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Hydrogen Production From Propionate by *Rhodopseudomonas capsulata*

XIAN-YANG SHI AND HAN-QING YU*

Laboratory of Environmental Biotechnology, Department of Chemistry, University of Science & Technology of China, Hefei, Anhui, 230026, China, E-mail: hqyu@ustc.edu.cn

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Abstract

Hydrogen production from propionate at various concentrations by *Rhodopseudomonas capsulata*, a purple nonsulfur bacterium, was studied at a temperature of 31°C, a pH of 7.0, and an illumination intensity of 3000 Lux. Among the six levels of propionate, 3.84 g/L was found to be the optimum propionate concentration for H_2 production in terms of substrate utilization efficiency, H_2 percentage, cumulative H_2 production, and H_2 yield. A modified Gompertz equation was able to describe properly the production of H_2 from propionate. A comparative study of H_2 production with acetate, propionate, and butyrate at 40 m*M* showed that, as a substrate for H_2 production by *R. capsulata*, propionate was better than butyrate, but less favorable than acetate.

Index Entries: Hydrogen; photosynthetic bacteria; propionate; *Rhodopseudomonas capsulata*; acetate; butyrate.

Introduction

Despite the green nature of H_2 as a fuel (no CO_2 is produced and H_2 is used as a fuel), it is still primarily produced from nonrenewable sources such as natural gas or petroleum hydrocarbons through the reforming of steam. For H_2 to become a more sustainable source of energy, it should be produced through biologic routes using cheaper resources *(1)*. Photosynthetic bacteria are able to utilize volatile fatty acids (VFA) to produce H_2 at the expense of light *(2)*. The combination of photosynthetic bacteria with anaerobic acidogenic bacteria can form a two-step biosystem for H_2 production from organic wastes. In such a system, organic materials in wastewaters are fermented to H_2 and VFA in the dark acidogenic reactor; VFA in the

*Author to whom all correspondence and reprint requests should be addressed.

effluent from the acidogenic reactor can be further converted into H_2 and CO_2 in the subsequent photosynthetic reactor. Thus, in such a two-step process, wastewater is treated efficiently, and clean energy, H_2, is generated.

The main aqueous products from dark acidogenesis are acetate, propionate, and butyrate, and formate, lactate, valerate, and caproate are also produced as minor acidogenic products *(3,4)*. Photosynthetic bacteria are therefore expected to convert these organic acids into H_2 effectively. Conversion of lactate, acetate, and butyrate into H_2 by photosynthetic bacteria has been well documented *(5–7)*. In an investigation into H_2 evolution from lactate by *Rhodopseudomonas capsulata* B10, a maximal H_2 evolution rate of 120 mL of H_2/(L·h) was obtained at a dilution rate of 0.03 L/h *(8)*. A recent study showed that four short-chain acids—lactate, malate, acetate, and butyrate—were all readily utilized to generate H_2 by *Rhodopseudomonas* sp., *Rhodopseudomonas palustris*, and a nonidentified strain *(5)*. *Rhodopseudomonas* sp. produced the highest volume of H_2 at a rate of 25 mL of H_2/(L·h) when acetate was used as the substrate.

Among the organic acids produced from acidogenesis of wastewaters, propionate should be paid special attention. When methanogens are inhibited and H_2 is produced in an acidogenic reactor, propionate is often found to be present at a high level in the effluent *(1,8–10)*. For instance, during the acidogenesis of lactose-rich wastewater, propionate was the predominate fatty acid in the effluent *(8)*. In an acidogenic reactor for starch conversion at pH 5.0, the concentrations of acetate, propionate, and butyrate in the effluent were 1.008, 0.8446, and 0.4950 g/L, respectively *(10)*. Since acetate and butyrate could be readily utilized for H_2 production by photosynthetic bacteria, in order to promote the high rate and stable H_2 production in an acidogenic-photosynthetic system treating wastewaters, efficient utilization of propionate for H_2 generation by photosynthetic bacteria becomes essential. However, little is known about the conversion of propionate into H_2 by photosynthetic bacteria. Therefore, the present study was conducted to investigate H_2 production from propionate at various concentrations by an identified photosynthetic bacterial strain, and to compare the H_2 production potential of propionate with those of acetate and butyrate by using the same photosynthetic strain.

