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Multiple automated minibioreactor system for multifunctional screening in biotechnology

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Abstract— **The current techniques applied in biotechnology allow to obtain many types of molecules that must be tested on cell cultures (High Throughput Screening *HTS*). Although such tests are usually carried out automatically on mini or microwell plates, the procedures in the preindustrial stage are performed almost manually on higher volume recipients known as bioreactors. The growth conditions in both stages are completely different. The screening system presented in this work is based on the multiwell test plates philosophy, a disposable multiple minibioreactor that allows reproduction of industrial bioreactor culture conditions: aeration, stirring, temperature, O_2, pH and visible range optical absorbance measurements.**

It is possible to reproduce the growth conditions for both suspended and adherent animal cell types using 1 to 10 ml vol. bioreactors. In the case of bacteria or yeast, it is not possible to achieve a high biomass concentration, due to the reduced head volume air supply.

I. Introduction

THE application of genomic and proteomic technologies and combinational chemistry have increased the development of improved organisms. The use of new substances and processes that are applied in fields such as biomedicine, biotechnology, food industry and environment, will without doubt increase in the near future. The potential, although vast, is yet unknown.

This scenario brings the need to explore, in a rational timing and with technically robust and standardized tools, both the potential application of the different cells and natural enzymes and the therapeutic and/or toxic effects of the different molecules developed using the above mentioned techniques. These systems are known as High Throughput Screening (HTS).

Currently, most of the work carried out during the *screening* stages, (the development of new cell lines or molecules), can be performed by robots, able to manage tens of thousands of samples per day, or alternatively, it can be performed manually using incubators in which are placed the well known Petri-plates, T-flasks or miniwell plates (1-2 ml). The main disadvantage of both systems is their incapacity to control and measure real time conditions and culture evolution, resulting in a failure to reproduce high scale, real growth conditions.

Completion of the screening stage is followed by what is known as the production stage. This entails the use of complex and costly high capacity bioreactors (from 2-3 l). Information obtained from the biotechnology and pharmaceutical sector suggests a lack of direct correlation between the screening stage and production in a 30% failure rate.

During the initial phases of a new substance development with biological activity or biotechnological processes, the determination of parameters (such as, dissolved oxygen concentration, pH, temperature, glucose, etc…) becomes of special interest. It may be nearly impossible to reproduce the environmental conditions in which a product has been produced without a clear knowledge of its values and variation ranges.

II. State of the art

Some systems including different levels of automation and using the multiple minibioreactors approach have appeared:

--Bioscreen C MBR (Oy Growth Curves AB Ltd, 2003), system intended for the work automation in microbiology (designed for bacteria and yeast), allows the use of microplates up to 100-200 culture wells with a capacity of 400 ml per each. It is equipped with a shaker, global temperature control and optical density measurement at several wavelengths. [1]

--Some commercial and experimental systems based

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on reusable multiple bioreactors (made of glass), have been developed. (Cellstation, Fluorometrix [2], [3]) These systems provide stirring, thermoregulation, and pH and oxygen measurement (measurements are taken sequentially, only one bioreactor at once).

--Other devices, still in phase of development, are equipped with microsensors in multiwell plates, however such systems require to be placed inside an incubator to keep the growth conditions. [4]

A low cost screening system, able to reproduce the culture conditions of the production bioreactors, is presented, such system is based on a sterile disposable plate.

III. System Design

A. Explanation

It becomes interesting to face the design of an intermediate volume bioreactor system, big enough to reproduce the production growth conditions and small enough to be used for experimentation and screening tasks, including also, functions of control and measurement on the metabolical culture parameters. The system design criteria to accomplish are:

--MiniBioreactor (10-15 ml) made in biocompatible plastic.

--Culture medium thermoregulation within a range from 25 to 40 ºC, with ±0,1 ºC resolution.

--Magnetic embedded stirring system up to 400 r.p.m.

--Capacity of electrovalves actuation for minibioreactor's aeration.

--Design of a miniaturized and non invasive set of probes to follow the culture's metabolical activity.

--Possibility to keep several experiments in parallel.

