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Characteristics and Microbial Activity of *Shewanella* and *Escherichia coli* under a Direct-Current Electric Field

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The effects of a direct-current electric field on cell viability and physicochemical properties of exoelectrogens and non-exoelectrogens, e.g., *Shewanella* and *Escherichia coli*, were studied. The results obtained through the analysis of differences in growth patterns and growth rates for *Shewanella* and *Escherichia coli* under electric fields with varying intensity showed that the direct-current electric field accelerated the growth of microbes. More *Escherichia coli* grew in the cathode, and more *shewanella* grew in the anode. When *Shewanella* and *Escherichia coli* were used as the culture, the activation resistance accounted for a large proportion of the total resistance, and the activation resistance with *Shewanella* was much less than that with *Escherichia coli*. The anode capacitance with *Shewanella* was higher than that with *Escherichia coli*, indicating that the anode capacitance is negatively correlated with the resistance.

Keywords: Electrochemistry analysis; *Shewanella*; *Escherichia coli*; Direct-current electric field; Bioelectrochemical system

1. INTRODUCTION

Bioelectrochemical system (BES) is a new technology for wastewater treatment and resources or valuable energy can be recovered at the same time, and has received great attention by converting the electrons generated from the degradation of organic matters directly into electrical current [1, 2]. BES combines microbial metabolism with electrodes and circuits through extracellular electron transfer (EET) [3]. It uses electrochemical active microorganisms (EAMs) or biomolecules, as the catalyst to form current with electron transfer between the electrodes and the microorganisms [4].

In bioelectrochemical systems, hydrogen evolution, oxygen reduction, heavy metal reduction and other various reduction reactions on biological cathodes are combined with the oxidation of organic matter on biological anodes [5, 6]. Exoelectrogenic bacteria, growing on bioanode surfaces, can create current by oxidizing organic matrix and transferring electrodes to the bioanode. Engineering bioelectrochemical systems can make most use of the current created by exoelectrogenic microorganism [1]. Bioelectrochemical system mainly includes microbial fuel cells (MFCs) [7, 8], microbial electrolysis cells (MECs) [9], and microbial desalination cells (MDCs) [10]. In MFCs or MECs, electricity and chemicals can be produced by electrons, which are generated by microorganisms oxidizing the organics in the anode [11]. The use of MFCs as degrading organic compounds and bio-electricity generation has been successfully demonstrated [12].

The bioelectrochemical system is a system that uses the microorganism's electrical activity as reaction energy; thus, exoelectrogens are very important to a bioelectrochemical system. At present, most of the exoelectrogens are heterotrophic bacteria, using organic matter as a matrix; a few are autotrophic bacteria, using H_2, S_0, $S^{2-}$and NH_3 as the matrix [13, 14]. Exoelectrogens include eukaryotic microorganisms and prokaryotic microorganisms, which also include bacteria and archaea [15, 16]. At present, most of the reported exoelectrogens are bacteria, which are distributed in *Proteobacteria*, *Firmicutes*, *Acidobacteria* and *Actinobacteria* [17], the *Shewanella* studied in this paper belong to *gammaproteobacteria*. Moreover, non-exoelectrogens also play an indispensable role in an electrochemical system. In 1911, Potter first discovered that *Escherichia coli* could produce a voltage of 0.3-0.4 V by oxidizing substrates with chemical electronic mediators [18]. In 1931, Cohen obtained a voltage of 0.5 V by using a mixed bacteria system [19]. Non-exoelectrogens also significantly affect the performance of bioelectrochemical systems. In a BES, electrogenic bacteria oxidize organic matter and provide electrons to the anode [20], while different electron acceptors can reduce biologically or abiotically at the cathode according to the function of the system [21, 22]. Exoelectrogens play an important role in BES, and the substrates and inoculum sources affect the types of exoelectrogens and the performance of the BES. The function of the non-exoelectrogens is to compete with the exoelectrogens, which affects the electrolytic performance of the exoelectrogens. Therefore, the activity and electrochemical characteristics of the exoelectrogens and non-exoelectrogens are very important to the operational performance of a BES.

Electric field stimulation is a common method to study the activity and electrochemical characteristics of microorganisms, and is an important topic in the electrochemistry and bioengineering fields. At present, this technology has been preliminarily applied in yeast fermentation system [23], biological nitrogen removal system [24, 25], sewage treatment and soil remediation [26, 27]. Nakanishi *et al*. [23] studied the effect of electric field stimulation on microbial glucose fermentation. The biomass of *saccharomyces cerevisiae* and the metabolite alcohol yield increased by 1~3 times after direct-current stimulation of 10 mA and 100 mA on *saccharomyces cerevisiae* by using platinum wire as an electrode. She *et al*. [28] used 5 V and 10 mA of direct current to stimulate the bacterial enterobacter dissolution for 12 h. The dehydrogenase activity and glucose degradation rate of the cells in the bacteria were 1.98 times and 1.48 times as much as that of the non-exoelectrogens, indicating that the electric field stimulation could promote the growth and metabolism of the bacterial glucose.

