EvoBot: towards a Robot-Chemostat for culturing and maintaining Microbial Fuel Cells (MFCs)

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Abstract. In this paper we present EvoBot, a RepRap open-source 3D-printer modified to operate like a robot for culturing and maintaining Microbial Fuel Cells (MFCs). EvoBot is a modular liquid handling robot that has been adapted to host MFCs in its experimental layer, gather data from the MFCs and react on the set thresholds based on a feedback loop. This type of robot-MFC interaction, based on the feedback loop mechanism, will enable us to study further the adaptability and stability of these systems. To date, EvoBot has automated the nurturing process of MFCs with the aim of controlling liquid delivery, which is akin to a chemostat. The chemostat is a well-known microbiology method for culturing bacterial cells under controlled conditions with continuous nutrient supply. EvoBot is perhaps the first pioneering attempt at functionalizing the 3D printing technology by combining it with the chemostat methods. In this paper, we will explore the experiments that EvoBot has carried out so far and how the platform has been optimised over the past two years.

Keywords: EvoBot, Microbial Fuel Cells, 3D-printer, Cathode, Rep-Rap

1 Introduction

The term robot has been defined by the International Organization for Standardization (ISO) as "*a programmable actuated mechanism with a degree of autonomy, which is able to move within its environment in order to perform intended tasks*". With this in mind a Rep-Rap 3D-printer was modified into a laboratory robot in order to inoculate and maintain Microbial Fuel Cells (MFCs), in a similar manner to the maintenance of bacterial cultures within a chemostat, and perform interactive experiments with minimum human supervision; this marked the start of EvoBot.

The first commercially available laboratory robot, the Robot Chemist, was marketed in 1959 with the aim to automate wet-chemical analytical procedures [1]. This was the first step towards laboratory automation practices. Twenty years later, lab robots were introduced into the pharmaceutical industry for drug analysis [2]. After a long

period of adoption, robots are today playing a significant role in all aspects of laboratory procedures; from routine chemical analysis to drug development and DNA analysis. Autonomous laboratory robotics have generally advanced lab procedures since they offer: accuracy, speed, convenience and they are cost effective compared to labour cost. One research area that can directly benefit from such advances in laboratory robotics is Microbial Fuel Cells (MFCs).

Microbial Fuel Cells (MFCs) are bio-electrochemical devices that use microorganisms to generate electrical current through the oxidation of organic matter (i.e. anaerobic sludge, urine, acetate). For the last 17 years, MFCs have been closely associated with autonomous robots due to their capabilities of providing energy autonomy to biologically inspired robots such as Gastrobot [3], Ecobot I, II, III [4–6] and Row-bot [7]. Even though the above examples are proof-positive that energy autonomy is both plausible and feasible through the use of MFCs, research is still needed to reach full MFC potential and increase the capabilities of these artifacts, which are known as Symbots [8].

Due to the increasing demand for alternative ways of powering robots, MFC research has recently attracted a lot of interest that led to breakthrough discoveries [9,10]; the research emphasis is now on the development of the next generation of improved and optimised MFCs for maximum power production. In this line of thought, EvoBot attempts to implement the 3D RepRap technology as a mechanism for printing organic and inorganic substrata as well as accurately dosing the biofilm (microbial community adhered to the anode electrode) of the fuel cell with organic matter in the same manner as a chemostat. The chemostat is a widely-used apparatus for culturing cells [11] that enables the experimental control of cell growth rate, in order to study the adaptive evolution of microbes and achieving dynamic steady-states [12]. The cell culture grows and evolves within the chemostat in the presence of a continuous flow of nutrients. The vessel retains a constant volume as an overflow system is in place (Fig. 1). The optimum aim of EvoBot is to enforce, monitor and interact with evolving systems and eventually produce optimally evolved/adapted MFCs which will have improved energy generation capabilities.

