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Effect of anaerobic digestate fuel pellet production on *Enterobacteriaceae* and *Salmonella* persistence

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Abstract

Production of digestate pellets for fuel has been identified as a promising circular economy approach to provide renewable energy and additional income to farms, while at the same time presenting the potential to divert raw digestate from nutrient-saturated land and reduce the risk to water quality. Although previous research has investigated the feasibility of pellet production, there has been little focus on the bio-safety aspects of the system. Little is currently known about the persistence of bacteria present in the digestate and the potential impacts on human health for those handling this product. The aim of the present research was to determine the effect that each step in the pellet production process has on bacteria numbers: anaerobic digestion, mechanical separation, solid drying, and pelletisation. *Enterobacteriaceae* enumeration by colony count method was used to quantify bacteria, and the presence of *Salmonella* at each stage was determined. The *Enterobacteriaceae* count reduced with each stage, and the final pelletisation step reduced bacteria numbers to below detectable levels (<10 colony forming units/g). *Salmonella* was only detected in the starting slurry and absent from digestate onwards. Storage of the pellets under winter and simulated summer conditions showed no reactivation of *Enterobacteriaceae* over time. The pelletisation process produces a digestate product with *Enterobacteriaceae* counts below the maximum threshold (PAS110 specification) for transport off the source farm, but care must still be taken when handling digestate pellets as complete sterilisation has not been confirmed.

KEYWORDS

anaerobic digestion, animal by-products, digestate, *Enterobacteriaceae*, fuel pellet, mechanical separation, phosphorus

1 | INTRODUCTION

Mechanical separation of farm slurries and anaerobic digestate has been identified as a nutrient management strategy in regions of intensive livestock agriculture (Drosg et al., 2015; Lyons et al., 2021). Over-saturation of phosphorus (P) in agricultural soils presents a risk to water quality (Carpenter, 2008) and represents wasteful utilisation of a non-renewable resource. Digestate separation differentially partitions plant macro-nutrients into high-solid and low-solid fractions. The de-watered solid fraction contains a higher proportion of P and a lower proportion of nitrogen (N) than the unseparated digestate (Möller & Müller, 2012) and has a lower associated transport cost (Herbes et al., 2020). The low-solid fraction (liquid fraction) is rich in N, and potassium (K) and is suitable as an organic fertiliser with reduced P content relative to un-separated digestate. The liquid fraction of digestate can be spread normally with reduced risk to water quality, whereas the high-P solid fraction can be transported to areas of P deficit (Herbes et al., 2020) or processed into value-added products such as fuel pellets.

Pelletisation has been identified as a method to densify mechanically separated solids for transport (Ronga et al., 2020) and as a potential fuel production pathway (Cathcart et al., 2021). Previous research has explored the suitability of the solid fraction as a fuel (Czekała et al., 2018; Nagy et al., 2018; Pedrazzi et al., 2015) but has not considered the biological health and safety risks associated with material handling. The base material of agricultural digestate is animal slurry, an animal by-product (ABP). Bacterial genera associated with pathogenicity are found in animal manures (Table 1) and, whereas the combustion of digestate pellets in solid fuel boilers will result in a sterilised ash product rich in P, contact with digestate during processing and transport could expose individuals to a potentially hazardous material (Johansson et al., 2005).

To the authors' knowledge, only one journal article has been published exploring the effect of pelletisation on pathogens in digestate. Pulvirenti et al. (2015) showed that *Clostridium* spp. was eliminated entirely in pelletised digestate from an AD plant processing silage and cattle slurry. Although the results are encouraging, only one genus of bacteria was considered, *Clostridium* spp., which are obligate anaerobes (Reece et al., 2011). Jiang et al. (2020) reported that the order of pathogen resistances is: spore forming Gram-positive bacteria > non-spore forming Gram positive bacteria > nematode > Gram-negative bacteria ≥ viruses. As the pellet press is effective in eliminating Spore-forming *Clostridium* spp., it is likely that it is also effective in eliminating the less robust gram-negative bacteria observed in this study. This study adds to the literature by providing data specifically on the effect of pelletisation on *Enterobacteriaceae* and *Salmonella* which are required for testing of animal waste digestate as outlined in PAS110.

