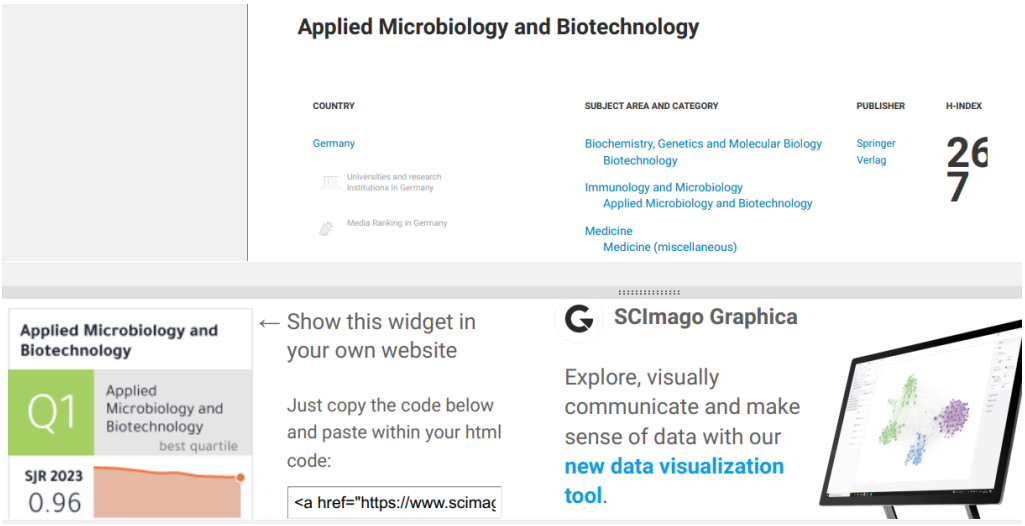
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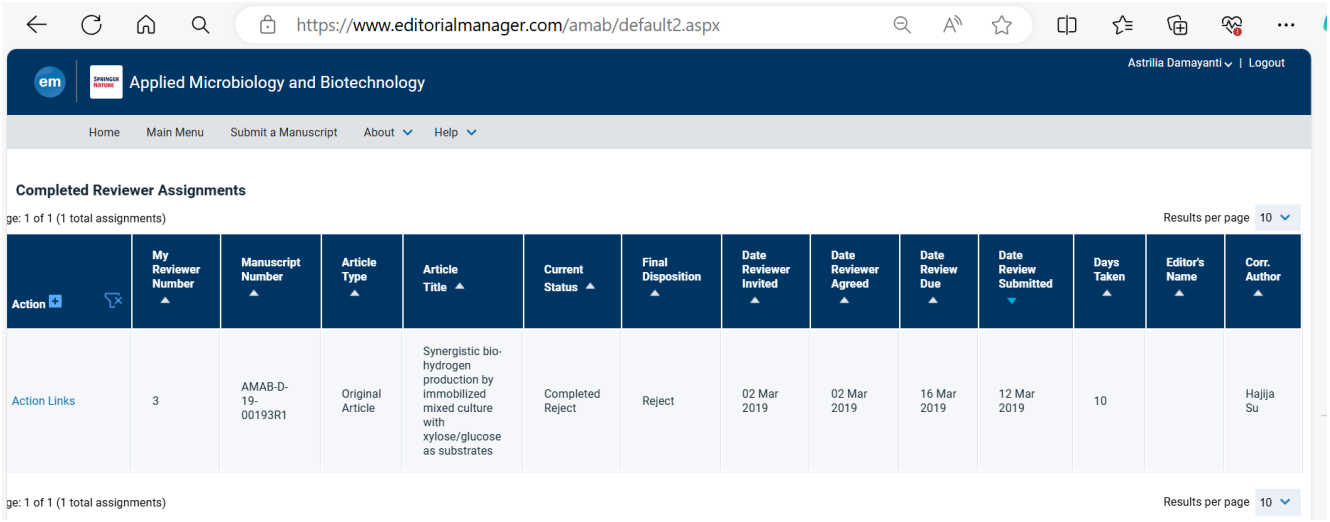
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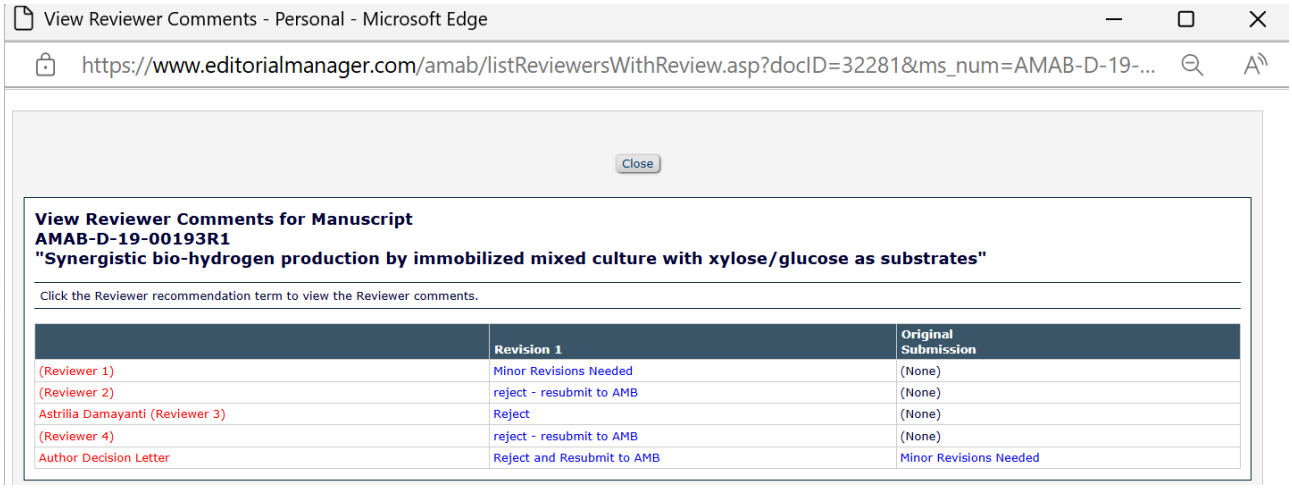
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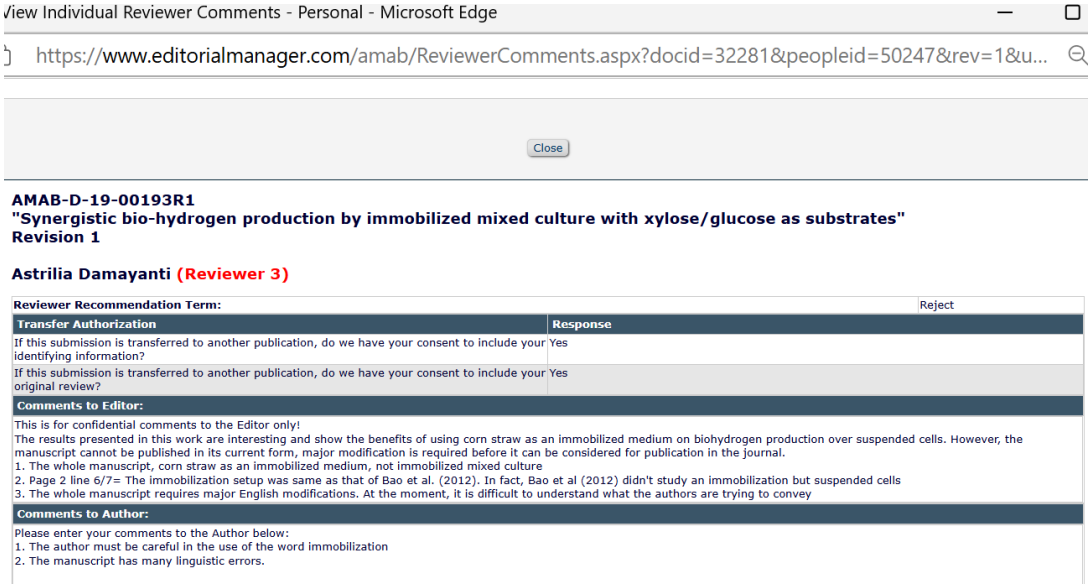

