Title	Enhancement Mechanism of Microbial Current Production by Conductive Iron Sulphides Biosynthesized by Sulphate Reducing Bacteria [an abstract of entire text]
Author(s)	Muralidharan, Murugan
Citation	北海道大学. 博士(理学) 甲第14011号
Issue Date	2020-03-25
Doc URL	http://hdl.handle.net/2115/81268
Type	theses (doctoral - abstract of entire text)
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File Information	Muralidharan_MURUGAN_summary.pdf

学 位 論 文 の 要 約

博士の専攻分野の名称　博士（理学）　氏名　Muralidharan MURUGAN

学 位 論 文 題 名

Enhancement Mechanism of Microbial Current Production by Conductive Iron Sulphides Biosynthesized by Sulphate Reducing Bacteria

（硫酸還元細菌により生合成された導電性硫化鉄による微生物生成電流増大機構に関する研究）

Microbial Fuel Cell (MFC) is one of the novel energy harvesting technology which are designed to provide renewable form of energy dependent on microbial metabolic activities. In these bio-electrochemical systems, the microbes oxidize organic substrates found in the natural environments and converts the energy stored in the chemical bonds into electricity. This system mimics the interactions of bacteria with insoluble electron donors and acceptors. Since there are different bacterial communities can be involved in the MFC current generation, they can use a wide range of substrates for their metabolism. This has given the use of various biodegradable substrates found in the waste water for the microbial metabolism and showing simultaneous electricity generation and municipal waste water treatment. Here the electrons that are produced by oxidation of substrates at the anode surface are transferred through the external circuit to reach cathode where reduction occurs. A proton exchange membrane allows the flow of protons across the anodic and cathodic chambers for charge balance.

Sediment Microbial Fuel Cell (SMFC) is a type of MFC which usually gets operated by microbial metabolisms in the sediments. Since the MFCs can utilize different kind of substrates, the solid phase substrates found in sediments, contaminated soil and sludge can also be utilized for energy harvesting. Especially, the aquatic sediments can provide sufficient organic substrates for bacterial metabolism for a long time. SMFC make use of the sedimental substrates to power electroactive microbes for electricity generation. This recently developing technology has been proven to be cost effective and can provide uninterrupted power supply for many low power marine devices like oceanographic sensors and deep-sea wireless devices. Here the anode part is usually submerged inside the anaerobic sediments where oxidation of organic substrates occurs and at the cathode which is suspended in the overlying water, which has relatively oxic environments.

Although the electricity production from the SMFCs was observed, efforts are being made to scale up the production and increase the electricity generation for longer durations. The interactions of microbial communities among themselves and their impact on anode current generation is now well understood. Even though many studies have been made on electrode materials, SMFC designs and operational conditions, a closer look at the microbial communities on the electrode surface seems to be important.

In addition to the interactions among bacterial communities, the minerals found in the sediments can also impact the electricity generation. Previous studies showed that the presence of conductive iron sulphide (FeS) nanoparticles actively increased the current production from the Iron Reducing Bacteria (IRB) Shewanella oneidensis MR-1. The marine sediments are rich in various metal sulphides having better electrical conductive properties. The anodic current production in the SMFC can be influenced by conductive minerals observed in the marine sediments. The sulphate reducing bacteria which usually dominate the marine sediments are capable of biomineralize various forms of iron sulphide minerals. Thus, in this work, the involvement of biomineralized conductive iron sulphides on the microbial current generation was studied.

The structure of the present thesis is as follows.

In Chapter 1, the general introduction for MFC, SMFC and their electricity generation mechanisms and designs are introduced. The mechanisms for direct and indirect extracellular electron mechanisms are briefly discussed followed by applications and challenges of SMFC systems. Biomineralization of conductive iron sulphides by sulphate reducing bacteria and past works based on microbial current enhancement by conductive nanoparticles are discussed.

In Chapter 2, the description of the experimental details such as the three-electrode electrochemical system design, microbial cultivation, conditions for electrochemical experiments, electrochemical measurements, characterization techniques like Scanning Electron Microscopy, Fluorescent In-Situ Hybridization and X-ray Photoelectron Spectroscopy Analysis are given.

