SUPPORTING INFORMATION

Pilot scale microbial fuel cells using air cathodes for producing electricity while treating wastewater

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Operation of the MFCs and BF units

The MFC was designed for intermittent or continuously fed wastewater flowing serially through the brush anode modules. The treatment system was tested at a site in northeastern Pennsylvania (Tobyhanna Army Depot). Wastewater, after screen filtering to limit the presence of large solids, was used as the influent to the MFC. The wastewater was collected with a pump from a wet well and stored in an influent tank (170 L plastic container) positioned outside of the building in which the MFC and the BF units were installed. A skimmer tank (416 L, cone bottom, flat top) was installed after the influent tank to minimize the content of solids fed to the MFC. Initially, the wastewater flow and level in the wet well was not sufficient during the holidays, weekends and overnight to allow a continuous feeding of wastewater to the MFC, thus the flow was switched off during those periods. To partially overcome the issue of insufficient flow, a 1900 L tank and submersible pump were installed after the initial startup period to provide enough wastewater for operating the MFC overnight during weekdays. Throughout the testing period, there were brief phases of temporary shutdown that occurred due to low occupancy and the lack of associated wastewater availability for extended periods, such as holiday breaks. There were also brief periods of inoperability due to slip stream hoses clogging due to debris or freezing conditions. These periods of inoperability were not due to issues with the performance or function of the MFC/BF system. Rather, they were associated with the slip stream system that was temporarily installed by the research team at the wastewater treatment plant facility. The wastewater flow was varied between 0.76 Lpm (theoretical hydraulic retention time (HRT) of 30 h, organic loading rate of 0.47 Kg_{COD} day^{-1} based on the average influent COD) and 3.78 Lpm (theoretical HRT of 6 h, organic loading rate of 2.31 Kg_{COD} day^{-1} based on the average influent COD) depending on the availability of wastewater. Higher flow rates resulted in the flooding of the cathode module closest to the influent manifold. The wastewater was heated to approximately 30 ^{o}C to avoid freezing damage to the reactor during winter months and allow the bacteria to operate close to their optimal temperature. The pH of the influent media and the H_2S and CH_4 in the building where the MFC and BF units were installed were continuously monitored.

The BF system was operated intermittently, whereby water is passed through the biologically activated carbon (BAC) media in an upflow manner at a surface loading rate of 4 L min^{-1} m^{-2} for 6 h, followed by a 6 h bioregeneration phase in which the filter was drained. The adsorption capacity of the media is recovered during the bioregeneration phase as microbes consume the adsorbed organic contaminants in the presence of air (oxygen) that flows into the filter bed after the liquid is drained. This approach promotes aerobic treatment without the high energy requirements of conventional aeration. Thus, it is a good complementary technology for the MFC as high levels of COD removal after the generation of electricity in the MFC can be achieved with little energy use. The two upflow columns were operated in parallel on alternate cycles of approximately 6 h each. The filters were automatically cleaned to remove accumulated biomass on daily scheduled and automated intervals. Automatic cleaning was achieved by cycling of water and compressed air in an upflow manner through the filter for five minutes and then draining the resulting biomass suspension to waste. To maximize startup rates and nutrient removal, the BF was seeded with a commercial starter culture enriched with nitrifying bacteria. Previous experiments using the intermittently operated BF systems to treat gray water demonstrated this system provide sustained (> 12 months) performance for the removal of organics, even when treating an NSF-350 standard solution of synthetic gray water that contained large quantities of dust, surfactants, biocides, oils, and other contaminants (Page et al., 2020; USAPHC, 2014). Pre-validation testing of the intermittently operated BF systems with wastewater was performed using mixed wastewater at the bench scale (Ward et al., 2015).

The field demonstration was conducted over six months to fully characterize the electrochemical and system performance of the MFC and BF units. The startup included inoculation of the bioreactors and initiation of biological activity. The MFC was inoculated with the effluent of several lab- and bench-scale bioelectrochemical systems at The Pennsylvania State University combined with the wastewater stream. The completion of the startup was assessed by measuring a steady voltage and current output for each MFC module over a three-day period. Once stable operation was achieved the performance of the MFC and BF were evaluated over a six-month period.

