Dissolved oxygen microelectrode sensors for analysis of trace amounts of dissolved oxygen

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Abstract

This research describes the development of a new microelectrode sensor (MES) for *in situ* measurements of dissolved oxygen (DO) using microfabrication technologies. A dynamic etching technique was used to fabricate 20 mm long sensor probes and sharpen them to micrometer dimensions. The sensors utilized a gold sensing electrode inside a recess fabricated at the tip of each microelectrode. Electrochemical performance of these (dissolved oxygen MES) sensors was fully characterized by measuring the oxygen concentration of saline solutions with a Ag/AgCl reference electrode. The DO MES exhibited a rapid 30 seconds linear response in the 0–9 mg/L range. The (dissolved oxygen MES) microelectrode sensor was successfully applied to evaluation of DO microprofiles in a multi-species aerobic bacterial film. The developed new sensors are able to measuring DO *in situ* analysis in many biological applications.

Introduction

Dissolved oxygen analysis measures the amount of gaseous oxygen (O_2) dissolved in an aqueous solution. Oxygen gets into water by diffusion from the surrounding air, by aeration (rapid movement), and as a waste product of photosynthesis [1]. Adequate dissolved oxygen is necessary for good water quality. Oxygen is a necessary element to all forms of life. Natural stream purification processes require adequate oxygen levels in order to provide for aerobic life forms. As dissolved oxygen levels in water drop below 5.0 mg/l, aquatic life is put under stress. The lower the concentration, the greater the stress. Oxygen levels that remain below 1-2 mg/l for a few hours can result in large fish

kills[1]. Dissolved oxygen (DO) sensors measure the amount of oxygen dissolved in liquid media, usually water. In medicine, dissolved oxygen reflects the percentage of hemoglobin binding sites in the bloodstream occupied by oxygen. Measurements of oxygen saturation in blood are used by physicians in medical diagnosis, since low saturation (hypoxemia) is indicative of a variety of medical conditions such as chronic obstructive pulmonary disease (COPD) or carbon monoxide poisoning. Thus, DO microelectrode sensors have been widely used in many research areas, including medicine and environmental engineering [2–5]. For example, microelectrodes can be used to provide DO measurements in ground water or wastewater, which are key tests in water quality and waste treatment process control. In a water quality application, where the goal is to maintain a fresh water stream fit for recreational purposes such as swimming and fishing, DO content must be kept high. If the DO level falls too low, the fish will suffocate and the water condition will become favorable for the growth of harmful bacteria. In wastewater treatment, solids are allowed to settle in large basins to which solutions rich in bacteria are added to accelerate decomposition of the solids. There is an optimum DO level for this process and the level is maintained by mechanically aerating the bacteria-impregnated content of the basins. If the DO level falls too low, the aerobic bacteria die and the decomposition ceases; if the DO level is excessive, more power is used for aeration, the bacteria may not settle properly and the process becomes cost inefficient [6]. Most commonly used DO sensors are typically a Clark-type cell consisting of an electrode system containing a selective membrane across which oxygen diffuses at a rate proportional to its partial pressure. DO sensors are typically polarographic and measure current as oxygen diffuses through the membrane and is reduced at the cathode. The cathode of an oxygen microelectrode sensor is made from a noble metal (Pt or Au) so that the electrode surface does not participate in the chemical reaction. The chemical reaction at the cathode surface can be expressed as [4]

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \quad (1)$$

A negative potential must be applied to the cathode for it to provide electrons to oxygen molecules. The anode is used only to establish the reference potential. Difference in potential must be more than 0.5 V. Selective membranes in DO sensors are typically formed from gas-permeable materials such as silicone [7], polyethylene [8], fluorinated ethylene-propylene (FEP), and Nafion [4]. However, such oxygen sensors suffer from fouling of membrane materials, leading to changes in sensitivity and the need to recalibrate for each use. Recessed DO

microelectrodes fabricated from pulled glass pipettes overcome these challenges by eliminating the selective membrane, yielding faster response times and increased sensor stability [5]. However, such microelectrodes are generally very difficult to manufacture, require trained personnel to use, and must be used in a laboratory under strictly controlled conditions. Further, due to the small signals involved, these microsensors are usually subject to electrical interference and must be used inside a well-grounded Faraday cage.

