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Menadione-Modified Anodes for Power Enhancement in Single Chamber Microbial Fuel Cells

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As anode fabrication with different materials has been proven to be a successful alternative for enhancing power generation in the microbial fuel cells, a new approach to improved performance of MFCs with the use of menadione/carbon powder composite-modified carbon cloth anode has been explored in this study. Menadione has formal potential to easily accept electrons from the outer membrane cytochromes of electroactive bacteria that can directly interact with the solid surface. Surface bound menadione was able to maintain an electrical wiring with the trans-membrane electron transfer pathways to facilitate extracellular electron transfer to the electrode. In a single chamber air cathode MFC inoculated with aerobic sludge, maximum power density of 1250 ± 35 mWm^{-2} was achieved, which was 25% higher than that of an unmodified anode. The observed high power density and improved coulomb efficiency of 61% were ascribed to the efficient electron shuttling *via* the immobilized menadione.

Key Words : Microbial fuel cells, Menadione, Electron transfer, Power density, Mediator

Introduction

Microbial fuel cells (MFCs) are the devices that generate electricity by the catalytic degradation of organic or inorganic matters with the help of living microorganisms inoculated on the electrode surface beforehand.[1-3] One reason that MFCs are not commonly considered a part of the energy portfolio for the future is that MFC technology is not yet sufficiently developed to produce substantial quantity of energy in a cost-effective manner.[4] One of the significant issues contributing to low performance is inefficiency of the anodic processes[5] that involve the interaction of bacteria and electron acceptor anode. Electrochemical communication between viable bacteria cell and electrodes is the recent research topic that explores different electron transfer pathways from organism to the inert electrode.[6,7]

There is a general agreement that the main obstacle to the power enhancement is the sluggish electron transfer (ET) from bacteria to the anode.[8] Several ET mechanisms have been identified: In ET relying on diffusion of artificial or endogeneous mediators, current density cannot increase beyond 0.16 Am^{-2} due to the slow diffusion rate.[9] The direct ET by a physical contact of the bacterial cells with the anode offers some advantage over the diffusion-type ET in the sense that no diffusional species are needed. This type of ET, which is found in electroactive bacteria, is achieved through the redox proteins located on the outer membrane of bacteria. Recently another ET mechanism has been identified in some strains such as *Shewanella oneidensis MR-1*, in which electronically conducting pili allow electrons to be transferred between bacteria and also between bacteria and the electrode.[10]

Although many of MFC works are directed to electron transfer, attempts using redox mediators have still been made aiming at increasing current density. Mediators used in the MFCs should possess properties that they are spontaneously reduced on the cell surface with simultaneous oxidation on the electrode surface. The mediator redox potential should be as low as possible to achieve maximum cell potential but higher than that of outer membrane protein.[11] Also mediators should not be toxic to bacteria as well as to humans. In this sense, quinone-type molecules are of special interest as they are abundantly present in biological systems most of which actively support the electron-relay system. Once they are extracellularly released, they participate in electron transport to the anode.[12] Bacteria use transmembrane electron transfer pathways to direct electrons from cell interior to the extracellular surface.[13] They excrete molecules such as phenazines, 2-amino-3-carboxy-1,4-napthaquinone, 1,2-dihydroxynapthalene and 2,6-di-*tert*-butyl-*p*-benzoquinone to facilitate extracellular electron transfer to the outside electron acceptor.[14,15] However, soluble mediators are easily lost from the site and flushed away in a continuous or semi-continuous fuel feed medium and therefore cannot be sustainable electron shuttles in the long run, unable to go towards electrodes but rather diffusing into the bulk. Furthermore most of the soluble mediators are not eco-friendly and exacerbate environmental condition with possible toxicity. On the contrary, a suitable mediator immobilization technique could overcome this hurdle, in that mediators coexist on the surface emerging as an un-detachable electrical wiring with the bacterial biofilm. Menadione and other quinine type mediators were proved to be efficient for electron transfer from bacteria to the electrode.[16] Park and Zeikus[17] observed fourfold increase in current when they added neutral Red as a mediator that effectively took electrons from NADH. Bond *et al.*[18] demonstrated that flavins secreted from a whole cell greatly increased ET rate. Other reports showed that surface-

immobilized redox molecules by means of polymeric species could act as a mediator. For example, MFCs constructed with polymer attached anthraquinone-2,6-disulphonic disodium salt (AQDS)-modified graphite electrode generated higher electricity compared to the unmodified electrode.[19,20]

