**SUPPORTING INFORMATION**

# Continuous flow microbial flow cell with anion exchange membrane for treating low conductivity and poorly buffered wastewaters

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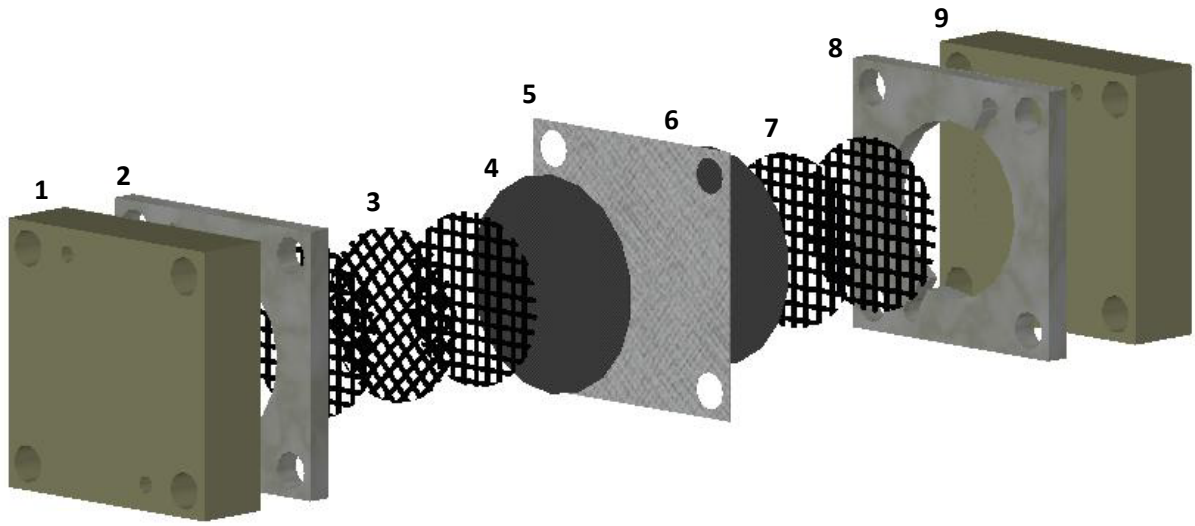


**Figure S1:** Schematic drawing of the MFC configuration used in this study. (1) Anode chamber endplate, solution-side; (2) silicon anode chamber gasket, solution-side; (3) plastic spacers, solution-side; (4) two layers carbon cloth anode; (5) AEM; (6) one layer carbon cloth cathode; (7) plastic spacers, air-side; (8) silicon cathode chamber gasket, air-side; and (9) cathode chamber endplate.

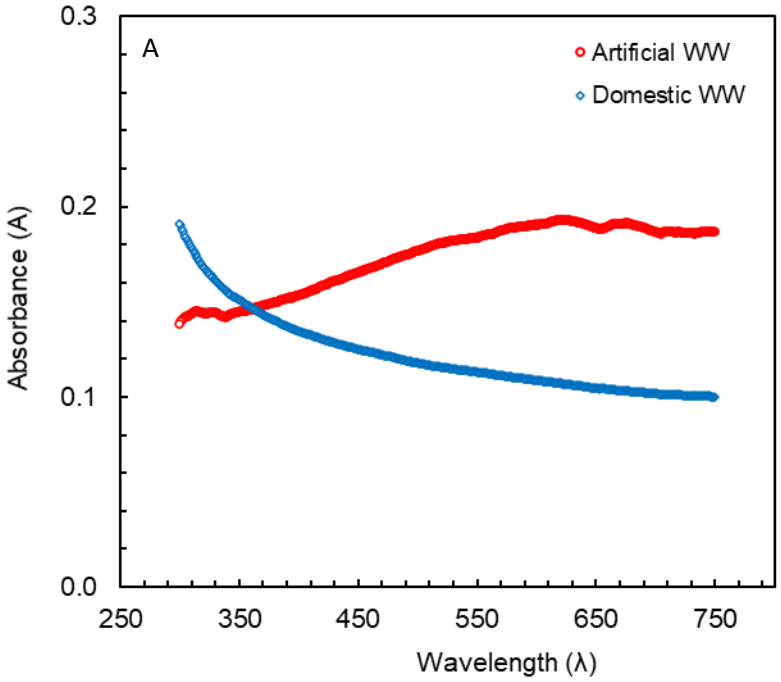



**Figure S2:** (A) Absorbance spectra of artificial wastewater compared to domestic wastewater collected from the primary clarifier at the Pennsylvania State University Wastewater Treatment Plant and (B) photo of the domestic wastewater (left) and artificial wastewater (right)

