



Evaluation of pathogen concentration in anaerobic digestate using a predictive modelling approach (ADRISK)



Rajat Nag^{a,*}, Agathe Auer^b, Stephen Nolan^c, Lauren Russell^{b,d}, Bryan K. Markey^b, Paul Whyte^b, Vincent O'Flaherty^c, Declan Bolton^d, Owen Fenton^e, Karl G. Richards^e, Enda Cummins^a

^a University College Dublin School of Biosystems and Food Engineering, Belfield, Dublin 4, Ireland

^b University College Dublin School of Veterinary Medicine, Belfield, Dublin 4, Ireland

^c National University of Ireland Galway, School of Natural Sciences and Ryan Institute, University Road, Galway, Ireland

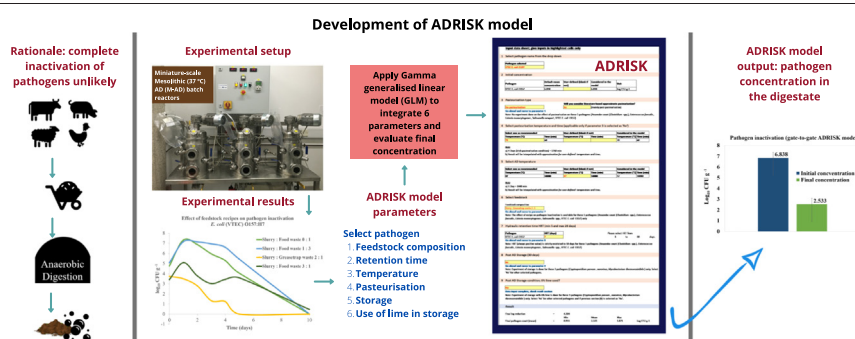
^d TEAGASC, Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland

^e TEAGASC, Environment Research Centre, Johnstown Castle, County Wexford, Ireland

HIGHLIGHTS

- Bespoke ADRISK tool is developed based on a gamma generalised linear model.
- Thermophilic AD/mesophilic AD & pasteurisation (PAS) may ensure the safety of digestate.
- Irish PAS specifications are more effective in eliminating pathogens but are substantially more energy-intensive than the EU equivalent.
- Post-AD literature-based PAS ensures safety.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 14 June 2021

Received in revised form 6 August 2021

Accepted 6 August 2021

Available online 10 August 2021

Editor: Damia Barcelo

Keywords:

Anaerobic digestion
Pathogen concentration
Pasteurisation
Spreadsheets
Exposure assessment
Gamma generalised linear model

ABSTRACT

Farmyard manure and slurry (FYM&S) is a valuable feedstock for anaerobic digestion (AD) plants. However, FYM&S may contain high concentrations of pathogens, and complete inactivation through the AD process is unlikely. Thus, following land application of digestate, pathogens may contaminate a range of environmental media posing a potential threat to public health. The present study aimed to combine primary laboratory data with literature-based secondary data to develop an Excel-based exposure assessment model (ADRISK) using a gamma generalised linear model to predict the final microorganism count in the digestate. This research examines the behaviour of a suite of pathogens (*Cryptosporidium parvum*, norovirus, *Mycobacterium* spp., *Salmonella* spp., *Listeria monocytogenes*, *Clostridium* spp., and pathogenic *Escherichia coli*) and indicators (total coliforms, *E. coli*, and enterococci) during mesophilic anaerobic digestion (M-AD) at 37 °C, pre/post-AD pasteurisation, and after a period of storage (with/without lime) for different feedstock proportions (slurry:food waste: 0:1, 1:3, 2:1, and 3:1). ADRISK tool simulations of faecal indicator bacteria levels across all scenarios show that the digestate can meet the EU standard without pasteurisation if the AD runs at 37 °C or a higher temperature with a higher C:N ratio (recipe 3) and a hydraulic retention time ≥ 7 days. The storage of digestate also reduced levels of microorganisms in the digestate. The Irish pasteurisation process (60 °C for 4 days), although more energy-intensive, is more effective than the EU pasteurisation (70 °C for 1 h) specification. Pre-AD pasteurisation was more effective for *C. parvum*, norovirus, *Mycobacterium thermoresistibile*. However, post-AD literature-based pasteurisation is most likely to assure the safety of the digestate. The information generated from this model can

* Corresponding author.

E-mail addresses: raj.nag@ucd.ie (R. Nag), agathe.auer@ucdconnect.ie (A. Auer), stephen@glasportbio.com (S. Nolan), lauren.russell@ucd.ie (L. Russell), bryan.markey@ucd.ie (B.K. Markey), paul.whyte@ucd.ie (P. Whyte), vincent.oflaherty@nuigalway.ie (V. O'Flaherty), declan.bolton@teagasc.ie (D. Bolton), owen.fenton@teagasc.ie (O. Fenton), karl.richards@teagasc.ie (K.G. Richards), enda.cummins@ucd.ie (E. Cummins).

inform policy-makers regarding the optimal M-AD process parameters necessary to maximise the inactivation of microorganisms, ensuring adverse environmental impact is minimised, and public health is protected.

© 2021 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Anaerobic digestion (AD) is a biological process that utilises organic matter such as food waste and FYM&S to produce biogas. This process yields a valuable co-product digestate, used as a soil conditioner and is widely used as a bio-fertilizer (Nag et al., 2019). Raw or stored FYM&S may contain high levels of pathogens (Hutchison et al., 2004). AD, which can include pasteurisation and storage stages, can inactivate pathogens present in the raw feedstock; however, complete inactivation is unlikely. Humans may be exposed to these pathogens through food (Nag et al., 2020a), water (Clarke et al., 2017), and air pathways (Nag et al., 2021b). As a result, there is a potential health risk associated with the spreading of digestate, necessitating a risk assessment. Foodborne illness or gastroenteritis is a global health concern (WHO, 2008; Thomas et al., 2013; Torgerson et al., 2015). The application of unprocessed animal slurry (AS) and anaerobic digestate on agricultural land could potentially play a role in pathogen transmission to humans

through the food chain, with ready-to-eat (RTE) crops being potential critical vectors as they may be eaten without further treatment or processing (Nag et al., 2020b, 2019).

The AD process is influenced by some independent and dependent parameters that impact pathogen inactivation. Independent parameters include the carbon:nitrogen ratio (a factor of feedstock recipe), pasteurisation conditions (pre, post or no pasteurisation), temperature, organic loading rate (OLR), hydraulic retention time (HRT), and solid retention time (SRT) (Nag et al., 2019). In conventional batch digesters (batch reactor, plug flow reactor (PFR), continuous stirred tank reactor (CSTR)), the fluids, solids and microbes are synchronously discharged; that is, HRT equals SRT (Liu et al., 2011). In contrast, sludge retention digesters (anaerobic contact reactor, up-flow anaerobic sludge blanket (UASB) reactor, expanded granular sludge bed (EGSB) reactor, internal circulation reactor (IC), up-flow solid reactor (USR), and anaerobic baffled reactor (ABR)) are operated at a relatively long SRT and short HRT, attributed to the separation of liquid and solid discharges (Liu et al.,

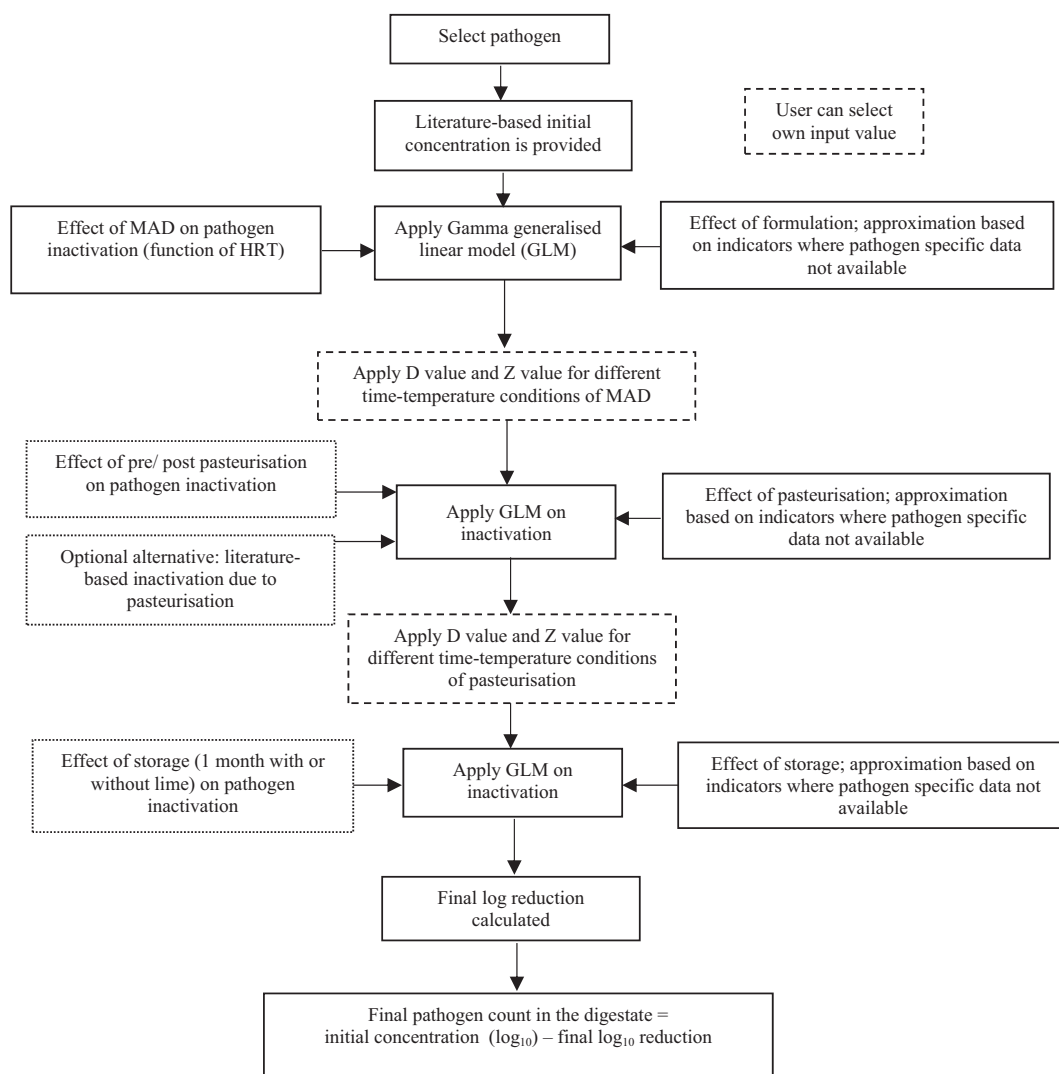


Fig. 1. Schematic of the methodology behind the ADRISK model (dotted boxes are optional inputs).

Table 1
Estimated Zvalue of pathogens (Nag et al., 2019).

Pathogen	Z value (°C)
<i>Clostridium</i> spp.	11.70
Coliform bacteria	9.15
<i>Cryptosporidium parvum</i>	5.00
<i>Enterococcus</i> spp.	7.24
<i>Enterococcus faecalis</i>	7.24
VTEC <i>E. coli</i> O157	9.15
<i>Escherichia coli</i>	9.15
Norovirus	14.43
<i>Listeria monocytogenes</i>	6.35
<i>Mycobacterium thermoresistibile</i>	4.36
<i>Salmonella</i> Newport	7.00

2011). Sludge retention digesters usually have a high biogas production rate. The dependent parameters of the AD plant can be monitored during the process, such as pH and toxicity development, caused by excessive free ammonia (Nag et al., 2019). This study aimed to assess these AD parameter-pathogen relationships and establish a predictive tool for estimating final pathogen counts using a Gamma Generalised Linear Model (GLM) while still recognising data limitations (Rosenfeld and Austbø, 2009). The predictive model was characterised by a combination of experimental results and available literature data.

