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土壤微生物产电信号评价芘污染毒性的研究*

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摘 要 通过向土壤中加入葡萄糖促进微生物产电过程,研究了芘污染条件下土壤产电变化规律。利用双室微生物燃料电池(Microbial fuel cells, MFCs),实时、连续记录芘污染土壤产电电压。产电 110 h 后结束 MFCs 运行,采用循环伏安法检测芘对土壤微生物电化学活性的影响;结合 PCR-DGGE 及测序技术,分析芘对 MFCs 阳极表面细菌群落结构的影响。结果显示,MFCs 产电电量随芘浓度增加显著降低。循环伏安检测显示芘降低了土壤微生物的电化学活性。DNA 序列分析表明,阳极细菌与已报道的产电细菌高度相似,包括 *Sporolactobacillus*、*Clostridium*、*Enterobacter*、*Bacillus* 及 *Ethanoligenens*。芘降低了 *Bacillus* 丰度。

关键词 微生物燃料电池;产电细菌;循环伏安;脱氢酶
中图分类号 X82 **文献标识码** A

多环芳烃(Polycyclic aromatic hydrocarbons, PAHs)是目前环境中主要污染物之一,主要来源于石油和煤炭的燃烧。在土壤中,PAHs 能够对微生物群落产生不利影响[1],包括抑制微生物功能和土壤酶活性[2-4]等。过量的 PAHs 还可以被植物吸收,进入食物链并影响人的健康。合适的生物指标可被用来监测 PAHs 在土壤中的毒性强弱,判断污染程度并为评价污染物生态学影响提供依据,例如:酶活性[5]、呼吸速率[6]、代谢熵[7]等,其中脱氢酶活性被认为是指示土壤微生物活性最灵敏的指标之一[8]。但是,上述传统方法操作较为繁琐,而且不能实现连续、实时监测。

微生物燃料电池(Microbial fuel cells, MFCs)是一类通过产电菌的催化作用,将有机质的化学能转化为电能的装置[9]。双室 MFCs 包含阳极室与阴极室,两室之间由离子交换膜分隔。阳极室中的产电菌厌氧分解有机物并将产生的电子转移给阳极,电子流过导线到达阴极并与电子受体(如 O_2、Fe(III))结合,从而形成电流。由于所产生的电流强度与产电菌的代谢活性密切相关,MFCs 可作为生物传感器对环境进行长时间连续实时的监测[10-11],例如监测废水中的生化需氧量(BOD)[12]、毒性物质如重金属[13-14]和酸污染[15]。上述 MFCs 生物传感器的建立需要采用培养基进行产电菌的接种、富集和驯化。待产电菌产电稳定后再通入废水,根据产生电流的升高或降低幅度判断污染物的毒性及其变化[16-17]。

Ringelberg 等[18]发现多种土壤具有产电能力。土壤中存在着多种产电菌,可以通过分解土壤有机质实现产电。参与产电的土壤微生物附着在阳极表面,主要属于变形菌门(*Proteobacteria*)和厚壁菌门(*Firmicutes*)[19-20]。产电菌广泛分布于土壤中并对环境变化十分敏感,所以污染物对土壤微生物产生胁迫的同时可能也会抑制产电菌活性,从而可以通过产电信号来反映污染物毒性,但目前相关研究较少。土壤含有产电所需的必备条件——产电微生物与有机质,因此土壤产电信号的形成无需通过外加培养基和产电细菌[21],从而在电信号产生机制上有别于基于 MFCs 的废水监测方法。此外,采用土壤产电信号评价污染毒性的优点在于实时、连续监测以及低成本和易操作。

为了弄清土壤产电信号是否能够指示土壤污染毒性,本研究向土壤施加芘胁迫并运行 MFCs 记录污染土壤产电电压,同时检测芘污染土壤的脱氢

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酶活性(dehydrogenase activity, DHA),以研究土壤产电信号对芘浓度的响应特征,以及检验产电信号与 DHA 的变化趋势是否一致,从而初步确定土壤产电信号指示土壤污染毒性的可行性,并运用分子生物学技术研究芘添加对 MFCs 阳极产电菌群落结构的影响。

1　材料与方法

1.1　供试土壤

供试土壤于 2013 年 6 月采自南京师范大学仙林校区的一处阔叶林地,采样深度 0 ~ 20 cm。样地气候条件为亚热带季风气候,年均降雨量 1 100 mm,年均气温 15 ℃。土样采集后经研磨过 2 mm 筛并充分混匀用于芘胁迫试验,少量过筛土壤继续风干进行理化分析。土壤结构参照吸量管法测定[22],土壤全碳和全氮通过元素分析仪(VARIO EL III, Elementar)测定,土壤 pH 按土水比 1∶2.5(w/v)采用 pH 计(FE20, Mettler Toledo)测定,电导率(EC)按土水比 1∶5(w/v)通过电导率仪(DDSJ-308F,上海雷磁)测定。土样的理化性质如下:土壤质地为黏壤土,全碳 1.81 mg g^{-1},全氮 0.62 mg g^{-1},土壤 pH 7.57,EC 76.6 μS cm^{-1}。

