



Anaerobic Digestion for Climate Change Mitigation: A Review

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Abstract

Since fossil sources for fuel and platform chemicals will become limited in the near future, it is important to develop new concepts for energy supply and production of basic reagents for the chemical industry. One alternative to crude oil and fossil natural gas could be the biological conversion of CO₂ or small organic molecules to methane via methanogenic archaea. Nowadays, the anaerobic digestion of energy crops has been widely applied in developed countries. It is reported that as high as 80–90% of the biogas plants in Germany used energy crops alone or with other waste biomass as substrate. Biogas production represents a fascinating process for the recovery of nutrients and renewable energy from various organic waste streams. The process is of interest for the production of value-added chemicals by mixed cultures and can also be applied in combined bioenergy production systems. Biochar is generated as a by-product of waste biomass pyrolysis, which is featured with a high proportion of carbon and porous structure and has been widely used as a soil amendment in agroecosystems. In Japan, about two million tons of rice husk are produced annually in the rice threshing process. Part of the rice husk is used in composting but a big fraction was still not properly used. Open burning of rice husks could cause serious climate change issues due to air pollution and has been prohibited by environmental regulations in Japan.

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Keywords

Anaerobic digestion · Bioaugmentation · Co-digestion · Direct interspecies ·
Psychrophilic anaerobes · Metagenomics · Microalgal biomass

Abbreviations

ACF	Accumulation continuous flow system
AD	Anaerobic digestion
AF	Anaerobic filter
AFP	Antifreeze proteins
AH	Anaerobic hybrid reactor
AHS	Anaerobic hybrid septic
AMBR	Anaerobic migrating blanket reactor
AnIBPR	Anaerobic immobilized bio-plate reactor
AnMBR	Anaerobic membrane bioreactor
AnSMBR	Anaerobic submerged membrane bioreactor
ASBR	Anaerobic sequencing batch reactor
BES	Bioelectrochemical systems
COD	Chemical oxygen demand
CPH	Cationic polymer equipped hybrid
DIET	Direct interspecies electron transfer
EGSB	Expanded granular sludge bed reactor
GAC	Granular activated carbon
HRT	Hydraulic retention time
HUSB	Hydrolytic up-flow sludge bed
LCA	Life cycle assessment
LCFA	Long-chain fatty acid
OLR	Organic loading rate
PTF	Polyurethane foam trickling filter
SBR	Sequencing batch reactor
UASB	Up-flow anaerobic sludge bed reactor
USFA	Unsaturated fatty acid
VFAs	Volatile fatty acids
ΔG	Gibbs free energy

4.1 Introduction

Energy, which is a crucial and vital ingredient for the modern development of economic activities of our society, is largely derived from fossil fuels causing accumulation of greenhouse gases (Kumar 2001, 2018, 2020a, b, c, 2021, Zandi et al. 2018; Kumar et al. 2018a, b, 2020, 2022a, b; IPCC 2021). About 600 Tg of

methane is produced largely (around 70%) per year due to the activity of methanogenic archaea (Conrad 2009). Thus, methanogenesis is important for the global carbon cycle. However, methanogens can be used for several applications, e.g., energy supply and the production of high-value compounds in the chemical industry (Enzmann et al. 2018). The potential contribution of anaerobic digestion to GHG reduction has been computed for the 27 EU countries on the basis of their 2005 Kyoto declarations and using life cycle data. Anaerobic digestion (AD) has been used widely as a form of energy recovery by biogas production from the organic fraction of municipal solid wastes (OFMSW) (Seruga et al. 2018).

The methanogens are also used in microbial electrosynthesis using CO₂ and electrical power to generate methane. As a result, anaerobic digestion (AD) has been emerging as an efficient and viable solution for fuel production from biowastes to alleviate environmental pollution and produce green energy (Wang et al. 2021a, b). Anaerobic digestion (AD) originated from dung, wood, sawdust, grass, and other plants, and paper waste (PW) is mainly composed of lignocellulosic matter and can be applied in AD (Ali et al. 2019; Li et al. 2020). However, the intrinsic lignocellulosic structure retards the decomposition of organic matter and negatively affects the biodegradation process (Abraham et al. 2020). Roy and Kumar (2013) reviewed the challenges faced during lignocellulose bioconversion and latest technologies to overcome one of the major hurdles in the process—the pretreatment procedure. The bioconversion process is however very complex and still requires many innovations.

4.2 Anaerobic Digestion

The process of composting is a source of greenhouse gases (GHG) that contribute to climate change. However, anaerobic digestion (AD) involves a consortium of microorganisms that convert substrates into biogas containing methane for renewable energy. Tilche and Galatola (2008) reported that anaerobic digestion has experienced several waves of technological development starting from wastewater treatment systems in the 1970s and showed promise as an alternative energy source in the 1980s. Subsequently, it became a standard for treating organic matter-rich industrial wastewater, and more recently returned to the market for its energy recovery potential, making use of different biomasses, including energy crops. Theuer et al. (2020) reviewed anaerobic digestion of livestock manure for the production of biogas. It provides a number of benefits creating interest in the technological application in many countries all over the world (Tabatabaei and Ghanavati 2018). They suggested several benefits of anaerobic digestion. Firstly, it produces methane (CH₄) that can be used for the generation of electricity, heat, and fuels, and the production of biogas remains independent of weather conditions. Secondly, it reduces greenhouse gas emissions from livestock husbandry (Meyer-Aurich et al. 2012; Agostini et al. 2015) at relatively low mitigation costs (Kalt et al. 2020; Scholz et al. 2011) by substituting fossil fuels, avoiding CH₄ and nitrous oxide (N₂O) emissions from manure storage, replacing synthetic fertilizers, and decreasing

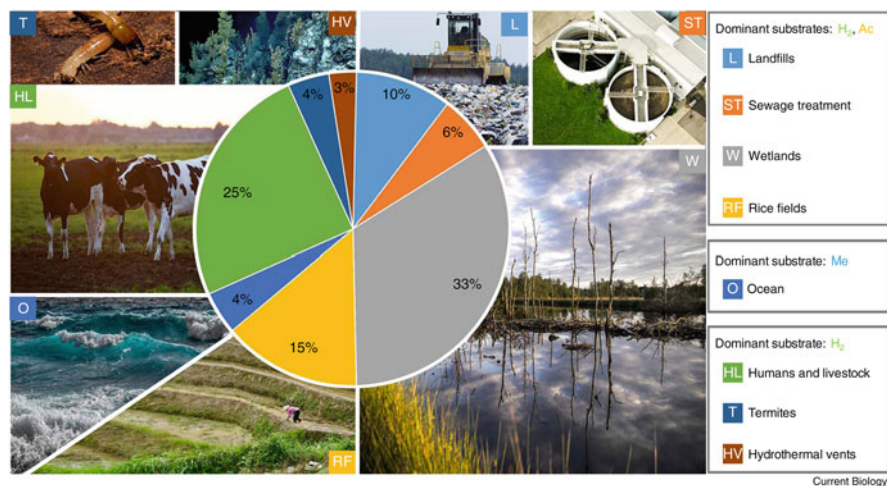


Fig. 4.1 Sources of biological methane emissions to the atmosphere. Methanogens are found in a wide range of habitats, where they actively contribute 70% of the 500–600 Tg of methane introduced to the atmosphere each year. Percentages are the contributions of each habitat to the biological portion of the global methane emission budget. The ecology of each habitat largely determines the dominant substrates and probably selects for the types of methanogens found therein. Hydrogenotrophic methanogens are found in H_2 -rich habitats, acetoclastic methanogens in acetate (Ac)-rich habitats, and methylotrophic methanogens in habitats rich in methyl compounds (Me). Source: Lyu, Z., Shao, N., Akinyemi, T., & Whitman, W. B. (2018). *Current Biology*, 28 (13). 727–732. Reproduced with permission License number 4970610534657 date 16.12.2020 from RightsLink

N_2O emissions after field application of digestates (Massé et al. 2011). Thirdly, anaerobic digestion of livestock manure improves organic fertilizer quality compared with undigested manure due to a better availability of important crop nutrients such as ammonium, phosphate, and potassium and simultaneously improves soil structure and increases the soil organic matter content (Arthurson 2009). Moreover, biological degradation during anaerobic digestion can decrease the concentrations of weed seeds (Baute et al. 2016), pathogens (Fröschle et al. 2015), and antibiotics (Massé et al. 2014). Conceptually, the microbial processes of AD can be described by the sequential steps of hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Bitton 2005; Venkiteswaran et al. 2015).

Anaerobic digestion is carried out in a variety of modes (i) batch or continuous; (ii) single, double, or multiple steps (staged digesters); and (iii) vertical or horizontal treatment units. Different mixing methods are used. For “dry” (high solids) or “wet” digestion (low solids) concentration, a simple digester design consists of a single, suitably shaped, static, or mixed digester which operates under selected conditions (Venkiteswaran et al. 2015). Different substrates can be used for AD (Fig. 4.1).

4.2.1 Pretreatment Methods

4.2.1.1 Wastewater Treatment

EU Water Framework Directive, the Oslo-Paris Convention, and oil operators in Norway are committed to the “zero discharge” of pollutants into the sea (Fakhru’l-Razi et al. 2009). According to Deng et al. (2021), the average limits of oil and grease discharge and chemical oxygen demand (COD) are 10 and 100 mg/L, respectively, in China.

Although oil and gas production is one of the most important industrial activities of modern civilization, the knowledge of microbial ecology and microbial interactions determining the efficiency of plants for oil-produced wastewater is limited (Deng et al. 2021). Microbial characteristics are important to the performance of oil-produced biological treatment processes using plants. They performed 16S rDNA amplicon sequencing of microbes that inhabited the plants from oil and marine associated environments. Biological wastewater treatment processes are regulated by many factors, such as local conditions, influent characteristics, reactor design, and operational parameters (Fig. 4.2).

