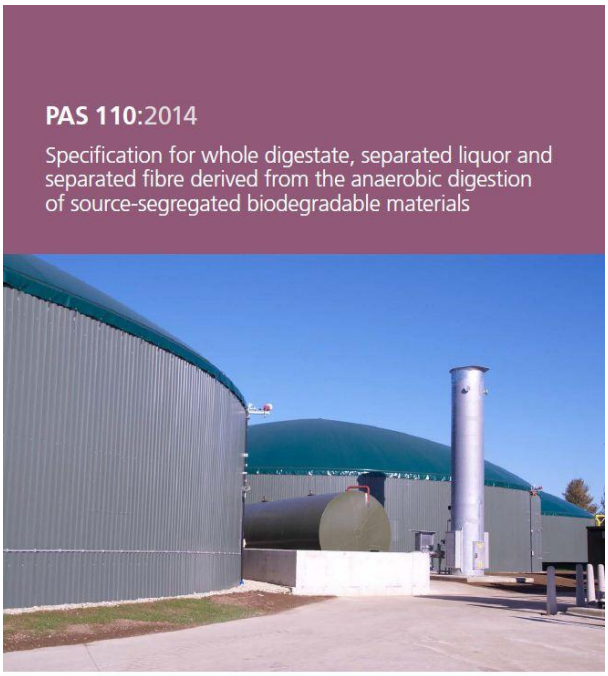
Supplementary report

Impacts of pasteurisation and mesophilic AD on some common crop pests and diseases in the UK



The susceptibilities of a number of common agricultural pests and diseases (including blackgrass, clubroot and late blight) to pasteurisation and/or mesophilic anaerobic digestion were investigated through literature review and laboratory experiments.

The results of the literature review are reported here, alongside discussion on possible approaches to demonstrating pasteurisation equivalence.

This report is of particular relevance to those seeking to understand the quality and safety of digestates from farm-fed AD facilities.

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Executive summary

AD operators – whether on or off-farm – must be aware of the range of phytohygiene hazards that could be associated with common AD feedstocks, and understand the impacts of AD with or without pasteurisation on these hazards to ensure that all digestate is used safely, with minimal phytohygiene risk.

Pasteurisation at 70°C for one hour has been shown to eliminate numerous plant pathogens of interest, and for this reason is included as a near-universal requirement in the BSI PAS 110 specification ('PAS110') for digestate. However, managing phytohygienic risk does not automatically require pasteurisation. For example, where inputs arise from, are digested on and returned to the same holding – plant pathogen risks will not increase as a result of the AD process. Even where feedstocks are imported for processing, any plant pathogens that are not eliminated by an AD process that has no pasteurisation stage will only present a subsequent risk to plant health if digestates are applied to land where susceptible crops are grown.

To inform operators' consideration of phytohygiene hazards in digestates this report examines the impact of pasteurisation on a range of common crop pests and diseases, and explores possible alternatives to the standard pasteurisation approach. This report also examines the approaches to pasteurisation taken in other sectors (particularly food processing) and considers how other European nations manage phytohygienic aspects of digestate. In addition to waste-fed AD facilities, this report is of relevance to farm-fed AD facilities accepting crop residues and other plant material for processing. The principles outlined may also be relevant to AD facilities considering alternative transformation approaches in compliance with the Animal By-Products Regulations.

In recommending alternatives to pasteurisation it is important to establish equivalence in terms of ability to reduce pathogen risk to an acceptable level. Since all digesters differ in operating parameters (for example: temperature, retention time, vessel design) and, crucially – feedstock – we are unable to recommend a pre-defined set of digestion parameters to obtain equivalence to pasteurisation, since these may not be equally effective across all digester types.

Alternatives phytohygiene control approaches include:

- Exemption of certain feedstocks where risk assessment indicates this is warranted or where the feedstocks have already undergone a similar procedure to the pasteurisation step;
- Use of alternative time/temperature regimes;
- Restriction of particularly high risk feedstocks (such as quarantine plant wastes) to defined 'special measures' or licensed facilities;
- Use of indicator organisms for process-specific validation.

Whilst other authors have proposed a number of pre-defined pasteurisation parameters, these are not proven for the wide range of potential crop pests and diseases that could be associated with some feedstocks (albeit at low levels), and they also require adherence to minimum hydraulic retention times within the digestion phase. Instead, we propose a validation process by which operators can demonstrate that their process, operated by their personnel, is sufficiently effective at reducing plant pathogen risk. This validation approach has the advantage of being in harmony with the approach taken in implementing Animal By-Products Regulations' requirements for demonstrating equivalence in pasteurisation (or

'transformation') processes that are intended to reduce animal pathogens to acceptable levels.

For validation we propose that any suggested alternative to the standard 70°C for one hour pasteurisation approach be tested for its ability to achieve a defined level of kill of indicator organisms introduced into the pasteurisation system. To aid analysis we propose that these organisms are introduced in sealed containers for ease of recovery and subsequent analysis, and because inoculating an entire reactor with a detectable level of indicator organism would not be practicable. Having considered various options, including those required by the German Biowaste Ordinance, we suggest that relatively persistent organisms for which viability assays can be conducted are chosen as indicators, and for this reason propose *Plasmodiophora brassicae* and tomato seed.

Contents

1.0 Introduction

Pasteurisation is a near-universal requirement in the voluntary digestate specification BSI PAS 110:2014, whether certified processes accept Animal By-Product (ABP) inputs – for which there are statutory pasteurisation (or 'transformation') requirements – or plant-based inputs, for which there are no statutory pasteurisation requirements (BSI, 2014).

Pasteurisation is designed to minimise the risks from microbiological hazards, and once validated with test organisms to demonstrate the required reductive effect on suitably robust test organisms, then it is not necessary to actually demonstrate the extent of pathogen kill during the subsequent operation of the process.

The degree of hygienic control afforded by pasteurisation is seen as favourable by stakeholders with an interest in (and an influence upon) the digestate market, for example food retailers. However, the capital and operational costs of pasteurisation can be significant, and this report outlines a number of options to pasteurisation that offer the potential to maintain digestate quality and safety whilst reducing costs.

2.0 First principles: Common AD feedstocks and potentially associated phytohygienic hazards

2.1 Common AD feedstocks

Only feedstocks not containing material that falls under the definition of animal by-products (ABP) are considered here. Anaerobic digestion of ABP material requires a pasteurisation step and subsequent testing for indicator bacteria under statute. There are currently no equivalent statutory controls over the use of non–ABP inputs (unless they are known to contain pathogens with designated quarantine status).

Non-ABP feedstocks for AD could be derived from a range of sources, including source-segregated household biowastes, source-segregated biowastes from commercial and industrial businesses, together with biowastes and 'non-wastes' from agricultural and associated activities (these could include vegetable offcuts from packing plants). Table 2-1 gives a summary of the range of non-ABP materials which may be present in AD feedstocks in the UK. Livestock manures and slurries are not included here, since they are considered an animal by-product[1].

Table 2-1 Plant waste materials typically used as feedstocks for AD

Source	Type of waste plant material
Primary food production and fresh retail waste	Fresh strawberries, raspberries, lettuce, tomatoes, apples, onions, potatoes, peas, beans, broccoli, avocado, citrus fruits, bananas etc
Food processing waste, food washing waste	Coffee, fruit, vegetables, tobacco, tea. Process water and food washing waste
Wastes from washing, cleaning and mechanical reduction of raw materials	Spent grains, fruit and potato pulp Materials unsuitable for consumption or processing Brewing waste – Malt husks, malt sprouts, malt dust
Wastes from forestry	Tree leaves, wood chips etc

[1] *It should be noted that, if the only ABPs that are used in anaerobic digestion are manure, digestive tract contents, milk, milk based products and colostrum, then no pasteurisation or APHA approval is normally required (APHA, 2014)*

Source	Type of waste plant material
Garden waste	Hedge and tree trimmings, grass cuttings and leafy materials
Animal husbandry wastes- farms, stables etc	Old Straw Husks, cereal dust, waste animal feeds Maize and grass silage, fodder beet

In addition to the materials described above, there is increasing interest in the use of crops grown specifically for anaerobic digestion ('purpose-grown crops' or PGCs). Grass, forage maize and forage wheat are often cited as potential PGCs (ADAS/AEA, 2011), and the fate of common pathogens associated with such materials during anaerobic digestion should also be considered – to ensure that appropriate phytohygiene controls are in place.

