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Nanotechnology to rescue the bacterial bidirectional extracellular electron transfer in bioelectrochemical systems

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Abbreviations	Expansions
BES	Bioelectrochemical system
MFC	Microbial fuel cell
MES	Microbial electrosynthesis
MEC	Microbial electrolysis cell
EET	Extracellular electron transfer
EAB	<i>Electrochemically active bacteria</i>
<i>S. oneidensis MR-1</i>	<i>Shewanella oneidensis MR-1</i>
<i>S. loihica PV-4</i>	<i>Shewanella loihica PV-4</i>
<i>S. putrefaciens</i>	<i>Shewanella putrefaciens</i>
<i>S. ovata</i>	<i>Sporomusa ovata</i>
<i>G. sulfurreducens</i>	<i>Geobacter sulfurreducens</i>
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
OM c-Cyts	Outer-membrane c-type cytochromes
RF	Riboflavin
FMN	Flavin mononucleotide
EPS	Extracellular polymeric substance
Si	Silicon
ITO	Indium tin oxide
CNT	Carbon nanotube
MWCNT	Multi-walled carbon nanotube
G-CNT	Graphene-carbon nanotube
3D	Three dimensional
EIS	Electrochemical impedance spectroscopy
RVC	Reticulated vitreous carbon
CV	Cyclic voltammetry
Fe ₃ O ₄	Magnetite
Fe ₂ O ₃	Hematite
NB	Nitrobenzene
GMS	Graphene modified stainless steel mesh
CHI/VSG	Chitosan/vacuum stripped graphene scaffold
PANI	Polyaniline
IL-GNS	Ionic liquid functionalized graphene nano-sheets
GO	Graphene oxide
rGO	Reduced graphene oxide
Au	Gold
Pd	Palladium
FeS	Iron sulfide
IET	Interspecies electron transfer
TiO ₂	Titanium dioxide
Ni	Nickel
NiO	Nickel oxide
PPy	Polypyrrole
PPy-NA	Polypyrrole nano-arrays
AQDS	Anthraquinone-2,6-disulphonic disodium salt
CB	Conduction band
VB	Valence band

Electrically active bacterium transports its metabolically generated electrons to insoluble substrates such as electrodes via a process known as extracellular electron transport (EET). Bacterial EET is a crucial process in geochemical cycling of metals, bioremediation and bioenergy devices such as microbial fuel cells (MFCs). Recently, it has been found that electroactive bacteria can reverse their respiratory pathways by accepting electrons from a negatively poised electrode to produce high-value chemicals such as ethanol in a process termed as microbial electrosynthesis (MES). A poor electrical connection between bacteria and electrode hinders the EET and MES processes significantly. Also, the bidirectional EET process is sluggish and need to be improved drastically to extend its practical applications. Several attempts have been undertaken to improve the bidirectional EET by employing various advanced nanostructured materials such as carbon nanotubes and graphene. This review covers the recent progress in the bacterial bidirectional EET processes using advanced nanostructures in the light of current understandings of bacteria-nanomaterial interactions.

1. Electrochemically active bacteria and extracellular electron transfer

Certain electrochemically active bacteria (EAB) commonly referred to as “exoelectrogens” (e.g., *Geobacter* and *Shewanella*) transport their metabolically generated electrons to insoluble substrates such as solid electrodes via a process known as extracellular electron transport (EET).^{1,2} Bacterial EET process plays a crucial role in bioremediation, energy recovery in systems known as microbial fuel cells (MFCs) and geochemical cycling of metals.³⁻⁵ Together, these systems are referred to as bioelectrochemical systems (BESs). Among the different BESs, MFCs have gained considerable attention over the years, owing to their dual functions in electricity generation and wastewater treatment.^{6,7} *Shewanella oneidensis* MR-1 and *Geobacter sulfurreducens* are the most extensively studied exoelectrogens to date.²

1.1. Electron transfer mechanism- Electron release

Bacterial EET involves the release of metabolically generated electrons through a series of protein networks to the external insoluble electron acceptors. Even though, numerous studies are available on the molecular mechanism involved in the EET, the actual mechanism still remains as enigmatic. There are three primary mechanisms available to describe the bacterial EET process namely, 1) direct electron transfer via outer membrane c-type cytochromes (OM c-Cyts),⁸ 2) shuttling of electrons via cell-secreted flavin⁹ and 3) via bacterial nanowires.^{1,10} A collective contribution of the above three mechanisms mostly notices in the bacterial EET process. In contrast to shuttling mechanism directed by cell-secreted flavins such as riboflavin (RF) and flavin mononucleotide (FMN), Okamoto et al. has recently proposed that RF, and FMN specifically bind to OM c-Cyts, OmcA and MtrC proteins, respectively and form bound-flavin semiquinones.¹¹⁻¹⁴ These bound-flavin semiquinones enhance the bacterial EET by 10³ to 10⁵-times more than the free flavin. These findings suggest that the interaction of flavin/OM c-Cyts is critical for the bacterial EET process.

Microbial nanowires have been considered as the sophisticated electrical cables produced by bacteria to transfer the electrons to solid electron acceptors. There is a misleading terminology regarding with bacterial

nanowires and pili in the existing literature. Most of the reports describe nanowires as the extensions of the bacterial pili.¹⁰ However, a recent study on *Shewanella* nanowires has proposed that the nanowires are extensions of the outer membrane and periplasm which is against the previous notion of pilin based structures.¹⁰ Multiheme cytochromes are localized on these nanowires which provide them excellent electrical conductivity. Deciphering the role of microbial nanowires on the EET process has recently received much attention among microbiologists, physicists, and chemists.¹⁵⁻¹⁹ A detailed understanding on the microbial nanowires will have tremendous implications in various nanobiotechnological applications, bioelectricity generation, corrosion and bioremediation.^{16-18, 20} A breakthrough work from Malvankar et al.^{21,22} demonstrated that nanowires produced by *G. sulfurreducens* behave like metallic conductors. The conductivity of the nanowires was significantly enhanced by cooling, which is a signature of metallic conduction. Also, there was two orders of high conductivity in the nanowires with decreasing pH. This observation suggested that pili were doped with protons and the protonation resulted in p-type carriers similar to the previous observations in organic metals such as polyaniline.²³ Similarly, nanowires produced by *S. oneidensis* MR-1 exhibit p-type, tunable electronic behavior, and holes are found to be the primary charge carriers in the bacterial nanowires.²⁴ However, a live debate is still going on for establishing a consensus in the molecular mechanism involved in the bacterial EET process.²⁵⁻³¹

1.2. Electron transfer mechanism- Electron uptake

Interestingly, some microbes sense negatively charged surfaces by up-taking electrons and use the surfaces as the electron donors for driving their metabolism. Recent studies have shown this exciting activity of the microbes that can accept electrons from a cathode to synthesize high-value chemicals and fuels such as acetate and methane via a process known as microbial electrosynthesis (MES, Figure 1).³²⁻³⁶ The MES is considered as a promising technology for the production of chemicals and fuels from CO₂.³⁷ Only limited studies are available for describing the bacterial electron uptake mechanism from a cathode and a better understanding on the molecular mechanism is needed for the practical application of this technology.³⁵ Electrochemical analyzes of *c*-Cyts in *Geobacter* species showed that the electron transfer ability of *c*-Cyts

may be reversible.³⁵ Also, some studies proposed the collective role of *c*-Cyts and hydrogenase enzymes in the bacterial electron uptake.^{35,38-40} A recent report revealed that *S. oneidensis* MR-1 reverse its respiratory pathway for reductive metabolism.⁴¹ *S. oneidensis* reduced fumarate to succinate by accepting electrons from the negatively poised graphite electrode. Deletions of OM *c*-Cyt anchoring protein-MtrB and periplasmic cytochrome MtrA severely reduced the fumarate reduction in *S. oneidensis*. Further investigations are needed to elucidate the exact mechanism involved in the bacterial electron uptake from the cathode.

1.3. Role of electrode material in EET

The finding of bacterial bidirectional EET has led to the development of MFC and MES technologies. However, these technologies are still far from practical applications due to many inherent obstacles such as reduced biofilm conductivity and poor bacteria-electrode electrical connection and limited understanding of bidirectional EET mechanism. Biofilm conductivity is believed to be a critical factor for the efficient EET process.⁴² Unfortunately, biofilm conductivity by itself is not enough to transfer electrons fluently to/from the electrode. Recently, material scientists have started to employ various advanced nanostructured materials such as carbon nanotubes, graphene, inorganic nanomaterials, noble metal nanoparticles, semiconductors and conducting polymers as bioanode or biocathode materials to enhance the bidirectional EET process. Especially, the efforts using nanostructured materials have led to a steep improvement in the bidirectional EET process. This review covers the recent progress in the bacterial bidirectional EET processes under the umbrella of nanostructured materials. A prior understanding of the fundamental mechanisms involved in the bacterial attachment to solid surfaces is essential as the biofilm formation is one of the critical process in the both MFCs and MES. Several attempts including theoretical and experimental studies have focused on investigating the bacterial attachment to various nanostructured surfaces. All the merits of using such nanomaterials in improving electron transfer in BESs are discussed along with current challenges and further perspectives.

2. Current understandings on bacteria-electrode interactions

In this section, the current understandings of the bacterial interactions with electrode surfaces are discussed. The knowledge evolved from the bacteria-electrode interactions can be directly utilized to set up strategies for the development of efficient bioanode and biocathode materials. Bacteria usually prefer to form biofilms on solid surfaces to shelter from various environmental perturbations and toxicity.⁴³ The bacterial biofilm formation has been extensively investigated, and it is known that bacteria secrete extracellular polymeric substance (EPS) to stick on a solid surface.⁴³ Genetic analyses have proposed that flagella and pili are also associated with the biofilm formation.⁴⁴ There are many factors that affect the formation of a biofilm such as surface topography, surface charge, wettability (of both the biofilm as well as the electrode) and the amount of EPS production, cell-cell signaling.⁴⁵⁻⁴⁸ Among them, the surface topography is known to play a critical role in the early stages of biofilm formation.⁴⁹

Nanotechnology has emerged as a potential tool to investigate bacterial life on nano-sized crevices.^{50,51} Nanomaterials provide us various possibilities to tune the surface of the electrode to understand the interaction of bacterial cells with the modified surfaces. For example, it is possible to investigate the role of surface porosity or roughness on the biofilm attachment by applying different porous nanomaterials as the electrodes. Bacterial cells are highly sensitive to electrode surfaces and hence a prior understanding on bacterial affinity towards electrode is necessary to develop efficient electrode materials. Recently, Silicon (Si) nanowire was employed to examine the bacteria-nanomaterial interaction as physical interactions between the bacteria and nanowires can be precisely measured. Sakimoto et al.⁵² submerged Si nanowires to a growth media containing *Sporomusa ovata* cells under a constant applied potential. The authors observed that *S. ovata* cells aligned parallel to the nanowires at high ionic concentration while at low ionic strength, bacterial cells attached to the nanowires in a random orientation (Figure 2). The results have suggested a salt-induced orientation and self-assembly of the bacterial cells on the nanowires. Similarly, a recent report has elegantly shown that how *S. oneidensis* cells sense Si nanowires during their swimming and attach to the Si nanowire surfaces.⁵³ The study revealed that *S. oneidensis* preferred to stay on the Si

nanowires rather than the planar bottom silicon substrate and demonstrated that nano-sized surfaces play a crucial role in the development of biofilms.

