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Effect of Aeration on the Growth and Nitrogen Fixation By *Azotobacter chroococcum* Beijerinck

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ABSTRACT

The ability of *Azotobacter chroococcum* to fix nitrogen as influenced by aeration was studied under three different conditions namely, continously shaken, continuously stationary and partially shaken cultures. Continuously shaken cultures showed six fold increase in population over the partially shaken one and twice as much over the continuously stationary one. Nitrogen fixed per gram of sugar consumed was 58.4 mg with continuously stationary culture, 84.3 mg with partially shaken culture and 68.4 mg with continuously shaken culture. Highest per cell fixation of nitrogen was obtained with partially shaken culture followed by continuously stationary and continuously shaken cultures.

INTRODUCTION

An important feature of N fixation reaction is its sensitivity to oxygen. The inhibition of nitrogen fixation by *Azotobacter* to higher oxygen tension has been demonstrated by Meyerhoff and Burk (1928). Nitrogen fixation was more efficient at low 0_2 tension. Three times as much N was fixed per unit of glucose consumed at a $p0_2$ of 0.04 atm as at $p0_2$ of 0.2 atm (Parker, 1954). Schmidt-Lorenz and Rippel-Baldes (1957) confirmed that $p0_2$ of 0.04 atm was optimal for rapid growth and efficient fixation of nitrogen. Tschpek and Giambiagi (1955) showed that at optimal $p0_2$ of 0.01 to 0.03 atm the energetic yield (number of cells/g of glucose) was three to four times larger than in air and the oxygen sensitivity increased with lowering of glucose concentration. According to Mulder and Brotonegoro (1974), for every g of glucose consumed *Azotobacter* was able to fix 46.5 mg of nitrogen. Simalar values have been found by Postgate(1971) in 0_2-limited continuous culture. Dalton and Postgate (1969) found that N-limited continuous cultures fixed about twice as much N per g of carbon source utilized at 0.03 atm of 0_2 as at the normal atmospheric value (0.2 atm). This paper reports the effect of partially and fully aerated and stationary cultural conditions on the growth and N fixation by *A. chroococcum*.

MATERIALS AND METHODS

A. chroococcum culture isolated from the paddy field was grown for one week in N-free broth (Mannitol B medium of Newton *et al.*, 1953) and one ml of this was inoculated into 50ml of N-free modified Burk's medium(B_m)

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which contained sucrose (5 g/l) as a carbon source in 250 ml Erlenmeyer flasks. One duplicate set of flasks was kept on the rotary shaker for 12 days, the second set was kept on rotary shaker for 24 hr and then transferred to stationary condition for 11 days and the third set was kept in stationary condition continuously for 12 days. After 12 days, the volume of the inoculated medium in the flask was measured and was made up to 50 ml with sterile distilled water. The population of *A. chroococcum* was estimated by dilution plate count method using B_5 medium. Total nitrogen in 5 ml of the culture was determined by microkjeldhal method and the sugar in the culture filtrate was estimated by Nelson's method (Nelson, 1944). The culture filtrate was obtained by centrifugation at 12,100 G for 10 min. A set of uninoculated flasks served as control.

RESULTS AND DISCUSSION

The results of the experiment on the population build-up and nitrogen fixation are presented in Table. There was three to six fold increase in population in case of continuously stationary and continuously shaken culture as compared to that in partially shaken culture. However, N fixed per gram of sugar consumed was more in the case of partially shaken culture (84.3 mg/g of sugar consumed), while it was 68.4 mg and 58.4 mg/g of sugar consumed in the continuously shaken and continously stationary cultures, respectively. Further, it was observed that N fixed per cell was high in partially shaken culture followed by continuously stationary and continuously shaken cultures.

It is well known that in the case of aerobic nitrogen fixing bacteria like *Azotobacter*, the efficiency of N fixation depends largely on the oxygen supply to growing organism.