Here we report a simple anode modification procedure using carbon powder and a proper redox molecule that are readily applicable to MFCs. We used menadione (2-methylnaphthalene-1,4-dione, known as vitamin K_3) as a modifier and demonstrated that immobilized menadione functioned as an electron shuttle between a bacterial biofilm and the electrode, producing higher power and current density compared to an unmodified anode under the identical cathodic conditions. This method is readily applicable to MFCs with a perspective of better performance and sustainability.

Experimental Section

Chemicals and Materials. Menadione, Nafion solution (Sigma-Aldrich), and PTFE (polytetrafluoroethylene) solution (Dupont) were used as received. 20% Pt/C powder on Vulcan XC-72 (De Nora North America, Inc., USA), carbon black (Vulcan XC-72, Cabot, USA), and carbon cloth (type A and type B, Fuel Cell Earth, USA) were purchased to prepare oxygen reduction catalyst layers and anode materials. Activated sludge was collected from Jungnang Sewage Treatment Center (Seoul, Korea). All other chemicals were of reagent grade.

MFC Construction and Electrode Preparation. MFCs were constructed as described by Cheng *et al.*[21] with a little modification. A cylindrical shape (3.0 cm ϕ × 2.1 cm L) reactor was made from plexiglas with a final volume of 14 mL and anode and cathode spacing of 2.0 cm. An air-cathode with oxygen diffusion and catalyst layers was prepared as descried in a previous report.[22] using Pt/C powder. Pt loading was maintained at 0.3 mg cm^{-2}. Menadione was dissolved in 0.2 mL DMF and sonicated. 10 mg of carbon powder was then soaked and sonicated for 15 minutes in menadione solution. 0.2 mL of Nafion solution was added to that mixture and mixed well. This composite paste was applied on the one side of carbon cloth (type A) anode with a paint brush and dried overnight at room temperature (modified anode). The same procedure without menadione was taken to prepare the anode for the comparison purpose (unmodified anode).

Enrichment and MFC Operation. Menadione-modified and unmodified anodes were inoculated from a mixture of treated sludge and culture media. Culture media were 1 g L^{-1} sodium acetate solution in 50 mM phosphate buffer (pH 7) containing 0.13 g L^{-1} KCl, 0.31 g L^{-1} NH_4Cl, 12.5 mL L^{-1} mineral solution, and 5 mL L^{-1} vitamin solution. The MFC was operated in a batch mode with 1 kΩ external resistance under controlled temperature at 30 °C. Batch change-over with the same culture media was done every time when the voltage drops below 50 mV. This process was repeated until at least five consecutive cycles exhibited stable and constant voltage output, and then data were taken. Power density curves were constructed from polarization curves which were obtained by varying external resistances. Three consecutive measurements were done for a single external resistance and the average was taken for power calculation.

