Chapter 2
Basics of Anaerobic Digestion Process

Abstract Basics of anaerobic digestion process is presented in this chapter. Principal reactions are Hydrolysis, Fermentation Acetogenesis/dehydrogenation, Methanogenesis. The critical step in the anaerobic digestion process is Methanogenesis.

Keywords Anaerobic digestion process · Hydrolysis · Fermentation acetogenesis · Dehydrogenation · Methanogenesis · Acetophilic · Methane bacteria · Hydrogenophilic

In the anaerobic digestion process the organic matter is broken down by a consortium of microorganisms in the absence of oxygen and lead to the formation of digestate and biogas which mainly consist of methane and carbon dioxide. This digestate which is the decomposed substrate resulting from biogas production can be used as a bio-fertilizer (Al Seadi 2001; Kelleher et al. 2000; Chen et al. 2008; Al Seadi et al. 2008). Figure 2.1 shows the anaerobic pathway.

Originally, anaerobic digestion was perceived as a two stage process involving the sequential action of acid forming and methane forming bacteria. Now, it is known to be a complex fermentation process brought about by the symbiotic association of different types of bacteria (Allen and Liu 1998; Edmond-Jacques 1986; Speece 1983; Kosaric and Blaszczyk 1992). The products produced by one group of bacteria serve as the substrates for the next group. The principal reaction sequences can be classified into four major groups involving the following (Fig. 2.1; Table 2.1):

- Hydrolysis
- Fermentation
- Acetogenesis/dehydrogenation
- Methanogenesis

In the first stage i.e. hydrolysis/liquefaction/solubilisation step, large organic polymers such as starches, cellulose, proteins and fats are broken down or

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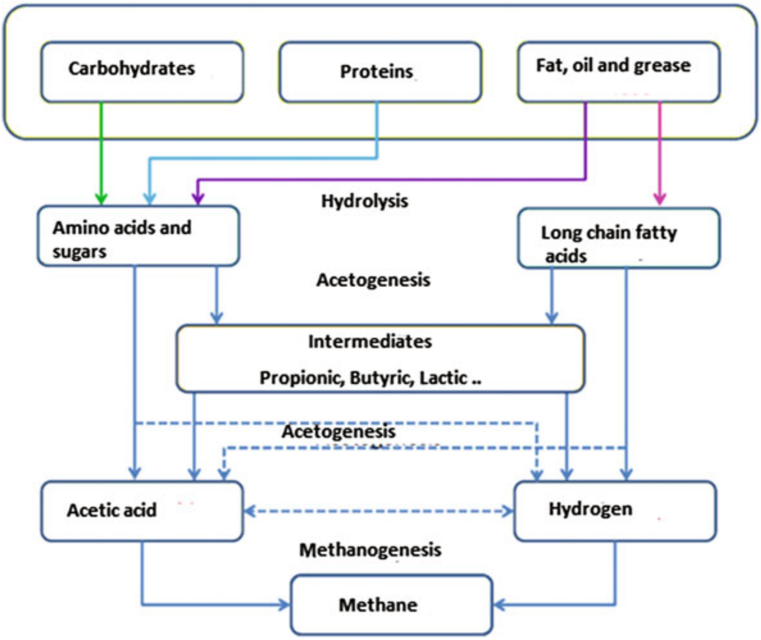

Fig. 2.1 Anaerobic pathway based on Wilson (2014)

Table 2.1 Steps involved in anaerobic oxidation of complex wastes

Hydrolysis $C_6H_{10}O_4 + 2H_2O \longrightarrow C_6H_{12}O_6 + H_2$
Acidogenesis $C_6H_{12}O_6 \leftrightarrow 2CH_3CH_2OH + 2CO_2$ $C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2H_2O$ $C_6H_{12}O_6 \rightarrow 3CH_3COOH$
Acetogenesis $CH_3CH_2COO^- + 3H_2O \leftrightarrow CH_3COO^- + H^+ + HCO_3- + 3H_2$ $C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 2CO_2 + 4H_2$ $CH_3CH_2OH + 2H_2O \leftrightarrow CH_3COO^- + 3H_2 + H^+$
Methanogenesis $CH_3COOH \rightarrow CH_4 + CO_2$ $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ $2CH_3CH_2OH + CO_2 \rightarrow CH_4 + 2CH_3COOH$

Zupančič and Grilc (2012), Biarnes (2013), Ostrem (2004), Bilitewski et al. (1997), Verma (2002), van Haandel and van der Lubbe (2007), EPA (2006)

depolymerized by acidogenic bacteria into sugars, amino acids, glycerol and long chain fatty acids by hydrolytic exo-enzymes (example, cellulase, amylase, protease, and lipase) excreted by fermentative microorganisms (EPA 2006; van Haandel and van der Lubbe 2007). The hydrolysis reaction is presented in Table 2.1.

