Microbial Community Dynamics in Anaerobic Digester Treating Human Waste: A Review

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Abstract

Microbial communities (bacteria and archaea) play the most important part in the production of biogas in anaerobic digesters. A comprehensive understanding of microbial diversity, composition, abundance, interactions and their behaviour is required to yield biogas optimally. Their active genes, metabolic products and proteins help to speed up the anaerobic digestion. High-throughput sequencing and appropriate bioinformatics analysis can easily assess the diversity and quantity of microbial communities, which is vital for the overall process. High-throughput sequencing provides detailed information on microbial diversity and resilience of anaerobic digester system. Sequencing tools like next-generation sequencing and 16S rRNA amplicon sequencing help in understanding the underlying causes of anaerobic digestion through exploring the microbial population in biogas reactors and interaction among microbiomes and process parameters. Anaerobic digestion of human waste has gained popularity due to its ability to transform organic waste into biogas. In the current chapter, the microbial community in anaerobic digesters and recent developments in biotechniques for assessing microbial diversity have been reviewed.

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6.1 Introduction

Understanding the microbial community dynamics is crucial to promote microbial interactions and to enable the metabolic co-dependencies. Microbial communities' physiology and ecology are influenced by temporal dynamics and spatial structure of its members. Microbial interactions can be facilitated by spatial structure, allowing metabolic co-dependencies to increase community resilience and homeostasis (Mark Welch et al. 2016; Ronda and Wang 2022). Microbial communities are sensitive to temporal dynamics, with changes in metabolism, community composition and function leading to phenotypically complex community trajectories. Understanding about the governing spatio-temporal principles within a microbiome is critical for its physiology.

Intrinsic and extrinsic variables influence the temporal dynamics of microbial communities (Ryo et al. 2019). Individual species metabolism and colonisation capacity and intra- and inter-species interactions are intrinsic determinants, while periodic alterations in ambient environments such as pH and nutritional availability are extrinsic impacts. Microbial communities have piqued researchers' interest because they decompose organic matter using carbon and nitrogen as energy sources, as well as oxygen, and produce CO_2 and soil-enriching compost. The resulting compost contains a high percentage of biologically stable humic compounds, making it an excellent soil addition (Białobrzewski et al. 2015).

Traditional systems such as aerobic and anaerobic digestion (AD) provide organic waste management and sustainable energy production (Choi et al. 2021). AD is commonly used in the treatment of organic waste, and it is gaining attention as biogas produced by AD is widely employed as a sustainable energy source. The AD process is used to treat a variety of organic wastes (liquid and solid), and it is increasingly being utilised to treat human waste.

In anaerobic digestion environments, microbes from a variety of taxonomic groups play an essential role in interactions that occur during biomass breakdown and methanogenesis (Li et al. 2017). A wide range of environmental conditions may have an impact on microbes (Table 6.1), viz. pH, alkalinity, organic matter, ammonia concentration and volatile fatty acids (VFA); also the variances in physiology, nutrition-dependent growth kinetics and sensitivities to environmental conditions affect the microbial population (Kovacs et al. 2015; Li et al. 2017).

Understanding the ecology of anaerobic digesters and how it relates to system's function necessitates the identification of active and numerous microorganisms, connecting their identities to their functional responsibilities. Several 16S rRNA gene amplicon analyses have showed that in comparably operating anaerobic digesters, there appears to be a group of abundant microorganisms that are stable throughout time (Venkiteshwaran et al. 2015; Werner et al. 2011). Other biological

Table 6.1 Environmental factors' influence on anaerobic digestion process

S. no.	Factors	Effects	Optimal range	Influence on AD process
1	pH, alkalinity	Biochemical metabolism	Acid-forming bacteria: pH 5 Methanogenic bacteria: pH 7	pH ranges distinguish the hydrolytic, acidogenic and methanogenic stages
2	Temperature	Microbial density and diversity	Mesophilic: 30–35 Thermophilic: 50–60	Influence acetoclastic and hydrogenotrophic methanogenesis processes
3	Ammonia	Methanogen community structure	AD—function up to 1000 mg TAN/L	Higher concentration inhibits the methanogenic activity
4	Hydraulic retention time	Process efficiency	Short/long	Short retention time favours hydrolytic-acidogenic phase. Long retention time aids methanogenic degradation
5	Organic loading rate	Microbial community structure	High/low	High OLR increases VFA production/accumulation. Organic shock loading condition favours hydrogenotrophs
6	Nutrients	Enzymatic activity	Macronutrients: Ca, K, Mg, Na, P and S Micronutrients: B, Co, Cu, Fe, Mn, Mo and Zn	Enrich the archaea community, faster VFA degradation, improved process stability

systems, such as wastewater treatment plants and the human digestive system, have also been linked to this activity (Saunders et al. 2015). However, in anaerobic digesters, a significant portion of the visible microbial population may come from dead or inactive cells that arrive with the influent biomass and retain DNA. As a result, reported microbial population dynamics are unlikely to accurately reflect changes in process performance or stability. This can lead to erroneous inferences and relationships (Fodor et al. 2012).

