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A new O_2-microelectrode and its application

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Abstract—A new oxygen microelectrode was introduced. An internal guard cathode was added besides its normal cathode sensor. It has a low zero measuring current, high signal stability and its easy to construct. As it is small in size and high in stability, it may be used not only for routine environmental application, but for other scientific research work as well.

Keywords: O_2-concentration; measurement; microelectrode; application.

Among the fundamental goals of microbial ecology is the development of methods that will enable the identification and counting of the important microorganisms in nature, the determination of their physical and chemical micro-environment, and the analysis of their metabolic processes and interactions. Due to the small size of organisms, it needs to develop high-resolution techniques for the observation and understanding of the world of bacteria on a microscale. The O_2-microelectrode is one which has been developed on this point.

Oxygen electrodes or oxygen sensors are already used quite extensively in many scientific fields and routine applications (Thomas, 1978). Nevertheless, they would be used more readily if they were developed to have a micro-size measuring tip and were easier to construct. Now, a new oxygen microelectrode has been introduced in some countries. The microelectrode contains two cathodes: a guard cathode which removes all the oxygen diffusing towards the tip from the internal electrolyte reservoir, and a sensing cathode. The microelectrodes exhibit better stability of the signal than do the older types of sensors. Furthermore, new procedures facilitate the construction of these microelectrodes.

1 The structure of the microelectrode and its features

The new microelectrode consists of a sensing cathode, a guard cathode, a Ag/AgCl anode, internal electrolyte and some glass tubes (Fig. 1). It is based on the oxygen microelectrode described by Revsbech and Ward (Revsbech, 1983). The major difference is the insertion of a tapered silver wire cathode with its tip 50—200μm from the tip of the oxygen-sensing cathode. The tip of the silver wire was shaped by electro-etching in 1 mol/L KCN solution.

The tip region of the sensing cathode was made of platinum covered with Schott 8533 glass.

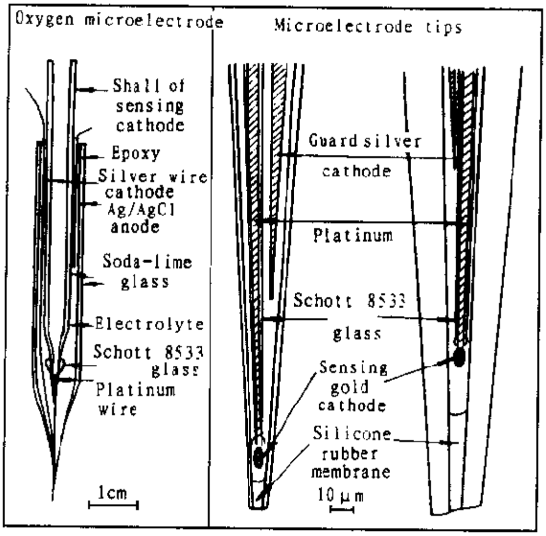

Fig. 1 O_2-microelectrode and its tip

This glass was used because of its good insulating characteristics and its stability under alkaline conditions. Etched platinum wires were inserted into glass capillaries of 8533 glass before these capillaries were fused in a flame with shafts of soda-lime glass (Schott 8418). The 8533 glass melts at a temperature about 70℃ lower than does soda-lime glass, so that fusion where the 8533 glass was outside the sodalime glass was very easy to make. The low melting point of the 8533 glass also facilitated glass coating of the platinum tip. When coated with glass, the platinum tip was exposed by bringing a hot platinum heating loop close to the tip. The glass thereby receded 10—20μm behind the platinum tip. After that, the exposed platinum tip was inserted into a saturated solution of $HAuCl_4 \cdot 4H_2O$ and electroplated with gold at 0.6V.

