MorVet Ltd

Consultancy services in health risk management, global disease control and food safety programs

Application of Biofertiliser to Pasture – Risk Management for Disease Exposure

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Executive Summary

The use of biowaste as feedstock for anaerobic digesters produces energy and biofertiliser, while reducing global warming. This system is used widely around the world, and the Bioenergy Association is promoting its increased use in New Zealand. However animal and human pathogens are typically present in the feedstock. Hazards to animal and human health from these pathogens are identified, and the effect of procedures specified in the BioEnergy Association "Guidelines for the production of digestate biofertiliser for application to land" (2025) and alternative procedures are described, including the evidence to support the conclusions.

If the entire amount of either feedstock or preferably biodigestate is heated to at least 70^0C for one hour or more, then risk attached to all organisms of concern other than spore-forming Gram positive bacteria will be effectively controlled, while the risk attached to spore-forming Gram positive bacteria can be effectively managed.

These findings apply to the application of biofertiliser to pasture or crops used for animal feed, but not to the application of biofertiliser to crops used for direct human consumption, especially those eaten raw or under-cooked.

Recommendations

The following recommendations are made on possible adjustments to the current Guidelines.

- Separately describe requirements for psychrophilic, mesophilic and thermophilic digesters.
- Specify more precisely pasteurisation options, particularly factors influencing the choice of pasteurisation before or after digestion.
- Consider changing the inclusion of animal manure in Group B when it is used on the farm of origin.
- Consider changing the monitoring system to a version approximating the EU policy, and identifying *Enterococcus spp*. to be monitored with *Salmonella spp*. This may require a quantitative evaluation of the appropriateness of the upper limit used by the EU.
- Require all results of monitoring to be reported, so that results of failed tests have to be included. Impose consequences for repeated failed tests, even if there are sufficient pass results to meet monitoring requirements.
- Consider advising farmers who use biofertiliser to vaccinate stock with a multivalent Clostridial vaccine before applying biofertiliser.

Benefits from Biowaste Utilisation

Biowaste from food waste and other sources generates methane and other undesirable products as it breaks down. It is currently a significant contributor to global warming, but there are efforts worldwide to utilise it for production of energy and fertiliser. There are currently estimated to be about 132,000 small to large digesters in operation worldwide, plus very large numbers of micro-scale digesters at household or local level. Although these produce over 400 TWh of energy and large quantities of fertiliser, current output still represents only an estimated 2% of the potential processing capacity (*1*).

New Zealand is evaluating potential expansion of biowaste utilisation. A search of the scientific literature was conducted to identify disease events for which a link to use of biofertiliser derived from anaerobic digestion of food waste and similar materials was suggested or confirmed, based on scientific evidence. None was found. However New Zealand places great importance on biosafety and biosecurity in relation to its export of animal products. This report has been commissioned to provide advice on the nature of any hazards involved in the process, and the adequacy of risk management strategies used in biofertiliser application.

Production of biofertiliser from source material

The system in New Zealand and worldwide uses anaerobic fermentation of food and agricultural waste leading to production of combustible gases which are used for generation of bioenergy, with the residual material after fermentation being available for the plant nutrients that it contains to be used as fertiliser. In this report, feedstock is the material entering the digester, biodigestate is the intermediate material emerging from the digester, and biofertiliser is the fully processed product ready for distribution to users.

While there are substantial benefits from this waste management system, there are also potential negative effects which need to be managed (*2*). Because there is a risk of pathogens being present at the end of the fermentation process, it is considered necessary to subsequently treat the material to manage the risk of pathogens being present at sufficient concentration to cause disease.

There are three digester types which operate at different temperature ranges – psychrophilic (temperature at ambient level or below), mesophilic (20 to 45^{0}C, usually 35-38^{0}C) and thermophilic (50 to 60^{0}C). This is one of the important factors determining whether pathogens survive the digestion process. Pasteurisation as described in this report requires heating the entire material to 70^{0}C for at least 60 minutes either before or after digestion.

This report evaluates the risk that pathogens may be present in the biofertiliser product at sufficient concentration to present a risk of producing disease in animals or people.

Hazard identification

A pathogen is considered to justify consideration for inclusion in the risk analysis if it can infect animals which are exposed to the fertiliser. This may cause disease in exposed animals, or cause infection in animals without causing disease - but subsequently result in human disease. A pathogen is also included if it is capable of causing infection in people who are directly exposed to the biofertiliser derived from biodigestate, such as farmers spreading the product on pasture.

Organisms are included in this hazard identification provided that they are known to be associated with food or other materials likely to be included in the biofertiliser. Organisms are excluded if their method of transmission means it would be unrealistic for them to be present in the biofertiliser in an infectious state – for example, organisms transmitted by an insect vector, or by the respiratory route. It is not realistic to consider every individual species of pathogen in detail, so organisms have been grouped for consideration based on their taxonomic group and their resistance to elimination from food waste during the production of biofertiliser.

Exotic organisms are only included if there is a significant risk that they could reach New Zealand and spread within the country by means which could allow them to subsequently be found in food waste.

Organisms included in the hazard assessment

In a recent paper (*3*), the concept of classifying diseases into epitypes was introduced. An **epitype** is defined as a description of the main epidemiological characteristics of diseases which allow a pathogen to be maintained and disseminated in a host population. In this context, maintenance means the mechanisms by which the pathogen persists in the animal population, the environment, and (for zoonoses) the human population.

Epitype 1 – directly transmitted from host to host, with maintenance entirely in hosts (including sexual and vertical transmission).

Epitype 2 – directly transmitted from host to host, with maintenance principally in hosts - but substantial environmental influence on frequency of transmission for some agents.

Epitype 3 – transmission mainly reliant on fomites as intermediates in transmission, maintenance in hosts.

Epitype 4 – transmission through food/feed or consumed water, long term maintenance in hosts and short term maintenance in environment.

Epitype 5 – transmission by mechanical vector, maintenance in hosts.

Epitype 6 – transmission by facultative biological vector, the vector phase does not involve a different replication phase for the agent, maintenance in hosts.

Epitype 7 – transmission through an obligatory cycle involving two different vertebrate species, maintenance shared between two types of hosts.

Epitype 8 – transmission through an obligatory cycle involving an invertebrate host in which the pathogen must go through a specific development stage before it can infect a vertebrate host. Maintenance shared between two types of hosts.

Epitype 9 – transmission through a non-animal biotic reservoir or through the abiotic environment, such as water or soil, no host to host transmission, maintenance entirely in environment.

All animal diseases included in the international reporting list of the World Organization for Animal Health (WOAH) plus other zoonoses considered of importance have been classified into epitypes. A small number of diseases have more than one transmission method, so are included in two or three epitypes.

Table 1 Number of diseases categorised in each epitype in the WOAH list of animal diseases (n = 120) and the author-curated list of important zoonoses (n = 41) . Diseases are counted multiple times if they fit into multiple epitypes.

Epitype	WOAH Animal disease list	Animal diseases including zoonoses	Zoonoses in people
1	12 (9%)	13 (7%)	7 (7%)
2	70 (53%)	92 (52%)	38 (37%)
3	8 (6%)	10 (6%)	10 (10%)
4	1 (1%)	10 (6%)	16 (16%)
5	4 (3%)	5 (3%)	3 (3%)
6	24 (18%)	30 (17%)	18 (18%)
7	4 (3%)	4 (2%)	4 (4%)
8	5 (4%)	6 (3%)	2 (2%)
9	4 (3%)	7 (4%)	3 (3%)
Total	**132**	**177**	**101**

The full list of diseases is given in Appendix A. Only disease agents which could potentially be present in feedstock to the anaerobic digester justify inclusion. All of these diseases have been evaluated to decide whether they should be covered in this assessment, and other diseases outside the list have been included where they are considered to represent a risk of inclusion in biofertiliser.

Gram negative bacteria

The cell wall and associated enzymes of different categories of bacteria determine their susceptibility and resistance to damage by various factors (*4, 5*). Gram negative bacteria have a cell envelope comprising an inner monolayer of peptidoglycan and a lipopolysaccharide outer layer (*6*). This is fairly fragile (*7*) and makes them more susceptible than other organisms to destruction by the processes involved in biofertiliser production and subsequent processing. Gram negative bacteria therefore are low risk pathogens for remaining infectious within biofertiliser produced from digestate.

It is however essential that strict measures are taken to protect the post-processing area of a biofertiliser production site from any way of being contaminated with material from earlier stages of processing, because Gram negative bacteria will grow readily in the biofertiliser if cross-contamination occurs.

The following Gram negative organisms which could be present in feedstock entering anaerobic digestion are capable of infecting animals and in some cases humans as well.

Campylobacteriacae

Campylobacter and *Arcobacter* are closely related organisms in this Family, and infect both animals and people. While infection is most common in poultry, *Campylobacter* is also common in ruminants and pigs (*8*). *Campylobacter jejuni* and *C. coli* are the most important human pathogens, and are thermophilic and microaerophilic organisms. Hence they are well adapted to surviving anaerobic fermentation. They can occur in feedstock to the anaerobic digestion process, and at lower concentration or absent in the biofertiliser product (*9*). Treatment at thermophilic temperatures was effective in eliminating Campylobacter, with increased effectiveness if combined with elevated hydrostatic pressure (*10*). However mesophilic digesters were not fully effective (*9*).

Arcobacter (recently renamed *Aliarcobacter*) is an emerging pathogen in this family which causes both human and animal diseases, and is likely to be present in food waste (*11, 12*). The organisms are rapidly killed at thermophilic temperatures (*13*), but survive much longer at lower temperatures (*11, 13*).

Enterobacteriaceae

This Family contains a wide range of organisms which can infect animals and people. This includes Shigatoxin-producing Escherichia coli, Aeromonas, Cronobacter (formerly Enterobacter), Klebsiella, Plesiomonas, Proteus, Salmonella,

They can be considered as a group (known as coliforms) since they have broadly similar low resistance to the conditions of anaerobic digestion and are not considered a significant risk group (*14*). They are also destroyed by heat treatment (*15*).

Moraxellaceae

Acinetobacter species are pathogens of growing importance and are involved in both human and animal infections (*16, 17*). These organisms persist in the environment for long periods. They are however expected to be destroyed during pasteurisation (*18, 19*), and are not considered to be a risk pathogen in relation to biofertiliser use.