Quantitative Microbial Risk Assessment (QMRA) is a widely accepted method to assess food quality and safety (U.S. EPA and USDA, 2012). The method is comprised of 4 tiers: hazard identification (HI), exposure assessment (EA), hazard characterisation (HC), and risk characterisation (RC). Nag et al. (2020b) carried out a HI study that ranked potential harmful pathogens based on survival potential during thermal inactivation, potential hazard pathways and severity in untreated patients. The results revealed that *Cryptosporidium parvum*, *Salmonella* spp., norovirus, pathogenic *E. coli*, *Mycobacterium* spp., *Clostridium* spp., and *Listeria monocytogenes* are among the top 10 pathogens of concern in Ireland following land application of digestate. Human norovirus is most likely found in food waste (Nag et al., 2020b) and is unlikely to be found in FYM&S (Mattison et al., 2007). The current study's focus was to develop a predictive model to assess the overall inactivation of pathogens following mesophilic-AD (M-AD) at 37 °C, including pasteurisation and storage

stages. Assessing the levels of pathogens in digestate before land application in agriculture is a critical step in evaluating potential anthropogenic sources of microbial pollution.

This study's principal hypothesis was "FYM&S, which is a valuable feedstock for AD plants, potentially contains high levels of pathogens and AD may not completely inactivate pathogens and thus trigger potential human health concerns". Therefore, this study's overall objective was to create a cursory predictive modelling tool to assess the survival of a suite of pathogens during the AD process using literature data and data from laboratory scale mesophilic AD batch reactors.

2. Materials and methods

A model framework is shown in Fig. 1, which highlights the main steps involved in developing the model and the data inputs for each step. Step 1 involves the quantification of the likely concentration of target pathogens in the feedstock of M-AD. This step allows for the insertion of an initial concentration (within certain realistic bounds). However, default values are suggested based upon a mix of primary experimental and secondary literature-based data. Step 2 looked at the inactivation of pathogens in M-AD (37 °C) based on four different recipes (ratios of Slurry:Food waste 0:1, Slurry:Food waste 1:3, Slurry:Grease-trap waste 2:1, and Slurry:Food waste 3:1). Pathogen inactivation is proportional to the hydraulic retention time (HRT) (Nag et al., 2019), with a 7-day HRT considered for this QMRA. The influence of pasteurisation was evaluated in Step 3. The last step (Step 4) is optional and involved the storage of digestate (with or without lime). Here, the inactivation of pathogens during one-month storage was assessed.

2.1. Initial concentration ($C_{initial}$)

Any initial concentration can be set in the model within a realistic range. However, default values are estimated to guide users. A Bayesian approach (Nag et al., 2021a) was adopted to estimate the concentration of several different pathogens (*Cryptosporidium*, *Mycobacterium*, *Salmonella*, and *Clostridium*) in FYM&S based on the prevalence of the disease in infected animals. The All Island Disease Surveillance Reports (AIDSR) from the last 10 years were considered as the primary data source for the prevalence of these diseases in cattle, pigs, and sheep in

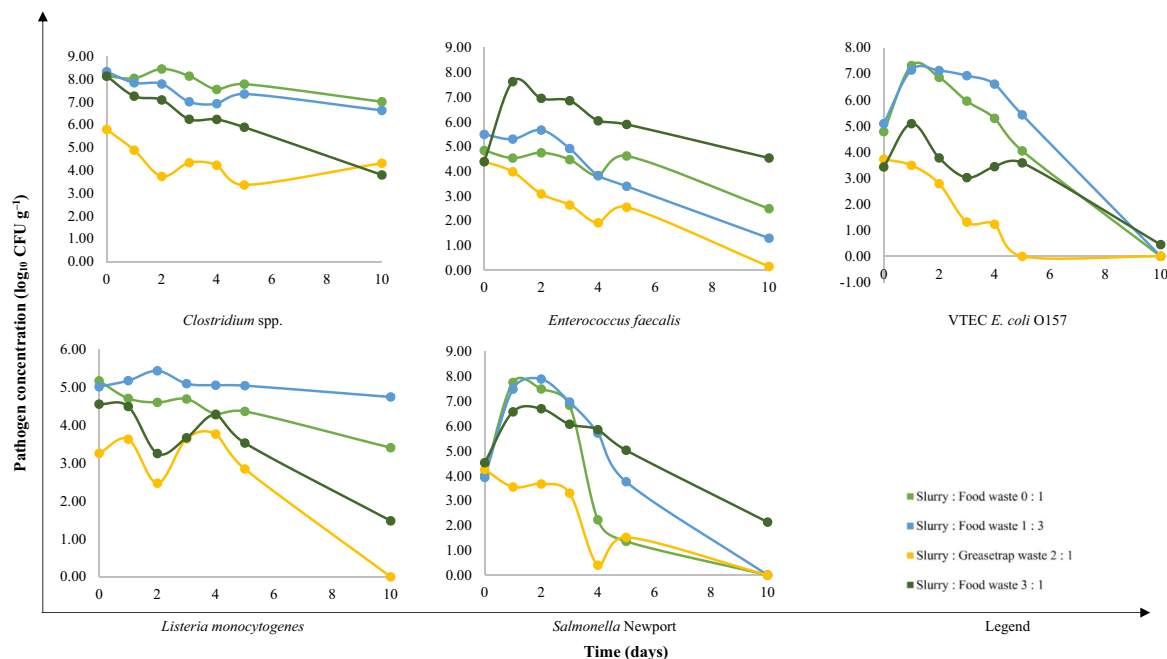


Fig. 2. Effect of different AD feedstock formulations on pathogen inactivation over time; raw data source: Russell et al. (2020).

Ireland. The concentration of pathogens in unprocessed AS from infected animals were taken from peer-reviewed journals. $C_{initial}$ was calculated by multiplying the probability of infection and pathogen concentration in infected animal manure and slurry. In addition, Nolan et al. (2018) recorded the concentration of total coliform, *Enterococcus* spp. and *E. coli* in cattle slurry. Based on Hutchison et al. (2004), the initial levels of VTEC *E. coli* O157 and *Listeria monocytogenes* were estimated. Norovirus concentration was adopted from DiCaprio et al. (2012). The summary of all $C_{initial}$ is presented in Table A1 (supplementary material).

2.2. Experiments: source of primary data

Data from the operation of miniature laboratory-scale mesophilic AD batch reactors (typical) were used to model the effect of AD conditions (Nolan et al., 2018). Four categories of experiments were conducted as follows:

- i. the survival (numbers \log_{10}) of key pathogenic bacteria at mesophilic temperature (37 °C) with different feedstock recipes (Russell et al., 2020).
- ii. the survival (numbers \log_{10}) of coliform bacteria, *Enterococcus* spp., and *E. coli* during AD at mesophilic temperature (37 °C) with either pre-AD pasteurisation (Slurry:Grease trap waste 2:1) or post-AD pasteurisation applied to digestate (Nolan et al., 2018).
- iii. the survival (%) of *Cryptosporidium parvum*, feline calicivirus (FCV), and *Mycobacterium thermoresistibile* during MAD (37 °C) with pre-AD pasteurisation (Slurry:Grease trap waste 2:1) or post-AD pasteurisation applied to digestate (Auer, 2019).
- iv. the survival (%) of *Cryptosporidium parvum*, FCV and *Mycobacterium thermoresistibile* during MAD (37 °C) with pre-AD pasteurisation (Slurry:Grease trap waste 2:1) or post-AD pasteurisation and storage, applied to digestate (Auer, 2019).

The completeness of data for use in the predictive model is documented in Table A2, where the availability of pathogen-specific data is indicated, or alternative indicators used where this information was not available.

2.3. Conversion of experimental time-temperature conditions for inactivation to literature-based Zvalue

The time-temperature conditions for thermal inactivation of each pathogen can be converted to the desired time-temperature conditions of the AD plant with Eq. (1) (Nag et al., 2020b).

$$New\ Dvalue\ (min) = Dref \div 10^{((Tnew - Tref)/Zvalue)} \tag{1}$$

where,

Tref (°C) = reference temperature at which the time-temperature inactivation experiments were done.

Dref (min) = duration of heating at Tref for the experiment necessary for the pathogen's complete inactivation.

Zvalue (°C) = temperature rise necessary to reduce decimal reduction time by one logarithmic cycle. The Zvalue of target pathogens is provided in Table 1.

Tnew (°C) = desired temperature at which the new inactivation duration is required to be known.

2.4. Literature-based pasteurisation

A literature-based assessment of the effect of pasteurisation on pathogen inactivation is presented in Table A3. Inactivation data for *Clostridium perfringens* spores was not found at 60 or 70 °C as temperatures of 70 °C to 110 °C are required to inactivate *Clostridium perfringens* vegetative cells and spores, respectively (Talukdar et al., 2017). The inactivation of indicators viz. total coliform, enterococci, and *E. coli*

Table 2
The derived equations for the effect of the feedstock formulation on pathogen inactivation.

Pathogens	Established equation to determine pathogen count 'y (log ₁₀)' based on different recipes			
	Slurry:Food waste 0:1	Slurry:Food waste 1:3	Slurry:Grease trap waste 2:1	Slurry:Food waste 3:1
<i>Clostridium</i> spp.	$y = 0.0059x^3 - 0.0912x^2 + 0.2131x + 8.0981$	$y = -0.0069x^3 + 0.1185x^2 - 0.6737x + 8.3975$	$y = -0.0065x^3 + 0.148x^2 - 0.966x + 5.696$	$y = -0.0076x^3 + 0.1155x^2 - 0.8296x + 8.1095$
<i>Enterococcus faecalis</i>	$y = -0.0066x^3 + 0.0735x^2 - 0.3161x + 4.8681$	$y = 0.0175x^3 - 0.2591x^2 + 0.4305x + 5.4108$	$y = -0.0109x^3 + 0.1705x^2 - 1.0516x + 4.5687$	$y = -0.0584x^2 + 0.4453x + 5.7459$
VTEC <i>E. coli</i> O157	$y = 0.0432x^3 - 0.6978x^2 + 2.1501x + 5.1086$	$y = 0.0276x^3 - 0.5251x^2 + 1.9616x + 5.2594$	$y = -0.0028x^4 + 0.0645x^3 - 0.4001x^2 + 0.0204x + 3.754$	$y = 0.0008x^3 - 0.0513x^2 + 0.0871x + 3.9415$
<i>Listeria monocytogenes</i>	$y = -0.0044x^3 + 0.0619x^2 - 0.3532x + 5.1251$	$y = 0.005x^3 - 0.0788x^2 + 0.2569x + 5.0349$	$y = 0.0069x^4 - 0.1254x^3 + 0.6131x^2 - 0.8788x + 3.4198$	$y = 0.0026x^4 - 0.0583x^3 + 0.3983x^2 - 1.0472x + 4.7026$
<i>Salmonella</i> Newport	$y = -0.0188x^4 + 0.4293x^3 - 3.1144x^2 + 6.602x + 3.9638$	$y = -0.0102x^4 + 0.2498x^3 - 2.03x^2 + 5.1587x + 3.9809$	$y = -0.0065x^4 + 0.1268x^3 - 0.7222x^2 + 0.6567x + 4.0713$	$y = 0.0319x^3 - 0.5357x^2 + 1.9072x + 4.7425$

Note. 'x' is the hydraulic retention time HRT (number of days) of the mesophilic anaerobic digestion (M-AD) such that $0 \leq x \leq 10$.

was collated from Lau et al. (2019). Longhurst et al. (2013) reported that a 6-log inactivation could be achieved for *E. coli* O157, *Salmonella*, *Listeria monocytogenes*, and *Cryptosporidium parvum*. Therefore, it is suggested that pasteurisation can inactivate 5–6 log (Table A3) when the digestate is pasteurised (post-pasteurisation). The inactivation information of norovirus and *Mycobacterium* spp. by pasteurisation is based on the findings of Baert et al. (2008) and McDonald et al. (2005), respectively.

