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Fabrication of a Novel, Cost-Effective Double-Sided Indium Tin Oxide-Based Nanoribbon Electrode and Its Application of Acute Toxicity Detection in Water

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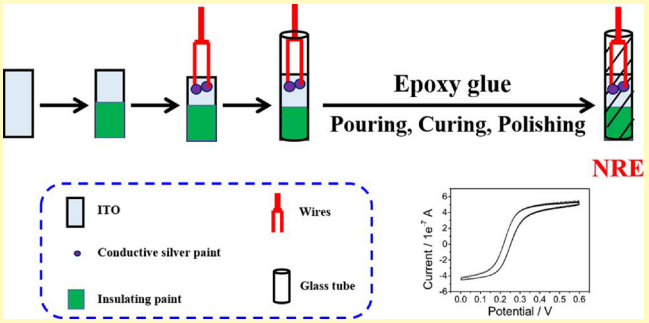
ABSTRACT: Microelectrode plays a crucial role in developing a rapid biosensor for detecting toxicity in water. In this study, a nanoribbon electrode (NRE) with amplified microelectrode signal was successfully prepared by electrodepositing 2-allylphenol on a double-sided indium tin oxide glass. The NRE provided a simple mean for obtaining large steady-state current response. Its advantages were discussed by contrasting the toxicity detection of 3,5-dichlorophenol (DCP) with single microelectrode, microelectrode array, and millimeter electrode as working electrodes in which potassium ferricyanide ($K_3[Fe(CN)_6]$) was adopted as a mediator, and *Escherichia coli* was selected as bioreceptor. At a constant potential of 450 mV, the current reached a steady state within 10 s. The biosensor was constructed using the NRE as working electrode, and its feasibility was verified by determining the toxicity of DCP. A 50% inhibitory concentration (IC_{50}) of 3.01 mg/L was obtained by analyzing the current responses of different concentrations of DCP within 1 h. These results exhibited that the proposed method based on the as-prepared NRE was a rapid, sensitive, and cost-effective way for toxicity detection in water.



KEYWORDS: *indium tin oxide (ITO), nanoribbon electrode (NRE), biosensor, toxicity, 3,5-dichlorophenol (DCP)*

During the rapid global industrialization, a large number of pollutants led to an increased potential risk to human health and environmental pollution. Therefore, there is an urgent need for a simple, rapid, and economical method to detect the toxicity of pollutants. Although physical and chemical analytical methods like gas chromatography−mass spectrometry (GC−MS) and high-performance liquid chromatography (HPLC) can accurately detect the species of chemical toxicants and their concentrations, they may not reflect the environmental risks of chemicals to living organisms. Therefore, it is important to develop effective methods for assessing chemical toxicity. Over the past few decades, various methods to evaluate the water toxicity from different trophic levels (such as fish,[1] daphnia,[2] algae,[3] mouse,[4] and microbes[5]) have flourished. The ubiquitous microbes are easy to cultivate compared to these relatively higher level organisms like fish, daphnia, and mouse. Due to their rapid response to toxic substances, short life cycle, and low cost, they have exerted great influence on chemical toxicity evaluation in recent years. The microbes are proposed as bioreceptor for detecting toxicity among standardized and commercialized methods. The method based on luminescent bacteria is the most successful and widely used one.[6] Several luminescent bacteria have been well commercialized.[7] *Vibrio fischeri* has been listed as an ISO standard method (ISO 11348-1/2/3:2007), and *Photobacterium phosphoreum* T3 has been listed as a Chinese standard method (GB/T 15441-1995). However, their application is limited owing to the disadvantages of these biometrics in the determination of turbid solutions due to reduced light intensity.[8]

