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Research Article

Influence of Different Cultural Conditions on Photoproduction of Hydrogen by *Rhodopseudomonas palustris* KU003

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Photoproduction of hydrogen by *Rhodopseudomonas palustris* KU003 under different cultural conditions with various carbon and nitrogen sources was investigated. Hydrogen production was measured using a Gas chromatograph. Malate promoted more amounts of hydrogen production under anaerobic light conditions than anaerobic dark conditions. Cumulative hydrogen production by the organism was recorded at various time intervals. Incubation period of 120 hrs was optimum for production of hydrogen. pH 7.0 ± 0.4 was optimum for production of hydrogen. L-glutamic acid was a good nitrogen source for production of hydrogen. Growing cells produced more amount of hydrogen than resting cells. Significance of the above results in presence of existing literature is discussed.

1. Introduction

Hydrogen is considered as a potential fuel for the future because it is renewable, ecofriendly, cheaper, transportable, has high energy content for per unit mass of any known fuel, it is easily convertible to electricity by fuel cells, and on combustion it gives water as the only byproduct. Compared to nonphototrophic bacteria, phototrophic bacteria produce threefold greater amounts of hydrogen per mole of substrate utilized. When the conversion efficiency of substrate into hydrogen by chemotrophs restricted to 33.3%, phototrophs can reach upto 100%.

A wide variety of organic substrates such as carbohydrates [1, 2], lactate, malate, benzoate [3], sucrose [4], and nitrogen sources Ethanolamine [5] and L-cysteine [6] were reported to promote higher amounts of hydrogen in phototrophic bacteria. However, the substrate specificity for hydrogen production varied with the species [7]. Stimulation of hydrogen production by spraying nitrogen gas in the presence of glucose has been reported by Mizuno et al. [8]. The efficacy of different organic substrates for hydrogen production in *R. rubrum* was investigated by Melnicki et al. [7]. In view of the above facts, influence of different cultural conditions on hydrogen production by anoxygenic phototrophic bacteria was studied and the results are discussed.

2. Material and Methods

The phototrophic bacteria were isolated from the effluent samples by enrichment techniques by inoculating into the medium and incubated anaerobically in visible light (2,000 lux). Bacteria thus isolated were identified with the help of cultural characteristics (colour, size, and shape), carbon and nitrogen requirement, vitamin requirements, absorption spectra analysis, bacteriochlorophylls, and carotenoids. Identification keys provided in Bergey's manual of systematic bacteriology (1994) [9] was adopted. Growth was determined by measuring optical density at 660 nm using UV-Vis spectrophotometer.

2.1. Preparation of Growing Cells. Logarithmic cultures of *R. palustris* were inoculated (1% v/v) into basal medium containing different carbon sources (1%) along with ammonium chloride (0.5%) as nitrogen source. When different nitrogen

Table 1: Effect of pH on hydrogen production (mL/15 mL vessel) by *R. palustris*.

pH		Incubation period (in hrs)							
		24	48	72	96	120	144	166	192
5.0	R	—	—	—	—	0.22 ± 0.06	—	—	—
	G	—	—	0.32 ± 0.14	0.50 ± 0.12	0.68 ± 0.04	0.44 ± 0.08	—	—
5.5	R	—	—	—	—	0.25 ± 0.04	0.38 ± 0.08	—	—
	G	—	0.24 ± 0.04	0.56 ± 0.24	0.94 ± 0.16	1.10 ± 0.18	0.78 ± 0.08	0.38 ± 0.04	—
6.0	R	—	—	—	0.38 ± 0.12	0.53 ± 0.04	0.44 ± 0.08	—	—
	G	0.58 ± 0.12	0.98 ± 0.08	1.54 ± 0.12	2.58 ± 0.04	3.36 ± 0.12	2.84 ± 0.18	1.92 ± 0.24	0.88 ± 0.18
6.5	R	0.24 ± 0.08	0.42 ± 0.04	0.54 ± 0.12	1.26 ± 0.08	1.08 ± 0.16	0.49 ± 0.10	0.32 ± 0.04	—
	G	0.36 ± 0.06	0.56 ± 0.14	2.62 ± 0.28	3.35 ± 0.12	4.66 ± 0.28	2.28 ± 0.18	1.88 ± 0.26	0.96 ± 0.14
7.0	R	—	0.32 ± 0.18	1.41 ± 0.22	1.88 ± 0.28	1.35 ± 0.12	0.96 ± 0.18	0.48 ± 0.18	—
	G	0.48 ± 0.06	0.88 ± 0.14	2.84 ± 0.22	4.62 ± 0.40	5.84 ± 0.32	3.56 ± 0.24	1.54 ± 0.12	0.98 ± 0.12
7.5	R	—	0.24 ± 0.06	0.48 ± 0.12	0.96 ± 0.12	1.54 ± 0.10	0.78 ± 0.36	0.32 ± 0.18	—
	G	0.88 ± 0.26	1.94 ± 0.16	2.86 ± 0.28	3.76 ± 0.22	4.88 ± 0.28	2.45 ± 0.24	1.76 ± 0.18	1.12 ± 0.16
8.0	R	—	—	0.34 ± 0.02	0.56 ± 0.08	0.86 ± 0.20	0.46 ± 0.12	—	—
	G	—	0.48 ± 0.14	0.84 ± 0.18	1.66 ± 0.10	2.56 ± 0.16	1.86 ± 0.22	0.92 ± 0.02	0.58 ± 0.20

