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From waste to energy: First experimental Bacterial Fuel Cells onboard the International Space Station

Bacterial Fuel Cells are innovative energy systems that use bacteria to transform carbohydrates anaerobically into free electrons and waste products. The bacteria deposit the electrons on the anode and hence create a potential difference between the anode and the cathode, yielding a 'bacterial battery'. This principle may be favourably influenced by enhanced bacterial productivity or bacterial growth in microgravity conditions, as is shown before in several other studies on bacteria in microgravity. Nonetheless, bacterial fuel cells have not been tested in space before. Currently foreseen applications are very promising for space flight and include waste disposal in manned space vehicles. This study describes a 'space-first' test of bacterial fuel cells onboard the International Space Station using the Rhodoferax ferrireducens strain. We test if it is possible to use a bacterial fuel cell in 1g and under both simulated (RPM) and real microgravity conditions. Due to differences in magnitude of the output the data had to be normalized and cumulatively plotted. In all, it can be concluded that bacterial fuel cells show similar phases in the output under different gravitational conditions. Hence it can be concluded from a biological point of view that bacterial fuel cells do operate in space.

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1 Introduction

Bacterial fuel cells are innovative energy systems that use bacteria that transform carbohydrates in anaerobic conditions into free electrons and waste products. The bacteria deposit the free electrons inside the fuel cells on the anode of the fuel cell. This creates a potential difference between the anode chamber and the cathode chamber and hence it yields a 'bacterial battery'.[1,2] The *Rhodoferax ferrireducens* strain thrives on the anaerobic transformation of carbohydrates and electron donation to an electron acceptor. This strain pulls of this feat with no use of a mediator and with a very high transformation efficiency. These characteristics makes the strain very interesting for the use in bacterial fuel cells.[3]

Bacterial fuel cells are very promising for future applications such as the use as batteries for pacemakers (using glucose from the bloodstream as permanent input for the system) or batteries for systems that are otherwise not easily accessible or where battery replacement is costly. [4] Furthermore, bacterial fuel cells could be very useful in manned space flight, as they may be convenient for converting organic waste into energy. An important question is whether those fuel cells have similar promising characteristics under microgravity conditions as they do on Earth.

Several studies performed to date have shown the effects of microgravity on certain bacterial strains [5]. These effects incorporate higher final population concentrations under microgravity and an increased transfer rate of the food which may increase the efficiency of fuel cells in sense of the produced out-

put. This is an important argument for studying bacterial fuel cells in space, although these positive gravity-effects may be limited to non-motile bacteria. Nonetheless it is yet unclear what effects could be expected for motile bacteria like *Rhodoferax ferrireducens* as these are motile but optimally settle themselves at the anode since this is the only place in the fuel cell where they can deposit free electrons. Up to now *Rhodoferax ferrireducens* is one of the most promising bacterial strains for bacterial fuel cells since they can transfer electrons directly to the electrode i.e. without the use of a mediator limiting the 'loss' of free electrons [1]. When applied for electricity production purposes, this strain will have a very high efficiency in transferring glucose into electricity inside bacterial fuel cells.

Bacterial fuel cells have not been tested under microgravity conditions before. Hence, this study describes first steps in getting insight in the operational aspects of bacterial fuel cells in space by testing them under different gravitational conditions. We tested whether it is possible to use a microbial fuel cell in space. The BugNRG experiment was flown during the DELTA mission to the International Space Station on Soyuz flight 8S to test this hypothesis. We proved that our bacterial fuel cells do work on Earth and under simulated and real microgravity conditions. Clearly, due to the low n-value and the inability to study the internal processes in the fuel cells in space, we recommend further research to refine the results.