In contrast, electrochemical microbial biosensors for toxicity measurement have drawn considerable attention lately due to some advantages such as fast response, low cost, no limitations for colored/turbid samples, etc.[9] For microbial electrochemical biosensor, the fast electron transfer between microbes and electrode is an essential prerequisite to achieve high sensitivity and rapid response.[6] There are two strategies for achieving this goal. On the one hand, redox mediators at appropriate concentrations are used to transform the respiration intensity to detectable electrochemical signals. On the other hand, microelectrodes are used as working electrode instead of millimeter-level electrodes. It took a long time to reach a

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steady state in the previous report.[10] Microelectrode has led to great advances with independence in terms of both time and potential in electrochemical studies especially for the analysis and determination of electroactive compounds in biological microenvironments since its introduction to electroanalytical chemistry in the last decade.[11] Microelectrode analysis shows that a steady-state current is obtained because the current is diffusion-limited due to the small surface area of a microelectrode.[12] Based on the advantage, microelectrodes have been recently applied in the fast toxicity detection fields.[13] Therefore, developing micro/nanoelectrodes can meet the requirements for electrode for rapid water toxicity detections.

Microelectrodes play an important role in current development of rapid electrochemical and environmental analysis techniques such as biochemical oxygen demand (BOD) and toxicity due to their inherent features.[14] However, the current intensity of a single microelectrode is so small that the detection signal is easy to be interfered by an environment under the same concentration of mediator. Amplifying the microelectrode current signal is one of the methods to solve this problem. Among, microelectrode array (MEA) can amplify the current signal of the microelectrode because its detection current is the algebraic sum of that of the single microelectrode for the same sample. MEA has been widely used for toxicity detection in our previous work.[15] However, its preparation procedure is time-consuming with high cost, thus it is difficult to apply to the massive production. In addition, a sufficient large current may not be obtained because of the limitation of the electrode's external dimensions.

Nanoribbon electrode (NRE) is a kind of nanometer electrode encapsulated with a thickness in nanometer scale and length in millimeter or centimeter scale. NRE has the advantages of fast mass transfer speed of microelectrode and large current of millimeter electrode. It is a promising candidate microelectrode with high current and rapid response. Traditional NRE often requires large instruments (such as magnetron sputtering) and professional technicians to prepare nanoscale conductive films. In addition, the shrinkage of the cured epoxy resin will lead to the separation between the conductive film and the epoxy resin, which would result in the prepared NRE becoming an unusable electrode.

Charrier et al. have developed a method for constructing probes that can be as small as 400 nm in overall physical size by the voltammetric polymerization of phenol and 2-allylphenol on the surface of flame-etched carbon fibers.[16] Kozitsina et al. have obtained insulating films with a thickness of a few micrometers on electrode surfaces by polymerizing 2-allylphenol.[17] Guo et al. have also insulated the carbon fibers by electrodeposition and cross-linking of 2-allylphenol copolymer.[18] Inspired by the work above, poly(2-allylphenol) insulation layer was electrodeposited by polymerizing 2-allylphenol on a double-sided indium tin oxide (ITO) glass in this work. This step was the key to the successful preparation of the NRE. Then, a strong mechanical strength, long-life NRE was fabricated by consolidating high-quality epoxy resin adhesive and being cured and polished. 3,5-Dichlorophenol (DCP) was used as a model toxic substance to investigate the NRE's response to toxic substances. DCP is a known inhibitor of respiration and is used in many types of test for inhibition/toxicity, whose 50% inhibitory concentration (IC_{50}) value is usually between 5 and 10 mg/L.[6,19] This result is consistent with the test results of OECD 209 (Activated Sludge, Respiration Inhibition Test (carbon and ammonium Oxidation))[20] and ISO 8192 (Water Quality-Test for inhibition of oxygen consumption by activated sludge for carbonaceous and ammonium oxidation).[21] Catterall et al. also reported that the model toxicant 3,5-DCP was used in most validation assays, though 2,4-DCP, Ag^+, Ni^{2+}, and Se(IV) were also utilized to make sure that a range of toxicant behaviors were investigated.[22] A biosensor was constructed using the NRE as the working electrode. $K_3[Fe(CN)_6]$ (40 mM) was used as the final mediator concentration because microbes may be damaged in higher concentration $K_3[Fe(CN)_6]$ solutions.[23] A high and steady-state current response was obtained based on the NRE in 10 s. The feasibility of water toxicity detection was verified with *E. coli* as bioreceptor, and an IC_{50} value of DCP was finally obtained.

