimated 2.6 billion people that lack improved sanitation throughout the world. In Environmental Systems Analysis, source separating sewage systems have proven to be of interest, since both energy and nutrients are saved compared with conventional systems. As the urine and faecal matter contribute with the majority of nutrients to wastewater but constitute a small part of the volume, these fractions are suitable for nutrient recycling to agriculture. The potential content of pathogenic (disease causing) microorganisms makes it a necessity to sanitise the material before use as a fertiliser, especially as many pathogens are zoonotic, infecting both man and animal. The main objective of this study was to evaluate ammonia based sanitation technology for source separated urine and faeces aiming for production of safe fertilisers. To achieve this objective, the inactivation kinetics of several groups of organisms was investigated in relation to concentration of free ammonia, NH_3, temperature and dry matter content. Inactivation of *Ascaris suum* eggs, S*almonella* spp. *Enterococcus* spp., *S.* Typhimurium phage 28B, an f-specific RNA phage MS2 and a coliphage ΦX 174 was monitored in spiked human urine and faeces. Storage of urine diluted 1:0, 1:1 and 1:3 with water was studied at 4, 14, 24, and 34°C. Faecal material, source separated dry, was treated with urea at concentrations ranging from 0.5% to 2% at 14, 24, and 34°C. Faecal material with ash amendments was studied at 24 and 34°C, separately and with supplementary addition of 1% urea. Temperature was found to be a key factor for the efficiency of the ammonia based sanitation, both through synergy and by affecting transformation of ammonia into NH_3. At 34°C the NH_3 concentrations in urine and faecal material resulted in short decimal reduction (D) values for microorganism concentrations, except for the bacteriophage 28B, which showed little inactivation in stored faecal material. At 24°C, treatments of both urine and faeces with NH_3 concentrations of 50 mM and above gave significant reductions whereas at lower concentrations (urine 1:3 and storage of faecal matter) little inactivation of bacteriophage 28B and ascaris eggs was observed. This means that urine must be collected as concentrated as possible in order to contain sufficient ammonia to reduce pathogens by storage. Treatment with urea, a 2% addition resulted in stable pH and NH_3 concentrations that resulted in fast *Salmonella* spp. inactivation even at 4°C and 14°C, and inactivation of ascaris and the bacteriophage at temperature 24°C and above. Coverage with ash and lime during collection can give an enhanced pathogen inactivation when later treated in closed containers. Accompanying urea treatment of faeces collected with ash is possible but with a high pH (>10) in the material urea will not be degraded and thus not contribute to inactivation.

Keywords: ammonia; *Ascaris*; bacteriophages; chemical disinfection; *Enterococcus* spp.; faeces; inactivation; model organism; nutrient recycling; pathogens; predictive microbiology; sanitation; *Salmonella* spp.; sanitation; temperature; urea, urine, urine diversion; zoonoses,

List of papers

The present thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Inactivation of bacteria and viruses in human urine depending on temperature and dilution rate

Björn Vinnerås, Annika Nordin, Charles B. Niwagaba, Karin Nyberg

Submitted to Water Research

II. Ammonia based sanitation of faecal sludge from source separating dry toilets

Annika Nordin, Jakob R Ottoson, Björn Vinnerås

Manuscript

III. Inactivation of ascaris eggs in source separated urine and faeces by ammonia at ambient temperatures

Annika Nordin, Karin Nyberg, Björn Vinnerås

Manuscript

Notes on the authorship of papers

I. A. Nordin and B. Vinnerås planned the study and A. Nordin, C. B. Niwagaba and K. Nyberg performed it. B. Vinnerås and A. Nordin did the writing with revision by co-authors.

II. A. Nordin and B. Vinnerås planned the study and A. Nordin performed it. A. Nordin and B. Vinnerås did the writing with revision by J.R. Ottoson.

III. A. Nordin and B. Vinnerås planned the study and A. Nordin and K. Nyberg performed it. A. Nordin and B. Vinnerås did the writing with revision by K. Nyberg.

Contents

Introduction

Humans generate waste, obviously at least urine and faeces, and disposal thereof was a matter of debate already in ancient literature (Deuteronomy 23:9, 12-13; (Carr and Strauss, 2001). In many societies, human waste material has been returned to agricultural land and the fertilising properties of excreta have long been recognised and utilised (Rockefeller, 2001). Urbanisation necessitated the use of water-borne sewerage in large cities and wastewater was discharged into recipient waters, where it was naturally dissipated and neutralised. With increasing population density, problems with *e.g*. odour and eutrophication of recipient waters have arisen and have been identified and partly managed. Current environmental pollution hazards from wastewater concern organic pollutants, *e.g*. pharmaceuticals and personal care products, which are to be found in both effluents and sludge (Gatidou *et al.*, 2007; Vieno *et al.*, 2007). Development of treatment strategies is an ongoing concern but 90% of the wastewater in developing countries is still discharged into recipient waters without any treatment (Langergraber and Muellegger, 2005). In Europe this figure is about 14% (EuroStat, 2006). The lack of treatment, and even of sanitation facilities, is a severe sanitary and public health problem in developing countries as it is estimated that 2.6 billion people lack improved sanitation (Ecosanres, 2007). Whether treatment of wastewater is undertaken or not, diluting contaminants with large volumes of water is a wasteful and in the long run non-viable solution. With water-borne sewerage, ideally the microorganisms are retained in the sewage sludge. The management of sewage sludge represents one of the major challenges in wastewater treatment today, with costs in many cases higher than for the water phase at treatment plants (Odegaard, 2004).

In most of the developing countries, *i.e*. the major part of the world, food production is mainly based on cereals and other vegetable products and the removal of nutrients with the harvested crop constitutes the majority of the nutrient flow out from arable land compared with meat and other animal products (Jönsson *et al.*, 2004). As mineral fertilisers are often not affordable, the continuous removal of nutrients from agricultural land results in less fertile soils and non-sustainable land use. Reuse of plant nutrients from human excreta can thereby contribute to a large proportion of the nutrient mass balance and can promote long-term soil fertility (Guzha *et al.*, 2005).

Deficient nutritional status is a major contributor to the global burden of disease and, together with diarrhoeal diseases, contributed *e.g*. 18.6% of the 2001 burden of disease measured in Disability Adjusted Life Years (DALYs) (Lopez *et al.*, 2006). Source separating sanitation systems that produce a safe human derived fertiliser can potentially contribute to great improvement in quality of life and life expectancy by enabling sustainable food production as well as proper waste management. Collection and treatment of human excreta are of great importance to safeguard human and animal health and there is a need for efficient sanitation techniques and more knowledge about pathogen survival and removal in the crude human excreta.

The most common treatment for source-separated faecal material is composting where the sanitisation is based on increase in temperature to above 50°C, which in many cases not are reached in all parts of the material, e.g. at air inlet. For urine, storage is currently the only large-scale practiced sanitation technique (Maurer *et al.*, 2006). In source separated urine and other nitrogen-rich materials, uncharged ammonia, NH_3 (aq), has been recognised as microbiocidal, and therefore manure, sewage sludge and faecal material have been amended with ammonia for the evaluation of microorganism inactivation (Allievi *et al.*, 1994; Höglund *et al.*, 1998; Park and Diez-Gonzalez, 2003; Vinnerås *et al.*, 2003b). The ammonia based sanitation method shows a high potential regarding both inactivation of pathogenic microorganisms and resource efficiency when used in fertiliser products. The resulting product has an increased fertiliser value as the ammonia is retained in the material.

Objectives

The main aim of the present study was to evaluate ammonia based sanitation technology for excreta fractions from source separating toilet systems, with the ultimate objective of reusing plant nutrients in human excreta by production of a safe fertiliser product.

Specific objectives were to evaluate possible treatment options and technologies for different fractions of toilet waste concerning inactivation of pathogenic microorganisms.

To accomplish these objectives, ammonia sensitivity and reduction rates for pathogens and model organisms were studied at different ammonia concentrations and treatment regimes, in both source separated urine and faeces, to estimate inactivation kinetics in relation to physical and chemical parameters.

Human excreta

Human metabolism and excreta

Human excreta are the waste products of the human metabolism. The ingested food is used as an energy source and as components in the synthesis of cells and tissues. As the foods are processed in the digestive system, essential molecules are absorbed by the intestines and passed to the blood stream, which transports them to target cells. Urine is the metabolic waste, excess ions and water, filtered away from the blood in the kidneys. The indigestible parts of the food, *e.g*. cellulose and chitin, pass through the intestines and are excreted in the faeces together with bacteria, digestion secretions such as bile, and exfoliated cells from the lining of the intestines (Petersen, 2007).

As the human organism has a limited maximum size, the flux of anabolic and catabolic reactions in adult human beings is in homeostasis and thus input to the metabolic chain results in a proportional output (Jönsson *et al.*, 2004). Although some elements are excreted with sweat, breathing (mainly H_2O and CO_2), hair, *etc*., most are excreted with the urine together with a large amount or water, about 550 kg per person and year, and with the faeces, about 50 kg per person and year (Lentner and Geigy, 1981; Vinnerås *et al.*, 2006). During growth and development, some of the elements ingested are incorporated into body parts such as bones, muscles, *etc*. As regards the macronutrients, approximately 2% N, 6% P and 0.6% K in intake are accumulated in the body during growth between the ages of 2 and 17 years (Jönsson *et al.*, 2004).

Since the same substances that are ingested are later excreted, the human excreta offer a complete source of plant nutrients but the different origin and function of the urine and the faeces influence their properties. Substances in urine have entered the metabolism and are water soluble (Lentner and Geigy, 1981; Kirchman H, 1995) whereas substances in faeces are partly incorporated into bacterial cells and indigestible material and are less plant available (Hotta *et al.*, 2007). Faeces therefore also contain recalcitrant carbon-rich material. The partitioning of plant nutrients between urine and faeces depends on the digestibility of the diet, as nutrients entering the metabolism are excreted in the urine, while the indigestible fraction is excreted with the faeces. In Sweden, around 88% of the N and 67% of the P excreted can be found in the urine, whereas in countries with less digestible diets, less of the N and P are found in the urine (Jönsson *et al.*, 2004; Vinnerås *et al.*, 2006).

Zoonotic pathogens restrict the use of human excreta

The anthropogenic delocalisation of resources may introduce both natural and synthetic compounds into new environments. Whether a substance is considered a contaminant is in many cases a matter of unnatural concentrations in the environment being considered. Plant nutrients, which cause eutrophication in aquatic environment but are a resource on arable land, are an example of this.

The major contaminant restricting the use of human excreta as a fertiliser is the possible presence of pathogenic microorganisms, *e.g*. bacteria and parasites (Ayres *et al.*, 1992; Jimenez *et al.*, 2007). Open defecation and disposal of untreated wastewater expose people and animals to infectious agents via contaminated water sources (Carr and Strauss, 2001). Similarly, the use of inadequately treated wastewater or toilet waste as a fertiliser is associated with increased prevalence of *e.g*. intestinal parasitic infections (Amahmid *et al.*, 1999; Moubarrad and Assobhei, 2007) and introduction of new routes for disease transmission must be prevented when using human derived fertilisers.

Of the diseases afflicting humans, approximately 50% are zoonotic, *i.e*. infectious to both man and animal and transferred between them (Cole and Oelschlegel, 2005), a property that affects risks with reuse as pathogens introduced by humans may be further spread by animals when biowaste is recycled (Albihn, 2001). Since zoonotic pathogens can persist in infected domestic and wild animals, good animal health status is important to not constitute a source of human infection for *e.g. Salmonella* ssp. and verotoxin-producing *Escherichia coli* (VTEC) (Albihn *et al.*, 2003; Boqvist and Vågsholm, 2005). In addition, some pathogens considered species-specific can occasionally be communicated between man and animal and target species for diseases may be affected by anthropogenic changes (Patz *et al.*, 2000). The zoonotic nature is debated for some pathogens and in Denmark, human ascaridiasis has been shown to originate from domestic pigs (Nejsum *et al.*, 2005).

Pathogenic organisms can be excreted at high concentrations even without any symptoms of disease. Therefore, human excreta must always be considered contagious and handled and treated accordingly to prevent disease transmission to man and animals and spread in the environment (Albihn, 2001; Schönning and Stenström, 2004). Thus, the major concern when using human derived fertilisers is to control and prevent the spread of pathogenic microorganisms into the environment in order to safeguard human and animal health.

Pathogens in human excreta

Pathogens are a diverse group of microorganisms and enteric infections can be caused by viruses, bacteria and parasites such as protozoa and helminths. Gastrointestinal pathogens are mostly shed with the faeces, where they can be excreted in high amounts, 10^9 units g^{-1} faeces, while only a few pathogenic microorganisms are shed from the human body via the urine (Bitton, 1999). The most probable source of pathogenic organisms in source separated urine is thus faecal cross-contamination in the sanitation system. Based on the content of faecal sterols in source separated urine, Schönning *et al.* (2002) estimated this contamination to be 9.1± 5.6 mg faecal material L^{-1} urine.

The prevalence of pathogens depends on region and sanitation status. The survival and spread of diseases depends on environmental factors such as climate, sanitation status and control programmes, with globalisation of trade and travel enhancing the distribution of pathogenic organisms (Macpherson, 2005). In many

developed countries viral infections are the most common cause of gastroenteritis while in developing countries, with lower levels of sanitation, parasites such as amoeba and helminths are more common (Koopmans *et al.*, 2002; Ashbolt, 2004).

Model and indicator organisms

To develop treatment options for human excreta that ensure human, animal and environmental safety, knowledge about inactivation and behaviour of pathogenic microorganisms in the material is required. For this, indicator and model organisms are used. An indicator organism is used to indicate the potential presence and decay of other, usually pathogenic, organisms. Faecal indicator organisms should ideally be present in high numbers in faeces and be easier to sample and measure than the actual pathogens. This means that they should be part of the normal intestinal flora (Bitton, 1999). When the relevant pathogens, or a good indicator organism, are absent or analyses difficult and costly, then model organisms can be added to the material for evaluation of treatment efficiency. Both indicator and model organisms should be equally or slightly more resistant than potential pathogens and be representative in their mode of inactivation. It is preferable, but not always possible, to use model and indicator organisms that are non- or weakly pathogenic.

