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Bioelectricity Generation and Sulfamonomethoxine Removal in Microbial Fuel Cells

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Simultaneous electricity generation and sulfamonomethoxine (SMM) wastewater treatment were investigated using a system of dual-chamber Microbial fuel cells (MFCs). SMM is one of the most common contaminants in some rivers and ground wastewater. *Shewanella putrefaciens* served as a model electroactive bacterium. In this work, the effect of four different substrates on the generation of bioelectricity with two-chamber MFCs based on nafion. 117. CV curve, power density, polarization curve, zeta potential and biofilm enrichment were determined on the anode carbon felt for *Shewanella putrefaciens* in these MFCs and at the studied SMM concentration range of 10-30 mg/L . The results showed that maximum output voltage and power density obtained from four carbon sources ranged from 296.26 to 899.17 mV and 15.88 to 157.57 mW/m^2, respectively, Zeta potential and Protein concentration obtained from four carbon sources ranged from -3.07 to -20.87 mV and 5.69 to 15.76 μg/cm^2. With 10 mg/L SMM, the highest degradation rates were 60.08%, 58.21%, 49.77%, and 31.42%. Relative to other substrates, the most electricity and the least internal resistance was produced using a sodium lactate substrate; also, sodium lactate obtained the highest SMM removal rate. These results indicate that wastewater contaminated with poisonous and refractory organic compounds such as antibiotics could serve as appropriate resources for power generation and contaminant removal using MFC technology.

Keywords: Microbial fuel cells (MFCs); Substrate; Power generation

1. INTRODUCTION

The problems of excessive fossil fuel consumption, serious environmental pollution and an energy shortage crisis have become the major bottlenecks restricting sustainable economic and social development worldwide. Environmental problems, and especially water pollution, are becoming more and more prevalent. Microbial fuel cells (MFCs) are new technology for treatment of pollutants and

simultaneous electricity generation, and have become increasingly popular. MFCs are of great concern and are have achieved greater prominence in the fields of environment, energy and material sciences [1-3]. MFCs can utilize various substrates including cellulose [4-5], small molecule organic matter [6-7] and pollutants [8] to generate electricity. Pant et al. [9] reported that the various substrates that have been explored for use in MFCs. The differing compositions and characteristics of substrates in MFCs will achieve different voltage or current responses. Operating conditions including pH, temperature, substrate species and concentration, also affect MFC performance [10-11].

In recent years, new developments have deepened the research in the field of MFCs: wetland microbial fuel cells [12] can treat different kinds of wastewater in wetlands and produce electricity, rice microbial fuel cell [13] can grow rice and produce electricity, and single-compartment air-cathode anaerobic fluidized bed microbial fuel cells [14]. Researchers have improved the electrical performance of microbial fuel cells by changing the cathode and anode [15], adding catalysts [16], changing reaction substrates [17] and microbial metabolic pathways [18]. Significant increases in power output of MCFs have been achieved by reducing the internal resistance and increasing the ionic strength of the electrolyte. However, there is limited data in the literature about the effects of the different substrates of power electronic circuits on MFC.

In the past fifty years, the number of studies on the application of microbial fuel cell for electricity generation has increased. The actual electrical energy generated by the MFC system operating in the laboratory is lower than the theoretical value [19]. Based on redox reactions catalyzed by microorganisms, microbial fuel cell can be designed to generate electric energy from sediments [20] and organic waste in water. They can also degrade sugar and some organic pollutants, converting them into carbon dioxide and other non-toxic substances. Sulfamonomethoxine (SMM) is one of the most bioactive sulfonamides and inhibits the growth of aquatic organisms [21]. It is widely used to treat and prevent diseases of animals and poultry. SMM have been detected in some rivers and groundwater in many locations worldwide, and the concentration of antibiotics have reached ng/L level [22]. These drugs and their metabolites can pose great risks to human health. Wang [35] successfully demonstrated single chamber MFCs for treatment of antibiotic wastewater and MFCs can perform biodegradation and produce electricity simultaneously.

For this study, we choose *Shewanella putrefaciens*. Our experimental study intended to determine how bacteria use an anode as an electron acceptor and to what extent they generate electrical output and pollutant removal. A two-chamber compartment cathode microbial fuel cell was constructed. Simulated antibiotic wastewater was used and the reactor was operated with sodium lactate as the substrate. After the MFC was stabilized, the substrate was replaced with glucose, sodium acetate and sodium propionate. The effectiveness of antibiotic biodegradation and the performance of power generation performance were determined for these MFCs. The power generation efficiencies of different substrates and the response mechanism of MFC to substrate replacement have important guiding significance for the treatment of production and living wastewater by MFCs in practical applications. This work might provide a new insight for an ecologically sound way to treat antibiotic wastewater. It provides theoretical support and the basis for MFC treatment of other refractory toxic substances.

