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Review

Enhanced Anaerobic Digestion by Stimulating DIET Reaction

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Abstract: Since the observation of direct interspecies electron transfer (DIET) in anaerobic mixed cultures in 2010s, the topic "DIET-stimulation" has been the main route to enhance the performance of anaerobic digestion (AD) under harsh conditions, such as high organic loading rate (OLR) and the toxicants' presence. In this review article, we tried to answer three main questions: (i) What are the merits and strategies for DIET stimulation? (ii) What are the consequences of stimulation? (iii) What is the mechanism of action behind the impact of this stimulation? Therefore, we introduced DIET history and recent relevant findings with a focus on the theoretical advantages. Then, we reviewed the most recent articles by categorizing how DIET reaction was stimulated by adding conductive material (CM) and/or applying external voltage (EV). The emphasis was made on the enhanced performance (yield and/or production rate), CM type, applied EV, and mechanism of action for each stimulation strategy. In addition, we explained DIET-caused changes in microbial community structure. Finally, future perspectives and practical limitations/chances were explored in detail. We expect this review article will provide a better understanding for DIET pathway in AD and encourage further research development in a right direction.

Keywords: direct interspecies electron transfer (DIET); conductive material (CM); external voltage (EV); microbial community change; anaerobic digestion

1. Introduction

Anaerobic digestion (AD) is a traditional biological process utilized for treating various organic wastes including wastewater and complex solid wastes. Via AD, organic matters are converted to biogas, rich in methane (CH_4), while nutrients-rich residues suitable for high-quality compost are generated as byproduct [1–3]. AD process consists of up to four consecutive steps, i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis, in which the rate-limiting step can be different depending on the type of treated feedstock [4]. However, the syntrophic acetogenesis was often considered as a bottleneck that is highly governing the whole rate of AD process [5,6]. Syntrophic acetogens convert C_2–C_6 organic acids/alcohols to electron donors of low-molecular weight, mainly H_2 and acetic acid, which would be consumed by methanogens. This is a well-known indirect interspecies electron transfer (IIET) route, and many attempts were made to enhance this reaction. In particular, the importance of keeping low H_2 partial pressure for acquiring stable IIET was addressed in many studies [7–9]. However, a new route, transferring electrons directly between acetogens or acidogens to methanogens, called direct interspecies electron transfer (DIET), was recently introduced (Figure 1).

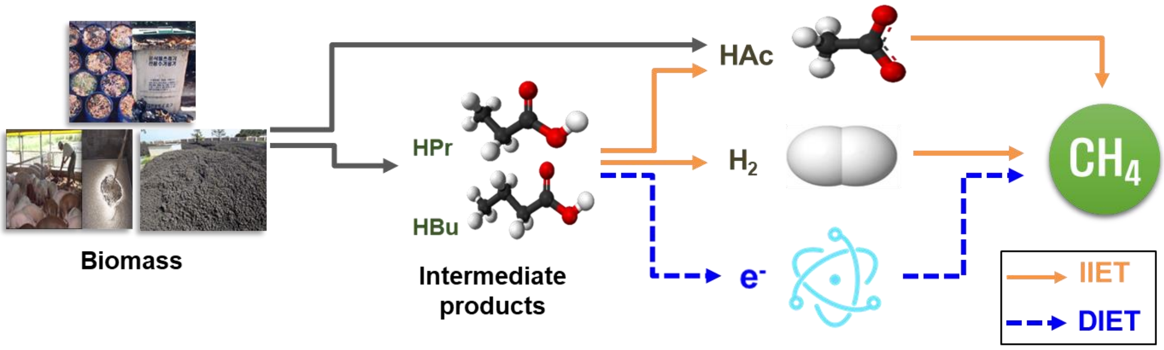

Figure 1. Indirect interspecies electron transfer (IIET) using acetate (HAc) and/or hydrogen (H_2) vs. direct interspecies electron transfer (DIET) in anaerobic digestion.

The first observation of DIET was done by Summers et al. [10], between two *Geobacter* sp. (*G. metallireducens* and *G. sulfurreducens*) in metabolizing ethanol. *Geobacter* is a well-known anaerobic metal oxidizer and can produce electrically conductive pili (e-pili), which can exchange electrons among different cells [11]. Later on, to our knowledge, the first clear evidence of DIET for CH_4 production was observed by Rotaru et al. [12]. When *G. metallireducens* was cocultured with *Methanosaeta harundinacea* fed with ethanol, it was possible to generate > 1 mole CH_4/mole ethanol, which cannot be done theoretically. From one mole of ethanol, *G. metallireducens* can generate 1 mole of acetate with 2 moles of H_2. *Methanosaeta harundinacea* cannot metabolize H_2, and thus, the theoretical maximum CH_4 yield is 1 mole CH_4/mole ethanol. From this result, the authors indicated DIET between two species, which was further confirmed by metatranscriptomic analysis. Thereafter, not only *Geobacter*, but the presence of many other electro-active bacteria (EAB) was detected in AD broth, and the research to stimulate DIET reaction is extensively going on [13–16].

