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Biohydrogen production by *Rhodobacter capsulatus* on acetate at fluctuating temperatures

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ABSTRACT

Hydrogen is a clean energy alternative to fossil fuels. Photosynthetic bacteria produce hydrogen from organic compounds under anaerobic, nitrogen-limiting conditions through a light-dependent electron transfer process. In this study, the hydrogen production efficiency of phototrophic bacteria, *Rhodobacter capsulatus* and its Hup mutant strain (an uptake hydrogenase deleted strain) were tested on different initial acetate concentrations at fluctuating temperatures with indoor and outdoor photobioreactors. Acetate was effectively metabolized and H_2 was produced at a high rate. Increasing the initial acetate concentration resulted in a shift in the utilization kinetics of acetate from first order to second order. The effects of fluctuating temperature and day/night cycles on hydrogen production were also studied in indoor and outdoor conditions using acetate as the carbon source. Temperature fluctuations and day/night cycles significantly decreased hydrogen production. It was found that the Hup mutant strain of *R. capsulatus* has better hydrogen productivity than the wild type parent in outdoor conditions.

1. Introduction

Hydrogen is a clean fuel as it produces only water upon combustion and is considered a main energy carrier of the foreseeable future. Currently most of the hydrogen is produced through electrolysis and from fossil fuels. However, for sustainable energy production, renewable resources must be used. The biological production of hydrogen offers an opportunity to utilize renewable resources such as biomass, water and sunlight.

Biological production of hydrogen can be achieved through biophotolysis, dark fermentation and photofermentation. Biophotolysis, in which solar energy is used to convert water to oxygen and hydrogen, is an inherently attractive process; however, it suffers from the insurmountable barriers of oxygen sensitivity, intrinsic limitations in light conversion efficiencies, problems with gas capture and separation, and very onerous economics (Hallenbeck and Benemann, 2002; Melis, 2002). On the other hand, the major bottleneck of the dark fermentation process is the low yield of hydrogen per mol of substrate due to the incomplete degradation of sugars to hydrogen and carbon dioxide.

In order to enhance hydrogen production, dark fermentation can be combined with photofermentation, a process by which organic acids resulting from the dark fermentation process can be completely decomposed to hydrogen and carbon dioxide by photosynthetic bacteria (Das and Veziroğlu, 2001). In such a two-step fermentation process, the concentration of acetate in the effluent of dark fermentation may vary significantly, affecting the growth and hydrogen production of photosynthetic bacteria in photofermentation. Hence, the concentration of acetate should be optimized for highly efficient hydrogen production. A combination of dark and photofermentation processes has been addressed in an EU 6th Framework project, HYVOLUTION, coordinated by Wageningen University, The Netherlands (Claassen and de Vrije, 2006; Wukovits et al., 2007). The goal of the project is to develop an integrated process through which biomass is utilized for biohydrogen production in two steps. In the first step biomass is fermented to acetate, carbon dioxide, and hydrogen by thermophilic dark fermentation, while in the second step, acetate is converted to hydrogen and carbon dioxide, thereby reaching the theoretical maximum hydrogen production of 12 mol/mol glucose.

Another way of improving hydrogen production is to utilize mutant bacterial strains. Purple non-sulfur (PNS) bacteria, which are capable of producing hydrogen under illumination by the action of the nitrogenase enzyme, are good candidates for photofermentative hydrogen production. However, they can also utilize molecular hydrogen as an electron donor by the membrane-bound H_2 uptake (Hup) hydrogenase enzyme, resulting in a decrease in the rate and

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Nomenclature

A	irradiated area (m^2)
C_{Ac}	calculated acetate concentration (mM)
C_{Ac_0}	initial acetate concentration (mM)
Hup	membrane-bound hydrogen uptake hydrogenase enzyme
I	light intensity (W/m^2)
PHB	polyhydroxy butyrate
PNS	purple non-sulfur (bacteria)
t	time (h)
TCA	tricarboxylic acid cycle
t_{H_2}	duration of hydrogen production (h)
V_{H_2}	volume of produced H_2 (l)
ρ_{H_2}	density of the produced hydrogen gas (g/l)
η	light conversion efficiency (%)

yield of H_2 production (Zorin et al., 1996). Hence, strains devoid of uptake hydrogenase activity (Hup-) were shown to have higher hydrogen productions (Franchi et al., 2004; Kim et al., 2006; Kars et al., 2008; Öztürk et al., 2006).

