Effect of Oxygen on Growth of *Azotobacter chroococcum* in Batch and Continuous Cultures

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**SUMMARY**

Initiation of growth of nitrogen-fixing Azotobacter species was prevented by efficient aeration but proceeded normally with gentle aeration; addition of CO_2 to the air did not relieve inhibition. The ratio of oxygen solution rate to concentration of organisms determined whether growth would be inhibited or not. Populations growing in media containing fixed nitrogen (NH_3) showed no unusual sensitivity to oxygen though inhibition could be induced at a P_O_2 value of 0.6 atm. Nitrogen-limited continuous cultures fixed about twice as much N_2/g. carbon source utilized at 0.03 atm. O_2 than at the atmospheric value (0.2 atm.); even at relatively high cell concentrations growth was inhibited at 0.6 atm. O_2. Carbon- and phosphate-limited continuous cultures showed even more sensitivity to oxygen when fixing nitrogen but none when growing with NH_3; excessive oxygen was lethal to phosphate-limited populations. These observations suggest that two mechanisms exist in the cell to protect the oxygen-sensitive components of nitrogenase from oxygen: augmented respiration to scavenge excess oxygen and a conformational state of nitrogenase that prevents damage by O_2.

**INTRODUCTION**

High oxygen tensions inhibit or delay the growth of many aerobic or facultatively anaerobic bacteria (Moore & Williams, 1911) including Azotobacter species (Meyerhof & Burk, 1928). However, the mechanisms of such oxygen toxicity remain, to a large extent, obscure. Barron (1955) proposed that harmful effects of oxygen on biochemical material were due mainly to non-specific oxidation of enzyme sulphhydryl groups. Chance, Jamieson & Coles (1965) found that hyperbaric oxygen inhibited energy-linked pyridine nucleotide reduction in mitochondria at 11 to 17 atm. and suggested that oxidation of sulphhydryl groups may be important. Certain nutrients may protect in the case of Achromobacter P6, since inhibition at high oxygen tensions depended on the nature of the carbon and energy source and could be reversed by amino acid supplements (Gottlieb, 1966).

Oxygen toxicity in Azotobacter at partial pressures of oxygen above a P_O_2 of 0.6 atm. seems fairly well established (Meyerhof & Burk, 1928; Tschapek & Giambiagi, 1955; Schmidt-Lorenz & Rippel-Baldes, 1957), although Fife (1943) reported that respiratory activity of Azotobacter increased up to a P_O_2 of 0.8 atm. Meyerhof & Burk originally regarded oxygen toxicity as an inhibition of nitrogen fixation, but the phenomenon was later considered to be a general inhibition of growth (Burk, 1930). Parker (1954) argued that, if metabolic hydrogen is involved in the reduction of nitro-

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gen to ammonia, then oxygen should compete for this hydrogen and hence depress fixation. Parker & Scutt (1960), assuming that spot analyses for N were measures of nitrogen assimilation rates, obtained kinetic data from growth experiments which showed oxygen to be a competitive inhibitor of nitrogen fixation. Parker (1954) found that nitrogen fixation was more efficient at low oxygen tensions: three times as much nitrogen was fixed per unit glucose consumed at a $P_{O_2}$ of 0.04 atm. than a $P_{O_2}$ of 0.2 atm. Tschapek & Giambiagi (1955) proposed that the influence of $P_{O_2}$ on nitrogen fixation was intimately linked with respiration but offered no explanation of the inhibition observed. Attempts to shift the inhibitory $P_{O_2}$ to above 0.6 atm., by using different substrates, were unsuccessful and served only to indicate that the mechanism was complex. Schmidt-Lorenz & Rippel-Baldes (1957) found that increased $P_{O_2}$ only affected nitrogen fixation and not assimilation of bound nitrogen; they came to the conclusion that the unfavourable influence of increasing $P_{O_2}$ on the efficiency of nitrogen fixation was due more to an effect on the $E_h$ of the environment than a direct effect on some enzyme system. Khmel, Gabinskaya & Ierusalimsky (1965) observed that yields of Azotobacter vinelandii fermentations were highest at low aeration rates and that maximum nitrogen fixation occurred at an aeration rate of 18.7 mmole O$_2$/l./hr. Dilworth (1962) found that increasing the $P_{O_2}$ above 0.6 atm. with A. vinelandii caused pyruvate accumulation and attributed the $O_2$ inhibition observed to inactivation of the pyruvic oxidase system.

