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Poly iron sulfate flocculant as an effective additive for improving the performance of microbial fuel cells

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HIGHLIGHTS

- Poly iron sulfate increases electric outputs from microbial fuel cells.
- Poly iron sulfate increases the abundance of *Geobacteraceae* and *Desulfuromonadaceae*.
- Poly iron sulfate suppresses the growth of methanogens.

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ABSTRACT

Laboratory microbial fuel cells were supplied with artificial wastewater and used to examine how supplementation with poly iron sulfate, an inorganic polymer flocculant widely used in wastewater-treatment plants, affects electricity generation and anode microbiomes. It is shown that poly iron sulfate substantially increases electric outputs from microbial fuel cells. Microbiological analyses show that iron and sulfate separately affect anode microbiomes, and the increase in power output is associated with the increases in bacteria affiliated with the families *Geobacteraceae* and/or *Desulfuromonadaceae*. We suggest that poly iron sulfate is an effective additive for increasing the electric output from microbial fuel cells. Other utilities of poly iron sulfate in microbial fuel cells are also discussed.

1. Introduction

Microbial fuel cells (MFCs) are devices that exploit microbial catabolic activities to generate electricity from organic matter (Logan, 2008). Using naturally occurring microbiomes as anode catalysts, MFCs are able to generate electricity from complex organic matter, such as waste biomass and wastewater pollutants (Zhi et al., 2014). MFCs are therefore expected as sustainable bioenergy devices that can also be used for saving energy for wastewater treatment (Watanabe, 2008; Li et al., 2014).

In recent years, numerous studies have been carried out to examine factors that may influence the MFC performance, and power outputs from MFCs have substantially increased (Wang et al., 2015). These studies include the development of anodes and cathodes (Zhou et al., 2011; Guo et al., 2015), modification of reactor configurations (Janicek et al., 2014), and optimization of electrolyte chemistry (Miyahara et al., 2016). In addition, several studies have attempted to supplement electrolytes in MFCs with additive materials that can stimulate the activity of exoelectrogens. To cite an instance, studies have shown that electricity generation from MFCs inoculated with *Shewanella* is accelerated by supplementing with small amounts of metals, such as iron (Wu et al., 2013) and copper (Xu et al., 2016). Another study has demonstrated beneficial effects of sulfur sources in activated sludge- and leachate-treating MFCs (Ieropoulos et al., 2013). In addition, it has been suggested that surfactants, e.g., Tween 80, increase electron-transfer rates between microbes and anodes and enhance power outputs from MFCs. (Wen et al., 2011). Although the supplementation with these additives is expected as easy methods for improving MFC performances during the operation, attention should be paid to whether or not they can be widely used in different types of MFC, particularly those that have been demonstrated useful in pure-culture MFCs. In addition, costs for additives need to be considered for the practical application.

A variety of chemicals are used in wastewater-treatment plants, including organic and inorganic flocculants (Lee et al., 2014), and these are also considered to be applicable to wastewater-treatment MFCs. Among these, the present study examined the

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utility of poly iron sulfate (PIS) in MFCs treating two types of artificial wastewater, artificial industrial wastewater (AIW) containing methanol and butanol, and artificial domestic wastewater (ADW) containing starch, peptone and yeast extract. PIS is an inorganic-polymer flocculant, in which iron atoms coordinate to oxygen atoms in sulfate to form a polymer (Supplementary Fig. S1). PIS has been widely used in wastewater-treatment plants for increasing the quality of treated water and promoting flocculation and precipitation of sludge (Mori and Takeda, 2003). We selected this flocculant, since positive effects of iron and sulfate on MFC performances have been reported (Wu et al., 2013; Ieropoulos et al., 2013). Electric outputs, effluent qualities and anode microbiomes were compared in MFCs in the presence and absence of PIS. In addition, other possible utilities of PIS in wastewater-treating MFCs are also discussed.

2. Materials and methods

2.1. Additives used in this study

PIS, an inorganic polymer flocculant (Polytetsu), was purchased from Nittetsu Mining. In a PIS solution, most iron is present as Fe^{3+}. In addition, for analyzing action mechanisms of PIS, sodium sulfate (Na_2SO_4) and ferric chloride ($FeCl_3$) were purchased from Wako Pure Chemicals and used as additives.

