Plugging in microbial metabolism for industrial applications

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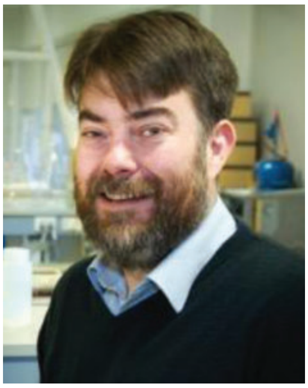

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The ability of electric microbes to electrically interact with electrodes is opening up a number of possibilities with industrial applications. Microbes are able to utilise the electrode as an electron source to reduce CO_2 for the production of organic compounds directly or produce H_2 as a reducing equivalent for partner microbes for the production of commodity chemicals. Electrodes can also allow redox unbalanced fermentation processes to occur through the addition or subtraction of reducing equivalents that remove bottle necks in these pathways. Electrodes are also providing a physical refuge for electric microbes to maintain anaerobic fermenter stability. It can be expected that the role for electric microbes will continued to be expanded as part of industrial applications in the future.

Microbes and electrons

Microbial electrochemical systems enable electrons to be removed and inserted directly into microbial metabolic processes by microbes that are capable of interacting with an electrode, which are commonly called electric bacteria[1]. While first gaining much attention due to their ability to generate electricity, the ability of microbes to externally transfer electrons both directly and indirectly, has seen interest in their use for environmental monitoring, bioremediation applications and industrial processes (see [1,2]). Processes, such as the production of commodity chemicals, such as acetate, butyrate and alcohols, using electrons as an energy source and unbalanced redox reactions in electrofermentation, has greatly expanded their industrial applications. These processes have the potential to revolutionise the industrial use of microbes into a new era of reduced inputs, stable outputs, and more customisation and design in bacterial metabolic processes.

Production of commodity chemicals utilising microbial electric systems

Acetogens and methanogens have long been of interest to industry due to their ability to reduce carbon dioxide to organic products such as acetate, methane, butanol, and ethanol[3]. When integrated into microbial electric systems, microbes capable of utilising electrodes as electron donors can use electricity to produce organic chemicals (Fig. 1*a*). An advantage of this process is the possibility for excess renewable or power production to be converted into organic compounds that are capable of acting as energy storage for later use, transport or as commodity chemicals[4]. A wide variety of microbes has been found to be capable of accepting electrons from an electrode to provide energy to drive the biological processes[5]. Termed microbial electrosynthesis, being likened to photosynthesis when using solar energy as an electron source[6], the rates of organic product formation using direct electron transfer is currently significantly less than those utilising *in situ* H_2 production as the electron donor to drive similar processes[3].

Multistep electrode production with multiple species

Interest has been shown in the use of multiple microbial species that associate with electrodes to enhance the production of commercially valuable products. The use of two species allows for the specialisation of both and removes the need to collect, concentrate and process H_2 gas from the system (Fig. 1*b*). A consortium was found to form a *Rhodobacter* sp. electrode-reducing biofilm in association with the electrode producing hydrogen and a planktonic culture comprising *Clostridium* sp., which reduced CO_2 to organic compounds and a small amount of alcohols[7]. Co-cultures

