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14. ABSTRACT

The overall goal of this project is to determine how electrode surface chemistry can be rationally designed to decrease resistance and increase power density in microbial fuel cells (MFCs). We now have 9 peer-reviewed journal publications from this project on this topic, with more in preparation. In this project period we applied electrochemical oxidation of activated carbon anodes to demonstrate that increasing roughness, oxygen containing groups, and hydrophilicity all tended to improve anode performance. Novel alkane-thiol self-assembled monolayer

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Report Title

Final Report: Rational Design of Anode Surface Chemistry in Microbial Fuel Cells for Improved Exoelectrogen Attachment and Electron Transfer

ABSTRACT

The overall goal of this project is to determine how electrode surface chemistry can be rationally designed to decrease resistance and increase power density in microbial fuel cells (MFCs). We now have 9 peer-reviewed journal publications from this project on this topic, with more in preparation. In this project period we applied electrochemical oxidation of activated carbon anodes to demonstrate that increasing roughness, oxygen containing groups, and hydrophilicity all tended to improve anode performance. Novel alkane-thiol self-assembled monolayer (SAM) anodes were also tested to show that more hydrophilic, higher surface energy SAMs (trimethylamine and carboxylic) provided the best performance as anodes. 454 pyrosequencing demonstrated that beta and gamma Proteobacteria were dominant on these anodes. Custom activated carbon cathodes were constructed with varying characteristics to show that cathodes with largest amounts of carbon oxides and larger pores tended to have the best performance. Non-Pt group metals were also evaluated as cathode materials, and it was determined that materials derived from iron salt and aminoantipyrine (Fe-AAPyr) or mebendazole (Fe-MBZ) provided superior or comparable performance to Pt at higher pH values. We also initiated a novel set of experiments evaluating pili, motility, and electron transfer using Shewanella mutants.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
08/24/2014	1.00	Baitao Li, Jun Zhou, Xiuxiu Zhou, Xiujun Wang, Baikun Li, Carlo Santoro, Matteo Grattieri, Sofia Babanova, Kateryna Artyushkova, Plamen Atanassov, Andrew J. Schuler. Surface Modification of Microbial Fuel Cells Anodes: Approaches to Practical Design, Electrochimica Acta, (07 2014): 116. doi: 10.1016/j.electacta.2014.04.136
08/24/2014	2.00	Carlo Santoro, Kateryna Artyushkova, Sofia Babanova, Plamen Atanassov, Ioannis Ieropoulos, Matteo Grattieri, Pierangela Cristiani, Stefano Trasatti, Baikun Li, Andrew J. Schuler. Parameters characterization and optimization of activated carbon (AC) cathodes for microbial fuel cell application, Bioresource Technology, (07 2014): 54. doi: 10.1016/j.biortech.2014.03.091
08/28/2014	3.00	J. N. Roy, K. E. Garcia, H. R. Luckarift, A. Falase, J. Cornejo, S. Babanova, A. J. Schuler, G. R. Johnson, P. B. Atanassov. Applied Electrode Potential Leads to Shewanella oneidensis MR-1 Biofilms Engaged in Direct Electron Transfer, Journal of the Electrochemical Society, (10 2013): 866. doi: 10.1149/2.001401jes
12/17/2015	8.00	Kateryna Artyushkova, Jose A. Cornejo, Linnea K. Ista, Sofia Babanova, Carlo Santoro, Plamen Atanassov, Andrew J. Schuler. Relationship between surface chemistry, biofilm structure, and electron transfer in Shewanella anodes, Biointerphases, (03 2015): 19013. doi: 10.1116/1.4913783
12/18/2015	14.00	Carlo Santoro, Sofia Babanova, Kateryna Artyushkova, Plamen Atanassov, John Greenman, Pierangela Cristiani, Stefano Trasatti, Andrew J. Schuler, Baikun Li, Ioannis Ieropoulos. The effects of wastewater types on power generation and phosphorus removal of microbial fuel cells (MFCs) with activated carbon (AC) cathodes, International Journal of Hydrogen Energy, (12 2014): 21796. doi: 10.1016/j.ijhydene.2014.09.167
12/18/2015	9.00	Carlo Santoro, Sofia Babanova, Benjamin Erable, Andrew Schuler, Plamen Atanassov. Bilirubin oxidase based enzymatic air-breathing cathode: Operation under pristine and contaminated conditions, Bioelectrochemistry, (04 2016): 1. doi: 10.1016/j.bioelechem.2015.10.005
12/18/2015	12.00	J. A. Cornejo, C. Lopez, S. Babanova, C. Santoro, K. Artyushkova, L. Ista, A. J. Schuler, P. Atanassov. Surface Modification for Enhanced Biofilm Formation and Electron Transport in Shewanella Anodes, Journal of the Electrochemical Society, (06 2015): 597. doi: 10.1149/2.0271509jes
12/18/2015	13.00	Alexey Serov, Claudia W. Narvaez Villarrubia, Sarah Stariha, Sofia Babanova, Andrew J. Schuler, Carlo Santoro, Kateryna Artyushkova, Plamen Atanassov. Double-Chamber Microbial Fuel Cell with a Non-Platinum-Group Metal Fe-N-C Cathode Catalyst, ChemSusChem, (03 2015): 828. doi: 10.1002/cssc.201402570

TOTAL: **8**

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received	Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

J.A. Cornejo, K. Artyushkova, C. Santoro, S. Babanova, L.K. Ista, A.J. Schuler, P. Atanassov. Microbial Anode Characterization for Electron Transfer and Biofilm Morphology Studies,37th Symposium on Applied Surface Analysis, AVS Rocky Mountain Chapter. Golden, CO, June 2015.

J.A. Cornejo, K. Artyushkova, C. Santoro, S. Babanova, L.K. Ista, A.J. Schuler, P. Atanassov. Surface Chemistry Enhanced Microbial Electrodes: Biofilm Modeling and Characterization. 249th American Chemical Society National Meeting. Denver, CO, March 2015.

L.K Ista. Interfacial interactions in bacterial physiology: motion, biofilm formation and charge transfer. Bioelectron Transfer Meeting (sponsored by ARL). 15-16th September 2015, Research Triangle Park, NC. Invited.

A.J. Schuler. BES's for removal of critical contaminants ♂by mixed culture biofilms: ♂Opportunities in electrode and reactor design♂. Bioelectron Transfer Meeting (sponsored by ARL). 15-16th September 2015, Research Triangle Park, NC. Invited.
L.K. Ista The interfacial processes of biofilms. Presentation to the Chemistry Department at New Mexico Tech. 13 November, 2015. Socorro, NM. Invited.

Number of Presentations: 2.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received		Paper
08/30/2014	5.00	Carlo Santoro, Kateryna Artyushkova , Sofia Babanova, Andrew Schuler, Plamen Atanassova. Surface-to-property characterization of activate carbon (AC) cathodes in biofuel cell, Surface Analysis Meeting, 36th Symposium on Applied Surface Analysis. 02-JUN-14, . : ,
08/30/2014	6.00	Linnea K Ista, , Jose A Cornejo, , Sofia Babanova, , Plamen B Atanassov , Andrew J Schuler. From Twitching to Nanowires Toward a Holistic Understanding of Motility Structures and Extracellular Electron Transfer, 225th Electrochemical Society Meeting. 11-MAY-14, . : ,
08/31/2014	7.00	Jose A. Cornejo , Carlo Santoro, Claudia Narváez Villarrubia , Kateryna Artyushkova , Sofia Babanova , Linnea Ista , Plamen Atanassov. Surface Modification of Carbon Felt Electrodes for Enhanced Biofilm Formation in Microbial Fuel Cells, 225th ECS Meeting, Orlando, Florida. 11-MAY-14, . : ,
12/21/2015	15.00	Jose A. Cornejo, , Carlo Santoro, , Claudia Narváez Villarrubia, , Kateryna Artyushkova, , Sofia Babanova, , Linnea Ista , Plamen Atanassov. Surface Modification of Carbon Felt Electrodes for Enhanced Biofilm Formation in Microbial Fuel Cells, Electrochemical Society National Meeting. 01-MAY-14, . : ,

TOTAL: **4**

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received		Paper
12/18/2015	11.00	Justin P Jahnke, James J Sumner, Andrew J Schuler, Jose A Cornejo , Plamen Atanassov , Linnea K Ista. Conjugated gold nanoparticles as a tool for probing the bacterial cell envelope: the case of Shewanella oneidensis MR-1, Biointerphases (09 2015)

TOTAL: **1**

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Jeremiah Houghton	1.00	
Jose Cornejo	0.42	
FTE Equivalent:	**1.42**	
Total Number:	**2**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Andrew Schuler	0.08	
Linnea Ista	0.52	No
FTE Equivalent:	**0.60**	
Total Number:	**2**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
John Matteson	0.04	
Erika Hernandez Hernandez	0.08	
FTE Equivalent:	**0.12**	
Total Number:	**2**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 2.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:...... 2.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:...... 2.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):...... 2.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:...... 1.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME	
Jose Cornejo	
Total Number:	**1**

Names of other research staff

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See Attachment.

