# Microbial fuel cells continuously fuelled by untreated fresh algal biomass.

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**Abstract:**

**Microbial fuel cells (MFCs) are energy transducers whereby organic matter is used as the electron donor to electroactive anaerobic microorganismes and the anode as their electron acceptor. An avenue of research in this field is to employ algae as the organic carbon source for the MFCs. However, in all studies demonstrating the feasibility of this principle, the algal biomass have always been pre-treated prior being fuelled to MFCs, e.g. centrifuged, dried and ground into powder, and/or treated by acid-thermal processes. The alternative presented here, is a flow-through system whereby the MFCs were continuously fed by the output of a photo-bioreactor. The system comprised of i) a culture of *Synechococcus leopoliensis* grown continuously in a photo-chemostat, ii) a pre-digester initiating the digestion of the phototrophs and producing a fuel devoid of oxygen, and iii) a cascade of 9 MFCs fluidicly and electricaly independant. At its best, this inline system produced 42 W of electrical power per cubic metre of fresh culture ($6.10^5$ cells $mL^{-1}$).**

**Key words:** Microbial fuel cell, ceramic membrane, in-line system, pre-digester, carbon-neutral energy.

1

2 **1 Introduction**

3 A recently developed avenue in the field of microbial fuel cells (MFCs) is the achievement of
4 self-sustainability by the introduction of photo-autotrophy in the system. MFCs are energy
5 transducers where electroactive microorganisms employ the anode as the electron acceptor for
6 their anaerobic respiration of organic matter [1-4]. Typically, MFC setups comprise two
7 electrodes: an anode and a cathode separated by a proton/cation exchange membrane. The
8 electrons are donated to the anode and flow through an external circuit, before reducing an
9 oxidising agent at the cathode (typically oxygen) combined with the incoming protons that
10 have passed through the exchange membrane. The general idea of photosynthetic MFCs is to
11 have a system whereby the energy comes from light and the carbon from carbon dioxide
12 [5,6]. Phototrophs can also be employed as the catalyst for the cathodic oxygen reduction
13 reaction [7-9], however this is not the scope of the present paper. The aim was to introduce
14 photoautotrophic microorganisms via a pre-digester, directly into a cascade of 8 MFCs as the
15 carbon source (fuel) for electroactive heterotrophs, without any energy-consuming pre-
16 treatment.

17 Phototrophs have previously been grown as biocatalysts for electron transfer using an added
18 mediator (e.g. 2-hydroxy-p-naphthoquinone) [3]. These phototrophs were selected to produce
19 hydrogen, as an endogenous mediator, which enables the development of a solar driven
20 hydrogen fuel cell [10]. Recently it was shown that algae could serve as fuel for MFCs, either
21 as an internally generated carbon-source, since they were growing within an illuminated
22 anodic compartment [6,11,12], or added as an external source of carbon [13-15]. The
23 incorporation of photoautotrophy within the MFC itself, even though easily implemented,
24 does not produce high voltage or power compared to more conventional MFCs containing
25 anodophilic bacteria. The important element of distinction from previous work is that
26 whenever algal biomass was employed as an external feedstock, it had always undergone

energy-consuming treatments [13-15] such as centrifugation, drying and grinding, and/or acid-thermal treatments.

The aim of this work was therefore to demonstrate that algal biomass could be employed as MFC fuel without the need for energy consuming pre-treatment, by recreating a simplified trophic chain [6], thus maintaining a continuous hydraulic flow and supporting a dynamic steady state [16]. The trophic chain was initiated by oxygenic photosynthesis that fixed organic carbon in a photo-bioreactor. Syntrophic fermenters then initiate digestion and transformation of the photosynthetic biomass into secondary fermentation products (short chain fatty acids) in a pre-digester. The processed digest was then further hydrolysed and utilised by the electroactive organisms within the MFCs [4,6,17]. The aim was to investigate the possibility of running a cascade of MFCs continuously via pre-digestion of fresh algal biomass, rather than create another photomicrobial solar cell, or photosynthetic microbial fuel cell [18].

