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Enhanced hydrogen production by immobilized cyanobacterium *Lyngbya perelegans* under varying anaerobic conditions

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ABSTRACT

Hydrogen (H_2) production was studied using a non-heterocystous, filamentous cyanobacterium, *Lyngbya perelegans* in free and immobilized forms, where cells were incubated under O_2 free anaerobic environment with alternate light and dark period (21 h light/3 h dark). For immobilization, agar and alginate systems were taken and it was revealed that hydrogen production was more sustained in alginate trapped cells as compared to agar trapped cells and free cells. Cell density and size of bound cell system were also important parameters influencing H_2 production in immobilized systems. The type of gas mixtures sparged for creating anoxic environment in the headspace had a significant influence on H_2 production showing methane (CH_4) to be most conducive component of the gaseous mixture.

1. Introduction

Photobiological hydrogen production using readily available substrates like water and carbondioxide achieved by different renewable technologies can provide a means of reducing our dependence on fossil fuels for energy [1]. Renewable and non-polluting features of photoproduction of H_2 makes it ideal, clean and sustainable energy source in contrast to chemical process [2]. Due to extreme sensitivity of the hydrogenase enzyme to molecular oxygen, which is produced by the cyanobacteria by photosystem II in the presence of light, production of H_2 is usually for a very short period in oxygenic environment [3]. It is therefore, necessary to design an operating system that would ensure stable and long term hydrogen production. For dealing with the problem of oxygen inactivation of H_2 evolving system and obligatory production of oxygen during photosynthesis, partially inactivated O_2 evolution system of algae was done by S-deprivation of the medium [4]. Another approach for increased H_2 production involves cell immobilization in some suitable matrix and creation of anoxic environment, especially in case of filamentous cyanobacteria, in which breakage of filaments and structural degeneration; normally occur due to agitation, resulting in decreased in hydrogen production. Cell immobilization provides protection to enzymes mediating metabolic

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activities against environmental disturbances, thus enabling the cells to be used for longer duration [5,6]. Besides this, immobilization effectively separates the cells from the liquid phase, significantly increases the cell density and as a consequence, allows for more efficient light utilization on a per unit area basis [7]. Effect of immobilization on facilitated hydrogen production has been reported mostly in unicellular microalgae [8,9].

The present study was aimed at evaluating H_2 photoproduction by a hitherto unexplored filamentous cyanobacterium *Lyngbya perelegans* in immobilized form using agar and alginate as the carriers for immobilization as there are very few reports on such aspects in filamentous cyanobacteria. These immobilization matrices with several advantages like natural origin, non-toxic nature and low cost are suitable for industrial use [10]. Also, the polymerization process does not shift the pH inside the matrix and the pH of the alginate solution used is near neutral [7], which is optimal for cyanobacterial growth. Further, the polymerization process takes place at room temperature involving non-toxic calcium ions.

The study further analyzes important variables of the bound cell system like size of the immobilized bead/cube and concentration of cyanobacterial inoculum on H_2 production. Photoproduction of H_2 by the cyanobacterial system in suspension and bound form was also studied under different anoxic environments created by sparging with gaseous mixtures of different types.

2. Materials and methods

2.1. Organism and growth conditions

L. perelegans, a non-heterocystous, filamentous cyanobacterium was isolated from sub-tropical fresh water environment of Faridabad district, Haryana, India, where it occurs as a predominant species. Pure culture of this strain was obtained by streaking on basal agar medium (BG-11) at pH 8.5 (buffered with 0.1 M tris amminomethane) using standard isolation and culturing techniques [11]. The cyanobacterium was grown in BG-11 medium for 7 days under fluorescent light (56.85μmol m^2 sec^{-1}) with 12/12 h light and dark photoperiod at 28 ± 3 °C temperature. Nitrogen supplement was given in the form of $NaNO_3$ (1.5 g/L) in the medium for growth. Initially nitrogen free medium was used for growth as some species of *Lyngbya* are reported to possess nitrogenase. However, the cells failed to grow after 2–3 days suggesting lack of nitrogenase in this species, which was later confirmed by performing acetylene reduction assay using gas chromatograph (Hardy et al., 1973).

2.2. Immobilization

Cells from 7 d old culture were harvested by centrifugation at 3000 rpm for 2 min and washed with distilled water prior to immobilization. Two types of gels, agar and alginate, were used for binding the cyanobacterial cells. For agar cubes, 4% agar was dissolved in 10 ml of distilled water at 100 °C and cooled to 50 °C. 10 ml of cyanobacterial suspension was then added to the flask, mixed and cooled to 30 °C on a cold glass plate. The gels of immobilized cyanobacterium were cut into small cubes of 1 mm^3. For alginate beads, cyanobacterial suspension and 4% sodium alginate (1:1) were mixed and the suspension was dropped into 2% $CaCl_2$ solution through a small syringe making small beads of 1 mm diameter.

For studying the effect of size of the immobilization matrix agar cubes of different sizes viz. $(0.5)^3$, $(1.0)^3$ and $(2.0)^3$ mm and alginate beads of 0.5, 1.0 and 2.0 mm diameter were used for hydrogen production experiments.

2.3. Measurement of hydrogen evolution

Hydrogen production at different time intervals (according to the experimental design) was estimated by withdrawing 1 ml aliquot of headspace gas phase with a gastight syringe and injecting it in a calibrated gas chromatograph (Agilent 6890N) equipped with a 13X molecular sieve column, thermal conductivity detector (TCD) and nitrogen as carrier gas. Temperature of the column was 75 °C while the injector and detector temperatures were maintained at 75 °C and 150 °C, respectively.

All the H_2 production assay experiments were performed in sealed vials (15 ml) in triplicates. For experiments with free cells, 2 ml (containing 0.03 g dry wt.) of 7 d old cyanobacterial culture in mid log phase was suspended in 1 ml of BG-11 medium and for experiments with bound cell system, 3.5 g of immobilized alginate beads/agar cubes (containing same amount of cyanobacterial cells by dry weight as in free cells) were added to the vials containing the medium.

Experiments were also performed by varying cyanobacterial dose (0.5, 1.0, 2.0 g) for determining the effect of cyanobacterial density per bead/cube on H_2 production. Anoxic conditions were maintained in H_2 assay by replacing the air in the vials with argon, an inert gas. For determining the effect of different anoxic environments on hydrogen production by *L. perelegans*, gas mixtures in different ratios (v/v) viz. only Ar (13), Ar:CO_2 (12:1), CO_2:N_2 (6:7), N_2:Ar (4:9), CO_2:Ar (2:11) and CH_4:Ar (11:2) were used.

Sealed vials containing immobilized or suspension culture were kept in orbital shaker (Orbitek LT) with continuous stirring at 100 rpm at 25 °C with alternate light and dark conditions (21 h light of 56.85 μmol m^2 sec^{-1}/3 h dark). These conditions were previously optimized in free culture of *L. perelegans* by changing the system variables [12].

3. Results and discussion

Table 1 shows the effect of size of both immobilized systems (agar and alginate) on the rate of H_2 production. Results show that 1.0 mm^3 agar cubes and or 1.0 mm diameter alginate beads are optimum for maximum H_2 production as compared to other two sizes of immobilized systems. Hence, 1.0 mm^3 agar cubes and or 1 mm diameter alginate beads were used further in all other experiments.

Hydrogen production by free and immobilized (agar and alginate) cyanobacterium showed marked variations (Fig. 1).