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# Effects of resazurin on perchlorate reduction and bioelectricity generation in microbial fuel cells and its catalysing mechanism

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ABSTRACT

Resazurin could improve the performance of microbial fuel cells (MFCs). Compared with a mediator-free MFC, MFCs with 3 μM resazurin, 6 μM resazurin, and 9 μM resazurin exhibited increases in perchlorate reduction ratio of 50.8%, 72.6% and 101.6%, respectively, and increases in the output voltage of 24.5%, 33.3% and 41.7%, respectively. An electrochemical analysis indicated that adding resazurin decreased the anode resistance and enhanced the biocatalytic activities of the anode. The content of humic acid increased, and the content of polysaccharide decreased in the resazurin-addition MFCs, which accelerated the transfer of electrons to the anode. Adding 9 μM resazurin to MFC with one of eight different inhibitors (6 μM) demonstrated that the acceleration sites of resazurin were NADH-ubiquinone reductase, NADH reductase and methylnaphthoquinone. A microbial analysis showed that adding resazurin changed the composition of the anodic microbial community and stimulated the growth of certain dominant species.



## 1. Introduction

Electricity generation using microbial fuel cells (MFCs) has recently drawn much attention as a new approach to wastewater treatment [1]. MFCs can utilize various organic substrates under anaerobic conditions to directly transform chemical energy to electricity [2]. A series of electrochemical reactions is catalysed by microorganisms [3,4]. Most studies on MFCs have used a mixed culture to degrade pollutants and generate electricity [5,6]. Few studies in the literature have focused on the use of MFCs for the disposal of perchlorate wastewater.

Perchlorate ($ClO_4^-$) is a major inorganic contaminant [7]. It is widely used in the manufacture of rocket propellants, missiles, road flares, automobile airbags and fireworks, and it ultimately enters water resources [8]. Perchlorate has been widely observed in groundwater, vegetables, rice, milk, and bottled water, and it has a direct effect on the uptake of iodine by the thyroid gland [9]. Therefore, the removal of perchlorate from wastewater has become an important part of the overall wastewater treatment process. Compared with a variety of physicochemical methods, the biological reduction of perchlorate is a better choice due to its economic and environmental advantages [8]. MFCs are considered as an effective biological treatment strategy for pollutant degradation [3]; however, few studies have reported the use of MFCs to treat perchlorate. Thus, further investigations of perchlorate reduction in MFCs are needed. The exploration of the electron transfer mechanism in MFCs treating perchlorate wastewater is also necessary.

The basic requirement for the establishment of an MFC is the electron transfer from a microbial cell to an electrode. Direct electron transfer is the physical contact between the bacterial cell membrane (or a membrane organelle) and the fuel cell anode [5]. The electron transfer from the bacteria to the anode is the rate-limiting step during the simultaneous removal of contaminant and bioelectricity generation in MFCs [6]. Mediated electron transfer occurs when exogenous (artificial) redox mediators, such as neutral red (NR, $C_{15}H_{17}ClN_4$), methylene blue (MB, $C_{16}H_{18}ClN_3S$), potassium ferricyanide (FeCN, $K_3[Fe(CN)_6]$), thionine (TH, $C_{14}H_{13}N_3O_2S$) and resazurin (RZ, $C_{12}H_7NO_4$) or secondary metabolites, accelerate the electron transfer from a primary electron donor to a terminal electron acceptor [5,10]. These redox mediators may increase the reaction rate by one to several orders of magnitude [6]. Therefore, the effect of mediated electron transfer on MFC performance has attracted the attention of researchers in recent years. Sund et al.

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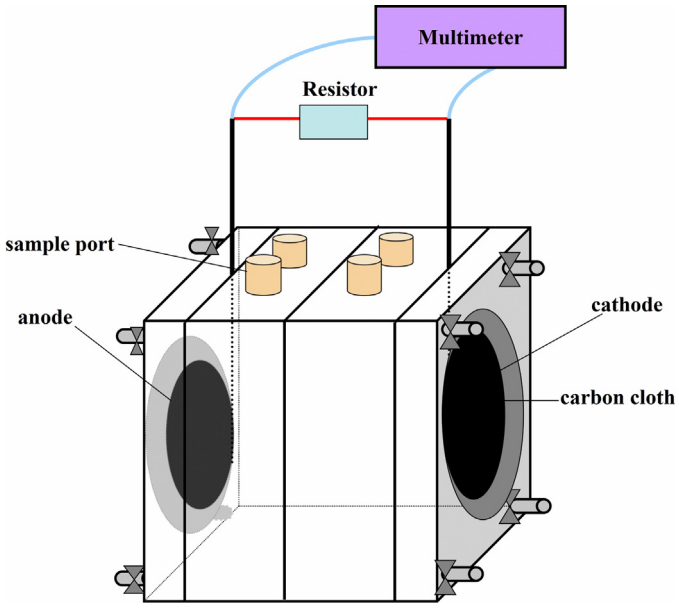


**Fig. 1.** Schematic diagram of the single-chamber microbial fuel cell used in this study.

showed the effect of resazurin on MFC performance in fermentation but not in perchlorate wastewater [10].

The present study aims to identify whether resazurin can act as a redox mediator to simultaneously enhance perchlorate reduction and electricity generation in MFCs. The performances of the MFCs were evaluated in terms of the perchlorate reduction rate and the output voltage. The electron transfer mechanism of resazurin on the anode was investigated using electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) and a component analysis of the extracellular polymeric substances (EPS). The electron transfer mechanism of resazurin was assessed by adding eight respiratory inhibitors. The microbial communities with and without the addition of resazurin were evaluated via an analysis of metabolic genes. This investigation could be used as a basis for future assessment and design of perchlorate-removing MFCs.

