INFLUENCE OF OXYGEN CONCENTRATION ON THE REDUCTION OF NITRATE BY A *PSEUDOMONAS* SP. IN THE GROWING CULTURE

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Summary

A method is described whereby oxygen tension in growing cultures of microorganisms may be assessed with reasonable accuracy at any time by means of a recording polarograph.

The method applied to a study of nitrate decomposition by a species of *Pseudomonas,* using unadapted cells as an inoculum, reveals that no apparent reduction occurs while oxygen is present in solution.

A method is also outlined for the rough assessment of substrate deficiency in cultures.

I. Introduction

Meiklejohn (1940) has investigated the effect of aeration on the reduction of nitrate, and she observed that reduction occurred under "aerobic" conditions in aerated cultures. In view of Stickland's (1931) observation on the effect of oxygen on the function of the enzyme nitratase, she was led to the conclusion that another enzyme not sensitive to oxygen was responsible for nitrate reduction under aerobic conditions. This point has been disputed by Sacks and Barker (1949) on the grounds that Meiklejohn's aeration proceduce was incorrect, inferring that the rate of oxygen uptake in portions of the culture fluid exceeded its rate of solution, and hence the steady state between the gas and liquid phases had not been achieved.

Using a constant mass of adapted cells, Sacks and Barker showed by manometric methods that the enzymes involved in the reduction of nitrate and nitrite were both sensitive to oxygen, and that the rate of nitrate reduction in air is 29 per cent. of that under anaerobic conditions, whilst the corresponding value for nitrite reduction was 25-35 per cent. A constant cell mass could not be expected to exist for any more than a momentary period in growing cultures. To study the effect of oxygen concentration on nitrate reduction in growing cultures it is necessary to know not only the actual oxygen concentration in the medium in relation to nitrate concentration but also the availability of oxidizable substrate at different time intervals during growth. Desaturation of the oxidative enzyme systems with substrate will reduce the ability of the organism to utilize both the oxygen and nitrate. This could give the impression that a high oxygen concentration coupled with lack of nitrate reduction indicated inhibition of nitrate reduction by oxygen, when, in effect, conditions were unsuitable for the reduction of either.

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As far as the authors are aware, there is no evidence to indicate that organisms are capable of utilizing oxygen from air without its prior solution in the supporting medium, whether the latter be liquid or solidified with agar. It seems reasonable to assume therefore that the important value to be determined is the actual oxygen concentration in solution.

All previous workers, including Meiklejohn (1940) and Sacks and Barker (1949) have employed an indirect method of assessing the oxygen status of the medium. All of the work has been based on the assumption that, by continuous passage of a gas mixture through a solution, equilibrium between the concentration of gas in the gas and liquid phases could be achieved. In a sterile fluid this must be the case but in a fluid containing actively metabolizing cells a second system is introduced, which is continually depleting the solution of oxygen, and as the mass of cells increases the rate at which this depletion occurs increases. Hence aeration of a solution at a constant rate, sufficient to maintain a steady state under sterile conditions, may fail to meet the demand of the deoxygenating system, resulting in the complete removal of oxygen from the solution.

To attack the problem directly it was necessary to develop a technique that would permit the determination of oxygen at any chosen time in a growing culture that was being constantly aerated, and at the same time to determine the availability of substrate and also nitrate and nitrite concentration. The apparatus and methods for achieving this are described in the following sections.

TABLE 1

OXYGEN CONCENTRATIONS IN PEPTONE YEAST EXTRACT BROTH UNDER VARYING DEGREES OF AERATION AT 25°C.

Stirring Rate (r.p.m.)	O_2 Concentration (p.p.m.)
500	8.1
300	8.0
200	8.0
100	7.9
Unstirred	7.2

II. Apparatus and Analytical Methods

(*a*) *Apparatus and Technique for Determination of Oxygen in Aerated Culture Fluids*

As will be evident from Table 2, 500 r.p.m. series, the rate of deoxygenation at times exceeded 8 p.p.m. per min. The steady state concentrations of oxygen in the medium at the temperature and the stirring rates employed are set out in Table 1. The low value obtained in the unstirred solution is due to the slow uptake of oxygen by components of the medium. If placed in a sealed vessel the oxygen concentration will gradually fall to zero.

Oxygen readings had therefore to be obtained within 10 seconds or less to be of any significance. The only method that presented any possibility of achieving this object was that employing the dropping mercury electrode (Skerman and Millis 1949). This method could determine 0.06 p.p.m. of oxygen with the apparatus employed. Quantities smaller than this are referred to in the text as "zero" oxygen concentration.

