# Microbiology Microbial Nanowires: An Electrifying Tale --Manuscript Draft--

Manuscript Number:	MIC-D-16-00123R3
Full Title:	Microbial Nanowires: An Electrifying Tale
Short Title:	Microbial Nanowires
Article Type:	Review
Section/Category:	Biotechnology
Corresponding Author:	Mandira Kochar, PhD The Energy and Resources Institute New Delhi, INDIA
First Author:	Sandeep K Sure
Order of Authors:	Sandeep K Sure
	Leigh M Ackland, PhD
	Angel A Torriero, PhD
	Alok Adholeya, PhD
	Mandira Kochar, PhD
Abstract:	Electromicrobiology has gained momentum in the last ten years with advances in microbial fuel cells and the discovery of microbial nanowires (MNWs). The list of MNWs producing microorganisms is growing and providing intriguing insights into the presence of such microorganisms in diverse environments and the potential roles MNWs can perform. This review discusses the MNWs produced by different microorganisms, including their structure, composition and role in electron transfer through MNWs. Two hypotheses, metallic-like conductivity and an electron hopping model, have been proposed for electron transfer and we present a current understanding of both these hypotheses. MNWs are not only poised to change the way we see microorganisms but may also impact the fields of bioenergy, biogeochemistry and bioremediation, hence their potential applications in these fields are highlighted here.

±

1	Review Paper
2	Title: Microbial Nanowires: An Electrifying Tale
3	Running Title: Microbial Nanowires
4	
5	Authors
6	Sandeep Sure, <sup>1</sup> M. Leigh Ackland, <sup>2</sup> Angel A.J. Torriero, <sup>2</sup> Alok Adholeya <sup>1</sup> and Mandira
7	Kochar <sup>1</sup>
8	
9	<sup>1</sup> TERI-Deakin Nanobiotechnology Centre, TERI Gram, The Energy and Resources Institute,
10	Gual Pahari, Gurgaon Faridabad Road, Gurgaon, Haryana 122 001, India
11	<sup>2</sup> Centre for Cellular and Molecular Biology, Deakin University, 221 Burwood Highway,
12	Burwood, Melbourne, Victoria 3125, Australia.
13	
14	Correspondence
15	Mandira Kochar
16	Email: mandira.malhotra@gmail.com or mandira.kochar@teri.res.in
17	
18	Word Count - 5466
19	
20	
21	
22	
23	
24	
25	

# 26 Abstract

Electromicrobiology has gained momentum in the last ten years with advances in microbial fuel cells and the discovery of microbial nanowires (MNWs). The list of MNWs producing microorganisms is growing and providing intriguing insights into the presence of such microorganisms in diverse environments and the potential roles MNWs can perform. This review discusses the MNWs produced by different microorganisms, including their structure, composition and role in electron transfer through MNWs. Two hypotheses, metallic-like conductivity and an electron hopping model, have been proposed for electron transfer and we present a current understanding of both these hypotheses. MNWs are not only poised to change the way we see microorganisms but may also impact the fields of bioenergy, biogeochemistry and bioremediation, hence their potential applications in these fields are highlighted here. 

# 49 Introduction

Microorganisms are known to produce sophisticated nanomachines like bacterial flagellar nanomotor that are made up of several proteins (Chalmeau *et al.*, 2009). Scientists are using peptides and proteins as building blocks for the construction of nanodevices including sensors and drug delivery vehicles (Petrov & Audette, 2012; Rosenman *et al.*, 2011; Scanlon & Aggeli, 2008). Several peptide nanotubes have been built and can be used as a casting module for synthesis of metal nanowires (Reches & Gazit, 2003; Scanlon & Aggeli, 2008).

One desired property of peptide/protein nanotubes is electrical conductivity. Such electrically 56 conductive nanotubes or nanowires are an essential requirement in the field of 57 nanoelectronics. Most proteins made of natural amino acids are insulating (Scanlon & Aggeli, 58 2008) and thus the efforts were made to build electrically conductive protein nanotubes that 59 60 in turn can act as nanowires (Creasey et al., 2015; Scanlon & Aggeli, 2008). However, Reguera et al. discovered extracellular electrically conductive protein nanofilaments in 61 62 Geobacter sulfurreducens and termed them microbial nanowires (MNWs) ((Reguera et al., 2005). This discovery opened many new avenues of research in nanotechnology and 63 microbiology. This review deals with MNWs produced by diverse microorganisms and 64 65 discusses several important aspects of MNWs including their types, role, mechanism of electron transfer and potential applications. 66

67

# **Discovery of microbial nanowires in different microbes**

As a part of anaerobic respiration, some bacteria are capable of transferring electrons to extracellular electron acceptors in a process termed extracellular respiration (Lovley, 2008). Extracellular respiration is commonly found in metal reducing bacteria like *G. sulfurreducen* and *Shewanella oneidensis*. There are three known strategies by which extracellular respiration is carried out by bacteria; first, bacteria transfer electrons directly to metals 74 through proteins present on the cell surface (Fig. 1a); second, metal chelators (citrate and nitrilotriacetic acid) deliver metals to intracellular metal oxidoreductases (Fig.1b) or finally 75 small molecules (humic substances) act as a shuttle to transfer electrons between the cell and 76 77 substrate (Fig.1b) (Gralnick & Newman, 2007; Richardson, 2000). An addition to this list is extracellular MNWs which act as a conduit of electrons between cell and distant substrates 78 (Fig. 1c) (Reguera et al., 2005). The conductivity of proteins has been studied earlier (Xu et 79 80 al., 2005) but their conductive behaviour and direct role in long range (upto µm distances) 81 extracellular electron transfer had not been reported. Similar studies done on extracellular pili-like structures (PLS) of S. oneidensis and Pseudomonas aeruginosa indicated PLS to be 82 non-conductive (Reguera et al., 2005). However, previously reported non-conductive PLS of 83 84 S. oneidensis were conclusively proved to be electrically conductive in the following year by a different research group (Gorby et al., 2006). There may be three reasons for the failure to 85 detect MNWs in S. oneidensis in the first attempt, the first being cultivation conditions. Luria 86 Bertani, a complex medium was used in the first study (Reguera et al., 2005), compared to 87 stressful culture conditions in the form of electron acceptor limiting conditions in the second 88 89 (Gorby et al., 2006), with the latter likely inducing formation of MNWs in S. oneidensis. The 90 second reason could be the delicate nature of bacterial pili (5-8nm in diameter) (Gorby et al., 2006; Pelicic, 2008; Simpson et al., 1976), while lastly, there may be a tendency of some 91 92 microorganisms to produce multiple pili like structures e.g. type II pseudopilus and thus the PLS probed by Reguera et al. may be different (Durand et al., 2003; Gorby et al., 2006). 93 MNWs have also been observed in the iron (Fe) reducing bacterium, Rhodopseudomonas 94 palustris strain RP2 (Venkidusamy et al., 2015), and in sulphate (SO<sub>4</sub><sup>2-</sup>) reducing bacterium 95 Desulfovibrio desulfuricans (Eaktasang et al., 2016). MNWs were not only observed in Fe 96

97 and  $SO_4^{2-}$  reducing bacteria as discussed above, but they were also identified in the Fe 98 oxidizing bacterium, *Acidithiobacillus ferrooxidans* (Li & Li, 2014; Valdes *et al.*, 2008). 99 With this discovery, it was hypothesized that, MNWs may connect cells to extracellular100 electron donors and acceptors.

Considering the role of MNWs in electron transfer, it was hypothesized that such conductive 101 102 structures might be present in pathogenic microbial biofilms residing in anaerobic zones of oral cavities (Rabaey, 2010). MNWs were observed for the first time in microbial biofilms 103 which causes bisphosphonate-related osteonecrosis of the jaw (BRONJ) (Wanger et al., 104 2013). These MNWs were found to interconnect different cells and appeared as PLS. This 105 biofilm was found to be colonized by around fifteen discernible bacterial morphotypes, 106 mostly anaerobic and facultatively anaerobic, constituting genera of Staphylococcus, 107 Bacillus, Fusobacterium, Actinomyces, Streptococcus, Selenomonas and Treponema but the 108 109 specific MNWs producing microorganisms amongst these could not be identified.

110 Apart from metal reducing and pathogenic microorganisms, MNWs have been observed in photosynthetic bacteria. Initial reports showed that Synechocystis, a unicellular 111 cyanobacterium, can produce MNWs in electron acceptor (CO<sub>2</sub>) limiting and high light 112 conditions (Gorby et al., 2006). Taking clues from this study, our group explored the 113 possibility of MNWs formation in other cyanobacteria. Some cyanobacteria become 114 electrogenic (transfer electrons extracellularly) under high light intensity. Synechocystis as 115 well as Nostoc sp. have been shown to exhibit such type of electrogenic behavior (Pisciotta et 116 al., 2010). Further, Microcystis aeruginosa also encounter CO<sub>2</sub> limitation and get exposed to 117 118 high light intensity when they form blooms. Thus, M. aeruginosa and N. punctiforme might be producing MNWs which has been confirmed by conductive atomic force microscopy 119 (AFM) analysis (Sure et al., 2015, Sure et al., 2016b). The discovery of MNWs in such 120 121 diverse microorganisms ranging from anaerobic, metal reducing bacteria to photosynthetic, aerobic cyanobacteria strengthen the viewpoint that they may be pervasive in the 122

environment. The MNWs producing microorganisms discovered to date are shown in Fig. 2and relevant description is given in Table 1.

