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Fabrication of Redox Potential Microelectrodes for Studies in Vegetated Soils or Biofilm Systems

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A new method for construction of redox potential microelectrodes has been successfully developed in this study. The new construction method consists of four major steps: (a) pulling a glass pipet; (b) trimming the micropipet tip to obtain a suitable size; (c) filling the micropipet with a low-melting point alloy; and (d) plating platinum onto the alloy surface. This method can be used to make redox potential microelectrodes with tip sizes as small as 1−3 μm for biofilm research and with tip sizes between 20 and 40 μm for in-situ measurements in plant-grown soil systems. The microelectrodes retain their characteristics for a few weeks. The high Nernst slope (57−61 mV/pH unit using pH buffer solutions saturated with quinhydrone to calibrate the electrodes), the short response time (ca. 0 s to 3 min), the high tolerance capacity for the potential interference ions, and the increased rigidity are features of this new method which make the redox potential microelectrodes suitable for studies in both biofilm and soil systems.

Introduction

Microscale measurements of the redox potential in physiology, medicine, microbial ecology, and environmental studies may provide information to explain many of the complex reactions which take place in the system tested (*1*−*4*). Few reports can be found on the reliability of microelectrodes for redox potential measurements in soil, biofilm, sediment, or other systems (*5*−*10*). Most measurements of soil redox potentials have been made with redox potential macroelectrodes. Flessa and Fischer (*7*) used Pt wires with a diameter of 0.5 mm as redox potential microelectrodes to measure redox potentials in microsites of the rhizosphere of flooded rice. The Pt wires were placed in several fixed points of soil and could not be used to measure other points of interest because the redox potential microelectrodes were not sturdy enough to again penetrate the soil after they were removed from the fixed positions.

One of the most accepted common methods to make microelectrodes is (a) to taper a metal wire (such as Pt or iridium), (b) to shield the metal wire with a glass micropipet, and (c) to plate the metal wire with another material so that the electrode can be used as a working probe. Plating materials include gold for oxygen electrodes (*11*, *12*), silver for Cl^- electrodes (*13*), iridium oxide for pH electrodes (*14*), hexachloroplatinic acid for glucose electrodes (*15*), or others. Usually, the metal wire tapering and glass micropipet shielding are two major steps that are difficult and time-consuming. Although a piece of Pt wire with a diameter of 5 μm or smaller can be obtained through commercial venders, it is very expensive to purchase a quantity of varying diameter Pt wires. In addition, it is difficult to shield the Pt wire with a glass pipet since (a) cracks often exist between the platinum wire and the glass pipet, considerably impairing the performance of the redox potential electrode (*4*), and (b) the total tip diameter is not easy to control (*12*).

The objective of this study was to find a new procedure to fabricate reliable redox potential microelectrodes that are small and sturdy enough for in-situ microscale measurements of redox potentials in soil or biofilm samples. This article will present the procedure to make the redox potential microelectrodes, to evaluate the performance of these microelectrodes and the redox potential distributions, measured by using a redox potential microelectrode, in a TNT- (2,4,6-trinitrotoluene) and RDX- (hexahdro-1,3,5-trinitro-1,3,5-triazine) contaminated vegetated soil.

Electrode Construction

Fabricating Redox Potential Microelectrodes. The new construction method consists of the following major steps: (a) pulling a glass pipet; (b) trimming the micropipet tip to the desirable size; (c) filling the micropipet with a low-melting point alloy; and (d) plating Pt onto the alloy surface. Generally, the first three steps take only 5−10 min, while the last step takes 1−3 h, depending on the tip size of the microelectrode.

The first step is to pull a glass pipet. Single-barreled borosilicate glass tubing (World Precision Instrument, Sarasota, FL) with O.D. 1.2 mm and I.D. 0.68 mm is used to make the micropipet. Untreated, uncleaned glass tubing is pulled on a vertical micropipet puller with a "loop" filament (P-30, Sutter Instrument Company, Novota, CA), using conditions of a high heat (990), normal onset of the hard pull (2.0), and moderate pull strength (650). This step usually takes only 1−2 min.

The second step is to trim the micropipet tip to obtain a suitable size. The pulled micropipet tip is broken to the suitable size using a forceps, which usually takes less than 1 min. For the soil tested in this study, the suitable tip size of the microelectrode was 20−40 μm (*16*). If a tip size of less than 3 μm is required, the glass micropipet can be bevelled using a micropipet beveler (BV-10, Sutter Instrument Co., Novota, CA). However, it takes 0.5−1 h to bevel a micropipet, depending on the tip size and the diamond beveling disk used.

