



Short communication

Improved microbial electrocatalysis with neutral red immobilized electrode

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ABSTRACT

Efficient electron transfer (ET) between microbes and electrodes is a key factor for electricity generation in microbial fuel cell (MFC). The utilization of reversible redox electron-mediator can enhance such extracellular ET but could result in environmental contamination and low cost-effectiveness. These limitations may be overcome by immobilizing electron-mediator molecules on electrode surface. In this paper, we present a stepwise amidation procedure to covalently immobilize neutral red (NR), which has been proved to be an appropriate mediator to harvest microbial metabolic electrons due to its excellent electrochemical reversibility and compatible redox potential to the major metabolic electron carriers (e.g., of NADH/NAD⁺), on carbon electrodes. In this procedure, immobilization of NR is realized by acylchlorination of the carboxylated carbon surface with thionyl chloride followed by amidation reaction with NR. It is shown that such a stepwise amidation procedure can significantly increase the amounts of NR molecules immobilized on carbon surface without altering their redox properties. In addition, the use of NR-immobilized carbon electrodes as MFC anode can significantly increase the power output and the utilization of carbon sources (organic fuel).

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1. Introduction

Microbial fuel cell (MFC) is a device that directly converts the chemical energy stored in organic carbon sources into electricity through extracellular electron transfer from microbes to solid electrodes [1,2]. Besides the abundance in nature, microbes mostly have versatile metabolic abilities to oxidize organic compounds from natural hydrocarbons to domestic and industrial wastes, which make MFC a potentially competitive solution of the worldwide crisis on energy shortage and environmental damage. Currently, the power density of MFC remain too low to be competitive to the conventional chemical fuel cell, mainly due to the very low rate of direct electron transfer from microbial cells to solid electrode [3]. Therefore, mechanism of electron transfer from microbial cells to extracellular solid electrode in MFC and methods to accelerate such transfer have been the subject of extensive studies. It has been previously shown that rapid electron transfer from microbes to electrodes might be realized by introducing proper electron-mediators such as thionin, azine, and 2-hydroxy-1,4-naphthoquinone (HNQ) in culture solution [4–6].

Ideally, a redox-mediator to deliver metabolic electrons to fuel cell anode should have good electrochemical reversibility so that

its own oxidation at electrode requires negligible activation barrier. It should also have a redox potential close to the redox potential of NAD⁺/NADH couple, which is the major electron carrier in microbial metabolism and locates on the top of the electron transfer chain in microbial respiration. As has been shown by Park et al. [7,8], neutral red (NR) was one of the redox molecules with these novel properties. The comparable redox potential of NR to NAD⁺/NADH will render metabolic electrons to be harvested at the top of respiration chain, therefore resulting in a relatively negative anode potential (high energy efficiency) in MFC.

Although employing soluble electron-mediators can significantly improve the performance of MFC, it could cause secondary environmental contamination as mediators flow out with the effluent. In addition, the continuous reinforcement of electron-mediators also brings about cost problem [9]. Such embarrassments might be overcome using immobilized electron-mediator. Previous studies have shown that it was difficult to physically immobilize a large amounts of mediator directly on electrode surface, and that mediator physically adsorbed on electrode showed little efficiency because it was easily removed from the electrode surface and flowed out with the effluent [10]. Adachi et al. [11] have introduced that the power output of MFC could be increased using a mediator-polymer-modified anode, in which a bioactive redox compound 9,10-anthraquinone-2,6-disulfate (AQDS) was immobilized on the anode surface with a thin layer of a functional polymer. But for the redox potential of AQDS (−0.184 V vs. NHE) was not compatible to NAD⁺/NADH couple, a little improved MFC performance

