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电活性生物膜: 形成、表征及应用*

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摘 要 电活性生物膜(Electrochemically active biofilms, EABs)是一类能够直接与胞外固态载体(铁氧化物、腐殖质及电极等)进行电子交换的生物膜. EABs的电子传递特性, 赋予了它在环境、能源和化工等领域的广泛应用前景, 已成为当前国际研究热点. 本文以革兰氏染色法为依据, 分别介绍了腐败希瓦氏菌(*Shewanella putrefaciens*)、硫还原地杆菌(*Geobacter sulfurreducens*)和丁酸梭菌(*Clostridium butyricum* EG3)为代表的阴性和阳性电活性微生物; 在普通生物膜的形成基础上, 讨论了EABs的两种主要培养方法; 分别从EABs输出电子与接受电子的角度, 详细论述了电活性微生物与胞外载体的电子传递机制; 重点阐述了利用电化学、光谱学、电子显微镜、分子生态学等多技术手段表征单个电活性微生物和整个EABs的形态、结构, 以及所揭示的胞外电子传递机制和相关影响因子; 对EABs在电能输出、污染物治理、有价品合成等方面应用作了详细介绍. 最后, 建议对EABs的研究建立一个统一、标准的表征方法, 同时应重点研究EABs接受电子的传递机制. 对这些机理的深入了解, 可使得EABs在污染物治理以及有机物的电合成等方面应用早日实现规模化、产业化生产. 图4 表1 参64

关键词 电活性生物膜; 电活性微生物; 胞外电子传递; 生物电化学系统; 微生物电合成
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Electrochemically active biofilms: formation, characterization and application*

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Abstract Microorganisms commonly form biofilms in order to strengthen their functions or survival in harsh environments. Electrochemically active biofilms (EABs) are special because they can donate electrons to, or accept electrons from, electrodes or natural analogs of electrodes such as Fe(Ⅲ) oxides and humid acids. Numerous promising applications can be developed based on EABs, including bio-remediation of polluted soils or water, electricity generation from waste materials, biosensors to monitor microbial metabolic activities, and biosynthesis of desirable products. This paper is organized as follows. Section 1 describes some Gram negative and Gram positive electroactive microbes, including *Shewanella putrefaciens*, *Geobacter sulfurreducens* and *Clostridium butyricum* EG3. Section 2 presents two principal approaches for EABs cultivation after describing the development of common biofilms that are not electroactive. Section 3 introduces the major electron-exchange mechanism, including how microorganisms get electrons from electrodes and how electrons from the decomposition of organic materials by microorganisms are conducted to electrode. Section 4 introduces electrochemical, spectroscopic, microscopic and molecular ecological techniques used to characterize the morphology and structure of a single microorganism or EABs to reveal the electron transfer mechanisms and influencing factors. Applications of EABs, which include energy production, wastewater and soil pollution remediation, and chemicals electrosynthesis, are introduced. Finally, we conclude that a uniform and standard method should be built up, more efforts should be put in revealing the electron-exchange mechanism between the microorganisms and the supporters, especially about how EABs accept electrons from electrodes. More understanding of the

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electron-transfer mechanism and its controlling factor, shall further promote the industrial application of EABs.

Keywords electrochemically active biofilms; electromicrobiology; extracellular electron transfer; bioelectrochemical systems; microbial electrosynthesis

在自然界中，绝大多数微生物是附着在有生命或无生命物体的表面，以群体即生物膜（Biofilms）的方式生长，而不是以浮游（Planktonic）状态生长. 与单细胞浮游态不同，微生物群体在整体上表现出一系列新的生物学特征：（1）更强的外界适应能力；（2）膜内微生物代谢低、存活期长；（3）在载体表面生长可诱导表达与浮游状态不同的基因等. 长期以来，对生物膜的研究要远落后于浮游微生物. 近年来随着生物膜学（Biofilmology）的兴起，人们逐渐认识到与浮游微生物相比，生物膜有更复杂的结构、更广泛的信息交流及更精密的调控机制，并且更紧密地影响着人类生活. 目前，生物膜学已成为医药、食品、环境等领域的研究热点. 然而直到现在，生物膜还是一个“黑箱”，膜内物质循环、群落结构和功能等有待探索. 2004年，Logan等发现从废水中富集形成的生物膜吸附在固体电极上的电子传递效率是浮游微生物的数百倍[1]. 这种可直接与胞外固态载体（铁氧化物、腐殖质及电极等）进行电子交换的生物膜，被称为电活性生物膜（Electrochemically active biofilms, EABs）. 与传统生物膜相比，EABs的最大特点是生物膜与胞外固态载体（铁氧化物、腐殖质及电极等）存在直接的电子交换过程.