EvoBot has been developed to perform similarly to the chemostat and the work has been broken down into different phases. During Phase 1, EvoBot demonstrated interface and interconnection with an MFC. Shortly after, during Phase 2 a long term experiment with 9 MFCs was performed. EvoBot was maintained by using a feedback loop to control the nutrient supply rate to the fuel cells based on reduction in power below a threshold [13]. Now EvoBot is in Phase 3 where it has been optimised to host more MFCs on to the platform, using two direct-current (DC) pumps to supply media using and two syringes to distribute the media into the cells. The main aim of this work was to demonstrate the development of EvoBot as a modern-day robotic biofilm bioreactor continuous culture system (based on the “chemostat” approach) for studying MFCs and their optimisation for producing power.

2 EvoBot

EvoBot is a modular, open source, versatile, and affordable robotic platform, which has been developed to perform liquid handling experiments; in the context of the EVOBLISS EU project (FP7-ICT). Evobot has been used in seven different laboratories around the world for diverse applications, such as interaction with MFCs [13], moving droplets, and improving the quality of artificial life experiments and performing OCT scans [14].

EvoBot consists of an actuation layer on top, an experimental layer in the middle, and a sensing layer at the bottom as can be seen in Fig. 2. The actuation layer comprises the robot head and modules mounted on it. The modules are plugged into the head and are usually designed to perform an action on the experiment. However, they may also have sensor functionality e.g. OCT (optical coherence tomography) scanning, an imaging technique which allows for optical sectioning of the sample. Such a sample can be the electroactive anodic biofilm of an active MFC with transparent anode chamber. The head which holds the modules can be moved in the horizontal plane. EvoBot's modularity allows for support of modules of different kinds for various applications. The experiment-dependent modules could entail syringe modules for liquid dispensing or aspirating, grippers to move the containers over the experimental layer or dispose dirty containers, an OCT scanner module to perform OCT scans, an extruder module to 3D print MFC parts, and other potential experiment-specific tools.

The experimental layer consists of a transparent Poly methyl methacrylate (PMMA) sheet on which reaction vessels (e.g. Petri dishes, well plates, beakers etc) and/or MFCs are positioned. The actuation layer interacts with the experimental layer by filling or emptying a specific volume to/from a syringe, washing a syringe, or/and disposing dirty containers.

The robot frame is built from Aluminium profiles, and the experimental layer and actuation layer are mounted on it. The layers can be easily moved up or down on the robot frame with a cam lever mechanism.

Configuring EvoBot for different experiments is easy, as different types of modules can be easily removed or plugged at the appropriate position. The head is responsible for moving the modules in the x-y plane, while the modules have motors to move vertically. The robot head can accommodate syringe modules to aspirate or dispense liquid at 17 potential positions, and up to 11 syringes can be used simultaneously as the socket positions overlap with adjacent ones.

EvoBot's design is based on open-source 3D printers using an Arduino board and a RAMPS shield. This electronics design allows building on existing software for open-source 3D printers. A computer controls the robot by communicating with the Arduino through serial communication. The Arduino is connected to the RAMPS shield. The RAMPS shield controls the three stepper motors to move the robot head, the modules mounted on the robot head, as well as the two DC pumps.

In the experiment presented below EvoBot was primarily used for MFC inoculation and maintenance as well as a characterization tool akin to chemostat. This is where the novelty of the project lies as the interaction of biochemical systems with the 3D printing technology can lead to a Robot-Chemostat.

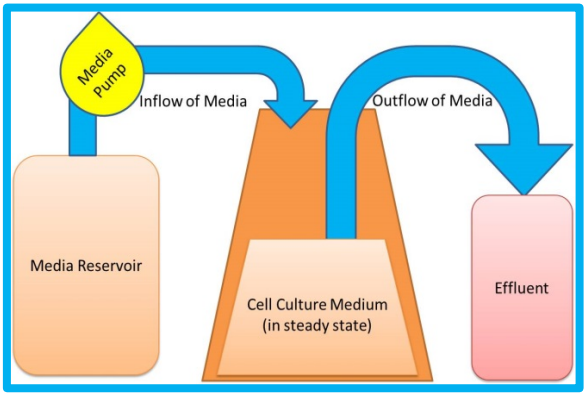

Fig. 1. Graphical representation of the chemostat method/biofilm reactor for culturing bacterial cells.

