Electricity generation by Shewanella sp. HN-41 in microbial fuel cells

Di Wu a, Defeng Xing a,*, Xiaoxue Mei b, Bingfeng Liu a, Changhong Guo b, Nanqi Ren a

aState Key Laboratory of Urban Water Resource and Environment, School of Municipal and Environmental Engineering, Harbin Institute of Technology, P.O. Box 2650, 73 Huanghe Road, Nangang District, Harbin, Heilongjiang Province 150090, China
bCollege of Life Science and Technology, Harbin Normal University, Harbin 150025, China

ABSTRACT

Microbial fuel cells (MFCs) provide new opportunities for energy generation through conversion of organic matter to electricity by electricity-generating bacteria. In this study, Shewanella sp. strain HN-41 was described as an exoelectrogen that had the ability of extracellular electron transfer in MFCs fed with lactate or glucose. The maximum power density produced by the strain HN-41 in lactate- and glucose-fed single-chamber MFCs reached 71.6 and 18.2 mW m⁻², respectively. The strain showed strong capability to reduce Fe(III) with lactate or glucose as electron donor during the initial incubation period, and secreted flavin mononucleotide (FMN), riboflavin, and traces of flavin adenine dinucleotide in MFCs. Addition of riboflavin and FMN as electron mediators contributed to 2–5 folds increase in power density. These findings on the ability of Shewanella sp. HN-41 to couple oxidation of glucose contributed to the expansion of our knowledge on utilization of carbon source by Shewanella sp.

1. Introduction

As a bioelectrochemical device, microbial fuel cells (MFCs) can efficiently convert organic compounds, wastewater, and renewable biomass to electricity. Bacteria that are capable of transferring extracellular electrons oxidize organic matter and transport the electrons to the anode in MFCs [1]. Shewanella sp. is the most-studied exoelectrogenic bacterium used for electricity production in MFCs [2,3]. These bacteria can respire a diverse range of organic substrates and reduce soluble or insoluble metal complexes during their catabolic metabolism. Although Shewanella oneidensis MR-1, a typical exoelectrogen, is not capable of metabolizing saccharides as a carbon source for extracellular electron transfer, later studies have demonstrated that several Shewanella sp., including S. baltica, S. frigidimarina, and S. japonica could oxidize glucose under aerobic conditions [4,5]. Nevertheless, electricity generation by Shewanella sp. in MFCs using glucose as the sole electron donor has been rarely reported [5].

The major mechanisms of extracellular electron transfer by exoelectrogens in MFCs are as follows. (1) The electrons are directly transferred from the outer surface of c-type cytochromes to the electrode [6,7]. (2) Some microorganisms could excrete soluble redox molecules that serve as “electron shuttles” to mediate electron transfer between the bacterial cells and electrode [8–11]. (3) The electrically
condutive pili or nanowires promote electron transfer across the multilayer biofilms on anodes [12,13]. As an electron shuttle, flavins can mediate extracellular electron transfer [8], and some microorganisms have been found to have the ability to use flavins for extracellular electron transfer [14]. Shewanella, as an exoelectrogen, can not only secrete flavins, but can also produce higher power output by utilizing exogenous flavins.

In the present study, electricity generation and iron reduction by an electricity-producing bacterium, Shewanella sp. HN-41, using glucose or lactate as the sole electron donor were investigated. Flavins secreted by strain HN-41 were measured under different culture conditions, and the effect of exogenous flavins as electron shuttle on extracellular electron transfer by strain HN-41 was examined.

2. Materials and methods

2.1. Strain and culture medium

Shewanella sp. HN-41 was obtained from Applied and Environmental Microbiology Laboratory of Gwangju Institute of Science and Technology. The strain was cultured aerobically at 30 °C in Luria–Bertani (LB) medium (pH 7.0) in a shake flask. Prior to inoculation into MFCs, the cells were harvested by centrifugation and washed thrice in 50 mM PIPES–NaOH buffer (pH 7.0). The washed cells were resuspended in Shewanella medium to the desired cell concentration (OD600 of 0.6). The Shewanella medium consisted of the following (per liter): 10 mM glucose or 10 mM lactate, 15.1 g of PIPES, 1.5 g of NH4Cl, 0.6 g of NaH2PO4, 3 g of NaOH, 0.1 g of KCl, 5.8 g of NaCl, 10 ml of trace mineral mix, and 10 ml of vitamin mix (pH 7.0).

