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Microtechnology in Space Bioreactors

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Abstract. Space biology is a young and rapidly developing discipline comprising basic research and biotechnology. In the next decades it will play a prominent role in the International Space Station (ISS). Therefore, there is an increasing demand for sophisticated instrumentation to satisfy the requirements of the future projects in space biology. Bioreactors will be needed to supply fresh living material (cells and tissues) either to study still obscure basic biological mechanisms or to develop profitable bioprocesses which will take advantage of the peculiar microgravity conditions. Since more than twenty years, the Space Biology Group of the ETHZ is carrying out research projects in space (Space Shuttle/Spacelab, MIR Station, satellites, and sounding rockets) that involve also the development of space-qualified instrumentation. In the last ten years we have developed, in collaboration with *Mecanex SA*, Nyon, and the Institute of Microtechnology of the University of Neuchâtel, a space bioreactor for the continuous culture of yeast cells under controlled conditions. Sensors, pH control, nutrients pump and fluid flowmeter are based on state-of-the-art silicon technology. After two successful space flights, a further improved version is presently prepared for a flight in the year 2000.

1. Introduction

Space biology has evolved from the scientists' need to better understand the effects of the space environment on living systems. The peculiarities of such an environment are a reduced gravity (*i.e.*, Shuttle: 10^{-2}–10^{-4} *g*), the almost absence of convection movements and cosmic radiation (10 mrad/d in average). At the beginning of human space exploration in the early sixties, the investigations were oriented essentially toward the health of the astronauts, so that medical and physiological experiments were predominant. Even if this aspect is still very important, today's investigators are increasingly interested in basic and applied research under microgravity. Also, the upcoming of the ISS is triggering the scientists to develop and plan new instruments for long-term experiments in space and for the recycling of components essential for life, such as oxygen, water, and air, from waste products. Biotechnology in space will become a daily process helping astronauts to live for several months far away from Earth.

For twenty years, our group has been active in this field focusing, first, on the study of immune-system cells, in particular T lymphocytes, and, second, on the development of 'space-qualified' (see below) instruments to carry out studies on single cells. We used 0 *g* as a tool to study the mechanism of activation of T lymphocytes. This mechanism is extremely complex and many of its steps still need clarification [1][2]. Our first two space experiments were performed in 1983 aboard the space shuttle *Challenger* [3] and *Columbia*, respectively [1]. We manufactured a tissue culture incubator that had to comply with the peculiar constraints of space flight.

During the past ten years, the development of continuous cell culture systems has also been an important aspect of our activity. Our research was conducted in orbit (Biokosmos, MIR, Spacelab, Spacehab), in sounding rockets (MASER, MAXUS) as well as on the ground in the centrifuge for hypergravity, and in clinostats (rotating devices that randomize Earth-gravity vector) for simulated microgravity. A summary of our experiments in space is given in the *Table*.

The purpose of this article is to describe the four generations of the bioreactor developed in collaboration with the Swiss aerospace companies *Contraves AG*, Zürich, *Mecanex SA* and the Institute of Microtechnology (IMT) of the University of Neuchâtel. We will also briefly present the data of two flight experiments.

2. Space-Qualified Instrumentation

All equipment to be installed in space laboratories must fulfil very strict requirements like crew safety, man-machine interaction, energy consumption, volume, electromagnetic contamination, and others. For instance, safety requires the use of non-offgassing, non-flammable, non-toxic materials. Handling operations by the crew shall be simple and not time-consuming. For biological samples, triple containment is necessary. On the other hand, materials have to be biocompatible with the biological specimen used.

There are two types of space instruments used for biology: Multi-user facilities and experiment-specific devices. A multi-user facility, such as the Biorack flown on several missions, consists of freezer, incubators, cooler, and reference centrifuges in which biological experiments can be performed. They are mostly built under supervision and financing of the space agencies. Experiment-specific