2.5. Effect of bioreactor feedstock formulation

Pathogen inactivation over time using different recipes is shown in Fig. 2. Polynomial equations are calculated based on the line of best fit for the data points. The fitted equations that are a function of time (days) are collated in Table 2 for pathogens such as *Clostridium* spp., *Enterococcus faecalis*, VTEC *E. coli* O157, *Listeria monocytogenes*, and

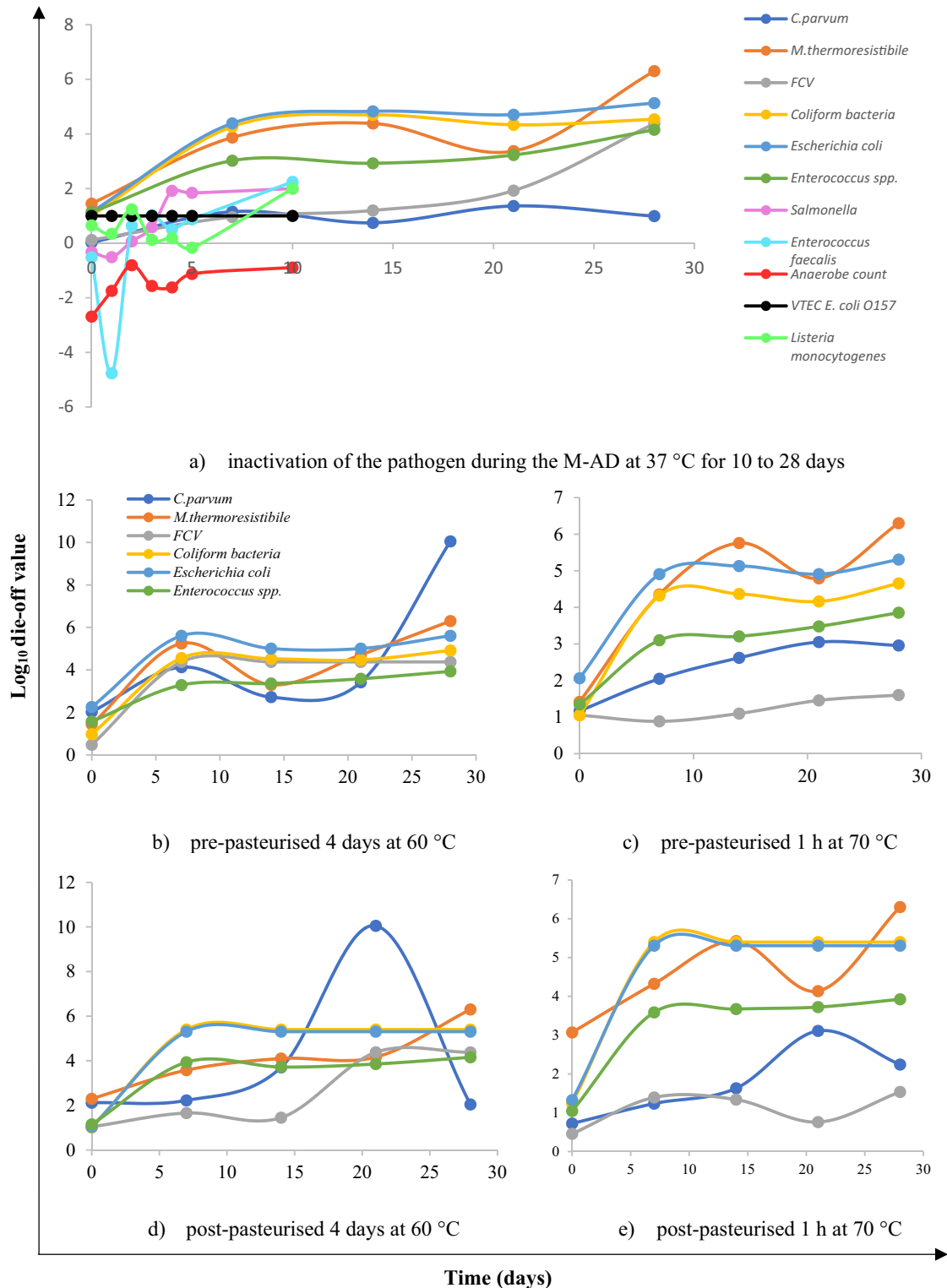


Fig. 3. Log₁₀ die-off value of pathogens in the digestate vs time (days).

Salmonella Newport (4 recipes). As the experiment was done for a maximum of 10 days, the equations are valid for 10 days only.

2.6. Effect of M-AD (37 °C) on pathogen inactivation (LR_{M-AD}) with or without pasteurisation and an optional 1-month storage

The growth and inactivation of pathogens during M-AD at 37 °C for 10 to 28 days was investigated. Four different feedstock formulations were used to check the influence of the carbon and nitrogen ratio on pathogen survival. Several of the species under investigation were cultivated under laboratory conditions for this study. In some cases, when the species could not be cultivated in the laboratory, including many protozoan parasites and viruses (such as norovirus (Haas et al., 2014)), alternative indicator microorganisms were used; feline calicivirus (FCV) was used as a surrogate for norovirus. Similar to Table 2, polynomial equations were fitted to the inactivation profile of

pathogens (Fig. 3), and the equations are displayed in Table 3. The effect of storage (at 10 °C for 30 days) on pathogen inactivation in the digestate was established by conducting tests on *Cryptosporidium*, FCV and *Mycobacterium thermoresistibile*. *M. thermoresistibile* was chosen as an indicator microorganism for bacteria during storage of digestate since it is more environmentally resilient compared to many other bacteria (Auer, 2019; Edwards et al., 2012). The inactivation values are presented in Table 3. In the absence of completeness of the laboratory test on all target pathogens, the inactivation value of *Mycobacterium thermoresistibile* was used for other target bacteria as an approximation.

2.7. Scenario analysis

A set of scenarios can be evaluated using ADRISK to look at the worst-case and most favourable conditions to maximise the log reduction. The baseline scenario looked at recipe 3 at 37 °C M-AD for 7 days without

Table 3
Derived equations based on experimental results MAD, pasteurisation (pre or post-AD) and storage (detection limit correction incorporated).

Pathogens	The predicted equation for log reduction (y) based on HRT (x)	Limitation	
No pasteurisation (MAD)			
<i>Clostridium</i> spp.	See Table 2	0 ≤ x ≤ 10 days	
Coliform bacteria	y = 0.0008 x ³ - 0.0446 x ² + 0.7294 x + 1.0253	0 ≤ x ≤ 28 days	
<i>Cryptosporidium parvum</i>	y = 0.0001 x ³ - 0.0085 x ² + 0.1675 x + 0.0904	0 ≤ x ≤ 28 days	
<i>Enterococcus</i> spp.	y = 0.0006 x ³ - 0.0288 x ² + 0.4177 x + 1.1674	0 ≤ x ≤ 28 days	
<i>Enterococcus faecalis</i>	See Table 2	0 ≤ x ≤ 10 days	
VTEC <i>E. coli</i> O157	See Table 2	0 ≤ x ≤ 10 days	
<i>Escherichia coli</i>	y = 0.0008 x ³ - 0.0433 x ² + 0.7161 x + 1.1565	0 ≤ x ≤ 28 days	
FCV (as surrogate norovirus)	y = 0.0006 x ³ - 0.0183 x ² + 0.2219 x + 0.1162	0 ≤ x ≤ 28 days	
<i>Listeria monocytogenes</i>	See Table 2	0 ≤ x ≤ 10 days	
<i>Mycobacterium thermoresistibile</i>	y = 0.0014 x ³ - 0.0604 x ² + 0.7511 x + 1.3764	0 ≤ x ≤ 28 days	
<i>Salmonella</i> Newport	See Table 2	0 ≤ x ≤ 10 days	
Pre-pasteurised 4 days at 60 °C			
Coliform bacteria	y = 0.001 x ³ - 0.0512 x ² + 0.7897 x + 1.0182	0 ≤ x ≤ 28 days	
<i>Cryptosporidium parvum</i>	y = 0.0023 x ³ - 0.0801 x ² + 0.7304 x + 2.0544	0 ≤ x ≤ 28 days	
<i>Enterococcus</i> spp.	y = 0.0004 x ³ - 0.0218 x ² + 0.3584 x + 1.597	0 ≤ x ≤ 28 days	
<i>Escherichia coli</i>	y = 0.0011 x ³ - 0.0536 x ² + 0.7536 x + 2.3201	0 ≤ x ≤ 28 days	
FCV (as surrogate norovirus)	y = 0.0009 x ³ - 0.0512 x ² + 0.8294 x + 0.5315	0 ≤ x ≤ 28 days	
<i>Mycobacterium thermoresistibile</i>	y = -0.0002 x ⁴ + 0.0134 x ³ - 0.2666 x ² + 1.8268 x + 1.4417	0 ≤ x ≤ 28 days	
Pre-pasteurised 1 h at 70 °C			
Coliform bacteria	y = 0.001 x ³ - 0.0485 x ² + 0.74 x + 1.0778	0 ≤ x ≤ 28 days	
<i>Cryptosporidium parvum</i>	y = -3E-05 x ⁴ + 0.0015 x ³ - 0.0289 x ² + 0.2563 x + 1.1677	0 ≤ x ≤ 28 days	
<i>Enterococcus</i> spp.	y = 0.0004 x ³ - 0.0216 x ² + 0.3621 x + 1.3724	0 ≤ x ≤ 28 days	
<i>Escherichia coli</i>	y = 0.0008 x ³ - 0.0409 x ² + 0.6428 x + 2.0778	0 ≤ x ≤ 28 days	
FCV (as surrogate norovirus)	y = -0.0001 x ³ + 0.0072 x ² - 0.0693 x + 1.0562	0 ≤ x ≤ 28 days	
<i>Mycobacterium thermoresistibile</i>	y = 1E-04 x ⁴ - 0.0045 x ³ + 0.0461 x ² + 0.2864 x + 1.4155	0 ≤ x ≤ 28 days	
Post-pasteurised 4 days at 60 °C			
Coliform bacteria	y = 0.0011 x ³ - 0.0576 x ² + 0.9332 x + 1.0723	0 ≤ x ≤ 28 days	
<i>Cryptosporidium parvum</i>	y = -0.0038 x ³ + 0.144 x ² - 1.0381 x + 2.444	0 ≤ x ≤ 28 days	
<i>Enterococcus</i> spp.	y = 0.0008 x ³ - 0.039 x ² + 0.5971 x + 1.1994	0 ≤ x ≤ 28 days	
<i>Escherichia coli</i>	y = 0.001 x ³ - 0.0561 x ² + 0.9084 x + 1.0942	0 ≤ x ≤ 28 days	
FCV (as surrogate norovirus)	y = -0.0002 x ⁴ + 0.0092 x ³ - 0.1426 x ² + 0.6941 x + 1.038	0 ≤ x ≤ 28 days	
<i>Mycobacterium thermoresistibile</i>	y = 4E-05 x ⁴ - 0.0015 x ³ + 0.0099 x ² + 0.1732 x + 2.2933	0 ≤ x ≤ 28 days	
Post-pasteurised 1 h at 70 °C			
Coliform bacteria	y = 0.001 x ³ - 0.0546 x ² + 0.8855 x + 1.2931	0 ≤ x ≤ 28 days	
<i>Cryptosporidium parvum</i>	y = -0.0005 x ³ + 0.0203 x ² - 0.0894 x + 0.789	0 ≤ x ≤ 28 days	
<i>Enterococcus</i> spp.	y = 0.0006 x ³ - 0.0335 x ² + 0.5435 x + 1.0771	0 ≤ x ≤ 28 days	
<i>Escherichia coli</i>	y = 0.001 x ³ - 0.0522 x ² + 0.8465 x + 1.3812	0 ≤ x ≤ 28 days	
FCV (as surrogate norovirus)	y = 0.0006 x ³ - 0.0252 x ² + 0.2964 x + 0.4366	0 ≤ x ≤ 28 days	
<i>Mycobacterium thermoresistibile</i>	y = 0.0001 x ⁴ - 0.007 x ³ + 0.0969 x ² - 0.2051 x + 3.0708	0 ≤ x ≤ 28 days	
Pathogens	Unpasteurised	Either pre-AD pasteurised or post-AD pasteurised	Limitation
Effect of 30 days storage without lime			
<i>Cryptosporidium parvum</i>	0.848	6.301	30 days
FCV (as surrogate norovirus)	10.051	10.051	30 days
<i>Mycobacterium thermoresistibile</i>	3.533	4.377	30 days
Effect of 30 days storage with 6% lime			
<i>Cryptosporidium parvum</i>	1.557		30 days
FCV (as surrogate norovirus)	10.051		30 days
<i>Mycobacterium thermoresistibile</i>	1.316		30 days

