



Review

Anaerobic digestion of agricultural manure and biomass – Critical indicators of risk and knowledge gaps



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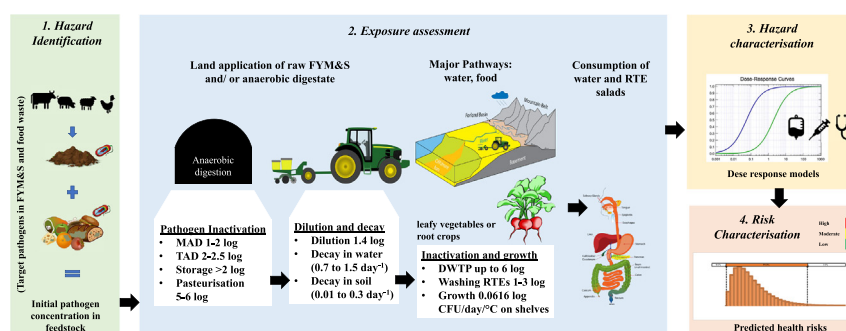
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HIGHLIGHTS

- Health risks associated with land-spreading of animal waste and anaerobic digestate
- Storage time and process parameters significantly influence pathogen inactivation.
- Pasteurisation, decay and dilution in soil reduce pathogen count.
- Need for process standardisation and unified risk assessment approach
- Need for enhanced AD farm-based modelling and data collation

GRAPHICAL ABSTRACT



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ABSTRACT

Anaerobic digestion (AD) has been identified as a potential green technology to treat food and municipal waste, agricultural residues, including farmyard manure and slurry (FYM&S), to produce biogas. FYM&S and digestate can act as soil conditioners and provide valuable nutrients to plants; however, it may also contain harmful pathogens. This study looks at the critical indicators in determining the microbial inactivation potential of AD and the possible implications for human and environmental health of spreading the resulting digestate on agricultural land. In addition, available strategies for risk assessment in the context of EU and Irish legislation are assessed. Storage time and process parameters (including temperature, pH, organic loading rate, hydraulic retention time), feedstock recipe (carbon-nitrogen ratio) to the AD plant (both mesophilic and thermophilic) were all assessed to significantly influence pathogen inactivation. However, complete inactivation of all pathogens is unlikely. There are limited studies evaluating risks from FYM&S as a feedstock in AD and the spreading of resulting digestate. The lack of process standardisation and varying feedstocks between AD farms means risk must be evaluated on a case by case basis and calls for a more unified risk assessment methodology. In addition, there is a need for the enhancement of AD farm-based modelling techniques and datasets to help in advancing knowledge in this area.

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1. Introduction

Livestock contributes approximately 40% of the global value of agricultural products, providing livelihoods and food security for almost 1.3 billion people worldwide (WHO, 2017). Sustainable agriculture and food production remain a major area of investigation to feed a growing population (Rockström et al., 2017). Likewise, the agri-food sector is one of the major contributors to the economic growth of Ireland; DAFM (2017) has reported that the food and beverages sector accounts for 24% of all industry turnover in Ireland causing a surplus in agriculture (in 2016) of €2587 million. A key element requiring ongoing management within the food production sector is the generation and safe disposal of waste streams.

The disposal of farmyard manure and slurry (FYM&S) is a challenge because of the growing numbers of animals, the resulting increased waste generation and limited disposal options. Carton and Magette (1999) estimated that 153 million tons (M t) of manure are produced annually in Ireland by farmed livestock. Singh et al. (2010) reported that slurry generation (from cattle, pig, sheep, and poultry) in Ireland was 34.14 M t in 2010 and estimated it to be 31.99 M t (6.3% drop) in 2020, of which 87% (mass fraction) will be generated by cattle. This FYM&S can be used directly as organic fertilizer for crops, including grass, or can also be potentially used as a feedstock in an AD plant for

biogas production. A mass balance performed by Banks et al. (2011) showed from total feedstock input, the outputs were 73.14% digestate and 13.62% gaseous material (methane 5.07%, carbon dioxide 8.32%, and water vapour 0.23%) (Fig. 1). This highlights the limited reduction by mass of the AD feedstock, resulting in the need for final digestate disposal.

Cederberg and Mattsson (2000) highlighted that the most important contributor to global warming in the dairy sector was methane (CH₄) generation. Purdy et al. (2018) showed that the rate of methane emission is amplified 3-fold if the annual manure mass is left uncovered over 4 months. The use of cow manure as an AD feedstock is a viable option and can help reduce carbon lost to the atmosphere in the form of methane (Purdy et al., 2018). Methane can be captured to produce energy and the digestate can be spread on land which can also help reduce greenhouse gas emissions (Purdy et al., 2018). It was reported that AD has the lowest impact on global warming, eutrophication and acidification and has a better carbon return on investment compared to incineration or composting (Oldfield et al., 2016). As an energy recovery mechanism, AD also fits within the waste hierarchy to reduce-reuse-recycle as described by the Irish Environmental Protection Agency (EPA, 2014).

The cost of imported fuels (oil, gas and coal) in Ireland was €5.7 billion in 2016 (Melia, 2016); with such high fuel costs, the Irish

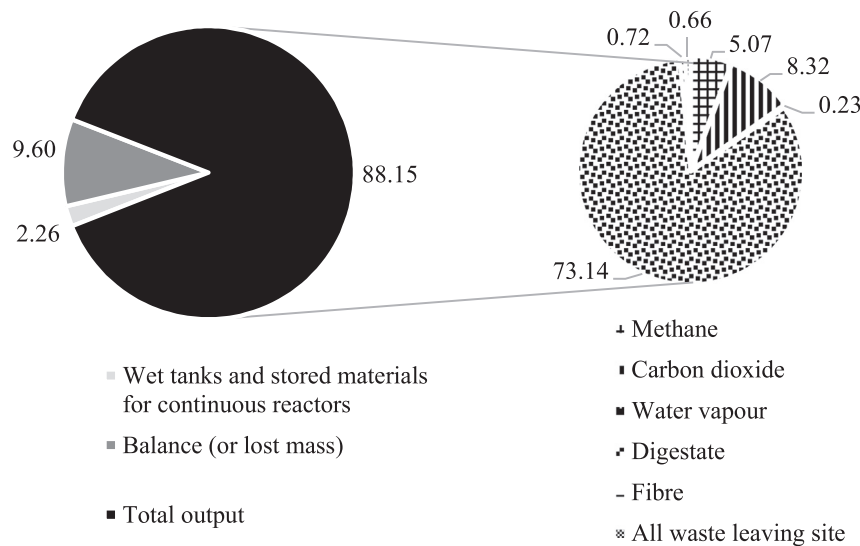


Fig. 1. Typical mass fraction of an AD plant (%). Data source: Banks et al. (2011).

government is developing instruments to change the fossil-based energy economy towards one based on sustainable energy (Vanegas and Bartlett, 2013). EU Directive 2008/28/EC directs that 20% of the energy consumed within the EU by 2020 must be generated from renewable sources. In Ireland, the target is 16% of the gross final consumption of energy and biogas (from residues) can make 7.6% of this target. O'Shea et al. (2016) illustrated that biomethane from wastes could supply 7% of energy in the transport sector. Singh et al. (2010) discovered that Ireland has significant sources of organic waste or residues in the form of agricultural slurry, the organic fraction of municipal solid waste (OFMSW), used cooking oil (UCO), slaughterhouse waste, tallow, in addition to surplus grass. These can all form primary inputs for an AD plant.

AD is a biological process which utilises the embodied energy in organic compounds to produce biogas (Manyi-Loh et al., 2013). Agricultural residues (including FYM&S, feed waste and bedding), crops which have high calorific value (e.g. maize silage, grass silage and whole crop cereals), industrial, commercial and domestic food waste (Green Generation, 2019), municipal waste, animal carcasses, garden waste (Manyi-Loh et al., 2013) are all considered potential feedstocks for AD. Biogas is one of the renewable resources of green energy and can be upgraded to 'biomethane' by scrubbing and then used as a fuel for transport and heating (Murphy et al., 2010). Currently, Irish AD plants only accept grass and FYM&S as their primary feedstock (Auer et al., 2016) in order to minimize potential biological hazards. However, one AD plant does accept industrial, commercial and domestic food waste (Green Generation, 2019). There are several potential pathogens (including bacteria, fungi, parasites, viruses) in such a variable feedstock. Processes with the potential for reducing the pathogen content of animal wastes were described by Gerba and Smith (2005) as facultative lagoons and storage, air-drying, composting, anaerobic digestion, aerobic digestion, and lime stabilization. Heat treatment (pasteurisation) is regularly used to inactivate pathogens in AD plants (Longhurst et al., 2019). There are differing reports regarding the effectiveness of pre-pasteurisation or post-pasteurisation used in conjunction with the AD process as there is the possibility of recontamination and regrowth if post-AD digestate residues are not strictly handled in a hygienic manner (Bagge et al., 2005; Allende et al., 2018) and spore-forming bacteria can still persevere after the pasteurisation and digestion process.

AD of complex organics can be described as a three-stage process involving; (a) Hydrolysis, liquefaction, and fermentation, (b) hydrogen and acetic acid formation, and (c) methane formation (Mukhuba et al., 2018). Manyi-Loh et al. (2013) proposed acidogenesis as an intermediate state between hydrolysis and acetogenesis. These steps (hydrolysis, acidogenesis, acetogenesis and methanogenesis) are inter-related (Manyi-Loh et al., 2013). MAD follows a temperature range of between 35 °C and 45 °C, whereas TAD is operated between 45 °C and 60 °C (it can be as high as 80 °C) (Sakar et al., 2009; Manyi-Loh et al., 2013; BSI, 2014). There are typically three types of AD process; mesophilic AD (MAD), thermophilic AD (TAD) and two-phase (hydrolysis and acidogenesis in the first phase then acetogenesis and methanogenesis in the second phase) (Demirer and Chen, 2005; Sakar et al., 2009; Rubio-Loza and Noyola, 2010). TAD is more effective in terms of pathogen inactivation as it operates under higher temperatures (Mahmud et al., 2016). However, AD plants in Ireland mostly follow the operational regime for MAD (Smyth et al., 2009) due to the higher costs involved in TAD. The controlling parameters of the AD process have an influence on pathogen inactivation (Manyi-Loh et al., 2013). There is legislation in place in many countries which recommends a set of operating conditions (time-temperature) for AD plants; however, conditions may vary between countries (Sahlström, 2003; DAFF, 2009).

Digestate is a co-product of the AD process and it can be applied to agricultural land to supplement nitrogen, phosphorus and potassium (NPK) in the soil. There are restrictions on the maximum amount of digestate (or indeed FYM&S) applied to agricultural land during

different seasons, due to concerns regarding surface runoff (EPC, 2002). Certain animals (cattle and pigs) are restricted from grazing on such lands where digestate or FYM&S were applied (21 to 60 days exclusion period) (The Commission of the European Communities, 2006). Ashkekuzzaman et al. (2018) supported the increased safety of an extended animal exclusion period (40 days exclusion period rather than 20 days) to reduce the risk of *E. coli* transmission to animals. Studies have also identified that there is a reduction of the pathogen concentration in digestate during storage (Hutchison et al., 2004).

As digestate and raw FYM&S are applied to land, the concentration of pathogens is diluted while there is also decay due to UV and heat from the solar radiation and stresses caused by desiccation and temperature fluctuations (Pepper et al., 2008). After this dilution and decay, the remaining pathogens in the field have the potential to spread through environmental media such as air, water (Kinyua et al., 2016) and also through direct contact with animals and ready to eat (RTE) crops grown on the land where bio-fertilizers are applied (Ashbolt, 2004; Thomas et al., 2013; Arfken et al., 2015; Klous et al., 2016; Van Leuken et al., 2016; Conrad et al., 2017). Potential pathogens in digestate and FYM&S land applications can cause serious illness (Liu et al., 2008) with mild or moderate effects on humans and animals and, in extreme instances, can potentially cause death (Hunter, 2003; Manyi-Loh et al., 2016). These concerns have prompted the need for risk assessment studies to assess the potential hazards associated with the spreading of digestate.

