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Preparation, performance, and application of a stable, sensitive and cost-effective microelectrode array

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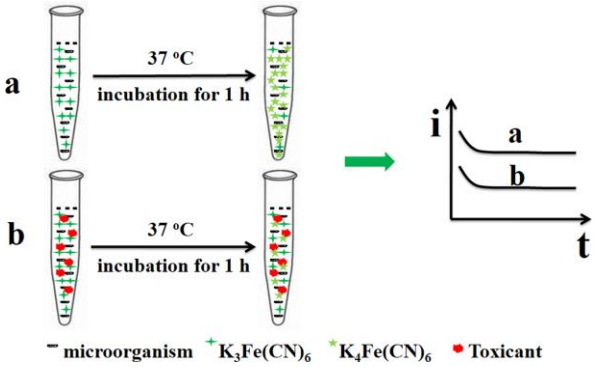
ABSTRACT:

The development of rapid toxicity detection technology has higher requirements for electrode. In this work, a stable, sensitive and cost-effective microelectrode array (MEA) was successfully prepared manually. The advantage of the as-prepared MEA was discussed by means of detecting toxicity of 3,5-Dichlorophenol (3,5-DCP) by contrasting with bulk electrode and single microelectrode, in which mixed microorganisms were selected as biocatalyst, and $K_3[Fe(CN)_6]$ was adopted as electron mediator. The current reached a stable state in 10 s under the constant potential of 450 mV. The feasibility of rapid detection of toxicity of formaldehyde with the MEA was further verified. The current responses were analyzed over formaldehyde of the final concentrations varied from 0.0036% to 1.0%, and the traditional parameter of 50% inhibitory concentration (IC_{50}) of 0.11% was obtained within 1 hrs. In brief, the as-prepared MEA has high sensitivity, good stability, strong anti-interference, and well corrosion resistance. It shows a very large application prospects in toxicity detection in water.

Graphical abstract



Keywords: *microelectrode array; biosensor; toxicity; 3,5-dichlorophenol; formaldehyde*

1. Introduction

Water environment safety is becoming a serious problem all over the world, and it is urgent to develop a simple, rapid and cost-effective assay for detecting water pollution degree and assessing aquatic ecological environment risk to human health. Recently, increasing biological assays are proposed and used to evaluate the effect of biochemical oxygen consumption or toxicant pollution on the environment[1-3]. Generally speaking, biosensors are composed of two essential elements, biocomponent and transducer[4]. Eukaryotic species, such as daphnia[5], algae[6], mouse[7] and fish[8], are used as biocomponent in conventional toxicity bioassays, but with some disadvantages such as time consuming, high cost, specialized equipment and trained personnel. Microorganisms with physiological parameters of short life cycle play the fundamental role in the aquatic ecological environment, with the adaption of analytical tools to toxicity studies for assessing organisms or ecosystem health. Based on the changes in the respiration chain activity[9], optical property[10] or morphology of microorganisms[11] induced by toxicants, a lot of toxicity bioassays have been designed and developed[12-14]. Among them, cellsense assay using *Pseucomonas putida* immobilized on screen printed electrodes shows more sensitive response to the toxicants, but the time spent on pretreatment and immobilization is too long and it is less reproducible compared to electrochemical

method[15]. The optical toxicity bioassays based on luminous bacteria have been successfully used to screen the toxicity of a large number of chemicals[16, 17], however, the drawback of these bioassays is not suitable for measurement of turbid solutions due to the decrease of light intensity[18].

In contrast, microbial toxicity assays based on the electrochemical measurement have caused considerable attentions lately because high concentrated cells can be used in the methods and it has no interference for turbid samples[19, 20]. However, the toxicity assays have strict demands for the working electrode. Microelectrodes have pivotal role to the recent development of rapid electrochemical techniques[21]. One successful model of such bioassays is MICREDOX, a rapid biosensor-based assay developed by Pasco et al. for monitoring biochemical oxygen demand (BOD) and toxicity with microelectrode[4, 14, 22]. But the single microelectrode is usually limited in the current intensity, less than the minimum detectability of conventional electrochemical instruments.

