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# Impact of cultural conditions on photoproduction of hydrogen by *Allochromatium* sp. GSKRLMBKU-01 isolated from marine water of Visakhapatnam

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ABSTRACT

In the present study, photoproduction of hydrogen by *Allochromatium* sp. strain GSKRLMBKU-01 isolated from marine water was measured under different cultural conditions. Hydrogen production was measured by using a Gas chromatography using argon gas as a carrier. Among different carbon and nitrogen sources used, succinate induced maximum hydrogen (5.68 ± 0.27 mL) production by immobilized cells, while free cells recorded 4.24 ± 0.30 mL of hydrogen under anaerobic light conditions. Immobilization of cells resulted in increased production of hydrogen. Ammonium chloride promoted more amounts of hydrogen production (2.68 ± 0.29 mL) by free cells, whereas glycine enhanced the hydrogen production upto 4.82 ± 0.36 mL in immobilized cells. In formic acid and urea, less hydrogen production was observed in both free and immobilized cells compared to other sources. Cumulative hydrogen production by the bacterium was recorded with the progress in incubation period. Incubation period of 192 h, pH of 7.0 and temperature at 30 °C were found to be optimum for the maximum hydrogen production. Significance of the above results was discussed in light of existing literature.



## Introduction

Marine habitats are ideal niches for the growth of anoxygenic phototrophic bacteria. Many anoxygenic phototrophic bacteria have been isolated from estuarine salt pans, salt marshes, coastal lagoons with elevated salt concentrations, tidal waters, brackish waters and marine coastal sediments [1–5]. Hydrogen is considered as a potential fuel as it is renewable, ecofriendly, cheaper, transportable and has high energy content 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 eco friendly by product [6]. In view of constant depletion of fossil fuels which are responsible for increase of $CO_2$ concentration in the atmosphere which leads to environmental decomposition. Hydrogen is considered to be safety fuel which is sustainable and economical [7]. Among biohydrogen production methods, photo-fermentative method could used for hydrogen production using organic waste water or biomass with solar energy proved to be more sustainable [8–10].

Hydrogen producing phototrophic bacteria were considered to be alternative energy source to non-conventional energy sources like solar, ocean, wind, waves, thermal, geothermal and thermonuclear energy. A wide variety of

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organic substrates such as carbohydrates [11], lactate [7], malate, benzoate [12], glucose [13] proved to be ideal for hydrogen production by variety of phototrophic bacteria. Ethanolamine as nitrogen source with sugars D-glucose, D-xylose and D-cellobiose enhanced the hydrogen production by *Rhodobacter capsulatus* and *Rhodospirillum rubrum* [14,15]. L-cysteine was reported to promote higher amounts of hydrogen production by the *Rhodobacter capsulatus* [7,14]. More substrate specificity was exhibited for hydrogen production by different phototrophic bacteria varied with the species [16]. Previous studies have demonstrated that photosynthetic bacteria produced hydrogen under inert gas as gas phase [17,18]. Hydrogen production under argon atmosphere in the presence of light will depend on nitrogenase [19].

Immobilization of phototrophic bacteria not only enhances hydrogen production but also used for the production of useful products and also protects the cells from inhibitory effect of oxygen, nitrogen, osmotic stress and pH [20–22]. *Rhodopseudomonas* sp., *Rsp. rubrum* [23], *Rhodobacter sphaeroides* [24], *Rba. capsulatus* KU002 [25,26]. *Rps. palustris* KU003 [27] and *Rps. acidophila* KU001 [28], *Rps. rutila* [29] and *Rhodocyclus tenuis* KU 017 [30] produced increasing amounts of hydrogen by immobilized cells of phototrophic bacteria. In view of the above facts, influence of different cultural conditions such as incubation period, pH, and various carbon and nitrogen sources on hydrogen production by *Allochromatium* sp., a purple sulfur bacteria using argon gas as carrier was studied and discussed in this paper.