2.2 Phytohygienic hazards potentially associated with common AD feedstocks, and their potential survival during AD

Lists of pathogens that could *potentially* be found in the range of feedstocks described in the Section 2.1 are very large. A list has been collated from current EU and EPPO lists for quarantine pathogens and reviews of UK pathogens (e.g. Noble *et al.* 2011), encompassing pathogens and pests likely to be of interest to the UK in terms of the economic, trade, agricultural and environmental damage that could be caused if a severe infection of an indigenous pest or a new outbreak of a quarantine pest were to occur (Table 2-2).

2.2.1 Synthesizing hazards: Is a pasteurisation step needed?

There are a number of organisms listed in Table 2-2 that are likely to be able to survive AD processes that do not include a pasteurisation stage, and may also be able to subsequently initiate a successful infection. The main categories of such organisms are those with hardy resting spores, e.g. *Spongospora,* clubroot, *Polymyxa betae, Synchytrium endobioticum, Tilletia indica, Verticillium dahliae* and sspp., and some of the more temperature resistant viruses, e.g. *TMV, Pepino mosaic potexvirus,* etc. Although some of these organisms are not indigenous to the UK and are therefore unlikely to be found in feedstocks, others are indigenous. For these organisms it is important not to spread these further or increase the background levels of inocula present in agricultural fields.

In the case of fill-and-draw AD systems, there is a possibility that these hazards could by-pass the MAD or TAD stage, and this would increase the number of potential phytohygienic hazards to include some of the bacteria, nematodes and less resilient fungi.

The experimental studies (presented in detail in the accompanying OMK007-002 report, with key summary data reproduced in Table 2-2 below) have confirmed that pasteurisation for 1 hour at 70°C eliminates the majority of the organisms of interest. Therefore the 'unavoidable control barrier' that a discrete pasteurisation step provides would be desirable in these circumstances. A possible exception to this would be where systems are fed from and provide digestate back to the same local holding.

Table 2-2 Evidence for survival of organisms through AD processes (without pasteurisation, unless stated). Experimental evidence is presented in the accompanying report (OMK002-007), whilst the expert opinions are those of the authors. MAD operating temperature = 37°C; TAD operating temperature = 57°C

Group	Pathogen	Disease	Host	From scientific literature –main points only	Experimental evidence	Expert view
Bacteria	*Xanthomonas axonopodis* pv. *citri*	Citrus canker	Citrus			Very low risk
	Ralstonia (Pseudomonas) solanacearum races 3 biovar 2	Brown rot	Potato	*R. solanacearum* was shown to survive MAD for more than 20 days although Ryckeboer *et al.* (2002) demonstrated that it could be destroyed to below detectable limits within one day during TAD of source separated household wastes at 52°C	Survives up to 8 days in MAD Eliminated by 1 hour at 70°C	Low risk
	Clavibacter michiganensis ssp. *sepedonicus*	Ring rot	Potato	Kaemmerer (2009) found thermosensitivity of *Cms* in AD rose considerably at temperatures above 35°C with a $\log_{10}$ decrease in population over 10 minutes for every 8°C increase in temperature between 35-55°C. Seigner *et al.*, (2010) showed that *Cms* in homogenized naturally infected potato tubers remained viable after 6	Survives up to 8 days in MAD Eliminated by 1 hour at 70°C	Low risk

Group	Pathogen	Disease	Host	From scientific literature –main points only	Experimental evidence	Expert view
				hours retention during experimental AD at 37°C with cattle slurry.		
	Erwinia amylovora	Fireblight	Apple			Low risk
	Acidovorax avenae var. *cittuli*	Bacterial fruit blotch	Melon			Low risk
	Xanthomonas campestris pv. campestris (strains overcoming napus resistance)	Black rot of crucifers	Oilseed rape			Low risk
	Erwinia carotovora ssp. *atroseptica / carotovora*	Potato blackleg disease	Potato, Tomato			Medium risk
	Streptomyces scabies				Survives 12 days in MAD and 1 hour at 70°C but this is based on DNA PCR	
Mycoplasmas	*Flavescence dorée /* vector *Scaphoideus titanus*	Leafhopper	Grapevine			Low to no risk
Fungi	*Claviceps purpurea* + ergot toxin, ergotpeptine	Ergot	Rice and other small grain cereals			Low risk
	Chrysomyxa arctostaphyli	Spruce broom rust	Spruce			No risk
	Leptosphaeria maculans (new strains)	Black leg of crucifers	Oilseed rape			Low risk

Group	Pathogen	Disease	Host	From scientific literature –main points only	Experimental evidence	Expert view
	Fusarium oxysporum f.sp. *pisi*	Fusarium wilt	Pea			Low-medium risk
	Fusarium culmorum				Survives 1 day in MAD and 1 hour in TAD	
	Fusarium oxysporum				Survives 1 day in MAD and 1 hour in TAD	
	Fusarium radicis lycopersicae				Survives 1 day in MAD and 1 hour in TAD	
	Helminthosporium oryzae	Rice brown-spot	Rice			No risk
	Magnaporthe grisea / Pyricularia oryzae	Rice blast	Rice			No risk
	Ustilago maydis	Corn smut	Maize			Low risk
	Xanthomonas axonopodis f.sp. *allii*	Seed-borne bacterial disease	Onion			Low risk
	Phakopsora pachyrhizi	Asian soybean rust	Soybean			No risk
	Penicillium patulum + patulin toxin	Blue mould rot	Apple			Low risk (mycotoxins may survive)
	Aspergillus flavus + aflatoxin, thremorgenic toxin	Aspergillus ear rot	Maize, soybean, peanut			Low risk (mycotoxins may survive)
	Pleospora papaveracea	Root disease	Poppy			Low risk
	Gibberella zeae (Fusarium	Corn stalk rot, Head scab of	Maize, wheat	Seigner et al (2010) found elimination of the		Low-medium risk

Group	Pathogen	Disease	Host	From scientific literature –main points only	Experimental evidence	Expert view
	graminearum) + deoxynivalenol and zearalenone toxins	wheat		fungus after 2 days at 38°C		(mycotoxins may survive)
	Aspergillus ochraceus + ochratoxine A		Maize, wheat, barley			Low risk (mycotoxins may survive)
	Penicillium verrucosum + ochratoxine A		Maize, wheat, barley			Low risk (mycotoxins may survive)
	Fusarium sp. + trichothécènes, etc.	Cereal foot rot	Wheat			Low risk
	Fusarium oxysporum f.sp. *albedinis*	Bayoudh	Date			No risk
	Tilletia indica	Karnal bunt	Wheat			High risk
	Tilletia tritici	Complete bunt	Wheat			Medium risk
	Tilletia laevis	Common bunt	Wheat			Low risk
	Tilletia contoversa	Dwarf bunt	Wheat			Low-medium risk
	Ustilago nuda	Barley loose smut	Wheat, barley			Low-medium risk
	Fusarium moniliforme / verticillioides + fumonisin toxin	Fusarium ear and stalk rot	Maize			Low-medium risk
Agaricomycetes	*Rhizoctonia solani*			Seigner *et al.*, (2010) reported survival at 0.3 days at 38°C	Survives up to 20 days in MAD with or without pre-pasteurisation	

