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Improving hydrogen generation from kitchen wastes by microbial acetate tolerance response

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ABSTRACT

The microbial acid tolerance response (ATR) was adopted to improve the hydrogen yield by relieving the organic acids inhibition. The results indicated that the hydrogen generation from kitchen wastes could be enhanced with the acetic acid stress of 6.0 g/L, and it reached 68.3 mL/gVS, which was 2.09 times of the control. The improvement was due to the increase of acetic acid tolerance with more ATR induction, since the increase of hydrogen yield was positive correlation with the concentration of acetic acid. H^+-ATPase activity system played an important role in the ATR. It reached maximum of 132.4 U/gTS at 6.0 g/L stress, which was 45.2% higher than the control. Dehydrogenase enzyme in 6.0 g/L group improved of 50.6% than the control, and it could indicate the microbial activity during hydrogen generation process.

1. Introduction

Hydrogen has been regarded as a clean energy carrier and is found to be one of the potential alternative to fossil fuel energy [1,2]. Dark fermentation technology is a favorable method for hydrogen generation, since the high hydrogen production rate can be achieved at low cost with various organic substrates [3,4].

Kitchen wastes contain a large amount of volatile organic compounds mainly in terms of single sugars, starch and protein, which are a potential feedstock for hydrogen production [5]. Hydrogen generation was accompanied by the production of organic acids and alcohols during its digestion process [6]. There are three known hydrogen fermentation types, namely butyric-type, propionic-type and ethanol-type, which could be characterized by the production of butyrate and acetate acids, propionate and acetate acids, ethanol and acetate acids, respectively [7]. The accumulation of such acids in digestion system will cause substrate inhibition and low hydrogen yield, since nonpolar undissociated acids can cross the cell membrane at a low pH value, dissociate within the cell, and uncouple the proton motive force [8]. To solve this problem, external regulatory methods of adjusting the hydrogen pressure [9], cogeneration methane [10] and photo fermentation [11] were proposed. However, few of them were involved in the intensive tolerance of hydrogen generation microorganism itself.

Previous studies have shown that many anaerobes can generate acid tolerance response (ATR) to adapt for acid stress during the reaction system [12]. It was also reported that this responses could effectively avoid cytoplasmic acidification, alleviate organic acid inhibition and will be beneficial to hydrogen evolution under acid pressure [13]. Moreover, this effect was very important for the degradation of kitchen wastes, as a lot of organic acids were formulated during the fermentation process. External acid stress could improve the tolerance of anaerobes, by activating an alternative microbial sigma factor of RNA polymerase or DNA-binding two-component response regulators [12].

Our previous study indicated that certain concentration of butyric acid stress could improve the acid tolerance of hydrogen generation microorganisms, and then enhance hydrogen accumulation [14]. However, the mechanism of ATR was not involved. Recent study showed that an H^+-ATPase (proton pumps) system is an important mechanism of inducible enzymatic ATR for the hydrogen-generation bacteria, since it could lessen the cell acidification by pumping the H^+ of cytoplasm to outer cell membrane [13]. Furthermore, dehydrogenase enzyme was found to be the crucial enzyme for inter conversion of the organic substrate resulting in generation of protons and electrons during hydrogen fermentation [15].

This study focused on understanding and optimizing the hydrogen generation from anaerobic microorganisms by acetate response. In addition, the volatile fatty acids (VFA), H^+-ATPase activity and dehydrogenase enzyme during hydrogen generation process were investigated.

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2. Materials and methods

2.1. Kitchen wastes and inoculum

The kitchen wastes were collected from dining hall in Jiangnan University. Kitchen wastes were made up of rice, meat, vegetable, bones, lipid, paper, etc., the wastes were grinded after bones, crushed paper and caps were singled out. The characteristics of substrates were shown in Table 1.

Anaerobic granular sludge was obtained from an anaerobic biogas digester of Xielian Thermal Power Plant Ltd. The digester was operated at about 35 °C at a hydraulic retention time (HRT) of 24 h, which disposal citric acid wastewater. After being autoclaved at 121 °C for 15 min to eliminate non-spore-harboring methanogens, the sludge was activated using synthetic wastewater, as described by Yan et al. [16], for 7 days. Finally, the hydrogen generation sludge was ready for acetate stress treatment.

