Photochemical & Photobiological Sciences

Purple bacteria photo-bioelectrochemistry: enthralling challenges and opportunities

Journal:	*Photochemical & Photobiological Sciences*
Manuscript ID	PP-PER-11-2019-000470.R1
Article Type:	Perspective
Date Submitted by the Author:	25-Jan-2020
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PERSPECTIVES

Purple bacteria photo-bioelectrochemistry: enthralling challenges and opportunities

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Purple non-sulfur bacteria are anoxygenic photosynthetic microorganisms characterized by extremely versatile metabolisms, allowing them to grow in a broad variety of conditions as well as in the presence of different contaminants. This characteristic motivates the interest in their employment in photo-bioelectrochemical systems applicable in environments with dynamic physico-chemical properties. While the photochemistry of purple bacteria has been intensively studied, their photo-bioelectrochemistry and extracellular electron transfer process with an electrode surface remain largely unexplored. Herein, the process of harvesting electrons from intact purple bacteria is reviewed, and the perspective of enthralling future research possibilities is presented, placing emphasis on the major challenges in the photo-bioelectrochemistry of purple bacteria.

Introduction

Photosynthetic bacteria offer the outstanding possibility to harvest sunlight through an elegant and fascinating molecular assembly: the photosystem. This system is comprised of light harvesting complexes ("antenna complex"), which are responsible for light (photons) absorption, and a "reaction center" where separation of charge occurs.[1, 2] These bacteria can be classified as oxygenic, such as cyanobacteria that absorb photons to carry out charge separation of water and metabolize carbon dioxide into energy-rich organic compounds (photoautotrophic metabolism);[3] or anoxygenic, such as purple bacteria that depend on organic substrates as photosynthetic electron donors (photoheterotrophic metabolism). Both types present specific advantages, enabling their application in the development of multiple biotechnologies. Specifically, cyanobacterial photochemistry and photo-bioelectrochemistry have been investigated for sustainable power generation systems and photo-bioelectrosynthesis (the reader is referred to recent literature focused on these topics).[3-8] Purple non-sulfur bacteria present an extremely versatile metabolism,[9] as they can grow both aerobically in the dark by using oxygen as the terminal electron acceptor, and anaerobically in the light by producing hydrogen.[10] Fermentative growth is also possible in the absence of light and oxygen.[11] Furthermore, besides utilizing a broad variety of organic acids, under illumination purple bacteria can also degrade organic compounds that are environmental pollutants, such as benzoic acid, nitrophenols, and halogenated aromatics.[12, 13] In view of this outstanding metabolic versatility, purple bacteria are of great interest for the development of bioelectrochemical systems (BESs) for sun-powered decontamination and monitoring of water environments. This *Perspective* specifically targets the process of harvesting photoexcited electrons from intact purple bacteria and their possible uses in BES, focusing on the genus *Rhodobacter* of the Rhodobacteraceae, with the species *Rhodobacter capsulatus* (*R. capsulatus*) and *Rhodobacter sphaeroides* (*R. sphaeroides*) being the model organisms to study bacterial photosynthesis. First, photochemistry of purple bacteria is briefly introduced to later focus on the less explored photo-bioelectrochemistry. The application of purple bacteria in photo-BESs is discussed to define new research opportunities and provide an outlook on this emerging research field.

(Photo)-bioelectrochemical systems

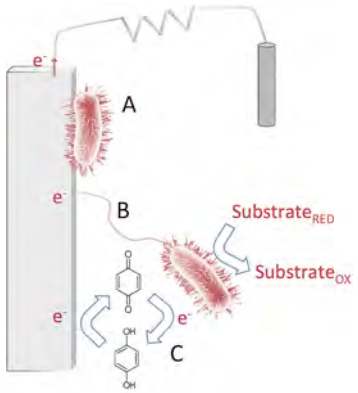

Figure 1. Scheme of the three extracellular electron transfer pathways in bioelectrochemical systems with intact bacterial cells as the biological catalyst for substrate oxidation. Direct extracellular electron transfer through membrane bound

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redox proteins (A), and through conductive nano-wires (B). Mediated extracellular electron transfer through exogenous (or endogenous) redox mediators (C).

A BES is an electrochemical device where biological entities are utilized as catalysts and undergo a bidirectional electron transfer with abiotic components (the electrodes) for remote power generation,[14-16] sensing,[17-25] synthesis purposes,[26-31] and sanitation.[32, 33] Such entities span from enzymes to intact bacterial cells capable of establishing an electrochemical communication (directly, or through the use of redox mediators) with an electrode surface.[34, 35] A scheme of intact bacterial cells and the mechanisms allowing electron transfer in a bioelectrochemical system is depicted in Figure 1, showing different electron transfer pathways.

