Supplementary materials and methods

Experiment protocol and data acquisition

For both anolyte and catholyte valve operation, DI water is filled into each valve control channel. The use of hydraulic-pressure for integrated microvalve control instead of pneumatic-pressure prevented gas bubbles from forming in the gas permeable PDMS chambers[1]. These bubbles, if not controlled, can move into the flow channel and eventually block the catholyte/anolyte flow. The use of hydraulic pressure successfully allowed reliable operation of the microfluidic MFC array for more than 2 weeks. Successful isolation between the neighboring cathode chambers during microvalve closure was confirmed through color dye testing and resistance measurement analysis between chambers (data not shown).

Before chip assembly, tubings for catholyte/anolyte inlet/outlet and control channel inlet/outlet were connected. De-ionized (DI) water was injected into the control channels gradually until no gas bubbles exist in the channel. Ferricyanide (100 mM) was first manually filled into the cathode chamber and then the proton exchange membrane (PEM) and anode chamber layer were aligned and assembled together with the cathode chamber layer. Microbes were loaded to each anode chamber and with all valves closed, then the anode chamber layer was enclosed with the anode electrode layer. The assembled device was then connected to a circuit board having series of load resistors. Voltages across external load resistors connected to each of the 24 miniature MFC units on the device were continuously recorded with a digital multimeter connected through a multiplexer controlled by a LabView™ (National Instruments, Austin, TX) interface.

During operation, catholyte was periodically replenished with a certain duty cycle by a syringe pump (Harvard Apparatus, Holliston, MA). During power measurement, pressure was applied

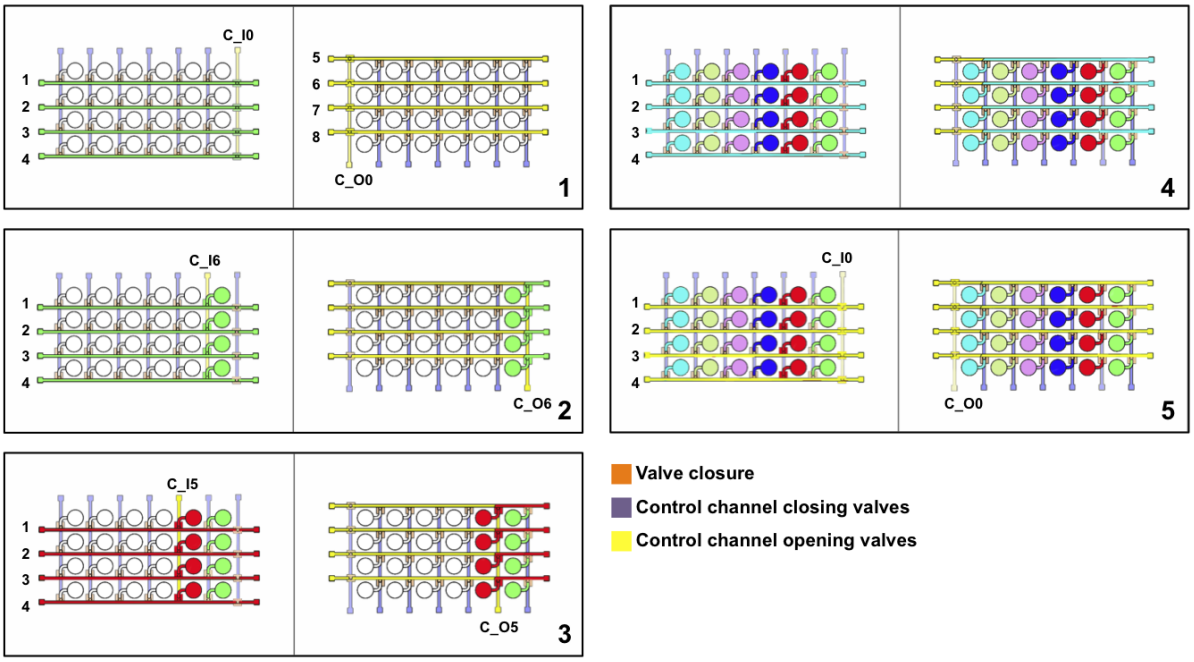
through the hydraulic-controlled channel so that all 24 cathode chambers are isolated, preventing cross-talk. Hence, the microfluidic MFC array worked as 24 individual miniaturized microfluidic MFCs. Polarization curves (voltage density vs. current density) were acquired by connecting each of the 24 miniature MFC units to varying load resistors and measuring the voltages across the resistors. This can be repeated over time, thus results in full polarization curves for each of the 24 microfluidic MFCs over time. Maximum power from each MFC unit was then identified from these polarization curves for further analyses. The microvalves were only opened when replenishing all 24 cathode chambers with fresh catholyte (flow rate: 200 µl/min, flow time: 15 min). Power measurements were taken both before and after each catholyte replenishment cycle to characterize how catholyte replenishment influenced the instant power output.

