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Modeling the inactivation of ascaris eggs as a function of ammonia concentration and temperature

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ABSTRACT

Ammonia sanitization is a promising technology for sanitizing human excreta intended for use as a fertilizer in agriculture. Ascaris eggs are the most persistent pathogens regarding ammonia inactivation and are commonly present in fecal sludge in low- and middle-income countries. In this study, a model for predicting ammonia inactivation of ascaris eggs was developed. Data from four previous studies were compiled and analyzed statistically, and a mathematical model for the treatment time required for inactivation was created. The inactivation rate increased with NH_3 activity to the power of 0.7. The required treatment time was found to decrease 10-fold for each 16 °C temperature increase. Dry matter (DM) content and pH had no direct effect on inactivation, but had an indirect effect due to their impact on NH_3 activity, which was estimated using the Pitzer approach. An additional model giving an approximation of Pitzer NH_3 activity but based on the Emerson approach, DM content and total ammonia (NH_{Tot}) was also developed. The treatment time required for different $\log_{10}$ reductions of ascaris egg viability can thus easily be estimated by the model as a function of NH_3 activity and temperature. The impact on treatment time by different treatment options can then be theoretically evaluated, promoting improvements of the treatment e.g. by adding urea or alkaline agents, or increasing the temperature by solar heating.

1. Introduction

There are currently 840 million people in the world suffering from chronic hunger, and with a growing world population there is a need for increased food production (FAO et al., 2013). Lack of fertilizer is often a limiting factor for food production, as the high cost of industrial fertilizers usually makes them unavailable to small-scale farmers. Moreover, global phosphorus resources are limited and nitrogen fixation is energy-demanding and the price fluctuates with energy prices (Steinbuks and Hertel, 2013).

An alternative to industrial fertilizers is to recycle plant-available nutrients in waste fractions such as food waste, animal manure, and human excreta. The content of pathogens is one of the major constraints for the use of human excreta as a fertilizer in food production. Thus, there is a need for technologies to inactivate pathogens in order to safely recycle the nutrients (Rijsberman and Zwane, 2012). One treatment option of interest is ammonia sanitization, as it is a simple and scale-independent alternative which can inactivate the pathogens in human excreta at a low cost if no or low volumes of flush water are used. Ammonia sanitization utilizes the pathogen-inactivating effect of uncharged ammonia (NH_3) (Warren, 1962), which has been shown to inactivate bacteria (Park and Diez-Gonzalez, 2003), viruses (Emmoth et al. 2011), protozoa (Jenkins et al. 1998), and helminth eggs (Nordin et al., 2009; Pecson and Nelson, 2005). At ambient storage temperature, helminth eggs are the most resistant pathogens to ammonia inactivation, with the exception of *Clostridium* spp. spores (Vinnerås et al. 2003), which are not considered to represent a health risk in this context (Sherpa et al. 2009).

Approximately 890 million people are infected annually with *Ascaris lumbricoides* (Pullan et al., 2014). Ascaris eggs are typically spread in the environment due to lack of adequate sanitation, as the fecal waste from 4.1 billion people is discharged to the environment without treatment, typically by indiscriminate emptying of sewage trucks or open defecation (Baum et al. 2013). The eggs are persistent and can remain infective in the soil for years (Papajova et al.,

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2008). Ascaris eggs are generally used as an indicator of helminth egg inactivation, as they have been found to be equally or more persistent than eggs of other genera, including whipworm (*Trichuris trichiura*) and hookworm (Ghiglietti et al. 1995). To enable the safe reuse of human excreta as a fertilizer in areas where helminth infections are endemic, it is critical to have a reliable treatment to inactivate helminth eggs.

Ammonia sanitization is a simple technology that only requires a source of ammonia and an airtight storage, so that the uncharged ammonia is not lost to the atmosphere. In fecal sludge from toilets with low or no water use, the concentration of intrinsic ammonia from the urine may be sufficient for inactivation (Fidjeland et al., 2013), but more ammonia in the form of urea can be added to enhance the treatment. The addition of urea increases the fertilizer value of the final product, but due to the cost it is desirable to add as little urea as possible. Another option to enhance the sanitization is to increase the temperature of the storage facility with solar heating (Nordin et al., 2013). Adding an alkaline agent such as lime or ash also enhances ammonia sanitization, because higher pH shifts the ammonia equilibrium such that a higher proportion of ammonia is uncharged (NH_3), which is the form that acts as a disinfectant. A major cost is the storage facility, so to reduce the cost the storage time should be as short as possible. Given the potential for ammonia sanitization as a low-cost disinfection method, it is important to be able to predict the time required for ascaris egg inactivation as a function of the main variables that affect treatment efficiency, such as temperature, ammonia concentration, and pH.