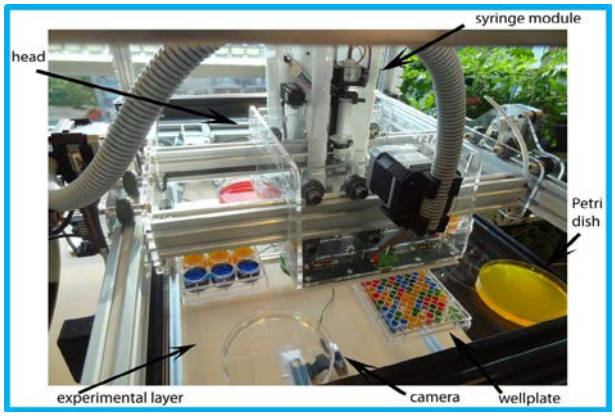

Fig. 2. Overview of the actuation, experimental, and sensing layer of the robot. EvoBot's actuation layer consists of a moving head on which various modules, such as syringe modules can be mounted. In this figure the experimental layer accommodates different vessels, and the camera at the bottom acts as the sensing layer by collecting experiment data.

3 Materials and Methods

Hardware

The EvoBot base was elongated from 620 cm (EvoBot V 0.5) to 1000 cm (EvoBot V 1.0). The syringe modules were adapted to host 20 ml syringe rather than 5 ml syringe (EvoBot V 0.5), the pumps were placed on the external profile of the robot rather than on the robot head and a dispensing module was added on board. The camera was placed on the side of the robot, to record the feeding/maintenance of the MFCs.

Microbial Fuel Cells

Interface and interconnection experiment

A standard analytical size two-chamber cubic MFC with anode and cathode liquid capacity of 25 mL and electrode total surface area of 270 cm^2 each, was placed on the EvoBot experimental arena. The anode and cathode electrode were connected with a 3 kOhm load (optimum load for that specific MFC).

Adaptation / Chemostat mimicking experiment

A set of 18 Nanocure® printed MFCs were adapted for this experiment (Fig. 3). The anode of the MFCs was constructed as previously described [6]. A 3mm custom made terracotta flat sheet, made following the same technique as previously reported, was used as the membrane [15]. For cathode electrode, a 5ml of alginate based custom made conductive paste was deposited manually using a syringe directly onto the membrane and the mixture was solidified after 24hours exposure to air (Fig. 3). After the inoculation period, the MFCs were loaded with 1kOhm.

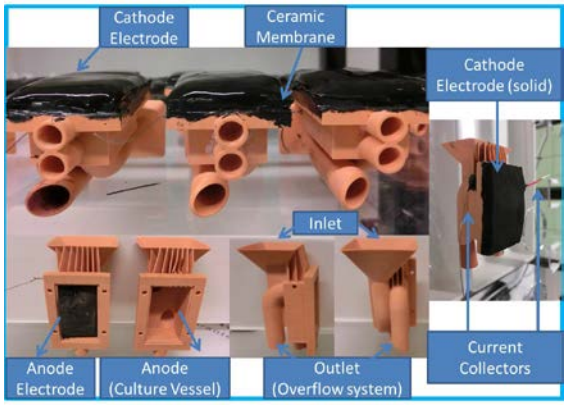

Fig. 3. Nanocure® printed Microbial Fuel Cells (MFCs). The anode compartment of the MFC acted like the culture vessel of a biofilm reactor/chemostat. Fresh medium entered through the inlet, whilst effluent was overflowing through the outlet.

Inoculation, Media and Delivery of Media

Interface and interconnection experiment

The MFCs were inoculated with activated sludge supplied by Wessex Water Scientific Laboratory (Cam Valley, Saltford, UK) supplemented with 5mM sodium acetate as the carbon energy (CE) source (Sigma-Aldrich, Dorset, UK). A DC pump was connected to the robot board and was used for pumping the enriched sludge into the anode chamber. The DC pump was calibrated and the activation time of the pump dictated the liquid volume which was dispensed. For this experiment, in order to fill the 50ml beakers the pump was activated once a day for 148 seconds in total.