Through the studies of last ten years, it was found that DIET reaction could be stimulated either by the supplementation of a conductive material (CM) and/or applying external voltage (EV) (Figure 2) [17–20]. Basically, EAB can oxidize organics, and then the released electrons are directly transferred through the extracellular cytochromes or the e-pili of EAB to methanogens, which reduce CO_2 into CH_4 [12]. Almost all of the DIET-related studies agreed that e-pili are composed of PilA protein [21–24]. Supplementing CM, specifically magnetite, could interestingly mediate the electron transfer among cells through substituting the pilus-associated cytochrome OmcS that are commonly found in *G. sulfurreducens* [25]. Li et al. [26] observed an increase in electron transfer rate from 0.0017 ± 0.0003 to 0.0056 ± 0.0015 s^{-1}, as a result of carbon cloth supplementation. Additionally, DIET could be stimulated through the establishment of two electrodes inside AD reactor, under slight EV with/without the presence of CM [3,27–29]. Such systems were called as bioelectrochemical systems merged with anaerobic digestion (BES-AD), which were initially applied for hydrogen generation [30–33]. However, the produced hydrogen was easily further converted to CH_4 on the cathode, while the overall CH_4 production rate was increased. The interesting thing found here was that the amounts of CH_4 generated through the electrodes in BES-AD systems were limited, while the majority of CH_4 production was assigned to the bulk of the broth, indicating active DIET reaction happening in the bulk [3,27–29].

Recent DIET-related review articles have mainly focused on the evidences of DIET and the addition of CM for stimulating DIET [34–37]; while the application of EV for stimulating DIET and corresponding microbial community change were not addressed. In this review, we are combing various points regarding DIET, i.e., DIET-caused thermodynamic favorability, recent researches that employed CM and/or EV for DIET stimulation, DIET-driven microbial community change, and future insights for DIET-related hot topics. Further, herein, we are giving a special focus for the contradictory results and debatable points that were found in recent DIET-related research articles. This review is expected to draw a panoramic understanding for various aspects related to DIET and figure out the weakness/challenging points in our knowledge about DIET.

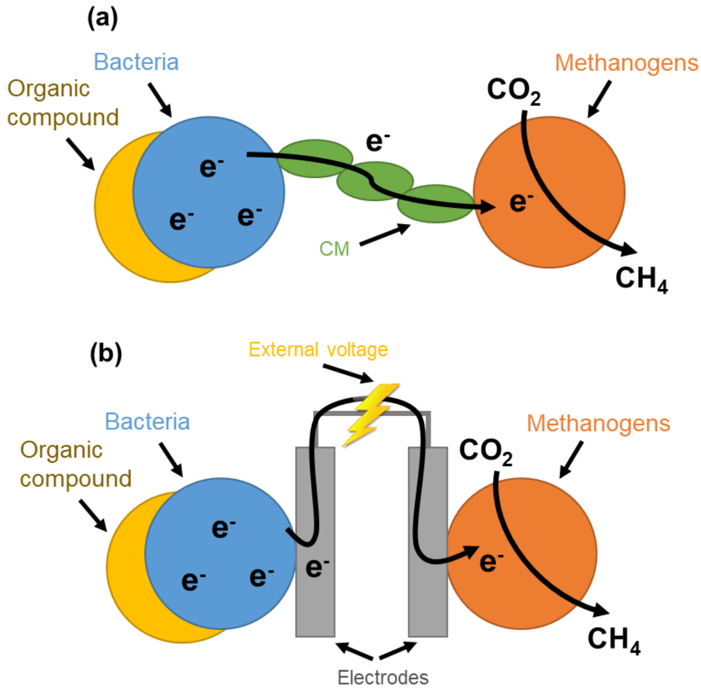

Figure 2. DIET stimulation mechanisms through (**a**) supplementation of conductive material (CM) and (**b**) applying external voltage (EV).