For anolyte replenishment, substrates were replenished at a flow rate of 1 ml/min, 1 ml of each anolyte were replenished into each of the anode chambers. Main inflow and outflow channels were flushed with sterialized DI water at a flow rate of 1ml/min with a total volume of 1 ml. The replenishment efficiency was characterized with color dye transmittance, showing that 91.5% of anolyte could be exchanged (data not shown).

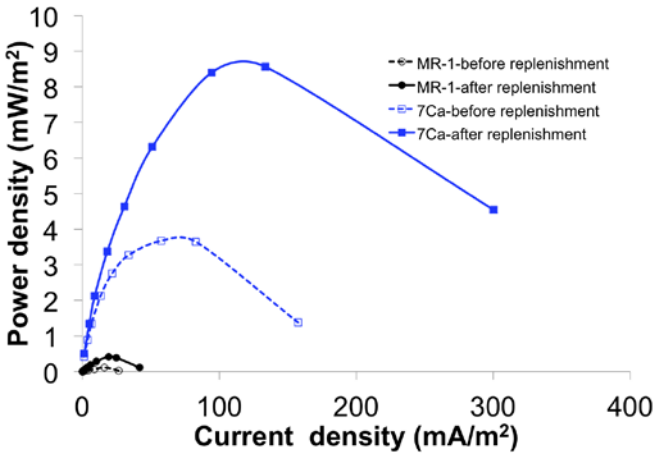
Microbe cultivation and biomass analysis

S. oneidensis MR-1 was used as a reference strain for the microfluidic MFC array characterization. A previously uncovered environmental species, *Shewanella* sp. Hac353 (DQ307734.1) (7Ca)[29], was used for parallel comparison studies. Cells stored in tryptic soy broth (TSB) supplemented with 15% glycerol at -80°C were streaked onto a TSB agar plate. The resultant colonies were inoculated into 5 ml of TSB liquid medium, and then cultured for 48 h at 30°C with agitation (150 rpm). Cells (OD_{600} = 1.7) were then ready to be used. Detailed microbial cultivation and preparation practices were described in our previous work[2]. For

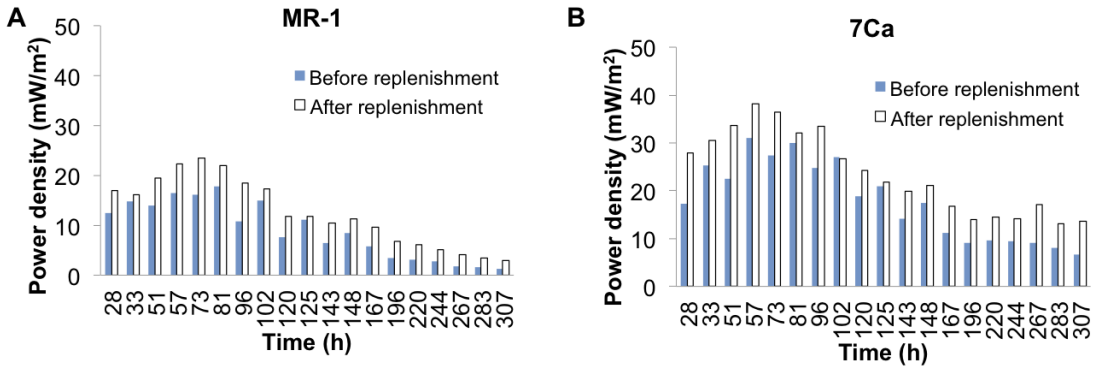
biomass analyses, cells were collected from anode chambers and lysed in 0.2 M NaOH and 1% SDS, followed by cell debris removal by centrifugation at 14,000 rpm for 10 min.



