

# Iridium Oxide pH Microelectrode

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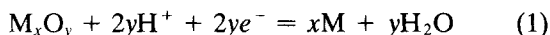
The manufacture, calibration, and signal conditioning during construction of an iridium/iridium oxide pH microsensor is described. The microsensor was designed to be used extracellularly, primarily in biofilm research. The sensing tip diameters were typically in the range of 3–15  $\mu\text{m}$ . The iridium oxide was formed by potential cycling in dilute sulfuric acid. A pH profile across a denitrifying biofilm was measured as an example of an application. The higher Nernstian slope (70–80 mV/pH for fresh electrodes), increased rigidity, and restriction of the sensing tip to the outermost end of the electrode are features which make the iridium/iridium oxide pH microelectrode superior to a glass microelectrode. © 1992 John Wiley & Sons, Inc.

Key words: pH measurement • microelectrodes • microenvironments • biofilms

## INTRODUCTION

Microscale measurements of pH in physiology, medicine, microbial ecology, and environmental studies<sup>31,33</sup> require microelectrodes with tip diameters below 10  $\mu\text{m}$  that are sturdy enough to resist breakage during penetration of tissue or when positioned at a hard surface. Fragility is the major drawback of pH sensing glass microelectrodes. Shielding to protect the glass increases total tip diameter and limits applications.<sup>21</sup> In this article we discuss the construction and application of a metal–metal oxide pH microelectrode that is more rigid than the pH sensing glass electrode.

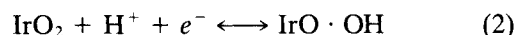
The potential of a reversible metal–metal oxide electrode  $\text{M}/\text{M}_x\text{O}_y\text{H}^+$  (aq), is determined by the reaction<sup>13</sup>



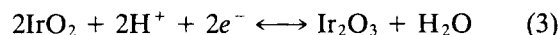
Ives and Janz<sup>15</sup> indicated that the metal of a properly working metal–metal oxide electrode must be sufficiently noble to resist attack by all solutions in which it is to be used. The metal oxide must also be stable. The latter statement appears to contradict the former because the more noble the metal, the less stable its oxide. The first successfully applied metal–metal oxide electrode was the antimony electrode developed by Uhl and Kestranek.<sup>34</sup> While there are a number of applications for the antimony pH electrode,<sup>18,34</sup> it is subject to interference by oxygen.<sup>17</sup> Materials that can also be used for pH electrodes

are  $\text{IrO}_2$ ,  $\text{TiO}_2$ ,  $\text{RuO}_2$ ,  $\text{RhO}_2$ ,  $\text{SnO}_2$ ,  $\text{Ta}_2\text{O}_5$ ,  $\text{OsO}_2$ , and  $\text{PtO}_2$ .<sup>12</sup> Electrodes based on  $\text{IrO}_2$  offer the best behavior based on fundamental electrochemical studies.<sup>13</sup>

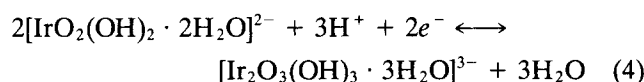
Iridium oxide electrodes can be prepared by sputtering<sup>16,32</sup> and thermal<sup>1,3,27</sup> or electrochemical oxidation of iridium wire.<sup>6,14,24,29,30</sup> Electrochemical oxidation of iridium substrate is the most frequently used technique and involves cycling iridium in sulfuric acid at potentials between  $-0.25$  and  $+1.25$  V saturated calomel electrode (SCE). The procedure forms an iridium oxide that is pH sensitive.<sup>24</sup> Sputtering and thermal oxidation produce predominately anhydrous iridium oxide  $\text{IrO}_2$ , while electrochemically oxidized iridium produces predominately hydrated  $\text{IrO}_2$  ( $\text{IrO}_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{Ir}(\text{OH})_4 \cdot 2\text{H}_2\text{O}$ ,  $[\text{IrO}_2(\text{OH})_2 \cdot 2\text{H}_2\text{O}]^{2-} \cdot 2\text{H}^+$ , etc.).<sup>6</sup> Anhydrous  $\text{IrO}_2$  responds to pH changes with a slope of  $\approx 59$  mV/pH unit. The mechanism of response can be explained as



or



Hydrated iridium oxides present super-Nernstian responses explained by mechanisms predicting 1.5 electrons transferred per  $\text{H}^+$  with a slope of  $\approx 90$  mV/pH unit<sup>6,7</sup>:



Iridium oxide pH sensors are typically used for electrochemical studies. This article presents a concise description of construction and application of an iridium oxide pH-sensitive microelectrode (tip diameter  $< 10$   $\mu\text{m}$ ). The design ensures that only the tip of the electrode is pH sensitive, an important requirement for the measurement of pH profiles across a tissue or through a biofilm.

