

# **Microbial Community in Waste Management Process of Anaerobic Digestion**

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## **Abstract**

Microorganisms have always been part of the human life both intrinsically and extrinsically. They are ubiquitous and have been a tool for genetic engineering, biotechnology and waste management. Anaerobic digestion is a process that is made possible by specific group of microorganisms which changes substrates into biogas, a renewable energy source. This paper aims to show the microbial community responsible for each stage of hydrolysis, acidogenesis, acetogenesis and methanogenesis in the anaerobic digestion (AD) process. Understanding the microorganisms involved in AD and how process parameters affect them is key in the optimization of the AD process for a high methane yield and as a tool for waste management.

## **Keywords**

Anaerobes, Anaerobic Digestion, Methanogens, Microbial community, Waste Management

## **1. Introduction**

Over hundreds of years, the process of anaerobic digestion (AD) has been in use. The use of biogas was first recorded in Syria during the 10th century b.c.e. Afterwards, 17th-century scientist Jan Baptist van Helmont concluded that decaying organic matter could turn into flammable gases. This idea was expanded by Count Alessandro Volta and he established a correlation between the amount of matter and the amount of gas produced. The first anaerobic digestion plant was built in Bombay (now Mumbai), India in 1859; after which more anaerobic digestion plants were built towards the end of the 19th century in England, where the fuel produced was used to light street lamps. Ever since then, anaerobic digestion plants have been built all over the world with improvement in its technology. (Fogle, R., 2016). Biotechnology of biogas generation is the degradation of different kinds of organic wastes, industrial (food) agricultural waste, wastewater, sewage sludge and organic fraction of municipal solid wastes (OFMSW) with the aid of microorganism under optimum process conditions. Anaerobic digestion culminates into biogas production, which entails conversion of the energy in biomass fuel. It aids in the recycling of organic wastes into stable liquid fertilizer and energy which helps to mitigate hazardous outcome of waste in the environment (Zieminski, K. and Frac, M., 2012). The resultant product of AD is biogas (both methane and carbon dioxide), and gases such as hydrogen sulfide and hydrogen. It is a process that involves different kinds of microorganisms. The intrinsic worth of anaerobic digestion includes low sludge production, increased organic loading rates, production of methane and reduced energy consumption. Other benefit of anaerobic digestion is the use of the slurry as fertilizer. The anaerobic process does not reduce the nutrient content of the slurry and thus making the fertilizer nutrient rich and a suitable replacement for chemical fertilizers. AD is also a waste

management tool which helps to reduce economic land area occupied by waste through degradation of the waste and helps prevent pollution of the environment and outbreak of diseases due to pathogenic organisms arising from poor waste management practice. Another major advantage of the AD process is its ability to resolve environmental waste management problems. AD aids in the stabilization of high strength wastes such as, domestic wastes, wastewater biosolids, industrial wastes, food processing residuals, and animal manure. In contrast, aerobic treatment requires aeration and more energy would be needed to generate the oxygen to oxidize the waste, thus, AD is more cost-effective for waste treatment and the heat required for the AD process is derived from the process. Also, the digestates are usually almost pathogen free, depending on the temperature and hydraulic retention time. Another way the AD process serves as a waste management tool is in the lowering of greenhouse gas emissions. With manure stored in open structure on livestock farms, AD occurs naturally resulting in the release of methane gas. If this gas is collected and flared, producing carbon dioxide, greenhouse gas evolution is reduced because carbon dioxide is 21 times less potent than methane (Safferman et al, 2012). In the light of environmental sustainability, AD is a renewable energy source and a waste stabilization technique (Nielsen et al, 2007). Anaerobic digesters help to reduce greenhouse gas emissions and create nutrient rich digestates that are effective fertilizers (Nielsen et al, 2009). AD combines both biochemical and physiochemical processes namely hydrolysis, acidogenesis, acetogenesis and methanogenesis to bring about energy generation and waste management.

