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# Influence of carbon-to-nitrogen ratio on the mixed-acid fermentation of wastewater sludge and pretreated bagasse

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ABSTRACT

In mixed-acid fermentation, carbon and nitrogen are critical nutrients for cell synthesis, growth, and metabolism. To study the effect of C/N ratio on the yield of carboxylic acids, wastewater sludge was co-digested with pretreated bagasse; the amount of sludge was varied from 0% to 100% (dry weight basis). Fermentation was performed at 55 °C at a solids concentration of 50 g dry solids/L, and Iodoform was used to inhibit methane formation. It was observed that C/N ratio significantly affects yield, especially at extreme ratios. The highest carboxylic acid yield (0.36 g acids/g VS fed) was obtained for C/N ratios ranging from 13 to 25 g C/g N. C/N ratio also affected the composition profile of carboxylic acids. In all mixtures, acetic acid was the major fraction, followed by butyric acid. However, *i*-butyric, valeric acid, and *i*-valeric acid increased with increasing sludge content, which likely resulted from protein degradation.

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## 1. Introduction

For all microbial processes, the right balance of nutrients is essential. In the case of anaerobic fermentations, the lack of nutrients can cause problems such as slow reaction rates, unstable processes, reduced product yield, and possible digester failure (Kayhanian and Rich, 1995; Yen and Brune, 2007). To overcome these problems, supplemental nutrients (e.g., magnesium sulfate, potassium phosphate) are generally added to the feedstock; however, nutrient addition can be expensive, and can reduce the cost-effectiveness of a plant (Kadam and Newman, 1997). Alternatively, co-digestion is an effective and economical method that seeks to optimize nutrient balance (e.g., carbon-to-nitrogen ratio) thereby enhancing product yield.

### 1.1. Co-digestion of wastewater sludge

Co-digesting mixed substrates offers more ecological, technological, and economic advantages than fermenting a single substrate. It involves combining two or more different types of waste with the aim of improving the efficiency of anaerobic digestion (Álvarez et al., 2010). The objective of co-digestion is to balance several parameters: macro- and micronutrients, C/N ratio, pH, inhibitors/toxic compounds, biodegradable organic matter, and dry matter (Hartmann et al., 2004). Additional benefits of co-digestion include synergistic effect of microorganisms, increased load of biodegradable matter, and better yields (Sosnowski et al., 2003). Sludge is a nutrient-rich substrate with high nitrogen content, but low in biodegradable carbon. To enhance digestion efficiency, sludge is usually combined with carbon-rich substrates, such as paper, municipal solid waste (MSW), or bagasse.

Co-digesting wastewater sludge with other substrates has been mainly studied in the context of anaerobic digestion for methane production. In most studies, municipal solid waste (MSW) was used as the carbon-rich substrate because MSW is generated in large amounts and is deficient in nutrients. For instance, Kayhanian and Rich (1995) conducted a pilot study to assess the biodegradable organic fraction of municipal solid waste (BOFMSW) as a substrate in high-solids anaerobic digestion. The results indicated that typical BOFMSW in the United States is deficient in most macro- and micro-nutrients required for robust and stable digestion; hence, wastewater treatment plant sludges have been used as nutrient source. Pilot-scale experiments performed by Kayhanian and Rich (1995) found that adding sludges as supplemental nutrients for MSW increased digester performance. A subsequent study showed that nutrients from the added sludge stabilized the digestion process. Kayhanian and Rich (1996) found that compared to digestion of sludge alone, commingled BOFMSW and primary sludge gave the highest gas production rates and was least likely to have reactor upsets. Furthermore, Hamzawi et al. (1998) studied the technical feasibility of anaerobic co-digestion of MSW and

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sewage sludge. A mixture of 25% MSW and 75% sewage sludge yielded the highest biogas production, but the study did not identify C/N ratio as a process variable. Demirekler and Anderson (1998) showed that adding sewage sludge to the organic fraction of MSW (OFMSW) decreased imbalances on start-up. A ratio of 80:20 of OFMSW to primary sewage sludge gave the highest specific gas production rate. Sosnowski et al. (2003) found that a mixture of sewage sludge (75 vol.%) and OFMSW (25 vol.%.) produced twice the biogas obtained from sewage sludge only. Sosnowski et al. (2008) performed a kinetic investigation of methane co-fermentation using sewage sludge and OFMSW. Adding co-substrates improved the buffering capacity of the fermentation broth. The highest biogas yield was obtained for 75% sewage sludge and 25% OFMSW by volume, which was better than mixed sludge (primary sludge and thickened excess activated sludge 1:1 vol.) or OFMSW only.

### *1.2. Importance of carbon-to-nitrogen ratio in fermentation*

All microorganisms require minerals for robust growth. Nutrients required by bacteria have been categorized as either macro- or micro-nutrients. Kayhanian and Rich (1995) summarized the function of macro- and micro-nutrients on the growth and metabolism of anaerobic bacteria, and stated that for proper bacterial metabolism and a stable anaerobic digestion, these nutrients must be present in the correct ratios and concentrations in the substrate. Macro-nutrients required by anaerobic bacteria are carbon, nitrogen, phosphorous, potassium, and sulfur; micro-nutrients include cobalt, copper, iron, molybdenum, nickel, selenium, tungsten, and zinc. Among these nutrients, organic carbon and inorganic nitrogen are critical for cell synthesis, growth, and metabolism in both aerobic and anaerobic biological decomposition processes, such as aerobic composting and anaerobic digestion (Kayhanian and Tchobanoglous, 1992).

