·综 述·

人工电活性微生物菌群的设计与应用

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摘 要：包括产电菌群和噬电菌群的人工电活性微生物菌群(synthetic electroactive microbial consortia)通过菌种间的物质能量级联反应介导化学能与(光)电能间的相互转化，其可利用底物来源广泛、双向电子传递速率快、环境稳定性强，在清洁电能开发、废水处理、环境修复、生物固碳固氮以及生物燃料、无机纳米材料、高聚物等高值化学品合成等多个领域具有广泛的应用前景。针对人工电活性微生物菌群设计、构建与应用，本文总结电活性微生物菌群界面电子传递和种间电子传递机制，概括基于“劳力分工”原理设计构建人工电活性微生物菌群物质能量级联反应基本架构，总结菌群关系与菌群生态位优化等人工电活性微生物菌群工程化策略，分类列举人工电活性微生物菌群在利用廉价生物质产电、生物光伏固碳产电，光驱噬电生物菌群固氮等相关应用。最后对人工电活性微生物菌群未来研究方向进行了展望。

关键词：人工电活性微生物菌群；电子传递；劳力分工；菌群关系；菌群生态位

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Design and applications of synthetic electroactive microbial consortia

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Abstract: Synthetic electroactive microbial consortia, which include exoelectrogenic and electrotrophic communities, catalyze the exchange of chemical and electrical energy in cascade metabolic reactions among different microbial strains. In comparison to a single strain, a community-based organisation that assigns tasks to multiple strains enables a broader feedstock spectrum, faster bi-directional electron transfer, and greater robustness. Therefore, the electroactive microbial consortia held great promise for a variety of applications such as bioelectricity and biohydrogen production, wastewater treatment, bioremediation, carbon and nitrogen fixation, and synthesis of biofuels, inorganic nanomaterials, and polymers. This review firstly summarized the mechanisms of biotic-abiotic interfacial electron transfer as well as biotic-biotic interspecific electron transfer in synthetic electroactive microbial consortia. This was followed by introducing the network of substance and energy metabolism in a synthetic electroactive microbial consortia designed by using the "division-of-labor" principle. Then, the strategies for engineering synthetic electroactive microbial consortiums were explored, which included intercellular communications optimization and ecological niche optimization. We further discussed the specific applications of synthetic electroactive microbial consortia. For instance, the synthetic exoelectrogenic communities were applied to biomass generation power technology, biophotovoltaics for the generation of renewable energy and the fixation of CO_2. Moreover, the synthetic electrotrophic communities were applied to light-driven N_2 fixation. Finally, this review prospected future research of the synthetic electroactive microbial consortia.
Keywords: synthetic electroactive microbial consortia; electron transfer; division of labor; intercellular communication; ecological niches

电活性微生物能够通过双向电子传递介导化学能与电能(包括太阳能电池板产生的光电子)相互转化。以电活性微生物为催化核心构建的微生物燃料电池、微生物电解池等多种生物电化学系统，在生物电能[1-3]与生物氢能[4-5]等清洁能源开发、废水处理[1,6]、环境修复[7-8]、生物固碳固氮[9-10]，以及生物燃料[11-12]、无机纳米材料[13-14]、高聚物[15-17]等高价值化学品合成等多个领域具有广泛的应用前景。近年来，通过对电活性微生物的挖掘筛选、机制解析、工程强化，使电活性微生物的研究取得了快速发展[18-20]。然而，纯培养体系中单一菌种可利用底物谱窄、电子传递速率慢、环境稳定性差，难以实现工业化应用。

相比于单菌，由多菌种构成的电活微生物群

落通过细胞代谢级联反应和有氧/厌氧呼吸链建立菌群物质能量代谢网络，可有效拓宽可利用底物谱、提高双向电子传递速率以及增强环境稳定性[21]。但天然电活性微生物菌群种类繁多、分支代谢复杂，导致天然电活性微生物菌群催化能量转化特异性差，且难以通过理性设计以工程手段强化其催化效率。基于“劳力分工”原则设计构建的人工电活性微生物菌群，由电活性微生物和发酵菌等非电活性微生物组成，包括将生物质化学能转化为电能的产电微生物菌群，和将电能(以及太阳能光电子)转化为化学能的噬电微生物菌群。其中，在产电微生物菌群中，发酵菌通过细胞物质代谢级联反应分解复杂生物质，生成小分子有机酸(如乳酸、乙酸等)作为产电菌的电子供体。在噬电微生物菌群中，噬电菌通过细胞能量代谢级联反应将外电极或太阳光伏输入的(光)电子储存在甲酸、乙酸、氢气等能量介体中，通过种间电子传递，这些能量介体可作为还原力载体驱动发酵菌合成高价值化学品。人工电活性微生物菌群结构清晰明确，旁支代谢路径少，物质代谢特异性强，因此在介导能量转化过程中具有更高的催化效率。

本文着眼于人工电活性微生物菌群设计、构建与应用的最新进展，首先总结概括了生物-非生物间界面电子传递和生物-生物间种间电子传递机制，为设计构建人工电活性微生物菌群提供理论基础。随后分类汇总人工电活性微生物菌群的设计构建原理与工程化策略，包括：(1) 基于“劳力分工”原理设计构建菌群物质能量级联反应基本架构；(2) 通过理性设计电活性微生物菌群的物质能量级联代谢，利用中间代谢产物理性构建菌群种间相互作用，优化电活性微生物菌群关系；(3) 结合气膜式生物反应器和 3D 打印多细胞活体材料空间工程策略，优化电活性微生物菌群氧气、空间生态位，提高人工电活性微生物菌群的鲁棒性。最后列举了产电微生物菌群利用发酵副产物甘油、纤维素以及木质纤维素等廉价生物质产电，生物光伏固碳产电，电/光驱噬电微生物菌群生物固碳/氮等人工电活性微生物菌群的应用(表 1)，并展望了人工电活性微生物菌群的未来研究与应用方向。

1 电活性微生物菌群电子传递机制

电活性微生物菌群包含了产电菌/噬电菌、发酵菌等生物成分以及电子受体/电子供体(如电极)等非生物成分，其中，生物(产电菌/噬电菌)-非生物(电子受体/电子供体)间界面电子传递和生物-生物(产电菌/噬电菌/发酵菌)间种间电子传递耦合了电活性微生物菌群的物质能量代谢，是设计人工电活性微生物菌群、驱动生物电化学系统高效运行的重要理论基础。

1.1 生物-非生物间的界面电子传递机制

1.1.1 细胞色素蛋白与导电菌毛介导的直接界面电子传递

含多血红素结构的 *c* 型细胞色素蛋白(*c*-type cytochrome, *c*-Cyts)在电活性微生物与环境电子受体(供体)间电子传递过程中发挥着重要作用[35](图 1A)。目前，针对细胞色素蛋白介导直接电子传递的研究主要集中在希瓦氏菌属(*Shewanella*)的金属还原 MtrABC 途径和地杆菌属(*Geobacter*)的类孔蛋白细胞色素蛋白复合物 Pcc 途径。在金属还原 MtrABC 途径中，位于细胞内膜的色素蛋白 CymA 兼具甲基萘醌氧化酶活性[36-37]，从而将内膜醌池中的电子转移至细胞周质色素蛋白(如 Fcc3、STC 等)，最后通过外膜色素蛋白复合体 MtrCAB 将电子传递到细胞表面[38-42]。金属还原 MtrABC 途径作为最为典型的直接电子传递路径，已经在沼泽红假单胞菌(*Rhodopseudomonas palustris* TIE-1)、嗜甜微生

表 1 人工电活性微生物菌群一览

Table 1 Summary of synthetic electroactive microbial consortia

No.	Microbial consortia	Division of labour	Bacterial relationships	Ecological niches	Results	Reference
1	*E. coli* *B. subtilis* *S. oneidensis*	(1) *E. coli* digested glucose to produce electron donor of lactate (2) *B. subtilis* digested glucose to produce riboflavin as electron shuttle (3) *S. oneidensis* acted as an exoelectrogen generating electricity (4) *S. oneidensis* catabolized lactate to acetate, feeding *E. coli* and *B. subtilis*	Cooperation	Undesigned	(1) Glucose was converted to electricity for more than 15 d (2) The coulomb efficiency reached 55.7%	[22]
2	*S. cerevisiae* *S. oneidensis* MR-1	(1) Engineered *S. cerevisiae* acting as a fermenter, converting glucose into lactate (2) *S. oneidensis* acting as an exoelectrogen, generating electricity from lactate	Commensalism	The spatial niche was optimized with enhancing the biofilm formation of exoelectrogen on anode surface	The maximum power density reached 123.4 mW/m^2 when feeding with glucose	[23]
3	*K. pneumoniae* *S. oneidensis*	(1) Engineered *K. pneumoniae* acting as a fermenter, converting glycerol into lactate (2) *S. oneidensis* acting as an exoelectrogen, generating electricity from lactate	Commensalism	Undesigned	The maximum power density reached 123.4 mW/m^2 when feeding with glycerol	[24]
4	*K. pneumoniae* *S. oneidensis*	(1) Engineered *K. pneumoniae* acting as a fermenter, converting glucose and xylose into lactate (2) *S. oneidensis* acting as an exoelectrogen, generating electricity from lactate	Commensalism	The spatial niche was optimized with enhancing the biofilm formation of exoelectrogen on anode surface	The maximum power density (104.7±10.0) mW/m^2 co-feeding with glucose and xylose from corn straw hydrolyzates	[25]
5	*B. subtilis* *S. oneidensis*	(1) *B. subtilis* acting as a fermenter, synthesizing electron shuttle riboflavin (2) *S. oneidensis* acting as an exoelectrogen, generating electricity facilitated by riboflavin	Commensalism	Undesigned	The maximum power density reached 277.4 mW/m^2 with coulombic efficiency of 5.6% (2) The discharge time was more than 500 h	[26]
6	*M. acetivorans* *P. denitrificans* *G. sulfurreducens*	(1) The engineered archaeal strain *M. acetivorans* with methyl-coenzyme M reductase for converting methane into acetate (2) The acetate acted as an electron donor of *G. sulfurreducens* for electricity generation	Commensalism	Undesigned	The coulombic efficiency reached 90%	[27]

(待续)

(续表 1)

No.	Microbial consortia	Division of labour	Bacterial relationships	Ecological niches	Results	Reference
7	*Paenibacillus* sp. *Klebsiella* sp. *G. sulfurreducens*	(1) *Paenibacillus* sp. acting as a fermenter, converting cellulose into glucose (2) *Klebsiella* sp. converting glucose into acetate (3) *G. sulfurreducens* acting as an exoelectrogen, generating electricity from acetate	Commensalism	Undesigned	(1) The maximum current density reached 621 mA/cm^2 (2) The maximum power density reached 1 146 mW/m^2	[28]
8	*E. coli* *S. oneidensis*	(1) The engineered *E. coli* acting as a fermenter, converting xylose into electron donor formate and electron shuttle flavins (2) The engineered *S. oneidensis* acting as an exoelectrogen, generating electricity from formate, facilitated by riboflavin	Commensalism	The spatial niche was optimized by enhancing the biofilm formation of exoelectrogen on anode surface	(1) The catalytic current reached 1.84 A/m^2 (2) The maximum power density reached 728.6 mW/m^2	[29]
9	*S. oneidensis* MR-1 *C. freundii* An1	(1) The *S. oneidensis* acting as an exoelectrogen, generating electricity from lactate (2) The *C. freundii* An1 acting as a competitor, competing with *S. oneidensis* for consuming lactate	Competition	Undesigned	The occurrence of competitor *C. freundii* An1 facilitated the EET of *S. oneidensis*, in which the maximum current density was improved by 6 times	[30]
10	*E. coli* *S. oneidensis*	(1) The engineered *E. coli* acting as a fermenter, synthesizing electron shuttle flavins (2) The *S. oneidensis* acting as an exoelectrogen, generating electricity facilitated by riboflavin	Commensalism	The spatial niche was optimized with immobilizing *E. coli* into beads, resulting in the *E. coli* was far from the anode surface	(1) The maximum current output reached 3 804.3 mA/m^2 (2) The maximum power density reached (434.2±3.4) mW/m^2	[31]
11	*S. elongatus* UTEX 2973 *S. oneidensis* MR-1	(1) Photosynthetic cyanobacteria directed solar energy from photons to D-lactate (2) *S. oneidensis* used the D-lactate for electricity generation	Cooperation	Spatial-temporal separation was setup with medium replenishment	(1) A spatial-temporal separation setup with medium replenishment enabled stable operation for over 40 d (2) The average power density reached 135 mW/m^2	[9]
12	*Synechocystis* sp. PCC 6803 *P. aeruginosa* PAO1 *S. oneidensis* MR-1	(1) *Synechocystis* sp. acting as a fermenter, fixing CO_2 into carbohydrate through photosynthesis (2) *P. aeruginosa* acting as a fermenter, synthesizing electron shuttle phenazine-1-carboxylic acid (3) *S. oneidensis* acting as an exoelectrogen, generating electricity from carbohydrate which was facilitated by phenazine-1-carboxylic acid	Commensalism	The spatial niche was optimized via building multilayer 3-D microbial communities	They were used as a gas sensor and powered wireless communication	[32]

