Effect of cathode electron acceptors on simultaneous anaerobic sulfide and nitrate removal in microbial fuel cell

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ABSTRACT

The current investigation reports the effect of cathode electron acceptors on simultaneous sulfide and nitrate removal in two-chamber microbial fuel cells (MFCs). Potassium permanganate and potassium ferricyanide were common cathode electron acceptors and evaluated for substrate removal and electricity generation. The abiotic MFCs produced electricity through spontaneous electrochemical oxidation of sulfide. In comparison with abiotic MFC, the biotic MFC showed better ability for simultaneous nitrate and sulfide removal along with electricity generation. Keeping external resistance of 1,000 Ω, both MFCs showed good capacities for substrate removal where nitrogen and sulfate were the main end products. The steady voltage with potassium permanganate electrodes was nearly twice that of with potassium ferricyanide. Cyclic voltammetry curves confirmed that the potassium permanganate had higher catalytic activity than potassium ferricyanide. The potassium permanganate may be a suitable choice as cathode electron acceptor for enhanced electricity generation during simultaneous treatment of sulfide and nitrate in MFCs.

Key words | anaerobic sulfide and nitrate removal, cyclic voltammetry, microbial fuel cell, potassium ferricyanide, potassium permanganate

INTRODUCTION

Sulfide-containing waste streams are generated by many industries, such as tanneries, petrochemical plants and viscose rayon factories (Mahmood et al. 2007a; Zhang et al. 2009). Sulfide is a toxic ionic species, which exerts various toxicological impacts on human health and environmental ecology (Jin et al. 2013). The sulfide treatment may involve various physical, chemical and biological processes. Among these technologies, the biological processes are relatively cost-effective as they operate under natural ambient conditions without any requirement for expensive chemicals and catalysts (Cirne et al. 2008). Nitrate can be used to control the sulfide generation under anoxic or anaerobic conditions by some bacterial species (Garcia-de-Lomas et al. 2007). For such reasons, the simultaneous anaerobic sulfide and nitrate removal process has been recently developed.

A few researchers have combined simultaneous anaerobic sulfide and nitrate removal process in microbial fuel cells (MFCs), which is a novel approach in the field of wastewater treatment (Logan et al. 2006). The novel process can generate electricity from the biotransformations of inorganic substrates in MFCs. There are only a few published reports on the simultaneous anaerobic nitrate and sulfide removal in MFCs. Lee et al. (2012) confirmed that the MFC was capable of the simultaneous sulfide and nitrate removal using monoculture of Pseudomonas sp. C27. A two-chambered MFC was operated for simultaneous sulfide and nitrate treatment using activated sludge (Cai & Zheng 2013; Cai et al. 2013). It was concluded that the electricity output enhanced through the microbial enrichment during the operation, suggesting that an active bacterial consortium capable of substrate removal and electricity generation had established in the MFC. The effect of electrode types and operating modes on the performance of the process has been investigated by our research group (Cai et al. 2014a, b).

The performance of MFCs is generally dependent on a number of factors, namely fuel cell configuration, operating temperature, proton exchange membrane, solution conductivity, pH, alkalinity, circuit resistance and cathode electron acceptor (Mohan et al. 2008; Gurung & Oh 2012). Being an important component of MFCs, the cathode electron acceptor has significant influence on electricity generation. Dissolved oxygen, potassium ferricyanide and
potassium permanganate have often been used as cathode electron acceptors in two-chamber MFCs (You et al. 2006; Gurung & Oh 2012; Wei et al. 2012; Chen et al. 2014). MFCs with dissolved air have attracted greater attention because of their simple structure without any by-product formation and easy management etc., but their major limitation is availability of cost-effective cathode catalyst and their low solubility (Kong et al. 2010). MFCs with potassium permanganate and potassium ferricyanide generate relatively higher voltage in comparison to those containing oxygen (Equations (1) and (2)).

\[
\begin{align*}
\text{MnO}_4^- + 4H^+ + 5e = \text{MnO}_2(s) + 2H_2O & \quad E^0 = 1.68 \text{ V} \quad (1) \\
\text{Fe(CN)}_6^{3-} + e = \text{Fe(CN)}_6^{4-} & \quad E^0 = 0.56 \text{ V} \quad (2)
\end{align*}
\]

The present investigation was aimed at exploration of two common cathode electron acceptors (potassium permanganate and potassium ferricyanide) for the simultaneous sulfide and nitrate removal process in MFCs. The effect of cathode electron acceptors on the substrate removal and power generation was compared under abiotic and biotic conditions of electron acceptors on the substrate removal and power generation. The effect of cathode electron acceptors, while the other used potassium permanganate as cathode electron acceptors during the operation of MFCs.