2. DIET-Caused Theoretical Advantages

From the thermodynamic perspective, it was basically expected that DIET can provide more energy benefits for the syntrophic partners than that secured by IIET. This is because in the case of DIET, there is no need for the generation and diffusion of metabolites, where these reactions consume energy [38]. In addition, under such conditions, there is no potential problems from accumulation of H_2, and as a result, faster electron transfer is expected [39]. As clearly shown in Table 1, DIET can provide a more thermodynamically favorable route for producing CH_4 from simple, toxic, and complex substrates, comparted to IIET. The reason for this difference is that Gibbs free energy of H_2 is zero, while H^+ has Gibbs free energy of −39.8 kJ/mol at pH 7 [40]. Therefore, DIET is advantageous for gaining more energy, facilitating cell growth and finally enhancing reaction rate. In addition, recent studies could calculate the external electron transfer rates per cell pair (cp) values, through mathematical modeling, which were 44.9×10^3 and 5.24×10^3 e^- cp^{-1} s^{-1} for DIET and H_2-based IIET, respectively [12,41,42].

Table 1. Gibbs free energies for various reactions, under indirect interspecies electron transfer (IIET) and direct interspecies electron transfer (DIET) conditions.

Reaction	Type of Reaction	Equation		ΔG^0 (kJ/mol)	Reference
Ethanol to CH_4	IIET	$CH_3CH_2OH + H_2O$	$\rightarrow CH_3COO^- + H^+ + 2H_2$	+9.7	[40]
	DIET	$CH_3CH_2OH + H_2O$	$\rightarrow CH_3COO^- + 5H^+ + 4e^-$	−149.6	[40]
Propionate to CH_4	IIET	$CH_3CH_2COO^- + 3H_2O$	$\rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$	+76.5	[40]
	DIET	$CH_3CH_2COO^- + 3H_2O$	$\rightarrow CH_3COO^- + HCO_3^- + 7H^+ + 6e^-$	−162.5	[40]
CH_4 oxidation	IIET	$CH_4 + 3H_2O$	$\rightarrow HCO_3^- + H^+ + 4H_2$	+135.6	[40]
	DIET	$CH_4 + 3H_2O$	$\rightarrow HCO_3^- + 9H^+ + 8e^-$	−183.1	[40]
Phenol to CH_4	IIET	$C_6H_5OH + 5H_2O$	$\rightarrow 3C_2H_4O_2 + 2H_2$	+150.5	[29]
	DIET	$C_6H_5OH + 5H_2O$	$\rightarrow 3C_2H_4O_2 + 4H^+ + 4e^-$	−9.0	[29]
Oleate to CH_4	IIET	$C_{18}H_{33}O_2^- + 16H_2O$	$\rightarrow 9C_2H_3O_2^- + 15H_2 + 8H^+$	+340.9	[43]
	DIET	$C_{18}H_{33}O_2^- + 16H_2O$	$\rightarrow 9C_2H_3O_2^- + 38H^+ + 30e^-$	−641.1	[43]

From energetic standpoint, some energy loss, however, was observed in the case of DIET occurrence; this was ascribed to the activation energy needed for electron donating and accepting redox cofactors (such as cytochromes) in order to transfer electrons [41]. It is worthy to highlight that the aforementioned electron transfer rate constants that were evaluated by Storck's group [41] were based on the respiration of *Shewanella* sp. on the electrodes. Considering that this *Shewanella* sp. has a limited electrical current density, and the transfer rate has high impact upon energy loss [44], it can be concluded that the above-stated values might be lower than the real values associated with electron transfer under DIET-stimulated conditions. Future advanced experiments and mathematical models are needed in order to accurately evaluate electron transfer constants for DIET and IIET.

The dominance of DIET over other potential syntrophic metabolism routes might happen as a response for the broth reactions. In other words, DIET can be a more favored route for microorganism in order to encounter environmental conditions [38]. For instance, when overall energy yield from a certain reaction is limited, the consumption of energy for generating metabolite, as electron shuttles, can be unfavored; instead, DIET in such case can be the optimal choice for energy saving [10,38]. For example, DIET could dominate over other IIET routes, when the system was subjected to acidic inhibition [45,46], toxic substrate [29,43], sulfate-rich wastewater [47], or ammonia containing wastewater [48].

So far, investigating the accurate number of electrons released from one organism and received by another one is still difficult, especially in the case of mixed cultures; therefore, the determination of the accurate energy gain for each microorganism is still limited. The determination of in-situ free energy potential, associated with DIET, is required for evaluating the portion of free energy that are available/consumed during DIET.

