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Lactic acid production from acidogenic fermentation of fruit and vegetable wastes

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HIGHLIGHTS

- Lactic acid with amount of 10–20 g/L was stably produced at pH 4.0.
- The fermentation type was finally converted into heterofermentation at pH 4.0.
- Hydrolysis was enhanced and lactic acid fermentation was improved at pH 5.0.
- *Bifidobacterium* played an important role for lactic acid production at pH 5.0.

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ABSTRACT

This work focused on the lactic acid production from acidogenic fermentation of fruit and vegetable wastes treatment. A long term completely stirred tank reactor (CSTR) lasting for 50 days was operated at organic loading rate (OLR) of 11 gVS/(L d) and sludge retention time (SRT) of 3 days with pH controlled at 4.0 (1–24 day) and 5.0 (25–50 day). The results indicated that high amount of approximately 10–20 g/L lactic acid was produced at pH of 4.0 and the fermentation type converted from coexistence of homofermentation and heterofermentation into heterofermentation. At pH of 5.0, the hydrolysis reaction was improved and the total concentration of fermentation products increased up to 29.5 gCOD/L. The heterofermentation was maintained, however, bifidus pathway by *Bifidobacterium* played an important role.

1. Introduction

In China, fruit and vegetable wastes (FVWs) are greatly generated approximately 1.3 million tons per day in Chinese cities, however, less than 20% were properly treated (Shen et al., 2013; Liu et al., 2012). Anaerobic digestion has been considered to be the most promising alternative technology for FVWs treatment. With the characteristics of high volatile solids and easy degradability, the FVWs could be rapidly hydrolyzed, which might lead to the acid accumulation and inhibition to methanogenesis if the reactor is overloaded. Ganesh et al. (2014) summarized the published literatures and concluded that the maximum OLR for single-phase anaerobic digestion of FVWs was within 3.6 KgVS/(m^3 d). According to the study of Shen et al. (2013), volatile fatty acids (VFAs) would accumulate to 2.3 g/L and pH would decrease to 6.8 if the OLR was increased to 3.5 KgVS/(m^3 d). And then, two-phase anaerobic digestion technology was applied for FVWs treatment to keep the stability of whole system at high OLR (Mtz-Viturtia et al., 1995; Bouallagui et al., 2004; Shen et al., 2013). However, the OLR of two-phase anaerobic digestion were only reported with limited increase to 5.7–7.7 KgVS/(m^3 d), which was summarized by Ganesh et al. (2014). In addition, the construction and operational cost were also increased for the additional reactors and alkaline addition.

Recently the concept of VFAs platform has been proposed and based on that, several end products other than methane such as polyhydroxyalkanoates (PHAs), medium chain fatty acids, biofuels or hydrogen gas are being researched (Chang et al., 2010; Reis et al., 2003; Agler et al., 2012; Steinbusch et al., 2000). There have been a lot of researches on the VFAs production from different wastes or wastewaters. Jiang et al. (2013) found that the optimal condition for VFAs production were pH 6.0, 35 °C, 11gTS/(L d) from food waste with acetate and butyrate accounting for 60%. Chen et al. (2007) reported that alkaline pH (8.0–11.0) could significantly improve the total VFAs concentration from waste activated sludge and acetate, propionate and isovaleric acid were the three main VFAs. Yu and Fang (2002) recommended that productions

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of butyrate and acetate were favored at pH of 6.0–6.5 while ethanol and propionate at pH 4.0–4.5 from dairy wastewater.

However, most of the studies focused on VFAs production (acetate, propionate, iso-butyrate, butyrate, iso-valerate, valerate), only a handful of studies paid attention to other fermentation products such as lactic acid, ethanol or succinate. Lactic acid has been verified as an available building block for PHAs and it is also widely used in industries such as the pharmaceutical, biomaterial, detergent, leather, and textile industries (Jiang et al., 2011; Kim et al., 2012; Mazzoli et al., 2014). Temudo et al. (2007) investigated the influence of pH on glucose fermentation by mixed culture and indicated that lactic acid with low yield rate of 0.2 mol/mol glucose was produced at pH of 4.0–5.0. Wang et al. (2014) evaluated the effect of pH on acidogenic end products from food waste in batch experiments and found that high concentration of 18 g/L lactic acid was produced at pH of 4.0. Selective production of lactic acid was tried with glucose as substrate by Itoh et al. (2012) and results showed that lactic acid was produced at low pH of 3.5, however, the lactic fermentation was not stable. Thus, the lactic acid could be produced from the acidogenic fermentation at low pH, however further investigation such as the fermentation stability in long term operation, fermentation mechanism and bacterial community are still necessary.

Therefore, a long term CSTR reactor was operated to investigate the possibility and stability of lactic acid production from acidogenic fermentation of FVWs treatment. The bacterial community and fermentation mechanism for lactic acid fermentation were also investigated. In addition, the method of increasing pH was also tried to improve the lactic acid fermentation.

2. Methods

2.1. Inoculums and substrate

The seed sludge was taken from anaerobic digester in Xiao Hongmen wastewater treatment plant in Beijing city, China. The pH, total suspended solids (TSS) and volatile suspended solids (VSS) concentration of the sludge were 7.8, 8.66 g/L, and 4.86 g/L, respectively.

