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Hydraulic retention time affects stable acetate production from tofu processing wastewater in extreme-thermophilic (70 °C) mixed culture fermentation

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HIGHLIGHTS

- Acetate production from TPW in extreme-thermophilic MCF was first investigated.
- Long HRT (>3 d) enhanced syntrophic acetate oxidation, causing less acetate production.
- Stable acetate production was achieved at a short HRT of 3 d.
- *Coprothermobacter* (main acetate-degraders) decreased when HRT dropped from 5 to 3 d.

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ABSTRACT

Acetate is an important industrial chemical and its production from wastes via mixed culture fermentation (MCF) is economic. In this work, the effect of hydraulic retention time (HRT) on acetate production from tofu processing wastewater (TPW) in extreme-thermophilic (70 °C) MCF was first investigated. It was found that long HRT (>3 days) could lead to less acetate production while stable acetate production was achieved at short HRT (3 days) with the yield of 0.57 g-COD/g-COD_{TPW}. The microbial community analysis showed that hydrogenotrophic methanogens (mainly *Methanothermobacter*) occupied up to 90% of archaea at both HRTs of 3 and 5 days. However, *Coprothermobacter*, the main acetate-degraders, decreased from 35.74% to 10.58% of bacteria when HRT decreased from 5 to 3 days, supporting the aggravation of syntrophic acetate oxidation in long HRT. This work demonstrated that HRT was a crucial factor to maintain stable acetate production from TPW in extreme-thermophilic MCF.

1. Introduction

Acetic acid is an important industrial chemical and its salts are building block compounds for polymers such as polyvinyl acetate and cellulose acetate, which are used to produce vinyl plastics and photographic films, respectively (Jang et al., 2012). In addition, acetate is considered to be one of the main intermediates in the carboxylate platform by mixed cultures, and it could be converted to other valuable metabolites, such as butyrate and ethanol. Steinbusch et al. proposed that acetate could be reduced to medium-chain fatty acids (C6 and C8) and ethanol with H_2 as an electron donor by mixed cultures (Steinbusch et al., 2008, 2011). Furthermore, the worldwide annual demand of acetic acid was estimated to be 10 million tons in 2007 (Sauer et al., 2008; Zhang et al., 2013). Currently, acetic acid is primarily produced from petrochemical based processes, such as methanol carbonylation, acetaldehyde oxidation, and butane liquidphase oxidation processes. However, with the limited nature of fossil oil, increasing oil prices, and global concerns on sustainability and the environment, acetate production from renewable resources by mixed culture fermentation (MCF) has attracted increasing attention (Jang et al., 2012; Kleerebezem and van Loosdrecht, 2007). For example, Zhang et al. reported that acetate could be produced with extreme-thermophilic MCF and its maximum concentration was 12.5 g/L (Zhang et al., 2013), 34.4 g/L (Zhang et al., 2014c) and 13.0 g/L (Zhang et al., 2015) with syngas, glucose and glycerol as the substrates, respectively.

Soybean curd (tofu) is a common favorite food in Asian and is typically processed by soybean grinding and boiling of soybeans,

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followed by filtration, protein coagulation, preservation, and packaging. During processing, up to 30% of the soybean is lost as waste, accounting for 8×10^5 tons per year in Korea and Japan (Kim et al., 2011). However, direct discharge of tofu processing wastewater (TPW) causes serious problems of environmental pollution (Zhu et al., 2002). TPW could be effectively treated biologically as it has a high organic content consisting of 40–60% carbohydrate. Besides, Kim et al. (2011) and Zhu et al. (2002) demonstrated that H_2 could be produced from TPW. Dong et al. (2010) reported TPW could be effectively converted to methane in a UASB reactor with MCF. So, TPW can also be an alternatively ideal substrate for acetate production in MCF.

In order to achieve high acetate production, the partial hydrogen pressure (P_{H2}) is kept below 60 Pa by thermodynamic consideration in MCF according to Zhang's reports (Zhang et al., 2015, 2014c). Higher temperature could enrich hydrogenotrophic methanogens which could keep the P_{H2} below 60 Pa. As reported by Zhang et al. the acetate yields were 1.5 mol/mol–glucose and 0.63–0.7 mol/mol-glycerol when the temperature was controlled at 70 ± 1 °C (Zhang et al., 2015, 2014c). However, some studies reported syntrophic acetate oxidation (SAO) bacteria were the dominant acetate utilizers in higher temperature conditions (Hao et al., 2010; Ho et al., 2013; Wett et al., 2014). Thermodynamically, the Gibbs free energy of SAO pathway is 104.45 kJ/mol at 25 °C and 83.23 kJ/mol at 70 °C, respectively (Eqs. (1) and (2)), which indicates SAO may need less energy for maintenance and growth at 70 °C. Therefore, the SAO pathway that leads to acetate consumption might happen in extreme-thermophilic MCF. Furthermore, Shigematsu et al. (2004) found that in a mesophilic acetate-fed chemostat, SAO was the main pathway at long hydraulic retention time (HRT) (40 d), whereas the aceticlastic pathway dominated at short HRT (1.7 d). Therefore, HRT would likely be a crucial factor to manipulate acetate production in extreme-thermophilic MCF.

