



Treatment alternatives of slaughterhouse wastes, and their effect on the inactivation of different pathogens: A review

Ingrid H. Franke-Whittle & Heribert Insam

To cite this article: Ingrid H. Franke-Whittle & Heribert Insam (2013) Treatment alternatives of slaughterhouse wastes, and their effect on the inactivation of different pathogens: A review, *Critical Reviews in Microbiology*, 39:2, 139-151

To link to this article: <http://dx.doi.org/10.3109/1040841X.2012.694410>



© 2013 The Author(s). Published by Taylor & Francis.



Published online: 13 Jun 2012.



Submit your article to this journal [↗](#)



Article views: 977



View related articles [↗](#)



Citing articles: 1 View citing articles [↗](#)

REVIEW ARTICLE

Treatment alternatives of slaughterhouse wastes, and their effect on the inactivation of different pathogens: A review

Ingrid H. Franke-Whittle and Heribert Insam

Institute of Microbiology, Leopold-Franzens University, Innsbruck, Austria

Abstract

Slaughterhouse wastes are a potential reservoir of bacterial, viral, prion and parasitic pathogens, capable of infecting both animals and humans. A quick, cost effective and safe disposal method is thus essential in order to reduce the risk of disease following animal slaughter. Different methods for the disposal of such wastes exist, including composting, anaerobic digestion (AD), alkaline hydrolysis (AH), rendering, incineration and burning. Composting is a disposal method that allows a recycling of the slaughterhouse waste nutrients back into the earth. The high fat and protein content of slaughterhouse wastes mean however, that such wastes are an excellent substrate for AD processes, resulting in both the disposal of wastes, a recycling of nutrients (soil amendment with sludge), and in methane production. Concerns exist as to whether AD and composting processes can inactivate pathogens. In contrast, AH is capable of the inactivation of almost all known microorganisms. This review was conducted in order to compare three different methods of slaughterhouse waste disposal, as regards to their ability to inactivate various microbial pathogens. The intention was to investigate whether AD could be used for waste disposal (either alone, or in combination with another process) such that both energy can be obtained and potentially hazardous materials be disposed of.

Keywords: Renewable energy, waste treatment, pathogen inactivation

Introduction

An in-depth review of available literature was conducted in order to compare three different methods of disposal of the waste products generated from the slaughtering of animals in abattoirs. The databases Medline and Web of Science were used, and search terms included slaughterhouse wastes, pathogens, composting, anaerobic digestion (AD) and alkaline hydrolysis (AH), as well as pathogen names. In particular, of interest was to determine the safety of using slaughterhouse waste in AD to produce methane, and as a method of disposal of such wastes. Slaughterhouse wastes are animal byproducts, and can contain different bacterial, viral, prion and parasitic pathogens. Composting and AH were also reviewed, with regard to their abilities to eliminate specific pathogens.

Slaughterhouse wastes constitute the inedible parts of animals derived from the production of meat, as well as blood and other animal byproducts. Inedible animal

tissues (organs, integument, ligaments, tendons, blood vessels, feathers, bone) can comprise up to 45% or more of the slaughtered animal. The remaining fraction, is, however, not only fit for disposal, and pet food companies are known to purchase large amounts of slaughterhouse wastes. These can be used either alone, or as a supplement for animal feed (Salminen and Rintala, 2002). Nonetheless, considerable amounts of slaughterhouse waste are produced worldwide, and their disposal poses a serious logistical challenge for meat/poultry processing plants. As a result of such high magnitudes of animal waste, and because of the legal restrictions and rising treatment costs of removal, improper and unsafe disposal of these wastes can occur. Such practices can then also lead to serious environmental problems (Arvanitoyannis and Ladas, 2008). Slaughterhouse wastes have, however, potential as an energy source, and could help to reduce the requirements of petroleum based fuels currently

Address for Correspondence: Ingrid Helene Franke-Whittle PhD, Institute of Microbiology, Leopold-Franzens University, Technikerstrasse 25, Innsbruck, 6020 Austria. E-mail: ingrid.whittle@uibk.ac.at

(Received 18 January 2012; revised 09 May 2012; accepted 14 May 2012)

used for the Earth's energy needs (Srivastava and Prasad, 2000).

As such alternative energy sources emerge, a number of questions will be asked concerning the safety of the end products generated. In the case of using slaughterhouse wastes in biogas production, the microbial quality of the end product is of major concern as slaughterhouse wastes can be contaminated with high numbers of microorganisms including bacteria, viruses, prions, fungi, yeasts and associated microbial toxins (Urlings et al., 1992). Such wastes pose a potential risk to animal and human health, unless handled and treated properly.

The management of animal carcasses has always been, and continues to be, a concern in animal production operations, slaughter plants, farms and other facilities that involve animals. Throughout history, burial and to a lesser extent, burning, have been the most commonly applied methods for the disposal of on-farm mortalities (Gwyther et al. 2011). However, the European Union Animal By-Product Regulation (EC) no. 1774/2002 (Anon, 2002) does not allow these practices to be conducted within the EU and limits the disposal routes to incineration (either on or off-farm), rendering, high temperature/pressure AH, disposal at maggot farms or through licensed waste collectors (Anon, 2002). The prevention of disposal by burial and burning was founded based on the perceived risk of an incomplete destruction of pathogens from mortalities during these processes, and thus the entering of infective agents into the animal feed chain (Anon, 2002). Similar risks are involved upon the slaughtering of animals. In contrast to burning, properly operated incineration facilities pose fewer pollution concerns. Also, bacteria (including spore-formers) and viruses should not survive the incineration process (NABC, 2004). There are, however, some concerns that transmissible spongiform encephalopathies (TSEs) such as BSE (bovine spongiform encephalopathy) can survive incineration processes if not conducted at a high enough temperature (NABC, 2004).

Rendering is another method used for the disposal of animal waste products. It involves the conversion of animal carcass/waste materials into three end products, carcass meal (proteinaceous solids), melted fat/tallow, and water. This is conducted using mechanical processes including grinding, mixing, pressing, decanting and separating, thermal processes including cooking, evaporating, and drying, as well as chemical processes such as solvent extraction (NABC, 2004). The rendering process simultaneously dries the material and separates the fat from the bone and protein. The fat obtained can be used as low-cost raw material in making grease, animal feed, soap, candles and biodiesel, and tallow is an important raw material in the steel rolling industry providing the required lubrication for compressing steel sheets. The protein meal produced can be used for animal feed. Thus the products provide significant additional income to the slaughterhouse. However, because of the problems with BSE, the feeding of meat and bone meal to cattle is

currently prohibited in developed countries. Thus rendering plants do not play as significant a role in the disposal of animal wastes today as they did in the past.

New risks have arisen from the implementation of the European Union (EU) Landfill Directive (EU, 1999) for more environmentally sensitive waste disposal methods. Evidence exists that certain pathogens and pests can survive composting or other waste treatment processes, sometimes through inadequate methods or failures in the process (Noble and Roberts, 2004). According to the European Union Animal By-Product Regulation (EC) no. 1774/2002, animal byproducts (ABP) are categorized in three classes. ABP of categories 2 and 3 can be treated by approved composting plants and by AD in approved biogas plants, while ABP of category 1 cannot. The guidelines do not stipulate allowances for AH. Most slaughterhouse wastes fall into categories 2 and 3.

This review focuses on three methods that can be used to dispose of slaughterhouse waste: composting, AD and AH. Particular emphasis has been placed on determining the inactivation successes of the three different methods on various bacterial, viral, parasitic and prion pathogens that can be encountered in animal carcasses and slaughterhouse wastes.

Treatment methods

Composting

Composting is an aerobic process by which organic materials are degraded through the activities of successive groups of microorganisms (Dees and Ghiorse, 2001). Substrates vary, and can include different types of organic and inorganic wastes, sewage sludge, pig, cattle or poultry manure, garden waste and municipal solid waste. The positive effects of composts on arable soil have been reported by many authors (Ibekwe et al., 2001; Bailey and Lazarovits, 2003).

An efficient and satisfactory composting process is dependent on the presence of a high microbial diversity (Beffa et al., 1996). Different microbial communities predominate during the four consecutive phases (Blanc et al., 1999; Alfreider et al., 2002; Ryckeboer et al., 2003) involving mesophilic, thermotolerant, and thermophilic aerobic microorganisms (Beffa et al., 1996). Continual change in environmental conditions in composts (temperature, pH, aeration, moisture, availability of substrates) results in stages of exponential growth and stationary phases for different organisms. Knowledge on the diversity of the microbial communities of compost is increasing rapidly through the use of molecular biology tools, such as the COMPOCHIP microarray (Franke-Whittle et al., 2005, 2009; Danon et al., 2008).

Among the concerns regarding the composting process and the use of composts in agriculture and horticulture, however, is the survival and spread of animal, human and plant pathogens. Thus, any composting process must be capable of the elimination of any health risk that may be present in the end product (Strauch, 1996;

Böhnel et al., 2002). An improperly managed composting process can induce the proliferation and dispersion of potentially pathogenic and/or allergenic thermotolerant/thermophilic fungi and bacteria (Beffa et al., 1996).