Adaptation / Chemostat mimicking experiment

Initially the fuel cells were inoculated with activated sludge and then with effluent from active urine fed MFCs. After a total of two weeks inoculation, the cells left to starve for a week to ensure that no traces of urine were left in the anode. The experiment started with the introduction of sterile sodium acetate medium to the 9 MFCs and with sterile casein medium to the other 9 MFCs. The pH of the media was buffered to 7. The media were contained within two bottles; each bottle was connected to a DC pump and had an air filter (Fig. 6B). The tubing of the pump leads to the dispensing module of the robot (Fig. 6A, 6D). For each experimental cycle the pump was set to deposit and distribute the required amount of feedstock (overall volume 50 ml) to the specified beakers around the arena and then the syringe was collecting the liquid from the beaker and distributed it to the anode chambers of the fuel cells.

Data acquisition

Interface and interconnection experiment

Electrical output (voltage) was measured in real time using a PicoLog ADC-24 interface (Pico Technology, Cambridgeshire, UK). Both the EvoBot and the data-logger were connected in the same HP laptop computer.

Adaptation / Chemostat mimicking experiment

The cells were individually connected and the data were logged using two multichannels Agilent 34972A, LXI Data Acquisition/Switch Units (Farnell, UK). Both data loggers are connected to the computer however only one of them communicates with the Python interface, the other is recording the data using the Benchlink Datalogger 3 software to ensure continuous monitoring and logging of the MFC's performance even in the unforeseen event of a software crash.

3.1 Software

Development Environment

Interface and interconnection experiment

Laboratory Virtual Instrument Engineering Workbench (LabVIEW) from National Instruments, Berkshire, UK was used to create the applications that interacted with the real-time data produced from MFCs. Using LabVIEW a multi-layer program was compiled in which a function was written that collected the data from the Picolog data logger and output that data as a string of values. LabVIEW was sampling the Picolog file every 1 minute for the MFC voltage reading. The feeding scripts were written in Python language (Python 3.6.0). In the case where the MFC voltage dropped below the preset threshold limit that was set in LabView, the python script was activating the robotic head (actuation layer) to move above the MFC anode and deposit by pump 12.5 mL of inoculum. After the disposing of the liquid, the robot head homed itself.

Adaptation / Chemostat mimicking experiment

Python script activates the pumps for a certain time frame in order to allow all the liquid to move from the end of the media tube to the tip of the dispensing module nozzle. Every 24 hours the robot initiates the pumps to fill the pre-specified beakers with the media and when full the syringe module draws the liquid (5 ml) from the beakers and it starts dispensing it to the anode compartment of the MFCs. The coordinates of each item on the experimental layer is stored into Python dictionaries.

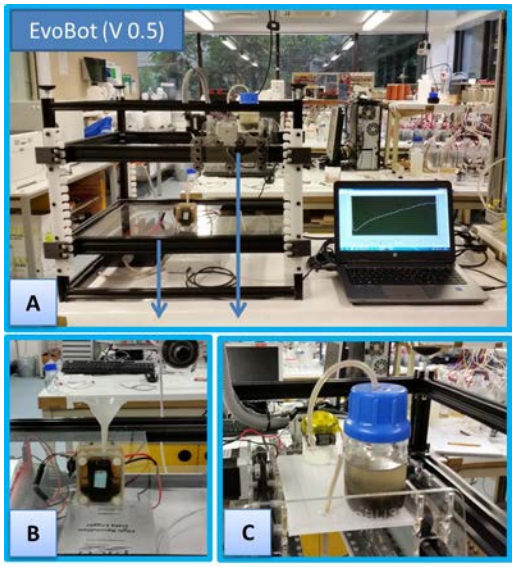

Fig. 4. Experimental set-up of Phase 1 Experiment (Interface and Interconnection). **[A]** The robot and data-logger are connected with the laptop computer which acts as a real-time voltage display. **[B]** The photo shows the anode of the MFC, pressed against the membrane using a rectangular piece of inert styrene material and **[C]** the DC pump connected to the bottle containing the sludge.

4 Results and Discussion

4.1 Interface and Interconnection of EvoBot with an MFC

In the first phase of the study, the work focused on investigating whether the voltage of an MFC can trigger the robotic arm of the EvoBot platform. To demonstrate this, a new abiotic MFC was set-up as described above. At the outset, the MFC was dry and contained no bacteria (therefore zero Voltage was recorded). The null voltage actuated the arm to move and deliver approximately 12.5 mL of activated sludge into the anode chamber (Fig. 4B). The 12.5 mL were deposited to the anode chamber of the MFC using a DC pump that was connected to a 50ml bottle of activated sludge (Fig. 4C).

