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

Serge Cosnier, Andrew Gross, Fabien Giroud, Michael Holzinger

► To cite this version:

Serge Cosnier, Andrew Gross, Fabien Giroud, Michael Holzinger. Beyond the hype surrounding biofuel cells: What's the future of enzymatic fuel cells?. *Current Opinion in Electrochemistry*, 2018, 12, pp.148-155. 10.1016/j.coelec.2018.06.006 . hal-02007139

HAL Id: hal-02007139

<https://hal.science/hal-02007139>

Submitted on 19 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

ELSEVIER

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

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

Address

Département de Chimie Moléculaire, CNRS-University of Grenoble Alpes, 38041, France

*Corresponding author: Cosnier, Serge
(serge.cosnier@univ-grenoble-alpes.fr)

Current Opinion in Electrochemistry 2018, XX:XX–XX

This review comes from a themed issue on Bioelectrochem

Edited by Anna Maria Oliveira Brett

For a complete overview see the Issue and the Editorial

Available online XX XXXX 2018

<https://doi.org/10.1016/j.coelec.2018.06.006>

2451-9103/© 2018 Elsevier B.V. All rights reserved.

Introduction

The development of sustainable and renewable energy sources with greater respect for the environment is a crucial challenge for the 21st century. Among various alternative energy sources, fuel cells have generated great interest for the production of clean electrical energy. Fuel cells generate power through electrochemical reactions via the oxidation of fuels such as hydrogen and alcohols at the anode, and the reduction of oxidants, typically oxygen, at the cathode. Fuel cells will produce electricity with heat and water as by-products providing that fuel and oxidant are available. Enzymatic fuel cells (EFCs) and microbial fuel cells (MFCs) are sub-categories of fuel cells that share similar operational principles for energy production [1,2]. 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 generation for powering microelectronic systems such as actuators 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 bacteria or yeast cells, for MFCs, to the higher cost of purified wild-type and engineered enzymes for EFCs. MFCs have been successfully employed as long-term power-generating systems, for example, for self-powered sensors placed in "hostile" environments or remote locations [9]. Although MFCs have successfully powered different generations of robots, this approach remains at the academic level because of limitations such as the large volume 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 opinion article attempts to shed light on some hitherto neglected facets of EFCs, to briefly revisit their possible applications, and to reveal new insights concerning future 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 electrodes include the hierarchically porous MgO-templated carbon and carbon nanotube (CNT) yarn textile electrodes [12,13].

2 Bioelectrochem

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 nanostructuration, 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

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

Influence of temperature on the operation of EFCs

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

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

stant physiological temperature of 37 °C. Nevertheless, this does not mean that relevant enzymes are unsuitable for operation at elevated temperatures. In fact, it appears that the power of EFCs can be increased by increasing the temperature, as demonstrated by Lojou and coworkers for H_2/O_2 EFCs using thermostable enzymes such as hydrogenase from *Aquifex aeolicus* and bilirubin oxidase from *Bacillus pumilus* [17[•],41]. High power densities have been obtained under these (relatively) mild conditions, making it possible to power a wireless transmission system [42] as illustrated in Figure 1. However, to accurately evaluate the performance and practicality of such hydrogen EFCs, it is necessary to also consider the power required to maintain the EFC at 60 °C. Rather than the possibility for improved EFC power output, the main advantage of this approach lies in the use of thermostable enzymes that allow the EFC to withstand significant temperature variations (e.g. from 30–80 °C) without denaturation.