Two types of composting systems exist, windrow and in-vessel systems. In-vessel composting has several advantages over windrow composting, namely, that it requires less space, provides better control than windrows, and offers a high process efficiency (Cekmecelioglu et al., 2005). The higher temperatures achieved however in windrow composting have been found to result in greater bacterial and pathogen reductions (Cekmecelioglu et al., 2005). According to Noble and Roberts (2004), the cool zones in in-vessel composting systems where there is no or little turning, are of concern for pathogen survival. Thus, windrow composting of slaughterhouse wastes is the more appropriate approach.

Composting provides an inexpensive alternative and environmentally acceptable method for the disposal of slaughterhouse wastes. The temperatures reached during the decomposition process can kill or greatly reduce most pathogens, thus reducing the chance to spread disease. Although the heat generated during composting results in a reduction of microorganism and pathogen numbers, it is not sufficient to completely sterilize the end product, thus leaving some potential for survival and (re)growth of pathogens. The levels of pathogenic bacteria remaining at the end of the composting process are dependent on the temperatures reached and the time the temperature was maintained. Legal requirements in various countries ask for a minimum temperature of 65°C for at least 6 consecutive days or two 3-day periods >65°C (e.g. Kompostverordnung, 2002). Achieving an average temperature of 55–60°C for 1–2 days is generally sufficient to reduce pathogenic viruses, bacteria, protozoa, and helminth ova to an acceptably low level. However, the endospores produced by spore-forming bacteria such as *Clostridium* and *Bacillus* would not be inactivated under these conditions (NABC, 2004). Typically, pH values exceeding 8 are also obtained in the composting process (Reuter et al., 2011) which also contributes to the inactivation of pathogens. Pathogens in compost can also be inactivated by the action of antibiotics produced by various microorganisms present in the compost (Hoitink and Boehm, 1999).

Composting can potentially serve as an acceptable method of disposal of slaughterhouse wastes. However, some additional treatments would be necessary to inactivate some pathogens in the end product. Composting can also serve as a method of post-treatment of AD wastes. This post-treatment helps to ensure a more complete breakdown of organic matter, and enriches the compost in terms of the nutrients. Composting of the anaerobic digestate would in addition act to reduce pathogen levels in such products.

AH

AH represents a relatively new technology for the disposal of animal carcass material, and other infectious wastes

(Kaye, 2003; NABC, 2004). The process uses sodium or potassium hydroxide to catalyze the hydrolysis of biological materials (proteins, nucleic acids, carbohydrates, lipids, etc.) into a sterile aqueous solution consisting of small peptides, amino acids, sugars, and soaps. In order to accelerate the hydrolysis, the process is usually conducted with pressure and temperature. For the inactivation of microbial pathogens, carcasses must be heated to 100°C and pressurized at 103 kpa for 3 h. To destroy prion containing material, carcasses must be heated to 150°C and pressurized at 486 kpa for 6–8 h (http://ssl-edss.tamu.edu/disposal/handbook/04_Alkaline.pdf).

Studies investigating the inactivation of pathogens using AH are limited, however, those that exist, indicate the method to be highly successful. Neyens et al. (2003) reported that an AH process conducted at 100°C, pH ≈ 10, 120 kpa and for 60 min was able to kill all pathogens. Unfortunately, it was not reported which pathogens were investigated. In a recent study, Dixon et al. (2012) investigated the use of AH at ambient temperature for the inactivation of the fish pathogens infectious salmon anaemia virus (ISAV) and *Lactococcus garvieae*. Both pathogens were inactivated after 48 h of treatment with 1 M NaOH at pH > 13 and at room temperature. The efficacy of AH as an alternative for the treatment and disposal of infectious waste was also evaluated by testing for the destruction of specific microorganisms in a study by Kaye et al. (2003). AH was found to destroy all representative classes of potentially infectious agents.

The sterile end product of AH can be released into a sanitary sewer, in accordance with local and federal guidelines regarding pH and temperature (Kaye, 2003). Studies conducted by the same authors have shown that by bubbling carbon dioxide into the hydrolysate at the end of the digestion, the pH of the end product can be reduced into the range of pH 8 or less. Considering that the hydrolysate is comprised of a mixture of single amino acids, small peptides, and fatty acids, all excellent growth nutrients for microorganisms (Kaye, 2003), alternative disposal methods for this waste product should be sought. One possibility is its use as a fertilizer (NABC, 2004), however, another possibility that would require further research, but which would serve in renewable energy production, would be its use in AD. Coupled with AD, an AH unit could produce significant amounts of energy, and at the same time, safely dispose of potentially contaminated slaughterhouse waste products.

An alkaline pretreatment with NaOH was used in a study to enhance biogas production from the AD of corn stover (Zhu et al., 2010). Different concentrations of NaOH (1–7.5% w/w) were tested and the preparations were subjected to AD. The highest biogas yields were obtained with 5% NaOH. This study shows that AH of slaughterhouse wastes followed by AD should be considered a potential route of treatment.

There are many advantages of using AH for the inactivation of disease agents in slaughterhouse wastes. These include the combination of a sterilization and digestion

step into one operation, the reduction of waste volume and weight by as much as 97%, the total destruction of pathogens including prions such as BSE and the lower emission of odors or public nuisances.

AD

AD is a biological process by which organic wastes are decomposed in the absence of oxygen, producing a sludge of agricultural value, as well as biogas, which can be used to generate energy (Lastella et al., 2002; Insam and Wett, 2008). In recent years, AD technology has been applied and used commercially in the treatment of farm, industrial, and municipal wastes. AD also represents an alternative method for the disposal of animal carcasses and slaughterhouse wastes, with the dual benefits of both eliminating waste material and producing energy (Insam et al., 2010), thus saving carbon credits. Concerns exist however as to whether an AD process can successfully inactivate pathogens. Often, a pasteurization step is used to help inactivate pathogens prior to AD. It is also common to include a secondary heat treatment process (e.g. composting or pasteurization) and a minimum storage period at the end of the process for the digestate as an additional measure (Sahlström, 2003). Swedish law for example, requires biogas plants that use animal waste to pasteurize the incoming substrate at 70°C for 60 min prior to digestion, to ensure a hygienically acceptable end product (Sahlström et al., 2008).

The anaerobic process itself requires specific environmental conditions and requires that the bacteria and archaea involved co-operate in a close and efficient syntrophism (Schink, 1997). Digestion occurs in four major stages (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) and complex polymers are degraded in a stepwise manner to yield CO₂ and CH₄.

AD can be run either under mesophilic (35°C) or thermophilic conditions (55°C), and the duration of the process and the effectiveness in destroying pathogens thus varies. A mesophilic process will typically run for 15–30 days, while a thermophilic process will run for 12–14 days (Vandeviere et al., 2002). Mesophilic AD processes are reportedly more robust and less sensitive to changes in process parameters than are thermophilic processes, however, gas production is lower and it is more likely that the end product poses a greater pathogen risk if applied directly to the field (NABC, 2004). Methane producing microorganisms are more sensitive to temperature alterations than other bacteria, and temperature variations as low as 2°C can reportedly have adverse effects on mesophilic AD processes (Gunnerson and Stuckey, 1986).

Thermophilic AD on the other hand offers higher methane production, faster throughput, and a better inactivation of pathogens. Despite its advantages, the thermophilic digestion process needs more expensive technology, and requires a higher degree of operation and monitoring (Vandeviere et al., 2002). Also, changes of only 0.5°C can affect thermophilic AD processes (Gunnerson and Stuckey, 1986).

Monteith et al. (1986) seeded a bovine enterovirus and parvovirus into liquid cattle manure, and noted that both viruses were rapidly inactivated by a thermophilic AD process, and not detected after 30 min. Under mesophilic conditions, however, the viruses were able to survive for 13 and 8 days, respectively. Spillman et al. (1987) also found that the rotavirus and coxsackievirus B5 animal viruses were rapidly inactivated by thermophilic AD. These findings indicate that viruses are inactivated during AD, but that the rate of inactivation is dependent on the virus, the temperature and the duration of digestion. Even when using a thermophilic AD process, it is advisable to use an additional heat treatment at the end of the process to fully inactivate pathogens capable of surviving AD (i.e. spore-formers). In fact, the European Commission Regulations (EC) no. 1774/2002 and no.208/2006 require a 70°C/60 min pasteurization step of animal waste prior to landspreading of sludges (Bagge et al., 2005). It should also be remembered however, that an additional heat treatment would still most likely not allow the inactivation of prions such as BSE (NABC, 2004).

The high lipid and protein contents of slaughterhouse waste products can cause inhibition of AD processes, as a result of an accumulation of ammonia produced from the degradation of proteins and long chain fatty acid accumulation from the breakdown of lipids (Cuetos et al., 2010). Also, lipids can form floating aggregates and foam that can cause stratification problems due to the adsorption of lipids into the biomass (Cuetos et al., 2010). However, if properly managed, AD can be conducted successfully on slaughterhouse wastes with high methane production, by allowing a progressive acclimatization of the microbial communities to an ammonia rich medium (Edström et al., 2003).

Pathogens

In this section, information on the survival and inactivation of a number of specific pathogens or indicator organisms is summarized.

Escherichia coli

Escherichia coli is a Gram negative, rod-shaped bacterium that is often found in the lower intestine of warm-blooded organisms. Most strains are harmless, although there are serotypes that cause food poisoning in humans, and strains such as O157:H7, which can cause serious illness or death in the elderly, the very young and the immuno-compromised (Sahlström et al., 2003).

