Cultivating electroactive microbes—from field to bench

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Cultivating electroactive microbes—field to bench

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Abstract

Electromicrobiology is an emerging field investigating and exploiting the interaction of microorganisms with insoluble electron donors or acceptors. Some of the most recently categorized electroactive microorganisms became of interest to sustainable bioengineering practices. However, laboratories worldwide typically maintain electroactive microorganisms on soluble substrates, which often leads to a decrease or loss of the ability to effectively exchange electrons with solid electrode surfaces. In order to develop future sustainable technologies, we cannot rely solely on existing lab-isolates. Therefore, we must develop isolation strategies for environmental strains with electroactive properties superior to strains in culture collections. In this article, we provide an overview of the studies that isolated or enriched electroactive microorganisms from the environment using an anode as the sole electron acceptor (electricity-generating microorganisms) or a cathode as the sole electron donor (electricity-consuming microorganisms). Next, we recommend a selective strategy for the isolation of electroactive microorganisms. Furthermore, we provide a practical guide for setting up electrochemical reactors and highlight crucial electrochemical techniques to determine electroactivity and the mode of electron transfer in novel organisms.

Supplementary material for this article is available online

Keywords: electroactive microorganisms, electrotroph, electrogen, microbial fuel cells, microbial electrosynthesis, bioelectrochemical systems

(Some figures may appear in colour only in the online journal)

Introduction

Living things conserve energy by translocating electrons from an organic food substrate (electron donor) to a terminal electron acceptor (e.g. oxygen) via redox reactions in a respiratory chain. During classical respiration, these redox reactions are intracellular. In electroactive microorganisms, electron transfer reactions extend beyond the cell surface in a process called extracellular electron transfer (EET) [1–4]. EET is a unique metabolic feature that enables electroactive microorganisms to use solid-state electron donors or acceptors located outside the cell, which would otherwise remain inaccessible. Electroactive microorganisms have the unparalleled capability to ‘release’ or ‘retrieve’ electrons from a solid-state extracellular substrate. Microorganisms ‘releasing’ electrons onto a solid-state extracellular electron acceptor are electrogens whereas microorganisms that ‘retrieve’ electrons from an extracellular electron donor are electrotrophs. Electrogens are capable of electron release onto an electrode/anode surface, which is quantifiable as a positive electric current whereas electrotrophs retrieve electrons from a poised electrode/cathode surface, which is quantifiable as a negative electric current [5].

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Electroactive microorganisms (electrogens and electro-trophs) employ different mechanisms of EET, which are direct EET or indirect-mediated EET (figure 1).

During direct EET, cells establish physical contacts usually via electron-conductive proteins, which transfer electrons across cell membranes [2–4, 6–8] such as outer-membrane multiheme c-type cytochromes (MHC) [1, 9–11], extracellular MHC wire-extensions [12, 13], [Fe–S] proteins [14], conductive pili [8, 15–19] or periplasmic extensions [20, 21] (figure 1). The mechanisms of direct EET vary significantly between different species of electroactive microorganisms suggestive of these organisms evolving comparable traits via convergent evolution in order to adapt to similar ecological niches in the environment.

During facilitated EET, diffusible redox-active molecules act as electron carriers and link redox reactions happening inside and outside of the cell [6, 22]. Facilitated EET includes EET mediated by redox shuttles, as well as EET mediated by extracellular enzymes producing diffusible chemicals (figure 1). Some examples of microorganisms that secrete and engage shuttles in EET are the Gram-positive Listeria monocytogenes [23], the Gram-negative Pseudomonas [24–26] and Shewanella [27–31]. Generally, secreted shuttles are two-electron carriers like flavins [23, 27–31] or phenazines [24–26]. However, an unusual soluble menaquinone (2-amino-3-carboxy-1,4-naphthoquinone) was recently linked to EET in Shewanella oneidensis [32]. Besides shuttles, EET can be facilitated by extracellular enzymes that prompt the recycling of electrons from an extracellular surface in certain methanogens and acetogens. For example, the methanogen Methanococcus maripaludis and acetogens of the genus Sporomusa, or Acetobacterium use Ni-Fe-hydrogenases and/or heterodisulfide reductase [33–36] to retrieve electrons from the surface of an electrode or of metalloferron (figure 1).

Electroactive microorganisms have earned considerable attention in the field of applied microbiology. Accordingly, bioengineering technologies have been developed to match the direction of the electron flow to cells (microbial electro-synthesis) and from cells (microbial fuel cells), independent of the electron transfer mechanism. There are two focus areas in the application of electroactive microorganisms that can be distinguished by the direction of electron flow: electron-releasing bioanodes when cells remove electrons from the feed to be ‘released’ onto an anode; and electron-retrieving bioanodes when cells ‘retrieve’ electrons from a cathode to use them as feed.

Some of the earliest bioelectrochemical systems dealt with bioanodes in microbial fuel cells (MFC) where microbes converted chemical energy from food substrates into electrical energy by transferring electrons to an anode [37, 38]. In MFCs, microorganisms oxidize simple/complex organics (e.g. glucose) or mixed organics from wastewaters [39], while producing high anodic current densities with coulombic efficiencies as high as 100% [38, 40–42]. The effectiveness of MFCs for the production of electrical energy remains a matter of debate [43, 44]. Nevertheless, MFCs were successfully applied to purify wastewater [39], to bioremediate toxic chemicals [45], or to adjust the redox balance of a fermentation broth [46–48]. These properties make MFCs a technology of interest, especially for remote geographic locations where access to water purification and bioremediation technologies is limited [49].

On the other hand, bioelectrochemical systems that deal with bioanodes are microbial electrosynthesis systems (MES), where microorganisms retrieve electrons from a cathode and convert electrical energy into chemical energy to be stored in synthesis products. In MES, autotrophic microorganisms use cathode-derived electrons to convert CO₂ to platform chemicals (e.g. acetate) [50–53], fuels (e.g. methane) [54–57], bioplastics (e.g. polyhydroxyalkanoates) [58], or bio-detergents (e.g. rhamnolipids) [59]. Additionally, cathodic electrons could be used to drive microorganisms to recover metals from metallurgy waste streams [60].

The success of bioelectrochemical applications depends on the electrochemical setup as much on the electroactivity of microorganisms. In 2016, Koch and Harnisch listed 94 species as electroactive [61] with electroactivity confirmed beyond the Geobacter and Shewanella genera, in almost all tested metal-reducers including the Betaproteobacterium—Rhodoferax ferrireducens [62]; the Chloroflexi—Ardentiacatenatamariitima [63] or the hyperthermophilic Archaea—
**Ferroglobus placidus** and **Geoglobus ahangari** [64]. Furthermore, electroactivity was confirmed in iron oxidizers like **Acidithiobacillus ferrooxidans** [65], nitrate reducers like **Pseudomonas alcaliphila** [66], sulfate reducers like **Desulfobulbus propionicus** [67], acetogens like **Sporomusa ovata** [51, 53, 68, 69], methanogens like **Methanosaeta barkeri** [56, 70] and photoautotrophs like **Rhodopseudomonas palustris** [71] or **Prostheochloris aestuarii** [72].

Until now, electroactivity testing and downstream biotechnology applications rely mostly on laboratory strains isolated and maintained on soluble substrates. Nevertheless, strains adjusted to soluble substrates adapt to a non-EET environment losing components for EET. On the other hand, their environmental analogs maintain EET competence in order to function in a selective EET environment (e.g. mineral rich sediments). For example, two **G. sulfurreducens** strains isolated with bioelectrochemical methods (table 1) led to higher power outputs than laboratory strains of the same species [73, 74]. Moreover, lab cultivation on diffusible substrates (H₂) led to diminished cathodic electron use in **M. maripaludis**, which lost the entire genomic island relevant for EET [36]. In extreme cases, we may be deceived on the electroactivity of a species by studying solely culture collection strains maintained on soluble substrates. This was the case for the culture-collection strain **Rhodopseudomonas palustris** ATCC 17001, which could not use an anode as electron acceptor whereas a same species isolate from an environmental analog with bioelectrochemical methods (table 1) led to higher power outputs than laboratory strains of the same species [73, 74].

Since electroactive microorganisms lose their EET-capabilities when grown under non-specific conditions in the laboratory, enrichment of biotechnologically relevant and effective electroactive microorganisms requires an electrochemical isolation approach. Throughout an electrochemical isolation procedure, electrical current (negative or positive) will provide the selective pressure for the isolation of electroactive microorganisms. In this review, we provide an overview of (table 1) and suggest a strategy for (figure 2) enrichment and isolation of environmental isolates with innate electroactivity. We provide a guide for the isolation of electroactive microorganisms in a standardized microbial electrochemical system (box 1), particularly suited for anaerobes, and finally offer an overview of essential methodologies to detect electroactivity and distinguish between direct and facilitated electron transfer.

**Niches for electroactive microorganisms**

It is anticipated that electroactive microorganisms occur in environments where a solid-state extracellular electron acceptor or donor is naturally abundant, offering a positive selective pressure for an electroactive metabolism to dominate such a specialized ecological niche. Surprisingly, in a previous review, authors did not find a specific ecological niche for electroactive microorganisms [61]. Most electroactive species described have been isolated on soluble substrates. However, their natural distribution is not suggestive of niche partitioning [61], likely because these species typically do not perform EET in their environment. In other words, species easily isolated on soluble substrates that preserve their EET properties are unsurprisingly not exhibiting EET-niche partitioning, probably because they adopted a generalist behavior and are adjusted to a variety of soluble substrates typical of their environment.

Environments with a predominance of solid-state electron donors and acceptors include:

(i) **Iron-rich minerals**. Iron is the most abundant metal on Earth, so microorganisms have adapted to use iron from minerals such as magnetite or pyrite, as a source or sink of electrons (see below) [76, 77];

(ii) **Metallic iron (Fe⁰)**. Unalloyed Fe⁰ is rare in the Earth’s crust (e.g. in serpentinite; iron ores), unless mined and enriched for human use (mild-steel infrastructure). Nevertheless, during the Anthropocene microorganisms adjusted to using Fe⁰ as an electron donor [78];

(iii) **Carbonaceous materials**. Some non-metallic materials occur in the environment, have the property to conduct charge and can therefore be used as donors or acceptors by microorganisms. Examples of carbonaceous materials are chars [79] and humic acids, the later includes the majority of undegradable organics in sediments and soils [80]. Chars are especially abundant in areas affected by forest fires [81] or are added to agricultural soils to stimulate plant or the decontamination of toxins [82];

(iv) **Other cells** act as electron donors and acceptors carrying thermodynamically synchronized metabolic interactions by sharing electrons via extracellular molecular electrical conduits (see below) [56, 83–85]. Cell-to-cell interactions in aquatic sediments may also strictly rely on naturally abundant conductive minerals to transfer electrons in between metabolically co-dependent microorganisms [86].