Materials and Methods

Strain and Culture Conditions

R. capsulata, obtained from Chenxin Microbial (Xizheng, China) was grown in a modified aSy medium, which was composed of a basal medium (inorganic salts), 0.1% yeast extract, 1.25 g/L of $(NH_4)_2SO_4$, and 9.8 g/L of sodium succinate, and a vitamin solution, as described by Miyake et al. *(11)*. One liter of the basal medium for this experiment contained 0.5 g of KH_2PO_4, 0.6 g of K_2HPO_4, 0.4 g of NaCl, 0.2 g of $MgSO_4$, 0.05 g of $CaCl_2{\cdot}2H_2O$, 1 mg of $FeSO_4{\cdot}7H_2O$, 0.5 mg of $(NH_4)_6Mo_7O_{24}$, 0.01 mg of $CoCl_2{\cdot}6H_2O$, 0.1 mg of $ZnCl_2$, 0.01 mg of $CuCl_2$, 2 mg of H_3BO_3, 2 mg of EDTA-2Na, 1 mg

of vitamin B_1, and 15 µg of biotin. The pH of the growth medium was adjusted to 7.4 using 1 *M* NaOH solution prior to autoclaving, and all media used were sterilized at 121°C for 15 min. The growth medium was illuminated using a tungsten lamp at a light intensity of 500 Lux. The culture was grown anaerobically in 300-mL reactors with a rubber stopper at 31°C.

H_2 Production Medium

After 72 h of growth, *R. capsulata* was harvested in the exponential phase and transferred into the H_2 production medium, which was composed of a basal medium plus carbon and nitrogen sources. Propionate at concentrations at 1.92, 2.88, 3.84, 4.80, 5.76, and 6.72 g/L was respectively used as the carbon source. In addition to propionate, acetate and butyrate at 40 m*M* were used as the carbon source, respectively, in this study for comparison. Sodium glutamate at 0.5 g/L was used as the nitrogen source. Temperature, pH, and illumination intensity for H_2 production were 31°C, 7.0, and 3000 Lux, respectively. The experiments were carried out in the same reactors used for growth with a volume of 150 mL. All the reactors were purged with argon for 10 min to ensure anaerobic conditions. The experiments were conducted until H_2 production in each reactor ceased.

Analytical Methods

Biogas production was determined using glass syringes following the approach proposed by Owen et al. *(12)*. The percentage of H_2 in the gas was analyzed by a gas chromatograph (Model SP-6800A; Lunan) equipped with a thermal conductivity detector and a 2-m stainless column packed with a 5-Å molecular sieve. The operational temperatures at the injection port, column oven, and detector were 100, 60, and 105°C, respectively. Argon was used as the carrier gas at a flow rate of 30 mL/min. The concentrations of acetate, propionate, and butyrate were determined by a second gas chromatograph (Model 6890NT; Angilent) equipped with a flame ionization detector and a 30 m × 0.25 mm × 0.25 µm fused-silica capillary column (DB-FFAP). Nitrogen was used as the carrier gas. Light intensity was measured using a digital luxmeter (ZDS-10F-2D; Jiading Xuelian, Shanghai, China).

Results

Role of Sodium Glutamate in H_2 Production

Figure 1 shows the H_2 production profiles of the two reactors, the control reactor with 0.5 g/L of glutamate as the sole carbon and nitrogen source, and the reactor with 3.84 g/L of propionate and 0.5 g/L of glutamate as the carbon and nitrogen source. No H_2 production was observed from the control reactor, whereas 139 mL of H_2 was produced from the reactor with 3.84 g/L of propionate and 0.5 g/L of glutamate. This result demonstrates that H_2 was produced only from propionate, and that sodium glutamate was not used as a carbon source for H_2 production by *R. capsulata*.

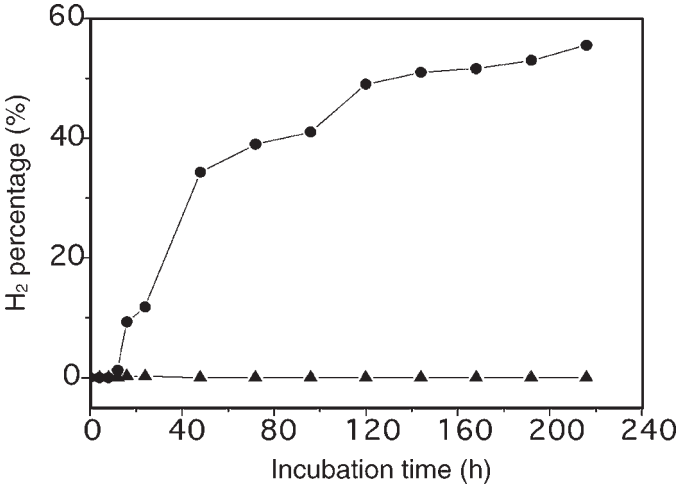

Fig. 1. H_2 percentage profiles in two reactors: (▲), 0.5 g/L of glutamate; (●), 0.5 g/L of glutamate + 3.84 g/L of propionate.

