The Mechanism of High Butanol Acetone Ratio in ABE Fermentation with Fern Root as Substrate

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The butanol acetone ratio (B/A ratio) is a critical index to evaluate the process of ABE fermentation. In this article, fern root starch with yeast extract added (FY) was used as substrate for ABE fermentation. The final B/A ratio in a 5 L anaerobic fermentor reached the highest levels of 3.42, which signified an increment of 41% compared with corn. The mechanisms for the high B/A ratio of FY substrate were as follows: 1) the weakened organic acid circuit resulted in decreased acetone synthesis; 2) the NADH level of butanol synthesis was high; 3) coA transferase activity was low and butanol dehydrogenase activity was high. The final butanol concentration and substrate utilization were examined in a 5 L anaerobic fermentor, and the results validated the feasibility of ABE fermentation with FY as substrate.

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INTRODUCTION

With the increasing depletion of fossil energy and the broad recognition of sustainable development, bioenergy has attracted more attention (Chiao and Sun 2007; Himmel *et al*. 2007; Lee *et al*. 2008; Ni and Sun 2009). Butanol is a promising biofuel and an important commodity chemical, and it has been applied in various occasions (Nigam and Singh 2011; Jiang *et al*. 2015).

Butanol fermentation is also known as acetone, butanol, and ethanol (ABE) fermentation. In ABE fermentation, researchers aim to increase the butanol concentration or its ratio over the total solvents. However, those operational strategies generally suffer from problems such as, lower ABE productivity, high refinery costs, expensive supplemental substrate usage, and operational complexity. The current methods to solve the above problems include: 1) screening of high-butanol-producing strains (Jiang *et al.* 2009); and (2) using cheap non-food biomass to reduce the cost of raw materials (Ibrahim *et al*. 2018; Mao *et al*. 2019). Conventional starch (corn) or sugar (molasses) substrates are expensive, which reduces the economic feasibility of ABE fermentation (Jones and Woods 1986; Ibrahim *et al*. 2018). (3) The traditional ABE fermentation product is a mixture, and the cost of product separation and refinement is very high. It is necessary to increase B/A ratio (mass ratio of butanol to acetone) or B/ABE (mass ratio of butanol to total solvent) ratio as much as possible while ensuring the concentration of butanol unchanged. The B/A ratio can be increased by improving the fermentation strains, adjusting the redox potential

(ORP), using mixed fermentation materials with strong reducing power, and adding trace electronic carrier materials (such as neutral red, methyl violet, *etc*.) (Wang *et al*. 2020). In a previous study, the proportion of butanol to total solvent reached 71.9%, and B/A ratio reached as high as approximately 3 with strain ea2018 (Tummala *et al.* 2003). However, the high B/A ratio was achieved by decreased acetone production, yet the butanol yield was basically unchanged. Another study used whey medium as substate, and a high B/A ratio of 100:1 was reached, but this is due to low butanol and acetone yield close to 0 (Bahl *et al*.1986). Besides the strain improvement, researches tried to introduce materials to help enhance B/A ratio: adding a small amount of electron carriers, such as neutral red, methyl violet and other pigment substances (Girbal *et al*. 1995); adding a small amount of pure butyric acid as a fermentation auxiliary material (butyric acid/glucose mass consumption ratio 4% to 8%) (Li *et al*.2014); aerating CO gas to inactivate the hydrogenase in the electron reciprocating shuttle system, and making full use of H^+ in the NADH synthesis pathway (Kim *et al*. 1984), *etc.* However, the above-mentioned methods did not significantly increase the concentration of butanol or B/A ratio, and there were disadvantages such as the need to discolor the fermentation broth, potential safety hazards, and expensive auxiliary materials. Luo *et al.* (2015) used the mixed culture of *Clostridium acetobutylicum/Saccharomyces cerevisiae* and added a small amount of synthetic butyric acid to successfully increase the butanol concentration and B/A ratio to 15.7 g/L and 2.83 respectively. However, the costs of these media are high and thus not feasible for industrial production. Therefore, despite many previous attempts, it is still urgent to develop new ways to optimize the B/A ration in ABE fermentation with cheap raw materials, high utilization of starch, cheap culture medium, and high utilization of NADH.

Fern root (FR) could be a promising substrate for ABE fermentation. Mao *et al*. (2019) reported that FR alone was not an effective substrate, but they produced butanol effectively when the material was supplemented with other organic nitrogen. In this study, B/A ratio in ABE fermentation using fern root with yeast extract added was investigated. The mechanism of high B/A ratio was explored by nitrogen content analysis, metabolic flux analysis (MFA), and enzyme analysis. 5 L fermentor tests were carried out to validate the feasibility.