Table 4
Details of parameters varied for scenario analysis.

Scenario	Change parameter from baseline	Recipe ^a	M-AD temp (°C)	Retention time (days)	Past	Pre-past temp (°C)	Pre-past duration (min)	Post-past temp (°C)	Post-past duration (min)	Post-past literature (temp, time)	Storage (without lime)	Storage (with lime)
Baseline	Baseline	3	37	7	No	NA	NA	NA	NA	NA	No	No
S1	Recipe	1										
S2	Recipe	2										
S3	Recipe	4										
S4	M-AD temp (°C)		10									
S5	M-AD temp (°C)		20									
S6	M-AD temp (°C)		30									
S7	T-AD temp (°C) ^b		50									
S8	T-AD temp (°C) ^b		60									
S9	M-AD time (days)			1								
S10	M-AD time (days)			5								
S11	M-AD time (days)			10								
S12	Pasteurisation				Pre-past	60	5760					
S13	Pasteurisation				Pre-past	60	2880					
S14	Pasteurisation				Pre-past	60	1440					
S15	Pasteurisation				Pre-past	60	60					
S16	Pasteurisation				Pre-past	65	60					
S17	Pasteurisation				Pre-past	70	60					
S18	Pasteurisation				Post-past			60	5760			
S19	Pasteurisation				Post-past			60	2880			
S20	Pasteurisation				Post-past			60	1440			
S21	Pasteurisation				Post-past			60	60			
S22	Pasteurisation				Post-past			65	60			
S23	Pasteurisation				Post-past			70	60			
S24	Pasteurisation				Literature ^c					70, 60		
S25	Storage										Yes	No
S26	Storage										No	Yes

Note.

- a) Recipe 1 = Slurry:Food waste 0:1, recipe 2 = Slurry:Food waste 1:3, recipe 3 = Slurry:Grease trap waste 2:1, recipe 4 = Slurry:Food waste 3:1.
b) Thermophilic AD (T-AD) temperature specification.
c) Recommended condition in literature.

pasteurisation and storage. A total of 26 scenarios were performed, changing one parameter at a time as compared to the baseline scenario (Table 4). The first 3 scenarios (S1–S3) looked at the change of feedstock formulations; the next 5 scenarios looked at the change of M-AD temperatures (S4–S8), the next 3 scenarios (S9–S11) compared the influence of retention time, the next 6 scenarios (S12–S17) are for 6 variations of pre-AD pasteurisation followed by 6 scenarios (S18–S23) for 6 variations of post-AD pasteurisation. Only one scenario (S24) looked at literature-based post-AD pasteurisation, as shown in Table A3. Finally, the last two scenarios (S25 and S26) looked at the effect of one-month storage with or without lime.

3. Results and discussion

3.1. ADRISK model interface for input parameters and comments on the outputs

The model was developed within Microsoft Excel (2016) with a series of drop boxes to allow user inputs and macros to perform the underlying calculations. A screenshot of the ADRISK spreadsheet-based tool is presented in Fig. 4, showing *Cryptosporidium parvum* (typical). With the conditions displayed in Fig. 4, for the given scenario, the final log reduction was calculated as 4.274 log₁₀ oocysts g⁻¹. The initial pathogen count was 2.690 log₁₀ oocysts g⁻¹, and the final mean pathogen count was evaluated as -1.584 log₁₀ oocysts g⁻¹. Simulated cursory mean log₁₀ reductions during the AD process under different process scenarios for all microorganisms are displayed in Table 5.

The EU Commission recommends pasteurisation at 70 °C for 60 min for treating waste before (or after) the AD process (Regulation (EC) no 1774/2002 (EPC, 2002); Commission Regulation (EU) No 142/2011 (The European Commission, 2011)). DAFM (2014) 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 particle size entering the unit has been restricted to 400 mm. The Irish regulation (DAFM, 2014) specifies that the thermal (e.g. pasteurisation) and chemical (e.g. use of lime) process must reduce either indicator bacteria *Enterococcus faecalis* or thermo-resistant bacteria *Salmonella* Senftenberg by at least 5 log₁₀ and thermo-resistant parvovirus must be decreased by 3 log₁₀, and there must be a 99.9% reduction (which is also 3 log₁₀) in parasitic eggs, namely *Ascaris* spp. A comparison of the simulated mean inactivation result of all organisms with the ADRISK tool with the recommended inactivation values of indicators such as *Enterococcus faecalis*, *Salmonella* Senftenberg, parvovirus, and *Ascaris* eggs mentioned above are also presented in Table 5.

Furthermore, according to Regulation (EC) No.1069/2009 (The European Parliament and Council, 2009) and Regulation (EU) No. 142/2011 (The European Commission, 2011), for AD digestate to be deemed safe for landspreading faecal indicator bacteria (FIB): total coliforms (TC), *E. coli* and enterococcal levels must be less than 1000 CFU g⁻¹. The final levels of FIBs are presented in Fig. 5. Overall observations suggest that none of the indicator levels has crossed the regulatory limit for the baseline scenario. Recipe 3 is therefore useful for pathogen inactivation as levels of coliform bacteria, *E. coli*, and enterococci have exceeded the regulatory limit in S1 & S3, S1 & S3, and S1 & S2 scenarios, respectively. Mesophilic AD at low temperature (30 °C), as well as psychrophilic AD (10–20 °C (Vanegas and Bartlett, 2013)), could not meet the criteria as the final count of FIBs in scenarios S4, S5, and S6 exceeded the regulatory limit. Scenarios S9 and S10 show that a minimum of 7 days or longer HRT is required for the inactivation of microorganisms. S14, S15, S16, S20, S21, and S22 suggest that a minimum of 2 days under the Irish pasteurisation condition is required to meet the standard. A comparison between S25 and S26 suggests that the effect of 1-month storage of the digestate on FIB inactivation is significant.

ADRISK is ideal for scenario analysis. Table 5 suggests that *Clostridium* spp. spores are very heat resistant, and maximum inactivation

Input data sheet; give inputs in highlighted cells only

1 Select pathogen name from the drop down

Pathogen selected

2 Initial concentration

Pathogen	Default mean concentration	User-defined (blank if not)	Considered in the model	Unit
Cryptosporidium parvum	2.690		2.690	Log Oocysts g-1

3 Pasteurisation type

Pre AD pasteurisation Will you consider literature-based approximate pasteurisation? (mainly post-pasteurisation)
 Go ahead and move to parameter 4
 Note: No experiment done on the effect of pasteurisation on these 5 pathogens (Anaerobe count (*Clostridium* spp.), *Enterococcus faecalis*, *Listeria monocytogenes*, *Salmonella newport*, VTEC *E. coli* O157)

4 Select pasteurisation temperature and time (applicable only if parameter 3 is selected as 'No')

Select one as recommended		User-defined (blank if not)		Considered in the model	
Temperature (°C)	Time (min)	Temperature (°C)	Time (min)	Temperature (°C)	Time (min)
60	5760			60	5760

Note
 a) 4 Days (Irish pasteurisation condition) = 5760 min
 b) Result will be interpolated with approximation for user-defined temperature and time.

5 Select AD temperature

Select one as recommended		User-defined (blank if not)		Considered in the model	
Temperature (°C)	Time (min)	Temperature (°C)	Time (min)	Temperature (°C)	Time (min)
37	10080	37	10080	37	10080

Note
 a) 1 Day = 1440 min
 b) Result will be interpolated with approximation for user-defined temperature and time.

6 Select feedstock

Feedstock composition

 Go ahead and move to parameter 7
 Note: The effect of recipe on pathogen inactivation is available for these 5 pathogens (Anaerobe count (*Clostridium* spp.), *Enterococcus faecalis*, *Listeria monocytogenes*, *Salmonella* spp., VTEC *E. coli* O157) only

7 Hydraulic retention time HRT (min 5 and max 28 days)

Pathogen	HRT (days)
Cryptosporidium parvum	7

Please select HRT from 1 to 28 days.
 Go ahead and move to parameter 8
 Note: HRT (always positive value) is strictly restricted to 10 days for these 5 pathogens (Anaerobe count (*Clostridium* spp.), *Enterococcus faecalis*, *Listeria monocytogenes*, *Salmonella* spp., VTEC *E. coli* O157)

8 Post AD Storage (30 days)

Go ahead and move to parameter 9
 Note: Experiment of storage is done for these 3 pathogens (*Cryptosporidium parvum*, norovirus, *Mycobacterium thermoresistibile*) only. Select 'No' for other selected pathogens.

9 Post AD Storage condition; 6% lime used?

Data input complete, check result section
 Note: Experiment of storage with 6% lime is done for these 3 pathogens (*Cryptosporidium parvum*, norovirus, *Mycobacterium thermoresistibile*) only. Select 'No' for other selected pathogens and if previous section (8) is selected as 'No'.

Result

Final log reduction	=	4.274			
Final pathogen count (mean)	=	Min -2.224	Mean -1.584	Max -1.334	Log Oocysts g-1

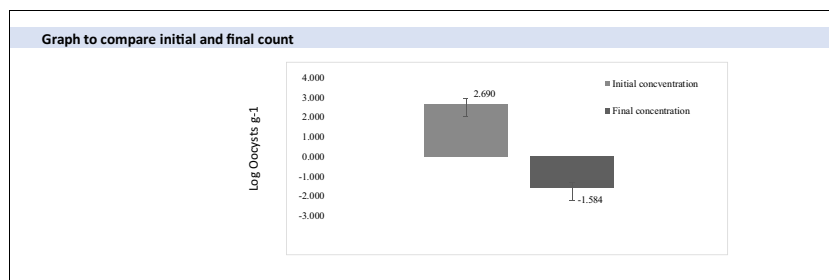


Fig. 4. A screengrab of ADRISK data input and result sheet; the operator is allowed to input the cells highlighted in yellow.