Mycoplasmataceae

It is most unlikely that *Mycoplasma bovis* would be present in feedstock for anaerobic digestion. However in view of the current eradication program, it is reassuring that it is readily inactivated by heat, and would not survive anaerobic digestion (*20, 21*).

Yersiniaceae

The genus *Yersinia* includes a range of different species, but the relevant ones here are *Y. pseudotuberculosis* and especially *Y. enterocolitica*. They cause disease in animals, including in New Zealand (*22*), and cause zoonotic infections in people. Infection can be transmitted in meat, especially pork, and also in milk (*22-24*).

They are unusually resistant to low temperatures but also show moderate resistance to elevated temperatures. They are found in feedstock (*25*) and survive in at least small numbers through psychrophilic (*26, 27*) and to a lesser extent through mesophilic anaerobic digestion (*15, 28, 29*). They are destroyed by heating at 70^{0}C for at least 40 to 60 minutes (*26, 30*).

Gram positive bacteria

Gram-positive bacteria do not have an outer membrane but are surrounded by layers of peptidoglycan many times thicker than is found in the Gram-negatives (*31*). Threading through these layers of peptidoglycan are long anionic polymers, called teichoic acids. They are therefore more resistant to biodigestate processing than Gram negative bacteria. Most Gram positive bacteria are not capable of forming resistant spores, which would further enhance their resistance to destruction.

Enterococcaceae

Bacteria in the Enterococcus genus are very widely distributed in people, animals and the environment (*32*). While traditionally considered of low significance, they have become increasingly significant as pathogens in recent decades, partly linked to their antibiotic resistance patterns (*33*). They mainly cause nosocomial infection and disease – both human (*32*) and animal (*34*). They are used as a measure of the level of faecal contamination of items in a wide range of circumstances. They are unusually heat tolerant (5 to 65 ^{0}C) and pH tolerant (4.5 to 10.0), so can survive mesophilic anaerobic digestion (*35*).

Listeriaceae

Listeria monocytogenes is an organism ubiquitous in the environment, and pathogenic to animals and people. It is facultatively anaerobic, has wide temperature tolerance for growth (0 to 45 ^{0}C) and is resistant to dehydration and acidic conditions (*36*). The organisms were detected in digestate product from mesophilic anaerobic digestion (*9, 35*), but could be destroyed by heating of digestate for 30 or 60 minutes at 70^{0}C (*15*).

Spore-forming Gram positive bacteria

Bacteria in the genera *Bacillus*, *Clostridium* and *Clostridioides* are typically capable of forming intracellular endospores when the environment is adverse and no longer conducive to population growth. These spores are released into the environment upon lysis of the parent cell from which they have developed through the sporulation process. The free spores have protective layers in the spore wall, are metabolically dormant and resistant to high temperatures, desiccation, radiation, and many chemicals. They can survive for extremely long periods in the environment before eventually germinating into vegetative bacterial cells again (*37*). Once this occurs, they again become susceptible to elimination by a range of methods.

Germination typically commences when the spore detects particular amino acids or sugars indicative of an improving environment, and this initiates a series of process leading back to vegetative cells (*38*).

These factors make these organisms the most resistant to control through the production process for biofertiliser.

Bacillaceae

Members of the genus *Bacillus* range from aerobic to anaerobic, occur exceptionally widely in the environment, and the relevant species within this genus are capable of long term persistence through the formation of endospores (*39*). Hence they are capable of persisting through anaerobic digestion and heat pasteurisation.

Bacillus anthracis is a serious human and animal pathogen, but is not considered to occur in New Zealand, and measures are in place to prevent its entry. It is therefore not considered further.

Bacillus cereus and related species have long been known as ubiquitous spore-forming environmental organisms but are now recognized as significant pathogens in people (*40, 41*), including infants (*42*), and animals (*43*). Some strains are aerobic, others are facultatively anaerobic. Unusually, some strains of *B. cereus* act as plant growth promoters in various ways (*44*). These organisms can survive anaerobic digestion (*45-47*).

Peptostreptococcaceae

Clostridioidies difficile (formerly *Clostridium*) is a spore-forming anaerobe which has become an important emerging pathogen over recent decades. While it was first identified in 1935 as a commensal of the lower digestive tract, with the increasing use of antibiotics since the 1950s it became important as a nosocomial infection in hospitals (*48*). Between 1999 and 2003 a more severe strain emerged in Canada (*49*), which has subsequently spread around the world. Infections have become more common in the community as well as in hospitals (*50*). Infection and in some cases disease occurs in a wide range of domestic (*51, 52*) and wild animals (*53*). *C. difficile* has been found in animals, food and wastewater in New Zealand (*54*). Transmission can occur between animals (*55*) as well as from environmental sources (*56-58*). The organism survives anaerobic digestion (*9*). Heating for 60 minutes at 70^0C in a laboratory experiment reduced the population but did not eliminate it. Following heat treatment, spore germination and proliferation of vegetative cells occured (*59*). Pretreatment of feedstock at 70^0C for one hour prior to anaerobic digestion resulted in a higher concentration of *C. difficile* (*60*).

Clostridiaceae

This family includes a range of anaerobes which cause diseases due to the toxins they produce.

Clostridium botulinum is a spore-forming anaerobe which produces a neurotoxin that causes serious and often fatal intoxication. It occurs when the person (*61*) or animal (*62*) consumes material which has been in anaerobic conditions sufficient to allow the organism to multiply and produce toxin (*63*). It survives in the environment for a long time, mainly in spore form (*64*). The organism itself does not cause disease.

Clostridium perfringens is an important cause of animal disease, and can also cause human disease (*65*). Within this species there are five different toxin types which cause different diseases of varying severity and prevalence in animals (the best known being enterotoxaemia in sheep) and also cause enterotoxaemias and gas gangrene in people. There are other similar members of the genus which cause animal disease and in some cases human disease, notably *C. chauvoei* (cause of blackleg in animals), *C. tetani* (cause of tetanus), *C. sordellii* (sudden death syndrome), *C. novyi* (Black disease), and *C. haemolyticum* (Redwater Disease).

Clostridium perfringens is the species which has been used as the example species for this genus in most investigations related to anaerobic digestion. Neither anaerobic digestion nor heat treatment can effectively reduce the concentration of C. perfringens in digestate (*9, 29, 46, 60, 66*). Reduction in some situations can be improved by the use of non-thermal methods (*67-70*). Germination-inactivation also has potential (*71, 72*).

Fungi

No studies could be found on either the effect of anaerobic digestion or heat treatment on pathogenic fungi. No firm conclusion can therefore be reached on possible contamination of biofertiliser with fungi. However it is unlikely that pathogenic fungi would survive pasteurisation, and be capable of causing increased risk of animal or human disease.

Mycotoxins

A variety of fungi are not directly pathogenic, but produce toxins which are highly thermostable and survive high temperatures (*73*), including pasteurisation. No evidence could be found on the effects of anaerobic digestion on levels of mycotoxins in biofertilisers. Mycotoxins only produce disease in animals and people when the product containing a mycotoxin is consumed in sufficient amounts. This is highly unlikely to occur as a result of biofertiliser application to pasture. Taking into account the extent of utilisation of biofertilisers in a wide range of countries without any reports of outbreaks of diseases caused by mycotoxins, it is considered reasonable to conclude that the circumstances during the production of biofertilisers are not favourable to mycotoxin production.

Protozoa

The genus *Cryptosporidium* contains multiple species, the main one *C. parvum*, and is responsible for both animal and human disease (*74-76*). Several outbreaks of human cryptosporidiosis have been reported, caused by access to untreated animal manure (*77*). Both anaerobic digestion (*78, 79*) and heat (*80, 81*) are effective in destroying the oocysts.

Giardia duodenalis is a zoonotic protozoal pathogen which causes human disease (*82, 83*). Only limited evidence is available, but shows that both anaerobic digestion (*79*) and heat will kill this organism (*84*).

Toxoplasma gondii is an important protozoal parasite, with felids as the definitive host and a wide range of mammals (*85*), birds (*86*) and people (*87, 88*) as intermediate hosts. It survives in the environment for extended periods (*89, 90*). It is readily destroyed by elevated temperature (*91, 92*). No information could be found on the effect of anaerobic digestion, but given its susceptibility to heat it would be expected to be inactivated by most common forms of anaerobic digestion.

Metazoan parasites

Nematode eggs are routinely found in feedstock for anaerobic digestion, and *Ascaris suum* is commonly used as the example parasite. A high proportion of eggs are destroyed at moderate temperatures, but a temperature of 53^0C is required for complete destruction (*66, 78*) in anaerobic digesters. It is likely that most other nematode eggs are similarly destroyed. There is limited data on survival of nematode eggs from different species in unprocessed animal manure (*93*), but not following anaerobic digestion. Eggs of trematodes such as the liver fluke *Fasciola hepatica* will also be destroyed in the production of biofertiliser (*94*).

Trichinella spiralis is an unusual parasite in that it is normally transmitted by consumption of meat containing encysted larvae. It is rare in New Zealand, absent from commercial pig farms, and has a very low probability of being present in meat included in food waste (*95*). Although the encysted larvae are reported to last longer in anaerobic digestion than other nematodes (*96*), the evidence supporting this is very limited and the probability of larvae being present in feedstock is low. The risk of occurrence in biofertiliser is not considered significant. Even if a few larvae reached biofertiliser, they would not result in animal infection.

Viruses

The virus population present in anaerobic digesters is substantial and complex (*97, 98*). The vast majority of these viruses are bacteriophages and other viruses which infect the organisms involved in the anaerobic digestion process (*99*). They are not relevant to disease risks resulting from use of biofertiliser. However viruses pathogenic for animals and/or people are regularly found in the feedstock to digesters and may potentially be present in the biofertiliser product unless they are destroyed during the production process.

However only a small proportion of the viruses of pathogenic importance are realistically likely to be found in feedstock – mainly those which are found in meat and/or milk, and some which could contaminate feedstock in sufficient quantities to be infectious. Vector-transmitted viruses, for example, do not represent a risk. All viruses have a minimum infective dose – the smallest number of virus particles that can initiate an infection (*100*). So merely having a virus present does not represent a risk unless the concentration is adequate to initiate infection in animals or people exposed to the biofertiliser.