EXPERIMENTAL

Microorganism and Media

The bacterial strain used in this study was *Clostridium acetobutylicum* CGMCC1.0134, which was obtained from the China General Microbiological Culture Collection Center (Beijing, China). Seed was stored as spore suspension in a 5% corn meal medium at 4 °C (Li *et al.* 2014; Mao *et al.* 2019). The FR/corn starch used for study was purchased from a local market. FR/corn starch was pretreated by adding a small amount of α-amylase (8 U/g-corn, heated in boiling water bath for 45 min) after being sieved up to a mesh size of 60. The medium was autoclaved in neutral pH at 121 °C for 15 min. Alpha-amylase (20,000 U/mL) was purchased from Aladdin Industrial Corporation (Shanghai, China).

ABE Fermentation

A 150 mL seed culture was prepared in a 250 mL fermentation bottle and was incubated in an anaerobic incubator (YQX-II, Shanghai Heng Yue Medical Devices

Corporation, Shanghai, China) at 37 °C for 20 h using corn meal (5 %, W/V) as the substrate. ABE fermentation was then carried out by adding 15 mL of seed liquid into 150 mL of fermentation medium (7 % corn medium or 5 % FR medium, W/V) in a 250 mL fermentation bottle. The cell growth, pH value, acetic acid (C_{ac}), butyric acid concentration (C_{by}), solvents concentration, glucose concentration (C_{glu}), and residual concentrations were measured during the fermentation process. The experiments were repeated in triplicate under the same conditions for each substrate.

Analytical Methods

Cell growth was measured by cell DNA. The DNA content was analyzed colorimetrically in deproteinized trichloroacetic acid (TCA, $C_2HCl_3O_2$) extracts (Martin and McDaniel 1975; Mao *et al*. 2019). The measurement of starch and C_{glu} followed the previous studies (Luo *et al*. 2016; Mao *et al*. 2019). Acetic acid, butyric acid, acetone, butanol, and ethanol were determined by gas chromatography (GC-2014C, Shimadzu Corporation, Kyoto, Japan) with a C_{18} column (ZKAT-FFAP). The above analysis was carried out under the following conditions: 1) oven temperature: the initial temperature was 40 °C. After retaining for 1 min, it was warmed to 70 °C with a heating gradient of 3 °C/min. The temperature was retained for another 1 min and then heated up to 140 °C at 5 °C/min. The temperature was kept constant again for 1 min, then heated up to 200 °C with a gradient of 15 °C/min and kept for 15 min; 2) injector temperature: 160 °C; 3) detector temperature: 220 °C; 4) carrier gas (nitrogen) flow rate: 2 mL/min; 5) hydrogen flow rate: 40 mL/min; 6) air flow rate: 400 mL/min. Free amino acid concentrations in the broth were determined by an automatic amino acid analyzer L-8900 (HITACHI Construction Machinery Corporation, Tokyo, Japan). Sample pretreatment was the same as described in a previous study (Li *et al*. 2012). The measurement of key enzyme gene transcription levels was described previously (Mao *et al*. 2019). The total gas concentration was measured by the drainage method. After several hours of fermentation, the total evolved gas amount was measured by collecting the gas in a graduated tube filled with water every hour. The hydrogen concentration was measured with a hydrogen analyzer (HGS-10C, Beijing Steidi Automatic Control Equipment Co., Ltd., Beijing, China). It was assumed that H_2 and CO_2 were the only two components in the gas.

Analysis of Metabolic Network of *Clostridium acetobutylicum*

In the calculation of metabolic flux in this paper, the absolute consumption rate of glucose was defined as 100 $mmol \cdot L^{-1} \cdot h^{-1}$, and the remaining calculations were based on this assumption. All the metabolic intermediates in the cell were treated by quasi-steady-state treatment for metabolic network analysis. In particular, it was assumed that the total consumption rate of *Clostridium acetobutylicum* in intracellular metabolites was equal to its total production rate.

Figure 1 is a simplified metabolic network for *Clostridium acetobutylicum*. There are 19 fluxes, including 7 transport fluxes (1 substrate in and 6 products out) and 12 intracellular fluxes (12 intracellular reactions in Table 1). The degrees of freedom in this system are calculated as 4 (the degrees of freedom are obtained by the calculation formula of metabolic flux analysis under overdetermined system). The glucose consumption rate was directly monitored and calculated, and the generation rates of acetic acid, butyric acid, acetone, butanol, ethanol, and hydrogen were decided by detecting the change of concentration with time.

