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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 (CO<sub>2</sub>) to methane (CH<sub>4</sub>) is commonly explained by interspecies electron transfer, i. e., direct interspecies electron transfer (DIET)-based CO<sub>2</sub> reduction or mediated interspecies electron transfer (MIET)-based CO<sub>2</sub> reduction. For DIET-based CO<sub>2</sub> reduction, or DIET-CO<sub>2</sub> reduction, where electrons are provided by electricigens and transferred to methanogenic archaea to complete CO<sub>2</sub> 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-CO<sub>2</sub> reduction and acetoclastic methanogenesis mediated by conductive materials. In the past decade, DIET-CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> reduction, or DIET-based CO<sub>2</sub> reduction, or DIET-CO<sub>2</sub> reduction. DIET-CO<sub>2</sub> 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-CO<sub>2</sub> 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-CO<sub>2</sub> 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 <sub>3</sub> O <sub>4</sub>	4.64 mM of Fe	NA	<i>M. barkeri</i>	[26]
Pure culture	Acetate	NanoFe <sub>3</sub> O <sub>4</sub>	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-CO<sub>2</sub> 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. CO<sub>2</sub> and acetate. For instance, hydrogenotrophic methanogens use H<sub>2</sub> and CO<sub>2</sub> as substrates to generate methane [18] (Eq. (1)):



Most cultivable methanogens are strict hydrogenotrophs, they use H electrons to reduce CO<sub>2</sub> [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-CO<sub>2</sub> 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 CO<sub>2</sub> 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-CO<sub>2</sub> and DIET-CO<sub>2</sub>), 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 CO<sub>2</sub> 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. CO<sub>2</sub> reduction to methane

Two mechanisms explain reduction of CO<sub>2</sub> 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 H<sub>2</sub> 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 H<sub>2</sub> 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>Methanoseta</i>	[132]
Anaerobic soil	Glucose	GAC	1 g/L	NA	<i>Methanoseta</i>	[120]
Waste activated sludge	Seed sludge	GAC	27 g/L	<i>anaerolineaceae</i>	<i>Methanoseta</i>	[77]
Organic wastewater	Anaerobic sludge/acetate	GAC	10 g/L	<i>gracilibacter</i>	<i>Methanoseta</i>	[99]
Packing media	Sludge/fructose and polyethylene glycol	GAC	NA	<i>Geobacter</i>	<i>Methanoseta</i>	[73]
Anaerobic sludge	Propionate	GAC	NA	<i>Syntrophobacte</i>	<i>Methanoseta</i>	[135]
Anaerobic sludge	Acetate	GAC	NA	<i>Syntrophobacte</i>	<i>Methanoseta</i>	[135]
Synthetic wastewater	Anaerobic sludge/glucose	GAC	10 g/L	NA	<i>Methanosetaceae</i>	[124]
Food waste	Seed sludge	Activated carbon	5,15, 25, 35 g/L	<i>Smithella</i>	<i>Methanoseta</i>	[142]
Synthetic wastewater	Seed sludge/VFAs	Activated carbon	5 g/L	<i>Syntrophomonas</i>	<i>Methanoseta</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>Methanoseta</i>	
Basic nutrient solution	Granular sludge/glucose	Biochar	10 g/L	<i>Enterobacteriaceae</i>	<i>Methanoseta</i>	[66]
Synthetic wastewater	Seed sludge/sucrose	Biochar	4 g/L	<i>Bacteroidetes</i>	<i>Methanosetaceae</i>	[52]
Food waste	Seed sludge	Biochar	10 g/L	<i>Fervidobacterium</i>	<i>Methanoseta</i>	[53]
Food waste	Activated sludge	Biochar	2, 6, 10 and 15 g/L	<i>Anaerolineaceae</i>	<i>Methanoseta</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>Methanosetaceae</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>Methanoseta</i>	[143]
Artificial wastewater	Seed sludge/butyrate	Graphite felt	NA	<i>Syntrophomonas</i>	<i>Methanoseta</i>	[143]
Propionate-butyrate medium	Anaerobic sludge/propionate and butyrate	Carbon felt particles	1583 m <sup>2</sup> /m <sup>3</sup>	<i>Desulfuromonas</i>	<i>Methanoseta</i>	[9]
Wastewater	Seed sludge	Nitrogen doped sewage sludge carbon	5 g/L	<i>Saccharofermentans</i>	<i>Methanoseta</i>	[52]
Synthetic wastewater	Anaerobic sludge/glucose	Graphene	0.1 g/L	NA	<i>Methanosetaceae</i>	[124]
Coal gasification wastewater	Seed sludge	Graphene	0.2, 0.5 and 1.0 g/L	<i>Saccharofermentans and atipia</i>	<i>Methanoseta</i>	[149]
Basal medium	Anaerobic granular sludge/Sucrose	Carbon nanotubes	1 g/L	<i>Clostridiales</i>	<i>Methanoseta</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>Methanoseta</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>Methanoseta</i>	[77]
Synthetic wastewater	Anaerobic sludge/glucose	Magnetite	4.62 g/L	NA	<i>Methanosetaceae</i>	[124]
Fresh leachate	Sludge	Magnetite	10 g/L	<i>Eubacteriaceae</i>	<i>Methanoseta</i>	[49]
Fischer-Tropsch wastewater	NA	Magnetite	0.1, 0.2 and 0.3 g/L	<i>Geobacter</i>	<i>Methanoseta</i>	[108]
Synthetic wastewater	Ethanol, acetate and propionate	Fe <sub>3</sub> O <sub>4</sub>	10 g/L	<i>Mesotoga</i>	<i>Methanoseta</i>	[61]
Whey	Anaerobic seed sludge	Magnetite	20 mM	<i>Clostridium</i>	<i>Methanoseta</i>	[7]
Basal medium	Anaerobic sludge/acetate	Magnetite	25 mM	<i>Geobacteraceae</i>	<i>Methanosarcina</i>	[151]
Synthetic wastewater	Anaerobic sludge/tryptone	Fe <sub>3</sub> O <sub>4</sub>	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 <sub>3</sub> O <sub>4</sub>	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>Methanoseta</i>	
Basal medium	Mixture of oil and water	Akaganéite	25 mM	<i>Halomonas</i>	<i>Methanoseta</i>	[75]

Note: Methane production was mainly from acetoclastic methanogenesis with a gray background. On the contrary, DIET-CO<sub>2</sub> 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  $\Delta G$  above 0. As a consequence, hydrogenase activity and metabolism of syntrophic bacteria are low.

