



活性污泥中电活性微生物的富集筛选研究进展

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摘要: 活性污泥含有多种微生物, 是废水处理系统中污染物降解转化的重要驱动者。电活性微生物(Electroactive microorganisms, EAMs)是活性污泥的重要微生物类群, 在废水资源化与能源化中发挥着核心关键作用。本文概括了活性污泥中 EAMs 的富集和筛选方法, 整理总结了目前已从活性污泥中获得的 EAMs 菌种资源信息, 在此基础上分析了从活性污泥中富集和筛选 EAMs 所存在的问题, 并进一步展望了未来的研究方向, 以期加速 EAMs 菌种资源的挖掘, 促进以 EAMs 为主导的废水资源化与能源化技术发展。

关键词: 电活性微生物, 活性污泥, 富集, 筛选, 废水资源化与能源化

Advances of enrichment and screening of electroactive microorganisms from activated sludge

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Abstract: Activated sludge contains a variety of microorganisms and is the key driver for pollutant degradation and transformation in wastewater treatment plants. Electroactive microorganisms (EAMs) is the important component of activated sludge and plays important roles in wastewater recycling and energy recovery. This review summarizes the methods for enrichment and screening of EAMs from activated sludge, and sums up the species of EAMs obtained from activated sludge so far. In addition, the existing problems of enrichment and screening for EAMs as well as the future research direction are also discussed, so as to promote the discovery of EAMs and the development of wastewater recycling and energy recovery

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technologies in which EAMs play the leading roles.

Keywords: Electroactive microorganisms, Activated sludge, Enrichment, Screening, Wastewater recycling and energy recovery

电活性微生物(Electroactive microorganisms, EAMs)是指能够通过细胞电子传递链与胞外电子供体或受体发生电子交换的一类微生物^[1-3]。目前所发现的 EAMs 主要分为两大类,一类可以将自身代谢过程中所产生的电子传递给胞外的电子受体,称作产电型微生物(Exoelectrogenic microorganisms);另一类可以接收胞外的电子来完成自身的呼吸代谢,称为电营养型微生物(Electrotrophic microorganisms)^[4-5]。这些微生物参与了多种重要的元素循环过程,在污染治理、生物能源和绿色化工等方面展现出广阔的应用前景^[6-12]。

活性污泥法利用活性污泥中微生物的呼吸代谢作用对废水中的污染物进行有氧或厌氧降解转化,是目前应用最为广泛的废水处理方法^[13]。EAMs 是活性污泥微生物种群的重要组成部分,在废水中污染物降解转化、资源化(再生水回用)和能源化(厌氧发酵产甲烷、生物制氢和电能产生)过程中扮演着重要角色^[14-21]。为了更好地认识和了解活性污泥中 EAMs 的种类及其功能活性特点,加速废水处理过程,促进其资源化和能源化进程,近年来科学家以活性污泥作为接种源,建立了多种 EAMs 富集选育方法,获得了多种新型的 EAMs。本文重点综述了活性污泥 EAMs 的富集和筛选方法,总结了从活性

污泥中分离获得的 EAMs 菌种资源信息,同时还分析了 EAMs 富集选育中所存在的问题,并展望了未来的研究方向,以期进一步提高 EAMs 菌种资源的挖掘效率,加速以 EAMs 为核心驱动力的废水资源化与能源化技术发展。

1 电活性微生物的富集和筛选

1.1 电活性微生物的富集

由于 EAMs 在活性污泥中的相对丰度较低,为了提高 EAMs 的分离选育效率,通常需要对活性污泥中的 EAMs 进行富集。已建立的 EAMs 富集方法主要是利用 EAMs 所具有的胞外电子传递功能特性,包括 Fe³⁺还原富集法、微生物燃料电池(Microbial fuel cells, MFCs)电极富集法、微生物电解池(Microbial electrolysis cells, MECs)电极富集法和恒电位仪-电化学系统富集法。这几种方法均具有各自的反应特点和优缺点,具体如表 1 所示。

