Effect of *Geobacter metallireducens* nanowire on electron transfer efficiency in microbial fuel cell

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Abstract: The inhibitory effect of electron mediator 2,6-anthraquinone disulphonate (AQDS) on *Geobacter metallireducens* nanowire in the microbial fuel cell (MFC) was studied. In the culture process of *G.metallireducens* with Fe(OH)₃ as an electron acceptor, the concentration of reduction product Fe (II) in solution without AQDS was higher than that with AQDS after 10 days, due to the formation of microbial nanowires. The effects of nanowire on electron transfer efficiency and electrical current characteristic were studied using a double chamber MFC reactor. The transfer efficiency between biofilm and electrodes was increased by nanowire, which increased the maximum output voltage of MFC was 442 mV. The nanowire biofilm electrode had a bigger cyclic voltammetry curve peak, smaller activation resistance, and a stronger current response signal through electrochemical measurement, which indicates that the nanowire enhanced the electrochemical activity of the electrode.

Key words: Microbial fuel cell; Electron mediator; Nanowire; Electron transport;

1 Introduction

Microbial fuel cells (MFC) can achieve both purposes of wastewater treatment and electrical energy production, through converting the chemical energy of organic compounds into electrical energy using microorganisms as catalysts (Hu et al.,2017). Because of the advantages of extensive substrates sources, easy reaction conditions, efficient processing capability, MFC have been widely researched to treat waste-water such as organic (Asefi et al.,2019; Priya et al,2019), cyanide (Wu et al,2019), and high-price metal (Li et al.2019). At present one of the limitations of MFC technology is the low output power, and various means have been used to improve the efficiency of electron transfer (S. Kalathil et al, 2018). For instance, the development of MFC electrode materials includes carbon felt (Feng et al, 2019), graphite foam (Chen et al, 2019), metal material (Liu et al, 2018) and natural biomass electrode material; Modification of electrode materials, such as graphene-modified electrode (Lin et al, 2019), non-metallic (Bajracharya et al, 2019) and metal element doped electrode (Palanisamy et al, 2019), carbon nanotubes modified electrode (Delord et al, 2019), conductive polymer (polyaniline (Zhai et al, 2019), polypyrrole (Anders et al, 2009), silane coupling agent) modified electrode materials; Adding electronic mediators such as humic acid (Anders et al, 2009), anthraquinone compounds (Tang et al, 2017), mercaptan-containing molecules, cysteine, melanin (Costa et al, 2009) to improve the electron transfer efficiency. Among them, quinone compounds are the preferred electronic medium in the process of biodegradation, which can not only transfer electrons in the microbial reaction and act as the REDOX medium but also the

hydroquinone decomposition products can further participate in the subsequent pure chemical reaction. Many studies have confirmed that AQDS acts as an electron shuttle, favoring the transfer of electrons between microorganisms and organic matter (Lovley D R et al, 1996; Luu et al, 2003), and also between electron donors and electron acceptors (Yang et al, 2009). Evidence from other studies suggests that only a small amount of AQDS (0.01–0.20 mM AQDS) is enough to “shuttle” electrons between microorganisms and oxides, nanocilia-like PANI grew uniformly on the surface of rGONF under the guidance of the low-level AQDS to improve successfully the specific capacitance and electrochemical stability of asymmetric supercapacitor (ASC) (Du et al, 2021), moreover positive effect that in the reductive dissolution of As(V)/Fe(III) during sediment supplementation with lower (0.05 mM) compared to the high level (0.10 mM) of AQDS, whereas an inhibitory effect resulting from even higher (1.00 mM) level of AQDS (Chen et al, 2017). However, at present, AQDS as an electronic medium to improve the efficiency of MFC electron transfer is less influential (Santoro et al, 2017). Nevertheless, how the electron shuttle compounds affect MFC electron transfer behavior and processes of the fabricate nanowire by microorganisms remains unknown.

