LITERATURE REVIEW: ANAEROBIC DIGESTION FOR FRUIT AND VEGETABLE PROCESSING WASTES

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Anaerobic Treatment Applications

Anaerobic digestion is most widely established as a waste treatment method in Western Europe (Angelidaki et al., 1999). Regulations there require strict abatement of greenhouse gases including methane and carbon dioxide (Carucci et al., 2005). As a treatment technology, anaerobic digestion is unique in that it both reduces waste and produces an energy resource in the biogas. Potential anaerobic feed substrates include manure (Al-Masri, 2001; Demirer and Chen, 2005), crop residues (Stewart et al., 1984; Weiland, 1993), organic municipal solid waste (i.e. solid food wastes) from restaurants or markets (Bouallagui et al., 2003; Han et al., 2005; Xu et al., 2002; Kiely et al., 1996), and wastewater from food processing plants (Tekin and Dalgic, 2000; Alvarez et al., 2005; Lepisto and Rintala, 1997; Viswanath et al., 1992).

Microbiology

There are four processing steps defined in anaerobic digestion, each attributed to different trophic groups. The first three steps are hydrolysis, acidogenesis, and acetogenesis, which are each performed by different bacterial groups. The final step is methanogenesis, performed by a type of archaea aptly termed methanogens (Bouallagui et al., 2004a).

In studying anaerobic digestion it is important to recognize that methanogens are archaea are yet often mislabeled as bacteria. Archaea make up a domain separate from bacteria and eukarya, and include methanogens, thermophiles, and halophiles (Schiraldi et al., 2002). Woese and Fox (1977) distinguished the domain by noting that archaea evince stark biochemical and genetic differences from bacteria.

Anaerobic bacteria have unique optima for pH and nutrient levels from methanogens, and exhibit different growth kinetics and stress responses (Bouallagui et al., 2004a). Methanogens are generally more sensitive than bacteria to environmental fluctuations. It is common for anaerobic digesters to manifest an unstable methanogen population: methane production may fluctuate significantly while bacterial volatile fatty acid (VFA) productions remain consistent (Bouallagui et al., 2005). The rate-controlling step is often acetogenisis though, as evidenced by VFA accumulation (Speece, 1996; Bouallagui et al., 2005).

Optimal environmental needs include pH and alkalinity, temperature, macronutrients (C/N/P), bioavailable micronutrients, and a lack of toxicity (Speece, 1996). Adequate conditions for all parameters must exist for a digester to flourish, as discussed below.

pH and Alkalinity

Optimal alkalinity and pH, between 6.5 and 8.2 (Speece, 1996), are critical for stable methanogensis, and yet confounded by the proceeding process steps that produce CO_2 and other acids. If acetogens are slowed by environmental stressors, then acids go unprocessed and pH will quickly drop, inhibiting methanogenesis entirely (Bouallagui et al., 2005; Speece, 1996). To counter this acidifying effect, alkalinity in the digester must be balanced with VFA levels relative to organic loading rate and hydraulic retention times (HRT) (Borja et al., 2004). A two-stage digester system was found to quell rising acid levels (Bouallagui et al., 2005). Biodegradation can also be limited by high pH though (Penaud et al. 1999), as ammonia is un-ionized above a pH of 8 and thus toxic (Speece, 1996).

Temperature

Anaerobic digestion can occur in three distinct temperature ranges: psychrophilic at 15°C to 25°C, mesophilic at 30°C to 37°C, and thermophilic at 50°C to 65°C (Bouallagui et al., 2004b; Speece, 1996). (It should be noted that thermophilic digestion is unrelated to the thermophile archaea that inhabit more extreme locales such as geysers and ocean heat vents). A distinct transition region between mesophilic and thermophilic digestion is evinced around 45°C where methane production notably decreases (Converti et al., 1999; Speece, 1996). Methanogens are also sensitive to abrupt temperature change (Bouallagui et al., 2004b). A 5°C drop resulted in a 34% decrease in methane production (Speece, 1996).

Thermophilic biomass has a higher metabolic rate than mesophilic and thus higher gas production (Speece, 1996; Converti et al., 1999), and protein assimilation (Speece, 1996). Thermophilic systems also exhibit greater pathogen elimination (Bouallagui et al., 2004b; Duran, 2006). Hydrogenotrophic methanogens exhibited improved activity with higher temperatures (Converti et al., 1999). The optimal temperature for acetoclastic methanogens was found to be 56 to 59°C (Speece, 1996).

