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A hydrogen peroxide microelectrode to use in bioelectrochemical systems

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ABSTRACT

Polarized electrodes are used in bioelectrochemical systems (BES) in which the electrodes are used to grow biofilms or remove biofilms. In either case H_2O_2 is generated as an intermittent product of oxygen reduction. Traditionally, a H_2O_2 microelectrode (HPM) consists of a sealed platinum wire with a tip a few μm in diameter. This microelectrode is connected to an external reference electrode (which is also used as the counter electrode) and polarized to +0.8 $V_{Ag/AgCl}$ to measure H_2O_2 concentration. This traditional H_2O_2 microelectrode (tHPM) works well for measuring H_2O_2 concentrations in biofilms growing on non-polarized surfaces. It is a common misconception that this tHPM will work correctly in an electric field. We observed experimentally that the tHPM produces artifacts in measurements when it is used in BES. This has not been noticed before. These undesired effects needed to be resolved before H_2O_2 concentration could be measured in BES. The goals of this work were to (1) describe the artifacts related to the use of tHPM in BES, (2) develop a H_2O_2 microelectrode which does not suffer from these artifacts, (3) verify the operation of the new microelectrode in BES, and (4) improve the sensitivity of the microelectrode. In this work, we built an all-in-one HPM (aHPM) in which the reference electrode and the working electrode were placed a few 100 μm away from each other in a sealed configuration. We verified that our aHPM, unlike the tHPM, is not affected by an interfering electric field and generates reproducible, correct measurements in BES. We compared H_2O_2 flux measurements from the electrode in a BES with depth profiles measured using aHPM and tHPM to identify artifacts and reliability. Finally, after proving the reliability of the aHPM in BES, we improved its sensitivity. The aHPM demonstrated a linear calibration in the range of 0.87 μM to 166.8 μM H_2O_2 concentration with a 1.69 μM limit of detection ($S/N = 2$). Although our goal was to develop an HPM which can operate in an electric field, the developed microelectrode can be used in many other applications requiring μM resolution.

1. Introduction

The microelectrodes used in biofilm research are needle-type electrodes with tips a few μm in diameter, and they are used to quantify microscale gradients noninvasively [1,2]. The traditional H_2O_2 microelectrode (tHPM) (Fig. 1A) consists of a sealed platinum wire with a tip diameter of 10–20 μm. The flat tip is coated with a cellulose acetate membrane. This glass-coated platinum wire is connected to an external reference electrode (RE) and polarized to +0.8 V against an external Ag/AgCl RE. The external RE is also used as a counter electrode (CE) since the current passed between the RE and the working electrode (WE) is very low and is not enough to damage the RE. The tHPM works well for measuring high H_2O_2 concentrations in biofilms growing on glass surfaces. Several studies have measured H_2O_2 concentrations in biofilms using tHPMs. For example, Liu et al. [3] investigated the penetration of H_2O_2 into biofilms with and without catalase inhibitor. Stewart et al. [4] measured the penetration of H_2O_2 into biofilms of wild-type and catalase-deficient *Pseudomonas aeruginosa* strains. We should note that in these studies the H_2O_2 concentrations were in the mM range (0–88 mM).

An interesting newer application of tHPMs is measuring low levels (μM) of H_2O_2 generated electrochemically on polarized-inert electrode surfaces. The inert electrodes are used to study the oxygen reduction mechanism, and one of the by-products of this reaction is H_2O_2. Traditionally, a rotating ring disk electrode [5–8] or a channel flow double electrode [9–11] is used to detect H_2O_2 from the oxygen reduction reaction; however, these cannot be used to detect H_2O_2 in biofilms growing on electrodes. Biofilms growing cathodically on polarized surfaces, or cathodic biofilms (biocathodes), can reduce oxygen during their growth phase [12,13]. In some

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Nomenclature

aHPM	all-in-one H_2O_2 microelectrode
Bare-HPM	bare/flat microelectrode tip
BES	bioelectrochemical system(s)
CE	counter electrode
HPM	H_2O_2 microelectrode
RE	reference electrode
Pt-Pt-HPM	platinized platinum microelectrode tip
tHPM	traditional H_2O_2 microelectrode
WE	working electrode

cases, to reduce cell attachment to a metal surface or to remove biofilms from a surface, the metal electrode is polarized to a potential at which oxygen is reduced to generate H_2O_2 [14]. Knowledge of the H_2O_2 concentrations in these biofilms and on these metal surfaces is critical to understanding the defense mechanism of biofilms against electrochemically produced H_2O_2 and to evaluating biocathode performance in microbial fuel cells. However, microelectrode measurements on polarized surfaces are difficult because of the interfering electric field caused by the external field [15]. For example, Babauta et al. [13] showed that the traditional pH microelectrode with separate working and reference electrodes measures unrealistic pH values in cathodic biofilms. For correct measurements, the distance between the pH microelectrode and the RE is minimized to a few hundred micrometers [13]. In some cases, the problem can be minimized by calibrating the microelectrode in the reactor where measurements are performed. For example, Wang et al. [16] calibrated pH and redox potential microelectrodes at the operating potential of the polarized electrode to offset the disturbance of the electric field.