2. EXPERIMENTAL SECTION

2.1. MFC constructions

Two-chamber MFCs (Fig. 1) are composed of an anode chamber, a cathode chamber and an ion exchange membrane (Nafion117 PEM, Dupont Co., USA,); the two identical chambers, with a working capacity of 100 mL. The anode and cathode chambers of the battery are cylindrical bottles made of plexiglass. Carbon felt (0.5cm thick, 2.8cm×4cm Three leaf carbon Co., Beijing) served as the reactor electrode in the experiment. The carbon felt was soaked in 1 mol/L acetone for 24 h before use and rinsed with deionized water until the aqueous solution was neutral. A 1000 Ω external resistor was maintained at 30°C for 30d to generate a stable voltage. This experiment used a three-electrode system. The anode used carbon felt as the working electrode. Titanium wire was used to connect the working electrode with the external circuit. The platinum wire electrode was the cathode, and Ag/AgCl served as the reference electrode.

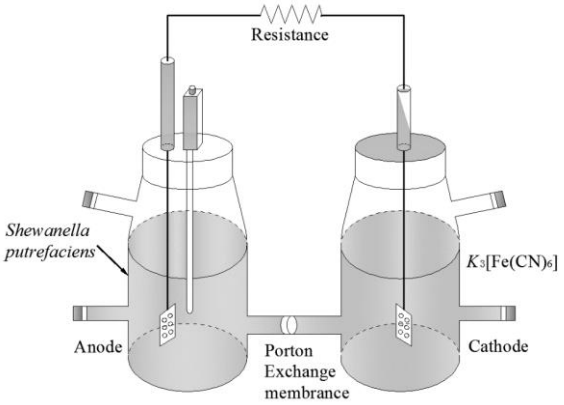

Figure 1. Schematic of the two-chambered MFC

2.2. Strain source and experimental operation

Source of the bacterium: The iron-reducing model bacteria *Shewanella putrefaciens* (ATCC 8071) was provided by China Marine microbial species conservation and management center (Xiamen, China).The strain was inoculated onto Nutrient Broth medium (pH=7.2) from bowei bio-technology (Shanghai, China) at 30°C. After 24 h of culture, the cells were collected by centrifugation (4000 rpm, 5 min) and washed three times in Physiological saline (0.9g NaCl into 1L deionized water)[23].

Anode matrix: Artificial simulated synthetic wastewater with the addition of SMM was used as the electrode liquid in the anode chamber. Different substrates for comparative experiments included four 50 mmol/L solutions of different organic matter (sodium lactate, sodium acetate, glucose, sodium propionate); these were used as the carbon source and added into the anode chamber. Three concentrations were used for the SMM concentration gradient: 10 mg/L, 20 mg/L, and 30 mg/L. Bacteria in logarithmic growth (OD_{600}=0.6) were placed in the anode.

Cathode matrix: Potassium ferricyanide is a commonly used cathode electron acceptor in laboratory studies. Potassium ferricyanide ($K_3Fe(CN)_6$ 16.45 g/L, K_2HPO_4 8.7 g/L) were added into PBS

(phosphate buffer solution) (pH 7.0) ($Na_2HPO_4 \cdot 12H_2O$, 10.32 g/L; $NaH_2PO_4 \cdot 2H_2O$, 3.32 g/L; NH_4Cl, 0.31 g/L; KCL, 0.13 g/L). [36].

All of these reagents were of analytical grade (purity>99%). SMM ($C_{11}H_{12}N_4O_3S$), molecular weight = 280.3.

2.3 Electrochemical and analytical measurements

2.3.1. Set-up of experiments

The simulated wastewater used in this work was synthesized to the same parameters ensure reproducible characteristics, and the experiments used four different carbon sources in the wastewater. N_2 was passed through the device for 15 min before startup to ensure the anode chamber had an anaerobic environment. MFC was prepared with lactic acid as the substrate, and the resistance ends were connected to the voltage acquisition system (PISO-813, ICP DAS Co., Ltd.) at 1 h intervals for real-time monitoring of voltage; after the voltage is generated, the color change of potassium ferricyanide solution is used to determine the need for cathode solution replacement; a color change from yellow to completely light green, indicates the cathode room solution needs to be replaced. After the two cycles, the MFC was prepared with addition of 10 mg/L SMM to the anode solution. If the MFC can obtain 3 stable output voltage cycles, the device can be considered to be successfully started. all tests were conducted at 30°C.

2.3.2. Cyclic Voltammetry

This study used Cyclic voltammetry (CV) curves to analyze the oxidation and reduction activity of bacteria under different substrates in MFCs. CV curves were used to determine the electrochemical activity of microbial strains whose range is -1.5 V to 1.5 V. The direction scanning was set at a scanning rate of 10 mV/s, and the potential change scanning of the working electrode was recorded.

2.3.3. Method for testing polarization curves and power density curves

Polarization curves are commonly used to analyze and describe battery characteristics [24]. The relationship between current and voltage using polarization curve was determined under different external resistance values. After 3 d of stable operation, the four groups of MFCs were subject to steady-state discharge tests. First, each MFC was run for 60 min at 1000 Ω conditions, and then the variable resistance box (ZX98A, Shanghai) was adjusted every 20 min. The slope was obtained by linear fitting of the ohmic polarization region data of the polarization curve. The slope is apparent internal resistance and the equation is as follows:

$$P_A = \frac{E_{MFC}^2}{R_{ext}A} \quad (1)$$

where A represents anode area, E_{MFC} refers to the battery voltage, and R_{ext} represents External resistance. Other electrochemical assays were performed using an electrochemical workstation (Chenhua CHI660E, Shanghai). The MFC was calculated according to the reported method [25].