In bioelectrochemical systems based on isolated enzymes, direct electron transfer has been reported in some cases,[36] with different approaches such as enzyme orientation utilized to facilitate the electron transfer from the active redox site to the electrode surface.[37, 38] Recently, multiprotein architecture was shown to allow the establishment of well-defined interprotein electron pathways.[39] However, for many proteins, the redox sites are buried inside the structure of the protein, making direct electron transfer not feasible or highly hindered. To overcome this limitation, reversible electron mediators are utilized to "shuttle" the electrons from the active sites to the electrode surface (or vice-versa). Such "electron-shuttles" are implemented both as soluble redox compounds (i.e., methyl viologen, quinones, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS))[29, 40, 41] or immobilized in side chains of polymer backbones.[42-45] When intact bacterial cells are utilized instead of enzymes, an additional challenge is posed to the process of harvesting electrons on an electrode surface, since the microbial cell envelope is not electrically conductive. The process of transferring electrons from the active sites of electron transport chains inside the bacterial cells to exogenous electron acceptors (i.e., an electrode surface, minerals, or exogenous redox mediators) is then defined as extracellular electron transfer (EET),[46] and BES relying on this process for power generation are classified as microbial fuel cells (MFCs). As a matter of fact, a detailed understanding of the EET process has been achieved for only a few bacterial species,[47-50] and thus EET remains a critical aspect to be elucidated for the majority of electroactive microorganisms reported in the literature.[51] Furthermore, recent focus has been placed on the role of extracellular polymeric substances on EET processes, underlining the importance of taking into consideration the components surrounding bacterial cells when studying the electron transfer process.[52] Extracellular polymeric substances determine the properties of biofilms and include polysaccharides, extracellular DNA, humic acids, and proteins.[53] It is then clear that future research efforts should be devoted to elucidating the EET processes with particular attention to all the components playing an active role in the process. This is of the utmost importance for the specific case of photosynthetic microorganisms, where little is known with regard to EET, resulting in limited harvesting of the photoexcited electrons and consequently lower current densities when compared to non-photosynthetic organisms.

Photochemistry of purple bacteria

The photosynthetic apparatus of purple bacteria, optimized during years of evolution, has at its core a membrane pigment-protein complex called the reaction center (RC), comprehensively studied and described in excellent literature.[1, 54, 55] A simplified scheme of the photosynthetic electron transfer chain taking place in the photosynthetic apparatus is reported in Figure 2. The process begins once light energy (photons, *hv*) is captured by light harvesting complexes (LHC), and the excitation energy is transferred to a dimer of bacteriochlorophyll molecules in the RC defined as the "special pair" (P870).

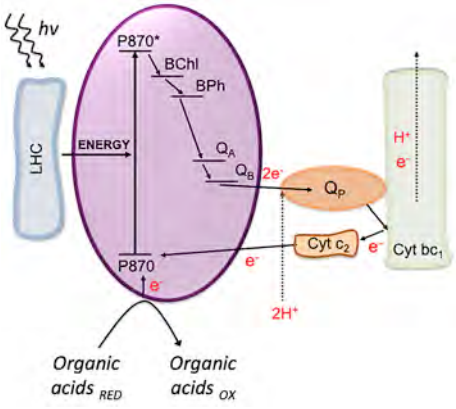

Figure 2. Scheme of the photosynthetic electron transport chain of purple bacteria.

The special pair absorbs at a wavelength of 870 nm, reaching its excited state indicated as P870*. It is after this excitation process that charge-separation can occur, meaning that an electron-hole pair separation is obtained. The primary electron acceptor is a monomeric bacteriochlorophyll (BChl), leaving the special pair in the oxidized state. The electron is then transferred to bacteriopheophytin (BPh) that subsequently transfers it to the primary quinone acceptor Q_A, and finally to the secondary quinone acceptor Q_B, which accepts two electrons. The electron transfer process is accomplished at the cost of a significant loss of energy. A critical aspect to be underlined is that in the RC the electron transfer is performed with a quantum yield of almost unity.[56] The quinone sitting in Q_B is loosely bound,[55] and once it reaches its doubly reduced doubly protonated state (using two protons obtained from the cytoplasm), it is exchanged for the quinone sitting outside the RC in the lipid soluble quinone pool (Q_P). From Q_P, the doubly reduced doubly protonated quinone reaches the binding pocket of cytochrome bc1 where electrons and protons are transferred, leading to a proton gradient that drives ATP synthesis by the ATP synthase enzyme. One electron is transferred from cytochrome bc1 to cytochrome c2 (cyt c2) that finally reduces the oxidized special pair, closing the

photosynthetic electron transfer chain. Under specific conditions (i.e., excess of illumination, organic substrates as electron donor, etc.), an over reduction of the RC can occur. Accordingly, bacterial cells developed alternative "electron sinks" to manage the excess of available electrons.[10, 57] As a result, the delicate redox balancing of the photosynthetic apparatus of *R. capsulatus* is maintained, avoiding negative effects on cell activity. Among the different electron sinks, the primary redox-balancing system under photoheterotrophic growth is through CO_2 fixation with the Calvin-Benson-Bassham (CBB) reductive pentose phosphate pathway. Interestingly, *R. capsulatus* is also capable of CO_2-dependant growth (photoautotrophic metabolism) thanks to the CBB system, which thus has a dual role of maintaining the redox potential of the cells and controlling the carbon metabolism.
Further electron sinks under photoheterotrophic growth are the N_2 fixation process accomplished by the dinitrogenase enzyme complex, and dimethyl sulfoxide reduction with dimethyl sulfoxide reductase. Interestingly, regulation of the different electron sinks has been proposed to take place in response to environmental stresses.[10]

