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Advantage of conductive materials on interspecies electron transfer-independent acetoclastic methanogenesis: A critical review

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ABSTRACT

Fossil-fuel overuse and global warming are calling for new techniques to provide sustainable fuels. Biomethane can be produced by anaerobic digestion of organic waste, yet microbial mechanisms involved are still debated. Traditionally, reduction of carbon dioxide (CO₂) to methane (CH₄) is commonly explained by interspecies electron transfer, i. e., direct interspecies electron transfer (DIET)-based CO₂ reduction or mediated interspecies electron transfer (MIET)-based CO₂ reduction. For DIET-based CO₂ reduction, or DIET-CO₂ reduction, where electrons are provided by electricigens and transferred to methanogenic archaea to complete CO₂ reduction for methane production. Methanogenesis is also executed and facilitated by acetoclastic methanogenesis in the presence of conductive materials, as evidenced recently. Here we compare DIET-CO₂ reduction and acetoclastic methanogenesis mediated by conductive materials. In the past decade, DIET-CO₂ reduction is considered as the backbone for methane production strategy in anaerobic engineering digestion. But increasing evidences propose the importance of acetoclastic methanogenesis strengthened by exogenous media. DIET-based CO₂ reduction has been extensively reviewed. Herein, we conclude the diverse microbial mechanisms affected by conductive materials to improve potential acetoclastic methanogenesis for the first time. Increasing electron transfer in methanogenic archaea and/or between bacteria and methanogens, microbial immobilization, pH buffering capacity, providing metal ions, reducing toxicity, regulation of oxidation-reduction potential are detailed reviewed. Possible future application based on acetotrophic methanogens is suggested via conductive materials in anaerobic digestion and natural ecological environment management.

1. Introduction

Methane is an abundant gaseous hydrocarbon produced by chemical, geological and biological processes. Methane is a both greenhouse gas contributing to global warming and a rather clean and efficient fuel. While emissions of fossil methane increase global warming, production of methane from actual biomass, e.g. organic waste, is considered carbon neutral for the climate (Fig. 1). Biomethane is produced solely by

microbial methanogens, which belong to the archaea kingdom, yet precise microbial mechanisms of biomethane production are not fully understood [114,21]. A better knowledge of factors controlling biomethane emission would be useful both to improve industrial biomethane production, and to design techniques of land management that limit methane emission to the atmosphere [24,79,80,96]. This manuscript reviews the latest reports on mechanisms of methanogenesis by anaerobic fermentation.

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Methanogenesis is the last step for anaerobic mineralization of organic matter [21]. Biodegradation of biomass to methane is performed by hydrolytic bacteria, acid-producing bacteria, acetogenic bacteria and methanogens [5]. The efficiency of methanogenesis is controlled by syntrophic interactions between bacteria and methanogens, which have complementary metabolisms [13,39,64,124]. In particular, bacteria and methanogens share electrons using two mechanisms: transfer of small compounds, e.g. hydrogen/formate, and direct electron transfer [64,92,121]. For instance, compounds such as hydrogen and formate act as electron shuttles in a process named interspecies hydrogen/formate transfer (IHT/IFT, [92]). Alternatively, electrons can be transferred directly between electroactive microorganisms and methanogens, named direct interspecies electron transfer (DIET). Electrically conductive pili (e-pili) and proteins are involved in this progress with methanogenic archaea as electron acceptor, yet relative research is still in infancy and the mechanism is still poorly known [27,64]).

Methane production by anaerobic fermentation can be improved by adding conductive materials, which favor DIET coupled with CO₂ reduction, or DIET-based CO₂ reduction, or DIET-CO₂ reduction. DIET-CO₂ reduction has been systematically proposed to explain methane production in studies lacking specific experiments allowing to decipher the mechanisms (Tables 1 and 2). This mechanism has been extensively reviewed [39,64,68,129]. Yet insights from recent reports point to another mechanism. For instance, Martins et al. [71] found no correlation between increased methane generation and electrical conductivity, suggesting that DIET is involved partly at most. Moreover, DIET has been evidenced unambiguously only in co-cultures of *Geobacter* with either *Methanosarcina* or *Methanosaeta* (*Methanotherix*) (Table 1; but *Geobacter* sp. are rarely detected in systems where methane production is enhanced [71,126]). Van Steendam et al. [101] concluded that many investigations lacked direct experimental evidence for the role of DIET in enhancing the performance of anaerobic digestion.

Another insight for an alternative mechanism is the predominance of acetotrophic methanogens to complete acetoclastic methanogenesis, which contribute more than 70% of methane during anaerobic

digestion [5,18]. Interestingly, the main methanogens using DIET-CO₂ reduction for methane generation are still acetotrophic methanogens, i. e., *Methanosaeta* and *Methanosarcina*, rather than hydrogenotrophic methanogens. Acetotrophic methanogens is thus suggesting a benefit from conductive materials. Recently, addition of conductive carbon nanotubes and nano-magnetite in pure cultures has favored acetoclastic methanogenesis (Table 1). These materials also accelerate direct acetate dismutation in anaerobic soils and anaerobic sludges [35,37,85,47,120] (Table 2). Similarly, biochar and granular activated carbon facilitate direct acetate dismutation, as firmly evidenced by isotope tracing [120,122,124]. Recent advances in methanogenesis through DIET-CO₂ reduction via conductive materials are substantially reviewed. Therefore, here we specially focus on the effect of conductive materials on acetotrophic methanogens and potential acetoclastic methanogenesis.

2. Methanogens and mechanisms of methane production

Methanogens are prokaryotic microorganisms belonging to the archaeal kingdom. Methanogenic archaea are obligate methane producers, which generate almost all biogenic methane as the end-product of anaerobic respiration to gain energy. All methanogens belong to the *Euryarchaeota* [62]. A genome-centric metagenomics has evidenced a methane metabolism in the archaeal phylum Bathyarchaeota [22], thus suggesting a wide phylogenetic and ecological diversity of microorganisms possessing methanogenic ability. The common habitats of methanogens include sediments, anaerobic soils, geothermal systems, landfill, livestock, termites, wastewater, sewage sludges and anaerobic digesters [5,22,35,119,125]. Accordingly, biomethane is both a renewable fuel source when produced in industrial plants, and a greenhouse gas contributing to global warming when methane is emitted into the atmosphere [4].

Methanogenic archaea actually includes 4 classes, 6 orders, 14 families, 29 genera and 155 species [34]. Known methanogenic pathways are few, they occur at the last steps of biomass decomposition. Substrates for methanogenesis are produced from macromolecules by



Fig.1. Biomethane production in an agricultural area in France. Copyright: Cedigaz 2020.

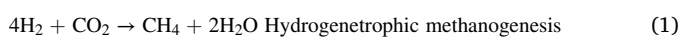
Table 1
Studies of conductive materials on pure culture or co-culture of methanogens.