**MATERIALS AND METHODS**

**Inoculum and enrichment of microbial communities**

Inoculum was collected from the anaerobic methanogenic reactor operated at Dengta wastewater treatment plant located near Hangzhou City in China. Its total solids (TS) and volatile suspended solids (VSS) were 95.03 g L\(^{-1}\) and 68.68 g L\(^{-1}\), respectively, with VSS/TS ratio of 0.72. The simultaneous anaerobic sulfide and nitrate removal reactor was operated under lithoautotrophic conditions where sulfide was used as electron donor and nitrate was employed as electron acceptor. For the initial 1 month, the reactor was fed with synthetic wastewater in order to acclimatize the bacterial consortia to the new substrates and to enrich the functional bacterial populations.

**Synthetic wastewater**

The MFC was fed with synthetic influent containing NaHCO\(_3\), MgCl\(_2\) and KH\(_2\)PO\(_4\) (1 g L\(^{-1}\) each); (NH\(_4\))\(_2\)SO\(_4\) (0.24 g L\(^{-1}\)) and trace element solution (1 mL L\(^{-1}\)). The trace element solution was prepared according to Mahmood et al. (2007b). The nitrate-nitrogen and sulfide-sulfur concentrations were added as potassium nitrate (KNO\(_3\)) and sodium sulfide (Na\(_2\)S·9H\(_2\)O), respectively, with their concentrations adjusted based on the type of experiment conducted.

**MFC construction**

The MFC consisted of anode and cathode chambers, and total volume of both was 350 mL (300 mL net volume) as reported in our previous study (Cai & Zheng 2013). The chambers were connected by a cation exchange membrane (CEM) (Ultran CMI-7000 Membrane International, USA). The electrodes were graphite rods (6 cm × φ1 cm, 18.80 cm\(^2\) net superficial area, Beijing Jixing Shengan Industry & Trade Co., Ltd), which were placed at the centers of each chamber and were parallel to the CEM. The external resistance was 1,000 Ω, applied to control electron flow from the anode to the cathode. Anaerobic sludge (100 mL) was inoculated in the anode chambers of biotic MFCs, and the solution in anodic chambers was circulated by a peristaltic pump. The cathode medium was 100 mg L\(^{-1}\) potassium permanganate (KMnO\(_4\), 50 mM PBS buffer, pH 7.0) or 625 mg L\(^{-1}\) potassium ferricyanide (K\(_3\)Fe(CN)\(_6\), 50 mM PBS buffer, pH 7.0). The concentrations of cathode electron acceptors are related to the electron transfers between anode and cathode chambers. Hence, the concentrations of potassium permanganate and potassium ferricyanide which could transfer the same number of electrons were calculated. The solutions were recycled by peristaltic pumps in the cathode compartment using a 2.5 L external buffer vessel, which maintained the stability of concentration of cathode electron acceptors during the operation of MFCs.

**MFC operation**

Two MFCs of same configuration were used in the experiment. One of the MFCs used potassium permanganate as the cathode electron acceptors, while the other used potassium ferricyanide.

The two MFCs were operated in batch mode at room temperature. The synthetic wastewater was fed into the anode of the MFC on a daily basis. The final concentration of sulfide added to the MFC was 100 mg L\(^{-1}\) after the anodic chamber was purged with N\(_2\) for 5 min in order to remove dissolved oxygen from the solution. The nitrate concentration was increased according to stoichiometry of the
chemical reaction (with S/N molar ratio of 5:2). The synthetic wastewater was fed to the anode chamber of the MFC every day. The MFC was operated under the circumstances until the effluent quality became stable, which was repeated three times at least. Subsequently, the influent substrate concentrations were increased to the next level. Three sulfide concentrations were studied: 100, 160 and 300 mg L\(^{-1}\).

The effluent substrate concentrations were analyzed 22 hours after the injection of the influent solution.

Analytical procedures

The physico-chemical parameters of influent and effluent, such as pH, nitrate, nitrite, sulfide and sulfate, were analyzed during the experiment. Nitrate was analyzed through the ultraviolet spectrophotometric screening method on a daily basis and nitrite was measured through the colorimetric method (APHA et al. 1998). The sulfide was determined by the iodometric method whereas sulfate was measured through the turbimetric method (APHA et al. 1998). The pH was determined following the standard method (APHA et al. 1998).

