The Influence of Microbial Community Dynamics on Anaerobic Digestion Efficiency and Stability: A Review

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ABSTRACT. An essential component in sustainable energy development is the production of bioenergy from waste. The most successful bioenergy technology worldwide is anaerobic digestion (AD), which is a microbially-mediated process of organic feedstock conversion into energy-rich compounds (volatile fatty acids (VFA) and biogas) for renewable energy generation. AD is deployed in a range of situations including systems for on-farm energy recovery from animal and plant waste to the processing of food and municipal solid waste (with the additional benefit of land-fill reduction). Anaerobic digesters rely on a diverse microbial community working syntrophically through a series of interrelated biochemical processes. Each stage in anaerobic digestion is carried out by different microbial groups. Thus, to optimise energy recovery from the AD process, the microbial community must have stable performance over time, balancing the various metabolic functions and taxonomic community composition in digesters. Complicating this balance, it has been found that the presence of ammonia, sulphate, and hydrogen sulphide in substantial concentrations often cause failure in the AD process. Thus, these substances cause adverse shifts in microbial community composition and/or inhibit bacterial growth, that influencing AD performance. ©2020. CBIORE-IJRED. All rights reserved

Keywords: Biogas, Methane, Sustainable Energy, Anaerobic Digestion, Microbial community

Article History: Received: September 14, 2019; Revised: December 12, 2019; Accepted: January 16, 2020; Available online: February 15, 2020

How to Cite This Article: Amekan, Y., (2020) The influence of microbial community dynamics on anaerobic digestion efficiency and stability: A Review. International Journal of Renewable Energy Development, 9(1), 85-95. https://doi.org/10.14710/ijred.9.1.85-95

1. Anaerobic Digestion for Renewable Energy Generation in Indonesia

Currently, the use of fossil fuels continues to increase globally reaching 88% of the total world energy needs (Bharathiraja et al. 2018). BP's Energy Outlook has even indicated that global energy demand will continue to grow to 35% by 2035. In Indonesia, its economic growth rate (5.05% in the first quarter of 2019; BPS – statistics Indonesia 2019) and population growth (1.10% in 2019; BPS – statistic Indonesia 2019) increased the need for fossil fuels as the main energy source. The use of fossil energy reaches 94% (Fig. 1; EBTKE 2016) of Indonesia's total energy consumption. This condition is exacerbated by the realization of reduced oil lifting making Indonesia a net oil importer (since 2003). High oil imports and the price of crude oil reaching $ 60/barrel had a large impact on the trade balance.

Meanwhile, greater global political pressure to reduce carbon emissions occurred due to increasing amounts of greenhouse gas emissions (with carbon dioxide (CO2) as the largest contributor) in the atmosphere as a result of burning fossil fuels. Indonesia’s energy sector greenhouse gas emissions reached 261.89 million tons of CO2 produced mostly by electricity, transportation and industry. This value continues to increase by 2.43% per year over the range 2000-2015. This is caused by the growth in energy consumption which continues to grow 2.35% per year (EBTKE 2016).

Fig 1. Indonesia's primary energy mix in 2015 (EBTKE 2016)

Also, the security of sustainable energy supply becomes a challenge in the global energy market because some oil and gas producing countries are in regions that are politically unstable and even experience military conflicts. Therefore, the utilization of alternative energy sources needs to be developed to ensure sustainable energy development, reduce dependence on fossil fuels and reduce adverse impacts on the environment.

Provision of alternative energy has become a concern of the Indonesian government through the issuance of Presidential Regulation No. 5 of 2006 concerning the

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National Energy Policy to develop alternative energy sources as fuel substitutes for oil (Amekan and Guntoro 2017). New energy sources that are developed must be renewable, environmentally friendly and have high energy yields to minimize the use of fossil-based energy in the total primary energy mix (Zhang, Hu and Lee 2016; Amekan et al. 2018; Bharathiraja et al. 2018; Nathia-Neves et al. 2018). Indonesia targets new renewable energy and bioenergy (biomass-based energy) to contribute 15% of the country’s total energy needs and reduce emissions by 26% by 2020 (EBTKE 2016).