## ELECTRODE CONSTRUCTION

Briefly, an iridium wire is tapered electrochemically and covered with glass (Fig. 1). Excess glass is ground off to expose the tip of the iridium wire. The wire is then recessed a few micrometers into the glass to provide a

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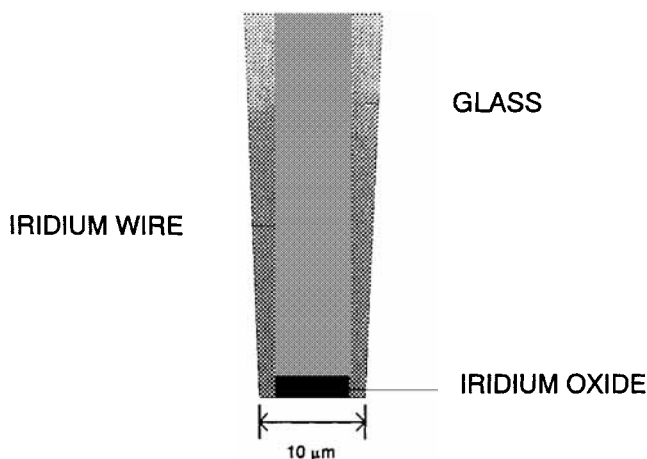


Figure 1. Tip of an iridium oxide pH microelectrode.

protected area for the formation of iridium oxide during potential cycling. The electrode tip is cleaned, aged in water, and calibrated. The following sections describe the individual steps in the process.

### Tapering Wire

Iridium is the most corrosion resistant metal known, resistant to common mineral acids at all temperatures and to cold and boiling aqua regia. Iridium wire is drawn at 600–700°C, which is below the recrystallization temperature. Wires that are drawn in such a way have a fibrous structure.<sup>2</sup> The nonuniform internal structure is the reason that the time required for tapering can vary from sample to sample in an unpredictable way. Several procedures for tapering were explored. Anodic polarization in 0.5M H<sub>2</sub>SO<sub>4</sub> produced the best results. Ten volts DC (vs. graphite) was applied to iridium wire (99%, 5 cm long, 75 μm diameter, Engelhard) for 5–10 min, causing

the iridium wire to taper to a 2–10 μm tip with a gradual decrease in diameter. The wire carried up to 1 A of current with ≈2 cm of wire submerged in acid and in some cases the iridium wire glowed. A minimum of 10 W must be available from the voltage source. The acid discolored after ≈3 min, turning dark purple after continued use.

The tapering process requires experience and constant attention. An electronic circuit was designed to improve the success rate during tapering (Fig. 2). The current in the wire decreases as the wire becomes thinner. An automatic switch (relay) shuts off the power supply when the current drops to a preset percentage of the maximum. The circuit has to be calibrated to obtain a required final tip diameter. A small resistor (0.22 Ω) was put in series with the voltage source to produce a voltage proportional to the current passing through the iridium wire. Maximum current flows at the beginning of the tapering process because of the maximum surface area of the iridium wire at that time. A LF398 Sample & Hold circuit was used to store the maximum current. Current decreases as the wire becomes thinner. When current drops to ≈80% of the maximum, a comparator circuit (LM311) turns off the power supply and the tapering process is complete. The percentage of the initial current at which the power supply is turned off controls the final tip diameter and can be adjusted by a potentiometer.

### Covering Wire with Glass

The center section of a 10-cm lead glass tube (Houde Glass Co., NJ) (1.3 mm i.d., 2.4 mm o.d.) was heated using propane flame and pulled apart to form two microcapillaries. The procedure produced a wide range of glass diameters for the capillaries. The tapered end

