Effect of Oxygen on Growth and the Synthesis of Bacteriochlorophyll in *Rhodospirillum molischianum*

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**ABSTRACT**

Sistrom, W. R. (University of Oregon, Eugene). Effect of oxygen on growth and the synthesis of bacteriochlorophyll in *Rhodospirillum molischianum*. J. Bacteriol. 89:403-408. 1965.—The basis of the strict anaerobiosis of the nonsulfur purple bacterium *Rhodospirillum molischianum* was investigated. The bacterium is unable to grow aerobically in the dark. In the light, the only immediate effect of oxygen is to inhibit the biosynthesis of bacteriochlorophyll and carotenoid pigments. Growth and protein synthesis are not inhibited directly, nor are the cells killed by oxygen. The inhibition of bacteriochlorophyll synthesis ultimately leads to inhibition of growth. Oxygen inhibits photopigment synthesis by means of a metabolic control system which is apparently identical with the one already known in facultatively aerobic species such as *R. rubrum*. Whole cells of *R. molischianum* respire in the dark; illumination inhibits respiration by about 50%. Cell-free extracts display significant levels of dark reduced nicotinamide adenine dinucleotide oxidase activity. Both these properties are in accord with the physiological behavior of the organism.

The nonsulfur purple bacteria (Athiorhodaceae) include both strict anaerobes and facultative anaerobes. Among the latter are the well-known species *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides*. The anaerobic group includes *Rhodospirillum molischianum* and other less well-known spirilla, as well as the morphologically anomalous *Rhodomicrobium vanニdii* (van Niel, 1963). The facultative species can grow either anaerobically in the light or aerobically in either light or darkness. The strict anaerobes are obligate phototrophs capable only of anaerobic growth in the light; routine observations of stab and shake cultures indicate that their growth is inhibited by air.

From what is known about aerobic and photosynthetic electron transport and adenosine triphosphate (ATP) generation in photosynthetic bacteria, it is not difficult to arrive at an explanation of the inability of the obligate phototrophs to grow aerobically in the dark. Since apparently the only components which are not common to both aerobic and photosynthetic electron-transport pathways are the terminal oxidases, it is likely that the obligate phototrophs lack an aerobic terminal oxidase which is coupled to ATP formation.

An explanation of the inhibition of growth by oxygen is not so obvious and, to my knowledge, no experiments have been performed which suggest one. I report here the results of some experiments with *Rhodospirillum molischianum* which indicate that the primary effect of oxygen is to prevent the synthesis of bacteriochlorophyll which, of course, is essential for continued photosynthetic growth. It appears that, in *R. molischianum*, the physiological basis of the inhibition of bacteriochlorophyll synthesis by oxygen is the same as in the facultative anaerobes, such as *Rhodopseudomonas spheroides* (Cohen-Bazire, Sistrom, and Stanier, 1956).

**MATERIALS AND METHODS**

A strain of *R. molischianum* kindly provided by C. B. van Niel was used throughout; this strain is one of Giesberger's (1947) original isolates.

The general methodology employed in this laboratory for the growth of photosynthetic bacteria has been described in detail elsewhere (Cohen-Bazire et al., 1956; Sistrom, 1960); the use of *R. molischianum* entailed certain minor modifications which are outlined below.

Suspensions of this organism have a disagreeable tendency to form small clumps which interferes with turbidity measurements and viable-cell counts. Vigorous mechanical agitation obviated this difficulty.
Table 1. Effect of oxygen on viable-cell count in cultures of *Rhodospirillum molischianum*.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Viable cells per ml (× 10⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>2.5</td>
</tr>
<tr>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>2.25</td>
</tr>
<tr>
<td>Dark</td>
<td>2.6</td>
</tr>
</tbody>
</table>

* A culture was grown overnight in medium CSu under photosynthetic conditions (N₂ 5% CO₂) at an intensity of 800 ft-c. The initial viable-cell count was determined, and the culture was divided in two portions. Both were aerated with air + 5% CO₂; one was illuminated at the same intensity, and the other was kept dark. Viable counts were made after 3.5 and 4 hr, respectively.