The third step is to fill the pulled micropipet with a low-melting point alloy. The trimmed glass micropipet is filled with an alloy (44.5% Bi, 22.45% Pb, 19.1% In, 8.3% Sn, 5.3% Cd; Belmont 2451, Belmont Metals Inc., Brooklyn, NY). Melting point of this alloy is 117 F. A piece of alloy wire, 2.5 or 3.5 cm long is inserted into the barrel and pushed forward as far as possible with the plunger of a gas chromatography (GC) syringe. The alloy wire is made from an alloy cake using the method reported by Zhang and Bishop (*17*, *18*) and Linsenmeier and Yancey (*11*). The diameter of the alloy wire is 0.5 mm, a little smaller than the I.D. of the glass pipet and therefore can be inserted into the barrel easily.

To heat the alloy and make it completely fill the tip of the micropipet, the micropipet inserted with a piece of the alloy is first mounted on a holder (Figure 1) so that the head of the alloy wire just enters the heating filament. Then, the heating circuit is turned on with a voltage of DC 0.5 V, supplied by a variable autotransformer (Staco Energy Product Co., Dayton, OH). When the filament is heated just enough to

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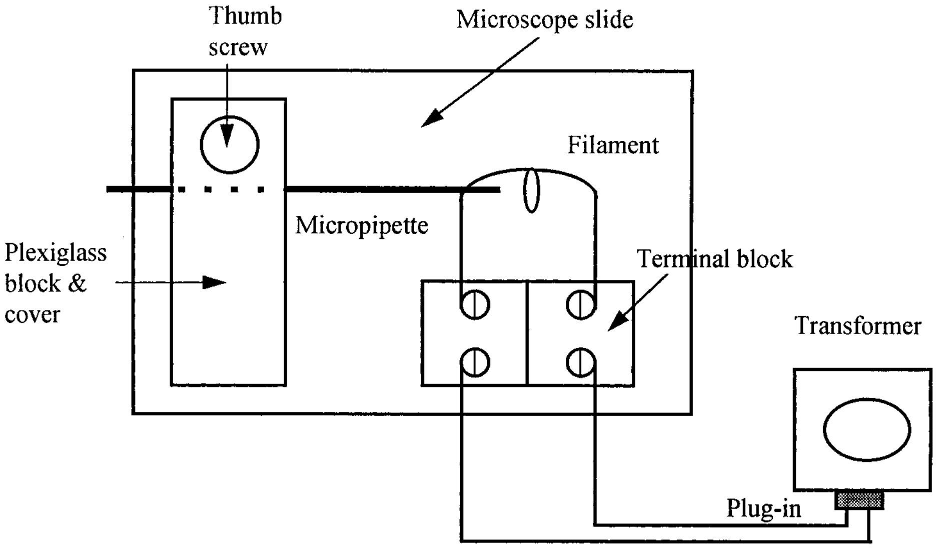

FIGURE 1. Electrical circuit used for filling a micropipet with alloy.

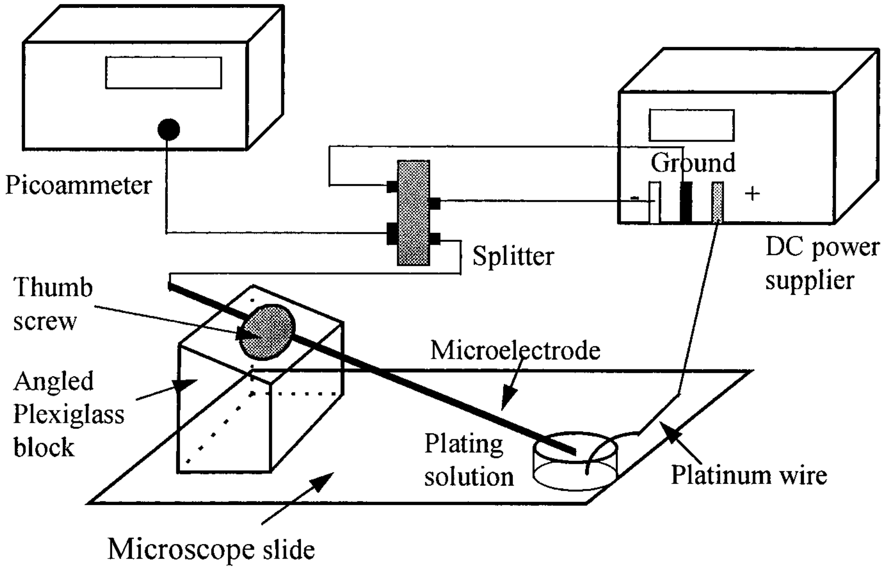

FIGURE 2. Pt plating system for microelectrodes.

melt the head of the alloy, a GC plunger is used to push the alloy toward the tip. The heat is shut down when a small ball of the alloy generates on the tip of the micropipet, showing that the alloy is overflowing from the tip of the glass micropipet. The small ball of the alloy is then blown off, and a micropipet filled with the alloy is ready. Generally, it takes only a few seconds to melt the alloy and fill the tip.