1.2　芘胁迫试验

取过筛土样,混合均匀后分为 5 份。从 5 份土样中各取 1/10 放入烧杯并加入芘的正己烷溶液,搅拌,对照仅加入正己烷溶液。待正己烷挥发完全后,将土样加回至大份土样中,充分混合均匀,即得到 5 个处理:芘添加量 60、120、180 和 240 mg kg^{-1} 的污染土样及不加芘的对照土样。将每个处理分为两份,一份调节含水率至 25%,常温下老化 72 h 后进行土壤脱氢酶活性测定,另一份用于产电并检测电信号。

1.3　土壤产电信号检测

采用双室构型 MFCs 检测不同芘浓度土壤的产电信号,每个处理运行 3 个 MFCs 平行,共运行 15 个 MFCs。产电检测方法如图 1 所示。MFCs 阳极室和阴极室均为边长 6 cm 立方体。阳极和阴极均采用边长 3 cm、厚度 0.5 cm 的正方形碳毡,与阳离子交换膜平行置于阳极室和阴极室中央,电极间距为 6 cm。为缩短启动时间并提升产电,土壤混合 4%(w/w)葡萄糖后加入阳极室并淹水,每个阳极室加入 120 g 土壤(干土重),阴极室加入 180 ml 铁氰化钾溶液(50 mmol L^{-1} 铁氰化钾溶于 50 mmol L^{-1} pH 7 的磷酸盐缓冲液)。阳极和阴极由钛丝连接并串联 1 000 Ω 电阻,采用数据采集卡(7660B,北京中泰研创)每隔 10 min 记录一次电压数据。所有 15 个 MFCs 于 30 ℃培养箱中运行 110 h。

1.4　土壤脱氢酶活性测定

土壤脱氢酶活性(DHA)通过比色法测定[23]。称取 2 g 土样(干土重),加入 5 ml 1% 氯化三苯基四氮唑(TTC)的 Tris 盐酸溶液。37 ℃培养 24 h 后,用甲醇定容,涡旋过滤,于 485 nm 测定吸光值。标准曲线由甲醇溶解的三苯甲臜(TPF)制备。

1.5　电化学活性测定

MFCs 阳极室土壤的电化学活性通过循环伏安法(CV)进行测定[24]。MFCs 运行 110 h 后采集阳极室土壤 50 g(干土重)于离心管中,4 000 r min^{-1} 离心 10 min,提取土壤溶液,采用 0.45 μm 滤膜过滤,同时按相同操作制备氯仿熏蒸灭菌土壤溶液。土壤溶液曝氮气 10 min 后进行 CV 检测。检测采用三电极体系,包括玻碳工作电极、铂丝对电极和 Ag/AgCl 参比电极,实验参数由恒电位仪(CHI 1040C,上海辰华)控制。扫描位宽为 −1 ~ +1 V (vs. SHE),扫速为 50 mV s^{-1},样品体积为 5 ml,实验过程保持静置。