Sampling method

The performance of the MFC was continuously evaluated through measurement of the current, voltage, and power output from each anode-cathode pairing (32 total channels). The power and current density were normalized by the cross-sectional area of the anode (0.62 m^2 each, 11 m^2 total) unless otherwise noted. The volumetric power was normalized by the active volume of the MFC (850 L), neglecting the inlet and outlet zones. During the initial three months of operation, voltage and current were monitored with a separate multimeter (Keithley 2700 voltmeter) by connecting each anode module to the two cathodes closer to it through a 4.9 ± 0.6 Ω external resistor. Following the startup and acclimation phase in the initial three months of operation, the control board was installed in the control panel allowing a continuous monitoring of the current, voltage and power output of the MFC. The wastewater flow rate, inlet pH and temperature were continuously monitored through the control panel. Total COD in grab samples was routinely measured at the MFC inlet and outlet and in the BF effluent using method 5220 (Hach COD system, Hach Company, Loveland, Colorado). The concentration of total nitrogen (method 10071, Hach), ammonia (method 10031, Hach), nitrate (method 10206, Hach), nitrite (method 8507, Hach), phosphorus (method 10209, Hach) and sulfide (method 8131, Hach) were measured at the same locations. At the end of the demonstration, the microbial biofilm on the anode and the cathode was sampled from the middle of the electrode. The microbial community analysis was analyzed by an external laboratory following a procedure previously described (Rossi et al., 2021). Turbidity of the solutions was measured with a spectrophotometer (LaMotte 1965-EPA LTC3000we). BOD_5 content, fecal coliforms and *Escherichia coli* were analyzed by an external laboratory. The total suspended solids (TSS) were measured at the MFC inlet and outlet as no solids were detected in the BF outlet. The sludge mass, TSS, volatile suspended solids (VSS) and dewaterability characteristics were measured at the end of the demonstration phase before demobilization. TSS analysis was performed by collecting the total solids portion on a Whatman glass microfiber GF/C (1822-042) passing a small volume of sample versus DI water through the filter (1.2 µm). The filter was weighed before the sample is filtered, and after the filter is dried to a constant mass at 105°C. The final concentration (mg L^{-1}) was calculated by weigh difference for the volume of samples filtered. After the TSS determination, the filters were baked at 550°C and the weight was measured again for VSS measurement. The total number and type of sample collected are reported in Table S1 and Table S2.

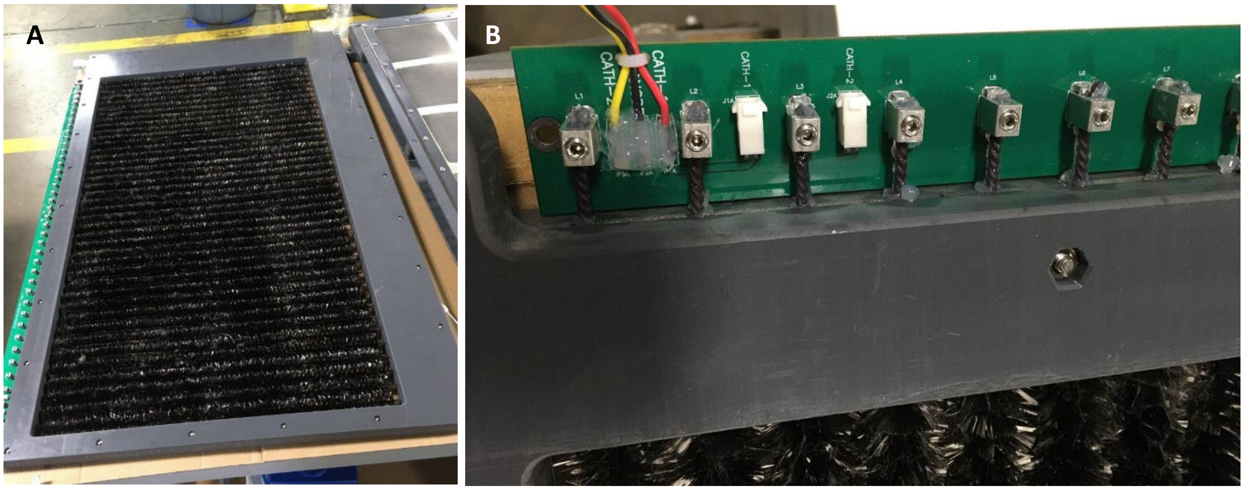


Figure S1. Photo of the (A) anode module comprising 40 individual brushes. The electroactive surface area of the anode module was 0.6 m^2. (B) Detail of the bus-bar used for connecting the brushes from the anode module and the cathodes on the two sides of the anode module.