Bacterial pathogens

Most species of pathogenic bacteria causing enteritis and diarrhoea in man are distributed worldwide, *e.g*. *Salmonella* spp., *Campylobacter* spp., *Yersinia* spp. and verotoxin producing *E. coli*, (Schönning and Stenström, 2004) Where sanitation is poor, *e.g*. in developing countries, *Salmonella typhi*, *Vibrio cholerae* and *Shigella* are common causes of diarrhoea from faecal contamination (Ashbolt, 2004; Schönning and Stenström, 2004). Some bacterial pathogens are excreted via the urine, *e.g*. *Salmonella typhi* and *paratyphi*, *Leptospira interogans* and occasionally *Mycobacterium spp*. (Schönning and Stenström, 2004; Wilson and Gaido, 2004). Bacteria may multiply outside the host and thereby increase the risk of exposure. This property, together with the, for many of the bacterial pathogens, zoonotic character, indicates their epidemiological importance (Albihn and Vinnerås, 2007).

Model and indicator bacteria

The human excreta contain a diverse microbial population not yet fully examined (Eckburg *et al.*, 2005) and the most frequently used faecal indicator bacteria belong to *Enterococcus* and *Enterobacteriaceae*. Concentrations of thermotolerant coliforms (*e.g. Escherichia coli*) are commonly used to denote water quality (Howard *et al.*, 2003). *E. coli* is more sensitive to treatment than many of the gastrointestinal pathogenic bacteria and thus its inactivation rates may not be representative (Stenström, 1996). *Salmonella*, also belonging to the family *Enterobacteriaceae*, is one of the most prevalent bacterial pathogen (Venglovsky

et al., 2006). *E. faecalis* and other enterococci have been studied as indicators of treatment efficiency regarding sewage sludge processing and are generally more resistant than faecal coliforms to environmental factors such as salt and desiccation, as well as to disinfection (Bitton, 1999).

In this work, a total of four salmonella strains were studied regarding sensitivity to ammonia treatment (Paper II) and a *Salmonella* Typhimurium, phage type 178, isolated from Swedish sewage sludge (Sahlström *et al.*, 2004) was studied in detail in both urine and faeces (Paper I & II). *Enterococcus faecalis* (ATCC 29212) and *Enterococcus* spp. originating from the material (Papers I & II) were also studied in detail and together they represent both Gram positive and Gram negative bacteria. In addition, *Escherichia coli* serotype H7:O157 (CCUG 44857) was used in some of the experiments (Papers I & II).

Viral pathogens

Viruses are a major cause of gastrointestinal infections in developed countries (Svensson, 2000) and are assumed to be responsible for many cases where no etiological agent is found (Höglund *et al.*, 2002). Based on their pathogenesis, viruses shed by faeces can be classified as enteropathogenic viruses, for which the gastrointestinal system is the principal site of infection (*e.g.* astroviruses, caliciviruses and rotaviruses), and non-enteropathogenic viruses, which can occur in the intestinal tract but not in association with gastroenteritis (*e.g.* most adenoviruses, enteroviruses and hepatitis A/E viruses) (Guardabassi *et al.*, 2003). Epidemic viral diarrhoea is caused primarily by the genus *Caliciviridae* (Vasickova, 2005) as a result of short-lived immunity (Ashbolt, 2004). Rotaviruses are by far the largest cause of diarrhoea in children and contribute significantly to child mortality in developing countries (Bitton, 1999), whereas hepatitis A is currently recognised as one of the most important food-borne pathogens in Europe (Vasickova, 2005).

Urine from unhealthy humans can contain pathogenic viruses (Vanchiere *et al.*, 2005) but due in general low pathogenisity and low frequency these are of minor concern compared with the large number of viruses that may enter the urine through faecal contamination in the sanitation system (Höglund *et al.*, 2002). Viruses are very small (20-300 nm) and of simple structure, with absence of organelles, metabolic activity and reproductive structures (Madigan and Martinko, 2005). Due to their lack of capability to replicate outside a host, viral numbers continuously decrease outside of the host. However, due to their low infective dose (from 1 to 10 viral particles), potential stability in the environment and ease of transport in aquifers (Höglund *et al.*, 2002), they are relevant disease agents.

Viral models

The use of bacteriophages, which infect bacterial cells, as a model for pathogenic viruses is common practice (Fraise *et al.*, 2004). Somatic coliphages, f-specific RNA coliphages and phages that infect *Bacteroides fragilis* are candidate model

phages for detection for faecal contamination in the environment (Havelaar *et al.*, 1990). They are naturally present in faecal material. In contrast, *Salmonella* Typhimurium phage 28B (Lilleengen, 1948), a phage that does not occur naturally in the environment, has previously been used as a model for viral survival in anaerobic digestion processes (Sahlström, 2006) and for tracing groundwater contamination (Carlander *et al.*, 2000).

In this work, *S*. Typhimurium phage 28B was used (Papers I & II) together with a bacteriophage Φx 174 (13706-B1) and a f-specific RNA coliphage MS2 (ATCC 13706-B1) (Paper I). The Φx 174 phage has been assessed and proposed as a model for viral disinfection (Bydzovská and Kneiflová, 1983). The MS2 coliphage has previously been used as an alternative to poliovirus in virucidal testing since it is structurally and genetically similar, *i.e*. it is a single stranded RNA, plus-sense virus (Fraise *et al.*, 2004). The phages used in Papers I & II represent different genome structures with *S*. Typhimurium phage 28B having double stranded (ds) DNA and bacteriophage Φx 174 single stranded (ss) DNA.

Parasites in human excreta

Protozoan parasites

Protozoa are a heterogenic group of single-celled, eukaryotic organisms defined as animals due to their motility. Protozoa that might be spread by excreta are parasites of the gastrointestinal tract and they do not spread via urine. Protozoa are responsible for the majority of enteric diseases and *Cryptosporidium hominis* and *Giardia intestinalis* are prevalent agents associated with water-borne outbreaks (WHO, 2006). Parasites are of greater concern in developing countries than in industrialised countries, with *Entamoeba histolytica* being an important cause of morbidity and mortality. The general importance of *Isospora* spp., *Cyclospora* spp. is currently debated (Schönning and Stenström, 2004).

Protozoa are most commonly excreted in cyst form, the infectious state where the cells are very resistant to environmental factors. Many of the protozoa mentioned are zoonotic, even though humans in most cases are the main reservoir of the organisms (Bitton, 1999; Acha and Szyfres, 2003; Ashbolt, 2004).

Helminths

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, with more than 25% of all humans being infected (Quilès *et al.*, 2006). Another helminthosis of importance is *Schistosomiasis* (bilhazia), 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 and Szyfres, 2003; WHO, 2006) Of the schistosomes, *S. haematobium* which is endemic in Africa, are excreted predominantly in the urine (WHO, 2006). Helminths with a direct lifecycle, *i.e*. no intermediate hosts, are more likely to infect humans by either eggs (*Ascaris*,

Trichuris, *Hymenolepsis nana*) or larvae (hookworms) compared with helminths with an indirect life cycle (Bethony *et al.*, 2006). However, the spread of helminths with indirect life cycle may affect animal health and meat quality *e.g.* tapeworms of *Taenia* spp. causing cysticercosis in cattle and pig.

Models for parasite inactivation

Oocysts of *Cryptosporidium* spp. are currently considered the microbial contaminant most resistant to drinking water treatment, *e.g.* chlorination, and are frequently studied as a conservative model for treatment efficiency (Fraise *et al.*, 2004). However, cryptosporidium wild cysts have been shown to be highly sensitive to treatment with ammonia in both aqueous solution (Jenkins *et al.*, 1998) and in stored human urine (Höglund and Stenström, 1999a).

Eggs of *Ascaris* spp. and of *Taenia* are regarded as being very resistant in the environment and to treatment, especially to chemical treatment. *Ascaris* is thereby regarded as a very conservative model organism. In screening of sewage sludge, eggs of the genus *Ascaridia* are the helminth eggs found most frequently both pre- and post-treatment (Gaspard *et al.*, 1997). *Ascaris suum*, which infects pigs, is often used as model for *A. lumbricoides* as pigs are infected all around the world and eggs and worms are easily available at slaughter. Comparison of the survival of *A. suum* and *A. lumbricoides* eggs harvested from worm uteri and eggs extracted from infected faeces in alkaline ammonia treatment (0.6 % NH_4OH w/w, pH 11.9) has shown equal inactivation independent of species or extraction method (Ghiglietti *et al.*, 1995). In this project, *A. suum* eggs were used as the parasitic model organism for inactivation in human faeces and urine (Paper III).

Treatment for sanitisation of human excreta

Factors affecting microbial inactivation

Pathogens in the environment will cease over time, although inactivation may be slow and unreliable. The main influence is by environmental factors such as moisture, temperature, carbon content and nutrient availability. However, changes in the external environment, *e.g.* seasonal shifts in temperature and humidity, can result in an increased number of pathogenic bacteria, a re-growth (Gibbs *et al.*, 1997). Biotic factors such as microbial competition for nutrients and antagonistic behaviour may also contribute to the reduction of pathogenic microorganisms. Factors that alone have a major effect on microbial survival and that are also quantifiable, and in the best case controllable, offer the possibility to predict sanitation levels in a way that storage alone can not.

Thermal inactivation of microorganisms is probably the most well known sanitation technique practised. The source of the heat can be external or generated within the material, *e.g.* composting. Temperature and time relationships have been examined for several pathogenic organisms and the combinations of time and

temperature sufficient for inactivation are reported by *e.g.* Vinnerås *et al* (2003a) and Feachem (1983). Composting is not a sanitation method *per se* – it is the high temperature in thermophilic composting that sanitises the material. Thus, a stable product that resembles soil is not a guarantee that the material is safe as regards pathogen content. Pasteurisation is commonly used as a pre-sanitation step to anaerobic digestion of animal-by products (Bagge *et al.*, 2005), but at present is not commonly included in the digestion of sewage sludge (Sahlström *et al.*, 2004). Incineration of faecal matter has been investigated as a sanitation method but is of limited interest as a low-tech sanitation method and approximated 90% of the nitrogen is lost (Niwagaba *et al.*, 2000).

Most enteric pathogens survive low pH and high salt concentration but strong alkalis or strong acids inactivate most of these organisms. Commonly used disinfectants for sewage and faecal sludge include lime and wood ash, where a pH of 12 for 3 months is recommended to inactivate persistent pathogens, such as helminth eggs (Eriksen *et al.*, 1995). To achieve this alkaline pH by ash amendment, large amounts of ash might have to be added (Boost and Poon, 1998). The activity of a disinfectant or preservative is usually increased when temperature is increased (Fraise *et al.*, 2004).

Ammonia as a chemical disinfectant

Chemical reactions and equilibria

Ammonia is a key intermediate in the nitrogen cycle in nature, and microbial production is the major ammonia source in the world. The toxic effects of ammonia on plants, animals and microorganisms have long been known and the biocidal effects are attributed to uncharged ammonia, NH_3 (Warren, 1962). Ionised ammonia, NH_4^+, is tolerated by most organisms, even at high concentrations, whereas NH_3 has a toxic effect even at low concentrations. In contrast to ionic NH_4^+, which needs active transportation through the cell membrane, the uncharged NH_3 molecule freely passes through cellular barriers by passive diffusion.

Ammonia is highly soluble in water which is partly explained by its polarity and ability to form hydrogen bonds. In aqueous solution, ammonia acts as a weak base producing hydroxide ions by the de-protonation of water (Eq. 1).

$$NH_3(aq) + H_2O(l) \leftrightarrow NH_4^+(aq) + OH^-(aq) \qquad \text{(Equation 1)}$$

Ammonia in solution is thus present both as dissolved ammonia gas, NH_3, and as ammonium ions, NH_4^+, and the relationship between them is quantified by the dissociation constant, K_a. The K_a of ammonia within the temperature range 0–50°C can be calculated by Equation 2 (Emerson, 1975), where T is the temperature in degrees Kelvin. The temperature dependence of the dissociation constant gives a pK_a, *i.e.* the pH at which the base and its conjugated acid are present in equal concentrations, of 9.9, 9.6, 9.3 and 9.0 for the temperatures 4, 14, 24 and 34°C respectively.

$$K_a = \frac{[NH_3]\cdot[H_3O^+]}{[NH_4^+]} = 10^{14-(\frac{2729.92}{T}+0.09018)} \quad \text{(Equation 2)}$$

$$[NH_3] = \frac{K_a \cdot ([NH_4^+]+[NH_3])}{K_a + [H_3O^+]} \quad \text{(Equation 3)}$$

The partitioning between NH_3 and NH_4^+ can be calculated according to Equation 3 and with H_3O^+ concentration being part of the equilibrium, the pH of the solution affects the fractionation of ammonia, with alkaline pH increasing the concentration of NH_3. As a consequence, ammonia is suggested to account for some of the variation that has been observed when stabilising ammonia-rich material with alkali (Allievi *et al.*, 1994; Mendez *et al.*, 2002). According to the influence of pH and temperature (Eq. 3), a pH of at least 8 is necessary for NH_3 to be present in any substantial fraction. The influence of temperature has a larger impact at moderate alkaline pH (8-10), whereas at pH 11 more than 90% of the ammonia is present as NH_3 regardless of temperature (Fig. 1).

