ARTICLE IN PRESS

Waste Management xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Anaerobic co-digestion of pig manure and food waste; effects on digestate biosafety, dewaterability, and microbial community dynamics

C. Dennehy [a], P.G. Lawlor [b], M.S. McCabe [c], P. Cormican [c], J. Sheahan [d], Y. Jiang [a], X. Zhan [a,*], G.E. Gardiner [e]

[a] Civil Engineering, College of Engineering and Informatics, National University of Ireland, Galway, Ireland
[b] Teagasc Pig Development Department, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland
[c] Teagasc Animal and Bioscience Research Department, Animal and Grassland Research and Innovation Centre, Grange, Dunsany, Co. Meath, Ireland
[d] School of Mathematics, Statistics and Applied Mathematics, National University of Ireland, Galway, Ireland
[e] Department of Science, Waterford Institute of Technology, Ireland

ARTICLE INFO

Article history:
Received 24 July 2017
Revised 26 October 2017
Accepted 27 October 2017
Available online xxxx

Keywords:
Indicator bacteria
Hydraulic retention time
Sequencing
Synergistaceae
Sphaerochaeta

ABSTRACT

This study assessed the effect of varying pig manure (PM)/food waste (FW) mixing ratio and hydraulic retention time (HRT) on methane yields, digestate dewaterability, enteric indicator bacteria and microbial communities during anaerobic co-digestion. Three 10 L digesters were operated at 39 °C, each with a PM/FW feedstock composition of 85%/15%, 63%/37% and 40%/60% (volatile solids basis). While the PM/FW ratio was different among reactors, the organic loading rate applied was equal, and increased stepwise with reducing HRT. The effects of three different HRTs were studied: 41, 29, and 21 days. Increasing the proportion of FW in the feedstock significantly increased methane yields, but had no significant effect on counts of enteric indicator bacteria in the digestate or specific resistance to filtration, suggesting that varying the PM/FW feedstock composition at the mixing ratios studied should not have major consequences for digestate disposal. Decreasing HRT significantly increased volumetric methane yields, increased digestate volatile solids concentrations and increased the proportion of particles >500 μm in the digestate, indicating that decreasing HRT to 21 days reduced methane conversion efficiency High throughput 16S rRNA sequencing data revealed that microbial communities were just slightly affected by changes in digester operating conditions. These results would provide information useful when optimizing the start-up and operation of biogas plants treating these substrates.

1. Introduction

On-farm biogas plants have been a key component of the renewable energy generation and energy security strategies of several EU countries for the past 25 years (Wilkinson, 2011). They typically operate with a feedstock comprised primarily of manures (generated on-farm) supplemented with energy crops or agricultural by-products (Dennehy, et al., 2017b; Wilkinson, 2011). The addition of the latter is undertaken to increase methane yields, thereby increasing the economic viability of such plants (Xie et al., 2012). However concerns regarding sustainable land use associated with energy crops (Fritsche et al., 2010) have led to increased interest in alternative low cost co-substrates in the past decade. Organic wastes such as catering waste, source segregated food waste (FW) and the organic fraction of municipal solid waste are increasingly used as co-substrates in on-farm biogas plants (Holm-Nielsen et al., 2009). On-farm plants may receive gate fees for digestion and subsequent disposal of these wastes (Dennehy et al., 2017a). Additionally, high specific methane yields (SMYs) of these wastes make them attractive as co-substrates (Holm-Nielsen et al., 2009).

As agricultural by-product-fed anaerobic digestion systems are utilized primarily for renewable energy generation, studies of co-digestion of manure with organic wastes have typically focused on identifying the co-substrates, operational conditions or reactor configurations which maximize methane yields (Mata-Alvarez et al., 2014). However, few studies have assessed the effects of changing operating conditions (such as hydraulic retention time

Abbreviations: BMP, biomethane potential; CFU, colony forming unit; FAN, free ammonia nitrogen; FW, food waste; LOD, limit of detection; OTU, operational taxonomic units; PCoA, principle component analysis; PCR, polymerase chain reaction; PM, pig manure; PSD, particle size distribution; SAO, syntrophic acetate oxidation; SAOB, syntrophic acetate oxidizing bacteria; SMY, specific methane yield; SRF, specific resistance to filtration; TAN, total ammonium nitrogen; VMY, volumetric methane yield.

* Corresponding author.
E-mail address: xinmin.zhan@nuigalway.ie (X. Zhan).

https://doi.org/10.1016/j.wasman.2017.10.047
0956-053X/

(HRT), organic loading rate (OLR), and substrate mixing ratio) on properties such as digestate dewaterability and pathogen removal, which determine the cost of digestate disposal.

The current regulatory environment within the EU is largely responsible for the paucity of studies on the effect of co-digestion on pathogen removal. The EU animal by-products regulation (EU Commission Regulation (EU) No 142/2011) stipulates that feedstock entering or digestate leaving biogas plants treating animal by-products (such as food wastes) must undergo one of a range of highly prescriptive sanitization processes (typically particle size reduction followed by heat treatment) prior to land application. This treatment must reduce pathogenic indicator organisms to below the regulatory limit in place for land application (the animal by-products regulation requires < 1000 CFU g^{-1} for *E. coli* or *Enterococcus* in digestate prior to land application) (Jiang et al., 2018). Therefore, the anaerobic digestion process *per se* is not relied upon to achieve a specific level of pathogen removal. However, the effect of varying feedstock composition and HRT (and consequently OLR) on the digestate pathogen content merits investigation in instances where such regulations are not in place. It is also important to assess whether changes in a digestate treatment regime would be required to ensure the land application standards are met when changes are made to digester HRT or feedstock composition. This is despite the increasing use, and significant potential associated with conversion of digestate to biochar (Maroušek, 2014) and energy (Maroušek et al., 2015).

