Avens Publishing Group
J Microbiol Microb Technol
May 2022 Volume:3, Issue:1

Microbes - The Key Players in Anaerobic Digestion for Biogas Production

Keywords: Anaerobic digestion; Biogas; Microbes; Molecular techniques; Meta-omics

Abstract

Microorganisms remain the powerhouse in the different processes of anaerobic digestion (AD). These organisms in a synergistic approach convert organic matters to biogas and other useful products. Over time, the study of these microbes involved in AD has been a challenge. This is because of the high limitations of the culture-based techniques in the isolation of microorganisms. The advent of molecular techniques in the study of microbes in AD has become a groundbreaking achievement. Meta-omics give very deep insights not only into the diversity of microbiota present but into the microbiome and pathways involved. More studies are required using molecular techniques to unlock more information about these tiny but powerful living machines that help in balancing and maintaining both the aerobic and anaerobic ecosystems.

Open Access **Review Article**

Journal of Microbiology & Microbial Technology

Ukaegbu-Obi KM* and Ifeanyi VO, Eze VC

Department of Microbiology, College of Natural Science, Michael Okpara University of Agriculture, Abia State, Nigeria

***Address for Correspondence:**

Ukaegbu-Obi KM, Department of Microbiology, College of Natural Science, Michael Okpara University of Agriculture, Abia State, Nigeria; E-mail: ukaegbu-obi.kelechi@mouau.edu.ng

Submission: 16 April, 2022
Accepted: 20 May, 2022
Published: 23 May, 2022

Introduction

The production of Biogas using anaerobic digestion has been in existence but the role of microbes was known in the 20th century [1]. Various microorganisms with specific properties take part in the AD process [2]. Microbiology of anaerobic transformation of organic wastes is a process that involves many different groups of bacteria in four main steps namely hydrolysis, acidogenesis, acetogenesis and methanogenesis [2-8].

The individual degradation steps are carried out by different consortia of microorganisms [4]. These organisms partly stand in syntrophic interrelation and place different requirements on the environment and in the final stage produce carbon dioxide and methane, as the main products of the digestion process [9-11]. Besides energy production, the degradation of organic waste also offers some other advantages including the reduction of odour release and decreased level of pathogens. Moreover, the nutrient-rich digested residue could be used as organic fertilizer for arable land instead of mineral fertilizer, as well as an organic substrate for greenhouse cultivation.

The Bioprocesses of Anaerobic Digestion

Anaerobic digestion is widely adopted in the world, but the microbial ecology of this process is still being studied [13]. Unfolding and proper understanding of the complex structural diversity are very important in understanding the functional relationship between the various metabolic groups of microorganisms (hydrolytic, acidogenic, acetogenic and methanogenic). Understanding this synergy will help improve and optimize the process of AD thereby making it more effective [13-14] (Figure 1).

Hydrolysis

Complex organic molecules like proteins, polysaccharides, and fat

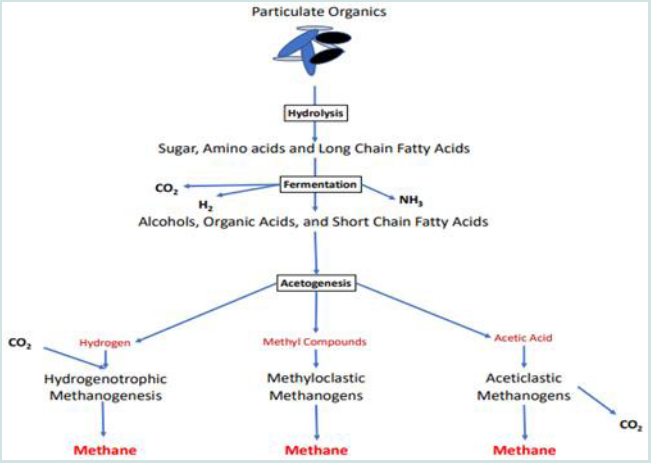

Figure 1: Stages of Anaerobic Digestion [12].

are converted into simpler ones or soluble monomers like peptides, saccharides, and fatty acids by exoenzymes like cellulase, protease, and lipase produced by hydrolytic and fermentative bacteria [13,15-17]. The enzymes required for hydrolysis can either be attached to microbial cells or secreted into the solution [18].