(3.95 log₁₀) was achieved for scenario S8, a thermophilic condition at 60 °C. Also, all selected microorganisms in this research satisfy the recommended log-reduction values (Table 5) for the literature-based post-AD pasteurisation scenario (S24) other than *Clostridium* spp. At 37 °C or below (M-AD), only coliform bacteria, *E. coli*, and *Mycobacterium thermoresistibile* showed significant reductions for S2 and S11, S2 & S11, and S3 & S11 scenarios, respectively. However, the inactivation of *M. thermoresistibile* was 4.65 log₁₀ (<5 log₁₀). Recipe 2 effectively reduced coliform and *E. coli* count, whereas Recipe 4 was useful for *M. thermoresistibile*. A gradual increase of inactivation of *M. thermoresistibile* was observed for Recipes 1 (slurry:food waste 0:1), 2 (slurry:food waste 1:3), 3 (slurry:greasetrap waste 2:1), 4 (slurry:food waste 3:1) producing log reductions of 2.52, 3.29, 4.40, 6.82 log₁₀, respectively. As the C:N ratio increases, an increase in the amount of microorganism inactivated was observed for *Clostridium* spp., *C. parvum*, *Enterococcus* spp., and norovirus.

The ratio of C:N in food waste is higher than the slurry; C:N ratio in FOG is 90:1 (Rashid and Voroney, 2004) whereas, the C:N ratio in cattle manure is 10:1 to 20:1 (Bernal et al., 2009; Huang et al., 2017) which means there is more carbon in food waste (specifically fat oil and grease, FOG) compared to animal manure. As the C:N ratio increased, methane potential initially increased and then declined (Wang et al., 2012). C:N ratios of 25:1 and 30:1 had better digestion performance (Abbasi et al., 2012; Puyuelo et al., 2011; Song et al., 2014; Zhou et al., 2015) with stable pH and low concentrations of total ammonium nitrogen and free NH₃, which is toxic to pathogen survival. That may explain why greater levels of coliform bacteria and *E. coli* inactivation were observed for recipe 3. Also, the maximum CH₄ potential was achieved with a C:N ratio of 27:1 after optimisation using response surface methodology (Wang et al., 2012).

Without pasteurisation, the maximum inactivation was observed in S8 (T-AD at 60 °C), followed by S7 (T-AD at 50 °C), i.e. thermophilic AD

conditions. Therefore, thermophilic AD treatment is a better way to inactivate pathogens than M-AD, which agrees with previous studies (Mahmud et al., 2016). The inactivation of norovirus was not significantly higher as compared to other microorganisms. When pasteurisation is considered, pre-M-AD pasteurisation was more effective compared to post-M-AD pasteurisation for *Cryptosporidium parvum*, norovirus, *Mycobacterium thermoresistibile*. The experimental inactivation value of pasteurisation was lower than the literature-based post-AD pasteurisation values. Therefore, no microorganisms other than *E. coli* and VTEC *E. coli* O157 met the criteria for log reductions during AD, although post-pasteurisation had been considered (scenarios S18–S23). If the literature-based pasteurisation is considered, all microorganisms satisfied this minimum log reduction criteria except *Clostridium* spp. spores, as indicated earlier. Also, Nolan et al. (2018) revealed that post-M-AD pasteurisation effectively reduced faecal indicator bacterial counts in the digestate compared to pre-M-AD pasteurisation.

3.2. Trade-off between economic aspect and ensuring microbial safety

According to Coultry et al. (2013) cited in Nag et al. (2019), pasteurisation at a higher temperature (70 °C) for a 60 min is more energy efficient compared to 60 °C for 48 continuous hours (two times) (DAFM, 2009) as the cost associated with heating the digestate twice to 60 °C for 48 h is 80 fold higher compared to heating to 70 °C for 1 h (Coultry et al., 2013). Similarly, post-digestion pasteurisation using the Irish national standard is almost 65 times more expensive to operate to meet the EU standard (Coultry et al., 2013). However, the alternative pasteurisation temperature of 60 °C for a longer period (96 h) set by (DAFM, 2014) provides better inactivation ability than the EU pasteurisation limit of 70 °C for an hour comparing scenarios S12 vs S17 and S18 vs S23 (Table 5).

Table 5

Simulated cursory mean log₁₀ reduction during the anaerobic digestion process under different process scenarios for all microorganisms; green highlighted cells represent appropriate process conditions ensuring recommended pathogen inactivation (log reduction).

Scenario	<i>Clostridium</i> spp.	Coliform bacteria	<i>Cryptosporidium parvum</i>	<i>Enterococcus</i> spp.	<i>Enterococcus faecalis</i>	VTEC <i>E. coli</i> O157	<i>Escherichia coli</i>	norovirus	<i>Listeria monocytogenes</i>	<i>Mycobacterium thermoresistibile</i>	<i>Salmonella</i> Newport
BS	1.98	4.46	1.12	3.13	2.99	4.30	4.56	1.22	2.80	4.40	3.15
S1	1.20	1.59	0.73	1.82	1.82	4.57	1.62	0.78	1.19	2.52	4.52
S2	1.52	5.90	0.89	2.36	3.92	2.77	6.04	0.96	0.59	3.29	2.41
S3	3.00	-0.15	1.64	4.81	-0.01	1.87	-0.16	1.79	1.81	6.82	2.20
S4	-0.33	1.51	-4.28	-0.60	-0.74	1.35	1.61	-0.65	-1.45	-1.79	-0.71
S5	0.53	2.61	-2.28	0.78	0.64	2.45	2.71	0.04	0.12	0.50	0.72
S6	1.38	3.70	-0.28	2.16	2.02	3.54	3.80	0.74	1.70	2.79	2.15
S7	3.09	5.88	3.72	4.92	4.78	5.73	5.99	2.12	4.85	7.38	5.01
S8	3.95	6.98	5.72	6.31	6.17	6.82	7.08	2.82	6.42	9.67	6.43
S9	0.22	1.11	-0.35	0.95	0.29	-0.28	1.23	-0.28	-0.22	1.47	-0.66
S10	2.04	3.75	0.82	2.71	2.45	3.68	3.85	0.94	0.53	3.89	3.08
S11	1.76	5.06	1.41	3.46	4.76	3.70	5.19	1.50	4.28	4.65	4.25
S12	2.27	4.62	4.27	3.42	3.28	5.33	5.59	4.38	3.09	5.52	4.17
S13	1.97	4.32	3.97	3.12	2.98	5.03	5.29	4.08	2.79	5.22	3.87
S14	1.67	4.02	3.67	2.82	2.68	4.73	4.99	3.78	2.48	4.92	3.57
S15	0.29	2.64	2.29	1.44	1.30	3.35	3.61	2.40	1.10	3.54	2.19
S16	0.90	3.33	2.89	2.21	2.07	3.97	4.23	2.35	1.94	4.45	2.92
S17	2.08	4.47	2.28	3.23	3.09	4.83	5.09	1.13	2.90	4.62	3.67
S18	2.27	4.62	1.17	3.42	3.28	5.33	5.59	1.83	3.09	3.82	4.17
S19	1.97	4.32	0.87	3.12	2.98	5.03	5.29	1.53	2.79	3.51	3.87
S20	1.67	4.02	0.57	2.82	2.68	4.73	4.99	1.23	2.48	3.21	3.57
S21	0.29	2.64	-0.81	1.44	1.30	3.35	3.61	-0.15	1.10	1.83	2.19
S22	0.90	3.33	0.20	2.21	2.07	3.97	4.23	0.43	1.94	3.05	2.92
S23	2.08	4.47	1.23	3.23	3.09	4.83	5.09	1.73	2.90	4.47	3.67
S24	2.18	10.66	7.12	8.53	8.39	10.30	10.56	7.22	8.80	10.40	8.15
S25	1.60	4.08	1.33	2.75	2.61	3.93	4.19	10.54	2.42	4.02	2.77
S26	-0.61	1.87	2.04	0.53	0.39	1.71	1.97	10.54	0.20	1.80	0.55
Safe limit ^a	5 ^b	5 ^b	3 ^c	5 ^d	5 (itself)	5 ^b	5 ^b	3 ^c	5 ^b	5 ^b	5 ^f

Note: Scenario S12 is M-AD with Irish pre-AD-pasteurisation condition, and S17 is M-AD with the European pre-AD-pasteurisation condition. Scenario S18 is M-AD with Irish post-AD-pasteurisation condition, and S23 is M-AD with the European post-AD-pasteurisation condition. S24 stands for M-AD with post-AD-literature-based pasteurisation scenario.

a safe limit for indicators, **b** assumed limit, **c** assumed limit based on *Ascaris* eggs, **d** assumed limit based on *Enterococcus faecalis*, **e** assumed limit based on parvovirus, **f** assumed limit based on *Salmonella* Senftenberg.

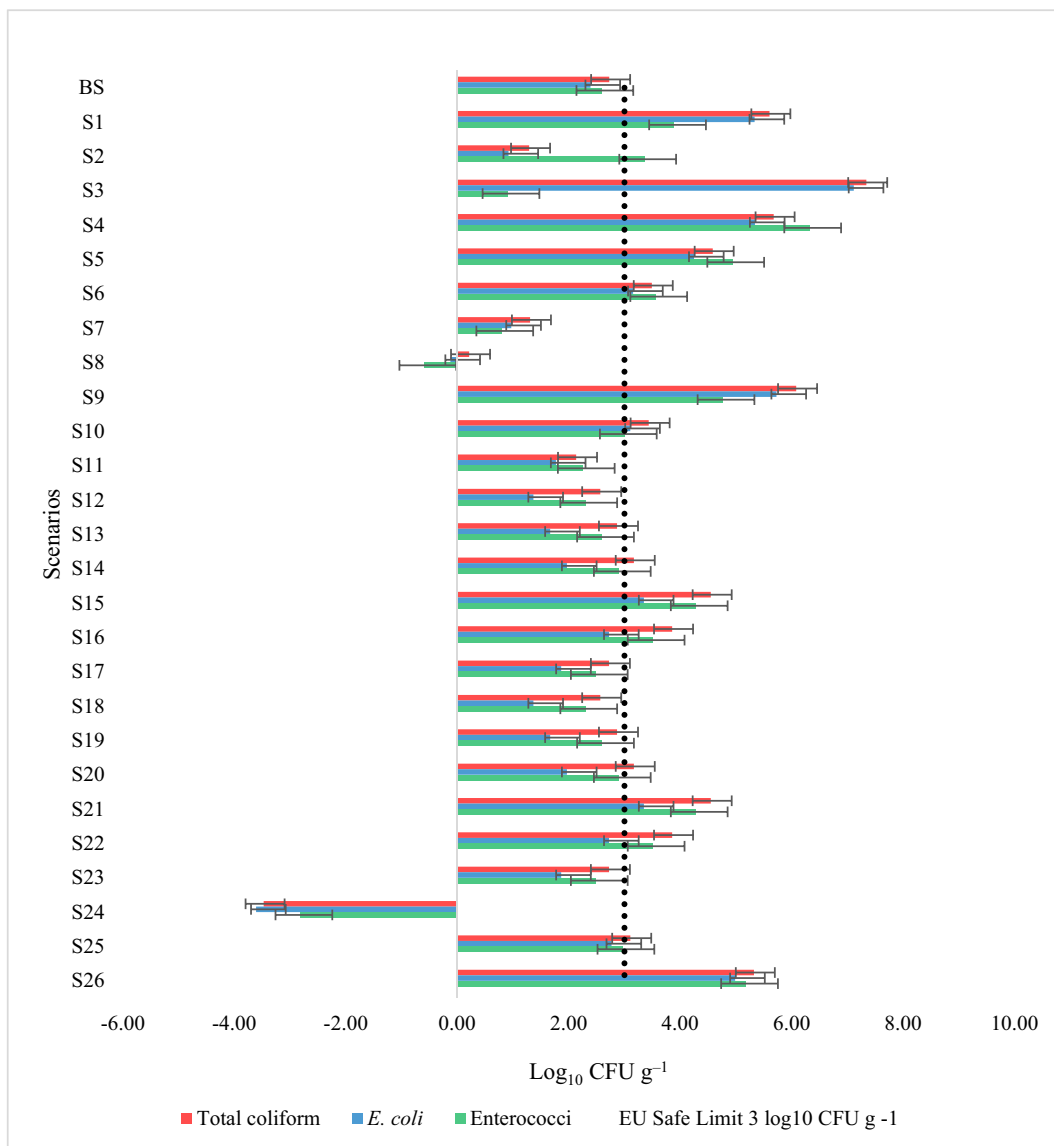


Fig. 5. The final concentration of the faecal indicator bacteria (FIB): total coliforms, *E. coli* and enterococci based on the default initial count of FIB in the raw farmyard manure and slurry and a suite of AD process conditions considered in the ADRISK tool. The dotted black line represents the safe level of FIBs as 1000 CFU g⁻¹ in the digestate for safe land spreading of digestate on the field as per the Regulation (EC) No.1069/2009 (The European Parliament and Council, 2009) and Regulation (EU) No. 142/2011 (The European Commission, 2011).

