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Methane Production and Conductive Materials: A Critical Review

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Supporting Information

ABSTRACT: Conductive materials (CM) have been extensively reported to enhance methane production in anaerobic digestion processes. The occurrence of direct interspecies electron transfer (DIET) in microbial communities, as an alternative or complementary to indirect electron transfer (via hydrogen or formate), is the main explanation given to justify the improvement of methane production. Not disregarding that DIET can be promoted in the presence of certain CM, it surely does not explain all the reported observations. In fact, in methanogenic environments DIET was only unequivocally demonstrated in cocultures of *Geobacter metallireducens* with *Methanosaeta harundinacea* or *Methanosarcina barkeri* and frequently *Geobacter* sp. are not detected in improved methane production driven systems. Furthermore, conductive carbon nanotubes were shown to accelerate the activity of methanogens growing in pure cultures, where DIET is not expected to occur, and hydrogenotrophic activity is ubiquitous in full-scale anaerobic digesters treating for example brewery wastewaters, indicating that interspecies hydrogen transfer is an important electron transfer mechanism in those systems. This paper presents an overview of the effect of several iron-based and carbon-based CM in bioengineered systems, focusing on the improvement in methane production and in microbial communities' changes. Control assays, as fundamental elements to support major conclusions in reported experiments, are critically revised and discussed.

1. INTRODUCTION

Methane is a renewable energy source that can be produced in controlled bioengineered systems from a wide range of organic substrates including diluted industrial wastewater, animal manure or the organic fraction of municipal solid waste, through a process generally called anaerobic digestion (AD). Fundamental knowledge and technology developments of AD processes have evolved significantly and in parallel in the last decades. The fact that the process relies on the activity of slow growing anaerobic microorganisms[1] results in low nutrient requirements and low amounts of sludge produced, which are strong advantages of the anaerobic treatment process. These microorganisms grow slowly because the energy gain from the anaerobic metabolism is low and has to be divided by different trophic groups, that is, the bacteria performing hydrolytic, acidogenesis, and acetogenesis reactions, and the methanogens converting intermediary degradation products into methane.[2]

Syntrophic interactions between bacteria and methanogens are the basis to maintain an AD system working efficiently. These microorganisms, with distinct, but complementary metabolic capabilities, exchange electrons for energy purposes, normally through the transfer of small soluble chemical compounds, such as hydrogen or formate, that act as electron shuttles. This interspecies hydrogen/formate transfer process is very important since the overall thermodynamics depends on the capacity of the microbial communities to maintain a low hydrogen partial pressure.[3] Thus, diffusion limitations of these metabolites, between anaerobic bacteria and methanogenic archaea, can be important bottlenecks in the anaerobic conversion process.[4,5]

Recent studies proposed that interspecies electron transfer (IET) can also be performed directly between bacteria and methanogenic archaea, or with the aid of conductive materials (CM), being potentially a more energy conserving approach, and thus improving the rate of methanogenesis.[6,7] However, clear evidence of direct interspecies electron transfer (DIET) was only observed in cocultures of electroactive bacteria, namely *Geobacter* species, and in cocultures of *G. metallireducens* with *Methanosaeta harundinacea*[3,7] or *Methanosarcina barkeri*.[8] Moreover, DIET seems to require outer membrane *c*-type cytochromes and pili,[6,7] but traditional syntrophic fatty acid-degrading bacteria (e.g., *Syntrophomonas wolfei* and *Syntrophus aciditrophicus*)[9] and most methanogens (e.g., all members of the orders *Methanopyrales*, *Methanococcales*, *Methanobacteriales* and *Methanomicrobiales*)[10] lack the genes for these cell components. Another indication that not all syntrophic bacteria are capable of DIET is the case of *Pelobacter carbinolicus*, a known syntrophic ethanol oxidizing bacterium, that could only establish syntrophic interactions

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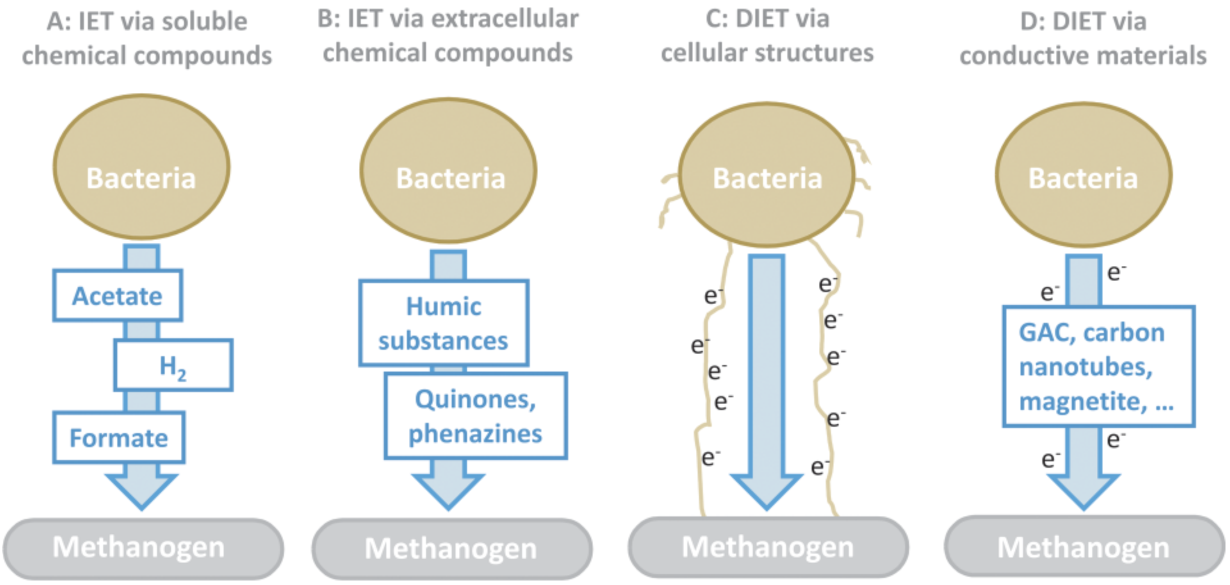

