Promotion of direct interspecies electron transfer and potential impact of conductive materials in anaerobic digestion and its downstream processing - a critical review

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Highlight

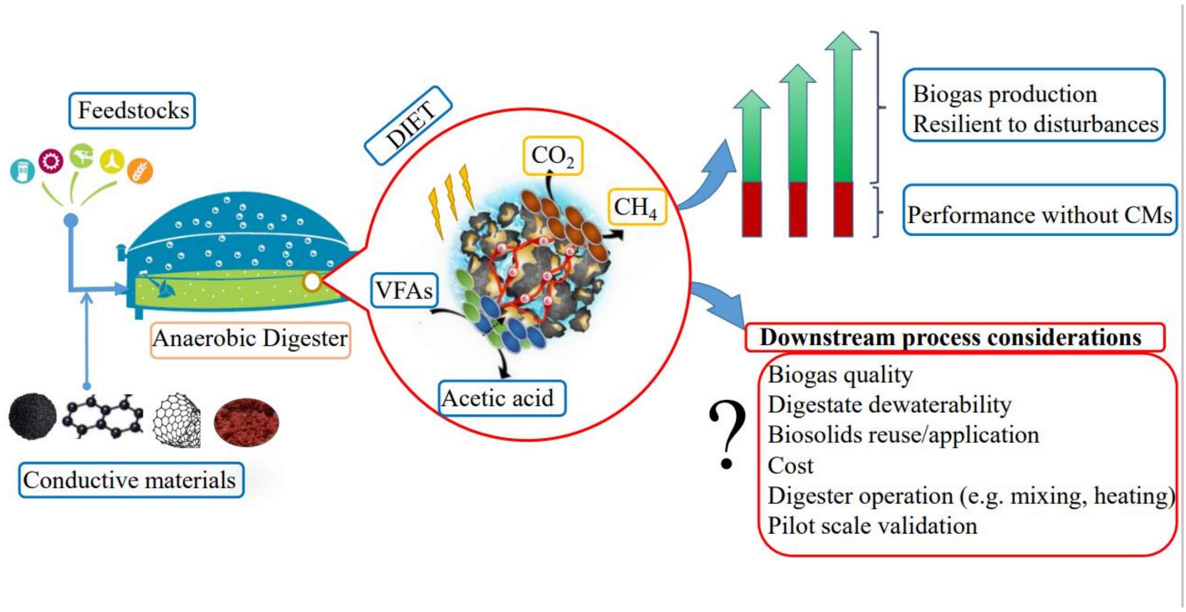
- CM addition for direct interspecies electrons transfer can enhance AD performance
- Research to date is limited to carbon and iron based CMs using small scale reactors
- Future work at pilot scale is recommended to validate the benefits of CM addition
- Future work is also to access the impact of CM addition on downstream processing

Abstract

Addition of conductive materials (CMs) has been reported to facilitate direct interspecies electron transfer (DIET) and improved anaerobic digestion (AD) performance. This reviews summaries the benefits and outlines remaining research challenges of the addition of CMs with focus on the downstream processing of AD. CM addition may alter biogas quality, digestate dewaterability, biosolids volume, and centrate quality. Better biogas quality has been observed due to the adsorption of H_2S to metallic CMs. The addition of CMs results in an increase in solid content of the digestate and thus additional requirement for sludge dewatering and handling and the final biosolids volume for disposal. This review highlights the need for more research at pilot-scale to validate the benefits of CM addition and to evaluate CM selection, doses, material costs, and the impact on downstream processes. The lack of research on the impact of CMs on downstream process of AD is highlighted.

Keywords: Conductive materials; anaerobic digestion; direct interspecies electron transfer; dewatering; biosolids.

Graphical abstract



1. Introduction

In the anaerobic digestion (AD) process, microorganisms acquire energy through fermentation to grow and reproduce and break down organic materials to produce biogas. The AD process is fundamentally the coupling between the oxidation and reduction of chemical compounds and concomitant electron transfer involving several stages (Gahlot et al. 2020; Nguyen et al. 2019). First, bacteria degrade particulate organic matter and complex organic compounds to soluble volatile fatty acids (VFAs). VFAs are then furthered transformed to acetic acids, H_2 and CO_2 for methanogenesis. In the methanogenesis, microbes in the domain of archaea known as methanogens reduce CO_2 and H_2 to form CH_4. Acetic acid is also transformed to CH_4 in the methanogenesis stage. In this syntrophic methanogenesis process, interspecies electron transfer is a key to accelerate the reaction kinetics (Park et al. 2018; Wu et al. 2020; Xie et al. 2020). Hydrogen and formate are electron donnor and act as a shuttle for interspecies electron transfers. Since the process is driven by molecular diffusion, methanogenesis is often the rate-limiting step in the AD process.

In the AD process, electron transfer can be achieved via the exchange of electron containing species such as hydrogen or formate. In the interspecies hydrogen transfer, H_2 is produced by the syntrophic fatty acids oxidizing bacteria (e.g. *Syntrophobacter wolinii* and *Syntrophomonas wolfei*). This process is only thermodyamically feasible at below the critical hydrogen partial pressure threshold which is quite low (Stams & Plugge 2009). On the other hand, a sufficient H_2 content is required for hydrogenotrophic methanogensis to produce CH_4 (Amani et al. 2010; Cazier et al. 2015). The delicate balance between hydrogen partial pressure in the digester and methanogesis is another reason for the slow kinetics of the AD process. Interspecies formate transfer was first demonstrated when the rate of methane production in syntrophic butyrate-degrading cultures (e.g. *Syntrophomonas wolfei* and *Methanobacterium formicicum*) could not be explained by interspecies hydrogen transfer alone (Stams & Plugge 2009; Thiele & Zeikus 1988). However, the electron transfer by formate shuttle is driven by diffusion, resulting in a slow overal reaction kinetics in AD process.