Different modes of AFM including conductive AFM, scanning tunneling microscopy (STM),
electrostatic force microscopy and specially designed nanofabricated electrodes are
established techniques for identification and electrical characterization of MNWs produced
by different microorganisms and use of these multiple techniques have been advocated to
unambiguously confirm the presence of MNWs in microorganisms (Castro *et al.*, 2014;
Gorby *et al.*, 2006; Li & Li, 2014; Malvankar *et al.*, 2014; Reguera *et al.*, 2005; Sure *et al.*,
2015; Venkidusamy *et al.*, 2015; Wanger *et al.*, 2013).

132

# 133 **Types of microbial nanowires**

Diverse microorganisms have been observed to produce MNWs (Fig. 2) and their composition and structure have been found to be different from each other. According to available data, MNWs can be categorized into three types (Fig. 3);

137 **Pili** 

138 MNWs in G. sulfurreducens, A. ferroxidans and Synechocystis sp. have been found to be TFP 139 which are the most widespread type of pili present among bacteria (Li & Li, 2014; Pelicic, 2008; Reguera et al., 2005; Sure et al., 2015). Apart from common functions like adhesion 140 and biofilm formation exhibited by most other bacterial pili, TFP possess unique functional 141 characteristics which include twitching motility, uptake of DNA in transformation and phage 142 attachment (Pelicic, 2008; Proft & Baker, 2009). In addition to these functions, their electron 143 carrying capacity further increases their significance as multifunctional extracellular 144 structures. MNWs in G. sulfurreducens are polymers of PilA subunit while same is PilA1 for 145 Synechocystis (Fig. 3a-b) (Reguera et al., 2005; Sure et al., 2015). Though MNWs from both 146 microorganisms are TFP, the molecular weight (MW) of their subunits (~10kDa for G. 147

148 sulfurreducens while ~22kDa for Synechocystis) and dimensions (width/length - 3-5nm/10-20µm and 4.5-7nm/2-10µm for G. sulfurreducens and Synechocystis, respectively) differ 149 from each other (Lovley et al., 2009; Lovley, 2011; Sure et al., 2015). In G. sulfurreducens, 150 cytochromes are found to be associated with MNWs and its role in electron transfer through 151 MNWs is disputed (Malvankar et al., 2011a; Malvankar et al., 2011b; Strycharz-Glaven et 152 al., 2011; Strycharz-Glaven & Tender, 2012). It needs to be explored whether Synechocystis 153 MNWs are embedded with cytochromes and the potential role of the latter in electron 154 transfer. MNWs in A. ferroxidans may be made up of PilV and PilW proteins (Li & Li, 155 156 2014). The MNWs from different microorganisms will not always look the same and vary in width and length due to two reasons; 1) TFP have bundle forming ability as a result of which 157 their observed width may vary; 2) Concerning the length, it may depend on age of culture and 158 159 sample preparation methods which may lead to breakage of long, delicate pili.

160

## 161 Extended periplasmic and outer membranes

S. oneidensis possess three different types of extracellular proteinaceous appendages;1) Msh 162 pili, 2) TFP and 3) flagella (Bouhenni et al., 2010), but it was not clear which one of these 163 acts as MNWs. Msh pili has been shown necessary for extracellular electron transfer 164 (Fitzgerald et al., 2012), while TFP and flagella have been shown to be dispensable 165 166 (Bouhenni et al., 2010). However, MNWs in S. oneidensis are made up of outer membrane 167 vesicle chains which subsequently elongates and become MNWs (Fig. 3c) (Pirbadian et al., 2014). Unlike pili and flagella, which are mostly homopolymers of single subunit, MNWs in 168 S. oneidensis are a concoction of different cytochromes and periplasmic as well as outer 169 170 membrane components. The formation of outer membrane vesicle chains and tubes has been reported in Myxococcus xanthus (Remis et al., 2014; Wei et al., 2014). Also, the ability of 171 peptide nanotubes to convert into vesicles and vice versa is well known (Scanlon & Aggeli, 172

173 2008). Thus, it would not be surprising to know that MNWs in *S. oneidensis* are formed in174 similar manners.

As discussed earlier, S. oneidensisis known to produce pili/flagella and it is puzzling why it 175 employs a completely different strategy to produce MNWs. The role of MNWs in S. 176 oneidensis physiology and metabolism is still largely unknown and deciphering it may help 177 us understand the reason behind its completely different make-up from other MNWs. 178 179 However, so far it was not ruled out that other extracellular structures (pili and flagella) in S. oneidensis cannot conduct electrons. Also in their study, electrical conductivity 180 181 measurements of extended membrane extensions were not done (Pirbadian et al., 2014). All extracellular structures produced by S. oneidensis should be isolated and studied 182 independently for their conductive behavior. Only then it would be appropriate to claim that 183 184 MNWs produced by S. oneidensisare outer and periplasmic membrane extensions only and not pili or flagella. 185

186

#### 187 Unknown type – Microbial nanowires whose identity need to be confirmed

Pili-like conductive structures have been identified in A. hydrophila, R. palustris, D. 188 desulfuricans and N. punctiforme but their identity has not been confirmed so far (Castro et 189 al., 2014; Eaktasang et al., 2016; Sure et al., 2016b; Venkidusamy et al., 2015). Two distinct 190 191 types of MNWs; first, short/thin MNWs of size 6-7.5nm in diameter and 0.5-2µm in length 192 and second, long/thick MNWs of size ~20-40nm in diameter and  $\geq 10 \mu m$  long were observed in N. punctiforme (Sure et al., 2016b). The identity of MNWs from multispecies biofilms 193 observed in BRONJ could also not be confirmed (Wanger et al., 2013). MNWs in M. 194 195 aeruginosa have been found to be composed of a protein similar to an unnamed protein (GenBank : CAO90693.1) whose amino acid sequence does not match with any known 196 197 protein (Sure et al., 2015). Unlike others, MNWs in M. aeruginosa are wider and may be made of two subfilaments or contain central channel inside it (Fig. 3d-f) (Sure *et al.*, 2015).
More elaborate studies are needed to further confirm the identities of above mentioned
MNWs.

201 Pelotomaculum thermopropionicum produces electrically conductive flagellum-like 202 appendages (10-20nm in diameter) in monoculture as well in coculture with 203 Methanothermobacter thermoautotropicus (Gorby et al., 2006). These flagellum-like 204 appendages may be indeed flagellas as subsequent study by other group have shown that P. 205 thermopropionicum in cocultures with M. thermoautotropicus produce flagella which are 206 involved in symbiosis (Shimoyama et al., 2009). G. sulfurreducens is also known to produce 207 flagella which were found to be non-conductive (Malvankar et al., 2014).

208

## 209 Physiological role of microbial nanowires

As discussed in the previous sections, each type of MNWs has unique structure and composition and they may have evolved as per the physiological requirements of the microorganisms. Some of the observed and hypothesized functions of MNWs are discussed below.

214 MNWs can act as a conduit between cell and extracellular electron acceptor/donors thereby mediating to and fro electron transfer. For instance, in metal reducing microorganisms like G. 215 sulfurreducens, it was observed that MNWs can help bacteria to transfer electrons to electron 216 217 acceptors (metals) available at a distance without the need of direct cell attachment or dissolved electron shuttles (Reguera et al., 2005). Also in metal oxidizing microorganisms 218 like A. ferrooxidans, MNWs may have the ability to transfer electrons to the cell surface thus 219 220 greatly helping cells to access electron donors at a distance (Li & Li, 2014). In anaerobic environments, photosynthetic microorganisms can use arsenic (As) as electron donor (Kulp et 221 222 al., 2008) and here MNWs can play an important role to bridge the gap between the cells and any available electron donor like As. Our preliminary studies have shown that *Synechocystis*MNWs can bind and immobilize As and thus may act as a conduit of electrons between cells
and As (Sure *et al.*, 2016a). Due to their ability to interact with metals, MNWs can act as a
protective cellular mechanism against toxic metals (Fig. 4) (Cologgi *et al.*, 2011).

Apart from extracellular electron acceptor/donor, MNWs can also conduit of electrons 227 between two different cells. For instance, occurrence of interspecies electron transfer in 228 229 Geobacter metallireducens and G. sulfurreducens was observed in coculture (Summers et al., 2010). Such interspecies electron transfer was also investigated in methanogenic wastewater 230 231 aggregates where it was hypothesized that microorganisms can directly transfer electrons to each other, rather than use hydrogen and formate as intermediate electron carrier (Morita et 232 al., 2011). It is hypothesized that MNWs may be involved in such type of interspecies 233 234 electron transfer. Apart from interspecies electron transfer, MNWs have also been implicated 235 in electron transfer between bacteria and archaea (Wegener et al., 2015). Further, cyanobacteria are an important constituent of the microbial mat and it is hypothesized that 236 cyanobacterial MNWs can transfer electrons to other microorganisms present in the microbial 237 mat (Gorby et al., 2006; Lea-Smith et al., 2015). Such MNWs mediated electron transfer 238 between two microorganisms can also be part of cell communication/signaling (Reguera, 239 2011). For instance, it has been observed that MNWs of G. sulfurreducens leads to the 240 241 formation of electronic networks which interconnect individual cells (Reguera, 2011). MNWs 242 in G. sulfurreducens have been found to be conductive even at low voltage which is in accordance with the electronic communication occurring between cells (Reguera et al., 2005; 243 Reguera, 2011). Furthermore, recent study has shown that MNWs production in G. 244 245 sulfurreducens is necessary for the formation of optimum electroactive and thick (more than 10µm) biofilms (Steidl et al., 2016). 246

Apart from these general roles, MNWs might be involved in specific functions. For example, in carbon limiting conditions, the component of photosynthetic apparatus- plastoquinone (PQ) gets over reduced due unavailability of carbon to sink electrons. It is hypothesized that MNWs may help cyanobacteria to release these extra electrons present on PQ so as to restrict cell damage (Gorby *et al.*, 2006; Lea-Smith *et al.*, 2015; Pisciotta *et al.*, 2010). MNWs produced in *M. aeruginosa* might be important for bloom formation while those of *N. punctiforme* may be involved in plant symbiosis (Duggan *et al.*, 2007; Sure *et al.*, 2015).