In this work, we successfully demonstrate a novel DO microelectrode sensor using microfabrication technologies. The micrometer tip size and the needle nature of the sensor permit analysis to be performed *in situ* by penetrating biological or environmental samples. Gold was selected as the electrode material because it could be used to directly measure DO [9]. Integrating such microelectrode sensors with an IC chip for signal acquisition and processing will permit the development of an integrated system for *in situ* analysis. Such a sensor system will be ideally suited for rapidly and accurately sensing analyte *in situ* for Industrial and bioapplications.

Experiments

Reagents and Chemicals

Nitric acid (BDH), hydrofluoric acid (BDH), Polystyrene (Fluka). HF-based glass etching solution was prepared by mixing HF, HNO_3, and H_2O in a 10:7:33 (v/v/v) ratios. The Au etchant was prepared by mixing a 1:4:40 (g/g/mL) mixtures of I_2, KI, and deionized water.

The saline solution was prepared by dissolving 8.5 g of NaCl in 1 L of water. The mineral salt solution was composed of 30mg/L of sodium nitrate ($NaNO_3$), 10 mg/L of ammonium chloride (NH_4Cl), 40 mg/L of disodium hydrogen phosphate (Na_2HPO_4), 10 mg/L of monopotassium phosphate (KH_2PO_4), 3.8 mg/L of magnesium sulfate ($MgSO_4$), 0.65 mg/L of ferric chloride ($FeCl_3$), 11.2 mg/L of manganese sulfate ($MnSO_4$), 0.7 mg/L of copper sulfate ($CuSO_4$) and 12 mg/L of zinc sulfate ($ZnSO_4$).

Apparatus

Ag/AgCl standard electrode, DO meter (Hana Inc.), Microscope (Olympus), Picoammeter, potentiostat , pH meter (Hana Inc.).

Fabrication

Micromachined microelectrode was fabricated from 200 μm thick, 100 mm × 80 mm borosilicate glass wafers. Initially, 1 cm long glass probes were formed by cutting at 700 μm center-to-center spacing with 250 μm thickness. The probes were annealed at 600 C^o to relieve stress. The etching process consists of three steps. In the first step, the probes were

etched for 10 min with agitation to smooth the diced surface and reduce the probe dimension using a 10:7:33 (v/v/v) mixture of HF, HNO_3, and H_2O with the etch rate of approximately 2 µm/min. In the second etch step, the glass beams were gradually tapered by withdrawal. In the final etch step, microelectrode tips were sharpened to approximately 300 nm using meniscus etching. Probes were coated by evaporation with 20 nm of Ti and 200 nm of Au to make the Au cathode. For easier handling and to establish electrical connections with individual microelectrodes, the array was packaged with carriers fabricated from copper-clad laminate glass-epoxy. Microelectrodes were fixed to carriers using epoxy. Conductive silver epoxy was used to establish the electrical connections to individual microelectrodes. To insulate individual microelectrode sensors, a 1.5-2 µm thick polystyrene insulating layer was coated over the entire substrate in a polystyrene coater.
A recess was formed at the tip of each microelectrode. Fig. 1 illustrates the recess structure.

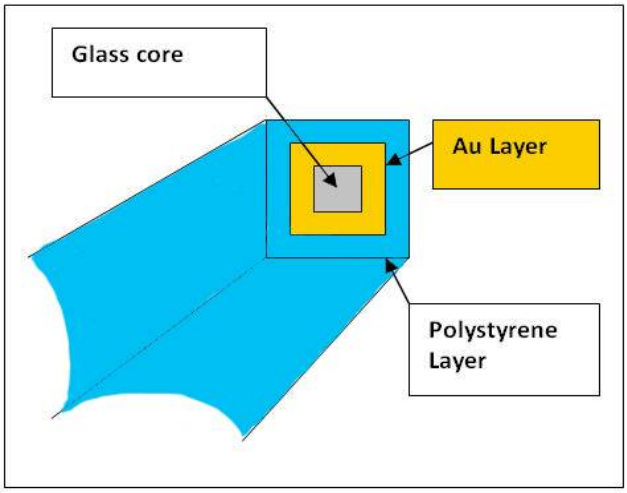

Figure (1): The recess structure (illustration)

To form the recess, microelectrode tips were first beveled at 45° above horizontal for 30 min on a rotating plate under visual control to remove polystyrene and Ti/Au layers and to expose the glass core. Then, the exposed glass core and Ti were etched using HF-based etchant for 5 min to form the recesses. These beveling and etching steps permitted control of the recess opening size and depth. In the final step, the exposed Au was etched with a 1:4:40 (m/m/v) mixture of I_2, KI, and H_2O for 3 min. This was necessary to relocate the Au sensing area inside the formed recess. Microelectrodes were cleaned ultrasonically in distilled water after each etching step.