A decade ago, Summers et al. (2010) discovered the direct interspecies electrons transfer (DIET) phenomenon in an anaerobic culture. DIET is a syntrophic metabolism amongst microorganisms in which electrons transfer from one microbial cell to another through an electrical connection without any involvment of reduced electron carriers (i.e. redox mediators) such as molecular hydrogen, formate, or protein (Gahlot et al. 2020). This discovery has sparked new strategies to improve AD process (Park et al. 2018; Wu et al. 2020; Yin & Wu 2019; Zhao et al. 2015). These include the introduction of the electrically conductive pili from microbes in the *Geobacter* genus and a range of other conductive materials (CMs) to the AD reactor (Gahlot et al. 2020; Liu et al. 2012; Rotaru et al. 2014a; Yan et al. 2017). In particular, simple and low cost CMs such as biochar, carbon nanotubes, iron-based oxide, granular activated carbon, powdered activated carbon, and magnetite have showed enhancement in methane yield, improved process stability, and shortened start phase, and recovered near failure AD. Althgough CM addition has shown to achieve some good results, to date, there have not been any studies to assess the potential impacts of CM upon biogas quality (e.g. methane content and impurities), biosolids quality (e.g. odour and volatile solid content), biosolids volume and the digested sludge processing (e.g. sludge dewaterability and sludge centrate treatment). The potential negative impacts of CM could restrict the implementation of the practice at full scale AD. For example, a significant operating cost (ca. 50%) is attributed to biosolids management (Semblante et al. 2014; Wickham et al. 2018). CM addition at high dose (e.g. 20 g/L of active digester volume) without retention could double the biosolids volume after digeste dewatering. Yuan et al. (2021) added 20 g/L biochar into the anaeobic bottle for biogas production. In addition, high total solid content due to CM addition could require more energy for digester mixing and heating as well as sludge pumping. A review of the literature on the impact of CMs on downstream process of AD is imperative for a better vision in the application of CMs.

This review summarises state-of-the-art knowledge on CM addition to facilitate DIET and enhance AD performance over the last ten years. The proposed DIET mechanisms via electrically conductive pili (e-pili), electrically conductive proteins (c-type cytochrome), and CMs in the AD process as well as the benefits of CM addition are summarised and discussed. A specific focus is on the impact of CMs within the AD process and the downstream processing of digested sludge with CMs. The collective information in this review provides a broader consideration for future research and practical application of CM addition to the AD process.

2. Direct interspecies electron transfer in anaerobic digestion

DIET was first observed when Summers el al. (2010) co-cultured two *Geobacter* species *Geobacter metallireducens* and *Geobacter sulfurreducens* under anaerobic condition. *G. metallireducens* oxidised an electron donor substrate (i.e. ethanol), but could not use fumarate as an electron acceptor. On the other hand, *G. sulfurreducens* could not oxidise ethanol as electron donor, but reduced the electron acceptor (i.e. fumarate) to succinate. Thus, these microorganisms have a different way of electron transfer. Summer et al. (2010) observed electrically conductive aggregates between these two *Geobacter* species for electron transfer. Furthermore, Summers et al (2010) reported these electrically conductive aggregates as c-type cytochromes and conductive type IV pili. In a subsequent study, Rotaru et al (2012) observed a direct electron transfer from acetogenic *Geobacter* species to methanogenic *Methanosarcina barkeri* via conductive pili or cytochrome.

Electron transfer via conductive pili and cytochrome does not reply on the diffusion of soluble electron carriers (i.e. H_2 and formate) (Fig. 1a&b). Thus, it is expected that the electron transfer is faster and more energy efficient for methanogenesis (Cheng & Call 2016; Cruz Viggi et al. 2014). Tan et al. (2016) demonstrated through a theoritical calculation that the conductivity of one e-pilus was sufficient to support half-maximal rates of electron transfer. Conductive pili also enables a long-range electron transfer between microorganisms (Malvankar & Lovley 2014). In the e-pili structure, aromatic amino acids are the key elements for electron transfer. However, the mechanism by which they conduct electricity is poorly understood. Currently, there are two hypotheses to explain the electron transfer mechanism: metal-like conductivity and superexchange conductivity (Yin & Wu 2019). In the metal-like conductivity hypothesis, e-pili is conductive due to the overlapping $\pi - \pi$ orbitals of the aromatic amino acids within its structure. For example, Rotaru et al. (2014b) observed that without e-pili regulating gene *Geobacter metallireducens* could not form co-cultures with methanognens *M. barkeri*.

