GLOBAL WATER PATHOGEN PROJECT

PART FOUR. MANAGEMENT OF RISK FROM EXCRETA AND WASTEWATER

CHEMICAL DISINFECTANTS

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Summary

Safe water, sanitation, and hygiene provision and promotion are critical elements of emergency response to ensure human safety, health, and dignity. Disinfectants, such as chlorine, are widely used in emergency response to treat water for drinking. However, excreta is rarely treated in emergencies; the current focus of response activities is to provide safe, clean, and private sanitation facilities. In this chapter, we provide a summary of knowledge on disinfection of excreta in emergencies and recommendations for future research. In particular, we recommend the need to prioritize disinfection of waste in emergencies to prevent ongoing transmission of disease and to work with responders and beneficiaries to develop appropriate, low-cost, transportable, acceptable, and easy-to-use excreta disinfection solutions.

Chemical disinfectants inactivate pathogens by chemically degrading their building blocks or disrupting their metabolism. The efficacy of chemical disinfectants thus depends strongly on their reactivity with biomolecules. In addition, both the disinfectant concentration throughout the treatment, as well as the duration of the disinfection treatment (exposure time) are important parameters determining the disinfection efficiency.

To be applicable in the context of sanitation, chemical disinfections must have several basic characteristics: they must be active against a wide range of pathogens; be sufficiently cost-effective to be applied frequently and in large quantities; be reasonably safe to produce, store and apply; and create a final product that is safe to be handled by humans or to be discharged into the environment.

This chapter focuses on two groups of chemicals that meet these requirements, namely oxidants (mainly free chlorine) and bases (ammonia and lime). Oxidants are well-studied in the context of drinking water disinfection, though less information is available for the disinfection of sanitation-relevant matrices. Ammonia and lime are treatments that are exclusively used in the context of sanitation. For these chemical disinfectants, we have conducted a literature survey and collected kinetic data on their inactivation efficiencies in sanitation-relevant matrices. We have considered all pathogenic organisms described in part 3 of the GWPP, though data were only available for a subset of these organisms. In addition, we have included indicator organisms typically used to mimic the fate of pathogens during disinfection.

The collected data were analyzed and visualized to provide an overview over the disinfection efficiency of chlorine, ammonia and lime toward different pathogen groups or individual organisms. In addition, whenever possible, the data were scrutinized with respect to the effects of important matrix properties, namely temperature, solids content, ammonia and organic matter content. We furthermore aimed to identify the least susceptible, process-limiting pathogens for each treatment, and to suggest suitable indicator organisms. Finally, treatment recommendations are provided, and we highlight major knowledge gaps that need to be addressed in order to refine these recommendations.

1.0 Oxidants

Chemical oxidants are reactive molecules that can efficiently degrade (oxidize) a broad range of substrates, including the building blocks of pathogens. Oxidants typically used for disinfection include chlorine (Cl_2 or hypochlorite, HOCl), chlorine dioxide (ClO_2), chloramines (NH_2Cl, $NHCl_2$, NCl_3), ozone (O_3), and peracetic acid. Compared to ammonia, chemical oxidants exhibit much higher reactivity toward pathogens. However, oxidants rapidly react with other matrix constituents, including organic matter, organic nitrogen compounds and ammonia. In matrices with a high organic matter or ammonia content, such as the matrices encountered in sanitation systems, the oxidant is thus rapidly consumed by the matrix. The extent of oxidant consumption by the matrix is termed “disinfectant demand”. Matrices with a high disinfectant demand strongly compromise the availability of oxidants for pathogen inactivation. Oxidants are therefore mostly used in matrices with comparatively low disinfectant demand, such as drinking water or secondary wastewater effluents. Applications to matrices with higher organic matter content, and thus higher disinfectant demand, would require large dosages and therefore are less practical.

As a result of the disinfectant demand, pathogens in the matrix are exposed to a decreasing oxidant concentration over time. The rate of decrease, and thus the residual oxidant concentration available for disinfection, is dependent on the specific matrix composition. It is therefore not possible to relate inactivation to the initial oxidant concentration. Instead, inactivation is typically evaluated as a function of the oxidant *exposure*. The oxidant exposure corresponds to the time-dependent concentration of the oxidant, integrated over the exposure time.

$$ exposure = \int_{0}^{t}[oxidant]dt\qquad(1) $$

To accurately assess the extent of inactivation in a matrix, the oxidant exposure, and hence the oxidant decay rate and the treatment time, must thus be known. While it may be possible to measure the decay of most oxidants during treatment, this is often impractical and costly. Alternatively, the oxidant decay can be determined in preliminary lab tests, or it can be estimated using empirical models (USEPA, 1986). The most pragmatic and conservative approach is to simply determine the terminal oxidant residual at the end of the treatment, or, in the case of a flow-through system, at the reactor effluent. This value, in conjunction with the treatment time, can then be used to estimate the minimal oxidant exposure and hence the minimal extent of treatment. The simplest form to express this relationship is the Chick-Watson law:

$$ -ln\frac{N}{N_{0}}= -k\textrm{C}_{final}^{n}t\qquad(2) $$

Here, the disinfectant exposure, defined in equation (1),

simply corresponds to $C^{n}_{final*}t$, where C_{final} is the terminal disinfectant residual, and t is the treatment time. The coefficient of dilution, n, frequently corresponds to 1, indicating a proportional contribution of the oxidant and the exposure time to disinfection. Finally, k is a pathogen- and oxidant-specific inactivation rate constant. For actual treatment systems, this approach needs to be adapted to the treatment-specific setup. For example, in flow-through systems, the treatment time t is not a constant, but corresponds to the residence time distribution; t is therefore typically approximated as the time needed for 10% of the volume to exit the flow-through reactor. Additionally, equation (2) can be modified to account for the time-dependence of the oxidant concentration (equation 1). Finally, departures from the Chick-Watson law are common, leading to tailing or shouldering in the disinfection curve (USEPA, 1986). Nevertheless, this law frequently provides a convenient first assessment of the disinfection susceptibility of pathogens.

Exposure values required to reach a given level of inactivation can be calculated from published, exposure-based rate constants or from tabulated Ct values (Guillot and Loret, 2005). These values, however, were mostly obtained in clean (e.g., drinking water) matrices. In sanitation-relevant matrices, the tabulated Ct values may overestimate the actual extent of inactivation, because of shielding interferences from particles, organic matter, or cell debris. Here, we thus focus only on the literature data obtained in sanitation-relevant matrices (primary and secondary wastewater effluent, sludge), and highlight the challenges associated with disinfection in the context of sanitation. We focus mainly on chlorine, which is the most widely applied chemical oxidant.

1.1 Free Chlorine (FC)

1.1.1 Disinfection mechanism and efficiency

Free chlorine is formed when gaseous chlorine, Cl_2, is dissolved in water to form the aqueous chlorine species hypochlorous acid (HOCl) and its conjugated base, hypochlorite (OCl^-). HOCl is a weak acid (pKa=7.54 at 25°C; Morris, 1966) and strong oxidant (E=1.49 V at 25°C; Copeland and Lytle, 2014), while ClO^- is a weaker oxidant in comparison (E= 0.9 V at 25°C; Copeland and Lytle, 2014). In addition, the neutral HOCl can penetrate the outer layers of microorganisms more readily compared to the negatively charged OCl^-, and hence act on the intracellular building blocks of microorganisms. Overall, HOCl acid is thus the more reactive aqueous free chlorine species. As such, the overall disinfection efficiency of FC is greatest at pH values where HOCl is the dominant species (pH < 7.5).

FC is effective against bacteria and viruses. It readily oxidizes their proteins, nucleic acids and lipids, and thereby disrupts vital bacterial and viral functions (McDonnell and Russell, 1999). For example, FC has been found inhibit DNA and RNA synthesis, interfere with host cell recognition, disrupt bacterial membranes, and disrupt the cellular activity of proteins. These actions make FC highly efficient against bacteria and reasonably efficient against most viruses. In contrast, FC is significantly less effective against *Cryptosporidium* oocysts (Korich et al., 1990) and bacterial spores, likely due to protection offered by these organisms strong cell walls or coats. These trends are well-reflected in Figure 1, which summarizes reported chlorine inactivation data of pathogens in wastewater and sludge. This figure confirms the relative susceptibilities of pathogens toward chlorine: for a given level of inactivation, a higher Ct is generally required for viruses and spores than for bacteria.

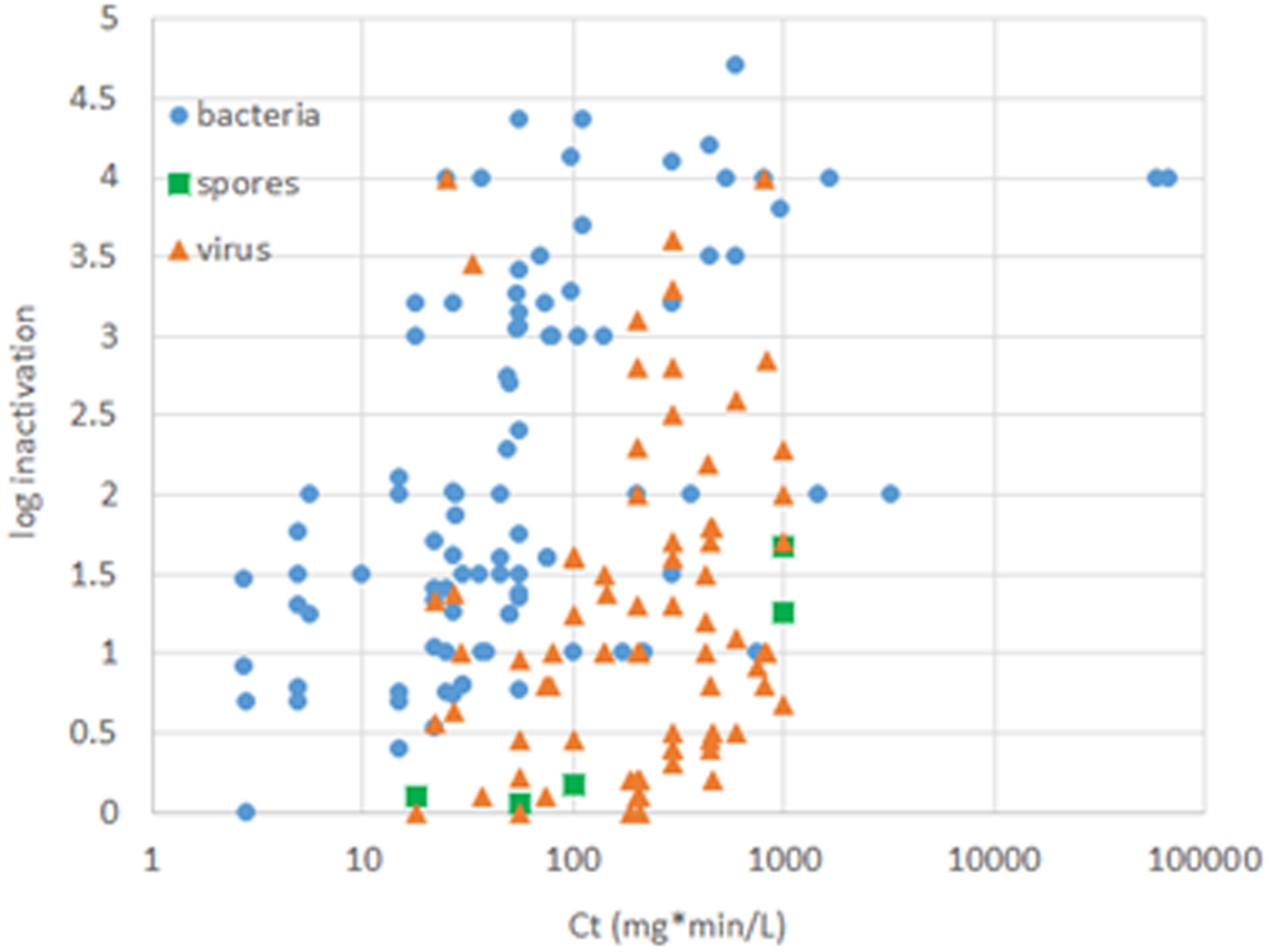


Figure 1. Overview over reported data on chlorine disinfection of bacteria, spores and viruses in waste-water and sludge matrices. For those studies that did not report measured exposures, Ct was estimated from the reported applied or measured chlorine concentrations and treatment times. The references used to create this plot are indicated in the reference section.