By introducing the guard cathode, the outer casing can be made very wide compared to the sensing cathode. One of the problems with the construction of Clark-type oxygen microelectrodes was the need to insert slim cathodes into slim outer casing to ensure a low diffusion supply of oxygen to the cathode tip from the electrolyte reservoir and hence a reasonably low residual (zero) current when exposed to anoxic environments. When a guard cathode was inserted the zero current remained low even if there was plenty of space between cathode and casing.

When the zero current is minimized and stabilized by using a guard cathode, the current in the measuring circuit is dependent only on the diffusion supply of oxygen to the surface of the membrane. Diffusion is a very efficient transport process over small distances. For this reason neither substrate uptake by free-living small bacteria, nor the oxygen signal from small microelectrodes is significantly affected by adjective transport ("stirring") of the medium. In fact, sensitivity to stirring is not only dependent on the size of the oxygen consuming sensor tip, but also on the oxygen concentration on the surface of the silicone membrane. The oxygen consumption of the sensor and hence also the sensitivity to stirring can by decreased by increasing the distance between the oxygen consuming cathode and the sensor tip. Sensors with long distances between tip and cathode, however, respond slowly to changes in oxygen concentration. The thin microelectrode tip shown in Fig. 1 had a 10μm thick membrane. The sensor with this tip gave currents for N_2, air and O_2, respectively of 11, 290 and 1347 pA when both the guard and the sensing cathode were polarized at 0.8V. Without a guard cathode, the current for N_2 was about 200 pA, and this high zero current varied continuously.

The primary reason for the variable zero current was a decrease in the O_2 concentration of the internal electrolyte due to the O_2 consumption of the cathode. The 90% response time of the microelectrode and the circuit for measuring changes in oxygen concentration was 0.4s. The current was 4% higher in vigorously stirred, aerated water than in stagnant water, and that deviation is unsatisfactory for some applications. Now, fast-responding microelectrodes have been manufactured with a lower effect of stirring. Such low stirring effects were achieved by reducing the respect to the thin tip shown in Fig. 1, a reduction in diameter of a factor of two (i.e., from about 3 μm to about 1.5 μm) would substantially decrease the discrepancy found between stagnant and stirred water. This action would also result in a lower current in the measuring circuit; yet even a reduction to 20% of the original value would give a signal which could be amplified without the problem of electrical noise. The alkaline resistance of the Schott 8533 glass makes it possible to use an internal electrolyte with a high pH, but such solutions can only be used in sensors with relatively thick and durable silicone membranes. A reasonable compromise between relatively high pH and tolerable decomposition rates of microelectrode components is obtained by a pH 10.3 electrolyte containing 0.5 mol KCl and buffered with 0.5 mol HCO_3^-/CO_3^-. A sensor with a 24μm thick membrane and filled with this electrolyte still worked after 24 days of continuous operation in aerated water, and the small variations in signal during that period could be explained by variations in temperature (19.7—22.1℃) and barometric pressure.

Often O_2-microelectrodes with guard cathodes have almost undetectable zero currents of less than 1 pA. The use of another highly insulating glass (Schott 8512) resulted in sensors with extremely low zero currents, but cathodes made from this glass are more fragile. Details of making of O_2-microelectrodes were described by Bai Aimin (Bai, 1992).

In Fig. 1, it is also shown a thick sensor tip made especially for use in coarse sediments. Such kind of tips were formed following the ways described by Revsbech and Jørgensen (Revsbech, 1986).

2 Principle of O_2-microelectrode

An electrode system (an electrode for short) is composed of an electron leading phase (anode and the cathode) and a ion leading phase, which is normally called the electrolyte (Fig.2).

In the electrolyte the charged particles are influenced by a force, which can be described as the product of the particle charge and the negative gradient of the potential field.