The theory of nanowire transfer in MFC refers to that some microorganisms produce a conductive pilin protein (nanowires) in the process of growth and metabolism, and the electrons can be transferred to the anode electrodes through nanowires (N. Alves et al, 2016). The microbes are connected to the electrodes through nanowires, thus releasing the transmission constraints between cell

membranes and electrodes. Not only the biofilm on the surface of the electrode can transfer electrons to the electrodes but also the outer microbes can transfer electrons through nanowires, which can increase the output current of MFC (Numfon et al, 2016). At present, many bacteria have been observed such as *Geobacterium*, *Schwarzschild*, *Shewanella oneidensis*, *Synechocysti*, *Pelotomaculum thermopropionicum* producing nanowires (Ganesh et al, 2017). Among them, the *Geolimetal Metallireducens* bacteria are very efficient in remote extracellular electron transfer, which can effectively explain the bridging relationship between cytoplasmic electron donor and extracellular receptor, and better demonstrate the influence of nanowires electronic media (Marisa et al, 2020).

To improve the electronic transfer efficiency of microbes and the output voltage of MFC, the generation mechanism of *Geobacter metallireducens* nanowire by the electronic mediator (AQDS) has been studied. Meanwhile, the effect of nanowires on the electron transfer speed, the metal reduction efficiency, and output voltage was explored with a double chamber MFC device. The effect of nanowire on MFC efficiency by electrochemical means was examined. This study investigated the inducing effect of insoluble electron acceptors on nanowires generation, which will be very important for understanding MFC, soil remediation, and solid-phase fermentation using solid-phase as electron acceptor.

2 Materials and methods

2.1 Growth medium and microorganism

Geobacter metallireducens (*G.metallireducens*), a German Collection of

Microorganisms and Cell Cultures preserved strain (DSMZ 7210, ATCC 53774), was used as an experimental strain. The composition of the synthetic medium used in the study was: sodium acetate (10 mmol/L), KCl (0.1 g/L), NH₄Cl (0.2 g/L), NaH₂PO₄ (0.6 g/L), NaHCO₃ (2.5 g/L), Wolfes' trace mineral solution (10 mL/L) and Wolfes' vitamin solution (10 mL/L), pH 6.8~7.0. The gas N₂-CO₂ (8:2) was used to remove oxygen from the growth medium, then the growth medium was separated into anaerobic culture tubes to be sterilized at 121 °C for 15 min.

2.2 MFC construction and operation

The structure of the double chamber MFC reactor is shown in Fig.1. The reactor consists of 250 mL cathode and 250 mL anode chamber connected by a proton exchange membrane (Nafion-117, DuPont). The electrode was a pure graphite electrode with a surface area of 75 cm², which was cleaned by 1.0 mol/L HCl and 1.0 mol/L NaOH to remove impurity ions and adsorbed microbes respectively. The mixed liquid with a ratio of bacterial solution to anolyte is 1:9 was added to the MFC anode chamber, and the mixture gas N₂-CO₂ (8:2) was slowly passed through to remove oxygen. The catholyte was added to the cathode chamber, and the air was continuously insufflated to maintain dissolved oxygen concentration. The anolyte contained KCl 0.1 g/L, NH₄Cl 0.2 g/L, NaH₂PO₄ 0.6 g/L, NaHCO₃ 2.5 g/L, NaCl 2.9 g/L, Wolfes' vitamin solution 10 mL/L, Wolfes' mineral solution 10 mL/L, electron donor NaAC 10 mmol/L. And the composition of catholyte is KCl 0.1 g/L, NH₄Cl 0.2 g/L, NaH₂PO₄ 0.6 g/L, NaCl 2.9 g/L, Tris-HCl (adjust pH to 7.0). 50 μmol/L and 0 μmol/L of AQDS will be adding to the corresponding MFC and

111 set 3 parallels for each concentration gradient under the experiment, respectively. The
 112 cell voltages were measured every 5 min using a data acquisition system (RBH8223H,
 113 Ribohua Co.) across an external resistance of 510 Ω . At the end of the MFC operation
 114 cycle, 10 mmol/L electron donor NaAc was added into the anode for the next cycle.