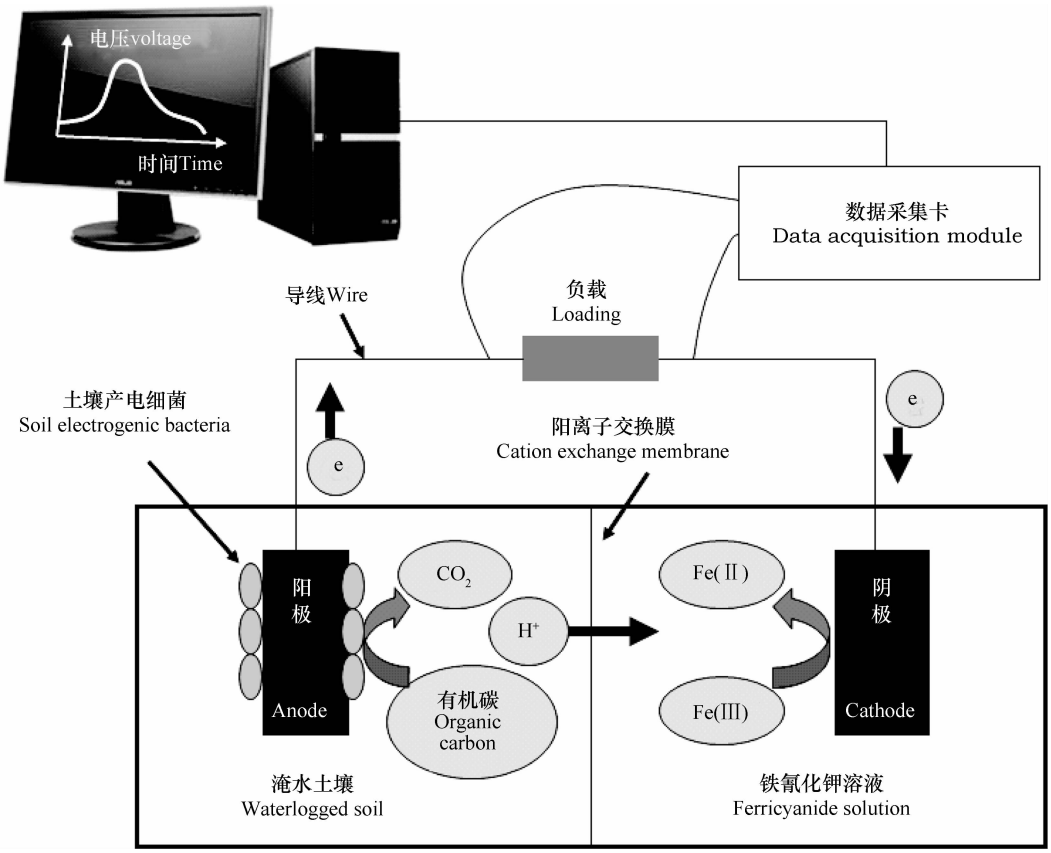
1.6　DNA 提取及 PCR 扩增

MFCs 运行 110 h 结束后,选取对照和 240 mg kg^{-1} 芘处理,取出阳极碳毡用无菌水冲洗掉表面土壤,剪下 1 cm 见方的阳极碳毡用于 DNA 提取[25]。使用土壤 DNA 快速提取试剂盒(Fast DNA® SPIN Kit for Soil, MP)按照制造商说明书分别提取阳极碳毡与未污染土壤的 DNA。

提取的 DNA 进行 16S rRNA 基因 PCR 扩增用于 DGGE 分析。所用引物为 341f(含 GC 夹)和 907r[26]。25 μl 的反应体系由 1 μl DNA 模版,0.5 μl、1 μmol L^{-1} 的上游与下游引物(Invitrogen),12.5 μl、2 × Taq-T SuperMix(Transgene)和 10.5 μl 超纯水组成。循环温度条件为:94 ℃预变性 4 min;94 ℃变性 1 min,54 ℃退火 30 s,72 ℃延伸 30 s,30 个循环;72 ℃延伸 5 min。

1.7　DGGE、测序及系统发育分析

DGGE 实验采用 8% 的聚丙烯酰胺凝胶,变性梯度为 40% ~ 80%(100% 为 7 mol L^{-1} 尿素,40%(v/v)去离子甲酰胺)。电泳条件为:0.5 × TAE 缓冲液,60 ℃,100 V 于 Bio-Rad Dcode™ 通用突变检测系统电泳 16 h。电泳完成后,使用 1∶10 000 稀释



注:每个 MFC 反应器由阳极室和阴极室组成,两室由阳离子交换膜分隔,阳极与阴极通过钛丝与 1 000 Ω 外阻连接。阳极室与阴极室分别加入淹水土壤和铁氰化钾的磷酸缓冲盐溶液。土壤中参与产电的细菌在阳极富集并降解葡萄糖,生成 CO_2、电子和质子。电子通过钛丝传递至阴极并将 Fe(III)还原为 Fe(II)。产生的电压每隔 10 min 由数据采集卡记录并得到电压随时间变化曲线 Note: An MFC reactor consists of an anode chamber and a cathode chamber separated by a cation exchange membrane. The anode and cathode are connected to a 1 000 Ω external resistance with titanium wire. The anode chamber and cathode chamber is filled with waterlogged soil and potassium ferricyanide in 50 mmol L^{-1} PBS, respectively. The soil bacteria involved in power generation get enriched on anode and degrade glucose to produce CO_2, electrons and protons. The electrons are transmitted to cathode through the wire and reduce Fe(III) to Fe(II). The produced voltage is recorded using a data acquisition module every 10 mins and the curve of voltage against time is presented using a computer

图 1 土壤产电信号检测示意图

Fig. 1 Sketch of the operation of monitoring of electrical signals generated in soil

的 SYBR™ Green I 核酸凝胶染液(Invitrogen)对胶染色 30 min 并脱色 10 min。将凝胶立即置于 ChemiDoc™ XRS + 凝胶成像系统(Bio-Rad)并采用 Quantity One 4.4.0 软件(Bio-Rad)分析 DGGE 图谱得到相似性矩阵数据,通过未加权算术平均对群法(unweighted pair group method with arithmetic averages, UPGMA)进行聚类分析。