## **2. Phases of Anaerobic Digestion and its Microbial Communities**

### **2.1 Hydrolysis**

Hydrolysis is a reaction that engages enzyme for the conversion of long chain organic matter such as lipids, polysaccharides, protein, nucleic acids and fats into soluble and smaller organic molecules that can be utilized by microorganisms in the fermentation stage. The soluble compounds formed are amino acids, simple aromatics, fatty acids, monosaccharides, and other short chain compounds (Myint et al., 2007). Hydrolytic bacteria are phylogenetically varied, and most of these are found in two main phyla, Bacteroidetes and Firmicutes (Venkiteshwaran et al, 2015). Hydrolysis is propelled by the extracellular enzymes secreted as by products of the microorganism active in this stage. (Yang et al., 2006). Some microorganisms can breakdown different kinds of organic polymers while some are specific in the likes of saccharolytic and proteolytic microorganisms which break down sugars and proteins respectively. Strict anaerobes like clostridia and facultative bacteria such as streptococci are involved at this stage of anaerobic digestion. Hydrolysis is a vital step before the fermentation stage, in that fermentative bacteria are unable to take in complex organic polymers into their cells. Examples of enzymes involved in hydrolysis are cellulase, cellobilase, xylanase and amylase which breakdown carbohydrates into sugars, protease degrades protein into amino acids, and lipase aids in the degradation of lipid into glycerol and long-chain fatty acids (LCFA) (Parawira et al., 2005). Enzyme production, diffusion, adsorption, reaction, and enzyme deactivation are stages that occur during hydrolysis (Batstone et al., 2002). The size, volume, shape, and surface area of the organic material, enzyme production, biomass concentration and adsorption have a great influence on the rate of the reaction at this stage.

### **2.2 Acidogenesis**

In acidogenesis, the monomers from hydrolysis are broken down to simpler compounds of volatile fatty acids (VFA) (e.g. acetic acid, propionic acid, and butyric acid), carbon dioxide, hydrogen and alcohol. This is made possible by various facultative and obligatory anaerobic bacteria like *Pseudomonas sp.*, *Bacillus sp.*, *Clostridium*, *Micrococcus sp.* and *Flavobacterium sp.* These resulting components are the products of anaerobic respiration of acidogenic biomass. In summary, simple sugars, fatty acids and amino acids are transformed into organic acids and alcohols. Also, hydrogen concentration produced in this stage as an intermediate product determines the kind of ultimate product produced in the fermentation stage in that if the partial pressure of the hydrogen is increased, then there is a consequent reduction in the amount of reduced compounds.