Singh et al.⁵⁴ reported that nanostructured titania film with a surface roughness of 20 nm enhances biofilm formation, and a further enhancement in the surface roughness inhibits the bacterial attachment. Mitik-Dineva et al.⁵⁵ showed that surface with a nano-scale roughness stimulated the bacterial attachment three-fold higher than the micro or macro-sized surface roughness. They investigated the role of nano-roughness on the bacterial attachment by forming a *Pseudomonas isachenkonni* (a proteobacterium) biofilm on a glass substrate with a modified nano-scale roughness. A higher number of bacterial cells was attached to the modified glass substrate compared to the unmodified glass. Also, there was a morphological transformation of the cells on the nano-sized rough surface by an increase in the cell size and EPS production. These results clearly indicated that nano-surface induced the cellular metabolism of the attached bacterial cells. Recently Ding et al.⁵⁶ demonstrated the crucial role of surface wettability on the biofilm attachment and the bacterial EET process. To investigate the role of wettability on the EET, tin-doped In₂O₃ (ITO) with different wettability were used to develop *Shewanella loihica* PV-4 biofilms at a constant applied potential. The biofilm formed on the hydrophilic ITO glass substrate delivered 5-times higher EET activity than the biofilm grown on the hydrophobic substrate (Figure 3). Electrochemical measurements revealed that the surface wettability enhanced the electrical connection between the microbes and electrode by altering local polarity at microbe/electrode interface. The alteration in the local polarity affected the redox state of OM *c*-Cyts, which allowed them to stay mostly in the reduced state. The redox state variation in the OM *c*-Cyts with the hydrophilic surface drastically enhanced the EET process. In short, high surface roughness or porosity allows the bacteria to attach firmly on the electrode surface that helps them to transfer the electrons easily to the electrode. Also, hydrophilic surface promote the biofilm formation and regulates the EET process.

3. Enhancement of bacterial EET by nanostructured materials

The previous decade has seen a lot of research efforts being made towards the development of newer electrode materials as both anode and cathode for BESs.^{57,58} These include different types of carbon and metal based catalysts.^{59,60} Of late, several nanomaterials have emerged as promising materials for both anode and cathode construction.⁶¹ These are believed to improve the BES performance in terms of bacterial adhesion, electron transfer and electrochemical efficiency. The application of various nano-structured electrode materials significantly enhanced the performance of the BESs and Table 1 shows a comparison of the performance of BESs with different nanostructured bioanode materials.

3.1. Carbon nanotube-based nanostructures

The combination of the remarkable conductivity, high specific surface area, and biocompatibility make carbon nanotube (CNT) as a suitable candidate for the development of bioanode materials in MFCs. CNT-based electrode materials significantly improved the performance of the bioanode by enhancing the bacterial EET. Xie et al.⁶² used a three-dimensional (3D) macro-porous CNT-textile anode to improve the MFC performance drastically as compared to traditional electrodes such as carbon cloths. 3D macro-scale porous structure enabled bacteria to colonize both interior and exterior of the anode allowing an efficient electroactive biofilm formation. In contrast, 2D structure precludes biofilm formation at the interior of the electrode making less surface area available for the bacterial attachment. The macroporous structure further allows unhindered diffusion of the substrate to the inner part of anode, which maintained internal bacterial colonization. Also, CNT-textile fibers facilitated bacterial EET by forming a compact attachment with the microbial nanowires (Figure 4). Electrochemical impedance spectroscopy (EIS) revealed a 10-fold lower charge transfer resistance of CNT-textile fibers as compared to a carbon cloth. A follow-up work using CNT coated-sponge as an anode electrode produced higher current density than that of the CNT-textile fiber electrode.⁶³ The increased performance of CNT-sponge electrode was attributed to lower internal resistance, controllable and tunable pore sizes and excellent mechanical stability. Similar to the CNT-textile fiber, 3D and macroporous structure of the CNT-sponge allowed enhanced diffusion of the electrolyte for the internal bacterial colonization. A crinkled CNT anode showed a better performance for the production

190 of bioelectricity relative to an uncrinkled CNT due to its enhanced interaction with exoelectrogens by
191 forming an intimate contact with microbial nanowires.⁶⁴ The performance of crinkled CNT as the bioanode
192 material was comparable with the CNT doped-sponge electrode.⁶³ Flexer et al.⁶⁵ developed an efficient
193 bioanode for MFCs by decorating CNTs on macroporous reticulated vitreous carbon (RVC) via chemical
194 vapor deposition (NanoWeb-RVC). The modified bioanode exhibited three times higher current density
195 over the control by mixed culture bacteria as biocatalysts. The macroporous structure of the electrode
196 provided excess electroactive surface area for the biofilm formation and scanning electron micrographic
197 (SEM) images clearly showed thick reddish colored biofilms on the electrode surface. A rich *c*-Cyts content
198 may be responsible for the red coloration of the biofilm and rod shape microbes were mainly identified in
199 the biofilm, which were very similar to *Geobacter* species. Cyclic voltammetry (CV) analysis showed that
200 the NanoWeb electrode greatly enhanced the bacterial EET by making a healthy interaction with OM *c*-
201 Cyts and bacterial nanowires.

202 The combination of CNTs with other conductive materials such as hematite and magnetite also exhibited
203 remarkable improvements in the efficiency of bioanodes Mono-dispersed magnetite (Fe₃O₄)/CNT nano-
204 composite modified carbon paper exhibited an increased current generation over the control using *E.coli* as
205 a biocatalyst.⁶⁶ Magnetic properties of Fe₃O₄ allowed CNTs to attach to the carbon paper firmly while CNTs
206 offered excellent conductivity for the bacterial EET process. CV analysis revealed the improved
207 electrochemical activity of *E.coli* biofilm due to the presence of Fe₃O₄/CNT nano-composite, which was
208 further confirmed by the impedance measurement. The role of Fe₃O₄ in the bacterial EET process will be
209 discussed in detail in the section 3.4. Vertically aligned, forest type multi-walled CNT with nickel silicide
210 anode produced a high power density in a micro-sized MFC (1.25 μ L).⁶⁷ Similarly, a CNT modified anode
211 showed a large current generation in a micro-sized MFC (75 μ L) due to high surface area and outstanding
212 conductivity of the CNT.⁶⁸ Advances in micro-sized MFCs will allow them to power ultra-low-power
213 electronics. A 3D hierarchical macroporous chitosan dispersed multi-walled CNT bioanode permitted to
214 form an efficient electroactive *G. sulfurreducens* biofilm.⁶⁹ The synergetic effect of macroporous structure

215 and the excellent conductivity of CNTs induced the EET process of *G. sulfurreducens* significantly. Liu et
216 al.⁷⁰ experimentally and theoretically demonstrated that CNT-networks can significantly enhance the EET
217 process in *S. oneidensis* MR-1 by producing a ten times larger current generation in an MFC as compared
218 to a carbon paper electrode. CV analysis showed that there was a strong interaction between CNT-network
219 and OM *c*-Cyts, which promoted the EET process. Mutant experiment lacking MtrC and OmcA
220 significantly decreased the current production indicating the inevitable role of OM *c*-Cyts in the EET
221 process. Molecular dynamic simulations confirmed the increased interaction of Fe²⁺ in reduced heme
222 groups of OM *c*-Cyts and CNTs. Peng et al.⁷¹ also observed a similar promoting effect of CNTs on the
223 direct electron transfer in *S. oneidensis* MR-1.

224 Doping of CNTs with different materials showed improved performance of the bioanodes. A CNT-doped
225 hydrogel deposited on a carbon paper produced larger current relative to the control.⁷² A high content of
226 oxygen-containing functional groups on the surface of conducting CNTs and large specific surface area
227 were responsible for the improved performance of the MFC. XPS analysis confirmed the presence of a
228 quinone structure on the surface of CNTs, which could act as a redox mediator for the EET process.
229 Recently, an MFC with CNT/MnO₂ modified anode produced a higher current generation than that of a
230 plain carbon paper.⁷³ Large surface area, excellent conductivity and supercapacitance behavior of MnO₂
231 were attributed to the enhanced performance of the CNT/MnO₂ modified electrode. Zhang et al.⁷⁴
232 demonstrated that thicker biofilm formation on CNT-modified graphite bioanode deteriorated the MFC
233 performance and thin biofilms (<5 μm) are only feasible to accelerate the EET process. This observation
234 raises a significant concern for the usage of CNT-modified bioanode as *G. sulfurreducens*, a high current
235 producing bacterium usually form a thick biofilm (> 20 μm).⁷⁵ Erbay et al.⁷⁶ systematically investigated the
236 role of the geometry of CNTs on the performance of MFCs. They demonstrated that longer CNTs delivered
237 higher current density than the smaller CNTs, which is possibly due to the large surface area of the former.
238 Also, densely packed CNT network showed a lower performance as compared to loosely packed CNTs.
239 The dense CNT networks hindered the passage of microbes into the interior, resulting in a biofilm formation

only on the top of CNT-assembly while loosely packed CNTs allowed bacterial colonization in both exterior and interior of the bioanode. An addition of amorphous carbon on the CNT-modified anode significantly reduced the current density due to the hindrance in the bacterial EET. These results are highly useful to design efficient CNT-modified bioanodes for high-performance MFCs.

