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ORIGINAL ARTICLE

Biogeochemistry of methanogenesis with a specific emphasis on the mineral-facilitating effects

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Abstract The Earth surface contains various oxic and anoxic environments. The later include natural wetlands, river and lake sediments, paddy field soils and landfills. In the last few decades, the biogeochemical cycle of carbon in anoxic environments, which leads to the production and emission of methane, a potent greenhouse gas in the atmosphere, has drawn great attentions from both scientific and public sectors. New organisms and mechanisms involved in methanogenesis and carbon cycling have been uncovered. Interspecies electron transfer is considered as a crucial step in methanogenesis in anoxic environments. Electron-carrying mediators, like H_2 and formate, are known to play the key role in electron transfer. Recently, it has been found that in addition to the conventional electron transfer via chemical mediators, direct interspecies electron transfer (DIET) can occur. In this Review, we describe the ecology and biogeochemistry of methanogenesis and highlight the effect of microbe-mineral interaction on microbial syntrophy. Recent advances in the study of DIET may pave the way towards a mechanistic understanding of methanogenesis and the influence of microbe-mineral interaction on this process.

Keywords Syntrophy · Methanogenesis · Direct interspecies electron transfer · Magnetite · Wetland · Paddy fields

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1 Introduction

Anoxic environments are widespread on Earth. Wetlands are poorly drained areas subjecting to permanent or periodic waterlogging. Though occupying about 3% of the terrestrial surface of Earth, wetlands retain significant amounts of CO_2-derived carbon in a recalcitrant form, amounting proximately 30% of the terrestrial reserve of carbon (Drake et al. 2009). Paddy fields occupy about 162 million hectares of the land, that is no more than 10% of the world arable lands but provides the staple food for nearly a half of the world populations (Haefele et al. 2014). Lakes, rivers and streams sculpt the terrestrial surface, forming aquatic networks like ecological arteries flowing through the continental landscape. They are vital resources for all life on this planet.

Plants and algae in anoxic habitats bloom in summer leaving deposit of huge amounts of organic materials. The plant-derived organic polymers are mineralized to CO_2 under oxic conditions. But under anoxic conditions, a complex microbial assemblage consisting of hydrolytic, fermenting, homoacetogenic, syntrophic, and methanogenic microorganisms are involved in the anaerobic decomposition of organic polymers. A series of intermediate byproducts comprising various short-chain fatty acids and alcohols are produced during the fermentation (Rui et al. 2009). Many of these intermediate products are chemically more reduced than the original form of organic polymer carbon. When the oxidants like nitrate, sulfate and ferric iron are present, the products are used as electron donors for anaerobic respirations. But in the absence of these oxidants, methanogenesis occurs producing CH_4 as the final product. Methanogenesis is the key step of biogeochemical C cycling in anoxic environments. An unwanted result of C cycling in these habitats is the emission of methane, a potent greenhouse gas next to CO_2 in the atmosphere (Conrad 2009).

A sophisticated interspecies electron-transfer (IET) network is involved in methanogenesis and H_2 and formate serve as the electron-transferring mediators. Very recently, however, direct interspecies electron transfer (DIET) in anaerobic microbes has been uncovered (Summers et al. 2010; Shrestha et al. 2013). The DIET activity among *Geobacter* species might not be very surprising as these organisms have been known to channel electrons outside cells for anaerobic respiration using insoluble ferric iron as electron acceptor. But it was not without surprising that DIET occurred in methanogenesis (Rotaru et al. 2014), because unlike *Geobacter* spp. methanogens were not known to possess the capability of extracellular respiration. DIET can take two forms. First, DIET is mediated by the biologically generated appendages like pili and molecular complexes like outer-membrane c-type cytochromes. Second, DIET can be mediated by the naturally-occurring or artificially supplemented conductive materials. These materials include magnetite, pyrite, black carbon and manufactured carbon nanotubes (CNTs). In this review, we will report the syntrophic methanogenesis in paddy field soils and lake sediments with a specific emphasis on the stimulation of microbial syntrophy by the electric-conductive mineral magnetite and the artificial nanomaterial CNTs (Li et al. 2015; Zhang and Lu 2016). These studies suggest the occurring of DIET in methanogenesis in the natural anoxic habitats.

