Bioresource Technology 113 (2012) 37–43

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Effect of carbonate on anaerobic acidogenesis and fermentative hydrogen production from glucose using leachate as supplementary culture under alkaline conditions

Qiang Liu [a,*], Xiao Lei Zhang [a], Zhao Jun [a], Ai Hua Zhao [b], Shan Ping Chen [b], Feng Liu [b], Jun Tai [b], Jian Yong Liu [a], Guang Ren Qian [a]

[a] School of Environmental and Chemical Engineering, Shanghai University, No. 99 Shangda Road, Shanghai 200444, PR China
[b] Shanghai Engineering Research of Municipal Solid Waste Treatment and Recycle, No. 345 Shilong Road, Shanghai 200232, PR China

ARTICLE INFO

Article history:
Received 23 September 2011
Received in revised form 23 February 2012
Accepted 24 February 2012
Available online 5 March 2012

Keywords:
Carbonate
Acidogenesis
Hydrogen production
Fresh leachate
Volatile fatty acid

ABSTRACT

Carbonate was added into a co-culture of glucose and fresh leachate under alkaline condition to enhance batched acidogenesis and fermentative hydrogen production simultaneously. Results indicated carbonate has positive effect on both H_2 production and acetic acid generation. The highest hydrogen yield (about 1.40 mol/mol glucose) was obtained at $[CO_3^{2-}]$ = 280 mg/L with pH 8.0 and $[CO_3^{2-}]$ = 560 mg/L with pH 9. The dominant liquid metabolites were ethanol, acetic and butyric acid. The highest total volatile fatty acid yield (0.38 g/g glucose) was achieved at $[CO_3^{2-}]$ = 560 mg/L with pH 9. In this case, the acetic acid yield reached 0.13 g/g glucose. Verification tests using simulated wastewater as substrate were also carried out at pH 9. Results demonstrated calcium ions inhibit hydrogenogens activity while carbonate addition can alleviate the suppression effect caused by Ca^{2+}.

1. Introduction

Hydrogen is a clean renewable energy carrier with zero pollutant emissions (Chong et al., 2009a). Among various hydrogen production methods, anaerobic digestion from organic wastes seems to be a promising and environmentally friendly alternative (Benemann, 1996).

Generally, anaerobic digestion consists of three major steps: hydrolysis/acidogenesis, acetogenesis and methanogenesis. Hydrogen is produced during the first two steps (Kotsopoulos et al., 2009). Meanwhile, valuable biochemical materials (such as acetate and butyrate) can also be generated in these processes. Due to this consideration, organic waste anaerobic digestion may not only lower the amount of generated pollution, but also be used to produce biochemicals (Nie et al., 2008). Reviewing literature indicates that a lot of studies have reported the anaerobic hydrogen production operation from organic wastes (Chong et al., 2009b; Ntaikou et al., 2009; Oh and Logan, 2005; Ozmihci and Kargi, 2011). However, most of these reports focused on producing hydrogen under acid or neutral condition, the reported anaerobic digestion operations under alkaline were scarce. The reason is that the activities of hydrogen producing bacteria (HPB) and hydrogenase are much higher under acid and neutral conditions. However, alkaline condition has been proved to be favorable for the volatile fatty acids (VFAs) formation (Chen et al., 2007; Wu et al., 2010). The increased VFAs generation could compensate the loss of hydrogen production under the alkaline condition.

Among all the bio-products during the anaerobic digestion, acetate is one of the key intermediates with high economic and application value (Domke et al., 2004). Acetogenesis and homoacetogenesis are two major pathways for acetate production. Homoacetogenesis requires CO_2 or bicarbonate for forming acetate as follow (Siriwongrungson et al., 2007):

$$4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 4H_2O \quad (1)$$

According to this principle, we could infer that adding carbonate into substrate might produce HCO_3^- and thus facilitate acetate production during anaerobic digestion.

In a previous study (Liu et al., 2011a), we used fresh compost leachate as nutrient source for fermentative hydrogen production from glucose under acid condition (pH 5.5) and found the over abundant Ca^{2+} contained in leachate reduce hydrogen production due to the inhibition effect of calcium ion on HPB (Hammes et al., 2003). Another previous study of us proved Ca^{2+} could be effectively removed by participating with carbonate, phosphate or carboxylate during the methane production from fresh leachate solely under neutral circumstance (Liu et al., 2011b).

* Corresponding author. Tel.: +86 21 6613 7743; fax: +86 21 6613 7761.
E-mail address: qliu@shu.edu.cn (Q. Liu).

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doi:10.1016/j.biortech.2012.02.115

Therefore, in this study, we added carbonate into the co-substrate of glucose and leachate for the aims of improving fermentative hydrogen production and acidogenesis simultaneously under alkaline condition. The effects of initial pH (in the range of pH 8–11) and carbonate concentrations on biohydrogen production and acidogenesis were investigated by batch tests. The optimal conditions of biohydrogen production as well as acidogenesis were explored. Based on the obtained optimalizing condition, the effects of carbonate and calcium during this process were verified via anaerobic fermentative tests using synthetic wastewater as substrate under the optimal pH condition and significantly high hydrogen production was achieved.

2. Methods

2.1. Fresh leachate

Fresh leachate used in this study was obtained from Pudong Compost Factory, Shanghai, China. This leachate is characterized by fairly high organic matter and calcium content and contains a certain amount of Mg, Fe and Zn ions. Other heavy metals such as Cr, Cd, Cu, Mn, Ni and Pb were negligible in this leachate. The detailed composition of the leachate was shown in Table 1.

