Supporting Information

Effect of Pre-Acclimation of Granular Activated Carbon on Microbial Electrolysis Cell Startup and Performance

Nicole LaBarge[a], Yasemin Dilsad Yilmazel[a], Pei-Ying Hong[b], and Bruce E. Logan[a,*]

[a] Department of Civil and Environmental Engineering, The Pennsylvania State University, University Park, PA, 16802

[b] King Abdullah University of Science and Technology (KAUST), Water Desalination and Reuse Center (WDRC), Biological and Environmental Sciences & Engineering Division (BESE), Thuwal, 23955-6900, Saudi Arabia

Stoichiometric conversion of substrate to methane

For GAC pre-acclimation to organic substrates, volumetric methane production was compared with theoretical stoichiometric methane production. Moles of methane generated from methanol, acetate, and propionate were calculated using the following equation for anaerobic digestion:

$$C_nH_aO_bN_c + \left(n - {}^{a}/_{4} - {}^{b}/_{2} + {}^{3c}/_{4}\right)H_2O$$

$$\rightarrow \left({}^{n}/_{2} + {}^{a}/_{8} - {}^{b}/_{4} - {}^{3c}/_{8}\right)CH_4 + \left({}^{n}/_{2} - {}^{a}/_{8} + {}^{b}/_{4} + {}^{3c}/_{8}\right)CO_2 + cNH_3$$

Moles of methane generated from digestion of hydrogen molecule was calculated by the equation:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

Theoretical methane generation for the substrates used during pre-acclimation is summarized in Table S1.

Table S1. Theoretical methane generated per mole of substrate

Substrate	Chemical formula	mol CH_4 / mol substrate
Methanol	CH_3OH	0.75
Acetate	CH_3COOH	1.0
Propionate	CH_3CH_2COOH	1.75
Hydrogen	H_2	0.25

The theoretical volume of methane generated from substrates (Table S2) including methanol, acetate, and propionate was calculated as:

$$V_{methane} = \frac{V_s\, \rho_s\, n_s}{M_s\, V_m}$$

where V_s is the volume of substrate added, ρ_s is the density of substrate, n_s is the moles of methane generated per mole of substrate, M_s is the molar mass of substrate, and V_m is the molar volume of an ideal gas at standard temperature and pressure.

The theoretical volume of methane generated from the hydrogen feed was calculated based on the hydrogen in the headspace and dissolved in the medium, and assuming equilibrium between the two phases. Methane generated from hydrogen in the headspace was calculated using:

$$V_{m,H} = \frac{f_s\, V_H\, P_s\, n_s\, V_m}{R\, T}$$

where $V_{m,H}$ is the theoretical methane generated from hydrogen in the headspace, f_s is the fraction of hydrogen gas in the feed, V_H is the headspace volume of the serum bottle, P_s is the pressure of hydrogen gas added, n_s is the moles of methane generated per mole of substrate, V_m is the molar volume of an ideal gas at standard temperature and pressure, R is the ideal gas constant, and T is room temperature. Theoretical methane generated by aqueous hydrogen was calculated using:

$$V_{m,L} = H_s \, V_L \, n_s \, V_m$$

where $V_{m,L}$ is the theoretical methane generated from hydrogen dissolved in the liquid, H_s is the solubility of hydrogen gas in water at 25 °C, V_L is the liquid volume in the serum bottle, n_s is the moles of methane generated per mole of substrate, and V_m is the molar volume of an ideal gas at standard temperature and pressure.

Table S2. Maximum methane production (mL at STP), and methane production based on stoichiometric conversion to methane (mL at STP) for GAC fed methane (M); a mixture of methanol, acetate, and propionate (MAP); and hydrogen (H).

Cycle	M		MAP		H	
	1	2	1	2	1	2
1	5.9	5.8	1.2	1.2	1.2	1.3
2	5.5	5.6	1.9	1.8	0.6	0.7
3	7.1	7.0	1.5	1.7	16.2	17.7
4	7.2	6.3	3.4	1.7	34.8	29.8
5	7.9	8.5	2.3	2.2	39.1	30.2
6	9.1	8.7				
7	8.6	8.3				
8	8.3	7.5				
9	8.8	8.4				
Average (all cycles)	7.4 ± 1.2		1.9 ± 0.6		17.1 ± 15.5	
Stoichiometric conversion	8.3		10.6		21.5	
% of stoichiometric conversion	90.0%		17.8%		79.7%	

