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

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A B S T R A C T

Self-assembled monolayers (SAMs) modified gold anodes are used in single chamber microbial fuel cells for organic removal and electricity generation. Hydrophilic (−N(CH₃)₂ −OH, −COOH) and hydrophobic (−CH₃) 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(CH₃)₂ 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 (−CH₃ 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.

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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], polyamine [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
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 diazonium salts. As a result, the surface of each anode has been altered distinctly to be hydrophilic (−OH, −SO3−, −N(CH3)3+) or hydrophobic (−CH3) 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 phenylphosphonium 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 chemistry, in this study we used gold anodes with ω-substituted alkanethiolates on gold terminated with functional groups (−N(CH3)3+, −COO−, −OH and −CH3). 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 anode cathode 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 −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-mercaptopoundecanol (OH; Aldrich, St Louis, MO), undecanethiol (CH3; Aldrich, St Louis, MO), 1-mercaptopoundecanoic acid (COOH, Aldrich, St Louis, MO) 1-mercaptopoundecyl trimethylamine (N(CH3)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 (Rame-Hart Instrument Co., Model No. 400-22-300 with DROPimage Standard, NJ). Ultrapure water droplets (1.5 μL) were deposited 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 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 (C2H3NaO2, 5gL−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 cm2) 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 m2 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 cm2 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 (PersonalDAQ/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 potentiotstat (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 × 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 cm2).
2.5. Biofilm characterization on the electrode

At the end of the 45 day experiments, anodic and cathodic biofilms were removed by scraping the electrodes. Pyrosequencing was done on one anode per each electrode material and on the planktonic biomass of the MFC equipped with N(CH3)3+-modified anode. DNA was extracted using sucrose lysis/cetyltrimethylammonium bromide (CTAB) as previously described [32], and its purity was evaluated from the ratio of absorbances at 260 and 280 nm. Samples were shipped on ice for DNA pyrosequencing by Research and Testing Laboratories (Lubbock, TX, USA). Bacterial tag-encoded FLX amplicon pyrosequencing was performed as described previously [33] with small modifications to utilize the titanium sequencing platform (Roche Applied Science, Indianapolis, IN). A single 35 cycle PCR step with Qiagen HotStar master mix and addition of 0.5 U of HotStar HifiFidelity polymerase was used in each reaction (Qiagen, Valencia, CA). The primers were 28f (5′-GAG TTT GAT CNT GGC TCA G-3′) and 519r (5′-GTN TTA CNG CGG CKG CTG-3′) (Escherichia coli 16S gene numbering). Pyrosequence reads were analyzed at UNM using AmpliconNoise 1.25 [34] to remove low quality sequences, which included sequences less than 200 bp in length, with an average quality score of less than 25, containing ambiguous characters, and/or without the correct primer sequence. A workflow script in QIIME 1.80 was used to pick operational taxonomic units (OTUs) at the 97% sequence identity level. Representative sequences from each OTU were identified by the Ribosomal Database Project44 classification method using QIIME, with assignment of taxonomic identities using the Greengenes 16S rRNA gene database [35].

3. Results and discussion

Gold electrodes were modified with self assembled monolayers (SAMs) having four different terminal groups, —CH3, —OH, —COOH and —N(CH3)3+. A stable molecular monolayer coated uniformly the surface as showed previously [27,28]. As the aim of this study was to compare the influence of the surface chemistry on biofilm formation, current and power generation in MFC rather than compare modified vs. unmodified anodes, no control experiment with bare gold electrode was carried out.

