DEVELOPMENT OF A SPACE BIOREACTOR USING MICROTECHNOLOGY

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Abstract

A miniature bioreactor for the cultivation of cells aboard Spacelab is presented. Yeast cells are grown in a 3 milliliter reactor chamber. Supply of fresh nutrient medium is provided by a piezo-electric silicon micropump. In the reactor chamber, pH, temperature and redox potential are monitored and the pH is regulated at a constant value. The complete instrument is fitted in a standard experiment container of 63×63×85mm. The bioreactor has been used on the IML-2 mission in July 1994 and is currently being refurbished for a reflight in the spring of 1996.

Introduction

Experiments with cells in space have shown that important cellular functions are changed in microgravity [1]. These findings are of great interest for fundamental research as well as for possible biotechnological applications. For the cultivation of cells aboard spacelab, a miniature bioreactor is developed. The objective is to evaluate in a controlled bioreactor experiment (chemostat cultivation) the effects of mixing and stirring on the growth characteristics of yeast cells. The experiment has been selected by ESA for the IML-2 mission which took place in July 1994. The experiment was located in Biorack, a multi-user facility developed by ESA to host biological experiments in Spacelab. The individual experiments are mounted in standard containers of various dimensions. The Type II container allocated for this experiment has internal dimensions of 63×63×85mm.

The design of the Space Bioreactor includes the following features;

- Supply of fresh medium to cells in the reactor chamber (working volume 3 ml) over a period of 9 days at an adjustable rate (0.2 - 1.5 ml/h)
- Measurement of pH, temperature and redox potential of the culture
- Control of pH of the culture
- On-line data transfer to ground control

In view of the complexity of the instrument and the limited dimensions of the Type II container, the application of silicon technology to provide both the sensors and a micromachined pump and flow sensor are of a distinct advantage.

During its first flight on IML-2 in July 1994, the technical feasibility of the bioreactor concept has clearly been demonstrated. Due to excessive temperatures in the laboratory where the flight experiment had to be prepared, the experiment has not yet given all the expected biological results. Therefore, a reflight opportunity has been granted and the bioreactor is currently being prepared for the STS-76 mission in the spring of 1996. In order to make the liquid handling more robust, a controlled flow system will be implemented.

Experimental Setup

Figure 1 shows the individual elements of the bioreactor and their interconnections. In the following section, these elements will be briefly described. It is interesting to note that the reactor chamber, the heart of the instrument, occupies less than 1% of the volume available in the experiment container. The largest volume is reserved for the nutrient medium reservoir. Both the fresh and used medium reservoir are flexible and while the first is emptied during the experiment, the second takes its place while filling up.