Molecular approaches have been developed to address this issue, but the complex matrix of anaerobic digester sludge samples will likely result in undesirable chemical reactions. As a result, monitoring the microbial composition of the influent to detect the abundant organisms sustained by immigration could be a viable alternative (Lee et al. 2015; Seib et al. 2016). Various microbial communities involved in the anaerobic digestion process for treating different organic waste with a special focus on human waste are reviewed here in this chapter.

6.2 Microbial Communities in Anaerobic Digestion Process

The microbiological processes of AD can be conceptualised as hydrolysis, acidogenesis, acetogenesis and methanogenesis. These four processes are carried out via guild of microbes, and it is necessary to uphold a balanced reaction rate for stable digestion. Table 6.2 depicts the microbial communities observed during the four phases of the anaerobic digestion process.

The impact of microbe's structure on digester operation and stability has received little attention. Researchers recently began to apply data on the community structure of microbes to better understand or forecast how it affects digester performance (Venkiteshwaran et al. 2015). Microbial diversity has been proven to play a crucial influence in natural and engineered ecosystem performance, as measured by species richness and relative abundance of species. It's a type of functional insurance that allows an ecosystem's richness and evenness to be maintained through compensating growth (Fernandez et al. 2000). System's perturbation may change in the population of one species within a functional group, i.e. one species decreased or

Table 6.2 Microbial community in anaerobic digestion process

S. no.	Anaerobic digestion process	Major microbial community	References
1	Hydrolysis	*Firmicutes* and *Bacteroidetes* *Acetivibrio* *Clostridium* *Bacteroides* *Thermotoga* (phylum *Thermotogae*)	Amekan (2020) De Vrieze et al. (2015) Hassa et al. (2018) Venkiteshwaran et al. (2015)
2	Acidogenesis	*Bacteroidetes* *Chloroflexi* *Firmicutes* *Proteobacteria*	Stiles and Holzapfel (1997) Balk et al. (2002) Dong et al. (2000)
3	Acetogenesis	*Smithllela Syntrophobacter Pelotomaculum Syntrophus Syntrophomonas*	Liu et al. (1999) de Bok et al. (2001) Imachi et al. (2007) Sousa et al. (2007)
4	Methanogenesis	*Methanobacterium Methanobrevibacter Methanoculleus Methanospirillum Methanothermobacter Methanosaeta Methanosarcina*	Amekan (2020) Hori et al. (2006) Leclerc et al. (2004) Savant et al. (2002) Cuzin et al. (2001)

eliminated; a different species belonging to the same functional group and more resistant to the perturbation may quickly take its place if it was there in sufficient numbers at the outset (Fernandez et al. 2000; Briones and Raskin 2003; Wittebolle et al. 2009; Werner et al. 2011).

In the operational phase, the AD process is used to treat municipal and industrial wastes based on their solid content. Han et al. (2017) examined AD's (full-scale) operating under wet condition (total solids $\leq$10%) and semi-dry condition (total solids $\leq$20%). In wet systems, *Methanobacteriaceae*, *Porphyromonadaceae*, *Sphingobacteriaceae* and *Syntrophomonadaceae* were the dominant bacterial and archaeal groups. In semi-dry digester, *Clostridiaceae*, *Lachnospiraceae*, *Methanomicrobiaceae*, *Patulibacteraceae*, *Pseudonocardiaceae* and *Rikenellaceae* species were predominant.

In single and two-stage thermophilic digesters, the effects of vegetable and fruit waste and swine manure co-digestion on microbial structure were compared by Merlino et al. (2013). The single-stage process produced highly diverse microbial population (archaea (Methanosarcinales); bacteria (*Bacilli*, *Clostridia* and *Firmicutes*)) than the two-stage method, which was linked to the increased substrate degradation and, as a result, better process performance.

