Optimization of volatile fatty acid compositions for hydrogen production by *Rhodopseudomonas capsulata*

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Abstract: The effects of volatile fatty acid (VFA) compositions on the cell growth, H2 production rate and H2 yield have been investigated using mathematical models. The results show that a modified logistic model indicates that the maximum cell growth of 0.03 h\(^{-1}\) was obtained at acetate fraction of 0.571, propionate 0.143 and butyrate 0.286 in the total amount of VFA. The response surface methodology appears to be a useful approach to select appropriate substrate compositions for H\(_2\) production. Under the conditions in this work, the fractions of 0.551–0.566 for acetate, 0.204–0.211 for propionate, and 0.230–0.238 for butyrate were optimal for H\(_2\) production by *Rhodopseudomonas capsulata*.

Keywords: hydrogen; mathematical models; photosynthetic bacteria; response surface methodology (RSM); volatile fatty acids (VFA)

INTRODUCTION

Hydrogen has been considered as a potential substitute for fossil fuel as it is a clean, renewable and efficient fuel.\(^1\) Production of H\(_2\) by thermochemical and radiolytic processes is not cost effective due to their intensive consumption of energy.\(^2\) Biological production of H\(_2\) is attracting extensive attention as an environmentally friendly process for H\(_2\) production, especially if wastewater or other biomass could be used as raw material.\(^3\) Photosynthetic and fermentative H\(_2\) productions are the two main biological routes. The latter process gives a higher rate and does not rely on the availability of light sources. However, its H\(_2\) conversion yield is much lower than that with the photosynthetic process.\(^4\) A more efficient and complete H\(_2\)-producing process has been proposed by combining anaerobic H\(_2\)-formers with photosynthetic bacteria (PSB). In this combined system, organic materials in wastewaters are fermented to H\(_2\), and volatile fatty acids (VFA) in the dark acidogenic reactor; VFA in the effluent from the acidogenic reactor can be further converted to H\(_2\) and CO\(_2\) in the subsequent photosynthetic reactor.\(^5,6\) In such a two-step process, wastewater is treated efficiently, and clean energy, H\(_2\), is generated. Thus, more investigations should be conducted on H\(_2\) production from VFA by PSB to improve their H\(_2\) production rate and yield.

The VFA composition has a significant effect on H\(_2\) production by PSB due to the various VFA being utilized with different efficiencies by PSB.\(^7\) A previous study has shown that *Rhodopseudomonas* sp produced a higher H\(_2\) yield of 0.73 for acetate and a lower one of 0.08 for butyrate.\(^5\) Another study has demonstrated that *Rhodopseudomonas palustris* could utilize 700 mg dm\(^{-3}\) of butyrate to produce more H\(_2\) than the same level of propionate.\(^8\) In acidogenic fermentation, in addition to H\(_2\), the major aqueous metabolites are acetate, propionate and butyrate.\(^5,9\) In order to promote high-rate and stable H\(_2\) production in an acidogenic–photosynthetic system treating wastewaters, manipulating the composition of the effluent from the acidogenic reactor, ie fractions of acetate, propionate and butyrate in the effluent VFA, is essential for enhancing H\(_2\) production by PSB. The desired composition of the acidogenic aqueous products could be achieved by properly controlling operational parameters, such as pH, hydraulic retention time, substrate concentration, temperature, etc.\(^2\)

A conventional ‘change-one-factor-at-a-time’ method has always been used to optimize the biological production of H\(_2\).\(^5,10\) Its disadvantages and that an excess number of experiments is required and the results are unreliable. An alternative and more efficient approach, response surface methodology (RSM), is...
based on statistical methods. The RSM is a useful tool for analyzing biological processes, designing the experiment, building models, evaluating the effects of several factors, and searching for optimum conditions to give desirable responses and reducing the number of experiments.\textsuperscript{11} This method has been used widely in food science and technology, and microbiology, as well as in enzyme applications.\textsuperscript{12} Thus, the RSM can be employed for analyzing the effect of a selected response of independent variables and for modeling of complex systems.

This study was designed to evaluate the H\textsubscript{2} production by \textit{R capsulata} with various ratios of a mixture of acetate, propionate and butyrate as its substrate, and to explore the optimum composition of the substrate for H\textsubscript{2} production. For this purpose, the RSM was applied.