Figure 1. Possible interspecies electron transfer mechanisms in methanogenic environments.[42−44]

with *Geobacter sulfurreducens* via interspecies hydrogen transfer or interspecies formate transfer,[11] although it has been reported to contain *c*-type cytochromes.[12]

Notwithstanding these facts, in the past five years, many studies have reported the improvement of methane production in anaerobic reactors amended with CM such as magnetite, granular activated carbon (GAC), carbon nanotubes (CNT), and biochar, among others.[1,8,13−27] In general, these CM are highly stable, have large surface area, good adsorption capacity, and high electric conductivity.[28−30] Some of these materials may act as redox mediators in microbial catalysis of compounds with electrophilic groups, such as dyes.[30]

The role of DIET is discussed in recent papers on anaerobic digestion processes amended with CM.[31−33] The authors of these papers interpret the enhancement of methane production as a direct consequence of DIET promoted by CM. However, exploring the results collected from a significant number of studies on this topic, turned clear that the effect of CM goes beyond the stimulation of DIET.

In this review, the recent findings on the effect of CM in AD bioengineered systems is summarized, exploring how these materials affect methane production rate and the structure of anaerobic microbial communities. This is a recent research field with a relevant impact in engineered and natural anaerobic microbial processes. Little is known about the mechanisms behind the improvement of methane production rates when CM are present. Apart from DIET, other elements of knowledge should be considered, and main interpretations of the researchers contributing to the knowledge in this field are herein addressed and discussed.

2. ELECTRON TRANSFER MECHANISMS IN METHANOGENIC ENVIRONMENTS

Anaerobic digestion is a biological process, where complex organic compounds are sequentially converted to simple compounds, by a wide variety of microorganisms. The rate and the route governing electrons transfer among the microbial community, determines the entire process efficiency.[34−37] Mineralization of waste to methane is dependent on the activity of hydrolytic, acidogenic, and acetogenic bacteria, which generate the substrates (i.e., hydrogen, formate and acetate) for methanogenic archaea, which ultimately produce methane in AD processes.[37−40] IET efficiency between syntrophic partners dictates the rates of conversion bioprocesses when methanogenic activity is guaranteed. In methanogenic systems microorganisms can transfer electrons between each other: (1) via soluble chemical compounds (e.g., hydrogen, formate, and acetate) which act as electron shuttles; (2) via other chemicals (e.g., humic substances); (3) via direct electron transfer by electrically conductive pili or direct contact; and (4) by direct electron transfer mediated by CM (Figure 1). In the following subsections the fundamentals and mechanisms of IET are briefly exposed.[33,41−43]

2.1. Interspecies Electron Transfer via Soluble Molecules. The most studied and well-known mechanism of electron transfer in methanogenic communities is the indirect interspecies electron transfer via hydrogen or formate.[9,44−46] Syntrophic bacteria produce hydrogen or formate as a way to dissipate the reducing power, i.e., the electrons formed during the degradation of organic compounds and, in turn, methanogens utilize those molecules as electron donors to reduce CO_2 to methane (Figure 1A). Therefore, hydrogen/formate act as shuttles between hydrogen/formate-forming bacteria and hydrogen/formate-utilizing methanogens. At high hydrogen concentrations (>10 Pa), the hydrogenase activity is inhibited and consequently the metabolism of syntrophic bacteria is inhibited as well, while that of the methanogens is stimulated, and vice versa.[3,47−49] Formate formation has been detected particularly in cocultures growing on proteins,[50] or fatty acids like propionate and butyrate.[3,51,52] Under certain conditions, interspecies formate transfer may prevail because formate has a higher diffusion coefficient, comparing to hydrogen.[53,54] Syntrophic interactions involving hydrogen or formate as electron shuttles are well described in cocultures degrading common compounds formed during the AD process such as butyrate,[55] propionate,[56] ethanol,[57] and acetate.[58]

2.2. Interspecies Electron Transfer via Extracellular Compounds. In numerous anaerobic environments, the interspecies electron transfer can be mediated also by insoluble compounds, such as humic acids present in humus.[59−62] Unlike soluble electron shuttles, such as hydrogen or formate, that can diffuse in and out of the cell,[63] insoluble compounds do not penetrate the cell surface. Lovley and co-workers[64] demonstrated that humus can mediate electron transfer between humics-reducing and humics-oxidizing microorganisms (Figure 1B). The electron acceptor properties have been related mainly with the redox active quinone moieties present in humic substances.[65] Several microorganisms were found to reduce humic acids or anthraquinone-2,6-disulfonate (AQDS) using hydrogen as electron donor (e.g., the halorespiring bacterium, *Desulfitobacterium* PCE1, the sulfate-reducing bacterium, *Desulfovibrio* G11 and the methanogenic archaea, *Methanospirillum hungatei* JF1) or lactate (*Desulfitobacterium dehalogenans* and *Desulfitobacterium* PCE1).[59]

Humus can also be reoxidized and act as an electron donor. For example, humic acids can be redox mediators in the anaerobic substrate oxidation coupled to the abiotic reduction of metal oxides such as Fe(III) and Mn(IV), being reoxidized and participating in many cycles.[59] Some bacteria of the genus *Geobacter* have been reported as quinone-reducing microorganisms using Fe(III) as the terminal electron acceptor[66−69] but other microorganisms share this ability, such as some *Shewanella*, *Desulfitobacterium*, *Desulphuromonas*, *Geospirillum*, *Wolinella*, and *Geothrix*[69] and the methanogenic archaea *Methanopyrus kandleri*, *Methanobacterium thermoautotrophicum*.[70] The anaerobic oxidation of lactate and hydrogen by *Desulfitobacterium dehalogenans* was obtained with AQDS as mediator, associated with the reduction of goethite.[59]