A pasteurization experiment was conducted by Sahlström et al. (2008) to determine the survival rates of *E. coli* O157 after heat treatment. In the experiment, *E. coli* O157 was found to survive after 30 min at 55°C, but not after 60 min at 55°C. Similarly, a treatment at 70°C for 30 min was sufficient to inactivate *E. coli*. Inactivation of non-pathogenic *E. coli* and pathogenic *E. coli* O157:H7 has been reported during composting of several types of waste, including animal manure and sewage sludge (Lemunier et al. 2005). The persistence

however of non-pathogenic *E. coli* during composting and the finding of non-pathogenic *E. coli* in mature composts have also been reported (Lemunier et al. 2005). It is possible that in these cases the composting process employed was not conducted for a long enough time at a high enough temperature.

Laboratory scale experiments conducted by Jiang et al. (2003) have shown that *E. coli* O157:H7 in bovine fecal matter are inactivated at thermophilic composting temperatures. In their study, Jiang et al. (2003) showed that large populations (10^4 – 10^7 CFU/g) of *E. coli* O157:H7 survived for 36 days during composting in a bioreactor at an external temperature of 21°C but were undetectable after 7–14 days of composting in the bioreactor at an external temperature of 50°C. In another study, Lung et al. (2001) investigated *E. coli* O157:H7 survival after cow manure composting. *E. coli* O157:H7 was not detected after 72 h of composting at 45°C. Thus, a composting process conducted at a minimum temperature of 50°C and lasting longer than a week was assumed to be able to successfully inactivate *E. coli* O157:H7 in manure, and could be assumed to inactivate other *E. coli* strains in slaughterhouse waste products.

The reductions of pathogenic and indicator bacteria (including *E. coli*) in animal slurry subjected to mesophilic (35°C) and thermophilic (53°C) AD was investigated in a study using both small-scale and full-scale reactors (Olsen and Larsen, 1987). At small-scale digestion at 35°C, a T90 value (time required for a 90% reduction in number of bacteria) of 1.8 days was measured for *E. coli*. At 53°C, the T90 value was reduced to 0.4 h for *E. coli*. Similar values were obtained at full-scale. Thus, we would hypothesize that a combination of a pasteurization step prior to AD, followed by an AD process should totally inactivate all *E. coli* cells present in any slaughterhouse waste material.

No information could be found concerning the use of AH to inactivate *E. coli*. However, considering that AH was found in a study by Kaye et al. (1998) to completely destroy all representative classes of potentially infectious agents as well as disposing of animal carcasses by solubilization and digestion, it can be assumed the process would also totally inactivate *E. coli*.

Salmonella

Salmonella are ubiquitous and cause illnesses such as typhoid and paratyphoid fever, and very frequently, food poisoning. All serovars of *Salmonella* are potentially pathogenic to both humans and animals, and the organism is spread in the environment by animal slurry, and sewage sludge (Sahlström et al., 2003). *Salmonella* is often used as an indicator organism to test whether a particular treatment process has been successful in the inactivation of microorganisms. Many studies have been conducted investigating the survival of *Salmonella* at various temperatures, for different periods of time. Generally, *Salmonella* is not able to survive at temperatures above 70°C. With the exception of *S. senftenberg*, *Salmonellae*

are destroyed at 56°C for 10–20 min, although there is better heat tolerance at low water activity and in high fat foods. In a study by Paluszak et al. (2011), *S. senftenberg* W775 introduced into meat material was found to be eliminated by a composting process within 30 h at 68°C. However, *Salmonella* has been reported to be able to re-colonize composts when temperatures are reduced near the end of the composting process if the process did not reach sufficiently high temperatures, or if the pile was not adequately aerated or turned (NABC, 2004).

Temperature is the most important factor affecting the survival of a pathogenic microorganism during AD. Studies by Plym-Forshell (1995) showed that *Salmonella* could be inactivated within 24 h in a thermophilic digestion process, while weeks-months were required in a mesophilic digestion process. However, in a study conducted by Gadre et al. (1986), all *Salmonella* species investigated were inactivated after 10 days at 37°C. Pasteurization is frequently used prior to AD in order to reduce pathogen numbers (Sahlström et al., 2003). As it is known that *Salmonella* in sludge cannot withstand more than 5 min of heating at 70°C, a pasteurization step should act as extra insurance for *Salmonella* inactivation from slaughterhouse wastes and in animal material after AD.

Literature does not reveal much information on the use of AH to inactivate *Salmonella*. A study by Mastroeni et al. (1994) found AH of crude whole *Salmonella* extracts reduced the lethal toxicity of the extracts on mice. Extracts were however only incubated with NaOH at a final concentration of 0.25 M for 3 h at 37°C. This is much lower a temperature than what is used in AH units. Considering this, and that AH was found in a study by Kaye et al. (1998) to completely destroy all representative classes of potentially infectious agents as well as disposing of animal carcasses by solubilization and digestion, it can be assumed that the process would also totally inactivate *Salmonella* species.

Clostridium (botulism, black quarter, tetanus)

Clostridium botulinum causes the disease botulism. The disease can occur following ingestion of contaminated food, from colonization of the infant gastrointestinal tract, or from a wound infection. Vegetative cells and spores of *C. botulinum* can be found in soil, decaying vegetation and manure (Bagge et al., 2010). The disease results from the production of a potent neurotoxin which is released when spores germinate (Berge et al., 2009).

Black quarter is an infectious and often lethal bacterial disease of sheep and cattle caused by *Clostridium chauvoei*. The bacteria can be found in soil and faeces (Bagge et al., 2010), and once a pasture has become contaminated, the disease usually occurs annually in susceptible animals that graze on the pasture (Timoney et al., 1988).

Clostridium tetani is the causal agent of tetanus, a disease characterized by muscular spasms that can lead to respiratory failure and, in up to 40% of cases, death. Spores of the bacteria can reside in soil or in the gastrointestinal

tract of animals. *C. tetani* usually enters a host through a wound to the skin. *C. botulinum*, *C. chauvoei* and *C. tetani* can cause serious diseases in farm animals, resulting in suffering or even death for the animals. Spore-forming bacteria including pathogenic *Clostridium* species can survive pasteurization and are reportedly not affected by the AD process (Olsen and Larsen 1987; Bagge et al., 2005; Sahlström et al., 2008). Couturier & Galtier (2000) report survival of *Clostridium* following thermophilic digestion. The survival of pathogenic Clostridia during the biogas process results in the need for careful assessment of the risk of application of digested residues onto agriculture. A study by Bagge et al. (2010) however failed to show the survival of pathogenic clostridia after AD.

It has been reported that *Clostridium* can survive the composting process (Berge et al., 2010). In a carcass-composting environment, it has been found that anaerobic zones do develop, despite attempts to keep the entire process aerobic. These anaerobic zones allow the growth of *Clostridium* cells. Eventually, as decomposition occurs, and the temperatures increase, *Clostridium* growth will slow, and sporulation will occur (Berge et al., 2010).

The AH process has been shown to destroy all pathogens listed as index organisms by the State and Territorial Association on Alternative Treatment Technologies (STAATT I and STAATT II), which require a 6-log (99.9999%) reduction in vegetative agents and a 4-log (99.99%) reduction in sporeforming agents (NABC, 2004). Thus, it could be assumed that *Clostridium* would be inactivated through AH, and that AH would appear to be the best approach that could be taken to inactivate spore-forming clostridial pathogens.

***Brucella abortus* (Brucellosis)**

Bacteria of the genus *Brucella* are the causative agents for the zoonotic disease brucellosis. *B. abortus* infects cattle primarily, although it can occasionally also infect sheep (Bertram-Shaw et al., 1976). *B. abortus* infects the placenta and fetus of gestating cows and causes the fetus to abort. When humans however, are infected by this organism they develop a severe fever, but do not abort (Corbel et al., 1997).

Bacteria of the genus *Brucella* have been reported to be very heat sensitive (Van den Heever et al., 1992; Jay, 2000). Pasteurization at 63°C for 30 min and at 72°C for 15 sec successfully removed *Brucella* from contaminated raw milk, making it safe for consumption, indicating that pasteurization of animal carcasses could be used to remove the risk of disease. These findings would also suggest that composting could also inactivate *Brucella*. Similarly, Harada et al. (1993) reported that a temperature of 61°C for 3 min would inactivate *Brucella abortis* or *suis*. It is thus likely that a thermophilic AD process would also inactivate this pathogen.

Again, although no literature reports regarding the survival of *Brucella* after a process of AH could be found based on the report of Kaye et al. (1998), it could be expected that AH would be capable of the inactivation of *Brucella* species.

Bacillus anthracis

Anthrax is an acute disease caused by *Bacillus anthracis*. The disease affects both humans and animals, and most forms of the disease are lethal. Infections occur when spores of *B. anthracis* enter the body via abrasion, inhalation, ingestion, and, on rare occasions, via insect vectors (Reuter et al., 2011). The endospores are extremely persistent in the environment and can remain viable in soils for up to 300 years (Reuter et al., 2011).

Reuter et al. (2011) conducted an experiment to speculate on the possible fate of *B. anthracis* within a field scale composting system. Because of regulatory requirements, safety considerations and the field scale design of the experiment, *B. anthracis* spores were not used, but rather spores of *B. licheniformis* and *B. thuringiensis* were chosen. The study showed that a one millionfold inactivation of *Bacillus* spores was achieved by exposure to compost and elevated temperatures over time. Variation in composting microclimates within the compost pile may explain why some spores remained viable in certain parts of the compost pile. A total inactivation of *Bacillus* spores was not possible. However, composting may still have merit as a means of bio-containment of infected carcasses and slaughterhouse wastes, reducing and diluting the transfer of infectious spores into the environment (Reuter et al., 2011).

