Sanitisation of Faecal Sludge by Ammonia

Treatment Technology for Safe Reuse in Agriculture

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Abstract

Faecal sludge contains valuable plant nutrients and can be used as a fertiliser in agriculture, instead of being emitted as a pollutant. As this involves a risk of pathogen transmission, it is crucial to inactivate the pathogens in faecal sludge. One treatment alternative is ammonia sanitisation, as uncharged ammonia (NH_3) inactivates pathogens. The aim of this thesis was to study how the pathogen inactivation depends on treatment factors, mainly NH_3 concentration, temperature and storage time, and based on this to make treatment recommendations that ensure pathogen inactivation.

Salmonella inactivation was rapid and could be eliminated within a few days. Reovirus and adenovirus were inactivated more slowly than that, but more rapidly than bacteriophages PhiX174, 28B and MS2. *Ascaris* eggs were generally inactivated more slowly than the other studied organisms, especially at low temperatures (<20 °C). *Ascaris* egg inactivation was modelled as a function of NH_3 concentration and temperature, which enabled the prediction of required treatment time. An assessment of health risk associated with consumption of crops eaten raw indicated that a 4.5 $\log_{10}$ reduction of *Ascaris* eggs and a 7.5 $\log_{10}$ reduction of rotavirus were required for unrestricted use of ammonia-treated faecal sludge as a fertiliser.

Faecal sludge contains some ammonia mainly due to the ammonia in urine, but the concentrations can be low due to dilution with flushwater and losses to air. Mixing source-separated urine and faeces from urine-diverting dry toilets will give a high enough NH_3 concentration for pathogen inactivation. Estimations of NH_3 concentrations in faecal sludge from vacuum, pour-flush and low-flush toilets indicated that the ammonia concentrations required for stable pH may not be reached without the addition of ammonia. The addition can be urea, which is a common mineral fertiliser that hydrolyses to ammonia and carbonate through the enzyme urease found in faeces.

Ammonia sanitisation of faecal sludge is a simple and robust technology enabling a high degree of pathogen inactivation. This can considerably reduce the health risk for farmers, food consumers and downstream populations. It is important to minimise flush water volumes in order to reduce the treatment costs.

Keywords: faecal sludge, ammonia sanitisation, treatment technology, *Ascaris*, *Salmonella*, virus, health risk, human excreta

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Dedication

To my sons Jonas and Daniel and my wife Kari

Above all else, guard your heart, for everything you do flows from it.
Proverbs 4:23

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Fidjeland, J., Magri, M.E., Jönsson, H., Albihn, A. and Vinnerås, B. (2013) The potential for self-sanitisation of faecal sludge by intrinsic ammonia. *Water Research* 47(16), 6014-6023.

II Fidjeland, J., Nordin, A., Vinnerås, B. (submitted) Inactivation of *Ascaris* eggs and *Salmonella* spp. in faecal sludge by treatment with ammonia and urea. Planned submission to *Journal of Water, Sanitation and Hygiene for Development*

III Magri M. E., Fidjeland J., Jönsson H., Albihn A. and Vinnerås B. (2015). Inactivation of adenovirus, reovirus and bacteriophages in fecal sludge by pH and ammonia. *Science of the Total Environment* 520(0), 213-21.

IV Fidjeland, J., Nordin, A., Pecson, B., Nelson, K., Vinnerås, B. (submitted) Modelling *Ascaris* egg inactivation as a function of ammonia and temperature. Submitted to *Water Research*

V Fidjeland, J., Ottoson, J., Vinnerås, B. Quantitative microbial risk assessment of agricultural use of faecal sludge sanitised by ammonia. Manuscript

The contribution of Jörgen Fidjeland to the papers included in this thesis was as follows:

I Planning of the study was performed by all authors; Fidjeland and Magri performed the study; Fidjeland did the data analysis and writing, with revision by co-authors.

II Fidjeland planned the study with input from co-authors, and performed the study; Fidjeland did the data analysis and writing, with revision by co-authors.

III Planning of the study was performed by all authors; Fidjeland and Magri performed the study and analysed the data; Magri wrote the paper with revision by the co-authors.

IV Fidjeland planned the study with input from co-authors, and did the data analysis; Fidjeland wrote the paper with revision by the co-authors.

V Planning of the study was performed by all authors; Fidjeland performed the risk assessment with input from co-authors; Fidjeland wrote the paper with revision by co-authors.

Abbreviations

ABP	Animal by-products
CFU	Colony-forming units
DALY	Disability-adjusted life years
DM	Dry matter
EHEC	Enterohaemorrhagic *E.coli*
ETEC	Enterotoxigenic *E.coli*
GHG	Greenhouse gases
GWP	Global warming potential
PFU	Plaque-forming units
LOORMSRE	Leave out one root mean square relative error
LRV	$\log_{10}$ reductions of viability
QMRA	Quantitative microbial risk assessment
TAN	Total ammonia nitrogen ($NH_3 + NH_4^+$)
UDDT	Urine-diverting dry toilet

1 Introduction

At present there are about one billion people in the world without access to toilets who are defecating in the open. The construction of toilets is only one part of the solution, as toilet waste often ends up in the environment without proper management (Opel, 2012). It is estimated that toilet waste from three billion people with access to toilets is not treated (Baum *et al.*, 2013). Faecal sludge is often dumped in waterways instead of being transported to sludge treatment facilities, causing outbreaks of diseases such as the cholera outbreak in Haiti in 2010 (Frerichs *et al.*, 2012). Many toilets are flushed by rainwater during the rainy season, and pathogenic microorganisms are therefore spread to downstream populations, as well as contaminating fresh water and groundwater sources (Rebaudet *et al.*, 2013; Howard *et al.*, 2003). Septic tanks are often unserviced and full, which reduces the treatment effect of the wastewater (Yen-Phi *et al.*, 2010).

Exposure to pathogens from human excreta causes severe diarrhoea and infections with parasitic worms, especially in children (Dold & Holland, 2011). It is estimated that the lack of safe drinking water, proper sanitation and handwashing is the cause of 0.3 million deaths every year, of which the majority are children (Lim *et al.*, 2012). In addition to direct transmission to the human population, some zoonotic pathogens also infect domestic animals and wildlife, causing economic losses and subsequent transmission to humans (Albihn & Vinneras, 2007). The lack of treatment of the excreta also causes eutrophication of many waterways, which has a severe impact on aquatic ecosystems. It is therefore crucial to contain and treat human excreta in order to prevent eutrophication and the spread of disease.

Lack of food and essential nutrients is a huge health problem in many parts of the world. It is estimated that 805 million people are chronically undernourished (FAO *et al.*, 2013). Poor nutrition causes 3.1 million deaths every year in children under five, representing almost half (47 %) of all deaths in this age group (Horton & Lo, 2013). Measured in disability-adjusted life

years (DALYs), in many places this is a much greater health problem than lack of proper sanitation. In sub-Saharan Africa, it is estimated that improper drinking water, sanitisation and handwashing are responsible for 3.4 % of total DALY loss, while mal- and undernutrition accounts for 15.3 % (Lopez *et al.*, 2006).

Human excreta contain plant nutrients and organic matter that, if used as fertiliser and for soil improvement purposes in agriculture, can increase agricultural food production. While mineral fertilisers are often unaffordable for smallholders (Kimetu *et al.*, 2004), human excreta are free and readily available, especially in urban areas. Local recycling of nutrients in human excreta can therefore contribute to increasing the availability of food and decrease mal- and under-nutrition. However, as human excreta contain pathogens, there is a risk that the recycled excreta may have a harmful effect on health, as pathogens may be recycled as well. Studies show that the use of human excreta as fertiliser is correlated with a higher prevalence of soil-transmitted helminths and diarrhoea (Phuc *et al.*, 2014; Pham-Duc *et al.*, 2013; Corrales *et al.*, 2006).

However, the problem with mal- and undernutrition is linked to sanitation, since diarrhoea and infections with intestinal parasitic worms decrease the uptake of nutrients from food (Crompton & Nesheim, 2002; Ahmed *et al.*, 1993; Brown *et al.*, 1980). Recycling of insufficiently treated human excreta may therefore potentially worsen the nutritional status of the affected population rather than improve it. Development of treatment methods for sufficient pathogen inactivation is therefore crucial in order to be able to reuse excreta safely as a fertiliser (Rijsberman & Zwane, 2012).

One alternative for treatment of human excreta is ammonia sanitisation, which utilises the ability of uncharged ammonia NH_3 to inactivate pathogens. Previous research on ammonia sanitisation has primarily focused on pathogen inactivation in source-separated urine and faeces, as well as wastewater treatment plant sludge. However, a summary of data collected by the Joint Monitoring Programme (JMP, 2012) indicates that approximately 1.1 billion people use water-flushed toilets that are not connected to septic tanks or a sewage pipe network. The focus in this thesis was therefore to investigate treatment alternatives for faecal sludge where urine and faeces are mixed and varying amounts of flushwater are used.

2 Objectives

The overall aim of this thesis was to facilitate the production of hygienically safe fertilisers from faecal sludge by ammonia sanitisation through the development of treatment recommendations that ensure pathogen die-off. Specifically, the aims were:

- to provide evidence on the inactivation of bacteria, viruses and helminth eggs in relation to treatment factors including urine content, flushwater volumes, urea dose, temperature and storage time, as well as physiochemical parameters such as pH and NH_3 (Papers I-III)
- to model the inactivation of *Ascaris* eggs as a function of physical and chemical treatment parameters (Paper IV)
- to quantify the microbial health risks associated with agricultural use of ammonia-treated faecal sludge, identify the most hazardous pathogens in this context, and evaluate the level of pathogen inactivation required for an acceptably low health risk (Paper V)

3 Structure of the thesis

The first studies of this thesis (Paper I-III) analysed pathogen inactivation in faecal sludge on a laboratory scale. This was done in order to collect more data on pathogen inactivation and the characteristics of faecal sludge. The inactivation data for *Ascaris* eggs were further processed and generalised in Paper IV, while Paper V evaluated the degree of pathogen inactivation required.

Paper I studied the potential for self-sanitisation, *i.e.* using urine as the only ammonia source for sanitisation of faecal sludge. The paper studied the inactivation of *Ascaris suum*, *Salmonella* spp. and *Enterococcus* spp. in faecal sludge depending on the content of urine, faeces and flushwater, and how the inactivation correlated to NH_3 concentration and temperature.

Paper II studied the inactivation of *Ascaris* eggs and *Salmonella* spp. inactivation in a more diluted faecal sludge, using urea to increase the ammonia concentration. The study also evaluated the impact of carbonate relative to ammonia for *Ascaris* inactivation, as both are present during ammonia sanitisation.

Paper III studied the inactivation of viruses and bacteriophages in the same types of sludge as studied in Papers I and II. The potential of using bacteriophages as indicators of virus inactivation was evaluated.

Paper IV modelled the inactivation of *Ascaris* eggs as a function of physiochemical parameters. The model was based on *Ascaris* egg inactivation data from Papers I and II and two other studies.

Paper V assessed the health risks associated with the use of treated faecal sludge as a fertiliser and the pathogen inactivation required for safe reuse. Inactivation data from Papers III and IV were used in the risk assessment.

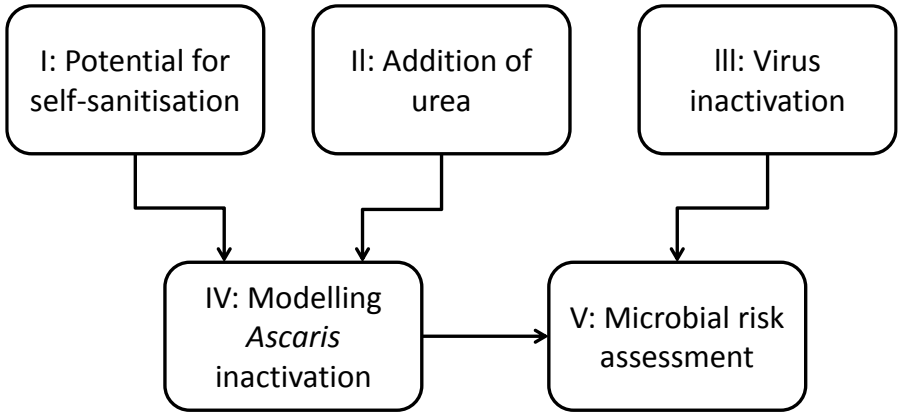


Figure 1. Structure of the papers in this thesis

In addition, the variation in the physiochemical characteristics of faecal sludge from different toilet systems was assessed based on literature data. Furthermore, these characteristics were used to estimate the storage requirements for different treatment alternatives, and the quantities of nutrients recycled were compared to the nutrients added to enhance pathogen inactivation.

4 Background

4.1 Human excreta as fertiliser

Human excreta contain a lot of plant nutrients and can therefore be used as fertiliser in agriculture. 90 % of the nitrogen in human excreta is found in urine and 10 % in faeces, while 60-75 % of phosphorous is found in urine and 25-40 % in faeces (Jönsson & Vinnerås, 2004). Urine and faeces also contain a significant amount of potassium and micronutrients that can enhance plant growth (Sakhare *et al.*, 2008). The organic matter in faeces can also be used as a soil conditioner since it can improve the structure and water-holding capacity of the soil.

The amount of nitrogen and phosphorous excreted per person per year varies depending on diet, and ranges between 2-4 kg N/p/year and 0.3-0.6 kg P/p/year for adults (Jönsson & Vinnerås, 2004). Comparing the nutrients in faeces and urine with mineral fertiliser use shows that, in the case of Uganda, the total input of N and P to the soil can be multiplied by a factor of 13 and 17 respectively by using human excreta as a fertiliser in agriculture (Vinnerås *et al.*, manuscript). This is mainly due to the low use of mineral fertilisers there. In Vietnam, which has a high use of mineral fertiliser due to subsidies, the reuse of human excreta in agriculture could replace 14 % of N and 24 % of P (Vinnerås *et al.*, manuscript).

For farmers who cannot afford mineral fertilisers, human excreta can be an alternative fertiliser for increasing food production and income. Human excreta may also replace the use of mineral fertilisers, which can reduce the environmental impacts of fertiliser production. With regard to the emission of greenhouse gases (GHG), the replacement of nitrogen from mineral fertilisers with nitrogen from excreta has the greatest potential for reducing emissions; the recycling of nitrogen accounts for 97 % of all GHG savings by using human excreta as a fertiliser (Vinnerås *et al.*, manuscript). Replacing mineral

fertilisers with excreta may also reduce fluctuations in food prices, as nitrogen fertilisers are mainly produced with natural gas, so when gas prices increase, the cost of food goes up (Steinbuks & Hertel, 2013).