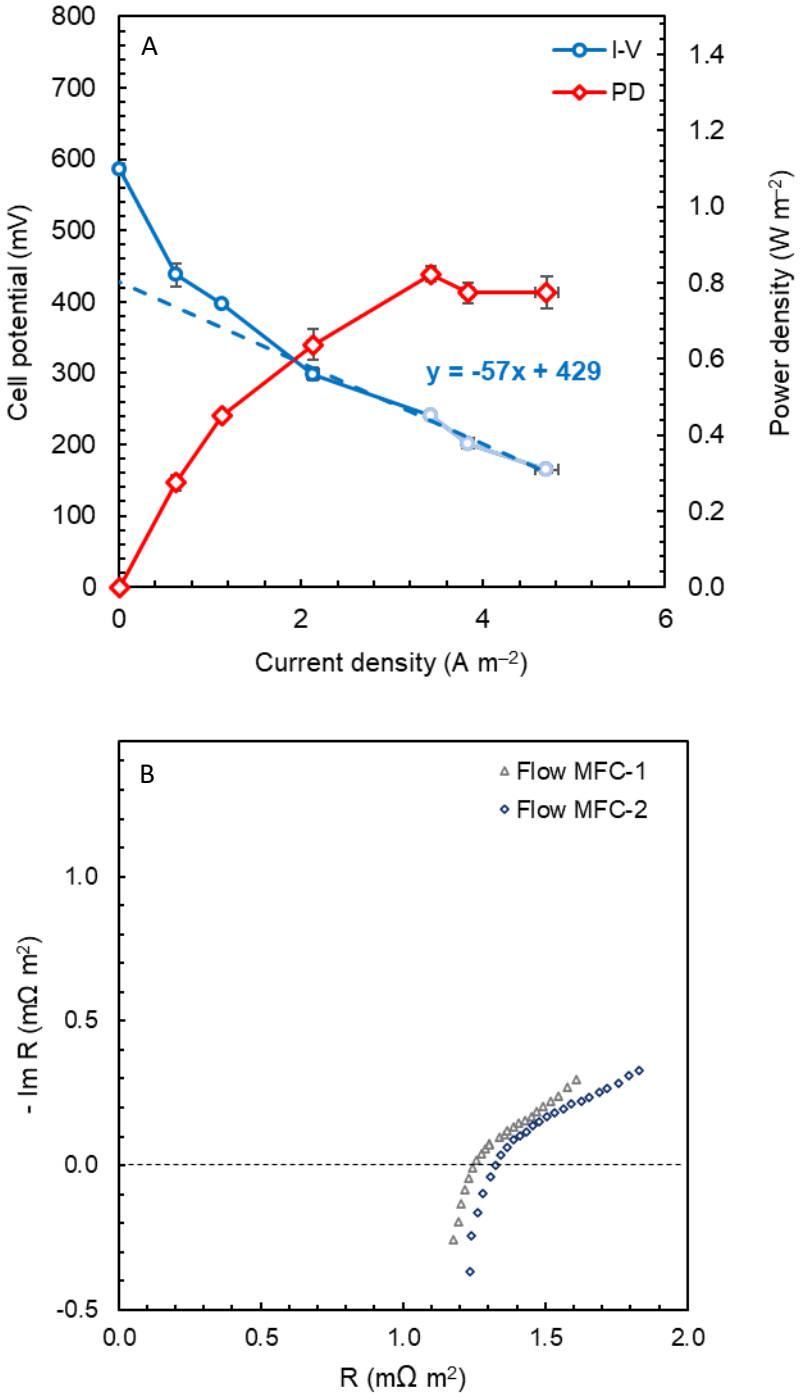


**Figure S3:** (A) Polarization curve obtained after 2h at OCP in wastewater. (B) EIS at high frequencies (from 100 kHz to 500 Hz, 5 mV amplitude, 10 points $s^{-1}$, ≈25 s $scan^{-1}$) used to calculate the solution resistance of the flow-MFC fed with wastewater.

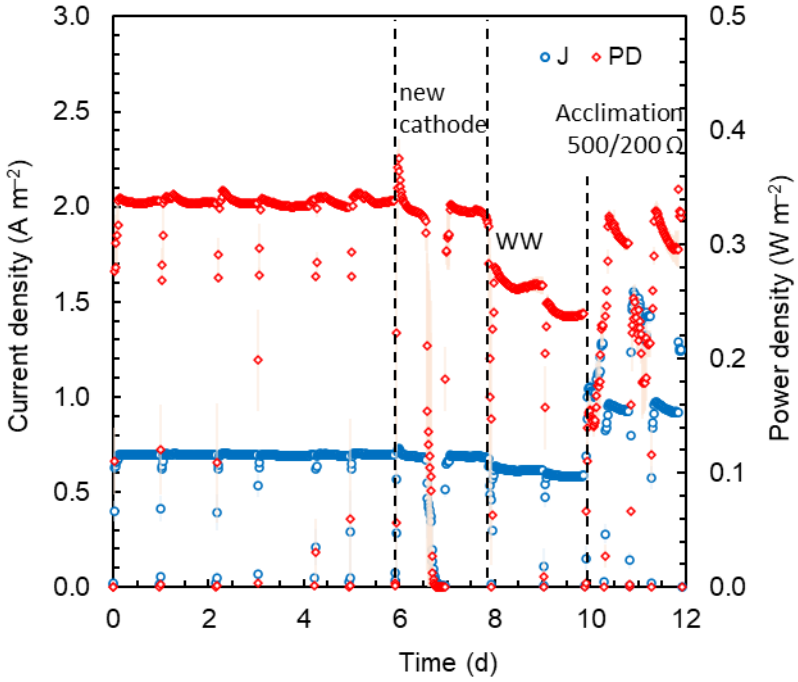


**Figure S4:** Current density (J) and power density (PD) of the cubic MFC during acclimation with wastewater. The dashed lines indicate when the solution was replaced with a new media.