3.1. Overall MFC voltage

The MFC potential under constant external load (1000 Ω) was monitored for 45 d (Fig. 1). As in many other MFC studies, the potential increased with time, indicating electrochemically active biofilm formation. However, the potential evolution with time was different for each material, indicating differences in electroactivity and electron transfer rate [36]. MFCs fitted with —N(CH3)3+-SAM anodes showed a rapid potential increase (after 15 d), followed by —COOH (17 d), —CH3 (17 d) and —OH (20 d) (Fig. 1). The —N(CH3)3+-MFCs showed the fastest potential slope (170–195 mV d−1) and achieved stable conditions after roughly 18 d. COOH-MFCs showed a slower potential slope (75–96 mV d−1) and stabilized after 22–23 d. The potential slope of OH-MFCs could be divided in different parts: i) at potential <0.15 V, the slope was of 21–25 mV d−1; ii) at potential 0.42 > V > 0.15 V with the slope increased to 50–56 mV d−1. Finally, the U slope of CH3-MFCs was much smaller than the other materials, only 2.2–2.3 mV d−1. These results show that anode coating affects both the start-up time of potential production and the rate of potential increase with time, which in turn correlates to the attachment and growth of electrochemically active biofilms. The relative start-up times recorded for the different functional groups mostly correlated to results reported by Guo et al. using chemically modified glassy carbon anodes (e.g. —N(CH3)3+ > —CH3 > —OH); however, our systems demonstrated overall faster start up times for each comparative functional group. For example, Guo et al. reported a startup time of 23 d for their —N(CH3)3+-modified electrodes poised at 0.2 V (vs. Ag/AgCl) [7]. The reported startup time is eight days slower than that observed in this study with —N(CH3)3+–n-modified gold electrodes operated under MFC mode in a single-chamber system. Similarly, the —OH and —CH3 modified electrodes in the Guo et al. study showed start up times of 25.4 d and 37.2 d, respectively [7]. Our results indicate significantly faster start-up times (5–20 d faster).

Despite the different start-up times for each of our well performing functional groups, the N(CH3)3+–, COOH– and OH–MFCs reached a similar stable voltage output (0.41–0.43 V) since the external resistance chosen was higher than the smaller sustainable resistance [37]. However, the potential of CH3–MFC increased slowly over time and stabilized after 40 d to only 0.06 V (Fig. 1).

3.2. Anode polarization curves

To study the anode behavior independently of the cathode, the anode electrochemical response was measured using potentiodynamic polarization curves after 45 d of MFC operation (Fig. 2). Despite the fact that the voltage achieved at 1000 Ω external resistance after day 45 of MFC polarization was approximately the same for N(CH3)3+–, COOH– and OH–MFCs, the different surface chemistry of the anodes results in a different anodic polarization behaviors. Maximum current densities of 225–230 μA cm−2 were achieved by the —N(CH3)3+ modified anodes at approximately −0.40 V vs. Ag/AgCl. The —N(CH3)3+–anode demonstrated another peak at higher anodic potential (−0.27 V vs. Ag/AgCl). The maximum current densities achieved for each SAMs-modified anode follow the same trends as the voltage startup conditions in that the —N(CH3)3+ systems showed the highest
current and power produced to assess full system performance. The performance followed by $-\text{COOH}$, $-\text{OH}$ and $-\text{CH}_3$, respectively (Fig. 2).

The $-\text{N}(\text{CH}_3)_2^+$-MFCs showed the highest anode current densities and the shortest start up time most likely due to bacteria preference towards hydrophilic and positively charged surfaces in agreement with previous studies [7].

We have previously demonstrated that different bacteria exhibit different attachment responses to SAMs [25–28]. $-\text{COOH}$ and $-\text{OH}$-SAM generated much lower current densities relative to the $\text{N}(\text{CH}_3)_2^+$-anodes. These data suggest that the surface chemistry directly affect microbial species and/or specific biofilm characteristics. Furthermore, the $-\text{COOH}$, $-\text{OH}$ and $-\text{CH}_3$-modified surfaces may limit biofilm development and electron transfer rate [7]. Cathode polarization curves showed that cathode performance was similar across all systems (Fig. S1), indicating that MFC current output was controlled by the variations in anode performance. The performance of the cathodes decreased slightly after 45 d of operation for all MFCs tested (Fig. S1). The decrease in the cathodic current might have resulted from biofilm development at the cathode (Fig. S2) and by salt precipitations at the cathode [38] which also could introduce diffusional limitations and induce biofouling of the catalytic sites at the cathode–liquid interface.