In this simplified metabolic network diagram of *Clostridium acetobutylicum*, the

measurable flux is higher than the system degree of freedom, which indicates that the system is an over-determined system. In this situation, the least square statistical method can be used to estimate the flux vector. The following equation can be obtained,

$$V_c=-[S_cTS_c]^{-1}[S_cTS_m]V_m \quad (1)$$

where V_m (S_m) is the measured transport fluxes (matrix), V_c (S_c) is the remaining unknown transport fluxes (matrix), and T is the measurement coefficient matrix (Stephanopoulos *et al*. 2001).

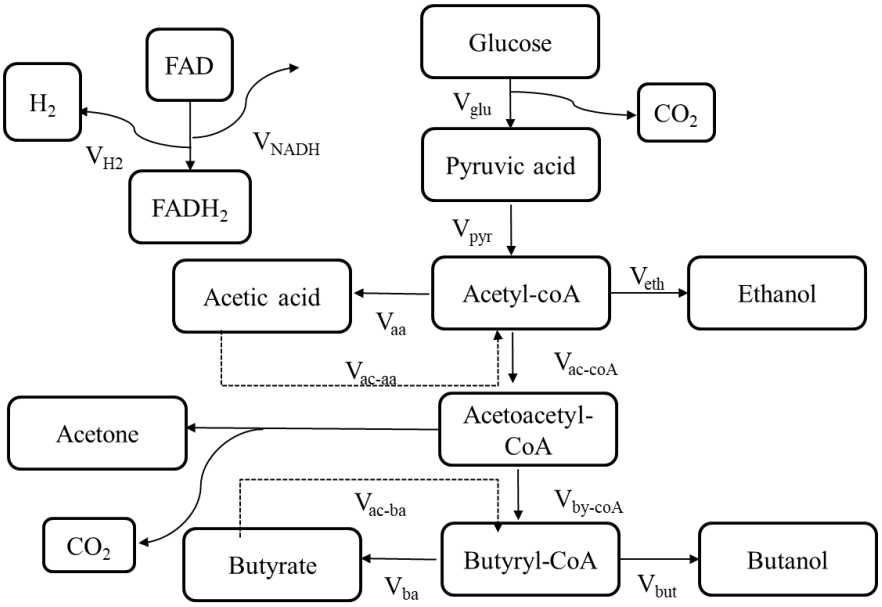

Fig. 1. Metabolic network diagram of *Clostridium acetobutylicum*

Table 1. Major Metabolic Reactions in Metabolic Networks

Reactions
1: glucose→2pyruvate+2NADH+2ATP
2: pyruvate→acetyl-CoA+CO_2+ReducedFerredoxin
3: acetyl-CoA+2NADH→ethanol
4: acetyl-CoA→acetate+ATP
5: 2acetyl-CoA→acetoacetyl-CoA
6: acetoacetyl-CoA+acetate→acetone+CO_2+acetyl-CoA
7: acetoacetyl-CoA+butyrate→acetone+CO_2+butyryl-CoA
8: acetoacetyl-CoA+2NADH→butyryl-CoA
9: butyryl-CoA→butyrate+ATP
10: butyryl-CoA+2NADH→butanol
11: $FADH_2$→H_2
12: $FADH_2$→NADH

$V_{ac\text{-}coA}$ and $V_{by\text{-}coA}$ are the reaction rates of acetyl-coA and butyryl-coA flowing through the main metabolic pathway of *Clostridium acetobutylicum*, respectively; V_{aa} and V_{ba} are the synthesis rates of acetic acid and butyric acid in the *Clostridium acetobutylicum*

metabolic pathway, respectively. $V_{ac\text{-}aa}$ and $V_{ac\text{-}ba}$ are the reabsorption rates of acetic acid and butyric acid in the *Clostridium acetobutylicum* metabolic pathway, respectively; V_{eth} and V_{but} are the generation rates of ethanol and butanol in the metabolic pathway of *Clostridium acetobutylicum*, respectively. V_{H2} and V_{NADH} are the synthesis rates of H_2 and NADH in the system of electron shuttle transport, respectively.

RESULTS AND DISCUSSION

Butanol Acetone Ratio in ABE Fermentation by FR

In the process of ABE fermentation, butanol yield and butanol acetone ratio (B/A ratio) are two main factors to evaluate the feasibility of fermentation. In previous studies, ABE fermentation performance with FR as substrate was improved after 3.0 $g \cdot L^{-1}$ YE solution was added into FR medium, while fermentation on FR without YE was low (Mao *et al*. 2019).