2.3. Direct Interspecies Electron Transfer. Recently, DIET has been described in anaerobic environments, involving the formation of an electric current between electron-donating and electron-accepting microbes (Figure 1C)[42,43,71,72] and without the need to produce and exchange electron carriers (i.e., hydrogen and formate). DIET is analogous to direct extracellular electron transfer, which consists in the electron transfer between cells and a solid-state electron acceptor such as iron and manganese oxides or electrodes.[72] Direct extracellular electron transfer is well studied in bacteria belonging to the genera *Shewanella* and *Geobacter*,[42,43,71] which are highly efficient in dealing with solid extracellular electron acceptors. DIET was first described in defined cocultures of *G. metallireducens*, an ethanol oxidizing bacteria, and *G. sulfurreducens*, a fumarate reducing bacteria.[71] These microorganisms establish a syntrophic relationship, where *G. metallireducens* metabolize the ethanol and the *G. sulfurreducens* reduces the fumarate. The ability of this culture for performing DIET was discovered when cocultures formed with *G. sulfurreducens* strains lacking the *hyb* gene (thus unable to utilize hydrogen), were able to oxidize ethanol and to reduce fumarate. Under these conditions, interspecies electron exchange between *G. metallireducens* and *G. sulfurreducens* occurred directly via conductive pili and without the formation of soluble intermediates.[71] Further, a recent metatranscriptomic analysis showed low abundance of transcripts for hydrogenase and formate dehydrogenase subunits, which provided strong evidence of DIET also between the wild-type of *G. metallireducens* and *G. sulfurreducens*.[73]

More recently, it was shown that DIET can occur in cocultures of *Geobacter* species and acetoclastic methanogens (i.e., *Methanosaeta* and *Methanosarcina* species).[6,8] Rotaru and co-workers[6] showed that *Methanosaeta harundinacea*, a strictly acetoclastic methanogen, can receive electrons directly from *G. metallireducens* to produce methane. The idea that *Methanosaeta* species are acetoclastic specialists, only producing methane from acetate, changed from this point on, since it seems to be able to activate the CO_2 reduction pathway for methane production. In this context, the electrons released by *Geobacter* species are transferred, via pili, directly to *Methanosaeta*, but the cell machinery involved in electrons uptake by the methanogen is not yet known.[6] These findings gave a new perspective on the interspecies interactions taking place in anaerobic bioreactors producing methane.

2.4. DIET Promoted by Conductive Materials. The presence of CM such as GAC, carbon cloth, and biochar appears to promote DIET via a conduction-based mechanism, in which electrons migrate through the CM from electron-donating to electron-accepting cells.[20,22,74]

Surprisingly, it was observed that the lack of pili and other cell component involved in the exogenous electron transfer can be compensated by the presence of CM, namely GAC,[8] carbon cloth[74] and biochar.[22] This was verified in defined cocultures of *pil*A-deficient strains of *G. metallireducens* with *Methanosarcina barkeri*, which could not convert ethanol to methane unless in the presence of CM. This methanogenic coculture in the presence of biochar were able to utilize 86% of the electrons released from ethanol oxidation for methane production, but without biochar no ethanol was consumed and no methane was produced.[22] Similarly, the lack of the pilin-associated *c*-type cytochrome (OmcS), necessary for extracellular electron transfer in *Geobacter* species, could be compensated by magnetite, another conductive material.[75] Without magnetite *Geobacter* strains lacking genes for OmcS were ineffective in forming viable cocultures,[71] but in the presence of magnetite, the OmcS-deficient mutants performed similarly to the wild type.[75]

2.5. Hydrogen and Formate IET versus DIET. An interesting approach toward the clarification of the importance of DIET in methanogenesis was presented by Storck and co-workers[72] who proposed a mechanistic framework that enabled the direct assessment of the relative feasibility of DIET, and mediated interspecies electron transfer (MIET) in a thermodynamically restricted syntrophic system (propionate conversion to acetate and methane), through mathematical modeling. They found that DIET could be more favorable than hydrogen-MIET, but substantially less favorable than formate-MIET (1 order of magnitude rate difference), assuming a default parameter set based on literature data. The model results also suggested that DIET may be a thermodynamically more feasible alternative to MIET for disperse communities limited by diffusion, which is contrary to experimental observations where nanowire-DIET is commonly observed in dense aggregates,[6,8,71] possibly indicating that coevolution and cometabolism are more important than external limitations in the simulated system.[72] These authors also suggested that CM reduce resistivity, and leave only activation losses, making long-range transport even more feasible.

3. EFFECT OF CONDUCTIVE MATERIALS ON METHANE PRODUCTION

3.1. CM Improving Methane Production. Several strategies have been implemented to improve AD. Empirical modification of process design, operational conditions, and application of substrate pretreatments, such as microaeration, thermal and mechanical treatments are some examples.[1,76,77] Recently, several authors have been reporting the enhancement