Bagge et al. (2010) investigated the diversity of spore-forming bacteria in cattle manure, slaughterhouse waste and samples from biogas plants. Of particular interest in their study, were *Clostridium* and *Bacillus*. Before digested residues are reused for agriculture, biowaste must be adequately treated and proven to be hygienically safe (Bagge et al., 2010). To reduce the risk of spreading pathogens, pasteurization of biowaste at 70°C for an hour prior to AD is recommended. Pasteurization however will not inactivate bacterial spores (Bagge et al., 2005; Sahlström et al., 2008). The results of Bagge et al. (2010) showed that *Bacillus* species seemed to pass the biogas process from biowaste to digested residues relatively unaffected. Due to their survival of pasteurization and the AD process, it is important to assess the risk of spreading digested residues from biogas plants containing *Bacillus* spores onto agricultural fields, and to take the necessary precautions.

The efficacy of AH for the inactivation of pure cultures of bacteria, including *B. subtilis*, for the disposal of infectious animal waste was evaluated in a study by Kaye et al. (1998). The tissue digester was operated at 110–120°C and approximately 103 kpa for 18 h. Samples were removed after a cooling phase at 50°C. The process was found to completely destroy *B. subtilis*. It can thus be expected that AH would be capable of the inactivation of the more deadly pathogen, *B. anthracis*.

***Mycobacterium bovis* (Beef tuberculosis)**

Mycobacterium bovis is the causative agent of bovine tuberculosis, a chronic disease of cattle and other species. The disease can cause major losses in animal

production and is a potentially debilitating zoonosis. *M. bovis* is capable of jumping the species barrier, and causing tuberculosis in humans, a disease normally caused by *M. tuberculosis*. Normally, *M. bovis* is transmitted to humans via infected milk, although it can also spread via aerosol droplets. However, actual infections in humans are rare, because of control measures put in place. Studies by Croshaw et al. (1971) showed that *M. bovis* was destroyed by the pasteurization of milk at 71.7°C for 15 sec, and at 62.8°C for 30 min.

Far more work has been conducted investigating the survival of *Mycobacterium avium subsp. paratuberculosis* after composting than *M. bovis*. *M. avium subsp. paratuberculosis* is a member of the same genus, and the causal agent of paratuberculosis, an intestinal infection of domestic and wild ruminants. Grewel et al. (2006) showed that after 3 days of composting at 55°C, *M. avium subsp. paratuberculosis* were no longer detectable. Also, Gobec et al. (2009) showed that *M. avium subsp. paratuberculosis* was not able to be isolated 24 h after the start of composting. However, a report by Haheesy (1996) indicated that farm yard (composted) manure must be exposed to a mean temperature of 60–70°C during the composting process for 3 weeks to destroy *M. bovis* bacilli. The authors concluded that as the majority of solid dung heaps do not reach this high a temperature, composted manure cannot necessarily be considered safe. These findings appear to be contradictory of the results of related *Mycobacterium* studies, and of the findings of pasteurization studies by Croshaw et al. (1971).

No studies investigating *M. bovis* in AD could be found upon intensive literature searches. In a mesophilic AD study by Olsen et al. (1985), *M. paratuberculosis* was detected and cultured up until 28 days of digestion. Under thermophilic conditions, however, *M. paratuberculosis* was inactivated after 24 h. In a study by Slana et al. (2011), viable *M. avium subsp. paratuberculosis* cells were detected using culture from fermenters up to 2 months. Therefore, the safety of the final products of digestion used for fertilization concerning the presence of *Mycobacterium* species cannot be certain.

Kaye et al. (1998) tested the survival of organisms after AH. All organisms tested, including *M. bovis* were completely destroyed by the AH process.

Erysipelothrix rhusiopathiae

Erysipelothrix rhusiopathiae is found all over the world and has been isolated from a variety of vertebrates as well as from soil. *E. rhusiopathiae* is primarily considered an animal pathogen, causing a disease known as erysipelas in turkeys, pigs, birds, sheep, fish, and reptiles (Brooke and Riley, 1999).

Erysipelothrix cultures are reportedly destroyed by exposure to moist heat at 55°C for 10 min (Timoney et al., 1988). A study conducted by Morrow et al. (1995) investigated composting as an approach to treat swine mortalities. The authors placed cultures of *E. rhusiopathiae* in compost piles. The temperatures reached in

the composting process were high enough to destroy *E. rhusiopathiae*, and the authors concluded that properly produced composts did not pose a risk for transmitting disease.

Olsen and Larsen (1987) published the results of experiments investigating the reductions of pathogenic and indicator bacteria in animal slurry subjected to mesophilic (35°C) and thermophilic (53°C) AD. Under mesophilic AD conditions, a T90 value of 1–8 days was obtained for *E. rhusiopathiae*, while at 53°C, the T90 value was reduced to 1–2 h. A study conducted by Han et al. (2011) investigated the effects of an anaerobic lagoon fermentation and an autothermal thermophilic aerobic digestion on bacterial pathogens contained in raw swine manure. *E. rhusiopathiae* was present in the raw manure, however was no longer detectable following a mesophilic anaerobic lagoon fermentation. Manure treated by thermophilic aerobic digestion also did not yield detectable levels of *E. rhusiopathiae*. These findings suggest that AD processes would be successful in the inactivation of *E. rhusiopathiae*.

Although no literature reports regarding the survival of *E. rhusiopathiae* after a process of AH could be found, the report of Kaye et al. (1998) would indicate that AH should be capable of the inactivation of *E. rhusiopathiae*.

BSE

BSE, also known as mad cow disease, is a relatively new disease that primarily affects cattle. BSE can also cause a corresponding disease in humans—Creutzfeldt-Jakob disease (Anon, 2003). There is still much controversy regarding the causes of BSE and Creutzfeldt-Jakob disease, however the most common belief is that the infectious agents are prions, an abnormal form of a type of protein (Anon, 2003). However, the prion hypothesis has also been challenged and an autoimmune response theory has been postulated (Ebringer et al., 1997).

Currently, the public considers BSE to be the greatest concern to any bovine-based product. The risk, however, of spreading BSE via composting of catering wastes in the UK has been shown to be 'remote', because there are many controls in place for keeping the disease from entering the food system, and hence the food residuals stream in the UK today. In the slaughterhouse, TSE management aims to prevent infected material from entering the food and feed chains. According to EU legislation, animals suspected of TSE infection are separated and safely disposed of. Prions such as BSE are more resistant to heat than many viruses (Gale et al., 2004). In fact, BSE infected material remains infected after cooking, rendering and long periods of incubation in the soil (Anon, 2003). According to Rohwer (1984), less than a 0.5 log (70%) destruction of scrapie agent was seen after 60 min at 60°C and 80°C. It can therefore be assumed that a standard composting process whereby the temperature was maintained at 60°C for 2 days would not reduce BSE infectivity.

TSEs have also been reported to survive the operational temperatures at which AD are conducted (Hinckley et al., 2008). Studies by Topper et al. (2006) showed AD under both mesophilic and thermophilic conditions to be incapable of reducing or eliminating BSE. In a study conducted by Brown et al. (2000), infected brain material was heated to 600°C. Despite brains being totally ashed, when reconstituted with saline to their original weights, ashed brain material was able to transmit disease to 5 of 35 inoculated hamsters.

Apart from incineration (where temperatures of >850°C are reached; Gwyther et al 2011), AH is the only effective method known for the destruction of prion material (NABC 2004). A study carried out by the Institute of Animal Health at the University of Edinburgh, investigated the ability of AH to destroy BSE prions grown in the brains of mice. Infected mice heads were subjected to AH for either 3 or 6 h, and after neutralization of the hydrolysates, aliquots were injected into mice. Evidence of TSE was found in some mouse brains of mice injected with hydrolysate taken from the 3 h digestion, but significantly, no disease was found in the brains of mice injected with hydrolysate from the 6 h digestion (NABC, 2004). Studies conducted by Murphy et al. (2009) also showed scrapie to be inactivated by AH.

Aphtho virus

Foot-and-mouth disease (FMD) is an infectious and sometimes fatal viral disease caused by the Aphtho virus that affects domesticated and wild ruminants and pigs. Most infected animals eventually recover but often develop sequelae (Guan et al., 2010). The highly contagious nature of the virus and the severity of economic impacts associated with disease have resulted in FMD being labeled as the most important disease limiting the trade of animals and animal products throughout the world (Arzt et al., 2011).

The virus can survive for weeks or months in refrigerated internal organs, bone marrow, lymph and hemal nodes, glands, and residual blood, however can be inactivated within 3 days in skeletal muscle after slaughter due to reduced pH. The FMD virus can be inactivated using heat, however, the amount of virus inactivation is determined by the temperature (Guan et al., 2010), and by access of the heat to the virus infected material. Blackwell et al. (1988) reported that heating to an internal temperature of 58.3°C inactivated FMD in minced beef containing virus-infected lymph nodes, while temperatures of up to 79.4°C were required for minced meat in nylon tubes. The American Association of Food Hygiene Veterinarians (1990) reported that core temperatures of 93°C were needed to inactivate the FMD virus in heart muscle.

