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Lactic acid accumulation from sludge and food waste to improve the yield of propionic acid-enriched VFA

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ABSTRACT

According to our previous study, propionic acid-enriched volatile fatty acids (VFA), the preferred carbon source of biological wastewater nutrient removal, can be produced via the lactic acid pathway during the two-stage fermentation of organic wastes using *Propionibacterium acidipropionici*. In the current study a new strategy, based on lactic acid accumulation from sludge and food waste, was introduced to significantly increase the yield of propionic acid-enriched VFA. First, the effect of different temperature on the two-stage fermentation was fully discussed. Both advantages and disadvantages of the fermentation in different temperatures were elaborated. Furthermore, the proposed metabolic pathway at 35 °C and 50 °C was introduced. It was observed that by initially controlling temperature at 50 °C for 4 h and subsequently at 35 °C for 2 d, the steady and maximal accumulation of lactic acid was obtained in the first stage, which resulted in the yield of propionic acid-enriched VFA in the second stage being increased to 15.3 g COD/L and the percentage of propionic acid in VFA reaching 69.9%.

1. Introduction

It has been widely recognized that nitrogen and phosphorus biological removal is highly depended on the concentration of the influent wastewater carbon source, especially VFA [1,2]. VFA can be produced by the fermentation of organic matters, such as waste activated sludge (WAS) and food waste [3,4]. Additionally, the previous study demonstrated that phosphorus removal efficiency was significantly improved with the increase of propionic/acetic acid ratio [5]. It is therefore promising to add VFA containing higher fraction of propionic acid rather than acetic acid to the influent wastewater which lack of carbon resource. Although extensively efforts have been made to convert WAS to acetic acid-containing VFA [6–8], few reference has reported the methods to improve propionic acid fraction in VFA from sludge fermentation.

In our previous finding, the addition of carbohydrate substrate to WAS fermentation increased the propionic acid percentage to approximately 48% [9]. Recently, a two-stage fermentation method based on lactic acid pathway was observed to further improve the propionic acid percentage in VFA to 68.4%, which was much higher than that reported in the literatures [10]. In the first stage the mixture of WAS and food waste was fermented to produce lactic acid, and then in the second stage *Propionibacterium acidipropionici* was inoculated to the first stage liquid to produce propionic acid [11]. Nevertheless, there were few references available regarding the strategy for improving the yield of propionic acid-enriched VFA.

According to our recent observation, lactic acid plays a key role in the two-stage fermentation of propionic acid-enriched VFA from WAS and food waste as it is the direct substrate for propionic acid production in the second stage. It is anticipated that a higher lactic acid production in the first stage would result in a greater propionic acid-enriched VFA production in the second stage. Nevertheless lactic acid is an intermediate metabolic product during the first stage which could be readily degraded by lactic acid consuming microorganisms present in WAS or food waste [12].

In this study, the effect of temperature on the two-stage fermentation from sludge and food waste was first studied. Further, the metabolic pathway in different temperature was discussed. Then, the new strategy was reported by inhibiting lactic acid consumption and optimizing lactic acid accumulation in the first stage. By the analysis of the carbon mass balance for the new strategy, the organic variations in the first stage was clearly exhibited. Finally, the yield of propionic acid-enriched VFA was significantly increased in the second stage by the optimal first stage liquid.

2. Methods

2.1. Microorganism, medium and culture, the mixture of sludge and food waste

P. acidipropionici ATCC 4875 used in this study was purchased from American Type Culture Collection. The bacterium was

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cultured in 400 mL synthetic medium in the serum bottle (working volume 500 mL) containing 5 g/L casein hydrolysate (Fluka), 10 g/L lactate sodium, 10 g/L yeast extract, 2.5 g/L $K_2HPO_4{\cdot}H_2O$, 1.5 g/L KH_2PO_4, 0.01 g/L $MnSO_4$, 0.2 g/L $MgSO_4{\cdot}7H_2O$, 0.01 g/L $FeSO_4{\cdot}7H_2O$. The initial pH was adjusted to 6.9 ± 0.2 by 3 M potassium hydroxide (KOH) or 3 M HCl, and the medium was sterilized at 121 °C and 15 psig for 20 min. To maintain the anaerobic condition, the serum bottle was sealed with a rubber cap in the anaerobic glove box after inoculation. Then, 40 mL *P. acidipropionici* inocula were inoculated to the serum bottle (unless otherwise noted, the inoculation scale was approx. 10%), and cultured in an air-bath shaker (160 rpm) at 30 ± 1 °C for 3 d for further use.

The waste activated sludge used in the current study was obtained from a municipal wastewater treatment plant in Shanghai China, and settled for 24 h. The main characteristics of the concentrated sludge were as follows: pH 6.6 ± 0.3, total suspended solids (TSS) 17.52 ± 2.17 g/L, volatile suspended solids (VSS) 12.12 ± 1.15 g/L, soluble chemical oxygen demand (SCOD) 0.29 ± 0.02 g/L, total chemical oxygen demand (TCOD) 18.85 ± 0.35 g/L. The food waste was collected from a dinning restaurant in Shanghai and eliminated tissue, chopsticks and bones. The food waste without sterilization was stored in the 4 °C refrigerator for the repeated tests. The characteristic was TSS 87.62 ± 5.32 g/L, VSS 85.28 ± 3.28 g/L, TCOD 131.21 ± 14.66 g/L, SCOD 41.32 ± 5.11 g/L. Unless otherwise noted, the mixture of sludge and food waste used for the first stage fermentation was added with tap water to make the final total chemical oxygen demand (TCOD) of 25.0 ± 1.5 g/L. The mixing ratio of sludge to the food waste was recorded as mass ratio (g/g) of 0.24:1 according to the previous study [10].