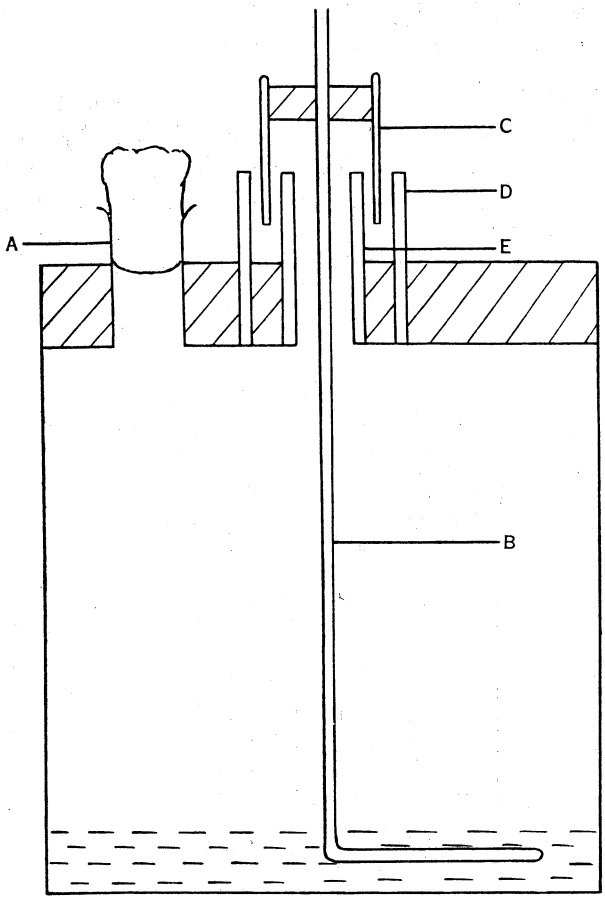

Fig. 1.—Vessel used in aeration of cultures. The vessel is of glass sealed with a rubber bung fitted with an inoculation inlet *A* and two metal cylinders *D* and *E*. The bent glass rod *B* is used for stirring. Sterility is maintained by the glass cylinder *C* spinning between the metal cylinders *D* and *E*.

The normal stationary electrode is useless in turbulent solutions unless the movement of the solution is constant both in speed and direction. It cannot, therefore, be introduced directly into a vigorously aerated solution. A possible alternative was the rotating platinum micro-electrode. The present authors

attempted to incorporate such an electrode in a stirring device used for aeration without success and eventually reverted to the following method, which provided readings that represented the oxygen status of the culture within 3 sec. of sampling.

(i) *Apparatus.*—The main components of the apparatus were a Cambridge photographic recording polarograph; a constant-speed stirring machine with the stirring rate adjustable at 100, 200, 300, 400, and 500 r.p.m.; a specially designed culture vessel (Fig. 1), a "Hyvac" pump, and a polarographic cell maintained in a constant-temperature water-bath.

When 75 ml. of the medium employed was placed in the culture vessel, a 2 cm. depth of fluid resulted. Aeration was achieved by stirring with the glass rod. No provision was made for replenishment of fresh air in the jar. Experimental evidence suggests that the supply of free air is adequate. In any case it is not necessary since correlation is made between nitrate reduction and the actual oxygen present in the solution, not with that in the gas phase.

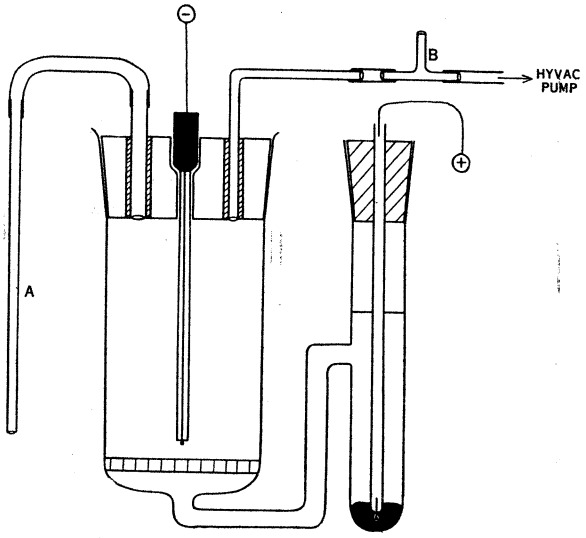

Fig. 2.—Polarographic cell assembly used for sampling and measuring oxygen concentration.

(ii) *Sampling.*—Reference to Figure 2 will show the type of apparatus employed. The cell is the same type as that employed in previous work (Skerman and Millis 1949). Removal of the sample from the aerated flask to the polarographic cell was achieved in less than two seconds by turning on the pump, inserting the pipette *A* into the flask while the stirring machine was still operating, and then pressing the thumb over the outlet *B*. Preliminary trials showed that in 2 sec. or less the cell was filled.