The practice of dewatering digestate from agricultural-based biogas plants is becoming more common. In the EU, this has been driven by regulations on soil nitrogen and phosphorus contents which limit the land available near farms on which manure or digestate is spread (Nolan et al., 2012). Dewatering generates a liquid fraction which can be treated or land-spread locally and a lower volume solid fraction which is more economical to transport (Nolan et al., 2012). The effect of co-digestion on digestate dewaterability has been the focus of some research (Habiba et al., 2009); however, much of this work focused on sewage sludge as a primary substrate. In terms of HRT, some authors found that longer HRTs resulted in more complete digestion, causing a uniform destruction of particles of all sizes and improved dewaterability (Habiba et al., 2009), while others found that shorter HRTs improved dewaterability by generating a digestate comprised of a greater proportion of larger particles (Lü et al., 2015). However there are few studies assessing the effect of varying the substrate mixing ratio on dewatering while controlling both OLR and HRT.

High throughput DNA sequencing has revolutionised the study of compositional changes in bacterial and archaeal communities in response to changes in the operating conditions within biogas plants (Campanaro et al., 2016). Applying techniques such as 16S rRNA sequencing to assess the effect of varying feedstock mixing ratios and HRT on microbial communities provides additional information on the stability within the reactor and can identify specific shifts in the microbial composition in response to changes in process variables (Carballa et al., 2015). While studies have shown that microbial populations change dynamically within anaerobic digestion systems even when operating parameters are unchanged (De Vrieze et al., 2016), linking changes in the abundance of organisms or the community structure to operational conditions can contribute to the development of microbial biomarkers for reactor stability and/or fermentation/methanogenic pathway shifts (Vanwonterghem et al., 2014).

Combining molecular analysis with chemical, physical and culture-based methods allows for an in-depth assessment of the effects that changes in HRT and substrate composition might have on anaerobic digester performance and stability. Therefore, the aim of this study was to assess the effect of varying substrate composition (PM/FW ratio) and HRT on; methane yields and process stability; digestate dewaterability; digestate biosafety; and microbial community dynamics.

2. Materials and methods

2.1. Experimental design

Three 10 L reactors (R1, R2, and R3) with a working volume of 7.5 L were used as anaerobic digesters. The reactors were subject to 4 phases; Phase I, a start-up phase, and three operational phases – Phase II at a HRT of 41 days, Phase III at a HRT of 29 days and Phase IV at a HRT of 21 days. Therefore the organic loading rate (OLR) was progressively increased from 1 kg volatile solids (VS) m^{-3} day^{-1} (Phase II) to 1.5 kg VS m^{-3} day^{-1} (Phase III) and to 3 kg VS m^{-3} day^{-1} (Phase IV). As reducing HRT resulted in increases in OLR, it is not possible to definitively conclude that the effects observed in this study were due to either factor, rather a combination of both. However, this remains a useful observation, as such a relationship between OLR and HRT exist in most on-farm biogas plants.

During the start-up phase (Phase I), all three reactors were fed with the same feedstock (85% PM and 15% FW on a VS basis). This was done in order to ensure that the operating conditions in each reactor were identical prior to varying the substrate composition. During Phase II the substrate composition of R2 and R3 were altered so that the proportion of PM in the feedstock mixture of each reactor (on a VS basis) was 85% in R1, 62.5% in R2 and 40% in R3, with the remaining portion comprised of FW. In order to ensure uniform OLR and HRT for each mixture, the feedstock mixture for each reactor was made up to the required volume with deionised water. OLR and HRT were coupled in order to ensure differences observed between substrate compositions were not confounded by differences in OLR.

The PM/FW mixing ratio range was chosen as higher FW ratios would result in a semi-solid feedstock due to the high total solids (TS) content of Irish PM (on average 58 g L^{-1} (McCutcheon, 1997)). On-farm biogas plants are usually completely stirred tank reactors (CSTRs) and therefore operate with a typical feedstock TS concentration of <150 g L^{-1}. This limits the mixing ratio between manure and solid co-substrates such as FW. Therefore, this study aimed to focus on PM/FW mixing ratios likely to be used at farm-scale.

The reactors were operated at 39 °C for the duration of the experiment. The temperature was maintained by water jackets and a thermostatically controlled water bath from which water was circulated to the reactor jackets. Mixing was undertaken for a period of 1 h per day by mechanical stirrers at 100 relative centrifugal force (RCF). After the inoculum was added, the reactors were sealed and the contents were flushed with N_2. Reactors were fed with substrate every weekday. Daily OLR was adjusted to account for the lack of feeding at weekends in order to ensure that OLR was maintained at a correct level. The feedstock mixture was prepared daily.

2.2. Substrates and inoculum

The PM used in this study was taken in batches every 4 weeks from the manure storage tanks of a pig farm in Co. Galway, Ireland. It was stored at 9 °C (in order to simulate typical manure storage temperatures in Ireland) prior to use during the subsequent 4 weeks. FW was collected weekly from 5 residences, combined and subsampled as described by Browne et al. (2014). After subsampling, the FW was blended using a food processor (Russell Hobbs 500 W 18087 Blender) and stored at 4 °C until use (for a maximum of 5 days). Unfrozen PM and FW samples were used during the feeding of the reactors in order to ensure that,