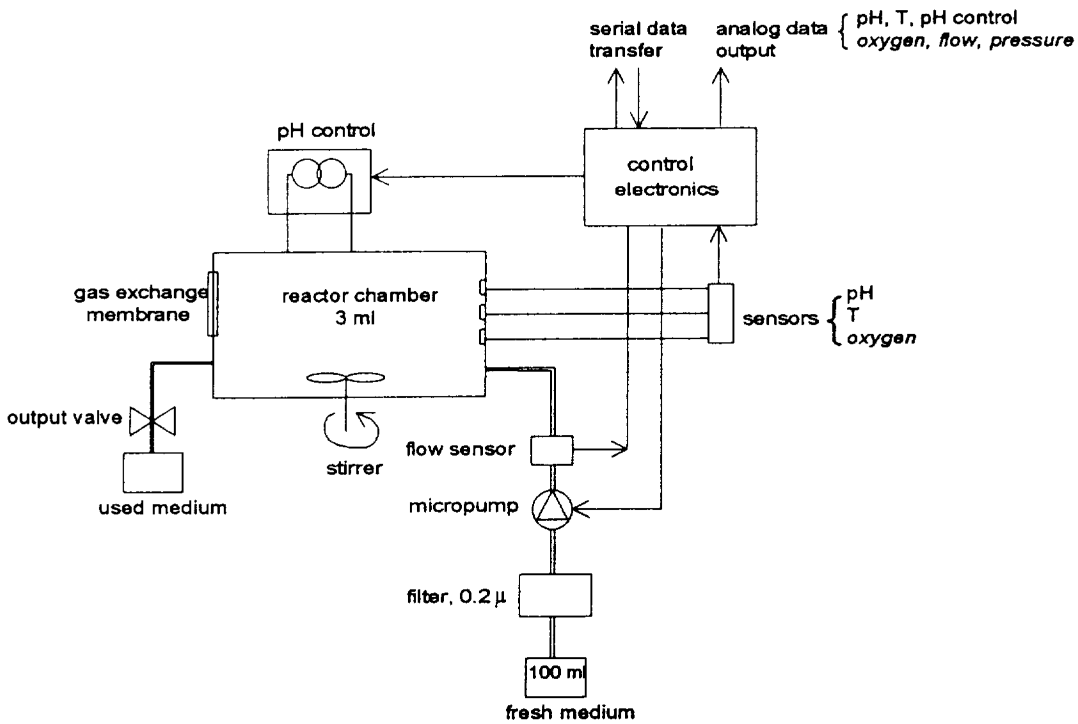

Figure 1. Principal elements of the miniature bioreactor

Pumping system

The supply of fresh medium to the reactor chamber is accomplished with a microfabricated pump [2, 3]. It is a membrane pump consisting of a structured silicon part sandwiched between two glass plates. The silicon forms two passive one-way valves that close on the thicker of the two glass layers. The thinner glass forms the membrane that is driven by a ceramic piezo-electric disk. When used as such, the flow rate of the pump is depending on the output pressure that has to be provided. Unforeseen variations in the bioreactor chamber pressure were the cause that during the first flight fluid delivery was not sufficiently controlled. For the reflight, a flow sensor is added so that the pump can operate in a closed loop control system and supply the required flow independent of output pressure [4].

Sensors

The sensors for the bioreactor, a pH-ISFET, a temperature sensitive diode and a thin film platinum redox electrode have all been integrated on a single chip that measures 3.5×3.5 mm. The sensor chip is mounted on a carrier that is inserted in the reactor chamber so that the sensor surface becomes part of the chamber wall without creation of dead angles. The reference electrode used in connection with the chemical sensors is a gel-filled Ag/AgCl electrode.

pH-control

For optimal growth conditions for the cells, the pH in the reactor has to be maintained at a constant value. During normal growth, yeast cells will acidify the medium for which a compensation is required. In view of the risk connected to the use of concentrated NaOH for this purpose, an electrochemical pH control is developed. A titanium electrode in the reactor chamber acts as a cathode for the electrolysis of water to produce hydroxyl ions. As a counter electrode a silver wire is used, embedded in a KCl loaded gel. The counter electrode compartment is separated from the reactor chamber by a Nafion membrane. Electrochemical compensation of the pH can be controlled very precise and avoids the need for an additional pump for NaOH dosage.

Electronic Circuits

The operation of the bioreactor is controlled by an Intel 87C51 microcontroller. The circuitry consists further of amplifiers for the sensors, a high voltage driver circuit for the micropump and a current source for pH-control. The pump operation is governed by an analog control system, the set-point of which is controlled by the microprocessor. The current source for pH-control is galvanically isolated from the rest of the circuit to avoid interference with the sensor signals. Relevant data are available on one multiplexed analog output channel. For preparation of the experiment before the flight a serial data connection is provided and the instrument can be directly interfaced to a standard personal computer. Figure 2 shows the basic electronic connections in the instrument. The circuits are built up with surface mounted components on two printed circuit boards