具有代表性的 DGGE 条带被切下并置于 40 μl 超纯水中,4 ℃过夜溶脱 DNA。吸取 2 μl 的溶脱 DNA 作为模版,341f/907r 为引物,反应体系与循环设置同上所述,进行 PCR 扩增。所得 PCR 产物使用 Peasy™-T3 克隆试剂盒(Transgene)进行克隆并导入 Trans1-T1 耐噬菌体感受态细胞 *E. coli*(Transgene)。采用特异性引物 M13f/M13r 对克隆进行 PCR 扩增和筛选[27]。每个 DGGE 条带挑选 5 个克隆子由 Invitrogen 公司测序。测序结果经 DNASTAR Lasergene 7.1 软件去除载体序列后,提交 BlastX (http://ncbi.nlm.nih.gov/blast)进行比对。系统发育分析通过 MEGA4 软件按 neighbor-joining 算法与距离计算完成。相似性 98% 以上的序列视作同一个 OTU。所比对的序列已全部提交于 GenBank 数据库,分配编号为 KJ128061 ~ KJ128073。

1.8 统计分析

电量(C)为土壤在 110 h 的产电过程中产生的总电量。本实验中电量按下式计算得出:

$$C = \sum_{n=1}^{m} \frac{(U_n + U_{n+1})}{2 \times 1\,000} \times 600$$

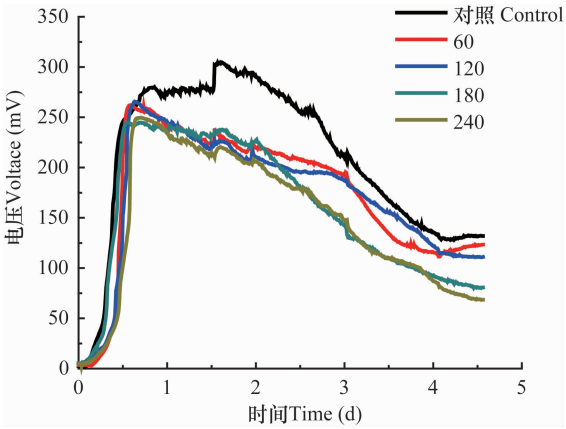
式中,U 为电压(V),1 000 为所用负载阻值(Ω),

600 为记录电压的间隔时间(s),n 为数据采集卡记录电压值的次数,m 为 110 h 中记录电压的总次数。处理间差异的显著性采用单因素方差分析(ANOVA),产电电量与脱氢酶活性之间相关性采用 Pearson 相关分析。所有统计分析采用 SPSS 16 软件。

2　结　果

2.1　产电信号对芘胁迫的响应

MFCs 运行过程的电压曲线如图 2 所示。所有处理均在 6 h 左右启动产电,并在 12 h 左右达到第一个产电峰值。加芘处理的 MFCs 峰值在 240 ~ 270 mV 之间,而对照处理的产电峰值为 36 h 出现的 305 mV。对照与芘污染处理的产电量计算结果(表 1)显示,不加芘对照 MFCs 的电量值显著高于芘污染处理,并且电量随芘浓度的增加显著下降($p < 0.05$)。此外,以氯仿灭菌的土壤运行 MFCs,其电压值始终低于 2 mV。作为参照的传统指标土壤脱氢酶活性也随芘含量的增加而显著减少,且产电电量与脱氢酶活性对毒性响应也具有显著的正相关关系($p < 0.01$)。



注:MFCs 运行时间 110 h,每隔 10 min 记录一次电压数据。图例中对照代表不加芘处理,60、120、180 和 240 分别代表向土壤中添加 60、120、180 和 240 mg kg^{-1} 芘含量的处理

Note: The MFCs operated for 110 hours and voltage data were recorded every 10 min. In the figure legend, control denotes soil without pyrene addition; the number 60, 120, 180 and 240 denote soil treatments added with 60, 120, 180 and 240 mg kg^{-1} pyrene, respectively

图 2　添加不同浓度芘的土壤产电电压

Fig. 2　Voltages generated by soilsadded with different pyrene content

表 1　添加不同芘浓度土壤的脱氢酶活性和产电电量

Table 1　Dehydrogenase activity and quantity of electrons generated of soils added with different pyrene content

芘浓度 Pyrene content(mg kg^{-1})	脱氢酶活性 Dehydrogenase activity(TPF mg kg^{-1})	电量 Quantity of electrons(C)
0	164.8 ± 12.4a	82.4 ± 4.7a
60	142.4 ± 13.5b	68.0 ± 5.5b
120	135.7 ± 9.3bc	67.5 ± 7.5bc
180	130.4 ± 6.4bc	62.9 ± 2.8bc
240	122.9 ± 3.6c	58.2 ± 2.9c

注:平均值 ± 标准偏差($n = 3$)。脱氢酶活性为芘污染并老化 3 d 后的检测值,电量为 MFCs 运行 110 h 的总产电量。同列中有相同字母表示处理间差异不显著($p > 0.05$)Note: Means ± SD ($n = 3$). Dehydrogenase activity was determined 3 d after pyrene addition; Quantity of electrons was produced by MFCs operated for 110 h. The same letters in the same column indicate insignificant differences between treatments ($p > 0.05$)