Anaerobic system	substrate	Conductive material	Applied concentration(dose)	Electron-donating microorganisms	Electron-accepting microorganism	Reference
Co-culture	Ethanol	Carbon cloth	10, 20 g/L	<i>Geobacter metallireducens</i>	<i>M. barkeri</i>	[15]
Co-culture	Ethanol	Ferrihydrite	25 mM	<i>Geobacter</i>	<i>M. barkeri</i>	[94]
Co-culture	Ethanol	GAC	25 g/L	<i>Geobacter metallireducens</i>	<i>M. barkeri</i>	[58]
Co-culture	Ethanol	Biochar	26 g/L	<i>Geobacter metallireducens</i>	<i>M. barkeri</i>	[16]
Pure culture	Acetate	Carbon nanotubes	0.1, 0.5, 1, 5 g/L	NA	<i>Methanosaeta concilii</i>	[88]
Pure culture	Acetate	Carbon nanotubes	0.1, 0.5, 1, 6 g/L	NA	<i>M. mazei</i>	[88]
Pure culture	Acetate	NanoFe ₃ O ₄	4.64 mM of Fe	NA	<i>M. barkeri</i>	[26]
Pure culture	Acetate	NanoFe ₃ O ₄	4.65 mM of Fe	NA	<i>M. mazei</i>	[110]
Pure culture	Acetate	Carbon nanotubes	0.2 g/L	NA	<i>M. barkeri</i>	[128]
Pure culture	Acetate	Carbon nanotubes	0.2 g/L	NA	<i>M. mazei</i>	[128]

Note: methane was from acetoclastic methanogenesis with a gray background. It was proposed that DIET-CO₂ reduction progress generated methane with white background. NA, not available. GAC: granular activated carbon.

hydrolysis, acidogenesis and acetogenesis in multi-community anaerobic environments (Fig. 2). Typically, complex organic matter is first cut into small particles by physicochemical and enzymatic processes. In particular, extracellular enzymes such as cellulases, hemicellulases, amylases, lipases and proteases are secreted by bacteria, e.g. *Bacteroides* and *Streptococcus*. Then, enzymes cut macromolecules into soluble substrates. During acidogenesis, soluble monosaccharides, amino acids and long-chain fatty acids are generated and further used by fermentative bacteria, involving both strict and facultative anaerobes. Then, during acetogenesis, metabolites such as butyrate and propionate are degraded into acetate. Overall, all these processes are controlling the efficiency of further methane generation.

During methanogenesis, methanogens gain energy by forming methane from 1-carbon and 2-carbon compounds, e.g. CO₂ and acetate. For instance, hydrogenotrophic methanogens use H₂ and CO₂ as substrates to generate methane [18] (Eq. (1)):



Most cultivable methanogens are strict hydrogenotrophs, they use H electrons to reduce CO₂ [34]. Some methanogenic archaea can also use electrons from other sources such as microbes and biocathodes as energy sources, a process named either direct electron transfer (DET), or direct interspecies electron transfer (DIET) when electrons are exchanged between two species [64]. For instance, DIET-CO₂ reduction is operated by electroactive bacteria coupled to methanogenic archaea [86] (Eq. (2)):



Acetotrophic methanogens directly break acetate to generate methane [114] (Eq. (3)):



Here, the carboxyl group of acetate is first oxidized into CO₂ and electrons. Electrons are then transferred to CoM-S-S-CoB that transforms the acetate methyl into methane [114]. This metabolism is responsible for 70–90% of the total methane produced during the anaerobic digestion of organic waste and in terrestrial environments [5,18]. Some methanogens use other methyl compounds such as methanol and methylamine as substrates to produce methane, which constitutes the third methanogenic pathway and contributes little to methane accumulation. Overall, three pathways are actually known to produce

methane: hydrogenotrophic methanogenesis (MIET-CO₂ and DIET-CO₂), acetoclastic methanogenesis and methyltrophic methanogenesis.

Despite its major contribution to biomethane production, acetoclastic methanogenesis is performed to only two genera, *Methanosarcina* and *Methanosaeta*. *Methanosarcina* is a versatile methanogen able to use the three metabolic pathways, whereas *Methanosaeta* feeds only on acetate. In 2014, it was observed that *Methanosaeta* can accept electrons provided by *Geobacter* to reduce CO₂ to generate methane [86]. *Methanosaeta* is able to process acetate very efficiently, even at acetate levels as low as 5 μM [114]. By contrast, *Methanosarcina* requires a minimum concentration of about 1 mM. The distinct acetate affinity may explain the differences in the first step of the acetate metabolism. The low-affinity acetate kinase-phosphotransacetylase system is used to activate acetate to generate acetyl-CoA in *Methanosarcina*, while *Methanosaeta* employs the high-affinity adenosine monophosphate forming acetyl-CoA [114]. In addition, genome sequence analysis suggests that *Methanosarcina* and *Methanosaeta* probably use different modes of electron transfer and energy conservation. Recent advances in acetoclastic methanogenesis have been recently summarized by Evans et al. [21] and Welte and Deppenmeier [114].

3. CO₂ reduction to methane

Two mechanisms explain reduction of CO₂ into methane, mediated interspecies electron transfer (MIET) and direct interspecies electron transfer (DIET).

3.1. Mediated interspecies electron transfer

Efficient methanogenesis is observed when there is a syntrophy between fermentative bacteria and methanogenic archaea. The syntrophic species take advantage of the metabolism of the partner species to overcome thermodynamic barriers, thus enabling to break intermediates that are otherwise difficult to break [92]. The two core processes, organic degradation and methane production, highly depend on the efficiency of interspecies electron transfer [92]. Historically, interspecies electron transfer has referred to mediated interspecies electron transfer with H₂ or formate as electron carriers, named respectively interspecies hydrogen transfer (IHT) and interspecies formate transfer (IFT). During anaerobic digestion, digestion and methanogenesis activities reach a balance where H₂ produced by the syntrophic bacteria is

Table 2

Studies of conductive materials on microbial consortium. (See below-mentioned references for further information.)

