6

Sanitation and Hygiene in Manure Management

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6.1 Hygiene Risks Associated with Manure Management

Manure management is essential for sustainable use of plant nutrients. The flows and treatments of manure should be as short as possible in order to optimise recycling of the plant nutrients in manure, as each treatment step causes losses of nutrients. However, to minimise the transmission of pathogenic microorganisms the flows should be as long as possible, as each additional step decreases the risk of disease transmission during management of manure and from infected crops.

There are several factors affecting the risk of disease transmission via the flow of plant nutrients in manure from the animal into the food or feed. The first is the health status of the animal, as the risk of disease transmission is lower from healthy animals than from sick animals. During management a risk factor is the age of the manure, as in most cases the pathogen load decreases over time. The disease-causing organisms can either be animal-specific or zoonotic and infect several species. The zoonotic diseases affect manure management by requiring greater awareness, as the risk of transmitting diseases along the food chain is higher than with non-zoonotic diseases. The animal-specific diseases can be controlled in manure management by application to food or feed crops consumed by other species, while the zoonotic diseases require other barriers. A typical example of this is the VAC farming system (VAC is the abbreviation of the Vietnamese words *vuon*, *ao*, *chuong*, which mean garden, pond, livestock pen), where the manure is used for production of algae, which in turn are used as fish food (Chapter 2). This practice has been shown to contaminate the skin of fish with *Enterobacteriaceae* (e.g. *Salmonella* spp.) that can affect people along the food management chain as the bacteria are transmitted to the hands of those handling the dead and live fish

Animal Manure Recycling: Treatment and Management, First Edition. Edited by Sven G. Sommer, Morten L. Christensen, Thomas Schmidt and Lars S. Jensen.

(Yajima and Kurokura, 2008). The fish meat is not affected by this (Son Thi Thanh *et al.*, 2011), but the risk of transmitting pathogenic bacteria to the fish meat during handling is high.

Method of transfer and application of manure also affect the risk of disease transmission (e.g. the risk of transmission to the feed or food crop with direct incorporation into the soil is 99% lower than with surface spreading). In the field, the crop or vegetable to which the manure is applied is a factor affecting the risk of disease transmission. The greatest risks are associated with plants where edible plant parts that are close to the soil are consumed raw (e.g. lettuce and cucumber), while the risks are lower with crops that are processed prior to consumption (e.g. barley for beer production) or even crops that are not consumed at all (e.g. energy crops).

The main barrier to controlling the pathogen level in the manure is treatment prior to application, to ponds or to fields. This lowers the risk of disease transmission later in the manure management chain considerably compared with untreated manure. The focus of current manure management is therefore on treatment before transferring manure to the end use in the field.

See Text Box – Basic 6.1 for definitions used in this chapter.

Text Box – Basic 6.1 Definitions

Animal byproduct (ABP): Term used in EU regulations for organic waste of animal origin that may be hazardous.

Zoonosis: An infectious disease that is transmitted between species (animals to humans).

D_T: Decimal reduction time value. Equal to reduction of 90% of the population, can also be written as 1 $\log_{10}$ reduction.

Pathogen reduction is often written as the number of logarithms it is reduced over a time period/treatment. This is often written as a reduction of 1,000 times is written as 3 $\log_{10}$, and is equal to 99.9% reduction.

Hydraulic retention time (HRT): A measure of the average length of time that a soluble compound remains in a constructed bioreactor:

$$\mathrm{HRT} = \frac{\text{Reactor volume } (\mathrm{m}^3)}{\text{Influent flowrate } (\mathrm{m}^3\ \mathrm{h}^{-1})} \tag{6.1}$$

Minimum retention time (MRT): A measure of the minimum time between feeding a reactor and detecting any part of the feedstock in the effluent.

6.2 Why Must the Pathogens in Manure be Managed?

When a disease is present in a herd on a farm, the main route of transmission is via animal-to-animal contact. When the disease has decreased in the herd, other transmission routes become important (e.g. fields, infected buildings). It is important to note in manure management that if the disease is zoonotic (i.e. can infect humans as well as animals, such as *Salmonella* spp.), there is a health risk to workers applying the manure to the field.

For the animal-specific diseases there is a barrier to disease transmission as the contaminated manure needs to find its way back to the original host animal. The major risk of transmission here is from animal to animal. However, at the farm level there is a risk of disease transmission via the manure when the disease in the herd has gone, as disease can come back via the manured fields and fodder crops. In addition, the management practice of placing dead animals on the manure heap or in the manure tank increases the risk of maintaining a high contamination level within the manure. However, there is a larger risk with selling the meat of the dead

animals, both regarding transmission of zoonotic and non-zoonotic disease, as the animals are transported longer distances and often distributed into multiple homes.

For controlling the spread of disease spread from dead animals, it is important to have proper management. The carcass can be composted at the farm level, as long as there is no risk of further spread of the disease. This can be performed by building a large compost heap that covers the animals, preferably with a large base combined with a plastic lining. This means that a high temperature is generated in the full compost mass and ensures that the fluids produced remain in the compost (Berge *et al.*, 2009). This can also be performed within the animal house if necessary, especially with avian flu, which is so highly contagious that transport of the contaminated dead animals and the manure should be avoided. However, the avian flu virus is readily inactivated even at mesophilic temperatures (Elving *et al.*, 2012).

Additional risks from applying contaminated manure are the risk of disease transmission to a larger animal group, including wild animals, and transmission back to farm animals. The microorganisms applied to fields with manure can be expected to survive for long periods. For example, after application of chicken manure containing *Salmonella* Typhimurium to soil in one study, large numbers of the pathogen were measured for 3 months, after which period the numbers declined by 99.99% (a 10 000-fold or 4 $\log_{10}$ reduction), while in cattle slurry and human urine applied to soil the survival was shorter, 60 and 15 days, respectively (Nyberg *et al.*, 2010). The study also showed that high concentrations of easily available carbon in the manure increased the survival of *S.* Typhimurium in the soil. However, when enrichment methods were used to look for salmonella in 25 g soil, *S.* Typhimurium was still detectable after 180 days in soil with a mean air temperature of 15–20 °C during the first 90 days, followed by decreasing temperatures down to near zero at day 180. This indicates that application of contaminated manure to the soil increases the risk of disease transmission both to crops and to water over a long time.