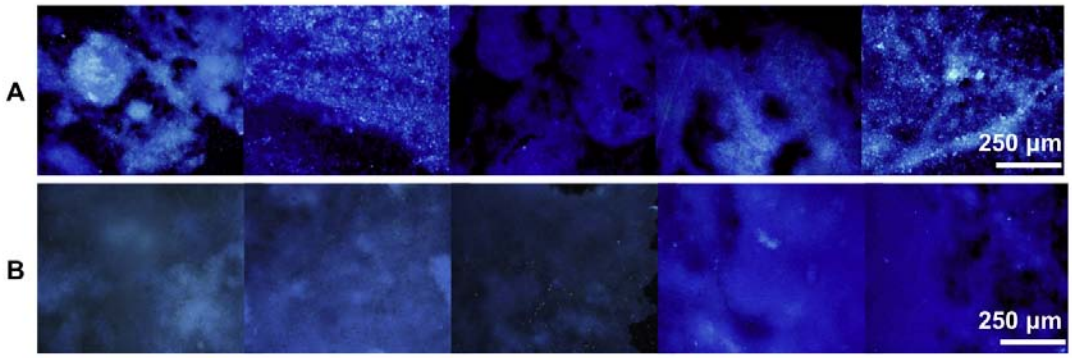
Supplementary Fig. S1. Anolyte replenishment procedure (1-5). **1**: Inflow channels are flushed with 4 types of substrates from channel inlets (1-4) and outflow channels are flushed with sterialized DI water from outflow inlet (5-8) with C_I0 and C_O0 controlled valves opened. **2**: 4 chambers in column 6 are replenished, with C_I6 and C_O6 controlled valves opened. **3**: Inflow channels are flushed with C_I0 and C_O0 controlled valves opened and then 4 chambers in column 5 are replenished, with C_I5 and C_O5 controlled valves opened. **4**: All anode chambers are replenished with identical procedure described in **3**. 5: Inflow channels and outflow channels flushed with sterialized DI water from outflow inlet (5-8) with C_I0 and C_O0 controlled valves opened. Finally all valves are closed for continuous voltage or power measurements.