Bacteria are commonly represented as $C_5H_7O_2N$, in which nitrogen comprises approximately 12% of bacterial cell mass. Nitrogen is used to synthesize new cells, including the synthesis of proteins, enzymes, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) (Parkin and Owen, 1986). Carbon is used as an energy source and to form new cells. Carbon-to-nitrogen ratios (C/N) ranging from 15 to 70 (on a mass basis) have been used for aerobic composting and anaerobic digestion (Kayhanian and Tchobanoglous, 1992). However, C/N ratios above or below the ideal can lead to problems.

A high C/N ratio is deficient in nitrogen and slows the digestion rate because there are insufficient cells to maintain active microbial biomass. A low C/N ratio has a high nitrogen content and can evolve ammonia, which is toxic to microorganisms and can completely halt the fermentation. Kayhanian and Tchobanoglous (1992) state that the optimum C/N ratio for anaerobic digestion is from 25 to 30 based on biodegradable carbon and nitrogen mass. Kim et al. (2006) studied the performance of a pilot-scale $H_2$ fermenter for different C/N ratios (20, 25 and 30), and found that C/N ratios higher than 20 g carbohydrate COD/g TKN decreased the digester performance and were accompanied by increased lactate and propionate production. Lin and Lay (2004) studied the effect of C/N ratio on fermentative hydrogen production by mixed microflora. They concluded that hydrogen production by anaerobic microflora in sewage sludge depended on the influent C/N ratio. The highest yield was obtained at a C/N ratio of 47, where the carbon concentrations were measured as g COD/L. Liu et al. (2008) studied the effects of initial carbon–nitrogen ratio on the bioconversion of volatile fatty acids from sewage sludge. Their results indicated that initial C/N ratio was one of the most important factors that influenced the yield and the distribution patterns of volatile fatty acids (VFAs). Maximum product yields (total VFAs-COD) and butyric acid were obtained from sludge mixtures with the highest initial C/N ratio of 15.1, compared to C/N ratios of 12.22 and 5.01 g C/g N for the other sludge mixtures.

For the MixAlco™ process, Holtzapple et al. (1999) uses 80% municipal solid waste and 20% sewage sludge. The same ratio of substrate mixtures has been used in studies by other researchers for example Domke et al. (2004) (80% paper/20% biosludge), Aiello-Mazzarri et al. (2005) (80% municipal solid waste/20% sewage sludge), and Fu and Holtzapple (2010) (80% lime-treated bagasse/20% chicken manure) among others. However, these studies did not quantify the C/N ratio of the substrate mixtures and did not relate C/N ratio with product yields. Furthermore, other studies on C/N ratio that were reviewed do not converge on a single optimum C/N ratio, but instead different optima have been suggested. Inconsistency of units further renders comparison difficult. The objective of this study is thus directed towards a better understanding of the influence of C/N on mixed-acid fermentation.

In this study, carbon-rich sugarcane bagasse and nutrient-rich sludge were blended to complement each other and increase the overall efficiency of the digestion process. Bagasse was chosen because it is rich in carbohydrates (hemicellulose, cellulose, and lignin) but low in nutrients, while sludge contains high amount of proteins and other nutrients thereby complementing each other. The aims of the study were to (1) study the effect of initial C/N ratio on the yields of carboxylic acids, and (2) determine the effect of C/N ratio on the composition profile of carboxylic acids.

## 2. Methods

### *2.1. Substrates*

Bagasse was obtained from Rio Grande Valley Sugar Growers, Inc. (Santa Rosa, TX, USA). Based on sieve test, bagasse particles were measured (by weight) to be 0.3% fine material (less than 0.18 mm), 42.4% between 0.18 and 2.0 mm, 42.8% between 2.0 and 4.75 mm, and 21.6% longer than 4.75 mm. To increase digestibility, long term lime pretreatment procedure recommended for high-lignin content biomass (Kim and Holtzapple, 2006) was adopted. The raw bagasse, as received, was mixed with 0.3 g $Ca(OH)_2$/g dry biomass and distilled water and pretreated at 50 °C for 8 weeks in the presence of air. The pretreated bagasse was then allowed to dry in the air until constant mass. The final moisture content of the bagasse was 0.06 g $H_2O$/g wet biomass (SD ± 0.07), and the volatile solids content was 0.84 g volatile solids/g dry solids (SD ± 0.31).

Mixed sewage sludge, comprising of primary and secondary sludge, was obtained from Burton's Creek Treatment Plant (Bryan, TX, USA). The sludge was centrifuged for 25 min at 4000 rpm to increase the solids content of the sludge. The centrifuged sludge contained 0.87 g $H_2O$/g wet biomass (SD ± 0.95) and 0.62 g volatile solids/g dry solids (SD ± 1.79). The sludge was used without any pretreatment because a previous study showed that lime-treating sludge negatively affects yield of carboxylic acids (Rughoonundun et al., 2010).

Table 1 shows the characteristics of lime-treated bagasse and mixed sludge. The C/N ratio is based on total carbon and total nitrogen as a percentage of the total dry mass. It was calculated according to Eq. (1), which is the most common method for determining C/N ratio (Kayhanian and Tchobanoglous, 1992).

$$\text{C/N ratio} = \frac{\text{total carbon mass}}{\text{total nitrogen mass}} \qquad (1)$$

### *2.2. Methods*

#### *2.2.1. The fermentor*

The fermentor (Fig. 1) was assembled from a 1-L polypropylene centrifuge bottle (98 × 169 mm), Nalgene brand NNI 3120-1010. The centrifuge bottle was capped with a rubber stopper that had

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