All the results so far have been obtained from studies with batch cultures. Under such conditions the environment is continually changing and reproducibility in the behaviour of populations is sometimes difficult to obtain (Malek, 1966). In this paper we have re-investigated the effect of $P_{O_2}$ on growth and nitrogen fixation in Azotobacter chroococcum by using both batch cultures and continuous cultures.

**METHODS**

**Organisms and media.** Stock cultures of Azotobacter chroococcum NCIB 8003, Azotobacter vinelandii NCIB 8660 and Azotobacter macrocytogenes NCIB 8700, were maintained on slopes of medium 'mannitol B' (Burk's medium as prescribed by Newton, Wilson & Burris (1953) with mannitol in place of sucrose) and stored at room temperature. For continuous cultures a modified Burk's medium ('mannitol B') was used which contained (g./l.): mannitol, 10; K$_2$HPO$_4$, 0.64; KH$_2$PO$_4$, 0.16; NaCl, 0.2; MgSO$_4$.7H$_2$O, 0.2; CaCl$_2$, 0.1; distilled water plus these trace elements (mg./l.): FeSO$_4$.7H$_2$O, 2.5; H$_3$BO$_3$, 2.9; CoSO$_4$.7H$_2$O, 1.2; CuSO$_4$.5H$_2$O, 0.1; MnCl$_2$.4H$_2$O, 0.09; Na$_2$MoO$_4$.2H$_2$O, 2.5; ZnSO$_4$.7H$_2$O, 1.2; nitritolriacetic acid, 100. NH$_4$Cl was 200 mg./l. when added; final pH, 7.4 ± 0.2. Concentrations of mannitol, phosphate and other constituents were altered occasionally as mentioned below.

Twenty-litre batches of medium were autoclaved at 121° for 45 min., after which time a precipitate was visible. This disappeared after several days at room temperature; the medium was not used until all the precipitate had dissolved.

**Viabilities.** Slide culture (Postgate, Crumpton & Hunter, 1961) on mannitol B$_4$ medium solidified with agar was used. Annuli were of stainless steel, 1.2 mm deep; incubation was for 18 to 24 hr at 25° and viabilities were reproducible to ± 3%.

**Apparatus.** Continuous culture apparatus with working volumes of approximately 200 ml. as described by Baker (1968) was used and maintained at 30°. For batch-
culture experiments similar vessels without side arms were used. Aeration was effected by magnetic stirring which gave oxygen solution rates of up to 180 mmole O$_2$/l./hr (see Results). Oxygen solution rates were determined by the sulphite oxidation method (Cooper, Fernstrom & Miller, 1944).

The oxygen concentration in the culture was continuously measured by using the membrane-covered Clark electrode (Protech Ltd., Rickmansworth). The partial pressures of gases in the gas stream were regulated by gas flowmeters (Rotameter Mfg Co., Croydon), corrected for differences in gas density, the rates of flow being taken as proportional to the partial pressures of gas in the final mixture. At $P_{O_2}$ values below atmospheric, the $P_{N_2}$ was kept constant at 0.8 atm. and argon was introduced into the gas stream to give the required $P_{O_2}$. Above atmospheric $P_{O_2}$, no argon was used and $P_{N_2}$ was equal to $(1 - P_{O_2})$. The buffering action of the medium was sufficient to maintain a constant pH value of 6.8 with nitrogen-fixing populations, so automatic pH control was necessary only with ammonia-grown bacteria.

Criteria of nutrient limitation. Chemostat cultures were considered limited by a given nutrient when: (1) reduction in concentration of the nutrient by 50% decreased bacterial concentrations proportionately; (2) a doubling in concentration of all other nutrients in the medium had no effect on bacterial concentration. In the case of N$_2$ limitation, the first criterion was not readily applicable, but a doubling of all medium constituents produced no increase in bacterial concentration. Electrode readings indicated that oxygen was present in excess and so, by elimination, N$_2$ gas was considered to be growth-limiting.

