Biochemical Engineering Journal 73 (2013) 80–85

Contents lists available at SciVerse ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

Adding Fe^0 powder to enhance the anaerobic conversion of propionate to acetate

Xusheng Meng, Yaobin Zhang*, Qi Li, Xie Quan

Key Laboratory of Industrial Ecology and Environmental Engineering (MOE), School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, PR China

ARTICLE INFO

Article history:
Received 3 September 2012
Received in revised form 31 January 2013
Accepted 3 February 2013
Available online 14 February 2013

Keywords:
Propionate
Fe^0
Acetate
Acetogenesis
Homoacetogenesis
Anaerobic

ABSTRACT

Propionate is an unfavorable substrate for the anaerobic digestion because it is thermodynamically difficult to be decomposed into acetate. An attempt to enhance the decomposition of propionate by adding Fe^0 powder (10 g) into an acidogenic reactor (A1) with propionate as the sole carbon source was made in this study. The results showed that the propionate conversion rate (67–89%) in A1 were higher than that in a reference reactor (43–77%) without dosing of Fe^0 (A2). The enhanced conversion of propionate caused both chemical oxygen demand removal (COD) (57–79%) and acetate production (178–328 mg/L) in A1 to increase significantly. Although Fe^0 contributed the H_2 production chemically, the H_2 content of A1 was less than that of A2. The reason was ascribed to the enhanced utilization of H_2 for the homoacetogenesis. It was calculated that the Gibbs free energy in the decomposition of propionate was decreased by about 8.0–10.2% with the dosing of Fe^0. Also, the activities of enzymes related to the acetogenesis were enhanced by 2–34-folds. Fluorescence in situ hybridization (FISH) and denaturing gradient gel electrophoresis (DGGE) analysis indicated that Fe^0 increased the abundance of microbial communities, especially propionate-utilizing bacteria and homoacetogenic bacteria.

1. Introduction

Propionate is a main intermediate in the stage of hydrolysis/fermentation, the degradation of which is thermodynamically unfavorable [1,2]. According to the calculation on the Gibbs free energy, the conversion of propionate to acetate does not occur until the partial pressure of hydrogen decreases below an extremely low level (<10 Pa) [1,3], but the partial pressure of hydrogen in practice usually exceeds this range. As a result, the accumulation of propionate is often observed in the anaerobic digesters, which may destroy the pH balance between acidogenesis and methanogenesis, further deteriorating the digestion [4,5]. Thus, the propionate production and/or propionic-type fermentation occurring in the acidogenesis stage should be reduced during the anaerobic digestion.

Anaerobic digestion includes three fermentation types: propionic-type, butyric-type and acetic-type [3,6]. A way to relieve the accumulation of propionate is to reduce the propionic-type fermentation. The fermentation type is closely related to oxidative–reductive potential (ORP) [7–9]. It is believed that the propionic-type fermentation is a facultative anaerobic process, occurring at ORP higher than −278 mV [7]. Wang et al. [8] reported that the fermentation was shifted from the propionic-type to the butyric-type and acetic-type under lower ORP level. In our previous study [5], Fe^0 powder was added into an acidogenic reactor to decrease ORP and reduce the propionate accumulation, and the results showed that the concentration of propionate dropped from 416 to 225 mg/L and the concentration of acetate increased from 222 to 408 mg/L. It suggested that more organics was not necessary to undergo the propionate stage and could directly be hydrolyzed and fermentated into acetate due to the addition of Fe^0.

Alternatively, the accumulation of propionate could probably be reduced by accelerating the decomposition of propionate. It was assumed that Fe^0 was likely to enhance the conversion of propionate to acetate because Fe^0 could serve as an electron donor in the microbial metabolism [10] and promote a number of key enzymes activities in the acetogenesis process [5,11]. For example, pyruvate–ferredoxin oxidoreductase (POR) that contains Fe—S clusters is a crucial enzyme to catalyze the decomposition of propionate, which meant that appropriate addition of Fe^0 was likely to enhance its activity [11]. So far the effects of Fe^0 on the conversion of propionate to acetate remained unknown. Thus, Fe^0 powder was dosed in an acidogenic reactor with propionate as the sole carbon source to attempt to accelerate the decomposition of propionate and reduce its accumulation in this study.

* Corresponding author. Tel.: +86 411 8470 6460; fax: +86 411 8470 6263.
E-mail addresses: zhangyb@dlut.edu.cn, yaobinzhang@163.com (Y. Zhang).

1369-703X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bej.2013.02.004

2. Materials and methods

2.1. Experimental reactors

Fe^0 powder (10 g, purity >98%, 0.2 mm in diameter, a BET surface area of 0.05 m^2/g) was added into A1 with a working volume of 2 L (φ 100 mm × 280 mm) (Fig. S1). The control reactor A2 was the same as A1 but without dosing Fe^0 powder. The hydraulic retention times (HRTs) of A1 and A2 were decreased stepwise from 6 to 4 h then to 2 h and finally recovered to 4 h again. During the experiment at the HRT of 6 h, 2-bromoethanesulfonate (BES, 40 mM), a specifically methanogenic inhibitor [12], was added into A1 and A2 every three days. The acidogenic reactors were operated in an upflow mode at 35 °C.

2.2. Sludge and wastewater

The seed sludge obtained from the sedimentation tank of a municipal sewage plant in Dalian (China). After removing large debris, the volatile suspended sludge (VSS) was 3.1 g/L and total suspended sludge (TSS) was 12.9 g/L. Each reactor was inoculated with the seed sludge of 1 L.

