Chapter 6
Electrochemical Interactions Between Microorganisms and Conductive Particles

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6.1 Introduction

Electrochemically active microorganisms have an ability to acquire energy through transferring electrons to or from extracellular solid materials. The ability is specifically termed as extracellular electron transfer (EET). Since its discovery, various types of microbial electrochemical systems (MESs), in which electrochemically active microorganisms function as biocatalysts, have been developed (Logan and Rabaey 2012; Kato 2015). Microbial fuel cells (MFC) and microbial electrosynthesis cells are the representatives of MESs, in which microbial cells interact with artificial, macroscopic electrodes. Considering natural environments where electrochemically active microorganisms are originally present, they would interact with naturally occurring conductive materials such as iron minerals. It is well-known that considerably large parts of iron minerals are present in the form of nano-sized particles in environments (Hochella et al. 2008). Hence, knowledge on the electrochemical interaction between microbial cells and small conductive particles is crucial for understanding the ecophysiology of electrochemically active microorganisms. Furthermore, such electrochemical interaction between calls and particles has led to development of new biotechnologies. In this chapter, recent progresses in the following three research topics are introduced from the viewpoints of both basic study and biotechnological application: facilitated long-range electron transfer in artificial conductive biofilm, electric syntrophy (interspecies electron transfer via conductive particles), and microbial photo-electrosynthesis with semiconductive nano-particles.

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6.2 Facilitated Long-Range Electron Transfer in Artificial Conductive Biofilm

Electrochemically active microorganisms perform EET via various mechanisms (Torres et al. 2010). The first mechanism is the direct electron transfer mediated by redox proteins such as *c*-type cytochromes (*c*-Cyts) located on cell surfaces. Microorganisms using this mechanism need to adhere to solid conductive materials, resulting in the limitation in number of microbial cells that participate in EET processes. If the cells away from the electrode can also participate in EET processes, the efficiency of the MESs can be improved. The second mechanism is EET via self-secreted, naturally occurring and/or artificially supplied electron mediators (Watanabe 2008). In this mechanism, microbial cells are able to perform EET without direct contact to solid surfaces. However, their EET efficiencies are generally limited by the diffusion of mediator compounds (Torres et al. 2010). The third mechanism is EET via conductive biofilm. *Geobacter sulfurreducens* is well-known to produce such conductive biofilm on both anodic and cathodic electrodes, in which conductive pili and secreted extracellular *c*-Cyts contribute its conductivity (Reguera 2018). EET based on conductive biofilm is the most efficient among the three mechanisms, since a large number of microbial cells in thick biofilm are able to interact with distant electrodes. However, the ability to produce conductive biofilms is limited to only some species in the genus *Geobacter* (Rotaru et al. 2015; Kato 2017).

Recent studies demonstrated that microbial EET reactions can be facilitated by formation of “artificial conductive biofilm” that consists of composites of electrochemically active microbial cells and conductive nano- and/or micro-particles. This concept was first proposed by Nakamura et al. (2009). They demonstrated that an anodic current-generating bacterium, *Shewanella loihica*, has the ability to self-organize an electrically conductive network using nano-particles of semiconductive iron oxides (hematite, α-Fe_2O_3), which largely increased the current generation capability. Photo-electrochemical and molecular genetic approaches revealed that electron hopping between outer-membrane *c*-Cyts of *Shewanella* and hematite particles is crucial for construction of the conductive network (Okamoto et al. 2012). The same research group showed that supplementation of various (semi) conductive particles including magnetite (Fe_3O_4) and biogenic iron sulfides can induce formation of artificial conductive biofilm by *Shewanella* spp. and also *Geobacter* spp. (Kato et al. 2010, 2013; Nakamura et al. 2010). Considering that (semi)conductive iron oxide and iron sulfide minerals are abundantly present in natural environments (Weber et al. 2006), long-range electron transfer mediated by iron minerals is considered to largely contribute to the functioning of microbial communities in various environments.

