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Review Article

Beyond the hype surrounding biofuel cells: What's the future of enzymatic fuel cells?

Serge Cosnier*, Andrew J. Gross, Fabien Giroud and Michael Holzinger

1 After a short comparison of biofuel cells based on enzymes
2 and microorganisms, several important developments and
3 applications of enzymatic fuel cells (EFCs) are discussed. This
4 discussion emphasizes how to evaluate the performance of
5 EFCs, and highlights the influence of temperature and how it
6 must be carefully considered for practical use of EFCs as
7 power sources. Some of the latest and most important
8 innovations in EFC design using buckypapers and redox
9 nanoparticles are briefly reviewed.

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Introduction

15 The development of sustainable and renewable energy
16 sources with greater respect for the environment is a cru-
17 cial challenge for the 21st century. Among various alterna-
18 tive energy sources, fuel cells have generated great inter-
19 est for the production of clean electrical energy. Fuel cells
20 generate power through electrochemical reactions via the
21 oxidation of fuels such as hydrogen and alcohols at the an-
22 ode, and the reduction of oxidants, typically oxygen, at the
23 cathode. Fuel cells will produce electricity with heat and
24 water as by-products providing that fuel and oxidant are
25 available. Enzymatic fuel cells (EFCs) and microbial fuel
26 cells (MFCs) are sub-categories of fuel cells that share
27 similar operational principles for energy production [1,2].
28 However, biological fuel cells are not intended to over-

come the depletion of fossil fuels and enter the category
of renewable energy resources such as hydroelectric, solar,
and wind energy. The scope of applications for EFCs and
MFCs is restricted, at least for now, to electricity genera-
tion for powering microelectronic systems such as actua-
tors and sensors [3,4,5,6] and for use in environmental
remediation and sanitation systems [7,8].

Enzymatic and microbial biofuel cells differ vastly in
terms of their volumetric size (cm^3 to m^3), power density
output (μW to mW) and their targeted applications (small-
to large-scale). Another important consideration lies in the
cost of the biocatalyst, which ranges from low-cost bacte-
ria or yeast cells, for MFCs, to the higher cost of puri-
fied wild-type and engineered enzymes for EFCs. MFCs
have been successfully employed as long-term power-
generating systems, for example, for self-powered sen-
sors placed in "hostile" environments or remote locations
[9]. Although MFCs have successfully powered different
generations of robots, this approach remains at the aca-
demic level because of limitations such as the large vol-
ume of MFCs required and their long amortization cost
[8]. Nevertheless, MFCs constitute an original and eco-
friendly system for producing electrical energy, e.g. from
waste, and have become an attractive bioreactor concept
for purification of wastewaters. EFCs on the other hand,
because of their superior power density and compactness,
are better suited to miniaturization and use as portable
power sources for wearable and implantable electronics
devices. EFCs appear to carry greater promise as battery-
free power solutions, but it is necessary to define if this is
a simple scientific curiosity or if their potentialities really
do have conceivable real-world applications. This opin-
ion article attempts to shed light on some hitherto ne-
glected facets of EFCs, to briefly revisit their possible
applications, and to reveal new insights concerning fu-
ture directions. It is emphasized that this review does
not cover all of the exciting facets and developments in
EFC research. Recent reviews are available for further
information on important topics such as the evolution of
bioinspired strategies for the electrical wiring of enzymes
and the development of high surface area bioelectrodes
[10,11]. Notable innovations on high surface area elec-
trodes include the hierarchically porous MgO-templated
carbon and carbon nanotube (CNT) yarn textile elec-
trodes [12,13].

