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# Evaluating the toxicity of food processing wastes as co-digestion substrates with dairy manure



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### ABSTRACT

Studies have shown that including food waste as a co-digestion substrate in the anaerobic digestion of livestock manure can increase energy production. However, the type and inclusion rate of food waste used for co-digestion need to be carefully considered in order to prevent adverse conditions in the digestion environment. This study determined the effect of increasing the concentration (2%, 5%, 15% and 30%, by volume) of four food-processing wastes (meatball, chicken, cranberry and ice cream processing wastes) on methane production. Anaerobic toxicity assay (ATA) and specific methanogenic activity (SMA) tests were conducted to determine the concentration at which each food waste became toxic to the digestion environment. Decreases in methane production were observed at concentrations above 5% for all four food waste substrates, with up to 99% decreases in methane production at 30% food processing wastes (by volume).

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# 1. Introduction

Anaerobic digestion is a biotechnology utilized for the treatment of organic wastes and the production of biogas, which can be used as a fuel for heating or co-generation of electricity and heat. In addition to renewable energy production, the utilization of anaerobic digestion technology in place of traditional manure lagoon/storage systems results in a number of other benefits, including: (1) improved water quality, (2) decreased odor and fly propagation, (3) reduced greenhouse gas emissions, and (4) effluent solids (Archer and Kirsop, 1990; Powers et al., 1999; USEPA, 2004; Clemens et al., 2006; Lansing et al., 2008).

Anaerobic digestion of animal manure has been widely demonstrated and researched. Digesters can generate income from the sale of electricity, digested fibers, and carbon credits. However, the return on investment from dairy manure digesters is not always favorable, especially for small to medium-scale dairy systems (<500 dairy cows), partly due to the relatively low biogas yield of dairy manure compared to other types of organic wastes, such as food waste (Klavon et al., 2013). One approach for improving the economics of dairy digesters is to increase biogas production by co-digesting manure with more degradable wastes, such as food waste, which often bring additional income in the form of a tipping fees received for accepting and treating off-farm food waste in the digester (Klavon et al., 2013). Food waste can be added to the dairy manure digesters provided that (1) appropriate off-farm wastes are available in the vicinity of the dairy farm, (2) the farmer's land is capable of incorporating the additional nutrients and salts from the off-farm wastes, and/or (3) a reduction in off-farm fertilizers is achieved in order to comply with the farmer's approved nutrient management plan. While much of the solids (30–70%) associated with off-farm food wastes can be degraded during digestion, the total nutrient concentrations (TN, TP) are largely unchanged during digestion (El Mashad and Zhang, 2010; Moss, 2012). When considering co-digestion with off-farm wastes, the type and ratio of food waste used in the co-digestion process needs to be carefully considered in order to prevent a reduction in methane production due to an adverse digestion environment.

A wide variety of food waste substances have been reported to be inhibitory to the anaerobic digestion processes. A material may be considered inhibitory when it causes an adverse shift in microbial populations or inhibition of microbial growth. Inhibition is usually indicated by a decrease in methane production rates and accumulation of organic acids (Kroeker et al., 1979). Ammonia can be produced to inhibitory levels during digestion as organically-bound nitrogen present in food waste, manure, and urea readily mineralize during digestion releasing ammonia (Zeeman et al., 1985; Hashimoto, 1986; Kayhanian, 1994; Krylova et al., 1997; Hansen et al., 1998). It has been suggested that ammonia







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toxicity can be avoided if an optimal digester pH is maintained (6.8–7.2), and the ammonia–nitrogen concentration remains below 3 g/L (Geradi, 2003). Additional studies have shown methane inhibition with NH<sub>4</sub>-N concentrations above 4.9 g/L, with 100% inhibition above 8 g/L (Sterling et al., 2001; Sung and Liu, 2003).

For example, meat processing wastes have high concentrations of grease, blood, feces, and recalcitrant organic matter such as straw and hair, which can lead to the accumulation of ammonia and long-chain fatty acids (LCFAs) as the protein and lipids are degraded, which inhibits the methanogens (Salminen and Rintala, 1999). Cirne et al. (2007) increased the concentration of lipids from 5% to 47% of the chemical oxygen demand (COD) of the manure substrate and found similar methane production rates for 5%, 10% and 18% lipids, with inhibition at 31%, 40% and 47% lipids, based on percent COD. Lansing et al. (2010) found a 50% reduction in the specific methane yield ( $m^3 CH_4/kg VS/day$ ) when increasing the volumetric concentration of used cooking grease co-digested with swine manure from 2.5% to 10%, with no significant increase in methane production (L/day) with increasing grease concentrations, even with the increased volatile solids (VS), with increases in cooking grease concentrations. Food wastes also frequently contains high concentrations of biocides and disinfectants, such as hypochlorite (Tritt, 1992). Tritt (1992) suggests that the difficult nature of these wastes could be overcome by manure co-digestion, which can result in improved C/N ratio, higher alkalinity, and dilution of the inhibitory compounds.

