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BIOSYSTEMS
ENGINEERING LTD.

NovaUCD, Belfield Innovation Park,
University College Dublin,
Belfield, Dublin 4, Ireland.
T +353 (0)1 716 3530 / 3531
F +353 (0)1 716 3532
W www.bioe.ie

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Sanitary Safety of Animal by-product Ash

European Sustainable Phosphorus Platform

Sanitary Safety of Animal by-product Ash

Research

Estefany Carolina Alquinta González

Prof Kevin McDonnell

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Abbreviations

ASP	Advanced Sterilization Products
aw	Water activity
ABP	Animal by-product.
BIOHAZ Panel	EFSA Panel on Biological Hazards
BSE	Bovine spongiform encephalopathy
BPV	Bovine Parvovirus
C-BSE	classical bovine spongiform encephalopathy
CE	Circular Economy
CJD	Creutzfeldt-Jakob disease
CPV	Canine Parvovirus
CWD	Chronic wasting disease in cervids
EFSA	European Food Safety Authority
ESPP	European Sustainable Phosphorus Platform
FFI	Fatal familial insomnia
GSS	Gerstmann-Sträussler-Scheinker disease in humans
H-BSE	H-type bovine spongiform encephalopathy
IEA	International Energy Agency
L-BSE	L-type bovine spongiform encephalopathy
MVM	Minute virus of mice
MRM	Mechanically recovered meat
PG127	Prion strain
PrP^{Sc}	Abnormal protease-resistant isoform of prion protein
PPV	Porcine Parvovirus
RML	Rocky Mountain Laboratories prions
SDS	Sodium dodecyl sulfate
SSA	Sewage Sludge Ash
TSE	Transmissible spongiform encephalopathy
tg338	Prion strain
22L	Prion strain
263K	Prion strain

Introduction

Combustion ashes of Category 2 and 3 animal by-products (e.g. of manure) are included in the EU Fertilising Products Regulation CMC13 “Thermal oxidation materials and derivates” (European Commission, 2021). European Sustainable Phosphorus Platform (ESPP) and other stakeholders consider that incineration ashes of Category 1 animal by-products, that is materials susceptible to transmit Bovine spongiform encephalopathy (BSE), should also be considered if shown to be safe.

The objective is therefore to collect available data, recent or not, on:

- ✓ Pathogen content of ashes resulting from combustion of animal by-products (Category 2 or Category 3, including manure).
- ✓ In particular, where combustion conditions respect the EU Industrial Emissions Directive incineration requirements (850°C for 2 seconds and 1,100 for 0.2 sec).
- ✓ Specifically to try to find any available data on pathogens in combustion ashes of Category 1 animal by-products, that is data on elimination of prions in combustion processes, where again the final aim is to assess whether safety is ensured by the EU Industrial Emissions Directive incineration requirements
- ✓ Identify any research centres carrying out recently such investigations, ongoing projects, etc

Therefore, this study was a literature review that assessed the available published work on the above topics. Fifty-one papers from Science Direct, EFSA Journal, PubMed Central, DOAJ, Molecular Medicine, Plos Pathogens, Wiley Online Library, MDPI were used for this research review. From them, twenty-two were related to ABP risk reduction using thermal/chemical treatment. Thirteen of them present relevant data of pathogen reduction using specifically thermal treatments, and two of them concern studies related to ABP ash. They were selected regarding the year of publication, prioritizing studies from the last fifteen years.

The search terms included “animal ash”, “category 1 ABP”, “Animal by-product”, “ash disposal”, “prions”, “incineration”, “indicator microorganisms”.

Assessment

Since 1995, over 140 patients with variant Creutzfeldt–Jakob disease (CJD) have died as a probable result of having consumed processed meat products contaminated by the agent of bovine spongiform encephalopathy (BSE) in mechanically recovered meat (MRM) that contained vertebral column nervous tissue. Although almost all cases have occurred in Great Britain, France and Italy have had indigenous cases, and future cases may appear in any country in which BSE exists.

Governments in Europe, as elsewhere, have taken steps to minimize the risk of exposure to BSE, both in terms of breaking the cycle of animal exposure to halt the spread of disease among cattle, and of prohibiting potentially infectious cattle tissue from entering the human food chain. However, implementation of these precautions has not been uniformed, and regulatory strategies, even when implemented, require continuous inspection to assure compliance.

1.1. Definition Animal by-products (ABP) - Category 1, 2, and 3.

Article 3 point 1 of the Regulation (EC) No 1069/2009 has defined that Animal by-product means "entire bodies or parts of animals, products of animal origin or other products obtained from animals, which are not intended for human consumption, including oocytes, embryos, and semen," while Article 3 point 2 indicate that derived products are “products obtained from one or more treatments, transformations or steps of processing of animal by-products." Moreover, Article 7 point 2 specifies that derived products shall be subject to the rules for the specific category of animal by-products from which they have been derived. The categorization of ABP reflects the level of risk to public and animal health arising from those ABP.

Therefore, categories 1, 2, and 3 shall compromise the ABP shown in *Table 1*.

Table 1 Categories 1, 2, 3 materials according to Regulation (EC) No 1069/2009. Source: (European Parliament, 2009)

Categorization	Article	Characteristics
Category 1	8	(a) entire bodies and all body parts, including hides and skins, of the following animals: **(i)** animals suspected of being infected by a TSE in accordance with Regulation (EC) No 999/2001 or in which the presence of a TSE has been officially confirmed; **(ii)** animals killed in the context of TSE eradication measures; **(iii)** animals other than farmed and wild animals, including in particular pet animals, zoo animals, and circus animals **(iv)** animals used for experiments as defined by Article 2(d) of Directive 86/609/EEC without prejudice to Article 3(2) of Regulation (EC) No 1831/2003; **(v)** wild animals, when suspected of being infected with diseases communicable to humans or animals; (b) the following material: **(i)** specified risk material; **(ii)** entire bodies or parts of dead animals containing specified risk material at the time of disposal; (c) animal by-products derived from animals which have been submitted to illegal treatment as defined in Article 1(2)(d) of Directive 96/22/EC or Article 2(b) of Directive 96/23/EC; (d) animal by-products containing residues of other substances and environmental contaminants listed in Group B(3) of Annex I to Directive 96/23/EC, if such residues exceed the permitted level laid down by Community legislation or, in the absence thereof, by national legislation; (e) animal by-products collected during the treatment of wastewater required by implementing rules adopted under point (c) of the first paragraph of Article 27: **(i)** from establishments or plants processing Category 1 material; or **(ii)** from other establishments or plants where specified risk material is being removed;

		(f) catering waste from means of transport operating internationally; (g) mixtures of Category 1 material with either Category 2 material or Category 3 material, or both.
Category 2	9	(a) manure, non-mineralised guano, and digestive tract content; (b) animal by-products collected during the treatment of wastewater required by implementing rules adopted under point (c) of the first paragraph of Article 27: **(i)** from establishments or plants processing Category 2 material; or **(ii)** from slaughterhouses other than those covered by Article 8(e); (c) animal by-products containing residues of authorised substances or contaminants exceeding the permitted levels as referred to in Article 15(3) of Directive 96/23/EC; (d) products of animal origin which have been declared unfit for human consumption due to the presence of foreign bodies in those products; (e) products of animal origin, other than Category 1 material, that are: **(i)** Imported or introduced from a third country and fail to comply with Community veterinary legislation for their import or introduction into the Community except where Community legislation allows their import or introduction subject to specific restrictions or their return to the third country; or **(ii)** (ii) dispatched to another Member State and fail to comply with requirements laid down or authorised by Community legislation except where they are returned with the authorisation of the competent authority of the Member State of origin; (f) animals and parts of animals, other than those referred to in Article 8 or Article 10, **(i)** that died other than by being slaughtered or killed for human consumption, including animals killed for disease control purposes;