Methane generation rate

Methane generation rates were calculated as nmol cm^{-3} d^{-1}. Weekly methane generation rates were calculated between each GC measurement of the cathode headspace according to the equation:

$$R_m \left[\frac{nmol}{cm^3 \times d}\right] = \frac{\Delta V_m[mL]\ 10^9\left[{}^{nmol}/_{mol}\right]}{\Delta t[d]\ 22.4\left[{}^{L}/_{mol}\right]\ 10^3\left[{}^{mL}/_{L}\right]\ V_h[cm^3]}$$

where R_m is the methane generation rate, ΔV_m is the change in methane volume between measurements, Δt is the time between measurements, and V_h is the cathode headspace volume. Weekly rates were averaged, starting after the lag phase (rates with a starting concentration of zero), and excluding the stationary phase (less than 10% increase in methane). An example of this calculation is shown in Figure , where the weekly production rates have been averaged to obtain overall methane production rates (slope of dotted line).

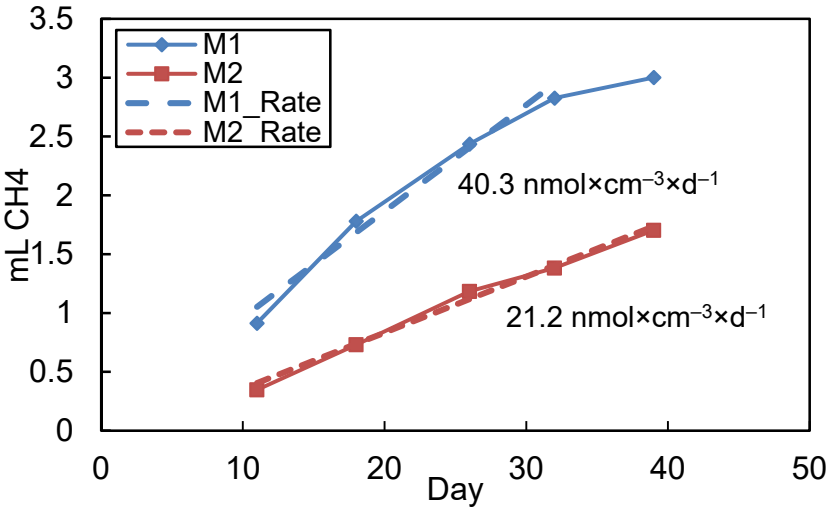

Figure S1. Methane in MEC cathode headspace for reactor M1 and M2, cycle 6. The figure shows averaged methane generation rates by the slopes of the fitted lines.

MEC operation

Table S3 displays methane generation rates from Figure 2, and also includes averaged values.

Table S3. Methane production rates (nmol cm^{-3} d^{-1}) for different MEC acclimations across successive cycles. Labeling is identical to that in **Error! Reference source not found.**.

Cycle	bog		GAC+bog		M		MAP		H	
	1	2	1	2	1	2	1	2	1	2
1	35.1	32.2	1.7	0.0	3.6	12.3	0.0	11.4	142.5	52.2
2	53.4	0.0	0.0	11.5	0.0	0.0	22.4	18.8	4.2	0.0
3	1.0	0.0	8.4	13.3	5.8	24.0	40.2	8.5	3.7	13.1
4	1.7	0.0	6.2	12.2	17.6	15.2	29.2	8.0	17.8	17.8
5			11.1	8.8	25.0	19.4	24.7	9.8	29.8	10.5
6			19.0	12.7	40.3	21.2	30.3	18.4	29.9	19.8
7			16.2	15.0	29.6	40.4	39.6	18.2	13.5	37.6
8					13.3	28.6				
Average (cycles 4-7)			12.7 ± 4.1		25.1 ± 9.7		22.3 ± 10.8		22.1 ± 9.3	

Charged transferred across the bog-only MECs was at least one order of magnitude lower than the other MECs tested, indicating that this reactor was unable to achieve effective charge transfer with the startup method used (Table S4). The lack of pre-acclimation or lack of GAC may have hindered the adaptation of the inoculum to adapt to the MEC environment.