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CHIMIA 1999, 53, No. 3

Table. *Experiments Conducted in Space by the Space Biology Group of the ETHZ*

Mission	Year	Experiment/instrument developed with Swiss aerospace industry
STS-8	1983	Incubator with human embryonic kidney cells
STS-9, Spacelab 1	1983	Incubator with human lymphocytes
STS-61-A, D1	1985	2 experiments: lymphocyte cultures, lymphocytes from astronauts
STS-40, Spacelab SLS-1	1991	2 experiments: lymphocyte cultures, lymphocytes from astronauts
STS-42, Spacelab IML-1	1992	Hybridoma cells, DCCS with hamster embryonic kidney cells (*Contraves AG*), Friend cells
STS-65, Spacelab IML-2	1994	3 experiments: lymphocyte activation and motion, bioreactor SBR I (*Mecanex SA*, Univ. Neuchâtel) with yeast cells
STS-76, 3rd Shuttle to MIR	1996	Bioreactor SBR I (*Mecanex SA*, Univ. Neuchâtel) with yeast cells
STS-107	2000	Bioreactor SBR II (*Mecanex SA*, Seyonic, Neuchâtel), tentatively scheduled
STS-81, 5th Shuttle to MIR	1997	Study on preservation of mammalian cells
MIR Mission 7	1988	Immunological 'skin-test' on cosmonauts with applicator
MIR Mission 8	1989	Immunological 'skin-test' on cosmonauts with applicator
MIR Mission 9	1990	Immunological 'skin-test' on cosmonauts with applicator
Kosmos Biosatellite 9	1989	Test of the DCCS (*Contraves AG*) with protoplasts
MASER 3	1989	Lymphocytes: mitogen binding, patching and capping
MASER 4	1990	Lymphocytes: cytoskeleton, mitogen binding, patching and capping
MAXUS 1	1991	Lymphocytes: motility, cytoskeleton, mitogen binding, patching and capping, rocket failure
MAXUS 1b	1992	Lymphocytes: cytoskeleton, mitogen binding, patching and capping
MAXUS 2	1995	Lymphocytes: motility, cytoskeleton, mitogen binding, patching and capping
Stratospheric balloon, program ODISSEA	1986	Cosmic radiation and lymphocyte activation, balloon failure
Stratospheric balloon, program ODISSEA	1987	Cosmic radiation and lymphocyte activation

hardware is mostly developed for and adapted to one specific biological experiment. The best way for scientists to have instruments optimally conceived for their experiments is to participate in their development and design. The use of silicon microtechnology was a key element to contend the extremely strict place restriction imposed by the standard (Type II) containers of the Biorack facility.

Here, we present a miniaturized and controlled bioreactor as an example for the utilization of microtechnology for space instruments.

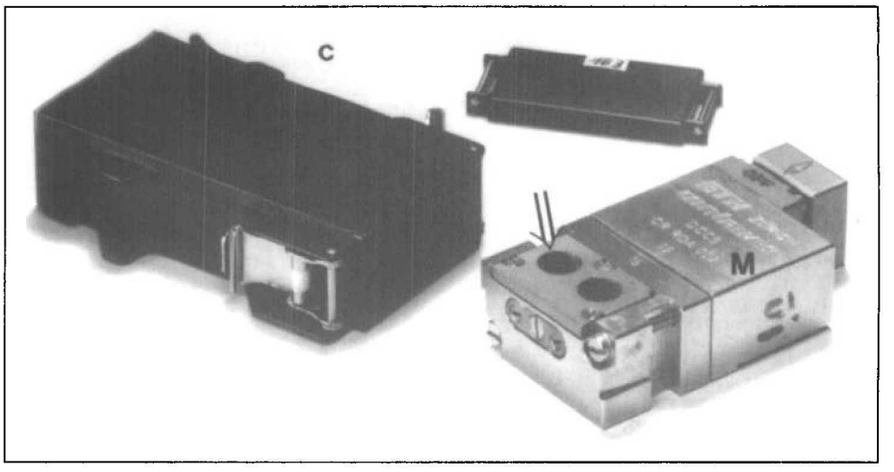

Fig. 1. *Dynamic cell cultivation system DCCS.* On the left is the container Type I (c). On the right the DCCS with the two circular windows (arrow) over the cultivation chambers. The osmotic pump was located inside of the metallic bloc (M).

2.1. *Dynamic Cell Culture System, DCCS*

With one exception (namely an automated device flown in Skylab in 1973), all the early experiments performed in space with single cells were so-called batch experiments. A batch experiment is an experiment in which the cells are cultivated in a fixed amount of nutrient solution (medium). As a consequence, such culti-