To avoid admixture of air with the sample as it surged into the cell, nitrogen was blown through the opening *B* to displace all air through the pipette *A* before the sample was taken.

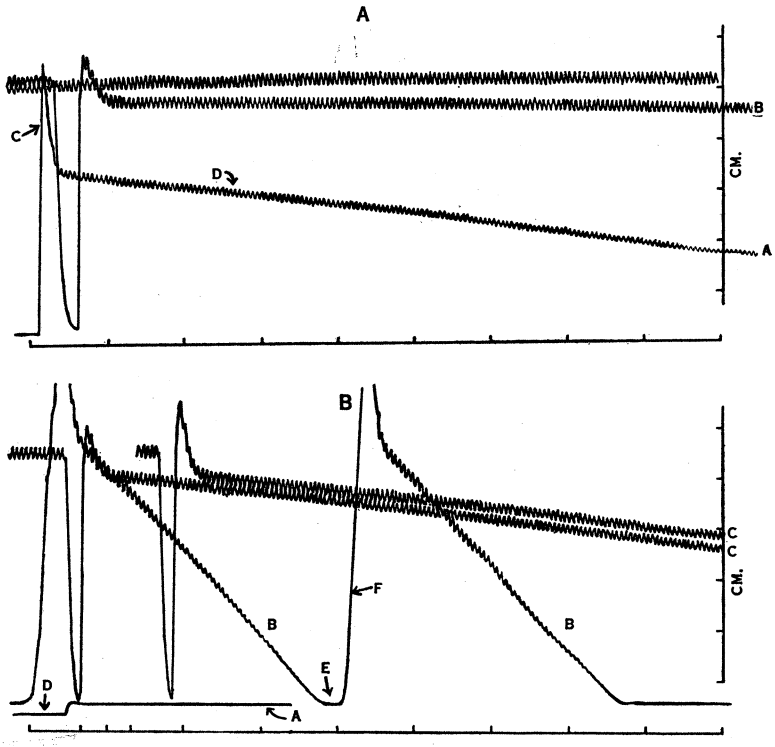

Fig. 3.—Polarograms from which figures in Table 1 were derived. In all cases the time interval (abscissa) is 57 sec. The oxygen concentration is expressed in cm. galvanometer deflection (1 cm. = 1.36 p.p.m. O_2). *A*, *B*, *C*, *D*, and *E* are polarograms for the 4, 8, 12, 16, and 20 hr. sampling periods respectively. For explanation see text.

(iii) *Recording of Oxygen Concentration in the Sample.*—Prior to the acquisition of a Cambridge recording polarograph, readings were made of galvanometer deflection visually. Most of the findings obtained by this method were subsequently confirmed by the recording instrument. However, where uptake rates were high or the oxygen concentration low, or both, as was often the case, accurate visual readings were impossible. The recording polarograph (see Kolthoff and Lingane 1946) overcame this difficulty. The machine was coupled with the polarographic cell in the usual manner and adjusted to record current flow at a fixed applied e.m.f. of 0.6 V. Just prior to sampling the machine was set in motion. Owing to the initial absence of an electrolyte in the cell the circuit was broken and hence the polarograph recorded the galvanometer zero reading as a black line running parallel to the abscissa (Fig. 3*B*, curve *D*).

When the culture fluid was sucked into the cell the circuit was closed with the resulting sudden deflection of the mirror galvanometer. The behaviour of the galvanometer needle at this instant depended on the existence and quantity of reducible ion in solution. With fluids devoid of oxygen and more than