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| **Reviewer Recommendation Term:** | Reject |
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**Comments to Editor:**

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The results presented in this work are interesting and show the benefits of using corn straw as an immobilized medium on biohydrogen production over suspended cells. However, the manuscript cannot be published in its current form, major modification is required before it can be considered for publication in the journal.
1. The whole manuscript, corn straw as an immobilized medium, not immobilized mixed culture
2. Page 2 line 6/7= The immobilization setup was same as that of Bao et al. (2012). In fact, Bao et al (2012) didn't study an immobilization but suspended cells
3. The whole manuscript requires major English modifications. At the moment, it is difficult to understand what the authors are trying to convey

**Comments to Author:**

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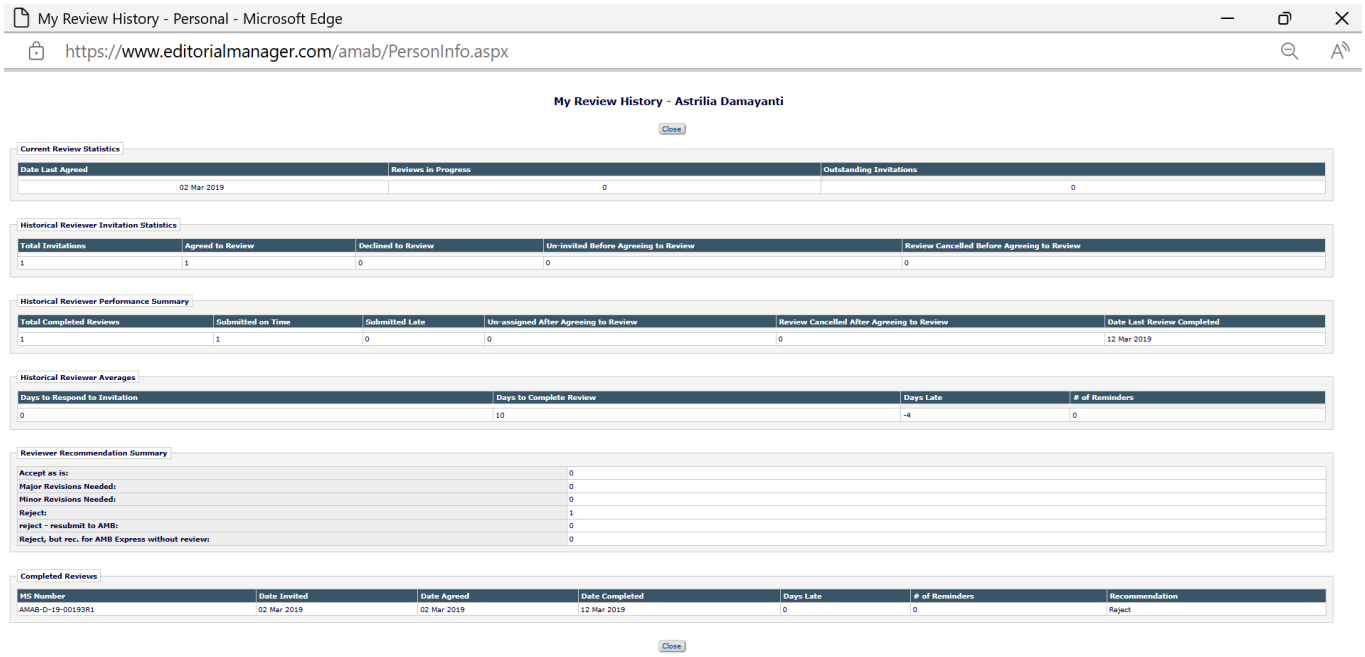
1. The author must be careful in the use of the word immobilization
2. The manuscript has many linguistic errors.



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# Synergistic bio-hydrogen production by immobilized mixed culture with xylose/glucose as substrates

--Manuscript Draft--

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| **Manuscript Number:** | AMAB-D-19-00193R1 |
| **Full Title:** | Synergistic bio-hydrogen production by immobilized mixed culture with xylose/glucose as substrates |
| **Article Type:** | Original Article |
| **Section/Category:** | Bioenergy and biofuels |
| **Abstract:** | An artificially immobilized mixed culture system was investigated to produce hydrogen by using xylose/glucose co-fermentation. The synergistic mechanism of Bacillus cereus A1 and Brevumdimonas naejangsanensis B1 was demonstrated. The results showed promising performance of this mixed culture system. The hydrogen yield of the immobilized mixed culture system was 1.54 mol H2/mol xylose, at an optimum xylose concentration of 10 g/L. The volume of hydrogen production of the immobilized fermentation increased by 29.9% compared with a suspended fermentation, while the maximum rate of hydrogen production could reach 49.9mL/h which was 4.5 times that of the suspended fermentation, thus significantly shortened the fermentation time. The average substrate utilization rate in this batch fermentation experiment was 96%, meanwhile, the fermentation performance was stable. The highest yield of hydrogen was 1.73 mol H2/mol mixed sugar when glucose and xylose were mixed in a 1:1 ratio. The results of this study demonstrated a considerable synergistic effect of immobilized mixed culture technology. |

Dear editor,

Thank you for the comments on this manuscript. We have checked and revised it according to the comments. The two strains A1 and B1 were isolated in our previous studies in 2014, and their serial number are CGMCC No. 9035, and CGMCC No.9036, respectively.

In addition, we have revised the manuscript as suggested since we consider that some sentences or descriptions in the Discussion part are not so accurate based on the results.

Thank you again, and we do expect the manuscript will be published in *Applied Microbiology and Biotechnology.*

Your sincerely,

All authors

# Synergistic bio-hydrogen production by immobilized mixed culture with xylose/glucose as substrates

**Fei Peng [1,2], Zhihong Ma [1,2], Ting Zhang [1,2], Haijiang Su [1,2,*]**

## Abstract

An artificially immobilized mixed culture system was investigated to produce hydrogen by using xylose/glucose co-fermentation. The synergistic mechanism of *Bacillus cereus* A1 and *Brevumdimonas naejangsanensis* B1 was demonstrated. The results showed promising performance of this mixed culture system. The hydrogen yield of the immobilized mixed culture system was 1.54 mol $H_2$/mol xylose, at an optimum xylose concentration of 10 g/L. The volume of hydrogen production of the immobilized fermentation increased by 29.9% compared with a suspended fermentation, while the maximum rate of hydrogen production could reach 49.9mL/h which was 4.5 times that of the suspended fermentation, thus significantly shortened the fermentation time. The average substrate utilization rate in this batch fermentation experiment was 96%, meanwhile, the fermentation performance was stable. The highest yield of hydrogen was 1.73 mol $H_2$/mol mixed sugar when glucose and xylose were mixed in a 1:1 ratio. The results of this study demonstrated a considerable synergistic effect of immobilized mixed culture technology.

**Keywords** Hydrogen production · Immobilized mixed culture system · Synergistic effects · Glucose · Xylose.

## Introduction

With the development of modern society, world energy consumption has grown rapidly. Hydrogen, as one of the most promising energy sources, has become a valid alternative for fossil energy, being environmentally-benign and with highly-efficient properties. Most of the current production methods of hydrogen are however energy intensive and fossil-fuel based (Jung et al. 2011), moreover consuming lots of energy and leading to secondary pollution. Compared with conventional thermo-chemical and electrochemical hydrogen production methods, bio-hydrogen production is energy-lean and less polluting, hence attracting widespread attention (Rahman et al. 2016).

The choice of fermentation substrate should be based on the principle of low cost, environmental protection and renewability. Biomass is a widely available renewable resource, yet direct combustion or other thermo-chemical transformations are of low energy efficiency, of high labor intensity and environmentally hazardous (Kumar et al. 2017). Biomass lignocellulose can be hydrolized into a glucose and xylose rich hydrolysate. The use of glucose as a substrate for hydrogen production by fermentation has been widely studied (Abdalla et al. 2018; Ghimire et al. 2015; Zhang et al. 2016; Wong et al. 2014; Zhang et al. 2017). Little research has been conducted in bio-hydrogen production from xylose (Goshima et al. 2013) for the lack of xylose-utilizing strains. In order to efficiently and cheaply convert lignocellulose into $H_2$, the key point of the current research is to screen xylose-utilizing bacteria.