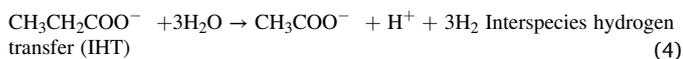
In the 2000 s, interspecies electron transfer was known to be mediated only by compounds, e.g. formate and bihydrogen. Later, in the past decade, DIET has been proposed as a more efficient and energy-saving mechanism, compared to MIET [64]. DIET involves electrically conductive pili as conduit to transfer 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,  $\Delta G^0$  of  $-26.4 \text{ kJ/mol}$ , 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-CO<sub>2</sub> reduction

DIET-CO<sub>2</sub> 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 CO<sub>2</sub> [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-CO<sub>2</sub> [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 CO<sub>2</sub> reduction [76]. We provided unambiguous evidence for promotion

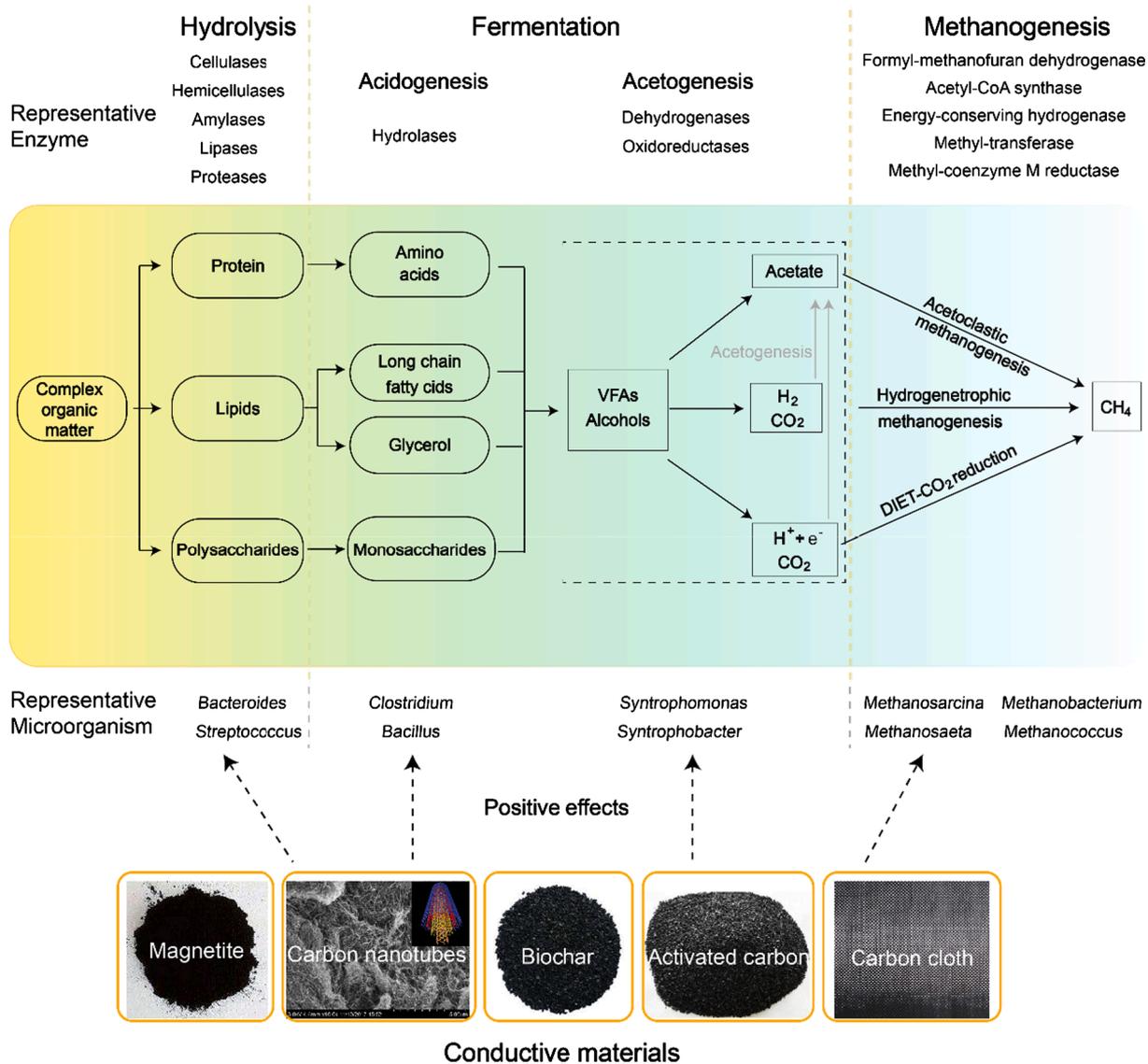


Fig. 2. Hydrolysis, fermentation and methanogenesis in anaerobic environments. Three mechanisms explain biomethane production: acetoclastic methanogenesis, hydrogenotrophic methanogenesis and direct interspecies electron transfer (DIET)-CO<sub>2</sub> reduction. Diverse conductive materials increase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

of acetoclastic methanogenesis rather than CO<sub>2</sub> reduction, in a *Methanosarcina* spp. culture with carbon nanotubes, using isotope tracing and CH<sub>3</sub>F 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, nanoFe<sub>3</sub>O<sub>4</sub> 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 NanoFe<sub>3</sub>O<sub>4</sub> 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 NanoFe<sub>3</sub>O<sub>4</sub>, thus promoting membrane electron transfer and acetoclastic methanogenesis. The redox cycling of nanoFe<sub>3</sub>O<sub>4</sub> 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-CO<sub>2</sub> 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-CO<sub>2</sub> was definitively provided in the study of anaerobic soils by <sup>13</sup>C tracing, CH<sub>3</sub>F 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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-Fe<sub>3</sub>O<sub>4</sub> 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-Fe<sub>3</sub>O<sub>4</sub> 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-CO<sub>2</sub> reduction, yet only by indirect evidence. Moreover, metatranscriptomic studies disclosed that genes coding for acetoclastic methanogenesis, not genes for CO<sub>2</sub> reduction, were abundantly expressed in *Methanosarcina* with nano-magnetite [35]. Overall the respective contributions of DIET-CO<sub>2</sub> 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 (Fe<sup>3+</sup>,Ni<sup>2+</sup>)<sub>8</sub>(OH,O)<sub>16</sub>Cl<sub>1,25</sub>nH<sub>2</sub>O 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].