The pellet production process includes steps with the potential to reduce bacteria numbers. Anaerobic digestion

TABLE 1 Pathogens associated with animal manures (Nicholson et al., 2004; Watabe et al., 2003)

Animal-related microorganisms in cattle slurry	Associated diseases[a]
Faecal coliforms (indicator bacteria, not all are pathogenic)	
Salmonella spp. (pathogenic)	Salmonellosis
Generic *Escherichia coli* (not all pathogenic), including 0157 (pathogenic)	Haemorrhagic diarrhoea
Enterococci (generally not pathogenic)	Urinary tract infections, meningitis, endocarditis
Listeria monocytogenes (pathogenic)	Listeriosis
Clostridium perfringens, Clostridium tetani, Clostridium botulinum, Clostridiodes difficile (pathogenic)	Botulism, tetanus, colitis
Mycobacterium avium subsp. *paratuberculosis* (MAP) (pathogenic)	Johne's disease (cattle)
Enterovirus (pathogenic)	Haemorrhagic diarrhoea
Campylobacter coli, Campylobacter jejuni (pathogenic)	Campylobacteriosis (diarrhoea)
Cryptosporidium (*C. parvum* considered pathogenic)	Cryptosporidiosis (gastrointestinal illness)

[a]Human diseases, unless otherwise stated, although there are unproven links between Johne's disesase and Crohn's disease (Agrawal et al., 2021).

has been demonstrated to greatly reduce the prevalence of various indicator species. Riungu et al. (2018) showed inactivation of *Escherichia coli* in single-stage and two-stage reactors, with greater inactivation in the two-stage systems. Costa et al. (2017) found a mean reduction in faecal coliform counts from 4.1×10^5 CFUs/g to 4.1×10^2 CFUs/g following mesophilic AD of both cattle and pig slurry (CFU = colony forming unit). After mechanical separation, drying of the solid fraction represents the next step with potential to reduce bacteria presence, as low moisture environments inhibit bacteria proliferation (Esbelin et al., 2018). Finally, the pellet pressing stage submits the solid fraction to pressures typically between 115 and 300 kPa (Whittaker & Shield, 2017) and temperatures of up to 90°C. There has been limited previous research on the effect of pellet production, and the storage of pellets, on pathogen persistence. This study will address these knowledge gaps.

In the United Kingdom, transport of digestate and digestate products off farms is controlled by the BSI PAS110 (British Standards Institute Publicly Accessible Specification 110) (BSI, 2014). For any digestate using ABPs (e.g. slurry), the standard requires testing in accordance with appropriate ABP regulations as set out by the competent authority. In Northern Ireland, The Animal By-Products (Enforcement) Regulations (Northern Ireland) 2011 lay down health rules regarding APBs. Similar European Union regulations exist dealing with the control of ABPs (Regulation [EC] No 1069/2009). The minimum ABP regulation requirements in Northern Ireland are for an *Enterobacteriaceae* count of fewer than 1000 CFUs/g and absence of *Salmonella* spp. in 5×25 g fresh matter samples. In the absence of specific guidelines for digestate pellets, testing was undertaken for *Enterobacteriaceae* and *Salmonellae* spp. in accordance with the PAS110 specification.

The *Enterobacteriaceae* is a family of Gram-negative rod-shaped bacteria, which includes various common pathogenic genera commonly found in the mammalian gut (e.g. *Escherichia, Salmonella, Shigella* and *Enterobacter* among others) (Reece et al., 2011). *Enterobacteriaceae* enumeration is commonly used as an indication of whether a material is likely to contain pathogenic bacteria. Due to the wide variety of bacterial genera and species associated with animal slurries and the commercial nature of anaerobic digestion, it would be unreasonable to test for every potential pathogen on a regular basis.

The aim of this paper was to determine the effect the pellet production process has on digestate *Salmonella* and *Enterobacteriaceae*. The evaluation of pathogen survival is important to enable recommendations to be made for production, storage and handling. To achieve this, the following objectives were set: (1) measure *Enterobacteriaceae* and *Salmonella* presence throughout the pellet production process; (2) determine *Enterobacteriaceae* and *Salmonella* presence in freshly produced pellets and (3) test for the presence of *Enterobacteriaceae* and *Salmonella* in stored pellets.