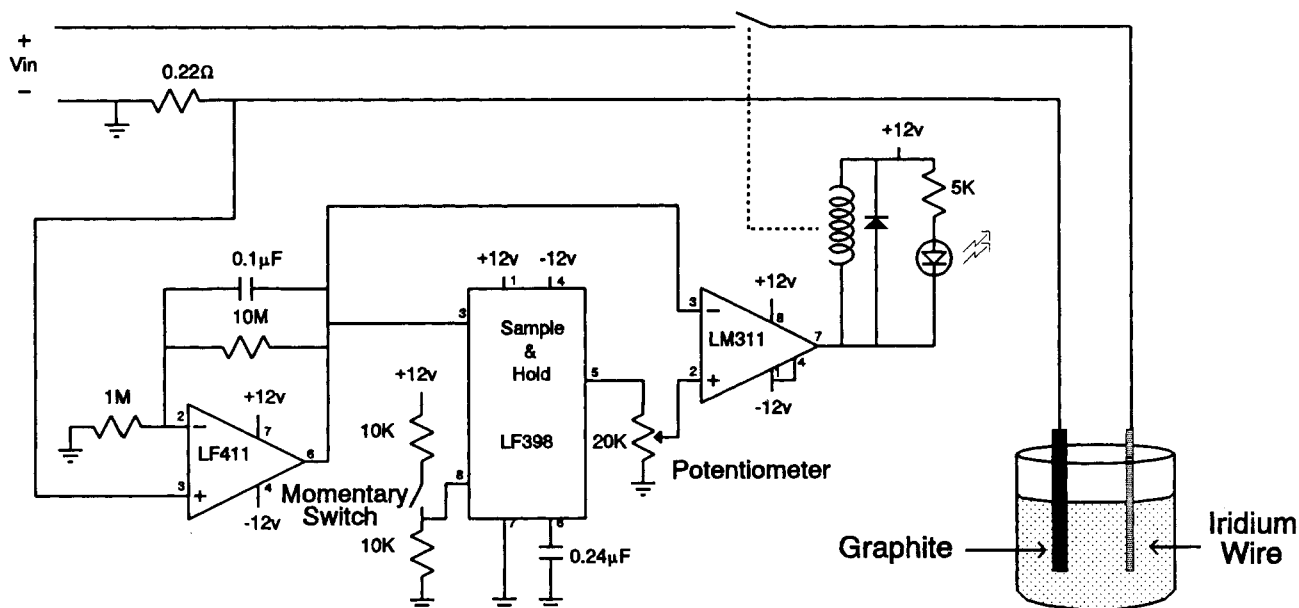


Figure 2. Auto-tapering circuit.

of the wire was carefully inserted into the glass and pushed into the capillary leaving  $\approx 1$  cm in the thicker shaft. The manipulation has been described in detail by Revsbech and Jorgensen.<sup>31</sup> The capillary was suspended with the narrow end pointing up and 5 g weight attached to the bottom of the thick shaft. An M-shaped electrical coil was used to apply heat  $\approx 1$  cm below the end of the wire. The heating coil was made of 1-mm-diameter nichrome wire. The power delivered by the heating coil was  $\approx 110$  W (2.29 V, 49 A). Soda lime and borosilicate glass were considered but have higher melting points than lead glass, making it more difficult to cover the iridium wire. A Micro Electrode Puller (Stoelting model no. 51217) is helpful but not essential. The electrode dropped into a beaker as the glass melted and separated. A small strand of glass extended from the tip of the electrode.

### Grinding Off Excess Glass

A Narishige EG-4 diamond grinding wheel with a self-contained micromanipulator was used to remove the excess glass from the tip and expose the iridium wire. The tip of the electrode was lowered onto the grinding wheel with the micromanipulator and ground until the iridium tip was exposed. Grinding was monitored with an Infinity model CFM microscope with attached camera (Edmund Scientific). Microvibrations were the main cause of electrode damage at this stage of construction. Vibrations decreased when the speed of the grinding wheel was reduced. Grinding the electrode at a slight angle, not absolutely perpendicular to the wheel, reduced damage during grinding. Another advantage of using a soft lead glass was that it was flexible and could absorb vibrations produced by imperfections of the grinding surface and imbalance in the motor and mechanical systems. Soda lime glass and borosilicate glass were too rigid and tended to shatter during grinding.

### Recessing Iridium Wire

A potential disadvantage of iridium oxide electrodes prepared by potential cycling can be poor adhesion of the oxide layer to the metal surface.<sup>6</sup> The recess as shown in Figure 1 mechanically reinforces the iridium oxide film and protects it from external stresses during use. The recess should be approximately 20% of the tip diameter. Too deep a recess will cause an increase in response time because of the increased diffusion path length. Deep recesses can also cause problems during the oxidation procedure if a bubble is trapped in the tip. To recess the iridium wire, the tip of the capillary was immersed in 0.5M  $\text{H}_2\text{SO}_4$  and +2 V DC (vs. graphite) was applied to the electrode for 1 min. Stirring was required to remove bubbles. The electrode was then rinsed with distilled water.