Microelectrode array (MEA) has made tremendous advances in electrochemical studies since the introduction of electroanalytical chemistry more than 40 years ago, their time and potential unaffected[23]. MEA is prepared by integrating multiple microelectrodes together, therefore, its current is the calculated sum of that of a single microelectrode. Being a very powerful tool for environmental analysis, MEA is of rapid widespread application in fields including biochemical and environmental analysis. Due to its inherent features[24, 25], MEA is allowed to be used in highly resistive media, at fast scan rates, and with high sensitivity[26]. Although the as-prepared MEA has been widely applied in toxicity testing in our previous work[27, 28], its preparation procedure and its performance, and the feasibility in toxicity test are still neither in detail expatiated nor in-depth studied.

In this study, microelectrodes array was manually prepared with different diameters and numbers of micron-grade Pt wire. A toxicity biosensor based on the as-prepared MEA was also proposed, in which mixed microorganisms were used as model microorganism, and $K_3[Fe(CN)_6]$ has been employed as the artificial electron mediator. The feasibility of toxicity detection was further demonstrated with 3,5-dichlorophenol (3,5-DCP) as the reference toxicant, which has been widely researched[29-31]. Formaldehyde plays an important role to worldwide economic development. However, more and more people are exposed to an environment of formaldehyde, as it exists everywhere in workshop, tobacco smoke, household

products, automobile exhaust and newly renovated buildings. Formaldehyde is widely found in wastewater owing to its solubleness of 55%, too. Therefore, it is necessary for us to carry out rapid detection of formaldehyde wastewater. The toxicity can be determined by measuring the difference of oxidation currents of the resultant $K_4[Fe(CN)_6]$ in the presence and absence of formaldehyde electrochemically. Finally, the currents were directly converted to inhibitory percentage through simple signal treatment. The greater advantage of MEA was validated in toxicity detection than single microelectrode and conventional bulk electrode.

2. Experimental

2.1. Materials and reagents

Glass tubes and glass capillaries were purchased from glass processing workshop of the Changchun Institute of Applied Chemistry. Pt wires (Φ=20.0, 25.0 and 50.0 μm) were purchased from Sino-platinum Metals CO., Ltd. Peptone was purchased from Beijing Aoboxing Bio-tech Co., Ltd. Yeast extract was purchased from OXOID Co. Ltd., UK. Glycerol was purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. 3,5-DCP was purchased from Sigma-Aldrich. Graphite powder and formaldehyde (0.815 g/cm³) were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. $K_3[Fe(CN)_6]$, KCl, NaCl, KH_2PO_4, Na_2HPO_4 and NaOH were purchased from Beijing chemical plant. Phosphate buffer solution (PBS, 0.08 M Na_2HPO_4 and 0.12 M KH_2PO_4) and LB broth (10 g/L peptone, 10 g/L NaCl and 5 g/L yeast extract) were prepared with deionized water and sterilized at 121 ^oC for 15 mins. The toxicant solutions (3,5-DCP and formaldehyde) with desired concentration were prepared by diluting the high-concentration stock solution (1000 mg/L and 37%~40%) with freshly-prepared deionized water. 3,5-DCP was replaced by deionized water in the control solution. 45 mM $K_3Fe(CN)_6$ was used as the final concentration because microbes may be damaged in higher concentration $K_3Fe(CN)_6$ solutions[32]. If not stated specifically, all chemicals used were received with no further purification and all solutions were prepared by using deionized water (18.25 MΩ/cm) purified with a Milli-Q Gradient System (Millipore).

2.2. Instrumentation and Procedures

Electrochemical measurements including cyclic voltammetry and chronoamperometry were performed with an electrochemical analyzer (CHI 832B, Shanghai Chen Hua Instrument Co., Ltd.). A conventional three-electrode system was