2.2. Operation of the two-stage fermentation system

From Fig. 1, the two-stage fermentation system includes: (1) the first stage reactor (working volume of 3.0 L) to produce lactic acid from the mixture of sludge and food waste; and (2) the second stage reactor (working volume of 1.1 L) to convert lactic acid to propionic acid-enriched VFA using *P. acidipropionici*. The first stage reactor (mechanically stirred at 100 rpm) was maintained at pH 8.2 ± 0.4 using 5 M NaOH or 5 M HCl. The mixture of sludge and food waste (characterized as above) was added to the first stage reactor, the temperature of which was controlled by water bath equipment. After certain days of fermentation, the first stage suspension liquid was obtained 0.9 L via centrifugation (× 3000 g by 5 min). Subsequently, it was autoclaved (121 °C and 15 psig for 20 min) in the second stage reactor. Then, the second stage reactor was inoculated with 0.1 L *P. acidipropionici* inoculums (cultivated for 3 d already) followed by nitrogen flush. The second stage fermentation was operated at 30 °C and pH 7.0 ± 0.5 for several days.

2.3. Effect of temperature on the two-stage fermentation

As mentioned above, four first stage reactors were operated, respectively at 20 °C, 35 °C, 50 °C and 65 °C water-bath during the entire first stage fermentation. The concentration of lactic acid in each reactor was assayed every 12 h during the time course of 96 h. The VFA content in the first stage fermentation was recorded, respectively at 36 h, 60 h and 96 h. But after 60 h the liquid phase from the first stage was obtained and the second stage fermentation was conducted for 6 d according to the above operational procedures. The concentration of the VFA in the second stage reactor was measured each day during the entire second stage fermentation. The data recorded are the averages of triplicate tests.

2.4. Study of lactic acid producing and consuming metabolic pathway

In the co-fermentation of lactic acid from sludge and food waste, lactic acid consuming bacteria were both come from WAS and food waste [10]. It is necessary to separate two substrates and investigate the performance, respectively in consuming lactic acid at different temperature. Thus, four serum bottles (600 mL each) were inoculated with 50 mL WAS and 450 mL synthetic wastewater containing 10 g/L lactic acid, 2.5 g/L $K_2HPO_4{\cdot}H_2O$, 1.5 g/L KH_2PO_4, and the pH value was adjusted to 8.2 ± 0.2 by adding 5 M hydrochloric acid (HCl) or 5 M sodium hydroxide (NaOH). Meanwhile, another four serum bottles were also prepared containing the mixture of 50 mL food waste and 450 mL synthetic wastewater as above. After flushed with nitrogen, the serum bottles were capped with butyl elastic rubber stoppers to separate oxygen from outside, and placed in the water-bath shakers (120 rpm) at different temperatures: 20 °C, 35 °C, 50 °C and 65 °C. To prevent the recovery of enzyme activity from the experimental procedure (like centrifuge, wash, suspension etc.) at the room temperature, relatively sufficient fermentation time of 2 d was conducted in all serum bottles. Thus, lactic acid and VFA were assayed after 2 d. Simultaneously, the activities of the key VFA-forming enzymes (acetate kinase (AK) and propionyl CoA to succinyl CoA transferase (CoAT)) and lactic acid production enzymes (lactate dehydrogenase (LDH), NAD independent lactate dehydrogenase (iLDH)) in the serum bottles sets with WAS were compared.

2.5. Optimization of lactic acid accumulation for improving the yield of propionic acid-enriched VFA

According to the procedure of the two-stage fermentation, four reactors were initially operated at 50 °C, respectively for 1 h, 2.5 h, 4 h and 5.5 h, which were named for R-1 h, R-2.5 h, R-4 h and R-5.5 h. Then, all reactors were operated at 35 °C for another 80 h. During this first stage fermentation, lactic acid was assayed every 12 h. The specific efficiency (E_a) of lactic acid production was calculated as $E_a\left(g/(g \times d)\right) = [\text{lactic}]/[\text{TCOD}] \times [\text{time}]$. The carbon mass balance was analyzed to record the variations of the fermentative matters, samples for which was taken before and after the 50 °C pretreatment, 48 h and 72 h at 35 °C fermentation. But after 48 h of the first stage fermentation, 0.9 L of the first stage suspension liquids were obtained, respectively from the four reactors and another four second stage reactors were conducted accordingly. After 5 d of the second stage fermentation (i.e., VFA content was stable), the yield of VFA from the four reactors were measured and the content of propionic acid in VFA was documented.

2.6. Analytical methods

Lactic acid was measured by high-performance liquid chromatography (Agilent 1200, USA) equipped with refractive index detector (RID-10A) and Bio-Rad Aminex HPX-87 (300 × 7.8 mm) analytic column. 5 mM (mmol/L) H_2SO_4 was used as mobile phase at a flow rate of 0.6 mL/min. The injection volume was 20 μL. The COD equivalent of lactic acid was 1.08 g COD/g lactic acid. The method for VFA assay was documented previously [10]. The VFA content was calculated as the sum of measured acetic, propionic, *n*-butyric, iso-butyric, *n*-valeric and iso-valeric acid. The cell extract for the assay of VFA-forming enzymes and lactic acid-forming enzymeswas prepared as follow: 50 mL of the fermentation mixture was taken out from each serum bottle, which was centrifuged, washed and resuspended to 15 mL of 30 mM Tris/HCl (pH 7.4). The suspension was sonicated at 35 kHz and 4 °C for 30 min to break down the cells. To remove the debris, 2 mL of the mixture was centrifuged at 10,000 rpm in 4 °C for 30 min. The supernatant