Simulated FVWs was used as substrate composing 57% watermelon, 29% apple and 14% potato by wet weight. After crushed by a food waste disposer (produced by In Sink erator company, Model 55, 410w) and fully mixed, the simulated FVWs was stored at −20 °C in a refrigerator and the frozen FVWs was thawed at −4 °C before use every day. Raw shredded FVWs were analyzed more than ten times, and the initial total solids (TS) concentration and volatile solids (VS) were approximately 100 g/kg and 87 g/kg, respectively. The total COD (tCOD), soluble COD (sCOD) and NH_4^+-N were 137.1, 79.3 and 0.07 g/kg, respectively, and the pH was 4.5–4.8.

2.2. Reactors operation design

A CSTR reactor with a working volume of 1.5 L (total 2 L) was operated at a greenhouse maintained at 35 °C. The reactor was fully stirred by magnetically stirring. The SRT of the CSTR reactor was set at 3 days and OLR was 11 gVS/(L d) by feeding diluted FVWs (200 g raw FVWs diluted to 500 ml with tap water). The pH of CSTR reactor was controlled at 4.0 (stage 1: 1–24 day) and 5.0 (stage 2: 25–50 day) by a pH controller with addition of HCl (2 M) and NaOH (2 M). The reactor was fed and drawn off once a day and the draw-off effluent was used for analysis. The characteristics of effluent were determined every day in the first 24 days and every two days in the 25–50 day.

2.3. Analytical methods

The production of gas was flowed to the gas counters manufactured by Bioprocess AB, Lund, Sweden. Gas samples were taken through the sampling port located on the top of the reactor while effluent samples were collected from the draw-off materials every day.

TS, VS, TSS and VSS were measured according to the standard methods (APHA, 1998). The tCOD and sCOD were determined by spectrophotometry (DR6000, HACH Company, Germany) and sCOD was measured after filtration (0.45 μm). The total COD of fermentation products marked as tCODp was calculated by the sum of COD of individual fermentation product.

Volatile fatty acids (acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate) and other fermentation products including caproate, lactate, succinic acid and formic acid were determined by high performance liquid chromatography (SHIMADZU Company, Japan), using an Aminex HPX-87H column (T = 50 °C) from Bio Rad coupled to an UV (210 nm) detector, while sulfuric acid 5 mM was used as eluent at a rate of 0.5 ml/min. Ethanol was determined by gas chromatography (Agilent 7890A, Agilent Technologies, America) equipped with a capillary column (DB-FFAP, 30 m × 0.53 mm × 1 μm) and a flame ionization detector. The temperature of the injector and detector were both 230 °C. The column temperature was increased from 70 °C to 180 °C at a rate of 20 °C/min and kept at 180 °C for an additional 5 min. The nitrogen gas was used as carrier gas. Gas content (hydrogen gas, carbon monoxide, carbon dioxide and methane) was determined by gas chromatograph (Agilent, 7890A) fitted with a thermal conductivity detector and Agilent Carboxen 1000 column (4.5 m × 2 mm; mesh size 60/80). The argon gas was used as carrier gas with a rate of 30 mL/min. The temperature of injector, detector and column were 150 °C, 250 °C and 150 °C, respectively.

2.4. Bacterial community analysis

2.4.1. Clone library analysis

To analyze the structure of bacterial communities in the CSTR, the sludge of the 24th day in the CSTR was used for clone library analysis and high throughput pyrosequencing. In addition, the sludge of the 50th day was used for high throughput pyrosequencing.

The deoxyribonucleic acids (DNAs) of different sludge samples were extracted using the Fast DNA SPIN Kit for Soil (MP Biomedicals LLC., Califonia, USA). The primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and1522R (5′-AAGGAGGTGATCCAGCCAGCCGCA-3′) were used for the amplification of bacterial 16S rRNA genes (Fuller et al., 2003). The polymerase chain reaction (PCR) was performed in a final volume of 50 μL containing 5 μL of DNA buffer, 4 μL of deoxy-ribonucleoside triphosphate (dNTP), 0.5 μL of each primer, 1 μL of extracted DNA solutions, 0.5 μL Taq DNA polymerase and 38.5 μL sterile water. The operations of amplification of bacterial 16S rRNA genes were as follows: 94 °C for 2 min, 30 cycles of 94 °C for 1 min, annealing at 50 °C for 1 min and extension at 72 °C for 1.5 min and final extension at 72 °C for 5 min. The PCR products were purified with QIA quick PCR purification kit (Qiagen Company, Hilden, Germany) and then cloned into PMD18-T vector (Takara Biotechnology Co., Ltd., Dalian, China). 50 Positive clones were sequenced (completed by Takara Biotechnology Co., Ltd., Dalian, China), which were randomly chosen using a 3730XLDNAAnalyzer (Applied Biosystems Company, USA) and all sequences were submitted to the BLASTN (NCBI, USA) to obtain the closest relatives.

2.4.2. High throughput pyrosequencing and sequence analysis

The samples were sent to GENEWIZ, Inc. (Jiangsu, China) for DNA extraction and sequencing on the Illumina MiSeq