(待续)

(续表 1)

No.	Microbial consortia	Division of labour	Bacterial relationships	Ecological niches	Results	Reference
13	*R. palustris* *M. barkeri*	(1) *R. palustris* harvested solar energy and performed anoxygenic photosynthesis (2) *R. palustris* transferred electrons to *M. barkeri* (3) *M. barkeri* used reducing power to drive methane generation	Commensalism	Undesigned	The methane production rate reached (4.7±0.2) μmol/(L·h) under light	[33]
14	*G. metallireducens* *R. palustris*	(1) *G. metallireducens* transferred electrons to *R. palustris* (2) *R. palustris* used reducing power to the dark CO_2 fixation	Commensalism	Undesigned	The previously unidentified dark CO_2 fixation mode enabled by electroautotrophy	[34]
15	*S. ovata* *R. palustris*	(1) *S. ovata* reduced CO_2 to acetate driven by light (2) *R. palustris* performed N_2 fixation and biopolyester generation by using acetate	Cooperation	Undesigned	(1) Establishing full solar to chemical conversion driven by a photovoltaic device (2) Solar to chemical efficiencies of 1.7%, 0.5%, and 0.1% for acetate, nitrogenous biomass, and ammonia	[10]

物(*Rhodofera ferrireducens*)、石质营养性微生物(*Sideroxydans lithotrophicus*) ES-1 等多种电活性微生物中发现其同源蛋白[43-46]。与金属还原 MtrABC 途径结构类似，地杆菌属类孔蛋白细胞色素蛋白复合物 Pcc 电子传递通道也包含了内膜色素蛋白 ImcH、CbcL[47-48]，周质色素蛋白的 PpcA、PpcD[49-50]以及外膜色素蛋白复合体 OmbB-OmaB-OmcB、OmbC-OmaC-OmcC 等[51-52]。相比于 MtrCAB 途径，*Geobacter* 类孔蛋白细胞色素蛋白复合物 Pcc 途径包含多个色素蛋白同源物，可组成多条电子传递跨膜传递通道，并且能够根据胞外电池受体(供体)的性质(如氧化还原电位)选择性地表达开启不同的电子跨膜传递路径[53]。

相比于细胞色素蛋白通过细胞与非生物界面直接接触介导的短距离电子传递，Lovely 等[54-55]发现硫还原地杆菌(*Geobacter sulfurreducens*)等电活性微生物还可通过导电菌毛介导长距离电子传递。这种导电菌毛由富含芳香族氨基酸的菌毛单体蛋白 PilA 组装而成，直径为 3−5 nm，因此又被称为“导电纳米线(nanowire)”(图 1B)。值得注意的是，希瓦氏菌(*Shewanella oneidensis* MR-1)也可生长出类导电纳米线结构介导电子传递，但这已被证实是含色素蛋白(如 MtrC、OmcA)的外膜延伸[56]。现在发现可使用导电菌毛介导长距离直接电子传递的菌株有 *Geobacter*、*R. palustris*、集胞藻(*Synechocystis*) PCC 6803、嗜热丙酸厌氧肠状菌(*Pelotomaculum thermopropionicum*)、变化赖氨酸芽孢杆菌(*Lysinibacillus varians*) GY32 等[57-59]。目前，电子沿导电纳米线的传递机制仍处于研究阶段[60-62]。最近 Malvankar 等利用冷冻电镜相继解析了导电纳米线的高分辨率蛋白结构，发现 *Geobacter* 的导电纳米线是由 OmcS、OmcZ、

OmcE 等细胞色素蛋白构成[63-65]。导电纳米线在介导电子传递过程中，电子以色素蛋白中的血红素为位点、通过电子跳跃的方式快速传递，而菌毛单体蛋白并不具有导电性，但可促进导电纳米线从周质空间延伸至细胞壁外部，从而加快导电纳米线的组装[66]。

1.1.2 电子传递载体介导的间接界面电子传递

除上述细胞色素蛋白、导电纳米线介导的直接电子传递，电活性微生物还可分泌或利用电子传递载体介导间接电子传递(图 1C)[67-68]。电子传递载体是具有氧化还原活性的可溶性小分子化合物，包括黄素类化合物(核黄素、黄素腺嘌呤二核苷酸等)、吩嗪类化合物(吩嗪-1-羧酸、绿脓菌素等)以及蒽醌类化合物(蒽醌-2,6-二磺酸，腐殖酸)。间接电子传递机制已经在 *S. oneidensis*[68-70]、*G. sulfurreducens*[71]、铜绿假单胞菌(*Pseudomonas aeruginosa*)[72-73]、李斯特菌(*Listeria monocytogenes*)[74]和噬汽水单胞菌(*Aeromonas hydrophila*)[75]等电活性微生物中相继发现。电子传递载体通过与电活性微生物细胞色素蛋白间的氧化还原作用实现电子传递[76]，其介导的间接电子传递速率与电子传递载体的种类和浓度密切相关[29,77]。最新的研究发现，吩嗪-1-甲酰胺(phenazine-1-carboxamide, PCN)、绿脓菌素(pyocyanin, PYO)吩嗪类电子传递载体可通过与电活性生物被膜基质中的胞外 DNA (extracellular DNA, eDNA)结合介导间接电子传递[78]。eDNA 能够与 PCN 和 PYO 结合，使其“滞留”在生物膜中，并作为电子传递通道，实现生物膜内的远距离的电子传递[78]。

1.2 生物-生物种间电子传递机制

电活性微生物菌群中生物-生物间种间电子传递机制包括细胞代谢产物或氧化还原活性分子介导的间接种间电子传递和导电菌毛、色素蛋白以及导电材料介导的直接种间电子传递[79]。

1.2.1 氢气、甲酸盐以及氧化还原活性小分子介导的间接种间电子传递

H_2、甲酸被最早发现可作为电子传递载体微生物的间接种间电子传递(图 2A)[80-82]。相比于 H_2，甲酸盐在溶液环境中具有更高的溶解度，但在缺乏足够浓度梯度势的条件下，其溶解扩散速率较 H_2 更慢[83]。除 H_2、甲酸盐等细胞代谢产物外，诸如核黄素、吩嗪、蒽醌以及腐殖酸等氧化还原活性分子也可介导间接种间电子传递[84-85]。例如，Huang 等发现，在金属还原地杆菌(*Geobacter metallireducens*)-*G. sulfurreducens* 两菌共培养体系中，两菌分泌的核黄素以游离电子传递载体氧化 *G. metallireducens* 色素蛋白 Gmet_2896、还原 *G. sulfurreducens* 色素蛋白 OmcS，从而将 *G. metallireducens* 电子传至 *G. sulfurreducens*[86]。

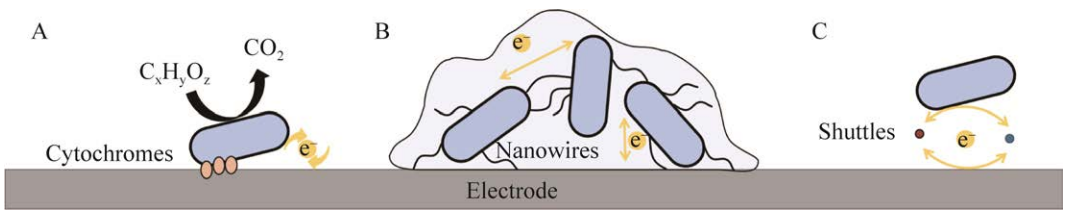

图 1 电活性微生物胞外电子传递机制

Figure 1 Mechanisms of extracellular electron transfer of electroactive microorganisms. A: Direct electron transfer mediated by *c*-Cyts. B: Direct electron transfer mediated by conductive nanowires. C: Indirect electron transfer mediated by electron transport carriers.

1.2.2 导电菌毛、色素蛋白以及导电材料介导的直接种间电子传递

在 *G. metallireducens-G. sulfurreducens* 电活性菌群中，敲除 *G. sulfurreducens* 色素蛋白 OmcS 和导电菌毛单体蛋白 PilA 后，共培养体系受到抑制，基于此 Summers 等率先提出导电菌毛(图 2B)及色素蛋白(图 2C)介导的直接种间电子传递机制[87]。随后，直接种间电子传递机制相继在 *G. metallireducens-M. barkeri*[88]、*G. metallireducens*-竹节状甲烷鬃菌(*Methanosaeta harundinacea*)[89]、*G. metallireducens-R. palustris*[34]等多个电活性微生物菌群中发现。同时近期研究表明，当距离足够近时细胞表面色素蛋白内部的血红素可进行蛋白间的电子传递[90]。Liu 等通过敲除地杆菌导电菌毛组装 ATP 酶 PilB、抑制导电菌毛 pili 的组装，发现导电菌毛缺失突变株 *G. metallireducens*、*G. sulfurreducens* 依旧可以共培养，并且证明了 *G. metallireducens* 色素蛋白 Gmet_2896 在菌群直接种间电子传递中发挥着重要作用[91]。而在通过直接种间电子传递共培养的 *P. aestuarri-G. sulfurreducens* 电活性菌群中，敲除 *G. sulfurreducen* 电子跨膜传递色素蛋白复合体 OmbB-OmaB-OmcB-OrfS-OmbC-OmaC-OmcC 后，菌群微生物的生长受到显著抑制，进一步证明细胞色素蛋白在直接种间电子传递过程中发挥着重要作用[92]。此外，诸如磁铁矿[93]、生物炭[94]、碳布[95]、石墨[96]等非生物体导电材料被证明可作为“导线”连接细胞，搭建细胞间电子传递通道，介导电活性微生物菌群的直接种间电子传递(图 2D)。相比于间接种间电子传递，直接种间电子传递因不涉及电子传递载体的溶解、扩散限制，具有更高的电子传递速率[97]。

2 人工电活性微生物菌群的设计构建原理与工程策略

2.1 “劳力分工”-人工电活性微生物设计构建原理

劳力分工是微生物菌群的基本特征，指在由多菌种组成的微生物菌群中，各菌种基于其生理形态特征，发挥各自功能、承担菌群部分任务，并通过物质能量代谢等级联反应建立菌种间的相互作用关系，以实现相互协作并完成整个菌群体系的既定功能目标。在人工电活性微生物设计构建过程中，基于劳力分工原理，通过对菌群中各菌种进行合理的功能定位和任务分工，可显著降低各个菌种的代谢负荷、提高菌种的催化效率，从而更加高效地实现电活性微生物菌群的复杂功能[98]。

在人工产电微生物菌群中，发酵菌通常以纤维素、五六碳糖等复杂生物质为底物，通过细胞分解代谢，将其降解为小分子有机酸，并合成核黄素、吩嗪等氧化还原活性化合物，为产电菌提

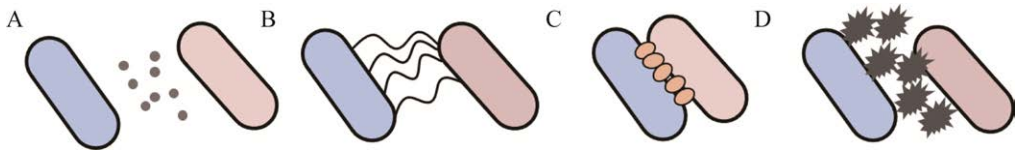

图 2 电活性微生物种间电子转移机制

Figure 2 Mechanisms of interspecies electron transport between electroactive microorganisms. A: Indirect interspecies electron transfer mediated by soluble electron shuttles such as H_2. B: Direct interspecies electron transfer mediated by conductive nanowires. C: Direct interspecies electron transfer mediated by *c*-Cyts. D: Direct interspecies electron transfer mediated by electrically conductive materials.