After the robot had introduced 12.5 mL of activated sludge in the MFC (Fig. 5A), the voltage increased and stabilised at approximately 27 mV. Once the command

"Feeding Threshold" was set to 20 mV the original experiment planned to let the voltage decrease as the microbes consumed the food in the sludge. However, since the time period for a freshly inoculated MFC, to fall under 20mV, can take up to 24 hours, 10 mL of sludge was manually removed to simulate food consumption (Fig. 5B); due to the disturbance when removing the anolyte a spike in voltage was observed. However, once the voltage decreased below the preset feeding threshold the robot arm was activated to move to the position above the MFC anode inlet and initiated the DC pump for 37 seconds, thus introducing 12.5 mL of new sludge medium into the MFC.

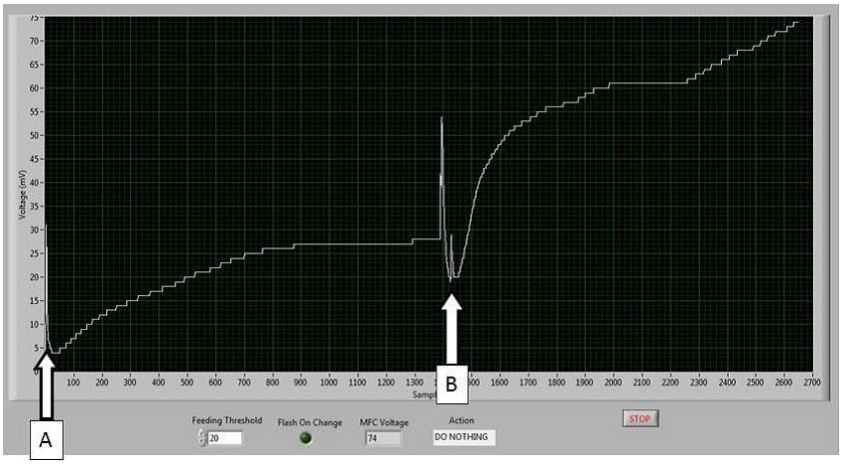

Fig. 5. **Screen capture of the voltage increase**. **[A]** Initially the MFC output was zero as the MFC was abiotic, this triggered the robotic arm to move to the fuel cell and activate the pump to feed the preset amount of inoculum (12.5 mL) to the anode. **[B]** At point B, 10 mL of the anolyte were manually removed to simulate food consumption and as a consequence, the voltage dropped. Since the MFC output fell below the preset threshold of 20mV the robotic arm was activated and another 12.5 mL of inoculum were deposited to the anode; as a result, the voltage of the MFC continued to increase.

This particular experiment demonstrated the feedback loop between the MFC output (the voltage and power to maintain that voltage) produced by the microorganism living inside an MFC and the robotic controller, was able to activate a python script that initiated a set of given tasks to increase the voltage above the minimum threshold. This novel use of the EvoBot demonstrated that the robot was able to maintain the MFCs by reacting to the carbon energy source depletion within the anode which reflects on the power output. To sum up, this experiment successfully demonstrated for the first time that the electrical output recorded from an MFC in real-time can establish a feedback loop with the EvoBot.

4.2 Adaptation / Chemostat mimicking experiment

Following the successful demonstration of the interconnection between MFCs and EvoBot, the research moved onto Phase 2 using the platform to optimise the environ-

mental parameters for faster growth of the cells on the electrode and maximum power transfer [13]. This work focused on optimising the substrate parameters that will be used to feed the MFCs and ultimately to improve their power performance. It is worth mentioning that the MFCs described here, are exactly the same as the ones used on EcoBot-III [6], which (as with all MFCs) are capable of relatively low, but continuous, levels of power. This is why for practical applications collectives of MFCs (i.e. stacks) are used and in the case of EcoBot-III the whole robot, including the central micro-controller, sensors and actuators, were powered by the 48 MFCs aboard (ca. 50uW/MFC). EvoBot platform was able to inoculate the otherwise empty/abiotic MFCs and note the power profiles developed over time with only EvoBot supporting the cells. This was the first long-term example of using the EvoBot platform for maintaining MFCs and operating interactive MFC experiments.