Photo-bioelectrochemistry of purple bacteria

Combining the metabolism of purple bacteria with an electrode as the solid electron acceptor allows for the development of photo-bioelectrochemical systems and provides an "artificial electron sink" for the excess of photoexcited electrons obtained under illumination. To accomplish the goal of harvesting electrons, a pathway for the transfer of photoexcited electrons obtained at the active sites of the RC (the Q_A/Q_B sites) and the Q_P to an electrode surface must be established. However, the RC and the entire photosynthetic machinery is embedded in the inner membrane of purple bacteria, surrounded by a periplasm layer and the outer membrane, posing critical challenges to the process of harvesting the photoexcited electrons. In the following sections, the different architectures utilized to develop biotic/abiotic photo-anodes are discussed, underlining their advantages and limitations. Later, applications of purple bacteria in photo-bioelectrochemical systems are reported, highlighting the opening for future research possibilities.

Purple bacteria direct extracellular electron transfer

The simplest architecture to study the EET process is by directly depositing intact purple bacteria cells on an electrode surface (as schematized in Figure 1A). Photo-bioelectrocatalysis for the oxidation of the reduced electron donors (i.e., malic acid), catalysed by the microorganisms, can be studied by performing electrochemical experiments, such as cyclic voltammetries (CVs) and amperometric i-t curves, in light/dark conditions in a three-electrode electrochemical cell. In a CV, the potential of an electrode (the working electrode, WE) is controlled versus a reference electrode (RE, i.e., saturated calomel electrode, SCE, or silver|silver chloride electrode). The experiment is performed by cycling the potential of the working electrode between two values, as shown in Figure 3A, and measuring the current passing between the WE and a counter electrode (CE, i.e., a Pt mesh). Accordingly, the potential applied to the working electrode can be considered as an excitation signal and the obtained current as the response signal. Conversely, in amperometric i-t curves the potential of the working electrode is fixed at a specific value (E_{app}, Figure 3B), and the resulting current passing between this electrode and the counter electrode is measured over time.[58] This technique is then specifically useful to measure photocurrent generation over time.

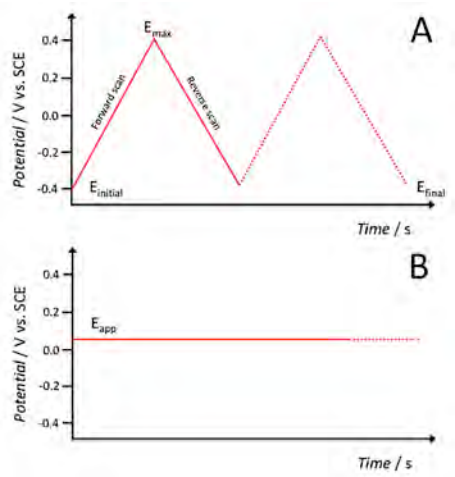

Figure 3. Cyclic voltammetry triangular potential waveform (A) for a scan between -0.4 and +0.4 V vs. SCE. Constant potential applied in amperometric i-t curves (B).

For the study of direct EET, intact purple bacteria cells are deposited on the surface of the WE utilized as a biotic/abiotic photoanode. Reports of *R. capsulatus* direct EET have been performed utilizing both bare-graphite electrodes[59] and carbon paper electrodes[60] as support for the photoanodes. A graphite electrode provides a considerably higher surface area compared to a carbon paper electrode (due to their different roughness). Accordingly, biophotocurrent obtained using graphite electrodes was considerably higher than photoanodes employing carbon paper support, reaching approximately 3 and 0.2 μA cm^{-2}, respectively, while being polarized at potentials higher than +0.3 V vs. SCE. The enhanced direct EET achieved on graphite electrodes was attributed to the interaction between the available quinones in the membrane of *R. capsulatus* cells and the porous electrode surfaces.[59] A similar response in direct EET conditions was obtained for cyanobacteria cells, where photoexcited electrons where harvested from the photosynthetic apparatus through the plastoquinone pool to carbon nanotubes modified electrodes.[4] While the direct EET between intact purple bacteria and abiotic electrode surface is possible, the low currents reported call for artificial approaches to enhance the process, as discussed in the following sections.

Soluble exogenous redox mediators and purple bacteria

Addition of exogenous redox mediators in solution has been proven to successfully mediate the EET for several bacterial species.[61-63] Redox mediators are molecules that can be reversibly oxidized and reduced, thus acting as electron carries among sequential redox reactions.[64] For a redox mediator accepting one electron from an active redox site in the electron transport chain of a bacterial cell, the following general reduction reaction can be considered:

$$Med^{n} + e^{-} \rightarrow Med^{n-1} \quad (1)$$

with Med^{n} indicating the oxidized state of the mediator, and Med^{n-1} indicating its reduced state. The reduced mediator is then re-oxidized at the electrode surface, completing the mediation of the EET process. It is important to note that in addition to being non-biodegradable and non-toxic for the bacteria, a mediator of choice should have a high electron transfer rate, and the bacterial membrane should be permeable to both its oxidized and reduced forms. Accordingly, a scheme of the mechanism of action of an exogenous redox mediator is reported in Figure 4.