3. CM as a Tool for DIET Pathway Stimulation

Based on their capability for DIET stimulation, CM are widely utilized for enhancing the efficiency and stability of CH_4 production [42,49]. Utilized CM can be classified into carbon-based conductive materials (CBCM) and iron-based conductive materials (IBCM). [49]. CM could compensate the lack of vital cell components, needed for completion of DIET, such as pili and c-type cytochrome [50–52]. In ethanol-fed methanogenic co-culture that consisted of *Methanosarcina barkeri* and *G. sulfurreducens*, which had deficiency of pili and pili-associated cytochrome OmcS, no CH_4 could be produced unless CM, i.e., biochar, was supplemented [52]. Chen's group inferred that the presence of biochar enabled 86% of the electrons produced during ethanol oxidation to be utilized for CH_4 generation; while in the absence of biochar, neither ethanol oxidation nor CH_4 generation was observed [52]. In addition, when the pilin-associated c-type cytochrome (OmcS), required for DIET, was not available in *Geobacter* species, magnetite could compensate such lack, and reduce the need for cell to produce OmcS [25]. Similarly, when PilA gene was deleted or yielded very limited pili, *Geobacter* strains grew and made co-cultures in the presence of granular activated carbon (GAC); this observation would never happen without GAC role [53,54].

Table 2 shows the positive/negative impact of CM upon CH_4 productivity for various wastes, treated in batch and continuous treatment systems, specifically up-flow anaerobic sludge blanket reactor (UASB). Generally, when CMs are added to batch tests, lag phase reduction and CH_4 production rate increase could always be observed. However, the increase in CH_4 production yield is not necessarily achieved [48,55–61]. Even, slight negative impact (−4%, compared to control) upon cumulative CH_4 production yield was observed by magnetite addition [62]. But, some reports showed that an increase in CH_4 yield can be also observed after CM supplementation [43,63]. DIET-stimulated digesters were also able to sustain against the harsh operational conditions, e.g., accumulation of sulfate [47] or ammonia [48] or phenol [29]. Consequently, previous studies highlighted that complex substrates, e.g., dog food, sugar industrial wastewater, and leachate of municipal solid waste, can be effectively treated under DIET-stimulated conditions [49,64–67]. For justifying these results, authors referred that CM's presence might secure higher stability for the reactors and resulted in preventing the inhibition-causing problems. Further reason was the enrichment of either DIET-capable or incapable methanogens. In addition, some of those studies referred to the negativity of reaction Gibbs free energy value as a potential reason for enhanced performance. Finally, all these studies highlighted that DIET pathway saves energy, which is reflected on either overcoming the harsh conditions and/or generating higher volumes of CH_4.

Table 2. Impact of various conductive material (CM) supplementation upon CH_4 productivity.

Conductive Material	Particle Size (μm)	Concentration	Substrate	Operation	Improvement/Deterioration (%) [a]	Reference
Granular activated carbon	1220–1430	10 g/L	Glucose	Batch	+168 (CH_4 production rate)	[55]
Single wall carbon nanotubes	0.001–0.002	1 g/L	Glucose Sucrose	Batch Continuous	+92 (CH_4 production rate) − −2 (CH_4 production)	[55] [63]
Carbon nanotubes	0.001–0.002	5 g/L	Glucose	Batch	+2 (CH_4 yield)	[56]
Stainless steel	500–2000	26 g/L	Sodium lactate	Continuous	+8–+25 (CH_4 production)	[47]
Red mud	-	20 g/L	Waste activated sludge	Batch	+36 (CH_4 production)	[57]
Ferric oxyhydroxide	-	20 mM Fe	Whey	Batch	+173 (CH_4 production Rate)	[58]
Ferrihydrite	-	25 mM	Acetate	Batch	+15 (CH_4 production)	[59]
Magnetite	0.05–0.70	5–25 mM	Whey, propionate, acetate, butyrate	Batch	+36–+203 (CH_4 production rate) −4–+44 (CH_4 production)	[48,58,60–62]

[a] Improvement/deterioration (%), compared to control.

The different enhancement performances, implemented by CBCM and IBCM can be explained based on the difference in size and structure of such CM [68]. In specific, nano-sized IBCM are probably attached to the bacterial cells, since CM have smaller size than bacterial cells [25,68]. On the other hand, microbes can be efficiently attached to the surface of CBCM, providing electron conduits for many microorganisms [49,51,52]. This strategy is more preferred for microbes, from metabolic standpoint, since there is no energy consumption for conductive pili synthesis [69]. This approach can be utilized for providing the efficiency of using the DIET-based syntrophy in anaerobic digestors with various configurations [70].