1.1.1 Fe³⁺还原富集法

铁是自然界中最丰富的具有氧化还原活性的金属元素,主要以铁氧化物的形式普遍存在于多种环境中^[22]。Fe³⁺还原富集法最早用于富集筛选铁还原菌,其基本原理是 Fe³⁺作为一种胞外电子受体可以接收来自 EAMs 呼吸代谢所产生的电子被还原为 Fe²⁺,从而通过检测 Fe³⁺的还原情况来判断 EAMs

表1 电活性微生物富集方法的比较

Table 1 Comparison of methods for enrichment of electroactive microorganisms

方法 Methods	特征 Feature	优点 Advantage	缺点 Disadvantage	参考文献 References
Fe ³⁺	Fe ³⁺ was reduced to Fe ²⁺ on anaerobic conditions	Convenient; Commercial	Poor specificity; Low efficiency	[22-23]
MFCs	The method based on current production from the bacterial metabolism	Obvious; High community diversity on enrichment	Time-consuming; Low efficiency on enrichment of new species	[24-25]
MECs	A potential is applied to the anode, hydrogen production from cathode	Strong selectivity	Noncommercial; Needs power supply	[26]
Potentiostat-controlled bioelectrochemical cells	The method based on three-electrode set-up	The potential of electrode can be controlled		[27]

的富集效果。由于前期研究发现的 EAMs 大部分是典型的铁还原菌^[4]，因此越来越多的研究团队选用该方法来富集筛选 EAMs。Zhang 等以蔗糖废水中的厌氧污泥作为接种源，利用 Fe_2O_3 作为电子受体，富集获得了以 *Desulfovibrio* 为优势物种、具有电活性的铁还原菌群^[28]。Shen 等则以污水处理厂的厌氧污泥作为接种物，使用 Fe(III)-EDTA 作为电子受体，富集获得了能够降解 1,4-二噁烷能力的具有电活性的铁还原菌群^[29]。尽管该方法成本较低，但是随着研究的深入，发现并非所有的 EAMs 都能进行 Fe^{3+} 还原^[30-31]，也并非所有的铁还原菌都具有电化学活性^[32]，该方法针对性差、效率低的缺点逐渐凸显^[22-23]。

1.1.2 MFCs 电极富集法

MFCs 电极富集法主要是以 MFCs 阳极室的电极作为微生物胞外呼吸的电子受体从而富集 EAMs (图 1)。MFCs 根据有无质子交换膜可分为两种构型：单室 MFCs 和双室 MFCs。Xing 等采用污水处理厂初沉池污泥作为接种物，利用单室 MFCs 作为富集

装置，获得了以 *Firmicutes* 为优势物种的电活性菌群，并从中分离出一株具有脱氮功能的 EAM——*Comamonas denitrificans*^[30]。Zhou 等选用生活污水处理厂的活性污泥作为接种源，利用双室 MFCs 富集分离到一株兼性厌氧的 EAM——*Citrobacter freundii* LZ-1^[33]。Park 等在探索底物预驯化过程对 MFC 阳极微生物群落结构影响的相关研究中发现，在没有任何底物进行预驯化的情况下，以活性污泥为接种源的 MFC 阳极富集到大量的 *Geobacter*^[34]。除此之外，Zhao 等在研究底物转化对 MFC 阳极微生物群落的影响时发现，乳酸作为底物也会使 *Geobacter* 在阳极得到富集^[35]。目前，多数实验研究主要采用双室 MFCs 来对活性污泥中的 EAMs 进行富集^[36-38]，但是常规双室 MFCs 装置需占用较大空间且运行时间较长^[24]。为此，Zuo 等开发设计了 U-型管双室 MFCs 装置，不仅克服了常规双室 MFCs 存在的不足，而且该装置可以使阳极室中原本呈悬浮态的 EAMs 也得到富集^[31]。Yu 等选取污水处理厂初沉池污泥作为接种源，采用自行设计的 U-型管 MFCs