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Selecting the suitable strains and the optimal experimental conditions to ferment carbohydrate into hydrogen, can effectively control the types of end products, so as to obtain a higher amount of hydrogen production. There are however some problems of pure culture hydrogen production in the practical application, such as being contaminated by other bacteria, applicable within a relatively narrow substrate range, and often needing the substrate to be sterilized (Bundhoo 2017). In contrast, hydrogen production by mixed culture fermentation has low requirements for substrates, can use a wide range of substrates, and has no need to use a pure bacterial culture. Bao et al. researched bio-hydrogen fermentation characteristics by mixed culture of *Bacillus* sp. and *Brevumdimonas* sp. The results showed that the hydrogen yield was doubled in mixed culture compared with pure culture (Bao et al. 2012; Bao et al. 2013). Maintinguer et al. obtained *Clostridium* and *Klebsiella* hybrid inoculants from the UASB reactor of slaughterhouse wastewater treatment, and used them to produce $H_2$ and volatile fatty acids for different sugar contents and obtaiend a maximum hydrogen yield of 0.8 mol $H_2$/mol xylose when the xylose concentration was 14% (2011). Qiu et al. demonstrated that co-fermentation by *Clostridium* sp. and *Thermoanaerobacterium* sp. can achieve optimal hydrogen production at pH of 7.0 and a xylose concentration of 7.5 g/L (2016).

The biomass concentration in a suspended fermentation reactor is low, which results in a slow rate of substrate utilization, even if cell co-fermentation is used. Immobilized-cell technology can increase the concentration of biomass in the reactor, improve the processing efficiency and the stability of the operation process (Ashwini et al. 2014). Applying immobilized-cell technology to bio-hydrogen production can not only remediate the easy elution of free cell from system, but will also improve the stability of continuous biological hydrogen production, including the tolerance to some extreme conditions (such as lower pH, higher temperature, or higher organic loading rate) (Massalha et al. 2015; Li et al. 2010; Han et al. 2012). Jamali et al. used granular activated carbon as a support carrier to form bio-films to immobilize cells. The experimental results showed that the largest hydrogen production rate was 2.0 mmol $H_2$/(L·h) when sludge/GAC was 1:2, while the HPR was only 0.5 mmol $H_2$/(L·h) in the unfixed suspension culture group, far below the previously tested immobilized group (2016). According to the principle of biocompatibility, many scientists use natural materials as carriers of immobilization, mainly agricultural waste and other biomass materials, to reduce the waste as well as to find ways to add value. Guevara et al. used dispersal loofah fiber, acrylic acid, aggregate loofah fiber, and high-density polyethylene as the carrier. The results showed that its final fixed biomass was 19.6 mg VS·$m^{-3}$ when the dispersed loofah fiber was used as a carrier, compared with 8.0 mg VS·$m^{-3}$ at the initial stage of fermentation (2015). In addition, some studies also used bagasse (Plangklang et al. 2012), crop stalks (Wang et al. 2018), tea waste (Gupta and Balomajumder 2015), and biomass carbon (Ma et al. 2017) as immobilization carriers.

From the previous research it is clear that the efficiency and rate of hydrogen production by xylose fermentation is relatively low, and that different inhibitors of cellulose hydrolysate have a certain impact on the growth of microorganisms. Therefore, the fermentation of cellulose hydrolyzate into hydrogen is still difficult (Lee et al. 2013).

To provide an answer to the basic above drawbacks, we used corn straw as an immobilized medium to study the effects of different xylose concentrations on immobilized hydrogen production, and compared the suspended fermentation and immobilized fermentation under the optimum xylose concentration. With xylose as a substrate, the immobilized mixed culture system can produce hydrogen by continuous 10 batches of fermentation. At the same time, the hydrogen production when glucose and xylose were mixed with 1:1 ratio as substrate was also studied, which lay a foundation for future developments of using immobilized complex bacteria to produce hydrogen by cellulose hydrolysis fermentation.

## Experimental materials and methods

### Microorganisms and medium compositions

In this study, the mixed culture of A1 and B1 was used. *Bacillus cereus* strain A1, CGMCC No. 9035 and *Brevumdimonas naejangsanensis* strain B1, CGMCC No.9036 were isolated in our previous studies (Zhang et al. 2014; Su et al. 2014)).

The compositions of the seed medium were beef extract (3 g/L), peptone (10 g/L), NaCl (5 g/L). The fermentation medium comprised peptone (2 g/L), NaCl (5 g/L), $KH_2PO_4$ (1 g/L), $K_2HPO_4$ (1 g/L), the concentrations of xylose and glucose varied within the experiments.

### Immoblization and experimental setup

The biomass adsorption material was cut into small pieces, and 9.0 g of biomass adsorption material was weighed into the sterilized fermentation medium, where the seed liquids of A1 and B1 cultured in advance were added to. The co-fermentation in the fermentation broth allows the A1 and B1 bacteria to adsorb onto the biomass adsorption material during the fermentation process.

The fermentation device used here was a 1.2 L jar with a total reaction volume of 1 L. In the suspended fermentation, 900 mL was used as the fermentation medium. Two kinds of strains were inoculated with 50 mL of 1:1 mixing ratio. The immobilization setup was same as that of Bao et al. (2012). Each reactor was filled with 0.9 L fermentation medium, and 9 g of carrier that had absorbed the microorganisms (in the immobilized fermentations) or 100 mL seed medium with the mixed strains (in the suspended fermentations). Further procedures accounted for the previous literature (Bao et al. 2013). Before fermentation, the bottle mouth was plugged with a rubber stopper, leaving a sampling port and an exhaust port, and argon was used to remove the air above the fermentation bottle and to provide an anaerobic environment for the fermentation of the bacteria. After purging, the sampling port was plugged, and a pre-vacuumed gas bag was attached to the exhaust to collect the gas generated during the fermentation process. Fermentation was then performed in a magnetic stirred water bath at 35 °C. Samples were taken every 12 h and each experimental parameter was determined.

## Analysis methods

In this study, pH, total sugar concentration, hydrogen production, volatile fatty acid (VFAs) and other major parameters were determined according to standard analytical procedures (Wang et al. 2018).

### Tolerance of immobilized complex bacteria to load fluctuation

4.5 g, 9.0 g, and 18.0 g of xylose were added to 900 mL of fermentation medium respectively to set the xylose concentrations at 5 g/L, 10 g/L, and 20 g/L.

### Xylose and glucose mixtures as substrates

The total sugar concentration was 10 g/L, and 4.5 g of xylose and 4.5 g of glucose were simultaneously added in 900 mL of the fermentation medium in order to ensure that the mass ratio of xylose to glucose was 1:1.

## Results

### Effect of immobilized mixed culture technology on bio-hydrogen fermentation

The fermentation system easily leads to failure at lower hydraulic retention time (HRT) in the continuous hydrogen production of the suspended fermentation, due to the loss of cells (Jiang et al. 2018; Ye et al. 2011). Immobilized mixed culture technology can improve the system hydrogen production rate and yield while solving the limitations of the suspended fermentation, which also improving the stability of the continuous biological hydrogen production, including some operating parameters such as pH, temperature, and organic loading rate. The cells bound to the carrier will stay attached for a long period of time, which makes the hydrogen production rate stable under long-term continuous operation.

As shown in Fig.1, both suspended and immoblilized system showed a linear increase in cumulative hydrogen production with 10 g/L xylose as a substrate. During the suspended fermentation, the fermentation time lasted 220 hours and the final cumulative hydrogen production was 1593 mL. The yield of hydrogen production was 1.33 mol $H_2$/mol xylose. On the contrary, the time of immobilized fermentation was only 72 hours, the final hydrogen production was 2070 mL, and the yield of hydrogen was 1.54 mol $H_2$/mol xylose. The time of immobilized fermentation was shortened by two-thirds compared with that of suspended fermentation, but the yield of hydrogen production was increased by 15.8% compared with suspended fermentation. With the immobilized mixed culture technology, the transfer rate of the intermediate metabolites increases, which was beneficial to the improvement of hydrogen production efficiency.

