VETBIONET

Veterinary Biocontained facility Network for excellence in animal infectiology research and experimentation

Deliverable D3.9

Position paper for policy makers on the biosafety of alkaline hydrolysis (biodigesters) as an alternative destruction technique to incineration based on the practical experience gained in the sector in Europe

Due date of deliverable: February 2021

Actual submission date: M56

Start date of the project: March 1st, 2017 **Duration:** 60 months

Organisation name of lead contractor: INRAE

Revision: V1

Dissemination level	
Public	√
Confidential, only for members of the consortium (including Commission Services)	
Classified, as referred to in Commission Decision 2001/844/EC	

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

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1. Summary

Objectives: Work package 3 (WP3, "Best practices for biosafety, biosecurity and quality management in high containment farmed animal facilities") centres on the elements and principles of the CWA 15793 workshop agreement drafted by the CEN (European Committee for Standardization) in September 2011 (CWA 15793:2011). The CWA 15793:2011 relates to "Laboratory biorisk management", and WP3 aims to inspect and highlight the specific requirements for the management of high containment farmed animal facilities (HCFAFs). One of the major challenges in HCFAFs is the large volume of post-mortem material being generated that must be disposed of in a safe and lawful manner.

The objective of WP3 Task 3.6 ("Best practice for the use of Tissue Digester") and the present deliverable report (D3.9) was to look at the biosafety and regulatory conformity of alkaline hydrolysis (biodigesters) as an alternative destruction technique to incineration. To replace the common incineration technique would help towards reducing CO_2 emissions of HCFAFs, so is desirable with the EU and national governments' plans to tackle global warming. To achieve this, the Task 3.6 participants (INRAE, WBVR, FLI, APHA, INIA, IRTA, EpiBioSafe) investigated the state of knowledge about biodigesters, the validation information available and the legal requirements for disposal of waste from HCFAFs and post-mortem (PM) rooms.

A workshop on the alkaline hydrolysis technique was organised with VetBioNet partners and two biodigester manufacturers to understand the theory and practical aspects of the process. Currently all biodigesters are produced outside the EU, and although alkaline hydrolysis as a method for biomaterial decontamination is broadly accepted, the Task identified that there are issues with the absence of validation data.

An investigation into EU regulations covering waste produced from PM rooms was undertaken. The current legislative position is based on an EU SSC Opinion on Alkaline digesters in 2002 that stated that the methodology presented at that time did not give sufficient log reduction in prions to allow it to be considered free of potential

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infectivity. Hence, the regulations required the waste material from biodigesters to be incinerated.

Currently the key legislation covering this is Regulation (EC) 1069/2009 which classifies the waste from PM rooms as Category 1 (high risk) which requires all material to be incinerated, whether it has been exposed to other sterilisation techniques beforehand or not. To remove this restriction, a solid scientific case must be presented by a competent authority to prove to the European Food Standards Authority (EFSA) that the material produced is safe (including prion inactivation). The VetBioNet work group identified two potential ways forward:

1) To validate the technology's ability to remove prions using a laboratory model. This would have to address the issues identified in the 2002 report. This was considered a large piece of work with a risk that the process itself might not be able to achieve the appropriate log reduction in prion infectivity, especially because VetBioNet partners have noticed that there is variability in the process. Therefore, any validation would have to demonstrate wide confidence intervals in the prion log reduction to cope with this variability.

2) To use a risk analysis methodology to demonstrate the absence of prions: EFSA's own Updated quantitative risk assessment (QRA) of the BSE risk posed by processed animal protein (PAP) uses their mathematical model for BSE that shows the risk is negligible. Therefore, it can be argued that prion reduction ability of the process is irrelevant as it is not there in the first place.

Option 2 was considered the most straightforward way for a competent authority should they wish to take this case forward as part of their CO_2 reduction commitment, but production of this information was considered outside the remit of the VetBioNet project.

2. Introduction

Alkaline hydrolysis has been used chemically for many years but has been marketed and developed for waste management over the past 20 years. The digestion system combines an alkaline solution (50% NaOH/KOH) and heat in a pressurized container to reduce animal, human and microbial tissue to a sterile aqueous solution. The total solids reduction is estimated at 97%. This sterile liquid (hydrolysate) includes an

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inorganic and metal concentrate. The residual solids are captured in a straining basket and are comprised of teeth and bone easily crushed into sterile bone meal (calcium phosphate powder).