Technology Transfer

We have continued our work with Dr. James Sumner at the Army Research Lab on developing nanoparticle probes to interrogate bacterial cel membrane characteristics. Dr Sumner is interested in the use of conjugated gold nanoparticles to specifically probe the organization of lipid islands on the surface of Shewanella oneidnesis. Using the same set of conjugated gold nanoparticles we employ (conjugations include amine, methyl and carboxylic acid), Dr. Sumner probed the surface of S. oneidensis treated with a range of concentrations of an amphiphilic distyrylstilbene oligoelectrolyte (DSSN+), which has been previously shown to integrate into membranes and facilitate electron transport across them in Saccharomyces cervisiae Garner et al., 2010. Dr. Sumner confirmed our results that carboxylic acid conjugated gold nanoparticles attached preferentially to the subterminal region of S. oneidensis but also Roy et al., 2012 showed that 100 nM DSSN+ disrupted this specific labeling of the cells by carboxylic acid conjugated nanoparticles. This is approximately the same concentration at which DSSN+ confers external electron transport in yeast. We are continuing this collaboration with Dr. Sumner and his group to examine the effect of mutations in cells structures thought to be involved in S. oneidensis nanowire production and external electron transfer (pili, the outer membrane cytochrome C complex) on both DSSN+ interaction and conjugated nanoparticle interactions. This work resulted in the recently accepted paper Jahnke et al. accepted.

We have also obtained a second year of funding from the Defense Threat Reduction Agency for our “bacterial chromatography” project. Although this proposal is independent of ARO work, the surface motility experiments on S. oneidensis obtained herein were deemed sufficient to warrant investigation. Linnea Ista, a co-PI on both grants, will head this investigation.

References

Garner, L.E., Park, J., Dyar, S.M., Chworos, A., Sumner, J.J. and Bazan, G., 2010. Modification of the optoelectronic properties of membranes via insertion of amphiphilic phenylenevinylene oligoelectrolytes. J Am Chem Soc 132, 10042-10052.

Justin P Jahnke, James J Sumner, Andrew J Schuler, Jose A Cornejo , Plamen Atanassov , Linnea K Ista. Conjugated gold nanoparticles as a tool for probing the bacterial cell envelope: the case of Shewanella oneidensis MR-1. Accepted to Biointerphases.

Roy, J., Luckarift, H., Lau, C., Falase, A., Garcia, K., Ista, L., Chellamuthu, P., Ramasamy, R., Gadhamshetty, V., Wanger, G., Gorby, Y., Nealson, K., Bretschger, O., Johnson, G., Atanassov. P., 2012. A study of the flavin response by Shewanella cultures in carbon-limited environments. RSC Advances 2, 10020–10027.

SCIENTIFIC PROGRESS AND ACCOMPLISHMENTS

Final Report
Rational Design of Anode Surface Chemistry in Microbial Fuel Cells for Improved Exoelectrogen Attachment and Electron Transfer

Army Research Office Proposal Number: 60173-EV-H
Agreement Number: W911NF-12-1-0208

A.J. Schuler (PI) and L. Ista (co-PI)

December 21, 2015

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FORWARD

This final report describes the the scientific progress and accomplishments during the second reporting period of this project (8/1/14 to 7/31/15). The project has now produced 9 peer-reviewed journal papers, with several more in preparation. This project period focused on Phases 2 *"Determine anode surface chemistry effects on function, performance, and biofilm structure in MFCs with mixed culture biofilms"* and *3 "Determine proton transport and pH effects on biofilm structure and develop a corresponding novel hybrid anode design"*, with reduced focus on Phase 3 reflecting the reduced budget as described in the Revised Scope of Work (September 30, 2011). Progress during this project period included advances on several fronts, including advanced in anode design (analyzing modifications of carbon cloth and application of self-assembled monolayers [SAMs]) and cathode design (modifications of activated carbon, analysis of the effects of different wastewater types on activated carbon, utilization of non-platinum group metals), as well as evaluation of surface motility an electron transfer in *Shewanella oneidensis.*

STATEMENT OF THE PROBLEM STUDIED

Microbial fuel cells (MFCs) rely on the activity of exoelectrogenic bacteria, which are able to generate energy by coupling the reduction of organic substrates to the delivery of electrons to an anode. The interactions between exoelectrogens and electrode (anode and cathode) surfaces are not well understood, and so the interface between the electrode surfaces and bacterial cells, through which electrons must pass, is an excellent target for improving the performance of MFCs. *The overall objective of this project is to determine how anode surface chemistry can be rationally designed to decrease resistance and increase power density in MFCs*.

SUMMARY OF RESULTS

Surface modifications of microbial fuel cell anodes for improved performance

Modification of carbon cloth anodes.

(Related publication: Li, et al. 2014.)

Modification of carbon cloth anodes by electrochemical oxidation was explored as an approach to enhance the startup and improve the performance of 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 the ratio of saturated/unsaturated carbon on the surface and consequently, a decrease in electrode resistance was observed (Li et al., 2014). A linear dependence between the MFCs operational characteristics and the anodes resistance indicated the dominating role of this parameter.

Electrochemical modification led to an increase in roughness that was higher than by chemical modification alone, and enhanced the bacterial attachment (wet and dry biomass) and biofilm formation compared to the control electrode (unmodified carbon cloth). CC-NS, CC-AS and CC-AN anodes accelerated the start up period of the MFCs and demonstrated higher current and power relative to the unmodified CC, with the CC-NS modification providing the highest current generation (Figure 1).

Principal Component 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 (Figure 2). Oxygen-containing groups on the surface were probably responsible for higher hydrophillicity of the surface that increased the bacteria attachment on the electrode and consequently improved the performance.

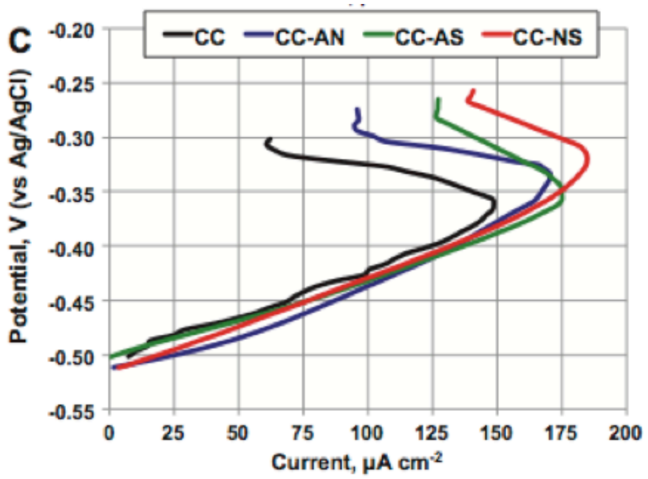

Figure 1. Effects of carbon cloth anode modification on current generation in mixed culture MFCs. Modification with nitric acid and sulfuric acid (CC-NS) yielded the highest levels of performance.

