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Current Opinion in Biotechnology

Title

Electrochemical biotechnologies minimizing the required electrode assemblies

Short title

Toward commercialization of microbial electrochemical systems

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2

Abstract

Microbial electrochemical systems (MESs) are expected to be put into practical use as an environmental technology that can support a future environmentally friendly society. However, conventional MESs present a challenge of inevitably increasing initial investment, mainly due to requirements for a large numbers of electrode assemblies. In this review, we introduce electrochemical biotechnologies that are under development and can minimize the required electrode assemblies. The novel biotechnologies, called electro-fermentation and indirect electro-stimulation, can drive specific microbial metabolism by electrochemically controlling intercellular and extracellular redox states, respectively. Other technologies, namely electric syntrophy and microbial photo-electrosynthesis, obviate the need for electrode assemblies, instead stimulating targeted reactions by using conductive particles to create new metabolic electron flows.

1 Introduction

2 Some of the redox reactions that occur in living cells, such as
3 organic-oxidation/oxygen-reduction in microbial respiration and water-oxidation/carbon
4 dioxide-reduction in photosynthesis, are important from the viewpoint of global
5 environmental and energy issues. Although catalysts composed of rare elements such as
6 platinum are generally required, microorganisms achieve these reactions at normal
7 temperature and pressure using earth-abundant elements. In developing environmentally
8 friendly energy systems that do not rely on fossil fuels, it is critical to use the energy
9 and material conversion abilities inherent in microbial metabolism. However,
10 microorganisms derived from nature do not necessarily perform the specific metabolic
11 reactions desired by human beings. Hence it is necessary to develop biotechnologies for
12 exploiting or controlling microbial metabolism.

13 In recent years, biotechnologies combining microbiology and electrochemistry,
14 namely microbial electrochemical systems (MESs), have attracted considerable
15 attentions [1*,2]. The representative technologies among MESs are microbial fuel cells
16 (MFCs) and microbial electrosynthesis cells (MECs) (Fig. 1A and B). In MFCs, the
17 respiratory electrons of microorganisms are transferred to an electrical circuit via an
18 anode, under conditions providing the coexistence of an appropriate cathode reaction
19 such as an oxygen reduction reaction, thereby forming a battery circuit [3,4*]. MFCs are
20 particularly appealing as a novel technology for energy-saving wastewater treatment
21 systems. On the other hand, in MECs, high-energy electrons are injected into the
22 microorganisms from a cathode, resulting in efficient microbial production of valuable
23 substances [5]. This mechanism can be regarded as a process for the conversion of
24 electrical energy to chemical energy; indeed, recent efforts have permitted the
25 production of high-energy chemicals from carbon dioxide using high-energy electrons
26 derived from an MEC cathode [6,7]. In addition, developments have lately yielded

1 hybrid technologies that couple MECs and anaerobic wastewater treatment systems to
2 stimulate degradation of recalcitrant substances on the electrodes [8,9,10,11].

3 In recent years, on-site and scale-up experiments with MFC/MEC technologies
4 have made large advances, and research on the scaling-up of these technologies has
5 reached a mature state [12,13,14]. However, these conventional MES technologies rely
6 on interfacial electron transfer at the electrode surfaces, and therefore inevitably require
7 a large number of electrode assemblies. Currently, constituents of electrode composites
8 such as electrode materials, electrocatalysts, and various functional membranes remain
9 too expensive to be economically feasible. This cost challenge represents a major
10 obstacle, necessitating both scientific and technological breakthroughs for practical
11 application of MFC/MEC technologies.

12 In this review, we introduce some developing biotechnologies that, which based on
13 a knowledge of microbial electrochemistry, permit the use of smaller numbers of
14 electrode assemblies. The first emerging technology is “electro-fermentation”, in which
15 the desired metabolic pathways are stimulated by electrochemically controlling the
16 microbial intracellular redox state (Fig. 1C). The second such technology is “indirect
17 electro-stimulation”, in which targeted microbial activities are promoted or suppressed
18 by controlling the redox state of the bulk solution (Fig. 1D). We also present examples
19 of electrochemical biotechnologies that do not require the use of any electrodes, namely
20 electric syntrophy and microbial electro-photosynthesis, in which new metabolic
21 electron flows are created by supplementation with conductive materials (Fig. 1E and
22 F).