An opinion was given by the EU Commission Services 2002 on the submission and accompanying dossier from a commercial company requesting endorsement of a process for the safe disposal of animal waste which may be contaminated by Transmissible spongiform encephalopathies (TSEs) (SSC Opinion on Alkaline digesters 2002). This process consists of a treatment of animal waste by means of high temperature (150°C, 3 hours) and corresponding high-pressure alkaline hydrolysis. The Scientific Steering Committee (SSC) was requested to address the following questions:

1. Can the treatment of animal waste, as described by the dossier, be considered safe in relation to TSE risk? Can the liquid residues be considered safe in relation to TSE risk?
2. Can the by-products resulting from this treatment (i.e. ash of the bones and teeth of vertebrates) be considered safe in relation to TSE risk?

Regarding the first question the SSC concluded that the liquid residue after a 3-hour digestion cycle could retain infective potential. Under controlled laboratory conditions in a single experiment the treatment of animal waste by means of high temperature (150°C, 3 hours) and high pressure alkaline hydrolysis has been shown to reduce the infectivity of TSE/BSE by a factor of $10^{3.5}$ – $10^{4.5}$. Due to constraints specific to this experiment, further studies on the combination of heat, pH and time in clearance studies are needed before any final assurance could be given regarding the safety of the process with respect to TSE risks. No infectivity was found after 6 hours. This may indicate that the clearance after 6-hours processing time is higher than after 3 hours. However, these experiments can only give a measure of the minimum clearance possible and do not permit the quantification of the clearance factor after 6 hours. Regarding the second question, the SSC concluded that, on the basis of the data available, by-products of the 3-hour process could carry a risk of BSE/TSE infectivity and that this risk may decrease with the duration of processing; further data would be needed in order to make a definitive statement.

It appears that no further data was forthcoming, so incineration remains a required step with material from alkaline tissue digester from HCFAFs.

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A VetBioNet workshop was held on 12th November 2018 with manufacturers PRI Digester Systems and BioSAFE Engineering, covering the theory and practicalities of the commercialized biodigester systems. VetBioNet partners attending the workshop included notably those using alkaline biodigesters (INRAE, FLI, WBVR, IRTA). The partners shared their practical experience of running these machines (Annex 1). It was stated that at least one partner found variability in the composition of the output, indicating variation in the process.

Subsequent work to the workshop (piloted by APHA) also gave clarity to the legal position of the biodigester issue. Initially biodigesters had been controlled by "Commission Regulation (EC) No. 92/2005, Annex I, Alkaline Hydrolysis Process", but this had been subsequently repealed and replaced with "Regulation (EC) 1069/2009". The investigation also revealed that to remove this requirement a national competent authority would have to present a scientific case to the European Food Safety Authority (EFSA) that prions or any other infectious agent used to infect animals in HCFAFs had been inactivated. EFSA would have to accept this case and make a recommendation to remove the incineration requirement to the EU.

3. Results

Although the VetBioNet workshop was informative, no additional information on the validation of this process against TSE agents was forthcoming, or any other category 1 (high risk) waste as defined in Regulation (EC) 1069/2009 (Annex 2) which can be produced by HCFAFs and/or the associated PM facilities.

An investigation was made into what would be required to remove this requirement. An application would have to be submitted to EFSA (Biological hazard applications: overview and procedure) by a competent national (MS) authority presenting a report with scientific evidence that alkaline digestion presented no infectious risk, particularly from TSEs.

This could be done in two ways:

1) To validate the technology's ability to remove prions using a laboratory model. This would have to address the issues identified in the 2002 report. This was considered a large piece of work with a risk that the process itself might not be able to achieve the appropriate log reduction in prion infectivity. Particularly

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because VetBioNet partners observed that there is variability in the process; therefore, any validation would have to demonstrate wide confidence intervals in the prion log reduction to cope with this variability.