1.1.2 Current practice in chlorine disinfection of wastewater

Free chlorine is typically applied either by dissolving chlorine gas (Cl_2) in water, or as a liquid hypochlorite or sodium hypochlorite solution (HOCl or NaOCl). Chlorine gas is toxic and must be stored under high pressure, and thus holds significant safety risks for operators. It should therefore be stored in a well-protected, secure area. Liquid chlorine solutions can either be stored, or produced on-site. Stored liquid chlorine solutions are high in concentration (10-15 % w/w) and are corrosive. Storage tanks should therefore be made of materials that do not corrode, both to protect the operator from exposure to liquid chlorine and to prevent the degradation of the chlorine by reaction with the tank. In contrast, on-site production of HOCl via the electrolysis of brine solutions allows operators to maintain only smaller amounts of HOCl solutions at lower concentrations. While the on-site generation of chlorine is probably the safest option, it still holds a small risk from the co-production of hydrogen gas, and it requires additional operator training. Independent of the source, all chlorinated matrices must be dechlorinated before discharge into the environment or reuse, due to the toxicity of residual chlorine to humans and ecosystems. The most commonly used chemical dechlorinating agents are sulfur dioxide, sodium sulfite, sodium bisulfite or sodium metabisulfite. Alternatively, activated carbon can be used for dechlorination. While the elimination of the chlorine residual is important from an ecotoxicological standpoint, it does enhance the potential for inactivated bacteria to reactivate or regrow (Blatchley et al., 2007; Li et al. 2013), which may partially reverse the disinfecting effects of chlorine. The likelihood of pathogen reactivation and regrowth in disinfected matrices or upon their discharge into the environment, and the associated public health significance, remains to be fully clarified.

Figure 1 illustrates the apparent variability in the efficiency of chlorine disinfection for sanitation: for a given extent of inactivation, reported Ct values for a given pathogen type can span several orders of magnitude. For example, for a 2 $\log_{10}$ inactivation of bacteria, Ct values of 5.6 up to > 1000 m*min/L have been reported. Given the

large variability in chlorine disinfection of sanitation-relevant matrices, the appropriate chlorine dose to achieve a given level of inactivation is difficult to estimate. Therefore wastewater chlorination typically follows standard practices. Specifically, chlorine doses of 5-20 mg/L are applied for a duration of 30-60 min to disinfect secondary effluent (USEPA, 1999; Lazarova et al., 1999). Higher chlorine exposures are needed for stronger effluents, such as primary wastewater and or trickling filter effluents. Furthermore, it has been found that applying either longer exposure times or higher chlorine concentrations are approximately equally effective (Linden et al., 2004). For sludge stabilization, much higher chlorine exposures must be applied. Typical chlorine concentrations range from 600 to 2000 mg/L, depending on the type of sludge being treated (Wang, 2006).

Ultimately, however, the dosing – and resulting FC exposure and inactivation – will depend on the properties of the matrix under consideration. In particular, ammonia and organic amines, organic matter and suspended solids content deserve particular consideration.

1.1.3 Influence of ammonia and organic amines

Organic nitrogen compounds present in fresh waste are a major sink for free chlorine. In particular organic amines react with FC at high rates (Deborde and von Gunten, 2008), and thus exert a high chlorine demand. Over time, organic nitrogen compounds such as urea are degraded to ammonia, which accounts for an important of chlorine demand in wastewater and stored waste products. Ammonia readily reacts with FC to form chloramines, or combined available chlorine (NH_2Cl, $NHCl_2$, NCl_3). While chloramines can also inactivate a range of pathogens, they are less potent than FC, in particular against viruses (Sobsey, 1989) . As such, the reaction of FC with ammonia lowers the disinfection efficiency of the applied free chlorine. Free chlorine is only present if all the ammonia and organic carbon is oxidized. To overcome the chlorine demand exerted by ammonia, chlorine doses are applied that exceed the ammonia-exerted demand (breakpoint chlorination). In practice, a mass ratio of 1:10 to 1:15 (ammonia N : chlorine as Cl_2) (Griffin and Chamberlin, 1941) is sufficient to oxidize all ammonia in the matrix as well as consume the demand exerted by organic matter. FC applied beyond this ratio is available as free chlorine for disinfection.

While well-understood in theory, the effect of ammonia on chlorine efficiency is not evident from the studies reviewed herein (Figure 2, left panel). No consistent trend between total ammonia content of the matrix and the Ct needed to achieve 1 $\log_{10}$ of inactivation was found for either bacteria or viruses. This may be due to the fact that most studies that reported ammonia contents of their matrices applied chlorination below the breakpoint (ammonia N : chlorine as $Cl_2 < 15$; Figure 2, right panel). As such, significant chloramine concentrations were likely present, while most of the reported or reported Ct values only include FC.

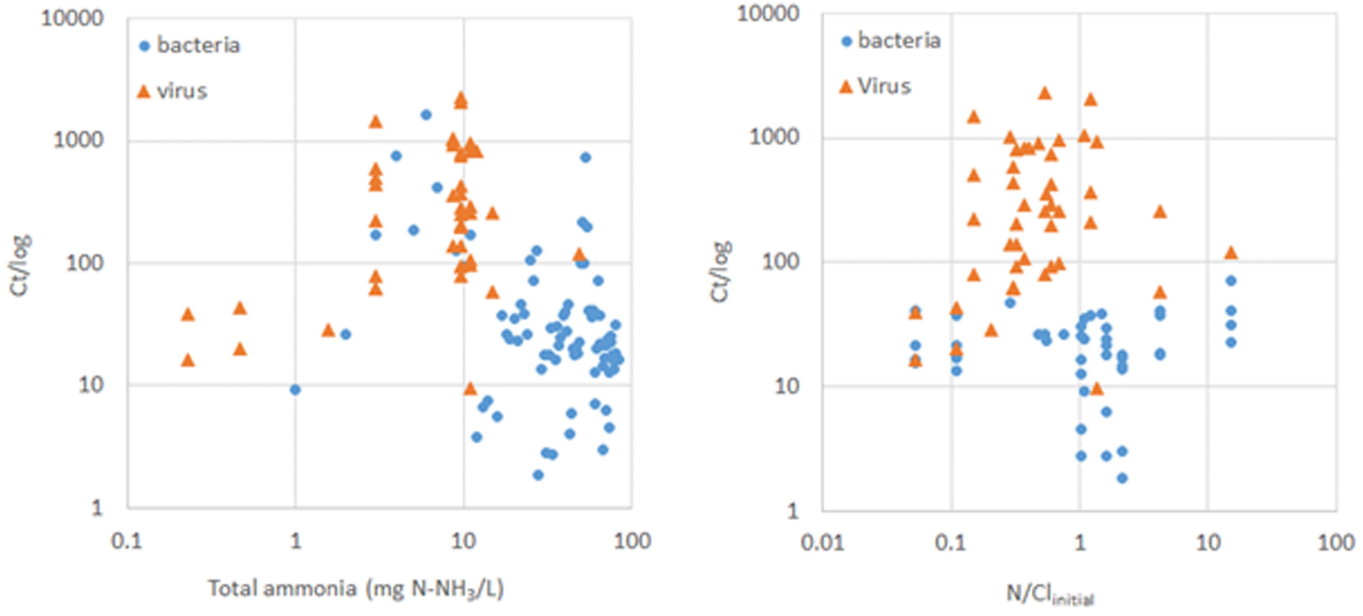


Figure 2. Required Ct (mg*min/L) to achieve a 1 $\log_{10}$ pathogen reduction, as a function of the total ammonia content of the matrix (left panel) and the applied $N/Cl_{initial}$ ratio (right panel).

Given the low reactivity of viruses toward chloramines, it is advisable to apply breakpoint chlorination if feasible, i.e., to supply sufficient chlorine such that all ammonia is oxidized and free chlorine is present. Breakpoint chlorination can typically only be applied in matrices with an ammonia concentration up to 3 mg/L (nitrified effluents),

as higher ammonia concentrations would require prohibitively high chlorine concentrations. It has been demonstrated that efficient virus inactivation can be achieved if breakpoint chlorination is applied to wastewater.

1.1.4 Influence of organic matter

Organic matter reacts with free chlorine to form a suite of chlorinated organic substances. In particular electron-rich moieties of organic matter, such as aromatic carbons, organic amines, and reduced sulfur groups, rapidly react with chlorine (Deborde and von Gunten, 2008). Chloramines also react with organic matter, albeit to a lesser extent (Vikesland et al., 1998). These reactions lower the concentration of free chlorine or chloramines available for disinfection. This trend is apparent from Figure 3, which shows that increasing concentrations of organic matter - here quantified as the chemical oxygen demand (COD) of the matrix – increase the Ct requirement for the disinfection of bacteria. For viruses, this correlation is not evident based on the data reviewed herein.

Reducing the organic load of the matrix prior to disinfection thus lowers the Ct requirement, and allows for the use of lower chlorine doses. A lower chlorine dose is additionally beneficial because it reduces the formation of hazardous disinfection byproducts, such as trihalomethanes and haloacetic acids. Given the high reactivity of organic matter with free chlorine, along with the formation of problematic products, organic-rich matrices such as sludge should consider alternative treatment methods.

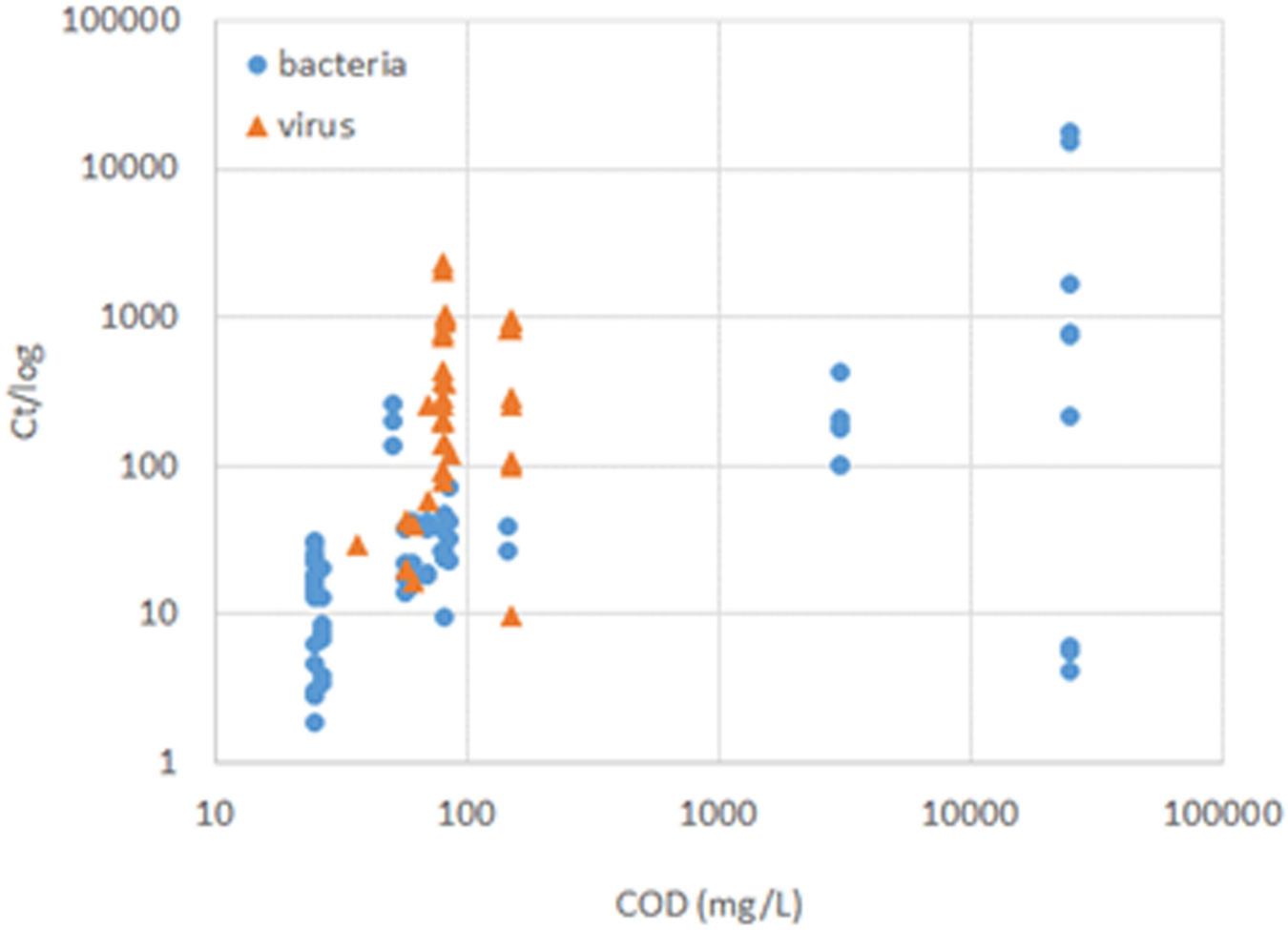


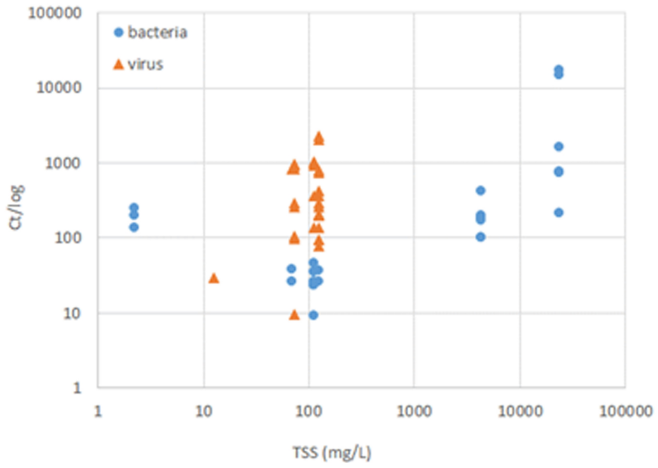
Figure 3. Required Ct (mg*min/L) to achieve a 1 log_{10} pathogen reduction, as a function of the chemical oxygen demand of the matrix. If COD was not reported, it was estimated based on total organic carbon (TOC) or bioavailable organic carbon (BOD), using the ratios COD/TOC = 3 and COD / BOD = 2.1 (Fuller, 2016, accessed 2016).

