The maximum specific hydrogen-producing activity of anaerobic mixed cultures: definition and determination

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Fermentative hydrogen production from wastes has many advantages compared to various chemical methods. Methodology for characterizing the hydrogen-producing activity of anaerobic mixed cultures is essential for monitoring reactor operation in fermentative hydrogen production, however there is lack of such kind of standardized methodologies. In the present study, a new index, i.e., the maximum specific hydrogen-producing activity (SHA<sub>m</sub>) of anaerobic mixed cultures, was proposed, and consequently a reliable and simple method, named SHA<sub>m</sub> test, was developed to determine it. Furthermore, the influences of various parameters on the SHA<sub>m</sub> value determination of anaerobic mixed cultures were evaluated.

Additionally, this SHA<sub>m</sub> assay was tested for different types of substrates and bacterial inocula. Our results demonstrate that this novel SHA<sub>m</sub> assay was a rapid, accurate and simple methodology for determining the hydrogen-producing activity of anaerobic mixed cultures. Thus, application of this approach is beneficial to establishing a stable anaerobic hydrogen-producing system.

Energy shortage is one of major global challenges, and thus there is an increasing interest in the search for renewable energy sources to meet the current and future energy requirements. Hydrogen is an attractive potential alternative energy source due to its advantages including clean, efficient and non-polluting characteristics. Compared with various chemical methods for hydrogen production, biological hydrogen production by fermentative process can be operated at ambient temperatures and normal pressures. Furthermore, this process can reuse a large amount of various waste materials from industries and agriculture. Therefore, biological hydrogen production facilitates both waste treatment and energy recovery.

During the start-up or steady-state operation of fermentative hydrogen-producing reactor, a sufficient quantity of active hydrogen-producing bacteria should be maintained within anaerobic reactors. Therefore, methodology for characterizing the hydrogen-producing bacteria and their activity is essential for monitoring reactors from the standpoint of the design and operation. Some techniques, such as microscopic counts, most probable number (MPN), adenosine triphosphate (ATP) and dehydrogenase activity, might be available to determine the level and activity of hydrogen-producing bacteria. However, the use of the MPN test was found to be not practical due to long doubling times, requirements of strict anaerobic conditions, and the difficulty in cultivating some of the species involved. Both ATP and dehydrogenase activity assays cannot be used as reliable methods for determining the level of active hydrogen-producing bacteria. In order to find out the optimum initial pH, substrate concentration, inocula, and enrichment procedure to start up a hydrogen-producing reactor, van Ginneken et al. adopted a modified Gompertz equation to simulate a batch hydrogen production process and determine the hydrogen production rate of anaerobic mixed cultures. In another study, Zheng et al. investigated the influence of illumination on the fermentative hydrogen production system, and also used a modified Gompertz equation to calculate the specific hydrogen production rate of anaerobic mixed cultures. However, three model parameters - lag time, H<sub>2</sub> production potential, and H<sub>2</sub> production rate, were adjusted to fit the experimental data in this empirical model. Even though this curve-fitting approach yields high correlation coefficients between the observed and fitted hydrogen evolution data, the three model parameters determined by curve-fitting were restricted to specific experimental conditions. In a methane-producing reactor, the specific methanogenic activity (SMA) assay has been demonstrated to be an effective method and therefore has been standardized and widely used to determine the methanogenic ability of anaerobic mixed cultures under various conditions.
However, there is no standardized methodology for biological hydrogen-producing activity assay. Similar with the SMA assay, development of such a standardized methodology is essentially required and important for evaluating the hydrogen-producing ability of anaerobic mixed cultures under various conditions, especially for comparison purpose to arrive at conclusions.

Therefore, the present study aimed at developing and standardizing the biological assay for determining the ability of anaerobic mixed cultures to convert substrate to hydrogen. Based on the kinetic relationships between substrate, product and microorganism, a reliable and simple approach was proposed to determine the hydrogen-producing activity of anaerobic mixed cultures in this study. This method gave a direct measurement of the maximum rate of hydrogen production per unit of microbial biomass per unit time, e.g., the maximum specific hydrogen-producing activity (SHA$_{m}$) of anaerobic mixed cultures, thus, was named as the SHA$_{m}$ test. Furthermore, the influences of various parameters, such as pH, substrate concentration and temperature, on the SHA$_{m}$ determination were also evaluated. Additionally, this SHA$_{m}$ assay was tested for different types of substrates and bacterial sources, and then verified with two case studies. The use of such an SHA$_{m}$ test could allow us to rapidly, accurately and simply determine the potential hydrogen-producing activity of anaerobic mixed cultures, which would be useful for establishing a stable anaerobic hydrogen-producing system.