Microbial communities determine the efficiency of biological treatment processes (Jiménez et al. 2018). Unlike information about microbial composition in municipal wastewater treatment plants investigated in many studies, information about the microbial composition characteristics, especially microbial origin, of oil-produced wastewater is limited (Deng et al. 2021).

The sequencing of 16S ribosomal (RNA) gene from ashore oil-water (AOW) microbial community indicated bacteria affiliated within the genera *Desulfovibrio*, *Flexistipes*, *Pseudomonas*, *Novispirillum*, *Halanaerobium*, *Sphaerochaeta*, *Acholeplasma*, *Marinobacterium*, and *Marinobacter* but often cannot explain microbial function variation (Deng et al. 2021).

4.2.1.2 Microbial Pretreatment

Roy and Kumar (2013) reviewed pretreatment methods. Wang et al. (2021a, b) reported that microbial pretreatment to lignocellulosic biomass for anaerobic digestion (AD) has achieved increasing attention. Microbial community analyses showed that microbial responses to oxygen varied significantly with microbial consortium, which consequently caused different AD performances.

4.3 Methane

Methane (CH₄) accounts for 14% of total global greenhouse gas emissions and is the second largest contributor to global warming (Intergovernmental Panel on Climate Change 2007). However, the atmospheric methane concentration has more than doubled since the start of the industrial era (Lyu et al. 2018). Methane is produced in the rumen and hindgut of animals by a group of Archaea known collectively as methanogens, which belong to the phylum Euryarchaeota (Hook et al. 2010). Ruminant livestock represent the single largest anthropogenic source of the potent

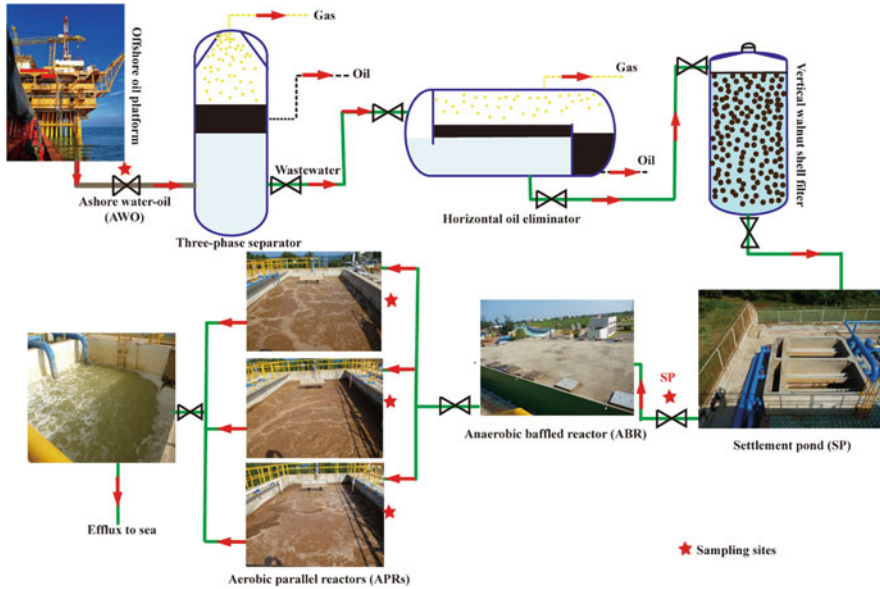


Fig. 4.2 A schematic overview of the full-scale offshore-produced water treatment plant, red stars showing the sampling sites. Wastewater from three separators is settled in a horizontal oil eliminator with an upper oil slick, and then the lower water phase is filtrated through a vertical walnut shell filter and flows into a settlement pond (SP). These processes are followed by downstream biological treatment processes with a maximum treatment capacity of 1000 m³/day. Wastewater flows into an ABR and is treated for 12 h; then the effluent from the ABR is treated in three APRs (APR1, APR2, and APR3) for 12 h. Finally, treated water is discharged to sea. Source: Deng S, Wang B, Zhang W, Su S, Dong H, Banat IM, et al. (2021) Elucidate microbial characteristics in a full-scale treatment plant for offshore oil produced wastewater. PLoS ONE 16(8): e0255836. 10.1371/journal.pone.0255836. Reproduced from Copyright: © 2021 Deng et al. as an open access article distributed under the terms of the Creative Commons Attribution License

greenhouse gas methane, which is generated by methanogenic archaea residing in ruminant digestive tracts (Shi et al. 2014). The majority of methane in nature is derived from acetate (Galagan et al. 2002). Methanogenesis, the biological production of methane, plays a pivotal role in the global carbon cycle and contributes significantly to global warming (Lyu et al. 2018). However, methane production for biogas components is good for the environment protection and greenhouse gas mitigation under controlled conditions.

Besides the canonical methanogenesis pathways described below and syntrophic interactions of methanogens and bacteria, methanogens are capable of methane generation from additional substrates (see review by Kurth et al. 2020). Kurth et al. (2020) provided extended list which is presented in Fig. 4.3.

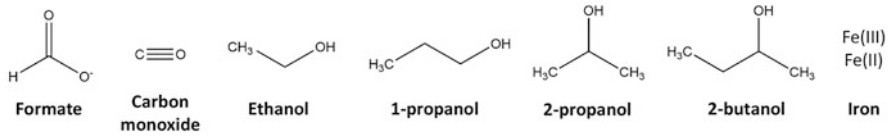
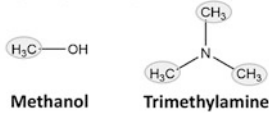
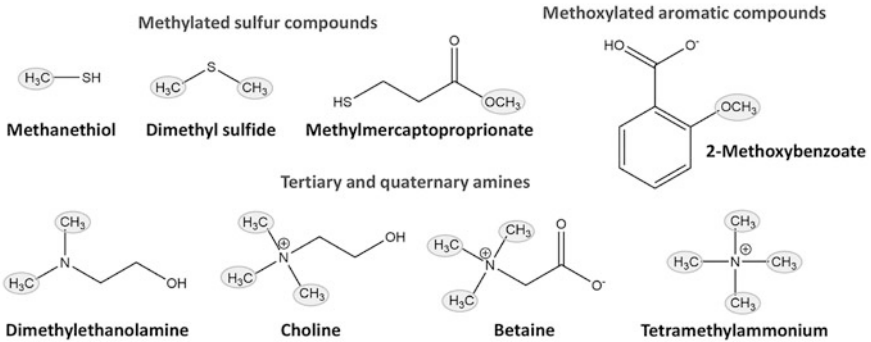
Additional/alternative substrates for hydrogenotrophic methanogenesis**Hydrogen-dependent methylotrophic methanogenesis****Additional/alternative substrates for methylotrophic methanogenesis**

Fig. 4.3 Extended substrate range of methanogens. 2-Methoxybenzoate is only one example for methoxylated aromatic compounds that can be used for methanogenesis (Mayumi et al. 2016). Source: Kurth, J.M., Op den Camp, H.J.M. & Welte, C.U. Several ways one goal—methanogenesis from unconventional substrates. *Appl Microbiol Biotechnol* 104, 6839–6854 (2020). <https://doi.org/10.1007/s00253-020-10724-7>. This is an open-access article distributed under the terms of the [Creative Commons CC BY](https://creativecommons.org/licenses/by/4.0/) license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

4.4 Methanogens

Methanogens are a relative diverse group of archaea and can be found in various anoxic habitats (Garcia et al. 2000). Methanogens are the only known microorganisms capable of methane production, making them of interest when investigating methane abatement strategies (Hook et al. 2010). Methanogens belong to the Euryarchaeota of Archaeobacteria and can convert inorganic-organic compounds into methane and carbon dioxide. The final product, biogas, is a mixture of methane (CH_4), carbon dioxide (CO_2), and other trace gases and is considered to be a suitable future replacement for fossil oil (Ren et al. 2008). Among livestock, methane production is greatest in ruminants, as methanogens are able to produce methane freely through the normal process of feed digestion. Much research has

been directed toward methane abatement strategies to be used in ruminants and has been reviewed elsewhere (Shibata and Terada 2010). Abatement strategies are often limited by the diet fed, the management conditions, physiological state, and use of the animals.

Methanogens are biocatalysts, which have the potential to contribute to a solution for future energy problems by producing methane as a storable energy carrier (Enzmann et al. 2018). According to Pfeifer et al. (2021), methanogens thrive in habitats from hot vents in the deep oceans to ice-cold permafrost soils; in rice field soils, freshwater, and marine sediments; as well as in the intestine and oral cavity of animals. Chaudhary et al. (2018) reported methane Archaea from the human skin microbiome, and they are proposed to play a role in ammonia turnover. Methanogens use a limited range of substrates, including CO_2/H_2 , formate, acetate, and methyl compounds (Hook et al. 2010). Shi et al. (2014) studied methane yield phenotypes linked to differential gene expression in the sheep rumen microbiome. Only a few rumen methanogens have been cultivated or characterized in detail, and their respective contributions to CH_4 production under in vivo conditions in live-stock remain poorly defined (Buddle et al. 2011). It was reported that almost a third (28%) of anthropogenic CH_4 emissions are due to enteric fermentation in livestock (Yusufa et al. 2012) which will rise further due to an increased worldwide demand for meat, milk, and other animal products. Measurements of ruminant CH_4 emissions are mainly from animal trials in which the effects of particular diets or inhibitors of CH_4 formation were assessed (Martinez-Fernandez et al. 2014).

