Acetylene Reduction by *Beijerinckia* under Various Partial Pressures of Oxygen and Acetylene

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SUMMARY

Acetylene reduction by *Beijerinckia indica* in shaken liquid cultures increased with increase of $p_{C_2H_2}$ up to 0.74 atm. Acetylene reduction was linear for at least 40 min. The oxygen partial pressure also affected activity with most acetylene reduction at a p_{O_2} of 0.15 atm for liquid cultures grown in air.

INTRODUCTION

The reduction of acetylene to ethylene (Dilworth, 1966; Schöllhorn & Burris, 1966) is now widely used as an index of biological nitrogen fixation (e.g. Stewart, Fitzgerald & Burris, 1967; Hardy, Holsten, Jackson & Burns, 1968; Spiff & Odu, 1972). Drozd & Postgate (1970) have reported that the oxygen sensitivity of the Azotobacteriaceae can lead to a false assessment of nitrogenase activity by the acetylene reduction test. We report here that different partial pressures of acetylene and oxygen greatly affect the level of acetylene reduction by *Beijerinckia*, a nitrogen-fixing bacterium found in some acidic tropical soils.

METHODS

Beijerinckia was obtained from the American Type Collection as *Azotobacter indicum* 9037. Cultures for subsequent inoculation were grown in 500 ml conical flasks, containing 100 ml of medium, at 30 °C on a reciprocating shaker (150 strokes/min). The medium contained: KH_2PO_4 , 1.0; sucrose, 20.0; $MgSO_4 \cdot 7H_2O$, 0.5; Na_2MoO_4 , 0.02; ferric citrate, 0.09; in g/l distilled H_2O , pH 5.5. The sugar and phosphate were autoclaved separately and added just before inoculating the flasks.

Nitrogenase activity was measured by the acetylene reduction technique: 5 ml serum bottles equipped with gas-tight rubber stoppers were evacuated, flushed (using a vacuum line) several times with high purity argon and refilled with the desired partial pressure of oxygen and acetylene and then with argon to 1 atm, the calculated volume of the gases being injected directly into the bottles with a plastic disposable syringe. Three replicate vials were used for each treatment as well as a blank without added culture, to determine the level of ethylene contamination in the added acetylene. A , O_2 and C_2H_2 were obtained as high purity cylinder gases (Matheson Co., U.S.A.) and were used without further purification. A 1 ml sample of *Beijerinckia* culture (or of water for blanks) was usually injected to initiate the reaction. The reaction was usually terminated by injecting 5 N- H_2SO_4 into the reaction mixture.

The bottles were incubated in a 30 °C water bath with reciprocal shaking (152 strokes/min). The ethylene produced was measured with a Varian aerograph model 600D gas

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Fig. 1. Ethylene production by *Beijerinckia* incubated at various partial pressures of acetylene for 30 min. The gas phase contained 20 % O₂, C₂H₂ as indicated and A to 1 atm.

Table 1. Effect of pO_2 on C₂H₄ production by *Beijerinckia* in liquid culture

Vials contained pC ₂ H ₄ 0.2 atm., balance A.								
pO_2 (atm.)								
0	0.05	0.1	0.15	0.20	0.25	0.30	0.40	0.50
nmol C ₂ H ₄ /g dry wt/30 min								
390	480	565	646	437	332	241	232	63

chromatograph equipped with a hydrogen flame ionization detector and a 3 mm × 2.5 m aluminium column packed with Porapak R; the gas sample, usually 1 ml, being withdrawn from the bottles and injected directly into the gas chromatograph.

RESULTS

The relationship between the partial pressure of acetylene and the amount of ethylene produced for *Beijerinckia* was irregular (Fig. 1). Ethylene production increased with increasing acetylene levels until pC_2H_2 of 0.74 atm. Nitrogen fixation by cell-free extracts of *Azotobacter vinelandii* increased as the pN_2 increased to 0.5 atm (Strandberg & Wilson, 1967).

Ethylene production was measurable after 5 min and increased linearly for at least 40 min. Crude cell-free extracts of *Beijerinckia* incubated with suitable cofactors reduced acetylene, and maximum activity was obtained with a pC_2H_2 of about 0.04 atm. The specific activity was considerably affected by the partial pressure of acetylene in the gas phase.

Beijerinckia cultures were assayed for their capacity to reduce acetylene under various pO_2 levels. The oxygen and acetylene (0.2 atm) were injected directly into an evacuated bottle and argon added to one atmosphere. Table 1 shows that considerable acetylene reduction took place under anaerobic conditions for short periods (30 min). Maximum acetylene reduction occurred at a pO_2 of 0.15 atm and was negligible at oxygen tensions greater than 0.50 atm.

DISCUSSION

The effect of $p\text{C}_2\text{H}_2$ is an important consideration in the acetylene reduction technique. Schöllhorn & Burris (1967), using cell-free preparations from *Azotobacter vinelandii* and *Clostridium pasteurianum*, obtained a K_m of 0.01 atm for acetylene reduction, similar to that obtained by Dilworth (1966). This was, however, low compared with a value of about 0.1 atm N_2 for nitrogen fixation. Schöllhorn & Burris only reported using partial pressures of acetylene up to 0.1 atm. Drozd & Postgate (1970) obtained K_m values of 0.0028 ± 0.0005 atm for *Azotobacter chroococcum*. Hardy *et al.* (1968) obtained a Michaelis constant of 0.003 to 0.008 atm C_2H_2 with an average of 0.006 atm for *Clostridium pasteurianum*, while for *A. vinelandii* incubated at 0.1 atm or less C_2H_2 they obtained 0.003 to 0.006 atm with 0.005 as average but showed 'an as yet unexplained increase in the rate of C_2H_2 reduction at 0.2 and 0.5 atm of C_2H_2 '.

Our present results suggest that the effect of $p\text{C}_2\text{H}_2$ on acetylene reduction by *Beijerinckia* should be examined further. The amount of acetylene in the gas phase used by various workers has varied considerably. Care should be taken to see that sufficient C_2H_2 is present to give maximum reduction rates.

The oxygen sensitivity of the Azotobacteriaceae is well known. Postgate (1969) and Dalton & Postgate (1969) have suggested that the high respiratory rate of *Azotobacter* protects functional nitrogenase from damage by oxygen. The results reported here agree with the view that nitrogen fixation by aerobic organisms may occur under partially or fully anaerobic conditions in natural environments.

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