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ASSESSMENT OF THE RISK FOR PATHOGENS IN PHOSPHORUS RECOVERED FROM SEWAGE SLUDGE ASH

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Summary

The company, EasyMining, has developed a process called Ash2Phos that recovers phosphorus from the ashes from incinerated sewage sludge. This phosphorus product has qualities that would make it suitable for use as a phosphate supplement in animal feeds. However, current EU legislation bans the use of any nutrients recycled from wastewater in animal feeds.

This report presents a qualitative risk assessment for the presence of pathogens in phosphorus recovered from sewage sludge ash. The specific risk question assessed in the report is "*What is the probability that phosphorus recovered from sewage sludge contains infectious animal pathogens following incineration and processing through the Ash2Phos process?*". The assessment does not include any evaluation of the consequences of exposure to pathogens.

Under the assumptions made in the assessment and with the information available today, the risk that phosphorus recovered from sewage sludge using the Ash2Phos process contains infections animal pathogens is assessed to be negligible. While it has been clearly shown that sewage sludge contains high levels of bacteria, viruses and parasites, both the incineration step in converting sewage sludge to ash and the chemical exposure steps in the Ash2Phos process are assessed to be effective barriers against these pathogens which will result in their complete inactivation. Regarding prions, the negligible (bovine spongiform encephalopathy) to very low (scrapie) risk that these transmissible spongiform encephalopathies are present in the European animal population makes it extremely unlikely that prions are present in wastewater streams. In addition, the available evidence supports that both the incineration process and the chemical treatment steps in the Ash2Phos process are able to significantly reduce the infectivity of prions.

During the risk assessment process, several important gaps in the knowledge about prions were identified. These include a lack of information about the presence and concentrations of prions in European wastewater streams and sewage sludge, imprecise data on the temperatures and residence times required to completely inactivate prions during incineration, and insufficient information about the effects of pH and chemical treatments on the infectivity of prions. Additional scientific evidence to fill these gaps would strengthen the assessment of the various steps in the process of recovering phosphorus from sewage sludge ash and thus the overall assessment.

Contents

Definitions

Pathogen	a microorganism that can cause disease
Prevalence	the proportion of a population that has a disease, condition or characteristic in a given time period
Zoonosis	a disease that can be transmitted between humans and animals

1 Introduction

The National Veterinary Institute (SVA) has been asked by the company, EasyMining, for a risk assessment of the probability of pathogens remaining in phosphorus recovered from sewage sludge ash for use in animal feed. EasyMining has developed a process (Ash2Phos) that recycles phosphate from the ashes from incinerated sewage sludge. The resulting product has characteristics that make it suitable for use as a phosphate supplement in animal feeds. However, under current EU legislation, any nutrients recycled from wastewater are banned from use in animal feeds, irrespective of processing method or origin of the wastewater (EU Feed Regulation 767/2009, Annex III, point 5).

1.1 RISK QUESTION

The company has formulated the following questions to SVA:

- Is the ash from sewage sludge a safe starting point for recovering phosphorus for animal feed?
- What is the probability that production animals fed phosphorus recovered using the Ash2Phos process are exposed to pathogens from the original sewage sludge?

To answer these questions, SVA has formulated the following risk question:

What is the probability that phosphorus recovered from sewage sludge contains infectious animal pathogens following incineration and processing through the Ash2Phos process?

For ease of assessment, the risk question will be assessed in three parts:

1. *What is the probability that raw sewage sludge contains animal pathogens?*
2. *What is the probability that sewage sludge ash contains animal pathogens?*
3. *What is the probability that phosphorus recovered from sewage sludge ash using the Ash2Phos process contains animal pathogens?*

1.2 SCOPE OF THE ASSESSMENT

This risk assessment will be limited to pathogens. No other hazards, such as heavy metals or pharmaceutical residues, will be assessed. Also, only the Ash2Phos method of recovery of phosphorus from sewage sludge ash will be assessed and no other available methods for phosphorus recovery. Only sewage sludge originating from countries within the European Union and no other countries will be assessed.

In the risk assessment it is assumed that all current regulations and recommendations for wastewater treatment and sewage sludge incineration are followed and that no operational disturbances or failures occur during either the incineration or the Ash2Phos process.

2 Background

2.1 PHOSPHORUS IN ANIMAL FEED

Phosphorus is an essential nutrient for all living organisms (Havukainen et al., 2016; Luyckx and Caneghem, 2021). The mineral is required for many biological processes including energy metabolism, protein synthesis, cell-signaling and maintaining acid-base balance, and it is an important component in the synthesis of DNA, RNA, cell membranes, muscle and bone (Singh et al., 2018; Li et al., 2016). In agriculture, phosphorus is routinely added to animal diets to ensure requirements are met in order to maintain animal health, welfare and productivity. It has been estimated that phosphorus is the second to third most costly component in animal feed supplements (Manopriya et al., 2022). A major source of the phosphorus used in animal feeds is inorganic phosphorus that is extracted through the mining, crushing and chemical processing of natural phosphate rock (Manopriya et al., 2022). Approximately 7% of the phosphorus extracted from phosphate rock is used in animal feed (Cieslik and Konieczka, 2017). However, phosphate rock is a finite resource, and it is expected that all known natural sources will be depleted or even exhausted within the next 50-100 years (Bloem et al., 2017; Rorat et al., 2019). Additionally, phosphate rock contains varying levels of heavy metals, which can be difficult to remove, creating both environmental and health concerns (Javied et al., 2009). Phosphorus has been classified as a critical raw material by the EU since 2017 (Di Giacomo and Romano, 2022). Therefore, interest in recovering and recycling phosphorus from alternative sources has increased in recent years.

2.2 SEWAGE SLUDGE AS A POTENTIAL SOURCE OF PHOSPHORUS

One alternative source of phosphorus is the ash that remains after the incineration of sewage sludge. Sewage sludge is a waste product produced during the treatment t of wastewater. It is made up of the solids and semi-solids that are removed during the wastewater treatment process. It is rich in organic matter and contains many valuable nutrients, including phosphorus (Bloem et al., 2017). Sewage sludge is also often heavily loaded with organic and inorganic contaminants including heavy metals, nanoparticles, pharmaceutical residues and pathogenic organisms (Bloem et al., 2017). As such, there are often stringent regulations that must be followed when handling and disposing of sewage sludge. A well-accepted and commonly used method of sewage sludge disposal is incineration, which is a combustion process that uses high heat and oxygen to destroy the organic fraction of the sewage sludge and convert it into ash (Di Giacomo and Romano, 2022). This ash has a high phosphorus concentration (4-12%) which is comparable to the concentration of phosphorus typically found in phosphate rock (2-18%) (Luyckx and Canaghem, 2021). Therefore, sewage sludge ash is seen as good alternative source of phosphorus and several processes have been developed to recover phosphorus from sewage sludge ash (Bloem et al., 2017; Luyckx and Caneghem, 2021). According to EasyMining, the phosphorus product recovered from sewage sludge ash using the Ash2Phos process is both free from heavy metal contaminants and has a digestibility comparable to that of traditionally sourced phosphorus, which makes it suitable for use as a phosphorus supplement in animal feeds.

2.3 WASTEWATER TREATMENT AND SEWAGE SLUDGE PRODUCTION

Urban populations generate large amounts of wastewater that is treated to remove contaminants before being released back into waterways so that it will not adversely affect human, animal or environmental health (Chahal et al, 2016). Wastewater entering treatment facilities comes from three sources: i) household wastewater (toilets, personal hygiene, food preparation etc), ii) industrial wastewater and iii) stormwater (rain run-off from surfaces) (Figure 1) (Chahal et al., 2016).

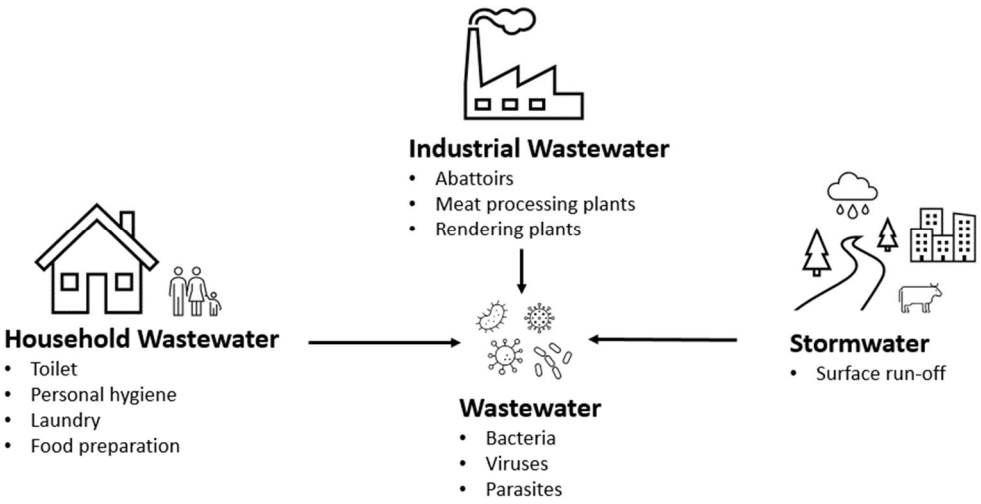

Figure 1. Potential sources of pathogens in wastewater

While wastewater treatment processes vary from facility to facility, most treatment plants use a combination of physical, chemical and biological methods to remove contaminants from wastewater (Chahal et al., 2016; Di Giacomo and Romana, 2022). These processes can generally be grouped together into preliminary, primary, secondary and tertiary stages (Figure 2).

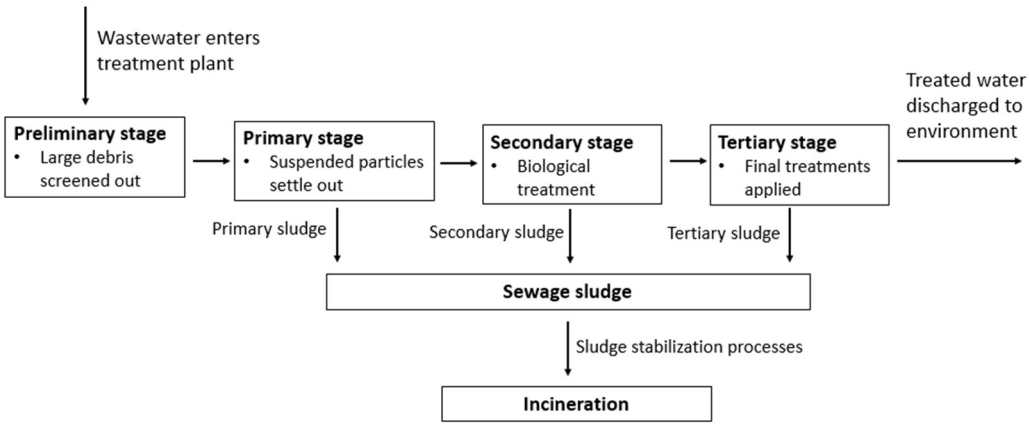

Figure 2. Steps in the wastewater treatment and sewage sludge production process

In the preliminary stage, large debris, gravel and grit that may clog or damage downstream equipment is screened out (Di Giacomo and Romana, 2022). In the primary stage, suspended solid waste particles settle out of the water under the force of gravity in large settling tanks, thereby clarifying the wastewater. Many systems add flocculants or coagulants to facilitate the settling process in the primary stage. In the secondary stage, nutrients and dissolved organic and inorganic solids are removed by applying various biological treatment processes coupled with solid/liquid separation (Chahal et al., 2016; Ottoson, 2004). During the tertiary stage, various chemical, biological or physical treatments, such as filters, chlorine, ozone or ultraviolet light, may be applied as a final measure to ensure the desired quality of treated water is achieved.