The metabolical parameters to be measured are: Amount of suspended biomass, medium's pH, dissolved O_2, and the velocity of oxygen consumption OUR (*Oxygen Uptake Rate*). The oxygen measurement is performed by a polarographic probe located into a port integrated in the bioreactor recipient. The probe's electrodes are in touch with an oxygen permeable silicone membrane that isolates the culture medium from the outer atmosphere, the OUR estimation is performed by means of the dissolved oxygen extinction time measurement when the air supply is closed and substituted by nitrogen.

In the figure 1 is shown the variation of the pO_2 measured for three different probes, being fast enough to follow the oxygen consumption in a bacteria culture, since bacteria respiration rate is faster than animal cells, will be possible utilization of Clark's probes to measure the OUR of any cell type.

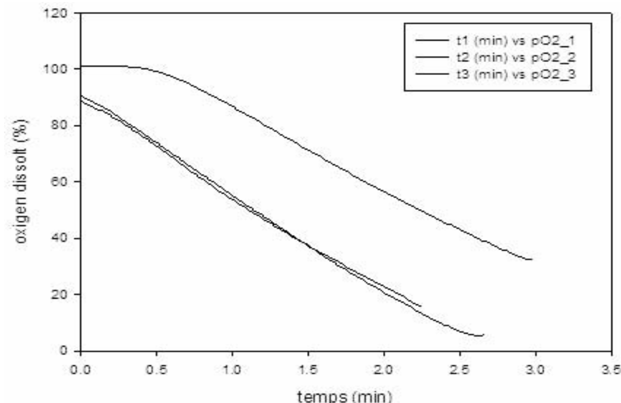

Fig. 1. Example of dissolved oxygen measurement for bacteria culture (Escherichia Coli)

It's been proved that Clark's probe is sensible to electrostatic spurious, so currently, we are working on its substitution by an optical probe based on fluorescence, the emitted light by a chemical compound integrated in the bioreactor's recipient at certain wavelength as response to the excitation wavelength may be modulated by the oxygen concentration. This measurement method will result more robust than the current one.

In the other hand, the pH and suspended biomass density measurement are performed through a fiber optic probe, by means of the culture medium optical absorbance at certain wavelengths.

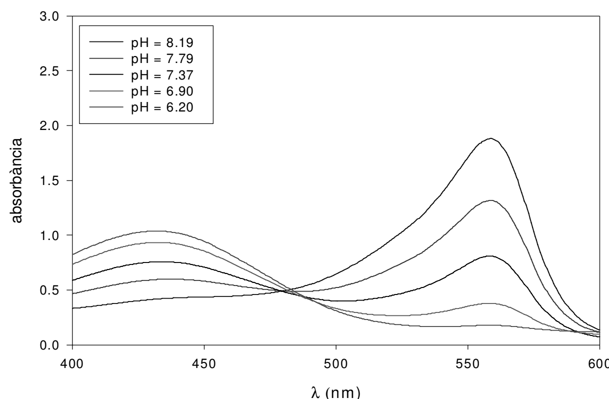

Fig. 2. Optical absorbance variation of a phenol red solution 15 mg/L (DMEM Medium) in function of the pH

The system is constituted by the following parts:

--A Control card that implements an Ethernet communications link, which allows the system's remote operation and connection of multiple systems to configure a network. It includes a spectrophotometer to perform the optical measurements, the drivers for the electrovalves and the excitation lamps actuation, the temperature control algorithm (PI) and the generation of the stirring signals.

--A thermoregulation system based on Peltier devices.

--Temperature acquisition circuits.

--Oxygen acquisition circuits.

--A magnetic stirrer through controlled electromagnets. The lack of mobile parts ensures its reliability.

--A set of optical probes and excitation lights.

--Electrovalves drivers.