6.2.1 Manure Treatment

Manure management differs between countries, as does the attitude towards the manure. In the Nordic countries, manure is considered a resource and is used as a fertiliser in agriculture, especially in organic farming where other plant nutrient sources are less available and most often costly. Worldwide, livestock production is becoming more specialised (Steinfeld *et al.*, 2006), and in many parts of the world it is becoming decoupled from plant production and manure is considered to be a waste and is managed accordingly.

Irrespective of how the manure is considered, it has to be managed and either used as a fertiliser or treated as wastewater. Treatment results in production of a solid fraction that is often sold as manure and a water fraction that ends up in the water recipient (Vanotti *et al.*, 2009). In other places the solid manure is just dumped; for example, Komakech *et al.* (2013) found that manure management in and around Kampala city, Uganda, comprises two main pathways: (i) one-third is used as a fertiliser on-farm or sold as a fertiliser (at a price just covering the removal costs), and (ii) between half and two-thirds are discarded locally (mainly for the rain to flush away) or dumped in landfill.

6.2.2 Expression of Pathogen Reduction

The standard commonly used for expressing the reduction in pathogens in animal waste and other organic waste products is the decimal reduction (90%) of the microorganisms over time (D_T). D_T has units of time, which can be seconds, minutes, hours and so on, depending on the units used when measuring the reduction. This value is determined empirically, by relating the reduction in the number of pathogens in a material over time (Figure 6.1), especially in a non-categorised material. However, most materials and organisms have been evaluated in different processes, which may include temperature treatment, addition of chemicals and so on,

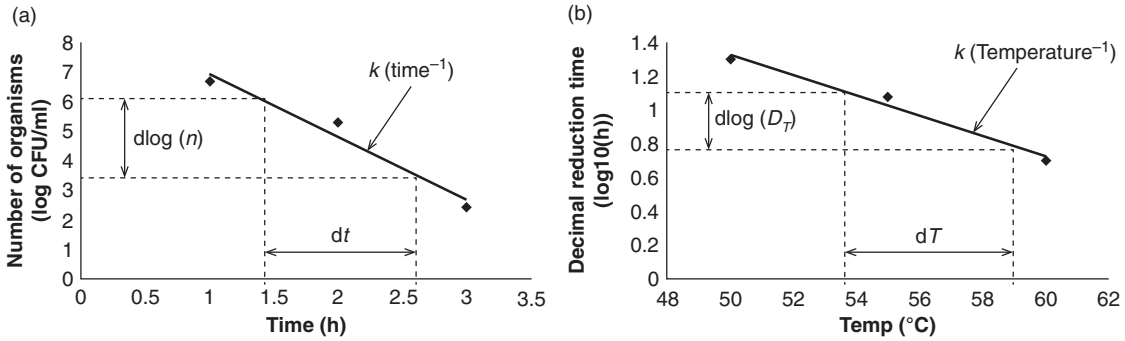

Figure 6.1 *Reduction in number of pathogens as affected by temperature during treatment and treatment time. (a) Log number of pathogens measured in relation to time. (b) The slope of log number of pathogens* (D_T) *as a function of temperature, giving Z.*

and the set-up is often not standardised. Consequently, different times of inactivation have been reported for similar treatments due to the design of the study:

$$k_d = \frac{d\log(n)}{dt}(\text{time}^{-1}) \tag{6.2}$$

$$D_t = \frac{1}{k_d}(\text{time}) \tag{6.3}$$

$$Z = \frac{dT}{d\log(D_T)} \tag{6.4}$$

The development of a D_T value is based upon the log reduction (90%) of a single organism over time at a specific temperature. The larger the number of analyses, the more accurate the estimated slope of the decay, which is determined by performing a curve fitting for a log-normal reduction curve (log number of pathogens versus time; Figure 6.1). The slope should be estimated based on at least three data points and the value of the correlation coefficient for the linearity of the survival curve should not be less than 80%. The k_d value (the slope of the reduction in organisms over time), with units time^{-1}, is calculated according to Equations (6.2) and (6.3). In Equation (6.2), $d\log(n)$ (the change in number of organisms between time t_0 and t_1) is divided by dt (the difference between time t_0 and t_1). The relationship between the decimal reduction time (D_T) value and the slope (k_d) of the reduction is according to Equation (6.3).

The D_T value is specific for each organism at each temperature and the relationship between the D_T values at different temperatures is called the Z value, which is defined as the number of degrees Celsius required to change the D_T value by a factor of 10 (i.e. a decimal reduction; Figure 6.1b). The correlation between log D_T and temperature is log-normal according to Equation (6.4).

As with the calculation of the D_T value, the Z value needs three D_T values for calculation and the slope of the curve k_z has the same relationship to the Z value as the D_T value has to k_d (Equation 6.3).

The time required for a decimal reduction can be determined in this way. In the EU regulations on animal byproducts (ABP) (EC1069/2009; EC, 2009) and on treatment of ABP (EC142/2011; EU, 2011), the recommendation for treatment of ABP category 3 and manure to be traded (category 2) is a treatment validated to reduce *Enterococcus faecalis* or *Salmonella* Senftenberg (775W, H_2S negative) by five decimals (i.e. by 5 $\log_{10}$). If viruses are considered a risk in the system, a thermotolerant animal virus (e.g. parvovirus) should be reduced by 3 $\log_{10}$.