254

# 255 Mechanism of electron transfer through microbial nanowires

With the discovery of MNWs, efforts to understand the mechanism of electron flow were 256 commenced. The mechanism of electron transfer in MNWs has been extensively studied in. 257 258 G. sulfurreducens and S. oneidensis and two major mechanism of conductivity have been proposed for MNWs; 1) Metallic like conductivity model and 2) Electron hopping model. For 259 260 G. sulfurreducens MNWs, both models have been advocated by different group of scientists while for S. oneidensis MNWs, electrons transfer is believed to occur by hopping mechanism. 261 Both major proposed mechanisms of electron transfer through MNWs have been discussed 262 263 here.

264

## 265 Metallic like conductivity model

Elaborate experiments done by Malvankar *et al.* in *G. sulfurreducens* showed that its MNWs have intrinsic metallic like electrical conductivity which is comparable to carbon nanotubes and some organic conductors (Malvankar *et al.*, 2011b; Malvankar *et al.*, 2012; Malvankar *et al.*, 2014; Malvankar *et al.*, 2015). This observation was distinct from an electron hopping mechanism observed in biological systems like photosynthetic reaction centres (Feliciano, 2012). Some of the important properties which support metallic like conductivity model for *G. sulfurreducens* MNWs and biofilms are their temperature and pH dependent electrical behavior (Malvankar *et al.*, 2011b). This observation was also supported by structural studies where lower pH was shown to induce conformational changes in aromatic amino acids which thereby causes higher conductivity in the *Geobacter* pili (Malvankar *et al.*, 2015).

In synthetic organic metals, electron transfer is attributed to overlapping  $\pi$ - $\pi$  orbitals of 276 aromatic rings present in it. Proteins also contain several aromatic amino acids whose 277 278 aromatic constituents can play a role similar to that of organic metals in electron transfer. To confirm the role of these aromatic amino acids in electron transfer, a *Geobacter* strain, Aro-5 279 280 was constructed (Vargas et al., 2013). In Geobacter Aro-5, five aromatic amino acids of PilA, the pili subunit were replaced with alanine and it was observed that the resultant 281 modified pili showed considerable diminished electrical conductivity and ability to reduce Fe 282 283 (III) compared to the pili of the control strain (Vargas et al., 2013). Though this study confirmed that aromatic amino acids play an important role in conductivity of G. 284 sulfurreducens pili and its biofilm, it is still unclear how modified pili are able to show 285 residual electrical conductivity. Further, the removal of aromatic amino acids may be altering 286 the 3D structure of pili and thereby the positioning of cytochromes on pili, which can 287 ultimately decrease pili conductivity (Boesen & Nielsen, 2013). This is important considering 288 the fact that tilting of molecules and interplanar distances have the potential to affect charge 289 290 transport. The pilus filament model by Yan et al. based on Neisseria gonorrhoeae concludes 291 that aromatics are too far to be involved in electron transport (Yan et al., 2015). However, experimental data generated using techniques like synchrotron X-ray microdiffraction and 292 rocking-curve X-ray diffraction have refuted this model and strongly supported the role of 293 294 aromatic amino acids in long distance electron transfer and reinforced metallic like conductivity mechanism in Geobacter pili (Malvankar et al., 2015). Along with experimental 295 296 data, modeling studies also supported the metallic conductivity model where lowest energy

models of *Geobacter* pili were observed to have no central channel and closely packed, core chain of aromatic residues facilitated electron transport along the length of the pilus and conferred the potentially electrically conductive geometry to it (Xiao *et al.*, 2016).

The importance of intrinsic pili structure of G. sulfurreducens in electron transfer was further 300 301 studied where the pilA gene of G. sulfurreducens was replaced with the pilA gene from P. aeruginosa (Liu et al., 2014). The resultant strain was able to produce and assemble P. 302 303 aeruginosa PilA subunits into pili and interestingly these hybrid pili had the same pattern of cytochromes as that of control cells. But the conductivity of these hybrid pili was found to be 304 305 14 times lesser than normal pili with significantly diminished ability to reduce iron and current generation. From these observations, authors suggested the intrinsic structures of 306 Geobacter pilus and not associated cytochromes, are important for electron transfer through 307 308 it. However, as the pili of *P. aeruginosa* have been found non-conductive in the earlier study 309 (Reguera et al., 2005), the hybrid pili here should also show non-conductive behaviour if the conductivity is 100% related to intrinsic structure of pili. Since the pili show diminished 310 conductivity, the basis needs to be worked out unambiguously to reach any conclusion. 311

The cytochromes located on Geobacter pili were hypothesized to be terminal reductases 312 which transfer electrons from pili to electron acceptors like Fe and not the one playing a role 313 in electron transport (Malvankar et al., 2014). Malvankar et al. stressed that electron hopping 314 does not meet the necessary biochemical requirement for electron transfer through pili. They 315 316 reported that OmcS cytochromes assumed to be involved in electron transfer along the length of pili are too far from each other to carry out electron transfer as per the electron hopping 317 model (Malvankar et al., 2012). They also reported that denaturing cytochromes in G. 318 319 sulfurreducens pili networks and biofilms does not affect the electrical conductivity, thus ruling out any role of cytochromes in electron transfer through pili and biofilms (Malvankar 320 et al., 2011b; Malvankar et al., 2012). Further, STM analysis of G. sulfurreducens MNWs 321

supported these findings where electron transfer is attributed to the intrinsic pili structure, and
not to the cytochromes (Veazey *et al.*, 2011).

From all above observed results (Leang *et al.*, 2010; Malvankar *et al.*, 2011b; Malvankar *et al.*, 2012), scientists refuted the electron hopping model for electron transfer in *G. sulfurreducens* MNWs and proposed metallic like conductivity model for same (Malvankar *et al.*, 2011a; Malvankar & Lovley, 2012).

328

#### 329 Electron hopping model

330 One view is emerging that electron transfer occurs by multistep hopping in *Geobacter* and 331 *Shewanella* MNWs and not by metallic conduction as proposed earlier. In *Geobacter* MNWs, 332 aromatic amino acids are supposed to be involved in such electron transfer whereas for 333 *Shewanella* MNWs, cytochromes are believed to play this role.

As discussed in earlier section, it has been proved unambiguously that aromatic amino acids 334 are indispensable for electron transfer through Geobacter MNWs. However, it is debatable 335 whether these aromatic amino acids transfer electrons by metallic conduction or by multistep 336 hopping. Multiple modeling studies strongly support the hypothesis that electron transfer 337 through Geobacter MNWs occurs by multistep hopping among aromatic amino acids 338 (Feliciano et al., 2015; Lebedev et al., 2015; Yan et al., 2015). This hypothesis was further 339 strengthened by a recent report where experimental evidence has been provided to support 340 341 multistep hopping in Geobacter MNWs where cryogenic STM of Geobacter pili showed thermal activation of the differential transversal conductance at low voltages which is in 342 accordance with electron hopping mechanism (Lampa-Pastirk et al., 2016). It has also been 343 shown that metals or redox organic cofactor free Geobacter pili show carrier mobility of 344  $3.2 \times 10^{-2}$  cm<sup>2</sup>/Vs which is too low for metallic conductivity regime where carrier mobilities of 345 more than  $1 \text{ cm}^2/\text{Vs}$  are required (Lampa-Pastirk *et al.*, 2016). 346

347 Quantitative measurement of electron transport across S. oneidensis MNWs showed that a complex electronic structure formed by its molecular constituents mediates electron transport 348 in it (El-Naggar et al., 2008). It has been proved that S. oneidensis MR-1 requires 349 350 cytochromes, MtrC and OmcA for production of MNWs (El-Naggar et al., 2010; Gorby et al., 2006). Both of these cytochromes are located on the outer membrane of the cell. 351 Scientists hypothesized that long range electron transfer through S. oneidensis MNWs takes 352 place by electron hopping where intricate cytochrome network may be involved (Strycharz-353 Glaven et al., 2011; Tender, 2011) and multiple experimental and modelling studies have 354 355 confirmed this hypothesis (El-Naggar et al., 2010; Gorby et al., 2006; Leung et al., 2013; Pirbadian & El-Naggar, 2012; Polizzi et al., 2012). A recent study has reported that MNWs 356 in S. oneidensis are composed of extended periplasmic and outer membranes embedded with 357 358 cytochromes (Fig. 3c) which further supports the electron hopping model (Pirbadian et al., 2014). However, in S. oneidensis MNWs, it is yet to be proved conclusively that cytochromes 359 360 are closely spaced enough (1-2nm) to carry out charge transport over µm distances. Interested readers are referred to specific reviews on this topic (Skourtis, 2013; Waleed Shinwari et al., 361 362 2010).