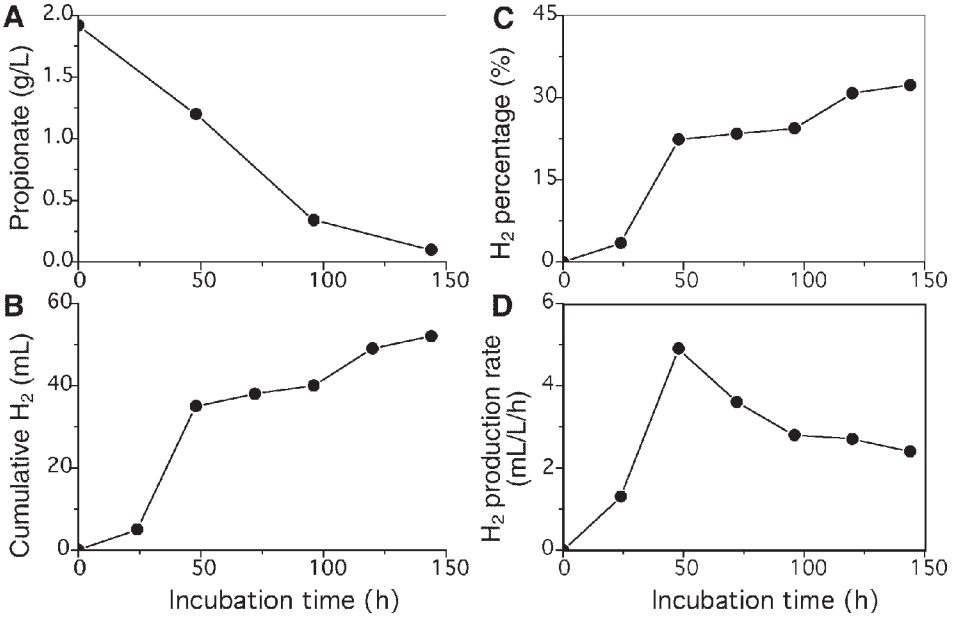

Fig. 2. H_2 production at 1.92 g/L of propionate: **(A)** residual propionate concentration; **(B)** cumulative H_2 production; **(C)** H_2 percentage; **(D)** H_2 production rate.

H_2 Production From Propionate at a Low Concentration

Figure 2 illustrates the changes in residual propionate concentration, cumulative H_2 production, H_2 percentage, and H_2 production rate at lower propionate levels, using 1.92 g/L as an example. Figure 3 illustrates the corresponding results at higher propionate levels, using 6.72 g/L as an example, for comparison.

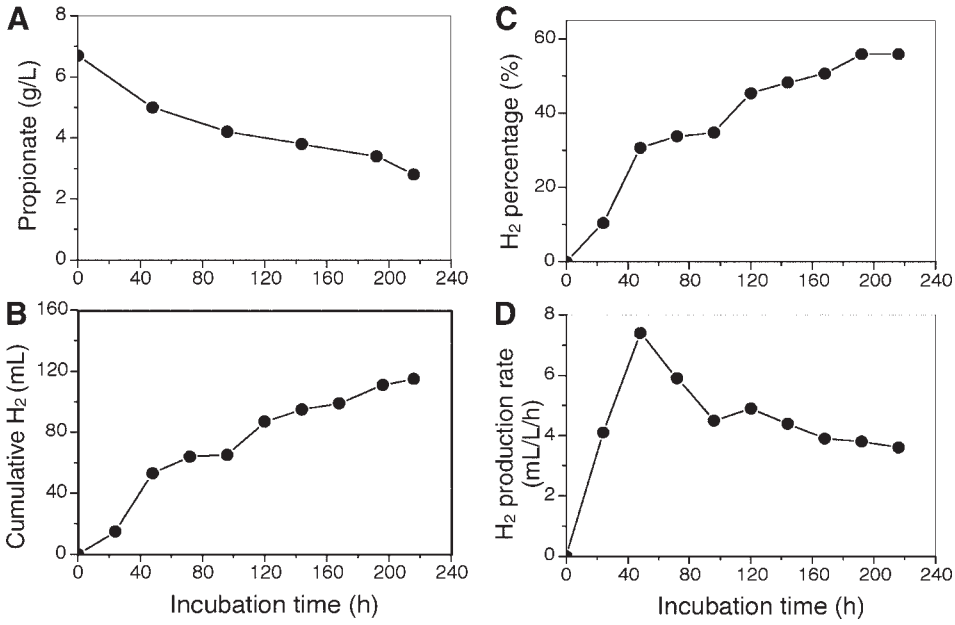

Fig. 3. H_2 production at 6.72 g/L of propionate: **(A)** residual propionate concentration; **(B)** cumulative H_2 production; **(C)** H_2 percentage; **(D)** H_2 production rate.