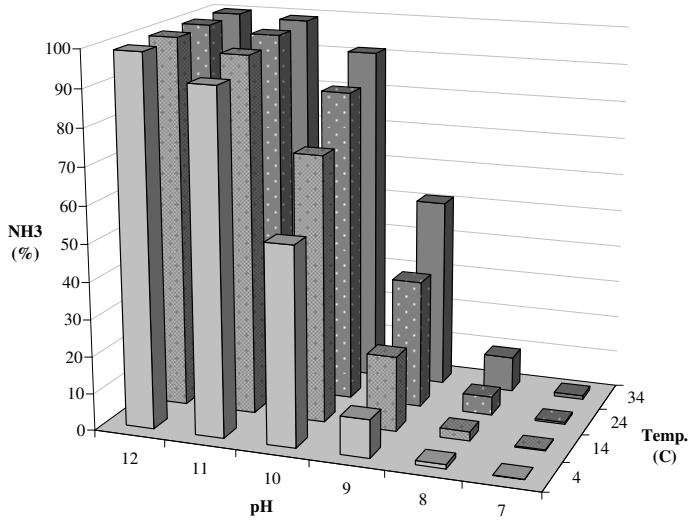

Figure 1. Fraction of total ammonia present as NH_3 (aq) at combinations of pH 7-12 and temperatures 4-34°C calculated from Equation 3.

Disinfectant action of ammonia

In source separated urine, as well as other nitrogen-rich materials, uncharged ammonia, NH_3 (aq), has been recognised to be microbiocidal and manure, sewage sludge and faecal material have been amended with ammonia for the evaluation of microorganism inactivation (Allievi *et al.*, 1994; Höglund *et al.*, 1998; Park and

Diez-Gonzalez, 2003; Vinnerås *et al.*, 2003b). Inactivation effects correlated to concentrations of uncharged ammonia have been reported for several types of microorganisms, bacteria, viruses and parasites, both in the mentioned biosolids and in pure ammonia solutions (Jenkins *et al.*, 1998; Pecson and Nelson, 2005). However, the mechanism and action of ammonia as a disinfectant are not totally clear. The small size of the ammonia molecule (molecular weight 17.03) and its high solubility, not only in water but also in lipids, enhances fast transport over membranes and other cellular structures. One hypothesis is that NH_3 causes rapid alkalinisation of the bacterial cytoplasm, as it easily penetrates the cell membrane by simple diffusion and reduces the intracellular proton concentration when NH_4^+ is formed (Park and Diez-Gonzalez, 2003). For virus inactivation, there are studies indicating that the inactivation is achieved by rupture of the RNA chain in otherwise intact viral particles (Burge *et al.*, 1983). The proposed inactivation mechanism for ssRNA viruses is cleavage of the RNA in intact particles with otherwise little structural alteration (Ward, 1978).

Methodology

Materials

Urine used in the present study originated from a source-separating, low-flush, sanitation system resulting in a dilution of approximately 1:1 (3.6-4.1 g NH_4 L^{-1} and pH 8.9-9.0). For additional evaluation of influences from the collection system, urine was collected directly from persons in a single household and urea was degraded with urease (Papers I & III).

The faecal matter containing some toilet paper used in this study was from two sources: collected from mainly men in late teens or early twenties doing their military service and from faecal bins in a housing area using dry urine diverting toilets. Water content was set to 80-83%, which corresponds to the water content of excreted faeces (Vinnerås *et al.*, 2006). Ash used for treatment of faecal matter was from a mix of hard and soft wood, incinerated in a heating furnace. For treatments at 34°C the ash was sieved (mesh 7 mm) (Paper II).

Uterus derived eggs of *A. suum* were studied in permeable nylon bags (mesh 35 µm), approximately 10^4 eggs each (Paper III). Bacteria and bacteriophages were inoculated into the urine and faeces (phage suspension 2 mL L^{-1} urine and bacterial solution 10 mL L^{-1} urine) resulting in initial concentrations of 10^6-10^8 cfu/pfu per mL urine or gram final faecal material (Papers I & II).

Experimental set-up

Faecal material was studied in soft plastic containers with minimal head space containing approximately 200 g each. Urine was studied either in 500 or 50 mL plastic flasks or centrifuge tubes. Urine was studied at 1:0, 1:1 and 1:3 dilutions with water at temperatures 4, 14, 24 and 34°C.

Two sets of faecal material were studied, non-amended and amended with ash, 0.1 L per 100 g faeces (17% DM), simulating surface coverage by ash after defecation. Ash amended faeces were adjusted to 17% DM and also further treated with 1% urea, and these treatments were studied at 24 and 34°C. Faecal material without ash was treated with urea from 0.5 to 2% addition w/w at temperatures 14, 24 and 34, although not all concentrations were studied at all temperatures. In addition, inactivation of all organisms was studied in ammonia-free controls (physiological saline solution, 0.8-0.9% NaCl) for the temperatures studied.

Microbial reduction kinetics

Modelling disinfection kinetics

Reduction kinetics based on empirical data enable estimation of the behaviour of microbial populations as a function of process parameters. These process parameters and reduction coefficients provide tools to predict microbial inactivation and to compare the impact of different process factors on various organism groups.

The use of consistent parameters across inactivation technologies simplifies comparison between different organisms and studies. The traditional approach to describe changes in microbial populations as a function of time is based on the assumption of first order reduction kinetics, *i.e*. that the inactivation is concentration dependent, with a halved microbial population at every equal time period. This inactivation gives a linear reduction for microbial log-concentrations over time. Equation 4 gives the microbial concentration N_t at time t dependent on N_0, the initial microbial population, and k, the decay coefficient. From the decay coefficient, the time for one (1) $\log_{10}$ reduction of the microbial population, mostly denoted D or t_{90}, can be derived. D-value is often used to compare microbial inactivation rates and for prediction of the degree of sanitation given a certain incoming pathogen concentration. Similarly is t_{99}, time for 2-$\log_{10}$ reduction, *i.e.* 99%, often used for the inactivation of helminth eggs.

$$N_t = N_0 \cdot e^{-kt} \qquad \text{(Equation 4)}$$

However, deviations to the first order kinetics model have repeatedly been noted. Phenomena such as shoulders and tails or sigmoid curves in log-linear diagrams have often been observed. Alternative models to explain the microbial inactivation kinetics when the log-linearity of the data is questionable have been developed, but their predictive power is lower since accurate D are difficult to derive for these (Xiong *et al.*, 1999).

Modelling deviations from first order kinetics

Increased inactivation rates

In the present work the bacterial inactivation in many cases followed first order kinetics with a good fit (R2>95%) but a general deviation from first order kinetics was observed for all the organisms studied, even though not in every treatment. Organisms reached the detection limit (10 pfu/100 cfu g^{-1} faeces and 1 pfu /10 cfu mL^{-1} urine) earlier than estimated from prior samplings assuming first order reduction. This behaviour was observed frequently for both *Salmonella* spp. and *Enterococcus* spp. at intense treatments and when detection limit values gave lower estimates of D-values they were included in the function (Fig. 2).

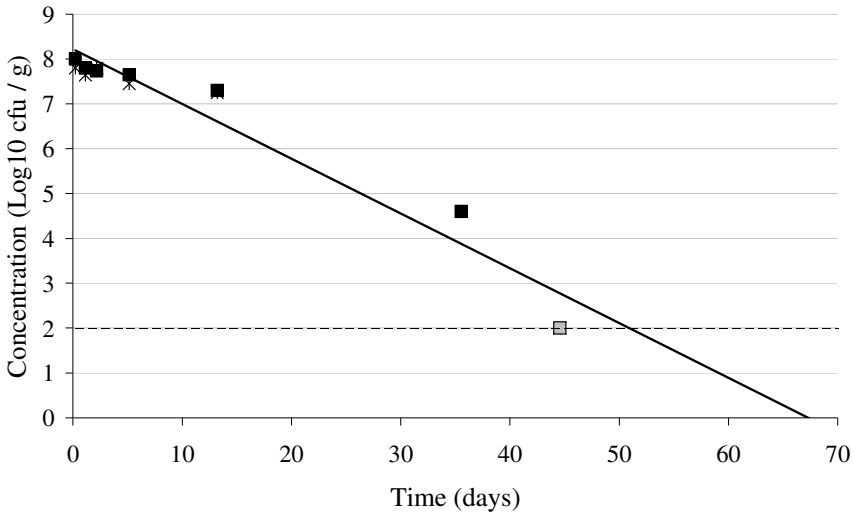

Figure 2. Log concentration (log_{10} cfu g^{-1} faeces) of *Salmonella Typhimurium*, sewage isolate, as a function of time in faecal material stored at 14°C, measured in duplicate. Non detected values included in the regression, are marked with grey symbols and the detection limit concentration with a broken line

Fluctuating concentrations of *Enterococcus* spp. (Papers I & II)

As the uncharged ammonia may also act as a substrate for bacteria, the pH is important for ammonia speciation and thus for ensuring that the amounts of uncharged NH_3 suffice to inactivate microorganisms. In some of the studies, *Enterococcus* spp. showed initial growth and fluctuating concentrations when temperature and ammonia concentrations were less intense (Fig. 3). Since growth was also observed in ammonia free controls at 24°C and 34°C (Paper I), it was concluded that ammonia was not the cause of the growth, but it may have been present at too low concentrations to give any direct inactivation.

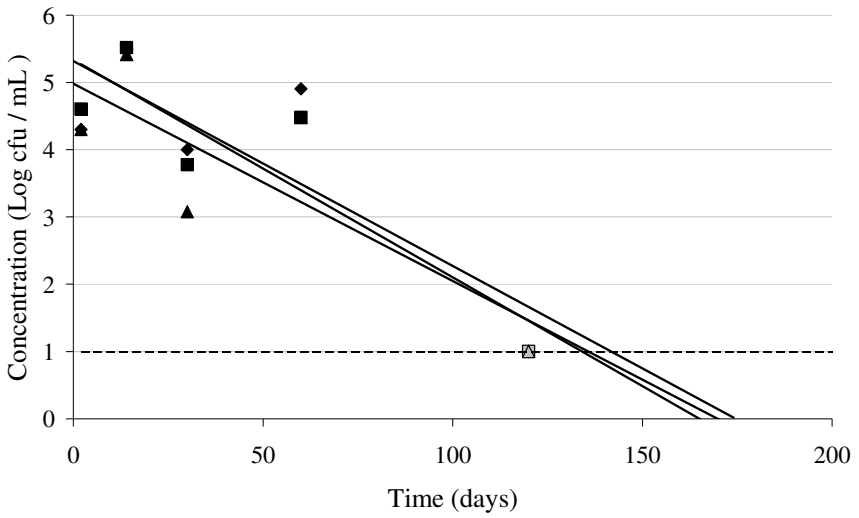

Figure 3. Log concentration (cfu mL^{-1}) of *Enterococcus* spp as a function of time in urine diluted 1:1 at 34°C, measured in triplicate, showing fluctuating concentrations over time. Measured non detected limit values, included in the regression, are marked with grey symbols and the detection limit concentration with a broken line

Initial decline in bacteriophage concentrations (Papers I & II)

In some of the treatments, the reduction kinetics for two of the bacteriophages, MS2 and Φx 174, deviated from first order by having an initially fast inactivation rate, which was followed by a phase with slower inactivation rate. The fast inactivation mostly lasted until concentrations of 10^3 to 10^4 pfu mL^{-1} were reached. In urine at high temperature (≥24°C), a clear breakpoint between the inactivation rates was observed (Paper I; Fig. 4). In order not to overestimate the sanitation efficiency, the initial fast reduction phase was excluded from the dataset when fitting the function of inactivation, *i.e.* estimating D-values.

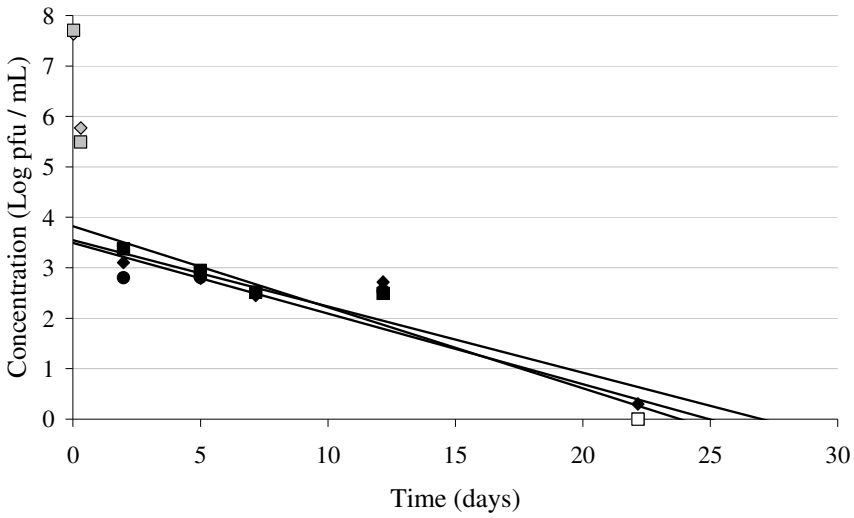

Figure 4. Log concentration (pfu mL^{-1}) of bacteriophage MS2 as a function of time in urine diluted 1:1 at 34°C, measured in triplicate. The data show two distinct inactivation rates, with the exponential regression based on the lower rate. Samples excluded from the regression are shown in grey and non detected concentration is 10^0 pfu mL^{-1}, marked with white symbol.

Lag phase periods for the inactivation of *A. suum* eggs (Paper III)

In the studies of inactivation of *A. suum eggs*, an initial phase with no significant reduction of egg viability was observed for some of the urine dilutions at 24 and 34°C. For the faecal storage at 34°C, egg viability on day 5 and 8 was higher than the initial viability. Similar delayed inactivation of *Ascaris* eggs when treated with ammonia can be observed in data presented by Ghiglietti *et al*. (1995; 1997) and by Pecson *et al*. (2007). Pecson *et al*. (2007) also proposed a model for shouldered inactivation of *Ascaris* eggs according to studies presented by (Harm, 1980). When fitting the data of Paper III, the model did not indicate any lag phase and the main reason was probably too low initial sampling frequency. The model based on exponential decay resulted in estimates of time for total inactivation exceeding the reduction time actually observed in Paper III. Consequently, for estimates of t_{99}, treatments with a lag phase were divided into two linear inactivation functions. In all cases these linear functions gave a better fit than the function proposed by Pecson *et al.* (2007) (Fig. 5).

