A review of interspecies electron transfer in anaerobic digestion

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Abstract. Anaerobic digestion (AD) is an effective way to recover energy from organic waste. About 70% of the methane emitted into the atmosphere is derived from the degradation of organic matter by microorganisms under anaerobic conditions. Interspecies electron transfer (IET) is the key link of syntrophic methanogenesis, an in-depth understanding of IET during AD contributes to the rational use of energy. IET mainly includes three modes, namely, interspecies hydrogen transfer (IHT), interspecies formate transfer (IFT) and interspecies direct electron transfer (DIET). This review summarized and analyzed the IET patterns in the AD process, and related metabolic mechanisms and existing studies were explained.

1. Introduction
Anaerobic digestion (AD) is an effective way to recover energy from organic waste[1]. About 70% of the methane emitting into the atmosphere is derived from the degradation of organic matter by microorganisms under anaerobic conditions[2]. In the methanogenic environment, due to the lack of inorganic electron acceptors (such as O₂), organic matter can only undergo fermentation degradation process using protons and HCO₃⁻ as electron acceptors[3]. This process is roughly as follows: (i) anaerobic bacteria decompose complex organic compounds into low molecular weight intermediates such as volatile fatty acids (VFAs); (ii) hydrogen-producing acetogens convert these VFAs to H₂ and acetate at low H₂ partial pressures; (iii) methanogens convert CO₂, acetate, H₂, etc. to CH₄[4].

The intermediate products produced by the fermentation bacterium-mediated complex organic matter in the degradation process are mainly volatile fatty acids (VFAs), and the further decomposition is an endothermic process under standard conditions and cannot be spontaneously carried out. On the other hand, VFAs degradation products, as precursors of methanogenesis, can also thermodynamically promote methanogenic processes more efficiently. This kind of cooperative symbiosis is called syntrophic methanogenesis process. Interspecies electron transfer (IET) is the key link of syntrophic methanogenesis, it determines whether the organic matter degradation and methanogenesis process can be carried out efficiently and orderly, and it is also an important means for mutual bacteria and methanogens to intervene to break through thermodynamic constraints to maintain growth. Therefore, an in-depth understanding of IET during AD contributes to the rational use of energy. IET mainly includes three modes, namely, interspecies hydrogen transfer (IHT), interspecies formate transfer (IFT) and interspecies direct electron transfer (DIET).

2. The interspecies hydrogen transfer (IHT)
AD is a complex microbial metabolic process that requires the participation of multiple microorganisms[5]. Meanwhile, H₂ is an important substrate for the production of CH₄, and this mechanism can be seen as the process by which H₂ acts as a carrier to transfer electrons from VFAs or
alcohol to CO₂[6]. This process is called IHT and it is the most common form of electron transfer in AD process. Propionate and butyrate are the two main sources of H₂, and in theory their degradation is thermodynamically feasible only when the H₂ partial pressure is kept low[7]. The degradation can be expressed by the following equations:

\[
\text{CH}_3\text{CH}_2\text{COO}^- + 3\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COO}^- + \text{HCO}_3^- + \text{H}^+ + 3\text{H}_2, \Delta G = +76.1 \text{ kJ/mol} (1)
\]

\[
\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COO}^- + \text{H}^+ + 2\text{H}_2, \Delta G = +48.1 \text{ kJ/mol} (2)
\]

In other words, IHT is usually a process of absorbing energy, and due to thermodynamic limitations, most of this mechanism can only be carried out when the partial pressure of H₂ is low[8]. This makes the AD process greatly inhibited. Once the methanogens slow down the rate of molecular H₂ utilization, due to environmental conditions, the acetogens will also slow down the use of propionate and butyrate, which is why once the anaerobic digestion system fails, the bioreactors will produce the phenomenon of accumulation of VFAs.

