Surface Modification of Microbial Fuel Cells Anodes: Approaches to Practical Design

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\textbf{A B S T R A C T}

Anode modification was explored as an approach to enhance the startup and improve the performance of microbial fuel cells (MFCs) inoculated with mixed cultures for wastewater treatment. Carbon cloth (CC) anodes were modified by electrochemical oxidation in three electrolytes: nitric acid + sulfuric acid (CC-NS), ammonium nitrate (CC-AN), and ammonium sulfate (CC-AS). The acidic modification of the anode material increased in the ratio of saturated/unsaturated carbon on the surface and consequently, a decrease in electrode resistance was observed. A linear dependence between the MFCs operational characteristics and the anodes resistance ($R^2 > 0.9$) indicated the dominating role of this parameter. This modification also enhanced the bacterial attachment (wet and dry biomass) and biofilm formation. CC-NS, CC-AS and CC-AN anodes accelerated the startup up period of the MFCs and demonstrated higher current and power compared to the unmodified CC. The differences in MFCs electrochemical behavior tended to decrease with time. Principal Components Analysis (PCA) was used to identify the parameters having major influence on the system performance, and the results underlined the positive effect of the surface modification on the MFCs output due to increases in the amounts of unsaturated and oxidized carbon, Electrochemical Accessible Surface Area (ECSA) and bacterial attachment.

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1. Introduction

The microbial fuel cell (MFC) is an emerging biotechnology utilizing bacteria to degrade organic compounds present in wastewater and convert the chemical energy into electricity [1]. An MFC reactor is composed of two solid electrodes (anode and cathode), and an electrolyte that is usually wastewater or a liquid organic waste [1]. Anaerobic electrochemically active bacteria growing on anode surfaces degrade organic compounds (i.e. acetate, glucose, and protein in wastewater), and produce electrons, protons, and CO\textsubscript{2}. The electrons are then transferred to the anode, from where they are moved through an external circuit to cathode surface and generate current. The protons are transferred through solution to the cathode, and combined with electrons and oxygen to produce water [1]. MFCs have been explored for wastewater treatment [2,3], biochemical oxygen demand (BOD) sensors [4], hydrogen producing bioreactors [5], toxic metal removal [6] and water desalination [7]. Although the electrochemical performance of MFCs has been enhanced in recent years and several small devices have been powered [8,9], the low power output is still the major obstacle for the utilization of MFCs as practical devices [10].

The electrochemical characteristics of electrodes greatly affect MFC performance [1,11,12]. Developing low-cost anode materials with high surface areas for bacterial attachment and high conductivity for electron transfer is important to enhance power generation of MFCs. Introducing charged functional groups for material surface modifications has been conducted to increase the available surface area, and to create hydrophobic surfaces for bacterial attachment [13–15]. Specially, ammonia gas treatment of carbon cloth [16], acid treatment of graphite felt or carbon mesh
materials [17,18] and nitric acid treatment of activated carbon fiber anode [19] were employed and the power output of MFCs was notably increased. Another approach to surface modification is heat treatment, which increases the surface area of anode electrodes (e.g. carbon mesh [18], graphite brush [16] and nanofibers [14]) and enhances power output.

Electrochemical oxidation is an emerging approach for electrode material modification. Similar to the chemical approaches, functional groups (e.g. carboxyl) are introduced onto electrode surfaces in order to enhance biofilm formation and facilitate electron transport from microorganisms to anode surfaces. Electrochemical oxidation has been demonstrated as an appropriate approach for surface modification in MFCs [20,21]. Compared with other modification techniques (e.g. ammonia treatment, composite coating method, and heating treatments), electrochemical oxidation is fast and convenient for modifying electrode materials. The main advantages are relatively low temperatures and short times of treatment. Moreover, electrochemical modification can be performed with the utilization of common and low-cost chemical compounds (e.g. nitric/sulfuric acid, ammonium nitrate and ammonium sulfate), and thus not affecting the overall cost of the materials.

The study aimed at modifying plain carbon cloth (CC) surfaces through chemical and electrochemical oxidation with: (a) a mixture of nitric and sulfuric acid; (b) ammonium nitrate and (c) ammonium sulfate. The surface chemistry of the modified (chemically and electrochemically) materials was investigated using X-ray Photoelectron Spectroscopy (XPS), and the roughness parameters were calculated by processing SEM images [22,23]. Electrochemical Accessible Surface Area (ECSA) and resistance were also evaluated. Biofilm attachment and development and anode performances were characterized over time in order to determine the influence of the modification procedure. Finally, the modified CC anodes and plain CC anodes were installed in single chamber MFCs (SCMFCs) to compare power generation.