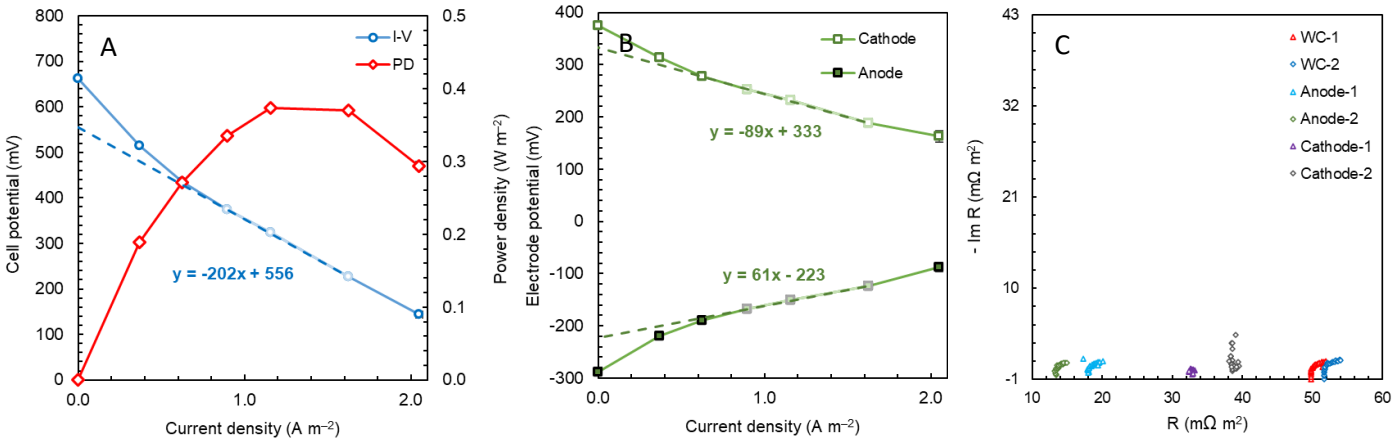


**Figure S5:** (A) Polarization curve of the cubic MFC and (B) respective anode and cathode potentials. (C) electrochemical impedance spectroscopy (EIS) at high frequencies (from 100 kHz to 500 Hz, 5 mV amplitude, 10 points $s^{-1}$, ≈25 s $scan^{-1}$) used to calculate the solution resistance of the whole cell (WC) and that between anode and cathode and reference electrode (RE).

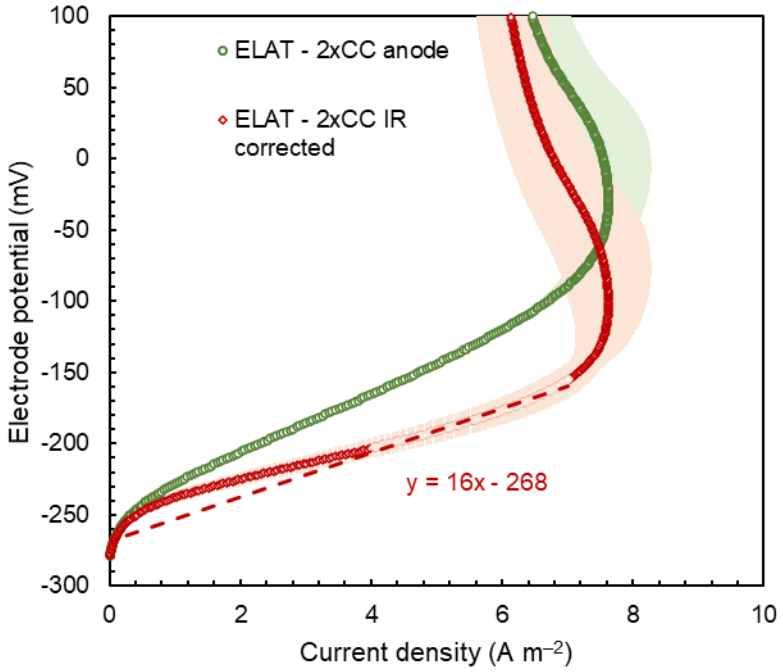


**Figure S6:** Carbon cloth anode linear sweep voltammetry (LSV) with potential corrected (IR corrected) and not corrected for the solution resistance between electrode and reference electrode.

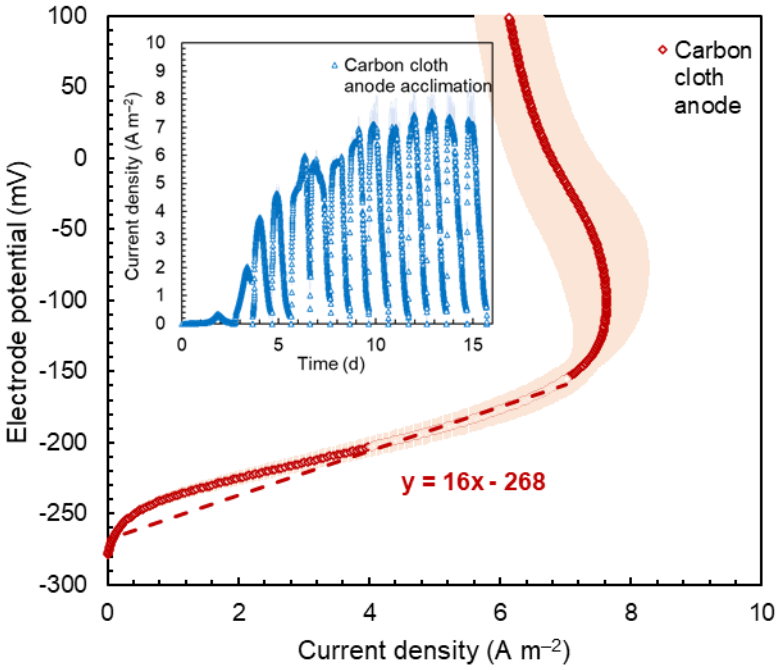


**Figure S7:** LSV of the carbon cloth anode acclimated at +200 mV in 50 mM PBS for 14 days. Current densities during the acclimation phase are shown in the inset. The dashed line represents the linearization of the data in the current density range typical for maximum power generation in MFCs (4-7 A $m^{-2}$).