2 | MATERIALS AND METHODS

2.1 | Pellet production process

There are four steps in the process from slurry to finished pellet (Figure 1): (1) anaerobic digestion, (2) mechanical separation, (3) solid drying, (4) pellet pressing. Slurry, along with grass silage was digested in the mesophilic anaerobic digestion plant in the Agri-Food and Biosciences Institute (AFBI) (Hillsborough, Northern Ireland). Feedstock consisted of 89% dairy slurry with 11% grass silage. The plant consists of two continuously stirred tank reactors in series. Slurry is stored in a $200 \, m^3$ feedstock tank upstream from the primary digester tank. The primary digester tank is a $650 \, m^3$ ($600 \, m^3$ working volume, $50 \, m^3$ headroom) recirculated gas-mixed reactor, insulated and heated to 39°C. The secondary digester tank is a $650 \, m^3$ recirculated gas-mixed reactor with no heating or insulation. The hydraulic retention time in each tank is 28 days. Digestate from the secondary tank was pumped to a $100 \, m^3$ storage tank prior to mechanical separation. Mechanical separation was carried out with a Fan Separator 3.2–780 screw press (FAN SEPARATOR GmbH, Marktschorgast, Bavaria, Germany) with a typical flow rate of 9–11 m^3/h. The resulting solid fraction was then dried from 80% moisture content to approximately 15% moisture content before being pelletised in a Farm Feed Systems Minipress (Farm Feed Systems Ltd., Cinderford, Gloucestershire, United Kingdom). The drying system consisted of a 22.5 kW centrifugal fan (Typhoon TC4 RO), which drew fresh ambient air through a 200 kW heat exchanger and delivered 650 m^3/min against a backpressure of 10 mb down a plenum and up through a drying floor with 50 mm vents spaced at 32 cm intervals. The heat delivered to the heat exchanger was generated by a woodchip fired boiler burning willow wood chip and spare heat from biogas combustion. The pellet press used in the study operates at approximately 80–90°C while applying pressure to the material to force it through a 6-mm pellet die. Pellets exit the die with a moisture content of approximately 8%.

2.2 | Sampling

Samples were taken at each stage of pellet production and of the final pellets (Figure 1), both freshly made and following storage for 1 month. Initially, five sampling
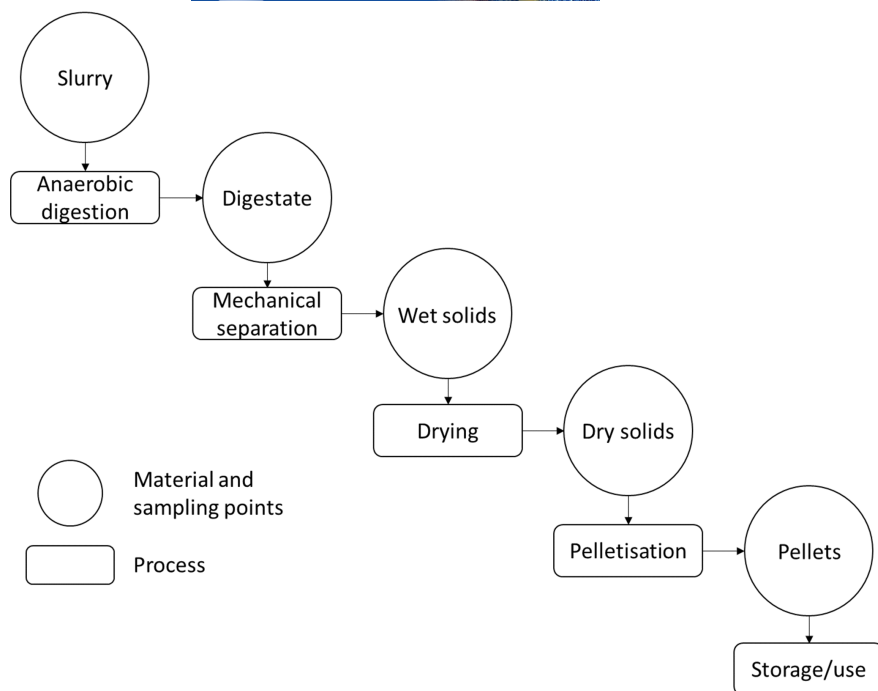
FIGURE 1 Schematic of pellet production process, identifying sampling points in the study.
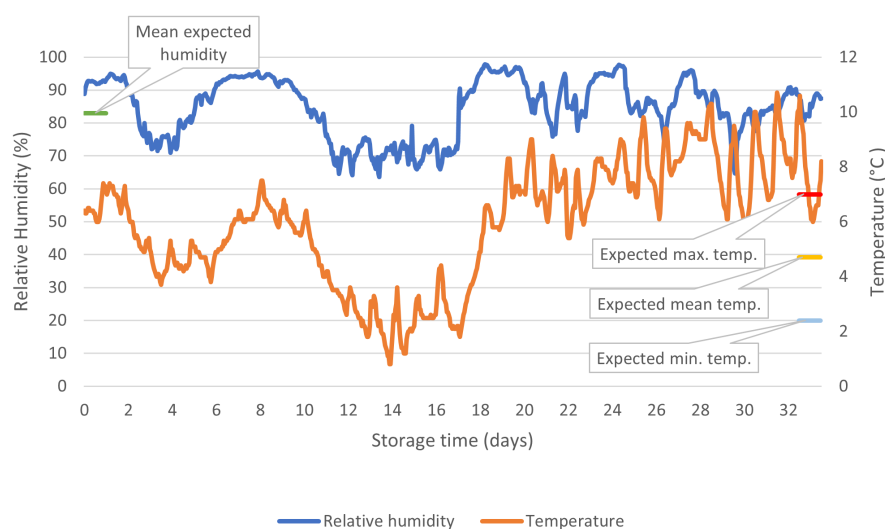