Thermophilic digestion requires disproportionately higher metal nutrients (Speece, 2006). Thermophilic biomass has a significantly slower growth rate than mesophiles and consequently a longer start-up period (Speece, 1996). A two-stage system with thermophlic and mesophilic in series utilized the advantages of both (Bouallagui et al., 2005).

A digester's temperature range is often selected to compliment the climatic region, as to avoid excessive heating demands (Bouallagui et al., 2004b). It follows then that as Europe is the prevailing region in anaerobic research and development and of a temperate clime, mesophilic systems are most commonly studied and pursued (Borja et al., 2004; Penaud et al., 1999;

Al-Masri, 2001; Bouallagui et al., 2003). An underlying reason may also be that acetogens and methanogens are equally active at a mid temperature range (Speece, 1996); acetogen activity dominates at lower temperatures, where methanogens are more active a higher temperatures.

Nutrient Availability

Methanogenesis is very sensitive to nutrient availability. Basic macronutrients and a library of micronutrients must be properly allotted and biologically available (Speece, 1996). Like most metabolic processes, anaerobic digestion requires an adequate C:N:P ratio, where carbon is accounted for in terms of COD at times. One source considered fruit and vegetable waste to be balanced with a COD/N/P of 100/4.3/0.9 (Bouallagui et al., 2004b). Nitrogen or phosphorus deficiency may be expressed by VFA accumulation (Speece, 1996). Ammonium is commonly used as a measure of digester performance (Carucci et al., 2005). Nitrogen concentrations must generally be above 40 to 70 mg/L NH_4-N (Speece, 1996).

Micronutrient availability can play a pivotal role in anaerobic digestion. When a food waste lacking in trace metals was co-digested with municipal sludge abundant in iron, manganese, copper and zinc, significantly more methane was produced than from food waste alone (Carucci et al., 2005). Low methane production initially attributed to toxicity may often be amended by trace metal addition, particularly iron, cobalt and nickel (Speece, 1996). To prevent such limiting conditions in batch digesters and bench scale studies, researchers commonly provide bulk nutrient solutions with a host of trace metals (Owen et al., 1979; Shelton and Tiedje, 1984; DiStefano and Ambulkar, 2006; Aquino and Stuckey, 2007). Iron, cobalt, nickel, zinc, copper, manganese, molybdenum, selenium, tungsten and boron have been shown to stimulate methanogenesis (Speece, 1996; Speece, 2006).

Among required nutrients, sulfide is especially noteworthy. While microbes require sulfide, it readily precipitates with various trace metals (especially iron), rendering all biologically unavailable (Speece, 1996; Isa et al., 1986). Sulfur also plays a role in the multifaceted competition between methanogens and sulfate reducing bacteria, which produce hydrogen sulfide in lieu of methane (Speece, 1996). Hydrogen sulfide gas is toxic to both anaerobes and humans. Sulfate and free sulfide are recognized as methanogen inhibitors at higher concentrations (Isa et al., 1986).

Toxicity

Anaerobic digestion can be hindered by certain compounds. At high concentrations, potassium and other salts can interrupt cell function (Carucci et al.; 2005; Speece, 1996). Sodium chloride was found to inhibit methanogenic activity (Dolfing and Bloemen, 1985). It was shown however, that in adding sodium hydroxide, the hydroxide anions caused the negative effect, not the

sodium cations (Penaud et al., 1999). Surfactants were shown to cause greater inhibition of anaerobic treatment than aerobic treatment (Mohan et al., 2006). As mentioned in section 2.3, ammonia is un-ionized above pH 8 and is toxic to anaerobes. Acetogens are particularly sensitive, and concentrations above 3000 mg/L NH_4-N are toxic irregardless of pH (Calli et al., 2005). While not necessarily 'toxic', increased total solids loading have a direct correlation with methanogen inhibition (Viswanath et al., 1992; Carucci et al., 2005; Tekin and Dalgic, 2000). General toxicity can be determined by an Anaerobic Toxicity Assay, based on a reduced gas production rate despite an abundance of acetate (Owen et al., 1979; Speece, 1996).