used, which consisted of an as-prepared Ag/AgCl electrode (saturated KCl) as the reference electrode, an as-prepared Pt plate as the counter electrode and an as-prepared Pt MEA (20.0 μm diameter Pt wire) as the working electrode, respectively. For toxicity tests, the current-time curve was used to make a quantitative measure of the reduced mediator. The working potential was set at 450 mV, which is highly sufficient to reoxidize the $K_4Fe(CN)_6$ produced by the reduction of $K_3Fe(CN)_6$ during cell respiration[32]. All potentials given here were versus the Ag/AgCl reference electrode, and all the tests were performed at room temperature. To exclude possible interference from cells, the culture supernatants were collected by centrifugation at a centrifuge (Eppendorf, Centrifuge 5804 R, Eppendorf Co. Ltd) before electrochemical assay. A new and small capacity full temperature oscillator (ZHWY-200B) was purchased from Shanghai Zhicheng Analytical Instrument Manufacturing Co. Ltd. (Shanghai, China). Rotary vane vacuum pump (FY-1C) was purchased from Wenling Mechanical and Electrical Co., Ltd. (Wenling, China). Ultrasonic cleaner was purchased from Kunshan Ultrasonic Instruments Co., Ltd. (KQ-100DE, Kunshan, China). Drying oven was purchased from Hubei Hengfeng Medical Pharmaceutical Equipment Co., Ltd. (SFG-02.500, Huangshi, China). Bunsen burner was purchased from Rocker Scientific Co., Ltd. (Rocker Dragon 200D, Taiwan, China).

2.3. Microorganisms preparation

Microbial strains were obtained from local groundwater as the parent strains. Bacteria were grown aerobically by shaking (220 rpm) in LB broth at 37 ^{o}C for 10 hrs. Cells were harvested by centrifugation at 6000 rpm for 10 mins at room temperature, then washed twice with PBS and resuspended in PBS. The concentration of cells was adjusted to an absorbance value of 24, measured at 600 nm (OD_{600}) using a Cary 500 Scan UV-vis-NIR Spectrophotometer. The bacterial suspension was used for the further experiments on the day of harvesting.

2.4. Preparation of MEA

A scale-up of the procedure was employed for preparing microdisk electrode. A glass capillary was pulled from 10 mm inside diameter glass tubing, with their inner diameter 65.0 to 100.0 μm. The capillary was cut in lengths of 2 cm, successively cleaned with acetone, ethanol and deionized water by ultrasonic cleaning, and placed in 80 ^{o}C drying oven for 2 hrs. Similarly Pt wire of 20.0 μm, 25.0 μm or 50.0 μm diameter were cut into 3 cm lengths, successively cleaned in acetone, ethanol and deionized water, and dried at 80 ^{o}C.

A Pt wire was then carefully inserted into each capillary until it reached the end. The portion of each capillary with the wire was sealed over about a 2 mm length with a small flame provided by an alcohol lamp. The holder for the array was a length of 80 mm glass tubing (6 mm outer diameter and 4 mm inner diameter). The desired number of these capillaries were placed in the end of the large glass tube, and the wires, capillaries, and tube were slowly fused with a small flame, then, the wires, capillaries, and tube were heated and slowly fused with a big flame provided by a bunsen burner after a vacuum was pulled for 5 mins on the end of the tube opposite to the bundle of capillaries.

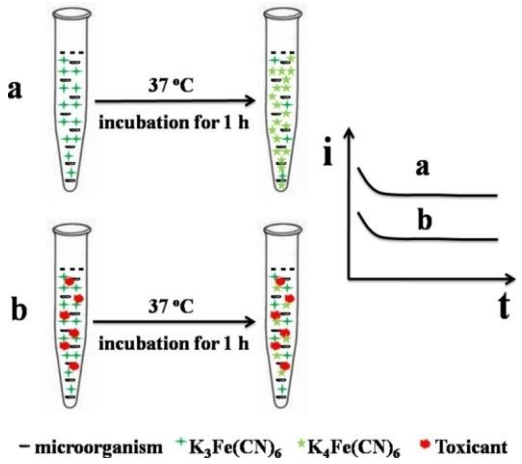
The assembly was placed above flame to remove strain, then, the tip of the assembly was cut with a diamond cutter to expose the Pt microdisks, polished with 280-grit alumina (Siliciumcarbid, 991A). A smooth finish was achieved by polishing with successively finer grades of silicon carbide abrasive paper (Siliciumcarbid, 991A, P600, P1000, P2000, P3000, P5000) and completed by polishing with 0.05 μm gamma alumina powder (SPI #1337), and thoroughly ultrasonic cleaned with ultra-pure water before use. The end of the tube was filled into the graphite powder, then inserted an electrical conductor and fixed with 502 glue. Connection to an electrical lead inside the tube was achieved by using a low-melting alloy, Wood's metal.