Voltage across the 1,000 \(\Omega\) resistor was recorded at an interval of 10 min using a digital acquisition system (Agilent 34970A data acquisition/switch unit). A cyclic voltammeter (660C, CH Instruments Inc., USA) was used to analyze the redox status of substrates. The potential range of \(-2.0\) V and \(+2.0\) V was applied for this purpose. Graphite rods were used as the working electrode and platinum (CHI115, CH Instruments) as the counter electrode. Ag/AgCl electrode was also utilized as reference electrode. Voltage rate of 10 mV s\(^{-1}\) was chosen as scan rate in CV analysis. All tests were repeated at least three times for quality assurance.

RESULTS AND DISCUSSION

Abiotic MFCs with different cathode acceptors

Two MFCs operating as abiotic MFCs without the anaerobic sludge in anode chambers were designated as control MFCs, to evaluate the chemical sulfide and nitrate removal under anaerobic conditions. The anode chambers of both abiotic MFCs were fed with 14 mg L\(^{-1}\) nitrate and 60 mg L\(^{-1}\) sulfide, while the individual cathode electron acceptors were potassium permanganate and potassium ferricyanide. The concentrations of substrates in the anode chambers were regularly measured. Although cathode electron acceptors in the two abiotic MFCs were different, the dynamics of substrate concentrations were almost the same (data not shown). The sulfide concentration decreased to lower than 1.0 mg L\(^{-1}\) after 5 hours, and it was undetectable after 7 hours. The sulfate and thiosulfate concentrations slightly changed during the process. With the passage of time, the solution in the anode chambers became turbid, and white precipitates were clearly observed at the bottom of the anode chamber, which suggested that element sulfur was generated in both MFCs. However, the nitrate and nitrite concentrations in the anodic chamber did not significantly change during that period.

Figure 1 shows the closed circuit voltage and cathode potential (vs. normal hydrogen electrode (NHE)) of two abiotic MFCs with different cathode acceptors in a typical batch test. At external resistance of 1,000 \(\Omega\), the maximum voltage of the abiotic MFC with potassium permanganate was 658 mV, which was observed at the feeding moment. Then the voltage rapidly dropped to 159 mV after 4 hours, a decrease of 75.1%. Afterwards, it slowly descended to 11 mV after 7 hours. The steady voltage of the abiotic MFC with potassium permanganate was approximately 3 mV. Conversely, the cathode potential of the MFC remained stable throughout the experiment, which was about 725 mV (vs. NHE).

The maximum voltage from the abiotic MFC with potassium ferricyanide was 283 mV, generated during the first few minutes (Figure 1). Subsequently it rapidly dropped and decreased to 10 mV after 4 hours, a decrease of 96.5%. It gradually decreased to lower than 1 mV after 6 hours. The steady voltage of the abiotic MFC with potassium ferricyanide was approximately 0.1 mV. The cathode...
potential of the MFC still remained constant; the value was about 480 mV (vs NHE).

Evidently, power generation in abiotic MFCs was related to spontaneous electrochemical oxidation of sulfi
de. Sulfi
de is an electrochemically active oxidizable com-
ponent which can directly donate electrons to the anodic
 electrode in MFCs. Previously, many researchers have
demonstrated sulfi
de oxidation into elemental sulfur,
which is an electrochemical reaction (Dutta et al. 2008;
demonstrated that sulfi
de oxidation was spontaneous in
an abiotic fuel cell reactor with K3Fe(CN)6 as cathode elec-
tron acceptor. It is well established that elemental sulfur is
the major end product of electrochemical sulfi
de oxidation.
A similar trend was observed in the results of the present
study.

The voltage is the difference in potentials at the anode
and cathode. Cathode potential is determined by charac-
teristics of the cathode electron acceptors, while anode
potential is dependent on the chemical reactions in the
anode chamber. The nature of sulfi
de oxidation in the
anode chambers was similar, implying that the distinction
between the voltages outputs of the two MFCs was caused
by the cathode electron acceptor. The cathode electron
acceptor has obvious bearing on the voltage output of
MFCs, which is directly related with redox potential of
materials. In the current experiment, the redox potential of
potassium permanganate was 725 mV while that of potas-
sium ferricyanide was 480 mV. It was suggested that the
MFC with potassium permanganate as cathode electron
acceptor showed better capacity to generate electricity with-
out anaerobic sludge.