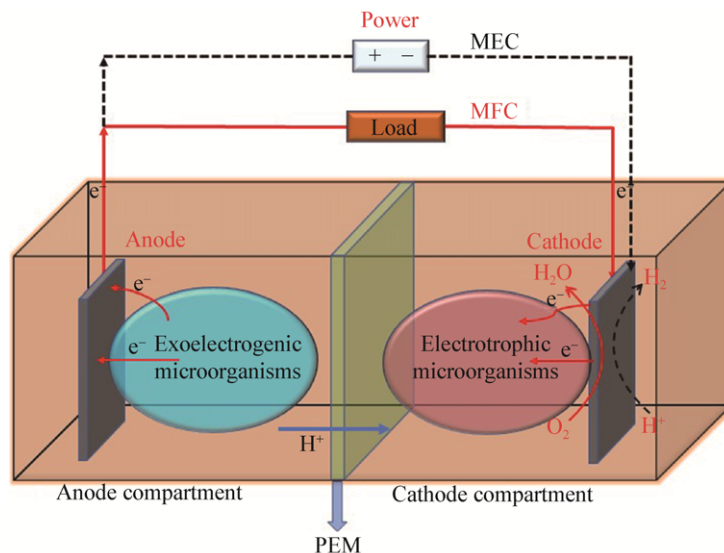


图 1 MFC/MEC 富集原理示意图

Figure 1 Schematic diagram of the MFC/MEC for enrichment

注：单室 MFC/MEC 不含质子交换膜，双室 MFC/MEC 被质子交换膜分隔成阴阳两极室；MFC 无需外界能量输入，MEC 需外界提供能量。

Note: Single-chamber MFC/MEC does not contain a proton exchange membrane, whereas dual-chamber MFC/MEC contains a proton exchange membrane to separate cathode compartment and anode compartment; The MEC needs power supply, while MFC does not.

装置, 富集获得了具有一定电活性的 *Ochrobactrum* sp.、*Achromobacter* sp. 和 *Acinetobacter* sp.^[39]。MFCs 电极富集法效果明显直观、针对性强。然而 MFCs 装置和材料的成本也较高, 运行时间较长, 且对于新的 EAM 的富集效果并不理想^[40]。

1.1.3 MECs 电极富集法

MFCs 电极富集法的基本原理是在 MECs 中微生物氧化其中的基质产生电子和质子, 在阴极室通过化学或者生物催化作用结合生成氢气, 而产电型 EAMs 主要被富集在阳极表面和阳极液体中, 而生物阴极中会存在少量的电营养型 EAMs^[26]。MECs 与 MFCs 的基本结构相似, 也具有阴阳两极, 以及连接阴阳两极的外电路^[41-42], 不同在于 MECs 需要一个外加电压, 因而 MECs 系统消耗电能(图 1)。MECs 也有 2 种不同构型, 分别为单室和双室(有无质子膜)。Zhao 等选取污水处理厂二沉池的剩余活性污泥作为接种物, 以单室 MECs 作为富集装置, 获得了高效促进活性污泥中甲烷产生以 *Geobacter* 为主要物种的产电菌群^[43]。Liu 等以污水处理厂的厌氧污泥(初沉池和二沉池污泥进行混合)为接种源, 乙酸钠为电子供体, 利用双室 MECs 装置富集获得了具有较高库仑效率的 EAMs 混合培养物(包括 *Desulfovibrio*、*Rhodospseudomonas*、*Shewanella* 和 *Geobacter*)^[44]。Nam 等的研究发现, 微生物电解池可以用来处理纤维素废水, 而且可以实现废水中蛋白质的去除, 其阳极富集到的细菌以 *Deltaproteobacteria* 为主, 古菌则主要是 *Methanobacterium*^[45]。Yin 等的研究发现, 当生物炭(Biochar, BC)作为催化剂投加到 MEC 后, 阳极上产电菌 *Thermincola* 的丰度则大幅度降低^[46]。该方法选择性较强, 然而富集过程中需要额外提供能量, 导致其运行成本较高, 同时也比较耗时^[40]。

1.1.4 恒电位仪-电化学系统富集法

恒电位仪-电化学系统富集法主要利用电化学系统的工作电极作为微生物胞外电子传递的电子受体而实现对 EAMs 的富集(图 2)。恒电位仪是用来控制工作电极(阳极或阴极)电位的一种设备。恒