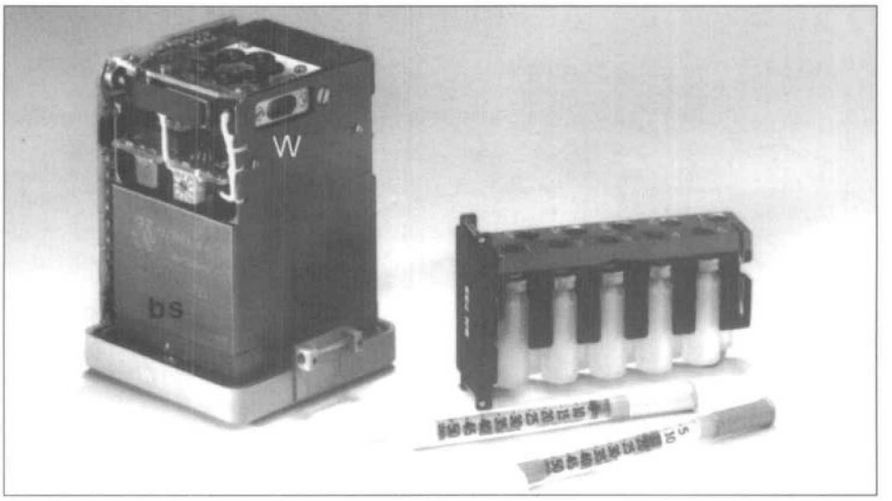

Fig. 2. *SBR I: Miniaturized bioreactor with, on its left, two syringes for the sampling and the sample bottles.* The cultivation chamber is on the top of the bioreactor. The inspection window is located on the right upper side (w). The sensors, which are integrated into the chamber wall at the opposite of the window, are not visible on this picture. The basis structure (bs) contains the fresh and the used medium. Total height: 8 cm.

vation is very limited in time due to the exhaustion of the nutrients and the accumulation of waste products. To overcome this problem, we decided in 1986 to develop, in collaboration with *Contraves AG* (Zürich, Switzerland), a totally automatic cultivation instrument for animal cells with continuous delivery of fresh medium, so that the cells can grow for several days without being starved. The development of the instrument was funded by the PRODEX program of the European Space Agency (ESA). This program supports the development of specific space hardware in countries which have not their own space agency such as Switzerland, Austria, Belgium and, since 1998, Hungary. The first system we developed was called Dynamic Cell Culture System (DCCS, *Fig. 1*). It is a completely closed system consisting of three main parts: the pump, housing, the pump and the culture chamber [4]. The fresh-medium reservoir has a capacity of 230 µl. The novelty of this system was the self-powered osmotic pump supplying the cells with fresh medium at a continuous flow rate of 1 µl h^{-1}. The DCCS was designed to fit into one standard ESA Type I container (81 × 40 × 20 mm). Its biological performance was tested on the Biokosmos 9 satellite (1989) with plant protoplasts [5]. The protoplasts were cultivated for 14 days in orbit. The results showed that the DCCS worked well under microgravity conditions. Aboard the IML-1 mission (STS-42, 1992) the DCCS was used for the cultivation of hamster kidney cells. These cells are interesting because they produce tissue plasminogen activator (t-PA). No difference in cell growth and t-PA secretion was found between flight and ground [6]. Though the DCCS, which does not need any external power supply or electronic controlling device, is suitable for continuous cultivation in microgravity, it has some limitations such as the very small working volume of the culture (200 µl), which does not allow the withdrawal of a sample during experiment, and the lack of control and on-line information.