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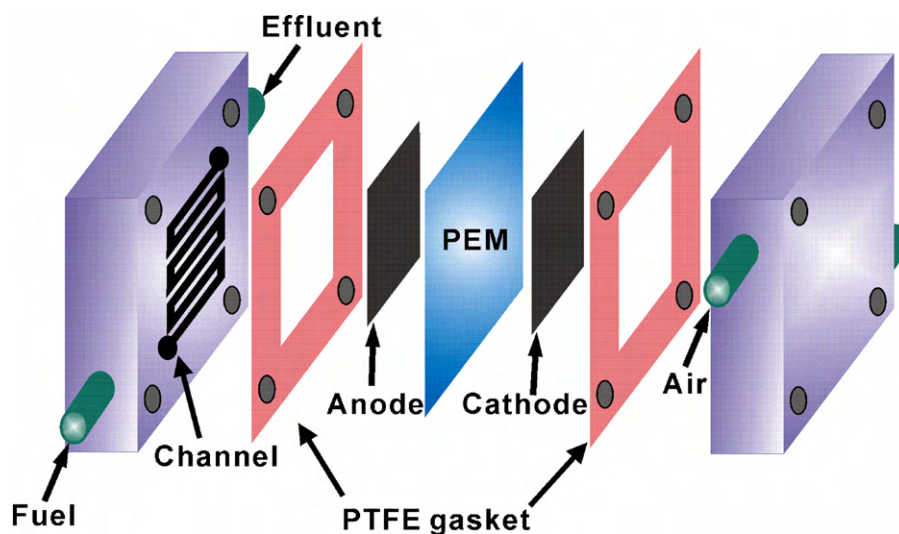


Fig. 1. The configuration of MFC.

was arrived. As stated above, NR was one of the ideal electron mediators for MFC to achieve high power output. Park et al. [12] and Park and Zeikus [13] have explored a chemical method for NR immobilization on carbon electrode, in which NR molecules were attached on carbon surface through a one-step amidation reaction between carboxylated carbon and NR molecules with *N,N'*-dicyclohexylcarbodiimide (DCC) as dehydration–condensation agent. The resulted covalent bond could prevent NR from being washed away during the MFC performance.

In this paper, we present a stepwise amidation procedure for covalent immobilization of NR on carbon electrode. Instead of direct amidation of the carboxylated carbon surface with NR, we first transfer carboxyl groups on oxidized carbon surface into acylchlorides. Acylamide bonds are then formed through the reaction between acylchlorides on carbon surface and amine groups of NR molecules. It is shown that, compared with the one-step direct amidation method, the present stepwise amidation procedure can significantly increase the amounts of NR molecules immobilized on carbon surface without altering its redox reversibility. In the mean-

time, significantly improved MFC performance is also achieved using NR-modified carbon anode fabricated with the present stepwise amidation procedure.

2. Materials and methods

2.1. MFC configuration

The MFC in present study has a similar structure to the conventional proton exchange membrane fuel cell (PEMFC), in which a membrane-electrode-assembly (MEA) is sandwiched between the two graphite plates with flowing channels on one side (Fig. 1). The MEA consists of an anode, a piece of PEM (Nafion 117) and a cathode, with the two electrodes pressed tightly on two sides of the PEM. The anode is a piece of 2×2 cm² carbon paper, and the cathode electrode is a conventional gas-diffusion-electrode fabricated by coating one side of a carbon paper with Pt/C catalyst.

