JOURNAL OF ENVIRONMENTAL SCIENCES 42 (2016) 196–201

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JES JOURNAL OF ENVIRONMENTAL SCIENCES

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Impact of undissociated volatile fatty acids on acidogenesis in a two-phase anaerobic system

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ARTICLE INFO

Article history:
Received 3 May 2015
Revised 16 June 2015
Accepted 17 June 2015
Available online 8 September 2015

Keywords:
Anaerobic process
Waste treatment
Substrate inhibition
Undissociated volatile fatty acids
Acidogenesis

ABSTRACT

This study investigated the degradation and production of volatile fatty acids (VFAs) in the acidogenic phase reactor of a two-phase anaerobic system. 20 mmol/L bromoethanesulfonic acid (BESA) was used to inhibit acidogenic methanogens (which were present in the acidogenic phase reactor) from degrading VFAs. The impact of undissociated volatile fatty acids (unVFAs) on "net" VFAs production in the acidogenic phase reactor was then evaluated, with the exclusion of concurrent VFAs degradation. "Net" VFAs production from glucose degradation was partially inhibited at high unVFAs concentrations, with 59%, 37% and 60% reduction in production rates at 2190 mg chemical oxygen demand (COD)/L undissociated acetic acid (unHAc), 2130 mg COD/L undissociated propionic acid (unHPr) and 2280 mg COD/L undissociated *n*-butyric acid (unHBu), respectively. The profile of VFAs produced further indicated that while an unVFA can primarily affect its own formation, there were also unVFAs that affected the formation of other VFAs.

Introduction

Anaerobic processes have been widely applied in waste and wastewater treatment and recovery of energy *via* biogas production. The process comprises hydrolysis, acidogenesis, acetogenesis and methanogenesis, with volatile fatty acids (VFAs) produced in the first three steps, including short-chain fatty acids with six carbons or fewer (acetic, propionic, butyric, *etc.*) (Parawira et al., 2004; Ucisik and Henze, 2008; Yu and Fang, 2003).

In the conventional single-stage anaerobic system, all microbes are kept in the same vessel. However, these microbes require different optimum growth conditions (*i.e.*, oxido-reductive activities, growth rates and pH values). The two-phase anaerobic process attempts to physically separate acid- and methane-formers in two reactors, so that each reactor can be operated optimally (Pohland and Ghosh, 1971). The acidogenic phase can be achieved with shorter solid retention time and at lower pH (5–6), so that methanogens would be suppressed and hydrolytic and acidogenic bacteria accumulated (Zhang and Noike, 1991). However, complete depletion of methanogens from the acidogenic phase reactor is not practical (Beccari et al., 1998). The presence of methanogens and the related acetic acid (HAc) degradation in the acidogenic phase reactor has been reported (Xiao et al., 2013). Researchers also reported minor propionic acid (HPr) degradation associated with a low abundance of propionate-oxidizing bacteria in the acidogenic phase reactor (Xiao et al., 2015). However, the acidogenic biomass ability for *n*-butyric acid (HBu) degradation, which is a syntrophic reaction of methanogens and HBu-oxidizing bacteria (Amani et al., 2011), remains unclear.

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http://dx.doi.org/10.1016/j.jes.2015.06.015

Table 1 – Initial volatile fatty acids (VFAs) (acetic acid (HAc), propionic acid (HPr), and *n*-butyric acid (HBu)) concentrations, pH values, and corresponding undissociated volatile fatty acids (unVFAs) (undissociated acetic acid (unHAc), undissociated propionic acid (unHPr), and undissociated *n*-butyric acid (unHBu)) concentrations in the mixed liquor.

HAc (mg COD/L)	pH	HPr (mg COD/L)	pH	HBu (mg COD/L)	pH	unHAc (mg COD/L)	unHPr (mg COD/L)	unHBu (mg COD/L)
3400	6.50	3000	6.50	1700	6.50	60	70	35
3400	5.50	3000	6.00	3400	6.50	520	215	70
900	4.50	3000	5.50	3400	6.00	580	590	205
3400	5.00	1500	5.00	3400	5.50	1240	655	575
3400	4.50	3000	4.50	3400	5.00	2190	2130	1335
				3400	4.50			2280

Although VFAs production is necessary for methane generation in the anaerobic process, high VFAs concentrations can inhibit the microbial activities (Siegert and Banks, 2005). Undissociated volatile fatty acids (unVFAs) had been reported to be more inhibitory than the related dissociated VFAs form; VFAs inhibition of microbes was mainly attributed to unVFAs (Esgalhado and Roseiro, 1998; Guldfeldt and Arneborg, 1998; Xiao et al., 2013). The effects of undissociated acetic acid (unHAc) and undissociated *n*-butyric acid (unHBu) on biohydrogen production in glucose fermentation have been investigated (van Ginkel and Logan, 2005). To date, little is known about the impact of unVFAs on VFAs production and composition. This study investigated the impact of undissociated acetic acid (unHAc), propionic acid (unHPr), and *n*-butyric acid (unHBu) on "net" VFAs production and the composition profile of such production.