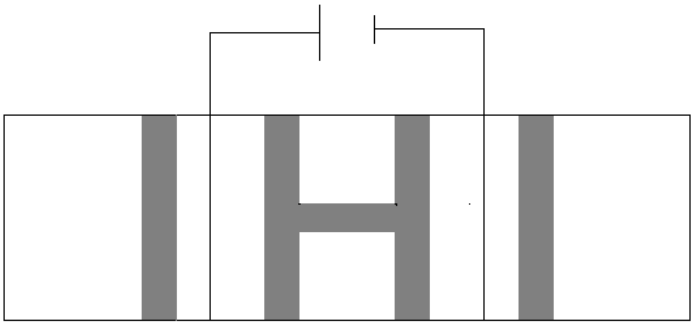
The ethanol yield and biological denitrification increased obviously under the direct-current

electric field, but the research on this topic only stayed at the level of phenomenological observation with no unified understanding of the mechanism. Therefore, *Shewanella* and *Escherichia coli*, which are typical exoelectrogens and non-exoelectrogens were used in this paper to study the growth and electrochemical characteristics in a direct-current electric field. In addition, the interaction between bacterial cells and electrodes and the mechanism for extracellular electron transfer were studied.

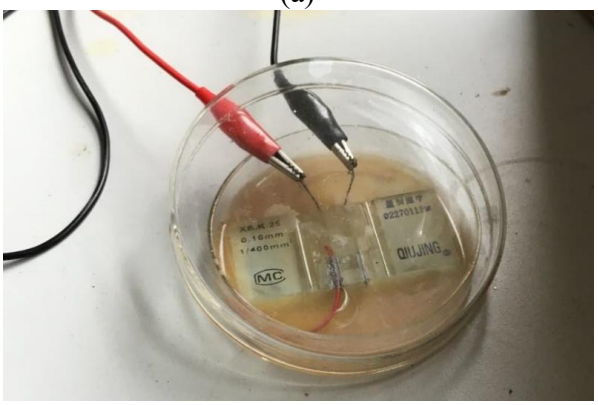
2. EXPERIMENTAL

2.1 Microelectrochemical bioreactor

In this experiment, the blood cell counting board was used as a microelectrochemical bioreactor, and titanium wire (diameter 0.3 mm) used as a collector. Both ends of the electrode were connected to a direct-current voltage (MS305D adjustable direct current power supply, Mai Sheng Power Technology Co., Ltd., China). The culture medium was placed in the groove of the blood cell count board, as shown in Figure 1.



(a)



(b)

Figure 1. Microelectrochemical bioreactor device consisted of blood cell counting board. Titanium wire (diameter 0.3 mm) were used as a collector to connect the anode and cathode: (a) schematic sketch of the device, (b) experimental device

2.2 Strain and culture medium

Escherichia coli and *Shewanella* were used in the experiment. The microorganism was placed

into an Luria–Bertani (LB) liquid medium. Tryptone (Shanghai chemical reagent procurement and supply center chemical plant), 10 g/L; Yeast extract (Chemical Reagent Co., Ltd.), 5 g/L; NaCl (Shanghai Ling Feng Chemical Reagent Co., Ltd.), 10 g/L; pH=7.0.

2.3 Microbial cultivation

Escherichia coli and *Shewanella* were inoculated in a conical bottle containing 100 mL LB liquid medium respectively and then placed in the concentrator (28 ℃, speed 130 r/min, SHA-82A digital bath constant temperature oscillator, Changzhou Wanhe Instrument Manufacturing Co., Ltd.). When the bacteria entered the logarithmic phase, 5 mL was inoculated into the blood count board, and the cover glass was covered and connected to the power supply.

2.4 Experimental methods

2.4.1 Microorganism growth under a microscope

In the experiment, the electric intensity was 0.2 V/cm, the two ends of the electrode were connected to a direct current voltage of 0.2 V, and the distance between the cathode and anode was 1 cm. During the training process, the blood cell count board was taken out every 24 h. After wiping with alcohol cotton, the device was placed under a microscope (XSP series biological microscope, Shanghai Moike Instrument Equipment Co., Ltd.) to observe the growth condition for the *Escherichia coli* and *Shewanella* in different positions and record the position and time. After that, the blood count board was cleaned and sterilized by alcohol cotton and reinserted into the culture dish, and then connected to the power supply. The experimental period was 72 h.

2.4.2 pH changes during the process

The pH of the medium was measured every 2 h. The cycle was used to measure 9 sets of data during 0-16 h. The pH curve was drawn after measuring all the data.

2.4.3 Cyclic voltammetric scanning analysis

A two electrode system was used, the anode was the working electrode and the counter electrode and reference electrode were both the cathode. The cyclic voltammetry was scanned by an electrochemical workstation (Shanghai Chen Hua Instrument Co., Ltd.). The scanning range used for *Escherichia coli* was -1 V-1.2 V, the scanning speed was 100 mV/s; the scanning range for *Shewanella* was -1 V-0.5 V and the scanning speed was 100 mV/s.

2.4.4 Alternating current impedance analysis

A two electrode system was used, the anode was the working electrode and the counter electrode

and reference electrode were both the cathode. The disturbance voltage was 10 mV, and the scanning frequency range was 10^{-5}-10^{5} Hz. After obtaining the Nyquist diagram, the equivalent circuit fitting was carried out through ZSimpWin software. Figure 2 shows an equivalent circuit diagram for the experiment, where R_0 is the ohmic resistance of the BES, and R_1 and R_2 are activation resistance. Q_1 and Q_2 are capacitance.