Initially, in Chapter 3, the interaction of the SRB pure culture *D. vulgaris* Hildenborough with the anode was observed. The presence of FeS bioagglomerate impacted the overall current generation. Many of the earlier reports showed the hydrogen sulphide mediated abiotic oxidization coupled electron transfer at the anodes. However, we observed that in addition to the above-mentioned mechanism, FeS precipitation also dominated the current generation. The anodic current generation was declined after the lactate depletion stage which was characterized by oxidative loss of FeS on the anodes. The iron sulphides were found to be attached to the SRB membrane. A new proposal for FeS mediated electron transfer to the anodes from the SRB was proposed in this study. Understanding of new pathways would help to improve the design and operation of SRB MFCs.

In Chapter 4, the symbiotic current generation in the SRB and IRB were studied along with the importance of bioagglomerates of FeS. The anodic current generation in the SRB and IRB coculture electrochemical system was many folds higher than that of the current produced by IRB pure cultures. In these synergetic current production mechanisms, the impact of conductive iron monosulphides formation was seemed to be evident. SEM observations showed formation of FeS bioagglomerates with the bacterial cells embedded in them. Much thicker bioagglomerates were observed in the coculture systems when compared with the SRB and IRB pure cultures separately. This opened up the importance of long-range electron transfer across the bioagglomerates which increased the electron transfer to the anodes. Performance of source-drain electrochemical experiments showed the importance of FeS based long-range electron conduction in the bioagglomerates. These observations showed that the presence of much higher conductive bioagglomerates can increase the power output of sediment microbial fuel cells.

Chapter 5 showed the symbiotic interactions between SRB and IRB on FeS bio precipitation and increased bacterial growth. The findings showed that the presence of IRB accelerated the FeS precipitation by the SRB which was higher when compared to that of SRB pure cultures. Similarly, the increase in total bacterial cell counts was higher in the SRB and IRB coculture systems which also showed increase in the total protein contents much higher than that of SRB pure cultures. With observations from the IRB mutant strain lacking outer membrane cytochromes, an interspecies electron transport model was proposed for the synergetic interactions where the metabolically generated electrons of IRB are coupled to sulphate reduction by SRBs.

Chapter 6 explained the improvement of microbial current generation by the precipitation of metal sulphides with better electrical properties. Addition of Ni^{2+} and molybdate both showed current generation although each showed different current generation profiles. The toxicity of Cu^{2+} to the bacterial cells prevented electricity generation which can be reduced in a system having more bacterial cell counts and active FeS bio precipitation.

In this thesis, it has been proved that the microbial current generation at the anodes can be increased by forming bioagglomerates having efficient conductive iron sulphides. The microbial interactions that happen at the anode surfaces can influence the iron sulphide precipitation and thereby impact the anodic current generation. This phenomenon can be applied in SMFCs to improve their performances. Even though FeS is the most abundant mineral observed at the anoxic sediments, inclusion of other metal sulphides to the sediments can increase the electricity production.

Future prospects

From the current study, it was emphasized that the microbial current generation can be enhanced in the presence of conductive bioprecipitates. With lower power densities, the cost of production for the SMFCs are higher as there is a need for larger surface area for the electrodes. However, by using systems having conductive bioprecipitates the current densities at the electrodes can be increased many folds. This can potentially reduce the surface area of the electrodes being used for the SMFC devices. Thus, the cost for establishing the SMFC units can be reduced. The findings of this work can be employed in developing subsurface electrical grids. New ideas are being proposed to develop underwater electrical interconnections to power various devices that require high electrical power. Thus, increasing the SMFC performances are always going to improve the development of subsurface electric grids.

The microbial interactions between the sulphate reducing bacteria and the iron reducing bacteria has been observed to be syntrophic increasing the microbial activities and subsequently the sulphate reduction to precipitate iron sulphides. According to the present ideas the dominant bacteria can outgrow at competing environments. However, our current observations showed that the symbiotic interactions between bacteria can actually benefit growth of different microbial communities. The electron transfer between SRB and IRB can favor efficient energy utilization in energy limiting environments.

The symbiotic interactions between IRB and SRB enhanced the sulphate reduction by SRB significantly. Provided that the microbial based sulphate reduction is impacting the global bio-geochemical sulphur cycles, the synergetic interactions of the Sulphate reducing bacteria with the other microbes will have a considerable shift in the sulphur cycles globally.