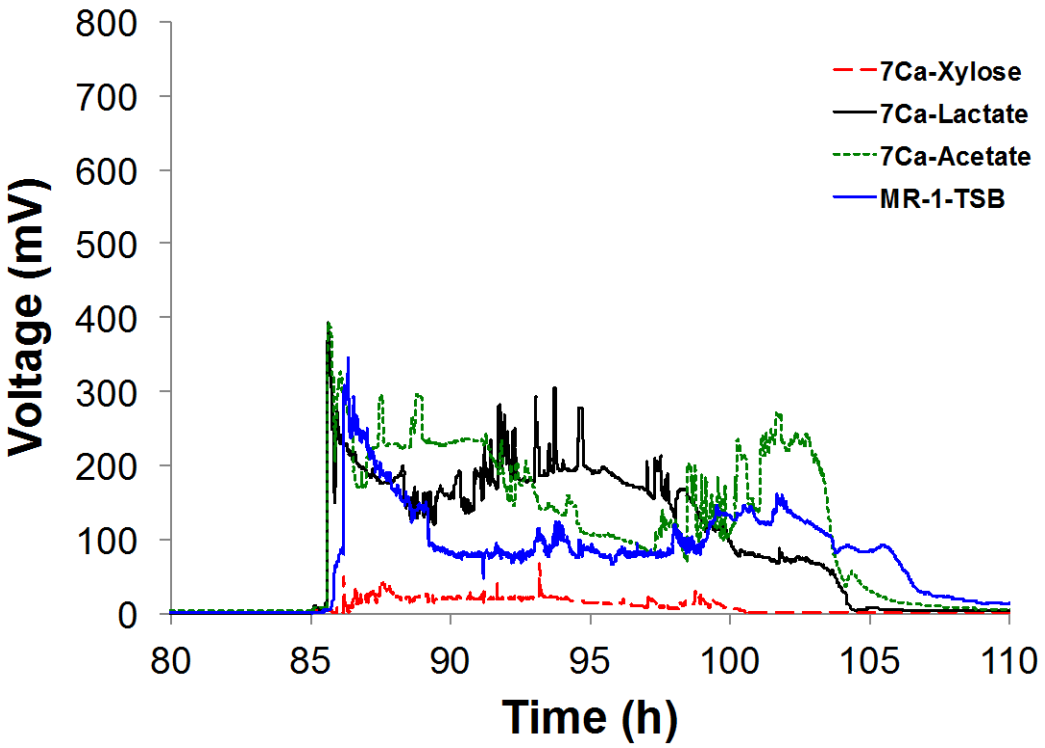
Supplementary Fig. S2. Power improvement of *S. oneidensis* MR-1 (MR-1) and 7Ca after a catholyte replenishment to previously unreplenished MFC unites. Catholyte was replenished at 195h, and power densities were measured both before (194h) and after (196h) the catholyte replenishment. (*N* = 3-5 for each case).



