Continuous photo-hydrogen production from acetate using *Rhodopseudomonas palustris* WP 3-5

Kuei-Ling Yeh¹, Chun-Yen Chen¹², Yung-Chung Lo¹ and Jo-Shu Chang¹²³

¹ Department of Chemical Engineering, National Cheng Kung University
² Sustainable Environment Research Centre, National Cheng Kung University
³ Microalgae Biotechnology and Bioengineering Laboratory, Center for Biotechnology and Biosciences, National Cheng Kung University, Tainan, Taiwan

Abstract

Phototrophic hydrogen production using components of dark fermentation metabolites (i.e. acetate) is an ideal biohydrogen production route since it enables the highest yield possible (i.e., 12 mol H₂/mol hexose) and could also reduce COD content in the dark fermentation effluent to mitigate the environmental burden. In this work, a purple nonsulfur photosynthetic bacterial isolate *Rhodopseudomonas palustris* WP3-5 was utilized to produce H₂. To assess the performance of the photo-H₂ production system, the continuous photobioreactor was operated at a hydraulic retention time (HRT) of 48 h under different acetate concentrations. The optimal H₂ production efficiency occurred when the acetate concentration was 3000 mg COD/l, leading to an overall H₂ production rate and a H₂ yield of 20.4 ml/h/l and 0.98 mol H₂/mol acetate, respectively. In all tests, the acetate conversion was nearly constant at 87.0±3.1%. Next, the preferable HRT leading to the best H₂ production performance was identified. The results show that at an acetate concentration of 3000 mg COD/L, H₂ production rate increased with a decrease in HRT, giving the best performance at HRT=12 h with an overall H₂ production rate of 42.05 ml/h/l and a H₂ yield of 1.39 mol H₂/mol acetate. Operation under the aforementioned optimal substrate concentration and HRT, the overall H₂ production rate could be enhanced two-fold higher when compared with that obtained from preliminary tests. In addition, the HRT setting was found to play a key role in influencing the performance of continuous H₂ production by *R. palustris* WP3-5. The proposed continuous cultures seem to be a favourable choice of bioreactor strategy, possessing the potential to achieve higher H₂ production efficiency with an excellent stability.

1 Introduction

Hydrogen is a clean energy since combustion of H₂ produces only water without greenhouse gases. Hydrogen can also be directly utilized by hydrogen fuel cell to generate electricity at very high efficiency [1], thereby being considered a promising alternative energy carrier of the future. To this end, developing hydrogen production technology leading to a sufficient and sustainable H₂ supply is highly demanded [2]. Biological H₂ production considered as the most environmentally friendly route of
producing H2 [3], thereby fulfilling the goals of recycling of renewable resources and clean energy production [4]. Phototrophic H2 production has the advantage of high theoretical substrate conversion efficiency and mineralization of organic substrates (e.g., organic acids), thereby being considered a critical step in the integrated fermentative hydrogen production [5-8]. This study was to identify the best carbon substrate (acetate) concentration since the organic loading often plays a critical role in affecting the kinetics of catabolism. Meanwhile, the stability of the continuous H2 production system using different HRT concentration was also assessed to improve the performance of phototrophic H2 production by R. palustris WP3-5.

2 Materials and methods

2.1 Bacterial strain and cultivation medium

The Rhodopseudomonas palustris WP3-5 isolated from a swine wastewater treatment plant located in central Taiwan [9] was used for phototrophic H2 production. The culture medium was using 2000 mg COD/l sodium acetate as a sole carbon substrate. The cells were cultivated at 32°C anaerobically for 48 h under a light intensity of approximately 50 W/m² (illuminated by tungsten filament lamp). The initial pH value of medium prior to incubation was adjusted to 7.0-7.1. Argon gas was used to create an anaerobic condition.

2.2 Fabrication and operation of photobioreactor

The photobioreactor was a 1.0-liter glass-made vessel illuminated with external light sources (100 W tungsten filament lamp). The total light intensity for each illumination system was adjusted to ca. 95 W/m². After the reactor apparatus was sterilized by autoclave, cells of R. palustris WP3-5 were inoculated into the reactor with a 10% inoculum. All the fermentation was operated at pH 7.1 with an agitation rate of 200 rpm. A gas meter (Type TG1; Ritter Inc., Germany) was used to measure the amount of gas products generated and the gas volumes were calibrated to 25 °C and 760 mmHg. Gas samples were taken from sampling port by gas syringe at desired time intervals to measure the gas composition. The liquid sample was also collected from the sealed glass vessel with respect to time to determine cell concentration, pH and residual acetate concentration.