Research subject	Inoculum/Substrates	Conductive materials	Dose	Predominant bacteria	Predominant methanogens	Reference
Synthetic brewery wastewater	Seed sludge/glucose	GAC	5 g/L	<i>Proteiniphilum</i>	<i>Methanosarcina</i>	[130]
Food waste	Anaerobic digester sludge	GAC	25 g /L	<i>Firmicutes</i>	<i>Methanosarcina</i>	[87]
Feeding medium	Seed sludge/glucose	GAC	10 g/L	<i>Actinobacteria</i>	<i>Methanosaeta</i>	[132]
Anaerobic soil	Glucose	GAC	1 g/L	NA	<i>Methanosaeta</i>	[120]
Waste activated sludge	Seed sludge	GAC	27 g/L	<i>anaerolineaceae</i>	<i>Methanosaeta</i>	[77]
Organic wastewater	Anaerobic sludge/acetate	GAC	10 g/L	<i>gracilibacter</i>	<i>Methanosaeta</i>	[99]
Packing media	and polyethylene glycol	GAC	NA	<i>Geobacter</i>	<i>Methanosaeta</i>	[73]
Anaerobic sludge	Propionate	GAC	NA	<i>Syntrophobacte</i>	<i>Methanosaeta</i>	[135]
Anaerobic sludge	Acetate	GAC	NA	<i>Syntrophobacte</i>	<i>Methanosaeta</i>	[135]
Synthetic wastewater	Anaerobic sludge/glucose	GAC	10 g/L	NA	<i>Methanosaetaceae</i>	[124]
Food waste	Seed sludge	Activated carbon	5,15, 25, 35 g/L	<i>Smithella</i>	<i>Methanosaeta</i>	[142]
Synthetic wastewater	Seed sludge/VFAs	Activated carbon	5 g/L	<i>Syntrophomonas</i>	<i>Methanosaeta</i>	
Flammulina velutipes residues	seed sludge/VFAs	Particle activated carbon	14.3 g /L	<i>VadinBC27</i>	<i>Methanosarcina</i>	[54]
Synthetic wastewater	Seed sludge/VFAs	Iron modified activated carbon	5 g/L	<i>Syntrophomonas</i>	<i>Methanosaeta</i>	
Basic nutrient solution	Granular sludge/glucose	Biochar	10 g/L	<i>Enterobacteriaceae</i>	<i>Methanosaeta</i>	[66]
Synthetic wastewater	Seed sludge/sucrose	Biochar	4 g/L	<i>Bacteroidetes</i>	<i>Methanosaetaceae</i>	[52]
Food waste	Seed sludge	Biochar	10 g/L	<i>Fervidobacterium</i>	<i>Methanosaeta</i>	[53]
Food waste	Activated sludge	Biochar	2, 6, 10 and 15 g/L	<i>Anaerolineaceae</i>	<i>Methanosaeta</i>	[108]
Chicken manure feed	Seed sludge	Biochar	NA	<i>Bacteroidia</i>	<i>Methanosarcina</i>	[74]
Anaerobic soil	Acetate	Biochar	1 g/L	<i>Lentimicrobiaceae</i>	<i>Methanosarcina</i>	
Synthetic wastewater	Anaerobic sludge/glucose	Biochar	1 g/L	NA	<i>Methanosaetaceae</i>	[124]
Dog food	Seed sludge	Carbon cloth	3 pieces/L	<i>Sporanaerobacter</i>	<i>Methanosarcina</i>	[19]
Anaerobic soil	Straw	Carbon cloth	3 pieces/Vial	<i>Mobilitalea</i>	<i>Methanosarcina</i>	[52]
Artificial wastewater	Seed sludge/propionate	Graphite felt	NA	<i>Smithella</i>	<i>Methanosaeta</i>	[143]
Artificial wastewater	Seed sludge/butyrate	Graphite felt	NA	<i>Syntrophomonas</i>	<i>Methanosaeta</i>	[143]
Propionate-butyrate medium	Anaerobic sludge/propionate and butyrate	Carbon felt particles	1583 m ² /m ³	<i>Desulfuromonas</i>	<i>Methanosaeta</i>	[9]
Wastewater	Seed sludge	Nitrogen doped sewage sludge carbon	5 g/L	<i>Saccharofermentans</i>	<i>Methanosaeta</i>	[52]
Synthetic wastewater	Anaerobic sludge/glucose	Graphene	0.1 g/L	NA	<i>Methanosaetaceae</i>	[124]
Coal gasification wastewater	Seed sludge	Graphene	0.2, 0.5 and 1.0 g/L	<i>Saccharofermentans and atipia</i>	<i>Methanosaeta</i>	[149]
Basal medium	Anaerobic granular sludge/Sucrose	Carbon nanotubes	1 g/L	<i>Clostridiales</i>	<i>Methanosaeta</i>	[53]
Anaerobic soil	Glucose	Carbon nanotubes	0.2 g/L	NA	<i>Methanosarcina</i>	[120]
waste activated sludge	Seed sludge	GAC/GMagnetite	27 g/L	<i>prolixibacteraceae</i>	<i>Methanosaeta</i>	[77]
Anaerobic paddy soil	Sodium acetate	Magnetite	20 mM	<i>Clostridium and Geobacter</i>	<i>Methanosarcina</i>	[41]
waste activated sludge	Seed sludge	Magnetite	27 g/L	<i>anaerolineaceae</i>	<i>Methanosaeta</i>	[77]
Synthetic wastewater	Anaerobic sludge/glucose	Magnetite	4.62 g/L	NA	<i>Methanosaetaceae</i>	[124]
Fresh leachate	Sludge	Magnetite	10 g/L	<i>Eubacteriaceae</i>	<i>Methanosaeta</i>	[49]
Fischer-Tropsch wastewater	NA	Magnetite	0.1, 0.2 and 0.3 g/L	<i>Geobacter</i>	<i>Methanosaeta</i>	[108]
Synthetic wastewater	Ethanol, acetate and propionate	Fe ₃ O ₄	10 g/L	<i>Mesotoga</i>	<i>Methanosaeta</i>	[61]
Whey	Anaerobic seed sludge	Magnetite	20 mM	<i>Clostridium</i>	<i>Methanosaeta</i>	[7]
Basal medium	Anaerobic sludge/acetate	Magnetite	25 mM	<i>Geobacteraceae</i>	<i>Methanosarcina</i>	[151]
Synthetic wastewater	Anaerobic sludge/tryptone	Fe ₃ O ₄	10 g/L	<i>Proteiniclasticum</i>	<i>Methanosarcina</i>	[138]
Inorganic medium	Seed sludge/acetate and propionate	Magnetite	5 and 10 mM	<i>Tepidanaerobacter syntrophicus</i>	<i>Methanosarcina</i>	[131]
Synthetic wastewater	Inoculated sludge/sucrose	Magnetite	10 g Fe/L	<i>Chloroflexi</i>	<i>Methanosarcina</i>	[106]
Anaerobic soil	Straw	Nano-magnetite	20 mM	<i>Bacillus</i>	<i>Methanosarcina</i>	[121]
Anaerobic soil	Acetate	Magnetite	25 mM	<i>Proteobacteria</i>	<i>Methanosarcina</i>	[148]
Basal medium	Anaerobic soil/straw	NanoFe ₃ O ₄	2.1 mM	NA	<i>Methanosarcina</i>	[83]
Dairy farm wastewater	Inoculated sludge	Magnetite	2.5 g/L	<i>Clostridium</i>	<i>Methanosarcina</i>	[85]
Fresh water enrichment medium	Anaerobic soil/acetate	Amorphous iron	100 mM	<i>serratia</i>	<i>Methanosarcina</i>	[126]
Inorganic medium	Anaerobic soil/acetate	Ferrihydrite	20 mM	<i>Geobacter</i>	<i>Methanosarcina</i>	[42]
Synthetic wastewater	Seed sludge/VFAs	Goethite	5 g/L	<i>Syntrophomonas</i>	<i>Methanosaeta</i>	
Basal medium	Mixture of oil and water	Akaganéite	25 mM	<i>Halomonas</i>	<i>Methanosaeta</i>	[75]

Note: Methane production was mainly from acetoclastic methanogenesis with a gray background. On the contrary, DIET-CO₂ reduction predominantly contributed to methane accumulation. GAC: granular activated carbon. VFAs: VFAs. NA, not available.

used timely by methanogens [141]. Nevertheless, electron diffusion is limited by electron carriers and, in turn, MIET efficiency is relatively low [63,64]. At high hydrogen pressure, above 10 Pa, fermentative bacteria is inhibited due to a thermodynamically unfavorable reaction, with ΔG above 0. As a consequence, hydrogenase activity and metabolism of syntrophic bacteria are low.

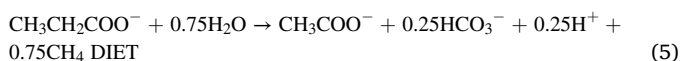
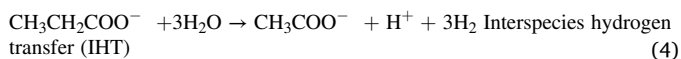
In the 2000 s, interspecies electron transfer was known to be mediated only by compounds, e.g. formate and bihydrogen. Later, in the past decade, DIET has been proposed as a more efficient and energy-saving mechanism, compared to MIET [64]. DIET involves electrically conductive pili as conduit to transfer electrons from one species to the other. DIET thus avoids energy consumption for the production and consumption of the MIET intermediates [63,64]. The stability of DIET is optimal in methanogenic biofilm aggregates [50,63]. DIET improves the competitiveness for electroactive microorganisms. For instance, volatile fatty acids (VFAs) are used by some anaerobic bacteria except for electroactive microorganisms. Here DIET provides a selective advantage for electron-donating bacteria over other anaerobic bacteria.

3.2. Direct interspecies electron transfer

DIET proceeds either biologically or by conductive materials. Biological DIET involves conductive pili (e-pili) and c-type cytochrome. A pilus is a hair-like appendage on the surface of many bacteria and archaea. Two mechanisms have been proposed to explain e-pili conduction: metal-driven conduction where charges spread across the entire filament by pi-pi orbital overlaps and charge delocalization [100,103]. For pi-pi orbital overlaps, electron density can be transferred from filled ligand orbital to the empty d-orbital of the metal center, or from the filled d-orbital of the metal to the empty orbital of the ligand. Charge delocalization is a stabilizing force because it spreads energy over a larger area rather than keeping it confined to a small area. Since electrons are charges, the presence of delocalized electrons brings extra stability to a system compared to a similar system where electrons are localized [100]. Electron tunneling in the pili-embedded cytochrome can be completed [64]. Co-cultures and anaerobic digestion thus benefit from e-pili-mediated DIET.