## The Biochemistry

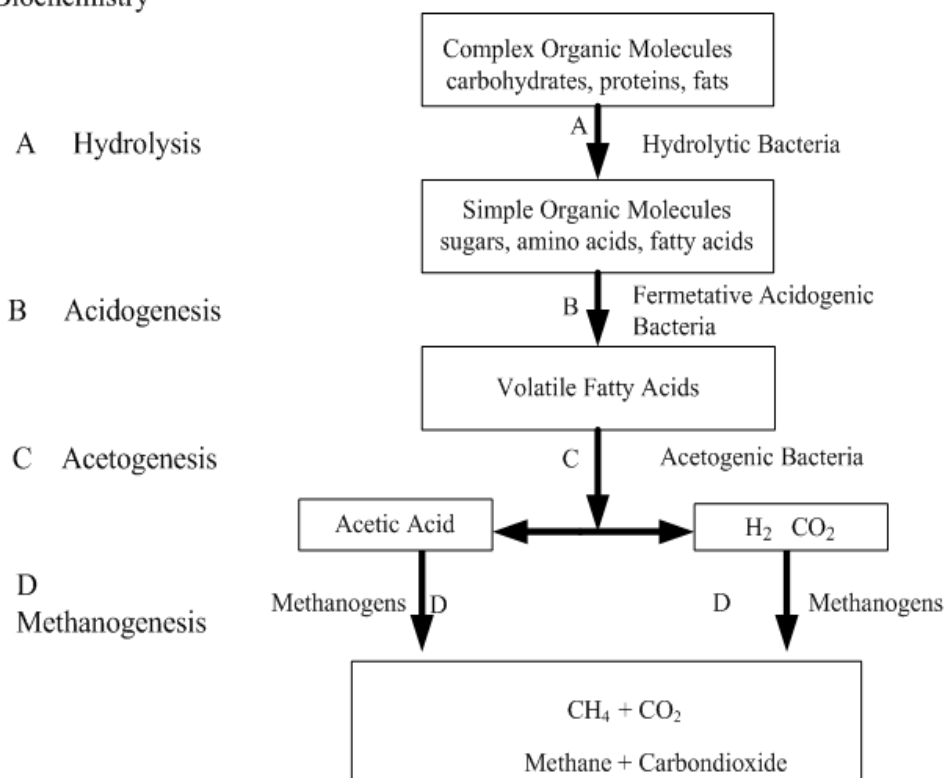


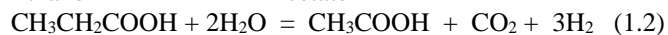
Figure 1. Anaerobic Digestion Process

### 2.3 Acetogenesis

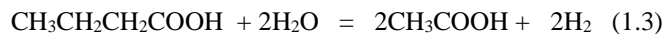
In this process, the acetate bacteria (genera of *Syntrophomonas* and *Syntrophobacter*) change the acidogenesis products (ethanol, propionic acid, butyric acid) into acetates and hydrogen to be used by the bacteria in the methanogenesis stage. *Methanobacterium suboxydans* degrades pentanoic acid to propionic acid and *Methanobacterium propionicum* degrades propionic acid to acetic acid. Hydrogen is also released and this is toxic on the microbial community in this stage. To solve this problem there is a symbiosis of acetogenic bacteria with autotrophic methane bacteria which uses hydrogen, thus, the hydrogen is mopped up to prevent toxicity of the microbial community. Most importantly, this stage determines the efficiency of biogas production because the acetate produced is the precursor for methane formation by a reduction process. (Fayyaz et al., 2014). The symbiotic inter -species hydrogen transfer solves the problem of toxicity at this stage. Products of acidogenesis are converted to acetate by acetogenic bacteria according to the following reactions:



Ethanol                      Acetate



Propionic acid                      Acetate



Butyric acid                      Acetate

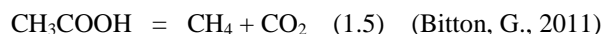
## 2.4 Methanogenesis

This is the concluding stage of AD in which methanogenic bacteria are active in the production of methane and this happens by the conversion of intermediate products (acetic acid, carbon dioxide, Hydrogen, etc.) into methane and carbon dioxide. Heterotrophic methane bacteria convert acetic acid into methane, autotrophic methane bacteria reduce carbon dioxide in the presence of hydrogen to produce methane. The bacteria in the domain archaea are generally involved in the production of methane. Methanogenic bacteria have a limited temperature resistance because of their enzymatic structures; thus, temperature is an important parameter for their functionality (Zeikus, 1977). Methanogens are classified into three groups:

a. Hydrogenotrophic methanogens (i.e. hydrogen using chemolithotrophs) use hydrogen or formate to reduce carbondioxide into methane. Most of the methanococcales and methanobacteriales use  $H_2$  and  $CO_2$ .



b. Acetotrophic methanogens also called the acetoclastic or acetate-splitting methanogens converts acetate into methane and  $CO_2$ .