In this study, *Clostridium acetobutylicum* CGMCC1.0134 was cultivated on FR with YE added (hereinafter referred to as FY) and corn to compare their fermentation performances in 250-mL fermentation bottles (taking the end of gas production as the end of fermentation).

Table 2 shows that when the initial C_s of corn (50 $g \cdot L^{-1}$) was the same as that of FY (50 $g \cdot L^{-1}$ of FY), the final C_{bu} (8.98 $g \cdot L^{-1}$) and the B/A ratio (1.85) of corn were both much lower than those of FY (12.82 $g \cdot L^{-1}$ and 2.98). Even when the C_s of corn was elevated to 70 $g \cdot L^{-1}$ and thereby enhanced the final yield and B/A ratio to 10.95 $g \cdot L^{-1}$ and 1.76, respectively, the effectiveness was still lower than that of FY. The results showed that the C_{bu}, B/A ratio, starch utilization rate, and butanol yield in FY medium were all higher than those in corn medium, which validated the high effectiveness of FY medium as substrate for ABE fermentation. Although FR without YE added showed poor performance in productivity probably due to lack of nitrogen source, the B/A ratio (2.23) was still higher than that of corn (1.85). This indicated that FR was more conducive to higher B/A ratio than corn.

Table 2. Comparison of ABE Fermentation Performance under Different Substrate Dosages in 250-mL Fermentation Bottle

Parameter	1. Corn	2. Corn	3. FR	4. FY
Corn dosage ($g \cdot L^{-1}$)	70.0	50.0	0.0	0.0
FR dosage ($g \cdot L^{-1}$)	0.0	0.0	50.0	50.0
YE dosage ($g \cdot L^{-1}$)	0.0	0.0	0.0	3.0
Butanol ($g \cdot L^{-1}$)	11.0±0.2	9.0±0.1	4.3±0.2	12.8±0.3
Acetone ($g \cdot L^{-1}$)	6.2±0.2	4.9±0.2	1.9±0.1	4.3±0.1
Ethanol ($g \cdot L^{-1}$)	2.8±0.1	2.0±0.2	1.6±0.1	1.6±0.2
ABE ($g \cdot L^{-1}$)	20.0±0.2	15.8±0.3	7.8±0.1	18.7±0.4
Gas released ($L \cdot L^{-1}$)	19.0±0.4	12.7±0.2	2.0±0.3	22.6±0.6
B/A ratio (W/W)	**1.8±0.1**	**1.9±0.1**	**2. 2±0.2**	**3.0±0.3**
Starch consumption ($g \cdot L^{-1}$)	55.6±0.9	40.0±0.8	29.0±0.2	46.0±0.7
Starch utilization (%)	79.4±0.8	79.9±0.9	58.0±0.4	91.9±0.9
Butanol productivity ($g \cdot L^{-1} \cdot h^{-1}$)	0.15±0.02	0.12±0.02	0.07±0.03	0. 21±0.01
Yield of butanol (g/g starch)	0. 20±0.02	0. 22±0.02	0.15±0.01	0. 28±0.02
Yield of ABE (g/g starch)	0.34±0.01	0.32±0.02	0. 27±0.02	0.49±0.01

The utilization of different substrates resulted in the differences of B/A ratio. Previous studies showed that carbon metabolic flux was impacted mainly by genetic modification and environmental change (Li *et al*. 2013). In this study, genetic modification was not applied, and the operating conditions were not changed for all batches. It could be inferred that the substrate difference between corn and FY resulted in the difference of carbon metabolic flux distributions and thereafter led to the higher B/A ratio for FY in the ABE fermentation process. The difference between FY and corn may be related to the difference of nitrogen content in substrates, or the effect of substrates on the carbon metabolic flow. This effect was investigated below.

Analysis of Nitrogen Contents in the Substrates

Nitrogen sources are mainly composed of amino acids, so the content of nitrogen source was characterized by amino acid concentration. The free amino acid contents in FY (5%, W/V) and corn (5%/7%, W/V) after complete hydrolysis are shown in Table 3. The free amino acid concentration in FY medium (8000 mg/100g substrate in total) was similar to that of corn medium (8326 mg/100 g substrate in total) after enzymatic pre-treatment (Table 2), and both of them were lower than that of 7% W/V corn. Comparing the result with the B/A ratios, there was no obvious correlation between nitrogen content and B/A ratio, which indicated that nitrogen had no major effect, if any, on the B/A ratio. The low nitrogen but high B/A ratio for FR alone validated this conclusion (Mao *et al*. 2019).