EXPERIMENTAL SECTION

Chemicals and Reagents. 2-Allylphenol was obtained from Sigma-Aldrich. Epoxy AB electronic sealant was purchased from Ausbond (China) Ltd. (150, Shenzhen, China). Glass tubes were provided by the glass processing workshop of Changchun Institute of Applied Chemistry, Chinese Academy of Science. Peptone was purchased from Beijing Aoboxing Bio-Tech Co., Ltd. Yeast extract was purchased from Oxoid Co. Ltd. (U.K.). Glycerol was purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. Each liter of lysogeny broth (LB) was prepared with 10 g of peptone, 10 g of sodium chloride (NaCl), and 5 g of yeast extract and sterilized by a high-pressure steam before use. Ammonium hydroxide, methanol, $K_3[Fe(CN)_6]$, potassium chloride (KCl), NaCl monobasic potassium phosphate (KH_2PO_4), and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Beijing Chemical Plant. A 200 mM phosphate buffer solution (PBS) was prepared as follows: 42.98 g of Na_2HPO_4 and 10.89 g of KH_2PO_4 per liter. DCP was purchased from Sigma-Aldrich. Toxic solutions with required concentration were prepared by diluting 1000 mg/L of high concentration stock solution with deionized water. The double-sided conductive ITO strips (transmittance >84% and sheet resistance <6 Ω/sq) were obtained from Zhuhai Kaivo Optoelectronic Technology Co., Ltd. Unless otherwise specified, all chemical reagents were of analytical reagent grade, and all solutions were prepared using deionized water (≥18.25 MΩ/cm) purified with a Milli-Q Gradient System (Millipore).

Instrumentations. Electrochemical measurements, including cyclic voltammetry and chronoamperometry, were performed using an electrochemical analyzer (CHI 832B, Shanghai Chen Hua Instrument Co., Ltd.) equipped with a three-electrode system (platinum wire as counter electrode, Ag/AgCl electrode (3 M KCl , Shanghai Chen Hua Instrument Co., Ltd.) as reference electrode, and the NRE prepared as working electrode). The oscillator (ZHWY-200B) for microbial culture was purchased from Shanghai Zhicheng Analytical Instrument Manufacturing Co. Ltd. Ultrasonic cleaner for solution formulation was purchased from Kunshan Ultrasonic Instruments Co., Ltd. (KQ-100DE, Kunshan, China). The culture solutions were centrifuged in a centrifuge (Eppendorf, 5804 R, Eppendorf Co. Ltd) before electrochemical detection. The DC power supply (MS-305D) for electrodeposition insulating film was purchased from Dongguan Maihao Electronic Technology Co., Ltd. Cell optical density at 600 nm (OD_{600}) was measured using a Cary 500 Scan UV−vis−NIR spectrophotometer.

Microbe Culture. *E. coli* (DH 5α) was obtained from the China General Microbiological Culture Collection Center (CGMCC) and maintained on the nutrient substrate at −80 °C to ensure its viability. Erlenmeyer flasks (500 mL) containing 300 mL autoclaved LB growth media was inoculated with the microbial strains and incubated. Bacteria were grown overnight aerobically by a shaking incubator (200 rpm) at 37 °C for 16 h. The suitable medium and incubation temperature were confirmed by CGMCC and Cole-Parmer Instrument Company guides. Cells were harvested by centrifugation at 5000 rpm for 5 min at room temperature. The cell pellets were washed twice with 50 mM PBS and resuspended in the PBS. The cells were

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adjusted to the desired optical density. The *E. coli* suspensions were used for further experiments on the day of harvesting.