## 2 Material and Methods

### 2.1 Strain and culture media

The anodes were inoculated with activated sludge (Wessex Water, Saltford UK). The microbial fuel cells (MFCs) were maintained in batch mode for 2 weeks under a 1.5kΩ load, and subsequently operated under continuous flow.

The strain employed as the primary element of this artificial system was *Synechococcus leopoliensis* (obtained from www.sciento.co.uk; A.591). The medium used for the growth of the oxygenic phototrophs in the photo-chemostat was BG-11 [19]. The pH was adjusted to 7.2 prior to autoclaving.

### 2.2 MFCs design and operation

A cascade of 9 MFCs was constructed as shown in Figure 1, as opposed to a single enlarged MFC of equivalent volume; the cascade is also operated in sequential mode, where the output of one MFC feeds the next one downstream. Such a setup allows for a better utilisation of the organic matter into electricity because of shorter diffusion distances [20,21]. The anodic

compartment (4.5 mL) was built in black acrylic material to avoid any development of phototrophic organisms. For the same reason all the tubing used was black ISO-Versinic (3 mm ID; Saint Gobain Performance Plastics, FR). The anodes were made from a 64 $cm^2$ sheet of carbon fibre veil (20 g $m^{-2}$) (PRF Composite Materials Poole, Dorset, UK). The cathode employed the same carbon fibre veil but with a 160 $cm^2$ total surface area. Both electrodes were folded down to a 3D structure with an exposed surface area of 3.3 $cm^2$. The membrane had a surface area of 6.8 $cm^2$ and consisted of 2mm thick terracotta (CTM potter supplies, UK). The water absorption (% of weight) of the terracotta membranes was 9.1 % ± 0.3 % [22]. Tap water was employed as the catholyte with continuous flow set at 5 mL $min^{-1}$. Light-tight gas-gap drippers were placed between each MFC to avoid any electrical cross-circuit via fluidic conduction from unit to unit, thus allowing each MFC to be electrically isolated for monitoring purposes. The total volume of the anodic compartment, tube and gas-gaps was approximately 6.5 mL.

### 2.3 Photo-chemostat design and operation

The photo-chemostat was implemented in order to have a continuous source of fresh algal biomass, as feedstock for the MFCs. Therefore, the optimisation of the growing conditions was not the aim of the present study. The photo-chemostat was a 1000 mL glass vessel with a rubber butyl septum (Glasgerätebau Ochs, Germany). The photo-chemostat was set on a 12-hour diurnal rhythm to simulate light/dark cycles typical of natural algal production systems. The 12 h light shift regime consisted of a light dose equivalent for 24 h of 40 μE $m^{-2}$ $s^{-1}$ ± 5 μE $m^{-2}$ $s^{-1}$ (34 W; Cool White, Sylvania), incubated at ambient room temperature (23°C ± 2°C), and under constant agitation. An aquarium pump was constantly pumping air into the vessel through an autoclaved air filter (Midisart® 2000 PTFE 17805; Sartorius). At first, the photo-chemostat was run in batch mode and inoculated with 10 mL of mother culture ($5.85x10^3$ cells $mL^{-1}$ final concentration). The algal growth was monitored by cell counts with a haemocytometer (AC1000 Improved Neubauer, Hawksley, UK) and the maximum growth rate calculated in order to select an appropriate input dilution rate for continuous operation as

a photo-chemostat by connecting it, through a peristaltic pump (Welco Co.,Ltd, Japan), to a 10 L tank of sterile media (BG-11). All tubing and connectors were autoclaved and assembled under sterile conditions. Moreover, to avoid any contamination of the photo-chemostat from downstream MFCs or pre-digester, two sterile anti-grow-back dripping mechanisms were present at the output of the photo-chemostat. Two identical dripping systems were also introduced between the 10 L tank of sterile media and the photo-bioreactor to prevent the former from grow-back contamination.