2.2. Reactor configuration and operation

MFCs used in the present study were cassette electrode- (CE-) MFCs that were described in our previous study (Miyahara et al., 2015a). The configuration of CE-MFC is presented in Supplementary Fig. S2. Each MFC was equipped with one CE that consisted of two sets of air cathodes, separators and graphite-felt anodes. The anode and cathode had projected areas of 68 and 65 cm^2, respectively, and the liquid capacity of each MFC was approximately 300 mL. The liquid surface was covered with polystyrene boards (Miyahara et al., 2015b), and the MFCs were placed in a water bath at 30 °C. MFCs were continuously supplied with either AIW or ADW at a flow rate of 300 mL day^{-1}. AIW was composed (L^{-1}) of methanol (350 mg), butanol (350 mg), urea (90 mg), NaCl (5.8 g), KH_2PO_4 (36.3 mg), $CaCl_2{\cdot}2H_2O$ (154 mg), $MgSO_4{\cdot}7H_2O$ (149 mg), KCl (70 mg), $NaHCO_3$ (28.9 mg), and trace-metal solution (3.3 mL; used in DSMZ medium 318, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH). A chemical oxygen demand (COD) of AIW was approximately 1300 mg L^{-1}. Major ingredients in ADW include starch, peptone and yeast extract, and its composition was described elsewhere (Miyahara et al., 2013). COD of ADW was approximately 500 mg L^{-1}. The operation of MFCs was initiated by inoculating with 0.3 g (wet weight) of sludge obtained from a wastewater-treatment facility in a chemical plant (Shiga, Japan). The anodes and cathodes of each MFC were connected via an external resister (R_{ext} [Ω]), and the voltage across the resister (E [V]) was monitored using a data logger (GL820, Graphtec).

2.3. Evaluation of MFC performance

COD was measured using a low-range (25–1500 mg L^{-1}) system (Hach Co.). A COD-removal efficiency (CRE) was calculated from the influent COD (COD_{in}) and effluent COD (COD_{ef}) as CRE = $[COD_{in} - COD_{ef}]/COD_{in}$, and current ($I$) was calculated using E and R_{ext} (Watanabe, 2008). Polarization curves were drawn using a potentiostat (HZ-5000, Hokuto Denko) at a scan rate of 0.5 mV s^{-1}, and power curves were generated based on polarization curves (Logan et al., 2008). Current and power densities (J [mA m^{-2}] and P [mW m^{-2}], respectively) were calculated based on the projected anode area. The maximum power density (the peak of a power curve, P_{max} [mW m^{-2}]), open-circuit potential (E_{op} [V]), maximum current density (J_{max} [mA m^{-2}]), and internal resistance (R_{int} [V]) were determined from these curves (Logan et al., 2008).

2.4. Analyses of anode microbiomes

Pieces of graphite felt were cut from anodes on both sides of a CE and were stored at −20 °C. To determine the total protein amount as an index for the total microbial biomass in anode biofilm, proteins were extracted from the anode pieces (0.25 cm^2) using B-PERII reagent (Pierce) and were quantified using a BCA protein kit (Pierce) as described previously (Shimoyama et al., 2009).

DNA was extracted from graphite-felt pieces (0.25 cm^2) using a Fast DNA Spin Kit for Soil (Q-Bio). The relative abundance of *Desulfuromonadales* bacteria to total bacteria in the anode microbiomes was evaluated based on copy numbers of 16S rRNA genes that were determined by quantitative real-time PCR (qPCR) as described previously (Miyahara et al., 2015b). Fragments of bacterial 16S rRNA genes were amplified from *Desulfuromonadales* bacteria using the primer pair Geo494F and Geo825R (Kato et al., 2010), while those of the total bacteria were amplified using the primer pair 341f and 534r (Watanabe et al., 2001). In addition, fragments of 16S rRNA genes of the total archaea were amplified using the primer pair ARC787f and ARC1059r (Shin et al., 2010) for the qPCR analysis.

For analyzing phylogenetic compositions of bacteria in anode microbiomes, 16S rRNA gene fragments were amplified by PCR using tagged bacterial universal primers as described elsewhere (Miyahara et al., 2015a). Amplicons from different samples were mixed at the same concentration (1 ng μl^{-1} each) and subjected to pyrosequencing using a Genome Sequencer FLX system (Roche Applied Science). Phylogenetic analyses were conducted using the Silva rRNA database (http://www.arb-silva.de/). Nucleotide sequences determined in the present study were deposited into the DDBJ Sequence Read Archive Database (accession numbers: DRA004918).

3. Results and discussion

3.1. PIS increases electric outputs from MFCs

In order to examine the effect of PIS on electric outputs from MFCs, CE-MFCs were operated by supplying with AIW containing different amounts of the PIS solution, and electric outputs from these MFCs (I series in Table 1) were compared. We used AIW containing methanol and butanol, since these alcohols are frequently detected in industrial wastewater (Patterson, 1985). The composition and COD concentration of AIW simulated those in a real industrial wastewater from a chemical plant. The operation of CE-MFCs was initiated at R_{ext} of 10,000 Ω, and it was changed according to increases and decreases in E (Fig. 1). As a result, CE-MFCs, except for I-P400, could be operated at relatively stable E values several weeks after commencing the operation (Fig. 1c and d). In the following experiments, MFCs were operated in similar ways.

For comparing performances of MFC suppled with different concentrations of PIS, the polarization analysis was conducted repeatedly after electric outputs from these MFCs became stable (after day 20). Supplementary Fig. S3 shows polarization behaviors of these MFCs, and data representing MFC performances are summarized in Supplementary Table S1. We found that, although the P_{max} values for I-C was low (57 ± 7 mW m^{-2}), it was substantially increased by supplementing with PIS and exceeded 300 mW m^{-2}