Analytical procedures. To determine organism concentration, duplicate 10 ml. samples of culture were filtered through weighed membrane filters (Oxoid Ltd.), the filters washed with an equal volume of distilled water, dried in an oven and weighed. Frequent determinations were made by nephelometric measurements, but these served only as a general indication of cell mass and were not always proportional to it. In continuous cultures a variation in the opacity ±2% or less over four doubling times was taken as an indication of steady state conditions. Mannitol was estimated by periodate oxidation (Neish, 1950). Cultures were sampled into a few drops of concentrated H$_2$SO$_4$ (to stop metabolism), centrifuged, and 0.2 to 1 ml. of supernatant fluid analysed. Nitrogen was determined by the micro-Kjeldahl technique, followed by colorimetric analysis of ammonia with Nessler's reagent (Meynell & Meynell, 1965). Respiratory activity was measured by conventional $Q_{O_2}$ estimations in Warburg manometers at 30°. The samples were transferred as rapidly as possible from the culture vessel to the Warburg flask and underwent no centrifuging or washing procedure. For cytochrome spectra, samples were centrifuged and the deposit suspended in medium B$_6$ to give a final concentration equivalent to 10 mg./ml. dry wt bacteria. Two ml. of the concentrated sample were transferred to a diffuse reflectance cell and extinction measured over the range 400-600 m$\mu$ in a Unicam S.P. 700 spectrophotometer. Sodium dithionite was added to obtain reduced spectra.

RESULTS

Effect of aeration on growth in batch cultures

Batch cultures of Azotobacter chroococcum did not grow after inoculation when subjected to vigorous aeration, but grew when the aeration was initially only slight (Dalton & Postgate, 1967). Inhibition by high aeration of various aerobic bacteria has
been generally attributed to lack of CO₂, which is necessary for the initiation of growth of many aerobes (Walker, 1932; Gladstone, Fildes & Richardson, 1935). Such an explanation did not apply to the nitrogen-fixing organisms A. chroococcum, A. vine-
lantii and A. macrocytogenes, which could be inhibited by efficient aeration even under an atmosphere containing 0.02 atm. CO₂. Azotobacter chroococcum was chosen for further investigation since its behaviour was also being studied in continuous cultures, which were a source of reproducible inocula.

Table 1. Aeration and growth of Azotobacter chroococcum

<table>
<thead>
<tr>
<th>O₂ solution rate (mmole O₂/l./hr)</th>
<th>N₂-grown</th>
<th>NH₄-grown</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₀₂ (atm.)</td>
<td>42</td>
<td>42(+CO₂)</td>
</tr>
<tr>
<td>0.08</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.2</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>0.4</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>0.6</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Three batch cultures were set up in vessels resembling those used for continuous culture and containing the regular medium with 10 g. mannitol/l. Two of these were stirred to give the relatively high aeration rate of 42 mmole O₂/l./hr, one of these had 0.02 atm. CO₂ in the atmosphere passed over the culture, the third was stirred to give the relatively low aeration rate of 3 mmole O₂/l./hr. The cultures were inoculated to equiv. 0.3 µg. dry wt Azotobacter chroococcum/ml. from a nitrogen-limited continuous culture and incubated for up to 4 days at 30°. The results are illustrated in Table 1; inhibition by high aeration rates was complete with nitrogen-fixing populations even at atmospheric P₀₂ values; only at very high P₀₂ values were NH₄-utilizing strains inhibited. Inhibition depended on inoculum size: at equiv. 0.75 µg. dry wt organism/ml. growth was not completely inhibited but merely delayed for about 20 hr; at 1.5 µg./ml. high aeration induced a lag of about 10 hr as compared with the normal lag of 4 to 5 hr. These observations showed that inhibition of growth, apparently due to oxygen toxicity and not to CO₂ deprivation, could occur among Azotobacter species in batch cultures. The problem was studied further in continuous flow culture systems in which various parameters influencing growth were more readily controlled.

Effect of aeration on growth and nitrogen fixation in continuous culture

A steady-state population of Azotobacter chroococcum growing at a dilution rate of 0.2 hr⁻¹ and P₀₂ 0.2 atm. was established (P₀₂ 0.8 atm.) in continuous culture. The culture was sampled on three separate days and analysed for nitrogen content, mannitol consumption, dry weight of organism and respiratory activity. The P₀₂ was then altered and the analyses repeated when the culture was considered to be in a new steady state. Respiratory activity increased with P₀₂ from Q₀₂ 250 µl.mg./hr at 0.025 atm. O₂ to a plateau of Q₀₂ 950 between 0.2 and 0.5 atm. O₂.