		(ii) fetuses; **(iii)** oocytes, embryos, and semen which are not destined for breeding purposes; and **(iv)** (iv) dead-in-shell poultry; (g) mixtures of Category 2 material with Category 3 material; (h) animal by-products other than Category 1 material or Category 3 material.
Category 3	10	(a) carcasses and parts of animals slaughtered or, in the case of game, bodies or parts of animals killed, and which are fit for human consumption in accordance with Community legislation, but are not intended for human consumption for commercial reasons; (b) carcasses and the following parts originating either from animals that have been slaughtered in a slaughterhouse and were considered fit for slaughter for human consumption following an ante-mortem inspection or bodies and the following parts of animals from game killed for human consumption in accordance with Community legislation: **(i)** carcasses or bodies and parts of animals which are rejected as unfit for human consumption in accordance with Community legislation, but which did not show any signs of disease communicable to humans or animals; **(ii)** heads of poultry; **(iii)** hides and skins, including trimmings and splitting thereof, horns and feet, including the phalanges and the carpus and metacarpus bones, tarsus and metatarsus bones, of: · animals, other than ruminants requiring TSE testing, and · ruminants which have been tested with a negative result in accordance with Article 6(1) of Regulation (EC) No 999/2001; **(iv)** pig bristles; **(v)** feathers; (c) animal by-products from poultry and lagomorphs slaughtered on the farm as referred to in Article 1(3)(d) of Regulation (EC) No 853/2004, which did not show any signs of disease communicable to humans or animals;

		(d) blood of animals which did not show any signs of disease communicable through blood to humans or animals obtained from the following animals that have been slaughtered in a slaughterhouse after having been considered fit for slaughter for human consumption following an ante-mortem inspection in accordance with Community legislation: **(i)** animals other than ruminants requiring TSE testing; and **(ii)** ruminants which have been tested with a negative result in accordance with Article 6(1) of Regulation (EC) No 999/2001; (e) animal by-products arising from the production of products intended for human consumption, including degreased bones, greaves, and centrifuge or separator sludge from milk processing; (f) products of animal origin, or foodstuffs containing products of animal origin, which are no longer intended for human consumption for commercial reasons or due to problems of manufacturing or packaging defects or other defects from which no risk to public or animal health arise; (g) petfood and feeding stuffs of animal origin, or feeding stuffs containing animal by-products or derived products, which are no longer intended for feeding for commercial reasons or due to problems of manufacturing or packaging defects or other defects from which no risk to public or animal health arises; (h) blood, placenta, wool, feathers, hair, horns, hoof cuts, and raw milk originating from live animals that did not show any signs of disease communicable through that product to humans or animals; (i) aquatic animals, and parts of such animals, except sea mammals, which did not show any signs of disease communicable to humans or animals; (j) animal by-products from aquatic animals originating from establishments or plants manufacturing products for human consumption;

		(k) the following material originating from animals which did not show any signs of disease communicable through that material to humans or animals: **(i)** shells from shellfish with soft tissue or flesh; **(ii)** the following originating from terrestrial animals: · hatchery by-products, · eggs, · egg by-products, including egg shells, **(iii)** day-old chicks killed for commercial reasons; (l) aquatic and terrestrial invertebrates other than species pathogenic to humans or animals; (m) animals and parts thereof of the zoological orders of Rodentia and Lagomorpha, except Category 1 material as referred to in Article 8(a)(iii), (iv), and (v) and Category 2 material as referred to in Article 9(a) to (g); (n) hides and skins, hooves, feathers, wool, horns, hair, and fur originating from dead animals that did not show any signs of disease communicable through that product to humans or animals, other than those referred to in point (b) of this Article; (o) adipose tissue from animals which did not show any signs of disease communicable through that material to humans or animals, which were slaughtered in a slaughterhouse and which were considered fit for slaughter for human consumption following an ante-mortem inspection in accordance with Community legislation; (p) catering waste other than as referred to in Article 8(f).

1.2.Operating condition - Incineration and Co-incineration.

Regulation (EU) No 142/2011 implements Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption. It also implements Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive.

Therefore, regulation (EU) No 142/2011 states in whereas that since the incineration and the co-incineration of certain animal by-products do not fall within the scope of Directive 2000/76/EC of the European Parliament and of the Council of 4 December 2000 on the incineration of waste, adequate rules for the prevention of health risks arising from such operations should be laid down in this regulation, taking into account the possible effects on the environment. Residues from the operation of the incineration or co-incineration of animal by-products or derived products should be recycled or disposed of, in accordance with Union environmental legislation, since in particular, that legislation allows for the use of the phosphorous component of ashes in fertilisers and for the handover of ashes from the cremation of pet animals to the owners.

Moreover, Annex III, Chapter 1, Section 2 indicates that incineration or co-incineration plants shall be designed, equipped, built, and operated in such a way that the gas resulting from the process is raised in a controlled and homogeneous fashion, even under the most unfavorable conditions, to a temperature of 850 °C for at least 2 seconds or to a temperature of 1,100 °C for 0.2 seconds, as measured near the inner wall or at another representative point of the chamber where the incineration or the co-incineration is carried out, as authorised by the competent authority.

It establishes different conditions between high- and low-capacity incineration and co-incineration plants, summarized in *Table 2.* However, the lack of specific test for assessment of ash contamination leads to the use of indicator microorganisms to demonstrate inactivation as per the requirements for the heat treatment of healthcare waste.

Table 2 Conditions for high- and low-capacity incineration and co-incineration plants according to Regulation (EU) No 142/2011. Source: (European Commission, 2011)

Chapter	Section	Capacity
		HIGH-CAPACITY INCINERATION AND CO-INCINERATION PLANTS
II	1	**Specific operating conditions** incineration or co-incineration plants treating only animal by-products and derived products with a capacity of more than 50 kg per hour (high-capacity plants) and which are not required to have a permit to operate in accordance with Directive 2000/76/EC shall comply with the following conditions: (a) The plants must be equipped for each line with at least one auxiliary burner. This burner shall be switched on automatically when the temperature of the combustion gases after the last injection of combustion air falls below 850 °C or 1,100 °C, as applicable. It must also be used during plant start-up and shut-down operations to ensure that the temperature of 850 °C or 1,100 °C, as applicable, is maintained at all times during these operations and as long as unburned material is in the chamber where the incineration or co-incineration is carried out. (b) When animal by-products or derived products are introduced into the chamber where the incineration or co-incineration is carried out by a continuous process, the plant must operate an automatic system to prevent the introduction of animal by-products or derived products at start-up until the temperature of 850 °C or 1,100 °C, as applicable, has been reached, and whenever the temperature is not maintained. (c) The operator must operate the incineration plant in such manner that a level of incineration is achieved such that the slag and bottom ashes total organic carbon content is less than 3 % or their loss on ignition is less than 5 % of the dry weight of the material. If necessary, appropriate techniques of pre-treatment shall be used.