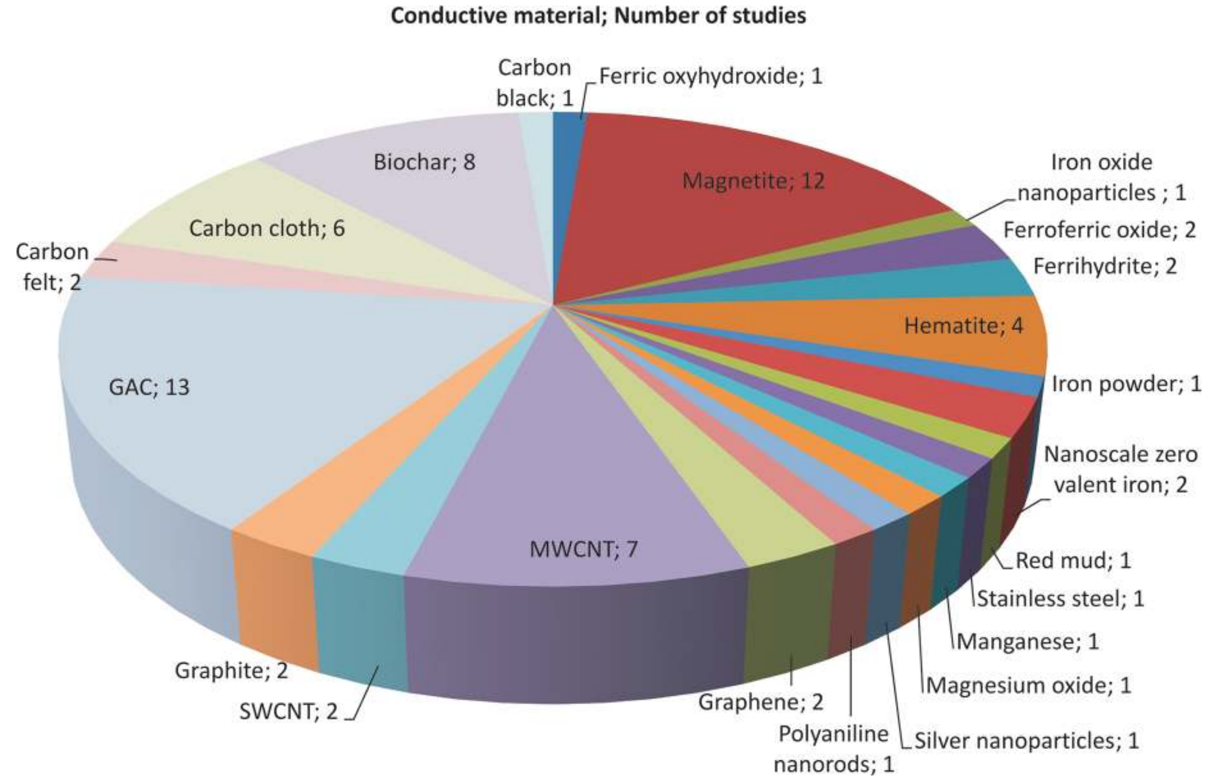

Figure 2. Number of studies reporting the effect of different types of CM on methane production.

of methane production by the use of CM (Figure 2), which is now considered a strategy to improve methane production efficiency and stability of anaerobic reactors.[7,20] The type of conductive material, the microbial substrates, the mode of operation and the main results reported in scientific communications are summarized in Table 1 and Supporting Information (SI) Table S1. In general, lag phases preceding methane production are reduced and methane production rates increase when CM are added to batch experiments.[8,16,18−20,27,74,78,79] In continuous anaerobic digesters, CM also accelerate methane production and contribute for a more stable operation, allowing higher applied organic loading rates while maintaining high COD removal rates.[21,80−83]

Only few studies used direct methanogenic substrates, such as acetate or H_2/CO_2,[18,24,84,85,98] while most studies used volatile fatty acids, such as butyrate and propionate, and alcohols as substrates to promote the syntrophic metabolism within microbial communities.[8,16−18,20,22−24,78,79,82,85,96,100] More complex organic matter, such as dairy wastewater,[1,83,86] brewery wastewater,[20] beet sugar wastewater,[87] food waste,[13] municipal solid waste,[19] waste activated sludge[89,90,99,103,104] or leachate[81] have also been investigated. When compared to control conditions, the addition of CM to bioreactors treating these complex wastewaters resulted in less accumulation of VFAs, thus improving the efficiency of the AD process.

GAC and magnetite were the most common CM applied in AD systems, followed by CNT, carbon cloth and biochar (Figure 2/Table 1).

3.2. CM Inhibiting Methane Production. From all CM tested, magnesium oxide, silver nanoparticles, ferrihydrite and carbon black (amorphous carbon) were the only ones inhibiting methanogenesis (Table 2).

The addition of carbon black nanoparticles (20 g/L) to anaerobic sludge completely inhibited methane production from glucose. In these cultures methanogens were more affected than bacteria, and additional pure culture studies showed that *Methanosarcina barkeri* completely lost activity when growing with carbon black.[105] The reasons behind the inhibitory effect of carbon black were not investigated but probably due to their small size, they may present antimicrobial properties, similar to those reported for single walled carbon nanotubes.[106] High concentrations of silver nanoparticles and magnesium oxide (500 mg/g TSS) inhibited methane production from waste activated sludge in 26.5% and 98.9% relatively to the control, respectively, although the microbial community composition was apparently not affected.[89] Both Zhou and co-workers[84] and Kato and co-workers[24] reported the inhibition of methanogenesis from acetate and ethanol (0.6−1.9 times comparing with the control) in the presence of ferrihydrite. Indeed, one of the applications of ferrihydrite is specifically the inhibition of methanogenesis, as it was previously reported in several studies.[107−112] Several reasons have been suggested to justify the inhibition by ferrihydrite: (1) the utilization of the primary electron donors (i.e., acetate or hydrogen) available for methane production by iron reducing bacteria,[109,111,113] and/or the diversion of electron flow from CO_2 reduction (methanogenesis) to Fe(III) reduction by methanogens capable of Fe(III) reduction,[114−116] thus shifting from methanogenic to iron-reducing conditions, and (2) the increase of the redox potential (to approximately −100 mV) caused by the presence of Fe(III) oxides, which does not benefit methanogenic reactions.[84,117,118] Nevertheless, the secondary iron minerals formed from microbial ferrihydrite reduction, might also benefit methanogenesis.[26,119] For example, when magnetite is formed as the predominant secondary iron mineral, the methane production rates from acetate and ethanol increased by 30% and 135%, respectively,