For chemical treatment, the relationship between temperature and added chemical is more complex, and in most cases cannot be estimated with the simple linear logarithmic reduction correlation found during high-temperature treatment. The effect of chemical treatment can often have an initial lag phase with little or no reduction before a faster reduction phase that is exponentially related to time, showing a linear logarithmic reduction versus time (Nordin *et al.*, 2009). Most tests of treatments do not provide enough data for development of two-phase models, so instead rather simpler models including both the lag and the log phase are used to present a complete reduction (e.g. the time required for a 3 $\log_{10}$ reduction in *Ascaris* spp. in a treatment). *Ascaris* spp. is often recommended to be used as a model for chemical treatment due to its high tolerance to chemicals; for example, in the ABP regulations (EC1069/2009) and on treatment of ABP (EC142/2011), a 3 $\log_{10}$ reduction in *Ascaris* spp. should be the outcome of a sanitising method using chemical treatment, if the treatment is to be accepted for treatment of the biomass.

6.3 Manure Treatment Alternatives

Treatment of slurry may be anaerobic, or aerobic through aeration of stored slurry, composting of solid manure or through treatment with chemical amendments. For all treatments, time is a most important additional parameter. Furthermore, temperature is a variable included in most studies evaluating the reduction in pathogen content in animal solids and slurries (Table 6.1).

Table 6.1 *Sanitising treatment of manure to be applied in fields according to EU and other regulations and recommendations for safe manure management.*

Management	Treatment	Regulation/recommendation
Transferring and selling manure between farms (e.g. solids from separated manure[a])	1 h at above 70 °C or treatment with similar effect	EC1069/2009 (EC, 2009)
ABP category 3[b]	1 h at above 70 °C or treatment with similar effect	EC1069/2009 (EC, 2009)
Composting	turning compost twice first week and reaching temperature >55 °C, composting >3 months	guidelines for growers to reduce the risks of microbiological contamination of ready-to-eat crops (Hickman *et al.*, 2009)
Composting of human manure	>1 wk at >55°C	WHO (2006)
Composting in reactors	4 h at >55 °C	USEPA (1994)
Storage	batch storage >6 months without addition of fresh manure	guidelines for growers to reduce risks of microbiological contamination of ready-to-eat crops (Hickman *et al.*, 2009)
Liming	addition of quick or slaked lime to reach pH 12 during >2 h	guidelines for growers to reduce risks of microbiological contamination of ready-to-eat crops (Hickman *et al.*, 2009)
Ammonia	addition of urea, 2% treatment for 1 wk	for *Salmonella* removal during outbreaks

[a]Slurry or solid manure that is not mixed with organic waste and not sold may be spread on agricultural land without sanitising treatment.
[b]ABP Animal by-product: Term used in EU regulations for describing waste from animals, including slaughter waste and manure.

6.3.1 Storage

Storage of manure can be considered a pathogen reduction measure, where the main treatment variable is time from initiation of storage to application of manure to the field. This time is affected by the timing of application of manure to ensure optimal nutrient utilisation of the plant nutrients (i.e. the manure should be applied prior to, or early in, the growing season). Therefore, depending on the number of crops that can be grown in 1 year, the storage time varies. For example, in Northern Europe, with one crop per growing season, the average time of storage is 6 months. However, if only one manure tank is used, there will be fresh manure in the tank on the day of spreading to the fields, leading to a minimal retention time of 1 day. In subtropical countries the storage time may be only a few months because three crops are grown per year and storage time may be zero when liquid manure is used to fertilise fish ponds in Asia in the VAC system (Chapter 2).

As storage is the main treatment option performed today, it is important to bear in mind that the main reduction occurs in the manure tank when no fresh manure is added. A constant stream of fresh manure can contaminate the remaining part of the tank with pathogens and provide fresh substrate to support their growth. Even if the count of pathogenic microorganisms in the manure has decreased below the detection limit, it is possible to have regrowth, especially in association with changes in the environment (e.g. during spring when temperatures are increasing) (Gibbs *et al.*, 1997). In addition, when adding fresh manure to the tank the regrowth effect increases with increasing amount of material with a high content of easily available carbon compared with material with little easily available carbon (Elving *et al.*, 2010). The inactivation of pathogenic bacteria is generally faster than inactivation of viruses and parasites, which have a longer survival time. One of the most stable organisms is *Ascaris suum* (intestinal worms in pigs), for which viable individuals have been measured in a pig house that had been empty for 14 years (P. Wallgren, personal communication; Per.Wallgren@SVA.se).

A first step in decreasing the risk of disease transmission is to have more than one slurry tank and thereby increase the minimum storage time prior to field application, as the survival of pathogenic microorganisms is higher in the soil than in the slurry tank. *Salmonella* spp. in stored cattle slurry have been shown to have a decimal reduction in the range of 2–3 weeks (Hutchison *et al.*, 2005), which is considerably faster than the 6- to 7-week decimal reduction time reported for inactivation in manured soil (Nyberg *et al.*, 2011).

6.3.2 Anaerobic Treatment

The degradation of organic matter in an anaerobic treatment does not produce a surplus of heat, as the energy of components ends up in the methane (Chapter 4). Therefore, the anaerobic process requires external heating to reach temperatures above the ambient temperature. It is possible to run the anaerobic treatment process from psychrophilic temperatures around 10 °C up to hyperthermophilic temperatures around 65 °C. The main difference is the speed of the process and some effect on the gas composition and the substances degraded. This treatment is mostly associated with biogas production using digester technology (Chapter 13).

Most anaerobic digesters are run at mesophilic operating temperatures. Pathogenic bacteria such as *Salmonella* spp. can take part in the first degradation step, acidification, and thereby show initial growth (Ottoson *et al.*, 2008b), resulting in higher numbers or a constant outflow of pathogenic *Enterobacteriaceae* (e.g. *Salmonella* spp.) in the effluent of an anaerobic psycrophilic or mesophilic reactor.