2) To use a risk analysis methodology to demonstrate the absence of prions: EFSAs own Updated quantitative risk assessment (QRA) of the BSE risk posed by processed animal protein (PAP) uses their mathematical model for BSE that shows the risk is negligible. The updated model (2018) estimated a total BSE infectivity four times lower than that estimated in 2011, with less than one new case of BSE expected to arise each year. In the hypothetical scenario of a whole carcass of an infected cow entering the feed chain without any removal of specified risk material (SRM) or reduction of BSE infectivity via rendering, up to four new cases of BSE could be expected at the upper 95th percentile. Therefore, a reasonable argument could be put forward that unless experimentally infected with BSE or other TSE agents, there is a negligible risk of TSE agents being in the experimental animal in the first place; thus, the inability of alkaline biodigestion to inactivate prions would not be an issue to the decontamination of Category 1 waste. Still, this would have to be underpinned by data over inactivation of other conventional Category 1 pathogens.

4. Conclusions

The use of biodigesters would allow HCFAF's to reduce their carbon footprint, provided that the requirement for biodigester-derived waste incineration under Regulation (EC) 1069/2009 could be removed. The concept of alkaline hydrolysis as a robust method of waste decontamination is generally accepted –apart from prion inactivation, for which a case was presented to the EU in 2002 and ultimately rejected. To remove this requirement now a national competent authority would have to present a scientific case to the EFSA that risks posed by prions/TSEs and all other infectious agents used for experimental infection in HCFAF's have been eliminated by biodigester treatment. EFSA would have to accept this case and then make a recommendation to remove the incineration requirement to the EU.

WP3 Task 3.6 gathered that there is no suitable validation data available for a competent authority to present to EFSA and that there would be a significant risk of

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failure of demonstrating a consistent log reduction of prions with a suitably wide confidence interval to allow practical operation of biodigesters.

Without manufacturers' support and suitable data, it was deemed not feasible for the VetBioNet partners (those with biodigesters) to produce sufficient data for drafting a risk assessment that could be submitted by a competent national authority in the time frame of the project. This would require tremendous partner efforts (Joint Research Activities) that were not foreseen in the DoA.

However, Task 3.6 could identify a potential way forward through a risk assessment based on EFSA's own mathematical model showing that (as of 2018) there is a negligible risk of BSE being present in the cattle population. The updated model estimated a total BSE infectivity four times lower than that estimated in 2011, with less than one new case of BSE expected to arise each year. In the hypothetical scenario of a whole carcass of an infected cow entering the feed chain without any removal of specified risk material (SRM) or reduction of BSE infectivity via rendering, up to four new cases of BSE could be expected at the upper 95th percentile.

Therefore, a reasonable argument could be put forward that unless experimentally infected with BSE or other TSE agents, there is a negligible risk of TSE agents being in the experimental animal in the first place; thus, the inability of alkaline biodigestion to inactivate prions would not be an issue to the decontamination of Category 1 waste. Still, this would have to be underpinned by data over inactivation of other conventional Category 1 pathogens.

While the present D3.9 report cannot serve as an evidence-based position paper for policy makers to push towards a change in the current EU regulation, it details and raises awareness about the alkaline hydrolysis/biodigester issue for HCFAFs and points to a solution to overcome this issue through a risk assessment integrating a recently established mathematical model.