During actual operation, the external resistance is sequentially connected to the circuit with resistance values set from high to low, and was selected from a range of 30000 Ω-50 Ω for 11 decreasing external resistance values.

2.3.4. Zeta potential and BCA protein concentration detection

In this experiment, Zeta potential was determined by electrophoretic light scattering. An electric field is applied at both ends of the electric pool Zeta. Under the applied electric field, negatively charged suspended particles gradually move toward the anode. For the test, 80 microliters of test solution was poured into a U-shaped plexiglass tube. Zeta potential (ZP) was determined by a Zetasizer Nano ZS90 (Melvin, England).

On the third day, 20 μL of the anolyte was taken from the MFC reactor and 200 μL of BCA reagent was added; these were mixed by gentle shaking, and incubated at 37℃ for 1h. After cooling to room temperature, the absorbance was measured at 562nm on the microplate reader, referencing to a blank control. To determine protein content, absorbance values were referenced against a standard curve created by measuring protein standards with BCA reagent.

2.4. Determination method of SMM

The concentration of SMM in solution was determined with the Agilent 1220 high performance liquid chromatography using an ultrasonic detector at a wavelength of 270nm at a flow rate of 1 mL/min. The Agilenthc-c18 chromatographic column (4.6 × 250mm, 5 μm) was used. 1mL sample was washed with methanol, and sonicated. The mobile phase was composed of 0.4% acetic acid and acetonitrile. After centrifugation, the supernatant passed through a 0.22μm water filter membrane, and the injection volume was 20 μL.

2.5. Data analysis and processing

The degradation rate (X%) of the SMM by *S. putrefaciens* were calculated using the equations:

$$C = C_0 e^{-kt} \quad (2)$$

where C_0 and C are the respective initial concentration and measured value of SMM at reaction time t, and k is the rate constant. The statistical and preliminary data analysis was run with Excel 2010(Microsoft Inc., Redmond WA, USA), and graphs were drawn with Origin 9.0 (Origin Software, Northampton, MA, USA), the following experiments took seven days for one running cycle. The data in the experiment was tested on the third day. All experiments were performed in triplicate and expressed as average ± standard deviation.

3. RESULTS AND DISCUSSION

3.1. MFC performance by Sodium lactate addition

The initiation of the MFC is divided into three stages: delay period, rise period, and stability period [26]. The electricity generation of MFC started after a lag phase and the output voltage rose slowly. Sodium lactate was used as the substrate during the start-up period. The voltage output of *Shewanella putrefaciens* rapidly increased in MFCs; At 99 h, it reached 325.586mv. In the third cycle, MFCs were prepared by adding 10 mg/L SMM to the anode solution. After the third substrate solution replacement, the current in the circuit was produced by the growth of microbes, which were transferring electrons onto the anode; the cathode accepts electrons and increases protons reduction ability, enhancing anode substrate degradation. As the power output increased, the output voltage correspondingly increases. After a decline in voltage, the substrate solution was replaced for the 4th time, and the MFC voltage peaks quickly; At 147h, it reached 578.3845mv. The maximum voltage with addition of 10 mmol/L SMM was 77.6% greater than that for the 0 mmol/L SMM treatment), 325.586 mV).

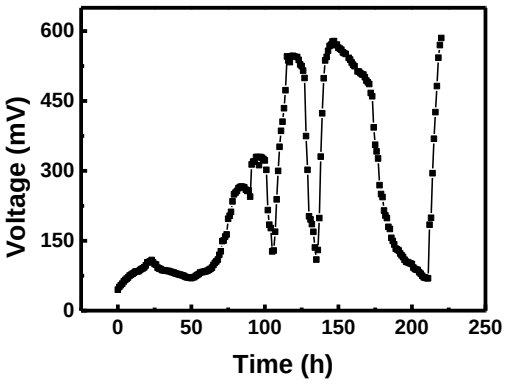

Figure 2. Start-up progress in MFC

Table 1. Maximum MFC voltage output for different substrates

Substrate	Concentration (mg/L)	Maximum Output Voltage (mV)	Starting Time (h)
Sodium lactate	10	578	147
Sodium acetate	10	495	160
Glucose	10	425	189
Sodium propionate	10	235	225

As shown in figure 2, it was maintained for 10 h on the sixth day, indicating that the generation of electric energy has nothing to do with the suspended microorganisms in the solution. The

microorganisms have attached to the anode to form a biofilm, effectively transferring the electrons generated by microbial respiration directly to the anode; this event is indicative of the successful start of the MFC. As can be seen from Table 1, the MFC using sodium lactate required 142h to start electricity production, and the output voltage was 2.45 times higher than that of the MFC with sodium propionate as substrate. Regarding to the more complex glucose and sodium propionate substrates, the starting times were 189 h and 225 h, respectively. The low voltage output could be explained by different electrogenic metabolism [9]. The low concentration of SMM ensures a relatively sufficient nutrient matrix and provides favorable conditions for the growth and enrichment of microorganisms with electrochemical activity in the anode.