23 24 **Electro-fermentation: stimulation of microbial metabolism by electrochemical** 25 **control of intracellular redox states**

26 Microorganisms alter their gene expression patterns and metabolic pathways in

1 response to shifts in the intracellular redox balances. Based on this knowledge, several
2 laboratories have generated biotechnologies that stimulate a specific metabolic pathway
3 by electrochemically controlling the intracellular redox states of microbial cells; these
4 processes are called “electro-fermentation” [15,16,17]. Compared with the
5 conventional MESs, these technologies require smaller numbers of electrodes, since the
6 quantities of electrons that need to be exchanged with the external circuits are smaller
7 than those flowing in the main metabolic processes (Fig.1C). The concept of
8 electro-fermentation is not new, having already been advocated in the 1970s. In
9 conventional studies, artificial electron mediators, including neutral red and methyl
10 viologen, were used to uptake/inject electrons from/into microbial cells [18,19,20].
11 These studies succeeded in improving production of valuable chemicals (e.g., fuel
12 compounds and amino acids) by electrochemical control of fermentative
13 microorganisms in laboratory experiments. However, the cost, stability, and cytotoxicity
14 of artificial mediators have hampered the practical application of this technology.

15 This section introduces two different methodologies that have been used to
16 overcome the issues encountered with artificial electron mediators. The first approach is
17 the development of novel, biocompatible electron mediators. Coman et al. [21]
18 developed cytocompatible osmium redox polymers that permit efficient electric
19 communication between electrodes and diverse microorganisms such as *Bacillus*
20 *subtilis*. This research group successfully constructed a solar/electricity energy
21 conversion system incorporating green algae and the osmium redox polymers [22].
22 Nishio et al. [23] developed electron-mediating co-polymers consisting of a hydrophilic
23 phospholipid-like domain and a hydrophobic, redox-active vinylferrocene domain. This
24 amphipathic mediator showed low cytotoxicity and enabled a diverse range of
25 microorganisms to exchange electrons with electrodes [23,24]. Using the new
26 amphipathic mediator, this laboratory achieved enhancement of polyhydroxybutyrate

production by *Ralstonia eutropha* [25] and control of the circadian rhythms of photosynthetic cyanobacteria [26], clearly demonstrating the practical feasibility of this technology.

The second methodology is electrical control of microorganisms that are innately electrochemically active, including microbial communities enriched on anodic/cathodic electrodes. In a proof-of-concept study in pure culture, electrochemical metabolic control without mediator compounds was demonstrated with *Shewanella oneidensis* and *Clostridium pasteurianum*, as well as genetically engineered *Escherichia coli* [27,28,29]. This concept has been successfully applied to complex microbial community systems. Steinbusch et al. [30] demonstrated enhancement of ethanol production via reduction of acetate by electrochemically active microbial communities enriched on electrodes with the aid of a cathodic supply of reducing equivalents. Zhou et al. [31] reported that conversion of glycerol into 1,3-propanediol can be improved by injecting trace amounts of electrons to cathodic microbial communities. This group reported that the efficiency coefficient (i.e., charge transferred between electrodes and microorganisms per charge required for increase in target products) was only 0.05 [16], which indicated that the improvement of 1,3-propanediol production was due to enhancement of specific metabolic pathways (and/or specific microbial species) via an alteration of cellular redox states, and not by direct supplementation of reducing power.

Indirect electro-stimulation: control of specific microbial metabolism/species via modification of redox state in bulk solution

Electric fermentation technologies still require electron transfer between microorganisms and electrodes. Since only microbial cells in close proximity to electrode surfaces can be controlled, the total number of electrodes still cannot be reduced substantially by such technology. Alternatively, techniques have been

developed for electrically adjusting the redox state of the bulk solution to promote or suppress specific microbial metabolism and/or species; this methodology has been termed “indirect electro-stimulation”. This technique has been intensively studied as a novel approach to regulate the metabolism of anaerobic microorganisms that are sensitive to redox conditions, particularly methanogenic archaea (Fig. 2). Methanogenic archaea play a pivotal role in some anaerobic wastewater treatment systems, such as those used in anaerobic digesters. In contrast, methanogenic archaea are undesirable microorganisms in other anaerobic wastewater treatment systems, such as biological hydrogen production reactors, since these organisms consume the target product, hydrogen gas.