EABs的发现改变了生物膜与接触界面相互作用的传统认识，为深入理解生物膜的结构和功能提供了全新视角. 目前，EABs的形成及其电子传递机制已成为环境领域关注的热点. 本文从EABs的形成入手，阐述EABs胞外电子交换途径、重要电活性微生物种类，总结EABs的研究方法，并对其应用前景进行详细介绍.

1 电活性生物膜的形成

1.1 EABs的形成过程

普通生物膜形成大致可分为4个过程：（1）微生物在固体表面的黏附以及微菌落形成，并融合成生物膜的基底层；（2）胞外聚合物的产生与释放；（3）结构复杂化并发育为成熟的生物膜. 生物膜的形成是一个动态过程，伴随着微生物的聚集与脱落（图1）.

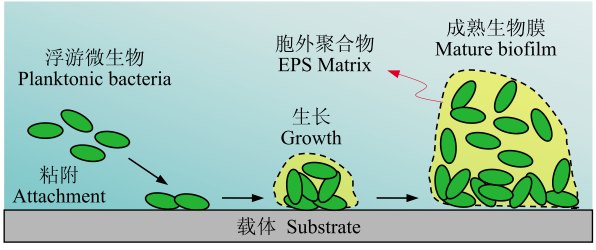

图1 生物膜的发展：初始粘附、生长、成熟[2].

Fig. 1 Biofilm development: attachment, growth, mature biofilms [2].

与普通生物膜相比，电活性生物膜（EABs）既可“输出”微生物氧化有机物过程产生的电子，也可“接受”环境中的胞外电子. 因此，电子交换能力成为EABs挂膜成与否和电活性高低的重要指标. 目前，培养高效的EABs主要依赖以下两种装置：（1）生物电化学系统（Bioelectrochemical systems, BES），主要是微生物燃料电池（Microbial fuel cell, MFC），以及以MFC为基础经改进或新组装的生物电化学系统，比较典型的有微生物电解池（Microbial electrolysis cell, MEC）[3]、微生物脱盐燃料电池（Microbial desalination cell, MDC）[4]、微生物太阳能电池（Microbial solar cell, MSC）[5] 等；（2）电化学工作站，该方法是利用电化学工作站的工作电极作为微生物的载体，利用恒定电位[6]、电位阶跃技术[7]、恒电流[8]等方法培养EABs.

1.2 电活性微生物的种类

电活性微生物是EABs研究的核心要素. 根据电子在电活性微生物与载体间的传递方向，可将EABs划分为阳极电活性生物膜（输出电子）和阴极电活性生物膜（接受电子）.

从Kim发现和分离*Shewanella putrefaciens* IR-1至今，研究者已经分离、鉴定出了近50 株电化学活性菌[9]. 对阳极EABs的研究发现，其微生物种类具有高度的多样性，几乎遍及所有的细菌门，尤以变形杆菌门（Proteobacteria）和硬壁菌门（Firmicutes）最为常见. 其中，地杆菌属的*Geobacter sulfurreducens*和希瓦氏菌属的*Shewanella oneidensis* MR-1是研究得最多的模式菌株，目前已完成全基因组测序. 这些电活性微生物大多为不形成芽孢的革兰氏阴性菌，关于革兰氏阳性菌产出电子的研究较少. 从能量代谢角度分析，革兰氏阳性菌的细胞壁含有大量肽聚糖，其厚度远大于阴性菌，阻隔电子的传导，因而一般认为革兰氏阳性菌的电化学活性不明显. 典型的革兰氏阴性电活性微生物主要有：腐败希瓦氏菌（*Shewanella putrefaciens*）[10]、硫还原地杆菌（*Geobacter sulfurreducens*）[11]、铁还原红育菌（*Rhodoferax ferrireducens*）[12]、嗜水气单孢菌（*Aeromonas hydrophila*）[13]、丙酸脱硫葱球菌（*Desulfobulbus propionicus*）[14]、肺炎克雷伯氏菌（*Klebsiella pneumoniae* L17）[15] 等. 革兰氏阳性菌株主要有：丁酸梭菌（*Clostridium butyricum* EG3）[16]、*Thermincola ferriacetica* Z-0001 [17]、*Bacillus subtilis* [18]、*Corynebacterium hum* MFC03 [19]、*Tolumonas osonensis* OCF 7^{T} [20]、*Thermincola Potens* JR [21]、*T. ferriacetica* DSM 14005 [22]、*Desulfitobacterium hafniense* [23]等.