3.2. Anodic Electrochemistry Performance

Different substrate electrochemical analyses were performed in operating MFC reactors. Cyclic voltammetry is a common method in electrochemical analysis. The effects of four different substrates (sodium lactate, sodium acetate, glucose, and sodium propionate) were tested to determine the electrical characteristics of MFCs.

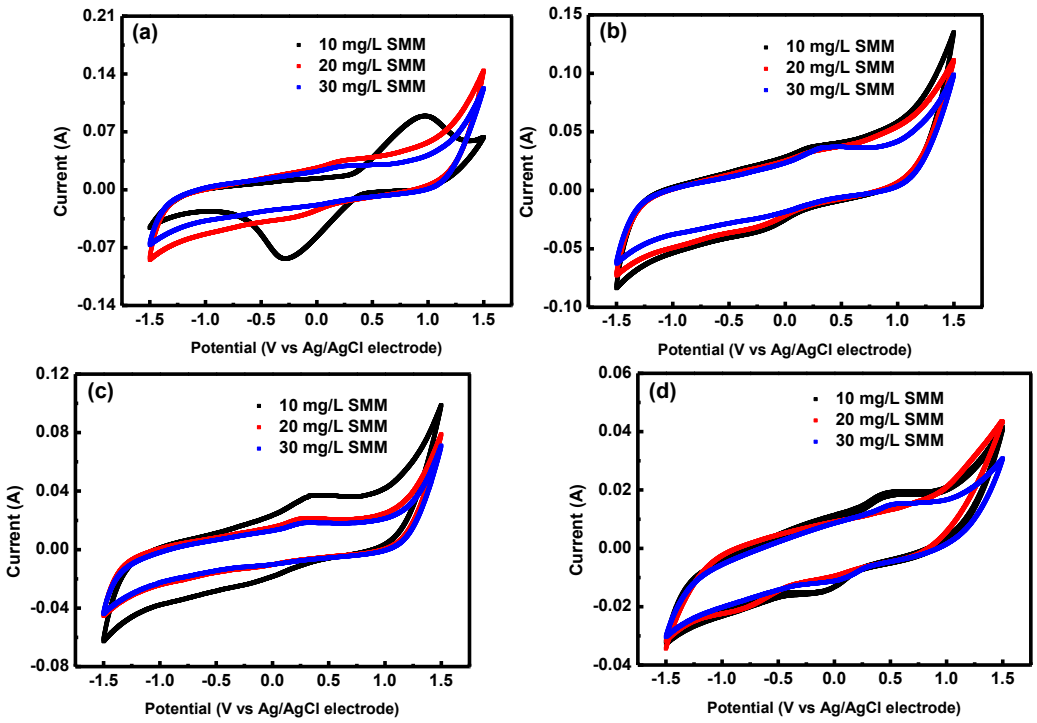

Figure 3. Cyclic voltammetry curve in 4 microbial fuel cells under different substrates of *Shewanella putrefaciens* cells treated for 3d with various doses of SMM. (a) Sodium lactate; (b) Sodium acetate; (c) Glucose; (d) Sodium propionate. The concentration gradients of SMM was set to 10, 20 and 30 mg/L

As shown in figure 3, the shape of the CV curve displays information about the processes of oxidation and reduction peaks place on the electrode surface. The results indicated that higher

concentrations of SMM reduce the areas of their respective CV curves; with a SMM concentration of 10 mg/L, the CV area in sodium lactate (Fig. 3a) was the largest of the experimental treatments. Fig. 3a presents the CV profiles of 10 mg/L SMM between 0.75 - 1.25 V appearing as an obvious redox peak, which indicates the strongest microbial oxidation-reduction activity. The redox peak position in Fig.3b and Fig.3d corresponds to 0.1 - 0.4 V and 0.25 - 0.75 V, respectively. No significant redox peaks were observed using glucose as a substrate. The overall power generation capacity corresponds to the same concentration of antibiotics according to the substrate, with a>b>c>d. This implies that some of these substrates were present in high concentration in the experiment, which gave low to the high current. Yu et al. [27] reported that *Shewanella oneidensis* (*S. oneidensis*) MR-1 has a pair of similar redox peaks at the center of the 0.28 V position. Rabaey et al. [28] used cyclic voltammetry to study the self-regulating ability of anodic microorganisms to move electrons through biofilm or extracellular electron shuttles.