Group	Pathogen	Disease	Host	From scientific literature –main points only	Experimental evidence	Expert view
					–on the basis of RNA PCR test	
Oomycetes	*Phytophthora infestans*	Potato late blight	Potato		Survives 1 day in MAD and 1 hour in TAD	
	Phytophthora ramorum	Sudden oak death	Oak			Low risk
	Phytophthora kernoviae		Beech, rhododendron, bilberry, etc			Low risk
	Phytophthora lateralis		Cypress, etc			Low risk
	Verticillium dahliae and sspp.		Potato,OSR	Seigner *et al.*, (2010) reported survival up to 28 days at 38°C and 8 days at 55°C		Medium risk
	Phytophthora pseudosyringae		Bilberry, various tree spp., etc			Low risk
	Aphanomyces euteiches		Pea			Low-medium risk
	Phytophthora cinnamomi				Survives 1 day in MAD and 1 hour in TAD	
	Phytophthora nicotianae				Survives 1 day in MAD and 1 hour in TAD	
Chytridiomycetes	*Synchytrium endobioticum*	Potato wart	Potato	Seigner *et al* (2010) reported very long survival times in the batch		Low-medium risk

Group	Pathogen	Disease	Host	From scientific literature –main points only	Experimental evidence	Expert view
				process: even after 137 days' incubation (the end of the trial) in fermentation substrate at 38°C, 99% of the resting sori were intact		
Phytomyxeae	*Spongospora subterranea*				Survives 3 days in MAD with pre-pasteurisation; 12 days in MAD without pre-pasteurisation	
Plasmodiophoro-mycetes	*Plasmodiophora brassicae*			In four studies on AD, eradication, based on the results of bioassay test plants, was achieved after 21 days at 40°C or after 10 hours at 52°C, whereas the results of Engeli *et al* (1993) indicate that the pathogen can survive in AD for 14 days at 55°C	Survives 1 day in MAD and 1 hour in TAD	
Viruses	Lettuce big vein virus		Lettuce			Low risk
	Plum pox potyvirus	Sharka	Plum, peach			Low risk
	Tomato yellow leaf curl virus (TYLCV) / *Bemisia*		Tomato			Low risk

Group	Pathogen	Disease	Host	From scientific literature –main points only	Experimental evidence	Expert view
	tabaci					
	Pepino mosaic potexvirus		Tomato			Medium-high risk
	Tomato spotted wilt virus (TSWV) / *Frankliniella occidentalis*		Tomato, tobacco			Low risk
	Tomato Mosaic Virus		Tomato	*TMV* was inactivated after a 74-day exposure in AD at 36°C, although it survived a similar treatment or 28-day exposure in thermophilic conditions (around 55°C) in two different studies		Low-Medium risk
	Potato Virus X		Potato			Low risk
	Potato Virus Y		Potato	*Potato Virus Y* in infected potatoes did not survive a MAD process for 6 hours		
	Potato mop top virus		Potato			Low risk
	Tomato bushy stunt		Tomato, pepper			Low risk
Viroids	Potato spindle tuber viroid		Potato			Low-medium risk
	Tomato chlorotic dwarf viroid					Low-medium risk
Nematodes	*Globodera rostochiensis* and *G. pallida*		Potato	Viable PCN cysts survived anaerobic digestion in sewage sludge although		Low-medium risk

Group	Pathogen	Disease	Host	From scientific literature –main points only	Experimental evidence	Expert view
				populations were reduced (Turner *et al.*, 1983). Similar studies by Catroux *et al.* (1983) showed that nearly 100% of the cysts of *G. rostochiensis* and *G. pallida* were killed during anaerobic digestion of sewage		
	Heterodera schachtii		Maize, beet, etc			Low-Medium risk
	Meloidogyne spp.		Potato, etc			Low-medium risk
	Pratylenchus spp.		Potato			Low-medium risk
Seeds	Tomato seed (*Lycopersicum esculentum*) Ailsa Craig			Ryckeboer et al. 2002 reported survival of seed for 1 day at 52°C. Engeli et al. 1993 reported survival for 14 days at 55°C and 21.5 days at 35°C	Not detected after 3-6 days in MAD Not detected after 1 day in TAD Eliminated by 1 hour at 70°C	
	Black grass seed (*Alopecurus myosuroides*)				Not detected after 3-6 days in MAD Not detected after 1 day in TAD Eliminated by	

Group	Pathogen	Disease	Host	From scientific literature –main points only	Experimental evidence	Expert view
					1 hour at 70°C	
Human pathogens/ indicators	*Escherichia coli*				1.8 log decrease at 30 days in MAD Eliminated by 1 hour at 70°C	
	Salmonella				3.5 log decrease at 30 days in MAD Eliminated by 1 hour at 70°C	
	Clostridium botulinum			No proliferation of human pathogenic Clostridia occurs under mesophilic or thermophilic conditions assessed in lab scale reactors (Dohrmann *et al.* 2011)		
	C. perfringens			Their survival during the AD process has been extensively studied and can be described as high in mesophilic AD systems (Puchajda & Oleszkiewicz 2003; Lepeuple *et al.* 2004; Watcharasukarn *et al.* 2009). Survival in TAD is less well investigated.		

3.0 Alternative approaches for phytohygiene management in digestate

3.1 Effects of various time-temperature regimes on pathogen eradication

There have been numerous studies examining the fate of animal pathogens following exposure in organic wastes to different time-temperature regimes. These generally show a linear relationship between the logarithm of the exposure time required to inactivate the pathogen and the temperature of the waste, i.e. as the temperature decreases, the time needed to achieve inactivation increases logarithmically. For example, Aitken et al. (2006) showed that the time needed for a 10-fold (or 1-$\log_{10}$) reduction in the *E. coli* count in AD increased by 15 times when the temperature was reduced from 55 to 50°C. Pandey & Soupir (2011) showed a similar increase in decimal reduction time for *E. coli* when the temperature in AD was reduced from 52.5 to 37°C. Smith et al. (2004) also examined the decay kinetics of several isolates of *E. coli* and *Salmonella* spp. in nutrient broth and centrifuged sewage sludge. At 70°C, all species and serotypes were inactivated within 10 seconds of exposure. At 55°C, inactivation occurred within 20-60 minutes of exposure, with a 1 $\log_{10}$ reduction in survivors every 3-8 minutes depending on the thermo-tolerance of the specific bacteria and the inoculation media. At 35°C, a 1.5 – 2 $\log_{10}$ reduction in survivors occurred during a 20 day test period, but none of the test organisms were inactivated.

Feacham et al. (1983) showed that the slope of the relationship between inactivation time and temperature differed for different pathogens in sewage sludge, with a steeper slope for enteric viruses than for *Salmonella* spp. and eggs of *Ascaris* spp. Burge (1983) also showed that the effect of a 5°C decrease in the temperature of sewage on the additional time required to achieve a 1 $\log_{10}$ reduction in enteric pathogens differed between pathogens. Reducing the temperature of the sludge from 60°C to 55°C increased the required time for achieving a 1 $\log_{10}$ reduction in Adenovirus by 65, whereas the same temperature reduction increased the required time for *Histolytica* cysts by only 1.8. Murphy et al (2002) also showed that the effect of reducing the temperature of pathogen-inoculated meat products from 70°C to 55°C on the time needed to achieve a decimal reduction of *Salmonella* spp. was different to that for *Listeria innocua*. For *Salmonella* spp., the time increased from 0.25 to 27 minutes, but for *L. innocua*, the time increased from 0.18 to 192 minutes.