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

Buckypaper-based EFCs for wearables and implantable devices

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

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.

different shapes and sizes compared to electrodes based on glassy carbon. Until now, buckypapers have mainly been modified via π -stacking interactions with pyrene derivatives, which are used for covalent or non-covalent binding of enzymes and/or mediators, and have led to high performance bioelectrodes for *in-vivo* biofuel cells. In particular, Katz and coworkers have highlighted the practical advantages of buckypaper for implantation compared to traditional bulk electrodes (e.g. glassy carbon), such as their ideal microscale thickness and tunable dimensions [54,55]. Owing to attractive properties such as their flexibility, Minter and coworkers developed a miniaturized buckypaper-based lactate/ O_2 EFC embedded in a contact lens for operation in human tears [56]. Besides increasing the length and alignment of CNTs, another possibility to improve the strength and flexibility of buckypapers lies with the incorporation of an organic crosslinker. An elegant approach is to “molecularly” cross-link the CNTs of buckypaper with bis-pyrene-2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (bis-pyrene-ABTS), which enhances the mechanical stability of the buckypaper and gives it redox properties for wiring multicopper enzymes [57,58]. Furthermore, Cosnier and coworkers reported a new approach for fabricating robust and flexible buckypaper bioelectrodes based on the intimate association of CNTs and linear polynorbornene polymers [59,60]. These polymers were functionalized with pyrene groups, for cross-linking, and activated esters, for covalent attachment of enzymes and/or molecules for electrical enzyme wiring. Besides improved flexibility and 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 limitations, 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 bottlenecks 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 related to the deactivation of immobilized enzymes, the intrinsic catalytic component, and this seems somewhat ineluctable. In particular, enzymes have a limited stability that depends on the environment in which they are used (temperature, pH, inhibitors...). Weak power output is related to factors including the sub-optimal fixation of enzymes and redox mediators to electrodes, which reduces markedly their mobility and flexibility, and in some cases, fixes enzymes in poor orientations for electron transfer. An attractive solution lies in the development of bioelectrodes based on confined solutions of enzymes and supramolecular assemblies of redox mediators or electron transfer-promoting molecules. The electrical wiring of enzymes in solution with electrodes forming 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.

6 Bioelectrochem

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 O_2 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.

Acknowledgement

The authors are grateful to the Agence Nationale de la Recherche for funding (ANR-15-JTIC-0002-01).