3.3. Assumptions, limitations, and future recommendations

- i. Data gaps where pathogen-specific data is unavailable are highlighted in Table A2, highlighting the need for further experiments to satisfy these gaps, mainly pertaining to the effect of pasteurisation and storage on pathogen inactivation.
- ii. The experiment where the effect of the AD feedstock formulation on pathogen inactivation was evaluated is currently valid for 10 days; it can be extended to 1 month. An HRT, which is a minimum of 10 times the doubling time of the bacteria, is recommended; as HRT increases, the bacterial count drops due to insufficient food present for their growth (Manyi-Loh et al., 2013; Zinder et al., 1984). Therefore, based on Section 3.3, this research proposes that future work to compare the economic impact of long HRT (month/s) without pasteurisation and short HRT with post-AD pasteurisation be carried out to evaluate which can satisfy the recommended minimum inactivation values during the AD process.
- iii. The findings of the current study are based on batch reactor experiments, and therefore in this case, HRT and SRT are equal. However,

this may differ for full-scale AD continuous reactors (sludge retention digesters) due to differences in inoculation methods, rheology and hydrodynamic factors (Hofmann et al., 2020). Therefore, a similar tool such as ADRISK should discriminate between HRT and SRT for continuous reactor-based data in the future.

- iv. Further validation is needed with a range of parameters as indicated in this study to corroborate this predictive ADRISK tool.
- v. A repeat of the experiments moving from miniature-scale prototype to large scale is highly recommended.

3.4. Comparison with similar studies

The gamma generalised linear model (ADRISK) presented in this study is based on the experimental findings of Russell et al. (2020), Nolan et al. (2018), and Auer (2019). The concept of gamma generalised linear models are well established. ADRISK is the first bespoke model; therefore, any comparison was hardly found for the modelling aspect. A summary of the inactivation of microorganisms during AD is presented here for comparison with the literature. Sahlström (2003)

revealed that AD in England runs either as batch or continuous processes. However, due to economic and practical reasons, most of the digesters are run continuously. A greater decline of viable counts of *E. coli*, *S. typhimurium*, *L. monocytogenes*, *Y. enterocolitica*, and *Mycobacterium avium paratuberculosis* was observed in batch compared to continuous AD. In bench-scale AD experiments 1–2 log₁₀ reductions in faecal coliforms were achieved under mesophilic conditions at 35–36 °C M-AD with 10–30 day SRT (Carroll and Long, 2016). With an SRT of 16 days, 1.87–2.76 log₁₀ reductions in *Salmonella* sp. was demonstrated previously in a mesophilic bench-scale reactor study (Chen et al., 2012). Carroll and Long (2016) also reported that *E. coli* might be more thermotolerant than other faecal coliforms, which other researchers have also observed (Ziamba and Peccia, 2012). Chen et al. (2012) demonstrated approximately 3 log₁₀ reductions of *E. coli* in mesophilic bench-scale AD with a 16-day SRT which is in line with the current research findings (Table 5). A slower rate of inactivation of enterococci was observed compared to faecal coliforms in the thermophilic reactors (k of 1–3 per day) but had comparable rate constants in the mesophilic reactors (Carroll and Long, 2016). This is also similar to ADRISK outputs (Table 5).

One of the oldest studies in this field (Olsen and Larsen, 1987) reported reductions of pathogenic and indicator bacteria in animal slurry subjected to M-AD (35 °C) and T-AD (53 °C) measured in small-scale, as well as full-scale, reactors. For all vegetative cells of bacteria, a time-dependent inactivation profile was observed. In a small-scale digestion study that applied conditions of 35 °C and T₉₀ values were 2.4 days for *Salmonella Typhimurium*, 2.0 days for *S. dublin* and *Streptococcus faecalis*, 1.8 days for *E. coli*, 3.2 and 3.1 days for faecal coliforms and total coliforms, respectively. Reductions of 2.3 log₁₀ and 2 log₁₀ were observed for *S. typhimurium* and *E. coli*, respectively, within seven days. At 53 °C, T₉₀ values of 0.7 h for *S. typhimurium*, 0.6 h for *S. dublin*, 0.4 h for *E. coli* were reported. Spores of *Clostridium perfringens* and *B. cereus* were not inactivated at 35 °C or 53 °C. Also, Xu et al. (2015) reported that at 36 °C, there was no significant ($P > 0.05$) change in the numbers of *Clostridium difficile* spored over the 60-day digestion period; however, on average, inactivation levels of 3.5 log₁₀ for *C. difficile* could be achieved after 10 days at 55 °C. The value of T₉₀ was significantly correlated to species of bacteria and temperature during digestion. Olsen and Larsen (1987) mentioned that in general, the inactivation of pathogens was not influenced by the type of slurry (cattle or pig), the reactor process (batch or continuous), the amount of gas produced during fermentation, the slurry dry-matter contents, the concentration of N-NH₃ and pH in the slurry. Thomas et al. (2019) indicated that the temperature and retention time were the main inactivation factors of AD. No direct correlation could be found between pH, volatile fatty acids (VFA) or ammonia (NH₃) and *E. coli* inactivation. However, Nag et al. (2019) reported that the inactivation of pathogens is influenced by not only temperature and HRT but also organic loading rate (OLR), pH (dependable parameter) and feedstock composition (C:N ratio). Dennehy et al. (2018) also examined the impact of feedstock composition (pig manure and food waste) and HRT on enteric indicator bacteria counts in digestate. Dennehy et al. (2018) found that decreasing HRT (from 41 to 21 days) did not appear to have any clear effects on the concentrations of total coliforms; however, decreasing the HRT from 29 to 21 days increased *E. coli* concentrations slightly. Decreasing the HRT from 41 to 21 days (resulting in an increase in OLR from 1 to 3 kg VS m⁻³ days⁻¹) may increase volumetric methane yield while not leading to a major rise in microbial counts in digestate (Dennehy et al., 2018). Scenarios BS, S1, S2, and S3 (Table 5) show that the inactivation of the microorganisms is significantly influenced by the feedstock composition (C:N ratio). Also, each of the microorganisms showed a unique inactivation pattern based on different feedstock compositions (Table 5, BS, S1, S2, and S3); therefore, any conclusion regarding which feedstock composition is the best for inactivation of all microorganisms could not be drawn.

At 37 °C, a 4 log₁₀ inactivation was achieved within 7 days with a steady slope, and beyond 7 days the curve flattened (Thomas et al., 2019), indicating that the major inactivation occurred within the first

week of the AD process. Jiang et al. (2018) reported that the dry co-digestion of food waste and pig manure at 37 °C effectively inactivated enteric indicator bacteria such as *E. coli* and total coliforms counts, which decreased below the limit of detection (LOD, 2 log₁₀ CFU g⁻¹) within 4–7 days. The inactivation of *E. coli* and total coliforms in the digestate was 3.5 and 2.5 log₁₀, respectively, with free VFA identified as a significant inactivation factor. Enterococci were more resistant, but the counts decreased below the LOD within 12 days in the digestate inoculum systems showing a 3 log₁₀ inactivation. Residence time was the most significant inactivation factor for enterococci, with the free VFA concentration playing a secondary role at a high food waste/pig manure ratio. A FW/PM ratio of 50:50 was preferable to achieve system stability, methane production and enteric indicator bacteria inactivation (Jiang et al., 2018). In another study, changes in *Salmonella* Senftenberg and *Enterococcus* spp. populations in laboratory-scale (54 ± 0.3 °C) suggests that 6 log₁₀ and 4 log₁₀ reductions can be achieved within 24 h, respectively (Seruga et al., 2020). These authors also reported a correlation between increased inactivation and increased VFA concentration. High VFA concentrations resulted in highly available acid molecules, and a high osmotic gradient across cell membranes led to increased toxicity to microorganisms (Seruga et al., 2020). Olsen and Larsen (1987) also indicated that the T₉₀ values for *S. Typhimurium* and *E. coli* at full-scale digestion were fully comparable to the values determined by small-scale experiments. One similar experimental study (Maynaud et al., 2016) on *Campylobacter coli*, *Salmonella enterica* Derby, and *Listeria monocytogenes* suggests that M-AD at 37 °C can inactivate those pathogens in the order of 1.9–5.3, 2.8–4.2, and 0–1 log₁₀ (mean values), respectively after 7 days using various feedstock compositions. The effect of stirring is also an important parameter as Yin et al. (2018) found that the combination of thermal pre-treatment (70 °C) and mixing was more effective than thermal pre-treatment alone for improving inactivation of faecal bacteria (faecal coliform, *Salmonella* spp., and faecal *Streptococcus* spp.).

There is little research on *Cryptosporidium parvum* and *Mycobacterium* spp., human norovirus, and *E. coli* O157:H7. Kato et al. (2003) investigated the viability of *Cryptosporidium parvum* oocyst eggs inoculated in anaerobic digesters. The digesters were maintained at 37 °C, 47 °C, and 55 °C, with 10-day retention. Oocysts were inactivated rapidly with reductions exceeding 2 log₁₀ after 10 days at 37 °C (which is higher than the current research finding), 4 days at 47 °C, and 2 days at 55 °C. At 37 °C, AD inactivated >75% of the eggs after 10 days (0.6 log₁₀ reduction), showing resilience compared to the oocyst. Franke-Whittle and Insam (2013) reported total inactivation of *Mycobacterium bovis* during pasteurisation at 70 °C whereas, partial inactivation occurred during M-AD (37 °C), and no information is available on inactivation at T-AD (55 °C) or M-AD (37 °C) of pre-pasteurised feedstock. Sassi et al. (2018) found that the M-AD (32 ± 3 °C for 21 days ± 8 h) and T-AD (55 ± 3 °C for 5 days ± 8 h) can inactivate murine norovirus with reductions of 2.2 ± 0.18 log₁₀ and >4.1 log₁₀ achieved, respectively. Also, M-AD (37 °C) and T-AD (52 °C) of pig slurry induced at least a 4 log₁₀ decrease in murine norovirus 1, used as a surrogate for porcine norovirus, after 13 and 7 days, respectively (Baert et al., 2010). Aitken et al. (2007) investigated the inactivation of *E. coli* O157:H7 during T-AD (50–55 °C) of dairy cattle manure, and a sharp decline of 4.2 log₁₀ was achieved within 50 h.

