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开闭路条件下沉积物微生物燃料电池阳极细菌群落差异解析

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摘要: 采用 Solixa 高通量测序技术对比了沉积物微生物燃料电池(sediment microbial fuel cells, SMFCs)开闭路条件下启动阳极生物膜细菌群落结构差异. SMFCs 开路条件下启动阳极生物膜获得优化序列3 936条,经过 97% 相似度归并后得到1 581个操作分类单元(operational taxonomic units, OTUs); SMFCs 连接 5 kΩ 电阻闭路启动阳极生物膜获得优化序列3 930条,聚类归并后产生1 551个 OTUs,α 多样性指数分析表明开路条件下启动阳极生物膜菌群多样性相对更丰富. SMFCs 开、闭路条件下启动阳极生物膜优势菌均为 Proteobacteria、Firmicutes 和 Bacteroidetes,它们在开路下阳极生物膜中的含量分别为 59.79%、12.54% 和 9.02%,而在闭路条件下分别为 63.02%、10.01% 和 3.60%,SMFCs 闭路条件下运行,地杆菌 *Geobacter* 在阳极生物膜中含量高达 16.55%. SMFCs 开、闭路条件下启动阳极生物膜细菌群落结构的差异说明 SMFCs 启动过程电子的传递影响电极生物膜微生物群落结构.

关键词: 沉积物微生物燃料电池; 开路; 闭路; 细菌群落多样性; 高通量测序
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Comparative Analysis of the Bacterial Community on Anodic Biofilms in Sediment Microbial Fuel Cell Under Open and Closed Circuits

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Abstract: To investigate the differences in microbial community of anodic biofilms under open and closed circuits, sediment microbial fuel cell (SMFCs) reactors connecting with a 5 kΩ external resistance and open circuit during the start-up period were operated individually. Anodic biofilms were collected and analyzed using Solexa high-throughput sequencing technology. A total of 3936 and 3930 operational taxonomic units (OTUs) were obtained from the anodic biofilms under open and closed circuits, respectively. After 97% similarity merging, 1 581 and 1 551 OTUs were finally determined from open and close circuit biofilms, respectively. The analysis of α diversity showed that bacterial diversity of anodic biofilm under open circuit was higher than that under closed circuit. The dominant bacterial were Proteobacteria, Firmicutes and Bacteroidetes for both open and closed circuits. They accounted for 59.79%, 12.54% and 9.02% under open circuit biofilm respectively; and these values were 63.02%, 10.01% and 3.60% in the closed circuit biofilm respectively, and *Geobacter* accounted for 16.55% in the closed circuit biofilm. The present study demonstrated that the electron transfer process during start-up period affected the microbial community structure of the anodic biofilms.

Key words: sediment microbial fuel cell; open circuit; closed circuit; bacterial community diversity; high throughput sequencing

沉积物微生物燃料电池(sediment microbial fuel cells,SMFCs)是利用电化学活性微生物代谢将河、湖或海底沉积物中有机质的化学能转化成电能的装置. SMFCs 在底泥原位修复[1]的同时,可为远程无线传感器[2]和水下超声波探测器[3]等水体监测仪器提供低功率的电源,应用前景广泛.

电极电位影响电化学活性微生物与电极间的电子传递,设置合适的电位有助于电化学活性微生物在电极上富集成膜,缩短微生物燃料电池的启动时间,并提高其产电性能[4~6]. Wang 等[7]发现 0.20 V (vs. Ag/AgCl)电位促进了阳极电化学活性微生物在电极表面形成生物膜. Finkelstein 等[8]发现 0.62 V (vs. Ag/AgCl) 电位能够促进电化学活性微生物在阳极生物膜的富集从而缩短微生物燃料电池启动时间. 外电阻可影响阳极电位,进而影响阳极生物

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膜的微生物群落结构. 李辉等[9]发现微生物燃料电池启动阶段外接电阻阻值越高,阳极生物膜微生物生物量越高,但产电微生物的丰度越低,阳极的电化学性能越差,同时也发现开路条件下电池启动时间更长. 研究开、闭路条件下 SMFCs 阳极生物膜微生物群落结构差异, 有助于加深电化学活性微生物与电极间电子传递对电池启动影响机制的认识.