The acidogenic reactors were fed with an artificial wastewater with a fixed COD of 3500 mg/L. Sodium propionate, NH_4Cl and KH_2PO_4 were added as the carbon, nitrogen and phosphorus sources, respectively, to give a COD:N:P ratio of 200:5:1. The trace elements were added according to Liu et al. [5]. The pH of influent wastewater was adjusted to 6.0 by 1:1 HCl solution.

2.3. Analysis methods

TSS, VSS and COD were conducted in accordance with Standard Methods for the Examination of Water and Wastewater [13]. The pH was recorded using a pH analyzer (Sartorius PB-20, Germany). The concentration of Fe^{2+} was determined by ortho phenanthroline spectrophotometry at 510 nm (UV-721, Techcomp, China). The composition of biogas was analyzed by a gas chromatograph (GC-14C/TCD, Shimadzu, Japan) and the concentrations of acetate and propionate were determined by another gas chromatograph (GC-2010/FID, Shimadzu, Japan) [14].

The dehydrogenase activity was analyzed by the method of Nybroe et al. [15]. The activity of POR was determined according to the reported method [16]. The activities of acetate kinase (AK) and phosphotransacetylase (PTA) were assayed with the method of Yan et al. [17].

FISH was used to determine the abundance of microbes in the acidogenic reactors. FISH was conducted according to the method of Wu et al. [18]. Fluorescence labels of the oligonucleotide probes used in this study included a Cy3-labeled Wol223 and MPOB222 (propionic-utilizing bacteria, red), and a FITC-labeled AW (homoacetogenic bacteria, green). 4′,6′-Diamidino-2-phenylindole (DAPI) was used for characterization of the entire bacterium (blue). The samples were observed under a confocal laser scanning microscope (FV1000, Olympus, Japan). The obtained FISH images were imported to Image-Pro Plus 6.0 for analyzing of the relative abundance of microorganisms.

The genomic DNA of the sample was extracted using an extraction kit (Bioteke Corporation, Beijing, China) according to the manufacturer's instructions. A primer combined GM341F with DS907R was used to selectively amplify the 16S rDNA sequences of eubacteria. A 40-nucleotide GC clamp was added to the forward primer at the 5′-end to improve the detection of sequence variation in amplified DNA fragments by subsequent DGGE. The polymerase chain reaction (PCR) products obtained were applied to DGGE analysis, which was conducted by the Dcode system (Bio-Rad). The concrete steps of DGGE analysis for bacteria were conducted according to the illustration by Zhang et al. [19]. Some dominant DGGE bands were excised and reamplified by PCR using the primers described above without the GC clamp. The PCR products were then sequenced by TaKaRa Biotechnology Co., Ltd. (Dalian, China). The obtained gene sequences were compared with the reference microorganisms available in the GenBank by BLAST search. The sequences were aligned using ClustalX program and the phylogenetic tree was constructed by a distance method (neighbor-joining) using Mega software [20].

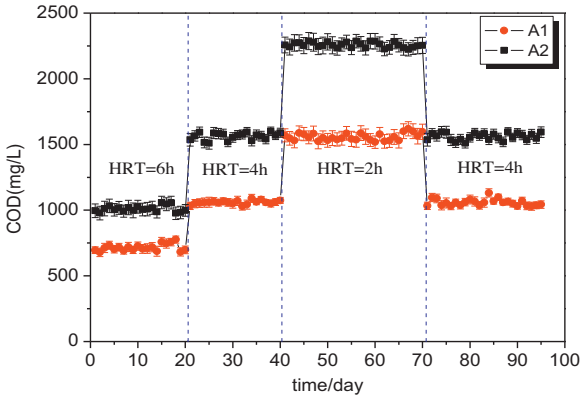

Fig. 1. Effluent COD from the acidogenic reactors under different HRTs. Each reactor was sampled three times in a given day for determination, namely, each point in the figure was the average value from three samples. Error bars represent standard deviations of triplicate tests.

2.4. Data analysis

The Gibbs free energies (ΔG) of propionate conversion and homoacetogenesis were calculated according to the Van't Hoff equation as follows: $\Delta G = \Delta G^\circ + RT \ln$ (products/reactants). The values of ΔG° were calculated from the standard Gibbs energies of formation [21,22] using the equations under standard conditions (1 M, 10^5 Pa, 298.14 K) as follows:

$$CH_3CH_2COO^-(aq) + 2H_2O(l) = CH_3COO^-(aq) + CO_2(g) + 3H_2(g) \quad \Delta G = +76.1\ kJ/mol \tag{1}$$

$$4H_2(g) + 2CO_2(g) = CH_3COO^-(aq) + H^+(aq) + 2H_2O(l) \quad \Delta G = -55.1\ kJ/mol \tag{2}$$

These two equations, namely acetification (or conversion of propionate) and homoacetogenesis, were the main processes in the acetogenesis stage, which could be combined into one equation (3):

$$4CH_3CH_2COO^-(aq) + 2H_2O(l) + 2CO_2(g) = 7CH_3COO^-(aq) + 3H^+(aq) \quad \Delta G = +139.1\ kJ/mol \tag{3}$$

3. Results and discussion

3.1. Effluent COD under different HRTs

It has been reported that the HRT was critical to successful operation of anaerobic system [23]. The acetogenesis was usually operated at a relatively low HRT to rush out methanogens for reducing their influence on acetogenesis [24]. To research the effects of Fe^0 powder on the decomposition of propionate, A1 and A2 were operated at the HRTs ranging from 6 to 2 h, and then recovering to 4 h. The influent COD was fixed at about 3500 mg/L. As Fig. 1