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# Selection of organisms for systems biology study of microbial electricity generation: a review

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**Abstract**

A microbial fuel cell (MFC) is a device that uses microorganisms as biocatalysts to transform chemical energy or light energy into electricity. However, the commercial applications of MFCs are limited by their performance. This review presents the perspective that *in silico* metabolic modelling based on genome-scale metabolic networks can be used for understanding the metabolisms of the anodic microorganisms and optimizes the performance of their metabolic networks for MFCs. This is in contrast to conventional research that focuses on engineering designs and study of biological aspects of MFCs to improve interactions of anode and microorganisms. Four categories of biocatalysts - microalgae, cyanobacteria, geobacteria and yeast - are nominated for future *in silico* constraint-based modelling of MFCs after taking into account the cell type, operation mode, electron source and the availability of metabolic network specifications. In addition, the advantages and disadvantages of each organism for MFCs are discussed and compared.

**Keywords:** Microbial fuel cell (MFC), Flux balance analysis, *Synechocystis*, *Chlamydomonas*, *Saccharomyces*, *Geobacter*, Cyanobacteria, Algae, Genome-scale metabolic network, Constraint-based modelling

## Review

### Introduction

The technology for extracting an electrical current for use in external circuits from the metabolic processes of living microbes has been in development for more than a century [1]. The resulting devices, termed microbial fuel cells (MFCs), have several potential advantages over more prominent sustainable energy technologies such as solar or wind power. For example, they can directly convert organic waste into electricity [2] without pollution or inefficient intermediate steps that involve mechanical generators. This feature, energy recovery from solid wastes, has been exploited in proposed national strategies for many Asian countries [3]. It may be possible to achieve the same goal by inorganic catalysts or enzymes, but using living cells makes it possible to exploit their adaptability to environmental conditions and avoids the high capital cost of installation for other waste-to-energy systems reviewed by Eddine and Salah [4]. The whole organisms used in MFCs contain various enzymes and therefore allow different substrates (or mixed substrates) to be used. The organisms in the fuel cell system can be considered as micro-reactors and provide optimal conditions for different enzymes. Because the organisms are self-replicating, the organic matter oxidation implemented by these bio-catalysts are self-sustaining [5] and not subject to catalytic poisoning like metallic catalysts or degradation of enzyme catalysts. By selecting photosynthetic microbes, solar energy could be converted at the same time. One can envisage portable electronics powered by MFCs that are 'charged' by feeding them nutrients rather than electric current, or medical implants that derive their power directly from nutrients circulating in the bloodstream. Perhaps the process can be reversed, and external electrical power supplied to an MFC converted into biomass, as a temporary storage, to overcome the intermittent nature of many other sustainable energy sources - a possibility currently under serious consideration [6-8].

However, these future possibilities are still severely hampered by the low energy yields per mass or volume that are currently achieved. Generally MFC energy output is reported in milliwatts per square metre of electrode area or per cubic metre of electrolyte volume [9].

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Scientific research has increased the densities of MFCs to over 1 kW $m^{-3}$ (reactor volume) and to 6.9 W $m^{-2}$ (anode area) under optimal conditions in the laboratory [10]. However, these values still cannot meet the needs of many applications, which require a power output larger than 100 kW $m^{-2}$ [11]. For this reason large-scale waste water treatment is the application closest to industrial realisation and is the domain of much current MFC research.

A variety of designs are under development to improve the efficiency and potential application of MFCs to industry. Areas under investigation include the selection of electrode materials for optimal electrochemical performance and maximising electrode surface to volume ratios; improving charge transfer between microbes and electrodes either chemically or by mechanical design; and finding and maintaining optimal living conditions for microbe colonies, efficient supply of nutrients and removal of effluent. Different configurations are being investigated for extracting current, sometimes in combination with production of hydrogen or other metabolites of further use in energy generation and with or without exploitation of photosynthesis. The choice of process configuration and engineering design is also closely linked with the selection of the most suitable organism for a particular design or for whether overall priority is given to energy generation, waste disposal or some other objective.

A schematic representation of MFC research activity is shown in Figure 1. While there is a large volume of biochemical research literature on, for example, electron transfer chains and redox processes in cell metabolism that is relevant to MFC, relatively few studies focus specifically on MFC. This is exacerbated by the fact that ongoing research continues to identify new mechanisms for electron exchange between microbes and electrodes, new design strategies to exploit these and consequently new candidate organisms. Such organisms have not necessarily been well studied experimentally before.

*In silico* modelling is well suited to bridge this gap and extend knowledge in the biochemical interface between MFC biology and engineering design. Externally, electron flow (in the external circuit) and the counterflow of protons in the electrolyte make up the current that carries useful electrical power. Internally, both of these are comprehensively woven into the fabric of metabolism: electrons being transported by redox carrier molecules such as reduced nicotinamide adenine dinucleotide (NADH) that participate in a large fraction of all biochemical reactions, and protons that, for example, drive adenosine triphosphate (ATP) synthesis needed for energy transport are also ubiquitously involved in a great many reactions. This clearly calls for a systems-level approach rather than the reductionist strategy of pathway-oriented, conventional biochemistry.

This is the domain of systems biology [12]. Systems biology provides *in silico* models that incorporate biological data, metabolic flux data and different physicochemical constraints such as the conservation of mass and energy, thermodynamics, redox balance, etc. [12] and thus provides an opportunity to identify the bottlenecks hidden in a complex network of interactions and cellular compartmentation [13].

The kinetic behaviour of a metabolic network at a whole-genome level can be constructed and analysed through a mathematical model [14,15]. However, the characterization of metabolic networks is still far from comprehensive in databases [16] and even in the best-understood organisms, the majority of kinetic parameters are undetermined.

The development of new computational methods allows for the whole-network modelling of metabolism and conduction of compelling and testable predictions even without many parameters. The key idea is to incorporate stoichiometry and other fundamental principles as mathematical constraints, which separate feasible and infeasible metabolic behaviours. Compared with kinetic parameters, these constraints are much easier to

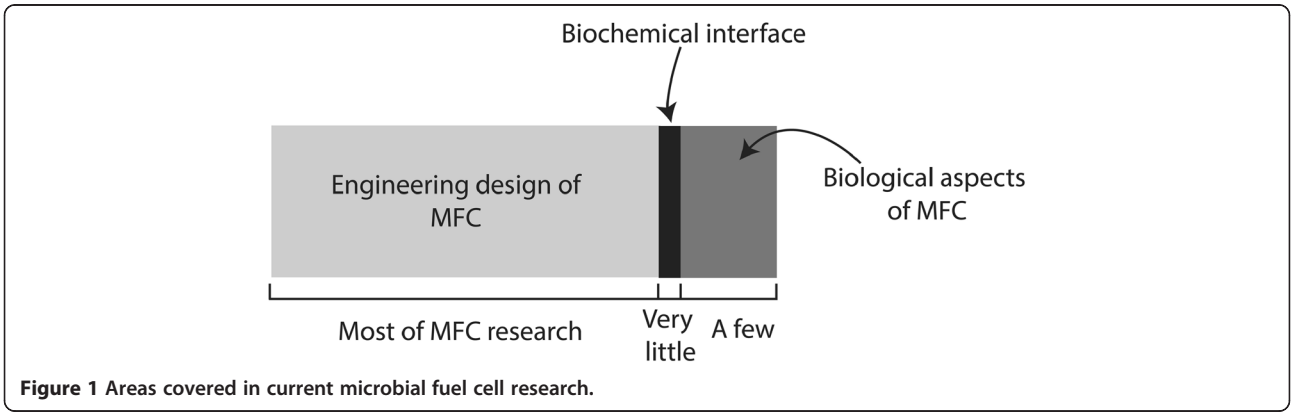


**Figure 1** Areas covered in current microbial fuel cell research.

identify and make it possible to build a large-scale model [17]. These constraint-based modelling approaches allow integration of high-throughput post-genomic data but describe steady states and generally offer no information about metabolite concentrations or the temporal dynamics of the system [18-20].