### Cleaning Tip

Problems were encountered early in the development of this microelectrode when improper cleaning techniques were used. Iridium would not oxidize or would exhibit irregular oxide growth if the surface was not clean. Although recession of the tip provides some measure of cleaning, a separate cleaning technique was incorporated in the design. The recessed tip was immersed in 0.05M  $\text{Na}_3\text{PO}_4$  and -5 V DC (vs. graphite) was applied for 10 min. Stirring was necessary to release bubbles from the tip as they formed. The electrode was then rinsed with distilled water.

### Forming Iridium Oxide

A programmable potentiostat (e.g., EG&G PARC 273, Princeton, NJ) was used to form iridium oxide on the tip of the electrode. However, only one electrode could be oxidized at a time and each one required 4 h of polarization. To facilitate the oxidation of more than one electrode, we constructed a series of oxidation circuits (Fig. 3). The three-electrode system was used to form iridium oxide at the tip of the electrode via voltammetric cycling.<sup>35</sup> TL084 FET input operational amplifiers were used because of their high input impedance ( $10^{12} \Omega$ ). This is necessary because very low currents are generated with tip diameters in the range 5–10  $\mu\text{m}$ . The amplifier on the left converts a two-electrode system into a three-electrode system. Voltage applied to the positive input terminal of the amplifier is essentially the same as the voltage on the negative terminal because of the virtual short circuit between them. Therefore, voltage applied by the function generator to the positive input terminal is the same as the voltage applied to the reference electrode. The working electrode is tied to ground through the virtual short circuit between the input terminals of the second amplifier. Therefore, the voltage supplied by the function generator is essentially the voltage between the reference and working electrodes. The counter electrode is connected to the output of the first amplifier, providing the oxidizing current for the work-

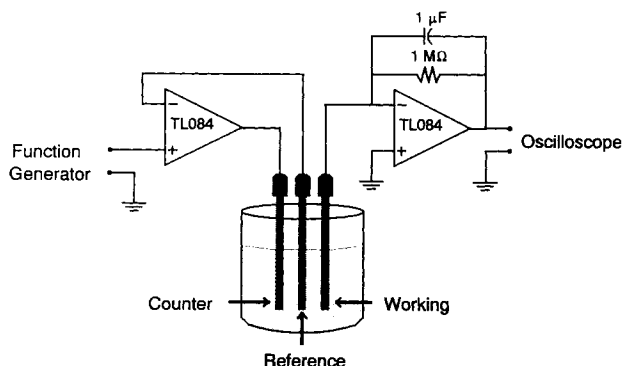


Figure 3. Iridium electrode oxidation circuit.

ing electrode. There is essentially no current flowing in the reference electrode.

The second amplifier does not contribute to the oxidation process directly. Instead it is used to monitor the current in the working electrode. The oxidation process would be unchanged if the amplifier were removed and the working electrode were connected to ground. The amplifier monitors current by producing a voltage which can be displayed on an oscilloscope. The proportionality constant is the value of the resistance ( $V = IR$ ). The amplifier provides a monitor for the oxidation/reduction process at the tip of the electrode.<sup>5,6,14,20</sup> The capacitor works with the resistor to provide an initial filtering of the signal, decreasing noise.

Low-level current measurements and high-impedance circuits are inherently noisy. Electrical noise is generated by all electrical devices including room lights, computers, and measuring equipment. Noise generated by someone entering the room was comparable to the signal generated by the electrode during the oxidation process. Noise dominated the unfiltered oxidation current. One way to reduce noise is to electrically filter the signals. For the oxidation circuit, a capacitor (Fig. 3) can serve this purpose. Signal noise is reduced as the value of the capacitance is increased. This technique becomes damaging if the capacitance is increased too far because the filter will begin to distort the signal of interest. Sometimes there is no value of capacitance that will effectively attenuate the noise without distorting the signal. Because of this problem, a more complex filter was designed and constructed. Sallen-Key and Star filters were used to realize the fourth-order hourglass characteristic.<sup>4,9</sup> The hourglass filter attenuates high-frequency noise much better than the capacitor while having almost no effect on lower frequencies.<sup>4</sup> The low-pass filter was designed with a notch at 60 Hz to attenuate noise generated by equipment and room lights. For the 6000-mV/s scan rate the unfiltered signal had a maximum amplitude of 800 mA. The filtered signal had an amplitude of only 140 mA. In laboratory measurements, noise was over 5 times larger than the signal of interest.