The composition of the main end products in the liquid phase when the suspended fermentation and the immobilized fermentation

used 10 g/L xylose as a substrate was shown in Fig.2. The acetic acid content of the fermentation broth in suspended fermentation was 0.843 g/L while the butyric acid content was 2.76 g/L, accounting for 76.6% of the mixed acid. The acetic acid content in the fermentation broth during immobilized fermentation was 0.883 g/L, and the butyric acid content was 3.27 g/L, accounting for 78.7% of the mixed acid: the butyric acid content in the immobilized fermentation process increased by 18.5%, indicating that the accumulation of butyric acid was closely related to the cumulative hydrogen production.

The modified Gompertz equation has been widely used in hydrogen production by batch dark fermentation (Rahman et al. 2016), which is defined as follows:

$$P_t = P_m \exp\left\{-\exp\left[\frac{R_m \times e}{P_m}(\lambda - t) + 1\right]\right\}$$

Several parameters involved in the model, including the cumulative hydrogen production at culture time t ($P_t$), the lag phase (λ), maximum hydrogen production potential ($P_m$), the maximum hydrogen production rate ($R_m$). "e" is a constant equal to 2.718.

From Table 1, it can be seen that the lag phase of fermentation shortened from 24.8 hours for suspended fermentation to 15.1 hours, which demonstrates that immobilized mixed culture had a high cell density and biological activity. The immobilized cells could quickly adapt to the environment. The maximum hydrogen production rate was increased from 11.1 mL/h in suspended fermentation to 49.9 mL/h, which was 4.5 times more than the former. In addition, the variance of immobilized fermentation was closer to 1 which means that it was more in line with the Gompertz equation.

## Effect of substrate fluctuation on tolerance of the artificial immobilized mixed culture system

Substrate concentration directly affects the hydrogen production efficiency of microorganisms as well as the stability and tolerance of the fermentation process (Park et al. 2015). Fig.3 shows the variation trend of pH value, total sugar concentration, cumulative hydrogen production with time, and content of liquid end product at the end of fermentation.

The variation of the content of total sugar and cumulative $H_2$ production is presented in Fig.3(a)(b). It can be clearly seen that the hydrogen production efficiency was reduced at concentration extremes. Fig.3(a) shows that the fermentation efficiency was reduced at a substrate concentration of 20 g/L, and the required fermentation time was much longer than the fermentation time at a substrate concentration of 5 g/L and 10 g/L, which was more than doubled. Moreover, substrate efficiencies were 98.9% and 99.6% at substrate concentrations of 5 g/L and 10 g/L, respectively, and the substrate was almost completely consumed. However, when the substrate concentration was 20 g/L, the substrate utilization rate was only 86.2%, and the substrate was not completely consumed. This might be because the high concentration of intermediate metabolites produced a certain inhibition on fermentation. Fig.3(b) further shows that the cumulative hydrogen production amounts from low to high substrate concentrations were 893 mL, 2070 mL, and 3193 mL, respectively. The hydrogen yields were 1.33 mol $H_2$/mol xylose, 1.54 mol $H_2$/mol xylose and 1.19 mol $H_2$/mol xylose, respectively. From the yield of hydrogen production, 10 g/L of the three substrate concentrations was the optimal concentration.

However, this result also further demonstrates that there was a higher substrate tolerance for the immobilized mixed culture system. The pH of fermentation broth is one of the most important factors affecting hydrogen production because pH affects the microbial metabolic pathway (Annous et al. 1996) and the activity of related enzymes such as hexokinase (Ma et al. 2017). The variation of the fermentation broth's pH also triggers changes in the population of hydrogen-producing microorganisms in the mixed micro-biota, including the structure and morphology of microorganisms (Ma et al. 2017). It can be seen from Fig.3 (c) that when the substrate concentration was 5 g/L, the pH decreases slowly. It might be that the substrate concentration was low, less VFAs was produced, and the microbial utilization efficiency was high. When the substrate concentration was 10 g/L and 20 g/L, the pH value decreased rapidly, but when the substrate concentration increased to 20 g/L, the pH drop rate did not continue to increase. It manifests that the biomass concentration was limited at this time, leading to a longer time for fermentation before the substrate can be fully utilized.

It can be seen from Fig.3(d) that when xylose was used as substrate, the liquid phase end products mainly contain acetic acid and butyric acid, with dominant butyric acid. The content of butyric acid at the end of fermentation was 1.81 g/L, 3.27 g/L and 3.85 g/L,

respectively, for the substrate concentration of the three groups from low to high, which accounted for 67.7%, 78.7% and 65.6% of the mixed acids, respectively. The results demonstrated that the production of hydrogen by complex bacteria with xylose as substrate was a butyric acid type fermentation, and the theoretical yield of hydrogen was 1.67 mol $H_2$/mol xylose. With the increase of the percentage of butyric acid, the yield of hydrogen production increased as well. When the substrate concentration was 10 g/L, the maximum hydrogen production was 1.54 mol $H_2$/mol xylose, which reached 92.2% of the theoretical value (Sivagurunathan et al. 2015). The optimal xylose substrate concentration for hydrogen production 10 g/L.

### Hydrogen production from continuous batch fermentation under optimum xylose concentration

In this experiment, the biological load and utilization rate of immobilized cells were maintained at a high level in a continuous fermentation process, so as to obtain a stable production rate and hydrogen yield. Immobilized cells can maintain a high biomass level of the reactor at low HRT and improve the efficiency of hydrogen production (Sivagurunathan et al. 2016). Therefore, after determining the optimum substrate concentration, 10 g/L xylose was used as the substrate and biomass adsorption material was used as the carrier to carry out 10 consecutive batches of fermentation. The cumulative production during continuous fermentation was studied. The changes of cumulative hydrogen production, substrate utilization and hydrogen production yield are shown in Fig.4. Although the fermentation yields fluctuate with the increase of fermentation batches, the fermentation effect of 10 batches of fermentation was better than that of suspended fermentation. The average substrate utilization rate for the 10 batches was 96%, the average hydrogen production was 1972 mL, and the average hydrogen production yield was 1.47 mol $H_2$/mol xylose.

### Hydrogen production by fermentation of mixed glucose and xylose

Since xylose and glucose are produced by hydrolysis of lignocellulose, a mix of both substrates was used. The hydrogen production characteristics of immobilized fermentation using the complex substrate were studied with the mass ratio of glucose to xylose as 1:1 and the total sugar concentration of 10 g/L as the raw material.

The changes of pH, total sugar concentration, and cumulative hydrogen production during fermentation of the mixed substrate can be seen in Fig.5. Compared with the use of sole xylose as the substrate, the fermentation cycle was shortened to 60 hours which means the time for hydrogen production was advanced, the pH value decreased faster. The final cumulative hydrogen production reached to 2054 mL. The total sugar concentration is essentially zero at the end of the fermentation. At 84 hours, a mixture of xylose and glucose was added directly to the fermentation broth, the ratio of them was still 1:1, the sugar concentration was restored to 10 g/L, and the pH was adjusted back to 6.5. The fermentation ended after another 60 hours. The cumulative hydrogen production of the secondary fermentation was 4270 mL and the hydrogen yield was 1.73 mol $H_2$/mol mixed sugar. The hydrogen production rate of mixed substrates is higher than that of xylose (1.47 mol $H_2$/mol xylose) and glucose (1.50 mol $H_2$/mol glucose) individually.

It can be seen from Fig.6 that when the mixed substrates of xylose and glucose were used, the dominant position of butyric acid in the liquid phase end product was more obvious. However, due to the simultaneous adjustment of the pH during the addition of the substrate, the final content of acetic acid and butyric acid was not further increased. The final cumulative amount of acetic acid was 0.65 g/L, and the cumulative amount of butyric acid was 3.37 g/L.