R: resting cells; G: growing cells; —: no hydrogen production.

Table 2: Effect of carbon sources on hydrogen production (mL/15 mL vessel) by *R. palustris* under anaerobic light.

Carbon source		Incubation period (in hrs)							
		24	48	72	96	120	144	166	192
Lactate	R	0.32 ± 0.18	0.66 ± 0.04	1.22 ± 0.10	1.65 ± 0.10	2.44 ± 0.14	1.72 ± 0.28	0.96 ± 0.24	0.48 ± 0.24
	G	0.78 ± 0.18	1.14 ± 0.22	2.88 ± 0.14	4.10 ± 0.36	5.94 ± 0.16	4.52 ± 0.28	3.96 ± 0.36	2.88 ± 0.14
Malate	R	0.38 ± 0.16	0.76 ± 0.26	0.94 ± 0.12	1.78 ± 0.22	2.86 ± 0.14	1.96 ± 0.08	1.16 ± 0.08	0.78 ± 0.16
	G	2.44 ± 0.18	3.74 ± 0.14	4.66 ± 0.18	5.14 ± 0.46	6.22 ± 0.28	5.42 ± 0.16	4.28 ± 0.32	3.66 ± 0.24
Succinate	R	—	0.22 ± 0.04	0.54 ± 0.24	0.88 ± 0.18	1.66 ± 0.08	1.10 ± 0.10	0.76 ± 0.02	0.38 ± 0.10
	G	0.96 ± 0.06	1.84 ± 0.18	2.62 ± 0.14	3.88 ± 0.36	4.28 ± 0.28	3.44 ± 0.18	2.86 ± 0.42	1.76 ± 0.32
Fructose	R	—	—	—	0.34 ± 0.08	0.56 ± 0.18	0.22 ± 0.06	—	—
	G	—	—	0.34 ± 0.04	0.66 ± 0.12	1.8 ± 0.28	0.82 ± 0.04	0.40 ± 0.06	—
Glucose	R	—	—	—	—	0.26 ± 0.06	—	—	—
	G	—	—	0.28 ± 0.12	0.74 ± 0.08	1.25 ± 0.04	0.36 ± 0.12	—	—
Lactose	R	—	—	0.38 ± 0.06	0.64 ± 0.06	1.4 ± 0.02	0.88 ± 0.06	0.52 ± 0.04	0.26 ± 0.08
	G	0.66 ± 0.04	1.22 ± 0.08	1.66 ± 0.06	2.78 ± 0.20	3.22 ± 0.12	2.56 ± 0.16	1.78 ± 0.14	0.94 ± 0.08
Maltose	R	—	—	—	0.28 ± 0.18	0.68 ± 0.10	0.38 ± 0.20	—	—
	G	0.46 ± 0.06	0.84 ± 0.10	1.12 ± 0.28	1.88 ± 0.32	2.46 ± 0.26	1.36 ± 0.32	0.92 ± 0.24	0.62 ± 0.12
Sucrose	R	—	—	—	0.20 ± 0.20	0.72 ± 0.16	0.38 ± 0.14	—	—
	G	—	0.40 ± 0.20	0.52 ± 0.12	0.88 ± 0.12	1.66 ± 0.20	1.22 ± 0.22	0.66 ± 0.22	0.28 ± 0.04
Mannitol	R	—	—	0.58 ± 0.04	0.98 ± 0.12	1.14 ± 0.14	0.76 ± 0.12	0.34 ± 0.06	—
	G	0.56 ± 0.18	1.34 ± 0.22	2.12 ± 0.20	2.68 ± 0.44	3.86 ± 0.28	2.78 ± 0.16	1.86 ± 0.08	0.78 ± 0.12
Sorbitol	R	—	—	—	0.28 ± 0.08	0.44 ± 0.12	—	—	—
	G	—	0.26 ± 0.10	0.52 ± 0.12	0.84 ± 0.08	1.54 ± 0.06	0.66 ± 0.18	0.44 ± 0.04	—