Contained storage is another effective way to reduce the microbial count in digestate, mainly for non-spore-forming bacteria such as faecal coliform and *Salmonella* spp. within two weeks (Bujoczek et al., 2002). However, a two-year study on spore-forming *Clostridium perfringens* showed that spores could not be inactivated during subsequent storage at 35–37 °C. Thus additional treatment such as the use of low dose alkaline disinfection with CaO (100 kg kg⁻¹ of TS of digestate) is required to inactivate this spore-forming bacteria (Bujoczek et al., 2002). Pascual-Benito et al. (2015) assessed the persistence of *E. coli*, clostridial spores, and *Salmonella* spp. in mesophilic and thermophilic digestate after different pasteurisation treatments and storage conditions. Pasteurisation

at 55 °C resulted in *E. coli*-injured cells that could be resuscitated during the first 24 h. Different sludge treatments altered the inactivation kinetics of *E. coli*, while *Clostridium* spp. showed lower die-off, and regrowth was observed in stored sludge for up to 60 days. No regrowth was observed for *Salmonella* spp.

The experimental results obtained during the current research regarding microbial inactivation during AD and storage are in line with the literature. However, the inactivation with the post-AD pasteurisation observed in our study contrasts with the literature-based pasteurisation values (Table A3). The experimental studies (Auer, 2019; Nolan et al., 2018) showed the lower potential of post-AD pasteurisation on microbial inactivation compared to the literature-based post-AD pasteurisation values. One possible reason to explain these observations could be differences in initial pathogen concentrations affecting final concentrations after M-AD and post-AD pasteurisation along with the limits of detection used between studies. Further investigations are required to determine the influence of pasteurisation on the digestate based on the full-scale AD process applied under Irish conditions.

4. Conclusion

This research collates a large data set (scientific literature and miniature-scale AD batch reactor data) on pathogen survival, growth, and inactivation during anaerobic digestion (AD) of slurry based on various feedstock recipes, retention time, temperature, pasteurisation (pre/post-AD), and/or storage (with/without lime) on selected microorganisms. The tool developed (ADRISK) has a simple interface with 12 input cells on a single tab of a Microsoft Excel spreadsheet to calculate a cursory concentration of organisms in the digestate. ADRISK can evaluate a multitude of scenarios by varying the process parameters with the scenario analysis facility. To the authors' knowledge, ADRISK is the first Excel-based tool for evaluating pathogenic loading in the digestate. However, the approximation of results suggests that further experiments are required to minimise the assumptions made for the gamma generalised linear model. The AD process is very complex, with a multitude of parameters and process conditions. The simulated mean of the faecal indicator bacteria across all scenarios shows that the digestate can meet the EU standard without pasteurisation if the AD runs at 37 °C or higher temperature with a higher C:N ratio (recipe 3) and hydraulic retention time equals or is longer than 7 days. The storage of digestate was found to be another way to reduce the microorganism count in the digestate. The Irish pasteurisation condition (60 °C for 4 days) was determined to be more effective than the EU pasteurisation (70 °C for 1 h) specification. However, the Irish pasteurisation process is more energy-intensive compared to the EU pasteurisation standard. Pre-AD pasteurisation was more effective compared to post-AD pasteurisation for *Cryptosporidium parvum*, norovirus, *Mycobacterium thermoresistibile*. Post-AD literature-based pasteurisation can ensure the safety of the digestate to the greatest extent. While many data gaps and uncertainties remain, the ADRISK model provides an initial framework and cursory attempt to assess the likely survival of pathogens through the AD process while paving the way for further development and characterisation of this predictive model.

CRedit authorship contribution statement

Rajat Nag: Conceptualisation, Methodology, Software, Data curation, Visualisation, Investigation, Writing - original draft. **Agathe Auer, Stephen Nolan, Lauren Russell:** Data curation, Laboratory experiments. **Paul Whyte, Bryan K. Markey, Vincent O'Flaherty, Declan Bolton, Owen Fenton, Karl G. Richards:** Project management, Funding, Writing - reviewing and editing. **Enda Cummins:** Project management, Funding, Conceptualisation, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by the Department of Agriculture, Food and the Marine (DAFM), Ireland [grant number 14/SF/847].

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.149574>.

References

- Abbasi, T., Tauseef, S.M., Abbasi, S.A., 2012. A brief history of anaerobic digestion and "biogas". *Biogas Energy*, pp. 11–24 <https://doi.org/10.1007/978-1-4614-1040-9>.
- Aitken, M.D., Sobsey, M.D., Van Abel, N.A., Blauth, K.E., Singleton, D.R., Crunk, P.L., Nichols, C., Walters, G.W., Schneider, M., 2007. Inactivation of *Escherichia coli* O157:H7 during thermophilic anaerobic digestion of manure from dairy cattle. *Water Res.* 41, 1659–1666. <https://doi.org/10.1016/j.watres.2007.01.034>.
- Auer, A.R., 2019. Investigation of health risks associated with the spread of anaerobic digestion residue. *UCD Sch. Vet. Med. Univ. Coll. Dublin*, pp. 1–182.
- Baert, L., Uyttendaele, M., Van Coillie, E., Debevere, J., 2008. The reduction of murine norovirus 1, *B. fragilis* HSP40 infecting phage B40–8 and *E. coli* after a mild thermal pasteurization process of raspberry puree. *Food Microbiol.* 25, 871–874. <https://doi.org/10.1016/j.fm.2008.06.002>.
- Baert, L., De Gussem, B., Boon, N., Verstraete, W., Debevere, J., Uyttendaele, M., 2010. Inactivation of murine norovirus 1 and *Bacteroides fragilis* phage B40–8 by mesophilic and thermophilic anaerobic digestion of pig slurry. *Appl. Environ. Microbiol.* 76, 2013–2017. <https://doi.org/10.1128/AEM.01657-09>.
- Bernal, M.P., Albuquerque, J.A., Moral, R., 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* 100, 5444–5453. <https://doi.org/10.1016/j.biortech.2008.11.027>.
- Bujczek, G., Oleszkiewicz, J.A., Danesh, S., Sparling, R.R., 2002. Co-processing of organic fraction of municipal solid waste and primary sludge – stabilization and disinfection. *Environ. Technol.* 23, 227–241. <https://doi.org/10.1080/0959332508618423>.
- Carroll, Z.S., Long, S.C., 2016. Bench-scale analysis of surrogates for anaerobic digestion processes. *Water Environ. Res.* 88, 458–467. <https://doi.org/10.2175/106143016x14504669768499>.
- Chen, Y., Fu, B., Wang, Y., Jiang, Q., Liu, H., 2012. Reactor performance and bacterial pathogen removal in response to sludge retention time in a mesophilic anaerobic digester treating sewage sludge. *Bioresour. Technol.* 106, 20–26. <https://doi.org/10.1016/j.biortech.2011.11.093>.
- Clarke, R., Peyton, D., Healy, M.G., Fenton, O., Cummins, E., 2017. A quantitative microbial risk assessment model for total coliforms and *E. coli* in surface runoff following application of biosolids to grassland. *Environ. Pollut.* 224, 739–750. <https://doi.org/10.1016/j.envpol.2016.12.025>.
- Coultry, J., Walsh, E., McDonnell, K.P., 2013. Energy and economic implications of anaerobic digestion pasteurisation regulations in Ireland. *Energy* 60, 125–128. <https://doi.org/10.1016/j.energy.2013.07.059>.
- DAFM, 2009. Conditions for Approval and Operation of Biogas Plants Treating Animal By-products in Ireland.
- DAFM, 2014. Conditions for approval and operation of biogas plants using animal by-products and derived products in Ireland [WWW document]. URL <https://www.agriculture.gov.ie/media/migration/foodindustrydevelopmenttrademarkets/animalby-products/applicationformsconditionsforabpprocessingoperations/conditionsforms/CN14160916.pdf> (accessed 4.21.20).
- Dennehy, C., Lawlor, P.G., McCabe, M.S., Cormican, P., Sheahan, J., Jiang, Y., Zhan, X., Gardiner, G.E., 2018. Anaerobic co-digestion of pig manure and food waste; effects on digestate biosafety, dewaterability, and microbial community dynamics. *Waste Manag.* 71, 532–541. <https://doi.org/10.1016/j.wasman.2017.10.047>.
- DiCaprio, E., Ma, Y., Purgianto, A., Hughes, J., Li, J., 2012. Internalization and dissemination of human norovirus and animal caliciviruses in hydroponically grown romaine lettuce. *Appl. Environ. Microbiol.* 78, 6143–6152. <https://doi.org/10.1128/AEM.01081-12>.
- Edwards, T.E., Liao, R., Phan, I., Myler, P.J., Grundner, C., 2012. *Mycobacterium thermoresistibile* as a source of thermostable orthologs of *mycobacterium tuberculosis* proteins. *Protein Sci.* 21, 1093–1096. <https://doi.org/10.1002/pro.2084>.
- EPC, 2002. REGULATION (EC) No 1774/2002: laying down health rules concerning animal by-products not intended for human consumption. *Off. J. Eur. Communities OJ L* 273, 1–163 <https://doi.org/10.1016/2004R0726-v.7.05.06.2013>.
- Franke-Whittle, I.H., Insam, H., 2013. Treatment alternatives of slaughterhouse wastes, and their effect on the inactivation of different pathogens: a review. *Crit. Rev. Microbiol.* <https://doi.org/10.3109/1040841X.2012.694410>.
- Haas, C., Rose, J., Gerba, C., 2014. *Quantitative Microbial Risk Assessment*. Second. ed. John Wiley & Sons Inc, New Jersey.

