Research Article

Photoproduction of Hydrogen under Different Cultural Conditions by Alginate Immobilized *Rhodopsedomonas palustris* KU003

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*Rhodopsedomonas palustris* KU003 was immobilized in alginate and its hydrogen producing abilities were assessed. Maximum hydrogen production took place between 120 and 144 hrs in most carbon sources. Alginate immobilization induced higher amount of hydrogen in malate-, lactate-, and succinate-containing medium in *Rps. palustris*. 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. Glucose and sorbitol were poorer substrates as they induced only limited amount of hydrogen. Anaerobic light induced comparatively more amount of hydrogen in the bacteria under investigation than in anaerobic dark. Thiourea was a poor nitrogen source for the production of hydrogen by *Rps. palustris*. Results of the above are discussed in the light of existing literature in this communication.

1. Introduction

Purple nonsulphur phototrophic bacteria are very well known for their hydrogen producing capabilities and bioremediation abilities [1–4]. They are also known to produce polyhydroxy butyrate which can be used as bioplastic [1, 5]. The unique ability of phototrophic bacteria to produce hydrogen is being tried to use as an alternative energy source compared to nonconventional energy sources like solar, wind, ocean, and waves, thermal, geothermal, and thermo-nuclear [6]. Enhancement and stabilization of hydrogen production could be achieved by immobilization as it protects the cells from inhibitory effect of oxygen, nitrogen, and osmotic stress and pH. Various immobilization techniques are being used to enhance and stabilize photoproduction of hydrogen by photosynthetic bacteria [7, 8]. von Felton et al. (1985) [9] used agar, agarose, alginate, pectin, and k-carrageenan for hydrogen production by *Rhodospirillum rubrum*. Cationic polyelectrolyte was used to entrap anoxygenic phototrophic bacteria for enhanced hydrogen production [10]. Hydrogen production by photoreactive nonporous latex coatings of *Rhodopsedomonas palustris* CGA 009 was investigated by Gosse et al. (2007) [11]. In the present study, effect of immobilization on production of hydrogen by *Rps. palustris* was investigated and 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 the light (2000 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 (1989) [12] was adopted. Growing cells were used in this study based on our earlier observation [4]. Preparation of growing cells was done as follows. Logarithmic cultures of...
Rps. 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 sources were tested, acetate was used as a source of carbon. Similar conditions were maintained devoid of light for investigations under anaerobic dark conditions.

Bacteria were separated by centrifugation at 5000 rpm at 4°C for 10 min. The cells were washed and resuspended in sterile saline. The alginate entrapments of cells were performed according to the method of Johansen and Flink (1986) [13]. Sodium alginate solution (3%) was prepared by dissolving 3 g of sodium alginate in 100 mL boiling water and autoclaved at 121°C for 15 minutes. It was allowed to cool and both alginate and cell suspension were mixed and stirred manually for 10 min to get a uniform mixture. The slurry was taken in a sterile syringe and added drop wise into ice-cold 0.2 M CaCl₂ solution from 5 cm height and kept for curing at 4°C for four hours. The cured beads were washed with sterile distilled water 3 to 4 times. When the beads were not being used, they were preserved in 0.9% sodium chloride solution and stored in a refrigerator.

The basic techniques used in the hydrogen production were those established by Vincenzini et al. (1986) [14]. Five mL of bacterial culture was harvested by centrifugation at 10,000 x g for 10 min, washed thrice with 0.3% saline, and the cells were suspended in the basal medium devoid of electron donor and nitrogen source at a pH of 7.0 ± 0.4. 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 sub- asals and anaerobic conditions were created by evacuating and flushing with nitrogen (100%).

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 5 A column (2 m x 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 bacteria was calculated from the peak height of the recorder with reference to calibration curve prepared using ultra-pure hydrogen.

### 3. Results and Discussion

Perusal of Table 1 reveals that maximum hydrogen production took place between 120 and 144 hrs in most carbon sources. There were variations in the initial pH and final pH but the variations were minute and not greater than 0.38 (±). Hence, they were not presented in the table. Alginate immobilization induced higher amount of hydrogen in malate followed by lactate and succinate. Glucose and sorbitol were poorer substrates as they induced only limited amount of hydrogen. There was a difference in the production of maximal hydrogen rates varying between 120 and 144 hrs. Response of immobilized cells of phototrophic bacteria towards nitrogen sources under different conditions was studied and the results are depicted in Table 2.

L-glutamic acid induced more production of hydrogen than other nitrogen sources. Rps. palustris produced more hydrogen in tyrosine-, aspartic-acid-, and asparagines-containing media. Similarly, thiourea was poor nitrogen source for the production of hydrogen by Rps. palustris. Highest production of hydrogen was observed by 144 hrs in L-glutamic acid containing medium. Anaerobic light induced comparatively more amount of hydrogen in the bacteria under investigation than in anaerobic dark (Table 3). Results of the study from Tables 3 and 4 clearly show that similar trends of preference towards carbon and nitrogen sources were seen except the production rate of hydrogen was less compared to anaerobic light. Figure 1 gives a comparison between hydrogen production between 120 and 144 hrs by Immobilized and Suspension cultures (unpublished data) of the same organism.

