Correlation between bio-hydrogen production and polyhydroxybutyrate (PHB) synthesis by \textit{Rhodopseudomonas palustris} WP3-5

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\textbf{A B S T R A C T}

The aim of this study was to determine the competition between H\textsubscript{2} production and polyhydroxybutyrate (PHB) accumulation of \textit{Rhodopseudomonas palustris} WP3-5 when grown on six different substrates. From the results, strain WP3-5 can utilize acetate, propionate, malate, and lactate to produce H\textsubscript{2} but can only synthesize PHB on acetate and propionate. The substrate conversion efficiency (SCE) on acetate and propionate increased significantly after the maximum PHB content was achieved, illustrating a competition for reducing power when PHB synthesis occurred. However, when strain WP3-5 was cultivated at suboptimal pH values on acetate, the synthesized PHB prevented strain WP3-5 from the stress of the inappropriate pH and retained H\textsubscript{2} producing efficiency as at optimal pH value. Consequently, although PHB synthesis does compete with H\textsubscript{2} production in \textit{R. palustris} WP3-5, it is still conducive to H\textsubscript{2} production when strain WP3-5 is in a stressful condition.

\textbf{1. Introduction}

Nowadays, fossil fuel combustion is faced with several obstacles, including exhausted sources, emission of greenhouse gases, and the release of hazardous pollutants into the environment. Hydrogen (H\textsubscript{2}) that is biologically produced by microorganisms has been extensively developed because of its high-energy content (122 kJ/g) and clean product after combustion. Moreover, the biological manufacture of H\textsubscript{2} shows lower cost and fewer pollutants released into the environment when compared with the chemical manufacture. Among the various biological processes to produce H\textsubscript{2}, the photobiological H\textsubscript{2} production via purple non-sulfur bacteria (PNSB) is becoming a promising technology attributed to its high-purity H\textsubscript{2}, lower energy requirement, and flexible applicability (Turner et al., 2008).

Several species of PNSB are capable of producing H\textsubscript{2} photobiologically by utilizing organic acids as the substrate, such as \textit{Rhodobacter sphaeroides}, \textit{Rhodospirillum rubrum}, \textit{Rhodobacter capsulatus}, \textit{Rhodobacter sulfidophilus}, and \textit{Rhodopseudomonas palustris}. These PNSB gain electrons and protons by degrading organic acids and generate ATP from photosystems. The harvest of the electrons, protons, and ATP can further support nitrogenase to achieve nitrogen fixation under nitrogen-limiting conditions. When both ammonia and nitrogen gas are absent, nitrogenase can act as a non-specific enzyme and converts the proton to H\textsubscript{2} with electrons and thus produce H\textsubscript{2}. H\textsubscript{2} production by PNSB is complex and is affected by many factors with many intercellular aspects such as cell growth, energy distribution, light harvesting efficiency, and enzyme activity. For instance, the nitrogenase activity is vulnerable to the oxygen and ammonium content, as oxygen and ammonium can seriously disrupt its capability to produce H\textsubscript{2}. It has been reported that when changing the nitrogen source from glutamate to ammonium chloride, the volume of H\textsubscript{2} produced by \textit{R. palustris} P4 decreased by approximately 50% (Oh et al., 2004). In addition to the influence on the nitrogenase activity, the biochemical properties of the substrate also play an important role in the H\textsubscript{2} producing efficiency. Various types of substrates result in different numbers of intermediate metabolites and varying energy costs due to the use of different metabolic routes, causing a large difference in the H\textsubscript{2}-producing behavior. Moreover, it has been indicated that the oxidation state of the substrate was another significant factor because it is related to the electron availability (Yilmaz et al., 2010). McKinlay and Harwood (2011) noted that a substrate with a negative oxidation state (e.g., butyrate or acetate) showed a higher energy flux in the biomass synthesis and metabolic energy cost than those substrates with positive oxidation states (e.g., fumarate or succinate). Additionally, the H\textsubscript{2} production of PNSB is also affected by several cultivation conditions, including inoculum age, light intensity, pH, temperature, and type of operation (Basak and Das, 2007).