![Graph](http://jb.asm.org/)

**Fig. 1.** Effect of semiaerobic conditions on growth and photopigment synthesis in *Rhodospirillum molischianum*. A culture was grown under anaerobic conditions (N₂ 5% CO₂) and in a light intensity of 600 ft-c; at the time indicated by the arrow, the aeration mixture was changed to 5% O₂ 5% CO₂ 90% N₂. Symbols: protein (+); bacteriochlorophyll (Δ); lycopene (○); and optical density at 1,000 μm (O).

Viable counts were performed by adding appropriate dilutions to the complete medium described by Griffiths and Stanier (1958) to which sodium sulfide was added (1 drop of 1% Na₂S: 9H₂O per 10 ml). After the inoculated medium had gelled, it was overlaid with 3 to 4 ml of the same medium, and the whole was finally covered with sterile Vaseline. The tubes were incubated at room temperature in the light.

Medium A of Sistrom (1960), with potassium succinate (0.2%) as carbon source and supplement, was used with casein hydrolysate (0.1% or 0.2%), was used; this is referred to as medium CSu.

Estimations of bacteriochlorophyll were carried out as described earlier (Cohen-Bazire et al., 1956). The major carotenoid pigments in *R. molischianum* are lycopene and hydroxylycopene (Goodwin and Land, 1956a; Sistrom, unpublished data). These pigments were estimated from the optical densities at 472 μm of methanol extracts after correcting for the absorption by bacteriochlorophyll at this wavelength. A value of 2,460 was used for the extinction coefficient (ε₅₄₆) in methanol; this is based on the value in petroleum ether given by Goodwin and Land (1956).

Protein was estimated by the method of Lowry et al. (1951) after digestion for 1 to 2 hr in 1 M NaOH at 40°C.

All cultures were incubated at 34°C.

Measurements of oxygen uptake were performed in an illuminated Warburg apparatus. The vessels were illuminated, as required, with a 100-W tungsten lamp; the light intensity at the level of the vessel was about 1,000 ft-c. Each vessel contained 0.2 ml of 10% KOH in the center well, 2 ml of cell suspension in 0.025 M potassium phosphate buffer (pH 6.8), and 10 μmoles of potassium succinate as indicated. The temperature was 34°C.

Reduced nicotinamide adenine dinucleotide (NADH₂) oxidase activity was assayed by measuring the decrease in absorbancy at 340 μm. The cuvettes contained, in a final volume of 3 ml: NADH₂, 0.2 to 0.3 μmoles; tris(hydroxymethyl)-aminomethane (Tris) buffer (pH 7.8), 60 μmoles; MgSO₄, 30 μmoles; NaCl, 240 μmoles; and 0.1 to 0.4 ml of cell extract. The assays were conducted at room temperature. The extracts were prepared by sonic disruption of cell suspensions in TSM buffer (0.02 M Tris, 0.01 M MgSO₄, and 0.08 M NaCl; pH 7.8) followed by centrifugation at 20,000 × g for 10 min to remove whole cells and debris.

**RESULTS**

Although *R. molischianum* develops only in the lower portions of stab or shake cultures, we found it unnecessary to protect anaerobically grown stock cultures from air. Furthermore, Giesberger (1947) was struck by the occurrence of this organism in the surface layers of the waters from which he isolated it. Therefore, it seemed unlikely that oxygen damages or kills the cells. This supposition was borne out by viable-cell counts of aerobic cultures (Table 1). In the presence of air, with or without light, the number of viable cells did not decline over a period of at least 3 hr.

I next studied the effect of oxygen on growth and photopigment synthesis. A typical experiment in which an illuminated culture was aerated with 5% O₂ is shown in Fig. 1. There was only a slight inhibition of growth. The effect on photopigment synthesis was much more drastic.
formation of bacteriochlorophyll was completely suppressed and that of lycopene nearly so. These results are strikingly similar to those reported previously for Rhodopseudomonas spheroides and Rhodospirillum rubrum. The similarity is also seen in the fact that, when a culture was returned to anaerobic conditions after a period of aerobic growth, there was an almost immediate resumption of bacteriochlorophyll synthesis at a high rate (Fig. 2). This recovery substantiates the conclusion from viable-cell counts that oxygen does not damage the cells.

In the dark, under either aerobic or anaerobic conditions, neither bacteriochlorophyll nor turbidity increased.

These experiments suffice to show that the strict anaerobiosis of R. molischianum is due to inhibition of bacteriochlorophyll synthesis by oxygen and not to a toxic effect of oxygen on cell growth. In an obligate phototroph, inhibition of photopigment synthesis will presumably lead to arithmetic growth; however, none of my experiments was continued long enough to demonstrate this point unambiguously.