2.2　芘胁迫对土壤电化学活性的影响

在扫描电位范围内,采用氯仿熏蒸灭菌后的土壤溶液未出现任何明显的氧化还原峰(图 3)。阳极室中不加芘对照的土壤溶液与加入 240 mg kg^{-1} 芘的土壤溶液在 -100 mV 左右出现明显的还原峰,在 +100 mV 左右出现微弱的氧化峰,并且芘添加处理弱于不加芘对照处理。

2.3　芘胁迫对土壤产电细菌群落结构的影响

应用 DGGE 技术分离 16S rRNA 基因片段 PCR 产物的图谱显示,不同样品均分离到 20 条以上的电泳条带(图 4),其中土壤的条带数远高于 MFCs 阳极。在阳极样品中,图中编号 1、2、3、4、6 和 8 的条带为主要条带,条带 5 和 7 可能代表对芘敏感的产电菌,同时条带 9 和 10 则代表耐受芘的菌种。对 DGGE 图谱的聚类分析结果(图 5)显示将土壤样品与 MFCs 阳极样品归为一类的相似值仅为 0.43,存在明显差异(一般认为相似值高于 0.60 的两个群体具有较好的相似性)。而在阳极样品中,不加芘对照样品与 240 mg kg^{-1} 芘处理样品相似性达到 0.83,但也存在差异,包括对照泳道中编号 5 和 7 的条带在芘污染样品的泳道中基本消失。

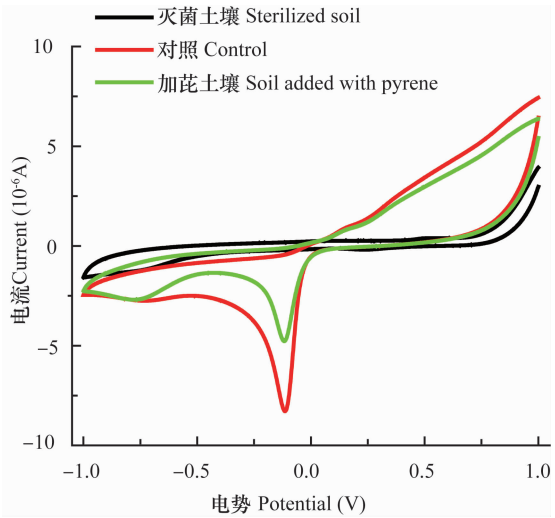
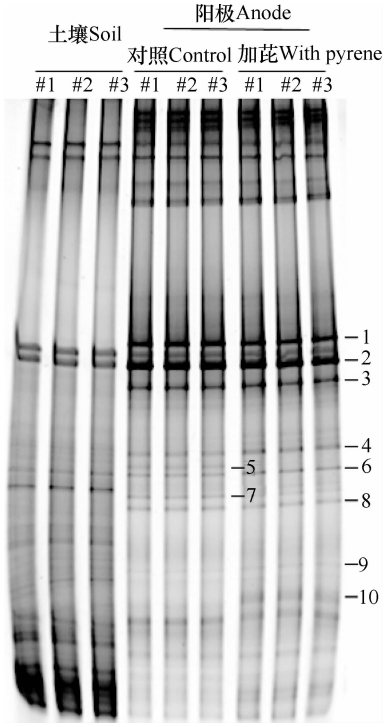


图 3 从加芘土壤(240 mg kg^{-1}芘)、对照(不加芘土壤)以及灭菌土壤中提取土壤溶液的循环伏安图

Fig. 3 Cyclic voltammograms of soil solutions fromsoil added with pyrene (240 mgkg^{-1} pyrene), control (soil without pyrene addition) and sterilized soil



注:编号 1 - 10 条带代表后续进行测序和系统发育分析的主要条带

Note: The numbers from 1 to 10 represent the major DGGE bands for sequencing and phylogenetic analysis

图 4 加芘土壤(240 mg kg^{-1}芘)和对照(不加芘土壤)产电110 h后阳极样品及未加芘土壤样品的 16S rRNA 基因 DGGE 图谱

Fig. 4 DGGE profiles of 16S rRNA gene fragments from anode of MFCs with pyrene treated soil (240 mg kg^{-1} pyrene) and control (soil without pyrene addition) after 110 h of operation and soil samples without pyrene addition