If virus particles are present at adequate concentration in the feedstock, then the next issue is whether processing to produce and apply the biofertiliser will destroy sufficient virus (*101*) to reduce the concentration below the minimum infectious dose (*102, 103*). There are differences between DNA and RNA viruses, and between enveloped and non-enveloped viruses (*101*). There are also differences due to the digestion method – thermophilic digestion will typically reduce virus concentration more than mesophilic (*104*). Pasteurisation following mesophilic anaerobic digestion was effective in substantially reducing virus concentration (*105*).

Relatively few viruses of importance in animal and human health are at significant risk of contaminating the feedstock (which requires that they are found in adequate concentration in animal products discarded into food waste), surviving the anaerobic digestion process and any pre or post-digestion pasteurisation that is carried out, being applied as biofertiliser, and being available in at least minimum infective dose to animals or people who come in contact with the fertiliser.

Moreover only a small number of viruses have sufficient evidence about the various probabilities involved in the steps of this pathway to make a judgement of the scale of the risk of this whole sequence of steps taking place. Therefore the scale of the risk will be discussed for four epidemiologically important viruses where at least some evidence is available. The examples are two animal diseases, one zoonosis, and one human disease.

Because of the importance of foot and mouth disease (FMD) as a risk to New Zealand animal production, it is the first example. There is an extremely large literature on FMD, but few papers relevant to this assessment. FMD virus is transmitted in meat and especially products which include offals (*106-108*), as well as in milk (*109*). Pasteurisation at 70^{0}C is effective in killing FMD virus (*110*), so thermophilic digestion will also be effective (*111*), but the effect of mesophilic or psychrophilic digestion does not appear to have been assessed. However various studies which approximate these conditions suggest that the virus is destroyed in these digesters (*112*).

African swine fever virus was considered to have been limited to a relatively small part of the world until in 2007 it was discovered in Georgia and subsequently has spread widely across Europe and Asia. Wild boars play a significant role in the spatial expansion that has occurred. The disease has appeared unexpectedly in a number of countries since 2007, due either to movement of wild boars or of food or feed items containing the virus (*113, 114*). While New Zealand is free and considered to be a relatively low risk country for introduction of the disease, raw pork is now imported from European countries which must be considered at risk of becoming infected. Transmission occurs by a number of routes (*113, 115*), but the relevant ones here are a variety of pigmeat products, imported legally or illegally.

While the virus can survive for very long times within the ambient temperature range, it is destroyed by heating for one hour at 55^{0}C. Therefore pasteurisation at 70^{0}C for one hour should eliminate any virus that is present (*116*). The effect of anaerobic digestion does not appear to have been investigated.

Hepatitis E virus (HEV) occurs in pigs, including New Zealand pigs (*117*). It can also occur in ruminants (*118*), and an unusual variant occurs in rats (*119*). It is an important cause of human hepatitis. The virus can be transmitted in both directions between people and pigs (*120*). Infection can be transmitted to people in meat products and milk (*118, 121*), and also in plant products that have been washed in HEV contaminated water. Hence feedstock for anaerobic digestion may contain the virus. The virus remains infectious for periods of weeks on dry surfaces (*122*).

Heating material containing HEV at 70^{0}C for one hour will eliminate virus (*123*). No evidence could be found on the effects of anaerobic digestion.

Norovirus is a highly infectious and very widespread pathogen of people. It may be present in some types of feedstock. Some strains can be transferred from people to pigs, but not in the other direction (*120*). A wide range of animal species carry noroviruses, and the evidence of zoonotic transfer of these viruses to people remains inconclusive (*124*).

Pasteurisation at 70^0C for one hour will eliminate the virus (*125*), and the probability of norovirus being present on material fertilised with biofertiliser is considered very low (*105*).

Prions

Prions represent a very different challenge for destruction because they contain no nucleic acid and are simply mis-folded proteins which are very difficult to destroy (*126*). Neither anaerobic digestion nor pasteurisation would eliminate infectious prions.

The three animal prion diseases of concern are bovine spongiform encephalopathy (BSE), scrapie of sheep and goats, and chronic wasting disease of deer (*127-130*). Transmission methods vary according to the particular disease (*131*). None of them are present in New Zealand, so no measures against them are required. The condition atypical scrapie/Nor98 is a spontaneous, non-contagious degenerative condition in older sheep. A single case was detected in a New Zealand sheep in 2009 (*127*), but it is not a prion disease, although it can lead to positive tests for scrapie.

There is some evidence suggesting that prions are involved in a wider range of disease of animals and people (*132-134*), but this does not raise the risk of prions being present in feedstock to anaerobic digestion in New Zealand.

Anaerobic digestion methods

There are many variations in the detail of how anaerobic digestion methods are implemented in a particular plant. While the fine details of individual methods will have some influence on the survival of organisms through the process, this discussion will be limited to the main factors.

The process of anaerobic digestion is as follows (*135*):

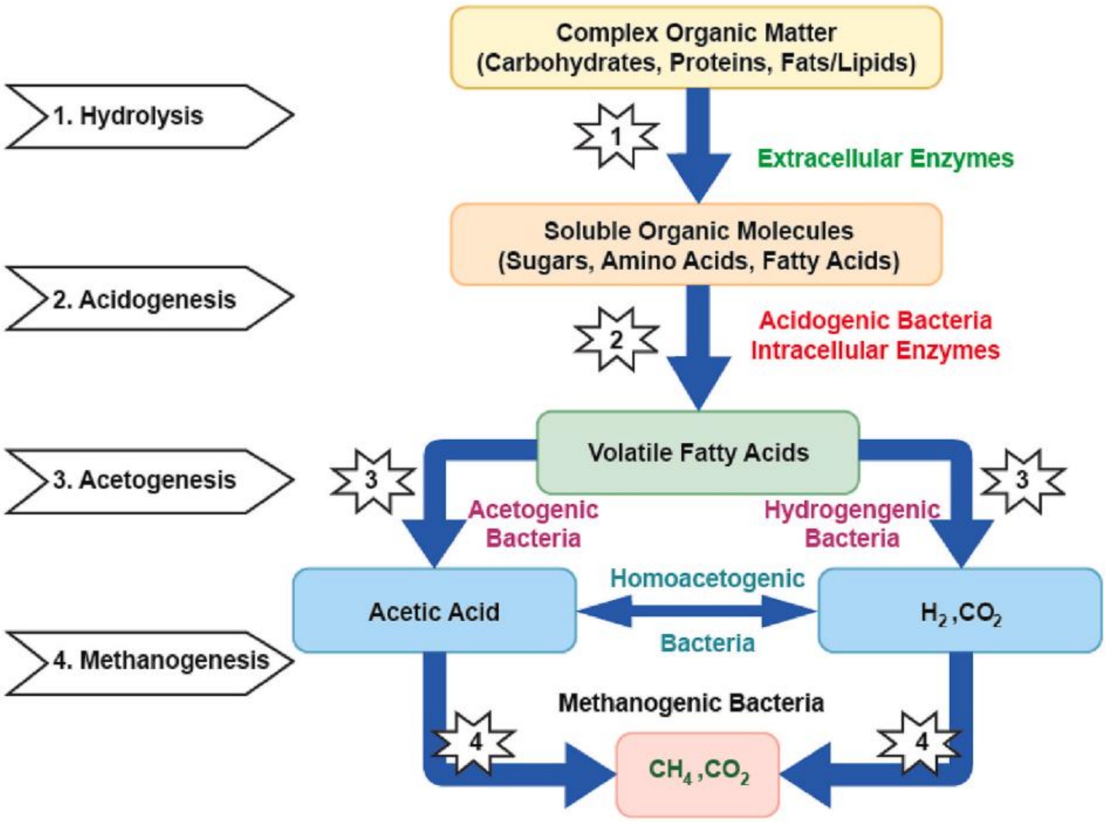

Figure 1 Anaerobic digestion process

The process can be carried out at three different temperature ranges – psychrophilic (temperature at ambient level or below), mesophilic (20 to 45^{0}C, usually 35-38^{0}C) and thermophilic (50 to 60^{0}C). The temperature level, duration of digestion and other influences such as pH and concentration of the digestate at various times through the digestion process can all influence survival of pathogens.

Effect of anaerobic digestion on organisms in feedstock

As described above, each of the major categories of pathogenic organisms vary in their susceptibility to different factors in their environment. The influences that are important are the process of anaerobic digestion, any pre-treatments that may be applied to the feedstock and any post-treatments that maybe applied to the biodigestate before it is distributed as biofertiliser.

A very recent meta-analysis (*136*) has evaluated 214 papers dealing with the pathogen reduction effects which result from anaerobic digestion, of which 121 met the criteria for inclusion in the study. Since this paper and its supplementary information cover the subject so fully, most of the evaluation used here makes use of the synthesis provided by this paper. An earlier review (*137*) and a meta-analysis (*138*) cover similar information and draw generally similar conclusions, but provide their results in extensive tables rather than in graphical form, so the latest meta-analysis with very informative graphical reporting has been used as the source of the analyses shown below.

Reduction in population of organisms from anaerobic digestion

Figure 2 shows box and whisker plots for the effect of anaerobic digestion in achieving log reductions in counts of different categories of organisms. Red graphs are pathogens, blue graphs are indicator organisms used to assess performance of the digester operation. Consistent with the earlier discussion, Gram negative organisms including E. coli show the greatest reduction, Gram positive spore forming bacteria show the least reduction, and others are inbetween.

