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Title

Electrochemical biotechnologies minimizing the required electrode assemblies

Short title

Toward commercialization of microbial electrochemical systems

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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.

Introduction

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

In recent years, biotechnologies combining microbiology and electrochemistry, namely microbial electrochemical systems (MESs), have attracted considerable attentions [1•,2]. The representative technologies among MESs are microbial fuel cells (MFCs) and microbial electrosynthesis cells (MECs) (Fig. 1A and B). In MFCs, the respiratory electrons of microorganisms are transferred to an electrical circuit via an anode, under conditions providing the coexistence of an appropriate cathode reaction such as an oxygen reduction reaction, thereby forming a battery circuit [3,4•]. MFCs are particularly appealing as a novel technology for energy-saving wastewater treatment systems. On the other hand, in MECs, high-energy electrons are injected into the microorganisms from a cathode, resulting in efficient microbial production of valuable substances [5]. This mechanism can be regarded as a process for the conversion of electrical energy to chemical energy; indeed, recent efforts have permitted the production of high-energy chemicals from carbon dioxide using high-energy electrons derived from an MEC cathode [6,7]. In addition, developments have lately yielded hybrid technologies that couple MECs and anaerobic wastewater treatment systems to stimulate degradation of recalcitrant substances on the electrodes [8,9,10,11].

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

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

Electro-fermentation: stimulation of microbial metabolism by electrochemical control of intracellular redox states

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

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

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

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

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

Some anaerobic processes proceed via cooperation of multiple microbial species through energy exchanges. This type of microbial symbiosis is termed “syntrophy”. Small molecules such as H_2 and formate usually function as the energy carriers in these systems. In contrast, recent studies have demonstrated that interspecies energy exchange can be mediated by electric currents flowing through conductive solid materials, including iron oxide minerals [37] and a biologically produced conductive apparatus [38]; these processes are termed as “electric syntrophy” or “direct interspecies electron transfer”. Kato et al. [39] demonstrated that supplementation with conductive iron oxide nanoparticles promoted syntrophic methanogenesis from acetate and ethanol, a step that has the potential of improving methane fermentation of organic wastewater. Subsequently, other research groups showed that methanogenesis from various organic substances (e.g., propionate, butyrate, and aromatic compounds) was accelerated by addition of conductive iron oxide nanoparticles [40,41,42,43]. Furthermore, inexpensive carbonaceous materials such as activated carbon and biochar have been shown to mediate electric syntrophy [44,45,46]; these processes have the potential to drastically reduce the cost in application to wastewater treatment. Improvement of efficiency and stability of methane fermentation of actual wastewater has been demonstrated by experiments using laboratory-scale bioreactors [47,48•,49,50]. This technology can use existing anaerobic digester reactors as is, thereby decreasing the initial investment cost.

In addition to methane fermentation, this technology also has been applied to stimulation of some bioremediation processes. It was reported that introduction of conductive iron oxides into contaminated soil or into a bioreactor containing chlorinated aromatic compounds facilitated microbial reductive dechlorination of trichloroethene or 2,4-dichloronitrobenzene, respectively, by promoting interspecies electron transfer processes [51,52]. Also, Cruz Viggi et al. [53] demonstrated that biodegradation of petroleum hydrocarbons in sediments was stimulated by electrically connecting the anaerobic sediment and the overlying O_2-containing surface water with centimeter-long graphite rods.

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

Conclusions

Research on the scale-up of MES technologies is approaching maturity. Considering the cost of electrode assemblies, however, there appears to be a limit to the practical application of MESs. The present review therefore examined three types of developing electrochemical biotechnologies, namely electro-fermentation, indirect electro-stimulation, and electric syntrophy. Since these technologies require few or no electron exchanges with external circuits, these processes are expected to bypass the 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 H_2-assimilating methanogens, respectively.