Guan et al. (2010) investigated the inactivation and degradation of FMD virus during composting of infected pig carcasses. FMD virus infected pig carcasses were composted in a mixture of chicken manure and wood shavings, and compost temperatures reached 50°C and 70°C by days 10 and 19, respectively. Results indicated

that the virus was inactivated in specimens in compost by day 10 and the viral RNA was degraded in skin and internal organ tissues by day 21.

Inactivation experiments conducted by Turner et al. (2000) in contrast, showed the FMD virus to be remarkably thermally stable, surviving with little or no loss of titre at 55°C and 60°C. Even at 65°C, the virus did not drop to below detectable levels within 5 min in a slurry. From these studies, the authors recommended treatment of slurry and water-containing FMD virus in full-scale mobile treatment plant operating at a minimum of 70°C.

As with other viruses, the bone marrow of infected animal carcasses can contain high loadings of FMD virus (Farez and Morley, 1997). Therefore, to effectively inactivate FMD virus from animal waste material, a composting process where the temperature was maintained at greater than 65°C for at least 2 days would need to be used. According to Gale et al. (2004), a composting temperature of 60°C for 2 days would give at least a 6-log reduction of all exotic pig viruses, including FMD, even in the bone marrow of whole pig carcasses discarded for composting.

No information could be found regarding the survival of FMD virus after either mesophilic or thermophilic AD. Considering the heat stability of the virus, it could be predicted that AD would not successfully inactivate FMD virus. However, the combination of a pasteurization step and an AD process could be expected to be successful in the inactivation of the virus.

No literature reports regarding the survival of FMD virus after a process of AH could be found. As the report of Kaye et al. (1998) states that the process was found to completely destroy all representative classes of potentially infectious agents, it can be expected that AH would be capable of the inactivation of this virus.

Lyssa virus (Rabies)

Rabies is a disease caused by the lyssa virus, and is an acute encephalitis in warm-blooded animals (Kopcha et al., 2010). In humans, rabies is almost always fatal if post-exposure prophylaxis is not administered prior to the onset of severe symptoms. The economic impact of the disease is significant, with the virus causing death of livestock in some countries.

The rabies virus is able to be inactivated by heat (Kopcha et al., 2010) and thus a pasteurization step at 70°C should be considered successful in rabies inactivation. No information was able to be found on composting inactivation of the virus, but based on the findings of Kopcha et al. (2010), it would be likely that composting would also successfully inactivate the virus. Studies by Kissling and Reese (1963) investigated virus stability at different temperatures. Rabies tissue culture-adapted virus in serum-free medium was reported to be sensitive to heat inactivation. At 56°C, almost all virus was inactivated within 30 min, while at 37°C, more than 99 % of the virus was inactivated within 24 h. At 37°C, no virus was viable after 32–48 h.

No information could be found upon extensive literature searching on the survival of the rabies virus after an AD process. Considering the heat sensitivity of the virus, it is likely that it would be inactivated after a thermophilic AD, but it is not certain whether a mesophilic AD process would have the same effect. When combined with a pasteurization step however, one could hypothesize that the virus would be inactivated.

Again, no literature reports regarding the survival of the rabies virus after a process of AH could be found. Based on the findings of Kaye et al. (1998), it would however be expected that inactivation of the virus would result from AH.

African swine fever virus

African swine fever (ASF) is a viral disease (genus *Asfivirus*) which causes a devastating haemorrhagic fever of pigs with mortality rates approaching 100% (Costard et al., 2009). Virus is excreted in the faeces of infected pigs, and as faeces mostly are transferred to a slurry store prior to the infection being identified, viral contamination of large quantities of pig slurry can occur inadvertently.

ASF virus has been reported to be quite resistant to inactivation, and can survive many freeze-thaw cycles (Turner et al., 1998). It is also resistant to changes in pH, and certain strains are resistant to complete inactivation at pH values between 4 and 13 (Turner et al., 1998). The virus is however very sensitive to drying, and can be inactivated by lipid solvents because of its lipid envelope (Turner et al., 1998).

ASF virus is however heat sensitive. In studies conducted by Plowright and Parker (1967), ASF virus was shown to be inactivated (5 log inactivation) by 90 min at 56°C. However, work done by Turner et al. (1998) indicated the virus to be inactivated at 50°C in 30 min, at 56°C within 90 sec and within seconds at 60°C.

The findings of Turner et al. (1998) described above, as well as projected costs of treatment for virus inactivation resulted in the authors deciding that heat was the most effective means of ASF virus inactivation. This is because heat treatment was found to be very rapid and consistent at a temperature of 56°C, while alkali inactivation was slower. Also, heat can be recovered, thus reducing costs. These authors designed a pilot scale treatment plant to treat pig slurry at a minimum temperature of 65°C, and maintained this temperature in at least 99.99% of the material for a minimum of 5 min. This was done so to provide a reasonable safety margin for virus inactivation.

The bone marrow of infected pig carcasses contains high loadings of ASF virus (Gale et al., 2004). When considering the composting of an animal carcass, the times and temperature of the process must be carefully considered. It has been estimated that a sphere of diameter 40 cm (which would be similar to a large leg or carcass of pork containing the bone) would require 40 h to reach a temperature of 56°C at the centre when the surrounding composting temperature is 60°C (Haug, 1993). Therefore, a composting process with a temperature of 60°C maintained for 2 days

would give at least a 6-log reduction of all exotic pig viruses, including ASF virus, even in the bone marrow of whole pig carcasses (Gale et al., 2004). As studies have shown ASF virus to be inactivated within seconds at 60°C, it would appear that a correctly performed composting process should be successful in inactivating ASF virus.

No information could be found regarding AD and the survival rates of ASF virus subjected to the process. While no predictions can be made as to the survival of the virus in a mesophilic process, it could be predicted that a thermophilic process may successfully inactivate the virus.

A 1% solution of both NaOH or Ca(OH)₂ was found to be able to result in a 4 fold inactivation of the virus at 4°C within 150 sec. More dilute solutions (0.5%) of both chemicals were however not found to be able to inactivate virus at 22°C in 5 min (Turner et al., 1998). It could be expected that a AH process, which uses alkaline solution as well as heat to inactivate microbial organisms would be successful in the inactivation of ASF virus.

Phlebo virus (Rift Valley fever)

Rift Valley fever (RVF) is a viral zoonosis resulting in fever. The disease is caused by the RVF virus, a member of the Phlebovirus genus. It is spread by the bite of infected mosquitoes, or by exposure to tissues or blood of infected animals (Zaki et al., 2006). Humans infected with the virus have self-limited febrile illness, but retinal degeneration (5–10%), hemorrhagic fever (<1%), or encephalitis (<1%) can also develop (Laughlin et al., 1979; Meegan and Bailey, 1989). The RVF virus is susceptible to low pH (≤ 6.2), lipid solvents and detergents, as well as solutions of sodium or calcium hypochlorite with residual chlorine content greater than 5000 ppm. In a neutral or alkaline pH, mixed with serum or other proteins, the virus can survive for as long as four months at 4°C and eight years below 0°C. The virus is also quickly destroyed by pH changes in decomposing carcasses (http://www.cfsph.iastate.edu/Factsheets/pdfs/rift_valley_fever.pdf). Thus, although no literature can be found on AH of animals infected with RVF virus, it would appear that AH would not be a recommended method of treatment of animal carcasses in order to inactivate the virus.

Unfortunately, no information could be found on composting or AD with respect to the inactivation of RVF virus, although Flory et al. (2010) report that composting approaches can be used to treat RVF diseased animals, thus preventing disease outbreaks.

Cysticercus bovis (beef measles)

Bovine cysticercosis is a zoonotic disease found worldwide affecting the muscles of cattle (Pawlowski and Schultz, 1972; Ogunremi and Benjamin, 2010). The disease is most prevalent in countries where poor sanitation practices on cattle farms are common and where cultural habits include eating undercooked meat. It does not represent a serious human health risk. Bovine cysticercosis is caused by *Cysticercus bovis*, the larval stage of *Taenia*

saginata, a species of human tapeworm (McFadden et al., 2011). Humans can acquire intestinal tapeworm infections by the consumption of beef containing the parasite. Disease in cattle is initiated by the ingestion of materials contaminated with tapeworm eggs originating from human feces larvae of a human tapeworm. Cattle become infected with bovine cysticercosis by ingesting materials contaminated with tapeworm eggs originating from human faeces (McFadden et al., 2011).

A study was conducted by Hughes et al. (2005) to investigate the survival of *T. saginata* ova after AD at 35°C for 10 days followed by lagooning for 15 days. Results indicated that viability and infectivity were completely destroyed. The authors also showed that a 3 h treatment at 55°C in crude sludge resulted in a 99 % reduction of ova infectivity. It could thus be assumed that a 70°C pasteurization step prior to AD treatment, as is the regulation in Europe, would successfully inactivate bovine cysticercosis.

Another study by Morris et al. (1986) showed 100% efficacy of sanitation of *T. saginata* at 35°C. A thorough review of the literature has failed to find studies whereby the inactivation of *T. saginata* by composting was investigated. However, the US Environmental Protection Agency (Part 503) sets standards for the destruction and concentration of pathogens in composted biosolids. These standards are commonly used as marketplace specifications for compost sold for public use. By maintaining these standards, various harmful pathogens should be destroyed (<http://ccpeat.com/literature/composttech.pdf>). According to the standards, *T. saginata* does not survive for more than a few minutes at 55°C. Thus, a standard composting procedure would successfully inactivate this organism.