The overall aim of this study is to document the different types of AD and pasteurisation conditions used in Ireland and the EU and to assess the influence of critical processes on the inactivation potential for different pathogens. In addition, available strategies for risk assessment and relevant EU and Irish legislation are considered in the context of guiding future risk assessment efforts.

2. Production of organic waste and the use of AD in Ireland

The safe utilisation of waste streams is a key objective for the sustainable development of many countries. The EPA (2014) recommends minimization of the volume of waste going to landfill and the extraction of energy from organic waste where possible. They suggest the sequential steps of 'minimization > reuse > composting > energy recovery' as preferred options when dealing with waste disposal. AD is an energy recovery process and typically uses the wet portion of waste such as municipal waste, animal manure and slurry.

According to the EPA (2016) composting and AD utilised 300 kilotons (kt) of organic and municipal waste (kitchen and canteen food waste, garden and park green waste, edible oils and fats) and was the main source of waste accepted (65%) in Ireland in 2015. The amount of manure produced during livestock housing periods requires management (Carton and Magette, 1999). Almost 43 M t of manure produced by cattle and sheep requires storage and spreading annually (Carton and Magette, 1999). Also, the amount of land used for agricultural purposes increased by 2.8%, to about 4.6 Mha in 2010 compared with 4.4 Mha in 2000 (Eurostat, 2017). Therefore, agricultural residues and grass can also be a valuable AD feedstock, used on its own or in co-digestion with other waste materials. Currently, grass is grown on 3.94 Mha which is >90% of the agricultural land of Ireland (Singh et al., 2010). If only 25% of this area (0.1 Mha) was used to produce grass as an input for AD; it would have the potential to generate 11.9 PJ energy each year to 2020 (Singh et al., 2010).

Within Europe, Germany was an early adopter of AD (since the early 1990s) and produces the highest amount of biogas (4 GW of energy) with approximately 8000 plants; in comparison, AD in Ireland is a relatively new technology (Auer et al., 2016; Bangalore et al., 2016) with only 10 AD plants (DAFM, 2019b). However, Smyth (2018) reported that there are 14 wastewater treatment plants in Ireland with AD facilities currently in operation. Composting is predominantly used for treating biological waste in Ireland with 31 composting plants (EPA, 2016). In Ireland, composting is economically preferable to AD;

however, AD has a greater reduction on greenhouse gas emissions (1451 kg CO₂ ton⁻¹ of bio-waste treated) compared to composting (1190 kg CO₂ ton⁻¹) (Murphy and Power, 2006).

3. Health concerns

The feedstock of the AD process potentially contains a high number of microorganisms (Hutchison et al., 2004). Most bacteria are not pathogenic and some of these microorganisms may be pathogenic and harmful to humans and animals (Alberts et al., 2014). They can have major impacts on the quality of our soil, food grown on that soil, and can potentially have an impact on human health. Humans and all types of livestock, pets and wildlife who have direct and indirect access to farmland have the potential to carry human pathogens (Guan and Holley, 2003; Public Health Agency of Canada, 2019). A pathogen is a biological agent that causes disease or illness in humans, animals, or crops. In some countries, slaughterhouse waste (blood, and other animal-by-products) may be used as a feedstock and represent another potential source of pathogens if not adequately treated before being used as a feedstock (Sahlström, 2003; Tirziu et al., 2011). Harmful pathogens may only represent a very small proportion of all microorganisms; however, they can be responsible for outbreak incidents (Teunis et al., 2010; Eurosurveillance Editorial Team, 2013).

3.1. Outbreaks in Europe and Ireland

Several disease outbreaks (country-wise other than 11 Europe wide outbreaks) have been observed in Europe over the last 20 years

(Eurosurveillance, 2018) as highlighted in Fig. 2. *Salmonella* (15 outbreaks), influenza virus (14), measles virus (9), *Cryptosporidium* (9), *E. coli* (8) and *Legionella* (8) are the top six pathogens which have been responsible for several human outbreaks in Europe. In Ireland, there has been a significant number of outbreak-associated cases of human illness (4215 in 2017) (Fig. 3) (HSE Health Protection Surveillance Centre, 2019) out of which 320 (7.6%) of the outbreak-associated cases were hospitalised with 40 incidents of deaths reported. From Fig. 3, it was understood that influenza and other seasonal respiratory viruses, brucellosis, campylobacteriosis, rotavirus, *E. coli* VTEC, hepatitis C, cryptosporidiosis, hepatitis B, *Streptococcus pneumoniae* (invasive) and *Salmonella* are top ten reasons for these human illnesses in Ireland. These pathogens may arise from contaminated environmental sources (Healy et al., 2017). Most transmission occurs through water, aerosol, soil ingestion, food and zoonotic contact (McAloon et al., 2017; Li et al., 2018; Dogan et al., 2019; Zhao and Liu, 2019). According to the HSE Health Protection Surveillance Centre (2019), the most prevalent infections intestinal disease (IID) causing pathogens identified were norovirus, *E. coli*, acute infectious gastroenteritis, respiratory infection, influenza, cryptosporidiosis, salmonellosis and infection due to *Clostridium difficile*. The challenge is to ensure the contribution of agricultural land treated with FYM&S and digestate does not add to such disease incidences. Ireland reports the highest human incidence of verotoxigenic *E. coli* (VTEC) infection in Europe (Óhaiseadha et al., 2017). Óhaiseadha et al. (2017) found that VTEC infection in the Republic of Ireland is particularly associated with rural areas, due to the high risk of exposure associated with contact with cattle and unregulated groundwater supplies. In another study, human sewage was identified as the likely

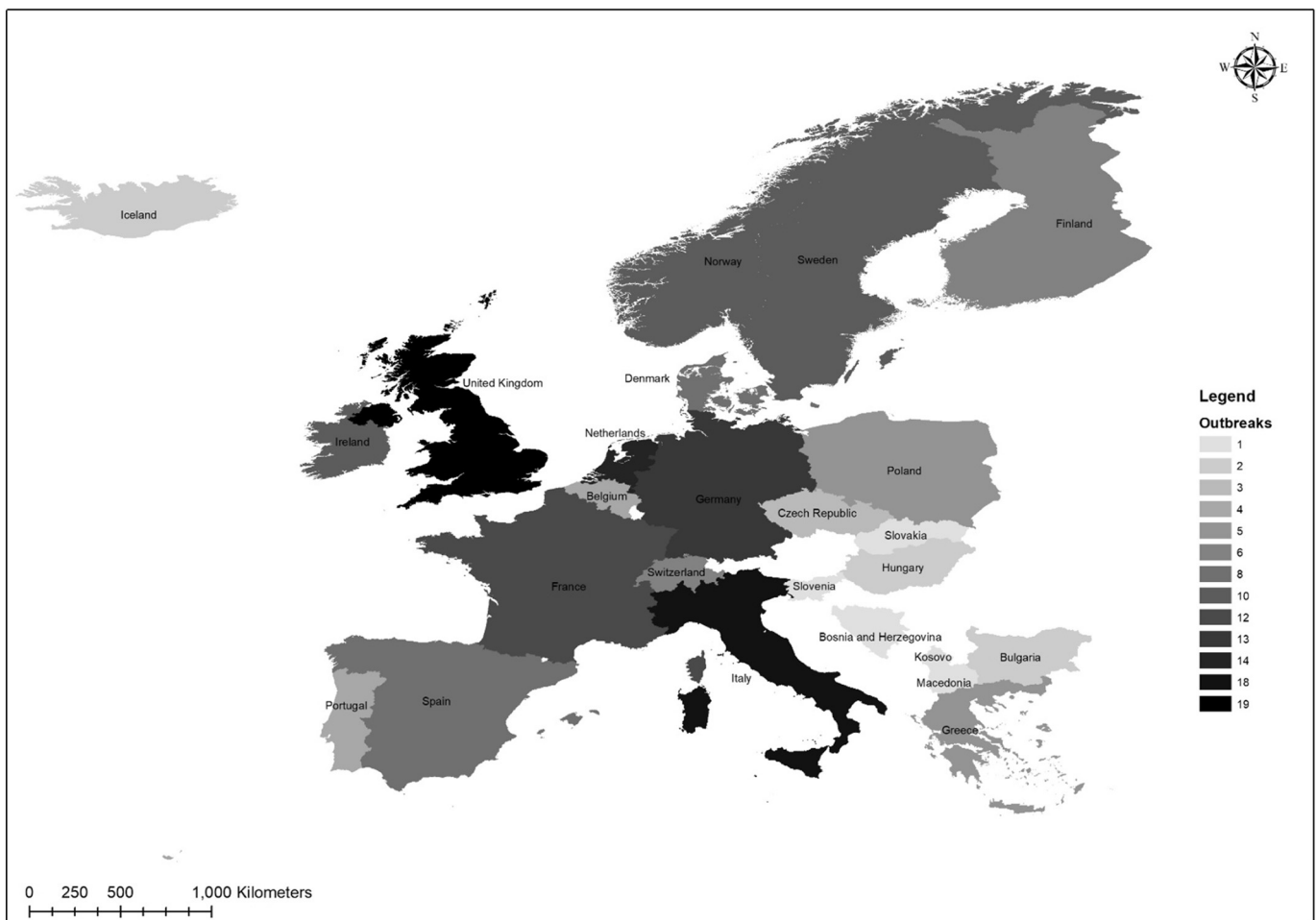


Fig. 2. Outbreaks observed in Europe (last 20 years). Data source: Eurosurveillance (2018).

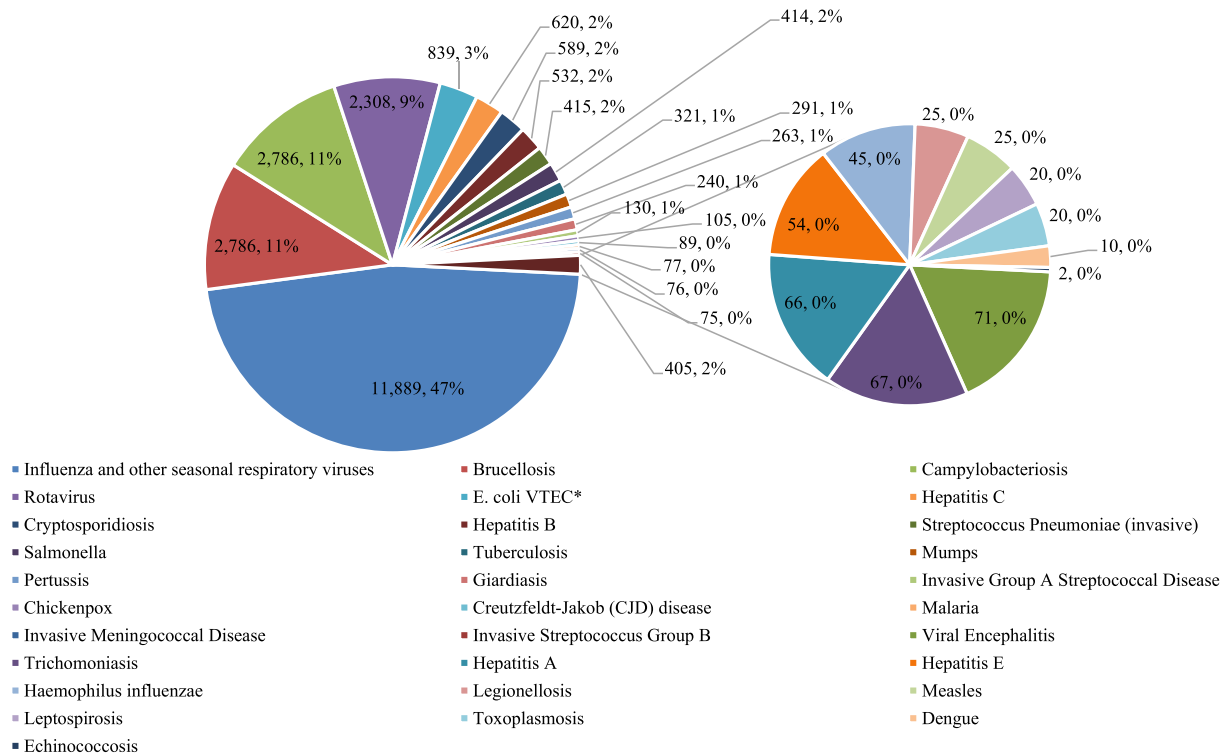


Fig. 3. Annual reported cases in 2017 (with %) in Ireland.
Data source: HSE Health Protection Surveillance Centre (2019).

contributor (Mac Kenzie et al., 1994) for the largest drinking water outbreak in U.S. history; the *Cryptosporidium* outbreak in Milwaukee in 1993. Potential biological hazards reportedly associated with spreading manure on agricultural land are detailed in Table 1.