Measurements and Calculation. The cell voltage (V_{cell}) over R_{ext} was recorded by an automatic battery cycler (WBCS300, WonAtech, Korea) as a function of time. Power (P) was calculated from $P = V^2/R_{ext}$. Power was normalized by the anode surface area (m^2). The coulombic efficiency (CE) was calculated according to the equation, CE (%) = $(Q_{act}/Q_{th}) \times 100$, where Q_{act} is actual charge obtained from the experiments and Q_{th} is theoretical charge calculated from the complete oxidation of acetate, $C_2H_3O_2^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^-$. Q_{act} was obtained by integrating the discharge curve with time, $Q_{act} = \int I dt$. The cell voltage was converted to current by the Ohm's law, $I = V_{cell}/R_{ext}$. Potential variation of anode and cathode during discharging was registered with respect to the standard calomel electrode as a reference that was placed in the cell using a multimeter. Cyclic voltammetry was employed to characterize electrochemical interactions between the anode and microbial biofilms after MFC measurements. An ordinary three-electrode system was employed using Pt counter and SCE reference electrodes.

Images of anodic biofilm were collected with SEM (JSM-6380, JEOL, Japan) after experiments. For the better images, the anode sample was immersed in 3% glutaraldehyde solution for 2 h and washed in 50 mM phosphate buffer for 15 min twice and treated with ethanol and then subjected to drying.

Results and Discussion

Start-up of an MFC. Figure 1 shows typical start-up parts of the MFC operation using unmodified (black) and menadione-modified (red) anodes. Discharge was done with the 1 kΩ external load. The start-up period of *ca.* 100 h was needed before the stable cell potential was developed for both electrodes. During that period electroactive bacteria are acclimated on the anode surface. Almost the same voltage

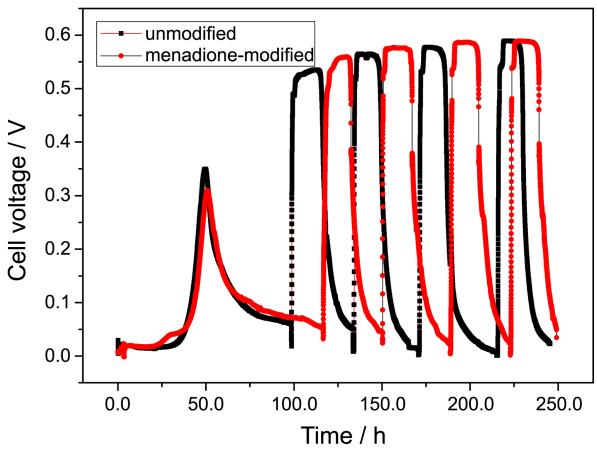


Figure 1. Charging and discharging pattern during a start-up period with a 1 kW external resistor. Acetate feed solution (1 g L^{-1}) was added after discharging.

patterns were observed regardless of modification, indicating that immobilized menadione molecules do not appreciably affect the MFC performance. This is understandable because a biofilm of electroactive bacteria have ability to directly transfer electrons to the anode even without mediators. However, the difference could be clearly seen from the power density curves.

Power Generation in MFCs. The plot of power density *vs*. current density was constructed from polarization curves (Fig. 2(a)). Individual electrode potentials as a function of current density were recorded for both anodes (Fig. 2(b)). While cathode potential variations are very similar each other MFCs indicating that the cathodic reaction is not affected by the anodic reaction, anode potentials show rather large difference particularly at high current density. This implies that menadione molecules function as an electron conduit. Power density curves more clearly show the difference between modified and unmodified anodes. Maximum power density at 998 mWm^{-2} at 3.1 Am^{-2} observed for an unmodified anode increased to 1250 mWm^{-2} at 4.7 Am^{-2} for modified one. About 25% and 52% increases in power and current densities respectively were resulted upon modification. From these observations, it may be said that surface confined menadiones more easily take electrons from cytochromes located in the outer membrane than the direct electron transfer and also they may help electron transfer from bacteria that are not directly attached to the electrode. In our experiments, however, increase in power and/or current density was not as great as in other cases. For example, Adachi and coworkers[20] applied polyethyleneimine-attached AQDS as an anode modifier to effectively transfer electrons from a bacterial biofilm using *Geobacter sulfureducens* as a biocatalyst. In a half-cell test using an anode only, they observed 60 times increase in current density compared to unmodified electrode. Feng *et al.*[19] performed nearly the same experiments as Adachi *et al.* using polypyrrole-attached AQDS as a surface-confined mediator and *Shwanella decolorationis* S12 as a biocatalyst. They reported P_{max} of 1300 mWm^{-2} with current density of 2.75 Am^{-2}. Although this power density is a 13 times increase compared to unmodified one, our P_{max} of 1250 mWm^{-2} is not much different from theirs with even higher current density at P_{max}.