To further develop the photobiological production of H\textsubscript{2} for commercial purposes, a variety of studies to enhance the H\textsubscript{2} production via PNSB have been conducted. The most investigated strategy was optimizing the basic parameters, including the competition between H\textsubscript{2} production and polyhydroxybutyrate (PHB) synthesis by \textit{Rhodopseudomonas palustris} WP3-5.
operating conditions, substrate selection, immobilization of PNSB cells for a higher retention time, and an integrated system by combining different types of H₂-producing microorganisms (Ergolu and Melis, 2011). On the other hand, genetic engineering approaches, such as inactivation of the uptake hydrogenase, exclusion of energy storage compounds, and improvement for ammonia tolerance, were also employed to increase the efficiency of H₂ production. Among these genetic strategies, the elimination of poly-β-hydroxybutyrate (PHB) notably increased the amount of H₂ produced by *R. sphaeroides* KD131 (Kim et al., 2006). PHB is an energy- and carbon-storage compound found in a wide variety of microorganisms when they are faced with a suboptimal environment. It can be mobilized and used for survival when the carbon source becomes a limiting resource. However, PHB synthesis consumes a large number of metabolites and reducing equivalents that are required for the H₂ production in PNSB. Therefore, several studies indicated that PHB accumulation in PNSB should compete with H₂ production or directly from the last liquid culture. After inoculation, the activated temperature was 32°C.

2.1. Microorganism and cell preservation

The purple non-sulfur bacterial species used in this study, *R. palustris* WP3-5, was isolated from an activated sludge of a hoggy wastewater treatment plant. The medium used for the cell preservation was prepared according to previous study (Lee et al., 2002). To preserve the cell in a solid culture, a single colony was streaked onto a fresh plate and incubated in an anaerobic jar for 15 min to maintain an anaerobic environment, and then resealed and re-sparged for further cultivation.

Because of the possible competition between PHB synthesis and H₂ production, the correlation between PHB formation and H₂ production of *R. palustris* WP3-5 was investigated in this study. Due to the effect of the substrate type and oxidation state on H₂ production, acetate, propionate, malate, lactate, glucose, and lactose were used as the substrates to extensively study the H₂ production and PHB formation by *R. palustris* WP3-5. By measuring the cumulative H₂ volume and PHB content, this study tried to evaluate whether a competition in the reducing power distribution between PHB synthesis and H₂ production is present.

2. Methods

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2.2. Batch experiments for substrates utilization and pH value influence

To prepare sufficient biomass for the batch experiments, *R. palustris* WP3-5 was precultured in a large volume for 3 days under the same cultivation conditions as mentioned earlier. After the preculture, cells were harvested by centrifugation at 4°C and 6000 rpm and washed twice by a sterile buffer solution (0.5 g/L KH₂PO₄, 0.5 g/L KH₂PO₄, 0.4 g/L NaCl, and 1 g/L CaCl₂·2H₂O at pH 6.8) to eliminate the spent medium. Substrates including acetate, propionate, malate, lactate, glucose, and lactose were individually added to a cell density of optical density (O.D.) 0.3 at 660 nm. The mixture was then divided into serum bottles, sealed with a rubber seal, sparged with argon gas for 5 min to maintain an anaerobic environment, and cultivated as above. The procedures for the pH value batch experiments were identical to those of the substrate utilization experiments. However, only the acetate and malate substrates were investigated for the pH value experiments. The pH value was controlled at 6.0, 6.8, and 8.0 for both substrates. The buffer system used for the pH 6.8 experiment was a phosphate buffer in the same concentration as the medium, whereas a 10 mM tris buffer was used for the pH 8.0 experiment and a 10 mM bis–tris buffer was used for pH 6.0 experiment. To maintain the pH value, 1 N NaOH or HCl was used to adjust the pH value after every sampling. The serum bottle for the next sampling was sterilely opened for the pH adjustment, then resealed and re-sparged for further cultivation.