Several studies on ammonia inactivation of ascaris eggs are using *Ascaris suum* eggs as a model organism, as they have been shown to have similar inactivation to *Ascaris lumbricoides* eggs (Ghiglietti et al., 1995). The inactivation observed for a treatment at a given temperature and ammonia concentration typically has an initial lag phase with slow inactivation, followed by an exponential phase with a more rapid inactivation. This two-phase inactivation is described by equation (1) (Harm, 1980), where the inactivation rate, k, is the inverse of the decimal reduction time (t_{90}) during the exponential phase. The parameter n determines the lag phase, and is the ratio between lag phase l and t_{90} (equation (2)).

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

$$n = \frac{l}{t_{90}} = l\cdot k \quad (2)$$

Several studies have used eqs. (1) and (2) to describe ascaris egg inactivation at different ammonia concentrations and temperatures, but the values of k, n, and lag phase duration have not yet been generalized as functions of treatment parameters (Fidjeland et al. (manuscript), Fidjeland et al., 2013, Nordin 2010, Pecson et al., 2007). Therefore, the aim of this study was to develop a model to predict the inactivation of ascaris eggs as a function of NH_3 concentration, temperature, and other potential physical or chemical parameters such as matrix, dry matter (DM) content, carbonate and pH, through regression analysis of previously published data. An important part of this analysis was to identify the factors which directly affect ascaris egg inactivation and the factors which indirectly impact inactivation through influencing the ammonia equilibrium.

The concentration of uncharged ammonia, NH_3, is often estimated by the Emerson approach, in which it is based on NH_{TOT}, temperature, and pH (Emerson et al., 1975). A more precise estimation of the actual NH_3 activity can be made using the Pitzer approach, in which it is based on the concentration of ionic species and their ion-specific interaction, in addition to pH and temperature (Pitzer, 1991). However, this method is less applicable, as it requires software, a library of Pitzer parameters, and knowledge of the actual concentration of the ions present in high concentrations in the solution. An additional aim was therefore to develop a mathematical relationship between NH_3 activity estimated with the Pitzer approach and with the Emerson approach.

2. Model construction

2.1. Inactivation model

2.1.1. Preliminary model

Data from several studies on ammonia inactivation of ascaris eggs (Table 1) were summarized in a dataset containing estimates of the inactivation rate k and parameter n in eq. (1) from time series of ascaris egg viability measurements with constant temperature and NH_3 concentration. The inactivation data were first reanalyzed by non-linear regression, fitting them to the lag phase inactivation formula (eq. (1)). Linear regression was used for cases with negative estimated lag phase, or only two data points per time series. The statistical analyses were performed with the statistical software R v. 2.14.0 (R Development Core Team, 2014).

The activity of uncharged ammonia, NH_3, was estimated with the Pitzer approach using PHREEQC Interactive v. 3.1.1 and an improved database with Pitzer parameters (Wächter et al. in prep.). Literature data were used to estimate the concentration of the most important ions in urine and feces (Jönsson et al., 2005; Nishimuta et al., 2006; Putnam, 1971). The concentrations of ions were assumed to be proportional to the dry matter content for feces and to the ammonium concentration for urine. This assumption was used to provide a rough estimate of the concentration of ions; the actual concentrations were not measured, but were assumed to be varied and diet-dependent. The ions included in the estimation, and their relative concentration in feces and urine, are listed in Table 2. The concentration of carbonate in urine was assumed to be half the concentration of ammonia, based on the stoichiometric relationship from degradation of urea. The concentrations of total ammonia (NH_{Tot}, mM) and total carbonate in the water phase of the matrix was calculated according to eq. (3), where TAN is Total Ammonia Nitrogen (mg/L), prior to using Pitzer approach for estimation of activity of species.

$$NH_{Tot} = \frac{TAN}{14.01}\cdot\frac{1}{(1-DM)} \quad (3)$$

The inactivation rate k was then plotted as a function of NH_3 activity for the different temperatures studied (Fig. 1). Linear regression with intercept at 0 was used to describe the inactivation rate k as a function of NH_3 activity. Data series with a non-optimal sampling frequency and no detected lag phase, or slow inactivation which never reached the exponential phase during the study period, could have resulted in the inactivation rate k being underestimated and therefore these data points were not included in the linear regression (Fig. 1).

By plotting the inactivation rate against NH_3 activity for each temperature separately, it was found that the inactivation rate could be described as increasing linearly with the NH_3 activity (Fig. 1). The inactivation rates could thus be described by equation (4):

$$k_T = v_T\cdot NH_3 \quad (4)$$

The parameter v_T showed a clear temperature trend, and a linear relationship was found between the logarithm of v_T and temperature in the range 5–42 °C ($R^2 = 0.99$) (Fig. 2). For temperatures above 42 °C, the v_T deviated from the trend (Fig. 2), and data from