Sewage sludge, which is the solid or semi-solid waste produced during the wastewater treatment process, may be generated in the primary, secondary and/or tertiary stages of wastewater treatment (Chahal et al., 2016). Sewage sludge from all three stages of wastewater treatment is typically combined and handled together (Gholipour et al., 2022; Schnell et al., 2020) (Figure 2). Because raw sewage sludge is composed primarily of organic matter, it is easily fermentable and must be stabilized before it can be further managed (Dumontet et al., 2001). A wide variety of processes can be used to stabilize raw sewage sludge, including various chemical treatments (eg lime, ozone), aerobic or anaerobic biological digestion (eg. biogas production), composting and/or thermal treatments (eg drying) (Dumontet et al., 2001; Di Giacomo and Romano, 2022). These processes reduce the organic matter and microorganisms in the sewage sludge which inhibits odour production and further decomposition. The stabilized sewage sludge can then either be utilized in further processes or sent for final disposal (Di Giacomo and Romano, 2022).

2.4 INCINERATION OF SEWAGE SLUDGE

A common method of sewage sludge disposal is through incineration. There are several methods to incinerate sewage sludge, but it is most commonly done through a process known as fluidized bed incineration (Kasina and Jarosz, 2023; Schnell et al., 2020). During this process, preheated air is used to fluidize a bed of sand. The fluidized sand bed mixes violently and serves to distribute and break up the sewage sludge as it is pumped in, while simultaneously providing a very large surface area and well-distributed supply of oxygen to promote combustion (Schnell et al., 2020). Directive 2000/76/EC of the European Parliament and of the Council of 4 December 2000 on the incineration of waste states that incineration plants must fulfil 850°C for at least two seconds in order to minimize the emission of environmental pollutants, such as dioxins, during the incineration process. These high temperatures destroy the organic contaminants in the sewage sludge leaving behind only the non-combustible, inorganic substances in the form of ash (Bloem et al., 2017). The volume of ash remaining after incineration is approximately 10% of the original sewage sludge volume (Kasina and Jarosz, 2023; Cieslik and Konieczka, 2017).

2.5 THE ASH2PHOS PROCESS

The Ash2Phos process is described as follows by the company:

Ash2Phos is a continuous, wet chemical process for P recovery from incinerated sewage sludge ash. The process is designed specifically for processing fly ash from sewage sludge mono-incineration using the fluidized bed incineration method. The main inputs in the process are incinerated sewage sludge ash, hydrochloric acid (HCl), lime (CaO) and sodium hydroxide (NaOH). The main products of the process are precipitated calcium phosphate, ferric chloride, sodium aluminate, silicate sand and heavy metal residue (Figure 3).

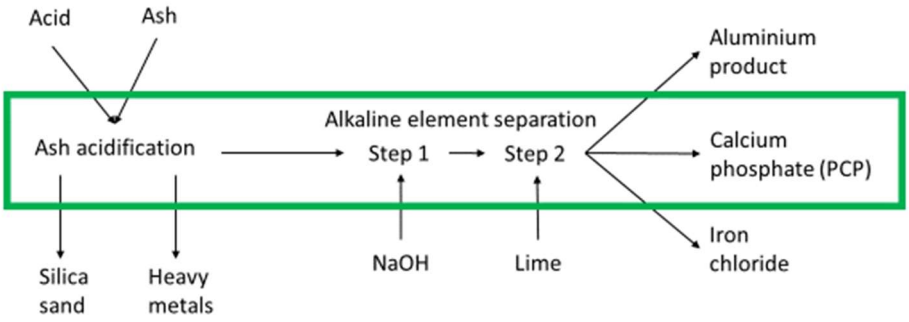

Figure 3. A simplified diagram of the steps involved in the Ash2Phos process including the main inputs and outputs.

The process of recovering phosphorus from ash involves several steps which are described in detail below.

STEP 1: Ash dissolution

The sludge ash is fed into a stirred dissolution reactor. Concentrated hydrochloric acid (30% by weight) is fed to the dissolution reactor together with process water. The operational conditions are: temperature 40°C, pH 0 – 0.5, residence time 30 minutes, liquid to solid ratio 3 liter per kg and effective HCl concentration 3M. The ash is composed of a mixture of inorganic minerals such as hematite, calcite, apatite, quartz etc. The hydrochloric acid breaks the covalent bonds in the crystal structure of the minerals and phosphorus is dissolved in a form of phosphate anion.

STEP 2: Filtration of the non-dissolved fraction (silicate sand)

The next step involves filtration of the non-dissolved fraction. This is done in a vacuum belt filter in which a filter cake is first formed on the belt filter. The filtrate from the first filtration is circulated and filtrated again through the filter cake itself. This process filters out particles larger than a range of 1-10 micrometers. The non-dissolved fraction has about 50% of the mass of the original ash.

STEP 3: Reaction of dissolved phosphorus with iron

The clear filtrate from step 2 is fed into a stirred reactor to which iron hydroxide is added. The operational conditions are: temperature 40°C, pH 0-2, retention time 33 minutes, liquid to solid ratio of 3 liter per kg.

STEP 4: pH adjustment

The slurry from step 3 is fed to an additional stirred reactor in which the slurry is reacted with a slurry of slaked lime (ca 22% by weight). The operational conditions are temperature 40°C, pH 2.5-3, and retention time of 48 minutes. Phosphate is precipitated mainly as ferric phosphate.

STEP 5: Separation of ferric phosphate mineral.

The precipitated phosphate mineral is separated from the solution by filtration. Filtration is done in a vacuum belt filter as in step 2.

STEP 6: Dissolution of separated ferric phosphate mineral.

The separated phosphate mineral is fed into an additional stirred reactor where it is dissolved under alkaline conditions. This is done by feeding a recycled sodium hydroxide solution into the reactor, along with a concentrated sodium hydroxide (50% by weight). The effective concentration of sodium hydroxide in the reactor is ca 3M, temperature 40°C, pH 12.5 -13 and retention time of 50 minutes. Phosphate is again dissolved in the form of an anion by breaking the covalent bonds in the ferric phosphate.

STEP 7: Separation of dissolved phosphorus

The phosphorus which is now dissolved in an alkaline solution is separated from the non-dissolved fraction by vacuum belt filtration as in step 2.

STEP 8: Precipitation of calcium phosphate

The clear solution from step 7 is fed into a stirred reactor in which milk of slaked lime (22% by weight) is fed into the reactor to precipitate the phosphate anion with the added calcium. This reaction generates sodium hydroxide. The operational conditions are pH 12.7 – 14, temperature 40 – 50°C, and retention time of 60 minutes.

STEP 9: Separation of precipitated calcium phosphate

The precipitated calcium phosphate is separated from the alkaline solution by filtration. Filtration is done in a vacuum belt filter as in step 2. Typical cutoff for the filtration 1 – 10 micrometers.

STEP 10: Drying of the precipitated calcium phosphate.

The separated calcium phosphate is dried in a dryer at 105ºC to achieve > 95% dry matter content.

3 Risk Assessment Method

This risk assessment is qualitative and follows the guidelines in the Codex Alimentarius (WHO, 2007). The report includes a qualitative assessment (Table 1) of the probability of the presence of pathogens but does not include any assessment of the consequences of exposure to pathogens.

Table 1: Terminology for probabilities in the risk assessment

Level	Interpretation
Negligible	So rare as to be inconsequential
Very low	Very unlikely, but cannot be ruled out
Low	Rare, but does occur
Medium high	Sometimes occurs
High	Often occurs
Very high	Almost certainly occurs

The assessment of probability of the presence of pathogens is based on the pathway shown in Figure 4.

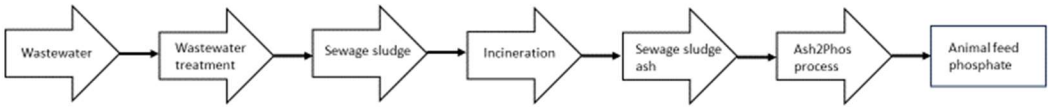

Figure 4. Pathway considered in the risk assessment.

The uncertainty in the results of the risk assessment is assessed qualitatively (Table 2) and reflects both natural variation and the uncertainty that originates in the knowledge base ie. the lack of knowledge about the actual situation.

Table 2: Terminology for uncertainty in the risk assessment

Level	**Interpretation**
Low	1. Solid and complete data available 2. Strong evidence from several sources **3.** Several authors report similar
Medium	4. Some, but not complete data available 5. Evidence from single references 6. Authors report differing conclusions
High	7. Little to no data available 8. Evidence drawn from unpublished reports, observations or personal communications rather than scientific references 9. Authors report conclusions that differ significantly from each other

The assessment is performed in three steps along the pathway corresponding to the three parts of the risk question presented above. Each step is first assessed separately assuming that pathogens are present in wastewater, raw sewage sludge and sewage sludge ash, respectively. In a second step, the outcomes of the assessment for the three separate steps are merged to an overall assessment to answer the original, comprehensive risk question. This is done using the matrix in Table 3. This procedure can be repeated, if needed, using the result from the first weighing step, to merge with the following step.

Table 3. Matrix for weighing together the probabilities along the pathway.

Probability of first step	Probability of second step					
	Negligible	Very Low	Low	Medium	High	Very High
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Very Low	Negligible	Very Low	Very Low	Very Low	Very Low	Very Low
Low	Negligible	Very Low	Low	Low	Low	Low
Medium	Very Low	Low	Low	Medium	Medium	Medium
High	Low	Low	Medium	Medium	High	High
Very High	Low	Medium	Medium	High	High	Very High

3.1 USE OF INDICATOR MICROORGANISMS

Determining the effect of the various processes on every single pathogen type that might be present in sewage sludge would be extremely difficult and time consuming. In such instances, the use of specific indicator microorganisms that represent the most resistant organisms within pathogen categories is routine. If the resilient indicator microorganisms are inactivated by a process, then all, less resilient pathogens can also be assumed to be inactivated (Koutsoumanis et al., 2021; McDonnell, 2022).

4 Hazard Identification and Characterization

4.1 PATHOGENS IN SEWAGE SLUDGE

Several factors will affect whether pathogens are present in wastewater or sewage sludge, including the prevalence of the pathogen in the population contributing to the wastewater, routes of entry of the pathogen into the wastewater stream and the pathogens' ability to survive in the waste treatment environment. Pathogens may enter the wastewater treatment stream from any of the sources shown in Figure 1. For example, domestic sources of pathogens include those found in waste flushed down the toilet or from contaminated bathing and laundry water. Industrial sources may include pathogens shed by livestock at abattoirs or from meat processing facilities. Storm water may contain pathogens shed by wildlife, pets or livestock in the outdoor environment. Therefore, the prevalence and concentration of pathogens in wastewater entering a treatment facility is highly dependent on the health status of the population the facility serves as well as the make-up of the sewers connected to the system (Bloem et al., 2017). The pathogen composition in wastewater will also vary from day-to-day, season-to-season and year-to-year (Di Giacomo and Romana 2022; Rorat et al., 2019).