Table 1. Summary of the Studies Reporting the Enhancement of Methane Production by CM

conductive material	particle size/μm	applied concentration/g/L	common substrates	methane improvement[a]	reference
ferric oxyhydroxide	1 to 2	1.8	real dairy wastewater	2.0	1
magnetite	0.01 to 0.3	0.01 to 25	acetate, ethanol, benzoate, butyrate, propionate, real and synthetic dairy wastewater	1.3 to 3.6	1,16,17,23−25,27,78,83−86
iron oxide nanoparticles	0.02	0.8	beet sugar industrial wastewater	1.3	87
ferroferric oxide	0.01 to 0.3	10	synthetic wastewater	1.2 to 1.8	14,88
ferrihydrite	~0.03	3.4 to 4.2	acetate, ethanol	1.1	24
hematite	0.02 to 0.3	0.022 to 10.9	acetate, ethanol, benzoate, sludge	1.3 to 2.2	24,25,84,89
iron powder		10	sludge	1.4	90
nanoscale zerovalent iron	0.001 to 0.1	0.02 to 10.9	sludge	1.3 to 2.2	89,90
red mud		20	activated sludge from municipal wwtp	1.4	91
stainless steel	500 to 2000	25.7	synthetic wastewater	1.3	92
manganese	0.07 to 1.5	2 to 8	synthetic wastewater	4.4	93
polyaniline nanorods	0.25 to 3	1.2	sucrose	2.0	94
graphene		0.03 to 2.0	ethanol, synthetic wastewater	1.2 to 1.5	95,96
multiwalled CNT	0.010 to 0.2	0.1 to 5.0	H_2/CO_2, acetate, butyrate, beet sugar industrial wastewater	1.1 to 17	16,18,87
single-walled CNT	0.001	1.0	sucrose, glucose	1.8 to 2	79,97
graphite	6 to 25	12 to 132 graphite rods	synthetic complex waste and wastewater	1.0 to 1.3	13,80
GAC	841 to 2000	3.3 to 50	acetate, ethanol, butyrate, propionate, glucose, synthetic brewery wastewater, synthetic dairy wastewater, activated sludge, organic fraction of municipal solid waste, synthetic complex waste	1.1 to 18	8,13,15,19−21,79,83,98−100
powered activated carbon	149 to 177	5	synthetic brewery wastewater	na	21
carbon felt		3 to 30 pieces	synthetic wastewater (glucose), and synthetic complex waste	1.1	13,101
carbon cloth		10 to 20 pieces	ethanol, synthetic wastewater, leachate from municipal solid waste incineration, organic fraction of municipal solid, waste, synthetic complex waste	1.1 to 10	13,19,74,80,81,102
biochar	60 to 700	0.3 to 42.7	ethanol, butyrate, propionate, sludge, synthetic wastewater	1.2 to 1.3	22,80,82,103,104

[a] Number of times that methane production increases relative to the control.

Table 2. Summary of the Studies Reporting the Inhibition of Methane Production by CM

conductive material	particle size/μm	applied concentration/g/L	substrate	methane inhibition[a]	reference
ferrihydrite	~0.03	2.2 to 4.2	acetate, ethanol	1.9 to 4.3	24,84
magnesium oxide	<0.05	0.02 to 10.9	sludge	92.6	89
silver nanoparticles	<0.1	0.02 to 10.9	sludge	1.4	89
carbon black	0.02 to 0.35	4 to 20	glucose	51.5	105

[a] Number of times that methane production decreases relative to the control.

compared with a control lacking ferrihydrite.[26] These results highlight the important role of iron biomineralization in the biogeochemical cycling of carbon in diverse anaerobic environments.

3.3. Relationship between CM, ORP, Electrical Conductivity and Methane Production.

Despite the high number of research papers reporting the effect of CM on methane production efficiency (Figure 2), the mechanisms by which CM enhance methanogenic activity are still not known. It was verified that in the presence of CM (specifically multiwalled CNT) the methanogenic activity of pure cultures of methanogens increased significantly, and that it was correlated with the variation of the redox potential (ORP).[18] Till date, this was the only study monitoring ORP during methanogenesis with CM. In that work, higher concentrations of multiwalled CNT resulted in a growth medium with a more negative ORP, which benefited methanogenesis that ideally occurs at ORP ranging from −200 mV and −400 mV.[118] Nevertheless, Salvador and co-workers[18] also observed that in the absence of a reducing agent, the ORP increased (to values reaching approximately −200 mV), having the opposite effect verified in the assays performed with the reducing agent. Surprisingly, without reducing agent, the methanogenic activity of *Methanobacterium formicicum* still increased with increasing concentrations of multiwalled CNT.

The electrical conductivity is another parameter that varies with the presence of CM in methanogenic systems. The electrical conductivity of anaerobic biomass, biofilms or granules was reported to increase in the presence of CM, namely CNT (27 times), stainless steel (14 times), GAC (3.5 times), ferroferric oxide (2.1 times), carbon cloth (2 times) and biochar (1.5 times).[78,79,81,92,98,103] This increase has been

Table 3. Summary of the Studies Using Non-Conductive Materials

material	inoculum	substrate	reported effects	reference
polyester cloth (three pieces; 8 × 20 × 0.1 cm^3)	anaerobic sludge	synthetic complex waste	-high start-up period -lower methane content (26%) -incomplete degradation of VFAs -lower total COD removal efficiencies (20% to 31%)	19
polyester cloth (3 pieces; 8 × 20 × 0.1 cm^3)	anaerobic sludge	organic fraction of municipal solid waste	-no differences in methane production between polyester cloth and nonamended control reactor	13
cotton cloth (20 × 10 × 0.05 cm, 400 cm^2)	anaerobic sludge	artificial wastewater (butanol)	-high start-up period	102
silica-coated nanoFe_3O_4 (1.1 g/L)	paddy soil	butyrate	-similar methane production in silica-coated nanoFe_3O_4 to the control assay without any amendment;	78
plastic threads (25.7 g/L)	anaerobic sludge	artificial wastewater	-4.5 times less methane production in the reactor with plastic than in the reactor with stainless steel -lower COD removal rate -slightly higher sulfate removal	92
zeolite (33.3 g/L)	anaerobic sludge	acetate	-no effect on methane production.	98