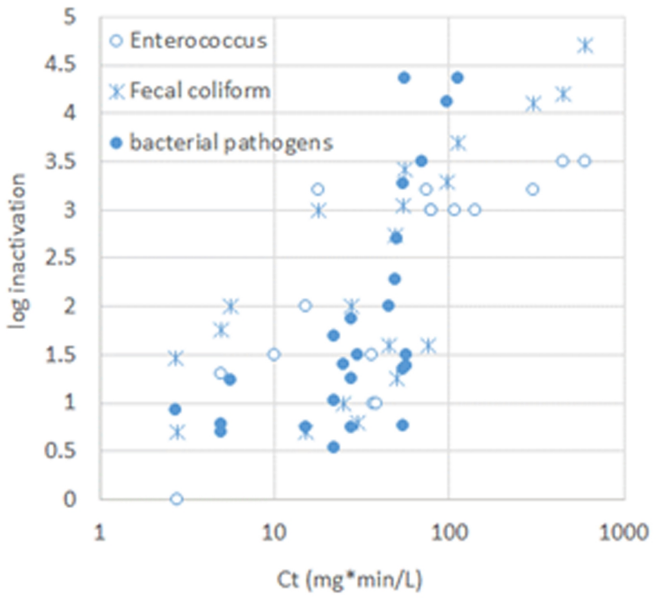
1.1.5 Influence of particulates

Particles may exert a protective effect on pathogens during disinfection, because they may occlude pathogens and shield them from free chlorine or chloramine. Correspondingly, increasing particle concentrations, or turbidity, leads to less efficient disinfection by free chlorine (Tsai and Lin, 1999; Dietrich et al., 2007; Camarillo et al., 2011). Interestingly, chloramines have been demonstrated to more efficiently inactivate pathogens occluded by

particles (Camarillo et al., 2011; Linden et al., 2004). This can be explained by their lower reactivity, which prevents them from being consumed by the particle before reaching the pathogen. Furthermore, it has been demonstrated that longer contact times are a more successful strategy to ensure disinfection compared to increased chlorine concentrations. The Ct approach is thus not fully valid for the disinfection of occluded pathogens.

Figure 4. Required Ct (mg*min/L) to achieve a 1 log_{10} pathogen reduction, as a function of the chemical oxygen demand of the matrix.





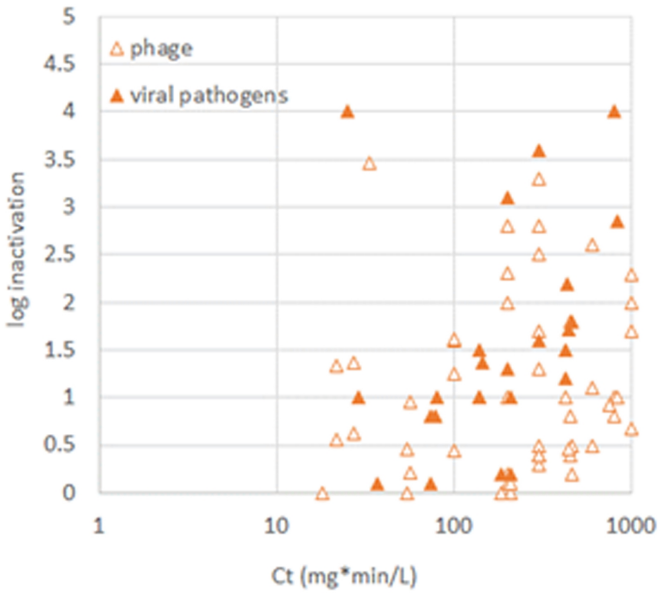


Figure 5. Comparison of the disinfection behavior of indicators (*Enterococcus* and fecal coliform for bacteria; coliphages for virus) and corresponding pathogen groups during wastewater disinfection by chlorine.

This is reflected in Figure 4, which shows an increasing trend in the Ct requirement for bacteria with increasing concentration of solids. It is thus recommended to filter the matrix prior to disinfection, to prevent pathogen shielding. Alternatively, nitrification of the wastewater has been found to lower the particle concentration and thereby enhance disinfection efficiency (Camarillo et al., 2011). Similarly, settling of sludge yielded better disinfection results compared to sludge thickening (Tsai and Lin, 1999).

1.1.6 Appropriate indicators

1.1.7 Conclusions and recommendations

Given the complex nature of the matrices and their effect on chlorine disinfection dynamics, it is not surprising that considerable scatter exists in reported data of pathogen inactivation in sanitation-related matrices (Figure 1). To provide useful guidance for chlorine disinfection, better data are urgently needed. In particular, disinfection experiments need to be carried out in the relevant matrices, and the matrix parameters must be rigorously reported, with respect to pH, temperature, and the contents of suspended solids, COD, and ammonia. In addition, Ct values with respect to both free and combined chlorine must be tracked and reported.

Despite the paucity and heterogeneity of the data

reviewed, some initial conclusions and recommendations can be drawn summarized for the application of free chlorine in sanitation:

Benefits of free chlorine:

1. Free chlorine is a cheap and readily available disinfectant

2. Free chlorine is effective against bacteria and viruses.

Detriments of chlorine:

1. Free chlorine rapidly reacts with ammonia to form less potent chloramines, which are not effective against viruses.

2. Free chlorine is not efficient against protozoa, helminths or bacterial spores

3. Chlorine application necessitates a dechlorination step

4. Free chlorine is readily consumed by organic matter to form hazardous byproducts.

5. Chlorination does not fully prevent regrowth of bacteria

Recommendations to ensure optimal chlorine disinfection performance:

1. Ct values should be chosen based on viruses or viral indicators (bacteriophages), which are more difficult to disinfect than bacteria

2. Maintain a pH below 7.5

3. Minimize organic loading prior to disinfection, for example by coagulation-flocculation

4. Remove particles by filtration or nitrification, to prevent pathogen shielding

5. If feasible, apply breakpoint chlorination to ensure a free chlorine residual and virus inactivation.

6. Avoid chlorination of matrices very high in solids and organic matter content.

1.2 Alternative oxidants

Given the drawbacks of chlorine and chloramines - mainly their inability to inactivate protozoa and the formation of hazardous byproducts - alternative oxidants, such as ClO_2, ozone and peracetic acid, have been applied. In particular ClO_2 and ozone are more effective against protozoa than free chlorine or chloramine (Korich et al., 1990), and all three oxidants are considered to yield fewer problematic byproducts (though this view is contested). Currently, there are not sufficient data available to thoroughly assess the efficiency of these three oxidants toward pathogens in sanitation-relevant matrices. Nevertheless, they all have some interesting features that make them interesting candidates as free chlorine alternatives:

ClO_2offers the great advantage of being insensitive to changes in pH and ammonia content. Furthermore, it produces a measurable residual, and it requires a shorter contact time and dose compared to chlorine (2-5 mg/L ClO_2 during 5-15 min, compared to instead of 5-20 mg/L FC for 30-60 min). However, ClO_2 is more expensive than chlorine, and must be generated onsite, therefore its application to date has been limited.

1.2.1 Ozone

Ozone is increasingly applied for wastewater treatment, albeit often with the main goal of reducing organic micropollutants. Ozone is particularly interesting because of its high efficiency against protozoa and all viruses. In terms of performance monitoring, however, ozone is a challenge. The ozone demand in wastewater is very high and ozone decay is rapid, such that residual ozone frequently cannot be detected, while significant pathogen inactivation may nevertheless be achieved. Instead of directly measuring ozone residual, alternative approaches have thus been suggested that correlate the extent of inactivation with surrogates for ozone exposure, such as the applied O3:TOC ratio (Gerrity et al., 2012). While promising, these approaches need further validation in the future. A detriment of ozone is that its application is energy intensive and requires a trained operator. As such, ozone is mainly of interest for large plants, or for applications targeted at *Cryptosporidium* control. Finally, even though ozone is more reactive toward all organisms than chlorine in demand-free solutions, comparisons in wastewater are

not as clear: while ozone has been shown to be more efficient toward viral indicators, it was less efficient compared to chlorine toward bacterial indicators, and neither disinfectant was successful at inactivating spores (Tyrell et al., 1995).

1.2.2 Peracetic Acid

Peracetic acid has frequently been applied in the food industry, and applications to wastewater, ballast water and sludge are increasingly gaining attention. Peracetic acid has been described as a viable alternative to chlorine (Baldry and French, 1989), though future studies will be needed to support this claim. The main advantages of paracetic acid are its ease of implementation, the low potential for harmful byproduct formation, and the short treatment times. Furthermore, peracetic acid is relatively insensitive to changes in pH and to the presence of organic matter and ammonia. However, while peracetic acid has frequently been reported to be effective against bacteria, viruses and spores (Kitis, 2004), its effect on protozoa and helminths has not been clearly established. In addition, peracetic acid is still expensive compared to free chlorine, which hinders its broader use.

In summary, alternative oxidants to chlorine are likely to gain importance for sanitation in the near future, either alone or in combination with other disinfection treatments (e.g., peracetic acid/UV). More data are needed, however, to better assess the efficiency of these treatments in sanitation-related matrices, and to identify the parameters that limit or promote their performance.

2.0 Ammonia

Ammonia treatment in sanitation is a relatively new treatment approach. Since early 2000, this approach has evolved into a treatment method for wastewater and manure products for intended agricultural use. The treatment can be applied to a wide range of volumes: it has been proven efficient for single faecal droppings (50-500g) in the self-sanitising, single use, biodegradable toilet the Peepoo (Vinnerås et al., 2009), but also for the treatment of up to 1000 m^3 of black water (Nordin and Vinnerås, 2015). In addition, ammonia treatment offers two major advantages over to other approaches discussed in this chapter: firstly, in contrast to lime and oxidants, the ammonia is not consumed during the treatment. Therefore, as long as the appropriate treatment conditions are maintained (see below), the hygienisation of ammonia effect is sustained for prolonged periods, while also reducing the risk for re-growth (Vinnerås et al., 2003). Secondly, ammonia treatment of matrices intended to be used in agriculture has the additional benefit that the treatment increases the fertiliser value, by providing additional nitrogen (Albihn and Vinnerås, 2007).