Table 3. Amino Acid Concentrations in FY and Corn Medium after Complete Proteolysis

Amino Acid Type	7% Corn (mg/100 mL medium)	5% Corn (mg/100 mL medium)	5% FY (mg/100 mL medium)
Asp	616.16±3.5	422.6858±3.5	628.52±3.5
Thr	674.25±3.5	462.5355±3.5	293.74±3.5
Ser	691.44±3.5	474.3278±3.5	396.57±3.5
Glu	1928.09±3.5	1322.67±3.5	1989.99±3.5
Gly	359.65±3.5	246.7199±3.5	294.73±3.5
Ala	1096.14±3.5	751.952±3.5	586.75±3.5
Cys	103.45±3.5	70.9667±3.5	157.3±3.5
Val	863.8±3.5	592.5668±3.5	365.7±3.5
Met	278.91±3.5	191.3323±3.5	107.4±3.5
Ile	737.93±3.5	506.22±3.5	269.36±3.5
Leu	1243.54±3.5	853.0684±3.5	873.85±3.5
Tyr	242.02±3.5	166.0257±3.5	387.71±3.5
Phe	895.88±3.5	614.5737±3.5	371.6±3.5
Lys	751.51±3.5	515.5359±3.5	284.3±3.5
His	233. 26±3.5	160.0164±3.5	201.9±3.5
Arg	620.38±3.5	425.5807±3.5	423.87±3.5
Pro	325.18±3.5	223.0735±3.5	693.14±3.5
Totals	11661.6±3.5	7999.851±3.5	8326.43±3.5

Asp: aspartic acid; Thr: threonine; Ser: Serine; Glu: glutamic acid; Gly: glycine; Ala: Alanine; Cys: tryptophan; Val: Valine; Met: methionine; Ile: Isoleucine; Leu: Leucine; Tyr: Tyrosine; Phe: phenylalanine; Lys: Lysine; His: histidine; Arg: arginine; Pro: Proline; Val: valine; Leu: leucine.

Distribution of Carbon Metabolic Flow in ABE Fermentation with FY or Corn as Substrate

Because nitrogen was not the main factor leading to the high B/A ratio of FY, the carbon metabolic flux distribution was examined to analyze the impact of substrates on the entire metabolic pathway. The carbon metabolic flows measured by the metabolic flux analysis (MFA) with corn/FY as substrate during fermentation are shown in Table 4.

ABE fermentation is characterized by the formation and re-assimilation of acetate/butyrate in several closed reaction rings during the solventogenic phase, and the change of strength of acetate/butyrate ring can affect the production of butanol and acetone in this phase (Girbal and Soucaille 1994). In the solventogenic phase, the carbon flows distributed to acetic acid and ethanol synthesis with FR substrate were lower than those with corn substrate. The butyric acid synthesis competes with butanol production for carbon source at the node of butyryl-CoA. In the reabsorption process of acetic acid and butyric acid, the amount of carbon metabolic flow to acetic acid and butyric acid (10.14 and 40.5 $mmol \cdot L^{-1} \cdot h^{-1}$) in FY were lower than those in corn (38.1 and 76.9 $mmol \cdot L^{-1} \cdot h^{-1}$). Compared with corn medium, butyric acid synthesis pathway was weakened in FY medium, and more carbon went to the butanol synthesis pathway. In addition, in FY fermentation, the rates of reuse of acetic acid and butyric acid ($V_{ac\text{-}aa}$, $V_{ac\text{-}ba}$) were lower than those of corn. These two pathways are coupled with the synthesis of acetone, so the synthesis of acetone in the FY fermentation process was weakened. This result explains the high B/A ratio in FY fermentation broth. When the pathway of reabsorption in the metabolic network of *Clostridium acetobutylicum* is inhibited, the B/A ratio in the fermentation broth increases (Girbal and Soucaille 1994).

The synthesis rate of NADH in FY in the electronic shuttle transfer system was 74.5 $mmol \cdot L^{-1} \cdot h^{-1}$, which was higher than that of corn (57.4 $mmol \cdot L^{-1} \cdot h^{-1}$) under the same operating conditions. Meanwhile, the metabolic flux of H_2 (104.5 $mmol \cdot L^{-1} \cdot h^{-1}$) in ABE fermentation was lower than that with corn as substrate (110.5 $mmol \cdot L^{-1} \cdot h^{-1}$). The formation of acetone neither consumes NADH nor produces H_2, but the formation of butanol consumes a certain amount of NADH accompanied with the production of H_2. Higher NADH synthesis increases the B/A ratio (Monot *et al.* 1984; Girbal *et al*. 1995). In the solventogenic phase, some electrons flow to H_2, and others are used to synthesize NADH. As shown in Fig. 2, the ratio of H_2/CO_2 in corn medium was higher than the corresponding value in FY medium in almost the whole fermentation process. This phenomenon verified the previous results. In the FY medium, more electrons went to the synthesis of NADH and reduced the production of H_2, which could be another reason for the high B/A ratio in the FY fermentation broth. The combination of the two mechanisms could result in the higher B/A ratio for FY substrate compared with corn.