Effect of Menadione Loading Amount on Power Density. We examined how the power density curve changes with a different loading amount of menadione (Fig. 3). Among test amounts of 0.05, 0.14, and 0.284 mg (menadione) cm^{-2}, 0.14 mg cm^{-2} gave the best result. At a small loading, power density is low because not enough menadione molecules are available to transfer electrons. Lower power density at higher loading is rather peculiar. It may be due to the hydrophobic nature of menadione in that high coverage increases hydrophobicity of the surface hence reducing the bacterial interaction with the anode surface.

Coulombic Efficiency. Coulomb efficiency (CE) is an important parameter for assessing an MFC performance since it determines the conversion efficiency of substrate into electricity. An efficient electron transfer can accelerate the substrate degradation to protons and electrons with high energy recovery from the system where bacteria use minimum energy in biomass production. We examined CE as a function of current density (Fig. 4). CE monotonically increased with current density. CE range of 10-61% was obtained at current range of 0.3-5.1 Am^{-2} for the modified anode, while 11-46% CE was observed for the unmodified one at 0.2-4.3 Am^{-2} current range. This result is comparable with other

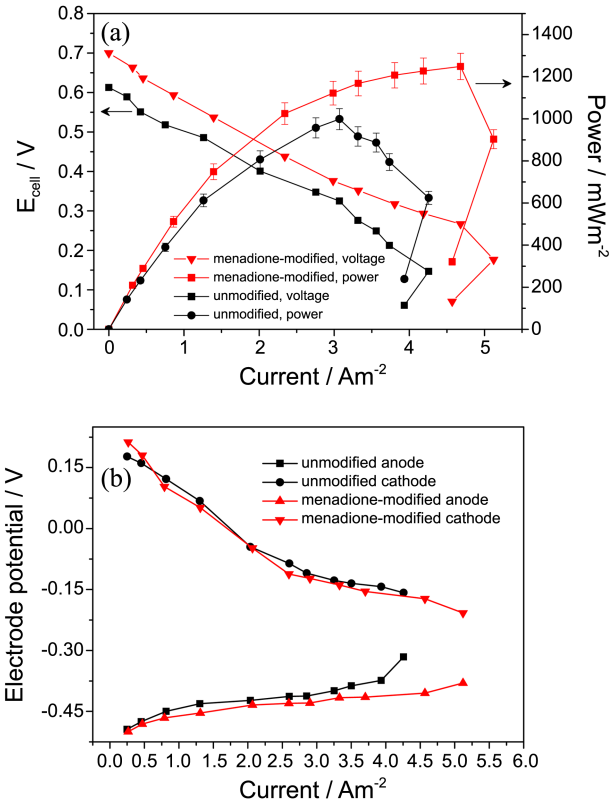


Figure 2. Polarization curve and power density plot as a function of current density for unmodified (black) and menadione-modified (red) anodes (Panel a). Panel b shows variation of individual electrode potentials measured during the MFC operation for unmodified (black) and menadione-modified (red) anodes.

