UNIVERSITAT POLITÈCNICA DE CATALUNYA BARCELONATECH

Departament d'**Enginyeria de Sistemes, Automàtica i Informàtica Industrial**

# Contribution to the development of methods and systems for the automatization during the early stages of Bioprocess development.

Thesis presented for the qualification of Ph.D.

Program: **Biomedical Engineering**

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Barcelona 2015

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# Agraïments.

El conjunt dels reptes i les dificultat afrontades durant el desenvolupament d'aquest treball no es restringeixen només al context de la tesi doctoral, sinó que han format part del dia a dia d'una aventura empresarial anomenada HEXASCREEN CULTURE TECHNOLOGIES s. l. amb l'ambiciós objectiu de desenvolupar i portar al mercat un producte tecnològicament avançat i capaç de donar solucions a un dels sectors industrials més exigents del món, la Biotecnologia mèdica i farmacèutica.

Malgrat que el projecte empresarial no ha tingut l'èxit esperat, cal posar en valor tant la feina com l'actitud amb la que els treballadors i alguns socis de la companyia van recolzar el projecte i que va permetre no només fer realitat el producte sinó també aconseguir èxits remarcables com ara la col·laboració amb el *Biotechnology Process Engineering Center* del *Massachusets Institute of Technology* i la reconversió del producte pel cultiu de cèl·lules mare embrionàries humanes per a la *University of Calgary*.

Agrair també l'ajuda sempre incondicional per part dels grups de recerca *d'Enginyeria Cel·lular i Tissular* de la *Universitat Autònoma de Barcelona* i de *Instrumentació i Enginyeria Biomèdica* de la *Universitat Politècnica de Catalunya*. Així com en especial al meu director de tesi, el Dr. Ramon Bragós qui a més de proporcionar un assessorament crític i didàctic ha estat sempre un exemple a seguir per la seva talla professional i humana.

Finalment adreço el meu agraïment més profund a la meva germana Laura i el meus pares Núria i Andreu, els quals sempre m'han recolzat, tant en els moments d'èxit com els més difícils.

# Contents:

# Abstract.

The present dissertation deals about some of the tools and methods used in Biomedicine and Biotechnology to discover new innovative therapeutic agents. Whose development and production still involve an important amount of manual labour. Currently, such fields are considered as a zone of frontier in science [1]. Therefore, it exists an urgent need for more efficient and reproducible manners to carry out experiments and get results. In this direction bioreactors and fermenters are being used not just for production but for testing the potentiality of some cell species to produce such therapeutic agents. The most widespread concept of a bioreactor is made of a vessel connected to a control unit by means of an ensemble of probes and actuators with the aim of monitoring the growth of some cell specie (the biocatalyst), its metabolic activity, and controlling some important physical and chemical conditions, for instance: temperature, pH, $pO_2$…

In order to make such systems useful for the early bioprocess development stages (Cell screening, Clone optimisation, and Process optimisation) engineers and biotechnologists have the challenge of designing the processes keeping in mind the following requirements:

- Capability to perform an statistically significant number of experiments (*High Throughput Screening*),
- Capability to produce results being representative of the future production stages (*Scalability*).
- Capability to produce reliable results at low cost.

That's why during the last decade an important scientific and commercial interest on miniaturised disposable cell culture systems has risen. Different approaches have been developed [2] [3] [4]. Some of them focused on increasing the experimental throughput by reducing the culture volume and others on offering bigger volumes but including stunning automation and monitorization features. Probably it doesn't still exist an optimum compromise between the experimental throughput and how bio-process significant the results are for every application. Therefore, the right measurement and control methods shall be chosen depending on the type of cells cultured and the type and size of the bioreactor used.

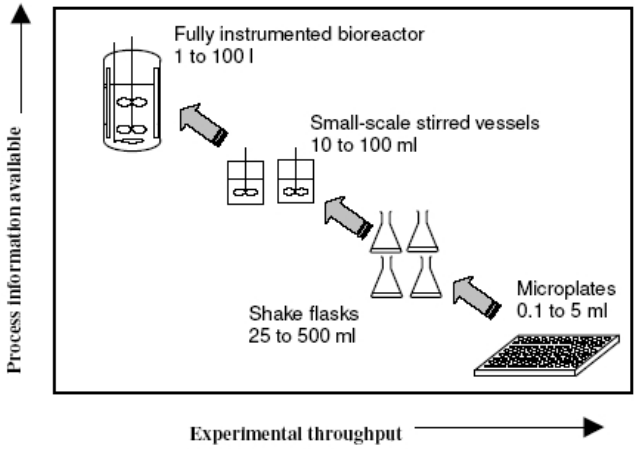


*Figure 1*
*Trade-off between the data throughput and how bio-process significant the data are.*
*From D. Doig S., I. Betts J. et al. [5] [6]*

The work here presented aims to be a contribution to the state of the art on cell culture monitoring and control techniques applied to the bioprocess development taking into account the requirements mentioned above. Specifically about the design and construction of instrumentation for the estimation of the *Oxygen Uptake Rate* (OUR) on miniaturized disposable bioreactors. OUR has been described as a key variable for tracking and monitoring the metabolic activity in animal cell culture [7][8]. This work proposal fits within this framework and tries to demonstrate the feasibility of a new method for the continuous estimation of the OUR. The proposed method is based on the accurate control of the oxygen concentration by means of *Pulse Width Modulated* (PWM) valves and the use of internal control loop variables to estimate the OUR. The method claims for a cheaper, continuous and accurate estimation, as well as being free of cell stress due to strong changes in the medium's oxygen concentration, as happens with the *Dynamic Method*, being this the most common technique.

To that end, after the study of the background and the system modelling and simulation three different testing platforms were built and every technical mean required to carry out the experimentation was developed. The first and the second testing platforms are experimental prototypes named as HexaScreen® Hexa-Batch and MonoScreen® Fed-Batch; they are disposable Minibioreactors (MBR's) manufactured by HEXASCREEN CULTURE TECHNOLOGIES S.L., the third platform is based on a Biostat® Bplus Bench-Scale bioreactor by SARTORIOUS AG. Such developments required some additional effort to design instruments and algorithms not just for measuring and control the Dissolved Oxygen (DO), but the pH, Temperature, Cell Concentration, and a wide set of means to allow the cell growth (such design will be specifically introduced in chapter 4. Therefore, although the main topics under focus are the OUR estimation and the Dissolved Oxygen (DO) measurement, due to the fact that the cultivation of cell species requires a number of considerations besides the oxygen consumption, the general scope of the present dissertation ranges from the state of the art in Minibioreactor design to different techniques applied for measure and control, as well as for monitoring the metabolic activity of the cell species.

A number of original hardware and instrumental contributions will be introduced. Below some of the main achievements:

- Development and construction of two state-of-the-art Minibioreactor platforms useful to essay the OUR estimation and DO measurement methods under study.
- Development and construction of a low cost, state-of-art. DO measurement instrument to perform a highly accurate control of the medium's DO concentration.
- Demonstration of the feasibility of a new method for the continuous estimation of the OUR, based on the accurate control of the DO concentration and especially suited for animal cell culture.

Therefore, the following chapters introduce a theoretical approach to a new method for the estimation of the OUR, which has been demonstrated by means of the experiments carried out with three different types of bioreactors, being two of them, as will be shown, an innovative embodiment within the field of disposable bioreactors.

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