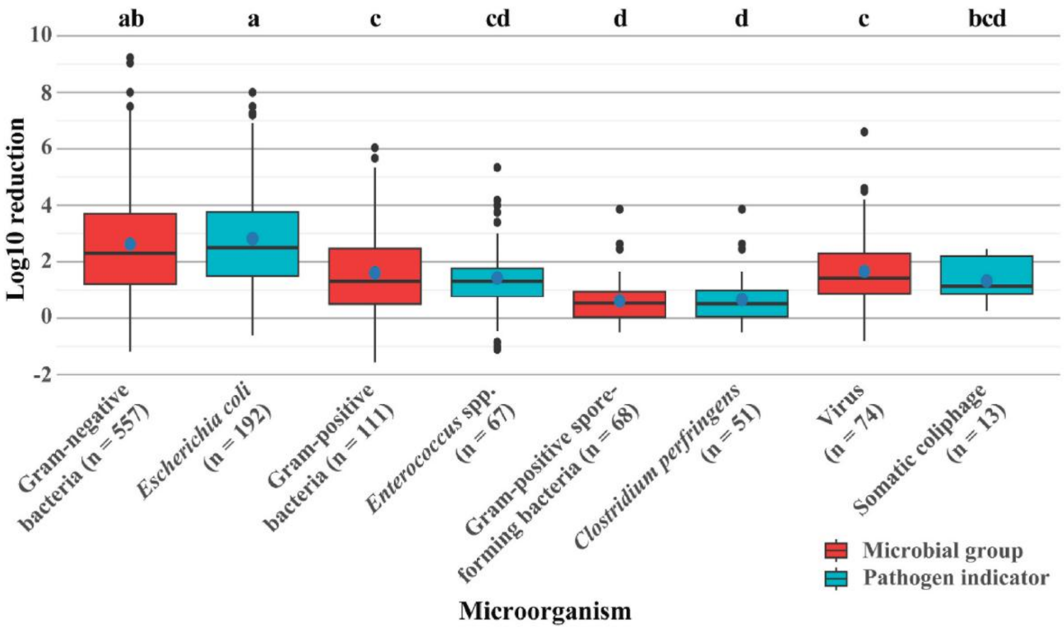

Figure 2 Microorganism Log10 reduction for different groups (red) of microorganisms and for their respective pathogen indicators (blue). Mean values are represented by blue dots. Only the microbial groups with three or more independent values (n ≥ 3) are presented. Identical letters above boxplots indicate homogeneous groups that are not significantly different. n is the number of source observations used to produce the graph.

Effect of digester temperature on reduction in organism populations

The temperature at which anaerobic digestion takes place is very influential on the effectiveness of the process in reducing the count of various types of organisms. Figure 3 shows that thermophilic digestion produces clearly the best result in reducing counts of non-spore forming bacteria and mildly better result than other temperature levels with viruses. However temperature has very limited effect on spore-forming bacteria, which show only small reduction at all temperatures.

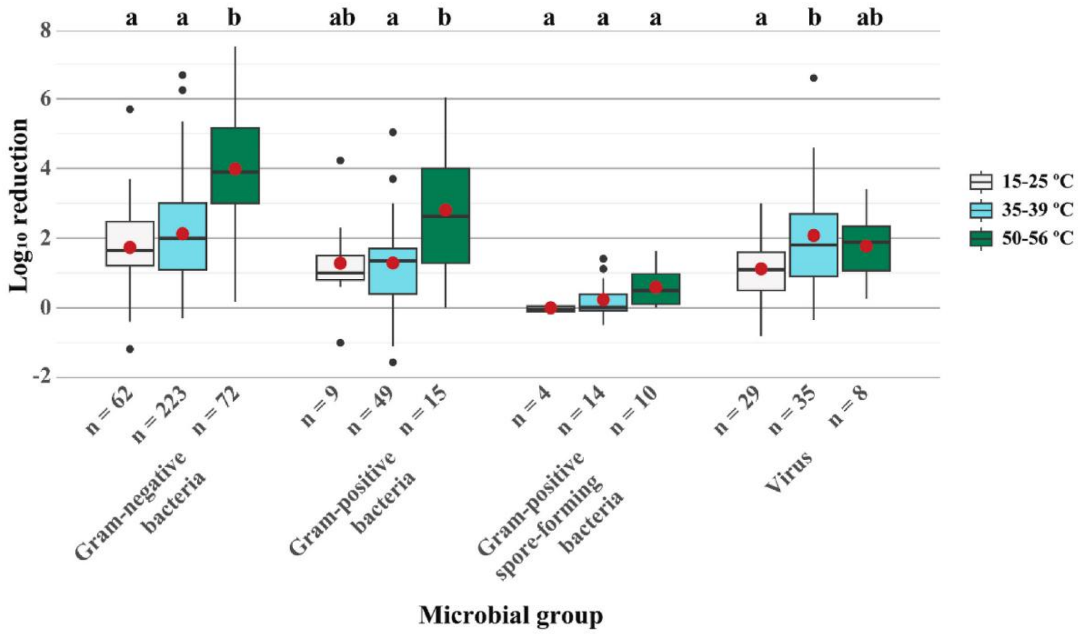

Figure 3 Microorganism Log10 reduction for different groups of microorganisms and for different temperature ranges. Mean values are represented by red dots. Identical letters above boxplots indicate homogeneous groups. n stands for the number of independent datapoints.

Duration of digestion process

The duration of the digestion process is also influential, but unfortunately there is insufficient data to assess this effect for spore forming bacteria. Longer duration generally achieves greater reduction,

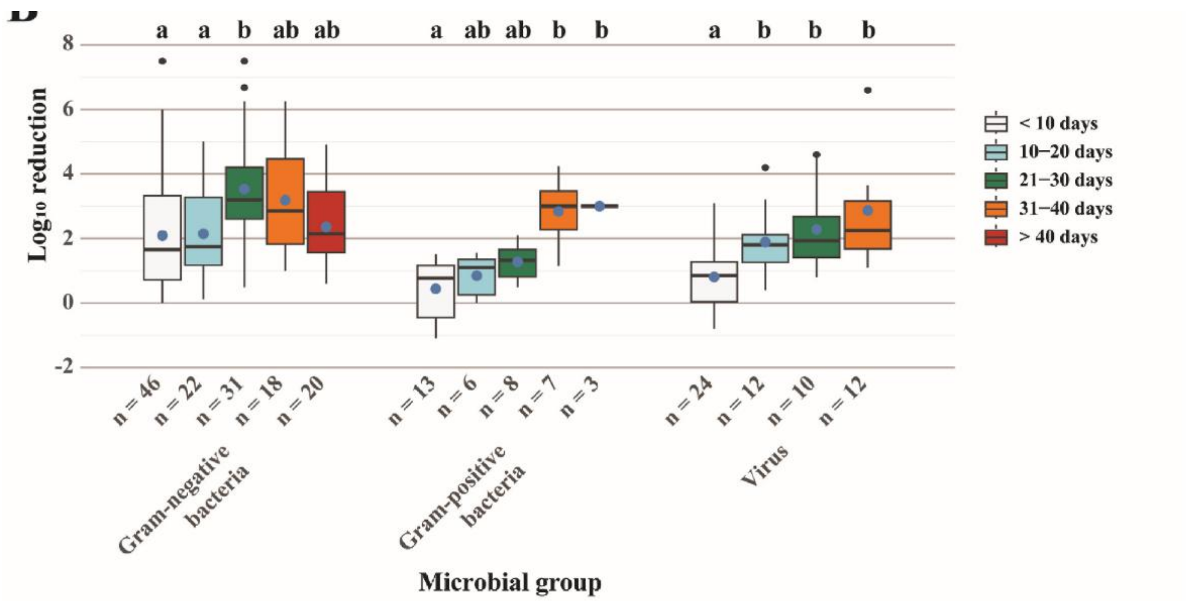

Figure 4 Effect of duration of digestion on reduction in organism count Microorganism Log10 reduction for each microbial group in batch reactors with different durations. Mean values are represented by blue dots. Only the conditions with three or more independent values (n ≥ 3) are presented, so spore-forming bacteria are absent.. Identical letters above boxplots indicate homogeneous groups. n stands for the number of independent datapoints.

Effect of digester type on organism reduction

Different digester types (Figures 5 and 6) have limited influence on reducing organism count. Within the batch system, sampling at different stages of the process produces different results because the nature of the digestion process is at a different phase.

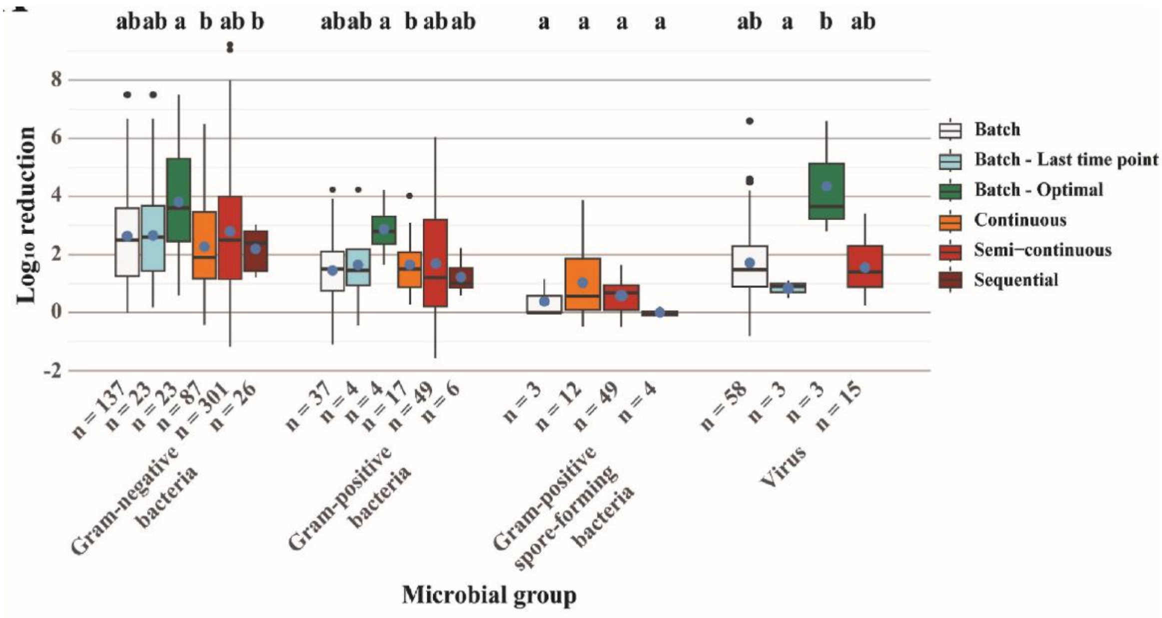

Figure 5 Effect of digester type on reduction in organism count. Microorganism Log10 reduction for batch processing with sampling at different stages, compared with non-batch methods. Mean values are represented by blue dots. Only the conditions with three or more independent values (n ≥ 3) are presented. Identical letters above boxplots indicate homogeneous groups. n stands for the number of independent datapoints.

