Bioresource Technology 173 (2014) 82–86

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Carbon cloth stimulates direct interspecies electron transfer in syntrophic co-cultures

Shanshan Chen [a], Amelia-Elena Rotaru [a,b,*], Fanghua Liu [a,c], Jo Philips [a], Trevor L. Woodard [a], Kelly P. Nevin [a], Derek R. Lovley [a]

[a] Department of Microbiology, University of Massachusetts, Amherst, MA 01003, USA
[b] Nordic Center for Earth Evolution, University of Southern Denmark, Odense S DK-5230, Denmark
[c] Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China

HIGHLIGHTS

- Conductive carbon cloth stimulated syntrophic metabolism in DIET co-cultures.
- Carbon cloth did not stimulate metabolism in a co-culture that relied on H_2 transfer.
- Non-conductive cotton cloth did not facilitate DIET.
- Carbon cloth restored DIET in *Geobacter* strains missing pili or OmcS cytochrome.

ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form 2 September 2014
Accepted 4 September 2014
Available online 22 September 2014

Keyword:
Carbon cloth
Syntrophy
Direct interspecies electron transfer
Geobacter
Methanosarcina

ABSTRACT

This study investigated the possibility that the electrical conductivity of carbon cloth accelerates direct interspecies electron transfer (DIET) in co-cultures. Carbon cloth accelerated metabolism of DIET co-cultures (*Geobacter metallireducens–Geobacter sulfurreducens* and *G. metallireducens–Methanosarcina barkeri*) but did not promote metabolism of co-cultures performing interspecies H_2 transfer (*Desulfovibrio vulgaris–G. sulfurreducens*). On the other hand, DIET co-cultures were not stimulated by poorly conductive cotton cloth. Mutant strains lacking electrically conductive pili, or pili-associated cytochromes participated in DIET only in the presence of carbon cloth. In co-cultures promoted by carbon cloth, cells were primarily associated with the cloth although the syntrophic partners were too far apart for cell-to-cell biological electrical connections to be feasible. Carbon cloth seemingly mediated interspecies electron transfer between the distant syntrophic partners. These results suggest that the ability of carbon cloth to accelerate DIET should be considered in anaerobic digester designs that incorporate carbon cloth.

1. Introduction

Materials that have the potential to support biofilm growth can enhance anaerobic digestion of organic wastes to methane (Adu-Gyamfi et al., 2012). One of the materials that has shown promise is carbon cloth (Sasaki et al., 2007, 2009, 2010; Tatara et al., 2008; Zhang et al., 2012; Zhao et al., 2013). In these studies the enhanced methane production in the presence of carbon cloth was attributed to its ability to promote microbial attachment. However, another possibility is that the conductive properties of carbon cloth will impact electron exchange between microorganisms similar to other conductive materials which were used to mediate electron transfer between cells and electrodes or other cells (Chen et al., 2014; Cruz Viggi et al., 2014; Kato et al., 2012; Liu et al., 2012, 2014; Rotaru et al., 2014a). It has been recently discovered that some methanogens can receive electrons from an electron-generating microorganism either directly – using molecular electric connections (Chen et al., 2014; Liu et al., 2012; Rotaru et al., 2014a,b), or indirectly – using conductive minerals (Chen et al., 2014; Kato et al., 2012; Liu et al., 2012, 2014).