2.2. MFC configuration and operation

Electricity generation by strain HN-41 was evaluated by using single-chamber air-cathode and two-chamber MFCs equipped with a carbon cloth anode (7 cm2). The air-cathode single-chamber MFCs (4 cm long, 3 cm diameter, 25 ml volume) were used as previously described [15]. The air-cathode was a (30% wet-proofed) carbon cloth (type B, E-TEK, 7 cm2) coated with NH4Cl, and 0.13 g L$^{-1}$ of NaH2PO4, 0.6 g of NaH2PO4, 3 g of NaOH, 0.1 g of KCl, 5.8 g of NaCl, 10 ml of trace mineral mix, and 10 ml of vitamin mix (pH 7.0).

2.3. Electrochemical analysis

The anode potential was successively poised at +200 mV against the Ag/AgCl reference electrode by connecting to the working electrode of a potentiostat (Model WMPG1000 Multichannel Potentiostat/Galvanostat, Korea), while the cathode was used as a counter electrode, with each potential operated for two consecutive cycles. One cycle period was defined as 24 h. Power densities of MFCs were determined based on linear sweep voltammetry (LSV) using a potentiostat (Model WMPG1000 Multichannel Potentiostat/Galvanostat, Korea) from −0.6 to −0.05 V at a rate of 0.1 mV s$^{-1}$, which allowed obtaining a peak curve of power density.

2.4. Chemical analysis

For the determination of protein concentrations, the bacterial cells were harvested by centrifugation at 12,000 rpm for 10 min and washed twice with 50 mM PBS (pH 7.0). The washed cells were resuspended in 0.1 M NaOH and heated in boiling water for 10 min to obtain cell lysate. This lysate was centrifuged at 12,000 rpm for 5 min and the suspension was used for protein determination [17]. Protein concentrations in the planktonic cells in MFCs and pure culture were measured according to the ferrozine assay, as previously described [9]. The concentrations of Fe(II) and Fe(III) were measured according to the ferrozine assay, as previously described [18]. Fe(III) was reduced to Fe(II) by using hydroxylamine hydrochloride, and then determined as described earlier.

3. Results and discussion

3.1. Cell growth and iron reduction using lactate or glucose as the sole electron donor

Strain HN-41 was found to have the ability to oxidize lactate, glucose, and yeast extract and simultaneously secrete flavins with oxygen as the electron acceptor (Fig. S1, Supporting Information). In order to measure the iron reduction capacity, strain HN-41 was grown at 30 °C in anaerobic culture tubes containing Shewanella medium consisting of lactate (10 mM), glucose (10 mM), or yeast extract (1.8 g/L) and ferric citrate (10 mM). The Fe(III) reduction rate showed a quick increase with lactate or glucose as the electron donor during the initial incubation period (0–20 h), followed by a slow increase after 24 h. The reduction rate with glucose as the electron donor
was similar to that with lactate, and was higher than that with yeast extract (Fig. 1).

3.2. Electricity generation with glucose or lactate

The maximum voltages recorded in the single-chamber MFCs with lactate and glucose as the electron donor were approximately 225.8 ± 0.5 and 40.3 ± 2.2 mV, respectively, while those in the two-chamber MFCs with lactate and glucose as the electron donor were 115 ± 3 and 84 ± 3 mV, respectively (Fig. 2A). On the other hand, the MFCs loaded only with the substrate and without bacterial cell suspensions did not produce any current. The pH changes over the fed-batch cycle were similar in the single- and two-chamber MFCs. The final pH of the effluent in the single-chamber MFCs with lactate and glucose as substrate was 7.04 ± 0.09 and 6.48 ± 0.01, respectively, while that in the anode chamber of the two-chamber MFCs with lactate and glucose as substrate was 6.2 ± 0.08 and 5.94 ± 0.11, respectively.