Having EvoBot driven experiments provides the fuel cells with automated feeding and hydration pulses, which are dictated by the voltage threshold, as well as continuous monitor and maintenance of the MFC, eliminating human intervention. This line of work provides a better understanding of what was needed in order to evolve the robot and increase its experimental capabilities. One of the needs was the enlargement of EvoBot's experimental arena, in order to hold more MFCs, allowing the parallel operation of more experiments as well as improvement of the syringe unit to avoid cross contamination. These issues were addressed and improved before performing the Phase 3 Adaptation / Chemostat mimicking experiment. The improved robot gave us the possibility to perform two different experiments using continuous feeding cycles (called the "chemostat" approach). The experimental set-up is presented below (Fig. 6).

In microbiology, the chemostat is the most common type of continuous culture device. It is an open system where its culture vessel maintains a constant volume to which fresh medium is added at a constant rate and an equal volume of spend culture is removed at the same rate and as a result, the growth rate is equal to the dilution rate ($\mu = D$) and the system reaches dynamic equilibrium. In the natural world, a plethora of biofilms are formed in continuous or periodic nutrient replenishing conditions and can be regarded as open systems as well [16]. Because the anodic biofilm electrodes are made from perfusible carbon veil the MFC falls within the general category of matrix perfusion systems. In the literature, it has been reported that such systems have similarities to a chemostat model [16]. Thus in this paper, we refer to the anode compartment of the fuel cell as the culture vessel (chemostat analogy).

Even though the system in the current study was a batch-fed culture system, i.e. not a continuous flow system like a conventional chemostat, the results nevertheless have shown that slow transitional repeat states can be maintained following further CE source supply following depletion (Fig. 7). These promising findings and this continuing line of work can provide useful insights into repeat batch fed microbial fuel cell systems, their behaviour as well as understanding of how to increase or optimize their power production capabilities.

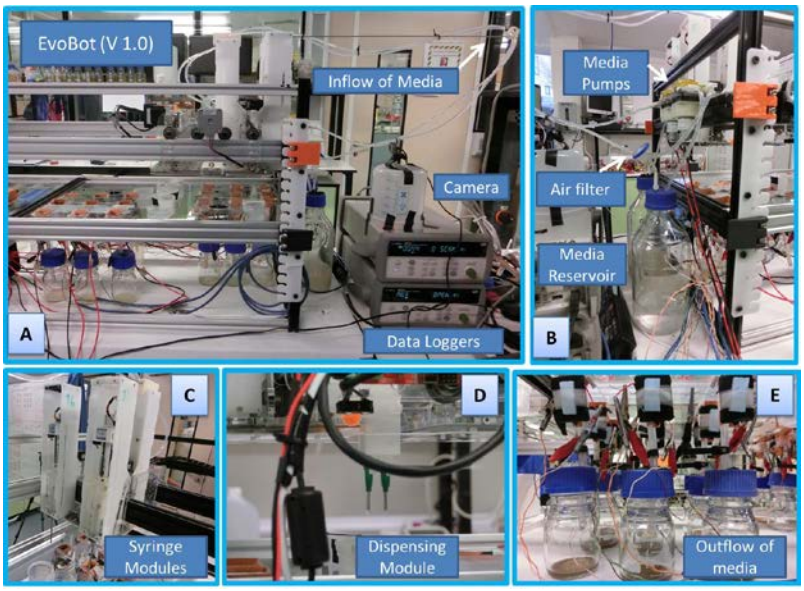

Fig. 6. EvoBot Version 1.0: Optimised based on the Chemostat approach for Phase 3 experiments. [A] EvoBot was able to host two experiments that count in total 18 MFCs. The data loggers were connected to the MFCs and the computer, and a camera was monitoring the experiment 24/7. **[B]** Similar to the chemostat the media reservoir was connected to the DC pumps and the tubes were connected to the **[D]** dispensing module. **[C]** The syringes were set to draw the liquid and dispense it to the culture vessel. **[E]** The waste perfusate was collected from the bottom into bottles (outflow stream) for further analysis.