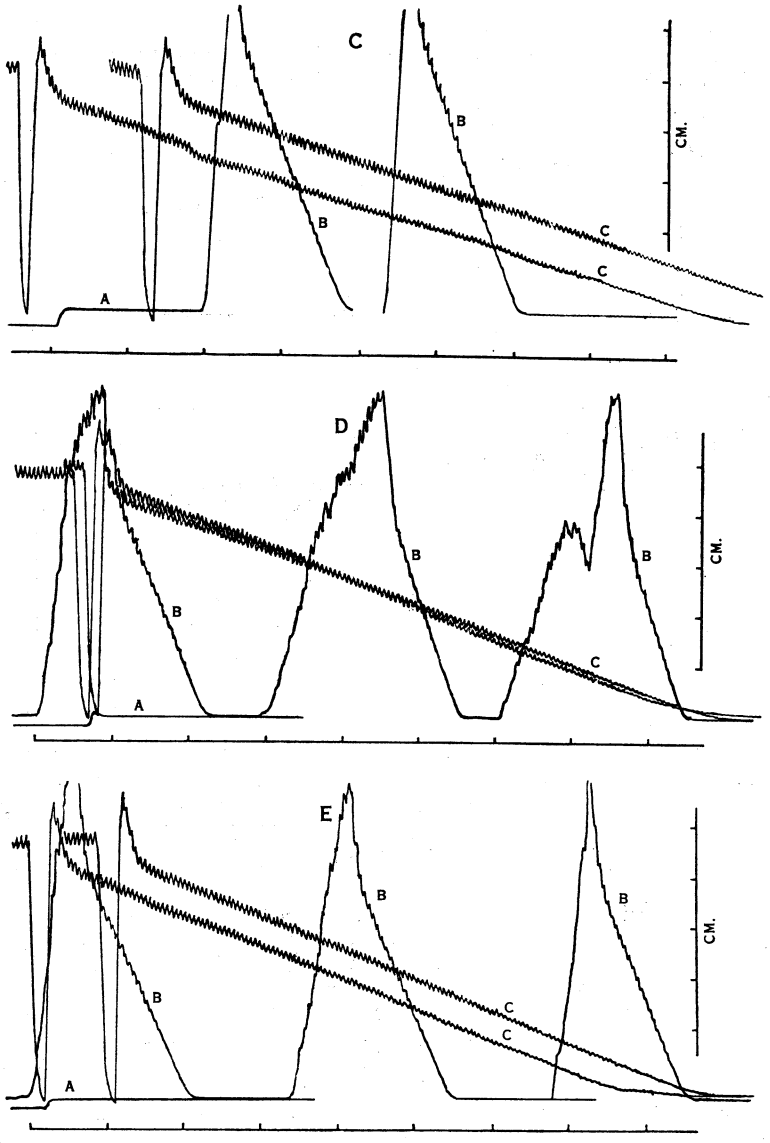

Fig. 3 (*C-E*).—For details see page 515.

traces of other reducible ions the galvanometer needle merely shifted position from the galvanometer zero to the residual current reading for the fixed applied e.m.f. employed (Fig. 3*B*, curve *A*). Turbulence alone would only be responsible for a very minor fluctuation in current if reducible ions were absent.

In the presence of reducible ions the turbulence of the solution caused the movement of the ions to the mercury drop by other than pure diffusion, with the result that the readings were extremely erratic and unduly high until turbulence subsided (Fig. 3*A*, curve *C*). Once turbulence subsided the current decreased at a uniform rate as the oxygen was removed from solution (Fig. 3*A*, curve *D*).

By extrapolating this slope back to zero time a reasonable assessment of the oxygen concentration at the sampling instant was obtained.

(*b*) *Estimation of Substrate Availability*

It is generally accepted that nitrate functions as an alternative to oxygen in respiration by cells containing the enzyme nitratase. The two major factors limiting its reduction are oxygen on the one hand and availability of oxidizable substrate on the other. If, after the removal of oxygen, the available oxidizable substrate should be depleted it is obvious that no further nitrate can be reduced and metabolism ceases. The present authors considered that, for the purposes of this problem, a rough assessment of substrate availability could be obtained by comparing the oxygen uptake rate of a portion of the culture itself with the oxygen uptake rate of a portion of the culture sown into a fresh sample of the same culture medium.

For example, in the absence of other governing factors, the uptake rate of 2 ml. of the culture when sown into 20 ml. of the fresh medium should be 1/11 of that of the whole population in the original culture provided the latter had plenty of readily oxidizable substrate. If on the other hand the substrate in the original culture was partially or wholly depleted then the ratio would become increasingly narrow and finally reversed as the availability of substrate was reduced to zero.

This method was therefore adopted. Where the initial oxygen concentration was large the rate of O_2 uptake was obtained from the graph (Fig. 3*A*, curve *D*). Where it was low, oxygen was blown into the sample of culture in the cell to bring the level as near to the concentration existing in the sterile medium as practicable and the uptake rate then determined (Fig. 3*B*, points *E*, *F*, *B*).

(*c*) *Estimation of Nitrate and Nitrite*

Both nitrate and nitrite yield the same colour when determined colorimetrically by the brucine method (Noll 1945). Where mixtures occur the colour densities are additive. Nitrite can be determined in the presence of nitrate by the method of Rider and Mellon (1946). In this work standard curves were prepared for nitrate and nitrite concentrations by the brucine method and for nitrite by the Rider and Mellon procedure.

In the analysis of mixtures, nitrite was estimated by the Rider and Mellon procedure and the equivalent colour density obtained for the brucine method from the standard curve. This, subtracted from the total density determined by the brucine procedure, yields the concentration of nitrate.

The method is based on the assumption that both standard curves are in accordance with Beer's Law. Actually neither was a straight line over the whole range employed. The results are not strictly accurate where high concentrations ($>$ 300 p.p.m.) of nitrite are present. However, on test mixtures of nitrate and nitrite, the method of correction gave the values for nitrate present.