Hirano et al. [32] investigated the correlation between the methane-generating activities of *Methanothermobacter thermautotrophicus* and the redox potential of a bulk solution that was controlled by poised graphite electrodes. This laboratory demonstrated that the methane-generation activities per cell were enhanced to up to 3.5-fold in cultures provided with negative electrode potential (-0.8 V vs. Ag/AgCl), and were suppressed over 10-fold in cultures provided with positive electrode potential (above -0.1 V vs. Ag/AgCl). This technology has already been applied to actual wastewater treatment systems that use complex microbial communities. Sasaki et al. [33] reported that methane production from thickened sewage sludge was greatly enhanced in the cathode compartment by applying a potential of -0.8 V vs. Ag/AgCl to a cathode placed in the methane fermentation reactor. Microbial community analysis revealed that the relative abundance of hydrogen-utilizing methanogens in such a reactor was increased 3.6- to 6.0-fold. Notably, the efficiency coefficient (i.e., charge transferred from electrodes to bulk solution per charge required for an increase in methane production) was only 0.001, a value significantly smaller than the efficiency coefficient observed with electro-fermentation (as discussed above in the **Electro-fermentation** section).

1 These results suggested that the electrons injected from the cathode increased the
2 metabolic flow of the entire microbial consortium through changes in both the
3 metabolic activities and the microbial community structure, rather than being used only
4 for methane production. Stimulation of methanogenesis by electrochemical control of
5 redox potential also has been confirmed in methane fermentation systems decomposing
6 synthetic wastewater [34] or garbage [35]. On the other hand, in systems that
7 bio-generate hydrogen gas from organic waste, it was reported that the microbial
8 production of hydrogen gas in the anode compartment is improved [36]. Those authors
9 speculated that growth of hydrogen-assimilating methanogen was inhibited by anodic
10 polarization.

11 12 **Electric syntrophy and microbial photo-electrosynthesis: generation of new** 13 **metabolic electron flow via conductive particles**

14 In the final segment of this review, we cover a newly developing biotechnology in
15 which supplementation with electrically conductive particles is used to improve
16 microbial activities by creating alternative electron flow between microbial cells. In this
17 technology, macroscopic electrodes and electric wiring are completely unnecessary,
18 since electron exchange with the external circuit is no longer required. Here, we
19 introduce two examples of these technologies. In the first, high-efficiency symbiotic
20 microbial metabolisms is induced using conductive particles (electric syntrophy, Fig.
21 1E); in the second, valuable substances are microbially generated from carbon dioxide
22 using photo-functional semiconductor nanoparticles (microbial photo-electrosynthesis,
23 Fig. 1F).

24 Some anaerobic processes proceed via cooperation of multiple microbial species
25 through energy exchanges. This type of microbial symbiosis is termed “syntrophy”.
26 Small molecules such as H₂ and formate usually function as the energy carriers in these

1 systems. In contrast, recent studies have demonstrated that interspecies energy exchange
2 can be mediated by electric currents flowing through conductive solid materials,
3 including iron oxide minerals [37] and a biologically produced conductive apparatus
4 [38]; these processes are termed as “electric syntrophy” or “direct interspecies electron
5 transfer”. Kato et al. [39] demonstrated that supplementation with conductive iron oxide
6 nanoparticles promoted syntrophic methanogenesis from acetate and ethanol, a step that
7 has the potential of improving methane fermentation of organic wastewater.
8 Subsequently, other research groups showed that methanogenesis from various organic
9 substances (e.g., propionate, butyrate, and aromatic compounds) was accelerated by
10 addition of conductive iron oxide nanoparticles [40,41,42,43]. Furthermore, inexpensive
11 carbonaceous materials such as activated carbon and biochar have been shown to
12 mediate electric syntrophy [44,45,46]; these processes have the potential to drastically
13 reduce the cost in application to wastewater treatment. Improvement of efficiency and
14 stability of methane fermentation of actual wastewater has been demonstrated by
15 experiments using laboratory-scale bioreactors [47,48,49,50]. This technology can use
16 existing anaerobic digester reactors as is, thereby decreasing the initial investment cost.