### 2.4 Pre-digester

In the second phase of the experiment, a pre-digester was added between the photo-chemostat and the cascade of MFCs (Figure 1). The pre-digester comprised a non stirred 1000 mL light-tight glass bottle with a rubber butyl stopper, separating the vessel from the outside environment and allowing inlet and outlet tubes from the top. The inlet extended 5 cm into the vessel whilst the outlet tube reached 0.5 cm from the floor of the vessel. The pre-digester had a working volume of 500 mL ± 15 mL (Figure 1). The pre-digester was first inoculated with 50 mL of MFC effluent.

### 2.4 Data capture

The electrical output of each MFC was measured in millivolts (mV) against time using a PicoTech data logger (ADC-24, Pico Technology Ltd). The voltage was recorded every 2 minutes. The current $I$ in Amperes (A) was calculated using Ohm's law, $I = V / R$, where $V$ is the measured voltage in Volts (V) and $R$ is the known value of the resistor. The power output $P$ in watts (W) was calculated as $P = I \times V$.

## 3 Results and discussion

Based on the maximum growth rate under 12 h light shifts ($\mu_{max}$= 0.086 $d^{-1}$ ± 0.005 $d^{-1}$; Figure 2), the calculation of the optimum flow rate resulted in a replacement rate of 86 mL per day. However, to avoid the culture from being washed-out, the flow rate applied to the photo-chemostat (Figure 1) corresponded to 87% of the optimum flow rate ($F_{87\%}$ = 75 mL $d^{-1}$ ± 4.6

mL $d^{-1}$). Due to the low flow rate, cells from the chemostat were settling in the tubes between each stage of the system. Therefore, a pulse-feed regime was applied to the whole in-line system, where 12.5 mL were pumped every 4 hours. At this flow rate, the hydraulic retention time (HRT) of each MFC was therefore 2 h, and for the stack of 9 MFC in cascade, 18 h.

Electricity production by the cascade of MFCs fed directly from the photo-chemostat was close to zero prior to the introduction of the pre-digester (Figure 3a). This was also confirmed by the measurements of the open circuit voltage ($V_{oc}$, no current produced) that was lower (34 mV; Figure 3b) than comparable MFCs (500 mV < $V_{oc}$ < 900 mV) when fed digest from TYE media [23]. Such a low $V_{oc}$ suggests that the difference of redox potential between the anodic and cathodic compartment was unusually small for generating good power output levels [9]. As the cascade was fed with fresh cells of oxygenic phototrophs, it was postulated that the low $V_{oc}$ was due to the high concentration of oxygen in the anolyte fuelling the MFCs. Thus, the cascade was disconnected from the photo-chemostat and connected to the mixed-culture pre-digester that was set at an HRT of 10 days and would deliver a fuel devoid of oxygen whilst containing more accessible organic matter (e.g. organic acids). As illustrated by the $V_{oc}$ (Figure 3c), following this step, the redox potential difference reached 795 mV ± 5 mV. Based on these results, a pre-digester was introduced between the photo-chemostat and the cascade of MFCs (Figure 1).

The introduction of the pre-digester, in-line between the photo-chemostat and the cascade, resulted in the production of power, which was consistent with the HRT of the pre-digester (160 h; Figure 4a). Because of the cascade configuration and of the pulse-feed regime, the anodic volume of each MFC was renewed 63 times during the last 127 h of the experiment. Therefore, it can be assumed that MFCs 7 and 8 had reached a dynamic metabolic steady state during the last 48 h, since the electrical output of these MFCs had reached a plateau. The electrical output of the MFCs, up to MFC 5, was lower or had not yet reached steady state, albeit some of them yet increasing (i.e. MFC 5). It is assumed that MFCs prior to MFC 7 were limited because of the quality of the organic feedstock (partially hydrolysed), whilst the lower