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At the very beginning, EFCs were developed to generate electrical energy mainly through the oxidation of saccharides at the anode coupled with the reduction of oxygen at the cathode [4,14]. In addition to glucose, which is by far the most exploited fuel at the anode, other fuels that have been explored include fructose, pyruvate, hydrogen, lactate, methanol and ethanol [3,4,6]. Since glucose is an essential and relatively abundant source of energy in living organisms, special attention has been given to the development of implantable EFCs for powering implanted medical devices from physiological fluids [15]. In parallel, EFCs are considered for powering portable electronic devices such as mobile phones, sensors, digital music players, laptops, and GPS systems. A fascinating flagship application, proposed by many authors for more than a decade, is the use of EFCs as a lifetime power supply for cardiac pacemakers. However, despite all of the promises, there are many hurdles and roadblocks to overcome. It is now time to recognize that the pacemaker application is unrealistic. Besides sterilization and biocompatibility problems, the major issue with implanted EFCs is their operational stability that ranges from a few hours to one year compared to 5–8 years for sealed batteries [16].

A recent and promising generation of biofuel cells concerns H_2/O_2 EFCs [17*,18**]. The adsorption, entrapment and chemical immobilization of NiFe and FeFe hydrogenases, and their “electrical wiring”, is at the origin of bioanodes for electrocatalytic interconversion of protons and hydrogen [19–23]. Hydrogenases entrapped in redox polymers are particularly active for bioelectrocatalytic hydrogen oxidation [24*,25,26]. Recent hydrogenase electrodes exhibit characteristics that are competitive to those of Pt-based electrodes such as long-term stability, mW power densities, insensitivity to fuel impurities such as CO and sulfides, and tolerance to O_2 . EFCs with hydrogenase bioanodes thus show great promise as noble metal-free hydrogen fuel cells [27–31]. Compared with glucose EFCs, a major advantage of hydrogen EFCs lies in the size of the fuel. Hydrogen is markedly smaller than glucose or various redox mediators used for electrical enzyme wiring. The small size can thus be exploited, via careful electrode design with permselective membranes, to prevent the leakage of redox mediators whilst allowing H_2 to efficiently diffuse into the cell. Hydrogen EFCs have been successfully developed with gas-diffusion electrodes, like air-breathing cathodes or H_2 -breathing anodes, which make it possible to increase the availability of dissolved O_2 and H_2 in quiescent solutions. This is an essential development for portable EFCs used outside of the body. Owing to electrode nanostructuring, and improvements in enzyme orientation and electrical wiring, great improvements in power densities have been achieved, which can match, or even surpass, the performance of glucose EFCs. However, the use of hydrogen for EFCs confronts us with problems of H_2 storage and transport. This is in contrast to the use of glucose, which

is a safe, abundant and easy-to-handle energy compound. Hydrogen can be physically stored as either a gas or a liquid but generally requires high pressure and large volume tanks. Practical high density hydrogen storage remains a major challenge for EFCs dedicated to portable applications.

How to define EFC power?

One of the problematic points for the comparison and continuous optimization of EFC performance concerns their power output and how it is defined. As already pointed out by Katz and coworkers, the power density of an EFC is an important parameter that must be associated with the real power generated by the EFC in order to determine which applications can actually be considered [32]. There are a multitude of electronic and wireless electrical products that consume microwatt to milliwatt powers during operation. Many electronic devices have multiple power modes such as a “standby” mode, where low power is consumed, and “active modes”, for example, for data transmission. High milliwatt powers, for example, may therefore only be required for very short periods of peak activity. Future advances in ultra-low power and low voltage electronics in the next decade are expected which will further valorise energy-harvesting devices with μW to mW outputs. For instance, an ultra-low power 3.5 μW -consuming cell phone without a battery is under development [33].

In the research field of biological fuel cells, power densities can be misleading. For example, microwatt power densities obtained using microelectrodes seem extremely attractive, but in reality, are almost useless when the actual power output is on the order of nanowatts. Another issue for bioelectrodes is that the power does not evolve proportionally to the surface area owing to the electrode morphology that plays, for example, on the continuous supply and removal of reactants and products. The power is highly dependent on electrode morphology, including the structure and thickness of the adsorbed enzymes, and the crucial mass transport and charge propagation processes that occur inside and outside of the biocatalyst layer(s) [34].