Electrolyte and Electrodeposition. The double-sided ITO strips (size: 3 × 50 mm) were sequentially washed with acetone, ethanol, and deionized water for 10 min in an ultrasonic bath and dried at 80 °C. The ITO strips were then immersed into a solution of 1 mol/L NaOH/ethanol 1:1 (v/v) for 15 min to activate the surface of the ITO strips. After the ITO strips were thoroughly rinsed with deionized water, they were dried by nitrogen gas flow. The electrodeposition and cross-linking of 2-allylphenol copolymer was used to insulate the ITO. An electropolymerization solution of 215 mM of 2-allylphenol was prepared by dissolving 520 μL of 2-allylphenol in 18.0 mL mixture solution of 1:1 methanol−water and adjusting pH to 9.0−9.5 with ammonium hydroxide. The ITO was immersed in the 2-allylphenol solution, and the polymer was deposited where 4 V positive voltage was applied to the ITO strips versus a platinum wire counter electrode for 5−10 min. The ITO strip-deposited insulating layer was thoroughly rinsed with deionized water. Polymer curing was then accomplished in an oven at 150 °C for 30−60 min.

Preparation of NRE. The NRE was fabricated as described in Figure 2. The holder for the NRE has a length of 80 mm borosilicate glass tube (outer diameter of 6 mm and inner diameter of 4 mm). Epoxy AB electronic sealant was added to the end of a glass tube and cured at room temperature for more than 24 h. The electrode tip was cut using an SYJ-160 low-speed diamond saw (MTI Corporation) to expose the electrode active surface and then polished successively with different grades of silicon carbide abrasive paper (Siliciumcarbid, 991A, P280, P600, P2000, P3000, and P5000) and completed by polishing with 0.05 μm gamma alumina powder (SPI #1337) and then thoroughly cleaned with deionized water in an ultrasonic bath.

Biotoxicity Assessment. The test samples were prepared by mixing 0.4 mL $K_3[Fe(CN)_6]$, 0.1 mL toxic solution, and 0.5 mL *E. coli* suspension (OD_{600} = 6). In the control sample, the toxic chemical solution (DCP) was replaced with deionized water. The samples were incubated at 37 °C for 1 h and then centrifuged at 10000 rpm for 10 min. The supernatants of the samples were detected by the traditional three-electrode system. The working electrode was placed at 450 mV. The inhibition ratio was calculated based on the current values at 10 s. Unless otherwise stated, Ag/AgCl electrode was used as reference electrode for all electrochemical data, and all tests were performed at room temperature. The electrochemical cell used is a 1.5 mL conical plastic centrifuge tube. All tests are performed in triplicate.

RESULTS AND DISCUSSION

Principle of the Toxicity Detection. In gram-negative bacteria, hydrophilic $K_3[Fe(CN)_6]$ can shuttle through the cell membrane. $K_3[Fe(CN)_6]$ was reduced to $K_4[Fe(CN)_6]$ by intracellular NADH. An amperometric method was used to quantify reduced mediator $K_4[Fe(CN)_6]$ produced during the microbial incubation. The schematic illustration of water toxicity assessment is shown in Figure 1. Figure 1a indicates that more $K_3[Fe(CN)_6]$ was reduced to $K_4[Fe(CN)_6]$ in the control sample. Therefore, a higher oxidation current was obtained. In Figure 1b, the respiration activity of a microorganism was inhibited in the presence of toxicants, and the production of $K_4[Fe(CN)_6]$ was decreased by reducing the $K_3[Fe(CN)_6]$ during the microbial incubation. Therefore, the detected current also decreased accordingly. Then, the inhibition ratio of toxicity would be calculated through the difference of the oxidizing currents of $K_4[Fe(CN)_6]$.