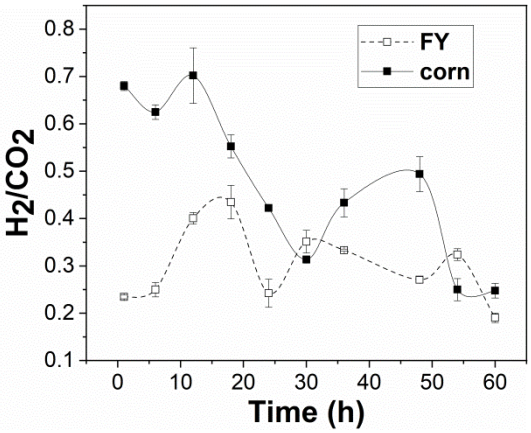

Fig. 2. H_2/CO_2 ratio during the fermentation medium of corn and FY

Table 4. Distribution of Carbon Metabolic Fluxes in Metabolic Pathways of FY and Corn ABE Fermentation

Metabolite	Metabolic flux of corn ABE fermentation broth (mmol·L^{-1}·h^{-1})	Metabolic flux of FY ABE fermentation (mmol·L^{-1}·h^{-1})
V_{glu}	100.0	100.0
V_{pyr}	200.0	200.0
$V_{ac\text{-}CoA}$	167.9	150.9
$V_{acac\text{-}CoA}$	54.9	37.8
V_{NADH}	67.4	74.5
V_{aa}	38.1	10.1
V_{ba}	76.9	40.5
$V_{ac\text{-}aa}$	25.3	32.9
$V_{ac\text{-}ba}$	19. 2	21.4
V_{eth}	6.5	7.6
V_{ace}	35.8	10.9
V_{but}	61.5	70.0
V_{H2}	110.5	104.5

Detection of Key Enzyme Genes in ABE Fermentation

In this study, ABE fermentation was conducted with *Clostridium acetobutylicum* CGMCC 1.0134 on FY with corn as control. Figure 3 shows the transcription level of key enzyme genes in corn and FY fermentation medium. CtfB gene encodes coA transferase, which is responsible for the reabsorption of acetic acid and butyric acid. The two acids are used in the synthesis of ethanol and butanol precursors (acetyl-CoA and butyryl-CoA), together with the synthesis of acetone.

In Fig. 3(a), throughout the fermentation process, the expression level of ctfB gene in corn ABE fermentation broth was much higher than that in FY ABE fermentation broth. This means that the activity of coenzyme A transferase in the corn medium was higher, which was more conducive to the synthesis of acetone. The coding gene of butanol dehydrogenase was bdhA, and butanol dehydrogenase catalyzes the cell to produce butanol. From Fig. 3(b), at the solventogentic phase, the relative expression levels of bdhA gene in corn ABE fermentation broth were significantly lower than that in FY ABE fermentation broth. Therefore, in FY medium, lower coA transferase activity and higher butanol dehydrogenase activity resulted in higher B/A ratio.