**Biotic MFCs with different cathode acceptors**

Two MFCs with 100 mL anaerobic sludge in anode
chambers were used as biotic MFCs (experimental MFCs).
These were operated to demonstrate the influence of cath-
ode electron acceptors on the performance of simultane-
utaneous anaerobic sulfi
de and nitrate removal under biological conditions.

In the case of potassium permanganate as cathode elec-
tron acceptor, the influent sulfi
de and nitrate concentrations
were in the range of 100 mg L\(^{-1}\) to 300 mg L\(^{-1}\) and 17.5 mg
L\(^{-1}\) to 52.5 mg L\(^{-1}\), respectively. The results showed that the
sulfi
de removal percentage remained higher than 99.5%,
and its effluent concentration was less than 2.1 mg L\(^{-1}\)
(Figure 2(a)). The effluent sulfate concentration went up with
the rise of influent sulfi
de concentration, and increased from
81.9 to 262.1 mg L\(^{-1}\). The nitrate removal percentage was
also higher than 98.0%, and the effluent nitrate and nitrite con-
centrations rose slightly from 0.02 mg L\(^{-1}\) and 0.55 mg L\(^{-1}\)
to 1.05 mg L\(^{-1}\) and 1.02 mg L\(^{-1}\), respectively. For potassium
ferricyanide as cathode electron acceptor, the sulfi
de and
nitrate removal percentages remained higher than 99.5%
and 97.2%, respectively (Figure 2(b)). The effluent sulfate
concentration also increased with the rise of influent sulfi
de concentration (increased from 86.1 to 231.9 mg L\(^{-1}\)). The
concentrations of nitrite in the effluent rose slightly from
0.01 to 3.61 mg L\(^{-1}\).

At the external resistance value of 1,000 \(\Omega\), the maxi-
mum voltage generated by the MFC during the first 5 min
with potassium permanganate was 91 mV (Figure 3(a)).
Then the voltage dropped rapidly and reached a steady
point (24 mV). The profiles of voltage in the MFC were simi-
lar at different influent concentrations. The voltage
generation in the MFC had direct relationship with the
increasing influent substrate concentrations, which could
reach 343 mV in the tested range. However, the maximum

![Figure 2 | Substrate removal in the MFCs: (a) with potassium permanganate; (b) with potassium ferricyanide.](https://iwaponline.com/wst/article-pdf/73/4/947/464238/wst073040947.pdf)
value appeared during the initial stages of the experiment but lasted for a short while. A subsequent rapid drop in the voltage output to a stable value was observed, which lasted for more than 10 h. The voltage in the 20th hour after the injection of influent solution was designated as steady state voltage. To evaluate the voltage generation, the steady voltage was used as an indicator. With the increase in the influent sulfide concentration from 100 to 300 mg L\(^{-1}\), the steady state voltage increased from 24 to 45 mV (the value being highest in the tested substrate concentration range). A similar situation was observed for the MFC using potassium ferricyanide as cathode electron acceptor. In the tested substrate range, the maximum voltage elevated from 94 to 279 mV, while the steady voltage increased from 9 to 22 mV (Figure 3(b)).

Figure 2 shows that both MFCs displayed slight differences in substrate removal and both of them showed good capacity for simultaneous sulfide and nitrate removal. For the tested substrate concentration range, about 70.3–87.4% sulfide converted to sulfate and about 91.6–99.4% nitrate converted to nitrogen (Figure 2). It was suggested that nitrogen and sulfate were the main end products in both MFCs. It was inferred that the nature of biological reactions was similar in the anode chambers. However, the steady voltages of the MFC with potassium permanganate cathode electron acceptor were 2.67 times, 2.29 times and 2.04 times higher than those of the MFC with potassium ferricyanide, when the influent sulfide concentrations were 100, 160 and 300 mg/L, respectively (Figure 3). The results were considered in alignment with the previous reported studies. Employing \textit{S. putrefaciens} as biocatalyst in the anode chamber with the addition of LB media as anolyte, Pandit \textit{et al.} (2011) found that the MFC with potassium permanganate generated higher voltage and power density (1.11 V and 116.2 mW m\(^{-2}\)) than that using potassium ferricyanide (0.78 V and 40.6 mW m\(^{-2}\)). Jafary \textit{et al.} (2013) used syrup with a high sugar content as a substrate in a dual-chambered MFC. When potassium ferricyanide was used as an oxidizing agent in the cathode side, the maximum output of power density was 65 mW m\(^{-2}\). Power density increased almost 2.5-fold and reached 234 mW m\(^{-2}\) when the electron acceptor in the cathode chamber was replaced with potassium permanganate.