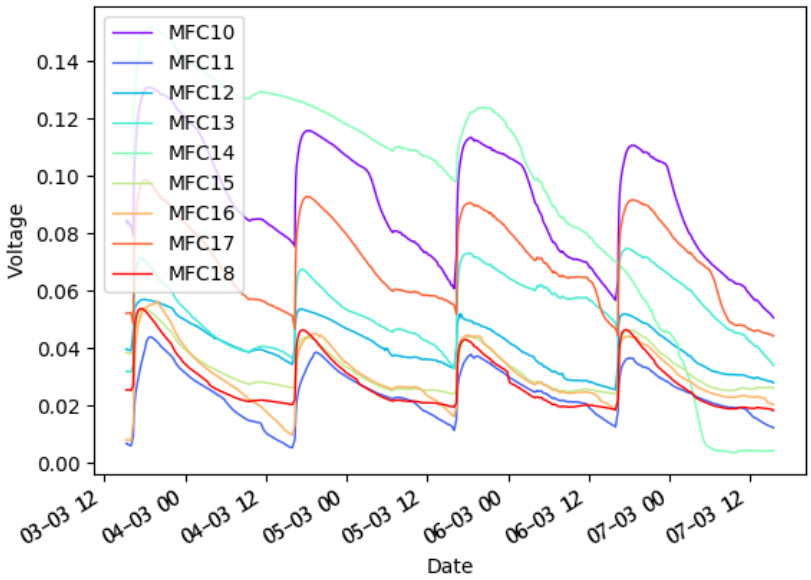

Fig. 7. Python generated graph based on the power output of the MFCs fed with casein. The graph shows the data produced within five days of EvoBot feeding the MFCs. The data show the reproducibility and stability of these cells when the fresh medium is fed to the biofilm daily. The non-periodic feeding of MFC 14, and consequent deterioration of performance was due to the liquid level of feedstock being below the lowest reach of the syringe needle; resulting in abnormal dispensing of volume (less frequent feeding). Undesired as this was, it demonstrates how depletion of the CE source affects bacterial metabolism, and therefore power output. In other words, it demonstrates the value-added of the automated maintenance, provided by EvoBot.

5 Conclusions

Robotic systems have many advantages for rapidly comparing MFC with different intrinsic factors (e.g. species of colonizing microbes and their ecological proportions; size/shape/design of MFC) as well as extrinsic physicochemical factors such as temperature, pH, pO_2, redox, osmotic pressure, type and concentration of nutrients. Biofilm reactors may offer many advantages compared to conventional chemostats so the two approaches (robot and biofilm bioreactor) can advance the biofilm and MFC research respectively. Thus, in order to continue developing the robot-chemostat, we need to more fully address the parameters of controlled physicochemical environments. At present, a pH module is being tested, which will be incorporated into the experiments. This will automatically test the inlet pH as well as the outlet pH of the anodic chamber in real time, to help understand the relationship between power output and anolyte pH. Also, it will give a new insight into the chemical and microbial transformations that take place within each chamber. Our aim is to continue using EvoBot to perform interactive experiments that will produce high quality reproducible data from multiple comparisons of conditions across a wide range of the physicochemical realm; data that would be difficult to achieve through conventional manual experimentation on MFCs and electroactive biofilms.

This work demonstrates the potential of using the EvoBot, an open-source modified RepRap 3D-printer, for the inoculation, maintenance and study of Microbial Fuel Cell (MFCs) systems. Furthermore, it presents the possibilities that this type of robot can be developed along the lines of robotically controlled environments integrated with MFC-based bioreactors with similarities as well as important differences to the well characterized planktonic chemostat. In addition, these experiments are the precursors to the development of a new class of living robots (Symbots), which can enforce, monitor and interact with evolving systems, such as MFCs. As a result, such a platform would not only benefit the MFC field but the robotics field as well since EvoBot can be used in the future as a maturing/optimising factory for MFCs that can ultimately be used as the power sources for small robots, including EvoBot itself that may be powered by the very same MFCs that it had built, inoculated and maintained; this would a step in the direction of truly Living Machines!

Acknowledgments
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