The superexchange conductivity hypothesis emphasised on the role of extracellular cytochrome-cytochrome for electron transfer in DIET pathway. Wang et al. (2019b) suggested that e-pili was made of polymerisation of multiheme c-type cytochromes, which allows continous electron flow paths. However, Ueki et al. (2018) reported that the co-culture of *G. metallireducens* and *G. sulfurreducens* without conductive pili but abundant cytochromes could not conduct DIET, suggesting the essential role of e-pili. Although the roles of e-pili and c-type cytochrome in DIET have not been fully understood, the finding of DIET appears to be an imporant form of syntrophic metabolism in diverse anaerobic enviroment, that could help to enhance the performance of the AD process.

CM addition to faciliate DIET has been demonstrated in a similar manner to that by e-pili or c-type cytochrome (Fig 1c). Liu et al. (2015) observed that a *Geobater* species without c-type cytochrome regulating gene could perform DIET in the presence of magnetite. Likewise, granular activated carbon (GAC) and biochar can substitute e-pili in DIET. Liu et al (Liu et al. 2012) deleted the e-pili and pili-associated cytochrome regulating genes of *G. sulfurreducens* and *G. metallireducens* observed only DIET in the presence of GAC. Two species colonised on the electrically conductive GAC, suggesting that GAC also allows for cell-to-cell electron transfer. DIET via abiotic CMs opens new strategy to boost biogas production in the AD process (Section 3). The CMs serve as substitues for biological connectors and its can be controlled (e.g. through different dose and type of CMs). Park et al. (2018) suggested that interspecies hydrogen transfer and interspecies formate transfer are less effective compared to DIET via CMs.

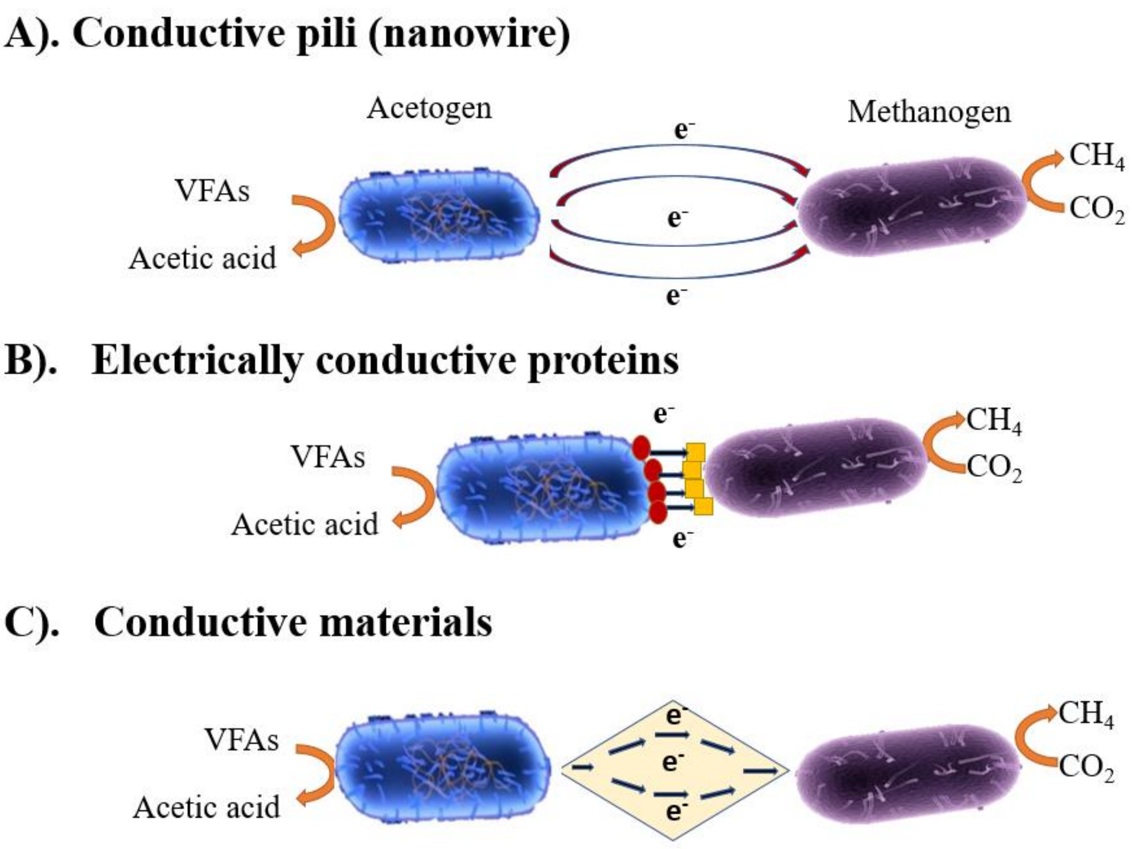


Figure 1: Three proposed mechanisms of DIET between acetogen and methanoge in AD process.

3. Conductive materials impacts on anaerobic digestion

3.1. Performance of AD with CM addition

Recent research findings from laboratory-scale digester bottles have demonstrated the effective of CMs on improving biogas production (i.e. biogas volume and biogas production rate) (Table 1). The observed improvement depends on number of factors including properties of CMs (i.e. surface area, pore size, shape and conductivity) and dose (Chiappero et al. 2020; Gahlot et al. 2020; Martins et al. 2018). For example, biogas production increases as the amount of GAC added in the digester increases (Table 1). The high dose of GAC provide more surface area for the microorganisms to colonise and then conduit electron transfer. Of a particular note is the significant difference in doses between high and low cost CMs. Expensive CMs such as graphene and CNT doses were below 2 mg/L, whereas GAC, PAC and biochar doses were up to 25 g/L digester active volume. Table 1 also shows that previously studies were mainly bacth test experiment (e.g. in biomethane production test and serum bottles) to demonstrate the benefit of CMs on biogas production.