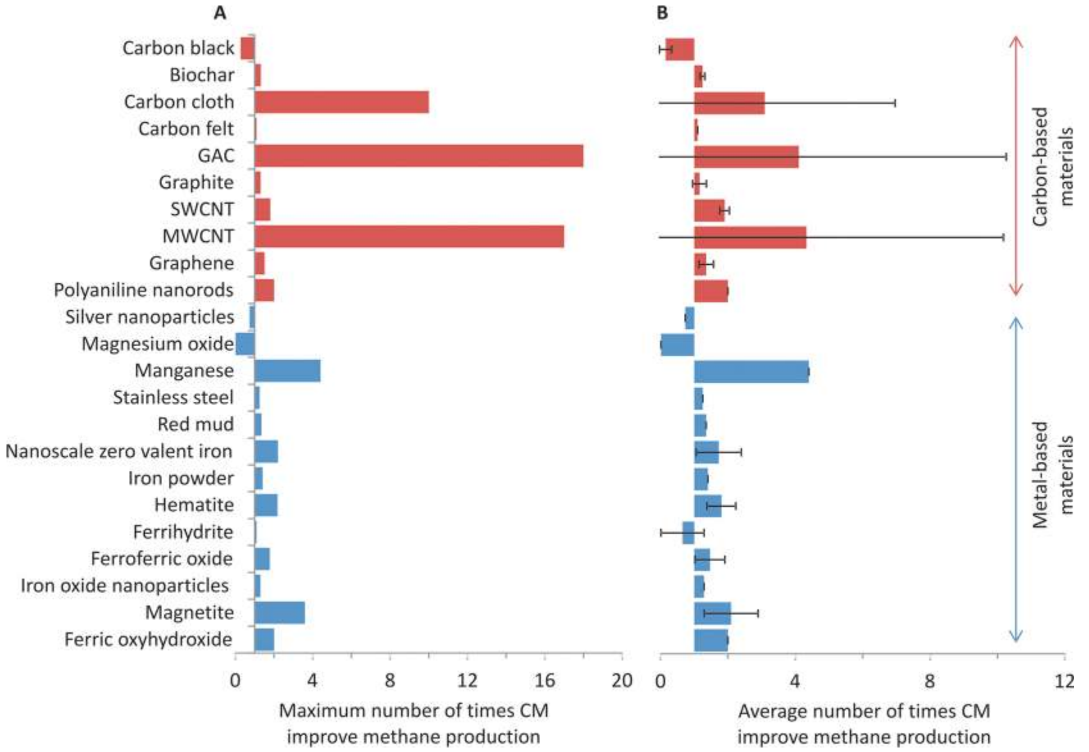

Figure 3. Number of times that methane production increases relatively to control conditions (data from the literature cited in Tables 1, 2 and SI Table S1); (a) the best result for each CM; (b) average calculated values for each CM.

justified by a presumable higher expression of electrically conductive pili produced by bacteria which perform DIET,[81] but also by cations released from these materials, as it was reported for the case of biochar.[103] However, the electrical conductivity also increased in the presence of non-CM (e.g., plastic threads),[92] which indicates that electrical conductivity of bulk sludge is affected by materials with high electrical conductivity but also by non-CM. Furthermore, the conductivity seems to vary depending on the metabolism and composition of anaerobic microbial communities, independently of the presence of CM. The conductivity of enrichment cultures previously stimulated with ethanol, and degrading propionate and/or butyrate, was 5 fold higher, for propionate, and 76 fold higher for butyrate, when compared with nonstimulated enrichments (without previous contact with ethanol).[100] Zhao and co-workers[100] related the increase in the conductivity to the enrichment of *Geobacter* species in ethanol-stimulated enrichments, which could transfer electrons directly to acetoclastic methanogens. However, although *Geobacter*'s relative abundance increased in enrichments stimulated with ethanol (a common electron donor for several *Geobacter* species), it represented less than 4% of the total bacterial community. In the same cultures, *Syntrophomonas* and *Smithella* species, well-known as butyrate and propionate degraders,[55,120] were far more abundant (representing approximately 30% of the total microbial community).

3.4. The Need of Control Assays. To clarify the role of the electrical conductivity on the enhancement of methane production, control experiments with non-CM are needed. However, to date, only few studies performed control experiments with non-CM (Table 3). For example, Li and co-workers[92] compared the efficiency of two UASB reactors treating sulfate-containing wastewater, one amended with plastic threads and the other with stainless steel. The results showed that more methane was produced (7.5−24.6%) in the reactor amended with conductive stainless steel, which was less affected by the sulfate reduction than the reactor with insulated plastic material, although the electric conductivity was higher in both conditions when compared to the initial inoculum.[92] In another study, the anaerobic digesters supplemented with conductive carbon cloth presented a higher capacity to resist the acidic impacts, and an improved methane production than digesters supplemented with nonconductive cotton cloth.[102] However, both studies lack control assays without any material. When both controls are performed, that is, controls with nonconductive material and without material, it seems that electrical conductivity is an important parameter. For example, Dang and co-workers[13,19] showed that in the presence of polyester cloth (nonconductive material), methane production was similar to the control with no material, and lower than when CM are applied. Similarly, the addition of zeolite, as a nonconductive control, did not affect methane production from acetate by comparing to the control without material.[98] Also, the syntrophic oxidation of butyrate to methane in a paddy soil enrichment was significantly accelerated in the presence of nanoFe_3O_4 (magnetite), but this effect disappeared when magnetite was coated with silica that insulated the mineral from electrical conduction.[78] Nevertheless, another study attributed the beneficial effect of carbon felt, during the AD of molasses, to the increase of biomass retention rather than to the conductive characteristics of the material.[121] This fact indicates that the support for biomass attachment provided by the materials could also be an important parameter in the enhancement of methane production.[121] Indeed, Salvador and co-workers[18] showed that the positive effect provided by multiwalled CNT on the methane production by *M. formicicum* is dismissed when the multiwalled CNT are removed from the culture media, once again pointing out for the importance of the physical presence of the carbon material.