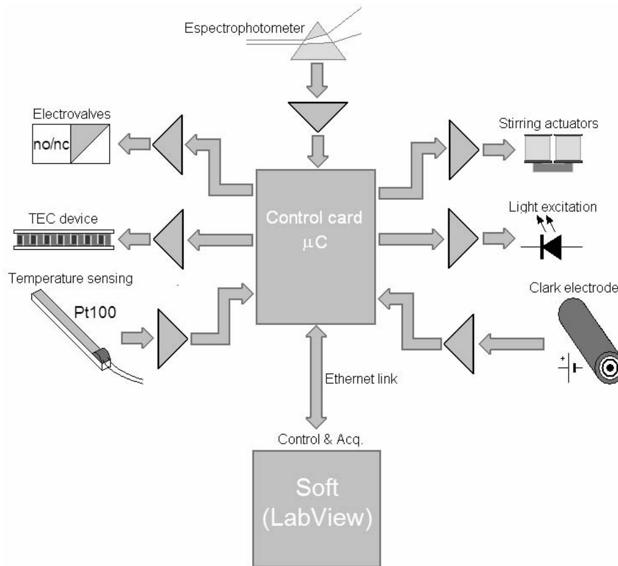

Fig. 3. System's blocks diagram

B. *Developed solutions*

The above explanation allowed us to take the initiative to build two prototypes on which we are still working in order to improve their performance and later industrialization [5]. Both systems accomplish the mentioned specifications and are made of two parts. The first one, the bioreactor's recipient by itself, manufactured in biocompatible plastic (glass polyesthirene) is a disposable part that provides all the elements of big bioreactors: the stirring element is a magnet suspended as a pendulum lined with silicone, ports for probes allocation, incoming and outgoing gas filters and finally a septum permits minibioreactor filling and inoculation. The second part is the whole acquisition and control system that executes the aeration, thermoregulation, stirring and measuring tasks.

All the control parameters and measured values are displayed on a graphic use interface developed with LabView^{TM}.

--MonoScreen System:

Figure 5 shows a detail of the designed minibioreactor, equipped with probes filters and gas tubing. The minibioreactor may optionally use different kinds of stirring elements, stirring bars or pendulum depending on the culture type (animal cells, yeasts or bacteria). Once the minibioreactor has been filled with fresh medium and inoculated in a sterile area, later it is installed within the system's actuation block, which dos not require to be placed in a sterile area.

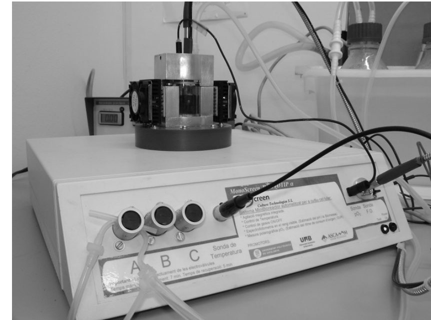

Fig. 4. MonoScreen system prototype

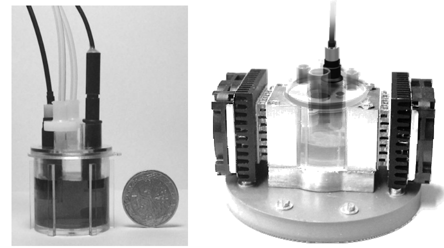

Fig. 5. Detail of the minibioreactor's mono version and the actuation block.

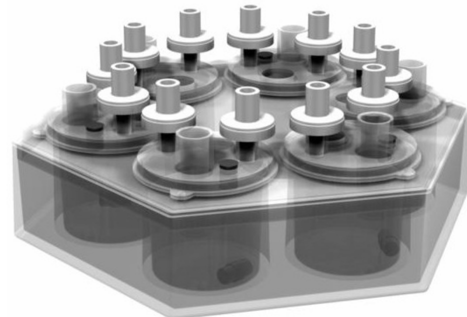

Fig. 6. 3D model of the minibioreactor's hexa version.

-- HexaScreen system:

The main feature of the screening stage is the high number of experiments that must be carried out. That is the reason why it has been designed and built a second version. In this case the recipient integrates six minibioreactors that share a thermal shell which is filled with water that ensures to have the same temperature in each bioreactor. Furthermore, electronics were redesigned to keep the culture conditions and to acquire data from six minibioreactors, So, it is possible to increase the experimentation capacity by means of executing up to six experiments in parallel, in the same stirring and temperature conditions, as well as following in real time the evolution of the metabolical variables.