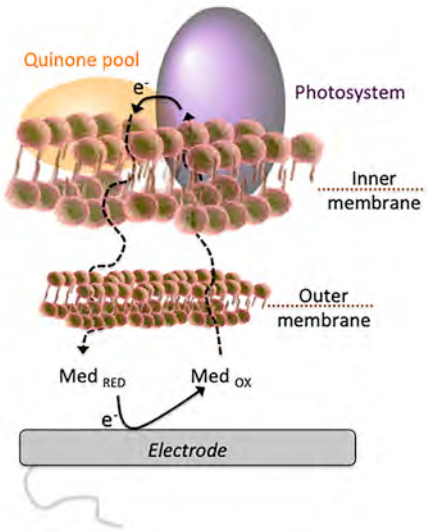

Figure 4. Scheme of the redox mediation mechanism for an exogenous redox mediator undergoing a one-electron transfer with intact purple bacteria. Once the oxidized exogenous mediator (Med_{OX}) diffuses through the outer and inner membranes, it is reduced accepting one electron from reduced endogenous quinones (located in Q_A/Q_B-Q_P). The reduced exogenous mediator (Med_{RED}) diffuses back to the electrode surface, where it is re-oxidized by transferring one electron to the electrode.

Cai et al. performed a pioneering study utilizing scanning electrochemical microscopy (SECM) to investigate the capability of hydrophobic and hydrophilic redox mediators to cross the cellular membranes of *R. sphaeroides* in their reduced state to be oxidized by redox moieties located in the periplasm (for hydrophilic mediators) or the cytoplasm of the cells (for hydrophobic mediators).[65] In SECM experiments, a tip electrode is placed at variable distances from the sample to be analysed (*R. sphaeroides* cells in this case), and the current resulting from the reaction of the reduced (or oxidized) redox mediator at the tip is recorded. A scheme of the setup utilized by Cai et al. is reported in Figure 5. They showed that the selected hydrophobic and hydrophilic redox mediators could cross the inner and the outer membrane, respectively. More interestingly, by measuring the oxidation or reduction rate constant of the various mediators, it was determined that the two classes of redox mediators react with different intracellular redox centers. In fact, a redox mediator is oxidized if its formal redox potential is more negative than the intracellular potential encountered, and the driving force for the reaction is determined by the difference between the two potentials.

Recently, with the aim to further clarify the extracellular electron transfer process taking place between purple bacteria and quinone-based hydrophobic soluble redox mediators, electrochemical experimental evidence was combined with density functional theory (DFT) calculations.[60] Specifically, DFT was utilized to determine the electronic and structural parameters of different quinone forms, which were then utilized for the study of multivariate linear regression relationships. The calculated reduction potential of the single-electron step in the reduction of benzoquinone yielded a direct linear correlation with the photo-biocatalytic current output experimentally obtained with the set of quinone-based mediators utilized. Accordingly, the combination of DFT calculations, multivariate linear regression, and electrochemical experimental evidence allowed unveiling the limiting electron transfer step in the EET process between purple bacteria and an electrode surface, being the proton decoupled, single electron transfer taking place in the lipophilic membrane of the bacterial cells. The result provides important insights for future research focused on enhancing the EET process of purple bacteria, allowing for the rational design of redox mediators, and overcoming the trial-and-error approach often used with exogenous redox mediators.

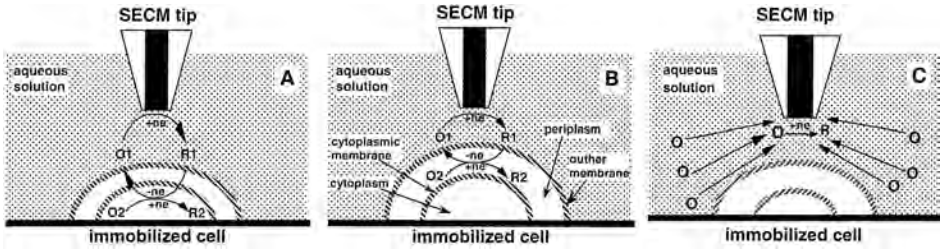

Figure 5. Scheme of the system utilized by Cai et al. to study the interaction between hydrophobic (A) and hydrophilic (B) exogenous redox mediators with *R. sphaeroides* cells by performing SECM experiments. The case of redox mediators not capable of crossing neither the outer nor the inner membrane is also reported (C). *O* indicates oxidized redox mediators; *R* indicates reduced redox mediators; *ne* indicates the number of electrons exchanged between redox moieties. Adapted with permission from C. Cai, B. Liu, M.V. Mirkin. Scanning Electrochemical Microscopy of Living Cells. 3. *Rhodobacter sphaeroides*. Anal. Chem. 2002, 74, 114-119.[65] Copyright 2002 American Chemical Society.