It is suggested that such larger amount of nitrogen can be fixed by the *Azotobacter*, if the oxygen supply to the growing culture is reduced (Meyerhoff and Burk, 1928). Dalton and Postgate(1969)obtained fixation of

TABLE. Effect of oxygen tension on the growth and N fixation by *Azotobacter chroococcum*

Treatment	population per ml X 10^3	N fixed per 100 ml in mg	Sugar consumed per ml in mg	N fixed per gram of sugar consumed in mg	N fixed per cell in $\mu\mu$ g $\times 10^{-9}$
Continuously aerated	297	34.2	5.00	68.4	1.15
Initial shaking for 24 hour and stationary thereafter	45	20.0	2.37	84.3	4.40
Continuously stationary	139	29.2	5.00	58.4	2.08

40 mg N/g mannitol consumed at pO_2 of 0.02 atm. Postgate (1974) stated that batch and continuous cultures of *A. chroococcum* were sensitive to excessive aeration while fixing nitrogen. In the present study approximately two-fold increase in N fixed/cell was obtained in the continuously stationary culture over the continuously aerated culture though the population of cells in the latter was almost twice as much as in the former. This might be due to high concentration of dissolved oxygen in the medium in the continuously aerated culture which might have affected the efficiency of N fixation despite two to six fold increase in population as compared to the continuously stationary and partially shaken cultures.

The highest per cell fixation of nitrogen and also high amount of N fixed/g of sugar consumed inspite of restricted growth of the *Azotobacter* observed in case of partially aerated culture could be due to the fact that after initial aeration of 24 hr, the concentrarion of dissolved oxygen in the medium might have attained the optimal level of partial pressure of oxygen for efficient N fixation.

The stationary culture has fixed about twice as much N/cell, though the population of the cells were half as compared to the continuously shaken culture. This could be due to low oxygen tension and slow diffusion of air into the culture especially into the lower layers under stationary conditions.

REFERENCES

DALTON, H. and J. R. POSTGATE, 1969. Effect of oxygen on growth of *Azotobacter chroococcum* in batch and coninuous culture. *J. Gen. Microbiol.* 54 : 463-73.

MEYERHOFF, O. and D. BURK, 1928. Uber die fixation des Luftstickstoffes durch *Azotobacter*. *Z. Phys. Chem.* (A) 139: 117-47.

MULDER, E. G. and S. BROTONEGORO. 1974. Free living heterotrophic nitrogen fixing bacteria. In: *The Biology of Nitrogen Fixation* (Ed.) A. Quispel, North Holland Pub. Co., Amsterdam, p. 37-85

NELSON, N. 1944. A photometric adaptation of the Somogyi's method for the determination of glucose. *J. Biol. Chem.* 153 : 275-80.

NEWTON, J.W., P. W. WILSON and R. H.BURRIS. 1953. Direct demonstration of ammonia as intermediate in nitrogen fixation by *Azotobacter*. *J. Biol. Chem.* 204 : 445-51.

PARKER, C. A. 1954. Effect of oxygen on the fixation of nitrogen by *Azotobacter*. *Nature*. 173: 780-81.

POSTGATE, J. R. 1971. Fixation by free living microbes-Physiology. In: *The Chmistry and Biochemistry of Nitrogen Fixation*. (Ed.) J. R. Postgate, Plenum Press, London, pp. 161-90.

POSTGATE, J. R. 1974. Pre-requisites for biological nitrogen fixation in free living heterophic bacteria. In : *The Biology of Nitrogen Fixation*. (Ed.) A. Quispel, North Holland Pub. Co, Amsterdam, pp. 663-86.

SCHMIDT-LORENZ, W. and A. RIPPEL - BALDES. 1957. Wirkung des Sauerstoff-Partialdrucks auf Wachstum und Stickstoffbindung von *Azotobacter chroococcum* Beij. *Arch. Microbiol.* 28 :45-68.

TSCHAPEK, M. and N. GIAMBIAGI. 1955. Nitrogen fixation of *Azotobacter* in soil, its inhibition by oxygen. *Arch. Microbiol.* 21: 367-90.