IV. RESULTS

The described system is being used to follow the concentration and the activity of different types of animal cell cultures (adherent and suspended). The following graphs are a sample of the collected data:

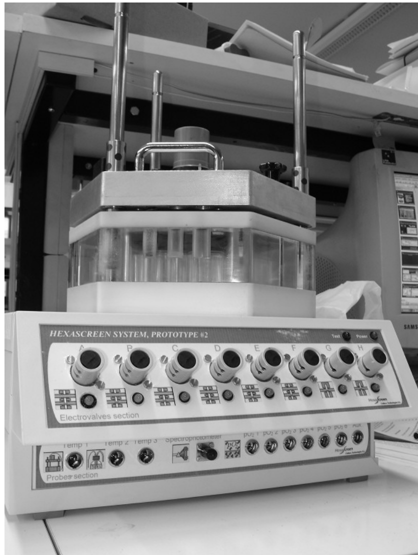

Fig. 7. HexaScreen system prototype

KB 26.5 10% FCS

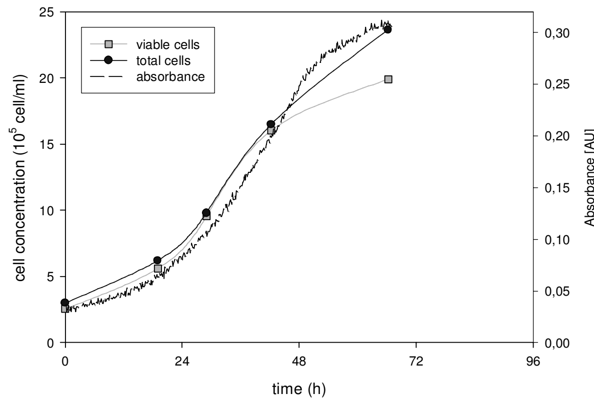

Fig. 8. Optical density measurement evolution (KB-26.5 hybridoma). Data obtained by the MonoScreen System and through inventory off-line.

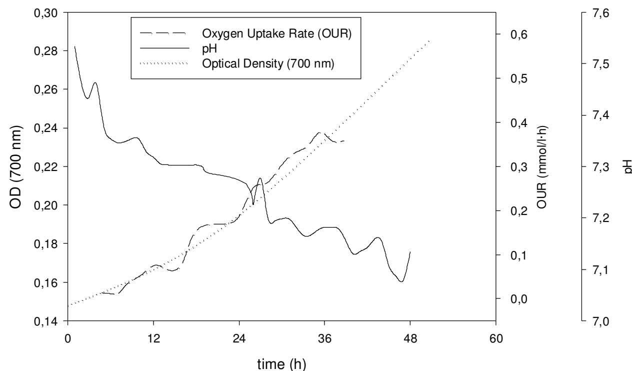

Fig. 9. OUR, pH and biomass concentration register obtained by the MonoScreen system. (KB-26.5 hybridoma).

Notice how for the suspended cells's graphs (fig. 8, 9), the measurements provided by the system are coherent with the control culture evolution, for both OUR measurement (the oxygen consumption increases as the culture grows) and Optical density - pH (the pH decreases as the lactic acid increases, which is directly proportional to the living cells concentration).

Until now, the results obtained for adherent cells are limited to the OUR. This is due to the optical interference produced by the non-homogeneous monolayer that grows at the minibioreactor's bottom surface. The system is being adapted to apply the bioimpedace measurement method, by means of printed microelectrodes to be used in these situations. [6]

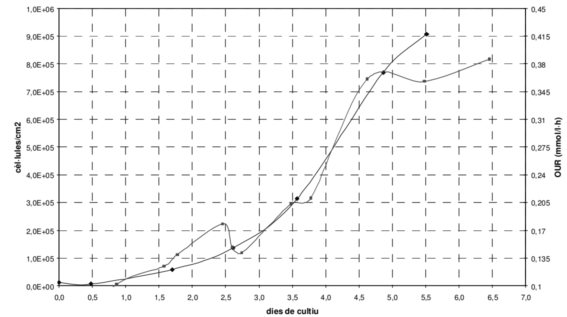

Fig. 10. OUR register (-▪-) obtained for a Vero cells culture vs. a biomass control obtained through tripsiniztion off-line (-♦-)

V. Conclusion

An automated minibioreactor system, able to reproduce within a volume of just 10 ml the bigger scale bioreactors operation conditions has been presented. Two complete prototypes have been developed as well as the disposable recipients for both versions. Some results obtained with bacteria, adherent and suspended cell cultures are shown.

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