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Review

Methods for understanding microbial community structures and functions in microbial fuel cells: A review

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HIGHLIGHTS

- Microbiological culture should be integrated with genomics for studying MFC.
- Genomic tools reveal the structure and function of microbial communities.
- Isotope-assisted phylogenetic analysis links taxonomy to microbial metabolisms.

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ABSTRACT

Microbial fuel cells (MFCs) employ microorganisms to recover electric energy from organic matter. However, fundamental knowledge of electrochemically active bacteria is still required to maximize MFCs power output for practical applications. This review presents microbiological and electrochemical techniques to help researchers choose the appropriate methods for the MFCs study. Pre-genomic and genomic techniques such as 16S rRNA based phylogeny and metagenomics have provided important information in the structure and genetic potential of electrode-colonizing microbial communities. Post-genomic techniques such as metatranscriptomics allow functional characterizations of electrode biofilm communities by quantifying gene expression levels. Isotope-assisted phylogenetic analysis can further link taxonomic information to microbial metabolisms. A combination of electrochemical, phylogenetic, metagenomic, and post-metagenomic techniques offers opportunities to a better understanding of the extracellular electron transfer process, which in turn can lead to process optimization for power output.

1. Introduction

Microbial fuel cell (MFC) is a promising technology that harnesses the power of microorganisms to minimize environmental pollution and at the same time generate renewable electricity (Ge et al., 2013; Logan et al., 2006; Rittmann et al., 2008). While the power output of MFCs has increased by several orders of magnitude in more than a decade (Logan, 2009; Logan and Regan, 2006), it is still too low for practical application and therefore increasing power production becomes one of the most important tasks for MFC development (Li et al., 2013; Lovley, 2008). Despite numerous studies has been published to examine the reactor design and configuration, operational parameters, and electrode materials (Logan et al., 2006; Qian and Morse, 2011; Qiao et al., 2010), the core element of an MFC, microbes, has not been well understood for maximizing power output.

Recent studies have greatly expanded the range of microorganisms known to be electrochemically active in electricity generation in MFC systems. Those organisms, in different terms such as "electrochemically active bacteria", "anode respiring bacteria", "electricigen" or "exoelectrogenic bacteria", are proven to be able to generate and transfer electrons from substrates to a working electrode without aid of external mediators. The representative microorganisms include metal reducing bacteria such as *Geobacter* spp. and *Shewanella* spp., and phototrophic bacteria like *Rhodopseudomonas* spp. (Malvankar and Lovley, 2012; Morishima et al., 2007; Myers and Nealson, 1988; Ringeisen et al., 2006; Xing et al., 2008). In an MFC, non electrochemically-active microorganisms are also critical for electricity generation through syntrophic cooperation, especially for self-sustained systems or complex substrates (Chae et al., 2009; Freguia et al., 2008; He et al., 2009; Parameswaran et al., 2010; Ren et al., 2007). Several electron transfer mechanisms, including direct electron transfer via membrane-bound c-type cytochrome or nanowires, and self-mediated electron transfer via endogenous redox-active metabolites have

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been proposed to guide us in developing or selecting the right and effective bacteria for enhanced power output.

A better understanding of these electrochemically-active bacteria and microbial community in an MFC will aid in our efforts to increase electricity generation required for our envisioned application of MFCs. To achieve this, appropriate and effective research methods will be very important, especially the fast-changing techniques and methods used in microbiology field. While the cooperation between researchers could alleviate the difficult situation to some extent for the microbial study in MFCs, it is still necessary for a researcher to comprehend the techniques for recognizing the limitation of methods, interpreting data, and discussing its implications. For example, the complexity and inappropriate use of molecular techniques could cause different and inconsistent results in microbial community analysis, increasing the potential risk for drawing deficient/biased and even wrong conclusion in the study. There have been various review articles on microbiology in MFCs (Logan, 2009; Logan and Regan, 2006; Lovley, 2006; Mao and Verwoerd, 2013; Rabaey et al., 2007); however, no review papers have been focused on the techniques for microbiology studies. Hence, a comprehensive review focusing on techniques will benefit the researchers with the choice of appropriate methods for studying the microbes in MFCs.

This paper aims to provide useful information on the status and application of various techniques for microbiological studies in MFCs, and how these techniques can contribute to advancing our understanding of the roles and potential of microorganisms for power enhancement. The advantages and limitations of the techniques will also be discussed.