Table S4. Coulombs transferred over each MEC cycle, including averages over cycles 1–4 and cycles 4–7. Labeling is identical to that in **Error! Reference source not found.**

Cycle	bog		GAC+bog		M		MAP		H	
	1	2	1	2	1	2	1	2	1	2
1	-6.9E+0	4.2E+0	-1.6E+2	-1.2E+2	-1.2E+2	-1.7E+2	-6.9E+4	-1.3E+2	-4.9E+1	-1.7E+2
2	9.0E+0	-1.9E+0	-2.1E+1	-1.5E+2	-5.8E+1	-6.4E+0	-1.4E+2	-4.2E+1	-4.6E+1	-5.5E+1
3	-5.5E+0	-2.0E+1	-2.1E+2	-1.1E+2	-7.0E+1	-1.5E+2	-3.4E+2	-1.8E+2	-1.1E+2	-2.2E+2
4	-4.7E+0	-3.5E+0	-2.4E+2	-1.7E+2	-1.4E+2	-4.7E+1	-1.3E+2	-5.8E+1	-2.5E+2	-1.7E+2
5			-1.9E+2	-1.7E+2	-1.1E+2	-1.3E+2	-1.4E+2	-1.1E+2	-8.3E+1	-4.1E+1
6			-1.4E+3	-8.6E+2	-2.0E+2	-1.1E+2	-1.6E+2	-1.1E+2	-2.3E+2	-1.3E+2
7			-7.8E+2	-5.8E+2	-5.4E+1	-1.4E+2	-1.3E+2	-7.8E+1	-3.4E+2	-1.1E+3
8					-3.4E+1	-9.3E+1				
Avg. (1-4)	-3.7E+0 ± 8.6E+0		-1.5E+2 ± 6.7E+1		-9.5E+1 ± 5.8E+1		-8.7E+3 ± 2.4E+4		-1.3E+2 ± 8.0E+1	
Avg. (4-7)			-5.5E+2 ± 4.4E+2		-1.2E+2 ± 5.0E+1		-1.1E+2 ± 3.4E+1		-2.9E+2 ± 3.3E+2	

Coulombic recovery (CR) was used to assess the effectiveness of acclimation method to promote methane generation (Fig. S2, Table S5). Trends in CR were similar to trends in methane production rate, with M, MAP, and H reactors showing higher CR than the GAC-bog reactors. However, CR of the bog-only reactor was artificially high due to low charge transfer.

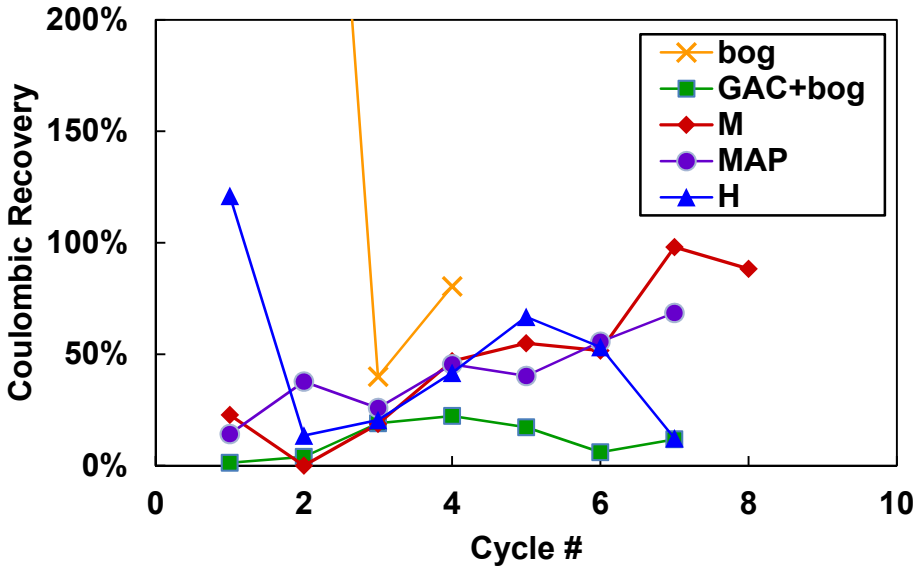

Figure S2. Coulombic recoveries for different GAC pre-acclimation methods. Labeling is identical to that in **Error! Reference source not found.**. Bog cycle 1 (2307%, 3635%) and bog cycle 2 (499%, 499%) are out of the range shown. The figure shows the same data as Fig. 3.

Table S5. Coulombic recoveries for duplicate MECs with different GAC pre-acclimation methods across successive cycles. Labeling is identical to that in **Error! Reference source not found.**.