2. Materials and methods

2.1. Electrodes

Carbon cloth (Hesen Electric Co., Ltd. China) (referred to here as CC) was used as anode material. Carbon cloths were soaked in acetone for 3 hrs, and then washed with deionized water before being dried at 120 °C for 2 hrs. The electrochemical modification of the anodes was carried out in two-electrode setup at ambient temperature. The CC (geometrical surface area: 16 cm²) was used as the working electrode and a graphite rod was the counter electrode, with an electrode distance of 2 cm. The electrolysis current was set at 1.25 mA/cm² and kept for 30 min. Three types of electrolyte solutions were used to modify the anodes: nitric acid + sulfuric acid (4:1.5 mol L⁻¹), ammonium nitrate (NH₄NO₃, 1.4 mol L⁻¹), and ammonium sulfate ((NH₄)₂SO₄, 0.7 mol L⁻¹). The molar concentration for nitric acid was selected as 4 mol L⁻¹ due to the destruction of carbon materials at higher acid concentrations, and the better effect on the surface was observed at the sulfuric acid concentration of 1.5 mol L⁻¹. The molar concentration of ammonium nitrate was twice that of ammonium sulfate in order to keep the same ammonium ions molar ratio.

The modified anodes were then washed three times with deionized water, dried at 120 °C for 30 min, and stored in a dryer before being installed in SCMFCs. Chemical modification of the same material was used as the control sample, and the chemical and morphological properties of the materials modified with two procedures were compared.

The cathodes were made of a mix of activated carbon (AC) (Calgon, 802 m²g⁻¹ as BET area) and 20%wt PTFE (60% dispersion in water, Sigma Aldrich) pressed for 2 minutes at 1400 psi on a CC (30%wt PTFE, Fuel Cell Earth) used as electron collector. The AC cathodes were thermally treated at 200 °C for 1 hour to optimize their performance [24].

2.2. Materials characterization

2.2.1. Surface chemistry

Initially, the surface chemistry of chemically and electrochemically modified materials was measured using X-ray photoelectron spectroscopy (Kratos Amicus, UK) with a monochromated Al Kα source (hv = 1486.6 eV). This screening was conducted to check the difference in surface chemistry between the electrochemical and the chemical modification. The surface chemistry of the electrochemically modified anodes materials was also analyzed before the MFC experiments. X-ray photoelectron spectrometer (Kratos Axis Ultra DLD) with a monochromatic Al Kα source operating at 300 W was used. Survey and high-resolution spectra C1s, N1s, O1s and S 2p spectra were acquired at pass energies of 80 and 20 eV, respectively. Three different areas on each sample were analyzed and no charge compensation was necessary. Spectra were processed in the CASAXPS program. A linear background was used for all spectra. The sensitivity factors provided by the manufacturer were used. The curve-fits were performed using a 70% Gaussian/30% Lorentzian (GL (30)) line shape.

2.2.2. Surface Morphology

The surface images of the plain CC and the modified (chemically and electrochemically) CC (CC-NS, CC-AN, and CC-AS) anodes were acquired using an SEM (S-3700, Hitachi, Japan) at 1k and 20k magnification. The images were taken using fixed experimental parameters (i.e. voltage, magnification, brightness, current and gain) in order to produce the images in way the differences to be caused by morphology features and not by instrumental variability [22,23]. Digital Image Processing was used to process the images using an in-house developed Graphical User Interface (GUI) in Matlab with the Image Processing Toolbox [25]. Six SEM images were processed for each magnification and modification. This technique had been used successfully to determine roughness parameters. [13,22,23,26].