Anaerobic co-digestion significantly balances the C/N ratio, maintains buffering of medium through pH/alkalinity equilibrium, supplements micro- and macronutrients, attenuates inhibitors or any toxic composites and enhances biodegradability of organic matter (Hartmann et al. 2002). Digestion of a wide range of feedstocks improves not only biogas production and process stability but also the diversity and dynamic range of microbial populations (Cuetos et al. 2008). C/N balance in anaerobic co-digestion has been shown to alter bacterial and archaeal association in previous investigations. Under varying operating circumstances, *Firmicutes* and *Chloroflexi* were found to be capable of degrading a wide range of organics (Tyagi et al. 2021). Both groups were found in abundance in a wide spectrum of anaerobic co-digesters and exhibited resistance to heavy organic loading (Rong et al. 2018).

Kirkegaard et al. (2017) used 16S rRNA gene sequencing to investigate the microbiota in anaerobic digesters (full-scale) processing suspended particles. In mesophilic, mesophilic plus thermal hydrolysis and thermophilic digesters, diverse microbial communities were discovered (Kirkegaard et al. 2017). *Candidatus Methanofastidiosa* (WCHA1-57) belonging to archaea is the dominant in mesophilic digesters. Acetoclastic methanogens, viz. *Methanothermobacter*, *Methanosarcina* and *Methanobrevibacter*, were dominant in thermophilic digestion. Abundance population of *Methanosaeta* and *Methanoculleus* were seen in mesophilic digestion combined with thermal hydrolysis process. *Methanoculleus* might be present in AD due to increased levels of ammonia in the system.

Microbial populations in AD, viz. mono-digesters, mesophilic co-digesters and thermophilic co-digesters, were examined by Sundberg et al. (2013). Two major elements that determine the organisation of microbial populations in digesters are the operational temperature and feedstock content. *Actinobacteria*, *Chloroflexi*, *Euryarchaeota*, *Proteobacteria* and *Spirochetes* remained dominant in mono-digesters, whereas *Firmicutes* dominated in co-digesters. The development of *Thermotogae* species existed in the thermophilic digesters. The makeup of the microbial population in a digester is substantially influenced by operational parameters and substrate type. A healthy and diverse microbial population that can endure process perturbations is aided by a good nutritional balance. The link between the functional microbial populations and process parameters can be exploited to generate tools for designing and operating and in controlling the process of AD (Tyagi et al. 2021; Supaphol et al. 2011).

6.3 Microbial Diversity: Biotechniques

The spectrum of microorganisms and their proportional abundance in a given community is referred to as microbial diversity. Microbial diversity is significant because it affects the resilience of processes (Torsvik et al. 1998; Mirmohamadsadeghi et al. 2021). It can provide detailed information about biological diversity in (1) genetic variation within the species, (2) the number and distribution of different species and (3) community diversity. The classification of unknown bacteria, on the other hand, can be the most difficult aspect of determining microbial diversity (Fakruddin and Mannan 2013).

The variance in the molecular features, i.e. nucleic acid homology, can be used to determine biodiversity. The community's stability is linked to the system's stability, and stress in the AD system can result in unstable system and fluctuation in species diversity (Yannarell and Triplett 2005). As a result, diversity analysis is appealing since it allows for a deeper understanding of (1) organisms' genetics and distribution in a community, (2) diversity and functional role, (3) species type and (4) specified amount of individual species in the system (Fakruddin and Mannan 2013).

Recently emerging molecular and chemical ecology approaches have opened up new possibilities for studying microbial diversity (Giovannoni et al. 1990; Akyol et al. 2019). These techniques can be used to look into the diversity and structure of microbial communities. Polymerase chain reaction amplification, a common molecular biology technique, allows specific DNA sequences to be amplified and used to assess the makeup of microbial communities. Many microbial systems use the rRNA genes (i.e. 16S rRNA) to investigate biodiversity and microbial composition (Vanwonterghem et al. 2014).

Assessment of microbial community in ADs has been successful using conventional molecular fingerprinting approaches or first-generation sequencing techniques. These procedures, however, are time-consuming and result in a low community resolution (Leclerc et al. 2004). High-throughput techniques, often known as next-generation sequencing (NGS), are newly developed sequencing technologies that can sequence numerous DNA molecules simultaneously at low cost, in a short amount of time and with high resolution (Churko et al. 2013). These characteristics result in the creation of enormous data sets, which can help with correlation analysis statistically (Vanwonterghem et al. 2014).

