Application of conductive polymers in biocathode of microbial fuel cells and microbial community

Chao Li, Lili Ding, Hao Cui, Libin Zhang, Ke Xu, Hongqiang Ren*

State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210046, Jiangsu, PR China

Abstract

Four kinds of conductive polymers, polyaniline (PANI) and its co-polymers poly (aniline-co-o-aminophenol) (PANOA), poly (aniline-co-2, 4-diaminophenol) (PANDAP) and poly (aniline-1, 8-diaminonaphthalene) (PANDAN) had been gradually synthesized, and these functional groups introduction can change the characteristics of primary PANI, which also endowed these co-polymers and the poly (aniline-co-2, 4-diaminophenol) (PANDAP) and poly (aniline-1, 8-diaminonaphthalene) (PANDAN) had been gradually synthesized, and these functional groups introduction can change the characteristics of primary PANI, which also endowed these co-polymers excellent performance in electrochemistry research (Mu, 2004; Li et al., 2011a).

Taking into account that PANI and its co-polymers have the ability of catalytic oxidation of oxygen and good biocompatibility (Khomenko et al., 2005), these materials seem to have the potential to improve the biocathode in MFCs.

Moreover, the biofilm on electrode is another crucial factor for the MFC performance. And the various electrode materials also influence the biofilm activities and the potential of attachment surface. However, electrode-oxidation bacteria which can catalyze cathodic reductions in cathode biofilm are rarely studied, and the information on the electron transfer mechanisms for biocathode is limited (Lovley, 2008).

In our previous study (Li et al., 2011b), conductive polymer modified anodes were prepared and their biodiversity were...
investigated. For the present work, PANI and its three co-polymers with different functional groups introduction, (PANO with −OH, PANDAP with both −OH and −NH₂, PANOA with −NH₂) were applied in modifying the abiotic cathodes and bioanodes, and their catalysis of oxygen reduction ability and biofilm characteristics were studied.

Therefore, the purposes of present study are to: (1) Investigate the effects of different conductive polymers modified cathodes (both abiotic cathode and biocathode) on MFC performance. (2) Discuss the relationship between the conductive polymer materials and cathode biofilm biodiversity. (3) Explore the further advantages of the functional groups on the conductive polymer modified MFC cathodes.

2. Methods

2.1. Cathode material preparation

The carbon felt with the size 4 × 3 × 0.3 cm was used as basic material for cathode. Before polymerization, all the carbon electrodes were soaked into 0.1 M HCl for 24 h, and washed by purified water, and then treated by ultrasonic for 60 min in order to drive away the air in carbon felt.

Refer to the previous literature (Zhang et al., 2007), the four kinds of conductive polymer coated cathodes were prepared as unmodified.

The PANI modified cathode: carbon felt electrodes was submerged in 200 mL HCl (1 M). Aniline (0.08 mol) and ammonium persulfate (APS, 0.08 mol) were added and then stirred constantly for 8 h of polymerization by a magnetic stirrer. Washed the coated electrode by distilled water till the filtrate to colorless and then dried in ovens at 50 °C for 24 h.

The other three co-polymer modified cathodes are prepared same as above except for (respectively):

- PANO: O-aminophenol (0.008 mol) was further added.
- PANDP: 2, 4-diaminophenol (0.004 mol) was further added.
- PANOD: 1, 8-diaminonaphthalene (0.004 mol) was further added.

2.2. Operation of MFC reactors

10 H-type two chamber MFC reactors were operated simultaneously in parallel. The MFC configuration is same as the previous described (Li et al., 2011b). Anode material was the primary carbon felt identical as cathodes (unmodified).

All the MFC anode chambers were inoculated by anaerobic digester sludge. The anode medium contained (per liter): KCl (0.13 g), NaH₂PO₄ (4.22 g), Na₂HPO₄ (2.75 g), (NH₄)₂SO₄ (0.56 g), MgSO₄·7H₂O (0.2 g), CaCl₂ (15 mg), FeCl₃·6H₂O (1 mg), MnSO₄·H₂O (20 mg) (You et al., 2009), and 1 g/L sodium acetate, and 1 ml/L of trace elements solution (Logan et al., 2005). The cathode medium was same as anode except for replacing the sodium acetate by 2 g/L NaHCO₃ (pH of both medium adjusted to 7.0 before use).