Pathogens in wastewater may be free in suspension or attached to particles in the water. While some of the pathogen load in wastewater is decreased through predation and competition during the secondary stage of treatment, many pathogens are removed from wastewater during the settling process in the primary, secondary and tertiary stages of treatment (Ottoson, 2004). Thus, many of the pathogens found in wastewater are partitioned into the sewage sludge (Bibby and Peccia, 2013), where they become concentrated (Gholipour et al., 2022, Olofsson et al, 2012). Even in countries where hygiene standards are generally high, the degree of pathogen prevalence in sewage sludge is significant (Dumontet et al., 2001). Because humans are the main contributors to wastewater, the pathogens in sewage sludge originate primarily from human waste. Most pathogens are species-specific so these pathogens pose the greatest risk to humans. However, some pathogens are zoonotic and therefore pose a health risk to animals as well (Bloem et al., 2017).

The main pathogens found in sewage sludge include bacteria, viruses, and parasites (Fijalkowski et al., 2017; Chahal et al., 2016; Bloem et al., 2017). The presence of prions in wastewater has never been reported (Hinckley et al, 2008), but because they are considered to be the most resistant pathogen (Koustoumanis et al., 2021) their presence must also be considered.

4.1.1 Bacteria in wastewater and raw sewage sludge

The presence of bacteria in both wastewater and sewage sludge has been well documented and bacteria are the most diverse and prevalent type of microorganism found in wastewater (Chahal et al., 2016; Strauch, 1991). One study identified 243 species of potentially

pathogenic bacteria in sewage treatment facilities (Huang et al., 2018), many of which are known to infect animal species. Some bacterial pathogens found in wastewater that can infect animals include *Escherichia coli*, *Salmonella* spp., *Listeria* spp., *Yersinia* spp., *Campylobacter* spp., *Clostridium* spp., *Leptospira* spp., and *Mycobacterium* spp. (Jiang et al., 2020, Chahal et al., 2016; Varela and Manaia, 2013). Much of the bacterial load in wastewater is partitioned into the sewage sludge during the water treatment process, making the bacterial concentration in raw sewage sludge higher than that in wastewater (Mocé-Llivina et al., 2003). Because sewage sludge is composed primarily of organic matter, it serves as an excellent medium where bacteria can not only survive but, under certain conditions, also multiply (Carrington, 2001; Strauch, 1991). Levels of some bacteria, such as *Salmonella* and streptococci, have been found to remain high in sewage sludge after a storage period of 12 months (Gibbs et al., 1995). It has also been found that some bacteria, including *Salmonella*, are able to regrow during sludge storage, despite bacterial numbers initially falling during the storage period (Gibbs et al., 1997; Zaleski et al., 2005). Bacterial survival time in sewage sludge is dependent on a large number of factors including the initial bacterial concentration, temperature, pH, moisture content and which stabilization process(es) is/are applied to the sludge.

4.1.2 Viruses in wastewater and raw sewage sludge

Unlike bacteria, viruses are not able to multiply outside of a living host cell (Carrington, 2001). However, given the correct conditions, many viruses are able to survive for long periods of time in the environment. Studies have shown that untreated wastewater can contain between 10^3-10^7 virus particles per liter of water (Chahal et al., 2016), and many of the viruses will end up concentrated in the sewage sludge during the treatment process (Gholipour et al., 2022). Sewage sludge is known to contain viruses, particularly those of intestinal origin (Rorat et al., 2019) and almost 100% of sewage sludge samples examined have been shown to contain viruses (Dumontet et al., 2001). One study identified 43 different types of viruses in samples of sewage sludge (Bibby and Peccia, 2013), many of which had the ability to infect animals. Some of the viruses known to be found in sewage sludge that are of concern for animal health include Rotavirus, Coronavirus, Influenza virus, and Parvovirus (Chahal et al., 2016; Dumontet et al., 2001). As with bacteria, survival of viruses in sewage sludge is dependent on many external factors such as temperature, pH and application of various stabilization treatments.

4.1.3 Parasites in wastewater and raw sewage sludge

Many parasites have evolved to have life cycle stages that are extremely resistant to adverse environmental conditions (Rocha et al., 2016). Thus, many are able to survive in both wastewater and sewage sludge. However, parasites are not able to reproduce outside of a suitable host (Carrington, 2001). Protozoan parasites known to infect animals, such as *Cryptosporidium* spp., *Giardia* and *Toxoplasma* are commonly detected in sewage sludge (Chahal et al. 2016; Jiang et al., 2020). For example, one study found 3810 *Cryptosporidium* oocysts and 11 800 *Giardia* cysts in 100 g of sewage sludge (Dumontet et al., 2001). *Giardia* cysts have been found to survive in sewage sludge for at least 12 months (Gibbs et al., 1995). It has been estimated that >99.99% of parasite eggs are eliminated from treated wastewater during the primary and secondary treatment phases, thus ending up in sewage sludge (Chilian et al., 2022). Parasite eggs that are commonly found in sewage sludge and that can potentially infect livestock include those of Ascaris, Trichuris and Toxocara (Rorat et al.,

2019). Parasite eggs can survive for many years in the environment, given the proper conditions (Strauch, 1991). In sewage sludge stored under conditions similar to those found in a sewage lagoons, *Toxocara* eggs remained infective for up to 16 months while *Ascaris* spp. eggs remained infective for up to 33 months (O'Donnell et al., 1984). As with bacteria, survival of parasites in sewage sludge is dependent on many external factors such as temperature, pH and application of various stabilization treatments.

4.1.4 Prions in wastewater and raw sewage sludge

Prions are proteinaceous, infectious particles that are the causative agents for a group of diseases known as transmissible spongiform encephalopathies (TSEs) (Appel et al., 2006). TSEs are fatal neurodegenerative diseases that affect a number of mammalian species, including humans (Saunders et al., 2008). The TSEs of importance to domestic animals are Bovine spongiform encephalopathy (BSE) in cattle, and scrapie in sheep and goats.

The presence of prions in wastewater or sewage sludge has never been reported nor has it been thoroughly investigated (Hinckley et al., 2008). However, there are several plausible ways BSE or scrapie prions could potentially enter the wastewater treatment stream. Scrapie prions are shed in both the urine and feces from infected sheep and goats (Maluquer de Motes et al., 2012; Rubenstein et al., 2011; Terry et al., 2011) and could therefore enter wastewater via surface runoff. Both BSE and scrapie prions are also found in various tissues in infected animals (Sawada et al., 2019, Kumagai et al., 2019; Acin et al., 2021; Saunders et al., 2008; Race et al., 1998) so leachate from whole carcasses or tissues of TSE-infected animals may therefore serve as a source of contamination for stormwater (Saunders et al., 2008). However, the presence of prions in stormwater has not been thoroughly examined. One study detected prions in one sample of surface water run-off entering a water treatment facility from an area where Chronic wasting disease, a TSE that affects deer species, was endemic in the wild deer population. However, the amounts detected were found to be below infectious levels (Nichols et al., 2009). No studies to detect the presence of the prions causing BSE or scrapie in stormwater have been done.

BSE or scrapie prions could also enter wastewater streams from industrial sources that might handle infected animals or carcasses, such as abattoirs, meat processors or carcass rendering facilities (Saunders et al., 2008, Maluquer de Motes et al., 2008, Yamamoto et al., 2005). These facilities generally have barriers in place that minimize the risk of entry of infectious prions into wastewater (Gale and Stanfield, 2001; Yamamoto et al., 2006; Adkin et al., 2012a; Quinn and Fabiansson, 2001). At abattoirs for example, any animals exhibiting signs of TSE disease during pre-slaughter inspection are deemed unfit and are not slaughtered (Gale and Stanfield, 2001). Tissues considered to be at high risk for containing infectious prions are typically removed and disposed of using methods considered appropriate to inactivate infectious prions, such as incineration (Gale and Stanfield, 2001; Yamamoto et al., 2006; Adkin et al., 2012a). Abattoirs and other facilities that handle animal carcasses and parts also typically have mesh screens over the drains to prevent small particles from entering the wastewater (Gale and Stanfield, 2001; Yamamoto et al., 2006; Adkin et al., 2012b). However, the effectiveness of these measures in preventing prions from entering wastewater has not been thoroughly investigated. Only one study where wastewater from industrial facilities was examined for the presence of prions has been published. These researchers found no prions in any of 17 wastewater samples collected

from 3 different abattoirs, 9 of which were collected within 24 hours after slaughtering a confirmed BSE-positive animal (Maluquer de Motes et al., 2008).

Several groups have made estimates of the amounts of infectious prions that might enter wastewater from industrial facilities from prion-infected carcasses, all of which have been very low. One group estimated that between 2-20 g of infective tissue would enter the wastewater stream from one BSE infected animal slaughtered at an abattoir (Quinn and Fabiansson, 2001). Another estimated that between 0.01-1% of high-risk tissues (brain and spinal cord) from a BSE-infected animal might enter the wastewater stream from an abattoir (Gale and Stanfield, 2001). A third group estimated that the highest amount of prion infectivity entering wastewater from different types of facilities handling animal carcasses came from BSE infected cattle carcasses handled at rendering plants or large incinerators, with an average infectivity of 7.4 Bovine Oral ID_{50} per infected carcass (one ID_{50} is the amount of infectivity that will cause infection in 50% of cattle exposed to it) (Adkin et al., 2012a). For classical scrapie, it has been estimated that an infectivity of 1 ID_{50} per infected small ruminant carcass would enter the wastewater from an abattoir (Adkin et al., 2018). All of these assessments made note of limitations in the estimations due to a lack of available scientific data.

Prions are extremely resistant to inactivation (Sakudo et al., 2011) and can survive in wastewater for long periods of time. One study showed that BSE and scrapie prions were able to survive in experimentally contaminated wastewater for at least 50 days (Maluquer de Motes et al., 2008). A similar study found that prions were detectable in artificially contaminated wastewater for 150 days with no reduction in infectivity (Maluquer de Motes et al., 2012). Yet another study showed that BSE and scrapie prions remained detectable and infective in wastewater for over 6 and 8 years, respectively, although their infectivity was reduced by several orders of magnitude during this time (Marin-Moreno et al., 2016).

Due to their resistant nature, the various processes applied during the wastewater treatment process have very little effect on prion infectivity (Yamamoto et al., 2006; Gale and Stanfield, 2001; Adkin et al., 2012a). One study showed that during a simulated wastewater treatment process, there was no significant degradation of prions that had been artificially added to wastewater samples (Hinckley et al., 2008). Prions are "sticky" and bind to particles in the environment (Gale et al., 1998). Therefore, any prions in wastewater are very likely to be partitioned into and concentrated in the sewage sludge fraction during the treatment process (Gale et al., 1998; Gale and Stanfield, 2001). One study that simulated the fate of prions during the wastewater treatment process using samples artificially "spiked" with prions, found that most of the prions (>99%) were removed from suspension and routed to the sewage sludge (Hinckley et al., 2008).