3.2. Notification rates

The total number of confirmed human illnesses (notification rate) (HSE Health Protection Surveillance Centre, 2019) is summarised in Fig. 4 which suggests *Clostridium*, *Cryptosporidium*, *E. coli*, *Salmonella* are the main pathogens of concern in Ireland. According to the European Food Safety Authority and European Centre for Disease Prevention and Control (2018), *Campylobacter* is the most frequently reported gastrointestinal bacterial pathogen in humans in the EU since 2005. In 2017, the number of reported cases of human campylobacteriosis was 246,158 with an EU notification rate of 64.8 per 100,000 population, which was a 10.01% increase compared with the rate in 2013. Similarly, a total of 91,662 confirmed salmonellosis cases were reported by 28 EU member states, representing a notification rate of 19.7 cases per 100,000 population (EFSA and ECDC, 2018) which is a 15.84% rise in notifications since 2013. These hazards are followed by Yersiniosis (6823), *E. coli* STEC infections (6073) and Listeriosis (2480) in 2017.

3.3. Disease transmission

Protozoan and bacterial zoonotic pathogens may survive and reproduce in the soil, water, and manure under favourable conditions (Guan and Holley, 2003). However, if transmitted to humans, the effects can be severe (Bank-Wolf et al., 2010). Havelaar et al. (2012) cited in Boqvist et al. (2018) report that about two-thirds of the food-borne diseases have been caused by foodborne infections of animal origin, followed by human-to-human transmission (secondary transmission) and environmental transmission in the Netherlands. Efstratiou et al. (2017) found that Europe is responsible for 9% of the worldwide reported

waterborne outbreaks including Ireland (2.4% and UK 5.5%). Brouwer et al. (2018) stated that, even if food is the means of transmission of the pathogen, it is not necessarily the source of contamination; contamination sources are identified as wastewater irrigation, food handlers, etc. through pork, beef, chicken, shellfish, eggs and dairy, contaminated vegetable crops (such as nuts and greens). Zoonotic pathogens can naturally transmit from animals to humans (Guan and Holley, 2003; Songer, 2010; Pestechian et al., 2014); and often asymptotically colonize the animal host (e.g. grazing animals). Zoonotic pathogens typically circulate in domestic animals without causing disease and can be frequently present in animal manure and slurry (Gajadhar et al., 2006; Youn, 2009; Bank-Wolf et al., 2010; Songer, 2010). However, there are limited studies available on the intensity and type of contact patterns of zoonotic transmissions leading to transmission (Klous et al., 2016). A study in the UK (Jones and Martin, 2003) suggests that zoonotic pathogens such as *Salmonella*, *E. coli* O157:H7, *Mycobacterium* spp., and animal pathogens such as bovine viral diarrhoea (BVD) virus, *Serpulina hyodysenteriae*, foot and mouth disease (FMD) virus, classical swine fever (CSF) virus and Aujeszky's disease virus can be transmitted by food waste. DAFM (2019a) highlight the increased risk from FMD or CSF by feeding animals food waste. Similarly, parasites are the most common zoonotic pathogen which can be spread from farm animals to humans (Gajadhar et al., 2006; Youn, 2009; Pestechian et al., 2014). Hence, for co-digestion, it is important to assess the safety of food waste in addition to manure and slurry as a potential source of zoonotic pathogens.

4. AD critical indicators for pathogen inactivation

The AD process depends on a number of critical indicators such as, temperature (Manyi-Loh et al., 2013), process stages (one step or two step), pH, carbon and nitrogen (C:N) ratio, free ammonia concentration, organic loading rate (OLR), hydraulic retention time (HRT) (Sakar et al., 2009), solid retention time (SRT) (Nges and Liu, 2010), and effect of pre-treatment and storage (Gruber-Brunhumer et al., 2016). Temperature,

Table 1
Reported potential biological hazards associated with spreading FYM&S and digestate on agricultural land.

Pathogen	Types	Possible effects	High hazard pathway	Host range/reservoir	Reference
<i>Brucella</i> spp.	Bacteria	Fever, may affect any organ system such as circulatory, respiratory, digestive, excretory and nervous; headache, weakness, profuse sweating, chills, arthralgia	Zoonosis. Unpasteurised milk and products or undercooked meat, contact with milk, urine, and genital organs	Sheep, cattle, Humans, swine, goats, deer, dogs	3, 6
<i>Campylobacter jejuni</i>	Bacteria	Acute enterocolitis in humans, diarrhoea that may contain red or white blood cells, fever, malaise, nausea and vomiting	Grazing contaminated land; Ready-to-eat crops, contaminated food (primarily chicken) or contaminated drinking water	Mainly pigs, humans, sheep, cattle	1, 2, 3, 4, 6
<i>Clostridium</i> spp.	Bacteria	Paralysis and sometimes fatal respiratory failure	Poor processing/contaminated food, wound contamination, absorption by the nose	Horses (infected), humans, fowl, fish, cows, dogs. Spores found in soil, agricultural products	1, 2, 3, 6
<i>E. coli</i> enterohaemorrhagic (verotoxin) O157:H7	Bacteria	Haemorrhagic colitis, intestinal disease, diarrhoea; fever, haemolytic uremic syndrome (HUS), thrombocytopenic purpura (TTP)	Grazing contaminated land; RTE crops, ingestion of contaminated food, faecal-oral transmission; person-to-person	Humans, animals (sheep, goats, pigs, poultry, calves, cattle)	1, 2, 3, 4, 6
<i>Enterobacter</i> spp.	Bacteria	Cerebral abscess, pneumonia, meningitis, septicæmia, and wound, urinary tract and intestinal infections	Direct or indirect contact of mucosal surfaces with an infectious agent	Plants, humans, and animals primarily colonizers of the lower gastrointestinal tract. Soil and water, dairy products	3, 6
<i>Listeria monocytogenes</i>	Bacteria	Listeriosis, particularly serious in pregnant women, and those at the extremes of age; fever, chills, malaise, arthralgia, back pain, and diarrhoea	Grazing contaminated land; RTE crops, contaminated food, trans-placentally from mother to child during pregnancy	Humans, animals, birds, soil, manure, vegetable, silage, water, poultry, processed meats, milk, cheese, slaughterhouse waste	1, 3, 4, 6
<i>Mycobacterium</i> spp.	Bacteria	Tuberculosis	Nosocomial, direct contact with a contaminated environment, grazing contaminated land; water; RTE crops	Humans, domestic and wild animals	1, 3, 4, 6
<i>Salmonella enterica</i> spp.	Bacteria	Gastroenteritis or “food poisoning”, Salmonellosis	Grazing contaminated land; RTE crops, contaminated foods and water, faeces, animal feed, or humans. Meat, poultry, milk, and egg	Horse, reptiles, cattle, swine, poultry, wild birds, flies and pets	1, 2, 3, 4, 6
<i>Salmonella typhi</i>	Bacteria	Typhoid fever	Grazing contaminated land; RTE crops, poor hygiene habits and public sanitation conditions, flying insects	Horse, reptiles, cattle, swine, poultry, wild birds, flies and pets	1, 2, 4, 6
<i>Vibrio cholerae</i> , serovar O1	Bacteria	Cholera	Water, contaminated with infectious faeces; infectious raw fish and seafood	Humans, water birds, shellfish, fish, and herbivores	1, 2, 3, 6
<i>Yersinia enterocolitica</i>	Bacteria	Yersiniosis, watery stools, abdominal pain and acute mesenteric lymphadenitis	Grazing contaminated land; water; RTE crops, faecal-oral, raw pork products, undercooked pork, tofu and unpasteurized milk, untreated water	Pigs, several warm-blooded animals, farm animals and pets	1, 2, 3, 6
<i>Aspergillus</i> spp.	Fungi	Clinical allergies, infections, infections associated with damaged tissue, and pulmonary infections	Inhalation of airborne conidia, contaminated water, hospital fabrics and plastics	Humans, cows, dolphins, birds, and horses	1, 3, 4, 6
<i>Cryptosporidium parvum</i>	Parasites: Protozoa	Acute gastroenteritis; diarrhoea, abdominal pain, cramps, fever, vomiting, myalgia, flatulence, nausea, anorexia, malaise, and fatigue. Opportunistic in AIDS patients	Grazing contaminated land; Silage; Water; RTE crops, faecal-oral route, direct contact with infected humans or animals, contaminated food or water and aerosols	Environment, many mammalian species	2, 3, 4, 5, 6
<i>Echinococcus multilocularis</i>	Parasites: helminths	Affects the liver and may predispose to liver cancer	Ingestion of infected eggs; vegetables and water contaminated with the parasite eggs	Foxes, wolves, dogs, cats, mice, occasionally humans	1, 3, 5, 6
<i>Taenia saginata</i> (beef tapeworm)	Parasites: helminths	Taeniasis, nausea, anorexia, or epigastric pain. Anxiety, headache, dizziness, urticaria diarrhoea, abdominal pain, weight loss and discomfort	Ingesting the eggs: infected flesh of raw or undercooked beef	Humans; definitive host. Cattle; intermediate host. Sheep	1, 2, 3, 5, 6
<i>Taenia solium</i> (pork tapeworm)	Parasites: helminths	Taeniasis, cysticercosis, neurocysticercosis (adult-onset epilepsy)	Ingesting the eggs; eating raw or undercooked, infected pork.	Humans; definitive host. Pigs and dogs; intermediate host	1, 2, 3, 5, 6
<i>Trichinella</i> spp.	Parasites: helminths	Trichinellosis syndrome: nausea, vomiting, epigastric pains, and diarrhoea and/or constipation	Consumption of raw or undercooked meat of infected animals (pigs)	Pigs, horses, bears, wild boars	2, 5, 6
<i>Giardia lamblia</i> , <i>Giardia intestinalis</i>	Parasites: Protozoa	Giardiasis, diarrhoea	Faeces of infected persons, contaminate hands, drinking water, swimming pool, faecal-oral route, or contaminated food, soil	Humans and mammals (primates, dogs, cats, cattle, sheep, pigs, rodents)	1, 2, 3, 5, 6
<i>Toxoplasma gondii</i>	Parasites: Protozoa	Toxoplasmosis, tachyzoites, fever, rash, headache, lymphadenopathy, weight loss, weakness, pneumonia, and myalgia	Poorly cooked infected meats (pork, mutton, beef), water, food, or milk, unwashed raw vegetables or fruits	Cats, humans, and warm-blooded vertebrates, including most mammals and birds	1, 3, 4, 5, 6
Foot-and-mouth disease (FMD) virus	Virus	Weight loss, vesicular stomatitis, lameness, myocarditis, death (neonates)	Grazing contaminated land, animal-to-animal, aerosol, fomites, standing water, and uncooked food scraps and feed supplements	Cattle, pigs, sheep, goats, deer	4, 6
Classical swine fever (CSF) virus	Virus	Hog cholera (acute to chronic disease of pigs)	Grazing contaminated land, infected pigs	Pigs	2, 4, 6
Hepatitis A virus	Virus	Infectious hepatitis: fever, malaise, anorexia, nausea, abdominal discomfort, dark urine, and jaundice	Faecal-oral route	Humans, owl, monkeys	1, 2, 3, 6
Norovirus (Norwalk virus)	Virus	Epidemic gastroenteritis; rapid onset of nausea, vomiting, diarrhoea, abdominal cramps, abdominal pain, malaise, headache,	Person to person, contaminated surfaces, food, water, fomites, and aerosols	Humans, pigs, cattle, shellfish, mice	1, 2, 3, 6

(continued on next page)

Table 1 (continued)

Pathogen	Types	Possible effects	High hazard pathway	Host range/reservoir	Reference
Parvovirus B19	Virus	and fever Encephalopathy, epilepsy, meningitis, myocarditis, dilated cardiomyopathy, and autoimmune hepatitis, fever, coryza, headache, and nausea	Grazing contaminated land	Humans	1, 2, 3, 6
Scrapie	Prion	A member of the transmissible spongiform encephalopathies (TSEs), affects sheep, and less frequently, goats	Grazing contaminated land; able to persist in soil for years without losing their pathogenic activity	Sheep, goats	2, 4, 6

Reference: 1 (Carrington, 2001); 2 (Jones and Martin, 2003); 3 (Lepeuple et al., 2004); 4 (Longhurst et al., 2013); 5 (Torgerson et al., 2015); 6 (Public Health Agency of Canada, 2019).