Declarations

None.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- Paper of special interest
- Paper of outstanding interest.

1. Zhao C, Gai P, Song R, Chen Y, Zhang J, Zhu J-J: **Nanostructured material-based biofuel cells: recent advances and future prospects.** *Chem Soc Rev* 2017, **46**:1545–1564.
2. Santoro C, Arbizzani C, Erable B, Ieropoulos I: **Microbial fuel cells: from fundamentals to applications. A review.** *J Power Sources* 2017, **356**:225–244.
3. Bandothkar AJ: **Review—wearable biofuel cells: Past, present and future.** *J Electrochem Soc* 2017, **164**:H3007–H3014.
4. Rasmussen M, Abdellaoui S, Minter SD: **Enzymatic biofuel cells: 30 years of critical advancements.** *Biosens Bioelectron* 2016, **76**:91–102.
5. Bandothkar AJ, You J-M, Kim N-H, Gu Y, Kumar R, Mohan AMV, Kurniawan J, Imani S, Nakagawa T, Parish B, Parthasarathy M, Mercier PP, Xu S, Wang J: **Soft, stretchable, high power density electronic skin-based biofuel cells for scavenging energy from human sweat.** *Energy Environ Sci* 2017, **10**:1581–1589.
6. Cosnier S, Gross AJ, Goff AL, Holzinger M: **Recent advances on enzymatic glucose/oxygen and hydrogen/oxygen biofuel cells: achievements and limitations.** *J. Power Sources* 2016, **325**:252–263.
7. Ieropoulos IA, Stinchcombe A, Gajda I, Forbes S, Merino-Jimenez I, Pasternak G, Sanchez-Herranz D, Greenman J: **Pee power urinal – Microbial fuel cell technology field trials in the context of sanitation.** *Environ. Sci. Water Res. Technol.* 2016, **2**:336–343.
8. Tommasi T, Lombardelli G: **Energy sustainability of microbial fuel cell (MFC): a case study.** *J Power Sources* 2017, **356**:438–447.
9. Pasternak G, Greenman J, Ieropoulos IA: **Self-powered, autonomous biological oxygen demand biosensor for online water quality monitoring.** *Sens Actuators B Chem* 2017, **244**:815–822.
10. Mazurenko I, de Poulpique A, Lojou E: **Recent developments in high surface area bioelectrodes for enzymatic fuel cells.** *Curr Opin Electrochem* 2017, **5**:74–84.
11. Saboe PO, Conte E, Farrell M, Bazan GC, Kumar M: **Biomimetic and bioinspired approaches for wiring enzymes to electrode interfaces.** *Energy Environ Sci* 2017, **10**:14–42.
12. Tsujimura S, Murata K, Akatsuka W: **Exceptionally high glucose current on a hierarchically structured porous carbon electrode with “Wired” flavin adenine dinucleotide-dependent glucose dehydrogenase.** *J Am Chem Soc* 2014, **136**:14432–14437.
13. Kwon CH, Lee S-H, Choi Y-B, Lee JA, Kim SH, Kim H-H, Spinks GM, Wallace GG, Lima MD, Kozlov ME, Baughman RH, Kim SJ: *Nat Commun* 2014, **5**:3928.
14. Mano N: **A. de Poulpique, ^{O2} reduction in enzymatic biofuel cells.** *Chem Rev* 2017, **5**:2392–2468.
15. Cosnier S, Goff AL, Holzinger M: **Towards glucose biofuel cells implanted in human body for powering artificial organs: review.** *Electrochem Commun* 2014, **38**:19–23.
16. Reuillard B, Abreu C, Lalaoui N, Le Goff A, Holzinger M, Ondel O, Buret F, Cosnier S: **One-year stability for a glucose/oxygen biofuel cell combined with pH reactivation of the laccase/carbon nanotube biocathode.** *Bioelectrochemistry* 2015, **106**:73–76.