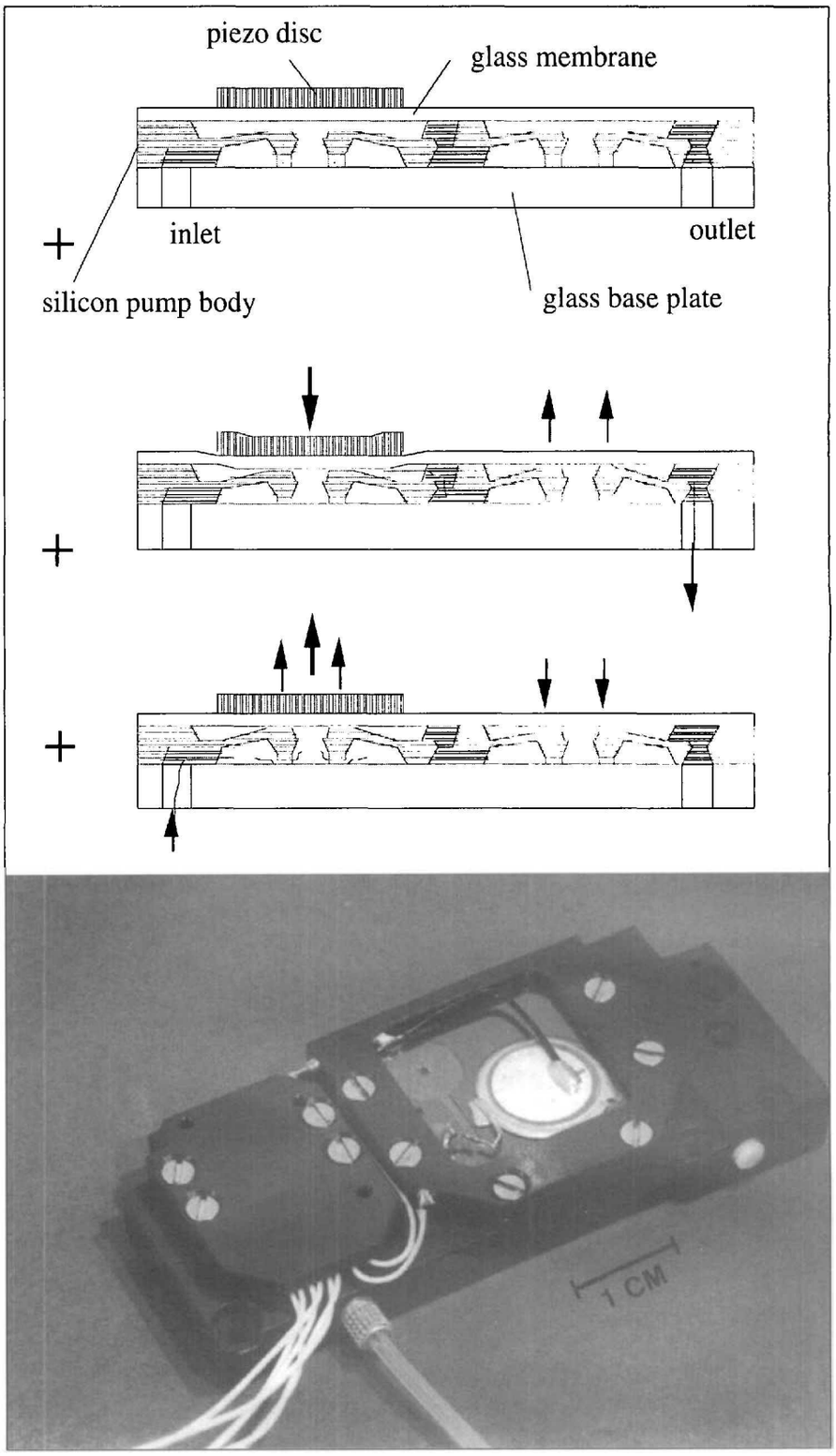

Fig. 3. *Micropump.* The piezo device is clearly visible as a disk on the right of the pump. The left part of the structure contains the flow sensor with the electrical connector and the tube bringing the medium to the culture chamber.

2.2. *Space Bioreactor SBR I: Miniaturized and Controlled*

Our next goal was to overcome the limitations of the DCCS and to develop a miniaturized bioreactor with sampling capability, pH control, gas exchange, continuous fresh-medium supply, and on-line measurements. The real challenge was to build a bioreactor with almost all the capabilities of a commercial one (total volume, electronics and mechanics included, of about 1 m^3 for a 1 l bioreactor) but fitting into a Type II/E Biorack container that has for volume 365 ml (size 87 × 63 × 63 mm, *Fig. 2*). This instrument was built in collaboration with *Mecanex SA* (Nyon, Switzerland) and the Institute of Microtechnology of the University of Neuchâtel with PRODEX funding. It is designed for yeast-cell cultivation [7]. We chose yeast cells because they are widely used in biotechnology (*i.e.*, beer and wine industry), in basic and cancer research, and as a model organism in molecular biology.

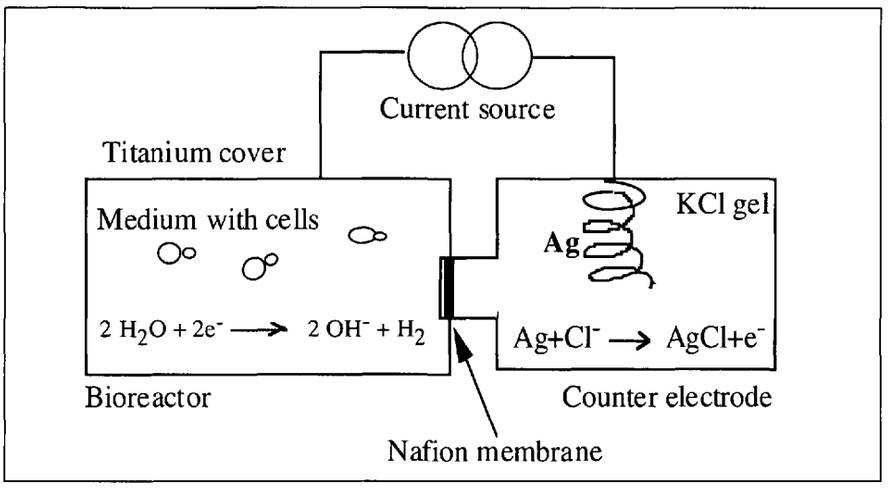

Fig. 4. *Principle of the electrochemical pH regulation.* K^+ ions are formed in the counter electrode, they pass through the Nafion membrane to the bioreactor chamber and combined with the hydroxyl ions to form KOH.