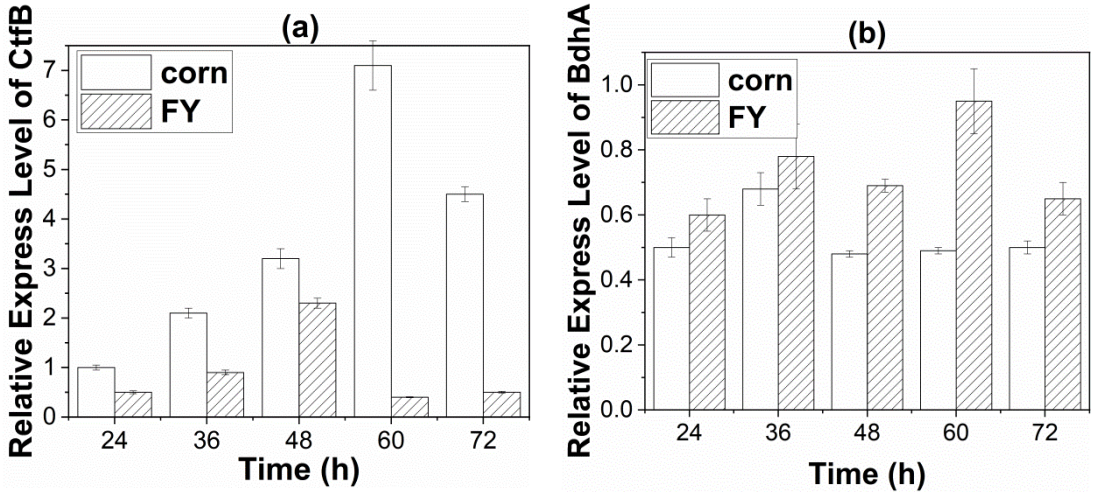

Fig. 3. Transcriptional levels of key enzyme genes in the fermentation medium of corn and FY

Validation Experiment of 5L Fermentor Tests

In this experiment, corn and FY medium were fermented in a 5L fermentation tank. The parameters of the fermentation process are shown in Fig. 4. In Fig. 4(a), the changing trends of pH were similar for corn substrate and FY substrate, both of which dropped first and then rose.

The turning point for FR (at approximately 12 h) occurred faster than corn (at approximately 30 h). This trend was comparable with previous studies (Luo *et al*. 2016; Luo *et al*. 2018). The pH changes indicated the phase shift in ABE fermentation. The gas yield of FR substrate (20700 mL) was slightly higher than that of corn substrate (18980 mL).

Figure 4(b) illustrates the evolution of acid concentration. The curve of acetic acid in FY/corn trended upward, downward, and then upward again, which was consistent with the curve of butyric acid. In FY/corn medium, the total organic acid concentration was approximately 2.5 $g \cdot L^{-1}$ at 12 h, where C_{by} was 2.3 $g \cdot L^{-1}$. Thus, the total acid concentrations in the two mediums were close to equal. However, during most of the time, the concentration of butyric acid in FR medium was higher than that in corn medium. Butyrate is an excellent substrate for butanol production under the existence of glucose, and a higher concentration of butyric acid within a certain range can stimulate the synthesis of solvents (Tashiro *et al*. 2004, 2007).

Figure 4(c) shows the changing trend of starch and glucose during the fermentation of FY/corn ABE fermentation. For FY/corn substrate, the total starch consumption was 88.6%/62.9%, respectively. In FY fermentation, the starch was almost completely consumed, which means the consumption in FY fermentation was much higher than in corn fermentation. Thus, the substrate utilization of FY fermentation was higher than that of corn fermentation.

Figure 4(d) shows the solvent concentration changes with fermentation time. The final concentrations were 12. 29 g $\cdot L^{-1}$ butanol for FY and 10.86 g $\cdot L^{-1}$ butanol for corn, 3.59 g $\cdot L^{-1}$ acetone for FY and 6.04 g $\cdot L^{-1}$ acetone for corn, and 18.86 g $\cdot L^{-1}$ total solvent for FY and 18.34 g $\cdot L^{-1}$ total solvent for corn. Obviously, the butanol yield of FY was higher than that of corn, while the acetone yield was lower.