Some natural occurrences of iron rich-minerals are the conductive structures found in hydrothermal vent chimneys or serpentinizing springs, aquatic sediments and soils [87–89]. Environments where conductive minerals abounded dominated throughout Earth’s history. Ancient oceanic environments were iron-rich [90] and likewise are present analogs (e.g. lake Matano Indonesia, lake La Cruz, Spain) [91, 92]. One environment where electroactive microorganisms may have adapted over long evolutionary time scales [93] to electrically conductive surfaces are hydrothermal chimney walls [94–96], which are thought to spontaneously generate electricity [94, 95]. In fact, hydrothermal vent isolates were capable of EET with insoluble electron donors like rocks/minerals [97–100] or electrodes [101, 102].

Last but not least, some microbial species can exchange electrons with each other by transferring electrons from an electron to an electrotroph via direct interspecies electron transfer (DIET) [56, 83–85]. During DIET, the electron is provided with an electron donor for oxidative metabolism, but without any of its electron acceptor, whereas the electrotrophs is provided only with an electron acceptor. Thus, by coupling
| Organism                          | Inoculum source       | Bioelectrochemical enrichment | Other enrichment approach | Isolation method                                                                 | Reference |
|----------------------------------|-----------------------|------------------------------|---------------------------|----------------------------------------------------------------------------------|-----------|
| *Clostridium butyricum* EG3      | Starch processing wastewater | Wastewater EDs and an anode no set potential (TEA) | None                      | Dilution plating on agar-media with glucose (ED) and Fe(III)citrate (TEA)        | [162]     |
| *Aeromonas hydrophila* PA3       | Undefined inoculum    | Acetate (ED) and an anode no set potential (TEA) | None                      | Dilution plating on agar-media with acetate (ED) and Fe(III)pyrophosphate (TEA) | [163]     |
| *Geopyscho bacter electro diphilus* | Marine Sediment       | Sediment organics (ED) and a sediment - anode no set potential (TEA) | Primary: Liquid dilution series with acetate (ED) and insoluble Fe(III)oxide (TEA) 3x Secondary: Plated with acetate (ED) and Fe(III)pyrophosphate (TEA) Tertiary: Liquid dilution with acetate/benzylate (ED) and Fe(III)pyrophosphate (TEA) | [164]     |
| *Ochrobactrum anthraci* YZ-1     | Primary clarifier wastewater | Acetate (ED) and an anode no set potential (TEA) | None                      | Serial dilution MFCs with acetate (ED) and an anode no set potential (TEA). 5x    | [126]     |
| *Rhodopseudomonas palustris* DX-1 | Undefined inoculum    | Acetate (ED) and an anode no set potential (TEA) | None                      | Dilution series (roll-tubes) with acetate (ED) and amorphous Fe(III) (TEA)     | [75]      |
| *Thermincola potens* JR          | Thermophilic anaerobic digestor | Anode no set potential (TEA) and acetate (ED) | None                      | Primary: Liquid dilution series with acetate (ED) and AQDS (TEA) Secondary: Dilution series (agar shakes) with acetate (ED) and AQDS (TEA) | [40, 165]|
| *Brevibacteria sp.* (2 strains) | Domestic wastewater   | Glucose / wastewater organics (EDs) and an anode no set potential (TEA) | None                      | Primary: Liquid dilution series on LB media, O2 (TEA); 6x Secondary: Dilution series in LB agar slants, O2 (TEA) | [139]     |
| *Arcobacter sp.* (2 species)     | Marine sediment       | Acetate (ED) and an anode no set potential (TEA) | None                      | Dilution plating with organisms (ED yeast, blood etc) under aerobic (O2; TEA) or anaerobic (fermentative) conditions | [140]     |
| *Aeromonsas sp.*                 | Wastewater            | Glucose (ED) and an anode no set potential (TEA) | None                      | Dilution plating with glucose (ED) and ferric citrate (TEA). 3x          | [166]     |
| *Shewanella marisflavi* EP1      | Marine Sediment       | Lactate (ED) and an anode no set potential (TEA) | Primary: Lactate (ED) and an acetate (TEA) Secondary: Anode biofilm + lactate (ED) and Fe(III)citrate (TEA) | Dilution series (roll-tubes) with lactate (ED) and ferric citrate (TEA). 3x | [146]     |
| *Comamonas denitrificans* DX-4   | Wastewater            | Acetate (ED); anode no set potential (TEA) | None                      | Serial dilution MFCs with acetate (ED) and an anode no set potential (TEA). 3x | [146]     |
| *Citrobacter sp.* SX-1           | Wastewater            | Acetate (ED); anode no set potential (TEA) | None                      | Dilution plating with acetate (ED) and ferric citrate (TEA)               | [167]     |
| *Geobacter brementis* (5 strains) | Garden compost        | Primary: acetate (ED) and 12 sediment-anodes at +0.7 V (TEA) | Secondary: Anode biofilm with ethanol or lactate (ED) and AQDS, Fe(III) citrate or Fe(III)NTA (TEA) | Primary: Liquid dilution series (3x) with ethanol/lactate (ED) and AQDS, Fe(III)citrate/Fe(III)NTA (TEA) Secondary: Dilution plating: same substrates as above | [168]     |
| *Bacillus pseudofirmus* MC02     | Undefined inoculum    | Primary: Unknown EDs; anode no set potential (TEA) | Secondary: Anode biofilm with acetate (ED) and AQDS (TEA). 3x | Dilution plating on LB-agar, O2 (TEA) followed by colony re-streaking on LB agar, O2 (TEA) | [141]     |
| *Tolunomas osnomensis* OCF 7     | Anaerobic sewage sludge | Glucose (ED); anode no set potential (TEA) | None                      | Dilution plating with glucose (ED) and Fe(III) citrate (TEA)               | [169]     |
| *Geobacter sulfurreducens* D8     | Rice paddy soil       | Primary: anode at +0.544 V (TEA) and undefined EDs from the soil | None                      | Dilution to extinction prior to plating onto agar-media with acetate (ED) and amorphous Fe(III) (TEA). 10x | [170]     |
| Organism                        | Inoculum source       | Bioelectrochemical enrichment | Other enrichment approach | Isolation method                                                                 | Reference |
|--------------------------------|-----------------------|------------------------------|--------------------------|---------------------------------------------------------------------------------|-----------|
| **Raoultella electrica**       | Wastewater            | Glucose (ED); anode no set potential (TEA) | None                     | Dilution plating onto LB agar, with O₂ (TEA)                                     | [142]     |
| **Enterobacter sp. R2B1**      | Precond. activated sludge | Acetate (ED); anode no set potential (TEA) | None                     | Dilution series (roll-tubes) with acetate (ED) and insoluble Fe(III)oxides (TEA) | [127]     |
| **Geobacter sp. SD-1**         | Domestic wastewater   | Formate (ED); anode no set potential (TEA) | None                     | Serial dilution MFCs with acetate (ED) and an anode 0.7 V across the circuit (TEA), 5x | [125]     |
| **Klebsiella sp. MC-1**        |Undefined inoculum     | Glucose (ED) + cyanide and an anode no set potential (TEA); | None                     | Dilution series in liquid and solid with glucose (ED) + cyanide and Fe(III)citrate (TEA) | [171]     |
| **Citrobacter freundii Z7**    | Aerobic sewage sludge | Primary: Glucose (ED) and an anode no set potential (TEA) | Secondary: anode biofilm in LB broth with Fe(III)citrate (TEA) and O₂ (TEA) | Dilution plating onto agar plates; with O₂ (TEA)                              | [137]     |
| **Desulfuromonas soudanensis WTL** | Deep subsurface aquifer | Primary: In situ borehole anode (TEA) | Secondary: Bionode transferred with acetate (ED) set at +0.24 V (TEA) | None                                                                             |           |
| **Delfia sp. WE1-13**          | Deep subsurface aquifer | Secondary: H₂ (ED); anode (TEA) at one of the voltages +0.272/0.372/0.472/0.572 V | Primary: Sponge reactor with H₂ (ED), ferrihydrite + Mn(IV)oxides (TEA) | Plating onto R2A agar; O₂ (TEA)                                                 | [122]     |
| **Azonexus sp. WE2-4**         |                  | Quaternary: acetate (ED); anode (TEA) at one of the voltages +0.272/0.372/0.472/0.572 V | Tertiary: Anode biomass with acetate (ED) and soluble Fe(III)NTA (TEA) |                                                             |           |
| **Aeromonas jandaei SCS5**     | Activated sludge     | Acetate (ED) anode no set potential (TEA) | None                     | Dilution series (roll-tubes) with acetate (ED) and insoluble Fe(III)oxide       | [172]     |
| **Cloacibacterium normanense RA1** | Rumen liquid         | Autoclaved hay (ED) and an anode no set potential (TEA) | None                     | Dilution plating onto nutrient agar with O₂ (TEA)                               | [138]     |
| **Micrococcus luteus RA2**     | Serpenizing spring    | Primary: In situ anode (TEA) in serpenizing spring containing H₂ (ED) | Secondary: Bionode at +0.4 V (TEA) with i.e. galactose (ED) | Dilutions series plating with galactose (ED) and manganese dioxide (TEA)       | [89]      |
| **Geobacter metallireducens, Aeromonas sp., Enterbacter sp.** | Urban canal sediment | Primary: An anode at +0.4/0.6 V (TEA) and sediment EDs | None                     | Plating with acetate (ED) and Fe(III) citrate (TEA)                             | [155]     |
| **Dietzia sp. RNV-4**          | River sediment       | Primary: Sediment organics (ED) and a sediment-anode no set potential (TEA) | None                     | Primary: Liquid dilution series with acetate (ED) and Fe(III)citrate (TEA). 4x | [119]     |
| **Geobacter sulfurreducens subsp. ethanolicus CL-1** | Rice paddy soil | Primary: Anode at +0.544 V (TEA) soil EDs and acetate (ED) | Secondary: Anaerobic enrichment (ED and TEA not disclosed) | Primary: Dilution to extinction | [173]     |

