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Dynamics of propionic acid degradation in a two-phase anaerobic system

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HIGHLIGHTS

- Propionic acid was produced and accumulated in the acidogenic reactor.
- 0.02% *Desulfacinum* and 0.08% *Desulfobulbus* were present in the acidogenic reactor.
- Propionic acid was degraded in the methanogenic reactor.
- 2.68% *Smithella* and 0.35% *Syntrophobacter* were present in the methanogenic reactor.
- ATP may be an optional indicator for process stability.

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ABSTRACT

This paper reports on propionic acid (HPr) degradation in a laboratory scale two-phase anaerobic system, where HPr was accumulated in the acidogenic reactor and degraded in the methanogenic reactor. Batch tests using biomass from the two-phase anaerobic system showed HPr degradation was rarely detectable in the acidogenic reactor when HPr concentration ranged from 639 to 4531 mg HPr L^{-1} and at pH 4.50 to 6.50. Biomass from the methanogenic reactor could, however, successfully degrade HPr at its initial concentration of up to 4585 mg HPr L^{-1} at pH 6.40–7.30. ATP results showed that differences in the degradation ability of HPr by the acidogenic and methanogenic biomass may be related with their respective different biomass activities. Results from pyrosequencing showed that the predominant propionic acid oxidizing bacteria (POB) in the methanogenic reactor were *Smithella* (2.68%) and *Syntrophobacter* (0.35%); while poor degradation of HPr in the acidogenic reactor may be associated with the low abundance of POB (0.02% *Desulfacinum* and 0.08% *Desulfobulbus*). This might have been induced by the long-term unfavorable environment for POB growth in the acidogenic reactor.

1. Introduction

HPr is one of the major substrates contributing to methane formation in the anaerobic process (Glissmann and Conrad, 2000). It contributes to 35% methane production with 20% of this resulting from conversion of acetic acid (HAc) generated from HPr oxidation and 15% from the concomitant oxidation of HPr to H_2, a precursor of methane (Mah et al., 1990). Considering the thermodynamic kinetics, oxidation of HPr is endergonic and difficult Eq. (1), thus leading to the accumulation of HPr and subsequent system failure (Pullammanappallil et al., 2001). Due to differences in configurations, substrates, and operational parameters, the maximum tolerance of HPr in the single-stage system had varied in previous reports (Ma et al., 2009). Generally, the range of HPr concentration tolerable for the stable operation of a single-stage system was 1500–2220 mg HPr L^{-1} (Barredo and Evison, 1991).

Abbreviations: ATP, adenosine triphosphate; CSTR, continuous stirred tank reactor; CTAB, hexadecyl trimethyl ammonium bromide; d, day; F/M, Food/Microorganism; h, hour; HAc, acetic acid; HPr, propionic acid; HRT, hydraulic retention time; OTUs, operational taxonomic units; PEG, polyethylene glycol; POB, propionic acid oxidizing bacteria; RDP, ribosomal database project; RLU, relative luminescence; s, second; TSS, total suspended solids; VFAs, volatile fatty acids; VS, volatile solids; VSS, volatile suspended solids.

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$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2 \ \Delta G_0' = +76.1 \text{ kJ mol}^{-1} \quad (1)$$

In the traditional single-stage anaerobic system, the imbalance of different microorganisms in the population, normally indicated as volatile fatty acids (VFAs) accumulation (especially HPr), would be one of the major causes for system failure (Nielsen et al., 2007). For this reason, the acid-formers and methane-formers are separated into different reactors, and this separation creates an optimal growth environment for the hydrolytic/acidogenic and methanogenic biomass, respectively (Pohland and Ghosh, 1971). The different operating pH and hydraulic retention time (HRT) in the acidogenic and methanogenic reactors may result in different microbial communities. Xiao et al. (2013) had reported the degradation of HAc by methanogens in the acidogenic and methanogenic reactors of a two-phase anaerobic system. Kim et al. (2002) had reported HPr removal in single-stage and daily fed-batch two-phase continuous stirred tank reactor (CSTR) systems. However, HPr degradation in these systems was not the focus, and they had only focused on using HPr concentration as an indicator for system operation during start-up, steady state, and system failure periods. There is, to date, little in the published literature on HPr degradation dynamics in a two-phase anaerobic sludge digestion system.

Adenosine triphosphate (ATP), associated with biomass viability, can potentially be an important parameter for tracking progress of anaerobic sludge digestion. It reflects fluctuations in the bacterial metabolic activities and provides more intracellular information than the conventional measurements such as total suspended solids (TSS) and volatile suspended solids (VSS) (Chung and Neethling, 1988; Chen, 2000; Eydal and Pedersen, 2007). However, there have been few reports on the relationship between ATP and anaerobic microbial activities in a two-phase anaerobic system. In this study, ATP of biomass from the acidogenic and methanogenic reactors of the two-phase CSTR anaerobic system was determined to evaluate the microbial responses to HPr.