尽管阳极EABs被广泛研究，但阴极EABs的研究却是近几年才出现，而且大多数是分析微生物群落，研究纯菌的例子较少. 既可输出电子又能接受电子的电化学活性细菌主要是*Geobacter*、*Shewanella*等属的几株细菌. 此外，在从电极接受电子的电化学活性细菌中，*Dechlorospirillum strain* VDY [24]能催化高氯酸盐还原菌，*Desulfovibrio desulfuricans*能对硫酸

盐还原[25]，*Geobacter metallireducens*能将硝酸盐还原为亚硝酸盐[26]，*Hlamydomonas reinhardtii* 能够捕获太阳能，同时在阴极产氧和催化氧气还原[27]，*Sporomusa ovata*能还原CO_2成乙酸和丁酸[28].

1.3 电活性生物膜电子传递机制

1.3.1 阳极EABs电子传递机制 阳极EABs电子由微生物传递到最终电子受体主要有以下4种机制（图2）：（1）直接接触机制：利用外膜上的Cyt-c直接将电子传递至受体.（2）纳米导线机制：利用细胞表面可导电的纤毛或菌毛（微生物纳米导线）将电子传递至受体.（3）电子穿梭体机制：利用外源介体或内生介体（自身分泌的代谢产物）将电子转移至电子受体. 外源介体有硫堇、可溶性醌、Fe(III)-EDTA、甲基紫精、中性红等. 内生介体包括微生物的初级代谢物（如H_2、H_2S和氨等）和次级代谢物（如吩嗪类色素和核黄素）.（4）应电运动机制（Electrokinesis）：微生物将氧化底物产生的电子储存在细胞表面，形成一个带电体，利用鞭毛运动快速撞击受体表面即离开的方式释放电子，然后重新氧化有机物参与下个循环. 上述几种电子传递方式不是孤立存在的. 在电子受体有限的自然环境中，微生物常运用上述4种机制，协同完成胞外电子传递.

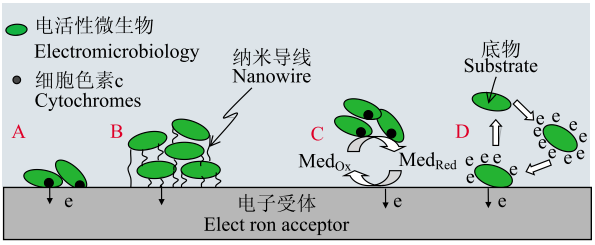

图2 阳极EABs电子传递机制[29]. （A）直接接触；（B）纳米导线；（C）电子穿梭；（D）应电运动.

Fig. 2 Schematics of anode EABs electron transfer mechanism [29]. (A) Direct contact through outer-membrane cytochrome; (B) Electron transfer through “nanowires”; (C) via exogenous or endogenous mediators; (D) via electrokinesis.