Addition of CMs to the feed can increase digester stability against disturbances (i.e. shock load, pH, and temperature vatiation) and shorten lag phase. It is expected that the hydrogen partial pressure and acidification increase under high loading rate and inhibit hydrogen-producing bacteria and methanogens. However, with DIET via CMs, the methane production could still be sustained. Luo et al. (2015) observed that biochar addition enhanced the degradation rate of VFAs and increased methane production rate by 70.6% at 6 g/L glucose loading. In another study, interspecies hydrogen transfer was reported to shift to DIET under carbon cloth addition (Zhao et al. 2017). This alternation supported the digester resilience to acidic condition pH 6.

Lag phase in AD is the time period from when a disturbance in perfomrance occurs until the digester fully recovers (e.g. methane production rate is consistent as before disturbance). In the presence of CMs, the lag phase has been reported to decrease by 10–75% (Luo et al.

2015; Park et al. 2018). Luo et al (2015) achieved 30.3% reduction in the methanogenic lag phase with 10 g/L biochar addition. Yan et al. (2017) reported the adsorption of acetate on CMs GAC, biochar, and CNT to reduce the lag phase and improve performance as well as stability of thermophilic AD. CMs facilitate the sludge decomposition to release protein and polysaccharide for microorganisms. This abiotic hydrolysis was observed during the addition of magnetite into sludge with 34% increase in soluble protein and 12.6% in polysaccharide (Peng et al. 2018).

Although CM addition has mainly reported to enhance AD performance and alleviated the inhibition impact by pH, VFA, ammonia, and sulfate concentrations, a few recently studies reported the negative impact of CMs when exploring some new materials (Yin et al. 2020). Nano-ZnO and nano-CuO addition at 6 g/L reduced 93% of methane production (Chen et al. 2020). This result was probably because the toxicity effect of nano-ZnO and nano-CuO on AD microbial community. The negative effect of CMs also found when applied at high dose, indicating the important of dose selection (Wang et al. 2018). Tian et al. (2017) observed an increase in 14% of methane volume with 0.03 g/L graphene addition whereas, at dose of 0.12 g/L inhibited methanogens (i.e. *Methanoregula, Methanosaeta and Methanospirillum* species).

Table 1: Performance of AD in batch and continous reactor types with different CMs

CMs	Dose (g/L)	Reactor type	Digestion time (day)	% increase CH_4 volume)	Reference
GAC	0.5 – 5	Batch	20	17.4	(Yang et al. 2017)
	1	Continuous	45	80	(Lee et al. 2016)
	10	Batch	30	70	(Yan et al. 2017)
	25	Batch	20	150	(Liu et al. 2012)
	5 - 15	Batch	30	13 – 22	(He et al. 2021)
	5	Batch	6	66	(Xu et al. 2020)
PAC	5	Batch	100	10	(Xu et al. 2015)
Biochar	1.25	Continuous	10	60	(Zhao et al. 2015)
	10	Batch		21	(Luo et al. 2015)
	25	Batch	12	12.8	(Lin et al. 2017)

	10	Batch	18	23	(Liang et al. 2021)
Carbon cloth	1.25	Continuous	10	70	(Zhao et al. 2015)
	100 cm^2/L	Continuous	50	30 - 45	(Lei et al. 2016)
Graphene	1	Batch	12	25	(Lin et al. 2017)
	0.03	Batch	55	13.4	(Tian et al. 2017)
	1	Batch	22	28	(Lin et al. 2018)
Graphite rods	200 cm^2/L	Continuous	10	40	(Zhao et al. 2015)
Nano-graphite	0.2	Batch	30	13 – 22	(He et al. 2021)
Carbon nanotube	1	Batch	7.5	100	(Li et al. 2015)
Stainless steel	25	Continous	60	7.5 – 24.6	(Li et al. 2017)
Al_2O_3	6	Batch	30	23.4	(Chen et al. 2020)
Fe_3O_4	5	Batch	50	50	(Zhang & Lu 2016)
	10	Batch	18	16	(Liang et al. 2021)
	10	Batch	11	78.3	(Yin et al. 2018)
Magnetite	0.01 – 1	Batch	20	44	(Jing et al. 2017)
	27	Batch	55	7.3	(Peng et al. 2018)
	0.2 – 0.6	Semi-continous	240	5	(Wang et al. 2018)
	2	UASB	36	30.4	(Wang et al. 2019a)
Red mud	20	Batch	28	35.5	(Ye et al. 2018)
Fe^0	10	Batch	18	12	(Liang et al. 2021)
Goethite	5	Batch	6	50	(Xu et al. 2020)

3.2. Conductive materials selection and dose

The selection of CMs depends on factors including potentially electrical conductivity, biocompatibility, chemical stability, lightweight, availability, and cost. CMs that have been applied in AD for DIET can be categorised into two groups: carbon-based CMs and iron-based nanoparticles (Gahlot et al. 2020). Carbon-based CMs are PAC, GAC, graphene, biochar, graphite, carbon cloth, and multiwall carbon nanotubes or single-wall carbon nanotubes. Apart from being electrically conductivity, carbon-based materials support the AD process in a number of ways. For example, GAC is an excellent redox mediator and provides large reactive surface area for microbial colonisation (Liu et al. 2012). Carbon-based materials also adsorb chemical compounds that can inhibit methanogenesis such as phenol and ammonia (Bertin et al. 2004). It is also noted that some expensive carbon-based materials (i.e. graphene and graphite) has showed the improvement in DIET and thus biogas production. However, the high cost of these materials would hinder application in a practical context. On the other hand, biochar can be cheaply obtained from agriculture residue and can be readily returned to agricultural land (section 4.3).

