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THE EFFECT OF TEMPERATURE AND LIGHT INTENSITY ON HYDROGEN GAS PRODUCTION BY DIFFERENT RHODOPSEUDOMONAS CAPSULATA STRAINS

P. Stevens, C. Vertonghen, P. De Vos and J. De Ley*

Laboratorium voor Microbiologie en microbiële Genetica,
Rijksuniversiteit, Ledeganckstraat 35, B-9000 Gent, Belgium

SUMMARY

Six strains of Rhodopseudomonas capsulata were tested for their ability for anaerobic light-dependent hydrogen gas production from acetate in different incubation temperatures and light intensities.

Certain strains show a higher efficiency of acetate conversion to H_2 at higher temperatures and higher light intensities, others on the other hand are insensitive or even show the opposite effect.

INTRODUCTION

The fact that photosynthetic bacteria are able to produce appreciable amounts of hydrogen gas from simple organic compounds as acetate, lactate, butyrate, etc. (Gest and Kamen, 1949; Siegel and Kamen, 1951; Gest et al., 1962) might lead to an interesting alternative process for the commercial production of hydrogen gas. In particular, sun light as a free energy source may make the process economically attractive. However, before an economically rewarding outdoor fermentor can be operative, much fundamental research on the stability and yield of the biological process in various conditions still has to be carried out. Outdoor experiments with photosynthetic bacteria are strongly affected by fluctuations in temperature and light intensity due to the day-night cycle and to seasonal, geographic and climatic conditions. No extensive data are available on the impact of all these parameters on the efficiency of the photosynthetic process. Therefore we screened six strains of Rhodopseudomonas

capsulata for hydrogen gas production at different temperatures between 20°C and 35°C and different light intensities between 9.5 mmol/m^2/s and 1.7 mmol/m^2/s corresponding to midday (full sun) daylight and overcast daylight respectively (Smith and Holmes, 1977).

MATERIALS AND METHODS

Bacterial strains. We used six strains of Rhodopseudomonas capsulata: ATCC 23782, ATCC 17013 (recently reclassified as Rhps. cap., De Ley, 1982), DSM 152 and three strains from the culture collection of Dr. J.D. Wall, B 100, ST 410, ST 407.

Medium. The mineral medium used in all our experiments was the L-glutamic acid-containing medium of Weaver et al. (1975) supplemented with 50 mM of acetate.

Fermentation vessel. To obtain reproducible results for light and temperature experiments we used our self-designed fermentation vessels (Stevens et. al., 1983), with 350 ml medium and flushed with He gas. Four fermentation temperatures (35, 30, 25 and 20°C) were tested. They were obtained by appropriately changing the temperature of the cooling water. The effect of various light intensities was obtained by changing the incandescent bulbs inside the vessel: 100 W = 9.5 mmol/m^2/s; 60 W = 6.0 mmol/m^2/s, 25 W = 1.9 mmol/m^2/s, 15 W = 1.7 mmol/m^2/s. The light intensities were measured with a IL 600A research photometer (International Light) equipped with SEE flat response silicon detector (300-1100 mm).

Hydrogen gas detection. Gas analyses were carried out gas-chromatographically as described earlier (De Vos et al., 1983).

Acetate detection. The acetate concentration was measured gas-chromatographically before and after each experiment as reported previously (Stevens et al., 1983).

RESULTS AND DISCUSSION

Most of our experiments on hydrogen gas production and those of others, were carried out at 30°C with an excess of light (Stevens et al., 1983; Jouanneau et al., 1980). The presently described screening was carried out to select the most appropriate strain for a given outdoor experiment taking into accound efficiency, temperature and light intensity.

1. Hydrogen gas production at different incubation temperatures. For all strains, the hydrogen gas evolution was followed at the four different incubation temperatures mentioned above with a constant light intensity of 1.9 mmol/m^2/s. Higher temperatures than 35°C are seldom reached in our countries, experiments at temperatures below 20°C were not applied because of the slow growth rate. The conversion efficiency of acetate to hydrogen gas

$$CH_3COOH + 2H_2O \longrightarrow 4H_2 + 2CO_2$$

at each temperature is listed in Table 1.