Schnürer & Schnürer (2006) also showed that the relative effectiveness of a 1-h treatment at 70°C or thermophilic anaerobic digestion at 55°C with a hydraulic retention time of 19 days in inactivating fungi in organic waste differed between species. For *Paecilomyces* species and *Trichocladium minimum*, the 1-h high temperature treatment was more effective, but for *Aspergillus* and *Cladosporium* species, the thermophilic AD treatment was more effective.

Carrington et al. (1982) demonstrated a more complex interaction between the effects of temperature and retention time in anaerobic digestion on *Salmonella* Dusseldorf populations. At 35°C, there was a constant logarithmic decay rate in the population of viable *S.* Dusseldorf with retention time, i.e. there was a 1-log reduction in the viable population every 40 minutes. However, at 48°C, the decay rate decreased with time, so there was a 3-log reduction in the viable population in the first two hours, but less than a 1-log reduction in the subsequent 6 hours. Whitmore & Robertson (1995) also found that the rate of decay in viable oocysts of *Cryptosporidium parvum* in sewage sludge was inconsistent with different time-temperature regimes. At temperatures between 4°C and 20°C, there was a near constant decline in the percentage viability of oocysts, whereas at 37°C, the rate of decline decreased with increasing retention time.

Jones (1976) found that rate of decline of *Salmonella* spp. in cattle slurry was influenced by the size of the viable population introduced. This could influence the relationship between temperature and time to extinction, with small populations of pathogens being eradicated in shorter time periods.

There are few plant pathogens for which there are comprehensive temperature–time matrices for inactivation, enabling the equivalence of different treatments to be compared under the same conditions (Noble & Roberts, 2004; Noble et al. 2009). Data obtained in different experiments, particularly in organic wastes (which are inherently variable), may be influenced by other factors than the time and temperature of exposure. Unless very frequent sampling is conducted in experiments, shorter times than those reported for achieving inactivation of pathogens may have been adequate. This makes determination of equivalent time-temperature inactivation treatments less precise.

3.1.1 Difficulties with transposing time-temperature regimes for pasteurisation equivalence in AD from other treatment systems

While there is a significant volume of data on the effects on pathogens of exposure to heat in compost, soil and abiotic systems, there are few studies examining pathogen destruction following exposure to equivalent time-temperature regimes in different biological or abiotic systems. This means that inferences on the fate of pathogens under one system are not necessarily accurate for the fate of those same pathogens under different systems.

Heat appears to be the most important factor for the elimination of plant pathogens during composting (Bollen & Volker, 1996; Noble et al., 2009). Moisture content, as well as other chemical and microbial factors may also influence pathogen destruction. Toxic volatile organic compounds such as hydrogen sulphide and organic acids in AD may also have significant effects on pathogen destruction (Termorshuizen et al., 2003).

Seigner et al. (2010) showed that plant pathogen kill was improved in anaerobic digestate compared with the same time-temperature treatments in water. Inactivation of *Plasmodiophora brassicae* in AD at 52°C has been achieved within 1 day (Ryckeboer et al., 2002) and within 21 days at 40°C (Termorshuizen et al., 2003). In compost, the same organism has been found to be viable after 7 days at 65°C (Ylimaki et al., 1983; Noble et al., 2011).

Rhizoctonia solani and *Verticillium dahliae* were inactivated in less than 8 hours at 40-50°C in water and moist systems (Miller & Stoddard, 1956; Pullman et al., 1981; van Loenen *et al.,* 2003), whereas reports for the same organisms in compost give eradication times of more than 7 days at equivalent compost temperatures (Yuen & Raabe, 1984; Bollen *et al.*, 1989; Christensen *et al.,* 2001; Noble et al., 2004).

Various data on the time required at different temperatures for inactivation of a range of plant pathogens in anaerobic digestion, composting and incubation studies are presented in Table 3-1. As expected, this shows viral plant pathogens to have the most tolerance to heat, whereas bacterial plant pathogens are generally more sensitive to heat than fungi. As with animal pathogens, there is a logarithmic relationship between the time to inactivation and the exposure temperature for plant pathogens. However, the effect of temperature on the inactivation time differs between plant pathogens and between different systems. For *Phytophthora nicotianae* and *Verticillium albo-atrum* (incubator studies) the inactivation time at 55°C was less than 1% of that at 40°C. For *Verticillium albo-atrum, V. dahliae* (anaerobic digester), *Fusarium oxysporum* (composting) and *Thielaviopsis basicola* (incubator), the inactivation time at 55°C was more than 7% of that at 40°C.

Table 3-1 Time-temperature treatments that resulted in plant pathogen inactivation in the same experiments

SYSTEM/ Pathogen type	Inoculum	Feedstocks	Detection method	Hours to extinction at (°C):											Reference
				35	40	45	50	55	60	65	70	75	80	85	
ANAEROBIC DIGESTION/ Fungi															
Verticillium albo-atrum	mycelium	silage	plating PCR	-	672	-	-	192	-	-	-	-	-	-	Seigner et al. 2010
Verticillium dahliae	hops	silage	plating PCR	-	672	-	-	192	-	-	-	-	-	-	Seigner et al. 2010
COMPOST/ Fungi															
Fusarium oxysporum				-	-	-	-	-	-	-	-	-	-	-	
f.sp. *cepae*	chlamydo-spores	green wastes	plating	-	-	168	24	-	-	-	-	-	-	-	Noble et al. 2011
f.sp. *radicis-lycopersici*	chlamydo-spores	green wastes	plating	-	336	168	96	24	-	-	-	-	-	-	Noble et al. 2011
Rhizoctonia solani	mycelium	green waste	plating	-	168	-	24	-	-	-	-	-	-	-	Noble et al. 2004
Sclerotium cepivorum	sclerotia	onion waste	plating	-	-	168	72	-	-	-	-	-	-	-	Coventry et al. 2002
COMPOST/ Oomycetes															
Phytophthora cinnamomi	mycelium	green wastes	plating	-	72	24	-	-	-	-	-	-	-	-	Noble et al. 2011
Phytophthora ramorum	mycelium	green wastes	plating	240	24	-	-	-	-	-	-	-	-	-	Noble et al. 2011
COMPOST/ Plasmodiophoromycetes															
Plasmodiophora brassicae	galls	green wastes	bioassay	-	-	-	168	-	24	-	-	-	-	-	Fayolle et al. 2006
COMPOST/ Viruses															
Tobacco Mosaic Virus	tobacco leaves	tobacco	bioassay	-	-	-	-	-	192	-	120	-	-	-	Ryckeboer et al. 2002

SYSTEM/ Pathogen type	Inoculum	Feedstocks	Detection method	Hours to extinction at (°C):											Reference
				35	40	45	50	55	60	65	70	75	80	85	
INCUBATOR or WATER BATH/ Bacteria															
Pectobacterium carotovorum	suspension	pepper wastes	plating	-	-	-	15	-	1	-	0.5	-	-	-	Elorrieta et al. 2003
Pectobacterium carotovorum pv. *atroseptica*	suspension		plating	-	1.33	-	0.3	-	-	-	-	-	-	-	Robinson & Foster 1987
Pseudomonas syringae pv. *syringae*	melon seedlings	pepper wastes	plating	-	-	-	15	-	1	-	0.3	-	-	-	Elorrieta et al. 2003
Xanthomonas campestris pv. *vesicatoria*	melon seedlings	pepper wastes	plating	-	-	-	15	-	1	-	0.3	-	-	-	Elorrieta et al. 2003
Ralstonia solanacearum		plant residues	bioassay	-	120	12	-	-	-	-	-	-	-	-	Date et al. 1993
Ralstonia solanacearum		water	plating	-	-	2	-	0.1	-	-	-	-	-	-	Termorshuizen et al. 2003
Botrytis cinerea		geranium leaves	plating	-	504	-	168	-	-	-	-	-	-	-	Hoitink et al. 1976
Fusarium oxysporum f.sp. *melonis*		Veg. residues	plating	-	-	288	-	80	-	1	-	-	-	-	Suarez-Estrella et al. 2003
Macrophomina phaseolina	mycelium	cellophane	plating	-	-	-	2	0.2	0	-	-	-	-	-	Bega & Smith 1962
Macrophomina phaseolina	mycelium	PDA	plating	-	-	-	48	24	-	-	-	-	-	-	Mihail & Alcorn 1984
Rhizoctonia solani	micro-sclerotia		plating	-	-	48	10	8	0.5	-	-	-	-	-	Grushevoi et al. 1940
Rhizoctonia solani		barley seed	plating	-	1176	-	168	-	-	-	-	-	-	-	Hoitink et al. 1976
Sclerotinia sclerotiorum	sclerotia		plating	-	-	36	-	1	-	-	-	-	-	-	Grushevoi et al. 1940