	2	**Water discharges** 1. Sites of high-capacity plants, including associated storage areas for animal by-products, shall be designed in such a way as to prevent unauthorised and accidental release of any polluting substances into soil, surface water and groundwater. 2. Storage capacity shall be provided for contaminated rainwater run-off from the plant site or for contaminated water arising from spillage or firefighting operations. 3. The operator shall, if necessary, ensure that such rainwater and such water can be tested and treated before discharge, when necessary.
III	-	**LOW-CAPACITY INCINERATION AND CO-INCINERATION PLANTS** Incineration and co-incineration plants treating only animal by-products and derived products with a maximum capacity of less than 50 kg of animal by-products per hour or per batch (low-capacity plants) and which are not required to have a permit to operate in accordance with Directive 2000/76/EC shall: (a) only be used for the disposal of: (i) dead pet animals referred to in Article 8(a)(iii) of Regulation (EC) No 1069/2009; or (ii) Category 1 materials referred to in Article 8(b), (e) and (f), Category 2 materials referred to in Article 9 or Category 3 materials referred to in Article 10 of that Regulation; (b) when Category 1 materials referred to in Article 8(b) of Regulation (EC) No 1069/2009 are introduced into the low- capacity plant, be equipped with an auxiliary burner; (c) operate in such a way that the animal by-products are completely reduced to ash.

1.3.Hazard Identification - Indicator Microorganisms

Indicator microorganisms, in thermal treatments, represent the most resilient or resistant organisms within specific categories (Koutsoumanis et al., 2021). Therefore, it is assumed that when these resilient pathogens are inactivated, any other less resilient hazards are also inactivated. This inactivation reduces the risk for human and animal health, which is mentioned in the regulation (EU) 142/2011 Annex VII, Chapter II, Section 1, Point (c) as follows:

The risk reduction for human and animal health which can be achieved by the process must be estimated on the basis of direct measurements. Where no direct measurement is available, modelling or extrapolation from other processes may also be used. In order to demonstrate effective risk reduction, the identified hazard (such as Salmonella) must be quantified both in the input (raw) material and in the resulting output material. For the purpose of this Chapter, output material comprises any end-products resulting from and by-products derived from the process.

Estimates must be accompanied by evidence. This includes – for measurements – information on the methodology used (sensitivity and reliability of the methods used), nature of samples that have been analysed, and evidence that samples are representative (relevant real samples, number of tests performed).

If surrogates for prion measurement are used, an explanation should be given of their relevance. An evaluation of the validity with the uncertainties involved must be provided;

Concerning organic fertilisers and soil improvers (from Category 2 and 3), Annex XI mentions in Chapter I, Section 2, Point (c) that the competent authority may authorise the use of other standardised process parameters than those referred to in point (b), provided an applicant demonstrates that such parameters ensure minimising of biological risks.

Furthermore, this point indicates in numeral (iii) that this validation must demonstrate that the process achieves the following overall risk reduction:

For thermal and chemical processes by reduction of Enterococcus faecalis by at least 5 log_{10} and by reduction of infectivity titre of thermoresistant viruses such as parvovirus, where they are identified as a relevant hazard, by at least 3 log_{10}.

Since currently Category 1 is not authorized to be used for manufacturing organic fertilisers or soil improvers, the mentioned legislation does not state a definitive specific reduction of prions for the incineration process. However, previous the European Food Safety Authority (EFSA) opinion established that a reduction of at least 6 $\log_{10}$ in transmissible spongiform encephalopathy (TSE) infectivity should be achieved by the approved biodiesel production process (EFSA, 2015; Ricci *et al.*, 2018; Koutsoumanis *et al.*, 2020). Consequently, for this study 6$\log_{10}$ was the validation reduction used as a parameter, which in the future would need to be discussed and approved by the BIOHAZ panel in case of utilizing Category 1 as a raw material.

1.4.Thermal Inactivation Data

There is no data available that evaluate the infectivity reduction achieved by applying incineration and co-incineration conditions (850 °C for 2 sec or 1,100 °C for 0,2 sec) for any of the categories. Hence, data and extrapolation from other processes were used as the legislation allows.

1.4.1. Category 1

Prions

Category 1 ABP materials contain different biological hazards, including some highly heat-resistant bacterial spores or viruses. However, Prions (PrP^{Sc}) is considered the most relevant hazard. (Koutsoumanis et al., 2021) . Prion diseases are fatal neurodegenerative diseases that affect numerous mammal species and include kuru, Creutzfeldt-Jakob disease (CJD), fatal familial insomnia (FFI) and Gerstmann-Sträussler-Scheinker disease (GSS) in humans, scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle and chronic wasting disease (CWD) in cervids (Marín-Moreno *et al.*, 2019).

The 'protein-only hypothesis' predicts that a prion conveys its infectious structural information to its normally folded non-infectious counterpart, leading to disease transmission. (Ma and Wang, 2014) . PrP^{C} conversion into PrP^{Sc} is a post-translational process where both isoforms share an identical amino acid sequence but differ in conformation (Marín-Moreno *et al.*, 2019). This conformational change confers distinct physicochemical properties such as a greater tendency to

aggregate, greater insolubility in non-ionic detergents, partial resistance to protease digestion, and high resistance to heat and chemical sterilization (Horiuchi and Caughey, 1999; Riesner, 2003)

For category 1 and due to the extreme thermostability of prions, it can be assumed that even thermoresistant viruses and bacterial spores are completely inactivated if the new method assures the inactivation of prions. (Koutsoumanis et al., 2021). *Table 3* shows data gathered from comprehensive review articles that applied thermal inactivation processes as part or full treatment in contaminated animal tissues. They evaluated seven variants of prions (22L, RML, CJD, Nor98, AS, C-BSE, L.BSE, H-BSE, and 263K), under 17 different methodologies, between 98 [°C] to 1,000 [°C]. The strain 263K was the only variant that presented data for ash in incineration conditions above 600 [°C] (Brown *et al,* 2000; Brown *et al,* 2004). The studies indicated that this strain was chosen because the concentration of infectivity in brain tissue of terminally ill animals is as high or higher than in any other TSE, natural or experimental, and thus allows the maximum measure of reduction (Brown *et al,* 2004). On top of that this strain shows resistance to heat that is comparable to BSE and superior to other tested TSE strains (Brown *et al,* 2004).

It is important to mention that in the cases the thermal treatment was only a part of the process, the level of inactivation in *Table 3* corresponds only to that specific part and not the complete process.

Table 3 Thermal Inactivation data for Prions. Source: Own elaboration

Pathogen	Matrix	Treatment	Temp [°C]	t[min]	Level of inactivation	Reference
22L	Brain homogenates of Tga20 mice infected	Heat treatment in a thermocycler (98 °C, 2 hours)	98	120	5log10	(Marín-Moreno *et al.*, 2019)
22L	Brain homogenates of Tga20 mice infected	Heat treatment in thermocycler plus PK digestion (98 °C, 2 hours, subjected to PK digestion)	98	120	5log10	
22L	Brain homogenates of Tga20 mice infected	Heat treatment in thermocycler plus PK digestion (subjected to PK digestion first, 98 °C, 2 hours)	98	120	5log10	
RML	Brain homogenates of Tga20 mice infected	Heat treatment in a thermocycler (98 °C, 2 hours)	98	120	6log10	
RML	Brain homogenates of Tga20 mice infected	Heat treatment in thermocycler plus PK digestion (98 °C, 2 hours, subjected to PK digestion)	98	120	6log10	

RML	Brain homogenates of Tga20 mice infected	Heat treatment in thermocycler plus PK digestion (subjected to PK digestion first, 98 °C, 2 hours)	98	120	6log10	
BSE	Brain homogenates of Tga20 mice infected	Heat treatment in a thermocycler (98 °C, 2 hours)	98	120	0log10	
BSE	Brain homogenates of Tga20 mice infected	Heat treatment in thermocycler plus PK digestion (98 °C, 2 hours, subject to PK digestion)	98	120	0log10	
BSE	Brain homogenates of Tga20 mice infected	Heat treatment in thermocycler plus PK digestion (subjected to PK digestion first, 98 °C, 2 hours)	98	120	0log10	
RML	Tallow	BDI RepCat process (200° C, 70 bar, and 15 min)	200	15	> 6log10	(Koutsoumanis et al., 2021)
CJD	Tallow	BDI RepCat process (200° C, 70 bar, and 15 min)	200	15	> 6log10	
RML	Tallow	RepCat Biodiesel process (200° C, 80 bar, and 30 min) in MeOH presence	200	30	> 6log10	