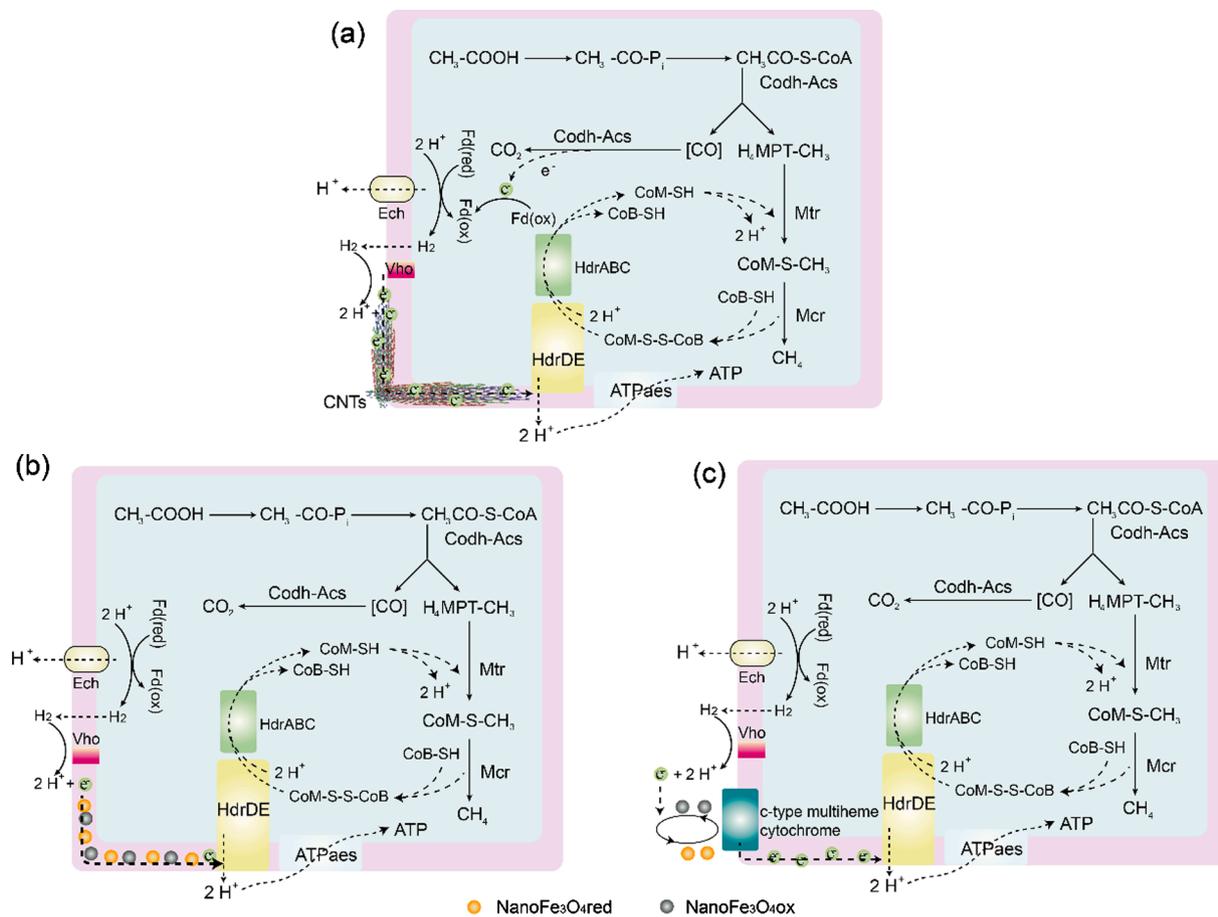
Some studies also reported that both *Methanosaeta* and *Methanosarcina* promoted anaerobic digestion and methane production. Here, conductive materials facilitated electron exchange via DIET in the syntrophic oxidation of VFAs, resulting in efficient methane production using biochar, graphite felt and 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 nanoFe<sub>3</sub>O<sub>4</sub> (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]. NanoFe<sub>3</sub>O<sub>4</sub> 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]. CO<sub>2</sub> 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 CO<sub>2</sub> by microbial carbonic anhydrase reduced the CO<sub>2</sub> 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 ( $\text{NH}_4^+$ ) and free ammonia ( $\text{NH}_3$ ) 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  $\text{Fe}_2\text{O}_3$ , 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  $\text{CO}_2$  [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  $\text{Fe}_3\text{O}_4$  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  $\text{CO}_2$  reduction and acetoclastic methanogenesis. Massive research papers and reviews focused on DIET-based  $\text{CO}_2$  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

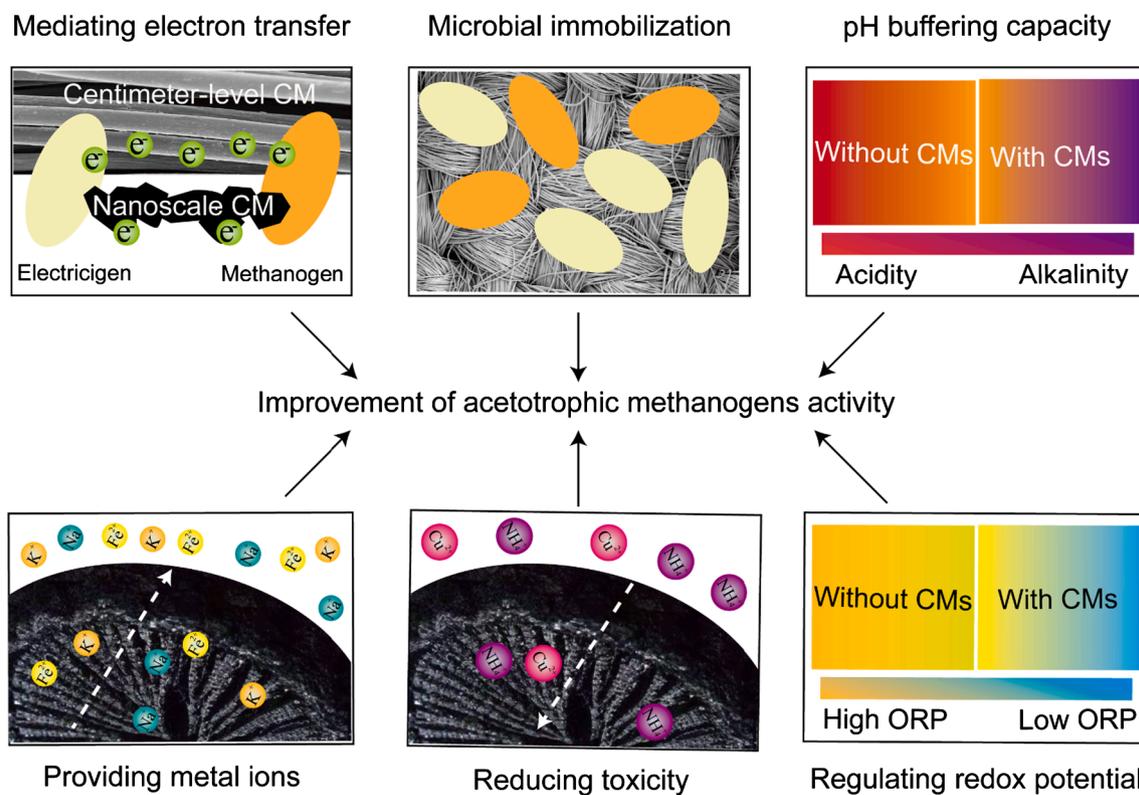


Fig. 4. Effects of conductive materials on acetotrophic methanogens. Conductive material mediate electron transfer, provide attachment for microbes, adsorb ionic nutrients, regulate pH and oxydoreduction potential (ORP), and reduce toxicity.