C:N ratio, OLR, HRT are independent parameters whereas; pH and free ammonia concentration are dependent parameters. Of these parameters, the temperature has the most significant influence on pathogen inactivation (Sakar et al., 2009; Abbasi et al., 2012a; Manyi-Loh et al., 2013). The likely influence of these parameters on pathogen inactivation is tabulated in Table 2. Sakar et al. (2009) mentioned that most of MAD experiments are carried out at 30 °C; however, the optimal

temperature for mesophilic growth is situated near 40 °C. The main objective of an AD plant is to produce biogas (mainly methane); however, the inactivation of pathogens is also an important function from a risk perspective. Methane production potential is directly proportional to the initial increase of C:N ratio; however, in higher C:N ratio, methane potential declines (Wang et al., 2012). C:N ratios of 25:1 and 30:1 had better digestion performance (Puyuelo et al., 2011; Abbasi et al.,

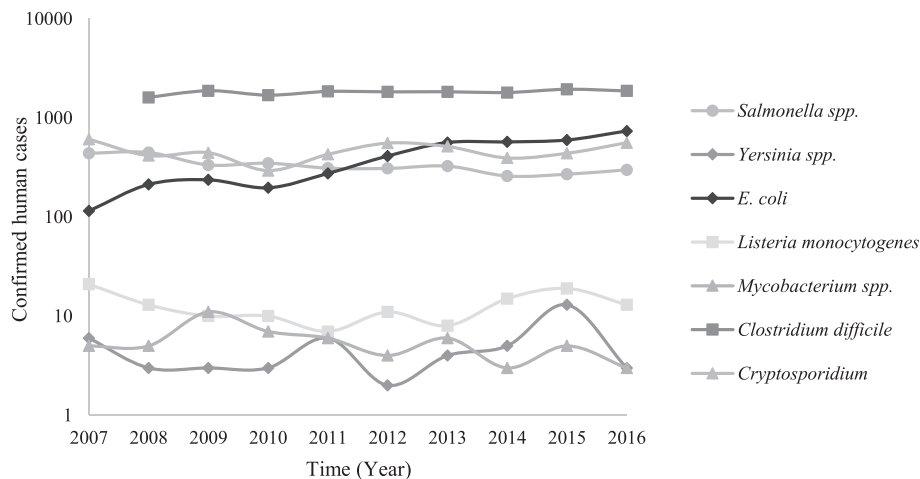


Fig. 4. Total number of confirmed human cases (notification rates) in Ireland (2007–2016).
Data source: HSE Health Protection Surveillance Centre (2019).

Table 2

Influence of AD process parameters on biogas production and pathogen inactivation.

Parameters	Optimum value	Parameters (effect of changing from optimal value)	Effect on		Reference
			Gas production	Pathogen inactivation	
Temperature	35 to 45 °C temperature as mesophilic and 45 to 80 °C for thermophilic	Increase	Increase (however, less stable)	Increase	(Kim et al., 2006; Sakar et al., 2009; Abbasi et al., 2012a; Bialek and Flaherty, 2013; Manyi-Loh et al., 2013; Vanegas and Bartlett, 2013; BSI, 2014)
pH	6.2 to 8.5	Both increase and decrease beyond optimum value	Decrease	Increase	(Murto et al., 2004; Clemens et al., 2005; Ward et al., 2008; Sakar et al., 2009; Abbasi et al., 2012a)
Organic loading rate (OLR)	Cattle waste (2.5 to 3.0 kg volatile solids m ⁻³ day ⁻¹) pig slurry (3.0 to 3.5 kg volatile solids m ⁻³ day ⁻¹) cattle waste combined with co-substrates (5 to 7 kg volatile solids m ⁻³ day ⁻¹)	Increase	Decrease	Increase in higher OLR (as pH drops)	(Weiland, 2000; Murto et al., 2004; Sakar et al., 2009; Nges and Liu, 2010; Abbasi et al., 2012b; Manyi-Loh et al., 2013; BSI, 2014; Song et al., 2014)
Toxicity: (cations Na, K, Ca, Mg) and heavy metals (Cu, Cr, Cd, Zn, Ni, Pb), chlorine, free ammonia NH ₃ , H ₂ S	Not recommended	Increase	Decrease	Increase	(Lin, 1992; Lin and Chen, 1999; Chen et al., 2008; Sakar et al., 2009; Li et al., 2016)
C:N ratio	20:1 to 30:1	Decrease drastically	Decrease	Decrease	(Puyuelo et al., 2011; Abbasi et al., 2012a; Song et al., 2014; Zhou et al., 2015)
Hydraulic retention time (HRT)	(5 to 7 days) >10 times the minimum doubling time of bacteria	Increase	Decrease	Decrease	(Zinder et al., 1984; Manyi-Loh et al., 2013)

Table 3

Recent studies on pathogen inactivation in MAD/TAD (with or without pasteurisation) and storage.

Reference	Studied organisms	Type of exp.	Experimental conditions										Inactivation			
			Vol.	Feedstock	Temp. (°C)	OLR	HRT (days)	pH	TS (%)	VS (%)	VFAs (g l ⁻¹)	NH ₃ or NH ₄ ⁺	MAD	TAD	Storage	AD + Pas. (pasteurisation)
Qi et al., 2018	<i>Salmonella</i> , <i>E. coli</i> , <i>Campylobacter</i> , <i>Enterococci</i>	Lab-scale continuously fed anaerobic digestions	11.2 l	Dairy manure	37 (MAD) and 55 (TAD)	Unit (g VS l ⁻¹ day ⁻¹) and 2.73 (MAD) and 4.30 (TAD)	28.6 (MAD) and 18.2 (TAD)	Initial 6.3 ± 0.2, MAD 7.7 ± 0.1, TAD 7.6 ± 0.2	Initial 10.1 ± 0.4, MAD 6.2 ± 0.7, TAD 6.5 ± 0.5	Initial 8.8 ± 0.7, MAD 4.7 ± 0.5, TAD 5.0 ± 0.3	Initial 1.197, MAD 0.038, TAD 0.103	NH ₄ -N (g kg ⁻¹): initial 5.3, MAD 9.8, TAD 12.2	<i>Salmonella</i> 86.5%, <i>E. coli</i> 96.9%, <i>Campylobacter</i> 72.2%, <i>Enterococci</i> 85.6%	<i>Salmonella</i> 99.3%, <i>E. coli</i> 99.7%, <i>Campylobacter</i> 90.1%, <i>Enterococci</i> 91.1%	-	-
Dennehy et al., 2018	Total coliform, <i>E. coli</i> , <i>Enterococci</i>	Batch (4 phases)	10 l (working vol. 7.5 l)	Pig manure (PM)/food waste (FW) 85%/15%, 63%/37% and 40%/60% (vs basis)	39	Unit (kg VS m ⁻³ day ⁻¹) 1 (Phase I and II) 1.5 (Phase III) and 3 (Phase IV)	41, 29, and 21	7.62 ± 0.1	8.4 ± 0.1	4.3 ± 0.02	7.84 ± 0.27	NH ₄ -N: 2.96 ± 0.01 g l ⁻¹	2 log	-	-	-
Costa et al., 2017	Coliforms, <i>Lactobacilli</i> , <i>Streptococci</i> , <i>Clostridia</i>	Storage only	20 l	Fresh dairy manure/pig slurry and digestate	43 and 37 (pig farms), 48 (dairy)	Storage only	56, 40 (pig farm), 90 (dairy)	8.5 to 8.7 (dairy), 7.9 to 8.7 (pig) mean values	5.5 to 9.8 (dairy), 3.6 to 8.4 (pig) mean values	4 to 6.8 (dairy), 2 to 5.1 (pig) mean values	5.5 to 9.8 (dairy), 3.6 to 8.4 (pig) mean values	NH ₃ -N (g kg ⁻¹ fresh matter): 2 to 1 (dairy), 2.9 to 0.6 (pig) mean values	Coliforms 2 log (cattle and pig), <i>Lactobacilli</i> 2 (cattle) to 3 log (pig), <i>Streptococci</i> 1.5 (cattle) to 2.5 (pig), <i>Clostridia</i> 0.3 log (cattle), growth > 0.5 log (pig)	-	6 months storage: coliforms (fresh) > 3 log (cattle), >2 log (pig), coliforms (digestate) no reduction (cattle), 1 log (pig); <i>Lactobacilli</i> (fresh) > 3 log, <i>Lactobacilli</i> (digestate) no reduction (cattle), 3 log (pig); <i>Streptococci</i> (fresh) > 3 log, <i>Streptococci</i> (digestate) no reduction (cattle), 2 log (pig); <i>Clostridia</i> (1.5 growth)	-
Nolan et al., 2018	Total coliforms, <i>E. coli</i> , <i>Enterococci</i>	Lab-scale (10 l) batch-fed miniature (50ml)	10 l (working vol. 7 l)	Slurry co-digested with fats, oils, and grease (FOG)	37	30 g VS l ⁻¹ in a 2:1 inoculum to feedstock ratio	28	7	7.47 to 7.27	5.83 to 5.65 (61 to 64% removal in 7 days)	-	NH ₃ : 0.865 to 1.233 g l ⁻¹	Total coliforms (>3 log in 7 days), <i>E. coli</i> (>3 log in 7 days), <i>Enterococci</i> (>1.5 log in 7 days, >2 log in 28 days)	-	-	T. coliforms (pre pas. 3 log in 7 days; post pas. 5 log in 7 days), <i>E. coli</i> (pre pas. 3 log in 7 days; post pas. 5 log in 7 days), <i>Enterococci</i> (pre pas. 2 log in 7 days; post pas.