2.3. Analytical methods

After measuring the gas volume in the serum bottle by the water replacement method, the gas composition can be correctly sampled due to the atmospheric pressure equilibration. The gas composition was analyzed by GC/TCD. The column was a Supelco 60/90 Carboxen-1000 stainless steel column and the carrier gas was argon, which operated at a flow rate of 19.2 mL/min. The temperature of the inlet and the detector were 100 and 190°C, respectively. The temperature program of the oven was held at 65°C for 2 min and increased at 20°C per min to 180°C.

The cell dried weight was determined based on a correlation equation between the cell density and dried weight. The cell density was measured by a UV/VIS spectrophotometer (HITACHI, U-2800) at the wavelength of 660 nm and the cell dried weight was analyzed according to Standard Methods (APHA, 1996). The pH was measured by a pH meter (WTW inoLab pH/ION LEVEL2) and was judged by pH indicator strips (Neutralit®, Merck, Germany) when adjusting the pH value during batch experiments. To determine the substrate concentration, acetate and propionate were analyzed by GC equipped with a flame ionization detector (FID). Then, 20 μL of 1 N H₂SO₄ was added to the liquid sample for acidification. The column used for GC/FID was a DB-WAXetr column (30 m × 0.35 mm with 0.1 μm film thickness) and the carrier gas was N₂, which operated at 3.0 mL/min. The sample volume for the injection was 1 μL. The temperature of the injector and the detector was controlled at 250°C, and the temperature program of the oven was held at 60°C for 5 min and rose by 15°C per min to 180°C. For malate and lactate analysis, the sample was treated according to the study of Wasselmaier et al. (2000) before being injecting into the high performance liquid chromatography (L7100, Hitachi, Tokyo, Japan). The column used to separate malate and lactate was the Mightysil RP-18 column and the mobile phase was phosphoric acid, which operated at 0.8 mL/min. The elution was monitored at 220 nm by a UV detector (L7400, Hitachi, Tokyo, Japan). Additionally, the glucose and lactose concentration was determined by the phenol–sulfur method (Dubois et al., 1956). The calculation of the substrate conversion efficiency (SCE) for all batch experiments was according to the study from Vincenzini et al. (1982).

The PHB content was analyzed according to the study by Satoh et al. (1996) but was modified as described below. To extract the intercellular PHB, the cell pellet was freeze-dried and then mixed with 2 mL of an acidic methanol solution (20% H₂SO₄) and 2 mL chloroform and was then heated to 100°C for 3.5 h. After the...
extract cooled down to room temperature, it was mixed with 1 mL aqueous ammonia solution (28%) and shaken vigorously for 1 min. After centrifugation at 3000 rpm for 10 min, the extracted PHB was harvested from the lower phase of chloroform solution and analyzed by GC/FID. The operating condition of the GC for the PHB analysis was identical to that for the acetate and propionate analysis, except for the temperature program of the oven. The oven was held at 80 °C for 4 min and increased at 8 °C per min to a final temperature at 160 °C. Methyl (R)-(-)-3-hydroxybutyrate (Aldrich–Sigma) was used as a standard for the PHB analysis.

3. Results and discussion

3.1. H₂ production on various substrates

To investigate the substrate utilization, *R. palustris* WP3-5 was cultivated with acetate, propionate, malate, lactate, glucose, and lactose. Fig. 1 shows the cell growth, H₂ production, and substrate utilization. In general, PNSB can decompose a variety of substrates as the electron donor and carbon source, depending on the specific species. From the results in Fig. 1, strain WP3-5 grew well on each organic acid, indicating that it can assimilate various organic acids for cell division (Fig. 1a). During the exponential phase, strain WP3-5 showed the highest growth rate on acetate. The cell density during the stationary phase was slightly lower when strain WP3-5 was cultivated with propionate and malate. Moreover, strain WP3-5 produced the highest volume of H₂ (131.3 mL) on lactate for 114 h, followed by acetate (105.5 mL), malate (104.3 mL), and propionate (79.2 mL), thus showing a wide capability of H₂ production on several organic acids (Fig. 1b). It was elucidated that PNSB, including *R. sphaeroides* and *R. palustris*, can produce H₂ from fermented waste products, which contain several types of organic acids (Keskin et al., 2011). Although strain WP3-5 can utilize these organic acids to produce H₂, propionate resulted in the lowest volume of H₂ produced in our study. The production of bio-hydrogen by PNSB has been well studied using acetate and butyrate as the carbon sources. In general, the H₂ production with propionate is lower than that with acetate or butyrate (Shi and Yu, 2004; Suwansaard et al., 2010).