供电子供体和电子传递载体，协同促进人工产电菌群的能量转化效率。例如，在 Yang 等构建了以木糖为底物的大肠杆菌(*Escherichia coli*)-*S. oneidensis* 双菌产电微生物菌群。其中 *E. coil* 以木糖为底物代谢生成甲酸，*S. oneidensis* 以甲酸为电子供体产电。并在此基础上，通过在 *E. coli* 中进一步引入外源核黄素合成路径 *ribADEH*，使工程 *E. coli* 同时能以木糖为底物合成电子传递载体核黄素，加速 *S. oneidensis* 电子传递过程(图 3A)[99]。综上可见，甲酸、乙酸、乳酸等电子供体与核黄素、吩嗪等电子传递载体是连接发酵菌与产电菌的重要中间代谢物。因此在人工产电菌群的设计构建过程中，可以通过工程改造发酵菌与产电菌的细胞膜转运蛋白来调节细胞通透性，从而加速中间产物的细胞间传质效率，提高人工产电微生物菌群能量转化。

噬电微生物菌群通常利用外源电能、太阳能，将 CO_2、N_2 等高氧化态、低能量密度化合物转化为高还原态、高能量密度的生物燃料等高值化学品。其中，噬电微生物通过其胞外电子传递通道(噬电通道，如 *R. palustris* TIE-1 细胞色素蛋白复合体 PioBAC)[35,43,100]，可将源于电极或太阳能(光电子)的电子吸收，随后通过种间电子传递，将电子转移至发酵菌，驱动发酵菌固碳、固氮以合成高值化学品。在噬电菌与发酵菌的种间电子传递过程中，噬电菌将吸收的电子转化为胞内还原力，随后通过导电菌毛、细胞色素蛋白等直接电子传递通道传递至发酵菌，或通过合成甲酸、乙酸、氢气等能量载体或还原黄素、吩嗪、蒽醌等电子传递载体等，利用间接种间电子传递通道传递至发酵菌。例如，在 *R. palustris*-巴氏甲烷八叠球菌(*Methanosarcina barkeri*)二元噬电菌群中，*R. palustris* 通过厌氧光合作用，吸收太阳能光电子，随后利用细胞合成的腐殖酸类化合物作为种间电子传递载体，将光电子传递至产甲烷菌 *M. barkeri*，驱动 *M. barkeri* 还原 CO_2 合成甲烷(图 3B)[33]。相比于产电微生物菌群，人工噬电微生物菌群的研究尚处于起步阶段，针对噬电菌群中噬电菌与发酵菌的劳力分工优化，如固碳固氮代谢路径分割、种间电子传递优化等尚未得到系统研究。

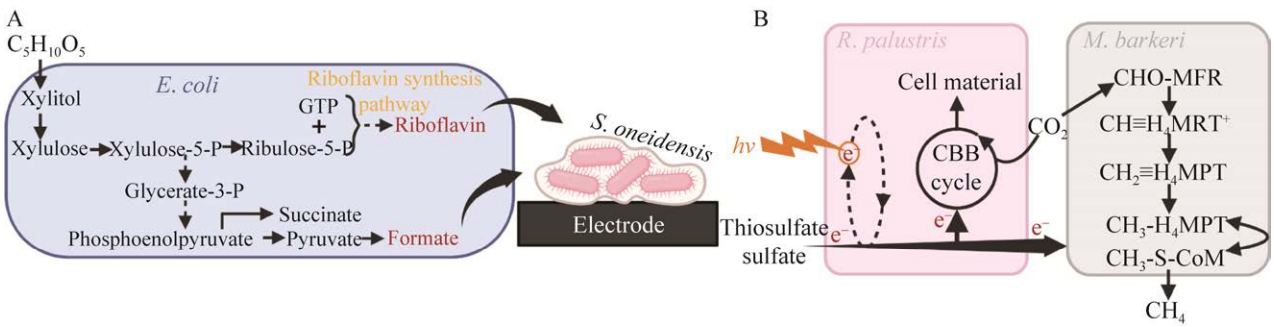

图 3　利用“劳力分工”设计构建电活性微生物菌群[33,99]

Figure 3　Designing electroactive microbial consortia basing on the principle of “division of labour”[33,99]. A: A synthetic microbial consortium composed of genetically modified *E. coli* and *S. oneidensis* was rationally designed and constructed to efficiently harvest energy from xylose[99]. B: *R. palustris* formed a direct electric syntrophic coculture with *M. barkeri*. *R. palustris* harvested solar energy, performed anoxygenic photosynthesis using sodium thiosulfate as an electron donor, and transferred electrons extracellularly to *M. barkeri* to drive methane generation[33].

2.2 人工电活性微生物菌群工程策略

在人工电活性微生物菌群中，维持菌种间稳定共存以保障菌群长期高效运行，是菌群工程强化的重要目标。高催化效率、高鲁棒性的电活性微生物菌群需各菌种间具备合理的相互作用，以建立多维度交互叠加的菌群通讯网络，在菌群内部形成抗外界干扰的自调控机制，以维持菌群的分布结构、种群丰度比例长期稳定。此外，各菌种在电活性微生物菌群中所处的生态位，即所在微环境的温度、溶氧、pH、营养分布、氧化还原电势、空间位(与电极相对位置)等，也对菌群的催化效率和稳定性具有重要影响[101]。因此，本节从优化电活性微生物种间相互作用和种群生态位两个角度，总结探讨电活性微生物的菌群的工程化策略。

2.2.1 优化电活性微生物菌群关系

电活性微生物菌群通过细胞分泌的代谢产物、蛋白、多肽、囊泡以及信号分子(如种群感应分子)进行菌种间相互作用，从而形成包括偏利共生、合作(互利共生)、竞争等菌群种间关系(图 4)[102-103]。通过理性设计电活性微生物菌群的物质、能量级联代谢，利用中间代谢产物调控细胞间相互作用，解除菌群相互作用单一、菌群鲁棒性差等限制。

(1) 偏利共生

偏利共生作为自然界中最为普遍的菌群关系，在人工电活性微生物菌群中最为常见，其中物质能量流自上游菌流向下游菌，下游菌受益生长但并不反哺上游菌(图 4A)。例如 Lin 等通过敲除酿酒酵母(*Saccharomyces cerevisiae*)乙醇脱氢酶，抑制其乙醇合成，并通过过表达 L-乳酸脱氢酶，使 *S. cerevisiae* 以葡萄糖为底物大量合成乳酸，乳酸进一步作为 *S. oneidensis* 电子供体产电[23]。在 *S. cerevisiae-S. oneidensis* 二元人工产电菌群中，与发酵菌 *S. cerevisiae* 为产电菌 *S. oneidensis* 提供电子供体乳酸相比，*S. oneidensis* 并未对 *S. cerevisiae* 产生反馈促进或抑制作用，因此二者间通过偏利共生种群关系维持菌群稳定共生。

(2) 合作(互利共生)

多菌种间合作以达到互利共生是微生物菌群最佳菌群关系，建立合作型菌群关系，有助于保持菌群密度平衡、提高菌群鲁棒性和催化转化效率，从而实现菌群长期稳定共存。菌种间底物/中间代谢物的交叉喂养(cross-feeding)是构建互利共生菌群关系的主要工程策略[104-106]。Liu 等通过多重劳力分工优化，设计构建了以葡萄糖为底物的枯草芽孢杆菌 *Bacillus subtilis-E. coli-S. oneidensis* 三菌人工产电微生物菌群(图 4B)。其中，葡萄糖作为 *E. coli*、*B. subtilis* 的共同底物，分别被 *E. coli* 和 *B. subtilis* 代谢生产乳酸和核黄素，生成的乳酸和核黄素分别作为产电菌 *S. oneidensis* 的电子供体和电子传递载体，加速希瓦氏菌的胞外电子传递过程。与此同时，*S. oneidensis* 代谢乳酸生成的乙酸可作为 *E. coli* 和 *B. subtilis* 底物被进一步代谢分解。在 *E. coli-B. subtilis-S. oneidensis* 三菌人工产电微生物菌群中，乳酸、核黄素、乙酸等代谢物通过交叉喂养联系了菌种间的相互作用，形成了互利共生的菌群关系。最终这一以葡萄糖为底物的三菌产电微生物菌群输出电压达到 550 mV、连续稳定放电 15 d，实现底物葡萄糖的完全代谢，库伦效率达到 55.7%[22]。同样地，在利用棕榈油炼制废水产电的 *P. aeruginosa* PA14-产气肠杆菌(*Enterobacter aerogenes*)二元人工产电菌群中，产电菌 *E. aerogenes* 中间代谢物 2,3-丁二醇可作为 *P. aeruginosa* 底物，诱导 *P. aeruginosa* 加速合成绿脓菌素(pyocyanine, PYO)。绿脓菌素反过来作为 *E. aerogenes* 的电子传递载体，显著增强了 *P. aeruginosa-E. aerogenes* 菌群的电能输出[107]。

(3) 竞争

竞争是微生物菌群普遍存在的菌群关系，这通常是由多种微生物共用同一底物而产生的对底物与生存空间的竞争。近期研究发现，竞争型菌群关系有助于电活性菌群维持稳定状态并提高菌群的催化效率。例如 Xiao 等利用均以乳酸作为碳源的 *S. oneidensis* MR-1 和弗氏柠檬酸杆菌(*Citrobacter freundii* An1)建立了竞争菌群关系模型，以研究竞争菌群关系对电活性微生物菌群的影响。结果发现，*S. oneidensis* MR-1-*C. freundii* An1 共培养菌群的输出电流达到 38.4 μA/cm^2，较 *S. oneidensis* MR-1 纯培养体系提高 6 倍。进一步分析发现，在 *S. oneidensis* MR-1-*C. freundii* An1 中，*S. oneidensis* MR-1 与 *C. freundii* An1 对底物碳源的竞争增强了 *S. oneidensis* MR-1 在电极表面的代谢活性，提高了对底物的摄取消耗，加速了核黄素等电子传递载体的合成，促进了电活性生物膜的形成，进而提高了 *S. oneidensis* MR-1 的电能转化效率与电子传递速率(图 4C)[30]。该研究证明了竞争型菌群关系对电活性微生物菌群催化效率的促进作用，为优化人工电活性微生物菌群、提高菌群稳定性拓宽了设计思路。

2.2.2 电活性微生物菌群生态位优化

在电活性微生物菌群中，产电菌、噬电菌需附着于电极表面从而与外界进行高效电子传递，同时为保证电子传递过程中电子不被氧气掠夺，电活性微生物通常需要厌氧生存环境。与之相比，电活性微生物菌群中的发酵菌等非电活性微生物则需远离电极表面为电活性微生物释放更多电活性位点，并且绝大部分发酵菌在富氧条件下能够更加高效地代谢、合成目标产物。因此，在电活性微生物菌群中，需调节控制菌群空间分布结构、设置溶氧梯度，以满足不同微生物对空间生态位、氧气生态位的个性化需求。