(continued on next page)

Table 3 (continued)

Reference	Studied organisms	Type of exp.	Experimental conditions										Inactivation				
			Vol.	Feedstock	Temp. (°C)	OLR	HRT (days)	pH	TS (%)	VS (%)	VFAs (g l ⁻¹)	NH ₃ or NH ₄ ⁺	MAD	TAD	Storage	AD + Pas. (pasteurisation)	
Thomas et al., 2019	Extended-spectrum-β-lactamase (ESBL)-/AmpC-producing <i>E. coli</i>	Lab-scale	2 l glass bottles (1.5 l of inoculum)	Broiler chicken faeces and wood pellets	MAD (37, 42) and TAD (55)	Organic dry matter ODM _{Substrate} to ODM _{Inoculum} of 0.49 (37 °C), 0.50 (42 °C) and 0.48 (55 °C)	35	8.24 to 7.70 (37 °C), 7.74 to 7.68 (42 °C), 8.57 to 7.87 (55 °C)	-	-	0.48 to <0.02 (37 °C), 0.08 to 0.22 (42 °C), 0.39 to 0.13 (55 °C)	NH ₄ -N (g kg ⁻¹ fresh matter): 0.753 to 1.131 (37 °C), 0.747 to 1.149 (42 °C), 0.753 to 1.294 (55 °C); NH ₃ (g kg ⁻¹ fresh matter): 0.137 to 0.0687 (37 °C), 0.066 to 0.090 (42 °C), 0.445 to 0.291 (55 °C)	At 37 °C: 3 log reduction in 8 days, 5 log reduction in 26 days; At 42 °C: 5 log reduction in 8 days	At 55 °C: 6 log reduction in 2 h	-	2.5 log)	
Iwasaki et al., 2019	<i>Pseudomonas</i> spp.	Lab-scale continuously fed AD	10 l	Cow manure	MAD (37) and TAD (55)	-	-	30 (MAD) and 15 (TAD)	-	10	-	-	Reduction 42.5% to 58.8% Increase 39.1% to 45.6% depending on subspecies	Reduction 54.0%, to 69.2% No increase	-	-	
Chiapetta et al., 2019 (study 1)	<i>E. coli</i> , Enterococci, MAP, and enterovirus	Full-scale Commercial AD: plug-flow design, continuous mix design	plug-flow 5.9 × 10 ⁶ l continuous mix 0.49 × 10 ⁶ l	Cow manure	35 to 38	-	-	20	-	-	-	-	Continuous mix: <i>E. coli</i> 1.8 log, Enterococci 0.9 log, MAP 0.8 log, enterovirus 3/10 to 1/10 detected; plug flow: <i>E. coli</i> 4 log, Enterococci 1.2 log, MAP 1.85 log, enterovirus 4/10 to 1/10 detected	-	-	-	
Chiapetta et al., 2019 (study 2)	<i>E. coli</i> GEC (including O157: H7), Enterococci, <i>Salmonella</i> spp., MAP and <i>Campylobacter</i>	Full-scale Commercial AD: plug-flow design	6.1 × 10 ⁶ l	Cow manure, food wastes, slaughter waste, biodiesel by-product, fat/grease, wood pulp fibre	38	-	-	17	-	-	-	-	<i>E. coli</i> GEC 2.22 log, Enterococci 0.92 log, <i>Salmonella</i> 10% increase, MAP 22%, <i>Campylobacter</i> 28%	-	-	-	
Venglovsky et al., 2018	<i>Salmonella typhimurium</i> , <i>E. coli</i> , total coliforms, faecal Enterococci, <i>Ascaris suum</i> eggs	Closed plastic containers	5 l	Raw pig slurry	(4, 20 and 42)	-	-	115 (storage)	Initial DM: 6.95 ± 0.11 increased to: (at 4 °C) 8.35 ± 0.58; (at 20 °C) 8.55 ±	Unit (g kg ⁻¹) 28.00 ± 6.26 to 15.09 ± 1.23 at 4 °C, 13.09 ± 4.12 at	-	-	NH ₄ ⁺ (g kg ⁻¹ DM) initial 50.00 ± 21.11 (slurry); at 4 °C (295.5 at day 40) decreased to 84.13 ± 21.23. At 20	-	-	Reduction at 4 °C, 20 °C and 42 °C: <i>S. typhimurium</i> > 5 log in 90, 32, 22 days, <i>E. coli</i> > 2 log in 90, 40, 40 days, total	-

								0.17; (at 20 °C and 42 °C)	9.35 ± 0.05	12.89 ± 1.05 at 42 °C		°C, (200.45 ± 50.83 day 12 and 225.45 ± 54.23 day 32), final concentration 27.11 ± 1.06. At 42 °C, (215.13 ± 14.12 day 32) at day 115, 0.56 ± 0.01		coliforms >3 log in 115, 55, 55 days, faecal <i>Enterococci</i> > 3 log in 115, 40, 40 day, <i>Ascaris suum</i> eggs 80% reduction in 12 days at 42 °C, no reduction at 4 °C and 20 °C
Jiang et al., 2018a	<i>E. coli</i> , total coliforms and <i>Enterococci</i>	Laboratory-scale batch experiment	1 l glass digesters	Co-digestion of food waste (FW) and pig manure (PM)	37	FW/PM ratios of 50:50 and 75:25	120	6.8–8.7	26.3 (FW), 23.8 (PM), 16.6 (inocul. digest.), 19.0 (inocul. sludge)	25.0 (FW), 19.4 (PM), 11.7 (inocul. digest.), 12.6 (inocul. sludge)	Calculated free VFAs (digestate), 0.035–0.375 (digestate), 147–895 (sludge)	Total NH ₃ -N (g l ⁻¹) 7.2 (digestate), 6.4–6.9 (sludge) Free NH ₃ -N (g l ⁻¹) 3.2 to 3.3 (digestate), 2.8 (sludge)	2.1–2.3 log (<i>E. coli</i>), 3.4–3.6 log (total coliforms) in 4 days, 3 log (<i>Enterococci</i>) in 12 to 30 days	
Jiang et al., 2018b	<i>Salmonella</i>	Laboratory-scale batch experiment	1 l glass digesters	Co-digestion of food waste (FW) and pig manure (PM)	37	FW/PM ratios 50:50	9	8.7 to 7 (max. growth at pH 7)	26.3 (FW), 24.0 (PM), 19.5 (inocul. digest.), 22.1 (inocul. sludge)	25.0 (FW), 19.4 (PM), 11.7 (inocul. digestate), 12.6 (inocul. sludge)	0 at beginning to (1.8 to 8.0) at ay 9	Total NH ₃ -N (g l ⁻¹) 1.50 to 3.75	4–6 log inactivation in 7 days	

2012a; Song et al., 2014; Zhou et al., 2015) with stable pH and low concentrations of total ammonium nitrogen and free NH_3 (the latter being toxic to pathogen's survival). The most advantageous pH for bacterial growth in AD is in the range of 6.5 to 8.5 (Sakar et al., 2009). NH_3 toxicity thresholds are very sensitive to a pH above 7 and the free NH_3 levels should be maintained below 80 mg l^{-1} (Sakar et al., 2009). Jiang et al. (2018b) mentioned the toxic effect of volatile fatty acids (VFAs) on *Salmonella* inactivation which was much greater than that of NH_3 . Burton & Turner (2003) cited in Sakar et al. (2009) proposed optimal OLR for cattle manure as 2.5 to 3.5 kg volatile solids (VS) $\text{m}^{-3} \text{ day}^{-1}$, for cattle manure with co-substrates 5.0 to 7.0 kg VS $\text{m}^{-3} \text{ day}^{-1}$, and for pig manure 3.0 to 3.5 kg VS $\text{m}^{-3} \text{ day}^{-1}$. In mesophilic conditions, they proposed average HRTs for cattle manure of 12 to 25 days, for cattle manure with straw bedding 15 to 35 days, and for pig manure 10 to 20 days. The AD process parameters are not standardised and there is the flexibility of selecting those within given ranges (optimum value in Table 2). Recent studies on pathogen inactivation during MAD and TAD have detailed the experimental conditions (the type of experiments, the volume of the digester, feedstock type, temperature, OLR, HRT, pH, total solids TS, VS, VFAs, NH_3 or NH_4^+) and are presented in Table 3. Given the multitude of variables a multi-variate study on pathogen inactivation selecting a range of parameters presented in Tables 2 and 3 is recommended. Other process-influencing parameters include the specific surface area of the substrate and the effect of dilution, mixing, agitation or stirring (Abbasi et al., 2012b). The inhibitors of an AD process can include the presence of heavy metals, oxygen, ammonia, volatile and long chain fatty acids and light (Kayhanian, 1999; Tada Tsukahara and Sawayama, 2006; Chen et al., 2008; Argun and Kargi, 2009).

The biological process of AD has the potential to inactivate pathogens. Horan et al. (2004) discovered that a two-stage MAD process (process parameters for the specific study; HRT = 12 days, temperature = $35 \pm 3 \text{ }^\circ\text{C}$) achieved more than a $2 \log_{10}$ reduction in *E. coli*, *Listeria monocytogenes* and *Salmonella senftenberg*. Smith et al. (2005) reported a $2 \log_{10}$ reduction of *E. coli* as a microbiological standard for sewage sludge and compost or digestate with conventional mesophilic waste treatment. In another study, Saunders et al. (2012) discovered that MAD did not affect the survivability of indicator bacteria. In addition, the land application method did not affect the rate of bacterial die-off highlighting the importance of implementing bacterial reduction measures prior to spreading.

At higher temperatures (thermophilic), TAD provides more rapid reaction rates (and faster biogas production) compared to mesophilic temperatures. TAD has the additional advantage of achieving higher inactivation of pathogens when compared to MAD (Beneragama et al., 2013; Mahmud et al., 2016). Pandey and Soupir (2011) estimated a $2 \log_{10}$ and $8 \log_{10}$ reductions of *E. coli* within 2 days and 4 days, respectively using a TAD ($52.5 \text{ }^\circ\text{C}$) process. In the same study, only $2.5 \log_{10}$ reduction of this bacterium over 40 days was possible with a MAD ($37 \text{ }^\circ\text{C}$) process. This further highlights the significant effect of process temperature on pathogen inactivation.

Pasteurisation was identified (Longhurst et al., 2019) as a heat treatment which can result in a $6.0 \log_{10}$ reduction in bacterial populations and a $5.0 \log_{10}$ reduction for exotic viruses such as foot and mouth disease (FMD) and classical swine fever (CSF) virus; however, pasteurisation will not denature prions and is ineffective against prions that cause diseases such as scrapie or Bovine Spongiform Encephalopathy (BSE). It is a cost-intensive process and can be used before waste goes into the AD system. Alternatively, it can be carried out after reducing the volume of digestate following the AD process. The Irish pasteurisation standard ($60 \text{ }^\circ\text{C}$ for 48 h, twice) was found to be prohibitively energy inefficient and expensive compared to the more common EU standard ($70 \text{ }^\circ\text{C}$ for 1 h) (Coultry et al., 2013). Alternative treatment options include pre-pasteurisation of only specified high-risk feedstock streams, which may prevent contamination of the entire feedstock mixture and saves on the extra costs associated with pasteurisation of the entire digester volume (Seadi and Lukehurst, 2012). The effect of

pasteurisation and its sequence (pre or post pasteurisation) is also important to determine the fate of the retained pathogens in the digestate; Bagge et al. (2005) showed that the pre-pasteurisation step before AD resulted in an adequate reduction of pathogens ensuring the hygienic standard of the digestate residue is maintained to avoid recontamination and regrowth. Lepeuple et al. (2004) reported that a combination of pasteurisation followed by MAD was two times more lethal to pathogens compared to MAD alone. In contrast, Nolan et al. (2018) found that post AD pasteurisation performed better compared to pre AD pasteurisation in terms of pathogen inactivation. Also, Coultry et al. (2013) reported that energy usage for pre-pasteurisation (average temperature of feedstock considered as $12 \text{ }^\circ\text{C}$) is nearly twice that compared to post pasteurisation (digestate temperature monitored as $40 \text{ }^\circ\text{C}$).