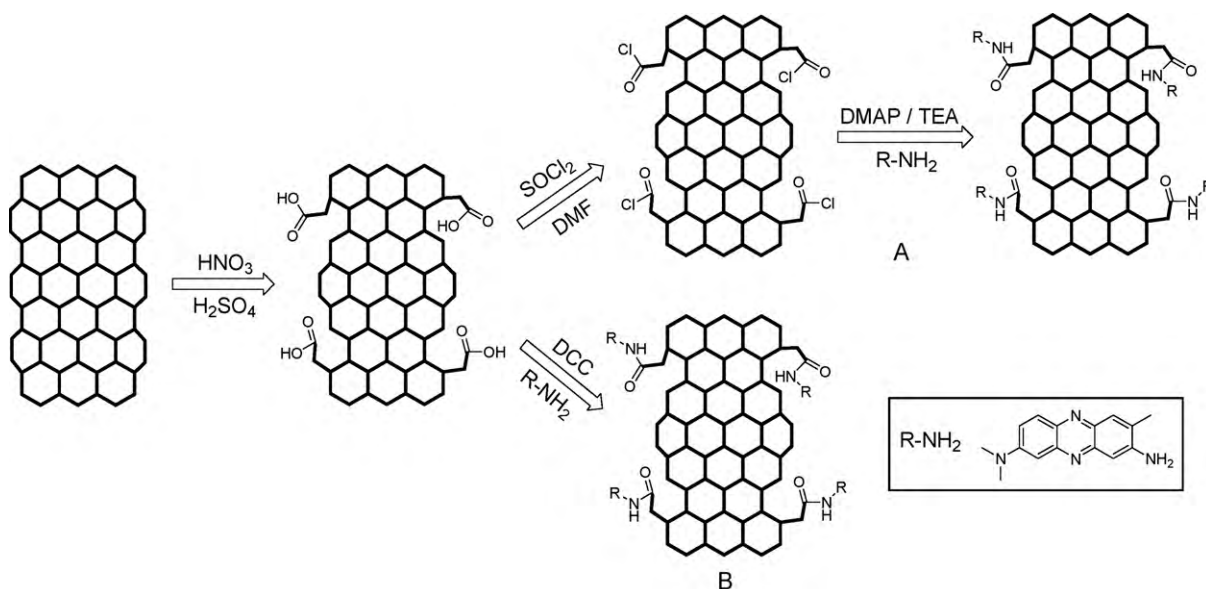


Fig. 2. The schematic illustration for two methods of covalent modification of carbon surface with NR. (A) The stepwise amidation procedure. (B) The one-step condensation amidation procedure.

2.2. MFC operation

Fuel cells were operated at 30 °C in continuous flow mode by connecting the anode flowing field with an external reactor. The potential of the anode was measured against a SCE reference electrode with a piece of Nafion ribbon serving as salt bridge. The fuel cells were discharged by connecting the anode and cathode with a constant loading resistance of 1 k Ω . The anode reactor was initially inoculated with wastewater. When obvious electricity generation appeared, sodium acetate solution (NaAC 1 g L⁻¹, NH₄Cl 0.31 g L⁻¹, KCl 0.13 g L⁻¹, Na₂HPO₄·12H₂O 6.93 g L⁻¹, NaH₂PO₄·2H₂O 5.62 g L⁻¹) was then used as carbon source. The reactor was continuously purged with N₂ gas to maintain an anoxic condition so that the aerobic oxidation of the organic matter in the wastewater was inhibited. Air was passed into the cathode chamber through an air pump. The anode potential and the cell voltage were recorded with a battery testing system (Neware CT-3008W).

2.3. Analysis method

The voltammetric measurements were carried out using a CHI 660B electrochemical workstation (CH Instruments, USA) in three electrodes configuration with a SCE reference electrode and a Pt sheet counter electrode. Fourier transform infrared (FT-IR) spectra were obtained in the range of 500–4000 cm⁻¹ on Nicolet NEXUS670 spectrophotometer at room temperature.