As was noted, many studies on VFAs production in the acidogenic phase reactor had not considered the effect of VFAs degradation (Jung et al., 2000; Salomoni et al., 2011). Bromoethanesulfonic acid (BESA) was reported to be effective at inhibiting methanogen activities (Chae et al., 2010). This study investigated the possibility of excluding the effect of VFAs degradation on the "net" VFAs production (net means VFAs production without VFAs degradation).

1. Materials and methods

1.1. Inoculum

The inoculum was drawn from the acidogenic phase reactor of a two-phase anaerobic sludge digestion system (working volume 7.5 L) operated in continuous stirred tank reactor (CSTR) mode with a hydraulic retention time of 3 days and pH of 5.50 ± 0.30, with details as described by Maspolim et al. (2015). The highest HAc, HPr and HBu concentrations experienced by the acidogenic biomass were 1200, 1960 and 1285 mg chemical oxygen demand (COD)/L, respectively.

1.2. Experimental set-up

Inoculum was drawn from the acidogenic phase reactor to investigate the impact of unHAc, unHPr and unHBu at various concentrations on VFAs production. The inoculum was treated to remove residual VFAs by centrifugation and re-suspension with an equivalent volume of synthetic feed media, with details as described by Xiao et al. (2013). To inhibit the activities of the acidogenic methanogens (which are present in the acidogenic phase reactor), 20 mmol/L BESA was chosen. The appropriate BESA concentration was identified *via* batch tests with details as described in (Appendix A Fig. S1). In this experiment, 500 mg/L glucose was used as the baseline substrate to stimulate acidogenesis, resulting in major acidogenesis products such as HAc, HPr, and HBu (Wang et al., 2011). The unVFAs concentrations were calculated from the total VFAs concentrations and pH values as described in Eqs. (1) and (2) (Guldfeldt and Arneborg, 1998; van Ginkel and Logan, 2005). The calculated unVFAs concentrations at different pH values are shown in Table 1.

$$\mathrm{pH} = \mathrm{p}K_a + \log(\mathrm{A}^-/\mathrm{HA}) \quad (1)$$

$$\mathrm{A}^- + \mathrm{HA} = \text{Total acid} \quad (2)$$

where, A^- (mg/L) and HA (mg/L) are the concentrations of dissociated and undissociated VFAs, respectively. pK_a values chosen for HAc, HPr and HBu were 4.76, 4.89 and 4.81 at 35°C, respectively (Fukuzaki et al., 1990; van Ginkel and Logan, 2005).

Briefly, 50 mL synthetic feed media (Labib et al., 1992) containing 500 mg/L glucose, 20 mmol/L BESA and different concentrations of unVFAs (unHAc, unHPr or unHBu) were mixed with 50 mL pretreated inoculum, filled into 120 mL serum bottles, and then placed on a shaker (Sartorius Stedim Biotech, Germany) at 35°C and 170 r/min. The unVFAs concentrations were achieved through addition of total VFAs and manipulating pH (Table 1). For example, 60 mg/L unHAc in the serum bottle was achieved through adding 3400 mg COD/L HAc and manipulating pH in the mixed liquor to 6.50. A similar procedure was adopted with other unHAc, unHPr and unHBu concentrations. pH was adjusted with 1 mol/L HCl and 1 mol/L NaOH. Incubation periods for the unHAc, unHPr and unHBu experiments were 23 hr, 19 hr, and 19.5 hr, respectively. These periods were chosen so as not to exhaust the pH buffering capability and to keep pH changes within the range of 0.10–0.30 pH units. VFAs production was calculated based on the net increase in total VFAs concentrations within the incubation periods and reported as mg COD/L. VFAs production rates were then normalized against volatile suspended solid (VSS) concentrations. To estimate the potential VFAs degradation, a carbon mass balance calculation was conducted in terms of total chemical oxygen demand (TCOD). At predetermined sampling intervals, 2 mL mixed liquor sample was drawn from the serum bottles for VFAs and COD tests. Each test was performed in triplicate.

1.3. Analysis

VFAs measurement was as described by Xiao et al. (2013). Briefly, a sludge sample was centrifuged at 12,857 ×*g* for 5 min.