4.1.4.1 Prevalence of prions in EU animal populations

When assessing the probability that prions are present in wastewater, the prevalence of TSEs in the population contributing to the wastewater must also be considered. A surveillance program for BSE has been in place in the EU for over 20 years and the results indicate that the prevalence of BSE in European cattle is extremely low. Between 2017-2021, over 5 million European cattle were tested for the presence of BSE prions within this program, with only 2 animals testing positive for classical BSE, the type transmitted via

consumption of contaminated feed (EFSA, 2022). The World Organisation for Animal Health considers the incidence of BSE in cattle to be negligible with the number of cases per million animals approaching zero (WOAH, 2023). As with BSE, a scrapie surveillance program for small ruminants in the EU has also been ongoing for over 20 years. Since 2002, over 10 million sheep and goats have been tested as part of this surveillance program (EFSA, 2022). Over the years, the number of cases of classical scrapie, the type which is transmitted via contagious prions, has been dropping. The results of the surveillance show that over the last 10 years, there has been a significant decreasing trend (approximately 3%/year) in the number of sheep with classical scrapie. Between 2011 and 2016, the number of sheep testing positive for classical scrapie more than halved, from 1416 cases to 554 cases. In 2021, 429 631 small ruminants were tested, of which 448 (0.1%) tested positive for classical scrapie (EFSA, 2022). Thus, the prevalence of classical scrapie in European small ruminants is very low.

4.2 EFFECT OF HEAT ON PATHOGENS

All pathogens are sensitive to heat and can be inactivated if the temperature is raised sufficiently (McDonnell, 2022). Incineration is considered a highly effective means to dispose of a variety of wastes that are potentially contaminated with pathogens. For example, the leading method of disposing of infectious medical waste in high income countries is through incineration, as it both reduces the waste volume and ensures its sterilization (Windfeld and Brooks, 2015). Similarly, incineration of animal carcasses at a temperature of at least 850^0C is considered to be an effective disposal method that is expected to destroy all infective agents (Gwyther et al., 2011; NABC, 2004). Incineration degrades nearly all organic matter present in sewage sludge (Bauer, 2020) and the resulting ash contains negligible levels of residual organic matter (Jama-Rodzenska et al., 2021). The organic matter content of sewage sludge ash produced through fluidized bed incineration at 850^0C has been found to be between 0.1 - 0.6% (Ki et al., 2021; Latosinska and Gawdzik, 2014).

While the incineration process is expected to destroy all pathogens in sewage sludge (Cieslik and Konieczka, 2017; Chilian et al., 2022; Luyckx and Caneghem, 2021), evidence that definitively shows this is lacking. Many studies have examined the effects of heat treatment on a wide range of pathogens, but none of them have examined the specific requirements set out in Directive 2000/76/EC for the incineration of sewage sludge (ie 850^0C for 2 seconds). As there is a lack of research examining the effect on pathogens of incineration at high temperatures for short time periods, others have relied on extrapolation from available data to determine its effectiveness (McDonnell, 2022; Koutsoumanis et al., 2021). Much of this available data comes from studies on thermal inactivation of pathogens that have looked at the effects of temperatures under 100^0C for time periods of 30 minutes or more.

4.2.1 Effect of heat on bacteria

Bacteria are generally sensitive to heat and cannot survive the temperatures reached during burning (Koutsoumanis, 2021). Most bacteria are killed at temperatures in excess of 70^0C over a relatively short period of time (Carrington, 2001). However, certain bacteria species are more resistant to heat inactivation. One of the most heat resistant bacteria is *Enterococcus faecalis*, a Gram-positive, non-spore-forming bacterium which has been known to survive

pasteurization (McAuley et al., 2012) and the manufacture of cooked processed meat products (Hugas et al., 2003). Therefore, this bacterium has been identified as an appropriate indicator organism when considering the effectiveness of various thermal inactivation processes (Lau et al., 2020). In an EFSA assessment of collated data from several sources, it was calculated that thermal treatment of *Enterococcus faecalis* at only 98^0C for 2 seconds would result in a reduction of $>5\log_{10}$, while at 110^0C a reduction of $>5\log_{10}$ would take only 0.2 seconds (Koutsoumanis et al., 2021). As these temperatures are well below (approximately 8-9 times lower) the required operating temperature for incineration, it is expected that incineration at 850^0C for 2 seconds will completely inactivate *Enterococcus faecalis* (McDonnell, 2022). Another bacterium that has been used as an indicator organism in assessments of thermal treatments is the Gram-negative, non-spore-forming *Salmonella* Senftenberg as the time needed to reduce the population of *Salmonella* Senftenberg at a given temperature by 1 $\log_{10}$ is 10 to 20 times longer than for other *Salmonella* serovars (Doyle and Mazzotta, 2000). Calculations have shown that treatment at 74^0C for 2 seconds will reduce the population of *Salmonella* Senftenberg by $>5\log_{10}$, while at 78.8^0C, a $>5\log_{10}$ reduction would take only 0.2 seconds (Koutsoumanis, 2021). If the treatment is able to inactivate *Salmonella* Senftenberg, it will be effective against all other salmonellas as well as other Gram-negative, spore-forming bacteria (Koutsoumanis, 2021).

4.2.2 Effect of heat on viruses

Like bacteria, most viruses are inactivated relatively quickly if subjected to temperatures above 71^0C (Knight et al., 2013). Non-enveloped viruses are more resistant to thermal inactivation than enveloped viruses (Koutsoumanis, 2021; Knight et al., 2013, Carrington, 2001). Among the non-enveloped viruses, animal parvoviruses have been shown to be the most heat-resistant viruses (Knight et al., 2013; Nims and Plavsic, 2013) and they have therefore been used as indicator organisms when assessing the effectiveness of thermal treatment processes (Koutsoumanis, 2021; Emmoth, 2010). If a treatment is sufficient to inactivate parvovirus, then all other non-enveloped viruses should also be inactivated by the treatment. Depending on parvovirus species and the treated matrix, it has been shown that treatment at temperatures ranging from 101-196^0C for 30 seconds results in a $4\log_{10}$ reduction in parvovirus (Nims and Plavsic, 2013).

4.2.3 Effect of heat on parasites

Among the parasites that are present in sewage sludge, eggs from the nematode *Ascaris* are the most refractory to thermal treatment processes, when compared to other helminth eggs (Rocha et al., 2016; Maya et al., 2012). *Ascaris* eggs can survive for more than a year at a temperature of 40^0C (Naidoo and Foutch, 2018). Therefore, eggs from *Ascaris* spp have been used to examine the effects of various thermal processes on contaminated wastes (Koutsoumanis, 2021; Sahlström et al., 2008). Although considered highly resistant to heat treatment when compared to other parasites, *Ascaris* eggs can be inactivated at relatively low temperatures. Thermal treatment at 45^0C is sufficient to completely inactivate *Ascaris* eggs under both aerobic and anaerobic conditions, although this does require a relatively long exposure time of 2 days (Harroff et al., 2019). Temperatures above 60^0C have been recommended to obtain complete inactivation of helminth eggs (Maya et al., 2012). At 80^0C, complete inactivation of Ascaris eggs is achieved in <5 seconds (Naidoo and Foutch, 2018).

4.2.4 Effect of heat on prions

Multiple studies have examined the effects of heat treatment processes on prions (Marin-Mareno et al., 2019; Koutsoumanis et al., 2021; Mohammadi et al., 2020; Muller and Reisner, 2005; Chapman et al., 2020; Spiropoulos et al., 2019). However, all of these have looked at the effects of much lower temperatures (98^0C -200^0C) than the 850^0C required for sewage sludge incineration. Only one study (Brown et al., 2004) has examined the potential to inactivate prions at incineration temperatures. This study found that incineration of prion-infected brain tissue at 600^0C did not completely inactivate prions. Two of 21 hamsters that were inoculated directly in the brain with ash remaining after the incineration became infected, indicating that exposure to 600^0C allows for a low level of infectivity to persist. No hamsters that were inoculated with ash remaining after the incineration of prion-infected brain tissue at 1000^0C became infected. This led to the conclusion that prions are completely inactivated at temperatures approaching 1000^0C (Brown et al., 2004). It should be noted however, that in this study, the incineration temperatures were maintained for 5 and 15 minutes, which is significantly longer than the 2 second requirement laid out in Directive 2000/76/EC. Others created a simulation model to estimate the amount of BSE infectivity contained in the ashes produced during incineration of meat-and-bone meal from potentially BSE-infected carcasses. The results suggested that one ton of ash would contain $\leq$1.8E-07 cattle ID_{50} 95% of the time and concluded that the risk from using the ash in the fertilizer or phosphate industry would be negligible (Paisley and Hostrup-Pedersen, 2005). A report from EFSA on the inactivation of microorganisms in animal by-products states that the risk of TSE infectivity from incineration ash would be extremely small if incineration is conducted at 850^0C (Koutsoumanis et al., 2021). Incineration is considered the most effective method of disposal of prion-contaminated waste (Paisley and Hostrup-Pedersen, 2005; Saunders et al., 2008; Gwyther et al., 2011; Koutsoumanis et al., 2021) and ash derived from incineration of waste is considered safe and may be disposed of in landfills (Koutsoumanis et al., 2021).

4.3 EFFECT OF CHEMICAL TREATMENTS ON PATHOGENS

During the process of recovering phosphorus from sewage sludge ash, the ash undergoes both an acid treatment (HCl) and an alkaline treatment (NaOH and lime). The pH during the acid treatment decreases to $\leq$0.5 for at least 30 minutes and during the alkaline treatment increases to >12 for at least 30 minutes. pH is one of the most significant environmental factors impacting the survival of microorganisms (Lund et al., 2020; Jin and Kirk, 2018) so the pH extremes encountered during the Ash2Phos process are likely to have an effect on any pathogens that may be present in the sewage sludge ash.

4.3.1 Effect of pH on bacteria

Most bacteria are neutrophiles, meaning that they survive within a neutral pH range of 5-9 (Jin and Kirk, 2018; Merino et al., 2019). Some pathogenic bacteria however, such as *Listeria monocytogenes* and *Salmonella typhimurium*, are adapted to survive at a slightly lower pH range and can, for example, survive in the acidic environment found in the stomach (ph 3.0-4.5) (Zhu et al., 2006). However, even these bacteria, are completely inactivated at a pH below 4.0 (Tiganitas et al., 2009). A pH of 2.0 or less will kill 90% of bacteria in less than 30 minutes (Zhu et al., 2006). Only extreme acidophiles, which are typically isolated from hyperacidic lakes, hotsprings and volcanoes, can survive at pH <3 (Merino et al., 2019).

These microorganisms are not known animal pathogens. There are also alkaliphilic bacteria that can survive at a high pH of >9 (Merino et al., 2019). Currently, the most extreme alkaliphile known can survive at a pH as high as 12.5 (Merino et al., 2019) and this is also not a known animal pathogen.