Genome-scale metabolic modelling requires high-quality metabolic network reconstructions [14]. The reconstructions are based on sequenced genomes and are generally built manually using information from metabolic databases (e.g. KEGG and BRENDA) and primary literature. The metabolic network reconstruction process is described in detail elsewhere [21]. Recently, due to the development of the high-throughput technologies, the reconstructions have now been built for various organisms [22].

Flux analysis can be combined with cell biology and sub-cellular biochemistry to reveal the functionality and efficiency of the enzymes associated with cell biological components or structures [23]. Metabolic regulation can be deeply understood only when multiple system components are examined simultaneously. This kind of analysis has been conducted in microbial and medical research in recent years.

Flux determinations can produce results that are hardly predictable from observed changes in transcript or protein levels because most of metabolic control takes place at post-translational levels and enzyme activities are often not correlated with changes in transcript or protein levels [23]. The incorporation of data from enzyme platforms should make the functionality of genomics strategies more clear. System-wide metabolic flux characterization is an important part of metabolic engineering [23].

Nevertheless, there is no published literature that uses genome-scale flux models to study the metabolic behaviour of biocatalysts in MFCs. The only attempt to use a genome-scale model to study biocatalyst behaviour in an MFC was presented as a conference abstract paper in the 17th European Symposium on Computer Aided Process Engineering (2007) [24]. However, this paper is an immature work that did not provide the source of the central metabolic network, describe the gene knockout methodology or discuss the results in relation to other MFC experimental work. Therefore, future research activities are urgently needed to fill the research gap indicated in Figure 1, with recently advanced constraint-based modelling approaches.

An essential first step in applying constraint-based analysis to microbial fuel cells is to choose appropriate organisms for further study. Due to the varied strategies and designs alluded to above, no single organism can serve as a suitable model, and a major advantage of *in silico* modelling is that different organisms can be studied in the same framework to facilitate mutual comparisons. This paper reviews the background against which such choices can be made and proposes a set of four organisms for the purpose.

The 'Microbial fuel cells' section explains the construction, operation and classification of current MFC designs, and the 'Current directions of MFC research' section reviews the issues being addressed in current MFC research. Based on this, the 'Microorganisms for *in silico* study of MFC functioning' section discusses a selection of organisms that are representative of various combinations of biological aspects that can be exploited in MFCs, while also featuring well-established metabolic network reconstructions, suitable for the computational analysis.

## Microbial fuel cells

MFCs are unique devices that can use microorganisms as catalysts for transforming chemical energy directly into electricity. The biggest advantage of an MFC is that it can generate combustion-less, pollution-free bioelectricity directly from the organic matter in biomass [2]. In an MFC the energy stored in chemical bonds in organic compounds is converted to electrical energy through enzymatic reactions by microorganisms. Thus, the electricity production by MFC is associated with the normal living processes of bacteria capturing and processing energy.

In a typical MFC configuration (Figure 2), microorganisms are situated in the anodic compartment and use the biomass for growth while forming electrons and protons [25]. The electrons are transported out of cells to an electrode using redox mediators or directly expelled by some microorganisms for reducing the substrate. The protons or $H^+$ ions are diffused through the electrolyte to the cathode where it is oxidised to water. The cathode can be in a separate chamber (i.e. double-chambered MFCs) or in the same chamber (i.e. single-chambered MFCs). A single-chambered MFC eliminates the need for the cathodic chamber by exposing the cathode directly to the air. The only by-product released by MFCs is carbon dioxide, which can be fixed by plants for photosynthesis.

MFCs require running under conditions predefined by the optimum growth and living conditions of the used microorganisms. Thus, factors affecting the MFC's efficiency include electrode material, pH buffer and electrolyte, proton exchange system and operating conditions in both the anodic chamber and the cathodic chamber. MFCs are usually operated at ambient temperature, at atmospheric pressure and at pH conditions that are neutral or only slightly acidic [26].

MFCs harness the electrons from these systems in three main operation modes: mediated electron transfer

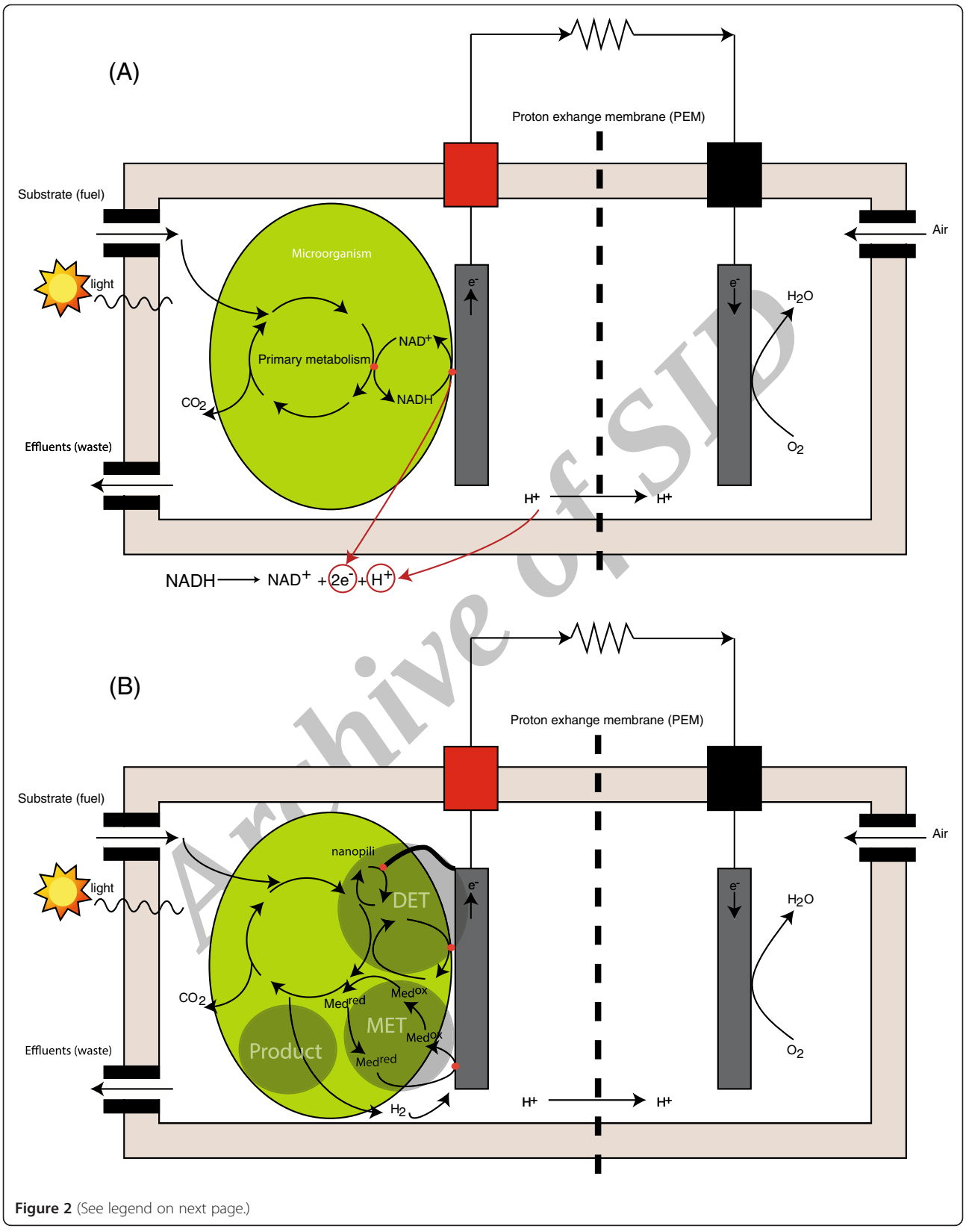


**Figure 2** (See legend on next page.)

(See figure on previous page.)
**Figure 2 The working principle of a microbial fuel cell.** (**A**) A bacterium in the anode compartment transfers electrons obtained from an electron donor (glucose or light in the case of photosynthetic organisms) to the anode electrode. Protons are also produced in excess during electron production. These protons flow through the proton exchange membrane (PEM) into the cathode chamber. The electrons flow from the anode through an external resistance (or load) to the cathode where they react with the final electron acceptor (oxygen) and protons. (**B**) Three electron transfer modes: (1) directly via membrane-associated components (DET), (2) mediated by soluble electron shuttles (MET) or (3) primary product (Product), e.g. $H_2$ can act as a fuel to be oxidised to provide electrons for the electricity circuit. Med, redox mediator; Red oval, terminal electron shuttle in or on the bacterium.