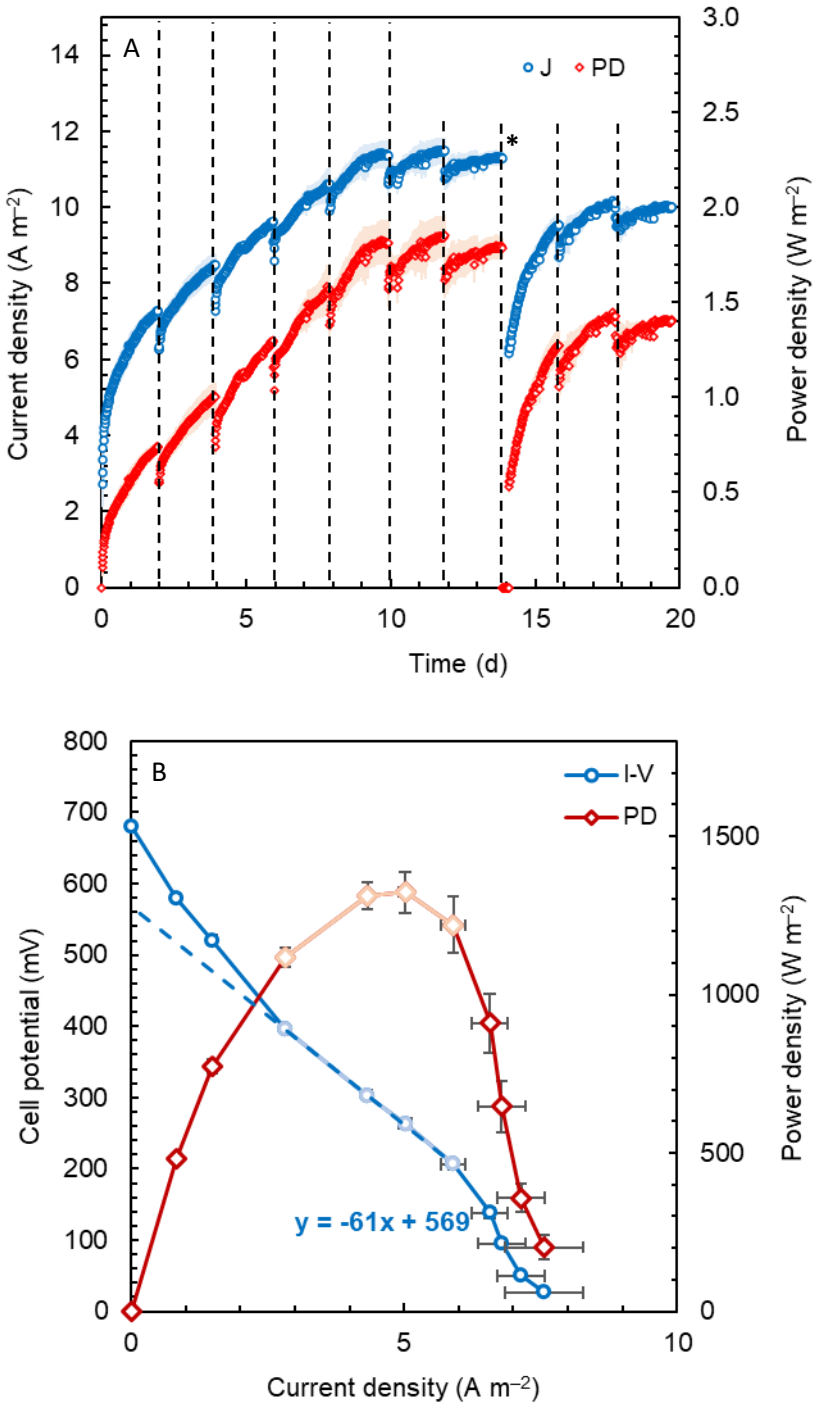


**Figure S8:** (A) Power density (PD) and current density (J) during acclimation in the flow cell with only one layer of carbon cloth and 20 Ω external resistance in PBS. The dashed lines indicate when the solution was replaced with a new media. *Polarization test. (B) Power density and polarization curve of the MFCs. The dashed lines represent the linearization of the data in the maximum power region.