FIGURE 2 Temperature and humidity data at AFBI-Hillsborough, Northern Ireland, during pellet winter storage experiment (27 January – 1 March 2021). Expected values taken from historical weather data (climate-data.org, 2021).

points were considered for bacterial enumeration (Figure 1). The storage conditions tested were based on practices for commercially manufactured wood pellets (6 mm diameter), which are sold both in bulk and in 10 kg bags. Both loose and bagged pellets were therefore tested. As silos and fuel storage sheds will experience temperature and humidity fluctuations throughout the year, winter and simulated summer storage experiments were carried out to compare how different conditions affect pellet bacteria numbers. A period of approximately 1 month was investigated in the storage experiments which is the expected storage period for the pellets from manufacture to end use.

In the winter storage experiment, two 10-kg samples of freshly made pellets were placed in a storage shed in AFBI-Hillsborough, one sample bagged and the other piled loose on the concrete floor. A data logger measured temperature and humidity every hour for 33 days (27th January – 1st March 2021) (Figure 2). The simulated summer storage experiment took place in a Weiss Technik Fitotron SGC 120 plant growth chamber (Schunk GmbH, Lauffen, Baden-Württemberg, Germany) at a fixed temperature of 14°C and humidity of 50%. The temperature of 14°C was chosen to match the mean temperature in Hillsborough in August according to UK Meteorological Office data (max. Temperature 18°C, min. Temperature 10.8°C) (Metoffice.gov.uk, 2021). The humidity was set to the maximum that the growth chamber could sustain (50%); however, it should be noted that the mean humidity in July is 78% (Climate-data.org, 2022). Two 10-kg pellet samples were placed in the growth chamber, with one sample bagged and the other piled on the chamber floor.

Storage time for the simulated summer storage was also 33 days (3 February – 8 March 2021).

Although the simulated summer storage experiment did not account for humidity and temperature fluctuations as were seen in the winter storage scenario, it was considered that the higher humidity and lower temperatures typical of winter would be more likely to induce dewing on the pellets which would partially rehydrate and potentially revive any inactive bacteria. Dewing is less likely in warmer months as warmer air can hold more water and therefore the simulated experiment was deemed a suitable approximation of summer conditions.

Triplicate samples (3 × 500 g for solid samples, 3 × 500 ml for liquid samples) were taken from the identified points in the pellet production process (Figure 1). Samples from active processes (AD, separation and pelletisation) were taken at 5-min intervals for 15 min (three samples) during normal operation. Dried digestate samples were gathered from three separate areas of a drying bay containing 5 t dried digestate. The digestate in the drying bay was well mixed prior to sampling. Solid samples were placed in sterile bags using aseptic technique to avoid any contamination from outside sources, whereas the liquid samples were tapped into sterile bottles directly from the digester feedstock tank (slurry) and secondary digester (digestate). The dried digestate samples and those from the stored pellet piles and bags were well mixed prior to triplicate sampling to ensure the triplicates were representative. Each 500 g/500 ml triplicate sample was then submitted to a United Kingdom Accreditation Service (UKAS) accredited lab where they were sub-sampled for Enterobacteriaceae enumeration and *Salmonella* detection.