(MET), direct electron transfer (DET) and product mode. Photosynthetic MFCs use photosynthesis as the electron source and can also be operated in the same modes.

#### *Mediated electron transfer*

MET is defined as where a mediator molecule acts an electron relay that repeatedly cycles between the reaction sites and the electrode [11]. MET is the most common electron transfer mode used in MFCs and can be classified into two sub-types [11]:

- Indirect transfer systems that involve freely diffusing mediator molecules (i.e. diffusive MET)
- Indirect transfer systems in which the mediator is integrated into the electrode or the cell membrane (i.e. non-diffusive MET)

In diffusive MET, the mediators enter the cell membrane and exchange electrons between cellular metabolism inside the cell and the electrode outside it. In the non-diffusive MET, the mediator can collect the electrons from the cell membrane without penetrating the cell.

Based on the type of mediators, diffusive MET can also be classified into three sub-categories:

- MET via exogenous (artificial) redox mediators
- MET via secondary metabolites
- MET via primary metabolites

The detailed mechanisms in those three classes are discussed in [27]. Because the terminal electron transfer to or from the electrode determines the overall cell potential, potential (voltage) losses can be minimised by using a mediator that has a reaction potential near that of the biological component.

#### *Direct electron transfer*

DET is defined as the case where electrons cycle directly between a microorganism and an electrode. DET can be achieved through two naturally occurring mechanisms:

- Membrane-bound c-type cytochromes, which exist in the cell membrane in some organisms [28,29] to provide electron transfer capacity. For example, multi-heme proteins have especially evolved in sediment-inhabiting metal-reducing microorganisms such as *Geobacter* [30], *Rhodoferax* [31] and *Shewanella* [32]. In their natural environment, iron (III) oxides act as the solid terminal electron acceptors, but in the case of MFC, the anode is used as the solid electron acceptor.
- Electronically conducting nanowires. The DET via outer membrane cytochromes requires the cytochrome (the bacterial cell) to be physically adhered to the fuel cell anode. When a biofilm is formed, only bacteria in the first monolayer at the anode surface are electrochemically active [30]. Thus, the maximum cell density in this bacterial monolayer usually influences the MFC performance. However, it has been shown that some *Geobacter* and the *Shewanella* strains can evolve electronically conducting molecular pili (nanowires of 2 to 3 μm long, made of fibrous protein structures [33]) that make the microorganism able to reach and use more distant solid electron acceptors [34,35]. The pili are connected to the membrane-bound cytochromes and allow transference of the electrons to the distant electron acceptors without cellular contact (Figure 2B). Thus, thicker electroactive biofilms can be formed to increase anode performance. It was shown that fuel cell performance can be increased up to tenfold upon nanowire formation of *Geobacter sulfurreducens* [35].

#### *Product type*

In product-type MFCs, microbes metabolize the substrate, releasing a secondary fuel product such as hydrogen that then diffuses to the electrode and is oxidised or reduced (as appropriate) to form a final waste product, which is discharged [11]. The product operation is similar to conventional fermentation processes, in that products of the microbial metabolism are used as the fuel at the electrode.

The product system is made up of two independent stages: one is storage of the microbial reaction product, and the other one is the product being fed to a conventional fuel cell process driven by non-biological catalysis, such as in the case of a proton exchange membrane fuel cell, where $H_2$ is converted into electricity [36]. These

stages may also be physically separated in different containment vessels. However, a product system only truly belongs to a biofuel cell system when the microbes and the electrode are together in the same anode compartment [11]. Nowadays, the fermentation (mostly to hydrogen gas) usually takes place in the fuel cell itself [37,38].

Product systems have two main drawbacks, one is that the efficiency of the conversion of the biological substrate to hydrogen is quite low, and the other is that hydrogen oxidation requires high fuel cell temperatures. Also, the produced biofuel gas is always contaminated with other by-products such as CO, $H_2S$ and (poly)siloxanes making it not sufficiently pure for direct use in a fuel cell [39].

#### *Photosynthetic MFCs*

Photosynthetic MFCs are MFCs that generate electricity from a light source rather than a fuel substrate and require the mediator involved to be light stable [40]. Conventionally two operating modes exist for photosynthetic MFCs:

- Energy is produced and stored by the microorganism during illumination and then released and processed in the same way as in a non-photosynthetic biofuel cell.
- The energy produced during illumination may be directly extracted in the form of electrons for creating an external electrical circuit.

A single photosynthetic MFC may possess both of these two modes of action. However, it is recently thought better to classify photosynthetic MFCs into categories based on seven approaches that integrate photosynthesis with MFCs - photosynthetic MFCs [40]:

1. Photosynthetic bacteria at the anode with artificial mediators
2. Hydrogen-generating photosynthetic bacteria with an electrocatalytic anode
3. A mixed culture, with photosynthetic bacteria supplying organic matter to heterotrophic electroactive bacteria at the anode
4. Photosynthesis in plants, supplying organic matter via rhizodeposits to heterotrophic electroactive bacteria at the anode
5. An external photosynthetic bioreactor, where only biomass or metabolic products are transferred to the anode compartment to feed heterotrophic electroactive bacteria
6. Direct electron transfer between photosynthetic bacteria and electrodes
7. Photosynthesis at the cathode to provide oxygen

These sub-types have been discussed in detail by Rosenbaum et al. [40].

#### *Microorganisms suitable for MFCs*

Most microorganisms are unable to donate sufficient electrons outside of cells to produce usable currents, because the outer layers of most microbial species are made up of non-conductive lipid membrane, peptididoglycans and lipopolysaccharides which restrain electron transfer to the anode [41]. Since the 1980s, it has been found that artificial water-soluble electron shuttles (i.e. methylene blue, thionine, neutral red and 2-hydroxy-1,4-naphthoquinone) can be used as mediators that transport the electrons from electron carrier molecules inside the cell (e.g. NADH, NADPH or reduced cytochromes) to the anode surface [41]. For example, an MFC based on *Proteus vulgaris* used thionine as a mediator to generate electricity from sucrose [42].

Since the 1990s, some bacterial species such as *Pseudomonas aeruginosa* [43] and *Clostridium butyricumcan* [44] have been found to be able to self-mediate extracellular electron transfer using their own metabolic products. Meanwhile, direct transfer of electrons (DET) that involves use of electrochemically active redox enzymes (i.e. cytochromes) has been discovered in a number of bacterial species such as *Shewanella putrefaciens* [28,29,45], *Shewanella oneidensis* [46], *Geobacter sulfurreducens*[a] [30,47], *Rhodoferax ferrireducens* [31], and the oxygenic phototrophic cyanobacterium *Synechocystis* sp. PCC 6803[a] [34]. These microorganisms are termed as exoelectrogens, and among them *S. oneidensis* and *G. sulfurreducens* have evolved electronically conducting molecular pili (nanowires) to further facilitate the DET [34,35]. Besides DET mode, *S. oneidensis* can conduct MET using a self-produced mediator [48]. The exoelectrogens in MFCs are thought to actively use electrodes to conserve electrochemical energy required for their growth and thus ensure high rates of fuel oxidation and electron transfer for the production of electrical energy [5].