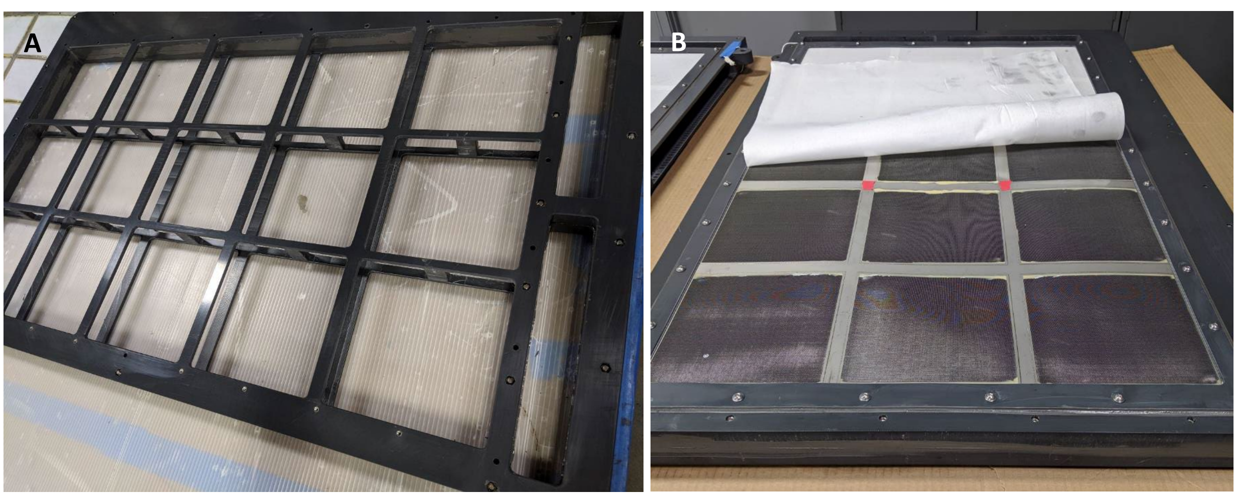


Figure S2. Photo of the (A) cathode module used to separate the two cathodes facing each other. A plastic structure was used to limit the cathode deformation due to the pressure of the water while allowing passive air flow in the cathode chamber. (B) Solution side of the cathode module, showing the stainless steel structure used to connect the 15 square cathode panels.

Figure S3. Close-up photo of the two GAC tanks of the BF unit.

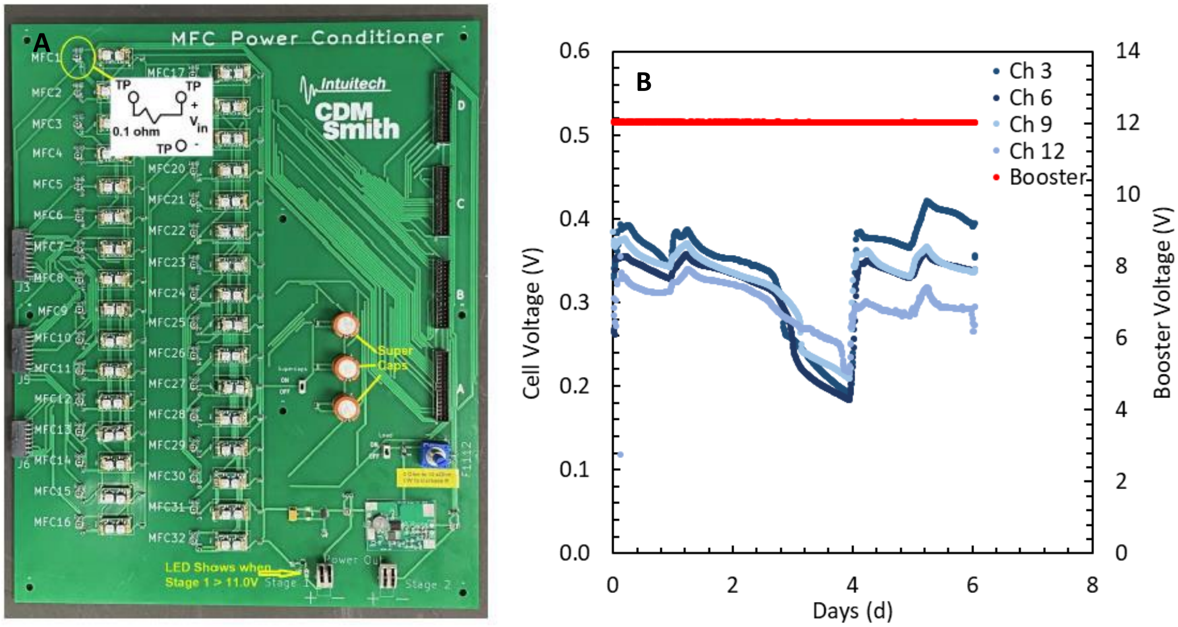


Figure S4. Power condition unit of the MFC. The (A) control board allowed a continuous measure of the power and current produced by each single module and boosted the voltage to 12 V with an efficiency of approximately 42%.

Figure S5. Photos of the MFC during operation. (A) Photo of the influent section and (B) of the side of the tank.