It can be seen that the combined bacteria can efficiently utilize xylose and glucose as a mixed substrates for fermentation to produce hydrogen. The hemicellulosic hydrolysate contains both five- and six-carbon sugars. The proportion of xylose and glucose can be adjusted according to the proportion of both kinds of sugars in the cellulose hydrolysate. This study provides comprehensive utilization of lignocellulose.

## Discussion

In this paper, an immobilized mixed culture system was used to study the characteristics of synergistic hydrogen production from xylose and glucose mixed substrate, based on the previous studies (Bao et al. 2012; Ma et al. 2017; Wang et al. 2018). The hydrogen yield of the immobilized mixed culture system was 1.54 mol $H_2$/mol xylose, and 92.2% of the theoretical value of acid fermentation was achieved when using immobilized mixed culture system to produce hydrogen by using 10 g/L of xylose as substrate. The lag period was shortened by immobilization. The volume of hydrogen production was 29.9% higher than that of suspended fermentation,

and the maximum rate of hydrogen production could reach 49.9mL/h which was 4.5 times higher than that of suspended fermentation. The average substrate utilization rate of the immobilized fermentation was 96% for the 10 batches, and the average hydrogen yield was 1.47 mol $H_2$/mol xylose. In addition, this system can utilize the mixed sugar of xylose and glucose as substrate, with a hydrogen yield of 1.73 mol $H_2$/mol mixed sugar, which was higher than the hydrogen production rate of the single substrate. This study provides a basis for further development of hydrogen production by complex cell substrates such as cellulose hydrolysate in a mixed culture system.

Further research and discussion are needed for the continuous hydrogenation of complex strains and the optimization of carriers. The influence of cofactors such as NADH on biohydrogen production. The process of biohydrogen production is a microbial metabolic process involving many enzymes, and the cofactor NADH affects the metabolic process by affecting enzyme activity. On the basis of continuous fermentation in this paper, continue to optimize HRT or increase the fermenter, so that the bacteria can maintain biological activity, improve the hydrogen production efficiency of the system, and provide support for industrial hydrogen production. Although the immobilized hydrogen production of the composite strain can significantly improve the hydrogen production efficiency of the system, some mechanisms in this process are not clear, so the further research can also be focused on the synergistic mechanism. While 4 mol of $H_2$ can be generated from 1 mol of glucose in dark fermentation, the actual $H_2$ yield is lower than 50% of the theoretical maximum result from thermodynamic limitation, by-products and the $H_2$-consuming reaction (Lalman et al. 2013). Since the photo-fermentative bacterium can utilize VFAs produced by the dark-fermentative bacteria, this process can release the inhibition of VFAs in the dark-fermentation, increase the substrate utilization and realize continuous and stable $H_2$ production.

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## Compliance with Ethical Standards

The authors declare that they have no conflict of interest.

This article does not contain any studies with human participants performed by any of the authors.

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Fig.1 Variation of cumulative $H_2$ production with time

Fig.2 Type and concentration of VFAs

Fig.3 Hydrogen production performance of immobilized mixed culture operated under different concentration of xylose. Line charts and bar chart include all data of the corresponding periods. **a** pH value **b** Total sugar **c** Cumulative $H_2$ production **d** End products

Fig.4 The comparison of multiple batch fermentation

Fig.5 Variation of pH, total sugar concentration and cumulative $H_2$ production with time

Fig.6 Variation of concentration of acetic and butyric acid with time

Table 1 The fitting coefficients of Gompertz equation

| Fermentation method | λ(h) | $R_m$(mL/h) | $R^2$ |
|---|---|---|---|
| Suspended fermentation | 24.8 | 11.1 | 0.991 |
| Immobilized fermentation | 15.1 | 49.9 | 0.996 |

Figure 1

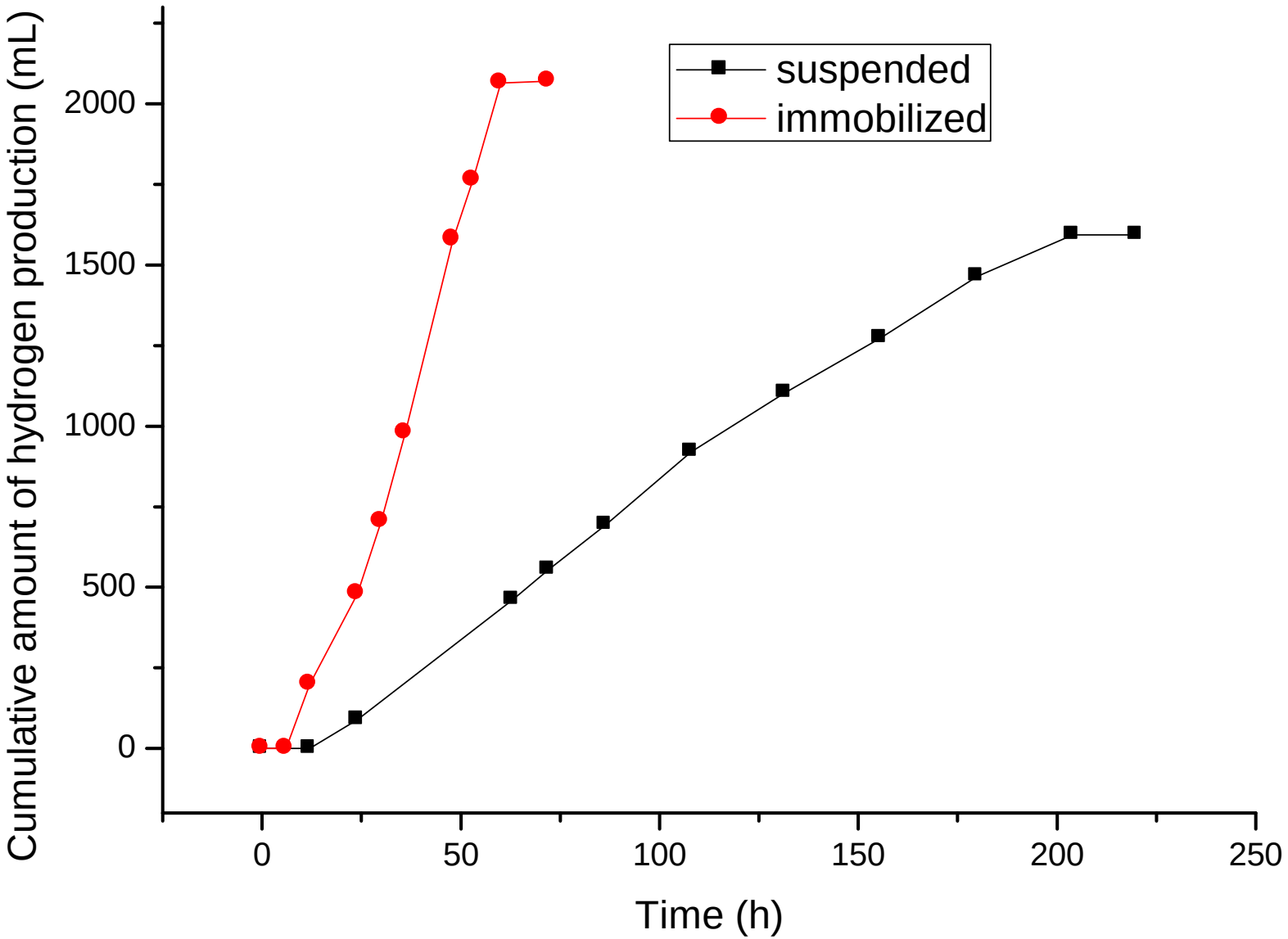

Cumulative amount of hydrogen production (mL)
2000
1500
1000
500
0
0
50
100
150
200
250
Time (h)
suspended
immobilized