2.3 | Salmonella detection

Detection of Salmonella was carried out according to ISO 6579-1:2017+A1:2020. Positive and negative controls were processed at each stage: *S. Lindi* NCTC 08494 (positive) and *Y. enterocolitica* NCTC 10460 (negative). For each triplicate sample submitted, 5 × 25 g samples were each added to 225 ml buffered peptone water and incubated for 18 h at 37°C producing a pre-enriched sample. Of this, 0.1 ml was added to 10 ml Rappaport-Vassiliadis Soya (RVS) broth and incubated for 24 h at 41.5°C. One ml of the pre-enriched samples was also added to separate 10 ml Muller–Kaufmann Tetrathionate Novobiocin (MKTTn) broths, enriched with iodine-iodide solution and oxoid novobiocin selective supplement, and incubated at 37°C for 24 h. The RVS and MKTTn broths were then streaked on Brilliant Green Agar and Xylose Lysine Desoxycholate Agar and incubated at 37°C for 24 h before being examined for typical *Salmonella* colonies. Serological testing was carried out by the Kauffman–White classification.
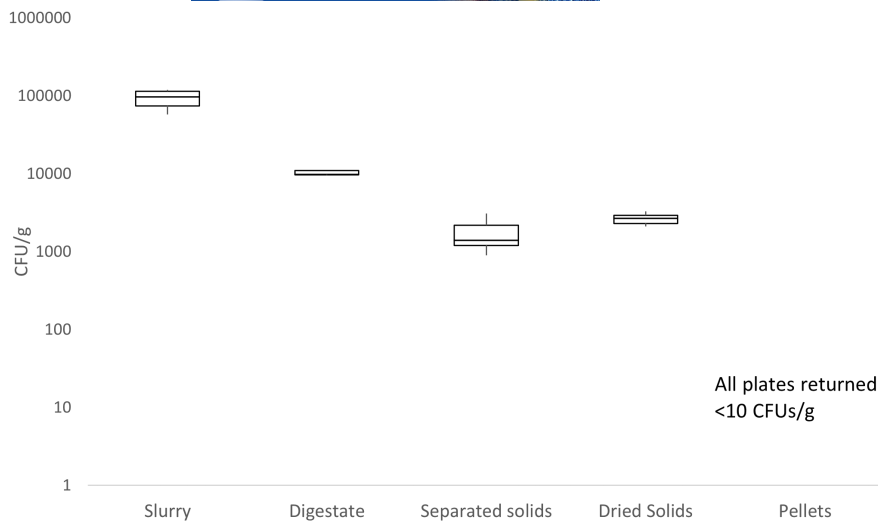
2.4 | Enterobacteriaceae enumeration

Enumeration was carried out using the colony count method on solid growth media in accordance with ISO 201528-2:2017. Briefly, 10 g solid samples (wet solids, dried solids and finished pellets) were added to 90 ml peptone saline diluent to form a primary dilution. 10-ml liquid samples (slurry, digestate) were added to 90-ml buffered peptone water. Each triplicate sample provided to the lab was sub-sampled five times. Further serial decimal dilutions of each sample were made depending on expected bacteria numbers to achieve between 50 and 150 colonies per dish. Fifteen ml molten violet red bile agar (VRBGA) was inoculated with 1 ml of the appropriate serial dilutions, mixed and then allowed to solidify. The inoculated medium was then overlaid with 10 ml sterile VRBGA and incubated at 37°C for 20 h, after which time colony counts were made. Positive (*E. coli* NCTC 10538) and negative (*Ps. Fluorescens* NCTC 10038) controls were processed during each stage of the test. Presence of *Enterobacteriaceae* colonies was verified using the oxidase test (negative) and glucose fermentation test (positive).

3 | RESULTS

3.1 | Salmonella detection and Enterobacteriaceae enumeration

The slurry samples submitted for *Salmonella* detection all returned positive. All isolated *Salmonella* colonies were identified as *S. enterica* serotype Typhimurium. All other samples returned negative Salmonella tests indicating that the AD process under investigation is effective in eliminating *Salmonella*.

The greatest decrease in bacterial numbers was following the anaerobic digestion stage (Figure 3). The mean *Enterobacteriaceae* colony count in slurry across the triplicate samples was 9.4×10^4 CFUs/g (wet mass basis unless otherwise stated), whereas the mean count for digestate was 1×10^4 CFUs/g. The separation process (screw press) produced separated solids with a mean *Enterobacteriaceae* count of 1.7×10^3 CFUs/g. Unexpectedly, drying of the solids from approximately 75% to 15% moisture showed an increase in mean bacteria number to 2.6×10^3 CFUs/g. Following pelletisation all *Enterobacteriaceae* counts were reduced to <10 CFUs/g.