2 Method and materials

As previously described, the BugNRG experiment utilised the *Rhodoferax ferrireducens* strain to produce electricity. *Rhodoferax ferrireducens* is capable of anaerobically transforming carbohydrates (i.e. glucose) and donating electrons to the carbon electrodes in the bacterial fuel cells according to net-reaction:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \quad (1)$$

In their natural environment the *Rhodoferax ferrireducens* strain needs metallic particles in its environment to donate the electrons to transform their food. Inside the fuel cells, the metallic particles have been replaced by a carbon electrode that transfers the electrons from the anode chamber to the cathode chamber that contains an electron accepting solution. The used electron-acceptor solution inside the cathode chambers was 0,25M $K_3Fe(CN)_6$.

Rhodoferax ferrireducens was cultivated under the optimum growth condition of 25ºC [3] and only the anode chambers of the bacterial fuel cell were injected with the strain under anaerobic conditions inside a portable glovebox. The anaerobic conditions inside the glovebox were created via gas packs and an oxygen level below 2% percent was maintained. The hardware was cleaned with ethanol. All experiments used a Basai 1001 growth medium inside the anode chamber, which was sterilized using an autoclave. This medium contained in total 0,19 mol of glucose per anode chamber. All liquids for the fuel cells were flushed with anaerobic gas to ensure the exposure of the system to oxygen is negligible and to avoid disturbance of the cultures by other bacterial strains in the fuel cells. Additional experiments were used to test that fuel cells without bacterial component or with bacteria but prepared aerobically do not generate any measurable output.

The BugNRG-experiment made use of specially designed closed bacterial fuel cells. These bacterial fuel cells are comprised of two cylindrical polycarbonate chambers that are compressed onto each other with a threaded ring. The anode and

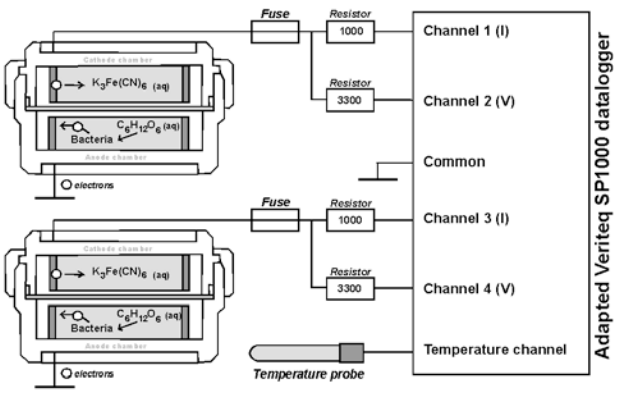

Fig. 1: A schematic overview of the hardware of the BugNRG experiment.The bacterial fuel cells are shown on the left and are annotated with the chemical solutions. The block on the right shows the datalogger that was used for measuring and datastorage. Depicted between the fuel cells and the datalogger are the electronics used to discharge the bacterial fuel cells.

Fig. 2. Disassembled BugNRG experiment facility. Counter clockwise: the experiment-bus also showing the temperature probe, 2nd level of containment and 3rd level of containment

cathode chambers are separated by a cation exchange membrane. To counteract leakage, a rubber o-ring has been incorporated into the design and to maintain proper air tightness. To facilitate the filling and purging of the chambers when assembled, a filling hole has been included. Each of the two chambers of a fuel cell has a total volume of 10,7 mL and contains a sleeved carbon-felt electrode. This electrode is connected to electrical wiring on the exterior of the fuel cell.

The output of the fuel cells can only be measured if the fuel cells are discharged across a load. If this is not done, a 'short circuit' of the fuel cell will result [1]. The bacterial fuel cells of the BugNRG experiment were discharged using a load of 1kΩ for measuring the produced current. Via the 1kΩ resistor the fuels cells are shorted to ground via the current measurement channel of a datalogger. The fuel-cell voltage is measured by the datalogger via a current limiting resistor with a load of 3k3Ω. A fuse separates the fuel cells from the rest of the circuitry. An adapted Veriteq SP1000 datalogger was used to log the output parameters (voltage and current) and the temperature. Figure 1 shows a schematic version of the bacterial fuel cells and the electronic wiring.

