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Ping ZHOU

En-Ren ZHANG

Li ZHOU

Guo-Wang DIAO

Jun-Le NIU

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绿脓杆菌 *Pseudomonas aeruginosa* BTE-1 直接电催化特征

周萍, 张恩仁*, 周立, 刁国旺, 牛俊乐

(扬州大学 江苏省环境材料与环境工程重点实验室, 化学化工学院, 江苏 扬州 225002)

摘要: 研究产电绿脓杆菌 *P. aeruginosa* BTE-1 的电化学催化特征. 结果表明, 在厌氧条件下, *P. aeruginosa* BTE-1 菌株不能分泌可充当电子介体的绿脓菌素, 但可依靠在电极表面形成生物膜而呈现直接电催化性能. *P. aeruginosa* BTE-1 在电极表面形成生物膜与其在特定电极电位下向电极传递电子的过程直接相关, 适宜的电位为 0.2 V (vs. SCE), 电位过高可能会损害 *P. aeruginosa* BTE-1 细胞. 室温范围内升高温度可增强 *P. aeruginosa* BTE-1 生物膜的电催化活性, 但过高的温度 (>60 °C) 会抑制生物膜电催化活性. 循环伏安曲线显示, 在厌氧条件下形成的 *P. aeruginosa* BTE-1 生物膜, 具有与典型产电菌株 *G. sulfurreducens* 相近的氧化还原电位 (-0.4 V ~ -0.2 V, vs. SCE). *P. aeruginosa* BTE-1 生物膜可电催化酵母抽取物和葡萄糖, 但不能电催化醋酸盐.

关键词: *Pseudomonas aeruginosa* BTE-1; 生物膜; 电化学催化

中图分类号: O646

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产电细菌是一类能够将底物氧化产生电子, 并以某种方式传递至电极进而产生电流的细菌. 这类细菌可构建微生物燃料电池或生物电化学反应器^[1-3], 因此, 该类细菌可作为有生命特征的新型电催化材料. 研究表明^[4-5], 在不同环境中(包括淡水及海洋沉积物、生活污水和活性泥等), 自然界众多菌群存在着代谢类型多样化的产电细菌. 目前, 分离培养的产电细菌纯化株只有十几株, 且其中仅几株模式菌株的催化特征(主要是 *Geobacter sulfurreducens* 和 *Shewanella oneidensis* 及其相关突变株)被研究^[6-8], 对于有关产电细菌-电极间相互作用的研究仍相当有限.

绿脓杆菌 (*Pseudomonas aeruginosa*) 属于机会致病菌, 多数纯化株在特定培养条件下可向细胞周围溶液分泌吩嗪类色素(主要是绿脓菌素), 其药理学研究倍受关注^[9-11]. 该菌也是 MFC 菌群中的常见细菌. 研究表明, 对于纯化株 *P. aeruginosa* KRP1 和 *P. aeruginosa* PA14, 其细菌自身分泌的绿脓菌素对细胞-电极间的电子传递至关重要, 若细菌不分泌色素, 其菌株的电催化活性极低^[12-13].

P. aeruginosa BTE-1 是从沉积物菌源接种的

MFC 阳极表面菌膜中分离纯化出来的绿脓杆菌菌株, 在好氧培养条件下, 该菌株可分泌大量绿脓菌素, 其培养体系呈深绿色, 而厌氧培养条件下, 则几乎不分泌绿脓菌素. 研究表明, 外加绿脓菌素后, 该菌株呈现出较高的产电水平, 但在厌氧电极室中, 菌株本身只分泌极少量的绿脓菌素, 且在较短时间内 (< 50 h), 因附着在电极表面的细胞量有限, 从而产电水平低^[14]. 本文深入研究在厌氧电极室中 *P. aeruginosa* BTE-1 生物膜的电催化特征, 考察某些参数(培养基更换次数及体系温度等)的影响, 以期改善该菌株的电催化性能.

