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Acetic acid inhibition on methanogens in a two-phase anaerobic process

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ABSTRACT

The inhibitory effect of acetic acid on methanogens in a two-phase anaerobic process was evaluated. The results in this study showed that some methanogens still existed in the acidogenic phase although their dominance in the total microbial community was only 1% compared to 9.6% in the methanogenic phase. The inhibition threshold of acetic acid on acidogenic phase methanogens was, however, higher than that on methanogenic phase methanogens. At pH 6.00, acetic acid inhibition on methanogenic phase methanogens was observed when acetic acid concentration was higher than 1619.47 mg HAc/L although there was no obvious inhibition on acidogenic phase methanogens in the range of 1646.47–2781.19 mg HAc/L. There was also no acetic acid inhibition on acidogenic phase methanogens at pH 5.50, 6.00 and 6.50 in the range of 565.29–2781.19 mg HAc/L. However, for methanogenic phase methanogens, the inhibition was obvious and a second order substrate inhibition model, $q_s = q_m S/[K_s + S + (S^2/K_i)]$, could be adapted to describe the inhibition kinetics and mechanism of undissociated acetic acid on methanogenic phase methanogens. The results showed substrate saturation constant K_s, substrate inhibition constant K_i, and maximum specific utilization rate of acetic acid q_m, were 1.66 mg unHAc/L, 145.17 mg unHAc/L, and 3.53 mg HAc/L g MLVSS h, respectively.

1. Introduction

Conventional bioconversion of sludge in anaerobic digestion systems is usually characterized by hydrolysis, acidogenesis, acetogenesis and methanogenesis [1]. The imbalanced growth of acidogens and methanogens in a single-stage anaerobic reactor can result in process failure due to accumulation of volatile fatty acids (VFAs), which would cause pH decrease and inhibition of methanogen activity. The two-phase anaerobic process has physical separation of hydrolysis-acidogenesis from methanogenesis in two reactors [2]. Complex organic compounds are converted into simpler forms becoming soluble chemical oxygen demand (COD) and thereafter as VFAs in the acidogenic phase; the VFAs are then converted into biogas by methanogenic phase methanogens [3]. In the two-phase system, the acidogenic phase protects the methanogenic phase from rapid acidification and sharp pH declines [4]. The two-phase process seeks to provide optimum conditions for acid- and methane-formers with its better control of acidogenesis; therefore, it can achieve high organic loading rates and higher volatile solids (VS) and COD removal efficiencies than the traditional single-stage system [5].

The activities of methanogenic communities are affected by VFA concentrations and pH [6]. During hydrolysis and acidogenesis, acetic acid is the main VFA product [7]. Many studies have been carried out to explore the inhibition effect of acetic acid on methanogens [8,9] and the inhibitory mechanisms caused by high concentrations of acetic acid in the single-stage anaerobic digester [10,11]. However, it is noteworthy that all previous studies and results were based on the single-stage anaerobic system.

It has been pointed out that it was difficult to completely separate acidogenesis from methanogenesis [12], and that some methanogenic activities in the acidogenic phase were necessary to support the syntrophic interaction between different trophic groups of microorganisms [13]. Researchers have identified the

Abbreviations: Ac_i, initial acetic acid concentration; COD, chemical oxygen demand; CSTR, continuous stirred tank reactor; C_T, total acetic acid concentration; F/M, food/microorganism; HAc, acetic acid; HRT, hydraulic retention time; MLVSS, mixed liquor volatile suspended solids; qPCR, quantitative polymerase chain reaction; TCOD, total chemical oxygen demand; unHAc, undissociated acetic acid; VFAs, volatile fatty acids; VS, volatile solids.

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presence of methanogens in the acidogenic phase of a two-phase anaerobic digestion system [14]. In this study, the two-phase anaerobic process referred herein also had some methanogens in the acidogenic phase. As is known, the amount of acetic acid-utilizing methanogens in the traditional single-stage anaerobic digesters was only 10–50% of that in the methanogenic phase of the two-phase system [5]. Thus, the acetic acid utilization by acidogenic phase methanogens (methanogens cultivated in the acidogenic phase) and methanogenic phase methanogens (methanogens cultivated in the methanogenic phase) of the two-phase system may be different from that cultivated in the conventional single-stage anaerobic digestion system. Previous research work has shown methanogens in the single-stage anaerobic system were severely inhibited by the action of undissociated VFAs [15] and undissociated acetic acid (unHAc) was the uncoupler of the plasma membrane [16]. The effect of acetic acid concentration on methanogens was through the undissociated acetic acid form. To date, the degradation of acetic acid and its effect on acidogenic phase methanogens and methanogenic phase methanogens of the two-phase system have not been studied in detail.

This study aims to (1) identify the existence of methanogens in the acidogenic phase and their abilities to degrade acetic acid; (2) explore the effect of pH and acetic acid concentration on acetic acid utilization by acidogenic phase and methanogenic phase methanogens in a two-phase anaerobic process; and (3) investigate the possible kinetic parameters associated with the effect of undissociated acetic acid on acidogenic phase and methanogenic phase methanogens.

