SHORT COMMUNICATION

Penetration of Oxygen into Bacterial Colonies

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Previous estimates of the depth of oxygen penetration into bacterial colonies were made after measuring actual and potential respiration rates of whole colonies, or by calculation from kinetic values determined from the growth of bacteria in liquid culture. This paper reports the use of microelectrodes to measure oxygen penetration directly. Oxygen became undetectable 25–30 μm below the surface of a 120 μm deep, 18 h colony of *Bacillus cereus*. The colony was grown on a nutrient-rich agar medium incubated at 30 °C in a water-saturated atmosphere.

INTRODUCTION

The bacterial colony is a heterogeneous structure in which organisms proliferate in gradients of growth-supporting solute molecules. In aerobically grown colonies, nutrients diffuse upwards from the agar whilst oxygen diffuses downwards into the structure from the atmosphere. The depth to which oxygen penetrates determines the physiological behaviour of organisms in the colony.

Pirt (1967) has estimated values for oxygen penetration into bacterial colonies using data derived from liquid culture experiments. For colonies of *Escherichia coli* growing at their maximum specific growth rate on a glucose/salts medium, it was concluded that oxygen should penetrate to a depth of 40 μm , whilst this value rises to 127 μm under maintenance conditions.

Wimpenny & Lewis (1977) calculated oxygen penetration after investigating the respiration of whole colonies. Assuming that an aerobic outer layer of the colony was responsible for the measured respiration of the whole colony, it was calculated that oxygen penetrated to a mean depth of 31 μm for *E. coli*, 41 μm for *Enterobacter cloacae* and 37 μm for *Bacillus cereus*. The value for *Staphylococcus albus* was much lower at 9 μm and this was attributed to a lower rate of diffusion through and between tightly-packed spherical organisms.

Despite previous calculations and estimates, no direct measurements of oxygen penetration have been made so far. In this paper, we report the use of microelectrodes to determine partial pressures of oxygen in and around a colony of *B. cereus*.

METHODS

Growth and maintenance of organisms. *Bacillus cereus* C11 was obtained from the departmental culture collection and maintained at 4 °C on slopes of TSBA medium containing (g l^{-1}): tryptic soy broth (Difco), 30; agar (Difco), 15. Petri dishes (9 cm diameter) containing 20 ml TSBA medium were prepared on a level surface and dried at 37 °C for 1–1.5 h. The plates were inoculated as follows: a freshly-drawn glass needle made from a Pasteur pipette was inserted into the surface growth of a confluent lawn plate of organisms and then allowed to touch the surface of the sterile agar without penetrating it. One to three colonies per plate were initiated in this way. After inoculation, the plates were incubated in a water-saturated atmosphere at 30 °C.

Measurement of oxygen. An oxygen-sensitive microelectrode (Model 723, Transidyne General Corporation, Ann Arbor, Mich., U.S.A.) was mounted on a Prior micromanipulator and lowered at measured intervals into the colony in a Petri dish on a level board. It was possible to lower the electrode at intervals which were accurate to 5 μm . The oxygen microelectrode and a silver/silver chloride reference electrode inserted into the agar were connected to a Transidyne chemical microsensor (Model 1201).

Calibration of the electrode. Before measurements were made, the microelectrode was lowered into a 0.4% (w/v) agar gel containing 4 ml amorphous ferrous sulphide slurry l^{-1} (the slurry contained approximately 2 g sulphide l^{-1}) (Brock & O'Dea, 1977) and adjusted to read 0% partial pressure of oxygen. Before each set of readings, the electrode was lowered so that it just touched the uninoculated surface of the agar. The latter was assumed to be fully saturated with air and the microsensor was adjusted to read 100% partial pressure of oxygen.

Interpretation of data. Oxygen microelectrodes will drift slightly, even after an initial stabilization period. This particularly affected the determination of the zero reading when oxygen was absent. To determine the extent to which electrode drift was significant, a comparison of measurements was made between those in a 0.4% (w/v) agar gel containing 4 ml amorphous ferrous sulphide slurry l^{-1} (as above), and what was assumed to be the anaerobic region of a 72 h colony of *B. cereus* C11. Twelve measurements were made alternately in ferrous sulphide and the colony. The degree of drift was noted, and the microsensor readjusted if necessary.

For $n = 6$ measurements in ferrous sulphide, $\bar{x} = 1.167$ (S.D. = 1.17); and for $n = 6$ in the colony, $\bar{x} = 1.0$ (S.D. = 1.79). Using 'Student's t test', $t = 0.35$ showing that there was no significant difference between each set of readings.

Measurement of colony profile. The microelectrode was located at a fixed position above the centre of the colony using the coarse control of the micromanipulator. The electrode was lowered from this position (using the fine control of the micromanipulator) until it just touched the surface of the colony. The distance from the original fixed position was noted. This procedure was repeated at measured intervals across one diameter of the colony and in part of the agar outside it.

RESULTS

The partial pressure of oxygen was measured in a colony of *B. cereus* and in the underlying agar medium. Figure 1 shows a series of individual oxygen profiles at different points across the colony. Oxygen tension dropped gradually in the agar near the edge of the growing colony, and much more rapidly when the electrode passed through the outer edge of the structure. Nearer the centre, oxygen tension dropped to zero both inside the colony and in the agar beneath it. Sub-surface anoxia was most pronounced at the centre of the colony.