Acetate is the major source of methane in nature (Wang et al. 2011). The majority of investigations have focused on acetotrophic methanogens for which energy-conserving electron transport is dependent on the production and consumption of H_2 as an intermediate, although the great majority of aceto-autotrophs are unable to metabolize H_2 . The presence of cytochrome c and a complex (Ma-Rnf) homologous to the Rnf (*Rhodobacter* nitrogen fixation) complexes is distributed in the domain. Bacteria distinguish non- H_2 -utilizing *Methanosarcina acetivorans* from H_2 -utilizing species suggesting fundamentally different electron transport pathways. The membrane-bound electron transport chain of acetate-grown *M. acetivorans* provides an understanding of acetotrophic methanogens.

4.4.1 Phylogeny and Habitats of Methanogens

Enzmann et al. (2018) reported that the methanogenic archaea belonged exclusively to the phylum Euryarchaeota. Earlier the methanogens were classified first into five orders, namely, Methanococcales, Methanobacteriales, Methanosarcinales, Methanomicrobiales, and Methanopyrales, but two more orders Methanocellales and Methanomassiliicoccales were added to it (Iino et al. 2013). Hydrogenotrophic methanogenesis from H_2 and CO_2 is found in almost all methanogenic orders with the exception of the Methanomassiliicoccales (Enzmann et al. 2018). Methane formation from acetate, called aceticlastic methanogenesis, can be found only in the order Methanosarcinales. In addition to phyla Euryarchaeota, two new phyla,

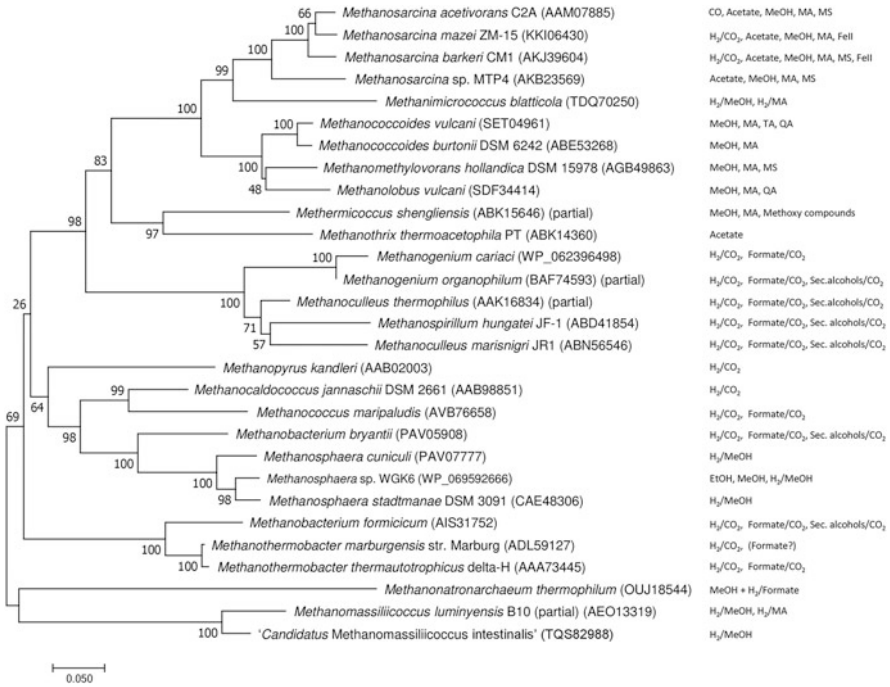


Fig. 4.4 Evolutionary relationships of methyl-coenzyme M reductase (subunit A) of different methanogens. The evolutionary history was inferred using the neighbor-joining method. The optimal tree with the sum of branch length = 3.29201331 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Dayhoff matrix-based method and are in the units of the number of amino acid substitutions per site. The analysis involved 29 amino acid sequences. All ambiguous positions were removed for each sequence pair. There were a total of 583 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2019). MA, methylamines; MS, methylated sulfur compounds; TA, tertiary amines; QA, quaternary amines. Source: Kurth, J.M., Op den Camp, H.J.M. & Welte, C.U. (2020) Several ways one goal—methanogenesis from unconventional substrates. *Appl Microbiol Biotechnol* 104, 6839–6854 . <https://doi.org/10.1007/s00253-020-10724-7>. This is an open-access article distributed under the terms of the Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

namely, Bathyarchaeota (Evans et al. 2015) and the Verstraetearchaeota (Vanwonterghem et al. 2016), have been postulated. Genome sequences from both phyla indicate a methylotrophic methane metabolism in these—as of yet uncultivated—potential methanogens.

Kurth et al. (2020) compared evolutionary relationships of methyl-coenzyme M reductase (subunit A) of different methanogens (Fig. 4.4).

Enzmann et al. (2018) reported that in biogas plants, due to hydrolysis of complex polymers to sugars and amino acids, followed by fermentation and acetogenesis, acetate, H_2 , and CO_2 are produced as substrates for methanogenesis. Therefore, hydrogenotrophic and acetoclastic methanogens are prevalent in mesophilic biogas plants, often dominated by species of *Methanosarcina* (*Methanotherix* at low acetate concentrations) or *Methanoculleus*. However, under certain conditions syntrophic acetate oxidation may be the dominant path toward methane (Westerholm et al. 2016).

4.5 Methanogenesis

Methanogenesis is a microbial-related process relevant to methane production, i.e., acidification, hydrolysis, and methanogenesis. The microbial community has the populations of key acidogens (e.g., *Longilinea* sp.) and methanogens (e.g., *Methanosaeta* sp.). According to Sowers (2009), biological methanogens are prokaryotic single-cell microorganisms, classified as methanogenic Archaea. The first photosynthetically active life developed in the Archean, about 2.4 billion years ago (Knesting et al. 2020). These microorganisms require highly reduced, anaerobic conditions for growth. Methanogenesis is ubiquitous in environments including deep subsurface rocks, submarine hydrothermal vents, freshwater and marine sediments, ruminants, and even the human colon.

Although most of the methane (CH_4) produced is oxidized to carbon dioxide (CO_2) by methane-consuming organisms, substantial quantities (ca. 1014 g/year) escape into the atmosphere where it acts as a potent greenhouse gas (Thauer et al. 2008). Methanogenesis is the terminal step in biomass degradation in many anaerobic environments and plays a central role in the global carbon cycle.

Most methanogens produce CH_4 by reducing CO_2 with hydrogen gas (H_2) (Deppenmeier 2002). However, some *Methanosarcina* species such as *M. barkeri* and *M. mazei* also are capable of using a variety of other substrates, including carbon compounds such as methanol, methyl sulfides, and methylamines (Deppenmeier 2004) and acetate, which accounts for ca. 2/3 of global CH_4 production (Ferry 1992).

The dominant source of CH_4 emissions from livestock is from ruminants (Naqv 2011), where CH_4 is formed as a by-product of feed fermentation in the forestomach (rumen) by CH_4 -producing archaea, known as methanogens (Boone et al. 1993). Thauer et al. (2008) focused on the energy metabolism of methanogenic archaea growing on H_2 and CO_2 , with the emphasis on differences between methanogens with and without cytochromes. The pathways are coupled to the generation of an electrochemical sodium ion gradient and an electrochemical proton gradient. Both ion gradients are used directly for ATP synthesis via membrane integral ATP synthases. The function of the abovementioned systems and their components in the metabolism of methanogens is described in detail by Enzmann et al. (2018). Molecular hydrogen is mainly produced by carbohydrate fermentation and then used by methanogens to produce CH_4 in the rumen (Ma 2019). Rumen methanogenesis mainly involves three types of methanogenic metabolic pathways, which includes hydrogenotrophic (reduction of CO_2 coupled to the oxidation of H_2), methylotrophic