The maximum power density produced by strain HN-41 in the lactate- and glucose-fed single-chamber MFCs reached 71.6 and 18.2 mW m⁻², respectively (Fig. 2B). On the other hand, the lactate- and glucose-fed two-chamber MFCs exhibited a lower maximum power density of 38.7 and 29.9 mW m⁻², respectively. The maximum current densities recorded with the anode potential successively poised at +200 mV against the Ag/AgCl reference electrode were approximately 321.5 ± 8.5 and 115.3 ± 4.7 mA m⁻² in the lactate- and glucose-fed single-chamber MFCs, respectively, and 255.9 ± 14.1 and 96.8 ± 3.2 mA m⁻² in the two-chamber MFCs fed with lactate and glucose, respectively (Fig. 3A and B). The current generated by the MFCs fed with lactate or glucose had an obvious peak curve, which subsequently decreased due to substrate consumption (Fig. 3A and B). It has been reported that glucose oxidation produced diverse soluble metabolites due to the inherent fermentable characteristic of glucose, resulting in significant electron loss within the MFC systems, along with a low power output [19]. Two recent studies have also demonstrated that the metabolite produced from sugar compounds in the anode chamber can reduce the performance of the MFCs [20,21].

3.3. Quantification of secreted extracellular flavins

Flavins accumulated over time in the suspension of the two-chamber MFCs during the operation of the reactor (Fig. 4A). The flavins secreted by strain HN-41 with both glucose and lactate as electron donors were predominantly flavin
mononucleotide (FMN) (maximum yield: 0.31 ± 0.01 and 0.25 ± 0.01 μM at 72 h, respectively), followed by riboflavin (maximum yield: 0.14 ± 0.02 and 0.16 ± 0.02 μM at 72 h, respectively). And yet flavin adenine dinucleotide (FAD) occurred only at trace level.

Except for the production of FMN with lactate as the electron donor, the relative amounts of all the flavins per unit of biomass gradually increased and subsequently leveled off or decreased in the two-chamber MFCs (Fig. 4B). The results obtained showed that cells fed with lactate (maximum FMN and riboflavin yield: 2.33 ± 0.05 and 1.47 ± 0.10 μM g⁻¹ protein, respectively) had greater ability to secrete extracellular flavins, when compared with those fed with glucose (maximum FMN and riboflavin yield: 2.27 ± 0.15 and 1.24 ± 0.30 μM g⁻¹ protein, respectively). Due to the short operation time of the single-chamber MFCs, the relative amounts of flavins per unit of biomass were assessed in the anode suspension of the single-chamber MFCs after 24 h. FMN was found to be the predominant flavin (2.52 ± 0.14 and 2.30 ± 0.10 μM g⁻¹ protein with lactate and glucose as electron donors, respectively), followed by riboflavin (1.53 ± 0.23 and 1.27 ± 0.12 μM g⁻¹ protein with lactate and glucose as electron donors, respectively). And FAD (0.15 ± 0.06 and 0.13 ± 0.01 μM g⁻¹ protein with lactate and glucose as electron donors, respectively) was detected only in trace amounts. The total amount of the secreted extracellular flavins in the supernatants of the single-chamber MFCs was higher than that in the supernatants of the two-chamber MFCs, presumably due to the influence of oxygen passing through the cathode into the reactors on flavin secretion by strain HN-41 [22].

3.4. Effect of exogenous flavins on power generation

At the beginning of a cycle, 4 μM riboflavin or FMN was added to the anode chamber in the two-chamber MFCs (Fig. 4C and D). In the presence of lactate or glucose as electron donor, the maximum power density increased by 2–5 folds with the addition of riboflavin or FMN, when compared with that observed in the control without flavin supplementation. The maximum power density with the addition of riboflavin increased from 43.6 ± 2.8 and 31.4 ± 1.5 mW m⁻² to 297.6 ± 10.6 and 125.7 ± 2.6 mW m⁻² in lactate- and glucose-fed MFCs, respectively, whereas that with the addition of FMN increased to 145.3 ± 3.2 and 105.9 ± 4.3 mW m⁻², respectively. These results implied that the more efficient electron transfer kinetics was observed with exogenous mediators added to the anode chamber, rather than relying solely on self-mediation or direct electron transfer from exoelectrogen [23].