Cycle	bog		GAC+bog		M		MAP		H	
	1	2	1	2	1	2	1	2	1	2
1	2307%	3635%	3%	0%	17%	28%	0%	28%	219%	23%
2	499%	499%	0%	8%	0%	0%	8%	67%	27%	0%
3	80%	0%	8%	30%	19%	18%	44%	8%	15%	26%
4	161%	0%	8%	37%	31%	62%	59%	32%	35%	48%
5			18%	17%	59%	50%	53%	28%	62%	72%
6			6%	6%	51%	52%	64%	48%	51%	55%
7			12%	12%	125%	71%	83%	55%	13%	11%
8					90%	86%				

DNA extracted from samples

The mass of sample taken for DNA extraction, as well as the mass of extracted DNA in a 100 µL volume, is shown in Table S6. DNA concentration should only be considered an accurate measure of order of magnitude, as precision of Nanodrop readings was low. The Bray-Curtis similarity between acclimation reactors is shown in Table S7, and similarity between GAC samples and brush samples in MECs is shown in Table S8.

Table S6. Mass of the sample used for DNA extraction, concentration of extracted DNA, and calculated DNA yield per gram of sample for the 100 µL of final fluid containing the extracted DNA.

Reactor type	Reactor	Sample location	g sample	ng/µL DNA	ng(DNA) /g(sample)
Bog sediment inoculum	for H acclimation rxtr	bog sediment	0.475	17.1	3,603
	for MAP acclimation rxtr	bog sediment	0.317	34.9	11,013
Acclimation reactor	M1	GAC	0.446	2.9	650
	M2	GAC	0.396	4.1	1,036
	H1	GAC	0.327	5.8	1,772
	H2	GAC	0.390	9.9	2,537
	MAP1	GAC	0.514	3.5	681
	MAP2	GAC	0.406	4.0	984
MEC	M1	GAC	0.260	2.0	769
	M1	carbon fiber	0.235	4.0	1,701
	M2	GAC	0.314	4.8	1,531
	M2	carbon fiber	0.283	23.7	8,372
	H1	GAC	0.264	2.4	910
	H1	carbon fiber	0.164	3.1	1,891
	H2	GAC	0.259	2.4	928
	H2	carbon fiber	0.235	3.4	1,444
	MAP1	GAC	0.287	1.6	557
	MAP1	carbon fiber	0.241	33.0	13,687
	MAP2	GAC	0.301	6.0	1,991
	MAP2	carbon fiber	0.271	10.3	3,797
	GAC+bog1	GAC	0.302	1.4	464
	GAC+bog1	carbon fiber	0.329	1.1	334
	GAC+bog2	GAC	0.360	2.3	638
	GAC+bog2	carbon fiber	0.304	1.0	329

Table S7. Bray-Curtis similarity (%) among acclimation reactor samples, taken at the end of reactor operation. Similarities were calculated from square-root transformed relative abundances. Darker shades correlate with more similar samples.

	M1	M2	MAP1	MAP2	H1	H2
M1		83	72	71	50	51
M2	83		69	65	49	49
MAP1	72	69		85	46	48
MAP2	71	65	85		47	50
H1	50	49	46	47		78
H2	51	49	48	50	78	

Table S8. Bray-Curtis similarity (%) among GAC carbon fiber brush DNA samples, taken from MECs at the end of their operation. Similarities were calculated from square-root transformed relative abundances. Darker shades correlate with more similar samples.

GAC	GAC +bog1	GAC +bog2	M1	M2	MAP1	MAP2	H1	H2
GAC+bog1		81	64	64	60	53	57	58
GAC+bog2	81		63	67	60	52	54	54
M1	64	63		82	81	70	66	64
M2	64	67	82		77	69	62	61
MAP1	60	60	81	77		79	65	61
MAP2	53	52	70	69	79		61	62
H1	57	54	66	62	65	61		82
H2	58	54	64	61	61	62	82	

Brush	GAC +bog1	GAC +bog2	M1	M2	MAP1	MAP2	H1	H2
GAC+bog1		71	57	62	58	52	48	48
GAC+bog2	71		57	61	55	50	44	46
M1	57	57		79	76	71	58	56
M2	62	61	79		71	66	50	49
MAP1	58	55	76	71		75	58	51
MAP2	52	50	71	66	75		59	60
H1	48	44	58	50	58	59		70
H2	48	46	56	49	51	60	70	