All necessary equipment for the experiment was integrated into a single piece of hardware that could be flown into orbit. The BugNRG-experiment facility consisted of two main sections; the experiment-bus and the experiment-payload. The experiment-bus contained the datalogger and discharge electronics. The bus was designed in such a way that the datalogger could be disconnected from the entire facility so it could be brought back to earth for data-analysis. Due to mass restrictions of the available return mass onboard the Soyuz, only the datalogger could be brought back to Earth and no fuel cells could be returned. The experiment-payload consisted of two fuel cells that were contained in two additional levels of containment. In this configuration it was possible to protect the ISS-crew from the bacterial and chemical contents of the fuel cells. The experiment-payload container also included a temperature sensor to allow the registration of the ambient temperature experienced by the fuel cells. The obtained temperature profile was used during the performance of the reference experiments. Figure 2 shows the different parts of the BugNRG-experiment facility as described above.

The flight-experiment was partially prepared on-site. Fuel cells containing cultivated bacteria were prepared inside the lab in Amsterdam and were taken to the *Baikonour Cosmodrome* via cooled transportation. Between 15 and 13 hours before launch a fresh solution of glucose and growth medium was added to the anode chambers and all the fuel cells were tested for their instant output. These preparations took place inside the portable glovebox of Bradford Engineering using gas packs to obtain an anaerobic environment. A level of >1% oxygen was measured inside the glovebox during preparations. After leakage testing, fuel cells fit for flight were selected and integrated into the experiment facility. Activation of the experiment took place 12 hours before launch. The experiment was stowed inside the launch vehicle several hours prior to launch to reduce possible influences of the 1g environment. Two fuel cells were launched onboard Soyuz-TMA4 and subjected to a ten-day period of microgravity onboard the ISS. At the end of the experiment run the datalogger was disconnected by cosmonaut André Kuipers and stowed for return to Earth.

After obtaining the flight data, including the temperature profile, several reference experiments were performed. The post-flight experiment needed to be performed under the exact same conditions as in the flight experiment. To this extend these

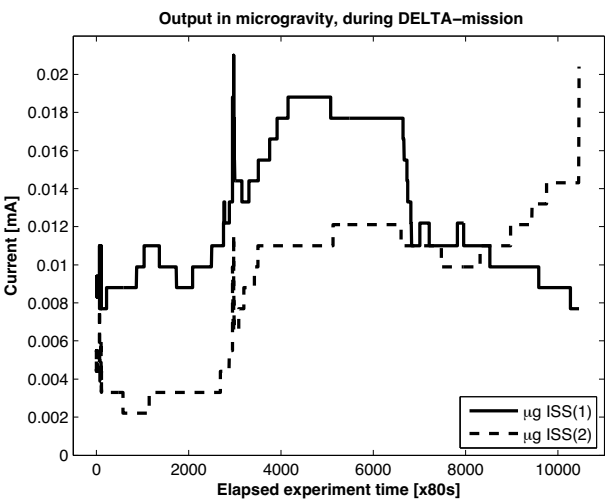

Fig. 3: The discharge-curves of the two fuel cells that were flown to the International Space Station.

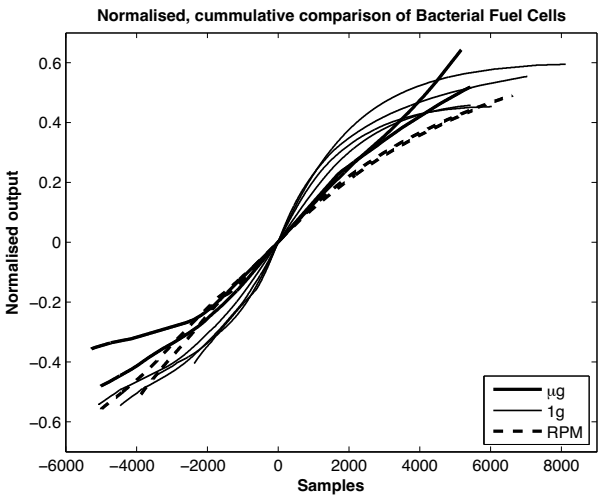

Fig. 4: Normalised cumulative presentation of the data of the microgravity, RPM and 1g experiments