Alternatively, electroactive microorganisms that lack e-pili and other electron-bridging organs can still perform DIET artificially by addition of conductive materials such as carbon-based conductors or metals to the medium [16,59]. Here electrons are transferred at the material surface, which is supported by Shrestha et al. [91] who found a correlation of r of 0.67 between abundance of *Geobacter* spp., where *Geobacter* can also accept electrons from abiotic resource [116], and granule conductivity. Other studies also showed that enhanced methane production by conductive materials is correlated with electron redox transfer [139]. As a consequence, direct electron transfer properties of conductive materials may contribute little for electromethanogenesis [102].

Magnetite is highly conductive, and is thus a common iron-bearing mineral used in DIET to enhance methane production. Guskos et al. [29] showed that magnetite electrical conductivity reaches $2.5 \times 10^2 \Omega \cdot \text{cm}^{-2}$, versus $2 \text{ M}\Omega \cdot \text{cm}$ for hematite and $16 \text{ M}\Omega \cdot \text{cm}$ for goethite. In systems involving propionate as substrate, propionate is oxidized to acetate via two possible reactions [13] (Eqs. (4) and (5)):



DIET is favored because the Gibbs free energy induced by magnetite in DIET, ΔG^0 of -26.4 kJ/mol , is much more negative than that in IHT, of $+72.7 \text{ kJ/mol}$ [38]. It suggests the obvious preference of DIET rather than IHT during anaerobic digestion in the presence conductive Fe-bearing mineral, which benefits methane production. Similarly,

magnetite induced a 3-fold increase of methane production with butyrate as substrate [51]. It was speculated that DIET-promoting effect of magnetite is due to magnetite conductivity. Wang et al. [104,110] proposed that magnetite had different promoting effects on methanogenic degradation of different substrates, and magnetite facilitated different syntrophic partners in different stages. Another mechanism involving Fe(II)/Fe(III) redox cycling may also accelerate the transfer of electrons. This was recently confirmed during acetoclastic methanogenesis, which was accelerated and correlated with redox cycling of magnetite Fe(II)/Fe(III) in pure cultures of *Methanosarcina* [26,110].

In the same vein, it is widely accepted that the high conductivity of conductive materials strengthens DIET. For example, the conductivity of biochar and granular activated carbon, of 2–5 and 3000 $\mu\text{S/cm}$, suggests their potential to perform DIET [16,58]. Yet the stimulation efficiencies of DIET by biochar and granular activated carbon for methane production are similar despite their different conductivities. Therefore, electrical conductivity is not the sole factor controlling improved methane production. Other possible factors include the redox potential, the specific surface area and the roughness of conductive materials.

4. Acetoclastic methanogenesis versus DIET-CO₂ reduction

DIET-CO₂ reduction has been proposed to explain enhanced methane production when conductive materials are added, as a result of high conductivity [129]. However, microbial diversity analysis in most studies reveal that *Geobacter* pp. were minor in abundance or even absent [71,128]. Table 2 confirms that the predominant bacteria in anaerobic fermentation are not electricity-producing microorganisms (electricigens) but the fermentative microorganisms. When electricigens are undetectable, other bacteria have been suggested to be involved in DIET: for example, Lei et al. [48] showed that *Syntrophomonas* sp. exchange electrons directly with *Methanospirillum hungatei* via a carbon cloth. Yet, a co-culture study showed that *M. hungatei* is unable to accept electrons directly from electricigen to reduce CO₂ [86].

DIET is still proposed as the main mechanism to interpret unexpected results of anaerobic digestion. Indeed, other possible mechanisms are overlooked by the actual research focus on *Geobacter* species and other electroactive bacteria. Several insights suggest the occurrence of another mechanism. For example, to study the contribution of electrodes and electric current to process stability and methane production during the electro-fermentation of food waste, the authors demonstrated that *Geobacter* occurred at the cathode with a low abundance [61]. Electric current stimulated the growth of hydrogenotrophic methanogens, but acetotrophic *Methanosaeta* still made up 27.6–61.9% of archaeal community. For the function of conductive materials, magnetite accelerates the oxidation of propionate by acting as an electron acceptor, rather than promoting DIET [136]. Moreover, carbon nanotubes promoted methanogenic activities of four different species of methanogenic archaea growing in pure cultures [88], thus proving that the effect of conductive materials are not solely due to interactions between different species. Recently, Lu et al. [65] proposed that redox-active biochar and conductive graphite stimulated methanogenic metabolism in anaerobic digestion of waste-activated sludge: beyond direct interspecies electron transfer. Both graphite and biochar increased methane production primarily by enhancing the acetoclastic pathway, while syntrophic acetate oxidation via DIET was not thermodynamically favorable. Therefore the mechanism by which conductive materials enhance methane generation is open to debate.

Findings in the last several years show that conductive materials can promote methane production by direct acetate dismutation (acetoclastic methanogenesis), alternatively to DIET-CO₂ [26,65,128]. Indeed, under the stressful conditions, which is often encountered during anaerobic fermentation, the rate limiting step is usually acetoclastic methanogenesis [5]. This is supported by experimental and modelled data of growth of acetoclastic methanogens, thus lowering the contribution of CO₂ reduction [76]. We provided unambiguous evidence for promotion