Discarding the endogenous respiration of microbes, the amperometric values were converted to inhibition by applying the following eq 1:[15c]

$$\text{inhibition}\% = (1 - i_{tox}/i_{con}) \times 100\% \quad (1)$$

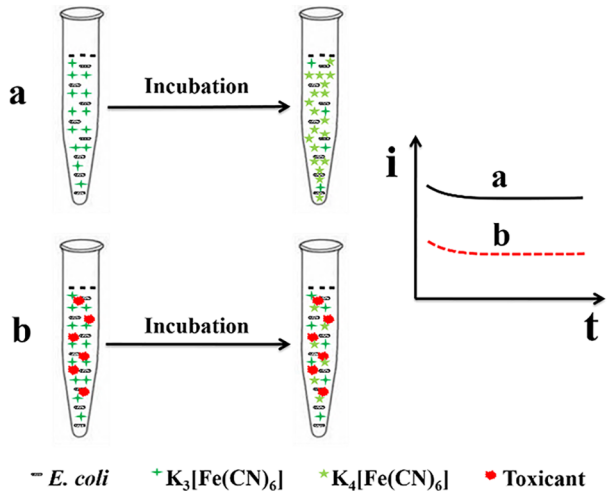

Figure 1. Schematic illustration of the $K_3[Fe(CN)_6]$-mediated water toxicity detection.

In eq 1, i_{tox} is the limiting current recorded for a toxicant sample, and i_{con} is the limiting current recorded for the control sample. The inhibition calculated at each toxicant concentration was graphed against its corresponding concentration. A best fit regression line was fitted against the linear section of the resulting curve to determine the traditional parameter of IC_{50} values. The IC_{50} values were interpolated from the intersection of the best fit regression line with the 50 percentile IC.

Preparation Process and Morphology Characterization of NRE. The preparation process of the NRE is shown in Figure 2, which involved four steps. First, 2-allylphenol was polymerized on the ITO surface at a voltage of 4 V. Polymerization reaction is shown in Figure S1. The electrochemical polymerization of 2-allylphenol gives a mixture of different repeating units instead of a simple polymer structure.[24] Coating the double-sided ITO with a dense insulating film was a critical step during preparation of the NRE. Figure 3A shows a cross section of polymer-insulated electrode in which the insulation layer and the conductive layer of ITO were closely combined. Second, the uninsulated end of ITO was attached to copper wire with conductive silver paint. Third, the insulated ITO connected to the wire was inserted into a glass tube after the conductive paint was dried. Finally, epoxy glue was added into the glass tube and then the NRE was obtained by curing and polishing.

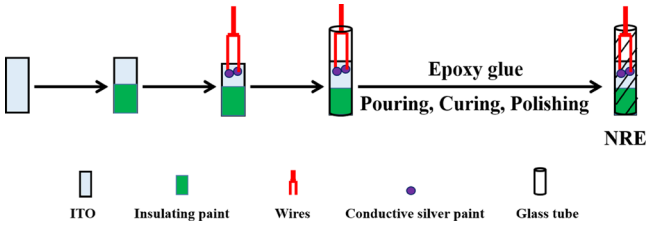

Figure 2. Preparation process diagram of the NRE.

A boundary between epoxy resin and ITO glass can be seen in the SEM image of small magnification (bar is 100 μm, Figure 3B). The SEM image of large magnification (bar is 2 μm, Figure 3C) indicates that there is about 0.655 μm gap between epoxy and ITO glass. It can be imagined that if there

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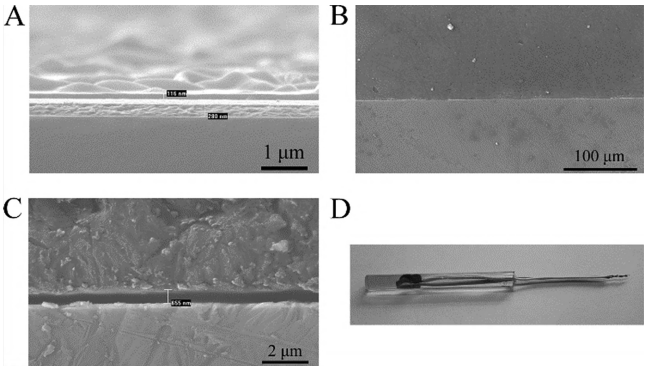