2.2.3. Electrochemical Accessible Area, SEM images and resistance measurements

The Electrochemical Accessible Surface Area (ECSA) of the anodes was calculated based on the capacitance determined by cyclic voltammetry. The cyclic voltammetry (CV) (-0.6 to 0.6 V vs. Ag/AgCl, 0.01 V s⁻¹) was performed in a three-electrode setup with the anodes used as working electrodes, a Pt-wire as counter electrode and saturated Ag/AgCl reference electrodes. Phosphate buffer solution (PBS) of 50 mM with 0.1 M KCl was used as electrolyte. The current at 0.45 V vs. Ag/AgCl was used for the ECSA calculation using the following equation [27]:

\[
ECSA = \frac{i_{ox} - i_{red}}{2V} \times \frac{1}{C_{sp}}
\]

where \(i_{ox}\) and \(i_{red}\) are the oxidation and reduction current at 0.45 V vs. Ag/AgCl, \(V\) is the scan rate in V s⁻¹ and \(C_{sp}\) as the specific capacitance. The specific capacitance for the CC was 35 μF cm⁻² [28]. The ECSA was used only for comparison of the electrodes tested in this study.

The material resistance was measured by connecting 2 alligator clips along the sample (length: 4 cm) and reading the ohmic value from a voltmeter (Fluke 45). Four samples of each type anode were examined.
2.2.4. Bacterial attachment

The two parameters, wet and dry biomass were used to indicate the changes in bacterial attachment to the anode surfaces. The anodes were immersed in a medium solution containing sodium acetate substrate (1.5 g L⁻¹, pH 7.4-7.5) and wastewater for 4 weeks, and the weights were periodically measured using a high precision balance (OHAUS Discovery, uncertainty 0.01 mg) to determine the extents of bacterial attachment, biofilm formation, other aggregates and bio fouling (termed as wet biomass) [13]. The balance resolution guaranteed high accuracy of the measurement. After 4 weeks, the anodes were dried (room temperature for 4 days) and the final weight was measured, which was used to determine the biomass attached on the anode surface, termed as dry biomass.

2.3. MFC configuration and electrochemical characterization

Membraneless single chamber microbial fuel cells (SCMFs) with gas-diffusion air-cathode, having a volume of 130 mL were used in this study [29,30]. Four MFCs were operated in parallel to investigate the power generation of the plain carbon cloth and the modified anodes (CC, CC-NS, CC-AN, and CC-AS). All MFCs were operated in a batch mode at room temperature of 21 ± 1°C using medium solution enriched with sodium acetate (1.5 g L⁻¹, pH 7.4-7.5). The wastewater was taken from a wastewater treatment plant (Albuquerque, NM, USA). The medium solution contained 50 mL of PBS (50 mM), 40 mL of activated sludge and 40 mL of primary wastewater [31]. Sodium acetate substrate (1.5 g L⁻¹) was added as organic substrate.

An external resistance was applied (1000 Ω) to the MFCs and the voltage was recorded (Personal DAQ/56) over time every 25 minutes. Single electrode (anode and cathode) potentiodynamic polarization curves (Gamry P600) were performed using a potentiostat (Gamry P600) at a scan rate of 0.2 mV s⁻¹ after leaving the electrodes in open circuit potential (OCP) for at least 1 hour in a three electrodes configuration as previously showed [32]. Pt wire was used as counter electrode, and a 3 M KCl Ag/AgCl electrode as a reference electrode [33]. The anode OCP was measured at the beginning of the polarization curve and corresponded at the potential with no current passing through the MFC system as showed previously [34].

Overall polarization curves (E-I) were measured using a potentiostat connecting the anode as working electrode (WE) and the cathode as counter (CE) and reference electrode (RE). The polarization curves were run starting from the potential between anode (WE) and cathode (CE and RE) at the OCP and going till 10 mV vs RE. The scan rate was 1 mV s⁻¹. Power density was obtained by multiplying the potential (E) and the current produced (I) and dividing the geometric anode area (16 cm²).

2.4. Principal Components Analysis (PCA)

PCA, using autoscaling as a preprocessing option (mean centering and scaling to unit variance), is a statistical tool to process large sets of variables in order to find patterns in data [35,36]. PCA transforms correlated or possibly correlated variables into new variables called principal components (PC). PC 1 separates the variables into two groups depending on their effect on the final output, positive or negative. PC 2 shows the deviation of the variables from the data trend. PLS_Toolbox 5.0 for Matlab was used for the multivariate analysis of the data.