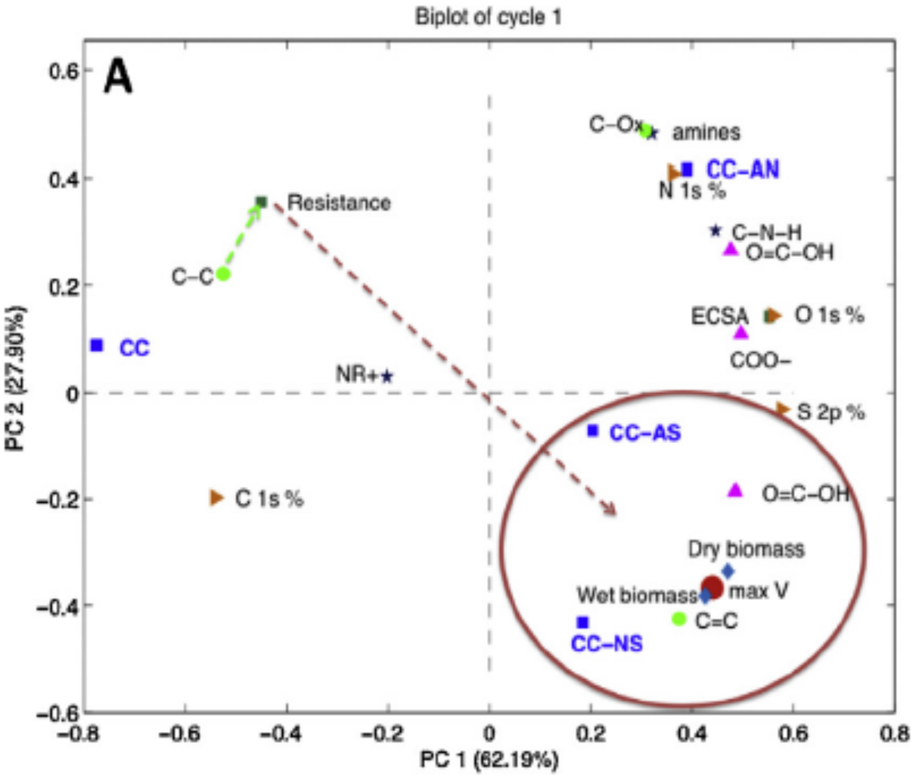

Figure 2. PCA relative of performances and surface chemistry of the samples of interest during the first working cycle.

Utilization of SAMs to evaluate anode surface chemistry effects in single chamber MFCs with mixed culture inoculum.

(Related publication: Santoro et al, 2015a)

Self-assembled monolayers (SAMs) modified gold anodes were used as anode electrode in single chamber microbial fuel cells (MFC) for organic removal and electricity generation. Hydrophilic (-$N(CH_3)_3^+$, -OH, -COOH) and hydrophobic (-CH_3) SAMs were 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. The main advantage of SAMs compared to other carbonaceous materials is that the surface is very uniform and roughness can be considered negligible. In fact, 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.

Single chamber MFCs (SCMFCs) were inoculated with activated sludge and phosphate buffer (0.1M) in 50% volume respectively. Voltage was recorded every 25 minutes and power curves were obtained at the end of the experiments after 45 days. MFCs fitted with -$N(CH_3)_3^+$-SAM anodes showed a rapid potential increase (after 15 d), followed by -COOH (17 d), -CH_3 (17 d) and -OH (20 d) (Figure 3). The -$N(CH_3)_3^+$-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. 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. Despite the different start-up times for each of our well performing functional groups, the $N(CH_3)_3^+$-, 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. However, the potential of CH_3-MFC increased slowly over time and stabilized after 40 d to only 0.06 V (Figure 3).

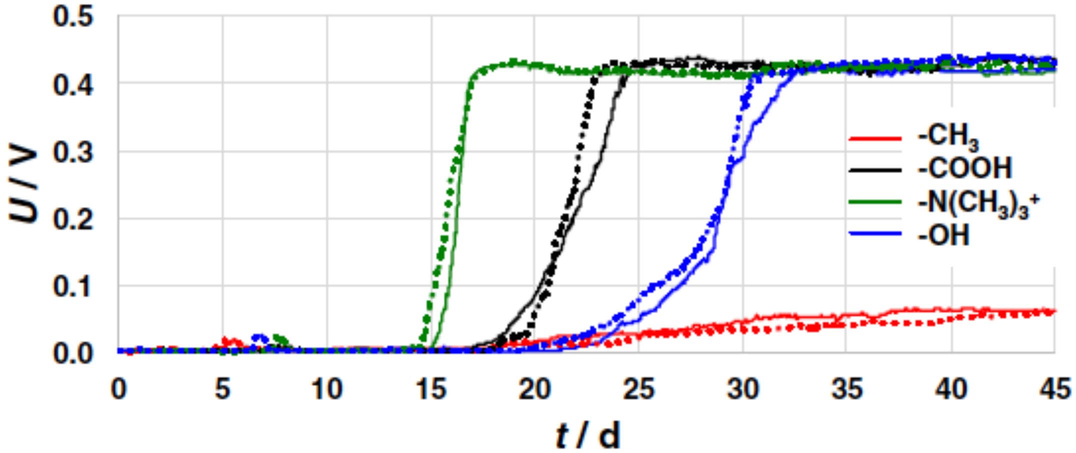

Figure 3. Voltage over 1000 ohm recorded for 45 days for four SAM-modified anodes.

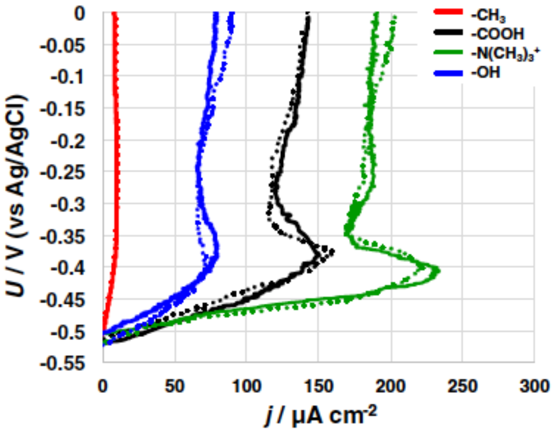

Figure 4. Anode polarization curves after 45 days.

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 (Figure 4). Despite the fact that the voltage achieved after day 45 of MFC polarization was approximately the same for $N(CH_3)_3^+$-, 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 $-N(CH_3)_3^+$ modified anodes at approximately −0.40 V vs. Ag/AgCl. The $N(CH_3)_3^+$- 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 $-N(CH_3)_3^+$ systems showed the highest performance followed by -COOH, -OH and $-CH_3$, respectively (Figure 4).

After electrochemical tests, biofilm from the different SAMs was analyzed using pyrosequencing. Pyrosequencing of 16S rRNA gene amplicons derived from the anode-associated biomass shows diverse bacterial populations at the different anode modified surfaces (Figure 5). Our findings show that the enrichment of electroactive biofilms on SAMs-electrodes operated in independent reactors yields different taxonomic compositions. A phylum-level analysis indicated that Proteobacteria comprised between 32% and 37% of the anode-associated biofilms. The $-N(CH_3)_3^+$ modified surface featured Proteobacteria in a relative abundance of 36%, the -COOH had 37% relative abundance, and -OH had 32% relative abundance. The $-N(CH_3)_3^+$ bulk solution (labeled as WW in Figure 5) also showed 37% relative abundance of Proteobacteria. However, Proteobacteria only accounted for approximately 6% of the biofilm associated with the $-CH_3$ 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 CH_3-modified electrode had the highest relative abundance of Firmicutes at 34%.

The phylum Bacteroidetes was present in all of the communities with relative community abundance of 22% ($-N(CH_3)_3^+$), 12% (-COOH), 6% (-OH), 13% ($-CH_3$) and 15% (WW). The $-CH_3$ modified anode also had a relatively high abundance of phyla Lentisphaerae (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, ε-Proteobacteria and α-Proteobacteria. Class δ-Proteobacteria had the highest relative abundance in the -N$(CH_3)_3^+$, -COOH and -OH anode biofilms, and very low relative abundance in the\\CH3 anode-associated biofilm and the -N$(CH_3)_3^+$ bulk solution (WW). Several members of class δ-Proteobacteria and γ-Proteobacteria have been reported as electrochemically active microbes in bioelectrochemical systems. The high relative abundance of class δ-Proteobacteria in all of the biofilms that showed good current and power output (-N$(CH_3)_3^+$, -COOH and -OH), suggests that these community members were active in electron transfer to the anode surfaces boosting up the current generated.