SYSTEM/ Pathogen type	Inoculum	Feedstocks	Detection method	Hours to extinction at (°C):											Reference
				35	40	45	50	55	60	65	70	75	80	85	
Sclerotinia sclerotiorum	sclerotia		plating	-	-	-	936	-	-	-	96	-	-	-	Hermann et al. 1994
Sclerotium cepivorum	sclerotia		plating	192	192	12	6	-	-	-	-	-	-	-	McLean et al. 2001
Sclerotium cepivorum	sclerotia		plating	-	-	41	19	-	-	-	-	-	-	-	Adams 1987
Sclerotium rolfsii	mycelium	PDA	plating	-	-	-	6	3	-	-	-	-	-	-	Mihail & Alcorn 1984
Thielaviopsis basicola	chlamydo-spores		plating	-	115	48	-	24	6	5	4	1.5	-	-	Grushevoi et al. 1940
Thielaviopsis basicola	mycelium		plating	-	-	15	0	-	-	-	-	-	-	-	Pullman et al. 1981
Verticillium albo-atrum	hop vine		plating	-	168	12	3	1	0.3	-	-	-	-	-	Talboys 1961
Verticillium albo-atrum	mycelium		plating	-	-	-	-	0.1	0.1	0.02	-	-	-	-	Miller & Stoddard 1956
Verticillium dahliae	mycelium		plating	-	-	8	2	-	-	-	-	-	-	-	Pullman et al. 1981
INCUBATOR or WATER BATH/ Oomycetes															
Phytophthora cinnamomi	mycelium	agar	plating	-	-	1	0.3	-	-	-	-	-	-	-	Gallo et al. 2007
Phytophthora nicotianae	mycelium	agar	plating	-	100	3.3	1.3	0.1	0.1	-	-	-	-	-	McGovern et al. 2000
Pythium ultimum	mycelium		plating	-	-	9	0.6	-	-	-	-	-	-	-	Pullman et al. 1981
INCUBATOR or WATER BATH/ Viruses															
Cucumber green mottle	residues		bioassay	-	-	-	840	-	-	-	72	-	-	-	Avgelis et al. 1992
Tobacco mosaic	plant juice		bioassay	-	-	-	-	-	-	-	528	-	0.3	0.17	Broadbent 1965

3.1.2 Energy implications of different time/temperature approaches

Ziemba and Peccia (2011) examined the net energy production associated with pathogen inactivation in sewage sludge for a range of thermal options that included: mesophilic anaerobic digestion (MAD), thermophilic anaerobic digestion (TAnD) at 50 and 55°C (all with a 15 day retention time), temperature phased anaerobic digestion at 50 and 55°C and a retention time of 5 days followed by MAD for 15 days, and batch pasteurisation of 5 hours at 60°C and 1 hour at 70°C both followed by MAD for 15 days. They found that at temperatures of 60°C or above there was a sharp increase in the inactivation rate coefficient of *E. coli* and that this was associated with permanent ribosome damage. At 55°C or below, although inactivation rates were high there was no associated change in the structure of the cell to indicate effective and permanent inactivation. Perhaps most surprisingly, they observed that net energy production was similar for all reactor configurations and thus concluded that energy consumption is not a significant barrier to improving the pathogen quality of biosolids.

3.2 The food industry approach to pasteurisation

Pasteurisation is the term given to a process used in the food industry whereby a foodstuff is heated to a temperature sufficient to significantly reduce microbial load while maintaining organoleptic qualities. The heat treatment is applied for a precise length of time and the foodstuff is then cooled immediately. There are several forms of pasteurisation, of which the principal ones are high temperature short time (HTST) and low temperature long time (LTLT). The temperature / time combinations within each form can vary with the type of foodstuff treated.

A common misconception is that pasteurisation is equivalent to sterilisation, in other words that it completely eliminates microorganisms, while in fact it actually only reduces the microbial load by several logs; for instance pasteurised milk still contains a viable natural microflora, and will spoil if not kept refrigerated. However the 5-log reduction which the pasteurisation process can mediate is expected to reduce the numbers of any contaminating pathogens to a level in which they do not pose a hazard (Pearce et al., 2012). Spores, e.g. of *Bacillus cereus*, are not affected by any form of pasteurisation (Griffiths, 1992).

Pasteurisation regimes in the food industry encompass a wide range of time and temperature options and it is difficult to compare their effect on microbiological hazards without resort to experimental validation.

3.2.1 Pasteurising milk

Several species of microbial pathogens are considered to have the potential to contaminate and be transmitted by milk or dairy products (Hudson et al., 2003), including *Bacillus* spp., *Brucella*, *Cryptosporidium*, *Campylobacter* spp., *Clostridium botulinum, Coxiella burnetii, Escherichia coli* O157:H7, *Listeria monocytogenes, Mycobacterium avium* subsp. *paratuberculosis* (MAP), *Mycobacterium bovis,* streptococci, *Salmonella*, *Staphylococcus aureus,* and *Yersinia enterocolitica*.

Pasteurisation of milk has been employed by the dairy industry for over a century, originally to kill the bacterial agent of tuberculosis, but with conditions changed subsequently to be able to mediate a 5-log reduction in viable *Coxiella burnetti*, the agent of Q-fever and the most heat-resistant non-sporulating pathogen which might contaminate milk (Holsinger et al., 1997). The theory is that if this bacterium is thus affected then the viability of other pathogenic types should be reduced to a similar or greater extent.

Currently in the UK, industrial practice conforms to European regulatory requirements. European Commission Regulation 2074/2005 states:

"When raw milk or dairy products undergo heat treatment, food business operators must ensure that this satisfies the requirements laid down in Chapter XI of Annex II to Regulation (EC) No 852/2004. In particular, they shall ensure, when using the following processes, that they comply with the specifications mentioned:

(a) Pasteurisation is achieved by a treatment involving:

(i) a high temperature for a short time (at least 72°C for 15 seconds);
(ii) a low temperature for a long time (at least 63°C for 30 minutes); or
(iii) any other combination of time-temperature conditions to obtain an equivalent effect, such that the products show, where applicable, a negative reaction to an alkaline phosphatase test immediately after such treatment."

Alkaline phosphatase is an enzyme naturally present in all raw milks, which is used as an indicator of correct milk pasteurisation. It has a thermal resistance greater than that of *Coxiella burnetti,* and therefore if it is inactivated by the pasteurisation process then the process is considered to have been effective in reducing the microbial load (Murthy et al., 1993).