随着分子生物学理论和技术的快速发展, T-RFLP[10]、DGGE[11,12]以及克隆文库技术[13,14]等基于 PCR 的分子指纹技术不需要对微生物进行分离培养,已广泛应用于电极生物膜微生物种群结构解析,但是这些传统的分子生物学方法获得样品微生物群落信息有限. 高通量测序技术具有较高的测序深度,能较全面地反映电极生物膜微生物群落结构[15,16]. 本研究采用 Solexa 高通量测序技术系统分析了开、闭路条件下启动的 SMFCs 阳极生物膜细菌群落结构差异,加深了产电微生物电子传递对 SMFCs 启动影响机制的认识.

1　材料与方法

1.1　实验装置及运行条件

实验装置如图 1 所示,50 L 圆柱形容器下层泥土区(取自厦门集美某池塘底泥)为阳极厌氧区域,阳极电极材料为碳毡,几何面积为 16 cm^2 (4 cm × 4 cm),埋于泥土约 15 cm 处,钛丝引出；上层水相深度约为 6 cm,阴极为 100 cm^2 (10 cm × 10 cm) 的碳毡,悬浮于好氧水面,阴阳极用钛丝引出后导线连接,外电阻为 5 kΩ；开路对照采用相同设置但未连接外部电阻. 电极使用前依次用 0.1 $mol\cdot L^{-1}$ HCl 和 0.1 $mol\cdot L^{-1}$ NaOH 浸泡去除表面吸附物. 电压 (U) 每隔 10 min 由数据采集器(Keithley2700,美国)自动采集保存.

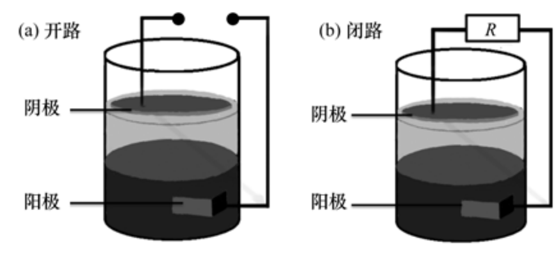

图 1　实验装置示意

Fig. 1　Diagram of experimental setup

1.2　Solexa 高通量测序

1.2.1　电极表面生物膜 DNA 提取

SMFCs 产生连续、稳定的输出电压(0.38 V)视为阳极生物膜发育成熟. 此后取出阳极并以磷酸钠盐缓冲液(50 $mmol\cdot L^{-1}$, pH 7.0)洗涤,采用土壤微生物 DNA 提取试剂盒(Mobio,美国)提取阳极生物膜基因组 DNA.

1.2.2　PCR 扩增

以提取的 SMFCs 阳极生物膜 DNA 为模板,用引物 27F 和 533R 对细菌 16S rDNA 的 V3 可变区进行 PCR 扩增,其中在作为测序端的通用引物 533R 上加标签标记[17]. PCR 反应体系组成：5 × FastPfu 缓冲液 4 μL, dNTP 2 μL, 正向引物 0.8 μL, 反向引物 0.8 μL, FastPfu 聚合酶 0.4 μL, 模板 1 μL, 灭菌蒸馏水补足至 20 μL. PCR 扩增程序为：95℃,预变性 5 min；95℃ 40 s, 56℃ 30 s, 72℃ 40 s, 30 个循环；72℃, 10 min；4℃保存.

1.2.3　PCR 产物定量及微乳液 PCR

PCR 产物经纯化后采用 QuantiFluor™-ST 蓝色荧光定量系统(Promega,美国)定量,将待测样品比例混合进行微乳液 PCR,产物上机测序.

1.2.4　序列分析

使用 QIIME 1.5.0 软件[18]处理所得序列,去除的冗余序列包括其平均质量低于 25,或其模糊碱基数大于 0,或长度短于 200 bp；序列比对采用 NCBI 数据库,使用香农(Shannon)指数、Chao1 指数、Phylogenetic diversity(PD)等表征样品菌落多样性.

2　结果与讨论

2.1　生物膜细菌群落结构分析

2.1.1　测序质量评价

利用高通量测序技术对 SMFCs 在开、闭路条件下启动阳极生物膜细菌 16S rDNA V3 区序列分析. 开路下阳极生物膜共获得4 238条原始序列,优化序列3 936条,97% 相似度聚类分析产生1 581个 OTUs；闭路启动阳极生物膜共获得4 198条原始序列,优化序列3 930条,经过 97% 相似度归并共产生1 551个 OTUs.