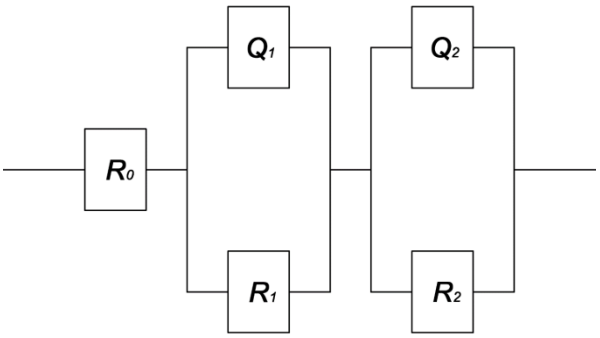


Figure 2. Equivalent circuit diagram used for fitting of Nyquist diagram of *Shewanella* and *Escherichia coli*: R_0 is the ohmic resistance, R_1 and R_2 are activation resistance, Q_1 and Q_2 are capacitance.

3. RESULTS AND DISCUSSION

3.1 Effect of a direct-current electric field on the growth rate of bacteria

As shown in Figure 3 (A), at 0 h, *Escherichia coli* was evenly distributed on the blood count plate, and the number of *Escherichia coli* increased over time. At 24 h, it was observed that the *Escherichia coli* on the cathode increased slightly comparing with anode, but the change was not obvious. At 72 h, the difference between the number of *Escherichia coli* on the cathode and anode was obvious, the *Escherichia coli* on the cathode was much better than that on the anode. According to Figure 3 (B), the growth of the cathode and anode were almost the same under the condition of no direct-current electric field. This illustrates the fact that the difference between the number of *Escherichia coli* on the cathode and anode is due to the existence of the direct-current electric field. Hydrogen produced by cathode helped the cells to transfer electrons, which changed the nature of the solution and was conducive to the growth of *Escherichia coli*, thus accelerating the rate of growth [29].

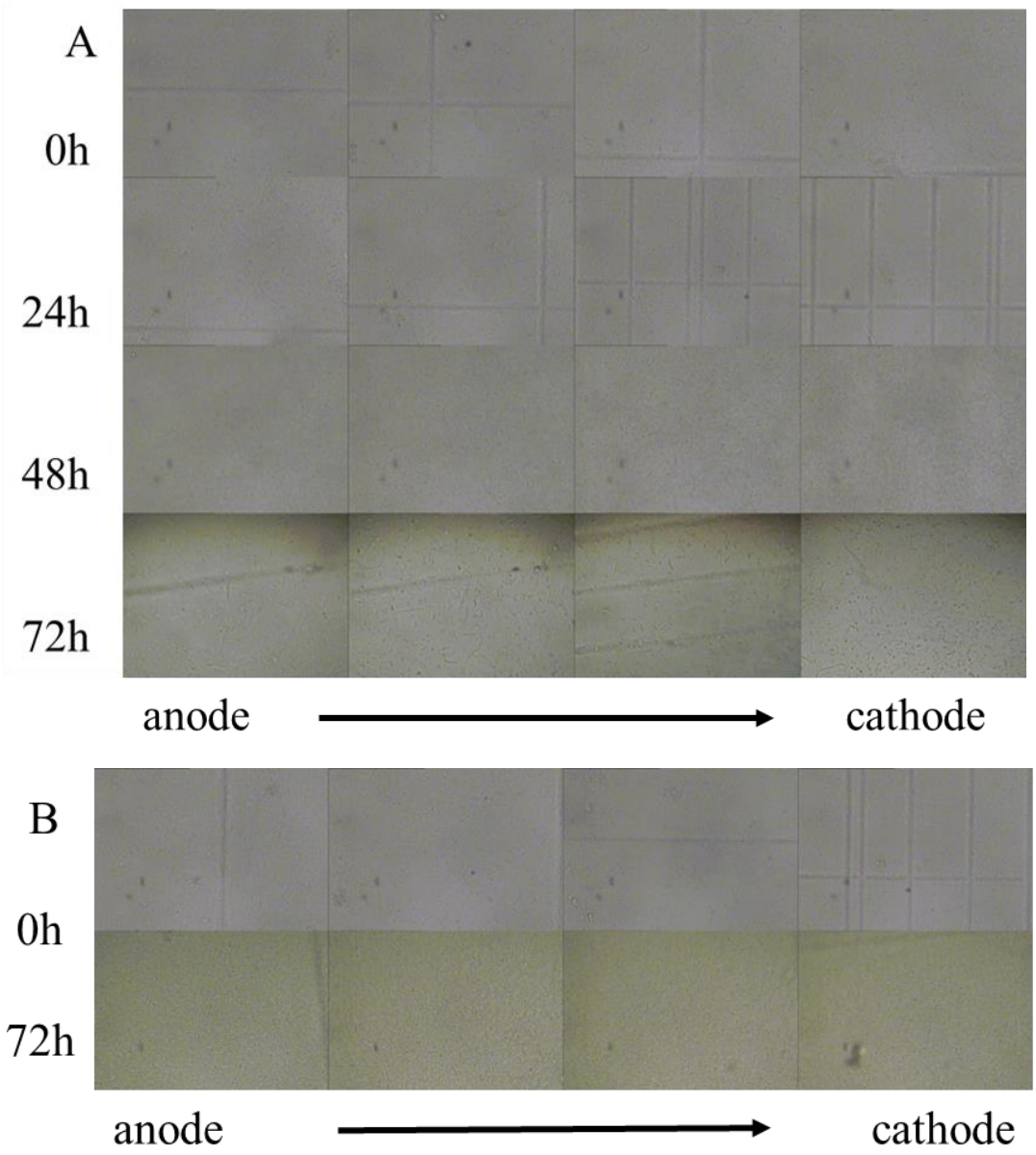


Figure 3. (A) Growth in anode and cathode of *Escherichia coli* versus time (h) in LB liquid medium under 0.2 V/cm direct-current electric field, (B) Growth in anode and cathode of *Escherichia coli* versus time (h) in LB liquid medium without a direct-current electric field