4.2 Constraints of using human excreta as a fertiliser

4.2.1 Heavy metals and organic pollutants

The concentrations of heavy metals in faecal sludge are usually much lower than in WWTP sludge or biosolids and manure (*Table 1*) (Winker *et al.*, 2009), although it should be noted that there is considerable variation in the heavy-metal content of WWTP sludge depending on the sources of the wastewater being treated. The concentrations of heavy metals in faecal sludge are generally low and similar to mineral fertilisers (*Table 1*). The exceptions are copper and zinc, which can be found in higher concentrations in faecal sludge than in mineral fertilisers. The high concentration of copper is mainly due to piping and is lower when other materials are used for water pipes (Tervahauta *et al.*, 2014). The heavy metal content in faecal sludge is due to the faecal matter, as urine has very low concentrations of heavy metals (Jönsson *et al.*, 2005).

Table 1. *Heavy-metal content in faecal sludge, WWTP sludge, cow manure and mineral fertiliser as mg/kg P. Adapted from Tervahauta* et al. *(2014)*

Element	Faecal sludge[a]	WWTP sludge[b]	Cow manure[c]	Mineral fertiliser (P)[d]
As	12	300	nd	33
Cd	13	39	33	91
Cr	731	1268	1145	1245
Cu	3720	12701	14397	207
Hg	0.12	23	nd	0.7
Ni	466	1025	1472	202
Pb	69	3519	695	154
Zn	13919	31166	25947	1923

nd: not detected
References: [a] Tervahauta *et al.* (2014), [b] CBS (2011), [c] van Dooren *et al.* (2005), [d] Remy and Ruhland (2006)

The levels of hormones and antibiotics are usually lower in faecal sludge than in manure, while the variety of pharmaceutical residues is larger in faecal sludge (Combalbert & Hernandez-Raquet, 2010; Winker *et al.*, 2008). The rich microflora in soil, as well as the availability of air and water, enhance the degradation of these substances in soil (Li *et al.*, 2013). However, some pharmaceutical substances have shown lengthy survival in soil as well as

uptake in plants, although at low levels (Winker *et al.*, 2010; Schluesener & Bester, 2006). An important ecological risk of organic pollutants is associated with endocrine disruption in fish and other aquatic organisms (Sumpter, 1998). The content of hormones and pharmaceutical residues in excreta-derived fertilisers that end up in the soil environment therefore probably represents a low risk compared to the content in wastewater ending up in water recipients.

Some antibiotics have shown slow degradation in soil (Christian *et al.*, 2003). Application of excreta to soil can cause a spread of bacteria with antibiotic resistance (Albihn & Vinneras, 2007). The resistance genes may be transferred to other indigenous bacteria in the soil, thus increasing the concentration of these genes in the environment (Goss *et al.*, 2013). A recent study has shown an increased concentration of antibiotic-resistance genes on crops grown in soil amended with treated biosolids, and also indicates that the genes are more persistent than the bacteria (Rahube *et al.*, 2014).

4.2.2 Pathogens in human excreta

Human excreta from infected individuals contain pathogenic microorganisms that may cause disease in humans and animals exposed to it. The majority of the pathogens are excreted in the faeces.

Several species of bacteria cause diarrhoea and are shed in considerable quantities in faeces from infected individuals. This includes *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, enterotoxigenic *E. coli* (ETEC) and enterohaemorrhagic *E. coli* (EHEC). These bacteria can regrow in the environment, which prolongs their survival (Nyberg *et al.*, 2011). *Vibrio cholerae* may also infect protozoa and thus extend their survival in water (Vezzulli *et al.*, 2010).

Protozoan parasites such as *Cryptosporidium* spp. and *Giardia lamblia* can cause severe gastroenteritis, and are responsible for a large proportion of water-borne gastroenteritis since they are not inactivated by chlorine. While human faeces are the main source of contamination of *Cryptosporidium hominis* in water sources, livestock are the main reservoir for *Cryptosporidium parvum*, which is a zoonotic parasite that can infect both humans and livestock (Hunter & Thompson, 2005). *Entamoeba histolytica* also shed their cysts in human faeces. Ingestion of cysts may cause amoebiasis, a severe disease that can destroy tissue in internal organs.

Several viruses such as rotavirus, calicievirus, adenovirus type 40 & 41 and astroviruses cause gastroenteritis and are mainly transmitted through faeces. Other viruses such as hepatitis A and E cause hepatitis and not gastroenteritis, but are still present in faeces and transmitted by the faecal-oral route (Greening, 2006). Hepatitis A outbreaks have been traced to the use of

wastewater for irrigation (Barrimah *et al.*, 1999). Hepatitis E is a zoonotic virus and can be transmitted between humans and pigs, making pigs a potential reservoir (Pavio *et al.*, 2010).

Helminths are a type of parasite whose eggs typically survive for a long time in the environment. *Ascaris lumbriocoides* (roundworm) is a very common parasite, with about 1.3 billion people infected worldwide. Hookworm is less common, but the disease is more serious and causes as many deaths as ascariasis (*Table 2*). *Taenia* spp. (tapeworm) is a zoonotic disease that spreads eggs in the faeces of infected humans. The eggs may cause cysticercosis in both humans and livestock, which is a serious condition for humans and can cause transmission to humans or economic losses when present in meat. *Trichuris trichuria* (whipworm) is also persistent in the environment, causes gastroenteritis in infected individuals and is common in low and mid-income countries.

Table 2. *Estimates of global mortality and burden of disease measured as disability-adjusted life years (DALYs) for selected sanitation-related diseases*

Disease	Mortality (1000 deaths/year)	Burden of disease (1000 DALYs/year)
Rotaviral enteritis	251	18650
Shigellosis	123	7052
Cryptosporidiosis	100	8372
Enteropathogenic E.coli infection	89	7542
Enterotoxigenic E.coli infection	121	6894
Typhoid and paratyphoid fever	190	12239
Other salmonella infections	81	4847
Ascariasis	2.7	1315
Trichuriasis	3[a]	638
Hookworm disease	3[a]	3231
Cysticercosis	1.2	503
Schistosomiasis	11.7	3309

References: Mortality: Lozano *et al.* (2012). Burden of disease: Murray *et al.* (2012). Hookworm disease, Trichuriasis (a): WHO (2004)

Several pathogens represent a greater risk for children than for adults. Ascariasis is mainly a problem for those under 15 years old. The main reasons for childhood diarrhoea globally is rotavirus, *Cryptosporidium* spp., *Shigella* spp. and ETEC, and together are estimated to be responsible for the majority of cases of severe to moderate diarrhoea in children (Kotloff *et al.*, 2013). *Campylobacter* spp., *Aeromonas* spp, *Vibrio cholerae*, calicievirus and

adenovirus type 40 and 41 are also major causes of childhood diarrhoea (Kotloff *et al.*, 2013; Ramani & Kang, 2009).

While many pathogens are excreted in faeces, urine does not normally contain pathogens, and the main risk when using source-separated urine as a fertiliser is therefore cross-contamination of faecal matter (Höglund *et al.*, 2002). However, infections with *Schistosoma haematobium* may cause the excretion of eggs in urine, sometimes during the whole life of the infected individual. Other pathogens such as *Leptospira interrogans*, *Salmonella* typhi and *Salmonella* paratyphi may also be excreted in urine (Feachem, 1983), but this transmission is probably not significant compared with other transmission routes (Höglund *et al.*, 2002).

4.2.3 Regulations

In order to regulate the reuse of human excreta as fertiliser, many countries have regulations concerning the required treatments and/or the maximum allowed pathogen concentration in treated material. The US EPA requires <1 helminth eggs and <3 MPN *Salmonella* spp. per 4 g dry matter (DM) for class A biosolids. Several other countries also have restrictions on the levels of *E.coli* and/or enterococci.

Few regulations carry restrictions on the concentrations of protozoa and viruses, but the treatment technologies used to achieve sufficiently low concentrations of bacteria and helminths are designed to have an inactivation effect on protozoa and viruses as well. EU regulations for animal by-products (ABP) require alternative thermal or chemical treatment technologies for category 3 (low-risk) waste and have to demonstrate a 5 $\log_{10}$ reduction of *Enterococcus* faecalis or *Salmonella* Senftenberg, and at least a 3 $\log_{10}$ reduction of thermoresistant viruses if the viruses are assumed to be a problem (European Union, 2006). Furthermore, a 3 $\log_{10}$ reduction of *Ascaris* eggs is required if the treatment is a chemical process.

4.3 Ammonia sanitisation

Ammonia has long been known to have antimicrobial properties (Warren, 1962). Several studies have shown that ammonia is capable of inactivating a wide range of pathogens, including bacteria (Nordin *et al.*, 2009b; Vinnerås *et al.*, 2008; Mendez *et al.*, 2004), viruses (Emmoth *et al.*, 2011; Emmoth *et al.*, 2007), protozoa (Jenkins *et al.*, 1998) and helminth eggs (Nordin *et al.*, 2009a; Pecson *et al.*, 2007; Pecson & Nelson, 2005). The only microorganism studied that showed no inactivation effect of ammonia is *Clostridia* spp. spores (Vinnerås *et al.*, 2003).

The mechanisms of pathogen inactivation by NH_3 are not well understood. Being uncharged, the molecule is probably able to pass through cell membranes, and as a weak base it may increase the internal pH of the cell to levels that make the cell deteriorate (Park & Diez-Gonzalez, 2003). In addition, it is suggested that bacterial cells with a high pH may pump out K^+ ions in exchange for H^+ ions in order to restore the pH, and that the lack of K^+ may eventually kill the cell (Bujozek, 2001). For viruses, it has been shown that the outer capsule of non-enveloped viruses is not damaged by NH_3, and that viruses inactivated by NH_3 may still inject their genome into attacked cells (Ward, 1978). This suggests that the genome is damaged by NH_3. In the case of RNA viruses, the suggested mechanism of genome destruction by NH_3 and other bases is transesterification of the RNA (Decrey *et al.*, 2015).

Uncharged ammonia (NH_3) is in equilibrium with ammonium (NH_4^+), and it is only NH_3 that inactivates pathogens (Emerson *et al.*, 1975). The fraction of NH_{TOT} that is uncharged is greater at higher pH levels and higher temperatures (*Figure 2*).

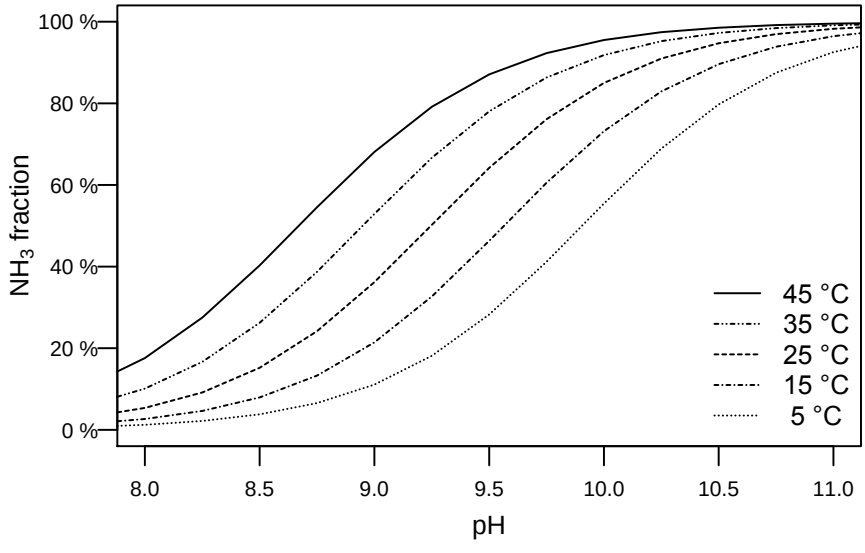


Figure 2. NH_3 fraction of NH_{TOT} as a function of pH and temperature, as estimated by the Emerson approach

4.3.1 Ammonia sanitisation systems for treatment of faecal sludge

Faecal sludge contains some intrinsic ammonia, mainly due to the urea content of urine. The ammonia concentration in the sludge is strongly dependent on the

volumes of water used to flush the toilet. Ammonia may also be lost to the air from collection chambers unless they are closed.

In cases where the concentration of intrinsic ammonia is not sufficiently high to achieve pathogen inactivation, or the pathogen inactivation is too slow, it is desirable to increase the concentration. This can be done by adding ammonia, increasing the pH or a combination of both. One alternative is to add the mineral fertiliser urea. Upon contact with the enzyme urease, which is present in faeces, the urea hydrolyses into ammonia and carbonate and increases the pH value to around 9. Another alternative is to add ammonia solution (25 %), which increases the pH more compared to addition of urea. While urea is harmless to handle, work with ammonia solution presents a health risk due to its alkalinity.

The process of urea hydrolysis may take weeks for faecal sludge from toilets using large amounts of flushwater, as the enzymes are then more diluted. One option to reduce the duration of urea hydrolysis might be to add urea to the collection tank connected to the toilet prior to filling, so that the urea can hydrolyse during filling. Another option is not to empty all of the solids when the treatment is finished, but rather transfer it to the next batch in order to increase the concentration of urease, which is probably higher in the solids fraction.

The pH of faecal sludge can be increased by adding lime or ash (McKinley *et al.*, 2012; Nordin *et al.*, 2009a), either directly into the toilet after defecation or prior to treatment. Too high initial pH (>10.5) prior to addition of urea can inhibit the hydrolysis of urea into ammonia and carbonate.

Ammonia spreads by diffusion in sludge, and it diffuses more quickly in sludge with a low dry matter (DM) content. In sludge with a DM content of ≤5 %, the ammonia concentration spreads at a rate of around 2.5 cm h^{-1} (Vinnerås *et al.*, 2009). While intrinsic ammonia from urine can be assumed to be evenly distributed, the addition of urea to faecal sludge may require mechanical mixing.

The ammonia concentration in faecal sludge mainly depends on the amount of water used for flushing the toilet. A pour-flush toilet is a low-tech toilet where the flushing is done manually with a bucket. The amount of water used depends on the user, but is mainly impacted by the design of the water lock at the outlet pipe, as the user will add as much water as required to make the excreta disappear. Reports state that the water use is 1-2 L per flush (Bhagwan *et al.*, 2014), but sludge characterisation is only performed on sludge in soak pits, making it impossible to estimate the actual average flushwater use. Vacuum toilets use water and vacuum suction to flush the toilet and use 0.5-1.5 L flushwater per flush, which is less than for pour-flush toilets. However, in

the case of vacuum toilets, some losses of ammonia may occur due to flushing with air. Low-flush toilets typically use 2 L for a low flush intended for use after urination, and 4 L in a flush intended for use after defecation.

In urine-diverting dry toilets (UDDTs), urine and faeces are separated, which enables separated treatment of the two fractions. However, one interesting treatment method is to mix urine and faeces after they are removed from the toilet. By doing this, the ammonia concentration in the urine can be used to inactivate the pathogens in the faeces. In a common pit latrine, urine and faeces are also mixed without the addition of water, but the pathogen inactivation is low as NH_3 is lost to air and leaking water.