Regarding the electrode architecture, which ranges from simple planar electrodes with monolayers of enzymes to 3D-structured mesoporous carbon, CNT pellets, and CNT yarns with embedded enzymes, the electroactive surfaces and enzyme loadings can be vastly different [12*,13]. Despite this, the power densities of biofuel cells are conventionally calculated only with respect to the geometric surface of the electrodes. So what about the fact that most bioelectrodes are now based on 3D architectures with huge differences in electrode thickness and surface area? Another difficulty in defining power also lies sometimes in the design of EFCs that exploit anodes and cathodes with different sizes. For example, a H_2/O_2

185 EFC combines a 6 cm^2 bilirubin oxidase-modified cathode
186 with a 1.2 cm^2 hydrogenase-modified anode to balance
187 the catalytic performance as a function of the H_2 -air
188 mixture used [18**]. The raw power output must surely
189 now be reported in all cases? It now seems valuable to define
190 the volumetric power of bioelectrodes in mW cm^{-3} ,
191 especially if 3D electrodes with thicknesses ranging from
192 micrometres and centimetres are being used. Indeed, we
193 have recently reported the raw power together with the
194 current density of an EFC in both cm^2 and cm^3 [35*].
195 A crucial power definition for the future, which will become
196 more relevant as EFCs emerge for practical device
197 powering, is the volumetric power density of the single
198 or stacked EFC in cm^3 , taking into account the space between
199 the anode(s) and the cathode(s) and the entire cell.
200 For a single EFC, the optimisation of distance between
201 the bioelectrodes, which also plays on the resistance, substrate
202 supply, and by-product formation, will become a key
203 consideration for the power/size ratio.

204 Influence of temperature on the operation of EFCs

205 Besides the difficulty of designing EFCs whose enzymatic
206 reactions at the anode and the cathode must operate at the
207 same pH, another major problem for EFCs concerns the
208 influence of temperature on the rate and stability of enzyme-
209 catalyzed reactions, and hence on EFC performance. This
210 key parameter has often been underestimated or neglected,
211 yet can drastically change the perspectives of EFCs for
212 different applications. Depending on the origin of the enzyme,
213 the temperature/activity profile may show optima ranging from
214 low temperatures ($3\text{--}5\text{ }^\circ\text{C}$) to high temperatures ($50\text{--}80\text{ }^\circ\text{C}$).
215 Regarding the more common glucose/ O_2 EFCs, cathodes are
216 usually based on immobilized bilirubin oxidase or laccase for
217 the reduction of oxygen. Laccase from *Trametes versicolor*
218 exhibits its greatest stability at $30\text{ }^\circ\text{C}$ and undergoes a
219 thermal deactivation above $50\text{ }^\circ\text{C}$, which for example, leads
220 to a loss in activity of 53% and 90% after 6 and 48 hrs,
221 respectively [36]. In contrast, bilirubin oxidase from
222 *Myrothecium verrucaria* is considered relatively stable in
223 the range of $40\text{--}60\text{ }^\circ\text{C}$ but loses 40% of its activity when the
224 temperature decreases from 40 to $25\text{ }^\circ\text{C}$ [37]. At the anode,
225 the oxidation of glucose is mainly catalyzed by glucose
226 oxidase. The latter has a maximum activity at $20\text{--}35\text{ }^\circ\text{C}$ but
227 retains only 65% of its activity at $45\text{ }^\circ\text{C}$, and exhibits total
228 thermal inactivation at $60\text{ }^\circ\text{C}$ [38–40].
229