Long-chain fatty acids (LCFAs) and polyphenols are natural antimicrobial compounds. In multiple studies of different phenol-rich substrates, anaerobic digestion was drastically inhibited unless some form of pre-treatment was employed, such as ozonation (Alvarez et al., 2005), fungal treatment (Dhouib et al., 2006), or electro Fenton reaction (Khoufi et al., 2006). Adding sulfate during treatment can also improve phenol biodegradability (Speece, 1996; Isa et al., 1986), though the negative affects of sulfate must then be abated. It is important to note that while a microbe population may express inhibition upon initial exposure to a compound, anaerobes have a strong ability to acclimate (Speece, 1996). Acclimation to LCFAs could not be achieved and the inhibiting affect was irreversible (Angelidaki and Ahring, 1992).

Methane Production Estimation

Simple anaerobic digestibility is often reported in terms of percent COD removal. Further testing is often desired to determine the biological methane potential**,** reported as volume methane produced per unit COD**.** Batch tests are commonly conducted using some variation of the Owen serum bottle method (Owen et al., 1979; Demirer et al., 2000; Han et al., 2005; Shelton and Tiedje, 1984). Based on preliminary batch test results, studies are often expanded to a semi-continuous bench scale reactor (Carucci et al., 2005; Hwang and Cheng, 1991). There are respirometers available for anaerobic applications. In anaerobic mode, a respirometer measures the individual gas production from multiple flasks, each approximately 500 ml in volume (Mohan et al., 2006). This combines the benefit of test controls and replicates from the serum bottle methods with the continuous gas monitoring of bench scale reactors (DiStefano and Ambulkar, 2006). In a hydrogen production study, the anaerobic respirometer produced 43% more gas than the Owen serum bottle method (Logan et al., 2002).

Many studies use synthetic substrates to observe the affects of specific parameters on anaerobic digestion (Converti et al., 1999; Conklin et al., 2006); Isa et al., 1986; Fitzgerald, 1996). However, to study wastewater biodegradability in the lab, substrate must be imported from the original processing waste stream. Processing wastewaters often include a

conglomeration of food wastes, disinfectants, anti-scaling and other chemicals, the interactions of which cannot be synthetically replicated.

Digester Start-up, Operation and Acclimation

Anaerobes have a relatively slow growth rate compared to their aerobic counterparts. Organic substrate must be processed through several steps before reaching simple carbon compounds appropriate for methanogenesis. Thus, a reactor of any size may require several weeks before significant methane production is observed. A two-liter lab reactor digesting pig slurry produced minimal biogas for the first 25 days of testing (Kiely et al., 1997). Start-up time is greatly improved when the reactor is seeded with adequate inoculate from an acclimated seed source (Totzke, 2006). Improved reactor performance, e.g. biomass retention and methane production, has been correlated with higher hydraulic retention time (HRT) (Han et al., 2005; Bouallagui et al., 2003; Viswanath et al., 1992).

Different methanogen species express distinct responses to environmental stimuli and stressors. Hourly versus daily feeding schedules were shown to select for methanogens with low and high growth rates, respectively (Conklin et al., 2006). The microbes with a higher growth rate made for a more stable digester, responding better to peak loads and feed shortages. Similarly, biogas production is influenced by organic loading, total solids and HRT (Viswanath et al., 1992; Tekin and Dalgic, 2000). HRT is of greater concern for a substrate with a high non-soluble fraction (Tekin and Dalgic, 2000). Switching feedstock between different fruit wastes evinced little effect on biogas production (Viswanath et al., 1992). This is significant for food processing applications as plants often switch seasonal commodities throughout the year. Table 1 includes a brief comparison of various sized reactors and feed sources and their subsequent methane production.

Biogas Products

Methane and carbon dioxide are the main constituents of anaerobic biogas. The methane fraction can range from 40 to 85% depending on methanogen vitality, substrate quality and reactor type (Alvarez et al., 2005; Stewart et al., 1984; Tekin and Dalgic, 2000; Yacob et al., 2006). A large percentage of the carbon dioxide remains in solution (Shelton and Tiedje, 1984). Being mostly methane, biogas can be collected and burned, like natural gas, for thermal or electric energy. In Denmark, community digesters produce both heating potential for local farms and electricity to the power grid (Angelidaki et al., 1999). Several engine types are adapted to run on biogas, and emerging technology in high-temperature fuel cells can convert and store energy from biogas (Bove and Lunghi, 2006; Sorge, 2006). The anaerobic digestion process can also be manipulated to produce a higher percentage of hydrogen gas, a sought-after resource for fuel cells (Han et al., 2005; Logan et al., 2002).