To connect the micropipet with a wire to make a connection to the polarizing source, the microelectrode is repositioned so that the other end of the alloy can be melted by heating, using an applied voltage of 1−1.5 V. This heating does not influence the tip when the initial length of the alloy is >2 cm. A coated copper wire of ca. 6 cm long (the insulated coating at the very end is sanded off) is soldered to the alloy. This step takes only 3 min. It should be pointed out that, if the wire is too short, the reheating will affect the tip of the micropipet filled with the alloy. Some gaps in the alloy within the microelectrode may form, which may impact the transferring effectiveness of the electrical signal; in the worst case, no signal will be transferred at all.

The fourth step is to plate Pt onto the surface of the alloy to make the alloy-filled micropipet be a redox potential microelectrode. As shown in Figure 2, a system of plating Pt onto the alloy surface was made based on the information provided by Linsenmeier and Yancey (*11*). The same microscopic slide, angled Plexiglas block, and bath of plating solution are used in the system. A platinum wire is used as an anode by soaking part of it in the plating solution and fixing the remaining part on the slide with a glue. The electrode is mounted on the angled Plexiglas block so that it dips into a small reservoir (0.2 mL) containing the plating solution. The plating solution consists of 18 mL of distilled water, 2 mL of 10% platinum chloride (Sigma, p5775, $H_2PtCl_6 \cdot 6H_2O$), and 0.2 mL of 1% lead acetate solution (Sigma, L3396). The plating current is provided by a dual DC power supply (Model 3015, Protek, Cole-parmer, Chicago, IL) monitored with a picoammeter (Model 485, Keithley Instruments, Cleveland, OH). Plating is conducted at a voltage of 0.5−0.8 V and a current of 0.3−0.6 μA. The applied voltage cannot be too high; otherwise, (a) bubbles will be generated at the tip of the microelectrode which prevents Pt from being plated on the alloy surface, or (b) the platinized platinum will deposit on the surface of alloy very quickly and roughly, which will result in a poor performance of the redox potential microelectrode. The low voltage and low current can guarantee Pt to be plated on the surface of the alloy gently,

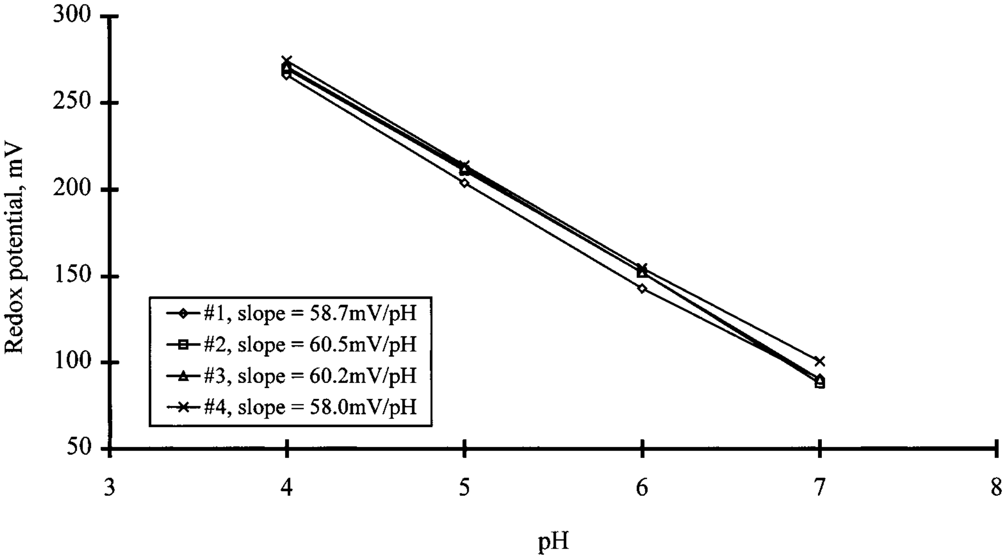

FIGURE 3. Redox potential readings measured by different microelectrodes in pH 4−7 buffer solutions saturated with quinhydrone.

evenly, and, thus, successfully. Usually, for microelectrodes with a small tip (e.g., 1−3 μm), the lower values of the recommended voltage and current should be used. For microelectrode with a tip size larger than 40 μm, the suitable values of the voltage and current can be determined based on the above-recommended values. Generally, after 1−2 h of heating, when the platinized platinum can be observed through a microscope, the plating can be terminated. The plated microelectrode is taken away from the plating solution and left to air-dry for 2−3 h before being used.