With too low initial sampling frequency, a lag phase might not be detected in treatments with a fast reduction due to few data points. This may explain why lag phases not was observed in all treatments. Similarly, at low temperature storage of urine, the sampling did not cover the span of the whole inactivation and thus the inactivation may still have been within the lag-phase, since at the most 20% inactivation occurred during the 6 months of measurement.

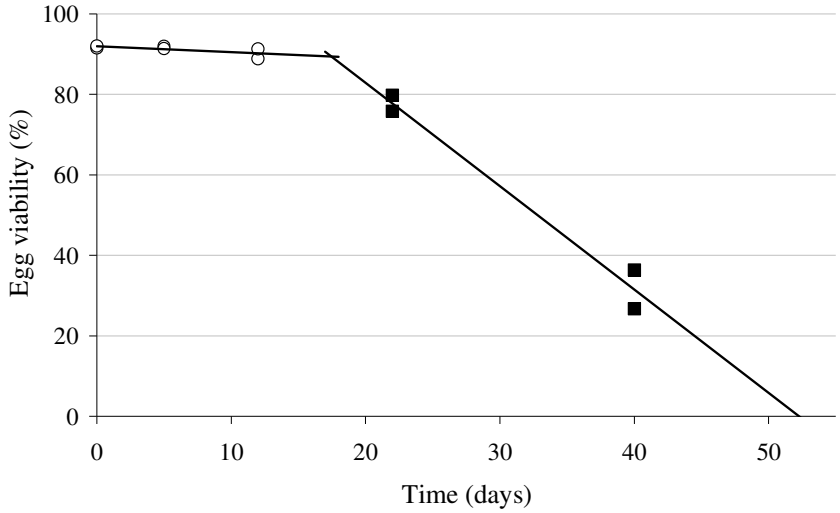

Figure 5. Inactivation of *Ascaris suum* eggs in urine diluted 1:1 stored at 24°C. The t_{99} was derived by linear regression of the two sets of inactivation, *i.e*. the initial stationary phase with no significant reduction (○) and the set of data with significant inactivation (■) from the initial viability.

When a lag phase was observed (Paper III), it constituted one third to half of the time taken for total inactivation (>2 $\log_{10}$ reduction). The initial lag phase, with only little inactivation of *Ascaris* eggs, seems to be proportional to the total time for inactivation. This is similar to observations for *Ascaris* eggs in sewage sludge at pH 7 and 12, irrespective of whether the inactivation took place during 24 days or 110 min (Pecson *et al.*, 2007). For *A. suum* and *A. lumbricoides* in ammonia treated sewage sludge, the lag phase constituted approximately half of the time taken for total inactivation (Ghiglietti *et al.*, 1997). The lack of a lag phase in stored faeces may be explained by bile from the faeces having induced development of the eggs (Han *et al.*, 2000) (observed for the faeces at 34°C), which may have made them more susceptible to the ammonia. In the literature, contradictory results have been reported regarding whether embryonated eggs are less or more susceptible to inactivation factors (Johnson *et al.*, 1998; Pecson *et al.*, 2007).

In conclusion, estimation of inactivation without considering lag phases will overestimate time for reduction. For the treatments with slow inactivation, *e.g*. storage of urine at temperatures 4 and 14°C (Paper I), little can actually be concluded about the reduction beyond the last sampling at six months.

Bacterial reduction

S. Typhimurium model for *Enterobacteriaceae (PaperI & II)*

Differences in sensitivity to ammonia between species were tested for four strains of salmonella: *S.* Typhimurium isolated from Swedish sewage (Sahlström *et al.*, 2004); *S.* Typhimurium (CCUG 3169), *S.* Typhimurium DT 104, isolated from cattle, and *S. Yoruba* isolated from fodder. The susceptibility was tested in stored faeces and faeces with 2% urea at 14 and 24°C. Little variation in reduction rate, and thus in sensitivity, was observed between the strains and species at 24°C, 2% urea gave a 6-7 $\log_{10}$ reduction in 24 hours for all salmonella strains studied. At 14°C *S.* Typhimurium isolated from sewage sludge proved more resistant to both the 2% urea treatment and the storage, having twice as long reduction time compared to the other strains studied (Paper II).

Escherichia coli O157:H7 (CCUG 44857) was studied at 14 and 34°C in urine 1:1 and in faeces. The *E. coli* were equally or more sensitive than *Salmonella* spp. This was true for all treatments and reduction at 34°C was rapid. After 6.5 hours the bacteria were not detected in any of the amended faeces at 34°C. This corresponded to more than a 7 $\log_{10}$ reduction. Storage of faeces at 34°C gave D-values of 1.06 days and 1.2 days for salmonella and *E. coli* respectively, indicating equal sensitivity. At 14°C, *E. coli* O157:H7 was more sensitive than salmonella, with 2 and 5 times faster reduction in faeces treated with 0.5% and 2% urea, respectively. The D-values in stored faeces at 14°C gave less difference, 32 and 44 days respectively.

This is in accordance with the studies on *S.* Typhimurium and *E. coli* O157 performed by Mendez *et al.* (2004) on ammonia treatment of sewage sludge. Inactivation in relation to ammonia concentration has not been studied for a wide range of bacterial pathogens. In alkaline treatment of *e.g.* sewage sludge the alkaline effects can never be totally separated from that of uncharged ammonia, and such studies thereby gives and indication on sensitivity to both pH and free ammonia. In general, *E. coli* is considered more resistant than *Shigella* (Stenström, 1996) and Boost & Poon (1998) found *E. coli* and *S.* Typhimurium to be more resistant to alkaline treatment of sewage sludge than *Salmonella* Typhi, *Shigella sonnei*, *Vibrio parahaemolytica* and *Campylobacter jejuni*. Despite not being specifically correlated to ammonia concentration, this indicates that *Salmonella* spp. can be an appropriate indicator organism for inactivation of *Enterobacteriaceae* in ammonia based sanitation.

Salmonella inactivation (Paper I & II)

The reduction of *Salmonella* spp. was very fast compared with that of the other organisms studied. The relationship between NH_3 concentrations and inactivation rates was less apparent (Paper I), but also less relevant due to the short inactivation times. In urine the longest D-values was 6.5±9 days, at 4°C Plotting D-values against NH_3 concentrations (Fig. 6) shows some inconsistencies when NH_3 concentrations were below 50 mM but may to some extent be explained by the underestimated D-values, i.e. presented as “less than” values in Paper I. At 50 mM NH_3 and above in the urine storage, the D-value was approximately 2 days or less.

The faecal treatments at 24°C, where ammonia was measured, do dot deviate from the urine reduction data despite the different constitution and dry matter content (Fig. 6).

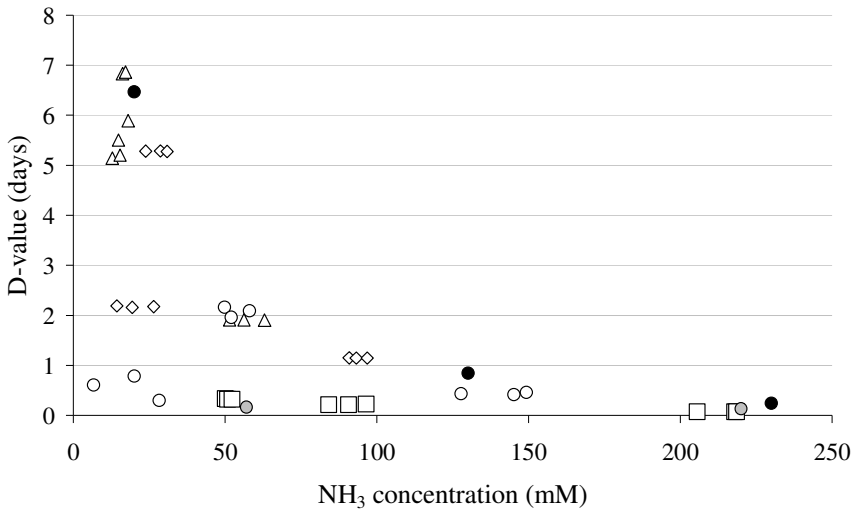

Figure 6. Reduction rates for *Salmonella* spp. (D-values in days) plotted against concentration of uncharged ammonia, NH_3, in urine (white) (4-14°C) and in faeces (black), DM 17%, (14-34°C). Faecal samples with ash amendment are marked with shaded symbols and temperatures 34°C (□), 24°C (○), 14°C (◇), 4°C (△).

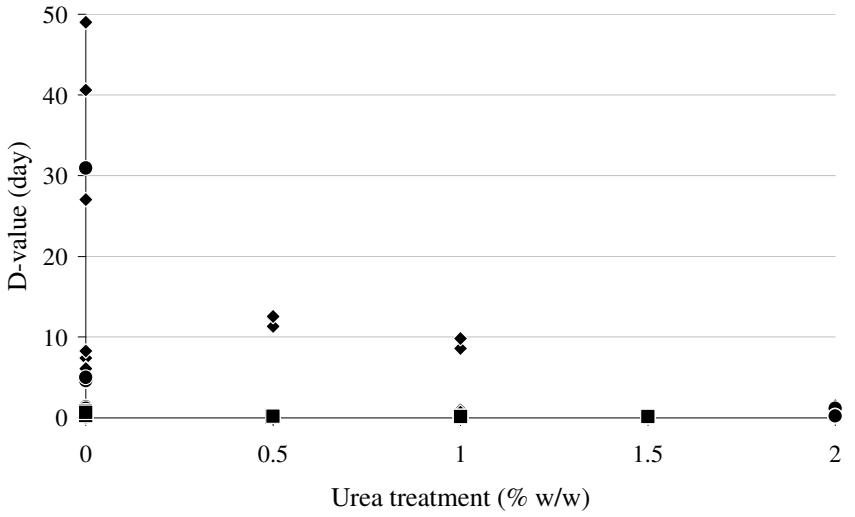

Figure 7. Reduction of Salmonella spp. (D-values) from faecal treatments with urea added as percentage w/w to faecal matter (DM 17%) at temperatures 34°C (■), 24°C (●), 14°C (♦) and 4°C (▲).

However, in stored faeces with no amendments and intrinsic ammonia concentrations of 210-250 mM, had a low and variable pH that resulted in 0-62 mM NH_3. The D-values followed the free ammonia concentration and varied from 1 to 50 days. The higher D-values were in most cases associated with lower temperatures (Paper II) (Fig. 7). In ammonia free temperature controls the salmonella stayed constand for a long time at all temperatures studied (4, 14, 24, 37°C), in some cases even growth were observed (Paper I).

When Park & Diez-Gonzales (2003) evaluated the threshold inhibitory concentration of NH_3 (from urea additions) with respect to *E. coli* O157:H7 and *S.* Typhimurium, reduction could be observed at 20°C in pure broth cultivation at 5 mM whereas 30 mM NH_3 (aq) was required when studied in cattle manure. This supports the results from the present study regarding NH_3 thresh hold concentrations and imply that faecal material require higher concentrations of NH_3, not only as a matter of pH and ammonia equilibrium. For NH_3 concentrations 40-45 mM, Park and Diez-Gonzales (2003) observed D-values less that one day for *S.* Typhimurium*, E. coli* O157:H7 and total coliforms and with 245 mM NH_3 D-values were less than half a day. This agrees well with results by Vinnerås *et al.* (2003b), who treated faecal material (DM 10%) with 6% urea at 20°C (470 mM NH_3) resulting in D-values less than 0.7 days for both *Salmonella* and *E. coli*. The findings on *Salmonella* spp. inactivation by ammonia (Papers I & II) and the studies mentioned above indicate that NH_3 affects the *Salmonella* spp. inactivation, and even at low concentrations (17 mM).

Enterococcus spp. (Paper I & II)

At 34°C the inactivation of *Enterococcus* spp. was fast, irrespective of NH_3 concentrations (lowest studied in urine was 40°mM (Paper I)) even in faecal storage, where the ammonia presumably was low, resulted in D-value of 5 days (Paper II). In ammonia free controls (0.8-0.9% NaCl) at 37°C, growth was observed, indicating the existence of a threshold concentration also at 34°C, which is below the concentrations that were analysed in this study.

At temperatures 24°C and lower, different ammonia concentrations resulted in big variety in reduction rates of *Enterococcus* spp. For free ammonia concentrations of 50 mM and above the D-values were 10 days and less, with the exception for some of the faecal treatments (without ash) that despite concentrations above 100 mM resulted in somewhat longer D-values (Fig. 8). However, the inactivation in faecal material (Paper II) was in line with the results from urine with D-values of 12 and 14 days for 1 and 2% urea, respectively. Concentrations of NH_3 in faecal treatments in Figure 8 may have been overestimated, since they were based on the highest pH achieved, which not was maintained during the whole studies.