Hydrolysis is a relatively slow step and it can limit the rate of the overall anaerobic digestion process, especially when using solid waste as the substrate [13,18]. The rate of the hydrolysis process depends on such parameters as size of particles, pH, Production of enzymes, diffusion and adsorption of enzymes on the particles of wastes subjected to the digestion process. Different compounds have different hydrolysis rates [19].

Several groups of hydrolytic microorganisms (strict anaerobes and facultative anaerobes) [20] are involved in the degradation of several substrate compositions, where the bacteria Bacteroides, *Clostridium* and *Staphylococcus* are significant drivers [21] others are *Streptococcus*, *Enterobacterium* [17,21]. Hydrolytic bacteria in AD

ISSN: 2474-4530

are found within five phyla: Firmicutes, Bacteroidetes, Fibrobacter, Spirochaetes, and Thermotogae [22]. Firmicutes and Bacteroidetes are typically the most abundant taxa of hydrolytic bacteria in AD, although the relative abundance of these taxa is often dictated by inoculum and reactor type, as reviewed by [22] (Table 1) (2015).

In order to improve hydrolysis and anaerobic digestion performance, several pretreatments (thermal, thermochemical ultrasonic alkaline) have been carried out, which cause the lysis or disintegration of sludge cells [23]. Hydrolytic bacteria can be inhibited by elevated levels of Volatile Fatty Acids and hydrogen partial pressure [22]. Acid accumulation and the process pH decrease usually occur when hydrolysis occurs too rapidly, and this inhibits methanogens [19].

Acidogenesis

Hydrolysis is immediately followed by the acid-forming step - acidogenesis [15].

In the acidogenesis stage, acidogenic bacteria such as *Lactobacillus, Streptococcus, Clostridium* etc transform hydrolysis products (amino acids and sugars) into volatile fatty acids (VFAs) (acetic acid, butyric acid, and propionic acid), organic acids (succinic acid and lactic acid), ammonia (NH3), hydrogen gas (H2), carbon dioxide (CO2), hydrogen sulphide (H_2S), and low alcohols [16,17].

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 acidogenic and acetogenic 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 that includes both facultative and obligate anaerobes. Facultative organisms are able to live in both aerobic and anaerobic environments. Acidogenic facultative anaerobes present make use of the oxygen that may be introduced into the digester during feeding [24]. This action is very important in creating favourable conditions for the obligate anaerobes.

As opposed to other stages, acidogenesis is generally believed to proceed at a faster rate than all other processes of anaerobic digestion, with acidogenic bacteria having a regeneration time of fewer than 36 hours. With the rapidity of this stage, the production of volatile fatty acids creates direct precursors for the final stage of methanogenesis; VFA acidification is widely reported to be a cause of digester failure [25]. During acidogenesis, the pH reduces [26]. When the acidogenesis rate is too high and the pH drop is significant, severe inhibition of methanogens (methane-forming bacteria) will be triggered [19]. The VFA other than acetate as well as some alcohols is subsequently oxidized by syntrophic bacteria to acetate, hydrogen and carbon dioxide.

Table 1: Bacterial groups involved in the hydrolysis stage of different substrate components.

Primary substrate Components	Hydrolyzed Products	Bacterial Group
Carbohydrates	Soluble sugars	*Clostridium, acitovibrio celluliticus, Staphylococcus, Bacteroids*
Lipids	Higher fatty acids or alcohols and glycerol	*Clostridium, Staphylococcus, Micrococcus*
Proteins	Soluble peptides and amino acids	*Bacteroids, Bacillus, Vibrio*

Species that have been isolated from anaerobic digesters include *Clostridium, Peptococcus, Bifidobacterium, Desulfovibrio, Corynebacterium, Lactobacillus, Actinomyces, Staphylococcus, Streptococcus, Micrococcus, Bacillus, Pseudomonas, Selemonas, Veillonella, Sarcina, Desulfobacter, Desulfomonas* and *Escherichia coli* [27]. These microorganisms are able to withstand low pH, high temperatures and a high organic loading rate.