Checking the Redox Microelectrode. Four standard solutions were used to check (calibrate) the redox potential microelectrode: (a) pH 4 buffer solutions (VWR) saturated with quinhydrone (Aldrich, 28,296-0), (b) pH 7 buffer solution saturated with quinhydrone, (c) 0.05 potassium biphthalate (Aldrich, 17,992-2) solution saturated with quinhydrone, and (d) 0.0033 M $K_3Fe(CN)_6$ (Aldrich, 20,801-9) and 0.0033 M $K_4Fe(CN)_6$ (Aldrich, 22,768-4) in 0.1 M KCl solution, respectively. Coupled with a Ag/AgCl electrode, the redox potentials for the pH 7 and 4 buffer solutions saturated with quinhydrone should be (i) 86 and 263 mV, respectively, at 25 °C; (ii) 97 and 268 mV, respectively, at 20 °C; and (iii) 79 and 258 mV, respectively, at 30 °C. Generally, if the reading for the standard solutions is ±10 mV from the ideal reading, the electrode can still be regarded as good. The ideal slope (Δredox potential/ΔpH unit) obtained from these two standard solutions is 59 mV/pH unit (*19*). The pH of the 0.05 M potassium biphthalate solution saturated with quinhydrone is calculated from the following eq 1 (*4*)

$$\mathrm{pH} = \frac{500 - 0.09(t - 25) - E}{59.1 + 0.2(t - 25)} \tag{1}$$

where E is the measured emf coupled with a Ag/AgCl electrode, mV, and t is the temperature, °C. The calculated pH should not deviate from the actual pH of that buffer solution at the given temperature by more than 0.04 pH unit. For 0.0033 M $K_3Fe(CN)_6$ and 0.0033 M $K_4Fe(CN)_6$ in 0.1 M KCl solution, the emf of a cell consisting of a redox potential electrode in this solution and a Ag/AgCl electrode is 231 mV at 25 °C (*4*). In this study, after a redox potential microelectrode was made, it would be checked (calibrated) by being coupled with a self-made Ag/AgCl microelectrode (*16*), using some or all of the above-mentioned standard solutions.

Figure 3 shows some examples of the observed slopes. All the slopes shown are close to 59 mV/pH unit, indicating that the electrodes are in good condition and can be used for redox potential measurements. Based on the authors' experience, the chance for obtaining a good redox potential microelectrode is approximately 70%. In this study, the microelectrode was considered to be good if it gave a slope higher than 56 mV/pH unit and lower than 62 mV/pH unit (<5% departure of the ideal value). Electrodes with a slope lower than 56 mV/pH unit were found in this study, most of which showed a good performance after being replated with Pt for several hours. No electrode with a slope higher than 62 mV/pH was found in this study. In fact, the highest slope found was 60.9 mV/pH unit.

Checking the Performance of Redox Potential Microelectrodes. To investigate the reliability of the redox potential electrode fabricated using the new method, the following characteristics were evaluated: (a) the response time and the drift of the redox potential microelectrode; (b) the time-course stability of the electrode (the lifetime of the electrode); and (c) potentially interfering (poisoning) ions for the redox potential microelectrode.

Figure 4 shows the response times of a redox potential microelectrode with a tip O.D. size of 35 μm in different standard solutions. The experiment was conducted by sequentially inserting the microelectrode into three different kinds of standard solutions. The ideal redox reading for the first solution (pH 4 buffer solution saturated with quinhydrone) is 263 mV (*19*). For the second solution, with the pH value of 3.9 and temperature of 22.6 °C, the ideal redox potential (based on eq 1) should be 271.6 mV. The redox potential for the third solution is 231 mV (*4*). Figure 4 shows that the redox reading for the first standard solution is 260.1 mV, 1.1% lower than the theoretical value (263 mV); for the second, the reading is 270.3 mV, 0.5% lower than the theoretical value (271.6 mV); and for the third, the measured reading is 231.8 mV, 0.3% higher than the ideal value. These results show that the redox potential microelectrode can be used to measure the sample precisely and accurately.

Figure 4 also shows that the response times (the time taken by the electrode to get 99% of the final stable reading) for the redox potential microelectrode in different solutions are 3, 0.5, and ca. 0 min, respectively, all of which are very short response times for the electrode. In addition, after the readings of the electrode become stable, there is no drifting at all. Therefore, when measuring redox potential distributions in TNT- and RDX-contaminated soil or biofilm systems, due to the low conductivity and more complicated equilibrium in the systems, the reading of one point was recorded 3−5 min after the microelectrode is inserted into the point.