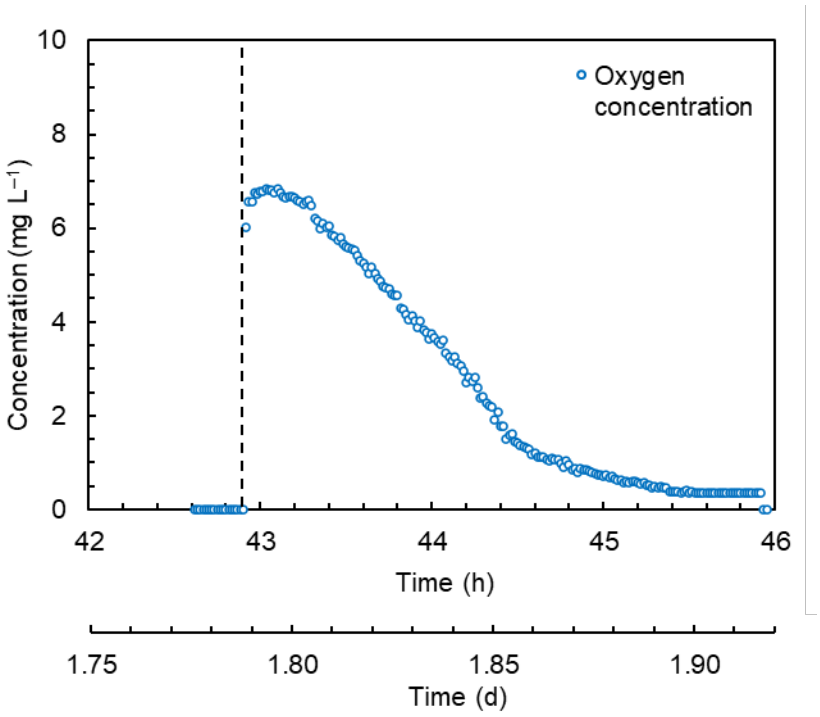


**Figure S9:** Oxygen concentration during reactor acclimation with 50 mM PBS after solution replacement.

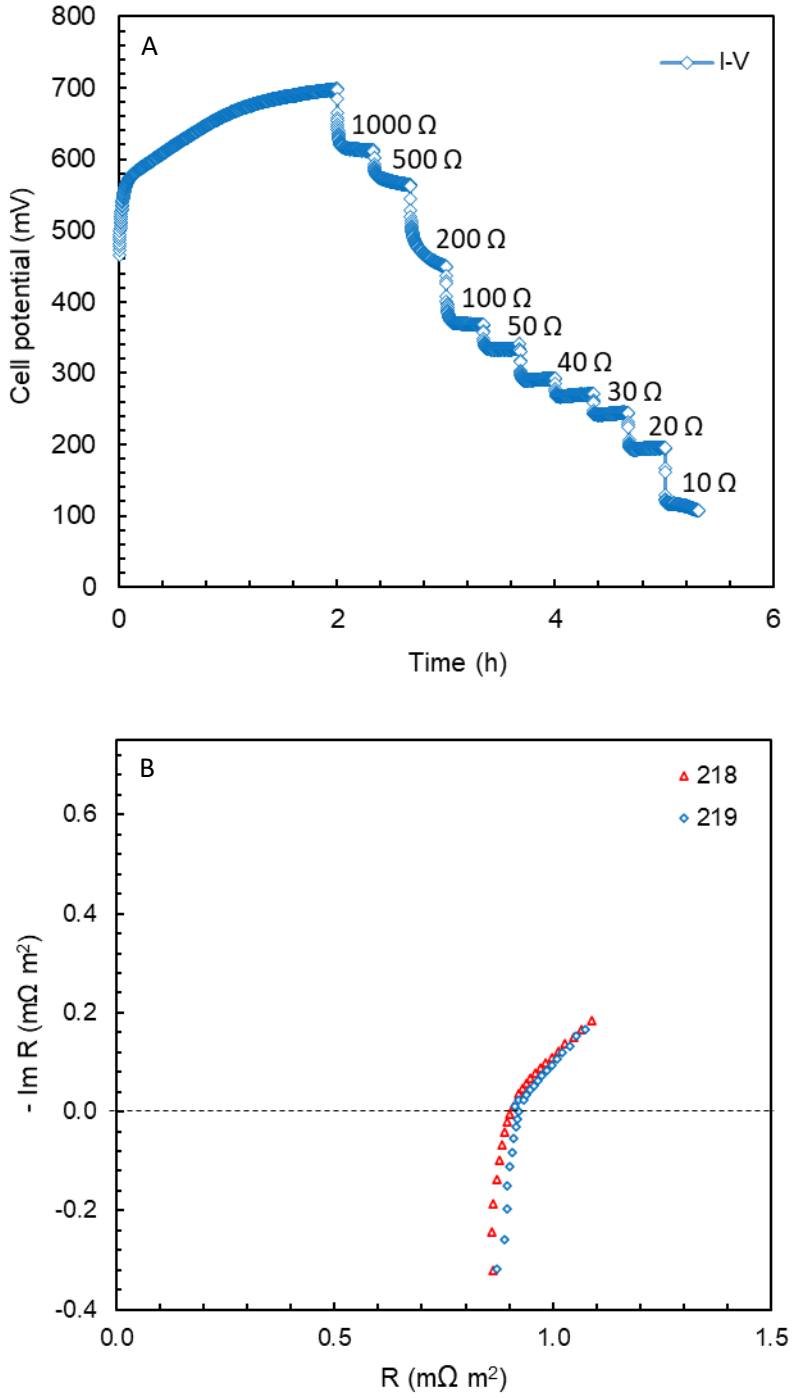


**Figure S10:** (A) Voltage output during polarization test and (B) EIS at high frequencies (from 100 kHz to 500 Hz, 5 mV amplitude, 10 points $s^{-1}$, ≈25 s $scan^{-1}$) used to calculate the solution resistance of the flow-MFC in 50 mM PBS.