2.1.2　细菌多样性评价

开、闭路启动阳极生物膜细菌序列信息及其多样性指数如表 1 所示. 从中可知,开路条件下启动阳极生物膜菌群 Shannon 指数、PD 指数及 Chao1 指数均大于闭路条件下运行的电极生物膜,说明开路条件下阳极生物膜菌群丰富度及多样性都高于闭路条件下阳极生物膜. 这一现象的产生可能是由于闭路条件下,微生物与阳极间存在着电子传递,影响了微生物在阳极的生长代谢,进而影响电池启动期的阳极成膜速度以及阳极菌群结构.

表 1 阳极生物膜 Solexa 测序信息及 α 多样性指数

Table 1 Solexa sequencing and α diversity statistics of the anode biofilm

样品	原始序列数	有效序列数	97% 相似性		
			Chao1 指数	PD 指数	Shannon 指数
开路	4 238	3 936	7 138	134.9	8.74
闭路	4 198	3 930	6 690	124.6	8.49

2.2 阳极生物膜菌群分类和群落结构分析

阳极生物膜在门分类水平组成如图 2 所示. 两个样品菌群表现出了较高的多样性,开路条件下生物膜中细菌相对含量超过 1% 的门有 5 个,闭路条件下有 6 个. 两个生物膜样品细菌群落在门分类水平最明显差异在于 Proteobacteria、Firmicutes 和 Bacteroidetes 在各自群落中所占比例的不同,这 3 个门的细菌序列分别占总序列数的 81.35%(开路)和 76.71%(闭路). Proteobacteria 在两个样品含量最为丰富,在开路和闭路启动阳极生物膜中的含量分别为 59.79% 和 63.02%. Firmicutes 是样品中含量第 2 多的门,在开路启动阳极生物膜中含量为 12.54%,在闭路条件下阳极生物膜中含量为 10.01%. Bacteroidetes 在闭路阳极生物膜中的含量只有 3.60%,而在开路阳极生物膜中占 9.02%.

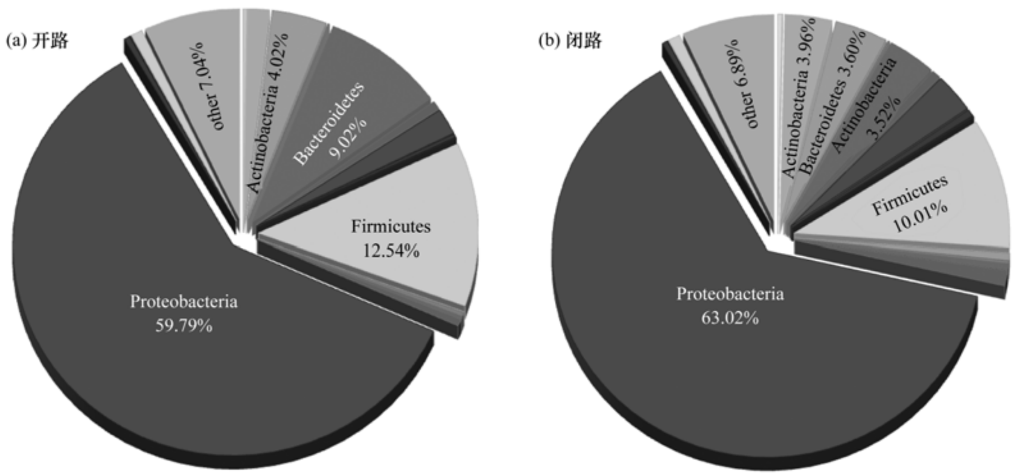

图 2 开闭路条件下电极生物膜细菌在门分类水平组成

Fig. 2 Relative abundance of bacterial community composition at phylum level under open and closed circuit conditions

电极生物膜在纲分类水平上组成如图 3 所示,两种条件下启动的阳极生物膜含量大于 0.1% 的细菌分布于 17 个纲,包括 α-、β-、γ-、δ-Proteobacteria、Flavobacteriia、Sphingobacteria、Bacteroidia 等. α-Proteobacteria 在闭路启动的 SMFCs 阳极生物膜中只有 5.63%,但在开路条件下启动阳极生物膜中含量达 19.52%. γ-Proteobacteria 在闭路条件下启动的 SMFCs 阳极生物膜中含量为 18.51%,远高于开路条件下启动的 4.32%. 已知的电化学活性菌如 *Shewanella*[19]、*Citrobacter*[20]、*Aeromonas*[21]、*Klebsiella*[22]、*Pseudomonas*[23] 等都属于 γ-Proteobacteria,说明闭路启动有利于产电微生物富集.

