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Response of anaerobes to methyl fluoride, 2-bromoethanesulfonate and hydrogen during acetate degradation

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Abstract

To use the selective inhibition method for quantitative analysis of acetate metabolism in methanogenic systems, the responses of microbial communities and metabolic activities, which were involved in anaerobic degradation of acetate, to the addition of methyl fluoride (CH_3F), 2-bromoethanesulfonate (BES) and hydrogen were investigated in a thermophilic batch experiment. Both the methanogenic inhibitors, i.e., CH_3F and BES, showed their effectiveness on inhibiting CH_4 production, whereas acetate metabolism other than acetoclastic methanogenesis was stimulated by BES, as reflected by the fluctuated acetate concentration. Syntrophic acetate oxidation was thermodynamically blocked by hydrogen (H_2), while H_2-utilizing reactions as hydrogenotrophic methanogenesis and homoacetogenesis were correspondingly promoted. Results of PCR-DGGE fingerprinting showed that, CH_3F did not influence the microbial populations significantly. However, the BES and hydrogen notably altered the bacterial community structures and increased the diversity. BES gradually changed the methanogenic community structure by affecting the existence of different populations to different levels, whilst H_2 greatly changed the abundance of different methanogenic populations, and induced growth of new species.

Key words: methanogenic inhibitor; hydrogen; thermophilic anaerobic digestion; microbial diversity; denaturing gradient gel electrophoresis (DGGE)

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Introduction

Anaerobic digestion of biomass for methane production is attracting increasing attention owing to its potential as a substitute for fossil fuels and to reduce carbon dioxide emissions. A better understanding of the mechanisms responsible for methane production will help to improve operational strategies and to solve the existing technical problems such as the accumulation of volatile fatty acids (VFAs) and the following inhibition of methanogenesis. Externally added selective inhibitors, therefore, present potentially powerful tools in the study of microbial processes.

In general, there are specific and nonspecific inhibitors. For example, chloroform ($CHCl_3$), which was firstly found to inhibit methanogenesis by Bauchop (1967), is known to block the function of corrinoid enzymes and to inhibit methyl-coenzyme M reductase of methanogens (Gunsalus and Wolfe, 1978). As a classic nonspecific inhibitor for methanogens, it can also inhibit the activity of homoacetogens and acetate-consuming sulfate-reducing bacteria (Scholten et al., 2000). By contrast, 2-bromoethanesulfonate (BES), as a structural analogue of coenzyme M, was used to block methane formation by methanogens (Zinder et al., 1984). A further inhibitor, methyl fluoride (CH_3F), has been widely used to specifically inhibit acetoclastic methanogenesis (AM) with hydrogenotrophic methanogenesis (HM) unaffected, if appropriate dose was applied (Janssen and Frenzel, 1997). Vinyl acetate also showed the potential as an AM inhibitor, which was recently reported to irreversibly inhibit AM (Durán et al., 2011). However, its inhibitory effect on various anaerobic metabolisms needs to be further confirmed. Thus, to separately investigate different methanogenic pathways, without significantly disturbing other non-methanogenic metabolisms, combined application of CH_3F and BES is the best choice. Quantitative information on the contribution of different metabolic pathways to methane generation and waste reduction is expected to be obtained by this method. In fact, these two classic inhibitors have been widely used as metabolic probes for quantitative analysis of the carbon and electron flow in natural and engineered methanogenic systems (Xu

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et al., 2010).

Although the inhibition of methanogenesis by CH_3F and BES in anaerobic environments has been well established, most studies only focused on the practical applications. Little is known about their effects on the *in situ* diversity of microbial community structures. Recently, BES has been reported to inhibit or promote the growth of certain bacteria (Chiu and Lee, 2001). Due to the potential nonspecific or unintended effects of the methanogenic inhibitors, further study is necessary to fully understand their influence on the ecology of microbial community in the target system.

Metabolism of acetate is a key link in the degradation of accumulated VFAs and initiation of methanogenesis. Two pathways could be responsible for the methanogenic conversion of acetate: AM and the syntrophic acetate oxidation (SAO) coupled with HM. Thermodynamically, SAO is inferior to AM and can only proceed if H_2 partial pressure (pH_2) is kept at a low level (**Table 1**). On the contrary, acetate production via homoacetogenesis (HA) is favorable at high pH_2. Although AM was generally considered as the main mechanism for methane production, the potential significance of SAO was gradually recognized recently (Hao et al., 2011). It has been found in various anaerobic environments (Zinder and Koch, 1984; Li et al., 2009; Liu and Conrad, 2010), and to be predominant under thermophilic or other stressed conditions (Schnürer et al., 1999; Karakashev et al., 2006; Qu et al., 2009). To study the metabolism involved in methanogenic degradation of acetate at high concentrations, the application of inhibitors to separate different pathways is a considerable choice.