2. Materials and methods

2.1. Culture source

The culture for the study was drawn from a laboratory-scale continuous stirred tank reactor (CSTR) two-phase anaerobic sludge digestion system. Nitrogen gas was sparged into the headspace to maintain anaerobic conditions whenever sludge was withdrawn. The system was fed with concentrated mixed primary sludge and secondary sludge (total chemical oxygen demand (TCOD) of 46.90 ± 9.00 g/L) collected from a local sewage treatment plant. The CSTR system has been operated for 113 days with a hydraulic retention time (HRT) of 3 days and pH of 5.50 ± 0.30 for the acidogenic phase, and a HRT of 17 days and pH of 7.00 ± 0.20 for the methanogenic phase. The system displayed good performance with VS reduction of 41.46% and biogas yield of 0.96 L/g $VS_{destroyed}$ before the experiments described in this paper were carried out. The highest concentrations of acetic acid that the acidogenic and methanogenic culture experienced prior to these experiments were 1125 and 1172 mg HAc/L, respectively. The term acetic acid is used here to indicate the chemical species in all its forms (generic form); i.e. dissociated acetic acid as well as undissociated acetic acid.

Table 1
Composition of stock solution of nutrients and trace elements (0.2 mL/L) for the synthetic feed [17].

Nutrient (g/L)		Trace element (g/L)	
$(NH_4)_2HPO_4$	0.024	$CoCl_2 \cdot 6H_2O$	1.25
NH_4HCO_3	0.34	H_3BO_3	1.25
KCl	0.002	$MnCl_2 \cdot 4H_2O$	3.057
$MgCl_2 \cdot 6H_2O$	0.166	$Na_2MoO_4 \cdot 4H_2O$	0.1
$CaCl_2 \cdot 2H_2O$	0.166	$NiCl_2 \cdot 6H_2O$	1.25
$FeCl_2 \cdot 4H_2O$	0.006	$ZnCl_2$	1.25
$NaHCO_3$	0.5	Thiamine	1.945

2.2. Experimental set-up: acetic acid inhibition on acidogenic and methanogenic phase methanogens

Sludge for this study was withdrawn from both acidogenic phase and methanogenic phase reactors. Serum bottles (120 mL) containing 50 mL culture and 50 mL synthetic feed media (Table 1) were incubated in an incubator (Sartorius Stedim Biotech, Germany) (35 ± 2 °C and 150 rpm). Prior to addition of the synthetic feed and acetic acid, the culture from the methanogenic phase was incubated at room temperature overnight without additional carbon source to allow degradation of residual VFAs (20–30 mg VFAs/L) in the culture. Residual VFAs from the acidogenic culture were removed by centrifugation ($12857 \times g$, 10 min) and washing (with COD free synthetic feed).

A baseline concentration of acetic acid which did not inhibit was chosen in order to evaluate the normal activity of the methanogens in the two phases. Previous researchers have demonstrated that 500 mg HAc/L did not show inhibitory effect on methanogens from the single-stage anaerobic digestion system [15]. Hence, 500 mg HAc/L acetic acid was added in each serum bottle as baseline carbon source for the two cultures. To determine the effect of initial acetic acid concentration (Ac_i) and pH on acetic acid utilization by the acidogenic phase and methanogenic phase methanogens, different amounts of additional Ac_i were then added into the serum bottles with various pre-set pH values (Table 2). The concentrations of acetic acid added to the serum bottles with culture from the acidogenic phase (Condition 1) varied from 65.29 to 2281.19 mg HAc/L with pH ranging from 4.50 to 6.50. The concentrations of acetic acid added to the serum bottles with culture from the methanogenic phase varied from 46.08 to 4279.01 mg HAc/L (Condition 2) with pH ranging from 6.00 to 7.70. The desired pH in each serum bottle was adjusted by addition of 1 N HCl or 1 N NaOH before the start of the experiment.

The reaction periods for sludge from the acidogenic phase and methanogenic phase were 97 h and 70 h, respectively. The sampling intervals for the acidogenic phase experiment were at 0th h, 22th h, 28th h, 53th h and 97th h and for the methanogenic phase experiment were at 0th h, 19th h, 26th h, 32th h, 44th h, 50th h, 56th h and 70th h, respectively. Acetic acid utilization rate was calculated using linear regression of the measured acetic acid concentrations

Table 2
Initial added acetic acid concentrations for the acidogenic and methanogenic tests.

Measured initial substrate and added acetic acid concentrations and pH under acidogenic conditions Condition 1			Measured initial substrate and added acetic acid concentrations and pH under methanogenic conditions Condition 2		
Substrate (mg HAc/L)	pH	Ac_i (mg HAc/L)	Substrate (mg HAc/L)	pH	Ac_i (mg HAc/L)
	4.50	65.29		6.00	46.08
	5.00	324.53		6.40	681.65
	5.50	603.11		6.80	1119.47
	6.00	1146.47		7.30	2203.23
$Ac_i = 500$	6.50	2281.19	$Ac_i = 500$	7.70	4279.01