Fig 1. The structure of the double chamber MFC reactor

118 2.3 Electrochemical tests

119 Electrochemical measurements include cyclic voltammetry (CV),
 120 electro-chemical impedance spectroscopy (EIS) and linear sweep chronoamperometry
 121 (LSC) were carried by CHI660D electrochemistry tester with a three-electrode
 122 electro-chemical system. The electrochemical tests were researched using the MFC
 123 anode as the working electrode, a platinum electrode as a counter electrode, and a
 124 saturated calomel electrode as the reference electrode. In a mixed buffer (10 mmol/L
 125 phosphates +10 mmol/L NaAc), CV spectra were recorded at the scanning rate of 5
 126 mV/s. And LSC spectra were recorded at the scanning voltage range of 0.8~0 V,

scanning rate of 0.01 mV/s. In a 10 mmol/L $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) + 0.1 mol/L KCl solution, EIS was measured for MFC anode in a frequency range of 0.1Hz~100kHz with an AC signal of 5 mV amplitude.

2.4 Scanning electron microscopy

The microbial structure of enriched bacteria and biofilm on the MFC anode surface were examined using scanning electron microscopy (JSM-7001F, JEOL Ltd). The electrodes were fixed with 2.5% glutaraldehyde at 4 °C for 2~4 h, and rinsed 3 times with 100 mmol/L sodium cacodylate buffer (pH 6.8) for 10 min. Then fixed electrode was dehydrated gradually with 50%, 70%, 80%, and 90% ethanol for 15 ~ 20 minutes, and gently washed twice with pure isoamyl acetate for 15 min. The samples were placed in the critical evaporator to be dried for 4 h, and the dried samples were coated with gold prior to SEM analysis.

3 Results and discussion

3.1 Generation of bacterial nanowires

Metal reducing bacteria take metal oxides as the final electron acceptor to form a complete electron transfer chain through extracellular electron transfer, which is the metal reduction process by microbes. Ineffective extracellular electron transport will impact intracellular electron transport negatively, which prevents bacterium to synthesize adenosine triphosphate (ATP), the energy source of activities for metabolism and growth (Okamoto et al, 2014). The low bioavailability of electron acceptors leads to inefficient extracellular electron transport and inactive vital movement of bacteria. And the efficiency of bioleaching and environmental

remediation is reduced (Liu et al, 2014).

Electron mediators act as electronic transport carriers in dissimilatory reduction system, which can accelerate the electron transfer rate between microbes and solid oxides. For example, intracellular reduction cytochromes lose electrons to free AQDS (9,10-anthraquinone-2,6-sulfonate) to form AHQDS molecules. The AHQDS containing electrons moved to the surface of $\text{Fe}(\text{OH})_3$ for transmitting electrons. Then by losing electrons AHQDS convert back to AQDS to transport electrons for the next round (Pruetsaji et al, 2018; Han et al, 2017). The process is shown in Fig. 2a. The equation is as following:

In recent years, it has been found that *G. metallireducens* can generate conductive nanowires similar to additional pili, due to the lack of soluble electron acceptors (Sébastien et al, 2017). Microbes transfer electrons far away from the cell surface through highly efficient conductive nanowires, thereby, microbes break through the electron transfer restriction which requires direct contact with solid electron acceptors or adds electronic mediators (Mónica et al, 2016). Microbial nanowires are an effective way that microorganisms evolve to improve the efficiency of extracellular electron transfer (Toshiyuki et al, 2018). The process is shown in Fig. 2b.

Fig.2 Electron transfer pathways for *G. metallireducens* during the reduction process

The growth of nanowires of reducing bacteria was inhibited by adding AQDS, and the effect of nanowires on the reduction efficiency was compared. Fig.3 shows nanowire production on the reduction efficiency of $\text{Fe}(\text{OH})_3$ (100mM) with an initial concentration of electron donor (NaAc) 10 mM.