3.2.2 Pasteurising egg

Egg products can also be subject to pasteurisation in the food industry. The Egg Regulations (Anon, 1993) state that liquid egg must be pasteurised prior to retail sale:

"Whole egg and yolk shall be pasteurised by being-
(a) retained at a temperature of not less than 64.4°C for at least 2 minutes and 30 seconds, or
(b) retained at another temperature and for another period of time to achieve at least the same degree of destruction of vegetative pathogenic organisms as if treated by the process specified in paragraph (a) above, and then as quickly as possible cooled to a temperature below 4°C and retained at that temperature unless otherwise preserved, save that the temperature of whole egg or yolk may be held above 4°C solely for the purpose of dissolving added sugar or salt after which the whole egg or yolk shall be immediately cooled to below 4°C."

The Regulations also state that an enzyme inactivation test (alpha-amylase test) shall be carried out as a means of assessing whether liquid egg has been pasteurised. The rationale which underlies this approach is that if the heat treatment is sufficient to denature the enzyme then it will also be sufficient to ensure that no bacterial pathogens, in particular *Salmonella* (the pathogenic species of major concern as regards contamination and transmission by eggs (Cogan and Humphrey, 2003)), survive. Alpha-amylase is a naturally occurring enzyme found in egg (Murthy, 1970). Heat inactivation of this enzyme has been found to parallel that observed for *Salmonella* Senftenberg, the most heat resistant of the salmonellas.

Other foodstuffs which are industrially pasteurised include fruit juices, and alcoholic beverages such as beer and wine. Generally, this is performed to eliminate spoilage organisms such as yeasts and moulds. There do not appear to be regulations or common industry standards for pasteurisation of these products.

3.2.3 Pasteurisation Units

The concept of Pasteurisation Units (PU) sets out to quantify the amount of pasteurisation that has taken place by expressing the process in terms of time, temperature and a Z-value which varies depending on the heat stability of the organism(s) in question. One PU is defined as the sterilising effect seen when a substrate is held at a base temperature for one minute. A minimum temperature is defined below which no contribution is made to PU and the effect on increasing temperature above the base is determined by the Z value, defined as the temperature change required to reduce the survival of target organism(s) by 10-fold (Del Vecchio et al., 1951).

Since a time-temperature regime for pasteurisation currently exists in the PAS110 specification, it is conceivable that the PU approach could be useful in determining acceptable alternative time/temperature combinations. However, the Z value is dependent on the persistence of the microbial hazard (and conceivably on the chemical environment during pasteurisation) so some validation would be required to ensure that an alternative regime proposed was equivalent to the PAS110 pasteurisation treatment as currently defined.

3.3 The use of indicator organisms

3.3.1 Setting alternative processing parameters within the ABPR

Measures to protect against potential animal and zoonotic hazards associated with ABP material are set out in EU regulations 1069/2009EC (the control regulation) and 142/2011 EC (the implementing regulation), in which the requirement for pasteurisation at 70°C for one hour with a maximum particle size of 12 mm is specified. However, the regulation allows AD operators flexibility in employing alternative transformation methods, provided that such methods "ensure adequate reduction of biological risks". Permitting for alternative methods is administered by APHA (the Animal and Plant Health Agency), and the UK's approach to demonstrating pasteurisation equivalence under the ABPR provides a useful background against which to consider alternatives to pasteurisation for reduction of risks from plant pathogens.

There are several conceivable methods for demonstrating effectiveness of alternative methods to pasteurisation. At the most basic level it is possible to seed a digester with a feedstock containing indicator organisms and assay for the reduction in viable count on completion of the transformation method being tested. While this may constitute a robust validation it has several drawbacks: firstly, addition of the indicator organisms specified in 142/2011EC (*Salmonella* Senftenberg or *Enterococcus faecalis*, and parvovirus) into the feedstock itself would result in severe restrictions on that plant under a zoonosis order, and is clearly impractical. Laboratory testing of the parameters in a contained small-scale system is more practical, and indeed can be employed to test a specific time/temperature regime on indicator organisms. However this alone is not considered adequate: the approach taken by APHA is that each biogas plant operating pasteurisation alternatives must seek separate approval, and that any validation experiments have to be conducted on the plant itself rather than small-scale analogous systems. The rationale is to ensure that each plant *and* its operators are able to ensure adequate reduction of biological risks, and that differences in feedstock between plants do not influence the effectiveness of the site-specific transformation process. Type approval, ie permitting a specific time/temperature regime in place of pasteurisation if it has been shown to provide adequate reduction of biological risks and providing the parameters can be adequately monitored and controlled, has not been considered sufficiently robust when granting approvals for alternative transformation methods.

To validate alternative transformation approaches, an inoculum of test organism(s) is placed into the digestion or composting system within a sealed recoverable container representing

the desired 'particle size', rather than the test organisms being added directly to the digestate feedstock. The contents of the test container are then assayed after completion of the test protocol. This approach has the advantage of testing both particle size and time/temperature within the actual facility seeking derogation from standard pasteurisation. Although relatively simple to perform in composting facilities, a validation that requires introduction of a sample capsule into an AD system may require some engineering modification such that the sample containers can be placed in appropriate locations and recovered after the required time has elapsed. Conducting tests in which indicator organisms are enclosed in semi-permeable membranes was not considered appropriate: although this would allow the effect of physicochemical conditions (for example pH, ammonia) to be assessed as well as those of time and temperature, the risk of rupture of containers or leaching of the contents was considered too great. Furthermore, whilst AD operations may well expose pathogens to a range of physicochemical conditions, these cannot be guaranteed at all times – unlike a discrete thermal process. However, semi-permeable membrane containers are recommended for use in the direct validation processes in Germany (see Section 3.6.1).

3.3.2 Possible approaches for non-ABP inputs

Spiking with known amounts of indicator organisms in recoverable containers offers an attractive solution to directly validating process efficacy. Although this would be more challenging in AD than in composting systems, operators who wish to validate alternatives to pasteurisation could consider engineering solutions which would allow such samples to be introduced to and recovered from appropriate points. Key to this approach would be selection of suitable indicator organisms to act as proxies for inactivation of pathogens. The German waste ordinance provides a precedent for this, using the highly persistent plant pathogens TMV and *Plasmodiophora brassicae* as indicators (as well as tomato seeds).

Various indicator organisms have been used for direct validation of composting processes, including the plant pathogens *P. brassicae*, TMV, *Fusarium oxysporum* and tomato seeds (Idelmann 2005; Noble et al 2011).

Although common in the natural environment, and likely to be present in many AD feedstocks and apparently offering the potential for indirect process validation, *E. coli* and *Salmonella* are not suitable indicators for inactivation of plant pathogens, as some of the spore-forming plant pathogens are significantly more likely to survive digestion (without pasteurisation) than these bacteria.

Other organisms which occur naturally in the AD process may also be considered suitable as indicator organisms for indirect validation. Schnürer & Schnürer (2006) present data on seven fungal species tested through a sanitisation process of 70°C for one hour. After this time, only spores of *Thermomyces lanuginosus* survived at levels above the detection limit. *Aspergillus flavus* and *fumigatus* were both inactivated, although naturally occurring *Aspergillus* species were shown to survive.

3.4 Ability to test for pathogens in digestate or feedstock (the 'continuous surveillance' option)

If feedstock were monitored and controlled there would be a low risk of the resulting digestate being contaminated and no further tests would be required. Feedstocks would have to be tested for specified plant pathogens before they were used in AD processes. However, such an approach has severe limitations.