1.3.2 阴极EABs电子传递机制 关于电子从微生物传递至固态电子受体的机制已经进行了大量研究. 但是，其相反过程，即电子如何从电极等传递至微生物胞内，目前相关研究比较缺乏，主要存在以下几种方式（图3）：（1）直接电子传递. 在MES系统中，最重要的电子传递方式是直接电子传递. 研究者们从*Geobacter metallireducens*催化还原硝酸盐[26]、*Sporomusa ovata*还原CO_2成乙酸和丁酸[28]等试验中，证实了电子可以不经过任何介体直接从电极传递至胞内.（2）H_2介导的电子传递. 在MES系统中，很容易在阴极产生氢气，氢气可以在不影响微生物完整性情况下驱动微生物的代谢[30].（3）电子穿梭体介导的电子传递. 与阳极电子传递过程相似，电子穿梭体也可以有效地将电子从固体电极传递至微生物胞内. 跟H_2介导的电子传递相比，电子穿梭体往往水溶性比较好，因而可以更加有效传递电子.（4）中间产物介导电子传递. 在以上电子传递基础上，微生物从电极获取了大量电子，合成一定量的甲酸或乙酸，这些小分量有机酸可以被体系中的其他微生物利用，用于产生分子量更大的产物[31].

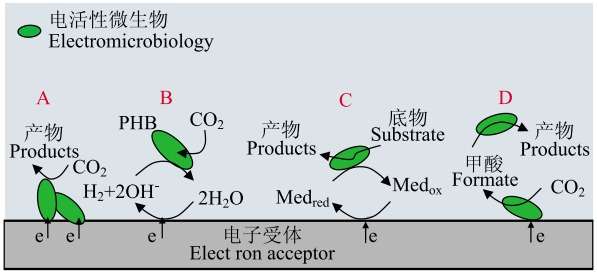

图3 阴极EABs电子传递机制示意图[32]. （A）直接接触；（B）H_2介导的电子传递；（C）电子穿梭体介导的电子传递；（D）中间产物介导电子传递.

Fig. 3 Mechanisms for electron transfer from electrodes to microorganisms [32]. (A) Direct contact; (B) H_2 as electron shuttle; (C) common electron shuttle; (D) middle products as electron shuttle.

2 电活性生物膜的表征

生物膜是个复杂的结构，其模型主要有以下几种：（1）平面二维同质结构，并具有相对恒定的厚度；（2）“异质镶嵌”结构，众多微生物借助胞外聚合物的作用聚集形成许多小的叠状体，叠状体再进一步地联结形成宏观可见的柱状物；（3）“蘑菇或郁金香”结构，由类似蘑菇或郁金香形状的微菌落组成，膜底比膜顶窄，外形类似蘑菇或郁金香的结构. 生物膜结构与其功能密切相关，因而生物膜结构表征，有利于对生物膜功能的深入理解. 对EABs结构的表征可从4个层面进行：（1）整个生物膜，（2）处于生物膜中的单个细胞，（3）分离的独立单个细胞，（4）细胞膜蛋白以及基因等[33]. 其表征技术多种多样，比较常用的EABs表征技术见表1.

全面了解EABs的结构、特性、功能须结合电化学、光谱学、显微镜、分子生态学等多种表征技术. 其中电化学方法是研究EABs电活性最直接、有效的方法，以伏安法、电化学阻抗谱、塔菲尔曲线等较为常见. 电化学方法（如伏安法）能表征EABs中参与电子转移过程的关键电活性组分性质（氧化还原性质、电子转移可逆性等）. 光谱电化学是基于电化学技术改进的方法，它能对生物膜进行活体、原位检测，已成为近年来得到极大发展的EABs表征技术. 其中，紫外/可见光谱是最简便的一种. 在微观技术方面，扫描电镜和激光共聚焦荧光显微镜是目前常用的直接观察分析生物膜结构的技术. 通过扫描电镜可对细菌形貌和结构、生物膜厚度、有无纳米导线等更加清晰的了解. 激光共聚焦荧光显微镜可在微观尺度实现对生物膜中有机物和微生物种类的组成及其空间分布进行原位、非破坏性表征. 在分子层面上，DNA/RNA、功能基因和蛋白质组学等比较常用的分子生物技术是研究微生物种类、群落结构、电子传递途径分子机制的重要工具.

3 电活性生物膜的应用

一直以来，对EABs的研究大多数是采用MFC装置，涉及产电和污水处理等方面. 近几年来，EABs的更多应用逐渐被人们发现，比如物质电合成、贵金属还原、CO_2还原等（图4）.