Acetogenesis

During the third stage, namely acetogenesis, the VFAs, especially acetic acids and butyric acids, are converted into acetate, H2 and CO2. Among the VFAs (acetic acid, butyric acid, and propionic acid), 65-95% of methane is directly produced from acetic acid [16]. As the end product of these two processes - acidogenesis and acetogenesis is acid, some researchers merge these two processes as acidogenesis [19].

An obligate, syntrophic relationship exists between the acetogens and methanogens. Syntrophy is the phenomenon in which one species lives off the products of another species. About 64% of the methane produced during anaerobic digestion comes from acetate, while the remaining 36% comes from hydrogen [18].

Acetogenesis refers to the synthesis of acetate, which includes the formation of acetate by the reduction of Carbon dioxide and the formation of acetate from organic acids. Hydrogen-utilizing acetogens, previously also termed homoacetogens, are strict anaerobic bacteria that can use the acetyl-CoA pathway as (i) their predominant mechanism for the reductive synthesis of acetyl-CoA from CO2, (ii) terminal electron-accepting, energy-conserving process, and (iii) mechanism for the synthesis of cell carbon from CO2 [28]. These bacteria compete with methanogens for substrates like hydrogen, formate, and methanol.

Organic acids (such as propionate and butyrate) and alcohols (such as ethanol) produced during the fermentation step are oxidized to acetate by hydrogen-producing acetogens. Electrons produced from this oxidation reaction are transferred to protons (H+) to produce hydrogen or bicarbonate to generate formate [29]. The production of hydrogen exerts toxic effects on the acetogenic bacteria [20]. Collaboration with the hydrogen-consuming (hydrogenotrophic) methanogens becomes essential, in a symbiotic process described as syntrophy, in which the methanogens constantly utilize hydrogen to produce methane [20].

Acetogens that oxidize organic acids obligately use hydrogen ions and carbon dioxide as electron acceptors. The acetogenic bacteria grow faster than the methanogens and the interaction of the two groups of bacteria is important for the performance of the anaerobic digester [28].

The coupling or syntrophic relationship of hydrogen producers and hydrogen consumers is called interspecies hydrogen transfer. Obligately syntrophic communities of acetogenic bacteria and methanogenic archaea have several unique features: (i) they degrade fatty acids coupled to growth, while neither the methanogen nor the acetogen alone is able to degrade these compounds, (ii) intermicrobial distances between acetogens and hydrogen-scavenging microorganisms influence specific growth rates [29], and (iii) the communities have evolved biochemical mechanisms that allow sharing of chemical energy. Typical homoacetogenic bacteria

ISSN: 2474-4530

are *Acetobacterium woodii* and *Clostridium aceticum* [21]. Bacteria that form the acetate by using butyrates and propionates respectively are known as *Syntrophobacter wolinii, Smithella propionica* and *Pelotomaculum schinkii. Clostridium aceticum* is another microorganism that develops H2 and CO2 acetate. The accumulation of hydrogen can inhibit the metabolism of acetogenic bacteria [30]. The maintenance of an extremely low partial pressure of hydrogen is, therefore, essential for the acetogenic and Hydrogen-producing bacteria [21].

Methanogenesis

In general and as mentioned earlier, the Anaerobic Digestion of organic material requires the combined activity of several different groups of microorganisms with different metabolic capacities [30-32]. To obtain a stable biogas process, all the conversion steps involved in the degradation of organic matters and the microorganisms carrying out these steps must work in a synchronized manner.

Methanogenesis is the last stage where methane and Carbon dioxide are derived from acetogenesis products (acetic acid, H2, CO2 and formate and methanol, methylamine or dimethyl sulfide) by methanogenic bacteria [5,30]. It is a critical step in the entire anaerobic digestion process, and its biochemical reactions are the slowest in comparison to those in other steps.

