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Evaluating analytical methods for the characterization of lipids, proteins and carbohydrates in organic substrates for anaerobic co-digestion

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ABSTRACT

This study provides insights into the characterization of lipids, proteins and carbohydrate content in substrates for codigestion, and evaluates their effects on biogas yield. Among the analytical methods evaluated, the Bligh and Dyer, Hach Total Nitrogen and the Anthrone method were found to be most suitable for lipids, proteins and carbohydrates analysis, respectively. The co-digestibility of ten co-substrate mixes prepared using various volume-to-volume ratios of foodwaste (FW), fats, oils and grease (FOG), and waste activated sludge (WAS) were tested using biomethane potential assays. The three main substrates were mono-digested as well. WAS mono-digestion yielded the lowest methane yield of 118 mL CH_4/g VS, while a 50:50 mix of WAS and FOG, containing 85% lipid and 15% protein produced the highest methane yield of 1040 mL CH_4/g VS. In general, lipid-rich samples yielded more biogas than samples rich in proteins and carbohydrates. However, samples rich in proteins and carbohydrates had faster biogas production rates.

1. Introduction

For several decades, anaerobic digestion has been used to stabilize wastewater residuals, mitigate greenhouse gas emissions, and recover energy in the form of biogas. Despite its proven potential to generate renewable energy and provide a range of other environmental benefits (Appels et al., 2008), the operational complexities (Nguyen et al., 2015) and perceived economic burden (Klavon et al., 2013) associated with anaerobic digestion technology has affected its widespread implementation, especially among small-scale wastewater treatment plants. A recent USDA study reported that out of the approximately 16,000 wastewater treatment plants in the United States, only 1,241 have anaerobic digesters, and only 860 beneficially utilize the biogas (USDA et al., 2014). One possible way of maximizing the prospects of anaerobic digestion is to employ *anaerobic co-digestion*: a process of adding supplemental high-strength organic substrates, such as foodwaste and fats, oils and grease (FOG) to an anaerobic digestion system to augment biogas production. Anaerobic co-digestion has received significant attention in recent years (Mata-Alvarez et al., 2014; Nghiem et al., 2017) because of the growing desire of wastewater treatment plants to become energy-neutral, coupled with increasing concerns and strict regulations against landfilling of organic waste (BioCycle, 2013; Edwards et al., 2015).

Although the co-digestion of high-strength organic waste with wastewater residuals yields higher biogas, the complex and variable characteristics of co-substrates can potentially inhibit the microbial communities that drive the process, thereby causing system upset or failure (Long et al., 2012). However, if the characteristics of the co-substrates are comprehensively analyzed and understood with respect to system operation and optimization, such failures may be avoided. Typically, most anaerobic co-digestion feasibility studies look into the bulk characteristics of the co-substrates (Belle et al., 2015; Esposito et al., 2012; Wang et al., 2013; Zhang et al., 2007). These studies have limited their characterization to chemical oxygen demand (COD), total solids (TS) and volatile solids (VS), and in some cases, rely on the carbon to nitrogen ratio (C/N). Meanwhile, key parameters such as lipids, proteins and carbohydrates, which are precursors for inhibitory compounds like long-chain fatty acids and ammonia, are not analyzed.

Wagner et al. (2013) investigated the impact of lipids, proteins and cellulose (a complex carbohydrate) on biogas production and found protein-rich substrates to be benign to the anaerobic digestion process, whereas lipid-rich and complex carbohydrates such as cellulose were problematic since excessive amounts of carbohydrates and lipids led to the accumulation of volatile fatty acids thereby upsetting the process. In a review by Chen et al. (2008), it is reported that, long chain fatty acids are able to adsorb unto cell membranes and disrupt their functionality, while ammonia within the range of 1.7–14 g/L caused process upsets in anaerobic digestion systems. Meanwhile, Labatut et al. (2011) showed

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that lipid-rich samples and easily degradable carbohydrates resulted in high methane yields. Elbeshbishy and Nakhla (2012) co-digested proteins with carbohydrates and observed synergistic benefits which improved biogas production.

In spite of the potential of lipids, proteins and carbohydrates to affect the anaerobic co-digestion process, very few studies (Agyeman and Tao, 2014; Kabouris et al., 2009; Labatut et al., 2011; Sun et al., 2014) have looked into a detailed characterization of their content in substrates for anaerobic co-digestion. This may partly be because of the lack of established standard methods for analyzing these parameters in wastewater sludge and other organic substrates like foodwaste and FOG used for anaerobic co-digestion (Raunkjær et al., 1994). Hence, there is limited information on the influence of lipids, proteins and carbohydrates on the anaerobic co-digestion of WAS, foodwaste and FOG.

Therefore, the objectives of this study were to (1) evaluate and optimize selected analytical methods for determining lipids, proteins and carbohydrates concentrations in wastewater sludge and foodwaste as substrates for anaerobic co-digestion and (2) evaluate the effects of lipids, proteins and carbohydrates on anaerobic co-digestion of wastewater residuals, foodwaste and FOG using Biomethane Potential (BMP) assay and different co-substrate mix ratios. These substrates were selected because they are among the most common substrates co-digested with wastewater residuals.