3 Results and discussion

3.1 Effect of sodium acetate concentration on the photo-hydrogen production performance

Literature shows that the sodium acetate concentration is critical factors affecting the fermentation kinetics of biohydrogen production [10]. Therefore, in this study, acetate was used as the sole carbon source for phototrophic H2 production. The effect of acetate concentration on photo-H2 production performance was examined in
continuous cultures containing different initial acetate concentrations (sodium acetate = 1000–4000 mg COD/l). The results are indicated in Table 1. The volumetric H$_2$ production rate and specific H$_2$ production rate appeared to increase with increasing acetate concentration from 1000 to 4000 mg COD/l in all tests. This trend is quite reasonable as higher acetate (carbon substrate) loading usually leads to higher H$_2$ production. The optimal H$_2$ production efficiency occurred when the acetate concentration was 3000 mg COD/l, gave the best overall H$_2$ production rate and H$_2$ yield of 20.4 ml/h/l and 0.98 mol H$_2$/mol acetate, respectively. Meanwhile, the acetate conversion was nearly constant at 87.0±3.1%. Therefore, the acetate concentration of 3000 mg COD/l could give the relatively higher overall H$_2$ production rate and H$_2$ yield than other sodium acetate concentration used.

3.2 Effect of HRT on the photo-hydrogen production performance

To assess the applicability of the phototrophic H$_2$ production system, continuous culture by using different HRT was performed for a prolonged period of time under the optimal conditions in the present work (i.e., sodium acetate concentration = 3000 mg COD/l, light source = TL, light intensity = 95 W/m$^2$). As shown in Table 2, at 48 h HRT, the continuous culture gave a volumetric H$_2$ production rate of 20.4 ml/l/h. When the HRT was decreased to 12 h, the volumetric H$_2$ production rate was rapidly increased from 20.4 ml/l/h to 42.05 ml/l/h. Meanwhile, the cell concentration reached a nearly constant value of 3.11 g/l. The acetate conversion efficiency was obviously decreased with a decrease in HRT. These results clearly indicate that HRT was an important effecting factor for the photo-H$_2$ production of continuous operation by R. palustris WP3-5. This suggests that continuous cultures seem to be a more favorable choice of bioreactor strategy, having the potential to achieve higher H$_2$ production. Meanwhile, the H$_2$ content in biogas was nearly constant at 81.2±2.9% at 12 h HRT, indicating easier separation in downstream processing to obtain purified H$_2$ products for fuel cell applications.

Table 1: Photo-H$_2$ production performance at different sodium acetate concentration (HRT=48 h)

<table>
<thead>
<tr>
<th>CH$_3$COOH (mg COD/L)</th>
<th>H$_2$ yield (mol H$_2$/mol acetate)</th>
<th>Volumetric H$_2$ production rate (mL/h/L)</th>
<th>Specific H$_2$ production rate (mL/h/g cell)</th>
<th>Acetate conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>0.86</td>
<td>6.86</td>
<td>2.65</td>
<td>98</td>
</tr>
<tr>
<td>2000</td>
<td>1.15</td>
<td>17.49</td>
<td>5.18</td>
<td>99</td>
</tr>
<tr>
<td>3000</td>
<td>0.98</td>
<td>20.40</td>
<td>5.46</td>
<td>87</td>
</tr>
<tr>
<td>4000</td>
<td>1.06</td>
<td>22.65</td>
<td>6.51</td>
<td>67</td>
</tr>
</tbody>
</table>
### Table 2: Photo-$H_2$ production performance at different HRT (sodium acetate concentration = 3000 mg COD/L)

<table>
<thead>
<tr>
<th>HRT (h)</th>
<th>$H_2$ yield (mol $H_2$/mol acetate)</th>
<th>Volumetric $H_2$ production rate (mL/h/L)</th>
<th>Specific $H_2$ production rate (mL/h/g cell)</th>
<th>Acetate conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>0.98</td>
<td>20.40</td>
<td>5.46</td>
<td>87</td>
</tr>
<tr>
<td>36</td>
<td>1.46</td>
<td>28.85</td>
<td>7.37</td>
<td>62</td>
</tr>
<tr>
<td>24</td>
<td>1.55</td>
<td>35.33</td>
<td>7.90</td>
<td>48</td>
</tr>
<tr>
<td>12</td>
<td>1.39</td>
<td>42.05*</td>
<td>9.50</td>
<td>31</td>
</tr>
</tbody>
</table>

### Reference