2 Methanogenesis in paddy field soil

Root and straw residues are the major source of organic matter in paddy field soils. It was estimated that annual input of organic matter into paddy field ranged from 1700 to 3470 kg ha^{-1}, and over 65% of them were derived from the plant residues (Kimura et al. 2004). Plant polymers are composed of cellulose, hemicellulose and lignin. The rate of decomposition depends on the type of residue, the chemical components, the temperature and other factors. Based on the formation of H_2, fatty acids and the activities of polysaccharolytic enzymes, five stages were proposed for the decomposition of plant residues, i.e. the production and consumption of reducing sugars, the production and consumption of H_2 and fatty acids, and the production of CH_4 (Glissmann and Conrad 2000). Butyrate is identified as an important intermediate product next to acetate and propionate during the anaerobic decomposition of plant residues (Rui et al. 2009). Molecular analysis using the DNA-based stable isotope probing (SIP) showed that the active organisms involved in the syntrophic oxidation of butyrate in paddy soil included *Syntrophomonas* spp. and methanogens *Methanosarcina* and *Methanocella* (Liu et al. 2011). However, the mechanism of syntrophic interaction in butyrate oxidation, and particularly the influence of environmental factors on this process remains poorly understood.

Recently, the effect of iron mineral, specifically magnetite nanoparticles, on the syntrophic oxidation of butyrate in a methanogenic enrichment of rice soil has been investigated using multiple approaches including molecular, isotopic and chemical analyses (Li et al. 2015). The soil used represents the typical paddy field in southern China where rice has been cultivated for thousands of years. Though the use of enrichment can be viewed as a serious compromise to in situ conditions, such treatment does not affect the capacity of the soil to produce CH_4 from syntrophic oxidation of butyrate.

The addition of magnetite nanoparticles (nanoFe_3O_4) significantly accelerated syntrophic production of CH_4 from butyrate oxidation and this stimulating effect retained throughout continuous transfers of the enrichment. Furthermore, the stimulatory effect increased with the increase of nanoFe_3O_4 concentration and the effect retained when nanoFe_3O_4 was replaced by graphite nanoparticles (Fig. 1). However, silica coating of the nanoFe_3O_4 particles, which was believed to insulate the capacity of electric conductivity, dismissed the effect. The analysis of microbial community by DNA-based stable isotope probing revealed that the bacterial lineages *Syntrophomonadaceae* and *Geobacteraceae*, and the archaeal lineages *Methanosarcinaceae*, *Methanocellales* and *Methanobacteriales* were involved in the syntrophic oxidation of butyrate and production of CH_4. Among them, the growth of *Geobacter* spp. strictly relied on the presence of nanoFe_3O_4 and its electrical conductivity in particular. Other organisms except *Methanobacteriales* were present in enrichments regardless of nanoFe_3O_4 amendment. Scanning electron micrography showed that the nanoparticles attached on microbial cell surfaces and cells of different morphologies were associated together and connected via the nanoFe_3O_4 particles.

It is noteworthy that *Geobacter* spp. were present in this enrichment and their activity was closely linked to the presence of nanoFe_3O_4 that stimulated the methanogenic syntrophy. *Geobacter* spp. are known as iron-respiring bacteria and they are distributed widespread in anoxic habitats (Lovley and Phillips 1986). But these organisms are not known for syntrophic oxidation of butyrate. However, it has been documented recently that *Geobacter* spp. perform DIET either directly (Summers et al. 2010; Morita et al. 2011; Shrestha et al. 2013; Rotaru et al. 2014) or using conductive materials resulting in cooperative metabolism (Kato et al. 2012a; Liu et al. 2012). An earlier study indicated that *Geobacter* spp. were involved in syntrophic