2.5. Principle of the toxicity detection

The basic principle of the toxicity detection is based on the changes in the respiration chain activity of microorganisms induced by toxicants. Schematic illustration of the $K_3Fe(CN)_6$-mediator rapid water toxicity assessment was shown in Scheme 1.

Microbial respiration and metabolic activity can produce large numbers of electrons. As clearly shown in Scheme 1(a), more $K_3Fe(CN)_6$ in the control sample was reduced to $K_4Fe(CN)_6$. $K_4Fe(CN)_6$ was oxidized to $K_3Fe(CN)_6$ on the surface of MEA under the 450 mV constant potential, and a larger oxidation current was detected. It was shown in Scheme 1(b) that the respiration activity of the microorganism was inhibited when the toxic substance was added to the sample, the number of generated electrons was decreased, and the amount of $K_4Fe(CN)_6$ obtained by reducing the $K_3Fe(CN)_6$ was also decreased. In other words, the current detected by the MEA was also decreased. In brief, the inhibition ratio of toxicity was calculated through the difference of electrons produced by the damaged microorganisms and non-damaged microorganisms, i.e., the difference of the oxidizing currents of the reduced

$K_4Fe(CN)_6$. In general, the smaller current obtained along with the greater toxicity and greater concentration of toxic substances added.



Scheme 1. Schematic illustration of the $K_3Fe(CN)_6$ -mediator rapid water toxicity detection.

Without considering the endogenous respiration of microorganisms, the toxicity could be expressed by the inhibition % shown in the following formula (1) [27]:

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

The inhibition ratio was calculated according to formula (1) when electrochemical assay was used, where, i_{tox} and i_{con} were the oxidation currents of $K_4Fe(CN)_6$ in the presence and absence of toxicants, respectively. The ratio of the electrochemical signals, recorded in the presence and absence of toxin, provided an index of inhibition.

3. Results and discussion

3.1. Preparation of MEA

The structure schematic and photo of MEA are shown in Fig. 1(A) and Fig. 1(B), respectively. The MEA was fabricated with 20.0 μm diameter Pt wire in a 6 mm outer diameter glass tube. It can be clearly seen that it contains many single microelectrodes, and its overall shape is the same as a bulk electrode. The electrode section is in length of about 6 mm and its surface can be repeatedly polished for many times, therefore, the electrode has a long life. The disks are widely separated in the array, the polished disk surfaces appear to be flush with the glass surface with no discernible fissures or cracks at the metal-glass seal.

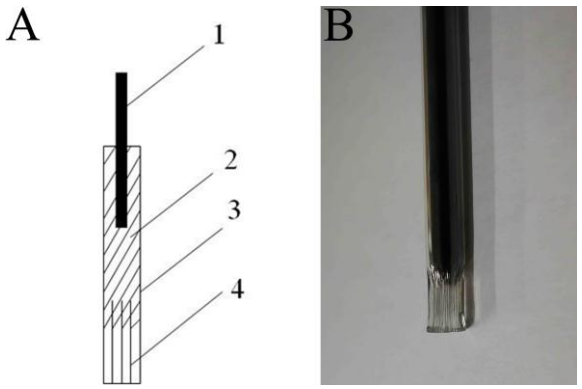


Fig. 1. (A) Structure schematic of the MEA (1-electrical conductor; 2-carbon powder; 3-glass tube; 4-Pt wire); (B) photo of the microelectrode array of 32 pieces of 20.0-μm-diameter Pt disks.

3.2. Effect of the diameter of electrode material, the number of microelectrode and the scanning speed

Cyclic voltammogram (CV) curves from three as-prepared single microelectrodes with diameter 20.0, 25.0 and 50.0 μm Pt wire were obtained in the same electrolyte. As shown in Fig. S1, the current increases with the increasing of wire diameter, however, CV curves of the electrode with 50.0 μm diameter have a pair of peaks similar to that of large electrodes, because the reaction products on the electrode surface cannot spread timely. The currents of electrodes with diameter 20.0 μm and 25.0 μm showed little difference, however, electrode with smaller diameter embraced faster mass transfer on the microelectrode surface, so, single microelectrode with diameter 20.0 μm was chosen to prepare MEAs in this work.