The results for a series of electrode measurements can be superimposed on the profile of a colony growing on an agar surface. Each measurement is indicated in Fig. 2 as a dot. Isopleths of oxygen partial pressure were constructed by joining regions of similar oxygen tension.

The following observations may be made: although the colony was 120 μm high at its centre, oxygen only penetrated to a depth of 25–30 μm . This appeared to be approximately true across most of the colony. The exact pattern of oxygen penetration was less clear in the leading edge region; oxygen may not penetrate as far in this region due to the high metabolic rates of organisms growing where nutrients are in excess (Wimpenny, 1979).

DISCUSSION

Microelectrodes have been used by animal physiologists since 1954 (Thomas, 1978) but have found few applications in microbiology or ecology. There are some notable exceptions. A number of studies have been done on marine sediments using microelectrodes (Jorgensen *et al.*, 1979; Sorensen *et al.*, 1979; Revsbech *et al.*, 1980*a, b*; Revsbech *et al.*, 1981). Microelectrodes have been used to determine oxygen profiles in microbial film (Whalen *et al.*, 1969; Bungay & Chen, 1981; Chen & Bungay, 1981) and by Wall & Bellinger (1982) to study pH and pO_2 in marine microenvironments. In this paper, we report the use of microelectrodes to determine pO_2 in and around a bacterial colony.

Oxygen was found to penetrate to a depth of 25–30 μm in an 18 h colony of *B. cereus* growing aerobically on a tryptic soy broth agar plate at 30 °C. This value contrasts with a mean value of 37 μm obtained by Wimpenny & Lewis (1977) from calculations based on actual and potential respiration rates of colonies. The mathematical calculations of Wimpenny & Lewis were based on the assumption that the shape of a non-spreading bacterial colony can be regarded as the segment of a sphere. Whilst this was a reasonable assumption, it was not strictly accurate and may account for part of the difference between observed and calculated values.

Further work will measure the penetration of oxygen into colonies of three different bacteria, *B. cereus*, *E. coli* and *S. albus* as a function of time, temperature and nutrition. Microelectrodes will also be used to measure respiration rates and oxygen diffusion coefficients in these colonies.

Fig. 1. Oxygen profiles in and around an 18 h colony of *B. cereus* incubated on TSBA in a water-saturated atmosphere at 30 °C. pO_2 is expressed as a percentage of the air-saturated value. The profiles A–F correspond to the labelled lines of points in Fig. 2.

Fig. 2. The distribution of oxygen in and around an 18 h colony of *B. cereus* incubated on TSBA in a water-saturated atmosphere at 30 °C. Values shown are a percentage of the air-saturated value, and isopleths connect points of similar partial pressure.

REFERENCES

- BROCK, T. D. & O'DEA, K. (1977). Amorphous ferrous sulfide as a reducing agent for culture of anaerobes. *Applied and Environmental Microbiology* **33**, 254–256.
- BUNGAY, H. R. & CHEN, Y. S. (1981). Dissolved oxygen profiles in photosynthetic microbial slimes. *Biotechnology and Bioengineering* **23**, 1893–1895.
- CHEN, Y. S. & BUNGAY, H. R. (1981). Microelectrode studies of oxygen transfer in trickling filter slimes. *Biotechnology and Bioengineering* **23**, 781–792.
- JORGENSEN, B. B., REVSBECH, N. P., BLACKBURN, T. H. & COHEN, Y. (1979). Diurnal cycle of oxygen and sulfide microgradients and microbial photosynthesis in a cyanobacterial mat sediment. *Applied and Environmental Microbiology* **38**, 46–58.
- PIRT, S. J. (1967). A kinetic study of the mode of growth of surface colonies of bacteria and fungi. *Journal of General Microbiology* **47**, 181–197.
- REVSBECH, N. P., JORGENSEN, B. B. & BLACKBURN, T. H. (1980a). Oxygen in the sea bottom measured with a microelectrode. *Science* **207**, 1355–1356.
- REVSBECH, N. P., SORESEN, J., BLACKBURN, T. H. & LOMHOLT, J. P. (1980b). Distribution of oxygen in marine sediments measured with microelectrodes. *Limnology and Oceanography* **25**, 403–411.
- REVSBECH, N. P., JORGENSEN, B. B. & BRIK, O. (1981). Primary production of microalgae in sediments measured by oxygen microprofile $H^{14}CO_3^-$ -fixation, and oxygen exchange methods. *Limnology and Oceanography* **26**, 717–730.
- SORESEN, J., JORGENSEN, B. B. & REVSBECH, N. P. (1979). A comparison of oxygen, nitrate, and sulfate respiration in coastal marine sediments. *Microbial Ecology* **5**, 105–115.
- THOMAS, R. C. (1978). *Ion Sensitive Intracellular Microelectrodes*. London: Academic Press.

- WALL, P. & BELLINGER, E. G. (1982). The application of microelectrodes in the study of pH and pO_2 in marine microenvironments. *Laboratory Practice* **31**, 889–892.
- WHALEN, W. J., BUNGAY, H. R. & SANDERS, W. M. (1969). Microelectrode determinations of oxygen profiles in microbial slime systems. *Environmental Science and Technology* **3**, 1297–1298.
- WIMPENNY, J. W. T. (1979). The growth and form of bacterial colonies. *Journal of General Microbiology* **114**, 483–486.
- WIMPENNY, J. W. T. & LEWIS, M. W. A. (1977). The growth and respiration of bacterial colonies. *Journal of General Microbiology* **103**, 9–18.