The latest data from the Directorate General of New, Renewable Energy and Energy Conversion (2016) shows that the energy potential so far has been utilized by the government including hydropower, solar, wind and marine energy. The amount of energy potentially produced is more than 300,000 MW. Energy supply generated from new renewable energy sources currently contributes significantly (6.2%) which continues to increase (average 0.36%/year) from the previous year, despite dependence on conventional fuels, such as gas, coal, and oil remains high (Figure 1). Therefore, the higher utilization of new renewable energy technologies is expected to play a role in the realization of reducing greenhouse gas emissions in Indonesia.

Another potential energy that Indonesia has also eyed in developing sustainable energy is the production of bioenergy from waste (animals, plants and domestic). One of the most successful bioenergy technologies throughout the world is anaerobic digestion (AD). The anaerobic digestion process involves microbes (bacteria and archaea) in the process of converting organic material under conditions without oxygen to produce biogas, especially methane, as an energy source for fuel and electricity (Werner et al. 2011; Vanwongterghem et al. 2014). AD can contribute to increasing the proportion of renewable energy based on biomass in Indonesia’s total energy mix. This technology can also be a solution for processing waste materials which reaches 8 million tons per year with an energy potential of 534.73 MW (EBTKE 2016). Currently, there are more than 300 biogas reactors throughout Indonesia that include biomass-based power plant, biogas and municipal solid waste, Bioenergy power plant and household-scale biogas. This number is still far lower than Germany (the highest biogas producing country in Europe) which has more than 8000 active biogas reactors operating and the total biogas produced is equivalent to 4 TW (terawatt) electricity capacity (Aehmas et al. 2017). Another example, in the United Kingdom (UK), the number of AD plants increased almost 50% in 5 years (106 AD plants in 2013 to 607 AD plants in 2018) according to a report from the Anaerobic Digestion & Bioresources Association (ADBA). These biogas plants use various feedstock, like industrial, agricultural, municipal and sewage sludge feedstocks. The biogas production continues to experience rapid development despite global economic pressure and the biogas that now being produced is enough to power over 1 million homes in the UK.

2. Conversion Steps in Anaerobic Digestion

Biogas is a gaseous product (contains 50 – 80% CH₄, 15 – 45% CO₂, 5% H₂O and some trace gases) that produced biologically through anaerobic digestion (AD) process that involved diverse of microbial communities that work syntrophically in supporting a series of interrelated biochemical reactions (Bond andTempleton 2011; Plugge 2017; Calusinska et al. 2018). Those four biochemical functions (Fig. 2; Heeg et al. 2014; Diaz et al. 2018) are (i) hydrolysis of complex polymers into simple soluble products, (ii) acidogenesis (fermentation of simple soluble products into short-chain fatty acids), (iii) acetogenesis (anaerobic oxidation of short-chain fatty acids/volatile fatty acids (VFAs) into acetates and hydrogen), and (iv) methanogenesis (methane production from acetate and hydrogen by methanotrophic/acetoclastic and hydrogenotrophic methanogens).

2.1 Hydrolysis

The complex substrates (polymers) that cannot be directly transported across the cell membrane of microorganisms, such as polysaccharides, lipids and proteins, are hydrolysed by hydrolyses (cellulose, xylanase, pectinase, amylase, lipase, and protease) excreted by hydrolytic bacteria. Hydrolytic bacteria are very diverse phylogenetically. Many studies support findings that Firmicutes and Bacteroidetes although their abundance varies depending on operational conditions are the two dominant phyla in AD that responsible for the breakdown of the polymers, such as Acetivibrio, Clostridium, Bacteroides, and Thermotoga (Phylum Thermotogae), etc. (Liebl 2001; O’Sullivan et al. 2005; Cirne et al. 2007; Zverlov et al. 2010; Strauber et al. 2012; De Vrieze et al. 2015; Hassa et al. 2018). Hydrolytic bacteria have rapid growth and can utilise hydrolysis products as the growth substrate, mainly by fermentation which produces VFAs.