A good linear relationship between the peak currents and the array numbers is a criterion in evaluating the MEAs characters. As shown in Fig. S2(A), six arrays have been constructed with disk numbers 1, 2, 4, 8, 16 to 32, and currents increases with the increasing of the number of arrays. It can be clearly seen from Fig. S2(B), a coefficient of correlation of r^2=0.99293, is obtained by linear fitting equation (y=0.13167x-0.00479) between peak current of linear sweep voltammetry (LSV) and the numbers of electrode wires.

Fig. S3(A) points out that currents increases with the increasing of the scanning speed, however, the CV curve shows that the shape of the large electrode as the reaction product on the electrode surface is too late to diffuse when the scanning speed exceeded 100 mV/s. As shown in Fig. S3(B), the coefficient of correlation, r^2=0.96912, obtained by linear fitting equation (y=-0.14613x-4.14927) between current peaks of LSV and $V^{1/2}$ in the scope of 1~100 mV/s, demonstrates that the electrochemical reaction is controlled by diffusion on the MEA surface.

3.3. Stability of MEA

Once the diameter of electrode reduced from millimeter to micron, a lot of electrochemical behaviors will change, too. For example, its steady-state current density is much higher than that of the conventional bulk electrode under forced convection. We further examined the stability of the prepared MEA in the absence of a shielding box, and had the result presented in Fig. 2(A). The bare Pt MEA not only exhibites the microelectrode characteristics of typical reversed "S" model for 5 mM $K_3Fe(CN)_6$ and 5 mM $K_4Fe(CN)_6$ (an equivalent concentration of potassium ferrocyanide reducted from 45 mM potassium ferricyanide by microbials) in 0.1 M KCl solution, but also has a good scanning repeatability after 20 cycles of potential scan. Fig. 2(B) explains that the oxidation current of $K_4Fe(CN)_6$ has little difference for 15 samples. And Fig. 2(C) presents that the peak currents of the MEA remain unchanged for the solution of 5 mM $K_3Fe(CN)_6$ and 5 mM $K_4Fe(CN)_6$ in 0.1 M KCl over more than 5-month investigation. In general, those experimental results indicate sufficiently that the prepared MEA has a very good stability.

The MEA is very robust to bear typical treatments of the arrays include sonicleaningor polishing with 0.05 μm alumina or P5000 silicon carbide abrasive paper between experiments. The electrodes are also not affected for long time exposing to organic solvents.

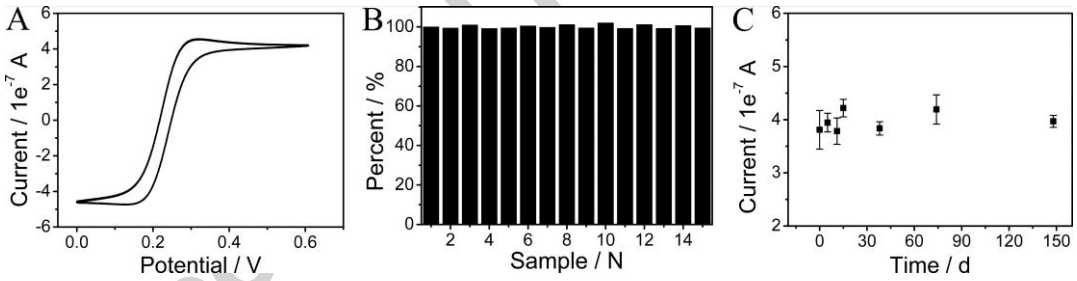


Fig. 2. The stability of the bare Pt MEA (32 Pt wires with 20.0 μm diameter) of (A) the CVs of 20 cycles of the sample at 50 mV/s; (B) the oxidation current relative value of the sample; (C) the life for more than 5-month (the CVs peak currents of the samples, each point plotted was the average of three samples). Each sample is a solution containing 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, and 0.1 M KCl.