230 The different thermal behavior of oxidoreductase enzymes
231 is a real problem for the performance and stability of EFCs,
232 especially considering that an EFC must employ two very
233 different enzymes at the anode and cathode. The influence of
234 temperature may call into question the use of EFCs for
235 powering portable electronics exposed to certain environmental
236 conditions with wide variations in temperature. This problem
237 actually becomes an attractive point for implanted biofuel
238 cells, of which one of the main features is their exposure,
239 by definition, to a constant

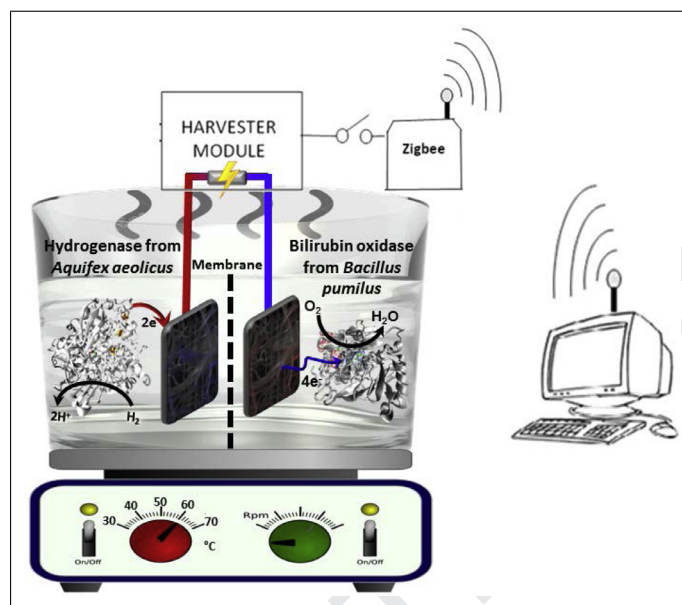
240 physiological temperature of $37\text{ }^\circ\text{C}$. Nevertheless, this does
241 not mean that relevant enzymes are unsuitable for operation
242 at elevated temperatures. In fact, it appears that the power
243 of EFCs can be increased by increasing the temperature, as
244 demonstrated by Lojou and coworkers for H_2/O_2 EFCs using
245 thermostable enzymes such as hydrogenase from *Aquifex
246 aeolicus* and bilirubin oxidase from *Bacillus pumilus* [17*,
247 41]. High power densities have been obtained under these
248 (relatively) mild conditions, making it possible to power a
249 wireless transmission system [42] as illustrated in Figure 1.
250 However, to accurately evaluate the performance and practicality
251 of such hydrogen EFCs, it is necessary to also consider the
252 power required to maintain the EFC at $60\text{ }^\circ\text{C}$. Rather than the
253 possibility for improved EFC power output, the main advantage
254 of this approach lies in the use of thermostable enzymes that
255 allow the EFC to withstand significant temperature variations
256 (e.g. from $30\text{--}80\text{ }^\circ\text{C}$) without denaturation.
257

258 As well as temperature considerations, the type of solution
259 used in the biofuel cell is another critical but often overlooked
260 factor in EFC research. Until now, relatively few studies have
261 investigated EFC performance in matrices for practical
262 applications such as biological fluids, environmental waters or
263 wastewater. The vast majority of experiments have simply
264 been performed in aqueous buffer solutions. Towards human
265 body-integrated applications, recent studies have started to
266 address bioelectrode and EFC performance directly in human
267 serum, urine, saliva and blood [43–47]. These works have
268 highlighted complications that reduce EFC power output and
269 stability due to low substrate concentrations and interferences
270 from ascorbic acid, chloride, uric acid and biophysical matter.
271 Reported strategies to address interferences include the use
272 of a dialysis bag as a protective membrane, a strategy first
273 reported by our group in 2010 for an EFC implanted in a rat
274 [48].
275