2.2. Acetic acid tolerance process and experiment setup

Acid stress was conducted in a 500 mL reaction bottle, the bottle contained different acetic acid concentration of 0, 2.0, 4.0, 6.0, 8.0 and 10.0 g/L, respectively, the hydrogen generation sludge was inoculated into the bottle, the sludge to solution ratio (based on weight) was 3:4, and then the bottles were agitated at 70 rpm and 35 ± 1 °C under anaerobic condition for 7 days. No nutrition was added to the bottles during the stress process. The sludge was washed with de-ionized water after stress process, and as inoculums for hydrogen production. The total solid (TS) and volatile solid (VS) of sludge were 11.26% (wet basis) and 5.06% (wet basis), respectively, while the diameter of sludge was about 2 mm.

Kitchen wastes and sludge with the inoculation rate of 1:2 (VS contents) were put into 500 mL reaction bottle, the total volume of kitchen waste and sludge was 200 mL, the initial pH was adjusted to 7.5 after adding 200 mL water. The headspace of the reaction bottles were purged with nitrogen to maintain an anaerobic environment, and then the bottles were agitated at 70 rpm and reaction temperature was kept constant at 35 ± 1 °C in a shaking water bath. Finally, batch fermentation for hydrogen generation was started.

2.3. Analytical methods

TS and VS were determined by the standards methods of SEPA [17], nitrogen and protein were analyzed by using the Kjeldahl method, lipids were determined by using the Soxhlet method [18], total carbon was monitored with a total organic carbon (TOC) analyzer (Elementar, Germany), carbohydrate was determined by phenol sulfuric acid method [19].

The composition of hydrogen was detected using a gas chromatograph equipped with a thermal conductivity detector and a stainless packed column. Operating temperature of column and detector was kept at 90 °C and 100 °C, respectively. Argon was used as the carrier gas at the flow rate of 0.25 mL/s.

VFA was analyzed by using an high performance liquid chromatography (HPLC) equipped with a UV detector with the wavelength of 210 nm and with a ZORBAX SB-A column (300 × 7.8 mm, Biorad, USA) at the column temperature of 30 °C. 0.5% of acetonitrile and 99.5% of KH_2PO_4 (0.02 mol/L) were used as the mobile phase with a flow rate at 0.5 mL/min.

H^+-ATPase activity was assayed by the method of our previous article [13]. Dehydrogenase enzyme activity assay was based on estimation of the triphenyltetrazolium chloride (TTC) reduction rate to triphenyl formazan [20].

3. Results and discussion

3.1. Hydrogen performance from kitchen wastes by acetate tolerance response

Fig. 1(A) showed the change of hydrogen accumulation during fermentation process with different acetate stress concentrations. Hydrogen generation started after about 5 h of lag phase in each bottle, and it increased rapidly with time up to 20 h, after which time it remained stable. The final hydrogen yield first increased with the stress concentration, but it decreased since 6.0 g/L group. The maximum yield reached 68.3 mL/gTS with 6.0 g/L stress, which was 2.09 times of the control. However, it was only 29.9 mL/gTS when stress concentration was 10.0 g/L, which was lower than the control.

The specific hydrogen generation rates in all groups firstly showed increased but then decreased. The maximum value occurred at 10th h (Fig. 1B). The 6.0 g/L group reached maximum of 5.92 mL/(g_TS h), which was 1.03 times higher than the control. The change of 4.0 g/L group was similar to the 8.0 g/L group.

It seemed that certain concentration of acetic acid stress could enhance the acid tolerance response of hydrogen-producing microorganism and then increase the hydrogen yield. However, the microorganism would be inhibited when the stress concentration surpassed 6.0 g/L, since high levels of undissociated acetic acids could pass through cell membranes and cause loss of activity of the relatively acid-sensitive glycolytic enzymes and damage macromolecules [21]. This finding was consistent with our previous study of butyric acid stress [14], however, the optimal stress concentration of acetic acid was higher than the butyric acid concentration (4.0 g/L). This indicated that acetic acid showed less toxic than butyric acid in the tolerance response. Undissociated butyric acid was more lipophilic and nonpolar than acetic acid, which could enter cells more easily and cause more cytoplasmic acidification. In addition, excess butyric acid could hamper NAD^+ regeneration, and further decrease the level of metabolic activity [8].

