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Review

Advances towards understanding and engineering direct interspecies electron transfer in anaerobic digestion

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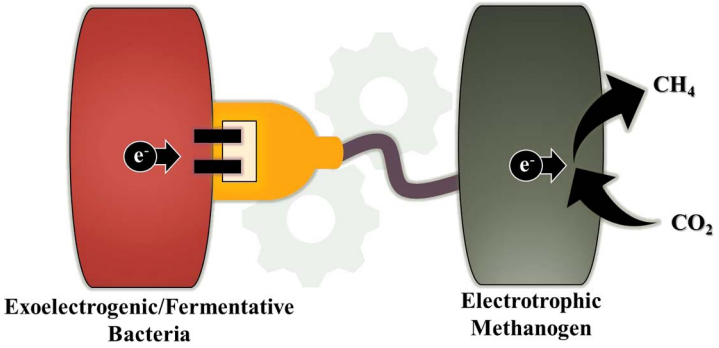
GRAPHICAL ABSTRACT



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ABSTRACT

Direct interspecies electron transfer (DIET) is a recently discovered microbial syntrophy where cell-to-cell electron transfer occurs between syntrophic microbial species. DIET between bacteria and methanogenic archaea in anaerobic digestion can accelerate the syntrophic conversion of various reduced organic compounds to methane. DIET-based syntrophy can naturally occur in some anaerobic digester via conductive pili, however, can be engineered via the addition of various non-biological conductive materials. In recent years, research into understanding and engineering DIET-based syntrophy has emerged with the aim of improving methanogenesis kinetics in anaerobic digestion. This article presents a state-of-art review focusing on the fundamental mechanisms, key microbial players, the role of electrical conductivity, the effectiveness of various conductive additives, the significance of substrate characteristics and organic loading rates in promoting DIET in anaerobic digestion.

1. Introduction

Methanogenesis represents a significant portion of carbon flow in both natural and engineered anaerobic environments. Methanogenesis in engineered systems, such as anaerobic digester and microbial electrolysis cell, is of great importance in sustainable management and bioenergy recovery from organic waste and high strength wastewater. Among these two engineered systems, anaerobic digestion for methane production has already been widely adopted at full-scale for stabilization of various organic waste streams (Carrere et al., 2016; Cavinato et al., 2013). The microbes mediating methane-forming reactions in anaerobic digestion are known as methanogens or methanoarchaea (Thauer, 1998). Methanogens can utilize simple organic substrates, such as acetate, CO_2/H_2, methanol, and formate (De Bok et al., 2004;

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Gujer and Zehnder, 1983; Thauer, 1998). Hence, methanogens build syntrophic associations with other microorganisms for methane production from short-chain volatile fatty acids and alcohols, such as ethanol, propionate, butyrate, etc., produced from biodegradation of complex organic compounds (De Bok et al., 2004; Shin et al., 2010). These reduced organic compounds are usually found to be degraded to acetate and H_2/CO_2 by syntrophic microorganisms (mainly fermentative bacteria), and then consumed by methanogens. Acetoclastic methanogens utilize acetate; H_2 is consumed by H_2-utilizing or hydrogenotrophic methanogens for methane production via CO_2 reduction. Thus, hydrogenotrophic methanogens maintain low H_2 partial pressures that provide a thermodynamically feasible condition for fermentative bacteria to continue fermentation of the reduced organic compounds, such as ethanol, propionate, butyrate, etc. (De Bok et al., 2004; Gujer and Zehnder, 1983; Shin et al., 2010). In some methanogenic environments, formate has also been identified as an electron carrier between methanogens and fermentative bacteria (Boone et al., 1989). For many years, H_2 and formate transfer between methanogens and their syntrophic partners were thought to be the most sustainable mechanisms for interspecies electron transfer between methanogens and fermentative bacteria. Recent discoveries revealed that some bacteria could directly transfer electrons to methanogens instead of interspecies H_2/formate transfer (Morita et al., 2011; Rotaru et al., 2014a,b). This unique cell-to-cell electron transfer mechanism allows the methane production from the reduced organic compounds in a thermodynamically and metabolically more efficient manner (Cheng and Call, 2016), which ultimately provides rapid conversion of organic wastes to methane. This newly discovered mechanism of electron transfer between species has been recognized in the literature as “direct interspecies electron transfer (DIET)” (Morita et al., 2011; Summers et al., 2010; Lovley, 2011a,b; Cheng and Call, 2016; Dubé and Guiot, 2015). Methanogens that can directly accept electrons from other species are called “electrotrophic methanogen” (Lovley, 2011a,b).

Studies have shown that a wide variety of electron donating bacteria and electrotrophic methanogens can build a DIET-based syntrophic partnership, possibly driven by digester operating conditions, such as substrate type, organic loading rate, reactor configuration, and so on (Zhao et al., 2015; Wang et al., 2016; Zhao et al., 2016a; Shrestha et al., 2014; Dang et al., 2017, 2016). As shown in Table 1, various DIET-active electron donating bacteria have been isolated from methanogenic digesters (Chen et al., 2014a,b; Rotaru et al., 2014a,b; Lin et al., 2017; Lee et al., 2016a,b; Zhuang et al., 2015; Jing et al., 2017; Dang et al., 2016, 2017; Lei et al., 2016). Among them, *Geobacter* and *Pseudomonas* species are known as “exoelectrogen” or “electrochemically active bacteria (EAB)” for their ability to produce electricity in microbial electrochemical systems via extracellular electron transfer (EET) (Logan 2009; Chang et al., 2006). In comparison, other species, such as *Sporanaerobacter*, *Bacteroides*, *Streptococcus*, and *Syntrophomonas* are typically known as fermentative bacteria (Dang et al., 2016, 2017; Lei et al., 2016), and their ability to conduct EET is not yet conclusive.