3. Results and discussion

3.1. Initial screening to identify anode properties after chemical and electrochemical treatment

Initial XPS data showed differences between chemical and electrochemical treatment (Table 1), with the biggest difference being in oxidation degree. For chemical modification using AS and AN, a slight increase in O and N was observed, and the carbon content changed only slightly. For electrochemical modification using AN and AS, large amounts of oxygen (more than double untreated and chemically treated samples) and nitrogen (from roughly 1% to roughly 2%) were detected, and this was also reflected in the greater amounts of carbon containing surface oxides groups CxOy in the electrochemically-treated samples. For the chemical modification of NS, a dramatic increase in O (from 0.3 to 23%) and N (from 0 to 4.0%) was detected. The electrochemical modification using NS had a similar amount of oxygen and less N compared with chemical modification, but the carbon had large amounts of unsaturated carbons.

This screening result showed that both chemical and electrochemical treatments changed the surface chemistries. Electrochemical treatment using AN and AS introduced larger increases in the degrees of oxidation relative to chemical modification, while electrochemical treatment using NS produced similar changes to chemical modifications.

The SEM images of different CC anodes were processed and the results were summarized (Fig. 1). The Gaussian filter was applied to the images at the 20 K magnification to separate the original images into two subsets, one with high-frequency roughness in the range of 20-100 nm (Fig. 1A) and the other with low-frequency roughness in the range of 0.9-5 μm (Fig. 1B). The same filter was applied to the images at the 1 K magnification to separate roughness into two other ranges, one in the range of 6.5-50 μm (high-frequency component, Fig. 1C) and the other in the range of 163-450 μm (low-frequency component, Fig. 1D). The chemical and electrochemical treatment with all types of chemicals (i.e., AN, AS and NS) increased the small scale roughness (20-100 nm) (Fig. 1A) and the intermediate roughness (6.5-50 μm (Fig. 1C) compared with the plain CC. In terms of macro-roughness (163-450 μm), both chemical and electrochemical treatment with NS increased roughness, while the treatment with AS and AN (both chemical and electrochemical) decreased the macro-roughness (Fig. 1D). The electrochemical treatment using AN and NS increased the roughness in the range 20-100 nm (Fig. 1A) and 6.5 and 50 μm (Fig. 1C) in comparison

<table>
<thead>
<tr>
<th>Table 1</th>
<th>XPS analysis of elemental composition of the plain CC and chemically and electrochemically modified CC (the amount of types of N and O: atomic %, the amounts of types of carbon: relative %).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>C 1s %</td>
</tr>
<tr>
<td>Plain CC</td>
<td>96.2</td>
</tr>
<tr>
<td>Chem_CC-AN</td>
<td>92.4</td>
</tr>
<tr>
<td>Chem_CC-AS</td>
<td>93.5</td>
</tr>
<tr>
<td>Chem_CC-NS</td>
<td>69</td>
</tr>
<tr>
<td>Elec_CC-AN</td>
<td>82.9</td>
</tr>
<tr>
<td>Elec_CC-AS</td>
<td>84.9</td>
</tr>
<tr>
<td>Elec_CC-NS</td>
<td>70.5</td>
</tr>
</tbody>
</table>
with the chemical treatment, while slightly decreasing the roughness between 0.9-5 µm (Fig. 1.B) and 163-450 µm (Fig. 1.D). The electrochemical treatment using NS increased the roughness in the range 0.9-5 µm (Fig. 1.B) and 6.5 and 50 µm (Fig. 1.C) compared to the chemical treatment, while it decreased slightly between 20-100 nm (Fig. 1.A) and 163-450 µm (Fig. 1.D). This opposite effect of NS and AN/AS on the morphology was the most obvious for macro-roughness, which was correlated with the highest chemical differences between NS and AN/AS modifications.

The measurement of the roughness on the chemical and electrochemical treatments underlined the increases in the surface roughness and the probable benefit for bacterial attachment [13]. The plain CC had the largest amount of functionalized carbon but the lowest nano- and macro-roughness. The increase in O and N functionalities introduced by chemical and electrochemical treatment was accompanied with the increase in nano-roughness (20-100 nm) that was the highest for electrochemical treatment with AN and AS. Both NS-treated samples had the highest amount of S and unsaturated C and the largest macro-roughness (160-450 µm). The difference in the surface chemistry and the increase
in O and N functional groups well demonstrated the benefits of electrochemical treatment for anode materials in MFCs.