As shown in Figure 4, at 0 h, *Shewanella* was evenly distributed on the blood cell counting chamber. After 72 h, it was observed by a microscope that the growth of the bacteria on the anode was better than that on the cathode. This phenomenon was contrary to the experimental results obtained for the *Escherichia coli*. The reason for this difference is that *Escherichia coli* is a non-exoelectrogen, and *Shewanella* is a kind of exoelectrogen; *Shewanella* used the extracellular electron acceptor to transfer electrons through the cell membrane to the extracellular region, thus transferring along the direction of the electron flow, electron flow from the cathode to the anode, resulting in a difference in the growth on the cathode and anode for the *Shewanella* [30].

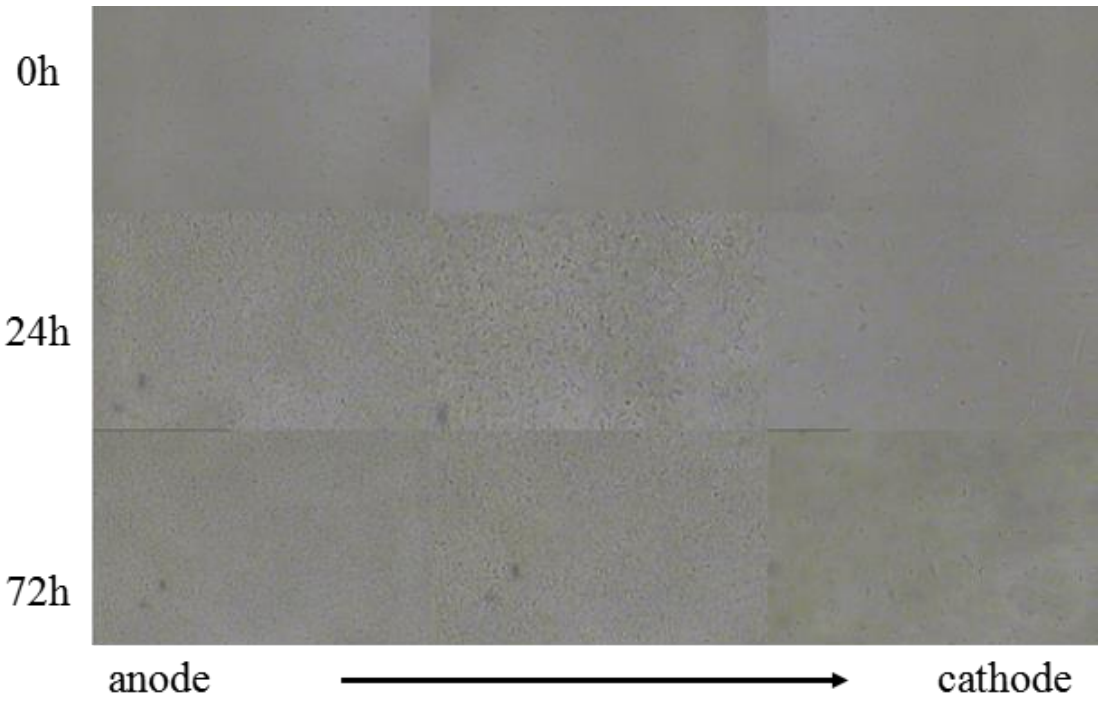
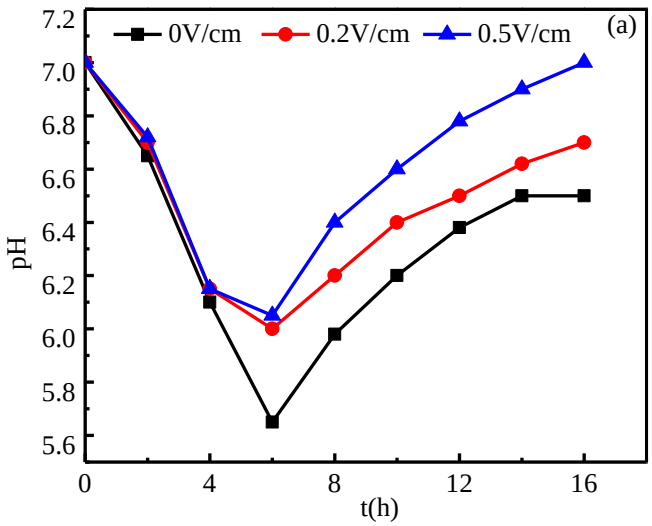


Figure 4. Growth in anode and cathode of *Shewanella* versus time (h) in LB liquid medium under 0.2 V/cm direct-current electric field

3.2. Analysis of the physicochemical properties of the culture

Figure 5 shows the influence of a direct-current electric field on the pH of *Escherichia coli* and *Shewanella*. The pH decreases in 0-6 h and increases gradually within 6 h-16 h, there is a significant difference in the pH curves under 0.5 V/cm and 0 V/cm direct-current electric field.