Each kind of cathode material (Four conductive polymers modified and one control) corresponds to two MFC reactors.

Group A (abiotic cathode), five MFC reactors with different cathode materials (mark as AR1, AR2, AR3, AR4 and AR5 for the unmodified, PANI, PANO, PANDP and PANOD modified cathode separately), operated in abiotic cathode mode (without microorganism inoculation in cathode) for the whole experiment process.

Group B (biocathode), another five MFCs (correspondingly, marked as BR1, BR2, BR3, BR4, BR5), were inoculated by aerobic sludge in cathode after 200 h startup period.

Except for cathode material, all the MFC reactors hold the totally same operated condition at room temperature 25 °C. The external resistor was fixed at 1000 Ω, and both anode and cathode electrolyte with substrates, were replaced every 4 days (before original substrate depleted).

2.3. Electrochemical calculations and analysis

The voltage was collected by a multimeter. And the current, the power density (normalized to the cathode projected surface area, unless stated otherwise) were calculated as previous (Logan et al., 2006). Polarization curves for each MFCs were generated by changing the external resistance in the range of 10–8000 Ω.

2.4. DO and pH influence

The pH of cathode electrolyte was adjusted to 7.0, 7.4, 7.8, 8.2, 8.6 by NaOH (but retain the same conductivity with KCl), and for each pH stage, the two groups of MFCs operated for at least 8 h.

Dissolved oxygen (DO) concentration of cathode chamber was stabilized at a certain value (from 8.0 to 2.0 mg/L) by controlling aeration, each stage operated for at least 8 h.

2.5. Characterization of modified cathode

Infrared spectra of each modified cathode surface were obtained by attenuated total reflection (ATR) method. And the morphology of all cathode surfaces was observed by scanning electron microscopy (SEM) before and after biofilm attachment.

2.6. Bacterial community analysis on cathode

For Group B, at the 60th day after cathode inoculation, biofilm samples of all cathodes were scraped. PCR was performed after DNA extraction (Zhou et al., 1996), with the primers 518F and 338R (with GC-clamp) under the following conditions: 94 °C/5 min denaturation step; 30 cycles of 94 °C/40 s, 58 °C/40 s, 72 °C/55 s; and a final extension step at 72 °C/10 min. DGGE was performed as described previously (Li et al., 2011b). Some representative DGGE bands were cut for sequencing and blasted with NCBI.

3. Results and discussion

3.1. Cathode characterization

The infrared spectrum fingerprint spectrum of modified cathodes was shown in Fig. S1. For all these four conductive polymers, the peaks around 1140 and 1580 cm⁻¹ provided the evidence of quinoid ring. The feature which appeared at around 1450 and 3200–3500 cm⁻¹ confirmed that phenolic hydroxyl groups were successfully introduced to PANOA and PANDAP. On the other hand, the peaks near 1240 cm⁻¹ indicated the four conductive polymers were at proton-doped state. These band positions in the spectrum were in agreement with previous literature (Karthikeyan et al., 2009). However, there were some shifts for these bands owing to the occurrence of copolymerization.

Besides molecular chain of PANI, the absorption peak around 3580 cm⁻¹ confirmed that phenolic hydroxyl groups were successfully introduced to PANOA and PANDAP. On the other hand, the peak 3200–3500 cm⁻¹, which corresponds to the characteristic N–H stretching vibration, demonstrated the introduction of amino groups to PANDAP and PANOD.

Therefore, from Fig. S1, the four kinds of materials were successfully coated on the carbon felt cathode surfaces.

In this experiment, all the conductive polymer materials were synthesized based on same amount of aniline (0.08 mol and 200 ml). It focused on the comparison of different conductive
polymers, with different functional groups (–OH or –NH$_3$). It is considered that different loading rate of the chemical modification material on anode probably further influence MFC performance (Zou et al., 2008). However, the effect of different loading rate for each material is not taken into consideration here.