While it may also be possible to test all digestate for the presence of proscribed pathogens before it is considered safe, this option would require extensive and continuous testing which could be highly costly. Tests would have to be capable of detecting the lowest inoculum

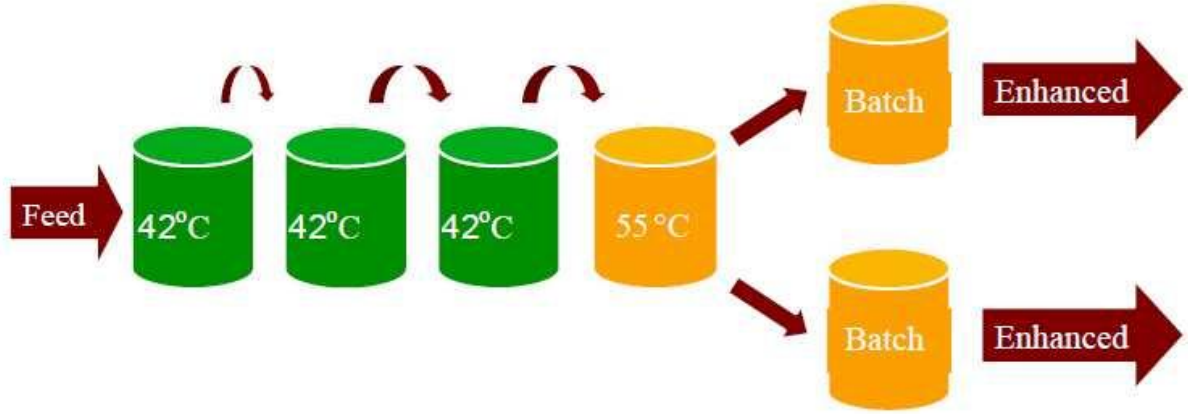
levels identified as a risk to crops. Highly sensitive methods, generally DNA-based, are available for major pathogens, although an enrichment step may be required for added sensitivity and to differentiate between viable and dead cells. Appropriate statistical sampling approaches would also need to be considered.

3.5 Enhanced Enzymic Hydrolysis (EEH)

The EEH process utilises a number of reactors operated in series to approximate a plug-flow mode of mixing and thus minimise any short-circuiting of feedstock. The process consists of five or six reactors operated in series. The reactors may be operated at different temperatures and Hydraulic Retention Times (HRTs) in order to carry out the dual role of hydrolysis and sanitisation. The first stage of the process is the hydrolysis stage and comprises three vessels in series, each operating at around 42°C and providing an overall retention time of approximately two days. This low retention time and associated high organic loading rate provides an environment with a pH of around 5.5, which together with the elevated temperature, provides ideal conditions for acid hydrolysis of the feedstock.

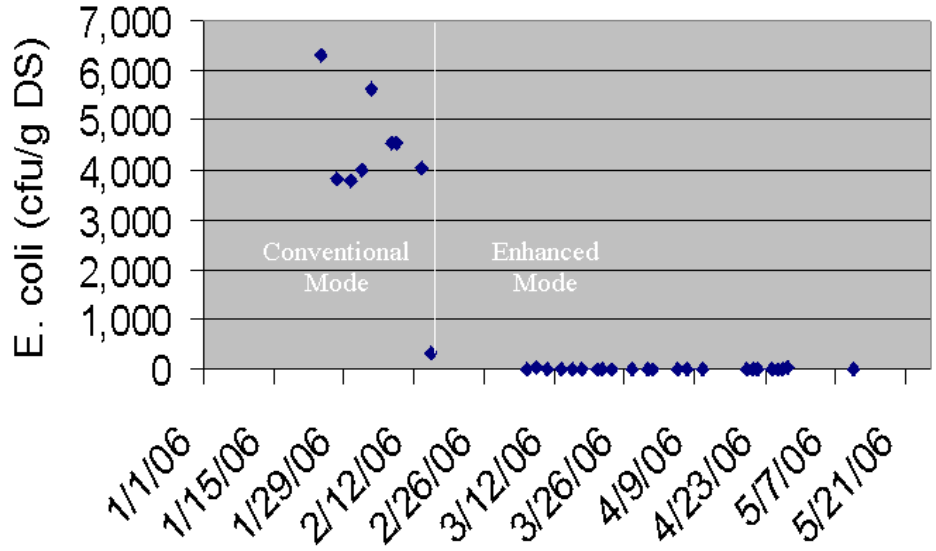
The second stage of the process involves two tanks at 55°C that are operated on a fill, hold and draw down basis to ensure a minimum of five hours retention time at this temperature, thus ensuring effective pathogen destruction. The temperature increase is achieved by recirculation of the first stage digestate through steam injection units. The hydrolysed and sanitised sludge from the EEH unit is then fed to a conventional MAD digester, which can be operated at a reduced HRT of between 11 to 18 days, since the first stage of hydrolysis stage has already been undertaken (Figure 3-1).

Figure 3-1 Typical layout of an EEH process with the reactors in green providing acid hydrolysis whereas those in yellow provide sanitisation (from Riches et al., 2011)



The first full-scale EEH plant was constructed at Macclesfield in 2002 followed shortly after by plants at Bromborough and Blackburn, both treating sewage sludge and operated by United Utilities. The efficacy of the EEH unit can be seen in Figure 3-2 in which greater than 6-log removal of *E. coli* was observed shortly after initiation of the enhanced mode of treatment at Blackburn, providing a digestate that was completely free from *E. coli.*

Figure 3-2 Rapid die-off of *E. coli* during the operation of the Blackburn EEH in enhanced mode, to provide a digestate free of this bacterium



More recently, EEH has been commissioned at King's Lynn and Great Billing (Figure 3-3) and operated by Anglian Water, with 6-log removal of *E. coli* reported by Riches *et al.,* (2011). At present this technology is operated predominantly using sewage sludge as a feedstock. However Wessex Water plan to treat up to 24,000 tonnes of food waste using existing wastewater digestion infrastructure, including the EEH process at their Avonmouth site near Bristol (Hills, 2011).

Figure 3-3 Enhanced enzymic hydrolysis reactors at the Great Billings sludge treatment centre operated by Anglian Water and achieving a 6-log removal of *E. coli*

With proper validation, EEH systems may offer a solution for microbiological hazard reduction by considering the AD process as a whole rather than reliance on a discrete pathogen kill step such as pasteurisation, however they may not achieve widespread use outside the waste water treatment sector as capital and operating costs are typically higher than single tank systems.

3.6 Controlling digestate phytohygiene in other EU countries

3.6.1 Germany

The German Biowaste Ordinance lays down requirements on plants processing biowaste destined for use in agriculture, silviculture and horticulture. Use within the same holding as the waste arises is excluded from these requirements.

Time/temperature requirements are laid down as either 55°C for 24 hours with a minimum retention time of 20 days or, if a lower temperature or shorter retention time are employed the process must include a pre- or post-treatment at 70°C for one hour.

Additionally each facility must undertake a direct process validation, which must be completed within 12 months of commissioning for new facilities and repeated on existing facilities where processes have changed substantially. Validation for the effectiveness of a process in reducing risks to plant health entails testing 36 samples, each sample being tested on three consecutive days.

Test organisms must be placed within the part of the process responsible for thermal inactivation, although a provision is made for circumstances where the design of the system makes this impractical, in which case the "efficacy of the process with regard to sanitisation must be demonstrated in other ways by suitable experts". Test organisms are introduced into the system inside semi-permeable membranes in non-decomposable containers.

For plant pathogens the test organisms specified in the ordinance are tobacco mosaic virus (TMV), *Plasmodiophora brassicae* (the causal agent of clubroot of brassicas) and tomato seed. Testing methods and acceptable limits for the indicator organisms are specified (Bruns, 1994; Pollman and Steiner, 1994).

The ordinance also sets out a requirement for indirect monitoring, whereby temperature measurements of the part of the system responsible for thermal inactivation are taken regularly (continuously if possible) in three representative zones. A further analysis of the final digestate is also required, and a limit for seeds or reproductive parts of plants of two per litre of substrate is specified.