Figure 3. (A) SEM image of ITO section after electrodeposition of insulation layer. SEM images of the NRE surface of small magnification (B) and large magnification (C). (D) Photo of the NRE.

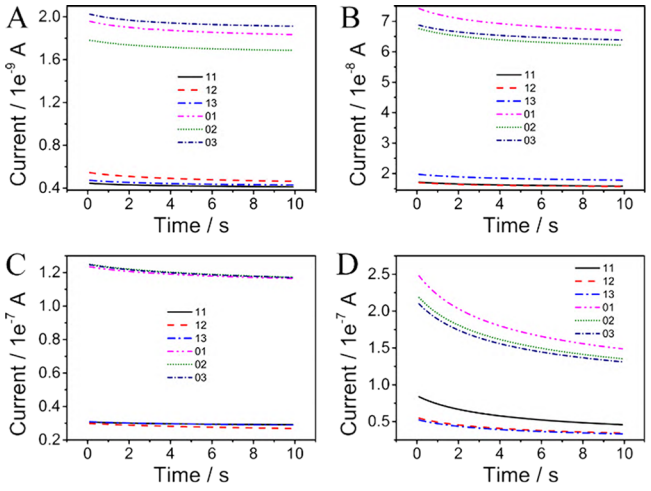

Figure 4. $I-t$ curves of control samples (01, 02, and 03) and test samples (11, 12, and 13) with (A) single microelectrode (diameter 20.0 μm); (B) MEA (32 Pt wires with a diameter of 20.0 μm); (C) NRE; and (D) millimeter electrode (diameter of 1.0 mm). Test sample: 45 mM $K_3[Fe(CN)_6]$, OD_{600} = 6 *E. coli*, and 10 mg/L DCP for 1 h incubation.

was no poly(2-allylphenol) insulation layer on the ITO surface, the test solution would leak into the electrode, and the NRE would not be a micro/nanoelectrode but a conventional millimeter electrode, which means that the preparation of the NRE electrode has failed. After an ITO stripe was prepared into an NRE through the aforementioned preparation process in this work, a pair of peaks of CV curve for ITO in Figure S2A has magically disappeared and turned into the standard "S" type steady-state CV curve of microelectrode for the NRE in Figure S2B, which proved the successful preparation of the NRE. As can be seen from Figure 3D, the cylindrical NRE is similar to millimeter electrodes, which can be reused after polishing and updating the surface of the electrodes. Therefore, it can suffer from typical treatments including mechanical polishing with 0.05 μm alumina or P7000 silicon carbide abrasive paper.

Toxicity Detection with Different Types of Electrodes. As shown in Figure S3A, currents increase with increasing scan rate. The linear sweep voltammetry (LSV) curve is similar to that of the millimeter electrode when scan rate exceeds 200 mV/s. This can be interpreted that the reaction product in the transient state does not have time to diffuse on the electrode surface. From Figure S3B, the coefficient of correlation (r^2 = 0.98643) is obtained by linear fitting equation ($y = 0.19708x + 4.77148$) between the peak currents of LSV and $V^{1/2}$ in the scope of 1−200 mV/s, which demonstrates that the electrochemical reaction is controlled by the diffusion on the NRE surface.