No information could be obtained regarding the inactivation efficiency of AH on *T. saginata*. However, Kaye et al. (1998) showed *Giardia* cysts to be completely destroyed by AH, an indication that slaughterhouse waste material infected by bovine cysticercosis might also successfully be treated by AH.

Conclusions

In the past, solid slaughterhouse wastes were most commonly treated by rendering, the process providing slaughterhouses with an additional source of income. However, because of the risk of TSEs, the economic value of such products has been reduced significantly, and in fact, such products must in many cases be treated as waste themselves (Palatsi et al., 2011). The cost for the safe disposal of slaughterhouse waste in recent years has thus considerably increased. This is primarily due to health risks from the presence of pathogens in such wastes. Several different possibilities for their disposal exist, as described in this review.

Composting is one alternative for the disposal of slaughterhouse wastes. The process has various benefits, including reduced environmental pollution, the generation of a valuable byproduct, and the destruction of

a majority of pathogens (NABC, 2004). The successful conversion of such wastes into good-quality compost however requires close control. When performed under stringent management, the final product should not pose a risk to animal and human health (Gale, 2004). There are however some pathogens that are not able to be destroyed by composting, such as prions and spore forming bacteria.

The process of AH of slaughterhouse wastes is relatively new. It uses a strong base, heat and temperature to catalyze the hydrolysis of biological materials into a sterile aqueous solution consisting of peptides, amino acids, sugars and soaps (Kaye et al., 1998). This effluent is highly alkaline and very rich in nutrients, and although it can be released into a sanitary sewer, it can also potentially pose problems (NABC, 2004). It has been found to be extremely effective in the elimination of many pathogens and prions from carcasses as well as from animal wastes. The waste from the process is however very rich in nutrients, and would thus offer high biogas generation potential.

AD is today one of the most promising methods for the disposal of slaughterhouse waste (Gwyther et al., 2011). This process not only produces a digestate which can be used as a valuable fertilizer, but it also produces heat and biogas, that in turn can be converted to energy. Moreover, slaughterhouse wastes are rich in proteins and nitrogen, and thus are ideal substrates for the AD process. Numerous studies have reported various levels of effectiveness in the removal of different pathogens using AD.

The results of our extensive literature review concerning the survival of pathogens after composting, AH and AD are summarized in Table 1. Although there would not appear to be a single approach that would inactivate all the pathogens investigated in this study, an AD process with either a pre- or post- pasteurization step would most likely inactivate the majority of microorganisms. Prions would however survive a pasteurization and an AD process, as would spore-forming bacteria. The survival of prions should however not be a cause for concern, as any biogas plant operator should be able to prevent diseased animals or suspected TSE diseased animals from entering the process.

Although spore-formers will also not be removed from the joint processes of AD and pasteurization, the numbers could be expected to be significantly reduced. The European Union has laid down various laws concerning the disposal of animal byproducts, including ABP-Regulation (Regulation (EC) no. 1774/2002), which defines new treatment possibilities and corresponding mandatory processing parameters. In Sweden, slaughterhouse wastes are treated with a 70°C pasteurization step prior to AD, and digestates are used in agriculture. No problems seem to have arisen with spore-forming pathogens such as *Clostridium* and *Bacillus* as a result of such treatment. The benefits of using AD to treat slaughterhouse wastes are immense,

Table 1. Summary of inactivation of different pathogens by different treatments.

Pathogen	Inactivation by					
	Pasteurization* (70°C)	Mesophilic AD (37°C)	Thermophilic AD (55°C)	Pre-asteurization and anaerobic digestion at 37°C **	Composting	Alkaline hydrolysis
<i>Escherichia coli</i>	++	+	+	(PI)	+	(PI)
<i>Salmonella</i>	++	+	++	(PI)	++	(PI)
<i>Clostridium</i>	-	-	-	(-)	-	(PI)
<i>Brucella abortus</i>	++	NI	(PI)	(PI)	NI	(PI)
<i>Bacillus anthracis</i>	-	-	-	(-)	-	(PI)
<i>Mycobacterium bovis</i>	++	(+)	(PI)	(PI)	C	++
<i>Erysipelothrix rhusiopathiae</i>	++	+	++	(PI)	++	(PI)
BSE prion	-	-	-	(-)	-	++
Aphtho virus	(PI)	NI	(+)	(PI)	C	(PI)
Rabies virus	++	NI	(PI)	(PI)	(PI)	(PI)
African Swine Fever Virus	++	NI	(PI)	(PI)	++	(PI)
Phlebo virus	(PI)	NI	NI	(PI)	(PI)	-
<i>Cysticercus bovis</i>	(PI)	++	++	(PI)	(PI)	(PI)

++, total inactivation; +, inactivation; -, survival; (PI), no information on process, but predicted inactivation of pathogen; (-), no information found, but predicted survival of pathogen; NI, no information found; C, contradictory information; *, Pasteurization for 60 min;

**, Pre-pasteurization for 60 min at 70°C.

and not only are the unpleasant waste products of the ever-growing meat industry disposed of, but renewable energy is produced. Alternatively, an AH process, combined with AD should result in the most efficient method possible for the elimination of pathogens, and at the same time, produce energy. This would however require some research to be conducted to investigate the best way to do this, to prevent any inhibitions and problems occurring from using the alkaline waste in the AD process.

Acknowledgements

Tobias Gundolf is acknowledged for his literature review which served as a reference point for preparing this manuscript.

Declaration of interest

I.H.F.W. was funded by the Fonds zur Förderung der wissenschaftlichen Forschung (FWF) Austria, P-200010. The Red Meat Abattoir Association of South Africa initiated and supported the study.

References

- Acha PN, Szyfres B. (1987). Zoonoses and communicable diseases common to man and animals. 2nd edition. Washington: Pan American Health Organization/World Health Organization.
- Alfreider A, Peters S, Tebbe CC, Rangger A, Insam H. (2002). Microbial community dynamics during composting of organic matter as determined by 16S ribosomal DNA analysis. *Compost Sci Util*, 10, 303–312.
- Anon. (2002). The Animal By-Products Regulations (EC) no. 1774/2002. European Commission, Brussels.
- Anon. (2003). Risk of BSE in food residuals compost is 'remote'. *Biocycle*, 44, 18–18.

- Arvanitoyannis IS, Ladas D. (2008). Meat waste treatment methods and potential uses. *Int J Food Sci Tech*, 43, 543–559.
- Arzt J, Juleff N, Zhang Z, Rodriguez LL. (2011). The pathogenesis of foot-and-mouth disease I: viral pathways in cattle. *Transbound Emerg Dis*, 58, 291–304.
- Bagge E, Sahlström L, Albihn A. (2005). The effect of hygienic treatment on the microbial flora of biowaste at biogas plants. *Water Res*, 39, 4879–4886.
- Bagge E, Persson M, Johansson KE. (2010). Diversity of spore-forming bacteria in cattle manure, slaughterhouse waste and samples from biogas plants. *J Appl Microbiol*, 109, 1549–1565.
- Bailey KL, Lazarovits LG. (2003). Suppressing soil-borne diseases with residue management and organic amendments. *Soil Till Res*, 72, 169–180.
- Beffa T, Blanc M, Lyon PF, Vogt G, Marchiani M, Fischer JL, Aragno M. (1996). Isolation of *Thermus* strains from hot composts (60 to 80 degrees C). *Appl Environ Microbiol*, 62, 1723–1727.
- Berg G, Berman D. (1980). Destruction by anaerobic mesophilic and thermophilic digestion of viruses and indicator bacteria indigenous to domestic sludges. *Appl Environ Microbiol*, 39, 361–368.
- Berge AC, Glanville TD, Millner PD, Klingborg DJ. (2009). Methods and microbial risks associated with composting of animal carcasses in the United States. *J Am Vet Med Assoc*, 234, 47–56.
- Bertram Shaw W. (1976). B. abortus infection in sheep. I. Field case. II. Experimental infection of ewes. *Brit Vet J*, 132, 18–26 and 132, 143–151.
- Blackwell JH, Nolan EJ, Rickansrud DA. (1998). Total caloric input of a thermal-process as an index of lethality for foot-and-mouth disease virus. *J Food Sci*, 53, 185–190.
- Blanc M, Marilley L, Beffa T, Aragno M. (1999). Thermophilic bacterial communities in hot composts as revealed by most probable number counts and molecular (16S rDNA) methods. *FEMS Microbiol Ecol*, 28, 141–149.
- Böhnelt H, Briese B-H, Gessler F. (2002). Methods for health risk assessment by *Clostridium botulinum* in biocompost. In: Insam H, Riddech N, Klammer S, eds. *Microbiology of Composting*. Berlin, Germany: Springer-Verlag, 517–526.
- Brooke CJ, Riley TV. (1999). *Erysipelothrix rhusiopathiae*: bacteriology, epidemiology and clinical manifestations of an occupational pathogen. *J Med Microbiol*, 48, 789–799.
- Brown P, Rau EH, Johnson BK, Bacote AE, Gibbs CJ Jr, Gajdusek DC. (2000). New studies on the heat resistance of hamster-adapted