电位仪控制的生物电化学系统主要是由一个工作电极(电活性生物膜形成)、一个用来平衡电荷的对电极以及一个参比电极组成^[27]。Yoon 等利用三电极电化学电池装置, 以污水处理厂的厌氧污泥为接种物, 人工合成废水作为电子供体, 短时间内(一周)就富集获得了具有稳定产电能力的 EAMs 混合培养物^[47]。Liu 等的研究发现, 通过连续的电化学选择和生物膜驯化过程可以提高生物膜的产电能力, 而且在其工作电极形成了以 *Geobacter* 为主的电活性生物膜^[48]。Doyle 等也利用此方法, 以乳酸盐作为底物, 在工作电极上富集获得了以 *Geobacter metallireducens* 为主要物种的电活性菌群, 并从中分离获得了 2 株纯培养物^[49]。相比于 MFCs, 该系统可以更好地对 EAMs 的特性进行量化, 而且能够稳定控制工作电极的电势, 因此理论上可以有效地富集到更多的 EAMs。但是该系统的成本要远远高于 MFCs, 所以目前在 EAMs 富集上的应用有限。

1.2 电活性微生物的快速筛选方法

为了更好地分离获取 EAMs, 需要对富集到的微生物群落中的功能菌株进行特异性地筛选, 以便进一步认识和了解 EAMs 的分类及其功能活性机理。MFCs 和 MECs 等生物电化学系统是筛选 EAMs 的经典方法, 已经有较多相关的文章^[25,40,50]报道。

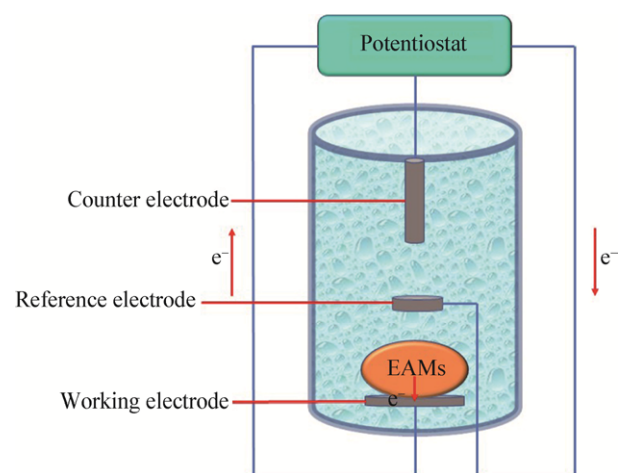


图 2 恒电位仪-电化学系统富集原理示意图

Figure 2 Schematic diagram of potentiostat-electrochemical system for enrichment

但由于该方法通常需要电化学分析设备, 成本较高, 操作较为繁琐, 且需要多学科知识背景, 近些年研究者们建立了越来越多的快速筛选方法。我们重点介绍 WO_3 纳米探针显色法、过氧化物酶显色法和染料还原法 3 种方法(表 2)。

1.2.1 WO_3 纳米探针显色法

WO_3 纳米探针显色 WO_3 是一种具有六角结构的纳米材料, 拥有独特的比色特性^[53], 因其具有良好的导电性、选择性和生物相容性, 已经被广泛用作电致变色探针^[54-55]。 WO_3 纳米探针显色法的基本原理是利用 WO_3 接收来自微生物的电子后会发生明显的颜色变化, 从而实现 EAMs 的检测和筛选。 WO_3 纳米探针可以快速捕获来自 EAMs 的电子, 使其颜色由白色变成蓝色, 捕获的电子越多则颜色就变得越深^[56]。而且从微生物到 WO_3 的胞外电子传递方式与微生物到电极的胞外电子传递方式十分相似。Yuan 等利用合成的 WO_3 纳米探针, 以 96 孔板作为反应装置, 对分离于活性污泥的 12 株菌进行电化学活性筛选^[51,57]。Yang 等采用 WO_3 纳米探针耦合 MPN (The most probable number) 法来实现对来自城市污水处理厂样品中 EAMs 的快速检测和计数^[56]。Marques 等则利用微波水热合成法合成 WO_3 纳米颗粒, 然后与未经处理的打印纸相融合形成一层活性传感器来检测 EAMs^[58]。利用 WO_3 纳米探针可以快速、直观和有效地对 EAMs 进行检测和筛选, 其成本较低且可实现对 EAMs 高通量的鉴定和分离, 但是 WO_3 纳米材料的合成相对复杂。