Iridium oxide can be produced using different scan rates of the oxidizing waveform and lengths of time. De Rooij and Bergveld produced the first iridium oxide pH sensor.<sup>13</sup> They cycled electrodes between  $-0.25$  and  $1.25$  V for 200 cycles in  $0.5M$   $H_2SO_4$  at a scan rate of  $150$  mV/s. Burke et al.<sup>6</sup> used  $6000$  mV/s for 5000 cycles in  $1.0M$   $H_2SO_4$ . Hitchman and Ramanathan<sup>14</sup> cycled at a scan rate of  $3000$  mV/s for 8000 cycles in  $0.5M$   $H_2SO_4$ . Glab et al.<sup>13</sup> stated that the rate of growth is fairly constant as long as the scan rate is less than  $100$  mV/s and additionally that growth rate decreases linearly with the log of the scan rate.<sup>30</sup>

Four scan rates,  $150$ ,  $1000$ ,  $3000$ , and  $6000$  mV/s, were used. Figure 4 presents the voltammograms taken for  $150$  mV/s. Waveforms were stored on a Tektronix 2211 digital storage oscilloscope and transferred to the com-

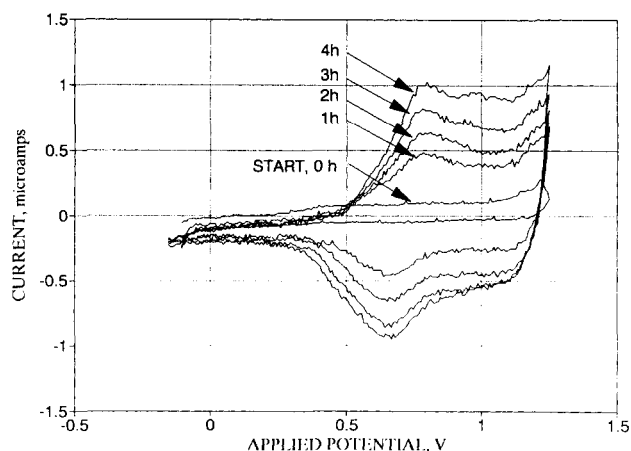


**Figure 4.** Series of cyclic voltammetry profiles ( $150$  mV/s) monitored after  $0$ ,  $1$ ,  $2$ ,  $3$ , and  $4$  h of cycling.

puter via Grabber II software (Tektronix). Waveforms were taken every  $10$  min for the first hour followed by hourly measurements for  $4$  h. The current profile for the  $150$ -mV/s scan rate was bounded by  $-1$  and  $1.5$  mA while the current profile for the  $6000$ -mV/s scan rate ranged from  $-60$  to  $140$  mA. A capacitor passes more current at higher frequencies for a given voltage, suggesting that there is a capacitance increase associated with the oxide formation. Positive current indicates oxidation and negative current, reduction.<sup>5,14,28</sup> The current profile for the  $150$ -mV/s scan rate has approximately equal areas of positive (oxidation) and negative (reduction) currents which result in the low oxidation/reduction ratio. Oxide coatings on the electrodes prepared at the  $150$ -mV/s scan rate were thin and inconspicuous when imaged in a scanning electron microscope.

Thick black oxide coatings formed on electrodes oxidized at higher scan rates. Current levels for faster scan rates were much higher than for the  $150$ -mV/s scan rate. The oxidizing current at faster scan rates exceeded the reduction current, corresponding to a significantly faster oxide growth rate. Thick black oxide coatings formed in  $2$ – $4$  h for the  $3000$ - and  $6000$ -mV/s scan rates while there was almost no oxide formation after  $4$ – $6$  h for the  $150$ -mV/s scan rate.