276 Buckypaper-based EFCs for wearables and implantable devices

277 Carbon nanotubes are a widely used material in bioelectrode
278 design [6*]. In the last 5–7 years, free-standing thin films of
279 CNTs, called buckypapers, have emerged for use in biofuel
280 cells [49*]. Buckypapers are in fact at the origin of remarkable
281 advances in the field of EFCs [50–53], particularly concerning
282 implanted biofuel cells [51**, 54**]. Buckypaper films are
283 self-supporting macroscopic sheets of entangled CNTs held
284 together by π - π stacking interactions with an average
285 thickness of $5\text{--}200\text{ }\mu\text{m}$. Buckypapers are typically obtained
286 either from a commercial source (e.g. NanoTechLabs Inc.)
287 via roll-to-roll processing, or via conventional vacuum
288 filtration of a dispersion of CNTs in organic or aqueous
289 solution. Compared with classical electrodes based on glassy
290 carbon, buckypapers constitute a break of concept since they
291 are the electrode itself. Furthermore, these electrodes are
292 lighter, more compact and flexible, and more easily processed
293 into
294

Figure 1



Schematic presentation of a H_2/O_2 biofuel cell setup with thermophilic biocatalysts producing sufficient power at elevated temperature for supplying a wireless transmission system.

295 different shapes and sizes compared to electrodes based
 296 on glassy carbon. Until now, buckypapers have mainly
 297 been modified via π -stacking interactions with pyrene
 298 derivatives, which are used for covalent or non-covalent
 299 binding of enzymes and/or mediators, and have led to
 300 high performance bioelectrodes for *in-vivo* biofuel cells.
 301 In particular, Katz and coworkers have highlighted the
 302 practical advantages of buckypaper for implantation com-
 303 pared to traditional bulk electrodes (e.g. glassy carbon),
 304 such as their ideal microscale thickness and tunable di-
 305 mensions [54,55]. Owing to attractive properties such
 306 as their flexibility, Minter and coworkers developed a
 307 miniaturized buckypaper-based lactate/ O_2 EFC embed-
 308 ded in a contact lens for operation in human tears [56].
 309 Besides increasing the length and alignment of CNTs,
 310 another possibility to improve the strength and flexibil-
 311 ity of buckypapers lies with the incorporation of an or-
 312 ganic crosslinker. An elegant approach is to “molecularly”
 313 cross-link the CNTs of buckypaper with bis-pyrene-2,2’-
 314 azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (bis-
 315 pyrene-ABTS), which enhances the mechanical stability
 316 of the buckypaper and gives it redox properties for wiring
 317 multicopper enzymes [57,58]. Furthermore, Cosnier and
 318 coworkers reported a new approach for fabricating robust
 319 and flexible buckypaper bioelectrodes based on the in-
 320 timate association of CNTs and linear polynorbornene
 321 polymers [59,60]. These polymers were functionalized
 322 with pyrene groups, for cross-linking, and activated esters,
 323 for covalent attachment of enzymes and/or molecules for
 324 electrical enzyme wiring. Besides improved flexibility and
 325 handleability, enzyme-modified buckypapers are proving

to be effective bioelectrodes for EFCs and will certainly
 have an important role to play in future implantable or
 wearable EFCs. To improve the limited performance of
 biocathodes, which commonly suffer from oxygen limi-
 tations, we note the cathode design from Atanassov and
 his colleagues that combined a gas-diffusion layer with a
 catalytic buckypaper such that the individual benefits of
 both materials could be exploited [61]. Some examples of
 buckypaper interfaces and their applications are sketched
 in Figure 2.