**Figure S11:** Photos of the (A, B) cathode side and (C, D) plastic spacer side of the carbon cloth anodes from duplicate reactors after 35 days of operation.

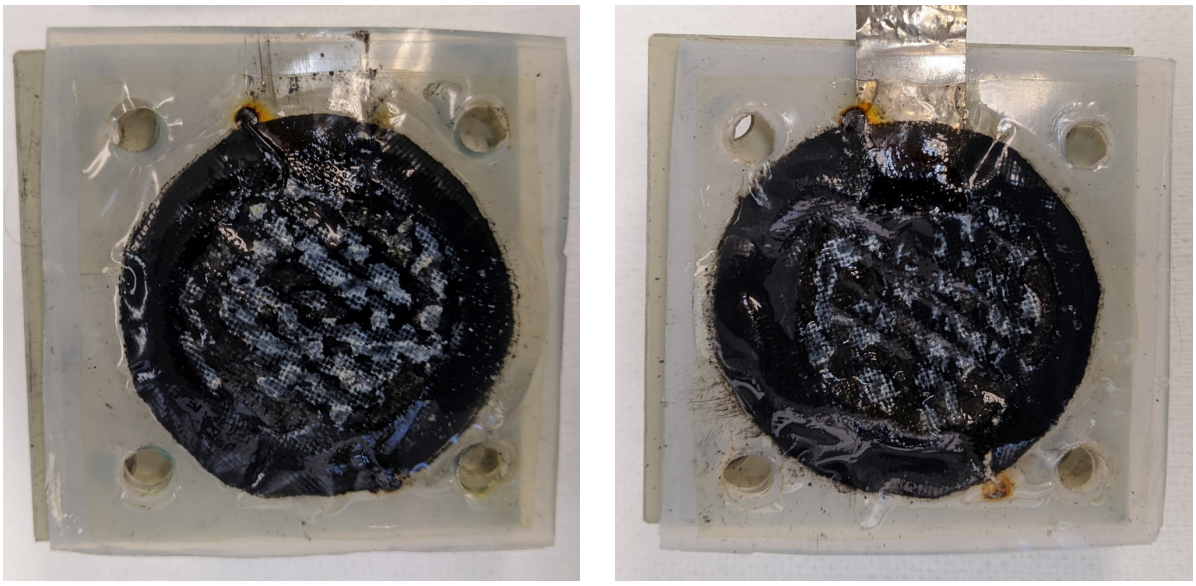

**Figure S12:** Photos of the anode side of the AEM from duplicate reactors after 35 days of operation.

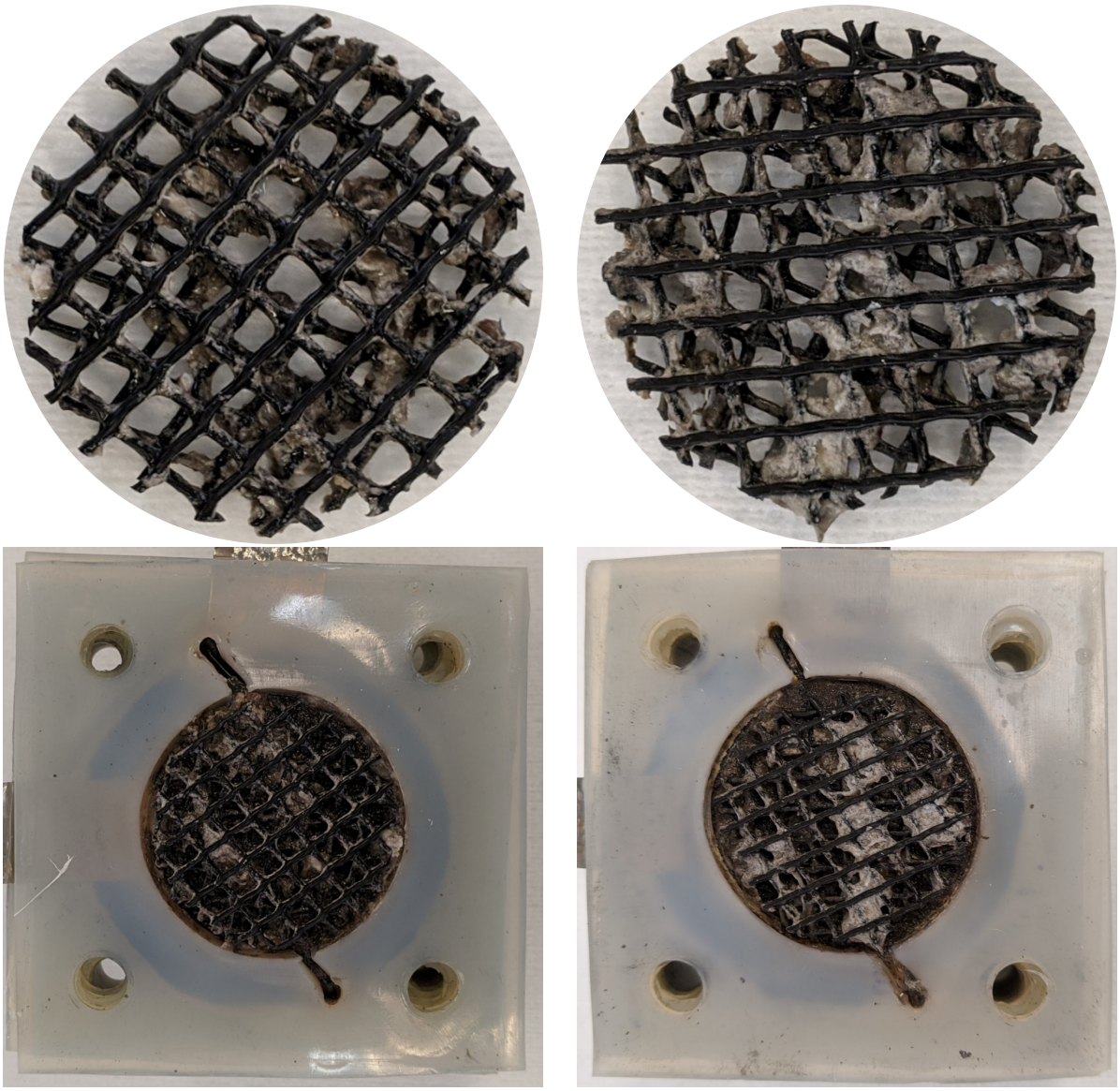

**Figure S13:** Photos of the plastic spacers used in the anode chamber from duplicate reactors after 35 days of operation.

**Microbial community analysis**

DNA: Archaea and Bacteria, 16S rRNA gene region V4 sequencing libraries were prepared by a custom protocol based on an Illumina protocol (Illumina, 2015). Up to 10 ng of extracted DNA was used as template for PCR amplification of the Archaea and Bacteria, 16S rRNA gene region V4 amplicons. Each PCR reaction (25 µL) contained (12.5 µL) PCRBIO Ultra mix (PCR Biosystems, USA) and 400 nM of each forward and reverse tailed primer mix. PCR was conducted with the following program: Initial denaturation at 95 C for 2 min, 30 cycles of amplification (95 C for 15 s, 55 C for 15 s, 72 C for 50 s) and a final elongation at 72 C for 5 min. Duplicate PCR reactions were performed for each sample and the duplicates were pooled after PCR. The forward and reverse tailed primers were designed according to (Illumina, 2015) and contain primers targeting the Archaea and Bacteria, 16S rRNA gene region V4: [515FB] GTGYCAGCMGCCGCGGTAA and [806RB] GGACTACNVGGGTWTCTAAT (Apprill et al., 2015). The primer tails enable attachment of Illumina Nextera adaptors necessary for sequencing in a subsequent PCR.

RNA: Archaea and Bacteria, 16S rRNA gene region V4 sequencing libraries were prepared by a custom protocol based on an Illumina protocol (Illumina, 2015). Up to 10 ng of extracted RNA was used as template for PCR amplification of the Archaea and Bacteria, 16S rRNA gene region V4 amplicons. Each PCR reaction (25 µL) contained (12.5 µL) 2X Platinum SuperFi RT-PCR Master Mix from the SuperScript IV One-Step RT-PCR System (Thermo Fisher Scientific, USA), (0.25 _L) SuperScrip IV RT Mix (Thermo Fisher Scientific, USA) and 400 nM of each forward and reverse tailed primer mix. PCR was conducted with the following program: Reverse transcription at 60 C for 10 min, an initial denaturation at 98 C for 2 min, 25 cycles of