In addition to the ability of CNT to enhance the EET, it can even alter the bacterial metabolism. Recently Yan et al.⁷⁷ reported that addition of CNTs into *S. oneidensis* MR-1 shifted the intracellular reduction of nitrobenzene (NB) to extracellular. It indicated that CNTs could alter the electron flow route in *S. oneidensis*. Wild-type and mutant strains lacking OM *c*-Cyts exhibited similar NB reduction rate. Intriguingly, the addition of CNTs to the wild-type MR-1 accelerated NB reduction while mutant strains were unaffected by CNTs. The extracellular NB reduction could slow down the diffusion of NB into the interior of the cells, allowing a high microbial activity due to the lower toxic effect of the NB. The results suggested the existence of an intimate contact of CNTs with OM *c*-Cyts to enhance the extracellular NB reduction and possibilities of other nanostructures to alter the electron transfer route in *Shewanella*. A very recent report demonstrated that CNTs have a strong affinity towards *Saccharomyces cerevisiae* cells, and the CNT/*S. cerevisiae* bioanode exhibited an increased current generation in the MFC.⁷⁸

3.2. Graphene-based nanostructures

Similar to CNTs, graphene has been extensively used for developing electrodes in MFCs due its outstanding electrical conductivity, high specific surface area, and excellent mechanical strength. Graphene modified stainless steel mesh (GMS) bioanode produced 18-times higher electricity in an MFC as compared to the bare stainless steel mesh.⁷⁹ The GMS provided large surface area for the bacterial adhesion and lowered the charge transfer resistance significantly to enhance the EET process. 3D graphene sponge bioanode prepared by an ice template method showed a better performance over graphite felts in MFCs owing to its high surface area and low charge transfer resistance.⁸⁰ A micrometer-sized MFC equipped with a graphene anode produced a power output of $\sim 1 \text{ nW}^{81}$, which is sufficient to run ultra-low power electronics.⁸² A

264 biocompatible chitosan/vacuum stripped graphene scaffold (CHI/VSG) bioanode produced a 78-times
265 higher current density over the control in a *Pseudomonas aeruginosa* bio-catalyzed MFC.⁸³ *P. aeruginosa*
266 usually secretes endogenous mediators such as phenazine to accelerate the EET process, and a previous
267 report demonstrated that graphene enhances the production of phenazine.⁸⁴ Here, the VSG significantly
268 stimulated the secretion of endogenous redox mediators and also provided large surface area for the biofilm
269 formation. The macroporous structure of the CHI/VSG composite permitted an undisturbed flow of the
270 substrate to the inner core of the anode, promoting internal bacterial colonization. Also, EIS measurement
271 revealed a reduced charge transfer resistance, which facilitated the EET process in *P. aeruginosa*.

272 Graphene like other carbon materials is highly hydrophobic, which makes less affinity towards the bacterial
273 attachment as compared to other carbonaceous materials.⁸⁵ Yong et al.⁸⁵ deposited a conducting polymer,
274 polyaniline (PANI) which is highly hydrophilic in nature on graphene nanostructures to make PANI
275 hybridized 3D graphene bioanode in MFCs. The surface hydrophilicity allowed a thicker biofilm formation
276 on the modified bioanode and a very recent study suggested that the surface hydrophilicity can stimulate
277 the EET process.⁵⁶ The PANI/graphene bioanode delivered improved current generation relative to
278 traditional carbon electrodes. SEM images revealed the formation of biofilm in both interior and exterior
279 of the modified electrode while bare 3D graphene possessed biofilm at exterior only. PANI is positively
280 charged in the neutral condition, which can have an intimate electrostatic contact with negatively charged
281 bacterial membrane surfaces.⁸⁶ CV analysis confirmed that PANI/graphene foam facilitated direct electron
282 transfer in *S. oneidensis*. This enhanced direct electron transfer mechanism depicts that OM *c*-Cyts created
283 a firm contact with PANI/graphene by developing an efficient electronic conduit. Ionic liquid
284 functionalized graphene nano-sheets decorated on a carbon paper anode (CP-IL-GNS) delivered 7-fold
285 higher bio-current generation over the control in a *S. oneidensis* MFC.⁸⁷ The IL-GNS created a positively
286 charged surface on the carbon paper for the electrostatic attraction of negatively charged *Shewanella* cells
287 (Figure 5). Also, the IL-GNS provided high conductivity and large surface area for the improved electron
288 transfer from bacteria to the electrode.

Graphene oxide (GO) nano-ribbons deposited on a carbon paper significantly enhanced direct electron transfer process in *S. oneidensis* MR-1.⁸⁸ The authors proposed that GO nano-ribbons behaved as conductive microbial nanowires to facilitate the bacterial EET process. Reduced GO decorated on nickel foam (rGO/Ni) showed an improved electricity production in a *S. oneidensis* MFC.⁸⁹ The enhanced performance was attributed to macro-porosity and high conductivity of the rGO/Ni. Yong et al.⁹⁰ developed a self-assembled 3D rGO/bacterial biofilm via in situ reduction of GO by *S. oneidensis* MR-1. As-formed rGO/*Shewanella* biofilm showed 25-fold enhancement in the EET as compared to naturally occurring biofilms. The 3D hybrid biofilm demonstrated an outstanding electrochemical activity and improved direct electron transfer rate between the cells and electrode. The rGO interconnected the cells in the biofilm, which lowered the charge transfer resistance significantly over the natural *Shewanella* biofilms. Similarly, in situ reduction of GO via microbial respiration enhanced the bacterial EET by forming a graphene/biofilm scaffold.⁹¹ Electrochemical measurements demonstrated that as-formed graphene structure induced exoelectrogens to accelerate the EET rate. A recent report from Zhao et al.⁹² demonstrated that *Shewanella* biofilm formed on a hybrid graphene-CNT electrode exhibited an improved EET activity for bioelectricity generation. The modified electrode was developed by inoculating *S. oneidenis* MR-1 cells to a solution containing GO and CNTs with lactate as the electron donor. After two days of the inoculation, the non-conducting GO was converted to conducting graphene structures by accepting electrons from *Shewanella* cells. As-formed G-CNT/biofilm was collected by centrifugation and deposited on an acid-treated carbon felt electrode. The acid-treatment created a positively charged surface on the carbon felt, which attracted the negatively charged G-CNT/biofilm. Figure 6 shows a schematic illustration of the formation of hybrid G-CNT/biofilm and the EET process from the biofilm to the electrode. The modified electrode resulted in an enhanced bacterial loading and improved direct electron transfer process via OM *c*-Cyts.

3.3. Noble metal nanomaterials

Platinum and platinum group metals have been used for a long time in MFC electrodes especially cathodes.^{59,60} It is not surprising therefore that noble metal nanoparticles have been explored as materials

for improved catalytic performance of these systems. Gold (Au) and palladium (Pd) nanoparticles decorated graphite electrodes delivered excellent current production in MFCs over the controls.⁹³ Significant enhancement in the current generation observed with particle sizes greater than 200 nm, possibly due to the solid direct contact with the bacterial cells. A negative correlation between the current density and the nanoparticle density was observed. In contrast, Alatraktchi et al.⁹⁴ found an increased current generation with Au nanoparticle density. DNA sequencing has shown that the high density of Au nanoparticles increased the population of exoelectrogenic bacteria. Similarly, Au nanoparticles deposited on a carbon paper produced 2.9 times higher current over the bare carbon paper.⁹⁵ Using single crystal approach, Maestro et al.⁹⁶ demonstrated that crystal geometry of Au nanoparticles is critical for the bacterial EET. Three different single crystal forms of Au - Au (110), Au (111) and Au (210) were employed to investigate the effect of the crystal orientation on the EET activity in *G. sulfurreducens*. The crystal orientation, Au (210) exhibited enhanced current generation over the remaining crystal orientations. The result sheds light on the fact that similar to the type of electrode materials, the crystallographic nature of the material is also crucial for the development of efficient bioanodes.

Beyond Au, Pd has also been investigated for its ability to improve EET in BESs. Wu et al.⁹⁷ demonstrated that membrane bound-Pd nanoparticles significantly improved the bacterial EET by facilitating electron transfer between cells and electrode. Similarly, membrane bound-Au nanoparticles in *S. oneidensis* cells greatly enhanced the direct electron transfer from cells to the electrode surface.⁹⁸ Outer membrane proteins MtrC and OmcA are believed to be the key redox proteins for the EET in *S. oneidensis* MR-1.⁸ Intriguingly, mutant strains lacking OmcA/MtrC showed a similar EET rate with the wild-type in the presence of membrane-bound Au nanoparticles implying that the EET chain in *S. oneidensis* could be repaired by the Au nanoparticles. A similar report proposed that magnetite nanoparticles can compensate *c*-Cyts (MtrC/OmcA) in *G. sulfurreducens*, and it will be discussed in the section 3.4 later.⁹⁹ The results led to the conclusion that nanomaterials can mimic membrane-bound proteins, which warrant further extensive research. A seminal work from Xiao et al.¹⁰⁰ demonstrated that Au nanoparticles wired-flavin adenine

dinucleotide (FAD) transferred electrons to a conductive electrode much faster (~ 5000 per second) compared with the rate at which molecular oxygen, the natural co-substrate of the enzyme, accepted electrons (~ 700 per second). Here, the Au nanoparticle acted as an “electrical nanoplug” to firmly attach the enzyme on the conductive surface, which enhanced the electrical conductivity of the redox enzyme. As a series of redox enzymes are involved in the bacterial EET, this information is crucial for the understanding of Au nanoparticle effect in the EET. However, depending on noble metals for the development of bioanode materials is not affordable for practical applications.

3.4. Inorganic nanomaterials

Various inorganic nanomaterials such as iron sulfide, goethite, magnetite, hematite, and nickel have been extensively used to accelerate the bacterial EET. *Shewanella* uses sulfur-mediated electron shuttling process for the metal reduction.¹⁰¹ In situ production of iron sulfide (FeS) nanoparticles in *S. loihica* PV-4 delivered two times larger current production in MFCs than the cells lacking the FeS nanoparticles.¹⁰² FeS is an abundant biogenic mineral existing in the nature. Mutant experiments lacking OM *c*-Cyts delivered 70% lower current density as compared to the wild-type. The substantial reduction in the current generation with mutant strains suggested that the Fe 3d orbital in the biogenic FeS nanoparticles interacted with OM *c*-Cyts forming an electrical conduit for the EET process. Similarly, *S. loihica* PV-4 with in situ generated biogenic FeS nanoparticles produced 500 pA current which was 3-4 times higher than the current generated from a single cell *Shewanella* or *Geobacter*.¹⁰³ The FeS nanoparticles established an efficient electronic conduit for the EET process by interconnecting the remote bacterial cells that were not in direct contact with the electrode surface.