1 output of MFC 9 could be because most of the available carbon energy fuel has been utilised
2 by all previous MFCs in the cascade (i.e. starvation). These results thus demonstrate the
3 potential power capability of the cascade stack, giving in total, by mathematically adding the
4 output of isolated units, up to 42.5 W $m^{-3}$ of fresh culture ($10^5$ cells $mL^{-1}$) that would be
5 expected to be produced if the units had been electrically connected together in series or
6 parallel.
7 Assuming that the system was reaching its steady state, during the last 127 h, the areas under
8 curve of each of the last MFCs illustrated sequential metabolic steady states, according to
9 their physical position in the cascade. Since all MFCs were identical in design and flow rate,
10 the feedstock quality is defined here as the content of accessible organic electron donors for
11 the electroactive respiration, which is then reflected as the electrical power output of each
12 MFC [24]. Based on the amount of power produced during the last 127 hours of the
13 experiment, MFCs 7 and 8 were the most powerful (Figure 4b). It can thus be hypothesised
14 that they were consuming feedstock that had sufficient retention time to give maximum
15 availability of nutrients as monomers or small molecular weight products of hydrolysis. This
16 also suggested that prior to MFC 7, the digestion of the feedstock was insufficient for
17 maximum power production, and by MFC 9, the feedstock was possibly depleted of most of
18 its total carbon-energy sources by all previous MFC (Figure 4). Therefore, these findings
19 suggest that the HRT of each MFC was too short, since it took 10 h for the biomass to reach a
20 level of quality suited for electro-active respiration (HRT of 2 h per MFC). Subsequently, in
21 the final experiments the HRT of each MFC was set to 12 h whilst maintaining the pre-
22 digester HRT at 160 h by decreasing the photobioreactor volume to 150 mL (dilution rate of
23 0.5 mL $h^{-1}$). Again, to avoid cell sedimentation within the tubing, the system was under a
24 pulse-feed regime of 4.2 mL every 8 h. Results of this second setup indicate that the power
25 production was slightly lower than in the previous case (Figure. 5; total potential power ± 29
26 W $m^{-3}$). Under this regime, it was the first MFCs of the cascade that produced higher power
27 (MFC 1 to 3), which implied that the HRT of each MFC was now too long. This was

evidenced by the behaviour of the power output of the first MFC: initially the power was increasing, then after a short plateau the power decreased until new fuel was pumped in. The maximum power output produced by each MFC indicated that, under this configuration, the feedstock-biomass reached the desired optimum quality at the $2^{nd}$ MFC. The empirical observation of the HRT being either too short or too long *in situ* is an important finding illustrating that the electrical profile of the stack output can be used as a tool for tuning practical MFC stacks in the field. The results thus demonstrate with a certain degree of accuracy that this is an impotant feature that only exists with a cascade system such as the one described here, since the pre-digester on its own could not generate a utilisable digest for electroactive anaerobic respiration (see Fig.4 and 5). Overall, the system gave a dynamic power output state over a period of 60 h (stability), thus confirming that fresh algal biomass can fuel a cascade of MFCs via a pre-digester, without any complicated and expensive pre-treatment.

The instability of the system during a period longer than 2-4 days was due to the instability of the fuelled digest from the pre-digester: once lysed the digest would typically sediment. This sedimentation resulted in a heterogeneous fuelling of the MFCs, which means they received a rich fuel at the beginning and then a more diluted substrate. Thus, the stabilisation over time of a continuously fed in-line system may require some removal of the top supernatant by a separate flow system in order to concentrate the lysed organic matter as a sediment, thus allowing a stable feeding of the stack.

## 4 Conclusions

The present study reports on the feasibility of producing light-driven carbon-neutral electricity with MFCs. It was demonstrated that algal biomass could continuously feed a cascade of MFCs in an in-line system consisting of a photo-chemostat, a pre-digester and an electro-active cascade. The advantage from the use of such an in-line system is that the algal biomass does not need any energy-consuming pre-treatment such as centrifugation, grinding and/or acid thermal treatment, since an adapted pre-digester is sufficient to fuel MFCs with

pre-processed algal biomass. However, for the longer-term stability of the system, the HRTs of each compartment would need further investigation and optimisation since this is a critical aspect of the system stability.