Power density and internal resistance are important indices of microbial fuel cell performance. In general, the maximum power is obtained when the highest point of the system power curve is equal to the external resistance R and the internal resistance r. If the output power is larger, the power generation capacity of the MFC is better. The reason why the power density decreases gradually is that the polarization inside the battery causes an approximate short circuit, which leads to the rapid decrease of the output power density. According to figure 4a, the decrease of the output voltage may be due to the bio toxicity of SMM, which inhibits the activity of electricity-producing microorganisms. When it increased from 0 to 10 mg/L SMM, the output voltage increased; this was because the electricity-producing bacteria were better adapted to a low concentration SMM environment, and the microorganisms began to use SMM as a carbon source to continuously produce electricity. With the continuous increase of SMM content, the power density and internal resistance of microbial fuel cells decreased sharply, which may have been due to the toxic effect of sulfanilamide on microbial activity of the anode. Also, some electrons could have been absorbed for self-reduction of sulfanilamide, which would hinder the proton-electron transfer process and negatively impact electricity generation. In terms of the overall effects of SMM concentration on power density and internal resistance values, lower levels resulted in an increasing trend, and thereafter, the trend was decreasing. The polarization curve obtained in this experiment is similar to that obtained in Logan [29] and other experiments in terms of the overall trend.

In order to further study the internal resistance of MFC, the steady-state discharge method was adopted. After drawing the voltage-current curve (Fig. 4), the nonlinear fitting of $y=ax^2+bx+c$ to the apparent internal resistance of the battery was applied. According to Fig. 5, the maximum power of different substrates and the internal resistance values of the corresponding four systems are obtained from ohm's law [24] [30]. In the MFC system, lower concentrations of pollutants result in less inhibition on microorganisms in the anode chamber; this lowers the activation loss (internal resistance) of MFCs, thereby increasing electric energy generated by the system. In reactor a, b, c, and d, the maximum output power occurred in the 10 mg/L SMM treatment, which corresponded to 152.57, 84.65, 26.48, and 15.88 mW/m^2. The maximum output power with sodium lactate substrate was about 10 times that with sodium propionate. With sodium lactate substrate, the internal resistance of the system (10 mg/L SMM) was 817.63 Ω, 943.59 Ω, 1212.8 Ω, respectively. The experimental results show that the MFC internal resistance increases with the increase of the same substrate pollutant concentration. The difference in

power density is caused by different internal resistances. The complexity of the substrate also affects the energy output of MFCs. The polarization curve of MFCs under different substrate conditions is shown in figures 4 and 5. When the solution conductivity is high, the substrate mass transfer process in the solution is accelerated, a higher charge is produced per unit time. With regard to the shape of the polarization curves, the good performance of the sodium lactate can be clearly seen as compared with the other carbon sources. Similar to the results of Wen et al. [31], co-substrates could enhance the energy output. From the above results, it can be seen that the activity of electron producing bacteria in MFCs are not inhibited, and the four organic compounds as substrates have a high tolerance to SMM within a certain range. The sodium lactate is an electron-withdrawing group when the hydroxyl group induces the effect, making H^+ more easily dissociated. Sodium acetate is easily ionized in the solution to reduce the internal resistance of the battery. The glucose fermentation products are mostly organic acids. Due to its long carbon chain, sodium propionate is not easy to ionize and generates the least electrical energy [32].These experimental results have certain practical significance, indicating the possibility of MFC technology being applied in sulfonamide industrial wastewater treatment.