Characterization

DO microelectrode sensors were polarized and calibrated with a commercial Ag/AgCl reference electrode. DO microelectrodes were polarized for at least several hours before calibration. The polarization voltage source and the current input were from a potentiostat. The −750 mV polarization voltage was applied to the oxygen microelectrodes against the Ag/AgCl reference electrode for at least several hours. Negative applied voltage can reduce the amount of O_2 on the cathode of the microelectrode surface, and given sufficient potential and time, O_2 concentration can be reduced to zero [9]. Following polarization, a test solution was prepared in both saline and mineral salt solutions by (0%, 10%, 21% O_2 or 0.0mg/L, 4.1mg/L, 8.7mg/L O_2) all test solutions were determined with DO meter (Hana Inc.). The oxygenated saline solutions in the 0–9 mg/L O_2 range were used for microelectrode characterization. Mineral salt solution was used to perform the biofilm tests because it consists of various nutrients needed for sustaining biofilms. A commercial DO meter (Hana Inc.) was used to verify the amount of oxygen in bulk solution and during calibration.

Results and discussion

DO microelectrode sensor (MES)

Fabrication technique was used to fabricate DO microelectrode sensors. Four-sensor microelectrode sensors were fabricated from a single 100 mm × 80 mm borosilicate glass wafer. Following the initial dicing step, individual sensors were not separated. Instead, the subsequent chemical etching and metallization process steps were performed on the entire wafer array. Only after metallization were the individual microelectrode sensors separated for packaging. This batch processing substantially simplified fabrication, permitting increased uniformity and consistency of microelectrodes, as well as savings in time and cost.

The fabricated recessed DO microelectrode sensors consisted of four 1-cm long probes at 700 µm center-to-center spacing. The sensor structure, which has four electrical isolated microelectrodes, increases robustness. The initial dimensions of the diced microelectrodes were 200 µm in width and 190 µm in thickness. Using the first etch step, diced probes were smoothed and reduced to approximately 100 µm wide and 90 µm high at the base. At the end of the etch process and prior to recess fabrication, dimensions of the resulting microelectrode tips were approximately 200 nm with a taper angle of 20°, as reported earlier [10]. There are two critical factors that determine sensitivity of the recessed oxygen sensors: the recess diameter and recess length [11]. To fabricate a recess, one of the four pyramidal faces in a probe was ground at 45° to expose the glass

core. Microscope was used to contact one face of the tip end on the rotated beveller surface and to control the contacted area while grinding the desired recess opening size. The over-contact area resulted in larger beveled area and bent microelectrodes. The angled sensors tips are expected to permit easier penetration of tissues and biofilms for biological applications. An etchant was used to form a recess at the beveled tips. The HF-based mixture was selected for this step due to its high selectivity towards glass and Ti. This process permitted removal of the glass core and the metal seed layer, but kept the conducing gold and the insulating polystyrene layers intact.To verify the recess functionality, oxygen microelectrode sensors were tested in oxygenated saline before and after the glass and gold etching steps. When the Au sensing area was exposed to saline before glass etching, a current of ~0.1 μA was measured with respect to a Ag/AgCl reference (standard deviations were on the order of 23 nA). The current remained constant independent of DO concentrations, without selectivity. The same results were observed for microelectrodes following glass etching, but prior to Au etching. Only following the Au etching to relocate the sensing area inside the recess did the DO microelectrode exhibit significant sensitivity to different DO concentrations and stable currents in the pA range with 1000 times smaller standard deviations (~20 pA). The recessed DO microelectrode following Au etching exhibited no stirring effect, which is in agreement with results previously reported by Henry and Fritsch for a disk-type microelectrode [12]. The recessed structure with inner cathode improved sensitivity and stability of the DO microelectrode.