**Table 1.** (Continued.)
Table 1. (Continued.)

| Organism | Inoculum source | Bioelectrochemical enrichment | Other enrichment approach | Isolation method | Reference |
|----------|-----------------|-------------------------------|---------------------------|-----------------|-----------|
| *Citrobacter sp.* KVM11 | Contaminated ground-water and sludge | Petroleum hydrocarbon mix (ED) and an anode no set potential | None | Dilution plating with acetate (ED) and ferric citrate (TEA) | [174] |
| *Kluyvera georgiana* MCC 3673 | Freshwater lake sediment | Primary: Oilseed cake (ED) and an anode no set potential (TEA); media replenished 15x | LB-broth; O₂ (TEA) | Dilution series on LB-agar media, O₂ (TEA) | [124] |
| *Enterococcus avium* strain Gut-S1 | Primary: Soil organics and an acetate | None | Anode biomass streaked on agar plates with acetate or lactate (ED) and manganese dioxide (TEA) | | [175] |
| *Klebsiella pneumoniae* strain Gut-S2 | None | | | | |
| *Citrobacter sp.* strain ND-2 | Rice paddy soil | Primary: Soil organics and a sediment-anode no set potential (TEA) | None | Dilution plating onto solid agar supplemented with acetate (ED) and FTO electrode poised at 0 V (TEA) | [143] |
| *Geobacter sp.* strain RPFA-12G-1 | Rice paddy soil | Secondary: Bioanode further cultivated in biocathode further cultivated in bioelectrochemical reactor with acetate (ED) and anode poised at −0.2 V (TEA) | None | | |

**CATHODIC**

| Organism | Inoculum source | Bioelectrochemical enrichment | Other enrichment approach | Isolation method | Reference |
|----------|-----------------|-------------------------------|---------------------------|-----------------|-----------|
| *Dechlorosporillum* strain VDY | Groundwater | Primary: Groundwater inoculated H cell reactor with cathode poised at ca. −0.3 mV (ED) and perchlorate (TEA) | Secondary: biocathode in media with acetate (ED) and perchlorate (TEA). Repeated once | Dilution series in agar shakes with acetate (ED) and perchlorate (TEA) | [135] |
| *Labrenzia aggregata* a (7 sp.) | Marine Sediment | Primary: A cathode under sunlight (EDs) and O₂ (TEA) | None | Primary: Dilution series with FeS (ED) and O₂ (TEA) | [110, 111, 129] |
| *Hyphomonas adhaerens* b (1 sp.) | Marine Sediment | Primary: A cathode under sunlight (EDs) and O₂ (TEA) | None | Secondary: Plating and re-streaking on marine agar broth Difco, aerobically (O₂; TEA) or plating on agar-media with acetate (ED) and O₂ (TEA) | Unsuccessful isolation | [121] |
| *Baccilllus firmus* b (1 sp.) | Marine Sediment | Secondary: Bioanode further cultivated in bioelectrochemical reactor with acetate (ED) and anode poised at −0.2 V (TEA) | | | |
| *Marinobacter* c (2 sp.) | Marine Sediment | Secondary: Bioanode further cultivated in bioelectrochemical reactor with acetate (ED) and anode poised at −0.2 V (TEA) | | | |
| *Phaeobacter daeponensis* d (4 sp.) | Marine Sediment | Secondary: Bioanode further cultivated in bioelectrochemical reactor with acetate (ED) and anode poised at −0.2 V (TEA) | | | |
| *Candidatus* Tenderia electrophaga | Marine Sediment | Secondary: Bioanode further cultivated in bioelectrochemical reactor with acetate (ED) and anode poised at −0.2 V (TEA) | | | |
| *H. aquamarina* (1 sp.) | Seawater | In situ stainless steel cathode ca. 0 V (ED) and O₂ (TEA) | None | Dilution plating on marine agar, aerobically (O₂ as TEA) | [136] |
| *Roseobacter* sp. (4 sp.) | Seawater | In situ stainless steel cathode ca. 0 V (ED) and O₂ (TEA) | None | Dilution plating on marine agar, aerobically (O₂ as TEA) | [136] |
| *Silicibacter* (2 sp.) | Seawater | In situ stainless steel cathode ca. 0 V (ED) and O₂ (TEA) | None || |
| *Winogradskyella poriferorum, Acinetobacter johnsonii* | Seawater | In situ stainless steel cathode ca. 0 V (ED) and O₂ (TEA) | None | Liquid dilution series and/or plating onto agar media (Difco); O₂ (TEA) | [123] |
| *Thiohydra electrothropa, Halomonas sp., Idiomarina sp., Marinobacter sp., Pseudomonas sp., Thalassospira sp.* | Marine Sediment | Primary: Sediment microorganisms with a cathode at −0.203 V and +0.2 V and undefined TEAs from the sediment | Secondary: Lab-reactor (conditions see above) | Tertiary: Cathode-biomass enriched using insoluble substrates Fe⁺ or S⁰ (ED) and nitrate (TEA) | Dilution series in agar shakes and/or plating with either Fe(II), S⁰ or thiosulfate (ED) and nitrate or Fe(II)-NTA (TEA) | [132, 133] |
| *Bacillus* sp. strain H | Anaerobic digestor sludge | Digestor sludge H-cell with Cr(VI) (TEA) and a cathode (ED) with an undefined potential from degradation of wastewater organics at the anode | None | Dilution plating of cathodic biofilm on LB agar with Cr(VI)₃ (TEA) | [131] |

Note: All the potentials reported here are against the standard hydrogen electrode. MFC microbial fuel cell; AQDS Anthraquinone-2,6-disulfonate; ED electron donor; TEA terminal electron acceptor; FTO fluorine-doped tin oxide. Primary, secondary, tertiary and quaternary refer to the succession during the enrichment/isolation procedure.

a *Labrenzia* species were inactive on a cathode. Only one showed an FeS-oxidation band for three successive transfers.
b Not tested on a cathode, however, it did not maintain FeS-oxidation activity over three successive transfers.
c One strain (Marinobacter adherens) was electroactive using a cathode as electron donor only when 2 mM acetate was provided as carbon source.
the two processes the dual-species consortia can thrive. During DIET, the electron requires the same EET conduit that is required for interactions with electrodes, which includes pili and extracellular MHC-cytochromes [1]. The dependency on the electrical conduit was verified with genetically manipulated partners, incapable to express an EET conduit, and with partners known to use other EET mechanisms (e.g. H₂ rather than DIET) [84, 85]. Conversely, it is challenging to demonstrate DIET in the environment, as we do not have a specific molecular or chemical fingerprint. Despite this, DIET has been reported by indirect measurements in environments such as anaerobic digesters [103, 104], rice paddies [105], or deep-sea sediments [106, 107]. In environmental consortia, DIET is often endorsed by indirect observations such as: (i) high conductivity of the consortia [103, 104]; (ii) failure to make use of diffusible formate or H₂ [103]; (iii) high expression of genes associated with EET [105–107]; (iv) stimulation of the metabolism by conductive materials [108]; (v) or by phylogenetic affiliation to DIET-species [109]. However, in these environments, the actual mechanism of interaction and partner co-dependency remain a matter for future inquiry.

Finally, the ability to exchange electrons with the extracellular milieu provides a selective advantage for electroactive microbes in a variety of ecological niches in the environment. Of these pre-adapted electroactive species we can selectively isolate novel strains, characterize them, and use their properties in sustainable technologies relying on bioelectrochemical systems.

Electrochemical enrichment and isolation

The challenge during the isolation of electroactive strains is that isolation on non-selective media was previously shown to lead to loss of electroactivity [110–112]. We reviewed the studies that employed electrochemical technologies to obtain electroactive strains (table 1). Sometimes, isolation was possible despite the use of unselective media during the procedure. Nonetheless, below we will focus on those studies, which maintained the selective pressure throughout the steps of enrichment and isolation, with the help of solid-state electron sinks or sources.

A suitable approach to enrich electroactive microorganisms involves the use of in situ electrodes, because it overcomes enrichment bias artifacts [113] that would otherwise lead to changes in cultivability [114] or viability [115], for example due to grazing [116]. In situ enrichment often leads to the isolation of new electroactive strains. For example, an anode inserted directly into a borehole of a deep underground mine provided a niche for the growth of the electroactive Desulfuromonas soudanensis [117]. Different approaches were used to enrich electroactive organisms from groundwater or sediments, in situ. Thus, for in situ colonization, the groundwater from 1478 m depth was passed through a self-designed electrochemical reactor equipped with four electrodes, two poised at oxidizing, and the other two at reducing potentials [118]. For in situ enrichment from sediments, naturally existing redox gradients can be exploited in benthic or sediment MFC (SMFC). SMFCs operate with the anode embedded in the anoxic sediment and the cathode in the oxic water above [44]. The organisms in the sediment provide the electron source, while O₂ in the water above acts as the electron sink. SMFCs can selectively enrich native electroactive microorganisms both at the anode and the cathode. This was the case of the electrogen Dietzia sp. RNV-4, which was isolated from the anode of a river sediment SMFC [119], whereas the electrotoph ‘Candidatus Tenderia electrophaga’ was enriched from the biocathode community of a marine phototrophic SMFC [110, 111, 120, 121].

Generally, the isolation of a species requires growing it from a single cell to ensure a single cell origin. Besides, isolation of a species with unique traits requires sustaining the selective pressure for the entire duration of the isolation. Attempts to isolate electroactive strains often involve unselective media such as solid-LB, due to the simplicity of the isolation procedure, which requires only aerobic streaking to attain single cell colonies [119, 122–124]. Conversely, only a few studies upheld selective conditions during enrichment and isolation by adding insoluble electron acceptors to the dilution series [125, 126]. Insoluble Fe(III)-oxides have been often used as electron acceptors to isolate electrogenic microbes [117, 127]. Nonetheless, by providing insoluble minerals as electrode replacements, we may restrict isolation to microbes skilled for example at insoluble Fe(III)-oxide-respiration, but unskilled at electrode-respiration, which was the case of Geobacter brementis [128].

Isolation of electrotophs by conventional methods is more challenging than that of electrogens which led to a low number of cathodic isolates (table 1). Electrotophs are of interest for biotechnology [112, 129, 130], but are usually isolated with soluble electron donors [131, 132]. Some exceptional strains were enriched with metallic iron (Fe0) as an extracellular source of electrons [132–134]. However, the researchers discontinued the use of a solid electron donor during the strain purification procedure and instead set up dilution series with H₂ or other soluble/diffusible substrates [120, 123, 135, 136]. Growth on soluble substrates could lead to incapacitation of the strains in using the solid surface at all, as was the case with M. maripaludis strains, which lost the genomic islands relevant for EET-constituents when grown on H₂ [36].