Photoanodes employing soluble redox mediators

The hydrophobic diffusible redox mediator 2,5-dichloro-1,4-benzoquinone was utilized by Kasuno et al. to develop bio-photoanodes with intact *R. sphaeroides* cells, showing the effects of different light intensity, mediator concentrations, and long-term storage of the electrodes on biophotocurrent generation.[66] Commenting on the latter aspect, the authors noted a severe loss of performance (approximately 60%) over a period of 30 days when the cells where stored at 4˚C in the dark. This interesting result merits a critical discussion on the suitability of the use of diffusible soluble redox mediators in bio-photoanodes for practical applications. In recent works, Longatte et al. showed that the long-term use of quinone-based mediators could lead to alteration and inhibition of the photosynthetic chain of unicellular algae,[67, 68] further confirming that this type of redox mediation might not be the preferred choice for long-term operation.

Furthermore, the release of soluble quinone mediators would lead to additional negative effects on aquatic environments due to their toxicity. All these aspects call for the development of novel mediation approaches independent of soluble diffusible quinone-based redox mediators.

Photoanodes employing polymer-confined redox moieties

The research group of Lo Gorton pioneered the use of polymer-confined redox moieties for the mediation of the extracellular electron transfer process between purple bacteria and an electrode surface.[59, 69] Specifically, the group focused on the use of different osmium (Os) redox polymers, where the redox moiety is constituted by the $Os^{3+/2+}$ functionalities located at the end of the side chains of a polymer backbone. In redox polymers, the backbone is nonconductive, and the electrons are shuttled via self-exchange-based conduction.[42] In the first work reporting the use of Os-polymer with *R. capsulatus*,[69] the utilized redox polymer, for which the structural representation is reported in Figure 6, presented a relatively high positive redox potential of +0.176 V vs. SCE. The redox polymer successfully mediated the EET when succinate was utilized as the carbon source for *R. capsulatus* cells under heterotrophic metabolism. The authors reported that the obtained current response (~ 4 μA cm^{-2} on graphite electrodes) was significantly lower compared to other non-photosynthetic microorganisms, and it was shown that the addition of freely diffusing hexacyanoferrate (III) (redox potential +0.191 V vs. SCE) as redox mediator in the presence of the Os-polymer resulted in a consistent increase in current response.

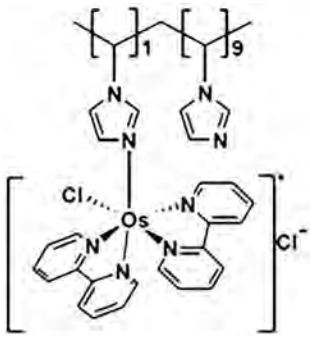

Figure 6. Structural representation of the pioneering Os-redox polymer utilized for mediation of the extracellular electron transfer process of purple bacteria. Reproduced with permission from K. Hasan, S.A. Patil, K. Gorecki, D. Leech, C. Hagerhall, L. Gorton. Electrochemical communication between heterotrophically grown *Rhodobacter capsulatus* with electrodes mediated by an osmium redox polymer. Bioelectrochem. 2013, 93, 30-36.[69] Copyright 2013 Elsevier.

The influence of the photo-heterotrophic metabolism and the use of malic acid as a carbon source was investigated in a later study by the same group.[59] It was reported that by modifying the chemical structure of the redox moiety (substituting H to the Cl bonded to the Os atom) and exposing bacterial cells to light excitation allowed enhancing the current response to ~ 8 μA cm^{-2} (on graphite electrodes) with the photoanode operating at a potential of +0.303 V vs. SCE. The selected potential was required to ensure a sufficient overpotential for electron transfer from the mediator to the electrode. Operating under such conditions results in a consistent loss of available potential if the photoanode is applied in a photo-BES for energy generation, as the operating potential of the anode and cathode determine the available potential difference of the device. With this issue in mind, the use of a bio-inspired redox polymer where naphthoquinone redox moieties having a relatively low redox potential (~ -0.180 V vs. SCE) are utilized to harvest photoexcited electrons from purple bacteria was recently reported.[70] A scheme of the developed system is reported in Figure 7. An important aspect is that electron transfer mediation through the naphthoquinone polymer was significantly affected by the geometry utilized to prepare the biotic/abiotic photoanodes. Specifically, 2-D photoanodes with the polymer immobilized on the surface of carbon paper electrodes and bacterial cells deposited on top of the modified electrode did not allow the efficient diffusion of naphthoquinone moieties through the cellular membrane of *R. capsulatus*. Conversely, controlling the ratio of bacterial cells and redox polymer in a 3-D photoanode retained the capability of the redox moieties to diffuse to the active redox sites of the cells. As a result, the utilized system for redox mediation allowed reducing the overpotential for harvesting photoexcited electrons, operating at +0.073 V vs. SCE. The

report opened new possibilities for the development of biotic/abiotic photoanodes applicable in BES for energy generation as well as self-powered photo-biosensors. However, an important aspect requiring future research efforts is the stability of the photoanode, which showed approximately a 20% decrease in biophotocurrent generation in one hour where two light and dark cycles were alternated.