## 2. Materials and methods

### 2.1. Chemicals

Resazurin ($C_{12}H_7NO_4$, RZ), cupric chloride ($CuCl_2$), capsaicin ($C_{18}H_{27}NO_3$), rotenone ($C_{23}H_{22}O_6$), dicyclohexylcarbodiimide (DCCD, $C_{13}H_{22}N_2$), sodium azide ($NaN_3$), dicoumarol ($C_{19}H_{12}O_6$), mesoxalonitrile 3-chlorophenylhydrazone (CCCP, $C_9H_5ClN_4$) and sfuinasfrine dihydrosfhlorid (QDH, $C_{23}H_{32}C_{l3}N_3O$) were purchased from Sigma Ltd. (Shanghai, China). Other chemical reagents were analytical grade and purchased from Xiandai Ltd. (Shijiazhuang, China).

### 2.2. MFCs construction

Single-chamber air cathode MFCs were constructed as shown in Fig. 1. Briefly, the total volume of the MFC was 30 mL. A carbon cloth anode (without wetproofing; ETEK, USA) with a surface area of 9 $cm^2$ was positioned in the chamber at a distance of 4 cm from the cathode. For the cathode, a carbon cloth (30% wetproofing; ETEK, USA) incorporating 0.5 mg/$cm^2$ Pt catalyst was used with an anode of the same size. The anode and cathode were connected using a titanium wire to an external resistor of 400 Ω. Four holes were drilled into the top of the reactor for substrate injection and sample extraction.

### 2.3. MFCs inoculation and operation

The anode chamber was inoculated with anaerobic sludge (25% v/v) from the Qiaoxi Municipal Wastewater Treatment Plant (Shijiazhuang City, China). In the growth medium, a phosphate buffer was used to maintain a pH of 7.0. Each litre of the growth medium contained 0.994 g sodium acetate, 0.212 g (150 mg $L^{-1}$) perchlorate, a vitamin solution (2 g folic acid, 1.0 g vitamin $B_6$, 0.5 g vitamin $B_2$, 0.5 g niacin, 0.5 g vitamin $B_5$, 0.05 g vitamin $B_{12}$, 0.5 g thioctic acid, and 0.5 g *P*-sulfamic acid) and a microelement solution (0.2 mg $N(CH_2COOH)_3$, 0.3 mg $MgSO_4$, 0.1 mg NaCl, 0.013 mg $ZnCl_2$, 0.01 mg $FeSO_4{\cdot}7H_2O$, 0.01 mg $CaCl_2{\cdot}2H_2O$, 0.01 mg $CoCl_2{\cdot}6H_2O$, 0.001 mg $CuSO_4{\cdot}5H_2O$, 0.001 mg $H_3BO_3$, 0.0025 mg $Na_2MoO_4$, 0.0025 mg $NiCl_2{\cdot}6H_2O$, and 0.0025 mg $NaWO_4{\cdot}2H_2O$). The voltages were stable at 25 d and all the internal resistances of MFCs were nearly 400 Ω, based on the power density and polarization curves.

Resazurin was selected as the redox mediator. In the first set of tests, four MFCs with different amounts of resazurin (3 μM, 6 μM, 9 μM and 12 μM) and a control MFC (mediator-free) were operated at 30 °C in fed batch mode. In the second set of tests, capsaicin, rotenone, DCCD, $NaN_3$, dicoumarol, CCCP, QDH and $CuCl_2$ were added as inhibitors, and eight MFCs with 6 μM of different inhibitors (a single MFC for each inhibitor) were operated at 30 °C in fed batch mode. In the third set of tests, 9 μM of resazurin was added to eight MFCs with 6 μM eight inhibitors, and the MFCs were operated at 30 °C in fed batch mode. The anode solution was refreshed when the output voltage decreased below 10 mV. All tests were conducted at least in duplicate, and all statistical analyses were performed using OriginPro 8.5.

### 2.4. Analysis and calculations

#### 2.4.1. Perchlorate measurements

The sample was first transferred into a centrifuge tube and centrifuged. The supernatant was then filtered with a 0.22 μM filter membrane and stored in a refrigerator at 4 °C for further measurement. The perchlorate was analysed by suppressed conductivity ion chromatography using a system (ICS-1100, ThermoFisher, MMAS) fitted with an AS20 analytical column (4 mm × 250 mm) and an AG20 guard column (4 mm × 50 mm). An eluent gradient concentration (KOH) from 15 mM to 40 mM was utilized (15 mM from 0 to 10 min; 40 mM from 10 min to 21 min).

#### 2.4.2. Electrochemical measurements

The output voltage across the cell was recorded every 30 min using a precise multimeter (9205T, Gamecyber, Nanjing, China) and a data acquisition system (the Altai data acquisition card, Shanghai, China). The coulombic efficiencies (CEs) based on the total acetate additive amount were calculated as previously described [11].

EIS and CV were measured using an electrochemical workstation (CHI660E, Chenhua, Shanghai, China). The EIS measurement was conducted for the anode in a frequency range of 100 kHz–0.1 mHz with an AC signal of 10 mV amplitude. Titanium wire of anode was used as the working electrode, titanium wire of cathode was used as the counter electrode and a saturated calomel electrode (SCE, +0.242 V vs. normal hydrogen electrode, NHE) was used as the reference electrode. The data were fitted and simulated using ZSimpWin3.10 software based on a predetermined equivalent electrical circuit. CV was performed in a three-electrode setup comprising a glassy carbon (2.0 mm) as a working electrode, a Pt foil as a counter electrode and SCE as the reference electrode.