3.2. Anodes surface characterization of materials used for electrochemical measurements

The SEM images showed the fibers of the modified CC anode materials and the plain CC at 20k magnification (Fig. 2). There was no visual difference in between the CC materials tested, but as discussed previously, there was a notable difference in the surface morphology at micro- and nanometers scale (See 3.1)

XPS analyses were performed before the electrochemical tests in order to evaluate the changes in the surface chemistry caused by electrochemical oxidation, in which the elemental composition and amount of N and O were determined based on the XPS spectrum (Fig. 3, Table 2). The plain CC had a significant amount of O present and 3–4% of N and S. The treatment procedures in ammonium sulfate (CC-AS) and ammonium nitrate (CC-AN) increased the amount of all of these elements. O increased from 15% to 30–35%, while N increased from 4% to 8–10% and S from 3 to 6%. The treatment in acids (CC-NS) did not significantly increase N, and increased S and O.

The high resolution spectra were curve fitted to evaluate the chemical changes of the modified CCs (Table 2). In the plain CC, different types of C were present with main component being from aliphatic and unsaturated (graphitic and aromatic) carbons. Surface oxides were also present - C-OH, C-OC and C=O (all summed and represented as C-Ox in Table 2). One type of N (at 401.8 eV) was either quaternary N or as N2O3 species. Surface oxides were confirmed by O 1s spectra where two peaks represented C-O (533.1 eV) and C=O (531.8 eV) in the plain CC.

The surface chemical compositions of CC-AS and CC-AN were similar, but different types of chemical species were formed by these two treatments. The major changes in carbon were simultaneous graphitization and oxidation, but the degree of graphitization was larger for CC-AS while the degree of oxidation was larger for CC-AN. Importantly, the amount of oxygenated carbons increased for both treatments. The electrochemical oxidation in CC-AS introduced some amounts of amino functional groups with hydrogen as registered by new peak at 401.2 eV. The originally present quaternary nitrogens stayed intact during this treatment.

Table 2
XPS analysis of elemental composition of the plain CC and modified CC (the amount of types of N and O; atomic %, the amounts of types of carbon: relative %).

<table>
<thead>
<tr>
<th>Sample</th>
<th>C 1s %</th>
<th>N 1s %</th>
<th>O 1s %</th>
<th>S 2p %</th>
<th>284.0-284.5</th>
<th>284.8</th>
<th>286.0-288.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>77.7</td>
<td>3.9</td>
<td>15.5</td>
<td>3.0</td>
<td>29.4</td>
<td>39.7</td>
<td>15.1</td>
</tr>
<tr>
<td>CC-AS</td>
<td>56.5</td>
<td>8.2</td>
<td>20.4</td>
<td>5.9</td>
<td>57.6</td>
<td>10.0</td>
<td>16.9</td>
</tr>
<tr>
<td>CC-AN</td>
<td>47.7</td>
<td>10.0</td>
<td>36.1</td>
<td>6.2</td>
<td>39.5</td>
<td>17.1</td>
<td>22.8</td>
</tr>
<tr>
<td>CC-NS</td>
<td>62.9</td>
<td>3.5</td>
<td>27.8</td>
<td>5.8</td>
<td>62.0</td>
<td>9.6</td>
<td>14.3</td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>amines</td>
<td>C-OH</td>
<td>NR4+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC-AS</td>
<td>0.2</td>
<td>0.0</td>
<td>3.7</td>
<td></td>
<td>3.7</td>
<td>17.8</td>
<td>8.0</td>
</tr>
<tr>
<td>CC-AN</td>
<td>0.5</td>
<td>2.2</td>
<td>5.5</td>
<td></td>
<td>10.8</td>
<td>18.8</td>
<td>6.5</td>
</tr>
<tr>
<td>CC-NS</td>
<td>1.1</td>
<td>8.0</td>
<td>0.9</td>
<td></td>
<td>7.6</td>
<td>13.2</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Fig. 3. High resolution C 1s spectra for the plain CC and three modified samples.
contrast, the CC-AN had a complete conversion of quaternary nitro-
gen into hydrogenated ones, and the amount of primary and sec-
dary amines was also higher than CC-AS. The modification in
acids (CC-NS) also caused similar rearrangements, but no additional
nitrogen was introduced.

Analysis of oxygen spectra also pointed to significant changes
in surface chemistry during treatments (Table 2). Acids treatment
(CC-NS) did not change the amount of initially present oxygen types
in the CC but introduced a significant amount of carboxylic oxy-
gen. Ammonium nitrate (CC-AN) and ammonium sulfate (CC-AS)
treatments increased all types of oxygen and most importantly,
introduced carboxylate.