Anaerobic digester's microbial communities can be studied using metagenomic and bioinformatics techniques. Next-generation sequencing-based metagenomics is a fast emerging study that aids in the knowledge of the diversity and functional complexity of biological systems such as the human body, animals, soil, ocean and anaerobic habitats. In anaerobic digester, a metagenomic technique can reveal the progress of a digester, i.e. ability to progress from the first phase to an acidic state in which volatile fatty acids build and come back to normal operation (Jünemann et al. 2017; Pore et al. 2016; Lei et al. 2019). The primary goal of metagenomic approaches, particularly in less complicated environments, is to reconstruct substantial portions of genomes from species found in the microbial community (Mirmohamadsadeghi et al. 2021).

Gene-centric metagenomics has demonstrated to be more effective in complex environments like anaerobic digester by delivering a snapshot of gene frequency (Fontana et al. 2018). Metagenomic approaches have shown a large quantity of gene reads, the majority of which have yet to be identified, limiting the functional information derived from these reads. Regardless, metagenomics has shed light on the evolutionary connections among diverse species as well as the microbial community's metabolic functionality in AD (Vanwonterghem et al. 2014). The microbial diversity and their function can be considerably affected by different feedstocks, pretreatment of substrate and operational conditions (Duan et al. 2021).

The functional redundancy can be estimated using an approach that combines metagenomics with AD performance data. Furthermore, by maintaining the amount of metabolic diversity, it is feasible to achieve a steady operational situation (Mirmohamadsadeghi et al. 2021). Future amplicon sequencing methods with a higher resolution and longer read length, as well as enhanced algorithms and genome binning procedures, may usher in future improvements in metagenomics (Muller et al. 2013; Albertsen et al. 2013). In the future, metagenomics paired with meta-omic approaches such as meta-proteomes and meta-transcriptomes will aid in the creation of genomic database for anaerobic digester and also offer information on various functional groups and interactions among them (Vanwonterghem et al. 2014). Metagenomic and bioinformatics methodologies include the following series of steps (Rudakiya and Narra 2021; Zhang et al. 2019; Sun et al. 2016).

1. Sample collection from different AD processes that are feed stock dependent.
2. Bioinformatics study of metagenomic data related to microbial populations requires DNA extraction.
3. Following that, polymerase chain reaction (PCR) is performed via 16S rRNA or particular primers.
4. Products from PCR can be cloned to appropriate vectors, and vector library is created through vector cloning techniques.
5. Roche GS FLX454 pyrosequencing platform forms the basis for DNA sequencing.
6. Following the capture of metagenomic data, raw next-generation sequencing reads are obtained.

7. Raw sequence pretreatment is a crucial step in obtaining high-quality readings for downstream processing (Tools: Trimmomatic software, ACE Pyrotag Pipeline, HMMER, MG-RAST, ChimeraSlayer (Campanaro et al. 2016; Ho et al. 2014; Azizi et al. 2016; Wirth et al. 2012; Martinez et al. 2014))
8. Eliminating adapters and linkers, without chimaeras and replication, de-multiplexing barcoded samples and quality control are all part of the sequence pretreatment.
   Sequences are allied through MOTHUR, INFERNAL aligner and ClustalW (Martinez et al. 2014; Cardinali-Rezende et al. 2016; Zhang et al. 2019).
9. Consequently, aligned sequences are grouped into operational taxonomic units (OTUs) using average neighbouring clustering algorithm (Tools: Usearch software, sequence classifiers – RDP Bayesian Classifier, UCLUST-RDP classifier and MEGA/MEGA5 (Cardinali-Rezende et al. 2016; Pope et al. 2013; Rudakiya et al. 2019)).
10. Investigation of biological diversity of microbial communities (Tools: MOTHUR package, R software package having VEGAN library and RDP Pipeline (Zhang et al. 2019; Oksanen et al. 2007; Cardinali-Rezende et al. 2016)).
11. Taxonomic composition analysis is a bioinformatics investigation used for the anaerobic microbial populations and performed via (1) filtering and comparing databases and (2) taxonomic groups of sequences.

Metagenomic techniques, though not optimal for online use due to long processing times and expensive costs, offer a wealth of information about the microbial phylogeny in anaerobic digester systems. However, a biomarker database must be built before these strategies can be fully realised in AD (Hashemi et al. 2021).