Consistently, many authors applied one ineffective strategy for the isolation of electroactive microorganisms, which is aerobic cultivation with nutrient-rich agar (table 1) [111, 122–124, 131, 136–142]. This strategy favors fast-growing, oxygen-respiring organisms over electrotophic ones, obscuring downstream electrochemical studies, and interpretation of data. For example, multiple isolates obtained from a phototrophic SMFC on rich-agar media were not electroactive [111], whereas the actual cathodic microorganism ‘Candidatus Tenderia electrophaga’ could not be enriched
**Preparation of the reference electrode**

**Principle:**
An electrochemical reactor consists of one or two chambers with at least two electrodes submerged in a conductive ionic solution (electrolyte). In bioelectrochemical systems, the electrolyte is usually the growth media of the microorganisms without an external electron donor and acceptor. The electrodes used are a working electrode (WE) and counter electrode (CE), with the redox reaction of interest happening at the WE. The circuit (WE/CE) can be closed when the two electrodes get connected to a potentiostat. For precise control of the potential, the WE can be calibrated against a reference electrode (RE) by the potentiostat. The chambers are preferably segregated to keep the oxidation and reduction reactions isolated. Typically, the two compartments are separated by a membrane selective for proton exchange. The following protocol is based on the set up used in our lab and has been tested successfully for cultivation of strict anaerobes such as methanogenic Archaea (e.g. [56]). The list of materials used available in the supplementary materials.

**Preparation of the working and counter electrodes**

1. Wash the graphite block. Soak in 1 M HCl overnight. Soak in 1 M NaOH overnight. Rinse with deionized water until the pH of the refuse is neutral. Air dry before proceeding
2. Drill a hole on top of each graphite block 2 cm (h) × 2 mm (ø) ([figure S1](https://stacks.iop.org/NANO/31/174003/mmedia))
3. Coat one end of a Ti-wire 2 mm (ø) × 12.5 cm (l) with conductive epoxy and insert the wire into the hole of the graphite block. Coat the wire–graphite junction with a biocompatible non-conductive epoxy
4. Cure the epoxy by baking the electrode-wire set up at 80 °C for 3 h
5. The electrode-wire connection is tested with a multimeter by examining the resistance between the graphite block and the wire. A good electrical connection gives an internal resistance below 10 Ω
6. To ensure anaerobiosis, reactors are secured with black GL45 rubber stoppers pierced to fit the disconnected end of the Ti-wire. Stoppers can be drilled or pierced with an 18 G heated needle to produce holes of ~2 mm (ø) ([figure S1](https://stacks.iop.org/NANO/31/174003/figure S1-red circle)). Seal the junction between the wire and the stopper with epoxy to avoid possible gas leaks or O₂-contamination

**Preparation of the reference electrode**

7. Pierce a hole 2 mm (ø) through a blue butyl septum 20 mm (h) using a heated 18 G needle
8. Insert the reference electrode (RE) from the top to protrude ~4 cm below the stopper and ensure close proximity to the working electrode ~1 cm (for a 500 ml chamber; see [figure S1](https://stacks.iop.org/NANO/31/174003/figure S1)). Do not seal the junction because sometimes RE must be changed
9. Sterilize the RE attached to the stopper by soaking 10 min in 10% NaOCl, dip in ethanol (98%) and then keep in sterile MQ until further use

**Preparation of the H-cell reactors**

10. Cut the membrane guided by the inner diameter of the outer O-ring ([figure S1](https://stacks.iop.org/NANO/31/174003/figure S1-red circle))
11. Place the membrane in miliQ water to allow expansion. Do not allow it to dry
12. Insert butyl stoppers and crimp seal all side ports of the H-cells
13. Place the wet membrane onto the assembled O-ring ([figure S1](https://stacks.iop.org/NANO/31/174003/figure S1)) and sandwich it between the two glass chambers. Proceed to the next steps immediately not to allow for the membrane to dry
14. Fill both chambers with ~500 ml miliQ water. Insert the WE and CE electrodes attached to the GL45 stoppers into the appropriate chambers and seal the bottles with an autoclavable open top screw cap
15. Before autoclaving, place a 22 G needle in the stoppers of each chamber to allow steam release during autoclaving, because the membranes cannot withstand the autoclave pressure. Autoclave 20 min at 121 °C. After autoclaving, quickly remove the needles and seal visible holes in the stoppers with quick-dry epoxy

*From this point forward work sterile. Use sterile tubing, connections, needles, syringes and filters. Ensure sterility by ethanol-flaming septa before needle insertion. Filter-sterilize the anaerobic gas (N₂:CO₂, 80:20). Work with only one chamber at a time.*

16. Chamber #1: Flush with sterile gas (N₂:CO₂) while vacuuming sterile water from the lowest port of the chamber. Use a sterile needle fitted to a Luer-lock adapter which is lodged in a sterile tube and connected to a vacuum pump ([figure S2](https://stacks.iop.org/NANO/31/174003/figure S2-red circle)).
17. For the chamber with the WE, replace the stopper for the middle port with a sterile RE joined to a rubber stopper ([figure 4](https://stacks.iop.org/NANO/31/174003/figure 4)). Work quickly and close to a flame to ensure sterility.
18. Degas the chamber for 10 min to reestablish anoxia.
19. Pressurize a bottle of sterile media with sterile N₂CO₂ ([figure S2](https://stacks.iop.org/NANO/31/174003/figure S2-red circle)). Use the pressure buildup to push ~550 ml media into the H-cell chamber. Connect the media bottle to the reactor chamber. For this use a sterile tube with Luer-lock adapter ends fitted with needles and controlled by a valve ([figure S2](https://stacks.iop.org/NANO/31/174003/figure S2-red circle)).
on any rich media [121]. Therefore, several researchers developed small-scale electrochemical reactors for the isolation of electroactive microbes. With this approach, Geobacter sp. SD-1 and Ochrobactrum anthropi YZ-1 were isolated via successive liquid dilutions to extinction series in electrochemical reactors exclusive of nutrient-rich media [125, 126]. Additionally, an ‘electrode-plate method’ has been successfully employed to isolate electrogenic microorganisms [141]. The authors used a diluted cell suspension streaked on agar-plates containing the soluble electron donor, however with a transparent anode at the top as a solid-state electron acceptor. Besides the anode, a reference and counter electrode were placed inside the agar for precise control of the voltage. It remains to be tested whether this electrode-plating method has applicability in the reverse direction in order to isolate electrotrophs. Published reports revealed challenges in finding an appropriate solid-state electron donor for electrotrophs. Solid-state electron donors like Fe\(^0\) although successfully applied in liquid media [132] pose two problems in solid media-one being the lack of specificity because Fe\(^0\) generates H\(_2\) gas abiotically, and secondly H\(_2\)-gas would induce fractures in the solid media rendering isolation of single cell colonies impossible. As an alternative to Fe\(^0\), we recommend to use other biocompatible materials that can store charge and be pre-reduced electrically, such as Prussian Blue (a low-cost hexacyano-Fe complex material [144]) or biochar [145], as solid-state electron donors for selective isolation on agar-plates.

Below we present an electrochemical cultivation strategy (figure 2) by combining strategies presented in previous studies, including in situ primary enrichment in bioelectrochemical setups (see table 1), followed by laboratory electrochemical enrichment and dilution to extinction series in liquid or solid media with electrodes as electron donor/acceptor, as described by four previous reports [125, 126, 143, 147]. Most electroactive microorganisms are anaerobes [61]; thus, we propose to conduct all steps under anoxia because many anaerobes get inhibited by exposure to O\(_2\). Anoxia can be achieved by working under a N\(_2\) gas-stream, or ideally inside an anaerobic bag/chamber. For isolation under selective conditions, we propose to follow five

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**Box 1.** (Continued.)

20. Flush the chambers for 15–30 min by bubbling sterile N\(_2\)CO\(_2\) via the lower ports. Use a needle outlet on the top port to allow a steady flow through the reactor.

21. Chamber #2: Repeat steps 16 and 18–20

22. Initiate electrochemical measurements

**Inoculation and sampling**

23. Use a side port to inject 5 ml of a 20x concentrated cell inoculum harvested under sterile and anoxic conditions. Afterwards, flush for 5 min with sterile N\(_2\)CO\(_2\) to ensure anoxia and removal of carry over gases

24. For gas-samples, extract headspace gas from the chamber via the G45/top port. Use sterile, flushed, gas-tight syringes with a gas-tight valve

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**Figure 2.** Strategy to isolate electroactive microorganisms from the environment. 1. In situ colonization of the electrode; 2. Laboratory enrichment of the bioelectrode in a bioelectrochemical system; 3. Mechanical separation of the electrode biofilm under anoxic conditions; 4. Liquid dilution series in bioelectrochemical reactors; 5. Transfer of the last grown dilution on solid media with electrodes as donor/acceptor; 6. Growth on/in conductive agar with an electrode as sole electron donor/acceptor. Electrotaxis may occur.
steps of which two are optional: (1) in situ electrode colonization; (2—optional) electrochemical enrichment; (3) biofilm detachment; (4—optional) liquid electrochemical dilutions to extinction; (5) dilution to extinction on solid-media via electrode-plating.

**Step 1**: Electrode colonization in situ (e.g. SMFC). The biofilm colonizing the electrode in situ would be the ideal source for single cell direct isolation on solidified media after biofilm detachment. Alternatively, electroactive microorganisms from an environment can be sorted based on polarizability [147] for downstream isolation.

**Step 2 (optional)**: Transfer the electrode-biofilm to a media with a chemical composition similar to the in situ water (enrichment). Enrichment biases are likely [113–115], and therefore this step could be discarded for the next step.

**Step 3**: There are two ways to carry out mechanical detachment by scraping the electrodes or by light sonication (few cycles at <20% intensity to ensure cell integrity) followed by gentle rinsing with a stream of anoxic media. This step should preferably occur in an anaerobic chamber.

**Step 4 (optional)**: The biofilm-suspension can be used for liquid dilutions to extinctions, ideally, in batch bioelectrochemical reactors (figure 3). In box 1, we illustrate a protocol for anaerobic batch bioelectrochemical reactors. This step could be repeated until one morphotype and 16 S phylotype becomes isolated. Membrane-less, small volume, high throughput electrochemical cells [148] were previously used to enrich anode respiring electrogens and reduce the costs of isolation associated with dual chamber BES. However, membrane-less BES cannot be used to isolate strict anaerobes because inhibitory O₂ is produced at the anode.

**Step 5**: Ultimately, for isolation of a new species the highest liquid dilution in which growth was observed is used as inoculum for a dilution series in solid media. Solid dilution series should provide us with colonies from a single cell. To maintain the selective pressure, we advise using the electrode-plate method [143]. Another possibility is to place the inoculum at a distance from the electrode, so electroactive cells use taxis towards a solid-state electrode [99, 100].

