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Inactivation of enteric indicator bacteria and system stability during dry co-digestion of food waste and pig manure☆

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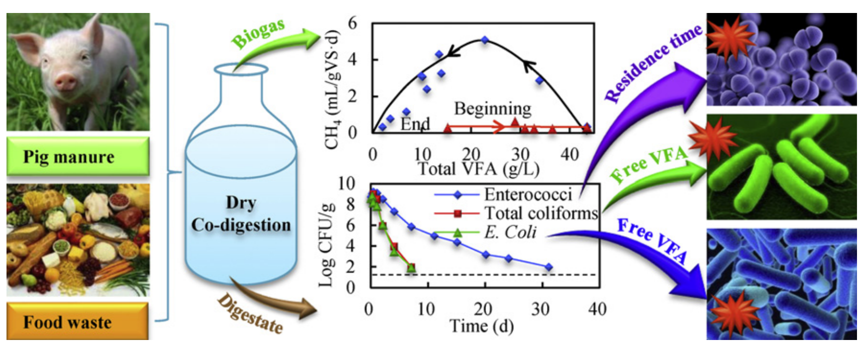
HIGHLIGHTS

- Dry co-digestion of food waste and pig manure inactivated pathogens effectively.
- Free VFA was the significant inactivation factor for *E. coli* and total coliforms.
- Residence time was the most significant inactivation factor for enterococci.
- Digestate inoculum and a FW/PM ratio of 50:50 led to preferable system stability.
- Stable dry co-digestion should be carried out with VFAs <20 g/L.

GRAPHICAL ABSTRACT



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ABSTRACT

Provision of digestate with satisfactory biosafety is critical to land application of digestate and to the anaerobic digestion approach to treating manure and food waste (FW). No studies have been conducted on digestate biosafety in dry co-digestion systems. The aim of this study was to assess the inactivation efficiency and possible inactivation mechanism for three enteric indicator bacteria and the system stability during dry mesophilic anaerobic co-digestion of FW and pig manure (PM). The effects of two different inocula were examined at a rate of 50% based on volatile solids (VS): digestate taken from existing dry co-digestion digesters and dewatered anaerobic sludge from a local wastewater treatment plant. The FW/PM ratios of 50:50 and 75:25 on a VS basis were also assessed. The results showed that using digestate as the inoculum and a FW/PM ratio of 50:50 led to stable dry co-digestion, with the specific methane yield (SMY) of 252 mL/gVS$_{added}$. Total volatile fatty acid (VFA) concentration was a significant inhibition factor for methane production during dry co-digestion ($P < 0.001$). The data also showed that dry co-digestion of FW and PM effectively inactivated enteric indicator bacteria. *E. coli* and total coliforms counts decreased below the limit of detection (LOD, 10^2 CFU/g) within 4–7 days, with free VFA identified as a significant inactivation factor. Enterococci were more resistant but nonetheless the counts decreased below the LOD within 12 days in the digestate inoculum systems and 26–31 days in the sludge inoculum systems. The residence time was the most significant inactivation factor for enterococci, with the free VFA concentration playing a secondary role at high FW/PM ratio in the sludge inoculum system. In conclusion, digestate as inoculum and the FW/PM ratio of 50:50 were preferable operation conditions to realize system stability, methane production and enteric indicator bacteria inactivation.

☆ The authors declare no competing financial interest.

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1. Introduction

It was estimated that 589,260 tonnes of biodegradable municipal waste (BMW) was generated in Ireland in 2012, comprised primarily of food waste (FW). Almost all this waste was treated by landfilling (589,000 tonnes) (McCoole et al., 2014). However, the EU Landfill Directive (1999/31/EC) requires a decrease in the amount of BMW landfilled from 916,000 tonnes in 2010 to 610,000 tonnes in 2013 and 427,000 tonnes in 2016. The landfill levy in Ireland was increased from €30/tonne of waste disposed to €50/tonne in 2011 and further to €65/tonne in 2012 and €75/tonne in 2013 (DECLG, 2012). These increasingly stringent regulations and ever-increasing costs are driving the need to find alternative non-landfill uses for BMW.

According to the national pig herd census, there were 152,906 breeding pigs in Ireland in October 2013 (DAFM, 2013). Each sow with progeny was estimated to produce approximately 21 m^3 of liquid pig manure (PM) at 4.8% dry matter (Nolan et al., 2012). Therefore ~3.19 million m^3 of liquid PM are produced annually in Ireland and land application is the preferred utilization method. The EU Nitrates Directive (91/676/EEC) sets a limit of 170 kg organic nitrogen per hectare per year on PM application, and due to the lack of proximity to suitable spread-lands in certain areas of the country, there is a demand for alternative uses for PM.

Anaerobic digestion is a method of treating bio-wastes such as FW and PM, which can recover energy, decrease waste volume, reduce odour production and mitigate greenhouse gas (GHG) emissions (Dennehy et al., 2017a; Orzi et al., 2015). However, previous studies have found that mono-digestion of FW or PM is prone to inhibition caused by high volatile fatty acid (VFA) or high ammonia concentrations, respectively, resulting in low biogas production or even system failure (Jiang et al., 2014). Co-digestion of FW and PM may provide an effective solution to address each of these issues as there should be a buffering interaction between the VFA and ammonia. Co-digestion is reported to be able to increase the methane yield, shorten the lag phase and improve the system stability of anaerobic digestion systems, due to the buffering effect, optimization of the carbon to nitrogen ratio (C/N) and addition of trace elements found in PM (Rodriguez-Verde et al., 2014; Xie et al., 2011; Xie et al., 2016). Previous studies found that wet co-digestion of FW and PM had obvious synergistic effects on methane production, with the specific methane yield (SMY) increased by ~20% at the PM/FW ratios of 3/2 and 2/3 (Dennehy et al., 2016a; Dennehy et al., 2017b). Compared with wet digestion (total solids (TS) content of substrate < 20%), dry digestion (TS of substrate > 20%) can reduce the digester volume significantly, thereby considerably decreasing the initial capital expenditure and the energy consumption required for heating. Dry digestion has been used in the industry but no studies on biosafety of digested FW and PM have been conducted to date.