RML	Tallow	RepCat Biodiesel process (200° C, 70 bar and 15 min) MeOH presence	200	15	> 6log10	(Mohammadi *et al.*, 2020)
CJD	Tallow	RepCat Biodiesel process (200° C, 80 bar, and 30 min) glycerine sample	200	30	> 6log10	
CJD	Tallow	RepCat Biodiesel process (200° C, 70 bar and 15 min) glycerine sample	200	15	> 6log10	
Nor98	Brain Tissue from Sheep	Autoclave (133 °C, 3 bar, 20 min)	133	20	10log10	(Spiropoulos *et al.*, 2019)
AS	Brain Tissue from Sheep	Autoclave (133 °C, 3 bar, 20 min)	133	20	10log10	
C-BSE	Brain Tissue from bovine	Autoclave (133 °C, 3 bar, 20 min)	133	20	5.78log10	(Chapman *et al.*, 2020)
L.-Type BSE	Brain Tissue from bovine	Autoclave (133 °C, 3 bar, 20 min)	133	20	9.40log10	
H.-Type BSE	Brain Tissue from bovine	Autoclave (133 °C, 3 bar, 20 min)	133	20	3.94log10	
263K	Brain Tissue from hamster	Heating (15 min)	150	-	2-3 log10	

263K	Brain Tissue from hamster	Heating (15 min)	300	-	~6 log10	(Brown *et al*, 2000)
263K	Brain Tissue from hamster	Heating (5 min)	150	-	2 log10	
263K	Brain Tissue from hamster	Heating (5 min)	300	-	4 log10	
263K	Brain Tissue from hamster	Heating (5 min)	1000	-	Total inactivation	
263K	Brain Tissue from hamster	Heating-air gas (15 min)	612	-	> 8log10	(Brown *et al*, 2004)
263K	Brain Tissue from hamster	Heating-N_2 (15 min)	598	-	Total inactivation	
263K	Brain Tissue from hamster	Heating-air gas (15 min)	996	-	Total inactivation	

263K	Brain Tissue from hamster	Heating-N_2 (15 min)	997	-	Total inactivation	
263K	Tallow	hydrolytic fat splitting (90% bovine edible tallow, 10%water, 200°C, 20 min)	200	20	7log10	(Müller and Riesner, 2005)
263K	Tallow	hydrolytic fat splitting (bovine edible tallow, 200°C, 27 min) glycerol regime	200	27	> 6log10	(Müller *et al.*, 2006)
263K	Tallow	fat hydrogenation (bovine edible tallow, 160 °C, 12 bar, 20 min)	160	20	> 5,9log10	(Müller *et al.*, 2008)

1.4.2. Category 2 and 3

Koutsoumanis et al., (2021) analyzed these two categories deeply. The data presented was extracted and consolidated from different scientific studies, and estimated the time needed to inactivate the pathogens as a function of the treatment temperature.

Enterococcus faecalis

Enterococcal infections became one of the most challenging nosocomial problems, with two species, Enterococcus faecium and Enterococcus faecalis, ranking among the leading causes of hospital-acquired infections (Suchomel *et al.*, 2019). The ability of enterococci to acquire and exchange plasmids and transposons that carry antimicrobial resistance and virulence genes has contributed to their role as multiresistant pathogens (Mundy *et al.*, 2000). They are the most thermotolerant of non-sporulating bacteria, and some can survive pasteurization temperatures. Tolerance to environmental extremes explains their survival during processing of cooked and uncooked cured meats and their ability to multiply during fermentation (Hugas *et al.*, 2003). Consequently, nowadays. E. faecalis is used for testing thermal disinfection processes (Koutsoumanis et al., 2021).

The data presented in *Table 4* estimate the times needed (5D) to inactivate 5 $\log_{10}$ units as a function of the treatment temperature for different studies.

Table 4 Thermal Inactivation for Enterococcus Faecalis. Source: (Koutsoumanis et al., 2021)

Hazard	Product group	Product/or medium	Treatment	T (°C)	D (min)	5D (min)	Reference
Enterococcus faecalis	Liquids	Mixed	Heat	55	23.22	116.1	Sörqvist (2003)
Enterococcus faecalis	Liquids	Mixed	Heat	60	6.92	34.56	
Enterococcus faecalis	Liquids	Mixed	Heat	65	2.05	10.25	
Enterococcus faecalis	Liquids	Mixed	Heat	72	0.38	1.9	
Enterococcus faecalis	Liquids	Whole milk	Heat	57	61.73	308.65	Aguirre et al. (2009)
Enterococcus faecalis	Liquids	Whole milk	Heat	59	34.84	174.2	
Enterococcus faecalis	Liquids	Whole milk	Heat	61	18.48	92.4	
Enterococcus faecalis	Liquids	Whole milk	Heat	64	5.91	29.55	
Enterococcus faecalis	Solid product	Whole raw almond kernels	Hot water	88	0.36	1.8	Harris et al. (2012)
Enterococcus faecalis	Solid product	Growth in BHI and treatment in aseptically prepared ground beef	Heating in water bath	55	57.53	287.65	Saucier and Plamondon (2011)
Enterococcus faecalis	Solid product	Growth in BHI and treatment in aseptically prepared ground beef	Heating in water bath	60	13.37	66.85	
Enterococcus faecalis	Solid product	Growth in BHI and treatment in aseptically prepared ground beef	Heating in water bath	65	1.93	9.65	
Enterococcus faecalis	Solid product	Growth in BHI and treatment in aseptically prepared ground beef	Heating in water bath	70	0.19	0.95	
Enterococcus faecalis	Solid product	Growth in ME2 and treatment in aseptically prepared ground beef	Heating in water bath	55	58.65	293.25	
Enterococcus faecalis	Solid product	Growth in ME2 and treatment in aseptically prepared ground beef	Heating in water bath	60	13.37	66.85	
Enterococcus faecalis	Solid product	Growth in ME2 and treatment in aseptically prepared ground beef	Heating in water bath	65	2.12	10.6	
Enterococcus faecalis	Solid product	Growth in ME2 and treatment in *aseptically* prepared ground beef	Heating in water bath	70	0.15	0.75	
Enterococcus faecalis	Semi-liquid	Digestion waste	Heat	55	8.3	41.5	Ugwuanyi et al. (1999)
Enterococcus faecalis	Semi-liquid	Digestion waste	Heat	60	6.61	33.05	
Enterococcus faecalis	Semi-liquid	Digestion waste	Heat	55	4.72	23.6	
Enterococcus faecalis	Semi-liquid	Digestion waste	Heat	60	5.24	26.2	

Salmonella Senftenberg

Salmonella Senftenberg 775 W is known to be exceptionally heat resistant in high-moisture foods (Podolak *et al.*, 2017). The heat resistance of the serovars is not solely dependent on the serovars themselves but also on the surrounding environments (Podolak *et al.*, 2017; Sekhon *et al.*, 2020). For instance, Salmonella Senftenberg has better survivability in high-moisture foods than other serovans, but due to its weaker adaptability to the low-water-activity environment, its heat resistance of it was found to be lower than other serovans in non-fat dry milk (Sekhon *et al.*, 2020).

As well as with the E. faecalis, the data was used by Koutsoumanis et al., (2021) to estimate the times needed to inactivate 5 $\log_{10}$ units of S. Senftenberg as a function of the treatment temperature. The material tested were eggs, beef, and other products derived from milk.