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**Figure caption:**

**Figure 1:** Illustration of the in-line system setup. Each MFC is separated from the previous one by an air-gap (not shown) avoiding electrical connection through the anolyte. The pre-digester was introduced in the second phase of the experiment. The catholyte was recycled.

**Figure 2:** Growth curve of the *Synechococcus leopoliensis* culture under the given conditions. The cell concentration of the first time point was 5.85 $10^3$ cells $mL^{-1}$. Error bars represent the variation in the samples determination.

**Figure 3:** Voltage evolution of the first MFC of the cascade prior to the introduction of the pre-digester. a) Closed circuit voltage (Vcc; 1.5 KΩ resistor). b) Open circuit voltage when the photo-chemostat directly fuelled the cascade of MFCs. c) Open circuit voltage when the cascade of MFCs was disconnected from the chemostat and fed with *Synechococcus leopoliensis* derived biomass taken from a separate (not connected in line) pre-digester (HRT of 10 days).

**Figure 4:** **a)**Evolution of the power produced by the cascade of 9 MFCs when placed in-line with a photo-chemostat and a pre-digester. **b)** Amount of energy per day produced by the last MFCs of the cascade over the last 48 h of the experiment. Error bars represent the Standard deviation (n=1440): the variation of the produced power per MFC (standard deviation over 48 h) was transposed into units of energy.

**Figure 5:** Power produced by the first MFCs of the cascade, when the HRT of each unit was set at 12 h. Arrows indicate pulse-feed points.

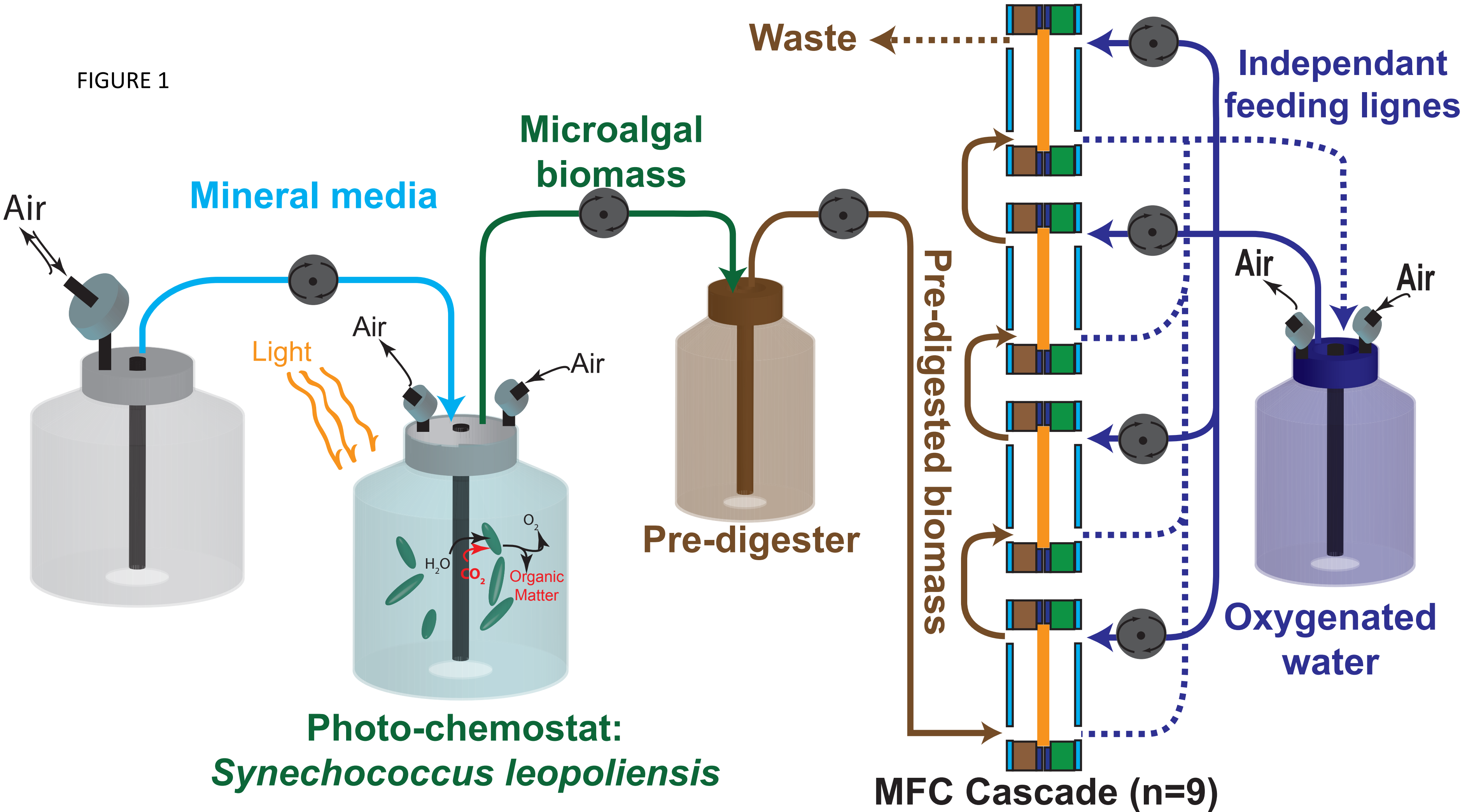
FIGURE 1
Waste
Independant feeding lignes
Microalgal biomass
Mineral media
Air
Air
Light
Air
Pre-digested biomass
Air
Air
Pre-digester
H2O
O2
CO2
Organic Matter
Oxygenated water
Photo-chemostat:
Synechococcus leopoliensis
MFC Cascade (n=9)