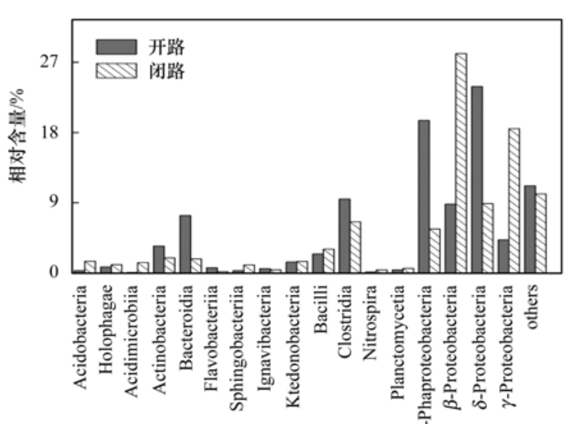

图 3 电极生物膜细菌在纲分类水平含量

Fig. 3 Relative abundance of bacterial community composition at class level

在属的水平上进行群落结构分析如表 2 所示. 开闭路条件下启动的生物膜中含量大于 0.5% 的属共有 17 个,这些属的细菌在开、闭路启动阳极生物

膜中含量分别为 15.60% 和 36.99%. 两种条件下启动的 SMFCs 阳极生物膜内菌群组成有相似构成，但各个菌属的含量差异较大，如地杆菌 *Geobacter* 在闭路电极生物膜中含量为 16.55% 远大于开路条件下的 2.31%. *Geobacter* 是广泛分布的电化学活性细菌，在元素生物地球化学过程中起重要作用[24,25]. 其中 *G. sulfurreducens* 基因组测序已经完成，是目前了解最清楚的电化学活性细菌之一，在生物电化学系统运行过程中，其库仑效率甚至能达到 99%，产电是其获得能量的主要途径[26].

表 2　阳极生物膜中相对丰度高于 0.5% 细菌组成

Table 2　Relative abundance of OTU composition above 0.5% in the anode biofilm

目	科	属	相对丰度/%	
			开路	闭路
Desulfuromonadales	Geobacteraceae	*Geobacter*	2.31	16.55
Rhodospirillales	Rhodospirillaceae	*Azospirillum*	0.20	10.27
Bacteroidales	Porphyromonadaceae	*Dysgonomonas*	0.42	3.94
Rhodospirillales	Rhodospirillaceae	*Magnetospirillum*	0.51	0.58
Bacillales	Planococcaceae	*Sporosarcina*	0.78	0.81
Clostridiales	Symbiobacteriaceae	*Symbiobacterium*	0.58	0.80
Actinomycetales	Dietziaceae	*Dietzia*	0.02	0.76
Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1.32	0.67
Burkholderiales	Oxalobacteraceae	*Massilia*	0.11	0.54
Burkholderiales	Oxalobacteraceae	*Herbaspirillum*	0.63	0.33
Burkholderiales	Comamonadaceae	*Hydrogenophaga*	0.08	0.52
Acidiferrobacterales	Acidiferrobacteraceae	*Acidiferrobacter*	0.71	0.03
Gallionellales	Gallionellaceae	*Gallionellaceae*	4.87	0.21
Bdellovibrionales	Bdellovibrionaceae	*Bdellovibrio*	1.41	0.02
Enterobacteriales	Enterobacteriaceae	*Escherichia*	0.98	0.16
Holophagales	Holophagaceae	*Geothrix*	0.67	0.47

相比传统 DGGE 和克隆文库技术等微生物群落研究的分子生物学手段，高通量测序技术显示出巨大优势，获得的微生物群落信息更高，能检测到传统技术很难发现的、丰度较低的电化学活性微生物；该技术可更准确、全面地反映电极微生物群落结构[15,27].

3　结论

(1) SMFCs 开、闭路条件下启动阳极生物膜细菌群落在门分类水平最明显差异在于 Proteobacteria、Firmicutes 和 Bacteroidetes 在各自群落中所占比例的不同.

(2) Chao1、Shannon 和 PD 指数分析表明 SMFCs 开路下电极生物膜内菌群的丰富度和多样性均高于闭路运行阳极生物膜.

(3) SMFCs 闭路条件下运行，地杆菌 *Geobacter* 在阳极生物膜中含量较高，说明闭路条件下地杆菌 *Geobacter* 在阳极表面选择性富集.

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