$$CH_3COO^- + 4H_2O = 2HCO_3^- + 4H_2 + H^+ \quad \Delta G^{0'} = +104.45\ \text{kJ/mol at } 25\ ^\circ\text{C} \tag{1}$$

$$CH_3COO^- + 4H_2O = 2HCO_3^- + 4H_2 + H^+ \quad \Delta G^{0'} = +83.23\ \text{kJ/mol at } 70\ ^\circ\text{C} \tag{2}$$

To our knowledge, the effect of HRT on acetate production from TPW in extreme-thermophilic (70 °C) MCF has not been reported. Thus, the objectives of this study were (i) determine the yields of metabolites and acetate purity in long-term operation of a continuous stirred tank reactor (CSTR) treating TPW; (ii) evaluating the effect of HRT on stable acetate production in CSTR; and (iii) analyzing the microbial community of bacteria and archaea in CSTR at different HRTs.

2. Materials and methods

2.1. Feedstock preparation and experimental setup

TPW was obtained from a local tofu company (located in Hefei, China) as a by-product from the filtration of boiled (95–105 °C) bean curd. TPW was stored at −20 °C until use. In order to reduce the effect of substrate concentration fluctuation, TPW was pretreated by centrifugation with 5000 rpm for 5 min and diluted with tap water at a 1:1 ratio (w/w). The characteristics of the raw and diluted TPW are listed in Table 1.

The inoculated anaerobic sludge was collected from a mesophilic expanded granular sludge bed (EGSB) reactor treating starch wastewater in Shandong, China (Chen et al., 2015). Firstly, the inoculated sludge was grinded and filtered through a 0.9-mm stainless steel mesh to remove large particles before purging with

Table 1
Characteristics of raw and diluted (×2) TPW.

Parameters	Raw TPW	Diluted TPW[a]
TCOD (g/L)	37.07 ± 2.99	16.08 ± 1.39
SCOD (g/L)	30.97 ± 2.75	15.24 ± 0.45
Total carbohydrate (g/L)	9.18 ± 0.78	4.32 ± 0.56
Protein (g/L)	3.42 ± 0.55	1.63 ± 0.28
pH	4.35 ± 0.12	5.25 ± 0.10
NH_4^+ (mg/L)	17.69 ± 0.53	8.58 ± 0.22
PO_4^{3-} (mg/L)	16.23 ± 0.23	7.89 ± 0.13

[a] Centrifuged at 5000 rpm for 5 min.

nitrogen (99.99%) for anaerobic conditions. The total volume of the reactor in this study was 2.5 L with a working volume of 2 L (Fig. 1). The temperature of the reactor gradually increased from 37 to 70 °C in the batch mode according to Zhang et al. (2015). Then the reactor temperature was maintained at 70 ± 1 °C with a water bath. The stirring velocity was 360 rpm and the pH was controlled automatically at 7.0 with 2 M NaOH.

After the temperature reached 70 °C, the fed-batch experiments were started to determine the maximum yields of acetate and methane from TPW. When acetate didn't accumulate further, the reactor operation mode was changed to a continuous mode. The influent substrate in CSTR was the raw TPW from 0 to 135 days and diluted TPW at days 136–210, respectively.

2.2. Batch experiments for acetate consumption at different HRTs

Batch experiments for determining the consumption of acetate and other metabolites with CSTR effluent at different HRTs were performed in serum bottles. The total volume of serum bottle was 160 mL with a working volume of 60 mL. After the addition of 60 mL effluents collected at Day 125 (HRT = 3 d) and Day 179 (HRT = 6 d), the bottles were purged with nitrogen (99.99%) for 20 min and then sealed with butyl-rubber stopper and aluminum cap. The bottles were placed in a water bath to keep the temperature of 70 °C. The hydrogen and methane contents in the headspace and the concentrations of acetate, ethanol, propionate, butyrate and other metabolites in liquid solution were measured every two days. All the tests above were carried out in duplicates.

2.3. Analytical methods

The samples from the reactors were immediately filtered through 0.45 μm membrane and analyzed for volatile fatty acids

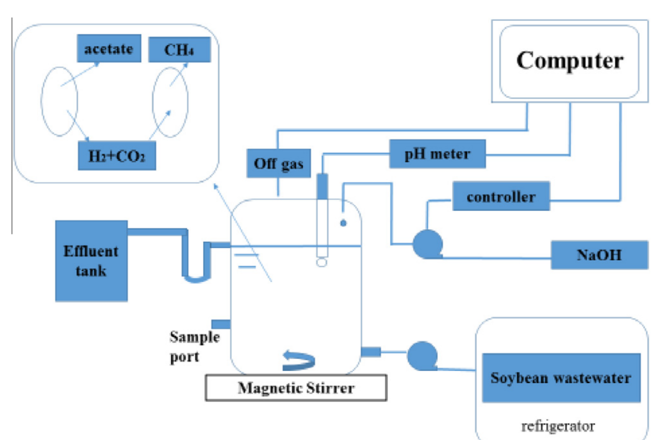

Fig. 1. Schematic diagram of acetate production from TPW.