In most of the previous MFC studies, bacteria have been used for electricity generation. On the other hand, since 2000, eukaryotes such as microalgae (e.g. *Chlamydomonas reinhardtii*[a]) and yeast (e.g. *Saccharomyces cerevisiae*[a]) have also emerged as good choices for MFC use, because they have been studied as model microorganisms in the lab and have been widely used in the industry for a long time.

### Current directions of MFC research

#### *Engineering design and biological aspects*

Most previous studies tended to improve power densities of MFCs by optimizing the reactor configuration and operation parameters [49,50], such as modifications of the electrode materials to incorporate metals that contain current collectors [51,52], use of metals highly optimized for bacterial adhesion and metals possessing high electrical conductivity to minimise ohmic losses

[10], and application of a biocathode that can increase MFC performance by improved oxidation of hydrogen at the cathode [53]. Applications of chemical treatments and precious metals to electrodes in order to increase power production in the laboratory have also been investigated [54,55]. However, these modifications, like the use of larger laboratory reactors, may increase the cost and lead to compromises on performance based on material costs. Many bottlenecks also exist for improving those physical and chemical properties.

Since the fundamental source of electrons is the cellular metabolism, it is particularly important to focus on biological processes that take place in the microbial cells. In this regard, further clarification is needed of the factors relevant to the anodic catalysis process, such as the diversity of the electrochemically active microorganisms [56] and especially the electricity generation mechanisms in relation to normal metabolic states.

#### *Mediator-less, mediator-self-producing and artificial mediator-based MFCs*

Mediator-less MFCs are a more recent development relying on evolved ability of exoelectrogens for disposal of electrons originating from substrate oxidation. This type of MFCs has been increasingly preferred, because use of mediators complicates the cell design and these mediators are usually toxic, costly and unsustainable, limiting MFC development [27,57-60]. In addition, mediator-involved MFCs usually produce low current densities (0.1 to 1 A $m^{-2}$) [27]. Unfortunately, mediator-less MFCs may not yet find a wide range of applications since the discovered exoelectrogens are still few in number and it is non-exoelectrogens that are largely used in the agricultural and industrial areas [61]. Thus, an important direction of MFC research is development of MFCs using non-exoelectrogens without exogenous mediators [61].

However, problems arise from the fact that redox molecules used in electron transfer reactions are not situated on the outer membrane, but in the cytoplasmic membrane. One way is to develop direct electron transfer using carbon nanoparticles that can contact the redox centres that are incorporated in the interior cell membrane [61]. Another way is to identify and develop self-produced mediators (e.g. in the case of *Shewanella* species mentioned above) through engineering methods [56].

#### *Conventional photosynthetic MFCs*

It is appealing to study whether phototrophic microbes can be used in an MFC generating electricity from sunlight, because sunlight is an unlimited energy resource and more solar energy reaches the Earth in 1 h ($4.1 \times 10^{20}$ J) than the energy consumed on the planet in a year [56,62,63]. In addition, the development of a self-sustainable photosynthetic MFC is important to meeting energy requirements at remote locations, where routine addition of fuel would be technically difficult and expensive [56].

Photosynthetic MFCs can generate electricity indirectly or directly. For example, in the indirect way, *Rhodobacter sphaeroides* in the MFC can produce $H_2$ that is oxidised at a platinum coated anode to generate electricity [64,65], whereas in the direct way, *Rhodopseudomonas palustris*, a photosynthetic purple non-sulphur bacterium, can generate electricity in a biofilm anode MFC by direct electron transfer [66].

There are also more traditional photosynthetic MFC configurations where photosynthetic organisms live with other microbes and supply products to heterotrophs [67]. Photosynthetic microorganisms (e.g. cyanobacteria or microalgae) and heterotrophic bacteria exhibit synergistic interactions [68] that can be used in self-sustained phototrophic MFCs [62]. An indirect synergistic relationship between photosynthetic organisms and electricigens has been exemplified in a recent study, in which algal photobioreactors were used to supply organic matter produced via photosynthesis to an MFC for electricity generation [69]. The operation of this type of photosynthetic MFCs is $CO_2$ neutral and does not need buffers or exogenous electron transfer mediators [67]. However, the photosynthetic MFC power densities obtained are quite low when compared with those that are currently reported for conventional MFCs, e.g. 0.95 mW $m^{-2}$ for polyaniline-coated and 1.3 mW $m^{-2}$ for polypyrrole-coated anodes [70] versus values in the watt per square metre range for conventional cells.

#### *MFCs based on the photosynthetic electron transfer chain*

Recently, the photosynthetic electron transfer chain is considered as a source of the electrons harvested on the anode surface, which is different from the previously designed anaerobic MFCs, sediment MFCs or anaerobic photosynthetic MFCs [70]. A single-chamber photosynthetic MFC based on two photosynthetic cultures, planktonic cyanobacteria *Synechocystis* sp. PCC 6803 and a natural freshwater biofilm, has shown a positive light response (i.e. immediate increase in current upon illumination) [70]. This phenomenon proves that it is possible to extract electrons directly from the photosynthetic electron transfer chain, and not only from the respiratory transfer chain or through oxidation of hydrogen [71].

### Microorganisms for *in silico* study of MFC functioning

#### *Categories and representatives*

Because the electricity generation in MFCs is based on the metabolic activity of living microorganisms, experimental screening of different microorganisms for better anodic activity has long been recognised as a fundamental way to

improve MFC performance. It is also possible to improve the performance by culturing microorganisms under selective pressure for enhanced power production [30,72].

Compared to experimental studies, *in silico* modelling is less constrained to a particular MFC design and operating mode. Clearly no single organism is likely to be optimal for all of the varied designs discussed before. To date, every microorganism used in previous MFC studies has advantages and disadvantages. Selection of the microorganism depends on a variety of factors such as types of application, the capability of power generation, the availability of types of energy source for bacterial survival and the ability of extracellular electron transfer, in that electrodes are not natural electron acceptors.

From the modelling perspective, a broader view is possible. Categories of microbes can be identified to cover the range of operating modes and, within these, individual organisms selected that will allow different modes to be individually studied and also compared quantitatively.

MFC microbial communities can be divided into three groups: heterotrophic cells, photoheterotrophic cells and sedimentary cells [9]. The distinction between phototrophic and heterotrophic metabolism is fundamentally important in determining the operating mode. Another key distinction is between prokaryotes and eukaryotes. Compared to prokaryotic species and mixed cultures that have been mostly studied for different MFC applications, fewer studies involve eukaryotes as biocatalysts in MFC operations [73]. This is because the metabolic processes of eukaryotic cells take place in the membrane surrounded cell organelles (e.g. chloroplasts) and is thus putative to be difficult for some commonly used redox mediators such as 2-hydroxy-1,4-nepthoquinone to get access to [57,74]. While prokaryotes have the advantage that their simpler cell membranes and internal structure are more amenable for physical electron extraction, the more complex metabolism of eukaryotes may be more efficient and be able to support a larger diversion of redox carrier flux without undue harmful effects on the organism.