Magnetite (Fe₃O₄), an abundant natural mineral plays critical roles in bioremediation and geochemical cycling of metals.^{104,105} Many studies have proposed that Fe₃O₄ can facilitate EET processes in iron-reducing microbes.^{104,105} Kato et al.¹⁰⁶ reported that nano-Fe₃O₄ enhanced interspecies electron transfer (IET) between *G. sulfurreducens* and *Thiobacillus denitrificans*, accompanying acetate oxidation coupled

363 to nitrate reduction. The Fe_3O_4 nanoparticles constructed an electrical bridge between these bacterial species
364 to facilitate the IET process, revealing the role of conductive minerals in the bacterial EET process. In
365 contrast, the addition of semiconductive-hematite (Fe_2O_3) did not exhibit any impact on the IET. Peng et
366 al.¹⁰⁷ also observed an enhanced current generation in MFCs by the addition of nano- Fe_3O_4 . The
367 investigation of the molecular mechanism behind the improved bacterial EET associated with Fe_3O_4
368 revealed that the Fe_3O_4 nanoparticles behave like c-type cytochrome, OmcS.⁹⁹ A repressed expression of
369 OmcS gene was observed in wild-type strains in the presence of Fe_3O_4 , suggesting that the cells need to
370 produce less OmcS, if Fe_3O_4 is available. The transmission electron microscopic images (Figure 7) depicted
371 that the nanoscale Fe_3O_4 were well dispersed in the *Geobacter* species by preferentially attaching on the
372 conductive pili, which was responsible for the improved EET rate in *G. sulfurreducens*.

373 Goethite nanowhiskers modified carbon paper bioanode delivered 60% higher current generation in an
374 MFC relative to the bare carbon paper.¹⁰⁸ The modified anode provided improved surface area for the
375 biofilm formation, and also exhibited a low charge transfer resistance for the EET in *S. loihica* PV-4. Carbon
376 coated titanium dioxide (TiO_2) core-shell 3D anode demonstrated a high performance in MFCs due to its
377 enhanced electrochemical capacitance.¹⁰⁹ Previous reports suggested that TiO_2 can increase the MFC
378 performance by facilitating the EET process.¹¹⁰⁻¹¹² Nickel oxide nanoflakes deposited on a carbon cloth
379 (NiO nanoflakes/carbon cloth) rendered an extensive electroactive surface area and low interfacial charge
380 transfer resistance to facilitate the EET in *S. putrefaciens*.¹¹³ Also, the deposition of NiO on the carbon cloth
381 created a hydrophilic surface for the enhanced bacterial adhesion. Some studies reported a decline in the
382 EET rate of *S. oneidensis* MR-1 over time due to the formation of dense biofilm, which hindered the
383 diffusion of substrate and electron mediators such as flavins.^{114,115} A very recent report from Zhang et al.¹¹⁶
384 demonstrated that a complete suppression of biofilm formation on a carbon electrode by hexagonal WO_3
385 nanorods promoted the flavin-mediated EET process in *S. oneidensis*. The smaller size and high
386 crystallinity of the WO_3 nanorods prevented the biofilm growth on the electrode surface, and planktonic
387 *Shewanella* cells used flavins as shuttling molecules to enhance the EET process. This observation is

concordant with a previous report from Lieber and co-workers.¹¹⁷ In this report, the authors designed a bio-electrochemical set up with two types of working electrodes to probe the EET activity in *S. oneidensis* MR-1. The first type of working electrode was developed by constructing nano-holes on a conducting ITO glass that can preclude cell attachment to the electrode surface. The second electrode was designed in such a way that the cells can easily attach to the electrode surface. Interestingly, *S. oneidensis* exhibited a similar EET activity with both electrodes. However, replacement of supernatant with fresh media severely reduced the EET activity in *Shewanella* and reintroduction of the original cell-free media caused a quick increase in the EET process. These observations clearly suggested that there is no need for direct contact of MR-1 cells to the electrode surface (no need of biofilm) and shuttling molecules are mainly responsible for the EET activity in MR-1 cells. However, these observations may not be applicable to *G. sulfurreducens* as they secrete very less shuttling molecules and prefer to make thick biofilms on the electrode surface for the direct electron transfer.

3.5. Conductive polymers

Conductive polymers such as polyaniline and polypyrrole are widely used in electronics and sensors due to their good conductivity and polymer character. Recently, conductive polymers has received great attention as the bioanode materials in MFCs with outstanding performances. Polypyrrole (PPy) nanotube coated carbon paper anode delivered 6-times larger bioelectricity relative to the bare carbon paper in *S. oneidensis* MR-1 MFC.¹¹⁸ The PPy modified electrode provided high conductivity and large surface area for the improved performance of the MFC and EIS measurement revealed that the modified anode exhibited a low charge transfer resistance for the enhanced EET process. Ding et al.¹¹⁹ proposed PPy nano arrays (PPy-NA) as bioanode materials for an enhanced performance in MFCs over the control carbon paper, Au and ITO electrodes. The authors systematically investigated the possibility of different EET mechanisms in the presence of modified bioanode. An addition of RF in the medium with wild type *Shewanella* showed little effect on the EET activity suggesting that PPy-NA bioanode promoted direct electron transfer (via OM c-Cyts) from *Shewanella* to the electrode instead of electron shuttling by RF. Mutant experiments lacking

major *c*-Cyt *shew2525* severely impaired the EET process, indicating the role of *c*-Cyts in the EET mechanism. PPy acted as a conductor to transfer the metabolically generated electrons from the bacterial cells to the electrode surface. Also, as PPy was positively charged in the neutral solution, the PPy-NA electrostatically attracted the negatively charged bacterial cells to form a thick biofilm. SEM images showed that *Shewanella* formed a 3D porous biofilm on the surface of PPy-NA where cells were bundled tightly by creating an electrical conduit to facilitate the EET process (Figure 8). Moreover, the macroporous 3D biofilm allowed a free passage of the substrate (lactate) for the improved bacterial respiration. An MFC equipped with a PPy/anthraquinone-2,6-disulphonic disodium salt (PPy/AQDS)-modified anode exhibited 13-times higher current output over the unmodified electrode in the presence of *S. decolorationis* S12.¹²⁰ CV and SEM analyses showed that the modified anode provided a large surface area for the bacterial adhesion and improved the EET process. Previous studies have already shown that AQDS can serve as an electron mediator to enhance the EET rate in *G. sulfurreducens*.^{121,122}

Besides PPy-NA, polyaniline nanoarrays also showed excellent performance in BESs. Ding et al.¹²³ reported that EET in *S. loihica* PV-4 can be controlled with polyaniline nanoarray (PANI-NA)-bioanode by varying applied potential. Due to the presence of quinone ring in PANI, it can act as a solid electron mediator for the EET process. PANI can exist in different oxidation levels according to the applied potential, which can dictate the electron transfer to the electrode surface. At an applied potential of -0.3 V vs. Ag/AgCl, PANI stayed in reduced state and hence it was not able to mediate the anodic EET, or it blocked the electron transfer from cells to the electrode. On the other hand, at an applied potential of -0.2 V vs. Ag/AgCl, PANI stayed in the oxidized state and mediated the EET process. This observation indicated that when the applied potential became more positive, the ability of PANI as the electron mediator enhanced for the EET process. Also, the PANI-NA electrode provided large surface area for the bacterial adhesion and enabled an intimate contact with microbial nanowires. It has already been demonstrated that 3D PANI-nanowire networks formed on an ITO glass significantly enhanced the EET rate in *S. loihica* PV-4.¹²⁴

3.6. Photo-active semiconductors

Photo-active semiconductors such as hematite (Fe_2O_3) were employed as the bioanode materials in MFCs.¹²⁵⁻¹²⁷ The irradiation of light to the photo-anode of MFC largely increased the photo-current in the presence of live bacterial cells. Photoexcitation of Fe_2O_3 -modified bioanode generated electrons in the conduction band (CB) in the presence of *S. loihica* cells.¹²⁵ Figure 9a shows that the Fe_2O_3 interconnected the bacterial cells (even remote cells), resulting in a highly electrically connected bacterial networks on the electrode surface. The photo-generated electrons in the CB transferred to the electrode surface via electron hopping where the bacterial cells acted as electrical bridges by establishing long-distance electron transfer conduits. Mutant experiments lacking OM *c*-Cyts significantly reduced the current production, indicating the involvement of OM *c*-Cyts in the electron hopping process. In contrast, Qian et al.¹²⁷ proposed that the photo-excited electrons in the CB of Fe_2O_3 directly transferred to the electrode while holes generated in the valence band (VB) accepted electrons from the OM *c*-Cyts of *S. oneidensis* cells, electron shuttles, or water (Figure 9d).

4. Enhancement of microbial electrosynthesis process by nanostructured materials

Bacterial extracellular electron uptake from a cathode to produce chemicals is a recent research trend compared to the bacterial EET process. Even though many reports are available for the development of anode materials for the EET process, only limited studies are available for the development of efficient cathode materials for microbial electrosynthesis (MES) process.¹²⁸ Uncertainty in the molecular mechanism involved in the MES process mainly hinders the progress in designing cathodes for the MES technology. Here, some recent reports on the development of biocathode materials for the MES process are discussed. Zhang et al.¹²⁹ employed various kinds of nanostructured materials to develop cathode electrodes to synthesize acetate from CO_2 by *Sporomusa ovata* biofilms. Polyaniline-poly acrylonitrile (PANI-PAN) microporous composite modified carbon cloth cathode exhibited 3-fold higher acetate production relative to the control. *S. ovata* biofilm produced 3.4-fold larger acetate production over the control with a cathode made of single walled CNTs deposited on the cotton and polyester textile fibers. Physical deposition of Au,

Pd or Ni nanoparticles on a carbon cloth also enhanced the acetate production by *S. ovata* biofilm over the controls. Nie et al.¹³⁰ developed an efficient cathode by microwave assisted deposition of Ni nanowires on a graphite surface for the improved acetate production from CO₂ using bio-catalytic activity of *S. ovata*. The deposition of Ni nanowires on the graphite surface without microwave assistance caused the detachment of Ni wires from the graphite surface to the medium during the microbial acetate production. CV analyzes showed that Ni nanowire deposited-graphite exhibited higher cathodic current density over the untreated graphite and Ni nanoparticles coated-graphite. Also, the CV analyses excluded the possibility of involvement of electron shuttles in the microbial production of acetate. SEM images (Figure 10) depicted thick biofilm formations on the Ni nanowire deposited-graphite surfaces while a thinner biofilm formation was observed on the Ni nanoparticle deposited-graphite surface. Here, Ni nanowires provided a large surface area and excellent conductive networks for the adhesion of *S. ovata cells*. Also, the negatively charged *S. ovata cells*^{131,132} electrostatically attached to the positively charged Ni nanowire deposited-graphite electrode¹²⁹, which facilitated the microbial acetate production.