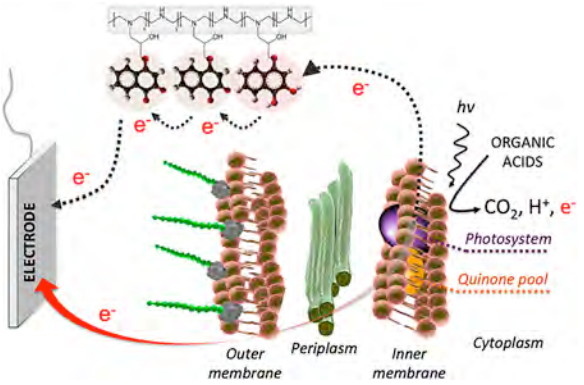

Figure 7. Scheme of the bio-electrocatalytic process allowing for the harvesting of photoexcited electrons through naphthoquinone redox moieties immobilized on a polymer backbone (black dashed arrows). The red arrow indicates the limited direct electron transfer from the active sites/quinone pool to the electrode surface. Reproduced from M. Grattieri, S. Patterson, J. Copeland, K. Klunder, S.D. Minteer. Purple bacteria & 3-D redox hydrogels for bioinspired photo-bioelectrocatalysis. ChemSusChem 2020, 13, 230-237.[70] Copyright 2019 Wiley-VCH Verlag GmbH & Co.

Bioengineering for enhanced direct EET

A very interesting research approach is the engineering of purple bacteria cells to introduce heterologous electron transport pathways to transport electrons across the periplasm and the outer membrane. While such an approach has not yet been explored for purple bacteria, the group of Caroline Ajo-Franklin successfully introduced different features of the electron transfer pathway of *Shewanella oneidensis* MR-1 (*S. oneidensis* MR-1) into *Escherichia coli* (*E. coli*) cells.[71, 72] The major components of the electron transfer pathway of *S. oneidensis* MR-1 are an inner membrane tetraheme cytochrome CymA, a periplasmic decaheme cytochrome MtrA, and two outer membrane decaheme cytochromes OmcA and MtrC. These electron carriers allow a series of intermolecular electron transfers terminating with the reduction of an extracellular electron acceptor. An important aspect that the authors unveiled is the effect of the single features on the enhancement of the EET process as well as cell viability and capability to grow under metal-reducing conditions. Focusing on the engineering of photosynthetic microorganisms to introduce a heterologous electron transfer pathway, Sekar et al. reported the expression of an outer membrane protein involved in the extracellular reduction of iron oxide by *Geobacter sulfurreducens* (cytochrome c, OmcS) in cyanobacteria.[73] The genetically modified *Synechococcus elongatus* generated a nine-fold higher current compared to wild type bacteria (approximately 350 and 40 mA m^{-2}).

Accordingly, bioengineering of purple bacteria could open exciting research opportunities thanks to the high metabolic versatility of these organisms. It remains clear that several challenges remain for the bioengineering of photosynthetic organisms, such as the demand of metabolic resources for foreign protein expression, and the limited synthetic biology tools available for photosynthetic organisms compared to other model organisms.[74]

Purple bacteria application in photo-BES

Purple bacteria have been found on the electrodes of operating MFCs by Xing et al., which then isolated the strain obtaining a close match to the purple non-sulfur bacterium *Rhodopseudomonas palustris* ATCC 17001.[75] The isolated strain designated as *Rhodopseudomonas palustris* DX-1 was tested for power generation in MFCs, showing that the pure culture was able to sustain power densities similar to those of mixed cultures MFCs (in the range of 1000 mW m^{-2}). In this preliminary report, light influence on the current generation and the EET mechanisms were not investigated in detail. A later study by Cao et al. focused on these topics, specifically investigating light/dark effects on the electrochemical performance of MFCs operated with the enrichment of a phototrophic consortium from wastewater.[76] A fourfold increase in power output was obtained under illumination, further rising to an eightfold increase when compared to MFCs operated without the enriched phototrophic community. Microbial community analysis showed that the enriched phototrophic community belonged to the genera *Rhodobacter* and *Rhodopseudomonas*. Furthermore, the appearance of redox peaks associated with a soluble redox couple in cyclic voltammetries performed utilizing the supernatant of the anode medium as the electrolyte revealed the presence of an endogenous redox mediator produced by the microbial community colonizing the anode. It is important to note that other bacteria colonizing the electrode could be responsible for the production of the endogenous mediator, making the possibility of a synergistic effect between phototrophic and heterotrophic microorganisms extremely interesting for the development of BES to be applied in the field.

Recently, a mixed culture enriched in purple bacteria was utilized at a bio-photocathode electrode (rather than a photoanode) for the bioelectrosynthesis of H_2 by Vasiliadou et al.[77] Specifically, the authors utilized a graphite electrode polarized at -0.5 V vs. Ag|AgCl as an electron donor for the purple bacteria community colonizing the electrode surface. Utilizing malic acid as the carbon source, and sodium-glutamate as a nitrogen source, the author showed that purple bacteria successfully utilized the electrode as an electron donor. While the H_2 production rate in the BES was similar to the production through classic biological processes, the application of the phototrophic bacterial community in the BES allowed for CO_2 fixation. Under such conditions, CO_2 emissions were drastically reduced compared to electrode-free biological

processes, elucidating a new bio-electrosynthetic route for H_2 production.