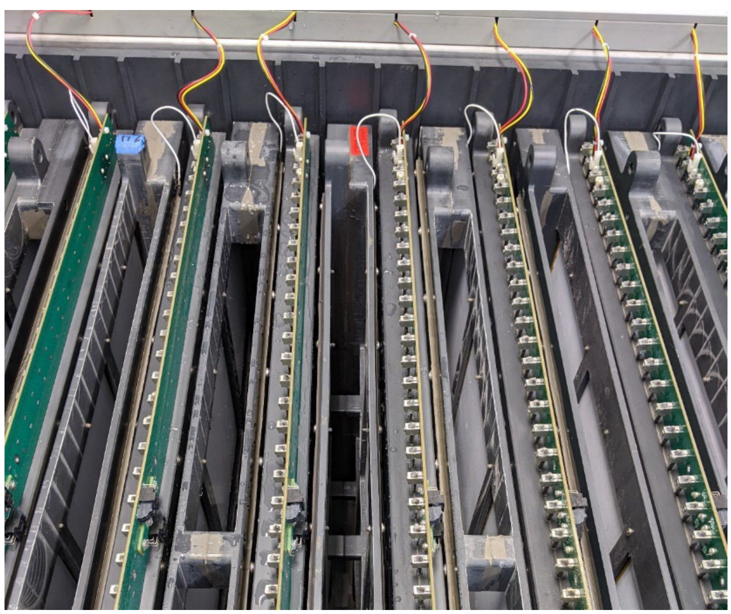


Figure S6. Detail of the MFC stacking assembly. The anode modules were facing one cathode on each side of the module. All the modules were wired to the control board.

Figure S7. Photo of the combined MFC/BF skid. The wastewater was flowing by gravity from the MFC to the BF.

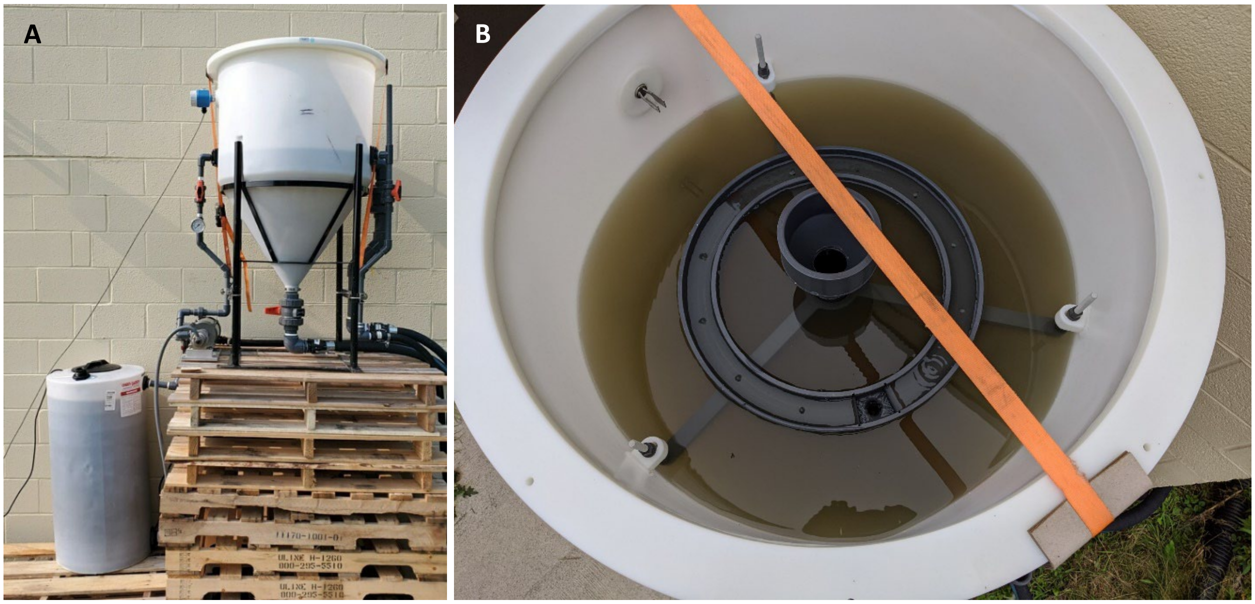


Figure S8. (A) Photo of the influent and skimmer tanks used to collect and filter the raw wastewater used in the MFC/BF skid. (B) Detail of the skimmer tank.