experiments used a nearly identical preparation protocol to the flight-protocol. All post-flight experiments were prepared in the same portable glovebox with gas packs as used in the preparation of the flight-fuel cells. Back-up and reference experiments used the same batch of bacteria and chemicals. After depletion of these supplies, a new batch of chemicals, as close to the used batch was used.

The post-flight experiment included reference experiments and Random Positioning Machine (RPM) experiments. All these post-flight experiments were performed inside an incubator using the exact ISS temperature profile. The reference experiments took place inside the RPM of the Dutch Experiment Support Centre (DESC). Half of the fuel cells were placed on a stable platform in order to simulate only the ISS temperature profile in a 1g environment. The other half of the experiments was performed under both simulated microgravity on the RPM and an ISS temperature profile. During the reference experiments the data of >8 RPM-fuel cells and >10 1g fuel cells was collected.

3 Results and discussion

The first observation of the data obtained from the flight experiment demonstrates the hardware operated well, and the data were recorded properly. The bacterial fuel cells that were flown to the International Space Station (n=2) however produced an overall low output. Figure 3 shows the discharge-curve of the two flight-fuel cells. The flight back-up fuel cells (n=2), which closest resemble the flight-BFCs, and the post-flight experiment (n>15) have neither produced similar low outputs. The output of bacterial fuel cells in 1g range from 0-0,4 mA with a majority clusterd in the range of 0-0,1 mA. Simulated microgravity ranges from 0-0,35 mA. Microgravity ranges from 0-0,02 mA. The origins of the large range in the magnitude of the overall output in the performed experiments is not clearly known, but may be attributed to the amount of bacterial cells present inside the bacterial fuel cells at the beginning of the experiment.

Discharge-curves of bacterial fuels can in general be described by several features that are related to the behaviour of the bacteria based on observations in 1g. In case of a normal functioning bacterial fuel cell, the discharge-curve will show three phases. The first phase of the curve is contributed to the lag-phase during which the produced output remains constant or only shows a slight increase. The lag-phase is succeeded by a growth-phase during which the output rises significantly and reaches a maximum after a variable amount of days. After reaching a maximum value the output is reduced during the extinction-phase. When taking the effects of convection and diffusion into account for the size of the bacterial fuel cells, the observed extinction is most likely the result of pollution caused by the bacterial waste products and possibly the depletion of the available nutrients for the bacteria.

The phases in the discharge-curves of the fuel cells flown to the ISS show similarities when compared to the discharge-curves of the post-flight fuel cells studied in the 1g environment. This demonstrates from a biological point of view that fuel cells work in a both a microgravity environment and a 1g environment.

In order to better compare the data to reflect on the use of bacterial fuel cells in microgravity, and to correct for the observed differences in magnitude, the data was normalized and presented a different, illustrative way such that comparing the discharge phases of the bacterial fuel cells in all the different gravitations conditions would be possible. To this extent a number of operations on the data were performed. The measurements were first cumulatively plotted meaning: adding a value to the sum of the previous values. This operation yields a stretched s-shaped graph that represents the different phases of the bacterial activities. Through time, the lag-phase, growth-phase and extinction-phase are visible. These phases can respectively be distinguished as the lowest left end of the graph, the rising almost linear part, and the right upper end of the graph that is levelling off. The stretched s-shaped graph is the shape for a normal functioning bacterial (or biological) system. With the use of this technique, one can eliminate bacterial fuel cells from further calculations if they do not show a lag-phase. The obtained graphs were subsequently fitted using a tenth order polynomial function. Using the fitted function, the points of inflection were calculated. The obtained cumulative graphs were normalized between values 0 and 1 and subsequently plotted with the points of inflection at coordinates (0,0). The results of all these operations are presented in figure 4 and apply to the data obtained in the microgravity, simulated microgravity and normal gravity experiments. This figure only presents comparable data. Non-comparable datasets (i.e. those not having a lag-phase) have been disregarded.