Environmental stress and photo-bioelectrocatalysis

An important aspect to be considered when developing BES for application in the field is that the biological catalyst will be exposed to changing environmental conditions, in comparison to the operation of such devices in a controlled laboratory environment. Some of the environmental stresses that could influence photo-bioelectrochemical performance include changes in temperature or light intensity,[78] lack of substrate, presence of contaminants,[79-81] and operation under continuous flow or batch conditions,[82] among others. The high metabolic versatility of purple bacteria could allow for minimizing the effects of external parameters, ensuring operation under stress conditions. Focusing on this issue, studies have been conducted to elucidate the capability of purple bacteria to cope with salt stress. It was shown that prolonged exposure of *R. capsulatus* to increasing salinity allowed high cell density as well as photo-bioelectrocatalysis for malic acid oxidation under photoheterotrophic metabolism comparable to salt-free conditions.[83] Furthermore, a recent study combined bioinformatic and electrochemical tools to discuss photo-bioelectrocatalysis under saline conditions in views of the metabolism shift resulting from adaptation to salinity. Transcriptome analysis provided evidence for a modified electron flux in the photosynthetic apparatus of cells adapted to salinity, under which the capability of an exogenous redox mediator to harvest photoexcited electrons was also altered.[84] The possibility to artificially tune salt adaptation of *R. capsulatus* was discussed in view of cell organization and biofilm formation. Contribution of the *R. capsulatus* gene transfer agent (rcGTA), allowing an expedited evolution through intercellular gene transfer and quorum sensing autoinducers was also considered. The rcGTA is a phage-like vesicle carrying approximately 4.5kb of double stranded DNA to neighbouring cells,[85] and a scheme of its mechanism of action is reported in Figure 8. Briefly, a cell adapted to the environment can self-lyse, transferring DNA to another live cell through the rcGTA. The DNA is then incorporated into the genome of the new cell, possibly allowing for the accumulation of additional traits facilitating adaptation to the environmental stress. Such a rapid evolutionary mechanism could play a critical role in the successful application of purple bacteria in BES operating in the field. A better understanding of the rcGTA involvement in stress response, as well as of other mechanisms of response is of the utmost importance and could allow for the tuning of purple bacteria photo-bioelectrocatalysis under environmental stresses. Accordingly, the results paved the way for future studies focused on the application of purple bacteria for seawater quality monitoring or sunlight-powered decontamination of saline wastewater.

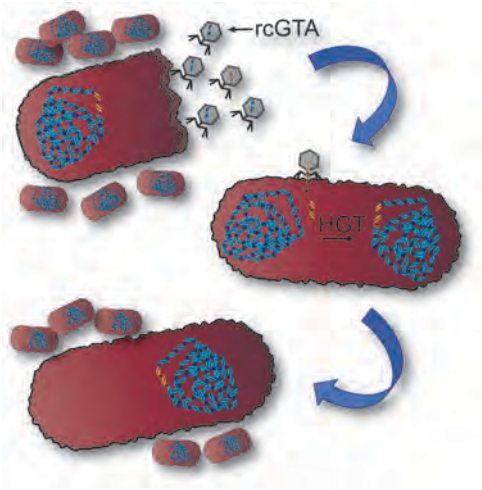

Figure 8. Mechanism of action of the *R. capsulatus* gene transfer agent (rcGTA). Among the random segments of the genome being carried by the rcGTA some could account for the trait leading to adaptation (represented in orange). M. Grattieri, K. Beaver, E. Gaffney, S.D. Minteer. Tuning purple bacteria salt-tolerance for photobioelectrochemical systems in saline environments. Faraday Discuss. 2019, 215, 15-25.[83] – Reproduced by permission of The Royal Society of Chemistry.

The environmental stress of heavy metals contaminants on purple bacteria has been extensively investigated by Trotta and collaborators.[79, 80, 86, 87] Specifically, the detailed cellular responses of *R. sphaeroides* to cobalt, chromium, mercury, nickel, and gold among other heavy metals were reported. A very interesting aspect is that, conversely from the adaptation to salt stress previously discussed, the ability to cope with the presence of these heavy metals was not the result of selection processes, as cells could be exposed to the stress without previous adaptation or selection procedures.[79, 80, 87] Furthermore, the same group explored the capability of *R. sphaeroides* to adapt to high concentrations of heavy metals at a metabolic level.[86] These studies provide an excellent knowledge base for the future development of sunlight-powered photo-bioelectrochemical systems for heavy metals detection based on purple bacteria. Additionally, another extremely interesting finding is that *R. sphaeroides* cells were capable of extracellular synthesis of spherical gold nanoparticles, in response to exposure to Au (III) in the range of 5 – 40 μM.[87] Transmission electron microscopy micrographs of the bacterial cells exposed to 10 μM Au(III) revealing the presence of gold nanoparticles are shown in Figure 9. Coupling of such an outstanding stress response with an electrode surface could open the pathway for the development of photo-BES for enhanced and optimized gold-nanoparticle synthesis. Furthermore, in a recently published work, Zannoni and collaborators reported the capability of *R. capsulatus* to extracellularly produce Tellurium nanoparticles.[88] The authors utilized 2-hydroxy-1,4-naphthoquinone as an exogenous redox mediator, which harvested the photoexcited electrons and acted as an electron shuttle for the reduction of tellurite

(TeO_3^{2-}) to elemental Te^0. These results further support the use of purple bacteria for developing sustainable approaches for the synthesis of different nanoparticles.