It is possible to imagine two ways by which oxygen could inhibit photopigment synthesis: (i) by inhibiting directly the biosynthetic reactions leading to bacteriochlorophyll and carotenoids, and (ii) indirectly by means of a control mechanism of the same sort as that known to occur in the aerobic species (Cohen-Bazire et al., 1956). It is possible to distinguish between these two alternatives experimentally; for the rationale of the experiments to be understood, it is necessary to recall briefly the salient features of the control of photopigment synthesis in the aerobic species.

In these organisms an increase in either light intensity or oxygen tension reduces or even entirely stops the synthesis of photopigments; conversely, a decrease in either light intensity or oxygen tension increases the differential rate of synthesis of these pigments. It was suggested that these results could be explained by assuming that the rate of pigment synthesis is inversely related to the concentration of a regulator substance within the cell, and that the concentration of this substance, in turn, is determined by the state of oxidation of the electron-transport pathway. A higher degree of oxidation, as would result from an increase in light intensity or oxygen tension, will raise the concentration of the regulator and thereby reduce the rate of bacteriochlorophyll synthesis and carotenoid synthesis (Cohen-Bazire et al., 1956). It follows that, in an illuminated, aerobic culture, the rate of pigment synthesis will depend on both the light intensity and oxygen tension. For example, an increase in light intensity can offset the effect of a decrease in oxygen tension and vice versa.

In the case of R. molischianum, if the first alternative mentioned above is correct, that is, if oxygen inhibits bacteriochlorophyll synthesis directly, the effect of oxygen should be independent of changes in the light intensity. On the other hand, if the second alternative is correct, the degree of inhibition should depend on changes in light intensity.

Accordingly, I studied the effects of both a sudden increase and a sudden decrease in light intensity on the inhibition of bacteriochlorophyll synthesis in cultures aerated with 5% O₂. In the first experiment, a culture was grown anaerobically at a light intensity of 1,200 ft-c, and at the start of the experiment the light intensity was reduced to 80 ft-c. A part of the culture was aerated with 5% O₂; the rest remained under anaerobic conditions as a control (Fig. 3). The data have been replotted in Fig. 4 to show the increase in bacteriochlorophyll relative to the increase in turbidity; the slopes of the curves give the differential rates of pigment synthesis directly. In the culture aerated with 5% O₂, synthesis of bacteriochlorophyll was only partially inhibited, in contrast to the results of the
The converse experiment was also performed (Fig. 5). In this case, the illumination was not reduced at the start. Figure 6 shows the differential rates of bacteriochlorophyll synthesis. In this experiment, aeration with 5% O₂ completely inhibited the formation of bacteriochlorophyll. When, after 3 hr, the light intensity was reduced to 120 ft-c, pigment synthesis immediately recommenced in the semiaerobic culture.

The synthesis of lycopene underwent similar changes; for clarity, these data have been omitted from the figures. These results are in complete accord with the idea that, in *R. molischianum*, the formation of photopigments is inhibited by oxygen through the action of a metabolic control mechanism similar to the one which occurs in *Rhodopseudomonas spheroides* and *Rhodospirillum rubrum*.

This implies that *R. molischianum* can respire; accordingly, some simple experiments were performed to test this implication. Cells of *R. molischianum* respire at a rate comparable to that of *Rhodopseudomonas spheroides* tested under similar conditions, and, furthermore, the respira-

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**Fig. 3.** Inhibition of bacteriochlorophyll synthesis by oxygen and light. A culture of *Rhodospirillum molischianum* was grown overnight in medium CSu with 0.2% casein hydrolysate under anaerobic conditions and in a light intensity of 1,800 ft-c. The culture was divided into two parts; one was kept under anaerobic conditions (closed symbols) and the other was aerated with 8% O₂ and 90% N₂ (open symbols). The light intensity on both cultures was reduced to 80 ft-c. At 190 min, the anaerobic culture was diluted with fresh medium; at 365 min (arrow), the light intensity was increased to 400 ft-c. Symbols: bacteriochlorophyll (Δ, ▲); optical density at 1,600 μm (○, ●).