**Results**

**Definition of SHA$_{m}$** The relationship between microbial growth and product formation for the anaerobic hydrogen production by mixed anaerobic cultures was simulated by the Luedeking-Piret model:

$$\frac{dP_i}{dt} = \alpha_i \frac{dX}{dt} + \beta_i X$$  \hspace{1cm} (1)

where $\alpha_i$ is growth-associated formation coefficient of product $i$; $\beta_i$ is non-growth-associated formation coefficient of product $i$ ($P_i$); $X$ is microorganism concentration (g-VSS/L); and VSS is volatile suspended solids.

Eq. (1) could be changed into:

$$\frac{dP_i}{dX} = \alpha_i \mu + \beta_i$$  \hspace{1cm} (2)

where $dP/dX/X$ is specific formation rate of product $i$; and $\mu$ (1/d) is specific growth rate of microorganisms. A straight line could be obtained with an intercept of $\beta_i$ and a slope of $\alpha_i$, if plotting $dP/dX$ against $\mu$.

In the anaerobic fermentative process, a typical fitted plot for hydrogen production was reported in Mu et al. and the estimated $P_{H_2}$ value was nearly equal to zero, suggesting that the formation of hydrogen in such a process was mainly growth-associated. Although hydrogen can be produced during stationary phase(s) of bacterial cultures, several studies have also suggested that hydrogen was mainly produced during the period of biomass growth compared to the stationary phase in batch test for anaerobic mixed cultures, further suggesting that the formation of hydrogen was (but not purely) growth-dependent in the anaerobic fermentative process by mixed cultures. As a consequence, the correlation between hydrogen and substrate could be expressed as Eq. (3):

$$\frac{dP_{H_2}}{dt} = -Y_{H_2} \times \frac{dS}{dt}$$  \hspace{1cm} (3)

where $S$ (mmol/L) is substrate concentration; and $Y_{H_2}$ (mL-H$_2$/mmol-substrate) is hydrogen yield coefficient.

In a batch fermentative hydrogen production process, Eq. (3) could be changed into:

$$\frac{dS}{dt} = -\frac{1}{Y_{H_2} \times V_R} \times \frac{dV_{H_2}}{dt} \times \frac{T_0}{T_1}$$  \hspace{1cm} (4)

where $V_{H_2}$ (mL) is accumulated volume of hydrogen; $V_R$ (L) is reactor volume; $T_0$ (K) is standard temperature, e.g., 273.15 K; and $T_1$ (K) is room temperature.

The degradation of substrate was expressed by Monod-type equation:

$$\frac{dS}{dt} = -\frac{U_{max} \times S \times X}{K_s + S}$$  \hspace{1cm} (5)

where $U_{max}$ (1/d) is maximum specific substrate degradation rate; and $K_s$ (mmol/L) is half-saturation constant.

The following Eq. (6) was obtained by combining Eqs. (4) and (5):

$$\frac{1}{Y_{H_2} \times V_R} \times \frac{dV_{H_2}}{dt} \times \frac{T_0}{T_1} = \frac{U_{max} \times S \times X}{K_s + S}$$

At the beginning of fermentation, $S \gg K_s$, thus Eq. (6) was simplified into:

$$\frac{1}{V_R} \times \frac{dV_{H_2}}{dt} \times \frac{T_0}{T_1} = Y_{H_2} \times U_{max}$$  \hspace{1cm} (7)

Here, we defined the SHA$_{m}$ of anaerobic mixed cultures (mL-H$_2$/g-VSS/d) as:

$$SHA_m = Y_{H_2} \times U_{max}$$  \hspace{1cm} (8)

Thus, Eq. (7) could be rewritten as:

$$\frac{1}{V_R} \times \frac{dV_{H_2}}{dt} \times \frac{T_0}{T_1} = SHA_m$$  \hspace{1cm} (9)

Eq. (9) indicates that the specific hydrogen production rate of anaerobic mixed cultures was a constant value at the beginning of fermentation, which was equal to the SHA$_{m}$.