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 dozes 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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#### References

- [1] Acharya BK, Mohana S, Madamwar D. Anaerobic treatment of distillery spent wash – A study on upflow anaerobic fixed film bioreactor. *Bioresour Technol* 2008;99(11):4621–6.
- [2] Ahmed E, Rothenberger A. Adsorption of volatile hydrocarbons in iron polysulfide chalcogels. *Microporous Mesoporous Mater* 2014;199:74–82.
- [3] Ammar Y, Swailes D, Bridgens B, Chen JJ. Influence of surface roughness on the initial formation of biofilm. *Surf Coat Technol* 2015;284:410–6.
- [4] Angel R, Claus P, Conrad R. Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. *ISME J* 2012;6(4): 847–62.
- [5] Arif S, Liaquat R, Adil M. Applications of materials as additives in anaerobic digestion technology. *Renew Sust Energ Rev* 2018;97:354–66.
- [6] Azman S, Khadem AF, Plugge CM, Stams AJM, Bec S, Zeeman G. Effect of humic acid on anaerobic digestion of cellulose and xylan in completely stirred tank reactors: inhibitory effect, mitigation of the inhibition and the dynamics of the microbial communities. *Appl Microbiol Biotechnol* 2017;101(2):889–901.

- [7] Baek G, Kim J, Cho K, Bae H, Lee C. The biostimulation of anaerobic digestion with (semi)conductive ferric oxides: their potential for enhanced biomethanation. *Appl Microbiol Biotechnol* 2015;99(23):10355–66.
- [8] Bagreev A, Bandosz TJ, Locke DC. Pore structure and surface chemistry of adsorbents obtained by pyrolysis of sewage sludge-derived fertilizer. *Carbon* 2001;39(13):1971–9.
- [9] Barua S, Zakaria BS, Dhar BR. Enhanced methanogenic co-degradation of propionate and butyrate by anaerobic microbiome enriched on conductive carbon fibers. *Bioresour Technol* 2018;266:259–66.
- [10] Beckmann S, Welte C, Li XM, Oo YM, Kroeninger L, Heo Y, et al. Novel phenazine crystals enable direct electron transfer to methanogens in anaerobic digestion by redox potential modulation. *Energy Environ Sci* 2016;9(2):644–55.
- [11] Bertin L, Lampis S, Todaro D, Scoma A, Vallini G, Marchetti L, et al. Anaerobic acidogenic digestion of olive mill wastewaters in biofilm reactors packed with ceramic filters or granular activated carbon. *Water Res* 2010;44(15):4537–49.
- [12] Capson-Tojo G, Moscoviz R, Ruiz D, Santa-Catalina G, Trably E, Rouez M, et al. Addition of granular activated carbon and trace elements to favor volatile fatty acid consumption during anaerobic digestion of food waste. *Bioresour Technol* 2018;260:157–68.
- [13] Cavalcante WA, Gehring TA, Zaiat M. Stimulation and inhibition of direct interspecies electron transfer mechanisms within methanogenic reactors by adding magnetite and granular active carbon. *Chem Eng J* 2021;415:128882.
- [14] Chauhan A, Ogram A. Phylogeny of acetate-utilizing microorganisms in soils along a nutrient gradient in the Florida everglades. *Appl Environ Microbiol* 2006;72(10):6837–40.
- [15] Chen SS, Rotaru AE, Liu FH, Phillips J, Woodard TL, Nevin KP, et al. Carbon cloth stimulates direct interspecies electron transfer in syntrophic co-cultures. *Bioresour Technol* 2014;173:82–6.
- [16] Chen SS, Rotaru AE, Shrestha PM, Malvankar NS, Liu FH, Fan W, et al. Promoting interspecies electron transfer with biochar. *Sci Rep* 2014;4:5019.
- [17] Chen Y, Cheng JJ, Creamer KS. Inhibition of anaerobic digestion process: a review. *Bioresour Technol* 2008;99(10):4044–64.
- [18] Conrad R. Quantification of methanogenic pathways using stable carbon isotopic signatures: a review and a proposal. *Org Geochem* 2005;36(5):739–52.
- [19] Dang Y, Holmes DE, Zhao ZQ, Woodard TL, Zhang YB, Sun DZ, et al. Enhancing anaerobic digestion of complex organic waste with carbon-based conductive materials. *Bioresour Technol* 2016;220:516–22.
- [20] Dang Y, Lei YQ, Liu Z, Xue YT, Sun DZ, Wang LY, et al. Impact of fulvic acids on bio-methanogenic treatment of municipal solid waste incineration leachate. *Water Res* 2016;106:71–8.
- [21] Evans PN, Boyd JA, Leu AO, Woodcroft B, Parks DH, Hugenholtz P, et al. An evolving view of methane metabolism in the archaea. *Nat Rev Microbiol* 2019;17(4):219–32.
- [22] Evans PN, Parks DH, Chadwick GL, Robbins SJ, Orphan VJ, Golding SD, et al. Methane metabolism in the archaeal phylum bathyarchaeota revealed by genome-centric metagenomics. *Science* 2015;350(6259):434–8.
- [23] Fagbohunge MO, Herbert BMJ, Hurst L, Li H, Usmani SQ, Semple KT. Impact of biochar on the anaerobic digestion of citrus peel waste. *Bioresour Technol* 2016; 216:142–9.
- [24] Feng H, Guo J, Han M, Wang W, Peng C, Jin J, Song X, Yu S. A review of the mechanisms and controlling factors of methane dynamics in forest ecosystems. *Forest Ecol Manag* 2020;455:117702.
- [25] Fey A, Conrad R. Effect of temperature on carbon and electron flow and on the archaeal community in methanogenic rice field soil. *Appl Environ Microbiol* 2000;66(11):4790–7.
- [26] Fu L, Zhou T, Wang JY, You LX, Lu YH, Yu LP, et al. NanoFe<sub>3</sub>O<sub>4</sub> as solid electron shuttles to accelerate acetotrophic methanogenesis by *Methanosarcina barkeri*. *Front Microbiol* 2019;10:388.
- [27] Gao K, Lu Y. Putative extracellular electron transfer in methanogenic archaea. *Front Microbiol* 2021;12:611739.
- [28] Glaser B, Wiedner K, Seelig S, Schmidt HP, Gerber H. Biochar organic fertilizers from natural resources as substitute for mineral fertilizers. *Agron Sustainable Dev* 2015;35(2):667–78.
- [29] Guskos N, Papadopoulos GJ, Likodimos V, Patapis S, Yarmis D, Przepiera A, et al. Photoacoustic, epr and electrical conductivity investigations of three synthetic mineral pigments: hematite, goethite and magnetite. *Mater Res Bull* 2002;37(6): 1051–61.
- [30] Habouzit F, Gevaudan G, Hamelin J, Steyer JP, Bernet N. Influence of support material properties on the potential selection of Archaea during initial adhesion of a methanogenic consortium. *Bioresour Technol* 2011;102(5):4054–60.
- [31] He PJ, Zhang HH, Duan HW, Shao LM, Lu F. Continuity of biochar-associated biofilm in anaerobic digestion. *Chem Eng J* 2020;390:124605.
- [32] Hirano S, Matsumoto N, Morita M, Sasaki K, Ohmura N. Electrochemical control of redox potential affects methanogenesis of the hydrogenotrophic methanogen *Methanothermobacter thermoautotrophicus*. *Lett Appl Microbiol* 2013;56(5): 315–21.