Calibration of microelectrode sensor (MES)

The developed DO sensors were characterized using oxygenated saline (0.85% NaCl solution) in the 0–9 mg/L O_2 range. DO microelectrode sensors were polarized before calibration in saline [1,5].The measurements after polarization were higher than those before polarization.This polarization process depletes oxygen from the oxygen cathode, any residual oxygen causes disturbances in the measurements [3]. Testing solutions were prepared in saline of 10% O_2 and air (21% O_2). The 21% O_2 which equals to 8.7 mg/L is near the maximum saturation of oxygen in water at 20 ^oC. Typical values for DO in surface or drinking waters are around 6–7 mg/L, we measured tap water as containing approximately 15% O_2 or ~6 mg/L concentration. Thus, our test range spans the maximum and minimum O_2 concentrations in solution. The commercial DO meter (Hana Inc.) was used to verify the oxygen concentrations in all test samples. Three-point calibration curves

of the DO MES in saline and mineral salt solutions with respect to a Ag/AgCl reference at 20 ºC are shown in Fig. 2.

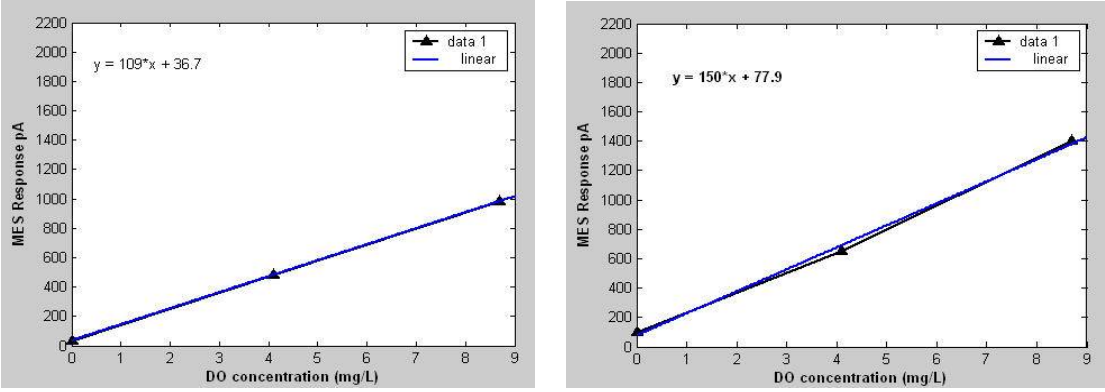

Figure (2): Calibration Graph shown Saline solution (0.85% NaCl slution) and Mineral solution

Correlation coefficients were calculated for each curve and found near (0.99). The sensor performed linearly (0 - 9 mg/L), and exhibited a high sensitivity of ~150 pA/(mg L) in saline. In mineral salt solution, the same sensor under the same test conditions exhibited a lower sensitivity, ~109 pA/(mg L), which is due to interference from the present mineral salts[12]. Dissolved oxygen solubility in saline waters is typically less than in clean water. The time for 90% response was typically less than 30 s, which is much shorter than that of macroscale commercial oxygen electrodes. These characteristics are a substantial improvement over the previously reported microelectrodes constructed from pulled glass pipettes which exhibited similar response times but lower sensitivities of ~13 pA/(mg L) [13]. This increased sensitivity is attributed to the differences in recess dimensions and electrode surface areas.

DO measurement in biofilm

Measurements of dissolved oxygen were performed in an aerobic biofilm. Biofilm was placed in a chamber with mineral salt solution. The mineral salt solution was injected into the biofilm chamber. The biofilm chamber was placed on microscope stage to facilitating vertical movement over fixed MES. The MES was held in a fixed arm of the stand to measure vertical microprofiles of dissolved oxygen while penetrating the biofilm. An Ag/AgCl reference electrode connected to the

ground of a picoammeter was situated in the biofilm chamber, and the MES was connected to the current input as a working electrode. By observing through an optical microscope set over the biofilm sample, the position of the electrodes was precisely controlled to perform all measurements at the same point. DO measurements were performed vertically at approximately every 200 µm step down into the mineral salt solution and in the biofilm using the microscope stage.

A typical microprofile of dissolved oxygen in a biofilm sample is shown in Fig. 3.