Figures for the steady state yields of organism and of nitrogen fixed under various partial pressures of oxygen are plotted in Fig. 1. The yield of organisms showed a
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plateau about the range of normal air and decreased at very low or high $P_{O_2}$ values. In contrast, the nitrogen fixed per g. mannitol increased at low $P_{O_2}$ values to uncommonly high values. The efficiency of nitrogen fixation at the ordinary atmospheric $P_{O_2}$ of 0.2 (37 mg. N/g. mannitol) is higher than most values recorded in the literature; we attribute this fact to the use of continuous culture, because the criteria given in Methods, together with other evidence, indicated that the growth-limiting substrate in these conditions was nitrogen gas, and cultures in such conditions would select for bacterial variants of high nitrogen-fixing activity.

![Graph](https://via.placeholder.com/150)

Fig. 1. Concentrations of bacteria and efficiencies of nitrogen fixation in continuous cultures of *Azotobacter chroococcum* under various partial pressures of oxygen at $D = 0.2$ hr$^{-1}$.

- ○, efficiency; ■, organism concentration.

These cultures were not particularly sensitive to high oxygen tensions until $P_{O_2}$ values in excess of 0.6 atm. were reached; this figure is comparable to figures given by earlier workers and discussed in the introduction. Steady-state populations were then established at $P_{O_2} = 0.2$ atm. and the mannitol concentration decreased until this substrate became formally the growth-limiting substrate and determined the steady-state yield of organisms. Such populations proved difficult to establish and unstable when established because they showed hypersensitivity to oxygen even at normal atmospheric $P_{O_2}$; at a dilution rate of 0.14 hr$^{-1}$ a mannitol-limited nitrogen-fixing population (grown with mannitol 0.15 g./l. medium) showed 30% inhibition of growth (yield declined from 0.35 mg./ml. to 0.24 mg./ml.) in 2 hr when the oxygen supply to the organism was increased, either by increasing the stirring rate from 680 to 1100 rev./min. or the $P_{O_2}$ from 0.2 to 0.5 atm. (the stirring rates quoted approximate to $O_2$ transfer rates of 16.0 mmole $O_2$/l./hr and 70 mmole $O_2$/l./hr respectively). The population recovered when the oxygen supply was brought back to normal.
Carbon-limited populations growing with fixed nitrogen (1.5 g/l. NH₄Cl under A+O₂ mixtures) did not show such sensitivity to aeration.

Tests of the carbon-limited nitrogen-fixing population by using oxygen electrodes showed that an ambient oxygen concentration in the actual culture of 10 μM (equivalent to 0.01 atm. O₂ at saturation) was the highest tolerated. When the dissolved oxygen concentration exceeded this value, growth was inhibited; when it fell much below this value, oxygen tended to become the growth-limiting substrate. Nitrogen-limited populations required an ambient O₂ of about 20 μM for maximum growth and were more tolerant of increased O₂ supply than C-limited populations. However, inhibition of growth did occur when the dissolved O₂ concentration was in excess of 30 μM. Oxygen inhibition was reflected in dramatic changes in the dissolved oxygen concentration when aeration conditions were altered. Figure 2 illustrates the effect of increasing the Pₒ₂ over a nitrogen-limited culture in a steady state at D = 0.15 hr⁻¹.

The hypersensitivity of carbon-limited populations to oxygen inhibition suggested that nitrogen-limited populations, if grown slowly so as to allow maximum use of available carbon, might show comparable hypersensitivity. Such a population, grown slowly at 0.05 hr⁻¹ but with mannitol (10 g/l.), which would normally be sufficient, showed inhibition by high aeration (190 mmole O₂/l/hr) under the normal Pₒ₂ of 0.2 atm.; it did not show such inhibition at faster growth rates. The question whether the population was shifted from a formal N₂ limitation to a formal mannitol limitation by an increase in the aeration rate will be considered in the Discussion.