R: resting cells; G: growing cells; —: no hydrogen production.

sources were tested, acetate was used as a source of carbon. Similar conditions were maintained devoid of light for investigations under anaerobic dark conditions.

2.2. Preparation of Resting Cell Suspensions. Cells of *R. palustris* were grown with acetate and ammonium chloride until mid-log phase and were harvested by centrifugation (16,000 ×g for 20 minutes). The pellet was washed twice and resuspended in basal salts medium. This suspension was then distributed into screw-cap test tubes (10 × 100 mm) to fill them fully (anaerobic) or into 20 mL medium in a 100 mL conical flask (aerobic) and incubated under light (2,400 lux) or dark conditions at 32°C. Similar conditions were maintained devoid of light for investigations under anaerobic dark conditions.

The basic technique used in the hydrogen production was that of Vincenzini et al. [10]. Five mL of bacterial culture was harvested by centrifugation at 10,000 ×g for 10 min, washed

Table 3: Effect of nitrogen sources on hydrogen production (mL/15 mL vessel) by *R. palustris* under anaerobic light.

Nitrogen source		Incubation period (in hrs)							
		24	48	72	96	120	144	166	192
Potassium nitrate	R	—	—	0.22 ± 0.04	0.42 ± 0.08	0.66 ± 0.10	0.36 ± 0.08		
	G	—	—	0.46 ± 0.08	0.88 ± 0.04	1.22 ± 0.10	0.62 ± 0.18	0.32 ± 0.04	—
Sodium nitrate	R	—	—	0.36 ± 0.06	0.52 ± 0.08	0.76 ± 0.10	0.48 ± 0.12	—	—
	G	0.42 ± 0.08	0.54 ± 0.14	0.84 ± 0.12	1.26 ± 0.14	1.86 ± 0.18	1.44 ± 0.28	1.08 ± 0.16	0.66 ± 0.06
Ammonium chloride	R	—	—	—	—	—	—	—	—
	G	—	—	—	—	—	—	—	—
Urea	R	—	—	—	—	0.34 ± 0.04	—	—	—
	G	—	—	0.38 ± 0.08	0.66 ± 0.08	0.88 ± 0.12	0.52 ± 0.16	0.38 ± 0.04	—
Thiourea	R	—	—	—	—	—			—
	G	—	—	—	0.38 ± 0.18	0.54 ± 0.28	0.34 ± 0.02	0.22 ± 0.06	—
Glycine	R		—	—	0.28 ± 0.08	0.48 ± 0.10	0.36 ± 0.06	—	—
	G	—	—	0.54 ± 0.20	0.96 ± 0.12	1.3 ± 0.18	0.74 ± 0.14	0.46 ± 0.10	—
L-asparagine	R	—	—	0.24 ± 0.16	0.44 ± 0.12	0.66 ± 0.08	0.36 ± 0.06	—	—
	G	—	0.46 ± 0.02	0.84 ± 0.12	1.26 ± 0.06	1.74 ± 0.18	1.46 ± 0.12	1.16 ± 0.06	0.68 ± 0.06
L-aspartic acid	R	—	—	0.36 ± 0.18	0.68 ± 0.18	0.96 ± 0.08	0.58 ± 0.20	0.26 ± 0.08	—
	G	0.52 ± 0.06	0.76 ± 0.08	1.34 ± 0.08	1.84 ± 0.08	2.2 ± 0.08	1.54 ± 0.08	1.08 ± 0.18	0.66 ± 0.12
L-glutamic acid	R	—	—	0.66 ± 0.04	0.88 ± 0.08	1.22 ± 0.10	0.98 ± 0.12	0.72 ± 0.04	—
	G	0.48 ± 0.10	0.96 ± 0.16	1.54 ± 0.12	1.86 ± 0.08	2.56 ± 0.06	2.22 ± 0.08	1.76 ± 0.08	0.84 ± 0.08
L-glutamine	R	—	—	—	0.48 ± 0.12	0.78 ± 0.04	0.56 ± 0.06	0.34 ± 0.10	—
	G	—	0.42 ± 0.08	0.76 ± 0.14	1.16 ± 0.24	1.58 ± 0.16	1.24 ± 0.28	0.88 ± 0.14	0.54 ± 0.02
L-tyrosine	R	—	—	0.32 ± 0.02	0.46 ± 0.22	0.84 ± 0.28	0.58 ± 0.06	0.28 ± 0.14	—
	G	0.38 ± 0.04	0.78 ± 0.06	1.18 ± 0.14	1.56 ± 0.14	2.30 ± 0.08	1.78 ± 0.12	1.34 ± 0.16	0.58 ± 0.04
L-cystine	R	—	—	0.32 ± 0.04	0.54 ± 0.08	1.10 ± 0.08	0.68 ± 0.12	0.38 ± 0.14	—
	G	0.30 ± 0.04	0.76 ± 0.10	1.34 ± 0.12	1.64 ± 0.14	1.94 ± 0.28	1.48 ± 0.24	1.10 ± 0.22	0.54 ± 0.16
L-methionine	R	—	—	—	0.34 ± 0.06	0.64 ± 0.04	0.40 ± 0.08	—	—
	G	—	—	0.46 ± 0.04	0.84 ± 0.32	1.24 ± 0.26	0.96 ± 0.10	0.56 ± 0.12	—
Nitrogen gas	R	—	—	—	—	0.28 ± 0.02	—	—	—
	G	—	—	0.28 ± 0.10	0.66 ± 0.18	1.12 ± 0.32	0.42 ± 0.16	—	—