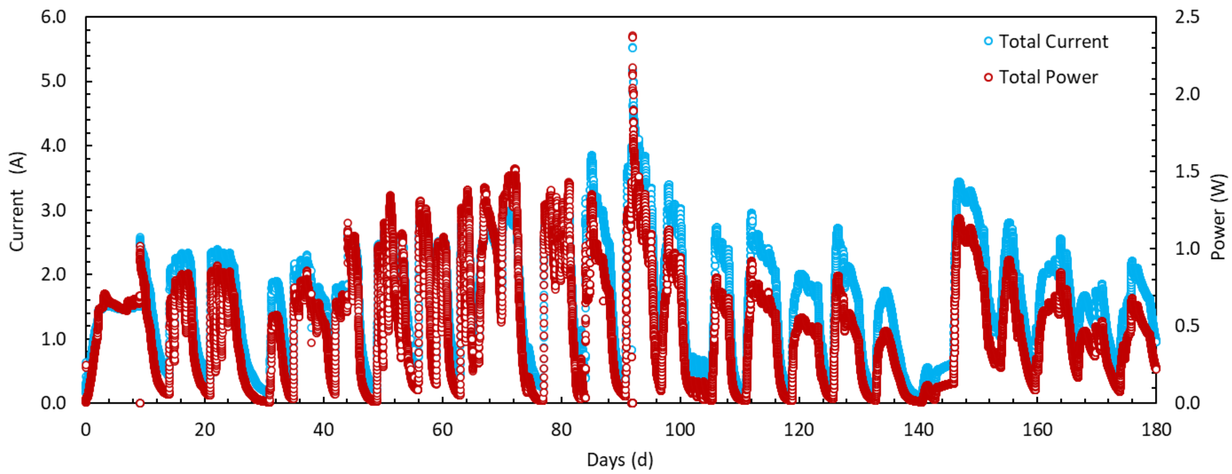


Figure S9. Performance of the pilot MFC over six months of operation in terms of total current and power generated.

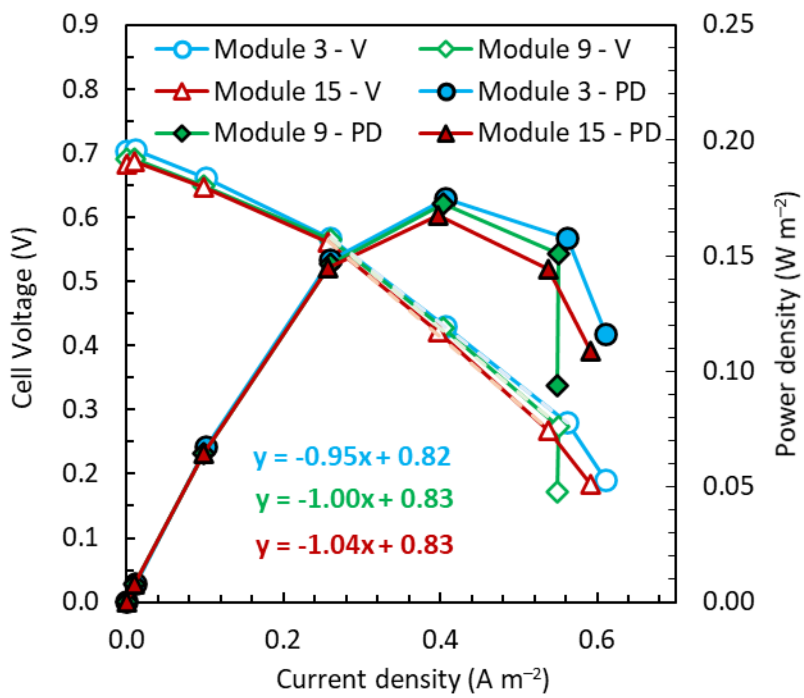


Figure S10. Polarization curve of the modules 3, 9 and 15 fed domestic wastewater. The maximum power density was 0.172 ± 0.003 W m^{-2} compared to an average of 0.151 ± 0.004 W m^{-2} (module 3: 0.184 ± 0.003 W m^{-2}; module 9: 0.152 ± 0.003 W m^{-2}; module 15: 0.116 ± 0.012 W m^{-2};), obtained for over 9 h after 81 days of continuous operation. The average internal resistance calculated from the slope of the polarization curve (faded line) was 1.00 ± 0.04 Ω m^2.