In addition to the four organic acids, strain WP3-5 cannot utilize glucose and lactose as the substrate for cell growth, H₂ production, and PHB accumulation (Fig. S1 in Supplementary data). Although the cell density on glucose and lactose increased to 0.29 and 0.27 mg/mL, respectively (Fig. S1a in Supplementary data), these slight increases might be attributed to the energy storage substances produced during the cell preculture. Strain WP3-5 was cultivated under acetate during the cell preculture, and thus there was a residual PHB content at 2–4% of the cell dried weight at the beginning of the batch experiments (Fig. S1d in Supplementary data). The rapid degradation of the intrinsic PHB illustrated that strain WP3-5 utilized the intracellular PHB as the carbon and energy source to survive on glucose and lactose. The limited capability to grow on glucose and lactose might be caused by the absence of sugar-specific transporters or hexokinase (Larimer et al., 2004). A deficiency in transporters or hexokinase disrupts the conversion of glucose to glucose-6-phosphate, thus discontinuing glycolysis and the citric acid (TCA) cycle. Nevertheless, some *R. palustris* strains are still capable of assimilating carbohydrates. *R. palustris* strain CQK 01 showed the highest H₂ production rate (2.61 mmol/L/h) when cultivated with 4 g/L glucose in immobilized cells (Wang et al., 2010). Oh et al. (2002) isolated a new chemoheterotrophic *R. palustris* strain P4.
that can utilize glucose and lactose as the carbon source for cell growth and H₂ production. Therefore, the utilization of carbohydrates by *Rhodopseudomonas palustris* was a strain-dependent capability.

Strain WP3-5 showed another notable characteristic as it produced the highest volume of H₂ on lactate. Although it is consistent with the characteristic of a newly isolated *Rhodopseudomonas palustris* strain AV33, the phenotype of strain WP3-5 was obviously different from strain AV33, which produces only a few milliliters of H₂ in the presence of acetate (Bianchi et al., 2010). Therefore, the high production of H₂ on lactate by strain WP3-5 might be contributed to by a higher substrate concentration (Fig. 1c). Strain WP3-5 showed the highest substrate conversion efficiency (SCE) of 14.2% on acetate (Table 1), revealing that it could convert acetate to H₂ more efficiently than on lactate (SCE of 9.3%). Barbosa et al. (2001) observed that the SCE of acetate was higher than that of lactate in both *Rhodopseudomonas* sp. and *Rhodopseudomonas palustris*. From other studies, acetate is considered to be a preferable substrate in the photofermentative H₂ production. A newly isolated *Rhodopseudomonas* strain TN1 showed the highest SCE of 62% on 20 mM acetate, compared with propionate and butyrate (Suwansaard et al., 2010). Therefore, acetate should be the optimal organic acid for strain WP3-5 to produce H₂ among these organic acids.