2. Techniques for studying exoelectrogenic microbes

Bacterial-derived electricity has been known for more than one hundred years (Potter, 1911). Since then, it took several decades to demonstrate that bacteria could carry out the "extracellular electron transfer" process without the aid of exogenous mediators (Lovley and Phillips, 1988; Myers and Nealson, 1988). Improvement and breakthrough in the design and performance during the past decade make MFCs at forefront to utilize exoelectrogenic microbes for sustainable energy production and electricity recovery.

In an MFC, exoelectrogenic microbes colonize on the anode to bio-catalyze the conversion of an array of substrates for electricity. Significant advancements have been made with a variety of techniques to understand their morphology, genetic potential, and metabolic capacity that are related to electricity generation in MFCs. Herein techniques are reviewed for studying these exoelectrogenic microbes and discuss their application in MFCs.

2.1. Isolation

Isolation is a traditional yet remains useful methods to study and characterize a bacterium. Even though it is well established that only a small minority (1%) of microorganisms are readily cultivated *in vitro*, many research endeavours are still trying to recover "unculturables" from soil and aquatic environments. This is because only through the isolation of bacteria in pure culture that a comprehensive characterization of their physiological, biochemical, genetic, and functional properties can be undertaken without interference from other bacterial species and uncontrolled environment.

To fully understand an exoelectrogenic bacterium, it is often necessary to isolate a pure culture from MFCs or other bioelectrochemical systems (BESs), where is mostly likely to habitat and enrich these exoelectrogenic bacteria. Even though many pure strains in history were firstly isolated from non-BES systems, such as marine sediment and wastewater treatment plant, and later on were demonstrated to be electrochemically active (Logan and Regan, 2006).

2.1.1. Dilution

One strategy used by many researchers for isolating pure cultures is the dilution-to-extinction method, which aims to isolate the most abundant member in the community. Currently there are several ways commonly used for the separation purpose: the streak plate, the spread plate, and the pour plate; all of them rely on dilution of the mixed bacteria to the point at which a single cell can grow into a single and visible pure colony. In these methods, the inoculum is diluted either in serial dilution tubes containing liquid medium or via successive streaks over the surface of a solid medium.

The dilution tubes are usually performed in an anaerobic environment for selecting anode-respiring bacteria. The dilution method has been proved to be effective for isolations of exoelectrogenic bacteria, such as *Pseudomonas aeruginosa* (Rabaey et al., 2004), *Thermincola* sp. (Wrighton et al., 2008), *Comamonas denitrificans* (Xing et al., 2010), and *Rhodopseudomonas palustris* (Xing et al., 2008). However, the limitation of the dilution method is that an enrichment of exoelectrogenic bacteria in samples is preferred and even required before isolation. Such enrichment is often done in an MFC system where the exoelectrogenic bacteria become dominant and other non-essential bacteria die out over time. Otherwise, isolation is difficult to proceed when the background flora is higher than the bacteria of interest in the inoculum. Hence, the dilution method is more useful for isolation of the dominant population from MFCs. Combined use with other methods is required if the target is exoelectrogenic bacteria. For example, a number of dissimilatory metal reducing bacteria (DMRBs), which can produce electricity in MFCs without addition of exogenous mediators, lead to a strategy of indirect selection of exoelectrogenic bacteria by isolating new DMRBs and then subsequently testing them in MFCs. The use of dilute nutrient media for isolating oligotrophic bacteria could provide an alternative method for acquiring novel electrogenic bacteria (Connon and Giovannoni, 2002).

2.1.2. Physical enhancement

A special U-tube MFC that allows bacteria to directly settle on the anode was developed to selectively isolate electrochemically active bacteria based on their ability to generate electricity, assisting researchers in acquiring additional electrochemically active bacteria that do not require metal oxides for respiration (Zuo et al., 2008). An MFC operated under laboratory light was successfully used for isolation of a phototrophic purple bacterium identified as *R. palustris* DX-1, which could produce a higher current density than mixed cultures under same conditions (Xing et al., 2008). Elevated temperature such as thermophilic conditions was used to enhance the isolation of electrochemically active bacteria (Fu et al., 2013; Wrighton et al., 2008). Therefore, physical enhanced methods offer the opportunity to identify and understand electrochemically active bacteria that was previously too difficult to be obtained by regular methods.

2.2. Cellular structures: nanowires and gram-stain properties

Using transmission electron microscopy (TEM) and atomic force microscope (AFM), the pili of *Geobacter sulfurreducens*, which was only 3–5 nm in width and can extend more than 20 micrometers in length, has been demonstrated to be highly conductive and served as "microbial nanowires" to transfer electron out of cell to insoluble electron acceptors (Reguera et al., 2005). Images from scanning electron microscope (SEM) and scanning tunneling