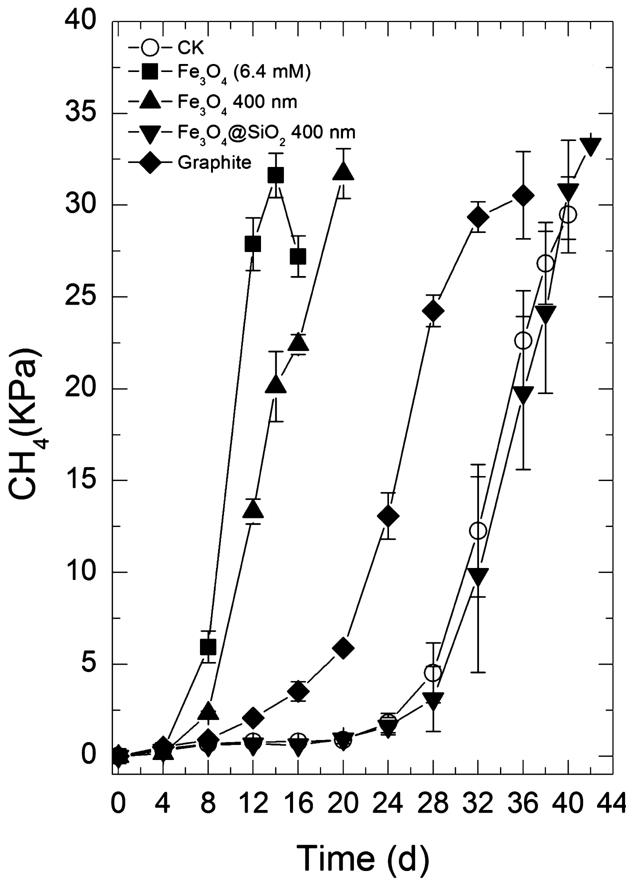

Fig. 1 The production of CH_4 in paddy soil enrichment was significantly accelerated in the presence of nanoFe_3O_4 either of 30 or 400 nm size. The stimulating effect was evident when nanoFe_3O_4 was replaced by graphite, but was completely dismissed if nanoFe_3O_4 was coated with silica (Fe_3O_4@SiO_2) that insulated its electric conductivity (Li et al. 2015)

production of CH_4 from ethanol and acetate in paddy soil in the presence of conductive iron minerals (Kato et al. 2012b). In line with these findings, it was probable that *Geobacter* spp. played the important role in the syntrophic oxidation of butyrate and production of CH_4 via DIET activity that was facilitated by conductive iron minerals.

3 Methanogenesis in lake sediment

It has been demonstrated that the biogeochemistry and function of lakes are greatly influenced by anthropogenic activities. A recent study compared the activity of methanogenesis in sediment between an urban pond and a natural lake with the attempt to assess the long-term anthropogenic effect (Zhang and Lu 2016). The effect of nanoFe_3O_4 and carbon nanotubes on the methanogenic syntrophy was also evaluated. Enrichment cultivation was conducted to simplify the microbial ecosystem and prevent from the interference of the complicated biological and environmental interactions.

The molecular analysis of microbial community revealed that the microbial composition was still complicated after continuous cultivation and was more diverse in the enrichment of natural lake sediment than the urban pond. The bacterial and archaeal populations in the urban pond enrichment consisted mainly of the bacterial *Syntrophomonas* and *Desulfovibrio* and the archaeal *Methanosarcina* and *Methanomicrobia*. Those of natural lake enrichment comprised the bacterial lineages of *Syntrophomonas*, *Sulfurospirillum*, *Paenibacillus*, and the archaeal lineages of *Methanoregula*, *Methanosarcina*, *Methanospirillum*, *Methanosaeta*, respectively. Albeit the difference in composition and complexity of microbial communities, the rate of CH_4 production did not show significant difference between two lake enrichments (Fig. 2), indicating that under enrichment conditions the microbial guilds of different origins could function similarly.

In consistence with the observation in paddy field soil enrichment, the addition of nanoFe_3O_4 substantially facilitated the syntrophic production of CH_4 from butyrate oxidation in the lake sediment enrichments. Interestingly, the addition of CNTs, a chemically-stable artificial nanomaterial with high electric conductivity, displayed a similar stimulatory effect. This study suggested that the electric conductivity of the added nanoparticles played the key role in facilitating the syntrophic oxidation of butyrate.