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5. Annex

Annex 1: Practical information on biodigester use by VetBioNet partners

User :	Wageningen Bioveterinary Research	IRTA-CReSA	PFIE-INRA	FLI
1. Which carcasses are treated in your digester?				
Q: Cattle? sheep? pig? chicken? mice?	A: Cattle, sheep, pigs, goats, chicken, mice	Mostly pig, but also sheep, goat, calves, and chicken. Rarely exotic species.	Cattle, sheep, pigs,	Cattle, sheep, goats, pig, poultry, lab rodents
Q: Infected animals?	A: YES	Yes, but from non-zoonotic pathogens	No (on hold)	YES
Q: Which pathogen (s)?	A: RVFV, HPAI, PEDV,	Non zoonotic ones as Porcine circovirus, PRRSV, Blue Tongue virus.	NA	up to RG 4 BUT NOT prions
Q: Carcasses waste coming from BSL2 or 3 environment ?	A: Carcasses from BSL2 and BSL3 experiments (veterinary and human BSL2 and BSL3)	Yes, only BSL3 environment	NA	YES; ABSL 2, 3, 4
2. Which digester ? supplier ? model ? starting date ? currently in use ?	Digester: Thermal Tissue Digester P&ID Supplier: PRI Model: 48 TTD-500M BS Starting date: 13 august 2012 Currently in use: yes	Progressive Recovery Inc (PRI); Capacity 1090 liters; serial number 2816;25.11.2010; In fully operation.	Digester: Thermal Tissue Digester Supplier: PRI Model: 60 TTD-P900M System P&ID Starting date: mai 2013 Currently in use: No	BIOSAFE Tissue Digesters; since 2014
3. Details on cycles				
Q: Weight of carcass ?	A: Load 500 kg	Above 200 kg and no more than 300 kgr.	Load: 900 kg	500 kg & 1000 kg
Q: Volume of NaOH/KOH ?	A: It is a calculated value depending of the weight.	20-25% of the organic load, regarding the type of species to be processed.	15-25% of the organic load, regarding the type of species to be processed.	A: KOH is a calculated value depending of the weight (20-25%).
Q: Volume of water ?	A: In a TTD, less than ½ the total weight of the load is additional water per cycle.	An amount of 125% of the organic load (carcasses weight).	40-50% of the organic load, regarding the type of species to be processed.	100-150%
Q: Duration of each cycle ?	A: 12 hours	At least 3 hours; no more than 5 hours. A complete cycle accounts for 4.5 to 7 hours, respectively.	8-10 hours	≥ 540 min
Q: Temperature and pressure during cycle ?	A: 150 °C	Temperature of 150ºC at 4 bars (3 bars overpressure above environment)	Temperature of 150ºC at 5 bars	150 °C
Q: At the end of each cycle, do you reduce ph to ph 7 ?	A: No. We add some vinegar for the smell.	No, It's kept at pH 13-14 and pumped to a truck of a homologated waste processing company.	No	No
Q: Do you sort liquid and solid at the end of the process ?	A: No. There is one residue. Thick and liquid	Yes, solid is transferred to the incinerator and liquid I accumulated and pumped to a scheduled truck form a homologated waste treatment company.	No. The residue is thick and liquid	Yes
4. What are you doing with digestate after process ?				
Q: Composting ? service provider to destroy digestate ?	A: The digestate is treated as animal waste category I. It is transported, processed and the residue is incinerated.	The solid digestate is submitted to incineration and autoclaving before exiting the facility. The liquid digestate is collected by and homologated waste treatment company.	The digestate is treated as animal waste category I. It is transported, processed and the residue is incinerated.	Fluid: waste water treatment plant; Solid: incinerated off site
5. Regulatory guidelines for use of digester				
Q: Which regulatory guidance are you following for use of digester (European / US/ international)?	A: Sterilisation and alkaline hydrolysis of the animal carcasses shall be performed in accordance with "Commission Regulation (EC) No. 92/2005, Annex I, Alkaline Hydrolysis Process" at 150°C for at least 3 hours		Sterilisation and alkaline hydrolysis of the animal carcasses shall be performed in accordance with "Commission Regulation (EC) No. 92/2005, Annex I, Alkaline Hydrolysis Process" at 150°C for at least 3 hours	Verordnung (EG) Nr. 1069/2009; Verordnung (EU) Nr. 142/2011

Annex 2: Animal by-products

Definition of "animal by-products"

Animal by-products are defined in Article 3 of Regulation (EC) 1069/2009 as "entire bodies or parts of animals, products of animal origin or other products obtained from animals that are not intended for human consumption". This includes catering waste, used cooking oil, former foodstuffs, butcher and slaughterhouse waste, blood, feathers, wool, hides and skins, fallen stock, pet animals, zoo and circus animals, hunt trophies, manure, ova, embryos and semen not intended for breeding purposes.

Categories of "animal by-products"

Under Regulation (EC) 1069/2009 animal by-products can fall into one of three categories. The issues treated in this report relate to Category 1 Material.

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Category 1 material

Category 1 material is defined in Article 8. Category 1 material presents the highest risk, and consists principally of material that is considered a TSE risk, such as Specified Risk Material (those parts of an animal considered most likely to contain infectious agents, e.g. BSE prions in bovine spinal cord). Pet animals, zoo and circus animals and experimental animals are also classified as Category 1 material due to the level of veterinary drugs and residues they are likely to contain and due to the fact that adequate diagnoses of the exact cause of death of exotic animals can be difficult to achieve. Several are known to harbour TSEs and may carry other (exotic) diseases. Likewise, wild animals may be classified as Category 1 material when they are suspected of carrying a disease communicable to humans or animals. Catering waste from means of international transport (coming from outside the EU) is also Category 1 material.

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