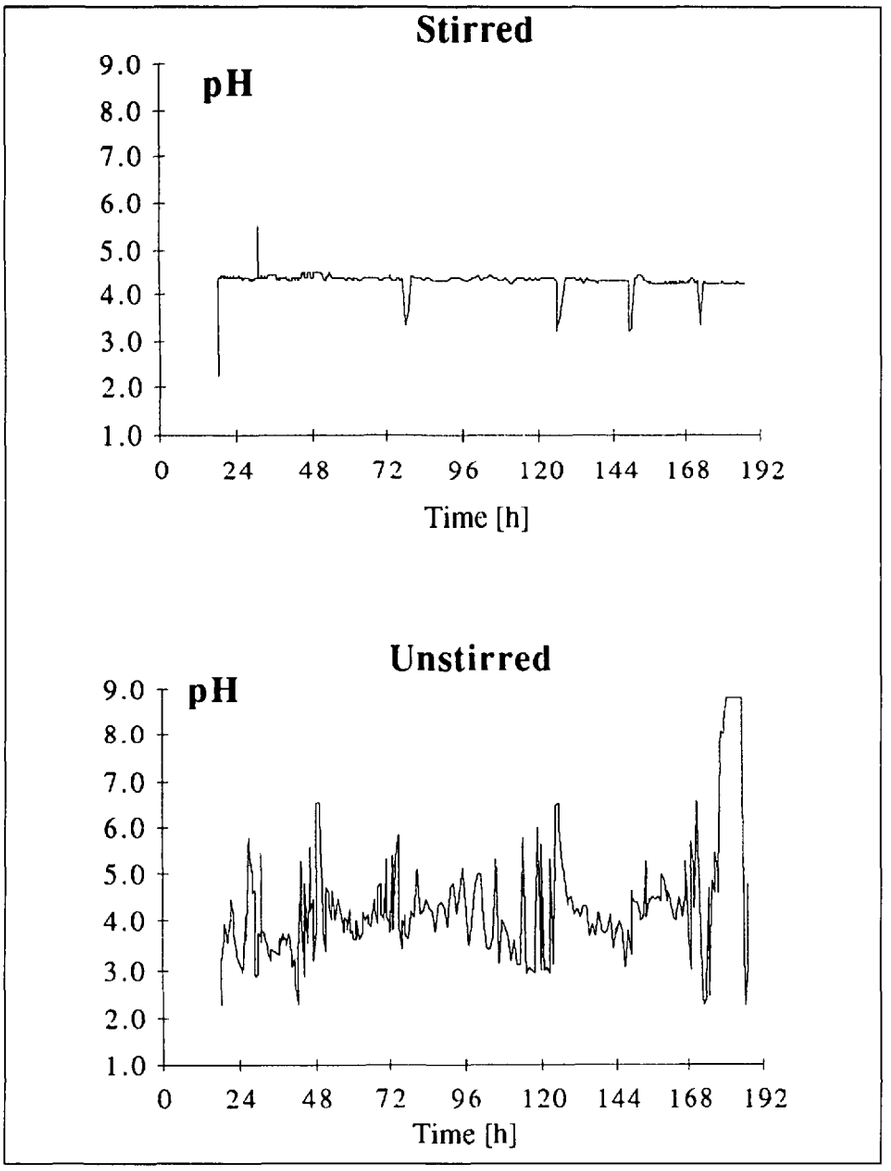

Fig. 5. *pH values for both ground bioreactors.* In the stirred one, the sampling times are very well visible (drops). About 1 ml of fresh medium at pH 2.5 is delivered to the chamber (pH 4.5) to replace the sampling volume, a pH drop is resulting especially because no compensation of pH is effected during sample withdrawal (no electrical connection of the bioreactor).