3. Results and discussion

3.1. Immobilization of NR on carbon electrode through amidation reaction

The covalent immobilization of NR molecules on carbon electrode can be realized via forming acylamide bond between the amino group in NR and the carboxyl group on carbon surface produced by oxidation. Two methods are explored in this study to form such acylamide bond, namely, the stepwise amidation procedure and the one-step condensation amidation procedure (Fig. 2). We first use carbon fibers (CFs) as model electrodes to demonstrate the two immobilization methods. Both procedures require a pre-oxidation of carbon surface to produce carboxyl groups. Before oxidation, CFs are cleaned in concentrated HCl solution by sonication for 30 min and rinsed thoroughly with double distilled water. After thermal treatment in a tube furnace at 225 °C for 18 h under argon atmosphere, the cleaned CFs are then oxidized by refluxing in a mixture of 12 M nitric acid/sulphuric acid solution for 12 h to produce -COOH groups on their surfaces [14]. The one-step condensation amidation method is very similar to that used by Park and his coworkers [12,13]. Briefly, the oxidized CFs are first soaked in DCC solution (2 mg mL⁻¹ in chloroform) at 4 °C for 6 h, and then continuously incubated in chloroform solution containing 2 mg mL⁻¹ DCC and 0.1 mM NR at 4 °C for 12 h. In this method, DCC serves as the dehydration–condensation agent for the direct amidation of the carboxyl groups on oxidized carbon surface.

In the stepwise amidation procedure, the oxidized CFs are first refluxed in thionyl chloride containing N,N-dimethylformamide (DMF) (3%, v/v) for 12 h to transform carboxyl groups to acylchlorides. DMF acts as catalyst to promote the formation of acylchlorides. The flask containing the acylchlorated CFs is then vacuumized, which is followed by addition of 4-dimethylaminopyridine (DMAP) and NR/triethylamine (TEA) anhydrous solution under anhydrous and anoxybiotic conditions. Upon addition of NR/TEA anhydrous solution, white smoke can be observed, indicating the occurrence of amidation process. After refluxing for 12 h, the functionalized CFs are rinsed thoroughly by

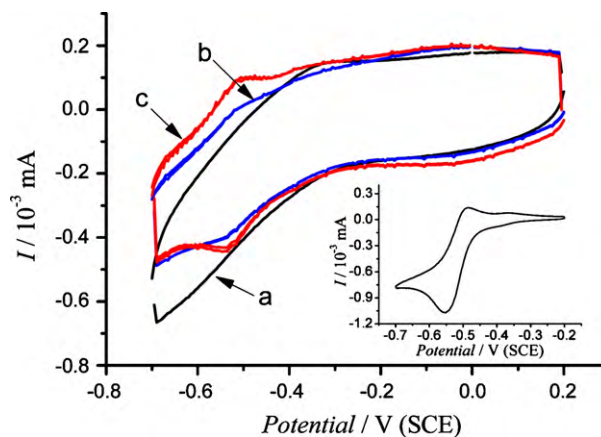


Fig. 3. The CVs of (a) carboxylated CF, (b) NR-modified CF through one-step condensation amidation reaction and (c) NR-modified CF through stepwise amidation procedure in 0.1 M PBS (pH 6.0). The inset: CV of bare CF in 0.25 mM NR solution (pH 6.0). Scanning rates: 50 mV s⁻¹.

sonication with double distilled water and then dried at 60 °C in an oven.

3.2. Characterization of NR-immobilized carbon electrodes

To compare the immobilization efficiency of these two amidation methods, NR-modified CFs are rinsed thoroughly to remove the physically adsorbed NR molecules and then subject to cyclic voltammogram (CV) measurements. Fig. 3 shows the CVs obtained in phosphate buffer solution (PBS) with bare CF, NR-immobilized CFs with the one-step condensation amidation procedure and the stepwise amidation procedure respectively. It can be seen that NR-immobilized CFs all exhibit a pair of redox peaks around 0.55 V vs. SCE which are not seen on the CV obtained with bare CF. As shown in the inset of Fig. 3, the bare CF in NR-contained PBS solution also exhibits a pair of redox peaks around 0.55 V vs. SCE. This indicates that NR molecules are indeed attached on CFs surface through these two amidation methods. However, the redox peaks given by NR-immobilized CF fabricated with the stepwise amidation procedure are much more defined and have much larger currents than that given by CF undergone NR immobilization through the one-step condensation amidation procedure. This implies that the amounts of immobilized NR molecules through the stepwise amidation procedure are much larger, although it involves relatively more complicated processes.