1.2.2 过氧化物酶显色法

外膜的细胞色素 c (Outer membrane c-type cytochromes, OMCs) 是胞外呼吸的核心组分, 它们可以将来自细胞质和内膜氧化酶的电子传递到胞外^[59-60]。OMCs 是含铁卟啉的酶蛋白, 可通过色原/过氧化物系统起到催化作用^[61]。铁卟啉的过氧化物酶活性已经被用来描述参与微生物胞外呼吸功能蛋白的特性^[62]。过氧化物酶显色法的基本原理是利用四甲基联苯胺 (3,3',5,5'-Tetramethylbenzidine, TMB) 与过氧化物酶反应发生明显的颜色变化(变成蓝色)来检测微生物的胞外呼吸能力, 完成对 EAMs 的检测筛选。本方法实验过程主要在 96 孔板上完成, 操作简单而且反应比较迅速, 因此可快速、高通量地对微生物的胞外呼吸能力进行测定, 从而检测筛选出具有胞外呼吸能力的微生物。Wen 等利用该方法实现了对电活性微生物 *Shewanella oneidensis* 的快速检测^[63]。Zhou 等采用此方法完成了对 *Escherichia coli* 和 *Geobacter sulfurreducens* PCA-1 电活性的快速鉴定^[52]。TMB 具有检测灵敏度高、稳定性好、安全无毒等优点, 然而该方法无法对微生物的胞外产电能力进行评估^[52]。

1.2.3 染料还原法

染料按照其分子结构性质可分为高极性染料和低极性染料, 根据相似相溶原理, 低极性染料可以进入到细胞内, 而高极性染料很难进入到细胞内。染料还原法筛选 EAMs 的基本原理是基于高极性染料在厌氧条件下可以作为胞外电子受体被微

表 2 电活性微生物快速筛选方法

Table 2 Rapid methods for screening electroactive microorganisms

方法 Methods	特征 Feature	优点 Advantage	缺点 Disadvantage	参考文献 References
WO_3 probe	WO_3 nanorods accepting electrons resulted color change	High throughput; Rapid; Sensitive	Complex synthesis of WO_3 nanorods	[51]
TMB	A colorimetric assay based on immunomagnetic capture and bacterial intrinsic peroxidase activity	High throughput; High efficiency; Quantitative	Unsuitable for evaluating exoelectrogenic ability	[52]
Dyes-reducing	A photometric assay based on high-polarity dyes degradation outside the cell	High throughput; Convenient; Commercial	Unable to evaluate exoelectrogenic ability of mixed cultures	[32]

生物还原脱色, 因此可通过颜色变化筛选具有胞外电子传递能力的微生物。我们团队利用这一方法, 从纺织印染废水活性污泥中分离获得了具有典型电活性的脱色希瓦氏菌(*Shewanella decolorationis* S12)^[64]。此外, 我们还运用荧光共振能量转移技术, 设计了高极性的染料荧光探针 NA-OG, 实现对 EAMs 的快速分离选育^[65]。Xiao 等也利用此方法实现了对 *Shewanella oneidensis* MR-1 及其突变株电活性的快速检测^[32]。该方法既可以借助 96 孔板也可以利用流式细胞仪开展工作, 通过监测染料的脱色情况评估微生物的胞外电子传递能力, 从而实现对 EAMs 的高通量快速筛选, 具有操作简便、成本低、效率高的优点^[32]。然而随着研究的深入, 研究发现并非所有能实现对高极性染料还原脱色的微生物都具有电活性^[66-68], 所以该方法也需要进一步的完善和优化。

