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Contributions of fermentative acidogenic bacteria and sulfate-reducing bacteria to lactate degradation and sulfate reduction

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Abstract

The roles of fermentative acidogenic bacteria and sulfate-reducing bacteria (SRB) in lactate degradation and sulfate reduction in a sulfidogenic bioreactor were investigated by traditional chemical monitoring and culture-independent methods. A continuously stirred tank reactor fed with synthetic wastewater containing lactate and SO_4^{2-} at 35 °C, 10 h of hydraulic retention time was used. The results showed that sulfate removal efficiency reached 99%, and sulfide and acetate were the main end products after 20 d of operation. 16S rRNA gene based clone libraries and single-strand conformation polymorphism profiles demonstrated that the proportion of SRB increased from 16% to 95%, and that *Desulfobulbus* spp., *Desulfovibrio* spp., *Pseudomonas* spp. and *Clostridium* spp. formed a stable, dominant community structure. The decreasing COD/SO_4^{2-} ratio had little effect on the community pattern except that *Pseudomonas* spp. and *Desulfobulbus* spp. increased slightly. The addition of molybdate to the influent significantly changed the microbial community, sulfate removal efficiency and the pattern of end products. *Clostridium* spp., *Bacteroides* spp. and *Ruminococcus* spp. became the dominant community members. The main end products switched from acetate to ethanol and then to propionate with the oxidation–reduction potentials increasing from −420 to −290 mV. A lactate degradation pathway was deduced: lactate served as the electronic donor for *Desulfovibrio* spp., or was fermented by *Clostridium* spp. and *Bacteroides* spp. to produce propionate or ethanol, which were subsequently utilized by *Desulfobulbus* spp. and *Desulfovibrio* spp. The acidotrophic SRB oxidized part of the acetate finally.

Keywords: Community structure; Lactate; Molybdate; Single-strand conformation polymorphism

1. Introduction

The presence of sulfate-reducing bacteria (SRB) often causes the failure of anaerobic high-sulfate wastewater digestion due to their ability to out-compete methane-producing bacteria (MPB) for similar substrates (Parkin et al., 1990). Therefore, early reports on the ecology of SRB were primarily focused on the relationship between SRB and MPB or hydrogen-producing acetogenic bacteria (HPAB). SRB have been frequently shown to out-compete MPB or HPAB for substrates under most conditions (Parkin et al., 1990; Uberoi and Bhattacharya, 1995), especially in the acidogenic phase of two-phase anaerobic digestion systems (Reis et al., 1988). During the acidogenic phase, the most abundant microbial group, as well as SRB, is the fermentative acidogenic bacteria (FAB) (Kalyuzhnyi et al., 1998), not MPB. The FAB play a key role in degrading macro-organics to hydrogen, ethanol and volatile fatty acids (VFAs), which are then utilized by SRB to reduce sulfate. The symbiotic relationship between SRB and FAB has been examined (Kalyuzhnyi et al., 1998); although no direct microbiological evidence has been presented previously. For example, some researchers have shown that

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changes in the COD/SO_4^{2-} (C/S) ratio and the addition of molybdate affected the sulfate removal efficiency (SRE), which was ascribed to changes in the microbial community structure (Cadavid et al., 1999; Vossoughi et al., 2003). Nemati et al. (2001) concluded that addition of molybdate could suppress the activity of SRB, but would not affect the overall composition of the microbial community. As such, further study using microbiological techniques is required to elucidate the actual relationship between the SRB and FAB in degrading organic substances.

At a low concentration, molybdate is a metabolic inhibitor of SRB and shows little effect on other microbes (Smith and Klug, 1981; Sørensen et al., 1981). Hence, it has been commonly used to investigate the relationship between the SRB and MPB in previous research. Smith and Klug (1981) and Nemati et al. (2001) showed that 0.095 mM and 0.2 mM molybdate was enough to suppress the production of H_2S by pure cultures and by eutrophic lake sediments. In this study, 0.1–0.4 mM molybdate was used to inhibit SRB in a bioreactor in order to illuminate the relationship between SRB and FAB using the polymerase chain reaction (PCR)-single-strand conformation polymorphism (SSCP) technique.

SRB can utilize more than 100 different organic substances, although lactate has been shown to be the preferred electron donor (Song et al., 1998; Kaksonen et al., 2004). FAB, such as some members of genera *Clostridium* and *Bacteroides*, can ferment lactate to propionate, acetate, ethanol and hydrogen (Macy et al., 1978; vander Wielen et al., 2002). Hence, lactate is a favorite substrate for both SRB and FAB and therefore was used in the present study.