R: resting cells; G: growing cells; —: no hydrogen production.

thrice with 0.3% saline, and the cells were suspended in the basal medium devoid of electron donor and nitrogen source. Depending on the experimental conditions, different electron donors and nitrogen sources were added at required concentrations. To test the hydrogen production activity, the washed cell suspension was inoculated into 8 mL of the medium in 15 mL capacity rimless test tubes sealed with subaseals, and anaerobic conditions were created by evacuating and flushing with nitrogen (100%). Cumulative hydrogen production was recorded at various time intervals. Hydrogen produced was measured by injecting 0.5 mL of the gas phase from the reaction vessels with an airtight syringe into a gas chromatograph (Mak Analytica make) fitted with a molecular sieve 5A column (2 m × 1/8″ ODSS) to a thermal conductivity detector (TCD). Gas analysis was done with oven temperature at 60°C with argon as carrier gas (flow rate 30 mL/min), 120 mA detector current. Integrator and recorder were used at highest sensitivity. Before withdrawing each sample, 0.5 mL of nitrogen was injected in the vessel to maintain positive pressure. The amount of hydrogen liberated by the photosynthetic bacterium was calculated from the peak height of the recorder with reference to calibration curve prepared using ultrapure hydrogen.

3. Results and Discussion

Perusal of Table 1 reveals that *R. palustris* could produce hydrogen over a narrow pH range of 5.0 to 8.0. Growth of the organism started from pH 4.00 but hydrogen production could not be recorded till pH 5.0, hence data was not shown. *Rps. palustris* opted for pH 7.0 for maximum production of hydrogen by growing cells and resting cells. Incubation period of 96–120 hrs was optimum for production of hydrogen. There were variations in the initial pH and final pH but the variations were minute and not greater than 0.28(±). Hence, they were not presented in the table. From Table 2, it is clear that the bacteria under investigation showed preference towards carbon source present in the medium. *Rps. palustris* preferred malate for maximum production of hydrogen at 120 hrs incubation. Lactate and Succinate also promoted more amounts of hydrogen production under anaerobic light. Glucose promoted lowest amounts of hydrogen production.

Table 4: Effect of carbon sources on hydrogen production (mL/15 mL vessel) by *R. palustris* under anaerobic dark.