### Electrochemical tests

Once isolated, the new strains must have their electrochemical properties tested because the mere association with an electrode is not proof of electroactivity. Nowadays, various types of high throughput methods demonstrate electroactivity, relying on electrochronic approaches with tungsten oxide (WO₃) [149], electrochemiluminescence [150], colorimetric [151, 152] and dielectrophoretic methods [153]. An example of a high-performance, eco-friendly approach for rapid electrochemical characterization is a paper-based 64-well sensing array containing MFC wirings (anode and cathode connected with a load) [154]. Nonetheless, these methods are not commercially available, so the use of conventional bioelectrochemical techniques is still necessary for standardization between laboratories. Some of the conventional bioelectrochemical techniques are chronopotentiometry and cyclic voltammetry. Chronoamperometry helps investigate the ability of a new isolate to facilitate electron transfer to and from an electrode [124, 132, 155]. Cyclic voltammetry helps distinguish between a direct and facilitated EET mechanism [117]. Hence the two must be used in combination to determine the type of EET mechanism employed by an electroactive microorganism.

Chronoamperometry (CA) is a technique in which the potential of a working electrode (exposed to microorganisms) stepped against a reference standard electrode gives a current response (mA), to be recorded over time (figure 4). For instance, microorganisms transfer electrons to the working electrode (anodic reactions) leading to the production of positive current, while their uptake of electrons from the working electrode (cathodic reactions) produces a negative one (figure 4). CA in a batch reactor is usually carried out until the current output stops and falls back to the baseline conditions when the soluble electrode acceptor or donor got depleted. From the current output, we can calculate current density and coulombic efficiency, which can then be used to compare performance with other studies [156]. For example, in an MES-system, the coulombic efficiency describes the recovery of the consumed current in the form of a synthesis product. For a methanogenic MES, the overall coulombic efficiency (η_CE, %) can be calculated from the amount of current consumed (I) for the formation of reduced products (CH₄, 8 electrons) for the given time (t) according to the equation (1) where F is the Faraday constant (96485.332 C mol⁻¹), m is the number of moles and n is the...
During cyclic voltammetry (CV), the potential is cycled between two setpoint potentials (V1 and V2), while the resulting current flow gets measured throughout the scan (figure 4). CV produces both an oxidative and a reductive current curve for the potential range between V1 and V2 (figure 4). Electroactive species carrying out reversible reactions between the electrode and the microbe may produce two current peaks, one for each direction (cathodic and anodic).

The CV technique can also be applied to distinguish the mode of electron transfer (direct or facilitated). Direct electron transfer should exhibit electrode-associated electroactivity, which we can assess by comparing current production rates of a microbial culture before and after exchanging the entire liquid volume. If the performance is similar in both conditions, the electroactive agent is localized at the electrode surface and not in solution. In the case of poor biofilm formers, we typically compare the CV of the grown culture to that of the spent cell-free filtered medium. This approach helps identify whether the planktonic cells or a soluble shuttle in the medium are involved in electron exchange with the electrode.

For detailed information on how to analyze cyclic voltammograms, as well as data from other electrochemical techniques, we recommend several excellent guides written by other research groups [157–160].

**Conclusions**

The field of electromicrobiology is rapidly emerging. Applications utilizing the ability of microorganisms to transport electrons extracellularly have moved far beyond its initial intended use in electricity generation. For example the development of hybrid bioelectrical systems with the ability to reduce carbon bonds with electricity or light [161]. With advances in several interdisciplinary fields, including electrochemistry, material science and biotechnology, microbial electrochemical systems have a real potential to provide meaningful solutions to current energy problems.
increasing interest in the biotech potential of microorganisms capable of EET. However, electroactive microorganisms could not get isolated via traditional means. Here, we have provided an overview of studies that isolated electroactive microorganisms from the environment; and supplied guidelines for bioelectrochemical isolation methods aspiring to promote the discovery of additional electroactive species for biotechnology.

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**References**

[1] Lovley D R *et al* 2011 Geobacter: the microbe electric’s physiology, ecology, and practical applications *Adv. Microb. Physiol.* 59 1–100

[2] Kracke F, Vassilev I and Krömer J O 2015 Microbial electron transport and energy conservation—the foundation for optimizing bioelectrochemical systems *Front. Microbiol.* 6 575

[3] Tremblay P-L, Angenent L T and Zhang T 2016 Extracellular electron uptake: among autotrophs and mediated by surfaces *Trends Biotechnol.* 35 360–71

[4] Shi L, Dong H, Reguera G, Beyenal H, Lu A, Liu J, Yu H and Fredrickson J K 2016 Extracellular electron transfer mechanisms between microorganisms and minerals *Nat. Publ. Gr.* 14 651–62

[5] Schröder U, Harnisch F and Angenent L T 2015 Microbial electrochemistry and technology: terminology and classification *Energy Environ. Sci.* 8 513–9

[6] Jiang Y, Shi M and Shi L 2019 Molecular underpinnings for microbial extracellular electron transfer during biogeochemical cycling of earth elements *Sci. China Life Sci.* 62 1275–86

[7] Karthikeyan R, Singh R and Bose A 2019 Microbial electron uptake in microbial electrosynthesis: a mini-review *J. Ind. Microbiol. Biotechnol.* 46 1419–26

[8] Lovley D and Walker D 2019 Geobacter protein nanowires *Front. Microbiol.* 10 2078

[9] Coursolle D and Granli J A 2010 Modularity of the Mtr respiratory pathway of Shewanella oneidensis strain MR-1 *Mol. Microbiol.* 77 995–1008

[10] Costa N L, Clarke T A, Philipp L A, Gescher J, Louro R O and Paquete C M 2018 Electron transfer process in microbial electrochemical technologies: the role of cell-surface exposed conductive proteins *Bioreour. Technol.* 255 308–17

[11] Deng X, Dohmae N, Nealson K H, Hashimoto K and Okamoto A 2018 Multi-heme cytochromes provide a pathway for survival in energy-limited environments *Sci. Adv.* 4 1–9

[12] Filman D J, Marino S F, Ward J E, Yang L, Mester Z, Bullitt E, Lovley D R and Strauss M 2019 Cryo-EM reveals the structural basis of long-range electron transport in a cytochrome-based bacterial nanowire *Commun. Biol.* 2 219

[13] Wang F *et al* 2019 Structure of microbial nanowires reveals stacked hemes that transport electrons over micrometers *Cell* 177 e10

[14] Bird L J, Saravia I H, Park S, Cañada E O, Salgueiro C A, Nitschke W, Louro R O and Newman D K 2014 Nonredundant roles for cytochrome c2 and two high-potential iron-sulfur proteins in the photoferroborhodopseudomonas palustris TIE-1 *J. Bacteriol.* 196 850–8

[15] Reguera G, McCarthy K D, Mehta T, Nicoll J S, Tuominen M T and Lovley D R 2005 Extracellular electron transfer via microbial nanowires *Nature* 435 1098–101

[16] Malvankar N S *et al* 2011 Tunable metallic-like conductivity in microbial nanowire networks *Nat. Nanotechnol.* 6 573–9

[17] Malvankar N S, Vargas M, Nevin K, Tremblay P L, Evans-Lutterodt K, Nykypanchuk D, Martz E, Tuomine M T and Lovley D R 2015 Structural basis for metallic-like conductivity in microbial nanowires *MBio* e00804

[18] Vargas M, Malvankar N S, Tremblay P, Leang C, Smith J A, Patel P, Snoeyenbos-West O L, Nevin K P and Lovley D R 2013 Aromatic amino acids required for pili conductivity and long-range extracellular electron transport in Geobacter sulfurreducens *MBio* 4 1–6

[19] Walker D J, Adhikari R Y, Holmes D E, Ward J E, Woodward T L, Nevin K P and Lovley D R 2018 Electrically conductive pili from pilin genes of phylogenetically diverse microorganisms *ISME J.* 12 48–58

[20] Pirbadian S *et al* 2014 Shewanella oneidensis MR-1 nanowires are outer membrane and periplasmic extensions of the extracellular electron transport components *Proc. Natl. Acad. Sci. USA* 111 12883–8

[21] Subramanian P, Pirbadian S, El-Naggar M Y and Jensen G J 2018 Ultrastructure of Shewanella oneidensis MR-1 nanowires revealed by electron cryotomography *Proc. Natl. Acad. Sci. USA* 115 E3246–55

[22] Sydow A, Krieg T, Mayer F, Schrader J and Holtmann D 2014 Electroactive bacteria—molecular mechanisms and genetic tools *Appl. Microbiol. Biotechnol.* 98 8481–95

[23] Light S H, Su L, Rivera-Lugo R, Cornejo J A, Louie A, Iavarone A T, Ajo-Franklin C M and Portnoy D A 2018 A flavin-based extracellular electron transfer mechanism in diverse Gram-positive bacteria *Nature* 562 140–4

[24] Rabaey K, Boon N, Höfte M and Verstraete W 2005 Microbial phenazine production enhances electron transfer in biofuel cells *Environ. Sci. Technol.* 39 3401–8

[25] Pham T H, Boon N, De Macer K, Höfte M, Rabaey K and Verstraete W 2008 Use of Pseudomonas species producing phenazine-based metabolites in the anodes of microbial fuel cells to improve electricity generation *Appl. Microbiol. Biotechnol.* 80 985–93

[26] Hernandez M E, Kappler A and Newman D K 2004 Phenazines and other reoxy-active antibiotics promote microbial mineral reduction *Appl. Environ. Microbiol.* 70 921–8

[27] Marsili E, Baron D B, Shikhare I D, Coursolle D, Granli J A and Bond D R 2008 Shewanella secretes flavins that mediate extracellular electron transfer *PNAS* 105 3968–73

[28] Kotsloski N J and Granli J A 2013 Flavin electron shuttles dominate extracellular electron transfer by Shewanella oneidensis *MBio* 4 e00553-12

[29] Newman D K and Kolter R 2000 A role for excreted quinones in extracellular electron transfer *Nature* 405 94–7
[30] Brutinel E D and Gralnick J A 2012 Shuttling happens: soluble flavin mediators of extracellular electron transfer in Shewanella Applic. Microbiol. Biotechnol. 93 41–8

[31] von Canstein H, Ogawa J, Shimizu S and Lloyd J R 2008 Secretion of flavins by shewanella species and their role in extracellular electron transfer Appl. Environ. Microbiol. 74 615–23

[32] Mevers E, Su L, Pishchany G, Baruch M, Cornejo J, Hobert E, Dimise E, Ajo-Franklin C M and Clardy J 2019 An elusive electron shuttle from a facultative anaerobe Elife 8 1–15

[33] Deutzmann J S, Sahin M and Spormann A M 2015 Extracellular enzymes facilitate electron uptake in biocorrosion and bioelectrosynthesis MBio 6 1–8