Example of correction.—Initial nitrate, density 0.420, concentration 1700 p.p.m.; final nitrate, density 0.367; nitrite by Rider and Mellon method, 460 p.p.m. In the material diluted 1/100 for the nitrate test there would be 4.6 p.p.m. of nitrite. From the nitrite brucine calibration this corresponds with a density of 0.18.

Subtracting this from the final nitrate density,

$$0.367 - 0.18 = 0.187.$$

From the nitrate brucine curve this corresponds with a nitrate concentration of 650 p.p.m. The nitrate loss is therefore 950 p.p.m.

(*d*) *The Organism*

The organism employed was a species of *Pseudomonas* isolated from a clay loam at Dookie, Victoria.

(*e*) *The Culture Medium*

The culture medium employed in the study consisted of "Difco" peptone 1 per cent., "Difco" yeast extract 0.5 per cent., NaCl 0.5 per cent., KNO_3 0.2 per cent. The medium was made up in tap water and had an initial pH of 6.8. Preliminary trials had shown that the fully oxygenated medium with and without nitrate supported luxuriant growth. In the presence of the organic nitrogen the nitrate was not reduced. It was assumed therefore that the organic nitrogen was preferentially used and that the nitrate was involved under anaerobic conditions only in respiratory function.

(*f*) *The Inoculum*

The organism was grown on "Difco" peptone yeast extract agar slopes at 25°C. for 48 hr. The growth was suspended in sterile Ringer's solution and diluted to a density that gave a deflection of 50 mm. with the Evelyn photoelectric colorimeter, employing a 660 filter and full illumination. This approximated 10^9 cells per ml. One ml. was used to inoculate 75 ml. of medium.

III. General Procedure and Results

Briefly, the general procedure adopted was to autoclave the nitrate medium in the flask, attach it to the stirring machine and run the machine at the desired speed for 24 hr. prior to inoculation to ensure saturation of the medium with air at the temperature employed (25°C.) and freedom from contamination. The medium was then inoculated and after a fixed interval of time samples withdrawn for analysis.

Varying degrees of aeration were obtained by stirring the culture at speeds of 100, 200, 300, and 500 r.p.m. The froth created, especially at 500 r.p.m. was beaten down to a great extent by the stirring rod. For each speed, determinations were made of the oxygen level; the availability of substrate, residual nitrate and nitrite; pH change, population density, viable count (8 replicates), and cell size (averaged from 20 cell measurements) at intervals of 4, 8, 12, 16, 20 hr. after inoculation. A fresh flask was assembled for each time interval. The results of these experiments are set out in Table 2.

The photographic recordings for the 100 r.p.m. series are shown in Figures 3*A*, *B*, *C*, *D*, and *E*.

(*a*) *Discussion of Results*

Reference to Table 2 will show that nitrate reduction did not commence before the oxygen concentration was reduced to "zero," at 100 r.p.m. The ratio in column 6, used to express the availability of substrate should theoretically be unity and remain so as long as substrate is readily available. In fact the ratio was nearer 2.0. The authors can offer no explanation for this at present. The fact that it remains constant at approximately 1.8 indicates that substrate was at no time a limiting factor.

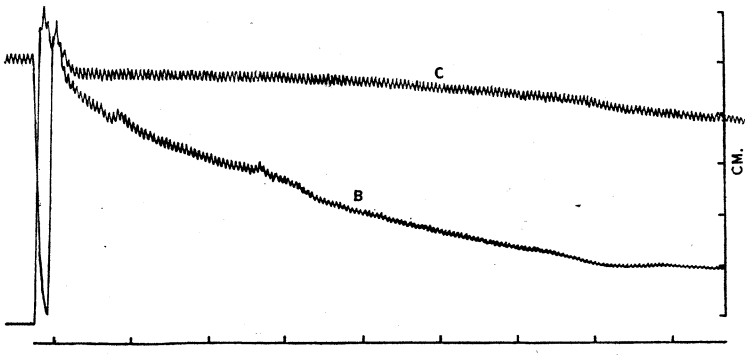

Fig. 4.—Polarogram showing rate of oxygen uptake in the original culture (curve *B*) and dilution culture (curve *C*) at 16 hr. in the 300 r.p.m. series. Abscissa, time intervals = 57 sec. Ordinate, 1 cm. = 1.36 p.p.m. O_2.

Reference to the 200 r.p.m. figures reveals a similar state up until the 16-20 hr. period. The change in ratio in column 6 to 6.7 indicates lack of substrate in the original culture medium sufficient to maintain the enzymes in a saturated state. However, the latter was maintained sufficiently long to effect complete reduction of the nitrate.

