Bioelectrochemistry 106 (2015) 186–193

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Effects of atmospheric air plasma treatment of graphite and carbon felt electrodes on the anodic current from *Shewanella* attached cells

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ARTICLE INFO

Article history:
Received 3 December 2014
Received in revised form 24 March 2015
Accepted 30 March 2015
Available online 1 April 2015

Keywords:
Shewanella loihica PV-4
Atmospheric air plasma
Electroactivity

ABSTRACT

The attachment of electrochemically active microorganisms (EAM) on an electrode is determined by both the chemistry and topography of the electrode surface. Pre-treatment of the electrode surface by atmospheric air plasma introduces hydrophilic functional groups, thereby increasing cell attachment and electroactivity in short-term experiments. In this study, we use graphite and carbon felt electrodes to grow the model EAM *Shewanella loihica* PV-4 at oxidative potential (0.2 V vs. Ag/AgCl). Cell attachment and electroactivity are measured through electrodynamic methods. Atmospheric air plasma pre-treatment increases cell attachment and current output at graphite electrodes by 25%, while it improves the electroactivity of the carbon felt electrodes by 450%. Air plasma pre-treatment decreased the coulombic efficiency on both carbon felt and graphite electrodes by 60% and 80%, respectively. Microbially produced flavins adsorb preferentially at the graphite electrode, and air plasma pre-treatment results in lower flavin adsorption at both graphite and carbon felt electrodes. Results show that air plasma pre-treatment is a feasible option to increase current output in bioelectrochemical systems.

1. Introduction

Shewanella sp. is a facultative aerobic proteobacterium typically encountered in aquatic environments [1]. *Shewanella* species have evolved dissimilatory metal reduction mechanisms via extracellular electron transfer (EET) to abundant electron acceptors such as iron, copper, magnesium, and environmental contaminants such as uranium and plutonium, as an adaptation to oxygen limited environments [2]. EET occurs over short interfacial distances through direct electron transfer or electron hopping via a chain of outer membrane cytochromes [3] or over large distances via mediated electron transfer either through microbially produced redox mediators [4] or through "nanowires", microbially conductive structures extruded by the cells [5]. Mediated electron transfer is primarily carried out via soluble flavins excreted by *Shewanella* on a species-wide level (with some exceptions, such as *S. denitrificans*, which lost its EET ability recently) [6]. *Shewanella* utilizes both routes of EET at a given time [7]. The relative contribution of each route depends on the surface chemistry and topography of the electrode material [8], the growth phase of the microorganisms, the biofilm microstructure [9], or cell encapsulation [10]. *Shewanella* sp. have been investigated for metal bioremediation due to its dissimilatory metal reducing capability, [11], metal nanoparticle biosynthesis capability [12] and bioelectricity production in microbial fuel cells [13]. The electroactivity of *Shewanella oneidensis* is limited by the substrate mass transfer at the biofilm/electrode interface and the concentration of microbially produced flavins in the supernatant or at the electrode. Therefore, *Shewanella* sp. strains that produce a high concentration of flavins are desirable.

Shewanella loihica PV-4 is a strain derived from an iron-rich microbial mat located at the Loihi seamount off the coast of Hawai'i. When grown for 30 days in potentiostat-controlled electrochemical cells alongside the model EAM *S. oneidensis* MR-1, *S. loihica* PV-4 biofilms produced a 30% higher current at higher coulombic efficiency (26% vs. 16%). Furthermore, *S. loihica* biofilms produced a sevenfold higher current than planktonic cells in the same conditions [14]. The EET mechanism in *S. loihica* PV-4 has been further investigated. As the deletion mutant of the *S. oneidensis* homologue of MtrC in *S. loihica* decreased current production to a negligible value, it is possible that the EET mechanism of *S. loihica* is favored in anode-attached cells. The direct electron transfer EET mechanism was subsequently correlated with biofilm age and increased thickness for *S. loihica* (as shown by electrodynamic methods) [9]. More recently, the effect of biofilm age and thickness on the balance of the two EET mechanisms was linked to the adsorption

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http://dx.doi.org/10.1016/j.bioelechem.2015.03.011
1567-5394/

of microbial produced flavins on the surface of the carbon electrode, which decreases the contribution of the planktonic cells (i.e., via non-adsorbed flavins) to the current output [15].

Both the chemical state and structure/topography of the electrode surface determine bacterial attachment and growth, as well as the prevalent EET mechanism at the interface cells/electrode [16]. For example, the use of rougher electrode surfaces (achieved using mechanical abrasion via sandpapers of known grit size) resulted in higher current output for the EAM *Geobacter sulfurreducens* [17]. However, it is not yet clear whether the higher current output at rough electrode surfaces is due to the thicker biofilm or to the high concentration of adsorbed flavins. A recent study from our group showed that *S. loihica* PV-4 growth cultures on smooth indium tin oxide electrodes favor the direct electron transfer mechanism yielding low current output. This is possibly due to flavins not adsorbing on the surface of a smooth indium tin oxide electrode or to the chemical properties of the indium tin oxide surface [8]. Biofilm formation has also been shown to cause an increase in the electrical resistance of carbon nanotube-treated graphite electrodes [18].