Perspectives on nanoparticle-based rechargeable EFCs

EFCs are faced with two major technological bottle-
 necks that currently block their development, namely
 their short lifetime, and to a lesser degree, their weak
 power output. The low stability of EFCs is largely re-
 lated to the deactivation of immobilized enzymes, the
 intrinsic catalytic component, and this seems somewhat
 ineluctable. In particular, enzymes have a limited stabil-
 ity that depends on the environment in which they are
 used (temperature, pH, inhibitors...). Weak power out-
 put is related to factors including the sub-optimal fixa-
 tion of enzymes and redox mediators to electrodes, which
 reduces markedly their mobility and flexibility, and in
 some cases, fixes enzymes in poor orientations for elec-
 tron transfer. An attractive solution lies in the devel-
 opment of bioelectrodes based on confined solutions of
 enzymes and supramolecular assemblies of redox medi-
 ators or electron transfer-promoting molecules. The elec-
 trical wiring of enzymes in solution with electrodes form-
 ing a conductive chamber only permeable to the enzyme

Figure 2

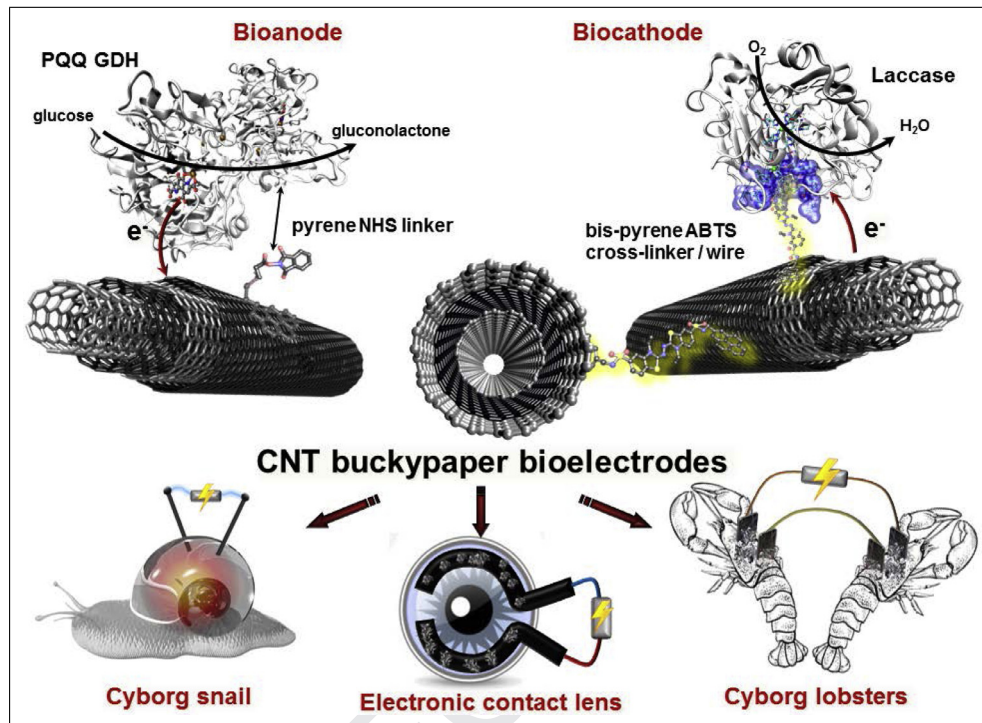
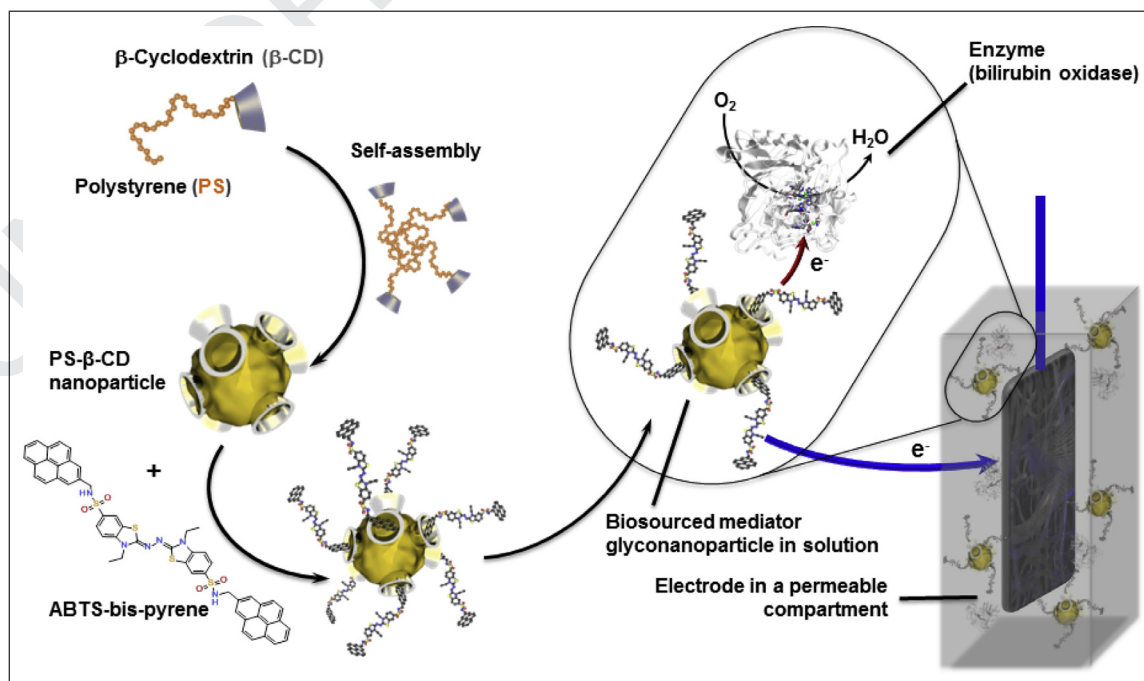