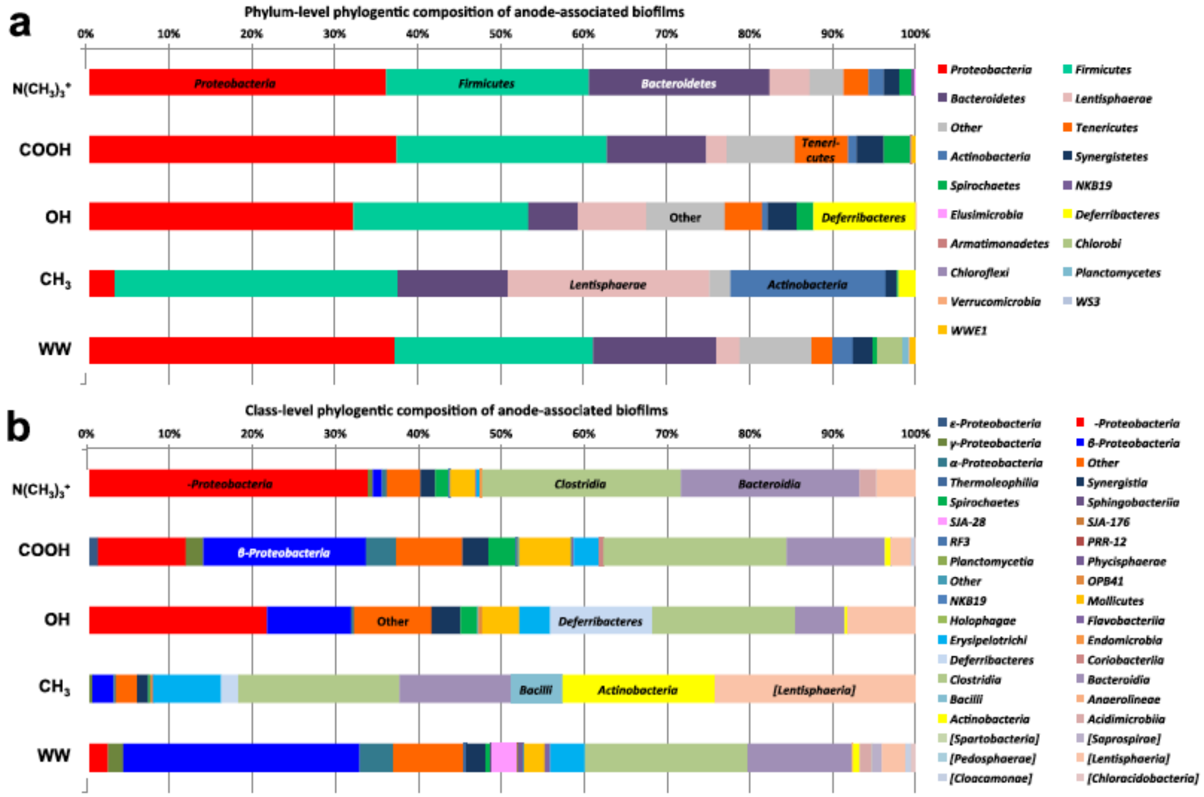

Figure 5. Phylum-level taxonomic distribution of 16S rRNA community profile within anode electrochemically active biofilm (a) and class-level taxonomic profile of 16S rRNA community profile within anode electrochemically active biofilm (b).

PCA was applied to better understand the relationships between the operational and surface characteristics, the samples, and performance. These were separated into three groups (Figure 6). The first group contains the -N$(CH_3)_3$ 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$(CH_3)_3$ anode and MFC, respectively, were the positive charge of the surface groups and the presence of higher amounts of bacteria from the classes of δ-Proteobacteria and Clostridia. So far, it has been considered that a high relative abundance of δ-Proteobacteria in the bacterial biofilm 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 were characterized with high hydrophilicity and lower relative abundance of δ-Proteobacteria and Clostridia. 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.

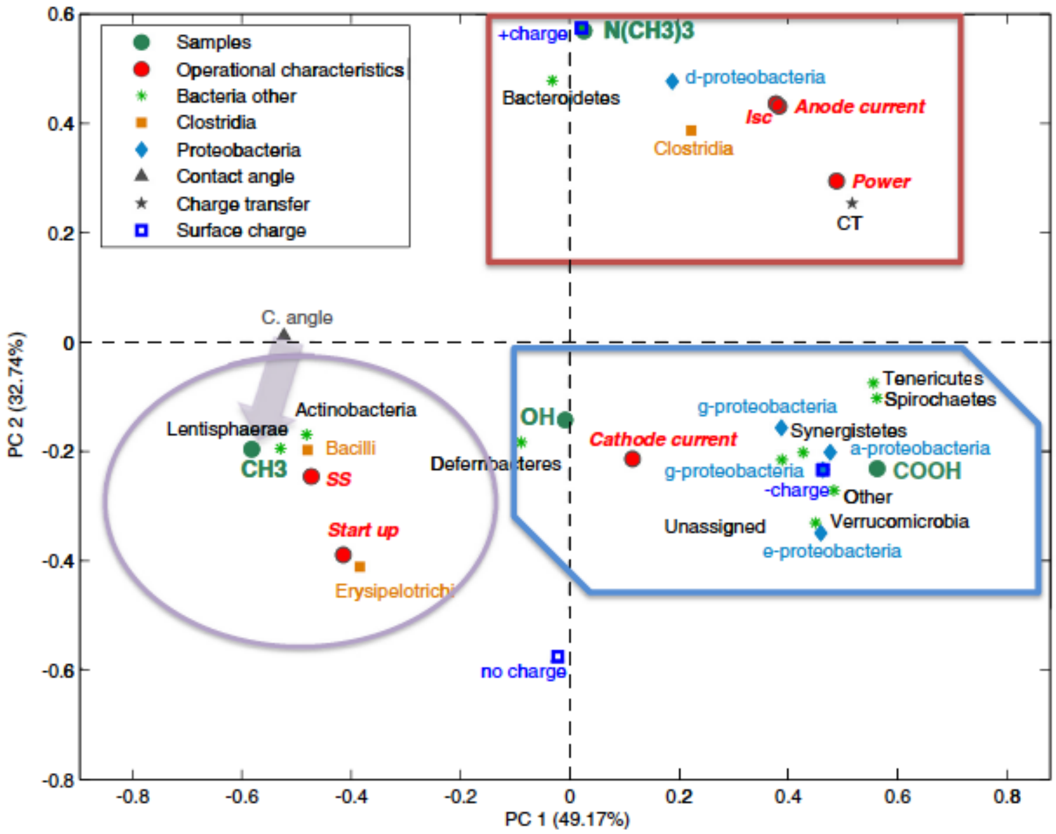

Figure 6. PCA biplot where scores (samples) and loadings (variables) for MFCs with different anode surface chemistries are plotted on the first two components, where Isc is the short circuit current of MFCs, SS time is steady-state time, C angle is contact angle, anode and cathode current and overall power generation.

Surface modifications of microbial fuel cell cathodes for improved performance

Modifications of activated carbon cathodes.

(Related publication: Santoro et al. 2014a.)

Activated carbon was also evaluated for use in MFC cathodes, with varied temperature and pressure during preparation of the activated carbon as experimental variables. The best performance was achieved when a pressure of 1400 psi was applied (175 to 3500 psi was evaluated) followed by heat treatment of 150–200 °C (25 to 340 °C was evaluated) for 1 h

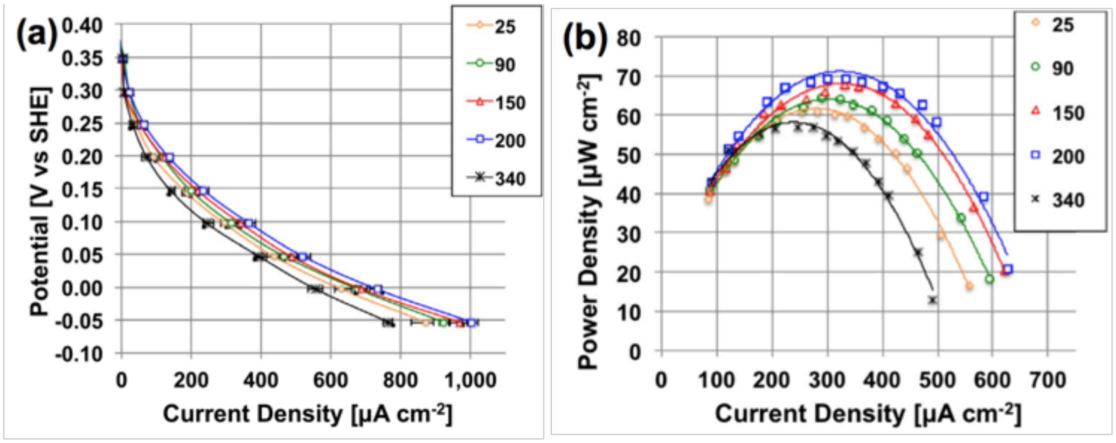

Figure 7. (a) Cathode polarization curves and (b) single chamber MFC power curves with activated carbon cathods prepared at different temperatures shown in legends (°C) (Santoro et al., 2014).

(Figure 7). The highest power density was observed with the cathodes treated at 200°C (70 µWcm^{-2}) and comparable power was reached when the cathode was treated at 150°C (69 µWcm^{-2}). The gained power decreases to 65 and 60 µWcm^{-2} with cathode treated at a temperature of 90°C and ambient temperature (25°C), respectively. The lowest performance (55 ±l µWcm^{-2}) was recorded with temperature treatment close to the PTFE melting point (343°C).