3.2. MFC performance

For the first 200 h, the 10 MFC reactors operated without cathode microorganism inoculation. After a lag period of about 50 h, all the MFCs quickly reached the voltage plateau, respectively. During this startup period without cathode microorganism, (both Group A and B), the voltage of the unmodified was 205 mV, while the four modified MFCs showed faster voltage increasing speed, and much higher voltage, at around 360 mV. However, there was no obvious difference among the four modified MFCs.

The five MFCs in Group A, which still operated with abiotic cathode, remained invariable voltage, compare with the value of the 200th hour. On the contrary, performance of another five MFCs in Group B which changed into biocathodes (from the 200th hour), began to raise and differentiate since the microorganism take part in (the arrow). However, biocathodes also need startup period for microorganism gradually grow, and play the role in electron transfer. Comparing the voltage rising speed, the fastest two were PANDAN and PANDAP, and the slowest was the unmodified.

Finally at the 700th hour, Group B, with cathode microorganism participate in, had better performance than Group A. The ranking of maximum voltage was (mV): 510, 482, 440, 389 and 319 for PANDAN, PANDAP, PANI, PANOA and unmodified.

During the first 200 h, Group A and B (both without inoculation in cathode) had very similar profiles (Fig. 1). In fact, Group A was inoculated by aerobic sludge afterward (after the 700th hour), which also obtained similar power generation situation and DGGE results as Group B (data not shown). Therefore, the whole operation of these reactors in this experiment can be duplicated.

3.3. Chemical and biological catalysis of the cathode

Polarization curves of the 10 reactors were obtained at the 700th hour by altering external resistance from 10 to 8000 Ω (Fig. 2).

Group A, the maximum power density (mW/m$^2$) was: 35.3, 121, 138, 140 and 131 for AR1, AR2, AR3, AR4 and AR5. There was no obvious difference among the four modified MFCs, however, the highest power outputs were PANDAP (AR4) and PANOA (AR3) modified MFCs, which were 297% and 291% higher than the unmodified one.

Group B, was: 99.7, 199, 143, 236 and 285 for BR1, BR2, BR3, BR4 and BR5. As microorganism growing on these materials, due to different functional groups (–OH, –NH$_3$, or both), the different adsorption of biofilm resulted in more different MFC performances. The highest are PANDAN (BR5) and PANDAP (BR4) modified MFCs, which were 186% and 137% higher than the unmodified one.

Since the potential of each anode was very similar in all MFCs (data not shown), the different performance of these MFCs mainly caused by different cathode.

At lower current densities (100 mA/m$^2$ for Group A, 200 mA/m$^2$ for Group B), there was obvious difference between modified and unmodified MFCs, which indicated that the activation energy for electron transfer in MFC cathode had been changed by these polymer materials (Rismani-Yazdi et al., 2008), i.e. the different performance of these MFCs mainly resulted from the difference of the catalytic activity in cathodes.

From Figs. 1 and 2, obvious difference was found in the startup speed and power output for all the 10 MFCs, which indicated both conductive polymer and cathode biofilm could influence MFC performance.

Since in Group A without microorganism took part in, conductive polymers can improve power densities by 240–300% compare with the control, the chemical catalysis of conductive polymers play an important role.
It is known that the conductive polymers themselves are catalyst which can lower the activation energy for O\textsubscript{2} reduction. Electrocatalytic activity toward the oxygen reduction of PANI was described as:

\[
(PANI)\text{ox} + n\text{H}^+ + ne = (PANI)\text{red},
\]

\[
(PANI)\text{red} + n\text{O}_2 = (PANI)\text{ox} + n\text{H}_2\text{O}_2
\] (Hourech et al., 1992).

Khomenko et al. (2005) pointed out the carbon atoms bonded to nitrogen, rather than that on pairs of side chain sites, are responsible for molecular oxygen adsorption, which can increase O–O bond length and make O\textsubscript{2} reduced easily.

Further, these conductive polymers also endowed the cathode more rough surface, which provided more reacting sites available for oxygen reduction. Therefore, only rely on their chemical catalysis ability, these PANI based polymers can be used as low-cost catalyst for MFC cathode.

