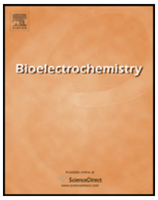
Bioelectrochemistry 106 (2015) 141–149

Contents lists available at ScienceDirect

Bioelectrochemistry

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



Influence of anode surface chemistry on microbial fuel cell operation

Carlo Santoro [a,b,1], Sofia Babanova [a,1], Kateryna Artyushkova [a], Jose A. Cornejo [a], Linnea Ista [c], Orianna Bretschger [d], Enrico Marsili [e], Plamen Atanassov [a,*], Andrew J. Schuler [b]

[a] Center for Micro-Engineered Materials (CMEM), Department of Chemical & Biological Engineering, University of New Mexico, Albuquerque, NM 87131, USA
[b] Center Emerging Energy Technologies (CEET), Department of Civil Engineering, University of New Mexico, Albuquerque, NM 87131, USA
[c] Center for Biochemical Engineering, Department of Chemical & Biological Engineering, University of New Mexico, Albuquerque, NM 87131, USA
[d] J. Craig Venter Institute, 4120 Capricorn Lane, La Jolla, CA 92037, USA
[e] Singapore Centre on Environmental Life Sciences Engineering (SCELSE), Nanyang Technological University, 60 Nanyang Drive, 637551 Singapore, Singapore

ARTICLE INFO

Article history:
Received 8 November 2014
Received in revised form 23 April 2015
Accepted 3 May 2015
Available online 6 May 2015

Keywords:
Microbial fuel cells
Self assembled monolayer
Surface modification
Bioelectrocatalysis
Anode biofilm analysis

ABSTRACT

Self-assembled monolayers (SAMs) modified gold anodes are used in single chamber microbial fuel cells for organic removal and electricity generation. Hydrophilic ($-N(CH_3)_3^+$, $-OH$, $-COOH$) and hydrophobic ($-CH_3$) SAMs are examined for their effect on bacterial attachment, current and power output. The different substratum chemistry affects the community composition of the electrochemically active biofilm formed and thus the current and power output. Of the four SAM-modified anodes tested, $-N(CH_3)_3^+$ results in the shortest start up time (15 days), highest current achieved (225 μA cm^{-2}) and highest MFC power density (40 μW cm^{-2}), followed by $-COOH$ (150 μA cm^{-2} and 37 μW cm^{-2}) and $-OH$ (83 μA cm^{-2} and 27 μW cm^{-2}) SAMs. Hydrophobic SAM decreases electrochemically active bacteria attachment and anode performance in comparison to hydrophilic SAMs ($-CH_3$ modified anodes 7 μA cm^{-2} anodic current and 1.2 μW cm^{-2} MFC's power density). A consortium of *Clostridia* and *δ-Proteobacteria* is found on all the anode surfaces, suggesting a synergistic cooperation under anodic conditions.

1. Introduction

Numerous bacterial species have shown the ability to oxidize organic compounds and use insoluble metal oxides and electrodes as their terminal electron acceptor [1,2]. Extracellular electron transfer in bacteria enables the construction and operation of bioelectrochemical systems called microbial fuel cells (MFCs) [3].

Electron transfer rate at the biofilm/electrode interface is one of the key factors determining the MFC's current and power output. Therefore, it is critical to optimize the electrode morphology and chemistry to promote fast electron transfer rate. This goal can be achieved through selection of specific electrode materials, enrichment with anode-respiring bacteria, morphological or chemical modifications of the electrode surface [4,5]. The electrode material and morphology should facilitate bacterial attachment and subsequent biofilm formation. At the same time, anode surface chemistry along with the biofilm formation should enhance electron transfer from bacteria to the electrode [6,7]. Several thermal or chemical treatments have been described to reduce MFC start-up time by facilitating rapid cell attachment and biofilm development for enhanced power output in MFC [3,8,9]. Thermal treatment of the electrodes leads to modification of the surface roughness and porosity and thus enhances cell concentration and biofilm development [10–13]. Depending on the gas atmosphere (e.g., nitrogen, oxygen, ammonia) used in thermal treatment, hydrophilic functional groups can be added on the electrode surface [14]. The main purpose of the chemical treatment is to introduce functional groups (typically nitrogen and oxygen containing groups) that improve cell attachment and biofilm development on electrode surface [15–18]. Several compounds, such as nitric acid [15,16], ethylenediamine [15], ammonium nitrate [16], ammonium persulfate [16], polyaniline [17], and 4(N,N-dimethylamino) benzene diazonium [18] have been used for surface chemical modification of carbonaceous electrodes. However, as previously shown [19], the chemical treatment of carbonaceous surfaces (e.g., carbon cloth) affects both the chemistry and morphology (e.g., roughness and porosity) of said surfaces, thus a clear discrimination of the benefits provided by chemical and surface effects is not straightforward [19]. The inability to distinguish the influence of only one parameter from the whole set of parameters that are usually altered through the commonly used surface modification techniques is a result of the intercorrelation between the introduced variances in the parameters' magnitudes [20]. Therefore, the impact of the anode surface chemistry on current and power output in MFC should be studied using flat electrode material, thus de-coupling chemical effects from change in surface morphology. In addition, proper statistical interpretation of the data sets should be provided. This