Carbon sources are known to influence hydrogen production through nitrogenase enzyme by causing variation in

### Table 1: Effect of carbon sources on hydrogen production (mL/15 mL vessel) by immobilized cells of Rps. palustris in anaerobic light.

| Carbon source | 24       | 48       | 72       | 96       | 120      | 144      | 166      | 192      |
|--------------|----------|----------|----------|----------|----------|----------|----------|----------|
| Fructose     | 0.26 ± 0.02 | 0.48 ± 0.10 | 0.98 ± 0.08 | 1.68 ± 0.06 | 1.86 ± 0.18 | 2.34 ± 0.28 | 1.22 ± 0.16 | 0.66 ± 0.08 |
| Glucose      | —        | 0.28 ± 0.06 | 0.48 ± 0.12 | 0.98 ± 0.20 | 1.54 ± 0.22 | 1.68 ± 0.12 | 1.14 ± 0.04 | 0.74 ± 0.28 |
| Maltose      | 0.42 ± 0.04 | 0.64 ± 0.08 | 1.22 ± 0.26 | 1.88 ± 0.12 | 2.42 ± 0.06 | 2.88 ± 0.14 | 2.08 ± 0.18 | 1.48 ± 0.02 |
| Sucrose      | 0.32 ± 0.14 | 0.54 ± 0.06 | 1.56 ± 0.14 | 1.94 ± 0.10 | 2.32 ± 0.24 | 2.56 ± 0.20 | 1.86 ± 0.14 | 0.66 ± 0.22 |
| Mannitol     | 0.22 ± 0.16 | 0.76 ± 0.12 | 1.86 ± 0.20 | 4.2 ± 0.12  | 3.52 ± 0.16 | 3.1 ± 0.14  | 2.8 ± 0.22  | 1.78 ± 0.16 |
| Lactate      | 0.24 ± 0.02 | 0.96 ± 0.08 | 1.64 ± 0.12 | 4.44 ± 0.56 | 6.14 ± 0.34 | 5.8 ± 0.22  | 3.88 ± 0.18 | 2.66 ± 0.12 |
| Malate       | 1.86 ± 0.04 | 3.84 ± 0.06 | 4.68 ± 0.10 | 5.34 ± 0.12 | 6.72 ± 0.08 | 4.28 ± 0.24 | 2.78 ± 0.16 | 1.64 ± 0.28 |
| Succinate    | 0.84 ± 0.14 | 1.44 ± 0.36 | 2.88 ± 0.04 | 3.78 ± 0.06 | 4.14 ± 0.12 | 4.56 ± 0.18 | 3.36 ± 0.06 | 2.54 ± 0.16 |
| Lactose      | 0.42 ± 0.06 | 0.68 ± 0.16 | 1.12 ± 0.26 | 1.88 ± 0.34 | 2.62 ± 0.22 | 3.58 ± 0.28 | 2.24 ± 0.44 | 1.78 ± 0.38 |
| Sorbitol     | —        | 0.22 ± 0.06 | 0.44 ± 0.16 | 0.92 ± 0.24 | 1.48 ± 0.32 | 1.88 ± 0.28 | 1.24 ± 0.34 | 0.72 ± 0.28 |

—No hydrogen.
Table 2: Effect of nitrogen source on hydrogen production (mL/15 mL vessel) by immobilized cells of Rps. palustris in anaerobic dark.

| Nitrogen source       | 24   | 48   | 72   | 96   | 120  | 144  | 166  | 192  |
|-----------------------|------|------|------|------|------|------|------|------|
| Potassium nitrate     |      |      | 0.26 | 0.48 | 0.92 | 1.48 | 0.76 | 0.52 |
| Sodium nitrate        |      |      | 0.10 | 1.10 | 1.66 | 2.10 | 1.46 | 0.78 |
| Ammonium chloride     |      |      |      |      |      |      |      |      |
| Urea                  |      |      | 0.38 | 0.66 | 1.14 | 0.78 | 0.54 | 0.24 |
| Thiourea              |      |      | 0.26 | 0.38 | 0.78 | 0.82 | 0.66 | 0.34 |
| Glycine               |      |      | 0.16 | 0.82 | 1.88 | 1.46 | 1.18 | 0.94 |
| L-asparagine          |      |      | 0.36 | 0.98 | 1.78 | 2.26 | 1.64 | 1.12 |
| L-aspartic acid       |      |      | 0.40 | 0.76 | 1.48 | 2.40 | 1.24 | 0.84 |
| L-glutamic acid       |      |      | 0.34 | 0.66 | 1.74 | 2.62 | 1.44 | 0.68 |
| L-glutamine           |      |      | 0.28 | 0.46 | 0.72 | 1.38 | 1.36 | 0.64 |
| L-tyrosine            |      |      | 0.16 | 0.82 | 1.86 | 2.48 | 1.18 | 0.94 |
| L-cystine             |      |      | 0.28 | 0.46 | 0.88 | 1.46 | 1.76 | 1.12 |
| L-methionine          |      |      | 0.40 | 0.68 | 0.88 | 1.52 | 1.1 | 0.74 |
| Nitrogen gas          |      |      |      |      |      |      |      |      |

— No hydrogen.