This study used traditional chemical monitoring and the culture-independent PCR-SSCP method to demonstrate the relationship between SRB and FAB, and their contributions to lactate degradation.

2. Materials and methods

2.1. Reactor operation and analytical methods

A continuously stirred tank reactor (CSTR) with a 1.35 l capacity and a 1 l working volume was used. Seed sludge was obtained from Shuangcheng moat sediment (Heilongjiang, China), which had suspended solids and volatile suspended solids of 166 and 27 g l^{-1}, respectively. The sludge was incubated in the bioreactor at 35 °C, with a 10 h of hydraulic retention time and a stirring speed of 200 rpm. The bioreactor operation involved three phases based on the composition of the influent. During startup (Phase I), the synthetic wastewater contained 4000 mg COD l^{-1} of lactate, 2000 mg sulfate (SO_4^{2-}) l^{-1} and a small quantity of $(NH_4)_2HPO_4$ at a final COD:N:P ratio of 100:5:1. After startup, COD in the influent decreased to 1000 mg l^{-1} for 15 d (Phase II) and then returned to Phase I. Subsequently, molybdate was fed to the influent with an increasing gradient of 0.1–0.4 mM (Phase III). Sludge from the CSTR was periodically sampled for total DNA extraction.

The COD, alkalinity, SO_4^{2-}, S^{2-} and pH in the bioreactor were determined using standard methods (APHA, 1998). Ethanol and VFAs, including acetate and propionate, were analyzed as described previously (Ren et al., 1997). The oxidation–reduction potential (ORP) in the reactor (E_h) was calculated by the equation $E_h = E_c + 249.1$ mV, where E_c is the observed ORP measured by an acidity voltmeter (pHS-25, Shanghai Analytical Apparatus Corporation, Shanghai China) and 249.1 is the potential value of the saturated calomel electrode.

2.2. DNA extraction and PCR amplification

Total DNA was extracted from 0.25 g (wet weight) activated sludge with a PowerSoil DNA kit (MoBio Laboratories, CA USA) according to manufacturer's instructions. Finally, the total DNA was suspended in 100 μl of 2 mM Tris–HCl (pH 8.0). The concentration and purity of the DNA were estimated by agarose gel electrophoresis and ultraviolet spectrometry (Beckman Coulter DU800, CA USA).

Two sets of primers were used to amplify the partial 16S rRNA genes of the bacterial community and SRB group of δ-Proteobacteria for SSCP analysis, respectively. For Bacteria, the forward primer BSF8/20 and the reverse primer BSR534/18 (Edwards et al., 1989) were utilized, corresponding to the 8–27 bp and 534–515 bp of the 16S rRNA gene of *Escherichia coli*. The second primer pair, SRB385F (Amann et al., 1990) and 926R (Schwieger and Tebbe, 1998), corresponded to the 385–402 bp and 926–907 bp of the 16S rRNA gene of *E. coli*. The SRB385F primer is group-specific for SRB of the δ-Proteobacteria as well as some Gram-positive bacteria. Both reverse primers were phosphorylated. For microbial composition analysis, the bacterial 16S rRNA gene based clone libraries were constructed with the primer pair BSF8/20 and 926R using the sludge on days 0, 19 and 47, respectively. All primers were acquired from Invitrogen (Shanghai, China). Each sample was amplified using four 25 μl volumes in PCR tubes containing 1×PCR buffer with Mg^{2+}, deoxynucleoside triphosphate solution (200 μM each), primers (0.6 μM each), and 0.125 U of EX *Taq* DNA polymerase (Takara, Dalian China). Approximately 5 ng of genomic DNA was added to each PCR mixture. A GeneAmp thermal cycler (Perkin–Elmer, MA USA) was used for PCR using the following program: 94 °C for 5 min, followed by 30 cycles at 94 °C for 40 s, 50 °C for 30 s and 72 °C for 40 s with a final extension step at 72 °C for 10 min. The purity and quantity of PCR products were estimated by running a 1% agarose gel and comparing their brightness to the quantitative marker DL2000 (Takara, Dalian China).

2.3. Construction of 16S rRNA gene based clone libraries

The PCR products of day 0 (seed sludge), day 19 (LM5) and day 47 (LM12) were purified with an agarose gel recov-