It is known that the direct amidation of carboxyl groups is a rather inefficient and low-yield process, especially when reacting with poorly reactive amino groups locating at high steric hindrance sites. The use of efficient dehydration–condensation agent, e.g., DCC, for direct amidation is a developed method in organic synthesis. The CVs in Fig. 3 seem to indicate that the usually used stepwise method via acylchloride is more efficient for amidation of carboxyl groups on carbon surface with NR. This is probably due to that the high activity of DCC would lead to the racemization of acylamide which finally limit the yield of product [15]. In the stepwise method, DMF with catalytic amounts can promote the formation of acylchloride which is more active than the carboxyl group. In addition, DMAP can efficiently catalyze the reaction between acylchloride and amino group.

Fig. 4 gives the FT-IR spectrum of NR-immobilized CFs prepared with the stepwise amidation procedure. The IR spectrum for CFs only undergone oxidation in HNO₃/H₂SO₄ mixture solution is also given for comparison. As shown by the spectrum b in Fig. 4, CFs before reacting with NR exhibits IR peaks at 1730 and 1588 cm⁻¹, which can be attributed to the stretching vibration of

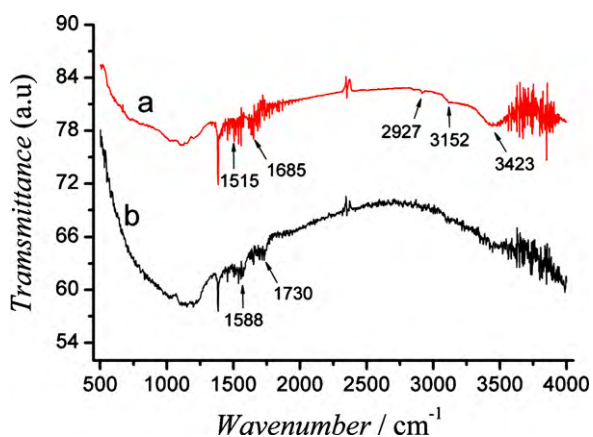


Fig. 4. FT-IR spectra of (a) NR-modified CFs and (b) carboxylated CFs.

C=O bond in $-\text{COOH}$ and $-\text{COO}^-$ groups respectively [16,17]. This indicates that carboxyl groups are produced on CF surface upon treatment in $\text{HNO}_3/\text{H}_2\text{SO}_4$ mixture solution. After NR modification with the two-step amidation procedure, CFs give characteristic IR absorption peaks associated with acylamide groups (spectrum a in Fig. 4), e.g., C=O stretching peaks in acylamide group at 1685 and 1515 cm^{-1} , C–H stretching peak of amine at 2927 cm^{-1} and N–H and O–H stretching peaks at 3152 and 3423 cm^{-1} respectively. This clearly indicates that NR molecules have been covalently linked to CF through acylamide bond.

3.3. Improved MFC performances using NR-immobilized carbon anode

Since carbon papers (CPs) are made of CFs, we immobilize NR on CP electrode by utilizing the above-mentioned stepwise amidation procedure and the resulted electrode is tested as MFC anode. Fig. 5 shows the variation of anode potential in two MFCs using CPs with and without NR immobilization respectively during a single discharge period, during which no carbon source is refurnished. Although the two MFCs were discharged with the same initial concentration of carbon source (calculated as chemical oxygen demand (COD): 780 mg L^{-1}), the MFC using NR-modified anode exhibits much more negative anode potential than that using ordinary PC as anode. Since the same Pt/C cathodes were used in the two MFCs, the cell voltages in two MFCs approximately follow the variation of their anode potentials. More negative anode potential in MFC