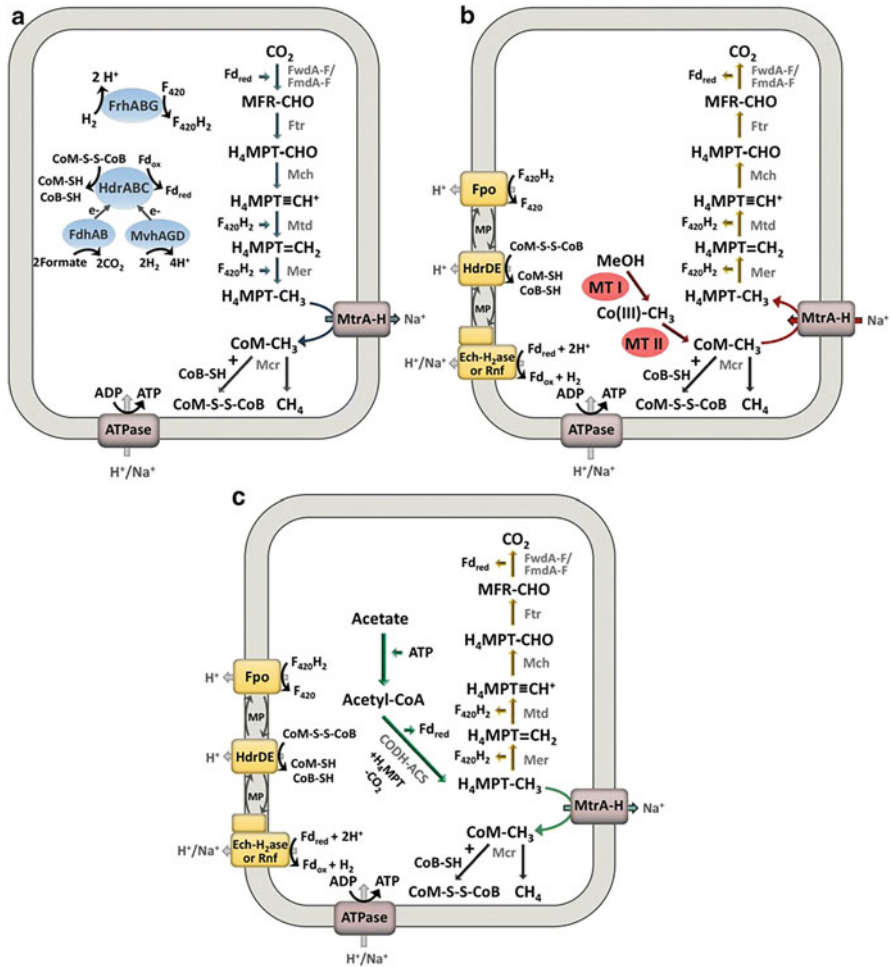


Fig. 4.5 Kurth et al. (2020) depicted three methods of methanogenesis: (a) hydrogenotrophic, (b) methylotrophic, and (c) acetoclastic methanogenesis pathways. The ferredoxin electron carrier is a two-electron carrier. Some methanogens use a H₄MPT derivative called tetrahydrosarcinopterin (H₄SPT). The Na⁺/H⁺ translocation stoichiometry is not represented in the figure. FwdA-F/FmdA-F, formylmethanofuran dehydrogenase; Ftr, formylmethanofuran-tetrahydromethanopterin formyl-transferase; Mch, methenyl-tetrahydromethanopterin cyclohydrolase; Mtd, methylenetetrahydromethanopterin dehydrogenase; Mer, 5,10-methylenetetrahydromethanopterin reductase; MtrA-H, tetrahydromethanopterin S-methyl-transferase; McrABCDG, methyl-coenzyme M reductase; FrhABG, coenzyme F₄₂₀-reducing hydrogenase; HdrABC, soluble heterodisulfide reductase; MvhAGD, F₄₂₀-nonreducing hydrogenase; FdhAB, formate dehydrogenase; FpoA-O, F₄₂₀H₂ dehydrogenase; HdrDE, membrane-bound heterodisulfide reductase; Ech-H₂ase, energy-converting hydrogenase; Rnf, Na⁺ -translocating ferredoxin; NAD⁺, oxidoreductase complex; ATPase, ATP synthase; CODH-ACS, acetyl-CoA decarbonylase/synthase; MT I and MT II, methyltransferase; CoB, coenzyme B; CoM, coenzyme M; H₄MPT, tetrahydromethanopterin; MFR, methanofuran; Fd, ferredoxin; F₄₂₀H₂, reduced coenzyme F₄₂₀; MP, methanophenazine; CO(III), cobalamin binding protein. Source: Kurth, J.M., Op den Camp,

(conversion of methyl group-containing compounds), and acetoclastic pathways (Rother and Krzycki 2010). Three main methanogenesis pathways (hydrogenotrophic, methylotrophic, and acetoclastic) share the core pathway of methanogenesis yet also differ in many aspects of their biochemistry and physiology (Fig. 4.5).

4.5.1 Hydrogenotrophic Archaea

According to Lyu et al. (2018), methanogenesis is an anaerobic respiration that uses oxidized carbon such as CO_2 as a terminal electron acceptor and generates methane as the final product of metabolism. $\text{H}_2 + \text{CO}_2$ and formate are converted to CH_4 via the CO_2 -reducing pathway, while methanol and methylamines are metabolized by the methylotrophic pathway. They are the predominant source of methanogenesis in deep marine sediments, termite hindguts, and human and animal gastrointestinal tracts, which altogether contribute a third of biologically generated methane emissions. Thus, methanogens are common in habitats that are poor in other electron acceptors, such as O_2 , NO_3 , Fe^{3+} , and SO_4^{2-} . Deppenmeier (2004) reported that members of the genus *Methanosarcina* are strictly anaerobic archaea, deriving their metabolic energy from the conversion of a restricted number of substrates to methane. During hydrogenotrophic methanogenesis, H_2 is oxidized to H^+ , and CO_2 is a terminal electron acceptor and generates CH_4 . The H_2 -dependent CO_2 reduction proceeds via carrier-bound C_1 intermediates which become stepwise reduced to methane (Fig. 4.5a).

4.5.2 Methylotrophic Methanogens

Methylotrophic methanogens are common in marine and hypersaline, sulfate-rich sediments where they utilize methylated compounds such as trimethylamine, dimethyl sulfate, and methanol. New members of this group have recently been found in habitats such as the bovine rumen. Methanomassiliicoccales occur in a large variety of anoxic habitats including wetlands and animal intestinal tracts especially in ruminant animals. They likely are among the major methane producers. Considering the role of methane as potent greenhouse gas, resolving the methanogenic nature of a broad range of putative novel methylotrophic methanogens and assessing their role in methane emitting environments are pressing issues for future research on methanogens (Söllinger and Urich 2019) (Fig. 4.5b).

Fig. 4.5 (continued) H.J.M. & Welte, C.U. Several ways one goal—methanogenesis from unconventional substrates. *Appl Microbiol Biotechnol* 104, 6839–6854 (2020). <https://doi.org/10.1007/s00253-020-10724-7>. This is an open-access article distributed under the terms of the [Creative Commons CC BY](#) license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

4.5.3 Aceticlastic Methanogens

Aceticlastic methanogens split acetate to form CH_4 and CO_2 . Biological methanogenesis from acetate is one of the most important processes for the maintenance of the carbon cycle on Earth. Galagan et al. (2002) analyzed the complete genome sequence of an acetate-utilizing methanogen, *Methanosarcina acetivorans* C2A. *Methanosaeta* are obligate aceticlastic methanogens that are known to use only acetate or acetate plus electrons obtained via direct interspecies electron transport (DIET) (Venkiteshwaran et al. 2015). Aceticlastic methanogens are the dominant methane producers in anaerobic digesters, rice fields, and wetlands. *Methanosarcina mazei* belongs to the group of aceticlastic methanogens and converts acetate into the potent greenhouse gases CO_2 and CH_4 . The aceticlastic respiratory chain involved in methane formation comprises the three transmembrane proteins Ech hydrogenase, F420 nonreducing hydrogenase, and heterodisulfide reductase. The energy-conserving transmembrane enzyme system used in the aceticlastic pathway of methanogenesis has been referred to as Fd/heterodisulfide oxidoreductase. The electron flow from Fdred to heterodisulfide reductase in *Methanosarcina mazei* has been reconstructed in recent years (Kurth et al. 2020) (Fig. 4.5c).

4.6 Improvement in Methane Production

4.6.1 Nano-Biochar

Zhang and Wang (2021) suggested catalytic use of biochar-supported nano-zerovalent iron (nZVI-BC) on anaerobic co-digestion (co-AD). Methane production potential (R_0) and daily methane production rate (G_m) of sewage sludge and food waste were enhanced with the catalyst as compared to normal biochar.

4.6.2 Bioaugmentation

The plant residues obtained after the phytoremediation process can be used in anaerobic digestion (Das and Kumar this volume). However, such residues still contain high concentrations of HMs which inhibit the digestion efficiency of plant residues. Mao et al. (2021) reported that bioaugmentation is an effective method to improve the degradation efficiency and methane yield of plant residues rich in HMs. They isolated cellulose-degrading anaerobic bacteria from cow dung, *Paracoccus* sp., termed strain LZ-G1 which degraded cellulose and simultaneously adsorbed Cd^{2+} . Thus, bioaugmentation provides an easy and a feasible method for the actual on-site treatment of HM-rich phytoremediation residues.

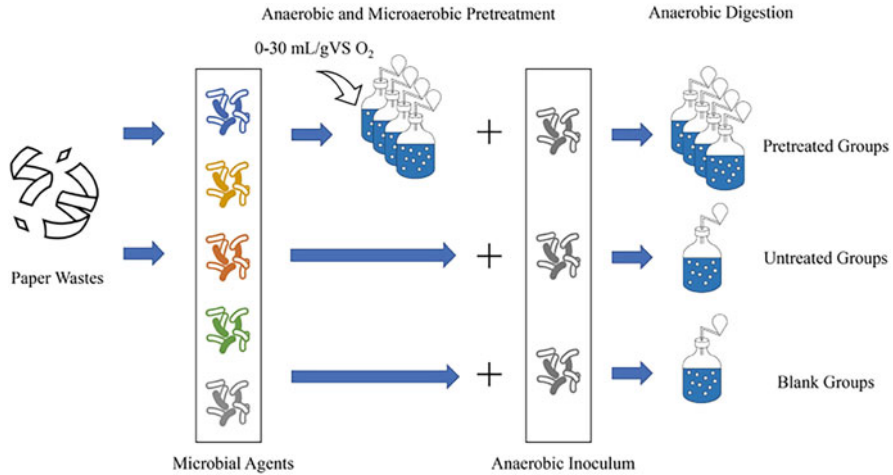


Fig. 4.6 Schematic diagram of the experimental setup. Song, C., Li, W., Cai, F., Liu, G., & Chen, C. (2021). Anaerobic and Microaerobic Pretreatment for Improving Methane Production From Paper Waste in Anaerobic Digestion. *Frontiers in Microbiology*, 12, 1520. <https://doi.org/10.3389/fmicb.2021.688290>. At Frontiers, the entire content of all present and past journals is immediately and permanently accessible online free of charge and published under the CC-BY license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original authors and the source are credited

4.6.3 Ultrasound Pretreatment

Zerrouki et al. (2021) investigated the use of ultrasound pretreatment as potential technique to solubilize organic matter and fermentation of fruit juice effluents in anaerobic batch reactor.

4.6.4 Micro-Oxygenic Treatment

Zhen et al. (2021) reported that micro-oxygen pretreatment, i.e., supplying a small amount of oxygen during microbial pretreatment of kitchen waste, shortened the fermentation cycle and improved the gas production efficiency of anaerobic fermentation. Recently, anaerobic and micro-aerobic pretreatment has attracted attention as it overcomes the obstacles of biogas production. In micro-aerobic condition, the stimulated microbes showed higher hydrolysis and acidogenesis activity (Xu et al. 2014). Microbial community analysis by Song et al. (2021) showed that *Clostridium sensu stricto 1* and *Clostridium sensu stricto 10* possessed high relative abundance after anaerobic pretreatment by straw-decomposing inoculum (SI), while *Bacteroides* and *Macellibacteroides* were enriched after micro-aerobic pretreatment by sheep manure (SM) which were all contributable to the cellulose degradation (Fig. 4.6). Zhen et al. stated that after the micro-aerobic pretreatment, *Firmicutes* and

Bacteroidetes were the predominant phyla during the AD of rice straw (Zhen et al. 2021).