- [33] Hoelzle RD, Virdis B, Batstone DJ. Regulation mechanisms in mixed and pure culture microbial fermentation. *Biotechnol Bioeng* 2014;111(11):2139–54.
- [34] Holmes DE, Smith JA. Biologically produced methane as a renewable energy source. *Adv Appl Microbiol* 2016;97:1–61.
- [35] Inaba R, Nagoya M, Kouzuma A, Watanabe K. Metatranscriptomic evidence for magnetite nanoparticle-stimulated acetoclastic methanogenesis under continuous agitation. *Appl Environ Microbiol* 2019;85(23):e01733–01719.
- [36] Jetten MSM, Stams AJM, Zehnder AJB. Methanogenesis from acetate - a comparison of the acetate metabolism in *Methanotheroxobacter* and *Methanosarcina* spp. *FEMS Microbiol Lett* 1992;88(3–4):181–97.
- [37] Jiang Q, Chen Y, Yu S, Zhu R, Zhong C, Zou H, et al. Effects of citrus peel biochar on anaerobic co-digestion of food waste and sewage sludge and its direct interspecies electron transfer pathway study. *Chem Eng J* 2020;398:125643.
- [38] Jing YH, Wan JJ, Angelidaki I, Zhang SC, Luo G. Itraq quantitative proteomic analysis reveals the pathways for methanation of propionate facilitated by magnetite. *Water Res* 2017;108:212–21.
- [39] Kang HJ, Lee SH, Lim TG, Lim TG, Park JH, Park HD. Recent advances in methanogenesis through direct interspecies electron transfer via conductive materials: a molecular microbiological perspective. *Bioresour Technol* 2021;322: 124587.
- [40] Kappler A, Benz M, Schink B, Brune A. Electron shuttling via humic acids in microbial iron(III) reduction in a freshwater sediment. *FEMS Microbiol Ecol* 2004;47(1):85–92.
- [41] Kato S, Hashimoto K, Watanabe K. Methanogenesis facilitated by electric syntrophy via (semi)conductive iron-oxide minerals. *Environ Microbiol* 2012;14(7):1646–54.
- [42] Kato S, Igarashi K. Enhancement of methanogenesis by electric syntrophy with biogenic iron-sulfide minerals. *Microbiol Biotechnol* 2019;8(3):e00647.
- [43] Komnitsas K, Zaharaki D, Pyloti I, Vamvuka D, Bartzas G. Assessment of pistachio shell biochar quality and its potential for adsorption of heavy metals. *Waste Biomass Valorization* 2015;6(5):805–16.
- [44] Kubota M, Nakabayashi T, Matsumoto Y, Shiomi T, Yamada Y, Ino K, et al. Selective adsorption of bacterial cells onto zeolites. *Colloid Surface B* 2008;64(1): 88–97.
- [45] Kumar G, Sivagurunathan P, Sen B, Kim SH, Lin CY. Mesophilic continuous fermentative hydrogen production from acid pretreated de-oiled jatropha waste hydrolysate using immobilized microorganisms. *Bioresour Technol* 2017;240: 137–43.
- [46] Lee J, Koo T, Yulisa A, Hwang S. Magnetite as an enhancer in methanogenic degradation of volatile fatty acids under ammonia-stressed condition. *J Environ Manage* 2019;241:418–26.
- [47] Lee S, Kang H, Lim T, Park H. Magnetite and granular activated carbon improve methanogenesis via different metabolic routes. *Fuel* 2020;281:118768.
- [48] Lei YQ, Sun DZ, Dang Y, Chen HM, Zhao ZQ, Zhang YB, et al. Stimulation of methanogenesis in anaerobic digesters treating leachate from a municipal solid waste incineration plant with carbon cloth. *Bioresour Technol* 2016;222:270–6.
- [49] Lei YQ, Wei LX, Liu TY, Xiao YY, Dang Y, Sun DZ, et al. Magnetite enhances anaerobic digestion and methanogenesis of fresh leachate from a municipal solid waste incineration plant. *Chem Eng J* 2018;348:992–9.
- [50] Li C, Lesnik KL, Liu H. Stay connected: electrical conductivity of microbial aggregates. *Biotechnol Adv* 2017;35(6):669–80.
- [51] Li HJ, Chang JL, Liu PF, Fu L, Ding DW, Lu YH. Direct interspecies electron transfer accelerates syntrophic oxidation of butyrate in paddy soil enrichments. *Environ Microbiol* 2015;17(5):1533–47.
- [52] Li JJ, Xiao LL, Zheng SL, Zhang YC, Luoc M, Tong C, et al. A new insight into the strategy for methane production affected by conductive carbon cloth in wetland soil: beneficial to acetoclastic methanogenesis instead of CO<sub>2</sub> reduction. *Sci Total Environ* 2018;643:1024–30.
- [53] Li Q, Xu MJ, Wang GJ, Chen R, Qiao W, Wang XC. Biochar assisted thermophilic co-digestion of food waste and waste activated sludge under high feedstock to seed sludge ratio in batch experiment. *Bioresour Technol* 2018;249:1009–16.
- [54] Li X, Li Q, He J, Zhang YF, Zhu NM. Application of activated carbon to enhance biogas production rate of *Flammulina velutipes* residues with composting pretreatment. *Waste Biomass Valorization* 2020:280–96.
- [55] Li Y, Chen YG, Wu J. Enhancement of methane production in anaerobic digestion process: a review. *Appl Energy* 2019;240:120–37.
- [56] Lim E, Tian H, Chen Y, Ni K, Zhang J, Tong Y. Methanogenic pathway and microbial succession during start-up and stabilization of thermophilic food waste anaerobic digestion with biochar. *Bioresour Technol* 2020;314:123751.
- [57] Liu FH, Rotaru AE, Shrestha PM, Malvankar NS, Nevin KP, Lovley DR. Promoting direct interspecies electron transfer with activated carbon. *Energy Environ Sci* 2012;5(10):8982–9.
- [58] Liu FH, Rotaru AE, Shrestha PM, Malvankar NS, Nevin KP, Lovley DR. Magnetite compensates for the lack of a pilin-associated c-type cytochrome in extracellular electron exchange. *Environ Microbiol* 2015;17(3):648–55.
- [59] Liu J, Liu F, Yu J, Wang Q, Li Z, Liu K, et al. Proteomics reveal biomethane production process induced by carbon nanotube. *Environ Res* 2021;200:111417.
- [60] Liu Y, Gu MQ, Yin QD, Wu GX. Inhibition mitigation and ecological mechanism of mesophilic methanogenesis triggered by supplement of ferrous oxide in sulfate-containing systems. *Bioresour Technol* 2019;288:121546.
- [61] Liu YC, Whitman WB. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Incredible Anaerobes From Physiol Genom Fuels* 2008; 1125:171–89.
- [62] Lovley DR. Happy together: microbial communities that hook up to swap electrons. *ISME J* 2017;11(2):327–36.
- [63] Lovley DR. Syntrophy goes electric: direct interspecies electron transfer. *Annu Rev Microbiol* 2017;71:643–64.
- [64] Lu C, Shen Y, Li C, Zhu N, Yuan H. redox-active biochar and conductive graphite stimulated methanogenic metabolism in anaerobic digestion of waste-activated sludge: beyond direct interspecies electron transfer. *ACS Sustainable Chem Eng* 2020;8:12626–36.
- [65] Lu F, Luo CH, Shao LM, He PJ. Biochar alleviates combined stress of ammonium and acids by firstly enriching *Methanoseta* and then *Methanosarcina*. *Water Res* 2016;90:34–43.