Fig.3 Effect of AQDS on the dissimilatory reduction of $\text{Fe}(\text{OH})_3$ (100mM)

It can be seen from Fig.3 that the reduction of $\text{Fe}(\text{OH})_3$ can be accelerated by AQDS in a short time (within 10 days) after inoculating microbes in the culture

178 system. Compared with using $\text{Fe}(\text{OH})_3$ as a microbial electron acceptor directly, the
 179 addition of AQDS can avoid the extracellular electron transfer process. The AQDS
 180 would act as an electron carrier to shuttle between the cell membrane and metal
 181 hydroxide, thus accelerating the dissimilatory reduction of $\text{Fe}(\text{OH})_3$. However, the
 182 accelerating effect of AQDS on microbial reduction was not significant after a long
 183 reaction time (10 days). Microbes grow nanowires without AQDS for a long time,
 184 which promoting the electron transfer exceeds AQDS in the reduction process (the
 185 concentration of reduction product $\text{Fe}(\text{II})$ is higher than that with AQDS). The
 186 addition of AQDS hinders the growth of microbial nanowires. Although the electron
 187 transfer is accelerated in the initial stage, the upper bound of transfer efficiency is
 188 worse than that of nanowires. Fig. 4 shows the microbial structure of
 189 *G.metallireducens* in the solution (reaction for 20 days). It can be observed that the
 190 addition of electronic mediator AQDS inhibit the production of nanowires (Fig.4a),
 191 while in a solution without electronic mediator AQDS, *G.metallireducens* is
 192 stimulated to produce nanowires (Fig.4b).

Fig.4 The microbial structure of *G.metallireducens* in the solution (reaction for 20 days)

3.2 Effect of nanowires in the MFC

To accurately research the effect of *G.metallireducens* nanowire on electron transfer efficiency, MFC is used to study the electron transfer process. Anodic graphite electrode was used as the final solid electron acceptor instead of Fe(OH)₃. Electrons reduce dissolved oxygen by anodic graphite electrode-external circuit-cathode, which maintaining the electron transfer process from microbes to solid electron receptors. By recording the current value of the external circuit, the process of electron transfer can be accurately reflected. The reaction process of MFC is as following:

The electricity voltage output obtained from the MFC inoculating *G.metallireducens* (10%) is shown in Fig.5(a). As can be seen from Fig.5(a), MFC can be activated quickly with 50μm AQDS, and the maximum voltage of MFC can be stabilized at about 500 mV. MFC was activated slow Without AQDS, and the maximum voltage of MFC was stabilized at 398 mV. Adding electronic mediator AQDS in starting period of MFC can significantly increase the output voltage of MFC, and AQDS acts as a transfer electron role between cells and solid electron acceptors. However, electron transfer efficiency of MFC without AQDS is low, due to the incomplete nanowires.

Fig.5 Electron current in a microbial fuel cell by *G.metallireducens*.

(a) In the microbial fuel cell, graphite electrode served as the sole electron acceptor, acetate as electron donor, 10% inoculum. (b) Voltage output of MFC which solution replaced after operation stability (10d)

To exclude the effect of AQDS on MFC, and study the relationship between microbial nanowire with electron transfer, the solid electron acceptor (anode graphite electrode) and the culture solution were separated. The new acetate solution was used to replace the culture solution in the anode chamber, the graphite electrode which formed the biofilm was retained, and then the MFC was reconnected. The Voltage output of the MFC solution was replaced after operation and stability were indicated in Fig.5(b). The output voltage of MFC without AQDS has a slight change, and the maximum output voltage was 442 mV. However, due to the removal of AQDS, the maximum output voltage of MFC with AQDS decreased significantly from 498 mV to 321 mV. The results showed that the electron transfer efficiency of nanowire biofilm is higher than that of biofilm without nanowires.

Fig.6 shows MFC anode biofilm with AQDS (Fig.6a) and without AQDS (Fig. 6b). It can be observed by comparing the figure that the *G.metallireducens* did not

234 generate nanowires in biofilm with AQDS. Microbes adhere to the graphite fibers of
235 electrodes such as granules. And the *G.metallireducens* generated nanowires in
236 biofilm without AQDS. Nanowires are interlaced to connect microbes in biofilm, or
237 microbes adhere to graphite fibers through nanowires.