2 从活性污泥中获得的 EAMs

为了更好地认识和掌握活性污泥中 EAMs 的功能活性特点, 充分发挥 EAMs 在废水资源化和能源化过程中的作用, 研究者已从活性污泥中富集分离了近 40 株 EAMs。到目前为止所获取的 EAMs 纯培养物基本上都归属于细菌, 且主要来自 *Proteobacteria*、*Firmicutes* 和 *Actinobacteria*, 尤其以 *Proteobacteria* 居多(图 3)。比如 Rezaei 等利用 U 型管 MFCs 富集分离到了可以利用纤维素作为底物进行产电的 *Enterobacter cloacae*^[70]; Xing 等从 MFCs 阳极生物膜上获得了具有脱氮功能的电活性菌 *Comamonas denitrificans*^[30]; Wu 等从 MFCs 的阴极获得了能够还原六价铬的电化学活性菌 *Bacillus* sp. 等^[89]。除细菌外, 研究者也从活性污泥中获得了具有电活性的真菌, 如 Lee 等以厌氧活性污泥作为接种源, 从 MFCs 的阳极生物膜上分离到了具有产电能力的酵母菌 *Candida* sp. IR11^[95]。总体而言, 到目前为止从活性污泥中分离选育的 EAMs 都以产电型微生物为主, 菌属种类仍相当有限, 产电效率也较低。这在很大程度上是由于活性污泥 EAMs 往往

需要比较独特的生长和营养条件, 已有的富集和筛选方法仍然存在一定的局限性所造成的。

近几年快速发展的高通量测序技术也为我们了解和获取活性污泥中尚未实现人工培养的 EAMs 基因组信息提供了很多便利^[96-98]。Eddie 等通过测定生物阴极微生物群落的宏基因组, 成功获得了尚未能进行人工培养的电营养型微生物 *Tenderia electrophaga* 的完整基因组, 并进一步结合宏转录组和宏蛋白组的结果, 推测该菌可以利用来自阴极的电子固定 CO₂^[99]。Mei 等通过测定不同温度下 MFCs 阳极生物膜的细菌 16S rRNA 基因序列, 发现在低温条件下生物膜的优势菌为 *Zoogloea*、*Simplicispira* 等未曾报道有电活性的菌属所组成^[97]。Xiao 等通过焦磷酸测序分析了 7 个不同阳极的微生物群落, 发现了 2 个未曾报道具有电活性的核心微生物, 并通过电活性测试验证了这 2 株菌均为电活性菌^[96]。虽然通过高通量测序技术不能获得电活性菌的实体培养物, 但是为进一步认识和了解活性污泥中的电活性微生物提供了新途径。随着流式细胞仪、微流控技术、单细胞分选等技术的发展和普及, 以及培养基组分的不断优化, 高通量测序技术将会为分离获取活性污泥中的电活性微生物提供有力的技术支撑。

3 问题与展望

尽管研究者们对活性污泥中 EAMs 的富集筛选进行了大量的努力和尝试, 但是目前所获得的 EAMs 菌种资源仍然十分有限, 种类也比较单一。一方面是由于所采用的富集选育方法仍不能很好地满足长期生长于活性污泥这个复杂群体中 EAMs 的生长和营养需求, 另一方面是由于活性污泥中存在着某些 EAMs 需要与周围的微生物互营共生。为了建立新型高效的 EAMs 富集选育技术, 提高活性污泥中 EAMs 菌种资源的挖掘效率, 重点需要从以下几个方面开展工作:

(1) 亟待发展基于废水资源化过程的 EAMs 富集培养技术

在废水资源化过程中, EAMs 需要协同反应器中的多种功能微生物, 将废水中有机污染物化学键中储存的化学能转化为电能, 进一步通过胞外呼吸将电子传递到电极上, 完成能量转换。脱离了特定反应条件所富集培养的 EAMs 往往难以与系统中的其它功能微生物建立紧密而有效的协同合作关系。

(2) 亟待建立基于高通量培养组的 EAMs 高效选育技术

在具有独特絮状结构的活性污泥中, EAMs 往往与周围的生物和非生物组成代谢网络共同发挥作用。利用近几年快速发展的微生物培养组学 (Microbial Culturomics), 通过结合原始菌群梯度稀释、培养基成分优化、MALDI-TOF 质谱检测、核糖体 RNA 基因全长测序等方法手段, 可以在保证代谢网络完整性的条件下, 大幅度提高核心功能微生物单细胞分离培养和物种鉴定的通量。