- [67] Lu JH, Chen C, Huang C, Zhuang HC, Leu SY, Lee DJ. Dark fermentation production of volatile fatty acids from glucose with biochar amended biological consortium. *Bioresour Technol* 2020;303:122921.
- [68] Lu JS, Chang JS, Lee DJ. Adding carbon-based materials on anaerobic digestion performance: a mini-review. *Bioresour Technol* 2020;300:122696.
- [69] Luo CH, Lu F, Shao LM, He PJ. Application of eco-compatible biochar in anaerobic digestion to relieve acid stress and promote the selective colonization of functional microbes. *Water Res* 2015;70:496.
- [70] A.N. Matheri M. Belaid T. Seodigeng J. Ngila The Role of Trace Elements on Anaerobic Co-digestion in Biogas Production 2016 London, U.K.
- [71] Martins G, Salvador AF, Pereira L, Alves MM. Methane production and conductive materials: a critical review. *Environ Sci Technol* 2018;52(18):10241–53.
- [72] Masebinu SO, Akinlabi ET, Muzenda E, Aboyade AO. A review of biochar properties and their roles in mitigating challenges with anaerobic digestion. *Renew Sust Energ Rev* 2019;103:291–307.
- [73] Mei R, Nobu MK, Narihiro T, Yu J, Sathyagal A, Willman E, et al. Novel Geobacter species and diverse methanogens contribute to enhanced methane production in media-added methanogenic reactors. *Water Res* 2018;147:403–12.
- [74] Pan JT, Ma JY, Zhai LM, Liu HB. Enhanced methane production and syntrophic connection between microorganisms during semi-continuous anaerobic digestion of chicken manure by adding biochar. *J Cleaner Prod* 2019;240:118178.
- [75] Pan P, Hong B, Mbadinga SM, Wang LY, Liu JF, Yang SZ, et al. Iron oxides alter methanogenic pathways of acetate in production water of high-temperature petroleum reservoir. *Appl Microbiol Biot* 2017;101(18):7053–63.
- [76] Paton M, Rodriguez J. A compilation and bioenergetic evaluation of syntrophic microbial growth yields in anaerobic digestion. *Water Res* 2019;159:176–83.
- [77] Peng H, Zhang YB, Tan DM, Zhao ZQ, Zhao HM, Quan X. Roles of magnetite and granular activated carbon in improvement of anaerobic sludge digestion. *Bioresour Technol* 2018;249:666–72.
- [78] Pham TH, Aelterman P, Verstraete W. Bioanode performance in bioelectrochemical systems: recent improvements and prospects. *Trends Biotechnol* 2009;27(3):168–78.
- [79] Prabhu AV, Raja SA, Avinash A, Pugazhendhi A. Parametric optimization of biogas potential in anaerobic co-digestion of biomass wastes. *Fuel* 2021;288:119574.
- [80] Putkinen A, Siljanen H, Laihonon A, Paasialo I, Porkka K, Tiirola M, et al. insight to the role of microbes in the methane exchange in trees: evidence from metagenomic sequencing. *New Phytol* 2021. <https://doi.org/10.1111/nph.17365>.
- [81] Qin Y, Wang HS, Li XR, Cheng JJ, Wu WX. Improving methane yield from organic fraction of municipal solid waste (ofmsw) with magnetic rice-straw biochar. *Bioresour Technol* 2017;245:1058–66.
- [82] Qiu B, Tao X, Wang H, Li W, Chu H. Biochar as a low-cost adsorbent for aqueous heavy metal removal: a review. *J Anal Appl Pyrol* 2021;11:105081.
- [83] Qiu CP, Feng YZ, Wu M, Zhang JW, Chen XF, Li ZP. Nano-Fe<sub>3</sub>O<sub>4</sub> accelerates methanogenic straw degradation in paddy soil enrichments. *Appl Soil Ecol* 2019;144:155–64.
- [84] Qiu L, Deng YF, Wang F, Davaritouchae M, Yao YQ. A review on biochar-mediated anaerobic digestion with enhanced methane recovery. *Renew Sust Energ Rev* 2019;115:109373.
- [85] Ren GP, Chen P, Yu J, Liu JB, Ye J, Zhou S. Recyclable magnetite-enhanced electromethanogenesis for biomethane production from wastewater. *Water Res* 2019;166:115095.
- [86] Rotaru AE, Shrestha PM, Liu FH, Shrestha M, Shrestha D, Embree M, et al. A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to Methanoseta for the reduction of carbon dioxide to methane. *Energy Environ Sci* 2014;7(1):408–15.
- [87] Ryue J, Lin L, Liu Y, Lu WJ, McCartney D, Dhar BR. Comparative effects of gas addition on methane productivity and microbial community in mesophilic and thermophilic anaerobic digestion of food waste. *Biochem Eng J* 2019;146:79–87.
- [88] Salvador AF, Martins G, Melle-Franco M, Serpa R, Stams AJM, Cavaleiro AJ, et al. Carbon nanotubes accelerate methane production in pure cultures of methanogens and in a syntrophic coculture. *Environ Microbiol* 2017;19(7):2727–39.
- [89] Sasaki K, Morita M, Hirano S, Ohmura N, Igarashi Y. Decreasing ammonia inhibition in thermophilic methanogenic bioreactors using carbon fiber textiles. *Appl Microbiol Biot* 2011;90(4):1555–61.
- [90] Shen YW, Linville JL, Urgun-Demirtas M, Schoene RP, Snyder SW. Producing pipeline-quality biomethane via anaerobic digestion of sludge amended with corn stover biochar with in-situ CO<sub>2</sub> removal. *Appl Energy* 2015;158:300–9.
- [91] Shrestha PM, Malvankar NS, Werner JJ, Franks AE, Elena-Rotaru A, Shrestha M, et al. Correlation between microbial community and granule conductivity in anaerobic bioreactors for brewery wastewater treatment. *Bioresour Technol* 2014;174:306–10.
- [92] Stams AJM, Plugge CM. Electron transfer in syntrophic communities of anaerobic bacteria and archaea. *Nat Rev Microbiol* 2009;7(8):568–77.
- [93] Stern N, Mejia J, He SM, Yang Y, Ginder-Vogel M, Roden EE. Dual Role of Humic Substances As Electron Donor and Shuttle for dissimilatory iron reduction. *Environ Sci Technol* 2018;52(10):5691–9.
- [94] Tang J, Zhuang L, Ma JL, Tang ZY, Yu Z, Zhou SG. Secondary mineralization of ferrihydrite affects microbial methanogenesis in geobacter-methanosarcina cocultures. *Appl Environ Microbiol* 2016;82(19):5869–77.
- [95] Tataru M, Makiuchi T, Ueno Y, Goto M, Sode K. Methanogenesis from acetate and propionate by thermophilic down-flow anaerobic packed-bed reactor. *Bioresour Technol* 2008;99(11):4786–95.