**3.2. PHB formation and competition with H₂ production on various substrates**

The formation of PHB and the competition phenomenon was only observed when strain WP3-5 was cultivated with organic acids because PHB was not synthesized on carbohydrate substrates. Fig. 1d illustrates the intracellular PHB formation on organic acids. The PHB accumulation was ambiguous when strain WP3-5 was incubated with malate and was small on lactate (1.4% increases). In contrast, WP3-5 was obviously synthesized on acetate and propionate, and the maximum PHB content that could be achieved was 10.2% and 4.2% of cell dried weight on acetate and propionate, respectively. The difference in the PHB formation by strain WP3-5 for the four organic acid substrates may be caused by the different metabolic routes of their assimilation and the precursor contents for PHB synthesis. Acetyl-coenzyme A (acetyl-CoA) is the main precursor for PHB synthesis in most PNSB (Kessler and Witholt, 2001). However, malate taken by *Rhodopseudomonas palustris* is generally converted to oxaloacetate by malate dehydrogenase or to pyruvate by the NADP-dependent malic enzyme through the TCA cycle (Sato et al., 2010). In the case of lactate, Horikiri et al. (2004) indicated that lactate dehydrogenase can convert lactate to pyruvate in *Rhodopseudomonas palustris* no. 7. Oxaloacetate and pyruvate, the main metabolite of malate and lactate, re-distributes only a small portion to synthesize acetyl-CoA during the H₂ production (McKinlay and Harwood, 2011). Therefore, strain WP3-5 cannot successfully synthesize PHB on malate and lactate due to the lack of sufficient acetyl-CoA as a precursor. In contrast, the metabolic routes of acetate and propionate provide accessible ways to generate acetyl-coA. *Rhodopseudomonas palustris* assimilates acetate through the glyoxylate cycle, where acetate is completely converted to acetyl-coA and then to malate (Laguna et al., 2011). For the assimilation of propionate, the methylmalonyl-CoA pathway is the most common metabolic route in microorganisms (Gottschalk, 1986). However, Choorit et al. (2011) observed that *Rhodopseudomonas* KG31 hydrolyzed propionate to acetate with an extended lag phase, speculating that propionate should be hydrolyzed by an extracellular enzyme through following equation:

\[
\text{CH}_3\text{CH}_2\text{COOH} + 3\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + \text{HCO}_3^- + \text{H}^+ + 2\text{H}_2
\]

Therefore, propionate can also be assimilated as acetate and provide sufficient acetyl-CoA to synthesize PHB.

In addition to the effect of the metabolic route, the oxidation state of the substrates is another factor that greatly affects the anabolism of PNSB (Yilmaz et al., 2010). The oxidation states of malate, acetate, lactate, and propionate are +1, 0, 0, and ~0.75, respectively. Although propionate has the most abundant electrons among these organic acids, strain WP3-5 requires HCO₃⁻ for cell growth through the Calvin cycle because the oxidation state of propionate is more reduced than the PNSB biomass (CH₁.₈N₀.₁₈O₀.₃₈). For the assimilation of propionate, the methylmalonyl-CoA pathway is the most common metabolic route in microorganisms (Gottschalk, 1986). In the case of lactate, Horikiri et al. (2004) indicated that lactate dehydrogenase can convert lactate to pyruvate in *Rhodopseudomonas palustris* no. 7. Oxaloacetate and pyruvate, the main metabolite of malate and lactate, re-distributes only a small portion to synthesize acetyl-CoA during the H₂ production (McKinlay and Harwood, 2011). Therefore, the propionate hydrolysis and the Calvin cycle performance should consume a portion of the electrons that were originally contributed to PHB synthesis, resulting in a lower PHB content than that on acetate. With the highest oxidation value, malate had the fewest electrons to allocate toward anabolism. Moreover, the oxidation state of the PHB monomer (C₄H₆O₂) is identical to the PNSB biomass, revealing that the unobvious PHB formation on malate might be caused not only by the metabolic route of malate but also by its oxidation state.

Because the H₂ production and PHB formation both require reducing power, the competition for reducing power between the H₂ production and PHB formation in PNSB has been widely studied (Hustede et al., 1993; Vincenzini et al., 1997). In this study, the competition for reducing power was also observed in *Rhodopseudomonas palustris* WP3-5. PHB synthesis on acetate and propionate was exuberant at the prime period of cultivation. When the cells were growing and accompanied by PHB synthesis during the exponential phase, H₂ was produced simultaneously. However, before the maximum PHB accumulation was achieved, the SCE of acetate and propionate for H₂ production was only 11.6% and 2.5%, respectively (Table 1). After the maximum content of PHB was achieved, the SCE of acetate and propionate increased to 17.1% and 11.8%, respectively. These results showed that the PHB synthesis in strain WP3-5 may have priority over H₂ production in receiving the harvested reducing power. In contrast, although PHB was also slightly accumulated on lactate during the exponential phase, the SCE of lactate remained almost the same regardless of whether the maximum PHB content was reached (Table 1). This result confirms that lactate might not be quite accessible for PHB synthesis.