Accumulated charge at the tip of the electrode for each of the four scan rates during a  $4$ -h period is plotted in Figure 5. Charge ( $Q$ ) is the product of current ( $i$ ) and time ( $t$ ). Accumulated charge was calculated by integrating anodic currents for each curve in Figure 4 to determine instantaneous charge for one cycle. Instantaneous charge was multiplied by the number of cycles in the time interval to get total charge for that interval. Finally, charge was accumulated over the  $4$ -h period. The assumption made was that the charge is constant over the time interval. This assumption may not be accurate but it was used for all four scan rates and should be a reliable means of comparison. Accumulated charge is a measure of oxide growth. The relationship between growth rate and scan rate is shown in Figure 5. Accumulated

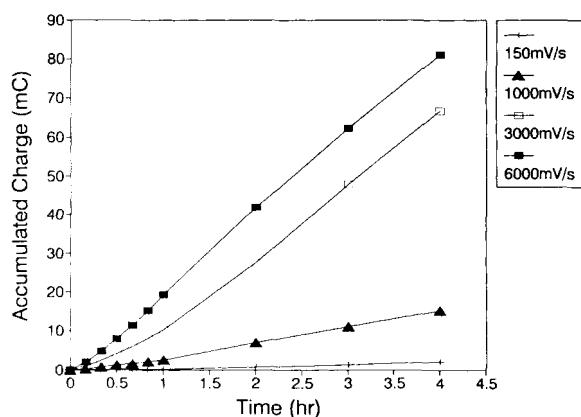
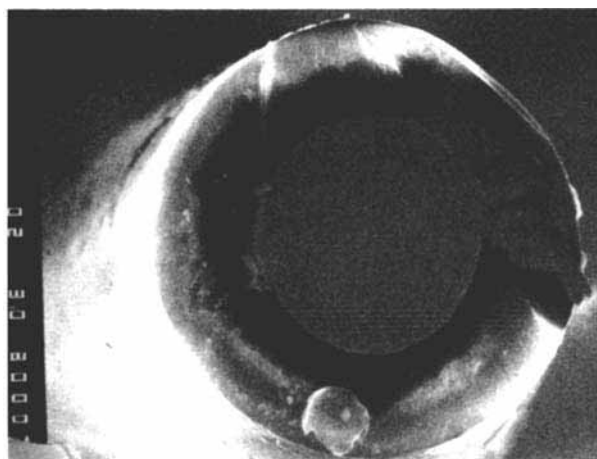
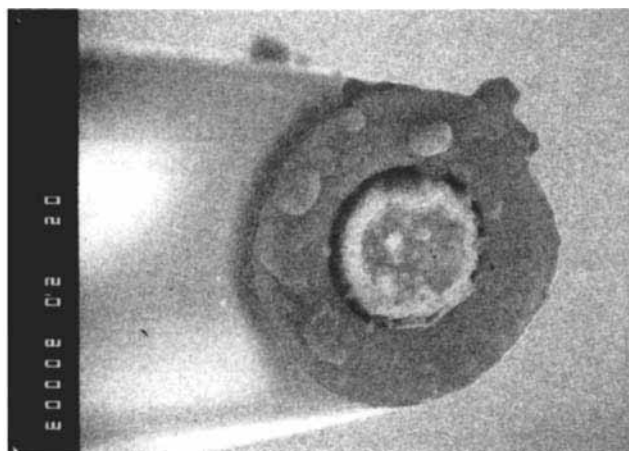


**Figure 5.** Accumulated charge for all scan rates (150, 1000, 3000, and 6000 mV/s).

charge for the 6000-mV/s scan rate was above 80 mC while it was below 5 mC for the 150-mV/s scan rate. Figure 6a is a scanning electron micrograph of an electrode cycled for 4 h at a scan rate of 150 mV/s. The electrode in Figure 6b was cycled for 1 h at a scan rate of



(a)



(b)

**Figure 6.** Scanning electron micrographs of the tip of a micro-electrode after cycling for (a) 4 h at a scan rate of 150 mV/s and (b) 1 h at a scan rate of 6000 mV/s.

6000 mV/s. The electrode cycled at the higher scan rate had a much thicker oxide coating than the electrode cycled at the slow scan rate. Mozota and Conway found similar results with their micrographs of iridium oxide growth.<sup>10,26</sup> The slope of the lines in Figure 5 indicate iridium oxide growth rate. Our study did not indicate any decrease in the oxide growth rate when the sweep rate exceeded 100 mV/s. On the contrary, there was a visible increase in growth rate associated with increase in scan rate up to 3000 mV/s, which simply demonstrated that more electrical work was put in at the faster cycling rate. Further increases in scan rate to 6000 mV/s did not increase oxide growth rate.