Reference to Table 2, 500 r.p.m., reveals that, at this high speed of stirring, complete oxidation of the substrate occurred before the population was able to reduce the oxygen to "zero" concentration. No nitrate was reduced.

In the 300 r.p.m. series the oxygen concentration, owing to the increased aeration rate, did not reach "zero" until 10-12 hr. The return to practically saturation level at 16 hr., only partial reduction of nitrate, and the reduction

TABLE 2

RESULTS OF EXPERIMENTS

Time of Sampling After Inoculation (hr.)	Oxygen Level (p.p.m.)	Oxygen Uptake Rates (p.p.m./min.) Of Original Culture: Initial	Of Original Culture: After Reoxygenation	Of 9.1% of Culture Inoculated into Fresh Medium	Oxygen Uptake Rate in Diluted Culture X11 / Oxygen Uptake Rate in Original Culture	Nitrate Lost (p.p.m.)	Nitrite Present (p.p.m.)	△ pH	Density $L = 2 - \log G$	Plate Count x10⁶	Average Cell Size (μ)
Stirring Rate 100 r.p.m.											
4	4.8	0.24	—	0.21	—	0	0	0.1	0.0434	—	1.8 × 1.0
4	5.7	0.3	—	—	—	0	0	—	0.0362	19	1.8 × 1.0
8	0	—	2.4	0.26	1.2	0	20	0.5	0.2676	500	1.7 × 0.8
8	0	—	3.4	0.26	0.8	0	57	0.8	0.2882	100	1.7 × 0.8
12	0	—	5.4	0.69	1.4	600	90	1.0	0.557	2800	1.6 × 0.5
12	0	—	4.5	0.75	1.8		280	1.2	0.545	4100	1.6 × 0.5
12	0	—	4.2	0.69	1.8	Total	460	1.0	0.512	4100	1.5 × 0.5
16	0	—	5.0	0.78	1.7	Total	0	1.2	0.699	6000	1.7 × 0.4
16	0	—	5.1	0.86	1.8	Total	0	1.4	0.716	—	1.4 × 0.5
20	0	—	5.0	0.85	1.9	Total	0	1.4	0.776	6000	1.5 × 0.4
20	0	—	5.0	0.85	1.9	Total	0	1.4	0.776	6000	1.5 × 0.4
Stirring Rate 200 r.p.m.											
4	6.8	0.21	—	—	—	0	0	0.05	0.0458	29	2.1 × 0.8
7	2.5	2.4	—	0.3	1.5	0	0	0.6	0.319	300	2.1 × 0.8
8	0	—	3.04	0.3	1.1	0	0	0.5	0.301	600	2.1 × 0.7
8	0	—	3.7	0.42	1.2	0	0	0.6	0.310	600	1.9 × 0.7
9	0	—	6.1	0.54	0.9	0	15	1.1	0.459	720	1.7 × 0.5
12	0	—	5.7	0.88	1.7	1100	380	1.2	0.699	2500	1.5 × 0.5
12	0	—	4.9	0.96	2.1	800	250	1.2	0.699	3500	1.4 × 0.6
16	0	—	14.2	1.57	1.2	1800 (Total)	0	1.5	0.912	9000	1.4 × 0.4
16	0	—	15.5	1.50	1.1	Total	0	1.3	0.912	10000	1.4 × 0.4
20	0	—	2.2	1.36	6.7	Total	0	1.6	0.912	9000	1.2 × 0.5

TABLE 2 (*Continued*)