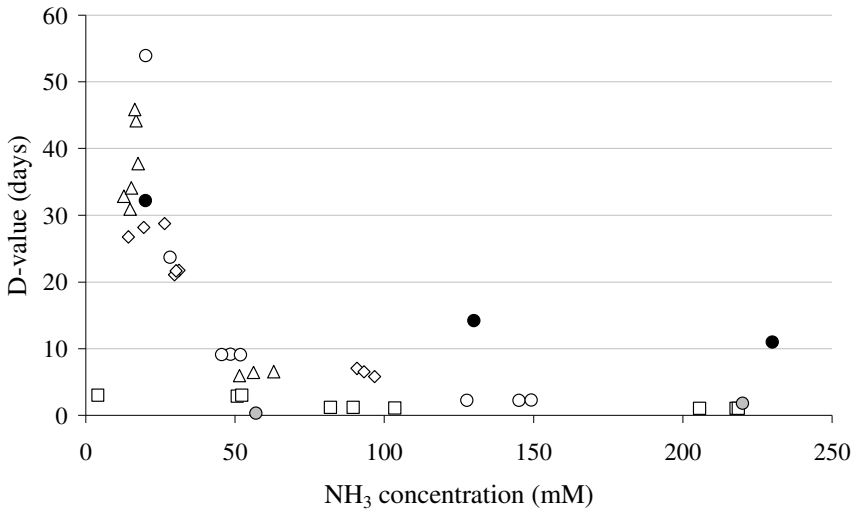

Figure 8. Reduction fates for *Enterococcus* spp. (D-values in days) plotted against concentration of uncharged ammonia, NH_3, in urine (white) (4-14°C) and in faeces (black), DM 17%, (14-34°C). Faecal samples with ash amendment are marked with shaded symbols and temperatures 34°C (□), 24°C (○), 14°C (◇), 4°C (△).

Below concentrations of 50 mM NH_3, D-values increased greatly when stored at 24°C and below, ranging from 20 to 55 days in urine (Fig. 8) and for storage of un-amended faeces a D-value of 412 days were estimated from repeated trials (Paper II). These results agree well with results reported by Vinnerås *et al.* (2004), who treated faecal material (DM 10%) with 6% urea at 20°C (470 mM NH_3) resulting in a D-value of less than 3 days for *Enterococcus* spp.

Inactivation of bacteriophages (Papers I & II)

For *S.* Typhimurium 28B phage (double-stranded DNA), no reduction was observed at 4°C over the six months of the study, though there was inactivation at 24°C and 34°C in the same range as that of Φx 174 (single-stranded DNA) and MS2 (single-stranded RNA) with a lower D-values for undiluted urine, at the most 15 days. At 4°C and 14°C, D-values for Φx 174 and MS2 ranged from 28 to 240 days, and could be related neither to temperature or NH_3concentrations. *S.* Typhimurium 28B phage showed similar behaviour, with D-values more or less randomly from 18 days to infinity, *i.e.* no reduction at all (Fig. 9).

No reduction in *S.* Typhimurium phage 28B was observed in stored faeces at 34°C for one month but when studied for a longer time (150 days) at 24°C, concentration started to decline, resulting in D-value of 170 days. Furthermore, slower reductions were observed in faecal samples compared with urine. Due to the deviating reduction of bacteriophages observed at 14°C and below, prediction of treatment efficiency at low temperature and low ammonia concentrations seems be difficult (Fig. 9).

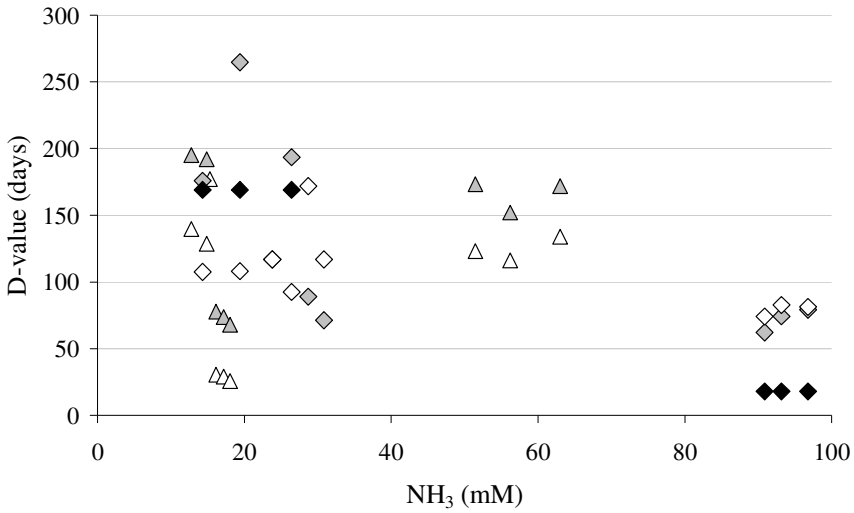

Figure 9. Reduction rates for bacteriophages (D-values in days) plotted against concentration of uncharged ammonia, NH_3, in urine at 14°C (◇), 4°C (△) with *S*. Typhimurium phage 28B (black), ΦX 174 (white) and MS2 (grey).

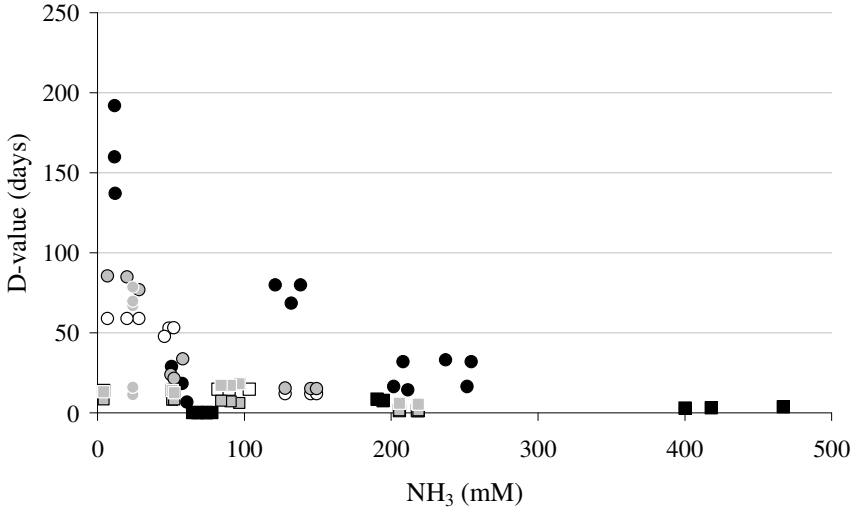

Figure 10. Reduction rates for bacteriophages (D-values in days) plotted against concentration of uncharged ammonia, NH_3, at 34°C (□) and 24°C (○), in faeces DM 17%, (black) studied for *S*. Typhimurium phage 28B and in urine (white or grey) also for ΦX 174 (grey-white out line) and MS2 (grey-black out line)

The inactivation of *S*. Typhimurium phage 28B in urine was also studied by Höglund *et al*. (2002) at temperatures 5 and 20°C, resulting in D-values similar to

the present (Paper I), *i.e.* 71 days at 20°C (60-70 mM NH_3) and little reduction in 200 days at 5°C. Höglund *et al.* (2002) also studied rhesus rotavirus (double-stranded RNA), which was considerably more sensitive than *S.* Typhimurium phage 28B.

However, no significant difference was observed between urine and physiological saline controls at either 4°C or 20°C, similarly to the present study (Paper I). Treatment of faecal material (DM 10%) with 6% urea at 20°C (470 mM NH_3) resulted in a D-value of 7.5 days for *S.* Typhimurium phage 28B (Vinnerås *et al.*, (2003b).

The viricidal effect of ammonia at 21°C has been tested on several polioviruses and other enteric viruses, including coxsackie and reovirus (Ward and Ashley, 1977; Ward, 1978) and the reduction at pH 9.5 (290 mM NH_3) was rapid for all viruses (>5 $\log_{10}$ reduction in 24 h), although reovirus (dsRNA) was less sensitive to the treatment (~2 $\log_{10}$ reduction in 24 h). Only faecal treatment with 2% urea resulted in this high NH_3 concentration but inactivation at 24°C for S. Typhimurium phage 28B was much longer, with D-value of 33 days. Similarly Ward (1978) tested inactivation of poliovirus and found faster reduction in water than in sewage sludge with same ammonia concentration but higher pH. The inactivation of poliovirus and bacteriophage f2, with structure comparable to that of MS2, was further examined by (Cramer *et al.*, 1983) at 20°C and both organisms showed a log-linear correlation between NH_3 (2-180 mM) and k (corresponding to D-values 1-90 days), with polio having a 4.5 faster inactivation than bacteriophage f2. The studies of the enteric viruses indicate that they may be reduced faster than the phages tested here. The most pathogenic viruses have single stranded (ss) RNA, whereas rotaviruses have double stranded (ds) RNA and adenoviruses double stranded (ds) DNA. Studies of the ammonia sensitivity of coated viruses, *e*.g. Avian influenza (ssRNA), indicate a much faster reduction compared with the reduction in the phages used in the present study (Emmoth *et al.*, 2007).

Ascaris (Paper III)

Ammonia based sanitation was potent in inactivating *A. suum*, an organism considered very resistant to alkaline and biological treatments. Ammonia was concluded to be an important factor for the efficient inactivation of *A. suum*, although synergistic effects were observed both from temperature and pH. In urine of all studied concentrations (1:0-1:3), at the most 20% inactivation of viable eggs occurred during 6 months when stored at 4 and 14°C, whereas all treatments at 34°C, both faecal and urine, resulted in a total inactivation (more than 3 $\log_{10}$) within one month.

At 24°C, treatment of faeces with 2% urea (230±20 mM NH_3) and 1% urea (130±9 mM NH_3) resulted in total inactivation (>99%) within one month, whereas the 1:3 diluted urine at 24°C (18 mM) did not contain enough ammonia to give any relevant/clear reduction of egg viability in 100 days. The results (Paper III) indicate some breakpoint concentration between 18 and 66 mM even at 24°C,

where the ~40 mM decrease in NH_3 concentration resulted in D-values an order of magnitude higher. In the absence of ammonia, egg viability was not reduced in one week at pH 13 at 24°C.

Synergy from temperature and ammonia was observed for temperatures 24 and 34°C (Fig. 11). Samples amended with ash all had shorter time for inactivation compared with ash free samples with similar NH_3 concentrations, indicating synergistic effects also from pH. Otherwise, the correlation between ammonia and inactivation time was linear at the two temperatures and at 24°C there was little difference between material (faecal or urine) at the same ammonia concentration. Compared with other data for ammonia inactivation of *Ascaris* spp. eggs in naturally contaminated sludge (Ghiglietti *et al.*, 1997; Pecson *et al.*, 2007), the inactivation rates were similar to the results in Paper III (Fig. 11).

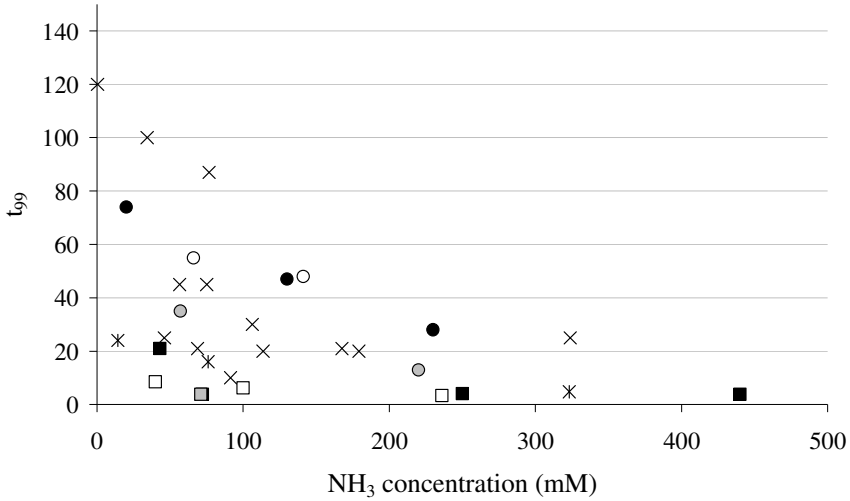

Figure 11. Inactivation of *Ascaris suum* egg viability (t_{99}) plotted against concentration of uncharged ammonia, NH_3, in urine (white) and in faeces (black), at temperatures 34°C (■), 24°C (●). Data from paper III is shown with filled symbols with faecal treatments with ash are marked with grey. The diagram include data on NH_3 inactivation of *Ascaris* spp. eggs in naturally contaminated sludge (Ghiglietti *et al.*, 1997; Pecson *et al.*, 2007) at 20-22°C (×) and 30°C (✳). The D-value of 780 days for 1:3 diluted urine at 24°C (18 mM) was excluded.

The t_{99} of 780 days for urine diluted 1:3 (24°C) was 10 times longer than for faecal storage at 24°C holding similar NH_3 concentrations (18±11 and 20±0.4 mM NH_3). Ghiglietti *et al.* (1997) studied the inactivation of ascaris eggs in both aqueous solution and sludge of 10 DM, at a NH_3 range similar to Paper III. Amendment with ammonia solution (0-0.4% v/v) resulted in a pH of 12.3 and above in water, whereas for sludge pH was at the most 10.5. In water solution at 29 mM, Ghiglietti *et al.* (1997) found no reduction in 20 days whereas in sludge, reduction was observed despite lower pH and NH_3 concentrations. These facts indicate that

substances other than ammonia can contribute to the inactivation of ascaris eggs in biosolids of faecal origin.

Of the protozoan pathogens, cryptosporidium oocysts are considered the most tolerant to disinfection. In a study by Jenkins *et al*. (1998), wild-type cryptosporidium oocysts were treated at 24°C with ammonium solution (7-148 mM NH_3) with exposure time from 10 minutes to 24 hours. Exposure to 148 mM for 24 hours reduced the viable oocysts by 80% and 5.8 days were estimated to be required for 99.999% inactivation. This indicates that *Ascaris* spp. can be regarded as a good model for parasite inactivation by ammonia based sanitation techniques as it seems to be one of the most resistant parasites of concern for human health.

Threshold levels for ammonia based sanitation

The above results show that the sensitivity to ammonia differed between the organisms studied. Also, at each temperature, the concentration of ammonia, at which a shift in inactivation was observed, varied. Threshold concentration can be defined as the concentration at which no inactivation is observed (*S*. Typhimurium phage 28B at 4°C) or as a concentration where a shift in inactivation function can be observed, where the dependence of inactivation on ammonia concentration sharply decreases. The threshold concentrations are important for deciding upon which treatments and dilutions that may be relevant to sanitise toilet wastes.