The fermentative microorganisms consist of both facultative and strict anaerobes (Broughton 2009). In the enzymatic hydrolysis step, the water-insoluble organics can be solubilized by using water to break the chemical bonds (Parawira 2004) and the resulted simple soluble compounds can be used by the bacterial cells (Gerardi 2003). While some products from hydrolysis such as hydrogen and acetate may be used by the methanogens in the anaerobic digestion process, the majority of the molecules, which were still relatively large, must be further converted to small molecules example acetic acid, so that they may be used to produce methane (Biarnes 2013). Hydrolysis is a relatively slow step and it can limit the rate of the overall anaerobic digestion process, especially when using solidwaste as the substrate (van Haandel and van der Lubbe 2007).

Hydrolysis is immediately followed by the acid-forming step—acidogenesis (Ostrem 2004). In this step, the organics are converted by acid-forming bacteria to higher organic acids such as propionic acid and butyric acid and to acetic acid, hydrogen and carbon dioxide. The higher organic acids are subsequently transferred to acetic acid and hydrogen by acetogenic bacteria. It is always not possible, to draw a clear distinction between acetogenic and acidogenic reactions. Acetate and hydrogen are produced during acidification and acetogenic reactions and both of them are substrates of methanogenic bacteria. The acidogenic and acetogenic bacteria belong to a large and diverse group which includes both facultative and obligate anaerobes. Facultative organisms are able to live in both aerobic and anaerobic environment and obligate are species for which oxygen is toxic. Species isolated from anaerobic digestors include *Clostridium*, *Peptococcus*, *Bifidobacterium*, *Desulfovibrio*, *Corynebacterium*, *Lactobacillus*, *Actinomyces*, *Staphylococcus*, *Streptococcus*, *Micrococcus*, *Bacillus*, *Pseudomonas*, *Selemonas*, *Veillonella*, *Sarcina*, *Desulfobacter*, *Desulfomonas* and *Escherichia coli* (Kosaric and Blaszczyk 1992). Characteristic of wastewater determine which bacteria predominate.

The hydrogen gas formed in acetogenesis step can be regarded as a waste product of acetogenesis because it inhibits the metabolism of acetogenic bacteria; however, it can be consumed by methane-producing bacteria functioning as hydrogen-scavenging bacteria and converted into methane (Al Seadi et al. 2008).

In the final reaction, methane is produced by methanogenic bacteria. These bacteria are capable of metabolizing formic acid, acetic acid, methanol, carbon monoxide, and carbon dioxide and hydrogen to methane. The methanogenic bacteria are crucial to anaerobic digestion process since they are slow growing and extremely sensitive to the changes in the environment and can assimilate only a narrow array of relatively simple substrates. Some of the notable species that have been classified are *Methanobacterium formicicum*, *M. bryantic* and *M. thermoautotrophicum*; *Methanobrevibacter ruminantium*, *M. arboriphilus* and *M. smithii*; *Methanococcus vannielli* and M. *voltae*; Methanomicrobium mobile; *Methanogenium cariaci* and *M. marinsnigri*, *Methanospirilum hungatei* and *Methanosarcina barkei* (Kosaric and Blaszczyk 1992). Two-third of the methane produced during anaerobic microbial conversion is derived from methyl moiety of acetate and about one-third is derived from carbon dioxide reduction. The methanogenic step is the point at which the organic pollution load, in terms of chemical oxygen demand or biochemical oxygen demand is significantly reduced by the anaerobic process since the preceding stages merely convert the organic matter from one form to another. Thus, efficient methanogenesis equates directly with efficient removal of carbonaceous pollution therefore anaerobic wastewater treatment processes have been designed and are operated primarily to satisfy the requirements of this group of bacteria.