According to Figs. 1 and 2, Group B performed better than Group A integrally, and they finally obtained different ranking for the four materials (the optimal material is: PANOA and PANDAP for abiotic cathode, PANDAN for biocathode). However, when cathode was inoculated (Group B), the materials with different functional groups introduction, as electron mediator (or produce mediators by themselves) or catalysts, which avoid the use of noble or non-noble catalysts for oxygen reduction, thereby enhancing the economic viability and environmentally sustainability of MFC systems. The microorganism has similar function as electron mediator (or produce mediators by themselves) or catalysts, which can improve MFC performance for both abiotic and biocathode. PANOA modified MFC (BR3) still showed a little more resistance to DO and pH change, but the difference among these five BRs was not obvious.

PANDAP, with phenolic hydroxyl group like PANOA also showed less sensitivity to DO and pH change (followed behind PANOA) than others, which indicated phenolic hydroxyl groups may be contributing.

The –OH, as an electron-withdrawing group, changes the π-electron density distribution on PANI chains and increases the heats of formation \((\Delta H)\) of the bonds between oxygen atoms and PANI, which made the adsorption \((PANI-O_2)\) more easily. On the other hand, –OH in the co-polymer chains can be oxidized to quinoline and reduced again in order to exchange proton between the material and the solution (Zhang et al., 2006). This “redox couple” endows the co-polymer pH buffering ability and its conductivity is less influence by pH change (Mu, 2004).

Therefore with –OH introduction, PANOA (and PANDAP) had tolerance of low DO and relative high pH, especially in Group A (abiotic cathode). However, when cathode was inoculated (Group B), cathode materials were covered by the biofilm, which reduced the exposure of functional groups –OH. The activities and biocatalysis of microorganism in cathode were also influenced by pH, DO change. Therefore, the superiority of –OH became not obvious in PANOA (BR3).

Figs. 1 and 2 (Group B) showed that when microorganism took part in the cathode, the four MFCs tended to have the similar downward trend to both DO and pH change. PANOA modified MFC (BR3) still showed a little more resistance to DO and pH change, but the difference among these five BRs was not obvious.

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3.4. Functional groups effects

In order to investigate the further advantages of different functional groups bring to conductive polymers, two factors: DO and pH variation in cathode chamber were used to check the effects on these MFC performance.

In two chambers MFC, the excessive DO in cathode may cross over the PEM to anode, which damages the anaerobic anode biofilm and causes the low cumblic efficiency. Reducing the aeration in cathode chamber is also expected to reduce the cost. However, reducing DO in cathode probably limits the power output of MFC. Moreover, the accumulation of hydroxyl (OH\textsuperscript{-}) in cathode often causes the pH gradient problem between anode and cathode chambers due to the limitation of PEM (Rozendal et al., 2008). So, the DO reducing and pH rising in cathode chamber are inevitable problem in practical application of MFC.

Therefore, the influence of DO reducing and pH rising in cathode chamber to MFC performance was studied in the two groups of MFCs (after polarization curves). Each result was repeated for three times (Fig. 3, n = 3).

For Group A, during the period of DO reducing from 8 to 2 mg/L (Fig. 3a), the voltage of AR1 (unmodified cathode) showed the largest decrease gradient of 66% (from 245 to 83 mV). The four modified MFCs descend much slower, and among these, AR3 (PANO modified) was the slowest, which only decreased by 16% (from 368 to 308 mV).

In the process of cathode pH rising from 7.0 to 8.6, AR3 also manifested the least sensitive to the pH change with only 7% decrease. Others were about 13–21% decrease.

In Group B (Fig. 3b and d), when the microorganism took part in the cathode, all the five MFCs tended to have the similar downward trend to both DO and pH change. PANOA modified MFC (BR3) still showed a little more resistance to DO and pH change, but the difference among these five BRs was not obvious.

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PANDAN with –NH₃ are more qualified for biocathode. PANDAP with both –OH and –NH₃, fall in between the two.