Furthermore, they have a very well-known metabolism and are very sensitive to environmental changes. The objective of the experiment was, besides the technical challenge, to investigate the effect of mixing in microgravity on biological parameters such as growth rate and metabolism. As yeast cells are non-motile it is necessary on Earth to stir the cultivation medium to avoid their sedimentation. In microgravity, they would not sediment, but the lack of convection would favour the formation of nutrient and waste gradients that could affect cell growth. To avoid the formation of such gradients, the bioreactor chamber (3 ml) was equipped with a magnetic stirring mechanism. A piezo-electric silicon micropump of 20 × 20 × 2 mm size was used for the delivery of fresh medium (*Fig. 3*). The flow rate was variable between 200 and 1200 µl per hour. The data were collected on-line by means of a sensor inserted directly into the cultivation chamber. The chip size was 3.5 × 3.5 mm. This sensor was an integration of: *i*) a pH-ISFET (ion-sensitive field-effect transistor) with Al_2O_3 gate insulator (sensitivity 51 mV/pH), *ii*) a temperature-sensitive diode in forward bias at 100 µA, and *iii*) thin-film platinum redox electrode [7]. The regulation of the pH was achieved by coulometric generation of hydroxyl ions at a titanium electrode in the bioreactor (*Fig. 4*). The counter electrode contained a chlorinated silver wire in a potassium chloride gel. The counter electrode was separated from the chamber by a cation-selective membrane. This type of pH control allows to avoid the use of concentrated NaOH. The pH sensor measured the level between pH 2 and pH 9 with an accuracy of ± 0.05 pH units. Its only disadvantage was its limited capacity due to the consumption of both the silver anode and the KCl in the counter electrode. The sampling port consists of a silicone-rubber septum and the gas exchange occurs by passive diffusion through seven *Silastic*® membranes. This bioreactor flew in 1994 and in 1996 aboard the shuttle missions STS-65 and STS-76. For the second flight, a flow sensor was implemented to improve the regulation of medium delivery. In fact, if the flow measured by the sensor was not nominal, the current delivered to the pump was adapted accordingly. This sensor was also measuring the pressure in the cultivation chamber. This measurement allowed detecting possible blockage of the outlet leading to an increase of pressure in the chamber. A new, improved version of the bioreactor is currently under development with *Mecanex* and *Seyonic SA*, Neuchâtel, a spin-off company of the

IMT of the University of Neuchâtel. It is scheduled to fly in a new instrument, called Biopack, in 2000 in the shuttle flight STS-107.

3. Results

As an example, in this section, we present the performance and part of the scientific results of the second flight of the SBR I in STS-76. The experiment consisted of two elements, namely cultivation of yeast cells in the bioreactor and, as a control, in batch chambers.

3.1. *Cultivation in the Bioreactor*

From the technical point of view, the second flight of SBR I was a success. The sensors worked according to the expectations and the pH was regulated correctly (*Fig. 5*). It was not a surprise to observe higher fluctuations of the pH under unstirred conditions in microgravity as well as on Earth. In fact, to obtain the correct pH at the sensor level (side of the chamber), more hydroxyl and K^+ ions have to be produced at the source (cover of the chamber and compensation electrode) when mixing is achieved only by diffusion and not by stirring. Moreover, in microgravity, no convection movements are occurring which leads to a larger fluctuation amplitude of the pH values.

From the biological point of view, as also observed in the previous flight [8], the yeast cells grew well in microgravity. Their metabolism was comparable to that on Earth as they consumed glucose and produced alcohol in similar ways (*Fig. 6*). Morphologically, no differences were observed between flight and ground samples. *Fig. 7* presents a transmission micrograph where the bud scars are visible.

Normally, the bud scars left on the mother cell after separation of the daughter cell are located bipolarly. We observed that the specific bipolar bud-scar positioning was altered under microgravity conditions. Interestingly enough, the percentage of cells with bud scars located randomly is much higher in space than on Earth when comparing the same cultivation conditions (*Fig. 8*). This might be due to: *i*) a higher mutation rate, the location being genetically defined [9], *ii*) the dysfunction of the cytoskeleton in space, the cytoskeleton playing an important role in the choice of the bud location [10], and/or *iii*) a disturbed metabolism because cells under starvation 'forget' where the bud has to be located [11]. With respect to the fact that the percentage of randomly located scars is higher in the unstirred bioreactors than in the stirred ones, we tend to assume that the last reason is relevant. Nevertheless, the fact that the percentage of randomly positioned scars is higher in both cultivation conditions in space than on Earth indicates that microgravity has indeed an effect on the cells. From this result we can conclude that both physico-chemical environment and microgravity have an effect on the bud location.