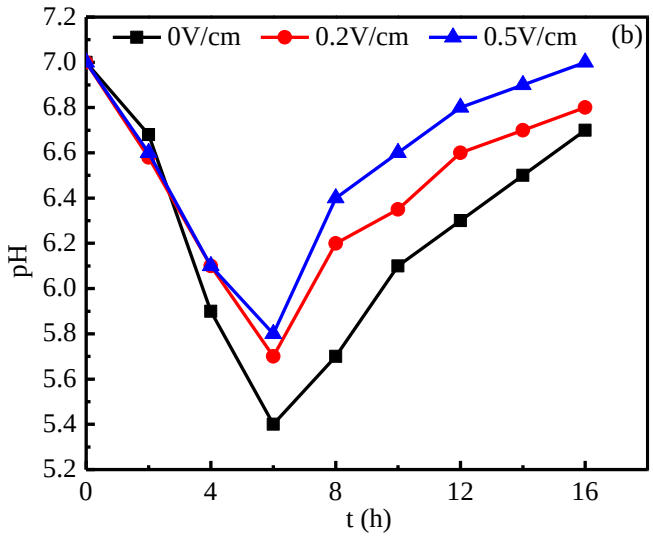


Figure 5. Effect of different direct-current electric field on pH of *Escherichia coli* and *Shewanella* in LB liquid medium at different time: (a) is the *Escherichia coli* and (b) is the *Shewanella*

The pH value decreased in the first six hours because *Escherichia coli* and *Shewanella* used sugar in the culture and then produced preferentially acidic metabolites [29]. When the carbohydrates in the medium were consumed, the bacteria used a high content of amino acids in the culture to carry out nitrogen metabolism, and the alkaline substance produced by the culture increased the pH value of the culture within 6 h-16 h. There is a significant difference in the pH curve under 0.5 V/cm and 0 V/cm direct-current electric field, indicating that the electrochemical reaction did change the pH value of the medium.

3.3. Cyclic voltammetry scanning analysis

Figure 6 shows cyclic voltammetric curves obtained for *Escherichia coli* and *Shewanella*. There were 3 main reactions in the system, of which 2 involved the reaction of adsorbing hydrogen ($H_2O+M+e^-=MH+OH^-$).

The voltage in this experiment was 0.2 V. Under this voltage, both *Escherichia coli* and *Shewanella* were in the stage of the hydrogen adsorption reaction. Because the adsorbed hydrogen only exists on the cathode surface, *Escherichia coli* tended to grow at the cathode. She *et al*. [28] pointed out that the hydrogen-rich environment produced by cathode reaction was the reason for accelerating cell growth. The generated hydrogen can be transferred to the solution through diffusion and convection, and the utilization rate for the microorganisms was high, which led to an increase in the overall growth of the microorganisms. The adsorption of hydrogen was both beneficial to the growth of *Escherichia coli* and *Shewanella*, and the high utilization of hydrogen. In addition, *Shewanella* is an electric bacterium and needs to lose electrons, so *Shewanella* is more likely to grow in the anode, which may cause the difference in the growth of the *Escherichia coli* and *Shewanella*.

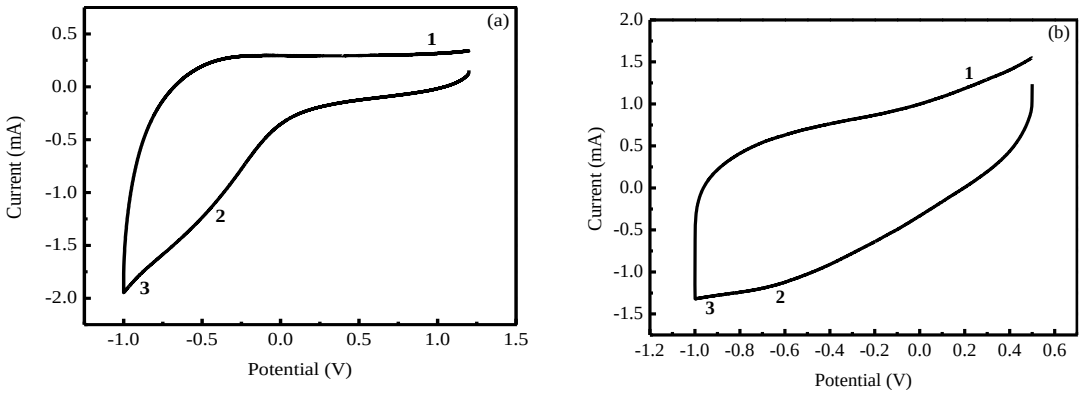


Figure 6. Cyclic voltammetric curve for *Escherichia coli* and *Shewanella* with bio- electro system: (a) the cyclic voltammetric curve of *Escherichia coli* at a scan rate of 100 mV/s in LB liquid medium. (b) the cyclic voltammetric curve of *Shewanella* at a scan rate of 100 mV/s in LB liquid medium

3.4 Alternating current impedance analysis

3.4.1 EIS analysis of Escherichia coli

Figure 7 shows the Nyquist diagram for the electrochemical impedance of *Escherichia coli*. By using the equivalent circuit diagram (Figure 2), the ohmic resistance R_0, activation resistance R_1, R_2 and anode capacitance Q_1 can be obtained, as shown in Table 1.