As shown in Fig. 2A, after incubation started, propionate concentration was reduced almost linearly with time. About 82% of propionate was utilized within 96 h. Thereafter, the utilization of propionate slowed down in the following 48 h. These results demonstrate that propionate was readily utilized at a low concentration by *R. capsulata*.

Figure 2B illustrates that little H_2 was produced in the initial 24 h. However, in the subsequent 24-h incubation, H_2 was produced sharply. From h 48 to h 96, no H_2 production was observed, although propionate concentration decreased substantially during this period. After h 96, there was a significant production of H_2. This might be attributed to the adaptation of the photosynthetic bacterium to the increase in pH in the reactor. Comparison of Fig. 2C and Fig. 2B shows that the H_2 percentage had a similar changing pattern to that of cumulative H_2 production. On the other hand, as illustrated in Fig. 2D, the H_2 production rate increased quickly 24 h after initiation of the experiment, reaching a peak of 4.9 mL/(L·h) at h 48; thereafter, the rate decreased considerably.

H_2 Production From Propionate at a High Concentration

H_2 production from propionate at high levels was also studied. As illustrated in Fig. 3A, at a concentration of 6.72 g/L, propionate was utilized quickly in the initial 96 h; after that, the propionate consumption became slow; and at the end of 216 h of incubation, 2.80 g/L of propionate was left unutilized. The low consumption efficiency of propionate by

R. capsulata might be associated with the inhibition caused by the high concentration of propionate.

Figure 3B,C shows that, at 6.72 g/L of propionate, the H_2 percentage had a similar changing pattern to that of cumulative H_2 production. However, these profiles were different from those at a lower propionate level, as shown in Fig. 2B,C. Production of H_2 at 6.72 g/L progressed more smoothly and had a shorter stationary phase. On the other hand, H_2 production at 6.72 g/L was slower, and H_2 production was completed after 216 h.

At 6.72 g/L of propionate, the H_2 production rate increased sharply during the period from h 24 to h 48. In the subsequent 48 h, the production rate decreased quickly but reached another peak at h 122 and thereafter the H_2 production rate decreased continuously.

Effect of Propionate Concentration

Performance of all the reactors is summarized in Table 1 to highlight the effect of propionate concentration on H_2 production. At 3.84 g/L or less, >99% of propionate was utilized by *R. capsulata*. However, a further increase in propionate concentration resulted in a significant decrease in propionate utilization efficiency.

Except at 1.92 g/L, the maximum H_2 percentage at other levels of propionate exceeded 50%, suggesting that the biogas produced from the utilization of propionate by *R. capsulata* was rich in H_2. The cumulative H_2 production did not increase with propionate concentration as expected but peaked at 3.84 g/L of propionate. In addition, at 3.84 g/L of propionate, the maximum H_2 production rate was of highest value.

One parameter, H_2 yield, could be employed to evaluate the H_2 production potential of a specific substrate *(13)*. This yield is defined as the ratio of the actual moles of H_2 produced to the theoretical moles of H_2, assuming that all the substrate is utilized to produce H_2 and CO_2 according to the following reaction:

$$C_xH_yO_z + (2x - z)H_2O \rightarrow (y/2 + 2x - 2)H_2 + xCO_2 \quad (1)$$

Thus, H_2 yield is expressed as

$$H_2 \text{ yield } (\%) = \frac{\text{actual hydrogen}}{\text{theoretical hydrogen}} \times 100 \quad (2)$$

As shown in Table 1, the H_2 yield at 3.84 g/L was of a similar level to the highest H_2 yield at 2.88 g/L. Taking the propionate utilization efficiency, H_2 percentage, cumulative H_2 production, and H_2 yield into account together, 3.84 g/L should be considered as the optimum propionate concentration for H_2 production by *R. capsulata* in the present study.