4.3.2 Effect of pH on viruses

Like bacteria, viruses are generally most stable at a neutral pH of around 7. Many enteric viruses are able to survive in more acidic conditions, such as those found in the gastrointestinal tract (pH 3 to 5) (Vasickova et al., 2010). Few viruses, however, are able to survive in conditions with a pH as low as 0.5. and acidic solutions are commonly used as disinfectants to inactivate viruses (Nishide et al., 2011). Viruses have been identified that can survive in extremely low pH (<3), but these are primarily viruses that infect acidophilic bacteria (Gil et al., 2021) and they are not known animal pathogens. Viral survival in extremely alkaline conditions is also limited and generally, viruses are less likely to survive in alkaline conditions than acidic (Vasickova et al., 2010). Again, those viruses that can survive in very high pH conditions are those that can infect alkalophilic bacteria (Gil et al., 2021) and are not known to be animal pathogens.

4.3.3 Effect of pH on parasites

Ascaris eggs are considered to be among the most resistant to chemical treatment and changes in pH (Capizzi-Banas et al., 2004; Izumi, 1952). As with many parasites, Ascaris eggs are relatively resistant to low pH as they are adapted to surviving in the acidic environment of the gastrointestinal tract. Treatment with HCl has been found to have little to no effect on the infectivity of Ascaris eggs (Izumi, 1952; Yoshida, 1920). There is conflicting evidence on the effect of alkaline pH on Ascaris eggs. One study found that lime treatment producing a pH of >12 completely inactivated Ascaris eggs, but only after a long period (10 weeks) of treatment (Eriksen et al., 1995). Others have shown that alkaline treatment with lime at a pH of $\geq$12 reduces viable Ascaris eggs to negligible levels in 75 mins at 55^0C or 8 mins at 60^0C (Capizzi-Banas et al., 2004). Another study found that, at temperatures of 30^0C and above, alkalinization results in complete inactivation of Ascaris eggs (Ghiglietti et al., 1995). However, others have found that alkaline pH has little effect on Ascaris eggs and even after > 2 months of treatment at pH $\geq$12.0, Ascaris eggs can remain viable (Senecal et al., 2020; Pecson et al., 2007).

4.3.4 Effect of pH on prions

There is little information available on the effect of pH alone on prions. However, other manufacturing processes that utilize two-step acid and alkaline treatments, such as the conversion of waste animal fats into biodiesel and the production of gelatine from animal bones, are considered effective methods to inactivate pathogens, including prions, and produce safe products (Mohammadi et al., 2020; Grobben et al., 2004). However, in these processes, the potentially prion-infected materials are typically exposed to the high and low pH for a longer period of time than in the Ash2Phos process. Because there is little information on the general effect of pH on prions, the effects of the specific chemicals used in the Ash2Phos process on prions has been assessed.

4.3.5 Effect of HCl treatment on prions

One study found that treatment of prion-infected brain tissue with a low concentration of HCl (1 N) for 2 hours at room temperature had no effect on prion infectivity (Tateishi et al., 1991). However, others found that treating prion-infected brain material with a relatively low concentration of 1 M HCl (3.6%) for 1 hour reduced infectivity by $10^{1.8}$ ID_{50} (Brown et al., 1986). Similar results were reported by Grobben et al. (2004) where treatment of scrapie infected brain material with 4% HCl produced a $10^{1.2}$ ID_{50} reduction in infectivity. However, treatment time in this study was 2 days. Another study found that treatment with low concentrations of HCl (1 M or 3M) for 1 hour at 25^0C reduced prion infectivity but did not completely inactivate them (Appel et al., 2006). However, if the temperature was increased to 85^0C, prions were completely inactivated by treatment with 1 M HCl for 1 hour. If exposed to a high concentration of HCl (8 M) all prions were also inactivated within 1 hour of treatment, even at room temperature (25^0C) (Appel et al., 2006).

4.3.6 Effect of NaOH treatment on prions

NaOH has been shown to reduce the infectivity of prions and 1-2 N NaOH solutions are recommended for prion decontamination (Sohn et al., 2019; CFSPH, 2016). Treatment of scrapie prion contaminated surfaces with 1N NaOH for 1 hour at 20^0C resulted in complete decontamination as shown by a 0% transmission rate to exposed hamsters (Fichet et al., 2004). Others have found that treatment of scrapie infected brain tissue with 1N NaOH at 25^0C for varying lengths of time up to 24hrs reduced, but did not completely eliminate, prion infectivity (Prusiner et al., 1984). It has also been reported that exposure of scrapie prions to 0.1 M NaOH for 15 minutes at room temperature resulted in infectivity log reduction of 5.0 (Brown et al., 1986). Others showed that 0.1 M NaOH at 60^0C for 2 mins and 0.25 M NaOH at 30^0C for 60 mins inactivated 3.96 and 3.93 logs of scrapie, respectively (Unal et al., 2007). This same study found that a higher concentration of NaOH (0.5 M) at 30^0C for 60 minutes or 75 minutes inactivated $\geq$4.23 and 4.15 logs of scrapie (Unal et al., 2007). Treatment of prion-contaminated soil with 2 N NaOH for 1 hour has been shown to completely eliminate prion infectivity (Sohn et al., 2019).

4.3.7 Effect of lime treatment on prions

One study found that lime treatment of prion-infected mouse brain resulted in a loss of infectivity of between $10^{2.1}$ to $10^{2.3}$ ID_{50} (Grobben et al., 2004). In another study, after 10 minutes of incubation in lime solution at 99^0C, prions were no longer detectable in samples of brain material from scrapie-infected sheep (Greenlee et al., 2008). Lime treatment of sewage sludge has been shown to be effective in inactivating infectious prions (Brooks et al., 2015). When digested sewage sludge was treated with lime (pH 12.9), a 2.9 $\log_{10}$ reduction in infectious prions occurred within 2 hours (Miles et al., 2013).

4.3.8 Effect of consecutive acid and alkaline treatment on prions

The recovery of phosphate from sewage sludge ash involves two process steps, acid treatment followed by alkaline treatment. It is possible that these two consecutive treatment steps may affect each other and either improve or impair the ability to inactivate prions. One study showed that the consecutive treatment of scrapie-infected mouse brain with 4% HCl and saturated lime solution resulted in a loss of infectivity of $10^{2.8}$ ID_{50}, whereas for the individual treatments it was $10^{1.2}$ ID_{50} for 4% HCl and $10^{2.1}$–$10^{2.3}$ ID_{50} for saturated lime (Grobben et al., 2004).

4.4 EFFECT OF FILTERING ON PATHOGENS

In several steps of the Ash2Phos process, solutions are passed through filters to separate dissolved fractions from non-dissolved fractions. This filtering process filters out particles larger than a range of 1-10 micrometers and may therefore act as a barrier to pathogens.

4.4.1 Effect of filtering on bacteria

The size of most bacteria is in the range of 1-10 micrometers. For example, Salmonellae are generally 2-5 x 0.5-1.5 microns in size (Andino and Hanning, 2015) while E.coli have dimensions of 1-3 x 0.4-0.7 microns (Basavaraju and Gunashree, 2022). The smallest pathogenic bacteria, the Mycoplasmas, typically have a diameter of 0.3 to 0.8 microns. Therefore, the filtering process will not act as a barrier for many bacteria types, should they be present during the Ash2Phos process.

4.4.2 Effect of filtering on viruses

Generally, viruses are smaller than bacteria. There is a wide range in virus size but they typically range from 0.02 - 0.4 microns. The largest viruses are approximately 1.25 microns in size (Edwards et al., 2021). Therefore, the filtering process will not act as a barrier to viruses, should they be present during the Ash2Phos process.

4.4.3 Effect of filtering on parasites

The eggs of many parasites are relatively large. For example, helminth eggs are typically between 20 – 80 microns in size (Mahapatra et al., 2022). The filtering in the Ash2Phos process would therefore act as a barrier to these parasites. However, other parasites are much smaller in size. For example, Giardia cysts are usually between 8 – 12 microns while Cryptosporidium oocysts range in size from 4 – 6 microns (Ratnayaka et al., 2009). The filtering process may therefore not act as a barrier to these parasites should they be present.

4.4.4 Effect of filtering on prions

It is estimated that the diameter of a prion is about 0.03 micrometers (Burrell et al., 2016). Therefore, the filtering that occurs during the Ash2Phos process would not act as a barrier to prions should they be present.

5 Assessment of Probabilities and Uncertainty

The risk question to be assessed is:

What is the probability that phosphorus recovered from sewage sludge contains infectious animal pathogens following incineration and processing through the Ash2Phos process?

The pathway from wastewater to recycled phosphorus includes several barriers that may contribute to reducing the probability that pathogens are present in the final phosphorus product, including the wastewater treatment process, sewage sludge incineration and the various steps in the Ash2Phos process.

To simplify the assessment of probability, the risk question has been broken down into three subquestions, based on the steps in the pathway of recycled phosphorus production, from wastewater to end product, shown in Figure 4 :

1. *What is the probability that raw sewage sludge contains infectious animal pathogens?*
2. *What is the probability that sewage sludge ash contains infectious animal pathogens?*
3. *What is the probability that phosphorus recovered from sewage sludge ash using the Ash2Phos process contains infectious animal pathogens?*

In this step of the risk assessment, the probability of each subquestion is assessed independently of the other subquestions. Therefore, the probability assessed for one subquestion has no bearing on the probability assessment for any of the subsequent subquestions. For each subquestion, it is assumed that pathogens are present in the first step of the pathway covered by that particular subquestion. Also, for each subquestion, each category of pathogen (bacteria, viruses, parasites, prions) is assessed separately as the steps in the pathway of phosphorus production may have varying effects the different pathogen categories.

5.1 WHAT IS THE PROBABILITY THAT RAW SEWAGE SLUDGE CONTAINS INFECTIOUS ANIMAL PATHOGENS?

This subquestion addresses the steps in the pathway of recycled phosphorus production shown in Figure 5.

Figure 5: Steps in the pathway from wastewater to sewage sludge

The presence of bacteria, viruses and parasites in wastewater treatment streams has been well-documented (Chahal et al., 2016; Jiang et al., 2020). There is excellent evidence to show that a large proportion of these three pathogens groups are removed from wastewater during treatment and become concentrated in raw sewage sludge (Gholipour et al., 2022; Chahal et al., 2016; Dumonet et al., 2001).

On the other hand, there is a lack of scientific data about the presence and/or concentrations of prions in wastewater or sewage sludge as this topic has not been thoroughly investigated. However, because the prevalence of transmissible encephalopathies in animals in Europe is negligible (BSE) to very low (scrapie) (WOAH, 2023; EFSA, 2022), the probability of prions entering wastewater streams in the first place will have the same magnitudes. Additionally, the measures in place at many facilities where prion-infected animals or carcasses are potentially handled will further reduce the risk of prions entering the wastewater stream. There is evidence to suggest that, should prions enter wastewater streams, the probability that they would survive the water treatment process and be partitioned into sewage sludge is high (Gale et al., 1998; Gale and Stanfield, 2001; Hinckley et al., 2008).

Assessment of the probability that raw sewage contains bacteria, virus and/or parasites: Very high

Uncertainty in the assessment: Low

Assessment of the probability that raw sewage contains prions: Negligible

Uncertainty in the assessment: Medium

Factors contributing to uncertainty in the assessment:

- Lack of information about the presence of and/or concentration of prions in wastewater streams and sewage sludge

5.2 WHAT IS THE PROBABILITY THAT SEWAGE SLUDGE ASH CONTAINS INFECTIOUS ANIMAL PATHOGENS?

This subquestion assesses the steps in the pathway of recycled phosphorus production shown in Figure 6.