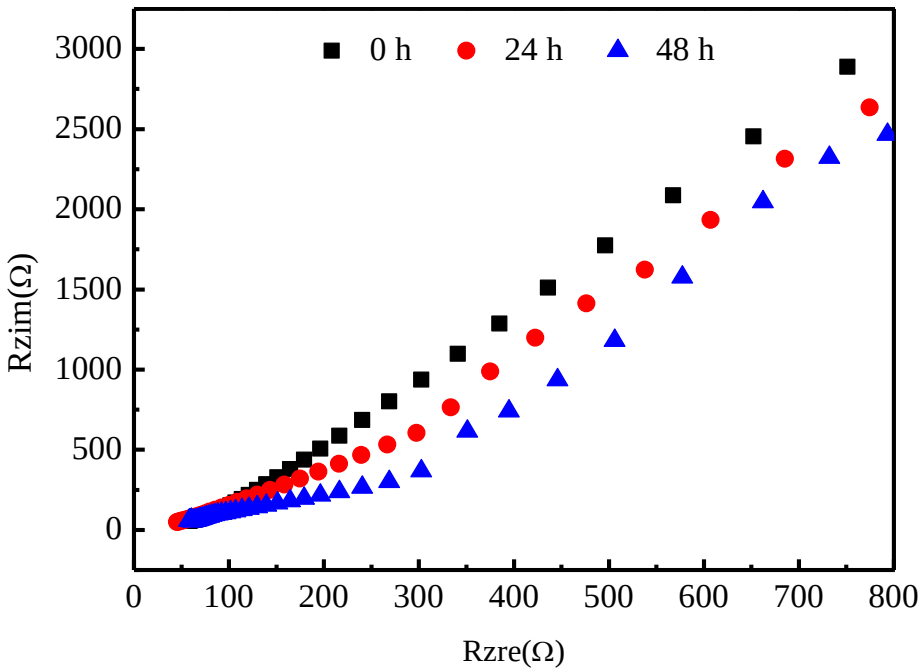


Figure 7. Electrochemistry impedance Nyquist diagram for *Escherichia coli* in LB liquid medium: the voltage was 10 mV and the scanning frequency range was 10^{-5}-10^{5} Hz

Table 1. Impedance parameters for *Escherichia coli* based on equivalent circuit diagram

t/h	R_0/Ω	R_1/Ω	R_2/Ω	R/Ω	Q_1/mF
0	65.53	2770	148.3	2983.8	2.5
24	64.81	2558	122.2	2745.01	2.8
48	64.22	2419	105.7	2588.92	2.93

The ohmic resistance, activation resistance and anode capacitance for *Escherichia coli* were analyzed based on the equivalent circuit fitting data. Figure 8 (a) shows a slight decrease in the ohmic resistance (R_0) after inoculation, but the range was very small and only 1.3 Ω. Because ohmic resistance was mainly related with the electrode material, electrolysis solution, diaphragm and contact of the parts rather than the bacteria [31, 32]. This is related to the composition, structure, size and assembly of the cell. In addition, both the activation resistance R_1 and R_2 decreased with time, which decreased from 2770 Ω to 2419 Ω and from 148.3 Ω to 105.7 Ω, respectively. The activation resistance R_1 accounted for more than 90% of the total internal resistance, indicating that the activation resistance R_1 affected the total internal resistance of the *Escherichia coli*. The change in the resistance showed the domestication of *Escherichia coli*. Figure 8 (b) illustrates the anode capacitance and resistance of the *Escherichia coli*. With the increase in the anode capacitance, the total internal resistance of *Escherichia coli* decreased gradually. After 48 h, the anode capacitance increased by 16.2% and the resistance decreased by 13.2%.

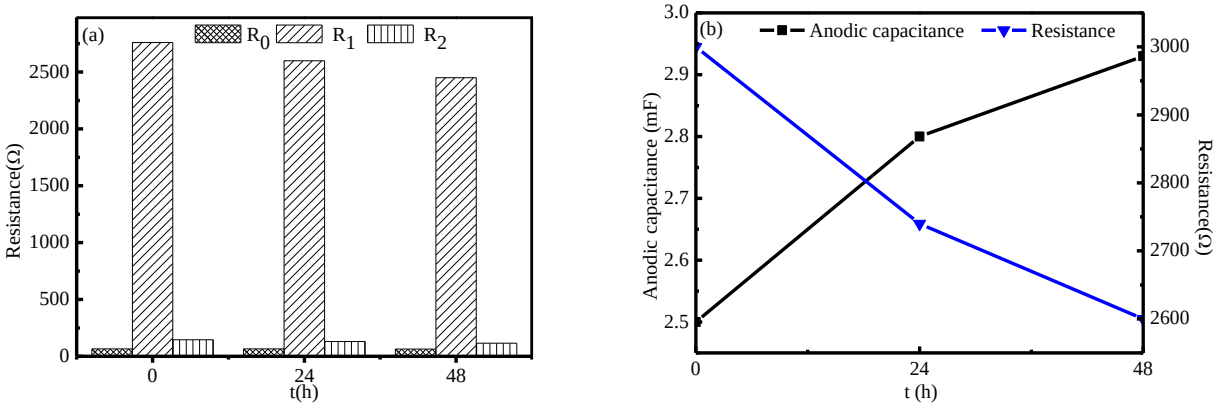


Figure 8. Resistance and anode capacitance of *Escherichia coli* based on the equivalent circuit fitting data: (a) R_0 is ohmic resistance and R_1, R_2 are activation resistance. (b) anode capacitance and total resistance

3.4.2 EIS analysis of Shewanella

Figure 9 shows the Nyquist diagram for the electrochemical impedance test of *Shewanella* within 72 h. The electrochemical impedance data were fitted with the equivalent circuit (Figure 2). The ohmic resistance R_0, activation resistance R_1, R_2 and anode capacitance Q_1 can be obtained, as shown in Table 2.