- Hofmann, J., Müller, L., Weinrich, S., Debeer, L., Schumacher, B., Velghe, F., Liebetrau, J., 2020. Assessing the effects of substrate disintegration on methane yield. *Chem. Eng. Technol.* 43, 47–58. <https://doi.org/10.1002/ceat.201900393>.
- Huang, J., Yu, Z., Gao, H., Yan, X., Chang, J., Wang, C., Hu, J., Zhang, L., 2017. Chemical structures and characteristics of animal manures and composts during composting and assessment of maturity indices. *PLoS One* 16. <https://doi.org/10.1371/journal.pone.0178110>.
- Hutchison, M.L., Walters, L.D., Avery, S.M., Synge, B.A., Moore, A., 2004. Levels of zoonotic agents in british livestock manures. *Let. Appl. Microbiol.* 39, 207–214. <https://doi.org/10.1111/j.1472-765X.2004.01564.x>.
- Jiang, Y., Dennehy, C., Lawlor, P.G., Hu, Z., Zhan, X., Gardiner, G.E., 2018. Inactivation of enteric indicator bacteria and system stability during dry co-digestion of food waste and pig manure. *Sci. Total Environ.* 612, 293–302. <https://doi.org/10.1016/j.scitotenv.2017.08.214>.
- Kato, S., Fogarty, E., Bowman, D.D., 2003. Effect of aerobic and anaerobic digestion on the viability of *Cryptosporidium parvum* oocysts and *ascaris suum* eggs. *Int. J. Environ. Health Res.* 13, 169–179. <https://doi.org/10.1080/0960312031000098071>.
- Lau, M., Monis, P., Ryan, G., Salvesson, A., Blackbeard, J., Gray, S., Sanciolo, P., 2019. Selection of surrogate pathogens and process indicator organisms for pasteurisation of municipal wastewater – a survey of literature data on heat inactivation of pathogens. *Process Saf. Environ. Prot.* <https://doi.org/10.1016/j.psep.2019.11.011>.
- Liu, X., Yan, Z., Yue, Z.B., 2011. *Biogas. Compr. Biotechnol.* Second Ed 3, pp. 99–114. <https://doi.org/10.1016/B978-0-08-088504-9.00165-3>.
- Longhurst, P., Tyrrel, S., Pollard, S., Sakrabani, R., Chambers, B., Taylor, M., Litterick, A., Gale, P., Goddard, A., Snary, E., 2013. *Risk-based Guidance for the Use of Source-segregated Anaerobic Digestates in GB Agriculture Oxon, UK*.
- Mahmud, Z.H., Das, P.K., Khanum, H., Hossainey, M.R.H., Islam, E., Mahmud, H.A.I., Islam, M.D., Shafiqul, Imran, K.M., Dey, D., Islam, M.D., Sirajul, 2016. Time-temperature model for bacterial and parasitic annihilation from cow dung and human faecal sludge: a forthcoming bio-fertilizer. *J. Bacteriol. Parasitol.* 07, 4–9. <https://doi.org/10.4172/2155-9597.1000284>.
- Manji-Loh, C.E., Mamphweli, S.N., Meyer, E.L., Okoh, A.I., Makaka, G., Simon, M., 2013. Microbial anaerobic digestion (bio-digesters) as an approach to the decontamination of animal wastes in pollution control and the generation of renewable energy. *Int. J. Environ. Res. Public Health* 10, 4390–4417. <https://doi.org/10.3390/ijerph10094390>.
- Mattison, K., Shukla, A., Cook, A., Pollari, F., Friendship, R., Kelton, D., Bidawid, S., Farber, J.M., 2007. Human noroviruses in swine and cattle. *Emerg. Infect. Dis.* 13, 1184–1188.
- Maynaud, G., Pouchet, A.M., Ziebal, C., Cuny, A., Druilhe, C., Steyer, J.P., Wéry, N., 2016. Persistence and potential viable but non-culturable state of pathogenic bacteria during storage of digestates from agricultural biogas plants. *Front. Microbiol.* 7. <https://doi.org/10.3389/fmicb.2016.01469>.
- McDonald, W.L., O'Riley, K.J., Schroen, C.J., Condon, R.J., 2005. Heat inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in milk. *Appl. Environ. Microbiol.* 71, 1785–1789. <https://doi.org/10.1128/AEM.71.4.1785-1789.2005>.
- Nag, R., Auer, A., Markey, B.K., Whyte, P., Nolan, S., O'Flaherty, V., Russell, L., Bolton, D., Fenton, O., Richards, K., Cummins, E., 2019. Anaerobic digestion of agricultural manure and biomass – critical indicators of risk and knowledge gaps. *Sci. Total Environ.* 690, 460–479. <https://doi.org/10.1016/j.scitotenv.2019.06.512>.
- Nag, R., Markey, B.K., Whyte, P., O'Flaherty, V., Bolton, D., Fenton, O., Richards, K.G., Cummins, E., 2020a. A quantitative risk assessment of *E. coli* O157:H7 on ready to eat foods following the application of biomaterials on land. In: Impe, J.F.M. Van, Polanska, M.E. (Eds.), *11th International Conference on Simulation and Modelling in the Food and Bio-Industry 2020 (FOODSIM'2020)*. EUROESIS-ETI, Ghent, Belgium, pp. 141–144.
- Nag, R., Whyte, P., Markey, B.K., O'Flaherty, V., Bolton, D., Fenton, O., Richards, K.G., Cummins, E., 2020b. Ranking hazards pertaining to human health concerns from land application of anaerobic digestate. *Sci. Total Environ.* 710, 1–17. <https://doi.org/10.1016/j.scitotenv.2019.136297>.
- Nag, R., Markey, B.K., Whyte, P., Flaherty, V.O., Bolton, D., Fenton, O., Richards, K.G., Cummins, E., 2021a. A bayesian inference approach to quantify average pathogen loads in farmyard manure and slurry using open-source irish datasets. *Sci. Total Environ.* 786, 1–11. <https://doi.org/10.1016/j.scitotenv.2021.147474>.
- Nag, R., Monahan, C., Whyte, P., Markey, B.K., Flaherty, V.O., Bolton, D., Fenton, O., Richards, K.G., Cummins, E., 2021b. Risk assessment of *Escherichia coli* in bioaerosols generated following land application of farmyard slurry. *Sci. Total Environ.* 791, 1–12. <https://doi.org/10.1016/j.scitotenv.2021.148189>.
- Nolan, S., Waters, N.R., Brennan, F., Auer, A., Fenton, O., Richards, K., Bolton, D.J., Pritchard, L., O'Flaherty, V., Abram, F., 2018. Toward assessing farm-based anaerobic digestate public health risks: comparative investigation with slurry, effect of pasteurization treatments, and use of miniature bioreactors as proxies for pathogen spiking trials. *Front. Sustain. Food Syst.* 2, 1–11. <https://doi.org/10.3389/fsufs.2018.00041>.
- Olsen, J.E., Larsen, H.E., 1987. Bacterial decimation times in anaerobic digestions of animal slurries. *Biol. Wastes* 21, 153–168. [https://doi.org/10.1016/0269-7483\(87\)90121-2](https://doi.org/10.1016/0269-7483(87)90121-2).
- Pascual-Benito, M., García-Aljaro, C., Casanovas-Massana, S., Blanch, A.R., Lucena, F., 2015. Effect of hygienization treatment on the recovery and/or regrowth of microbial indicators in sewage sludge. *J. Appl. Microbiol.* 118, 412–418. <https://doi.org/10.1111/jam.12708>.
- Puyuelo, B., Ponsá, S., Gea, T., Sánchez, A., 2011. Determining C/N ratios for typical organic wastes using biodegradable fractions. *Chemosphere* 85, 653–659. <https://doi.org/10.1016/j.chemosphere.2011.07.014>.
- Rashid, M.T., Voroney, R.P., 2004. Land application of oily food waste and corn production on amended soils. *Agron. J.* 96, 997–1004. <https://doi.org/10.2134/agronj2004.0997>.
- Rosenfeld, I., Austbø, D., 2009. Effect of type of grain and feed processing on gastrointestinal retention times in horses. *Am. Soc. Anim. Sci.* 87, 3991–3996. <https://doi.org/10.2527/jas.2008-1150>.
- Russell, L., Whyte, P., Zintl, A., Gordon, S., Markey, B., de Waal, T., Cummins, E., Nolan, S., O'Flaherty, V., Abram, F., Richards, K., Fenton, O., Bolton, D., 2020. A small study of bacterial contamination of anaerobic digestion materials and survival in different feed stocks. *Bioengineering* 7, 1–12. <https://doi.org/10.3390/bioengineering7030116>.
- Sahlström, L., 2003. A review of survival of pathogenic bacteria in organic waste used in biogas plants. *Bioresour. Technol.* 87, 161–166. [https://doi.org/10.1016/S0960-8524\(02\)00168-2](https://doi.org/10.1016/S0960-8524(02)00168-2).
- Sassi, H.P., Ikner, L.A., Abd-Elmaksoud, S., Gerba, C.P., Pepper, I.L., 2018. Comparative survival of viruses during thermophilic and mesophilic anaerobic digestion. *Sci. Total Environ.* 615, 15–19. <https://doi.org/10.1016/j.scitotenv.2017.09.205>.
- Seruga, P., Krzywonos, M., Paluszak, Z., Urbanowska, A., Pawlak-Kruczek, H., Niedzwiecki, L., Pinkowska, H., 2020. Pathogen reduction potential in anaerobic digestion of organic fraction of municipal solid waste and food waste. *Molecules* 25, 1–13. <https://doi.org/10.3390/molecules25020275>.
- Song, Z., Yang, G., Liu, X., Yan, Z., Yuan, Y., Liao, Y., 2014. Comparison of seven chemical pretreatments of corn straw for improving methane yield by anaerobic digestion. *PLoS One* 9, 1–8. <https://doi.org/10.1371/journal.pone.0093801>.
- Talukdar, P.K., Udombijitkul, P., Hossain, A., Sarkar, M., 2017. Inactivation strategies for *Clostridium perfringens* spores and vegetative cells. *Appl. Environ. Microbiol.* 83, 1–13.
- The European Commission, 2011. *Commission regulation (EU) no 142/2011. Off. J. Eur. Union L 54*.
- The European Parliament and Council, 2009. *Regulation (EC) no 1069/2009. Off. J. Eur. Union 300, 1–33*.
- Thomas, C., Idler, C., Ammon, C., Herrmann, C., Amon, T., 2019. Inactivation of ESBL-/AmpC-producing *Escherichia coli* during mesophilic and thermophilic anaerobic digestion of chicken manure. *Waste Manag.* 84, 74–82. <https://doi.org/10.1016/j.wasman.2018.11.028>.
- Thomas, M.K., Murray, R., Flockhart, L., Pintar, K., Pollari, F., Fazil, A., Nesbitt, A., Marshall, B., 2013. Estimates of the burden of foodborne illness in Canada for 30 specified pathogens and unspecified agents, circa 2006. *Foodborne Pathog. Dis.* 10, 639–648. <https://doi.org/10.1089/fpd.2012.1389>.
- Torgerson, P.R., Devleeschauwer, B., Praet, N., Speybroeck, N., Willingham, A.L., Kasuga, F., Rokni, M.B., Zhou, X.-N., Fevre, E.M., Sripa, B., Gargouri, N., Furst, T., Budke, C.M., Carabin, H., Kirk, M.D., Angulo, F.J., Havelaar, A., de Silva, N., 2015. World Health Organization estimates of the global and regional disease burden of 11 foodborne parasitic diseases, 2010: a data synthesis. *PLoS Med.* 12, 1–22. <https://doi.org/10.1371/journal.pmed.1001920>.
- US EPA and USDA, 2012. *Microbial Risk Assessment Guideline: Pathogenic Microorganisms With Focus on Food and Water*.
- Vanegas, C., Bartlett, J., 2013. Anaerobic digestion of *laminaria digitata*: the effect of temperature on biogas production and composition. *Waste Biomass Valoriz.* 4, 509–515. <https://doi.org/10.1007/s12649-012-9181-z>.
- Wang, X., Yang, G., Feng, Y., Ren, G., Han, X., 2012. Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. *Bioresour. Technol.* 120, 78–83. <https://doi.org/10.1016/j.biortech.2012.06.058>.
- WHO, 2008. *Foodborne Disease Outbreaks; Guidelines for Investigation and Control*.
- Xu, C., Salsali, H., Weese, S., Warriner, K., 2015. Inactivation of *Clostridium difficile* in sewage sludge by anaerobic thermophilic digestion. *Can. J. Microbiol.* 62, 16–23. <https://doi.org/10.1139/cjm-2015-0511>.
- Yin, F., Dong, H., Shang, B., Zhang, W., 2018. Effect of time and mixing in thermal pretreatment on faecal indicator bacteria inactivation. *Int. J. Environ. Res. Public Health* <https://doi.org/10.3390/ijerph15061225>.
- Zhou, S., Zhang, J., Zou, G., Riya, S., Hosomi, M., 2015. Mass and energy balances of dry thermophilic anaerobic digestion treating swine manure mixed with Rice straw. *Biotechnol. Res. Int.* 2015, 1–11. <https://doi.org/10.1552015/895015>.
- Ziemba, C., Peccia, J., 2012. Fecal coliform population dynamics associated with the thermophilic stabilization of treated sewage sludge. *J. Environ. Monit.* 14, 2755–2761. <https://doi.org/10.1039/c2em30373f>.
- Zinder, S.H., Cardwell, S.C., Anguish, T., Lee, M., Koch, M., 1984. Methanogenesis in a thermophilic (58°C) anaerobic digester: methanotroph sp. as an important acetate-methanogen. *Appl. Environ. Microbiol.* 47, 796–807.