Along with the changes in the surface chemistry, the electro-
chemical oxidation of the anode materials changed the anodes
surface areas. The ECSA analysis of the anodes using cyclic voltam-
metry (Table 3) clearly indicated that the ECSA of the modified CCs
was two orders of magnitude higher after the oxidation process,
which partially explained the higher electrochemical performance
of these anodes than the plain CC. The increase in the ECSA was
proportional to the increase in the amount of O and N (as shown in

<table>
<thead>
<tr>
<th>Sample</th>
<th>ECSA</th>
<th>R (Ω)</th>
</tr>
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<tbody>
<tr>
<td>CC</td>
<td>3.67</td>
<td>13.87</td>
</tr>
<tr>
<td>CC-AS</td>
<td>394.52</td>
<td>12.66</td>
</tr>
<tr>
<td>CC-AN</td>
<td>596.56</td>
<td>13.08</td>
</tr>
<tr>
<td>CC-NS</td>
<td>704.36</td>
<td>13.33</td>
</tr>
</tbody>
</table>

**Table 3**
Electrochemical Accessible Area (ECSA) values and resistance of the anodes materials.
Table 2) as a result of the introduced functional groups and defects during the electrochemical oxidation. In addition, surface modification also affected the material resistance (Table 3). The plain CC had the highest material resistance (13.87Ω) while the modification decreased the resistance with the lowest resistance (12.66Ω) obtained for CC-NS.

3.3. Bacterial attachment on the plain CC and modified CCs.

Previous studies found that the modification procedures altered the surface hydrophilic/hydrophobic ratio and surface chemistry [13], and it was hypothesized that electrochemical oxidation could influence the bacteria-electrode interactions and ultimately vary the extents of bacterial attachment. In addition, our previous study compared the dynamic of biofilm formation and development with “nucleation phenomena”, and proposed two different dynamics (instantaneous and progressive) [13]. The main difference between these two dynamics was caused by the surface properties, in which hydrophilic surfaces accommodated and enhanced the biofilm attachment (instantaneous) while hydrophobic surfaces discouraged the biofilm settlement [13].

Previous studies had shown that biofilm attachment was promoted by the electrode polarization [37]. In this study, the biomass attachment was performed without polarizing the electrode in order to elucidate the attachment phenomena only due to the surface chemistry properties of the materials. In fact, the attached biomass weights (wet weight, Fig. 4A, and dry weight, Fig. 4B) were measured on each electrode materials during 4-week incubation. The CC materials used were hydrophilic and the addition of functional groups to the CC surfaces further enhanced the materials wettability. The trend of “wet” mass variation of the anodes showed a dynamic biofilm attachment that was considered progressive. All the materials investigated showed dramatic increase in mass at the first 2 days of incubation (Fig. 4A), and the modified CCs enhanced the amount of “wet” biomass 10-15% compared to the plain CC. The amount of “dry” biomass showed the same trend (Fig. 4B), with CC-NS having the highest “dry” biomass, CC-AS and CC-AN next, and the plain CC having lowest “dry” biomass. Those results underlined that the surface chemistry modification enhanced biomass attachment and promoted biofilm formation.

3.4. SCMFC electrochemical performance

3.4.1. Discharge curves

The anodes were then installed in SCMFCs with activated carbon air cathodes. Since these SCMFCs had the same volume, inoculum, cathode materials, and operational conditions, variations in power generation in these SCMFCs were mainly caused by differences in the anode materials (plain CC, CC-NS, CC-AS and CC-AN). The voltage trend of batch-mode SCMFCs indicated that the modified CC anodes shortened the start-up period (Fig. 5), and reached stable potential output in the 2nd cycle, while the plain CC voltage steadily increased and the gap with the modified anodes shrank over time. In the 1st cycle (13 days), the increase in potential output followed approximately exponential dependence with time, which was in agreement with the trend of biomass attachment (Fig. 4A) and indicated a correlation between biomass accumulation on anodes and voltage generation. The peak in voltage obtained during the 1st cycle was different for each SCMFC, with a fast peak obtained with CC-NS (after 5 days, 0.37 V) followed by CC-AS (5.5 days, 0.34 V), CC-AN (6 days, 0.32 V), and a slow peak for the plain CC (7 days, 0.26 V) (Fig. 5). The peak potential was related with the wet and dry biomass attached on the anodes, which was demonstrated in later section.