The SHA$_{m}$ determination of anaerobic mixed cultures could be useful in a fermentative hydrogen production process. At the beginning of the start-up period of a new reactor, the activities of the seeding anaerobic mixed cultures and its amount are of great importance. Therefore, the present study aimed at developing and standardizing the biological assay for determining the ability of anaerobic mixed cultures to convert substrate to hydrogen. Based on the kinetic relationships between substrate, product and microorganism, a reliable and simple approach was proposed to determine the hydrogen-producing activity of anaerobic mixed cultures in this study. This method gave a direct measurement of the maximum rate of hydrogen production per unit of microbial biomass per unit time, e.g., the maximum specific hydrogen-producing activity (SHA$_{m}$) of anaerobic mixed cultures, thus, was named as the SHA$_{m}$ test. Furthermore, the influences of various parameters, such as pH, substrate concentration and temperature, on the SHA$_{m}$ determination were also evaluated. Additionally, this SHA$_{m}$ assay was tested for different types of substrates and bacterial sources, and then verified with two case studies. The use of such an SHA$_{m}$ test could allow us to rapidly, accurately and simply determine the potential hydrogen-producing activity of anaerobic mixed cultures, which would be useful for establishing a stable anaerobic hydrogen-producing system.

**Determination of SHA$_{m}$** A typical curve of the accumulated hydrogen concentration and temperature, on the SHA$_{m}$ determination was also evaluated. Additionally, this SHA$_{m}$ assay was tested for different types of substrates and bacterial sources, and then verified with two case studies. The use of such an SHA$_{m}$ test could allow us to rapidly, accurately and simply determine the potential hydrogen-producing activity of anaerobic mixed cultures, which would be useful for establishing a stable anaerobic hydrogen-producing system.

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Effects of various operational parameters on SHA

As shown in Figure 2, the SHA of the anaerobic mixed cultures increased from 419 ± 11 to 896 ± 8 mL-H_{2}/g-VSS/d as pH was increased from 4.0 to 5.5, then decreased to 496 ± 38 mL-H_{2}/g-VSS/d with a further increase to 7.0. A maximum SHA value was obtained at pH 5.5. The microbial activities may be controlled by the overall enzymatic activity, which is pH dependent, as shown in the followings:

\[
E^+ \leftrightarrow E + H^+ \\
E^{-} \leftrightarrow E^- + H^+ 
\]

where \(E\) represents the active enzyme, and \(E^+\) and \(E^-\) are the less active forms of charge-carrying enzyme. Assuming \(K_{H}\) and \(K_{OH}\)

\[
SHA_{m} = \frac{OSHA_{m}}{1 + K_{OH}/[H^+] + [H^+] / K_{H}} = \frac{OSHA_{m}}{1 + K_{OH}/10^{-pH} + 10^{-pH} / K_{H}} 
\]

where OSHA_{m} is the optimum SHA \(m\) (mL-H_{2}/g-VSS/d). As shown in Figure 2, the relationship between SHA_{m} and pH was well simulated by Eq. (12) with a high correlation coefficient of 0.930. The values of \(K_{H}, K_{OH}\), and OSHA_{m} were determined as 9.0 \times 10^{-3} mol/L, 1.1 \times 10^{-3} mol/L, and 905 mL-H_{2}/g-VSS/d, respectively. The estimated maximum SHA_{m} of 905 mL-H_{2}/g-VSS/d was much closer to the experimental maximum value of 896 ± 8 mL-H_{2}/g-VSS/d whereas the predicted optimum pH [(pK_{OH} + pK_{H})/2] was equal to the experimental optimum pH of 5.5. Additionally, the final pH was in a range of 4.5–4.0 due to the production of volatile fatty acids in the fermentative hydrogen production process.

The effect of substrate concentration on the SHA of anaerobic mixed cultures is presented in Figure 3. The SHA_{m} increased from 555 ± 30 to 1400 ± 49 mL-H_{2}/g-VSS/d with the increasing sucrose concentration from 2.92 to 17.54 mmol/L then decreased to 1049 ± 50 mL-H_{2}/g-VSS/d as the sucrose concentration was further increased to 20.47 mmol/L. The dependence of SHA_{m} on the initial substrate concentration could be described using the generalized Haldane equation:

\[
SHA_{m} = \frac{OSHA_{m} \times S}{(K_{s} + S) \times (1 + T / K_{i})^{n}}
\]

where \(K_{i}\) (mmol/L) is inhibition constant; \(I\) (mmol/L) is inhibitor concentration; and \(n\) is a constant (order of inhibition).