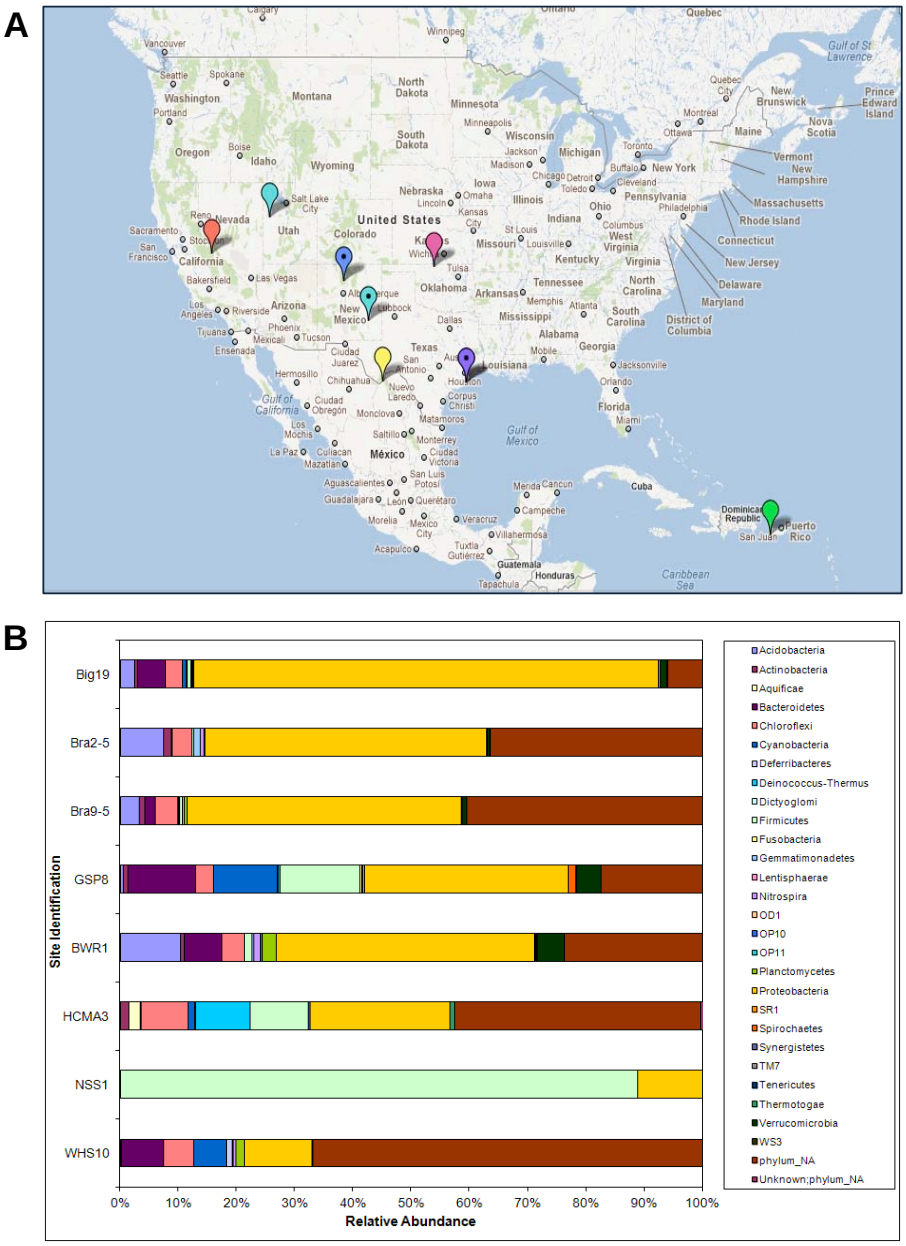
Supplementary Fig. S3. Power density improvement induced by catholyte replenishment for both (A) *S. oneidensis* MR-1 and (B) 7Ca. Measurements were taken right before (empty markers) and after catholyte replenishment (filled markers) in a sequence of: 1) power measurement, 2) catholyte replenishment, and 3) power measurement. Maximum power densities calculated from polarization curves obtained at each time point were plotted. Power density before and after each catholyte replenishment cycle were compared side by side. *(N* = 9-11 for each case).



Supplementary Fig. S4. Biofilm staining of anode electrodes with *Shewanella oneidensis* MR-1 loaded in the anode chambers (A) with and (B) without catholyte replenishment after 234 h of MFC array operation. Biofilm on anode electrodes were fixed with 4% paraformaldehyde and then stained with 2 µg/ml DAPI (4', 6-diamidino-2-phenylindole). Microscopy images were obtained with Olympus BX51 fluorescent microscope (5 of each type).



Supplementary Fig. S5. Substrate screening with *S. oneidensis* MR-1 (MR-1) and *Shewanella sp.* Hac353 (7Ca) with xylose, lactate, acetate and TSB (Substrate concentration: 50 mM). (Data published in proceedings of MicroTAS 2011)



Supplementary Fig. S6. Geographic and bacterial diversity associated with soil samples used as sources for natural communities in this study. (A) Locations in the United States and Puerto Rico where thermal and/or saline soils were sampled (Google Maps®) (accessed 20 February 2011). (B) Phylum level diversity for bacterial communities from 8 sites used in this study.

Supplementary Table 1. Two tail t-test results comparing differences between catholyte replenishment and no replenishment when using *S. oneidensis* MR-1 and 7Ca (up to 167 hours of operation). 'x' represents $p > 0.05$ where the two series of data were statistically not different, and 'o' represents $p < 0.05$ where the two series of data were statistically different. Shaded measurements indicate measurements after catholyte replenishment and others indicate measurements before catholyte replenishment. MR-1-unrep and 7Ca-unrep were measured simultaneously with MR-1-rep and 7Ca-rep. (N = 9-11 for each case)