1 实验

1.1 装置及材料

三电极体系, 温度可控, 厌氧, 由碳布工作电极 (7.5 cm², H2315, 德国 Freudenberg 公司)、饱和甘汞参比电极 (SCE) 和铂对电极组成.

菌株 (*P. aeruginosa* BTE-1) 由淡水沉积物 MFC 阳极表面生物膜分离^[14]. 该菌株在 LB 培养基中好氧培养, 能稳定分泌绿脓菌素.

LB 培养基: 由 5.0 g · L⁻¹ 酵母抽取物, 10 g · L⁻¹

氯化钠和10 g·L⁻¹蛋白胨组成.

无机盐: 2.5 g·L⁻¹ NaHCO₃、0.1 g·L⁻¹ KCl、1.5 g·L⁻¹ NH₄Cl、0.6 g·L⁻¹ NaH₂PO₄·H₂O、0.1 g·L⁻¹ NaCl、0.1 MgCl₂·6H₂O、0.1 g·L⁻¹ MgSO₄·7H₂O、0.005 g·L⁻¹ MnCl₂·4H₂O 和 0.001 g·L⁻¹ NaMoO₄·2H₂O. 培养基使用时应将该溶液调至中性,121 °C 灭菌 20 min.

1.2 菌株接种

将 *P. aeruginosa* BTE-1 菌株解冻、活化,在 LB 培养基中好氧培养 16 h. 用离心法收集 *P. aeruginosa* BTE-1 细胞,以新鲜 LB 培养基洗涤(3 次)该细胞. 尔后,按 10⁸ 个细胞/mL 的接种量在厌氧电极室(装 30 mL 厌氧 LB 培养基)接入 *P. aeruginosa* BTE-1. 使用电化学工作站(CHI1000a,上海辰华)测试菌株的电化学性能(37 °C),场发射扫描电镜(Philips XL30 ESEM)观察电极表面形貌.

2 结果与讨论

2.1 电位影响

分别测定了在-0.6、-0.4、-0.2、0、0.2、0.4 和 0.6 V 下 *P. aeruginosa* BTE-1 电流~时间变化,图 1 仅选取 0.6 V(A)、0.2 V(B)和-0.6 V(C)的曲线. 结果表明,*P. aeruginosa* BTE-1 在-0.6 和-0.4 V 细菌接种前后电流不甚变化;在-0.2、0 及 0.2 V,电流随时间延长而增大,在较正电位(>0.2 V),*P. aeruginosa* BTE-1 能将其细胞内呼吸链末端的电子传递到电极,形成阳极氧化电流,呈现传递电子的能力;在 0.4 或 0.6 V 电位下,其氧化电流迅速下降,过高的电位下,该细菌细胞因过氧化而损伤.

不同绿脓杆菌菌株的电催化性能存在差异. 在厌氧条件下,*P. aeruginosa* BTE-1 菌株电催化的适宜电位范围为-0.2 ~ 0.2 V,这与典型直接电子传递的 *Geobacter sulfurreducens* 菌相似^[8]. 如图 1B,在 0.2 V 电位下,*P. aeruginosa* BTE-1 的阳极氧化电流较大,重现性好,故选取该电位为宜.