Storage, either pre- or post-AD, can also result in pathogen inactivation in the feedstock or digestate, respectively. However, post-AD storage is typically recommended, as fresh manure should be added into the AD reactor as soon as possible to avoid methane loss (Purdy et al., 2018). Furthermore, Nolan et al. (2018) discovered that the bacterial (coliforms, *E. coli* and *Enterococci*) die off rates in digestate are higher compared to those observed during the storage of raw slurry alone. After 7 days the concentration of pathogens in the digestate was found to be $<3 \log_{10} \text{ CFU g}^{-1}$ (EU limit) while the concentration in the stored slurry was above the limit and was found to be at least $2.75 \log_{10}$ higher compared to digestate. According to Paavola and Rintala (2008), the effect of storage over a long period of time (>100 days) can have a substantial impact on pathogen reduction after the MAD process. Luo et al. (2017) reported that the number of total coliforms and *E. coli* in the digestate slurries (chicken and pig) decreased continuously during the storage period. More than a $2 \log_{10}$ reduction in *E. coli* levels was observed during 60 days of storage time. However, the rate of die-off was comparatively low for pig slurry and it was also reported that the higher reduction rate in the storage process of the chicken manure-digestate slurry was possibly due to the higher concentration of ammonia. Biswas et al. (2016) investigated pathogen decay in liquid dairy manure in anaerobic and limited aerobic storage conditions and identified that the ranges of *E. coli*, *Salmonella*, and *Listeria* levels over the 14 day incubation period at $30 \text{ }^\circ\text{C}$ were of the order 10^8 , 10^8 to 10^7 , and 10^7 to 10^6 , respectively; and both growth and inactivation patterns were driven by temperatures during the storage period.

Digestate is typically spread on land without any storage. Longhurst et al. (2013) indicated that the top layer of soil (top 300 mm) contains the highest concentration of pathogens. Therefore, as the volume of digestate is increased by a new larger soil volume, the concentration of pathogens is diluted in the soil ($1/30$ calculated as $1.477 \log_{10}$ reduction in concentration). A similar dilution effect for pathogens (water medium) has also been observed by other researchers (Kinyua et al., 2016; Clarke et al., 2017; Healy et al., 2017). After the application of FYM&S and/or digestate on land, pathogens have the potential to contaminate surface water (streams and lakes) and groundwater as a result of surface runoff due to rainfall. The EC (2003) recommended that a default dilution rate of 10 is used for sewage from a municipal water treatment plant where the surface runoff is mixed with river water. Consequently, this dilution factor has moderate to high importance in the evaluation of pathogen counts as a part of exposure assessment.

Pathogens decay over time following land application as a result of sunlight exposure (ultraviolet ray and heat) (Mtapuri-Zinyowera et al., 2009). Peng et al. (2008) concluded that temperature is the most important factor affecting the survival of *C. parvum* oocysts in the environment. Clarke et al. (2017) reported that the time required to travel from one point to another in a stream is the most sensitive parameter in bacterial decay (due to ultraviolet light, varying temperature and pH) modelling. Benham et al. (2006) reported that the first order decay equation is often used to describe the bacterial die-off (Chick's Law) rate of total coliform and *E. coli* in the soil, manure, streams and groundwater over time. Clarke et al. (2017) reported that the first

Table 4

Risk assessment studies on waste, digestate and FYM&S with details of pathogens assessed, source, pathways, AD condition, exposure, dose-response characteristics and sensitivity of key parameters of the model.

Country	Hazard identification (contaminants)	Methodology	Source	Pathway	AD condition	Exposure (pathogen concentration)	Hazard characterisation: minimum infectious dose (ID), (dose-response)	Most sensitive parameter mentioned in the study	Reference
United Kingdom	Scrapie	Stochastic and probabilistic (Monte Carlo simulation)	Carcass	Oral intake of sludge by sheep	Not AD; based on closed carcass storage	Not clearly mentioned; with 10 g ingestion, equating to an estimated mean of 13% risk of infection	Annual $10^{3.6}$ Ovine Oral ID ₅₀	a) Variability and uncertainty of infected sheep b) higher temperatures, alkalinity, storage for decay	(Adkin et al., 2014)
Costa Rica	<i>Cryptosporidium parvum</i>	Stochastic and probabilistic (Monte Carlo simulation)	Livestock waste	Fomite, soil and crop contamination, livestock waste management	21 °C, removal rates 0.023 log removal day ⁻¹	(Raw cattle) 9.09×10^4 to 3.89×10^{10} cysts g ⁻¹ TS, (digestate) 2.81×10^1 to 1.40×10^5 cysts g ⁻¹ TS	Dose: amount of soil ingested (0.48 kg soil/day); a single agent causing infection (r) = 5.72×10^{-2}	Soil contamination	(Kinyua et al., 2016)
Costa Rica	<i>Giardia lamblia</i>	Same as above	Livestock waste	Fomite, soil and crop contamination, livestock waste management	21 °C, removal rates 0.065 log removal day ⁻¹	(Raw cattle) 8.14×10^3 to 1.99×10^5 cysts g ⁻¹ TS, (digestate) 3.00×10^{-3} to 9.26×10^4 cysts g ⁻¹ TS	Dose: amount of soil ingested (0.48 kg soil/day); a single agent causing infection (r) = 1.99×10^{-2}	Soil contamination	(Kinyua et al., 2016)
USA	Many	Review on the mostly deterministic experimental studies	Class A and B biosolids and manure	Contamination of manure and biosolids to ground and surface water	Mesophilic; reduction in pathogen by 1 log	Varies with biosolids and manure (reference paper: Table 1 for biosolids and Table 2 for manure)	Various pathogens (Table 3 of the reference paper)	High livestock density and lack of advanced treatment systems for animal and human waste, and wastewater in a rural area of USA	(Oun et al., 2014)
Australia	<i>Campylobacter</i> spp.	Fate (stochastic) and transport model and probabilistic	Animal faeces, sewage	Foodborne (contaminated poultry, red meat, unpasteurised milk, unwashed fruit & vegetables), surface water, ground and drinking water	Mesophilic; 37 °C, varies with different barriers, sources and conditions (Table 2 of the reference paper)	Concentration in various sources of excrement, 10^3 to 10^8 CFU g ⁻¹ of faeces	500 organisms	Storage, raw meat and correct handling procedures	(Whiley et al., 2013)
USA	Human enteric viruses; echovirus, enterovirus, adenoviruses, rotaviruses, and noroviruses	Four-step QMRA methodology: stochastic and probabilistic	Class B biosolids; dewatered and MAD	Soil ingestion	Mesophilic	Many (Table III of the reference paper), daily soil ingestion rate: 50 mg day ⁻¹ (child) and 480 mg day ⁻¹	Exponential or Beta-Poisson models (Table II of the reference paper)	Virus concentration and ingestion rate, enteroviruses concentration was the most important parameter	(Kumar et al., 2012)
USA	Many	Four-step QMRA methodology: stochastic and probabilistic	Municipal Class B biosolids and manure	Soil ingestion, fresh crop ingestion, inhalation of bio-aerosols	Mesophilic	Varies with pathogen and species (Table 1 of the reference paper)	Many (Table 1 of the reference paper)	Time and dilution: mitigating factors in reducing all risks	(Brooks et al., 2012)
Globally	<i>Ascaris lumbricoides</i>	Monte Carlo (QMRA-MC) probabilistic model	Wastewater	Wastewater-irrigated carrots; inside carrot and residue soil on the skin	NA; Waste stabilization pond WSP 1-day anaerobic pond and 5-day facultative pond	1 to 10 eggs/l of treated wastewater	Dose N ₅₀ = 859; 1 egg/l results in an Ascaris infection risk of 6×10^{-4} per person per year (pppy) for children under 15	Type of wastewater (raw, treated, comply with the 1989 and 2006 WHO guidelines, highly treated) and the type of area (hyperendemic, endemic and non-endemic)	(Mara and Sleight, 2010)
United Kingdom	Prions; such as transmissible spongiform encephalopathies (TSEs), bovine spongiform encephalopathy (BSE),	Monte Carlo probabilistic model using @Risk (©Palisade)	Wastewater handling animal carcass; specified risk material (SRM-ABP category 1)	Soil ingestion	Not AD; the spread of wastewater derived from SRM handling facilities (abattoirs)	The maximum application rate of treated wastewater 0.025 tons m ⁻³ year ⁻¹ ; the amount of soil consumed per day 0.23 to 0.38 kg	Uncertain; assumed that the probability of infection by every single agent is independent of the size of the dose; the proportion of a population that becomes infected from a single dose =	For BSE in cattle, abattoir and rendering effluent; for TSEs in sheep, effluent from small incinerators and rendering plants; for scrapie, rendering plants major contributors	(Adkin et al., 2013)

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Table 4 (continued)

Country	Hazard identification (contaminants)	Methodology	Source	Pathway	AD condition	Exposure (pathogen concentration)	Hazard characterisation: minimum infectious dose (ID), (dose-response)	Most sensitive parameter mentioned in the study	Reference
USA	scrapie Human enteric viruses	Stochastic and probabilistic	Class A biosolids (indicator virus post-treatment limit 1 plaque-forming unit PFU (4 g) ⁻¹), for class B the condition is not strong	Direct ingestion, groundwater, inhalation of Bio-aerosols	One or two mesophilic digesters; log removal of single digester 1/15, double digester 1/7.5 and with lime treatment 1.1	0 to 31 PFU (4 g) ⁻¹ of raw sludge, <1 PFU (4 g) ⁻¹ in treated sludge	0.5 Beta-Poisson dose-response model; $\alpha = 0.026$ $\beta = 0.4$	Type of treatment, nonporous groundwater scenario (2×10^{-3})	(Eisenberg et al., 2008)
Italy	<i>Cryptosporidium</i> , <i>Campylobacter</i> and rotavirus	Four-step QMRA methodology: mostly deterministic	Wastewater treatment plants (WWTP) effluent	Bio-aerosols	Not mentioned (whether mesophilic or thermophilic)	Raw wastewater <i>Cryptosporidium</i> (1 to 10^4 oocysts l ⁻¹), <i>Campylobacter</i> (10^2 to 10^5 CFU l ⁻¹), and rotavirus (10^2 to 10^5 MPN l ⁻¹); treated wastewater <i>Cryptosporidium</i> (57.87 oocysts s ⁻¹), <i>Campylobacter</i> (578.70 CFU s ⁻¹) and Rotavirus (578.70 MPN s ⁻¹)	Beta-Poisson dose response model 9day value; <i>Cryptosporidium</i> D ₅₀ = 73, $\alpha = 0.115$, $\beta = 0.176$, <i>Campylobacter</i> D ₅₀ = 3.84×10^{10} , $\alpha = 0.024$, $\beta = 0.011$ rotavirus D ₅₀ = 6.17, $\alpha = 0.2531$, $\beta = 0.4265$	Weather conditions: most unfavourable (high humidity and low solar radiation, moderate to extremely stable atmospheric conditions and slight breeze)	(Stellacci et al., 2009)
Vietnam	<i>E. coli</i> O157:H7, <i>G. lamblia</i> , <i>C. parvum</i>	Four-step QMRA, Monte Carlo probabilistic model using @Risk (©Palisade)	Wastewater effluent	Accidental ingestion of wastewater by splashing directly into the mouths or indirectly on hands and then to the mouth	Mesophilic 37 °C	Mean values: (Biogas effluent) <i>E. coli</i> 14.7×10^5 CFU (100 ml) ⁻¹ , <i>G. lamblia</i> 19 cysts (100 ml) ⁻¹ , <i>C. parvum</i> 18 oocysts (100 ml) ⁻¹ , (drains) <i>E. coli</i> 9.3×10^5 CFU (100 ml) ⁻¹ , <i>G. lamblia</i> 4 cysts (100 ml) ⁻¹ , <i>C. parvum</i> 12 oocysts (100 ml) ⁻¹ ; ingestion dose 1 ml	Beta-Poisson dose-response model (<i>E. coli</i> O157:H7): ID ₅₀ = 214.94, $\alpha = 0.373$; exponential dose-response model (<i>G. lamblia</i> : $r = 0.0199$ and <i>C. parvum</i> : $r = 0.0042$)	High pathogen concentration in wastewater effluent (e.g., <i>E. coli</i> concentration was 14.7 to 147 times higher than the recommended standard (10^3 to 10^5 CFU (100 ml) ⁻¹) by WHO	(Le-Thi et al., 2017)
Ireland	Total coliform (TC) and <i>E. coli</i>	Stochastic fate model, Monte Carlo probabilistic model using @Risk (©Palisade)	Anaerobically digested biosolids	Effluent (surface-runoff)	Not mentioned (whether mesophilic or thermophilic)	Mean viable TC values 16.83 (24-hour rainfall) and 26.75 (48-hour rainfall) MPN day ⁻¹ ; for <i>E. coli</i> it was 5.20×10^{-1} and 2.34×10^{-1} MPN day ⁻¹	Exponential dose-response model; $r = 0.01$ for immunocompromised; 0.0000005 for immuno-competent population	'Time in stream' parameter in 'die-off rate in stream' model; risk of illness for immuno-compromised populations exceeded the thresholds of acceptable risk by a factor of 3 for TC and 1 to 3 for <i>E. coli</i>	(Clarke et al., 2017)
United Kingdom	Bacteria; <i>Campylobacter jejuni</i> , <i>Listeria monocytogenes</i> , <i>E. coli</i> O157, <i>Salmonella</i> , parasite; <i>Cryptosporidium parvum</i> , viruses; FMD, CSF prions; scrapie, and plant pathogens	Four-step QMRA, mostly stochastic (decay in soil) and deterministic approach based on literature-based data, a large report covering all indicators	Source-segregated anaerobic digestate	The soil on RTE crops (individual ingestion 0.35 g day ⁻¹)	Mesophilic	<i>Campylobacter jejuni</i> 5.7×10^{-10} CFU day ⁻¹ ; <i>Listeria monocytogenes</i> 2.11×10^{-11} CFU day ⁻¹ ; <i>E. coli</i> O157 3.2×10^{-9} CFU day ⁻¹ ; <i>Salmonella</i> 1.48×10^{-11} CFU day ⁻¹ ; <i>Cryptosporidium parvum</i> 7.98×10^{-11} oocysts day ⁻¹ ; FMD oral ID ₅₀ : cattle 2.6×10^{-12} , sheep 1.56×10^{-12} , pig 8.88×10^{-13} ; CSF porcine oral ID _{50s} 4.25×10^{-9} ; scrapie ovine oral ID ₅₀ 1.9×10^{-6} to 1.3×10^{-7}	Dose-response model types i) Beta-Poisson mathematical model for <i>Campylobacter jejuni</i> , <i>Listeria monocytogenes</i> , <i>E. coli</i> O157 ii) negative exponential model for <i>Salmonella</i> , <i>Cryptosporidium parvum</i> iii) $0.69 \times ID_{50}$ for viruses	The proportion of meat going to anaerobic digestion has little effect on the overall risks; conditions: 6.0 log reduction of the bacterial pathogens during pasteurisation 5.0 log for viruses	(Longhurst et al., 2013)