- [96] Tena M, Perez M, Solera R. Effect of hydraulic retention time on the methanogenic step of a two-stage anaerobic digestion system from sewage sludge and wine vinasse: microbial and kinetic evaluation - sciencedirect. *Fuel* 2021;296. <https://doi.org/10.1016/j.fuel.2021.120674>.
- [97] Thanh PM, Ketheesan B, Yan Z, Stuckey DC. Trace metal speciation and bioavailability in anaerobic digestion: a review. *Biotechnol Adv* 2016;34(2):122–36.
- [98] Tuomikoski, S., Runtti, H., Romar, H., Lassi, U., Kangas, T., 2021, Multiple heavy metals removal simultaneously by a biomass-based porous carbon. *Water Environ. Res.* <https://doi.org/10.1002/wer.1514>.
- [99] Usman M, Hao SL, Chen HH, Ren S, Tsang DCW, O-Thong, Luo G, Zhang SC. Molecular and microbial insights towards understanding the anaerobic digestion of the wastewater from hydrothermal liquefaction of sewage sludge facilitated by granular activated carbon (gac). *Environ Int* 2019;133:105257.
- [100] Valadbeigi Y. Comparison of effects of charge delocalization and  $\pi$ -electron delocalization on the stability of monocyclic compounds. *J Mol Graph Model* 2018:104–12.
- [101] Van Steendam C, Smets I, Skerlos S, Raskin L. Improving anaerobic digestion via direct interspecies electron transfer requires development of suitable characterization methods. *Curr Opin Biotechnol* 2019;57:183–90.
- [102] Viggli CC, Simonetti S, Palma E, Pagliaccia P, Braguglia C, Fazi S, et al. Enhancing methane production from food waste fermentate using biochar: the added value of electrochemical testing in pre-selecting the most effective type of biochar. *Biotechnol Biofuels* 2017;10:303.
- [103] Vollhardt P, Neil S. Organic chemistry: structure and function. 5th. New York: W. H. Freeman and Company; 2007.
- [104] Wang C, Wang C, Liu J, Han Z, Zhu L. Role of magnetite in methanogenic degradation of different substances. *Bioresour Technol* 2020;314:123720.
- [105] Wang CQ, Liu Y, Gao XY, Chen H, Xu XY, Zhu L. Role of biochar in the granulation of anaerobic sludge and improvement of electron transfer characteristics. *Bioresour Technol* 2018;268:28–35.
- [106] Wang CQ, Qiao WL, Chen H, Xu XY, Zhu L. A short-term stimulation of ethanol enhances the effect of magnetite on anaerobic digestion. *Appl Microbiol Biotechnol* 2019;103(3):1511–22.
- [107] Wang D, Ai J, Shen F, Yang G, Zhang YZ, Deng SH, et al. Improving anaerobic digestion of easy-acidification substrates by promoting buffering capacity using biochar derived from vermicompost. *Bioresour Technol* 2017;227:286–96.
- [108] Wang DX, Han YX, Han HJ, Li K, Xu CY, Zhuang HF. New insights into enhanced anaerobic degradation of fisher-tropsch wastewater with the assistance of magnetite. *Bioresour Technol* 2018;257:147–56.
- [110] Wang H, Byrne JM, Liu PF, Liu J, Dong XZ, Lu YH. Redox cycling of Fe(II) and Fe (III) in magnetite accelerates acetoclastic methanogenesis by Methanosarcina mazei. *Environmental Microbiology Reports* 2020;12(1):97–109.
- [111] Wang J, Hao X, Liu Z, Guo Z, Zhu L, Xiong B, et al. Biochar improves heavy metal passivation during wet anaerobic digestion of pig manure. *Environ Sci Pollut R* 2021;28:635–44.
- [112] Wang L, Rinklebe J, Tack F, Hou D. A review of green remediation strategies for heavy metal contaminated soil. *Soil Use and Manage* 2021. <https://doi.org/10.1111/sum.12717>.
- [113] Watanabe K. Recent developments in microbial fuel cell technologies for sustainable bioenergy. *J Biosci Bioeng* 2008;106(6):528–36.
- [114] Welte C, Deppenmeier U. Bioenergetics and anaerobic respiratory chains of acetoclastic methanogens. *Biochimica Biophysica Acta-Bioenerget* 2014;1837(7):1130–47.
- [115] Xiao LL, Hao JC, Wang WY, Lian B, Shang GD, Yang YW, et al. The up-regulation of carbonic anhydrase genes of *Bacillus mucilaginosus* under soluble Ca<sup>2+</sup> deficiency and the heterologously expressed enzyme promotes calcite dissolution. *Geomicrobiol J* 2014;31(7):632–41.
- [116] Xiao L, Li J, Lichtfouse E, Li Z, Liu F. Augmentation of chloramphenicol degradation by *Geobacter*-based biocatalysis and electric field. *J of Hazard Mater* 2021;410:124977.
- [117] Xiao LL, Lian B, Dong CL, Liu FH. The selective expression of carbonic anhydrase genes of *aspergillus nidulans* in response to changes in mineral nutrition and CO<sub>2</sub> concentration. *Microbiologyopen* 2016;5(1):60–9.
- [118] Xiao LL, Lian B, Hao JC, Liu CQ, Wang SJ. Effect of carbonic anhydrase on silicate weathering and carbonate formation at present day CO<sub>2</sub> concentrations compared to primordial values. *Sci Rep* 2015;5:7733.
- [119] Xiao L, Lichtfouse E, Kumar PS, Wang Q, Liu F. Biochar promotes methane production during anaerobic digestion of organic waste. *Environ Chem Lett* 2021. <https://doi.org/10.1007/s10311-021-01251-6>.
- [120] Xiao L, Liu FH, Lichtfouse E, Zhang P, Feng DW, Li FB. Methane production by acetate fermentation stimulated by *Shewanella oneidensis* and carbon materials: an alternative to classical CO<sub>2</sub> reduction. *Chem Eng J* 2020;389:124469.
- [121] Xiao LL, Liu FH, Liu JC, Li JJ, Zhang YC, Yu JF, et al. Nano-Fe<sub>3</sub>O<sub>4</sub> particles accelerating electromethanogenesis on an hour-long timescale in wetland soil. *Environmental Science-Nano* 2018;5(2):436–45.
- [122] Xiao L, Liu FH, Xu HD, Feng DW, Liu JC, Han GX. Biochar promotes methane production at high acetate concentrations in anaerobic soils. *Environ Chem Lett* 2019;17(3):1347–52.
- [123] Xiao L, Sun Q, Yuan H, Lian B. A practical soil management to improve soil quality by applying mineral organic fertilizer. *Acta Geochim* 2017;36:198–204.
- [124] Xiao L, Sun R, Zhang P, Zheng SL, Tan Y, Li JJ, et al. Simultaneous intensification of direct acetate cleavage and CO<sub>2</sub> reduction to generate methane by bioaugmentation and increased electron transfer. *Chem Eng J* 2019;378:122229.