### MATERIAL AND METHODS

#### Strain and pre-cultivation

\textit{R capsulata} provided by Chenxin Microbial Co, Yizheng, China, was used in this experiment. A medium based on the modified aSy medium was used as its growth medium, which included 1 g yeast extract; 1.25 g (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}; 9.8 g sodium succinate in 1 dm\textsuperscript{3} of basal medium. One dm\textsuperscript{3} of the basal medium contained 0.5 g KH\textsubscript{2}PO\textsubscript{4}; 0.6 g K\textsubscript{2}HPO\textsubscript{4}; 0.4 g NaCl; 0.2 g MgSO\textsubscript{4}; 0.05 g CaCl\textsubscript{2}.2H\textsubscript{2}O; 1 mg FeSO\textsubscript{4}.7H\textsubscript{2}O; 0.5 mg (NH\textsubscript{4})\textsubscript{6}Mo\textsubscript{7}O\textsubscript{24}; 0.01 mg CoCl\textsubscript{2}.6H\textsubscript{2}O; 0.1 mg ZnCl\textsubscript{2}; 0.01 mg CuCl\textsubscript{2}; 2 mg H\textsubscript{2}BO\textsubscript{3}; 2 mg EDTA–2Na; 1 mg vitamin B\textsubscript{i}; 15 \mu g biotin.

Glass vials with 300 cm\textsuperscript{3} of working volume were used for the growth of inocula. The gas phase part of the vial was flushed with argon for 10 min to ensure anaerobic conditions. The pre-cultivation was illuminated using tungsten lamps at a light intensity of 500 lux. The initial pH and temperature were kept at 6.8 and 32 ± 1 °C, respectively.

#### Experimental design

The culture medium used for H\textsubscript{2} production was composed of a basal medium plus carbon and nitrogen sources. A VFA mixture of acetate, propionate and butyrate with various ratios was used as a carbon source. The total VFA concentration was maintained at 2.8 g dm\textsuperscript{-3}, which was a similar level to that in the effluent from anaerobic acidogenesis of organic wastes.\textsuperscript{9} The experimental region was delimited by the range values of each component as follows (g g\textsuperscript{-1} mixture): 0.429 ≤ acetate fraction ≤ 0.875; 0 ≤ propionate fraction ≤ 0.429; 0.125 ≤ butyrate fraction ≤ 0.571. The selection of the experimental region was mainly dependent upon the actual compositions from an acidogenic reactor. The simplex-centroid design was applied to this type of model system to determine the effect of the mixture’s components on the defined variables.\textsuperscript{13} The proportions of each component in the mixture are not independent of each other. The compositions fulfill the following equation:

$$\sum X_i = 1$$  \hspace{1cm} (1)

where \(X_i\) values are the fractions of acetate, propionate and butyrate in total VFA.

The experimental compositions of mixtures were calculated by using a simplex-centroid design.\textsuperscript{13} The resulting compositions and experimental region are presented in Table 1 and Fig 1 respectively. The H\textsubscript{2} production rate and H\textsubscript{2} yield were selected as the dependent output variables. After the pre-cultivation was finished, 5% pre-culture was inoculated into the H\textsubscript{2} production medium and the initial cell concentration was adjusted to 0.4 g dm\textsuperscript{-3}. Sodium glutamate (0.5 g dm\textsuperscript{-3}) was used as a nitrogen source. The light intensity was adjusted to 4000 lux. The other conditions were the same as the pre-cultivation conditions. The experiments were conducted until the H\textsubscript{2} production in each vial ceased.

#### Table 1. Influence of the mixture composition on H\textsubscript{2} production rate and H\textsubscript{2} yield of \textit{R capsulata}