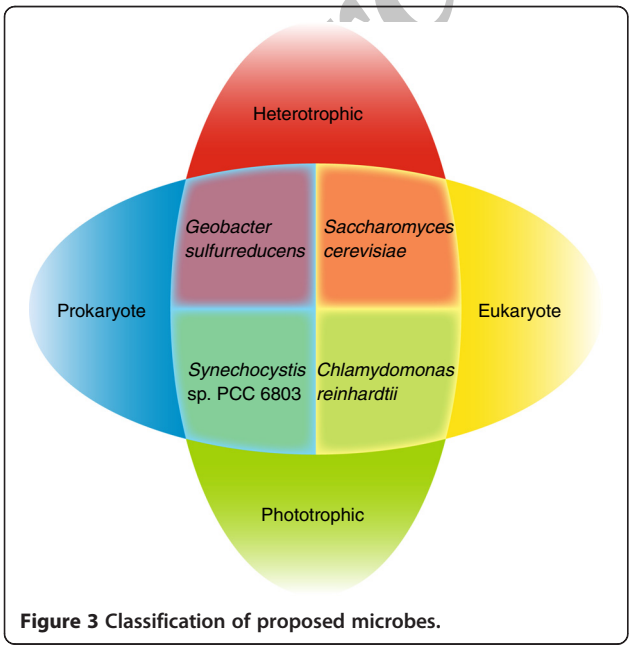


**Figure 3** Classification of proposed microbes.

The four anodic microorganisms: *C. reinhardtii*, *Synechocystis* sp. PCC 6803, *S. cerevisiae* and *G. sulfurreducens*, each combine a different pair of key features and are proposed as good candidates for MFC *in silico* characterization. As illustrated in Figure 3, *C. reinhardtii* and *S. cerevisiae* are eukaryotes, whereas *Synechocystis* sp. PCC 6803 and *G. sulfurreducens* are prokaryotes. The four organisms also cover the three groups of the MFC microbial communities mentioned above, i.e. *C. reinhardtii* and *Synechocystis* sp. PCC 6803 are photoheterotrophic cells, *S. cerevisiae* belongs to heterotrophic cells and *G. sulfurreducens* is a typical sedimentary cell.

*C. reinhardtii* and *Synechocystis* sp. PCC 6803 are photosynthetic organisms that are also capable of producing hydrogen. The comparison of organisms with/without photosynthesis can be used to study exploitation of photosynthetic and respiratory electron transport chains to supply MFC current.

A further consideration in a modelling study is whether the data are available and computationally manageable. All four microorganisms have been studied extensively as model organisms and used in various industries for a long time, and thus, related molecular tools and biological mechanisms are abundant. In particular, genome-scale metabolic networks have been reconstructed and are regularly updated for these four organisms. Based on a literature review, the most updated models are shown in Table 1. These models include the natural redox mediators (i.e. NADH) that are well balanced for the cellular energy metabolisms (e.g. oxidative phosphorylation, glycolysis, Calvin cycles and tricarboxylic acid (TCA) cycles) and are thus practicable for MFC modelling.

It is noted that two models were published in the same period for *C. reinhardtii* [75,76] and *Synechocystis* [77,78]. These models for the same organisms are not compared with each other by the authors, and thus, their limitations can only be revealed during the MFC modelling.

The biological features of the four microorganisms are summarized in Table 2, and the relevance of each of these microbes is reviewed in more detail in the following sections.

### *Chlamydomonas reinhardtii*

*C. reinhardtii* is a unicellular green alga that belongs to the Chlorophytes division, which diverged from the

**Table 1 The scope of the genome-scale models of the four selected organisms**

| | *Chlamydomonas reinhardtii* | | *Synechocystis* sp. PCC 6083 | | *Geobacter sulfurreducens* | *Saccharomyces cerevisiae* |
|---|---|---|---|---|---|---|
| Gene | 2,249 | 1,080 | 1,811 | 678 | 617 | 918 |
| Metabolite | 1,862 | 1,068 | 465 | 795 | 644 | 1,655 |
| Reaction | 1,725 | 2,190 | 493 | 863 | 709 | 2,110 |
| Compartment | 4 | 10 | 3 | 4 | 2 | 17 |
| Date | December 2011 | August 2011 | October 2011 | January 2012 | 2009 | 2012 |
| Reference | [75] | [76] | [77] | [78] | [79] | [80] |

The number indicates the counts of relative items in the network models.

Streptophytes division (including land plants) more than one billion years ago [85]. *C. reinhardtii* is an approximately 10-μm, unicellular, soil-dwelling green alga with an eyespot, a nucleus, multiple mitochondria, two anterior flagella for motility and mating, and a single cup-shaped chloroplast that accommodates the photosynthetic apparatus [86,87].

Like plants, *C. reinhardtii* has a cell wall and can grow in a medium lacking carbon and energy sources when illuminated [87]. Unlike angiosperms (flowering plants), this microorganism has functional photosynthetic apparatus even when in dark conditions and with an organic carbon source [87]. In the dark, acetate is the sole carbon source used by wild-type *C. reinhardtii in vivo* [88].

Because of the relative adaptability and quick generation time, *C. reinhardtii* has been used as a model to study eukaryotic photosynthesis, eukaryotic flagella and basal body functions and the pathological effects of their dysfunction [89,90], and investigated for water bioremediation and biofuel generation [87,91-93]. The cDNA, genomic sequence and mutant strains of *C. reinhardtii* are publicly available through the *Chlamydomonas* Center [94].

**Advantages of algae** *C. reinhardtii* inherits all potential advantages of algae for industrial use and scientific study, including [95-97] the following: (1) Algae biomass can potentially be produced at extremely high volumes, and this biomass can yield a much higher oil (1,000 to 4,000 gal/acre/year) than soybeans and other oil crops [97]. (2) Algae do not compete with traditional agriculture because they are a non-food-based resource which can be cultivated in large open ponds or in closed photobioreactors located on low-productive or non-arable land. (3) Algae have a good adaption to different climate and water conditions and can be grown in a wide range of water sources, such as brackish, saline, or fresh and waste water. (4) Algae can make use of resources that would otherwise be considered waste as substrate for growth [97]. (5) Algae can use and sequester $CO_2$ from

**Table 2 Comparison of facts of four selected microorganisms**

| Name | *Chlamydomonas reinhardtii* | *Synechocystis* sp. PCC 6083 | *Saccharomyces cerevisiae* | *Geobacter sulfurreducens* |
|---|---|---|---|---|
| Domain | Eukaryote | Prokaryote (Gram-negative) | Eukaryote | Prokaryote (Gram-negative) |
| Mitochondria | Multiple | N/A | Multiple | N/A |
| Chloroplast | Single chloroplast occupies two thirds of the cell | Chloroplast analogy | N/A | N/A |
| Hydrogen synthesis enzyme | Fe hydrogenase | NiFe hydrogenase | N/A | N/A |
| MFC mode | Product mode | Photosynthetic DET | MET DET (output extremely low) | DET |
| MFC performance | 0.4 W $m^{-2}$ | 0.02 W $m^{-2}$ | 1.5 W $m^{-2}$(MET) | 1.88 W $m^{-2}$ |
| | 3.3 W $m^{-3}$ | 0.007 W $m^{-3}$ | 90 W $m^{-3}$(MET) | 43 W $m^{-3}$ |
| | | | 0.003 W $m^{-2}$ (DET) | |
| Optimum growth temperature | 20°C to 25°C [81] | 30°C to 33°C [82] | 25°C to 35°C [83] | 30°C to 35°C [84] |
| Growth mode | Autotrophic | Autotrophic | Heterotrophic | Heterotrophic and sedimentary (soil inhabitant) |
| | Heterotrophic | Heterotrophic | | |
| | Mixotrophic | Mixotrophic | | |

N/A, not applicable.

many sources such as flue gases of fossil fuel power plants and other waste streams. (6) Algae can be processed into a wide range of products such as biodiesel, bioethanol, methane, bio-oil and biochar, and high-protein animal feed. (7) The 'simple' photosynthetic alga *C. reinhardtii* is an excellent model organism for a systems biology approach compared to a complex vascular plant [12].