The hydrogen yield was also affected by the component of kitchen wastes, since the complex nature of kitchen wastes may adversely affect its biodegradability. Bio-hydrogen production in the dark fermentation is performed by the Embden–Meyerhof pathway, the carbohydrate, protein and lipids of the kitchen wastes was first hydrolyzed to monosaccharides, peptides and amino acids, free fatty acids and glycerol, which were further converted to hydrogen, volatile fatty acids and carbon dioxide, respectively [22]. Lay et al. [23] indicated that the hydrogen producing potential of carbohydrate was more effective than protein and lipids. The concentration of carbohydrate took a large proportion of kitchen wastes in this study, and this made it a potential feedstock for the biological hydrogen generation.

Table 1
The characteristics of kitchen wastes.

Substrate	Total solids (TS/%)	Volatile solids (VS/%)	Total carbon (mg/gTS)	Total nitrogen (mg/gTS)	Carbohydrate (/TS)	Protein (/TS)	Lipids (/TS)
Kitchen wastes	15.76	14.35	726.78	30.18	52.32	18.45	10.65

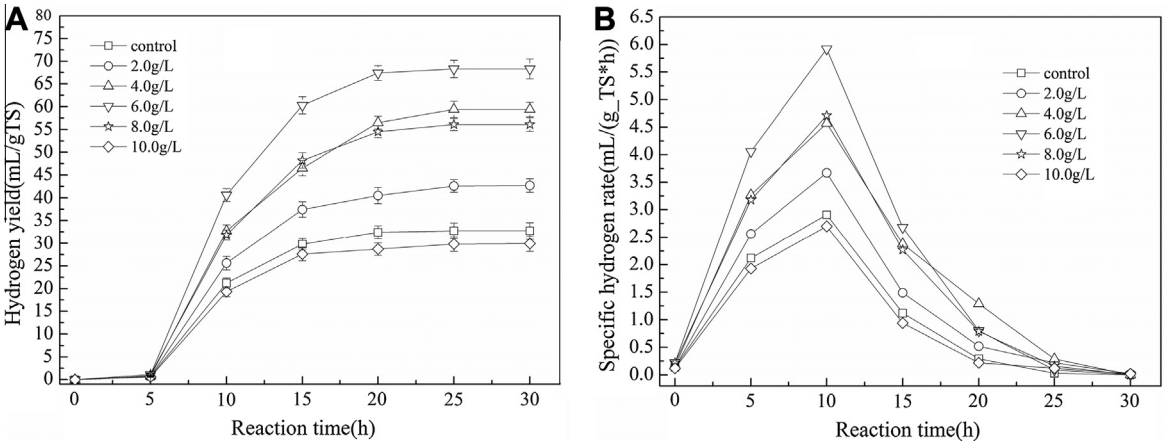

Fig. 1. Hydrogen variation with different acetic acid response (A) hydrogen accumulation yield; (B) specific hydrogen rate.

3.2. Effect of acetate response on VFA accumulation

Hydrogen accumulation was accompanied with VFA generation, and the variation of VFA could reflect the hydrogen yield. The total VFA was first increased then decreased in each group (Fig. 2). The largest improvement phase was occurred from 15th h to 20th h, and total VFA concentration increased of 4371.7, 4289.6, 3711.8, 4445.5, 4202.2 and 3772.8 mg/L during this period, respectively. The maximum concentrations were achieved at 20th h of 8755.2, 8835.9, 8428.2, 9939.0, 9319.1 and 8099.2 mg/L, respectively. However, the concentration decreased afterward, and the final concentrations were 8343.8, 8415.8, 7897.0, 9420.4, 8733.4 and 7321.9 mg/L, respectively.