For interpreting the impact of CM upon methanogenesis, two important factors need to be considered, i.e., electrical conductivity and oxidation redox potential (ORP). Electrical conductivity of the utilized microbial consortia is supposed to be higher in CM-amended reactors, compared to control [16,53,55,67]. Such increases can be related to the improved e-pili secretion, which is implemented by EAB that are enriched in the presence of CM [67,71,72]. Furthermore, ORP could be lowered down, to more negative values, as a result of CM addition [73]; this is beneficial for CH_4 generation process, which need ORP of (−200 to −400 mv) in order to be successfully implemented [74]. From thermodynamic standpoint, the supplementation of CM, which are featured of their negative redox potential, into AD broth can lead to directing reactions to DIET route [75]. In addition, it was found that the types and activities of e-pili and cytochromes, expressed from *Geobacter* and *Pelobacter* species for electron transfer, are different based on the surface potential, which is linked to the supplemented CM [76].

Notwithstanding, the enhancing impact, implemented by CM, might be also assigned to the enhanced biomass colonization, since CM usually have large surface that is capable for cell adhesion, especially in the case of nano-sized materials [77–79]. This can justify the enhanced CH_4 productivity, as CBCM are supplemented (Table 2). In case of IBCM, the electrical conductivity might have more remarkable impact than surface area [43,80]. Further study concluded that magnetite could play double role, i.e., (i) stimulating the up-regulated secretion of key enzymes that are involved in the substrate degradation and (ii) inducing the expression of DIET-related proteins [60]. Other studies confirmed that IBCM, specifically, magnetite and ferrihydrite, can act as oxidizing agents and enhance substrate degradation [20,81]. The latter role is not indeed related to DIET stimulation. Instead, it might be achieved at the expense of DIET stimulation, since IBCM are going to be consumed in such reactions instead of stimulating DIET. More research is needed in the future in order to highlight the relation between the enhancement, done by IBCM, and the characteristics of this CM, such as morphology and crystallinity.

4. Electrical Energy Input as Tool for DIET Pathway Stimulation

In BES-AD, slight EV is applied in order to induce the vital reactions [82], and as a consequence, DIET reaction, and the growth of DIET-capable species are stimulated, directly transferring electrons from EAB species to anode surface [83]. Table 3 shows the impact of various EV upon CH_4 from various wastes. Clearly, the slight EV application (−0.3–−1.8 V vs. standard hydrogen electrode (SHE)) led to significant enhancements in CH_4 production (20–1360%). Previous studies showed that optimal range can be (−0.3–−1.4 V vs. SHE) [84] or (−0.2–−0.8 V vs. SHE) [85]. Variation in the optimal EV was found in the literature. For instance, EV of −0.3 V vs. SHE was found to be effective for the treatment of phenol [29] and waste activated sludge (WAS) [86]; however, when EV was changed to -0.6 V vs. SHE, no change in the enhanced performance was observed in the case of phenol, while negative impact was denoted in the case of WAS. Other studies showed different results while optimizing EV. In specific, EV ranges of (−0.5–−1.5) and (−0.6–−1.2 V vs. SHE) were tested for treating glucose and WAS, where optimal EV was found to be −1.0 and −0.8 V vs. SHE, respectively [87,88].

Table 3. Impact of various voltages' application upon CH_4 productivity.

Electrode Type	Applied Voltage (V vs. SHE)	Electrode Surface Area (cm^2)	Substrate	CH_4 Production Improvement (%)	Reference
Carbon felt	−0.5	60	Waste activated sludge + Molasses	50	[89]
Carbon fiber brush	−1.0	ND [a]	Glucose	36	[84]
	−1.1	ND [a]	Glucose	30	[88]
Carbon nanotubes	−1.1	ND [a]	Food waste	20	[90]
Carbon cloth + Cobalt phosphorous catalyst	−0.8	12	Mixture of glucose, starch granule, beef extract, xylose, and cellulose	48	[91]
Ti/Ru alloy mesh plates	−1.8	20	Sewage sludge	1360	[32]
Carbon cloth + Pt	−0.8	162	Waste activated sludge	200	[92]
Fe tube + Graphite pillar	−0.3	45	Waste activated sludge	22	[86]
Graphite carbon + Ni	−0.3	ND [a]	Food waste	70	[93]
Titanium mesh	−0.3	40	Glycerol	60	[3]
Stainless steel	−0.8	ND [a]	Glycose	52	[94]

[a] Not determined.