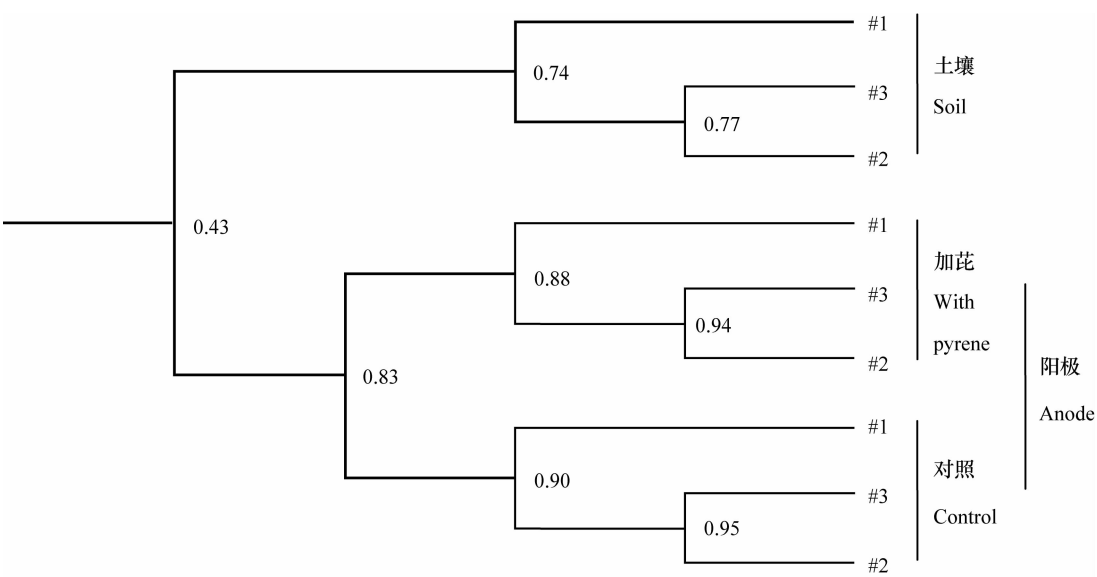
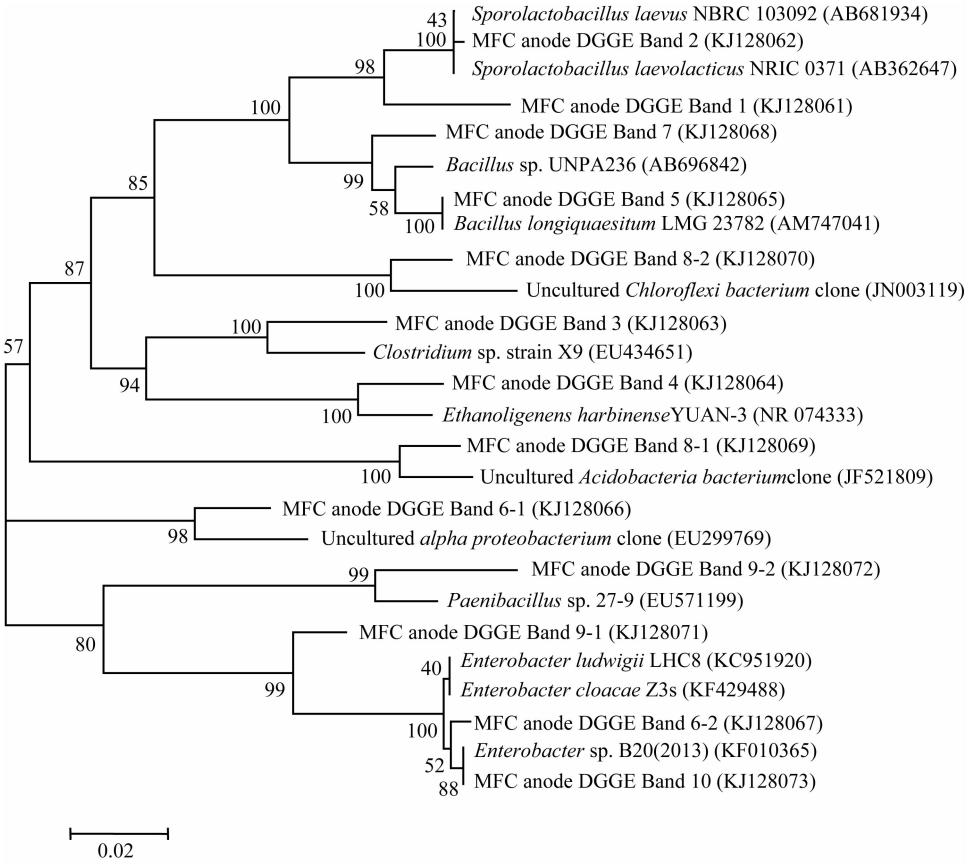


图 5 加芘土壤(240 mg kg^{-1}芘)和对照(不加芘土壤)的 MFCs 阳极样品与未加芘土壤样品16S rRNA基因 DGGE 图谱的聚类分析

Fig. 5 Cluster analysis of DGGE profiles of 16S rRNA gene from anode of MFCs with pyrene treated soil (240 mg kg^{-1}pyrene) and control (soil without pyrene addition), and from soil without pyrene addition