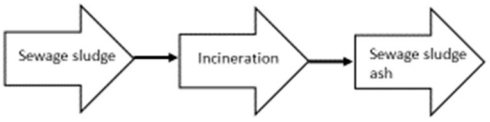

Figure 6: Steps in the pathway from sewage sludge to sewage sludge ash

Studies have found that temperatures 8 and 10 times lower than the required operating temperature for incineration (850^0C) will completely inactivate the indicator bacteria *Enterococcus faecalis* and *Salmonella* Senftenberg, respectively (Koutsoumanis et al., 2021; McDonnell, 2022). Because these bacteria are considered to be among the most heat resistant, all bacteria are expected to be completely inactivated at incineration temperature (McDonnell, 2022). Similarly, it has been determined that temperatures 4-8 times lower than the incineration temperature of 850^0C will completely inactivate parvoviruses (Nims and Plavsic, 2013), which are considered among the most heat-resistant viruses. Therefore, all virus types are expected to be completely inactivated at the legislated incineration temperature (McDonnell, 2022; Koutsoumanis et al., 2021). Complete inactivation of Ascaris eggs, which are the most refractory of all parasite eggs to heat, occurs at a temperature more than 10 times lower than incineration temperature (Naidoo and Foutch, 2018), which supports the complete inactivation of parasites at incineration temperatures. Scientific data on the heat inactivation of prions, particularly at incineration temperatures is sparse. The available evidence suggests that incineration at 600^0C allows a low level of prion infectivity to remain and that the complete inactivation of prions occurs somewhere within the temperature range of 600^0C -1000^0C (Brown et al., 2004). However, the exact temperature point at which complete inactivation occurs has not been confirmed. Therefore, it is not certain that incineration at the legislated temperature of 850^0C will result in complete prion inactivation. However, the extremely low (<1%) organic matter content of sewage sludge ash following fluidized bed incineration suggests that there is near complete destruction of all organic matter, including pathogens, during the incineration process.

Assessment of the probability that sewage sludge ash contains bacteria, viruses and/or parasites: Negligible

Uncertainty in the assessment: Low

Assessment of the probability that sewage sludge ash contains prions: Very low

Uncertainty in the assessment: Medium

Factors contributing to uncertainty in the assessment:

- Limited information about the effect of heat treatment on prion inactivation is available, particularly at the legislated temperature and residence time for incineration of sewage sludge ash (850^0C for 2 seconds)

5.3 WHAT IS THE PROBABILITY THAT PHOSPHORUS RECOVERED FROM SEWAGE SLUDGE ASH USING THE ASH2PHOS PROCESS CONTAINS INFECTIOUS ANIMAL PATHOGENS?

This subquestion assesses the steps in the pathway of recycled phosphorus production shown in Figure 7.

Figure 7: Steps in the pathway from sewage sludge ash to recycled phosphate for animal feed

During the Ash2Phos process, the ash undergoes both an acid treatment with HCl and an alkaline treatment with both NaOH and lime. During the acid treatment, the pH is maintained at $\leq$0.5 while during the alkaline treatment, pH is maintained at >14. There is good evidence to show that neither bacteria or viruses that are pathogenic to animals can survive at these extremes of pH.

For pathogenic parasites, the evidence suggests that the low pH achieved during the acid step of the Ash2Phos process is likely to have little effect on the most resistant parasite eggs. Although there is some conflicting research, the bulk of the evidence suggests that the high pH during the alkalinization steps is likely to at least significantly reduce, if not completely eliminate, pathogenic parasites that may be present in the ash. Additionally, the filtration steps during the Ash2Phos process are likely to remove any helminth eggs that may be present, but smaller parasites may be able to pass through this barrier.

HCl is capable of completely inactivating infectious prions, but either a high concentration or high temperature is required (Appel et al., 2006). In the Ash2Phos process, 3M HCl is added to the ash and this concentration of HCl has been shown to reduce, but not completely inactivate, prion activity (Appel et a., 2006). During this step of the Ash2Phos process, the temperature is raised to between 40-50^{0}C. This temperature is below that shown (85^{0}C) to completely activate prions with low concentrations of HCl treatment (Appel et al., 2006) so it is difficult to interpret the effect that the temperature increase during the acidification step might have on prion inactivation.

NaOH has also been shown by many to have an infectivity-reducing effect on prions (Fichet et al., 2004; Prusiner et al., 1984; Unal et al., 2007; Sohn et al., 2019), with higher concentrations having a greater effect (Unal et al., 2007). During the Ash2Phos process, 3M NaOH is added during the step to dissolve phosphate from ferric phosphate mineral. At

this concentration of NaOH, prions on both surfaces and in soil have been shown to be completely inactivated (Fichet et al., 2004 ; Sohn et al., 2019).

Treatment with lime has been shown to reduce the infectivity of prions (Grobben et al., 2004, Miles et al., 2013), but it is only when the temperature has been increased to 99^0C that it has been shown to result in complete inactivation (Greenlee et al., 2008). This temperature is not achieved during the lime treatment step of the Ash2Phos process.

With the information available, it cannot be confirmed that the Ash2Phos process will result in the complete inactivation of any prions that might be present in sewage sludge ash. However, because there is good evidence to support that each of the three chemical treatments on their own are capable of reducing the infectivity of prions, the combined effect of the HCl, NaOH and lime treatments in the Ash2Phos process are likely to substantially reduce the infectivity of any prions that might be present in sewage sludge ash.

Assessment of the probability that phosphorus recovered from sewage sludge ash using the Ash2Phos process contains bacteria, viruses and/or parasites: Negligible

Uncertainty in the assessment: Medium

Factors contributing to uncertainty in the assessment:

- Contradictory information on the effect of high pH on certain parasites

Assessment of the probability that phosphorus recovered from sewage sludge ash using the Ash2Phos process contains prions: Negligible

Uncertainty in the assessment: Medium

Factors contributing to uncertainty in the assessment:

- Contradictory information on the effect of both HCl and NaOH on prion infectivity.

6 Overall Assessment of Probability

What is the probability that phosphorus recovered from sewage sludge contains infectious animal pathogens following incineration and processing through the Ash2Phos process?

With today's level of knowledge and under the assumptions that all current regulations and recommendations for wastewater treatment and sewage sludge incineration are followed and that no operational disturbances or failures occur during either the incineration or the Ash2Phos process, the probability that phosphorus recovered from sewage sludge using the Ash2Phos process contains infectious animal pathogens is assessed to be negligible.

For bacteria, viruses and parasites, the likelihood is very high that the various processes in the pathway of phosphorus recovery from sewage sludge using the Ash2Phos process will result in complete inactivation of these pathogens. There is a clear scientific basis to support that no bacteria, viruses or parasites can survive the incineration step, making sewage sludge ash a safe substrate in terms of these pathogens. The extremes in pH, from approximately 0 to 14, that occur during the chemical treatments in the Ash2Phos process serve as additional barriers that will inactivate bacteria and viruses and significantly reduce, if not completely inactivate, any viable parasites.

The overall probability that phosphorus recovered from sewage sludge using the Ash2Phos process contains infectious prions was also assessed to be negligible. This level of probability is based primarily on the known negligible (BSE) to very low (scrapie) prevalence of TSEs in the European animal population, which makes it extremely unlikely that prions are present in wastewater streams. In addition, the evidence that is available clearly shows that both the incineration process and the chemical treatment steps in the Ash2Phos process alone are able to significantly reduce the infectivity of prions.
The presence of prions in wastewater and sewage sludge has not been thoroughly investigated and such studies would further support the assessment. Also, additional scientific evidence on the exact temperature and time needed for prion inactivation and the effect of sequential use of extreme pH and chemical treatment on inactivation of prions would strengthen the assessment of these steps of the procedure and thus the overall assessment.

It should be noted that any changes in either the assumptions or in the data on which the assessment was based could have a significant effect on the results of the risk assessment and in such case would call for an updated assessment.

6.1 KNOWLEDGE GAPS

During the course of this assessment, several important gaps in knowledge were identified:

1. There is a lack of scientific information about whether prions are present in European wastewater streams and/or sewage sludge and if so, at which concentrations they are found in these matrices.
2. Sufficient data to support the precise temperature and residence time required to ensure complete inactivation of prions during incineration is not available.
3. There is a lack of scientific information on the effect of the sequential use of extremes of pH, specifically using HCl and NaOH, on the infectivity of prions.

References

Acin C, Bolea R, Monzon M, Monleon E, Moreno B, Filali H, Marin B, Sola D, Betancor M, Guijarro IM, Garcia M, Vargas A, Badiola JJ. 2021. Classical and atypical scrapie in sheep and goats. Review on the etiology, genetic factors, pathogenesis, diagnosis and control measures of both diseases. *Animals*, 11, 691.

Adkin A, Donaldson N, Kelly L. 2012a. A quantitative assessment of the prion risk associated with wastewater from carcass-handling facilities. *Risk analysis*, 33(7), 1212-1227.

Adkin A, Donaldson N, Kelly L. 2012b. A quantitative assessment of the amount of prion diverted to Category 1 materials and wastewater during processing. *Risk analysis*, 33(7), 1197-1211.

Adkin A, Horifan V, Rajanayagam B, Arnold M, Konold T, Spiropoulos J, Kelly L. 2018. Estimating the impact on food and edible materials of changing scrapie control measures: the scrapie control model. Preventive Veterinary Medicine, 158, 51-64.

Andino A and Hanning I. 2015. *Salmonella enterica*: survival, colonization, and virulence difference among serovars. *The scientific world journal*, 2015, 520179.

Appel TR, Lucassen R, Groschup MH, Joncic M, Beekes M, Riesner D. 2006. Acid inactivation of prions: efficient and elevated temperature or high acid concentration. *Journal of general virology*, 87, 1385-1394.

Basavaraju M and Gunashree BS. 2022. *Escherichia coli:* An overview of main characteristics. *In*: MS Erjavec ed. Eschericha coli – old and new insights.

Bauer T. 2020. *Can new treatment combinations make sewage sludge management more sustainable?* Thesis, Luleå University of Technology, Luleå

Bibby K and Peccia J. 2013. Identification of viral pathogen diversity in sewage sludge by metagenome analysis. *Environmental science and technology*, 47(4), 1945-1951.

Bloem E, Albihn A, Elving J, Hermann L, Sarvi M, Schaaf T, Schick J, Turtola E, Ylivainio K. 2017. Contamination of organic nutrient sources with potentially toxic elements, antibiotics and pathogen microorganisms in relation to P fertilizer potential and treatment options for the production of sustainable fertilizers: a review. *Science of the total environment*, 607-608; 225-242.

Brooks JP, Gerba CP, Pepper IL. 2015. Land application of organic residuals: municipal biosolids and animal manures. *In*: ML Brusseau, IL Pepper, CP Gerba eds. *Environmental Microbiology*, pp 607-620.

Brown P, Rohwer RG, Gajdusek DC. 1986. Newer data on the inactivation of scrapie virus or Creutzfeldt-Jakob disease virus in brain tissue. *Journal of infectious disease*, 153(6), 1145-1148.

Brown P, Rau EH, Lemieux P, Johnson BK, Bacote AE, Gajdusek C. 2004. Infectivity studies of both ash and air emissions from simulated incineration of scrapie-contaminated tissues. *Environmental science and technology*, 38, 6155-6160.