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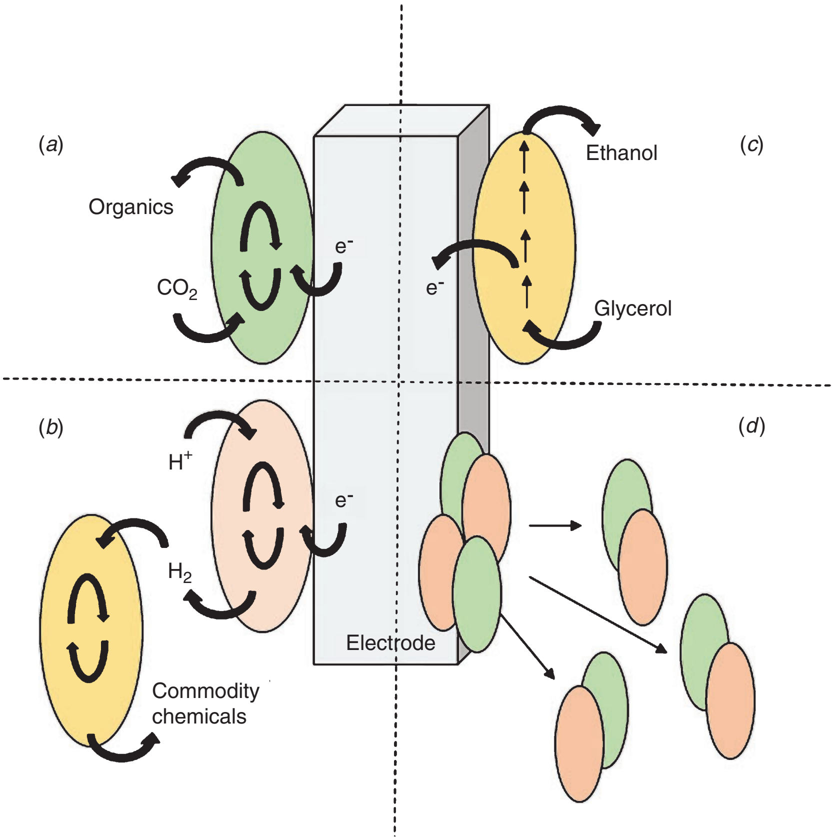

Figure 1. Linking electric microbial metabolism to electrodes for industrial processes can allow: (*a*) the use of an electrode as an electron donor for the reduction of CO_2 for the production of organic compounds; (*b*) electrode reduction for the production of H_2 by one species to provide reducing equivalents for bioproduction by a second microbial species; (*c*) removal or addition of electrons allowing for unbalanced fermentations to occur; and (*d*) provision of a refuge for syntrophic communities members in anaerobic reactors for enhanced function and stability.

of *Methanococcus maripaludis* and *Acetobacterium woodii* demonstrated that dual species, specialising in either cathodic reduction or bioproduction, are currently able to outperform monocultures attempting to do both[8]. While electron-transfer pathways occurring in biocathodes have still not been fully elucidated, increases in production that occur have been linked to biologically induced hydrogen production[9].

The use of co-cultures coupling cathodic electron uptake and biosynthesis reactions through interspecies electron transfer, either directly or through use of intermediate products, is proposed to be a useful strategy to allow modular systems to be established for production, and is a potential area of interest in the future utilising Synthetic Biology[10]. As Synthetic Biology principles are more widely accept by the general community, extensive effort will be placed on the production of a wide range of commodity chemicals to be introduced into commercial markets[11].

Fermentation and electrons

Brewing beer is one of the most widely recognised fermentation processes, and has been intertwined with much of human history from being mentioned in cuneiform, the oldest known form of writing from ancient Mesopotamia, to speculation that it may have been a driver of modern agriculture[12]. Fermentation utilises microorganisms to convert organic compounds to acids, gases, or alcohols, and has been utilised for food preservation increasing product storage and stability. Fermentations now occur on industrial-level scales producing biomass, primary and secondary metabolites, enzymes, recombinant products, and products for consumption[13]. Industrial fermentation processes are often constrained due to the need for sterility, the use of specific strains, substrate selectivity, maintenance of redox balance, environmental control, and product recovery[14].

The use of electrodes, and microbes capable of interacting with them, is now providing the possibility to control microbial cell growth and microbial density, increase product concentrations, allow imbalanced redox fermentations to occur, and provide energy requirements for organic carbon production from the reduction of carbon dioxide during microbial fermentation processes[6,15,16]. Fermentation can be benefited through microbial interactions with electrodes due to enhancement of electron transport pathways and improved energy production (Fig. 1*c*). Depending on the electrode potential, an excess of redox cofactors (such as NADH and NADPH) may be produced when an electrode is utilised as an electron acceptor, or in ATP production when being utilised as an electron acceptor[17].