238
239 Fig.6 The microbial structure of *G.metallireducens* in anode biofilm

240 Fig.7 illustrates the electron transfer in the biofilm of the MFC electrode. The
241 *G.metallireducens* of biofilm did not generate nanowires in MFC with electronic
242 mediators, microbes in the inner layer of biofilm transmit electrons to the electrodes
243 through direct contact, microbes in the outer layer of biofilm or solution transport
244 electrons to the surface of electrodes through electronic mediators. The
245 *G.metallireducens* of biofilm would generate nanowires in MFC without electronic
246 mediators, microbes in the inner and outer layers of the biofilm are connected by
247 nanowires to transfer electrons to the electrodes efficiently (Telma et al, 2015).

Fig.7 Electron transfers pathways for *G. metallireducens* in MFC.

3.3 Electrochemical characteristics of nanowire biofilm in MFC

Fig. 8. (a) CV of the ordinary biofilm electrode (dominated biofilms at constant electron mediator concentration ($50\mu\text{mol L}^{-1}$ AQDS)) and nanowire biofilm electrode (dominated biofilms electrodes at the without electron mediator (AQDS)) in a mixed buffer (10 mmol/L phosphate+10 mmol/L NaAC) at scan rate of 5 mV/s, respectively; (b) EIS of the ordinary biofilm electrode and

nanowire biofilm electrode in $\text{K}_3\text{Fe}(\text{CN})_6$ (5 mmol/L)+ $\text{K}_4\text{Fe}(\text{CN})_6$ (5 mmol/L)+KCl (0.1 mol/L) solution (Frequency range of 0.1 Hz to 100 kHz with an AC signal of 5 mV amplitude); (c) LSC plots of the ordinary biofilm electrode and nanowire biofilm electrode (0.8~0 V and with a scan rate of 0.01 mV/s).

Cyclic voltammetry (CV), electrochemical impedance spectrum (EIS), and linear step chronoamperometry (LSC) were used to characterize the electrochemical activity of the biofilm electrode. Fig.8a showed the CV profiles of ordinary biofilm electrodes (with AQDS) and nanowire biofilm electrodes (without AQDS). In a mixed buffer solution, a pair of redox peaks were observed clearly in the respective electrodes. But the nanowire biofilm electrodes showed higher CV peak current and smaller cathodic/anodic peak separation, indicating nanowire could increase the electrochemical activity of electrodes. The nanowire can accelerate the electron transfer between the microbes with the electrodes. The EIS plot consists of a semicircle (high-frequency region) and a straight line (low-frequency region). The diameter of the semicircle represents charge-transfer resistance (R_{ct}), which value is negatively correlated with the electron transfer speed. As shown in Fig.8b, the semicircle of nanowire biofilm electrodes is smaller than that of ordinary biofilm electrodes, suggesting lower interfacial charge-transfer resistance (R_{ct}) and a greater charge transfer rate. Linear step chronoamperometry is used to detect the electron transfer activity of the electrode. The results are shown in Fig.8c. Nanowire biofilm electrodes showed a stronger and broader current response than ordinary biofilm electrodes, which indicates nanowire biofilm electrodes could conduct stronger

current under the same voltage condition. Here, it seemed to limit the metabolism of the biofilms has a similar impact as added to AQDS. This could be a consequence of the dominating electron mediator of the AQDS, changed diffusion regimes, or increased charge transfer resistance due to reduced metabolic activity. The above results suggested that the nanowire could significantly enhance the charge transfer rate and electrode current signal. The output voltage and performance of the MFC were improved.

3 Conclusion

Compared with using $\text{Fe}(\text{OH})_3$ as a microbial electron acceptor directly, the addition of AQDS can accelerate the dissimilatory reduction of $\text{Fe}(\text{OH})_3$ in a preliminary stage. But the addition of AQDS hinders the growth of microbial nanowires, limits the upper bound of electron transfer efficiency. The accelerating effect of AQDS on microbial reduction was not significant after a long reaction time. By comparing microbial structure on electrode biofilm, the *G.metallireducens* did not generate nanowires with AQDS, microbes adhere to the graphite fibers of electrodes such as granules. And the *G.metallireducens* generated nanowires without AQDS. Nanowires are interlaced to connect microbes in biofilm, or microbes adhere to graphite fibers through nanowires. Microbial nanowires could significantly enhance the charge transfer rate and improve the performance of the MFC.

