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Comparing the inhibitory thresholds of dairy manure co-digesters after prolonged acclimation periods: Part 1 – Performance and operating limits

J.G. Usack, L.T. Angenent*

Biological and Environmental Engineering, Cornell University, 214 Riley-Robb Hall, Ithaca, NY 14853, USA

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ABSTRACT

Co-digestion has been used to improve biogas yields and the long-term stability of anaerobic digesters compared to mono-digestion; however, less is known about the ultimate inhibition from co-substrates at their maximum loading rates and mixing ratios because these limits cannot be practically tested by existing facilities. Here, we performed a controlled experiment with long operating periods to ensure sufficient acclimation with the goal to observe ultimate inhibition and the full benefit that can be gained from co-digestion. The three substrates: 1) food waste (FW); 2) alkaline hydrolysate (AH); and 3) crude glycerol (GY) were individually co-digested with dairy manure (MN) for more than 900 days using continuously stirred anaerobic reactors at mesophilic temperatures. Food waste caused no reduction in performance or stability when co-digested with manure up to a total organic loading rate (OLR) of 3.9 g volatile solids (VS)·L⁻¹·Day⁻¹ (MN:FW = 51:49; VS basis), resulting in a specific methane yield (SMY) of 297 ± 3 mL CH₄·g VS⁻¹ for the combined wastes. Alkaline hydrolysate was co-digested with manure up to a total OLR of 2.7 g VS·L⁻¹·Day⁻¹ (MN:AH = 75:25) with a corresponding SMY of 299 ± 6 mL CH₄·g VS⁻¹. However, the free ammonia concentration reached levels previously reported as inhibitory, and may have led to the observed accumulation of volatile fatty acids at higher loading rates. Crude glycerol co-digestion resulted in an optimum SMY of 549 ± 25 mL CH₄·g VS⁻¹ at a total OLR of 3.2 g VS·L⁻¹·Day⁻¹ (MN:GY = 62:38). Stable digestion beyond this level was prohibited by an accumulation of long-chain fatty acids and foaming. These results can be used to implement effective co-digestion strategies. Co-substrates that possess similar inhibiting characteristics should be monitored to prevent severe instability at high loading rates and mixing ratios.

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Abbreviations: ABW, animal by-product waste; ANOVA, analysis of variance; BMP₁₀₀, 100-day biochemical methane potential; BMP₂₅, 25-day biochemical methane potential; C/N, carbon to nitrogen ratio; COD, chemical oxygen demand; CT, co-digestion treatment level; FID, flame ionization detector; FW, food waste; FS, fixed solids; GC, gas chromatograph; GY, crude glycerol; HRT, hydraulic retention time; MLR, mass loading rate; MN, manure; LCFA, long-chain fatty acid; LCFA_{liquid}, liquid-associated LCFA; LCFA_{solid}, solid-associated LCFA; OLR, organic loading rate; ORG-N, organic nitrogen; SCOD, soluble chemical oxygen demand; SMY, specific methane yield; SS, steady-state; SU, start-up; TCD, thermal conductivity detector; TCOD, total chemical oxygen demand; TKN, total kjeldahl nitrogen; TS, total solids; VFA, volatile fatty acid.

* Corresponding author.

E-mail addresses: jgu3@cornell.edu (J.G. Usack), la249@cornell.edu (L.T. Angenent).<http://dx.doi.org/10.1016/j.watres.2015.05.055>

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1. Introduction

The use of anaerobic digestion (AD) for the treatment of agricultural wastes, such as animal manures, is an effective means to stabilize and control emissions from these wastes while simultaneously producing useful energy on farms. Co-digestion on farms, which blends one or more organic wastes with livestock manure, has made AD more feasible in the United States by increasing methane production, improving system stability, and providing tipping-fees that are paid to the farmer in return for accepting this waste (Bishop and Shumway, 2009). Several factors are cited for the realized benefits of co-digestion, which include optimizing the carbon–nitrogen ratio (C/N) of the substrate mixture (Wang et al., 2012), remedying nutrient or trace element limitations (Zhang et al., 2012), improving buffering capacity (Murto et al., 2004), diluting/neutralizing inhibitory compounds (Fang et al., 2011), and

more efficiently utilizing the long sludge retention times in farm-based anaerobic digestion with easier-to-degrade substrates to improve biogas yields without the need for additional capital costs (Jensen et al., 2014).

Co-digestion has been successfully implemented at many biogas facilities; however, cases of decreased performance and instability have also been reported for various co-substrate types. For example, abrupt changes in substrate composition by including a co-substrate without appropriate microbiome acclimation periods may adversely affect performance and stability (Regueiro et al., 2014; Zhang et al., 2014). Even after extended acclimatization periods, co-substrates that possess inhibitory or toxic chemical compounds, such as long-chain fatty acids (LCFAs) (Hwu et al., 1998; Neves et al., 2009a; Palatsi et al., 2011; Shin et al., 2003), ammonia (Angelidaki and Ahring, 1994; Callaghan et al., 2002; Garcia and Angenent, 2009; Regueiro et al., 2012), and other inorganic salts (de Baere et al., 1984; Fang et al., 2011), may contribute to decreased performance. Also, certain physical mechanisms, such as foaming and floatation, have disrupted the AD process and caused biomass washout (Hejnfelt and Angelidaki, 2009; Hwu et al., 1998). Recent developments in high throughput gene sequencing technology has drastically reduced the cost and processing time needed for microbial community surveys (Li et al., 2013), and has paved the way for new research that attempts to explain, or at least correlate, process performance and inhibition with underlying microbial community dynamics (Jensen et al., 2014; Regueiro et al., 2014; Werner et al., 2014; Zhang et al., 2014). However, drawing meaningful conclusions is difficult, if not impossible, without the use of large datasets comprising extensive operational and sequencing data over long operating periods, as from full-scale facilities where monitoring is routinely performed (Werner et al., 2011). However, full-scale facilities do not always allow for properly controlled experiments, especially those that push the operational threshold of the digester, so such investigations must be conducted in the laboratory.