The Nanoweb-RVC electrode, which showed excellent performance as a bioanode in the MFC⁶⁵ was employed as a biocathode for the microbial acetate production by mixed culture bacteria.¹³³ The NanoWeb cathode produced 2.6-higher acetate as compared to a carbon plate electrode. The 3D structure of the NanoWeb electrode allowed thick biofilm formations in both exterior and interior sides of the electrode and the nanostructure enhanced the microbe-electrode interaction significantly. Surprisingly, acetate production was not detected with unmodified RVC and also no biofilm was observed on the bare RVC electrode surface. These observations suggested that the nanostructure is essential for both microbial acetate production and biofilm formation. Yong et al.⁹⁰ constructed a biocathode by in situ reduction of non-conductive GO to conducting rGO using *S. oneidensis* MR-1. The rGO/*Shewanella* hybrid biofilm-cathode exhibited improved reduction of fumarate to succinate. The hybrid 3D cathode provided a huge electroactive surface area for the bacterial attachment and enhanced the microbe-electrode interaction by lowering the interfacial electron transfer resistance.

5. Conclusions and future perspectives

In summary, various advanced nanostructures significantly improved both bacterial EET and microbial electrosynthesis processes. The synergetic effect of large surface area, high conductivity, and low interfacial charge resistance provided by most of the nanostructured electrodes were mainly attributed to the improved bacterial bidirectional EET process. The nanostructures developed an intimate contact with OM *c*-Cyts and bacterial nanowires that established an active electronic conduit for the efficient EET process. For instance, some nanomaterials such as CNTs induced cellular metabolism and even altered the electron transfer route in *S. oneidensis* MR-1.⁷⁷ Graphene stimulated the production of phenazine, a well-known electron shuttling molecule for the EET process in *Pseudomonas*.⁸⁴ Also, Au and Fe₃O₄ nanoparticles mimicked OM *c*-Cyts for enhancing the bacterial EET process.^{98,99} These findings suggest that in addition to traditional understandings on nanomaterials such as their high electrical conductivity, a molecular level understanding of how nanomaterials affect the cellular metabolism is also needed to establish strategies for the development of efficient nanomaterials to enhance the bidirectional EET process.

There is still a lot of scope for the significant enhancement in the bacterial bidirectional EET process. Currently, nanomaterial science is well-developed which can provide various advanced nanostructures as suitable candidates for the electrode development, thanks to nanotechnology. For example, anisotropic nanomaterials with exotic shapes such as prisms and octahedral can be employed to enhance the EET rate as the edges in the nanostructures may be preferable to attach the OM *c*-Cyts and bacterial nanowires compared to spherical nanomaterials. A very recent study revealed that hydrophilic surfaces can facilitate the EET process⁵⁶ and hence application of nanostructures with super-hydrophilic surfaces may further promote the bacterial EET process. The nanostructures used for the bioanodes may also be suitable for biocathodes in the MES process as recent studies demonstrated that EET active bacteria such as *Shewanella* reverse their respiratory pathway for reductive metabolism by following the same EET pathway.^{41,134} Currently expensive materials such as CNTs, graphene, and noble metals are mostly employed for developing electrode materials and also the electrode fabrication is sometimes extremely complicated.

Hence, cheaper materials with simpler fabrication techniques are needed for the bulk production of efficient electrodes for the bacterial bidirectional EET process.

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References

1. G. Reguera, K. D. McCarthy, T. Mehta, J. S. Nicoll, M. T. Tuominen and D. R. Lovley, *Nature*, 2005, **435**, 1098-1101.
2. D. R. Lovley, *Annu. Rev. Microbiol.*, 2012, **66**, 391-409.
3. S. J. Fuller, D. McMillan, M. B. Renz, M. Schmidt, I. T. Burke and D. I. Stewart, *Appl. Environ. Microbiol.*, 2014, **80** (1), 128-37.
4. S. Kalathil, M. M. Khan, J. Lee and M. H. Cho, *Biotechnol. Adv.*, 2013, **31**, 915-24.
5. S. Kalathil, J. Lee and M. H. Cho, *Bioresource. Technol.*, 2012, **119**, 22-27.
6. S. Kalathil, J. Lee and M. H. Cho, *ChemSusChem.*, 2013, **6**, 246-250.
7. D. Pant, G. V. Bogaert, L. Diels and K. Vanbroekhoven, *Bioresource. Technol.*, 2010, **101**, 1533-43.
8. M. Estevez-Canales, A. Kuzume, Z. Borjas, M. Füeg, D. Lovley, T. Wandlowsk T and A. Esteve-Núñez, *Environ. Microbiol. Rep.*, 2015, **7**, 219-226.
9. E. Marsili, D. B. Baron, I. D. Shikhare, J. A. Gralnick and D. R. Bond DR, *Proc. Natl. Acad. Sci. USA*, 2008, **105**, 3968-3973.
10. S. Pirbadian, S. E. Barchinger, K. M. Leung, H. S. Byun, Y. Jangir, R. A. Bouhenni, S. B. Reed, M. F. Romine, D. A. Saffarini, L. Shi, Y. A. Gorby, J. H. Golbeck, M. Y. El-Naggar, *Proc. Natl. Acad. Sci. USA*, 2014, **111**, 12883-12888.
11. A. Okamoto, K. Hashimoto, K. H. Nealson and R. Nakamura, *Proc. Natl. Acad. Sci. USA*, 2013, **110**, 7856-7861.
12. A. Okamoto, S. Klathil, X. Deng, K. Hashimoto, R. Nakamura and K. H. Nealson, *Sci. Rep.*, 2014, **4**, 5628.
13. S. Kalathil, K. Hashimoto and A. Okamoto, *ChemElectroChem.*, 2014, **1**, 1840-1843.
14. A. Okamoto, K. Hashimoto and K. H. Nealson, *Angew. Chem. Int. Ed.*, 2014, **53**, 10988-91.
15. Y. A. Gorby, S. Yanina, J. S. McLean, K. M. Rosso, D. Moyles, A. Dohnalkova, T. J. Beveridge, I. S. Chang, B. H. Kim, K. S. Kim, D. E. Culley, S. B. Reed, M. F. Romine, D. A. Saffarini, E. A. Hill, L. Shi, D. A. Elias, D. W. Kennedy, G. Pinchuk, K. Watanabe K, S. Ishii, B. Logan, K. H. Nealson and J. K. Fredrickson, *Proc. Natl. Acad. Sci. USA*, 2006, **103**, 11358-11363.
16. A. G. Smart, Two experiments, two takes on electric bacteria, *Phys. Today.*, 2010, **63**, 18-20.
17. N. S. Malvankar and D. R. Lovley, *ChemSusChem.*, 2012, **5**, 1039-1046.

- 562 18. N. S. Malvankar and D. R. Lovley, *Curr. Opin. Biotechnol.*, 2014, **27**, 88-95.
- 563 19. F. Qian and Y. Li, *Nat. Nanotechnol.*, 2011, **6**, 538-539.
- 564 20. C. Hauser and S. Zhang, *Nature*, 2010, **468**, 516-517.
- 565 21. N. S. Malvankar, M. Vargas, K. P. Nevin, A. E. Franks, C. Leang, B. H. Kim, K. Inoue, T. Mester, S.
- 566 F. Covalla, J. P. Johnson, V. M. Rotello, M. T. Tuominen and D. R. Lovley, *Nat. Nanotechnol.*, 2011, **6**,
- 567 573-579.
- 568 22. N.S. Malvankar, S. E. Yalcin, M. T. Tuominen and D. R. Lovley, *Nat. Nanotechnol.*, 2014, **9**, 1012-
- 569 1017.
- 570 23. T. A. Skotheim and J. R. Reynolds, *Handbook of Conducting Polymers* (CRC, 2007).
- 571 24. K.M. Leung, G. Wanger, M. Y. El-Naggar, Y. Gorby, G. Southam, W. M. Lau and J. Yang, *Nano Lett.*,
- 572 2013, **13**, 2407-2413, DOI: 10.1021/nl400237p.
- 573 25. P. S. Bonanni, D. Massazza and J. P. Busalmen, *Phys. Chem. Chem. Phys.*, 2013, **15**, 10300-10306.
- 574 26. N. S. Malvankar, M. T. Tuominen and D. R. Lovley, *Energy Environ. Sci.*, 2012, **5**, 6247-6249.
- 575 27. D. R. Bond, S. M. Strycharz-Glaven, L. M. Tender and C. I. Torres, *ChemSusChem.*, 2014, **5**, 1099-
- 576 1105.
- 577 28. S. M. Strycharz-Glaven, R. M. Snider, A. Guiseppi-Elie A and L. M. Tender, *Energy Environ. Sci.*,
- 578 2011, **4**, 4366-4379.
- 579 29. S. M. Strycharz-Glaven and L. M. Tender, *Energy Environ. Sci.*, 2012, **5**, 6250-6255.
- 580 30. S. Pirbadian and M. Y. El-Naggar, *Phys. Chem. Chem. Phys.*, 2012, **14**, 13802-13808.
- 581 31. D. R. Lovley and N. S. Malvankar, *Environ. Microbiol.*, 2015, **17**, 2209-2215.
- 582 32. D. R. Lovley, *Environ. Microbiol. Rep.* 2011, **3**, 27-35.
- 583 33. K. P. Nevin, T. L. Woodard, A. E. Franks, Z. M. Summers and D. R. Lovley, *mBio*, 2010, **1**:e00103-
- 584 10.
- 585 34. K. Rabaey and R. A. Rozendal, *Nat. Rev. Microbiol.*, 2010, **8**, 706-716.
- 586 35. M. Rosenbaum, F. Aulenta, M. Villano and L. T. Angenent, *Bioresource. Technol.*, 2011, **102**, 324-33.
- 587 36. M. Sharma, N. Aryal, P. M. Sarma, K. Vanbroekhoven, B. Lal, X. Dominguez Benetton and D. Pant
- 588 *Chem. Commun.*, 2013, **49**, 6495-6497.