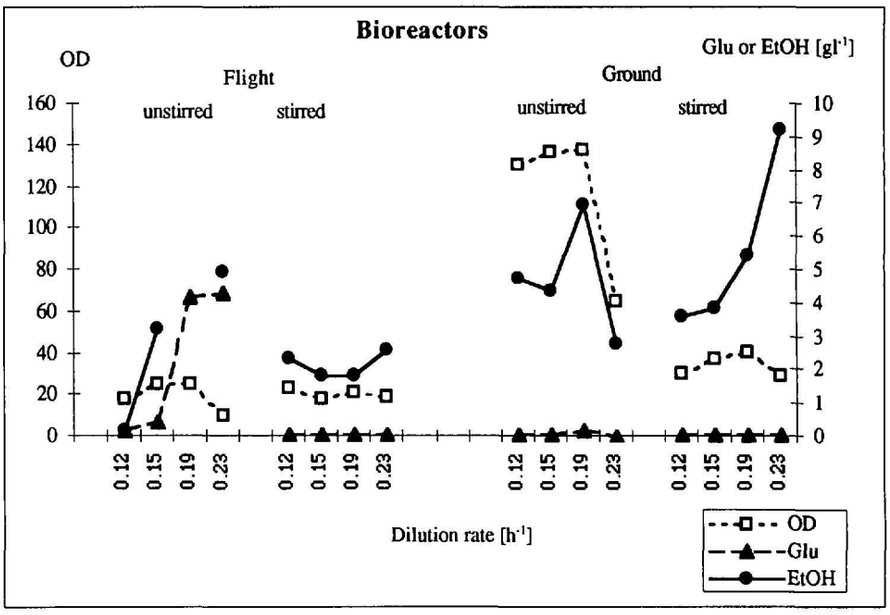

Fig. 6. *Optical density at 610 nm, glucose and ethanol concentration in the bioreactors' samples.* This diagram shows the evolution of the culture parameters (growth, glucose consumption, and ethanol production) during a continuous cultivation at increasing dilution rates. Mean values of dual measurements of a single sample are shown.

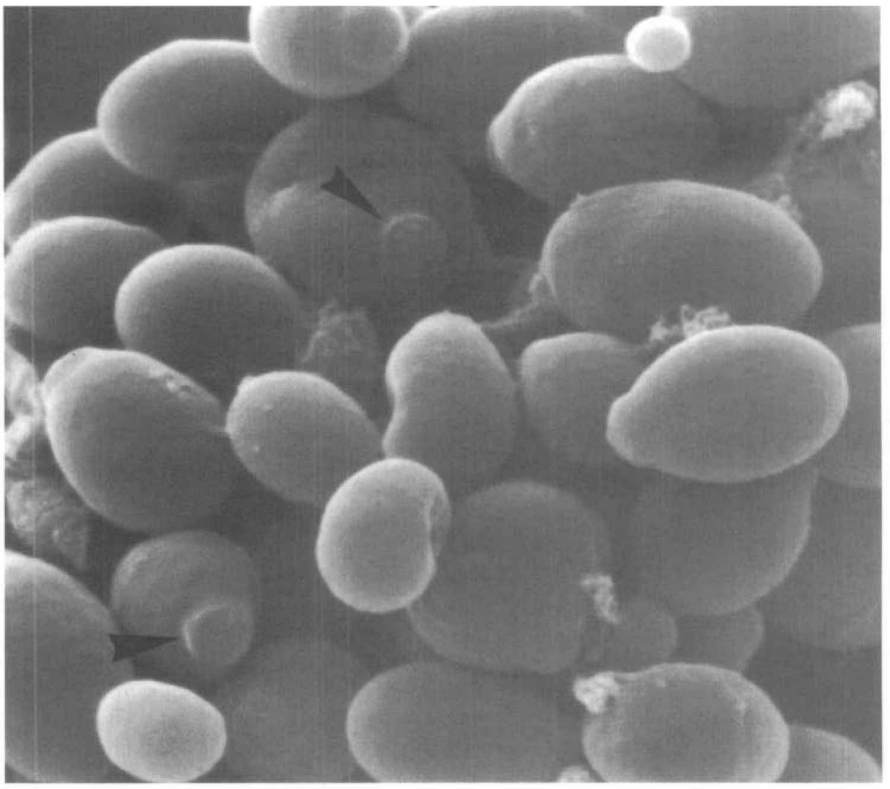

Fig. 7. *Scanning electron micrograph of yeast cells with budding scars* (arrows). Magnification 5000 ×.