Here, our primary objective was to perform a long-term treatability study at the laboratory scale: 1) to determine the additional methane yield that can be achieved through co-digestion; 2) to identify the maximum loading rate and mixing ratio of the co-substrate before a reduction in performance occurs; and 3) to find the causes of the performance reductions for each co-substrate at high loading rates and mixing ratios. Our secondary objective was to collect biomass samples and sufficient operational data to allow subsequent microbial community analysis. We used consumer food waste, alkaline hydrolysate, and crude glycerol as potential co-substrates for digestion with dairy manure because they: i) have high biochemical methane potentials relative to dairy manure and thereby have the capacity to improve methane yield compared to manure-only digestion; and ii) possess different inhibitory characteristics capable of reducing the performance or stability of the AD process.

2. Background on co-substrates

2.1. Food waste

Food waste is often used as a co-substrate with animal manures in farm-based AD systems due to its high biodegradability and its wide availability (Labatut et al., 2011). Indeed, an estimated 25 million tons of food scraps are sent to landfill in the U.S. every year, leading to ~13% of anthropogenic GHG emissions, yet only 2.2% is recovered for energy (EPA, 2009). Studies have demonstrated that co-digestion of animal manure with food wastes results in greater performance and stability than can be achieved by the digestion of either substrate individually (Alvarez and Lidén, 2008; Zhang et al.,

2012). However, food wastes characterized by high hydrolysis rates and low inherent alkalinity could lead to acidification of the AD process if added in excess of the buffering capacity of manure (Murto et al., 2004).

2.2. Alkaline hydrolysate

The alkaline hydrolysis process is used to treat deceased livestock or other animal-tissue wastes and involves exposing the animal tissue to a strong alkali solution at high temperature and pressure. Although few installations presently exist in the U.S., it represents a possible alternative treatment strategy for waste streams requiring hygienization (Das, 2008). The process yields a sterile aqueous solution (i.e., hydrolysate) of high organic strength, which is comprised mostly of sugars, amino acids, fatty acids, and their soaps (Das, 2008), suggesting that it could be treated by anaerobic digestion. However, the high protein and lipid content of animal-tissue wastes may lead to instability of the AD process through an accumulation of their degradation products, which are ammonia and long-chain fatty acids (LCFA), respectively (Hejnfelt and Angelidaki, 2009; Palatsi et al., 2011). In addition, the high residual salt concentration resulting from the alkali treatment process (KOH or NaOH) may inhibit poorly acclimated microbiomes (de Baere et al., 1984; Fang et al., 2011). Finally, soap formation from this substrate may cause excessive foaming, which could lead to system instability by entrapping and removing biomass (Hejnfelt and Angelidaki, 2009; Hwu et al., 1998).

2.3. Crude glycerol

Crude glycerol is a by-product of the biodiesel manufacturing process, which reached a record breaking 111 million gallons in 2013 in the U.S. (EIA, 2013), resulting in approximately 10 million gallons of crude glycerol. Due to the high-energy density, biodegradability, and long-term storage potential of crude glycerol, anaerobic digestion has been identified as a candidate end-use technology (Castrillón et al., 2013). The glycerol molecule is readily degraded in AD systems and studies have demonstrated the dramatic increase in methane yield that can be realized when glycerol is used as co-substrate (Astals et al., 2012; Castrillón et al., 2013; Jensen et al., 2014). However, the compositional differences found in crude glycerol, particularly the carbohydrate to lipid ratio, make estimating the optimum loading rate difficult. Moreover, the interplay of the rapidly degraded glycerol component, and the LCFA producing lipid component in crude glycerol poses multiple operational challenges, such as acidification and foaming, and one may exacerbate the other (Hejnfelt and Angelidaki, 2009; Jensen et al., 2014).

3. Methods and materials

3.1. System set-up and inoculation

We used four identical, cylindrical continuously-stirred anaerobic reactors made of glass with an inner-diameter of 12 cm, and an effective volume of 4.5 L. Mixing of the reactor contents was achieved using a 6.2 cm diameter internal impeller (Model: Lightnin A-310, Rochester, NY) that was set to 200 RPM, equivalent to a spatially-averaged velocity gradient (G) of 150 s^{-1} . A heated water jacket surrounding the reactor was maintained at $37 \pm 1 \text{ }^\circ\text{C}$. Specific information regarding the anaerobic digestion system, including a step-by-step video of system construction and equipment, is provided by Usack et al. (2012). Active inoculum was obtained from a farm-based, plug-flow anaerobic digestion system treating dairy manure at $38 \text{ }^\circ\text{C}$ and a hydraulic retention time (HRT) of 37 days.

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