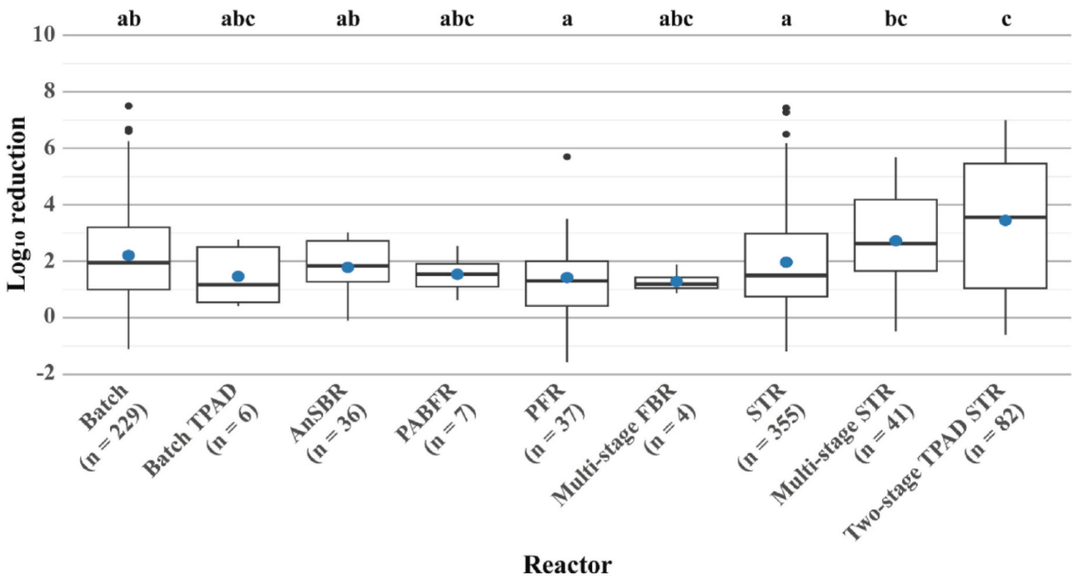

Figure 6 Overall microorganism Log10 reduction for different reactor types. Mean values are represented by blue dots. Only the reactors with three or more independent values (n ≥ 3) are presented. Identical letters above boxplots indicate homogeneous groups. TPAD stands for temperature phased anaerobic digestion, AnSBR for anaerobic sequencing batch reactor, PABFR for panelled anaerobic baffle-cum-filter reactor, PFR for plug flow reactor, FBR for fixed bed reactor, and STR for stirred tank reactor. n stands for the number of independent datapoints.

Effect of pH on organism reduction

Organism count is reduced by low or high pH to a greater extent than when pH is close to neutral.

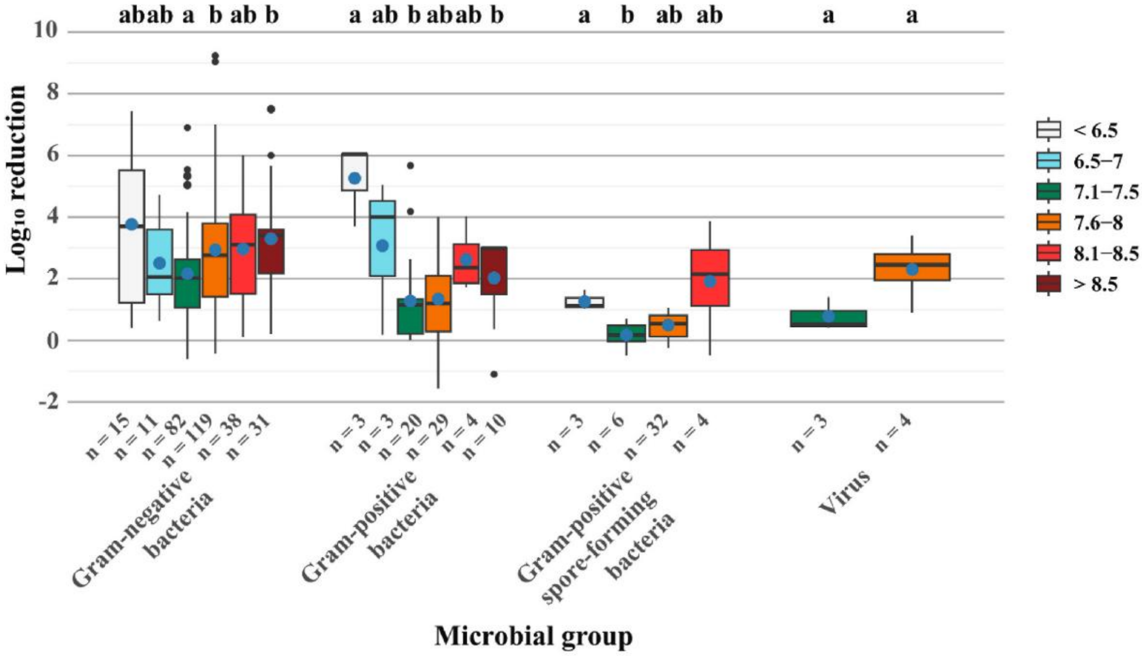

Figure 7 Microorganism Log10 reduction for different groups of microorganisms and for different pH ranges. Mean values are represented by blue dots. Only conditions with three or more independent values ($n \geq 3$) are presented. Identical letters above boxplots indicate homogeneous groups. n stands for the number of independent datapoints.

Effect of hydraulic retention time

Hydraulic retention time is the average length of time that a liquid spends in a reactor such as an anaerobic digester. Extended hydraulic retention time in the digester has limited effect on organism reduction.

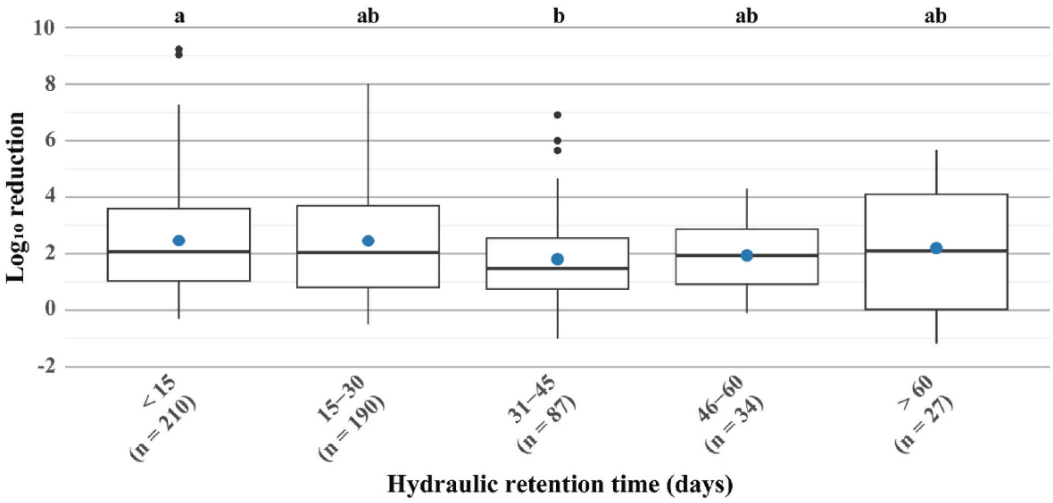

Figure 8 Overall microorganism Log10 reduction for different hydraulic retention time (HRT) ranges. Mean values are represented by blue dots. Identical letters above boxplots indicate homogeneous groups. n stands for the number of independent datapoints.

Effect of various forms of pretreatment and post-treatment

Ozonation prior to digestion is the most effective treat for reducing the count of undesirable organisms, with heat as the second best option, best after digestion but also effective prior to digestion.

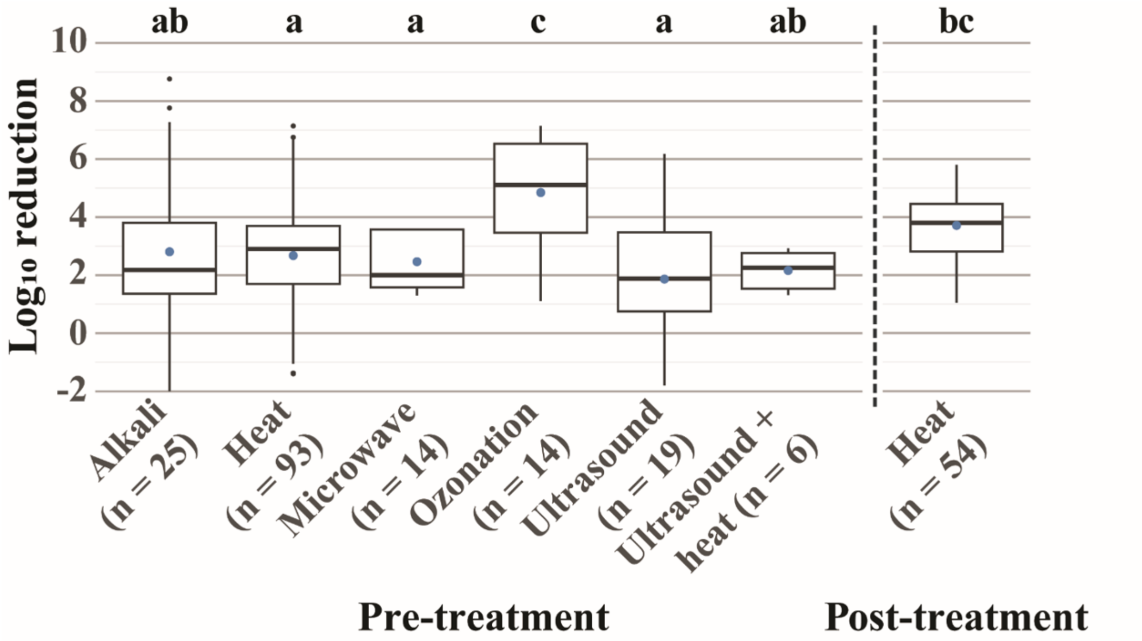

Figure 9 Effect of various forms of treatment prior to digestion and after digestion on reduction in organism count

Effect of combining anaerobic digestion with pre- or post-hygienization

In relation to EU or US EPA standards, thermophilic digestion meets both standards for all organisms except Clostridium perfringens, used as representing spore forming Gram positive bacteria. Mesophilic digestion fails or barely meets the standards when used alone, but can meet the EU standard when used in conjunction with pre- or post-hygienization. It fails the US standard. Psychrophilic digestion fails to meet either standard, even with pre- or post-treatment.