3.5. Cathode biofilm characterization

The SEM images of the cathode surfaces were shown in Fig. S2. It can be observed that the PANDAN and PANDAP modified cathodes, which had much active amino introduced to the surfaces, possessed the thickest cathode biofilm. And the weakest biofilm is on the unmodified one (compare among B1–B5).

Biofilm acts as catalysts or mediators in the cathode, which can greatly decrease the high cathodic overpotential. And for mixed culture, the synergetic effects of different sorts of bacteria in biofilm are important for power generation (Erable et al., 2010). Therefore, it is necessary to investigate the structure of microbial community of the biocathode.

Fig. 4 showed DGGE analysis of each biocathode in Group B, after 60 days' operation from the cathode inoculation. Approximately 30 detectable bands were observed on each cathode, which indicated that autotrophic or cathode-driving bacteria widely existed in biocathode since no organics were offered. According to the blast, the bacterial community of these cathodes mainly consisted of Proteobacteria and Bacteroidetes, which was in accord with the results of Rabaey et al. (2008). Among these, Nitrosonomas (No. 1) is a kind of autotrophic ammonia-oxidizing bacteria, and others bands such as Thiobacillus denitrificans (No. 6), and Rhodopseudomonas (Nos. 7 and 8) are bacteria belonging to Sphingobium which are the potential denitrifying bacteria (Elmore et al., 2007). The enrichment of these bacteria was beneficial to the utilization of nitrate on the cathode. Therefore, similar as the result of Chen et al. (2008), these bacteria together formed an autotrophic biofilm with the function of denitrification on cathodes (NH₄⁺ existing in cathode medium).

Rhodopseudomonas palustris (Nos. 7 and 8) and Pseudomonas aeruginosa (No. 4), which are known as efficient anode-reducing microorganism (Xing et al., 2008; Rabaey and Verstraete, 2005), were found on all the cathodes in this experiment (Fig. 4), especially the highly enrichment of P. aeruginosa on the PANI modified cathode. It can be suggested that these two kinds of bacteria may function as catalyst for both anodic and cathodic reaction. However, this is not a new phenomenon among electroactive bacteria yet (Rabaey et al., 2008; Cheng et al., 2010).

With the assistance of conductive polymers, the biodiversity of the four modified biocathode changed obviously, compare to the unmodified. Such as the four bands of Nos. 2, 4, 5 and 9 became more intense, which meant Rhodospirillum centenum, P. aeruginosa, Sphingobium chlorophenolicum and Thauera selenatis obtained more enrichment after modified. On the contrary, band Nos. 1 and 6 weakened compare with the control (unmodified) which indicated Nitrosomonas europaea and T. denitrificans were not suitable for growing on conductive polymers or eliminated after long period of higher current densities operation.

In general, due to the different biocompatibility and catalytic activity which conductive polymers brought to biocathode, the predominant phyla of cathode biofilm were changed from β-Proteobacteria (unmodified) to α and γ-Proteobacteria (modified).
The four modified cathodes had relative similar biodiversity, compared to the unmodified. However, some differences gave the demonstration that even detail distinction, such as different functional groups introduction in conductive polymers can alter the biodiversity of cathode biofilm. For instance, S. chlorophenolicum (No. 5) preferred to adhere on the polymers with –OH (PANOAc and PANDAP) compared to other materials.

3.6. Relationship between conductive polymers and biofilm

For all the cathode limitations which decrease MFC performance (Rismani-Yazdi et al., 2008), cathodic activation loss is the dominated one. Many methods were applied to optimize the reduction kinetics in cathode, such as decreasing the activation barrier by catalysts, increasing the reaction interface area or oxidant concentration. In this study, two methods: PANI based conductive polymers and cathode biofilm were considered.

According to Section 3.3, both the chemical catalysis of conductive polymers and biological catalysis of cathode biofilm can improve MFC performance because they all function as catalysts or mediators to help O2 reduction. However, the relationship of these two promotors (conductive polymers and biofilm) needs to be further discussed.

The formation of biofilm plays an important part in electrode potential in MFCs, since there is close interaction between microorganisms and cathodes (Huang et al., 2011). As environmental friendly material, conductive polymers in present works can provide cathode more biocompatible surface for biofilm attachment and the increased surface area, which can influence the biofilm activities, mass transfer dynamic, and the potential of the attachment surface.