6.4 Human Waste Anaerobic Digestion: Microbial Dynamics

Anaerobic digestion is a wastewater treatment method that converts organic matter into biomethane (Lettinga et al. 2001). AD is commonly used to treat a wide range of faecal wastes since it can be a cost-effective solution to lessen the environmental impact of faeces storage while simultaneously releasing methane. Human waste has been found to be a worthy substrate for generating biogas in many investigations, with equal performance in laboratory conditions (Duan et al. 2020; Lalander et al. 2018; Colon et al. 2015; Zhang and Angelidaki 2015). Human waste is high in organic matter and nutrients, making it a sustainable feedstock for a variety of applications (Singh et al. 2017) to yield biofuels, viz. methane, bioethanol and biodiesel, by pyrolysis, AD, hydrothermal liquefaction etc. (Gomaa and Abed 2017).

The ability of a large number of microbes capable of degrading complex organic polymers to work together is critical to the success of the AD process (Bedoya et al. 2020). In anaerobic digester, the microbial community gets influenced by temperature, organic load, amount of toxins, sludge retention duration, influent's composition, topographical location and annual seasons. However, little is known about the diversity and functional features of microbes in anaerobic digesters (Hao et al. 2016; Bedoya et al. 2020). A greater understanding of the dynamics and ecology of microbes in these systems can help predict their performance better and also throw the limelight on the desirable microbial structure for improved organic matter decomposition, biogas generation and pathogen control (Hao et al. 2016; De Francisci et al. 2015).

In anaerobic reactor, almost 90% of microbial population is represented by bacteria and archaea (Bedoya et al. 2020; Guo et al. 2015). Bacteria play a role in the early stages of AD, such as pathogen race, whereas archaea are in charge of the final step, which creates methane, a useful renewable energy source (Ariesyady et al. 2007). Samples from soil, ocean, human gut and sewage sludge have all been effectively used to describe phylogenetic compositions and functional potentiality of complex microbial populations using high-throughput sequencing technologies (Li et al. 2018; Nascimento et al. 2018). Bacterial communities were studied commonly in wastewater treatment plants, by sequencing of 16S rRNA gene amplicon libraries (metataxonomic method) (Iwai et al. 2016). Despite the fact that a large number of research have already focused on human waste AD, little is known about the process's stability and inhibitor variables during human waste AD treatment. Sequencing from 16S rRNA has been developed for functional inference (Duan et al. 2020; Iwai et al. 2016).

Sequencing tools like next-generation and 16S rRNA amplicon sequencing might help researchers in understanding the fundamental causes of AD by exploring the microbial populations in biogas reactors and the interaction among microbiomes and process parameters. In a recent batch experiment, researchers compared the microbial population composition of former and latter anaerobic digestion process of human faeces and showed that *Methanomicrobia* and *Cloacimonetes* were the most abundant archaea and bacteria, respectively (Gomaa and Abed 2017).

Aeration, nitrification and denitrification technologies have been designed to reduce COD and eliminate nitrogen from wastewaters in existing wastewater treatment plants (Khoshnevisan et al. 2018; Shirzad et al. 2019). Life cycle assessment (LCA) is a well-established instrument for assessing a variety of environmental effects over the course of a product or process's lifespan (Khoshnevisan et al. 2020). This method can be utilised to evaluate the entire environmental effects of anaerobic digestion of human waste, as it eliminates issues that arise, that is, the formation of intermediate elements. A life cycle energy and environmental assessment method was employed by Chen et al. (2012) to investigate the performance of the biogas-digestive system in China. Arafat et al. (2015) investigated the treatment technologies of municipal solid waste having energy recovery potential and its environmental impacts. Gao et al. (2017) compared present human excreta sanitation machinery to comprehensive Chinese rural toilet designs, which included rainwater harvesting flushing systems, standard flushing, urine segregation and composting schemes, using LCA. However, the environmental benefits of a well-designed human waste anaerobic digestion system have yet to be explored, and their evaluation is urgently required.

Duan et al. (2020) studied the AD of human waste at higher influent feedstock concentrations, ideal conditions, inhibitory variables and changes in microbial population in biogas reactors fed continuously. *Methanosaeta* and WSA2 were the dominant archaeal species among microbial populations during stable period. Microbial groups (WWE1 and WSA2) that were uncharacterised were observed, and the possible syntrophic interaction among the two groups would be critical in producing a high-performing process (Duan et al. 2020).