Magnetite and hematite are examples of iron-based CMs to enhance DIET in the anaerobic digestion. Recently, iron-rich wastes from mining and steel industry have also been utilised for DIET. Iron-based materials induce DIET via syntrophic association amongst bacteria and methanogens in the aggregates (Liu et al. 2015). During the culture, microorganisms attached on magnetite and thus magnetite conducted electron transfer amongst microorganisms.

The dose of CMs varies significantly in the literature. Currently, the dose is reported in g/L of reactor volume or g/g VS of sludge. Although in principle, CM doses in different studies can be converted to the same unit, some authors do not report the reactor volume or VS content; thus, it is not possible to normalise the unit. We aim to provide indirect guideline for the selection of conductive material dose. Lin et al. (2017) determined the dose based on the electric conductivity of graphene and biochar. The doses of graphene were 0.5 to 2 g/L significantly lower compared to 5 to 30 g/L of biochar. Yang et al. (2017) utilised dose-response relationship to establish the best CM dose in 150 mL digested bottles. The authors varied the GAC dose from 3.3 to 33 g/L. Similarly, Johnravindar et al. (2020) applied 5, 10, 15 g/L GAC and identified the optimal dose. Chen et al. (2020) reported the application of nano-carbon powder, nano-Al_2O_3 at 50 mg/g TS of sludge. Although the biogas production depends proportionally on the CM dose (Section 3.1), a high dose of CM can be problematic for sludge handling (i.e. mixing and pumping) and subsequent disposal.

It is also noticed that the techno-economic analysis of CM addition in AD has not been reported in the current literature. It is probably the current studies are small laboratory scale and batch-mode operation. The cost of CMs is likely a significant factor to consider when scale up the practice of CM addition. For example, at a dose of 1 g/L graphene (Table 1), it would need 1 kg per m^3 of digester volume. The graphene costs approximately US$140 per kg. Carbon based CMs such as GAC and PAC are cheaper than graphene and CNT. However, their high dose also contributes a significant cost to the overall operating budget. A comprehensive techno-economic analysis is needed to identify the CM that is suitable for full-scale operation.

3.3. Specific microorganisms associated with DIET

Addition of CMs altered the AD microbial community towards the proliferation of species that can participate in direct interspecies electron transfer (Table 2). The most commonly identified bacteria which have been enriched in the AD with CMs addtion includes *Geobacter sp.*, *Thauera sp.*, *Gordonia sp.*, *Syntrophomonas*, *Clostridium*, *Spirochaeta*, and *Bacteroides* (Table 2). *Geobacter sp.* is able to transfer electrons to other insoluble minerals and microorganisms via the conductive pili and c-type cytochromes (Gahlot et al. 2020; Summers et al. 2010). *Sporanaerobacter sp.* can transfer electrons to elemental sulfur that may support them in DIET with *Methanosarcina. Clostridiales sp.* can transfer extracellular electron to the insoluble Fe_2O_3 oxides by type IV pili. These species also have syntrophic metabolisms with methanogens, suggesting the DIET amongst them. Ye et al. (2018) suggested that higher conductivity enhanced the electron transfer between the syntrophic bacteria G*eobacteraceae* and methanogens (*Methanosaeta* and *Methanosarcina*), and then improved the methanogenesis. The relative abundance of these bacteria were significantly higher on the conductive material surface compared to that of suspended sludge in the AD reactor.

Conductive material addition also enriched the archaea species capable of transferring electrons amongst different species. *Methanospirillum*, *Methanosarcina*, *Methanosaeta* and *Methanolinea* were dominant in the AD with CM addition (Table 2). Indeed, Rotaru et al. (2014b) have demonstrated DIET between *Methanosarcina sp.* and *Geobacter metallireducens.* Likewise, Wang et al. (2018) observed the correlation amongst *Geobacter* and *Methanosaeta* species. The addition of magnetite also shifted the methanogenic pathway towards hydrogenotrophic methanogenesis. Carbon based CMs could also accelerated the proliferation of *Methanosaeta harundinacea* or *Methanosarcina barkeri* (Martins et al. 2018). Fe_3O_4 addition at 10 g/L enriched the acidogenics (i.e. *Proteiniclasticum* and *Prolixibacter*) and *Methanosarcina* which faciliated the hydrogenotrophic methanogenesis (Yin et al. 2018).

Table 2: List of enriched bacteria and archaea species in AD with CMs.