So far many research groups have attempted to improve the efficiency of MESs using induction of artificial conductive biofilm by supplementation with conductive nano-and micro-particles. Yu et al. largely improved a performance of MFC with pure-culture of *Shewanella oneidensis* by forming conductive biofilm with micro-sized graphite particles (Yu et al. 2011). Improvements of anodic current generation have been achieved by supplementation with diverse conductive materials, including magnetite (Peng et al. 2013), iron sulfides (Jiang et al. 2014), antimony-doped tin oxide (Zhang et al. 2015), gold (Chen et al. 2018), and also biologically reduced graphene oxides (Yong et al. 2014; Yoshida et al. 2016). Furthermore, this approach is also effective for enhancement of cathodic reactions. For example, artificial conductive biofilm composed of microbial cells and biologically reduced graphene oxides exhibited improved reduction of oxygen (Zhuang et al. 2012) and fumarate (Yong et al. 2014) in MFC bio-cathodes. Induction of artificial conductive biofilm is expected as a promising approach for improvement of diverse MESs, since it can promote microbial electrochemical reactions relatively easily, inexpensively, and environmentally friendly compared to other approaches such as genetic modification of microorganisms and supplementation with artificial mediator compounds.

6.3 Electric Syntrophy: Interspecies Electron Transfer via Conductive Particles

Some important microbial metabolic processes, in particular those proceed under anoxic conditions, are achieved by cooperation of multiple microbial species through energy/electron exchanges, which is specifically termed as syntrophy. Small chemicals such as organics, nitrogen and sulfur compounds, and formate/H_2 usually function as the energy/electron careers. In addition, recent studies demonstrated that interspecies energy/electron exchange is also mediated by electric currents flowing through conductive materials, which is specifically termed as electric syntrophy or direct interspecies electron transfer (Kouzuma et al. 2015; Lovley 2017). Electric syntrophy requires “electrical wires” that connect cells of two different electrochemically active microorganisms. Summers et al. demonstrated that conductive pili produced by microorganisms themselves work as electrical wires that connect metabolisms of *Geobacter metallireducens* (oxidation of ethanol) and *G. sulfurreducens* (reduction of fumarate) (Summers et al. 2010). Furthermore, Kato et al. demonstrated that nano-particles of conductive iron minerals such as magnetite can also facilitate electric syntrophy, using model microbial consortium consisting of two electrochemically active bacteria, namely, *G. sulfurreducens* and *Thiobacillus denitrificans* (Kato et al. 2012a).

Since electric syntrophy can enhance known microbial syntrophic processes and potentially can design new syntrophic processes, it has received considerable attention for their application to biotechnologies. Enhancement of methane fermentation processes, which has been already utilized for energy-saving waste(water) treatments, has been most intensively investigated. Since methanogenic archaea produce methane only from limited substrates (acetate, methanol, and H_2/CO_2), methane production from organic waste(water) requires the cooperation of multiple microbial species. Syntrophic interaction of organic acid-oxidizing bacteria and methanogenic archaea is regarded as the rate-limiting step of methanogenic processes (Kato and Watanabe 2010). This syntrophic reaction is generally mediated by interspecies electron transfer with formate/H_2 as the electron career. In contrast, it was demonstrated that electric current flowing through conductive materials can facilitate syntrophic methanogenesis. Morita et al. proposed for the first time that electric syntrophy via conductive biofilm produced by *Geobacter* spp. could facilitate methanogenesis in an anaerobic digester (Morita et al. 2011). Similarly, Kato et al. showed that supplementation with conductive iron oxide particles induces electric syntrophy and enhances methanogenesis in microbial communities derived from rice paddy soil (Kato et al. 2012b). Recent studies using a pure and defined co-cultures of *Geobacter* spp. and methanogens in the order *Methanosarcinales* revealed that conductive pili and outer-membrane and/or extracellular *c*-Cyts play pivotal roles in the direct interspecies electron transfer and conductive nano-particles can compensate for (at least part of) their functions and further facilitate electric syntrophy processes (Rotaru et al. 2014a, b; Ueki et al. 2018; Holmes et al. 2019). It has been reported that methanogenesis via electric syntrophy can be facilitated by diverse range of conductive materials, including iron oxides (Cruz Viggi et al. 2014; Yamada et al. 2015), iron sulfides (Kato and Igarashi 2019), graphite (Chen et al. 2014a), and carbon nanomaterials (Lin et al. 2017; Tian et al. 2017). In particular, finding that inexpensive carbon materials such as activated carbon (Liu et al. 2012; Rotaru et al. 2014b) and biochar (Chen et al. 2014b) facilitate methanogenesis would make it possible 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 (Barua and Dhar 2017; Baek et al. 2016; Dang et al. 2016; Zhao et al. 2016, and reviewed in Martins et al. 2018). Further investigation on microbial physiology and development of low-cost materials with high biocompatibility will proceed applicational use of electric syntrophy for wastewater treatment.