2. Materials and methods

2.1. Evaluating analytical methods for lipids, proteins and carbohydrates

Palmitic acid, bovine serum albumin and glucose were used as standards for lipids, proteins and carbohydrates, respectively. These standards were selected based on availability and as suggested by literature (Cui and Brummer, 2005; Raunkjær et al., 1994; Walker, 2002). The Bligh and Dyer method (Bligh and Dyer, 1959) and Folch method (Folch et al., 1957) were evaluated for lipids analysis; the Hach Total Nitrogen method 10071 (HACH Company, 2013) and ASTM D5176-08 (ASTM, 2008; Shimadzu, 2016) were evaluated for protein content analysis; and the Anthrone (Morris, 1948) and Phenol Sulfuric acid (Dubois et al., 1956) method were evaluated for total carbohydrates analysis of samples. All methods were tested over a range of concentrations of standards to verify sensitivity and reproducibility. Following that, the methods were applied to analyze lipids, proteins and carbohydrates content in WAS and foodwaste. Moreover, to evaluate if the complex matrix of WAS and foodwaste interfered with analyte detection and recovery, the samples were spiked with known concentrations of standards, and% recovery was determined.

2.1.1. Lipids analysis

The main differences between the Bligh and Dyer and Folch method are the ratios and volume of solvent used during the lipid-extraction procedure. While the Bligh and Dyer method employs a chloroform-to-methanol ratio of 1:2 (v/v), the Folch method employs a 2:1 (v/v) ratio. In determining the% lipid, both the Bligh and Dyer and Folch methods were evaluated using palmitic acid (> 99% pure lipid, Sigma Aldrich) as a standard. The sensitivity and accuracy of the methods were tested for 1% (w/w), 5% (w/w), 10% (w/w), 25% (w/w), 50% (w/w) and 100% (w/w) total lipid mixtures of palmitic acid and water. The method was also tested on WAS and foodwaste samples individually and spiked with 30 mg of the palmitic acid to determine recovery and matrix interferences.

2.1.1.1. Bligh & dyer lipids extraction procedure. 1 g of the standard (i.e., palmitic acid) or sample (i.e., WAS and foodwaste) was used in all analyses conducted in triplicates. To this, 3.75 mL (1:2 v/v) chloroform: methanol was added and vortexed for 2 min. Then, 1.25 mL of chloroform was added and vortexed for another 2 min. Finally, 1.25 mL of deionized water was added and vortexed for 1 min. The sample was then centrifuged to enable separation of the organic and aqueous layer. A known volume of the bottom organic layer (chloroform extract) was recovered carefully using a Pasteur pipette.

2.1.1.2. Folch lipids extraction procedure. For the Folch method, a 20 mL (2:1 v/v) chloroform: methanol mixture was added to 1 g of sample and vortexed for 2 min. The sample was then centrifuged to recover the liquid phase, to which 4 mL of 0.9% NaCl solution was added and vortexed briefly. The sample was further centrifuged to enable separation of the organic and aqueous layer, and a known volume of the organic layer was recovered as previously described for the Bligh and Dyer procedure.

2.1.1.3. Gravimetric analysis. Following extraction, a known volume of the chloroform extract (aliquot) was placed into a pre-weighed (W_1) aluminum pan (Fisher Scientific). The extract was allowed to dry at room temperature until a constant weight (W_2) was obtained. Percent lipid was estimated as:

$$\%Lipids = \frac{(W_2 - W_1) \times Volume\ of\ organic\ layer}{Aliquot\ volume\ \times\ Sample\ weight} \times 100$$

2.1.2. Protein analysis

The Hach Total Nitrogen method (10071) and the ASTM D5176-08 using a Shimadzu Total Nitrogen analyzer were evaluated for protein analysis, following published protocols (ASTM, 2008; HACH Company, 2013; Shimadzu, 2016). Bovine serum albumin (Sigma Aldrich) was used as a standard for these tests. Different concentrations of the standard were prepared and analyzed for its total nitrogen concentration, and the protein content was estimated to be 16% of the total nitrogen concentration (Jones, 1941)

Protein content in WAS and foodwaste samples was analyzed in a similar manner. These samples were also spiked with 64 mg/L and 56 mg/L bovine serum albumin, respectively, and the results obtained from spiked vs. unspiked samples were compared for recovery and matrix interferences.

2.1.3. Total carbohydrate analysis

According to literature, the two most common methods for total carbohydrate analysis are the phenol-sulfuric acid and anthrone methods. The former was tried, but ultimately not used because of health and environmental toxicity (Albalasmeh et al., 2013; Noyes et al., 2014). Therefore, a detailed procedure for the anthrone method is presented here.

It should be noted that although conventionally 0.2% anthrone solution is used (Morris, 1948), in this study a modified 0.1% anthrone solution (50 mg anthrone dissolved in 50 mL of 95% conc. H_2SO_4) was found to yield more consistent results. The method was tested on WAS and foodwaste samples, as well as those spiked with 100 mg/L of D-glucose (Fisher Scientific) standard solution.

2.1.3.1. Standard calibration curve. Since the anthrone method is a colorimetric technique that develops a green color complex that can be measured at 630 nm, a five-point calibration curve was developed by transferring 0.2, 0.4, 0.6, 0.8 and 1 mL of the 100 mg/L standard solution into a series of test tubes. Deionized water was added to bring the volume in each tube to 1 mL to yield final concentrations of 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L and 100 mg/L. To each tube containing a known standard, 3 mL of 0.1% freshly prepared anthrone solution was added, vortexed for 3 s, and heated at 100 °C for 5 min. The standards were then cooled in an ice bath for 5 min, and the absorbance of the greenish color developed was measured at 630 nm wavelength using a spectrophotometer. All analyses were carried out in triplicates and absorbance was measured within 10 min of color development. A standard curve was then developed using the absorbance obtained

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