Methanogens have longer duplication times (of up to 30 d) and are generally considered the most sensitive group to process disturbances [33]. The methanogens are the dominant species and are strict anaerobes. They are vulnerable to even small amounts of oxygen. They also require a lower redox potential for growth than most other anaerobic bacteria [21]. These microorganisms are particularly sensitive to changes in temperature and pH [19], their development is inhibited by a high level of volatile fatty acids and other compounds, that is, hydrogen, ammonia, sulphur hydrogen in the environment [5].

The end product can be formed, either by means of cleavage of acetic acid molecules to generate Carbon dioxide and methane or by the reduction of Carbon dioxide with hydrogen to form methane and water [20]. Besides the above two groups, some methane can also be produced by the methylotrophic methanogens [5,16,17].

There are three groups of methanogens, namely acetotrophic, hydrogenotrophic, and methylotrophic [16]. The majority of the methane is produced by acetotrophic methanogens, which transform the acetate (resulting from acetogenesis) into methane and carbon dioxide. The hydrogenotrophic group converts hydrogen and carbon dioxide into methane [20].

74.5 % of archaea species utilize hydrogen and carbon dioxide, 33 % utilize methyl compounds and 8.5 % utilize acetate. The utilization of methyl compounds (mainly micro-organisms belonging to the genera *Methanosarcina* and *Methanolobus*) is seldom accompanied by an ability to utilize hydrogen and carbon dioxide [34]. Some members of acetoclastic methanogens include *Methanosarcina, Methanothrix, Methanosaeta*, etc. Hydrogenotrophic methanogens include *Methanospirillum, Methanoculleus, Methanobrevibacter, Methanocorpusculum*, etc [18]. However, the *Methanosarcina* species could employ both the acetoclastic and the hydrogenotrophic methanogenesis pathways [20]. Methanogens are the most important microorganisms in the anaerobic digestion process converting organic matter to methane.

Microbial Identification in Ad

Identifying the species in a sample is crucial in microbiology research. Most of the microorganisms involved in AD are anaerobes and their cultivation in the laboratory is one of the most challenging areas of microbial research [35].

A. Culture-based techniques of Microbial Identification in AD

Before the advent of molecular tools such as metagenomics, the microbial ecology of various environments including those of the anaerobic world was largely elusive [14]. The culture-dependent methods have various limitations which lead to incomplete or even incorrect information on microbes isolated. It is usually only a small fraction of the microbes in anaerobic digestion plants that can be cultured because the artificial growth media may not adequately simulate the environment in the anaerobic digestion plants or provide all the nutrients required for the growth of the microbes [36]. Many microbes require syntrophic interactions with others, and thus they cannot be cultured individually, they require co-culturing of the microbes. Some microorganisms have similar physiological, biochemical and/or morphological characteristics; therefore, they cannot be distinguished from one another with certainty. As a result, it has been estimated that only one per cent of the microorganisms present in anaerobic environments had been isolated or characterized [37].

B. Molecular biology techniques of Microbial Identification in AD

Only a few per cent of bacteria and archaea have so far been isolated, but little is known about the dynamics and interactions between these microorganisms. The lack of knowledge results sometimes in malfunctions and unexplainable failures of biogas fermenters. With molecular techniques, more information can be received about the community structures in anaerobic processes [4].

A variety of 16S rRNA-based techniques have been developed and applied to microbial ecological studies of biogas-producing microbiomes. Cloning of PCR-amplified 16S rRNA gene, a fragment or the entire gene, followed by sequencing of individual clones with the Sanger sequencing technology has been used for decades in the analysis of microbiomes, including biogas-producing microbiomes [36-38]. The recent advancements in next-generation sequencing (NGS) technologies have made this traditional method obsolete.

Metagenomics, a culture-independent method allows for the direct examination of microbial community structure and function in an ecosystem using various bioinformatics pipelines [39]. The application of omics-based studies has revealed a number of things previously unknown to the anaerobic microbial world such as new taxa and their roles in various anaerobic systems [39].