**Biofuel and electricity production by algae** Due to the advantages listed above, algae have been examined in many studies for the generation of energy products, such as bio-oil, methane, methanol and hydrogen [98]. Nevertheless, these technologies have one disadvantage in that the fuel produced must be stored, transported and further processed to produce electricity. To circumvent these problems, MFC is used as an alternative way to directly generate electricity in only one process unit by means of hydrolysis and fermentation of algae and makes use of energy originating from sunlight.

However, algae are not exoelectrogens and the conventional mediators do not perform well in the extraction of the redox potential for algae-based MFCs because the redox species are produced through the metabolic mechanisms that take place in membrane-surrounded cell organelles in algae such as mitochondria and chloroplasts [74]. Thus, neither DET nor MET mode has been applied in MFCs using algae.

Previous studies tend to use algae in MFCs of the product mode that depend on the production of hydrogen molecules, which is then oxidised at the anode for electron transfer to the MFC circuit. Another mechanism is where algae produce organic matter that is used as a substrate for electrochemically active bacteria, which then supply electrons for MFCs from oxidization of the organic matter [69].

In one landmark study of MFC using algae, C*. reinhardtii* was used in a product-mode MFC to produce hydrogen for oxidation at the anode. A maximum hydrogen production rate of 7.6 ml/l culture $h^{-1}$ [99] was achieved, at a current yield of 9 mA at a constant electrode potential of 0.2 V. Using the culture volume and electrode dimensions, this corresponds to power densities of 0.4 W $m^{-2}$ and 3.3 W $m^{-3}$. In another study [98], *Chlorella vulgaris* microalgae were used as a biomass source to feed a mixed microbial culture, producing a maximum power density of 0.98 W $m^{-2}$ (277 W $m^{-3}$).

Furthermore, algae have been inoculated into a bioelectrode to generate oxygen as the electron acceptor [100]. Under illumination, algae produced oxygen as the electron acceptor for the MFC cathodic reactions, changing the bioelectrode into biocathode mode, while in darkness, the algal oxygen production stops and the bioelectrode mainly functioned as the bioanode. The reversible bioelectrode can relieve the pH membrane gradient generated by the acidification at the anode and the alkalisation at the cathode during normal MFC operation [100,101].

**Hydrogen production by C. reinhardtii** Only a specific group of green microalgae and cyanobacteria, e.g. microalga *C. reinhardtii*, have evolved the additional ability to harness the huge solar energy resource to drive molecular $H_2$ production [102-108]. The release of hydrogen by *C. reinhardtii* under light exposure was first reported in 1942 [105]. In 2000, sustained hydrogen production was achieved using induced sulphur depletion in a culture medium containing acetate, a carbon source that is used to cause the shift from aerobic to anaerobic state [107].

*C. reinhardtii* is one of the best eukaryotes for $H_2$ production [109]. The available experimental information, including genomics, indicates that *C. reinhardtii* possesses a complex metabolic network containing aerobic respiration and molecular flexibility associated with fermentative metabolism. The molecular flexibility is accomplished with adjustments in the rates of accumulation of organic acids, ethanol, $CO_2$ and $H_2$ [86,110-115] and underlies the cell's adaptive ability for hypoxic and anoxic conditions.

Compared to other $H_2$-producing organisms such as chemotrophic and phototrophic bacteria, *C. reinhardtii* is more practical for $H_2$ production as it can be easily and efficiently grown in bioreactors using solar light, grows rapidly (doubling times of the order 6 h or less) and has a flexible metabolism [116]. The genome of this model microorganism was fully sequenced in 2007 [86], which makes it possible to increase production yields of $H_2$ from water by optimization of cell metabolism.

**Limitation of hydrogen production by C. reinhardtii** In fact, hydrogen production by *C. reinhardtii* can still not meet the commercial requirement because of several biochemical and engineering shortcomings, for example, hydrogen production demands anoxia because oxygen can suppress transcription and function of hydrogenase(s). However, the anoxia is constrained by the function of the photosystem II (PSII), which provides electron and protons from water and conducts oxygen evolution in the photosynthetic electron transport chain. Economic assessments have suggested that microalgae should achieve an efficiency of 10% in the conversion of solar energy to bioenergy to be competitive with other $H_2$ production methods, such as biomass gasification or photovoltaic electrolysis [117]. This is a more than a fivefold increase in efficiency from current levels. Exploiting hydrogen production directly in a product-type MFC may help to bridge this gap.

### *Synechocystis sp. PCC 6803*

*Synechocystis* sp. PCC 6803 is a unicellular cyanobacterium, one of the earliest groups of microbes to evolve on earth. The first primitive bacteria on Earth are dated at 3.8 to 3.6 billion years ago [118]. It is thought that cyanobacteria flourished during the period from 3.5 to 1.8 billion years ago, consuming $CO_2$ and providing Earth with oxygen, making possible the development of the different forms of aerobic life. At present, cyanobacteria deliver amounts of oxygen to the atmosphere similar to those that are produced by higher plants [118]. Moreover, cyanobacteria harness 0.2% to 0.3% of the total solar energy (178,000 TW) that reaches the Earth [106] and convert the solar energy into biomass-stored chemical energy at the rate of approximately 450 TW, contributing to 20% to 30% of Earth's primary photosynthetic productivity [119].

Until 1982, the cyanobacteria were called blue-green algae because they can photosynthesize and look like chloroplasts. Since then, cyanobacteria were re-classified as prokaryotes [120]. It is suggested that cyanobacteria entered into a symbiosis with cells, which were not capable of absorbing $CO_2$ and releasing oxygen, and later became photosynthetic organelles of plants [121]. Nowadays many species of cyanobacteria, e.g. *Synechocystis* sp. PCC 6803, are widely distributed in nature.

*Synechocystis* sp. PCC 6803 and plants have similar oxygen-evolving apparatus and are thus used for studying photosynthesis in plant cells. The difference is that *Synechocystis* sp. PCC 6803 grow much faster than plants, and they are relatively easy organisms for genetic manipulations [118]. Also, plants are fixed at places where they grow and they have less adaptation abilities for their growth and propagation than cyanobacteria.

*Synechocystis* sp. PCC 6803 grow photoautotrophically on carbon dioxide and light, as well as heterotrophically on glucose. Like *C. reinhardtii*, *Synechocystis* sp. PCC 6803 is one of several hydrogen-yielding species of cyanobacteria [122]. After its genome was fully sequenced in the 1990s [123,124], this cyanobacteria species has become a popular model photosynthetic organism studied by many researchers.

**Advantages of cyanobacteria** Cyanobacteria, besides other photosynthetic microorganisms such as microalgae, can establish synergistic relationships with heterotrophic bacteria, for instance, in a microbial mat [68]. Thus, they could be potentially manipulated to establish an indirect synergistic relationship with electricigens in phototrophic MFC [69]. However, phototrophic MFCs usually have low conversion efficiency [62], and the study of phototrophic MFCs is in its nascent stages [62].

Since *Synechocystis* sp. PCC 6803 is a photoautotroph that divides rapidly, it has been enlisted as a platform for production of biofuels by using sunlight as an inexpensive energy source [125,126]. This feature makes this species suitable as a candidate for MFCs of product mode.