Sludge from pit latrines, septic tanks, soak pits, treatment ponds, and wastewater treatment plants can also be sanitised by ammonia. The urea doses required for sanitisation of these types of sludge have not been evaluated in this thesis. The buffer capacity is normally higher for sludge with a higher dry matter content, and the microbiological degradation may also cause some change in buffer capacity compared with raw faeces. Studies on sludge from wastewater treatment plants show that higher urea doses are required compared with faecal sludge (Fidjeland *et al.*, 2013).

4.3.2 Ammonia inactivation kinetics

It is generally assumed that the pathogen inactivation observed in studies can be used to predict pathogen inactivation under similar environmental conditions, *i.e.* similar ammonia concentration, pH and temperature. Usually the pathogen inactivation is log-linear, meaning that the treatment time required is proportional to the $\log_{10}$ reduction of viability wanted. However, there is often an initial lag phase in inactivation of more persistent microorganisms such as *Ascaris* eggs, with very slow inactivation, followed by an exponential phase with a log-linear inactivation.

Previous studies have shown that the inactivation of most pathogens is more rapid at higher temperatures and higher ammonia concentrations. However, the temperature dependency is not the same for all pathogens. *Ascaris* egg inactivation by ammonia is highly temperature dependent and is very slow at low temperatures (Nordin *et al.*, 2009a), while enterococci inactivation, for example, is less affected by temperature (Vinnerås *et al.*, 2008).

In addition to ammonia, carbonate concentration is also a relevant parameter in cases where the ammonia source is urine or urea. As urea is hydrolysed into two molecules of ammonia and one molecule of carbonate, the concentration of carbonate also increases proportionally. Carbonate has been shown to inactivate several species of bacteria, including *Salmonella* spp. (Diez-Gonzalez *et al.*, 2000).

5 Materials and methods

5.1 Pathogens and indicators

The inactivation of *Salmonella* Typhimurium phage type 178 was studied as a model for *Salmonella* spp. and enterohaemorrhagic *E.coli* (EHEC), as several *Salmonella* spp. strains and a non-toxin-producing EHEC strain have shown similar inactivation by ammonia as the *Salmonella* Typhimurium strain used here (Nordin, 2010). *Enterococcus faecalis* were studied as a treatment indicator and as a model for gram-positive bacteria. Three bacteriophages – 28B (Lilleengen, 1948), MS2 (ATCC15597-B1) and PhiX174 (ATCC13706-B1) – were studied as treatment indicators. Canine adenovirus type 1 (strain vacc-98) and human reovirus type 3 (ATCC-VR-232, strain Abney) were studied as models for human adenovirus and human rotavirus respectively. *Ascaris suum* eggs extracted from the intestines of slaughtered pigs were studied as an indicator of *Ascaris lumbricoides* eggs, which infect humans.

5.2 Impact of urine content and flushwater volumes (Papers I & III)

Urine, faeces and water were mixed in different proportions in order to study the impact of urine and water dilution on pathogen inactivation. Flushwater volumes of 1.5-2 L per person per day simulated the sludge from vacuum toilets, while a flushwater volume of 6 L per person per day was used to simulate sludge from pour-flush latrines. Urine and faeces was mixed without the addition of water to simulate the result of mixing source-separated faeces and urine in order to sanitise the faeces. Faeces and urine were collected from volunteers. The combinations of temperature, pathogens and sludge compositions studied are summarised in *Table 3* and *Table 4*.

Table 3. *Treatments studied for the inactivation of Ascaris, reovirus, adenovirus, 28B at 10 (only UDDT1), 23 and 28 °C*

Code	Sanitation system	Flushwater (L/day)	Faeces (kg/day)	Urine (L/day)
UDDT1	Source-separated faeces + urine	0	0.2	1
V4	Vacuum toilet	2	0.2	2
V5	Vacuum toilet	2	0.2	3
PF1	Pour-flush latrine	6	0.2	1
PF2	Pour-flush latrine	6	0.2	2

Table 4. *Treatments studied for the inactivation of Salmonella, Enterococcus, PhiX174, MS2 at 14, 26 and 28 °C*

Code	Sanitation system	Flushwater (L/day)	Faeces (kg/day)	Urine (L/day)
UDDT2	Source-separated faeces + urine	0	0.2	0.5
V1	Vacuum toilet	1.5	0.2	1
V2	Vacuum toilet	1.5	0.2	2
V3	Vacuum toilet	2	0.2	1
V4	Vacuum toilet	2	0.2	2
V5	Vacuum toilet	2	0.2	3
PF1	Pour-flush latrine	6	0.2	1
PF2	Pour-flush latrine	6	0.2	2

For the first treatment setup (*Table 3*), the viability of *Ascaris suum* eggs, reovirus, adenovirus and 28B were monitored. In the second setup (*Table 4*), the viability of *Salmonella* Typhimurium, *Enterococcus faecalis*, PhiX174 and MS2 were monitored. The time between samplings increased exponentially, *i.e.* typically on day 0 (startup), 1, 2, 4, 8 and so on. The sampling frequency was lower at lower temperatures.

5.3 Impact of the addition of urea or ammonia solution (Papers II & III)

A mixture of 97.7 % water, 1.6 % urine and 0.7 % faeces was made to simulate faecal sludge from a toilet using 30 L flushwater person^{-1} day^{-1}. Additions of doses from 0.05 to 1.5 % urea and ammonia solution (0.25 %) doses from 2-10 % were added to study the impact on pathogen inactivation. The microorganisms studied were *Ascaris suum* eggs, *Salmonella* Typhimurium, adenovirus and reovirus, except for the treatment with ammonia solution where virus viability was not monitored.

Table 5. *Treatments studied for inactivation of Ascaris suum eggs,* Salmonella *Typhimurium, adenovirus and reovirus in faecal sludge*

Treatment	Temperatures (°C)	Microorganisms
1.5 % urea	28, 23, 17, 10, 4	*Ascaris*, *Salmonella*, adenovirus, reovirus
0.75 % urea	28, 17	*Ascaris*, *Salmonella*, adenovirus, reovirus
0.4 % urea	32, 28	*Ascaris*, *Salmonella*, adenovirus, reovirus
0.15 % urea	32, 28	*Ascaris*, *Salmonella*, adenovirus, reovirus
0.05 % urea	32, 28	*Ascaris*, *Salmonella*, adenovirus, reovirus
10 % ammonia solution	32,23,10,4	*Ascaris*, *Salmonella*
5 % ammonia solution	10,4	*Ascaris*, *Salmonella*
3 % ammonia solution	17	*Ascaris*, *Salmonella*
Untreated	32, 28, 23, 17, 10, 4	*Ascaris*, *Salmonella*, adenovirus, reovirus

The pathogen concentration was also monitored in ammonia- and carbonate-free buffers at pH 7 and 9 at the studied temperatures. *Ascaris* egg inactivation was also studied in buffers with ammonia only and carbonate only at pH 9, and in ammonia- and carbonate-free buffers at pH 12.

5.4 Microbiological analysis

The concentration of bacteria was analysed using the plate-spreading technique after serial dilution. The viability of *Ascaris* eggs was analysed by viewing the eggs under a microscope after 30-40 days of incubation at 28 °C. Eggs that had developed to larvae were regarded as viable, while eggs that had not were regarded as non-viable. Bacteriophages were analysed using the double-layer agar technique after serial dilution, where the sample was mixed with host bacteria and agar, and the bacteriophages were enumerated as plaques. Virus concentration was determined by cell infection array, where host cell cultures and different dilutions of the sample were mixed in 96 well plates, and each

well was analysed under a microscope after seven and ten days' incubation to determine whether the cell was infected or not.

5.5 Physiochemical analysis

The concentration of total ammonia nitrogen (TAN) was determined spectrophotometrically using ammonia kit reagents (Merck). The pH was measured using a pH electrode and a pH meter (Meterlab pH meter 210, Copenhagen, Denmark). The storage temperatures were logged by Tinytag® loggers. The dry matter of faeces was measured by drying at 105 °C overnight.

The concentration of total ammonia NH_{TOT} (NH_3 + NH_4^+) (M) was estimated based on total ammonia nitrogen (TAN, g/L) and adjusted to the wet fraction according to eq. 1, where DM is the dry matter content. The concentration of uncharged ammonia, NH_3, was estimated in two different ways. In papers I and III, it was determined by the Emerson approach (Emerson *et al.*, 1975), using equations 2 and 3.

$$NH_{TOT} = \frac{TAN}{14.01} \cdot \frac{1}{(1 - DM)} \quad (1)$$

$$pKa = \frac{0.09018 + 2729.92}{273.15 + \mathrm{T}} \quad (2)$$

$$f_{Emerson} = \frac{10^{-pKa}}{10^{-pH} + 10^{-pKa}} \quad (3)$$

In Papers II and IV the concentration of NH_3 was estimated using the Pitzer approach (Pitzer, 1991). This gives a more precise estimation of substances in a solution as it takes into account the ionic strength of the solution and the interaction between different species of ions. Literature data on the composition of urine and faeces were applied in Paper IV in order to estimate the concentration of ions other than ammonium and carbonate. The activity of CO_3^{2-} was estimated instead of its concentration, as the activity is a better representation of ion interaction with other chemicals. The activity of carbonate was also estimated using the Pitzer approach. In this thesis, all NH_3

concentrations were estimated using the Pitzer approach unless otherwise stated.

5.6 Statistical analysis

The inactivation observed for most microorganisms could be described as log-linear inactivation (eq. 4), where N_0 is the initial concentration of microorganisms, N is the concentration of microorganisms after time t, and k is the inactivation rate describing how fast the inactivation is occurring. The time required for 1 $\log_{10}$ or 90 % reduction of viable microorganisms, called decimal reduction time, is denoted T_{90} and is the inverse of k (eq. 5).

$$N = N_0 \cdot 10^{-k \cdot t} \tag{4}$$

$$T_{90} = \frac{1}{k} \tag{5}$$

The inactivation of *Ascaris* eggs, enterococci and phage 28B in most cases had an initial lag phase with very slow inactivation, and this was modelled with eq. 6, where n is a parameter determining the ratio between the lag phase l and the decimal reduction time (eq. 7).

$$N = N_0 \left[1 - \left(1 - 10^{-k \cdot t} \right)^{10^n} \right] \tag{6}$$

$$\text{Lag period: } l = \frac{n}{k} \tag{7}$$

All statistical analyses were performed using the statistical software R v. 2.14.0 (R Development Core Team 2014) and the nls-package for the non-linear analysis.

5.7 *Ascaris* modelling (Paper IV)

The *Ascaris* egg inactivation model was developed through a two-stage process. In step 1, the values of the inactivation rate k and n (Eq. 6) that were obtained in Papers I and II and also from two other published papers (Nordin *et al.*, 2009a; Pecson & Nelson, 2005) were plotted *versus* the ammonia concentration and temperature in order to make a model describing the correlations mathematically. In step two, each individual *Ascaris* egg viability measurement was modelled as a function of ammonia concentration, temperature and time in order to refine the model. Furthermore, this allowed a test to be conducted on whether the effect of NH_3 was linear or not, and on the impact of other parameters such as carbonate, dry matter content and pH on inactivation. This was done by comparing several models with regards to the leave out one root mean squared relative error (LOORMSRE), which is a measurement of how well the model could predict the treatment time required for the observed *Ascaris* egg inactivation. The calculation of this is described in detail in Paper IV.

5.8 Quantitative microbial risk assessment (Paper V)

Literature data was used to estimate the health risk of using ammonia-treated faecal sludge as a fertiliser for growing crops eaten raw. Carrot was used as a representative of crops where the edible part is below soil, while data for several crops such as salad and spinach were used to represent leaf vegetables. The health risk was estimated for the ingestion of uncooked vegetables and how this is affected by the extent of treatment regarding pathogen inactivation.

There were limited data available for estimating the virus risk. The initial concentration of virus in the sludge was estimated based on the concentration of viral particles in faeces from infected individuals, the duration of infection and the prevalence of virus infection.

5.9 Evaluation of treatment systems

The volume and composition of sludge from four different types of toilets were estimated based on flushwater volume, ammonia concentration in urine and faeces, quantities of urine and faeces, and the number of defecations and urinations per person per day. In order to obtain an idea of the variation of these factors, three sub-scenarios were estimated per toilet by using values giving the maximum, minimum and average values (*Table 6*). The ammonia concentrations in urine and faeces were estimated from FAO data on food consumption (Jönsson & Vinnerås, 2004), and values for Uganda, Haiti and

Sweden were used for the median, minimum and maximum sub-scenarios respectively.

Table 6. *Assumptions for the estimation of median, minimum and maximum NH_3 concentrations in faecal sludge*

		Median NH_3	Min. NH_3	Max. NH_3	Ref.
Urine, TAN	g N/day	5.6	4.8	10	1
Urine, volume		1.4	2.6	0.6	2
Urinations per day		6	8	4	2
Faecal mass		250	796	51	2
Faeces, TAN	g N/day	0.16	0.16	0.3	1
Faeces, dry weight	g DM/day	29	81	12	
Defecations per day		1	1	1	2
Pour-flush toilet	l/flush	2	3	1	
Vacuum toilet	l/flush	1	2	0.6	
Low-flush toilet, full flush	l/flush	4	4	4	
Low-flush toilet, urine flush	l/flush	2	2	2	

1: Jönsson and Vinnerås (2004)
2: Rose *et al.* (2015)

6 Results and discussion

6.1 Impact of urine content and flushwater volumes

Using urine as the only source of ammonia was sufficient to inactivate the studied microorganisms, except at the lowest temperatures (10/14 °C) where 500 and 330 days were required for a 3 $\log_{10}$ reduction of *Ascaris* eggs and bacteriophages respectively (*Table 7* and *Table 8*). With the exception of the bacteriophages, the *Ascaris* eggs were the most persistent microorganism studied. According to the EU ABP requirements for treatment, a 3 $\log_{10}$ inactivation of *Ascaris* eggs would be the dimensioning criteria for chemical treatment for the temperature range studied. At 23 °C, a 3 $\log_{10}$ reduction of *Ascaris* egg viability was achieved within one month of the UDDT post-mix treatment, within 1.5 months for the vacuum toilet sludge, and within six months for the pour-flush toilet sludge. However, these storage times cannot be considered treatment recommendations for faecal sludge from different toilet systems, since the ammonia concentration in urine is diet dependent and varies considerably during the day, and the urine used in this study had a very high ammonia concentration (~15 g N/L) compared to Swedish average (~7 g N/L) (Jönsson *et al.*, 2005).

Salmonella Typhimurium was inactivated very rapidly and a 5 $\log_{10}$ reduction could be achieved within one week in all treatments except treatment PF1, which had the highest flushwater volume and a low amount of urine. The inactivation of *Enterococcus faecalis* was much slower than for *Salmonella* Typhimurium, probably due to the more complex membrane of gram-positive bacteria compared with gram-negative bacteria.