At 34°C, all organisms studied were inactivated even with the lowest NH_3 concentration (11-37 mM) in the faecal storage treatment, at significantly higher rates than with ammonia free controls, which had 10-100 times longer reduction times. Except bacteriophage 28B.

At 24°C a slower reduction was observed for the phages and for *Enterococcus* spp. at concentrations of free ammonia <40mM. The threshold of 40 mM free ammonia seemed to be accurate for all the organisms investigated except for *Salmonella* spp. where the threshold was much lower (<25 mM) and for *S*. Typhimurium phage 28B which seemed to have slightly higher threshold concentration, somewhere near 60 mM.

At temperatures 14°C and 4°C, where inactivation was studied mostly in urine, the bacteriophage reduction was badly related to NH_3 concentrations and thus inactivation in this temperature range seems difficult to predict. The bacteria studied were inactivated but for *Enterococcus* the concentrated urine resulted in three-fold shorter reduction compared with the 1:1 dilution, which at 14°C had 33 mM NH_3.

Organism correlations

The reduction (D-values) in *Salmonella* spp. was not well correlated to that in *Enterococcus* spp. (R^2=0.4). *Enterococcus faecalis* as indicator organism for the reduction of *Enterobacteriaceae* such as *Salmonella* spp. and *E. coli* O157 will be very conservative, especially at low ammonia concentrations, *i.e*. <40 mM NH_3,

where the difference in D-value was almost ten-fold between *Enterococcus* spp. and *Salmonella* spp.

Enterococcus faecalis is a very conservative indicator organism for the reduction of *Enterobacteriaceae,* e.g *Salmonella* spp. and *E. coli* O157. This is especially so for low ammonia concentrations, *i.e.* <40 mM NH_3, where the difference in D-value was almost ten-fold between *Enterococcus* spp. and *Salmonella* spp. Additionally, the ammonia sensitivity between the two groups of organisms was not linear. This makes the conclusion about the reduction at one concentration not comparable to the difference in reduction at another ammonia concentration.

The *S*. Typhimurium phage 28B and *A. suum* eggs both proved resistant to ammonia at low temperatures and indicated that the bacteriophage could act as a model for ascaris inactivation, which means that hygiene standards may be monitored much more easily both *in vitro* and *in vivo*. However, the relationship between ammonia and inactivation for the two organisms then have to be repeatedly thoroughly.

Barriers to disease transmission

The use of insufficiently treated human and animal wastes in agriculture may result in disease transmission (Venglovsky *et al.*, 2006; Albihn and Vinnerås, 2007). Mode of disease transmission is largely dependent on the scale, the interactions that take place within the system boundaries and the kinds of barriers present. A barrier can be defined as an event or a physical barrier that prevents transmission, reduces infectivity or decreases pathogen numbers, *e.g.* primary and secondary treatment, personal hygiene and fertilisation regimes.

Collection and treatment

Enclosed collection of human excreta and animal manure prevent the spread of pathogenic organisms into the environment and offers a possibility reduce pathogen number before introduction into the environment. In addition, ammonia present in the material will be retained. Treatment of the collected excreta needs to be efficient regarding the removal of pathogens, after which the treated material should be a valuable fertiliser that can be recycled in a safe way.

Fertilising strategies and crop selection

As most microbial pathogens decrease over time, pathogen concentration is highest in freshly excreted material and further handling and spread of the material in the environment result in reduced concentrations. However, regrowth of pathogenic bacteria in biomaterial such as sewage sludge have frequently been observed (Pepper *et al.*, 2006). In the environment as well as during storage and treatment, biotic and abiotic factors such as microbial competition, drying and UV radiation affect pathogen survival. The application technique therefore affects

exposure and survival (*e.g*. injection into soil can minimise human exposure from soil surface and aerosols but pathogen survival in the soil, when incorporated, may be prolonged (Gibbs *et al.*, 1997).

The selection of crops to be fertilised creates different risks for disease transmission. Use can be restricted to feed and food that is further processed. Fertilisers used for fodder production or pasture do not need the same high level of sanitation regarding viruses since most are species specific. A time delay between application and harvest or grazing also lowers the risk for disease transmission when using manure of human and animal origin as fertilisers in feed and food crops. However will the inactivation in the field depend on many factors including humidity and temperature.

System size

The size of the system determines the relevant restrictions on use. At family level, the risk of disease transmission from food cultivated with home produced fertiliser is negligible compared with transmission from person to person contact within the family (Schönning and Stenström, 2004; WHO, 2006). The larger the system, *e.g*. community up to municipal scale, the larger the risk of pathogens being present within the material (Sahlström *et al.*, 2004) and the greater the epidemiological importance of spread of contaminated fertilisers. Similarly, on-farm nutrient circulation via manure may also circulate diseases but within a more limited loop compared with to-farm fertilising, *e.g*. importing manure from extensive cattle holdings and biowaste from society to agricultural land. However, for on-site nutrient recycling systems diseases can persist or re-infect herds (Petersen *et al.*, 2007).

Material composition and treatment

Urine – dilution and storage temperature (Papers I & III)

The general recommended hygiene treatment for urine is storage based on its intrinsic concentration of ammonia (WHO, 2006). When excreted, urine holds a pH of 6.2-7.0 and 80% of the nitrogen is excreted as urea (Lentner and Geigy, 1981), which normally is transformed into ammonia during collection, increasing the pH to 8.8-9.2 in the collection tank due to the presence of urease producing bacteria in the pipes (Vinnerås *et al.*, 2006 ; Jönsson and Vinnerås, 2007). This sanitation method has been evaluated concerning inactivation of pathogenic microorganisms and indicator organisms at 5°C and at 20°C in urine with a concentration of approximately 3.3 g NH_3-N per litre (Höglund and Stenström, 1999b; Höglund *et al.*, 2002; Schönning *et al.*, 2002). Based on these studies and a risk assessments, guidelines recommend storage of collected urine for six months at 20°C to satisfactorily reduce its microbial content, covering a wide range of pathogenic bacteria and some viral agents and *Cryptosporidium parvum* (Schönning and Stenström, 2004).

Papers I and III showed that for urine the degradation of urea gave a stable pH even at 1:3 dilutions with water giving ammonia concentrations of 1.6 g NH_3-N L^{-1}.with water. The pH was stable for over 6 months, with only a slight decrease (at the most 0.2 units) over time at 24 and 34°C. The 1:1 dilution of urine that originated from a sanitation system also maintained slightly lower pH than the pH developed in urine collected fresh. It did however inactivate both *A. suum* and bacteriophages at temperatures of 24°C and above.

At 24°C the 1:3 dilute urine had too low concentrations (24±6 mM NH_3) for inactivation of *A. suum* eggs. The bacteria studied was inactivated both at lower ammonia concentrations and temperatures although *Enterococcus* spp. at ≤14°C had a D-values considerably longer for the 1:3 dilution compared with concentrated urine. These results stress the importance of keeping the urine as concentrated as possible during collection and storage.

A threshold for inactivation was identified at approximately 40mM free ammonia, below which inactivation was slow or showed little dependence on ammonia concentrations. This NH_3 concentration was reached for all urine dilutions at 34°C and for 1:0 and 1:1 at 24°C, whereas at 4 and 14°C only the concentrated urine (1:0) reached this concentration of free ammonia. As regards ascaris and bacteriophage 28B, the concentrations of 57-109 mM were insufficient to give a good inactivation at 14°C and below, indicating that even if urine is collected concentrated, the intrinsic ammonia will not be sufficient to inactivate those pathogens at that temperature. The breakpoint NH_3 concentration between 14 and 24°C needs to be more thoroughly examined to improve the accuracy in predicting the reduction efficiency of urine storage at ambient temperature in this range.

Faeces - Ammonia nitrogen and pH (Paper II)

Water make up 70 to 86% of the faecal matter when excreted (Lentner and Geigy, 1981) and a default dry matter content (DM) of 20% was proposed by Vinnerås *et al.* (2006). Many source separating systems collect the faecal fraction dry and amendment with soil and/or ash during collection is common to cover and dry the material and prevent odour and exposure to vectors such as flies. Drying during collection and storage and by amendments may produce a faecal material with a higher DM content. The faecal matter used in this study (Papers II & III) was from different sources and treatments were performed on different batches of material. Concentration of ammonia in solution was 214 -247 mM NH_3 when faecal material was adjusted to a DM of approximately 20%. This corresponds to 2-2.3 g N kg^{-1} faecal material w/w. Thus, faecal material from source separating sanitation can hold concentrations of total ammonia similar to that of urine from low flush systems (7 g N kg^{-1}). If the ammonia would all be present in the uncharged form, NH_3, intrinsic ammonia would be highly sufficient to inactivate the pathogens studied (Paper I; II; III).

Amending with ash or soil produces a mix with lower ammonia concentration compared with non-amended faeces but with alkaline additions the dilution might be compensated by increased concentration of free NH_3 (Paper III; II). Ash from which charcoal pieces had been removed by sieving gave a higher pH compared with un-sieved ash, which indicates that pathogen inactivation could be improved

by sieving the ash prior to use (Fig. 12). However, the risk is large that ammonia is lost as gas emission during open collection with ash, since its pH is high. In this study ash was added after collection and storage. Furthermore, the results show that urea addition to faecal material with a high pH (>10) will not improve the sanitation, as no hydrolysis producing ammonia will take place (Kabdasli *et al.*, 2006) (Paper II & III).

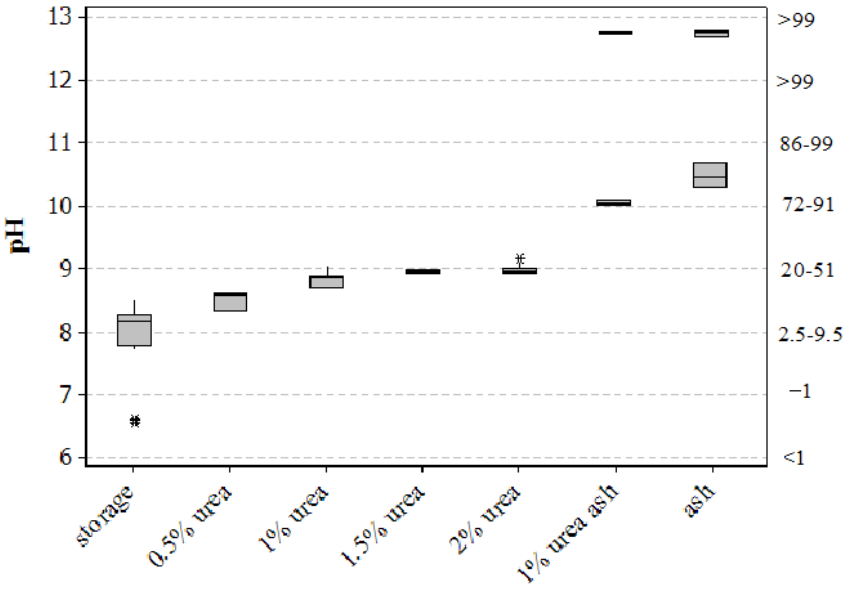

Figure 12. The pooled (n 4-19) initial pH (from day1-3), disregarding temperature (except for treatments with ash), for each treatment shown as inter-quartile range boxes with outliers marked (✳) and whiskers showing the outer range. Sample size (n) is indicated in the labels in parenthesis. On the right hand vertical axis, fraction of ammonia present as NH_3 is given for every pH for the temperature range 14 - 34°C.

In untreated faecal material different pH were measured, varying from 6.6 to 8.5. This variations are due to diet (Lentner and Geigy, 1981), due to degradation during the collection producing organic acids and due to misplaced urine (Fig. 12).Since the efficiency of ammonia based sanitation needs an alkaline pH the pathogen inactivation will depend on the properties of the faecal material. Therefore, to have an efficient sanitation, pH must be both increased and stabilised.

Ammonia producing additives (Papers II; III)

To reach sufficient levels of free ammonia in faecal material, an increase in pH is necessary. At pH 8 and temperatures 14-34°C, only 2.5-9.5% of the ammonia is present as NH_3, whereas at pH 9, 20-51% is present as NH_3. When increasing the

ammonia concentration in the faecal material by adding more than 1.5%, wet weight, of urea, the pH increases to a stable level around 9. Since the different storage temperatures for the faecal treatments not did result in significantly different pH, Figure 12 show the initial pH (highest from day 1-3) as pooled values for each treatment irrespectively temperature. The only exception is the faecal treatments with ash amendments (ash and 1% urea with ash) which are divided upon treatments with sieved ash (higher pH) and as that was added un-sieved (the lower pH).

In general, the pH declined over time, with the largest decrease observed when faecal material with initial low pH was further treated (the outlier in Fig. 12). However, stable pH (<0.2 decrease in 60 days) was observed in faeces (DM 17%) treated with 1-2% urea. The largest urea addition tested was 2% on a wet weight basis, which resulted in approximately 12 g NH-N kg^{-1} faecal material. The minimum dose of slurry that can be applied with current equipment is approximately 10 m^3 per hectare. Applying faecal material treated with 2% urea will result in 120 kg N ha^{-1}. With manual application, the spreading can be adapted to the nitrogen content, but high nitrogen content increase the risk of leaching and volatile losses and has to be managed by incorporation of the fertiliser soon after application.

Ammonia based sanitation

Low to high technology implementation

Ammonia based sanitation has the potential to inactivate pathogens in biowaste aimed for fertiliser use (Papers I, II & III). In most places nitrogen is the growth limiting plant nutrient. Since nitrogen in human excreta and manure is partly in the form of ammonia or urea, a shift in the waste management can enhance both nitrogen preservation and pathogen inactivation. As observed in Paper II, the inactivation of ascaris in stored faeces was considerably shorter than that reported for open storage (Corrales *et al.*, 2006). Implementing the results from these studies opens up the possibility for giving guidelines for safe reuse of animal and human manure based on the pH, ammonia content and dry matter content. Analysing these factors in the incoming material and taking into account limitations caused by the planned use and by storage capacity, an optimized treatment regime can be proposed for production of a safe fertiliser (Fig. 13).