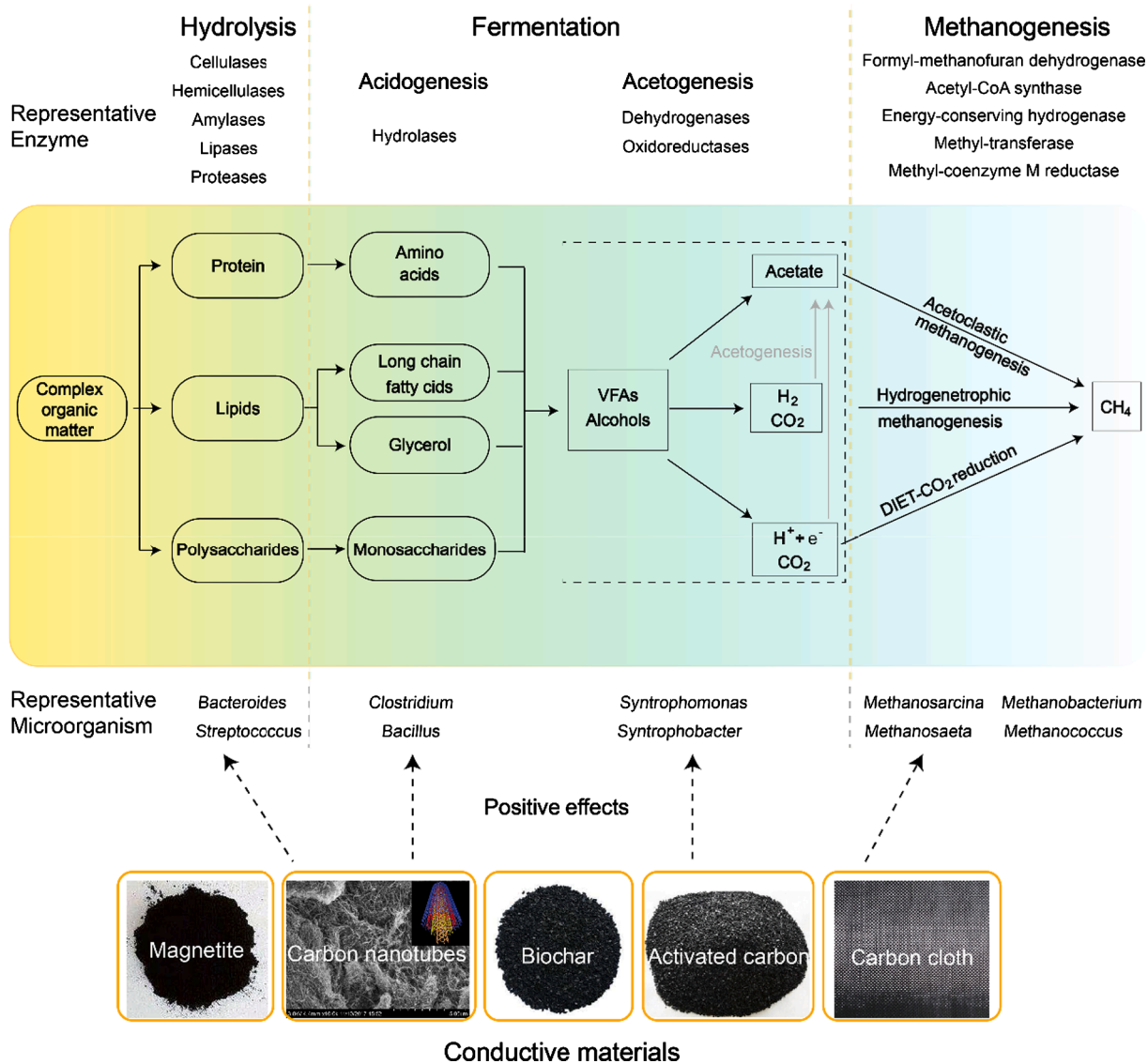


Fig. 2. Hydrolysis, fermentation and methanogenesis in anaerobic environments. Three mechanisms explain biomethane production: acetoclastic methanogenesis, hydrogenotrophic methanogenesis and direct interspecies electron transfer (DIET)-CO₂ reduction. Diverse conductive materials increase methane production and mineralization.

of acetoclastic methanogenesis rather than CO₂ reduction, in a *Methanosarcina* spp. culture with carbon nanotubes, using isotope tracing and CH₃F inhibition [128]. Here, the metabolism involved in the electron donating half-reaction – the carboxyl oxidation – is up-regulated (Fig. 3a). Furthermore, nanotubes were stuck tightly to the cell surfaces, suggesting that electron transfer mediated by nanotubes may accelerate the entire chain of electron transfer (Fig. 3a).

Moreover, magnetite can act as an electron shuttle to accelerate intracellular electron transfer, thus improving direct acetate dismutation of *M. barkeri* [26] (Fig. 3b). Here, nanoFe₃O₄ passes through the cell membrane of *M. barkeri*, and transfers electron in a similar manner as membrane-bound methanophenazine. Another investigation also showed that magnetite accelerated the production of methane by acetoclastic methanogenesis, which was interpreted by magnetite redox ability [104,110] (Fig. 3c). Interestingly, the authors showed that NanoFe₃O₄ did not enter into cells, contrary to Fu et al. [26]. Wang et al. [104,110] thus proposed that an outer-surface multiheme cytochrome and surface proteins accepted electrons from NanoFe₃O₄, thus promoting membrane electron transfer and acetoclastic methanogenesis. The redox cycling of nanoFe₃O₄ delivered a positive effect via the multiheme

c-type cytochrome to the membrane electron transfer and hence promoted acetoclastic methanogenesis. It was also recently verified by our group via proteome analysis [60]. Therefore the mechanisms by which conductive materials promote the acetoclastic methanogenesis are probably not only the three mechanisms presented in Fig. 3.

In complex microbial communities we found that conductive materials promote both DIET-CO₂ reduction and acetoclastic methanogenesis [124]. Acetoclastic methanogenesis was also evidenced by addition of magnetite in wastewater [85]. Here, improved methane production was attributed to the formation of dense planktonic cell co-aggregates glued by magnetite, which facilitated interspecies electron exchange. Meta-transcriptomic data further showed that genes involved in acetoclastic methanogenesis were highly expressed after addition of granular activated carbon, indicating that *Methanosaeta* was metabolically active and contributed to methane accumulation via acetoclastic methanogenesis [135]. About 4/5 of the methane rise was explained by acetoclastic methanogenesis. Evidence for the predominance of acetoclastic methanogenesis rather than DIET-CO₂ was definitively provided in the study of anaerobic soils by ¹³C tracing, CH₃F methanogenic inhibition, proteome analysis, thermodynamic analysis, electrochemical analysis,

modelling and microscopy [128]. Indeed, isotope tracing gives solid proof that methane originates from acetoclastic methanogenesis in the presence of conductive materials [26,120,124,128].

5. Acetotrophic methanogens and conductive materials

The major methanogenic archaea capable of using acetate and CO₂ are the acetotrophic methanogens *Methanosarcina* and *Methanosaeta* [34]. Recent advances on the use of conductive materials with *Methanosarcina* and *Methanosaeta* are summarized in Table 2.

5.1. *Methanosarcina*

Enhanced anaerobic digestion and methane production has been explained by the fact that *Methanosarcina* is enriched by the presence of conductive materials. For instance, addition of granular activated carbon increased the efficiency of dry anaerobic digestion of swine manure and methane generation [87]. Similarly, biochar strengthened the synergy between electricigens and *Methanosarcina* [74,57]. Here, Viggi et al. [102] demonstrated that biochar favored the growth of *Methanosarcina* over *Methanosaeta* during anaerobic digestion and methane production. Carbon cloth is also improving co-cultures and anaerobic digestion. For instance, *Sporanaerobacter* and *Methanosarcina* were enriched at the surface of carbon cloth surface during digestion of organic wastes [19]. Findings from our group also confirmed the acceleration of carbon cloth on methane generation [52]. Here, isotope tracing showed that higher methane production was mainly due to acetoclastic methanogenesis by *Methanosarcina*. We further showed that carbon nanotubes and graphene of high electrical conductivity stimulated methane generation by both CO₂ reduction and direct acetate dismutation, with a predominant contribution of *Methanosarcina* [124].

Metal-based materials also stimulate methane generation by *Methanosarcina*. Conductive iron oxides facilitated methane generation from propionate and acetate, and enhanced hydrolysis and acidification [131]. Magnetite induced the enrichment of electricigens and *Methanosarcina* efficiently in anaerobic sludges and soils [106]. Stimulation of methanogenesis by syntrophy between electroactive microorganisms and *Methanosarcina* with magnetite was revealed in anaerobic soils. For instance, nano-Fe₃O₄ stimulated the degradation of methanogenic straw in paddy soils, with a concomitant rise of *Methanosarcina* abundance [83]. The conductivity of Fe(III) oxides was proposed as a major factor explaining methanogenesis [148]. In wetland soils, nano-Fe₃O₄ particles accelerated electromethanogenesis rapidly by *Methanosarcina*, within several hours [121].

In situ biogenic magnetite located in a red clay horizon was also proposed to promote DIET for generating methane by *Methanosarcina* [126]. Similarly, the co-occurrence of sulfate and iron oxide promoted methane production by biogenic, semiconductive iron sulfides, which in turn induced faster methane production via electric syntrophy [42]. Moreover, biogenic magnetite was identified as the main contributor to promote DIET between *Geobacter* and *Methanosarcina* in a manner similar to that of c-type cytochrome [94]. Overall, most investigations attribute rising methane production to DIET-CO₂ reduction, yet only by indirect evidence. Moreover, metatranscriptomic studies disclosed that genes coding for acetoclastic methanogenesis, not genes for CO₂ reduction, were abundantly expressed in *Methanosarcina* with nano-magnetite [35]. Overall the respective contributions of DIET-CO₂ reduction and direct acetate dismutation requires further investigations.