Time of Sampling After Inoculation (hr.)	Oxygen Level (p.p.m.)	Oxygen Uptake Rates (p.p.m./min.) Of Original Culture: Initial	Of Original Culture: After Reoxygenation	Of 9.1% of Culture Inoculated into Fresh Medium	Oxygen Uptake Rate in Diluted Culture X11 / Oxygen Uptake Rate in Original Culture	Nitrate Lost (p.p.m.)	Nitrite Present (p.p.m.)	△ pH	Density $L = 2 - \log G$	Plate Count x10⁶	Average Cell Size (μ)
Stirring Rate 300 r.p.m.											
4	8.9	0.21	—	—		0	0	0	0.0458	28	2.0 × 0.8
8	8.6	0.43	—	0.3	0.8	0	0	0.5	0.382	800	2.0 × 0.6
10	2.8	2.1	—	1.1	5.7	0	0	1.1	0.699	4900	1.4 × 0.5
12	0.2	0	9.7	1.6	1.8	750	300	0.8	0.912	6000	1.3 × 0.5
12	0.2	0	3.2	0.69	2.2		460	0.7	0.912	7700	1.3 × 0.4
16	7.3	1.36	Not done	0.069	0.6	950	460	1.1	0.912	5000	1.5 × 0.5
16	5.6	0.96	Not done	0.136	1.6	900	510	1.0	0.912	8300	1.5 × 0.4
20	6.9	0.54	Not done	0.136	2.7	1000	460	1.3	1.000	7000	1.1 × 0.5
20	6.2	0.27	Not done	0.136	5.4	1150	600	1.3	1.126	9000	1.0 × 0.5
Stirring Rate 500 r.p.m.											
4	6.7	0.09	—	0.02	2.5	0	0	0.02	0.0605	9	1.7 × 0.9
4	7.7	0.18	—	0.01	0.6	0	0	0.2	0.0757	13	2.0 × 0.8
8	5.5	0.8	—	0.22	3.0	0	0	1.0	0.1427	200	1.9 × 0.7
8	5.1	1.1	—	0.26	2.6	0	0	1.0	0.2291	500	2.0 × 0.7
12	5.0	1.5	6.1	1.8	1.3	0	0	1.1	0.838	9500	
13	0	—	8.1	1.9	2.6	0	24	1.0	0.921	9000	
16	5.1	0.98	—	1.3	14.0	0	0	1.0	0.886	6000	1.3 × 0.5
16	5.8	0.77		1.1	16.0	0	0	1.5		11000	1.4 × 0.6
20	5.6	0.47	—	0.89	21.0	0	10	1.8		13000	0.8 × 0.5
20	8.0	0.7	—	0.98	15.0	0	14	1.0		15000	0.7 × 0.5

in oxygen uptake rate by the original culture strongly suggested deficiency of substrate. In a duplicate series in which the substrate concentration was doubled the oxygen concentration was reduced to "zero" and maintained there until the 20 hr. period. Nitrate completely disappeared.

The failure of the ratio in column 6 to change sharply during the 12-16 hr. period suggested that the nitrite that had accumulated acted adversely on the organism and partially inhibited oxygen uptake in the diluted culture.

Reference to Figure 3 will show that at all oxygen concentrations, both in the original (*B* curves) and diluted (*C* curves) samples, the rate of uptake of oxygen was uniform. This also applies to the 200 r.p.m. and 500 r.p.m. series.

In the 300 r.p.m. series the rate was virtually uniform until the 12 hr. period, i.e. until the appearance of nitrite. Subsequent to this the polarograms take the form shown in Figure 4.

In the original culture (curve *B*) the rate of oxygen uptake diminishes as the oxygen concentration diminishes. In the diluted sample (*C*) the rate is relatively constant for one minute and then gradually increases. This also suggested that nitrite inhibited oxygen utilization.

To test the effect of nitrite the following experiments were conducted:

(i) Cells were grown in Roux bottles on a "Difco" peptone yeast extract agar and washed off with sterile Ringer's solution. The suspension employed contained approximately 10^{10} cells per ml. One ml. of suspension was added per 10 ml. of medium in the polarographic cell. The oxygen uptake curves were recorded with the Cambridge polarograph, in the following media:

(1) The peptone yeast extract medium alone,
(2) The same medium plus 0.2 per cent. KNO_3,
(3) The same medium plus 0.2 per cent. KNO_2.

The result is shown in Figure 5.

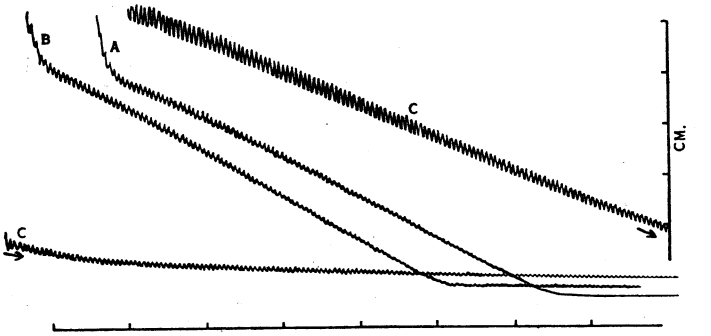

Fig. 5.—Relative rate of oxygen uptake by identical cell suspensions in *A*, broth alone; *B*, broth + nitrate; *C*, broth + nitrite.

There is virtually no difference between the broth alone (*A*) and broth plus nitrate (*B*). The latter was usually a shade faster than the former. With nitrite (*C*), however, the time taken to remove all the oxygen exceeded twice that for broth alone. (Note: In the recording polarograph the paper rotates on

a drum. Since curve *C* was incomplete at the end of one revolution the drum was returned to the starting point for continuation of the curve.)

(ii) Cells were grown, suspended in saline, and washed twice. Two ml. of the cell suspension were added to 16 ml. saline containing 500 p.p.m. nitrite. After varying time intervals 2 ml. of a solution containing 10 per cent. peptone and 5 per cent. yeast extract were added, the solution was mixed and then placed in the polarographic cell and reoxygenated. Oxygen uptake rates were then determined. The results are shown in Figure 6.