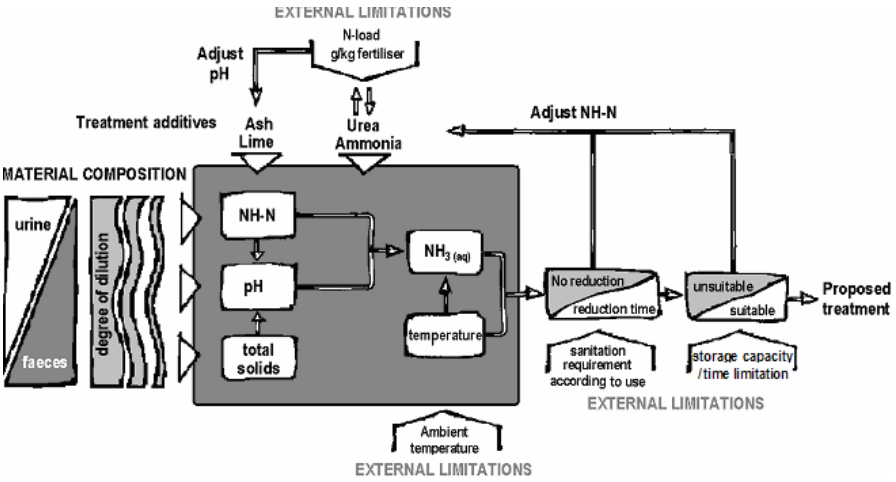

Figure 13. Schematic representation of a decision support tool to propose treatment of biowaste aimed for fertiliser use (Albihn and Vinnerås, 2007).

The ambient temperature has a major effect on the treatment efficiency, as it influences both concentration of free ammonia and the pathogens susceptibility to this free ammonia. From the constitution of incoming material and temperature, a reduction rate and thus treatment time can be estimated according to the pathogen reduction required as decided by intended use. Whether the proposed treatment time is suitable depends on storage capacity and/or seasonal use of fertiliser. Required treatment times can be regulated by amendments that increase the free ammonia concentration. The NH_3 concentration can be regulated both by alkali and ammonia producing substances. The selection of amendment may be determined by ammonia levels in the material, as too high ammonia concentration may be undesirable as regards N application rates and crop requirements. In the case of high nitrogen load, alkali may be the most suitable amendment. Using these factors in an iterative process, addition of urea/ammonia or lime/ash can be proposed together with a required time of treatment for production of a safe fertiliser.

Ammonia amendments

As the sanitation efficiency is directly connected to NH_3 concentration, an ammonia amendment that also increases the pH would optimise the ammonia sanitation. Aqueous ammonia possesses such properties but is not as easy to handle as urea due to the high volatility and human toxicity at high concentrations.

However, where these risks are properly managed, implementation of ammonia sanitation by use of ammonia or aqueous ammonia could be optimal, especially at large scale. Ottoson *et al.* (2007) evaluated both ammonia solution and urea as an amendment to cattle manure and Nordin (2006) in faecal material. At equimolar additions of total ammonia, a higher pH was reached with ammonia solution and thus a higher inactivation rate though less ammonia added. Urea is the most

common artificial nitrogen fertiliser in the world. It is a common ingredient in cosmetic products and toothpaste and a compound harmless to man. As urea is enzymatically degraded by urease, which is abundant in the faecal material, the risk of ammonia losses due to volatilisation is decreased, and healt hazards less. Therefore, urea is a more user-friendly ammonia amendment that seems the more suitable under most conditions.

Implementation of ammonia based sanitation

The ammonia based sanitation technology provides new possibilities to sanitise different types of toilet waste, liquid as well as solid, at both small and large scale, with low tech or automatised technology. For source separated urine and faeces, treatment times can be specified according to NH_3 concentration at temperatures above 20°C. With the current dry sanitation systems it is still optional to collect urine and faeces separately to avoid both odour and ammonia losses since air exchange is unavoidable. It is important to note that there is no single appropriate technology for all circumstances and all socio-economic segments of a society.

The main part of the 2.6 billion people estimated to lack improved sanitation is poor people in societies with weak infrastructure. Despite low technical demand, investment in a treatment container may be difficult due to poverty, land tenure issues or high population densities. For these situations a single use, biodegradable self sanitising toilet, *Sanibag*, is under development (www.peepoople.com). As its appearance resembles a plastic bag, it takes advantage of the widespread habit, in the absence of sanitations systems, to defecate in plastic bags. Important though, it adds the property of sanitising the material by use of urea integrated in the bag. Urea will degrade to ammonia upon contact with faeces and after sanitation also the bag will be degraded.

The investment cost in the Sanibag system is low and an ordered collection and treatment of the used bags might be economically self sustaining. Such system will both remove and inactivate the faecal pathogens and produce a safe fertiliser for increased crop production and food security. Thus, the Sanibag has the potential to be instrumental in accomplishing Target 10 under the Millenium Development goals: to halve by 2015 the proportion of people without sustainable access to safe drinking water and basic sanitation, and Target 2: to halve by 2015 the proportion of people who suffer from hunger.

Conclusions

Ammonia sanitation can provide controlled pathogen inactivation in both source separated faeces and urine across a variety of organism groups with very different characteristics, *e.g*. bacteria, parasites and viruses. However, temperature proved to be a key factor for the NH_3 (aq) toxicity on the studied organisms, especially for viruses and ascaris eggs. The NH_3 concentrations in concentrated urine, 7 g L^{-1}, were at ≤14°C not efficient to inactivate these organisms. Ascaris eggs and bacteriophage inactivation were not studied in faecal material below 24°C but the present result indicates that they will not be inactivated by the tested faecal treatments either at this low temperatures.

At temperatures above 34°C, the reduction in the organisms investigated was rapid even at moderate concentrations of free ammonia. This indicates that both source separated faecal matter and urine, even when diluted three parts water, intrinsically can contain sufficient concentrations of ammonia to inactivate pathogens within a reasonable time span (<6 months), provided that the pH is ≥9 and the temperature is >34°C. The exception is the *S.* Typhimurium phage 28B which showed little reduction in stored faeces even at 34°C. However, addition of 1% urea to the faecal matter at 34°C is enough for production of a safe fertiliser within two months of treatment, regarding a 6 $\log_{10}$ reduction of viral pathogens.

At 24°C the inactivation rates were slower than at 34°C and a threshold concentration of between 18-60 mM NH_3 was identified in urine for the inactivation of ascaris and bacteriophages which stresses the importance to avoid diluting urine more than 1:1 with water during the collection. When faecal material was amended with 2% urea at 24°C, also the most persistent pathogens were reduced whereas in un-amended faeces the reduction of bacteriophages was small during several months.

Addition of urea, 2%, to faecal matter produce a safe fertiliser within seven months regarding viruses and S*almonella* spp. in faecal matter can be inactivated in 2 months at 14-34°C with 1% urea treatment.

As long as the ammonia is retained in the material, *i.e*. treated in closed containers, it will inhibit microbial activity, both in urine and in faeces. However, the lower and variable pH in faeces, and higher content of organic material results in much slower reduction of pathogens during storage, especially for the bacteriophages the survival was longer in faecal material than in urine at same NH_3 concentration. Therefore, additional treatment of faeces will assure the pathogen reduction and urea is a user friendly ammonia amendment. When ash or lime is used as cover material an alkaline pH can enhance the effect of intrinsic and added ammonia, but at pH >10 further supplementing with urea will not be useful since enzymatic degradation will be hampered at this pH.

The present study confirmed current guidelines that storage of urine for 6 months at 20°C or higher is safe for unrestricted use and at temperatures below, the use is restricted to products that will be further processed. The result at 24°C, however, stresses the need of keeping the urine as concentrated as possible as the NH_3

concentrations in urine diluted 1:3 with water was not be sufficient to inactivate *Ascaris* at 24°C. However at ammonia levels that correspond to concentrated urine (7 g NH_3-N L^{-1} *i.e.* >40mM NH_3), the D-value for *Salmonella* spp was less than 2 weeks.

This study showed that safe sanitation of faecal matter can be achieved by ammonia sanitation at pH ≥9 and temperatures of ≥24°C. Above this temperature, the dose needed for sanitation decreases sharply with increasing temperature. Below this temperature, the treatment is still very efficient for pathogenic bacteria (*Enteroccoccus* spp. and *Salmonella* spp. investigated) but its efficiency on viruses and parasites is not sufficiently known, as the reduction of the investigated organisms in urine seemed unpredictable.

Future research

For a widespread application of ammonia based sanitation technology, its inactivation efficiency of parasites and viruses at temperatures below 24°C needs to be further studied. A more detailed study of inactivation could reveal breakpoint concentrations and treatment strategies, which can result in specified and optimized treatment recommendations of special interest in temperate climates.

The current knowledge of pathogen inactivation with regard to ammonia has been investigated for a range of resistant organisms. However, one resistant organisms not studied is *Mycobacterium tuberculosis*. This emerging pathogen can occasionally be excreted in urine and is of interest due to severity of disease and difficulty to treat.

Considering faeces collected dry, the diffusion of ammonia in organic material should be examined as a function of dry matter content to provide important knowledge for developing practical approaches to sanitising these fractions. Studies with different dry matter content regarding pathogen inactivation would provide further knowledge of *e.g.* viral affinity to solid material that might result in less efficient inactivation.

The possibility to sanitise blackwater, *i.e.* wastewater from toilets, with its intrinsic ammonia by minimising the amonunt of flushwater needs to be further investigated, as this, if it is possible, can offer a user and environmentally friendly sanitation system

As the temperature proved to be an important factor regarding the inactivation of pathogens, sanitation systems that keeps the urine and faecal matter warm ought to be investigated. Solar heating could be a resource-efficient technique for increasing and maintaining temperatures in urine and faecal material.

References

Acha, P.N. & Szyfres, B. 2003. *Zoonoses and communicable diseases common to man and animals*. PAHO, Washington, D.C.

Albihn, A. 2001. *Recycling biowaste--human and animal health problems.* Acta veterinaria Scandinavica. Supplementum 95, 69-75.

Albihn, A., Eriksson, E., Wallen, C. & Aspan, A. 2003. *Verotoxinogenic Escherichia coli (VTEC) O157:H7-a nationwide Swedish survey of bovine faeces.* Acta veterinaria Scandinavica 44, 43-52.

Albihn, A. & Vinnerås, B. 2007. *Biosecurity and arable use of manure and biowaste —Treatment alternatives.* Livestock Science. In press.

Allievi, L., Colombi, A., Calcaterra, E. & Ferrari, A. 1994. *Inactivation of fecal bacteria in sewage sludge by alkaline treatment.* Bioresource Technology 49, 25-30.

Amahmid, O., Asmama, S. & Bouhoum, K. 1999. *The effect of waste water reuse in irrigation on the contamination level of food crops by Giardia cysts and Ascaris eggs.* International Journal of Food Microbiology 49, 19-26.

Ashbolt, N.J. 2004. *Microbial contamination of drinking water and disease outcomes in developing regions.* Toxicology 198, 229-238.

Ayres, R.M., Stott, R., Mara, D.D. & Lee, D.L. 1992. *Waste-Water Reuse in Agriculture and the Risk of Intestinal Nematode Infection.* Parasitology Today 8, 32-35.

Bagge, E., Sahlström, L. & Albihn, A. 2005. *The effect of hygienic treatment on the microbial flora of biowaste at biogas plants.* Water Research 39, 4879-4886.

Bethony, J., Brooker, S., Albonico, M., Geiger, S.M., Loukas, A., Diemert, D. & Hotez, P.J. 2006. *Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm.* Lancet 367, 1521-1532.

Bitton, G. 1999. *Wastewater microbiology*. Wiley-Liss, New York.

Boost, M.V. & Poon, C.S. 1998. *The effect of a modified method of lime-stabilisation sewage treatment on enteric pathogens.* Environment International 24, 783-788.

Boqvist, S. & Vågsholm, I. 2005. *Risk factors for hazard of release from salmonella-control restriction on Swedish cattle farms from 1993 to 2002.* Preventive veterinary medicine 71, 35-44.

Burge, W.D., Cramer, W.N. & Kawata, K. 1983. *Effect of heat on virus inactivation by ammonia.* Applied and Environmental Microbiology 46, 446-451.

Bydzovská, O.O. & Kneiflová, J.J. 1983. *Assessment of viral disinfection by means of bacteriophage OX 174.* Journal of hygiene, epidemiology, microbiology, and immunology 27, 60-68.

Carlander, A., Aronsson, P., Allestam, G., Stenstrom, T.A. & Perttu, K. 2000. *Transport and retention of bacteriophages in two types of willow-cropped lysimeters.* Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering 35, 1477-1492.

Carr, R. & Strauss, M. 2001. *Chapter 5: Excreta-related infections and the role of sanitation in the control of transmission*. In: Fewtrell, L.,Bartram, J. (Eds.), Water quality - Guidelines, standards and health: Assessment of risk and risk management for water-related infectious disease,WHO, IWA Publishing.

Cole, J. & Oelschlegel, S. 2005. *Zoonoses Resources on the Internet.* Journal of Consumer Health on the Internet 9, 51.

Corrales, L.F., Izurieta, R. & Moe, C.L. 2006. *Association between intestinal parasitic infections and type of sanitation system in rural El Salvador.* Tropical Medicine & International Health 11, 1821-1831.

Cramer, W.N., Burge, W.D. & Kawata, K. 1983. *Kinetics of virus inactivation by ammonia.* Applied and Environmental Microbiology 45, 760-765.

Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nelson, K.E. & Relman, D.A. 2005. *Diversity of the Human Intestinal Microbial Flora.* Science 308, 1635-1638.

Ecosanres, w. 2007, 2007-10-01. http://www.ecosanres.org/.