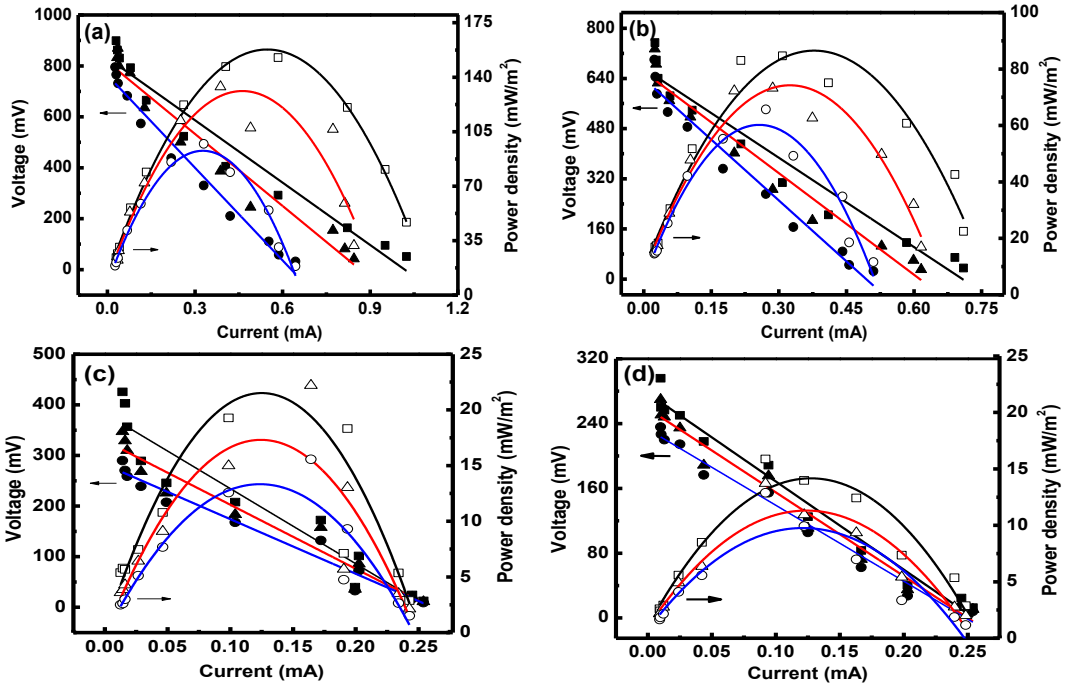

Figure 4. Polarization curves (hollow symbol) and power density curves (solid symbol) in 4 microbial fuel cells treated for 3d with various doses of SMM under different substrates. (a) Sodium lactate; (b) Sodium acetate; (c) Glucose; (d) Sodium propionate. The concentrations of SMM were 10, 20 and 30 mg/L.
(10 □, 20△, and 30 ○ mg/L, 10 ■, 20▲,and30● mg/L).

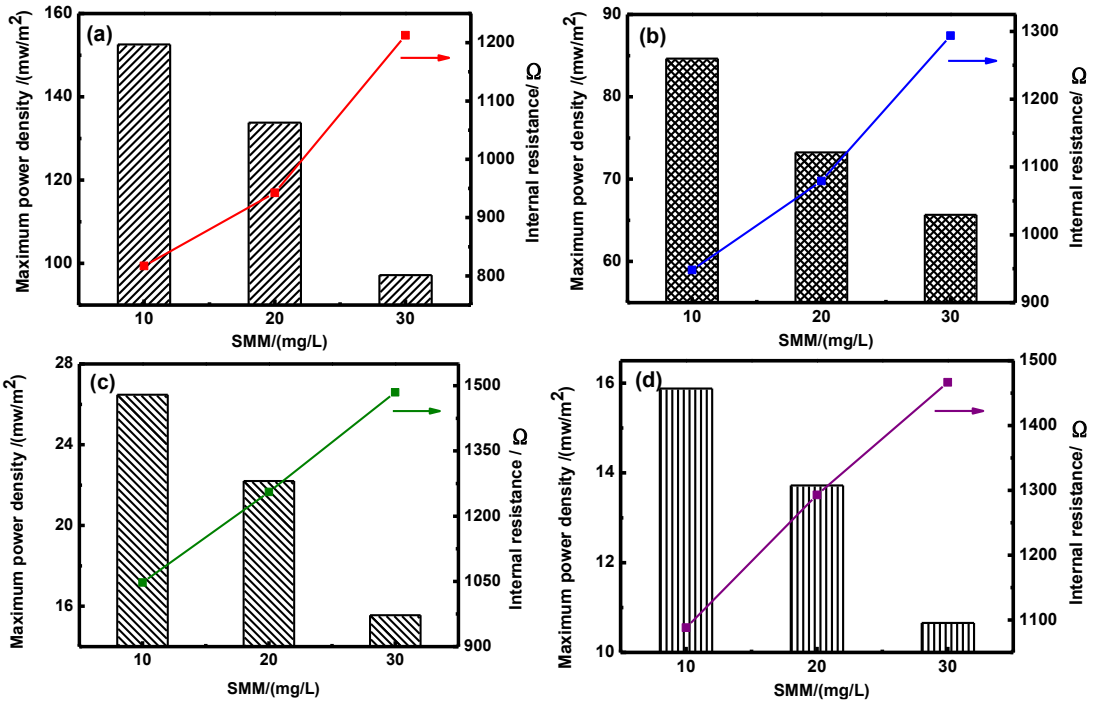

Figure 5. The maximum power density and internal resistance in 4 microbial fuel cells under different substrates of *Shewanella putrefaciens* cells treated for 3d with various doses of SMM. (a) Sodium lactate; (b) Sodium acetate; (c) Glucose; (d) Sodium propionate. The concentrations of SMM were 10, 20 and 30 mg/L.

3.3 Effect of different substrate on cell growth and Zeta potential analysis

Zeta potential is used to evaluate and predict the physical stability of the particle dispersion system. Generally, a greater absolute value of the Zeta potential implies a stronger electronegativity of the interface film [33]. A stronger electrostatic repulsion force, increases the strength of the droplet anti-coagulation ability, leading to stronger physical stability of the simulated emulsion. Also, the influence of SMM concentration on zeta potential is a downward trend after the initial increase, which is caused by flocculation at the dispersion limit in a state of flux.

Zeta potential values for when MFC filtrates of the four different substrates reached stable states are shown in Fig. 6. The absolute value of Zeta potential of the same substance with antibiotics added to different concentrations first increases and then decreases. When the SMM was 10 mg/L in the Zeta potential measurement experiment, the Zeta potential value was larger than the absolute value of the blank control group. As the concentration of SMM in the solution increases, the amount of charge increases, thereby increasing the action of charge, the effect of compression of the double electric layer is more obvious with increased charge, leading to the decrease of the absolute value of Zeta potential. The maximum zeta potential values with SMM concentration at 10 mg/L in MFCs were -20.86, -17.63, -15.87, and -14.57mV respectively. The chemical composition of the bacterial cell wall imparts a negative charge at their cell surfaces, and the electrophoresis phenomenon occurs under the action of an electric field. The zeta potential generated shows that bacteria can combine with low-concentration

antibiotics to generate electricity. At the same concentration, sodium lactate has the highest utilization rate and propionic acid Sodium solution has the weakest stability.