Methanogenesis is a critical step in the entire anaerobic digestion process, and its biochemical reactions are the slowest in comparison to those in other steps (Al Seadi et al. 2008). Methane-producing bacteria are strict anaerobes and are vulnerable to even small amounts of oxygen. The methane-producing bacteria can be subdivided into two groups: acetoclastic methane bacteria (acetophilic) and methane bacteria (hydrogenophilic). Another group of methane-producing bacteria is the methyltrophic bacteria which is also able to create methane from methanol (Paul and Liu 2012; Gerardi 2003).

In anaerobic processes where inorganic sulphur is constituent of the wastewater, the sulphate-reducing bacteria—*Desulfovibreo* are also of importance. Sulphate and/or sulphite is present in most effluents from acid sulphite, neutral sulphite semichemical (NSSC), Kraft, chemimechanical (CMP) and chemithermomechanical pulp mills and where aluminum sulphate is used as a sizing agent for paper production. The sulphur-reducing bacteria use sulphate and sulphite as electron acceptors in the metabolism of organic compound to produce hydrogen sulphide and carbon dioxide as end products. Sulphur reduction can become a significant factor in the performance and operation of pulp and paper anaerobic treatment systems. The hydrogen sulphide produced can be both toxic and corrosive. The sulphur reducing and the methane bacteria use and compete for the same organic compounds, reducing methane yield per unit of substrate removed. The methanogenic step is often the most critical one. Disturbance often result in an inhibition or depression of methane formation followed by an excess formation of fatty acids. A small part of the degraded organic matter is converted into new cellulose material. The sludge production rate is low compared with aerobic processes. This means that the sludge retention time must be relatively long if a sufficient amount of biomass is to be obtained in the system. A certain amount of biomass is required for high treatment efficiencies and a stable process.

An obligate, syntrophic relationship exists between the acetogens and methanogens. Synthrophy is the phenomenon that one species lives off the products of another species. The hydrogen partial pressure should be very low so that the thermodynamics become favorable for conversion of volatile acids and alcohols to acetate. Under standard conditions of 1 atm of hydrogen, the free energy change is positive for this conversion and thus precludes it. For example, the free energy change for conversion of propionate to acetate and hydrogen does not become negative until the hydrogen partial pressure decreases below 10^{-4} atm. This relationship has been shown by McCarty (1982). Therefore, it is obligatory that the hydrogen-utilizing methanogens maintain these extremely low hydrogen partial pressures in the system or else, the higher volatile acids, such as propionic and butyric acid, will accumulate in the system. Fortunately, the hydrogen utilizing methanogens in this physiological partnership are adept at this and normally perform this service with ease to allow the reaction to proceed efficiently all the way to methane production. This phenomenon of interspecies hydrogen transfer, which is important to anaerobic biotechnology is a very interesting symbiosis discovered by Brynt et al. (1967).

It is common for the bacterial population concentration to be higher than 1016 cells/ml in case of a well-functioning anaerobic digester (Amani et. al. 2010). This population is typically made of saccharolytic, proteolytic and lipolytic bacteria and methanogens (Gerardi 2003). Of these organisms, the methanogens are known to be highly sensitive to their environment in terms of temperature, pH, and the concentrations of certain chemical compounds (ammonia, volatile fatty acids) (Manser 2015). These are also the slowest growing organisms in the anaerobic digestion reactor. Methanogens are totally dependent on the acetogens and acidogens to survive, as these two organisms convert simple monomers produced during the hydrolysis step into volatile fatty acids and then into acetic acid, carbon dioxide and hydrogen (lipolytic) to supply the methane production process. This relationship is symbiotic as methanogens maintain the digester environment by consuming the protons and volatile fatty acids produced during acidogenesis and acetogenesis, which otherwise would become inhibitory to the biodegradation process.

The supply of hydrogen is often the limiting step in methane production in anaerobic digestion systems (Gerardi 2003). Currently, several research projects are being performed to optimize this aspect of anaerobic digestion system design and operation. Another limiting step in the production of methane is the accumulation of volatile fatty acid in the reactor produced during the acidogenesis step. This balance can be difficult to manage on a large scale because acidogens and acetogens continuously produce compounds that reduce the pH of the system below the preferred range of 6.4–8 for methanogens if sufficient buffering capacity is not available (Speece 1996; Rittmann and McCarty 2001). This type of inconsistency can promote ineffective biogas production in reactors which do not have strict control over the operating environment. Overall, the methanogens sensitivity to the reactor environment also creates an ideal setting for microorganisms, including some pathogens, to survive and possibly multiply during their residence in the system.

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