For further understanding of the electrochemical system it is necessary to consider the standard state. A standard state of a given electrode couple is the condition where activities of all the participating species in the electrochemical reaction are at unit activity at a temperature of 25℃. The equilibrium potential of this system under these conditions is the standard electrode potential, E_0, and is equal to the

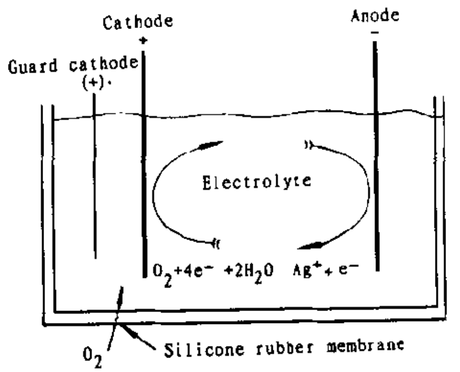

Fig.2 Schematic electrolytic cell reactions in microelectrode

electro-motorial force of a cell composed of the given electrode and a standard hydrogen electrode.

The electrode reaction for the oxygen electrode is

$$O_2 + 2H_2O + 4e^- \Leftrightarrow 4OH^-, E_0 = 0.401V,$$

in alkaline solutions, if the oxygen couple is measured against the Ag/AgCl anode

$$Ag(s) \Leftrightarrow Ag^+ + e^-, E_0 = 0.779V,$$

it is seen that the overall cell reactions are

$$O_2 + 2H_2O + 4e^- \Rightarrow 4OH^-$$

and $$4OH^- + 4Ag^+ \Rightarrow 4AgOH$$

and $$4Ag(s) \Rightarrow 4Ag^+ + 4e^-.$$

These reactions require the transfer of 4 electrons, which are provided by the anode. In this particular case the reaction takes place on the surface of the golden cathode tip, which itself is inert to the chemical system. Thus, the cathode is merely a catalytic surface. The cell system and the reactions are shown in Fig.2.

In fact oxygen interacts with all metal surfaces, but the noble metals make the closest approximation to an inert electrode material. Most work has been carried out on Pt, though some research has concerned Au and Pd electrodes.

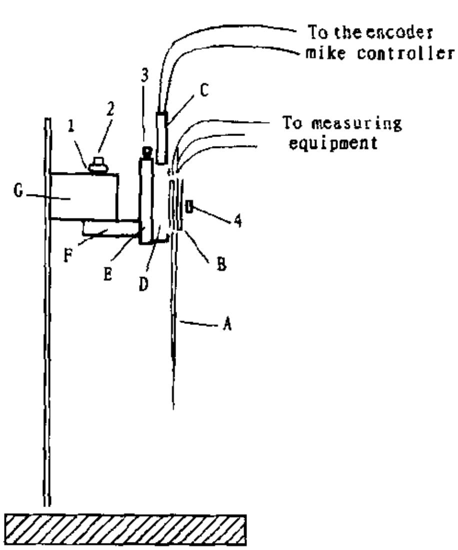

Fig.3 The microelectrode and its bearing part for measurement

1. knob which can adjust motor-hold position leftward and rightward;

2. knob which can adjust motor-hold position forward and backward;

3. knob which can make motor up and down;

4. Clamp which holds microelectrade

A. microelectrode; B. electrode-holder; C. motor; D. motor-holder; E. motor beating; F. motor beating; G. motor beating

3 The way to use a O_2-microelectrode

Basically, one should have two electric cell boxes, a current meter of Keithly Picoammeter Model 485, a manipulator of Marzhauser (type MD4), an Oriel motor (1-1825AF) and an Oriel controller (Encoder-Mike Controller Model 1-18011) in his measurement system (Bai, 1996). He may have a recorder to collect measuring data, or he could use a computer to collect data and do data treatment work automatically. The simplest way is to record every data from Picoammeter's display screen manually. After that, treat the data in a computer and get expected figures from it. The electric cells is used to polarize sensing cathode and guard cathode. The manipulator, Oriel motor and Oriel controller are necessary to hold a microelectrode and control its going into the measuring media slowly. The step length of Oriel motor can be adjusted to meet the measure needs.