3.6.2 Sweden

The Swedish Certification Rules for Digestate[2] do not include specific requirements for plant pathogens, but do specify parameters in accordance with EU regulations for ABP inputs (pasteurisation at 70°C for one hour and a maximum particle size of 12 mm). Instead, plants processing non-ABP material must incorporate a treatment at a minimum of 55°C for at least six hours and a hydraulic retention time of at least seven days. More stringent requirements are placed on plants that do not have adequate mixing and an even temperature distribution.

3.6.3 Proposed pan-European approach

Technical discussions in preparation for proposed End of Waste Criteria for Biowaste under the Waste Framework Directive (2008/98/EC) incorporate a suggested time/temperature regime of 55°C for 24 hours and a hydraulic retention time of 20 days for effective management of biological risks in digestate not containing ABP material (Anon 2012). The same document also proposes that requirements should not be overly restrictive such that competent authorities in member countries can authorise other treatments providing that effectiveness in reducing biological risk can be demonstrated.

[2] http://www.avfallsverige.se/fileadmin/uploads/Rapporter/Biologisk/B2009b.pdf

4.0 Summary: An alternative phytohygiene control approach for the UK?

MAD without a discreet pasteurisation step cannot be guaranteed to eliminate plant pathogens of interest, but the practice of operating digesters without pasteurisation where inputs arise from, are digested on and returned to the same holding is acceptable, so long as users of the digestate are made aware of the potential phytohygienic risks associated with this approach.

For this reason, pasteurisation remains a key requirement for the majority of AD processes seeking certification to BSI PAS110:2014. However, it is recognised that not all AD processes will wish to obtain certification to the PAS – but may nonetheless wish to understand and demonstrate the phytohygienic quality of their digestates. This report has examined various options that could serve to demonstrate digestate quality in the absence of standard 70°C / one hour pasteurisation:

- Exemption of certain feedstocks where risk assessment indicates this is warranted or where the feedstocks have already undergone a similar procedure to the pasteurisation step;
- Use of alternative time/temperature regimes;
- Restriction of particularly high risk feedstocks (such as quarantine plant wastes) to defined 'special measures' or licensed facilities;
- Use of indicator organisms for process-specific validation.

4.1 Exemption of specific feedstocks from a pasteurisation requirement

There are certain circumstances where specific non-ABP feedstocks could be exempted from the PAS110 pasteurisation requirement. For example, where all non-ABP feedstocks arose from food or drinks manufacturing they must have been treated through a thermal process that gives equivalent PU to 70°C for one hour. Where there is any doubt, operators could implement the new validation approach (Section 4.4).

4.2 Use of alternative time/temperature regimes

The use of alternative temperature and time regimes has been suggested, for example see Table 4-1. In principle these could be acceptable but would require validation as suggested in Section 4.4.

Table 4-1 Combinations of temperatures and Minimum Guaranteed Retention Times (MGRTs) for sanitisation, equivalent to 70°C for 1 hour – example from Denmark (Bendixen, 1994 & 1999; Bendixen & Bennetzen 1995)

Thermophilic temperature (°C)	***MGRT at thermophilic temperature[a] (hours)***	***MGRT by treatment in a separate pasteurisation tank[b] (hours)***	
		before thermophilic digestion[c]	before mesophilic digestion[d]
52.0	10		
53.5	8		
55.0	6	5.5	7.5
60.0		2.5	3.5

a) The hydraulic retention time (HRT) in the digester must be at least 7 days
b) Digestion may take place either before or after pasteurisation
c) See point a)
d) The mesophilic digestion temperature must be between 20°C and 52°C. The hydraulic retention time must be at least 14 days.

4.3 Restriction of high risk feedstocks

High risk feedstocks would include material known to contain pathogens for which there is zero tolerance (such as quarantine plant pathogens that are under statutory control), for example the potato ring rot and brown rot pathogens, *Clavibacter michiganensis* ssp. *sepedonicus* and *Ralstonia solanacearum*. Quarantine pathogens are highly unlikely to be present in material destined for AD except in crop waste arising from an interception of infected plant material or outbreak of quarantine diseases. In such cases it is conceivable that AD may be used as a route for safe disposal of infected plant material.

If a statutory body recommended treatment of high risk feedstocks containing plant quarantine organisms under statutory control through AD, then it would be essential that any process intended to achieve pathogen kill was completed as a batch process that could not be bypassed, and that it had been validated for the particular microbial hazards in question. The most effective way to ensure this would be to incorporate a pasteurisation step.

4.4 Process-specific validation

In recommending alternatives to pasteurisation it is important to establish equivalence to pasteurisation in terms of ability to reduce pathogen risk to an acceptable level. Since all digesters differ in operating parameters (for example temperature, retention time, vessel design) and, crucially, feedstock, we are not recommending a defined set of digestion parameters to obtain equivalence to pasteurisation since these may not be equally effective across all digester types. Instead we propose a validation process by which operators can demonstrate that their process, operated by their personnel, is sufficiently effective at reducing pathogen risk. This approach has the advantage of being in harmony with the approach taken in implementing ABP requirements.

For validation we propose that any suggested alternative to pasteurisation be tested for its ability to achieve a defined level of kill of indicator organisms introduced into the digester. To aid analysis we propose that these organisms are introduced in sealed containers for ease of recovery and subsequent analysis, and because inoculating an entire reactor with a detectable level of indicator organism would not be practicable. We suggest that more persistent organisms for which viability assays can be conducted are chosen as indicators, and for this reason propose *Plasmodiophora brassicae* and tomato seed.

Our suggested indicators differ slightly from other process validation approaches, for example the German Biowaste Ordinance (Anon, 2006b) which specifies *Plasmodiophora*, tomato seed and tobacco mosaic virus (TMV) as indicators. We do not propose TMV since we consider this too persistent to be a workable indicator but suggest the adoption of *Plasmodiophora*.

4.4.1 Validation protocol[3]

Direct validation requires AD processes to accommodate introduction and subsequent recovery of a capsule containing the indicator organisms *Plasmidiophora brassicae* (at a minimum of 3 g gall tissue/capsule) and tomato seed (cultivar Ailsa Craig, a minimum of 15 seeds/capsule). The capsule must be of sufficient size to contain the required titre of indicators while allowing sufficient thermal conductance to ensure that the centre of the capsule reaches the required temperature during the specified duration of the sanitisation step. The precise dimensions and construction of the capsule may need to be determined experimentally for each validation.

[3] *Please note that a more detailed protocol is available on the WRAP website under project code OIN002-017: http://www.wrap.org.uk/sites/files/wrap/OIN002-017%20Final%20Report%20(protocol).pdf*

Sufficient test capsules must be used to ensure that validation is achieved throughout the process in which the treatment is taking place, but we recommend a minimum of three capsules.

Capsules must be in place within the process for the period of time specified by the treatment under investigation. On removal, capsules must be subjected to tests as described below, commencing within 24 h of removal from the facility.

The process will be considered to have passed if viable tomato seed are undetectable and if *P. brassicae* bait plant tests do not result in infection as determined by real-time PCR assays (see below).

Tests for indicator organisms must be capable of ascertaining the viability of organisms. For *P. brassicae* this must involve incubation with bait plants prior to detection. Viability of the *P. brassicae* test material must be ascertained either before the test is carried out or by inclusion of appropriate control material (ie material not subjected to the treatment under validation) in testing. Proposed assay methods for *P. brassicae* are based on the methodology set out in the German Biowaste Ordinance (Anon, 2006b) with the addition of a real-time PCR assay to identify infected bait plants (Noble *et al.*, 2011).

For tomato, viability must be determined by germination or by methods described by Anon (2006a) and Pollmann and Steiner (1994). The germinating capacity of the batch of tomato seed used in tests must be measured before tests commence and must be at least 90%.

A detailed validation protocol has been published under WRAP project code OIN002-017 (WRAP, 2014).

5.0 References

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