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EFFECT OF GAS PHASE ON THE PHOTOPRODUCTION OF HYDROGEN AND SUBSTRATE CONVERSION EFFICIENCY IN THE PHOTOSYNTHETIC BACTERIUM *RHODOBACTER SPHAEROIDES* O.U. 001*

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Abstract—Photoproduction of hydrogen and substrate conversion efficiency by resting cells of a photosynthetic purple non-sulfur bacterium *Rhodobacter sphaeroides* O.U. 001 varied with the gas phase of the assay. Both exogenous and endogenous substrates could support photoproduction of hydrogen by this organism. Substrate conversion efficiency of more than 100% was observed when Ar, Ar + 10% H_2 and Ar + 10% N_2 + 10% H_2 were gas phases. This excess substrate conversion efficiency is attributed to hydrogen production from endogenous substrates. Dinitrogen (100%) and air in the gas phase totally inhibited hydrogen production. Hydrogen production was maximum in the presence of 10% H_2 in the gas phase. However, 100% H_2 atmosphere inhibited hydrogen production partially.

INTRODUCTION

Hydrogen is considered to be an ideal and pollution-free fuel for the future [1]. Since the discovery of photoproduction of hydrogen by green algae [2], photosynthetic bacteria [3] and cyanobacteria [4], a lot of work was carried out both on the basic and applied aspects of photobiological hydrogen formation which is a potential source of large scale generation of hydrogen.

Photosynthetic bacteria offer many advantages over other biological systems in this respect [5, 6]. Both growing and resting cells of purple non-sulfur bacteria were shown to dissimilate organic compounds into hydrogen and carbon dioxide [7, 8]. A substrate conversion efficiency of 20–100% from organic acids to hydrogen was shown among purple non-sulfur bacteria [9, 10].

Hydrogen photoproduction varies with the gas phase during assay [6, 11]. The difference in hydrogen production observed under various gas phases was attributed to the effect of these gases on hydrogen producing system (nitrogenase/hydrogenase) [11]. However, no attempt was made to study the possible effect of the gas phases on substrate utilization and conversion efficiencies. In this study, the effect of various gas phases on photoproduction of hydrogen (from both exogenous and endogenous substrates) in relation to substrate utilization and conversion efficiencies was investigated.

*Dedicated to the loving memory of Drs (Mrs) B. Renuka Rao and M. Vinayakumar.

MATERIALS AND METHODS

Organism and growth conditions

A purple non-sulfur photosynthetic bacterium *Rhodobacter sphaeroides* O.U. 001, isolated locally, was used in the present investigation. The organism was grown anaerobically in light (2000 lux) at a temperature of 30 ± 2°C, on the medium described by Biebl and Pfennig [12] with malate (30 mM) and sodium glutamate (10 mM) as carbon and nitrogen sources, respectively (pH 7.0).

Preparation of resting cell suspension

Log phase cells were harvested by centrifugation and washed repeatedly with saline (0.3%) till no traces of ammonia were detectable in the supernatant. The pellet was suspended in the basal medium (pH 6.9, devoid of nitrogen source) without and with malate (3 mM).

Hydrogen assay

The cell suspension was distributed (2 ml in each) into 15 ml capacity test tubes, sealed with suba seals, evacuated and replaced with the required gas phase. (Gases used in the experimentation were of ultrapure grade.)

Hydrogen in the gas phase was detected gas chromatographically by injecting 0.5 ml of the gas extracted from the reaction vessel into a CIC gas chromatograph equipped with molecular sieve 5A column with nitrogen as a carrier gas. The amount of hydrogen produced is calculated from a standard graph prepared using ultrapure hydrogen.

Cell mass determination

The cell mass was determined by measuring the absorption of a bacterial suspension at 660 nm. The bacterial dry wt was calculated with the following empirical relation determined for this organism, absorption at 660 nm of $0.1 \equiv 0.37$ mg dry wt ml^{-1}.

Calculation of substrate conversion efficiency

The conversion yields (%) were determined by comparing the hydrogen obtained to the theoretical maximal conversion as 100%

$$(C_4H_6O_5 + 3H_2O \rightarrow 4CO_2 + 6H_2)$$

using the following formula:

$$\% \text{ Substrate conversion efficiency} = 100 \times O/T$$

where,

O = Observed hydrogen production from substrate (mol);

T = Theoretical maximal hydrogen production (mol) $= s \times n$;

s = substrate consumed (mol);

n = number of moles of hydrogen to be produced theoretically per mole of substrate used.

Estimation of malate

Malate was estimated in the culture supernatant after hydrogen assay by the chromotropic acid method [5].

RESULTS

Photoproduction of hydrogen was observed both in the presence and absence of malate under all gas phases tested except air and 100% N_2. Hydrogen uptake was observed in the presence of N_2 + 10% H_2 as gas phase. Dinitrogen has partially or totally inhibited hydrogen production. An atmosphere of 100% H_2 inhibited hydrogen production partially in the presence of malate, while in its absence uptake was observed. Hydrogen production was maximum under gas phases containing 10% H_2 (Ar + 10% H_2; Ar + 10% H_2 + 10% N_2).