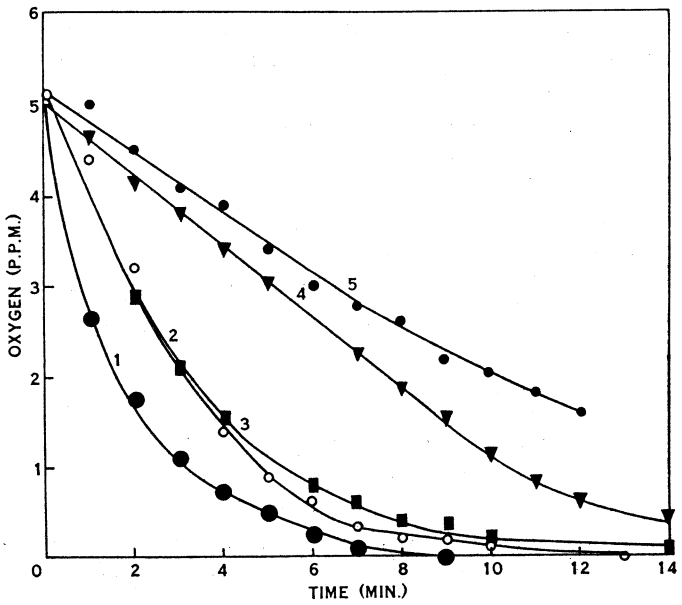

Fig. 6.—Relative rates of oxygen uptake by cells exposed to 500 p.p.m. nitrite prior to inoculation into broth. Curves 1, 2, 3, 4, 5 were obtained with cells exposed for 0, ½, 1, 2, and 4 hr. respectively.

Curve 1 is similar to curve *B* in Figure 4. As the time of exposure increases, the effect of nitrite poisoning becomes more pronounced. This poisoning effect explains the sudden inability of the cells to utilize oxygen in the dilute samples (Table 2, column 5, 200 r.p.m.) after the appearance and accumulation of nitrite in the 300 r.p.m. series and hence explains the absence of higher ratios in column 6 (200 r.p.m.) from the 12 hr. period onwards.

IV. Discussion

The work reported in this paper emphasizes the need for correlating nitrate reduction with substrate availability and oxygen concentration. Although unadapted cells were used for an inoculum, the reduction of nitrate, once the

oxygen concentration was reduced to "zero," was not long delayed. In view of the fact that "aerobic" conditions depress the formation of nitrate-reducing enzymes, one would not expect to find much, if any difference between cultures inoculated with adapted and unadapted cells. The initial population used in these experiments, 10^7 cells per ml., forms only about 2 per cent. of the cells present at the time the oxygen concentration reaches "zero." Ninety-eight per cent. of the population at this stage developed largely under conditions that would preclude enzyme formation.

According to Sacks and Barker, the nitrite-reducing enzyme(s) are not produced until the oxygen concentration is reduced to a level in equilibrium with 5 per cent. in the gas phase (approx. 2 p.p.m. at 25°C.). Even at this level they report 50 per cent. inhibition of activity. An increase in nitrite-reducing activity that would parallel a further reduction in oxygen concentration on the one hand and increase in cell mass (in growing cultures) on the other, would be expected to result in an increase in the amount of nitrite reduced in a given time interval. However, in actively growing cultures the time elapsing between the establishment of conditions satisfactory for the formation and function of the enzyme and the depletion of oxygen from the medium is so small that one could expect little, if any, nitrate reduction to occur during this period.

An important aspect of bacterial metabolism that seems to be little appreciated is the fact that an organism may use two alternative respiratory mechanisms at the same time, provided conditions are satisfactory for both. In the foregoing experiments it is obvious that oxygen must still be entering the solution and be utilized by the cells even though the oxygen concentration in solution is at a "zero" level, which permits simultaneous utilization of nitrate.

It seems possible that the so-called oxygen inhibition of nitratase activity may be more apparent than real. Information on this point has been gained by comparing the amount of nitrate reduction occurring under various "aerated" conditions with that occurring under strictly anaerobic conditions. Exactly similar curves to those reported, for instance, by Sacks and Barker could be obtained by gradually increasing the amount of oxygen entering a culture having an initial "zero" oxygen tension. The non-aerated culture would represent the "anaerobic condition." As aeration increases, the competitive action between oxygen and nitrate would, according to Stickland's observations, result in greater utilization of oxygen in preference to nitrate. The quantity of nitrate decomposed would diminish as the amount of available oxygen was increased, whilst the concentration of oxygen in solution at all times remains at "zero."

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VI. References

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