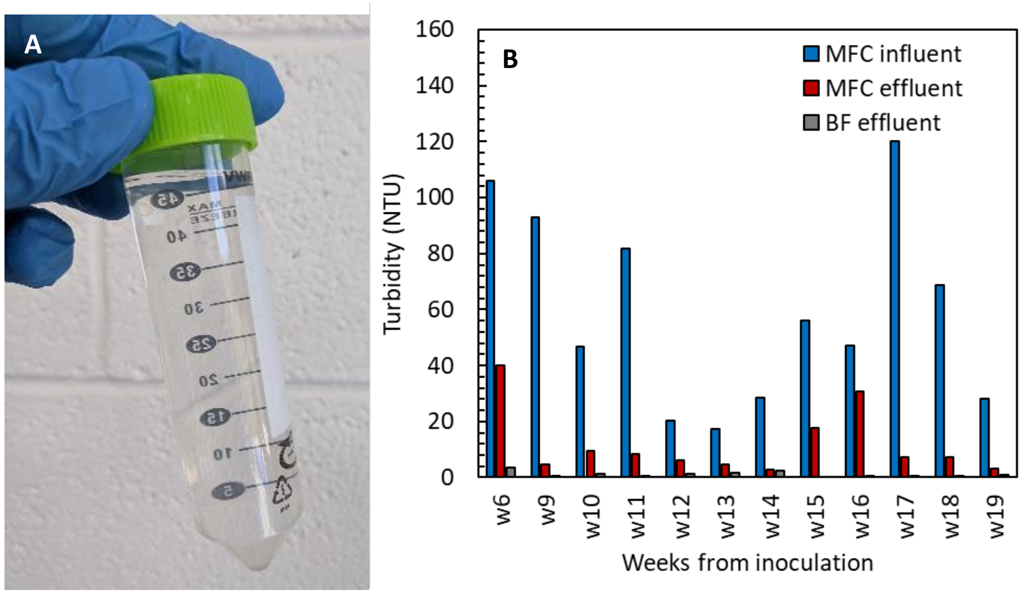


Figure S11. (A) Photo of a vial containing the BF effluent. No solids and a small amount of COD were detected in the BF effluent during the demonstration. (B) Turbidity measurement of the MFC influent, effluent and BF effluent.

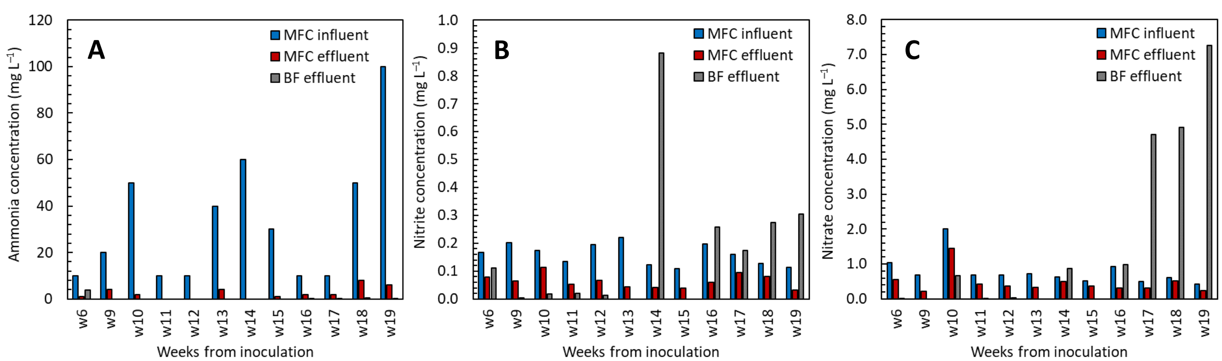


Figure S12. Concentration of (A) ammonia, (B) nitrite and (C) nitrate in the MFC influent, MFC effluent and post BF treatment.

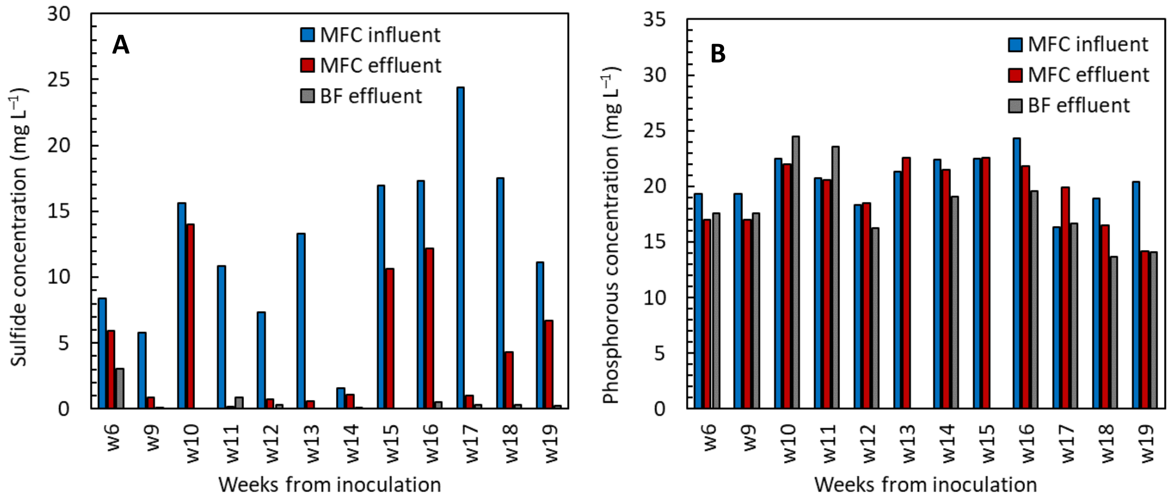


Figure S13. Concentration of (A) sulfide and (B) phosphorus in the MFC influent, effluent and post BF treatment.