- scrapie agent: threshold survival after ashing at 600 degrees C suggests an inorganic template of replication. *Proc Natl Acad Sci USA*, 97, 3418–3421.
- Bruggemann H, Baumer S, Fricke WF, Wiezer A, Liesegang H, Decker I, Herzberg C, Martinez-Arias R, Merkl R, Henne A, Gottschalk G. (2003). The genome sequence of *Clostridium tetani*, the causative agent of tetanus disease. *Proc Natl Acad Sci USA*, 100, 1316–1321.
- Cekmecelioglu D, Demirci A, Graves RE, Davitt NH. (2005). Applicability of optimized in-vessel food waste composting for windrow system. *Biosyst Eng*, 91, 479–486.
- Corbel MJ. (1997). Brucellosis: an overview. *Emerging Infect Dis*, 3, 213–221.
- Costard S, Wieland B, de Glanville W, Jori F, Rowlands R, Vosloo W, Roger F, Pfeiffer DU, Dixon LK. (2009). African swine fever: how can global spread be prevented? *Philos Trans R Soc Lond, B, Biol Sci*, 364, 2683–2696.
- Couturier C, Galtier L. (2000). Etat des connaissances sur le devenir des germes pathogènes et des micropolluants au cours de la méthanisation des déchets et sous-produits organiques (Programme ADEME, N° contrat: 9893024). http://www.solagro.org/site/im_user/034impacts.pdf. Annex: http://www.solagro.org/site/im_user/034annexes_sanitaire.pdf.
- Croshaw B. (1971). The destruction of Mycobacteria. In: Hugo WB, ed. *Inhibition and destruction of the microbial cell*. New York: Academic press, 419.
- Cuetos MJ, Gómez X, Otero M, Morán A. (2010). Anaerobic digestion and co-digestion of slaughterhouse waste (SHW): influence of heat and pressure pre-treatment in biogas yield. *Waste Manag*, 30, 1780–1789.
- Danon M, Franke-Whittle IH, Insam H, Chen Y, Hadar Y. (2008). Molecular analysis of bacterial community succession during prolonged compost curing. *FEMS Microbiol Ecol*, 65, 133–144.
- Dees PM, Ghiorse WC. (2001). Microbial diversity in hot synthetic compost as revealed by PCR-amplified rRNA sequences from cultivated isolates and extracted DNA. *FEMS Microbiol Ecol*, 35, 207–216.
- Dixon PF, Algoët M, Bayley A, Dodge M, Joiner C, Roberts E. (2012). Studies on the inactivation of selected viral and bacterial fish pathogens at high pH for waste disposal purposes. *J Fish Dis*, 35, 65–72.
- Ebringer A, Thorpe C, Pirt J, Wilson C, Cunningham P, Ettelaie C. (1997). Bovine spongiform encephalopathy: is it an autoimmune disease due to bacteria showing molecular mimicry with brain antigens? *Environ Health Perspect*, 105, 1172–1174.
- Edström M, Nordberg A, Thyselius L. (2003). Anaerobic treatment of animal byproducts from slaughterhouses at laboratory and pilot scale. *Appl Biochem Biotechnol*, 109, 127–138.
- Farez S, Morley RS. (1997). Potential animal health hazards of pork and pork products. *Rev - Off Int Epizoot*, 16, 65–78.
- Flory GA, Peer RW. (2010). Verification of poultry carcass composting research through application during actual avian influenza outbreaks. *ILAR J*, 51, 149–157.
- Franke-Whittle IH, Klammer SH, Insam H. (2005). Design and application of an oligonucleotide microarray for the investigation of compost microbial communities. *J Microbiol Methods*, 62, 37–56.
- Franke-Whittle IH, Knapp BA, Fuchs J, Kaufmann R, Insam H. (2009). Application of COMPOCHIP microarray to investigate the bacterial communities of different composts. *Microb Ecol*, 57, 510–521.
- Gadre RV, Ranade DR, Godbole SH. (1986). A note on survival of salmonellas during anaerobic digestion of cattle dung. *J Appl Bacteriol*, 60, 93–96.
- Gale P. (2002). Risk assessment: use of composting and biogas treatment to dispose of catering waste containing meat (Final Report to the Department for Environment, Food and Rural Affairs).
- Gale P. (2004). Risks to farm animals from pathogens in composted catering waste containing meat. *Vet Rec*, 155, 77–82.
- Gobec I, Oceppek M, Pogacnik M, Dobeic M. (2009). Inactivation of *Mycobacterium avium* paratuberculosis in sheep manure. *Slov Vet Res*, 46, 105–113.
- Grewal SK, Rajeev S, Sreevatsan S, Michel FC Jr. (2006). Persistence of *Mycobacterium avium* subsp. paratuberculosis and other zoonotic pathogens during simulated composting, manure packing, and liquid storage of dairy manure. *Appl Environ Microbiol*, 72, 565–574.
- Guan J, Chan M, Grenier C, Brooks BW, Spencer JL, Kranendonk C, Copps J, Clavijo A. (2010). Degradation of foot-and-mouth disease virus during composting of infected pig carcasses. *Can J Vet Res*, 74, 40–44.
- Gunnerson, CG, Stuckey DC. (1986). *Anaerobic digestion: principles and practice of biogas systems* (World Bank Technical Paper No. 49). Washington, DC: World Bank.
- Gwyther CL, Williams AP, Golyshin PN, Edwards-Jones G, Jones DL. (2011). The environmental and biosecurity characteristics of livestock carcass disposal methods: A review. *Waste Manag*, 31, 767–778.
- Haehy T. (1996). A survey of temperatures in cattle manure recorded on five farms in Co Dublin. Selected papers. Tuberculosis Investigation Unit, University College, Dublin, pp. 72–79.
- Han I, Congeevaram S, KiDW, Oh BT, Park J. (2011). Bacterial community analysis of swine manure treated with autothermal thermophilic aerobic digestion. *Appl Microbiol Biotechnol*, 89, 835–842.
- Harada Y, Haga K, Osada T, Koshino M. (1993). Quality of compost produced from animal wastes. *JARQ* 26, 238–246.
- Haug RT. (1993). Kinetics of heat inactivation. In Haug RT, ed. *The Practical Handbook of Compost Engineering*. London: Lewis Publishers, 161–203.
- Hinckley GT, Johnson CJ, Jacobson KH, Bartholomay C, McMahon KD, McKenzie D, Aiken JM, Pedersen JA. (2008). Persistence of pathogenic prion protein during simulated wastewater treatment processes. *Environ Sci Technol*, 42, 5254–5259.
- Hoberg EP. (2002). *Taenia* tapeworms: their biology, evolution and socioeconomic significance. *Microbes Infect*, 4, 859–866.
- Hoitink H, Boehm M. (1999). Biocontrol within the context of soil microbial communities: A Substrate-Dependent Phenomenon. *Annu Rev Phytopathol*, 37, 427–446.
- Hughes DL, Morris DL, Norrington IJ, Waite WM. (1985). The effects of pasteurisation and stabilisation of sludge on *Taenia saginata* eggs. In: Strauch D, Havelaar AH, Hermite PL, eds. *Inactivation of microorganisms in sewage sludge by stabilization processes*. Essex, England: Elsevier, 75–82.
- Ibekwe AM, Papiernik SK, Gan J, Yates SR, Crowley DE, Yang CH. (2001). Microcosm enrichment of 1,3-dichloropropene-degrading soil microbial communities in a compost-amended soil. *J Appl Microbiol*, 91, 668–676.
- Insam H, Franke-Whittle IH, Goberna M. (2010). *Microbes at work. From wastes to resources*. Springer, Heidelberg.
- Insam H, Wett B. (2008). Control of GHG emission at the microbial community level. *Waste Manag*, 28, 699–706.
- Jay J. (2000). *Modern Food Microbiology*, 6th ed. Gaithersburg, MD: Aspen Publishers.
- Jiang X, Morgan J, Doyle MP. (2003). Fate of *Escherichia coli* O157:H7 during composting of bovine manure in a laboratory-scale bioreactor. *J Food Prot*, 66, 25–30.
- Kaye G, Weber P, Evans A, Venezia R. (1998). Efficacy of Alkaline Hydrolysis as an Alternative Method for Treatment and Disposal of Infectious Animal Waste. *Contemp Top Lab Anim Sci*, 37, 43–46.
- Kissling RE, Reese DR. (1963). Anti-rabies vaccine of tissue culture origin. *J Immunol*, 91, 362–368.
- Kompostverordnung. (2002). Österreichisches Bundesgesetzblatt II, Nr. 292/2001. (Austrian Compost ordinance).
- Kopcha M. (2010). Rabies: A rare but important disease. *Mich Dairy Rev*, 15, 1–3.
- Lastella G, Testa C, Cornacchia G, Notornicola M, Voltasio F, Sharma VK. (2002). Anaerobic digestion of semi-solid organics waste: biogas production and its purification. *Energ Convers Manage*, 43, 63–75.
- Laughlin LW, Meegan JM, Strausbaugh LJ, Morens DM, Watten RH. (1979). Epidemic Rift Valley fever in Egypt: observations of the spectrum of human illness. *Trans R Soc Trop Med Hyg*, 73, 630–633.
- Lemunier M, Francou C, Rousseaux S, Houot S, Dantigny P, Piveteau P, Guzzo J. (2005). Long-term survival of pathogenic and sanitation indicator bacteria in experimental biowaste composts. *Appl Environ Microbiol*, 71, 5779–5786.