## Materials and methods

### *Isolation and identification of Allochromatium sp.*

*Allochromatium* sp. strain GSKRLMBKU-01 used in this study was isolated from the marine samples collected at coastal region of Ramakrishna Beach, Vishakhapatnam, India by enrichment techniques [31]. Nitrogen gas was flushed into the test tubes to maintain the anaerobic condition. Bacteria thus isolated were identified morphologically with the help of Bergey's manual of systematic bacteriology (1994) [32]. Growth was determined by measuring optical density at 660 nm using UV-Vis spectrophotometer. The morphologically identified bacterium was further confirmed by precise molecular identification by 16S rRNA sequencing analysis. Sequence thus obtained was submitted in National Centre for Biotechnological Information (Gen Bank Accession number HF677171.1).

### *Media*

Basal medium used for the isolation of purple sulfur bacteria was (g/L) $KH_2PO_4$ 1.0, $MgCl_2 \cdot 5H_2O$ 0.5, $NH_4Cl$ 1.0, Organic carbon ($CH_3COONa$) 1.0, NaCl 25.0, Yeast extract 0.1, $H_2S$ 0.25, Ascorbic acid 0.5, Sodium bicarbonate 3.0, Trace element solution 1 mL (modified SL4 of Pfennig and Lippert) [33] (mg/L): $ZnCl_2$ 70.0, $MnCl_2 \cdot 4H_2O$ 100, $CoCl_2 \cdot 6H_2O$ 190, $H_3BO_3$ 300, $NiCl_2 \cdot 6H_2O$ 24.0, $CuCl_2 \cdot 2H_2O$ 0.2, $NaMO_4.2H_2O$ 10, $Na_2$-EDTA 3.0, Vitamin solution 1 mL (mg/L) (Biotin 10.0, Niacinamide 35.0, Thiamine dichloride 30.0, PABA 20.0, Pyridoxal HCl 10.0, Calcium pantothenate 10.0 and vitamin $B_{12}$ 5.0), Distilled water 1.0 L, pH 8.0, sterilization at 121 °C for 15 min. The pH of the medium was adjusted to 8.0 by using 1 M HCl or NaOH solution. Basal medium supplemented with different carbon and nitrogen sources in equimolar concentration was used to study influence of carbon and nitrogen sources on hydrogen production.

### *Immobilization*

Fresh log phase cultures of *Allochromatium* sp. were harvested by centrifugation at 10, 000 × g for 10 min and washed thrice with 0.3% saline and suspended in basal medium and immobilized by alginate entrapments as suggested by Johnsen and Flink [34]. Sodium alginate solution (3%) was prepared by dissolving 3.0 g of sodium alginate in 100 mL boiling water and autoclaved at 121 °C for 15 min. On cooling, both alginate and cell suspension were mixed in the ratio of 1:1 (v/v) and stirred manually for 10 min to get a uniform mixture. Then the suspension was taken in a sterile syringe and added drop wise into ice-cold 0.2 M $CaCl_2$ solution from 5 cm height and kept for curing at 4 °C for 4 h. The cured beads were washed with sterile distilled water for 3 to 4 times. The beads were preserved in 0.9% sodium chloride solution and stored in a refrigerator to be used for experimentation at later stage.

### *Hydrogen production analysis*

The amount of hydrogen produced by the bacterium was determined as suggested by Vincenzini et al. [35] and Ramchander et al. [7].

For measuring hydrogen production, the washed cell suspension of free cells or immobilized cells were inoculated into 8 mL medium in 15 mL capacity rim less test tubes and sealed with subaseals to create anaerobic conditions by evacuating and flushing with 100% nitrogen gas. All the tubes were incubated under visible light (2000 lux) at 30 ± 2 °C for hydrogen production studies. The hydrogen produced 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 air tight syringe into a gas chromatography (Mak Analytica make) fitted with a molecular sieve 5A column (2 m × 1/8″ ODSS column, 60–80 mesh) to a thermal conductivity detector (TCD). Gas analysis was done at 60 °C with argon as carrier gas (flowrate 30 mL/min), 120 mA detector current. Integrator and recorder were used at highest sensitivity. The amount of hydrogen liberated by the *Allochromatium* sp. was calculated from the peak height of the recorder with reference to calibration curve prepared using ultra pure hydrogen.

### *Statistical analysis*

The results were statistically analyzed to mean and standard deviations by using GraphPad Prism.InStat Version 6 (GraphPad Software, Inc.,) to test the significance of treatment at $P \leq 0.05$.

## Results and discussion

### *Effect of incubation period*

Fig. 1 reveals that the hydrogen production recorded an increase upto 192 h of incubation period by both free and immobilized

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