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Correlation between circuital current, Cu(II) reduction and cellular electron transfer in EAB isolated from Cu(II)-reduced biocathodes of microbial fuel cells

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ABSTRACT

The performance of four indigenous electrochemically active bacteria (EAB) (*Stenotrophomonas maltophilia* JY1, *Citrobacter* sp. JY3, *Pseudomonas aeruginosa* JY5 and *Stenotrophomonas* sp. JY6) was evaluated for Cu(II) reduction on the cathodes of microbial fuel cells (MFCs). These EAB were isolated from well adapted mixed cultures on the MFC cathodes operated for Cu(II) reduction. The relationship between circuital current, Cu(II) reduction rate, and cellular electron transfer processes was investigated from a mechanistic point of view using X-ray photoelectron spectroscopy, scanning electronic microscopy coupled with energy dispersive X-ray spectrometry, linear sweep voltammetry and cyclic voltammetry. JY1 and JY5 exhibited a weak correlation between circuital current and Cu(II) reduction. A much stronger correlation was observed for JY3 followed by JY6, demonstrating the relationship between circuital current and Cu(II) reduction for these species. In the presence of electron transfer inhibitors (2,4-dinitrophenol or rotenone), significant inhibition on JY6 activity and a weak effect on JY1, JY3 and JY5 was observed, confirming a strong correlation between cellular electron transfer processes and either Cu(II) reduction or circuital current. This study provides evidence of the diverse functions played by these EAB, and adds to a deeper understanding of the capabilities exerted by diverse EAB associated with Cu(II) reduction.

1. Introduction

Microbial fuel cells (MFCs) are emerging as new, sustainable and effective technologies for the recovery of heavy metals from waste and wastewater [1]. Among diverse heavy metals, Cu(II), which is common in the electroplating and mining industries [2–3], has attracted significant attention due to the potential for its recovery and simultaneous wastewater detoxification. The recovery of Cu(II) with abiotic cathode MFCs has been demonstrated over a wide range of operating conditions and cell architectures [4–9]. However, abiotic cathodes often require the use of costly noble or non-noble co-catalysts in addition to an acidic medium. Therefore, the development of biocathodic MFCs, which are based on the catalysis of self-regenerating electrochemically active bacteria (EAB) under a neutral environment, may provide a sustainable and green alternative to abiotic systems. Biocathodic MFCs avoid the use of expensive materials and toxic organic reagents, and reduce the consumption of energy and acids [10–14]. Biocathodes are also able to reduce electrode overpotentials, the production of sludge, and the overall cost and the maintenance of MFCs. MFCs, utilizing mixed cultures, have been shown to be efficient in the recovery of Cu(II) from mixed metal influent and also a promising system for the synthesis of copper [15]. In contrast, MFCs with biocathodes operated with pure cultures have been used to study specific EAB and their electrochemical performance [10–14]. The exogenous EAB of *Shewanella* is one of the few examples which is able to reduce a metal (Cr(VI)) in MFCs [16–17].

Microorganisms possess endurance to aqueous Cu(II) through a variety of mechanisms due to their intrinsic abilities and habitat sites [18–20]. Therefore, well adapted microorganisms could provide new insights with regards to heavy metal reduction in MFCs. A limited number of microorganisms exhibiting efficient rates of Cu(II) reduction have been cultivated under Cu(II)-adaptive conditions [20–24]. Similar considerations, in principle, hold true for their use in cathodic reductive environments in MFCs, under which the activities of different indigenous EAB may exhibit various Cu(II) reduction rates. In parallel, EAB immobilized on the surface of cathodes may also be able to utilize cathodic electrons for their internal metabolism [11,25]. The operation of MFCs at different concentrations of Cu(II) in the catholyte is expected

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to clarify the correlation between the circuital current and the rate of Cu(II) reduction associated with the use of isolated EAB.

The bacteriological reduction of Cu(II), in the absence of a circuital current, is believed to be associated with cellular electron transfer processes through the cytoplasmic membrane and with the flux of protons through ATP-synthase [26–27]. Such mechanisms can be unravelled using cellular electron transfer inhibitors, such as 2,4-dinitrophenol (DNP) and rotenone ($C_{23}H_{22}O_6$). DNP dissipates the proton motive force associated with cellular electron transfer processes, and decreases hydrogen production by inhibiting the ATP synthesis by photophosphorylation, in the absence of a circuital current [28]. In contrast, rotenone inhibits the activity of NADH-dehydrogenase and blocks the reduction of U(VI) by facultative anaerobic bacteria in the absence of a circuital current [29]. The utilization of rotenone and DNP, in the presence and absence of a circuital current passing through the EAB cathodes of MFCs is thus expected to establish whether the circuital current and Cu(II) reduction are associated with cellular electron transfer processes in the EAB species.

In this study, we elucidate from a mechanistic point of view the performance and impact of four indigenous different EAB on Cu(II) reduction in MFCs. The EAB tentatively identified as *Stenotrophomonas maltophilia* JY1, *Citrobacter* sp. JY3, *Pseudomonas aeruginosa* JY5 and *Stenotrophomonas* sp. JY6, were isolated from well adapted mixed cultures grown on the surface of MFC cathodes operated for Cu(II) reduction [15]. X-ray photoelectron spectroscopy (XPS), scanning electronic microscopy (SEM) coupled with energy dispersive X-ray spectrometry (EDS), linear sweep voltammetry (LSV) and cyclic voltammetry (CV) were used to investigate the effect of circuital current on the speciation of the deposited copper, the morphologies of the cathodes and the cathodic redox reactions for each EAB species. The relationship between circuital current, the rate of Cu(II) reduction and the cellular electron transfer processes associated with each of the four EAB were clarified through the system response to a step change of Cu(II) concentration in the catholyte and reaction mechanisms elucidated in the presence or absence of DNP or rotenone electron transfer inhibitors.