Table 5 Thermal Inactivation for Salmonella Senftenberg. Source Source: (Koutsoumanis et al., 2021)

Hazard	Product group	Product/or medium	Treatment	pH	T (°C)	D (min)	5D (min)	Ref
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid whole eggs	Heat		55	34.3	171.5	Doyle and Mazzotta (2000)
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid whole eggs	Heat		60	5.6	28	
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid whole eggs	Heat		64	2.8	14	
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid egg yolks	Heat		55	42	210	
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid egg yolks	Heat		60	11.8	59	
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid egg whites	Heat		55	3	15	
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid egg whites	Heat		60	0.8	4	
Salmonella ser. Senftenberg 775W	Liquid food product	Raw milk	Heat		60	0.122	0.61	
Salmonella ser. Senftenberg 775W	Liquid food product	Raw milk	Heat		61.5	0.107	0.535	
Salmonella ser. Senftenberg 775W	Liquid food product	Raw milk	Heat		63	0.067	0.335	
Salmonella ser. Senftenberg 775W	Liquid food product	Raw milk	Heat		64.5	0.067	0.335	
Salmonella ser. Senftenberg 775W	Liquid food product	Raw milk	Heat		67.5	0.046	0.23	
Salmonella ser. Senftenberg	Solid food product	Ground beef	Heat		53	53	265	
Salmonella ser. Senftenberg	Solid food product	Ground beef	Heat		58	15.2	76	
Salmonella ser. Senftenberg	Solid food product	Ground beef	Heat		63	2.08	10.4	
Salmonella ser. Senftenberg	Solid food product	Ground beef	Heat		68	0.22	1.1	
Salmonella ser. Senftenberg	Liquid culture medium	PO_4	Heat		55	13	65	
Salmonella ser. Senftenberg	Liquid culture medium	PO_4	Heat		65	0.29	1.45	
Salmonella ser. Senftenberg	Liquid culture medium	PO_4	Heat		54.4	14.23	71.15	
Salmonella ser. Senftenberg	Liquid culture medium	PO_4	Heat		57.2	6.23	31.15	
Salmonella ser. Senftenberg	Liquid culture medium	PO_4	Heat		60	2.69	13.45	
Salmonella ser. Senftenberg S_2	Liquid culture medium	PO_4	Heat		54.4	17.13	85.65	
Salmonella ser. Senftenberg S_2	Liquid culture medium	PO_4	Heat		57.2	7.14	35.7	
Salmonella ser. Senftenberg S_2	Liquid culture medium	PO_4	Heat		60	2.88	14.4	
Salmonella ser. Senftenberg R_1	Liquid culture medium	PO_4	Heat		54.4	19.32	96.6	
Salmonella ser. Senftenberg R_1	Liquid culture medium	PO_4	Heat		57.2	3.72	18.6	
Salmonella ser. Senftenberg R_1	Liquid culture medium	PO_4	Heat		60	3.06	15.3	
Salmonella ser. Senftenberg R_2	Liquid culture medium	PO_4	Heat		54.4	12.77	63.85	
Salmonella ser. Senftenberg R_2	Liquid culture medium	PO_4	Heat		57.2	5.39	26.95	
Salmonella ser. Senftenberg R_2	Liquid culture medium	PO_4	Heat		60	2.31	11.55	
Salmonella ser. Senftenberg R_6	Liquid culture medium	PO_4	Heat		54.4	13.14	65.7	

Hazard	Product group	Product/or medium	Treatment	pH	T (°C)	D (min)	5D (min)	Ref
Salmonella ser. Senftenberg R_6	Liquid culture medium	PO_4	Heat		57.2	5.56	27.8	
Salmonella ser. Senftenberg R_6	Liquid culture medium	PO_4	Heat		60	1.92	9.6	
Salmonella ser. Senftenberg	Liquid culture medium	HI	Heat		50	268	1340	
Salmonella ser. Senftenberg	Liquid culture medium	HI	Heat		55	36.2	181	
Salmonella ser. Senftenberg	Liquid culture medium	HI	Heat		60	6.3	31.5	
Salmonella ser. Senftenberg	Liquid culture medium	HI	Heat		50	146	730	
Salmonella ser. Senftenberg	Liquid culture medium	HI	Heat		55	4.9	24.5	
Salmonella ser. Senftenberg	Liquid culture medium	HI	Heat		60	0.62	3.1	
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid egg whites	Heat	9	52.2	28.6	143	
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid egg whites	Heat	9	55	7.2	36	
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid egg whites	Heat	9	56.7	3.1	15.5	
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid egg whites	Heat	9	52.2	3.1	15.5	
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid egg whites	Heat	9	55	0.78	3.9	
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid egg whites	Heat	9.5	52.2	19.3	96.5	
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid egg whites	Heat	9.5	55	4.9	24.5	
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid egg whites	Heat	9.5	56.7	0.34	1.7	
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid egg whites	Heat	9.5	52.2	1.47	7.35	
Salmonella ser. Senftenberg 775W	Liquid food product	Liquid egg whites	Heat	9.5	55	0.36	1.8	
Salmonella ser. Senftenberg 775W	Semi-liquid food product	Chocolate	Heat		70	440	2200	
Salmonella ser. Senftenberg 775W	Semi-liquid food product	Chocolate	Heat		71	276	1380	
Salmonella ser. Senftenberg 775W	Semi-liquid food product	Chocolate	Heat		80	116	580	
Salmonella ser. Senftenberg 775W	Semi-liquid food product	Chocolate	Heat		90	36	180	

Parvovirus

The family Parvoviridae is divided into two subfamilies, Parvovirinae and Densovirinae, which infect vertebrates and invertebrates, respectively (Qiu *et al.*, 2017). They are small, single-stranded DNA viruses that have been classified into eight genera: Protoparvovirus, Amdoparvovirus, Aveparvovirus, Bocaparvovirus, Dependoparvovirus, Erythroparvovirus, Copiparvovirus, and Tetraparvovirus. Parvoviruses that infect humans are B19V, HBoVs, BuV, and PARV4, which belong to the Erythroparvovirus, Bocaparvovirus, Protoparvovirus, and Tetraparvovirus genera, respectively (Qiu *et al.*, 2017; de Souza *et al.*, 2018).

Many parvoviral pathogens of medical, veterinary, and ecological importance have been identified (de Souza *et al.*, 2018). Furthermore, Parvoviridae is by far the most heat-resistant viral family, followed by Caliciviridae and Picornaviridae, according to the study done by Nims and Plavsic (2013), where they compared four different viral families.

The data shown in *Table 6* includes information from avian strains, mice, bovines, porcine, and canines. In this case, the data was used to estimate > 3 $\log_{10}$ reductions when possible.