3.1 阳极生物膜的应用

（1）污水处理：从简单的小分子有机酸到复杂的木质

表1 电活性生物膜的常用表征技术

Table 1 Common analysis methods for electrochemically active biofilms

方法 Method		简要描述 Brief description	在电活性生物膜上的主要用途 Applications in electrochemically active biofilms
电化学分析方法 Electrochemical Methods	循环伏安法 Cyclic Voltammetry (CV)	循环伏安法是电化学的一种，它控制电极电势以不同的速率，随时间一次或多次反复扫描，并记录电流–电势曲线. CV is an electrochemical technique that applies a polarization potential scan from an initial polarization potential to a final polarization potential and measures the current.	CV可用于测量EABs的催化特性，确定氧化还原物质的氧化和还原电位，确认EABs是否具有电子转移能力[34]. CV is used to measure the catalytic activity of EABs, to identify the biofilms electrode potential at which active redox couples are oxidized or reduced, and to examine whether extracellular electron transfer occurs in EABs [34].
	Tafel曲线 Tafel equation	Tafel方程描述了电极表面电子传递动力. The Tafel equation simplifies the kinetics of electron transfer.	Tafel曲线用于研究EABs的电荷转移动力学参数，如氧化还原斜率和极化电阻，从而揭示电子转移过程，也能用于确认膜中活菌的存在是否[35]. Tafel plots were plotted to calculate the kinetic parameters such as oxidative and reductive slope and polarization resistance, to reveal the electron transfer process. It also can be used to identify the living cells in the biofilms [35].
	电化学阻抗谱 Electrochemical Impedance Spectroscopy (EIS)	交流阻抗谱是给系统一个频率不同的交流电势波，测量交流电势与电流信号的比值，从而反应系统的阻抗实部（电阻）和虚部（电容）电化学特性. EIS applies a sinusoidal potential waveform to measure the real impedance (resistance) and imaginary impedance (capacitance) of an electrochemical system over a range of frequencies.	交流阻抗谱用于测量生物膜生长、生物膜点到电导、电子转移机理. 测试过程中反应器运行不中断，不干扰微生物的培养，用于测量内阻的数值,区分内阻的3个组成部分：欧姆电阻、极化电阻和扩散阻力[34]. 研究产电菌分泌的氧化还原介体的电化学行为. EIS is a solid analytical technique for examining biofilm growth, biofilm conductivity and electron transfer mechanisms. It has been used as a nondestructive electrochemical technique to study the development of charge transfer resistance in EABs [34].
光谱学 Spectroscopy	表面增强红外吸收光谱 Surface Enhanced Infrared Absorption Spectroscopy (SEIRAS)	SEIRAS是利用金属纳米岛状膜受红外辐射激发的表面等离子共振与吸附物种振动的拐合使其红外吸收增强的一种光谱技术. SEIRAS is a strictly surface sensitive technique that exploits the electromagnetic properties of nanostructured metal films to enhance the vibrational bands of a molecular adlayer.	Busalmen J等首次报道了电化学结合傅里叶变换–表面增强红外吸收光谱方法用于研究*Geobacter sulfurreducens*与电极间的相互作用[36]. SEIRAS can be used to measure the redox state of c-type cytochromes on *G. sulfurreducens* cells attached to gold electrodes and determine that c-type cytochromes are directly oxidized/reduced by the biofilm electrode [36].