**Electrogenic activity of cyanobacteria** Unlike other exoelectrogens, such as *G. sulfurreducens*, in which the electrons are derived from biochemical oxidation of organic compounds via the respiratory electron transfer chain [127], cyanobacterial electrogenic activity does not need exogenous organic fuel and is entirely dependent on the energy of light, which drives the biophotolysis of water through the photosynthetic electron transfer chain in the cyanobacteria, releasing electrons [128,129]. The electrogenic activity of cyanobacteria may represent a form of overflow metabolism to protect cells under high-intensity light [70,129]. This light-driven electrogenic activity is conserved in diverse genera of cyanobacteria and is an important microbiological channel of solar energy into the biosphere [129].

The electrogenic activity of *Synechocystis* sp. PCC 6803 has been captured in an MFC for electricity generation. The MFC can achieve a steady power density of 6.7 mW $m^{-3}$ (peaking at 7.5 mW $m^{-3}$) [130,131]. These power densities are still much lower than the values achieved by the other microbes under discussion. Despite that, it is included in the selection list because it offers a unique combination of photosynthetic activity that is plausibly accessible to direct-mode electron transfer. The quoted measurements are quite recent, and it is worth exploring if this organism has the potential to deliver competitive power densities in the future.

**Hydrogen production by cyanobacteria** Cyanobacteria have a similar process for hydrogen production as algae, except that they use NiFe hydrogenases rather than Fe hydrogenases in microalgae and the hydrogenase of cyanobacteria is 100 times less active than those of the green algae *C. reinhardtii* [106]. These hydrogenases contain [Ni-Fe] catalytic centres that are extremely sensitive to inactivation by $O_2$, one of the major barriers to hydrogen production. Natural mechanisms such as consumption by respiration, chemical reduction via PSI and reversible inactivation of PSII $O_2$ evolution can reduce intracellular $O_2$ content and thereby increase $H_2$ production.

### *Saccharomyces cerevisiae*

The *Saccharomyces* genus currently contains eight species [132]. *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces pastorianus* are associated with anthropic environments, whereas *Saccharomyces paradoxus*, *Saccharomyces kudriavzevii*, *Saccharomyces cariocanus*, *Saccharomyces mikatae* and the recently

described *Saccharomyces arboricolus* are mostly isolated from natural environments [133,134]. These *Saccharomyces* species can play a major role in food or beverage fermentation. However, the ale yeasts involved in alcoholic fermentation mostly belong to the species *S. cerevisiae* [132]. Besides its important role in baking and brewing, this yeast species has been used as a eukaryotic model organism in molecular and cell biology, for example, the characteristic of many proteins can be discovered by studying their homologs in *S. cerevisiae*.

*S. cerevisiae* cells are round to ovoid, are 5 to 10 μm in diameter, reproduce by a budding process and can grow aerobically on glucose, maltose and trehalose but not on lactose or cellobiose. In the presence of oxygen, it is even able to operate in a mixed fermentation/respiration mode. The ratio of fermentation to respiration varies slightly among strains but is approximately 80:20 [135]. Furthermore, *S. cerevisiae* can be processed to produce potential advanced biofuels such as long-chain alcohols and isoprenoid- and fatty acid-based biofuels, which have physical properties that more closely resemble petroleum-derived fuels [136].

**Advantages of yeast for MFC** Yeast is sometimes thought to be impractical as a biocatalyst, due to difficulties with transferring electrons out of cellular organelles [137]. However, since yeasts are robust, are easily handled, are mostly non-pathogenic, have high catabolic rates and grow on substrate spectrum, they are well worth considering as promising biocatalysts for MFCs [138]. In addition, several other merits may exist for using *S. cerevisiae* in MFCs. First, *S. cerevisiae* can survive and function in an anaerobic condition that is required for the anode compartment of traditional MFC. Second, the optimal growth temperature for *S. cerevisiae* is around 30°C, which is a convenient ambient temperature. Third, the metabolism of this species is well understood, which helps locate mechanisms responsible for electricity generation in MFCs. Lastly, yeast-based fuel cells could be retrofitted into ethanol plants for *in situ* power generation [139].

**Yeast for in situ power generation** In an anaerobic condition, yeasts usually switch to fermentation reactions where one glucose molecule is consumed for the production of two molecules of pyruvates. Pyruvate is further transformed into alcohol or organic acid by recycling NADH to $NAD^+$, which is a key step to sustain the glycolysis process [140]. This glycolysis reaction takes place in the cytosol of the cell rather than in the mitochondria, so NADH could be easily accessed by the mediator molecule present in the cell membrane of the yeast [73]. The glycolysis and the oxidation of NADH to $NAD^+$ are not influenced by the energy extraction process in the MFCs. Based on these characteristics, MFCs using yeast can be directly applied in fermenters for *in situ* power generation [139].

**Limitation of S. cerevisiae for MFC use** Limitations exist for *S. cerevisiae* to be used in MFCs. First, *S. cerevisiae* has a weak ability to oxidise the substrate to supply the maximum number of electrons available for yeast-based MFC. In the mitochondrial process of *S. cerevisiae*, there is a total of only 14 ATP per glucose molecule produced, which is much less than a net of 28 to 30 ATP typically achieved by most aerobes [138]. Also, mediators are commonly required to facilitate the transfer of electrons to the anode, which makes exogenous mediators necessary to MFCs based on *S. cerevisiae* because this yeast is thought incapable of producing such mediators indigenously [139].

**The output of S. cerevisiae-based MFCs** In general, yeast-based MFCs perform better than cyanobacteria but still have a lower power output than bacterial fuel cells [138]. It was shown that methylene blue-mediated *S. cerevisiae* MFC can give a power density of 1.5 W $m^{-2}$ [141], which is less than the maximum of 6.86 W $m^{-2}$ reported by Fan et al. [135] for a mixed-culture MFC. The corresponding volumetric density, based on the specified anodic chamber volume of 10 ml, is 90 W $m^{-3}$.

A recent MFC that employs *S. cerevisiae* as the electron donor in the anodic half-cell and *C. vulgaris* as the electron acceptor in the cathodic half-cell can reach a maximum power at 90 mV and a load of 5,000 Ω, giving a power density of 0.95 mW $m^{-2}$ of electrode surface area [142]. This power density is still very low.

Another study investigated the possibility of *S. cerevisiae* to transfer electrons to an extracellular electron acceptor through DET mode and found that the cells that adhered to the anode were able to sustain power generation in a mediator-less MFC. However, the power performance of this MFC was extremely low (0.003 W $m^{-2}$) [143].

#### *Geobacter sulfurreducens*

*G. sulfurreducens* are comma-shaped Gram-negative, anaerobic bacteria capable of coupling oxidization of organic compounds to reduction of metals. This organism is one of the predominant metal-reducing bacteria in soil and hence plays an important ecological role in biotechnologically exploitable bioremediation. The activity of *Geobacter* species in sub-surface can be stimulated to remove organic and metal contaminants such as aromatic hydrocarbons and uranium from groundwater [144-146].

The genome sequence of *G. sulfurreducens* is available, and a system for genetic manipulation has been developed for this organism [147]. Since it was discovered in

1994 [148], this bacterium has been extensively studied for MFC applications. It has been reported that (1) *G. sulfurreducens* can completely oxidise electron donors by using only an electrode as the electron acceptor, (2) it can quantitatively transfer electrons to electrodes in the absence of electron mediators, and (3) this electron transfer is similar to those observed for electron transport to Fe(III) citrate [47].

**Advantages of G. sulfurreducens** *G. sulfurreducens* is the most abundant species on anode surfaces in MFCs grown with more than one bacterial species [149-151]. It can form biofilms on the anodes, which make all the cells participate in electron transport to the anode and thus increase the current production [152]. *G. sulfurreducens* is an anaerobe but can withstand low levels of oxygen and may use oxygen as an electron acceptor to support growth under aerobic conditions [153].