Illustration of buckypaper-based bioelectrodes with various immobilization and enzyme wiring functions (pyrroloquinoline quinone glucose dehydrogenase (PQQ GDH) and laccase) for efficient energy harvesting wearable and implantable biofuel cells.

Figure 3



Scheme illustrating the formation and modification of redox-active glyconanoparticles used for construction of an "all in solution" biocathode.

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substrates is envisaged. For enzymes that require or benefit from the use of a redox mediator, bioelectrocatalysis can be ensured using supramolecular assemblies of redox mediator freely diffusing in this chamber. This strategy will overcome the low operational stability of EFCs by allowing the renewal of solubilized enzymes and redox mediators, leading thus to rechargeable EFCs. This concept can also enhance the electrical wiring process, for example, via rotation and redox-hopping effects, and should lead to increased fuel cell performance. Recently, the first step of this unconventional approach was conceived with biosourced nanoparticles functionalized with β -cyclodextrin groups [62]. For construction of a biocathode, the nanoparticles were modified via host-guest interactions with bis-pyrene-ABTS and used as electron shuttles to facilitate electron transfer between the electrode and bilirubin oxidase in solution (Figure 3). The redox nanoparticles led to enhanced catalytic currents and improved stability for O₂ reduction compared to the same electro-enzymatic system with ABTS in solution [63*].

Conclusion

A significant number of emerging directions for EFCs are now under exploration, such as metabolon systems for cascade reactions of sequential enzymes [64,65**], hybrid fuel cells that seek to combine the advantages of metals and enzymes [66], engineered enzymes with enhanced thermal stability [67], the use of molecular catalysts that mimic enzyme activity with very large turnover frequencies and high stability [68*], and the integration of supercapacitors into fuel cell devices [69]. Another key emerging area concerns biofuel cell power management systems and the incorporation of energy storage systems such as capacitors to prolong biofuel cell lifetime for practical applications [70,53]. The promises and challenges of this exciting research field now require truly interdisciplinary research efforts between chemists, surface scientists, bio-scientists, electronic engineers and materials scientists. Despite the presence of technological obstacles to circumvent, there are real potential applications for EFCs ranging from implantable EFCs, for periodic monitoring of diseases during short periods (1–3 months), to totally biodegradable EFCs for disposable medical devices.

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Declarations

None.

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