Carbon source		Incubation period (in hrs)							
		24	48	72	96	120	144	166	192
Lactate	R	—	—	0.24 ± 0.02	0.48 ± 0.10	0.66 ± 0.14	0.38 ± 0.18	—	—
	G	—	—	0.38 ± 0.16	0.68 ± 0.18	1.24 ± 0.12	0.84 ± 0.28	0.44 ± 0.12	0.32 ± 0.18
Malate	R	—	—	0.42 ± 0.06	0.68 ± 0.32	0.98 ± 0.02	0.36 ± 0.16	—	—
	G	0.36 ± 0.14	0.58 ± 0.14	0.98 ± 0.14	1.26 ± 0.24	1.68 ± 0.20	1.34 ± 0.32	0.84 ± 0.14	0.40 ± 0.18
Succinate	R	—	—	—	0.26 ± 0.18	0.54 ± 0.12	0.36 ± 0.02	—	—
	G	—	0.26 ± 0.06	0.52 ± 0.12	0.78 ± 0.04	1.10 ± 0.28	0.82 ± 0.18	0.68 ± 0.06	0.38 ± 0.20
Fructose	R	—	—	—	—	0.22 ± 0.08	—	—	—
	G	—	—	0.46 ± 0.14	0.56 ± 0.20	0.78 ± 0.28	0.38 ± 0.12	0.28 ± 0.08	—
Glucose	R	—	—	—	—	0.28 ± 0.06	—	—	—
	G	—	—	—	0.38 ± 0.10	0.56 ± 0.14	0.26 ± 0.04	—	—
Lactose	R	—	—	—	0.48 ± 0.08	0.74 ± 0.12	0.36 ± 0.03	—	—
	G	—	0.34 ± 0.16	0.68 ± 0.06	1.04 ± 0.28	1.48 ± 0.16	0.92 ± 0.14	0.76 ± 0.28	0.54 ± 0.18
Maltose	R	—	—	—	—	0.38 ± 0.10	—	—	—
	G	—	—	0.22 ± 0.14	0.44 ± 0.32	0.94 ± 0.14	0.56 ± 0.10	0.32 ± 0.18	—
Sucrose	R	—	—	—	0.24 ± 0.12	0.48 ± 0.06	0.28 ± 0.18	—	—
	G	—	—	0.34 ± 0.02	0.66 ± 0.18	1.18 ± 0.24	0.78 ± 0.32	0.46 ± 0.12	0.26 ± 0.18
Mannitol	R	—	—	—	—	0.40 ± 0.04	—	—	—
	G	—	—	—	0.42 ± 0.06	0.64 ± 0.16	0.38 ± 0.08	—	—
Sorbitol	R	—	—	—	0.36 ± 0.22	0.68 ± 0.20	0.42 ± 0.12	0.26 ± 0.06	—
	G	—	—	0.32 ± 0.02	0.54 ± 0.08	0.86 ± 0.14	0.40 ± 0.08	0.24 ± 0.04	—

R: resting cells; G: growing cells; —: no hydrogen production.

L-glutamic acid followed by L-tyrosine and L-aspartic acid were good promoters of hydrogen in *Rps. palustris* in anaerobic light (Table 3). Ammonium chloride failed to produce hydrogen. Glycine and potassium nitrate were of equal nutritive value for the production of hydrogen. Growing cells produced more amount of hydrogen than resting cells. Thiourea promoted less amounts of hydrogen and was a poor nitrogen source for hydrogen production by *Rps. palustris*. Hydrogen production started from 24 hrs and considerable amount still could be recorded by the end of 192 hrs in glutamic-acid-containing medium. Perusal of Table 4 shows that malate and Lactose were preferred over other carbon sources under anaerobic dark conditions. Maltose and sorbitol were of same nutritive value for the production of hydrogen. In glucose, fructose, and maltose medium production of hydrogen was observed only at 120 hrs in growing cells. Lowest amounts of hydrogen were recorded in glucose medium similar to that in anaerobic light conditions. Hydrogen production was less in anaerobic dark conditions when compared to anaerobic light conditions. Growing cells could produce more amount of hydrogen in malate medium over extended periods. The hydrogen production started early in growing cells than in resting cells in most of the carbon sources tried.

Ammonium chloride failed to produce hydrogen production in *Rps. palustris* under anaerobic dark (Table 5). Inhibition of hydrogen in presence of ammonium ions has also been reported by Salerno et al. [11]. Thiourea, urea, and nitrogen gas were responsible for inhibition of hydrogen production in resting cells. In all other nitrogen sources tried, growing cells produced more hydrogen than resting cells. Resting cells in general showed a lag in hydrogen production than growing cells of *Rps. palustris*. Highest production of hydrogen was recorded in growing cells of L-glutamic acid and tyrosine media.