363

# **Potential applications of microbial nanowires**

365 One reason behind the widespread attention gained by MNWs is the potential applications of 366 this in several fields. Below we discuss some fields where MNWs can play an important role;

367 Bioenergy

For production of highly efficient microbial fuel cells, electron transfer should occur through biofilms so that even microorganisms which are away from anode can transfer electrons to it, thereby increasing total current output (Nwogu, 2007). Even for planktonic cells, long range electron transport is necessary to improve the efficiency of microbial fuel cells. Soluble electron shuttles (natural as well as artificial) and MNWs can be useful for such long range
electron transfers (Fig. 1). Electron shuttles to be used for long range electron transfer have
their own disadvantages - natural electron shuttles have slow diffusion rate which limits total
electron flux rates while lack of long term stability and toxicity to humans are the issues for
artificial electron shuttles (Malvankar & Lovley, 2012).

MNWs thus can play important role in improving the overall efficiency of microbial fuel 377 378 cells. In G. sulfurreducens, these MNWs help cells to make efficient contact with electrodes by acting as a bridge between cells and electrodes, thus enabling long range electron transfer 379 380 through biofilm (Steidl et al., 2016). This long range electron transfer thus have been shown to increase electricity production by 10 times (Reguera et al., 2006). On the same line, 381 MNWs producing photosynthetic microorganisms (Gorby et al., 2006; Sure et al., 2015; Sure 382 383 et al., 2016b) may be helpful in improving the efficiency of photosynthetic MFC and microbial solar cells (Rosenbaum et al., 2010; Strik et al., 2011). 384

Methane is considered as important renewable energy source which can be generated by anaerobic digestion of wastewater and biomass (Angenent *et al.*, 2004; De Mes *et al.*, 2003; Prochnow *et al.*, 2009). MNWs have been believed to play a role in methane production in syntrophic microbial communities (Morita *et al.*, 2011; Rotaru, 2014; Summers *et al.*, 2010; Wegener *et al.*, 2015), which can be exploited further for improved methane production in anaerobic digesters. Interested readers are referred to specific reviews on this topic (Lovley, 2011; Malvankar & Lovley, 2014).

392

#### **Bioremediation**

394 *Shewanella* and *Geobacter* have been extensively studied for bioremediation of heavy metals 395 and discovery of MNWs in these microorganisms have further increased their potential in this 396 field. It has been shown that MNWs can play important role in bioremediation of a heavy

metal like uranium (Cologgi et al., 2011). Presence of MNWs in G. sulfurreducens has been 397 shown to significantly mineralize more uranium per cell than a MNW deficient mutant 398 (Cologgi et al., 2011). The MNWs also increase cellular tolerance to uranium by preventing 399 400 its periplasmic accumulation as suggested in Fig. 4 (Cologgi et al., 2011). Further, such MNWs considerably increase the total surface area available for heavy metal adsorption and 401 subsequent detoxification. MNWs in Synechocystis also have been observed to precipitate 402 arsenic (Sure et al., 2016a) and chromium (Sure et al., unpublished data) and thus may be 403 helpful in their bioremediation. Readers may refer to specific review on this topic (Lovley, 404 405 2011).

406

#### 407 **Bioelectronics**

408 Scientists believe that MNWs will allow us to develop instruments usable in water and moist environments (Malvankar & Lovley, 2012). Furthermore, Leung et al. characterized S. 409 oneidensis MNWs and showed that they have enough mechanical strength (Young's modulus 410 ~1 GPa) to use it as a building block for construction of electronic devices (Leung et al., 411 2011). The MNWs can be modified using genetic and protein engineering, so different 412 ligands (metals) can be attached to it which may help to modulate its electric behavior 413 (Lovley et al., 2009) or can increase its electrical conductivity significantly (Tan et al., 2016). 414 415 In this direction, MNWs in G. sulfurreducens have been modified to have better conductive 416 and adhesive properties (Reguera et al., 2014). A recent study by Tan et al. has shown that, in G. sulfurreducens MNWs, replacing C-terminal phenylalnine and tyrosine of PilA with 417 tryptophan decreases its diameter by half and increases its conductivity by ~2000 fold (Tan et 418 419 al., 2016). MNWs may also be used in bionanosensors (Lovley et al., 2009), however no such prior art studies have been reported yet. Interested readers are referred to specific 420 reviews on this topic (Amdursky et al., 2014; Patolsky & Lieber, 2005; Patolsky et al., 2006; 421

Waleed Shinwari *et al.*, 2010; Wang *et al.*, 2014; Ziadan, 2012) which may sensitize them
about how different nanowires, including MNWs can be used for practical applications.

424

#### 425 **Potential target for pathogenic microorganisms**

MNWs have been found in pathogenic biofilms causing BRONJ and supposed to play 426 important role in maintenance and survival of it (Wanger et al., 2013). This discovery is very 427 428 important considering the fact that various human pathogenic microorganisms like Neisseria gonorrhoeae and Vibrio cholera produce pili which are actively involved in pathogenesis 429 430 (Heckels, 1989; Tacket et al., 1998; Zhang et al., 2000). Exoelectrogenic microbes with putative MNWs play specific role in host immune response (Ericsson et al., 2015). It needs to 431 be studied whether pili are conductive in different pathogenic bacteria and if so, what role 432 433 they play in pathogenesis. In the phenomenon called "bioelectric effect", electrically stimulated pathogenic biofilms showed increased susceptibility to antibiotics and this may 434 happen because of disruption of conductive filaments within them as a result of electrical 435 stimulation (Costerton et al., 1994; Wanger et al., 2013). The bioelectric effect also supports 436 the hypothesis that MNWs might be playing important role in maintenance of pathogenic 437 biofilms. Thus MNWs can be a potential target for prevention and treatment of relevant 438 diseases and future research in this direction may yield some exciting results. 439

440

# 441 Gaps in current research and future directions

442 Above examples suggest that microbes may have developed multiple strategies to produce 443 MNWs as per their niche and physiological requirement. Hence, more extensive screening of 444 microorganisms from diverse habitats needs to be done to establish their ability to produce 445 MNWs which may help to completely understand their abundance and role in environment. The physiological function of most known MNWs is not identified so far except that of *G*. *sulfurreducens*. This is another area which can be the focus of future studies.

Comparative characterization of all known MNWs for their conductive, biochemical and 448 449 mechanical properties should be done. This will significantly help to identify the best candidate for practical applications and may also help to produce hybrid MNWs with better 450 functionality than any individual one. It is also of utmost importance that mechanisms of 451 electron transfer through MNWs should be studied in MNWs produced by diverse 452 microorganisms (other than G. sulfurreduces and S. oneidensis). Apart from aromatic amino 453 454 acids, sulfur containing amino acids (methionine and cysteine) are also known to act as a relay in electron transfer (Sun et al., 2015; Wang et al., 2009). The probable role of these 455 sulfur-containing amino acids (if present) in electron transfer through MNWs has not been 456 457 studied so far and any involvement of these amino acids in conductivity of MNWs needs to be explored. 458

There is also a need to develop simple methods that will allow maximum production of 459 460 MNWs which will be important from application point of view. For example, method for MNWs production in Synechocystis was sophisticated earlier (Gorby et al., 2006) but in 461 recent times simple methods for maximum production of Synechocystis MNWs have been 462 identified (Sure et al., 2015). Most of the findings in this field till date have been generated 463 from few labs. Reproducibility, authenticity and credibility of particular data gets 464 465 strengthened when identical or similar results are obtained from different labs. This is especially applied to the advanced characterization of MNWs involving determination of 466 electron transfer mechanisms through them where ambiguity still exists among researchers. 467 468 So it is essential that further research be carried out to harness the true potential of this field and use it to tackle contemporary problems. 469

# 471 Conclusions

The ability of microorganisms to produce MNWs increases their potential to influence their 472 surrounding environment and thus further enhances their status as "tiny but powerful 473 organisms". Occurrence of MNWs in microorganisms may be widespread and they may be 474 employing it for diverse functions like extracellular electron transfer to metals, tolerance to 475 toxic metals, preventing photo damage, and cell communication depending upon their niche 476 and physiological needs. The discovery of new MNWs producing microorganisms and the 477 identification of specific environmental conditions leading to production of MNWs is 478 479 extremely important along with rigorous biochemical and electrical characterization of same. This will help in identification of most suitable MNWs for specific practical applications in 480 the field of bioremediation, bioenergy, bioelectronics and possibly bio therapeutics. More 481 482 efforts are needed to explore the mechanism of electron flow through different MNWs which would greatly help in modulation of electro conductive and other properties of MNWs. 483

484

## 485 **Conflict of Interest**

486 No conflict of interest to declare

487

# 488 Acknowledgement

The authors are thankful to the TERI-DeakinNanobiotechnology Centre for providing necessary infrastructure to carry out required research work. SS acknowledges PhD fellowship (Candidate ID – 212082401) provided by Deakin University, Australia. We are also grateful to the anonymous reviewers for their detailed and valuable inputs which helped to improve the quality of this review paper.