amplification (98 C for 15 s, 55 C for 15 s, 72 C for 50 s) and a final elongation at 72 C for 5 min. Duplicate PCR reactions were performed for each sample and the duplicates were pooled after PCR. The forward and reverse tailed primers were designed according to (Illumina, 2015) and contain primers targeting the Archaea and Bacteria, 16S rRNA gene region V4: [515FB] GTGYCAGCMGCCGCGGTAA and [806RB] GGACTACNVGGGTWTCTAAT (Apprill et al., 2015). The primer tails enable attachment of Illumina Nextera adaptors necessary for sequencing in a subsequent PCR.

Sequencing libraries were prepared from the purified amplicon libraries using a second PCR. Each PCR reaction (25 µL) contained PCRBIO HiFi buffer (1x), PCRBIO HiFi Polymerase (1 U/reaction) (PCRBiosystems, UK), adaptor mix (400 nM of each forward and reverse) and up to 10 ng of amplicon library template. PCR was conducted with the following program: Initial denaturation at 95 C for 2 min, 8 cycles of amplification (95 C for 20 s, 55 C for 30 s, 72 C for 60 s) and a final elongation at 72 C for 5 min. The resulting sequencing libraries were purified using the standard protocol for Agencourt Ampure XP Beads (Beckman Coulter, USA) with a bead to sample ratio of 4:5. DNA was eluted in 25 µL of nuclease free water (Qiagen, Germany). DNA concentration was measured using Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, USA). Gel electrophoresis using Tapestation 2200 and D1000/High sensitivity D1000 screentapes (Agilent, USA) was used to validate product size and purity of a subset of sequencing libraries.

The trimmed forward and reverse reads were merged using FLASH v. 1.2.7 (Magoč and Salzberg, 2011) with the settings -m 10 -M 250. The trimmed reads were dereplicated and formatted for use in the UPARSE workflow (Edgar, 2013). The dereplicated reads were clustered, using the usearch v. 7.0.1090 -cluster_otus command with default settings. OTU

abundances were estimated using the usearch v. 7.0.1090 -usearch_global command with -id 0.97 -maxaccepts 0 - maxrejects 0. Taxonomy was assigned using the RDP classifier (Wang et al., 2007) as implemented in the parallel_assign_taxonomy_rdp.py script in QIIME (Caporaso et al., 2010), using –confidence 0.8 and the SILVA database, release 132 (Quast et al., 2013).