There is a wide range and concentration of inhibition factors in food processing wastes, making it difficult to determine effective and useful co-digestion substrates. The anaerobic toxicity assay (ATA) was developed by Owen et al. (1979) as a screening method to determine the level at which a specific substrate causes an adverse effect on predominant methanogens, providing with useful screening information prior to continuous bench-scale or full-scale implementation. The purpose of this study was to determine the potential toxicity of various food waste substrates using the ATA method in order to determine acceptable inclusion limits and give valuable results to agricultural waste managers and researchers on the processes used to select potential food waste substrates for codigestion.

# 2. Methods

# 2.1. Sample collection

Food processing waste samples were taken from four food processing waste influents received weekly (or bi-weekly) by the Kilby dairy farm in Rising Sun, MD, USA for digestion in a covered lagoon dairy manure digester. The food processing wastes were from the manufacturing of cranberry sauce (CS), ice cream (IC), chicken fat for marinades (CF), and meatball fat from frozen food processing (MB). Samples were collected at the farm before being added to the digester, and brought to the laboratory on ice.

## 2.2. Sample characterization

The food waste and manure samples were analyzed within 24 h for pH, COD, total solids (TS) and volatile solids (VS), according to Standard Methods (APHA et al., 2005). The pH of the sample was measured directly with an Accumet Basic AB 15 pH Meter. For TS analysis, triplicate 10.0 ml samples were placed in crucibles that were pre-weighed and pre-dried to 550 °C. The crucibles were dried at 105 °C until a constant weight was maintained for TS concentration. Subsequently, the crucibles were placed in a furnace oven at 550 °C for one hour and weighed to determine VS concentration. COD concentration was determined using the EPA-approved Hach<sup>®</sup>

8000 Method for High Range COD utilizing a Hach<sup>®</sup> DRB200 digester and DR5000 spectrophotometer.

#### 2.3. Specific methanogenic activity test (SMA)

Specific methanogenic activity (SMA) tests were developed by de Zeeuw (1984) and Sørensen and Ahring (1993) to characterize available inoculum sources prior to incubation. SMA tests were utilized to determine the best inoculum source for the subsequent ATA testing. The inoculum sources collected and tested were (1) flushed manure at the Kilby dairy farm, (2) digester effluent from the covered lagoon at the Kilby dairy farm, which currently co-digests manure with food waste, and (3) inoculum from inside a dairy manure digestion system at the USDA Beltsville Agricultural Research Center (BARC) in Beltsville MD, USA, which does not co-digest with food waste. The samples were transported to the laboratory on ice and analyzed within 24 h of collection using SMA methods of Sørensen and Ahring (1993).

The SMA test determined accumulated methane in triplicate serum bottles (70 ml) spiked with acetate over a 48-h test period. Triplicate bottles without the acetate substrate were included as controls. Triplicate test bottles were filled with 50 ml of the respective test inoculum source and either 2 ml of sodium trihydrate acetate salt (30 mmol/L) to serve as the easily degradable substrate for the three inoculum sources (9 total bottles) or 2 ml deionized water to serve as the inoculum controls (9 bottles), resulting in 18 bottles tested. The bottles were purged with a mix of 30%  $CO_2$ and 70% N<sub>2</sub> to ensure anaerobic conditions, sealed with a butyl rubber stopper and aluminum crimp and placed on a platform shaker at 120 rpm (Innova 2300, New Brunswick Scientific) inside a 35 °C environmental chamber. Gas sampling began three hours after incubation and was collected every three hours for the first 24 h and three times during the second and third day of the 72-h test period. Biogas production was measured via volume displacement using a 50-ml wetted glass, gas-tight graduated syringe with 2 ml gradations. The methane content of the biogas was determined using a TCD gas chromatography (Agilent 7890A-GC) with an injection temperature of 250 °C, a detector temperature of 250 °C, and helium as the carrier gas at a flow rate of 8.6 ml/min. The TS and VS concentrations of each inoculum substrate were determined in triplicate prior to incubation. The pH of each bottle was measured at the beginning and end of the experiment.

#### 2.4. Anaerobic toxicity assay (ATA)

ATAs were developed by Owen et al. (1979) to determine the level at which a substrate causes an adverse effect on the predominant methanogens by introducing increasing levels of a potential toxicant to multiple assays containing inoculum and a standard feedstock, such as glucose or acetate. The standard feedstock provides an excess of easily degradable substrates during the assay period. A control, containing only inoculum and the standard feedstock, is assayed at the same time. The ATA protocol states that the standard feedstock and inoculum volume are kept constant, while the volume of potential toxicant is increased to determine the level at which toxicity occurs (Owen et al., 1979; International Standard ISO 13641-1, 2003; Moody et al., 2011). Methane production from the standard feedstock in the controls is then compared to methane production from the standard feedstock with the potential toxicant to determine the percent inhibition (or non-inhibition) of methane production from the standard feedstock at each concentration of the potential toxicant (Moody et al., 2011). As suggested by Moody et al. (2011) and ISO (2003), the period of the ATA study is limited (3-6 days) in order to isolate methane inhibition of the readily available feedstock (acetate + inoculum) without substantial

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