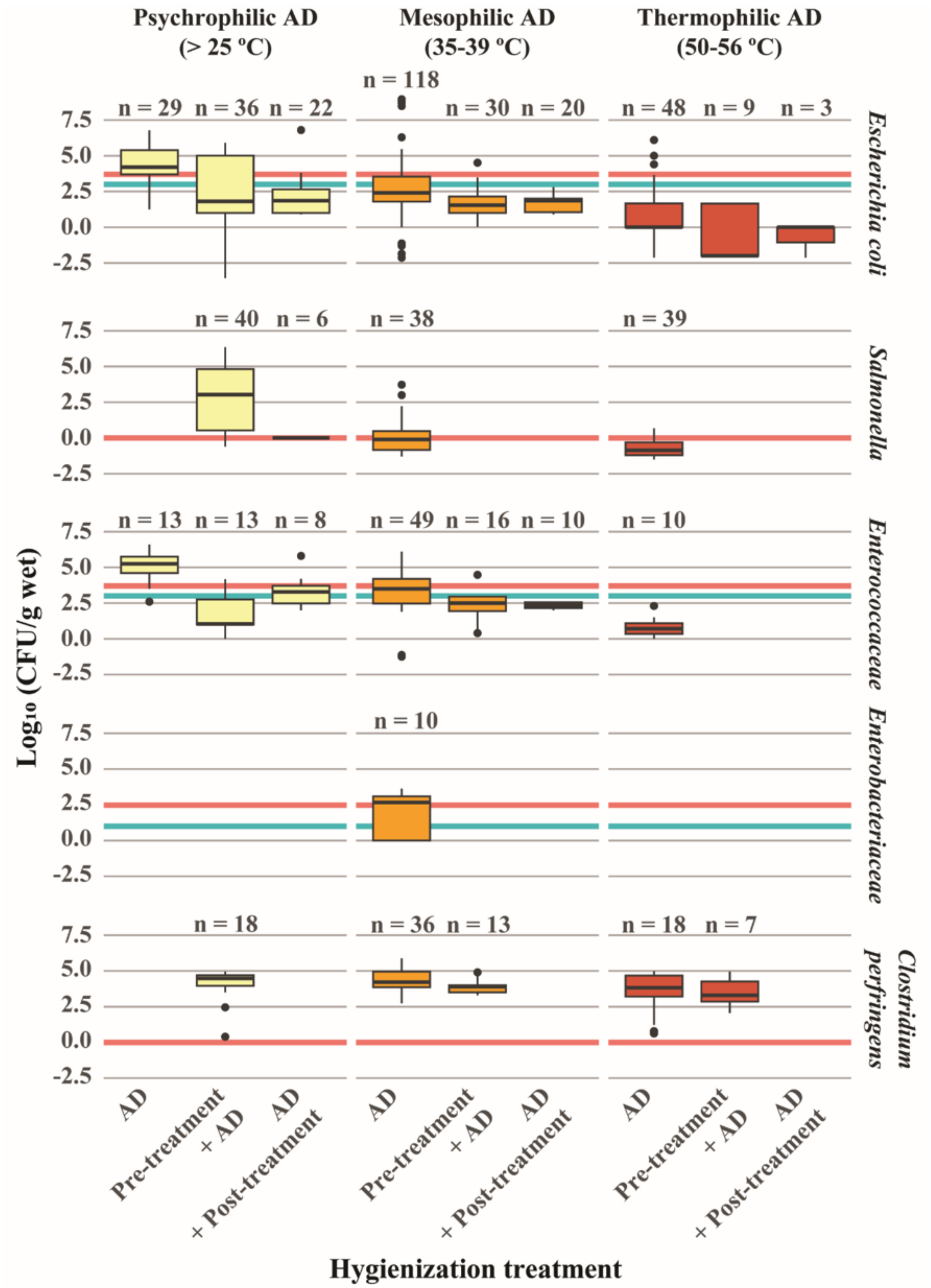

Figure 10 Effect of combining anaerobic digestion with either pre- or post-digestion treatment of any type on the count of different specific organisms and comparison with upper limit (red line) or lower limit where applicable (blue line) in EU regulatory limits described in CE142/2011.

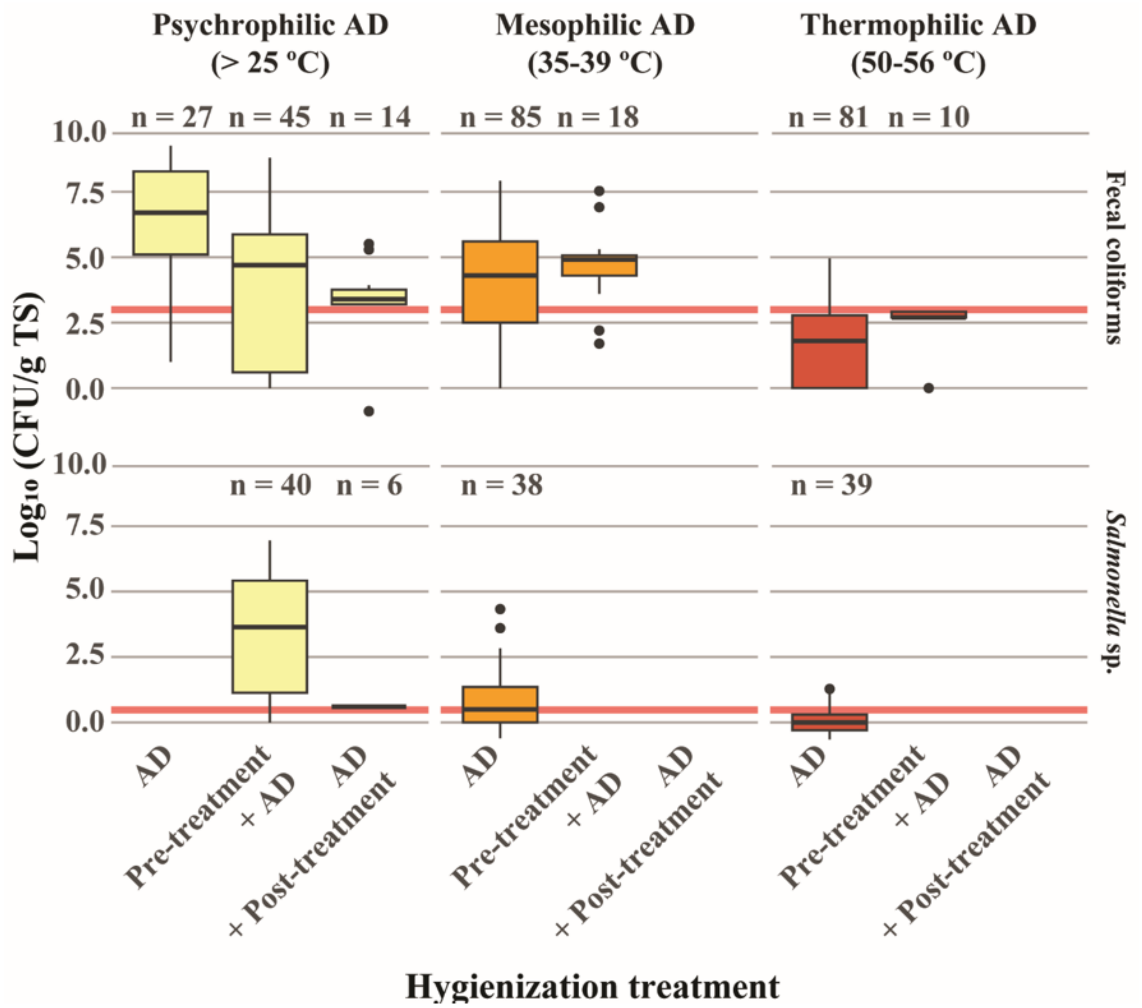

Figure 11 Effect of various forms of hygienization on count of specific organisms as required by US EPA Class A biosolids regulatory limits (EPA/600/R-22/194).

Summary of effects of factors on reduction in organisms by treatments

Table 2 provides a summary of the information provided earlier in the report concerning the susceptibility of different pathogens to reduction or elimination by each of the potential components of the production process for biofertiliser. While there is a degree of uncertainty about some items due to a lack of sufficient evidence, the table provides an overview of the expected position. It shows the risk that the particular organism will be present in feedstock supplied to the digester, which determines the initial level of risk. This is then modified by the temperature of the digester, and by the use of pasteurisation.

With the exception of exotic disease agents in the list, all other organisms listed occur naturally on a proportion of New Zealand farms. Therefore the use of biofertiliser as produced in New Zealand may increase the risk of an endemic disease occurring in animals on a farm if it raises the concentration of organisms on pasture consumed by animals. Table 2 shows that in New Zealand this is only the case for animal diseases caused by Gram positive spore forming bacteria. The risk is low for animals, and very low to negligible for people who may be exposed to the biofertiliser. Methods for managing this risk are described in the next section of the report.

Pathogen group	In feedstock	Psychrophilic digestate	Mesophilic digestate	Thermophilic digestate	After pasteurisation	Animal disease risk effect	Human disease risk effect
Campylobacteriaceae	Present	Present	Present	Possible	Absent	Nil	Nil
Enterobacteriaceae	Present	Present	Present	Absent	Absent	Nil	Nil
Moraxellaceae	Present	Unknown	Unknown	Present	Absent	Nil	Nil
Mycoplasmataceae	Unlikely	Unlikely	Unlikely	Absent	Absent	Nil	Nil
Yersiniaceae	Present	Present	Present	Possible	Absent	Nil	Nil
Enterococcaceae	Present	Present	Present	Present	Absent	Nil	Nil
Listeriaceae	Present	Present	Present	Possible	Absent	Nil	Nil
Bacillaceae	Present	Present	Present	Present	Present	Low	Very low
Peptostreptococcaceae	Present	Present	Present	Present	Present	Low	Very low
Clostridiaceae	Present	Present	Present	Present	Present	Low	Very low
Fungi	Present	Unknown	Unknown	Unlikely	Absent	Nil	Nil
Thermostable mycotoxins	Possible	Possible	Possible	Possible	Possible	Very low	Nil
Protozoan parasites	Present	Possible	Unlikely	Absent	Absent	Nil	Nil
Metazoan parasites	Present	Present	Possible	Absent	Absent	Nil	Nil
Trichinella species	Unlikely	Unlikely	Unlikely	Absent	Absent	Nil	Nil
Foot and mouth disease	Absent	Unknown	Unknown	Absent	Absent	Nil	Nil
African swine fever	Absent	Unknown	Unknown	Absent	Absent	Nil	Nil
Hepatitis E	Present	Unknown	Unknown	Absent	Absent	Nil	Nil
Norovirus	Present	Unknown	Unknown	Absent	Absent	Nil	Nil
Prions	Absent	No effect	No effect	No effect	No effect	Nil	Nil

Table 2 Expected risk level following different treatments to produce biofertiliser

Evaluation of risk management strategy

The Bioenergy Association report "Guidelines for the production of digestate biofertiliser for application to land" May 2025 lays out a strategy for procedures to be used. The document does not differentiate between procedures required for psychrophilic, mesophilic or thermophilic digestion. Thermophilic digestion achieves effective reduction of most organisms of concern, but mesophilic and psychrophilic digestion does not, and absolutely requires pasteurisation to achieve the same result.