- 589 37. S. A. Patil, S. Gildemyn, D. Pant, K. Zengler, B. E. Logan and Rabaey, *Biotechnol. Adv.* 2015, **33**, 736-
590 744.
- 591 38. L. L. Barton, F. Goulhen, M. Bruschi, N. A. Woodards, R. M. Plunkett and F. J. M. Rietmeijer,
592 *BioMetals*, 2007, **20**, 291-302.
- 593 39. M. Guiral-Brugna, M. T. Giudici-Orticoni, M. Bruschi and P. Bianco, *J. Electroanal. Chem.*, 2001, **510**,
594 136–143, [doi:10.1016/S0022-0728\(01\)00502-2](https://doi.org/10.1016/S0022-0728(01)00502-2)
- 595 40. A. S. Pereira, R. Franco, M. J. Feio, C. Pinto, J. Lampreia, M. A. Reis, J. Calvete, I. Moura, I. Beech,
596 A. R. Lino and J. J. G. Moura, *Biochem. Biophys. Res. Commun.*, 1996, **221**, 414–421.
- 597 41. D. E. Ross, J. M. Flynn, D. B. Baron, J. A. Gralnick and D. R. Bond, *PLoS ONE*, 2011, **6**, e16649.
- 598 42. N. S. Malvankar, M. T. Tuominen and D. R. Lovley, *Energy Environ. Sci.*, 2012, **5**, 5790-5797.
- 599 43. R. M. Donlan, *Emerging Infectious Diseases*, 2002, **8**, 881-890.
- 600 44. V. B. Trans, S. J. M. Fleiszig, D. J. Evans, C. Y. Radke, *Appl. Environ. Microbiol.* 2011, **77**, 3644-
601 3652.
- 602 45. B. Li and B. E. Logan, *Colloid Surfaces B: Biointerfaces*, 2004, **36**, 81-90.
- 603 46. C. H. Bell, B. S. Arora and T. A. Camesano, *Environ. Eng. Sci.*, 2005, **22**, 629-641.
- 604 47. P. Stoodley, K. Sauer, D. G. Davies and J. W. Costerton, *Annu. Rev. Microbiol.*, 2002, **56**, 187-2009.
- 605 48. E. K. Eboigbodin, A. R. J. Newton, F. A. Routh and A. C. Biggs, *Langmuir*, 2005, **21**, 12315-12319.
- 606 49. Y-W. Chang, A. A. Fragkopoulou, S. M. Marquez, H. D. Kim, T. E. Angelini, and A. Fernández-Nieves,
607 *N. J. Phys.*, 2015, **17**, 033017.
- 608 50. J. H. Felix and C. Dekker, *Science*, 2014, **346**, 1251821-9.
- 609 51. A. I. Hochbaum and J. Aizenberg, *Nano Lett.*, 2010, **10**, 3717-3721.
- 610 52. K. K. Sakimoto, C. Liu, J. Lim and P. Yang, *Nano Lett.*, 2014, **14**, 5471-5476.
- 611 53. H. E. Jeong, I. Kim, P. Karam, H-J. Choi and P. Yang, *Nano Lett.*, 2013, **13**, 2864-2869.
- 612 54. A. V. Singh, V. Vyas, R. Patil, V. Sharma, P. E. Scopelliti, G. Bongiorno, A. Podesta, C. Lenardi, W.
613 N. Gade and P. Milani, *PLoS ONE*, 2011, **6**: e25029.
- 614 55. N. Mitik-Dineva, J. Wang, R. C. Mocanasu, P. R. Stoddart, R. J. Crawford and E. P. Ivanova,
615 *Biotechnol. J.*, 2008, **3**, 536-544.
- 616 56. C-M. Ding, M-I. Lv, Y. Zhu, L. Jiang and H. Liu, *Angew. Chem. Int. Ed.*, 2015, **54**, 1446-1451.

- 617 57. M. Zhous, M. Chi, J. Luo, H. He and T. Jin, *J. Power Sources*, 2011, **196**, 4427-4435,
618 [doi:10.1016/j.jpowsour.2011.01.012](https://doi.org/10.1016/j.jpowsour.2011.01.012).
- 619 58. J. Wei, P. Liang and X. Huang, *Biorsource Technol.* 2011, **102**, 9335-44.
- 620 59. Y. Alvarez-Gallego, X. Dominguez-Benetton, D. Pant, L. Diels, K. Vanbroekhoven, Inge Genne and
621 P. Vermeiren, *Electrochimica Acta*, 2012, **82**, 415-426.
- 622 60. X. Zhang, D. Pant, F. Zhang, J. Liu, W. He and B. E. Logan, *ChemElectroChem* 2014, **1**, 1859-1866.
- 623 61. Mustakeem, *Mater. Renew Sustain. Energy*, 2015, **4**, 22.
- 624 62. X. Xie, L. Hu, M. Pasta, G. F. Wells, D. Kong, C. S. Criddle and Y. Cui, *Nano Lett.*, 2011, **11**, 291-
625 296.
- 626 63. X. Xie, M. Ye, L. Hu, N. Liu, J. R. McDonough, W. Chen, H. N. Alshareef, C. S. Criddle and Y. Cui,
627 *Energy Environ. Sci.*, 2012, **5**, 5265-5270.
- 628 64. X. Xie, W. Zhao, H. R. Lee, C. Liu, M. Ye, W. Xie, B. Cui, C. S. Criddle and Y. Cui, *ACS Nano*, 2014,
629 **8**, 11958-11965.
- 630 65. V. Flexer, J. Chen, B. C. Donose, P. Sherrell, G. G. Wallace and J. Keller, *Energy Environ. Sci.*, 2013,
631 **6**, 1291-1298.
- 632 66. I. H. Park, M. Christy, P. Kim and K. S. Nahm, *Biosens. Bioelectron.*, 2014, **58**, 75-80.
- 633 67. J. E. Mink, J. P. Rojas, B. E. Logan and M. M. Hussain, *Nano Lett.*, 2012, **12**, 791-795.
- 634 68. J. E. Mink and M. M. Hussain, *ACS Nano* 2013, **7**: 6921-6927.
- 635 69. K. Katuri, M. L. Ferrer, M. C. Gutiérrez, R. Jiménez, F. D. Monte and D. Leech, *Energy Environ. Sci.*,
636 2011, **4**, 4201-4210.
- 637 70. X-W. Liu, J-J. Chen, Y-X. Huang, X-F. Sun, G-P. Sheng, D-B. Li, L. Xiong, Y-Y. Zhang, F. Zhao and
638 H. Q. Yu, *Sci. Rep.*, 2014, **4**, 3732.
- 639 71. L. Peng, S-J. You and J-Y. Wang, *Biosens. Bioelectron.*, 2010, **25**, 1248-51.
- 640 72. X-W. Liu, Y-X. Huang, X-F. Sun, G-P. Sheng, F. Zhao, S-G. Wang and H-Q. Yu, *ACS Appl. Mater.*
641 *Interfaces.*, 2014, **6**, 8158-8164.
- 642 73. S. Kalathil, N. V. Hoa, J-J. Shim, M. M. Khan, J. Lee and M. H. Cho, *J. Nanosci. Nanotechnol.*, 2013,
643 **13**, 7712-7716.
- 644 74. X. Zhang, M. Epifanio and E. Marsili, *Electrochimica Acta.*, 2013, **102**, 252-258,
645 [doi:10.1016/j.electacta.2013.04.039](https://doi.org/10.1016/j.electacta.2013.04.039)

- 646 75. D. R. Bond, S. M. Strycharz-Glaven, L. M. Tender and C. I. Torres, *ChemSusChem.*, 2012, **5**, 1099-
647 1105.
- 648 76. C. Erbay, X. Pu, W. Choi, M-J. Choi, Y. Ryu, H. Hou, F. Lin, P-D. Figueiredo, C. Yu and A. Han, *J.*
649 *Power Sources*, 2015, **280**, 347-354, [doi:10.1016/j.jpowsour.2015.01.065](https://doi.org/10.1016/j.jpowsour.2015.01.065)
- 650 77. F-F. Yan, Y-R. HE, C. Wu, Y-Y. Cheng, W-W. Li and H-Q. Yu, *Environ. Sci. Technol.*, 2014, **1**, 128-
651 132.
- 652 78. S. Matsumoto, K. Yamanaka, H. Ogikubo, H. Akasaka and N. Ohtake, *Appl. Phys. Lett.*, 2014, **105**,
653 083904-4.
- 654 79. Y. Zhang, G. Mo, X. Li, W. Zhang, J. Zhang, J. Ye, X. Huang and C. Yu, *J. Power Sourc.*, 2011, **196**,
655 5402-5407.
- 656 80. W. Chen, Y-X. Huang, D-B. Li, H-Q. Yu and L. Yan, *RSC Adv.*, 2014, **4**, 21619-21624.
- 657 81. K. E. Mink, R. M. Qaisi and M. M. Hussain, *Energy Technol.*, 2013, **1**, 648-652.
- 658 82. D. M. Karabacak, S. H. Brongersma and M. Crego-Calama, *Lab Chip.*, 2010, **10**, 1976-1982.
- 659 83. Z. He, J. Liu, Y. Qiao, C. M. Li and T. T. Yang Tan, *Nano Lett.*, 2012, **12**, 4738-4741.
- 660 84. J. Liu, Y. Qiao, C. X. Guo, S. Lim, H. Song and C. M. Li, *Bioresourc Technol.*, 2012, **114**, 275-80.
- 661 85. Y-C. Yong, X-C. Dong, M. B. Chan-Park, H. Song and P. Chen, *ACS Nano*, 2012, **6**, 2394-2400.
- 662 86. B. Lai, X. Tang, H. Li, Z. Du, X. Liu and Q. Zhang, *Biosens. Bioelectron.*, 2011, **28**, 373-377,
663 [doi:10.1016/j.bios.2011.07.050](https://doi.org/10.1016/j.bios.2011.07.050)
- 664 87. C. Zhao, Y. Wang, F. Shi, J. Zhang and J-J. Zhu, *Chem. Commun.*, 2013, **49**, 6668-6670.
- 665 88. Y-X. Huang, X-W. Liu, J-F. Xie, G-P. Sheng, G-Y. Wang, Y-Y. Zhang, A-W. Xu and H-Q. Yu,
666 *Chem. Commun.*, 2011, **47**, 5795-5797.
- 667 89. H. Wang, G. Wang, Y. Ling, F. Qian, Y. Song, X. Lu, S. Chen, Y. Tong and Y. Li, *Nanoscale* 2013, **5**,
668 10283-10290.
- 669 90. Y-C. Yong, Y-Y. Yu, X. Zhang and H. Song, *Angew. Chem. Int. Ed.* 2014, **53**, 4480-4483.
- 670 91. Y. Yuan, S. Zhou, B. Zhao, L. Zhuang and Y. Wang, *Bioresourc. Technol.*, 2012, **116**, 453-458.
- 671 92. C-E. Zhao, J. Wu, Y. Ding, V. B. Wang, Y. Zhang, S. Kjelleberg, J. S. Chye Loo, B. Cao and Q.
672 Zhang, *ChemElectroChem.*, 2015, **2**, 654-658.
- 673 93. Y. Fan, S. Xu, R. Schaller, J. Jiao, F. Chaplen and H. Liu, *Biosens. Bioelectron.*, 2011, **26**, 1908-12.
- 674 94. F. A. Alatraktchi, Y. Zhang and I. Angelidaki, *Appl. Energy.*, 2014, **116**, 216-222.