3.5. The Physical and Chemical Characteristics of CM and Microbial Colonization. Independently of the application, the physical and chemical characteristics of CM are of utmost importance. Assays with similar CM with slightly different modifications may return significantly different results (Figure 3, Table 1). All materials are unique and even when they present similar general characteristics, their physical and chemical behavior and therefore the way they influence the biological reactions, can be potentially different. Some of the physicochemical characteristics of CM used in AD, namely the BET surface area, the electrical conductivity and the pH of point zero charge (pH_{pzc}), are summarized in SI Table S3. The basicity and acidity of the materials are correlated with the chemical groups present at their surface, that is, materials with high content of acidic groups at the surface such as phenols and carbonyl/quinone groups are more acidic and materials with low oxygen containing groups, present basic character. The pHpzc gives the net charge of the material, as a function of the solution pH: the surface of the materials becomes positively charged at pH < pHpzc and negatively charged at pH > pHpzc. In the case of magnetic nanomaterials, smaller particle sizes increase their performance, due to the increase of atoms on the surface and near the surface of the material, which are prone to adsorb and react with other atoms or molecules so as to attain surface stabilization. However, smaller particles sizes increase the surface energy, decreasing stability and leading to agglomeration and precipitation.[122]

It is known that bacterial activity may increase in the presence of solids/physical supports, especially in very dilute nutrient solutions.[123] This may be related with the concentration of nutrients in the surface of the solid by adsorption,[123,124] and by the retardation of the diffusion of exoenzymes and hydrolyzates away from the cell,[123] thereby promoting the assimilation of nutrients which must be first hydrolyzed. However, the fact that higher substrate and nutrient concentrations can be found at the solid surface does not explain per se better microbial activities.[125] In addition, the effect of the solids toward microbial activities depend on the nature of the microorganisms, the type and concentration of the substrates, and the nature of the solid surfaces.[125]

Surface roughness and surface free energy of solid materials have a potential role on microbial activity of adhered cells.[126] In general, microorganisms tend to adhere in larger extent to roughest surfaces which provide a higher specific surface area for cell adhesion. The protrusions from the rougher surfaces also increase the contact frequency between cells and surfaces.[127] Surface free energy has a significant role in the adsorption, adhesion, and wetting,[128] affecting the phenomena occurring at the solid−liquid interface. Habouzit and co-workers[129] found that low surface free energy materials, such as polypropylene, stainless steel, and polyethylene, facilitate the first stage of colonization of the support by the anaerobic digester biomass. The low surface free energy materials were found to be more easily colonized by methanogens.[129]

Regarding the application of CM in methane driven systems, major improvements in methane production rates were obtained with carbon based-CM, specifically with GAC, multiwalled CNT and carbon cloth (Figure 3B). Indeed, best results with CM were obtained with carbon-based materials that present higher surface areas, although lower electrical conductivities, when compared with metal-based CM (Figure 3, SI Table S3). However, there is no correlation between the electrical conductivity and the enhancement in methane production ($R^2 < 0.1$, data not shown). Nevertheless, it is important to note that the available values for electrical conductivity are scarce which limits this analysis. On the other hand, the initial rate and the long-term extent of synthetic iron(III) oxides reduction were linearly correlated with the oxide surface area.[130] However, other factors such as crystal structure, morphology, free energy, and particle aggregation also had an important influence on microbial metal oxide reduction rates.[130]

In addition, carbon based-CM had a similar role of humic substances and soluble quinones during the biological degradation of organic pollutants.[131] Small amounts of activated carbon, carbon xerogels, CNT, carbon fibers, biochar and graphene, accelerated the electron transfer from an electron donor to organic pollutants (as the final acceptors), improving significantly the reduction rates. Some examples include the reduction of azo dyes,[30,131−136] nitrocompounds,[137−141] herbicides[142] and pharmaceutical compounds.[143,144] The key factor is that these materials act as electron shuttles by receiving and donating electrons either biotically or abiotically. The electron shuttling via carbon materials was initially ascribed to the existing quinone moieties but recent studies showed that the shuttling effect is uncoupled from the existence of quinones in the surface of the materials.

Pereira and co-workers[131] showed that higher rates of azo dye reduction were obtained with activated carbon containing less oxygen-containing groups, and that it was due to the high content of electron rich sites on their basal planes (electrons π) and by a low concentration of electron withdrawing groups.

4. EFFECT OF CONDUCTIVE MATERIALS ON COMPLEX MICROBIAL COMMUNITIES

It is unquestionable that generally CM enhance the efficiency of methane production, but the reasons why it happens remain unclear. To better understand this phenomenon, the microbial communities are often studied with the objective to (1) identify the most important microbial players, (2) investigate the interspecies microbial interactions; and (3) get insights into the interspecies electron transfer mechanisms.

In most studies performed with CM, the microbial community analysis is based on the taxonomic composition obtained by sequencing the 16S rRNA genes. When using next generation sequencing technologies such as Illumina sequencing or 454-pyrosequecing, also the relative abundance of the microorganisms identified can be obtained. However, changes in community composition do not directly inform changes in the mechanisms of interspecies electron transfer. Nevertheless, in a significant number of studies the improvement of microbial activities in the presence of CM is usually justified by the shift of IET to DIET. This conclusion appears as a consequence of the detection of *Geobacter* species, which are known to exchange electrons directly with acetoclastic methanogens.[6,8] In some studies, the enrichment of *Geobacter* was verified closely attached to CM,[1,15,23,80,145−147] suggesting that the electron transfer mechanism in the consortia could change to, or include DIET. However, in the majority of the studies *Geobacter* species are present in low percentages[21,99−101] or even absent.[14,17,19,81,94] Notwithstanding these results, the conclusion that methane production is enhanced by shifting the IET to DIET is often maintained. Even when electroactive bacteria are not detected, other microorganisms have been suggested to participate in DIET. For example, it was suggested that *Syntrophomonas* sp. could exchange electrons directly with *Methanospirillum hungatei*, based on the fact that they were the most abundant microorganisms in a carbon cloth stimulated consortia.[81] However, this conclusion had no experimental support, and fundamental studies performed with *M. hungatei* revealed its inability to receive electrons directly from *Geobacter*.[6]