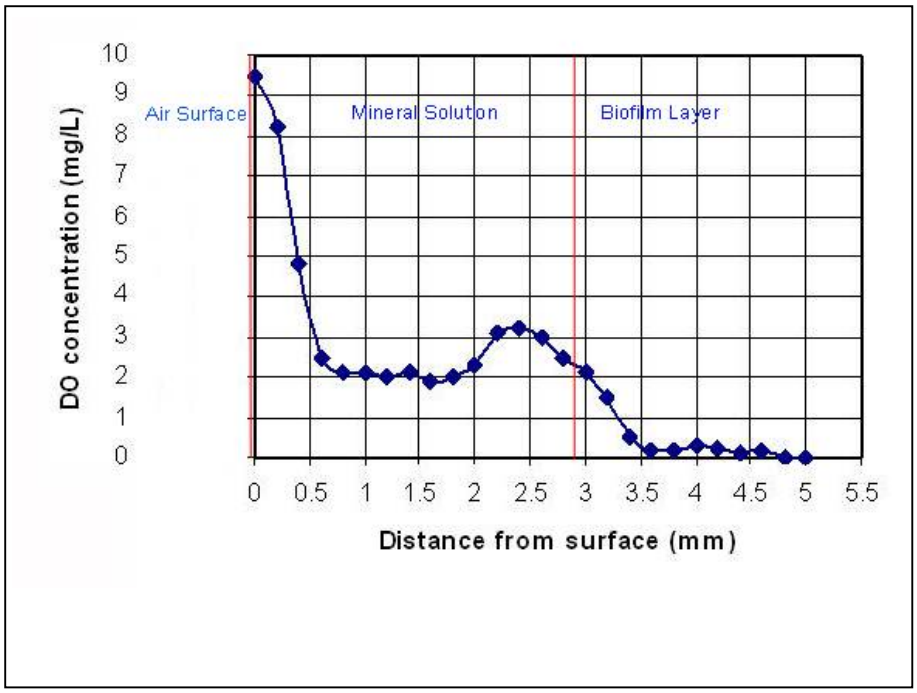

Figure (3): Microprofile of DO concentration measured by MES in an aerobic biofilm with mineral solution.

The *x*-axis shows distance from the solution surface to the biofilm bottom. The DO concentration at the interface between air and the mineral salt solution was high due to oxygen diffusion from air. The oxygen concentration decreased to about 2-3 mg/L in the mineral salt solution at the liquid/biofilm interface, beyond the initial 500 µm thick diffusion layer. Upon penetrating the aerobic biofilm surface, the DO concentration decreased dramatically from ~3.5 mg/L to zero near the bottom of the biofilm layer. The oxygen concentration microprofile demonstrates that oxygen was depleted at approximately 500 µm for the surface. Bishop and coworkers [5, 13] reported similar results using conventional pulled-glass-pipette microelectrodes in the same type of biofilm.

Conclusion

DO microelectrode sensors (MES) were successfully demonstrated using microfabrication technologies. These 1-cm long, micrometer tip size four-probe array sensors exhibited fast response times (~ 30s) and high sensitivity(~150 to ~109 pA/mg). Electrochemical tests showed the sensors to be stable and reliable. The sensors were successfully applied to DO microprofile measurements in an aerobic biofilm. This may be extending the microelectrode sensor concept to analytes beyond dissolved oxygen, developing a reliable and accurate system for *in situ* multi-analyte measurement in bioapplications

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الخلاصة

" متحسس قطبي دقيق لقياس الكميات النزرة من الأوكسجين المذاب "

ا.د.جاسم حلو نعمة ، و م. زيد عبد المجيد نعمة ، و هدى عبد الكريم حسين

الجامعة التكنولوجية / قسم العلوم التطبيقية

تم في هذا البحث وصف متحسس دقيق للقياسات الموقعية للأوكسجين المذاب بإتباع تقنيات التصنيع الدقيق . اعتمدت تقنية النخر المتحرك لتصنيع متحسس بطول ٢٠ ملم وتم تنحيفه لأبعاد دقيقة. يستعمل المتحسس قطبا" حساسا" من الذهب داخل ثقب في قمته الدقيقة. كما تم توصيف العمل الكهروكيميائي لهذا المتحسس بشكل تام باستعمال محاليل ملحية مع قطب (الفضة /كلوريد الفضة). أعطى المتحسس استجابة سريعة حوالي ٣٠ ثانية في المدى الخطي (صفر-٩)ملغم/لتر. تم تطبيق قياسات الأوكسجين باستعمال المتحسس لتقييم كمية الأوكسجين المذاب في فيلم من نمو البكتريا الهوائية. أن هذا المتحسس المحضر له القابلية على قياس كميات الأوكسجين المذاب موقعيا" في العديد من التطبيقات الحياتية.