DIET is an alternative to interspecies H_2/formate transfer for syntrophic electron exchange between microbial species (Rotaru et al., 2014a,b; Summers et al., 2010). DIET was initially described in co-cultures of *Geobacter metallireducens* and *Geobacter sulfurreducens* growing in medium in which ethanol was the electron donor and fumarate was the electron acceptor (Summers et al., 2010). *G. metallireducens* can metabolize ethanol, but cannot use fumarate as an electron acceptor (Lovley et al., 1993), whereas

* Corresponding author at: Nordic Center for Earth Evolution, University of Southern Denmark, Odense S DK-5230, Denmark.
E-mail address: arotaru@biology.sdu.dk (A.-E. Rotaru).

http://dx.doi.org/10.1016/j.biortech.2014.09.009
0960-8524/

G. sulfurreducens cannot metabolize ethanol, but can respire fumarate, which is then reduced to succinate (Caccavo et al., 1994). The co-culture adapted to metabolize ethanol with the reduction of fumarate (Summers et al., 2010). Multiple lines of evidence (Rotaru et al., 2012; Shrestha et al., 2013a,b; Summers et al., 2010) suggested that the electron transfer between the species was via the *Geobacter* pili that have metallic-like conductivity (Malvankar et al., 2011; Reguera et al., 2005). The possibility of interspecies H_2/formate transfer was ruled out by the fact that *G. metallireducens* is unable to metabolize ethanol with the production of H_2 or formate (Rotaru et al., 2012; Shrestha et al., 2013a,b), and the fact that interspecies electron exchange remained effective when the co-cultures were initiated with a *G. sulfurreducens* strain incapable of H_2 and formate uptake, because the genes encoding formate dehydrogenase and an uptake hydrogenase were deleted (Rotaru et al., 2012).

Methanosaeta and *Methanosarcina* species, which are often abundant in anaerobic digesters (Angenent et al., 2004; De Vrieze et al., 2012; McMahon et al., 2004; Morita et al., 2011; Steinhaus et al., 2007), are also capable of receiving electrons via DIET (Rotaru et al., 2014a,b). *Methanosaeta harundinacea* or *Methanosarcina barkeri* grew in defined co-cultures with ethanol-metabolizing *G. metallireducens* (Rotaru et al., 2014a,b), but only with strains of *G. metallireducens* that could produce pili which are electrically conductive (Malvankar et al., 2011; Reguera et al., 2005). Metatranscriptomic analysis, as well as an assessment of metabolic potential and granule conductivity suggested that *Methanosaeta* species in a digester treating simulated brewery wastes also reduced carbon dioxide to methane with electrons derived from DIET (Morita et al., 2011; Rotaru et al., 2014b).

Although the biological electrical connections necessary for DIET are sufficient for effective syntrophic metabolism, studies with granular activated carbon (GAC), biochar, or nano-magnetite minerals demonstrated that DIET could be promoted via the conductive materials (Chen et al., 2014; Liu et al., 2012, 2014). For example, amending *G. metallireducens–G. sulfurreducens* or *G. metallireducens–M. barkeri* co-cultures with GAC greatly accelerated the initial rate of interspecies electron exchange (Liu et al., 2012). In the presence of GAC, digester granules in which *Methanosaeta* species were the predominant methanogens produced methane 2.5-fold faster than in GAC-free controls (Liu et al., 2012). GAC is 3000-fold more conductive than the *Geobacter* pili and in the presence of GAC even pili-deficient strains can participate in DIET (Liu et al., 2012; Rotaru et al., 2014a). Electron-donating and accepting cells attached onto GAC, which served as a conduit for electron transfer between species.

This study aimed to reveal if carbon cloth, often used in rector design, presumably because of its biomass retention properties (Sasaki et al., 2007, 2009, 2010; Tatara et al., 2008; Zhang et al., 2012; Zhao et al., 2013) would rather serve as an electrical conduit to promote DIET. As control we tested non-conductive cotton cloth with similar biomass retention properties. Additionally, we examined if the conductivity of carbon cloth affected interspecies H_2 transfer. Learning about the impact of carbon cloth on electron transfer mechanism will assist future reactor designs and improve methane production during anaerobic digestion.