In parameter estimation, \(n\) was given values of 1 (referred to as Eq. (13a)) and 2 (referred to as Eq. (13b)). The fitted curves by Eqs. (13a), (13b) and (14) are shown in Figure 3 and the estimated values of various parameters are listed in Table 1. Although all of equations gave a well description about the relationship between SHA_{m} and substrate concentration, the \(K_{s}\) value, being higher than \(K_{i}\) estimated by both Eq. 13a and Eq. 13b, was not likely to be correct, on the basis of the values of \(K_{s}\) and \(K_{i}\) for methane production process found in
with a high correlation coefficient value of 0.986. Following estimates for OSHAm, Kc and Ks were selected as 4878 mL-H2/g-VSS/d, 22.77 mmol/L and 22.77 mmol/L, respectively. In literatures very limited information about the values of Kc and Ks for fermentative hydrogen production processes is available. Although in general the value of Kc should be much higher than the Ks value, a previous study also reported that the value of Kc was close or even equal to the Ks value in biological methane production. The reasons behind such an inconsistence need further investigations.

The effect of temperature on the SHAm of anaerobic mixed cultures was investigated in a range of 20.0 to 55.0°C, as presented in Figure 4. An increase in SHAm of anaerobic mixed cultures was observed from 864 ± 80 to 6400 ± 160 mL-H2/g-VSS/d with the increase in temperature. The temperature dependence of anaerobic mixed cultures SHAm was described by an Arrhenius type equation with a high correlation coefficient value of 0.986:

\[ \text{SHAm} = K \times e^{-\frac{E_a}{RT}} \]  

(15)

where K (mL-H2/g-VSS/d) is frequency factor; \( E_a \) (kJ/mol) is apparent activation energy; \( R \) (0.008314 kJ/mol/K) is gas constant; and T (K) is absolute temperature. As shown in Figure 4, the values of K and \( E_a \) were respectively estimated as 7.0 \times 10^{19} \text{ mL-H2/g-VSS/d} and 44.2 kJ/mol.

**Comparison of SHAm for different substrates and bacterial sources.** As shown in Table 2, the SHAm values of anaerobic mixed culture were similar for different types of substrates and in a range of 900–1000 mL-H2/g-VSS/d. This might be due to that all of substrates used were soluble and their hydrolysis was not a rate-limiting step for these substrates.

The SHAm values had a notable difference for various bacterial sources, as summarized in Table 3. The anaerobic microflora from the upflow anaerobic sludge blanket (UASB) reactor had a highest SHAm value of 1632 ± 288 mL-H2/g-VSS/d, while a lowest value of 935 ± 12 mL-H2/g-VSS/d for the anaerobically digested sludge from the municipal wastewater treatment plant. This result suggests that the method developed in this study could be applicable to assess the specific hydrogen-producing ability of different mixed bacterial inocula.

**Application for two case studies.** The methodology developed in this study was applied to describe the results reported in two cases. Different factors were intensively examined for their effects on the H2-producing activity of anaerobic mixed cultures in Case 1. As shown in Figure 5(A), the relationship between the microbial growth and the product formation in their study was also well simulated by the Luedeking-Piret model with a regression coefficient higher than 0.8. Moreover, the estimated \( \beta_{H2} \) value was equal to zero, suggesting that the formation of hydrogen in such a process was mainly growth-dependent. Consequently, the SHAm values of the anaerobic mixed culture were estimated using Eq. (10), and the results at various pH values are shown in Figure 5(B). A maximum SHAm value was observed at pH 6.0 for the anaerobic mixed cultures in their study. The estimated \( \beta_{H2} \) value was also equal to zero in Case 2 (Figure 6A), where the activated sludge was adopted as the seed for hydrogen production, further suggesting that hydrogen production using mixed cultures was mainly growth-dependent. Additionally, the calculated SHAm value was about 186 mL-H2/g-VSS/d in Case 2 (Figure 6B).