The methods of metagenomics provide extensive insight into microbial phylogeny in AD [40]. Meta-omics techniques and gene amplicon sequencing methods can fill this gap in the understanding of AD and have been developed in order to link the function and activity of the microbial community.

Metagenomic approaches are appropriate strategies for different

ISSN: 2474-4530

objectives such as identifying and isolating key players of an anaerobic culture [40]. Provide greater information than amplicon gene sequencing approaches [13].

Molecular biology techniques provide valuable tools for improved understanding of microbial communities and their function in connection with different aspects of AD, which in turn may help optimize the biogas production process more efficiently. A broad range of studies was published recently on investigations of microbial community structures in biogas reactors. The methodologies applied included analysis of total bacteria and archaeal community by targeting 16S rRNA using 454 next-generation sequencings (NGS) technique; as well as detection and quantification of methanogenic Archaea by quantitative real-time polymerase chain reaction (qPCR) [41]. The traditional molecular biology technologies help with identifying only the most abundant microbial populations present in the reactor. Due to their high sequencing depth, the newly developed sequencing techniques make the determination of both the most abundant and also the minor populations possible. The NGS-based metagenomic approach enables following up on changes in the microbial community structure starting from the very initial stage to the souring of the digester [42].

Investigations of the microbial community in 21 full-scale anaerobic digestion plants using 454 pyrosequencing of 16S rRNA gene sequences showed that the bacterial community was always more abundant and more diverse than the archaeal community in all reactors.

Similarly, the denaturing gradient gel electrophoresis (DGGE) technique is still among the promising methods to perform a preliminary analysis of the microbial community profile and monitor the various experimental stages during the biogas production process [43].

Moreover, the high-throughput Illumina Miseq approach is also widely considered a promising culture-independent method to perform microbial community analysis of AD systems. By the application of this method, the specific syntrophic relationships between acetogens and methanogens could be better understood, especially in terms of how they can be related to disturbances occurring in the biogas production process [44,45].

Conclusion

The role of microbes in anaerobic digestion cannot be overemphasized. These tiny creatures use a combined effort to transform different biomasses into valuable materials, maintaining the ecosystem in a unique and equilibrium state. Microbes which are ubiquitous can be harnessed to help man solve his numerous needs. The microbiota involved in anaerobic digestion should therefore be elaborately studied and exploited as more biomasses are used. These microbes can be further bioengineered to breakdown xenobiotics in anaerobic conditions to produce valuable materials. Unraveling the synergy amongst the microbiota involved in AD will not only help in the improvement and efficiency of the AD process but will also lead to the optimization of biogas production.