Phosphate-limited populations were also hypersensitive to oxygen. A phosphate-limited, nitrogen-fixing population (1.78 mg/P/l.), growing at a dilution rate of 0.115 hr⁻¹ with an oxygen solution rate of 15 mmole O₂/l/hr (Pₒ₂ = 0.2 atm.), gave
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a yield of 0.95 mg. dry wt organism/ml. Increasing the oxygen solution rate to 70 mmole O₂/l./hr, by increasing the stirring rate, decreased the yield to 0.35 mg./ml. over a 14 hr period. Similarly, an increase in P₂O₅ from 0.08 to 0.20 atm. at the higher stirring rate (1050 rev./min.) caused a decrease in yield from 0.7 mg./ml. to 0.25 mg./ml. over a 24 hr period at a dilution rate of 0.14 hr⁻¹. As with mannitol-limited populations, the effect was reversible; however, the culture could be sterilized completely when high aeration was continued for 3 days at a dilution rate of 0.05 hr⁻¹. Populations using NH₄⁺ instead of fixing atmospheric N₂ again showed no such oxygen sensitivity.

The lethality of oxygen to sensitive populations was studied by obtaining steady-state carbon-limited and phosphate-limited populations of similar population densities to avoid population effects on survival (Postgate & Hunter, 1963). Then, simultaneously, the flow of medium was stopped and aeration was increased just over twofold; the viability was then monitored by slide culture during several hours. This procedure gave a measure of the lethality of oxygen to the populations, uncomplicated by 'cryptic' growth which occurred when the cultures were left running. Figure 3 shows that oxygen was lethal to 70 % of a phosphate-limited population over 6 hr;
it was much less lethal to the carbon-limited population, though the decline in viability recorded is significant. Nitrogen-limited populations showed no decline in viability in comparable conditions.

**DISCUSSION**

Increased efficiency of nitrogen fixation at low $P_{O_2}$ values observed with batch cultures of Azotobacter by Parker (1954) was attributed to competition between oxygen and nitrogen for electrons by Parker & Scutt (1960), nitrogen fixation being regarded as a form of respiration. Our findings confirm the observations of Parker and his colleague and extend them to organisms in continuous culture; the complete inhibition of growth by high levels of oxygenation which we have observed presumably reflects an extreme state of oxygen competition, since it is specific to nitrogen-fixing populations. Inhibition was most marked with carbon-limited and phosphate-limited populations when these were fixing nitrogen and in these circumstances oxygen could be lethal to the organisms. We present an interpretation of the effects of oxygen on the physiology of Azotobacter on the basis of these findings and present knowledge of the behaviour of cell-free nitrogenase preparations.

Nitrogen-fixing systems have been extracted from *Azotobacter vinelandii* (Bulén, Burns & LeComte, 1965) and *A. chroococcum* (Kelly, 1966) in particulate forms which, unlike the soluble extracts of *Clostridium pasteurianum* (Carnahan, Mortenson, Mower & Castle, 1960), are reasonably stable in air. They can be further resolved into two soluble components, both of which are necessary for nitrogen fixation but which have become extremely sensitive to oxygen and readily damaged unless handled in anaerobic conditions (Bulén & LeComte, 1966; Kelly, Klucas & Burris, 1967). Nitrogen-fixing cell-free extracts of blue-green algae also appear to be oxygen-sensitive (Fay & Cox, 1967). These observations provide evidence that, even in aerobic bacteria, the components of nitrogenase are intrinsically sensitive to oxygen. The living organism, since it grows in air, must possess a mechanism for protecting the nitrogen-fixing site from oxygen. The oxygen-tolerance of the particulate preparations suggests that some steric arrangement of the components makes for oxygen tolerance and, in principle, this 'conformational protection', as we shall term it, could operate in two ways. Either the oxygen-sensitive sites could be inaccessible to oxygen, or they could be stabilized by conformational features of the nitrogenase complex so that, though accessible to oxygen, they would be undamaged by it.