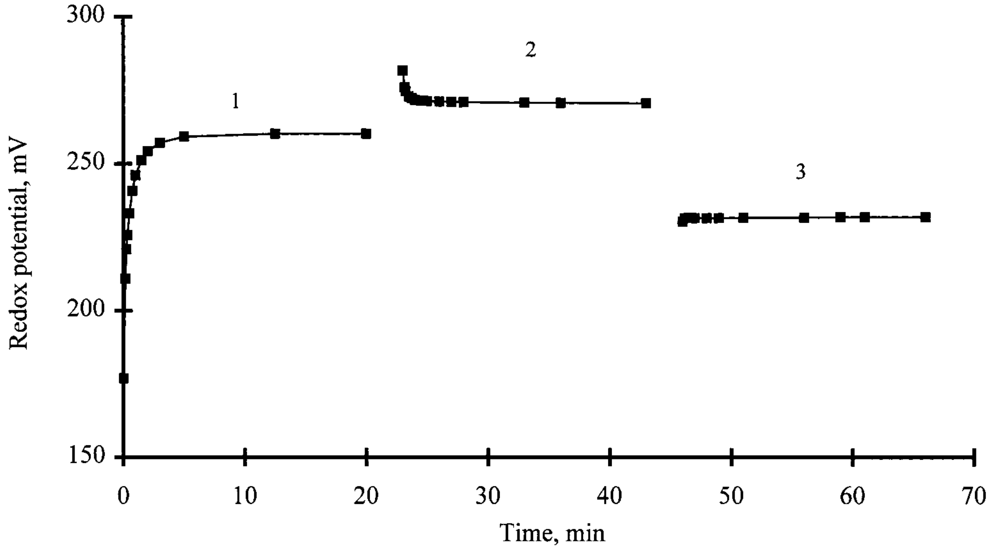

FIGURE 4. Response time of the redox potential microelectrode in different standard checking solutions: 1. pH 4 buffer solution saturated with quinhydrone; 2. potassium biphalate saturated with quinhydrone; 3. 0.0033 M $K_3Fe(CN)_6$, 0.0033 M $K_4Fe(CN)_6$ in 0.1 M KCl solution.

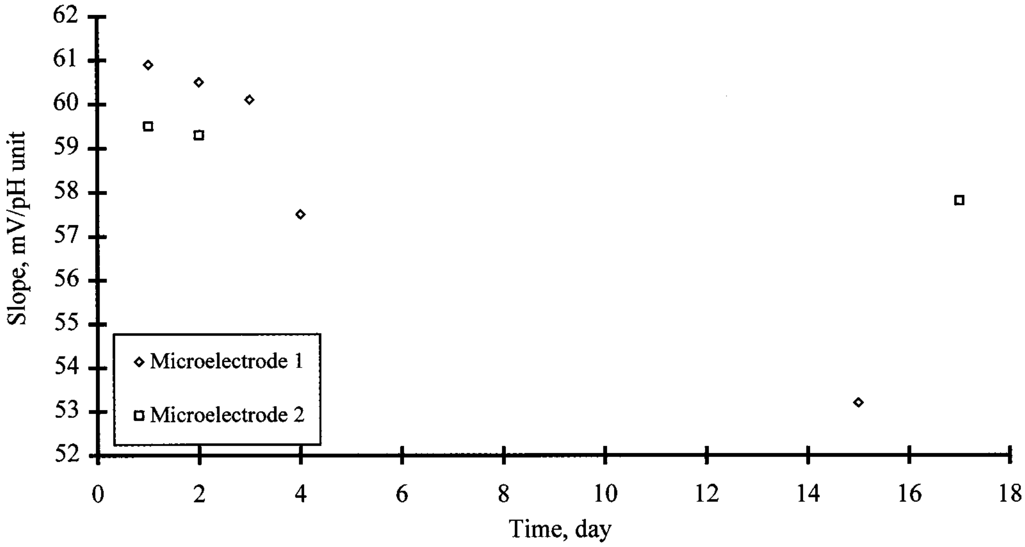

FIGURE 5. Time course of the stability of redox potential microelectrodes.

Figure 4 also indicates that former readings have no effect on the first reading of the following sample, which demonstrates that the Pt plated onto the alloy surface is “inert” enough that the microelectrode has no “memory” of the ions measured previously. This is also an important criterion to judge whether the redox potential microelectrode is good.

Figure 5 shows the stability of two microelectrodes over time. The readings of these microelectrodes were checked using pH 4 and 7 buffer solutions saturated with quinhydrone. For the pH 4 buffer solution, the readings ranged between 266.7 and 274.1 mV; for the pH 7 buffer solution, between 84 and 111.7 mV. Figure 5 shows the slopes of the microelectrode obtained at different days for a period of 17 days. For microelectrode 1, the slopes for the first 4 days were all in the permissible range (60.9, 60.5, 60.1, and 57.5 mV/pH unit, respectively). The slope at the 15th day, 53.2 mV/pH unit, was lower than 56 mV/pH unit. For microelectrode 2, the slope at the 17th day after it was made was 57.8 mV/pH. These results demonstrate that the lifetime of the redox potential microelectrode made under new method developed in this study can be at least a few days to several weeks. Generally, a lifetime of a few days is long enough for most measurements. In the cases where comparisons among different readings measured by the different microelectrodes are needed, the calibration readings of these microelectrodes in the same standard solution should be recorded; the differences of their readings should be used to convert all measured readings based on the “same” microelectrode. For example, if the calibration reading is *m* for electrode 1 and *n* for electrode 2 for the same standard solution, and if the measured reading is m' for point 1 using electrode 1 and n' for point 2 using electrode 2. To convert all readings based on electrode 1, the final reading of point 2 should be $n' + (m - n)$, while that for point 1 is still m'.