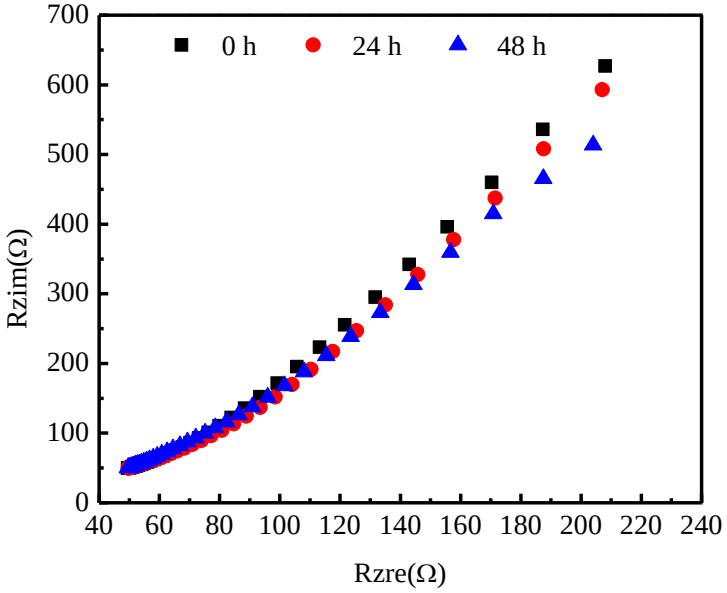


Figure 9. Electrochemistry impedance Nyquist diagram for *Shewanella* in LB liquid medium: the voltage was 10 mV and the scanning frequency range was 10^{-5}-10^{5} Hz

Table 2. Impedance parameters for *Shewanella* based on equivalent circuit diagram

t/h	R_0/Ω	R_1/Ω	R_2/Ω	R/Ω	Q_1/mF
0	35.21	526.8	98.32	660.33	3.4
24	35.09	505.7	87.22	628.01	3.7
48	34.92	443.7	73.66	552.28	4.5

The ohmic resistance (R_0), activation resistance (R_1, R_2) and the anode capacitance for the *Shewanella* are shown in Figure 10. After inoculation, the ohmic resistance decreased slightly by only 0.33 Ω. It was generally considered that the ohmic resistance had not changed significantly. Similarly, we drew that ohmic resistance was related to the structure of BES [31, 32]. The activation resistance R_1 and R_2 of *Shewanella* are listed in Figure 10 (a). It can be seen that both R_1 and R_2 decreased with time, which decreased from 526 Ω to 443 Ω and 98.3 Ω to 73.6 Ω respectively. In addition, R_1 occupied approximately 80% of the total resistance, indicating that R_1 affected the total resistance of the *Shewanella*. The change in the resistance showed the acclimation process of *Shewanella*. The anode capacitance and resistance are shown in Figure 10 (b). These indicated that the anode capacitance increased by 32.4% and the resistance decreased by 16.7% after 48 h.

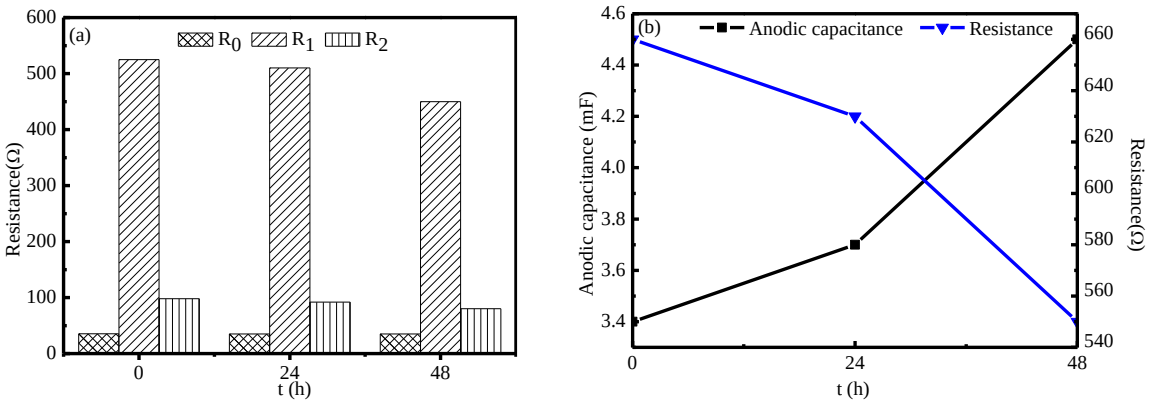


Figure 10. Resistance and anode capacitance of *Shewanella* based on the equivalent circuit fitting data: (a) R_0 is ohmic resistance and R_1, R_2 are activation resistance. (b) anode capacitance and total resistance

3.5 Comparative analysis of Escherichia coli and Shewanella

Figure 11 (a) shows the change in ohmic resistance (R_0), activation resistance (R_1 and R_2) for *Escherichia coli* and *Shewanella* with time. R_0 for the two bacteria did not change with time, and the ohmic resistance of *Escherichia coli* was higher than that of the *Shewanella* [33]. In addition, the R_1 value of *Escherichia coli* and *Shewanella* decreased, and the change in R_1 for *Escherichia coli* was greater than that for *Shewanella*, indicating that the domestication rate for *Escherichia coli* was faster. The R_2 value of *Escherichia coli* and *Shewanella* had a similar trend. Summarily, the activation resistance R_1 and R_2 of *Escherichia coli* and *Shewanella* decreased with time. The activation resistance decreased significantly because the number of *Escherichia coli* and *Shewanella* increased and began to produce electricity [34]. The resistance of *Escherichia coli* was greater than that of the *Shewanella*. The reason for this is that the *Shewanella* can use the extracellular electron acceptor to transfer the electron through the cell membrane to the extracellular region, resulting in a difference in the resistance between the two bacteria. Through electrochemical analysis, the resistance and capacitance characteristics of the *Escherichia coli* and *Shewanella* under the direct-current electric field were studied to determine the mechanism for extracellular electron transport of the bacteria [35].