Concerning H_2 evolution, the six strains tested may be subdivided in two groups of three strains each. The first group shows optimal acetate conversion at the higher temperatures: strains ATCC 23782 and ATCC 17013 at 30°C with efficiencies of 76.4% and 75.6% respectively; strain DSM 152 at 35°C with an efficiency of 73.3%. The total hydrogen gas production time at the higher temperatures is about three times shorter in comparison to the lower temperatures. The second group with strain B 100, ST 410 and ST 407 gives optimal acetate conversion at the lowest temperature (20°C) with efficiencies of 75.6, 78.4 and 81.2% respectively. Here, the total hydrogen gas production time at low temperatures takes only twice the time at high temperatures.

2. Hydrogen gas production at different light intensities. Obviously, the available light affects the growth and hence the hydrogen gas production of photoheterotrophic organisms. Most of the experiments with an artificial light source use an excess of light energy. The effect of the light intensities on hydrogen gas production is produced by changing the incandescent bulbs (100 W, 60 W, 25 W, 15 W) inside the fermentation vessel. The cultures were incubated at 30°C. The data are compiled in Table 2. Among the tested strains, two groups may be recognized. The first group contains Rhps. capsulata ATCC 23782, ATCC 17013, DSM 152 and ST 407 where the efficiencies of acetate conversion increase with increasing light intensities. The highest conversion efficiency (85.5%) was

Table 1. Hydrogen gas production from 50 mM acetate by six different Rhodopseudomonas capsulata strains at different incubation temperatures and a constant light of 1.9 mmol/m^2/s. The efficiency is expressed in %, calculated on the amount of acetate converted to H_2

°C	35	30	25	20	35	30	25	20	35	30	25	20
Strain	ATCC 23782				ATCC 17013				DSM 152			
Total H_2 production time (in days)	5	6	9	15	5	7	8	12	6	7	9	17
H_2 production (in mmol)	46.3	53.4	49.3	48.8	49.7	52.9	50.9	50.2	51.3	46.4	41.9	41.2
Efficiency	66.1	76.4	70.4	69.7	71.0	75.6	72.8	71.7	73.3	66.3	59.9	58.8
Strain	B 100				ST 410				ST 407			
Total H_2 production time (in days)	4	5	7	9	4	5	7	8	4	5	7	9
H_2 production (in mmol)	49.4	48.9	52.9	52.9	46.5	48.5	50.2	54.9	44.0	50.1	51.1	56.8
Efficiency	70.6	69.8	75.5	75.6	66.4	69.2	71.8	78.4	62.8	71.6	73.0	81.2

Table 2. Hydrogen gas production from 50 mM acetate by six different Rhodopseudomonas capsulata strains at different light intensities at 30°C. The efficiency is expressed in %, calculated on the amount of acetate converted to H_2

Watt	100	60	25	15	100	60	25	15	100	60	25	15
Strain	ATCC 23782				ATCC 17013				DSM 152			
Total H_2 production time (in days)	5	6	6	5	6	7	7	6	6	7	7	6
H_2 production (in mmol)	59.9	54.6	53.4	51.3	53.8	53.7	52.9	48.6	52.1	48.8	46.4	46.1
Efficiency	85.5	78.0	76.4	73.3	76.9	76.7	75.6	69.4	74.4	69.7	66.3	65.9
Strain	B 100				ST 410				ST 407			
Total H_2 production time (in days)	6	5	5	5	6	5	5	5	6	5	5	5
H_2 production (in mmol)	49.4	49.1	48.9	49.5	48.8	47.9	48.5	48.8	59.3	52.2	50.1	51.6
Efficiency	70.6	70.2	69.8	70.7	69.7	68.4	69.2	69.7	84.7	74.5	71.6	73.7

reached at a light intensity of 9.5 mmol/m^2/s (100 W) with strain ATCC 23782. In the second group, with strain B 100 and ST 410, the acetate conversion turns out to be insensitive to the different light intensities. However, the average efficiency is about 10% to 15% lower than the maximal efficiency of the other four strains. For all strains the light intensity range used seems not to affect the total hydrogen gas production time.

CONCLUSION

Efficiencies of acetate (and probably of other suitable substrates) conversion to hydrogen gas for the strains used were differently affected by temperature and light intensity. Therefore, the choice of the organism for outdoor experiments will largely have to depend on the climatic conditions.

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