The voltage output of each SCMFC reached stable status after the 2nd cycle (Fig. 5). The duration of the cycles shortened over time from 204-264 hours (cycle 2) to 140-170 hours (cycle 4), which was probably caused by the adaptation of electrogenic bacteria onto anode surfaces [13,37]. Moreover, the SCMFCs with the modified anodes had shorter cycles duration than those with the plain CC, most likely due to the higher biomass attachment (as shown in Fig. 4) and faster consumption of organic substrates. The voltage produced by the SCMFC with CC-NS had the highest output (401-413 mV), while the plain CC anode had the lowest voltage (345-383 mV) (Fig. 5).

3.4.2. Anode polarization behavior

Potentiodynamic polarization curves of the anodes were performed in SCMFCs over time to better understand the effects of surface modification on anode performance. The modification of the anode CC materials fastened SCMFCs start up as indicated by the anode polarization curve (Fig. 6). The electrochemical oxidation with NS enhanced electrodes performance. The current generated from the anodes was low during the 1st cycle, since bacterial biofilms had not been well developed on the electrode.
surface, which could also be confirmed by the higher anode OCP (-170/-370 mV, Fig. 6) than the typical values for strictly anaerobic conditions (-500 mV vs Ag/AgCl, [38]) (Fig. 6 A). With the proceeding of cycles, the current steadily increased as a result of biofilm formation and development on anodes (as shown in Fig. 4). In the 2nd cycle, the anodes polarization curves showed a peak around -380 mV (vs Ag/AgCl), probably due to the production and excretion of electrochemically active metabolic products that may act as endogenous mediators (Fig. 6 B) [39,40]. In the 4th cycle, the peak slightly increased (-350 mV) most likely due to much higher bacteria activity (Fig. 6 C). The modified CC anodes had high current production, with CC-NS having highest performance (150 μA cm⁻² in the 2nd cycle and 183 μA cm⁻² in the 4th cycle), followed by CC-AS and CC-AN, and the plain CC having the lowest current (101 μA cm⁻² in the 2nd cycle 2 and 153 μA cm⁻² in the 4th cycle).

3.4.3. MFCs polarization and power Curves

Polarization curves and power curves of SCMFCs with different anodes were performed in order to evaluate the effect of the anodes modification on MFC performance (Fig. 7). The maximum power densities for SCMFCs increased over time, which was consistent with anode polarization curves (as shown in Fig. 6). The cathode polarization curves did not change over time (data not shown), indicating that the increase in SCMCs output was mainly caused by the anode variations over time. The highest power densities of 17.2 μW cm⁻² (2nd cycle), 18.5 μW cm⁻² (3rd cycle) and 20.1 μW cm⁻² (4th cycle) were achieved with the SCMFCs with CC-NS. The SCMFCs with CC-AS and CC-AN anodes had slightly lower power densities, 15.6-19.7 μW cm⁻² and 15.1-19.2 μW cm⁻², respectively. The SCMFCs with plain CC had the lowest power densities (13.5-17.5 μW cm⁻²). It should be pointed out that the advantage of anode modification over plain CC decreased over time (Fig. 4), indicating that the surface property variation enhanced the initial bacterial attachment (Fig. 4) and shortened the start-up duration (Fig. 5), but once the anode surface was covered with bacteria, the surface influence gradually diminished over time. Actually, CC-NS had 25% higher power density than the plain CC in the 2nd cycle. The difference decreased to roughly 15% in the 3rd cycle, and was only 10% in the 4th cycle. The advantage decreased over four cycles, despite the faster startup of the modified anodes.

3.5. Principal Components Analysis (PCA)

The electrochemical measurement results (maximum voltage (Vmax), maximum anodic current (i_max), maximum power (Pmax), and anode resistance), the XPS analysis data, and anode surface property results (ECSA, wet biomass, and dry biomass) were comprehensively processed through PCA to determine the key factors defining the anodes behavior. The data were processed separately for each SCMFC cycle and the corresponding biplots were built (Fig. 8). The highest differences in anodes performance were observed during the initial start-up period (1st cycle) of SCMFC operation. The PCA biplot for the 1st cycle showed that the main parameters influencing the anodes performance were the amount of aliphatic and unsaturated carbons (C=C) and the dry and wet biomass attached to the electrodes surface (Fig. 8 A). The increased amount of graphitic and aromatic carbons decreased material...
resistance and therefore, increased anode output. In contrast, the higher amount of saturated carbons increased electrode resistance and lowered anode performance. It is well known that the current produced by MFCs is generally proportional to the amount of bacteria immobilized on electrode surfaces [41]. Therefore, it is not surprising that the higher values of wet and dry biomass are important parameters for higher voltage output. The presence of carboxylic oxygens (COO-) also had a positive effect most likely due to the increased hydrophilicity of the materials, which explained the higher bacteria attachment on those materials [13,24].