During photofermentation, using sunlight instead of artificial illumination further improves the process by decreasing the cost. The biological production of hydrogen at a competitive cost in industrial scales can only be possible in photobioreactors which are illuminated by sunlight. Therefore, outdoor application of the photobiological hydrogen process is a critical step. Photobacterial growth and the hydrogen productivity of photosynthetic bacteria in an outdoor reactor system is strongly affected by fluctuations in temperature and light intensity due to the day/night cycle and due to seasonal, geographic, and climatic conditions (Eroglu et al., 2008). Most photoheterotrophic bacteria produce hydrogen at 30–35 °C (Sasikala et al., 1993). However, depending on the climatic conditions, the temperature of a photobioreactor deviates significantly from the optimum temperature for hydrogen production, decreasing the yield. This brings about problems relative to cooling or heating and the potential for significant energy expenditures.

The aim of this study was to highlight the effect of acetate concentration and temperature fluctuations on hydrogen production by photosynthetic bacteria *Rhodobacter capsulatus*. Outdoor application was also implemented to compare the hydrogen production of the Hup^- mutant strain to the wild type strain in photobioreactors illuminated by sunlight.

2. Materials and methods

2.1. Bacterial strains, media and growth conditions

Wild type and mutant (uptake hydrogenase deleted, hup-) strains of *R. capsulatus* were grown photoheterotrophically on a modified medium of Biebl and Pfennig (BP) (1981) containing 7.5 mM of malate and 10 mM of glutamate as carbon and nitrogen sources, respectively. The cultures were incubated at 30 °C, under an illumination of 200 W/m^2. A 10% inoculation was done from the grown bacterial culture into the hydrogen production medium.

2.2. Effect of initial acetate concentration on hydrogen production

The effect of the initial acetate concentration on growth and hydrogen production was studied on a BP minimal medium containing 10–50 mM of acetate as the sole carbon source and 2 mM of glutamate as the nitrogen source. Bacteria were grown anaerobically in 50 ml bottles at 30 °C under an illumination of 200 W/m^2. The anaerobic environment was generated by flushing with pure argon gas. Growth, hydrogen production, pH and substrate consumption were monitored.

2.3. Effect of fluctuating temperature on hydrogen production

The effect of changes in daily temperature on growth and hydrogen production from the wild type strain of *R. capsulatus* was investigated in indoor conditions. In order to simulate the daily temperature changes occurring in outdoor conditions, an indoor experiment was designed in which temperature was altered daily between 15 °C and 40 °C. The bacteria were grown both under continuous light and utilizing a dark/light cycle (16 h light/8 h dark). Bacteria grown at a constant temperature (30 °C) under continuous illumination were used as the control. The growth medium contained 40 mM of acetate and 7.5 mM of lactate as the carbon sources and 2 mM of glutamate as the nitrogen source. Growth, hydrogen production, pH and substrate consumption were monitored.

2.4. Outdoor photobioreactors

A modified medium of BP containing 30 mM of acetate and 7.5 mM of lactate as the carbon sources and 2 mM of sodium glutamate as the nitrogen source was used for the hydrogen production from the wild type and mutant strains of *R. capsulatus* in outdoor conditions. A 10% inoculation by volume of the fresh medium was made into the bioreactors and they were run on batch mode. Bacteria were grown in 550 ml glass bottles under an anaerobic condition which was provided by flushing with pure argon gas. The initial pH of the photobioreactor was 6.6–6.8. The photobioreactors were kept under natural sunlight; hence, temperature and light intensity changed depending on outdoor conditions. The experiments were carried out from August to October, in Ankara, Turkey. The hydrogen gas produced by the bacteria was collected from the top by a thin hollow tube into a graded glass cylinder initially filled with water which was replaced by the hydrogen produced during the process.

2.5. Analytical methods

Light intensity and spectrum measurements were made by a luxmeter (Lutron) and a spectroradiometer (StellarNet EPP2000-VIS-50). The bacterial cell concentration was determined spectrophotometrically at 660 nm and converted to dry weight per liter of culture data. Evolved gas was analyzed by gas chromatography (Agilent Technologies 6890N) using a Supelco carboxen 1010 (150 mm × 4.1 mm, Part no: 79464) column. HPLC analysis for organic acid utilization was carried out by a Hamilton PRP-X300 (150 mm × 4.1 mm, Part no: 79464) column.

2.6. Calculation of light and substrate conversion efficiencies

Substrate conversion efficiency for acetate is determined as the ratio of moles of hydrogen that have actually been produced per moles of hydrogen that would have been produced if all of the substrate had been converted to hydrogen through the stoichiometric equation:

$$CH_3COOH + 2H_2O \rightarrow 4H_2 + 2CO_2 \quad (1)$$

Light conversion efficiency is determined as the ratio of the total energy value of the hydrogen that has been obtained to the total energy input to the photobioreactor by light radiation. It is calculated by

$$\eta = \left[\frac{33.61\rho_{H_2}}{IAt_{H_2}}\right] \times 100 \quad (2)$$