References

1. Demirel B, Scherer P (2008) The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. Rev Environ Sci Technol 7: 173-190.
2. Amani T, Nosrati M, Sreekrishnan TR (2010) Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects - a review. Environ Rev 18: 255-278.
3. Skiadas IV, Gavala HN, Lu J, Ahring BK (2005) Thermal pre-treatment of primary and secondary sludge at 70 degrees C prior to anaerobic digestion. Water Sci Technol. 52: 161-166.
4. Weiland P (2010) Biogas production: Current state and perspectives. Appl Microbiol Biotechnol 85: 849-860.
5. Ziemiński K, Frąc M (2012) Methane fermentation process as anaerobic digestion of biomass: Transformations, stages and microorganisms. Afr J Biotechnol 11: 4127-4139.
6. Maus I, Bremges A, Stolze Y, Hahnke S, Cibis KG, et al. (2017) Genomics and prevalence of bacterial and archaeal isolates from biogas-producing microbiomes. Biotechnol Biofuels 10: 264.
7. Pampillón-González L, Ortiz-Cornejo NL, Luna-Guido M, Dendooven L, Navarro-Noya YE (2017) Archaeal and bacterial community structure in an anaerobic digestion reactor (Lagoon Type) used for biogas production at a pig farm. J Mol Microbiol Biotechnol 27: 306-317.
8. Caruso MC, Braghieri A, Capece A, Napolitano F, Romano P, et al. (2019) Recent updates on the use of agro-food waste for biogas production. Appl Sci 9: 1217.
9. Angelidaki I, Ellegaard L, Ahring BK (1993) A mathematical model for dynamic simulation of anaerobic digestion of complex substrates: focusing on ammonia inhibition. Biotechnol Bioeng 42: 159-166.
10. Chasnyk O, Sołowski G, Shkarupa O (2015) Historical, technical and economic aspects of biogas development: Case of Poland and Ukraine. Renewable Sustainable Energy Rev 52: 227-239.
11. Abdeshahian P, Lim JS, Ho WS, Hashim H, Lee CT (2016) Potential of biogas production from farm animal waste in Malaysia. Renewable Sustainable Energy Rev 60: 714-723.
12. Hashemi S, Hashemi SE, Lien KM, Lamb JJ (2021) Molecular Microbial Community Analysis as an Analysis Tool for Optimal Biogas Production. Microorganisms 9: 1162.
13. Amha YM, Anwar MZ, Brower A, Jacobsen CS, Stadler LB, et al (2018) Inhibition of anaerobic digestion processes: Applications of molecular tools. Bioresour Technol 247: 999-1014.
14. Chinwendu OA, Bukola CO, Edet UO (2020) Metagenomics in-sight into the microbial structural diversity of anaerobic digester utilising fruit waste as substrate. J microb biotech food sci 9: 965-969.
15. Ostrem K, Themelis NJ (2004) Greening waste: Anaerobic digestion for treating the organic fraction of municipal solid wastes. Earth Engineering Center Columbia University. 6-9.
16. Sarker S, Lamb JJ, Hjelme DR, Lien KM (2019) A review of the role of critical parameters in the design and operation of biogas production plants. Appl Sci 9:1-37.
17. Mir MA, Hussain A, Verma C (2016) Design considerations and operational performance of anaerobic digester: A review. Cogent Engineering. 3: 1181696.
18. Angelidaki I, Karakashev D, Batstone DJ, Plugge CM, Stams AJM (2011) Biomethanation and Its Potential. Methods Enzymol 494: 327-351.
19. Leung DYC, Wang J (2016) An overview on biogas generation from anaerobic digestion of food waste. Int J Green Energy 13: 119-131.
20. Manyi-Loh CE, Mamphweli SN, Meyer EL, Okoh AI (2019) Microbial anaerobic digestion: process dynamics and implications from the renewable energy, environmental and agronomy perspectives. Int J Environ Sci Technol 16: 3913-3934.
21. Nayono SE (2010) Anaerobic digestion of organic solid waste for energy production.

ISSN: 2474-4530

22. Azman S, Khadem AF, van Lier JB, Zeeman G, Plugge CM (2015) Presence and Role of Anaerobic Hydrolytic Microbes in Conversion of Lignocellulosic Biomass for Biogas Production. Crit Rev Environ Sci Technol 45: 2523-2564.

23. Zhang L, Lee Y-W, Jahng D (2011) Anaerobic co-digestion of food waste and piggery wastewater: focusing on the role of trace elements. Bioresour Technol 102: 5048-5059.

24. Kothari R, Pandey AK, Kumar S, Tyagi VV, Tyagi SK (2014) Different aspects of dry anaerobic digestion for bio-energy: An overview. Renewable Sustainable Energy Rev 1: 174-195.

25. Masinde BH, Nyaanga DM, Njue MR (2020) Effect of Total Solids on Biogas Production in a Fixed Dome Laboratory Digester under Mesophilic Temperature. Annals of Advanced Agricultural Sci. 4: 26-33.

26. Kondusamy D, Kalamdhad AS (2014) Pre-treatment and anaerobic digestion of food waste for high rate methane production - A review. J Environ Chem Eng 2: 1821-1830.

27. Kosaric N, Blaszczyk R (1990) Microbial aggregates in anaerobic wastewater treatment. Adv Biochem Eng Biotechnol 42: 27-62.