There are also minor gas constituents which can be troublesome for certain applications. Biogas often includes a small fraction of hydrogen sulfide produced from sulfate-reducing bacteria (Speece, 1996). The specific percent fraction depends on the substrate components. Digestion of citric acid factory wastewater with 600 mg/L sulfate resulted in the biogas having 4% H_2S (Isa et al., 1986). Internal combustion engines are especially sensitive to hydrogen sulfide impurities (Speece, 1996) which can be expensive to purge (Isa et al., 1986).

Table 1 Biogas Production from Various Commodities and Reactor Types

Reference	Commodity	Reactor Type/ Size	Temperature	Biogas Production	Methane fraction
Bouallagui et al. 2004b	Raw fruit & vegetable waste (shredded)	Tubular/ 18L	Psychrophilic Mesophilic Thermophilic	0.64-1.05 l/l/d 0.83-2.34 l/l/d 1.7 -3.17 l/l/d	56-58% 54-65 % 58-62%
Alvarez et al. 2005	Cherry stillage	Sequencing Batch Reactor / 1.8 L	Low mesophilic (30°C)		58-71%
Stewart et al. 1984	Bananas (fruit and stem) Potatoes (peelings, rejects) Oats	Continuous/ 20 L	Mesophilic	497 L/kg TS 350-410 L/kg TS 227-257 L/kg TS	53% 44-50% 51-54%
Yacob et al. 2006	Palm oil mill effluent	Closed digester/ 500 m^3	High mesophilic (37-42°C)	650-1000 kg/d	
Tekin and Dalgic 2000	Olive pomace	Semi-continuous/ 1 L	Mesophilic	0.39-0.69 l/l/d	79.5-84%
Viswanath et al. 1992	Sequential feedings: mango, orange, pineapple, tomato processing, jackfruit and banana waste	Semi-continuous/ 45 L	Low mesophilic (30°C)	0.61-1.96 l/l/d	22-61.2%
Lepisto and Rintala 1997	Carrot processing wastewater. Potato and swede processing wastewater	UASB/ 2-3 L	Thermophilic (55°C)	7.3 l/l/d (315 cm^3/g COD) 347 cm^3/g COD	49% (carrots)

Economic and Environmental Considerations

There are several economic considerations in choosing anaerobic treatment. While primary attention goes to methane as an energy source, those with experience in the field have found equal or greater benefit in the stabilized sludge end product (Tekin and Dalgic, 2000). There is also the opportunity to apply for carbon credits through such organizations as the Chicago Climate Exchange, for actively reducing methane and carbon dioxide emissions (Jensen, 2006).

There are various operating costs associated with an anaerobic digester. If the influent substrate has a low pH or alkalinity, an alkalinity source must be metered in to support a neutral pH. To maintain digester stability, it is necessary to extensively monitor various parameters. There has been recent research to develop in-line infrared monitoring equipment to limit the over-sizing of digesters and lower capital and operating costs (Spanjers et al. 2006). There had been some trouble of the monitoring equipment clogging with higher solids content though.

Advantages and Disadvantages

Anaerobic treatment allows for higher organic loading rates and requires lower nutrient levels than traditional aerobic treatment (Speece, 1996). There is no aeration required, resulting in lower operating costs. While an anaerobic digester requires heating to maintain a mesophilic (or thermophilic) environment, this can often be met by reapplying 25% of the biogas as a heat source. Overall, anaerobic treatment can provide an energy resource while reducing a waste product. Due to the anaerobe's low growth kinetics, an anaerobic reactor can remain viable in a dormant state during periods of low flow or plant shut-down.

There are disadvantages to anaerobic wastewater treatment. It does not achieve significant nutrient treatment such as denitrification or phosphorus removal (Speece, 1996). If effluent discharge regulations require lower limits than can be met by anaerobic treatment, secondary or tertiary treatment such as aerobic polishing or phosphorus treatment would need to be considered. While a low growth rate can provide some advantages, it also equates to a slow start-up period and arduous recoveries from organic overloading or biomass washout.

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