[34] Milton R D, Ruth J C, Deutzmann J S, Milton R D, Sahin M and Spormann A M 2018 Methanococcus maripaludis employs three functional heterodisulfide reductase complexes for flavin-based electron bifurcation using hydrogen and formate Biochemistry 57 4848–57

[35] Lienemann M, Deutzmann J S, Milton R D, Sahin M and Spormann A M 2018 Mediator-free enzymatic bioelectrosynthesis of formate by the Methanococcus maripaludis heterodisulfide reductase supercomplex Biorecosr. Technol. 254 278–83

[36] Tsurumaru H et al 2018 An extracellular [NiFe] hydrogenase mediating iron corrosion is encoded in a genetically unstable genomic island in Methanococcus maripaludis Sci. Rep. 8 15149

[37] Potter M C 1911 Electrical effects accompanying the decomposition of organic compounds Proc. R. Soc. B 84 260–76

[38] Santoro C, Arbizzani C, Erable B and Leropoulos I 2017 Microbial fuel cells: from fundamentals to applications. A review J. Power Sources 356 225–44

[39] Pandey P, Shinde V N, Deopurkar R L, Kale S P, Patil S A and Pant D 2016 Recent advances in the use of different substrates in microbial fuel cells toward wastewater treatment and simultaneous energy recovery Appl. Energy 168 706–23

[40] Wrighton K C, Agbo P, Warnecke F, Weber K A, Brodie E L, DeSantis T Z, Hugenholtz P, Andersen G L and Coates J D 2008 An elusive electron shuttle from a facultative anaerobe from the Thermodesulfobacterium vulgare strain CH30 Environ. Microbiol. 10 2505–14

[41] Rahimnejad M, Adhami A, Darvari S, Zirepour A and Oh S E 2015 Microbial fuel cell as new technology for bioelectricity generation: a review Alexandria Eng. J. 5 745–56

[42] Wang H and Ren Z J 2013 A comprehensive review of microbial electrochemical systems as a platform technology Biotechnol. Adv. 31 1796–807

[43] Fernando E Y, Keshavzart T and Kyazze G 2019 The use of bioelectrochemical systems in environmental remediation of xenobiotics: a review J. Chem. Technol. Biotechnol. 94 2070–80

[44] Kracke F, Lai B, Yu S and Krömer J O 2018 Balancing cellular redox metabolism in microbial electrobiosynthesis and electro fermentation—a chance for metabolic engineering Metab. Eng. 45 109–20

[45] Schievan A, Pepe Sciarria T, Vanbroekhoven K, De Wever H, Puig S, Andersen S J, Rabaey K and Pant D 2016 Electro-fermentation—merging electrochemistry with fermentation in industrial applications Trends Biotechnol. 34 866–78

[46] Moscoviz R, Toledo-Alarcón J, Trably E and Bernet N 2016 Electro-fermentation: how to drive fermentation using electrochemical systems Trends Biotechnol. 34 856–65

[47] Reddy C N, Suthakar M P, Min B and Shammugam P 2018 Future perspectives on cost-effective microbial fuel cells in rural areas Microbial Fuel Cell Technology for Bioelectricity (Cham: Springer International Publishing) pp 283–302

[48] Gallego R, Puig S, Battle-Vilanova P, Balaguer M D and Colprim J 2015 Microbial electrobiosynthesis of butyrate from carbon dioxide Chem. Commun. 51 3235–8

[49] Aryal N, Tremblay P L, Lizak D M and Zhang T 2017 Performance of different Sporomusa species for the microbial electrobiosynthesis of acetate from carbon dioxide Biorecosr. Technol. 233 184–90

[50] Marshall C W, Ross D E, Fichot E B, Norman R S and May H D 2012 Electrolysis of commodity chemicals by an autotrophic microbial community Appl. Environ. Microbiol. 78 8412–20

[51] Nevin K P, Henley S A, Franks A E, Summers Z M, Ou J, Woodard T L, Snoeyenbos-West O L and Lovley D R 2011 Electrolysis of organic compounds from carbon dioxide is catalyzed by a diversity of aceticogenic microorganisms Appl. Environ. Microbiol. 77 2882–6

[52] Cheng S, Xing D, Call D F and Logan B E 2009 Direct biological conversion of electrical current into methane by electromethanogenesis Environ. Sci. Technol. 43 3953–8

[53] Villano M, Aulenta F, Ciucci C, Ferri T, Giuliano A and Majone M 2010 Bioelectrochemical reduction of CO2 to CH4 via direct and indirect extracellular electron transfer by a hydrogenophilic methanogenic culture Biorecosr. Technol. 101 3085–90

[54] Yee M O, Snoeyenbos-West O, Thamdrup B, Ottosen L D M and Rotaru A-E 2019 Extracellular electron uptake by two Methanosarcina species Front. Energy Res. 7 1–10

[55] Li H et al 2012 Integrated electromicrobial conversion of CO2 to higher alcohols Science 335 1596

[56] Ishizaki A and Tanaka K 1991 Production of poly-β-hydroxybutyric acid from carbon dioxide by Alcaligenes eutrophus ATCC 17697T J. Ferment. Bioeng. 71 254–7

[57] Schmitz S, Nies S, Wierckx N, Blank L M and Rosenbaum M A 2015 Engineering mediator-based electroactivity in the obligate aerobic bacterium Pseudomonas putida KT2440 Front. Microbiol. 6 1–13

[58] Wang H and Ren Z J 2014 Bioelectrochemical metal recovery from wastewater: a review Water Res. 66 219–32

[59] Koch C and Harnisch F 2016 Is there a specific ecological niche for electroactive microorganisms? ChemElectroChem 3 1282–95

[60] Chaudhuri S K and Lovley D R 2003 Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells Nat. Biotechnol. 21 1229–32

[61] Kawachi S and et al 2018 Anodic and cathodic extracellular electron transfer by the filamentous bacterium Ardenticaena maritima 1108 Front. Microbiol. 9 68

[62] Yilmazev Y D, Zhu X, Kim K Y, Holmes D E and Logan B E 2018 Electrical current generation in microbial electrolysis cells by hyperthermophilic archaea Ferroglobus placidus and Geoglobus anhangari Bioelectrochemistry 119 142–9

[63] Carbajosa S, Malik M, Caillard R, Lopez M F, Palomares F J, Martin-gago J A, Rodriguez N, Amils R, Fernandez V M and De L A L 2010 Biosensors and bioelectrochemical growth of Acidithiobacillus ferrooxidans on a graphite electrode for obtaining a
biocathode for direct electrocatalytic reduction of oxygen. 

Biosens. Bioelectron. 26 877–80

[66] Su W, Zhang L, Li D, Zhan G, Qian J and Tao Y 2012

Dissimilatory nitrate reduction by Pseudomonas alcaliphila with an electrode as the sole electron donor. 

Biotechnol. Bioeng. 109 2904–10

[67] Holmes D E, Bond D R and Lovley D R 2004 Electron transfer by Desulfovibulbus propionicus to Fe(III) and graphite electrodes. 

Appl. Environ. Microbiol. 70 1234–7

[68] Faraghiparapari N and Zengler K 2017 Production of organics from CO2 by microbial electrosynthesis (MES) at high temperature. 

J. Chem. Technol. Biotechnol. 92 375–81

[69] Nevin K P, Woodward T L and Frank A E 2010 Microbial Electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. 

MBio 1 1–4

[70] Rowe A R, Xu S, Gardel E, Bose A, Girguis P, Amend J P and El-Naggar M Y 2019 Methane-linked mechanisms of electron uptake from cathodes by Methanosarcina Barkeri. 

MBio 10 e02448-18

[71] Bose A, Gardel E, J. Vidoudez C, Parra E A and Girguis P R 2014 Electron uptake by iron-oxidizing phototrophic bacteria. 

Nat. Commun. 5 1–7

[72] Ha P T, Lindemann S R, Shi L, Dohnalkova A C, Fredrickson J K, Madigan M T and Beyenal H 2017

Syntrophic anaerobic photosynthesis via direct interspecies electron transfer. 

Nat. Commun. 8 13924

[73] Yi H, Nevin K P, Kim B-C, Franks A E, Klimes A, Tender L M and Lovley D R 2009 Selection of a variant of Geobacter sulphurreducens with enhanced capacity for current production in microbial fuel cells. 

Biosens. Bioelectron. 24 3498–503

[74] Cao Y, Mu H, Liu W, Zhang R, Guo J, Xian M and Liu H 2019 Electrocigens in the anode of microbial fuel cells: pure cultures versus mixed communities. 

Microb. Cell Fact. 18 39

[75] Xing D, Zuo Y, Cheng S, Regan J M and Logan B E 2008

Electrocity generation by Rhodopseudomonas palustris DX-1. 

Environ. Sci. Technol. 42 4146–51

[76] Richter K, Schickberger M and Gescher J 2012

Dissimilatory reduction of extracellular electron acceptors in anaerobic respiration. 

Appl. Environ. Microbiol. 78 913–21

[77] Melton E D, Swanen E D, Behrens S, Schmidt C and Kappler A 2014 The interplay of microbially mediated and abiotic reactions in the biogeochemical Fe cycle. 

Nat. Rev. Microbiol. 12 797–806

[78] Kato S 2016 Microbial extracellular electron transfer and its relevance to iron corrosion. 

Microbiot. 9 141–8

[79] Rotaru A-E, Posth N R, Loscher C R, Miracle M R, Vicente E, Cox R P, Thompson J, Poulton S and Thamdrup B 2019 Interspecies interactions mediated by conductive minerals in the sediments of the iron rich meromictic lake La Cruz. 

Sp. Limnietica 38 21–40

[80] Weber J, Chen Y, Jamroz E and Miano T 2018 Preface: humic substances in the environment. 

J. Soils Sediments 18 2665–7

[81] Schmidt M W I and Noack A G 2000 Black carbon in soils and sediments: analysis, distribution, implications, and current challenges. 

Glob. Biogeochem. Cycles 14 777–93

[82] Thomas S C and Gale N 2015 Biochar and forest restoration: a review and meta-analysis of tree growth responses. 

New For. 46 931–46

[83] Summers Z M, Fogarty H E, Leang C, Franks A E, Malvankar N S and Lovley D R 2010 Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. 

Science 330 1413–5

[84] Rotaru A-E, Shrestha P M, Liu F, Markovaite B, Chen S, Nevin K P and Lovley D R 2014 Direct interspecies electron transfer between Geobacter metallireducens and Methanosarcina Barkeri. 

Appl. Environ. Microbiol. 80 4599–605

[85] Rotaru A E, Shrestha P M, Liu F, Shrestha M, Shrestha D, Embree M, Zengler K, Wardman C, Nevin K P and Lovley D R 2014 A new model for electron flow during anaerobic digestion: 2904–10

[86] Rotaru A-E et al 2018 Conductive particles enable syntrophic acetate oxidation between Geobacter and Methanosarcina from coastal sediments. 