5.2. *Methanosaeta*

Methanosaeta has a much higher affinity for acetate than *Methanosarcina* [36], making *Methanosaeta* more competitive in anaerobic environments where acetate levels are low [14,25]. Studies on DIET showed a substantial enrichment of 16S rRNA gene sequences closely related to *Geobacter* and *Methanosaeta* species on biochar surface [105].

Syntrophy of *Anaerolineaceae* and *Methanosaeta* on biochar was proposed to explain higher methane production during anaerobic digestion of organic wastes [108]. Moreover, conductive carbon felt favored the formation of biofilms on anaerobic granules, the formation of *Methanosaeta*-containing microbiome, to reduce propionate accumulation, and enhance methanogenic co-degradation of propionate and butyrate [9].

Granular activated carbon was also used to enrich *Methanosaeta* species and to improve anaerobic sludge digestion by converting acetate to methane [77]. Molecular and microbial evidence showed that granular activated carbon enriched simultaneously *Methanosaeta* and fermentative bacteria, *Syntrophorhabdus* and *Synergistes*, during the degradation of organic compounds, which in turn facilitated methane production [99]. Granular activated carbon addition also favored syntrophy between recently-identified *Geobacter* species and *Methanosaeta*, thus enhancing methane generation [73]. This syntrophy was confirmed by bioinformatic analysis, which showed that activated carbon should induce the formation of a dense syntrophic microbial network [142]. Furthermore, graphene addition induced the enrichment of *Geobacter* and *Methanosaeta* during anaerobic degradation of coal gasification wastewater, and improved both methane production and the removal of the chemical oxygen demand [149].

Magnetite was found particularly effective in promoting degradation of butyrate, propionate and acetate; it was suggested here that magnetite bridged exoelectrogens and *Methanosaeta* species [108]. Magnetite also probably triggered DIET between *Syntrophomonas*, or *Trichococcus*, and *Methanosaeta* when dairy wastewater was digested anaerobically with activated sludges [7]. Addition of akaganéite, an iron oxide/hydroxide mineral of (Fe³⁺,Ni²⁺)₈(OH,O)₁₆Cl_{1,25}nH₂O formula, into a high-temperature petroleum reservoir, promoted methane production by shifting the methanogenic pathway from hydrogenotrophic methanogenesis fueled by syntrophic acetate oxidizers to acetoclastic methanogenic [75].

Some studies also reported that both *Methanosaeta* and *Methanosarcina* promoted anaerobic digestion and methane production. Here, conductive materials facilitated electron exchange via DIET in the syntrophic oxidation of VFAs, resulting in efficient methane production using biochar, graphite felt and goethite [53,132,143]. Noteworthy, biochar was used to alleviate the stress of ammonium and acids by firstly enriching *Methanosaeta*, then *Methanosarcina* [66]. *Methanosarcina*, *Methanosaeta* and magnetite further enhanced the anaerobic treatment of solid and liquid wastes [46,49]. Moreover, conductivity and levels of extracellular polymeric substances of anaerobic sludges were remarkably increased by adding N-doped sewage sludge C, suggesting that DIET was involved with *Methanosaeta* and *Methanosarcina* as terminal contributors [150].

6. Benefits of conductive materials

It has been widely observed that conductive materials improve methane production by facilitating DIET, yet precise DIET mechanisms are still obscure [152]. Addition of conductive materials immobilize microorganisms, thus promoting biofilm growth, regulating pH and oxidation-reduction potential, which favors acetotrophic methanogens and potential acetoclastic methanogenesis [72,84,152] (Fig. 4). Several mechanisms seem to be better than electron transfer: the activation on iron-containing enzymes involved in methanogenesis and acidogenesis, selective enrichment on functional microorganism, and high electron donating capacities. Some limitations of these materials, however, was proposed as well, such as sterilization properties of carbon nanotubes, high cost of graphene, and not reusable for almost conductive materials. Here we present the positive effects of adding conductive materials.

6.1. Microbial immobilization

The binding and colonization of microbes on solids is known to

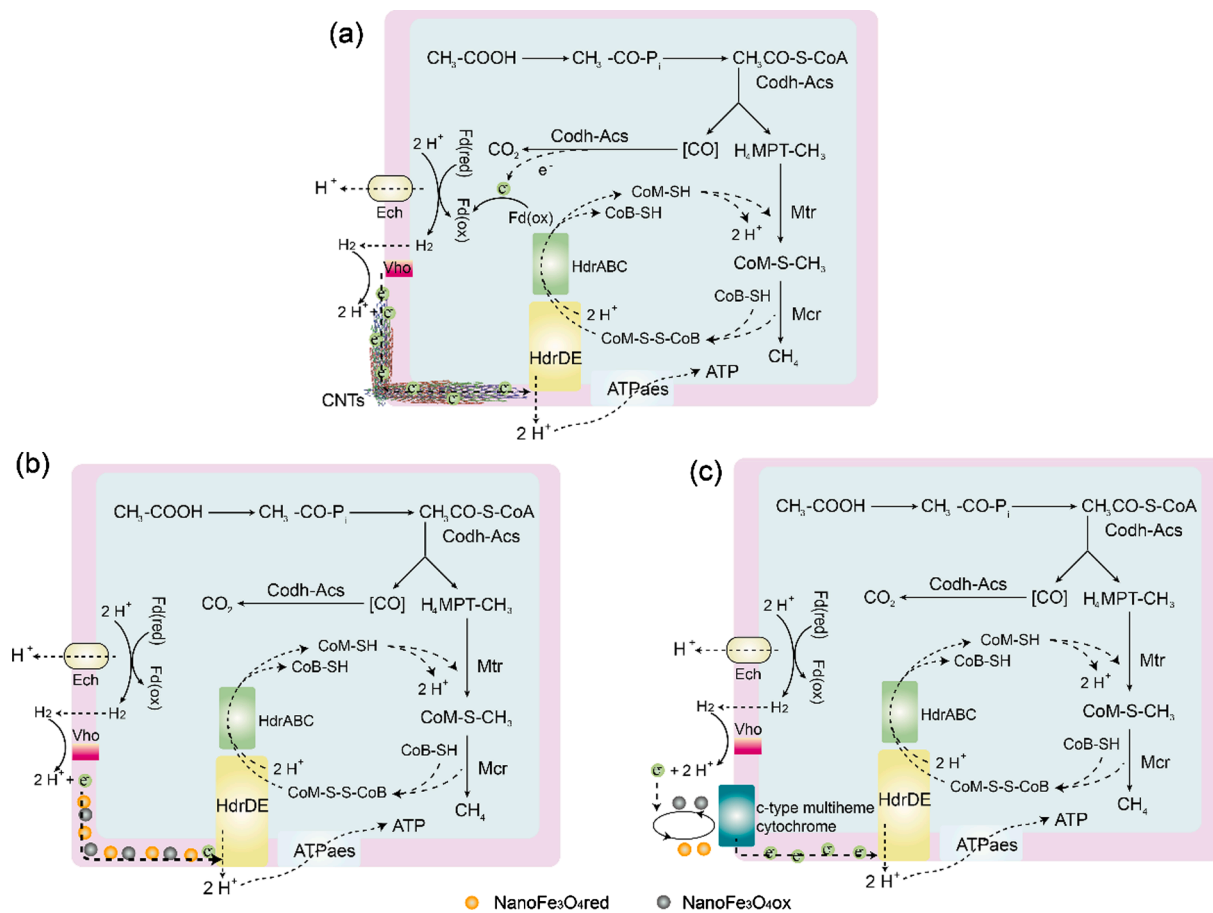


Fig. 3. Potential mechanisms by which conductive materials, carbon nanotubes (a) and nanoFe₃O₄ (b,c) accelerate acetoclastic methanogenesis. a: Carbon nanotubes attach to the cell surface, replace membrane components, and mediate electron transfer to accelerate CoM-S-S-CoB reduction inside the cell [128]. NanoFe₃O₄ particles act as solid electron shuttles to mediate electron transfer inside the cell (b, [26] and outside the cell (c, [110].

increase activity and syntrophy [15,58,67]. Microbial adherence is controlled by the adsorption capacity of materials, which in turn depends on surface morphology, pore volume and size, hydrophobicity and ion-exchange capacity [44,72]. Kumar et al. [45] showed that solids decreased biomass washout by trapping microbial communities inside the pores. Sequestration into the solid surface further allows to slow down the diffusion of exoenzymes and hydrolyzates away from the cell, thus saving substrates for anaerobic fermentation and methane production.