FIGURE 2

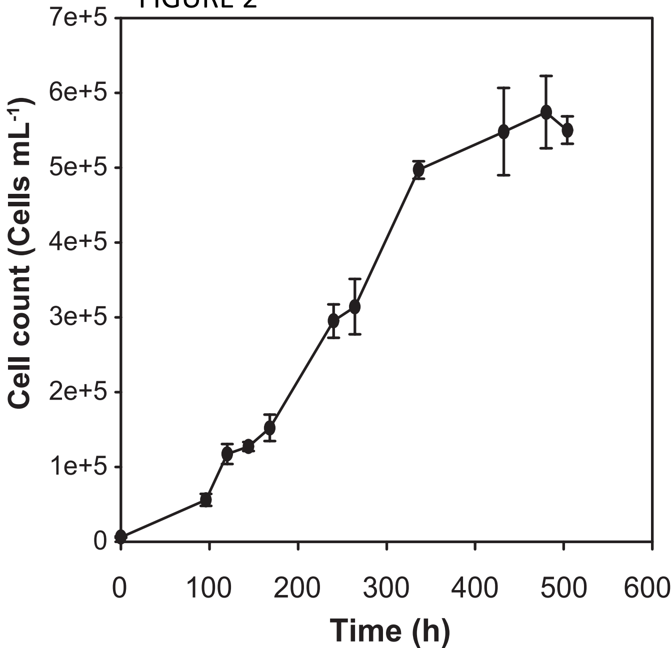

FIGURE 3

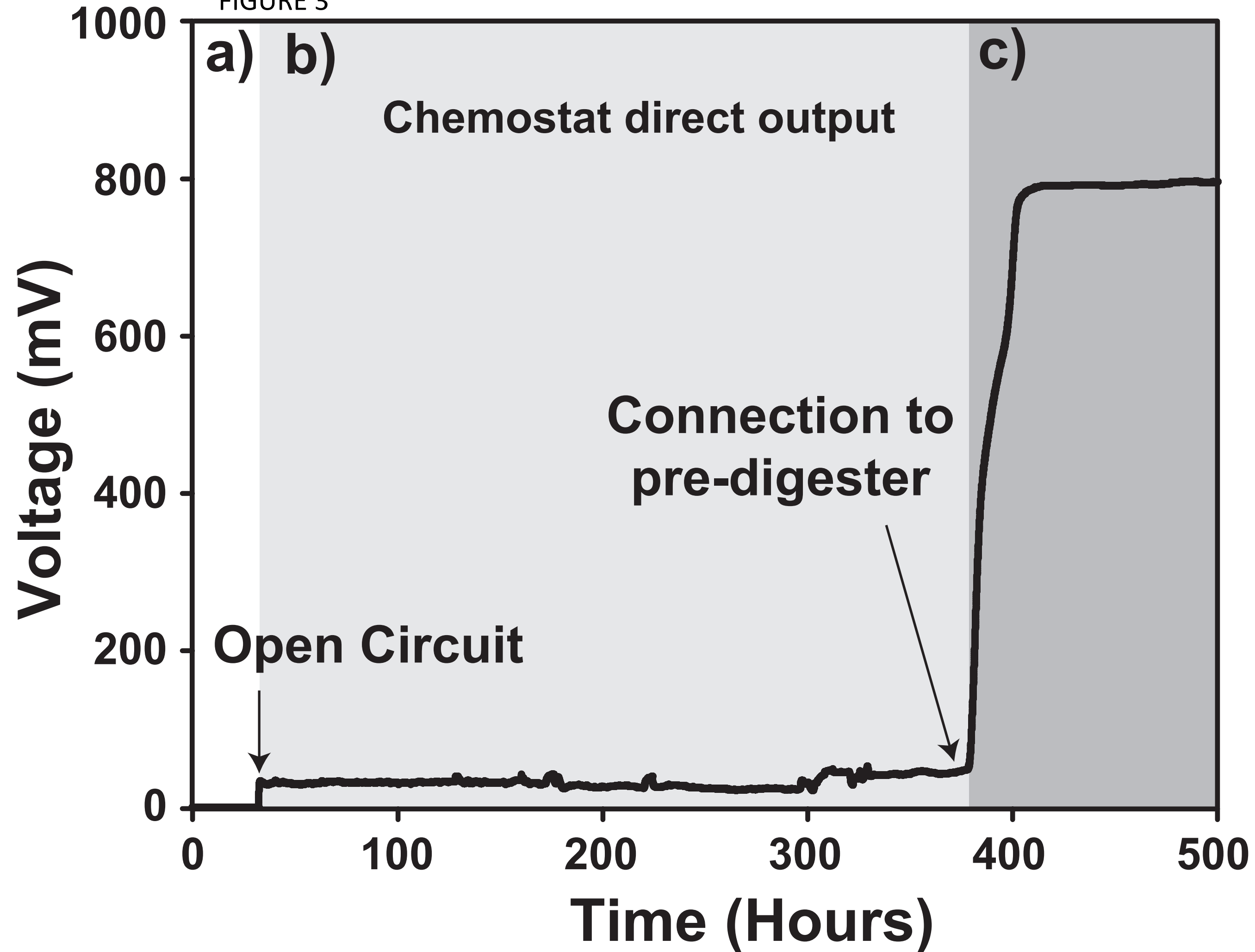

FIGURE 4

a)

Power (μW)

Time (days)

MFC 1
MFC 2
MFC 3
MFC 4
MFC 5
MFC 6
MFC 7
MFC 8
MFC 9

Pre-digester
Hydraulic retention time
(160 h)

b)

Energy produced ($J\ d^{-1}$)