17. Mazurenko I, Wang X, de Poulpique A, Lojou E: **H₂/O₂ enzymatic fuel cells: from proof-of-concept to powerful devices.** *Sustain Energy Fuels* 2017, **1**:1475–1501.
- This review summarizes the latest advances in the field of H₂/O₂ EFCs in terms of hydrogenase immobilization, hydrogenase structure, electrode nanostructuring, enzyme engineering, and the use of thermostable enzymes
18. Xu L, Armstrong FA: **Pushing the limits for enzyme-based membrane-less hydrogen fuel cells – achieving useful power and stability.** *RSC Adv* 2014, **5**:3649–3656.
- This paper describes “3D” porous hydrogenase electrodes combined in an EFC stack design that operates in parallel or in series
19. Jones AK, Sillery E, Albracht SPJ, Armstrong FA: **Direct comparison of the electrocatalytic oxidation of hydrogen by an enzyme and a platinum catalyst.** *Chem Commun* 2002, **0**:866–867.
20. Alonso-Lomillo MA, Rüdiger O, Maroto-Valiente A, Velez M, Rodríguez-Ramos I, Muñoz FJ, Fernández VM, De Lacey AL: **Hydrogenase-coated carbon nanotubes for efficient H₂ oxidation.** *Nano Lett* 2007, **7**:1603–1608.
21. Ciaccavava A, Infossi P, Ilbert M, Guiral M, Lecomte S, Giudici-Orticoni MT, Lojou E: **Electrochemistry, AFM, and PM-IRRA spectroscopy of immobilized hydrogenase: Role of a hydrophobic helix in enzyme orientation for efficient H₂ oxidation.** *Angew Chem Int Ed* 2012, **51**:953–956.
22. Liu J, Wu W-J, Fang F, Zorin NA, Chen M, Qian D-J: **Immobilization of hydrogenase on carbon nanotube polyelectrolytes as heterogeneous catalysts for electrocatalytic interconversion of protons and hydrogen.** *J Nanoparticle Res* 2016, **18**:220.
23. Karyakin AA, Morozov SV, Karyakina EE, Varfolomeyev SD, Zorin NA, Cosnier S: **Hydrogen fuel electrode based on bioelectrocatalysis by the enzyme hydrogenase.** *Electrochem Commun* 2002, **4**:417–420.
24. Morozov SV, Vignais PM, Cournac L, Zorin NA, Karyakina EE, Karyakin AA, Cosnier S: **Bioelectrocatalytic hydrogen production by hydrogenase electrodes.** *Int J Hydrog Energy* 2002, **27**:1501–1505.
- This is the first report of a redox polypyrrole-hydrogenase electrode. This paper also demonstrates the production of hydrogen by mass spectrometry and electrochemistry
25. Morozov SV, Voronin OG, Karyakina EE, Zorin NA, Cosnier S, Karyakin AA: **Tolerance to oxygen of hydrogen enzyme electrodes.** *Electrochem Commun* 2006, **8**:851–854.
26. Karyakin AA, Morozov SV, Voronin OG, Zorin NA, Karyakina EE, Fateyev VN, Cosnier S: **The limiting performance characteristics in bioelectrocatalysis of hydrogenase enzymes.** *Angew Chem Int Ed* 2007, **46**:7244–7246.
27. Pandelia M-E, Fourmond V, Tron-Infossi P, Lojou E, Bertrand P, Léger C, Giudici-Orticoni M-T, Lubitz W: **Membrane-bound hydrogenase I from the hyperthermophilic bacterium *aquifex aeolicus*: enzyme activation, redox intermediates and oxygen tolerance.** *J Am Chem Soc* 2010, **132**:6991–7004.
28. Vincent KA, Cracknell JA, Lenz O, Zebger I, Friedrich B, Armstrong FA: **Electrocatalytic hydrogen oxidation by an enzyme at high carbon monoxide or oxygen levels.** *Proc Natl Acad Sci* 2005, **102**:16951–16954.
29. Xu L, Armstrong FA: **Optimizing the power of enzyme-based membrane-less hydrogen fuel cells for hydrogen-rich H₂-air mixtures.** *Energy Environ Sci* 2013, **6**:2166–2171.
30. Plumeré N, Rüdiger O, Oughli AA, Williams R, Vivekananthan J, Pöller S, Schuhmann W, Lubitz W: **A redox hydrogel protects hydrogenase from high-potential deactivation and oxygen damage.** *Nat Chem*, vol 6 2014.
31. Fourmond V, Stapf S, Li H, Buesen D, Birrell J, Rüdiger O, Lubitz W, Schuhmann W, Plumeré N, Léger C: **Mechanism of protection of catalysts supported in redox hydrogel films.** *J Am Chem Soc* 2015, **137**:5494–5505.