c. Methylotrophic methanogens produce  $CH_4$  from methyl compounds such as methanols, methylamines, methylsulfides (Liu and Whitman, 2008). Considering a municipal anaerobic digester, 70% of the methane is derived from acetate and the rest from hydrogen and carbondioxide, with a very small quantity of methane generated by methylotrophic methanogens (Ferry, 1993). Hydrogenotrophic methanogens are crucial for the AD process because of their ability to use hydrogen and keep the partial pressure low. *Methanobacterium*, *Methanobrevibacter*, *Methanoculleus*, *Methanospirillum* and *Methanothermobacter* are examples of hydrogenotrophic methanogens involved in the AD process in Anaerobic digesters (Leclerc et al., 2004). The genera *methanosaeta* and *methanosarcina* are acetoclastic methanogens. *Methanosaeta* are obligate acetoclastic methanogens which uses acetate or direct electron transport as the substrate or electron donor and has a comparable slow growth but a high affinity for acetate and thus prominent at low acetate concentration (Liu and Whitman, 2008). While *Methanosarcina* are facultative acetoclastic methanogens which use hydrogen, carbondioxide or one carbon compound, in addition to acetate; apart from its varied options of substrate, they possess a higher growth rate and lower affinity for acetate, thus it dominates over *Methanosaeta* in digesters, when the concentration of acetate is high. ( Leclerc et.al., 2004).

## 3. Parameters Affecting Microbial Community in Anaerobic Digestion

Substrate composition is very imperative for anaerobic microorganisms in the whole process; this also determines the stability of the process and gas production. Carbon to nitrogen ratio plays an important role in the decomposition of organic matter, thus anaerobic process has been shown to be improved by using substrates from different sources (Pages et al, 2011). Yadvika et al., 2004 reported that co -digestion of substrates produces more gas than using a single substrate. Methanogens show higher diversity at mesophilic temperature ( $37^\circ C$ ). A downward shift in temperature to psychrophilic values changes the population from acetoclastic to hydrogenotrophic methanogens (Liu and Whitman, 2008). Higher concentrations of volatile fatty acids lead to a drop in pH and a shift from the optimum pH of 7 to 8 and a consistent increase of VFA's inhibits methanogenesis. Also, inoculum to substrate ratio is another factor that determines the size of microorganism available for degradation in the anaerobic process; a non-optimum ratio leads to high concentration of VFA's in the process. Process parameters affect the stability of the AD process, biogas generation and stabilization of the pollutants.

### 3.1 Temperature ( $T^\circ C$ )

AD process is usually carried out at either mesophilic (optimum  $T^\circ C$  of  $35^\circ C$ ) or thermophilic (optimum  $T^\circ C$  of  $55^\circ C$ ) temperatures. Temperature drives the rate of the reaction of the whole process. Optimum thermophilic temperature allows for higher organic loading rate and destruction of pathogens. Temperature changes affect the growth of methanogens. In utilization of volatile acids by methanogens, a decrease in temperature causes a decrease

of the maximum specific growth rate ( $\mu_{max}$ ), whereas the half - saturation constant  $K_s$  increases (Lawrence and McCarty, 1969). Thus, mesophilic digesters must be designed to operate at a temperature of 30 -- 35 ° C for their optimal functioning.

### **3.2 pH**

Most methanogens function optimally at a pH range of 7.0 -- 7.2, a reduction to a pH about 6.0 can lead to a process failure. Bacteria in the acidogenic phase generate organic acids, which decreases the pH of the bioreactor and this reduction is buffered by bicarbonate produced by methanogens. In harsh environmental conditions, the buffering capacity of the system can be disturbed and this can lead to the stop of methane production. Acidic conditions hinder the functionality of methanogens compared to acidogenic bacteria. An increase in volatile acids level provides an early indicator of system upset. Monitoring the ratio of total volatile acids (as acetic acid) to total alkalinity (as calcium carbonate) has been suggested to ensure that it remains below 0.1. One method for restoring the pH balance is to increase alkalinity by adding chemicals such as lime, anhydrous ammonia, sodium hydroxide, or sodium bicarbonate (Bitton, G., 2011).