Figure 2

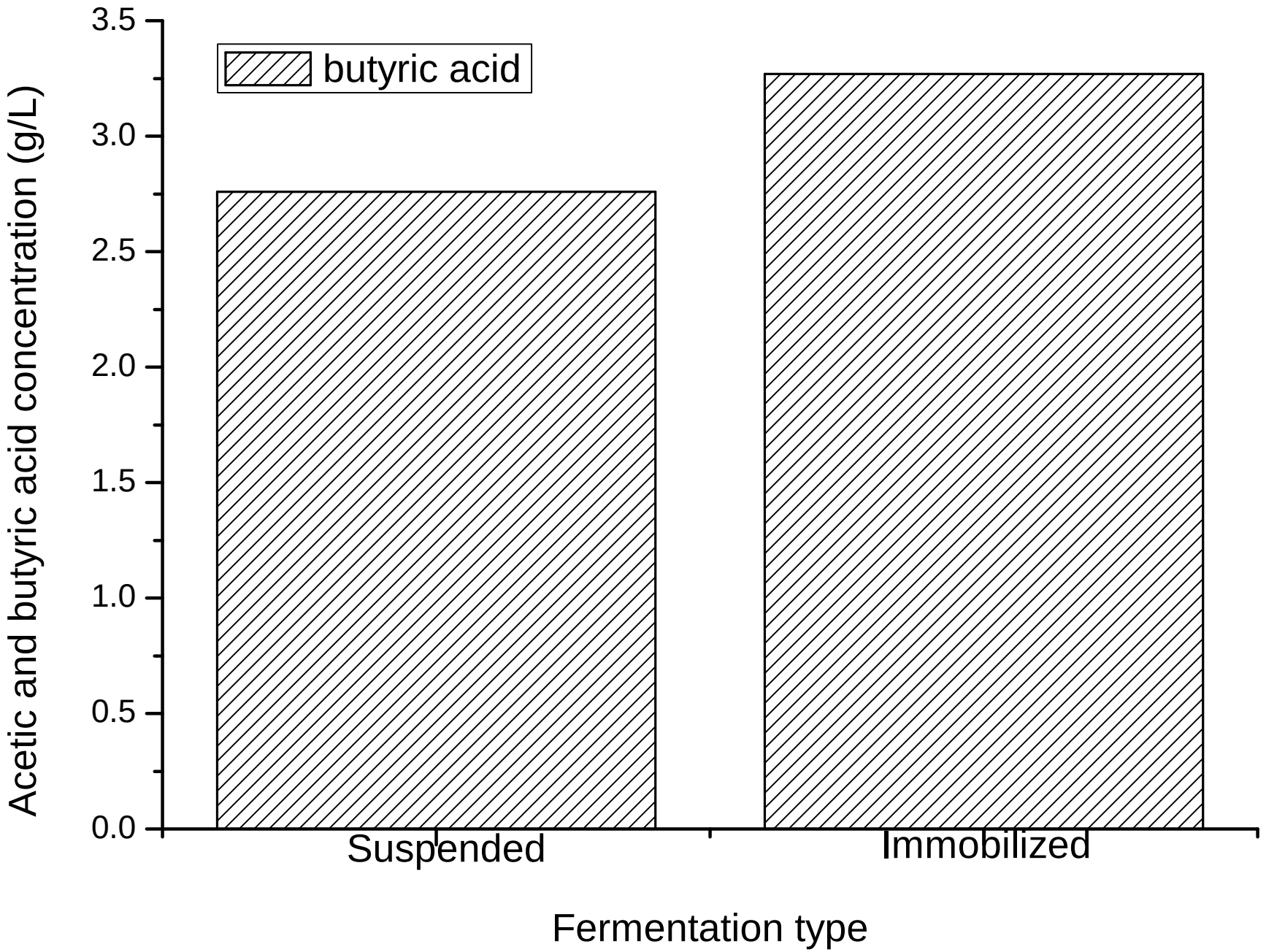
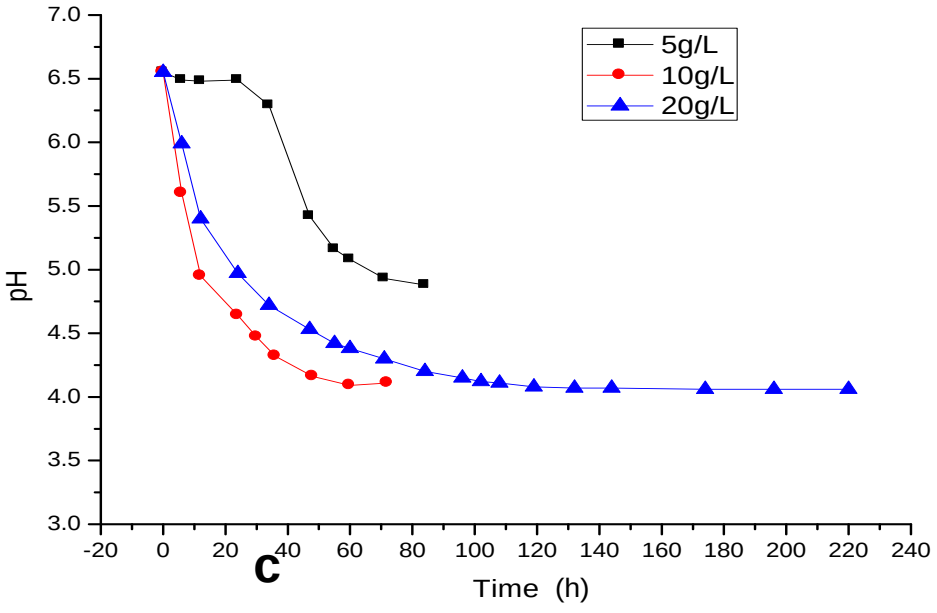
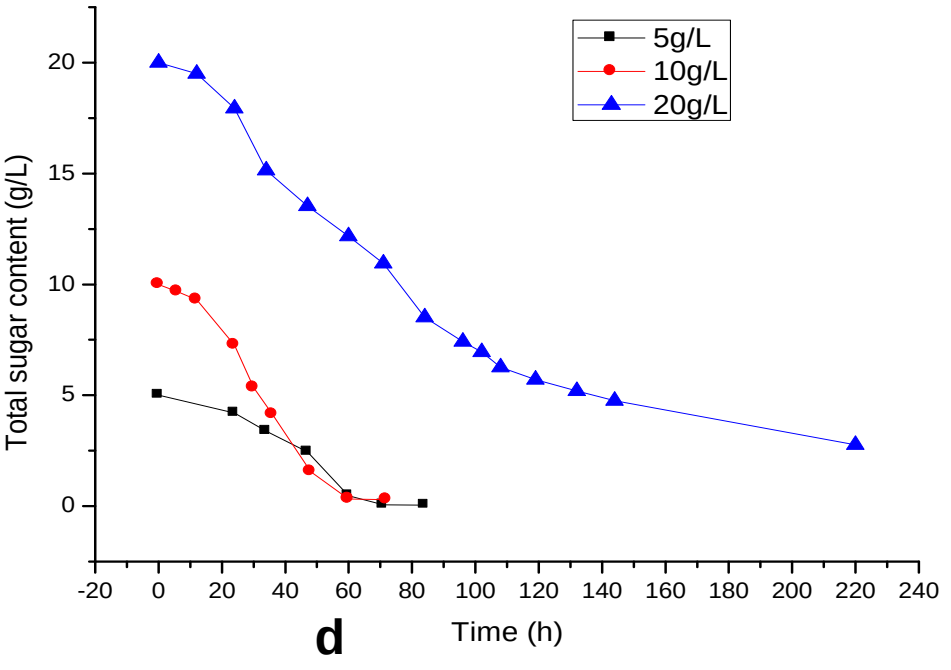
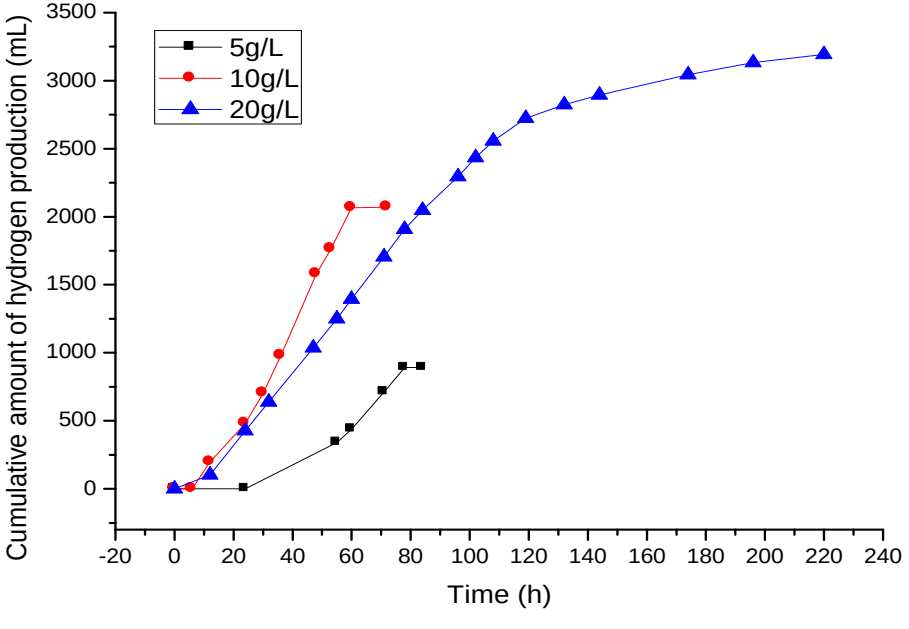