Note: ID₅₀ means infectious dose required to cause infection in 50% of an exposed population.

Four-step QMRA: hazard identification, exposure assessment, hazard characterisation and risk characterisation (Quantitative Microbiological Risk Assessment).

order inactivation constant for *E. coli* in water varies from 0.7 to 1.5 day⁻¹. Likewise, Saunders et al. (2012) evaluated the die-off rate of faecal coliform and *E. coli* in the soil as varying from 0.019 to 0.312 day⁻¹ and 0.014 to 0.328 day⁻¹, respectively.

Ireland has a low water stress index (Bixio et al., 2005); about 75% of Irish drinking water is abstracted from surface water, with the balance sourced from wells and boreholes. Roughly 1000 public water supply schemes deliver in excess of 1.2 Mm³ of water per day (Angelakis and Bontoux, 2001). Ireland is situated in North Atlantic Europe where rainfall in agricultural areas negates the need for irrigation and reuse of wastewater for irrigation is not typical in Ireland (Angelakis and Bontoux, 2001). Thus, the main source of contamination is likely to be through the use of organic fertilizers. In addition, decay over time (from sowing to harvest) in different environmental media (soil, water and air) can influence final pathogen reduction (Gurian et al., 2012; Longhurst et al., 2016; Clarke et al., 2017).

5. Risk assessment

The Codex Alimentarius Commission (Codex Alimentarius, 2011) provides a methodology for risk analysis. Risk analysis provides a structured framework for guiding policymakers and assisting in decision-making. The steps are hazard identification, exposure assessment, hazard characterisation and risk characterisation. In general terms, risk depends on three factors: (a) the level of pathogens present in the medium (e.g., soil, water and air), (b) level of exposure to the medium and finally, (c) the inherent harmfulness of the pathogen (U.S. EPA, 2019). Both qualitative (categorical outcome as low, moderate, high) and quantitative (deterministic, probabilistic or stochastic models) methods can be used for risk assessment (Schowanek et al., 2007; Adkin et al., 2014; Teunis and Schijven, 2019). Even, the term semi-quantitative risk assessment is used, as an intermediate stage between qualitative and quantitative risk assessments. In semi-quantitative risk assessment, risk factor categories are typically given a score (0: good, 1: medium, 2: bad) and calculations are based on score arithmetic (probability-impact matrix) (Teunis and Schijven, 2019).

Previous environmental risk assessment studies (Table 4) have focused on evaluating risks from spreading biosolids, manure and anaerobic digestion sludge (digestate) from municipal solid waste (MSW) management plants. Studies have reported varying effects of the AD process parameters on pathogen inactivation, including storage, temperature and pH level (Adkin et al., 2013, 2014; Whiley et al., 2013), highlighting the need for further investigation in this area. On the one hand, Brooks et al. (2012) reported that there is a potential risk associated with bacteria and parasites after immediate application of digestate on land; on the other hand, fomite, ground and surface water contamination, bioaerosols, secondary contamination and direct ingestion were also identified as possible significant hazard pathways (Eisenberg et al., 2008; Kinyua et al., 2016). Climatic conditions, including wind and rainfall, may also influence potential risk (Stellacci et al., 2009; Healy et al., 2017). The drinking water treatment plant plays a vital role to mitigate the problem of high levels of pathogen concentration in the water extraction points. According to Clarke et al. (2017), up to 6 log₁₀ reduction can be achieved in drinking water treatment plant through coagulation or flocculation and sedimentation (up to 2 log), filter reduction (rapid sand: up to 2 log) and disinfection (up to 2 log). Also, washing can reduce pathogen concentration on leafy greens by 1 log and 2 to 3 log using water and chlorine, respectively (Danyluk and Schaffner, 2011). The shelf life of RTE salads is generally, 1 to 5 days (FSAI, 2015) and Danyluk and Schaffner (2011) reported that the pathogens can grow on shelves at the rate of 0.0616 log CFU day⁻¹ °C⁻¹ which can be an important aspect of assessing the risk to the consumers.

The pathogenic risk to humans and animals can be incurred in different ways, including direct contact (Jackson et al., 1992; Jones and Martin, 2003), contact with zoonotic pathogens (fomites) (Harder

et al., 2014), assimilation (Fernandes et al., 2011), and inhalation (Fournier and Marrie, 1998; Pandey et al., 2013; Reed, 2014) or even person to person (Weinstein et al., 2001). A threshold level may be set to ensure safety, for example, the limit for *E. coli* and *Enterococci* in drinking water is 0 CFU (100 ml)⁻¹ (the European Communities, 2007). Ultimately the risk is dependent on the efficacy seriousness of the hazard, the dose and vulnerability of the exposed population. A hazard characterisation model determines the relationship between various doses and the effect (response) (Gibson III et al., 1998; U.S. EPA, 2019). The microbial dose-response relationship (Table 4) quantifies the probability of infection in relation to the exposure (ingestion) of pathogens; bacteria (colony forming units), viruses (plaque forming units), and protozoa (cysts or oocyst counts) (Gibson III et al., 1998). In addition, the risk from biological hazards can vary for immunocompromised individuals, including pregnant women children and the elderly (Mara and Sleight, 2010; Healy et al., 2017). A number of dose-response models are available in CAMRA (2018). A detailed dose-response model is needed to assess hazard characterisation in this area with particular emphasis on ensuring the safety of the both immunocompetent and vulnerable immunocompromised population.

6. Indicator organisms

Indicator organisms are commonly used to give an insight into the possible presence or survival of pathogens and are typically used in environmental risk assessment (Ferguson et al., 1996). Indicator bacteria such as faecal coliform and enterococci are part of the intestinal flora of warm-blooded animals (U.S. EPA, 2013) and help to identify the potential presence of faecal material and associated pathogens (Sahlström, 2003). Wilkes et al. (2009) contend that no one indicator is appropriate for all environmental systems and pathogens, even within a common geographic boundary (state or country). A list of indicator organisms which may be useful to consider when assessing potential hazards in AD digestate and FYM is presented in Table 5. According to the U.S. EPA (1990) cited by Aitken et al. (2005), the upper limit of indicator bacteria, viruses and helminth parasites in treated digestate sludge (4 g of dry solids) are set as 3 most probable number (MPN), 1 plaque-forming unit (PFU) and 1 ovum, respectively. According to (USEPA 2003), the AD process can have an inactivation range of 0.5 to 4.0 log₁₀, 0.5 to 2.0 log₁₀ and 0.5 log₁₀ of bacteria, viruses and parasites (protozoa and helminths), respectively. Thus, indicator organisms can be useful for initial risk screening of pathogens which can be present in the digestate.

Table 5
List of available indicator pathogens.

Name	Indicator for	Reference
<i>Escherichia coli</i>	Gram -ve, non-spore forming coliform bacteria	(Johansson et al., 2005)
<i>Salmonella</i> <i>Senftenberg</i>	Gram -ve, non-spore forming bacteria	(Wheeler et al., 1943; Mocé-Ilivina et al., 2003)
<i>Enterococcus faecalis</i>	Gram +ve, non-spore forming bacteria	(McFeters et al., 1974; Mocé-Ilivina et al., 2003; Sahlström, 2003; Anderson et al., 2005; Sidhu and Toze, 2009; Sahlström, 2003)
<i>Clostridium</i> spp.	Gram +ve, spore-forming bacteria	(Payment and Franco, 1993; Ferguson et al., 1996; WHO, 2001)
<i>Mycobacterium</i> spp.	Acid-fast, thermoresistant bacteria	(Deb et al., 2009)
Feline calicivirus (FCV)	Non-enveloped virus; resistant. enteric virus	(Wong et al., 2010; Cook, 2013; Cromeans et al., 2014)
<i>Ascaris</i> spp.	Parasites	(Nelson et al., 2004; Aitken et al., 2005)
<i>Cryptosporidium parvum</i>	Parasites	(Harwood et al., 2005)

7. EU and Irish legislative restrictions

Animal by-products (ABP) are defined (BSI, 2014) as 'entire bodies or parts of animals or products of animal origin referred to in Articles 2 and 3 of EU Regulation No 1069/2009 that are not intended for human consumption, including ova, embryos and semen'. Within the EU, the feedstock (waste material) is categorised based on the concentration and probability of the presence of hazards. Waste-Category 1 includes brain, spinal cord; Category 2 includes fallen animals, manure, contaminated milk; and Category 3 represents catering waste, brown or black bin waste (European Parliament and Council, 2009). However, the maximum concentration (allowable limit) of the indicator pathogens in digestate ABP is not clearly defined in (BSI, 2014). DAFF (2014a, 2014b) defines the type of AD plants (Table ST1, Supplementary materials) in Ireland according to the different categories of waste that they can use as the feedstock, different temperature regimes for heat treatment, and application condition of the digestate. For plant type 1 and 2, pasteurisation is essential whereas plant type 3 (if imported ABP is <5000 tons per annum) and 4 (deals with only waste category 3), pasteurisation is not required. The rules for plant type 5 (can accept waste category 2 and 3) are under review at present.