---

[*] Corresponding author at: Center for Micro-Engineered Materials (CMEM), Department of Chemical & Biological Engineering, MSC01 1120, Farris Engineering Center 247, 1 University of New Mexico, Albuquerque 87101-0001, USA.
E-mail address: plamen@unm.edu (P. Atanassov).

[1] The two authors have contributed equally to the manuscript.

http://dx.doi.org/10.1016/j.bioelechem.2015.05.002
1567-5394/

statistical tool should have the ability to identify correlation between particular factors and the final output of the studied system. Such a statistical technique is Principal Component Analysis (PCA), which application in MFCs and material analysis has been successfully demonstrated [20–22].

Recently, Guo et al. [7] studied the influence of the surface charge and hydrophobicity on the biomass accumulation, taxonomic distribution and electrochemical activity of multiple surface-modified anodes operated in half-cell bioelectrochemical systems. Anodes consisted of modified glassy carbon electrodes through electrochemical grafting with aryl dyazonium salts. As a result, the surface of each anode has been altered distinctively to be hydrophilic (—OH, $—SO_3^-$, $—N(CH_3)_3^+$) or hydrophobic ($—CH_3$) with positive, negative or neutral charge [7]. The researchers found that the most positively charged and hydrophilic surfaces were associated with improved biofilm formation and selection of electroactive microbes such as *Geobacter spp*. [7]. A similar conclusion has been reported by Picot et al., who observed significant increases in anode current output when the surface was amended with positively charged phenylphosphanium cations [23].

While these studies have added new knowledge relative to how anode surface modifications impact biofilm development and electrochemical activity under poised-potential conditions, the specific effects that functional groups may have on MFC operation, startup time, current and power output have not been described. Further, previous reports used modified carbon substrates including glassy carbon [7] and graphite plates [23]; however, these modifiable carbon substrates still have porous structures and therefore differential surface areas [24]. To separate the effects of surface area and surface chemistries, in this study we used gold anodes with ω-substituted alkanethiolates on gold terminated with functional groups ($—N(CH3)_3^+$, —COO-, —OH and $—CH_3$). We have previously shown that self-assembled monolayers (SAMs) improve cell attachment and early biofilm formation [25,26]. Here, we extend beyond poised-potential studies and use MFCs operated with a fixed resistance and equipped with activated carbon air cathodes to demonstrate how surface modified anodes can work specifically to enrich an electrocatalytic biofilm under less controlled operational conditions. The correlation between the surface chemistry of the anode and the MFC current and power output is investigated. Each anode material was tested in a separate MFC in order to: i) avoid undesired shunt-current losses, alternative current path through the ionically conductive electrolyte that can interfere with the current/power output, ii) avoid electrochemical and microbiological interaction among the different electrodes exposed to the same electrolyte, iii) study the air-breathing cathode performances related with different anodes used and show the overall output, iv) evaluate the microbial community developed on the electrodes (anode and cathode) starting from the same initial solution (in separate reactors), and v) use activated sludge (no pretreatment or previous enrichment) as inoculum to capture how biofilms establish on SAMs-modified electrodes under different selective pressures than have been reported previously (e.g. single chamber MFCs with air-breathing cathodes). Following electrochemical characterization for 45 days, DNA from anodic biofilms was sequenced to characterize the electrogenic communities and identify any phylogenetic differences that might have occurred as a function of the unique anode surface modifications.