3.4. Toxicity detection with different type of electrodes

Fig. 3 shows the differentiation of oxidation currents by detecting the microbes suspension in a mediator solution containing 4 mg/L 3,5-DCP for 1 h incubation using single microelectrode, MEA and conventional bulk electrode, respectively. As shown in Fig. 3(A, B, C), the oxidized currents in the $K_4Fe(CN)_6$ have a clear decrease

compared with that of the control sample. The oxidation currents intension also increase with the order of single microelectrode, MEA, and conventional bulk electrodes, respectively. The steady state current can be easily obtained in 10 s at single microelectrode and the MEA, except bulk electrode. More importantly, the repeatability of the MEA is the best among all tests. Therefore, the advantage is obvious when a conventional electrochemical instrument without high sensitivity was used in detecting toxicity. The smooth current signals further indicated that no interference from other electroactive species occurred in the potential range during measurement without a Faraday cage. All results indicated that the influence of the toxicant on microbe respiration could be estimated by chronoamperometry. In a word, the current signal of $K_4Fe(CN)_6$ produced from the metabolism was proven to be directly related to the toxicity, which could be effectively amplified by MEA.

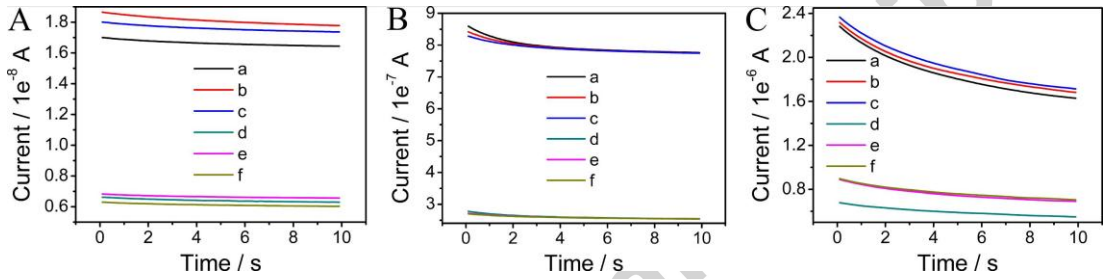


Fig. 3. The oxidation current differentiation for (A) single microelectrode (20.0 μm diameter); (B) MEA (32 Pt wires with diameter 20.0 μm) and (C) conventional bulk electrode (1.0 mm diameter) depending upon time for three parallel control samples (d, e, and f) and three parallel test samples (a, b, and c). Test sample: 45 mM $K_3Fe(CN)_6$, OD_{600} = 6, and 4 mg/L 3,5-DCP for 1 h incubation).

3.5. Feasibility of toxicity detection with MEA

In order to demonstrate the feasibility of toxicity detection with MEA, the changes in $K_3Fe(CN)_6$ mediated respirator activity of bacteria were measured in Fig. 4. It should be noted that the final concentrations of cells, $K_3Fe(CN)_6$ and formaldehyde (v/v) were OD_{600}=6, 2.25 mM and 0.05%, respectively. The solution samples d and e were incubated for 1 h in a 37 ^oC shaker at 220 rpm.

It is clear from Fig. 4 that the detection time of each sample is less than 10 s, which is helpful for rapid detection. Anodic currents are not observed for $K_3Fe(CN)_6$ (Fig. 4A, -curve a) and the mixture containing $K_3Fe(CN)_6$ and formaldehyde (Fig. 4A, -curve b). The color of the samples a and b in Fig. 4(B) has no change, which indicates that formaldehyde can't be oxidized by $K_3Fe(CN)_6$ or at 450 mV. $K_3Fe(CN)_6$ can accept electrons from cells easily, which is proved evidently by the immediate appearance of anodic current after mixing with cells, as shown in Fig. 4A, -curve c).

Then the same resultant mixture was incubated for 1 h at 37 ^{o}C, and the anodic current increased dramatically because of the accumulation of $K_4Fe(CN)_6$ (Fig. 4A, -curve d). The mixture containing $K_3Fe(CN)_6$ and cells is also incubated in the presence of formaldehyde, although the anodic current still increases, the current is smaller than that obtained without formaldehyde (Fig. 4A, -curve e).