- Lung AJ, Lin CM, Kim JM, Marshall MR, Nordstedt R, Thompson NP, Wei CI. (2001). Destruction of *Escherichia coli* O157:H7 and *Salmonella enteritidis* in cow manure composting. *J Food Prot*, 64, 1309–1314.
- Mastroeni P, Villarreal-Ramos B, Harrison JA, Demarco de Hormaeche R, Hormaeche CE. (1994). Toxicity of lipopolysaccharide and of soluble extracts of *Salmonella typhimurium* in mice immunized with a live attenuated *Salmonella* vaccine. *Infect Immun*, 62, 2285–2288.
- McFadden AM, Heath DD, Morley CM, Dorny P. (2011). Investigation of an outbreak of *Taenia saginata* cysts (*cysticercus bovis*) in dairy cattle from two farms. *Vet Parasitol*, 176, 177–184.
- Meegan JM, Bailey CJ. (1989). Rift Valley Fever. In Monath TP, ed. *The arboviruses: epidemiology and ecology*, vol. IV. Boca Raton, FL: CRC Press Inc, 51–76.
- Mock M, Fouet A. (2001). Anthrax. *Annu Rev Microbiol*, 55, 647–671.
- Monteith HD, Shannon EE, Derbyshire JB. (1986). The inactivation of a bovine enterovirus and a bovine parvovirus in cattle manure by anaerobic digestion, heat treatment, gamma irradiation, ensilage and composting. *J Hyg (Lond)*, 97, 175–184.
- Morgan Morrow WE, O'Quinn P, Barker J, Erickson G, Post K, McCaw M. (1995). Composting as a suitable technique for managing swine mortalities. *Swine Health Product*, 3, 236–243.
- Morris DL, Hughes DL, Hewitt RJ, Norrington IJ. (1986). Pathogens in sewage sludge: (ii) effects of sludge stabilization and treatment processes on viability and infectivity of beef tapeworm eggs. *Water Pollut Control*, 85, 476–481.
- Murphy RG, Scanga JA, Powers BE, Pilon JL, Vercauteren KC, Nash PB, Smith GC, Belk KE. (2009). Alkaline hydrolysis of mouse-adapted scrapie for inactivation and disposal of prion-positive material. *J Anim Sci*, 87, 1787–1793.
- NABC. (2004). Carcass disposal: a comprehensive review. Report written for the USDA Animal and Plant Health Inspection Service. National Agricultural Biosecurity Centre, Kansas State University, USA.
- Neafie RC, Marty AM, Johnson LK. (2000). Taeniasis and cysticercosis. In: Meyers WM, Neafie RC, Marty AM, Wear DJ, eds. *Helminthiasis, Pathology of Infectious Diseases*, vol. 1. Washington, DC: Armed Forces Institute of Pathology, 117–136.
- Neyens E, Baeyens J, Creemers C. (2003). Alkaline thermal sludge hydrolysis. *J Hazard Mater*, 97, 295–314.
- Noble R, Roberts SJ. (2004). Eradication of plant pathogens and nematodes during composting: a review. *Plant Pathol*, 53, 548–568.
- Ogunremi O, Benjamin J. (2010). Development and field evaluation of a new serological test for *Taenia saginata* cysticercosis. *Vet Parasitol*, 169, 93–101.
- Olsen JE, Larsen HE. (1987). Bacterial decimation times in anaerobic digestions of animal slurries. *Biol Waste*, 21, 153–168.
- Olsen JE, Jørgensen JB, Nansen P. (1985). On the reduction of *Mycobacterium paratuberculosis* in bovine slurry subjected to batch mesophilic or thermophilic anaerobic digestion. *Agr Wastes*, 13, 273–280.
- Paavola T, Syväsalto E, Rintala J. (2006). Co-digestion of manure and biowaste according to the EC animal by-products regulation and Finnish national regulations. *Water Sci Technol*, 53, 223–231.
- Palatsi J, Viñas M, Guivernau M, Fernandez B, Flotats X. (2011). Anaerobic digestion of slaughterhouse waste: main process limitations and microbial community interactions. *Bioresour Technol*, 102, 2219–2227.
- Paluszak Z, Bauza-Kaszewska J, Skowron K. (2011) Effect of animal by-products composting process on the inactivation of indicator bacteria. *BVet I Pulawy*, 55, 45–49.
- Plowright W, Parker J. (1967). The stability of African swine fever virus with particular reference to heat and pH inactivation. *Arch Gesamte Virusforsch*, 21, 383–402.
- Plym-Forsell L. (1995). Survival of salmonellas and *Ascaris suum* eggs in a thermophilic biogas plant. *Acta Vet Scand*, 36, 79–85.
- Pawlowski Z, Schultz MG. (1972). Taeniasis and cysticercosis (*Taenia saginata*). *Adv Parasitol*, 10, 269–343.
- Reuter T, Alexander TW, McAllister TA. (2011). Viability of *Bacillus licheniformis* and *Bacillus thuringiensis* spores as a model for predicting the fate of bacillus anthracis spores during composting of dead livestock. *Appl Environ Microbiol*, 77, 1588–1592.
- Rohwer RG. (1984). Virus like sensitivity of the scrapie agent to heat inactivation. *Science*, 223, 600–602.
- Ryckeboer J, Mergaert J, Coosemans J, Deprins K, Swings J. (2003). Microbiological aspects of biowaste during composting in a monitored compost bin. *J Appl Microbiol*, 94, 127–137.
- Sahlström L. (2003). A review of survival of pathogenic bacteria in organic waste used in biogas plants. *Bioresour Technol*, 87, 161–166.
- Sahlström L, Bagge E, Emmoth E, Holmqvist A, Danielsson-Tham ML, Albin A. (2008). A laboratory study of survival of selected microorganisms after heat treatment of biowaste used in biogas plants. *Bioresour Technol*, 99, 7859–7865.
- Salminen E, Rintala J. (2002). Anaerobic digestion of organic solid poultry slaughterhouse waste—a review. *Bioresour Technol*, 83, 13–26.
- Schink B. (1997). Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol Mol Biol Rev*, 61, 262–280.
- Slana I, Pribylova R, Kralova A, Pavlik I. (2011). Persistence of *Mycobacterium avium* subsp. *paratuberculosis* at a farm-scale biogas plant supplied with manure from paratuberculosis-affected dairy cattle. *Appl Environ Microbiol*, 77, 3115–3119.
- Spillman SK, Taub E, Schwyzer M, Wyler R. (1987). Inactivation of animal viruses during sewage sludge treatment. *Appl Environ Microbiol*, 53, 2077–2081.
- Srivastava A, Prasad R. (2000). Triglycerides-based diesel fuels. *Renew Sust Energ Rev*, 4, 111–133.
- Strauch D. (2002). Occurrence of microorganisms pathogenic for man and animals in source separated biowaste and compost importance, control, limits and epidemiology. In: De Bertoldi M, Sequi P, Lemmes B, Papi T, eds. *The Sciences of Composting*. Glasgow, UK: Blackie Academic and Professional, 224–232.
- Timoney JF, Gillespie JH, Scott FW, Barlough JE. (1988). Hagan and Bruner's Microbiology and Infectious Diseases of Domestic Animals. 8th ed. New York: Cornwell University Press.
- Topper P, Graves R, Richard T. (2006). The fate of nutrients and pathogens during anaerobic digestion of dairy manure. *Penn State Cooperative Extension. Agriculture and Biological Engineering. Extension Bulletin*.
- Turnbull PCB. (1998). Guidelines for the surveillance and control of anthrax in humans and animals. WHO/EMC/ZDI/98.6. <http://www.who.int/csr/resources/publications/anthrax/whoemc&di986text.pdf>.
- Turner C, Williams SM, Burton CH, Farrent JW, Wilkinson PJ. (1998). Laboratory scale inactivation of pig viruses in pig slurry and design of a pilot plant for thermal inactivation. *Water Sci Technol*, 38, 79–86.
- Turner C, Williams SM, Burton CH, Cumby TR, Wilkinson PJ, Farrent JW. (1999). Pilot scale thermal treatment of pig slurry for the inactivation of animal virus pathogens. *J Environ Sci Health B*, 34, 989–1007.
- Turner C, Williams SM, Cumby TR. (2000). The inactivation of foot and mouth disease, Aujeszky's disease and classical swine fever viruses in pig slurry. *J Appl Microbiol*, 89, 760–767.
- Urlings HA, van Logtestijn JG, Bijker PG. (1992). Slaughter by-products: problems, preliminary research and possible solutions. *Vet Q*, 14, 34–38.
- Van den Heever LW, Katz KW, Te Brugge LA. (1982). On the inactivation of *Brucella abortus* in naturally contaminated milk by commercial pasteurisation procedures. *J S Afr Vet Assoc*, 53, 233–234.
- Vandeviere P, De Baere L, Verstraete W. (2002). Types of anaerobic digester for solid wastes. In: Mata-Alvarez J, ed. *Biomethanization of the organic fraction of municipal solid wastes*. London: IWA Publishing, 111–137.
- Zaki A, Coudrier D, Yousef AI, Fakeeh M, Bouloy M, Billecocq A. (2006). Production of monoclonal antibodies against Rift Valley fever virus Application for rapid diagnosis tests (virus detection and ELISA) in human sera. *J Virol Methods*, 131, 34–40.
- Zhu J, Wan C, Li Y. (2010). Enhanced solid-state anaerobic digestion of corn stover by alkaline pretreatment. *Bioresour Technol*, 101, 7523–7528.