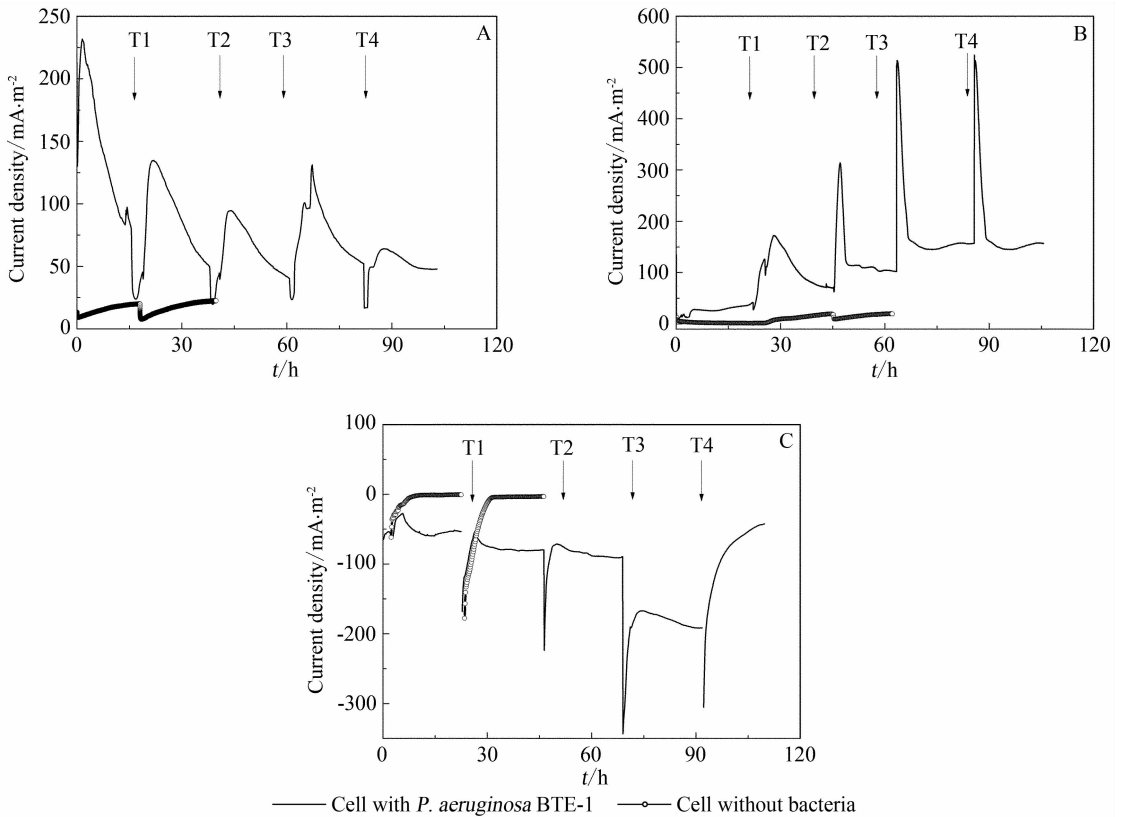


图 1 不同电位下 *P. aeruginosa* BTE-1 产生的电流~时间曲线(箭头代表更换培养基)
A. 0.6 V; B. 0.2 V; C. -0.6 V

Fig. 1 Current~time curves by *P. aeruginosa* BTE-1 at different potentials(arrows indicate medium replacement)
A. 0.6 V; B. 0.2 V; C. -0.6 V

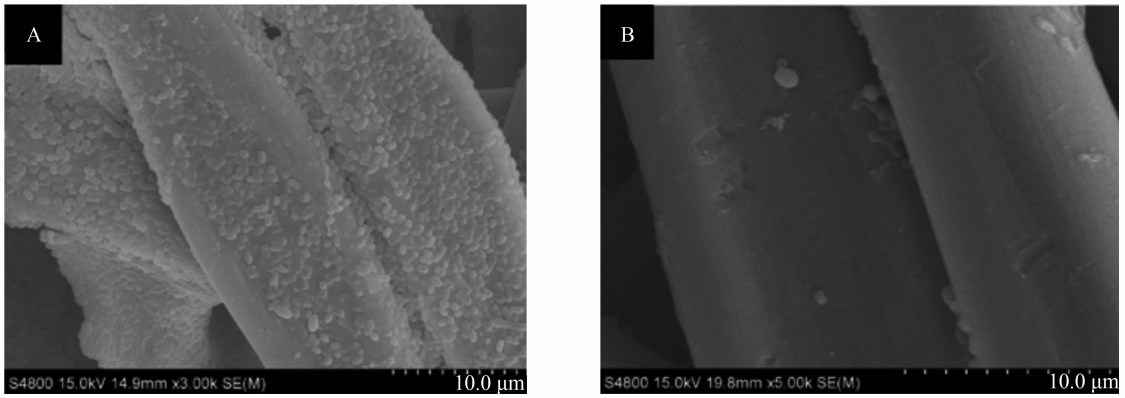


图2 碳布电极表面 SEM 照片(7次更换培养基,A. 通电;B. 未通电)