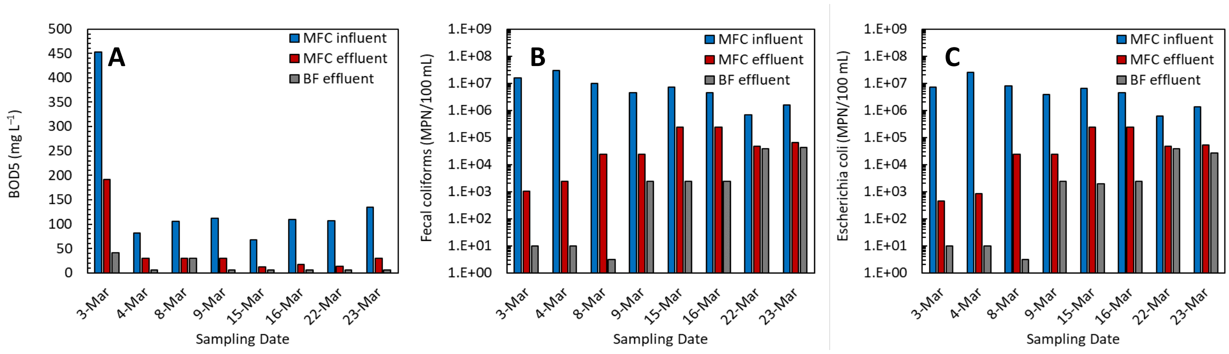
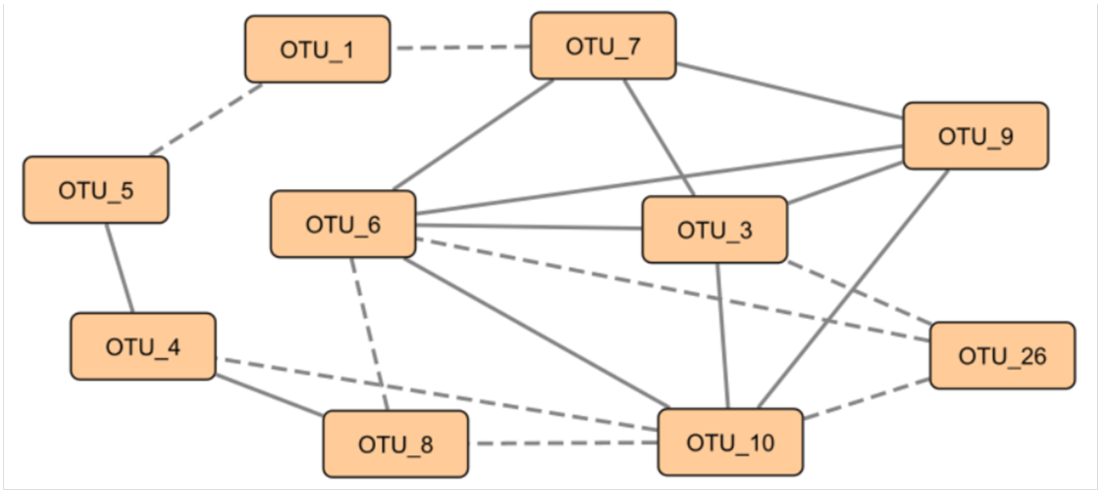


Figure S14. (A) BOD, (B) fecal coliforms and (C) *E. coli* concentration in the MFC influent, effluent and post BF treatment.



OTU	Phylum	Class	Order	Family	Genus
OTU_1	*Proteobacteria*	*Deltaproteobacteria*	*Desulfobacterales*	*Desulfobacteraceae*	*Desulfobacter*
OTU_3	*Proteobacteria*	*Deltaproteobacteria*	*Desulfuromonadales*	*Geobacteraceae*	*Geobacter*
OTU_4	*Proteobacteria*	*Gammaproteobacteria*	*Betaproteobacteriales*	*Rhodocyclaceae*	*Thauera*
OTU_5	*Epsilonbacteraeota*	*Campylobacteria*	*Campylobacterales*	*Thiovulaceae*	*Sulfurimonas*
OTU_6	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Carnobacteriaceae*	*Trichococcus*
OTU_7	*Bacteroidetes*	*Bacteroidia*	*Sphingobacteriales*	*Lentimicrobiaceae*	*Lentimicrobium*
OTU_8	*Proteobacteria*	*Gammaproteobacteria*	*Betaproteobacteriales*	*Rhodocyclaceae*	-
OTU_9	*Proteobacteria*	*Deltaproteobacteria*	*Desulfobacterales*	*Desulfobacteraceae*	-
OTU_10	*Epsilonbacteraeota*	*Campylobacteria*	*Campylobacterales*	*Sulfurovaceae*	*Sulfurovum*
OTU_26	*Proteobacteria*	*Gammaproteobacteria*	*Betaproteobacteriales*	*Rhodocyclaceae*	*Thauera*

Figure S15. Correlation network among 10 most abundant OTUs. Solid edge (positive) and dotted edge (negative) represent the correlation type between OTUs. Only statistically significant correlations (p-value < 0.05) were shown in the figure.