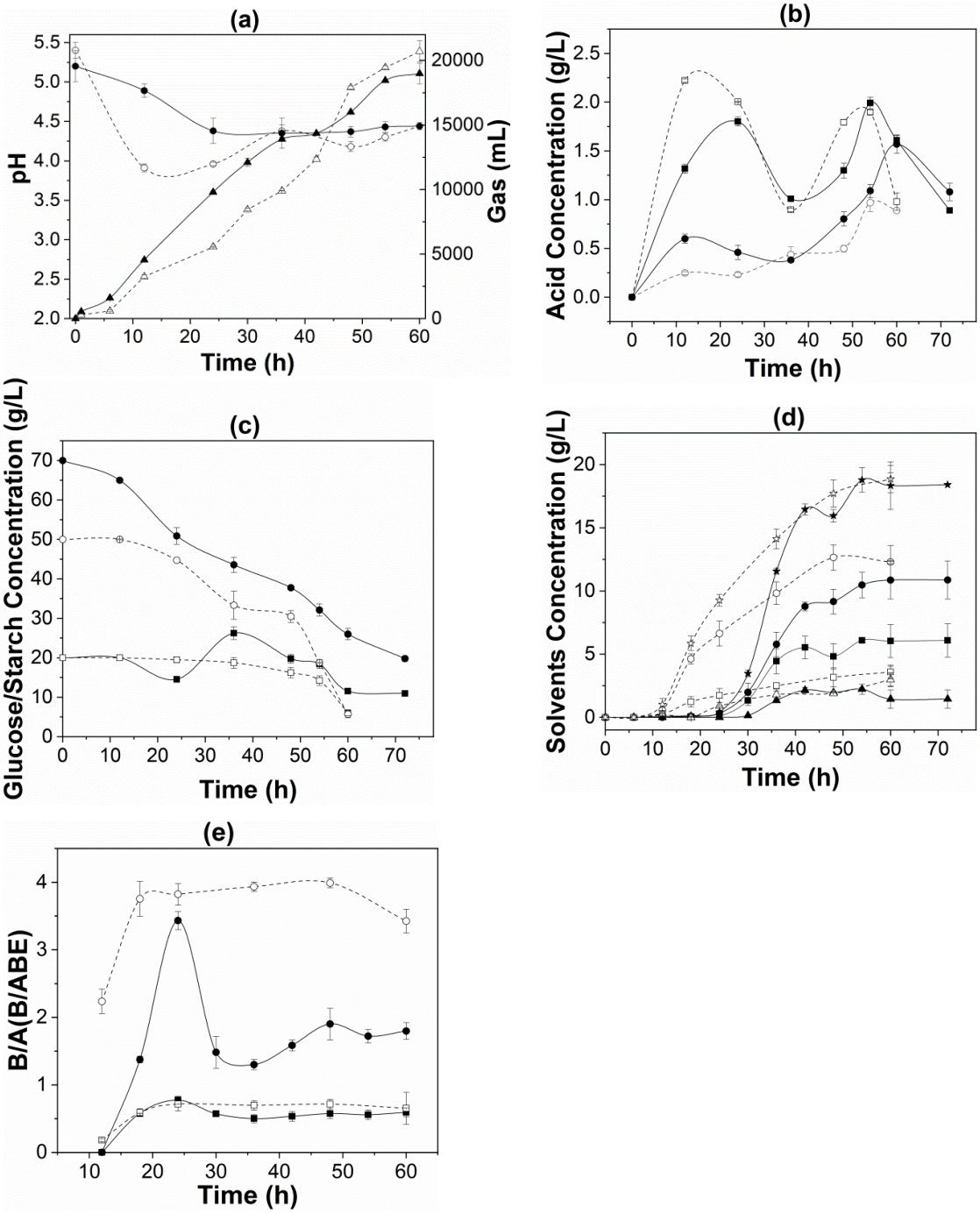

Fig. 4. Profiles of fermentation for butanol production using corn or FY.
(a) ●/○: changes of pH in corn/FY ABE fermentation medium; ▲/△: gas production during corn/FY ABE fermentation;
(b) ■/□: C_{by} in corn/FY ABE fermentation medium; ●/○: C_{ac} in corn/FY ABE fermentation medium;
(c) ■/□: C_{glu} in corn/FY ABE fermentation medium; ●/○: C_s in corn/FY ABE fermentation medium;
(d) ■/□: C_{ace} in corn/FY ABE fermentation medium; ●/○: C_{bu} in corn/FY ABE fermentation medium; ▲/△: ethanol concentration during corn/FY ABE fermentation; ★/☆: total solvent concentration during corn/FR ABE fermentation;
(e) ■/□: B/A ratio in corn/FY ABE fermentation medium; ●/○: B/A ratio in corn/FY ABE fermentation medium

In Figure 4(e), the B/A ratio of FY was higher than that of corn during the whole fermentation process and finally reached 3.42. In summary, in the ABE fermentation process with FY as the substrate, the substrate utilization rate was higher, the B/A ratio was higher, and the solvent yield was statistically equivalent compared to corn.

The feasibility of ABE fermentation with FY as substrate was validated through this study at a laboratory scale. Fern root as a cheap raw material does not compete with cultivated land, and the higher B/A ratio combined with other parameters indicates higher economic benefits in ABE fermentation.

CONCLUSIONS

1. Compared with the traditional corn substrate, FR with YE added had higher B/A ratio and substrate utilization.
2. Nitrogen showed no significant impact on the B/A ratio difference, while carbon metabolic pathway analysis showed weakened butyrate circuit and strengthened NADH synthesis for FY substrate, which could explain the mechanism of the high B/A ratio for FY substrate.
3. Enzyme detection and analysis demonstrated that the weaker coA transferase activity and the stronger butanol dehydrogenase activity promoted the B/A ratio in the FY medium.
4. A 5L fermentor test examined the critical parameters in the process and validated the feasibility of FY. Economic estimation showed that FY could be a cost-efficient substrate for ABE fermentation.
5. These results demonstrated the industrial potential of FY for ABE fermentation.

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