3.3. MFC polarization curves and power generation

The overall performances of the MFCs were evaluated in terms of current and power produced to assess full system performance. The $\text{N}(\text{CH}_3)_2^+$-, $\text{COOH}$- and $\text{OH}$-MFCs had similar OCV values at 550–565 mV and similar current output at potentials close to OCP until roughly 70 $\mu$A cm$^{-2}$ (Fig. 3a). As the current densities increase, the I–U curves showed diffusion or metabolic limitations going into overshoot conditions [39]. Particularly, the OH-MFCs showed mass transfer control at roughly 70 $\mu$A cm$^{-2}$ while the COOH-MFCs showed diffusion or metabolic limitations at roughly 125 $\mu$A cm$^{-2}$ and the $\text{N}(\text{CH}_3)_2^+$-MFC was limited by mass transfer at roughly 200 $\mu$A cm$^{-2}$.

The MFCs with $-\text{CH}_3$ modified anodes had significantly lower OCPs (450 mV) and significantly lower short circuit currents of 5–7 $\mu$A cm$^{-2}$. The $\text{N}(\text{CH}_3)_2^+$-MFCs had a maximum power density of 40–41 $\mu$W cm$^{-2}$ followed by COOH-MFC with 35–37 $\mu$W cm$^{-2}$ and by OH-MFCs with 25–28 $\mu$W cm$^{-2}$. The CH$_3$-MFCs had the lowest performances with a $P_{\text{max}}$ of only 1.2 $\mu$W cm$^{-2}$ (Fig. 3b). Comparison between Figs. 2, 3 and S1 shows that MFC polarization measurements followed the same trends as the anode polarization curves, indicating that the short-circuit current in this configuration was limited by the anode rather than the cathode.

3.4. Anodic biofilm analysis

Pyrosequencing of 16S rRNA gene amplicons derived from the anode-associated biomass shows diverse bacterial populations at the different anode modified surfaces (Fig. 4). Our findings show that the enrichment of electroactive biofilms on SAMs-electrodes operated in independent reactors yields different taxonomic compositions relative to previously reported studies [7]. A phylum-level analysis (Fig. 4a) indicates that phylum Proteobacteria comprised between 32% and 37% of the anode-associated biofilms. The $-\text{N}(\text{CH}_3)_2^+$ modified surface featured Proteobacteria in a relative abundance of 36%, the $-\text{COOH}$ had 37% relative abundance, and $-\text{OH}$ had 32% relative abundance. The $-\text{N}(\text{CH}_3)_2^+$ bulk solution (labeled as WW in Fig. 4) also showed 37% relative abundance of Proteobacteria. However, Proteobacteria only occupied approximately 6% of the biofilm associated with the $-\text{CH}_3$ modified anode.

The phylum Firmicutes had a high relative abundance in all of the communities, between 21% and 34% of the biofilm. The biofilm associated with the $-\text{CH}_3$-modified electrode had the highest relative abundance of Firmicutes at 34%. Interestingly, Firmicutes have been identified in many different microbial fuel cell reports, including a particular...
strain (*Firmicutes Thermocinclia* sp. strain JR) that was isolated from thermophilic microbial fuel cells and was able to directly transfer electrons to an anode surface [40].

The phylum *Bacteroidetes* was present in all of the communities with relative community abundance of 22% (—N(CH₃)₂⁺), 12% (—COOH), 6% (—OH), 13% (—CH₂) and 15% (WW). The —CH₃ modified anode also had a relatively high abundance of phyla *Lentisphaeraea* (24%) and *Actinobacteria* (19%) as compared to the other samples.