An interspecies electrical connection between species has been found to be critical for DIET. *Geobacter* species can make a biologically wired connection to methanogens by producing filamentous protein appendages called electrically conductive pili or microbial nanowire (Shrestha et al., 2014; Rotaru et al., 2014a,b). However, aggregation of species would be essential for such electrical connection, which may be possible in some specific configuration of anaerobic digesters, such as upflow anaerobic sludge blanket (UASB) reactor. It has now been shown that the addition of non-biological conductive materials, such as granular activated carbon (GAC), biochar, carbon cloth, iron nanoparticles, carbon nanotubes, etc., in methanogenic bioreactors can induce DIET-ability within a wide range of bacteria that cannot produce conductive pili or nanowires like *Geobacter* species (Liu et al., 2012; Chen et al., 2014a,b). Syntrophic partners can attach to the surface of these conductive materials and utilize them as electrical conduits for electron exchange. This approach can be metabolically more favorable since these conductive additives may alleviate the energy investment by microbes for the synthesis of these conductive pili (Zhao et al., 2015). Furthermore, this approach will allow sustainable engineering of DIET-based syntrophy in many configurations of anaerobic digesters.

Several studies have shown that facilitating DIET in anaerobic digestion can significantly enhance methanogenesis kinetics, thus, increases methane production rates (Liu et al., 2012; Dang et al., 2016; Zhao et al., 2015; Chen et al., 2014b; Rotaru et al., 2014a,b). It was also apparent from these studies that a significant portion of electrons from reduced organic compounds (mainly volatile fatty acids and alcohols) can be efficiently recovered as methane via DIET, which may not be possible via interspecies H_2/formate transfer. Mathematical modeling has shown that DIET-based interspecies electron transfer rate can be 8.6 folds higher than that of interspecies H_2 transfer rate (Storck et al., 2016). Therefore, DIET-active digesters can handle relatively higher organic loading rates (OLRs) over conventional digester (Zhao et al., 2015; Dang et al., 2016).

This review article summarizes scientific and engineering advances in promoting DIET-based syntrophy in anaerobic digestion. Particular attention is given to the developments of various non-biological conductive additives for engineering DIET. Furthermore, research gaps are highlighted to suggest directions for future studies.

2. DIET-active microbial communities

The current knowledge of DIET in methanogenic environments has been established based on the discovery of electric syntrophy between exoelectrogenic *Geobacter* species and methanogens (Morita et al., 2011; Rotaru et al., 2014a,b; Shrestha et al., 2014; Liu et al., 2012). An evidence of DIET between bacteria and methanogens was first found in upflow anaerobic sludge blanket (UASB) reactors treating brewery wastewater (Morita et al., 2011). Microbial community composition analysis showed a dominance of *Methanosaeta concilii* and *Geobacter* species in these aggregates. *Methanosaeta* species are known as strictly acetoclastic methanogen (Garcia et al., 2000); *Geobacter* species can degrade simple organic acids and extracellularly exchange electrons with their syntrophic partners in the absence of any conductive solids or insoluble electron acceptors (Summers et al., 2010). To understand the syntrophy between these two species in brewery digester, Morita et al. (2011) incubated the UASB aggregates with different electron donors, such as ethanol, hydrogen, formate, and acetate. The highest methane production rate was observed for ethanol, although acetoclastic methanogens were dominant in the aggregates. Methane production from hydrogen and formate were trivial. This finding suggested that an unknown methanogenesis pathway could be more dominant than methanogenesis via direct acetate utilization or interspecies H_2/formate transfer. Interestingly, the aggregates were electrically more conductive than a DIET-active co-culture aggregates of two *Geobacter* species (7.2 vs. 1.4 μS/cm). Hence, it was hypothesized that *Methanosaeta* species could be the syntrophic partner of *Geobacter* species, and aggregation of these two species in UASB granule might allow direct electron transport via *Geobacter* pili (see Fig. 1a). Thus, this study first provided indirect evidence that DIET could happen in methanogenic digesters.

More direct evidence of DIET in methanogenic communities was found by Rotaru et al. (2014a). In their study, DIET was investigated with defined co-cultures of *Geobacter metallireducens* and *Methanosaeta harundinacea*, isolated from UASB aggregates (Rotaru et al., 2014a). *G. metallireducens* is unable to grow via interspecies H_2/formate transfer (Summers et al., 2010); *Methanosaeta harundinacea* can not utilize H_2/formate (Smith and Ingram-Smith, 2007). Despite these facts, co-cultures of these species successfully produced methane from ethanol with 96% electron recovery. A stoichiometric analysis of methane production suggested that *M. harundinacea* produced methane from both acetate and electrons released from the oxidation of methanol by *G. metallireducens*; 1.5 mol of methane produced from 1 mol of ethanol (see Fig. 2). Metatranscriptomic analysis of co-cultures showed high