- [125] Xiao LL, Wang YP, Lichtfouse E, Li ZK, Kumar S, Liu J, et al. Effect of antibiotics on the microbial efficiency of anaerobic digestion of wastewater: a review. *Front Microbiol* 2021;11:611613.
- [126] Xiao L, Wei WC, Luo M, Xu HD, Feng DW, Yu JF, et al. A potential contribution of a Fe(III)-rich red clay horizon to methane release: biogenetic magnetite-mediated methanogenesis. *Catena* 2019;181:104081.
- [127] Xiao LL, Xie BH, Liu JC, Zhang HX, Han GX, Wang OM, et al. Stimulation of long-term ammonium nitrogen deposition on methanogenesis by methanocellaceae in a coastal wetland. *Sci Total Environ* 2017;595:337–43.
- [128] Xiao LL, Zheng SL, Lichtfouse E, Luo M, Tan Y, Liu FH. Carbon nanotubes accelerate acetoclastic methanogenesis from pure culture to anaerobic soil. *Soil Biol Biochem* 2020;150:107938.
- [129] Xu H, Chang JL, Wang H, Liu YC, Zhang XY, Liang P, et al. Enhancing direct interspecies electron transfer in syntrophic-methanogenic associations with (semi)conductive iron oxides: Effects and mechanisms. *Sci Total Environ* 2019; 695:133876.
- [130] Xu SY, He CQ, Luo LW, Lu F, He PJ, Cui LF. Comparing activated carbon of different particle sizes on enhancing methane generation in upflow anaerobic digester. *Bioresour Technol* 2015;196:606–12.
- [131] Yamada C, Kato S, Ueno Y, Ishii M, Igarashi Y. Conductive iron oxides accelerate thermophilic methanogenesis from acetate and propionate. *J Biosci Bioeng* 2015; 119(6):678–82.
- [132] Yan WW, Shen N, Xiao YY, Chen Y, Sun FQ, Tyagi VK, et al. The role of conductive materials in the start-up period of thermophilic anaerobic system. *Bioresour Technol* 2017;239:336–44.
- [133] Yan WW, Sun FQ, Liu JB, Zhou Y. Enhanced anaerobic phenol degradation by conductive materials via eps and microbial community alteration. *Chem Eng J* 2018;352:1–9.
- [134] Yang G, Wang ZH, Xian QM, Shen F, Sun C, Zhang YZ, et al. Effects of pyrolysis temperature on the physicochemical properties of biochar derived from vermicompost and its potential use as an environmental amendment. *RSC Adv* 2015;5(50):40117–25.
- [135] Yang PX, Tan GYA, Aslam M, Kim J, Lee PH. Metatranscriptomic evidence for classical and RuBisCO-mediated CO<sub>2</sub> reduction to methane facilitated by direct interspecies electron transfer in a methanogenic system. *Sci Rep* 2019;9:4116.
- [136] Yang ZM, Guo RB, Shi XS, Wang CS, Wang L, Dai M. Magnetite nanoparticles enable a rapid conversion of volatile fatty acids to methane. *RSC Adv* 2016;6(31): 25662–8.
- [137] Yenigun O, Demirel B. Ammonia inhibition in anaerobic digestion: a review. *Process Biochem* 2013;48(5–6):901–11.
- [138] Yin QD, He K, Liu AK, Wu GX. Enhanced system performance by dosing ferrous iron during the anaerobic treatment of tryptone-based high-strength wastewater. *Appl Microbiol Biot* 2017;101(9):3929–39.
- [139] Yuan HY, Ding LJ, Zama EF, Liu PP, Hozzein WN, Zhu YG. Biochar modulates methanogenesis through electron syntrophy of microorganisms with ethanol as a substrate. *Environ Sci Technol* 2018;52(21):12198–207.
- [140] Yuan JH, Xu RK, Zhang H. The forms of alkaline in the biochar produced from crop residues at different temperatures. *Bioresour Technol* 2011;102(3):3488–97.
- [141] Zabranska J, Pokorna D. Bioconversion of carbon dioxide to methane using hydrogen and hydrogenotrophic methanogens. *Biotechnol Adv* 2018;36(3): 707–20.
- [142] Zhang JX, Mao LW, Zhang L, Loh KC, Dai YJ, Tong YW. Metagenomic insight into the microbial networks and metabolic mechanism in anaerobic digesters for food waste by incorporating activated carbon. *Sci Rep* 2017;7:11293.
- [143] Zhang MY, Ma YQ, Ji DD, Li XY, Zhang JS, Zang LH. Synergetic promotion of direct interspecies electron transfer for syntrophic metabolism of propionate and butyrate with graphite felt in anaerobic digestion. *Bioresour Technol* 2019;287: 121373.
- [144] Zhang YC, Liu FH, Hao QQ, Xiao LL. Target-oriented recruitment of *Clostridium* to promote biohydrogen production by nano-ferrihydrite. *Fuel* 2020;276:118049.
- [145] Zhang YC, Liu FH, Xu HD, Xiao LL. Extraction of electrons by magnetite and ferrihydrite from hydrogen-producing *Clostridium bifermentans* by strengthening the acetate production pathway. *Sci China Technol Sc* 2019;62(10):1719–25.
- [146] Zhao ZQ, Zhang YB, Li Y, Dang Y, Zhu TT, Quan X. Potentially shifting from interspecies hydrogen transfer to direct interspecies electron transfer for syntrophic metabolism to resist acidic impact with conductive carbon cloth. *Chem Eng J* 2017;313:10–8.
- [147] Zhou GW, Yang XR, Marshall CW, Li H, Zheng BX, Yan Y, et al. Biochar addition increases the rates of dissimilatory iron reduction and methanogenesis in ferrihydrite enrichments. *Front Microbiol* 2017;8:589.
- [148] Zhou SG, Xu JL, Yang GQ, Zhuang L. Methanogenesis affected by the co-occurrence of iron(III) oxides and humic substances. *FEMS Microbiol Ecol* 2014; 88(1):107–20.
- [149] Zhu H, Han YX, Ma WC, Han HJ, Ma WW, Xu CY. New insights into enhanced anaerobic degradation of coal gasification wastewater (cgw) with the assistance of graphene. *Bioresour Technol* 2018;262:302–9.
- [150] Zhuang HF, Zhu H, Shan SD, Zhang LT, Fang CR, Shi Y. Potential enhancement of direct interspecies electron transfer for anaerobic degradation of coal gasification wastewater using up-flow anaerobic sludge blanket (uasb) with nitrogen doped sewage sludge carbon assisted. *Bioresour Technol* 2018;270:230–5.
- [151] Zhuang L, Ma JL, Yu Z, Wang YQ, Tang J. Magnetite accelerates syntrophic acetate oxidation in methanogenic systems with high ammonia concentrations. *Microb Biotechnol* 2018;11(4):710–20.
- [152] Xiao Leilei, Lichtfouse Eric, Kumar Senthil, Wang Quan, Liu Fanghua. Biochar promotes methane production during anaerobic digestion of organic waste. *Environ Chem Lett* 2021. <https://doi.org/10.1007/s10311-021-01251-6>.