Carbon sources are known to influence hydrogen production through nitrogenase enzyme by causing variation in electron donation capabilities of the cofactor compounds to nitrogenase [12]. Hence, differences in hydrogen production rates with different carbon sources were observed. In our study, organic nitrogen sources produced more amounts of hydrogen compared to inorganic nitrogen sources. Organic nitrogen sources are directly incorporated into proteins or transformed into other cellular nitrogenous constituents [13]. In contrast, cell spends more time in synthesizing amino acids for protein synthesis from inorganic nitrogen sources. This might be the reason for the variation observed in hydrogen production rates. Further studies are required to understand the molecular mechanism behind these observed variations in production of hydrogen with different carbon and nitrogen sources.

4. Conclusions

The present study show the ability of this phototrophic bacterium to produce hydrogen in different cultural conditions which can be exploited. Anaerobic dark conditions required to be explored more as it is more economically attractive. Investigations are on to find out more suitable and cheaper

Table 5: Effect of nitrogen sources on hydrogen production (mL/15 mL vessel) by *R. palustris* under anaerobic dark.

Nitrogen source		Incubation period (in hrs)							
		24	48	72	96	120	144	166	192
Potassium nitrate	R	—	—	—	—	0.22 ± 0.06	—	—	—
	G	—	—	0.38 ± 0.04	0.68 ± 0.06	0.98 ± 0.08	0.74 ± 0.18	0.30 ± 0.18	—
Sodium nitrate	R	—	—	—	—	0.23 ± 0.10	—	—	—
	G	—	—	—	0.30 ± 0.04	0.46 ± 0.18	0.22 ± 0.08	—	—
Ammonium chloride	R	—	—	—	—	—	—	—	—
	G	—	—	—	—	—	—	—	—
Urea	R	—	—	—	—	—	—	—	—
	G	—	—	—	0.24 ± 0.08	0.38 ± 0.02	—	—	—
Thiourea	R	—	—	—	—	—	—	—	—
	G	—	—	—	—	0.24 ± 0.08	—	—	—
Glycine	R	—	—	—	—	0.28 ± 0.06	—	—	—
	G	—	—	—	0.38 ± 0.12	0.74 ± 0.16	0.56 ± 0.04	0.22 ± 0.18	—
L-asparagine	R	—	—	—	—	0.36 ± 0.04	—	—	—
	G	—	0.38 ± 0.06	0.76 ± 0.12	0.98 ± 0.10	1.04 ± 0.08	0.74 ± 0.18	0.42 ± 0.06	—
L-glutamic acid	R	—	—	—	—	0.36 ± 0.08	—	—	—
	G	—	—	0.64 ± 0.18	0.94 ± 0.08	1.6 ± 0.28	0.72 ± 0.14	0.54 ± 0.10	—
L-glutamine	R	—	—	—	—	0.52 ± 0.08	0.30 ± 0.02	—	—
	G	0.48 ± 0.14	0.72 ± 0.08	0.98 ± 0.16	1.22 ± 0.08	1.16 ± 0.30	1.14 ± 0.06	0.86 ± 0.26	0.44 ± 0.12
L-tyrosine	R	—	—	—	0.32 ± 0.02	0.48 ± 0.10	0.24 ± 0.04	—	—
	G	—	—	—	0.28 ± 0.06	0.46 ± 0.08	0.34 ± 0.04	—	—
L-cystine	R	—	—	—	—	0.28 ± 0.08	—	—	—
	G	—	—	—	0.38 ± 0.14	0.56 ± 0.04	0.34 ± 0.02	—	—
L-methionine	R	—	—	—	—	0.38 ± 0.18	—	—	—
	G	—	0.20 ± 0.02	0.44 ± 0.08	0.72 ± 0.12	0.86 ± 0.14	0.56 ± 0.22	0.38 ± 0.06	—
Nitrogen gas	R	—	—	—	—	—	—	—	—
	G	—	—	—	—	0.42 ± 0.12	—	—	—
L-aspartic acid	R	—	—	—	0.38 ± 0.12	0.48 ± 0.02	0.58 ± 0.06	0.26 ± 0.08	—
	G	—	0.46 ± 0.04	0.74 ± 0.08	1.04 ± 0.10	0.96 ± 0.12	0.76 ± 0.22	0.54 ± 0.26	0.34 ± 0.08

R: resting cells; G: growing cells; —: no hydrogen production.

carbon and nitrogen sources for hydrogen production. Further, the organism should be investigated for simultaneous production of PHB (polyhydroxy butyrate) and ALA (amino levulinic acid) along with the production of hydrogen.

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