Fig. 2 SEM observations of the carbon cloth electrode surface after the medium replacement of seven times

A. with current flow; B. without current flow

2.2 *P. aeruginosa* BTE-1 生物膜

据图1B,在0.2 V电位下,当电极转移时,依靠附于碳布电极表面的 *P. aeruginosa* BTE-1 细胞可明显促使电流增大,多次更新培养基时,阳极电流仍不断升高,其阳极最终电流(峰电流达 $480(\pm 30) \text{ mA}\cdot\text{m}^{-2}$, 稳态电流 $125(\pm 25) \text{ mA}\cdot\text{m}^{-2}$) 达到稳定值.这时附着于电极表面的 *P. aeruginosa* BTE-1 细胞可趋于饱和,形成比较稳定的生物膜.

图2为碳布电极表面的 SEM 照片.可以看出,在0.2 V 电位下通电流经7次更换培养基的电极表面已经覆盖着一单层 *P. aeruginosa* BTE-1 生物细胞膜(图2A),而未通过电流的电极表面则无细胞附着(图2B).在缺少溶解态电子介体的情况下, *P. aeruginosa* BTE-1 为了能够直接向电极传递电子,细胞需要与固体电极保持直接接触.随着电子传递过程的进行,可逐渐在电极表面形成由单层细胞构成的生物膜.而当电极未接入电路,电极无法连续接受电子,仅依靠 *P. aeruginosa* BTE-1 细胞与碳材料电极表面的随机接触并不能形成可见的生物膜.由此可见,电极表面 *P. aeruginosa* BTE-1 生物膜的形成与该菌株电催化过程直接相关.

2.3 温度的影响

图3为步进升温过程中 *P. aeruginosa* BTE-1 生物膜的电催化氧化电流.从图看出,在适宜细菌生长的温度范围内,升温可提高其阳极氧化电流.但当温度达到 $60\text{ }^{\circ}\text{C}$ 时,阳极氧化电流反而迅速下降,与微生物电化学反应器升温过程的电流特征类似^[15].但在未接种细菌的电极体系中,相同的步进升温过程却对阳极氧化电流影响甚小.细菌细

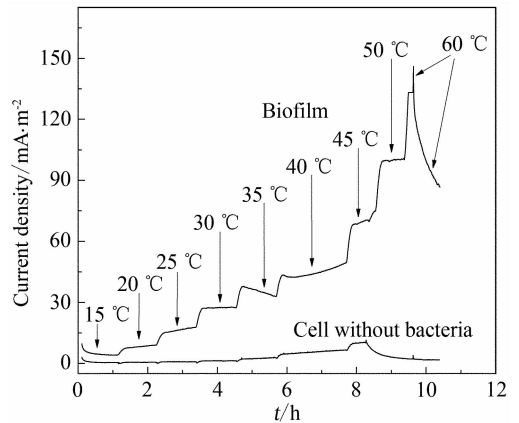


图3 温度对 *P. aeruginosa* BTE-1 生物膜催化活性的影响

Fig. 3 The effect of temperature on biocatalysis of *P. aeruginosa* BTE-1

胞的电催化能力,本质上是细胞内特有酶体系特征的体现.酶的活性受温度影响甚大,室温下,酶有活性,温度过高则受损害. *P. aeruginosa* BTE-1 生物膜的细胞活性显然与温度密切相关.

2.4 生物膜的循环伏安曲线

图4示出在不同培养基中,附着 *P. aeruginosa* BTE-1 生物膜电极和无菌碳布电极的循环伏安曲线.如图,在无机盐及含有有机电子供体(酵母提取物,葡萄糖和醋酸钠)的培养基中,生物膜电极于 $-0.4 \sim -0.2 \text{ V}$ 区间出现1对氧化还原峰,其峰电位与 *Geobacter sulfurreducens* 生物膜电极上的氧化还原峰电位相近^[6-7],而无菌电极则不出现对应的氧化还原峰.