The class-level analysis of the anode communities shows that the phylum *Proteobacteria* featured class *δ-Proteobacteria*, *β-Proteobacteria*, *γ-Proteobacteria*, *α-Proteobacteria* and *ε-Proteobacteria*. Class *δ-Proteobacteria* had the highest relative abundance in the —N(CH₃)₂⁺, —COOH and —OH anode biofilms, and very low relative abundance in the —CH₂ anode-associated biofilm and the —N(CH₃)₂⁺ bulk solution (WW). Several members of class *δ-Proteobacteria* and *γ-Proteobacteria* have been reported as electrochemically active microbes in bioelectrochemical systems [41–45].

The high relative abundance of class *δ-Proteobacteria* in all of the biofilms that showed good current and power output (—N(CH₃)₂⁺, —COOH and —OH), suggests that these community members are active in electron transfer to the anode surfaces. The —COOH anode-associated biofilm and the WW also featured a small percentage of class *γ-Proteobacteria*, which suggests that these community members might be active in multiple functions, including electron transfer to the anode and possibly fermentation of complex substrates in the bulk solution.

The presence of class *β-Proteobacteria* in wastewater-enriched microbial fuel cells has also been reported by several MFC researchers [41–43,46]; however, the functional role(s) of these microbes have not been comprehensively defined, and members of this class may perform multiple functions within the biofilm.

Interestingly, all the reactors featured a high relative abundance of fermentative microbes including class *Clostridia, Bacteroidia, Deferribacteres* and *Lentisphaeraea*. This result suggests that acetate was not the sole carbon source for the community and that residual complex carbon substrates from the activated sludge inoculum may also have been used as electron donors for the biofilm and planktonic communities. Members of class *Clostridia, Bacteroidia, Deferribacteres* and *Lentisphaeraea* have been reported in several wastewater-enriched microbial fuel cells [41–47], and we speculate that these organisms are critical for converting sugars and other complex substrates to simple volatile fatty acids that are the preferred carbon sources for electrochemically active *δ-* and *γ-Proteobacteria*.

A more detailed sequencing effort is required to identify strain-level associations with the various electrode surface chemistries; however, the class-level analysis suggests that there may have been different community members contributing to electron transfer at the various chemically-modified surfaces. The results strongly suggest that the surface chemistry (charge and hydrophobicity) of the —CH₃-modified anode had the most effect on microbial taxonomic enrichment, which negatively impacted the overall system function. The lack of *δ-Proteobacteria* members and a higher relative abundance of diverse fermentative members (e.g. *Bacilli, Lentisphaeraea* and *Actinobacteria*) have been reported as electrochemically active microbes in the presence of class *β-Proteobacteria* in wastewater-enriched microbial fuel cells [41–43,46].

The amount of pressure for *δ-Proteobacteria* varied significantly between the different electrode surfaces. The results strongly suggest that the surface chemistry (charge and hydrophobicity) of the —CH₃-modified anode had the most effect on microbial taxonomic enrichment, which negatively impacted the overall system function. The lack of *δ-Proteobacteria* members and a higher relative abundance of diverse fermentative members (e.g. *Bacilli, Lentisphaeraea* and *Actinobacteria*) correlate with the low electrochemical performance of the —CH₃-modified system, and suggest that fermentation was the primary function of this community. The data suggest that the hydrophilic and positively charged SAM-electrode (—N(CH₃)₂⁺) induced a stronger selective pressure for *δ-Proteobacteria*. The amount of *δ-Proteobacteria* was...
higher on the —N(CH₃)₃⁺ anode, followed by COOH, OH and CH₃ modified anodes. A lower relative abundance of electrogenic bacteria may have induced metabolic limitations on the rest of the community (e.g. build up of volatile fatty acids will slow fermentation) leading to fast decrease of the generated current at lower potentials. Therefore in the case of OH and COOH MFCs the region in the polarization curves controlled by methabolic limitations starts earlier at lower current densities in comparison to the N(CH₃)₃⁺ system (Figs. 2 and 3).