32. Gamella M, Koushanpour A, Katz E: **Biofuel cells – activation of micro- and macro-electronic devices.** *Bioelectrochemistry* 2018, **119**:33–42.
33. Talla V, Kellogg B, Gollakota S, Smith JR: **Battery-Free Cellphone.** *Proc ACM Interact Mob Wearable Ubiquitous Technol*, vol 1 2017 25:1–25:20.
34. Rajendran L, Kirthiga M, Laborda E: **Mathematical modeling of nonlinear reaction-diffusion processes in enzymatic biofuel cells.** *Curr Opin Electrochem* 2017, **1**:121–132.
35. Gross AJ, Chen X, Giroud F, Abreu C, Le Goff A, Holzinger M, Cosnier S: **A high power buckypaper biofuel cell: exploiting 1,10-phenanthroline-5,6-dione with FAD-dependent dehydrogenase for catalytically-powerful glucose oxidation.** *ACS Catal* 2017, **7**:4408–4416.
- This report demonstrates the preparation of lab-made buckypaper electrodes which exhibit excellent voltammetry and their use for the construction of a high power glucose/O₂ biofuel cell with the emerging FAD-GDH enzyme at the anode
36. Stoilova I, Krastanov A, Stanchev V: **Properties of crude laccase from *Trametes versicolor* produced by solid-substrate fermentation.** *Adv Biosci Biotechnol* 2010, **01**:208.
37. Tasca F, Farias D, Castro C, Acuna-Rougier C, Antiochia R: **Bilirubin oxidase from *Myrothecium verrucaria* physically adsorbed on graphite electrodes. Insights into the alternative resting form and the sources of activity loss.** *PLoS One*, vol 10 2015 e0132181.
38. Gouda MD, Singh SA, Rao AGA, Thakur MS, Karanth NG: **Thermal inactivation of glucose oxidase: mechanism and stabilization using additives.** *J Biol Chem* 2003, **278**:24324–24333.
39. El-Sherbeny GA, Shindia AA, Sherif YMMM: **Optimization of various factors affecting glucose oxidase activity produced by *Aspergillus niger*.** *Int J Agric Biol* 2005, **7**:953–958.
40. Singh J, Verma N: **Glucose oxidase from *Aspergillus niger*: Production, characterization and immobilization for glucose oxidation.** *Adv Appl Sci Res* 2013, **4**:250–257.
41. de Poulpique A, Ciaccavava A, Gadiou R, Gounel S, Giudici-Orticoni MT, Mano N, Lojou E: **Design of a H₂/O₂ biofuel cell based on thermostable enzymes.** *Electrochem Commun* 2014, **42**:72–74.
42. Monsalve K, Mazurenko I, Lalaoui N, Le Goff A, Holzinger M, Infossi P, Nitsche S, Lojou JY, Giudici-Orticoni MT, Cosnier S, Lojou E: **A H₂/O₂ enzymatic fuel cell as a sustainable power for a wireless device.** *Electrochem Commun* 2015, **60**:216–220.
43. Cadet M, Gounel S, S-Chaumeil C, Brilland X, Rouhana J, Louerat F, Mano N: **An enzymatic glucose/O₂ biofuel cell operating in human blood.** *Biosens Bioelectron* 2016, **83**:60–67.
44. Kwon CH, Lee JA, Choi Y-B, Kim H-H, Spinks GM, Lima MD, Baughman RH, Kim SJ: **Stability of carbon nanotube yarn biofuel cell in human body fluid.** *J Power Sources* 2015, **286**:103–108.
45. Milton RD, Lim K, Hickey DP, Minter SD: **Employing FAD-dependent glucose dehydrogenase within a glucose/oxygen enzymatic fuel cell operating in human serum.** *Bioelectrochemistry* 2015, **106**:56–63.
46. Ó Conghaile P, Falk M, MacAodha D, Yakovleva ME, Gonaus C, Peterbauer CK, Gorton L, Shleev S, Leech D: **A fully enzymatic membrane-less glucose/oxygen fuel cell provides 0.275 mA cm⁻² in 5 mM glucose operates in human physiological solutions and powers transmission of sensing data.** *Anal Chem* 2016, **88**:2156–2163.
47. Gobel G, Beltran ML, Mundhenk J, Heinlein T, Schneider J, Lisdat F: **Operation of a carbon nanotube-based glucose/oxygen biofuel cell in human body liquids-performance factors and characteristics.** *Electrochim Acta* 2016, **218**:278–284.
48. Cinquin P, Gondran C, Giroud F, Mazabrard S, Pellissier A, Boucher F, Alcaraz J-P, Gorgy K, Lenouvel F, Mathé S, Porcu P,