The effect of the electric field becomes more problematic when the microelectrode measurements need to be coupled with voltammetric analysis such as cyclic voltammetry (CV). In this case, to use a tHPM, it is necessary to calibrate at all the potentials at which the potential scan is employed. This is not practical. In some cases, the reactor configuration does not allow the RE to be placed close to the WE and the distance between WE and RE may lead to erroneous readings due to the presence of an electric field. To avoid these problems, the distance between microelectrode tip and RE should be minimized so that the measurements are not affected by the operating potential of the polarized electrode.

The electrochemical reactions used to detect H_2O_2 are:

Working electrode (platinum tip):

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (1)$$

Reference electrode:

$$2AgCl + 2e^- \rightarrow 2Ag + 2Cl^- \quad (2)$$

The rate of H_2O_2 oxidation (current) is linearly correlated with the H_2O_2 concentration in the vicinity of the tip. The current is also controlled by the polarization potential of the WE, which is the platinum tip. One of the factors affecting the polarization potential is the distance between the WE and RE electrodes. For example, Klett et al. [17] used Eq. (1) to describe the open circuit potential difference between WE and RE (ΔE (V)) as a function of the distance from the electric field [17]:

$$\Delta E = \frac{RT}{nF} \ln\left(\frac{\varepsilon_{ref}}{\varepsilon_{work}}\right) \quad (3)$$

where ε_{ref} and ε_{work} are the distances (cm) of RE and WE from the electric field, respectively, R (J mol^{-1} K^{-1}) is the gas constant, T (K) is the temperature, n is the number of electrons transferred, and F (C mol^{-1}) is the Faraday constant. According to Eq. (3), when WE and RE are located 6 or 12 cm from the electric field, ΔE is 23 or 32 mV, respectively. The deviations in the equilibrium potential for the tHPM affect the overpotential, as shown in the following equation [18]:

$$E_{app} = E_{eq} + \eta - iR_s \quad (4)$$

where E_{app} is the applied potential (V), E_{eq} is the equilibrium potential (V), which is the equilibrium value at open circuit, η is the overpotential (V), i is the current (A), and R_s is the solution resistance. However, for microelectrodes passing small currents (on the order of nA), the iR_s term is negligibly small. Since the limiting current is observed between +500 and +1000 $mV_{Ag/AgCl}$ (Fig. SI1), small variations in the equilibrium potential or applied potential are not expected to affect the measured current or measured H_2O_2 concentration. Interestingly, several studies have reported that the interference of the electric field induces a shift in the half-wave potential of the redox couples which results in poor reproducibility due to the sensitivity to the position of the working electrode

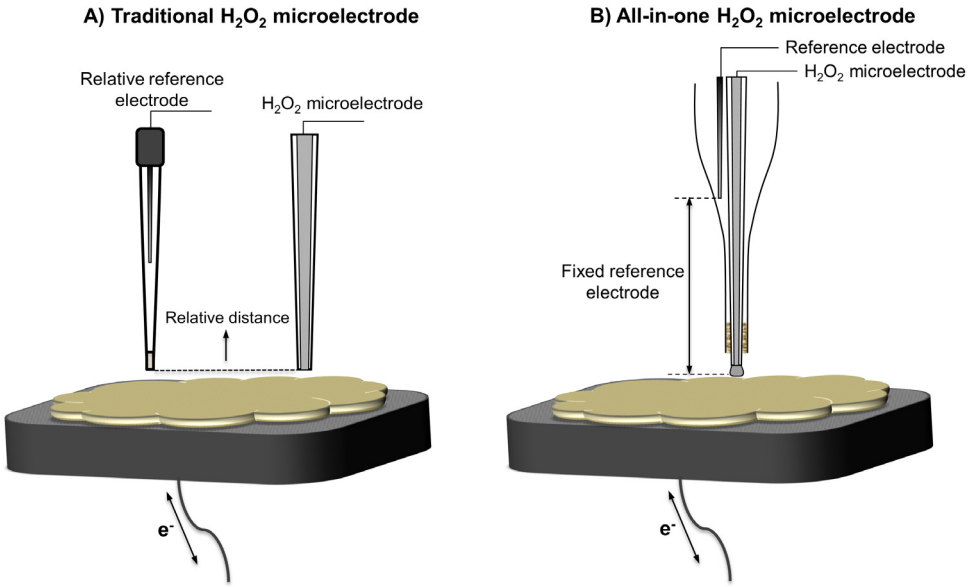

Fig. 1. Diagram of (A) the tHPM and (B) the proposed aHPM for use in biofilm studies.