The surface area of conductive materials is a major factor controlling acetotrophic methanogens. For instance, methanogenic archaea belonging to *Methanosarcina* occurred at much higher abundance in powdered biochar amendments than in granulated biochar amendments [147]. Surface roughness and surface free energy of solid materials also controlled the activity of fixed cells [3]. Microorganisms tended to adhere better to roughest surfaces with a higher specific surface area to form biofilms. Materials having low surface free energy were more easily colonized by methanogens [30].

Granular activated carbon is widely used in anaerobic systems to improve fermentation. This has been partly attributed to the large granular activated carbon surface area that promotes microorganism attachment [78,113]. Here, the rapid degradation of VFAs provided ample feeding substrate for methanogenic archaea, and was explained by the formation of biofilms on granular activated carbon surfaces [12]. Comparison of granular activated carbon and powdered activated carbon showed that VFAs were better metabolized using the powder, thus suggesting that a highly porous structure allowed better colonization. It was also observed that acetate adsorbed on granular activated carbon is

more available to methanogenic archaea [34].

The improvement of anaerobic digestion by biochar addition was attributed to selective colonization of functional microorganisms [31]. Biochar also facilitated biofilm formation on the surface and inside the pore channel, thus immobilizing microbes [69]. Porous biochar was also found to absorb ammonia, thus decreasing microbial inhibition by ammonia and fixing methanogens [66,69]. The microbial contact distance decreased in systems where magnetic carbon hosts microorganisms that are able to generate acetate and methane [81]. Carbon fibers were also used as supporting media for microbial growth in anaerobic fermentation systems. Biofilms easily formed on carbon fibers surfaces, thus increasing biomass retention time, which in turn improved of anaerobic digestion. Consequently, the methanogens population was more abundant in the biofilm fraction that developed on the packed bed than that in the liquid fraction [95]. The abundant genera in carbon fibers-amended bioreactors were acetoclastic *Methanosaeta* and *Methanosarcina*, suggesting that the enhancement of methanogenesis is explained partly by microbial immobilization.

6.2. pH buffering capacity

In practice, anaerobic digestion of easy-acidified substrates always displayed low stability, VFAs conversion and methane production [107]. CO₂ absorption and carbonation can improve anaerobic digestion, in particular the growth and activity of methanogenic archaea [90]. Biological and abiotic changes maintain the system stability. For instance, carbonation of CO₂ by microbial carbonic anhydrase reduced the CO₂ concentration [115,118,117]. Adding conductive carbon-based

materials is beneficial on several grounds. For instance, biochar is an acid-buffering material by nature [107,152]. The pH was in the range of 4.0–3.0 with acetate as substrate for the control. By comparison, vermicompost biochar maintained it from 9.0 to 5.0. Biochar pH and pyrolytic temperature are positively correlated [43], suggesting that biochar can be used to correct an acidified or alkaline system depending on the pyrolytic temperature. Furthermore, the high pyrolytic temperature promoted the contribution of inorganic carbonate to alkalinity, whereas organic anions were the dominant contributors to the alkalinity for biochar produced at lower temperatures [140].

The release of alkali and alkaline earth cations from biochar was found to maintain the alkalinity of anaerobic digestion, suggesting that biochar can also be used as lime fertilizer in agriculture [28]. The pH value was about 5.2 when mineral fertilizer was used. The addition of biochar can enhance it to and even over 10.0. A practical soil management to improve soil quality by applying mineral organic fertilizer seemed to play similar role [123], with obvious pH increase from 6.5 to 7.5. Conductive carbon cloth also showed the ability to resist acidity when the pH was adjusted to 5.0 and 7.0 [146]. Thus pH buffering capability contributes to feasible anaerobic digestion.

6.3. Providing metal ions

Biochar with high calcium, magnesium and iron provides essential elements for fermentative bacteria and methanogenic archaea [17,134]. Indeed, methanogenic archaea require more trace elements such as Fe, Cu, Ni and Co, than fermentative bacteria [55]. These trace elements are important cofactors of enzymes controlling the acetoclastic methanogenic pathway [33]. Therefore, the impact of metal ion limitation on methanogens may be higher than that of bacteria. Specially, acetotrophic methanogens and acetate oxidizing bacteria directly compete for acetate, which is the most important anaerobic fermentation product. Trace elements, however, may be inhibitory and even toxic to anaerobic digestion, where the extent depended on concentration of these elements [70]. The concentration of trace metals during anaerobic digestion should be monitored under retention time to guarantee the efficiency of methane formation. A study showed that biochar increased the concentrations and ionization of aqueous Co and Ni, which were thus more easily taken up by methanogenic archaea [97]. Kato et al. [41] observed a marked increase in methane production from ethanol when magnetite was supplied at 20 mM Fe, compared to the control without addition of iron. The authors proposed that the leaching of iron ions and their contribution as a nutrient to enhance methanogenesis was insignificant due to low concentration of Fe (II), below 2 mM, in cultures. However, as iron is the active site of the enzyme, 2 mM iron ion is sufficient to play a vital role [144,145]. Further comprehensive research to determine the optimum concentration of metal ions and their impact on microbial activity in anaerobic digestion is required.

6.4. Reducing toxicity

Ammonium ion (NH_4^+) and free ammonia (NH_3) are known to inhibit methanogenesis in anaerobic environments [127,137]. To circumvent the ammonia toxicity, diverse natural and synthetic materials have been extensively tested as adsorbents to decrease toxicity. For instance metal oxides such as Fe_2O_3 , CaO and MgO adsorb $\text{NH}_4^+\text{-N}$, thus lowering leaching and diffuse pollution. Biochar is also used as an effective adsorbent for *in situ* removal. Biochar can also adsorb organics, phosphate, nitrate, nitrite, metals and CO_2 [8,23]. Biochar was also found to alleviate acid and ammonium stress, which in turn favored colonization by *Methanosaeta* and *Methanosarcina* [66]. Coconut coir and charcoal reduced the total ammonia nitrogen in bioreactors by harboring the denitrifying and ammonia oxidizing microorganisms with the methanogens [1]. Sasaki et al. [89] found that carbon fiber textiles stabilized thermophilic methanogenic bioreactors at elevated concentrations of ammonia nitrogen of 3 g/L. Carbon fibers also favored the

colonisation of *Methanosarcina* sp.

Granular activated carbon of large surface area was reported to promote adsorption of toxic chemicals such as organic solvents and heavy metals [2]. Application of granular activated carbon was reported to both absorb phenolic compounds and increase methane production [11]. Magnetite and carbon nanotubes enriched phenol-degrading bacteria such as *Syntrophorhabdus* and *Brooklawnia*, and acetoclastic methanogens *Methanosaeta*, suggesting that *Syntrophus* and *Methanosaeta* were the species involved in methanogenic phenol degradation [133]. Conductive materials also enhance the resistance to sulfate stress in anaerobic fermentation. For example, adding Fe_3O_4 alleviated sulfate toxicity during mesophilic methanogenesis [61].