2.1 Properties of Ammonia

Ammonia is a weak base with a pK_a of 9.25 at 25°C. Consequently, ammonia treatment typically operates in the range of pH 8-10.5. Ammonia has a low boiling temperature of -33°C, therefore all treatments using ammonia are based on aqueous solutions, whereby up to 28% w/w ammonia can be dissolved in water. In aqueous solutions, ammonia is found in equilibrium between the protonated (NH_4^+) and neutral aqueous species (NH_3), and the neutral gaseous form. Note that inhalation of NH_3 gas is hazardous to humans, therefore appropriate precautions must be taken during ammonia treatment to ensure operator safety.

$$ NH_{3}(g)\leftrightarrow NH_{3}(aq) + H_{2}O\leftrightarrow NH^+_{4}+OH^-\qquad(3) $$

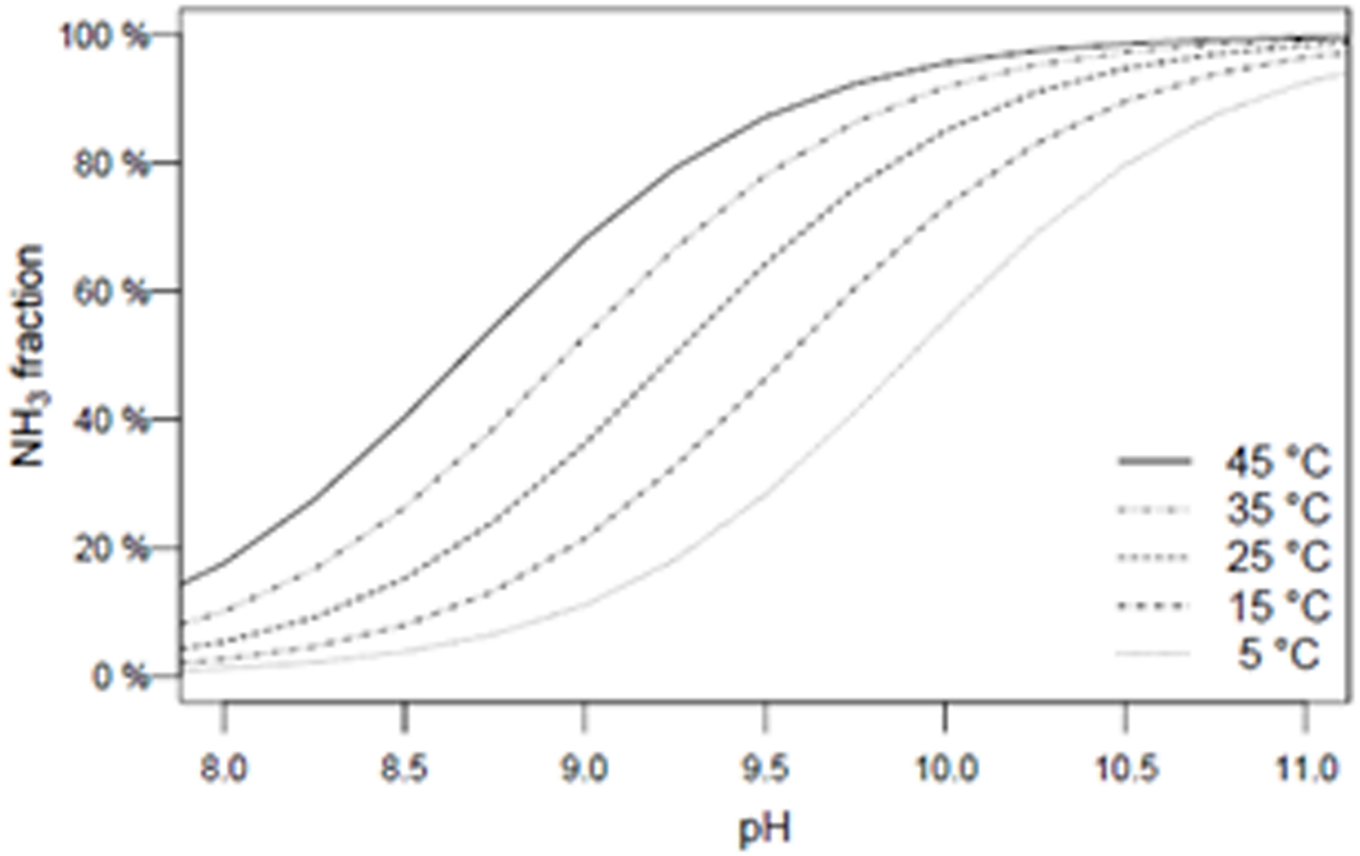


Figure 6. NH_3(aq) fraction of total aqueous ammonia (NH_3+NH_4^+), plotted as a function of pH and temperature, based on equilibrium calculation according to Emmerson.

2.2 Mechanisms of Inactivation

Ammonia treatment utilizes two inactivating factors: first, the neutral ammonia (NH_3) is a biocidal agent, and second, the elevated pH arising from ammonia addition further promotes inactivation (see section on lime for details). The mechanisms underlying the inactivation of microorganisms by NH_3 are not fully understood. The effect of ammonia on bacteria likely involves the passage of uncharged ammonia through the cell membrane. Similarly, NH_3 can diffuse through the lipid membranes of helminth eggs (Pecson and Nelson, 2005) and through the complex wall of protozoan oocysts (Jenkins et al., 1998). This causes the internal pH of the organisms to increase. In bacteria, the elevated internal ammonia concentration has been linked to a disruption of the internal pH regulation, where the cell exchanges K^+ for H^+ to decrease the pH, which ultimately causes a lack of K^+ and consequently cell death (Bujoczek et al., 2001). The effect of ammonia on ssRNA viruses involve ammonia-promoted cleavage of the viral genome. Hereby NH_3 acts as a catalyst in the alkaline transesterification of ssRNA (Decrey et al., 2015). Its effect on dsRNA or DNA viruses, in contrast, is much less pronounced and not understood (Decrey et al., 2016). Finally, NH_3 can diffuse through the lipid membranes of helminth eggs and through the complex wall of protozoan oocysts to further damage the microorganisms cells inside by the mechanisms mentioned above.

2.3 Sources of Ammonia

The ammonia used for sanitation can either be the intrinsic ammonia present in the matrix (Fidjeland et al., 2013), ammonia added as urea (Nordin et al., 2009; Ottoson et al., 2008), or ammonia added as in form of an aqueous solution (Emmoth et al., 2011; Pecson and Nelson, 2005). Intrinsic ammonia mainly stems from the hydrolysis of excreted urea. Once excreted, urea is rapidly enzymatically hydrolyzed by urease into ammonia and carbonate. Ureases are very common enzymes, which are found in various forms in bacteria, plants, animals and humans. Consequently, ureases are always present in sanitation-relevant matrices (Krajewska, 2009a; Krajewska, 2009b).

Enzymatic hydrolysis also converts externally added urea to ammonia. Urea is a common and harmless chemical; it is the most used chemical fertiliser worldwide, as well as a household chemical used as skin softener in many personal care products such as hand cream and toothpaste. As such, the addition of urea can thus be considered a very safe method supplementing ammonia, as it is does not convert to the more toxic ammonia until it is added to the material to treat. One added benefit of urea

addition is the inactivating effect of the carbonate formed during urea hydrolysis. Carbonate has been found to promote the inactivation of bacteria, e.g. *Salmonella* spp. (Park and Diez-Gonzalez, 2003) and of ssRNA viruses (Decrey et al, 2015, 2016), but not of helminths (Fidjeland et al., 2016). Addition of urea leads to an increase in pH to about 9.0-9.5, depending on dosage and buffering capacity of the matrix. Specifically, a low total solids content of the matrix and high urea dosage typically produce higher pH values (Figure 7). In contrast to lime treatment, the pH remains relatively stable over time (Nordin, 2010).

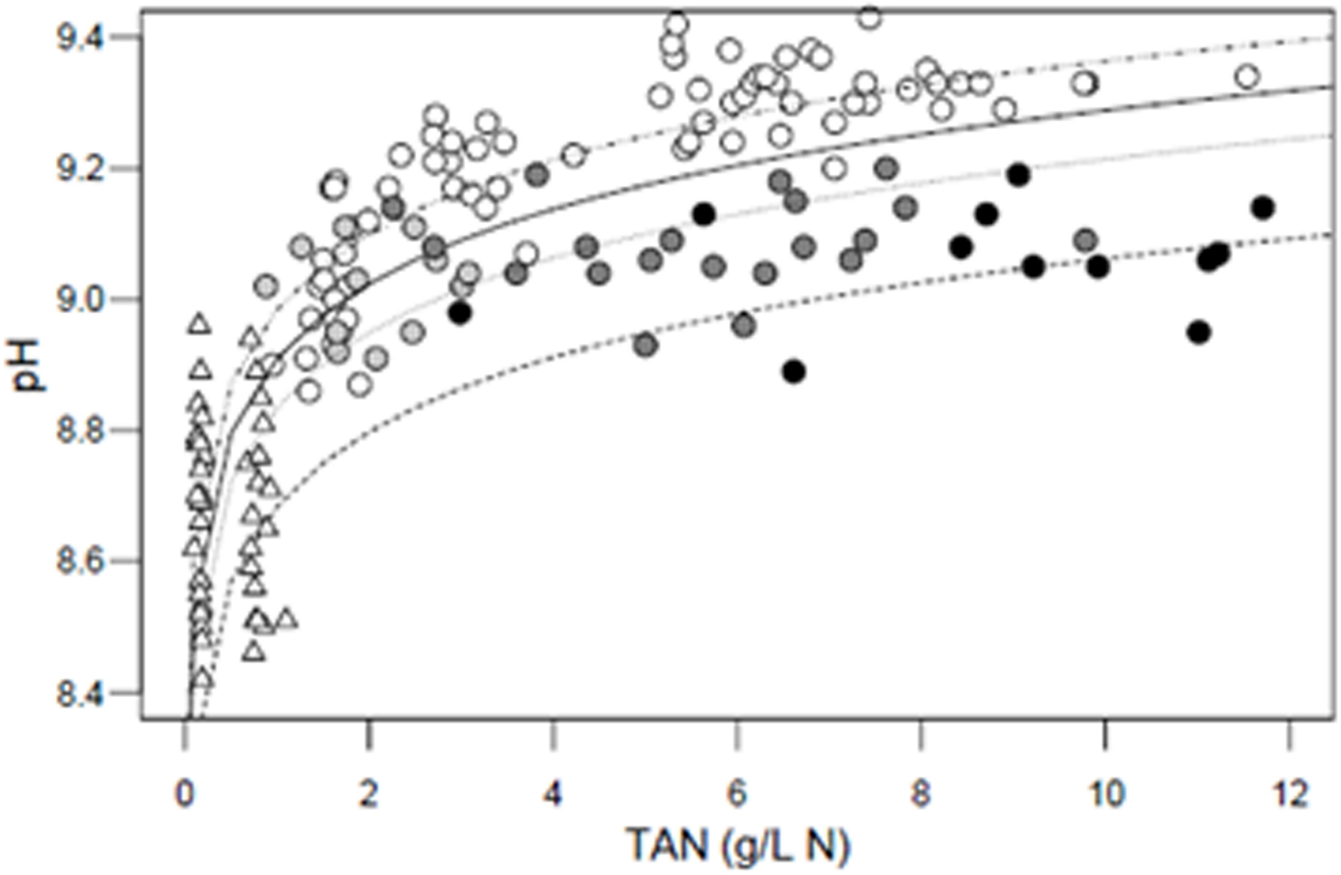


Figure 7. pH of fecal sludge after addition of different doses of urea, expressed as total ammonia nitrogen (TAN) and as a function of total solids content. White dots: 0.2% solids; light grey dots: 0.4% solids; dark grey dots: 0.7-1.2% solids; black dots: 3-5% solids. White triangles are treatments with decreasing pH. The lines represent model fits to the data as explained in Fidjeland, 2015 (Figure source: Fidjeland, 2015).

Using aqueous ammonia solutions, which can contain up to about 30% ammonia by weight (most commercially available concentrated bulk solutions are found at 25% w/w concentration), results in higher pH compared to urea addition, as it does not contain any pH-buffering carbonates. A final pH above 11 can be reached, depending on the dosage and the matrix properties (Fidjeland et al., 2016). This high pH alone can result in significant inactivation of some pathogens (see section on lime), though synergistic effects of pH and ammonia are required for others. The higher treatment pH also allows for a lower ammonia dose, as a larger fraction of the total ammonia is present in the biocidal NH_3 form. Finally, the disinfection process is more rapid compared to urea addition, as one does not rely on an initial enzymatic degradation of the added substance. However the process is more hazardous to the operator, as the ammonia solution is caustic, and gaseous NH_3 is highly irritating and malodorous.

2.4 Treatment Consideration

Given the volatility of NH_3, ammonia treatment needs to be performed in a sealed tank for assuring low air exchange, to minimise the losses of NH_3 gas. Furthermore, when adding ammonia or urea to the material, initial mixing is required where after the material can remain untouched until its discharge or application in the field. Proper initial mixing, along with NH_3 concentrations above 10 mM, are also necessary to minimize the risk for biogas production. This risk, however, can be readily controlled, as most biological activity is hampered when the concentration of NH_3 is above 10 mM (Vinnerås et al., 2008; Nordin et al., 2009; Magri et al., 2013). For example, only minimal emissions of N_2O and methane were measured from ammonia-treated sewage sludge during covered storage compared to untreated sludge (Willen, 2016). At the same time there is no risk of re-contamination or

regrowth of pathogenic bacteria as the treatment continues until the material is neutralized in the environment, e.g. when it is allied as a fertiliser.

To ensure that treatment is still active, it is advisable to periodically check both pH and the presence of ammonia. Frequently it is sufficient to evaluate if the matrix still has a strong ammonia odor, which is indicative of active treatment conditions. For a more quantitative approach, yet still simple approach, the concentration of NH3 can be estimated by the Emerson approach (Fidjeland et al., 2015; Jenkins et al., 1998), using the total ammonia nitrogen concentration (TAN), the pH and the temperature (equations 4 to 7).

$$ NH_{TOT}=\frac{TAN}{14.01}\times\frac{1}{1-DM}\qquad(4) $$

Where NH_{TOT} is the total ammonia concentration in solution; TAN is the total ammonia nitrogen (g/L); DM is the dry matter content (%), and T is the temperature in °C.

$$ pKa = \frac{0.09018+2729.92}{273.15+T}\qquad(5) $$

$$ f_{Emerson}=\frac{10^{-pKa}}{10^{-pH}+10^{-pKa}}\qquad(6) $$

$$ [NH_3]NH_{TOT}\times f_{Emerson}\qquad(7) $$

2.5 Effect of Ammonia on Different Pathogen Groups

Finally, not enough reliable treatment data in sanitation-relevant matrices are available on the inactivation of protozoa by ammonia, therefore no conclusive statements can be made regarding the effect of ammonia on this important pathogen group. The few studies performed to date indicate that protozoa are more resistant than gram negative bacteria and less resistant than *Ascaris* spp. (Hoglund and Stenstrom, 1999; Jenkins et al., 1998).