Table 7. *Time required for a 3 log_{10} reduction of* Ascaris suum *egg viability, reovirus, adenovirus and 28B, together with pH, concentration of $NH_{3,Pitzer}$ and activity of CO_3^{2-} in faecal sludge from different sanitation systems stored at different temperatures. Codes: UDDT – source-separated urine + faeces, V – faecal sludge from vacuum toilets, PF – faecal sludge from pour-flush toilets*

Code	Temp (°C)	*Ascaris*, 3 log_{10} (days)	Reovirus, 3 log_{10} (days)	Adenovirus 3 log_{10} (days)	28B, 3 log_{10} (days)	pH	NH_3 (mM)	CO_3^{2-} (mM)
UDDT1	28	24	6	9	30	9.0	257	7
V5	28	21	11	11	39	9.0	184	6
V4	28	19	11	15	44	8.9	141	5
PF2	28	30	14	11	71	8.9	77	3
PF1	28	42	20	15	174	8.9	38	2
UDDT1	23	32	17	21	55	8.9	176	6
V5	23	43	16	26	70	9.0	143	6
V4	23	42	17	22	90	9.0	117	5
PF2	23	78	21	17	234	9.0	63	3
PF1	23	164	19	35	415	8.9	41	2
UDDT1	10	495	77	74	336	9.0	75	5

Adenovirus and reovirus were inactivated fairly rapidly and a 3 log_{10} reduction could be achieved within a month at 23 and 28 °C, while longer treatment times were needed at 10 °C (*Table 7*). The bacteriophages were inactivated much more slowly than the viruses studied. Unless other relevant viruses are more persistent then the ones studied here, this makes bacteriophages a very conservative indicator for virus inactivation by ammonia.

The treatments with a higher urine:faeces ratio and low flushwater volumes had a higher pH compared to the treatments with a lower urine:faeces ratio. The treatments with a high flushwater volume obviously had lower NH_{TOT} concentrations, and hence a lower NH_3 concentration.

Table 8. *Time required for a 5 log_{10} reduction of* Salmonella *Typhimurium and* Enterococcus faecalis*, and 3 log_{10} reduction of PhiX174 and MS2, plus pH, concentration of NH_3 and activity of CO_3^{2-} in faecal sludge from different sanitation systems stored at different temperatures. Codes: UDDT – source-separated urine + faeces, V – faecal sludge from vacuum toilets, PF – faecal sludge from pour-flush toilets*

Code	Temp.	*Salmonella*, 5 log_{10}, (days)	*Enterococcus*, 5 log_{10} (days)	PhiX174, 3 log_{10} (days)	MS2, 3 log_{10} (days)	pH	NH_3 (mM)	CO_3^{2-} (mM)
UDDT2	14 °C	1.9	60	244	1,148	9.1	105	6
V5	14 °C	2.2	40	198	993	9.1	73	5
V2	14 °C	1.4	42	245	5,527	8.9	55	3
V4	14 °C	2.4	52	262	1,221	8.9	51	3
V3	14 °C	5.3	44	257	496	9.0	51	3
V1	14 °C	4.2	100	366	868	9.0	47	3
PF2	14 °C	6.3	91	306	385	9.0	34	2
PF1	14 °C	14.8	110	128	455	8.9	24	2
UDDT2	28 °C	1.1	13	60	31	9.1	232	7
V5	28 °C	1.2	17	79	34	9.1	159	6
V2	28 °C	1.4	18	63	40	8.9	127	4
V4	28 °C	1.6	14	48	39	8.9	118	4
V3	28 °C	1.5	20	76	63	9.0	113	4
V1	28 °C	1.9	18	63	47	9.0	106	4
PF2	28 °C	1.2	16	80	160	9.0	75	3
PF1	28 °C	16.3	32	39	NR	8.9	54	2

6.2 Impact of addition of urea or ammonia solution

Additions of low urea doses down to 0.4 % had a very strong impact on pathogen inactivation in the dilute faecal sludge compared with the untreated controls (*Table 9*). A 3 log_{10} reduction of virus concentration and *Ascaris* egg viability was achieved within 40 days with 0.4 % urea at 28 °C, while in the untreated controls no reduction of *Ascaris* egg viability was observed and 200 days would be required for a 3 log_{10} reduction of viruses. However, as the buffer capacity of faecal sludge depends on flushwater volumes, this treatment may not be sufficient for more concentrated faecal sludge.

At 10 and 4 °C, no *Ascaris* egg inactivation was observed in the faecal sludge treated with 1.5 % urea. However, the *Ascaris* eggs were inactivated by high doses of ammonia solution, showing that *Ascaris* egg inactivation is possible at low temperatures. A comparison of the inactivation of *Ascaris* eggs at different temperatures and NH_3 concentrations showed a very high

temperature dependency; at 32 °C a 3 $\log_{10}$ reduction was achieved within 17 days for 50 mM NH_3, while more than 100 days were required at 4 °C with over 1000 mM NH_3.

For the treatment with 0.05 or 0.15 % urea and the untreated controls, the pH of the treatments decreased during the experiment (*Figure 3*), which reduced the NH_3 concentration to very low levels. The decrease was more rapid at higher temperatures. A smaller pH decrease was observed for treatments with a higher urea dose, but the impact on NH_3 concentration was low. This was probably due to a higher buffer capacity caused by the ammonia and carbonate equilibriums for the treatments with higher urea doses. The mechanism of the pH decrease was not clear, but it could be due to enzymatic degradation of the organic faecal material into fatty acids and carbonate. For the treatments with 0.05 % urea or less, the rapid initial drop in pH was an indication of biological activity (*Figure 3*). The lower NH_3 limit for sanitisation of faecal sludge at high temperatures (>28 °C) seemed to be determined by the stability of the pH and not by the ability to inactivate *Ascaris*, as all the treatments with a stable pH also achieved a 3 $\log_{10}$ reduction of *Ascaris* within 40 days.

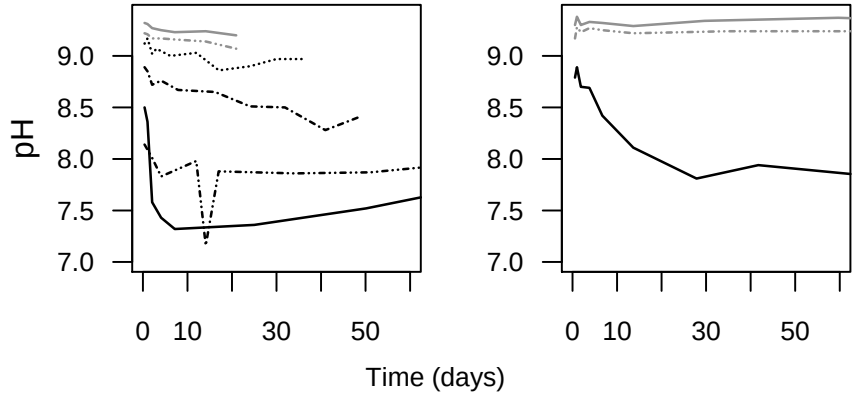


Figure 3. Development of pH at 28 °C for different urea doses: 1.5 % (grey solid line), 0.75 % (grey dashed line), 0.4 % (dotted line), 0.15 % (dotted dashed line), 0.05 % (dashed line), untreated (solid line)

Table 9. Treatment of faecal sludge with urea (U) or ammonia solution (A), time required for 3 log_{10} reduction in *Ascaris* egg viability, 5 log_{10} reduction of *Salmonella* Typhimurium viability, 3 log_{10} reduction of reovirus and adenovirus, plus pH, ammonia concentration (NH_3, mM) and carbonate activity (CO_3^{2-}, mM)

Treatment	Temp.	*Ascaris*, 3 log_{10} (days)	*Salmonella*, 5 log_{10} (days)	Reovirus, 3 log_{10} (days)	Adenovirus, 3 log_{10} (days)	pH	NH_3 (mM)	CO_3^{2-} (mM)
10 % A	32 °C	3	<0.15	ND	ND	11.2	915	4
0.4 % U	32 °C	17	1.6	17	9	9.0	50	2
0.15 % U	32 °C	NR	80	27	27	8.6	13	0.5
0.05 % U	32 °C	NR	NR	ND	ND	7.9	2	0.1
NT	32 °C	NR	NR	58	168	7.7	1	0.02
1.5 % U	28 °C	15	1.0	12	6	9.3	196	8
0.75 % U	28 °C	25	1.8	21	12	9.2	92	4
0.4 % U	28 °C	39	2.8	33	34	9.0	39	2
0.15 % U	28 °C	NR	283	33	120	8.6	12	0.5
0.05 % U	28 °C	NR	NR	ND	ND	7.9	1	0.0
NT	28 °C	NR	NR	197	122	7.7	1	0.03
10 % A	23 °C	6	<0.6	ND	ND	11.3	1,205	4
1.5 % U	23 °C	42	1.2	22	15	9.3	171	9
NT	23 °C	NR	NR	453	453	8.1	1	0.05
3 % A	17 °C	62	1.1	ND	ND	10.7	355	2
1.5 % U	17 °C	108	1.2	49	28	9.3	149	8
0.75 % U	17 °C	181	1.9	72	43	9.2	62	4
NT	17 °C	NR	NR	214	641	8.3	1	0.08
10 % A	10 °C	75	1.5	ND	ND	11.3	1,108	3
5 % A	10 °C	112	2.9	ND	ND	11.0	571	3
1.5 % U	10 °C	NR	2.7	180	55	9.3	94	7
NT	10 °C	NR	15	811	406	8.2	1	0.07
10 % A	4 °C	111	1.6	ND	ND	11.3	1,064	3
5 % A	4 °C	152	5.1	ND	ND	11.0	620	2
1.5 % U	4 °C	NR	2.4	182	43	9.4	71	7
NT	4 °C	NR	20	2,421	NR	8.6	1	0.07

NR: No reduction of viability observed during experiment
ND: Pathogen/treatment combination not done

6.3 Pathogen inactivation

6.3.1 *Salmonella* inactivation

Salmonella spp. inactivation was more rapid at higher concentrations of ammonia and carbonate and a higher pH. However, the correlation between pH and inactivation rate k was weaker compared with the correlations between ammonia and carbonate concentrations and k (*Figure 4*). More importantly, controls at pH 9.4 revealed that pH levels up to 9.4 did not show a direct inactivation effect on *Salmonella* spp. (*Figure 4*).

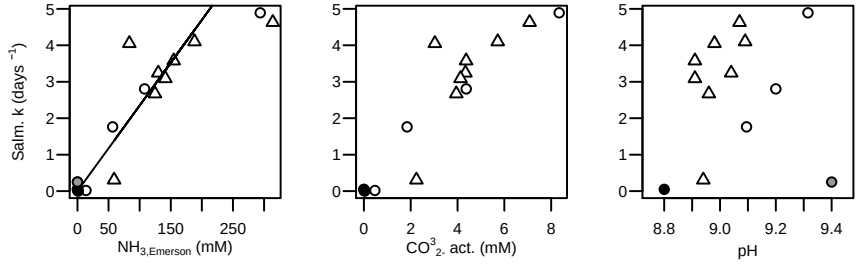


Figure 4. Inactivation rate *k* of *Salmonella* at 28 °C as a function of $NH_{3,Emerson}$ concentration, CO_3^{2-} activity and pH. Treatment with urine: Δ, with urea: O. Filled points indicate controls (ammonia and carbonate free buffers) at pH 9 (black) and 9.4 (grey). Line indicates predicted values by inactivation model reported by Nordin (2010)

At 28 °C the inactivation observed was almost identical with the inactivation predicted by a model of *Salmonella* spp. inactivation as a function of NH_3 concentration and temperature (*Figure 4*) (Nordin, 2010). However, at lower temperatures (4-17 °C) the inactivation was observed to be more rapid than that predicted by the model by a factor of ~2.

At the low temperatures of 4 and 10 °C, the inactivation resulting from the addition of 5 % ammonia solution was lower than the inactivation observed for the addition of 1.5 % urea, despite having a far higher ammonia concentration and pH of 11 (*Table 9*). As the urea-treated sample had a higher carbonate concentration than the treatment with ammonia solution, this strongly indicated that carbonate was more important than ammonia for *Salmonella* spp. inactivation at low temperatures. At 17 °C, treatment with 3 % ammonia (350 mM NH_3) had a similar inactivation as the treatment with 1.5 % urea (150 mM NH_3), showing that the impact of NH_3 increased relative to CO_3^{2-} at higher temperatures, but was still much lower than the effect of CO_3^{2-} at 17 °C. A study by Park and Diez-Gonzalez (2003) reported that equal concentrations of CO_3^{2-} and NH_3 have a similar inactivating effect on *Salmonella* Typhimurium at 37 °C. As the resulting concentration of NH_3 is several times higher than the

concentration of CO_3^{2-} when using urine or urea as the source of ammonia, this indicates that NH_3 is more important than CO_3^{2-} at high temperatures.

In the study of pathogen inactivation using urine as the ammonia source, the viability of *Salmonella* spp. was monitored by enrichment after falling below the detection limit for plate spreading. By doing this, it was possible to follow a ~7.5 $\log_{10}$ inactivation. The results from the enrichment test showed that the inactivation observed by plate spreading could be extrapolated with a good fit, and did not show any sign of tailing or prolonged survival of subpopulations.

Nordin (2010) reports that four strains of *Salmonella* were inactivated at the same rate when exposed to the same temperature and NH_3 concentration. This indicates that *Salmonella* Typhimurium can serve as a model for *Salmonella* species in general.

6.3.2 Enterococci inactivation

The inactivation of *Enterococcus faecalis* increased with higher pH and higher concentrations of ammonia (*Figure 5*) and carbonate. However, as these variables were correlated, it was not possible to separate out their effect. Preliminary results indicated that inactivation at high temperatures was due to both CO_3^{2-} and NH_3, while CO_3^{2-} was most important at low temperatures (data not shown).

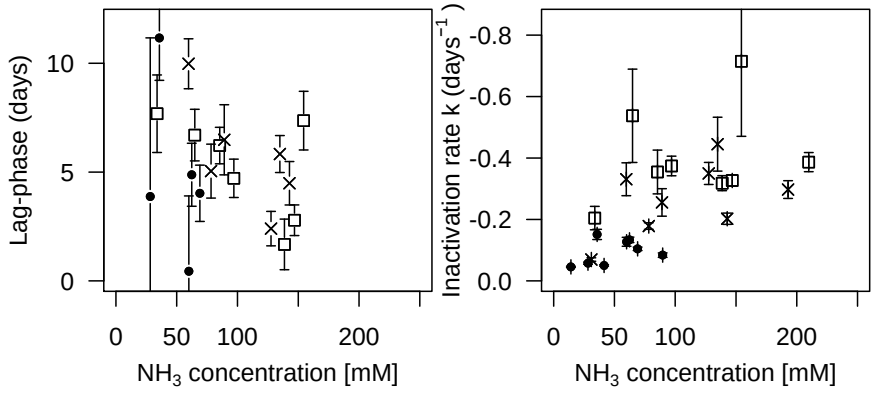


Figure 5. Enterococcus faecalis lag phase and inactivation rate as a function of $NH_{3,Emerson}$ concentration at (mM) at 28 °C (□), 26 °C (x) and 14 °C (●).