With the presence of large particles of conductive material, these heavy metals can be adsorbed on the surface of conductive materials. The immobilization of heavy metals significantly reduces the heavy metal ions in the solution, thus reducing the influence on microorganisms of the fermentation system [82,98]. Similarly, conductive materials can improve heavy metal passivation during anaerobic digestion of organic matter. For example, Wang et al. [111] showed that biochar improved heavy metal passivation during wet anaerobic digestion of pig manure. Application of biochar is also a green remediation strategy for heavy metal contaminated soil [112].

6.5. Regulation of oxidation–reduction potential

Humic substances can act as shuttles between *Geobacter* and other species [40,93]. In particular, humic substances stimulated interspecies electron transfer between *Geobacter* and *Methanosarcina* species in rice paddy soils [148]. By contrast, some studies of anaerobic digesters demonstrated that humic substances were not able to promote syntrophy between bacteria and methanogens [6,20]. Specially, the 2,6-anthraquinone disulphonate (AQDS) analog of humic substances did not mediate electron transfer between *G. metallireducens* and *M. barkeri* in co-culture [58]. In addition to mediating electron transfer, the electron donating capacity of conductive materials such as humic substances should be considered. Few studies have monitored the oxidation–reduction potential during methanogenesis. Results showed that addition of conductive materials benefited methanogenesis at the final oxidation–reduction potential ranging from -200 mV to -400 mV [32]. Addition of diverse biochar was tested on methanogenesis in anaerobic digestion revealed no correlation between biochar conductivity and methanogenic capacity [102]. Authors concluded that the electron donating capacity was more essential. Yuan et al. [139] showed that biochar promotion of methanogenesis depends mainly on redox properties and charge–discharge capacity, rather than conductivity.

Salvador et al. [88] found that high concentrations of carbon nanotubes, of 5 g/L, decreased the oxydo-reduction potential of the anaerobic system, which in turn benefited methanogenesis. Beckmann et al. [10] proposed that neutral red in soluble form served both as electron donor and acceptor in the membrane-bound respiratory chain of *Methanosarcina mazei*. Neutral red was reduced when an excess hydrogen was the electron donor, but oxidized when CoB-S-S-CoM heterodisulfide was the electron acceptor. Overall the oxydoreduction potential can be modified sometimes by conductive materials, but molecular mechanisms remain unclear.

7. Conclusion and perspective

The production of biomethane in anaerobic digester is actually explained by several main mechanisms: hydrogenotrophic methanogenesis, DIET-based CO_2 reduction and acetoclastic methanogenesis. Massive research papers and reviews focused on DIET-based CO_2 reduction, but acetoclastic methanogenesis deserves more attention. Conductive materials accelerate methane generation, yet underlying mechanisms are not fully deciphered. More than DIET, benefits to acetotrophic methanogens and acetoclastic methanogenesis and the

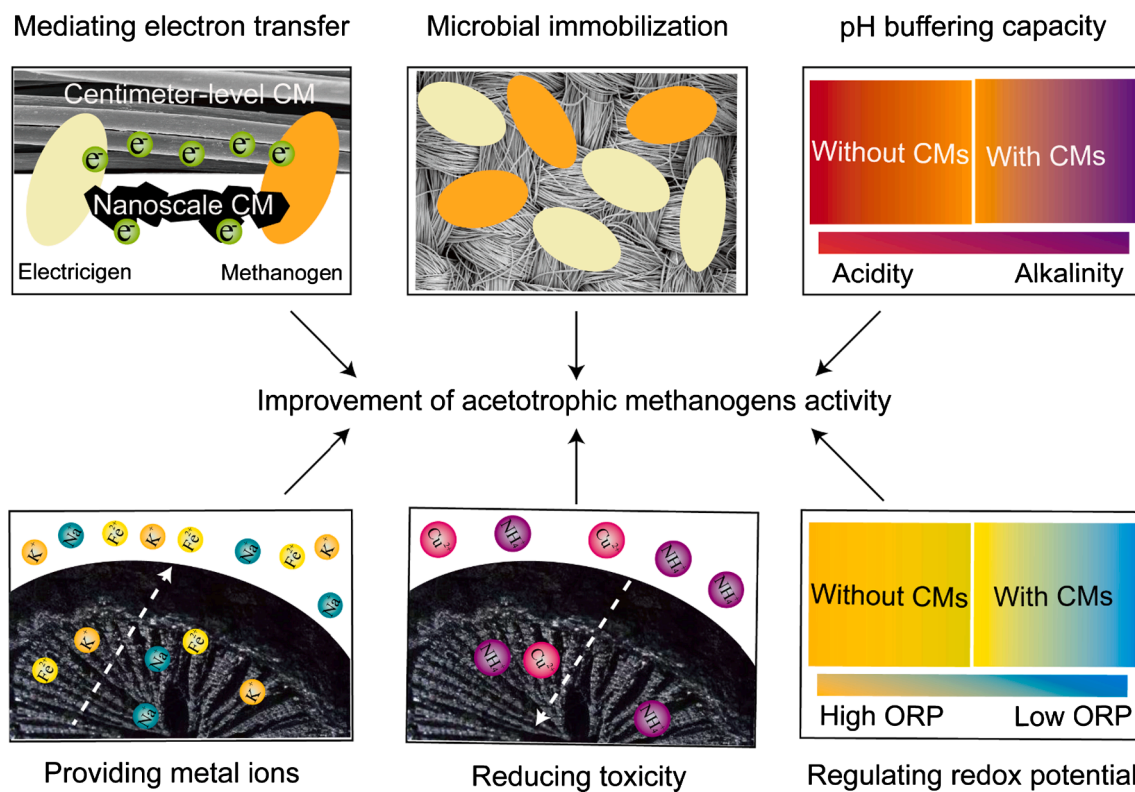


Fig. 4. Effects of conductive materials on acetotrophic methanogens. Conductive material mediate electron transfer, provide attachment for microbes, adsorb ionic nutrients, regulate pH and oxydoreduction potential (ORP), and reduce toxicity.

responding mechanism mediated by conductive materials should also be taken seriously. Some rules of conductive materials' impact on anaerobic digestion are summarized as follows:

Different types of conductive materials or even different doses of the same type have discrepant influences on the anaerobic digestion process, the methane-production capacity, and the related microbial community.

Besides the type of conductive materials, the duration of action and operation temperature altogether are considered to be the key factors affecting the effects of conductive materials on anaerobic digestion and methane production.

The long-term effects reserve more attention. Short-term experiments may be difficult to accurately reflect the potential effects of conductive materials on complex microbial consortia due to the lack of microbial adaptation. By comparison, long-term observation and comprehensive analysis are to be given topmost priority.

As a non-culture-based method, metagenomics becomes a vital tool to comprehend microbial communities sufficiently. Various omics techniques are more and more applied in understanding the effect of conductive materials on microbial communities. For example, metagenomics can be applied in identifying potential functional microbes. Furthermore, metatranscriptome is an alternative tool to identify microbial mechanism. Consequently, the application of omics methods will bring revolutionary improvements to the study of anaerobic digestion and methanogenic performance affected by conductive materials. In particular, omics can discover true functional microorganisms and related metabolic pathways. Collectively, the further research needs for comprehending the interactions between the conductive materials, substrate and intermediate metabolite, functional microorganisms, and the real impacts of adopting these additives on full-scale operations.

CRediT authorship contribution statement

Leilei Xiao: Data curation, Validation, Supervision. **Eric Lichtfouse:** Conceptualization, Data curation, Resources. **P. Senthil Kumar:** Conceptualization, Methodology, Validation, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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