The color changes of each sample in Fig. 4(B) show the consistency of the current changes in Fig. 4(A), which further confirms the above conclusions. These results demonstrate that the $K_3Fe(CN)_6$ mediated respirator activity of cells can be inhibited by formaldehyde, and show the feasibility of the proposed bioassay based on MEA in toxicity detection. In view of this, the steady current could be provided by the MEA in less than 10 s. The toxicity could be effectively distinguished by electrochemical method with MEA in Fig. 4(A) as the toxicity detection by colorimetric process in Fig. 4(B), the inhibition ratio of 0.05% formaldehyde is calculated to be 38.56% according to the currents.

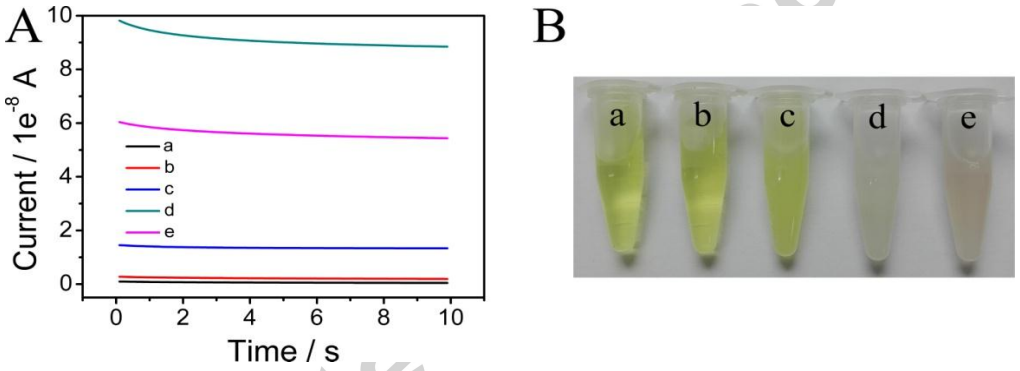


Fig. 4. (A) i-t curves recording electrochemical signals of MEA to the samples of (a) $K_3Fe(CN)_6$; (b) $K_3Fe(CN)_6$ and formaldehyde; (c) $K_3Fe(CN)_6$ and microorganisms (without cultivation); (d) $K_3Fe(CN)_6$ and microorganisms (cultivation for 1 h); and (e) $K_3Fe(CN)_6$, microorganisms and formaldehyde (cultivation for 1 h); (B) the sample pictures corresponding to the i-t curves in (A).

3.6. Toxicity detection

The soluble formaldehyde was selected here as the tested toxin because it has a fast biological toxicity to microorganisms and stable chemical properties in samples. The toxicity of formaldehyde is further tested at the optimized conditions containing final concentration of 45 mM $K_3Fe(CN)_6$, final cells concentration of OD_{600}=6 and 1 h incubation[32]. Fig. 5 shows the respiration ratio obtained at different concentrations of formaldehyde, and the IC_{50} value is 0.11%. The same trend was also observed in the duplicate experiment.

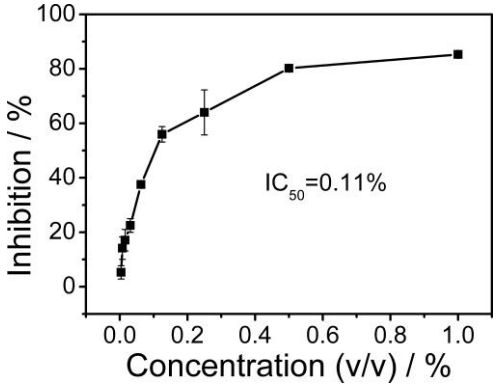


Fig. 5. Inhibitory curve of bacterial respiration at different concentrations of formaldehyde. Replicate samples containing 45 mM $K_3Fe(CN)_6$ and microorganisms solution of OD_{600}=6. Each point plotted was the average of three samples.