Burrell C, Howard C, Murphy F. 2016. Fenner and White's Medical Virology (5th ed.). Elsevier Science. Available at: https://www.perlego.com/book/1834716/fenner-and-whites-medical-virology-pdf.

Carrington EG. 2001. *Evaluation of sludge treatments for pathogen reduction – final report*. European Commission report no. CO 5026/1. Office for Official Publications of the European Community, Luxembourg.

Capizzi-Banas S, Deloge M, Remy M, Schwartzbrod J. 2004. Liming as an advanced treatment for sludge sanitization: helminth eggs elimination – *Ascaris* eggs as model. *Water research*, 38, 3251-3258.

Chahal C, Van den Akker B, Young F, Franco C, Blackbeard J, Monis P. 2016. Pathogen and particle associations in wastewater: significance and implication for treatment and disinfection processes. *Advances in applied microbiology*, 97, 63-119.

Chapman GE, Lockey R, Beck KE, Vickery C, Arnold M, Thorne L, Thorne JK, Walker SR, van Keulen L, Casalone C, Griffiths PC, Simmons MM, Terry LA, Spiropoulos J. 2020. Inactivation of H-type and L-type bovine spongiform encephalopathy following recommended autoclave decontamination procedures. *Transboundary and emerging diseases*, 67(5), 1872-1878.

Chilian A, Bancuta OR, Bancuta J, Popescu IV, Gheboianu AR, Tanase NM, Tuican M, Zaharia M, Zinicovscaia I. 2022. Extraction of heavy metals and phosphorus from sewage sludge with elimination of antibiotics and biological risks. *Chemical engineering journal*, 437, 135298.

Cieslik B and Konieczka P. 2017. A review of phosphorus recovery methods at various steps of wastewater treatment and sewage sludge management. The concept of "no solid waste generation" and analytical methods. *Journal of cleaner production*, 142, 1728-1740.

CFSPH (The Center for Food Safety and Public Health). 2016. Bovine Spongiform Encephalopathy Factsheet. Available at: https://www.cfsph.iastate.edu/Factsheets/pdfs/bovine_spongiform_encephalopathy.pdf

Di Giacomo G and Romano P. 2022. Evolution and prospects in managing sewage sludge resulting from municipal wastewater purification. *Energies*, 15, 5633.

Dumontet S, Scopa A, Kerje S, Krovacek K. 2001. The importance of pathogenic organisms in sewage and sewage sludge. *Journal of the air and waste management association*, 51, 848-860.

Doyle, ME and Mazzotta AS. 2000. Review of studies on the thermal resistance of Salmonellae. *Journal of food protection*, 63(6), 779-795.

EasyMining. 2023. *Ash2Phos technical details*. Available at: https://www.easymining.com/technologies/ash2phos/technical-details/ (Accessed 3 august 2023).

Edwards KF, Steward GF, Schvarcz CR. 2021. Making sense of virus size and the tradeoffs shaping viral fitness. *Ecology Letters*, 24, 363-373.

EFSA (European Food Safety Authority). 2022. The European Union summary report on surveillance for the presence of transmissible spongiform encephalopathies (TSE) in 2021. *EFSA Journal*, 20(11), 7655.

Emmoth E. 2010. *Virus inactivation – evaluation of processes used in biowaste management*. Thesis, Swedish University of Agricultural Sciences, Uppsala.

Eriksen L, Andreasen P, Ilsoe B. 1995. Inactivation of *Ascaris suum* eggs during storage in lime treated sewage sludge. *Water research*, 30(4), 1026-1029.

Fichet G, Comoy E, Duval C, Antloga K, Dehen C, Charbonnier A, McDonnell G, Brown P, Lasmezas CI, Deslys JP. 2004. Novel methods for disinfection of prion-contaminated medical devices. *Lancet*, 364, 521-26.

Fijalkowski K, Rorat A, Grobelak A, Kacprzak MJ. 2017. The presence of contaminants in sewage sludge – the current situation. *Journal of environmental management*, 203, 1126-1136.
Gale P, Young C, Stanfield G, Oakes D. 1998. Development of a risk assessment for BSE in the aquatic environment. *Journal of applied microbiology*, 84, 467-477.

Gale P and Stanfield G. 2001. Towards a quantitative risk assessment for BSE in sewage sludge. *Journal of applied microbiology*, 91, 563-569.

Ghiglietti R, Rossi P, Ramsan M, Colombi. 1995. A. Viability of Ascaris suum, Ascaris lumbicoides and Trichuris muris eggs to alkaline pH and different temperatures. *Parasitologia*, 37(2-3), 229-232.

Gholipour S, Ghalhari MR, Nikaeen M, Rabbani D, Pakzad P, Miranzadeh MB. 2022. Occurrence of viruses in sewage sludge: a systematic review. *Science of the total environment*, 824, 153886.

Gibbs RA, Hu CJ, Ho GE, Phillips PA, Unkovich I. 1995. Pathogen die-off in stored wastewater sludge. *Water science and technology*, 31, 91-95.

Gibbs RA, Hu CJ, Ho GE, Unkovich I. 1997. Regrowth of faecal coliforms and salmonellae in stored biosolids and soil amended with biosolids. *Water science and technology*, 35, 269-275.

Gil JF, Mesa V, Estrada-Ortiz N, Lopex-Obando M, Gomez A, Placido J. 2021. Viruses in extreme environments, current overview, and biotechnological potential. *Viruses*, 13, 81.

Greenlee JJ, Nicholson EM, Hamir AN, Noyes GP, Holtzapple MT, Kehrli ME. 2008. Ablation of prion protein immunoreactivity by heating in saturated calcium hydroxide. *BMC research notes*, 1(99).

Grobben AH, Steele PJ, Somerville RA, Taylor DM. 2004. Inactivation of the bovine-spongiform-encephalopathy (BSE) agent by the acid and alkaline processes used in the manufacture of bone gelatine. *Biotechnology and applied biochemistry*, 39, 329-338.

Gwyther CL. Prysor Williams A, Golyshin PN, Edwards-Jones G, Jones DL. 2011. The environmental and biosecurity characteristics of livestock carcass disposal methods: a review. *Waste management*, 4, 767-778.

Harroff LA, Liotta JL, Bowman DD, Angenent LT. 2019. Current time-temperature relationships for thermal inactivation of Ascaris eggs at mesophilic temperatures are too conservative and may hamper development of simple, but effective sanitation. *Water research*, 5, 100036.

Havukainen J, Nguyen MT, Hermann L, Horttanainen M, Mikkilä M, Deviatkin I, Linnanen L. 2016. Potential of phosphorus recovery from sewage sludge and manure ash by thermochemical treatment. *Waste management*, 49, 221-229.

Hinckley GT, Johnson CJ, Jacobson KH, Bartholomay C, McMahon KD, McKenzie D, Aiken JM, Pederson JA. 2008. Persistence of pathogenic prion protein during simulated wastewater treatment processes. *Environmental science and technology*, 42(14), 5254-5259.

Huang K, Mao Y, Zhao F, Zhand XX, Ju F, Ye L, Wang Y, Li B, Ren Q, Zhang T. 2018. Free-living bacteria and potential bacterial pathogens in sewage treatment plants. *Applied microbiology and biotechnology*, 102, 2455-2464.

Hugas M, Garriga M, Aymerich MT. 2003. Functionality of enterococci in meat products. *International journal of food microbiology*, 88(2-3), 223-233.

Izumi S. 1952. Biological studies on Ascaris eggs. *Japanese journal of medical science and biology*, 5(1), 21-36.

Jama-Rodzenska A, Sowinski J, Koziel JA, Bialowiec A. 2021. Phosphorus recovery from sewage sludge ash based on cradle-to-cradle approach – mini-review. *Minerals*, 11, 985.

Javied S, Mehmoud T, Chaudhry MM, Tufail M, Irfan N. 2009. Heavy metal pollution from phosphate rock used for the production of fertilizer in Pakistan. *Microchemical journal*, 91(1), 94-99.

Jiang Y, Xie SH, Dennehy C, Lawlor PG, Hu ZH, Wu GX, Zhan XM, Gardinger GE. 2020. Inactivation of pathogens in anaerobic digestion systems for converting biowastes to bioenergy. *Renewable and sustainable energy reviews*, 120, 109654.

Jin Q and Kirk MF. 2018. pH as a primary control in environmental microbiology: 1. Thermodynamic perspective. *Frontiers in environmental science*, 6:21.

Kasina M and Jarosz K. 2023. Chemical and microbiological techniques for recovery and/or removal of elements from incinerated sewage sludge ash – a review of basic methods. *Energies*, 16, 2840.

Ki D, Kang SH, Park KM. 2021. Upcycling of wastewater sludge incineration ash as 3D printing technology resource. *Frontiers in sustainability*, 2:697265.

Knight AI, Haines J, Zuber S. 2013. Thermal inactivation of animal virus pathogens. *Current topics in virology*, 11, 103-119.

Koutsoumanis K, Allende A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Bottari B, Cummins E, Ylivainio K, Guajardo I, Ortiz-Pelaez A, Alvarez-Ordonez A. 2021. Inactivation of indicator microorganisms and biological hazards by standard and/or alternative processing methods in Category 2 and 3 animal by-products and derived products to be used as organic fertilisers and/or soil improvers. *EFSA Journal,* 19(12), 6932.

Kumagai S, Daikai T, Onodera T. 2019. Bovine spongiform encephalopathy – a review from the perspective of food safety. *Food safety*, 7(2), 21-47.

Latosinska J and Gawdzik J. 2014. The impact of combustion technology of sewage sludge on mobility of heavy metals in sewage sludge ash. *Ecological chemistry and engineering S*, 28(1), 465-475.

Lau M, Monis P, Ryan G, Salveson A, Fontaine N, Blackbeard J, Gray S, Sanciolo P. 2020. Selection of surrogate pathogens and process indicator organisms for pasteurisation of municipal wastewater – a survey of literature data on heat inactivation of pathogens. *Process safety and environmental protection*, 133, 310-314.

Li X, Zhan D, Yan TY, Bryden WL. 2016. Phosphorus bioavailability: a key aspect for conserving this critical animal feed resource with reference to broiler nutrition. *Agriculture*, 6(2), 25-35.

Lund PA, De Biase D, Liran O, Scheler O, Mira NP, Cetecioglu A, Fernandez EN, Bover-Cid S, Hall R, Sauer M, Obyrne C. 2020. Understanding how microorganisms respond to acid pH is central to their control and successful exploitation. *Frontiers in microbiology*, 11, 556140.

Luyckx L and Caneghem J. 2021. Recovery of phosphorus from sewage sludge ash: influence of incineration temperature on ash minerology and related phosphorus and heavy metal extraction. *Journal of environmental chemical engineering,* 9, 106471.

Mahapatra S, Ali MD, Samal K, Moulick S. 2022. Diagnostic and treatment technologies for detection and removal of helminth in wastewater and sludge. *Energy nexus*, 8, 100147.

Maluquer de Motes C, Cano MJ, Torres JM, Pumarola M, Girones R. 2008. Detection and survival of prion agens in aquatic environments. *Water research*, 42, 2465-2472.