Table 6 Thermal Inactivation data for Parvoviridae. Source (Koutsoumanis et al., 2021):

Virus	Matrix/substrate	Initial load	Treatment	T (°C)	t(min)	Level of inactivation	D (min)	Reference
Canine parvovirus (CPV)	Human serum protein solution	$10^{5.5}$ $TCID_{50}$ mL^{-1}		103	1.5	Total inactivation		Lelie et al. (1987)
		10^{5} $TCID_{50}$ mL^{-1}		65	10	1.3 log $TCID_{50}$ mL^{-1}		
		$10^{4.9}$ $TCID_{50}$ mL^{-1}		65	40	2.3 log $TCID_{50}$ mL^{-1}		
		$10^{5.5}$ $TCID_{50}$ mL^{-1}		65	600	Total inactivation		
Porcine parvovirus (PPV)	Manure (25% pig manure and 75% cow manure) and bleaching clay	$10^{5.7}$ $TCID_{50}$ 50 mL^{-1}		70	60	0.6 log_{10}		Lund et al. (1996)
				55	660	4 log_{10}(Initial)		
				55	3,240	4 log_{10} (Terminal)		
Porcine parvovirus (PPV)	Manure, bleaching clay and household waste	$10^{5.7}$ $TCID_{50}$ 50 mL^{-1}		70	60	1.4 log_{10}		
				55	720	4 log_{10} (Initial)		
				55	3,240	4 log_{10} (Terminal)		
Porcine parvovirus (PPV)	Manure and bleaching clay	$10^{5.7}$ $TCID_{50}$ 50 mL^{-1}		55	8,880	4 log_{10}		
Bovine parvovirus (BPV)	Human plasma		Dry heat	100	300	4 log_{10}		Bräuniger et al. (2000)
			Moist heat	60	600	4 log_{10}		
Parvovirus B19	Human serum albumin			60	10	> 4 log_{10}		Blümel et al. (2002)
Porcine parvovirus (PPV)	Human serum albumin			60	60	Infectivity retained		Blümel et al. (2002)
Canine parvovirus (CPV)	Human serum albumin	8.2 log_{10} $TCID_{50}$ mL^{-1}		60	60	0.7 log_{10}	276.75	Yunoki et al. (2003)
				60	300	1.5 log_{10}		
				60	600	2.5 log_{10}		
		8.1 log^{10} $TCID_{50}$ mL^{-1}		60	60	0.3log_{10}		
				60	300	1.2log_{10}		
				60	600	2.4 log_{10}		

Virus	Matrix/substrate	Initial load	Treatment	T (°C)	t(min)	Level of inactivation	D (min)	Reference
Canine parvovirus (CPV)	0.5% Urinastatin solution	8.9 log_{10} $TCID_{50}$ mL^{-1}		60	60	1.6 log_{10}	80	
				60	300	5.1 log_{10}		
				60	600	> 6.4 log_{10}		
Canine parvovirus (CPV)	0.5% Urinastatin solution	8.2 log_{10} $TCID_{50}$ mL^{-1}		60	60	1.3 log_{10}		
				60	300	3.8 log_{10}		
				60	600	5.7 log_{10}		
Parvovirus B19			Heated in liquid	60		2–6 log_{10}		
Porcine parvovirus	Untreated mixed waste		Heat under laboratory conditions	55	60		60	Sahlström et al. (2008)
				70	30		19.8	
Parvovirus, avian strains				65	30	Infectivity retained		EFSA BIOHAZ Panel (2011)
Bovine parvovirus (BPV)			Dry heat	95	120	Infectivity retained		EFSA BIOHAZ Panel (2011)
Canine parvovirus (CPV)				80	420	Infectivity retained		EFSA BIOHAZ Panel (2011)
Minute virus of mice (MVM)	Culture media			141	0.5	1 log_{10}		Nims and Plavsic (2013c)
				196	0.5	4 log_{10}		
Minute virus of mice (MVM)	Water			104	0.5	1 log_{10}		
				117	0.5	4 log_{10}		
Bovine parvovirus (BPV)	Water			94	0.5	1 log_{10}		
				101	0.5	4 log_{10}		
Canine parvovirus (CPV)	Water			102	0.5	1 log_{10}		
				112	0.5	4 log_{10}		
Parvoviridae spp.				110	0.5	1 log_{10} [(b)]		
Parvoviridae spp.				131	x0.5	4 log_{10} [(b)]		
Porcine parvovirus (PPV)	Saline solution			70	72	1 log_{10}		Elving et al. (2014)
		6.7 log_{10} $TCID_{50}$ g^{-1}		70	60	0.9 log_{10}		
				55		1 log_{10}	1,372	

Virus	Matrix/substrate	Initial load	Treatment	T (°C)	t(min)	Level of inactivation	D (min)	Reference
Porcine parvovirus (PPV)	Dairy cow faeces			49		1 log_{10}	1,019	
				52		1 log_{10}	1,006 ($CI_{95\%}$ 828.6–1,280.4)	
				52	3,840	3 log_{10}		
				55		1 log_{10}	650 ($CI_{95\%}$ 531–839.4)	
				55	2,520	3 log_{10}		
Minute virus of mice *(Protoparvovirus)*	Culture medium		(35, 45, 60, 100°C)				4 $(D_{80})^{(a)}$	Nims and Zhou (2016)
Minute virus of mice *(Protoparvovirus)*	Water		(70, 80, 90°C)				14.3 $(D_{80})^{(a)}$	
Canine parvovirus *(Protoparvovirus)*	Water		(56, 80, 100°C)				21.4 $(D_{80})^{(a)}$	
Bovine parvovirus *(Bocaparvovirus)*	Water		(75, 80, 85, 90°C)				23.6 $(D_{80})^{(a)}$	
Parvovirus B19 *(Erythroparvovirus)*	5% Albumin		(52, 53, 54, 55.5, 57.5, 59, 60°C)				< 0.017 $(D_{80})^{(a)}$	
Parvovirus B19 *(Erythroparvovirus)*	Culture medium		(50, 60, 70°C)				1.8 $(D_{80})^{(a)}$	

(a): D-values from Nims and Zhou (2016) were estimated based on the reported data for other temperatures.
(b): Average temperature value for 1 log_{10} and 4 log_{10} *reduction in 30 s.*

1.5. Recent Advances in Prion Inactivation.

Prions are known to bind strongly on steel surfaces (Zobeley *et al.*, 1999; Flechsig *et al.*, 2001). Therefore, experiments have been performed using prion-contaminated steel wires to represent certain surgical instruments (Zobeley *et al.*, 1999; Flechsig *et al.*, 2001; Peretz *et al.*, 2006; Giles *et al.*, 2008; Sakudo *et al.*, 2019) . However, apart from these materials, there are various surface materials used in medical devices, which may influence prions inactivation.

This is not the only complication faced on the inactivation process. As it was shown in the data previously presented, the resistance of prions to inactivation differs among species or sources. For example, human CJD prions can be 100,000 times more difficult to inactivate than scrapie prion Sc237 during acidic sodium dodecyl sulfate (SDS) treatment (Peretz *et al.*, 2006). Similarly, the BSE prion is reportedly 1000 times more resistant than the mouse-passaged BSE strain (Giles *et al.*, 2008). Therefore, it has been suggested that inactivation technologies should be tested against all type of prions (Sakudo *et al.*, 2022).

Langeveld *et al.*, (2021) applied in their study a heat treatment in the presence of detergent and proteolysis by a keratinase from Bacillus licheniformis. After heating at 115°C with or without subsequent proteolysis, the original BSE infectivity of 6.4 $\log_{10}$ was reduced to a remaining infectivity of 4.6-5.7 $\log_{10}$ while strain characteristics were unaltered, even after precipitation with methanol. Similar treatment was applied on other prion strains, CWD1 in bank voles, 263 K scrapie in hamsters and sheep PG127 scrapie in tg338 ovinized mice. These strains infectivity was destroyed by heat only, which confirmed the heat resistant of the BSE variant.