Modeling on H_2 Production From Propionate

The cumulative H_2 production (H) data were fitted to a modified Gompertz equation *(14)*, which has been found to be an appropriate model

Table 1
H_2 Production at Various Propionate Concentrations

Propionate level (g/L)	Propionate utilization (%)	Maximum H_2 production rate (mL H_2/[L·h])	H_2 yield (%)	H_2 percentage (%)	Cumulative H_2 (mL)	Duration of H_2 evolution (h)
1.92	99.5	8.5	12.9	32	52	144
2.88	99.6	8.3	18.8	59	114	216
3.84	99.6	10.8	17.2	60	139	216
4.80	75.0	9.4	10.2	53	104	216
5.76	70.0	9.2	11.1	60	134	216
6.72	50.0	10.6	8.1	56	115	216

Table 2
Parameters Calculated From Nonlinear Regression of Eq. 3

Propionate concentration (g/L)	P (mL H_2)	r_m (mL H_2/[L·h])	λ (h)	R^2
1.92	47	1.13	18.7	0.956
2.88	126	0.66	5.7	0.977
3.84	149	0.86	0.2	0.977
4.80	98	1.01	6.3	0.977
5.76	148	0.79	4.4	0.981
6.72	99	0.93	15.1	0.935

for describing the progress of cumulative biogas production in dark anaerobic batch tests *(13)*:

$$H = P \cdot \exp\left\{-\exp\left[\frac{r_m \cdot e}{P}(\lambda - t) + 1\right]\right\} \quad (3)$$

in which P is the H_2 production potential (mL), r_m is the maximum H_2 production rate (mL/[L·h]), λ is the lag phase time (h), and e equals 2.718. The three parameters P, r_m, and a λ were nonlinearly evaluated using the function of Microsoft Origin 6.1 by converting the residual sum of squares between the experiment and the estimation into a minimum value. Table 2 summarizes the values of the calculated parameters.

According to Table 2, H_2 production should have a maximum potential of 149 mL at 3.84 g/L of propionate. The modeling results confirm that 3.84 g/L was the optimum propionate concentration for H_2 production. The high values of the correlation coefficients (R^2) listed in Table 2 imply that the modified Gompertz equation was able to describe properly the H_2 production from propionate by *R. capsulata*.

Discussion

In the effluent of an acidogenic reactor, acetate, propionate, and butyrate are the main aqueous components, accounting for approx 70–80% of the total VFA *(8)*. These VFA should be utilized by photosynthetic bacteria to produce H_2 in the subsequent photosynthetic reactor. To harvest H_2 in this acidogenic-photosynthetic system, highly efficient production of H_2 from these three VFA by photosynthetic bacteria is essential. However, photosynthetic bacteria are capable of utilizing various substrates as a carbon source for growth, but only part of these substrates is suitable for H_2 production. Previous studies demonstrate that both acetate and butyrate are good substrates for H_2 generation by various photosynthetic bacteria. In our study, acetate and butyrate could be used for H_2 production by *R. capsulata*. For comparison, Table 3 summarizes the H_2 production from

Table 3
H_2 Production From Acetate, Propionate, and Butyrate at 40 m*M*

Carbon source	Substrate level (g/L)	Maximum H_2 production rate (mL H_2/[L·h])	H_2 yield (%)	H_2 percentage (%)	Cumulative H_2 production (mL)
Acetate	3.28	10.9	43.8	70	236
Propionate	3.84	10.8	14.6	60	138
Butyrate	4.40	3.0	10.5	63	142

acetate, propionate, and butyrate at 40 m*M*, corresponding to 3.28 g/L of acetate, 3.84 g/L of propionate, and 4.4 g/L of butyrate, respectively.

Acetate, propionate, and butyrate were all readily utilized for H_2 production by *R. capsulata*, as shown in Table 3. The maximum H_2 production rates for the optimum acetate and propionate concentrations were two times higher than that for the optimum butyrate concentration. The H_2 yield for acetate, 43.8%, was much greater than those for propionate (14.6%) and butyrate (10.5%), indicating that acetate had the highest H_2 production potential among the three organic acids. The cumulative H_2 production for acetate was approximately one time higher than those for propionate and butyrate. Again, the maximum H_2 percentage for acetate was greater than those for propionate and butyrate. Figure 4 illustrates the consumption patterns of the three VFA. The consumption of acetate was the fastest, followed by propionate and then butyrate. Furthermore, the consumption of butyrate was not completed. These comparative results suggest that the suitability of the three VFA for H_2 production followed the order of acetate > propionate > butyrate.