PTFE is a common binding material used in the design of gas diffusion oxygen reducing electrodes, due to its hydrophobic properties that facilitate the oxygen permeability and diffusion. PTFE improves porosity and enhances the air permeability. According to the manufacturer specifications (Sigma Aldrich) the glass-transition temperature for PTFE used in this study was 131°C, which indicates that the activity of the cathode increases after the glass transition state of the polymer and significantly decreases after its melting point (327 °C). The phase transition of PTFE can vary the interaction between PTFE and AC particles when the cathodes are prepared. The increase in temperature treatment from ambient temperature (25°C) to 90°C removes the water from the PTFE emulsion, and a better electrical contact among the AC particles could be created. A further increase to 150–200°C leads to vitrification of PTFE and the slight expansion of the PTFE crystals probably enhances the complete coverage with AC particles. On the other hand, the heat treatment of the AC/PTFE at 340°C leads to the complete disintegration of the structure of PTFE, and the AC particles become covered with PTFE and thus substantially losses the porosity and the conductivity of the material.

Data of current density and resistance values for each carbon treated at different temperature were combined along with roughness and skewness data and XPS data for statistical structure-to-property correlations (Figure 8). Before heat treatment, the cathodes have a higher amount of oxidized tetrafluoroethylene with higher overall roughness, high similarity, and mesocale pores (10 nm – 1 um). With the treatment temperature increasing, the amount of fully fluorinated carbons increases while the amount of surface oxides decreases. For the sample with the best performance, the largest pores were dominant along with a higher amount of carbon oxides. These samples also have the highest charge transfer resistance, which was closely correlated with high amount of fully fluorinated carbons. Further heat treatment changes the composition by introducing a larger amount of partially fluorinated carbons and graphitic carbon. It also causes an increase of intermediate pores and uniformity on macroscale. The value of similarity on a scale between 10 nm and 1 lm embraces the values of similarity on a scale between 10 and 800 nm. Negative correlation between these two parameters points to the difference in the similarity on the scale between 800 nm and 1 um, which was the highest for pristine carbon. The highest current was observed for samples with largest amounts of carbon oxides, which was inversely related to the charge transfer resistance for cathodes with the highest amount of fully fluorinated carbons. Higher amounts of large pores were present at lower resistances and higher current densities.

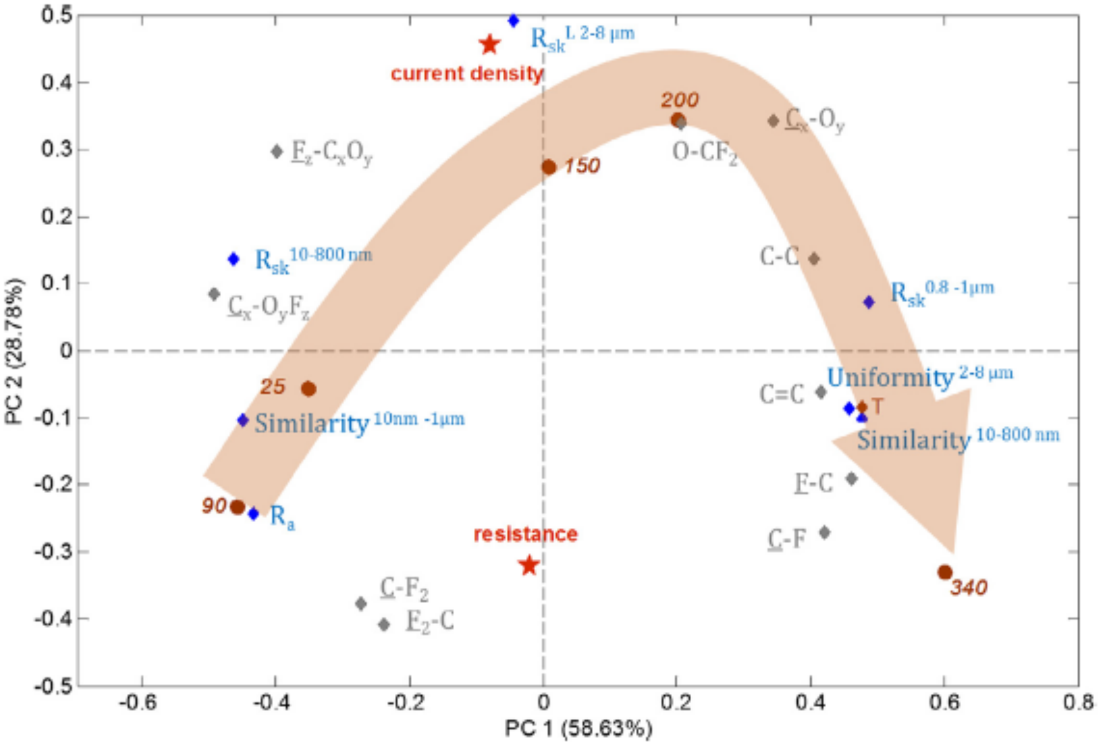

Figure 8. PCA plot of performance, surface chemistry and surface morphology of the AC based cathodes.

Activated carbon cathodes in MFC fed with different wastewaters.

(Related publication: Santoro et al. 2014b)

Cost-effective activated carbon (AC) material was investigated for the development of gas diffusion cathode employed in membraneless single chamber microbial fuel cells (SCMFCs) treating different feeding solutions. The electrocatalytic activity of AC cathodes was monitored in synthetic wastewater containing phosphate buffer saline solution and sodium acetate (PBS and NaOAc) and compared with several types of wastewaters (e.g. fresh urine (FU), hydrolysed urine (HU), wastewater and sodium acetate (WW + NaOAc) and raw wastewater (WW)). Synthetic wastewater (PBS) outperformed real wastewater in terms of cathode current and SCMFC power output (Figure 9). The results showed that the SCMFCs fed with urine generated 3 times higher power densities than those with raw WW and 25% higher than those with WW + NaOAc, most likely due to the high amount of electrons generated from organic substances.

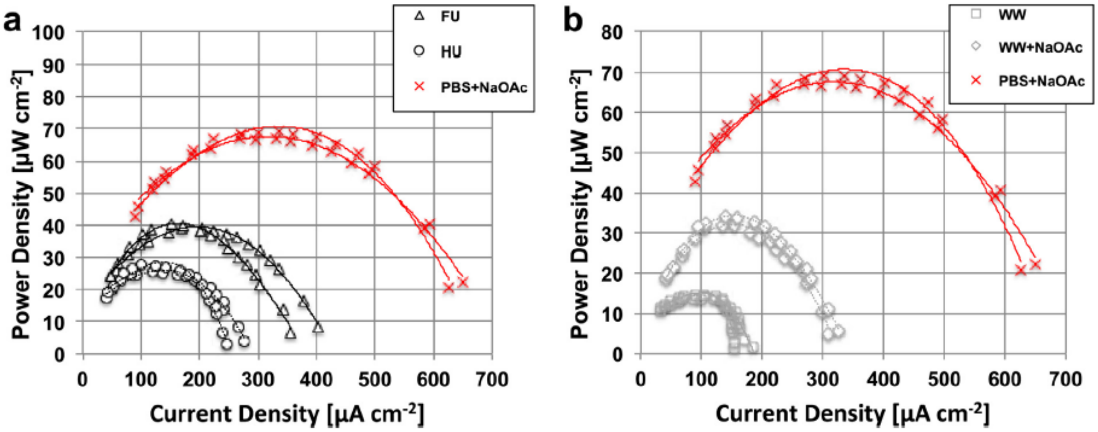

Figure 9. Comparison of SCMFCs Power Curves with AC cathodes and different feeding solutions: synthetic wastewater (PBS) and real wastewaters (FU and HU) (a) and WW and WW + NaOAc (b).

Utilization of non platinum groups metal as cathode catalyst in double chamber and single chamber MFC

(Related publications: Santoro et al. 2015b, Santoro et al., accepted.)

Non-Pt-group metal (non-PGM) materials based on transition metal–nitrogen–carbon (Me–N–C) and derived from iron salt and aminoantipyrine (Fe-AAPyr) of mebendazole (Fe-MBZ) were studied for the first time as cathode catalysts in double chamber microbial fuel cells (DCMFCs) (Figure 10). The pH value of the cathode chamber was varied from 6 to 11 to elucidate the activity of those catalysts in acidic to basic conditions.