It was reported that EV application could reduce the time for stabilizing reaction. However, this is not necessarily leading to higher CH_4 yield. In this regard, Park group [93] treated highly concentrated food waste in AD reactor, where EV of −0.3 V vs. SHE was applied. Expectedly, both stabilization time and CH_4 production rate were accelerated by 4.0 and 1.7 times faster than control, respectively. However, the final CH_4 production was the same for EV-supplemented reactor and control. Similarly, our research group treated phenol and could not observe significant difference in CH_4 productivity between EV-applied UASB and control UASB, at low OLR (<5.3 kg chemical oxygen demand (COD)/m^3/d). However, the significant impact was observed at higher OLRs [29]. Another research group highlighted that the electrode polarization could boost volumetric CH_4 production and CH_4 content by 53% and 20.6%, respectively [19]. This implies that EV application can be the key for various merits in AD. The proposed mechanisms for the achieved enhancements under DIET-stimulated conditions were linked mainly to the increased abundance of EAB, along with methanogens, and the advanced electron transfer rate. The effective contribution to DIET enhancement seems to be more related to broth consortia than electrode biofilm, since the amount of CH_4 released from electrodes was very limited, compared to that from the broth [3,27–29]. Specifically, CH_4 generation from the electrodes was found to be lower than 5% of the total generated CH_4 [3,29].

Generally, the required EV was decided by considering both electrode potential for H_2 evolution, which differs based on the electrode material, and electrode potential at which the reduced products could be harvested [95,96]. For instance, when CH_4 production was implemented using a pure culture of *Methanobacterium*-like archaeon, attached to graphite cathode, the selected EV was −0.4 V vs. SHE [97]. Such EV was more positive than the potential for H_2 generation using graphite as electrode material [98,99].

The effectiveness of BES-AD was found to be cardinally linked to the utilized electrode material. Generally, the utilized electrodes are required to have high particular characteristics, such as large surface area, electrochemical active surface, and acceptable electrical conductivity [100,101]. Further, biocompatibility and low cost are additional recommended characteristics [102]. Carbon materials seem to be the most utilized electrode material (Table 2). This might be because of carbon characteristics, e.g., low cost, high conductivity, good biocompatibility, and high capability for biofilm formation [103,104]. For enhancing the aforementioned features, carbon-based electrodes were modified by nano-sized particles of transition elements as (Co and P) [91], Pt [92], Fe [86], and Ni [93] (Table 2).

5. Microbial Community Change after DIET Stimulation

Generally, the most commonly studied DIET-implicated genera are *Geobacter* and *Shewanella* [10,68,105]. In their pioneering work, Summers et al. [10] observed DIET occurrence in a defined co-culture that consisted of *G. metallireducens*, as an exoelectrogenic bacteria, and *G. sulfurreducens*, a fumarate reducer. Later on, metatranscriptomic analysis was employed for confirming DIET occurrence between *G. sulfurreducens* and *G. metallireducens*, [106] or *G. sulfurreducens* and *Methanothrix* sp. [107]. These studies considered the limited abundance for hydrogenase and/or formate dehydrogenase transcript as strong indicator for DIET occurrence among the syntrophic partners. Further, DIET-capable syntrophic co-culture, which consisted of *Methanosaeta* sp. and *Geobacter* sp., was detected in BES-AD system [108].

In addition, the potential DIET occurrence was previously claimed between *Geobacter* sp. and acetoclastic methanogen (*Methanosaeta* sp.) [12], hydrogentrophic methanogen (*Methanobacterium* sp.) [7], or methanogen that can work as both acetoclastic and hydrogentrophic (*Methanosarcina* sp.) [50]. Apparently, various types of methanogens exhibited DIET capability. However, so far, there is no conclusion regarding which methanogen must be enriched as a result of DIET-stimulating condition, either by CM supplementation and/or EV application. For instance, when the low strength wastewater, which contained glucose as a main carbon source, was treated through BES system, equipped with graphene/polypyrrole electrode, hydrogentrophic methanogens' dominance was recorded [109]. However, the usage of nano-graphene for the treatment of the same wastewater could induce the presence of *Methanosaeta* sp. (acetoclastic methanogen) [110] (Table 4). Another informative example can be obtained through exploring the impact of ethanol, utilized as DIET stimulator, upon the syntrophic metabolism of propionate and butyrate [72]. Results exhibited that ethanol's presence enriched the abundance of *Methanosarcina* sp. and *Methanosaeta* sp., irrespective of whether the substrate was propionate or butyrate. However, in case of propionate, both of *Methanosarcina* sp. and *Methanosaeta* sp. could be enriched by almost the same ratio. On the other hand, when the substrate was propionate, ethanol's presence could mainly augment the abundance of *Methanosarcina* sp. [72]. Like the relation between the supplemented CM and enriched methanogens, the relation between the value of the applied EV and the type of methanogens that can be enriched is still unclear. In addition, the change in the composition of microbial community cannot directly refer to the active type of interspecies electron transfer [80]. Another unclear point is related to the electron receptors of enriched methanogens. Basically, there is an agreement that *Geobacter* sp. is exporting electrons through its conductive pili [50,111,112]; however, the mechanism by which electrons are received by methanogen is still vaguely understood [12].