Steps in the procedure

Although it is not explicitly stated as a requirement, it appears that pasteurisation of feedstock for one hour at 70^0C prior to anaerobic digestion is the recommended measure. Figure 9 shows that using pasteurisation on the biodigestate after anaerobic digestion is more effective, and this also contributes to preventing any possible contamination of digestate with material from feedstock.

The 21 day lay off period between application of biofertiliser to pasture and the introduction of grazing animals to the pasture does not appear to be based on scientific evidence, but is a precautionary approach. The main benefit is likely to be washing of any organisms off pasture and into soil, or destruction of organisms by sunlight.

Pathogens likely to be present in biofertiliser

The only group of organisms of importance which are likely to be present in the biofertiliser in significant quantities are the Gram positive spore forming bacteria. The members of the *Clostridium* genus which will be present and can cause animal disease are already present on virtually all farms. Most farmers vaccinate against all of them using clostridial vaccines which provide cover against up to ten different species/toxin types. This will effectively avoid any risk attached to the use of fertiliser. Therefore consideration could be given to recommending that farms which use biofertiliser ensure that their animals receive a clostridial vaccine before they begin using biofertiliser. Of the clostridial diseases, the only one considered a practical risk to people involved in handling biofertiliser is tetanus, and children are vaccinated with a series of doses between 6 weeks of age and 11years, which will give them effective lifetime protection against any wound which allows *Clostridium tetani* to produce toxin.

Bacillus cereus and related organisms can produce disease in both animals and people, but the risk is extremely low, the organisms are ubiquitous in the rural environment anyway, so no specific measures are considered necessary.

This leaves only *Clostridioides difficile* as an organism of concern which is expected to be in the biofertiliser product. As described above, this is an emerging pathogen of growing importance worldwide (*139, 140*). Infection is mainly nosocomial, but community transmission is becoming more common. Older age groups are at particular risk. Infection occurs in a wide range of animals (*54, 141-143*), but disease is uncommon. People are extensively exposed to the organism in products (*54, 141, 144*), but disease mainly occurs in people undergoing antibiotic treatment. There is active research currently to develop a vaccine for human use, especially in older age groups (*145-147*). Management strategies for human cases have evolved in recent years (*148*). Therefore it is concluded that the use of biofertiliser will make minimal contribution to the already common exposure of animals and people to this pathogen.

Feedstock pasteurisation requirements

The guidelines separate feedstock types into two categories.

Biodegradable Organic Material	
Group A (requires pasteurisation)	**Group B (does not require pasteurisation)**
Kerbside food waste collections including FOGO	Agricultural break crops
Food manufacturing that is not solely plant based	Fruit, orchard and packhouse waste
Primary processing, i.e. approved abattoirs	Vegetable, paddock waste including stems, roots
Post consumer foodstuffs • Food approved for human consumption, i.e. spoiled • Restaurant, café, catering waste (including milk, milk product, egg products)	Forage, grass, pasture Food manufacturing that is solely plant based Brewery waste Vineyard – grape marc
Fats, oils, grease, grease trap	Animal manure (biofertiliser must be used on same farm from which the manure is sourced)
Paunch grass	
DAF ex abattoir, dairy farm or factory	
Animal manure (biofertiliser can be used on farms from which the manure is not sourced)	

Items in Group B do not require pasteurisation. All the items except animal manure are derived from plants, and not expected to include animal or human pathogens. Animal manure is allowed if the biofertiliser is used on the same farm from which the manure is sourced.

While this will not introduce new organisms to the farm, it may increase the risk of disease outbreaks among stock on the farm, and could potentially also put people on the farm at greater risk. This is because the digestion process can potentially increase the concentration of pathogens in the biofertiliser as a direct consequence of the digestions process. As examples, *Campylobacter* spp. (*149*) and *Listeria monocytogenes* (*150, 151*) are both recognized causes of significant outbreaks of disease in human populations due to contamination of food or water with ruminant faeces, as well as causing disease outbreaks in animals. They are both facultative anaerobes, which can multiply under the conditions of anaerobic digestion at the temperatures of the digestion process. Therefore if the organisms are present in the manure used as feedstock and the product is not pasteurised, the concentration on pasture to which the biofertiliser is applied may be considerably higher than would otherwise be the case. This could potentially increase the risk of disease occurring in animals or people on the farm as a result of biofertiliser application.

Microbiological monitoring of biofertiliser

The guidelines require microbiological monitoring to be used on biofertiliser to ensure that the production process is achieving a level of occurrence of particular organisms less than a stated maximum.

Parameter	Standard	Authorised Analysis Methodology
E coli	Less than 100 MPN/g	Part 9221 F (modified) Standard Methods for the Examination of Water and Wastewater (APHA, 23rd ed. 2017)
Campylobacter	Less than 1/25g	Enumeration of Thermotolerant Campylobacter in Biosolids (A. Donnison, AgResearch Limited) Appendix 1 Biosolids Guidelines
Salmonella	Less than 2 MPN/g	Salmonella sp bacteria: Part 9260 D, Standard Methods for Examination of Water and Wastewater, (APHA, 1988), or Detection and enumeration of salmonella and Pseudomonas aeruginosa (Kenner and Clark, 1974)

[Source: PAS 110:2014 Specification for whole digestate, separated liquor and separated fibre derived from the anaerobic digestion of source-segregated biodegradable materials]

During the initial product monitoring phase, for Group A products there must be at least five sequential samples which meet these criteria. After verification has been completed, 5 samples must be taken during each 12 month period, with gaps of at least two months between samples. There does not appear to be a provision to prevent resampling within the two month period if the first test is a fail, but the product which gave the fail must be processed in one of the ways described in 9.6.

The monitoring requirement is derived from PAS 110:2014, a voluntary British Standards Institution (BSI) specification, developed by the British Waste and Resources Action Program (WRAP).

Much more recently the European Parliament has issued Regulation (EU) 2019/1009, updated to 20 November 2024, laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003.

This provides the following monitoring requirement:

Micro-organisms to be tested	Sampling plans			Limit
	n	c	m	M
Salmonella spp.	5	0	0	Absence in 25 g or 25 ml
►**M10** ↓ *Escherichia coli* or enterococci ◄	5	5	0	1 000 in 1 g or 1 ml

Where:

n = number of samples to be tested,

c = number of samples where the number of bacteria expressed in colony forming units (CFU) is between m and M,

m = threshold value for the number of bacteria expressed in CFU that is considered satisfactory,

M = maximum value of the number of bacteria expressed in CFU.

This protocol has advantages over the PAS 110: 2014 one. The PAS one uses three Gram negative enterobacteria, which have very similar resistance to each of the three forms of anaerobic digestion, and have low resistance to pasteurisation. Hence if one is absent from the digestate, the other two are also likely to be absent. In addition, while *E. coli* and Salmonella are relatively easy to culture and to quantify, *Campylobacter* presents significantly more complicated challenges for culture and enumeration (*152*).

The updated EU protocol is easier to undertake rapidly at low cost. The *Enterococcus* genus is a Gram positive organism that is more robust than E.coli, so using Salmonella and Enterococcus provides a two-level assessment of process effectiveness. Since the Enterococcus limit is not as restrictive as the Salmonella limit, it also provides a way of monitoring trends in count, not just presence/absence. Methods for conducting monitoring of Enterococcus are available and straightforward (*153, 154*). I therefore suggest reconsidering the organisms to be tested, and the limits required. The EU protocol is likely to be lower cost than the PAS one.

It is possible to use PCR methods for detection of these species in biofertiliser, but they are not in all cases an adequate replacement for more traditional culture and enumeration methods (*155, 156*).

Risks of using biofertiliser on human food crops

The assessment undertaken in this report relates to managing the use of biofertiliser on pasture and feed crops consumed only by animals, and risks to people who are handling the biofertiliser or to people consuming products from animals which had consumed feed to which biofertiliser had been applied. Direct application of biofertiliser to human food crops, especially those consumed in raw or under-cooked form, may enhance the risk level (*105, 143*). This may result from either seemingly minor failures of correct processing practices, or multiplication of very small numbers of organisms which survived processing and found a favourable environment on the food crop, or even perhaps in post-harvest handling. This would require a more comprehensive risk analysis (*125*), which would be greatly complicated by the difficulty of accurately defining critical control points in the production and processing of human food crops across New Zealand, and the challenges of making quantitative evaluations of the risk attached to these points. Use of biofertiliser on human food crops has not been considered as an option in this report.

Conclusions

Provided that pasteurisation at 70^0C for 60 minutes is undertaken before or preferably after anaerobic digestion of Group A feedstock, microbiological risks (other than spore-forming Gram positive organisms) associated with the use of the digestate as biofertiliser can be eliminated. With regard to the various species of spore-forming Gram positive organisms, the risk cannot be eliminated, but it can be adequately managed.

There are two potential causes of pathogens being present in biofertiliser in sufficient concentration to be infectious for animals or people. The first is failure to follow required practices which totally prevent contact between feedstock and biofertiliser, such as by use of the same clothing or tools on both sides of the process. The second is failure to comply with the full requirements for eliminating organisms, such as by not using the correct temperature or time in pasteurisation. Both errors would represent failure to correctly follow the guidelines, and should be detected by microbiological monitoring – although not necessarily within the desired time frame.

If the guidelines are correctly followed, biofertiliser will provide a valuable product for use on pasture and feed crops.

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Appendix A

Animal Diseases and Zoonoses in International Documents Considered for Inclusion in Biofertiliser Report

This list provides the epitypes which were used to create Table1. It shows epitypes for diseases when transmitted between animals, and epitypes for transmission of zoonoses between animals and people. In total 161 diseases are listed. The 120 diseases of mammals and birds are taken from the list of diseases reported biannually to the World Organization for Animal Health. Some are zoonoses.