## Aging

Neither thermally nor electrochemically prepared electrodes are exclusively covered with either anhydrous or hydrous iridium oxide. As previously discussed, thermal oxidation and sputtering produce more anhydrous iridium oxides while potential cycling produces more hydrous iridium oxides. The pH-sensitive oxide is composed of hydrous and anhydrous oxides. The degree of hydration changes with time causing a drift in calibration.<sup>6</sup> Furthermore, there is no consistency in the measured slope (mV/pH) for electrodes prepared by identical processes. For electrochemically prepared electrodes, Kinoshita et al.<sup>18</sup> report 69.7 mV/pH unit and Hitchman and Ramanathan<sup>14</sup> report 81.9 mV/pH unit at 25°C. Our measurements indicated an initial slope of 77.3 mV/pH unit that decreased after 1 week to 65.8 mV/pH. Because the greatest change in oxide hydration occurs during the first 12 h of equilibration,<sup>6</sup> electrodes were aged in distilled water for 12 h followed by aging in air for 2 h. Electrodes were stored in air.

## Calibration

A set of commercial buffer solutions pH 1–12 (Fisher) was used to calibrate the electrodes. An electrometer with a minimum input impedance of  $10^{12} \Omega$  was used to ensure that the meter did not load the electrode. Experimentation showed that the internal impedance of the electrodes can be as much as  $10^{10} \Omega$ . A meter with an input impedance comparable to or less than  $10^{10} \Omega$  would produce inaccurate results and damage the sensor. The sensor should never be short-circuited to the reference electrode while both are in solution because energy stored in the tip of the sensor will discharge and damage the electrode.

A World Precision Instruments model FD 223 electrometer (impedance  $10^{15} \Omega$ ) was used to measure pH from 1 to 12. Data was collected four times during the course of 1 week (Fig. 7). The original Nernstian slope was 77.3 mV/pH unit and  $E_0$  was 751 mV. Slopes of 70–80 mV/pH unit were typical of freshly cycled electrodes. Slopes usually settled to  $\approx 65$  mV/pH unit after several calibrations and uses. Response was immediate for most pH buffers.

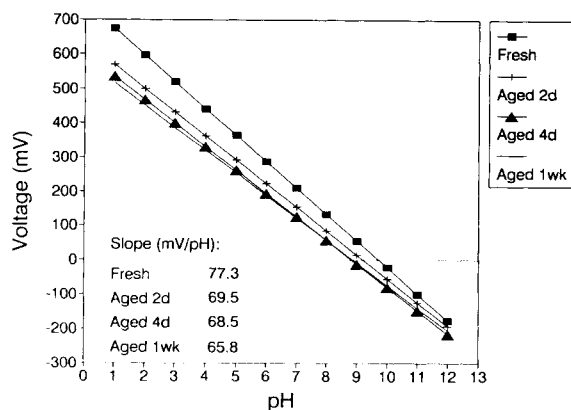


Figure 7. The effect of aging on the pH response.

## APPLICATION

### pH Measurement in a Biofilm

When surfaces are placed in aquatic environments, bacteria attach to the surface where they reproduce and excrete extracellular polymers to form viscoelastic layers called biofilms.<sup>8</sup> Activities of microorganisms within the biofilm produce chemistries within the strata and at the biofilm/surface interface that are radically different from that of the bulk solution in terms of dissolved oxygen, pH, and organic and inorganic species.<sup>11,22</sup> In some cases, the resulting interfacial conditions cannot be maintained in the bulk medium at ambient temperatures near atmospheric pressures.<sup>23,25</sup> Interfacial chemistry cannot be predicted from measurement of bulk solution parameters. The complexity of interfacial chemistry may further increase if a biofilm is deposited on a chemically active substratum (e.g., metal surface). Prediction of pH at an active substratum is impossible because of the complexity of such systems. Direct measurements with microelectrodes are required.

The iridium oxide microelectrode was used to measure a pH profile across a mixed-population biofilm known to contain denitrifying bacteria on a polycarbonate disc (Fig. 8). The biofilm accumulated in a rotating