为实现这一目标，气膜式生物反应器和 3D 打印多细胞活体材料空间工程策略相继被用于优化人工微生物菌群氧气生态位与空间生态位。

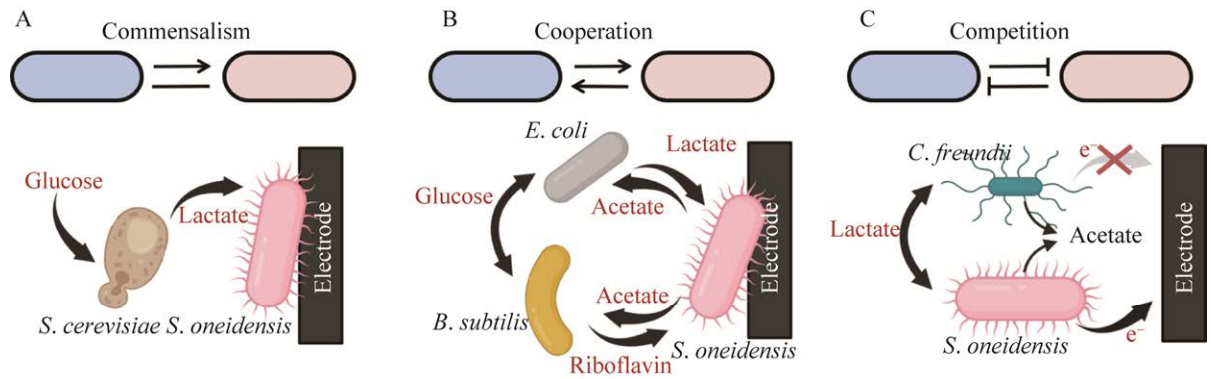

图 4 电活性微生物菌群的相互作用关系[22-23,30]

Figure 4 Interaction relationships of electroactive microbial consortia[22-23,30]. A: Commensalism. In *S. cerevisiae*-*S. oneidensis* consortium, the glucose was metabolized by engineered *S. cerevisiae* to produce lactic acid, which was in turn used by *S. oneidensis* as an electron donor for EET[23]. B: Mutualism. In *E. coli*-*B. subtilis*-*S. oneidensis* consortium, *E. coli* digested glucose to produce lactate as a carbon source and an electron donor and *B. subtilis* produced riboflavin as an electron shuttle, both of which facilitated the electron transfer of *S. oneidensis*[22]. C: Competition. The competition enhanced the metabolic activity of *S. oneidensis* MR-1 on electrode, which facilitated the biofilm formation and therefore helped *S. oneidensis* MR-1 to gain a competitive advantage over *C. freundii* An1[30].

气膜式生物反应器利用气体渗透膜管道，物理分隔气相组分与液相组分。在运行过程中，气体渗透膜管道作为曝气通道，氧气可通过气体渗透膜，如聚二甲基硅氧烷膜(polydimethylsiloxane, PDMS)进入液相体系，增加气膜附近液体中的溶氧量，但受限于氧气在液体中的传质速率，远离气膜附近的液体区域溶氧量降低。由此在液体中形成以气体渗透膜管道为中心，逐级降低的溶氧浓度梯度(图 5A)。利用气膜式生物反应器培养微生物菌群时，好氧菌占据气体渗透膜管周围的富氧区生长，贴附在气体渗透膜表面形成菌膜。菌膜内的好氧菌消耗大量的氧气，进一步截留氧气向远离气体渗透膜管道方向的液体区域扩散，从而使气体渗透膜管道的远端形成贫氧、厌氧区域，为菌群内厌氧微生物生长创造生存条件[108-109]。

利用气膜式生物反应器优化电活性微生物菌群生态位，需要将气膜式生物反应器与生物电化学系统相耦合，增加了反应器的设计开发难度。为此，通过 3D 打印多细胞活体材料的空间工程策略随即被提出，用于理性设计菌群空间分布结构、优化氧气生态位。其中，电活性微生物菌群中的各菌种首先分别与琼脂糖[32]、海藻胶(海藻酸钠)[110]、纤维素[111]等细胞交联剂混合形成多细胞活体材料，随后作为“生物胶水”通过 3D 打印技术逐层打印在生物电化学系统的电极表面，形成具有特定空间分布结构的电活性微生物菌群[112-114]。例如，Liu 等[32]利用 3D 打印多细胞活体材料空间工程策略，将 *Synechocystis* sp. PCC 6803、*P. aeruginosa* PAO1、*S. oneidensis* MR-1 逐层打印在电极表面，并通过优化 3 个菌株的空间相对位置，最终将 *Synechocystis* sp. PCC 6803 定位于空间最上层、*P. aeruginosa* PAO1 定位于中层、*S. oneidensis* MR-1 定位于靠近电极的最下层(图 5B)。在这一具有特定空间结构的三菌产电微生物菌群中，*Synechocystis* sp. PCC 6803 在光照下通过光合作用将 CO_2 转化为有机物，随后被位于中层的 *P. aeruginosa* PAO1 利用合成吩嗪类电子传递载体，从而加速底层 *S. oneidensis* MR-1 与电极间的电子传递速率。3D 打印多细胞活体材料空间工程策略能够使菌群内各菌种间实现可控的空间分离，优化菌群的空间生态位，促进电活性微生物菌群稳定高效运行。

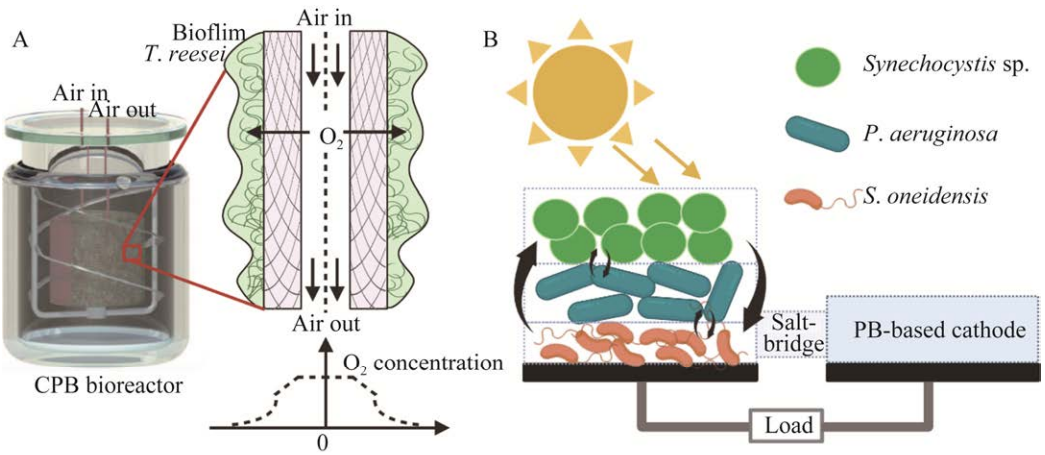

图 5　电活性微生物菌群生态位优化[32,108]

Figure 5　Optimization of ecological niches in electroactive microbial consortia[32,108]. A: Schematic representation of the membrane-aerated bioreactor with dissolved oxygen concentration profile[108]. B: Spatially structured 3-D microbial communities in solid-state agar-based microcompartments[32].

3 人工电活性微生物菌群的应用

3.1 利用甘油产电的人工产电微生物菌群

甘油作为生物柴油炼制过程中的主要副产物，将其转化为清洁电能对实现工业废料的环保处理具有重要意义。然而甘油作为一种高还原态的生物质能源，在厌氧发酵过程中需要额外加入高氧化态物质以保持其微生物胞外氧化还原电位平衡。为此，研究人员开发了非平衡发酵，将甘油厌氧发酵产生的过剩还原力以电子的形式传递给电极，以平衡微生物胞外氧化还原电位，在实现甘油底物高值转化的同时产生清洁电能。在产电微生物菌群在以甘油为底物的非平衡发酵过程中，可通过菌群劳力分工，减少代谢副产物的负反馈抑制，提高电能转化效率。例如，在以甘油为底物非平衡发酵产乙醇的*G. sulfurreducens*-纤维状梭状芽孢杆菌(*Clostridium cellobioparum*)二元产电微生物菌群中，*C. cellobioparum* 将甘油高效转化为乙醇的同时产生乙酸、乳酸等副产物，乙酸等副产物可作为 *G. sulfurreducens* 的电子供体产电，从而减少了甘油发酵副产物的积累，缓解了对 *C. cellobioparum* 反馈抑制，最终提高了乙醇的产量[115]。相较于厌氧菌 *G. sulfurreducens*，兼性厌氧产电菌 *S. oneidensis* 在实际甘油发酵过程中更易于操作。在克雷伯氏菌(*Klebsiella pneumoniae* J2B)-*S. oneidensis* MR-1 产电微生物菌群中，*K. pneumoniae* 作为模式甘油降解菌，将甘油转化产为乙酸、乳酸和琥珀酸等小分子物质，这些小分子有机酸作为 *S. oneidensis* MR-1 电子供体被 *S. oneidensis* MR-1 进一步代谢产电。然而野生型 *K. pneumoniae* 转化甘油到乳酸等电子供体产率较低，限制了 *K. pneumoniae*-*S. oneidensis* 产电微生物菌群的电能输出[116]。为此，Li 等通过合成生物学工程化策略，敲除 *K. pneumoniae* KG1 乙醇脱氢酶基因以破坏其乙醇合成途径、异源表达乳酸脱氢酶基因和乳酸转运子基因强化乳酸生物合成，同时强化 *S. oneidensis* MR-1 核黄素类电子传递载体合成以增强其胞外电子传递速率，最终显著提高了工程 *K. pneumoniae* KG1-*S. oneidensis* MR-1 产电微生物菌群以甘油为底物的电能输出[24]。

3.2 利用纤维素及木质纤维素产电的人工产电微生物菌群

纤维素与木质纤维素是自然界中分布最广、储量最丰富的有机生物质，全球每年合成的纤维素、木质纤维素达到数千亿吨。以纤维素、木质纤维素为底物产生清洁电能，实现纤维素与木质纤维素的高效开发利用，是解决当前能源短缺、环境污染等问题的重要途径。然而，绝大部分电活性微生物不具备纤维素、木质纤维素代谢路径，无法高效转化并利用此类生物质原料。为此，Ren 等基于劳力分工设计了 *C. cellulolyticum*-*G. sulfurreducens* 二元人工产电微生物菌群，其中 *C. cellulolyticum* 将纤维素发酵为乙酸盐、乙醇、氢气和二氧化碳。随后，*G. sulfurreducens* 以乙酸作为电子供体产电，从而实现将纤维素生物质化学能转化电能[117]。同样地，Jiang 等设计了由类芽孢杆菌(*Paenibacillus* sp.)-*Klebsiella* sp.-*G. sulfurreducens* PCA 三菌人工产电微生物菌群，以将纤维素作为微生物燃料电池的唯一底物。其中 *Paenibacillus* sp.将纤维素转化为纤维二糖和葡萄糖，*Klebsiella* sp.代谢纤维二糖和葡萄糖分解为乙酸等小分子有机酸，最后 *G. sulfurreducens* PCA 以乙酸等有机酸作为电子供体。该微生物燃料电池以纤维素为底物产生的电流密度达到 621 mA/cm^2[28]。通过代谢耦合与劳力分工设计、构建人工产电微生物菌群，拆解纤维素的分解代谢过程，有助于实现纤维素向清洁电能的高效转化。