实质上生物膜电极显示的氧化还原峰是细胞

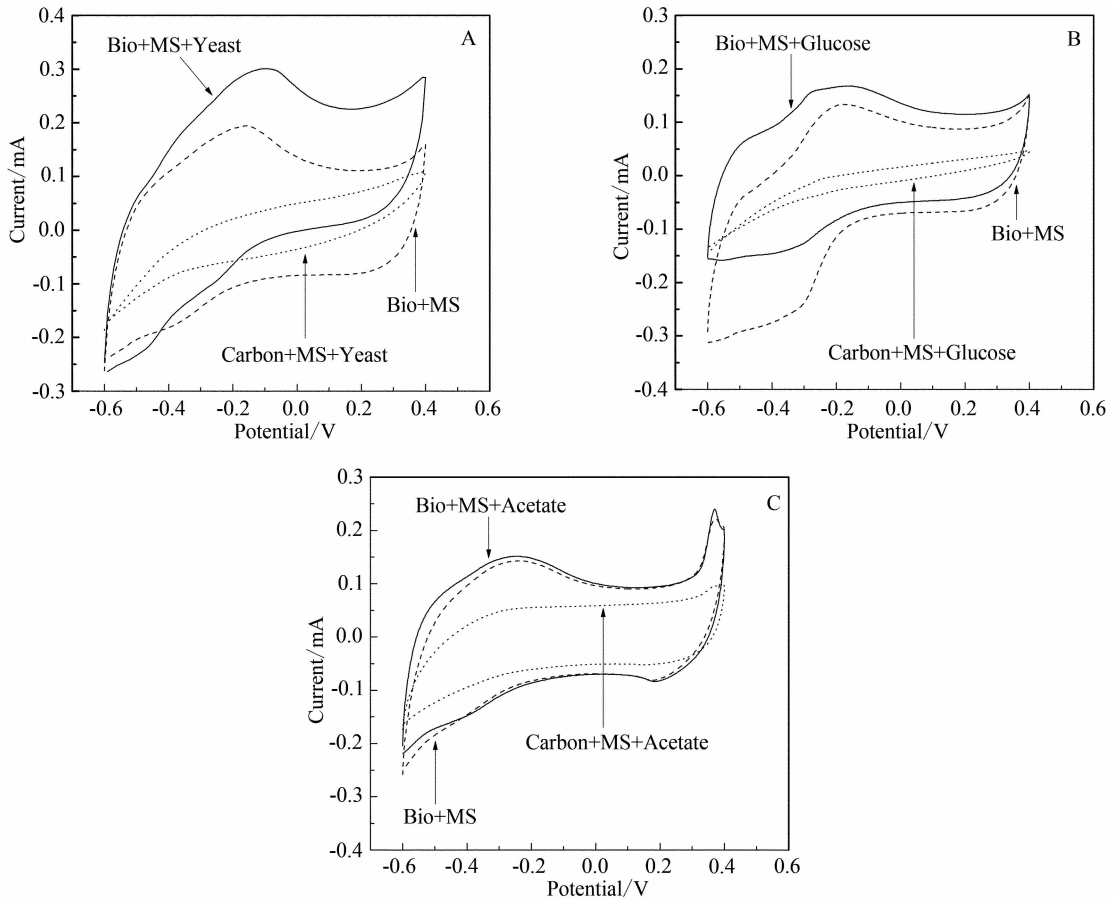


图 4 生物膜和无菌碳布电极在不同培养基中的 CV 曲线(扫速: $1 \text{ mV} \cdot \text{s}^{-1}$)

Bio:生物膜电极;Carbon:空白碳布电极;MS:无机盐溶液;Yeast: $5 \text{ g} \cdot \text{L}^{-1}$ 酵母提取物(A);Glucose: $5 \text{ g} \cdot \text{L}^{-1}$ 葡萄糖(B);Acetate: $5 \text{ g} \cdot \text{L}^{-1}$ 醋酸盐(C)