Emerson, K.R., RC; Lund, RE; Thurston, RV 1975. *Aqueous ammonia equilibrium calculations: Effects of pH and temperature.* Journal of the Fisheries Research Board of Canada 32, 2379-2383.

Emmoth, E., Vinnerås, B., Ottoson, J., Albihn, A. & Belák, E., 2007. *Ammonia treatment of hatchery waste for elimination of avian influenza virus*. In, International Congress in Animal Hygiene XIII. June 17-21 2007, Tartu, Estonia.

Eriksen, L., Andreasen, P. & Ilsoe, B. 1995. *Inactivation of Ascaris suum eggs during storage in lime treated sewage sludge.* Water Research 30, 1026-1029.

EuroStat, 2006. *90% of EU25 population connected to waste water collection systems*. ES News relaease, Vol. 37/2006, European Commission.

Feachem, R.G. 1983. *Sanitation and disease: health aspects of excreta and wastewater management*. Wiley, Chichester.

Fraise, A.P., Lambert, P.A., Maillard, J.-Y., Russell, A.D. & Ayliffe, G.A.J. 2004. *Principles and practice of disinfection, preservation and sterilization*. Blackwell, Malden.

Gaspard, P., Wiart, J. & Schwartzbrod, J. 1997. *Parasitological contamination of urban sludge used for agricultural purposes.* Waste Management & Research 15, 429-436.

Gatidou, G., Thomaidis, N.S., Stasinakis, A.S. & Lekkas, T.D. 2007. *Simultaneous determination of the endocrine disrupting compounds nonylphenol, nonylphenol ethoxylates, triclosan and bisphenol A in wastewater and sewage sludge by gas chromatography-mass spectrometry.* Journal of Chromatography A 1138, 32-41.

Ghiglietti, R., Rossi, P., Ramsan, M. & Colombi, A. 1995. *Viability of Ascaris suum, Ascaris lumbricoides and Trichuris muris eggs to alkaline pH and different temperatures.* Parassitologia 37, 229-232.

Ghiglietti, R., Genchi, C., Di Matteo, L., Calcaterra, E. & Colombi, A. 1997. *Survival of Ascaris suum eggs in ammonia-treated wastewater sludges.* Bioresource Technology 59, 195-198.

Gibbs, R.A., Hu, C.J., Ho, G.E. & Unkovich, I. 1997. *Regrowth of faecal coliforms and salmonellae in stored biosolids and soil amended with biosolids.* Water Science and Technology 35, 269-275.

Guardabassi, L., Sobsey, M. & Dalsgaard, A., 2003. *Occurrence and survival of viruses in composted human faeces.* Technical Report. Sustainable Urban Renewal and Wastewater Treatment No. 32., The Danish Environmental Protection Agency.

Guzha, E., Nhapi, I. & Rockstrom, J. 2005. *An assessment of the effect of human faeces and urine on maize production and water productivity.* Physics and Chemistry of the Earth 30, 840-845.

Han, Q., Eriksen, L., Boes, J. & Nansen, P. 2000. *Effects of bile on the in vitro hatching, exsheathment, and migration of Ascaris suum larvae.* Parasitology Research 86, 630-633.

Harm, W. 1980. *Biological effects of ultraviolet radiation*. Cambridge U.P., Cambridge.

Havelaar, A.H., Pot-Hogeboom, W.M., Furuse, K., Pot, R. & Hormann, M.P. 1990. *F-specific RNA bacteriophages and sensitive host strains in faeces and wastewater of human and animal origin.* The Journal of applied bacteriology 69, 30-37.

Höglund, C., Stenström, T.A., Jönsson, H. & Sundin, A. 1998. *Evaluation of faecal contamination and microbial die-off in urine separating sewage systems.* Water Science and Technology 38, 17-25.

Höglund, C., Ashbolt, N., Stenström, T.A. & Svensson, L. 2002. *Viral persistence in source-separated human urine.* Advances in Environmental Research 6, 265-275.

Höglund, C.E. & Stenström, T.A. 1999. *Survival of Cryptosporidium parvum oocysts in source separated human urine.* Canadian Journal of Microbiology 45, 740-746.

Hotta, S., Noguchi, T. & Funamizu, N. 2007. *Experimental study on nitrogen components during composting process of feces.* Water Science and Technology 55, 181-186.

Howard, G., Pedley, S., Barrett, M., Nalubega, M. & Johal, K. 2003. *Risk factors contributing to microbiological contamination of shallow groundwater in Kampala, Uganda.* Water Research 37, 3421-3429.

Jenkins, M.B., Bowman, D.D. & Ghiorse, W.C. 1998. *Inactivation of Cryptosporidium parvum oocysts by ammonia.* Applied and Environmental Microbiology 64, 784-788.

Jimenez, B., Austin, A., Cloete, E., Phasha, C. & Beltran, N. 2007. *Biological risks to food crops fertilized with Ecosan sludge.* Water Science and Technology 55, 21-29.

Johnson, P.W., Dixon, R. & Ross, A.D. 1998. *An in-vitro test for assessing the viability of Ascaris suum eggs exposed to various sewage treatment processes.* International Journal for Parasitology 28, 627-633.

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

Jönsson, H. & Vinnerås, B. 2007. *Experiences and suggestions for collection systems for source-separated urine and faeces.* Water Science and Technology 56, 71-76.

Kabdasli, I., Tunay, O., Islek, C., Erdinc, E., Huskalar, S. & Tatli, M.B. 2006. *Nitrogen recovery by urea hydrolysis and struvite precipitation from anthropogenic urine.* Water Science and Technology 53, 305-312.

Kirchman H, P.S. 1995. *Human urine-chemical composition and fertilizer use efficiency.* Fertilizer research 40, 149-154.

Koopmans, M., Bonsdorff, C.-H., Vinje, J., Medici, D. & Monroe, S. 2002. *Foodborne viruses 1.* FEMS Microbiology Reviews 26, 187-205.

Langergraber, G. & Muellegger, E. 2005. *Ecological Sanitation--a way to solve global sanitation problems?* Environment International 31, 433-444.

Lentner, C. & Geigy. 1981. *Geigy Scientific tables*. Ciba-Geigy, Basle.

Lilleengen, K. 1948. *Typing of Salmonella typhimurium by means of bacteriophage*. Royal Veterinary College, Stockholm.

Lopez, A.D., Mathers, C.D., Ezzati, M., Jamison, D.T. & Murray, C.J. 2006. *Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data.* Lancet 367, 1747-1757.

Macpherson, C.N.L. 2005. *Human behaviour and the epidemiology of parasitic zoonoses.* International Journal for Parasitology 35, 1319-1331.

Madigan, M.T. & Martinko, J.M. 2005. *Brock Biology of Microorganisms, 11th edn*. Pearson Prentice Hall, Upper Saddle river.

Maurer, M., Pronk, W. & Larsen, T.A. 2006. *Treatment processes for source-separated urine.* Water Research 40, 3151-3166.

Mendez, J.M., Jimenez, B.E. & Barrios, J.A. 2002. *Improved alkaline stabilization of municipal wastewater sludge.* Water Science and Technology 46, 139-146.

Mendez, J.M., Jimenez, B. & Maya, C. 2004. *Disinfection kinetics of pathogens in physicochemical sludge treated with ammonia.* Water Science and Technology 50, 67-74.

Moubarrad, F.-Z.L. & Assobhei, O. 2007. *Health risks of raw sewage with particular reference to Ascaris in the discharge zone of El Jadida (Morocco).* Desalination 215, 120-126.

Nejsum, P., Parker, E.D., Jr., Frydenberg, J., Roepstorff, A., Boes, J., Haque, R., Astrup, I., Prag, J. & Skov Sorensen, U.B. 2005. *Ascariasis Is a Zoonosis in Denmark.* Journal of Clinical Microbiology. 43, 1142-1148.

Niwagaba, C., Nalubega, M., Vinnerås, B. & Jönsson, H. 2000. *Incineration of Faecal Matter for Treatment and Sanitation.* Water Practice & Technology

Nordin, A. 2006. *Nitrogen based sanitation of source separated faecal matter*. Master Thesis. Swedish University of Agricultural Sciences, Uppsala.

Odegaard, H. 2004. *Sludge minimization technologies - an overview.* Water Science and Technology 49, 31-40.

Ottoson, J., Nordin, A., von Rosen, D. & Vinnerås, B. 2007. *Salmonella reduction in manure by the addition of urea and ammonia.* Bioresource Technology.

Park, G.W. & Diez-Gonzalez, F. 2003. *Utilization of carbonate and ammonia-based treatments to eliminate Escherichia coli O157:H7 and Salmonella*

Typhimurium DT104 from cattle manure. Journal of applied microbiology 94, 675-685.

Patz, J.A., Graczyk, T.K., Geller, N. & Vittor, A.Y. 2000. *Effects of environmental change on emerging parasitic diseases.* International Journal for Parasitology 30, 1395-1405.

Pecson, B.M. & Nelson, K.L. 2005. *Inactivation of Ascaris suum eggs by ammonia.* Environmental Science Technology 39, 7909-7914.

Pecson, B.M., Barrios, J.A., Jimenez, B.E. & Nelson, K.L. 2007. *The effects of temperature, pH, and ammonia concentration on the inactivation of Ascaris eggs in sewage sludge.* Water Research 41, 2893-2902.

Pepper, I.L., Brooks, J.P., Gerba, C.P. & Donald, L.S. 2006. *Pathogens in Biosolids*, Advances in Agronomy Vol. Volume 90, Academic Press.

Petersen, O., H. (ed.), 2007. *Human physiology*. Malden, Mass.: Blackwell Pub. .

Petersen, S.O., Sommer, S.G., Béline, F., Burton, C., Dach, J., Dourmad, J.Y., Leip, A., Menzi, H., Misselbrook, T., Nicholson, F., Poulsen, H.D., Provolo, G., Sørensen, P., Vinnerås, B., Weiske, A., Bernal, M.-P., Böhm, R., Juhász, C., Mihelic, R. & Martinez, J. 2007. *Recycling of livestock wastes and other residues - a whole-farm perspective.* Livestock Science. In press.

Quilès, F., Balandier, J.-Y. & Capizzi-Banas, S. 2006. *In situ characterisation of a microorganism surface by Raman microspectroscopy: the shell of Ascaris eggs.* Analytical and Bioanalytical Chemistry 386, 249-255.

Rockefeller, A.A. 2001. *Chapter 1: Civilization and sludge: Notes on the history and management of human excreta*. In: Goodland, R., Orlando, L.,Anhang, J. (Eds.), Toward sustainable sanitation, The International Association of Impact Assessment, Fargo.

Sahlström, L., Aspan, A., Bagge, E., Danielsson-Tham, M.L. & Albihn, A. 2004. *Bacterial pathogen incidences in sludge from Swedish sewage treatment plants.* Water Research 38, 1989-1994.

Sahlström, L. 2006. *Recycled biowaste as a source of infection*. Department of Biomedical Sciences and Veterinary Public Health Swedish University of Agricultural Sciences, Uppsala.

Schönning, C., Leeming, R. & Stenström, T.A. 2002. *Faecal contamination of source-separated human urine based on the content of faecal sterols.* Water Research 36, 1965-1972.

Schönning, C. & Stenström, T.A., 2004. *Guidelines on the safe use of urine and faeces in ecological sanitation systems*. Stockholm Environment Institute, EcoSanRes publications series, 2004:1

Stenström, T.A., 1996. *Sjukdomsframkallande mikroorganismer i avloppssystem: riskvärdering av traditionella och alternativa avloppslösningar*. Rapport Naturvårdsverket, Socialstyrelsen, Smittskyddsinstitutet, Stockholm.

Svensson, L.L. 2000. *Diagnosis of foodborne viral infections in patients.* International journal of food microbiology 59, 117-126.

Vanchiere, J.A., White, Z.S. & Butel, J.S. 2005. *Detection of BK virus and simian virus 40 in the urine of healthy children.* Journal of Medical Virology 75, 447-454.

Vasickova, P. 2005. *Viruses as a cause of foodborne diseases: a review of the literature.* Veterinární medicína 50, 89.

Venglovsky, J., Martinez, J. & Placha, I. 2006. *Hygienic and ecological risks connected with utilization of animal manures and biosolids in agriculture.* Livestock Science 102, 197-203.

Vieno, N., Tuhkanen, T. & Kronberg, L. 2007. *Elimination of pharmaceuticals in sewage treatment plants in Finland.* Water Research 41, 1001-1012.

Vinnerås, B., Björklund, A. & Jönsson, H., 2003a. *Thermal composting of faecal matter as treatment and possible disinfection method--laboratory-scale and pilot-scale studies*. In, Bioresource Technology, Vol. 88, pp. 47-54.

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

Vinnerås, B., Palmquist, H., Balmér, P. & Jönsson, H. 2006 *The characteristics of household wastewater and biodegradable solid waste - a proposal for new Swedish design values.* Urban Water Journal 3, 3-11.

Ward, R.L. & Ashley, C.S. 1977. *Identification of the viricidual agent in wastewater sludge.* Applied and Environmental Microbiology 33, 860-864.

Ward, R.L. 1978. *Mechanism of poliovirus inactivation by ammonia.* . Journal of Virology.

Warren, K.S. 1962. *Ammonia toxicity and pH.* Nature 195, 47-49.

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

Wilson, M., L. & Gaido, L. 2004. *Laboratory diagnosis of urinary tract infections in adult patients* Copyright, the Infectious Diseases Society of America. 38, 1150-1158.

Xiong, R., Xie, G., Edmondson, A.E. & Sheard, M.A. 1999. *A mathematical model for bacterial inactivation.* International Journal of Food Microbiology 46, 45-55.

Acknowledgements

These studies were financed by SLU, FORMAS and Sida SAREC and performed at the National Laboratory Institute, SVA.

I would also like to acknowledge my supervisors, Håkan Jönsson, Björn Vinnerås and Ann Albihn together with my colleagues at SVA and SLU assisting me to accomplish this thesis.

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