494

## 495 **References**

- Amdursky, N., Marchak, D., Sepunaru, L., Pecht, I., Sheves, M. & Cahen, D. (2014).
  Electronic Transport via Proteins. *Adv Mater* 26, 7142-7161.
- 498
- Angenent, L. T., Karim, K., Al-Dahhan, M. H., Wrenn, B. A. & Domíguez-Espinosa, R.
  (2004). Production of bioenergy and biochemicals from industrial and agricultural
  wastewater. *Trends Biotechnol* 22, 477-485.
- 502
- Boesen, T. & Nielsen, L. P. (2013). Molecular dissection of bacterial nanowires. *mBio* 4,
  e00270-00213.

- Bouhenni, R. A., Vora, G. J., Biffinger, J. C. & other authors (2010). The Role of
  Shewanella oneidensis MR-1 Outer Surface Structures in Extracellular Electron Transfer.
  Electroanalysis 22, 856-864.
- 509
- Castro, L., Vera, M., Munoz, J. A., Blazquez, M. L., Gonzalez, F., Sand, W. & Ballester,
  A. (2014). Aeromonas hydrophila produces conductive nanowires. *Res Microbiol* 165, 794802.
- 513
- Chalmeau, J., Dagkessamanskaia, A., Le Grimellec, C., Francois, J. M., Sternick, J. &
  Vieu, C. (2009). Contribution to the elucidation of the structure of the bacterial flagellum
  nano-motor through AFM imaging of the M-Ring. *Ultramicroscopy* 109, 845-853.
- 517
- 518 Cologgi, D. L., Lampa-Pastirk, S., Speers, A. M., Kelly, S. D. & Reguera, G. (2011).
- 519 Extracellular reduction of uranium via Geobacter conductive pili as a protective cellular
- 520 mechanism. *Proc Natl Acad Sci U S A* **108**, 15248-15252.

E	2	1
J	2	т

522	Costerton, J. W., Ellis, B., Lam, K., Johnson, F. & Khoury, A. E. (1994). Mechanism of
523	electrical enhancement of efficacy of antibiotics in killing biofilm bacteria. Antimicrob
524	Agents Chemother 38, 2803-2809.
525	
526	Creasey, R. C., Shingaya, Y. & Nakayama, T. (2015). Improved electrical conductance
527	through self-assembly of bioinspired peptides into nanoscale fibers. Mater Chem Phys 158,
528	52-59.
529	
530	De Mes, T., Stams, A., Reith, J. & Zeeman, G. (2003). Methane production by anaerobic
531	digestion of wastewater and solid wastes. Bio-methane & Bio-hydrogen. Dutch Biological
532	Hydrogen Foundation, Netherlands.
533	
534	Duggan, P. S., Gottardello, P. & Adams, D. G. (2007). Molecular analysis of genes in
535	Nostoc punctiforme involved in pilus biogenesis and plant infection. J Bacteriol 189, 4547-
536	4551.
537	
538	Durand, E., Bernadac, A., Ball, G., Lazdunski, A., Sturgis, J. N. & Filloux, A. (2003).
539	Type II protein secretion in <i>Pseudomonas aeruginosa</i> : the pseudopilus is a multifibrillar and
540	adhesive structure. J Bacteriol 185, 2749-2758.
541	
542	Eaktasang, N., Kang, C. S., Lim, H., Kwean, O. S., Cho, S., Kim, Y. & Kim, H. S.
543	(2016). Production of electrically-conductive nanoscale filaments by sulfate-reducing
544	bacteria in the microbial fuel cell. <i>Bioresource Technol</i> DOI:
545	10.1016/j.biortech.2015.12.090.

El-Naggar, M. Y., Gorby, Y. A., Xia, W. & Nealson, K. H. (2008). The molecular density
of states in bacterial nanowires. *Biophys J* 95, L10-12.

- 550 El-Naggar, M. Y., Wanger, G., Leung, K. M., Yuzvinsky, T. D., Southam, G., Yang, J.,
- Lau, W. M., Nealson, K. H. & Gorby, Y. A. (2010). Electrical transport along bacterial
  nanowires from *Shewanella oneidensis* MR-1. *Proc Natl Acad Sci U S A* 107, 18127-18131.

Ericsson, A., Davis, D., Franklin, C. & Hagan, C. (2015). Exoelectrogenic capacity of host
microbiota predicts lymphocyte recruitment to the gut. *Physiol Genomics*DOI:10.1152/physiolgenomics.00010.2015.

557

Feliciano, G. T. (2012). Molecular and Electronic Structure of the Peptide Subunit of *Geobacter sulfurreducens* Conductive Pili from First Principles. J Phys Chem A 116, 80238030.

561

Feliciano, G. T., Steidl, R. J. & Reguera, G. (2015). Structural and functional insights into
the conductive pili of *Geobacter sulfurreducens* revealed in molecular dynamics simulations. *Phys Chem Chem Phys* 17, 22217-22226.

565

Fitzgerald, L., Petersen, E., Ray, R., Little, B., Cooper, C., Howard, E., Ringeisen, B. &
Biffinger, J. (2012). *Shewanella oneidensis* MR-1 Msh pilin proteins are involved in
extracellular electron transfer in microbial fuel cells. *Process Biochem* 47, 170-174.

<sup>549</sup> 

570	Gorby, Y. A., Yanina, S., McLean, J. S. & other authors (2006). Electrically conductive
571	bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other
572	microorganisms. Proc Natl Acad Sci U S A 103, 11358-11363.
573	
574	Gralnick, J. A. & Newman, D. K. (2007). Extracellular respiration. Mol Microbiol 65, 1-11.
575	
576	Heckels, J. (1989). Structure and function of pili of pathogenic Neisseria species. Clin
577	Microbiol Rev 2, S66.
578	
579	Kulp, T. R., Hoeft, S. E., Asao, M. & other authors (2008). Arsenic(III) fuels anoxygenic
580	photosynthesis in hot spring biofilms from Mono Lake, California. Science 321, 967-970.
581	
582	Lampa-Pastirk, S., Veazey, J. P., Walsh, K. A., Feliciano, G. T., Steidl, R. J., Tessmer,
582 583	<ul><li>Lampa-Pastirk, S., Veazey, J. P., Walsh, K. A., Feliciano, G. T., Steidl, R. J., Tessmer,</li><li>S. H. &amp; Reguera, G. (2016). Thermally activated charge transport in microbial protein</li></ul>
582 583 584	<ul> <li>Lampa-Pastirk, S., Veazey, J. P., Walsh, K. A., Feliciano, G. T., Steidl, R. J., Tessmer,</li> <li>S. H. &amp; Reguera, G. (2016). Thermally activated charge transport in microbial protein nanowires. <i>Sci Rep</i> 6, doi:10.1038/srep23517</li> </ul>
582 583 584 585	<ul> <li>Lampa-Pastirk, S., Veazey, J. P., Walsh, K. A., Feliciano, G. T., Steidl, R. J., Tessmer,</li> <li>S. H. &amp; Reguera, G. (2016). Thermally activated charge transport in microbial protein nanowires. <i>Sci Rep</i> 6, doi:10.1038/srep23517</li> </ul>
582 583 584 585 586	<ul> <li>Lampa-Pastirk, S., Veazey, J. P., Walsh, K. A., Feliciano, G. T., Steidl, R. J., Tessmer,</li> <li>S. H. &amp; Reguera, G. (2016). Thermally activated charge transport in microbial protein nanowires. <i>Sci Rep</i> 6, doi:10.1038/srep23517</li> <li>Lea-Smith, D. J., Bombelli, P., Vasudevan, R. &amp; Howe, C. J. (2015). Photosynthetic,</li> </ul>
582 583 584 585 586 587	<ul> <li>Lampa-Pastirk, S., Veazey, J. P., Walsh, K. A., Feliciano, G. T., Steidl, R. J., Tessmer,</li> <li>S. H. &amp; Reguera, G. (2016). Thermally activated charge transport in microbial protein nanowires. <i>Sci Rep</i> 6, doi:10.1038/srep23517</li> <li>Lea-Smith, D. J., Bombelli, P., Vasudevan, R. &amp; Howe, C. J. (2015). Photosynthetic, respiratory and extracellular electron transport pathways in cyanobacteria. <i>BBA-Bioenergetics</i></li> </ul>
582 583 584 585 586 587 588	<ul> <li>Lampa-Pastirk, S., Veazey, J. P., Walsh, K. A., Feliciano, G. T., Steidl, R. J., Tessmer, S. H. &amp; Reguera, G. (2016). Thermally activated charge transport in microbial protein nanowires. <i>Sci Rep</i> 6, doi:10.1038/srep23517</li> <li>Lea-Smith, D. J., Bombelli, P., Vasudevan, R. &amp; Howe, C. J. (2015). Photosynthetic, respiratory and extracellular electron transport pathways in cyanobacteria. <i>BBA-Bioenergetics</i> 1857, 247-255.</li> </ul>
582 583 584 585 586 587 588 588	<ul> <li>Lampa-Pastirk, S., Veazey, J. P., Walsh, K. A., Feliciano, G. T., Steidl, R. J., Tessmer, S. H. &amp; Reguera, G. (2016). Thermally activated charge transport in microbial protein nanowires. <i>Sci Rep</i> 6, doi:10.1038/srep23517</li> <li>Lea-Smith, D. J., Bombelli, P., Vasudevan, R. &amp; Howe, C. J. (2015). Photosynthetic, respiratory and extracellular electron transport pathways in cyanobacteria. <i>BBA-Bioenergetics</i> 1857, 247-255.</li> </ul>
582 583 584 585 586 587 588 588 589 590	<ul> <li>Lampa-Pastirk, S., Veazey, J. P., Walsh, K. A., Feliciano, G. T., Steidl, R. J., Tessmer,</li> <li>S. H. &amp; Reguera, G. (2016). Thermally activated charge transport in microbial protein nanowires. <i>Sci Rep</i> 6, doi:10.1038/srep23517</li> <li>Lea-Smith, D. J., Bombelli, P., Vasudevan, R. &amp; Howe, C. J. (2015). Photosynthetic, respiratory and extracellular electron transport pathways in cyanobacteria. <i>BBA-Bioenergetics</i> 1857, 247-255.</li> <li>Leang, C., Qian, X., Mester, T. &amp; Lovley, D. R. (2010). Alignment of the c-type</li> </ul>
582 583 584 585 586 587 588 589 590 591	<ul> <li>Lampa-Pastirk, S., Veazey, J. P., Walsh, K. A., Feliciano, G. T., Steidl, R. J., Tessmer, S. H. &amp; Reguera, G. (2016). Thermally activated charge transport in microbial protein nanowires. <i>Sci Rep</i> 6, doi:10.1038/srep23517</li> <li>Lea-Smith, D. J., Bombelli, P., Vasudevan, R. &amp; Howe, C. J. (2015). Photosynthetic, respiratory and extracellular electron transport pathways in cyanobacteria. <i>BBA-Bioenergetics</i> 1857, 247-255.</li> <li>Leang, C., Qian, X., Mester, T. &amp; Lovley, D. R. (2010). Alignment of the c-type cytochrome OmcS along pili of <i>Geobacter sulfurreducens. Appl Environ Microbiol</i> 76, 4080-</li> </ul>
582 583 584 585 586 587 588 589 590 591 592	<ul> <li>Lampa-Pastirk, S., Veazey, J. P., Walsh, K. A., Feliciano, G. T., Steidl, R. J., Tessmer,</li> <li>S. H. &amp; Reguera, G. (2016). Thermally activated charge transport in microbial protein nanowires. <i>Sci Rep</i> 6, doi:10.1038/srep23517</li> <li>Lea-Smith, D. J., Bombelli, P., Vasudevan, R. &amp; Howe, C. J. (2015). Photosynthetic, respiratory and extracellular electron transport pathways in cyanobacteria. <i>BBA-Bioenergetics</i> 1857, 247-255.</li> <li>Leang, C., Qian, X., Mester, T. &amp; Lovley, D. R. (2010). Alignment of the c-type cytochrome OmcS along pili of <i>Geobacter sulfurreducens. Appl Environ Microbiol</i> 76, 4080-4084.</li> </ul>
582 583 584 585 586 587 588 589 590 591 592 593	<ul> <li>Lampa-Pastirk, S., Veazey, J. P., Walsh, K. A., Feliciano, G. T., Steidl, R. J., Tessmer, S. H. &amp; Reguera, G. (2016). Thermally activated charge transport in microbial protein nanowires. <i>Sci Rep</i> 6, doi:10.1038/srep23517</li> <li>Lea-Smith, D. J., Bombelli, P., Vasudevan, R. &amp; Howe, C. J. (2015). Photosynthetic, respiratory and extracellular electron transport pathways in cyanobacteria. <i>BBA-Bioenergetics</i> 1857, 247-255.</li> <li>Leang, C., Qian, X., Mester, T. &amp; Lovley, D. R. (2010). Alignment of the c-type cytochrome OmcS along pili of <i>Geobacter sulfurreducens. Appl Environ Microbiol</i> 76, 4080-4084.</li> </ul>