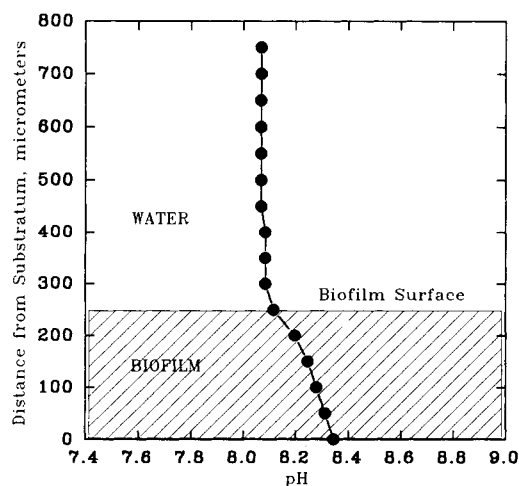
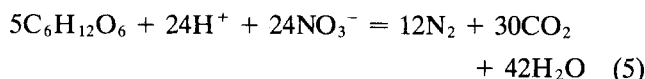


Figure 8. pH profile in a denitrifying biofilm.

biological contactor using a nutrient medium of glucose and mineral salts. The rotating polycarbonate discs were equipped with removable slides that were removed from the reactor and placed in a Petri dish. The Petri dish was filled with the feeding solution and deoxygenated with  $\text{Na}_2\text{SO}_3$ . Nitrate (100 mg/L) was added as a terminal electron acceptor in respiration and the system was forced to denitrification. The microelectrode was mounted in a micromanipulator (World Precision Instruments, Inc.) and positioned directly above the film. The pH was measured in 50- $\mu\text{m}$  increments as the electrode penetrated the film. An Ag/AgCl microelectrode (World Precision Instruments, Inc.) was used as a reference. Based on the respiration reaction [Equation (5)], the pH is expected to increase:



Three major factors influence pH in this system: (1) microbial denitrification rate (proton consumption), (2) reactions with the water buffering system (proton consumption changes the position of the equilibrium), and (3) diffusion (local proton consumption creates concentration gradients).

Measurements through the 250- $\mu\text{m}$ -thick denitrifying biofilm with the iridium oxide microelectrode indicate a continuous increase in pH from 8.16 to 8.36.

### Comparison with Glass Microelectrodes

Miniaturized iridium pH sensors are comparable in performance to commercial glass electrodes. The Nernst slope is usually slightly higher for the iridium electrode, ranging from 70 to 80 mV/pH unit for a freshly cycled sensor to  $\approx 65$  mV/pH unit after several uses. This compares to the 59-mV/pH unit Nernst slope for glass electrodes. The value of  $E_0$  is typically higher for the iridium electrode. For both glass and iridium oxide electrodes  $E_0$  varies between measurements. For the iridium electrode,  $E_0$  will usually be at its highest initially and will drop  $\approx 200$  mV before stabilizing. For glass microelectrodes  $E_0$  values do not exhibit such a wide range of change. However, glass electrodes must also be calibrated before each use. The correlation coefficients for both glass and iridium oxide electrodes are  $\approx 0.998$  or greater so that a two-point calibration is sufficient for either.

The only application we encountered where a glass microelectrode had to be used instead of the iridium oxide was measuring pH in the presence of  $\text{H}_2\text{S}$ . In such an environment the iridium oxide pH microelectrode demonstrated unstable, continuously drifting potential.

## CONCLUSIONS

Iridium oxide pH electrodes can be miniaturized to have tip diameters below 10  $\mu\text{m}$  using the design specified in this article. The microelectrodes are sturdy enough to be

positioned at solid surfaces and measure pH without the shielding required for glass microelectrodes.<sup>21</sup> The tip of the electrode is oxidized with voltammetric cycling in 0.5M sulfuric acid. Typical parameters for voltammetric cycling were 4 h with a scan rate of 1000 mV/s between the limits -0.25–1.25 V. Linearity was greater than 0.99 over the 1–12 pH range and the response time was almost instantaneous and at worst 1 min. This is better than a palladium oxide pH microelectrode<sup>19</sup> which shows linear response between pH 3 and 9. For each electrode  $E_0$  varies and can change with time and use, necessitating calibration before each use. A two-point calibration is sufficient because of the high degree of linearity in response to pH. After voltammetric cycling, the electrode must be aged in distilled water for 12 h. The iridium/iridium oxide electrode can then be used as a pH sensor and stored in air between uses for weeks. Noise is a significant problem when dealing with miniaturized electrode signals and electrical filtering is essential. Low current measurements require meters with high input impedances. Almost all the equipment used should have an input impedance of at least  $10^{12} \Omega$ . Miniaturized iridium pH electrodes can be used in many microscale applications such as determination of pH gradients within microbial biofilms formed on solid surfaces. The development of a working solid-state pH microelectrode has implications reaching beyond pH measurements. Other microsensors based on the pH sensor can be constructed.

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