4.6.5 Role of Temperature

4.6.5.1 Mesophilic and Thermophilic Temperature

Jiang et al. (2021) worked on in situ hydrogen biomethanation technology to upgrade biogas production. They reported that efficiency of biomethanation relies on various parameters, e.g., temperature, gas supplement, and hydrogenotrophic methanogens. They further reported that the hydrogenotrophic methanogenesis was performed better at thermophilic condition, while the dominant archaea genera *Methanobacterium* and *Methanothermobacter* performed better at mesophilic and thermophilic temperature, respectively. Jiang et al. (2021) concluded that the highest CH₄ content (greater than 90%) was obtained when H₂ and CO₂ were feeding at ratio of 4:1 and *Methanothermobacter* was dominant.

4.6.5.2 Psychrophilic Temperature

Tiwari et al. (2021) presented a future outlook on psychrophilic anaerobic digestion (AD). In this technique, the low temperature acclimated microbial biomass are used to overcome thermodynamic constraints through expression of cold-adapted enzymes obtained by genotypic and phenotypic variations. They suggested that bioaugmentation with psychrophilic strains could reduce start-up time and ensure daily stable performance for wastewater treatment facilities at low temperatures (Fig. 4.7).

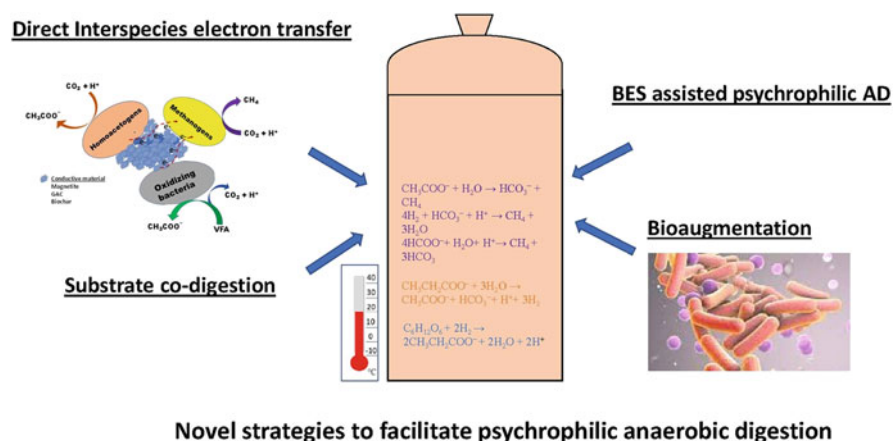


Fig. 4.7 Novel strategies to facilitate psychrophilic anaerobic digestion. Source: Tiwari, B. R., Rouissi, T., Brar, S. K., & Surampalli, R. Y. (2021). Critical insights into psychrophilic anaerobic digestion: novel strategies for improving biogas production. Waste Management, 131, 513–526. 10.1016/j.wasman.2021.08.041. Reproduced with license no. 5126880444981 from RightsLink dated 13 August 2021.

4.6.6 Effects of Silver Nanoparticles

Grosser et al. (2021) investigated the effects of nanoparticles on performance and stability of anaerobic digestion of sewage sludge in four reactors. They included sewage sludge (control reactor), and remaining ones were fed with sewage sludge with the addition of the following additives: (1) silver nanoparticles (NPs reactor), (2) ionic silver (AgNO_3 reactor), and (3) diluent used for nanoparticles (DIS reactor). They reported a fivefold increase in the number of *Methanosarcina* genus in Ag-NPs reactor compared to the control reactor.

4.7 Biotechnology of Archaea

According to Pfeifer et al. (2021), biotechnology can be defined as any technological application using biosystems, organisms, or derivatives thereof, to manufacture or modify bioproducts or to develop and engineer processes for specific application. Extracellular electron exchange in *Methanosarcina* species and closely related Archaea plays an important role in the global carbon cycle and enhances the speed and stability of anaerobic digestion by facilitating efficient syntrophic interactions (Holmes et al. 2019). During recent years, genetic tools for methanogens have been improved, opening a new field of research on these important microorganisms. Archaea represent a novel domain of life distinct from bacteria and eucarya (formerly known as eukaryotes). Nayak and Metcalf (2017) described the development of a Cas9-mediated genome-editing tool that allows facile genetic manipulation of the slow-growing methanogenic archaeon *Methanosarcina acetivorans* (see also Neumann et al. 2020).

Hassa et al. (2018) have reported that taxonomic profiling of biogas-producing communities by means of high-throughput 16S rRNA gene amplicon sequencing provided high-resolution insights into bacterial and archaeal structures of AD assemblages and their linkages to fed substrates and process parameters (Figs. 4.8 and 4.9).

They further characterized specific marker genes (Fig. 4.6) for direct and immediate insights into microbial community compositions and the phylogenetic relationship of community members by their PCR amplification from whole community (metagenomic) DNA. A widely and commonly used approach for microbial community profiling without prior cultivation is the analysis of the 16S small subunit ribosomal RNA (rRNA) gene sequence (Lebuhn et al. 2014; Simó et al. 2014).

4.7.1 Synthetic Genes for Industrial Products Production

The rapid autotrophic growth of the methanogenic archaeon *Methanococcus maripaludis* on H_2 and CO_2 makes it an attractive microbial chassis to inexpensively produce biochemicals (Lyu et al. 2018). Lyu et al. (2018) reported that a synthetic gene encoding geraniol synthase (GES) derived from *Ocimum basilicum* was cloned

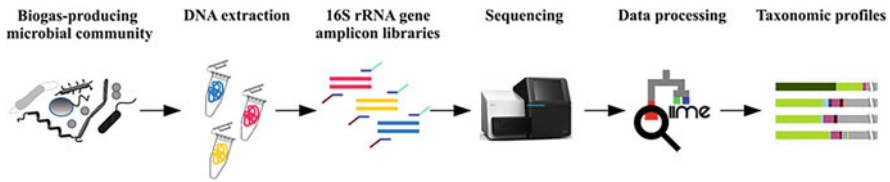


Fig. 4.8 Schematic overview on taxonomic profiling of biogas-producing microbial communities applying 16S rRNA gene amplicon sequencing. After extraction of whole community DNA, 16S rRNA gene amplicon libraries were constructed and subsequently sequenced. Obtained sequences were processed with the program QIIME (Caporaso et al. 2010) to calculate taxonomic community profiles. Source: Hassa J, Maus I, Off S, et al. 2018. Metagenome, metatranscriptome, and metaproteome approaches unraveled compositions and functional relationships of microbial communities residing in biogas plants. *Appl Microbiol Biotechnol.*; 102(12):5045–5063. <https://doi.org/10.1007/s00253-018-8976-7>. **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made

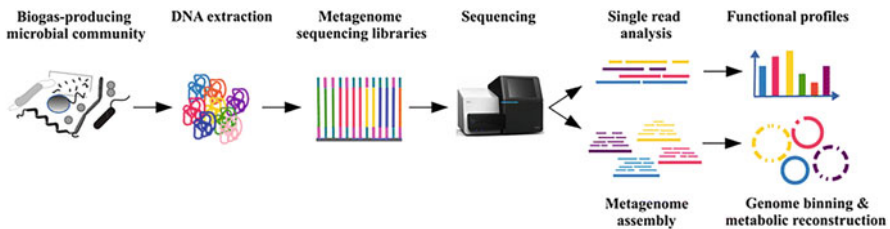


Fig. 4.9 Hassa et al. (2018) depicted workflow for functional profiling of microbial biogas communities exploiting metagenome sequence data. After sampling at biogas reactors, total DNA was extracted for construction of whole metagenome shotgun libraries which were subsequently sequenced on high-throughput sequencing platforms. Resulting sequencing data were quality checked and functionally characterized based on single read sequences in order to deduce functional profiles of the underlying biogas community. Moreover, metagenome assembly followed by a binning approach was applied to compile MAGs, which were then analyzed for their metabolic potential. Source: Hassa J, Maus I, Off S, et al. 2018 Metagenome, metatranscriptome, and metaproteome approaches unraveled compositions and functional relationships of microbial communities residing in biogas plants. *Appl Microbiol Biotechnol.*; 102(12):5045–5063. <https://doi.org/10.1007/s00253-018-8976-7>. **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made

into a *M. maripaludis* expression vector. Recombinant expression of GES in *M. maripaludis* during autotrophic growth on H₂/CO₂ or formate yielded geraniol at 2.8 and 4.0 mg g⁻¹ of dry weight, respectively. A conceptual model centered on the autotrophic acetyl coenzyme. Thus, a biosynthetic pathway alteration strategy can divert more autotrophic carbon flux to geraniol production (Lyu et al. 2018).

The discovery of a methanogen that can conserve energy to support growth solely from the oxidation of organic carbon coupled to the reduction of an extracellular electron acceptor expands the possible environments in which methanogens might thrive (Holmes et al. 2019), e.g., introduction of a bacterial esterase allowed *M. acetivorans* to grow on methyl esters (like methyl acetate and methyl propionate) (Lessner et al. 2010). Expression of the gene encoding 3-hydroxybutyryl-CoA dehydrogenase (Hbd) from *Clostridium acetobutylicum* resulted in formation of L-lactate (0.59 g/g methane) from methane with acetate as intermediate, possibly by Hbd exhibiting lactate dehydrogenase activity in the heterologous host (McAnulty et al. 2017). Thus, the principal possibility might exist to engineer *M. acetivorans* for industrial production.