Substrate utilization also varied with the gas phase (Table 1). Substrate utilization rates were in general higher in the presence of dinitrogen in the gas phase. Maximum utilization was observed when N_2 (100%) was the gas phase followed by Ar + 10% N_2, N_2 + 10% H_2 and air. Substrate utilization was at minimum in Ar, Ar + 10% H_2 + 10% N_2 and Ar + 10% H_2 as gas phases.

When air and N_2 were the gas phases, though substrate utilization was quite high, hydrogen formation was not observed. Substrate conversion efficiency was low with H_2 (100%) as the gas phase. When Ar, Ar + 10% H_2 and Ar + 10% H_2 + 10% N_2 were gas phases, a substrate conversion efficiency of over 100% was observed.

DISCUSSION

The results show that not only hydrogen production but also substrate utilization varies with the gas phase used. The organism could not photoevolve hydrogen when air was the gas phase, which could be due to (a) the inhibition of the enzymes (nitrogenase/hydrogenase) by molecular oxygen [13] (b) inhibition of the hydrogen evolving activity of nitrogenase by dinitrogen [11] in the air and (c) nitrogenase inhibition by ammonia leaching into the medium which was observed under these conditions (unpublished results).

Presence of dinitrogen, a known inhibitor of nitrogenase mediated hydrogen evolution [4] had inhibited hydrogen formation and substrate conversion efficiency also decreased either totally or partially depending upon its concentration. However, the simultaneous presence of hydrogen (10%) in a total nitrogen atmosphere had resulted in a net uptake of hydrogen both in the presence and absence of malate in the medium. Hydrogen is a good electron donor for nitrogenase activity [14, 15] and the hydrogen must have been utilized for the reduction of nitrogen under this atmosphere.

Hydrogen (100%) had inhibited hydrogen production in this strain. The results are similar to those observed in *Clostridium cellobioparum* where growth and hydrogen production were shown to be inhibited by hydrogen [16]. However, hydrogen production in *R. rubrum* was not affected even in the presence of 99% hydrogen [8].

Table 1. Photoproduction of hydrogen by *Rhodobacter sphaeroides* O.U. 001 under various gas phases

Gas phase	% Malate consumed	H_2 evolved (n moles (mg dry wt)$^{-1}$ h^{-1}) Presence of malate (A)	Absence of malate (B)	From malate (C = A − B)	% Conversion efficiency From A	Actual conversion efficiency From C
Air	92.0	0.0	0.0	0.0	0.0	0.0
Ar	86.0	165.0	18.0	147.0	122.2	100.0
N_2	97.6	0.0	0.0	0.0	0.0	0.0
H_2	87.5	53.0	−23.4	53.0	43.0	43.0
Ar + 10% N_2	92.2	128.0	9.8	118.2	90.3	83.4
Ar + 10% H_2	85.5	204.0	56.0	138.0	138.0	100.0
Ar + 10% N_2 + 10% H_2	86.5	196.0	52.0	144.0	136.0	100.0
N_2 + 10% H_2	93.7	−2.0	−18.0	0.0	0.0	0.0

The results expressed are after 72 h of incubation. For experimental details see text. Temperature, pH and light intensity of the assay were 30 ± 2°C, 6.9 and 2000 lux, respectively.

The enhanced hydrogen production in the presence of 10% H_2 could be explained as due to enhancement in nitrogenase activity as observed in *Anabaena cylindrica* and *Nostoc muscorum* [17].

Substrate conversion efficiency varied from 0 to 100% depending on the gas phase of the assay. The presence of N_2 in the gas phase resulted in decreased conversion efficiency when compared to that in the presence of Ar as the gas phase. Substrate conversion into hydrogen of more than 100% was obtained when assayed under Ar, Ar + 10% H_2 and Ar + 10% H_2 + 10% N_2 atmospheres. This excess hydrogen could be explained by assuming simultaneous involvement of endogenous substrates in hydrogen production. Endogenoous substrates are known to act as electron donors for hydrogen formation [8, 18]. When hydrogen production due to endogenous substrates (that obtained in the absence of malate) was deducted from the hydrogen production observed in the presence of malate, a conversion of 100% was obtained in all the three cases (Table 1). So it is concluded that in our strain, under the assay conditions used, the endogenous substrate played a role in hydrogen formation even in the presence of exogenous substrates. It should be noted here that the substrate concentration used is only 3 mM. At higher concentrations, which are normally employed by other workers [7, 8, 10] the involvement of endogenous substrates may or may not be there.

Segers and Verstraete [9] reported a conversion efficiency of 100% from acetate, however, these authors did not investigate the simultaneous involvement of endogenous substrates in hydrogen production. With our strain and under the experimental conditions employed, we found it essential to take into consideration the hydrogen production from endogenous substrates too, for calculation of substrate conversion efficiency.

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