TABLE 2 Statistical significance of the differences in Enterobacteriaceae counts between sample sets

Data set 1	Data set 2	Mann–Whitney U-test (p value)
Slurry	Digestate	3.1×10^{-6}
Digestate	Wet solids	3.1×10^{-6}
Wet solids	Dried solids	2.0×10^{-3}
Dried solids	Fresh pellets	6.7×10^{-7}

3.2 | Statistical analysis of results

Statistical analysis of the data was carried out using R (R Core Team, 2021). Levene's test for homogeneity of variance was carried out to determine whether ANOVA was a suitable statistical test. Because the *Enterobacteriaceae* counts for all the pellet counts were reported as <10 (i.e. anywhere between 0 and 9) all values were imputed to 4.5 CFUs/g for the statistical analysis. The null hypothesis for Levene's test is as follows: 'variances are equal across all data sets'. Levene's test yielded a p-value of 2.2×10^{-16} meaning the null hypothesis was rejected and the variances cannot be said to be equal across all sets (i.e. the data are heteroscedastic). This ruled out the use of ANOVA to compare the means of each data set and determine whether differences were statistically significant. Kolmogorov–Smirnoff tests were carried out on each data set to determine if the data followed normal distribution. All data sets were deemed not to follow a normal distribution therefore non-parametric Mann–Whitney *U* tests were used to determine significant difference (Table 2). All differences in *Enterobacteriaceae* counts were statistically significant ($p < 0.05$), indicating each step in the process had a significant impact on the number of colonies found.

4 | DISCUSSION

4.1 | Impact of the pellet production process stages

4.1.1 | Anaerobic digestion

Reduction in indicator species numbers from the AD process is well documented (Costa et al., 2017; Saunders et al., 2012), however it has been noted that complete elimination of bacteria through AD is unlikely (Nag et al., 2019). This is corroborated by our results which show AD has the greatest impact on Enterobacteriaceae numbers with a reduction of 89%. The digester in this study operates at mesophilic temperature (39°C) in the primary digester with an unheated secondary digester (temperatures between 12–15°C in winter). Our results show that a reduction in *Enterobacteriaceae* numbers can be achieved even without artificial heating of the secondary digester.

4.1.2 | Separation of digestate

Mechanical separation of digestate through a screw press showed further reduction in *Enterobacteriaceae* numbers, likely due to the de-watering effect of the process. During separation, digestate fibre is trapped behind a $1\,mm^2$ screen, whereas the liquid effluent and smaller particles are allowed to pass through. Free-floating bacteria and any adhering to smaller particles will easily pass through the screen, while a proportion will remain in the filter cake, which has a water content of approximately 70%–80%. The further reduction in *Enterobacteriaceae* count relative to the starting slurry material was 9%.

Chiapetta et al. (2019) observed a 1 log unit reduction in *Enterococci* in screw press separated solids from a plug

flow digester, but the reduction was not observed for generic *E. coli,* which maintained a similar concentration post separation. Additionally, an increase in *E. coli* was observed for separated solids of digestate from a continuous mix digester. A 0.5 log reduction was observed for *Mycobacterium avium* subsp. *paratuberculosis* (MAP) following separation (Chiapetta et al., 2019) indicating that the effect of mechanical separation on bacteria concentration may be dependent on species, genus and/or family.

Orzi et al. (2015) found that solid–liquid separation by screw press resulted in a reduced faecal coliform concentration in the solid fraction (2.0×10^2 CFUs/g) relative to the unseparated digestate (2.15×10^3 CFUs/g). In contrast, faecal *Streptococci* concentration was higher in the separated solids (1.55×10^3 CFUs/g) than the unseparated digestate (9.0×10^2 CFUs/g) when sampled in 2014. When sampled in 2013 they observed a decrease in faecal *Streptococci* concentration (3.5×10^2 CFUs/g vs. 5.0×10^1 CFUs/g). The reason for the difference observed is unclear however the physical characteristics of the digestate sampled at each time point were different (e.g. 5.45% total solids in 2013 vs. 4.59% total solids in 2014). It is possible that the difference in total solids impacted the mechanical separation performance which in turn affected the partitioning of faecal *Streptococci*. Similar to our findings, Orzi et al. (2015) found a decrease in *Enterobacteriaceae* concentration, following mechanical separation from 1.2×10^3 CFUs/g to 2.0×10^2 CFUs/g. The differences observed in these bacteria concentrations further indicates that the effect of mechanical separation on bacteria is likely species/genus/family specific, and observations of one bacterial species cannot be extrapolated to others.