2.2. Source of seed sludge

Anaerobic granular sludge obtained from a full-scale internal circulation bioreactor of a citric acid production plant was used in this study. The seed sludge consisted of well-settled black granules with the size from 0.28 to 2 mm in diameter and had a mixed liquor volatile suspended solids (MLVSS) content of 30.5 g/L as well as a mixed liquor suspended solids (MLSS) content of 41.4 g/L. Prior to use, the seed sludge was washed five times with tap water and then sieved to remove stone, sand and other coarse matters. Heat pretreatment was conducted by heating the sludge at 99 °C for 90 min to harvest hydrogen-producing anaerobes and inhibit hydrogentrophic methanogenesis.

2.3. Batch experiments

The batch tests were conducted in 500 mL conical flasks connected with 500 mL biogas collectors. The conical flask was filled with 50 mL granular sludge and 400 mL liquid and sealed with a butyl rubber stopper. The liquid contained the optimal glucose (6200 mg/L) and leachate (3380 mg COD/L) concentrations which were obtained from previous study (Liu et al., 2011a). The total nitrogen (TN), total phosphorous (TP) and Ca^{2+} concentrations of these diluted liquors were about 610, 4, and 195 mg/L, respectively. The initial pH of liquids was adjusted to 8, 9, 10, and 11 by HCl and NaOH solution, respectively. After that, different amounts of sodium carbonate (0, 280, 560, and 1120 mg/L, based on CO_3^{2-}) were separately added into the fermentation liquor. No extra nutrient was supplemented in all batch tests.

Table 1
Characteristics and Composition of fresh leachate used in this study (mg/L).

Component	Value	Component	Value
pH	4.9	VFA	3077
COD_{Cr}	55,689	Ca	2667
TN	10,056	Mg	364.8
TP	71	Fe	969.9
TS	968	Zn	15.8
Protein	14,400	Cr	nd

Before anaerobic fermentation, the void space of flasks was flushed by a mixed gas of nitrogen (80%) and carbon dioxide (20%). The sealed flasks were placed in a reciprocal shaker (reciprocation: 150 strokes/min) at 35 ± 1 °C. Anaerobic fermentation in each flask continued until the total biogas volume kept constant for consecutive 4 h. All batch tests were carried out in triplicate.

2.4. Analytical methods

COD, TN, TP concentrations in liquid were determined according to the standard method (APHA, 2005). pH value was measured by a pH-meter (Delta 320, Mettler-toledo). Metal ion contents in liquid were analyzed with an inductively coupled plasma atomic emission spectrometer (ICP-AES, Shimadzu, ICPS-7510). Total sugar (carbohydrate) concentration was measured using the phenol sulfuric acid assay (Dubois et al., 1956). Protein concentration was measured according to Lowry's method (Lowry et al., 1951). The volume of generated biogas was measured by the water displacement method. Biogas composition was analyzed by a GC9800 gas chromatography (Kechuang Chromatograph Instruments Co., Ltd., Shanghai, China) equipped with thermal conductivity detection (TCD). Soluble metabolites were analyzed by a gas chromatograph (GC-900A, Kechuang Chromatograph Instruments Co., Ltd., Shanghai, China) equipped with a flame ionization detector (FID). Details of analytical method can refer to Liu et al. (2011a).

Cumulative hydrogen volume was obtained from headspace measurements and the total volume of biogas produced for each time interval. The molar hydrogen yield was calculated by the moles of cumulative hydrogen volume at the end of fermentation process and the moles of glucose consumed in the fermentation. The calculation formulas of the cumulative hydrogen volume and molar hydrogen yield can be accessed from Logan et al. (2002). TVFA (total volatile fatty acid) and acetic acid yield were calculated as follows:

$$Y_{TVFA} = (C_{TVFA,f} - C_{TVFA,i})/(C_{g,i} - C_{g,f}) \quad (2)$$

$$Y_{HAc} = (C_{HAc,f} - C_{HAc,i})/(C_{g,i} - C_{g,f}) \quad (3)$$

where Y_{TVFA} and Y_{Hc} are TVFA and acetic acid yield, respectively. $C_{TVFA,f}$ and $C_{HAc,f}$ represent the concentrations of TVFA and acetic acid at the end of fermentation in each test. $C_{TVFA,i}$ and $C_{HAc,i}$ denote the initial concentrations of TVFA and acetic acid in each test. $C_{g,i}$ and $C_{g,f}$ stand for the initial and final glucose concentrations in each test.

3. Results and discussion

3.1. Effects of carbonate concentration and initial pH on cumulative hydrogen production

In all batch tests, the generated biogas contained carbon dioxide and hydrogen and was free of methane. As shown in Fig. 1, adding different amounts of carbonate into the co-culture led to various hydrogen production performances at different initial pH.

When initial pH was 8 (Fig. 1a), all the batch tests underwent 5–7 h of lag times. Under this circumstance, the maximal cumulative hydrogen volume reached 397.7 ± 13.5 mL at $[CO_3^{2-}]$ = 280 mg/L. This value was much higher than those at $[CO_3^{2-}]$ = 0, 560, and 1120 mg/L (with the maximal cumulative hydrogen volume being 128.9 ± 13.2, 296.6 ± 23.8, and 179.0 ± 11.6 mL, respectively).

As for initial pH 9 (Fig. 1b), the lag times of four batch tests ranged from 5 to 9 h. Unlike the case at pH 8, the highest cumulative hydrogen volume was obtained at $[CO_3^{2-}]$ = 560 mg/L (398.9 ± 11.3 mL) while the cumulative hydrogen volumes for