Table 4. The enrichment of methanogenic consortia after direct interspecies electron transfer (DIET) stimulation.

Main Carbon Source	DIET Stimulator	Enriched Methanogens	Metabolic	Reference
Butyrate, propionate, and acetate	Magnetite nanoparticles	*Methanobacterium* sp.	Hydrogentrophic	[113]
Glucose	Bioelectrochemical system with graphene/polypyrrole	*Methanoregula* sp.	Hydrogentrophic	[109]
Food waste and Sewage sludge	Sawdust-derived biochar	*Methanothermobacter* sp.	Hydrogentrophic	[114]
Glucose	Magnetite	*Methanobacterium* sp.	Hydrogentrophic	[7]
Acetic acid	Granular active carbon	*Methanospirillaceae* sp.	Hydrogentrophic	[115]
Glycerol	Magnetite	*Methanomassiliicoccus* sp.	Hydrogentrophic	[3]
Swine manure	Granular active carbon	*Methanosaeta* sp.	Acetoclastic	[116]
Glucose	Nano-graphene	*Methanosaeta* sp.	Acetoclastic	[110]
Ethanol and glucose	Powered activated carbon	*Methanosarcina* sp.	Acetoclastic	[117]
Sodium acetate, sodium propionate, and sodium butyrate	TiO_2 nanoparticles	*Methanobacterium* sp. and *Methanosarcina* sp.	Hydrogentrophic and acetoclastic	[118]

In BES-AD studies, various species could be enriched, such as *Methanococcus maripaludis* [119] and *Methanobacterium congolense* [120]. The type of enriched consortia might be related to the utilized electrode and the utilized catalyst [121]. For instance, mixed culture of anaerobic consortia could have better respiration condition when stainless-steel was used as cathode, than that while using pure conventional carbon-based materials [122]. Further, microbial growth/colonization on the surface of the electrode is governed by the electrode surface properties since they affect microbes–electrode interaction, which depends upon the formed bond, e.g., Van der Waals force, hydrogen bonding, or electrostatic interaction [101]. For instance, a faster growth for biofilm could be achieved, when the utilized electrode material has a positive charge. This is because of microorganisms' negative charge [123]. Further, extra-porous and nano-sized materials are perfect scaffold for improved microbial attachment/growth, enhancing biofilm formation [124]. A point of debate is the impact of DIET stimulation upon microbial diversity; some researches confirmed diversity lowering, as a result of DIET stimulation [29,125]; however, other researches referred to increased microbial diversity under DIET-stimulated condition [86,126].

Among the 11 studies shown in Table 2, only 4 research articles detected *Geobacter* sp. [47,48,57,59]. Furthermore, those four studies revealed very slight abundance for *Geobacter* sp. (<5% of the total bacterial community detected). Previous review article also highlighted that most of the studies that claimed DIET occurrence could not detect *Geobacter* sp. [34]. This dilemma might have two possible explanations. Firstly, if the claimed DIET did not really happen, then the enhancements of CH_4 productivity, in terms of yield and/or rate of production, might be assigned to other roles played by the utilized DIET-stimulator, such as enhancing biomass colonization [77–79] or stimulating organics' degrading microbes for enzymes' secretion and substrate utilization [29,60]. Secondly, if the claimed DIET really happened, then the applied DIET stimulation strategy could induce DIET-capable syntrophic bacteria, yet to be confirmed as EAB [34,127]. The latter explanation might agree with recent findings that confirmed DIET can be a common electron transfer option for not only *Geobacter* and *Shewanella* but also for various syntrophs such as *Pelobacter* sp. and *Syntrophorhabdus* sp., which are potential EAB [3,32]. Future studies need to confirm DIET capability/incapability of the detected species in DIET-stimulated reactors, in order to assure DIET occurrence; in addition, the enrichments in the abundance of other species, different from EAB, must be involved in explaining CH_4 productivity and substrate utilization efficiency profiles.

6. Conclusions and Future Perspectives

This review tried to give comprehensive explanation for the recent advanced knowledge regarding DIET, as an alternative metabolic approach for enhancing CH_4 productivity from various wastes. DIET reaction could provide theoretically advantageous route due to its more negative value of ΔG^0, and there is no need for the generation and diffusion of metabolites. It could be stimulated through the supplementation of CM and/or the application of EV. CBCM could compensate the lack of *Geobacter* conductive pili, and IBCM could reduce the bacterial need for OmcS production. In applying EV, carbon was the commonly utilized electrode material, because of its high conductivity and low cost. The increased amount of CH_4 production from the electrodes was negligible, indicating active DIET reaction occurring in the broth. DIET establishment leads to enriching acetoclastic and/or hydrogentrophic methanogens. *Geobacter* sp. was absent or slightly present in most of DIET studies that utilized mixed culture as inocula. Away from EAB, other bacterial species that are enriched after DIET stimulation might be implicated in the detected CH_4 productivity improvements.