4.1.3 | Digestate drying

Unexpectedly, there was a greater *Enterobacteriaceae* concentration in the dried solids (2.6×10^3 CFUs/g) than the wet solids (1.7×10^3 CFUs/g). The increase in reported bacteria concentration may be explained by the reduction in mass due to water evaporation. Water content was reduced to approximately 15% from 70% during the drying process as was required for pellet formation. When *Enterobacteriaceae* concentration was expressed on a dry matter basis (CFUs/g_{DM}), the decrease in bacteria count was visible. The mean Enterobacteriaceae concentration for wet solids was 5.7×10^3 CFUs/g_{DM}, whereas for dry solids it was 3.1×10^3 CFUs/g_{DM}. A Mann–Whitney *U* test yielded a *p*-value of 4.7×10^{-5} indicating there was a significant difference in the Enterobacteriaceae concentrations on a DM basis.

We can infer that the drying process and removal of water had some ability to reduce *Enterobacteriaceae*

numbers. Drying of bacteria and the resulting reduction in viability (Esbelin et al., 2018) is a well understood mechanism and many bacterial species have poor desiccation tolerance (Potts, 1994). Drying of pig slurry has been shown to significantly reduce viable bacteria counts in pig slurry (Kabelitz et al., 2020) when dried to a moisture content of 15%. Although the digestate investigated in this study was derived from cattle slurry, the same principal holds that reduction of moisture has an inhibitory effect on microbial growth.

4.1.4 | Pelletisation of solids

Pelletisation of solids resulted in a reduction of *Enterobacteriaceae* to below detectable levels. It is likely that the combination of temperature, pressure and desiccation is sufficient to reduce bacteria numbers to below detectable levels. Pulvirenti et al. (2015) found complete elimination of *Clostridia* spp., a resilient endospore-forming Gram-positive genera, in their pelletisation experiment.

4.1.5 | Storage of pellets

Bulk wood pellet deliveries are made by lorry and pumped into fuel silos or storage sheds, whereas bagged pellets can be purchased from hardware stores and other retailers. All pellet storage experiments returned colony counts of <10 CFUs/g indicating either (1) the pellets were free of *Enterobacteriaceae*, or (2) *Enterobacteriaceae* are present in numbers below the detection threshold and the water content of the pellets is sufficiently low to inhibit proliferation. A longer storage period may give time for any remaining bacteria to reactivate provided there is enough moisture available for growth; such moisture could be present as a result of dew or water ingress from a leak in the storage facility, highlighting the importance of proper storage conditions for the pellets. Pellets sold in bags have some protection from water ingress, whereas stored bulk pellets may be more susceptible to atmospheric moisture uptake and due to their size, are more likely to be kept in warmer conditions compared with bulk purchases.

4.2 | Practical implications for pellet production, storage, transport and handling

Rehydration of the pellets was not explored in the experiments as in real-world use the pellets must be protected from water ingress to maintain their suitability as a fuel. It

is possible that bacteria below detectable threshold could re-establish following contact with water, provided the bacterial species present are sufficiently tolerant to desiccation (Potts, 1994). However, in the case of contamination with water it would be difficult to ascertain whether the bacteria had survived the pelletisation process and were reactivated or were carried to the pellets in the water or through the air. Regardless of the source of potential bacteria, pellets contaminated with water would need to be discarded due to loss of structure and reduction in fuel quality.

As the *Enterobacteriaceae* were undetectable in the finished pellets, the pelletisation process fulfils the requirements of the PAS110 specification, meaning transport off the site they were produced on is permitted. Mechanical separation of digestate and slurries has been identified as a method to reduce risk to water bodies by partitioning phosphorus in the separated solid fraction and avoiding its application to nutrient vulnerable zones (Steinfeld et al., 2006). This study focused on the use of digestate pellets as a renewable fuel, but there is also the possibility for them to be used as fertiliser. The observed reduction in bacteria numbers alleviates concerns that ABP-derived products can introduce pathogens to arable and grassland soils, which may then be passed down the food chain (Nicholson et al., 2004).