3. The interspecies formate transfer (IFT)

Formate as an electron carrier was discovered when studying the *Syntrophobacter fumaroxidans*. It has been found that when the oxidation of butyrate was carried out in a reactor with whey and butyrate as a medium, the detected rate of methane formation could not be explained by the IHT, suggesting that formate may also be an electron carrier[6]. In the mutual system of *Syntrophomonas wolfei* and *Methanobacterium formicicum*, it was found by diffusion model that the diffusion rate of H₂ could not sufficient to explain the synthesis rate of methane at the H₂ natural concentration, but the rate of diffusion of formate could be explained. Moreover, both strains contain formate dehydrogenase, which proved the existence of IFT between species. The reaction equations for the degradation of propionate and butyrate with formate as an electron carrier was as follows:

\[
\text{CH}_3\text{CH}_2\text{COO}^- + 2\text{H}_2\text{O} + 2\text{CO}_2 \rightarrow \text{CH}_3\text{COO}^- + 3\text{HCOO}^- + 3\text{H}^+, \Delta G = +65.3 \text{ kJ/mol} (3)
\]

\[
\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + 2\text{H}_2\text{O} + 2\text{CO}_2 \rightarrow 2\text{CH}_3\text{COO}^- + 3\text{HCOO}^- + 3\text{H}^+, \Delta G = +38.5 \text{ kJ/mol} (4)
\]

The IHT were usually endergonic through the above equation. Whether H₂ or formate as the main interspecies electron carrier varies with different syntrophic systems and culture conditions. The diffusion rate of H₂ is 30 times that of formate, but the solubility of formate was much higher than that of H₂, which tends to form a higher concentration difference, about 1000 times higher than that of H₂, so it could be understood as the premise of achieving the same diffusion rate. Under the H₂, H₂ may be mainly involved in short-range electron transfer, and formate may be involved in long-distance electron transfer[10].

4. The interspecies direct electron transfer (DIET)

In addition to the two electron transfer methods of IHT and IFT, previous studies have shown that there was still DIET in the AD system, and it could directly transfer electrons to related methanogens, thereby reducing CO₂ to CH₄ with lower energy[11]. Some studies indicated that DIET was an alternative pathway to transfer electron between VFA oxidizing bacteria and methanogens during AD process, and it was confirmed in co-cultures of *Geobacter metallireducens* and *Methanoseta*[12] or *Methanosarcina*[13], *Geobacter sulfurreducens* and *Geobacter metallireducens*[14], and other co-cultured anaerobic systems[8]. There were three types of DIET mechanisms have been identified to date, i.e. DIET via membrane-bound electron transport proteins, DIET via conductive pili and DIET via abiotic conductive materials.

4.1. DIET via membrane-bound electron transport proteins

Studies have shown that *Prosthecochloris aestaurii* could accept electrons released by *G. sulfurreducens*, while transmission electron microscopy images showed no significant tight junctions of conductive pili between the two strains. The multiheme outer-surface cytochrome OmcZ responsible for the transfer of electrons to the electrodes[15] was believed to be associated with this type of DIET[10]. A similar physical association was also observed in the co-culture of methanogens archaea with sulfate-reducing bacterium[17]. DIET by membrane-bound electron transport proteins (such as OmcZ) may also be a
mechanism for methane formation, however there was no report on this. This suggests that DIET by membrane-bound electron transport proteins was not common for methanogens.

4.2. DIET via conductive pili

The pili of *G. sulfurreducens* was highly conductive and could act as a bio-nanowire to transfer electrons on the cell surface to extracellular Fe(III) oxides, and predicted that electrons could also be transferred between cells via pili[18]. Related studies have shown that DIET phenomenon has been found in the co-culture system of *Geobacter metallireducens* (electron-accepting bacteria) and *G. sulfurreducens* (electron-accepting bacteria)[14]. Successive transfer of the coculture evolved the bacteria to form electrically-conductive aggregated via conductive pili with enhanced OmcS (a multiheme c-type cytochrome) production, which was identified to facilitate DIET by association with conductive pili. Other studies have also shown that the methanogenic agglomerates founded in the beer wastewater anaerobic reactors were electrically conductive, *Geobacter* was the most abundant aggregate bacteria in the reactor, the archaea was dominated by *Methanosaeta*, and the rate of methanogenesis in the agglomerates was far below the rate of ethanol degradation, these results demonstrated for the first time that methanogenesis agglomerates were electrically conductive, and DIET between species was likely to be an important electron transfer mechanism for methanogenic systems[19].

4.3. DIET via abiotic conductive materials

Kato et al. first proposed that conductive materials could stimulate DIET to produce methane. They observed that supplementation of conductive iron oxide minerals (such as magnetite) in anaerobic serum bottles inoculated with paddy soil could enrich specific microbial populations (such as *Geobacter* and *Methanosaeta* species), thereby increasing methane production. They believed that conductive materials could establish electrical bridges between *Geobacter* and *Methanosaeta* species (i.e. DIET)[20]. Liu et al. demonstrated that the addition of granular activated carbon (GAC) to an anaerobic reactor with ethanol as a substrate could enhance DIET in the coculture of *G. metallireducens* and *M. barkeri*[21].