选择 DGGE 胶上具有代表性的 10 条带(分别对应图 4 中编号 1 ~ 10 条带)切割并纯化。所割条带均获得克隆子,对成功克隆的白色单菌落进行 DNA 测序,所得到的序列大小在 523 ~ 547 bp 范围内。将测序结果与 GenBank 核酸数据库比对,应用 MEGA 4 软件进行系统进化分析,结果(图 6)显示条带 1 和 2 序列与产芽孢乳杆菌属(*Sporolactobacillus*)相似,条带 3 序列与梭状芽孢杆菌属(*Clostridium*)相似,条带 4 序列与产乙醇杆菌属(*Ethanoligenens*)相似,条带 5 和 7 序列与芽孢杆菌属(*Bacillus*)相似,条带 6 和 10 序列与肠杆菌属(*Enterobacter*)相似,条带 8 序列与未获得纯培养的酸杆菌门(*Acidobacteria*)相似,条带 9 序列与肠杆菌属(*Enterobacter*)及类芽孢杆菌属(*Paenibacillus*)相似。



注:系统发育树中 16S rRNA 基因长度为 525 bp,DGGE 条带序列已提交至 GenBank 数据库,编号为 KJ128061 ~ KJ128073 Note: The tree represents the alignment of a 525-bp region in 16S rRNA gene sequences The DGGE bands were assigned the accession numbers KJ128061-KJ128073 after the sequences of the bands were submitted to the Genbank database

图 6　DGGE 条带序列与 GenBank 数据库比对序列的系统发育关系

Fig. 6　Phylogenetic relationships between the DGGE band sequences and the reference sequences in in the GenBank database

3　讨　论

本研究采用土壤产电信号指示土壤污染毒性,并从污染物影响土壤微生物电化学活性以及产电细菌群落的角度揭示了土壤产电对污染敏感的机理。在产电实验中,灭菌土壤产电电压极低,证明了土壤产电是土壤微生物的功能。实验中土壤产电电量随着土壤中芘浓度的增加而下降,并且变化趋势与土壤脱氢酶活性这一传统指标相一致,显示出土壤产电电量这一指标在指示土壤污染毒性方面具有较好的灵敏性和可靠性。土壤产电检测方法简便,成本较低,不需要消耗能源,还能产生电能。

通常 MFCs 运行开始阶段需要一定时间让产电菌在电极表面富集和驯化,之后产电才会逐渐上升达到检测的要求[28]。本实验为了缩短富集和驯化的时间,向土壤中添加了葡萄糖。葡萄糖和乙酸钠等易被代谢的小分子有机底物能够被产电菌快速

利用[29],从而在大幅度缩减土壤产电启动时间的同时,又提升了 MFCs 电压,便于显示不同芘处理间的差异。MFCs 阳极土壤溶液的 CV 图谱中,在 -100 mV出现了明显的还原峰,而在 +100 mV 出现了氧化峰,该对氧化还原峰可视为一个准可逆(quasi-reversible)反应[30]。并且灭菌的土壤溶液在扫描过程中始终未检测到氧化还原活性,说明该电化学活性极可能由土壤微生物产生。其中 240 mg kg^{-1}芘处理的峰高低于不加芘对照处理,表明芘的添加直接抑制了土壤产电菌的电化学活性。芘的添加也抑制了土壤脱氢酶活性,产电菌作为土壤微生物中的一个类群,其分解代谢有机底物的速率也将受影响,使得产电能力随着芘的添加而降低。

DGGE 图谱的聚类分析表明,土壤经过产电之后,土壤微生物群落中参与产电的微生物在阳极表面得以富集,从而造成土壤和阳极微生物群落结构的差异。而芘的添加也改变了 MFCs 阳极产电菌的群落组成,尤其是明显抑制了芽孢杆菌属(*Bacillus*)的细菌。本研究通过对阳极样品相对于土壤样品的特异性条带进行测序来确定参与产电的细菌的群落组成。目前在土壤中发现的产电菌主要集中在变形菌门(*Proteobacteria*)和厚壁菌门(*Firmicutes*)[16]。与本实验阳极样品特异性的 DGGE 条带序列有较高同源性的细菌种群中,芽孢杆菌属(*Bacillus*)、芽孢乳杆菌属(*Sporolactobacillus*)、类芽孢杆菌(*Paenibacillus*)、梭状芽孢杆菌属(*Clostridium*)、肠杆菌属(*Enterobacter*)和产乙醇杆菌属(*Ethanoligenens*)均属于这两个菌门。其中,芽孢杆菌(*Bacillus* sp.)主要通过可溶性的氧化还原介质将电子传递给阳极[31-32],而森林土壤中的可溶性 Fe(III)/Fe(II)和腐殖质可以作为电子传递介质[33]。因此芘对芽孢杆菌的抑制会削弱土壤产电能力。梭状芽孢杆菌属(*Clostridium*)[34]、类芽孢杆菌属(*Paenibacillus*)[35]、肠杆菌属(*Enterobacter*)[36]则已被证实具有直接产电能力。而芽孢乳杆菌属(*Sporolactobacillus*)[37]、产乙醇杆菌属(*Ethanoligenens*)[38]以及酸杆菌门(*Acidobacteria*)[39]不具有产电能力,而只是厌氧发酵葡萄糖。以往的研究认为,由于葡萄糖容易利用,其作为底物能够实现快速产电,但同时部分葡萄糖会被非产电菌发酵,从而降低了库伦效率[40]。