### Table 1

The SCE of the organic acid substrates and incubated with acetate substrate at various pH by *Rhodopseudomonas palustris* WP3-5.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>pH 6.9–7.4</th>
<th>pH 6.9–6.9</th>
<th>pH 6.9–7.2</th>
<th>pH 6.8</th>
<th>pH 6.0</th>
<th>pH 8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average SCE (%)</td>
<td>14.2</td>
<td>6.4</td>
<td>9.3</td>
<td>13.8</td>
<td>24.9</td>
<td>17.8</td>
</tr>
<tr>
<td>SCE before maximum PHB content (%)</td>
<td>11.6</td>
<td>2.5</td>
<td>–</td>
<td>8.3</td>
<td>19.7</td>
<td>9.4</td>
</tr>
<tr>
<td>SCE after maximum PHB content (%)</td>
<td>17.1</td>
<td>11.8</td>
<td>–</td>
<td>24.4</td>
<td>31.7</td>
<td>25.8</td>
</tr>
</tbody>
</table>

* No PHB accumulation (the detection limit of the PHB content ranges from 0.17% to 0.5% of the cell dried weight).
* The SCE means substrate conversion efficiency.

### 3.3. Effect of pH value on competition between H₂ and PHB

The external pH around the cell is an important growth factor for microorganisms, especially when the pH is outside of the
optimal range for cell growth. The optimal pH value for H₂ production of strain WP3-5 was at pH 6.8, indicating that it is a neutralophile (data not shown). Therefore, strain WP3-5 was also cultivated at pH 6.0 and 8.0 to observe the competition between H₂ production and PHB formation. Fig. 2 shows the cell growth, H₂ production, and PHB content when strain WP3-5 was incubated with acetate at various pH values. For cell growth, although the initial acetate concentration was not fixed, the maximum cell density in the stationary phase was disproportionate to the initial acetate concentration. The lowest cell density (0.44 mg/mL) was observed at pH 8.0, revealing that external pH significantly affects the cell growth of strain WP3-5. Moreover, inappropriate pH values also greatly affected the PHB content in the cell, resulting in a lower PHB content (maximum 5–7%) than that at the optimal pH 6.8 (maximum 9.7%). Indeed, the distinction between the external and internal pH of the cell is a key component associated with the proton motive force (PMF). The PMF, consisting of the transmembrane pH gradient and electrical potential, is an alternative energy source to produce adenosine triphosphate (ATP) or drive flagella. In general, the cell tends to keep its cytoplasmic pH value slightly higher than the outside pH to generate a negative PMF toward the cell. When the external pH becomes lower than the cytoplasmic pH, the PMF would be increased depending on differential level, but not vice versa (Krulwich et al., 2011). Consequently, the cell growth of strain WP3-5 showed the highest cell density at pH 6.0 but the lowest at pH 8.0, which might be caused by a difference in the intrinsic PMF rather than the initial acetate content. However, inappropriate pH can switch on a pH homeostasis via primary proton pumps or secondary active transporters to balance the transmembrane proton gradient in order to mitigate an unfavorable PMF (Slonczewski et al., 2009). Certain strategies for pH homeostasis are energy-consuming, such as F₁F₀-ATP synthase, and thus might result in a low PHB accumulation in strain WP3-5 during pH homeostasis. Furthermore, neutralophiles tend to pump protons out of the cell to maintain a constant level of the PMF when facing an acid environment. This mechanism reduced the intracellular proton content and resulted in a low H₂ production at pH 6.0 (Fig. 2b).