<table>
<thead>
<tr>
<th>Run</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
<th>H\textsubscript{2} production rate (cm\textsuperscript{3} dm\textsuperscript{-3} h\textsuperscript{-1})</th>
<th>H\textsubscript{2} yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.875</td>
<td>0.000</td>
<td>0.125</td>
<td>13.0</td>
<td>0.401</td>
</tr>
<tr>
<td>2</td>
<td>0.429</td>
<td>0.000</td>
<td>0.571</td>
<td>14.2</td>
<td>0.421</td>
</tr>
<tr>
<td>3</td>
<td>0.429</td>
<td>0.429</td>
<td>0.142</td>
<td>19.1</td>
<td>0.489</td>
</tr>
<tr>
<td>4</td>
<td>0.643</td>
<td>0.000</td>
<td>0.357</td>
<td>17.0</td>
<td>0.466</td>
</tr>
<tr>
<td>5</td>
<td>0.429</td>
<td>0.214</td>
<td>0.357</td>
<td>18.4</td>
<td>0.500</td>
</tr>
<tr>
<td>6</td>
<td>0.643</td>
<td>0.214</td>
<td>0.143</td>
<td>20.2</td>
<td>0.590</td>
</tr>
<tr>
<td>7</td>
<td>0.571</td>
<td>0.143</td>
<td>0.286</td>
<td>21.5</td>
<td>0.528</td>
</tr>
<tr>
<td>8</td>
<td>0.714</td>
<td>0.071</td>
<td>0.215</td>
<td>18.0</td>
<td>0.516</td>
</tr>
<tr>
<td>9</td>
<td>0.500</td>
<td>0.071</td>
<td>0.429</td>
<td>20.9</td>
<td>0.530</td>
</tr>
<tr>
<td>10</td>
<td>0.500</td>
<td>0.286</td>
<td>0.214</td>
<td>21.2</td>
<td>0.526</td>
</tr>
</tbody>
</table>

\*Expressed in mass fractions.

#### Experimental region

![Figure 1. Experimental region (small triangle area) and substrate compositions of the mixtures containing (g g\textsuperscript{-1} mixture): acetate, propionate and butyrate during H\textsubscript{2} production by \textit{R capsulata}. Lower and upper limits for the mass fraction of each component are shown.](image-url)
Analytical methods
Biogas production was determined with glass syringes following the approach proposed by Owen et al. The percentage of H₂ in the gas was analyzed by using a GC (Model SP-6800A, Lunan Co, Shandong, China) equipped with a thermal conductivity detector and a 2 m stainless column packed with 5Å molecular sieve. The operational temperatures at the injection port, the column oven and detector were 100, 60 and 105°C, respectively. Argon was used as carrier gas at a flow rate of 30 cm³ min⁻¹.

The concentrations of VFA were determined by a second GC (Model 6890NT, Agilent Inc, Palo Alto, CA, USA) equipped with a flame ionization detector and a 30 m × 0.25 mm × 0.25 µm fused-silica capillary column (DB-FFAP). The liquor samples were first centrifuged at 9000 x g for 15 min, and were then acidified by formic acid and filter through a 0.2 µm membrane and finally measured for free acids. Nitrogen was used as carrier gas. The sodium glutamate was estimated in terms of ammonia of glutamate by the phenol hypochlorite method.

RESULTS AND DISCUSSION
Cell growth
A modified logistic model, proposed by Koku et al., was used to describe the cell growth of R capsulata with various levels of acetate, propionate and butyrate as follows:

\[ X = \frac{X_0 \times \exp(k_c \times t)}{1 - (X_0/X_{\text{max}})(1 - \exp(k_c \times t))} \]  

where \( X \) is the cell dry weight concentration (g dm⁻³), \( X_0 \) is initial cell dry weight concentration (g dm⁻³), \( K_c \) is the apparent specific growth (h⁻¹) and \( X_{\text{max}} \) is the maximum cell weight concentration (g dm⁻³).

Figure 2 shows the cell dry weight as a function of incubation time for Run 3. The experimental data fitted well with the modified logistic model. The \( K_c \) and \( X_{\text{max}} \) values of this model for all runs are listed in Table 2. All the correlation coefficients (\( R^2 \)) were greater than 0.945, suggesting the model was able to describe the cell growth well. As shown in Table 2, the maximum cell dry weight concentration reached 2.15 g dm⁻³ in Run 3, at acetate fraction of 0.429, propionate fraction of 0.429 and butyrate fraction of 0.142. On the other hand, the maximum specific growth rate of 0.03 h⁻¹ was achieved in Run 7, corresponding to acetate fraction of 0.571, propionate fraction of 0.143 and butyrate fraction of 0.286.