17 In addition to methane fermentation, this technology also has been applied to
18 stimulation of some bioremediation processes. It was reported that introduction of
19 conductive iron oxides into contaminated soil or into a bioreactor containing chlorinated
20 aromatic compounds facilitated microbial reductive dechlorination of trichloroethene or
21 2,4-dichloronitrobenzene, respectively, by promoting interspecies electron transfer
22 processes [51,52]. Also, Cruz Viggi et al. [53] demonstrated that biodegradation of
23 petroleum hydrocarbons in sediments was stimulated by electrically connecting the
24 anaerobic sediment and the overlying O₂-containing surface water with centimeter-long
25 graphite rods.

26 Another promising approach is the microbial production of valuable substances

1 from carbon dioxide using light-absorbing semiconductor nanoparticles. Although
2 microbial production of valuable substances from carbon dioxide utilizing high-energy
3 electrons supplied from the cathode (as in MEC) has attracted attention in recent years
4 [54,55], such systems require large numbers of macroscopic electrode assemblies.
5 Therefore, as pointed out in the **Introduction**, there will be limitations when
6 considering widespread commercialization. Sakimoto et al. [56"] showed that an
7 acetogenic bacterium, *Moorella thermoacetica*, produces acetate from carbon dioxide
8 using photo-excited electrons obtained from semi-conductive cadmium sulfide
9 nano-particles that are formed by the microorganism itself. Although cysteine was used
10 as a sacrificial electron source in this research, the same group also succeeded in
11 microbial photosynthesis of organic compounds using water as an electron source,
12 employing titanium dioxide doped with manganese(II) phthalocyanine catalysts [57].
13 That research is still at the proof-of-concept stage in the laboratory. However, this novel
14 approach for the photosynthesis of organic matter using semiconductor materials and
15 microorganisms has the potential to outperform the energy conversion efficiency of
16 carbon dioxide fixation by photosynthetic organisms [58]; further research on the
17 efficiency and scale-up of these processes are anticipated.

19 **Conclusions**

20 Research on the scale-up of MES technologies is approaching maturity. Considering the
21 cost of electrode assemblies, however, there appears to be a limit to the practical
22 application of MESs. The present review therefore examined three types of developing
23 electrochemical biotechnologies, namely electro-fermentation, indirect
24 electro-stimulation, and electric syntrophy. Since these technologies require few or no
25 electron exchanges with external circuits, these processes are expected to bypass the
26 issue of the cost of electrode assemblies. Currently, these technologies are in the early

stage of development, typically existing as a proof-of-concept using pure culture of model microorganisms or as demonstration studies using laboratory-scale reactors with microbial communities. Further research on reactor engineering, enlargement of reactor systems, and improvement of long-term durability will aid practical application of these new-generation electrochemical biotechnologies.

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Figure legends

Figure 1

Schematic diagrams of the conventional and next-generation microbial electrochemical systems (MESs) introduced in this review. Microbial fuel cells (A) and microbial electrosynthesis cells (B) are representative of conventional MESs, which require a large numbers of electrode assemblies to exchange metabolic electrons with external circuits. In electro-fermentation (C), microbial metabolism is stimulated by electrochemical modification of the intracellular redox state, a process that can decrease the required number of electrode assemblies. In indirect electro-stimulation (D), there is no electron exchange between microorganisms and electrodes; microbial metabolism is controlled indirectly, via redox states in bulk solution. Electron exchanges with external circuits are no longer necessary in electric syntrophy (E) and microbial photo-electrosynthesis (F); in both of these processes, new electron flows are created by supplementation with (semi)conductive particles. Red and black arrows represent electron and carbon flows, respectively. Blue arrows represent promotive and/or suppressive effects. S; substrates, P; products; Red; reductive forms; Ox; oxidative forms.