The dominant components were acetic acid and butyric acid in all groups since 10th h. The concentrations of acetic acid and butyric acid account for 87.9%, 89.7%, 89.9%, 91.2%, 89.3% and 82.7% of the total VFA, which was higher than that reported (75%) by Li et al. [24], suggesting this was butyric-type fermentation. It only had acetic acid, propionic acid and butyric acid in each reaction bottle except the 10 g/L stress group at the end of reaction. It was found that the maximum acetic acid after reaction was 3799.4 mg/L in 6.0 g/L group, which improved 30.7% compared to the control, while it decreased 14.1% in 10 g/L group. The concentration of lactic acid could be negligible when compared to the other organic acids, and it disappeared at the end of reaction except 10 g/L group. Propionic acid took 9.35% of the residual acids in 10 g/L group,

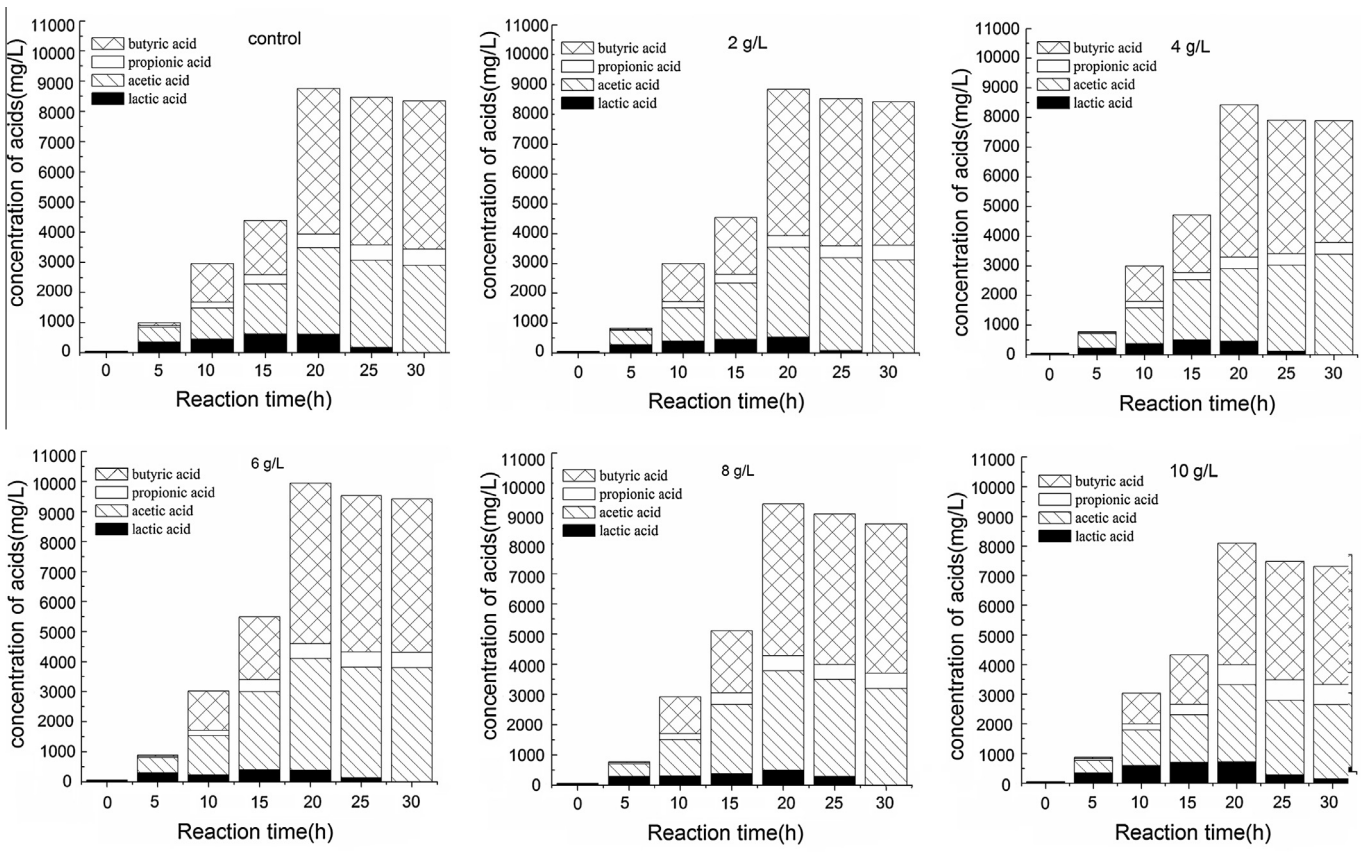

Fig. 2. The change of VFA under different acetate response.

which was higher than other groups. Propionic acid was considered to be a product of hydrogen consumption during hydrogen generation [7], and high concentration of propionic acid in the system was usually with low hydrogen yield.

It was interesting to find that the change of hydrogen yield was positive correlated with the final acetic acid concentration (Fig. 3). Hydrogen yield increased maximum of 109.0% to the control in 6.0 g/L group, at the same time its acetic acid also improved maximum of 30.7% to the control. However, the hydrogen yield and the concentration of acetic acid decreased of 8.6% and14.1% in 10 g/L group, respectively. This suggested that the suitable acetic acid response could improve the tolerance of microorganism and at the same time enhance the hydrogen yield, which confirmed the results of Huang et al. [13].

3.3. Effect of acetate response on H^+-ATPase activity in the anaerobic system