In some treatments, the concentration of enterococci increased at the end of the experiment. This was probably due to regrowth of other *Enterococcus* species, as different species of enterococci have shown different persistence during treatment and in the environment. Son Thi Thanh *et al.* (2011) showed that

enterococci species of non-faecal origin could grow at NH_3 concentrations that inactivated *Enterococcus faecalis*. This makes enterococci less applicable as an indicator, as biochemical tests are required to differentiate enterococci species.

6.3.3 Virus inactivation

Adenovirus and reovirus were inactivated at approximately the same speed in most treatments. The inactivation was generally faster at higher temperatures and higher ammonia concentrations (*Figure 6*). However, the concentration of ammonia was correlated to both pH and carbonate concentration, making the inactivating factor difficult to identify. As the inactivation in the ammonia-free buffer of pH 9 was faster than in the buffer of pH 7, it was clear that high pH had an effect, even in the absence of ammonia. The inactivation observed was therefore most likely due to both pH and ammonia content. A similar observation has previously been observed in the case of reovirus by Ward and Ashley (1977), who also found a higher inactivation at pH 9.5 compared with neutral pH, and an even higher inactivation with 287 mM NH_3 at pH 9.5 compared with the ammonia-free buffer at the same pH. A recent study showed that carbonate and to some extent other bases also inactivate MS2 (Decrey *et al.*, 2015), and it is possible that some of the inactivation observed in Paper III was also due to carbonate. According to the Bronsted catalysis law, is the effect of CO_3^{2-} higher per mM than NH_3 as CO_3^{2-} has a higher pka value (Decrey *et al.*, 2015), but on the other hand is the activity of carbonate much lower than the concentration of NH_3.

The temperature effect on virus inactivation was weak in the interval studied (4-33 °C), especially in the case of adenovirus. The inactivation rate for adenovirus in faecal sludge treated with 1.5 % urea was seven times higher at 28 °C compared with 5 °C, but much of the difference can be attributed to the NH_3 concentration being three times higher at 28 °C.

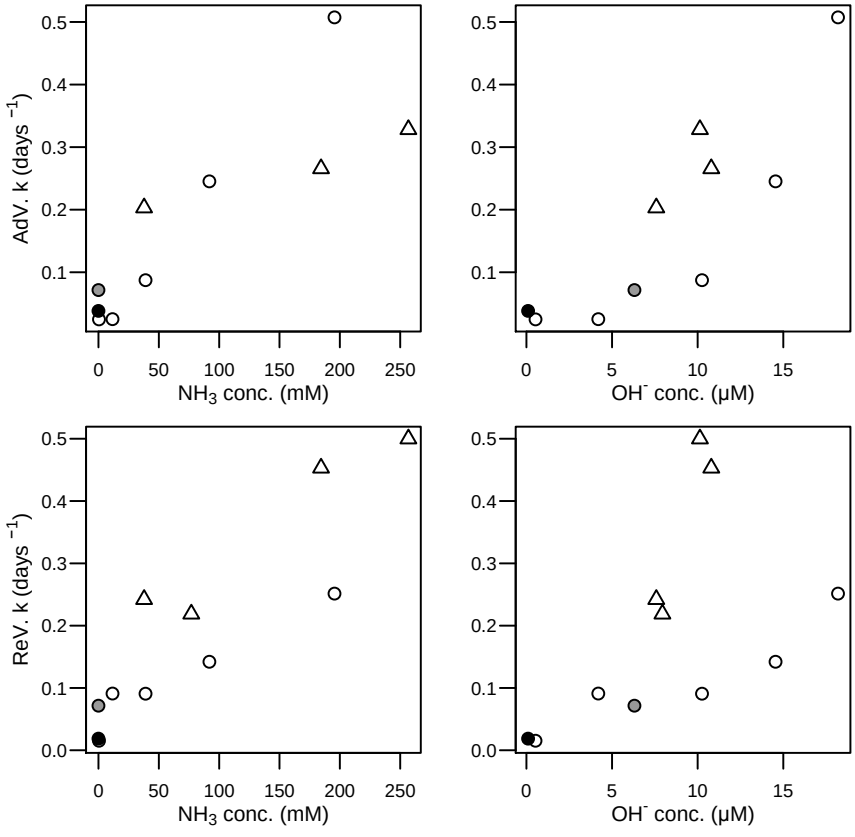


Figure 6. Inactivation rates of adenovirus (AdV) and reovirus (ReV) as a function of NH_3 concentration (mM) or OH^- (µm) concentration at 28 °C. Treatment with urine: Δ, with urea: O. Filled dots indicate controls (ammonia and carbonate free buffers) at pH 7 (grey) and pH 9 (black).

6.3.4 Bacteriophage inactivation

The inactivation rate of bacteriophages rose with increasing concentrations of ammonia, but also in this case it was difficult to distinguish the effect of ammonia from those of pH and carbonate (*Table 7* & *Table 8*).

The inactivation of bacteriophages was slower than that of viruses, especially at low temperatures. At lower temperatures the inactivation rate was also less correlated to physiochemical parameters such as pH and NH_3. As the influence on physiochemical parameters was different for viruses and bacteriophages, the value of bacteriophages as indicators is limited. However, they may serve as very conservative treatment indicators.

The effect of NH_3 on the inactivation rate k was estimated by linear regression for the data in Paper III and other published data. However, as NH_3

and pH are correlated in most studies, this should be interpreted as a combined effect of NH_3 and pH and possibly other bases. The treatment effect on MS2 inactivation was similar to that observed in previous studies on urine, but about 100 times lower than studies performed on hatchery waste and buffers (*Table 10*). The inactivation of reovirus type 3 reported by Ward and Ashley (1977) was also around ten times more rapid than in the present study. This may indicate that some components in faeces and urine act as a protective matrix for bacteriophages and viruses, and that inactivation studies performed in buffers may overestimate virus inactivation by ammonia in faecal sludge. As the concentration of the dry matter content of sludge is greater at the bottom of storage tanks, the effect of the protective matrix may be larger in full-scale treatments compared to laboratory-scale experiments. Future research should investigate this further.

Table 10. *Treatment effect on MS2 inactivation in different matrices*

Matrix	Temperature (°C)	Treatment effect (day^{-1} mM $NH_3{}^{-1}$)	Reference
Faecal sludge	28	$3.0x10^{-4}$	Paper III
	26	$3.0x10^{-4}$	
	14	$-2.0x10^{-4}$	
Buffer	28	$1.6x10^{-2}$	Decrey *et al.*, 2015
	20	$1.3x10^{-2}$	
Hatchery waste	25	$4.8x10^{-2}$	Emmoth *et al.*, 2011
	14	$1.6x10^{-2}$	
Urine	24	$3.9x10^{-4}$	Vinnerås *et al.*, 2008
	14	$8.0x10^{-5}$	

6.3.5 *Ascaris* egg inactivation model

The inactivation of *Ascaris* eggs was generally much slower than the other microorganisms studied. This was especially true for the low temperatures studied, as *Ascaris* egg inactivation showed a much higher temperature dependency than bacteria and viruses.

Ascaris eggs were unaffected by pH 12, showing that pH was not an inactivation factor. The study on inactivation in carbonate and ammonia buffers (Paper II) suggests that carbonate does have an inactivating effect on *Ascaris* eggs, but that the effect of carbonate is low compared to the effect of ammonia. It was therefore hypothesised that the inactivation of *Ascaris* eggs could be modelled as a function of NH_3 concentration and temperature.

The *Ascaris* inactivation can be described by eq. 6, where k is the inactivation rate and n determines the lag phase. The aim of the modelling below was to generalise the values of n and k in order to predict the inactivation of *Ascaris* eggs at varying ammonia concentrations and temperatures. First, the values of the parameters n and k from Papers I and II and two more studies by Pecson *et al.* (2005) and Nordin *et al.* (2009a) and their relation to NH_3 and temperature were investigated. The parameter n did not show any significant correlation to either temperature or NH_3 concentration. It was therefore initially hypothesised that the parameter n is a constant. The inactivation rate k increased with increasing NH_3 concentrations (*Figure 7*). The slopes of the regression lines, V_t, which represent the inactivating effect of ammonia, were steeper at higher temperatures. Plotting the logarithm of the slopes (V_t) against temperature revealed a linear relationship in the temperature range 5-42 °C, while data from higher temperatures deviated from this linear relationship (*Figure 8*).

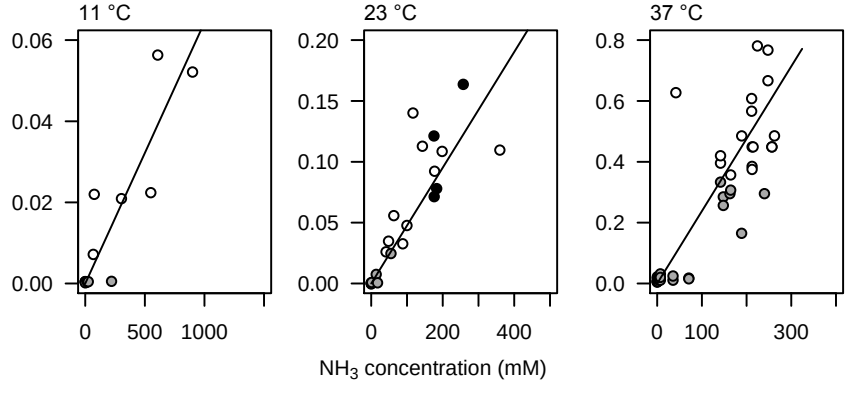


Figure 7. Ascaris inactivation rate k as a function of NH_3 concentration for selected temperatures. Grey dots indicate that inactivation did not reach the exponential phase and that the inactivation rate k may have been underestimated. Black dots indicate that frequency of sampling was not optimal for detecting the lag phase and exponential phase inactivation, and thus the inactivation rate may have been underestimated.

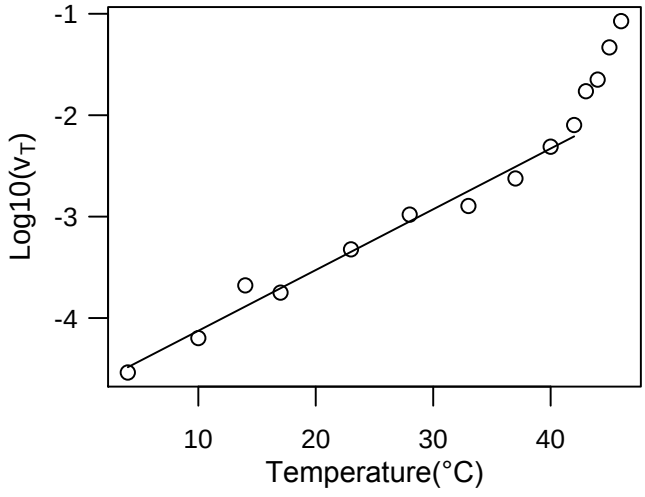


Figure 8. The logarithm of the slope V_t as a function of temperature

Based on the regression line from *Figure 8*, the inactivation rate k could therefore be described as eq. 8, where T is the temperature in °C:

$$k\left(T, \mathrm{NH_3}\right) = 10^{(-4.8+0.062T)} \cdot NH_3 \qquad (8)$$

The *Ascaris* egg inactivation could be predicted based on eq. 8 and eq. 6, assuming that parameter n in eq. 6 is constant. However, in order to refine the model parameters and investigate the effect of other physiochemical parameters, further modelling was undertaken. In this step, the intention was to model each individual *Ascaris* viability measurement.

Combining the assumption of n being constant, the expression of k from eq. 8 and eq. 6 solved for treatment time (t), this yielded eq. 9, hereafter called the linear model:

$$t = \frac{\log\left(1-10^{\frac{\log(1-\frac{N}{N_0})}{10^n}}\right)}{-k} = \frac{\log\left(1-10^{\frac{\log(1-\frac{N}{N_0})}{10^n}}\right)}{-10^{A+BT}\cdot NH_3} \quad (9)$$

The values of the parameters A, B and n in eq. 9 were fitted to the data using non-linear regression. However, plotting the residuals of the regression *versus* NH_3 concentration revealed a strong correlation (*Figure 9*). This indicated that the effect of ammonia on pathogen inactivation was not linear. By evaluating several mathematical models for the non-linear effect of NH_3, it was found that the power function gave a best fit to the data, where the inactivation rate k was a function of the NH_3 concentration to the power of parameter C, and the best fit value for the parameter C was 0.7.

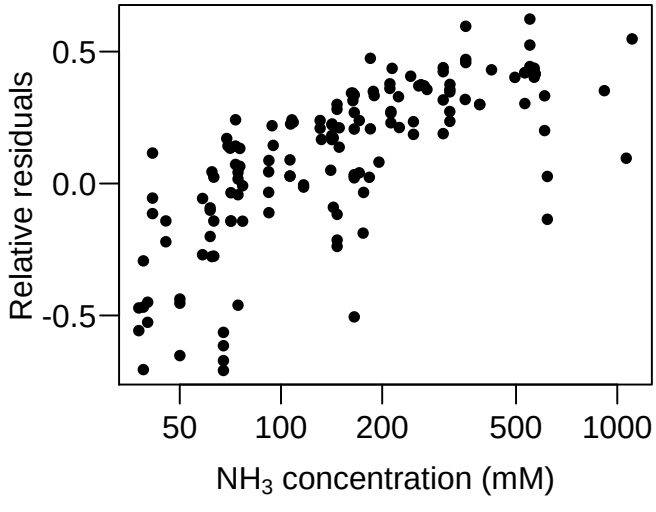


Figure 9. Relative residuals for the regression of eq. 9 *vs.* NH_3 concentration

Several other models were evaluated, including models where the parameters n and k were functions of DM content, matrix, CO_3^{2-} activity and pH, and models where the parameter n was a function of NH_3 or temperature (*Table 11*). The models were compared based on their model fit (LOORMSRE) where a lower value indicates a better fit, and the significance of the added parameter D.