Microbial aggregation was evident in the sediment enrichments (Fig. 3). It has been suggested that the formation of microbial aggregates increases the efficiency of interspecies H_2/formate transfer due to the reduction of cell-to-cell distance (Stams and Plugge 2009). However, the spatial architectures of microbial aggregation were substantially interrupted in the presence of nanoFe_3O_4 or CNTs nanoparticles (Figs. 3, 4). The consensus of FISH images showed that in the absence of nanomaterials the bacterial and archaeal cells were packaged closely with the bacterial cells forming the dense core and archaeal cells allocating peripherally. But in the presence of nanoFe_3O_4 or CNTs, the bacterial and archaeal cells were more separated. Resultantly, the microbial aggregates displayed a larger cell-to-cell distance on the average in the presence than in the absence of nanoparticles. If the intercellular distance were the key factor determining the syntrophic efficiency, a lower rate of CH_4 production would have been expected in the presence of nanoparticles. The discrepancy between the rate of CH_4 production (Fig. 2) and the microbial aggregate configuration (Figs. 3, 4) suggest that mechanisms other than the interspecies H_2 transfer must play a role.

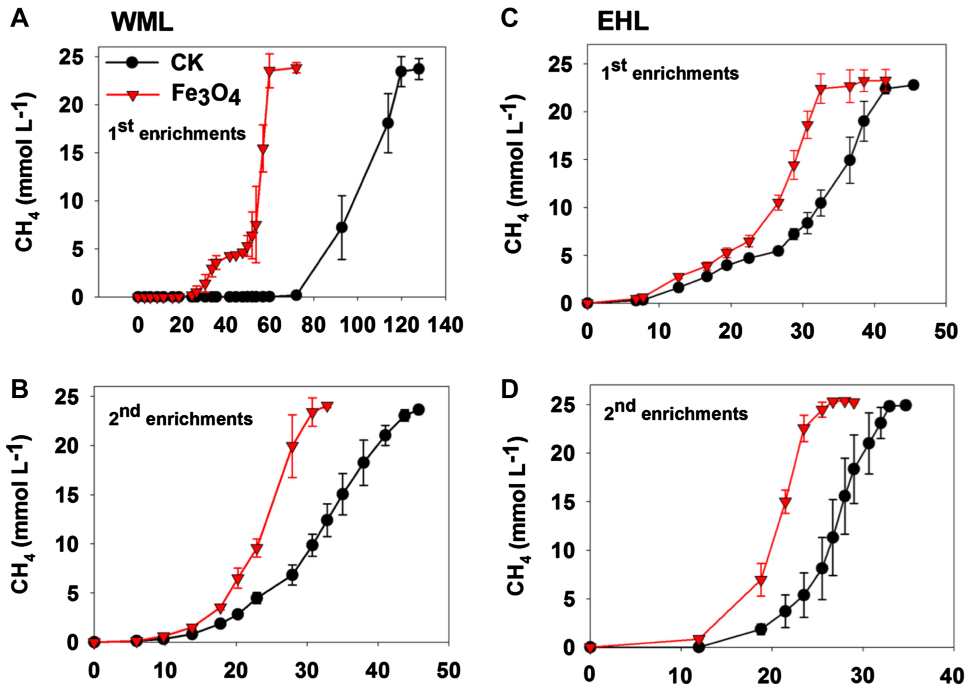

Fig. 2 The production of CH_4 in sediment enrichments of an urban pond (**a**, **b**) and a natural lake (**c**, **d**) was significantly accelerated in the presence of nanoFe_3O_4 (Zhang and Lu 2016)