Products of acidogenesis have to be consumed in the subsequent photosynthetic reactor. Thus, operational conditions for an acidogenic reactor should be maintained for more production of products suitable for H_2 generation by *R. capsulata*. Since the H_2 production rate of butyrate is much slower compared with acetate and propionate, butyrate is regarded not to be a desirable acidogenic product for the subsequent photosynthetic reactor seeded with *R. capsulata*. The engineering implication of this result is that appropriate conditions should be maintained for low production of butyrate and high production of acetate and propionate.

For various photosynthetic strains, their capacities for H_2 production from VFA are different. Table 4 compares the H_2 yields and maximum H_2 production rates of *R. capsulata* used in the present study with those of three other strains found in the literature. When acetate was used as the substrate, the H_2 yield of the four strains followed the order of *R. monas* sp. > *Rhodobacter* 8604 *R. capsulata* > *R. palustris*, and their maximum H_2 production rate was in the order of *R. monas* sp. > *R. capsulata* > *R. palustris*. On the other hand, when butyrate was used as the substrate, their H_2 yields followed the order of *R. capsulata* > *R. monas* sp. > *Rhodobacter* 8604, and the maximum H_2 production rate of *R. monas* sp. was higher than that of

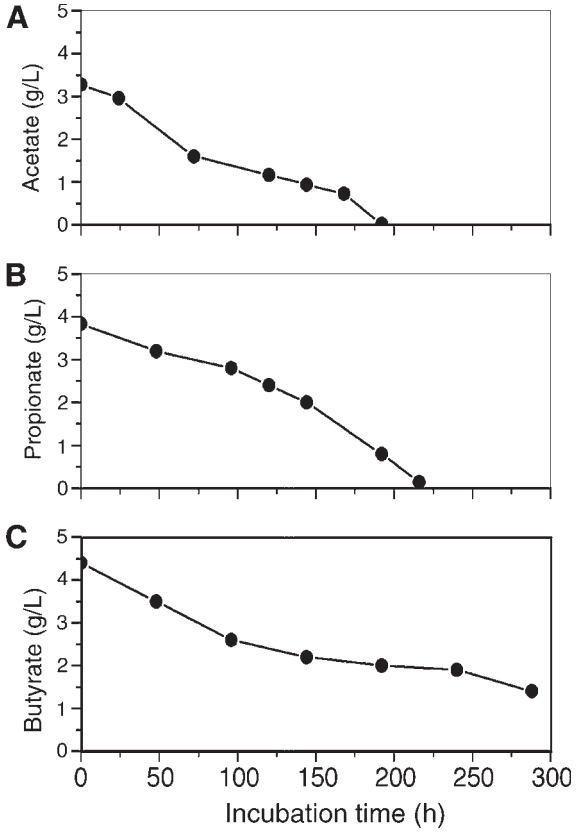

Fig. 4. Degradation patterns of **(A)** acetate, **(B)** propionate, and **(C)** butyrate at 40 m*M*.

R. capsulata. However, *R. palustris* was unable to utilize butyrate to produce H_2 *(5)*. When acetate or butyrate was used as a substrate, the H_2-producing capacity of *R. capsulata* was comparable with those of the well-known strains.

Our study demonstrates that *R. capsulata* was able to utilize acetate, propionate, and butyrate to produce H_2, although these three VFA are exactly the major acidogenic products of wastewaters *(3,15)*. Since it is not clear whether the other photosynthetic strains are able to utilize propionate to generate H_2, so far *R. capsulata* is the only photosynthetic bacterium reported to be able to convert these three VFA to H_2. Hence, *R. capsulata* was an appropriate photosynthetic culture for seeding the H_2-producing reactor in the acidogenic-photosynthetic system treating wastewaters.

Acknowledgments

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Table 4
Comparison of H_2 Production From Acetate and Butyrate by Different Strains

	Acetate			Butyrate			
Strain	Initial concentration (m*M*)	H_2 yield (%)	Maximum production rate (mL/[L·h])	Initial concentration (m*M*)	H_2 yield (%)	Maximum production rate (mL/[L·h])	Reference
Rhodobacter 8604	24	44.0	—	60	3.0	—	*12*
R. monas sp.	22	72.8	25.2	22	8.4	7.6	*5*
R. palustris	22	14.8	2.2	—	[a]	[a]	*5*
R. capsulata	20	41.3	8.3	40	10.5	3.0	Present study

[a]*R. palustris* was unable to utilize butyrate to produce H_2 *(5)*.

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