Once the electrode diameter size was reduced from millimeter to micron or nanometer, a lot of electrochemical behaviors would be changed. For example, its steady-state large current density is obtained because the effect of the electric double-layer capacity is small.[12] Current density would be much higher than that of the millimeter electrode under forced convection. DCP used as the reference toxicant has been widely researched.[10a,22] The feasibility of detection toxicity of DCP was demonstrated using the NRE as working electrode in Figure S4. The steady current could be provided by the NRE in less than 10 s. The toxicity could be effectively distinguished by electrochemical method with the NRE in Figure S4A. The inhibition ratio of 10 mg/L DCP was calculated to be 75.27% according to the currents. Figure 4 shows the differentiation of oxidation currents by detecting current signal and its reproducibility for sample solutions with and without 10 mg/L DCP using single microelectrode, MEA, NRE, and millimeter electrode, respectively. The oxidation currents increased in the order of single microelectrode, MEA, NRE, and millimeter electrodes. Steady-state current can be rapidly obtained in 10 s for a single microelectrode, MEA, or NRE, except for the millimeter electrode. The oxidized currents of samples with DCP decreased compared with that of the control sample. More importantly, the repeatability of the NRE is the best among all electrodes. All results suggest that the influence of the toxicant on microbe respiration could be estimated with the NRE. In a word, the current signal of $K_4[Fe(CN)_6]$ produced could be effectively detected and amplified by the NRE.

Stability of NRE. The stability of the prepared NRE in the absence of a shielding box was further examined. As can be seen from Table S1, although the thickness of the NRE is only 185 nm, its considerable width (3 mm) makes its geometry area (1110 $(\mu m)^2$) reach more than 3.5 times that of the disk microelectrode with a diameter of 20.0 μm (314 $(\mu m)^2$). This makes the NRE have a much better anti-interference performance than the microelectrode. Figure S5A shows that $i-t$ curve is smooth for the NRE, while $i-t$ curve for the microelectrode greatly fluctuates in Figure S5B. The above results show that the NRE has better anti-interference ability, which could ascribe to its large geometric area relative to the microelectrode.

As shown in Figure S6A, the CV curves for the same sample of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ with three NREs prepared in parallel had a good reproducibility. The photo of the three NREs is shown in Figure S6B. As shown in Figure 5A, the NRE exhibited not only the microelectrode characteristics of typical "S" model CV curve for 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ in 0.1 M KCl but also had good repeatability after 100 CV cycles. In addition, a good stability with a response difference of 94.67−102.99% was also obtained for 20 parallel samples with the same NRE (Figure 5B). It can also be seen from Figure S7 that the currents remained stable with the same NRE in a solution containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ in 0.1 M KCl for at least six months. These results indicate that the NRE is of excellent stability.

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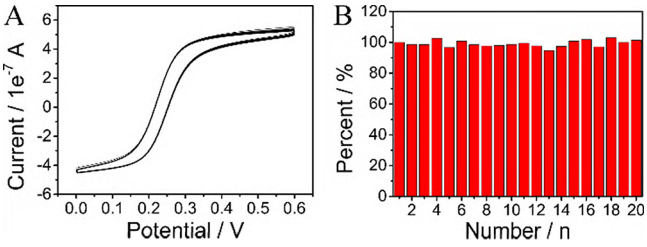

Figure 5. The stability of the NRE of (A) 100 cycle CV curves of a sample at a scan rate of 50 mV/s, each sample containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ in 0.1 M KCl. (B) Oxidation current relative values of 20 parallel samples, each sample containing 40 mM $K_3[Fe(CN)_6]$, OD_{600} = 6 *E. coli*, and incubating for 1 h.

The excellent stability is closely related to the electrodeposition of 2-allylphenol on ITO. The polymerization mechanism was described as follows. At a potential of 4 V, the phenolic group on the monomers was oxidized via a one-electron, one-proton transfer process, generating a free radical, which can then initiate a free radical, step-growth polymerization. The rate where new chains were generated reaches a steady state indicated by the leveling out of the current response. Upon heating, these discrete chains were cross-linked via the allyl groups, creating a network polymer system, which electrically insulated the ITO. As a result, the NRE was successfully prepared with good stability.