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References

[1]A.Divya Priya,Y.Pydi Setty (2019) Cashew apple juice as substrate as substrate for microbial fuel cell.Fuel,246:75-78. Doi:10.1016/2019.02.100.

[2]Anders Thygesen, Finn Willy Poulsen, Booki Min, Irini Angelidaki, Anne Belinda Thomsen (2009) The effect of different substrates and humic acid on power generation in microbial fuel cell operation. Bioresource Technol,100(3):1186-1191. Doi:10.1016/ 2008.07.067.

[3]A. Okamoto, R. Nakamura, K. H. Nealson, K. Hashimoto(2014) Bound Flavonoid Model Suggests Similar Electron-Transfer Mechanisms in Shewanella and Geobacter. ChemElectroChem,1:1808-1812. Doi:10.1002/201402151.

[4]Bahareh Asefi, Shiue-Lin Li, Henry A. Moreno, Viviana Sanchez-Torres, Anyi Hu, Jiangwei Li,Chang-Ping Yu (2019) Characterization of electricity production and microbial community of food waste-fed microbial fuel cells. Process Saf Environ,12-5:83-91. Doi:10.1016/2019.03.016.

[5]Brigitte Delord, Wilfrid Neri, Karen Bertaux, Alain Derre, Isabelle Ly, Nicolas Mano, Philippe Poulin (2017) Carbon nanotube fiber mats for microbial fuel cell electrodes. Bioresource Technol,243:1227-1231. Doi:10.1016/2017.06.170.

[6]Carlo Santoro, Catia Arbizzani, Benjamin Erable, Ioannis Ieropoulos (2017) Microbial fuel cells: From fundamentals to applications. A review. J Power Sources, 356:225-244. Doi:10.1016/2017.03.109.

322 [7]Dan-Dan Zhai, Zhen Fang, Hongwei Jin, Ming Hui, Christopher Joseph
323 Kirubakaran, Yang-Yang Yu, Yang-Chun Yong (2019) Vertical alignment of
324 polyaniline nanofibers on electrode surface for highperformance microbial fuel cells.
325 Bioresource Technol, 288:121499. Doi: 10.1016/2019.121499.

326 [8]Guiyu Liu, Jinlong Liu, Beibei Liu, Chunming Yang, Dong Qian, Junhua Li (2018)
327 Ketjen black carbon supported CoO@Co-N-C nanochains as an efficient
328 electrocatalyst for oxygen evolution. Int J Hydrogen Energy, 43(51):22942-22948.
329 Doi:10.1016/2018.10.142.

330 [9]Gowthami Palanisamy, Ho-Young Jung, T. Sadhasivam, Mahaveer D. Kurkuri,
331 Sang Chai Kim, Sung-Hee Roh (2019) A comprehensive review on microbial fuel cell
332 technologies: Processes, utilization, and advanced developments in electrodes and
333 membranes. J Clean Prod, 221:598-621. Doi:10.1016/2019.02.172.

334 [10]Ganesh Dattatraya Saratale, Rijuta Ganesh Saratale, Muhammad Kashif Shahid,
335 Guangyin Zhen, Gopalakrishnan Kumar, Han-Seung Shin, Young-Gyun Choi,
336 Sang-Hyoun Kim (2017) A comprehensive overview on electro-active biofilms, role
337 of exoelectrogens and their microbial niches in microbial fuel cells (MFCs).
338 Chemosphere, 178:534-547. Doi:10.1016/2017.03.066.

339 [11]H Wu, Yi Feng, Hr Li, Hj Wang, Jj Wang (2018) Co-metabolism kinetics and
340 electrogenesis change during cyanide degradation in a microbial fuel cell. RSC Adv,
341 8:40407-40416. Doi:10.1039/C8RA08775J.