Measurements	3	5	7	9	11	13	15	26	28	31	33	49	51	55	57	71	73	79	81	94	96	100	102	117	119	123	125	141	143	146	148	165	167
MR-1-rep&unrep	x	x	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	X	o
7Ca-rep&unrep	x	x	x	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	O	o

Supplementary Table 2. Two tail t-test results comparing differences between *Shewanella oneidensis* MR-1 and 7Ca with catholyte replenishment and without catholyte replenishment (up to 167 hours of operation). 'x' represents $p > 0.05$ where the two series of data were statistically no different and 'o' represents $p < 0.05$, where the two series of data were statistically different. Shaded measurements indicate measurements after catholyte replenishment and others indicate measurements before catholyte replenishment. MR-1-unrep and 7Ca-unrep were measured simultaneously with MR-1-rep and 7Ca-rep. (N = 9-11 for each case)

Measurements (h)	3	5	7	9	11	13	15	26	28	31	33	49	51	55	57	71	73	79	81	94	96	100	102	117	119	123	125	141	143	146	148	165	167
MR-1-rep&7Ca-rep	o	x	o	o	x	o	o	x	o	o	o	o	o	o	o	o	o	o	x	o	x	o	x	o	x	x	x	o	x	x	x	x	x
MR-1-unrep&7Ca-unrep	o	o	x	x	x	x	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o

Supplementary Table 3. Geographic, chemical, and physical properties of soils harboring microbial communities assayed in this study.

Site Identification	Sampling Date	Latitude	Longitude	State or Territory	Temperature (C°)[a]	pH[b]	EC (mmol/cm)	Organic Carbon (%)	Soil Classification	Sand (%)	Silt (%)	Clay (%)	Permit #
APHW2	05/04/10	19° 51' 10"	155° 55' 38"	HI	26.20	7.33	2.97	0.09	Sand	96	0	4	VP
Big19	03/17/09	29° 8' 59"	103° 0' 12"	TX	31.00	7.30	1.75	0.38	Sandy Loam	66	17	17	BIBE-2008_SCI-0036
Bra2-5	06/04/10	29° 3' 38"	95° 15' 37"	TX	20.60	7.71	4.26	2.08	Clay Loam	34	37	29	21543-8-101;21543-2-09-2
Bra9-5	06/04/10	29° 2' 16"	95° 16' 1"	TX	22.50	7.79	27.10	2.39	Clay Loam	30	35	35	21543-8-101;21543-2-09-2
BWR1	06/01/09	18° 0' 35"	67° 10' 15"	PR	ND	7.10	1.58	4.87	Clay Loam	35	27	38	9010;station#41521
GSP8	10/09/08	36° 47' 60"	98° 14' 58"	OK	19.10	7.40	37.10	0.40	Sand	93	0	7	RSS-08-0910;station#21630
HCMA3	08/06/09	37° 39' 41"	118° 49' 44"	CA	73.30	8.98	1.07	0.24	Loam	48	35	17	LOA
KKHW2	05/04/10	19° 46' 52"	156° 2' 31"	HI	27.50	ND	ND	20.29	ND	ND	ND	ND	H72152
LL2	11/15/08	33° 15' 14"	104° 20' 29"	NM	13.89	7.40	31.70	2.02	Sandy Loam	47	48	5	SU 09-02-01
NSS1	07/20/09	35° 54' 25"	106° 36' 58"	NM	58.70	2.58	3.65	2.20	ND	ND	ND	ND	VP
WHS10	05/01/09	39° 54 24"	113° 25' 51"	UT	48.40	7.20	30.50	1.48	Loamy Sand	79	13	8	LOP

Abbreviation: EC, Electrical Conductivity; ND, No Data.

[a]Soil temperature at the time of collection

[b]Soil pH is detailed salinity pH

References

1. A. M. Leach, A. R. Wheeler and R. N. Zare, *Analytical Chemistry*, 2003, **75**, 967-972.

2. H. Hou, L. Li, Y. Cho, P. de Figueiredo and A. Han, *PLoS ONE*, 2009, **4**, e6570.