**Table 2** | **SHAm values for various types of substrates**

| Substrate            | SHA (mL-H2/g-VSS/d) |
|----------------------|---------------------|
| Glucose              | 896 ± 8             |
| Sucrose              | 896 ± 15            |
| Starch               | 864 ± 53            |
| Brewage wastewater   | 1036 ± 133          |

Note: pH 5.5, temperature 20.0°C, substrate concentration 3.4 g-COD/L, biomass concentration 3 g-VSS/L, and anaerobic sludge from a UASB reactor as inocula.
Discussion

Biological hydrogen fermentation is a microbial process, which requires careful design and control. In practice, the process design is usually based on sludge loading rate and VSS is usually used to characterize the concentration of hydrogen-producing sludge. However, the VSS is undefined as it includes active biomass, as well as dead cell and particulate organic matter. The SHA m test proposed in this study is able to accurately quantify the hydrogen-producing activity of sludge in a simple and quick way under different conditions. Thus, the SHA m of sludge is likely to be more useful than VSS as a parameter for the design and operation of anaerobic hydrogen-producing reactors.

Hydrogen partial pressure in the liquid phase is one of the key factors affecting hydrogen production. Tanisho et al. observed an increase in residual NADH by sparging with argon in the reactor, which expected to give an increased hydrogen production. Logan et al. measured hydrogen production in two types of batch tests (Owen and respirometer) and found that the respirometric method resulted in the production of 43% more hydrogen from glucose than the Owen method. Therefore, a continuous gas release method (similar to respirometric one) was adopted in the SHA m test, in order to reduce the influence of hydrogen partial pressure on hydrogen production in reactors.

Hydrogen production with anaerobic mixed cultures has been shown to be affected by many factors, such as pH, substrate concentration and temperature. Many studies have demonstrated that proper control of pH is crucial to the hydrogen production and the reported optimum pH value is in a wide range of pH 5.0 to pH 9.0 for different types of hydrogen-producing cultures. This implies that the optimum pH for the SHA m was likely different for various bacteria, although a peak value of SHA m was observed at pH 5.5 for the anaerobic mixed cultures used in this study.

The substrate concentration is also critical in the microenvironment of the hydrogen-producing bacteria. A high substrate concentration could ensure a more rapid diffusion of substrate into microorganisms. However, as shown in Figure 3, when the substrate concentration was higher than 5.6 g-COD/L, the hydrogen production rate decreased.

Table 3 | Comparison of SHA m values for different bacterial sources

| Bacterial sources                                           | SHA m  
|-------------------------------------------------------------|--------|
| Anaerobic microflora from a UASB treating soybean-processing wastewater | 1632 ± 288 |
| Anaerobically digested sludge from the Zhuzhuangjing Municipal Wastewater Treatment Plant | 935 ± 12 |
| Anaerobic sludge from a CSTR treating chicken manure | 990 ± 58 |
| Cow dung compost from a farm | 1051 ± 35 |
| Sediments from Chaohu Lake | 1081 ± 94 |

Note: pH 5.5, temperature 30.0°C, sucrose concentration 3.4 g-COD/L, and biomass concentration 3 g-VSS/L.

Figure 5 | (A) Luedeking-Piret model plot for the relationship between the hydrogen formation rate and the specific growth rate of microorganisms and (B) the estimated SHA m values of the anaerobic mixed cultures at various pHs from Case 1.

Figure 6 | (A) Luedeking-Piret model plot for the relationship between the hydrogen formation rate and the specific growth rate of microorganisms and (B) the estimated SHA m value of the anaerobic mixed cultures from Case 2.
Alcohols are also produced in fermentative hydrogen production. The correlation between SHA\textsubscript{m} and RIHP.

In this study five different bacterial sources were respectively taken as inocula, including (1) aerobic microflora from a full-scale UASB reactor treating soybean-processing wastewater located in Benpu City, China; (2) anaerobically digested sludge from Zhuzhuanjing Municipal Wastewater Treatment Plant in Hefei City, China; (3) anaerobic sludge from a 600-m\textsuperscript{3} CSTR (continuous stirred tank reactor) treating chicken manure located in Jinhua City, China; (4) cow dung compost from a farm in Benpu City, China; and (5) sediments from Chaohu Lake, China. Prior to use, these inocula were sieved to remove stone, sand and other coarse matters. Thereafter, the inocula were heated at 102\degree C for 90 min to inactivate the methanogens and to enrich the hydrogen-producing bacteria\textsuperscript{12}. The initial concentration of biomass in the fermentor was about 3 g-VSS/L for all experiments.