The use of atmospheric air plasmas for surface treatment is well established [19,20]. The combination of the main plasma parameter values, such as discharge voltage (kV), power density (W cm^{-2}), discharge gap (mm) and treatment time (s), determines the nature of the possible plasma surface processes. These processes can be broadly classified as etching, cleaning, film deposition, ion implantation, oxidation or functionalization. The last two processes are relevant to the present work and are also dependent on the nature of the surface e.g., metal, ceramic, polymer or glass. The formation of functional groups results from chemical reactions between gaseous plasma active species, e.g., OH radicals, ozone and atomic oxygen species, and reactive surface species/sites over a depth of 1 nm. Functionalization is known to significantly improve the wettability and adhesion properties of a plasma-treated surface as it generally increases the surface energy. The effects of atmospheric air and oxygen plasmas on graphite [22,23], carbon-based [24], e.g., carbon nanotubes [25–27] or carbon felt [28,29] and hydrocarbon polymer [30] surfaces have been studied extensively. All these works report significant oxidation of the surfaces with the formation of oxygen-rich polar groups like carbonyl, acetals or carboxyl groups, depending on the experimental conditions, e.g., relative humidity and plasma parameters [22–24,30]. A notable concomitant effect of plasma surface treatment is the increase in surface roughness [21,30]. The effects of plasma processing/treatment on biomaterials are similar to those just described [31]. Previous works have considered the effects of plasma treatment of surfaces or electrodes for increased adhesion of bacterial cells, notably in the context of biofuel cell applications. For example, Bax et al. [32] have applied plasma pre-treatment to polymeric surfaces to improve eukaryotic biofilm formation and bio-adhesion, thus improving the tissue–polymer interface. Kamgang et al. showed that treatment with atmospheric air plasma rendered the polymer surface more hydrophilic, thus improving bacterial cell attachment and electricity production at anodes, despite electrostatic repulsion between cells and the electrode [33]. Radio-frequency generated-plasmas have also been beneficially used for electrode surface treatments. For example, Flexer et al. [34] demonstrated that radio-frequency oxygen and nitrogen 25 W plasma pre-treatment of electrodes increased the initial anodic current from a mixed microbial consortium, with faster cellular adhesion on the electrode surface and higher biofilm growth [34]. Using radio-frequency oxygen-plasma treatment, Okajima et al. showed that surface functionalization with hydrophilic groups on a carbon fiber surface also increased its surface capacitance by 28% [35] for a specific oxygen gas feed concentration. To the best of our knowledge, this effect has not been reported in works using atmospheric air plasmas. Interestingly, He et al. used plasma-based N^+ ion implantation to treat the carbon paper anode in a microbial fuel cell and showed significantly enhanced electricity production as a result [36]. In spite of the promising results achieved with both radio-frequency plasma reactors and plasma-based ion implanters, atmospheric air plasma seems to be a more viable technique for the routine pretreatment of large electrodes as it does not require vacuum chambers/systems and gas manifolds, and thereby minimizes the overall cost.

In this study, we investigate the effects of atmospheric air plasma pre-treatment on the current output from attached *S. loihica* PV-4 cells on polished graphite and carbon felt electrodes. We demonstrate that plasma pre-treatment increases the maximum current output and adsorption of microbially produced flavins is the main driver for electricity production.

2. Materials and methods

2.1. Bacterial strain

S. loihica PV-4(DSM 17748) was purchased from DSMZ (Germany). Stock cultures were prepared transferring 1 mL of actively growing culture to a cryo-tube containing 0.5 mL of 50% DMSO and stored at −80 °C.

2.2. Microbiological methods

S. loihica PV-4 was cultured in Luria-Bertani medium and incubated at 30 °C for 24 h on an aerobic rotary shaker at 150 rpm. 2 μM of $MgSO_4$ and 0.1 μM of $CaCl_2$ were added as growth stimulants for the early exponential phase. 3 mL of the culture at optical density $OD_{600} = 1.0$ was then added to 30 mL of defined medium containing per liter: $NaHCO_3$ 2.5 g, $CaCl_2 \cdot H_2O$ 0.08 g, NH_4Cl 1 g, $MgCl_2 \cdot 6H_2O$ 0.2 g, NaCl 10 g, HEPES buffer 7.2 g, yeast extract 0.5 g, trace mineral solution 10 mL, and vitamin solution 1 mL [2]. pH was adjusted to 7.6 through the addition of 0.1 M of HCl, 20 mM of lactic acid was used as the carbon source and 5 g L^{-1} filter-sterilized casaminoacids was used as a growth stimulant. The cells were grown aerobically in defined medium at 30 °C for 24 h, under shaking condition at 150 rpm. In the unwashed inoculum experiments, 5 mL of the bacterial suspension were then directly injected in each electrochemical cell and added with 5 mL of fresh defined medium with 40 mM lactate and no added riboflavin. In other 'washed inoculum' experiments, 5 mL of the bacterial suspension was spun down for 10 min at 5000 rpm, the bacterial pellet was resuspended in 10 mL of fresh defined medium with 20 mM lactate and no added riboflavin, and then injected into the electrochemical cells. The washing procedure ensures removal of microbially-produced soluble redox mediators.

2.3. Electrode preparation

The carbon felt and graphite (isotropic graphite Grade 347 from Tokai Carbon Co. Japan) sheets were both cut into 2 × 1 × 0.2 cm electrodes, defining a geometric surface area of 5.2 cm^2. Current output values were normalized to the electrode surface area. The graphite electrodes were sanded with either P240 (grit diameter 58.5 μm), P400 (grit diameter 35 μm) or P600 (grit diameter 25.8 μm) P-graded sandpapers in order to obtain different surface roughness. The roughest graphite electrode surface was thus obtained by polishing with P240 sandpaper. All electrodes were cleaned overnight in 1 M of HCl and then stored in deionized water. Some of the P240 graphite and carbon felt electrodes were also plasma pre-treated.

2.4. Plasma pre-treatment

The plasma apparatus used in this work has been described in detail previously [37]. Oscilloscope traces of the discharge current patterns show that the atmospheric air plasma is typical of a dielectric barrier discharge (DBD). Such DBD plasmas are out of equilibrium (non-thermal) plasmas characterized by electron temperatures of the order