![Diagram: Conversion Steps in Anaerobic Digestion](image)

**Fig 2.** The interrelated biochemical functions in AD (adapted from Wirth et al. 2012).

2.2 Acidogenesis and Acetogenesis

Acidogenesis takes the hydrolytic products and ferments them, creating VFAs (such as acetate, propionate, butyrate, and valerate), carbon dioxide, hydrogen and ammonia. Acidogenic bacteria (acidogens) include hydrolytic bacteria and fermentation bacteria that do not produce extracellular hydrolyses and are therefore reliant upon the hydrolytic bacteria for primary
metabolites. Bacteroidetes, Chloroflexi, Firmicutes and Proteobacteria are dominant taxa that have many species of acidogens. There are a number of non-hydrolytic acidogens that successfully identified so far, including Bifidobacterium (phylum Actinobacteria), Anaeroliniaceae (phylum Chloroflexi), and some thermophilic bacteria belonging to the Thermotogae phylum (Stiles and Holzapfel 1997; Balk et al. 2002; Dong et al. 2000; Yamada et al. 2006; De Vrieze et al. 2015). Acidogenesis progresses rapidly and can lead to VFA accumulation as well as a decrease in pH when given a substrate that is easily digested.

Some end products of hydrolysis and acidogenesis can be used directly by methanogens (acetoclastic and hydrogenotrophic) for biogas production, but other intermediates (such as VFAs and other simple alcohols) are metabolised and converted to the necessary substrates for methanogenesis (acetate, CO₂, H₂).

Syntrophic acetogenesis is the degradation/oxidation stages of intermediates into acetate, H₂, and CO₂. The term syntrophy refers to the symbiosis between acetogenic bacteria with hydrogenotrophic methanogens (de Bok et al. 2001). For example, acetogenesis carried out by Methanobacterium suboxydans (specializes in oxidizing 4-C and 6-C fatty acids to propionate and acetate), and Methanobacterium propionicum (convert propionate to acetate) will release hydrogen (H₂) that exhibits toxic effects to them, so it needs to be directly used by autotrophic methanogens (de Bok et al. 2005).

The oxidation of syntrophic propionate is significant because of almost 30% of the electrons generated from the complex substrate flow through propionate during the anaerobic digestion process (Speece et al. 2006). This stage is thermodynamically unfavourable unless the partial pressure of H₂ is maintained below 10⁻³ atm (McCarty and Smith 1986; Lowe et al. 1993). In anaerobic digesters, hydrogenotrophic methanogens live near syntrophic acetogens and consume the hydrogen released. This syntrophic relationship is based on the transfer of hydrogen from the producing microbes to the hydrogen consumption, called interspecies hydrogen transfer (Boone 1985; Schink 1997; Stams and Plugge 2009), which keeps the H₂ partial pressure low. Syntrophic acetogens found in anaerobic digesters include species in the genus Smithihella, Syntrophobacter, and Pelotomaculum for propionate oxidation (Liu et al. 1999; de Bok et al. 2001) and the genus Syntrophus and Syntrophomonas for the oxidation of butyric and longer chain fatty acids (Jackson et al. 1999; Imachi et al. 2007; Sousa et al. 2007). Syntrophic acetogenesis is a crucial step that determines the stability of anaerobic digester operations because some VFA, especially propionate, potentially inhibit methanogenesis even at neutral pH (Barredo and Evison 1991; Pullamanappil et al. 1998; Demrel and Yenigün 2002; Nielsen et al. 2007). Moreover, the efficiency of biogas production depicted by acetogenesis (approximately 25% acetates and 11% of H₂ is produced) because approximately 70% of CH₄ generated through acetate reduction (Schink 1997).

2.3 Methanogenesis

Methanogenesis is an anaerobic metabolic stage responsible for methane formation. The final stage of anaerobic digestion is carried out by methanogens, commonly called methanoochaeae (phylogenetically belonging to the phylum Euryarchaeota), grouped into methylotrophic and hydrogenotrophic based on the substrates they use to form methane (Thauer et al. 2008). Hydrogenotrophic methanogens are represented by 5 orders (e.g. Methanomicrobiales, Methanospyrales, Methanocellales, Methanococcales, and Methanobacterales) and almost all species depend on CO₂ reduction to CH₄. Hydrogen becomes the dominant compound of electron donors, but several other electron sources can also be used. Methylotrophic methanogen is represented by Methanosarcinales and Methanosaetae. Generally, species belonging to this group have characteristics capable of producing CH₄ from various methyl compounds (-CH₃), or methyl groups on acetate. Hydrogenotrophic methanogens are believed to have lived since antiquity along with the emergence of life on earth, while methylotrophic methanogens began to develop over 500 million years ago (Fournier and Gogarten 2008; Liu et al. 2012; Sousa et al. 2013; Costa and Leigh 2014). Methanogens community changes (diversity and species richness) in the anaerobic digester is not affected by temperature or hydraulic retention time applied, but predominantly afflicted by the composition of the substrate/feedstock, availability of nutrients and ammonia/ammonium contents (Hassa et al. 2018).