In principle, an “inert” electrode should react only with electrons, and its behavior toward all the oxidation−reduction system should be the same. However, in practice, no absolutely inert electrode exists. The platinum electrode can be oxidized by oxygen to form platinum oxides, such as PtO and PtO_2, etc., and can adsorb hydrogen and oxygen molecules as well as some organic and inorganic substances (*4*). To investigate potentially interfering ions for the microelectrode, several chemicals were selected based on literature search (*4, 18*). The chemical concentrations were as follows: 1000 mg/L KCl, 1000 mg/L NaCl, 1600 mg/L $NaHCO_3$, 100 mg/L $CaCl_2$, 1200 mg/L KNO_3, 1500 mg/L NH_4-

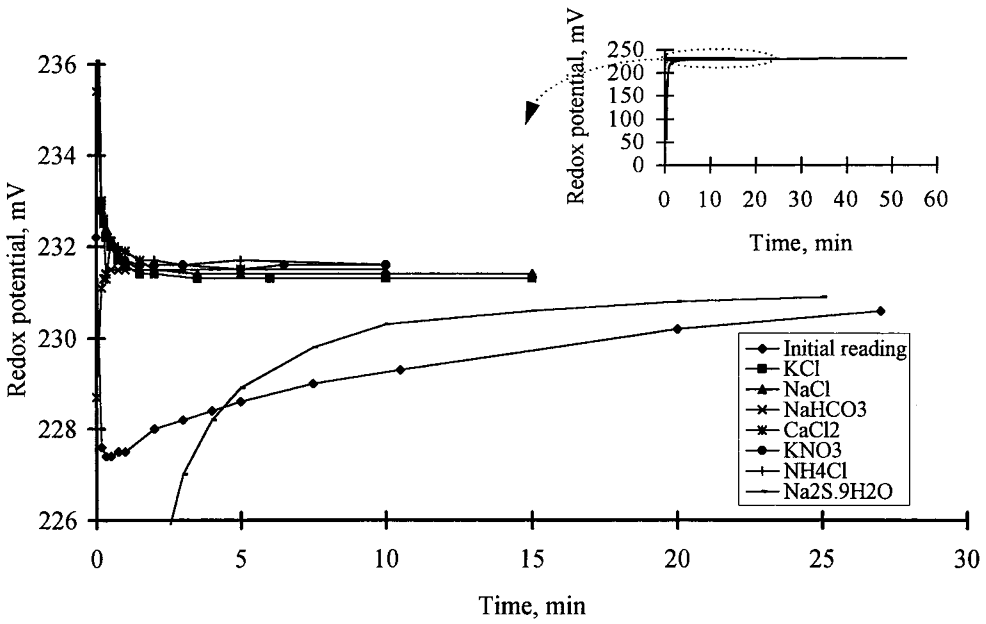

FIGURE 6. Experimental results of investigating potentially interfering ions for the redox potential microelectrode.

Cl, and 2000 mg/L $Na_2S{\cdot}9H_2O$. The chemical compounds selected are all common compounds that may be involved in aquatic or soil systems. It is not necessary for these ions to participate in the redox reaction. Precipitation is of major concern. Sulfide was selected because it was reported that in a reducing medium containing sulfide or ferrous ions, these ions can be adsorbed on the platinum surface, rendering the platinum "poisoned." Fe^{2+} ions were not selected because the standard redox solution used contained Fe^{2+} ions.

To investigate the poisoning phenomenon, the time course of the redox potential of a standard solution (0.0033 M $K_3Fe(CN)_6$, 0.0033 M $K_4Fe(CN)_6$ in 0.1 M KCl solution) was measured using a redox potential microelectrode and recorded as the initial readings shown in Figure 6. Then, the tip of the microelectrode was inserted into one of the aforementioned chemical solutions for 30 min. This step is called the "poisoning treatment". Finally, the microelectrode was taken out of the chemical solution and put back into the above-mentioned standard solution to measure the redox potential again. The readings obtained after the poisoning treatment were compared with the initial readings to see the influence of this chemical on the performance of the redox potential microelectrode. The experiments were conducted within 1 day by switching from one chemical solution to another.

Figure 6 shows the results of the poisoning tests, indicating the redox potential readings and after the tip of the redox potential electrode was inserted into the chemical solution. Figure 6 also shows that the presence of K^+, Cl^-, Na^+, HCO_3^-, Ca^{2+}, NO_3^-, and NH_4^+ does not interfere with the performance of the redox potential microelectrode. As shown in Figure 6, the readings of the redox microelectrode in the first 2 min were lower than 200 mV. This was not due to the hysteresis of the redox microelectrode, instead, it might be due to the low redox potential of Na_2S itself. When the redox microelectrode was moved from the Na_2S container to the container containing the standard redox solution, the tip of the redox microelectrode might contain some Na_2S that could cause very low readings at the very beginning. After 10−15 min, the microelectrode can be restored to its original reading. Therefore, the redox potential microelectrode fabricated using the new method developed in this study is capable of tolerating some common ions, making the redox potential microelectrode very enticing when dealing with some complicated oxidation−reduction systems such as biofilm or vegetated soils.