Maluquer de Motes C, Espinosa JC, Esteban A, Calvo M, Girones R, Torres JM. 2012. Persistence of the bovine spongiform encephalopathy infectious agent in sewage. *Environmental research*, 117, 1-7.

Manopriya S, Aberathna AAAU, Satharasinghe DA, Jayasooriya LJPAP, Mantilaka MMMGPG, Fernando CAN, Jayaweera BPA, Weerathilake WADV, Prathapasinghe GA, Liyanage JA, Premarathne JMKJK. 2022. Importance of phosphorus in farm animals. *Iranian journal of applied animal science*, 12(2), 203-210.

Marin-Moreno A, Aguilar-Calvo P, Moudjou M, Espinosa JC, Beringue V, Torres JM. 2019. Thermostability as a highly dependent prion strain feature. *Scientific reports*, 9(1), 11396.

Maya C, Torner-Morales FJ, Lucario ES, Hernandez E, Jimenez B. 2012. Viability of six species of larval and non-larval helminth eggs for different conditions of temperature, pH and dryness. *Water research*, 46, 4770-4782.

McAuley CN, Gobius KS, Britz ML, Craven HM. 2012. Heat resistance of thermoduric enterococci isolated from milk. *International journal of food microbiology*, 154(3), 162-168.

McDonnell K. 2022. Sanitary safety of animal by-product ash. Report for the European Sustainable Phosphorus Platform.

Merino N, Aronson HS, Bojanova DP, Feyhl-Buska J, Wong ML, Zhang S, Giovannelli D. Living at the extremes: extremophiles and the limits of life in planetary context. *Frontiers in microbiology,* 10:780.

Miles SL; Sun W, field JA, Gerba CP, Pepper IL. 2013. Survival of infectious prions during anaerobic digestion of municipal sewage sludge and lime stabilization of Class B biosolids. *Journal of residuals science and technology*, 10(2) 69-75.

Mocé-Llivina L, Muniesa M, Pimenta-Vale H, Lucena F, Jofre J. 2003. Survival of bacterial indicator species and bacteriophages after thermal treatment of sludge and sewage. *Applied and environmental microbiology*, 69(3), 1452-1456.

Mohammadi B, Raudner R, Shafiq M, Ahn E, Altmeppen HC, Glatzel M. 2020. Influence of methanol on prion reduction during high temperature and high pressure oleochemical processes. *European journal of lipid science and technology*, 122(9), 200136.

Muller H and Riesner D. 2005. Thermal degradation of prions in the presence of fats: implication for oleochemical processes. *European journal of lipid science and technology*, 107(11), 833-839.

NABC (National Agricultural Biosecurity Center). 2004. Carcass disposal: a comprehensive review. Report for the USDA Animal and Plant Health Inspection Service. Kansas State University.

Naidoo D and Foutch GL. 2018. The time-temperature relationship for the inactivation of *Ascaris* eggs. Journal of water, sanitation and hygiene for development, 8(1), 123-126.

Nichols TA, Pulford B, Wyckoff AC, Meyerett C, Michel B, Gertig K, Hoover EA, Jewell JE, Telling GC, Zabel MD. 2009. Detection of protease-resistant cervid prion protein in water from a CWD-endemic area. *Prion*, 3(3), 171-183.

Nims RW and Plavsic M. 2013. Intra-family and inter-family comparisons for viral susceptibility to heat inactivation. *Journal of microbial and biochemical technology*, 5(4), 136-141.

Nishide M, Tsujimoto K, Uozaki M, Ikeda M, Yamasaki H, Hajime Koyama A, Arakawa T. 2011. Effects of electrolytes on virus inactivation by acidic solutions. *International journal of molecular medicine*, 27(6), 803-809.

O'Donnell CJ, Meyer KB, Jones JV, Benton T, Kaneshiro ES, Nichols JS, Schafer FW. 1984. Survival of parasite eggs upon storage in sludge. *Applied and environmental microbiology*, 48(3), 618-625.

Olofsson U, Bignert A, Haglund P. 2012. Time-trends of metals and organic contaminants in sewage sludge. *Water research*, 46, 4841-4851.

Ottoson J. 2004. *Comparative analysis of pathogen occurrence in wastewater – management strategies for barrier function and microbial control.* Thesis, Royal Institute of Technology, Stockholm.

Paisley LG and Hostrup-Pedersen J. 2005. A quantitative assessment of the BSE risk associated with fly ash and slag from the incineration of meat-and-bone meal in a gas-fired plant in Denmark. *Preventive veterinary medicine*, 68, 263-275.

Pecson BM, Barios JA, Jimenez BE, Nelson KL. 2007. The effects of temperature, pH, and ammonia concentration on the inactivation of *Ascaris* eggs in sewage sludge. *Water research*, 41, 2893-2902.

Prusiner SB, McKinley MP, Bolton DC, Bowman KA, Groth DF, Cochran SP, Hennewwey EM, Braunfeld MB, Baringer JR, Chatigny MA. 1984. Prions: Methods for assay, purification, and characterization. *Methods in virology*, 8, 293-345.

Quinn DT and Fabiansson SU. 2001. *Risk assessment of abattoir effluent should BSE be found in cattle in Australia.* Report to the NHMRC special expert committee on TSEs – June 2001.

Race R, Jenny A, Sutton D. 1998. Scrapie infectivity and proteinase K-resistant prion protein in sheep placenta, brain, spleen, and lymph node: implications for transmission and antemortem diagnosis. *The journal of infectious diseases*, 178, 949-953.

Ratnayaka DD, Brandt MJ, Johnson KM. 2009. Water filtration: granular media filtration. *In*: Water Supply, 315-350.

Rocha MC, Barés ME, Braga MCB. 2016. Quantification of viable helminth eggs in samples of sewage sludge. *Water research*, 103, 245-255.

Rorat A, Courtois P, Vandenbulcke F, Lemiere S. 2019. Sanitary and environmental aspects of sewage sludge management. *Industrial and municipal sludge*, 155-180.

Rubenstein R, Chang B, Gray P, Piltch M, Bulgin MS, Sorensen-Melson S, Miller MW. 2011. Prion disease detection, PMCA kinetics and IgG in urine from sheep naturally/experimentally infected with scrapie and deer with preclinical/clinical chronic wasting disease. *Journal of virology*, 85 (17), 9031-9038.

Sahlström L, Bagge E, Emmoth E, Holmqvist A, Danielsson-Tham ML, Albihn A. 2008. A laboratory study of survival of selected microorganisms after heat treatment of biowaste used in biogas plants. Bioresource technology, 99, 7859-7865.

Sakudo A, Ano Y, Onodera T, Nitta K, Shintani H, Ikuta K, Tanaka Y. 2011. Fundamentals of prions and their inactivation (review). *International journal of molecular medicine*, 27, 483-489.

Saunders SE, Bartelt-Hunt SL, Bartz JC. 2008. Prions in the environment: occurrence, fate and mitigation. *Prion*, 2(4), 162-169.

Sawada K, Suzuki A, Yamaski T, Iwamaru Y, Matsuura Y, Miyazawa K, Masujin K, Atarashi R, Horiuchi M. 2019. Estimation of prion infectivity in tissues of cattle infected with atypical BSE by real time-quaking induced conversion assay. *The journal of veterinary medical science*, 81(6), 846-850.

Schnell M, Horst T, Quicker P. 2020. Thermal treatment of sewage sludge in Germany: a review. *Journal of environmental management*, 263, 110367.

Senecal J, Nordin A, Vinnerås B. 2020. Fate of *Ascaris* at various pH, temperature and moisture levels. *Journal of water and health*, 18, 375-382.

Singh J, Hundal JS, Sharma A, Udeybir SC, Sethis APS, Singh P. 2018. Phosphorus nutrition in dairy animals: a review. *International journal of current microbiology and applied sciences*, 7(4), 3518-3530.

Sohn HJ, Park KJ, Roh IS, Kim HJ, Park HC, Kang HE. 2019. Sodium hydroxide treatment effectively inhibits PrP^{CWD} replication in farm soil. *Prion*, 13(1), 137-140.

Spiropoulos J, Lockey R, Beck KE, Vickery C, Holder TM, Thorne L, Arnold M, Adreoletti O, Simmons MM, Terry LA. 2019. Incomplete inactivation of atypical scrapie following recommended autoclave decontamination procedures. *Transboundary and emerging diseases*, 66(5), 1993-2001.

Strauch D. 1991. Survival of pathogenic micro-organisms and parasties in excreta, manure and sewage sludge. *Scientific and technical review of the office international des epizooties,* 10(3). 813-846.

Tateishi J, Tashima T, Kitamoto T. 1991. Practical methods for chemical inactivation of Creutzfeldt-Jakob disease pathogen. *Microbiology and immunology*, 35(2), 163-166.

Terry LA, Howells L, Bishop K, Baker CA, Everest S, Thorne L, Maddison BC, Gough KC. 2011. Detection of prions in the faeces of sheep naturally infected with classical scrapie. *Veterinary research*, 42, 65.

Tiganitas A, Zeaki N, Goundaki AS, Drosinos EH, Skandamis PN. 2009. Study on the effect of lethal and sublethal pH and a_w stresses on the inactivation or growth of *Listeria monocytogenes* and *Salmonella* Typhimurium. *International journal of food microbiology*, 134(1-2), 104-112.

Unal A, Thyer J, Uren E, Middleton D, Braun M, Maher D. 2007. Investigation by bioassay of the efficacy of sodium hydroxide treatment on the inactivation of mouse-adapted scrapie. *Biologicals*, 35(3), 161-164.

Varela AR and Manaia CM. 2013. Human health impact of clinically relevant bacteria in wastewater habitats. Environmental science and pollution research, 20, 3550-3569.

Vasickova P, Pavlik I, Verani M, Carducci A. 2010. Issues concerning survival of viruses on surfaces. *Food and environmental virology*, 2, 24-34.

WHO (World Health Organisation). 2007. Codex Alimentarius – working principles for risk analysis for food safety for application by governments.

Windfeld ES and Brooks MSL. 2021. Medical waste management – a review. *Journal of environmental management*, 163, 98-108.

WOAH (World Organisation for Animal Health). 2023. *Bovine spongiform encephalopathy*. Available at: https://www.woah.org/en/disease/bovine-spongiform-encephalopathy/#ui-id-2. Accessed 8 august 2023.

Yamamoto T, Kobayashi S, Nishiguchi A, Nonaka T, Tsutsui T. 2006. Evaluation of bovine spongiform encephalopathy (BSE) infection risk of cattle via sewage sludge from wastewater treatment facilities in slaughterhouses in Japan. *Journal of veterinary medical science*, 68(2), 137-142.

Yoshida S. 1920. On the resistance of Ascaris eggs. *The journal of parasitology*, 6(3), 132-129.

Zaleski KJ, Josephson KL, Gerba CP, Pepper IL. 2005. Potential regrowth and recolonization of Salmonellae and indicators in biosolids and biosolid-amended soil. *Applied and environmental microbiology*, 71(7), 3701-3708.

Zhu H, Hart CA, Sales D, Roberts NB. 2006. Bacterial killing in gastric juice – effect of pH and pepsin on *Escherichia coli* and *Helicobacter pylori*. *Journal of medical microbiology*, 55, 1265-1270.

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