342 [12]Jianjun Hu, Quanguo Zhang, Duu-Jong Lee, Huu Hao Ngo (2018) Feasible use of
343 microbial fuel cells for pollution treatment. Renew Energ, 129:824-829.

- 344 Doi:10.1016/-2017.02.001.
- 345 [13]Luye Chen, Youzhi Li, Jiani Yao, Gaoming Wu, Bin Yang, Lecheng Lei, Yang
346 Hou, Zhongjian Li (2019) Fast expansion of graphite into superior three-dimensional
347 anode for microbial fuel cells.J Power Sources,412:86-92. Doi:10.1016/2018.11.033.
- 348 [14] Lovley D R , Bluntharris E L , Ejp P , et al (1996) Humic substances as electron
349 acceptors for microbial respiration[J]. Nature. 382(6590):445-448.
350 DOI:10.1038/382445a0.
- 351 [15]Luu, YS., Ramsay, J.A. (2003) Review: microbial mechanisms of accessing
352 insoluble Fe(III) as an energy source.World Journal of Microbiology and
353 Biotechnology, 19: 215–225. Doi.org/10.1023/A:1023225521311.
- 354 [16]Meng Li, Shaoqi Zhou, Yuting Xu (2019) Performance of Pb(II) reduction on
355 different cathodes of microbial electrolysis cell driven by Cr(VI)-reduced microbial
356 fuel cell, J Power Sources,418:1-10.Doi: 10.1016/2019.02.032.
- 357 [17]M.C. Costa, S. Mota, R.F. Nascimento, A.B. Dos Santos (2010)
358 Anthraquinone-2,6-disulfonate (AQDS) as a catalyst to enhance the reductive
359 decolourisation of the azo dyes Reactive Red 2 and Congo Red under anaerobic
360 conditions.Bioresource Technol,101(1):105-110. Doi:10.1016/2009.08.015.
- 361 [18]Mónica N. Alves, Ana P. Fernandes, Carlos A. Salgueiro, Catarina M. Paquete
362 (2016) Unraveling the electron transfer processes of a nanowire protein from
363 *Geobacter sulfurreducens*.BBABioenergetics, 1857(1):7-13.
364 Doi:10.1016/2015.09.010.
- 365 [19]Numfon Eaktasang, Christina S. Kang, Heejun Lim, Oh Sung Kwean, Suyeon

- 366 Cho, Yohan Kim, Han S. Kim (2016) Production of electrically-conductive nanoscale
367 filaments by sulfate-reducing bacteria in the microbial fuel cell. *Bioresource*
368 *Technol*,210:61-67. Doi:10.1016/2015.12.090.
- 369 [20]Marisa R. Ferreira, Tomás M. Fernandes, Carlos A. Salgueiro (2020)
370 Thermodynamic properties of triheme cytochrome PpcF from *Geobacter metallired-*
371 *ucens* reveal unprecedented functional mechanism. *BBA – Bioenergetics*, 1861(11);,
372 148271. Doi:10.1016/2020.148271.
- 373 [21]Mónica N. Alves, Ana P. Fernandes, Carlos A. Salgueiro, Catarina M. Paquete
374 (2016) Unraveling the electron transfer processes of a nanowire protein from
375 *Geobacter sulfurreducens*. *BBA Bioenergetics*, 1857(1):7-13.
376 Doi:10.1016/2015.09.010.
- 377 [22]Pruetsaji Winaikij, Paiboon Sreearunothai, Korakot Sombatmankhong (2018)
378 Probing mechanisms for microbial extracellular electron transfer (EET) using
379 electrochemical and microscopic characterisations. *Solid State Ionics*,320:283-291.
380 Doi:10.1016/2018.07.001.
- 381 [23]Rui Han, Xiaomin Li, Yundang Wu, Fangbai Li, Tongxu Liu (2017) In situ
382 spectral kinetics of quinone reduction by c-type cytochromes in intact *Shewanella*
383 *oneidensis* MR-1 cells. *Colloids and Surfaces A*,520:505-513.
384 Doi.10.1016/2017.02.023
- 385 [8]S. Kalathil, S. A. Patil, D. Pant (2018) Encyclopedia of Interfacial Chemistry. In: K.
386 Wandelt (Ed). Elsevier, Oxford, pp:309-318.
- 387 [24]S. Bajracharya, A. ElMekawy, S. Srikanth, D. Pant (2016) Cathodes for microbial

388 fuel cells Microbial Electrochemical and Fuel Cells.Woodhead Publishing,2179-213.