In addition to methanogenesis, several microbial processes, including reductive dichlorination (Aulenta et al. 2013) and anaerobic methane oxidation (McGlynn et al. 2015), are known to be driven by electric syntrophy, which suggests that stimulation of microbial syntrophy has the potential to be applied to various biotechnologies. For example, it has been reported that supplementation with conductive iron oxides into contaminated soil or a bioreactor containing chlorinated aromatic compounds facilitates reductive dechlorination of trichloroethene or 2,4-dichloronitrobenzene, respectively (Aulenta et al. 2013; Wang et al. 2017). Also, Cruz Viggi et al. showed that biodegradation of petroleum hydrocarbons in sediments can be stimulated by introduction of cm-long graphite rods into contaminated sediment to electrically connect the anaerobic sediment and the overlying O_2-containing surface water (Cruz Viggi et al. 2015).

6.4 Microbial Photo-electrosynthesis with Semiconductive Nano-particles

Microbial electrosynthesis is a biotechnology in which electrochemically active microorganisms convert CO_2 into valuable organics with the aid of high-energy electrons supplied from cathodic electrodes (Igarashi and Kato 2017; Prévoteau et al. 2019). Although microbial electrosynthesis has attracted much attention in recent years, such systems require a large number of macroscopic electrode assemblies, which will be limitations when considering widespread commercialization (Sasaki et al. 2018). As a new biotechnology that can solve this issue, "microbial photo-electrosynthesis" was proposed (Sakimoto et al. 2016a). They demonstrated that a non-photosynthetic bacterium (an acetogen *Moorella thermoacetica*) converts CO_2 into acetate using photo-excited high-energy electrons in the conduction band of semiconductive CdS nano-particles that formed by the microorganism itself. Although cysteine was used as a sacrificial electron source in this research, the same research group then achieved production of acetate using water as an electron source by combining the CdS-*M. thermoacetica* system with TiO_2 nano-particles carrying Mn(II) phthalocyanine catalysts (Sakimoto et al. 2016b). Although these studies are still at the stage of the proof of concept with small scale reactors, it has been estimated that this approach, namely, microbial photosynthesis using semiconductor materials and non-photosynthetic microorganisms, has a potential to exceed the energy conversion efficiency of CO_2 fixation by general photosynthetic organisms (Liu et al. 2016).

6.5 Concluding Remarks

In this chapter, several types of electrochemical interactions between microorganisms and conductive particles are introduced from both basic and applied perspectives. Further understanding of this type of interaction will help to elucidate the unknown ecophysiology of electrochemically active microorganisms. Furthermore, most of the technologies introduced here are currently in the early stage of development. Further research on reactor engineering, enlargement of reactor systems, and improvement of long-term durability will achieve the new and efficient MESs.

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