The degradation of pathogens in the anaerobic process is the result of three factors. The main effect comes via heat inactivation, as in the case of composting. This can be achieved either via pre-pasteurisation by heating the animal manure fed to the reactor (e.g. 1 h at 70 °C) or from the thermophilic process. Post-pasteurisation of the digestate should be avoided because this treatment is often associated with large risks of regrowth of unwanted microorganisms in the sanitised material and, as a consequence, the level of pathogenic bacteria can be higher than the initial values in the incoming raw material. To ensure inactivation by the

thermophilic process a temperature of 50 °C is required, as lower temperatures give a considerably slower reduction (Elving *et al.*, 2012). For estimating the inactivation by temperature, the calculations mentioned in Section 6.2.2 can be used.

The second regulating factor is the ammonia (NH_3) content of the digestate. After a longer adaptation period it is possible to run an anaerobic digester at high NH_3 concentrations (e.g. with high protein load) (Schnürer and Nordberg, 2008). As the microorganisms in the feed biomass are not adapted to the high NH_3 environment, the high NH_3 concentration has a strong lethal effect on incoming microorganisms (Ottoson *et al.*, 2008b).

In a fully mixed continuous anaerobic reactor, which is the most common reactor type found in Europe, the inactivation of pathogens can be considered to be low. The minimum retention time (MRT) in such a reactor is short, because the continuous feeding of the reactor and stirring cause a fraction of the fresh feed to bypass the reactor and be discharged within a short time. The proportion of the material discharged after the minimum retention time is affected by the incoming feed and the hydraulic retention time (HRT) (Equation 6.1). Mesophilic anaerobic treatment generally results in an average inactivation corresponding to a 1–2 $\log_{10}$ reduction in the incoming pathogens. When the hydraulic retention time is increased, the reduction in pathogens in the system increases (Yen-Phi *et al.*, 2009). In a plug flow reactor, the HRT and the MRT (Text Box – Basic 6.1) are closely related, as the mixing of the material is low. This kind of reactor is common in low and middle income countries. One common reactor type is the bag reactor, which is a polyethylene-covered pit or channel containing manure, or the Chinese dome digester, where transport of the material in the reactor is achieved by addition of fresh material, which pushes the old material further into and out of the reactor. In these reactors organic matter and pathogens may sediment and have a long HRT and, as a consequence, the inactivation of pathogens increases. Yen-Phi *et al.* (2009) found that increasing the HRT and MRT from 3 to 30 days resulted in a decrease in the monitored pathogens and indicator organisms by a factor of 2 $\log_{10}$. In such systems with low mixing and laminar flow, larger pathogens such as parasitic eggs sediment and have an even longer retention time.

As with composting, there is a risk of regrowth in the digestate. However, when the material is stabilised the risk of growth of pathogenic bacteria is less than in the raw material (Sidhu *et al.*, 2001; Elving *et al.*, 2010).

6.3.3 Composting

Composting can be performed for a solid or a liquid manure. The main difference is the method of aeration, with the solid manure being composted requiring a dry matter content larger than 35% to have pores for air transport into the compost (Haug, 1993). However, higher water content results in waterlogging of the pores, which impedes aeration. Aeration of liquid manure is treatment of manure with a dry matter content less than 12%, where air is pumped into the slurry to keep the oxygen level high.

Several processes are responsible for the inactivation of pathogenic microorganisms (e.g. competition, nutrient deficiency, etc.). However, the main inactivation effect comes from the heat generated during oxidative degradation of organic matter (Vinnerås *et al.*, 2010). The actual heat released during the build-up phase of composting is less than the potential heat production, since much of the energy released is spent on the growth of the bacterial population.

Some pathogens are easily inactivated at mesophilic temperatures; for example, avian influenza virus undergoes a decimal reduction within less than 30 min at 35 °C, while increasing the temperature by 10 °C more, to 45 °C, has been shown to result in a decimal reduction in this organism in less than 10 min (Elving *et al.*, 2012). However, the recommended temperature for general pathogen inactivation is treatment above 50 °C. The reason for this recommendation is that several organisms show very slow or no reduction at lower temperatures, and in some cases there is even growth of pathogenic bacteria at temperatures close to 50 °C

(e.g. *Salmonella* spp. has been reported to grow in the temperature range 6–47 °C) (Mitscherlich and Marth, 1984).

The World Health Organisation recommendation for thermal treatment of faecal compost is to keep the temperature above 55 °C for more than 1 week for safe sanitisation of the material (Table 6.1). The challenge is that the treatment must be performed so that all of the material reaches a high temperature. If this is achieved by aeration of liquid manure in reactors where the temperature is homogeneously distributed, then the treatment time can be shorter. The US Environmental Protection Agency (USEPA, 1994) recommends that slurry treatment in a reactor should be at least four hours at 55 °C to ensure safe sanitisation.

Vinnerås *et al.* (2010) found that applying repeated heating peaks, a process known as Tyndallisation, can have an enhanced effect on inactivation, as the repeated heat peaks add more stress to the organisms. Furthermore, in some cases this treatment has a strong effect on the spore-formers, as the spores can be encouraged to germinate during the periods with lower temperature. Therefore, applying an irregular temperature pattern to compost that is mixed can actually have a positive effect on sanitisation compared with having a constant high temperature.

In a solid composting process, the temperature and the moisture are generally not evenly distributed. The degradation process produces heat that is lost, mainly via evaporation of water (Haug, 1993). The temperature of the compost is determined by the heat production and the amount of heat lost via the surface. To increase the heat of the compost, more easily available organic substances can be added, thereby increasing the heat production, and/or the surface of the compost may be insulated to decrease the heat loss. However, the areas with the highest incoming air flow and areas connected to the surface are still most often colder than the core of the compost (Figure 6.2). The cold zones of a compost pile/windrow can be significant in size and even in compost heaps higher than 1.2 m, up to 35% of the compost can be below the required temperature of 50 °C. As a rule of thumb for composting, a windrow or pile should be mixed five times and have a temperature above 55 °C (USEPA, 1994).