The methanogenic pathway of all methanogenic species is essentially the same i.e. converting methyl groups into methane, but the only difference is the source of the methyl group they use as a source of carbon and energy. Most successfully isolated species can reduce CO₂ to methyl by utilizing H₂ or formic acid as a reducing agent. Some other species use CO and a small fraction utilizes short-chain aliphatic compounds as reducing agents (Guneratnam et al. 2017; Zabranaka and Pokorna 2018). Other species obtain methyl groups directly from the substrate, such as acetate, methanol or methylamine. Although most isolates can reduce CO₂, biologically only 30% of methane is obtained from this pathway. The majority (± 70%) comes from converting methyl acetate groups into methane (Ferry 2002; Welte and Deppenmeier 2014).

3. Effect of process parameters and inhibitory substances on microbial communities in the anaerobic digester

Over the last decade, so many researches were conducted to help us understand the efficiency and stability of AD that relies on the syntrophic activity of diverse microorganisms performing hydrolysis, acidogenesis, acetogenesis and methanogenesis. Although AD has been subjected to substantial process engineering, the underpinning microbial community has been treated largely as a ‘black box’ and presents a significant opportunity for additional optimisation. Fortunately, rapid development in sequencing technology (next-generation sequencing (NGS)) that can provide more comprehensive information at a much cheaper cost, making it easier for researchers to identify and understand not only community composition and their metabolic functions but also how operational conditions (such as type of feedstock and temperature) influence microbial system (structure and dynamics) that can be linked to AD performance efficiency and stability (Talbot et al. 2010).
et al. 2008; Nelson, Morrison and Yu 2011; Wilkins et al. 2015; Bocher et al. 2015; De Vrieze et al. 2017; De Vrieze et al. 2018; Kirkegaard et al. 2017; Hardegen et al. 2018).

Each stage in anaerobic digestion is carried out by different microbial groups, and its composition in the consortium depends on several factors, such as the type of substrate, temperature, pH, mixing and applied digester geometry (Yu and Mohan 2001; Insam et al. 2010; Francisci et al. 2015; Nathia-Neves et al. 2018). Therefore, to maintain process stability, it is crucial to keep the balance between the acid and methane forming microorganisms to optimising methane generation from the AD process. Previous studies also demonstrated that the presence of inhibitory substances in substantial concentrations (such as sodium chloride (Zhao et al. 2017), ammonia (Siles et al. 2010), sulphate (Siles et al. 2010), hydrogen sulphide (Hilton and Archer 1988), heavy metals (Dokulilova et al. 2018), and some organic compounds (Chen et al. 2008)) often cause failure in AD process. The inhibitors causing adverse shifts in microbial community composition and/or inhibit bacterial growth that influences AD performance.

3.1 Temperature

There are three major temperature operating ranges applied in anaerobic digester: psychrophilic (4 – 15 °C), mesophilic (20 – 40 °C) and thermophilic (45 – 70 °C) (Kim et al. 2017; Nathia-Neves et al. 2018). The operational temperature has a strong effect on the microbial communities involved in AD which results in huge differences in the types and abundance of microbes carrying out the process (De Vrieze et al. 2015; Kirkegaard et al. 2017). Temperature also affects the reaction thermodynamics, which the high temperature will favour oxidative reactions (i.e. acetate oxidation) while homoacetogenesis becomes more favourable at psychrophilic temperature (van Lier 1995; Schnurer et al. 1999).