Being a reduced ionic species, sulfide seemed to have some relationship with the voltage generation in the MFCs as the steady voltage and electron quantity of the MFCs increased with the increasing influent sulfide concentration (Figure 4). The curves show that steady voltage in the MFC was linearly related with the influent sulfide concentration (\(R^2 = 0.9919\) and \(R^2 = 0.9963\)) (Figure 4(a)). The slope of the curve is the ratio of steady voltage to sulfide concentration and represents the conversion efficiency of influent substrate converted to the output voltage. The steeper was the slope; the higher was the conversion efficiency. The slopes of the curves were 0.10 (potassium permanganate) and 0.061 (potassium ferricyanide), respectively. It implied that 1 mg L\(^{-1}\) sulfide conversion could generate 0.10 mV voltage (potassium permanganate) and 0.061 mV voltage (potassium ferricyanide), respectively. The number of electrons was calculated to represent the actual electricity production, which was better than steady voltage considering the variations in its output with the passage of time. Figure 4(b) shows that the number of electrons in the MFC was also linearly related with the influent sulfide concentration (\(R^2 = 0.947\) and \(R^2 = 0.986\)). At that time, the slopes of the curves were 0.026 (potassium permanganate) and 0.014 (potassium ferricyanide), respectively. It was
inferred that the conversion efficiency with potassium permanganate as cathode electron acceptor was higher than that with potassium ferricyanide in biotic MFCs, and the potassium permanganate was a better cathode electron acceptor to elevate the conversion efficiency of the biotic MFC.

**Electrochemical behavior of abiotic and biotic MFCs**

CV is an effective electrochemical analysis method, which is widely used to characterize and evaluate the redox activities of components in MFCs (Xu et al. 2013; Kondaveeti et al. 2014; Lee et al. 2014), and was adopted in the current study. Electrochemical behaviors of abiotic MFCs and biotic MFCs with different cathode electron acceptors were evaluated by CV (Figure 5). Voltammograms were recorded against Ag/AgCl visualized marked variation in redox peaks and electron transfer mechanism (the potential at which the reduction reaction takes place) in MFCs.

The voltammograms of abiotic MFCs using different cathode electron acceptors are shown in Figure 5(a). The voltammogram of the abiotic MFC with potassium permanganate had two oxidation-reduction peaks, which were approximately at 0.1 V (vs Ag/AgCl) and 0.9 V (vs Ag/AgCl), respectively, with the peak current of about 0.027 A. In the other abiotic MFC with potassium ferricyanide as cathode electron acceptor, the voltammogram also had two oxidation-reduction peaks, which were approximately at 0.3 V (vs Ag/AgCl) and 1.0 V (vs Ag/AgCl), respectively, and the peak current was about 0.008 A.

Voltammograms of biotic MFCs were obtained by using different cathode electron acceptors, which are shown in Figure 5(b). The voltammogram of the biotic MFC using potassium permanganate had only one oxidation-reduction peak (approximately at 0.3 V (vs Ag/AgCl)) with the peak
current of about 0.029 A, while the voltammogram of MFC with potassium permanganate also had one oxidation-reduction peak at 0.3 V (vs Ag/AgCl) with the peak current value of 0.023 A.

Thus, abiotic MFCs and biotic MFCs both displayed electrochemical activity. However, both differed in the redox components. It was indicated that microorganisms attached at the anode had higher electrochemical performances in MFCs. Although the CVs of both biotic MFCs with different cathode electron acceptors were similar, the maximum current of MFC using potassium permanganate was higher than that with potassium ferricyanide. It suggested that potassium permanganate had better catalytic activity than potassium ferricyanide in the MFC treating sulfide and nitrate, which was consistent with the power generation.

CONCLUSION

The current investigation explored the suitability of cathode electron acceptors in MFCs treating sulfide and nitrate simultaneously. The electricity generation in abiotic MFCs was suggested to be related with spontaneous electrochemical oxidation of sulfide. The biotic simultaneous sulfide and nitrate removal in MFCs was promising and produced nitrogen and sulfate as the main end products. The steady voltage of biotic MFC using potassium permanganate was nearly twice that of with potassium ferricyanide. The CV curves supported that potassium permanganate was a better choice regardless of using anaerobic sludge. Potassium permanganate was suggested to be a more suitable cathode electron acceptor for biotic MFC simultaneously treating sulfide and nitrate. The findings are useful in actual engineering facilities involving simultaneous treatment of sulfide- and nitrate-rich wastewaters in MFCs.

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REFERENCES