相比于纤维素，木质纤维素更难以被微生物

分解利用，以木质纤维素为底物构建的人工电活性微生物菌群鲜有报道。但值得注意的是，Shahab 等利用里氏木霉(*Trichoderma reesei*)-戊糖乳杆菌(*Lactobacillus pentosus*)双发酵菌群开发了木质纤维素-乳酸炼制平台。其中 *T. reesei* 通过有氧发酵代谢分泌纤维素水解酶，催化木质纤维素分解为纤维二糖或葡萄糖、木糖等多种小分子可溶性糖，这些小分子可溶性糖作为 *L. pentosus* 底物，通过厌氧发酵进一步转化为乳酸。为实现好氧菌 *T. reesei* 与厌氧菌 *L. pentosus* 稳定共生，研究人员利用膜曝气生物反应器在 *T. reesei-L. pentosus* 菌群中构建了溶氧梯度生态位。其中，好氧菌 *T. reesei* 紧靠膜生长，在膜表层形成菌膜，厌氧菌 *L. pentosus* 远离隔膜。当气体从膜的另一侧通过时，渗透过膜的氧气在膜附近形成富氧区，氧气优先被附着在膜上的 *T. reesei* 有氧发酵消耗殆尽，从而在培养体系内部形成厌氧环境，以支撑 *L. pentosus* 厌氧发酵，从而实现了 *T. reesei-L. pentosus* 的共培养[108]。该乳酸炼制平台可将木质纤维素高效转化为平台化合物乳酸，乳酸作为 *S. oneidensis* 等大部分产电微生物的电子供体，进一步被转化为电能。因此，将 *T. reesei-L. pentosus* 菌群与产电菌群相结合，能够快速构建出以木质纤维素为底物的生化电催化系统，以实现木质纤维素的高效转化(图 6B)。

3.3 电活性微生物菌群用于生物固碳

随着现代工业的快速发展，CO_2 等温室气体被大量排放，对全球气候变化产生严重影响。减碳固碳，努力实现碳达峰、碳中和是当前人类社会的共同目标。基于产电微生物菌群开发的生物光伏技术(biophotovoltaics)，利用自然光照将二氧化碳转换为电能，在固定二氧化碳的同时获得清洁能源，有望实现负碳电力工业。Zhu 等设计构建了聚球藻(*Synechococcus elongatus*)-*S. oneidensis* 双菌人工产电微生物菌群开发生物光伏技术，通过细胞代谢，分两阶段实现光驱二氧化碳固定与产电(图 6A)[9]。在第一阶段“充电”阶段，*S. elongatus* 通过光合作用吸收光子并产生激发电子，激发电子通过质子醌(plastohydroquinone, PQH2)、NADPH 等高能中间体运输，经 CO_2 固定途径将 CO_2 转化为乳酸，实现 CO_2 固定和太阳能储存；在第二阶段“放电”阶段，*S. oneidensis* 以乳酸为电子供体，通过细胞代谢转化为电能。基于以上劳力分工，研究人员依次通过培养基优化、开发双菌共培养培养基，过表达 D-乳酸脱氢酶、强化 *S. elongatus* 以 CO_2 为底物的乳酸生成效率，敲除 *S. oneidensis* 硝酸根还原酶基因、加速胞外电子传递速率，以及开发时-空间分离培养技术、优化菌群光照、溶氧与空间生态位，建立了连续培养的双菌人工固碳产电微生物菌群。率先开发出以人工产电微生物菌群为生物催化核心的生物光伏技术，促进了二氧化碳固定与清洁能源开发的研究。

除生物光伏技术产外，Liu 等发现，噬电微生物菌群可通过种间电子传递，驱动电能自养微生物在黑暗条件下固碳(图 6C)。其中，在 *R. palustris* 和 *G. metallireducens* 组成的双菌噬电微生物菌群中，*G. metallireducens* 氧化乙酸盐，产生的电子通过 *c* 型细胞色素蛋白、纳米导线以及核黄素电子传递载体传递给 *R. palustris*。*R. palustris* 随即提高硝酸盐还原酶等表达水平，产生跨膜质子梯度以驱动泛醌氧化还原，生成胞内还原力 NADPH、激活 ATP 酶产生 ATP。随后 NADPH 和 ATP 作为胞内能量载体，驱动 *R. palustri* 通过卡尔文循环(Calvin cycle, CBB)固定 CO_2[34]。该研究证明电能可作为微生物固碳的唯一还原力，揭示了厌氧黑暗的地下沉积环境中电能自养微生物利用电能驱动的黑暗固碳新模式。并且考虑到缺氧黑暗的沉积物是地球上最普遍和最丰富的生态位之一，该研究为深化全球

碳循环提供了新的重要认识。因此，可利用微生物电能自养代谢方式，通过种间电子传递或电极还原力驱动厌氧非光照环境下微生物固碳，这对减少碳排放、实现“双碳”目标具有重要意义。

3.4 光驱噬电人工微生物菌群实现生物固氮

在过去的 100 多年间，哈珀法合成氨一直是氮肥生产的主流工艺，然而这一在高温高压条件下的 N_2 还原工艺每年消耗全球近 1%能源并且释放大量温室气体，对全球气候、生态环境产生严重影响。近年来，噬电微生物菌群以光能或电能为驱动力，实现了同时还原 CO_2、N_2，合成生物有机质、胺类化合物。该技术凭借其环境友好、能量效率高的优势，为替代以哈珀法合成氨的传统固氮技术提供了可行方案。Cestellos-Blanco 等针对单一固氮微生物菌株活力弱、固氮效率低等问题，开发了由噬电菌卵形孢子菌(*Sporomusa ovata*)和固氮菌 *R. palustris* 组成的双菌噬电微生物菌群[10]。其中，*S. ovata* 可附着于太阳能硅纳米线电极表面，在光伏电能驱动下以 CO_2 为底物合成乙酸。乙酸同时作为碳源和能量载体，经 *R. palustris* 细胞代谢后产生胞内还原力 NAD(P)H 等，从而驱动 *R. palustris* 固氮酶还原 N_2 合成氨(图 6D)。在 *S. ovata-R. palustris* 噬电微生物菌群中，固氮菌 *R. palustris* 对乙酸的分解代谢成功地解决了乙酸累积导致的细胞毒性问题，而 *R. palustris* 固氮产生的氨分子可进一步作为固碳

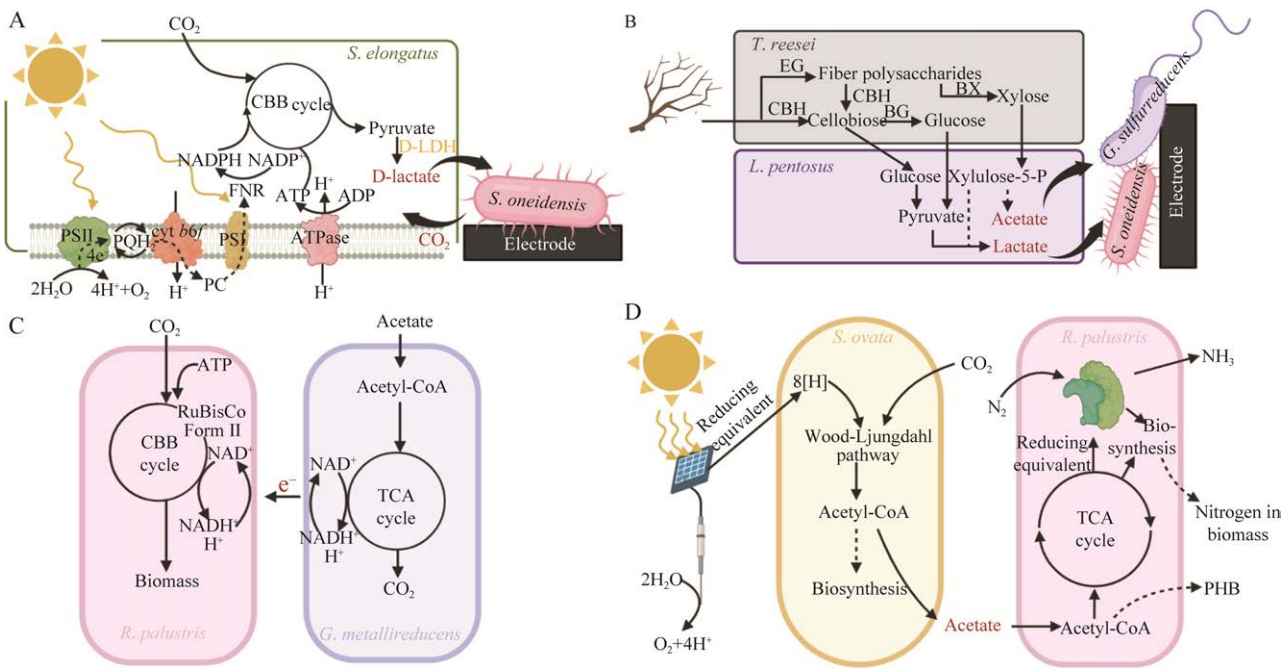

图 6 人工电活性微生物菌群的应用[9-10,34]

Figure 6 The application of synthetic electroactive microflora[9-10,34]. A: Schematic diagram of the biophotovoltaics system based on a two-species microbial consortium with constrained electron flow[9]. B: To harbor artificial cross-kingdom microbial consortia for the consolidated bioprocessing of lignocellulose to short-chain fatty acids. C: A dark CO_2 fixation mode enabled by the electrosyntrophic interaction between *G. metallireducens* and *R. palustris*[34]. D: Designed a biohybrid coculture for tandem and tunable CO_2 and N_2 fixation to value-added products[10].

菌 *R. palustris* 氮源以促进固氮菌高效固氮。因此，*S. ovata-R. palustris* 噬电微生物菌群通过产物的交叉代谢建立起合作(互利共生)的菌群关系。在太阳能驱动下，同时还原 CO_2、N_2 生成高聚物聚羟基丁酸酯(polyhydroxybutyrate, PHB)和铵根离子，实现了微生物固碳合成高值化学品和生物固氮合成氨，有望推动光电生物化学的发展并为气候变暖和环境问题提供解决方案。

4 总结与展望

人工电活性微生物菌群作为电活性微生物与微生物菌群形成的新型交叉领域，其可利用底物广泛、体系鲁棒性强、旁支代谢少而具有较高的能量催化效率，因而在能源、环境、化工等多个领域具有广阔的应用前景。本文总结概括了电活性微生物菌群中的电子传递机制，汇总分析人工电活性微生物菌群的工程策略，分类列举了产电菌群和噬电微菌群在诸多领域的应用，为人工电活性微生物菌群的设计构建与应用提供了重要参考。

然而，目前针对人工电活性微生物菌群的研究仍处于起步阶段，人工电活性微生物菌群的发展面临诸多挑战。比如：(1) 现有人工产电与噬电菌群双向电子传递速率慢严重限制了菌群的物质能量代谢，导致电活性微生物菌群介导的能量转化效率低。并且当前对电活性微生物菌群的物质能量代谢、细胞间互作等复杂机制理解不深入，缺乏工程改造菌群的工具、技术，这些严重阻碍了电活性微生物菌群的实际应用。(2) 目前对人工电活性微生物菌群的设计构建仍处于“劳力分工保证多菌种共生以维持其基本功能”的初级阶段。对于如何设计、构建、调控菌群中各种群分工、生长、分布及比例以实现催化效率最大化，仍没有研究开展深入探索。(3) 由于缺乏模块化标准与工程平台，人工电活性微生物菌群的设计构建过程效率低、耗时长，且在实际应用中难以与上下游工艺相互兼容，这将会阻碍人工电活性微生物菌群的未来工业化应用。

针对以上问题，未来可从以下几方面进一步深入研究。

(1) 开发菌群适应性共进化与理性设计改造相结合策略，工程人工电活性微生物菌群，提高其催化效率。尽管当前已有相关研究通过合成生物学技术方法针对人工电活性菌群中非电活性微生物与电活性微生物物质、能量代谢进行工程强化。但由于对菌群协同催化机理认知有限，传统的工程化策略方法成效不显著，并逐渐成为人工电活性微生物进一步发展的限制瓶颈。微生物菌群适应性共进化策略可使菌群在特定环境压力或任务目标的诱导下，实现菌群内各菌种的共进化，从而使菌群获得优良性状。尽管菌群适应性进化周期长、耗时多，但通过共进化菌群可分析获得诸多未知靶点，为建立电活性微生物菌群工程化理论基础和进一步理性设计改造提供重要参考。