- 675 95. F. A. Alatraktchi, Y. Zhang, J. S. Noori and I. Angelidaki, *Bioresour. Technol.*, 2012, **123**, 177-83.
- 676 96. B. Maestro, J. M. Ortiz, G. Schrott, J. P. Busalmen, V. Climent and J. M. Feliu, *Bioelectrochem.*, 2014,
677 **98**, 11-19.
- 678 97. X. Wu, F. Zhao, L. Rahunen, J. R. Varcoe, C. Avignone-Rossa, A. E. Thumser and R. C. T. Slade,
679 *Angew. Chem. Int. Ed.* 2011, **50**, 427-430.
- 680 98. R. Wu, L. Cui, L. Chen, C. Wang, C. Cao, G. Sheng, H. Yu and F. Zhao, *Sci. Rep.*, 2013, **3**, 3307.
- 681 99. F. Liu, A-E. Rotaru, P. M. Shrestha, N. S. Malvankar, K. P. Nevin and D. R. Lovley, *Environ.*
682 *Microbiol.*, 2015, **17**, 648-655.
- 683 100. Y. Xio, F. Patolsky, E. Katz, J. F. Hainfeld and I. Willner, *Science* 2003, **299**, 1877-81.
- 684 101. T. M. Flynn, E. J. O'Loughlin, B. Mishra, T. J. DiChristina and K. M. Kemner, *Science* 2014, **344**,
685 1039-1042.
- 686 102. R. Nakamura, A. Okamoto, N. Tajima, G. J. Newton, F. Kai, T. Takashima and K. Hashimoto K,
687 *ChemBioChem.*, 2010, **11**, 643-645.
- 688 103. X. Jiang, J. Hu, A. M. Lieber, C. S. Jackan, J. C. Biffinger, L. A. Fitzgerald, B. R. Ringeisen and C.
689 M. Lieber, *Nano Lett.*, 2014, **14**, 6737-6742.
- 690 104. S. Kato, R. Nakamura, F. Kai, K. Watanabe and K. Hashimoto, *Environ. Microbiol.*, 2010, **12**, 3114-
691 3123.
- 692 105. S. Kato, K. Hashimoto and K. Watanabe, *Environ. Microbiol.*, 2012, **14**, 1646-1654.
- 693 106. S. Kato, K. Hashimoto and K. Watanabe, *Proc. Natl. Acad. Sci. USA.*, 2012, **109**, 10042-10046.
- 694 107. X. Peng, H. Yu, L. Ai, N. Li and X. Wang, *Bioresour. Technol.*, 2013, **144**, 689-692.
- 695 108. L. Wang, L. Su, H. Chen, T. Yin, Z. Lin, X. Lin, C. Yuan and D. Fu, *Mat. Lett.*, 2015, **141**, 311-314,
696 [doi:10.1016/j.matlet.2014.11.121](https://doi.org/10.1016/j.matlet.2014.11.121).
- 697 109. J. Tang, Y. Yuan, T. Liu and S. Zhou S, *J. Power Sources.*, 2015, **274**, 170-176,
698 [doi:10.1016/j.jpowsour.2014.10.035](https://doi.org/10.1016/j.jpowsour.2014.10.035).
- 699 110. Z. Wen, S. Ci, S. Mao, S. Cui, G. Lu, K. Yu, S. Luo, Z. He and J. Chen, *J. Power Sources.*, 2013,
700 **234**, 100-106.
- 701 111. Y. Qiao, S-J. Bao, C. M. Li, X-Q. Cui, Z-S. Lu and J. Guo, *ACS Nano*, 2008, **2**, 113-119,
702 DOI: 10.1021/nn700102s.
- 703 112. T. Yin, Z. Lin, L. Su, C. Yuan and D. Fu, *ACS Appl. Mater. Interfaces* 2015, **7** (1), 400-408,
704 DOI: 10.1021/am506360x

113. Y. Qiao, X-S. Wu and C. M. Li, *J. Power Sources.*, 2014, **266**, 226-231, [doi:10.1016/j.jpowsour.2014.05.015](https://doi.org/10.1016/j.jpowsour.2014.05.015).
114. J. N. Roy, S. Babanova, K. E. Garcia and J. Cornejo, L. K. Ista and P. Atanassov, *Electrochimica Acta.*, 2014, **126**, 3-10, [doi:10.1016/j.electacta.2013.07.075](https://doi.org/10.1016/j.electacta.2013.07.075)
115. S. T. Read, P. Dutta, P. L. Bond, J. Keller and K. Rabaey, *BMC Microbiol.*, 2010, **10**, 98.
116. F. Zhang, S-J. Yuan, W-W. Li, J-J. Chen, C-C. Ko and H-Q. Yu, *Electrochimica Acta.*, 2015, **152**, 1-5.
117. X. Jiang, J. Hu, L. A. Fitzgerald, J. C. Biffinger, P. Xie, B. R. Ringeisen and C. M. Lieber, *Proc. Natl. Acad. Sci. USA.*, 2010, **107**, 16806-16810.
118. C-E. Zhao, J. Wu, S. Kjelleberg, J. S. Chey Loo and Q. Zhang, *Small*, 2015, **11**, 3440-3443.
119. C. Ding, H. Liu, M. Lv, T. Zhao, Y. Zhu and L. Jiang, *Nanoscale*, 2014, **6**, 7866-7871.
120. C. Feng, L. Ma, F. Li, H. Mai, X. Lang and S. Fan, *Biosens. Bioelectron.*, 2010, **25**, 1516-1520, [doi:10.1016/j.bios.2009.10.009](https://doi.org/10.1016/j.bios.2009.10.009)
121. M. Adachi, T. Shimomura, M. Komatsu, H. Yakuwa and A. Miya, *Chem. Commun.*, 2008, 2055-2057.
122. O. Schaetzle, F. Barrie`re and K. Baronian, *Energy Environ. Sci.*, 2008, **1**, 607-620.
123. C. Ding, H. Liu, Y. Zhu, M. Wan and L. Jiang, *Energy Environ. Sci.*, 2012, **5**, 8517-8522.
124. Y. Zhao, K. Watanabe, R. Nakamura, S. Mori, H. Liu, K. Ishii and K. Hashimoto, *Chem. Eur. J.*, 2010, **16**, 4982-4985.
125. R. Nakamura, F. Kai, A. Okamoto, G. J. Newton and K. Hashimoto, *Angew. Chem. Int. Ed.*, 2009, **48**, 508-511.
126. R. Nakamura, F. Kai, A. Okamoto and K. Hashimoto, *J. Mater. Chem. A*, 2013, **1**, 5148-5157.
127. F. Qian, H. Wang, Y. Ling, G. Wang, M. P. Thelen and Y. Li, *Nano Lett* 2014, **14**, 3688-3693.
128. S. Bajracharya, A. ter. Heijne, X. Dominguez Benetton, K. Vanbroekhoven, Cees. J.N. Buisman, David P.B.T.B. Strik and D. Pant, *Bioresource Technol.* 2015, **195**, 14-24.
129. T. Zhang, H. Nie, T. S. Bain, H. Lu, M. Cui, O-L. Snoeyenbos-West, A. E. Franks, K. P. Nevin, T. P. Russell and D. R. Lovley, *Energy Environ. Sci.*, 2013, **6**, 217-224.
130. H. Nie, T. Zhang, M. Cui, H. Lu, D. R. Lovley and T. P. Russell, *Phys. Chem. Chem. Phys.*, 2013, **15**, 14290-14294.

131. T. J. Beveridge, *J. Bacteriol.*, 1999, **181**, 4725-4733.

132. M. Sa'ra and U. B. Sleytr, *J. Bacteriol.*, 2000, **182**, 859-868.

133. L. Jourdin, S. Freguia, B. C. Donose, J. Chen, G. G. Wallace, J. Keller and V. Flexer, *J. Mater. Chem. A* 2014, **2**, 13093-13102.

134. A. Okamoto, K. Hashimoto and K. H. Nealson, *Angew. Chem.*, 2014, **126**, 11168-11171, DOI: 10.1002/ange.201407004

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Table 1 shows performance of bioelectrochemical system (BES) with different nanostructured bioanode materials.

Figure Legends

Figure 1 A cartoon representation of microbial electrosynthesis of acetate from CO₂.