- Lebedev, N., Mahmud, S., Griva, I., Blom, A. & Tender, L. M. (2015). On the electron
  transfer through *Geobacter sulfurreducens* PilA protein. *J Polym Sci B: Polym Phys* 53,
  1706-1717.
- 597
- Leung, K. M., Wagner, G., Guo, Q., Gorby, Y. A., Southam, G., Laue, W. M. & Yang, J.
- (2011). Bacterial nanowires: conductive as silicon, soft as polymer. *Soft Matter* 7, 6617-6621.
- 601 Leung, K. M., Wanger, G., El-Naggar, M. Y., Gorby, Y., Southam, G., Lau, W. M. &
- Yang, J. (2013). *Shewanella oneidensis* MR-1 bacterial nanowires exhibit p-type, tunable
  electronic behavior. *Nano Lett* 13, 2407-2411.
- 604
- Li, Y. & Li, H. (2014). Type IV pili of *Acidithiobacillus ferrooxidans* can transfer electrons
  from extracellular electron donors. *J Basic Microbiol* 54, 226-231.
- 607
- 608 Liu, X., Tremblay, P.-L., Malvankar, N. S., Nevin, K. P., Lovley, D. R. & Vargas, M.
- 609 (2014). A Geobacter sulfurreducens strain expressing Pseudomonas aeruginosa type IV pili
- 610 localizes OmcS on pili but is deficient in Fe (III) oxide reduction and current production.
- 611 *Appl Environ Microbiol* **80**, 1219-1224.
- 612
- Lovley, D. R. (2008). Extracellular electron transfer: wires, capacitors, iron lungs, and more. *Geobiology* 6, 225-231.
- 615

Lovley, D. R., Reguera, G., McCarthy, K. D. & Tuominem, M. T. (2009). Providing a
bacterium such as *Geobacteraceae* expressing a conductive proteinaceous pilus; culturing in
medium containing an electron acceptor such as iron III oxide; coupling to circuit; self-

619	assembling;	no	need	for	metallization.	US	Pat.	US	7,498,155	B2,	University	of
620	Massachuset	ts.										

622	Lovley, D. R. (2011). Live wires: direct extracellular electron exchange for bioenergy and
623	the bioremediation of energy-related contamination. Energy Environ Sci 4, 4896-4906.

624

625	Malvankar, N. S., Vargas, M., Nevin, K. P. & other authors (2011b). Tunable metallic-
626	like conductivity in microbial nanowire networks. Nat Nanotechnol 6, 573-579.

627

Malvankar, N. S. & Lovley, D. R. (2012). Microbial nanowires: a new paradigm for
biological electron transfer and bioelectronics. *ChemSusChem* 5, 1039-1046.

630

Malvankar, N. S., Tuominen, M. T. & Lovley, D. R. (2012). Lack of Cytochrome
Involvement in Long-Range Electron Transport through Conductive Biofilms & Nanowires
of *Geobacter sulfurreducens*. *Energy Environ Sci* 5, 8651-8659.

634

- Malvankar, N. S. & Lovley, D. R. (2014). Microbial nanowires for bioenergy applications. *Curr Opin Biotechnol* 27, 88-95.
- 637

Malvankar, N. S., Yalcin, S. E., Tuominen, M. T. & Lovley, D. R. (2014). Visualization of
charge propagation along individual pili proteins using ambient electrostatic force
microscopy. *Nat Nanotechnol* 9, 1012-1017.

642	Malvankar, N. S., Vargas, M., Nevin, K., Tremblay, PL., Evans-Lutterodt, K.,
643	Nykypanchuk, D., Martz, E., Tuominen, M. T. & Lovley, D. R. (2015). Structural Basis
644	for Metallic-Like Conductivity in Microbial Nanowires. <i>mBio</i> 6, e00084-00015.
645	
646	Morita, M., Malvankar, N. S., Franks, A. E., Summers, Z. M., Giloteaux, L., Rotaru, A.
647	E., Rotaru, C. & Lovley, D. R. (2011). Potential for direct interspecies electron transfer in
648	methanogenic wastewater digester aggregates. mBio 2, e00159-00111.
649	
650	Nwogu, N. G. (2007). Microbial Fuel Cells and Parameters Affecting Performance
651	When Generating Electricity. MMG 445 Basic Biotechnol eJ 3, 73-79.
652	
653	Patolsky, F. & Lieber, C. M. (2005). Nanowire nanosensors. Materials today 8, 20-28.
654	
655	Patolsky, F., Zheng, G. & Lieber, C. M. (2006). Nanowire sensors for medicine and the life
656	sciences. Nanomedicine 1, 51-65.
657	
658	Pelicic, V. (2008). Type IV pili: e pluribus unum? Mol Microbiol 68, 827-837.
659	
660	Petrov, A. & Audette, G. F. (2012). Peptide and protein-based nanotubes for
661	nanobiotechnology. Wiley Interdiscip Rev Nanomed Nanobiotechnol 4, 575-585.
662	
663	Pirbadian, S. & El-Naggar, M. Y. (2012). Multistep hopping and extracellular charge
664	transfer in microbial redox chains. Phys Chem Chem Phys 14, 13802-13808.
665	

666	Pirbadian, S., Barchinger, S. E., Leung, K. M. & other authors (2014). Shewanella
667	oneidensis MR-1 nanowires are outer membrane and periplasmic extensions of the
668	extracellular electron transport components. Proc Natl Acad Sci U S A 111, 12883-12888.
669	
670	<b>Biggiotte I M. Zou V. &amp; Bogkakov I V. (2010)</b> Light dependent electrogenia estivity of