Application

Bioremediation is gaining more and more interest in hazardous waste site treatment due to its characteristics of in-situ treatment and low cost. The success of a bioremediation strategy is determined by the interrelationships among organisms (including both microbes and plants), substrate (contaminant), and the soil−water environment. Therefore, to advance bioremediation into a predictable and controllable technology, it is imperative to understand the complex interplay of chemistry, physics, and microbial ecology and how the environment permits and ultimately limits remediation. Measurement of soil microenvironments, such as pH, redox potential, microbial communities, and other parameters, may provide information to explain many of the complex reactions taking place in soil systems. The traditional method without using microelectrode technologies can only measure the bulk soil parameters, which cannot reveal the microscale variability in the soil system. Therefore, it is imperative to measure microscale environments, such as pH, redox potential, etc. using microelectrode technologies.

The redox potential microelectrode fabricated using the new method developed in this study was used to measure the redox potential distributions of a vegetated soil contaminated with TNT and RDX, which were collected from the former Nebraska Ordnance Plant. The redox potentials, together with the pH value (the redox potential is a pH-dependent parameter), at different points with different heights and different distances to the plant were measured. Figure 7 shows the redox potential profiles of the soil sample based on pH 7, which shows that the redox potential increases as the distance between the measured point and the plant decreases. Therefore, the presence of a plant may increase the redox potential of the soil around the plant.

Discussion

To make a redox potential electrode, the critical part is the inert material which can only transfer electrons and has no impact on the oxidation−reduction system (*4, 19*). Although various conducting inert materials, such as platinum, platinized platinum, gold, palladium, iridium, rhodium, mercury,

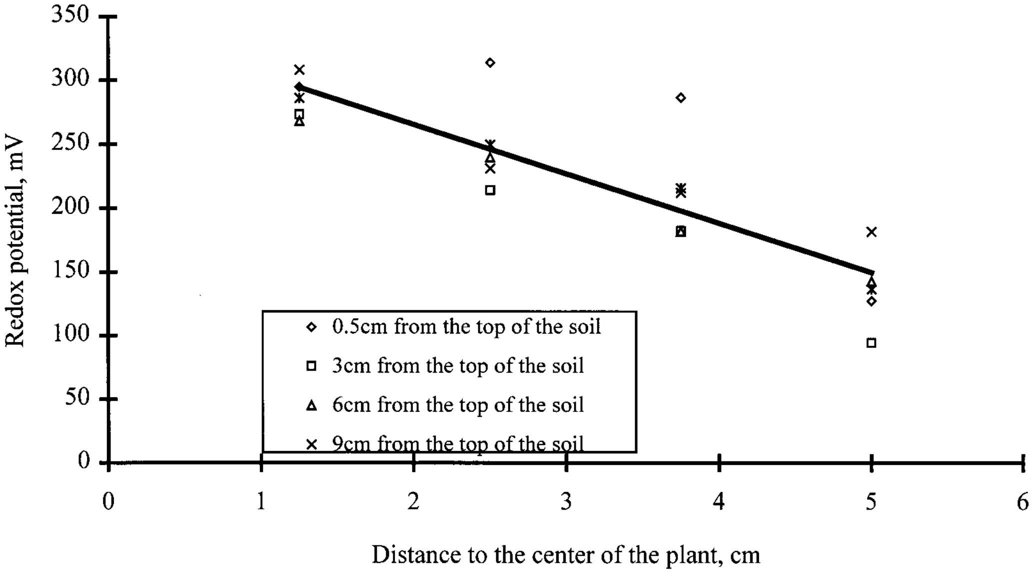

FIGURE 7. Redox potential distributions in a vegetated soil contaminated with TNT and RDX.

mercury amalgam, tungsten, solid graphite, graphite paste, titanium boride, boron nitride, and glassy carbon, have been tested, the platinum electrode is still the most commonly used electrode for the measurement of redox potentials in soil and water systems (*4*). When a platinum electrode is immersed in the soil or water containing an oxidation–reduction system, a potential difference will be generated at the interface of electrode–solution due to the transfer of electrons from the solution to the electrode or vice versa. Therefore, a redox potential electrode is an indicator electrode that reflects the tendency of liberating or gaining electrons in oxidation–reduction reactions.

