Biological photovoltaics: intra- and extra-cellular electron transport by cyanobacteria

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Abstract
A large variety of new energy-generating technologies are being developed in an effort to reduce global dependence on fossil fuels [1], but the contribution of their combustion products to the greenhouse effect, coupled with diminishing and increasingly unreliable sources of fuel, is driving the innovation of diverse alternative energy technologies. Using solar power is particularly attractive because of the amount of energy available: the amount of light energy striking the Earth in 1 h is approximately equal to the yearly global energy consumption [1]. The technology under review in the present paper, BPV (biological photovoltaics), is a close relation of the MFC (microbial fuel cell) – some forms have been described as photosynthetic MFCs – and it shares many of the same benefits and disadvantages. MFCs use living microbes to catalyse the oxidation of organic substrates and transfer electrons to an anode, and/or use electrons supplied by a cathode to reduce a substrate; the potential difference between the two reactions can be used to drive current through an external circuit [2]. Using microbes as catalysts for these redox reactions has a number of advantages over using synthetic/inorganic catalysts: they are comparatively cheap to produce, they can self-repair, and they can cope better with a broader range of substrates and tolerate impurities. However, the biological mechanisms behind these qualities require an energy input, making biological catalysts less efficient at converting chemical (or in the case of a BPV, solar) energy into electrical energy under optimal conditions. Hardiness and low cost can be of more value than simple efficiency in many situations: small-scale, ‘low-tech’, distributed power production is well suited to developing countries, where energy infrastructure may be poor, and the energy demand per person is low. These systems may also have potential additional environmental benefits, such as concomitant detoxification of wastewater [3] and carbon capture. BPV devices use photosynthetic micro-organisms as their biological component, meaning they require no chemical energy input (i.e. organic substrates) to operate, instead making use of solar energy to produce electrical power without producing CO₂ [4]. Unlike artificial photovoltaics, the electrons generated at the anode of a BPV could also be used to supply an electrosynthetic cathode; the reducing power supplied by the electrode is used in anabolic reactions by micro-organisms [5].

Light in MFCs
True BPV devices use only autotrophic organisms (or fractions thereof) to capture light and carry out charge separation concomitant with water oxidation, supplying some of the resulting electrons to an anode. However, light has been used as an energy input in a wide variety of MFCs (see reviews by Rosenbaum et al. [6,7]) and some examples are briefly described in the present paper.

Photosynthetic organisms may contribute to the cathodic reactions of an MFC. For example, sediment photomicrobial fuel cells have oxygenic photosynthetic organisms in the water column near the cathode. The oxygen produced is beneficial to a cathodic reaction where water is being regenerated [8], although penetration of dissolved oxygen to the anode is problematic as it inhibits electrolytic...
activity by the organisms in the sediment [9,10]. Autotrophic photosynthetic organisms may also contribute to reactions at the anode. For example, photosynthetic bacteria might be grown to feed heterotrophic bacteria, either by batch addition of the autotrophs as fuel into an MFC [11] or by combining the two in a mixed consortium where dead autotrophs and exuded organic compounds feed heterotrophs [12]. The use of heterotrophic microbes with known electrogenic properties and the ability to keep the anodic chamber anoxic are both beneficial to power production. Plant MFCs, where microbes are fed with exudates from the roots, are also being investigated [13]. The ability to harvest light has even been introduced into a non-photosynthetic organism: Shewanella oneidensis genetically engineered to produce the light-driven proton pump proteorhodopsin showed light-dependent current increases when used in an MFC, due to increased lactate uptake resulting from the increased protonotive force across the cytoplasmic membrane [14]. Purple bacteria have been used in MFCs for light-dependent production of hydrogen gas to mediate electron transfer to the anode [15], although this requires an input of electrons in the form of organic fuel. Some eukaryotic algal and cyanobacterial species can also produce hydrogen gas, allowing transfer of photosynthetically derived electrons to the anode, although the sensitivity of hydrogenase enzymes to oxygen means current production and photosynthesis must be temporally separated [6]. Current production without hydrogen generation by the molecular components of photosynthesis [16,17], from subcellular fractions of photosynthetic organisms [18] or pure cultures of phototrophs [19], is also possible. These biological materials are used in the anode of a BPV, and offer the most direct transduction of solar to electrical energy.