MBio 49 1–14

[87] Kato S, Nakamura R, Kai F, Watanabe K and Hashimoto K 2010 Respiratory interactions of soil bacteria with (semi) conductive iron-oxide minerals. 

Environ. Microbiol. 12 3114–23

[88] Lyew D and Sheppard J 2001 Technical note use of conductivity to monitor the treatment of acid mine drainage by sulphate-reducing bacteria. 

Water Res. 35 2081–6

[89] Rowe A R, Yoshimura M, LaRowe D E, Bird J I, Amend J P, Hashimoto K, Neutral K H and Okamoto A 2017 In situ electrochemical enrichment and isolation of a magnetite-reducing bacterium from a high pH serpentinizing spring. 

Environ. Microbiol. 19 2272–85

[90] Poulton S W and Canfield D E 2011 Ferruginous conditions: a dominant feature of the ocean through Earth’s history. 

Elements 7 107–12

[91] Camacho A, Miracle R, Romero-viana L, Picazo A and Vicente E 2017 Lake La Cruz, an iron-rich karstic meromictic lake in central Spain. 

Ecology of Meromictic Lakes (New York: Springer) pp 187–233

[92] Crowe S A, Jones C, Katsiv S, Neill A H O, Sturm A, Canfield D E, Haffner G D, Mucci A, Sundby B and Fowle D A 2008 Photoferrothroms thrive in an Archean Ocean analogue. 

Proc. Natl Acad. Sci. 105 15938–43

[93] Teske A and Särsen K B 2008 Uncultured archaea in deep marine subsurface sediments: have we caught them all? 

ISME. J. 3 3–18

[94] Takai K et al 2008 Variability in the microbial communities and hydrothermal fluid chemistry at the newly discovered Mariner hydrothermal field, southern Lau Basin. 

J. Geophys. Res. 113 G02031

[95] Nakamura R, Takashima T, Kato S, Takai K, Yamamoto M and Hashimoto K 2010 Electrical current generation across a black smoker chimney. 

Angew. Chemie Int. Ed. 49 7692–4

[96] Gartman A, Picard A, Clarke D R and Girguis P 2015 Characterizing interactions between electrical potential, microbial activity and mineralogy at hydrothermal vents. 

Goldschmidt 12637

[97] Edwards K J, Rogers D R, Wirsén C O and Mccollom T M 2003 Isolation and characterization of novel psychrophilic, neutrophilic, –proteobacteria from the deep sea. 

Appl. Environ. Microbiol. 69 2906–13

[98] Hafenbradl D, Keller M, Dirmeier R, Rachel R, Roßnagel P, Burggraf S, Huber H and Stetter K O 1996 Ferroglobus placidus gen. nov., sp. nov., a novel hyperthermophilic archaea that oxidizes Fe3+ at neutral pH under anoxic conditions. 

Arch. Microbiol. 2 308–14

[99] Kawaichi S, Ito N, Kamikawa R, Sugawara T, Yoshida T and Takai K 2008 Variability in the microbial communities and forest restoration: a review and meta-analysis of tree growth responses. 

New For. 46 931–46

[100] Makita H, Tanaka E, Mitsuobu S, Miyazaki M, Nunoura T, Uematsu K, Takaki Y, Nishi S, Shimamura S and Takai K 2017 Mariprodufusoid micogutta sp. nov., a novel iron-oxidizing zetaproteobacterium isolated from a deep-sea hydrothermal field at the Bayonnaise knoll of the Izu-Ogasawara arc, and a description of Mariprodufusoides ord.
nov. and Zetaproteobacteria class. *Arch. Microbiol.* **199** 335–46

[101] Kawaiachi S *et al* 2018 Anodic and cathodic extracellular electron transfer by the filamentous bacterium Ardenicatenaa maritima. *Front. Microbiol.* **9** 1–11

[102] Summers M Z, Granfield J A, Bond D R 2013 Cultivation of an obligate Fe(II)-oxidizing lithoautotrophic bacterium using electrodes *MBio* **4** 1–5

[103] Morita M, Malvankar N S, Franks A E, Summers Z M, Giloteaux L, Rotaru A E, Rotaru C and Lovley D R 2011 Potential for direct interspecies electron transfer in methanogenic wastewater digester aggregates *MBio* **2** e00159-11

[104] Shrestha P M *et al* 2014 Correlation between microbial community and granule conductivity in anaerobic bioreactors for brewery wastewater treatment. *Bioresour. Technol.* **174** 306–10

[105] Holmes D E *et al* 2017 Metatranscriptomic evidence for direct interspecies electron transfer between Geobacter and Methanothrix species in methanogenic rice paddy soils. *Appl. Environ. Microbiol.* **82** A02323-17

[106] McGlynn S E, Chadwick G L, Kempe C P and Orphan V J 2015 Single cell activity reveals direct electron transfer in methanotrophic consortia *Nature* **526** 531–5

[107] Wegener G, Krukenberg V, Riedel D, Tegtmeier H E and Boetius A 2015 Intercellular wiring enables electron transfer between methanotrophic archaea and bacteria *Nature* **526** 587–90

[108] Cheng Q and Call D F 2016 Hardwiring microbes: via direct interspecies electron transfer: mechanisms and applications *Environ. Sci. Process. Impacts* **18** 968–80

[109] Mei R, Nobu M K, Narhiro T, Yu J, Sathyagala A, Willman E and Liu W T 2018 Novel Geobacter species and diverse methanogens contribute to enhanced methane production in media-added methanogenic reactors *Water Res.* **147** 403–12

[110] Struycharz-Glaven S M, Glaven R H, Wang Z, Zhou J, Vora G J and Tender L M 2013 Electrochemical investigation of a microbial solar cell reveals a nonphotosynthetic biocathode catalyst *Appl. Environ. Microbiol.* **79** 3933–42

[111] Wang Z *et al* 2015 A previously uncharacterized, nonphotosynthetic member of the Chromatiaceae is the primary CO2-fixing constituent in a self-regenerating biocathode *Appl. Environ. Microbiol.* **81** 699–712

[112] Rabaey K and Rozendal R A 2010 Microbial electrosynthesis — revisiting the electrical route for microbial production *Nat. Rev. Microbiol.* **8** 706–16

[113] Dunbar J, White S and Forney L J 1997 Genetic diversity through the looking glass: effect of enrichment bias *Appl. Environ. Microbiol.* **63** 1326–31

[114] Ferguson R L, Buckley E N and Palumbo A V 1984 Response of marine bacteriochloroplast to differential filtration and confinement *Appl. Environ. Microbiol.* **47** 49–55

[115] Jürgens K, Bond M J and Vaque D 2000 Bacteria-flagellate coupling in microcosm experiments in the Central Atlantic Ocean *J. Exp. Mar. Bio. Ecol.* **245** 127–47

[116] Muniesa M, Blanch A R, Lucena F and Jofre J 2005 Bacteriophages may bias outcome of bacterial enrichment cultures *Appl. Environ. Microbiol.* **71** 4269–75

[117] Badalamenti J P, Summers Z M, Chan C H, Granfield J A and Bond D R 2016 Isolation and genomic characterization of ‘Desulfurimonas soudanensis WTL’, a metal- and electrode-respiring bacterium from anoxic deep subsurface brine *Front. Microbiol.* **7** 1–11

[118] Jangir Y, Karbelkar A A, Beedle N M, Zinke L A, Wanger G, Anderson C M, Reese B K, Amend J P and El-Naggar M Y 2019 *In situ* electrochemical studies of the terrestrial deep subsurface biosphere at the sanford underground research facility, South Dakota, USA *Frontiers Eng. Res.** **7** 121

[119] Sacco N J, Bonetto M C and Cortón E 2017 Isolation and characterization of a novel electrogenic bacterium, Dietzia sp. RNV-4 *PLoS One* **12** e0169950

[120] Malik S, Drott E, Griessler P, Lee J, Lee C, Lowy D A, Gray S and Tender L M 2009 A self-assembling self-repairing microbial photoelectrochemical solar cell *Energy Environ. Sci.* **2** 292–8

[121] Eddie B J, Wang Z, Malanoski A P, Hall R J, Oh S D, Heiner C, Lin B and Strycharz-Glaven S M 2016 ‘Candidatus Tenderia electrophaga’, an uncultivated electroautotroph from a biocathode enrichment *Int. J. Syst. Evol. Microbiol.* **66** 2178–85

[122] Jangir Y, French S, Monner L M, Moser D P, Amend J P and El-Naggar M Y 2016 Isolation and characterization of electrochemically active subsurface Delftia and Azonexus species *Front. Microbiol.* **7** 756

[123] Erable B, Vandecandelaire I, Faimali M, Delia M L, Etchevery L, Vandamme P and Bergel A 2010 Marine biofuel biofilm as biocathode catalyst *Bioelectrochemistry* **78** 51–6

[124] Sen T B and C T S 2019 Kluvera georgiana MCC 3673: a novel electron enriched in microbial fuel cell fed with Oilsed Cake *Curr. Microbiol.* **76** 650–70

[125] Sun D, Call D, Wang A, Cheng S and Logan B E 2014 Geobacter sp. SD-1 with enhanced electrochemical activity in high-salt concentration solutions *Environ. Microbiol. Rep.* **6** 723–9

[126] Zuo Y, Xing D, Regan J M and Logan B E 2008 Isolation of the exoelectrogenic bacterium Ochrobactrum anthropi YZ-1 by using a U-tube microbial fuel cell *Appl. Environ. Microbiol.* **74** 3130–7

[127] Feng C, Li J, Qin D, Chen L, Zhao F, Chen S, Hu H and Yu C P 2014 Characterization of exoelectrogenic bacteria enterobacter strains isolated from a microbial fuel cell exposed to copper shock load *PLoS One* **9** e113379

[128] Rotaru A-E, Woodard T L, Nevin K P and Lovley D R 2015 Link between capacity for current production and syntrophic growth in Geobacter species *Front. Microbiol.* **6** 744

[129] Rabaey K, Girgus P and Nielsen L K 2011 Metabolic and practical considerations on microbial electrocystis *Carr. Opin. Biotechnol.* **22** 371–7

[130] Bajracharya S, Srikant S, Mohanakrishna G, Zacharia R, Strik D F and Pant D 2017 Biotransformation of carbon dioxide in bioelectrochemical systems: State of the art and future prospects *J. Power Sources* **356** 256–73

[131] Wu X, Ren X, Owens G, Brunetti G, Zhou J, Yong X, Wei P and Jia H 2018 A facultative electroactive chromium(VI)-reducing bacterium aerobically isolated from a biocathode microbial fuel cell *Front. Microbiol.* **9** 2883