(2) 构建人工电活性微生物菌群数学模型，通过模拟计算精准调控菌群劳力分工、菌群关系与菌群结构分布，实现人工电活性微生物设计构建相关参数的“最优解”。例如，在“串联型”劳力分工菌种群，代谢途径应如何分割、中间代谢物应如何选择以实现菌群最高催化效率，应如何调控各菌种间相互作用、构建何种菌群关系以实现菌群菌种分布结构最优化，以及如何调控各菌种相对比例以实现最高物质与能量的最高转化效率。目前，诸如以上针对人工电活性微生物设计构建过程相关参数的“最优化”调控仍未得到有效研究。通过构建人工电活性微生物菌群数学模型，开发先进算法，结合人工智能机器学习，通过“设计-构建-测试-学习-优化”多轮迭代，模拟计算获得精准调控人工电活性微生物菌群的“最

优解”参数。

(3) 开发人工电活性微生物菌群模块化技术平台，实现菌群构建的“即插即用”，提高人工电活性微生物菌群的构建效率。标准模块化操作是实现广泛工业应用的重要前提。为了提高混菌电化学系统工业化应用水平，可通过将人工电活性微生物菌群设计为模块化技术平台，在工业生产过程中实现灵活的安插利用。例如，以木质纤维素、纤维素为底物的乙酸、乳酸等标准化炼制平台，可与 *Shewanella*、*Geobacter* 等产电菌无缝衔接，可直接构建获得以木质纤维素、纤维素为底物的产电微生物菌群。未来，可针对噬电菌群中的标准模块平台，如光电驱动微生物固碳产甲酸乙酸平台、光电驱动微生物固氮平台以及气体发酵固碳固氮等平台投入更多研究，以推动人工噬电微生物菌群的设计构建效率，实现以 CO_2、N_2 等为底物高效合成高值化学品。

REFERENCES

[1] LOGAN BE, RABAEY K. Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies[J]. Science, 2012, 337(6095): 686-690.

[2] CAO BC, ZHAO ZP, PENG LL, SHIU HY, DING MN, SONG F, GUAN X, LEE CK, HUANG J, ZHU D, FU XY, WONG GCL, LIU C, NEALSON K, WEISS PS, DUAN XF, HUANG Y. Silver nanoparticles boost charge-extraction efficiency in *Shewanella* microbial fuel cells[J]. Science, 2021, 373(6561): 1336-1340.

[3] LIU XM, GAO HY, WARD JE, LIU XR, YIN B, FU TD, CHEN JH, LOVLEY DR, YAO J. Power generation from ambient humidity using protein nanowires[J]. Nature, 2020, 578(7796): 550-554.

[4] LOGAN BE, SHI L, ROSSI R. Enabling the use of seawater for hydrogen gas production in water electrolyzers[J]. Joule, 2021, 5(4): 760-762.

[5] CHEN X, LOBO FL, BIAN YH, LU L, CHEN XW, TUCKER MP, WANG YX, REN ZJ. Electrical decoupling of microbial electrochemical reactions enables spontaneous H_2 evolution[J]. Energy & Environmental Science, 2020, 13(2): 495-502.

[6] LU L, GUEST JS, PETERS CA, ZHU XP, RAU GH, REN ZJ. Wastewater treatment for carbon capture and utilization[J]. Nature Sustainability, 2018, 1(12): 750-758.

[7] LOVLEY DR, PHILLIPS EJP, GORBY YA, LANDA ER. Microbial reduction of uranium[J]. Nature, 1991, 350(6317): 413-416.

[8] WILLIAMS KH, BARGAR JR, LLOYD JR, LOVLEY DR. Bioremediation of uranium-contaminated groundwater: a systems approach to subsurface biogeochemistry[J]. Current Opinion in Biotechnology, 2013, 24(3): 489-497.

[9] ZHU HW, MENG HK, ZHANG W, GAO HC, ZHOU J, ZHANG YP, LI Y. Development of a longevous two-species biophotovoltaics with constrained electron flow[J]. Nature Communications, 2019, 10: 4282.

[10] CESTELLOS-BLANCO S, CHAN RR, SHEN YX, KIM JM, TACKEN TA, LEDBETTER R, YU S, SEEFELDT LC, YANG PD. Photosynthetic biohybrid coculture for tandem and tunable CO_2 and N_2 fixation[J]. Proceedings of the National Academy of Sciences of the United States of America, 2022, 119(26): e2122364119.

[11] LI H, OPGENORTH PH, WERNICK DG, ROGERS S, WU TY, HIGASHIDE W, MALATI P, HUO YX, CHO KM, LIAO JC. Integrated electromicrobial conversion of CO_2 to higher alcohols[J]. Science, 2012, 335(6076): 1596.

[12] LIU XB, YU XB. Enhancement of butanol production: from biocatalysis to bioelectrocatalysis[J]. ACS Energy Letters, 2020, 5(3): 867-878.

[13] CHOI Y, LEE SY. Biosynthesis of inorganic nanomaterials using microbial cells and bacteriophages[J]. Nature Reviews Chemistry, 2020, 4(12): 638-656.

[14] TIAN LJ, LI WW, ZHU TT, CHEN JJ, WANG WK, AN PF, ZHANG L, DONG JC, GUAN Y, LIU DF, ZHOU NQ, LIU G, TIAN YC, YU HQ. Directed biofabrication of nanoparticles through regulating extracellular electron transfer[J]. Journal of the American Chemical Society, 2017, 139(35): 12149-12152.

[15] FAN G, DUNDAS CM, GRAHAM AJ, LYND NA, KEITZ BK. *Shewanella oneidensis* as a living electrode for controlled radical polymerization[J]. Proceedings of the National Academy of Sciences of the United States of America, 2018, 115(18): 4559-4564.

[16] FAN G, GRAHAM AJ, KOLLI J, LYND NA, KEITZ BK. Aerobic radical polymerization mediated by microbial metabolism[J]. Nature Chemistry, 2020, 12(7): 638-646.
[17] CHEN XL, CAO YX, LI F, TIAN Y, SONG H. Enzyme-assisted microbial electrosynthesis of poly(3-hydroxybutyrate) via CO_2 bioreduction by engineered *Ralstonia eutropha*[J]. ACS Catalysis, 2018, 8(5): 4429-4437.
[18] LOGAN BE, ROSSI R, RAGAB A, SAIKALY PE. Electroactive microorganisms in bioelectrochemical systems[J]. Nature Reviews Microbiology, 2019, 17(5): 307-319.
[19] KUMAR A, HSU LHH, KAVANAGH P, BARRIÈRE F, LENS PNL, LAPINSONNIÈRE L, LIENHARD V JH, SCHRÖDER U, JIANG XC, LEECH D. The ins and outs of microorganism-electrode electron transfer reactions[J]. Nature Reviews Chemistry, 2017, 1: 24.
[20] CHEN H, SIMOSKA O, LIM K, GRATTIERI M, YUAN MW, DONG FY, LEE YS, BEAVER K, WELIWATTE S, GAFFNEY EM, MINTEER SD. Fundamentals, applications, and future directions of bioelectrocatalysis[J]. Chemical Reviews, 2020, 120(23): 12903-12993.
[21] TSOI R, DAI ZJ, YOU LC. Emerging strategies for engineering microbial communities[J]. Biotechnology Advances, 2019, 37(6): 107372.
[22] LIU Y, DING MZ, LING W, YANG Y, ZHOU X, LI BZ, CHEN T, NIE Y, WANG MX, ZENG BX, LI X, LIU H, SUN BD, XU HM, ZHANG JM, JIAO Y, HOU YN, YANG H, XIAO SJ, LIN QC, et al. A three-species microbial consortium for power generation[J]. Energy & Environmental Science, 2017, 10(7): 1600-1609.
[23] LIN T, BAI X, HU YD, LI BZ, YUAN YJ, SONG H, YANG Y, WANG JY. Synthetic *Saccharomyces cerevisiae-Shewanella oneidensis* consortium enables glucose-fed high-performance microbial fuel cell[J]. AIChE Journal, 2017, 63(6): 1830-1838.
[24] LI F, YIN CJ, SUN LM, LI YX, GUO XW, SONG H. Synthetic *Klebsiella pneumoniae-Shewanella oneidensis* consortium enables glycerol-fed high-performance microbial fuel cells[J]. Biotechnology Journal, 2018, 13(5): e1700491.
[25] LI F, AN XJ, WU DG, XU J, CHEN YY, LI WC, CAO YX, GUO XW, LIN X, LI CF, LIU SX, SONG H. Engineering microbial consortia for high-performance cellulosic hydrolyzates-fed microbial fuel cells[J]. Frontiers in Microbiology, 2019, 10: 409.
[26] LIU T, YU YY, CHEN T, CHEN WN. A synthetic microbial consortium of *Shewanella* and *Bacillus* for enhanced generation of bioelectricity[J]. Biotechnology and Bioengineering, 2017, 114(3): 526-532.
[27] MCANULTY MJ, G POOSARLA V, KIM KY, JASSO-CHÁVEZ R, LOGAN BE, WOOD TK. Electricity from methane by reversing methanogenesis[J]. Nature Communications, 2017, 8: 15419.
[28] JIANG YJ, SONG RB, CAO LB, SU ZY, MA YM, LIU Y. Harvesting energy from cellulose through *Geobacter sulfurreducens* in unique ternary culture[J]. Analytica Chimica Acta, 2019, 1050: 44-50.
[29] YANG Y, DING YZ, HU YD, CAO B, RICE SA, KJELLEBERG S, SONG H. Enhancing bidirectional electron transfer of *Shewanella oneidensis* by a synthetic flavin pathway[J]. ACS Synthetic Biology, 2015, 4(7): 815-823.
[30] XIAO Y, CHEN G, CHEN Z, BAI R, ZHAO BY, TIAN XC, WU YC, ZHOU X, ZHAO F. Interspecific competition by non-exoelectrogenic *Citrobacter freundii* An1 boosts bioelectricity generation of exoelectrogenic *Shewanella oneidensis* MR-1[J]. Biosensors and Bioelectronics, 2021, 194: 113614.
[31] TAO L, WANG HB, XIE MS, THIA L, CHEN WN, WANG X. Improving mediated electron transport in anodic bioelectrocatalysis[J]. Chemical Communications: Cambridge, England, 2015, 51(61): 12170-12173.
[32] LIU L, MOHAMMADIFAR M, ELHADAD A, TAHERNIA M, ZHANG YX, ZHAO WF, CHOI S. Spatial engineering of microbial consortium for long-lasting, self-sustaining, and high-power generation in a bacteria-powered biobattery[J]. Advanced Energy Materials, 2021, 11(22): 2100713.
[33] HUANG LY, LIU X, ZHANG ZS, YE J, RENSING C, ZHOU SG, NEALSON KH. Light-driven carbon dioxide reduction to methane by *Methanosarcina barkeri* in an electric syntrophic coculture[J]. The ISME Journal, 2022, 16(2): 370-377.
[34] LIU X, HUANG LY, RENSING C, YE J, NEALSON KH, ZHOU SG. Syntrophic interspecies electron transfer drives carbon fixation and growth by *Rhodopseudomonas palustris* under dark, anoxic conditions[J]. Science Advances, 2021, 7(27): eabh1852.
[35] SHI L, DONG HL, REGUERA G, BEYENAL H, LU

AH, LIU J, YU HQ, FREDRICKSON JK. Extracellular electron transfer mechanisms between microorganisms and minerals[J]. Nature Reviews Microbiology, 2016, 14(10): 651-662.

[36] ZHONG YH, SHI L. Genomic analyses of the quinol oxidases and/or quinone reductases involved in bacterial extracellular electron transfer[J]. Frontiers in Microbiology, 2018, 9: 3029.