Our findings lead us to assume that a second protective mechanism also operates in the living organism: that respiration is used to scavenge oxygen from the neighbourhood of the nitrogen-fixing site. Such a mechanism was proposed by Phillips & Johnson (1961) as a result of their observations that *Azotobacter vinelandii*, given excess oxygen, consumed sugar at rates greater than those necessary to supply the energy requirement. They suggested that respiration functioned as an 'oxygen-wasting system' which maintained a low $E_h$ value within the cell, presumed to be necessary for nitrogen fixation. Evidence pointing to respiration as a protective process is provided by numerous other publications, including Meyerhof & Burk (1928), Burk (1930), Parker (1954), Schmidt-Lorenz & Rippel-Balides (1957), Parker & Scutt (1960), Dilworth & Parker (1961) and Khmel et al. (1965), even though their findings were not always interpreted as implying a protection process. Our reasons for regarding respiration as protective to the nitrogenase complex are the following. (1) Respiration
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in nitrogen-limited nitrogen-fixing cultures is adjusted to balance oxygen supply and leaves nitrogen fixation largely unaffected. In conditions of high aeration or high Po, nitrogen fixation became remarkably inefficient in terms of carbon substrate consumed; in near anaerobic conditions it became highly efficient, though the growth yield of the population was then not impressive. (2) In carbon-limited nitrogen-fixing cultures, which could not adjust their respiration to match oxygen tension, extreme oxygen sensitivity was observed. (3) The inhibitory Po in the gas phase varied according to the population density but the maximum tolerated molarity of oxygen in the culture medium remained much the same, indicating that a balance between solution and consumption of oxygen was being actively maintained. (4) Hypersensitivity to oxygen was not shown by populations that were not fixing nitrogen.

Our direct measurements of Qo values showed a trend in the direction of high Qo corresponding to high Po, as we should expect on this hypothesis, but we do not attach great significance to the actual Qo values because of the rapid alteration in Qo that follows handling of bacterial populations. Nevertheless, it seems logical to accept that the high Qo values for which the Azotobacteriaceae are famous (4000 to 5000 µl. Oa/mg. dry wt/hr; Williams & Wilson, 1954) represent a mechanism for excluding oxygen from parts of the cell.

The existence of some kind of conformational protection, supplemented in the actively growing and fixing organism by respiratory protection, accounts for the behaviour of nitrogen-limited and putatively carbon-limited populations. It raises the question whether the so-called carbon-limited populations obtained here were truly carbon-limited. The yield certainly depended on the carbon concentration in the inflowing medium, but it was likely that the rates of carbon and nitrogen assimilation were finely balanced such that a small excess of carbon was available for the respiratory protection mechanism. The concept of limitation by single nutrients in chemostats thus becomes rather involved in a circumstance in which oxygen concentration is critical and access of nitrogen to nitrogenase is limited by some intracellular mechanism.

Ammonia-grown organisms showed no abnormal sensitivity to oxygen; the failure of such inocula to grow at Po values of 0.6 is probably analogous to the sensitivity to hyperbaric oxygen shown by other bacteria. Many microbes respond to plentiful availability of oxygen by bringing in non-cytochrome respiratory pathways (Lenhoff, Nicholas & Kaplan, 1956; Rosenberger & Kogut, 1958). Reflectance spectra (Giovannelli, 1957), as adapted to Azotobacter by Moss & Tchan (1958), were determined on bacteria grown with high and low aeration. No impressive change in the heights of the cytochrome peaks between 400 and 650 mµ appeared, suggesting that a non-cytochrome pathway was not used by *Azotobacter chroococcum*. Moreover, the sensitivity of phosphate-limited populations to oxygen suggests that the respiratory protective mechanism operates through the cytochrome pathway, since otherwise there is no reason for such cells to be sensitive. The ATP:ADP ratio is known to exert a controlling effect on mitochondrial respiration (Klingenberg & Schollmeyer, 1960), and there are indications that phosphate compounds and nucleotides exert some respiratory control in bacteria (Ishikawa & Lehninger, 1962; Revsin & Brodie, 1967); ADP will stimulate and ATP inhibit the respiration of *A. chroococcum* (M. G. Yates, personal communication). If, in the phosphate-limited population, respiration is suddenly augmented to compensate for an increase in oxygen concentration, then ADP would be converted to ATP. If the control mechanism were analogous to that in
mitochondria, the ATP:ADP ratio would thus be shifted in favour of decreased respiration and, because the respiratory protective mechanism had been thwarted, the nitrogenase would no longer function properly and growth would cease. In these circumstances permanent damage to the nitrogenase might ensue and O₂ might become lethal; the exceptional lethality of oxygen to phosphate-limited populations might well be a reflexion of this condition.

REFERENCES
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