When the material is not degraded but rather preserved (e.g. by high temperature and low pH; Vinnerås *et al.*, 2010), changes in the composition (e.g. buffering the compost) can lead to high regrowth of pathogenic bacteria (Elving *et al.*, 2010). The growth in unmatured compost can actually increase the total number of organisms (e.g. growth of 2 $\log_{10}$ can occur in fresh compost material and this will offset the inactivation in the high-temperature section). However, as the material matures and is mixed several times, the risk of regrowth decreases (Sidhu *et al.*, 2001; Elving *et al.*, 2010).

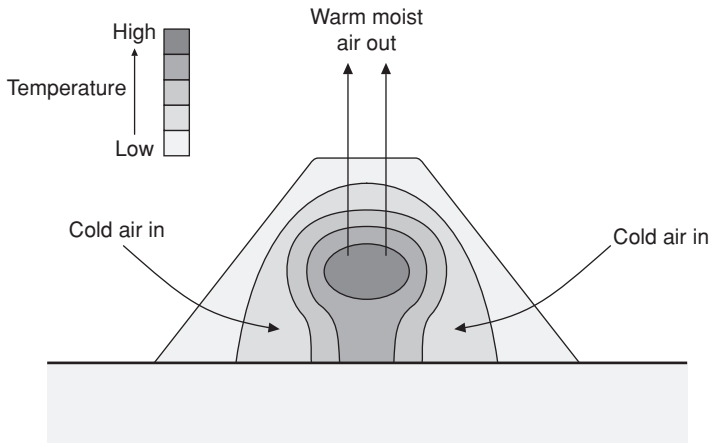

Figure 6.2 *Heat distribution within a windrow compost. (© University of Southern Denmark.)*

Even if there is a risk for growth of pathogenic bacteria in the cold zones, little or no growth of pathogenic bacteria can most often be expected in these zones. However, to achieve homogeneous sanitisation of the composted manure, a large proportion of the material needs to be treated at a high temperature. A general method for management of the temperature distribution in the compost material is mixing during the high-temperature phase. With temperature measurements, it is possible to map the temperature distribution in the compost and obtain a diagram similar to Figure 6.2. This diagram can then be used to determine the proportion of compost that can be considered to have reached sanitising temperature, which can be used to determine the proportion of compost that has not reached sanitisation temperature and inactivation of the pathogens of interest in the remainder of the compost. When several days are left between turnings, it is seldom necessary to calculate the exact inactivation in the warm areas. However, with shorter treatment times (e.g. a reactor with higher temperature prior to a long low-temperature treatment, it can also be interesting to know the inactivation in the high-temperature area) The total survival can be calculated by using the heat distribution between areas with high temperature (f_h) and areas with low temperature (f_l). The inactivation in the high-temperature area can then be calculated by using the inactivation rate (k_d) at a certain temperature over a set time period (d_t; e.g. the period during which this temperature prevails in the zone). The k_d used here is the same k_d as calculated in Equation (6.2). The high-temperature areas can be divided into several areas depending on the number of temperature zones determined in the compost, according to Figure 6.2. The reduction in the initial organisms (n_0) during the treatment time (t) to n_t is set based on the number of turnings of the compost N, plus the initial construction of the compost:

$$n_t = n_0 \left(f_l + f_h \cdot 10^{(-k_d \cdot \Delta t)}\right)^{N+1} \tag{6.5}$$

If the decrease in the number of organisms in the high-temperature zone is large, the reduction in that zone can be removed from Equation (6.5) and the reduction in the compost can be calculated based only on the number of organisms remaining in the cold zone after each complete mixing of the material as:

$$n_t = n_0 \left(f_l\right)^{N+1} \tag{6.6}$$

However, if the organisms are still viable in the cold zone (see above), the total number of microorganisms can actually increase, while if the number of organisms in the cold area decreases, the inactivation of the treatment is underestimated.

6.4 Chemical Treatment

Conventional chemical treatment aims to achieve a high pH that inactivates the microorganisms. However, some organisms are very stable even at high pH (e.g. the recommended lime treatment for sludge in the US is treatment at a pH above 12 during 3 months). If reactive lime (CaO) is used there will also be much production of heat, so in that case the treatment can be performed faster (i.e. if the treatment causes a temperature increase to 55–70 °C) (USEPA, 1994).

6.4.1 Ammonia Treatment

The alternative treatment is with NH_3, which can achieve good inactivation of microorganisms already at pH 8.5 and above (Text Box – Basic 6.2). Ammonia is highly soluble in water (1500 mol NH_3(aq) l^{-1}/mol NH_3(g) l^{-1}) and the dissolved NH_3(aq) is proportional to the partial pressure of NH_3(g) above the solution in a closed container, as given by the Henry's law equilibrium constant (Chapter 8). Ammonia is a weak base,

with a pK_a value of 9.25 at 25 °C (where 50% of the molecules are in the charged form (NH_4^+) and the other 50% in uncharged form (NH_3)). The relationship of (NH_4^+) to (NH_3) is:

$$NH_3(g) \rightleftharpoons NH_3(aq) + H_2O(l) \rightleftharpoons NH_4^+(aq) + OH^-(aq) \quad (6.7)$$

Text Box – Basic 6.2 Ammonia (NH_3) chemistry

- Solubility = 7020 g l^{-1} (1500 mol NH_3(aq) l^{-1}/mol NH_3(g) l^{-1}).
- pK_a (25 °C) = 9.25 when 50% is present as NH_3 and 50% as NH_4^+.
- When used for sanitisation, addition as: NH_3(aq) 25–28%.
- Urea (47% total NH_3-N by weight) enzymatically degraded to NH_3 in material; each urea molecule is degraded into two NH_3 molecules (i.e. 1 mol of urea gives 2 mol of NH_3).

The ammonium ion (NH_4^+) is harmless to microorganisms and the substance that performs the inactivation is NH_3, which has long been known to have this effect (Warren, 1962). The mechanism by which the NH_3 acts on the organisms is not fully described in the literature. However, the NH_3 molecule is small and has a high solubility, in water and in lipids, which allows transport over membranes and other cellular barriers by simple diffusion.