Timmers et al. (2017) reported “trace methane oxidation” (i.e., “reverse methanogenesis”) in wild-type methanogens during net methane production. Heterologous expression in *M. acetivorans* of genes encoding methyl-CoM reductase from anaerobic methanotrophic archaea (ANME-1) resulted in a strain that converted methane to acetate three times faster than the parental strain (Soo et al. 2016). The same holds true for the production of other high-value products like amino acids or vitamins with methanogens, and due to their slow growth, a technical application is not yet developed (Schiraldi et al. 2002). However, new processes may emerge using heterologous methanogens.

4.8 Extracellular Electron

Anaerobic digestion (AD) of organic waste to produce biogas is a mature biotechnology commercialized for decades (Feng et al. 2021). Some of the recent advances are presented here. Extracellular electron exchange in *Methanosarcina* species and closely related Archaea plays an important role in the global carbon cycle and enhances the speed and stability of anaerobic digestion by facilitating efficient syntrophic interactions.

4.8.1 Mineralization

Iron corrosion is an electrochemical process involving oxidation of metallic iron [Fe (0)] to Fe(II) (anodic reaction; Eq. (1)) and reduction of external electron acceptors (cathodic reaction) (Fig. 4.10).

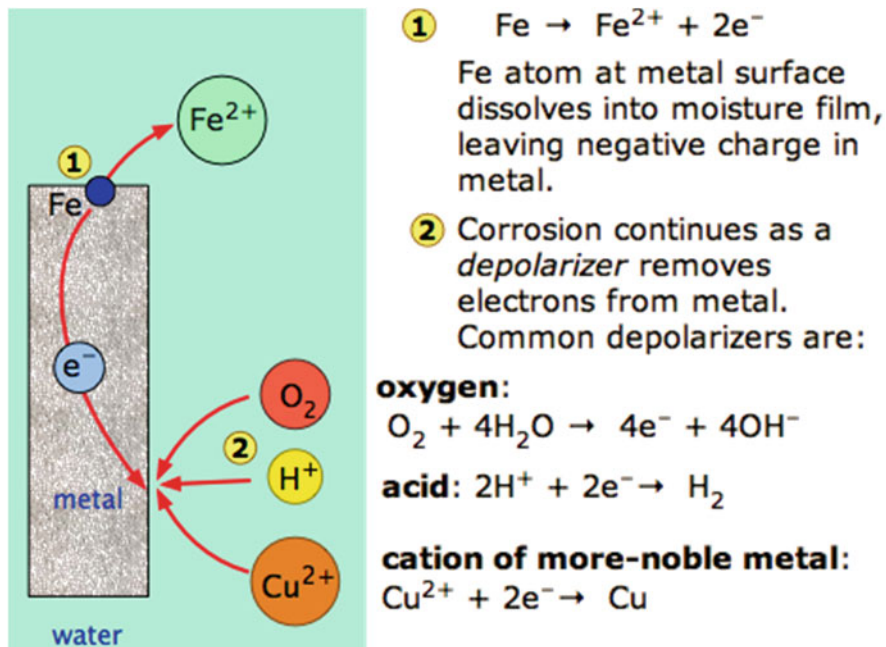


Fig. 4.10 Electrochemical corrosion of iron. Corrosion often begins at a location (1) where the metal is under stress (at a bend or weld) or is isolated from the air (where two pieces of metal are joined or under a loosely adhering paint film.) The metal ions dissolve in the moisture film and the electrons migrate to another location (2) where they are taken up by a depolarizer. Oxygen is the most common depolarizer; the resulting hydroxide ions react with the Fe^{2+} to form the mixture of hydrous iron oxides known as rust. (CC BY 3.0 Unported; Stephen Lower) [https://chem.libretexts.org/Bookshelves/General_Chemistry/Book%3A_Chem1_\(Lower\)/16%3A_Electrochemistry/16.08%3A_Electrochemical_Corrosion](https://chem.libretexts.org/Bookshelves/General_Chemistry/Book%3A_Chem1_(Lower)/16%3A_Electrochemistry/16.08%3A_Electrochemical_Corrosion)

4.8.2 Biomineralization: Microbiologically Influenced Corrosion (MIC)

Biomineralization refers to biologically induced mineralization in which an organism modifies its local microenvironment creating conditions such that there is chemical precipitation of mineral phases extracellularly.

Microbiologically influenced corrosion (MIC) or biocorrosion that enhances corrosion under anoxic conditions via uptake of electrons from metallic iron [Fe(0)] has been regarded as one of the major causative factors (Kato et al. 2015). Iron-corroding microorganisms were enriched from rice paddy field soil using a sulfate-free freshwater medium supplemented with Fe(0) granules as the sole electron donor under an N_2/CO_2 gas atmosphere. Kato et al. (2015) demonstrated acetogenesis-dependent MIC in pure cultures of acetogenic bacteria. Besides this newly isolated acetogen *Sporomusa* sp. GT1 and *S. sphaeroides* enhanced iron corrosion by generating acetate with Fe(0) granules as the sole electron donor. The enriched

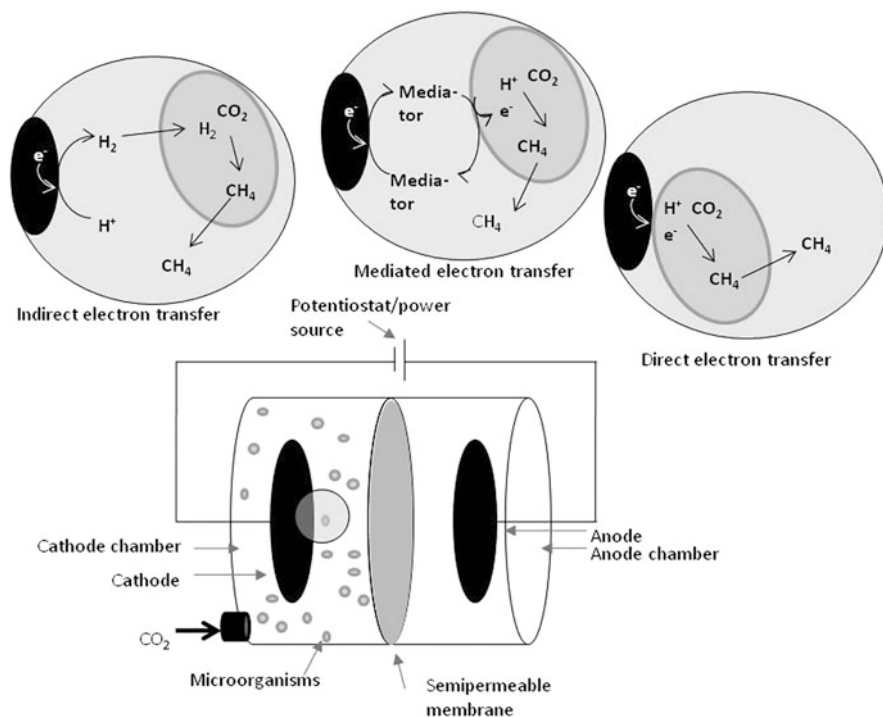


Fig. 4.11 Extracellular electron transfer. Means of electron transfer within a separated, electro-methanogenic system at the cathode: indirect electron transfer (IET), mediated electron transfer (MET), and direct electron transfer (DET). Enzmann, Mayer F., F., Rother, M. et al. Methanogens: biochemical background and biotechnological applications. *AMB Expr* 8, 1 (2018). <https://doi.org/10.1186/s13568-017-0531-x>. Reproduced under Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), Attribution 4.0 International (CC BY 4.0) which permits unrestricted use, distribution, and reproduction in any medium, provided appropriate credit is given to the original

communities with *Sporomusa* sp. and *Desulfovibrio* sp. produced larger amounts of acetate coupled with Fe(0) oxidation prior to CH₄ production. In addition to sulfate-reducing bacteria and methanogenic archaea in marine environments, acetogenic bacteria in freshwater environments, e.g. *Sporomusa sphaeroides* DSM2875 and *Acetobacterium woodii* DSM1030, have recently been suggested to cause MIC under anoxic conditions (Kato et al. 2015; Enzmann et al. 2018) (Fig. 4.11).

These observations demonstrated that growth of microorganisms utilizing Fe(0) as the sole electron donor occurred in the Fe(0) enrichments and that most of the reducing equivalents for the CH₄ generation were derived from Fe(0) oxidation (Enzmann et al. 2018).

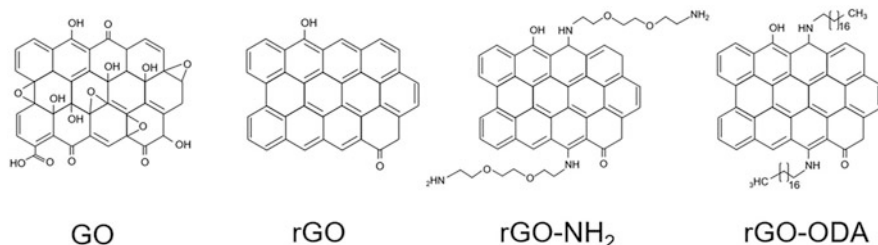


Fig. 4.12 Graphene oxide (GO)-based materials, including GO, and reduced GO (rGO), to anaerobic microbial communities, can promote DIET. Source: Igarashi, K., Miyako, E., & Kato, S. (2020). Direct Interspecies Electron Transfer Mediated by Graphene Oxide-Based Materials. *Frontiers in Microbiology*, 10, 3068. <https://doi.org/10.3389/fmicb.2019.03068> Frontiers open access

4.8.3 Direct Interspecies Electron Transfer (DIET)

Conductive materials are known to promote direct interspecies electron transfer (DIET) by electrically bridging microbial cells (Igarashi et al. 2020). Previous studies have suggested that supplementation of graphene oxide (GO)-based materials, including GO, and reduced GO (rGO), to anaerobic microbial communities, can promote DIET (Igarashi et al. 2020) (Fig. 4.12).