This *Geobacter* species can produce large amounts of electrical energy since it possesses multiple mechanisms that involve either pili or c-type cytochromes to facilitate the electron transfer to electrode in MFCs (discussed before in the 'Microbial fuel cells' section) [149]. Also, with the electron transfer to electrodes, the *Geobacter* species can effectively oxidise acetate [47,154]. A current density of 4.56 A $m^{-2}$, corresponding to power densities of 1.88 W $m^{-2}$ and 43 W $m^{-3}$, measured for *G. sulfurreducens* is among the highest reported for a pure culture [155]. By reducing the anode compartment volume to a fraction of a millilitre, the volumetric density was in fact increased to 2.15 kW $m^{-3}$. While the lower value is more realistic for comparison to other studies, this does show that very high densities are achievable in principle with this organism. In addition, *G. sulfurreducens* converts acetate to current with coulombic efficiencies of over 90% [151,155].

Previous studies have shown that when a high selective pressure for high rates of current production at high coulombic efficiencies is imposed on complex microbial communities, it is the organisms closely related to *G. sulfurreducens* that are routinely enriched on anodes of the MFCs [55,154,156-158]. Thus, *G. sulfurreducens* can also be used to study adaptation for enhanced power production.

**Metabolism of Geobacter species** The metabolism of *G. sulfurreducens* was investigated by constraint-based modelling [159]. In contrast to *Escherichia coli*, which primarily produces energy and biosynthetic precursors through sugar fermentation, *Geobacter* completely oxidises acetate and other electron donors via the TCA cycle [160,161], which makes it necessary to transfer electrons to terminal electron acceptors for regeneration of cytoplasmic and intramembrane electron acceptors and ATP synthesis. In *G. sulfurreducens*, this is accomplished by electron transfer to extracellular electron acceptors, i.e. Fe(III) oxides [162].

Since the rate of cytoplasmic proton consumption is lower than that of proton production during the reduction of extracellular electron acceptors such as Fe(III), the energy consumption with extracellular electron acceptors is lower compared to that associated with intracellular acceptors [159]. The use of extracellular electron shuttles makes the *Geobacter* species circumvent the metabolic cost of producing the electron shuttles and consequently more energetically competitive than shuttle-producing Fe(III) reducers in sub-surface environments [159].

*In silico* analysis suggested that the metabolic network of *G. sulfurreducens* contains pyruvate-ferredoxin oxidoreductase, which catalyzes synthesis of pyruvate from acetate and carbon dioxide in a single step, indicating that the synthesis of amino acids in *G. sulfurreducens* is more efficient than in *E. coli* [159].

**Limits and applications** MFCs powered by *G. sulfurreducens* are far away from being commercialized as a practical biofuel source [152], because up until now the current levels of these MFCs are around 14 mA which could be used to power very simple components [149] but still not big enough to drive complex mechanisms. However, the actual current densities that could be generated from MFCs based on *G. sulfurreducens* are still unclear and require further investigation [151].

**Comparison of Geobacter sp. and Shewanella sp.** Geobacteraceae and Shewanellaceae are classic models in MFC research as their metabolism and versatility have been studied extensively [72,163]. As mentioned before in the 'Microbial fuel cells' section, they are both capable of being used for DET mode in MFCs, because both *Shewanella* sp. and *Geobacter* sp. possess nanowires, electrically conductive bacterial appendages, to transport electrons from cells to solid electron acceptors such as graphite anodes in MFCs [34,35,162]. Despite those similarities, differences also exist when compared regarding the engineering design and performance of the MFCs.

*Geobacter*-based MFCs generate high coulombic efficiencies [164] but require strict anaerobic conditions which limit their applicability. In contrast, *Shewanella*-based MFCs can be operated with air-exposed cultures [27]. Unlike *Geobacter* sp. that requires direct contact to the electrode surface [72], *Shewanella* sp. can use additional mediators to facilitate electron transfer outside the cell membrane [27]. Importantly, besides utilizing nanowires to mediate the electron transport [165], they can synthesize their own redox mediators (i.e.

flavins) for extracellular electron transfer under diverse environmental conditions [163,166]. These two electron-mediated mechanisms determine the efficiency of the current generation in *Shewanella*-containing MFCs [167].

A maximum power density of 24 mW $m^{-2}$ (in the presence of an additional mediator) was reported for *Shewanella* [168]. This value appears low in comparison to bacterial cells, but that is because it was referred to the true microscopic area of a porous electrode. When expressed, as customary, in terms of macroscopic MFC dimensions, the equivalent power densities are 3 W $m^{-2}$ and 500 W $m^{-3}$. These values compare favourably with *Geobacter*. When dissolved oxygen was deliberately fed into the anode chamber, *Shewanella*-based MFC was still able to produce a power output of 6.5 mW $m^{-2}$ and 13 mA $m^{-2}$. The MFC used lactate as the fuel source and relied on self-excreted mediators of *Shewanella* [169].

In fact, the previously described *Shewanella*-based system would not be directly applicable to powering electronics and is required to use aerobic water [170]. For instance, a complex pumping system is necessary to continuously recirculate the anolyte between the anode and the large anolyte reservoir, but this pumping system at the anode could consume more power than the *Shewanella*-based MFC produces. Since *Shewanella* sp. cannot use oxygen as the electron acceptor, ferricyanide needs to be added as catholyte. However, ferricyanide is a non-renewable and toxic electron acceptor and can thus not be deployed in the field in the long term. Moreover, the coulombic efficiencies were found to be low (<6%), when calculated based on the incomplete oxidation of lactate to acetate [170].

Conversely, *G. sulfurreducens* can effectively oxidise acetate with electron transfer to electrodes [47,154] and convert acetate to current with coulombic efficiencies of more than 90% [151,155]. *G. sulfurreducens* is an anaerobe that can withstand low levels of oxygen and may use oxygen as an electron acceptor [153]. It has recently been shown that with a new configuration, MFCs based on *G. sulfurreducens* can become 100% aerobic, allowing for floating and/or untethered applications. At the same time, the performance of the MFCs is similar to their anaerobic/aerobic counterparts [170]. It is expected that with this aerobic configuration, power could be produced in a *G. sulfurreducens* MFC suspended in aerobic seawater [170].

## Conclusions

Electricity generation in MFCs is based on the metabolic activity of living microorganisms at the anode. The selection of microorganisms is based on many criteria, but the power output, electron transfer ability and biological functions such as photosynthesis and hydrogen production are particularly important. These important properties, in different combinations, are exemplified by the four representative microorganisms discussed above, and their referential facts for modelling are compared in Table 2. Studying these individually, and in combination, should reveal significant insights in the quest for higher power output MFCs.

Most MFC researchers have been active in engineering designs, i.e. how to create scalable and economical architectures and engineer more efficient hardware and how different microbes interact with the anodes/cathodes when transporting electrons [127]. Such research covers optimizing anodic conditions, housing constructs and component materials, learning more about microbial community ecology and isolating vigorous biocatalysts [9]. Biological aspects of MFCs have also received some attention, such as the anodic activity of different organisms. However, very little research has been done on the biochemical interface between the engineering design and the biological aspects (see Figure 1).

We conclude that future studies are required to work on that interface, i.e. how to enhance the anodic activity by means of adjusting the metabolic activity of biocatalysts, for example, utilizing metabolic network analysis. The genome-scale metabolic networks are quite new concepts and have only been produced in the last few years. The analysis of the metabolic network through modelling approaches, such as flux balance analysis, plays an important role in filling the gap between genotypes and phenotypes of microorganisms to provide a full picture of the biological system.

## Endnote

[a]Representative microorganisms chosen by this article and discussed in the 'Microorganisms for *in silico* study of MFC functioning' section.

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

LM and WV co-conceived the idea. LM conducted the review and drafted the manuscript. WV supervised the work and corrected the manuscript. Both authors read and approved the final manuscript.

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