The PCA biplots for the 2nd, 3rd, and 4th cycles followed the same trend (Fig. 8.B-8.D). PC 1 separated the samples into the modified anodes and the plain CC. The modification procedure had positive effect on the anodes performance due to higher amount of unsaturated carbon (C = C), oxidized carbon, higher ECSA, higher bacteria attachment, while the negative effects were the higher materials resistance and higher amount of saturated carbon (C - C). Afterwards, the two parameters were intra-correlated, showing a positive natural logarithmic correlation between the resistance and the amount of C-C bonds (Fig. 9.A), while a negative linear correlation between the amount of C - C bonds and the electrodes resistance was observed (Fig. 9.B), indicating an increase in unsaturated carbon bonds is an effective approach to lower material resistances.

The PCA analysis revealed that there was a correlation between the voltage generation of SCMFCs and the anode resistance. The importance of the anode resistance for the electrochemical behavior of SCMFCs was confirmed through dependence analysis (Fig. 10.A). The sensitivity of the voltage dependence on the electrode resistance decreased over cycles (1st to 4th) indicating that this parameter played significant role at the start-up (1st cycle) of SCMFCs and other parameters might start to take over as the key factors after the 1st cycle. Linear correlation for the maximum current and power densities of SCMFCs (Fig. 10.B and 10.C) showed that they increased over cycles (2nd to 4th) and the slope of the linear dependence with the resistance remained relatively constant, which indicated consistent influence of anode resistance to SCMFCs performance.

The second most important parameter was the biomass attached to the anode surface. Since the dry biomass was correlated with the wet biomass, a linear correlation between the maximum SCMFC voltage and both the dry and wet biomass was demonstrated (Fig. 11). The change in the biomass content at the 1st cycle was more dynamic than at other cycles, and consequently, small increases in the biomass amount led to a detectable increase in voltage generation in the startup period.

There was no clear correlation between the anode ECSAs and the SCMFCs performance, demonstrating that this parameter did not play a major role in the electrochemically oxidized anodes tested in this study. Greater influences might be caused by the surface chemistry modification and the attached functional groups rather than the ECSA. Opposite to expectation [11,42], the lower amounts of quaternary nitrogen did not decrease the anode performance.

Based on PCA and the plotted correlations, three parameters were important for enhancing SCMFC operation: i) higher amount of C = C bonds, ii) lower anodes resistance, and iii) more attached biomass. Electrochemical oxidation can effectively enhance anode properties. The differences in the anode performance were mainly
caused by carbon bonds, and as a result, altered material resistance and influenced hydrophilic/hydrophobic ratio, which determined the amount of biomass attached to the anodes.

4. Conclusions

This study thoroughly investigated electrochemical oxidation using nitric acid + sulfuric acid (CC-NS), ammonium nitrate (CC-AN), and ammonium sulfate (CC-AS) as methods for anode modification to enhance the startup and power generation of MFCs. Chemical and electrochemical treatment of CC anodes were compared in terms of surface chemistry. There were four major conclusions from the batch-mode SCMF tests.

First, the anode surface modification increased the amounts of nitrogen, sulfur, oxygen, as well as the amounts of unsaturated and oxidized carbon. The modification procedure decreased anodes resistance and promoted higher and faster bacteria adhesion and biofilm formation.

Second, the modified anodes had higher ECSA. The start-up period of the SCMFs with the modified anodes was shorter than those with the plain CC anode.

Third, the power densities and current densities increased over time with higher values generated by the modified anodes following the order: CC-NS > CC-AS > CC-AN > CC. The highest power density of SCMFs with CC-NS anode was 20.1 μW cm⁻².

Forth, the comprehensive PCA analysis clearly showed the positive effect of the anode modification on SCMF operation in the start-up duration, but this advantage diminished over time, indicating that the influence of other parameters, most likely bacteria activity and speciation, started to play major roles after biomass attached on the anode surfaces.

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References