Table 11. *Models of k (temperature effect excluded) and n evaluated and the resulting model fit measured as leave one out root of mean squared relative error (LOORMSRE), and the value and significance P_D of the parameter D*

Model	K	n	D	Model fit	P_D
Linear	NH_3	n	-	0.326	-
Power	NH_3^C	n	-	0.217	-
N(temp)	NH_3^C	n1+D*temp	0.09	0.217	0.0004
n(NH_3)	NH_3^C	n1+D*NH_3	-0.003	0.207	<0.0001
n(10^temp)	NH_3^C	10^(n1+D*temp)	0.01	0.215	0.03
n(pH)	NH_3^C	n1+D*pH	-0.08	0.218	0.4
n(CO_3^{2-})	NH_3^C	n1+D*CO_3^{2-}	-0.08	0.216	0.03
n(faeces)	NH_3^C	n1+D*faeces	-0.7	0.215	0.01
n(DM)	NH_3^C	n1+D*DM	-4	0.216	0.01
k(NH_3 * pH)	NH_3^C*(1+pH*D)	n	0.02	0.218	0.4
k(NH_3 + CO_3^{2-})	NH_3^C+D*CO3	n	1.0	0.219	0.09
k(NH_3 * faeces)	NH_3^C*(1+D*faeces)	n	0.2	0.216	0.01
k(NH_3 * DM)	NH_3^C*(1+D*DM)	n	1.1	0.217	0.01

Both temperature and NH_3 concentration had a significant impact on the parameter n. However, higher NH_3 concentrations were studied for lower temperatures, making NH_3 and temperature correlated variables. It was therefore not possible to identify which of the two factors impacted the parameter n. However, the improvement of model fit by having n as a function of NH_3 or temperature was minimal. DM content and a matrix effect of faeces also had a significant impact on both n and k, but the improvement in model fit was also very low in this case. This indicated that effect of matrix on ammonia inactivation of *Ascaris* egg is low, as the dataset contained *Ascaris* egg inactivation done in buffers, urine, faeces and faecal sludge.

Therefore, the model expressing k as a factor of temperature and a power function of NH_3 and keeping n as a constant was found to be the most useful model for predicting *Ascaris* egg inactivation. The treatment time required for a desired $\log_{10}$ reduction of *Ascaris* egg viability (LRV) could be estimated by

eq. 10, where T is the temperature in °C and $NH_{3,Pitzer}$ is the ammonia concentration in mM estimated by the Pitzer approach.

$$t = \frac{3.2 + LRV}{10^{-3.7+0.062 \cdot T} \cdot NH_{3,Pitzer}^{0.7}} \cdot 1.13 \quad (10)$$

The inverse of the parameter 0.062 is 16.1, which means that for every 16 °C increase in storage temperature, the treatment time required for a given $\log_{10}$ reduction (LRV) is reduced ten-fold. This showed that using solar heating, for example, to increase the storage tank temperature could reduce the storage time greatly.

The model gave a good fit when it was used to predict other published *Ascaris* egg inactivation data based on NH_3 concentration and temperature (*Figure 10*). The main limitation was primarily due to uncertainty in the concentration of NH_3, for example due to varying pH (*Figure 10*a). It was of particular interest to compare the prediction with the observed inactivation of *Ascaris lumbricoides* eggs that infect humans, as the model is based on *Ascaris suum* eggs that mainly infect pigs. The predicted inactivation of *Ascaris lumbricoides* eggs also fits reasonable well with the observed inactivation, but had a more rapid inactivation initially (*Figure 10*c). This could have been due to exposure to ammonia prior to the start of the experiment.

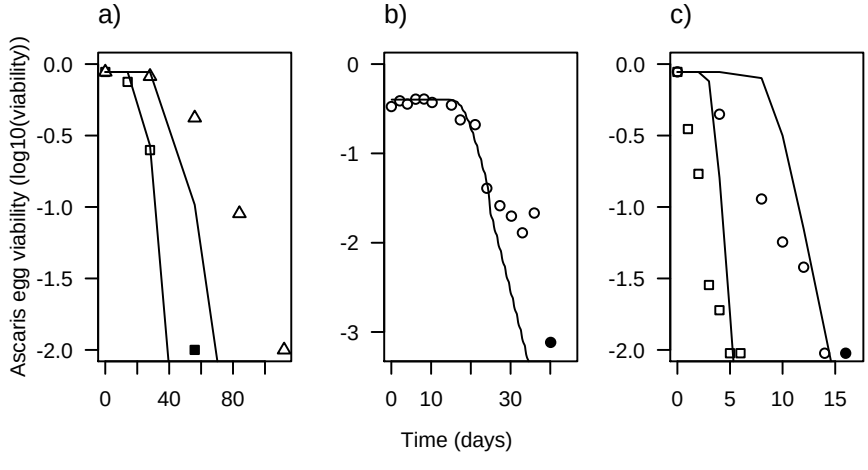


Figure 10. Reported viability (dots) and viability estimated by the model (lines). a) McKinley *et al.* (2012): matrix 4 (faeces + stored urine + ash) (□); matrix 6 (faeces + fresh urine + ash) (□). b) Nordin *et al.* (2013): solar-heated urine (O). c) Pecson *et al.* (2007): sludge + 1 g TAN/L (O), sludge + 5 g TAN/L (□). Filled points indicate zero viable eggs found (detection limit).

Ascaris is shown to be more persistent than eggs from *Taenia* spp., *Trichuris muris* and hookworm (Nordin *et al.*, submitted; Ghiglietti *et al.*, 1995), and inactivation of *Ascaris* eggs therefore indicates that other helminth eggs are inactivated to. There have been no studies on the effect of NH_3 on eggs of other genera, but a study by Maya *et al.* (2010) compared the inactivation of *Ascaris* spp., *Toxocara canis*, *Trichuris trichuria*, *Taenia solium* and *Hymenolepis nana* eggs by temperature and lime in sludge. It was found that *Ascaris* spp. was more persistent than the others, although only a small difference was found compared to *Toxocara canis* and *Taenia solium* eggs. It therefore seems unlikely that other parasite genera are more persistent to NH_3 than *Ascaris* spp.

The value of parameter n of 3.2 means that the lag phase lasts for the same amount of time as 3 $\log_{10}$ reduction of viability in the linear phase. Due to the relatively long lag phase, an increase from 3 to 5 $\log_{10}$ reduction will have a small impact on the treatment time (32 % longer).

6.4 Modelling pH and $NH_{3,Pitzer}$

6.4.1 Modelling pH as a function of DM and TAN

The pH of the different treatments of faecal sludge was found to be dependent on the amount of dry matter and concentration of TAN. Whether the ammonia came from urine or added urea did not affect the pH, but ammonia solution had a far higher pH-increasing effect as it does not contain carbonate which buffers the pH. After excluding treatments with a large decrease in pH, *i.e.* treatments in *Table 9* with 0.15 % urea or less, it was found that for the treatments with urine and urea as source of ammonia, the pH could be modelled by linear regression as a function of TAN and DM content according to eq. 11. TAN here is the concentration of total ammonia nitrogen given as g/L N, and DM content is given as a number between 0 and 1 (*e.g.* 5 % = 0.05). It should be noted that the model is not valid outside the ranges of DM (0.2 – 5 %) and TAN (1-13 g/L) used in the regression.

$$pH = 8.3 + 0.38 \cdot \log_{10} TAN - 0.26 \cdot \log_{10} DM \qquad (11)$$

While eq. 11 can be useful for predicting pH in faecal sludge, it should be remembered that for treatments with low NH_3 concentrations, there is a risk that biological activity may cause a drop in pH. The exact NH_3 concentration

threshold to avoid this is not known, but based on the initial NH_3 concentrations in Paper II it is somewhere between 5 and 20 mM.

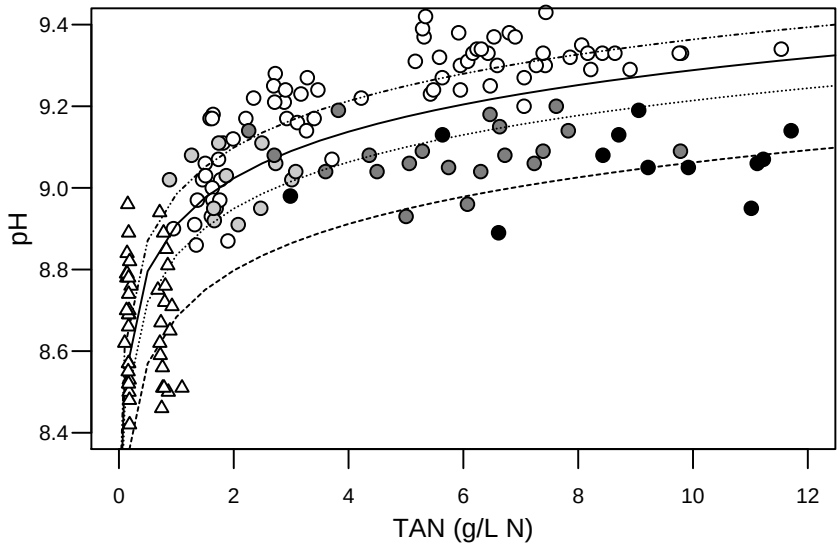


Figure 11. Faecal sludge pH plotted *vs.* TAN concentration. Lines indicate predicted values by eq. 11 for different TS levels: 0.2 % (long-dashed line, white points), 0.4 % (solid line, light grey points), 0.7-1.2 % (dotted line, dark grey points), 3-5 % (dashed line, black points). White triangles are treatments with decreasing pH not included in the model

6.4.2 Simplified estimation of $NH_{3,Pitzer}$

It is rather complicated to estimate the concentration of NH_3 with the Pitzer approach, as it requires software, a Pitzer parameter database and estimated concentrations of several ions. Therefore, a set of equations was developed to simplify the estimation. First, the $NH_{3,Pitzer}$ concentration was estimated for a large number of hypothetical faecal sludge batches with a range of values for pH (8.3-9.5), temperature (5-45 °C) DM matter (0-20 %) and NH_{TOT} (5-2000 mM). The concentrations of ions were estimated based on the concentration of ions in urine and faeces (details shown in Paper IV). Non-linear modelling was then used to predict the $NH_{3,Pitzer}$ as a function of DM content and NH_{TOT} concentration and the Emerson equation. It was found that the concentration of $NH_{3,Pitzer}$ could be estimated by eqs. 12 to 15, including the Emerson approach (eqs. 2 & 3):

$$NH_{3,Pitzer} = NH_{Tot} \cdot f_{Pitzer} \qquad (12)$$

$$f_{Pitzer} = f_{Emerson}\left(\alpha + (1-\alpha)\cdot f_{Emerson}{}^{\beta}\right) \quad (13)$$

$$\alpha = 0.84 - 0.012\sqrt{NH_{Tot} + 2000\cdot DM} \quad (14)$$

$$\beta = 0.89 + 0.049\sqrt{NH_{Tot} + 870\cdot DM} \quad (15)$$

where NH_{TOT} is the total ammonia concentration in mM and DM is the dry matter content (unitless, number between 0 and 1).

This resulted in an acceptable fit, giving conservative values compared to the values estimated by software (*Figure 12*). The NH_3 concentrations estimated by eqs. 12-15 were between 0 and 46 % lower than the NH_3 concentration estimated by software. The difference was less than 25 % for 90 % of the simulated dataset. Equations 12-15 might therefore be useful for predicting the $NH_{3,Pitzer}$ when the full Pitzer approach cannot be applied. The equations can be applied for faecal sludge where the source of ammonia is mineral fertiliser, urea or urine. It should be noted that the approach shown here will not give a correct result for pH values outside the pH range of 8.3-9.5 or if additives of ash or lime are used.

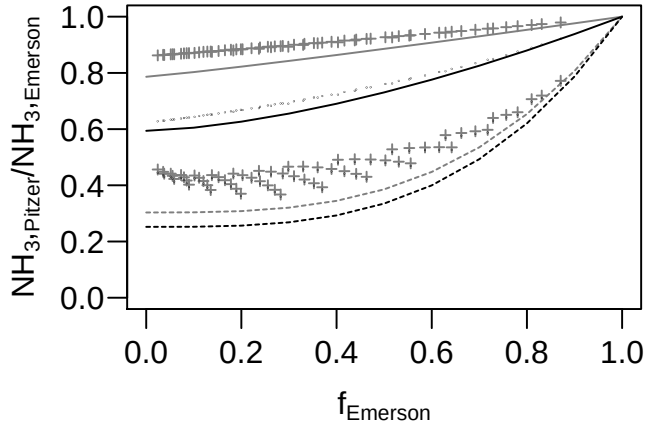


Figure 12. The ratio between $NH_{3,Pitzer}$ and $NH_{3,Emerson}$ as a function of $f_{Emerson}$ as estimated by full Pitzer approach (dots) and predicted values by eq. 12-15 (lines). NH_{TOT}:20 mM (solid), 2000 mM (dashed). DM content: 20 % (black lines, dots), 0 % (grey lines, +)

6.5 Quantitative microbial risk assessment

6.5.1 Hazard identification

Ascaris lumbricoides was identified as an important pathogen due to its persistence during ammonia sanitisation and high prevalence in low and mid-income countries. Rotavirus and adenovirus 40 & 41 have low infectious doses and are major causes of childhood diarrhoea (Kotloff *et al.*, 2013; Crabtree *et al.*, 1997). Their model organisms reovirus type 3 and canine adenovirus type 1 were equally persistent during ammonia sanitisation (Magri *et al.*, 2013), and have also been shown to be an important pathogen responsible for diarrhoea. However, due to its impact on public health, rotavirus was selected as an indicator of viral risk.

Gram-negative bacteria such as *Salmonella* spp., *Shigella* spp. and ETEC are also important causes of diarrhoea, but all gram-negative bacteria studied have been inactivated rapidly by ammonia and carbonate (Nordin, 2010). At 23 °C the treatment required for 1 $\log_{10}$ inactivation of *Ascaris* egg viability will yield a >40 $\log_{10}$ inactivation of *Salmonella* spp. Gram-negative bacteria were therefore not included in this risk assessment.

The ammonia inactivation of *Cryptosporidium* in urine and buffers is also rapid compared with *Ascaris* eggs (Höglund & Stenstrom, 1999; Jenkins *et al.*, 1998). At 23 °C the treatment required for 1 $\log_{10}$ inactivation of *Ascaris* egg viability will yield a >25 $\log_{10}$ inactivation of *Cryptosporidium parvum*. However, recent research on *Cryptosporidium* spp. in sludge indicates that the ammonia inactivation may be significantly slower in slurry, possibly due to a matrix effect (Petersen *et al.*, manuscript). The inactivation in faecal sludge may therefore be slower than for the studies involving urine and buffers.

6.5.2 Exposure assessment and dose-response assessment

The exposure to rotavirus and *Ascaris* eggs associated with the consumption of carrots and leaf crops grown in soil fertilised with ammonia-sanitised faecal sludge was estimated based on literature data on pathogen concentration in faecal sludge, attachment of pathogens to the crops, pathogen inactivation during growth, reduction of pathogen concentration by produce washing, and vegetable consumption quantities. Furthermore, the infection risk and disease outcome associated with ingestion of pathogens were estimated by dose-response models, infection-disease ratio and DALY losses per case, all from literature. A detailed description of this can be found in Paper V.

The health risk was estimated for two different types of faecal sludge in addition to urea-treated source-separated faeces. The exposure was estimated for several degrees of *Ascaris* egg and rotavirus inactivation in order to

estimate the health risk dependent on the degree of pathogen inactivation. Furthermore, for each $\log_{10}$ reduction of *Ascaris* eggs, the equivalent rotavirus inactivation was estimated for treatment at 23 and 28 °C (*Table 12*). This was based on the ammonia inactivation of reovirus (Paper III) and the *Ascaris* inactivation model (Paper V).