As can be seen in Figure 8, there appear to be two separate fractions among viruses that vary in their susceptibility to ammonia. Further refinement of the virus data reveal the strong influence of genome type and host range on NH_3 susceptiblility (Figure 9). ssRNA viruses, especially the enveloped viruses (e.g. avian influenza virus) are the most sensitive, followed by ssRNA phages (e.g. MS2). This is followed by dsRNA and dsDNA viruses (i.e. reovirus and adenovirus, respectively) and finally ssDNA phages (e.g. phiX174) and other dsDNA phages (e.g. 28B). Among the viruses of public health concern, treatment conditions should thus be chosen that target human viruses that do not possess a ssRNA genome, such as rotaviruses or adenoviruses.

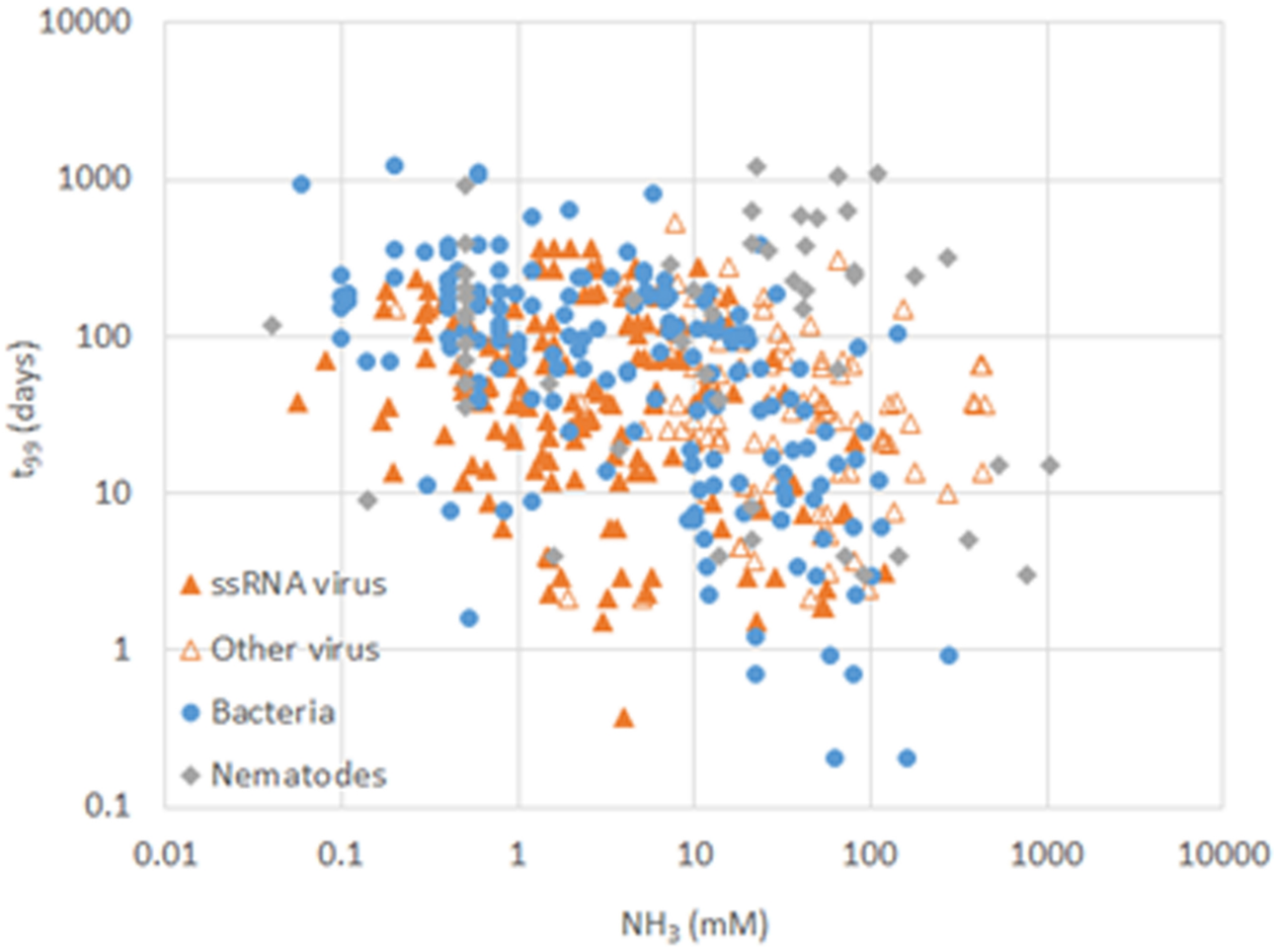


Figure 8. Treatment time to achieve a 2 log_{10} inactivation (t_{99}) vs. NH_3 concentration, for all pathogen types (n=526). Where T99 was not reported, it was extrapolated from the data. The references used to create this plot are indicated in the reference section.

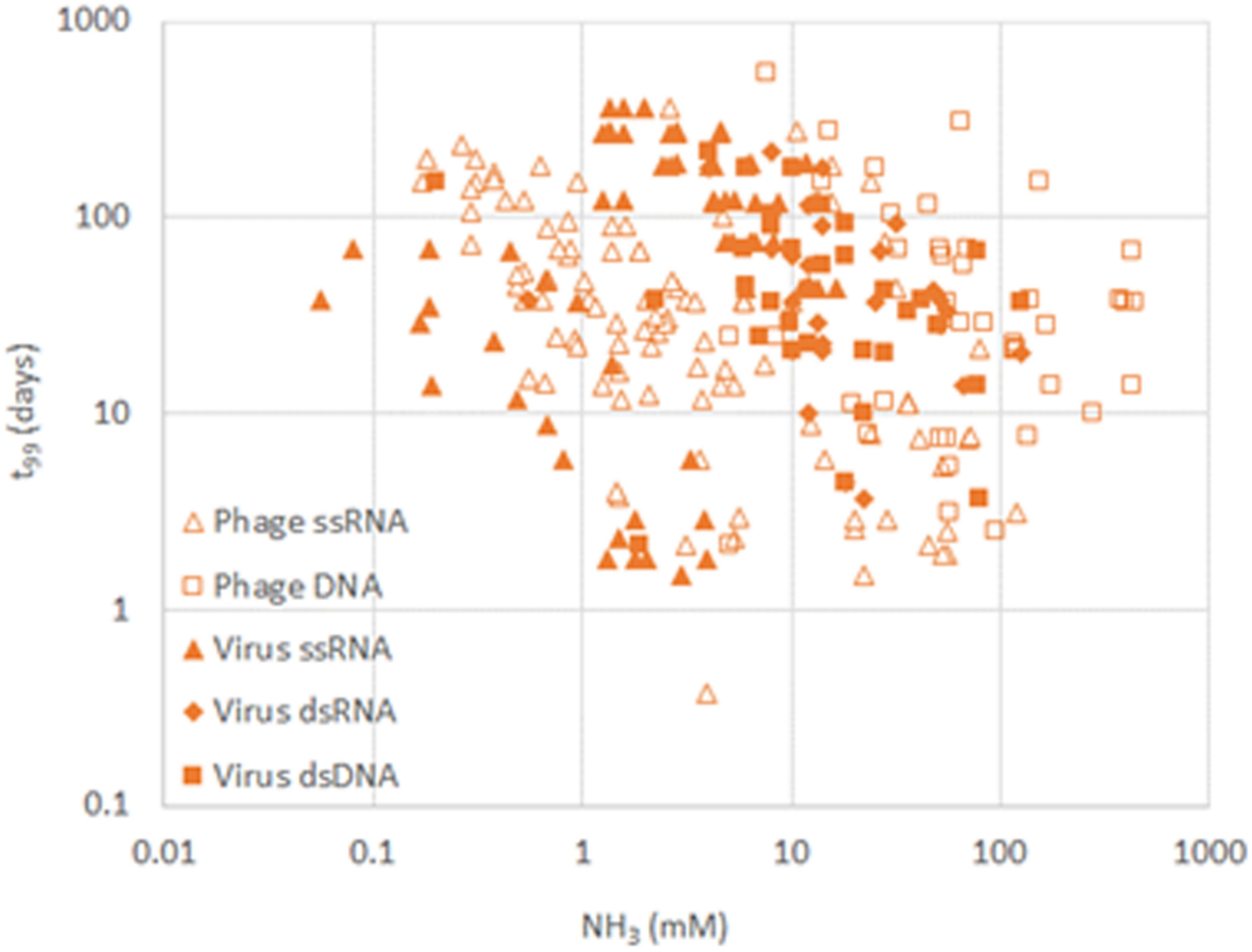


Figure 9. Treatment time to achieve a 2 log_{10} inactivation (t_{99}) of mammalian viruses and phages, separated by genome type.

2.6 Effect of Matrix Conditions

Ammonia treatment depends not only on the ammonia concentration and treatment time, but also on the temperature, pH and the total solid content. These three parameters are further evaluated in the following.

2.7 Temperature

The effect of temperature on the efficacy of ammonia can be assessed by comparing the NH_3 exposure (NH_3 concentration*t_{99}) as a function of temperature. Figure 10 shows such an evaluation for *Ascaris*. It is evident that the ammonia sensitivity increases as a function of increasing temperature: the Ct at temperatures below 10°C is considerably higher (>10,000) compared to temperatures above 20°C where the Ct is less than 1,000. The underlying reason for this effect may include that lower temperatures reduce the reaction kinetics of ammonia with biomolecules, but also change the characteristics of the lipids in the membranes of the organisms, which makes them less permeable (Fidjeland et al., 2016).

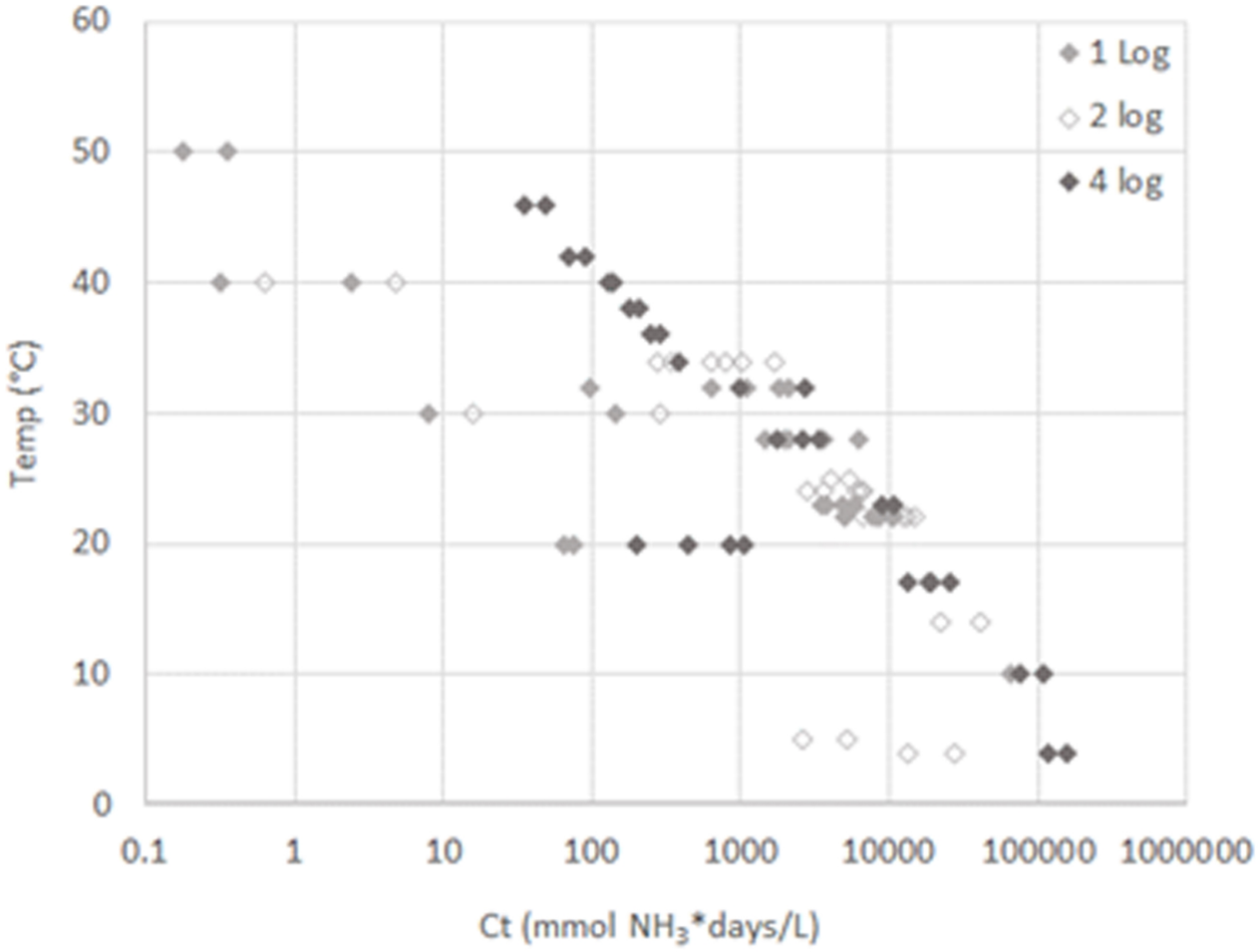


Figure 10. NH_3 exposure (NH_3 concentration*T99) required to reach a 1, 2, or 4 log_{10} inactivation of Ascaris spp. as a function of the treatment temperature.