[132] Rowe A R, Chellamuthu P, Lam B, Okamoto A and Nealon K H 2015 Marine sediments microbes capable of electrode oxidation as a surrogate for lithrophic insoluble substrate metabolism *Front Microbiol.* **6** 1–15

[133] Chang R, Bird L, Barr C, Osburn M, Willbanks E, Nealon K and Rowe A 2018 Thioclavla electrotroph sp. Nov., a versatile electrode and sulfur-oxidizing bacterium from marine sediments *Int. J. Syst. Evol. Microbiol.* **68** 1652–8

[134] Phillips J *et al* 2019 An *Acetobacterium* strain isolated with metallic iron as electron donor enhances iron corrosion by a similar mechanism as *Sporomusa sphaeroides* *FEMS Microbiol. Ecol.* **95** 1–13

[135] Thrash J C, Van Trump J I, Weber K A, Miller E, Achenbach L A and Coates J D 2007 Electrochemical stimulation of microbial perchlorate reduction *Environ. Sci. Technol.* **41** 1740–6
[136] Parot S, Vandecondelae I, Courret A, Délia M L, Vandamme P, Bergé M, Roques C and Bergel A 2011 Catalysis of the electrochemical reduction of oxygen by bacteria isolated from electro-active biofilms formed in seawater *Bioreour. Technol.* **102** 304–11

[137] Huang J, Zhang Y, Cao Y, Peng N, Wu P and Dong W 2015 Electrochemical bacterium phylogenetically related to Citrobacter freundii, isolated from anodic biofilm of a microbial fuel cell *Appl. Biochem. Biotechnol.* **175** 1879–91

[138] Aparna P P and Meignanalakshmi S 2016 Comparison of power generation of electrochemically active bacteria isolated from the single chambered multi-electrode microbial fuel cell developed using Capra hircus rumen fluid *Energy Sources A** **38** 982–8

[139] Feng Y, Lee H, Wang X and Liu Y 2009 Electricity generation in microbial fuel cells at different temperature and organic loading of electroactive bacteria *Asia-Pacific Power and Energy Engineering Conf., APPEEC* (Piscataway, NJ) (IEEE) pp 1–5

[140] Fedorovich V, Knighton M C, Pagaling E, Ward F B, Free A and Goryanin I 2009 Novel electrochemically active bacterium phylogenetically related to Arcobacter butzleri, isolated from a microbial fuel cell *Appl. Environ. Microbiol.* **75** 7326–34

[141] Ma C, Zhuang L, Zhou S G, Yang G Q, Yuan Y and Xu R X 2012 Alkaline extraacellular reduction: Isolation and characterization of an alkaliphilic and halotolerant bacterium, Bacillus pseudomicros *J. Appl. Microbiol.* **112** 883–91

[142] Kimura Z I, Chung K M, Ioh H, Hiraishi A and Okabe S 2014 Raoultella electra sp. nov., isolated from anodic biofilm of a glucose-fed microbial fuel cell *Int. J. Syst. Evol. Microbiol.* **64** 1384–8

[143] Ueoka N, Kouzuma A and Watanabe K 2018 Electrode plate-culture methods for colony isolation of exoelectrogens from anode microbmes *Bioelectrochemistry* **124** 1–6

[144] Rengasamy K, Ranaivorsoa T, Singh R and Bose A 2018 An insoluble iron complex coated cathode enhances direct electron uptake by Rhodopseudomonas palustris TIE-1 *Bioelectrochemistry* **122** 164–73

[145] Joseph S et al 2015 The electrochemical properties of biochars and how they affect soil redox properties and processes *Agronomy* **5** 322–40

[146] Xing D, Cheng S, Logan B E and Regan J M 2010 Isolation of the electroexoelectrogen denitrifying bacterium Comamonas denitrificans based on dilution to extinction *Appl. Microbiol. Biotechnol.* **85** 1575–87

[147] Fiedler S, Shirley S G, Schnelle T and Fuhr G 1998 Dielectrophoretic sorting of particles and cells in a microsystem *Anat. Chem.* **70** 1909–15

[148] Call D F and Logan B E 2011 A method for high throughput electrochemical research based on standard scale microbial electrosynthesis cells *Biosens. Bioelectrochem.* **26** 4526–31

[149] Yuan S-J, He H, Sheng G-P, Chen J-J, Tong Z-H, Cheng Y-Y, Li W-W, Lin Z-Q, Zhang F and Yu H-Q 2013 A photometric high-throughput method for identification of electrochemically active bacteria using a WO3 nanocluster probe *Sci. Rep.* **3** 1315

[150] You L-X, Chen N-J, Wang L, Chen J, Qin S-F, Rensing C, Lin Z-Y and Zhou S-G 2019 Electroluminescence for the identification of electrochemically active bacteria *Biosens. Bioelectrochem.* **137** 222–8

[151] Wen J, Zhou S and Chen J 2015 Colorimetric detection of Shewanella oneidensis based on immunomagnetic capture and bacterial intrinsic peroxidase activity *Sci. Rep.* **4** 5191

[152] Zhou S, Wen J, Chen J and Lu Q 2015 Rapid measurement of microbial extracellular respiration activity using a high-throughput colorimetric assay *Environ. Sci. Technol. Lett.* **2** 26–30

[153] Wang Q, Jones A A D, Gralnick J A, Lin L and Buic C R 2019 Microfluidic dielectrophoresis illuminates the relationship between microbial cell envelope polarizability and electrochemical activity *Hist. da Historiogr.* **11** eaat5064

[154] Taherinia M, Mohammadiar M, Hassan D J and Choi S 2019 A fully disposable 64-well paperpore sensing array for screening electroactive microorganisms *Nano Energy* **65** 104026

[155] Doyle L E, Yung P Y, Mitra S D, Wuertz S, Williams R B H, Lako F and Marsili E 2017 Electrochemical and genomic analysis of novel electroactive isolates obtained via potentiostatic enrichment from tropical sediment *J. Power Sources* **356** 539–48

[156] Blasco-Gómez R, Batlle-Vilanova P, Villano M, Balaguer M D, Colprim J and Puig S 2017 On the edge of research and technological application: a critical review of electromethanogenesis *Int. J. Mol. Sci.* **18** 874

[157] Kissing P 2002 Electrochemistry for the non-electrochemist *Curr. Sep.* **2** 51–3

[158] Fricke K, Harmisch F and Schro U 2008 On the use of cyclic voltammetry for the study of anodic electron transfer in microbial fuel cells *Energy Environ. Sci.* **14** 7

[159] Harmisch F and Freguia S 2012 A basic tutorial on cyclic voltammetry for the investigation of electroactive microbial biosilms *Chem.—An Asian J.* **7** 466–75

[160] Marsili E, Rolletson J B, Baron D B, Hozalski R M and Bond D R 2008 Microbial biofilm voltammetry: direct electrochemical characterization of catalytic electrode–attached biofilms *Appl. Environ. Microbiol.* **74** 7329–37

[161] Su L and Ajo-Franklin C M 2019 Reaching full potential: bioelectrochemical systems for storing renewable energy in chemical bonds *Curr. Opin. Biotechnol.* **57** 66–72

[162] Park H S, Kim B H, Kim H S, Kim H J, Kim G T, Kim M, Chang I S, Park Y K and Chang H I 2001 A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to Clostridium butyricum isolated from a microbial fuel cell *Anaerobe* **7** 297–306

[163] Pham C A, Jung S J, Phung N T, Lee J, Chang I S, Kim B H, Yi H and Chun J 2003 A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to Aeromonas hydrophila, isolated from a microbial fuel cell *FEBS Microbiol. Lett.* **223** 129–34

[164] Holmes D E, Nicoll J S, Bond D R and Lovley D R 2004 Potential role of a novel psychrotolerant member of the family Geobacteraceae, Geospirbacber electrodiphilus gen. nov., sp. nov., in electricity production by a marine sediment fuel cell *Appl. Environ. Microbiol.* **70** 6023–30

[165] Byrne-Bailey K G, Wrighton K C, Melnyk R A, Agbo P, Hazen T C and Coates J D 2010 Complete genome sequence of the electricity-producing ‘Thermimcola potens’ strain JR *J. Bacteriol.* **192** 4078–9

[166] Chung K and Okabe S 2009 Continuous power generation and microbial community structure of the anode biofilms in a three-stage microbial fuel cell system *Appl. Microbiol. Biotechnol.* **83** 965–77

[167] Xu S and Liu H 2011 New exoelectrogen Citrobacter sp. SX-1 isolated from a microbial fuel cell *J. Appl. Microbiol.* **111** 1108–15

[168] Nercessian O, Parot S, Délia M L, Bergel A and Achouak W 2012 Harvesting electricity with Geobacter bremensis phylogenetically related to Tolumonas osonensis and power performance in MFCs *Bioreour. Technol.* **139** 141–8

[169] Lu J, Yang J, He H, Jin T, Zhou L, Wang M and Zhou M 2013 A new electrochemically active bacterium phylogenetically related to Tolumonas osonensis and power performance in MFCs *Bioreour. Technol.* **139** 141–8

[170] Deng D, Zhang Y and Liu Y 2015 A Geobacter strain isolated from rice paddy soil with higher bioelectricity generation
capability in comparison to Geobacter sulfurreducens PCA

[171] Feng Y L, Wang W D, Tang X H, Li H R, Zhuwei D, Yang Z C and Du Y L 2014 Isolation and characterization of an electrochemically active and cyanide-degrading bacterium isolated from a microbial fuel cell RSC Adv. 4 36458–63

[172] Sharma S C D, Feng C, Li J, Hu A, Wang H, Qin D and Yu C-P 2016 Electrochemical characterization of a novel exoelectrogenic bacterium strain SCS5, Isolated from a Mediator-Less microbial fuel cell and phylogenetically related to Aeromonas jandaei Microbes Environ. Environ. 31 213–25

[173] Jiang Z, Zhang Y, Liu Z, Ma Y, Kang J and Liu Y 2018 Isolation and characterization of an exoelectrogenic strain CL-1 from soil and electron transfer mechanism by linking electrochemistry and spectroscopy Electrochim. Acta 292 982–9

[174] Venkidusamy K, Hari A R and Megharaj M 2018 Petrophilic, Fe(III) reducing exoelectrogen Citrobacter sp. KVM11, isolated from hydrocarbon fed microbial electrochemical remediation systems Front. Microbiol. 9 349

[175] Naradasu D, Miran W, Sakamoto M and Okamoto A 2019 Isolation and characterization of human gut bacteria capable of extracellular electron transport by electrochemical techniques Front. Microbiol. 10 3267