Experiment (Fig. 1) in which the light intensity was not reduced. The inhibition became progressively less during the course of the experiment, undoubtedly because cell growth resulted in a constantly decreasing oxygen tension. When, after 4.5 hr, the light intensity was increased to 400 ft-c, synthesis of bacteriochlorophyll in the semiaerobic culture was completely inhibited; however, this increase in light intensity did not significantly affect the differential rate of pigment synthesis in the anaerobic culture. At the time of the increase in light intensity, the anaerobic culture had a higher specific bacteriochlorophyll content than did the semiaerobic culture. Therefore, the increase in light intensity by itself would have caused a greater inhibition of bacteriochlorophyll synthesis in this culture than in the semiaerobic one. Thus, it is clear that the effects of oxygen and light intensity are additive.

**Fig. 4.** Differential rate of synthesis of bacteriochlorophyll in presence of oxygen and light. The data of Fig. 3 have been replotted to show the increase in bacteriochlorophyll relative to the increase in cell mass. The open symbols are for the semiaerobic culture (8% O₂): for the first 180 min (○); after 190 min (△). The closed symbols are for the anaerobic culture: for the first 180 min (●); after 190 min (▲). The light intensity was increased at the points indicated by the arrows. The differential rates (micrograms of bacteriochlorophyll per milliliter per 0.1 optical density unit) are shown on the figure.
been shown that oxygen inhibits bacteriochlorophyll synthesis by means of a metabolic control mechanism which is apparently identical to the one previously shown to occur in the facultatively anaerobic Athiorhodaceae (Cohen-Bazire et al., 1956).

If it is accepted that *R. molischianum* is unable to form ATP by respiration, then the fact that the effect of oxygen on bacteriochlorophyll synthesis is the same in this organism and in the aerobic species means that the control mechanism responds to the state of oxidation of an electron carrier and does not depend on the intracellular level of ATP. This conclusion corroborates the results of an earlier study (Sistrom, 1963) on the effects of various metabolic inhibitors on bacteriochlorophyll synthesis by *Rhodopseudomonas spheroides*.

The assumed inability of *R. molischianum* to

![Graph](http://jb.asm.org/)

**FIG. 5.** Inhibition of bacteriochlorophyll synthesis by oxygen and light. A culture of *Rhodospirillum molischianum* was grown overnight in medium CSu with 0.8% casein hydrolysate under anaerobic conditions and in a light intensity of 1,200 ft-c. The culture was divided into two parts; one was kept under anaerobic conditions (closed symbols), and the other was aerated with 8% O₂ + 8% CO₂ + 90% N₂ (open symbols); the light intensity was kept at 1,200 ft-c. At 145 min, the anaerobic culture was diluted with fresh medium. At 190 min (arrow), the light intensity was decreased to 120 ft-c. Symbols: bacteriochlorophyll (Δ, ▲); optical density at 1,000 μm (O₂, ★).

![Graph](http://jb.asm.org/)

**FIG. 6.** Differential rates of synthesis of bacteriochlorophyll in the presence of oxygen and light. The data of Fig. 5 have been replotted to show the increase in bacteriochlorophyll relative to the increase in cell mass. The open symbols are for the semiaerobic culture (8% O₂); for the first 145 min (O); after the light intensity was decreased (Δ). The closed symbols are for the anaerobic culture: for the first 145 min (★); after the light intensity was decreased (▲).

**TABLE 2.** Oxygen uptake by *Rhodospirillum molischianum* and *Rhodopseudomonas spheroides* in light and darkness

<table>
<thead>
<tr>
<th>Substrate</th>
<th><em>R. molischianum</em></th>
<th><em>R. spheroides</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dark</td>
<td>Light</td>
</tr>
<tr>
<td>None</td>
<td>26*</td>
<td>--</td>
</tr>
<tr>
<td>Succinate</td>
<td>66</td>
<td>33</td>
</tr>
</tbody>
</table>

* Results expressed as microliters of O₂ per hour per milligram of protein.
generate ATP by respiration can also explain the slight inhibition of growth by oxygen (see Fig. 1). In the presence of oxygen, the photosynthetic electron-transport pathway will be depleted of electrons, and, accordingly, the rate of ATP formation will fall.

It will be of interest to see if the strict anaerobiosis of other photosynthetic bacteria can be explained on the same basis as that of *R. molischianum*.

**Acknowledgments**

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I wish to thank Dorothy E. Sistrom for her skillful technical assistance.

**Literature Cited**