	表面增强拉曼散射光谱 Surface Enhanced Raman Scattering: SERS	SERS技术是利用吸附在粗糙化金属表面的化合物由于表面局域等离子激元被激发所引起的电磁增强，以及粗糙表面上的原子簇及吸附其上的分子构成拉曼增强的活性点，这两者的作用使被测定物的拉曼散射产生极大的增强效应. SERS is a surface-sensitive technique that enhances raman scattering by molecules adsorbed on rough metal surfaces or by nanostructures such as plasmonic-magnetic silica nanotubes.	Millo D等建立电化学结合表面增强共振拉曼光谱原位表征电活性生物膜的方法，利用表面增强共振拉曼光谱对电子转移过程中膜结合细胞色素结构分析，进一步证明了其在胞外直接电子转移过程的重要作用[37]. SERR can be used to probe selectively the heme groups solely of the proteins in the electrode surface, and to provide further valuable contributions as it is the only *in situ* methodology that allows probing of the structure and function of outer membrane cytochromes in biofilms [37].
	紫外/可见光谱 Ultraviolet–Visible Spectroscopy (UV/Vis)	紫外/可见光谱是利用物质的分子或离子对紫外和可见光的吸收所产生的紫外可见光谱及吸收程度可以对物质的组成、含量和结构进行分析、测定、推断. UV/Vis refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region.	活体细菌中的血红素铁具有很高的摩尔吸光系数，因而紫外/可见光谱能有效表征活体细胞色素[34]. 活体内氧化/还原的细胞色素c与纯化的细胞色素c吸收峰位置具有较大的差异[38]. UV/vis spectroscopy can be used to analyze the redox state of c-type cytochromes in biofilms [34]. And it can be used to compare the *in vivo* oxidation/reduction of c-type cytochromes (attached to whole cells) with the oxidation/reduction of purified c-type cytochromes [38].
微电极 Microelectrode	微电极 Microelectrode	微电极是指尖端的直径小于10 μm的一类电极，是一种原位、无破坏技术. Microelectrodes have a tip diameter less than 10 μm. So it is a nondestructive *in situ* technique.	对被测物体最少的扰动，可以利用微电极检测阴极生物膜反应过程中内部微环境OH-和O_2变化情况[34]. Microelectrodes have been used to measure concentrations of oxygen, hydrogen, hydrogen sulfide, and carbon dioxide, as well as pH, redox potential, and local flow velocities [34].
显微镜技术 Microscope	激光扫描共聚焦显微镜 Confocal Laser Scanning Microscopy (CLSM)	激光扫描共聚焦显微镜是利用激光点作为荧光的激发光并通过扫描装置对标本进行连续扫描，并通过空间共轭光阑阻挡离焦平面光线而成像的一种显微镜. CLSM scans the three dimensional surface of an object point-by-point by means of a focused laser beam, and creates the over-all picture by electronic means similar to those used in scanning electron microscopes.	激光共聚焦显微镜能在微观尺度对生物膜进行原位、非破坏性的观察，如：表面覆盖度、生物量、厚度、粗糙度等[39]. 该技术成功的被用于评价地杆硫还原菌分别以富马酸和电极作为电子受体的不同结构[40]. 同时也可结合荧光染色技术观察活菌在生物膜中的分布情况等[41]. CLSM is used to monitor biofilm parameters such as surface coverage, biovolume, thickness, and roughness with current [39]. It has been used to monitor the difference in biofilm structure between *G. sulfurreducens* biofilms grown on fumarate and an electrode[40], and to locate individual strains in a mixed culture biofilm [41].
	扫描电子显微镜 Scanning Electron Microscope (SEM)	扫描电子显微镜是利用电子束扫描样品表面从而获得样品信息的电子显微镜. SEM is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons.	从微观的角度观察微生物形貌和结构、生物膜厚度、有无纳米导线等. SEM provides a two-dimensional projection or a two-dimensional image of a sample in nanometer range.
	原子力显微镜 Atomic Force Microscopy (AFM)	原子力显微镜是通过检测样品表面和一个微型力敏感元件之间的极微弱原子相互作用力来研究物质的表面结构及性质. AFM provides a 3D surface profile, that does not require any special treatments that would irreversibly change or damage the sample.	利用原子力显微镜结合导电探针可以对微生物纳米线的导电率进行测定[42]. AFM can be used to study biological macromolecules and even living organisms. It can also be combined with a conductive probe to determine the conductivity of nanowire [42].