Figure 11 (b) reflects the anode capacitance of the *Escherichia coli* and *Shewanella* with time. The anodic capacitance of *Shewanella* was larger than that of the *Escherichia coli*, which indicated that the anode capacitance is negatively correlated with the resistance.

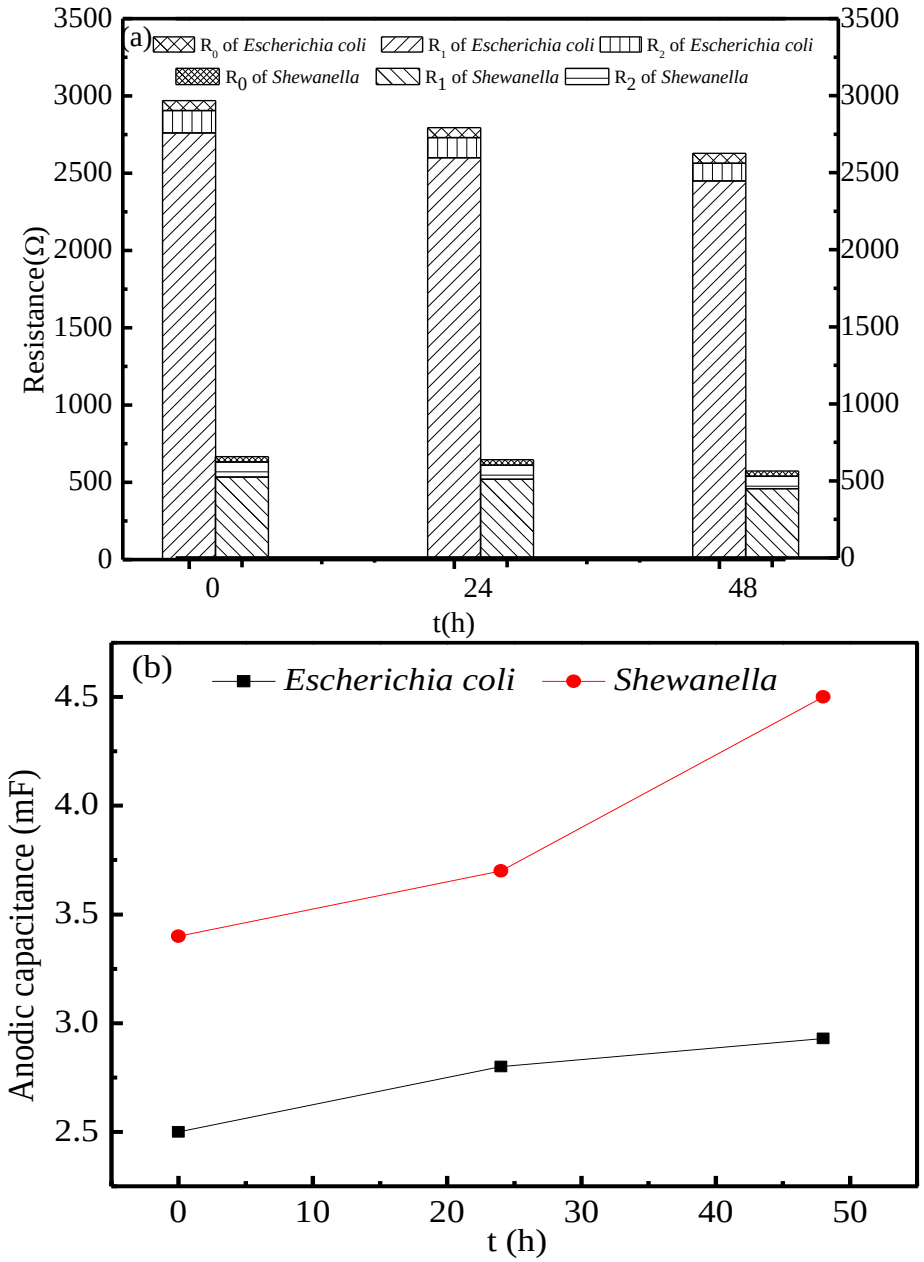


Figure 11. Comparison of the resistance and anode capacitance between *Escherichia coli* and *Shewanella*: (a) R_0 is ohmic resistance and R_1, R_2 are activation resistance; (b) anode capacitance

4. CONCLUSIONS

The microbial activity and electrochemical characteristics of *Shewanella* and *Escherichia coli* under a direct-current electric field were studied. The direct-current electric field can significantly accelerate the growth of microorganisms and have a dramatic influence on the pH value of the medium, resulting in the growth of *Escherichia coli* on the cathode and *Shewanella* on the anode. In addition, the activation resistance (R_1) is determined to constitute a large proportion of the total internal resistance. The activation resistance (R_1 and R_2), ohmic resistance (R_0) and total internal resistance for *Shewanella*

were found to be significantly lower than those for *Escherichia coli*, in contrast to the anode capacitance, indicating that the anode capacitance has a negative correlation with the resistance.

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