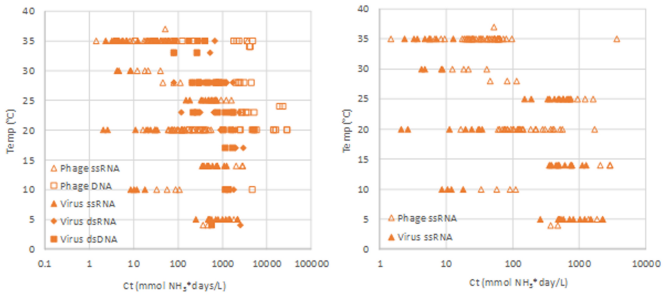
Fidjeland et al. (2016) presented an empirical equation to estimate the treatment time t to achieve a given log_{10} removal (LRV) of *Ascaris* in relation to the ammonia concentration and temperature (equation 8).

$$ t=\frac{3.2+LRV}{10^{-3.7+0.0062\ast T}\ast NH_3^{0.7}}\qquad(8) $$

At a temperature of 20°C, an ammonia concentration above 50 mM is required to inactivate *Ascaris* spp. by 3 log_{10} within one year. At temperatures below 20°C, ammonia concentrations above 100 mM are recommended, while higher temperatures are efficient with lower concentration. At temperatures above 40°C, thermal inactivation is the dominant inactivation process, such that additional ammonia does not significantly enhance inactivation. Finally, it should be noted that the inactivation of *Ascaris* by ammonia includes an initial lag-phase (Fidjeland, 2015; Nordin, 2010), during which only very slow inactivation is achieved. Shorter treatment times thus do not lead to proportionally lower inactivation, but may result in no inactivation at all.

Inactivation-enhancing effects of temperature have also been reported for viruses (Decrey et al., 2015, 2016). These effects are not immediately evident if all virus types are considered (Figure 11, left panel), likely because the NH_3 susceptibility of different virus types is affected by temperature to different extents (Decrey et al., 2016). If ssRNA viruses are considered separately, however, a trend toward lower NH_3 exposures is evident as the temperature increases from 5-35ºC (Figure 11, right panel). These effects are, however, less pronounced than the temperature effects on *Ascaris* spp.

Figure 11. NH_3 exposure (NH_3 concentration*T99) required to reach a 2 log_{10} inactivation of mammalian viruses and bacterial phages as a function of the treatment temperature. Left panel: all genome types. Right panel: ssRNA viruses and phages only.



A similarly small effect of temperature was also observed for the inactivation of bacteria by ammonia (Figure 12). As for viruses, temperature thus does not appear to be an important design variable for the inactivation of gram negative bacteria. It should be noted, however, that some of the Ct values for high temperatures may be overestimated, as inactivation was too rapid to be quantified accurately in some studies.

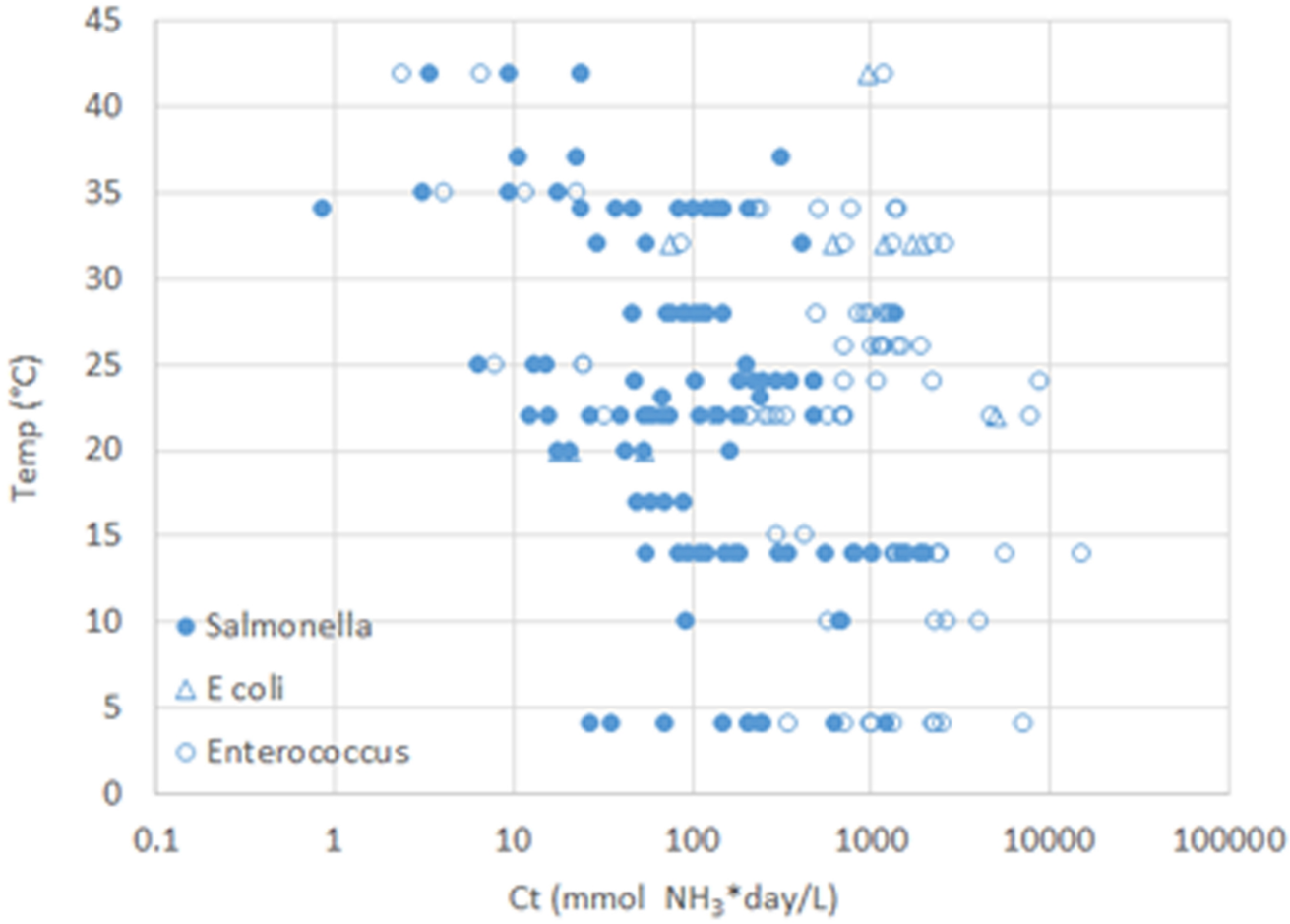
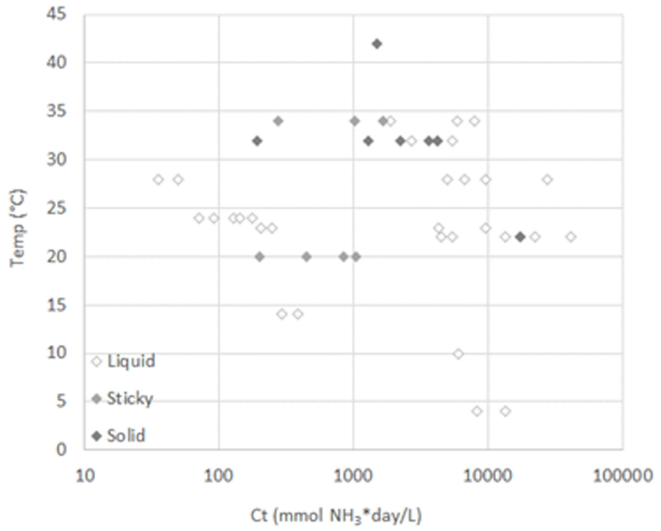


Figure 12. NH_3 exposure (NH_3 concentration*T99) required to reach a 2 log_{10} inactivation of bacteria as a function of the treatment temperature.

2.8 Solids Content

Most of the studies on ammonia treatment were performed on liquid matrices (total solids <12%). Solids, however, may shield pathogens from inactivation; they furthermore influence the pH and hence the ammonia content of the treatment (Figure 7). Here, we therefore present an assessment of the influence of the solids content on inactivation by ammonia. For this purpose, matrices were divided into three groups: liquid (total solids <12%), sticky (12-30%) and dry (>30%). For Ascaris, no effect of solids was observed (Figure 13). This is consistent with findings that only levels of >90% solids will promote *Ascaris* inactivation, due to desiccation. Similarly, bacterial inactivation was not influenced by the solids content. For viruses, insufficient data were available to assess the effect of solids.

Figure 13. NH_3 exposure (NH_3 concentration*T99) required to reach a 2 log_{10} inactivation of Ascaris, as a function of solids content and temperature of the matrix. Note that the effect of solids on pH, and hence NH_3 content, is not visible from this graph, as these effects are accounted for by using Ct, rather than added ammonia, on the x-axis.



2.9 Appropriate Indicators

Given the relatively tight trends in susceptibility to ammonia within the individual groups of organism, it appears feasible to track the inactivation of pathogens by using an indicators of the same organism group. For example, *Salmonella* spp. and *Escherichia coli* are very similar in their response to ammonia, and *E. coli* could therefore serve as a model/indicator for the treatment regarding the inactivation of *Salmonella* spp. (Figure 12).

For the inactivation of viruses, ssRNA phages (e.g. MS2) may serve as indicators for the treatment of ssRNA mammalian viruses, albeit as conservative ones: while phages follow the same overall trends in ammonia inactivation as mammalian viruses, they are generally somewhat more resistant (Figures 9 and 11). For non-ssRNA viruses, we recommend using a DNA phage as a conservative indicator, as ssRNA phages are frequently more labile in comparison.

2.10 Conclusions and Treatment Recommendations

Even though the data presented herein were taken from different studies and a wide range of matrices, the data is surprisingly consistent across most studies with respect to the treatment efficiency of ammonia on different organisms. Furthermore, the compiled data set is fairly large and includes a wide range of species. As such, we can formulate some conclusions regarding the use of ammonia for sanitation, and formulate some treatment recommendations:

Benefits of ammonia:

1. Treatment that is easy to apply, can be applied safely (using urea or intrinsic ammonia), and yields a high-value fertilizer product

2. Treatment can be applied to both small and large volumes

3. Treatment can be applied to matrices with different solid contents

4. Ammonia is effective against all organism groups studied to date, in particular ssRNA viruses and gram-negative bacteria

Detriments of ammonia:

1. Treatment can be slow and requires long treatment times, especially for Ascaris and non-ssRNA viruses.

2. Low treatment temperatures <20°C require even longer time of treatment, especially for the above mentioned organisms.

Recommendations to ensure optimal ammonia treatment:

1. Ammonia treatment should be designed to inactivate *Ascaris* spp. If *Ascaris* is not a pathogen of concern, then viruses with non-ssRNA genomes or gram-positive bacteria are limiting pathogens.

2. Ammonia treatment is most efficient at high NH_3 concentrations and high pH

3. Due to high buffering capacity of organic material, matrices with a high solids content requires higher ammonia additions to reach ideal pH and NH_3 concentration.

4. Temperatures should be maximized if *Ascaris* is the limiting organism. For bacteria and viruses, temperature is a secondary design variable

5. Recommended treatment conditions

   a. NH_3 concentrations for efficient treatment on a wet weight basis is 0.75% NH_3 (corresponding to 1.5% urea or 3% water solved ammonia (25%)),

   b. pH control is recommended to assure

pH>8.8.

c. Recommended treatment times are

i. T <20°C 6 months

ii. T <30°C 2 months

iii. T >30°C 0.5 month.