butyric acid
Acetic and butyric acid concentration (g/L)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Suspended
Immobilized
Fermentation type

Figure 3


pH
Time (h)
5g/L
10g/L
20g/L
c


Total sugar content (g/L)
Time (h)
5g/L
10g/L
20g/L
d


Cumulative amount of hydrogen production (mL)
Time (h)
5g/L
10g/L
20g/L

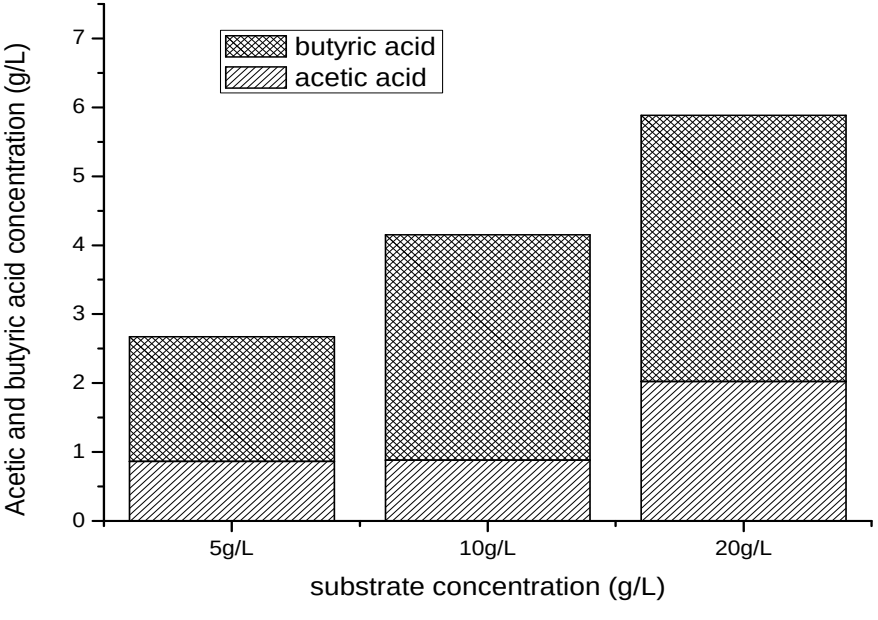

Acetic and butyric acid concentration (g/L)
substrate concentration (g/L)
butyric acid
acetic acid
5g/L
10g/L
20g/L

Figure 4

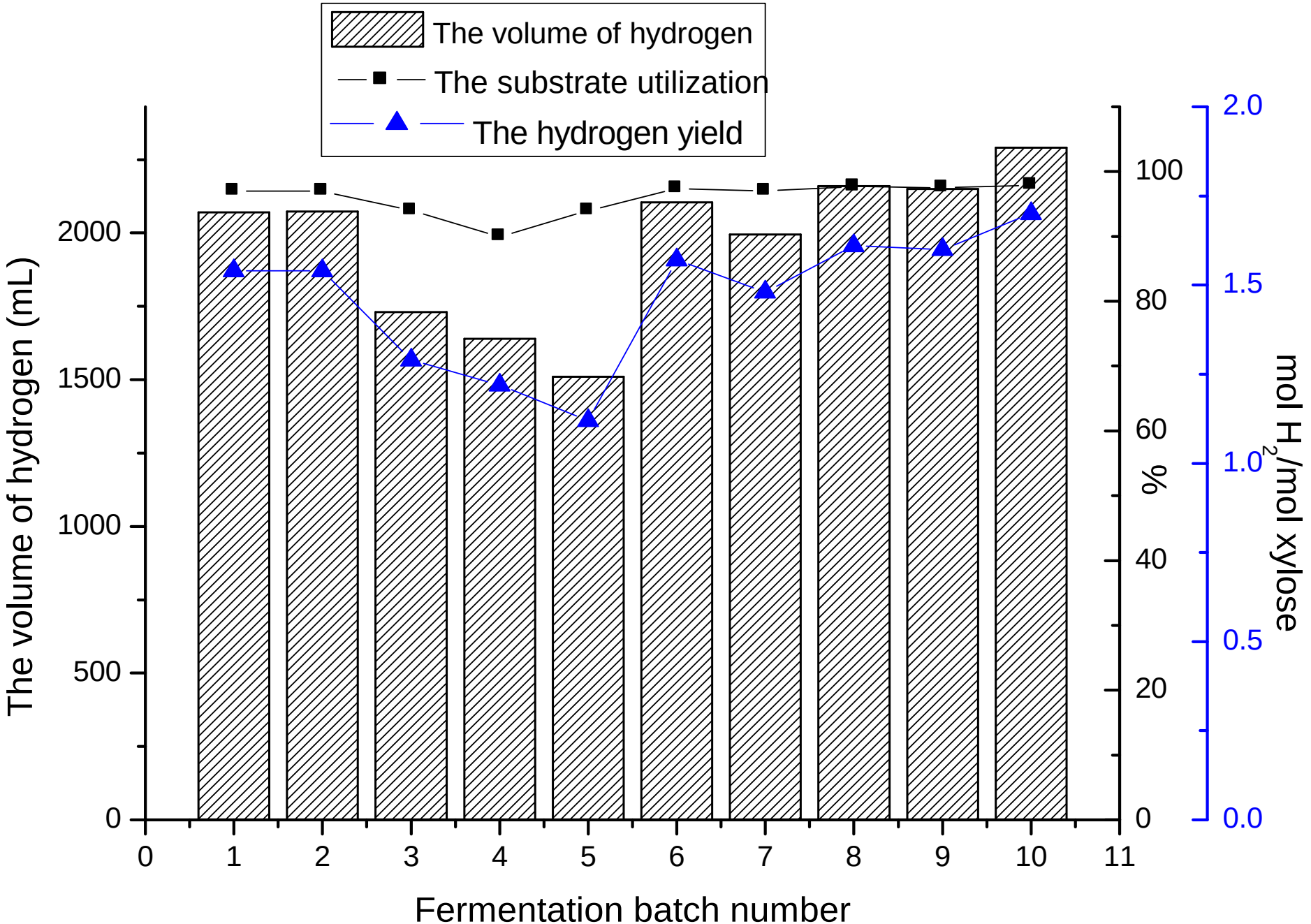

The volume of hydrogen
The substrate utilization
The hydrogen yield
The volume of hydrogen (mL)
0
500
1000
1500
2000
%
0
20
40
60
80
100
mol H2/mol xylose
0.0
0.5
1.0
1.5
2.0
0
1
2
3
4
5
6
7
8
9
10
11
Fermentation batch number