3.2 pH

Changes in pH can disrupt cell homeostasis and affects the microbial communities severely (Chen et al. 2008). Hydrolysis can be inhibited at either low or high pH because of the enzyme denaturation (Boon 1994). Hydrolytic bacteria and acidogens can tolerate the changes in pH, but acetogens and methanogens cannot (Amani et al. 2010; Nathia-Neves et al. 2018). The free acids (associated organic acids, H2S) cause inhibition at lower pH, and free bases (NH3) cause inhibition at higher pH values. It causes a change in pH then affect the passive transport of the free acid or base across the cell membrane and subsequent dissociation which later leads to process imbalance (Henderson 1971; Gerardi 2003). The organisms that mostly affected by this inhibition are methanogens (acetoclastic and hydrogenotrophic) and syntrophic acetogens in the digester (Requeiro et al. 2014; Montanes et al. 2014; de Jonge et al. 2017).

3.3 Ammonia (NH3)

Ammonia generated during breakdowns of nitrogen-rich organic feedstocks and can cause inhibition to the anaerobic digestion process through passive diffusion into the microorganism cell then induce proton imbalance and/or potassium deficiency (Kroeke et al. 1979; de Baere et al. 1984; Gallert et al. 1998). Some researcher has proposed the mechanism of ammonia inhibition, such as the change in the intracellular pH, increase of maintenance energy requirements, and inhibition of specific enzyme reaction (Whittman et al. 1995; Rajagopal et al. 2013).

Ammonia is known as an essential substance for anaerobic microorganisms growth if the concentration 50 – 200 mg/L (McCarty 1964; Liu and Sung 2002), but if the concentration reaches 1.7 – 14 g/L can caused 50% reduction in methane production (Sung and Liu 2003; Chen et al. 2008; Chen et al. 2016). The toxicity effect increases as the pH increase (Borja et al. 1996) because the high ratio of ammonium was shifting to its ionised form at higher pH when the concentration 1.5 – 3.0 g/L (Angelidaki and Ahring 1993). If the amount of ammonia exceeds, 3.0 g/L will cause complete inhibition of the AD process at any pH (Prochazka et al. 2012).

Methanogens are the most susceptible group of microorganism due to ammonia inhibition because other groups irrelevant with methanogenesis were enriched as a consequence of ammonia concentration increasing (Chen et al. 2016). Galert et al. (1998) have proposed two mechanisms of ammonia inhibition against methanogens, i.e. direct inhibition on methane-producing enzyme and inducing proton imbalance or potassium deficiency through passive diffusion of hydrophobic ammonia into the cell. According to Koster and Letinga (1988), 56.5% of methanogens lost their activity, and acidogenic bacteria in the granular sludge get severe impact when the concentration of ammonia 4.05 – 5.73 g NH3-N/L. Methanospirillum hungatei is the most sensitive methanogens because it is being inhibited at 4.2 g NH3-N/L, while Methanosarcina barkeri, Methanobacterium thermoautotrophicum, and Methanobacterium formicicum were inhibited at 10 g NH3-N/L (Jarrell et al. 1987; Chen et al. 2008). Chen et al. (2016) show that the hydrogenotrophic methanogens (Methanobacterium and Methanospirillum) were inhibited when the ammonia concentrations exceed 6 g/L. Moreover, it was found that increasing ammonia concentration caused a shift in the methane production process from acetoclastic methanogenesis towards syntrophic acetate oxidation paired with hydrogenotrophic methanogenesis (Chen et al. 2016).

3.4 Sulphate and Sulphide

High levels of sulphate present in the feedstock can cause inhibition to the generation of methane from anaerobic digestion systems. The inhibition occurs because of sulphate reduction always predominates methane production in anaerobic digester treating sulphate-rich feedstock as a consequence of thermodynamic and kinetic differences between the two processes (Abram and Nedwell 1978). Sulphate favours the growth and metabolism of sulphate reducing bacteria (SRB) which are competitors for the methanogens substrates acetic acid and hydrogen (Bryant et al. 1967; Abram and Nedwell 1978; Chen et al. 2008; Vilela et al. 2014). The SRB is more versatile in the range of substrate used than the methanogens and VFA other than acetic acid, propionic for example, can serve as the substrate for certain species of SRB. Furthermore, the Sulphur
reduction products, particularly hydrogen sulfide (H₂S), are also inhibitory to methanogenesis (Hulshof et al. 1998; Lens et al. 1998; Chen et al. 2008; Vilela et al. 2014; Camiloti et al. 2014).