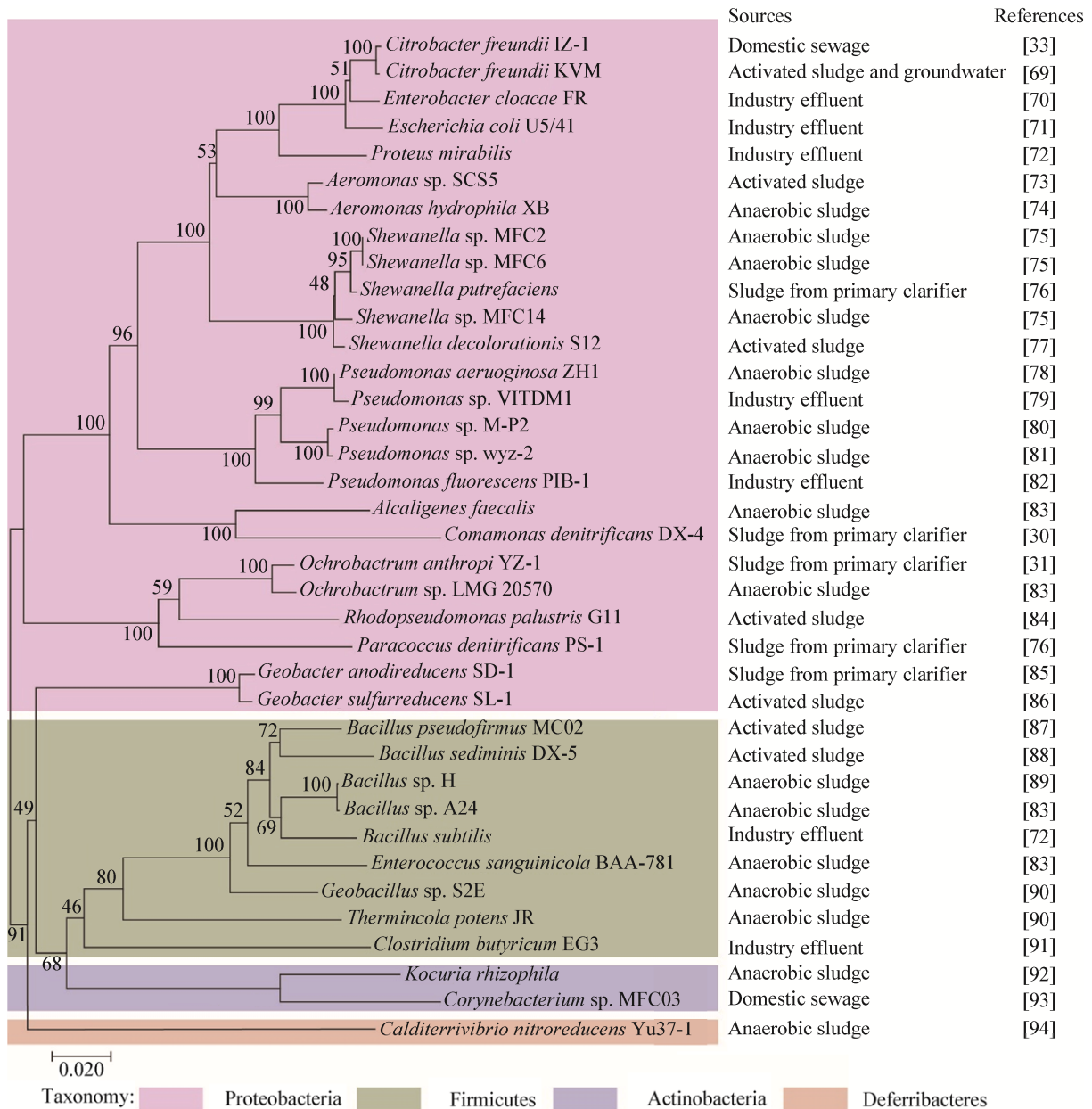


图 3 从活性污泥中已分离的电活性细菌

Figure 3 Electroactive bacteria isolated from activated sludge

(3) 亟待开展基于合成生物学的复合功能 EAMs 理性设计研究

由于废水资源化过程涉及底物分解转化及电能传递等多个环节, 需要复杂多样的代谢途径共同参与。随着高通量组学技术的广泛应用, 将逐渐阐明废水资源化过程的微生物代谢网络特点, 明确其核心关键功能基因或蛋白质。在此基础上, 借助合成生物学技术手段, 通过理性设计和构建废水资源化复合功能 EAMs, 有望突破传统微生物分离选育技术的局限性。

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