The NH_3 may act as an uncoupler, destroying the membrane potential of bacterial cells, or destroying (denaturing) proteins of the cell, both in the membranes and inside the cell (Bujozek, 2001). As NH_3 can easily be transported across membranes into the cell, it may also cause damage by rapid alkalinisation of the cytoplasm (Diez-Gonzalez *et al.*, 2000). To compensate for this and to maintain optimum internal pH, protons are taken up from outside the cell while at the same time potassium ions (K^+) are released and the loss of this essential element eventually leads to death of the bacterial cell (Bujozek, 2001). Few studies have been performed on the inactivation mechanisms of viruses, but studies on poliovirus have concluded that the inactivation is due to cleavage of the RNA, as the virus cells did not lose their capacity to attach and inject their genome into the host cell, but no reproduction occurred in the host cell (Ward, 1978; Burge *et al.*, 1983). The mechanism for inactivation of larger organisms such as parasites has not yet been identified. The function of NH_3 treatment is still unclear and so far the inactivation is empirically decided for each set of organisms to be used as treatment recommendations for sanitisation.

The inactivation of pathogens and indicator organisms by NH_3 treatment has been evaluated in a number of different substrates, including human urine (Vinnerås *et al.*, 2008), human faeces (Nordin *et al.*, 2009a, 2009b), blackwater (Fidjeland *et al.*, 2013a), sewage sludge (Pecson *et al.*, 2007; Fidjeland *et al.*, 2013b) and cattle manure (Ottoson *et al.*, 2008a) over a temperature range of 4–34 °C. All these studies report decimal reduction data for the organisms at different NH_3 concentrations, temperatures and pH. These data can be useful for a specific setting where the exact conditions of a planned treatment are known. However, when combining these data it is possible to estimate the time required for inactivation according to the ABP treatment regulations (EU142/2011; EU, 2011) at different NH_3 concentrations (Table 6.2). *Ascaris* spp. is included in Table 6.2, as the regulations state that when ABPs are treated chemically, *Ascaris* spp. has to be inactivated by 3 $\log_{10}$ by the treatment. The basic data for the inactivation at the different treatment alternatives include a lag and a log phase for *E. faecalis* and for *Ascaris* spp. (Figure 6.3). When performing the inactivation calculations according to the ABP regulations, it is possible to include both phases in the estimation of the required time. Using the simpler decimal reduction does not include the lag phase of the inactivation, resulting in an inaccurate time of inactivation. To develop the treatment recommendations, the data on inactivation were plotted according to the time for a 5 $\log_{10}$ reduction in the two bacteria species and 3 $\log_{10}$ reduction

Table 6.2 *Time required for fulfilment of EU regulations on ABP (EC142/2011) by chemical treatment of animal manure at different NH_3 concentrations and at temperatures from 4 °C.*

Ammonia concentration [NH_3] (mM)	*Salmonella* spp. (days for 5 $\log_{10}$ reduction)	*E. faecalis* (days for 5 $\log_{10}$ reduction)	*Ascaris* spp.[a] (days for 3 $\log_{10}$ reduction)
50	4	150	200
75	1	80	150
100	0.5	50	100
150	0.5	30	80
200	0.5	20	60
250	0.5	10	40

[a]Data only given for inactivation at temperatures above 20 °C. The reduction in *Ascaris* spp. at lower temperatures is significantly longer, as the lag phase increases in time and there are not sufficient data for generalising the inactivation at this concentration.

in the parasite. From this plot, the time for inactivation was determined at the different NH_3 concentrations, independent of the substrate and the temperature. This provided a conservative estimate for treatments at higher temperatures, as a higher speed of inactivation has been reported for a particular NH_3 concentration with increased treatment temperature (Vinnerås *et al.*, 2008; Nordin *et al.*, 2009a). The lowest temperature given for recommended time of treatment at different NH_3 concentrations is 4 °C for *Salmonella* spp. and *E. faecalis*. However, for *Ascaris* spp. there are only sufficient data for generalisation at temperatures above 20 °C, as most studies of *Ascaris* spp. inactivation at lower temperatures have not lasted long enough to pass the initial lag phase.

The effect of NH_3 treatment depends on the concentration of dissolved NH_3, the uncharged NH_3 molecule. The relationship between NH_3(aq) and TAN ($NH_3 + NH_4^+$) in a solution is quantified by the dissociation constant, K_a (Chapters 4 and 8), which is exponentially related to temperature:

$$[NH_3] = \frac{[TAN] \cdot K}{K + [H^+]}, \; pK_a = \frac{2728.92}{T} + 0.090181 \tag{6.8}$$

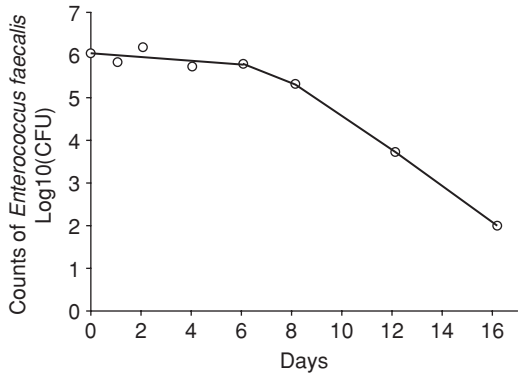

Figure 6.3 *A typical biphasic inactivation curve for NH_3 treatment of* E. faecalis *with an initial lag phase followed by a linear logarithmic decay. This is most clear for treatments with a low concentration of NH_3.*

To determine the concentration of uncharged NH_3 present in the material, the temperature, total ammoniacal concentration and pH of the sample must be determined. Having this information, the NH_3(aq) concentration can be determined and the treatment time of an infected manure to become sanitised can be determined (Table 6.2).

