Water Research 102 (2016) 475–484

Contents lists available at ScienceDirect

Water Research

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

# Communities stimulated with ethanol to perform direct interspecies electron transfer for syntrophic metabolism of propionate and butyrate

Zhiqiang Zhao [a], Yaobin Zhang [a, *], Qilin Yu [a], Yan Dang [b, c], Yang Li [a], Xie Quan [a]

[a] Key Laboratory of Industrial Ecology and Environmental Engineering (Dalian University of Technology), Ministry of Education, School of Environmental Science and Technology, Dalian University of Technology, Dalian, 116024, China
[b] Beijing Key Lab for Source Control Technology of Water Pollution, Beijing Forestry University, 35 Tsinghua East Road, Beijing, 100083, China
[c] Department of Microbiology, University of Massachusetts, Amherst, MA, 01003-9298, USA

ARTICLE INFO

*Article history:*
Received 7 March 2016
Received in revised form
18 June 2016
Accepted 3 July 2016
Available online 5 July 2016

*Keywords:*
Direct interspecies electron transfer (DIET)
Syntrophic metabolism of propionate and/or butyrate
Up-flow anaerobic sludge blanket (UASB)
*Geobacter* species

ABSTRACT

Direct interspecies electron transfer (DIET) has been considered as an alternative to interspecies $H_2$ transfer (IHT) for syntrophic metabolism, but the microorganisms capable of metabolizing the key intermediates, such as propionate and butyrate, via DIET have yet to be described. A strategy of culturing the enrichments with ethanol as a DIET substrate to stimulate the communities for the syntrophic metabolism of propionate and/or butyrate was proposed in this study. The results showed that the syntrophic propionate and/or butyrate degradation was significantly improved in the ethanol-stimulated reactor when propionate/butyrate was the sole carbon source. The conductivity of the ethanol-stimulated enrichments was as 5 folds (for propionate)/76 folds (for butyrate) as that of the traditional enrichments (never ethanol fed). Microbial community analysis revealed that *Geobacter* species known to proceed DIET were only detected in the ethanol-stimulated enrichments. Together with the significant increase of *Methanosaeta* and *Methanosarcina* species in these enrichments, the potential DIET between *Geobacter* and *Methanosaeta* or *Methanosarcina* species might be established to improve the syntrophic propionate and/or butyrate degradation. Further experiments demonstrated that granular activated carbon (GAC) could improve the syntrophic metabolism of propionate and/or butyrate of the ethanol-stimulated enrichments, while almost no effects on the traditional enrichments. Also, the high $H_2$ partial pressure could inhibit the syntrophic propionate and/or butyrate degradation of the traditional enrichments, but its effect on that of the ethanol-stimulated enrichments was negligible.



## 1. Introduction

Anaerobic digestion is a cost-effective strategy to treat the industrial wastewater with high-concentration organic matter (Appels et al., 2011; Holm-Nielsen et al., 2009). An important factor limiting the efficiency of anaerobic digestion is the relatively slow syntrophic metabolism of the key fermentative intermediates, such as propionate and butyrate (Stams and Plugge, 2009; Müller et al., 2010). During the past 40 years, the predominant working mode for interspecies electron exchange during syntrophic metabolism of propionate and/or butyrate was via interspecies $H_2$ transfer (IHT) (Stams and Plugge, 2009; Sieber et al., 2012). During IHT, $H_2$ serves as electron carrier between the substrate-oxidizing syntrophs and $H_2$-utilizing methanogens (Dong and Stams, 1995; McInerney et al., 2009; de Bok et al., 2004; de Bok et al., 2002). Formate may also act as an electron carrier for interspecies formate transfer (IFT) (Dong and Stams, 1995; Thiele and Zeikus, 1988). These reducing equivalents ($H_2$ and/or formate) should be efficiently consumed by methanogens in order for syntrophic partners to grow. This is particularly important for the syntrophic oxidation of substrate, since this process is endergonic under standard conditions and thermodynamically feasible only when the $H_2$ partial pressure (or formate concentration) is kept low (Stams and Plugge, 2009).