Previous researchers have showed that HPr degradation depended on the activities of POB (Mah et al., 1990; Kim et al., 2002), which are slow-growth microorganisms and so can be sensitive to reactor operating conditions (Pind et al., 2003). Several strains that could degrade HPr in the single-stage anaerobic system have been reported, such as *Syntrophobacter wolinii* (Boone and Bryant, 1980), *Smithella propionica* (Liu et al., 1999), *Desulfobulbus* (Kremer and Hansen, 1988) and *Desulfacinum hydrothermale* (Sievert and Kuever, 2000). It had also been reported that operational conditions could affect activities of the POB. Boone and Xun (1987) reported that pH was an important parameter affecting growth of a mixed POB culture and the optimal pH range for its growth was 6.80–8.50. Temperature and HRT also affected both biokinetic parameters and bacterial communities of POB in a temperature-phased two-phase anaerobic system (Zamanzadeh et al., 2013). There have, however, been few reports on differences of POB abundance in the separate acidogenic and methanogenic reactors.

This paper aims to describe: (1) microbial activity on HPr degradation in a two-phase anaerobic system; (2) the effect of pH and initial HPr concentration on HPr degradation; and (3) POB abundance in the acidogenic and methanogenic reactors.

2. Materials and methods

2.1. The two-phase CSTR anaerobic system

The laboratory scale two-phase continuous-flow CSTR anaerobic system is illustrated in Fig. 1. R1 (6 L) was the feed tank while the hydrolytic/acidogenic and methanogenic reactors were R2 (8 L) and R3 (45 L), respectively. Operating parameters for this system had been described in Xiao et al. (2013). Briefly, HRT for the acidogenic and methanogenic reactors were 3 d and 17 d, respectively. pH values of the above two phases were controlled at 5.50 ± 0.30 and 7.00 ± 0.20, respectively. The system was fed with a mixture of primary and secondary sludge (pH 5.7–6.0), which was collected weekly from a local sewage treatment plant and stored in 4 °C cold room before use. It had been operated for 113 d with volatile solids (VS) reduction of 31.6 ± 4.8% and methane production yield of 0.22 ± 0.01 L CH_4 g^{-1} VS_{fed} before the following studies were carried out. Detailed performance can be found in Maspolim et al. (2014).

2.2. Experimental set-up: HPr degradation and ATP of the acidogenic and methanogenic biomass

Biomass was drawn from the acidogenic and methanogenic reactors. The highest HPr concentration that the biomass was exposed to prior to the experiments had been 1474 mg HPr L^{-1} in the acidogenic reactor and 407 mg HPr L^{-1} in the methanogenic reactor, respectively. The term propionic/acetic acid is used to indicate the generic form, such as dissociated propionic/acetic acid and undissociated propionic/acetic acid.

The pretreatment methods to remove residual VFAs from the biomass were described in Xiao et al. (2013). Briefly, residual VFAs were removed by incubating (overnight) or centrifugation (12 857 × *g*, 10 min) the biomass.

Tests for determination of HPr degradation and ATP of the acidogenic and methanogenic biomass were done in serum bottles (120 mL), which contained 35 mL pretreated biomass and 35 mL synthetic media comprising nutrients and trace elements (0.2 mL L^{-1} synthetic media) (Labib et al., 1992). To test HPr degradation rates, a baseline concentration of 500 mg HPr L^{-1} was added as carbon source in the 35 mL synthetic media, which had been reported to have no inhibitory effects on acetic and propionic acids degradation (Mawson et al., 1991). Pre-set pH and HPr concentrations for the acidogenic biomass were 4.50, 5.00, 5.50, 6.00, 6.50 and 639, 1076, 1567, 2584, 4531 mg HPr L^{-1}, respectively. Pre-set pH and HPr concentrations for the methanogenic biomass were 6.40, 6.80, 7.30, 7.70 and 512, 942, 1495, 2565, 4585 mg HPr L^{-1}, respectively. Biomass withdrawn from the acidogenic and methanogenic reactors was incubated up to 109.50 h and 106.50 h, respectively. The utilization rate of HPr for the methanogenic tests was calculated using linear regression and normalized by biomass concentration (g-VSS) during 20^{th}–61^{th} h.

To test ATP content of biomass from the acidogenic and methanogenic reactors during HPr degradation, different amounts of HPr were added in the 35 mL synthetic media for the acidogenic biomass test (491, 961, 1467, 2696, and 4437 mg HP L^{-1}) and the methanogenic biomass test (416, 919, 1917, 2362, and 4616 mg HPr L^{-1}). The concentrations chosen covered the highest HPr concentration in the acidogenic (1474 mg HPr L^{-1}) and methanogenic (407 mg HPr L^{-1}) reactors and the concentration ranges described in HPr degradation experiments as listed above. pH values chosen for the ATP content test of acidogenic and methanogenic biomass were 5.50 and 6.80, respectively, which were close to the values maintained for the two-phase CSTR anaerobic system. Biomass from the acidogenic and methanogenic reactors was incubated with HPr at its chosen concentrations for 97 h and 68 h, respectively. ATP reduction rate was defined as the decrease of ATP concentration during a certain period. In this study, for the methanogenic biomass, ATP reduction rate was calculated based on data obtained between 20^{th} h and 68^{th} h, as a linear disappearance of HPr concentration was observed within this period. For the acidogenic biomass, as detectable degradation of HPr was rarely observed during the whole