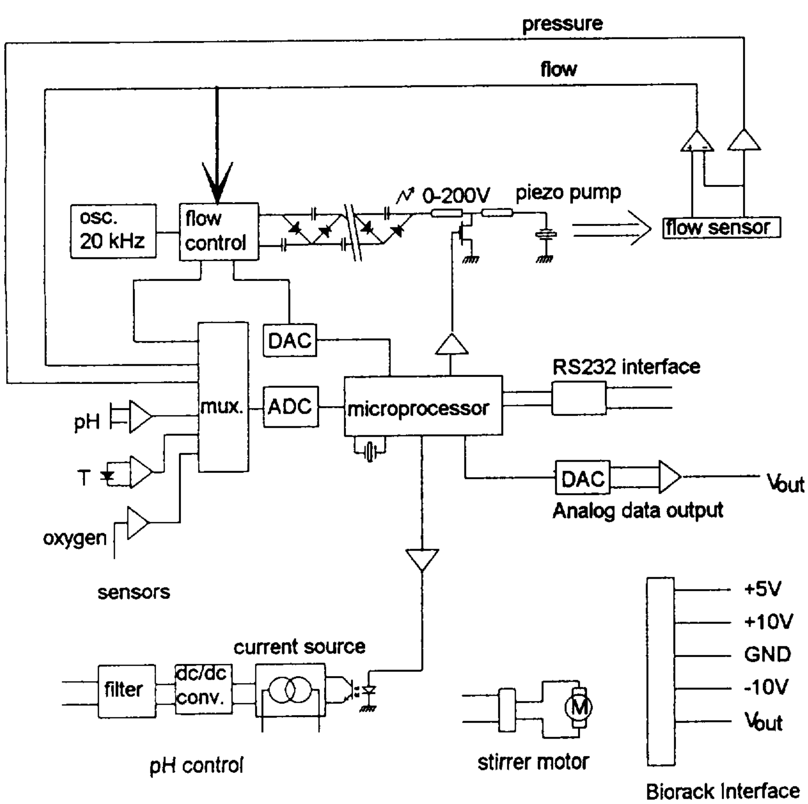

Figure 2. Electronic circuit of the bioreactor

Results

This project has started in 1991. After 15 months a breadboard model was presented to show the feasibility. After that the bioreactor has been qualified in vibration and EMC tests and, above all, in biological performance experiments as described in [5]. Figure 3 shows a photograph of an engineering model of the bioreactor that has been used for a number of the qualification tests. The first flight of the bioreactor has been relatively successful and currently the second flight is in preparation.

Conclusion

The development of a miniature bioreactor with microfabricated components for fluid handling and silicon sensors shows a promising outlook for their application in micro gravity experiments. In view of the special requirements for this kind of application, the bioreactor is an attractive showcase for microsystem technology.

Acknowledgment

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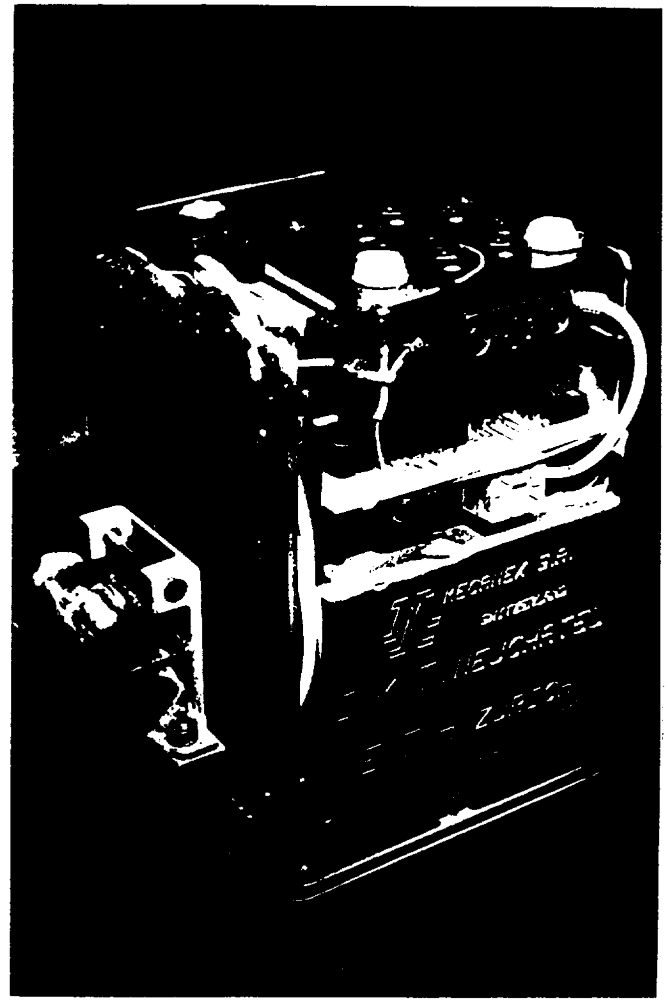

Figure 3. Engineering model of the space bioreactor mounted on the base of a Type II experiment container.

References

1 F. K. Gmünder, C.-G. Nordau, A. Tschopp, B. Huber and A. Cogoli, *J. Biotechnol.* 7 (1988) 217-228

2 H. T. G. van Lintel, F. C. M. van der Pol and S. Bouwstra, *Sensors and Actuators*, 15 (1988) 153-167

3 B. H. van der Schoot, S. Jeanneret, A. van den Berg and N. F. de Rooij, *Sensors and Actuators* B, 6 (1992) 57-60

4 M. A. Boillat, A. J. van der Wiel, A. C. Hoogerwerf, N. F. de Rooij, *Proceedings IEEE MEMS 1995*, pp 350-352, Amsterdam, the Netherlands, January 29-February 2, 1995

5 I. Walther, B. H. van der Schoot, S. Jeanneret, Ph. Arquint, N. F. de Rooij, V. Gass, B. Bechler, G. Lorenzi, A. Cogoli, *J. Biotechnology* 38 (1994) 21-32