Table 12. *Equivalent inactivation of* Ascaris *eggs and reovirus by ammonia at 23 and 28 °C*

Ascaris $\log_{10}$ red.	Reovirus $\log_{10}$ red.	
	23 °C	28 °C
1	6	3.5
2	7.5	4
3	9	5
4	10.5	6
5	12	7
6	13.5	8

6.5.3 Risk Characterisation

The consumption of leaf crops had a higher estimated risk than the consumption of carrots. In the case of leaf crops, a 4-5 $\log_{10}$ reduction of *Ascaris* egg viability was required to achieve an acceptable risk (*Table 13*), defined as lower than 10^{-6} DALYs per person per year. Viruses required a much higher pathogen inactivation, a 7-8 $\log_{10}$ reduction, depending on which sludge was used.

A higher degree of treatment was required for urea-treated faeces compared with the faecal sludge. The reason for the different $\log_{10}$ reduction requirements was primarily the different dry matter content. Dilution with water gives a lower risk per person, but is not an improvement from a public health perspective as the pathogens will be spread over a larger area, causing approximately the same number of infections.

It should be noted that the uncertainty was high for the estimation of virus risk. It is possible that the models for virus survival in crops and soil overestimate the surviving fraction, which in turn causes the risk to be overestimated. The estimated virus risk should therefore be interpreted as the potential virus risk, as this has not been studied in any depth. While the *Ascaris* infection risk has also been studied in epidemiological studies, there is limited evidence on the health risk associated with pre-harvest contamination of crops (Blumenthal & Peasey, 2002; Hernandez *et al.*, 1997).

Table 13. *Required log_{10} reductions of* Ascaris *egg viability and rotavirus depending on treatment system. Upper 95 % confidence interval given in brackets.*

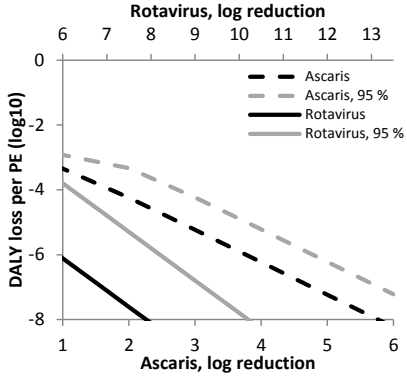
	DM content (%)	*Ascaris* (log_{10})		Rotavirus (log_{10})	
		Carrots	Leaf crops	Carrots	Leaf crops
Urea treated faeces	25 %	4.4 (5.5)	5.0 (6.0)	6.6 (8.9)	7.8 (10.1)
FS from vacuum toilet	4 %	3.9 (5.0)	4.5 (5.5)	6.1 (8.4)	7.3 (9.6)
FS; urea treated	0.7 %	3.7 (4.7)	4.2 (5.2)	5.8 (8.1)	7.0 (9.3)

The median sludge concentration of *Ascaris* eggs in the sludge after 3 log_{10} reduction of *Ascaris* eggs was 0.6 eggs/g TS. This is acceptable according to WHO guidelines for reuse of human excreta (WHO, 2006), while a 4 log_{10} reduction is required to achieve class A biosolids according to the US EPA, which requires > 1 egg/4 g TS. This supports previous risk assessments, which also suggests that the WHO guidelines may be too relaxed in the case of growing salad crops (Navarro & Jimenez, 2011).

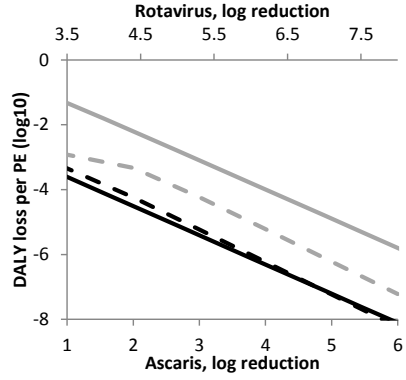
The acceptable risk of 10^{-6} DALYs per person per year has been criticised as being too strict for low- and mid-income countries, and it is claimed that it is stricter than that being applied even in high-income countries. Furthermore, at *Ascaris* concentrations equivalent with 3-5 log_{10} reductions, the disease outcome due to fertilisation with faecal sludge is low compared with other types of *Ascaris* transmission pathways such as lack of handwashing and post-harvest contamination of food. On the other hand, the disease outcome per intestinal worm is probably higher for individuals with an already high worm burden compared with healthy uninfected individuals, due to the risk of intestinal obstruction (Aydin *et al.*, 2014).

Comparing the DALY loss due to rotavirus infection with *Ascaris* infection shows that *Ascaris* represented the largest health burden after treatment at 23 °C, while rotavirus represented the largest health burden at 28 °C for both carrots and leaf vegetables (*Figure 13*). This was due to the different impact of temperature on pathogen inactivation; the effect of temperature on virus inactivation was low, while the effect on *Ascaris* inactivation was high. The equivalent virus inactivation was therefore much higher at 23 °C than at 28 °C (*Table 12*).

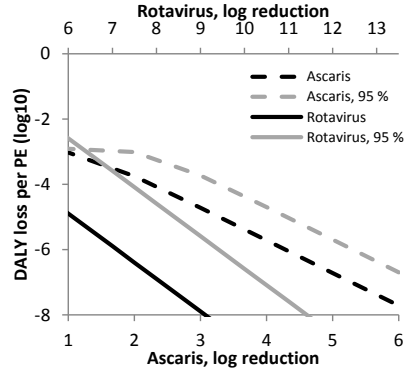
23 °C, carrots:



28 °C, carrots:



23 °C, leaf crops :



28 °C, leaf crops:

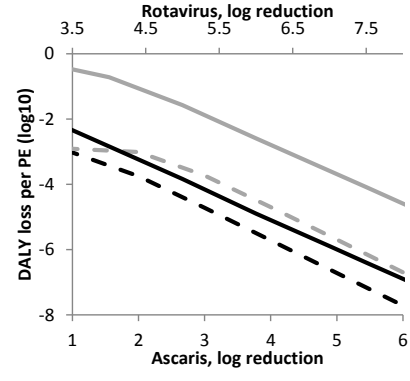


Figure 13. Comparison of health risks from rotavirus and *Ascaris* as DALY loss per person per year for consumption of carrots and leaf crops for ammonia treatment at 23 °C and 28 °C. Health risk is estimated based on treatment scenario 3: urea treatment of faecal sludge. Dashed line indicates DALY losses due to *Ascaris* infection and solid line indicates DALY losses due to rotavirus. Grey lines indicate upper 95 % confidence limit.

6.6 Evaluation of treatment systems

The TAN concentration, pH and NH_3 concentration for sludge from different types of toilets, as well as different urea doses, were estimated by applying the assumed characteristics of excreta and flushwater volumes listed in *Table 6*, the pH model previously described (eq. 11), and the Pitzer approach. Furthermore, the required treatment time for a 4 $\log_{10}$ reduction of *Ascaris* egg viability was estimated using the *Ascaris* inactivation model (eq. 10).

The difference between the maximum and minimum NH_3 concentrations was considerable (*Table 14*), which showed that it is difficult to predict physiochemical parameters of sludge based on toilet and treatment system alone. The location of the toilet, *i.e.* if it is a private toilet, public toilet or workplace toilet, will also greatly affect the composition. Furthermore, the time span before collection and treatment may have an impact, as anaerobic degradation may increase the buffer capacity due to production of carbonate and fatty acids, which may result in a lower final pH.

Table 14. *Estimated physiochemical properties of faecal sludge from different toilet technologies and with different urea addition, and the required storage time for 4 $\log_{10}$ reduction of* Ascaris *egg viability. Estimated minimum and maximum values in brackets.*

		NH_3 concentration (mM) at	Storage time for 4 $\log_{10}$ reductions of *Ascaris* egg viability (days)	
System	pH	23 °C	23 °C	28 °C
Urine + faeces	9.0 (8.8-9.2)	59 (20-304)	88 (188-28)	36 (76-12)
Vacuum, no urea	8.9 (8.7-9.1)	11 (3-65)	280 (711-82)	114 (286-34)
Pour flush, no urea	8.8 (8.7-9.1)	6 (2-42)	430 (922-111)	176 (371-46)
Low-flush, no urea	8.8 (8.7-9.1)	4 (2-14)	582 (945-239)	237 (380-100)
Vacuum, 0.25 % U	9.0 (9.0-9.2)	38 (27-95)	120 (154-63)	50 (63-26)
Pour flush, 0.25 % U	9.1 (9.0-9.2)	34 (27-74)	130 (153-75)	54 (63-32)
Low flush, 0.25 % U	9.1 (9.0-9.2)	33 (27-48)	132 (153-102)	55 (63-43)
Vacuum, 0.4 % U	9.1 (9.0-9.2)	55 (43-114)	93 (110-56)	39 (46-23)
Pour flush, 0.4 % U	9.2 (9.1-9.2)	65 (56-106)	82 (91-58)	34 (38-25)
Low flush, 0.4 % U	9.2 (9.1-9.3)	66 (56-85)	81 (91-68)	34 (38-29)

While the mixing of urine and faeces from UDDT toilets showed considerable potential for self-sanitisation using only the intrinsic ammonia, the estimation of intrinsic NH_3 in sludge from other toilets indicated that the addition of urea may be required to achieve sanitisation within a practical treatment time (<0.5

year). In addition, there is a risk of biological degradation causing a fall in pH in sludge from vacuum, pour-flush and low-flush toilets if urea is not added, as the treatments in Paper II with initial NH_3 concentrations below 20 mM experienced a fall in pH. The low intrinsic NH_3 concentrations in faecal sludge from toilets using water were mainly due to the high number of urinations, which were estimated to be four, six or eight times per day for the different scenarios.

The estimated storage capacity needed was obviously larger for systems using larger flushwater volumes (*Table 15*). This effect was due to both the larger volumes per day and the lower ammonia concentration resulting in longer storage times being required. The storage capacity requirement was greatly reduced by small additions of urea, due to the reduced treatment time.

The volumes of faecal sludge from the systems described here were large compared with sludge volumes from infiltration systems and soak pits. This increases the cost of treatment as well as the cost of transport. In soak pits and infiltration systems, most of the nitrogen is lost and may deteriorate the groundwater quality, and pathogens may also be transmitted to wells through groundwater, especially if the water table is high.

Table 15. *Estimated yearly sludge production per person and year, and storage capacity required to achieve 4 log_{10} reductions of* Ascaris *egg viability at 23 °C or 28 °C. Estimated maximum and minimum values in brackets.*

	Volume (m^3/p/year)	Storage capacity require for 4 log_{10} red. of *Ascaris* egg viability (m^3/p)	
		23 °C	28 °c
Urine + faeces	0.6 (1-0.2)	0.1 (0.6-0.02)	0.06 (0.3-0.008)
Vacuum, no urea	3 (8-1)	2 (15-0.3)	1.0 (6-0.1)
Pour flush, no urea	6 (11-2)	7 (28-0.6)	3 (11-0.3)
Low-flush, no urea	6 (9-5)	10 (22-3)	4 (9-1)
Vacuum, 0.25 % U	3 (8-1)	1 (3-0.2)	0.4 (1-0.10)
Pour flush, 0.25 % U	6 (11-2)	2 (5-0.4)	0.8 (2-0.2)
Low flush, 0.25 % U	6 (9-5)	2 (4-1)	1.0 (1-0.5)
Vacuum, 0.4 % U	3 (8-1)	0.8 (2-0.2)	0.3 (1.0-0.09)
Pour flush, 0.4 % U	6 (11-2)	1 (3-0.3)	0.5 (1-0.1)
Low flush, 0.4 % U	6 (9-5)	1 (2-0.9)	0.6 (0.9-0.4)

Large sludge volumes also increase fuel consumption for transportation. Recycling of nitrogen from human excreta may reduce the consumption of natural gas for production of mineral nitrogen fertiliser. However, if the nitrogen is diluted by large volumes of flushwater and the transport distance is long, the fuel used during transport can potentially be higher than the fuel

saved by recycling of nitrogen. In urban areas where the majority of the agricultural land is located outside of the city centres, long transports distances may be required to utilise all the nutrients in human excreta (Ushijima *et al.*, 2014). Addition of urea to faecal sludge increases the area needed for utilisation of the nitrogen in agriculture, which generates even longer transport distances.

A comparison of the amount of recycled ammonia nitrogen and the ammonia nitrogen added as urea shows that for even doses as low as 0.25 % urea, the amount of added ammonia nitrogen was greater than the recycled nitrogen (*Table 16*). The amount of added urea was greater for sludge from toilets using large volumes of flushwater.

The increased addition of urea also causes larger losses of nitrogen. Measurements of ammonia losses following urine application suggested that 1-10 % of already hydrolysed urea, *i.e.* ammonia, is lost after application, depending on the application method (Rodhe *et al.*, 2004).

Table 16. *Quantities of recycled intrinsic total nitrogen (TN) and total phosphorous (P) from urine and faeces compared with TAN added as urea. Estimated minimum and maximum values in brackets.*

System	TAN added kg N/p/yr	TN recycled kg N/p/yr	P recycled kg P/p/yr
Urine + faeces	0	2.5 (2.2-4.6)	0.4 (0.3-0.5)
Vacuum, no urea	0	2.5 (2.2-4.6)	0.4 (0.3-0.5)
Pour flush, no urea	0	2.5 (2.2-4.6)	0.4 (0.3-0.5)
Low-flush, no urea	0	2.5 (2.2-4.6)	0.4 (0.3-0.5)
Vacuum, 0.25 % U	4 (9-2)	2.5 (2.2-4.6)	0.4 (0.3-0.5)
Pour flush, 0.25 % U	7 (13-2)	2.5 (2.2-4.6)	0.4 (0.3-0.5)
Low flush, 0.25 % U	7 (10-5)	2.5 (2.2-4.6)	0.4 (0.3-0.5)
Vacuum, 0.4 % U	6 (14-2)	2.5 (2.2-4.6)	0.4 (0.3-0.5)
Pour flush, 0.4 % U	13 (26-5)	2.5 (2.2-4.6)	0.4 (0.3-0.5)
Low flush, 0.4 % U	15 (20-11)	2.5 (2.2-4.6)	0.4 (0.3-0.5)

The treatment system of mixing urine and faeces from UDDTs is better than the others in terms of economic and resource-use aspects. However, urine and faeces may also be treated separately by urine storage and addition of urea to faeces (Nordin *et al.*, 2009b; Vinnerås *et al.*, 2008). This requires a smaller storage capacity than the mixing of urine and faeces, as urine makes up most of the volume and requires a lower degree of pathogen inactivation when it is not mixed with faeces. However, from a resource perspective it may be more beneficial to use the urine as the source of ammonia.

In summary, it is clear that toilet systems using large volumes of water perform poorly in terms of economics and resources. The addition of low volumes of urea can improve pathogen inactivation and hence reduce the storage capacity required, but low-flush water volumes are still crucial to reduce transport and urea addition.