3.0 Lime

Lime treatment is a classic high pH treatment, which has been used on sludge, surfaces and liquids for over a century. The goal of lime treatment is to raise the pH of the matrix above 10, a value at which most organisms cannot survive. However, even if only a lower pH value is achieved by liming, the treatment can still be effective, because even a small increase in pH leads to higher concentrations of basic, biocidal matrix constituents, such as NH_3 and carbonate. These bases in turn contribute to pathogen inactivation (see section on ammonia treatment). Lime treatment thus has multiple routes by which inactivation can be achieved, though they are all ultimately based on raising the pH.

3.1 Properties of Lime

There are three main types of lime available: lime stone ($CaCO_3$), slaked lime ($Ca(OH)_2$) and burnt lime or quicklime (CaO). Lime stone occurs naturally, mostly in the form of a stone, but also in living organisms such as the shell of snails. Limestone is not ideal for disinfection, because it is slow reacting, and its dissolution leads to the liberation of carbonate, which only yields a small increase in pH. In contrast, slaked and burnt lime are more reactive and liberate hydroxide ion, which is a stronger base. The solubility of lime corresponds to 1.7 g/L of water at 20°C. Typically lime is added in oversaturation, such that a stable pH of 12.5 is reached. Adding more lime will not increase the pH further, but will stabilize the pH over a longer time period. When using burnt lime, the pH increases in the same manner as when adding slaked lime. In addition, however, the use of burnt lime results in an increase in temperature due to an initial exothermic reaction of CaO with water to yield $Ca(OH)_2$, temperatures above 70°C can be reached.

3.2 Mechanisms of Inactivation

High pH inactivates bacteria and parasites by raising their intracellular pH, which ultimately leads to a collapse of the intracellular functions. Alternatively, the organisms may starve to death because all their energy must be spent on actions that compensate for the high extracellular pH. For example, most non-extremophilic bacteria maintain a stable internal pH of about 7.4-7.9, but survive or even grow over a considerably larger external pH range of 5.5-9 (Padnan et al., 2005). At higher pH levels, cation/proton antiporters in the bacterial cell membrane export cations such as Na^+, in exchange for importing H^+. This energy consuming process is employed to maintain the intracellular pH within an optimal range. In combination with other stress factors such as low redox potential and high temperatures, the sensitivity to high extra cellular pH increases. Therefore the treatment with burnt lime (CaO) can be considerably more efficient compared to slaked lime ($Ca(OH)_2$) as the burnt lime increases not only the pH, but also the matrix temperature.

Viruses does not have any metabolic activity outside their host cell, and as such, the effects of high pH must involve a direct effect on the viral building blocks. Specifically, for ssRNA viruses, hydroxide ions can pass through the protein capsid and can catalyze the cleavage of the genome, in a process analogous to that described for ammonia above. This process is less relevant for DNA viruses and dsRNA viruses (see ammonia section), because these genome types are less prone to base-catalyzed degradation. At pH levels around 10, the dominant inactivation mechanism becomes protein denaturation, which affects viruses of all genome types (Decrey et al., 2016).

3.3 Treatment Considerations and Current Practice

Both slaked and burnt lime are alkaline and highly reactive. In addition, CaO also has strong oxidizing power. As such, both sources of lime can cause a hazard to treatment operators during handling and storage.

For an efficient treatment of enteric bacteria and viruses, a pH above 10 is required, while a considerably higher pH is needed for the inactivation of nematodes. The most pH-resistant pathogen known is *Ascaris* spp., which is only inactivated upon exposure to a pH above 12 for an extended period of time (see “nematodes” in Figure 14). To achieve these high pH values, large quantities of lime are necessary. For example, for the treatment of sludge using $Ca(OH)_2$, at least 12% slaked lime on dry matter basis is used to achieve a long term high pH and efficient hygienisation. For CaO as the lime source, doses of between 20-40% burned lime on dry matter basis must be used for achieving an efficient thermal and high pH.

While alkaline initially, the added lime reacts with carbon dioxide throughout the treatment to form calcium carbonate, which results in neutralization of the pH over time. To test if the treatment is still active, the pH should therefore be monitored periodically. The carbon dioxide mainly stems from biological activity in the matrix, though absorption of atmospheric carbon dioxide can also play a role in the neutralization of the added lime. Furthermore, the addition of CaO dries the material considerably, as the

exothermic reaction of CaO with water consumes approximately 32% by weight of the water in the matrix. To avoid re-wetting of the matrix, minimize the input of carbon dioxide from the atmosphere, and prevent ammonia losses due to volatilization at high pH, the matrix should be treated in a covered reactor.

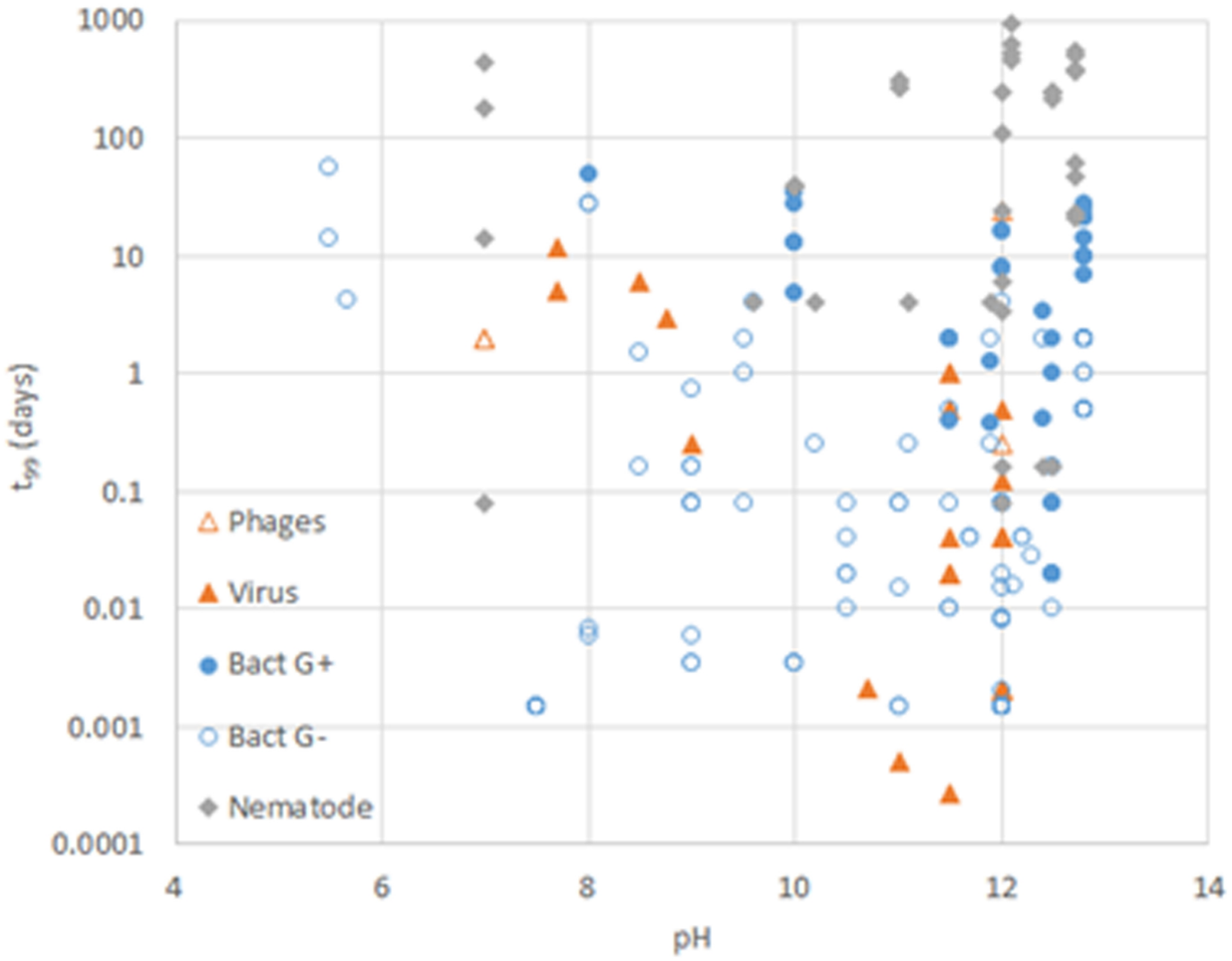


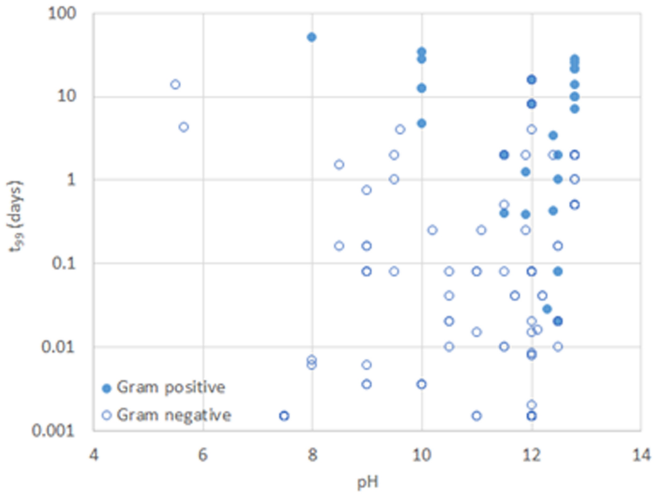
Figure 14. Time needed to achieve a 2 $\log_{10}$ inactivation (T99) of different organisms groups (phages, mammalian virus, gram posistive bacteria (Bact G+), gram negative bacteria (Bact *G*–) and nematodes), as a function of pH. The references used to create this plot are indicated in the reference section.

3.4 Effect of Lime on Different Pathogen Groups

Figure 14 summarizes the lime inactivation for different pathogen groups compiled from the literature. The data reveal a high degree of variability. Yet, some trends can be identified: It is readily evident that for most bacteria and viruses, inactivation above pH 10 is very rapid, with a 2 $\log_{10}$ reduction in infective pathogens reached within a day or less. In contrast, nematodes, mostly *Ascaris* spp., are considerably more resistant, such that higher pH values and longer treatment times are needed and the recommendation for treatment is at least three months of treatment above pH 12.

A more detailed look at bacterial inactivation by pH (Figure 15) again reveals a great variability in the reported data. Nevertheless it can be seen that gram negative bacteria tend to be more sensitive toward high pH than gram positive ones; they often require treatment times of less than 0.1 days to achieve a 2 $\log_{10}$ inactivation.

Figure 15. Time needed to achieve a 2 $\log_{10}$ inactivation of gram positive bacteria (mainly Clostridium spp. and Enterococcus spp.) and gram negative bacteria (G–; mainly Enterobacteriaceae), as a function of pH.



The high variability in the data shown in Figures 14 and 15 likely stems from the multiple effects of lime treatment discussed above: for pH values below 9, a direct effect of pH on any of the organisms is unlikely, as several studies have shown that a pH of 9 alone does not affect the survival of bacteria (Nordin et al., 2009b; Nordin and Vinnerås, 2015; Vinnerås et al., 2008), viruses (Decrey et al., 2016; Decrey et al., 2015; Emmoth et al., 2011; Magri et al., 2015) or the nematode Ascaris spp. (Fidjeland et al., 2013; Nordin et al., 2009a; Pecson et al., 2007). Nevertheless, in some studies included in Figures 14 and 15, rapid inactivation of the investigated organisms was reported at pH 9 or lower. While not explicitly stated in these studies, the observed inactivation at these lower pH values can likely be attributed to the biocidal action of ammonia and carbonate, which is found in most sanitation-relevant matrices, and which is enhanced by increasing pH.

3.5 Effect of Temperature and Lime Type

Differences in treatment temperature during liming often stem from the source of the lime: if burnt lime is used, the exothermic reaction of CaO with water leads to a heating of the matrix. As discussed above, higher temperatures are expected to lead to shorter treatment times. This is also reflected in treatment recommendations by the US EPA (EPA 625-R-92_013, 1992, revised 2003), which state that a treatment with burned lime that reaches 70°C during 30 minutes should be enough for a class A classification of biosolids. A comparison of the efficiency of lime treatment as a function of temperature and lime type is shown in Figures 16 to 18. It should be noted that not all burnt lime treatments successfully elevated the matrix temperature, and none of the treatments were performed at temperatures close to the recommended 70°C.