1.5.1. Plasma Inactivation of Prion Agents

Prions, which cause transmissible spongiform encephalopathies (TSEs), are a notorious group of infectious agents with possibly the highest resistance to complete inactivation. Although various gas plasma instruments have been developed, studies on prion inactivation using gas plasma instruments are limited. Among them, the hydrogen peroxide gas plasma instrument, STERRAD® (Advanced Sterilization Products; ASP, Johnson & Johnson, Irvine, CA, USA), is recommended for prion inactivation of heat-sensitive medical devices. However, STERRAD® is not a plasma

sterilizer but a hydrogen peroxide gas sterilizer. In STERRAD®, plasma generated by radio frequency (RF) discharge removes excess hydrogen peroxide gas and does not contribute to sterilization. This is also supported by evidence that the instrument was not affected by the presence or absence of RF gas plasma. However, recent studies have shown that other gas plasma instruments derived from air, nitrogen, oxygen, Ar, and a mixture of gases using corona, dielectric barrier, microwave, and pulse discharges can inactivate scrapie prions. As inactivation studies on prions other than scrapie are limited, further accumulation of evidence on the effectiveness of gas plasma using human-derived prion samples is warranted for practical purposes. (Sakudo A et al 2022)

1.5.2. Biodegradability of Prions in Compost

This study (Xu et al 2014) investigated the degradation of prions associated with scrapie (263K), chronic waste disease (CWD), and bovine spongiform encephalopathy (BSE) in lab-scale composters and PrP263K in field-scale compost piles. Western blotting (WB) indicated that 263K, CWD, and BSE were reduced by at least 2 log10, 1−2 log10, and 1 log10 after 28 days of lab-scale composting, respectively. Further analysis using protein misfolding cyclic amplification (PMCA) confirmed a reduction of 2 log10 in 263K and 3 log10 in CWD. Enrichment for proteolytic microorganisms through the addition of feather keratin to compost enhanced degradation of 263K and CWD. For field-scale composting, stainless steel beads coated with 263K were exposed to compost conditions and removed periodically for bioassays in Syrian hamsters. After 230 days of composting, only one in five hamsters succumbed to TSE disease, suggesting at least a 4.8 log10 reduction in 263K infectivity. Our findings show that composting reduces TSE, resulting in one 50% infectious dose (ID50) remaining in every 5600 kg of final compost for land application. With these consideration the authors conclude that composting may be a viable method for SRM disposal.

1.6. Ash Disposal

The International Energy Agency roadmap ‘Net Zero Emissions by 2050’ recognizes bioenergy as an important option. IEA estimates that it will account for 18% of total energy supply in 2050. It will play a major role to reach carbon neutrality of the global energy system, either through the

direct replacement of fossil fuels, or to offset emissions indirectly through the combined use of bioenergy with carbon capture and storage/utilization (International Energy Agency, 2021).

In this context circular economy (CE) as a concept has gained more and more importance. Zink and Geyer, (2017) defines CE as "an economic system of closed loops in which raw materials, components and products keep their quality and value for the longest possible and systems are fuelled by renewable energy sources". CE represents a promising strategy for supporting sustainable, restorative, and regenerative agriculture, considering the current global climate emergency, resource scarcity, environmental degradation, and increasing food demand (Velasco-Muñoz *et al.*, 2021).

Biomass then will have an increasing role in the process of industrial combustion (Fuller *et al.*, 2015), and with it the biomass ash produced will increase too. The biomass ash can be divided in: bottom ash, which is the ash extracted from the bottom part of the furnace, and fly ash consisting of small sized, low density particles that have been entrained with the combustion gases and fall out in various parts of the boiler and the flue gas cleaning system (Haglund, 2008). Its management depends on their chemical composition and safety (Zhai et al.,2021).

The most common management practice for it is landfill disposal, which poses relevant economic and environmental drawbacks (Silva *et al.*, 2019) Costly landfill taxes aim to encouraging companies to divert their wastes from landfills and to seek alternative management options (European Union Agency, 2009).

For instances, Plants growth depend on the availability of some essential primary and secondary macronutrients Nitrogen (N), Calcium (Ca), Potassium (K), Phosphorus (P), Magnesium (Mg) and Sulphur (S) (White and Brown, 2010). P and K in soils are not replenished on human timescales by mobilization from primary minerals or atmospheric deposition, to maintain soil productivity, they must be added in the form of organic matter and inorganic fertilizers (Cleveland *et al.*, 2013; Tipping *et al.*, 2014). Ca, Mg and S can also be growth limiting in some soil types and are added to agricultural soils when required (Zhao *et al.*, 1999; Gransee and Führs, 2013). In this context, Zhai et al.,(2021) found that ash from the three categories of woody biomass (temperate hardwood, tropical hardwood, and softwood) could be an interesting source of nutrients due to it contains Ca, K, P, Mg, and it also contains relatively low levels of toxic trace elements and organic contaminants.

Other application includes the use of wood ash in the manufacture of construction materials (e.g. as partial replacement of lightweight aggregate or, exploiting their pozzolanic properties in cement blends or directly in mortars), which reduce the CO_2 emissions associated with the cement clinker production process (Fuller *et al.*, 2015).

According Jastrzębska et al., (2022) study, a new generation of fertilizers can be yield by recovering waste streams of biological origin flow from municipal and industrial wastewater treatment systems and slaughterhouses which are abundant in P. The scarcity of phosphate rock is a serious problem for Europe, making it dependent on importing virtually the entire raw material needed. In 2014, phosphate rock was placed by the European Union (EU) on the list of critical raw materials, to which P was also added in 2017 (European Commission, 2017).

Jastrzębska et al., (2022) analyzed the performance of two fertilizer from sewage sludge ash (SSA) and animal blood (AF and BF) under field conditions in comparison with a commercial P fertilizer, superphosphate (SP). The difference between the AF and BF fertilizer was that BF was considered a Biofertilizer due to it incorporated lyophilized cells of P-solubilizing bacteria, Bacillus megaterium. In the experiments with spring or winter wheat, BF showed the same yield-forming efficiency as SP, and under poorer habitat conditions, performed slightly better than AF in increasing yield and soil available P.

Since SSA is also a carrier of other macro-and micro-nutrients (Wyciszkiewicz *et al.*, 2019) and possible toxic elements (Herzel *et al.*, 2016), further studies were carried out. It was found that biobased fertilizers did not affect the soil pH, did not increase As, Cd, Cr, Ni, and Pb content, and did not alter the abundance of heterotrophic bacteria and fungi in the soil. Despite the positive results, it was indicated that the research into strain selection and the proportion of P-solubilizing microorganisms introduced into fertilizers should be continued.

In a report by Leng et al., (2019) the researchers stated that meat & bone meal ash (MBMA), that is the bottom ash collected from a UK industrial-scale incinerator in a power plant with meat & bone meal (MBM) as the mono-energy source, was characterised in terms of its elemental and crystalline compositions. MBMA has high phosphorus concentration (13.48% P, or 31.31% P_2O_5) and low hazardous element content. The phosphorus present in it, which mainly in the form of hydroxyapatite (HAP), would only release at initial acid leaching pH lower than 2.7 at solid: liquid

ratio of 1:1 (wt.). The researchers stated that the large amount of MBM, which has a heating value ranging from 13,000 to 30,000 MJ tonne^{-1}, is mostly treated as waste and is disposed of or exploited by incineration as fuel to remove any risk of BSE transmission (Cascarosa *et al.*, 2012; Leng *et al.*, 2018; Leng and Huang, 2018). This produces a large amount of MBM incineration ash (MBMA) in Europe. However, although with P content as high as ~15% in MBMA (Coutand *et al.*, 2008), only limited amount of this ash is utilized as fertiliser (because of its less effectiveness (Alotaibi *et al.*, 2013) or as raw materials for P industry, and with most of them end up in the landfill. The research was focussed on the feasibility of MBMA for phosphorus recovery and did not deal with any tissue infectivity levels within the ash.

Regarding other animal origin ashes, Maj et al., (2022) characterized cow manure and chicken litter ashes in terms of combustion-related problems and ash properties. It was found that, in comparison with the current EU law regulations, the concentrations of Hg, Cu, As, Ni, Cd and Pb in all samples were below the limits. However, concentrations of Cr in all samples and Zn in industrial chicken litter exceed the standards. It was observed that the ash content depended on the farming style, where free-range raw materials are characterized by higher ash contents than industrial farming ones. Moreover, the phosphorus concentration presented higher values in industrial chicken litter samples.