H^+-ATPase proton pump could be responsible for the survival of microorganisms in acidic environments [21]. Fig. 4 presented the profile of H^+-ATPase activity in the reaction process. All groups showed first increasing then decreasing trend. Each group rapidly increased from initial about 70 U/gTS to the maximum value of 91.2, 102.3, 112.4, 132.4, 119.2 and 88.1 U/gTS, respectively. The peak time all occurred at 5th h. After which time, the activity decreased to 48.2–88.1 U/gTS at the end.

The motivation of removing electrons and acids out of cells by bacteria will be enhanced to avoid metabolism cessation or solventogenesis initiation when organic acids was accumulated [25], as a result, the H^+-ATPase activity improved in all groups. The data also indicated that after appropriate acetate response, it could obtain more H^+-ATPase than the control. This difference could probably be attributed to the bacteria population change with adaptive evolution.

The bacteria was threatened when the organic acids was dramatically increased in the system (Fig. 2), since self-produced acids were more toxic than externally added acids [25]. The decreasing in H^+-ATPase activity with the accumulation was greater in acid-intolerance group than in tolerant group. This reflected that the H^+-ATPase in acid-intolerant bacteria was more sensitive to the low pH than acid-tolerant bacteria, which was the same opinion as Miwa et al. [26]. It also indicated that ATR through H^+-ATPase system could be induced efficiently by acetate-tolerance bacteria via adaptive evolution, and then improve hydrogen yield. This also may help to explain the increasing of sludge tolerance by butyric acid stress and the enzyme activation improvement in our previous study [14].

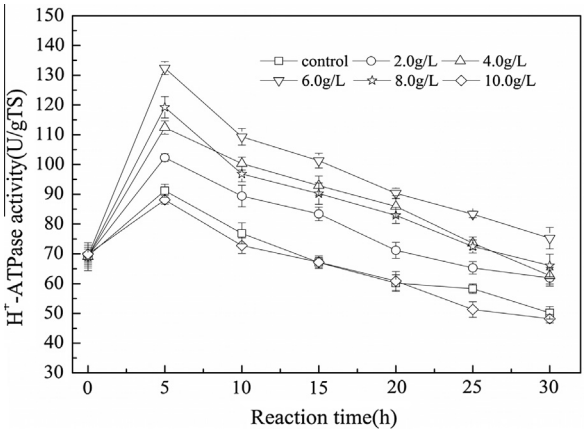

Fig. 4. The change of H^+-ATPase activity during the fermentation process.

