EFFECTS OF OPERATIONAL CONDITIONS AND CHARACTERISTICS OF FLOCCULATION AT HYDROGEN PRODUCTION BY USING IMMOBILIZED BED BIOREACTOR

Akira SANO 1, Shigeharu TANISHO 2

Abstract

A strain HN001 was shown to be effective in hydrogen production. In this study, the effects of operational conditions on hydrogen production were investigated using an immobilized bed bioreactor. The characteristics of flocculation were figured out in case of continuous cultivation.

Saran fiber, saran mesh and polypropylene nonwoven and polyethylene nonwoven were used as a sample of selectable supporter for immobilization. A cylindrical vessel (1.1 L) made from acrylic plastic was used as a cultivation tank. A circulation method was adopted for agitation. Temperature and pH of the cultivation were kept constant at 47 °C and 6.0, respectively. A molasses produced from a sugar plant was used as a sample of organic waste. The sugar concentration of molasses was adjusted from 5 g-glucose L\(^{-1}\) to 30 g-glucose L\(^{-1}\), while HRT was controlled at 1.0 - 1.5 h. Over the sugar concentration of 30 g-glucose L\(^{-1}\), H\(_2\) production rate decreased significantly. It is assumed that the molasses included many metal ion such as Na, K, Ca, Mg, etc. and the metal restrained bacteria from growth. The maximum rate of H\(_2\) production was 0.86 L-H\(_2\) h\(^{-1}\) L-culture\(^{-1}\) under the sugar concentration of 10 g L\(^{-1}\) and HRT of about 1.5 h. Addition of FeCl\(_3\) inhibit lactic acid production and enhance acetic acid and ethanol production. Iron ion had the effect on performance of H\(_2\) production. From the results of cultivation for 1 week, The flocculation on saran fiber formed many layers. Saran fiber was an effective supporter for immobilization of the strain HN001.

1. Introduction

Global warming becomes a matter of public knowledge. People’s awareness of environmental conservation is rising such as Kyoto protocol that obliged industrialized countries to reduce greenhouse gas emissions. A fuel cell that emits only water is effective in CO\(_2\) reduction from cars which moves emitting CO\(_2\). H\(_2\) used as a fuel of fuel cell is said to be an alternative future energy. Thus, the technology of the hydrogen production has been developed in various categories [1, 2]. A hydrogen production by bacteria using a biomass differs from the other method, and the biohydrogen production is sustainable technology.

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1 Venture Business Laboratory, Yokohama National University, Yokohama 240-8501, Japan
Tel.: +81-45-339-4467; fax: +81-45-339-4467; e-mail: sano@ynu.ac.jp

2 Department of Environmental Sciences, Yokohama National University, Yokohama 240-8501, Japan
The hydrogen production by anaerobic bacteria has been researched widely. The strain HN001 is a high performance bacterium for hydrogen production [3], it converts starch directly. In previous study, the effect of cultivation conditions such as temperature and pH on fermentation characteristics was clarified [4]. High-efficiency bioreactor is necessary to develop at advanced research of HN001. Anaerobic bioreactor has been applied to methane fermentation from long times ago, and the bioreactor for methane fermentation has been developed under various conditions. The hydrogen fermentation was controlled under very different conditions from the methane fermentation in the point of HRT and gas production rate. It is important that the bioreactor suitable for HN001 is designed stably and continuously to keep high bacterial concentration.

The continuous bioreactor for biohydrogen production was adopted in various methods for anaerobic bacteria [5, 6]. There are two factors by which make bioreactor efficient from the viewpoint of chemical engineering [7]. One is solid-liquid contact between bacteria and substrate, the other is three-phase separation among produced gas, soluble metabolites and bacteria. In this study, a supporter for immobilization was packed in a bioreactor to keep the bacterial concentration higher and contact between bacteria and substrates frequently.

In this study, the effects of operational conditions on hydrogen production were investigated by using an immobilized bed bioreactor. The characteristics of immobilization and flocculation were figured out.

2. Materials and methods

2.1. Experimental apparatus

Figure 1 shows the outline of experimental apparatus for immobilized bed bioreactor (1.2 L). A cylindrical vessel (φ 70 mm, 250 mm) made from acrylic plastic was used as a cultivation tank. A gas-liquid separator was set on the top of bioreactor. A circulation method was adopted for agitation of liquid and gas separated from the immobilized supporter by using liquid flow. A culture liquid was circulated after gas separation, and pH of culture liquid was controlled at 6.0. 2N NaOH aqueous solution for controlling pH was injected by using a roller pump. The concentration of casamino acids and yeast extract was adjusted to be 2.5 g L⁻¹ constantly. A thermocouple was put into the center of cultivation tank, and the temperature was kept at 47 °C by using a ceramic heater.

The sample held HRT of 1.0 - 1.5 h was fed from the bottom of fermentation tank through the sterilizer kept at 65 °C. The supporter for immobilization was packed in the cultivation tank. The operational conditions such as sample concentration and HRT were changed. A molasses produced from a sugar plant was used as a sample of organic waste. The estimated glucose concentration of molasses was adjusted from 5 g L⁻¹ to 30 g L⁻¹. The volume of produced gas and the concentrations of H₂ and CO₂ were measured.
by using wet gas meter and TCD, respectively. $\text{H}_2$ production rate was calculated at steady-state period in each condition. The concentration of soluble metabolites was measured by using HPLC. The bacterial concentration in the culture liquid was estimated by optical density from absorbance spectrometer. The effect of operational conditions on biohydrogen production was clarified by using the immobilized bed bioreactor.