4 结 论

土壤产电电量随土壤中芘浓度的增加显著减小,表明土壤产电信号指示土壤芘污染的毒性可行。芘抑制了土壤产电菌的电化学活性及底物代谢能力,并且改变了 MFCs 阳极所富集的产电菌的群落结构。由于本研究尝试采用土壤产电信号指示土壤污染毒性,所选择的污染物种类、浓度范围有限,将来的研究可以考察土壤产电信号对更多污染物及其浓度的响应特征,还可以优化 MFCs 构型和操作方法,缩短产电时间,进一步提高产电对污染响应的灵敏度。

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EVALUATION OF TOXICITY OF PYRENE POLLUTION BY ELECTRICAL SIGNALS GENERATED BY SOIL MICRO-ORGANISMS

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Abstract Polycyclic aromatic hydrocarbons (PAHs), being nowadays the major environmental pollutants, can bring about adverse effects on soil microbial community. Classical biological indicators, such as dehydrogenase activity, substrate induced respiration rate and community level physiological profiles, are widely used to evaluate toxicity of PAHs to soil ecosystems. However, it is not feasible to use them to monitor soil pollution in a real-time continuous way. Microbial fuel cells (MFCs) are a kind of devices that convert chemical energy of organic substrates into electrical power through the catalysis of electrogenic bacteria. As the current which is closely related to metabolic activity of the electrogenic bacteria can be recorded immediately and continuously, MFCs have already been used as a biosensor to monitor wastewater treatment. Electrogenic bacteria are known to be widely distributed in soil. They generate electricity while decomposing soil organic matter and are extremely sensitive to environmental change. Pollutants creating stress on soil microbes may also inhibit the activity of electrogenic bacteria. Thus electrical signals generated by soil micro-organisms could be used as an indicator to detect soil pollution. The objectives of the study are to determine feasibility of the use of the electrical signals to evaluate pyrene toxicity in soil, and understand how soil electrogenic bacteria respond to pyrene pollution.

Synthetic PAHs contaminated soils were prepared, different in amount of pyrene spiked, i. e. 0 (as control), 60, 120, 180 and 240 mg kg^{-1} pyrene and then packed into MFCs anode chambers, separately. To accelerate the electricity generation, 4% (w/w) glucose was thoroughly mixed into the soil before packing. Voltage of MFCs was real time monitored every 10 min for 110 h. To examine reliability of the use of electrical signals in toxicity detection, dehydrogenase activity in the pyrene contaminated soil was also measured simultaneously. After the MFCs operated for 110 h, electrochemical activity of the soil microbes was determined using cyclic voltammetry. Bacterial community diversity on the MFCs anode biofilm was determined through phylogenetic analysis of 16S rRNA genes with the PCR-DGGE and sequencing methods. All the DGGE band sequences were submitted to the GenBank and assigned with accession number, KJ128061-KJ128073.

Results show that the cells started to generate electricity after 6 h of operation. Peak voltages from MFCs of pyrene-contaminated soils were monitored varying between 240 ~ 270 mV, while that from control reached 305 mV. The coulomb production of the MFCs within the 110 h decreased significantly with the rate of pyrene added, and significantly ($p < 0.01$) correlated with soil dehydrogenase activity. Cyclic voltammogram shows that the soil of control treatment had higher redox peaks than the soil spiked with 240 mg kg^{-1} pyrene with ranging at potentials around – 100 mV and 100 mV while no peaks were observed in the fumigated and sterilized soil, illustrating that pyrene addition weakened electrochemical activity of the soil micro-organisms. DGGE patterns show that after 110 h of operation, the bacterial community on the MFCs anode biofilm differed significantly from that in the soil in structure. Sequencing and phylogenetic analysis of the DGGE bands reveal that the bacteria on the anode biofilm was highly similar to the known electrogenic bacteria, including *Sporolactobacillus*, *Clostridium*, *Enterobacter*, *Bacillus* and *Ethanoligenens*. Pyrene addition decreased the abundance of *Bacillus*.

This study demonstrates that the electrical signals generated by soil micro-organisms could satisfactorily be used to

evaluate pyrene toxicity in the soil. The mechanisms of pyrene reducing electricity generation include inhibition of electrical activity of soil micro-organisms and alteration of the structure of the electrogenic bacterial community on the anode. In future the study in this field should be oriented toward monitoring of more pollutants varying in a wider range of concentrations, and optimization of the configuration and operation of MFCs to shorten their startup time and to improve their sensitivity of electrical signals.

Key words　Microbial fuel cells (MFCs); Electrogenic bacteria; Cyclic voltammetry; Dehydrogenase

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