An additional 41 zoonoses are derived from lists provided by the Centers for Disease Control and Prevention in the United States, https://www.cdc.gov/healthy-pets/diseases/index.html#cdc_facts_stats_trends-a-to-z-list-of-zoonotic-diseases

and the United Kingdom government https://www.gov.uk/government/publications/list-of-zoonotic-diseases/list-of-zoonotic-diseases

plus additional zoonoses considered important.

Because some diseases fit more than one epitype due to two or three separate transmission methods, there are 177 epitypes listed for the 161 diseases in animals. There are 101 epitypes describing transmission methods to people for 87 zoonotic diseases. The list of zoonoses is far from comprehensive, but intended to provide examples of zoonoses recognized as important.

Epitype	Species mainly affected	Zoonotic epitype	Disease
			WOAH List
9	Multiple		Aeromonas hydrophila (Inf. with)
8	Equids		African horse sickness virus (Inf. with)
2	Suids		African swine fever virus (Inf. with)
9	Multiple	3	Anthrax
2	Pig		Atrophic rhinitis of swine (-2005)
2	Multiple		Aujeszky's disease virus (Inf. with)
2	Multiple	2	Avian chlamydiosis
2	Poultry		Avian infectious bronchitis
2	Poultry		Avian infectious laryngotracheitis
2	Poultry		Avian mycoplasmosis (M.synoviae) (2006-)
2	Poultry		Avian mycoplasmosis (Mycoplasma gallisepticum)
3	Poultry		Avian tuberculosis (-2005)
8	Ruminants		Bluetongue virus (Inf. with)
9	Multiple	4	Botulism (-2014)
8	Cattle		Bovine anaplasmosis
8	Cattle		Bovine babesiosis

7	Cattle	7	Bovine cysticercosis (-2014)
1	Cattle		Bovine genital campylobacteriosis
4	Cattle	4	Bovine spongiform encephalopathy
2	Multiple	2	Bovine tuberculosis (-2018)
2	Cattle		Bovine viral diarrhoea (2006-)
2	Cattle	3,4	Brucella abortus (Inf. with)
2	Sheep, goat	3,4	Brucella melitensis (Inf. with)
2	Suids	3,4	Brucella suis (Inf. with)
2	Equids	2	Burkholderia mallei (Inf. with) (Glanders)
2	Camels		Camelpox (2006-)
2	Goat		Caprine arthritis/encephalitis
2	Sheep, goat	3	Chlamydia abortus (Inf. with) (Enzootic abortion of ewes, ovine chlamydiosis)
2	Pig		Classical swine fever virus (Inf. with)
2	Sheep, goat		Contagious agalactia
2	Goat		Contagious caprine pleuropneumonia
1	Horse		Contagious equine metritis
6	Multiple	3,6	Crimean Congo haemorrhagic fever (2006-)
3	Multiple	3	Dermatophilosis (-2005)
1	Equids		Dourine
2	Ducks		Duck virus enteritis (-2005)
2	Ducks		Duck virus hepatitis
7	Multiple	7	Echinococcosis/hydatidosis
7	Multiple	7	Echinococcus granulosus (Inf. with) (2014-)
7	Multiple	7	Echinococcus multilocularis (Inf. with) (2014-)
6	Dog		Ehrlichia canis (Inf. with)
2	Pig		Enterovirus encephalomyelitis (-2005)
1	Cattle		Enzootic bovine leukosis
6	Ruminants		Epizootic hemorrhagic disease virus (Inf. with)
9	Equids		Epizootic lymphangitis (-2005)
2	Equids		Equid herpesvirus-1 (Inf. with) (Equine rhinopneumonitis) (2014-)
1,2	Equids		Equine arteritis virus (Inf. with)
6	Equids	6	Equine encephalomyelitis (Eastern and Western)(-2005)
6	Equids	6	Equine encephalomyelitis (Eastern)(2006-)
6	Equids	6	Equine encephalomyelitis (Western)(2006-)
6	Equids		Equine encephalosis virus (Inf. with)
5,1,3	Equids		Equine infectious anaemia
2	Equids		Equine influenza virus (Inf. with)
6	Equids		Equine piroplasmosis
2	Equids		Equine rhinopneumonitis (-2013)
2,3	Multiple		Foot and mouth disease virus (Inf. with)
2,3	Poultry	2,3	Fowl cholera (-2011)
2,3,6	Poultry		Fowl pox (-2005)
2,6,1	Poultry		Fowl typhoid
3	Bovids		Haemorrhagic septicaemia
6	Ruminants		Heartwater

2	Horse	2	Hendra viruses (Inf. with)
2	Poultry	2	Highly pathogenic avian influenza (poultry)
2	Birds	2	Highly pathogenic influenza A viruses (Inf. with)(non-poultry including wild birds)(2017-)
2	Horse		Horse mange (-2005)
2	Horse		Horse pox (-2005)
2	Bovids		Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis
3	Poultry		Infectious bursal disease (Gumboro disease)
2	Multiple	2	Influenza A virus (Inf. with)
6	Multiple	6	Japanese encephalitis
8	Multiple	8	Leishmaniosis
2	Multiple	2	Leptospirosis
2	Multiple	2	Low pathogenic avian influenza (poultry)(2006-)
6	Bovids		Lumpy skin disease virus (Inf. with)
2	Sheep, goat		Maedi-visna
2	Cattle		Malignant catarrhal fever (wildebeest only)(2006-2008)
2	Poultry		Marek's disease (-2011)
2	Camels	2	Middle East respiratory syndrome coronavirus (MERS-CoV)(Inf. with)
2	Monkeys	1,2	Mpox
2	Multiple	2	Mycobacterium tuberculosis (Inf. with)(-2017)
2	Multiple	2	Mycobacterium tuberculosis complex (Inf. with)(2019-)
2	Bovids		Mycoplasma mycoides subsp. mycoides SC (Inf. with) (Contagious bovine pleuropneumonia)
6,2	Rabbits		Myxomatosis
6	Sheep, goat	6	Nairobi sheep disease
5	Multiple	5	New world screwworm (Cochliomyia hominivorax)
2	Poultry		Newcastle disease virus (Inf. with)
5	Multiple	5	Old world screwworm (Chrysomya bezziana)
1	Sheep, deer		Ovine epididymitis (Brucella ovis)
2	Sheep, goat		Ovine pulmonary adenomatosis (-2005)
2	Ruminants		Paratuberculosis
2	Sheep, goat		Peste des petits ruminants virus (Inf. with)
2	Birds		Pigeon rotavirus
2	Pig		Porcine epidemic diarrhoea virus (Inf. with)
2	Pig		Porcine reproductive and respiratory syndrome virus (Inf. with)
2	Pig		Postweaning Multisystemic Wasting Syndrome (PMWS)
2	Multiple		Pox viruses (other than those listed by the OIE)(Inf. with)(2011-)
1,2	Poultry		Pullorum disease
2	Ruminants	2	Q fever
2,5	Rabbits		Rabbit haemorrhagic disease
1	Multiple	1	Rabies virus (Inf. with)
6	Multiple	6	Rift Valley fever virus (Inf. with)
2	Sheep		Salmonellosis (S. abortusovis)
2	Multiple	2	SARS-CoV-2 in animals (Inf. with)

6	Ruminants		Schmallenberg virus (Inf. with)
2	Sheep, goat		Scrapie
2	Sheep, goat		Sheep pox and goat pox
2	Pig	2	Streptococcus suis (Inf. with)
6	Multiple		Surra (Trypanosoma evansi)
2	Pig		Swine vesicular disease (-2014)
6	Pig	6	Taenia solium (Inf. with) (Porcine cysticercosis)
6	Ruminants		Theileriosis
2	Pig		Transmissible gastroenteritis
1	Pig	4	Trichinella spp. (Inf. with)
1	Bovids		Trichomonosis
6	Bovids	6	Trypanosomosis (tsetse-transmitted)
6	Multiple	6	Tularemia
2	Turkey		Turkey rhinotracheitis (2006-)
6	Equids	6	Venezuelan equine encephalomyelitis
2	Pig		Vesicular stomatitis (-2014)
6	Multiple	6	West Nile Fever
			Additional zoonoses, mainly from US CDC and UK lists, not included above
2,3	Dog, cat	2,3	Ancylostoma brazilienze
2,3	Dog, cat	2,3	Ancylostoma caninum
2	Cat	1	Bartonella henselae
2	Monkeys	2	B virus (Herpes B)
6	Multiple	6	Borrelia burgdorferi
9	Multiple	9	Cyptococcus neoformans
2	Cat, dog	1	Capnocytophaga spp.
2	Birds	2	Chlamydia psittaci
9	Multiple	9	Clostridioides difficile
2	Multiple	2	Contagious ecthyma (parapox)
2	Multiple	2,4	Corynebacterium ulcerans
2,4	Multiple	2,4	Cryptosporidium
2	Multiple	2	Dermatophytes (Microsporum, Trichophyton)
5	Dog, cat	5	Dipylidium
2	Multiple	2	Ebolavirus
2	Pig, fish	2	Erysipelothrix
4	Multiple	4	Escherichia coli (specific strains)
8	Multiple	8	Fascioliasis
4	Multiple	4	Giardia
2	Rodents	2	Hantavirus
4	Pig	4	Hepatitis E
9	Multiple	9	Histoplasma
2,6	Multiple	2,6	Kyasanur Forest Disease
2	Rodents	2	Lassa fever
4	Multiple	4	Listeria monocytogenes
6	Multiple	6	Louping ill
2	Rodents	2	Lymphocytic choriomeningitis
2	Bats	2,4	Nipah virus

6	Rodents	6	Orientia tsutsugamushi (scrub typhus)
2	Multiple	2	Pasteurella spp.
1	Rodents	1	Rat bite fever (Streptobacillus moniliformis, Spirillum minus)
6	Multiple	6	Rickettsia rickettsiae
2	Multiple	1,2	Sarcoptes scabiei
2	Cat	2	Sporothrix schenkii (Sporotrichosis)
2	Horse	2	Streptococcus zooepidemicus
4	Dog, cat	4	Toxocariasis
4	Multiple	1,4	Toxoplasma gondii
4	Canids	2	Uncinaria stenocephala
2	Primates	2	Yellow fever virus
4	Multiple	4	Yersinia enterocolitica and Y. pseudotuberculosis
6	Rodents	6	Yersinia pestis