7 Concluding discussion

7.1 Treatment monitoring and verification

Microbiological sampling of sludge is generally expensive and time-consuming. Furthermore, there may be a considerable spatial variation in pathogen concentration within a batch of sludge, and one negative sample cannot guarantee that the level of pathogens is low throughout the material. In the case of ammonia sanitisation, it may therefore be a good option to ensure that the physiochemical parameters are sufficient for pathogen inactivation. However, as previously discussed, there may be a considerable variation in sludge characteristics, even for toilets using the same amount of flushwater. It is therefore recommended to measure the physiochemical parameters and use them to predict pathogen inactivation in the material.

For ammonia sanitisation, the most important parameters to be measured are temperature, pH and total ammonia nitrogen (TAN). The concentration of NH_3 can then be estimated based on these parameters using eq. 12 to 15, or with software using the Pitzer approach directly, which gives a better estimate. An option for direct measurement of NH_3 is to use ammonia electrodes. However, the maximum range for their application is usually far below what is required for pathogen inactivation. Dilution with water impacts the chemical equilibrium and cannot be used for direct measurement of NH_3 with electrodes.

An option for monitoring the NH_3 concentration is to use an acid trap with a pH indicator, connected to airspace inside the storage facility. The transmission of NH_3 from the sludge to the air and then to the acid trap depends on the NH_3 concentration in the sludge. Hence the buffer capacity can be adjusted so that the indicator changes colour after a sufficient combination of NH_3 concentration and time. As both pathogen inactivation and the transmission of NH_3 through air are temperature dependent, the buffer capacity of the acid trap needs to be adjusted based on the storage temperature.

The variation of physiochemical factors during the treatment period is also important to consider. The decrease in pH observed in Paper II may reduce the concentration of NH_3 significantly, and measurement of pH at the start of the process may overestimate the average NH_3 concentration. Furthermore, the time required for hydrolysis of urea, both intrinsic and added urea, may be considerable if the dry matter content is low, as the concentration of urease is also lower in that case. While the theoretical concentration of NH_{TOT} can be estimated stoichiometrically based on the urea dose, the hydrolysis should be confirmed by NH_{TOT} measurements.

7.2 Social acceptability and health impact of different toilets

The ranking of toilet and sanitation systems from an economic and an environmental perspective shows that systems using low amounts of flushwater are preferable. However, these aspects conflict with the common desire for a normal flush toilet. While some implementations of UDDTs show high user satisfaction and usage (Tumwebaze *et al.*, 2011), other studies of user satisfaction have shown that UDDTs are associated with smell, clogging and difficult maintenance, and that traditional pit latrines are sometimes preferred instead of installed UDDTs (Roma *et al.*, 2013). In areas where urine-diverting dry toilets (UDDT) have been installed, this has been regarded by some users as a preliminary solution while waiting to be able to afford a flush toilet (Roma *et al.*, 2013).

The progress in sanitation status is often described as climbing the sanitation ladder. There is no common standard for the sanitation ladder, but it is often described as the improvement from open defecation to pit latrine, pour-flush latrine and then a flush toilet as the best alternative (*Figure 14*). There is little epidemiological evidence showing that the flush toilet is better than a pour-flush toilet or a UDDT from a health perspective. However, misplacement of faeces in the urine chamber of UDDT and hence clogging of the urine pipes requires maintenance and represents a health risk (Stenström *et al.*, 2011). In the case of the pour-flush toilet, there is a risk that children may ingest water intended for flushing, which may cause infections, especially if untreated greywater is used for flushing. Poorly designed pour-flush toilets are also more prone to blocking, which may require more maintenance. In general, the health risks associated with UDDT and pour-flush toilets can be minimised by good design to reduce the frequency of blocking, good training in maintenance, and the use of clean water for flushing.

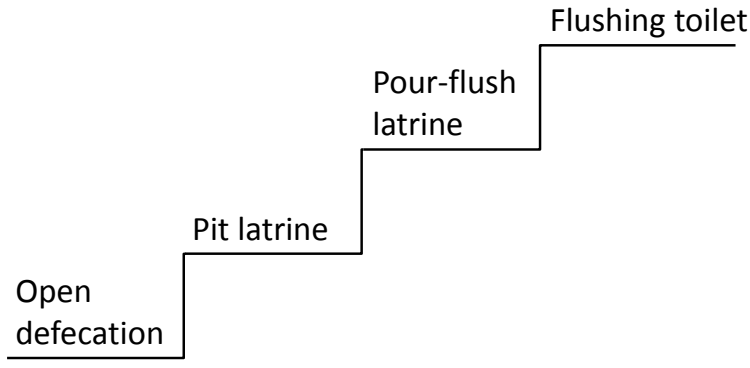


Figure 14. The sanitation ladder as an illustration of sanitation alternatives, with typically more desirable options to the right. Adapted from Keraita *et al.* (2010).

At present, the vacuum toilet probably represents the most promising compromise between the conflicting concerns of flushwater, economy and environment on one side, and user acceptability and health risk on the other. Vacuum toilets have a low flushwater volume and a similar user interface to normal flush toilets. However, due to the relative high investment costs of vacuum toilets compared with UDDTs, the latter may be a more economically sustainable option for many people. Future improvements in the designs of UDDTs may also reduce odour issues and maintenance demands, making it a more desirable option for users and from a health perspective.

Urine-diverting toilets allow separate volume reduction of urine and faeces prior to ammonia sanitisation. This can reduce the costs related to storage and transport considerably. One alternative is the No-Waste toilet, which uses urine-drying and vermicomposting of faeces to concentrate the excreta prior to ammonia sanitisation (Senecal & Vinnerås, 2014).

For toilets using water for flushing, the majority of the flushwater consumption is due to flushing after urination. Development of flush, vacuum and pour-flush toilets with urine diversion may potentially significantly reduce the flushwater volumes, as less water is required for flushing urine, and still have a high user acceptance. The use of separate urinals could also reduce the flushwater volumes significantly. Faeces may be separated from flushwater by using the Aquatron (Vinnerås & Jönsson, 2002), which can reduce the faecal sludge volume but requires a separate treatment of the flushwater. New toilet designs that treat and recirculate the flushwater within the toilet, such as the Blue Diversion Toilet, are also promising alternatives, which may provide low

cost and highly acceptable toilets as well as low sludge volumes (Larsen *et al.*, 2012).

Generally, a more functional approach to the sanitation ladder is needed in order to focus on the actual benefits of the different toilet technologies instead of having a pre-defined ranking (Kvarnström *et al.*, 2011). Sanitation systems should protect the health of both users and downstream populations, and facilitate recycling of the nutrients in the excreta.

7.3 The role of sanitisation in faecal sludge management

The WHO guidelines for the safe reuse of human excreta recommend a multi-barrier approach to avoid transmission of diseases from human excreta (WHO, 2006). In addition to pathogen inactivation during treatment, the barriers suggested include crop restrictions, protective clothing, preventing children from participating in agricultural work, and food processing. While these barriers can potentially replace some of the pathogen inactivation during treatment, it is very difficult to ensure they are followed.

Fertilisation with faecal sludge with a high concentration of pathogens may also cause a high risk for the food consumer. This risk can be reduced by growing crops where the edible part is above ground, or by washing, peeling, and/or boiling the crop. However, farmers tend to grow crops that have the highest economic benefit, regardless of their potential to transmit diseases, and salad crops are among crops generating the highest income. Even if crop restrictions are followed in the first growing season after application of faecal sludge, helminth eggs may be viable in the soil for years (Sanguinetti *et al.*, 2005) and may contaminate crops and workers in subsequent seasons if crop restrictions are not followed. Even if the crops are washed, peeled or boiled, the pathogens may very well have entered the kitchen at this stage, and good hygiene practices are required to avoid transmission of diseases.

The work applying faecal sludge as a fertiliser represents an occupational hazard for the farmer if the pathogen concentration in the sludge is still high. Several interventions are suggested for reducing this risk, and include protective clothing, gloves and facemasks. Furthermore, children are most at risk of diarrhoea and helminth egg infections. Studies have shown that protective clothing distributed free is not used, as it is less comfortable to work in gloves and facemasks at high temperatures (Seidu *et al.*, 2008). Even when protective clothing is used, it may be the children's work to clean the clothes afterwards. Children have a tendency to take part in agricultural work after sludge application as well, although this is discouraged.

Fertilisation with faecal sludge may also increase the risk of zoonotic infections in wild and domestic animals, both through ingestion of soil and through consumption of crop residues used as fodder. This can lead to subsequent transmission to humans as well as economic losses related to poor animal health (Zambrano *et al.*, 2014). The animals may act as reservoirs of diseases, and may cause outbreaks of diseases such as *Cryptosporidium* spp. (Smith *et al.*, 2007) and *Campylobacter* spp. (El-Tras *et al.*, 2015). Sanitisation of faecal sludge as well as manure can reduce the prevalence of diseases in both humans and animals. Some zoonotic parasites infecting both humans and animals are dependent on a human host to fulfil the lifecycle, *e.g. Taenia* spp. By sanitising human excreta, the eggs cannot develop cysticerci in their animal hosts (pigs or cattle), and this reduces the subsequent risk of human infection through meat consumption.

Application of faecal sludge may also contaminate groundwater aquifers and surface water sources due to run-off or infiltration of rainwater (Oun *et al.*, 2014; Ashbolt, 2004; Howard *et al.*, 2003; Scandura & Sobsey, 1997). A low degree of pathogen inactivation in the sludge before application therefore increases the risk for infection of humans and animals during water consumption. *Vibrio cholerae* may survive for a long time in water bodies due to its association with other organisms (Vezzulli *et al.*, 2010). Household-level treatment of drinking water is an important barrier in preventing waterborne transmission of faecal pathogens, but some protozoans such as *Giardia lamblia* and *Cryptosporidium* spp. are resistant to treatment with chlorine.

In conclusion, a high level of pathogen reduction in the faecal sludge treatment reduces health risks both in the whole food chain and in the environment. Consequently, the risk of pathogen reservoirs in the environment and the risk for farmers of all ages is reduced as the pathogen content in the soil is reduced, and the risk for food consumers is decreased since the crops contain fewer pathogens. Multiple barriers should still be advocated as a means of reducing the health risk, and practices involving washing, peeling and boiling may also reduce the infection risk associated with post-harvest contamination. However, a justification of lower pathogen inactivation requirements during treatment based on a dependency on other barriers may jeopardise both human and animal health.

8 Conclusions

- Adenovirus and reovirus are inactivated much more rapidly than bacteriophages during ammonia sanitisation. It is not clear how much of the virus inactivation is due to pH and how much to NH_3.

- *Salmonella* Typhimurium, which can be used as a model organism for most bacteria that cause diarrhoea, was inactivated more quickly than the other organisms studied. CO_3^{2-} may be a more important factor than NH_3 for inactivation of *Salmonella*, especially at low temperatures.

- The treatment time required for *Ascaris* egg inactivation can be estimated as a function of temperature and NH_3 concentration.

- At high temperatures (>25 °), the limiting factor for treatment with low ammonia concentrations may be pH stability, as treatments with too low ammonia concentration are vulnerable to decreasing pH. The studies indicate that an NH_3 concentration of at least 5-20 mM is required to inhibit biological degradation which causes a large pH decrease.

- *Ascaris* egg inactivation by ammonia is possible at low temperatures (<20 °C), but very high concentrations of ammonia and long storage times are required for inactivation.

- Viruses and *Ascaris* eggs are the main risks when using ammonia-sanitised faecal sludge in agriculture. For unrestricted use, faecal sludge should have a 4.5 $\log_{10}$ reduction for *Ascaris* eggs and 7.5 $\log_{10}$ reduction of rotavirus. After treatment at high storage temperatures (>28 °C) the virus represents a potentially greater health concern than *Ascaris*. However, the estimation of the risk of virus infection was uncertain.

- Urine may be sufficient as ammonia source for sanitisation of faecal sludge, but pathogen inactivation depends greatly on the quantities of flushwater used and ammonia concentration in urine. For faecal sludge with a low ammonia concentration and low pH, the studied pathogens may survive for a long time.

- For faecal sludge with a too low concentration of ammonia, low doses of urea (e.g. 0.4 %) can greatly increase the pathogen inactivation.

- Mixing urine and faeces from urine-diverting dry toilets gives a high concentration of ammonia and a low sludge volume. However, so far user acceptance of dry urine-diverting toilets is often lower than for toilets using water for flushing.

- Treatment of faecal sludge from toilets using large flushwater volumes requires larger storage volumes and longer storage times, which in turn considerably increase storage and transport costs.

9 Future research and development

- The NH_{TOT} concentration and pH in faecal sludge from different toilet systems should be measured. The variation in NH_{TOT} concentration and pH and how this relates to diet, toilet design and air-flushing system (in the case of vacuum toilets) should be evaluated. The required sampling frequency for treatment monitoring should be determined.

- The microbiological degradation of faecal sludge prior to treatment probably affects the buffer capacity of the sludge when the ammonia concentration is not sufficient to inactivate the microorganisms responsible for this. The resulting change in buffer capacity should be analysed to estimate the doses of urea needed for treatment. The buffer capacity of other types of sludge, such as pit latrine sludge and septic tank sludge, should also be measured.

- Hydrolysis of urea into ammonia and carbonate may take weeks, and this may prolong the storage times required. The time required for hydrolysis and how it depends on dry matter content and temperature should be quantified.

- *Vibrio cholerae* outbreaks are common in refugee camps. While other gram-negative bacteria are inactivated rapidly by ammonia, *Vibrio cholerae* can enter a dormant stage to prolong its survival. The ammonia inactivation of *Vibrio cholerae* should therefore be studied.

- Studies indicate that inactivation of viruses and *Cryptosporidium* oocysts depend on the substrate, with slower inactivation in faecal sludge and slurry compared with buffers. The effect of high-density sludge in the bottom of storage tanks on the inactivation of viruses and C*ryptosporidium* oocysts should be studied.

- The effect of ammonia, carbonate and pH on inactivation of virus, bacteria and protozoa should be separated. There is no direct effect of pH <12 on *Ascaris* egg inactivation, and effect of carbonate is low compared with the effect of ammonia in faecal sludge with urine and urea. However, the *Ascaris* egg inactivation by carbonate should be quantified in order to predict inactivation in substrates with higher carbonate concentration, such as e.g. digestate.

- There is a need to generalize the inactivation of more pathogens, especially viruses, to enable prediction of inactivation as functions of relevant treatment factors including but not limited to temperature, pH, ammonia, carbonate and dry matter content.

- The mechanisms of pathogen inactivation by ammonia, carbonate and pH are in many cases not well understood and should be studied further.

- The concentration of viruses in faecal sludge and their fate during vegetable growth and washing should be quantified, as these are major uncertainties for estimation of health risk associated with the use of faecal sludge as a fertilizer.

- There is a need to develop toilet technologies with a very low water use, high user acceptance and low maintenance requirements, as low flushwater consumption is critical for the economy and resource use during faecal sludge treatment and transport prior to agricultural use.

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