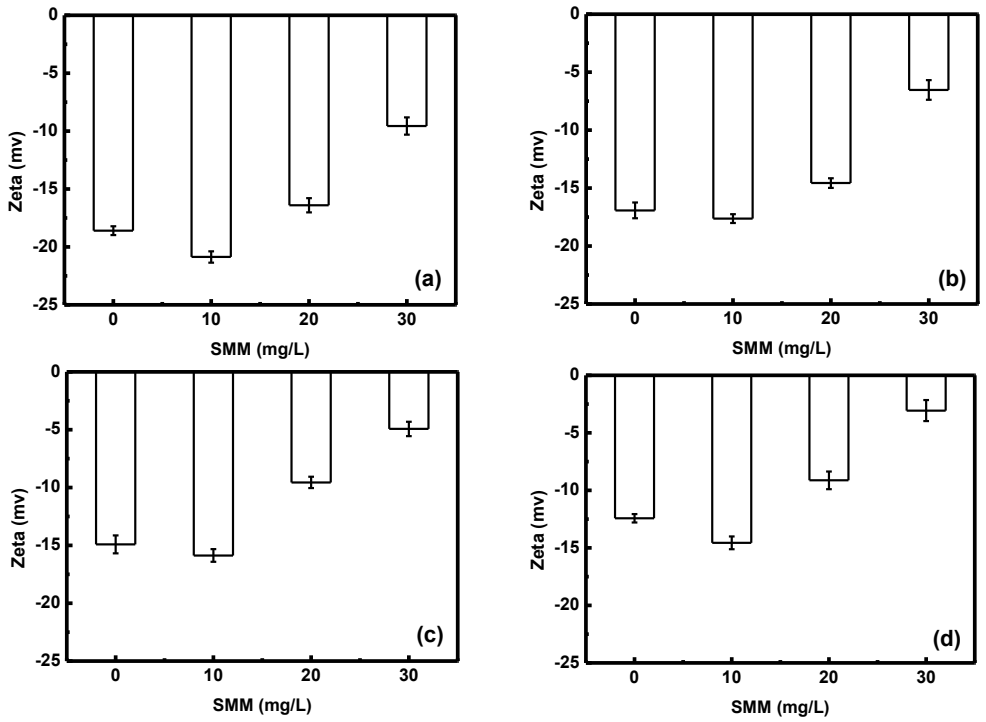

Figure 6. Determination of Zeta potential in 4 microbial fuel cells under different substrates of *Shewanella putrefaciens*; cells were treated for 3d with various doses of SMM. (a) Sodium lactate; (b) Sodium acetate; (c) Glucose; (d) Sodium propionate. The concentrations of SMM were 10, 20 and 30 mg/L

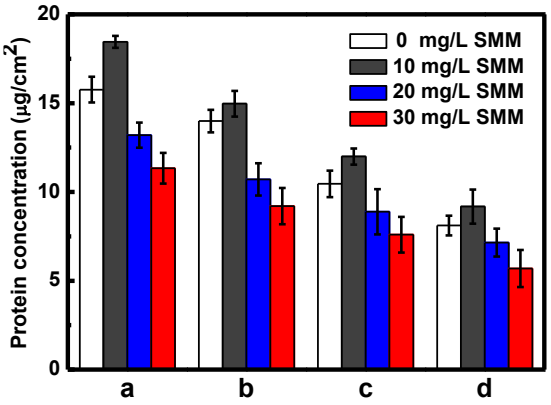

Figure 7. Determination of protein concentration of the anodes in 4 microbial fuel cells under different substrates of *Shewanella putrefaciens* cells treated for 3d with various concentrations of SMM. (a) Sodium lactate; (b) Sodium acetate; (c) Glucose; (d) Sodium propionate. The concentrations of SMM were 10, 20 and 30 mg/L

We also approximately calculated the relative amount of the biomass both on the anode and in the solution. (Fig. 7) The maximum accumulative amount of biomass of the anode biofilm was in sodium

lactate with 10 mg/L SMM concentration. Protein concentration detection further demonstrated previous experimental results. There may be two main reasons for such low output: (1) The inhibitory effect of sodium propionate on bacteria may greatly reduce the production of bioelectricity. (2) The MFC system with a substrate of sodium propionate has a high internal resistance value, and R_i may cause a significant reduction in operating potential due to ohmic limitation, thereby limiting power output.