Table 3: Effect of carbon sources on hydrogen production (mL/15 mL vessel) by immobilized cells of Rps. palustris in anaerobic dark.

| Carbon source  | 24   | 48   | 72   | 96   | 120  | 144  | 166  | 192  |
|---------------|------|------|------|------|------|------|------|------|
| Fructose      |      |      | 0.22 | 0.44 | 0.78 | 1.12 | 0.86 | 0.56 |
| Glucose       |      |      | 0.34 | 0.56 | 0.56 | 0.84 | 0.48 | 0.26 |
| Maltose       |      |      | 0.36 | 0.56 | 0.96 | 1.54 | 1.12 | 0.78 |
| Sucrose       |      |      | 0.28 | 0.68 | 1.18 | 1.62 | 0.78 | 0.38 |
| Mannitol      |      | 0.22 | 0.46 | 0.78 | 0.98 | 1.12 | 0.84 | 0.46 |
| Lactate       |      | 0.36 | 0.66 | 1.10 | 1.78 | 1.38 | 0.96 | 0.12 |
| Malate        |      | 0.38 | 0.66 | 1.28 | 1.76 | 2.12 | 1.54 | 0.78 |
| Succinate     |      | 0.38 | 0.56 | 0.98 | 1.48 | 0.78 | 0.44 | 0.12 |
| Lactose       |      | 0.24 | 0.46 | 0.64 | 0.94 | 1.92 | 1.34 | 0.96 |
| Sorbitol      | 0.44 | 0.68 | 0.74 | 0.94 | 1.24 | 0.64 | 0.32 | 0.28 |

— No hydrogen.

Table 4: Effect of nitrogen source on hydrogen production (mL/15 mL vessel) by immobilized cells of Rps. palustris in anaerobic dark.

| Nitrogen source     | 24   | 48   | 72   | 96   | 120  | 144  | 166  | 192  |
|---------------------|------|------|------|------|------|------|------|------|
| Potassium nitrate   |      |      | 0.32 | 0.56 | 1.10 | 0.78 | 0.56 | 0.38 |
| Sodium nitrate      |      |      | 0.28 | 0.44 | 0.78 | 0.36 | 0.24 | —   |
| Ammonium chloride   |      |      |      |      |      |      |      |      |
| Urea                |      |      | 0.20 | 0.42 | 0.62 | 0.38 | —   | —   |
| Thiourea            |      |      |      |      |      |      |      |      |
| Glycine             |      |      | 0.28 | 0.52 | 0.98 | 0.64 | 0.38 | 0.24 |
| L-asparagine        |      | 0.34 | 0.86 | 1.24 | 0.78 | 0.42 | 0.24 | 0.16 |
| L-aspartic acid     | 0.48 | 0.94 | 1.44 | 0.76 | 0.44 | 0.16 | —   | —   |
| L-glutamic acid     |      | 0.26 | 0.48 | 1.94 | 1.24 | 0.94 | 0.56 | 0.18 |
| L-glutamine         |      | 0.36 | 0.78 | 1.34 | 0.62 | 0.34 | —   | —   |
| L-tyrosine          |      | 0.28 | 0.86 | 0.54 | 0.54 | 0.22 | —   | —   |
| L-cystine           |      | 0.22 | 0.48 | 0.78 | 0.52 | 0.26 | —   | —   |
| L-methionine        | 0.20 | 0.76 | 1.18 | 0.84 | 0.58 | 0.26 | —   | —   |
| Nitrogen gas        |      |      |      |      |      |      |      |      |

— No hydrogen.
electron donation capabilities of the cofactor compounds to nitrogenase [15]. Hence, differences in hydrogen production rates with different carbon sources was observed. In our study, organic nitrogen sources produced more amounts of hydrogen compared to inorganic nitrogen sources. Inorganic nitrogen compounds like nitrite, nitrate, and ammonia have been reported to inhibit nitrogenase enzyme thus influencing hydrogen production. Organic nitrogen sources are directly incorporated into proteins or transformed into other cellular nitrogenous constituents [16]. Enhancement using immobilized cells of phototrophic bacteria was also reported by Singh et al. (1994) [17], Zhu et al. (1999) [10], Yokoi et al. (1997) [18], Lozinsky et al. (2003) [19], and many other workers. Immobilized, sulfur-deprived algal cultures of Chlamydomonas reinhardtii could photoproduce hydrogen for longer periods of time [20, 21]. Nitrogen limitation can be used to increase the hydrogen productivity in purple nonsulfur bacteria [22]. Present studies on this organism are in agreement with that of the earlier studies where there was an increment in the hydrogen production of the organism. Investigations are on to find out more suitable and cheaper carbon and nitrogen sources for hydrogen production. Further, the organism is also being investigated using various immobilization matrices which could be useful for continuous production of hydrogen for longer periods of time.

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