### **3.3 Inoculum Substrate Ratio (ISR)**

Inoculum is a medium of bacteria ingested into a digester to start a biological action and in this case to aid the anaerobic digestion process in the biodegradation of the substrate. Another term for the introduction of inoculum into the AD process is called seeding. Inoculum for the AD process can be obtained from slurry from waste water treatment plants, pig manure, chicken or cattle manure or sewage sludge from previous anaerobic digestion process. The ratio of introduction of the inoculum to the substrate is referred to as inoculum substrate ratio (ISR). Zeng et.al, 2015 published that an increase in biogas production was recorded as the ISR value decreased. The collective biogas production after 30 days of digestion for the ISR value of 2.0, 1.0 and 0.5 were 70.38, 126.57 and 153.51mL, respectively. In the same vein, the percentage of methane in total biogas volume was increased as the ISR decreased. The percentages of methane in the biogas were 35.92%, 38.74% and 45.19% with ISR value of 2.0, 1.0 and 0.5, respectively.

### **3.4 Organic Loading Rate (OLR)**

The amount of solid added into the digester per unit volume per day is the organic loading rate and it is denoted as OLR kg VS/L. d. Zingashina et al, 2015 investigated the organic loading rate increase at a constant HRT of 35 days in anaerobic digestion of ammonium rich substrate (chicken waste and cattle manure) in a mesophilic continuously stirred tank laboratory reactor. He reported that increasing the OLR from 1.0 to 3.5gVSL<sup>-1</sup>d<sup>-1</sup>, accompanied by ammonia, VFA and increase in pH, the proportion of all dominant Bacteroidales phylotypes decreased significantly from about 64 to 21%, while the abundance of the Firmicutes phylotypes increased from around 16 to 16% of the pyrotags. Thus, bacteria in an AD system respond differently to the organic loading rate.

### **3.5 Hydraulic Retention Time (HRT)**

The hydraulic retention time is affected by wastewater characteristics and environmental conditions and it must be long enough to allow metabolism by anaerobic bacteria in the digester. Aramrueang et al, 2016, reported a greater biogas and methane yield was achieved with longer HRT. The highest biogas (0.502 l [g VS]<sup>-1</sup>) and methane (0.342 l [g VS]<sup>-1</sup>) yield was produced when the digester was operated at OLR of 1.0 [g VS]<sup>-1</sup>d<sup>-1</sup> and 25-d HRT. The results show that more substrate conversion into biogas was achieved with longer HRT and lower OLR.

### **3.6 Surface Area/ Particle Size**

Substrate size has an influence on the gas production rate, thus it is imperative that the particle size of the substrate should be reduced to prevent digester clogging and a longer duration of degradation of the substrate by the microorganism. Adequate contact between biomass and substrate is a requirement for hydrolysis because the organisms secreting hydrolytic enzymes profit from adsorption to the surface of particulate substrate. Reduction of substrate particle size through pre- treatment for example grinding will increase surface area presented for adsorption of hydrolytic enzymes and consequently increased biogas production. (Agyeman and Tao, 2014). It was reported also by Agyeman and Tao, 2014, that reduction of food waste particle size from 8 to 2.5 mm increased

methane production rate by 10-28% and specific methane yield by 9-34% in co-digestion of dairy manure and food waste. Dewaterability of digester effluent was significantly improved by reducing food waste particle size.

#### 4. Conclusion

Understanding the biochemical reactions of the microorganism at each stage of anaerobic digestion is key to the optimization of methane yield and gaining the maximum benefit from the AD process as a waste management tool. Process parameters like temperature, pH, organic loading rate, inoculum to substrate ratio play a major role in the effectiveness of anaerobic digestion as a renewable energy generation source and a waste management tool. Though the AD process takes place in an engineering design, understanding the microbial community and how they are affected by process parameters are indispensable to a successful high methane yield from anaerobic digestion, a waste management tool.

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