A large advantage of the use of electrodes in fermentation reactions is the ability to balance the redox reactions. Electrodes have allowed the stoichiometric conversion of glycerol to ethanol using an engineered strain of *Shewanella oneidensis*, removing redox bottle necks and allowing unbalanced redox reactions to occur[18]. Similarly, an electrode allowed a shift toward more reduced products during fermentation with *Clostridium acetobutylicum*[19]. *In silico* elementary mode analysis predicts an increase in yield of 18 out of 20 different valuable products using anaerobic electrically enhanced fermentation. These included compounds such as succinic acid, lysine, and diaminopentane, which are already produced on an industrial scale, as well as other valuable products like *p*-aminobenzoic acid, *p*-hydroxybenzoic acid, and isoprene[17]. Increase in product formation by electrical enhancement is not necessarily dependent on the degree of reduction of the product, but rather the metabolic pathway it is derived from. The method of electron transport in the enhanced fermentation has a major impact on theoretical biomass and product yields.

Engineering industrial relevant microbial communities using electrodes

Anaerobic digestion is commonly utilised for the biotransformation of organic wastes to either carbon dioxide or methane. Electric microbes have been demonstrated to be present in these systems, helping overcome energy barriers and allowing the degradation of compounds otherwise not thermodynamically possible by an individual, a process occurring commonly in anaerobic digesters[20,21]. The direct transfer of electrons is advantageous in syntrophic communities as it maintains concentrations of key intermediates below key threshold levels, does not require reverse electron flow for the formation of H_2, and increases the Gibbs free energy of the intermediate reactions[21]. By enhancing the electrical link of these communities, such as the addition of conductive particles, or the addition of electrodes, it is possible to enhance the function, stabilise the microbial community structure, and increase the reactor performance overall[22].

The addition of electrodes to an anaerobic digester had the overall effect of enriching *Geobacter* species in association with the electrode and the reactor sludge, which correlated to an increase in methane production[23]. The enhancement of methane production is hypothesised to have occurred through direct interspecies electron transfer between *Geobacter* and *Methanosaeta* species. These species have previously been utilised in co-culture to demonstrate the direct transfer of electrons between species without the reliance, or formation of a bottle neck, on H_2 or formate in the anaerobic community[24]. Interestingly, in complex communities the electrode-associated communities are able to bring stability to the complex anaerobic digester community, helping prevent large fluctuations and preventing collapse and failure (Fig. 1*d*)[25]. It has been suggested that biomass retention on electrodes, rather than electrical current, enhances stability of the anaerobic digester community[26].

Future perspectives

Electric microbiology is expected to play an increasingly important role in industrial microbiology. The ability to provide energy in the form of electrons and carry out unbalanced fermentation reactions expands the range of possibilities for bioproduction. Currently, technological developments are lagging behind requirements for large scale production. With continued advancements, and with systems utilising microbial produced H_2 as a feedstock, dual culture systems are expected to be amongst the first widespread adoption of industrial electric microbiology.

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Biographies

Carolyn Bell is an Associate Lecturer in the Department of Physiology, Anatomy and Microbiology at La Trobe University. Her research interest areas include environmental microbiology, antibiotic resistance mechanisms, and applied plasmid biology.

Associate Professor Ashley E Franks is head of the Applied and Environmental Microbiology Laboratory at La Trobe University. He conducted his doctorate research as part of the Centre of Marine Biofouling and Bioinnovation at the University of New South Wales by investigating antifungal compounds produced by marine bacteria in biofilms. During his PhD he spent 4 months at the University of Exeter in the UK on an Adrian Lee Fellowship to develop dual bacterial/yeast biofilm systems. On graduating he moved to the Biomerit Research Centre in Cork, Ireland to work on bacterial plant interactions as a Government of Ireland Fellow in Science Technology and Engineering. This research looked at how to use bacteria to help plant growth. He then took a position as a Senior Scientist and Research Professor within the Geobacter Project at the University of Massachusetts Amherst in the USA where he worked on microbes that make electricity. He currently serves as the Chair of the Awards Committee for the International Society Microbial Electrochemical Technology and Secretary of Synthetic Biology Australasia.