8 Bioelectrochem

- 614 Cosnier S: **A glucose bioFuel cell implanted in rats.** *PLOS ONE* 2010, **5**:e10476. 668
- 615 49. Gross A, Holzinger M, Cosnier S: **Buckypaper bioelectrodes: 669**
- 616 • **Emerging materials for implantable and wearable biofuel cells.** 670
- 617 *Energy Environ Sci* 2018. <https://doi.org/10.1039/C8EE00330K>. 671
- 618 This paper is the first review on buckypapers in the field of 672
- 619 (bio)electrochemistry and biofuel cell research for body-integrated 673
- 620 applications 674
- 621 50. Villarrubia CWN, Soavi F, Santoro C, Arbizzani C, Serov A, 675
- 622 Rojas-Carbonell S, Gupta G, Atanassov P: **Self-feeding paper 676**
- 623 **based biofuel cell/self-powered hybrid μ -supercapacitor 677**
- 624 **integrated system.** *Biosens Bioelectron* 2016, **86**:459–465. 678
- 625 51. Halámková L, Halámek J, Bocharova V, Szczupak A, Alfonta L, 679
- 626 •• Katz E: **Implanted biofuel cell operating in a living snail.** *J Am 680*
- 627 *Chem Soc* 2012, **134**:5040–5043. 681
- 628 This was the first example of an EFC based on commercial buckypaper 682
- 629 implanted in a live animal in a natural environment. The power was 683
- 630 demonstrated continuously over 2 weeks and could be metabolically 684
- 631 recharged upon feeding or resting of the snail 685
- 632 52. Fokina O, Eipper J, Winandy L, Kerzenmacher S, Fischer R: 686
- 633 **Improving the performance of a biofuel cell cathode with 687**
- 634 **laccase-containing culture supernatant from *pycnoporus 688***
- 635 **sanguineus.** *Bioresour Technol* 2015, **175**:445–453. 689
- 636 53. Hou C, Liu A: **An integrated device of enzymatic biofuel cells 690**
- 637 **and supercapacitor for both efficient electric energy 691**
- 638 **conversion and storage.** *Electrochim Acta* 2017, **245**:303–308. 692
- 639 54. MacVittie K, Halámek J, Halámková L, Southcott M, Jemison WD, 693
- 640 •• Lobel R, Katz E: **From “cyborg” lobsters to a pacemaker 694**
- 641 **powered by implantable biofuel cells.** *Energy Environ Sci* 2013, 695
- 642 **6**:81–86. 696
- 643 The first paper to address the problem of interfacing electronics with 697
- 644 implantable glucose/O₂ EFCs. This paper highlighted the unfortunate 698
- 645 issue of tissue conductivity which prevented biofuel cell operation in 699
- 646 series in the same organism. Successful activation of a watch was 700
- 647 nevertheless demonstrated from in-vivo fuel cells in multiple organisms 701
- 648 55. MacVittie K, Conlon T, Katz E: **A wireless transmission system 702**
- 649 **powered by an enzyme biofuel cell implanted in an orange.** 703
- 650 *Bioelectrochemistry* 2015, **106**:28–33. 704
- 651 56. Reid RC, Minter SD, Gale BK: **Contact lens biofuel cell tested in 705**
- 652 **a synthetic tear solution.** *Biosens Bioelectron* 2015, **68**:142–148. 706
- 653 57. Bourourou M, Elouarzaki K, Holzinger M, Agnès C, Le Goff A, 707
- 654 Reverdy-Bruas N, Chaussy D, Party M, Maaref A, Cosnier S: 708
- 655 **Freestanding redox buckypaper electrodes from multi-wall 709**
- 656 **carbon nanotubes for bioelectrocatalytic oxygen reduction via 710**
- 657 **mediated electron transfer.** *Chem Sci* 2014, **5**:2885–2888. 711
- 658 58. Elouarzaki K, Bourourou M, Holzinger M, Goff AL, Marks RS, 712
- 659 Cosnier S: **Freestanding HRP-GOx redox buckypaper as an 713**
- 660 **oxygen-reducing biocathode for biofuel cell applications.** 714
- 661 *Energy Environ Sci* 2015, **8**:2069–2074. 715
- 662 59. Cosnier S, Haddad R, Moatsou D, O'Reilly RK: 716
- 663 **Biofunctionalizable flexible bucky paper by combination of 717**