The inhibition percentages, IC_{50} and EC_{50} obtained for formaldehyde in present study and literature are reported in Table 1. It is observed that the inhibition ratio of 38.56% in this study in 60 mins is comparable to that of 36.4% in Microbial three-electrode cell in 34 mins for 0.05% formaldehyde. The biosensor of this work has more sensitive than that of silicon-based MFC with the lowest tested concentration of 0.1%. The volume percentage (v/v) of 0.11% is converted into the mass concentration (mg/L) of 0.3317~0.3586 mg/L to compare with other methods as Microtox® and respirometer. It is obvious that the IC_{50} of 0.3317~0.3586 mg/L in this study is much smaller than EC_{50} of 8.1 mg/L with the microtox® and 9.2 mg/L with respirometer. Although the EC_{50} decreased from 62.69 mg/L to 9.2 mg/L with respirometer when the contact time increased from 15 mins to 24 hrs, its inhibition is more than that of Microtox® and Electrochemical assay. This different response can be explained considering that the Microtox® method uses a pure culture of marine microbial species, whose habitat is very different from the growth environment of activated sludge and mixed microorganisms. Similar results have been reported in Gutierrez et al.[33], which tested an electrolytic respirometry device and the Microtox® on inorganic wastewater contaminants and several organic.

It is worth noting that the sensitivity of the toxicity bioassay is relevant to the mediators, microorganisms and types of toxic substances. This toxicity biosensor has the advantage of broad spectrum over traditional enzyme based biosensor since the electrochemical response is based on the activity of living cells. The results demonstrate the promise of this bioassay in determination of toxicity of organic pollutant. An extension of this work in future needs to be assessed under different conditions of biological systems, and the toxicity of both traditional and emerging

toxins will be also evaluated by this biosensor.

Table 1. Comparison of values of IC_{50}, EC_{50} or inhibition ratio obtained by the electrochemical assay with that of other methods obtained in references.

Method and equipment	Microorganism	Volume percentage (v/v)	Inhibition	IC_{50}/ EC_{50}	Duration of test	Reference
Electrochemical assay	Mixed microorganisms	0.05%	38.56%	*0.11%	60 min	Present study
Microbial three-electrode cell	Electrochemical active bacteria	0.05%	36.4%	--	34 min	[2]
Microbial three-electrode cell	*Shewanella oneidensis MR-1*	0.05%	--	--	19.5 h	[34]
Silicon-based MFC	*Geobacter sulfurreducens* (DSM 12127)	0.1%	Lowest tested concentration	--	1.06 h	[35]
Microtox®	*Vibrio fischeri*	--	--	#8.1 mg/L	30 min	[36]
Respirometer	Activated sludge	--	--	#62.69 mg/L	15 min	[36]
Respirometer	Activated sludge	--	--	#9.2 mg/L	24 h	[37]

*: $IC_{50;}$

#: $EC_{50;}$

--: Not found in the literature.

4. Conclusions

In this work, a manual fabrication MEA method was proposed and its character and application in rapid toxicity detection were carried out. The as-prepared MEA was validated to have good stability, strong anti-interference, high sensitivity, corrosion resistance and long life in the application of rapid detection of toxicity of 3,5-DCP. Besides, the inhibition ratio of 0.05% formaldehyde was 38.56% and the IC_{50} was 0.11% .The research presented an alternative method for assessing the impact of water pollutants on microorganisms. These results confirmed the proposed electrochemical method used based on the as-prepared MEA is a sensitive, rapid and inexpensive way for toxicity assays of organics. In general, the method described here has shown great

potential as an initial toxicity-screening tool. Further research works on the on-line toxicity monitoring with the as-prepared MEA is underway.

Acknowledgements

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Associated content

Supporting Information

The Supporting Information is available free of charge on the Elsevier website at DOI: ××××.

Effect of the diameter of electrode material, the number of microelectrode and the scanning speed on the current strength.

Conflicts of interest

There are no conflicts of interest to declare.

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Highlights:

- A stable, sensitive and cost-effective microelectrode array (MEA) was successfully prepared.
- The advantage of MEA was discussed versus bulk electrode and single microelectrode.
- A toxicity biosensor based on the as-prepared MEA was proposed.
- The feasibility of the toxicity detection was verified with the MEA.
- IC_{50} of 0.11% for formaldehyde was obtained within 60 mins.