Electrogenesis from photoautotrophs?
The biological component of BPV systems has many possible electron-transfer steps between the initial photolysis of water and the donation of electrodes to an anode. A BPV containing eukaryotic algae (where photolysis occurs in thylakoid membranes located in a subcellular compartment, the chloroplast) could be considered the most complicated type of system. In contrast, individual PSII (Photosystem II) complexes attached directly to an electrode are an example of a very simply organized system. More complicated systems are less efficient: energy is lost each time the electron is passed between carrier molecules, and the system must expend energy to maintain itself. However, a complicated system such as a bacterial cell has the advantage that it can assemble and repair itself, and the ability to store metabolites may allow power generation in the dark. For this reason, most work in this field has been based on cyanobacteria (oxygenic photosynthetic bacteria, ‘blue-green algae’), as they have a comparatively simple internal structure. In these organisms, the thylakoid membranes where charge separation occurs are directly exposed to the cytoplasm, and the organism is enclosed by a typical Gram-negative cytoplasmic membrane/cell wall/outer membrane arrangement (Figure 1). Cyanobacterial metabolism is well studied, and genetic manipulation of many species (e.g. Synechocystis sp. PCC 6803) is well established [20].

The capacity for electron transfer from cyanobacteria to an electrode is very low compared with an anaerobic MFC operating with an electrogenic culture. Coulombic efficiencies of ~100% for harvesting electrons generated by oxidation of acetate by Geobacter sulfurreducens have been reported [21], whereas the coulombic efficiency of photosynthetic electron generation to external current was calculated to be less than 0.5% on the basis of data reported for Phormidium sp. [22]. By definition, autotrophic photosynthetic organisms use the reducing equivalents generated by the light reactions of photosynthesis to fix carbon and grow. Unlike anaerobic microbes, there is no need to use an external terminal electron acceptor for the photosynthetic light reactions, so excretion of electrons by a cyanobacterium would be wasteful under physiological conditions. An exception to this would be high light stress, when the photosynthetic pathway downstream of light-harvesting and water photolysis becomes rate-limiting. It has been suggested, and there is tentative evidence [23,24], that, under these conditions, electrogenic activity provides an alternative exit for electrons which would otherwise be trapped in the PETC (photosynthetic electron-transfer chain) and may lead to photodamage. However, given the tiny fraction of photosynthetic electron transfer that is diverted into external current, the contribution this makes to avoidance of photodamage may be minor. In order to make cyanobacteria useful organisms for direct production of electrical current, we need to understand (i) how electrons are transferred from the cell to the anode, (ii) what intracellular electron-transfer pathways take electrons to their point of exit, and (iii) how electron flux out of the cell can be improved so that the full light-harvesting potential of the organism can be exploited.

Getting the electrons out
The low rate of electron excretion from cyanobacteria in MFCs meant that almost all early studies used an added chemical mediator to harvest electrons from inside cells and transfer them to an anode. Ochiai et al. [22] reported that current outputs from immobilized Phormidium sp. cells were improved 2.5–3-fold by adding viologens or vitamin K$_3$ (a naphthoquinone derivative). HNQ (2-hydroxy-1,4-naphthoquinone) has also been shown to be an effective mediator, allowing Synechococcus sp. to generate current densities up to 3.25 A/m$^2$ [25], although the anodic compartment had to be sparged with N$_2$ to prevent oxidation of the mediator by O$_2$ [26].