Interestingly, the relative abundance of Proteobacteria associated with the —N(CH₃)₃⁺ anode found in this study (37%) was considerably lower than what was reported by Guo et al. (68%) [7]. We also observed that Firmicutes and Bacteroidetes phyla were much more abundant (one order of magnitude) relative to Guo et al. [7]. Despite different relative abundances of bacterial phyla found on the surfaces, current production and start up times observed in this study were improved relative to Guo et al. [7]. This finding suggests that a diverse consortium might be more electroactive and efficient at substrate conversion relative to Geobacter spp., which is in agreement with Ishii et al. [48].

Further, in the Guo et al. study [7] all anodes were immersed in the same electrochemical cell with a modified M9 medium and acetate as the electron donor. The described reactor was inoculated with the effluent of an operational BES that was described in Dennis et al. and had been enriched for over one year [49]. Accordingly, the anode-associated biofilm described by Dennis et al. featured a high relative abundance of Geobacter spp. (>62% relative abundance in the community), which was transferred to the reactor operated by Guo et al., having multiple electrodes with different surface properties exposed to the same inoculum and medium. The surface-modified anodes were consistently polarized at a constant potential of −0.2 V vs. Ag/AgCl (3.5 M KCl) and Guo et al. found more biomass attachment on hydrophilic and positively charged surfaces, which was directly related to the current output [7]. However, all the microbial biofilms found on the anodes contained over 68% of Geobacter spp. known to be electrochemically active microorganisms. While our experimental setup was different to reflect a less electrochemically controlled enrichment, our study found similarities, in that the most positively charged and hydrophilic surface attracted a higher relative abundance of δ-Proteobacteria. Interestingly, this finding was observed in duplicate MFC reactors inoculated with activated sludge (not previously enriched inoculum) and operated without poised potential addition or the interference of shunt-currents, which are induced when anode electrodes are operated in a shared media.

A 16S-rRNA sequence analysis of the cathode biofilms showed that the taxonomic composition of each cathode was fairly similar across the experimental conditions (Fig. S2); however, the cathode populations were significantly different than the anode biofilms analyzed from the same reactors.

3.5. PCA, relationship between surface chemistry, electrochemical output and anodic microbial community

As has been previously discussed, due to the complexity of MFCs and the abundance of factors determining the overall system performance and their intercorrelation, ascribing the observable variations in the MFC output to only one parameter is highly incorrect as also previously showed [27]. Thus, although in this study we intentionally varied only one parameter (anode surface chemistry), the differences in the MFC behavior cannot be attributed to the impact of only this variable. As we demonstrated, the variations in the anode surface chemistry led to differences in surface hydrophobicity (Table S1), surface charge as well as taxonomic composition on the anodes (Fig. 4), all leading to different MFC behaviors.

Principal Component Analysis was used to estimate correlations between the different factors affecting the tested MFCs and their operational characteristics, such as current and power output, start up and steady-state time. Steady-state time is defined as the time necessary for the system to reach steady-state current when the MFC is subjected to a constant external load in abundance of a carbon source. Since the cathode was not the limiting electrode, parameters associated with the anode operation were taken into account for the PCA analysis.

PCA separates the variables and the samples into three groups (Fig. 5). The first group contains the —N(CH₃)₃⁺ MFC with the highest current and power densities and the shortest start up and steady-state times. The parameters contributing to the higher performance of the —N(CH₃)₃⁺ anode and MFC, respectively, are the positive charge of the surface groups and the presence of higher amounts of bacteria from the classes of δ- Proteobacteria and Clostridia. Although, among the tested hydrophilic surfaces the N(CH₃)₃⁺ has the least pronounced hydrophilicity, it can be seen that its positive charge facilitates attachment of negatively charged bacteria at circumneutral pH values. So far it has been considered that a high relative abundance of δ-Proteobacteria in the bacterial biofilm [7,41,42] leads to enhancement of the anode electrical output, as this bacterial class includes several electrochemically active species. However, in this study due to the complexity of the carbon compounds in wastewater, a consortium between Clostridia and δ-Proteobacteria has been identified as a key aspect for increased electrochemical performance of the MFCs.