Application for Toxicity Assessment. Biotoxicity method of water detection has developed rapidly in recent years in which suitable species for use as biometric identifiers. As shown in Table S2, compared with fish, water flea, and algae, the toxicity detection method using microbes as bioreceptor has several advantages due to its simple cultivation, small individual differences, no moral justice, and so on. The bioelectrochemical method has caught a lot of focus of scientific researchers in recent years because it is not interfered by turbidity and chroma of water, and the instrument for signal detection is simple. However, the sensitivity is relatively low (ppm), which may be related to the toxicity of the mediator itself.[25]

Before the water toxicity test, the culture temperature and $K_3[Fe(CN)_6]$ concentration were optimized as shown in Figures S8 and S9. Higher currents can be obtained at a mild temperature of 37 °C and an appropriate concentration of 40 mM $K_3[Fe(CN)_6]$. The toxicity of DCP was further tested at the optimized conditions containing a final concentration of 40 mM $K_3[Fe(CN)_6]$, final cell concentration of OD_{600} = 6, and 1 h of incubation at 37 °C. Figure 6 shows the inhibitory curve obtained for different concentrations of DCP, and the 3.01 mg/L of the IC_{50} value was obtained. The same trend was also observed in the duplicate experiments. IC_{50} values obtained for DCP in this work and literature are reported in Table S3. The proposed biosensor has higher sensitivity than other detection methods. The high sensitivity obtained in this work may be mainly attributed to the use of more suitable 40 mM $K_3[Fe(CN)_6]$ concentration.

CONCLUSIONS

In summary, a novel, low-cost, and simple method of manual fabrication of the NRE has been proposed in this work. The current signal is amplified while the electrode dimension is reduced from micron to nanometer. The as-prepared NRE is validated to have strong anti-interference ability and good

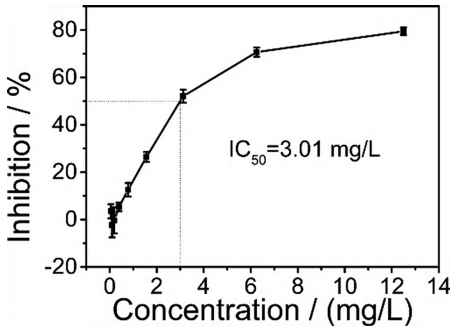

Figure 6. Inhibitory curve of microbe respiration for different concentrations of DCP. Replicate samples containing 40 mM $K_3[Fe(CN)_6]$, OD_{600} = 6 *E. coli*, and incubating for 1 h. Each point plotted was the average of three samples.

stability. A toxicity biosensor based on the NRE was built in which *E. coli* was used as bioreceptor, and $K_3[Fe(CN)_6]$ was employed as electron mediator. The NRE showed a more acute toxicity detection than single microelectrode, MEA, and millimeter electrode. A smooth current curve amplified by the NRE reached a steady state in 10 s under a constant potential of 450 mV. The toxicity could be determined electrochemically by measuring the difference of oxidation currents of the resultant $K_4[Fe(CN)_6]$ in the presence and absence of DCP. An IC_{50} of 3.01 mg/L for DCP was eventually obtained. These results confirm that the proposed electrochemical method based on the as-prepared NRE is a sensitive, rapid, and inexpensive way for toxicity assays.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssensors.0c01566.

Polymerization reaction of 2-allylphenol, comparison of CV curves between ITO and NRE, effect of the scan rate, feasibility of toxicity detection with NRE, comparison of anti-interference between NRE and microelectrode, reproducibility of three electrodes, long-term stability of NRE, optimization of incubation temperature, optimization of $K_3[Fe(CN)_6]$ concentration, comparison of geometric area between NRE and microelectrode, comparison of various methods of toxicity detection, and comparison of the IC_{50} values of DCP (PDF)

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Notes

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