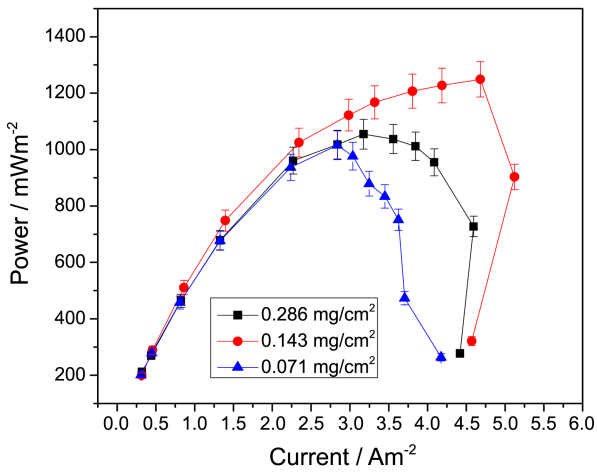


Figure 3. Effect of different loading amount of menadione on the fuel cell performance.

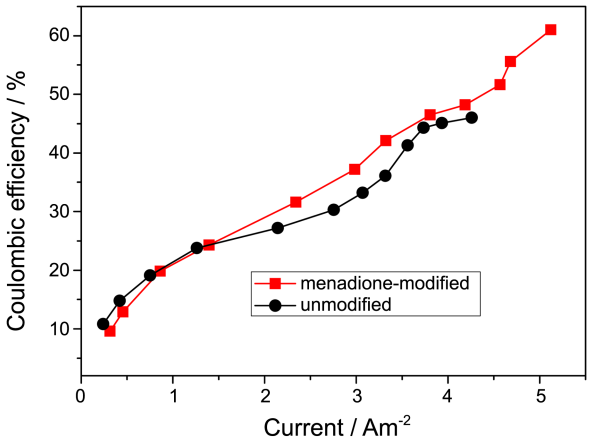


Figure 4. Variation of coulombic efficiency for unmodifed anode (black) and menadione-modified anode (red) as a function of current density. Discharging was done through a 1 kΩ external resistor.

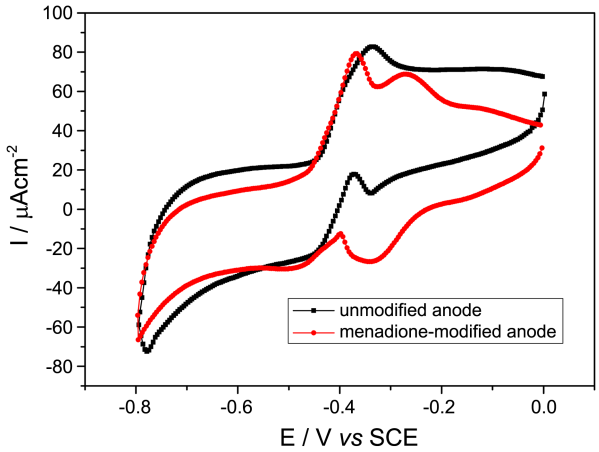


Figure 6. Cyclic voltammograms of carbon powder covered (black) and menadione-modified (red) anode after running MFC experiments in pH 7 PBS buffer. Scan rate = 1 mV s^{-1}.

MFC reports.[23,24] Generally CE for the unmodified anode is lower than that of modified anode except at the lower current range where high activation overpotential dominates the electrode process. Some balance between CE and P_{max} should be considered when MFCs are to be applied to the real world, however.

Surface Morphology and Electrochemical Results. A SEM image was taken after MFC running more than two months (Fig. 5). A well-formed bacterial film is visible. Since the whole surface is covered by the bacteria multilayer, the underlying carbon particles are not seen.

Menadione shows a reversible electron transfer with formal potential of –0.29 V *vs*. SCE at pH 7 (data not shown) from cyclic voltammetry. This potential is higher than that of NADH ($E^{0'}$ = –0.566 V *vs*. SCE). Therefore it is quite possible for electrons are transferred from NADH to menadione. This kind of electron transfer is, however, not plausible because immobilized menadione cannot have access to NADH in a bacterial cell. Electrons are first transferred to the outer membrane enzymes through the electron transfer chain then to menadione. Figure 6 shows voltammograms of unmodified and modified anodes in substrate-depleted condition at slow scan rate (1 mV s^{-1}) after the MFC experiments. At the outset of MFC operation, no special feature was observed. Both unmodified and modified anode show voltammetric features characteristic of electroactive bacteria that are ascribed to the outer membrane cytochrome.[25,26] Two reversible redox peaks could be identified. For an unmodified anode, the more negative redox process shows cathodic and anodic peaks at –0.464 and –0.337 V, respectively. Another prominent cathodic peak appears at –0.340 V whose anodic pair is not very clearly seen, however. The presence of redox peaks indicates that the biofilm formed on the carbon black-covered anode has ability to directly transfer electrons to the anode. A more negative redox pair could be attributed to the outer membrane cytochrome B while a peak at more positive potential indicates another electron transfer path through the membrane associated species.[27]