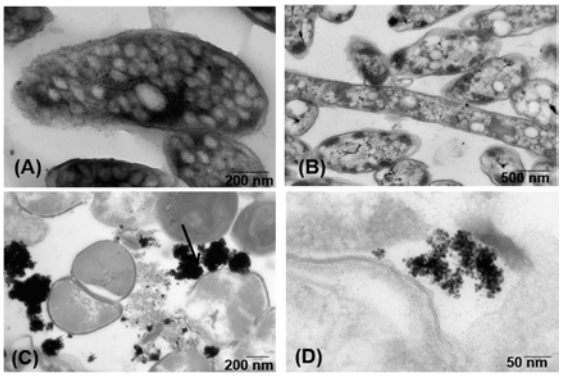

Figure 9. Transmission electron microscopy micrographs of *R. sphaeroides* cells not exposed (A) and exposed to 10 μM Au(III) (B) showing the significant cell elongation obtained after exposure to Au(III). Large extracellular aggregates of gold nanoparticles are shown at two different magnifications (C and D). Reproduced from F. Italiano, A. Agostiano, B.D. Belviso, R. Caliandro, B. Carrozzini, R. Comparelli, M.T. Melillo, E. Mesto, G. Tempesta, M. Trotta. Interaction between the photosynthetic anoxygenic microorganism *Rhodobacter sphaeroides* and soluble gold compounds. From toxicity to gold nanoparticle synthesis. Colloids Surf. B Biointerfaces 2018, 172, 362-371.[87] Copyright 2018 Elsevier.

Summary, conclusions, and future outlook

While purple bacteria have served as the model organisms for studying the photochemistry of photosynthetic organisms, the exploration of their photo-bioelectrochemistry began only recently and can be considered in its infancy. Enhancing the capability to harvest photoexcited electrons from intact purple bacteria cells will play a critical role in the development of photo-bioelectrochemical systems based on these organisms. To achieve this goal, several research challenges remain. First, a detailed understanding of the extracellular electron transfer process and the role of extracellular polymeric substances and the components surrounding bacterial cells must be achieved. Different architectures have been used to study the process of harvesting photoexcited electrons, showing limited current response in the absence of exogenous redox mediators. The use of diffusible redox mediators has provided critical insights into the interaction between the electron shuttle and the active redox sites of purple bacteria. Such a mediating approach, however, is not preferred for the application of bio-photoanodes in BES operating in the field due to loss of performance over time and the unwanted release of soluble mediators having negative effects on aquatic environments. In view of this issue, two new promising research approaches emerge, with the use of redox polymers where the redox moieties are immobilized on the polymer backbone avoiding their release in the environment, or the bioengineering of purple bacteria to introduce heterologous electron transfer pathways.

Furthermore, the capability of purple bacteria to cope and adapt to different environmental stresses could allow for the development of novel BES for on-line monitoring of contaminants, as well as new bio-electrosynthetic approaches for more sustainable production of valuable chemicals and nanoparticles. Therefore, interest in purple bacteria photo-bioelectrochemistry is growing, with several enthralling research opportunities requiring multidisciplinary approaches and collaborative research.

Acknowledgements

I would like to gratefully thank the European Society for Photobiology, particularly the Education and Training Committee, for honouring me with the 2019 Young Investigator Award, and giving me the possibility to contribute this *Perspective*. Additionally, special thanks go to Shelley Minteer and all my collaborators at the University of Utah (in alphabetical order): Kevin Beaver, Erin Gaffney, Kamrul Hasan, David Hickey, and Zayn Rhodes for being intrigued and passionate in the scientific journey of elucidating purple bacteria photo-bioelectrochemistry and photobiology with me.

Conflicts of interest

There are no conflicts to declare.

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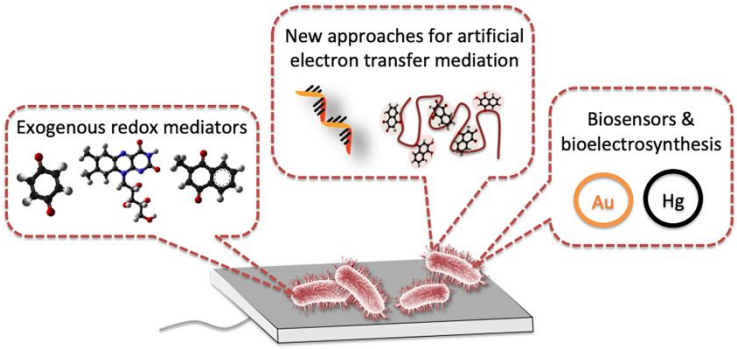

Perspective of research directions exploring purple bacteria photo-bioelectrochemistry: from harvesting photoexcited electrons to bioelectrochemical systems development