The EU Commission recommends pasteurisation at 70 °C for 60 min for treating waste before (or after) the AD process (EPC, 2002; The European Commission, 2011). DAFF (2009) approved an alternative method for feedstock (including catering waste mixed with manure, milk and colostrum) using a lower treatment temperature (60 °C) for a longer duration; 48 continuous hours (two times) where the maximum size entering the unit has been restricted to 400 mm. The Irish regulation (DAFF, 2009) specifies that the thermal (e.g. pasteurisation) and chemical (e.g. use of lime) process must reduce either *Enterococcus faecalis* or *Salmonella* Senftenberg by 5 log₁₀ and thermo-resistant viruses such as parvovirus must be decreased by 3 log₁₀ and there must be a 99.9% reduction in parasitic eggs, namely *Ascaris* spp. According to Coultry et al. (2013), pasteurisation at higher temperature (70 °C) for a 60 min (EPC, 2002; The European Commission, 2011) is more energy efficient compared to 60 °C for 48 continuous hours (two times) (DAFF, 2009) as the cost associated with heating the undigestate organic material twice to 60 °C for 48 h is 80 fold higher compared to heating the same to 70 °C for 1 h (the EU standard) (Coultry et al., 2013). Similarly, post-digestion pasteurisation using the Irish national standard is an almost 65-fold increase on the cost of pasteurisation meeting the EU standard (Coultry et al., 2013). Hence, due to the variability of time-temperature conditions, lab-scale experiments are required to check whether pasteurisation parameters are sufficient to meet the recommended levels of pathogen inactivation or if any additional chemical treatment (such as lime addition) may also be required.

There is a restriction on animal grazing and sowing after the application of organic fertilizer on to agricultural land in Ireland (DAFF, 2009). EPC (2002) suggests a minimum restriction for grazing animals (other than pigs) of 21 days (while for pigs it is 60 days), to avoid the potential of disease transmission. Similarly, in the case of new crop plantations, the restriction is also 21 days to avoid a high concentration of pathogen assimilation through water intake. The digestate can be spread on agricultural land in the same manner as is done with FYM&S and the same regulations and guidance apply to their application. FAI (2017) report the application rate of manure and slurries are restricted (DAFF, 2014c) depending on pathogenic concerns, soil nutrient levels (N, P, K) and crop intake capacity. Animal manure application rates are based on crop requirements; for example, root crops can take cattle-slurry up to 44 m³ ha⁻¹ in one application and for pig slurry, 11 m³ ha⁻¹ year⁻¹ is adequate for grass and cereals. The maximum application rate of cattle slurry is recommended as 55 m³ ha⁻¹ which reduces to 33 m³ ha⁻¹ if applied in a single application while 27.5 m³ ha⁻¹ for first cut silage and root crops is recommended for pig slurry (DAFF, 2014c; Government of Ireland, 2014). The shortfall between the nutrients applied in the manure and crop requirements should be made up

with inorganic fertilizers. The slurry should be applied when plants require the nutrients i.e., at the start or during the growing season. FYM should be stored in a manner that minimizes nutrient loss and applied to soil according to soil conditions and recommendations (Teagasc, 2017). Application of slurry is not permitted when heavy rain is forecast within 48 h and when the soil is saturated, frozen or snow-covered (The Council of the European Communities, 1991; Government of Ireland, 2014). When applying slurry, direct contamination of watercourses should be avoided by leaving adequate buffer zones (DAFF, 2014c) (Table ST2, Supplementary materials). The restriction limits may help in the reduction in human and animal health risks.

8. Knowledge gaps and recommendations

There are significant gaps identified in the literature which require further study before any potential risks from AD can be assessed. A recommended framework (Fig. 5) for potential risk assessment of the AD process is presented along with identified knowledge gaps in this area. Significant gaps exist with regards to:

1. Hazard identification by qualitative or semi-quantitative screening approaches (e.g. WRAP (2016) cited in Longhurst et al., 2019) are limited. They rank potential hazardous pathogens (Table 1 and Sections 3.1 and 3.2) and their ability to survive the AD process, possible routes (water, aerosol, soil ingestion, food and zoonotic contact) of transmission and potential severity of illness. An appropriate scoring system can be used to classify pathogen pathways and survival during AD/pasteurisation allowing for greater characterisation of hazard potential.
2. AD inactivation behaviour under several typical process conditions needs to be investigated (Table 3). While significant work has been done for some pathogens (e.g. *E. coli*, total coliform, *Enterococci*, *Mycobacterium*, *Salmonella*) there is limited work for others (e.g. *Clostridium* spp., *Ascaris* spp., enterovirus).
3. The cumulative effect of all AD parameters on indicator pathogens is unknown. A multivariate analysis is recommended to check the effect of independent AD parameters (from Tables 2 and 3) such as type of recipes, OLR, HRT and dependent parameters pH, free NH₃ on at least one indicator such as *E. coli*, total coliforms and heat resistant *Enterococci* as these were widely used as the indicator pathogens in many studies (Table 3).
4. The effect of pasteurisation sequence (pre- or post-AD) is established (Nolan et al., 2018) for some indicators (*E. coli*, total coliforms and *Enterococci*) but is unknown for others (e.g. *Mycobacterium* spp., Feline calicivirus (FCV), *Cryptosporidium parvum*).
5. While general decay constants are available for 'soil' (Longhurst et al., 2013), this may vary according to different soil texture classes (sandy, loam, clay etc.), pH, moisture content and the effect of soil organic carbon on the growth of pathogens needs to be established (Lang and Smith, 2007).
6. Environmental contamination routes (accidental oral intake of soil, fomite, contamination of FYM&S to ground and surface water, food (contaminated poultry, red meat, unpasteurised milk, unwashed fruit & vegetables), inhalation of bio-aerosols, wastewater-irrigated RTE crops as mentioned in the 5th column of Table 4) need to be investigated further to establish potential human exposure routes to pathogens following the application of AD digestate on land. Included in this are potential effects of process treatments (e.g. drinking water treatment) on abstracted water sources.

9. Conclusion

In this review, the focus was on pathogens originating from FYM&S and anaerobic digestate which can be transmitted to humans via aerosols, water, soil, crop, and fomites. There is significant potential to use AD as a renewable energy source and to use the resulting digestate as a valuable co-product on land. It is evident from the literature that a

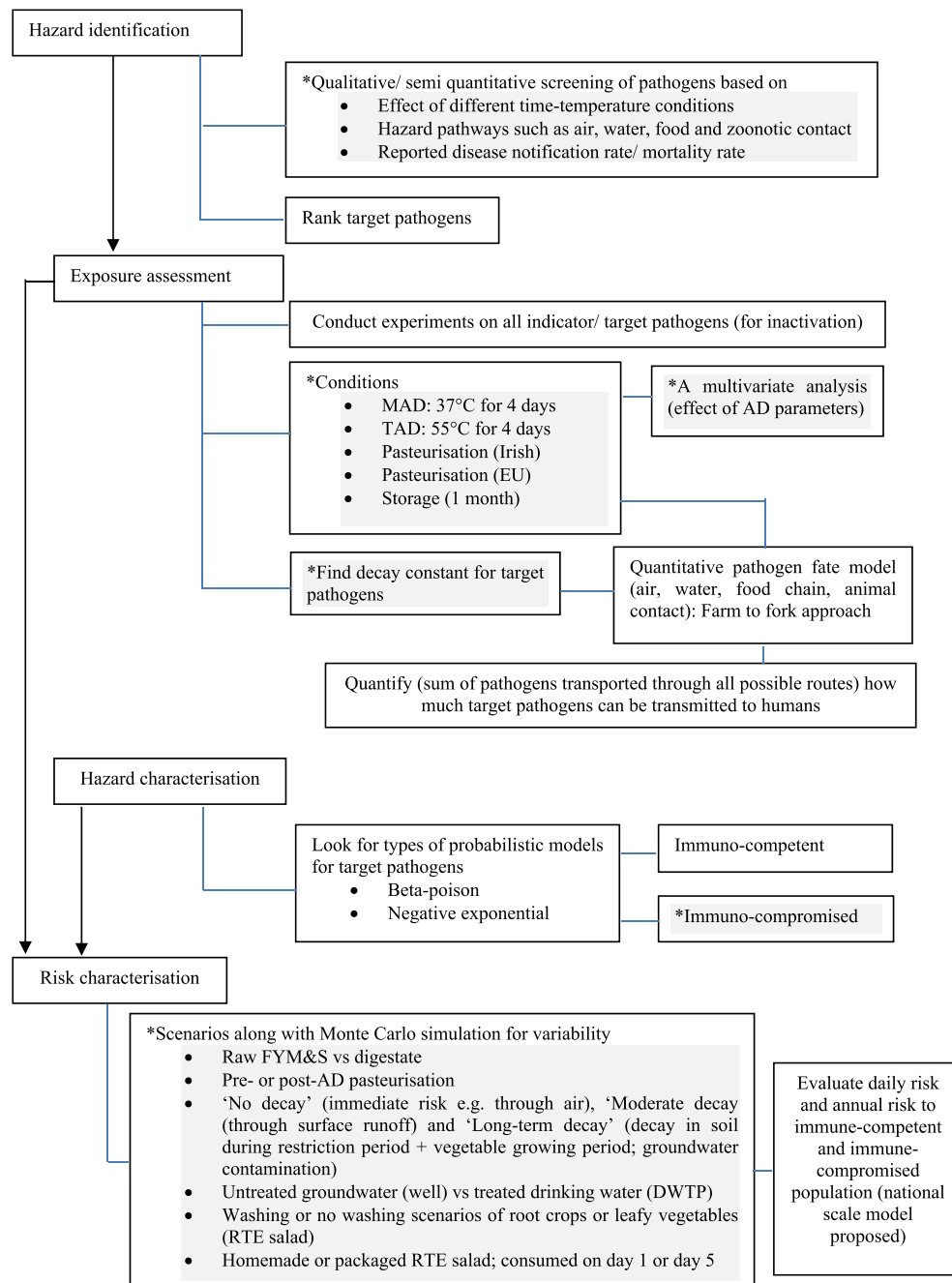


Fig. 5. Framework for risk assessment of AD showing knowledge gaps (*).

multitude of different pathogens are present in different quantities in FYM&S and anaerobic digestate but the influence of spreading FYM&S and digestate on agricultural land and resulting potential human exposure requires further research. Such an evaluation should be based around the principles of risk assessment and consider critical parameters including temperature (MAD or TAD), pH, OLR, toxicity, C:N ratio and HRT. This study highlights the significance of high temperature on pathogen inactivation. The optimal temperature for mesophilic growth was reported as near 40 °C whereas the most advantageous pH for bacterial growth in AD ranges from 6.5 to 8.5. Free NH₃ levels should be maintained below 80 mg L⁻¹ and optimal OLR varies from 2.5 to 7.0 kg VS m⁻³ day⁻¹ depending on organic proportions (C:N ratio ranging from 20:1 to 30:1) of feedstock. HRT for cattle manure, cattle manure with straw bedding and pig manure of 12 to 25 days, 15 to 35 days, and 10 to 20 days, respectively could be advantageous for

both the gas production and subsequent reduction in pathogen count. Very high OLR can result in high NH₃ (free ammonia) generation which further makes the environment toxic for bacterial growth. In higher HRT, a significant amount of inactivation can be achieved due to insufficient food and nutrition. The influence of different feedstock recipes (C:N ratio) and process stages, pre or post pasteurisation on pathogen lethality has also been highlighted. Pasteurisation is the most important parameter to decrease pathogen count (6 log₁₀ reduction). The controlling temperature for AD is the second most important parameter for pathogen inactivation; TAD can inactivate up to 8 log₁₀ of *E. coli* whereas, MAD can only inactivate approximately 2.5 log₁₀. More than a 2 log₁₀ reduction in *E. coli* levels was observed during 60 days of storage time. From the risk assessment point of view, these parameters must be recorded in the farms as AD farms do not use standard time-temperature conditions for the AD, pasteurisation (which is not

mandatory for all plant types) and storage facilities. Thus, the biological risk originating from digestate produced in AD plants may vary. Decay of pathogens during the restriction period and the crops' growing time could be a governing criterion for environmental fate modelling. In managing human exposure pathways, drinking water treatment, washing of RTE crops, shelf life of food and storage time-temperature of RTE salads could also be important parameters for risk assessments. The development of a more unified risk assessment methodology and further enhancement of AD farm-based modelling techniques and datasets would help in advancing knowledge in this area.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2019.06.512>.

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