Figure 2 Scanning electron microscopy (SEM) and fluorescence micrographs of *S. ovata* on nanowire arrays. (a,b) SEM and (c,d) fluorescence images were taken of *S. ovata* cells grown on Si nanowire arrays of 4 μm periodicity under (a,c) normal ionic concentrations ($\sim 100\text{ mM}$) and 100 kPa H₂:CO₂, showing random orientations with respect to the nanowire axis, and (b,d) with +200 mM NaCl and 500 kPa H₂:CO₂, showing alignment with the nanowire. Both (a,b) side view SEM and (d,e) top down in situ fluorescence microscopy show alignment, with (c) unaligned bacteria appearing as rods in fluorescence micrographs, and (d) aligned bacteria appearing as circles. Scale bars are (a,b) 2 and (c,d) 5 μm (Reproduced from Ref. 52 with the permission from American Chemical Society).⁵²

Figure 3 Graphical illustration showing a higher current generation from a *S. PV-4* biofilm developed on a (a) hydrophilic electrode surface (more electrons moving towards the resistor (light bulb)) (a) than (b) a hydrophobic surface (less electrons are shown moving) (color image only) (Reproduced from Ref. 56 with permission from John Wiley and Sons).⁵⁶

Figure 4 Carbon nanotube-textile (CNT-textile) composite. (A) Schematic of the electrode configuration and electron-transfer mechanisms for the CNT-textile anode (right), compared with the widely used carbon cloth anode (left). (B) Scanning electron microscope (SEM) image of the CNT-textile showing the open macroscale porous structure. The inset is a 15 cm $\times$ 15 cm piece of CNT-textile with a sheet resistance of 4 Ω square⁻¹. (C) SEM image of a textile fiber conformally coated with CNTs. (D, E) Cross section of a

773 CNT-textile fiber. The diameter of the CNT-textile fiber is about 20 μm , and the thickness of the CNT
774 coating is about 200 nm (Reproduced from Ref. 62 with permission from American Chemical Society).⁶²

775
776 **Figure 5** Schematic illustration of the interactions between the CP/IL-GNS anode and *S. oneidensis* cells
777 (Reproduced from Ref. 87 with permission from Royal Society of Chemistry).⁸⁷

778
779 **Figure 6** Fabrication of hybrid electroactive biofilm with built-in bacteria, and electron transfer process
780 from hybrid biofilm to electrode (Reproduced from Ref. 92 with permission from John Wiley and Sons).⁹²

781
782 **Figure 7** Transmission electron micrograph of (A) magnetite-free and (B) magnetite-amended *G.*
783 *metallireducens*/*G. sulfurreducens* co-cultures. The white arrows point to magnetite associated with pili
784 (Reproduced from Ref. 99 with permission from John Wiley and Sons).⁹⁹

785
786 **Figure 8** (a) SEM image of the as-prepared PPy-NA film showing the homogeneous distribution of PPy
787 nanowires at a large scale. Inset: high magnification SEM image of the PPy nanowires where the conical
788 structure is clearly seen. (b) A side-view SEM image of the as-prepared PPy-NA, suggesting its highly
789 ordered and aligned nature. (c) Bacterial cells on a PPy-NA electrode after 60 hours of electrochemical
790 culture at 0.2 V, exhibiting a typical porous structured bacterial network on the whole PPy-NA electrode.
791 The microbes were labeled in red. (d) Enlarged SEM image of microbes on the PPy-NA electrode, where
792 several microbes are bundled tightly together and are connected with neighboring microbe bundles, forming
793 a conduit to facilitate the electron transfer (Reproduced from Ref. 119 with permission from Royal Society
794 of Chemistry).¹¹⁹

795
796 **Figure 9** (a) Photocurrent generation from the bacteria/colloid network. (b) Energy levels of the outer-
797 membrane c-Cyts in the presence of $\alpha\text{-Fe}_2\text{O}_3$ colloids. E_{CB} is the energy level of the conduction band edge

of α -Fe₂O₃. (c) Proposed model for the bacterial long-distance ET process mediated by semiconductive α -Fe₂O₃. (d) Schematic and (e) corresponding energy diagram of the hybrid interface of the hematite photoanode in a solar microbial photoelectrochemical system. The CB, VB, and E_F represent the conduction band, valence band, and Fermi level, respectively. Solid and empty circles represent electrons and holes, respectively (Reproduced from Ref. 125 and 127 with permission from Wiley and Sons and Royal Society of Chemistry).^{125,127}

Figure 10 SEM micrographs of *Sporomusa* cells adhered (A–C) on nickel nanowire-coated graphite electrode and (D) on nickel nanoparticle-coated graphite (Reproduced from Ref. 130 with permission from Royal Society of Chemistry).¹³⁰

Type of BES	Type of electrode materials	Inoculum	Current density (mA/cm ²)	Reference
H-shaped MFC	CNT-textile anode, carbon cloth (0.5 mg cm ⁻² 10wt. % Pt on XC-72) cathode	Domestic wastewater	0.72	62
H-shaped MFC	CNT-sponge anode, CNT-sponge-Pt cathode	Domestic wastewater	2.13	63
H-shaped MFC	Crinkled CNT anode, carbon cloth (0.5 mg cm ⁻² 10wt. % Pt on XC-72) cathode	Domestic wastewater	0.24	64
Three-electrode system	NanoWeb-RVC anode, Ag/AgCl reference electrode, Ti mesh counter electrode	Anodic effluent from an existing BES	6.8	65
Two chamber MFC separated by Nafion membrane	Fe ₃ O ₄ /CNT anode, 20wt % Pt coated carbon cloth cathode	<i>E. coli</i> K12	0.19	66
Micro-sized MFC (1.25 µL)	MWCNTs anode, carbon cloth cathode	Mixed culture	0.0197	67
Single chamber MFC	CNT-hydrogel anode, Pt-coated (0.05 mg cm ⁻²) carbon cloth cathode (air cathode)	Anaerobic sludge	0.032	72
H-type MFC	CNT/MnO ₂ anode, Pt coated carbon paper cathode	Anaerobic sludge	0.0262	73
Micro-sized MFC	Graphene sheet on nickel anode, Pt coated carbon cloth cathode (air cathode)	Domestic wastewater	0.0034	81
Two chamber MFC separated by Nafion membrane	Chitosan/vacuum stripped graphene anode, plain carbon cloth cathode	<i>Pseudomonas aeruginosa</i>	0.255	83
Three electrode system	Ionic liquid functionalized graphene nano-sheets anode, Ag/AgCl reference electrode, Pt mesh counter electrode	<i>Shewanella oneidensis</i> MR-1	0.28	87
Three electrode system	Graphene oxide nanoribbons anode, Ag/AgCl reference electrode, Pt wire counter electrode	<i>Shewanella oneidensis</i> MR-1	0.19	88
H-type MFC	Graphene-CNT hybrid anode, carbon felt cathode	<i>Shewanella oneidensis</i> MR-1	0.123	92

Type of BES	Type of electrode materials	Inoculum	Current density (mA/cm ²)	Reference
Single chamber MEC	Au nanoparticle-coated graphite disc anode, carbon cloth (0.5 mg cm ⁻² 10wt. % Pt on XC-72) cathode	<i>Shewanella oneidensis</i> MR-1	0.0744	93
Single chamber MEC	Pd coated graphite disc anode, carbon cloth (0.5 mg cm ⁻² 10wt. % Pt on XC-72) cathode	<i>Shewanella oneidensis</i> MR-1	0.0088	93
H-type MFC	Au nanoparticle-coated carbon paper anode, ferricyanide solution catholyte	Domestic wastewater	0.118	94
Three electrode system	Goethite nanowhiskers modified carbon paper anode, Ag/AgCl reference electrode, Pt wire counter electrode	<i>Shewanella loihica</i> PV-4	0.048	108
Single chamber MFC	Carbon coated titanium dioxide core-shell nanoparticle anode, carbon cloth (0.5 mg cm ⁻² 10wt. % Pt on XC-72)air- cathode	Anaerobic sludge	1.2	109
H-type MFC	NiO nanoflakes/carbon cloth anode, ferricyanide catholyte	<i>Shewanella putrefaciens</i>	0.25	113
H-type MFC	Polypyrrole membrane anode, ferricyanide catholyte	<i>Shewanella oneidensis</i> MR-1	0.21	118
Three electrode system	Polypyrrole nano-arrays anode, Ag/AgCl reference electrode, Pt wire counter electrode	<i>Shewanella loihica</i> PV-4	0.32	119
Three electrode system	Nano-colloidal Fe ₂ O ₃ coated ITO photo bioanode, Ag/AgCl reference electrode, Pt wire counter electrode	<i>Shewanella loihica</i> PV-4	0.033	126
Three electrode system	Fe ₂ O ₃ coated ITO photo bioanode, Ag/AgCl reference electrode, Pt wire counter electrode	<i>Shewanella oneidensis</i> MR-1	0.25	127

Table 1

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Biography

Dr. Shafeer Kalathil received his master degree from the Dept. of Chemical Oceanography, Cochin University of Science and Technology, India in 2009. He has a Ph.D. Degree (2013) in Chemical Engineering & Technology from Yeungnam University, South Korea and later he moved to The University of Tokyo as a Japan Society for Promotion of Science (JSPS)-Postdoctoral Fellow. His research interest encompasses both the fundamental electrochemistry involved in bacterial extracellular electron transport and bionanotechnology. He has published 19 international peer-reviewed papers (h-index: 15) and has 8 patents to his credit. Currently he is working as a Postdoctoral Fellow at KAUST.

989

990 **Dr. Deepak Pant** is a Senior Scientist at the Flemish Institute for Technological Research
991 (VITO), Belgium. His research focuses on bioenergy, specifically, the design and optimization of
992 bio-electrochemical systems for energy recovery from wastewater and microbial electrosynthesis
993 for production of value-added chemicals through electrochemically driven bio-processes. He has
994 1 book (published by Springer), 1 Patent, 55 peer-reviewed publications with >2750 citations (h-
995 index 26) and 16 book chapters to his credit. He is a member of scientific communities including
996 ISMET, ISE, BES, BRSI, IFIBiop and AMI. He serves as Editorial board member for the
997 Journals: 'Bioresource Technology', 'Electronic Journal of Biotechnology', 'Biofuel Research
998 Journal', 'Heliyon' and 'Frontiers in Environmental Science'.

999

TOC

Advanced nanostructured electrode materials largely improve the bacterial bidirectional extracellular electron transfer in bioelectrochemical systems.