3.2. *Cultivation in Batch Chamber*

Additionally to the bioreactors that could not be placed on the 1 *g* reference

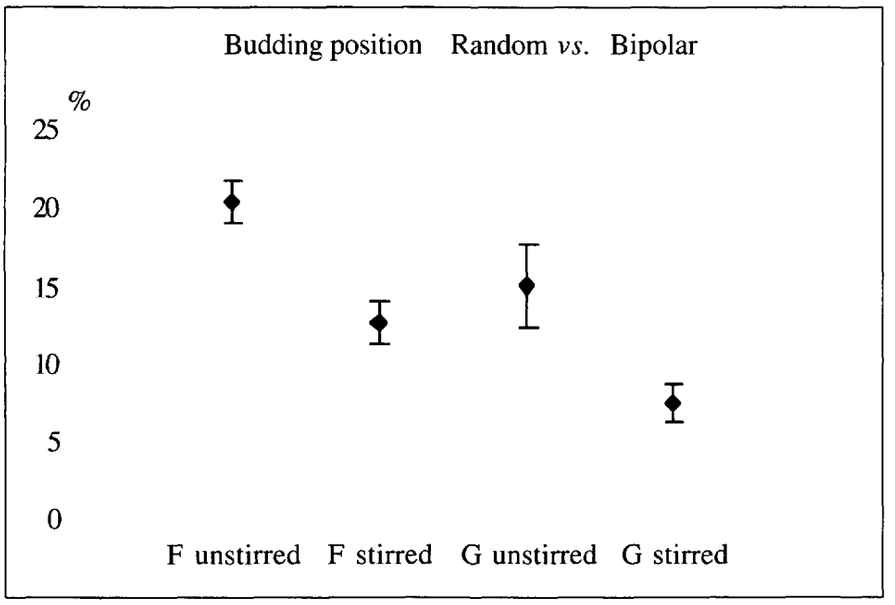

Fig. 8. *Percentage of the randomly located bud scars* vs. *the normally bipolar positioning.* The values were obtained counting 500 cells per sample; the standard deviation was calculated on the four samples available per bioreactor. *F*: Flight, *G*: Ground.

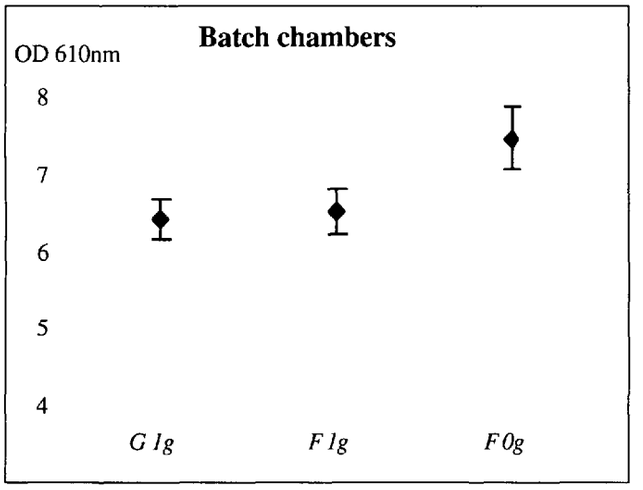

Fig. 9. *Optical density in the batch chamber after 32 h growth.* The standard deviation was calculated on duplicate measurements on quadruplicates per position. *F*: Flight, *G*: Ground.

centrifuge onboard due to their size, small batch chambers were incubated for 32 h at 22° in static position in the flight (0 *g*), on ground (1 *g*) and in the centrifuge in the flight (1*g*). After this time, the chambers were frozen to stop the growth of the cells. The analyses were performed after the flight. Optical density (OD), glucose consumption and ethanol production were measured. As expected, the glucose was fully consumed and ethanol was produced in every culture with no noticeable difference. At the OD level, a difference was observed between the cultures grown at 1 *g* and at 0 *g*. Both 1 *g* cultures (on board and static on ground) had a lower OD than the 0 *g* culture (*Fig. 9*). One could, thus, conclude that microgravity has a positive effect on the growth of the cells. However, experiments performed on the ground with agitation demonstrated that the cells grew also better than in a static position. Therefore, the growth difference observed in the batch cultures is rather caused by a negative effect of the sedimentation of the cells during centrifugation than by a positive microgravity effect. Static or centrifuge conditions are certainly not particularly favourable due to the accumulation of waste and the formation of gradients. This leads to the conclusion that for certain types of cells, at least for those not used to grow in static conditions, the centrifuge might not furnish the ideal control conditions.

4. Conclusions and Outlook

The application of silicon microtechnology for the development of instrumentation for space laboratories, the ISS in particular, will be of primary importance in the next decades. The trend to miniaturization and automation will be a technological challenge that will probably also favour spin-offs for Earth applications. Several types of bioreactors will be required: small-sized reactors (3–50 ml) of the type describe here for basic research and pilot bioprocesses, medium-sized reactors (1 l) for established bioprocesses, and large bioreactors (100 l) for closed ecological life-support systems to support long-duration human life in space. Thus, stable and reliable chemical and biological sensors for the measurement of parameters such as glucose consumption, concentration of dissolved O_2 and CO_2, metabolite production (*i.e.*, alcohol, lactate) as well as environmental control systems (pH, temperature, waste management) satisfying the requirements of manned space missions, will be needed. Depending on the specific purpose, both optical and electrochemical sensors might be of interest. The most important thing is that they cope with the space requirements for safety (*i.e.*, non-flammable, non-corrosive), power consumption (normally only few Watts available per experiment), and that their size is compatible with the restricted volume available.

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