Dealing with hydrogen sulphide in the AD process presents an area of interest to many researchers. Many studies have been conducted to investigate some methods to control sulphate reduction activity in anaerobic digesters to increase methanogenesis (Visser et al. 1993; Chaiprapat et al. 2011; Moestedt, Paledal and Schnurer 2013), precipitation with iron salts (Zhang, Keller and Yuan 2009), off-gas scrubbing (Ravishanker and Hills 1984; Nisimura and Yoda 1997; Mesa et al. 2002) and using SRB inhibitors, such as sodium nitrite (Nemati et al. 2001; Greene et al. 2003) and molybdate (Nemati et al. 2001; Isa and Anderson 2005).

Sodium molybdate (MoO₄²⁻) is a structural analogue of sulphate and known to be an effective inhibitor of sulphate reduction in sediments (Peck 1959; Oremland and Taylor 1978; Nedwell and Banat 1981; Biswas et al. 2009) and it was hypothesised that it could be used to increase methanogenic production from the sulphate-rich waste (Table 1). Additions of 2 – 20 mM molybdate were sufficient to control the growth of SRB and generation of H₂S by SRBs with 85 – 100% of inhibition.

It has suggested previously (Peck 1959; Peck 1961; Biswas et al. 2009) that MoO₄²⁻ plays its vital role, as a competitive inhibitor for sulphate in the ATP sulphurylase, by inhibits ATP sulphurylase (the first enzyme in sulphate activation) through the formation of unstable molecule equivalent to adenyl sulphate (APS). The inhibition causes an appropriate electron acceptor has not generated even though energy (ATP) was consumed. Moreover, the addition of molybdate to inoculums confirmed competition for common substrates between the two bacterial groups.

The molybdate stimulates the enhancement of methane production. According to Abraham and Nedwell (1978), the presence of molybdate can inhibit the hydrogen consumption and made it available for hydrogenotrophic methanogen to use it as sources of the electron for CO₂ reduction to methane. However, molybdate addition also shows some detrimental effects on methane production. Smith and Klug (1981) shows that high concentration of molybdate (200 mM) can cause 50% inhibition on methane production. Moreover, Zabedi et al. (2014) also found that low concentration of molybdate (2.5 mM) can affect methanogenesis (Table 1).

### Table 1.
The use of sodium molybdate in different concentrations to inhibit sulphate reduction.

| Inoculum                          | Molybdate conc. | % inhibition of sulphate reduction | % inhibition of methane production | Ref.                                      |
|-----------------------------------|-----------------|------------------------------------|-----------------------------------|------------------------------------------|
| Sediments of a shallow eutrophic lake | 0.2 mM          | 100                                | 14                                | Smith and Klug, 1981                     |
|                                   | 2 mM            | 100                                | 9                                 |                                          |
|                                   | 20 mM           | 100                                | 20                                |                                          |
|                                   | 200 mM          | ND                                 | 51                                |                                          |
| Salt marsh sediment               | 20 mM           | 96.27; 95.09                       | ND; 0                             | Banat et al., 1981; Nedwell and Banat 1981 |
| Sulphide rich sediment from shallow coastal lagoon | 20 mM          | 180                                | 0                                 | Sorensen et al 1981                     |
| Waterlogged Alder Swamp           | 1.5 mM          | 97.43                              | 0                                 | Westermann and Ahring 1987              |
| AD active sludge                  | 3               | 100                                | 0                                 | Tanaka and Lee 1997                     |
| Active sludge form anaerobic digester | 3              | 100                                | 0                                 | Ranade et al 1999                      |
| Enriched biomass from two-phase anaerobic digester | 2.5 mM        | 100                                | 50                                | Isa and Anderson 2005                   |
| SRB enriched biomass from         | 3               | 85                                 | 0                                 | Patidar and Tane 2005                   |
| Swine manure slurry               | 2               | 99.33                              | ND                                | Predicava et al 2008                    |
| Anaerobic digester effluent       | 2.5 mM          | 100                                | 11                                | Zabedi et al 2014                      |