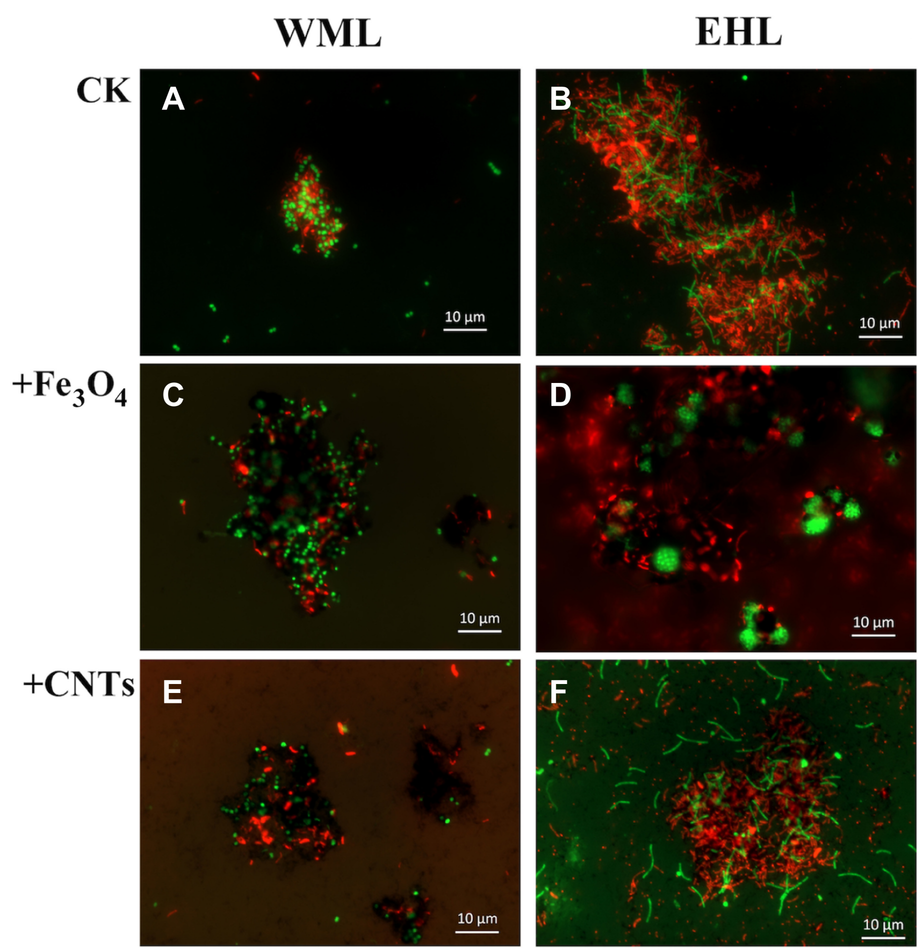

Fig. 3 The FISH images showed the formation of microbial aggregates in the enrichments of an urban pond (WML) and a natural lake (EHL). The bacterial (in *red*) and archaeal (in *green*) cells were closely packed in the absence of nanomaterials (**a**, **b**; CK) whereas the architectures of microbial aggregates were interrupted in the presence of nanoFe_3O_4 (**c**, **d**) or CNTs (**e**, **f**). The *dark* areas in **c**, **d**, **e**, **f** indicated the nanomaterials of nanoFe_3O_4 and CNTs, respectively (Zhang and Lu 2016)