An alternative to IHT for interspecies electron exchange during syntrophic metabolism is direct interspecies electron transfer (DIET) which required the electrically conductive pili (Rotaru et al., 2014a) and outer surface *c*-type cytochromes (Summers et al.,

* Corresponding author.
*E-mail address:* zhangyb@dlut.edu.cn (Y. Zhang).

http://dx.doi.org/10.1016/j.watres.2016.07.005
0043-1354/

2010) as the biological electrical connections. Up to now, DIET with ethanol as electron donor has been documented in defined co-cultures of two *Geobacter* species (Summers et al., 2010) as well as in defined methanogenic co-cultures of *Geobacter metallireducens* with *Methanosaeta* (Rotaru et al., 2014a) or *Methanosarcina* (Rotaru et al., 2014b) species. Further study (Kato et al., 2012) indicated that DIET with acetate as electron donor coupled with nitrate reduction could be proceeded in defined co-cultures of *Geobacter sulfurreducens* to *Thiobacillus denitrificans* in the presence of magnetite. However, it is unknown if the syntrophic metabolism of propionate and/or butyrate can proceed via DIET.

Recent studies reported that the syntrophic propionate and/or butyrate degradation could be improved by the addition of magnetite to the sludge taken from the methanogenic digesters (Cruz Viggi et al., 2014) and paddy soil (Li et al., 2015a, b). These studies suggested that mineral-mediated DIET was taking place in these incubations. However, the evidence was insufficient. For example, in the study by Li et al., 2015a, b, the presence of *Geobacter* species enriched with magnetite was not the necessary evidence for DIET, because not all *Geobacter* species were capable of DIET (Rotaru et al., 2015). Furthermore, the abundant *Syntrophomonadaceae* reported in Li's study (Li et al., 2015a, b), are usually involved in metabolizing butyrate to acetate via IHT rather than DIET. Nevertheless, these reports brought the enlightenment that microbial diversity in mixed cultures posed a great possibility that more microorganisms might participate in DIET. Therefore, the enrichment strategy of microorganisms capable of metabolizing propionate and/or butyrate via DIET deserves to be explored.

Multiple lines of evidence suggested that DIET was the primary mechanism for interspecies electron exchange in the aggregates of up-flow anaerobic sludge blanket (UASB) reactor treating brewery wastes (Morita et al., 2011; Shrestha et al., 2014). This may be because ethanol as a predominant substrate involved in brewery wastes can sustain the growth of *Geobacter* species which are capable of DIET. Besides, the long solid retention in the UASB reactor may makes the energetic investment required for producing biological electrical connection favorable. Based on these considerations, in this study we investigated if the syntrophic propionate and/or butyrate degradation could be enhanced by stimulating the microbial communities with ethanol as the specific substrate to perform DIET.

## 2. Materials and methods

### 2.1. Reactor design

Culture experiments were conducted in four UASB reactors (internal diameter of 70 mm and height of 300 mm) with the working volume of 1000 mL. At the top of the reactor, a 2 L gas sampling bag was connected with the gas outlet of the three-phase separator as description in our previous study (Zhao et al., 2015b). All the feedings were deoxygenated by $N_2$ for 1 h before use and pumped into the reactors with a peristaltic pump (ISMATEC). All the reactors were operated at 37.0 °C.

### 2.2. Seed sludge and feedings

Seed sludge obtained from a wastewater treatment plant based on the anaerobic activated sludge process in Dalian, China. The ratio of volatile suspended sludge to total suspended sludge (VSS/TSS) was 0.72 with the initial TSS of 17,100 mg/L. It was stored at 4 °C prior to use. Each of the UASB reactors received a 500 ml sludge inoculum before the experiments.