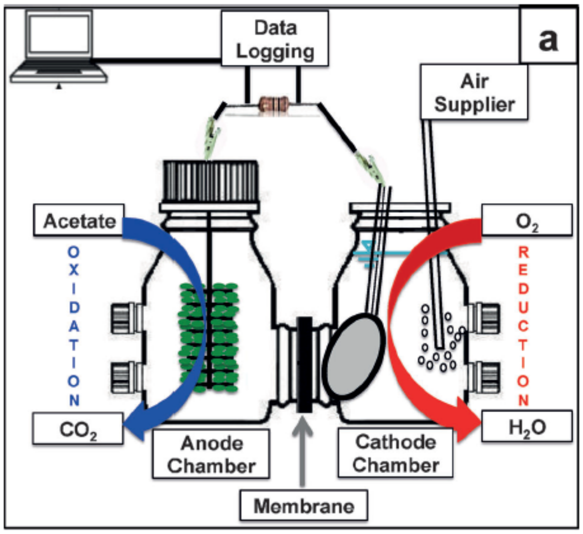

Figure 10. Configuration double chamber MFC.

The Fe-AAPyr- and Fe-MBZ-based cathodes were compared to a Pt-based cathode used as a baseline. Pt cathodes performed better at pH 6–7.5 and had similar performances at pH 9 and a substantially lower performance at pH 11 at which Fe-AAPyr and Fe-MBZ demonstrated their best electrocatalytic activity (Figure 11). The power density achieved with Pt constantly decreased from 94–99 uWcm^{-2} at pH 6 to 55–57 uWcm^{-2} at pH 11. In contrast, the power densities of DCMFCs using Fe-AAPyr and Fe-MBZ were 61–68 uWcm^{-2} at pH 6, decreased to 51–58 uWcm^{-2} at pH 7.5, increased to 65–75 uWcm^{-2} at pH 9, and the highest power density was achieved at pH 11 (68–80 uWcm^{-2}). Non-PGM cathode catalysts can be manufactured at the fraction of the cost of the Pt-based ones. The higher performance and lower cost indicates that non-PGM catalysts may be a viable materials choice in large-scale microbial fuel cells (Figure 11).

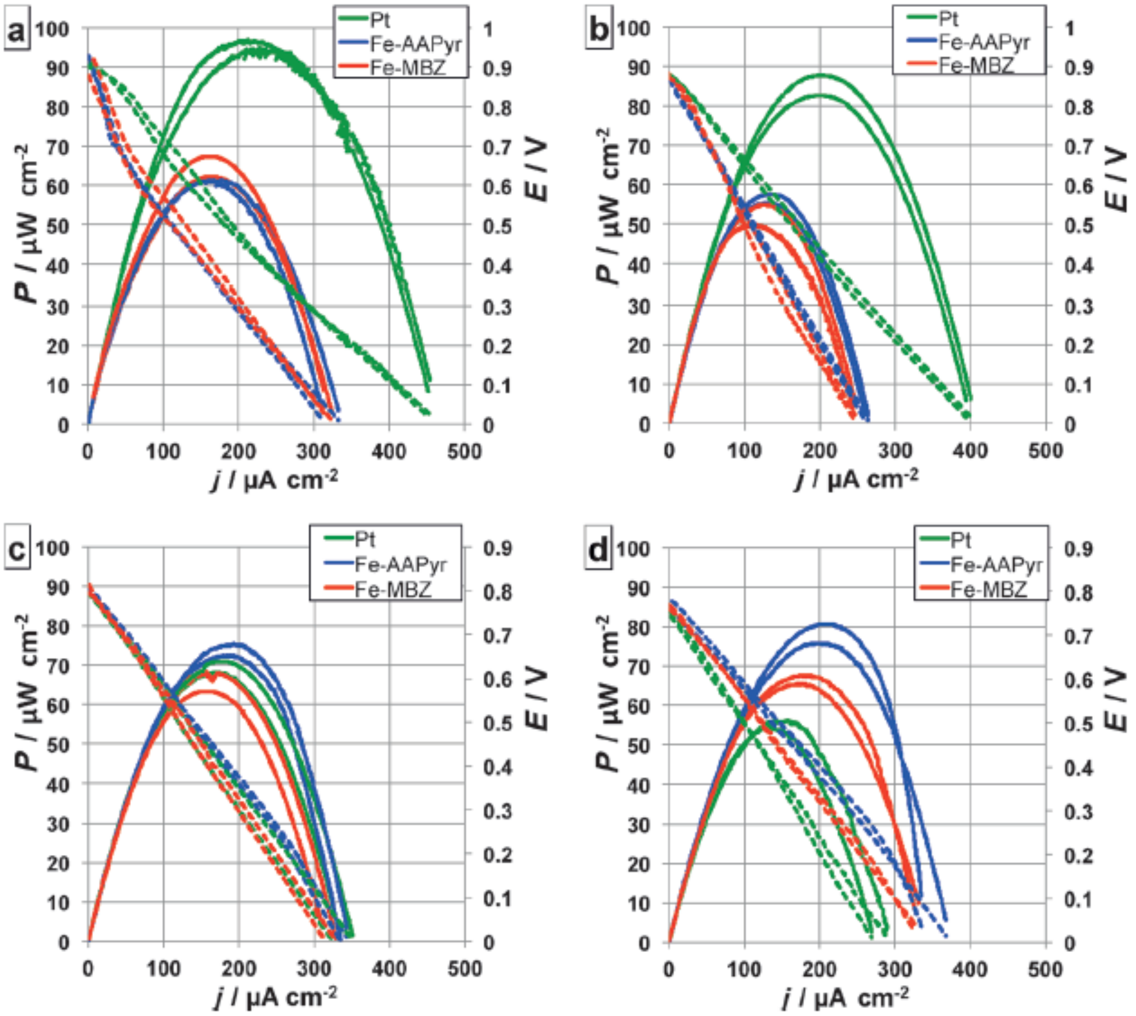

Figure 11. DCMFC power generated by different cathodes at different pH values: a) 6, b) 7.5, c) 9, and d) 11.

Fe-AAPyr was also tested in single chamber MFC. In fact, a new generation of innovative non-platinum group metal catalysts based on iron and aminoantipyrine as precursor (Fe-AAPyr) has been utilized in a membraneless single-chamber microbial fuel cell (SCMFC) running on wastewater. Fe-AAPyr was used as an oxygen reduction catalyst in a passive gas-diffusion cathode and implemented in SCMFC design. This catalyst demonstrated better performance than platinum (Pt) during screening in "clean" conditions (PBS) (Figure 12), and no degradation in performance during the operation in wastewater (Figure 13). The maximum power density generated by the SCMFC with Fe-AAPyr was 167 ± 6 μW cm^{-2} and remained stable over 16 days, while SCMFC with Pt decreased to 113 ± 4 μW cm^{-2} by day 13, achieving similar values of an activated carbon based cathode. The presence of S^{2-} and SO_4^{2-} showed insignificant decrease of ORR activity for the Fe-AAPyr. The reported results clearly demonstrate that Fe-AAPyr can be utilized in MFCs under the harsh conditions of wastewater.

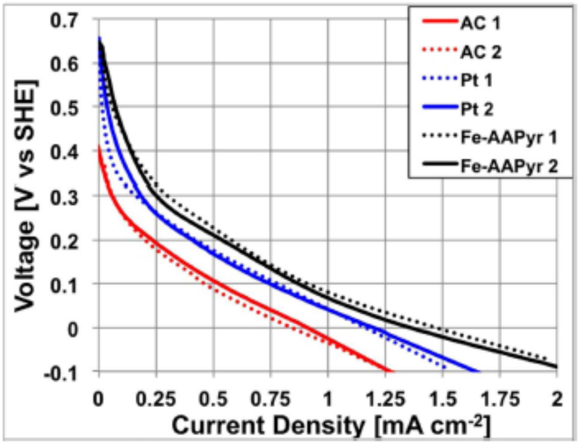

Figure 12. Linear Sweep voltammetry of AC, Pt and Fe-AAPyr in phosph

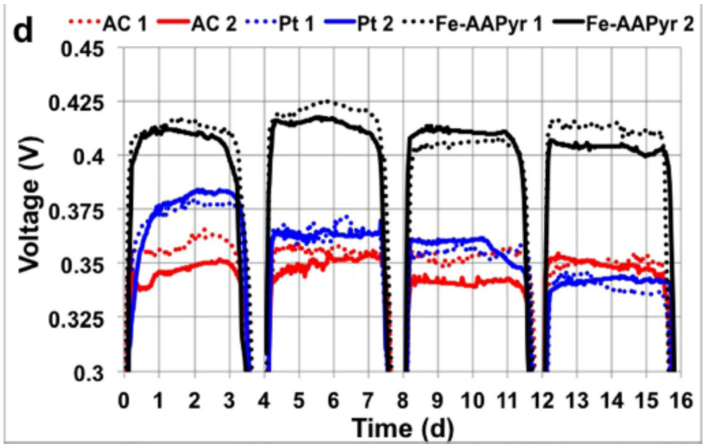

Figure 13. Voltage trend of MFCs over 16 days period of Fe-AAPyr (black), Pt (blue) and AC (red) cathode catalysts.