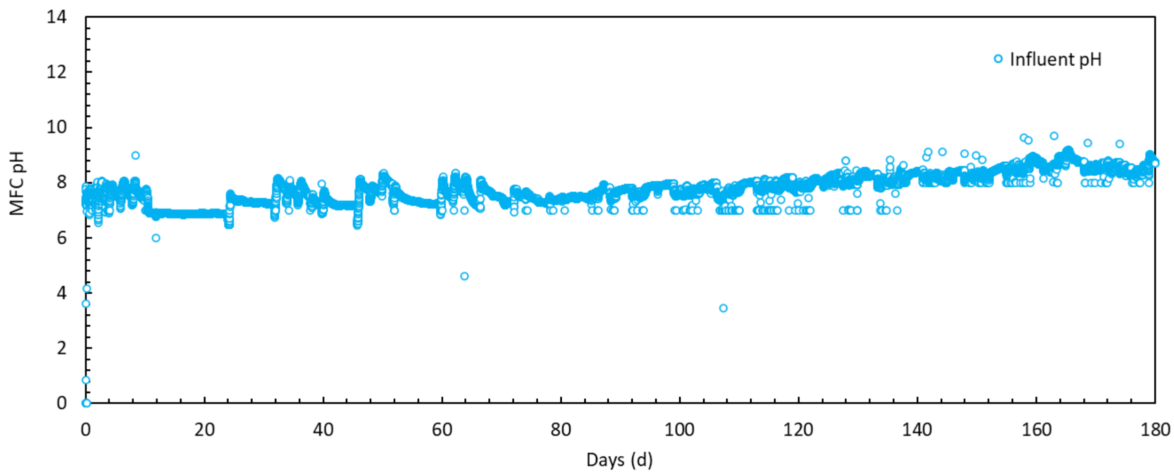


Figure S16. Influent pH over the course of the demonstration. The solution pH measured throughout the MFC and from the BF effluent did not appreciably change compared to the influent pH.

Table S1 Sample types and quantities

Matrix	Number of Samples	Analyte	Location
Wastewater	90	COD	MFC inlet, outlet, BF outlet
Wastewater	24	TSS	MFC inlet, outlet
Wastewater	36	Turbidity	MFC inlet, outlet, BF outlet
Wastewater	36	Ammonia	MFC inlet, outlet, BF outlet
Wastewater	36	Nitrate	MFC inlet, outlet, BF outlet
Wastewater	36	Nitrite	MFC inlet, outlet, BF outlet
Wastewater	36	Total phosphorus	MFC inlet, outlet, BF outlet
Wastewater	36	Sulfide	MFC inlet, outlet, BF outlet
Wastewater	90	pH	MFC inlet, outlet, BF outlet
Wastewater	Continuous	Temperature	MFC inlet
Wastewater	Continuous	Flow rate	MFC inlet
Wastewater	Continuous	Voltage	MFC
Wastewater	Continuous	Current	MFC
Wastewater	Continuous	Power	MFC
Wastewater	24	BOD_5	MFC inlet, outlet, BF outlet
Wastewater	24	fecal coliform	MFC inlet, outlet, BF outlet
Wastewater	24	*E. coli*	MFC inlet, outlet, BF outlet
Wastewater	1	Sludge wet mass	MFC
Wastewater	1	Sludge TSS	MFC
Wastewater	1	Sludge VSS	MFC
Wastewater	1	Sludge dewaterability	MFC

Table S2 Sample analysis methods

Matrix	Analyte	Method	Container	Preservative	Holding Time
Wastewater	COD	5220 (Hach)	Glass vial	None	Analyzed immediately / 72 h
Wastewater	TSS		Brass sleeve[2]	None	Analyzed immediately
Wastewater	Turbidity		Brass sleeve[3]	None	Analyzed immediately
Wastewater	Ammonia	10031 (Hach)	Glass vial	None	Analyzed immediately
Wastewater	Nitrate	10206 (Hach)	Glass vial	None	Analyzed immediately
Wastewater	Nitrite	8507 (Hach)	Glass vial	None	Analyzed immediately
Wastewater	Total phosphorous	10209 (Hach)	Glass vial	None	Analyzed immediately
Wastewater	Sulfide	8131 (Hach)	Glass vial	None	Analyzed immediately
Wastewater	BOD5	Analyzed by External Laboratory.			
Wastewater	fecal coliform	Analyzed by External Laboratory.			
Wastewater	*E. coli*	Analyzed by External Laboratory.			
Wastewater	Sludge wet mass		Plastic container	None	Analyzed immediately
Wastewater	Sludge TSS		Plastic container	None	Analyzed immediately
Wastewater	Sludge VSS		Plastic container	None	Analyzed immediately
Wastewater	Sludge dewaterability		Plastic container	None	Analyzed immediately

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