CMs	Dose (g/L)	Bacteria (relative abunance %)	Archaea (relative abunance %)	Ref
GAC	0	*Geobacter sp.* (0.06)	*Methanosarcina* (3)	(Yang et al. 2017)
GAC	5	*Geobacter sp.* (0.86)	*Methanosarcina* (8.7)	(Yang et al. 2017)
GAC	0	*Geobacter sp.* (0.03) *Thauera sp.* (2.5) *Gordonia sp.* (0.05)	*Methanospirillum* (7.5) *Methanosarcina* (6) *Methanolinea* (0.07)	(Lee et al. 2016)
GAC	1	*Geobacter sp.* (18) *Thauera sp.* (32.8) *Gordonia sp.* (8.3)	*Methanospirillum* (20.6) *Methanosarcina* (0.5) *Methanolinea* (5.2)	(Lee et al. 2016)
Activated carbon		*Chloroflexi (77.9)*	*Methanobacterium (39.4)*	(Wang et al. 2021)
Activated carbon		*Syntrophomonas* (19)	*Methanosaeta* (24) *Methanosarcina (*16.6)	(Xu et al. 2020)
Graphene	0	*Geobacter sp.* (8.43)	*Methanobacterium* (24.02) *Methanospirillum* (2.15)	(Lin et al. 2017)
Graphene	1	*Geobacter sp.*(9.94)	*Methanobacterium* (34.87) *Methanospirillum* (7.76)	(Lin et al. 2017)
Carbon cloth	0 cm^2/L	*Syntophomonas* (1.6) *Streptococcus* (1.4) *Bacteroides* (4.6)	*Methanosarcina* (14) *Methanospirillum* (2.1)	(Lei et al. 2016)
Carbon cloth	100 cm^2/L	*Syntophomonas* (4.1) *Streptococcus* (5.5) *Bacteroides* (11.2)	*Methanosarcina* (23) *Methanospirillum* (31)	(Lei et al. 2016)
Graphene	0.03	*Geobacter (1.64)*	*Methanoregula* (66.7) *Methanosaeta* (18.7) *Methanospirillum* (9.7)	(Tian et al. 2017)
Fe_3O_4	10	*Proteiniclasticum* (24.9) *Prolixibacter* (13.5)	*Methanosarcina* (13.7)	(Yin et al. 2018)
Zerovalent iron	0.25	*Firmicutes* (43.7)	*Methanobacterium* (30.4)	(Wang et al. 2021)

3.4. Impact of conductive materials on the anaerobic environments

CMs increase the electrical conductivity of anaerobic biomass, biofilms, or granules in the anaerobic digester. Yan et al. (2017) reported that the anaerobic sludge conductivity increased 27 and 3.5 times with carbon nanotube and GAC addition, respectively. CMs (i.e. stainless steel, ferroferric oxide, carbon cloth, and biochar) have been reported to increase the electrical conductivity by 1.5 to 14 times (Lei et al. 2016; Li et al. 2017; Zhang et al. 2017). Enhancement in the expression of electrically conductive pili amongst microorganisms during the DIET and release of cations from CMs are two intuitive reasons to increase electrical conductivity (Martins et al. 2018; Yan et al. 2017). Therefore, the increase in electrical conductivity can be measured as use to indicate the establishment of DIET.

CM addition provides solid surface for microbial colonisation. Since the discovery of DIET by Summers et al (2010) in the aggregates of two *Geobacter* species, it has been indicated that direct contact between CMs and microorganism is necessary for establishing DIET. Pytlak et al. (2020) observed the microbial colonisation on the biochar in anaerobic digestion and the interspecies electron transfer within the colony.

CM addition could alter the oxidation-reduction potential (ORP) in the AD. ORP is an important parameter, regulating the anaerobic metabolisms. A more negative ORP promotes methanogenesis (Hirano et al. 2013). A range of ORP from -200 mV and -400 mV provides the most favourable condition for methanogenesis (Hirano et al. 2013). Salvador et al. (2017) observed an increase in ORP with carbon nanotube addition at 5 g/L from -240 mV to -189 mV, promoting the growth of *Methanobacterium formicicum*.

4. Downstream processing of AD products

Products of the AD process are biogas and digestate. Biogas is a renewable fuel and digestate is a slurry sludge (i.e. 2 to 6% solid content) rich in ammonia and phosphorus (Fig. 2). Processing of these products is necessary for beneficial uses and safe disposal. The downstream processes include pre-treatment and upgrading biogas to use in combined heat power engine for heat and power at the plant or to supply in natural gas grid for other beneficial uses and sludge dewatering to produce high solid content biosolids and nutrient rich centrate. These downstream processes are integral of overall anaerobic digestion process. For example, better sludge dewatering could reduce chemical addition for sludge preconditioning, energy consumption for sludge centrifuge and increase solid content of the final biosolids to reduce the cost of transportation. Therefore, potential negative impact of CM addition should be considered prior to full scale applications.