- 670 Pisciotta, J. M., Zou, Y. & Baskakov, I. V. (2010). Light-dependent electrogenic activity of
  671 cyanobacteria. *PLoS One* 5, e10821.
- 672
- Polizzi, N. F., Skourtis, S. S. & Beratan, D. N. (2012). Physical constraints on charge
  transport through bacterial nanowires. *Faraday Discuss* 155, 43-61.
- 675
- Prochnow, A., Heiermann, M., Plöchl, M., Linke, B., Idler, C., Amon, T. & Hobbs, P.
  (2009). Bioenergy from permanent grassland–A review: 1. Biogas. *Bioresource Technol* 100, 4931-4944.
- 679
- Proft, T. & Baker, E. N. (2009). Pili in Gram-negative and Gram-positive bacteria structure, assembly and their role in disease. *Cell Mol Life Sci* 66, 613-635.
- 682
- Rabaey, K. (2010). Bioelectrochemical Systems: From Extracellular Electron Transfer to
  Biotechnological Application: IWA Publishing, U.K.
- 685
- Reches, M. & Gazit, E. (2003). Casting metal nanowires within discrete self-assembled
  peptide nanotubes. *Science* 300, 625-627.
- 688
- 689 Reguera, G., McCarthy, K. D., Mehta, T., Nicoll, J. S., Tuominen, M. T. & Lovley, D. R.
- 690 (2005). Extracellular electron transfer via microbial nanowires. *Nature* **435**, 1098-1101.

- Reguera, G., Nevin, K. P., Nicoll, J. S., Covalla, S. F., Woodard, T. L. & Lovley, D. R. 692 (2006). Biofilm and nanowire production leads to increased current in Geobacter 693 sulfurreducens fuel cells. Appl Environ Microbiol 72, 7345-7348. 694 695 Reguera, G. (2011). When microbial conversations get physical. Trends Microbiol 19, 105-696 697 113. 698 699 Reguera, G., Cologgi, D., Worden, R. M., Castro-forero, A. A. & Steidl, R. (2014). Microbial nanowires and methods of making and using. US Pat. US 2014/0239237 A1, 700 701 Michigan State University. 702 Remis, J. P., Wei, D., Gorur, A. & other authors (2014). Bacterial social networks: 703 structure and composition of Myxococcus xanthus outer membrane vesicle chains. Environ 704 705 Microbiol 16, 598-610. 706 Richardson, D. J. (2000). Bacterial respiration: a flexible process for a changing 707 environment. Microbiology 146 (Pt 3), 551-571. 708 709 710 Rosenbaum, M., He, Z. & Angenent, L. T. (2010). Light energy to bioelectricity: photosynthetic microbial fuel cells. Curr Opin Biotechnol 21, 259-264. 711 712 Rosenman, G., Beker, P., Koren, I., Yevnin, M., Bank-Srour, B., Mishina, E. & Semin, 713
- 714 S. (2011). Bioinspired peptide nanotubes: deposition technology, basic physics and
  - nanotechnology applications. *J Peptide Sci* **17**, 75-87.

717	Rotaru, A.E., Shrestha, P.M., Liu, F., Shrestha, M., Shrestha, D., Embree, M., Zengler,
718	K., Wardman, C., Nevin, K.P. & Lovley, D.R. (2014) A new model for electron flow
719	during anaerobic digestion: direct interspecies electron transfer to Methanosaeta for the
720	reduction of carbon dioxide to methane. Energy Environ Sci 7,408-415
721	
722	Scanlon, S. & Aggeli, A. (2008). Self-assembling peptide nanotubes. <i>Nano Today</i> 3, 22-30.
723	
724	Shimoyama, T., Kato, S., Ishii, S. & Watanabe, K. (2009). Flagellum mediates symbiosis.
725	Science <b>323</b> , 1574.
726	
727	Simpson, C. F., White, F. H. & Sandhu, T. S. (1976). The structure of pili (fimbriae) of
728	Moraxella bovis. Can J Comp Med 40, 1-4.
729	
730	Skourtis, S. S. (2013). Probing protein electron transfer mechanisms from the molecular to
731	the cellular length scales. <i>Peptide Sci</i> 100, 82-92.
732	
733	Steidl, R. J., Lampa-Pastirk, S. & Reguera, G. (2016). Mechanistic stratification in
734	electroactive biofilms of Geobacter sulfurreducens mediated by pilus nanowires. Nat.
735	<i>Commun.</i> <b>7</b> , 1-7.
736	
737	Strik, D. P., Timmers, R. A., Helder, M., Steinbusch, K. J., Hamelers, H. V. & Buisman,
738	C. J. (2011). Microbial solar cells: applying photosynthetic and electrochemically active
739	organisms. Trends Biotechnol 29, 41-49.
740	

- Strycharz-Glaven, S. M., Snider, R. M., Guiseppi-Eliec, A. & Tender, L. M. (2011). On
  the electrical conductivity of microbial nanowires and biofilms. *Energy Environ Sci* 4, 43664379.
- 744
- Strycharz-Glaven, S. M. & Tender, L. M. (2012). Reply to the 'Comment on 'On
  electrical conductivity of microbial nanowires & biofilms'' by N. S. Malvankar, M. T.
  Tuominen & D. R. Lovley. *Energy Environ Sci* 5, 6250-6255.
- 748
- 749 Summers, Z. M., Fogarty, H. E., Leang, C., Franks, A. E., Malvankar, N. S. & Lovley,

**D. R. (2010).** Direct exchange of electrons within aggregates of an evolved syntrophic
coculture of anaerobic bacteria. *Science* 330, 1413-1415.

- 752
- Sun, W., Shao, M., Ren, H., Xiao, D., Qin, X., Deng, L., Chen, X. & Gao, J. (2015). A New Type of Electron Relay Station in Proteins: Three-Piece S:  $\pi$ : S  $\leftrightarrow$  S:  $\pi$ : S Resonance Structure. *The J Phys Chem C* **119**, 6998-7005.
- 756

Sure, S., Torriero, A. A., Gaur, A., Li, L. H., Chen, Y., Tripathi, C., Adholeya, A.,
Ackland, M. L. & Kochar, M. (2015). Inquisition of *Microcystis aeruginosa* and *Synechocystis* nanowires: characterization and modelling. *Antonie van Leeuwenhoek* 108,
1213-1225.

- 761
- Sure, S., Ackland, M. L., Gaur, A., Gupta, P., Adholeya, A. & Kochar, M. (2016a).
  Probing *Synechocystis*-arsenic interactions through extracellular nanowires. *Front Microbiol*764 7, doi: 10.3389/fmicb.2016.01134
- 765

766	Sure, S., Torriero, A. A., Gaur, A., Li, L. H., Chen, Y., Tripathi, C., Adholeya, A.,
767	Ackland, M. L. & Kochar, M. (2016). Identification and topographical characterisation of
768	microbial nanowires in Nostoc punctiforme. Antonie van Leeuwenhoek, 109, 475-480.
769	
770	Tacket, C. O., Taylor, R. K., Losonsky, G., Lim, Y., Nataro, J. P., Kaper, J. B. &
771	Levine, M. M. (1998). Investigation of the roles of toxin-coregulated pili and mannose-
772	sensitive hemagglutinin pili in the pathogenesis of Vibrio cholerae O139 infection. Infect
773	<i>Immun</i> <b>66</b> , 692-695.
774	
775	Tan, Y., Adhikari, R. Y., Malvankar, N. S. & other authors (2016). Synthetic Biological
776	Protein Nanowires with High Conductivity. Small. doi: 10.1002/smll.201601112
777	

778 Tender, L. M. (2011). From mud to microbial electrode catalysts & conductive
779 nanomaterials. *MRS Bulletin* 36, 800-805.

780

Valdes, J., Pedroso, I., Quatrini, R., Dodson, R. J., Tettelin, H., Blake, R., 2nd, Eisen, J.
A. & Holmes, D. S. (2008). *Acidithiobacillus ferrooxidans* metabolism: from genome sequence to industrial applications. *BMC Genomics* 9, 597.

784

Vargas, M., Malvankar, N. S., Tremblay, P. L., Leang, C., Smith, J. A., Patel, P.,
Synoeyenbos-West, O., Nevin, K. P. & Lovley, D. R. (2013). Aromatic Amino Acids
Required for Pili Conductivity and Long-Range Extracellular Electron Transport in *Geobacter sulfurreducens. mBio* 4, e00105-00113.

790	Veazey, J. P., Reguera, G. & Tessmer, S. H. (2011). Electronic properties of conductive
791	pili of the metal-reducing bacterium Geobacter sulfurreducens probed by scanning tunneling
792	microscopy. Phys Rev E Stat Nonlin Soft Matter Phys 84, 060901.

- Venkidusamy, K., Megharaj, M., Schröder, U., Karouta, F., Mohan, S. V. & Naidu, R.
  (2015). Electron transport through electrically conductive nanofilaments in *Rhodopseudomonas palustris* strain RP2. *RSC Advances* 5, 100790-100798.
- 797
- Waleed Shinwari, M., Jamal Deen, M., Starikov, E. B. & Cuniberti, G. (2010). Electrical
  conductance in biological molecules. *Adv Funct Mater* 20, 1865-1883.