(a) Engineering perspectives

From an engineering perspective, the economic feasibility of DIET stimulation in AD reactors is still questionable. It can be roughly considered that the cost of CM supplementation, as DIET stimulation approach, might be lower than applying EV, which requires the installation of two electrodes that are recommended to be fabricated using CM. However, further studies need to be implemented in order to compare the economic feasibility of the two DIET stimulation approaches, considering the maintenance cost and CM loss during EV application and CM supplementation, respectively. For reducing the overall cost needed for DIET stimulation, two approaches are suggested. First approach is the usage of relatively cheap CM, such as red mud, or to recover that CM from other wastes, e.g., biochar recovery from digestate by pyrolysis.

Second approach depends on DIET establishing through two steps: (i) ethanol-type fermentation of easily biodegradable substrate, such as polysaccharide and (ii) methanogenesis, where, the ethanol-rich effluent, obtained from the first step was utilized for providing better conditions for DIET stimulation in the second step. Such approach was based on the special favorability of DIET-implicated microorganisms to ethanol, as a substrate [128]. Zhao group denoted that sludge fed with ethanol had higher abundance for *Methanothrix* sp. and gene for pilA, compared to that when propionate and butyrate were used as feeds. This strategy caused increase in CH_4 production from WAS by 36% [129] and 30% [130]. On the other hand, fabrication of electrodes that have low cost can support EV-based DIET performing. These perspectives can help in the commercialization of DIET-based reactors; however, more successful tests for bench-scale reactors are needed. In this regard, Yee et al. [131] provided a detailed practical protocol for the setting of the electrochemical reactors. Future tests have to consider calculating benefit/cost ratio for DIET-stimulated digestor and comparing it to control digestors. Thereafter, large scale application will just be an issue of time.

(b) Scientific perspectives

Undoubtedly, there is a literature chaos, specifically around CM-based DIET stimulation topic, since DIET occurrence has been claimed in many researches, without revealing vital experimental evidences. Therefore, recent review articles indicated that more experimental observations are required before claiming DIET stimulation [36,80]. Future researches should direct more effort for verifying DIET activation and/or other potential changes in anaerobic growth, which can be implicated in the observed enhancement in CH_4 production rate and/or yield. Recently, Van Steendam et al. [132] evaluated some advanced DIET identification methods, including fluorescence in situ hybridization, cyclic voltammetry, scanning, transmission electron microscope, and various (meta-)omic approaches; the usage of combined methods for confirming DIET occurrence was finally recommended. Additionally, Neu et al. [133] presented temperature-resolved THz spectroscopy that can easily measure the conductivity of wild-type pili. Further, Yee et al. [131] explained some electrochemical techniques that can be utilized for determining the electroactivity and the mode of electron transfer during the reactions. The aforementioned studies are referring to the right track of research that is worthy to be followed. More studies are needed to describe strict and simple verification strategy for validating DIET occurrence/absence.

Since they have noticed the electric conductivity of syntrophic aggregates that contained *Geobacter* sp., Lovley's team explained the observed long-distance electron transfer based on e-pili, composed of PilA protein, which had metallic-like conductivity and could act as conduit [10,21,22,134,135]. However, a recent work by Wang et al. [136] gave proofs to indicate that the conduit for such long-distance electron exchange process is not e-pili, rather, it can be cytochrome OmcS, in its polymerized chain form. Wang's group concluded that the role played by PilA is stimulating the excretion of OmcS. This view around the mechanism of electron transfer needs further research to be established. From the way how the e-pili were formed, it seems that we need to understand more about the characteristics of those e-pili. Very recently, an astonishing research work proved that the protein nanowires that were harvested from *G. sulfurreducens* can form a thin-film device, which is able to generate continuous electric power (0.5 V across a 7 μm thick film) in the ambient environment [137].

The rate of electron transfer seems to be one of the hot points that needs further experimental research and predictive models, since available information in literature about the efficiency and energetic losses, during DIET, are limited. Moreover, more models are needed to witness the response of microbial consortia to harsh environmental changes, and the mechanism of microbial cooperation for metabolism under such conditions. In other words, the question that needs to be answered is to what extent DIET can be the way for the best diet?

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