6.4.2 Ammonia Sanitisation at the Farm Level

Ammonia sanitisation can be used during disease outbreaks or as part of the daily regime on farms, especially if the manure or a separated fraction of the manure is intended for sale outside the farm and treatment is required according to the EU ABP regulations (See Text Box – Basic 6.1). Ammonia treatment relies on achieving a sufficiently high concentration of uncharged NH_3 for pathogen inactivation (Table 6.2). According to Equations (6.7) and (6.8), it is possible to increase the NH_3 content by increasing the temperature, increasing the pH or increasing the TAN concentration. The easiest way of increasing the NH_3 concentration is to add NH_3, which increases both the TAN and the pH. The most common method of NH_3 addition is to add a solution of NH_3, 25% NH_3(aq) being the most common commercially available product. The other alternative is to add NH_3 in the form of urea ($CO(NH_2)_2$). The urea itself is not toxic when added to the manure, but the naturally occurring enzymes (urease) transform urea into carbonic acid (H_2CO_3) and NH_3 (Equation 6.9). Urea addition can give a pH increase of up to approximately 9.2 depending on the concentration added and the buffering capacity of the treated material. As the carbonate produced from urea degradation buffers the material, the pH reached after urea addition is lower than that after addition of a solution of NH_3(aq):

$$CO(NH_2)_2 + 2H_2O \rightarrow H_2CO_3 + 2NH_3 \tag{6.9}$$

The treatment needs to be performed under cover to avoid losses of NH_3 (Chapters 8 and 9) As long as the NH_3 concentration is high in the manure there is no risk of recontamination, as the NH_3 keeps the growth of microorganisms low. This probably also results in lower greenhouse gas emissions from the manure, as the general biological activity decreases and thus no CH_4 or N_2O is produced during storage (Chapter 10). Ammonia sanitisation has been proven to function well in liquids (e.g. urine (Vinnerås *et al.*, 2008) and hatchery waste (Emmoth *et al.*, 2011)) and in solids (e.g. sewage sludge (Pecson *et al.*, 2007) and compost (Adamtey *et al.*, 2009)). As the NH_3 is not consumed during the treatment, the sanitisation can continue throughout the treatment and there is no risk of regrowth or recontamination as long as the NH_3 is kept in the material. When applying the treated manure to the soil, the dilution and buffering of the NH_3 leads to decreased pH, and the NH_3 is converted into NH_4^+ and acts as a fertiliser. Thus, the cost of the treatment can be allocated to the fertiliser. The fertiliser should be applied in such a way that large NH_3 losses are avoided (Chapter 9).

6.5 Summary

During disease outbreaks among farm animals, there is always a risk of disease transmission via the manure either directly to humans or animals or indirectly from manured crops, for example. Therefore, it is of major importance that the disease transmission chain is broken already at the manure management level. In addition, when manure is transported outside the farm (e.g. sold as a separated solid fertiliser fraction or soil improver) it must be treated to ensure that no pathogens are exported from one area to another.

Composting is the most common treatment of manure for removal of unwanted microorganisms. The main effect of composting is the heat, which ensures appropriate inactivation of unwanted microorganisms. For efficient removal of pathogenic organisms in the compost, the temperature has to reach above 50 °C, as the

efficiency of inactivation decreases considerably at lower temperatures. To ensure that all the material has been exposed to high temperature, the compost heap or windrow compost should be turned 5 times during high temperature treatment. Slurry should be mixed 3 times when treated in aeration reactors with smaller low-temperature zones.

Anaerobic treatment in biogas reactors or during storage of slurry is not a sanitisation treatment per se. Increasing the hydraulic retention time in biogas digesters increases the inactivation of pathogens, especially in plug flow reactors where the minimum retention time and the hydraulic retention time are similar. To ensure full sanitisation effect in anaerobic digestion, the treatment should be performed at temperatures above 50 °C or the incoming material should be pre-pasteurised.

The most efficient chemical treatment for pathogen removal from manure and its solid and liquid fractions is NH_3 treatment. This must be performed at pH above approximately 8.5, the requirement being that sufficient uncharged NH_3 is present in the material for microbial inactivation. With increased temperature and pH, NH_3 treatment becomes more efficient, as a larger proportion of the ammonium and NH_3 is present in the form of NH_3. As NH_3 is a volatile gas, the treatment should be performed in a closed system in order to avoid gaseous losses. The NH_3 is not consumed during the treatment, so there is no risk of recontamination of the treated manure, and upon application to soil the NH_3 acts as a fertiliser.

References

Adamtey, N., Cofie, O., Ofosu-Budu, G.K., Danso, S.K.A. and Forster, D. (2009) Production and storage of N-enriched co-compost. *Waste Manag.*, **29**, 2429–2436.

Berge, A.C.B., Glanville, T.D., Millner, P.D. and Klingborg, D.J. (2009) Methods and microbial risks associated with composting of animal carcasses in the United States. *J. Am. Vet. Medic. Assoc.*, **234**, 47–56.

Bujozek, G. (2001) Influence of ammonia and other abiotic factors on microbial activity and pathogen inactivation during processing of high-solid residues, *Dissertation*, University of Manitoba, Manitoba.

Burge, W.D., Cramer, W.N. and Kawata, K. (1983) Effect of heat on virus inactivation by ammonia. *Appl. Environ. Microbiol.*, **46**, 446–451.

Diez-Gonzalez, F., Jarvis, G.N., Adamovich, D.A. and Russell, J.B. (2000) Use of carbonate and alkali to eliminate *Escherichia coli* from dairy cattle manure. *Environ. Sci. Technol.*, **34**, 1275–1279.

EC (2009) Regulation (EC) No. 1069/2009 of the European parliament and of the council of 21 October 2009. Laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal By-products Regulation), *Official Journal of the European Union*, European Community, Brussels.

Elving, J., Ottoson, J., Vinnerås, B. and Albihn, A. (2010) Growth potential of faecal bacteria in simulated psychrophilic/mesophilic zones during composting of organic waste. *J. Appl. Microbiol.*, **108**, 1974–1981.

Elving, J., Emmoth, E., Albihn, A., Vinnerås, B. and Ottoson, J. (2012) Composting for avian influenza virus elimination. *Appl. Environ. Microbiol.*, **78**, 3280–3285.