[37] ZARGAR K, SALTIKOV CW. Lysine-91 of the tetraheme *c*-type cytochrome CymA is essential for quinone interaction and arsenate respiration in *Shewanella* sp. strain ANA-3[J]. Archives of Microbiology, 2009, 191(11): 797.

[38] MCMILLAN DGG, MARRITT SJ, FIRER-SHERWOOD MA, SHI L, RICHARDSON DJ, EVANS SD, ELLIOTT SJ, BUTT JN, JEUKEN LJC. Protein-protein interaction regulates the direction of catalysis and electron transfer in a redox enzyme complex[J]. Journal of the American Chemical Society, 2013, 135(28): 10550-10556.

[39] FIRER-SHERWOOD MA, BEWLEY KD, MOCK JY, ELLIOTT SJ. Tools for resolving complexity in the electron transfer networks of multiheme cytochromesc[J]. Metallomics, 2011, 3(4): 344-348.

[40] STURM G, RICHTER K, DOETSCH A, HEIDE H, LOURO RO, GESCHER J. A dynamic periplasmic electron transfer network enables respiratory flexibility beyond a thermodynamic regulatory regime[J]. The ISME Journal, 2015, 9(8): 1802-1811.

[41] LEYS D, MEYER TE, TSAPIN AS, NEALSON KH, CUSANOVICH MA, van BEEUMEN JJ. Crystal structures at atomic resolution reveal the novel concept of “electron-harvesting” as a role for the small tetraheme cytochrome *c*[J]. Journal of Biological Chemistry, 2002, 277(38): 35703-35711.

[42] EDWARDS MJ, WHITE GF, BUTT JN, RICHARDSON DJ, CLARKE TA. The crystal structure of a biological insulated transmembrane molecular wire[J]. Cell, 2020, 181(3): 665-673.e10.

[43] JIAO YQ, NEWMAN DK. The pio operon is essential for phototrophic Fe(II) oxidation in *Rhodopseudomonas palustris* TIE-1[J]. Journal of Bacteriology, 2007, 189(5): 1765-1773.

[44] FREDRICKSON JK, ROMINE MF, BELIAEV AS, AUCHTUNG JM, DRISCOLL ME, GARDNER TS, NEALSON KH, OSTERMAN AL, PINCHUK G, REED JL, RODIONOV DA, RODRIGUES JLM, SAFFARINI DA, SERRES MH, SPORMANN AM, ZHULIN IB, TIEDJE JM. Towards environmental systems biology of *Shewanella*[J]. Nature Reviews Microbiology, 2008, 6(8): 592-603.

[45] SHI L, ROSSO KM, ZACHARA JM, FREDRICKSON JK. Mtr extracellular electron-transfer pathways in Fe(III)-reducing or Fe(II)-oxidizing bacteria: a genomic perspective[J]. Biochemical Society Transactions, 2012, 40(6): 1261-1267.

[46] EMERSON D, FIELD EK, CHERTKOV O, DAVENPORT KW, GOODWIN L, MUNK C, NOLAN M, WOYKE T. Comparative genomics of freshwater Fe-oxidizing bacteria: implications for physiology, ecology, and systematics[J]. Frontiers in Microbiology, 2013, 4: 254.

[47] LEVAR CE, CHAN CH, MEHTA-KOLTE MG, BOND DR. An inner membrane cytochrome required only for reduction of high redox potential extracellular electron acceptors[J]. mBio, 2014, 5(6): e02034.

[48] ZACHAROFF L, CHAN CH, BOND DR. Reduction of low potential electron acceptors requires the CbcL inner membrane cytochrome of *Geobacter sulfurreducens*[J]. Bioelectrochemistry, 2016, 107: 7-13.

[49] LLOYD JR, LEANG C, HODGES MYERSON AL, COPPI MV, CUIFO S, METHE B, SANDLER SJ, LOVLEY DR. Biochemical and genetic characterization of PpcA, a periplasmic c-type cytochrome in *Geobacter sulfurreducens*[J]. The Biochemical Journal, 2003, 369(Pt 1): 153-161.

[50] SANTOS TC, SILVA MA, MORGADO L, DANTAS JM, SALGUEIRO CA. Diving into the redox properties of *Geobacter sulfurreducens* cytochromes: a model for extracellular electron transfer[J]. Dalton Transactions: Cambridge, England: 2003, 2015, 44(20): 9335-9344.

[51] LIU YM, WANG ZM, LIU J, LEVAR C, EDWARDS MJ, BABAUTA JT, KENNEDY DW, SHI Z, BEYENAL H, BOND DR, CLARKE TA, BUTT JN, RICHARDSON DJ, ROSSO KM, ZACHARA JM, FREDRICKSON JK, SHI L. A trans-outer membrane porin-cytochrome protein complex for extracellular electron transfer by *Geobacter sulfurreducens* PCA[J]. Environmental Microbiology Reports, 2014, 6(6): 776-785.

[52] BUTLER JE, YOUNG ND, LOVLEY DR. Evolution of electron transfer out of the cell: comparative genomics of six *Geobacter* genomes[J]. BMC Genomics, 2010, 11: 40.

[53] LEVAR CE, HOFFMAN CL, DUNSHEE AJ, TONER BM, BOND DR. Redox potential as a master variable controlling pathways of metal reduction by *Geobacter sulfurreducens*[J]. The ISME Journal, 2017, 11(3): 741-752.

[54] REGUERA G, MCCARTHY KD, MEHTA T, NICOLL JS, TUOMINEN MT, LOVLEY DR. Extracellular electron transfer via microbial nanowires[J]. Nature, 2005, 435(7045): 1098-1101.

[55] MALVANKAR NS, YALCIN SE, TUOMINEN MT, LOVLEY DR. Visualization of charge propagation along individual pili proteins using ambient electrostatic force microscopy[J]. Nature Nanotechnology, 2014, 9(12): 1012-1017.

[56] PIRBADIAN S, BARCHINGER SE, LEUNG KM, BYUN HS, JANGIR Y, BOUHENNI RA, REED SB, ROMINE MF, SAFFARINI DA, SHI L, GORBY YA, GOLBECK JH, EL-NAGGAR MY. *Shewanella oneidensis* MR-1 nanowires are outer membrane and periplasmic extensions of the extracellular electron transport components[J]. Proceedings of the National Academy of Sciences of the United States of America, 2014, 111(35): 12883-12888.

[57] YANG YG, WANG ZG, GAN CF, KLAUSEN LH, BONNÉ R, KONG GN, LUO DZ, MEERT M, ZHU CJ, SUN GP, GUO J, MA YX, BJERG JT, MANCA J, XU MY, NIELSEN LP, DONG MD. Long-distance electron transfer in a filamentous Gram-positive bacterium[J]. Nature Communications, 2021, 12: 1709.

[58] BHAYA D, BIANCO NR, BRYANT D, GROSSMAN A. Type IV pilus biogenesis and motility in the cyanobacterium *Synechocystis* sp. PCC6803[J]. Molecular Microbiology, 2000, 37(4): 941-951.

[59] GORBY YA, YANINA S, MCLEAN JS, ROSSO KM, MOYLES D, DOHNALKOVA A, BEVERIDGE TJ, CHANG IS, KIM BH, KIM KS, CULLEY DE, REED SB, ROMINE MF, SAFFARINI DA, HILL EA, SHI L, ELIAS DA, KENNEDY DW, PINCHUK G, WATANABE K, et al. Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms[J]. Proceedings of the National Academy of Sciences of the United States of America, 2006, 103(30): 11358-11363.

[60] MALVANKAR NS, VARGAS M, NEVIN KP, FRANKS AE, LEANG C, KIM BC, INOUE K, MESTER T, COVALLA SF, JOHNSON JP, ROTELLO VM, TUOMINEN MT, LOVLEY DR. Tunable metallic-like conductivity in microbial nanowire networks[J]. Nature Nanotechnology, 2011, 6(9): 573-579.

[61] VARGAS M, MALVANKAR NS, TREMBLAY PL, LEANG C, SMITH JA, PATEL P, SYNOEYENBOS-WEST O, NEVIN KP, LOVLEY DR. Aromatic amino acids required for pili conductivity and long-range extracellular electron transport in *Geobacter sulfurreducens*[J]. mBio, 2013, 4(2): e00105-113.

[62] LEANG C, QIAN XL, MESTER T, LOVLEY DR. Alignment of the c-type cytochrome OmcS along pili of *Geobacter sulfurreducens*[J]. Applied and Environmental Microbiology, 2010, 76(12): 4080-4084.

[63] WANG FB, GU YQ, O'BRIEN JP, YI SM, YALCIN SE, SRIKANTH V, SHEN C, VU D, ING NL, HOCHBAUM AI, EGELMAN EH, MALVANKAR NS. Structure of microbial nanowires reveals stacked hemes that transport electrons over micrometers[J]. Cell, 2019, 177(2): 361-369.e10.

[64] YALCIN SE, O'BRIEN JP, GU YQ, REISS K, YI SM, JAIN R, SRIKANTH V, DAHL PJ, HUYNH W, VU D, ACHARYA A, CHAUDHURI S, VARGA T, BATISTA VS, MALVANKAR NS. Electric field stimulates production of highly conductive microbial OmcZ nanowires[J]. Nature Chemical Biology, 2020, 16(10): 1136-1142.

[65] WANG FB, MUSTAFA K, SUCIU V, JOSHI K, CHAN CH, CHOI S, SU ZL, SI D, HOCHBAUM AI, EGELMAN EH, BOND DR. Cryo-EM structure of an extracellular *Geobacter* OmcE cytochrome filament reveals tetrahaem packing[J]. Nature Microbiology, 2022, 7(8): 1291-1300.

[66] GU YQ, SRIKANTH V, SALAZAR-MORALES AI, JAIN R, O'BRIEN JP, YI SM, SONI RK, SAMATEY FA, YALCIN SE, MALVANKAR NS. Structure of *Geobacter* pili reveals secretory rather than nanowire behaviour[J]. Nature, 2021, 597(7876): 430-434.

[67] NEWMAN DK, KOLTER R. A role for excreted quinones in extracellular electron transfer[J]. Nature, 2000, 405(6782): 94-97.

[68] MARSILI E, BARON DB, SHIKHARE ID, COURSOLLE D, GRALNICK JA, BOND DR. *Shewanella* secretes flavins that mediate extracellular electron transfer[J]. Proceedings of the National Academy of Sciences of the United States of America, 2008, 105(10): 3968-3973.

[69] von CANSTEIN H, OGAWA J, SHIMIZU S, LLOYD

JR. Secretion of flavins by *Shewanella* species and their role in extracellular electron transfer[J]. Applied and Environmental Microbiology, 2008, 74(3): 615-623.

[70] MEVERS E, SU L, PISHCHANY G, BARUCH M, CORNEJO J, HOBERT E, DIMISE E, AJO-FRANKLIN CM, CLARDY J. An elusive electron shuttle from a facultative anaerobe[J]. eLife, 2019, 8: e48054.

[71] OKAMOTO A, SAITO K, INOUE K, NEALSON KH, HASHIMOTO K, NAKAMURA R. Uptake of self-secreted flavins as bound cofactors for extracellular electron transfer in *Geobacter* species[J]. Energy & Environmental Science, 2014, 7(4): 1357-1361.

[72] WANG Y, KERN SE, NEWMAN DK. Endogenous phenazine antibiotics promote anaerobic survival of *Pseudomonas aeruginosa* via extracellular electron transfer[J]. Journal of Bacteriology, 2010, 192(1): 365-369.

[73] GLASSER NR, KERN SE, NEWMAN DK. Phenazine redox cycling enhances anaerobic survival in *Pseudomonas aeruginosa* by facilitating generation of ATP and a proton-motive force[J]. Molecular Microbiology, 2014, 92(2): 399-412.