Fuel cells in a 1g and (simulated) microgravity environment all show a lag phase that results in a growth-phase. During the lag-phase, the output of the fuel cells is generally higher in the case of (simulated) microgravity. However, after the point of inflection, the fuel cells subjected to microgravity do not show the same kind of extinction-phase and thereby differ from the observed behaviour of the 1g fuel cells. The fuel cells in microgravity show a tendency to remain producing output on an almost ‘stable’ level while the 1g bacterial fuel cells already start to level off as a result of their extinction-phase. It has to be noted that due to the low n-value, such a difference in behaviour cannot be statistically proven. Fuel cells on the RPM under simulated microgravity also show a delayed response to level off.

4 Conclusion

Data from the BugNRG experiment has provided the first insights on the operational behaviour of bacterial fuel cells in microgravity.

The large variety in the magnitude of the output of the bacterial fuel cells, regardless of the gravitational environment, makes it difficult to simply compare the output of bacterial fuel

cells. To overcome this problem, the used technique of normalizing and cumulative plotting of data facilitates a better comparison of the discharge-curves of bacterial fuel cells. When applied, differences are present in the behaviour of bacterial fuel cells in different gravitational conditions. In (simulated) microgravity, the lag-phase of bacterial fuel cells is more productive and after the point of inflection of the cumulative normalized graphs no, or at least a very slow extinction-phase is present. The observed difference in behaviour can however not be statistically proven due to the low n-value of the microgravity-experiments. What can be concluded is that bacterial fuel cells show similar phases in microgravity as in normal gravity. This proves from a biological point of view that bacterial fuel cells operate in space.

Bacterial fuel cell technology is still in its infancy. Many technical hurdles to transform waste into energy still have to be overcome such as high internal (ohmic) resistance [6]. These first insights in the operation of bacterial fuel cells in space show that bacterial fuel cells are promising for the use in systems inside (manned) space vehicles.

In all, the results of the performed experiments pave the way for a new hypothesis that bacterial fuel cells in space have the same phases in the discharge curves as in 1g but the lag-phase and extinction-phase can differ in shape when subjected to a (simulated) microgravity environment. However, the low n-value of the microgravity experiments endorses the need for more experiments in microgravity. Further research would hence be recommendable to get more profound knowledge of the influences of gravity on the output of bacterial fuel cells.

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References

[1] *Chadhuri, S.K., Lovley, D.R.:* Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. Nature Biotechnology, vol. 21, nr.10, 1229-1232 (2003)

[2] *Rabaey I., Ossieur W., Verhaege M., Verstraete W.:* Continuous microbial fuel cells convert carbohydrates to electricity. Water Science and Technology 52(1-2): pp515-523 (2005)

[3] *Finneran K.T., Johnsen C.V., Lovley D.R.: Rhodoferax ferrireducens* sp. nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe(III). International Journal of Systematic and Evolutionary Microbiology. May;53(Pt 3):669-73 (2003)

[4] *Lovly, D.R.:* Microbial fuel cells: novel microbial physiologies and engineering approaches. Current Opinion in Biotechnology. 17(3): pp. 327-332 (2005)

[5] *Klaus, D.M.:* Space Microbiology: Microgravity and Microorganisms. The Encyclopedia of Environmental Microbiology, G. Britton (ed.), John Wiley & Sons, NY, pp. 2996-3004 (2002)

[6] *Logan, B.E., Regan, J.M.:* Electricity-producing bacterial communities in microbial fuel cells. Trends in Microbiology, vol. 14, issue 12, pp 512-518 (2006)