Emerson, K., Russo, R., Lund, R. and Thurston, R. (1975) Aqueous ammonia equilibrium calculations: effects of pH and temperature. *J. Fish. Res. Board Can.*, **32**, 2379–2383.

Emmoth, E., Ottoson, J., Albihn, A., Belak, S. and Vinnerås, B. (2011) Ammonia disinfection of hatchery waste for elimination of single-stranded RNA viruses. *Appl. Environ. Microbiol.*, **77**, 3960–3966.

EU (2011) EU 142/2011. Regulation (EU) No. 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive, *Official Journal of the European Union*, European Community, Brussels.

Fidjeland, J., Magri, M.E., Jönsson, H., Albihn, A. and Vinnerås, B. (2013a) The potential for self-sanitization of faecal sludge by intrinsic ammonia. *Water Res.*, in press.

Fidjeland, J., Lalander, C., Jönsson, H. and Vinnerås, B. (2013b) Ammonia sanitisation of sewage sludge using urea. *Water Sci. Technol.*, in press.

Gibbs, R.A., Hu, C.J., Ho, G.E. and Unkovich, I. (1997) Regrowth of faecal coliforms and salmonellae in stored biosolids and soil amended with biosolids. *Water Sci. Technol.*, **35**, 269–275.

Haug, R.T. (1993) *The Practical Handbook of Compost Engineering*, Lewis, Boca Raton, FL.

Hickman, G., Chambers, B. and Moore, T. (2009) *Managing Farm Manures for Food Safety – Guidelines for Growers to Reduce the Risks of Microbiological Contamination of Ready-to-Eat Crops*, Food Standards Agency, London.

Hutchison, M.L., Walters, L.D., Moore, A. and Avery, S.M. (2005) Declines of zoonotic agents in liquid livestock wastes stored in batches on-farm. *J. Appl. Microbiol.*, **99**, 58–65.

Komakech, A., Banadda, N., Gebresenbet, G. and Vinnerås, B. (2013) Feed and manure management for city animals in Kampala Uganda. *Agron. Sustain. Dev.*, in press.

Mitscherlich, E. and Marth, E.H. (1984) *Microbial Survival in the Environment – Bacteria and Rickettsiae Important in Human and Animal Health*, Springer, Berlin.

Nordin, A., Ottoson, J. and Vinnerås, B. (2009a) Sanitation of faeces from source-separating dry toilets using urea. *J. Appl. Microbiol.*, **107**, 1579–1587.

Nordin, A., Nyberg, K. and Vinnerås, B. (2009b) Inactivation of *Ascaris* eggs in source-separated urine and faeces by ammonia at ambient temperatures. *Appl. Environ. Microbiol.*, **75**, 662–667.

Nyberg, K., Vinnerås, B., Ottoson, J., Aronsson, P. and Albihn, A. (2011) Inactivation of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in manure-amended soils studied in outdoor lysimeters. *Appl. Soil Ecol.*, **46**, 398–404.

Ottoson, J., Nordin, A., von Rosen, D. and Vinnerås, B. (2008a) *Salmonella* reduction in manure by the addition of urea and ammonia. *Bioresour. Technol.*, **99**, 1610–1615.

Ottoson, J., Schnürer, A. and Vinnerås, B. (2008b) *In situ* ammonia production as a sanitation agent during anaerobic digestion at mesophilic temperature. *Lett. Appl. Microbiol.*, **46**, 325–330.

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

Sidhu, J., Gibbs, R.A., Ho, G.E. and Unkovich, I. (2001) The role of indigenous microorganisms in suppression of salmonella regrowth in composted biosolids. *Water Res.*, **35**, 913–920.

Schnürer, A. and Nordberg, Å. (2008) Ammonia, a selective agent for methane production by syntrophic acetate oxidation at mesophilic temperature. *Water Sci. Technol.*, **57**, 735–740.

Son Thi Thanh, D., Dung Van, T., Madsen, H. and Dalsgaard, A. (2011) Survival of faecal indicator bacteria in treated pig manure stored in clay-covered heaps in Vietnam. *Vet. Microbiol.*, **152**, 374–378.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M. and de Haan, C. (2006) *Livestock's Long Shadow: Environmental Issues and Options*, FAO, Rome.

USEPA (1994) *A Plain English Guide to the EPA Part 503 Biosolids Rule*, US Environmental Protection Agency, Washington, DC.

Vanotti, M.B., Szogi, A.A., Millner, P.D. and Loughrin, J.H. (2009) Development of a second-generation environmentally superior technology for treatment of swine manure in the USA. *Bioresour. Technol.*, **100**, 5406–5416.

Vinnerås, B., Nordin, A., Niwagaba, C. and Nyberg, K. (2008) Inactivation of bacteria and viruses in human urine depending on temperature and dilution rate. *Water Res.*, **42**, 4067–4074.

Vinnerås, B., Agostini, F. and Jönsson, H. (2010) Sanitisation by composting, in *Microbes at Work from Wastes to Resources* (eds H. Insam, I. Franke-Whittle and M. Goberna), Springer, Berlin, pp. 171–191.

Ward, R.L. (1978) Mechanism of poliovirus inactivation by ammonia. *J. Virol.*, **26**, 299–305.

Warren, K.S. (1962) Ammonia toxicity and pH. *Nature*, **195**, 47–49.

WHO (2006) *Guidelines for the Safe Use of Wastewater, Excreta and Greywater. Volume 2: Wastewater Use in Agriculture*, WHO, Geneva.

Yajima, A. and Kurokura, H. (2008) Microbial risk assessment of livestock-integrated aquaculture and fish handling in Vietnam. *Fish. Sci.*, **74**, 1062–1068.

Yen-Phi, V.T., Clemens, J., Rechenburg, A., Vinnerås, B., Lenßen, C. and Kistemann, T. (2009) Hygienic effect of plastic bio-digesters under tropical conditions. *J. Water Health*, **7**, 590–596.