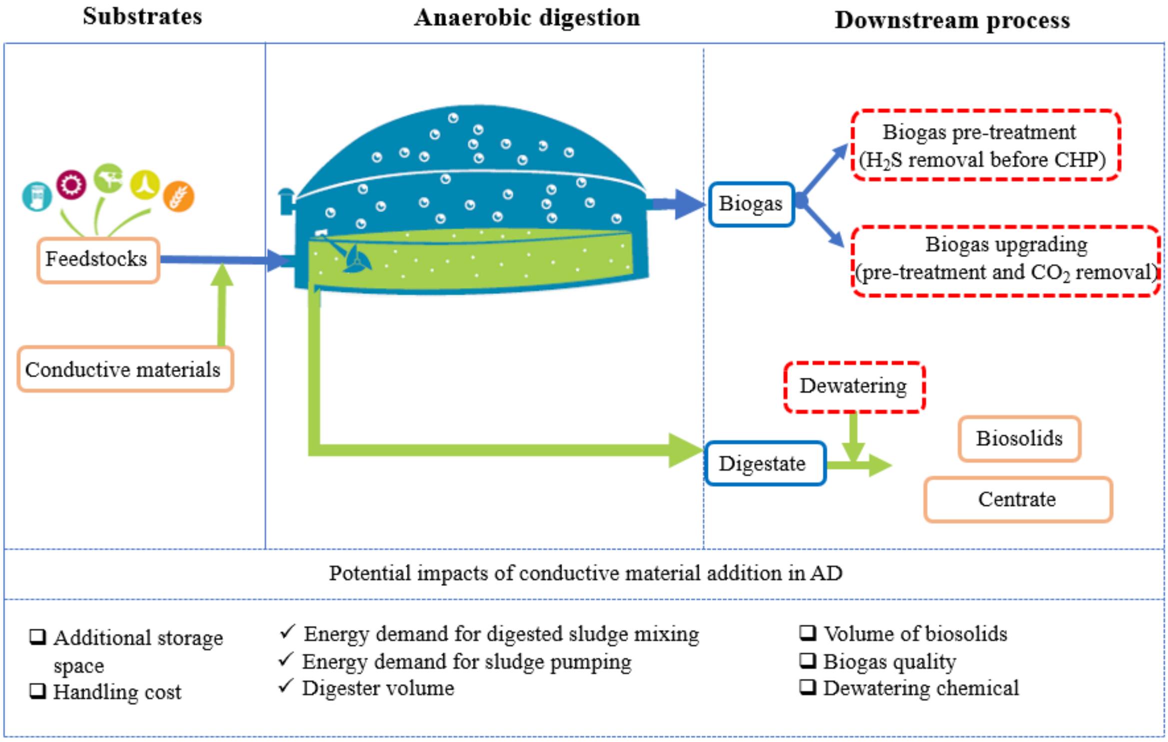


Figure 2: A schematic diagram of the anaerobic digestion and downstream process and possible impacts of CMs on full-scale AD.

4.1. Biogas quality

While DIET via CMs can increase biogas production volume and rate, added CMs has potential to increase biogas quality (i.e. high CH_4, low CO_2 and other impurities content). First, DIET via CMs provide thermodynamically favorable conditions for methanogenesis (e.g. enhanced converstion of CO_2 to CH_4) to reduce CO_2 level in biogas. Second, Carbon based materials such as PAC, GAC and biochar can physically absorb CO_2. Shen et al. (2016) reported that adding biochar from pine enhanced the CH_4 content to 92.3% in biogas from a mesophilic AD. When the biochar was from white oak, the CH_4 content in biogas of 79.0% was observed from a thermophilic AD. However, it is noted that these results are from small lab scale (i.e. 550 mL volume) experiment and the amount of biochar added to the digester was significant. The doses were 2.49 and 4.97 g/g dry matter of sludge for pine biochar and 2.20 and 4.40 g/g dry matter of sludge for white oak biochar. Pytlak et al. (2020) observed an increase in CH_4 content from 54 to 62% due to biochar addition at 1.25 g/L. Carbon based material addition also helped to reduce H_2S concentration in biogas (Wang et al. 2019c). Wang et al. (2019c) observed 78% reduction in H_2S levels from the addition of 3 g biochar in 500 g manure in the biomethane potential test. It is probably that carbon based materials adsorbs the sulfate (SO_4^{2-}) and diverted it from conversion to H_2S by sulfur reducing bacteria and diverted it from conversion to H_2S by sulfur reducing bacteria. H_2S is a corrosive gas, thus, its reduction increases the economic value of biogas (Nguyen et al. 2021).

Likewise, iron based materials such as iron oxide, iron powder, and steel making slag utilised for DIET can decrease H_2S level in biogas. When adding into the digester for DIET, iron based materials influence H_2S formation in biogas in three different ways. Iron oxide inhibits sulfate reducing bacteria and thus, sulfide production. It can react with sulfide to form FeS precipitate. Iron oxide can increase digester pH and lower the H_2S dissociation in the aqueous form. Fetra et al. (2018) achieved a removal of 50% H_2S by mixing iron powder of 1 g/L into the digester. The removal of H_2S was proportional to the iron powder dose. A dose of 2 g/L was reported to remove 89% H_2S without negatively influence on anaerobic digestion process. Farghali et al. (2019) investigated the removal of H_2S by adding Fe_2O_3 (20-100 mg/L) and TiO_2 (100 – 500 mg/L) in 0.6 L digester bottles. In comparison to the control digester bottle, a reduction of 53 to 62% of H_2S in biogas was achieved daily with Fe_2O_3 and TiO_2, respectively. Absorption of H_2S on TiO_2 and FeS deposition were the mechanisms for H_2S removal in the digester bottles (Farghali et al. 2019). The reduction of H_2S and CO_2 in raw biogas could facilitate the biogas pre-treatment and biogas upgrading processes (Nguyen et al. 2021) . For example, chemical absorption or iron sponge and activated carbon adsorption in scrubbing or adsorption column remove H_2S in biogas. High level of H_2S in raw biogas increase the cost of its removal process (i.e. shortens the lifetime of adsorption column and increase chemical usage).