Similar voltammograms were obtained for the menadione-modified anode. In this case, two redox pairs are more well-

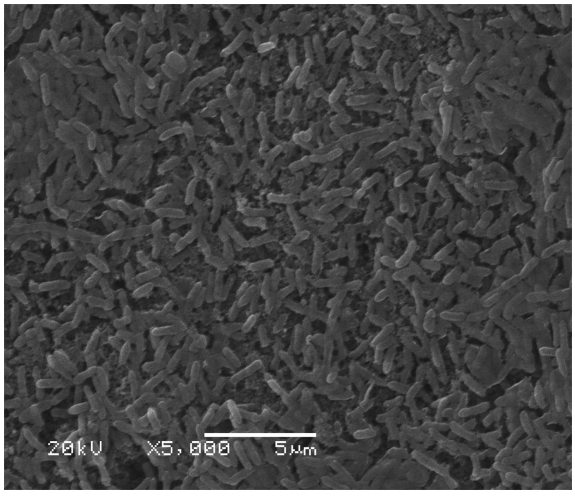
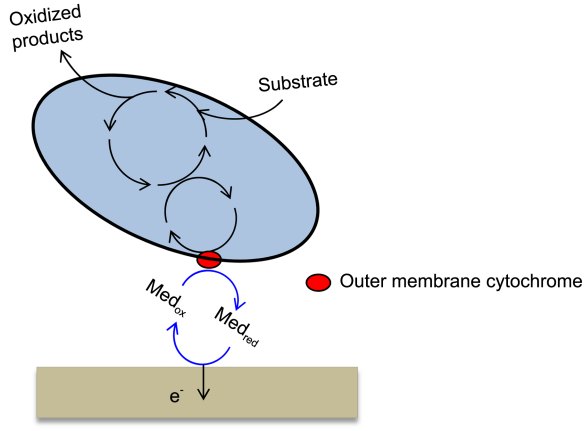


Figure 5. SEM image of a menadione-modified anode surface after running MFC experiments.



Scheme 1. Schematic illustration of surface-immobilized menadione mediated electron transfer for a bacterial biofilm.

defined with formal potentials at −0.397 V and −0.302 V, indicating that electrons are readily transferred to the anode. Notable is the cathodic peak at −0.340 V. It is very much broadened compared to the counter part for the unmodified anode. Since the only difference is the presence of menadione molecules on the surface, peak broadening is attributed to the overlapped menadione redox process as the formal potential of menadione is −0.29 V. Based on this observation, electron transfer to the anode *via* menadione is illustrated in Scheme 1, where outer membrane species come into the direct contact to menadiones immobilized on the surface.

Conclusion

In this study, we have tried to enhance the performance of microbial fuel cells by simply immobilizing menadione molecules on the anode surface aiming at facile electron transfer. Maximum power enhancement by 25% compared to the unmodified anode was achieved with increase in current density as well. The optimum amount of menadione loading on the surface was 0.19 mg cm^{-2}. Coulombic efficiency was monotonically increased with current density, reaching 61% at 5.1 Am^{-2}. It is believed that menadione molecules function as mediators transferring electrons to the anode from outer membrane redox species. This result shows one of possibilities to enhance MFC performance by simple modification of the anode surface with redox mediators.

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