Mediators have fallen out of favour in BPV studies because of concerns over their sustainability in scaled-up devices. Although toxicity to the cyanobacterium in the device may not be a problem with certain mediators (e.g. vitamin K$_3$ [27]), the system may still need to be kept anaerobic and
Electrons enter the plastoquinone pools of the thylakoid and cytoplasmic membranes from either the photosynthetic or respiratory complexes (electron flow is shown by thick grey arrows) and exit when they are used to reduce NADP⁺ or oxygen. Lipid-soluble mediators (e.g. BQ) can diffuse through membranes and be reduced by either thylakoid or cytoplasmic electron-transfer components. Non-lipid-soluble mediators (e.g. flavins) must pass through outer membrane porins and be reduced at the cytoplasmic membrane. An exogenous non-lipid-soluble mediator is shown being reduced by an unknown transmembrane protein which uses either plastoquinone or NAD(P)H as an electron donor. Mediators shuttle electrons to an electrode where they are oxidized. Unidentified proteins in the outer membrane may allow direct electron transfer to an electrode (e.g. pil); associated periplasmic and inner membrane components are not shown. Protons have been omitted for clarity. Membranes: OM, outer membrane; PM, cytoplasmic membrane; TM, thylakoid membrane. Mediators (octagonal shaded boxes): LS, lipid-soluble; NLS, non-lipid-soluble. Inhibitors (square boxes): DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; CCCP, carbonyl cyanide m-chlorophenylhydrazone; MV, Methyl Viologen. Photosynthetic electron-transfer components (white): OEC, oxygen-evolving complex; PSI/PSII, Photosystem I/II; b₆f, cytochrome b₆f complex; Cyt-c/PC, cytochrome c/plastocyanin; FD, ferredoxin; FNR, ferredoxin-NADP⁺ reductase. PQ, plastoquinone pool. Respiratory electron-input complexes (light grey): NDH-1, type I NADPH dehydrogenase; NDH-2, type II NADH dehydrogenase; SDH, succinate dehydrogenase. Terminal oxidases (black): cyd, cytochrome bd quinol oxidase; COX, cytochrome c oxidase; ARTO, alternative respiratory terminal oxidase. Other (dark grey): porin, non-specific outer membrane porins; TMP, unidentified transmembrane protein which reduces ferricyanide; direct transfer, unidentified protein(s) for direct electron transfer.

Closed to minimize the amount of mediator required and prevent pollution. Electrodes containing immobilized mediators may be a more acceptable means of improving electron export. For example, Zou et al. [28] added fibrillar polypyrrole to their anodes, which allowed approximately 15 times more current to be extracted. Electron transfer without the use of any artificial mediators may also be possible. Power generation with an unmodified indium/tin oxide electrode was demonstrated by McCormick et al. [19], who recorded a current response to ambient sunlight for over 30 days using naturally forming biofilms of pure cultures. The mechanism of natural cell–electrode electron transfer is still unknown; natural mediator compounds may be produced by the organism (e.g. quinones [29]), and there is a report that the model cyanobacterium *Synechocystis* sp. PCC 6803 can produce electrically conductive ‘nanowires’ [23]. Possible evidence of direct electron transfer between organism and electrode was reported by Cao et al. [30], who used a photosynthetic cathodic biofilm with bicarbonate as the terminal acceptor. However, the study used an undefined culture, so phototrophs may not have been responsible for the uptake of electrons from the cathode.
Intracellular electron transfer

Molecular components within cyanobacteria can contribute to current generation by generating reducing equivalents from light or stored metabolites, by being part of the mechanism for electron export (e.g., a hypothetical NADPH/mediator oxidoreductase), or possibly both. Chemical inhibitors (or knockout mutants) can be used to quantify the contribution of certain proteins to overall electrogenic activity. By normalizing the rate of reduction of a mediator (or an electrode) to the rate of oxygen evolution, it is possible to determine whether the inhibitor/knockout alters external current production by changing the underlying physiological electron-transfer rate or by affecting the electron-export mechanism. For example, if a protein only involved in electron export were knocked out, the mutant would have an essentially wild-type oxygen evolution rate, but decreased external current output. An important question that can be addressed in this manner is whether the ‘light effect’, i.e. the increase in electrogenic current upon illumination, is generated by electrons coming directly from the PETC, or whether light stimulates another part of the metabolism. However, interpretation of the results is complicated by the fact that the cyanobacterial respiratory complexes also feed electrons into the plastoquinone pool of the thylakoid membranes, allowing PSI (Photosystem I) to produce NADPH whether or not PSII is functioning [31] (Figure 1). Establishing the point where electrons exit the electron-transport chain is also important as it provides information about the energy of the electron before it is exported, and hints as to how the electron may leave the cell.