Direct interspecies electron transfer (DIET) plays a crucial role in the anaerobic biodegradation process, in which electrons released from electron-producing microorganisms (e.g., *Geobacter* species) are transferred directly to electron-consuming microorganisms (e.g., *Methanosarcina* species) (Reguera et al. 2005; Lovley 2011; Kato et al. 2015; Kouzuma et al. 2015) DIET utilizes defined coculture system composed of electroactive microorganisms, such as *Geobacter* species and members of *Methanosarcinales methanogens* (Rotaru et al. 2014a, b). Rotaru et al. (2014a) used DIET coculture of *Geobacter metallireducens* and *Methanosarcina barkeri* to report basic characteristics of the model DIET coculture and determined that long-range electron transfer via pilin is necessary for DIET-mediated process of methane production by ethanol oxidation followed by acetate disproportionation (Eqs. (4.1) and (4.2)) and DIET-mediated CO₂ reduction (Eq. (4.3)). Biodegradation of ethanol results in production of CH₄ and CO₂ (Eq. (4.4)).

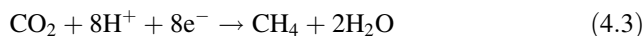
Ethanol oxidation to acetate:



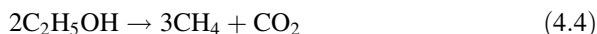
CH₄ production by acetate disproportionation:



CH₄ production by DIET-mediated CO₂ reduction:



Overall reaction (Eqs. (4.1) + (4.2) + (4.3)):



Igarashi et al. (2020) examined the DIET-promoting efficiency of GO on model coculture of *G. metallireducens* and *M. barkeri*. Amendment of GO induced methane production and ethanol consumption, while coculture without GO (Fig. 4.3) or monocultures of either microorganism in the presence of GO showed no methane production during the cultivation period tested. DIET brings a new opportunity to improve the efficiency of biogas technology as it may replace mediated interspecies electron transfer (MIET) by efficient electron transfer between exoelectrogens and electrorophic methanogens, thereby enhancing yields and rates of biogas production. It is concluded that supplementation of Graphene oxide (GO) and reduced GO (rGO) to a defined DIET coculture composed of an ethanol-oxidizing electron producer *Geobacter metallireducens* and a methane-producing electron consumer *Methanosarcina barkeri* promoted methane production from ethanol (Igarashi et al. 2020). Ethanol, as the initial electron donor in the discovery of the DIET pathway, is now a “hot topic” in the literature (Feng et al. 2021).

4.9 Applications

The most successful application so far at the commercial scale has been anaerobic digestion, which has been widely adopted for waste treatment. Methane recovery from waste activated sludge (WAS) through anaerobic digestion is generally restricted by the poor degradability of WAS. Wu et al. (2021) suggested a novel sludge pretreatment technology by using the calcium hypochlorite ($\text{Ca}(\text{ClO})_2$) in enhancing the methane production from WAS anaerobic digestion. In an anaerobic digester, methanogens work together with a consortium of other microorganisms to break down organic waste and produce methane-containing biogas as an energy product. Pure cultures of methanogens are capable of H_2 production from formate and methane production from coal. On the other side, genetically modified methanogens have been developed to produce methane from methyl esters, geraniol from H_2 and CO_2 or formate, isoprene from methanol, and acetate or lactate from methane.

According to Rother and Krzycki (2010) among the archaea, they are also the only known group synthesizing proteins containing *selenocysteine* or *pyrrolysine*. All but one of the known *archaeal pyrrolysine*-containing and all but two of the confirmed *archaeal selenocysteine*-containing proteins are involved in *methanogenesis*. Synthesis of these proteins proceeds through suppression of translational stop codons but otherwise the two systems are fundamentally different. Rother and Krzycki (2010) summarized the recent developments in selenocysteine-

and pyrrolysine-related research on archaea and aimed to put this knowledge into the context of their unique energy metabolism (Fig. 4.1).

In the hydrogenotrophic pathway of methanogenesis, CO₂ is sequentially reduced to methane in seven steps via coenzyme-bound intermediates using H₂ as the electron donor (Fig. 4.1) (Rother and Krzycki 2010). If the methanogenic growth substrate is formate, it is first oxidized to CO₂ via (sometimes Sec-containing) formate dehydrogenase (FDH, Fig. 4.1) (Jones et al. 1979).

4.9.1 Sweet Sorghum as a Source of Hydrogen and Methane

Antonopoulou et al. (2008) reported exploitation of sweet sorghum biomass as a source for hydrogen and methane. They investigated fermentative hydrogen production from the sugars of sweet sorghum extract at different hydraulic retention times (HRT). The subsequent methane production from the effluent of the hydrogenogenic process and the methane potential of the remaining solids after the extraction process were assessed as well (Antonopoulou et al. 2008). They demonstrated that biohydrogen production can be very efficiently coupled with a subsequent step of methane production and that sweet sorghum could be an ideal substrate for a combined gaseous biofuel production.

4.9.2 Anaerobic Digestion

Kim et al. (2004) performed studies to overcome the low efficiency of anaerobic digestion of sewage sludge and food waste by combining temperature-phased digestion, sequencing batch operation, and co-digestion technology. They demonstrated that the temperature-phased anaerobic sequencing batch reactor (TPASBR) system for the co-digestion of sewage sludge and food waste resulted in enhanced volatile solid (VS) reduction and methane production rate (Kim et al. 2006). This study aimed to evaluate the performance of the unified high-rate anaerobic digestion (UHAD) system treating co-substrate of sewage sludge and food waste. They reported enhanced performance could be attributed to longer retention time of active biomass, faster hydrolysis, higher CH₄ conversion rate, and balanced nutrient conditions of co-substrate in the UHAD system.

4.9.3 *Clostridium butyricum*

Junghare et al. (2012) isolated a mesophilic alkaline tolerant fermentative microbe from estuarine sediment samples and designated as *Clostridium butyricum* TM-9A, based on 16S rRNA gene sequence. This TM-9A strain produced hydrogen efficiently from a range of pentose and hexose sugars including di-, tri-, and polysaccharides like xylose, ribose, glucose, rhamnose, galactose, fructose, mannose, sucrose, arabinose, raffinose, cellulose, cellobiose, and starch. Optimization of

process parameters improved molar hydrogen yield of TM-9A strain (Junghare et al. 2012).

4.9.4 Reactor System

He and Osborne 2012 reported that hardened and insoluble fat, oil, and grease (FOG) deposits are the primary cause of sewer line blockages leading to sanitary sewer overflows (SSOs). They studied physicochemical characteristics of full-scale grease interceptors (GIs), the first “line of defense” against FOG buildup in sewer lines. They further assessed the physicochemical characteristics of two full-scale GI pipes over a 1-year period. Statistically significant differences between bio-augmented and untreated cycles were detected for several chemical and physical properties. The treated cycles had lower BOD and COD at the grease interceptor outlet. Hu et al. (2018) studied effects of lipid concentration on thermophilic anaerobic co-digestion of food waste and grease waste in a siphon-driven self-agitated anaerobic reactor. High-strength lipid wastes FOG (fat, oil, and grease) normally could not be directly released to the collection system in many metropolitan areas (He and Osborne (2012)).

4.9.5 Biogas

Natural degradation of organic material results in the production of biogas by microorganisms under anaerobic conditions. Anaerobic digestion converts organic material into biogas, a renewable fuel that could be used to produce electricity and heat or as vehicle fuel. In recent years, anaerobic digestion (AD) of waste and residues from agriculture and industry, municipal organic waste, sewage sludge, etc. has become as one of the most attractive renewable energy pathway. Biogas typically consists of methane (50–75%) and carbon dioxide (25–50%), minor amounts of other gases, and water vapor (<http://www.biogas-renewable-energy.info>). Biogas is produced from complex organic material that is decomposed by microorganisms in the anaerobic digestion (AD) process. Anaerobic digestion of energy crops, residues, and wastes is of increasing interest in order to reduce the greenhouse gas emissions and to facilitate a sustainable development of energy supply (Weiland 2010; Dornelas et al. 2017).

For anaerobic digestion, two substrates have been taken into account: (1) agricultural resources and (2) municipal organic waste (Pertl et al. 2010). Einarsson and Persson (2017) reviewed potential for biogas production from crop residues and manure, accounting for key technical, biochemical, environmental, and economic constraints. Maragkaki et al. (2018) reviewed biogas production from sewage sludge by adding small amount of agro-industrial by-products and food waste residues. They investigated co-digestion of sewage sludge (SS) and food waste (FW), grape residues (GR), crude glycerol (CG), cheese whey (CW), and sheep manure (SM), in a small ratio of 5–10% (v/v). Weiland (2010) suggested that the digestate from

anaerobic fermentation is a valuable fertilizer due to the increased availability of nitrogen and the better short-term fertilization effect. Anaerobic treatment minimizes the survival of pathogens which is important for using the digested residue as fertilizer.

The various methods of biogas production can be classified in wet and dry fermentation systems. Most often applied are wet digester systems using vertical stirred tank digester with different stirrer types dependent on the origin of the feedstock. In comparison to the fossil reference system, the electricity production using biogas saves GHG emissions from 0.188 to 1.193 kg CO₂ eq per kWh (e) (Bacenetti et al. 2013).

According to Plugge (2017), it is important to have in-depth knowledge and understanding of the anaerobic microbiome. Anaerobic co-digestion of sewage sludge and other organic wastes at a wastewater treatment plant (WWTP) is a promising method for both energy and material recovery. Maragkaki et al. (2018) demonstrated improving biogas production from anaerobic co-digestion of sewage sludge with a thermal dried mixture of food waste, cheese whey, and olive mill wastewater.