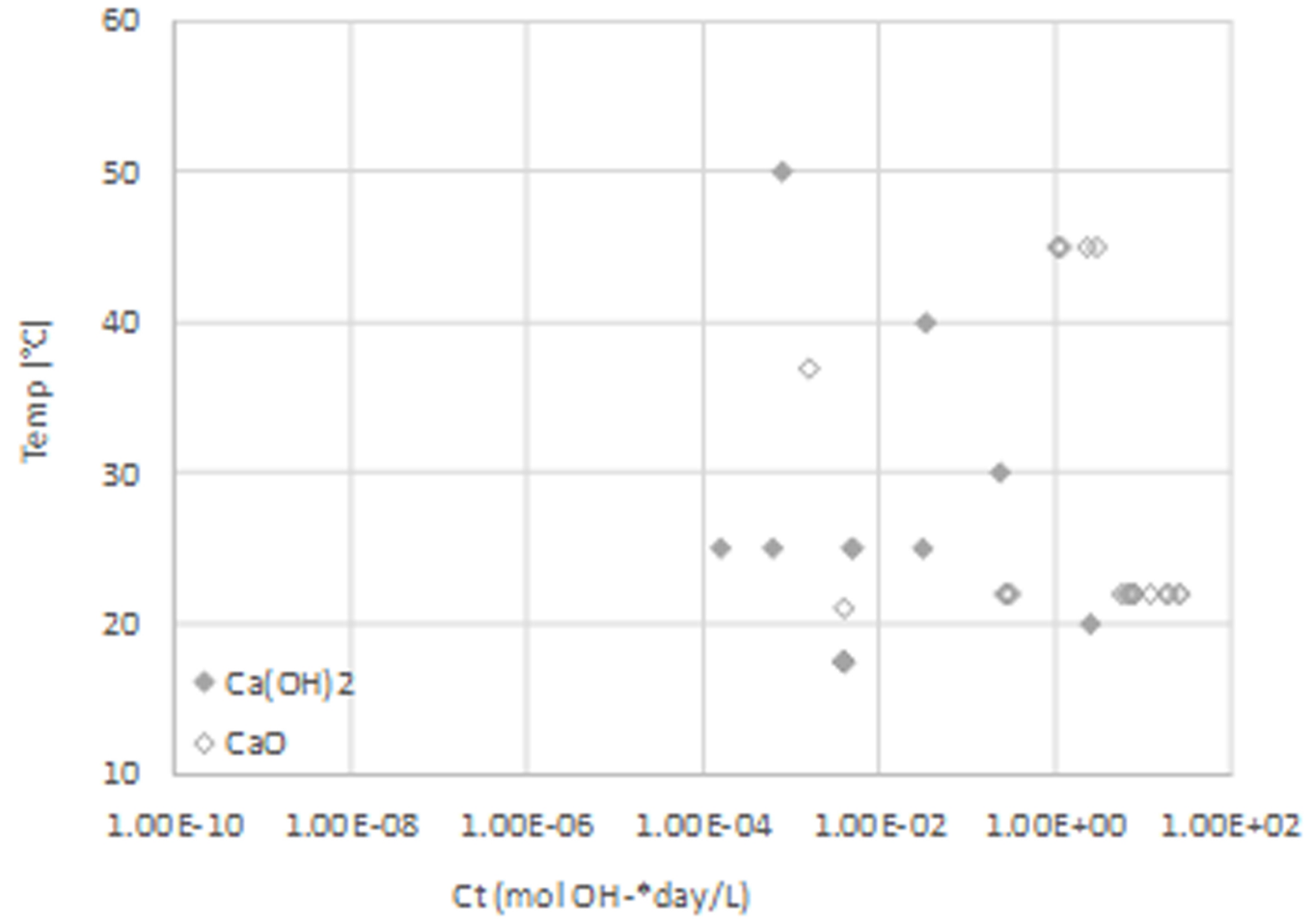


Figure 16. Lime exposure (OH^- concentration added*T99) needed to achieve a 2 log_{10} inactivation of nematodes (mainly *Ascaris* spp.) as a function of temperature and type of lime added.

Contrary to expectations, the data reviewed herein indicate that $Ca(OH)_2$ tends to inactivate nematodes more efficiently compared to CaO (Figure 16), whereas no obvious difference in the inactivation efficiency could be identified for bacteria (Figure 17). This is likely a result of the fact that the studies using burnt lime did not achieve a significant increase in temperature. If the main benefit of burnt lime – the increase in temperature – is not achieved, $Ca(OH)_2$ is thus equally or more efficient than CaO. For most bacteria, lime treatment at lower temperatures is effective enough such that higher temperatures may not be needed to achieve substantial bacterial reduction.

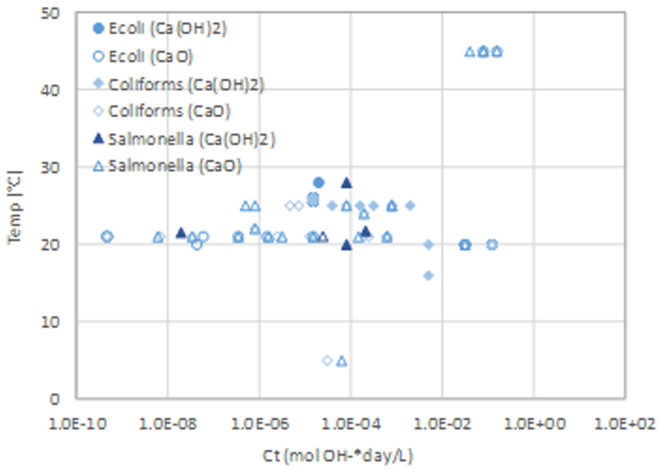


Figure 17. Lime exposure (OH^- concentration added*T99) needed to achieve a 2 log_{10} inactivation of *E.coli*, coliforms (including faecal coliforms) and *Salmonella* spp., as a function of temperature and type of lime added.

For virus inactivation, data were only available for studies using $Ca(OH)_2$. As for bacteria, lime treatment efficiently inactivates most mammalian viruses and phages (Figure 18), even at low temperatures. Inactivation at higher temperatures may be too fast to be experimentally measured, which could explain the lack of reported data.

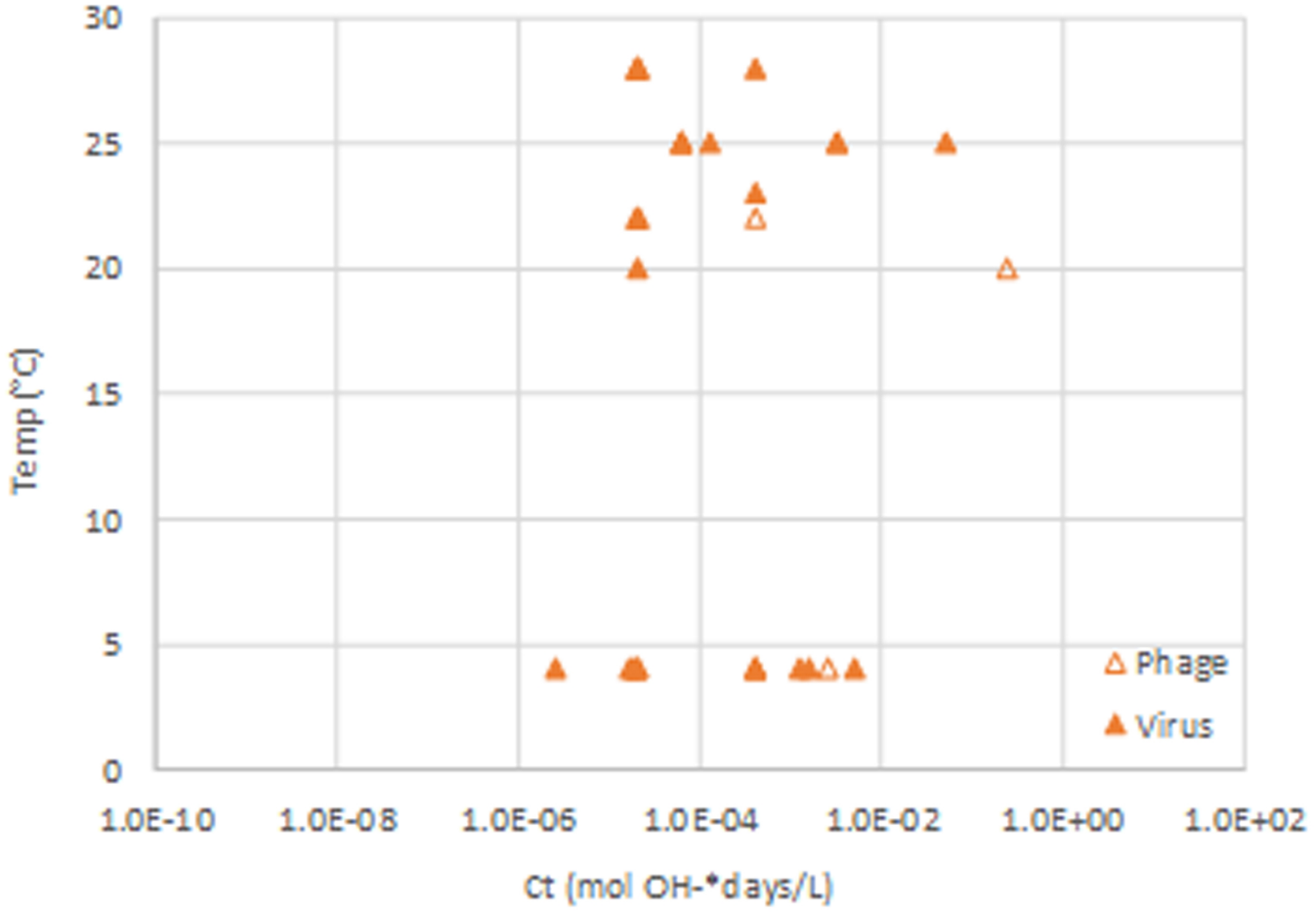


Figure 18. Lime exposure (OH^- concentration added*T99) needed to achieve a 2 log_{10} inactivation of mammalian viruses and phages as a function of temperature.

3.6 Appropriate Indicators

To date, there are insufficient data on the inactivation of pathogenic or indicator viruses and bacteria by lime, such that a conclusive assessment regarding the choice of appropriate indicator organisms is not possible. Data are particularly lacking for the inactivation of phages, which are often used to represent mammalian viruses. Future studies on lime treatment shouldtherefore include ssRNA and non-ssRNA phages, to assess if their inactivation behaviour is representative of the inactivation of the corresponding pathogenic mammalian viruses.

For bacteria, both gram positive (excluding gram positive spores) and gram negative bacteria show some overlap in their inactivation behaviour (Figure 15). Either organism group may thus work as an indicator for the inactivation of any of the other bacteria. Hereby *E.coli*, a gram negative organism, may be more representative of fast inactivating bacteria, whereas *Enterococcus* spp. could serve as a conservative indicator. Further work is needed, however, to confirm these suggestions.

3.7 Conclusions and Treatment Recommendations

While there are a lot of data on lime treatment of sanitation-relevant matrices available, many of these studies could not be included herein, due to a lack of essential data for the treatment and exposure evaluation. Nevertheless, we tentatively state some lime treatment recommendations based on the available literature and the data complied herein:

Benefits of lime:

1. Lime treatment that is easy to implement and apply, as no special conditions other than the high pH must be met.

2. The treatment is size independent.

3. When using burnt lime (or quicklime; CaO), the combination of increased temperature and pH result in a fast treatment (recommendation is 30 min when reaching above 70°C).

4. Some soils benefit from the addition of lime addition and the lime can then be considered to have improved fertiliser value.

Detriments of lime:

1. Lime treatment requires a very high and stable pH in order to be effective against all organisms. This requires a large quantity of lime.

2. Both types of applicable lime are strong, corrosive bases. CaO is additionally a strong oxidant. As such, these additives are hazardous to both humans and the environment.

3. Calcium easily reacts with matrix constituents, e.g. carbonate or phosphate, forming crystals that turn into sediments, which are sometimes hard to mix with the remainder of the material.

4. The high treatment pH risks losses of ammonia and thereby decreases the fertiliser value; therefore coverage is recommended.

5. The pH decreases over time due to reaction of lime with CO_2, which increases the risk for re-growth or recontamination.

Recommendations to ensure optimal lime treatment:

1. Lime treatment should be designed to inactivate *Ascaris* spp., which is the limiting organism. As such, a pH above 12 is required. When *Ascaris* is not an organism of concern, a pH above 10 is sufficient for inactivation of viruses and bacteria. However, it is important to recall that in a non-saturated lime treatment, i.e. pH ≤12, the pH decreases rapidly over time, which limits the exposure time at high pH, and presents a risk for regrowth of bacteria.

2. The temperature should be increased, for example by using burnt lime, to optimize the treatment, in particular of *Ascaris* spp.

3. It is recommended to monitor the pH in the matrix throughout the treatment to ensure that the high pH treatment is still active.

4. Coverage of the treatment is recommended to minimize the loss of ammonia within the substrate and to reduce the introduction of atmospheric carbon dioxide, both to improve the efficiency of the treatment and to keep a higher fertiliser value.

5. $Ca(OH)_2$ dosing: typical doses of at least 12% slaked lime on dry matter basis are used to achieve a long term high pH and efficient disinfection of sludge.

6. CaO dosing: typical doses of between 20-40% burned lime on dry matter basis are used for achieving an efficient thermal and high pH disinfection of sludge.

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