Enhancing selective butyric acid production by controlling fermentation conditions

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Introduction

The concepts of waste valorization and circular economy have driven many studies focusing on volatile fatty acids (VFAs) production through anaerobic fermentation because of their economical benefits. Many efforts have been made to explore efficient VFAs production, such as control of the temperature, organic loading rate and ratio of inoculum to substrate (ISR) (Wainaina et al., 2019; Feng et al., 2022). However, the high cost of recovery and purification hinders the subsequent reuse of the mixture of VFAs products. Therefore, VFAs selective production is very critical. Butyric acid is considered to be a valuable fermentation product because of its low solubility, high global market value and a vast of applications (Chen et al., 2022). In this study, batch experiments were carried out to explore the optimal operating conditions (temperature, pH and ISR) for the butyric acid selective production and investigate the metabolic pathway of VFAs in different fermentation conditions.

Materials and methods

Batch experiments were performed using serum bottles with a working volume of 400 ml. Glucose (3.0 g COD/L) was added as the carbon sources, and the inoculum was pre-activated anaerobic sludge. The parameters assessed were temperature (37 ºC and 55 ºC), pH (4.5 – 7.0, 8.5 – 11.0) and ISR (1:3, 1:2, 1:1, 2:1 and 3:1). The pH and temperature experiments were conducted first, whose results decided the pH and temperature in the ISR experiment (pH 5.5 and 37 ºC). pH was adjusted using 2M HCl or 2M NaOH every two days. All tests were carried out in triplicates. VFAs (acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid and valeric acid), ethanol, and lactic acid concentrations were measured in the samples taken from the reactors at intervals. pH and oxidation-reduction potential (ORP) were determined using pH/ORP meter. At the end of the reaction, biomass samples were collected for metagenomic sequencing.

Results

The butyric acid selective production was investigated under different temperature, pH and ISR. As shown in Fig.1, the results indicated that butyric acid selective production was related to temperature, pH and ISR. When pH ranged from 4.5 to 7.0, acetic acid, propionic acid and butyric acid were the predominate products at 37 ºC. The highest proportion of butyric acid occurred at pH 4.5, 5.0, 5.5 and 6.0 were 74%, 69%, 59% and 51%, respectively and butyric acid concentrations were up to 1260.5 mg COD/L, 1326.7 mg COD/L, 1314.8 mg COD/L and 1113.9 mg COD/L, respectively. When temperature was 55 ºC, the dominate products were changed to ethanol, butyric acid and acetic acid and the proportion of butyric acid varied from 9% to 28% with pH from 4.5 to 7.0. Alkaline conditions (pH from 8.5 to 11.0) were not favourable for butyric acid production. Butyric acid concentrations were lower than 500 mg COD/L, especially, almost no butyric acid production when pH was 9.5 - 11.0. Notably, lactic acid was detected with pH 10.5 and 11.0 at 55 ºC. Therefore, in the subsequent ISR experiment, pH and temperature were controlled at 5.5 and 37 ºC, respectively. Fig.1e indicates that the predominate products in fermentation were only acetic acid and butyric acid in ISR experiment except little propionic acid was detected when ISR was 1:2. The butyric acid proportion and butyric acid concentration were increased with ISR decreasing from 3:1 to 1:3. Butyric acid proportion was as high as 79% and butyric acid concentration was 1513.2 mg COD/L when ISR was 1:3.

ORP affected the distribution of cell metabolism by changing the ratio of NAD+/NADH, thereby changing the distribution of fermentation end products, and the optimal ORP range for butyric acid production was from -350 to -200 mv (Wang et al., 2021). The change of ORP values for different pH, temperature and ISR is present in Fig.2. The ORP values decreased with the pH increasing at 55 ºC. However, at 37 ºC, the ORP values were fluctuated with pH value ranging from 4.5 to 7.0. As shown in Fig.2c, the ORP value decreased from -219.4mv to -411.3 mv with the ISR decreasing, resulting in butyric acid concentration increasing. Compared to acetic acid, butyric acid production needed a lower ORP value. Controlling optimal ORP value is beneficial for butyric acid production.

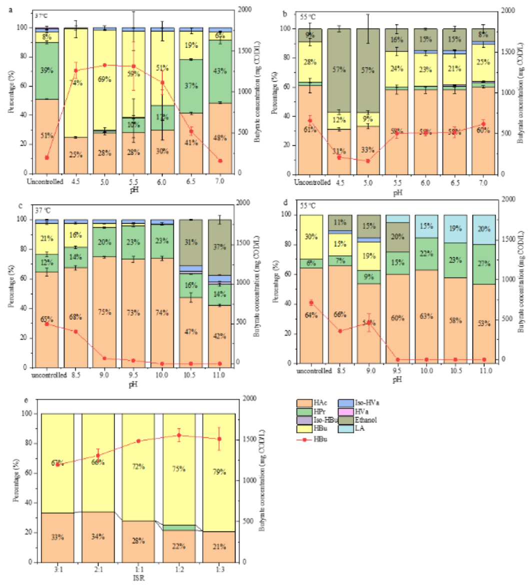

Fig.1 The percentage of VFAs and butyric acid concentration in different conditions

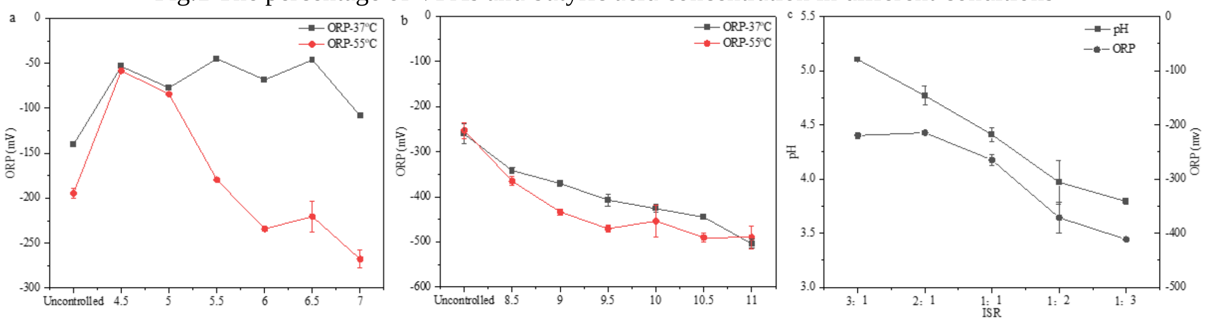

Fig.2 Change of pH and ORP at different conditions

Conclusions

The study investigated the influence of environmental factors (pH, temperature and ISR) on the production of butyric acid through fermentation. The results showed that pH, temperature and ISR significantly affected the butyric acid production. The optimal condition for butyric acid selective production was pH 5.5, 37 ºC and ISR 1:3. Controlling the ORP value may be a good option for further improving butyric acid production. The results of this study would provide technical support for the regulation of butyric acid production pathway.

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