Different physiological groups of microorganisms are involved as follows: hydrolytic bacteria, fermenting bacteria, organic acid-oxidizing bacteria, and methanogenic archaea, and these microorganisms degrade organic matter via cascades of biochemical conversions ultimately to biogas (Weiland 2010). Syntrophic relationships between hydrogen producers (acetogens) and hydrogen scavengers (homoacetogens, hydrogenotrophic methanogens) are critical to the process (Carballa et al. 2015).

The production of biogas by anaerobic digestion (AD) of agricultural residues, organic wastes, animal excrements, municipal sludge, and energy crops has a firm place in sustainable energy production and bioeconomy strategies, and biogas production has increased rapidly in many countries over the last 20 years. In biogas plants, complex polymers are hydrolyzed to sugars and amino acids, followed by fermentation and acetogenesis, and acetate, H₂, and CO₂ are produced as substrates for methanogenesis. Therefore, hydrogenotrophic and acetoclastic methanogens are prevalent in mesophilic biogas plants, often dominated by species of *Methanosarcina* (*Methanotherix* at low acetate concentrations) or *Methanoculleus* (Sundberg et al. 2013; Lucas et al. 2015). However, under certain conditions, syntrophic acetate oxidation may be the dominant path toward methane (Westerholm et al. 2016) (Fig. 4.13).

According to Theuer et al. (2020), efforts to integrate biogas plants into bioeconomy concepts will lead to an expansion of manure-based (small) biogas plants, while their operation is challenging due to critical characteristics of some types of livestock manure. Due to varying digester types, feedstocks, and process conditions, 16S rRNA gene amplicon sequencing showed differences in the taxonomic composition.

Wirth et al. (2021) suggested that biogas production through co-digestion of second- and third-generation substrates is an environmentally sustainable approach. They reported that co-digestions in anaerobic digestion experiments the combined

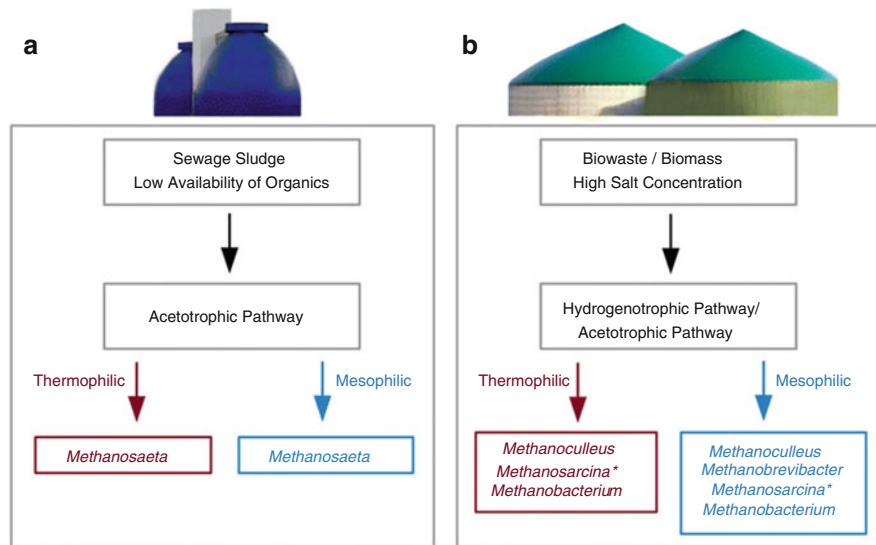


Fig. 4.13 Metagenome, metatranscriptome, and metaproteome approaches unraveled compositions and functional relationships of microbial communities residing in biogas plants. Source: Hassa J, Maus I, Off S, et al. 2018 Metagenome, metatranscriptome, and metaproteome approaches unraveled compositions and functional relationships of microbial communities residing in biogas plants. *Appl Microbiol Biotechnol.*;102(12):5045–5063. <https://doi.org/10.1007/s00253-018-8976-7>. **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made

substrates like green willow biomass, chicken manure waste, and microalgae biomass, the biogas yield was significantly higher as compared to the yield when energy willow was the sole substrate. Wirth et al. (2021) applied genome-centric metagenomics approach to gain functional insight into the complex anaerobic decomposing process. They enumerated the importance of *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes* phyla as major bacterial participants, while Methanomicrobia and Methanobacteria represented the archaeal constituents of the communities. The carbohydrate hydrolyzes the representatives of long-chain carbohydrate hydrolyzing microbes Bin_61: *Clostridia* is followed by action of hydrogenotrophic methanogen species *Methanoculleus* (Bin_10) and *Methanobacterium* (Bin_4). Wirth et al. (2021) reported that a sensitive balance between H_2 producers and consumers was shown to be critical for stable biomethane production and efficient waste biodegradation.

4.10 Discussion

Ma et al. (2018) suggested that anaerobic digestion, widely considered as a promising waste biomass disposal treatment approach, is attracting increasing interest in all corners of the globe. Production of biogas provides a versatile carrier of renewable energy, as methane can be used for replacement of fossil fuels in both heat and power generation and as a vehicle fuel (Weiland 2010). Messineo et al. (2019) reviewed the anaerobic digestion as important available route to recover energy from waste via production of biogas while reducing organic load and pollutants to the environment. The use of farming and agro-industrial wastes as co-substrate in anaerobic digestion can induce benefits related to the simultaneous treatment of different wastes. This co-digestion can significantly enhance the process stability as well as the biomethane generation.

The Conference on Sustainable Development of Energy, Water and Environment Systems (SDEWES) conferences of 2021 debated the issue of state of the art and future directions and priorities in the various areas of sustainable development. One of the important areas of sustainable development includes adoption of renewable energy sources by integrating heating, transport, industry, buildings, water, cooling, electricity, waste, wastewater, forestry, and agriculture systems (Østergaard et al. 2021). Wang et al. (2021a, b) discussed bioenergy potential from manure-generated biogas projects and the bottlenecks of AD technology. According to them, reducing energy input in AD projects and enhancing the efficiency of methanogenesis of livestock manure are key factors for achieving a high net output of biogas projects.

Thauer et al. (2008) suggested that although methanogenic archaea can reduce CO_2 with H_2 to methane, this does not take into account the fact that methanogens with cytochromes have considerably higher growth yields and threshold concentrations for H_2 than methanogens without cytochromes. These and other differences have been explained by Thauer et al. (2008) in his review. The methanogens with cytochromes, the first and last steps in methanogenesis from CO_2 , are coupled chemiosmotically, whereas in methanogens without cytochromes, these steps are energetically coupled by a cytoplasmic enzyme complex that mediates flavin-based electron bifurcation (Thauer et al. 2008). Wang et al. (2011) reported that overwhelming majority of methanogens capable of growth via conversion of the methyl group of acetate to methane do not metabolize H_2 suggesting they employ an electron transport pathway distinct from that proposed for the few acetotrophic methanogens in which H_2 is an obligatory intermediate, e.g., *M. acetivorans* which is acetotrophic methanogen incapable of metabolizing H_2 .

Söllinger and Urich (2019) reported that Methanomassiliicoccales occur in a large variety of anoxic habitats including wetlands and animal intestinal tracts of ruminant animals, i.e., in the major natural and anthropogenic sources of methane emissions, respectively. Considering the role of methane as potent greenhouse gas, resolving the methanogenic nature of a broad range of putative novel methylotrophic methanogens and assessing their role in methane emitting environments are pressing issues for future research on methanogens (Ferry 1999; Kumar et al. 2018a, b, 2020).

Biogas production from sewage sludge volatile solids (VS) by anaerobic digestion slows down toward the end of the process, among inhibitory factors being pH increase upon ammonia accumulation, poorly digestible biomaterials, and high fixed solid (FS) content (Kouzi et al. 2020). According to Kouzi et al. (2020), the risk of hazardous compounds limits the utilization of sewage treatment plant sludge, biogas production being one of the most common applications (Chen et al. 2014).

However, the bioenergy conversion efficiency of this process is not ideal. They further suggested that another problematic aspect of anaerobic digestion is the nutrient-rich effluent which needs to be treated before discharge. According to Liu et al. (2018), the biological processes for treating municipal wastewater have been developed based on the philosophy of biological oxidation with high energy consumption and generation of waste sludge. Thus, the energy self-sufficient biological reclamation of municipal wastewater needs to be addressed urgently.

4.11 Conclusion

Biogenic methane is a significant greenhouse gas, and the conversion of organic wastes to methane is an important bioenergy process. *Methanosarcina* species play an important role in methane production in many methanogenic soils and sediments as well as anaerobic waste digesters. Extracellular electron exchange in *Methanosarcina* species and closely related Archaea plays an important role in the global carbon cycle and enhances the speed and stability of anaerobic digestion by facilitating efficient syntrophic interactions. The discovery of a methanogen that can conserve energy to support growth solely from the oxidation of organic carbon coupled to the reduction of an extracellular electron acceptor expands the possible environments in which methanogens might thrive. Progress has been made in the past few decades to identify key microorganisms influencing AD. Yet, more work is required to realize robust, quantitative relationships between microbial community structure and functions such as methane production rate and resilience after perturbations. Venkiteshwaran et al. (2015) suggested other promising areas of research for improved AD may include methods to increase/control (1) hydrolysis rate, (2) direct interspecies electron transfer to methanogens, (3) community structure-function relationships of methanogens, (4) methanogenesis via acetate oxidation, and (5) bioaugmentation to study community-activity relationships or improve engineered bioprocesses. For developing sustainable climate change mitigation technology, it is essential to develop better reactors for high yield of fuel production through methanogenesis with controlled conditions. RE technology policies need to be in place to ensure that the adoption of environmentally sustainable biogas energy production becomes popular all over the world. Combined heat and power (CHP) unit is a very sustainable energy source which can help us combat global warming and related climate change issues. The actual targets can be achieved by infrastructural growth and systemic perspectives.

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