MFC 4
MFC 5
MFC 7
MFC 8
MFC 9

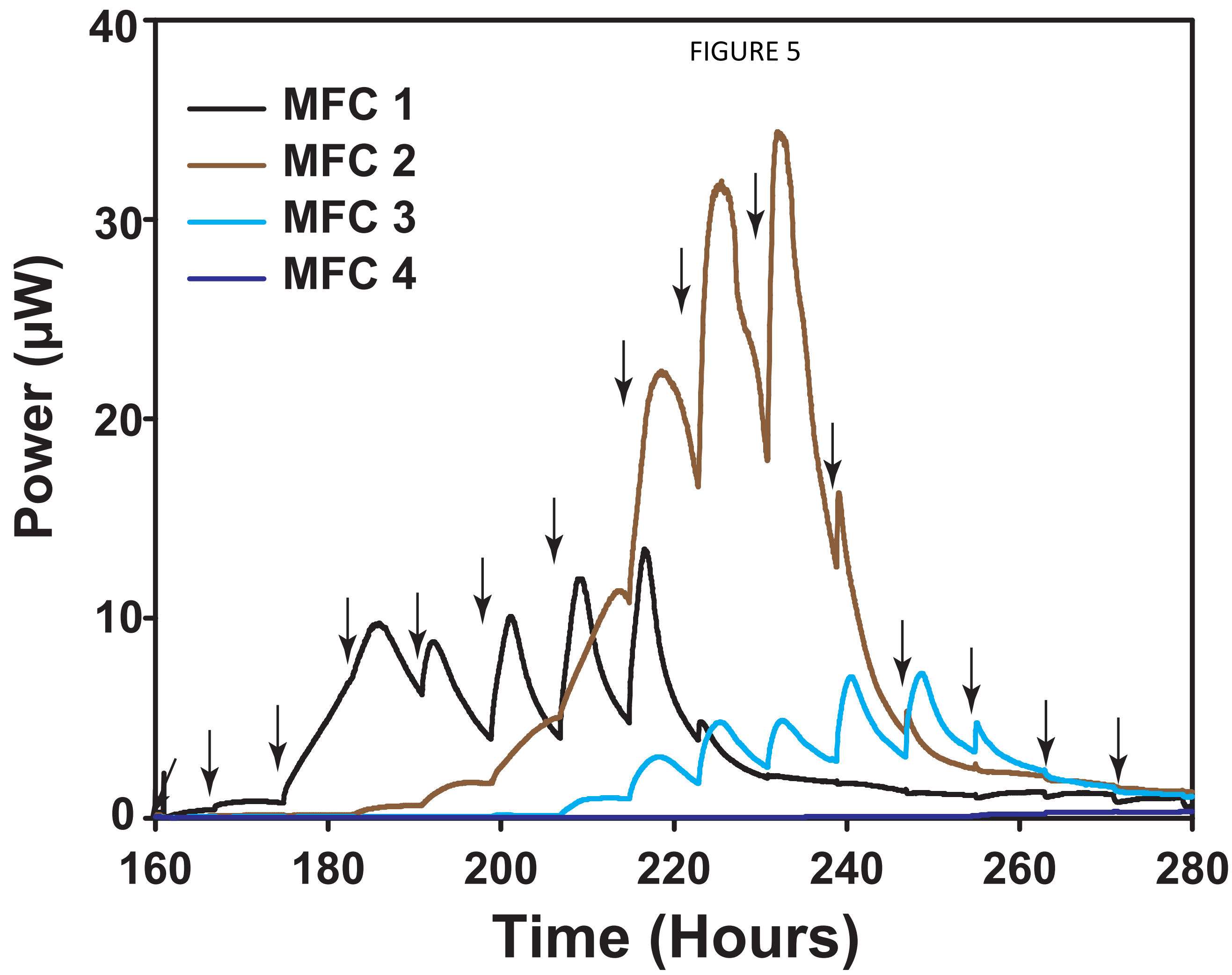
FIGURE 5
MFC 1
MFC 2
MFC 3
MFC 4
Power (μW)
0
10
20
30
40
160
180
200
220
240
260
280
Time (Hours)