Regarding the competition between H₂ and PHB, before reaching the maximum PHB content, the SCE was still obviously lower irrespective of the cultivated pH (Table 1). The SCE then increased from 8.3% and 9.4% to 24.4% and 25.8% at pH 6.0 and 8.0, respectively. When combining the results of the H₂ production and PHB accumulation on acetate and propionate, the SCE consistently increased for both substrates after the PHB content reached the maximum values, indicating that *R. palustris* WP3-5 preferred to provide reducing power toward PHB synthesis rather than H₂ production when the supplied substrate was suitable for PHB synthesis. However, when the substrate was changed from acetate to malate which is unable to synthesize PHB by strain WP3-5, the cell growth and H₂ production were both decreased at an inappropriate pH (Fig. 3). Slow increases of the cell density demonstrated that strain WP3-5 was under environmental stress when cultivated at pH 6.0 or 8.0. The pH effect on strain WP3-5 was also significant in H₂ production and even in malate utilization. Under pH 6.0 or 8.0, the cumulative H₂ volume was only half of that at pH 6.8 (111.0 mL), and malate was still present at concentrations above 2.1 mM at the end of the cultivations. Welander et al. (2009) demonstrated that a pH-homeostasis-deficient mutant of *R. palustris* TIE-1 grew much slower than the wild type at pH 6.0 or 8.0, indicating that the cytoplasmic pH value of *R. palustris* ranged between pH 6.0 and 8.0. Consequently, when the pH was maintained at

![Fig. 2](image_url)
inappropriate values, it became another environmental stress to strain WP3-5 when it was cultivated on the malate substrate.

From the results of cultivation on malate and acetate, a major difference in the response of strain WP3-5 was the availability of the substrate for PHB synthesis. In general, PHB can provide a variety of functions for microorganisms, and the majority of functions can assist microorganisms in overcoming environmental stresses, such as limited nutrient conditions, temperature fluctuation, UV irradiation, and osmotic shock (Zhao et al., 2007). Indeed, during H2 production, R. palustris WP3-5 was already under environmental stress due to a deficient nitrogen source. Therefore, in the use of reducing power, PHB synthesis may tend to predominate against H2 production under this nitrogen-limited stress, resulting in the observed competition between PHB and H2. However, when an inappropriate pH occurs as an extra environmental stress, the intracellular PHB can protect strain WP3-5 from the influence of pH stress and retain utilization of the substrate and production of H2. Furthermore, although the correlation between PHB synthesis and nitrogen fixation was less discussed in PNSB species, related issues have been widely studied in the symbiosis of rhizobia with legumes (Trainer and Charles, 2006). In rhizobia, the effect of the PHB synthesis on the nitrogen fixation efficiency differs significantly from one species to another, depending on the types of forming nodules and the environmental conditions. However, the nitrogenase activity was reduced by deleting the PHB synthesis gene (phbC) in the symbiosis of Sinorhizobium meliloti with two Medicago sp. (Wang et al., 2007). This reduction of the nitrogenase activity was caused by repression of the nifA gene in response to the nitrogenase expression as a transcriptional activator (Mandon et al., 1998). Moreover, Franchi et al. (2004) illustrated that the H2 production of R. sphaeroides RV was decreased when the PHB synthesis gene was knocked out. Nitrogenase is an essential component for H2 production by R. palustris WP3-5; thus, it can be postulated that the presence of the intracellular PHB of strain WP3-5 was still beneficial to its H2 production despite an obvious competition for reducing power. Consequently, because of the interference during H2 production, strain WP3-5 may have a tendency to synthesize PHB preferentially for extended survival. Even though the synthesis of PHB would divide a part of the reducing power, the natural function of PHB for cell protection should be valuable to the H2 production in PNSB.

4. Conclusions

The H2 production and PHB formation of R. palustris WP3-5 were discussed in this study. Strain WP3-5 can utilize acetate, propionate, lactate, and malate to produce H2 and exhibits the highest SCE on acetate (14.2%). Moreover, carbohydrates were improper substrates for strain WP3-5. Although the PHB synthesis reduced the SCE of the H2 production while synthesizing PHB (17.1–11.6% for acetate; 11.8–2.5% for propionate), the intracellular PHB was beneficial to the H2 production in strain WP3-5 in a pH-stress environment, indicating that PHB likely functions intricately rather than only competing with the H2 production for reducing equivalents.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2012.01.090.

References