The focus on *Geobacter* species and the assumption that DIET prevails in methanogenic environments amended with CM, disregards the importance of other potential microbial players. In fact, microorganisms closely related to typical hydrogen forming and syntrophic bacteria were enriched in assays performed with CM where methane production was enhanced. For example, *Syntrophomonas*, *Desulfotomaculum*, and *Smithella* were enriched with GAC;[19,82,100] *Syntrophomonas* with magnetite and hematite;[16,24,78] *Clostridium* (∼50% of the community) with polymerized conductive polyaniline nanorods[94] and with conductive carbon felt (from 28% to 41% of the community);[101] *Syntrophobacter* with magnetite; *Sporanaerobacter* (representing approximately 30% of the bacterial community) with carbon cloth,[13] and *Syntrophomonas* also with carbon cloth (SI Table S2).[81]

Not all microorganisms are able to perform DIET, and the research available suggests that this may be the case of the vast majority of the anaerobic microorganisms. Experimental evidence collected until date, point out for the inability of some *Syntrophomonas*[18] and *Pelobacter* species[11] to participate in DIET.

DIET does not always explain the enhancement of methane production. For example, magnetite was found to accelerate the oxidation of propionate by acting as an electron acceptor, rather than promoting DIET.[27] Similarly, Kato and co-workers[24] reported ferrihydrite reduction to ferrous ions by *Geobacter* in methanogenic cultures.

It was also demonstrated that increasing concentrations of multiwalled CNT, a carbon based conductive material, promoted increasing methanogenic activities of four different species of methanogens growing in pure cultures.[18] Methane production rates increased up to 17 times in pure cultures of *Methanobacterium formicicum*.[18] This finding proved that the effect of CM toward microbial communities goes beyond the interactions between different species of microorganisms. The fact that these materials affect the activity of methanogens in pure cultures, should be considered when interpreting the effect of similar materials toward the same microorganisms when growing in complex microbial communities.

As it was mentioned before, CM inhibited the methanogenic communities in a minor number of cases (Table 2). High concentrations of Ag and MgO nanoparticles (i.e., 500 mgAg/gTSS and 500 mgMgO/gTSS), reduced the abundance of bacterial and archaeal populations in 84% and 32% relatively to the control, respectively.[89] In addition, *α-Proteobacteria*, *β-Proteobacteria*, *Bacteroidetes*, and *Methanosaeta* were less active, and a significant decrease on enzymatic activities (e.g., proteases, acetate kinases and coenzyme F420) could be detected in the reactors exposed to Ag and MgO nanoparticles.[89] The supplementation of ferrihydrite also lead to microbial community changes.[24,84] For example, while *Methanosarcina* spp. were abundantly detected both in ferrihydrite[24,84] and magnetite[24] cultures, *Methanobacterium* was only detected in the control and in ferrihydrite supplemented cultures, being apparently inhibited by magnetite.[24]

5. CONCLUSIONS AND FUTURE PERSPECTIVES

Generally, CM improve the conversion of organic waste to methane by anaerobic microbial communities. Nevertheless, some CM (i.e., ferrihydrite, carbon black, Ag, and Mg nanoparticles) were reported to inhibit methane production. Carbon-based CM stimulated methanogenic communities in a larger extent than metal-based CM. However, it is hard to compare the effect of different CM since they present different physical and chemical characteristics (e.g., surface area, shape, pore size and volume, electrical conductivity, pH_{pzc}) and were applied in different systems with distinct substrates, inocula, and operational conditions.

The mechanisms behind the effect of CM are not well understood but it seems that more than one factor is responsible for the changes toward microbial activities. The electrical conductivity of biofilms, the redox potential of the growth medium, the specific surface area and the roughness of the materials seem to be important factors. Control assays with nonconductive materials are important for understanding the effect of CM, but such controls were rarely performed.

More studies with pure cultures and cocultures need to be conducted, as well as control assays. For example, studies with defined cocultures and different CM with distinct electrical conductivities and surface areas should help in the clarification of the role of these characteristics in the enhancement of methane production. Additional molecular biology methodologies should also be employed in order to get more functional data in addition to taxonomic information. For instance, analyzing the taxonomy based in 16S rRNA collected from RNA extracts in alternative to DNA extracts, measuring enzymes activities or following protein expression, would help to draw more reliable conclusions and to link microbial identity to activity. Metagenomics, metatranscriptomics and metaproteomics may be used to unravel microbial interactions and activities in uncultivable microbial communities. In addition, more studies should be performed in order to understand which trophic groups are directly affected by CM, if the methanogens, the bacteria or both.

A deeper microbial community analysis however, does not discard the need to explore more extensively the system. Measuring as much parameters as possible (e.g., redox potential, electric conductivity, methane, hydrogen, formate, among other parameters) and performing all necessary controls.

Another important issue that should be addressed is the economic and environmental impact of the continuous addition of CM to bioreactors. In some cases, the price of CM is very high, and the continuous addition is not economically feasible. The use of magnetic CM may be an interesting alternative since they can be easily recovered and reutilized due to their magnetic properties. These nanocomposites of carbon and magnetic nanomaterials present a high surface area, proper pore size and excellent catalytic properties. Also, the growth of carbon nanofibers in the surface of ceramic monoliths, and the immobilization of CM (eventually chemically and/or physically modified) in silicon foam may result in a superior macrostructured catalyst with tailored properties for specific applications.

Despite the yet limited knowledge on this topic, the application of CM appears as a good strategy to improve the methane production rates. Their application turns anaerobic systems more resilient to upsets caused by high organic loading rates or toxic compounds, and increase the competitiveness of AD in waste treatment and bioenergy production.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b01913.

> Three supporting tables that summarize the key studies regarding the enhancement of methane production by CM. Table S1 presents the conductive material, the type of reactor, the inoculum and the substrate used, as well as the reported effect on methane production. Table S2 presents the methods used for microbial community analysis and the reported microbial compositions. Table S3 summarize some of the physicochemical characteristics of CM used in AD (PDF)

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Notes

The authors declare no competing financial interest.

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