Consumption of VFA
Figure 3 illustrates the consumption patterns of acetate, propionate and butyrate in all runs. It can be observed that VFA concentration decreased rapidly in the initial 64 h, but later the consumption rate slowed down gradually. At 160 h, the VFA became almost depleted. Figure 4 illustrates the change of cumulative

<table>
<thead>
<tr>
<th>Run</th>
<th>( K_c ) (h⁻¹)</th>
<th>( X_{\text{max}} ) (g dm⁻³)</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0176</td>
<td>1.87</td>
<td>0.979</td>
</tr>
<tr>
<td>2</td>
<td>0.0229</td>
<td>1.95</td>
<td>0.998</td>
</tr>
<tr>
<td>3</td>
<td>0.0223</td>
<td>2.15</td>
<td>0.992</td>
</tr>
<tr>
<td>4</td>
<td>0.0217</td>
<td>1.75</td>
<td>0.978</td>
</tr>
<tr>
<td>5</td>
<td>0.0199</td>
<td>2.00</td>
<td>0.994</td>
</tr>
<tr>
<td>6</td>
<td>0.0193</td>
<td>2.07</td>
<td>0.945</td>
</tr>
<tr>
<td>7</td>
<td>0.0300</td>
<td>1.60</td>
<td>0.975</td>
</tr>
<tr>
<td>8</td>
<td>0.0218</td>
<td>1.80</td>
<td>0.992</td>
</tr>
<tr>
<td>9</td>
<td>0.0193</td>
<td>1.82</td>
<td>0.967</td>
</tr>
<tr>
<td>10</td>
<td>0.0217</td>
<td>1.94</td>
<td>0.992</td>
</tr>
</tbody>
</table>

Figure 2. Cell growth patterns in Run 3.

Figure 3. Degradation patterns of VFA in all runs.
H₂ with time in Run 3. Close examination of Figs 3 and 4 reveals a peculiar feature of the relationship of VFA consumption with H₂ production. After the VFA became completely depleted at 160 h, H₂ production continued for approximately 48 h. This might be associated with the endogenous metabolism of PSB, which was also observed by Koku et al.¹⁶ PSB are capable of accumulating large amounts of reserves such as poly-β-hydroxy butyrate (PHB), which may be degraded and used for H₂ production. Maeda et al.¹⁷ demonstrated that *Rhodovulum sulfidopholum* synthesized PHB at an early stage of the cultivation, and then used PHB as sole substrate to produce H₂. The highest H₂ production rate from PHB reached 33.0 cm³ dm⁻³ h⁻¹.

**H₂ production rate**

Statistical analysis was performed using MATLAB 6.5 (Mathworks Inc, Natick, MA, USA). The following model was applied to calculate the response variables, eg the maximum H₂ production rate:¹²

\[ \eta = \sum_{i=1}^{p} \beta_i X_i + \sum_{i<j}^{p} \beta_{ij} X_i X_j \]  

(3)

For the case of three components (ie acetate, propionate and butyrate), the model can be written for the maximum H₂ production rate (\( R_{H_2} \)) as follows:

\[
R_{H_2} = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3
\]  

(4)

\( X_1, X_2 \) and \( X_3 \) are the respective fractions of acetate, propionate and butyrate in the total VFA. \( \beta_i \)-values are the regression coefficients of the model. In the case of a mixture of three components, the sum of the mass fraction of each component must be equal to 1, ie \( X_1 + X_2 + X_3 = 1 \).

Table 1 shows the compositions of the experimental mixtures and the observed maximum H₂ production rates. \( R_{H_2} \), with a unit of cm³ dm⁻³ h⁻¹, can be estimated from the total volume of gas produced versus time data by simulating with a polynomial curve fitting and differentiation with respect to time. As shown in Table 1, the maximum \( R_{H_2} \) was achieved at the mass fraction of 0.571 (acetate), 0.143 (propionate) and 0.286 (butyrate). \( R_{H_2} \) data were subjected to multiple regression, giving:

\[
R_{H_2} = 5.5689X_1 - 20.584X_2 - 16.754X_3 + 99.162X_1 X_2 + 88.983X_1 X_3 + 65.955X_2 X_3
\]  

(5)

The high value of the correlation coefficient (0.937) indicates a good agreement between the model and experimental data of \( R_{H_2} \) for the range of the defined experimental zone. Since the sum of \( X_1, X_2 \) and \( X_3 \) is equal to 1, the following three equations were derived from eqn (5):

\[
R_{H_2} = -16.75 + 111.30X_1 + 62.12X_2 - 88.98X_1^2 - 65.95X_2^2 - 55.77X_1 X_2
\]  

(6)

\[
R_{H_2} = 5.569 + 73X_2 + 66.66X_3 - 99.16X_2^2 - 88.98X_2^2 - 122.19X_2 X_3
\]  