![Outline of experimental apparatus for immobilized bed bioreactor](image)

2.2. Characteristics of immobilization and flocculation

Polyethylene nonwoven, polypropylene nonwoven, saran fiber and saran mesh were used as the sample of selectable supporter for immobilization. Each supporter was set in the cultivation tank, the continuous cultivation was performed for a week to compare the characteristics of immobilization. Then, the immobilized supporter was observed from the point of flocculation and gas peeling.

After cultivation, each supporter for immobilization was removed from the reactor to measure the bacterial concentration for immobilization. The removed supporter was soused in an ion-exchanged water, and the immobilizing bacteria were washed off the supporter by using a ultrasonic cleaner at 80 °C for 15 min. The washed water including bacteria of 40 mL was settled out by using centrifugal sedimentation at 10,000 rpm for 5 min and the bacterial weight was measured after drying at 105 °C for 6 h.

3. Results and discussion

3.1. Effect of operational conditions

Saran fiber (200 mm x 4), saran mesh (200 mm x 2) and polypropylene nonwoven (200 mm x 150 mm) were set as the supporter for immobilization, and the continuous experiment was carried out. **Figure 2** shows the operational conditions of the immobilized bed bioreactor at constant pH of 6.0. Estimated glucose concentration was
adjusted in the range from 5 g L\(^{-1}\) to 30 g L\(^{-1}\). HRT was changed in the range from 1.0 h to 1.5 h after 4 days. At the cultivation time of about 6 days, FeCl\(_3\) of 5.0 g was added in the tank for enhancement of H\(_2\) production.

Figure 2. Operational conditions of the immobilized bed bioreactor

Figure 3. Changes in H\(_2\) production rate and optical density with

Figure 4. Changes in concentration of soluble metabolites and sugar consumption with cultivation time
**Figure 3** shows the changes in H$_2$ production rate and optical density (OD) with cultivation time. Under the estimated glucose concentration of 20 g L$^{-1}$, the H$_2$ production rate became steady at about 0.4 L-H$_2$ h$^{-1}$ L-culture$^{-1}$ until 3 days. In this stable period, OD became high from 1.9 to 2.1. Under the estimated glucose concentration of 10 g L$^{-1}$, H$_2$ production rate became the highest at about 0.5 L-H$_2$ h$^{-1}$ L-culture$^{-1}$ and OD became high from 2.1 to 2.9. OD became high with increasing bacterial concentration in culture liquid, and the increase of H$_2$ production rate corresponds to the increase of OD. Over the sugar concentration of 30 g-glucose L$^{-1}$, H$_2$ production rate decreased significantly. It is assumed that the molasses included many metal ion such as Na, K, Ca, Mg, etc. and the metal restrained bacteria from growth. In comparison of HRT, H$_2$ production rate decreased with decreasing HRT because of high metal ion. The maximum rate of H$_2$ production was 0.86 L-H$_2$ h$^{-1}$ L-culture$^{-1}$ under the sugar concentration of 10 g L$^{-1}$ and HRT of about 1.5 h.

**Figure 4** shows the changes in concentration of soluble metabolites and sugar consumption with cultivation time. The sugar consumption became low with increasing sugar concentration. This result corresponded to the result of H$_2$ production rate as shown in Fig. 3. In all conditions, ethanol and lactic acid were rich in the metabolites and H$_2$ yield was low. The maximum H$_2$ yield was 1.14 mol-H$_2$ mol-glucose$^{-1}$. At the cultivation time of about 6 days, addition of FeCl$_3$ inhibited lactic acid production and enhance acetic acid and ethanol production. Iron ion had the effect on performance of H$_2$ production.

### 3.2. Characteristics of immobilization and flocculation

**Table 1** shows the comparison of immobilized bacterial concentrations at different supporters. Amount of supporter per unit volume was shown in Table 1, too. In this immobilized bed reactor, the strain HN001 grew on the supporter, a part of floc was flaked and washed out in cultivation liquid. The bacterial concentration of saran fiber was higher than that of saran mesh. The bacterial concentration of polyethylene nonworen was relatively higher than that of polypropylene nonworen. These results indicated that high specific surface area such as saran fiber and polyethylene nonworen affected an immobilization performance of bacteria and material of supporter was less affected. From the observation, the gas peeling from immobilized supporter was better under high agitation flow rate. The flocculation on saran fiber formed many layers. It is clear that saran fiber was an effective supporter immobilizing the strain HN001.

**Table 1. Comparison of immobilized bacterial concentrations at different supporters**

<table>
<thead>
<tr>
<th>Supporter</th>
<th>Saran mesh</th>
<th>Saran fiber</th>
<th>Nonworen</th>
<th>Polypropylene nonworen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 mm×2</td>
<td>200 mm×2</td>
<td>(500 mm×100 mm)×2</td>
<td>(100 mm×150 mm)×4</td>
</tr>
<tr>
<td>#1 [g/L]</td>
<td>1.10</td>
<td>1.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#2 [g/L]</td>
<td>0.40</td>
<td>2.90</td>
<td></td>
<td>0.38</td>
</tr>
</tbody>
</table>
4. Conclusion

In this study, the effects of operational conditions on hydrogen production were investigated by using an immobilized bed bioreactor. The characteristics of immobilization were clarified. The followings were obtained.

1. The maximum H\textsubscript{2} production rate was 0.86 L-H\textsubscript{2} h\textsuperscript{-1} L-culture\textsuperscript{-1} under the sugar concentration of 10 g L\textsuperscript{-1} and HRT of about 1.5 h.
2. Specific surface area of supporter for immobilization affected an flocculation performance of bacteria.
3. Saran fiber was an effective supporter for immobilization of strain HN001.

References