3.4 Influence of substrates and Removal Rate

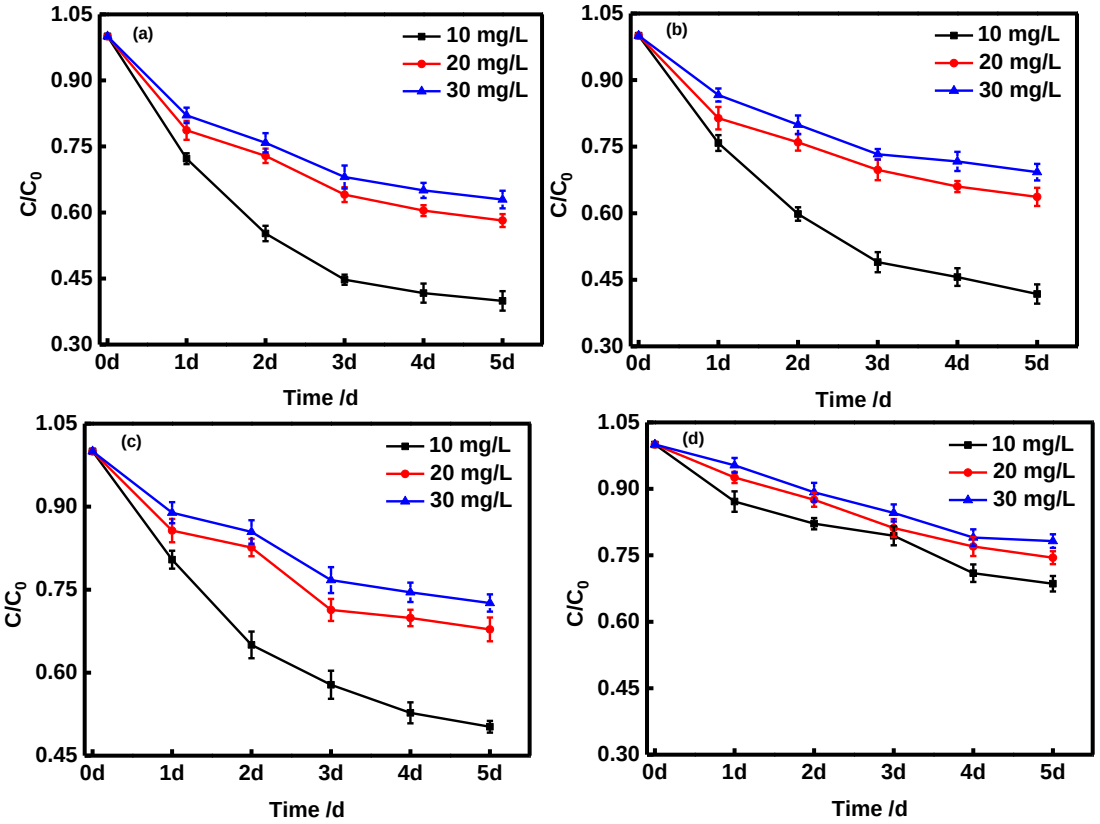

Figure 8. Degradation curves of SMM under different substrates of *Shewanella putrefaciens* cells treated for 3d with various SMM concentrations. (a) Sodium lactate; (b) Sodium acetate; (c) Glucose; (d) Sodium propionate. The SMM concentrations were 10, 20 and 30 mg/L

The MFCs were run for 5 days with different substrates (sodium lactate, sodium acetate, glucose, and sodium propionate) and different concentrations of SMM; the SMM degradation rate is shown in Figure 8. With 10 mg/L SMM, the highest degradation rates were 60.08%, 58.21%, 49.77%, and 31.42%, respectively. The figure shows that as the initial concentration of SMM increases, the degradation rate of SMM gradually decreases. It is also verified that the initial concentration of SMM not only has an effect on the power generation performance of MFCs, but also has a great impact on the degradation rate of SMM. This is consistent with the reported results of MFCs degrading other refractory organics. These results were similar to findings by Zhou et al [34].

4.CONCLUSIONS

In this study, Electricity generation in dual-chamber MFCs tested as a biosensor for SMM monitoring was achieved using different carbon sources. SMM can be used as a substrate to generate electrical energy under simulated indoor culture conditions. In this study, the start-up process of the two-compartment MFC reactor and its electrical energy production were tested with four different carbon sources as fuel. MFCs energy output correlated with the types of substrates used; at a temperature of 30 ℃, the best MFC electrochemical activity was obtained with sodium lactate used as the substrate and 10 mg/L of the antibiotic; the maximum output voltage was 899.17 mV, the maximum output power density was 152.57 mW/m^2, and the internal resistance was about 948.27 Ω. In contrast, sodium propionate substrate and 30 mg/L SMM yielded an output voltage of 81 mV, power density of 1.17 mW/m^2 and internal resistance of 1466.73 Ω. Furthermore, zeta potential and protein concentration detection validation of previous experimental results were presented; these were used to determine that that sodium lactate had the highest utilization rate, whereas sodium propionate solution had the weakest stability and the lowest bacterial protein content. Among the four substrates in the experiment, sodium lactate as the MFC anode matrix has higher wastewater treatment efficiency and production efficiency. This experiment successfully determined the influence of different anode substrates on the electrical efficiency of MFCs and their effects on antibiotic wastewater treatment; this provides the possibility for sustainable development of resources.

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