(7)

\[
R_{H_2} = -20.58 + 125.32X_1 + 69.78X_3 - 99.16X_1^2 - 65.95X_2^2 - 76.13X_1 X_3
\]  

(8)

The response surface model could be used to search for the optimum values of the variables and maximize the response. Once experimental designs were established, the response surface model gave an optimum condition where the maximum response was observed. This technique has been adopted for optimizing substrate composition for mycelial growth of *Pleurotus ostreatus*,¹² determining the optimum operational conditions for biodegradation of hydrocarbons,¹³ analyzing the interactions between medium components and optimal conditions for riboflavin production,¹⁸ etc. According to eqns (6), (7) and (8), the maximum \( R_{H_2} \) of 21.30 cm³ dm⁻³ h⁻¹ was estimated by setting the partial derivatives of these functions at zero with respect to the corresponding variables located at fractions of 0.551 for acetate, 0.211 for propionate, and 0.238 for butyrate. The response surface and contours of \( R_{H_2} \) were plotted using eqns (6), (7) and (8), and are shown in Fig 5.

The response surface of \( R_{H_2} \) (Fig 5) showed a clear peak, indicating that the optimum conditions were inside the triangle of the experimental shaded region (Fig 1). Thus, \( R_{H_2} \) could be maximized by selecting appropriate fractions of different VFA in a mixture. The shapes of the contour plots, circular, elliptical or saddle, indicate whether or not the interactions between the variables are significant. A circular contour plot shows that the interactions between the corresponding variables are negligible, whereas an elliptical or saddle nature indicates that the interactions between the corresponding variables are significant.¹⁹ As shown in Fig 5, a saddle contour...
Two-dimensional contour plots and three-dimensional graphs of the effects of VFA compositions on H₂ production rate. The H₂ yield data were also subjected to multiple regression, giving the following equation with a high correlation coefficient of 0.919:

\[ \text{H}_2 \text{ yield} = 0.2689X_1 - 0.4898X_2 - 0.2238X_3 + 2.1467X_1X_2 + 1.7461X_1X_3 + 1.6771X_2X_3 \]  

(10)

Similarly, in order to effectively evaluate the effect of the VFA fractions of acetate, propionate and butyrate on H₂ yield, three equations derived from eqn (10) are given as:

\[ \text{H}_2 \text{ yield} = -0.2238 + 2.2388X_1 + 1.4111X_2 - 1.7461X_1^2 - 1.6771X_2^2 - 1.2765X_1X_2 \]  

(11)

\[ \text{H}_2 \text{ yield} = -0.4898 + 2.9054X_1 + 1.9431X_3 - 2.0777X_1^2 - 2.1467X_3^2 - 1.6771X_1X_3 \]  

(12)

\[ \text{H}_2 \text{ yield} = 0.2689 + 1.388X_2 + 1.2534X_3 - 2.1467X_2^2 - 1.7461X_3^2 - 2.2167X_2X_3 \]  

(13)

Based on eqns (11), (12) and (13), the optimum condition for the maximum H₂ yield was estimated as: acetate fraction of 0.566, propionate 0.204 and butyrate 0.230. The calculated maximum H₂ yield was 0.555. Two- and three-dimensional response surfaces of H₂ yield are shown in Fig 6(A–C respectively). The response surfaces of H₂ yield also show a clear peak. A saddle contour plot implies that the three components (acetate, propionate and butyrate) and their interaction had a significant effect on H₂ yield. Thus, H₂ yield could be maximized by selecting appropriate fractions of different VFA in a mixture.

**CONCLUSIONS**

The following conclusions can be drawn from this study:

1. A modified logistic model was able to properly describe the cell growth for H₂ production by *R capsulata*. The results show that the maximum cell growth of 0.03 h⁻¹ was obtained in Run 7, corresponding to acetate fraction of 0.571, propionate 0.143 and butyrate 0.286.

2. The RSM was a useful approach to evaluate the relationship between substrate composition and H₂ production. The maximum H₂ production rate was achieved at acetate fraction of 0.551, propionate 0.211, and butyrate 0.238, whereas the highest H₂ yield was obtained at acetate fraction of 0.566, propionate 0.204, and butyrate 0.230.
3. By selecting appropriate fractions of different VFA in mixtures, cell growth, H₂ production rate and H₂ yield all could be maximized. This provides useful information about the desired composition of aqueous products from an acidogenic reactor.

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