- 664 **multi-walled carbon nanotubes and polynorbornene-pyrene -**
- 665 **Application to the bioelectrocatalytic reduction of oxygen.**
- 666 *Carbon* 2015, **93**:713–718.
- 667 60. Gross AJ, Robin MP, Nedellec Y, O'Reilly RK, Shan D, Cosnier S: 668
- 668 **Robust bifunctional buckypapers from carbon nanotubes and 669**
- 669 **polynorbornene copolymers for flexible engineering of 670**
- 670 **enzymatic bioelectrodes.** *Carbon* 2016, **107**:542–547. 671
- 671 61. Ciniciato GPMK, Lau C, Cochrane A, Sibbett SS, Gonzalez ER, 672
- 672 Atanassov P: *Electrochim Acta* 2012, **82**:208–213. 673
- 673 62. Gross AJ, Haddad R, Travelet C, Reynaud E, Audebert P, Borsali R, 674
- 674 Cosnier S: **Redox-active carbohydrate-coated nanoparticles: 675**
- 675 **self-assembly of a cyclodextrin-polystyrene glycopolymer with 676**
- 676 **tetrazine-naphthalimide.** *Langmuir* 2016, **32**:11939–11945. 677
- 677 63. Gross AJ, Chen X, Giroud F, Travelet C, Borsali R, Cosnier S: 678
- 678 • **Redox-active glyconanoparticles as electron shuttles for 679**
- 679 **mediated electron transfer with bilirubin oxidase in solution.** *J 680*
- 680 *Am Chem Soc* 2017, **139**:16076–16079. 681
- 681 The first enzyme wiring by organic redox nanoparticles is described and 682
- 682 demonstrated for O₂ reduction via mediated electron transfer. This 683
- 683 paper reports the self-assembly, characterization and 684
- 684 bioelectrocatalysis of β -cyclodextrin-based nanoparticles with enzyme 685
- 685 in solution 686
- 686 64. Liu Y, Hickey DP, Guo J-Y, Earl E, Abdellaoui S, Milton RD, 687
- 687 Sigman MS, Minter SD, Calabrese Barton S: **Substrate 688**
- 688 **channeling in an artificial metabolon: a molecular dynamics 689**
- 689 **blueprint for an experimental peptide bridge.** *ACS Catal* 2017, 690
- 690 **7**:2486–2493. 691
- 691 65. Wheelodon I, Minter SD, Banta S, Barton SC, Atanassov P, 692
- 692 •• Sigman M: **Substrate channelling as an approach to cascade 693**
- 693 **reactions.** *Nat Chem* 2016, **8**:299–309. 694
- 694 This work describes different natural and non-natural approaches for 695
- 695 channeling substrates between enzyme active sites for cascade 696
- 696 reactions for catalyzing sequential reactions 697
- 697 66. Majdecka D, Draminska S, Janusek D, Krysinski P, Bilewicz R: **A 698**
- 698 **self-powered biosensing device with an integrated hybrid 699**
- 699 **biofuel cell for intermittent monitoring of analytes.** *Biosens 700*
- 700 *Bioelectron* 2018, **102**:383–388. 701
- 701 67. K. Sode, K. Mori, Glucose dehydrogenase, US Pat., 8999691B2, 702
- 702 2015. 703
- 703 68. Gentil S, Lalaoui N, Dutta A, Nedellec Y, Cosnier S, Shaw WJ, 704
- 704 • Artero V, Le Goff A: **Carbon-nanotube-supported bio-inspired 705**
- 705 **nickel catalyst and its integration in hybrid hydrogen/air fuel 706**
- 706 **cells.** *Angew Chem Int Ed* 2017, **56**:1845–1849. 707
- 707 The most efficient example of a hybrid H₂/O₂ EFC to date which exploits 708
- 708 a bioinspired complex at the anode with an enzymatic biocathode 709
- 709 69. Xiao X, Conghaile PÓ, Leech D, Ludwig R, Magner E: **A symmetric 710**
- 710 **supercapacitor/biofuel cell hybrid device based on 711**
- 711 **enzyme-modified nanoporous gold: an autonomous pulse 712**
- 712 **generator.** *Biosens. Bioelectron* 2017, **90**:96–102. 713
- 713 70. Abreu C, Nedellec Y, Gross AJ, Ondel O, Buret F, Le Goff A, 714
- 714 Holzinger M, Cosnier S: **Assembly and stacking of flow-through 715**
- 715 **enzymatic bioelectrodes for high power glucose fuel cells.** 716
- 716 *ACS Appl Mater Interfaces* 2017, **9**:23836–23842. 717