In order to investigate the origin of the light effect, various groups used CCCP (carbonyl cyanide m-chlorophenylhydrazone) to block electron transfer at the luminal side of PSII [32–34]. The light effect was strongly inhibited, demonstrating that electrons generated by photolysis of water are responsible for the current produced upon illumination. PSII is also targeted by DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea], which inhibits electron transfer between the Q&A and Q&A proteins. The same researchers found that DCMU also strongly inhibited, but did not completely abolish, the light effect. Tanaka et al. [32] and Yagashita et al. [33] used the lipid-soluble mediator HNQ and concluded that the residual light effect was due to HNQ accepting some electrons before Q&A. However, incomplete inhibition of the light effect by DCMU was also seen when using the membrane-impermeant [35] mediator ferricyanide [18], suggesting that respiratory electrons may feed into PSI and exit as NADPH. In the same study, Bombelli et al. [18] used MV (Methyl Viologen) to accept electrons from PSI, which completely removed the current produced upon illumination, supporting the idea of electrons leaving the PETC as NADPH. NADPH might then be used as a substrate for enzymes in the cytoplasmic membrane to reduce ferricyanide, or, if no exogenous mediator were present, NADPH might provide reducing equivalents to proteins which contact the electrode or to an endogenous mediator. Pisciotta et al. [24] used DBMIB (2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone) to block electron flow out of the quinol pool, and, upon seeing an increase in current output with DBMIB, concluded that, in their system, electrons were leaving the electron-transfer chain from the plastoquinol pool. However, without controlling for the electron shuttling behaviour of DBMIB [33], or normalizing current output to O2 evolution, this conclusion may be premature.

Improving the power

The photocurrent-generating capacity of cyanobacteria is large. Photosynthetic oxygen evolution rates in the order of 500 μmol of O2/mg of chlorophyll per h are typical [36], equivalent to current production of 50 mA/mg of chlorophyll, or (assuming approximately 2 x 10^-18 g of chlorophyll per cell [37]) 1 pA/cell. Water photolysis and electron excretion would need to be tightly coupled to maximize the photocurrent; the downstream carbon fixing metabolism should ideally be bypassed. Torimura et al. [34] used BQ (1,4-benzoquinone) to extract electrons directly from the PETC [34]; by comparing BQ reduction with O2 evolution, they showed that 68% of electrons produced by photolysis were collected, equivalent to 11 pA/cell. The same study noted that O2 evolution increased 1.8-fold with mediator present, demonstrating the benefits to current generation of bypassing downstream metabolic limitations. If the PETC is linked directly to an electrode, mass transport losses and futile cycling of reducing equivalents can be avoided. Ryu et al. [38] used a nanoprobe to extract electrons directly from the chloroplast of the eukaryotic alga Chlamydomonas reinhardtii; 20% of the electrons generated by photosynthesis could be captured, producing a current of 1.2 pA per cell (approximately equivalent to 6 A/m² or 320 μA/mg of chlorophyll). These rough numbers are of a similar magnitude to those for anaerobic fuel cell strains, e.g. G. sulfurreducens KN400, which can produce approximately 5 pA/cell [39].

Harvesting a large proportion of the current available in cyanobacteria without using mediators is likely to require genetic modification to redirect electron flux out of the cell. When using ferricyanide as a mediator, current output varies with ferricyanide concentration, but not directly with the light level [18], showing that electron excretion is the main bottleneck in this set-up. Increasing electron export out of the cell is key to improving the electrogenic activity of cyanobacteria, and a necessary step before other changes to the metabolism are made. A central principle of the anaerobic Shewanella- or Geobacter-based systems is that the electrode is required as the terminal electron acceptor for the electron-transport chains. Bearing this in mind, the aim of any metabolic alterations to cyanobacteria would be to make it energetically beneficial to the cells to use an electrogenic pathway, or necessary to retain viability. Removing cyanobacterial terminal oxidases would force the organism to use an alternative terminal acceptor for electrons,
as would reducing the capacity for biomass generation (i.e. using CO₂ as an electron sink) by limiting supplied CO₂ or down-regulating Calvin cycle components. Cyclic electron transfer allows photosynthetic organisms to generate ATP by reusing reducing equivalents; reducing this capacity might necessitate the increased use of linear electron flow in order to create enough ATP.

Summary

BPV devices are a hybrid of MFCs and photosynthetic organisms; sunlight and water are used as the ‘fuel’ to generate electrical power or provide a source of reducing equivalents for a cathodic reaction. Using cyanobacteria as the biological component is a good compromise between having simple electron transfer from water photolysis to electrode, and enough sophistication to be able to assemble and repair themselves. BPV devices show an increase in power output upon illumination, and metabolic inhibitors have been used to show that most of these electrons come directly from photolysis of water. Conversion of light into electrical energy is inefficient; the transfer of electrons out across the cytoplasmic and outer membranes is currently the limiting step. Genetic alterations will be necessary if cyanobacteria are to produce current densities comparable with traditional MFCs, and these modifications will aim to uncouple photolysis of water from carbon fixation.

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