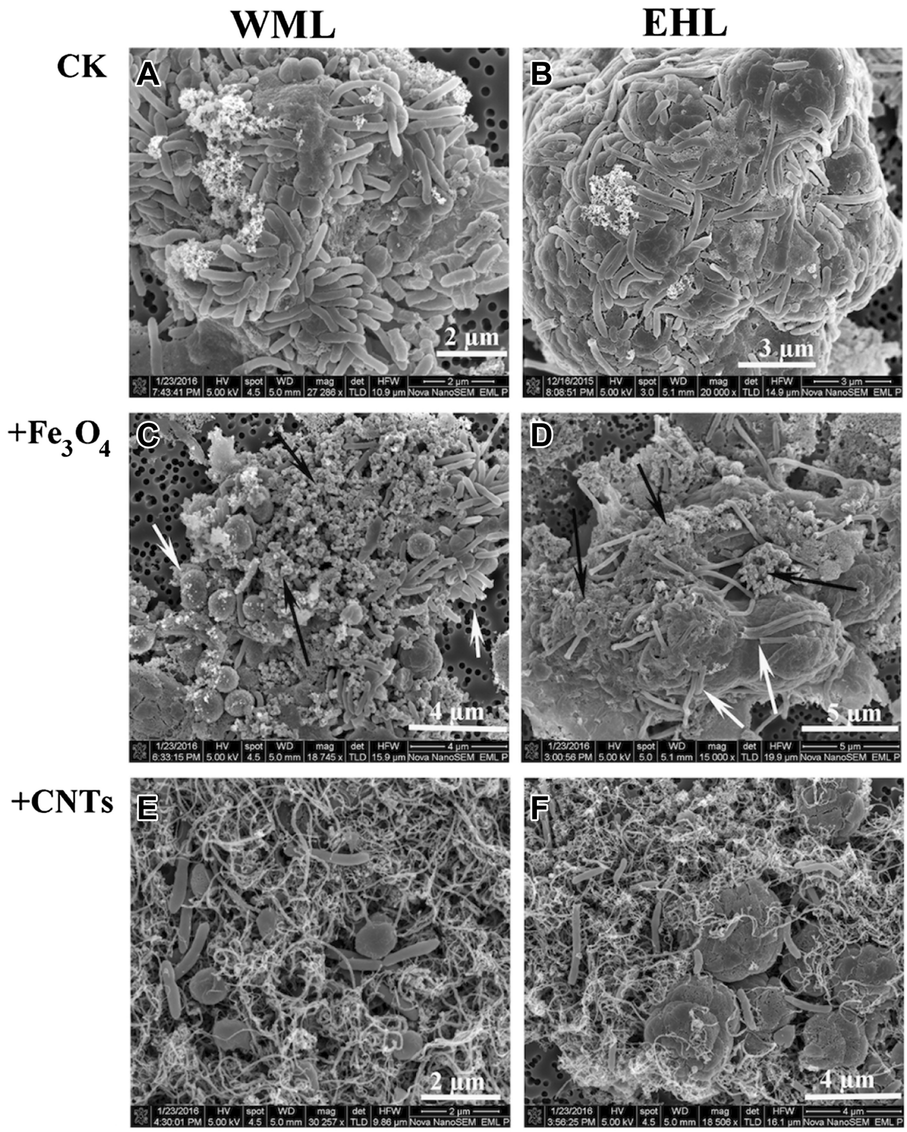

Fig. 4 The distinct architectures of microbial aggregation were more evident in scanning electron micrographs. **a**, **b** showed that the cells were densely packaged in the absence of nanomaterials (CK) while microbial aggregations were interrupted to a certain extent due to the presence of nanoFe_3O_4 (**c**, **d**) and CNTs (**e**, **f**) (Zhang and Lu 2016)

Considering that nanoFe_3O_4 and CNTs caused similar stimulating effects while except the common property in electric conductivity, two materials differ greatly in physico-chemical properties, it was plausible to assume that the electric conductivity of nanomaterials supported the syntrophic oxidation of butyrate. Moreover, both FISH and scanning electron microscopy (Fig. 4) indicated the formation of cell-nanomaterial-cell networking that could provide the physical basis for DIET activity. The discrepancy between cell-to-cell distance and the syntrophic activity has also been used to explain the involvement of DIET in the anaerobic oxidation of CH_4 in ocean sediments (McGlynn et al. 2015). Theoretical calculation suggested that the electron transfer rate among the syntrophic partners via direct electric conduction was substantially higher (10^6 times) than via interspecies H_2 diffusion (Viggi et al. 2014). Thus, it might be hypothesized that albeit the absence of *Geobacter* spp. in the lake sediment enrichments the syntrophic methanogenesis was facilitated by activating DIET in the presence of conductive nanomaterials. Nanomaterials could be either naturally occurring or artificially supplemented.

4 Conclusive remarks

Methanogenesis is the core machinery for biogeochemical C cycling in anoxic habitats. The influence of environmental factors on this process remains largely unexplored. Here we discussed the possible involvement of DIET in methanogenesis and the facilitation of this activity by electric-conductive mineral and artificial nanomaterials.

DIET has been uncovered very recently as an important mechanism for microbial interactions in environment.

Based on the origin of mediators, DIET can be categorized into two forms, DIET of biological origin, which employs biological structure or molecules for electron transfer, and DIET of environmental origin, which utilizes electric-conductive materials in environment. The DIET of biological origin has been a focus of recent studies, especially using the defined co-cultures with *Geobacter* spp. (Lovley 2012; Kouzuma et al. 2015). However, DIET of environment origin has been less investigated. DIET of environment origin shall confer an ecological advantage to microbes due to the reduction in cost for biosynthesis of molecular conduits (Kato et al. 2012a; Li et al. 2015). Furthermore, biological mediators are probably not ubiquitous among microbes, while the conductive-semiconductive minerals are widespread in nature. Additional studies shall shed light on the significance of DIET of environment origin in methanogenesis and C cycling in various environments.

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