续表1 Table 1 (Continued)

方法 Method		简要描述 Brief description	在电活性生物膜上的主要用途 Applications in electrochemically active biofilms
分子生物技术 Biotechnology	DNA/RNA	DNA/RNA是物种的区别标志. DNA/RNA is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms.	目前为止，希瓦氏菌和硫还原地杆菌是产电微生物研究的模式菌，已完成全基因组测序，有很好的遗传背景,对产电微生物和电子传递机理的深入研究基本都是以希瓦氏菌和硫还原地杆菌为模式菌进行研究. Until now, the DNA sequencing of *G. sulfurreducens* and *Shewanella oneidensis* MR-1has made them the reference type strains for electromicrobiology.
	变性梯度凝胶电泳 Denatured Gradient Gel Electrophoresis (DGGE)	变性梯度凝胶电泳是利用不同梯度的变性胶，将不同双链DNA 分开，从而区别不同的物种. DGGE is a microbial fingerprinting technique that separates amplification of roughly the same size based on sequence properties.	研究电活性生物膜上微生物的多样性及其功能优势菌种[43]. DGGE can be used for a rapid analysis of microbial diversity in biofilms. The information of the relative abundance of the different genotypes can be yielded by DGGE. Many lineages previously unknown can be detected by purifying and sequencing the characterized bands [43].
	末端限制性片段长度多样性 Terminal-Restriction Fragment Length Polymorphism (T-RFLP)	将DNA末端荧光物质标记的PCR产物进行限制性内切酶酶切，检测末端带有荧光性标记的片段.通过这些末端标记的片段就可以反应微生物群落组成情况. T-RFLP is a molecular biology technique for profiling of microbial communities based on the position of a restriction site closest to a labeled end of an amplified gene.	利用末端限制性片段长度多样性可以对不同的pH和不同的接种来源形成的电活性生物膜的微生物结构和组成多样性进行分析，研究对优势菌形成和电池性能起决定的作用的影响因素[44]. T-RFLP is used to prove the presence of certain bacteria in the biofilms, and to analyze the variation of community in biofilms under different conditions like pH-value and buffer capacity/ ionic [44].
	功能基因和蛋白质组学Proteomics and Functional Genomics	从基因组的角度解释微生物的宏观特性（如产电等）. Proteomics is the large-scale study of proteins, particularly their structures and functions. Functional genomics is a field of molecular biology that attempts to make use of the vast wealth of data produced by genomic projects to describe gene (and protein) functions and interactions.	从功能基因的角度研究电活性微生物产电的最本质控制因子，从而可以从根本上提高电子传递效率[45]. In order to improve the performance of microbial fuel cell radically, the genome and proteome information that control the electron transfer rate between the electroactive microorganism and supporter should become a focus of research [45].

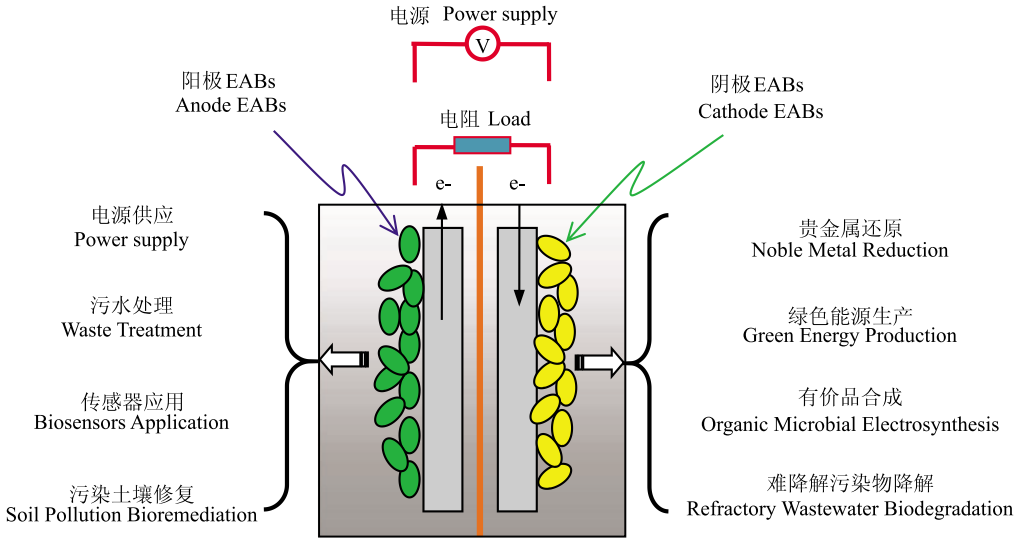

图4 电活性生物膜的应用示意图.

Fig. 4 Schematic representation of applications of EABs.