2. Methods

2.1. Microorganisms, media and growth conditions

All pure cultures and co-cultures were incubated in 27 mL pressure tubes with 10 mL medium under anoxic conditions with a gas phase of 80:20 of N_2:CO_2. *G. sulfurreducens* strain DL1 (ATCC 51573) and various mutant strains were transferred routinely on NBF medium with 10 mM acetate as the electron donor and 40 mM fumarate as the electron acceptor (Coppi et al., 2004). *G. metallireducens* strain GS-15 (ATCC 53774) and mutant strains were transferred routinely on FC medium with 10 mM ethanol as electron donor and 55 mM ferric citrate as the electron acceptor (Lovley et al., 1993). Co-cultures of *G. sulfurreducens* and *G. metallireducens* were initiated with a 5% inoculum of each microorganism into NBF medium which contained 10 mM ethanol as the electron donor and 40 mM fumarate as the electron acceptor. To determine whether pure cultures of *G. sulfurreducens* and *G. metallireducens* could grown in the same medium, each strain was also inoculated separately into NBF medium with 10 mM ethanol as the electron donor and 40 mM fumarate as the electron. The incubation temperature for the *Geobacter* co-culture studies was 30 °C.

To prepare co-cultures of *Desulfovibrio vulgaris* and *G. sulfurreducens* we inoculated NBF medium with 10 mM ethanol as electron donor with 5% of each strain and incubated at 30 °C. Prior to incubations, *D. vulgaris* was grown routinely in NB medium with 20 mM sulfate and 10 mM ethanol.

To prepare co-cultures of *G. metallireducens* and *M. barkeri*, *M. barkeri* type strain DSM 800 (ATCC 43569) was grown in a modified DSMZ methanogenic medium DSMZ medium 120 with 20 or 30 mM acetate as substrate as previously described (Rotaru et al., 2014a). The medium modifications were adopted to improve growth of *G. metallireducens* on this medium as well. Co-cultures of *G. metallireducens* and *M. barkeri* were initiated with 5% inoculum of each microorganism in the modified DSMZ methanogenic medium 120 with 10 mM ethanol as sole electron donor (Rotaru et al., 2014a). To determine whether the strains were capable of utilizing ethanol alone, *G. metallireducens* and *M. barkeri* were inoculated separately into the same medium. The incubation temperature for all studies with *M. barkeri* was 37 °C.

2.2. Culturing with carbon cloth

Carbon cloth named (Zoroflex; buyactivatedcharcoal.com) was cut into strips of 1.5 × 4 cm or 1.5 × 2 cm in order to provide 0.2 or 0.1 g per tube in 10 mL of medium, respectively. The carbon cloth strips were wet-sterilized by autoclaving in pressure tubes in 2 mL of the same NBF culture medium, under a N_2:CO_2 atmosphere for 30 min. Then 7.5 mL of NBF medium (for *Geobacter* cocultures) or 7.5 mL of modified 120 media (for *Geobacter–Methanosarcina* cocultures) were added to the cloth-containing tubes under anaerobic conditions along with 10 mM final concentration ethanol. Cotton cloth, which served as a non-conductive control, was treated in a similar manner. The conductivities of carbon cloth and cotton cloth were measured with a voltmeter by connecting the negative and positive probes to the diagonal ends of 1.5 × 2 cm cloth sheets. Co-cultures were initiated and incubated as described in the previous section.

2.3. Analytical techniques

Liquid and headspace samples were withdrawn with hypodermic needles and syringes under strict anaerobic conditions. Liquid samples were passed through 0.2 μm Acrodisc filters. Concentrations of volatile fatty acids (butyrate, propionate, succinate, malate, fumarate, acetate, formate) were analyzed with high performance liquid chromatography, ethanol and methane were analyzed with gas chromatography as previously described (Rotaru et al., 2014a).

To quantify protein in the planktonic phase, 0.5 mL of the culture medium was removed from early stationary cultures. To quantify the proteins attached to carbon cloth the entire carbon cloth was separated from the liquid with a tweezer and cell protein was extracted from the cloth using NaOH 0.5 mM as previously described (Liu et al., 2012) and additional bead beating with sterile