Currently, carbon-based conductive materials have been widely used in the research of DIET stimulation. These included biochar (BC), GAC, powdered activated carbon (PAC), carbon cloth, carbon nanotubes, graphite and graphene. Carbon-based materials generally have high electron conductivity, while large specific surface area and porosity were beneficial to the growth and electron transfer of microorganisms[22, 23]. Previous studies have shown that BC addition effectively increased maximum CH₄ production rate by 22.4% to 40.3%, and shortened the lag time by 27.5-64.4%, alleviating pH decrease due to VFAs accumulation[24]. The addition of GAC significantly enriched functional bacteria capable of participating in DIET, such as *Geobacter*, H₂-utilizing methanogens, etc., which promoted the efficiency of electron exchange between syntrophs. At the same time, methanogens enhanced substrate degradation and CH₄ production[25]. It has been reported that AD reactors supplemented with conductive carbon cloth could have higher adaptability by strengthening DIET to resist the effects of acid shock. In addition, when the H₂ partial pressure of the anaerobic system increases, the reactor without carbon cloth almost fails[26]. Results showed that carbon nanotubes at a concentration up to 1000 mg/L could induced much faster substrate utilization and methane production rates, exposure enhanced the electrical conductance of the sludge, which might promoted the DIET among anaerobic fermentative bacteria and methanogens in the AD process[27]. The addition of 1 g/L of graphene increased the degradation rate of ethanol by 29.1%, which increased CH₄ yield and productivity by 25% and 19.5%, respectively[28]. In addition to carbon-based materials, some non-carbon based materials have also found similar functions in the AD process, such as magnetite (Fe₃O₄), hematite (Fe₂O₃), stainless steel and polyaniline nanorods. For example, the optimal amount of Fe₃O₄ additive in the Fischer-Tropsch wastewater treatment process can make COD removal efficiency and cumulative CH₄ yield much better than the control group[29].

5. Conclusion

This review summarized and analyzed the IET patterns in the AD process. At the same time, it explained
the principle and application of this mechanism and cites published research. Related functional microorganisms (e.g. *Geobacter* and *Methanosarcina* species), and effects of DIET (e.g. substrate degradation rates and methane formation rates) were also identified.

Reference

[1] Zhao Z, Zhang Y, Woodard T L, Nevin K P, Lovley D R. Enhancing syntrophic metabolism in up-flow anaerobic sludge blanket reactors with conductive carbon materials[J]. Bioresource Technology, 2015, 191:140-145.

[2] Conrad R. The global methane cycle: recent advances in understanding the microbial processes involved[J]. Environmental Microbiology Reports, 2009, 1(5): 285-292.

[3] Stams A J M. Metabolic interactions between anaerobic bacteria in methanogenic environments[J]. Antonie van Leeuwenhoek, 1994, 66(1/3): 271-294

[4] Lv W, Schanbacher F L, Yu Z. Putting microbes to work in sequence: Recent advances in temperature-phased anaerobic digestion processes[J]. Bioresource Technology, 2010, 101(24):9409-9414.

[5] Lee S H, Kang H J, Lee Y H, et al. Monitoring bacterial community structure and variability in time scale in full-scale anaerobic digesters[J]. Journal of Environmental Monitoring, 2012, 14.

[6] Stams A J M, Plugge C M. Electron transfer in syntrophic communities of anaerobic bacteria and archaea[J]. Nature Reviews Microbiology, 2009, 7(8):568-577.

[7] Zhao Z, Zhang Y, Yu Q, et al. Communities stimulated with ethanol to perform direct interspecies electron transfer for syntrophic metabolism of propionate and butyrate[J]. Water Research, 2016, 102:475-484.

[8] Cruz Viggi C, Rossetti S, Fazi S, et al. Magnetite Particles Triggering a Faster and More Robust Syntrophic Pathway of Methanogenic Propionate Degradation[J]. Environmental Science & Technology, 2014, 48(13):7536-7543.

[9] Boone D R, Johnson R L, Liu Y. Diffusion of the interspecies electron carriers H₂ and formate in methanogenic ecosystems and its implications in the measurement of Kₘ for H₂ or formate uptake[J]. Applied and Environmental Microbiology, 1989, 55(7):1735-1741.