Figure 5

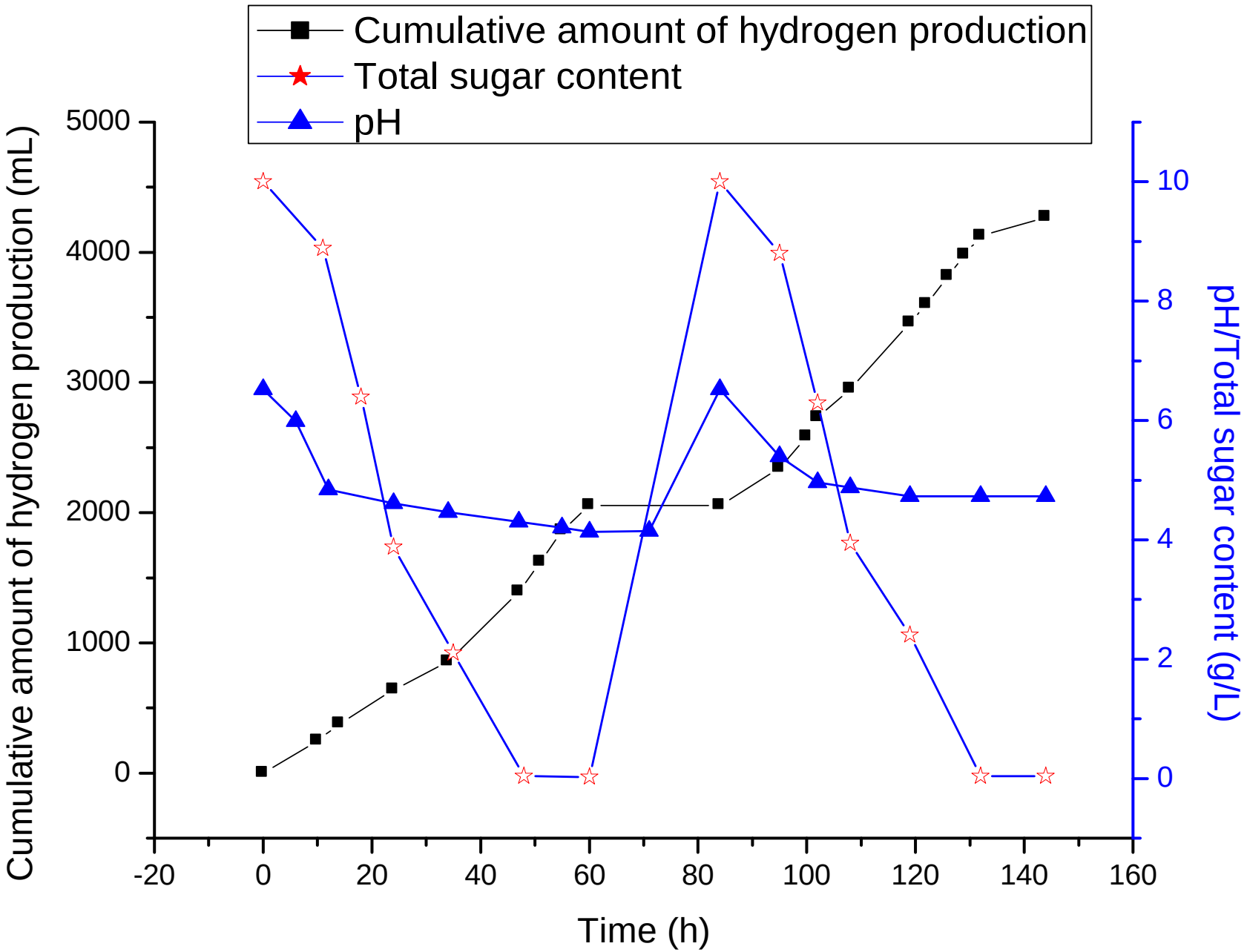
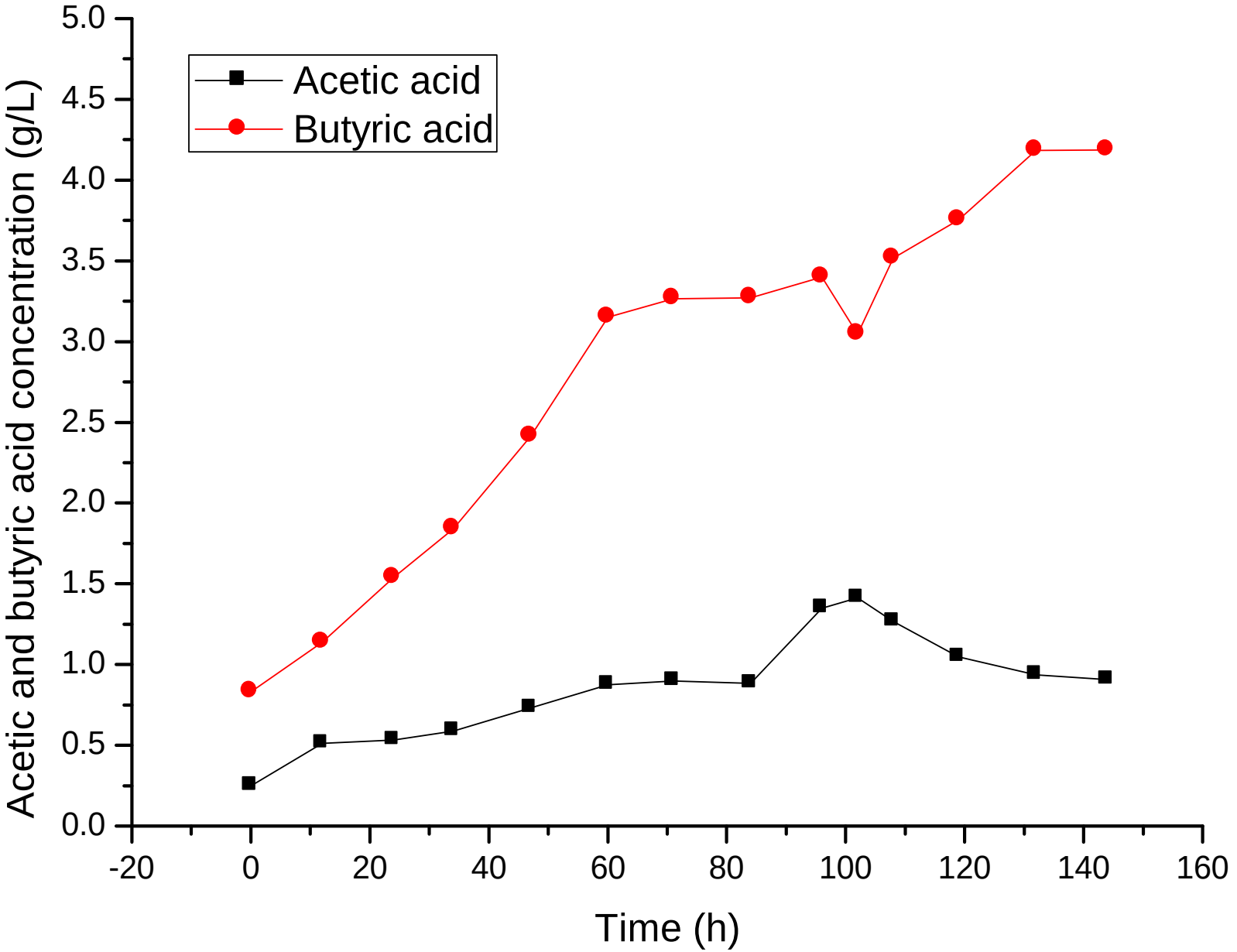

Cumulative amount of hydrogen production
Total sugar content
pH
Cumulative amount of hydrogen production (mL)
pH/Total sugar content (g/L)
Time (h)
5000
4000
3000
2000
1000
0
10
8
6
4
2
0
-20
0
20
40
60
80
100
120
140
160

Figure 6


Acetic acid
Butyric acid
Acetic and butyric acid concentration (g/L)
Time (h)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-20
0
20
40
60
80
100
120
140
160