In the present work, CH_3F and BES were used to inhibit AM pathway and total methanogenesis respectively. Since there are not specific inhibitors for SAO, we created conditions with high pH_2 to thermodynamically block SAO. Their effects on the microbial diversity and metabolic activities of the involved reactions were studied during the incubation with high concentration of acetate, by using denaturing gradient gel electrophoresis (DGGE) fingerprinting of the bacterial and archaeal communities, and by monitoring the carbon flow and thermodynamic analysis.

1 Materials and methods

1.1 Experimental set-up

All experiments were performed with freshly collected methanogenic granular sludge cultivated at 55°C in the dark. Twenty milliliters of sludge mixture was transferred into each 1.2-L reactor with 450 mL basal medium. Acetate was added as the substrate to reach a final concentration of 100 mmol/L to simulate the VFAs accumulation. Detailed information on the seed sludge, added solution and gas phase control was described by Hao et al. (2011). The pH range was 6.8–7.8, regulated by using 5 mol/L H_3PO_4 solution.

Total methanogenesis was inhibited by addition of 2-bromoethanesulfonate (BES) (98.5%, Aladdin, China) to final concentration of 50 mmol/L. For specific inhibition of AM, CH_3F (99%, Shanghai Chunyu Special Gas Corporation Limited, China) was injected to reach a content of 2.5% (V/V) in the headspace after equilibrium between the gaseous and liquid phases. To provide high pH_2

Table 1 Reactions involved in methanogenic conversion of acetate

Pathway	Reaction	$\Delta G^{0\prime}$ (kJ/mol)
(1) Acetoclastic methanogenesis (AM)	$CH_3COOH \rightarrow CH_4 + CO_2$	–31.0
(2) Syntrophic acetate oxidation (SAO)	$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 4H_2$	+104.6
(3) Hydrogenotrophic methanogenesis (HM)	$4H_2 + CO_2 \rightarrow CH_4 + H_2O$	–135.6
(4) Homoacetogenesis (HA)	$4H_2+2CO_2 \rightarrow CH_3COOH + 2H_2O$	–104.6
(5) Sum (2) + (3) (SAO-HM)	$CH_3COOH \rightarrow CH_4 + CO_2$	–31.0

The standard Gibbs free energy change ($\Delta G^{0\prime}$) values were calculated from Thauer et al. (1977).

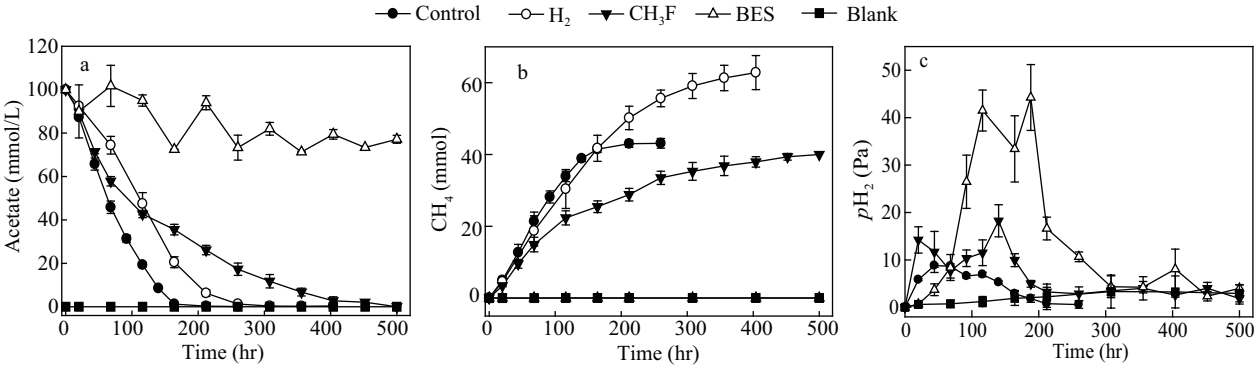

Fig. 1 Temporal change in acetate concentration (a), accumulated methane (b) and hydrogen partial pressure (pH_2) (c). Gas sampling was stopped three days after the acetate could not be detected.