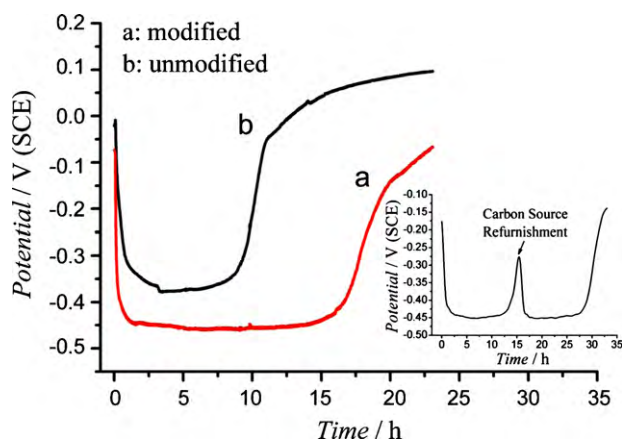


Fig. 5. The comparison of anode potentials of MFCs using CP electrodes with (a) and without (b) NR immobilization during a single discharge period under a certain equivalent quantity of carbon source. The inset: the changed potential of NR-modified anode with carbon source refurnishment.

corresponds to higher cell voltage, therefore larger discharge current under the constant-load discharge mode. With the same initial COD, the larger discharge current obtained with the NR-modified anode should be due to the facilitated electron transfer between microbial cells and electrode by NR immobilization, although the exact electron transfer mechanism between the immobilized NR molecules and the microbial cells is not clear.

It is also seen in Fig. 5 that the anode potentials in both MFCs maintain relatively stable negative values for certain periods, following which a rapid rise to much more positive values occurs. The NR-modified anode produces a potential plateau lasting for much longer time than the ordinary PC anode. The rise of anode potentials to positive values means that the currents flowing through MFCs decrease, i.e., the large discharge current at the potential plateaus cannot be sustained after a certain period. As shown in the inset of Fig. 5, the anode potentials will go back to the plateau values upon reinforcing carbon source. This indicates that the potential rise (current decrease) is due to the depletion of carbon source. Since the mechanism of electron transfer between the anodes and microbial cells is not clear, we cannot give a clear image on how the carbon source concentration may affect the discharge process. However, the much larger current and the longer potential plateau clearly indicate that the coulombic efficiency (the carbon source utilization) is significantly increased by NR-modification. We have monitored the COD values in solutions of the two MFCs in the course of discharge process. It was found that the COD values decrease to 241 mg L^{-1} (for ordinary CP anode) and 62 mg L^{-1} (for NR-immobilized CP anode) after having been discharged for 9 and 15 h respectively, i.e., at the end of the anode potential plateaus. According to these COD values, the carbon source utilizations are estimated to be 92 and 69% respectively for MFCs using NR-modified anode and bare CP anode. The significantly increased coulombic efficiency (the carbon source utilization) suggests that the accelerated electron-transfer rate in the presence of NR can speed up the metabolism of microbes.

The current density and power density associated with the discharge plateau for NR-modified anode are typically 1500 mA m^{-2} and 900 mW m^{-2} , which are higher than that achieved by Park et al. with NR-modified woven graphite anode fabricated using the one-step condensation amidation reaction (1100 mA m^{-2} , 800 mW m^{-2}) [14], despite the real specific surface area of woven graphite might be larger than CP anode used in present study. This further suggests that the stepwise amidation procedure may be more efficient than the one-step condensation amidation reaction for NR immobilization on carbon surface.

4. Conclusion

We demonstrate that NR, an ideal electron-mediator for microbial electrocatalysis, can be covalently immobilized on the surface of carbon electrode via acylamide bonds between the amino group in NR and the carboxyl group on carbon surface produced by oxidation. It is shown that the stepwise amidation procedure via an acylchlorination intermediate is more efficient to immobilize NR on carbon electrode surface than the one-step condensation amidation reaction with DCC as dehydration–condensation agent. The immobilization brings about little change in the redox properties of NR. The use of NR-immobilized carbon electrode fabricated with the present stepwise amidation procedure can lead to significantly increased power output and utilization of carbon sources in MFC.

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