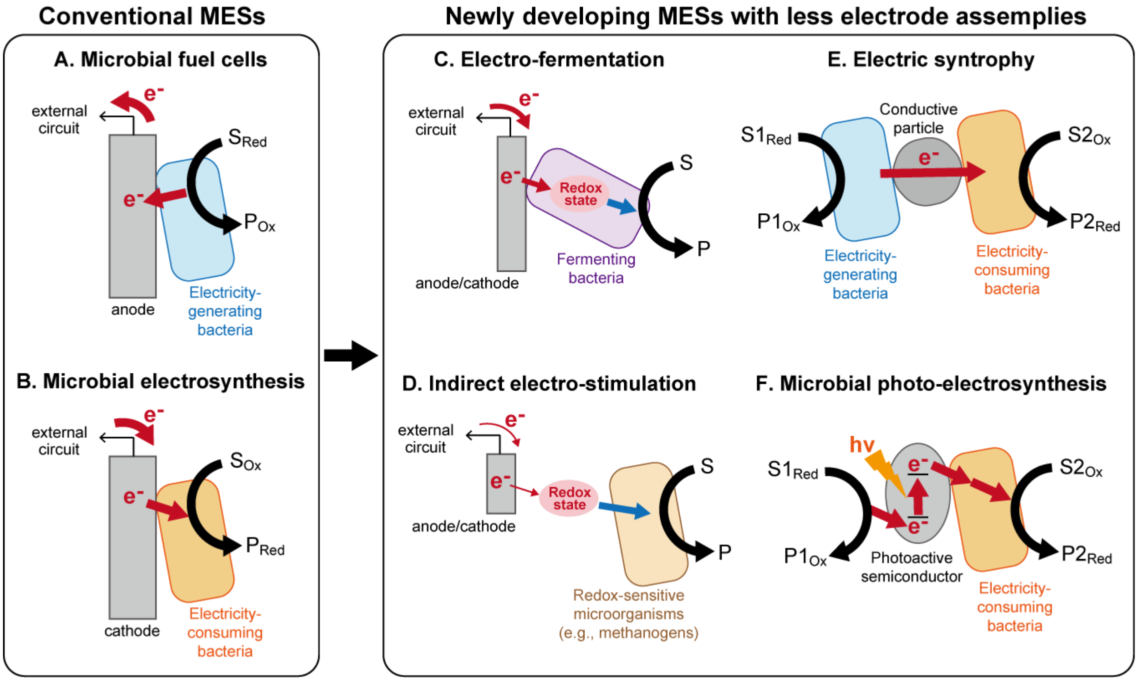
Conventional MESs
Newly developing MESs with less electrode assemplies
A. Microbial fuel cells
external circuit
e-
S Red
P Ox
anode
Electricity-generating bacteria
B. Microbial electrosynthesis
external circuit
e-
S Ox
P Red
cathode
Electricity-consuming bacteria
C. Electro-fermentation
external circuit
e-
Redox state
S
P
anode/cathode
Fermenting bacteria
E. Electric syntrophy
Conductive particle
S1 Red
P1 Ox
S2 Ox
P2 Red
e-
Electricity-generating bacteria
Electricity-consuming bacteria
D. Indirect electro-stimulation
external circuit
e-
Redox state
anode/cathode
S
P
Redox-sensitive microorganisms (e.g., methanogens)
F. Microbial photo-electrosynthesis
hv
S1 Red
P1 Ox
S2 Ox
P2 Red
Photoactive semiconductor
Electricity-consuming bacteria

Figure 1

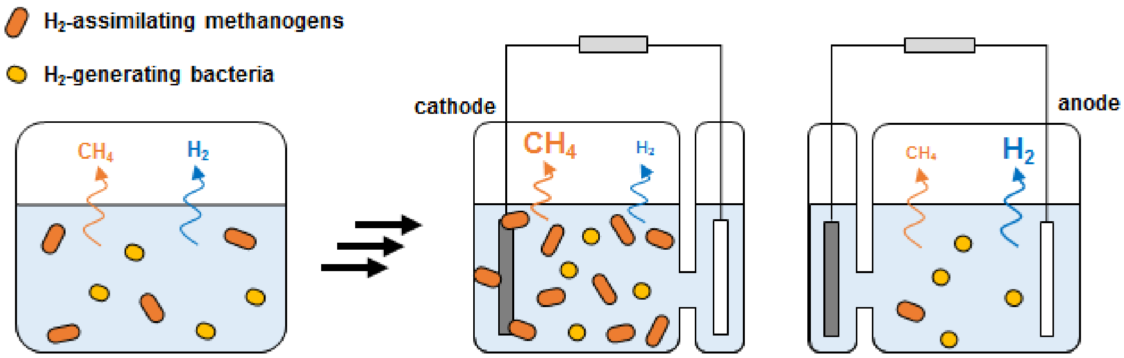
H_2-assimilating methanogens
H_2-generating bacteria
CH_4
H_2
cathode
CH_4
H_2
CH_4
H_2
anode

Figure 2