Fig. 4 CV with biofilm electrode and carbon cloth electrode in different media (scan rate: $1 \text{ mV} \cdot \text{s}^{-1}$)

Bio: biofilm electrode; Carbon: plain carbon cloth electrode; MS: mineral solution; Yeast: $5 \text{ g} \cdot \text{L}^{-1}$ (A); Glucose: $5 \text{ g} \cdot \text{L}^{-1}$ (B); Acetate: $5 \text{ g} \cdot \text{L}^{-1}$ (C)

膜上细胞色素的氧化还原反应,其氧化还原电位与某些细胞色素的氧化还原电位($-0.43 \sim -0.38 \text{ V}$)相近^[16-18].图4还显示,补充酵母提取物和葡萄糖,可使氧化电流增大,而补充醋酸钠,则其氧化还原电流未见明显变化,说明 *P. aeruginosa* BTE-1 生物膜对酵母提取物和葡萄糖有电催化氧化作用.

3 结 论

在厌氧电极室中,*P. aeruginosa* BTE-1 生物膜呈现直接电催化作用,如果仅依靠细胞与电极表面的接触.*P. aeruginosa* BTE-1 不能在电极表面形成生物膜,生物膜的形成与 *P. aeruginosa* BTE-1 在特定电位(例如 0.2 V)下直接向电极传递电子紧密相关.*P. aeruginosa* BTE-1 生物膜电催化活性与

温度有关,温度大于 $60 \text{ }^\circ\text{C}$,该膜电催化活性受到抑制;在 $-0.4 \sim -0.2 \text{ V}$ 电位区间,其电催化活性与文献报道的典型菌株的直接电子传递能力相近.

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Electrocatalytic Study of *Pseudomonas Aeruginosa* BTE-1 Strain

ZHOU Ping, ZHANG En-ren*, ZHOU Li, DIAO Guo-wang, NIU Jun-le

(*Jiangsu Provincial Key Laboratory of Environmental Materials and Engineering, Department of Chemistry and Chemical Engineering, Yangzhou University, Yangzhou 225002, Jiangsu, China*)

Abstract: The aim of the present study is to investigate the electrocatalytic activity of electricity-producing *Pseudomonas aeruginosa* BTE-1 strain under anaerobic conditions. *Pseudomonas aeruginosa* BTE-1 was inoculated into anaerobic three-electrode electrochemical cells, and the electrocatalytic activity was measured at poised potentials. HPLC and cyclic voltammetry were used to detect potential electron mediators in solutions. Experimental results showed that no detectable pyocyanine was excreted by *P. aeruginosa* BTE-1 strain in the anaerobic electrochemical cells, and *P. aeruginosa* BTE-1 exhibited direct electrocatalytic activity through the formation of biofilm on the electrode surface which was induced by the electron transfer from the cells of *P. aeruginosa* BTE-1 to the electrode at poised potentials. Suitable potential for biofilm formation was found to be 0.2 V (vs. SCE), and more positive potentials would lead to a potential harm to *P. aeruginosa* BTE-1 cells. At room temperature, the electrocatalytic activity of the *P. aeruginosa* BTE-1 biofilm could be enhanced by increasing temperature, however, the temperatures higher than 60 °C reduced the electrocatalytic activity of the biofilms quickly. Cyclic voltammetry analysis indicated that *P. aeruginosa* BTE-1 biofilms formed under anaerobic conditions exhibited an electrochemical catalytic wave in the potential range of -0.4 V to -0.2 V (vs. SCE), similar to that observed with a typical electricity-generating strain, *Geobacter sulfurreducens*. Organic substrates, such as compounds in yeast and glucose, could be oxidized through the catalysis of *P. aeruginosa* BTE-1 biofilms, whereas acetate could not be catalyzed to oxidize.

Key words: *Pseudomonas aeruginosa* BTE-1; biofilm; electrocatalysis