The first two of the four UASB reactors were fed with the wastewater mainly composed of ethanol and propionate/butyrate (ethanol-stimulated reactors). The initial composition of this feeding (per liter) was as follows (Zhao et al., 2015b): ethanol, 2.45 mL; sodium propionate/butyrate, 0.87 g/0.69 g (ethanol: propionate or butyrate = 4:1, as the ratio of chemical oxygen demand [COD]); $KH_2PO_4$, 0.11 g; $K_2HPO_4$, 0.17 g; $Na_2SO_4$, 0.05 g; $MgCl_2 \cdot 6H_2O$, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.05 g; trace element solution, 10 mL; vitamin solution, 10 mL. The detailed composition of trace element solution and vitamin solution was same as our previous study (Zhao et al., 2015b). After 10 days experiments, the ethanol content in these feedings gradually decreased from 80% to 60%, 40%, 20% and 0% (propionate/butyrate as the sole carbon source) (**ethanol-stimulated mode**) (see Fig. 1). Each feeding was fed for 10 days experiments, and this stage was totally lasted for 50 days. The COD concentration of these feedings was always kept at 5031.0 mg/L. The hydraulic retention time (HRT) of the two UASB reactors was 9.6 h.

The other two of the four UASB reactors used as the controls were fed with the wastewater only composed of propionate/butyrate with a COD of 5031.0 mg/L (control reactors) (**traditional mode**). Except for no addition of ethanol, the other composition of these feedings was same as the above-mentioned feedings (see Fig. 1). These feedings were continuously fed to the two UASB reactors for 50 days experiments. The HRT of the two UASB reactors was also 9.6 h.

After 50 days culture experiments the volume of the suspended sludge in the four UASB reactors was roughly the same (about 450 mL) and the volatile suspended solid (VSS) of the suspended sludge was measured (Fig. S1). In the study with butyrate, the VSS of the ethanol-stimulated and traditional enrichments was 8740 ± 332 mg/L (mean ± standard deviation, n = 3) and 9120 ± 455 mg/L, respectively. There was no significant difference ($P > 0.05$) in the VSS of these two enrichments. In the study with propionate, the VSS of the ethanol-stimulated and traditional enrichments was 7643 ± 256 mg/L and 7232 ± 331 mg/L, respectively. There was also no significant difference ($P > 0.05$) in the VSS of these two enrichments.

After 50 days culture experiments the HRT of the four UASB reactors gradually decreased from 9.62 to 4.56 h (corresponding to organic loading rate [OLR] increased from 12.55 to 26.48 KgCOD/$m^3$d) from day 51–100 as described in Fig. 1.

### 2.3. Batch experiments with GAC

To assess the effects of granular active carbon (GAC, 8–20 mesh,

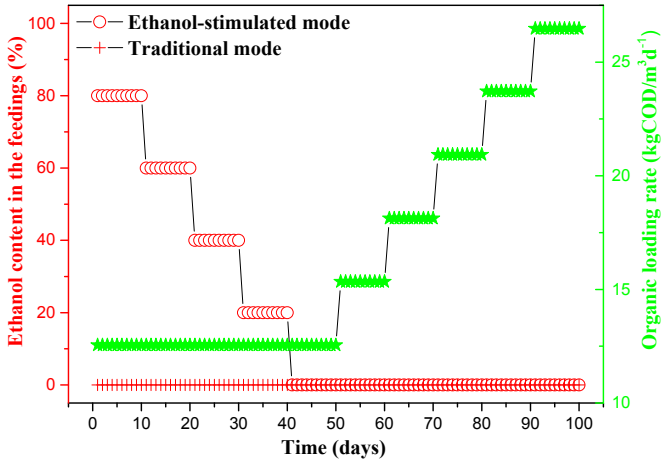


**Fig. 1.** Ethanol content in the ethanol-stimulated and traditional feedings (propionate and/or butyrate) and change of organic loading rate (OLR) in the four UASB reactors throughout the whole experiments.