Exogenous riboflavin was found to be more effective in enhancing electricity generation than FMN. The above-mentioned results demonstrated that riboflavin and FMN as electron shuttle could mediate the electron transfer from strain HN-41 to the electrode in MFCs. When the culture solution was replaced with fresh medium lacking flavins, the voltage output dropped to initial levels. It has been reported that removal of riboflavin from biofilms led to more than 70% reduction in the rate of electron transfer to the electrodes [8]. Furthermore, addition of flavins has been observed to obviously enhance power generation in MFCs [24]. Similarly, other studies have reported that different behaviors of Lactococcus lactis with different flavins may be ascribed to the selective

Fig. 4 – Concentrations (A) and the relative amounts of FMN, riboflavin and FAD secreted by Shewanella sp. HN-41 in two-chamber MFCs during a cycle. Effect of flavin addition on power density production in two-chamber MFCs fed with glucose (C) or lactate (D) as the electron donor.
binding of specific flavins to the flavin transporter in the cell membrane [14]. The ability of strain HN-41 to rapidly shuttle flavins through the uptake and secretion process indicated the presence of a flavin transport system.

Riboflavin and FMN are regarded as equally effective electron shuttles, and the small difference in the current produced may be attributed to the following two reasons: (1) the positive reduction potential of riboflavin is more (~208 mV vs. normal hydrogen electrode (NHE)) than that of FMN (~219 mV vs. NHE) and (2) riboflavin (376.4 Da) is a smaller molecule than FMN (456.3 Da), and can more rapidly diffuse across the dialysis membrane (cutoff: 7000 Da) [24]. The phenomenon observed in the present study confirmed that Shewanella sp. HN-41 had the potential to efficiently use flavins to increase power output, and produce higher power density with the addition of riboflavin. The ability of electrochemically active species to utilize flavins as efficient extracellular redox mediators is of great significance for the enhancement of the performance of MFCs.

A recent study reported genomic evidences of sugar utilization pathways in the Shewanella sp. [4]. Although electricity generation by S. oneidensis MR-1 reached 148 ± 20 mW m⁻² in MFCs fed with lactate [25], the wild strain lacked the ability to metabolize glucose as the sole carbon source, which was most likely due to a frame shift in the glcPMal gene [26]. S. oneidensis MR-1 and Lactococcus lactis co-culture condition can utilize glucose to produce the current in BES system [27]. After the mixed anodic microflora was augmented with Shewanella halotis, significant improvement in power output as well as biochemical behavior was observed [28]. In recent year, the mutants of S. oneidensis MR-1 also emerged to have the capability of using glucose as a sole carbon source to growth [29], but electricity generation by these mutants had not been tested. In the present study, strain HN-41 showed an acceptable power output in MFCs fed with lactate, when compared with that observed in previous studies [23, 30]. Furthermore, glucose was used as the sole electron donor, and electricity generation by strain HN-41 in MFCs was higher than that by S. japonica [5]. Thus, the study of Shewanella sp. HN-41 can contribute to the understanding of the mechanism of extracellular electron transfer in MFCs fed with fermentable substrates.

4. Conclusions

Shewanella sp. HN-41 was observed to have the capability of utilizing lactate or glucose as the sole electron donor to grow and exhibit electrochemical performance in MFCs. Thus, Shewanella sp. may have great potential to use diverse substrates to produce electricity. In addition, the present study confirmed that strain HN-41 can not only secrete flavins with FMN as the predominant flavin, but can also utilize them as electron shuttles to increase power output of MFCs.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (No. 31270004), the Fundamental Research Funds for the Central Universities (No. HIT-BRETIII.201232), the Fok Ying-Tong Education Foundation for Young Teachers in the Higher Education Institutions of China (No. 131076), and the Science Fund for Creative Research Groups of the National Natural Science Foundation of China (No. 51121062).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijhydene.2013.04.081.

References