As the measure media could be quite different, the way to use microelectrode is also different. But the purpose to use O_2-microelectrode is to measure micro-size oxygen profile, it is necessary to setup a specific measuring system to meet a specific requirement. In our research, we have successfully used O_2-microelectrode (which is also constructed in the same lab) and a specific system to proceed a measure of O_2 profile in biofilm ($\leqslant 200\mu m$ thick, Fig.3 and Fig.4).

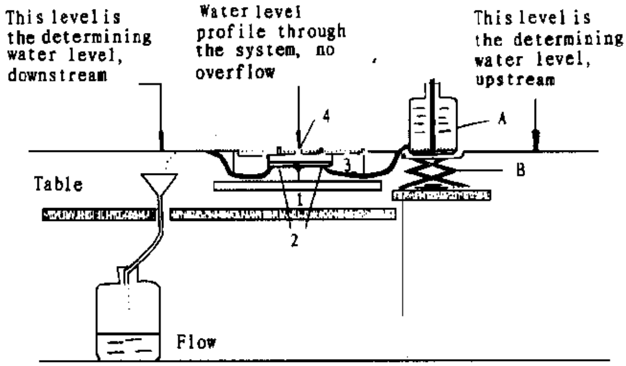

Fig.4 Experimental setup used for measuring O_2-profile in biofilm

4 Application of O_2-microelectrodes

The ability to construct microelectrodes with an eligible effect of stirring may be an advantage in several fields. The most common application of oxygen microelectrodes in environmental research is the measurement of oxygen profiles across substrate-water interfaces, where there is a transition from stirred to stagnant conditions, and often also a pronounced decrease in diffusivity. Because of a decrease in adjective transport of oxygen to the microelectrode tip, stirring-sensitive microelectrodes will show an erroneous decrease in oxygen concentration across such interfaces. A lower diffusivity of oxygen within the substrate as compared to water will cause an additional erroneous decrease in the signal from a stirring-sensitive microelectrode. These problems associated with the use of oxygen microelectrodes near interfaces were described by Gust *et al*. (Gust, 1987), who used microelectrodes with very high stirring effects (20%—50%). Such microelectrodes are not representative for ones in this paper, but stirring effects of a few percentage are often encountered.

In a research of biofilm waste water treatment, it is important to know O_2 profile in biofilm located in a reactor. When we master the skill of doing oxygen measurement in the biofilm, growth of the biofilm can be controlled and efficiency for treating waste water can be increased. When utilizing technology of biofilm waste water treatment, as limited by thickness of biofilm, prediction is often done theoretically in some cases. By the use of new microelectrode, it can not only do proof work to theory, but find the best status for biofilm in water treatment. Denmark Technical University has advanced in this field.

Oxygen is related directly with so many other factors, such as in photooxidation reaction. So when the oxygen profile has been obtained, we might understand related factors as luminous flux in micro-environment.

Microelectrodes with tips similar to the thick one in Fig. 1 would be sufficiently stable and mechanically rugged for applications where large oxygen sensors are normally used. In oceanography and limnology, polarographic oxygen sensors are used in benthic flux chambers and other submersible monitoring devices, and the ability of the microelectrodes to measure oxygen without a requirement for stirring (i. e., power consumption and disturbance of natural water stratification) may be an advantage.

5 Conclusion

Researchers in the fields of microbial ecology and other micro-environment study are paying more and more attention to oxygen microelectrodes. It is very important to understand the microbial micro-environments and the nature of the microorganisms that carry out the measured metabolic activities. Due to the small size of organisms, it is needed to have a high-resolution technique for the observation and understanding of the world of microscale. Along with development of a new type of oxygen microelectrode, which has a very stable measure signal and is easy to construct, it can be used in researches and applications of many fields related with micro-environment. Even if it needs some special tools and devices to construct and it needs to setup special system to do measurement, it will, of cause, improve our research work in micro-environments when introduced to our country.

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