2. Materials and method

2.1. Self assembled-monolayer production

Microscope glass coverslips (24 × 60 mm, #1, VWR, USA) were cleaned under UV ozone. The vacuum chamber was evacuated to $\sim 10^{-6}$ mTorr and a 15 Å Cr layer was deposited followed by 300 Å gold. Immediately after gold deposition, samples were incubated in 1 mM ethanolic solutions of 1-mercaptoundecanol (OH; Aldrich, St. Louis MO), undecanethiol (CH_3; Aldrich, St. Louis, MO),1-mercaptoundecanoic acid (COOH, Aldrich, St Louis, MO) 1-mercaptoundecyl trimethylamine ($N(CH_3)_3^+$; Prochimia, Poland) [27].

2.2. Contact angle measurement

After an immersion to the specific solution of at least 24 h, the regularity of the SAM surface chemistry was checked using X-ray Photoelectron Spectroscopy (XPS) as shown previously [27]. Contact angle was measured in order to quantify the wettability of the different solid SAMs. The contact angles were determined utilizing ultrapure water at room temperature and ambient humidity using the sessile drop technique with a goniometer (Ramé-Hart Instrument Co., Model No. 400-22-300 with DROPimage Standard, NJ). Ultrapure water droplets (1.5 μL) were deposed on each substrate, recorded in a video and then analyzed to obtain the contact angles. Each measurement was repeated at least 8 times at different locations on each SAM surface [28].

2.3. MFC configuration and cathode material

Single chamber microbial fuel cells (MFCs) with a volume of 130 mL were assembled as previously described [29]. The anolyte was 50% 0.1 M phosphate buffer solution (PBS) with 0.1 M KCl and 50% of activated sludge from the Albuquerque Wastewater Treatment Plant. Sodium acetate ($C_2H_3NaO_2$, 5 g L^{-1}) was used as a substrate and introduced periodically in each MFC to maintain non-limiting substrate concentration. The pH of the anolyte was 7.4–7.5 and remained constant along the entire experiment [30]. SAM-modified gold anodes (geometric area 14.4 cm^2) were assembled with two coverslips using a titanium wire with the functionalized surface facing the medium solution. The anode was connected to the cathode through an external resistance of 1000 Ω. The cathode used in this work has been previously described [22]. Briefly, activated carbon (Calgon, USA) with a surface area of 802 m^2 g^{-1} was grinded with 20%wt PTFE (60% dispersion in water, Sigma Aldrich). 60 ± 2 mg cm^{-2} of the obtained mixture was pressed at 1400 psi for 2 min on a carbon cloth (30% wt wet proof, Fuel Cell Earth) used as the electron collector. The cathode assembly was then heated at 200 °C for 1 h before utilization. The cathode had a geometric surface area of 3.5 cm^2 directly exposed to the electrolyte [22]. The SMFCs were operated in duplicate for each SAM-modified anode material at room temperature (21 ± 1 °C).

2.4. Electrochemical measurements

The overall single chamber microbial fuel cell (MFC) potential was recorded every 25 min using a datalog system (Personal DAQ/56, USA) connected to a PC for over 45 days. At the end of the experiment, anode and cathode potentiodynamic polarization curves were taken using a three-electrode configuration as previously described using a VersaStat potentiostat (Princeton Applied Research, USA) [31]. Briefly, the electrode under investigation (anode or cathode) was used as the working electrode, Ag/AgCl 3 M KCl (+0.21 V vs. SHE) was used as the reference electrode and a stainless steel A316 mesh with the same area of the working electrode was used as a counter electrode. The anode potential was scanned from open circuit potential (OCP) to 0 V vs. Ag/AgCl 3 M KCl. The cathode potential was scanned from OCP to −0.3 V vs. Ag/AgCl 3 M KCl. The scan rate utilized for the potentiodynamic curve was 0.2 and −0.2 mV s^{-1}, respectively [31]. The cell polarization curves were carried out after the single electrode polarizations in two electrode modes, connecting the anode as working and the cathode as counter and reference electrode. The polarization was started after the OCP stabilized (approximately 1 h), and then the polarization curve was measured from the open circuit cell potential (OCP) to 0.01 V. The power (P) was obtained using the equation $P = U \times I$ where U and I are the MFC voltage and current, respectively. Power and current are normalized to the anode geometric surface area (14.4 cm^2).