Surface motility and electron transfer in *Shewanella oneidensis.*

(Manuscript in preparation)

We also conducted a set of unique experiments to explore the motility of the exoelectrogen *Shewanella oneidensis* and role of various pili, with the ultimate goal of relating to exoelectrogenic activity. We demonstrated that *S. oneidensis* exhibits two different types of surface motility: type IV-pili driven twitching and flagellar driven swarming. Swarming motility requires a highly hydrated surface (*e.g.*, 0.5%-0.7% agar) and moves cells in a raft over the surface (Kearns, 2010). We have demonstrated swarming in *S. oneidensis* and shown that it was both dependent on the presence of flagella and also on the nutrition provided to the organisms. Figure 14 shows the extent of swarming of wild type (MR-1), and mutants lacking flagella (*Δflg*) Bouhenni et al., 2010 and unable to extrude type IV pili *(ΔpilD*) (Bouhenni et al., 2010) on different media. We observed slightly more motility on plates made from Luria-Burtani broth (LB) and those made from tryptic soy broth (TSB). Significantly, only growth and no swarming was observed on the *Δflg* mutant confirming that the motility observed was swarming. In contrast, the *ΔpilD* mutants showed increased swarming, unlike in other organisms (Barken et al., 2008). We suspect that this may be due to reduced surface attachment in these mutants (see below).

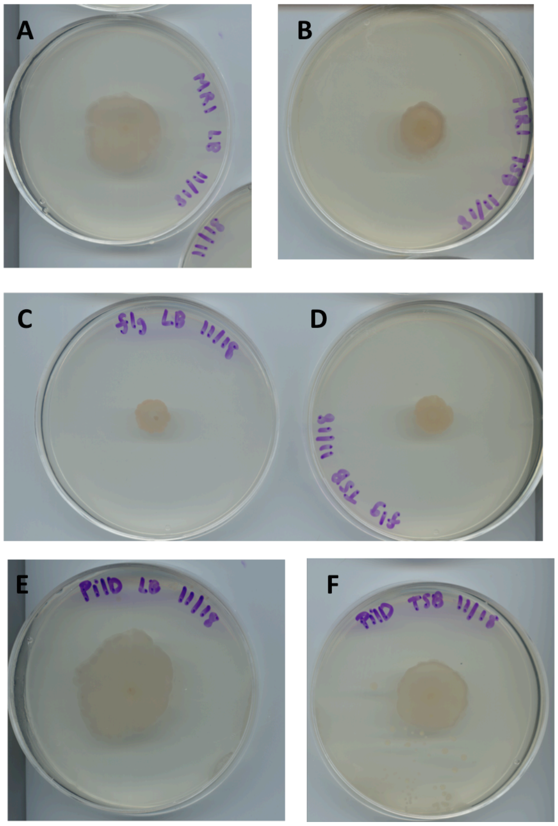

Figure 14: Swarming motility of *S. oneidensis* wild type (**A, B**), *Δflg* (**C,D**) and *ΔpilD* (**E,F**) on plates made with 0.7% agar with either Luria-Burtani broth (LB, **A,C,E**) or tryptic soy broth (TSB, **B,D,F**). Representative images.

The flagellar motor in *S. oneidensis* has two different stator systems: PomAB, is a sodium-dependent stator homolgous to that in *Vibrio* species, whereas MotAB is proton-dependent with the highest homology to that found in *Aeromonas hydrophila.* Of Mot AB seems to be predominant only under low sodium (*i.e.* <5 mM) conditions, and even at that concentration the amount of MotAB is only 1/3 of the amount of PomAB. We were curious if one of these stators was more likely to produce swarming than the other. To that end, we conducted a statically designed experiment which varied both the salt and the carbon/electron source concentration from 1-3 g/L NaCl and 0.5-5.0 mM lactate. Twitching was supported at these relatively high levels of salt, and was largely independent of the carbon source concentration, suggesting that the swarming motility was dependent on the PomAB stator. We are engaging in follow up studies to see whether this holds at lower concentrations salt and with *Δflg* controls.

Twitching motility has previously been shown to play a role in biofilm formation in *S. oneidensis* (Saville et al., 2010; Thormann et al., 2004) Like *Vibrio* species, *S. oneidensis* has two types of type IV pili that have potential to be involved in twitching (Saville et al., 2010; Thormann et al., 2004). One of these, the mannose-sensitive hemaglutinin A (MSHA) is similar to that found in *Vibrio* species and on glass, is suspected to be primarily responsible for adhesion. We have reported in previous years that in two hour attachment studies, mutants deficient in MSHA lose their specificity for attachment to hydrophilic SAMs. The second, called PilA, is homologous to the twitching pilus from *Pseudomonas aeruginosa*; we have previously reported that mutants in this pilus exhibit a slight reduction in attachment to hydrophilic surfaces.

The presence of pili seemed to be a requirement for extracellular electron transfer (EET) in this organism, and implicated in conductive nanowire formation (El-Naggar et al., 2010; Gorby et al., 2006). Subsequently, it was shown that nanowires are composed only of outer membrane extensions and the presence of type IV pili is not required (Pirbadian et al., 2014). So what is the role of type IV pili in EET? One possibility is that these structures are required for biofilm formation leading to sufficient biomass and of appropriate structure for EET. We have recently demonstrated that *S. oneidensis* forms different biofilm structures on self-assembled monolayers (SAMs) of different chemistry and, thus, investigated the ability of *S. oneidensis* to twitch on SAMs with different chemistry. Our investigations on wild type *S. oneidensis* on SAMs showed that substratum chemistry does, indeed affect the ability of *S. oneidensis* to twitch. Figure 15

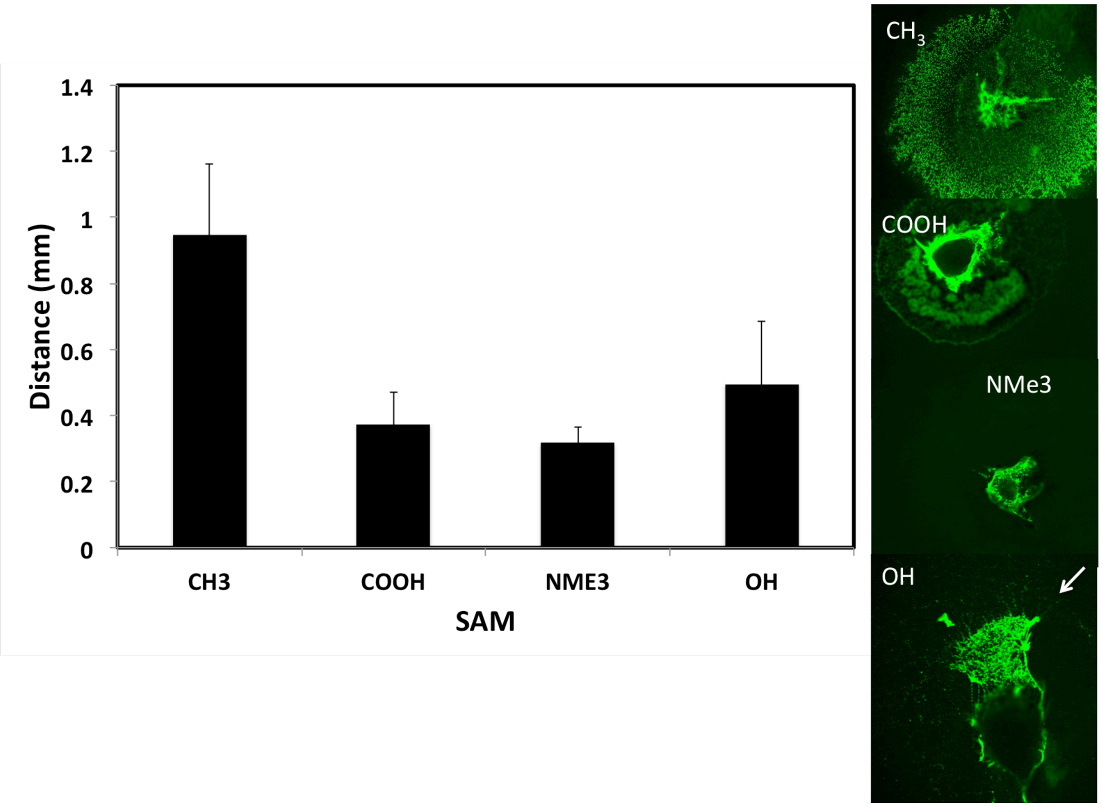

Figure 15. Twitching motility on SAMs of different chemistry. Left: Quantitative assay from 4 different SAMs on three different days. Data are average ± standard error. Right; representative twitching assay images on different SAMs. On OH-SAM the extent of diffused twitching is noted with an arrow.