纤维素都可作为被EABs利用，因此，EABs可处理各种类型的废水，从低浓度的生活污水到高浓度养殖废水[46-47]、皮革废水[48]、难生物降解的污水[49]等.（2）电源供应：EABs的最基本特征是产生电能，虽然目前的产电规模还未能达到商业利用，但是用于传感器等耗电较低的仪器当做电源还是足够的. 特别是为偏远地区和极端环境下的远程环境监测提供了电源[50-51].（3）传感器应用：根据EABs与溶液中物质的种类、浓度、有毒离子等的关系，可用作底物含量的测定、制作各种生物传感器，如BOD传感器、毒性传感器等[52-53].（4）土壤原位修复：生物修复通过活性微生物将有害物质分解、脱毒，采用这种简单易行、环境友好和低成本的方法替代物理化学治理已经得到普遍认可. 研究表明*G. sulfurreducens*能利用电极作为电子供体将U(Ⅵ)还原为U(Ⅳ)，生成不溶性沥青铀矿，然后将电极从受污染的地区拔出，从而实现铀沉积物的回收，使得污染土壤得到修复[54].

3.2 阴极生物膜的应用

（1）难降解污染物处理：在体系中存在Fe^{2+}的情况下，利用氧气作为阴极EABs的电子受体，可构造生物电 Fenton 体系，从而可对RhB等物质进行脱色处理[55]. 近年来，发现EABs生物膜可以通过电子穿梭机制直接或间接接受电子，从

而还原难降解有机物，如高氯酸[56]和氯取代基有机物[57]等，这为土壤污染治理和原位生物修复提供了一条新途径.（2）绿色能源生产：在外加电源的作用下，阳极EAs微生物氧化有机物，输出的电子经外电路到达阴极，产生的质子通过质子膜扩散到阴极，两者在阴极产生氢气[58]. 与传统制氢技术相比，EABs制氢具有：基质利用率高、纯度高等优势[59].（3）贵金属还原：EABs还原贵金属是一种低成本、环境友好、快速可控方法. 利用Cu^{2+}、Au^{+}、Pd^{2+}接受EABs氧化有机物释放的电子，实现金属离子的还原回收[60-62]. EABs介导合成的金属和金属–半导体纳米复合材料有望为纳米材料合成提供一种新途径.（4）有机品电合成："微生物电合成（Microbial electrosynthesis）"这一概念最早由Lovley研究组于2010提出. 研究发现，电活性微生物可以接受电极的电子，将CO_2还原为乙酸以及少量的2-羰基丁酸[28]. 另外，通过EABs系统富集产甲烷古菌，也可以而将CO_2转化为甲烷[63]. 在自然条件下，产乙酸菌通过Wood-Ljungdahl路径利用H_2作为电子受体将CO_2还原成乙酸[64]. 这些发现为解决能源危机提供新思路. 它不但提供一种捕获温室气体CO_2的新方法，也提供一种将CO_2转化为能源的新途径.

4 研究展望

目前，对于EABs的了解极其有限，并且缺乏一个统一的、标准的表征方法. 对它的组成、电子传递机制和功能的认识还有很多疑问，特别是阴极EABs的种类、电子传递机制等还处于起步阶段. 因此，需结合电化学、微电极、蛋白组学等技术对其电子传递途径进行进一步的探索. 在应用方面，虽然已经证实EABs有广泛的应用，而且有一些已经用于实际生活，比如，利用EABs的产电能力为海上分析检测设备、气象学浮标提供电源. 但是，要将这些应用规模化、产业化，要使得EABs生物传感器适应实际污水，并且稳定运行，要使得EABs真正原位修复污染土壤，还面临极大的挑战. 因此这些问题的解决将是今后研究的主题之一. 另一方面，阴极EABs可还原贵金属、电合成有价品等应用的发现，给我们的研究工作带来极大的鼓舞，特别是利用EABs还原CO_2制甲烷，不但提供了一种捕获温室气体CO_2的新方法，也提供了一种将CO_2转化为能源的新途径. 这个发现将给人类的生存、生产带来重要的影响，未来有望缓解全球范围内日益严峻的能源问题. 由于这些探索还处于起步阶段，如何降低能耗并提高产甲烷效率、提高EABs制氢系统的稳定性等，尚有待深入研究.

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