**Literature cited**


Apprill, A., Mcnally, S., Parsons, R., Weber, L., 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat. Microb. Ecol. 75, 129–137. https://doi.org/10.3354/ame01753

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., Mcdonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. correspondence QIIME allows analysis of high- throughput community sequencing data Intensity normalization improves color calling in SOLiD sequencing. Nat. Publ. Gr. 7, 335–336. https://doi.org/10.1038/nmeth0510-335

Illumina, 2015. 16S Metagenomic sequencing library. Illumina.com 1–28.

Magoč, T., Salzberg, S.L., 2011. FLASH: Fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963. https://doi.org/10.1093/bioinformatics/btr507

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 41, 590–596. https://doi.org/10.1093/nar/gks1219

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267. https://doi.org/10.1128/AEM.00062-07

**Table S1.** Characteristic of the primary effluent wastewater collected at the Pennsylvania State University Wastewater Treatment Plant.

| | PRIMARY SETTLING EFFLUENT | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 11/16/2012 6:45:00 AM | 11/17/2012 7:00:00 AM | 11/18/2012 7:00:00 AM | 11/19/2012 7:00:00 AM | 11/20/2012 6:50:00 AM | 11/21/2012 6:40:00 AM | 11/22/2012 6:50:00 AM | | |
| Flow Rate (mgd) | 2.13 | 2.02 | 1.78 | 1.17 | 1.33 | 1.37 | 1.22 | Average 7 days | S.D. |
| Day of the week | Friday | Saturday | Sunday | Monday | Tuesday | Wednesday | Thursday | | |
| Calcium (mg/L) | 69 | 10 | 66 | 71 | 73 | 66 | 62 | 60 | 21 |
| Copper (mg/L) | 0.09 | 0.14 | 0.08 | 0.11 | 0.18 | 0.10 | 0.12 | 0.12 | 0.03 |
| Mercury (mg/L) | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Potassium (mg/L) | 13 | 12 | 15 | 10 | 11 | 10 | 8 | 11 | 2 |
| Magnesium (mg/L) | 31 | 29 | 28 | 29 | 31 | 28 | 27 | 29 | 1 |
| Sodium (mg/L) | 190 | 196 | 169 | 199 | 223 | 147 | 222 | 192 | 25 |
| Zinc (mg/L) | 0.07 | 0.08 | 0.07 | 0.08 | 0.09 | 0.09 | 0.06 | 0.08 | 0.01 |
| Biochemical Oxygen Demand (mg/L) | 167 | 189 | 169 | 108 | 182 | 95 | 174 | 155 | 35 |
| Biochemical Oxygen Demand (Soluble) (mg/L) | 100 | 83.1 | 89.8 | 42.7 | 67.7 | 27.2 | 52.5 | 66 | 25 |
| Chemical Oxygen Demand (mg/L) | 321 | 287 | 307 | 246 | 356 | 228 | 258 | 286 | 42 |
| Chemical Oxygen Demand (Soluble) (mg/L) | 127 | 138 | 149 | 87.5 | 140 | 67 | 78.4 | 112 | 31 |
| Total Alkalinity (mg/L) | 386 | 380 | 390 | 350 | 320 | 280 | 310 | 345 | 40 |
| Chloride (mg/L) | 318 | 338 | 286 | 327 | 399 | 241 | 349 | 323 | 46 |
| Sulfate as SO4 (mg/L) | 37 | 35 | 35.2 | 37.9 | 33.3 | 32.5 | 33.7 | 35 | 2 |
| Phosphorus (mg/L) | 5.6 | 6.0 | 6.2 | 4.6 | 5.2 | 6.3 | 3.8 | 5.4 | 0.9 |
| Phosphorus (Dissolved) (mg/L) | 3.7 | 4.0 | 4.0 | 2.6 | 3.0 | 4.9 | 1.1 | 3.3 | 1.1 |
| Orthophosphate as P (mg/L) | 3.1 | 3.6 | 4.1 | 2.5 | 2.7 | 4.0 | 2.2 | 3.2 | 0.7 |
| Nitrate as N (mg/L) | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Nitrite as N (mg/L) | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Total Kjeldahl Nitrogen (mg/L) | 51.7 | 51.11 | 57.01 | 39.34 | 29.94 | 30.69 | 17.06 | 40 | 13 |
| Ammonia as N (mg/L) | 38.9 | 35.35 | 45.16 | 28.89 | 27.7 | 23.79 | 18.11 | 31 | 9 |
| Total Kjeldahl Nitrogen (Soluble) (mg/L) | 41.46 | 38.28 | 48.96 | 25.49 | 21.91 | 22.16 | 20.2 | 31 | 11 |
| Total Dissolved Solids (mg/L) | 890 | 899 | 780 | 898 | 1000 | 770 | 927 | 881 | 75 |
| Total Suspended Solids (mg/L) | 64 | 60 | 60 | 73 | 118 | 49 | 57 | 69 | 21 |
| Volatile Suspended Solids (mg/L) | 64 | 52 | 56 | 69 | 106 | 46 | 52 | 64 | 19 |
| pH | 7.9 | 7.3 | 7.6 | 7.6 | 7.4 | 7.7 | 7.4 | 7.6 | 0.2 |