2. Materials and methods

2.1. EAB isolation, incubation and identification

Bacterial isolates were obtained from mixed culture cultivated in the Cu(II)-reduced biocathodes of MFCs [15]. The isolation and incubation processes are detailed in the Supplementary material (SM). The DNA of these EAB isolates was extracted using a Qubit2.0 DNA kit (Sangon Biotech (Shanghai) Co. Ltd., China) according to the manufacturer's procedure. The 16S rRNA gene was amplified by PCR using universal primers 518 F (5′ CAGAGTTTGATCCTGGCT3′) and 1540R (5′ AGGAGGTGATCCAGCCGCA3′), as described in SI. The sequence data were compared with the GenBank database using the Blast server at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) to accurately identify the bacterial strains. All tests were performed in duplicate.

2.2. MFC reactors setup and operation

Identical two-chamber MFCs with cylindrical chambers 4.0 cm long by 3.0 cm in diameter were used in all experiments. The anodic and cathodic chambers were separated by a cation exchange membrane (CEM) (CMI-7000 Membrane International, Glen Rock, NJ) with a projected surface area of 7.1 cm^2. Both the anode and cathode were filled with graphite felt (1.0 cm × 1.0 cm × 0.5 cm, 8 pieces, Sanye Co., Beijing, China) and carbon rods were used as current collectors in both anode and cathode. For each of the duplicate reactors three replicate experiments were performed. The MFCs were operated at a fixed external resistance of 510 Ω.

The anodes were inoculated with suspended bacteria collected from a previous acetate-fed MFC reactor and an equivalent volume of nutrient solution containing acetate (1.0 g/L) was added [30–31]. The cathodes were fed using the same medium, except acetate was replaced by $NaHCO_3$ (10 mg/L), with further addition of Cu(II) (5 mg/L) to the cathodic chambers. Cathodes were quantitatively inoculated with the isolates with a total of 3×10^8 colony forming unit. The anolyte and catholyte were sparged with N_2 gas for 15 min prior to adding the solutions into the electrode chambers. No measurable Cu(II) in the anolyte and acetate in the catholyte were observed during each cycle operation, excluding the possibility of Cu(II) and acetate diffusion between the chambers, although the retention of Cu(II) on the ion exchange membrane could not be precluded [15]. Other catholyte and operational conditions are described in SM.

The performance of the biocathodes was evaluated against four control experiments, including (i) the operation of the reactors under the open circuit condition (OCC), (ii) the operation with a closed circuit but without the inoculation of the EAB (abiotic control), (iii) the cathodes covered with the EAB but in the absence of Cu(II), and (iv) the cathodes tested in the absence of both EAB and Cu(II). The last two control experiments were used to evaluate the CVs performance of the cell.

2.3. Measurement, analysis and calculation

The circuital current, dissolved oxygen, biomass, organics and Cu(II) concentration were determined according to the methodology reported in SM. The rate of Cu(II) removal and charge distribution were calculated as detailed in SM.

Maximum power was obtained by running LSV at a scan rate of 0.1 mV/s [30–31]. Power and current densities were normalized to the projected surface area of the membrane. EAB cathode redox behavior was studied using CV (CHI 650, Chenhua, Shanghai). The potential was scanned between −0.36 V and +0.46 V (vs. SHE) at a scan rate of 1.0 mV/s using a standard three-electrode arrangement with the biocathode as the working electrode, platinum plate as the counter electrode, and Ag/AgCl as the reference electrode. One-way ANOVA in SPSS 19.0 was used to analyze the statistical variation of the data, and all of the data indicated significance levels of $p < 0.05$.

The surfaces of the Cu-laden biomass were analyzed by X-ray photoelectron spectroscopy (XPS, Thermo Fisher Scientific, ESCALAB 250, US) with a mono-Al Kα X-ray source (1486.6 eV of photons). The X-ray source was run at a reduced power of 150 W. The morphology of the electrodes after Cu(II) reduction were examined with a SEM (QUANTA450, FEI company, USA) equipped with an EDS (X-MAX 20 mm^2/50 mm^2, Oxford Instruments, UK) according to the method described previously [15,30].

3. Results and discussion

3.1. EAB isolation

Four EAB were successfully isolated from well adapted mixed cultures grown on the surface of MFC cathodes operated for Cu(II) reduction [15] (Table S1). All bacteria were gram-negative and major opportunistic to facultative or survived from an anaerobic environment. JY1 matching *Stenotrophomonas maltophilia*, is known to convert Cu(II) into Cu(0) on the cell surface, in the absence of cathodic electrons [22–24]. *S. maltophilia* has been isolated previously from a copper polluted area [22], and has never been reported in MFCs. JY3 closely related to *Citrobacter* sp., is able to remove Cu(II) in a medium of SO_4^{2-} through the formation of CuS precipitate in the absence of cathodic electrons [20]. It has been isolated previously from anodic MFC biofilms in the absence of Cu(II) [32]. JY5 matching *Pseudomonas aeruginosa*, is able to remove Cu(II) in the absence of cathodic electrons [21]. It has been reported in either denitrification autotrophic biocathodes of MFCs [33], or in MFCs operated for the degradation of phenol and glycerol through mediated electron transfer [34–37]. Finally, JY6 corresponding to *Stenotrophomonas* sp., can tolerate high concentrations of metals including Ag, Cu, Cd, Hg and Mn [38–39] and has been isolated previously from a wide range of environments.