Up-flow anaerobic sludge blanket reactors to treat orthodox toilet and vacuum toilet black water with loading increments were effectively operated by Gao et al. (2019). The archaeal and bacterial populations clearly diverged between the conventional and vacuum toilet reactors, indicating that archaeal community evolved at a slower rate compared to bacterial community. Archaea members were hydrogenotrophic methanogens: *Methanolinea* in the conventional toilet reactor accounted for 56.6% and *Methanogenium* in the vacuum toilet reactor for 62.3%. Bacterial members were *Porphyromonadaceae* in both conventional (15.9%) and vacuum (13.4%) toilet reactors, sulphate-reducing bacteria in conventional and *Fibrobacteraceae* in vacuum toilet reactor (Gao et al. 2019).

Mesophilic AD (full scale) to treat sewage sludge and food wastewater was examined to investigate microbial communities and the effects of total ammonia nitrogen concentration and sodium ion concentration on changes in these communities (Lee et al. 2018). The addition of food waste and sewage sludge formed very distinct microbial community structures; and the variation among these two digesters was mainly influenced by total ammonia nitrogen and sodium ions. The bacterial populations of sewage sludge digesters are greatly influenced by microorganisms from influent sludge. *Methanoculleus* may be tolerant to high ammonia levels in AD.

High-solids AD, a promising approach having a smaller reactor and reduced heating energy consumption, has shown poorer digesting efficiency and increased tolerance to certain inhibitors in some cases. Archaeal and bacterial populations in anaerobic digesters handling sewage sludge having 10–19% of total solids were studied to learn more about the phenomenon (Liu et al. 2016). Genus *Methanosarcina* drove the acetoclastic methanogenesis in producing methane, and their total ratio decreased with increased total solids, which are contrary to the relative abundance of hydrogenotrophic methanogens. Microbial communities of different waste treatment in anaerobic digestion process are shown in Table 6.3. Understanding the prevalent microbial population is critical for improving biogas production and, as a result, the overall process efficacy. However, research on bacterial populations and abundance is relatively restricted. Precise databases for bacterial identification and sequencing methodologies should be developed. Validation of sequencing data is essential, and the isolation and screening of genes and proteins with potential industrial applications should be investigated.

Table 6.3 Microbial communities of different wastes in anaerobic digester

S. no.	Type of waste	Microbial dynamics	Reference
1	Toilet flushed black water	*Methanospirillaceae Methanoculleus Methanospirillum Methanogenium Porphyromonadaceae Fibrobacteraceae Ruminococcaceae Bacteroidaceae Clostridiales*	Gao et al. (2019)
2	Food waste and animal waste	*Methanobacterium beijingense Methanobacterium petrolearium Methanoculleus bourgensis Methanoculleus receptaculi*	Koo et al. (2017)
3	Food wastewater or sewage sludge	*Methanoculleus Methanobacterium Methanomassiliicoccus Methanomethylophilaceae Candidatus methanoplasma Methanosarcina Methanimicrococcus*	Lee et al. (2018)
4	Food waste-recycling wastewater	*Fastidiosipila Petrimonas* vadin BC27 *Syntrophomonas Proteiniphilum*	Kim et al. (2018)
5	Rice straw	*Enterobacteriaceae Clostridiaceae Prevotellaceae Peptostreptococcaceae*	Wachemo et al. (2019)
6	Human waste	*Methanosaeta* and WSA2	Duan et al. (2020)
7	Raw food wastewater	*Methanomicrobiales Methanosarcinales Methanobacteriales*	Kim et al. (2014)
8	Dairy manure	*Methanobacterium Methanoculleus*	Lv et al. (2013)

6.5 Conclusion

Human waste poses a threat to the environment and public health, making its long-term management a severe concern. Anaerobic digestion (AD) has long been promoted as a waste management process that is both environmentally beneficial and sustainable, producing biomethane as a by-product. AD covers a wide range of communities with a high level of functional interdependence among individual or group of organisms. Combination of meta-omics, virtualisation techniques and chemical analysis could be a potent device for extracting very important information from anaerobic digester. It is critical to recognise distinct species, understand their roles during the process, separate their functions and establish a stable AD process. Microbial populations of AD can be analysed via metagenomic and bioinformatics methodologies. Next-generation sequencing-based metagenomics is a fast emerging study that aids in the knowledge of the diversity and functional complexity of biological systems

Human waste is rich in organic matter and nutrients, making it a sustainable feedstock to yield biofuels like methane, bioethanol and biodiesel. The performance of AD process greatly depends on the synergic interactions of numerous microorganisms capable of degrading complex organic polymers. Understanding microbial dynamics and their ecology allied with the systems may forecast their performance better and also throw the limelight on the desirable microbial population structure for better organic matter degradation, biogas production and pathogen reduction.

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