In a conference paper presented at the international conference on nutrient recovery at Vancouver in 2009, (Kabbe and Wolfgang, 2009) discuss a patented incineration process to yield a combustion product (and hence comply with the required sterilisation process) that has a readily plant available phosphate compound (mono-phosphate) that will have no organic and hence pathogenic contamination.

1.6.1. EU Research Projects

Currently there are 1611 EU projects that has deal with ash management/disposal within EU. Of these, 69 were develop or studied in the period 2018 to 2022, and from them the most relevant for this study are:

- **AshCycle** (Finland June 2022 – May 2026) is dealing with the recovery of raw materials from a range of ashes from the incineration of biomass, municipal solid waste and sewage

sludge. The objective is to produce raw material suitable for industrial use. Its is not clear if phosphorus is one of the end products.

- **eThrough** (Portugal 2018-2022) Thinking rough towards sustainability. This is a Marie Sklodowska-Curie project looking at sustainable mining, production, recovery from secondary resources and recycling of critical raw materials. One of the outputs from the project is to look at phosphorus recovery from sewage sludge ash under kinetic control.
- **SusPhos** (Netherlands 2022 – 2023) European innovation ecosystem

  The SusPhos solution upcycles P-rich waste like Sewage Sludge Ash (SSA) or struvite into phosphates, iron & magnesium salts, and silica mixtures. In this project high-quality, pathogen-free compounds are proposed to be suitable for formulating fertilisers and phosphate-based flame retardants (FR).
- **B-ferST** (Spain 2019-2024) Bio-based fertilising products as the best practice for agricultural management and training.

  In this project the team is proposing an integrated bio-waste valorisation system in agricultural management. The project is introducing a more sustainable resource management solution via tailor-made nutrient dosing adapted to farmer systems. Central to the effort to reverse soil nutrient loss is the reuse of bio-waste to replace non-renewable, non-domestic and energy-intensive raw materials. B-FERST aims at changing the market uptake of fertilisers in intensive agriculture as well as reducing the carbon footprint of the fertilisers' production by at least 10 %, thus minimising their environmental impact.

  Hence while not dealing with ash per se, the use of fertiliser products is of interest.
- **HTCycle** (Germany 2018-2020) Sewage sludge reuse with phosphate recovery and heavy metal absorption with an innovative HTC technology.

  Within this project, incineration is considered the safest disposal method of sewage sludge but is relatively expensive and presents challenging technical issues. The EU-funded HTCycle project will demonstrate a better alternative to incineration. It will commercialise proprietary technology for hydrothermal carbonisation that should increase the amount of sludge converted into high-value products such as fuel, activated carbons for water treatment, phosphorus and soil remediation materials.

  The project produced an user's guide (in German) for the process works and its outcomes - this could be useful link to the technology provider to see if they can treat MBM ash.

Conclusions

An important amount of pathogens can be found in animal by-products' raw materials. Therefore, applying treatment processes to reduce the risk that ABP could represent for human and animal health is critical.

In accordance with the current legislative instrument, incineration and co-incineration require operating temperatures of 850 [°C] for at least 2 seconds or 1,100 [°C] for 0,2 seconds. Bacteria, viruses, and prions are sensitive to heat. However, its thermostability varies between pathogens, even between the same microbiological family.

Twenty two studies were found related to ABP risk reduction using thermal/chemical treatment, from them thirteen reports presented relevant data regarding risk reduction of pathogens after the application of thermal processes. No one of them evaluated the precise and specific incineration and co-incineration conditions indicated by the legislation, yet two of them analyses ABP ash. Therefore, data and extrapolations from other processes were used as the legislation allows.

For category 2 and 3, Enterococcus Faecalis, Salmonella Senftenberg, and Parvovirus were identified as indicator microorganisms, as they are the most resilient. Nevertheless, when category 1 is included, prions are considered the most resistant biological hazards. Therefore, it is assumed that when these resilient pathogens are inactivated, any other less resilient hazards are also inactivated.

Prions

It was assumed that the reduction needed was at least 6 $\log_{10}$. The most thermal unstable prions study were by far for L-BSE that at 133 [°C], 3 [bar] in 20 [min] presented 9,4 $\log_{10}$ of reduction, while Nor98 and AS presented 10 $\log_{10}$, under similar conditions. They were followed by RML, CJD, and 263K prions which reached >6 $\log_{10}$ at 200 [°C], under 70-80 [bar], applied for 15-30 [min].

In the case of 263K, the data also shows that the heating exposure or residence time does affect the reduction level. For instance, lower reductions are reached (4 $\log_{10}$) when the temperature is increased at 300 [°C] for only 5 [min].

There were three variants that did not reached the 6 log_{10} reduction: 22L which registered 5 log_{10} at 98 [°C]. C-BSE that presented 5.78 log_{10} at (200 [°C], 3 [bar], for 20 [min]), and H-BSE that hit 3.94 log_{10} at the same conditions as C-BSE.

Regarding incineration conditions, the 263K prions was the only scrapie that presented data using temperatures above 600 °C and ash samples. They determined >8 log_{10} reduction at 612 [°C] for 15 [min] and total inactivation at 1,000 [°C] at for 5 and 15 [min]. Considering that 263K and BSE has comparable heat resistance, it is expected to find similar results for C-BSE and H-BSE prions, at the above conditions.

Even though, the reduction level was reached at 600 [°C] and total inactivation was found at 1,000 [°C], the residence time are longer in comparison to the operation condition indicated in the legislation. For instance, in the study total inactivation was reached at 1,000 [°C] for 5 [min], while the legislation indicate 0,2 [sec]. which is 1,500 times faster. Therefore, for the author of this study is not possible to certainly determined that incineration and co-incineration processes inactivate the most thermostable prions at the operation conditions indicated in the legislation.

Enterococcus Faecalis and Salmonella Senftenberg

For Enterococcus Faecalis, according to the calculation done by Koutsoumanis et al., (2021), for a period of 2 seconds a reduction of >5 log_{10} at 98 [°C], while in 0,2 seconds, this reduction can be achieved by thermal treatment of 110.5 [°C].

Lower temperatures were observed by Koutsoumanis et al., (2021) for Salmonella Senftenberg. A reduction of >5 log_{10} in a period of 2 seconds will be reached at 74 [°C], while in 0,2 seconds, this reduction will be achieved at 78.8 [°C].

In both cases, the temperatures required are around 10 times lower than the incineration and co-incineration operation conditions. Therefore, it is expected that both thermal treatments completely reduce the risk associated with these bacteria.

Parvovirus

The most intense conditions were used by Nims and Plavsic (2013). In their study, 4log_{10} reductions were reached in 0,5 min at different temperatures depending on the pathogen. Bovine Parvovirus (BPV) achieved the reduction needed at 101 [°C], followed by Canine Parvovirus

(CPV) at 112 [°C], and Minute Virus of Mice (MVM) at 117 [°C] when the matrix was water. It was found that MVM thermostability increases to 196 [°C] when it is in culture media. Therefore, considering that the temperature of the incineration conditions is 4,3 times higher, the risk of infectivity from ash would be extremely small.

As it was mentioned the use of animal biomass as a source of bioenergy is growing, considering it positive impacts to the climate and environmental emergency related to decreasing the use of fossil fuel. However, the increasing generation of biomass ashes, that is commonly managed through landfilling disposal, poses relevant economic and environmental drawbacks that requires the evaluation of new options. These alternatives should secure the safe and health of human, animals, and ecosystems.

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