28. Drake HL (2012) Acetogenesis. Springer Science & Business Media. p647.

29. Stams AJM, Plugge CM (2009) Electron transfer in syntrophic communities of anaerobic bacteria and archaea. Nat Rev Microbiol 7: 568-577.

30. Uddin MN, Siddiki SYA, Mofijur M, Djavanroodi F, Hazrat MA, et al. (2021) Prospects of Bioenergy Production From Organic Waste Using Anaerobic Digestion Technology: A Mini Review. Frontiers in Energy Research 9: 1-8.

31. Mirmohamadsadeghi S, Karimi K, Azarbaijani R, Parsa Yeganeh L, Angelidaki I, et al. (2021) Pretreatment of lignocelluloses for enhanced biogas production: A review on influencing mechanisms and the importance of microbial diversity. Renewable Sustainable Energy Rev 135: 110173.

32. Shen R, Jing Y, Feng J, Zhao L, Yao Z, et al. (2021) Simultaneous carbon dioxide reduction and enhancement of methane production in biogas via anaerobic digestion of cornstalk in continuous stirred-tank reactors: The influences of biochar, environmental parameters, and microorganisms. Bioresour Technol 319: 124146.

33. Griffin ME, McMahon KD, Mackie RI, Raskin L (1998) Methanogenic population dynamics during start-up of anaerobic digesters treating municipal solid waste and biosolids. Biotechnol Bioeng 57: 342-355.

34. Jabłoński S, Rodowicz P, Łukaszewicz M (2015) Methanogenic archaea database containing physiological and biochemical characteristics. Int J Syst Evol Microbiol. 65: 1360-1368.

35. Mori K, Kamagata Y (2014) The challenges of studying the anaerobic microbial world. Microbes Environ. 29: 335-337.

36. Lim JW, Park T, Tong YW, Yu Z (2020) The microbiome driving anaerobic digestion and microbial analysis. In: Advances in Bioenergy 5:1-61.

37. Fang HHP (2010) Environmental Anaerobic Technology: Applications and New Developments. World Scientific. p404.

38. Nelson MC, Morrison M, Yu Z (2011) A meta-analysis of the microbial diversity observed in anaerobic digesters. Bioresour Technol. 102: 3730-3739.

39. Manyi-Loh CE, Mamphweli SN, Meyer EL, Okoh AI, Makaka G, et al. (2013) Microbial anaerobic digestion (bio-digesters) as an approach to the decontamination of animal wastes in pollution control and the generation of renewable energy. Int J Environ Res Public Health 10: 4390-4417.

40. Alireza Hashemi S, Mojtaba Mousavi S, Reza Naderi H, Bahrani S, Arjmand M, et al. (2021) Reinforced polypyrole with 2D graphene flakes decorated with interconnected nickel-tungsten metal oxide complex toward superiorly stable supercapacitor. Chem Eng J 418: 129396.

41. Zhang Q, Wang M, Ma X, Gao Q, Wang T, et al. (2019) High variations of methanogenic microorganisms drive full-scale anaerobic digestion process. Environ Int 126: 543-551.

42. Hassa J, Maus I, Off S, Pühler A, Scherer P, et al. (2018) Metagenome, metatranscriptome, and metaproteome approaches unraveled compositions and functional relationships of microbial communities residing in biogas plants. Appl Microbiol Biotechnol 102: 5045-5063.

43. Dias MF, Colturato LF, de Oliveira JP, Leite LR, Oliveira G, et al. (2016) Metagenomic analysis of a desulphurisation system used to treat biogas from vinasse methanisation. Bioresour Technol 205: 58-66.

44. Aydin S (2016) Enhancement of microbial diversity and methane yield by bacterial bioaugmentation through the anaerobic digestion of Haematococcus pluvialis. Appl Microbiol Biotechnol 100: 5631-5637.

45. Sárvári Horváth I, Tabatabaei M, Karimi K, Kumar R (2016) Recent updates on biogas production-a review. Biofuel research journal 3: 394.