On the other hand, various types of substrates, including glucose, sucrose, starch and brewage wastewater, were also compared in this study. Except for the brewage wastewater, all of other substrates were supplemented with buffering chemicals and balanced nutrients as follows: (unit in mg/L): NH\textsubscript{4}HCO\textsubscript{3} 405; K\textsubscript{2}HPO\textsubscript{4} 25; NaCl 10; CoCl\textsubscript{2} 5; Na\textsubscript{2}SO\textsubscript{4} 1; MnCl\textsubscript{2} 4H\textsubscript{2}O 5; AlCl\textsubscript{3} 2.5; (NH\textsubscript{4})\textsubscript{6}Mo\textsubscript{7}O\textsubscript{24} 15; CuCl\textsubscript{2} 50; MgCl\textsubscript{2} 6H\textsubscript{2}O 100; FeCl\textsubscript{3} 25; Na\textsubscript{2}CO\textsubscript{3} 6H\textsubscript{2}O 5; MnCl\textsubscript{2} 4H\textsubscript{2}O 5; ZnCl\textsubscript{2} 5. The characteristics of the brewage wastewater were similar to those reported by Yu et al\textsuperscript{11}. The initial substrate concentration was about 3.4 g-COD/L for all experiments except for the tests with various substrate concentrations, where the amounts of all organic and inorganic constituents were adjusted pro rata.

**Methods**

**Hydrogen-producing mixed cultures and substrate.** In this study five different bacterial sources were respectively taken as inocula, including (1) aerobic microflora from a full-scale UASB reactor treating soybean-processing wastewater located in Benpu City, China; (2) anaerobically digested sludge from Zhuzhuanjing Municipal Wastewater Treatment Plant in Hefei City, China; (3) anaerobic sludge from a 600-m\textsuperscript{3} CSTR (continuous stirred tank reactor) treating chicken manure located in Jinhua City, China; (4) cow dung compost from a farm in Benpu City, China; and (5) sediments from Chaohu Lake, China. Prior to use, these inocula were sieved to remove stone, sand and other coarse matters. Thereafter, the inocula were heated at 102\degree C for 90 min to inactivate the methanogens and to enrich the hydrogen-producing bacteria\textsuperscript{12}. The initial concentration of biomass in the fermentor was about 3 g-VSS/L for all experiments.

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**Test procedures of SHA\textsubscript{m}.** Using a continuous gas release apparatus, the setup of the SHA\textsubscript{m} test consisted of a 300-ml fermentor (serum vial), washing flask, gas collector, graduated flask, and vibrator with constant temperature. A required amount of anaerobic mixed cultures and substrate were respectively added into the serum vial, graduated flask, and vibrator with constant temperature. A required amount of anaerobic mixed cultures and substrate were respectively added into the serum vial, graduated flask, and vibrator with constant temperature. A required amount of anaerobic mixed cultures and substrate were respectively added into the serum vial, graduated flask, and vibrator with constant temperature. A required amount of anaerobic mixed cultures and substrate were respectively added into the serum vial, graduated flask, and vibrator with constant temperature.

**Analysis.** Sucrose concentration was determined by using anthrone-sulfuric acid method\textsuperscript{6}, while COD and VSS were measured using the standard methods\textsuperscript{46}. The percentage of H\textsubscript{2}, N\textsubscript{2}, and CH\textsubscript{4} in the gas was analyzed by using a GC (Model SP-6800A, Lunan Co., China) equipped with a thermal conductivity detector and a 3 m stainless steel column packed with 5 A molecular sieve. The operational temperatures at the injection port, the column oven and detector were 70, 80 and 80\degree C, respectively.

In anaerobic fermentative hydrogen production process, the RHIHP was defined as:  
\[ \text{RHIHP} = \frac{X - X_0}{X_0} \times 100\% \]  
where \( X_0 \) (g-VSS/L) is initial hydrogen-producing biomass concentration; and \( X \) (g-VSS/L) is hydrogen-producing biomass concentration at fermentative time \( t \) (h).

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Author contributions

Y.M. carried out the experiments, analyzed the data, and wrote the paper; H.Y.Y., Y.Z.W., C.S.H; carried out the experiments; Q.B.Z. and Y.W. analyzed the data, and wrote the paper; H.Q.Y. designed the experiments, analyzed the data, and wrote the paper.

Additional information

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