It is known that multiple electrons transfer process between bacteria and electrode were found in a variety of microorganisms, which benefit from the cytochromes. Nevertheless, the mechanism of biocathode is less known except for that the combination of c-type cytochrome and its oxidase takes charge for O2 reduction (Ramanavičius and Ramanavičienė, 2009). Generally, the electrons transfer process may be blocked by phospholipids and polysaccharides in the non-conducting cell wall (Park and Zeikus, 2002). The hydrophobic conductive polymers may act as the bridge or mediator, which played the role of bonding bacteria and carbon cathode more tightly, and facilitated or improved the electron transfer process from cathode to bacteria.

β-proteobacteria are known to participate in the biotransformation of metallic oxides and are an important part of MFC ecosystems (Kim et al., 2004). With the help of conductive polymers, the predominated phyla changed from β-proteobacteria (unmodified) to α-, γ-proteobacteria (modified), which provided the evidence that the whole electrons transfer process of cathode biofilm probably changed after modified.

In the four modified cathode biofilms, more enrichment of P. aeruginosa, S. chlorophenolicum and T. selenatis, were probably responsible for the improved performance. Although there is little report for the electroactivity of Rhodospirillum, Sphingobium, and Thauera, they were found widespread in MFC ecosystems (Lee et al., 2010). With the assistance of conductive polymers, these kinds of bacteria may achieve potential electricity catalytic activity or be helpful to the electrode-oxidizing bacteria, which can enhance the MFC performance.

To sum up, the conductive polymers not only increased the cathode biofilm thickness (Fig. S2) but also altered the biofilm biodiversity (Fig. 4), even may change the electron transfer mechanism for O2 cathodic catalysis reduction. Therefore, these materials help cathode microorganism to form a more developed and efficient biofilm, and the biofilm in return worked as a further coat like conductive polymers, together enhance MFC performance.

However, given that conductive polymers and biofilm played similar role in cathode, they might also be contradictory with each other. The biofilm covered the chemical catalyst sites of conductive polymers and reduce the ability of chemical O2 catalyst reduction. And the biofilm layer reduced the exposure of –OH groups, which made BR3 became less obvious resistance for DO and pH change (compare with AR3, Fig. 3).

Yuan et al. (2010) reported that in the polypyrrole modified biocathode single chamber MFC, the chemical catalysis rather than cathode biofilm, played the whole role to catalyze O2 cathodic reduction, because the same power generation was obtained with or without biofilm. This differs from the result of ours that the cathode biofilm can further contribute to power output promoting besides the chemical catalysis of conductive polymers (compare Group B to Group A). It may be because the cathode biofilm in single chamber MFC can consume organic carbon source (unlike the cathode without organic in two chamber MFC), which led to
considerable growth of aerobic heterotrophs, and turned these microorganism into an inefficiency biofilm for oxygen reduction. According to biodiversity analysis of biofilms, with the help of conductive polymers, some kinds of bacteria became more enrichment, even so, biodiversity of cathode biofilm did not become higher like our previous study on anode (Li et al., 2011b), indeed become lower since T. denitrificans almost disappeared on the modified cathodes. This is probably because much higher current density for the modified bioanode can decrease the biodiversity (Katuri et al., 2011), cause of the elimination of the electroactive bacteria with poor efficiency.

4. Conclusions

PANI, PANOA, PANDAP and PANDAN showed good oxygen catalytic reduction ability, and acted as favorable biofilm supporter, which indicated they are suitable for both abiotic cathodes and bioanodes in MFC, with greatly enhanced power densities. Functional groups introduction to the polymers can bring new advantages. The –OH contributed to be less sensitive to the variation of DO and pH in cathode; while –NH3 further helped the bioanode to be more adaptability for biomass adhesion, which enriched the biofilm thicker and more rapidly. After modified by conductive polymers, the predominated phyla in cathode microbial population were changed from β-proteobacteria to α, γ-proteobacteria.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in online version, at doi:10.1016/j.biortech.2012.03.115.

References