3.4. Dehydrogenase enzyme characterization during the reaction process

Dehydrogenase enzyme function was important for transferring the proton between metabolic intermediates [27]. The concentration of dehydrogenase enzyme was initially increased sharply in the first 10 h, but decreased afterward in each group (Fig. 5). The peak time was occurred at 10th h, with the concentration of 3256.7, 3984.3, 4409.4, 4903.4, 4598.4 and 2875.4 μg/(g_TS h), respectively. The maximum enzyme in 6.0 g/L group improved of 50.6% than the control. However, the enzyme decreased afterward in each group, and final concentration was 687.3–1434.5 μg/(g_TS h). The variation of enzyme in this study was similar with our previous study [14].

Kitchen wastes was fast hydrolysis during the initial time, and the activity of microorganisms kept relatively high, as a result the enzyme increased rapidly. However, the enzyme declined with the deficient of substrate and the drop of pH (final pH was 4.0–4.5).

The data showed that the change of dehydrogenase enzyme was in accordance with the hydrogen accumulation, since the high hydrogen output also occurred during the first 10 h and subsequently weakened (Fig. 1), this revealed that the enzyme might indicate the microbial activity, which in agreement with Yang et al. [28].

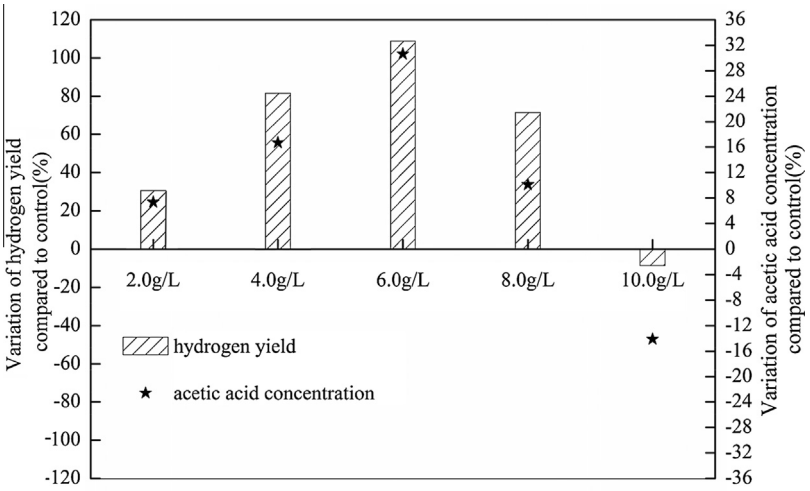

Fig. 3. The change relationship between acetic acid concentration and hydrogen yield.

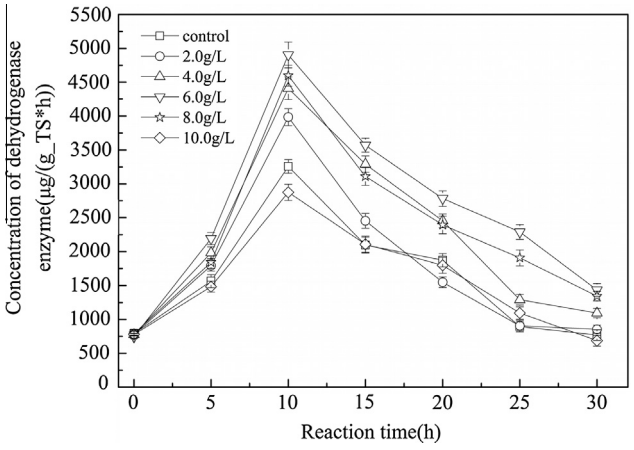

Fig. 5. Variation in the enzymatic activity of dehydrogenase enzyme.

4. Conclusions

The effect of microbial acetate tolerance response on hydrogen generation from kitchen wastes was investigated in this study. The results indicated that the hydrogen yield reached maximum of 68.3 mL/gVS under 6.0 g/L stress, which improved 109% of the control. The change of hydrogen yield was positive correlated with acetic acid concentration. H^+-ATPase activity system played an important role in the ATR. The peak time of dehydrogenase enzyme occurred at 10th h, and the maximum concentration was 4903.4 μg/(g_TS h) in 6.0 g/L group.

Acknowledgements

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