[10] Bok F A M D, Plugge C M, Stams A J M. Interspecies electron transfer in methanogenic propionate degrading consortia[J]. Water Research, 2004, 38(6):0-1375.

[11] Storck T, Virdis B, Batstone D J. Modelling extracellular limitations for mediated versus direct interspecies electron transfer[J]. Isme Journal, 2016, 10(3):621.

[12] Rotaru A E, Shrestha P M, Liu F, et al. A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to Methanosaeta for the reduction of carbon dioxide to methane[J]. Energy & Environmental Science, 7.

[13] Rotaru A E, Shrestha P M, Liu F, et al. Direct Interspecies Electron Transfer between Geobacter metallireducens and Methanosarcina barkeri[J]. Applied and Environmental Microbiology, 2014, 80(15):4599-4605.

[14] Summers Z M, Fogarty H E, Leang C, et al. Direct Exchange of Electrons Within Aggregates of an Evolved Syntrophic Coculture of Anaerobic Bacteria[J]. Science, 2010, 330(6009):1413-1415.

[15] Richter H, Nevin K P, Jia H, et al. Cyclic voltammetry of biofilms of wild type and mutant Geobacter sulfurreducens on fuel cell anodes indicates possible roles of OmCB, OmCZ, type IV pili, and protons in extracellular electron transfer[J]. Energy & Environmental Science, 2009, 2(5):506.

[16] Lovley, Derek R. Syntrophy Goes Electric: Direct Interspecies Electron Transfer[J]. Annual Review of Microbiology, 2017, 71(1):annurev-micro-030117-020420.

[17] Mcglynn S E, Chadwick G L, Kempes C P, et al. Single cell activity reveals direct electron transfer in methanotrophic consortia[J]. Nature, 2015.

[18] Reguera G, McCarthy K D, Mehta T, et al. Extracellular electron transfer via microbial nanowires[J]. Nature, 2005, 435(7045):1098-1101

[19] Morita M, Malvankar N S, Franks A E, et al. Potential for Direct Interspecies Electron Transfer in
Methanogenic Wastewater Digester Aggregates[J]. mBio, 2011, 2(4):e00159-11.

[20] Kato S, Hashimoto K, Watanabe K. Methanogenesis facilitated by electric syntrophy via (semi)conductive iron-oxide minerals[J]. Environmental Microbiology, 2012, 14(7):0-0.

[21] Liu F, Rotaru A E, Shrestha P M, et al. Promoting direct interspecies electron transfer with activated carbon[J]. Energy & Environmental Science, 2012, 5(10):8982.

[22] Pham T H, Aelterman P, Verstraete W. Bioanode performance in bioelectrochemical systems: recent improvements and prospects[J]. Trends in Biotechnology, 2009, 27(3):168-178.

[23] Watanabe K. Recent Developments in Microbial Fuel Cell Technologies for Sustainable Bioenergy[J]. Journal of Bioscience & Bioengineering, 2008, 106(6):528-536.

[24] Wang G, Li Q, Gao X, et al. Synergetic promotion of syntrophic methane production from anaerobic digestion of complex organic wastes by biochar: performance and associated mechanisms[J]. Bioresource Technology, 2017:S0960852417321132.

[25] Yang Y, Zhang Y, Li Z, et al. Adding granular activated carbon into anaerobic sludge digestion to promote methane production and sludge decomposition[J]. Journal of Cleaner Production, 2017, 149(Complete):1101-1108.

[26] Zhao Z, Zhang Y, Li Y, et al. Potentially shifting from interspecies hydrogen transfer to direct interspecies electron transfer for syntrophic metabolism to resist acidic impact with conductive carbon cloth[J]. Chemical Engineering Journal, 2016:S1385894716317375.

[27] Li L, Tong Z, Fang C, et al. Response of anaerobic granular sludge to single-wall carbon nanotube exposure[J]. Water Research, 2015, 70:1-8.

[28] Lin R, Cheng J, Zhang J, et al. Boosting biomethane yield and production rate with graphene: The potential of direct interspecies electron transfer in anaerobic digestion.[J]. Bioresource Technology, 2017, 239:345-352.

[29] Wang D, Han Y, Han H, Han H, Li K, Xu C, Zhuang H. New insights into enhanced anaerobic degradation of Fischer-Tropsch wastewater with the assistance of magnetite[J]. Bioresource Technology, 2018:S0960852418302815.