Although our results suggest that *Enterobacteriaceae* spp. are eliminated through pelletisation of digestate, there are other microorganisms that could potentially still be present in the pellets (Table 1). During handling and transport, digestate pellets will crumble to some extent and form dust that may become airborne when agitated. The risk of dust inhalation from digestate pellets is similar to the risk from the inhalation of wood dust from wood pellets, but the hazard might be more severe if reactivation of potentially pathogenic bacteria occurs in an individual's respiratory or gastric tracts. Personal protective equipment such as face masks (FFP3 rated face masks conforming to BS EN 149:2001) and gloves should, therefore, be used when handling the pellets to mitigate the potential risk from inhalation or ingestion of digestate.

As our results confirm Enterobacteriaceae are present in dried digestate solids, it is important that proper precautions are taken in its handling through the pellet production process. Dried digestate solids have low density and structural integrity and are prone to forming dust when agitated, more so than the finished pellets. The pellet production process itself results in significant quantities of dust when dried solids are being fed to the pelletising chamber. As with the handling of the final pellets, it is important that face masks are used when handling dried digestate solids to reduce the chance of inhalation. There is

less risk associated with wet digestate solids as dust is not formed during handling, but proper sanitary precautions should still be taken, such as the use of gloves, to prevent accidental ingestion.

4.3 | Limitations and further work

This study focussed on the two key parameters for digestate handling regulations as set out on the PAS110 specification: Enterobacteriaceae enumeration and *Salmonella* detection. Enterobacteriaceae are Gram-negative, facultative anaerobes but various other important human pathogens found in animal manures are Gram-positive, for example, *Clostridia* spp. and *Enterococci* spp., and these were not tested for in this study. Various bacterial species such as those in the *Salmonella* genus also release endotoxins when destroyed (Reece et al., 2011) which may be of concern due to the elimination of *Salmonella* in AD. Endotoxins are lipopolysaccharides that can require temperatures of up to 300°C to degrade (Reece et al., 2011). It is unknown whether the combination of temperature and pressure in the pellet press is sufficient to destabilise these molecules, and it is recommended that this is investigated in future research.

There were limitations associated with the simulated summer storage experiment as the maximum relative humidity achieved by the growth chamber used was 50%. The mean relative humidity expected during the summer (Jun-Aug) ranges from 77 to 80% (Climate-data.org, 2022). The mean temperatures during this period (13.2–14.7°C with highs of 17.6°C) would be more conducive to bacterial growth provided there is sufficient moisture present. It is currently unknown if moisture content of the pellets can reach level where bacterial growth is encouraged, while still maintaining structural integrity, which is an important indicator of fuel quality.

Testing of the pellets to determine water activity would indicate whether proliferation of bacteria is possible in the long term. Water activity is the ratio of the vapour pressure of unbound water in a substrate to the vapour pressure of pure water (Esbelin et al., 2018). *E. coli* and *Salmonella* spp. require a water activity of 0.95 for growth, while more tolerant species such as *Staphylococcus aureus* can grow with a water activity of 0.86 (Sperber, 1983). An extension of the storage period experiments, over a period of several months, and with sterilised water addition would help to confirm the presence or absence of bacteria in the pellets. Additionally, the detection of a wider range of potentially pathogenic bacteria would give further information on the level of bacterial kill achievable through pelletisation.

5 | CONCLUSION

Each step in the digestate pellet production process plays a role in the reduction of Enterobacteriaceae numbers. The final pelletisation step reduces Enterobacteriaceae numbers to below detectable levels and the produced pellets conform to the PAS110 specification for digestate transport off farms. Finished pellets therefore pose a reduced risk to human health compared with unpelletised material, but the handling of pellets should still be risk assessed and appropriate personal protective equipment used. Storage of the pellets for 33 days under winter and summer conditions saw no reactivation of Enterobacteriaceae numbers which indicates proliferation of microbes is restricted in the final product and the pellets do not pose an increased risk following storage. The findings support the proposal that pellets produced from agricultural anaerobic digestates could be a promising renewable fuel and highlight the need for further research into their combustion characteristics.

CONFLICT OF INTEREST

The authors confirm that they have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Data used in this paper is available in the supporting material and in Dryad: doi: 10.5061/dryad.dncjsxm2s.

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