Biosafety should be borne in mind when considering the treatment of FW and PM, as both of them are reservoirs of zoonotic pathogens, which can lead to diseases such as gastroenteritis, meningitis and pneumonia (Böhm et al., 1999; McCarthy et al., 2013). *Escherichia coli* (*E. coli*), total coliforms and enterococci are commonly used as indicator bacteria to assess the inactivation of enteric pathogens. The upper limits for *E. coli* and *Enterococcaceae* in digestate are 1000 CFU/g fresh matter according to Regulation (EU) No 142/2011. The previous study found that varying the FW/PM ratios in wet co-digestion of FW and PM and decreasing the HRT from 41 days to 21 days had no clear effect on pathogen inactivation (Dennehy et al., 2016b). Resende et al. (2014) studied wet co-digestion of sludge, cattle slurry and the organic fraction of municipal solid waste (OFMSW), and measured *E. coli* and enterococci concentrations in the final digestate of 3.6–4.4 and 3.8–4.8 $\log_{10}$ CFU/g, respectively, which were higher than the 1000 (3 $\log_{10}$) CFU/g limits. Subsequent land application of digestate may lead to pathogen contamination of soil, as well as contamination of underground and surface water, thereby posing a risk to human and animal health. Therefore digestate microbiological quality must be strictly controlled. Regulation (EU) No 142/2011 requires Category 3 animal by-products used as raw material in a biogas plant must be pasteurized at 70 °C for at least 1 h, which inevitably increases the capital and operational costs associated with anaerobic digesters.

Compared with wet digestion, the high substrate concentration in dry digestion is likely to result in high VFA and ammonia concentrations. VFA and ammonia are commonly reported as pathogen inactivation factors in mesophilic digestion (Kunte et al., 1998; Pecson et al., 2007). Watcharasukarn et al. (2009) reported 2.6 and 3.1 $\log_{10}$ reductions in *E. coli* and *Enterococcus faecalis* counts at VFA and total ammonia concentrations of 5.02–8.71 g/L and 1.59–1.67 g/L, respectively, during wet mesophilic digestion of cow manure. Ottoson et al. (2008) observed a 4-fold increase in the inactivation rate of enterococci at high free ammonia nitrogen (FAN) concentration of 644 mg/L compared to the control of 22 mg/L. Therefore, high VFA and ammonia concentrations may effectively inactivate enteric pathogens. If this is the case, the capital and operational costs associated with a heat-treatment step could potentially be avoided. However, the efficiency, mechanism and kinetics of pathogen inactivation during dry co-digestion of FW and PM have not been studied to date. While, on the other hand, high VFA and ammonium concentrations would inhibit the digestion system and affect its system stability.

Therefore, in the present study, dry co-digestion of FW and PM was conducted to assess: (1) the effects of different inocula and FW/PM ratios on system performance and pathogen inactivation, (2) the inhibition of high VFA concentration on methane production, and (3) the efficiency and possible mechanism of pathogen inactivation. The results obtained should provide a reference for large-scale engineering practices of dry co-digestion systems.

2. Materials and methods

2.1. Experimental design

Fresh FW was collected from 10 local residences in Galway City, Ireland, and was ground in a food processor (Kenwood FPP210 Multipro Food Processor, Havant, UK) to <2 mm and mixed before use. PM was obtained from a local pig farm and was centrifuged at 1500 ×*g* for 5 min to obtain the solid fraction. Two inocula were used in order to assess the effect of inoculum. One was solid digestate taken from a laboratory-scale batch FW/PM dry co-digestion system. The digesters had been operated for ~5 months, with no methane production any more. The other was dewatered anaerobic sludge obtained from a local wastewater treatment plant. The sludge was placed at 11 °C for one month to completely release biogas prior to use (Dennehy et al., 2016a). The physicochemical properties of the FW, PM, digestate and sludge are shown in Table 1.

In a previous study in which dewatered anaerobic sludge was used as the inoculum, the effects of the inoculum rate (25% and 50% based

Table 1
Physicochemical properties of food waste and pig manure substrates and the digestate and sludge used as inocula.

Feature	Food waste	Solid fraction of pig manure	Digestate	Sludge
pH	5.0	8.6	8.9	8.2
Moisture content (MC, %)	73.8	76.2	83.4	81.0
Total solids (TS, %)	26.3	23.8	16.6	19.0
Volatile solids (VS, %)	25.0	19.4	11.7	12.6
VS/TS (%)	95.4	81.5	70.2	66.3
Total volatile fatty acid (VFA, mg/L)	4657	5314	ND[a]	1758
Free VFA calculated (mg/L)	1907	1	ND	1
Total ammonia nitrogen (TAN, mg/L)	621	5052	7149	2773
Free ammonia nitrogen calculated (FAN, mg/L)	0	904	1570	218

[a] ND: Undetectable.