800

Wang, K., Wu, H., Meng, Y. & Wei, Z. (2014). Conducting polymer nanowire arrays for
high performance supercapacitors. *Small* 10, 14-31.

803

Wang, M., Gao, J., Müller, P. & Giese, B. (2009). Electron transfer in peptides with cysteine and methionine as relay amino acids. *Angew Chem Int Edit* **48**, 4232-4234.

806

Wanger, G., Gorby, Y., El-Naggar, M. Y., Yuzvinsky, T. D., Schaudinn, C., Gorur, A. &
Sedghizadeh, P. P. (2013). Electrically conductive bacterial nanowires in bisphosphonaterelated osteonecrosis of the jaw biofilms. *Oral Surg Oral Med Oral Pathol Oral Radiol* 115,
71-78.

811

Wegener, G., Krukenberg, V., Riedel, D., Tegetmeyer, H. E. & Boetius, A. (2015).
Intercellular wiring enables electron transfer between methanotrophic archaea and bacteria. *Nature* 526, 587-590.

612	
816	Wei, X., Vassallo, C. N., Pathak, D. T. & Wall, D. (2014). Myxobacteria produce outer
817	membrane-enclosed tubes in unstructured environments. J Bacteriol 196, 1807-1814.
818	
819	Xiao, K., Malvankar, N. S., Shu, C., Martz, E., Lovley, D. R. & Sun, X. (2016). Low
820	Energy Atomic Models Suggesting a Pilus Structure that could Account for Electrical
821	Conductivity of Geobacter sulfurreducens Pili. Sci Rep 6, doi:10.1038/srep23385
822	
823	Xu, D., Watt, G. D., Harb, J. N. & Davis, R. C. (2005). Electrical conductivity of ferritin
824	proteins by conductive AFM. Nano Lett 5, 571-577.
825	
826	Yan, H., Chuang, C., Zhugayevych, A., Tretiak, S., Dahlquist, F. W. & Bazan, G. C.
827	(2015). Inter-Aromatic Distances in Geobacter Sulfurreducens Pili Relevant to Biofilm
828	Charge Transport. Adv Mater 27, 1908-1911.
829	
830	Zhang, XL., Tsui, I. S., Yip, C. M., Fung, A. W., Wong, D. KH., Dai, X., Yang, Y.,
831	Hackett, J. & Morris, C. (2000). Salmonella enterica serovar Typhi uses type IVB pili to
832	enter human intestinal epithelial cells. Infect Immun 68, 3067-3073.
833	
834	Ziadan, K. M. (2012). Conducting Polymers Application: INTECH Open Access Publisher,
835	Croatia.
836	
837	

**Table 1:** List of MNWs producing microorganisms

Sr. No	Microorganisms	Component Protein of MNWs	Physiological Role	Conductivity Measurement (Along width/length)	Description	References
1	Geobacter sulfurreducens	pilin subunit PilA	Extracellular electron transfer to insoluble electron acceptor [e.g. Fe(III)]	Along width as well as length	Metal reducing, anaerobic	(Malvankar <i>et al.</i> , 2011b; Malvankar <i>et al.</i> , 2014; Reguera <i>et al.</i> , 2005)
2	Shewanella oneidensis MR-1	Periplasmic and outer membrane extensions embedded with cytochromes	Not known yet	Along width as well as length	Metal reducing, facultative anaerobic	(El-Naggar <i>et al.</i> , 2010; Gorby <i>et al.</i> , 2006; Pirbadian <i>et al.</i> , 2014)
3	Synechocystis sp. PCC 6803	pilin subunit PilA1	Not known yet	Along width	Photosynthetic, aerobic	(Gorby <i>et al.</i> , 2006; Sure <i>et al.</i> , 2015)
4	Pelatomaculum thermopropionicum	Not known yet	Not known yet	Along width	Anaerobic, thermophilic	(Gorby <i>et al.</i> , 2006)
5	Multispecies biofilms observed in bisphosphonate-related osteonecrosis of the jaw (BRONJ)	Not known yet	Not known yet	Along width as well as length	Unkonwn	(Wanger <i>et al.</i> , 2013)
6	Acidithiobacillus ferrooxidans	Not known yet	Not known yet	Along width	Chemolithoautotrophic, acidophilic	(Li and Li, 2013)
7	Aeromonas hydrophila	Not known yet	Not known yet	Along width	Facultative anaerobic, heterotroph	(Castro <i>et al.</i> , 2014)

8	Microcystis aeruginosa	Unnamed protein product (GenBank : CAO90693.1)	Not known yet	Along width	Photosynthetic, aerobic, forms toxic blooms	(Sure <i>et al.</i> , 2015)
9	Nostoc punctiforme	Not known yet	Not known yet	Along width	Photosynthetic, aerobic, filamentous	(Sure <i>et al.</i> , 2016b)
10	Rhodopseudomonas palustris strain RP2	Not known yet	Not known yet	Along width as well as length	Anoxic photosynthetic, iron respiring	(Venkidusamy <i>et al.</i> , 2015)
11	Desulfovibrio desulfuricans	Not known yet	Not known yet	Along width	Anaerobic, sulfate reducing	(Eaktasang <i>et al.</i> , 2016)

#### **Figure Legends**

Fig. 1. Strategies by which bacteria can transfer electrons extracellularly to electron acceptors (metals or anode of microbial fuel cell). Bacteria can transfer electrons extracellularly by direct attachment to metal or anode surface of microbial fuel cell (a) or employ metal chelators or small molecules as a mediator for electron transfer (b) or can use microbial nanowires (red lines) for same (c).

864

Fig. 2. Diverse microorganisms that can produce microbial nanowires (MNWs). (a) 865 866 Geobacter sulfurreducens, an anaerobic, dissimilatory metal reducing bacteria (DMRB). Inset shows nanowires at higher magnification. Scale bar, 0.2µm (TEM image) (Reprinted by 867 permission from Macmillan Publishers Ltd: Nature, Reguera et al., 2005@2005); (b) 868 869 Shewanella oneidensis, a facultative anaerobic, DMRB (AFM image). Inset shows in vivo 870 fluorescence image of same cell. The image has been color edited (Adapted from Pirbadianet al., 2014@National Academy of Sciences); (c) Rhodopseudomonas palustris strain RP2, 871 photosynthetic DMRB, metabolically versatile (Reproduced from Venkidusamyet al., 2015 872 with permission from The Royal Society of Chemistry); (d) Synechocystis PCC 6803 and (e) 873 Microcystis aeruginosa PCC 7806 which are aerobic, unicellular photosynthetic 874 microorganisms (TEM images) (With kind permission from Springer Science+Business 875 Media: Sureet al., 2015@Springer Science + Business Media); (f) Nostoc punctiformePCC 876 877 73120 an aerobic, multicellular and filamentous photosynthetic microorganism(Sureet al., 2016) (TEM image); (g) Desulfovibrio desulfuricans, anobligate anaerobe, sulphate 878 reducing(Adapted from Eaktasang al., 2016©Elsevier); (h) Pelotomaculum 879 et thermopropionicum and Methanothermobacter thermoautotrophicus (shown with filled 880 arrow) which are syntrophic methanogenic cocultures (SEM image) (Adapted from Gorbyet 881 882 al., 2006©National Academy of Sciences); (i) Unknown bacteria from BRONJ affected bone (AFM image). The image has been colour edited (Adapted from Wanger *et al.*,
2013©Elsevier). MNWs are also produced by *Acidithiobacillus ferroxidans* (Li & Li, 2014)
and *Aeromonas hydrophila* (Castro *et al*, 2014) (not shown here). MNWs have been shown
with open arrows in all images.

887

Fig. 3. Three types of microbial nanowires (MNWs) observed to date. 1) MNWs made of 888 type IV pili (TFP) as observed in Geobacter sulfurreducens and Synechocystis are made up of 889 subunits, PilA (a) and PilA1 (b), respectively. Both these subunits differ in their structure at 890 891 C-terminal and number and positioning of aromatic amino acids (shown with red colour) (With kind permission from Springer Science+Business Media: Sure et al., 2015@Springer 892 Science + Business Media); 2) MNWs made of extended periplasmic and outer membranes 893 894 along with cytochromes (e.g. MtrC, OmcA) as observed in Shewanella oneidensis (c) 895 (Adapted from Pirbadianet al., 2014@National Academy of Sciences); 3) Unknown MNWs as observed in Microcystis aeruginosa and other microorganisms. In M. aeruginosa, MNWs 896 was found to be made up of unknown protein (GenBank : CAO90693.1) and from its TEM 897 image (d), it seems that it is either made of two subfilaments (e) or contains central channel 898 (f) (With kind permission from Springer Science+Business Media: Sure et al., 2015©Springer 899 Science + Business Media). 900

901

Fig. 4. Potential role of Type IV pili (TFP) in cell-metal interaction. Schematic representation
of how TFP/microbial nanowires can reduce interaction between cell membrane and toxic
metals and can act as a protective barrier against latter.

905

906



Downloaded from www.microbiologyresearch.org by IP: 101.173.176.186 On: Tue, 06 Dec 2016 21:02:39



IP: 101.173.176.186 On: Tue, 06 Dec 2016 21:02:39





Nanowire avoid periplasmic accumulation of toxic metals and provide greater surface area for its reduction/oxidation

Downloaded from www.microbiologyresearch.org by IP: 101.173.176.186 On: Tue, 06 Dec 2016 21:02:39