Figure 2

The concept for indirect electro-stimulation of methane production. Redox states in bulk solution are negatively or positively shifted by cathodic or anodic electrodes, which promote or suppress respectively the metabolic activity and growth of H₂-assimilating methanogens, respectively.

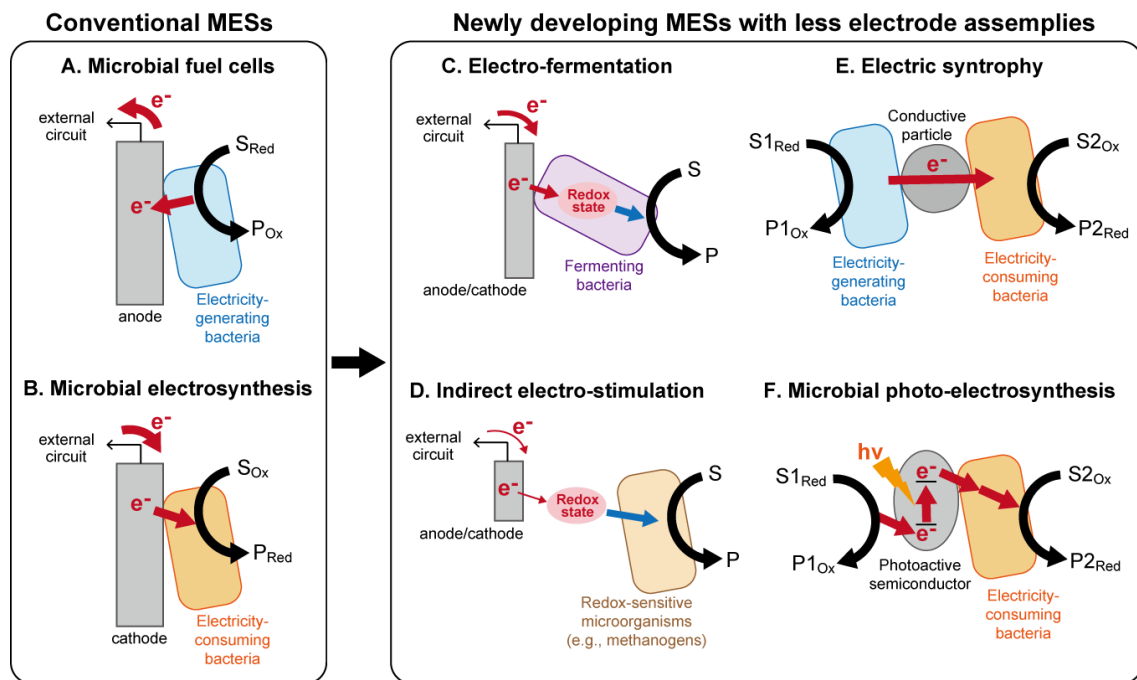


Figure 1

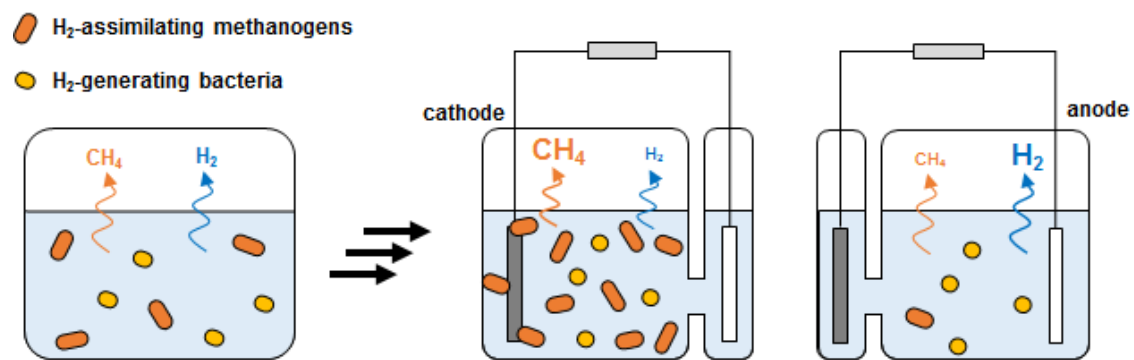


Figure 2