The new method for manufacturing the redox potential microelectrode developed in this study has two key technical improvements compared to the traditional methods. The first improvement is the use of a low-melting point alloy to fill the glass micropipet. This improvement is based on the technique of fabricating recessed cathode oxygen microelectrodes (*11*, *17*). Since the alloy is melted at 117 F, the microelectrode can be made with any tip size as long as the glass pipet puller can pull the glass pipet to that size. In addition, the rigidity of the micropipet is enhanced due to the filling of the alloy, which makes it possible for the microelectrode to be able to be used for soil and biofilm or other studies. The second improvement is to plate Pt onto the alloy surface so that Pt will serve as an inert material, while the alloy is used only for conducting electrons. It also ensures that there is no crack between the glass wall and the alloy plated with Pt. This improvement originated from one of the authors' experiences making the recessed cathode oxygen microelectrode. However, finding the suitable plating solution and plating conditions did involve a great deal of creativity. It should also be pointed out that the authors do not know the exact material that is plated onto the alloy surface. It may be Pt or some other oxidized Pt compounds. In this paper, Pt is used only as a descriptive convenience. The full understanding of the plated material(s) needs further study.

As mentioned in the application section, a redox potential microelectrode made using the new method developed in this study was used successfully to measure the redox potential distributions in TNT- and RDX-contaminated soils with plants. Due to the complicated characteristics of soil, the tip size of the microelectrode used in this study was increased to be 20–40 μm (*16*), sturdy enough to resist breakage during penetration of soils. However, in other fields, such as in biofilm studies, it is required that the tip size of the microelectrode be only a few micrometers, generally less than 5 μm. The redox potential microelectrode can still be made in this way as long as the tip size of the micropipet is pulled and trimmed to be smaller.

TABLE 1. Effect of the Distance between the Working and Reference Microelectrodes on Redox Potential

distance, cm	0.5	1.5	2.5	1.5
voltage, mV	285	302	308	277

In this study, it was found that the distance between the redox potential microelectrode (the working probe) and the reference electrode affected the redox potential readings. Table 1 shows the different readings of the redox potential at the same point, corresponding to the changes of the position of the reference electrode. It shows that the distance between the working probe and reference probe is a factor which can greatly affect the final reading. Therefore, the distance between the working probe and reference probe must be fixed and, if possible, reduced, which is extremely important for the soil sample due to the low conductivity and low moisture content. In applying the redox potential to the measurements of redox potentials in the TNT- and RDX-contaminated soil, a self-made microreference probe was bound together with the working probe using a regular tape. In that case, the distance between the two probes was 1.5 mm. Currently, further research is being conducted to make the double-barreled redox potential microelectrode. The major difficulty met so far is that the second barrel, which is empty for the reference electrode, affects the Pt plating onto the alloy surface such that the finished redox potential microelectrode cannot obtain a stable reading. The reason for this is not clear, but the authors believe that some special treatment of the secondary barrel may be necessary. If double-barreled redox potential microelectrodes can be made successfully, the distance between the working probe and reference probe can be reduced as small as the tip size of a double-barreled microelectrode, which can be smaller than 1–3 μm.

Finally, it was found that suitable cleaning of the tip of the redox potential microelectrode can restore the performance of the electrode and prolong the lifetime of the electrode. Before the redox potential microelectrode was made in the authors' laboratory, a commercial redox electrode (Microelectrodes Inc., Bedford, NH) with a tip size of 800 mm was used to test samples of interest. In the process of using it, it was found that the measured readings for standard solutions were much higher than the theoretical values after the electrode had been used for several days. The performance of the electrode was restored immediately after using a chromic sulfuric solution (VWR, 9845) to wash the tip of the electrode several times. Being maintained in this way, the commercial electrode still works very well after being used for more than 10 months. This cleaning technology will be used to wash the tip of the redox microelectrodes fabricated using the new method developed in this study in order to prolong their life.

Summary

A new method of constructing a redox potential microelectrode has been successfully developed in this study. The two key technical improvements compared to the traditional methods are (1) to use a low-melting point alloy to fill the glass micropipet, which makes it possible to make the tip size of a redox potential microelectrode as small as that which a micropipet puller could pull, and (2) to plate Pt onto the alloy surface so that Pt serves as the inert material while the alloy is used only for conducting electrons. The plating solution consists of 18 mL of distilled water, 2 mL of 10% platinum chloride, and 0.2 mL of 1% lead acetate solution. Plating was done at a voltage of 0.5–0.8 V and a current of 0.3–0.6 μA. Using this method, it takes only 1–3 h to make a redox microelectrode, depending on the tip size of the electrode.

The microelectrodes made in this study can retain their characteristics for at least a few days to several weeks; suitable cleaning technologies can restore the performance of the electrodes if the surface of the electrode is contaminated and can prolong the life of the electrodes. The high Nernstian slope (57–61 mV/pH using quinhydrone to calibrate the electrodes), the short response time (ca. 0 s to 3 min), the high tolerant capacity for the potential interference ions, such as K^+, Na^+, Ca^{2+}, HCO_3^-, NO_3^-, S^{2-}, and the increased rigidity are features which make the redox potential microelectrodes made by the new method suitable for studies in both biofilm and soil systems.

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