shows the differences in 2-day twitch diameters on different SAMs. The twitching was most extensive on CH_3 SAMs, which correlates with our previous observation that the biofilm formed on such a SAM is thick and covers most of the surface. The SAM on which the least twitching occurred was that with NMe_3^+ chemistry. On this SAM, we observed the most structure and the most EET. We posit that this is because on this SAM, most of the surface motility is directed not on the attachment substratum, but over the surface of cells in the developing biofilm, as has been observed in other organisms (Klausen et al., 2003a; Klausen et al., 2003b).

The combination of different mutants and SAM chemistry is under investigation currently, in order to determine whether or not different pilus types are active on different SAMs and also see how each affects biofilm formation.We have established, however, that the ability to twitch on

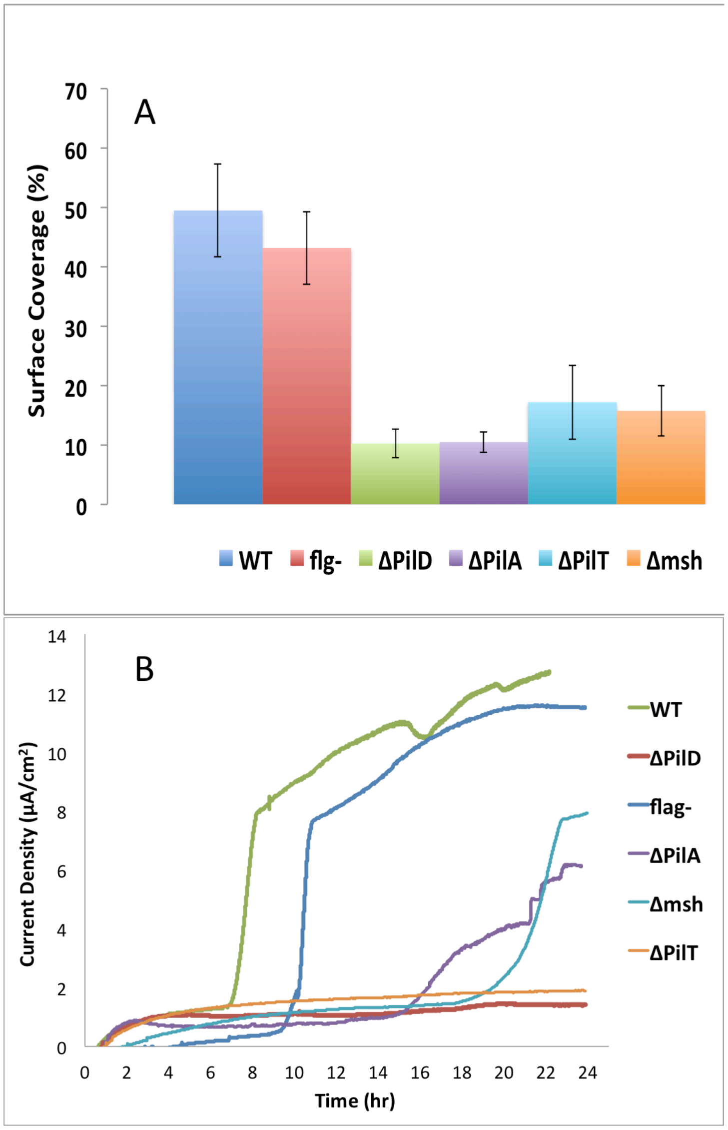

Figure 16. Accumulation (A) and current density over time (B) of *S. oneidnesis* wild type and motility mutants on ozonated carbon felt electrodes. (A) Data are averages of 3 experiments ± standard error.

ozonated carbon felt affects both biofilm formation and EET. For these studies, we used wild type, *ΔpilD* and *Δflg* mutants as before, but also evaluated the effect of loss of either pilus type (*i.e.ΔpilA* and *ΔmshA-D*) or the retraction motor required for twitching motility (*ΔpilT*). In Figure 16A, we observe that the loss of either, or bother pili or the pilus motor results in decreased biofilm formation on these this surface, suggesting that twitching motility is required for competent biofilm formation. In contrast, loss of flagella has no statistically significant effect on bacterial accumulation on ozonated carbon, suggesting that swarming motility is unimportant for biofilm formation on this surface. These results are also reflected in development of EET over time. Figure 16B shows that *Δflg* and wild-type cells show similar effects of starting EET, whereas those lacking pili altogether (*ΔpilD*) or are unable to twitch (*ΔpilT*) never exhibit EET. In contrast those lacking either MSHA (*Δmsh*) or PilA (*ΔpilA*) show delayed EET development on this surface. These results are consistent with previous observations on attachment to OH and COOH SAMs (Figure 17). While *ΔpilD* reduces the ability of *S. oneidensis* to attach to OH- and COOH-SAMs, the effects of the loss of individual pili types is less significant. We should also note that although we know that there are a high concentration of carbonyl and hydroxyl moieties on these ozonated surfaces (Cornejo et al., 2015) direct comparison with OH- and COOH-SAMs is not possible. Taken together, however, these data suggest that twitching motility is important for biofilm formation and EET in *S. oneidensis.*

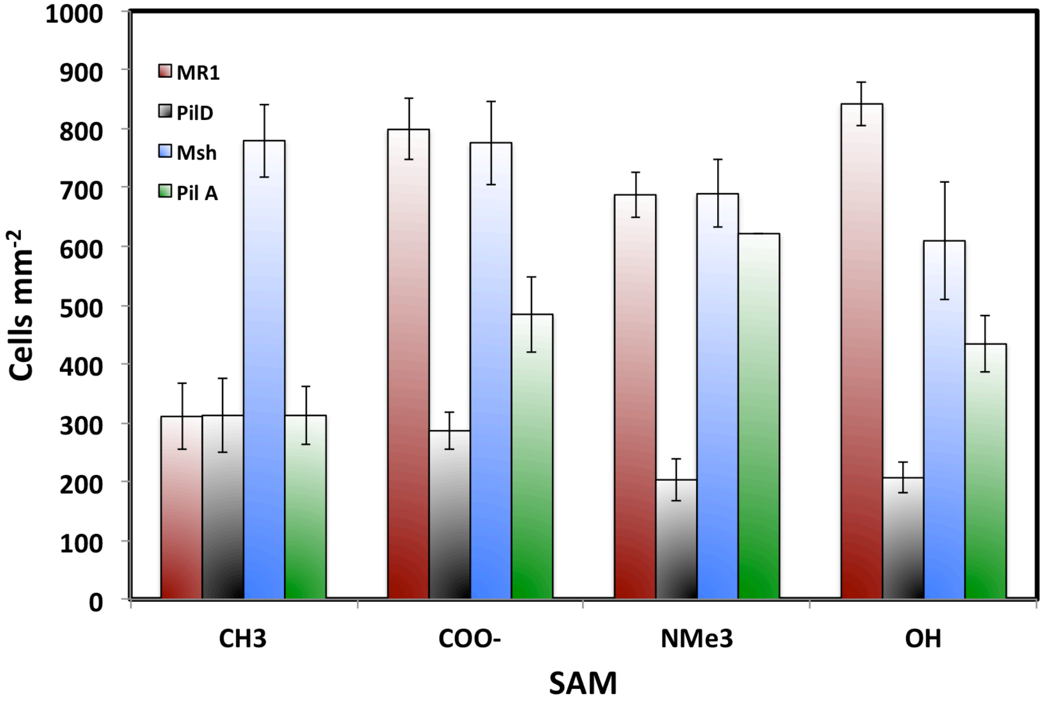

Figure 17: 2 hour attachment of *S. oneidensis* wild type (MR-1) and structural pilus mutants to SAMs of different chemistries. Data are average ± standard error; *n*=3).

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