4.2. Digestate treatment

4.2.1 Dewaterability

Anaerobic digested sludge can be viewed as a colloidal system that contains microorganisms embedded in a biopolymeric network, which has great affinity for water and poor dewatering ability. Dewatering is an integral part of digestate treatment. It includes two steps: pre-conditioning and physical separation. Pre-conditioning includes the addition of high molecular, highly charged cationic polymer (e.g. polyacrylamide) into digested sludge to neutralise the negative charge and bridging sludge colloidal. In the separation step, techniques such as centrifuge, screw press, belt press can be used to filtrate sludge. The dewatering process aims to achieve 15 to 30% solid content biosolids. While the selection of physical separation techniques is analogous, polymer demand is influenced by the anaerobic digested sludge physicochemical and biological properties. The content of extracellular polymeric substances (EPS), surface charge, rheological behaviour and disintegration of flocs are directly related to polymer demand and dewaterability (Cao et al. 2019). CMs addition has potential to influence these conditions, and thus, sludge dewatering.

Sludge dewaterability depends on adhesion of water molecule to sludge particles, especially the affinity between water molecule and polysaccharides. CMs increase the migration of bound water to free water, and thus, improving sludge dewaterability. Wang et al (2019d) observed that cations Na^+, K^+, and Ca^{2+} released from biochar addition in to the suspended liquid in AD cause the osmotic effects difference between the sludge flocs and suspended liquid, supporting transfer of free water. Thus, sludge dewatering under hydrothermal method is increased.

The presence of CMs changes the chemical composition and morphology of the sludge flocs. There can be negative or positive impact on sludge dewatering (i.e. polymer demand) depending on type of CMs. Sobeck et al (2002) suggested that a high content of multivalent cations would require a high polymer demand (i.e. charge neutralisation is less effective) during sludge precondition with cationic polymer. It is noted that discussion in this section is still preliminary. There is currently very little data regarding the impact of CMs on sludge dewatering as well as sludge rheology.

4.2.2 Centrate treatment

When the CMs can be solubilised during the AD process, solubilised materials such as Fe could end up in the centrate. Liu et al. (2015) recycled the centrate into bioreactor to transform Fe^{2+} to Fe^{3+} and subsequently utilised for chemical phosphorus removal. Shen et al. (2016) reported that centrate from the AD with biochar addition has high level of macro and micronutrients such as K (up to 300 mg/L), Ca (up to 750 mg/L), Mg (up to 1800 mg/L) and Fe (up to 390 mg/L). They suggested that this centrate could be used as agricultural lime fertiliser.

4.3. Biosolids

CM addition could generate a significant amount of biosolids, depending on the dose (Table 3). The solid content in the AD process without CMs are in the range of 30 to 50 g/L. Addition of CMs e.g. GAC at 27 g/L could result in 57 to 77 g/L total solid content. In addition, the presence of CMs in biosolids derived from AD could affect the disposal practices and the environment (i.e. land application, ocean disposal, incineration, and biofertiliser). For example, metal oxide nanoparticles (i.e. nano-ZnO, nano-CuO) in sludge could contaminate soil with heavy metals. The potential impacts have not been evaluated in the literature.

The application of CMs in full-scale AD plants remains a challenge due to CMs wash out during digestate discharge. Because of CMs wash out, continuous addition of CMs would be required to support DIET. This practice will not only increase biosolids volume for disposal and handling but also increase operating cost (Gahlot et al. 2020). Techniques to recover CMs prior to digestate discharge are suggested in future research to address this issue. These techniques may be developed as fixed- or moving-bed reactors and the magnetic properties of iron based CMs can be utilised to retain them prior to digestate discharge. However, the performance of recycled CMs as compared to the original CMs is questionable.

Table 3: Dose of CMs in recent studies

CMs	Dose (g/L)	TS of feed and inoculum (g/L)	Final TS in digester (g/L)	Reference
GAC	0.5 - 5	50	50.5 - 55	(Yang et al. 2017)
GAC	27	10-14	37 - 41	(Peng et al. 2018)
GAC	5 - 15	22.8	27.7 – 37.8	(Johnravindar et al. 2020)
Biochar	5 - 20	133	138 - 153	(Yuan et al. 2021)
Nano-Al_2O_3	2.5	50	52.5	(Chen et al. 2020)
Nano-ZnO	2.5	50	52.5	(Chen et al. 2020)
Nano-CuO	2.5	50	52.5	(Chen et al. 2020)
Red mud	20	32	52	(Ye et al. 2018)
Zero-valent iron	0 - 10	11.8	21.8	(Zhang et al. 2015)

5. Conclusion

Benefits of CM addition to enhance AD performance have been demonstrated in the literature but mostly from small-scale studies. CMs selection and dose varied significantly in previous studies. The impact of CMs on AD downstream processing is likely and has not been sufficiently examined. Small-scale studies in the current literature could not be used to evaluate the impacts of CMs on downstream digestate processing. CMs handling, mixing and pumping sludge have not been considered. Future work is recommended to validate the benefits of CM addition at pilot-scale, assess the impact on downstream digestate processing, and standardise selection criteria for CMs.

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