4. Microbial structure and dynamics residing in anaerobic digesters

To optimising energy recovery from AD process, the microbial system must have stable performance over time although there are various metabolic functions and taxonomic community composition in bioreactors (Werner et al. 2011; Louca et al. 2018). There are three ecological factors that very influential in maintaining a stable and robust community function in bioreactors: (i) functionally diverse microbial community in the sense that there are set of organisms capable of performing each metabolic function based on their genetic content (Werner et al. 2011; Louca et al. 2016; Louca et al. 2018). (ii) Evenness in functional structures (relative abundance of various functional groups of genes that associated with specific biochemical function) of the communities ensures that the microbial system has more capacity to use various pathways to induce methane production (Werner et al. 2011; Rivett and Bell 2018; Louca et al. 2018). Finally, (iii) based on their identical end-products (ex: CH₄ and CO₂), the coexistence of multiple distinct organisms (taxonomically) that exhibit high functional redundancy allow the community to maintain focal function overtime under perturbation and disturbances at a given place and times (Allison and Martiny 2008; Werner et al. 2011; Louca et al., 2018).

Many studies have sequenced and analysed through PCR amplification of conserved marker genes of the most abundant organisms in the anaerobic digesters to understand microbe community composition, but still view information about the dynamics in microbial systems and the influence on maintaining metabolic function and
process stability (Briones and Raskin 2003; Riviere et al. 2009; Werner et al. 2011; Vanwonterghem et al. 2014).

Microbial community shifts in bioreactors may be shaped by some environmental factors, such as temperature, pH, organic loading rate (OLR), hydraulic retention time (HRT), sulphate, ammonia concentration and feedstock composition (Dollhopf et al. 2001; Rademacher et al. 2012; Franke-Whittle et al. 2014; Li et al. 2015; Langer et al. 2015; Hulsen et al. 2016). Many researchers showed that change in microbial community during AD process does not affect the biogas production rates (Fernandez et al. 2005; Briones and Raskin 2003; Langer et al. 2015), suggest that functional stability of AD actively controlled by the environment and not the taxonomic variations because the microbial systems carried out focal biochemical functional at similar rates, regardless of differences in composition. A possible reason for this phenomena is alternative microbes can perform the same focal biochemical functions, or there is a functional redundancy (Louca et al. 2018), thus the microbial system better buffered against microbial shift caused by perturbation or disturbance. Several studies indicate the high resilience of microbial communities in diverse anaerobic digesters during AD through shifts within the microbial community structure, in terms of the species and their abundance, did not impact the biogas production rates. It means that the microbial communities residing in the digester adjust to applied conditions and optimised their metabolism in a way that assure efficient biogas production (Allison and Martiny 2008; Bengelsdorf et al. 2013; Langer et al. 2015).

5. Conclusion

Fossil energy is depleted, making the use of green technology increasingly important. The advantage of anaerobic digestion technology is that it can be used as a method for waste management. This is important to say because the process of solid waste disposal and liquid waste treatment requires huge costs, so any technology that can prevent the accumulation of solid material that is disposed of at landfills is very important. Every by-product released from the anaerobic digestion system, called digestate, can be further processed and used as fertilizer because it contains a lot of nitrogen (Bhattiraja et al. 2018), which means it will reduce the use of synthetic fertilizers. Moreover, anaerobic digestion systems are flexible because they can treat various types of waste, which means this technology can be widely applied. Its flexibility includes solid and liquid waste derived from the food and beverage industry (such as milk and beer) and agriculture. Another advantage is the amount of sludge (biomass) produced is far less when compared to aerobic waste treatment (Chen et al. 2008), only requires low nutritional input and relatively low operational and maintenance costs (Wijekoon et al. 2011; De Vrieze et al. 2012; Nathia-Neves et al. 2018).

The main challenge in the application of this technology is the efficiency and stability of anaerobic digestion which depends on the syntrophic activity of various microorganisms that carry out hydrolysis, acidogenesis, acetogenesis and methanogenesis. Not only the composition of the community and its metabolic functions but also operational conditions (such as the type of feedstocks, pH and temperature) also influence the microbial system (structure and dynamics) which can be linked to the efficiency and stability of anaerobic digestion process.

Acknowledgements

Thanks to Indonesian Endowment Fund for Education (LPDP) for the support.

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