The second group contains —COOH and —OH modified MFCs. Both are characterized with high hydrophilicity and lower relative abundance of δ-Proteobacteria and Clostridia. Contact angle measurements in ultrapure water showed similar values for —OH (27 ± 1°), —COOH (19 ± 1°) and slightly higher, but still in hydrophilic range for —N(CH₃)₃⁺ (51 ± 4°). SAMs despite having differently charged surfaces while the —CH₃ SAM had a much higher values (102 ± 2°) (Table S1). At the pH of wastewater used in this study, it is not entirely clear if the —COOH group from the SH(CH₃)₃COOH is deprotonated or not. The pKa₃ of HS(CH₃)₃COOH has been found to be 7.4 and the pH of the electrolyte in this study was 7.4–7.5. Crittenden et al. suggested that the carboxylic acid terminus of SAMs can interact with the peptide bonds in proteins via strong hydrogen bonds [50]. Thus, —COOH could bind outer membrane and enhance electron transfer in electrochemically active microorganisms.

The —CH₃ modified anode surface represents the third group. This group exhibits the longest start up and steady-state times, no δ-Proteobacteria, and most importantly, a hydrophobic character of the anode surface. The electron transfer rate at the biofilm/electrode interface along with the bacteria attachment depends on the hydrophilicity of the electrode surface. The variations in the surface hydrophilicity affect the electrolyte–electrode interactions, as shown by using a ferricyanide probe (Table S1), where increased hydrophobicity decreases the contact between the electrode and the electrolyte and thus lower the charge transfer rate at the bio-support interface. As a result of the decreased charge transfer ability of the electrode surface the generated current also decreases.

There is not a clear separation between the impact of the microbial population and the physical properties of the electrode surface on the MFCs’ final output. Therefore, it should be appreciated that both of these factors are equally important when MFCs are designed and studied. It is also reasonable to say that the physical properties of the electrodes are affecting the microbial community in the formed biofilm. Fig. S3 shows a qualitative path analysis of the factor intercorrelations, where the electrode surface chemistry directly determines electrode hydrophobicity and surface charge. Consequently the hydrophobic/hydrophilic properties of the surface define the electrode–electrolyte contact and thus charge transfer ability as well as bacterial attachment and biofilm formation. Electrode surface charge on the other hand also influences bacteria attachment. Variation in charge transfer abilities of the electrodes implies the generated current and power densities. In parallel, the types of bacteria present in the wastewater microbial population will greatly influence biofilm formation and the types of bacteria able to participate in the electron transfer, both very important parameters for successful MFC’s operation.
4. Conclusions

Hydrophilic/hydrophobic SAM-modified gold anodes, harboring positive or negative functional groups, determine the current and power output of MFCs. Electrochemically active microorganisms attach preferentially on hydrophilic and positively charged surfaces. In fact, $\text{N}^\text{+}(\text{CH}_\text{3})_\text{3}$ modified anodes showed the shortest start-up time, the highest current and power densities and the fastest electron transfer rates among the materials investigated. Pyrosequencing showed the highest percentage of $\delta$-Proteobacteria on $\text{N}^\text{+}(\text{CH}_\text{3})_\text{3}$ modified anodes, most likely responsible for direct electron transfer at the biofilm/electrode interface. A consortium of Clostridia and $\delta$-Proteobacteria was identified as...
a key aspect for increased MFCs' electrochemical performance under the conditions tested, which warrants further investigation. A clear separation between the impact of the microbial population and the physical properties of the electrode surface on the MFCs' final output cannot be made due to the intercorrelation of these factors. Both these factors are equally important when MFCs are designed and studied.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bioelechem.2015.05.002.

References


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