389 [25]Sébastien Duperron (2017) 3-Symbiosis and Other Functions, Sébastien
390 Duperron(-Ed).Microbial Symbioses,Elsevier, 45-59.

391 [26]Toshiyuki Ueki, Kelly P. Nevin, Amelia-Elena Rotaru, Li-Ying Wang, Joy E.
392 Ward, Trevor L. Woodard, Derek R. Lovley (2018) Geobacter Strains Expressing
393 Poorly Conductive Pili Reveal Constraints on Direct Interspecies Electron Transfer
394 Mechanisms.MBio, 9: 01273-01218. Doi: 10.1128/01273-18.

395 [27]Telma C. Santos,Marta A. Silva,Leonor Morgado,Joana M. Dantasa,Carlos A.
396 Salgueiro (2015) Diving into the redox properties of Geobacter sulfurreducens
397 cytochromes: a model for extracellular electron transfer.Dalton T,44: 9335-9344.
398 Doi:10.1039/C5DT00556F.

399 [28]Xiao-Qiu Lin, Zhi-Ling Li, Bin Liang, Jun Nan, Ai-Jie Wang (2019)
400 Identification of biofilm formation and exoelectrogenic population structure and
401 function with graphene/polyaniline modified anode in microbial fuel
402 cell.Chemosphere,219:358-364. Doi:10.1016/2018.11.212.

403 [29]Xinhua Tang, How Yong Ng (2017) Anthraquinone-2-sulfonate immobilized to
404 conductive polypyrrole hydrogel as a bioanode to enhance power production in
405 microbial fuel cell.Bioresource Technol,244(1):452-455. Doi:10.1016/2017.07.189.

406 [30]Xiaobo Liu, Liang Shi, Ji-Dong Gu (2018) Microbial electrocatalysis: Redox
407 mediators responsible for extracellular electron transfer.Biotechnol
408 Adv,36(7):1815-1827. Doi:10.1016/2018.07.001.

409 [31]Y. Feng, D. Li, J. Liu, W. He(2019)in Microbial Electrochemical Technology. S. V.

410 Mohan, S. Varjani, A. Pandey (Eds), Elsevier,pp:49-74.

411 [32] Yafei Yang, Hong Peng, Junfeng Niu, Zhiqiang Zhao, Yaobin Zhang (2019)

412 Promoting nitrogen removal during Fe(III) reduction coupled to anaerobic ammonium

413 oxidation (Feammox) by adding anthraquinone-2,6-disulfonate (AQDS),

414 Environmental Pollution, 247:973-979. Doi.org/10.1016/j.envpol.2019.02.008.

415 [33]Yuanyuan Du, Xiaobing Wang, Baoming Li, Haixia Lü, Yu Shen (2021)

416 AQDS-guided growth of nanocilia-like polyaniline on graphene nanofiber as cathode

417 material for high-performance asymmetric supercapacitors, Synthetic Metals,

418 272:116660.-Doi.org/10.1016/j.synthmet.2020.116660.

419 [34]Zheng Chen, Yuanpeng Wang, Xiuli Jiang, Dun Fu, Dong Xia, Haitao Wang,

420 Guowen Dong, Qingbiao Li (2017) Dual roles of AQDS as electron shuttles for

421 microbes and dissolved organic matter involved in arsenic and iron mobilization in

422 the arsenic-rich sediment, Science of The Total Environment,574:1684-1694.

423 Doi.org/10.1016/-j.scitotenv.2016.09.006.