[74] LIGHT SH, SU L, RIVERA-LUGO R, CORNEJO JA, LOUIE A, IAVARONE AT, AJO-FRANKLIN CM, PORTNOY DA. A flavin-based extracellular electron transfer mechanism in diverse Gram-positive bacteria[J]. Nature, 2018, 562(7725): 140-144.

[75] MIN D, LIU DF, WU J, CHENG L, ZHANG F, CHENG ZH, LI WW, YU HQ. Extracellular electron transfer via multiple electron shuttles in waterborne *Aeromonas hydrophila* for bioreduction of pollutants[J]. Biotechnology and Bioengineering, 2021, 118(12): 4760-4770.

[76] OKAMOTO A, HASHIMOTO K, NEALSON KH, NAKAMURA R. Rate enhancement of bacterial extracellular electron transport involves bound flavin semiquinones[J]. Proceedings of the National Academy of Sciences of the United States of America, 2013, 110(19): 7856-7861.

[77] LIN T, DING WQ, SUN LM, WANG L, LIU CG, SONG H. Engineered *Shewanella oneidensis*-reduced graphene oxide biohybrid with enhanced biosynthesis and transport of flavins enabled a highest bioelectricity output in microbial fuel cells[J]. Nano Energy, 2018, 50: 639-648.

[78] SAUNDERS SH, TSE ECM, YATES MD, OTERO FJ, TRAMMELL SA, STEMP EDA, BARTON JK, TENDER LM, NEWMAN DK. Extracellular DNA promotes efficient extracellular electron transfer by pyocyanin in *Pseudomonas aeruginosa* biofilms[J]. Cell, 2020, 182(4): 919-932.e19.

[79] DESMOND-LE QUÉMÉNER E, MOSCOVIZ R, BERNET N, MARCUS A. Modeling of interspecies electron transfer in anaerobic microbial communities[J]. Current Opinion in Biotechnology, 2021, 67: 49-57.

[80] BRYANT MP, WOLIN EA, WOLIN MJ, WOLFE RS. *Methanobacillus omelianskii*, a symbiotic association of two species of bacteria[J]. Archiv Fur Mikrobiologie, 1967, 59(1): 20-31.

[81] THIELE JH, ZEIKUS JG. Control of interspecies electron flow during anaerobic digestion: significance of formate transfer versus hydrogen transfer during syntrophic methanogenesis in flocs[J]. Applied and Environmental Microbiology, 1988, 54(1): 20-29.

[82] ROTARU AE, SHRESTHA PM, LIU FH, Ueki T, NEVIN K, SUMMERS ZM, LOVLEY DR. Interspecies electron transfer via hydrogen and formate rather than direct electrical connections in cocultures of *Pelobacter carbinolicus* and *Geobacter sulfurreducens*[J]. Applied and Environmental Microbiology, 2012, 78(21): 7645-7651.

[83] de BOK FAM, PLUGGE CM, STAMS AJM. Interspecies electron transfer in methanogenic propionate degrading consortia[J]. Water Research, 2004, 38(6): 1368-1375.

[84] CAI GJ, ZHU GF, ZHOU MD, LV N, WANG RM, LI CX, LI JJ, PAN XF. Syntrophic butyrate-oxidizing methanogenesis promoted by anthraquinone-2-sulfonate and cysteine: distinct tendencies towards the enrichment of methanogens and syntrophic fatty-acid oxidizing bacteria[J]. Bioresource Technology, 2021, 332: 125074.

[85] LOVLEY DR, FRAGA JL, COATES JD, BLUNT-HARRIS EL. Humics as an electron donor for anaerobic respiration[J]. Environmental Microbiology, 1999, 1(1): 89-98.

[86] HUANG LY, LIU X, YE Y, CHEN M, ZHOU SG. Evidence for the coexistence of direct and riboflavin-mediated interspecies electron transfer in *Geobacter* co-culture[J]. Environmental Microbiology, 2020, 22(1): 243-254.

[87] SUMMERS ZM, FOGARTY HE, LEANG C,

FRANKS AE, MALVANKAR NS, LOVLEY DR. Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria[J]. Science, 2010, 330(6009): 1413-1415.

[88] ROTARU AE, SHRESTHA PM, LIU FH, MARKOVAITE B, CHEN SS, NEVIN KP, LOVLEY DR. Direct interspecies electron transfer between *Geobacter metallireducens* and *Methanosarcina barkeri*[J]. Applied and Environmental Microbiology, 2014, 80(15): 4599-4605.

[89] ROTARU AE, SHRESTHA PM, LIU FH, SHRESTHA M, SHRESTHA D, EMBREE M, ZENGLER K, WARDMAN C, NEVIN KP, LOVLEY DR. A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to *Methanosaeta* for the reduction of carbon dioxide to methane[J]. Energy & Environmental Science, 2014, 7(1): 408-415.

[90] MCGLYNN SE, CHADWICK GL, KEMPES CP, ORPHAN VJ. Single cell activity reveals direct electron transfer in methanotrophic consortia[J]. Nature, 2015, 526(7574): 531-535.

[91] LIU X, ZHUO SY, RENSING C, ZHOU SG. Syntrophic growth with direct interspecies electron transfer between pili-free *Geobacter* species[J]. The ISME Journal, 2018, 12(9): 2142-2151.

[92] HA PT, LINDEMANN SR, SHI L, DOHNALKOVA AC, FREDRICKSON JK, MADIGAN MT, BEYENAL H. Syntrophic anaerobic photosynthesis via direct interspecies electron transfer[J]. Nature Communications, 2017, 8: 13924.

[93] CRUZ VIGGI C, ROSSETTI S, FAZI S, PAIANO P, MAJONE M, AULENTA F. Magnetite particles triggering a faster and more robust syntrophic pathway of methanogenic propionate degradation[J]. Environmental Science & Technology, 2014, 48(13): 7536-7543.

[94] LUO CH, LÜ F, SHAO LM, HE PJ. Application of eco-compatible biochar in anaerobic digestion to relieve acid stress and promote the selective colonization of functional microbes[J]. Water Research, 2015, 68: 710-718.

[95] ZHAO ZQ, ZHANG YB, LI Y, DANG Y, ZHU TT, QUAN X. Potentially shifting from interspecies hydrogen transfer to direct interspecies electron transfer for syntrophic metabolism to resist acidic impact with conductive carbon cloth[J]. Chemical Engineering Journal, 2017, 313: 10-18.

[96] ZHAO ZQ, ZHANG YB, WOODARD TL, NEVIN KP, LOVLEY DR. Enhancing syntrophic metabolism in up-flow anaerobic sludge blanket reactors with conductive carbon materials[J]. Bioresource Technology, 2015, 191: 140-145.

[97] STORCK T, VIRDIS B, BATSTONE DJ. Modelling extracellular limitations for mediated versus direct interspecies electron transfer[J]. The ISME Journal, 2016, 10(3): 621-631.

[98] ROELL GW, ZHA J, CARR RR, KOFFAS MA, FONG SS, TANG YJ. Engineering microbial consortia by division of labor[J]. Microbial Cell Factories, 2019, 18(1): 35.

[99] YANG Y, WU YC, HU YD, CAO YX, POH CL, CAO B, SONG H. Engineering electrode-attached microbial consortia for high-performance xylose-fed microbial fuel cell[J]. ACS Catalysis, 2015, 5(11): 6937-6945.

[100] BOSE A, GARDEL EJ, VIDOUDEZ C, PARRA EA, GIRGUIS PR. Electron uptake by iron-oxidizing phototrophic bacteria[J]. Nature Communications, 2014, 5: 3391.

[101] JOHNS NI, BLAZEJEWSKI T, GOMES AL, WANG HH. Principles for designing synthetic microbial communities[J]. Current Opinion in Microbiology, 2016, 31: 146-153.

[102] BASSLER BL, LOSICK R. Bacterially speaking[J]. Cell, 2006, 125(2): 237-246.

[103] DETER HS, LU T. Engineering microbial consortia with rationally designed cellular interactions[J]. Current Opinion in Biotechnology, 2022, 76: 102730.

[104] BERNSTEIN HC, PAULSON SD, CARLSON RP. Synthetic *Escherichia coli* consortia engineered for syntrophy demonstrate enhanced biomass productivity[J]. Journal of Biotechnology, 2012, 157(1): 159-166.

[105] HARCOMBE WR, RIEHL WJ, DUKOVSKI I, GRANGER BR, BETTS A, LANG AH, BONILLA G, KAR A, LEIBY N, MEHTA P, MARX CJ, SEGRÈ D. Metabolic resource allocation in individual microbes determines ecosystem interactions and spatial dynamics[J]. Cell Reports, 2014, 7(4): 1104-1115.

[106] SHOU WY, RAM S, VILAR JMG. Synthetic cooperation in engineered yeast populations[J]. Proceedings of the National Academy of Sciences of the United States of America, 2007, 104(6): 1877-1882.

[107] VENKATARAMAN A, ROSENBAUM MA, PERKINS SD, WERNER JJ, ANGENENT LT. Metabolite-based mutualism between *Pseudomonas aeruginosa* PA14

and *Enterobacter aerogenes* enhances current generation in bioelectrochemical systems[J]. Energy & Environmental Science, 2011, 4(11): 4550-4559.

[108] SHAHAB RL, BRETHAUER S, DAVEY MP, SMITH AG, VIGNOLINI S, LUTERBACHER JS, STUDER MH. A heterogeneous microbial consortium producing short-chain fatty acids from lignocellulose[J]. Science, 2020, 369(6507): eabb1214.

[109] SHAHAB RL, BRETHAUER S, LUTERBACHER JS, STUDER MH. Engineering of ecological niches to create stable artificial consortia for complex biotransformations[J]. Current Opinion in Biotechnology, 2020, 62: 129-136.

[110] QI X, WANG SY, JIANG Y, LIU PP, LI QC, HAO W, HAN JB, ZHOU YX, HUANG X, LIANG P. Artificial electrochemically active biofilm for improved sensing performance and quickly devising of water quality early warning biosensors[J]. Water Research, 2021, 198: 117164.

[111] FREYMAN MC, KOU TY, WANG SW, LI Y. 3D printing of living bacteria electrode[J]. Nano Research, 2020, 13(5): 1318-1323.

[112] GAO H, LI MH, YANG L, JIANG YJ, JIANG WK, YU ZY, ZHANG WM, XIN FX, JIANG M. Spatial niche construction of a consortium-based consolidated bioprocessing system[J]. Green Chemistry, 2022, 24(20): 7941-7950.

[113] HE FK, OU YT, LIU J, HUANG Q, TANG B, XIN FX, ZHANG J, JIANG M, CHEN S, YU ZY. 3D printed biocatalytic living materials with dual-network reinforced bioinks[J]. Small: Weinheim an Der Bergstrasse, Germany, 2022, 18(6): e2104820.

[114] CHEN XL, LAWRENCE JM, WEY LT, SCHERTEL L, JING QS, VIGNOLINI S, HOWE CJ, KAR-NARAYAN S, ZHANG JZ. 3D-printed hierarchical pillar array electrodes for high-performance semi-artificial photosynthesis[J]. Nature Materials, 2022, 21(7): 811-818.

[115] SPEERS AM, YOUNG JM, REGUERA G. Fermentation of glycerol into ethanol in a microbial electrolysis cell driven by a customized consortium[J]. Environmental Science & Technology, 2014, 48(11): 6350-6358.

[116] KIM C, SONG YE, LEE CR, JEON BH, KIM JR. Glycerol-fed microbial fuel cell with a co-culture of *Shewanella oneidensis* MR-1 and *Klebsiella pneumonae* J2B[J]. Journal of Industrial Microbiology & Biotechnology, 2016, 43(10): 1397-1403.

[117] REN ZY, WARD TE, REGAN JM. Electricity production from cellulose in a microbial fuel cell using a defined binary culture[J]. Environmental Science & Technology, 2007, 41(13): 4781-4786.

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