BIOREACTOR-ON-A CHIP: APPLICATION TO BAKER'S YEAST FERMENTATION

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***Abstract* – This paper presents a miniaturized bioreactor (bioreactor-on-a-chip) applied to baker's yeast fermentation. The bioreactor-on-a-chip is fabricated using a silicon and glass wafers applying micromachining technology (wet-etching techniques) in order to create microchannels, mixerchannels, microvalves. The miniaturization and integration allows smaller volumes to be used, which can be often rather challenging from the analytical point of view. Also, optical detection by absorption, electrochemical detection using microelectrodes and membranes are used. Moreover, electroanalytical techniques used in bioelectrochemistry (e.g. microdialysis, capillary electrophoresis and HPLC) are employed.**

Experimental results obtained from a macro laboratory set-up with a five-liter fermenter and on-line measurement of temperature, pH, dissolved oxygen and ethanol, and exhausted gases analyses are available. Biomass and glucose concentrations are estimated on-line by observation algorithms and confirmed by off-line measurements. An ethanol set-point control is implemented.

I. INTRODUCTION

Mainly living cells of *Saccharomyces cerevisiae* form baker's yeast, used in bakery and beer industries. Apart from its industrial importance there is a significant scientific interest in baker's yeast fermentation, which is complementary to the economical interest.

Baker's yeast production is a fed-batch fermentation with an inoculum of *Saccharomyces cerevisiae* (ATCC 32167) culture and as substrate feed (carbon) a glucose solution. We may distinguish three metabolic pathways: respirative growth on glucose, fermentative growth on glucose and respirative growth on ethanol. Respirative pathways occur in presence of oxygen and the fermentative one in its absence (with production of ethanol) [1].

A macro experimental set-up for studies of modeling and control of fermentation processes is expensive and complex. Moreover, on-line measures of biomass and glucose are, still nowadays, difficult to handle, expensive and not very reliable. This situation prevents the complete knowledge of the state system and makes more difficult to implement the control laws. Usually the analysis of some state variable is made off-line with a large response time [2].

Microtechnology enables the fabrication of precise and small structures (such as microchannels, micromixers, microfilters, micropumps, microvalves, microchambers) in glass, quartz or silicon wafers [3]. The bioreactor-on-a-chip has the potential to highly automate the sample preparation procedures, drastically reduce costs associated with bulky experiments. Moreover, the bioreactor-on-a-chip can be used for experimental study on the dynamical analysis and operation of bioreactors; laboratorial costs can be extraordinarily reduced in several ways, since nanoliter quantities of reagents and samples are needed.

The bioreactor-on-a-chip also provides a significant improvement in lab safety. Spills, explosions, and other laboratory accidents that can occur with conventional sample preparation techniques are not a problem. Since with the bioreactor-on-a-chip untrained personnel can accurately and precisely perform a complete analysis.

This paper presents a bioreactor-on-a-chip applied to baker's yeast fermentation, which includes: optical detection of biomass and microelectronics allowing parameter estimation and fermentation control. The principle used to force fluids to move through the microchannels is Electrokinetics.

II. MACRO EXPERIMENTAL SET-UP

The macro experimental set-up (Fig. 1) uses a five-litre fermenter with temperature control, aeration and agitation [4]. It is equipped with sensors for on-line measurement of environment variables, such as: temperature, pH and concentration of dissolved oxygen. These variables are monitored and controlled by a direct digital control unit (DCU). Each variable has its own control loop, with appropriate parameters, which can be modified by the user or through the supervisor computer. The DCU actuates in each final control element, as for example, acid and base pumps for pH control. The pumps and electrodes calibration is done with help of this control unit.

The fed-batch fermentation starts with a two and a half litre volume substrate medium and an inoculum of 0.5 l.

A 2.5 l of medium is added through a peristaltic pump in a profile dictated by a control law. The substrate addition is monitored with the help of a balance, by means of mass variation of the glass that contains the glucose solution. The feeding ends when the maximum volume is attained (the fermentation time is about 15 to 20 hours).

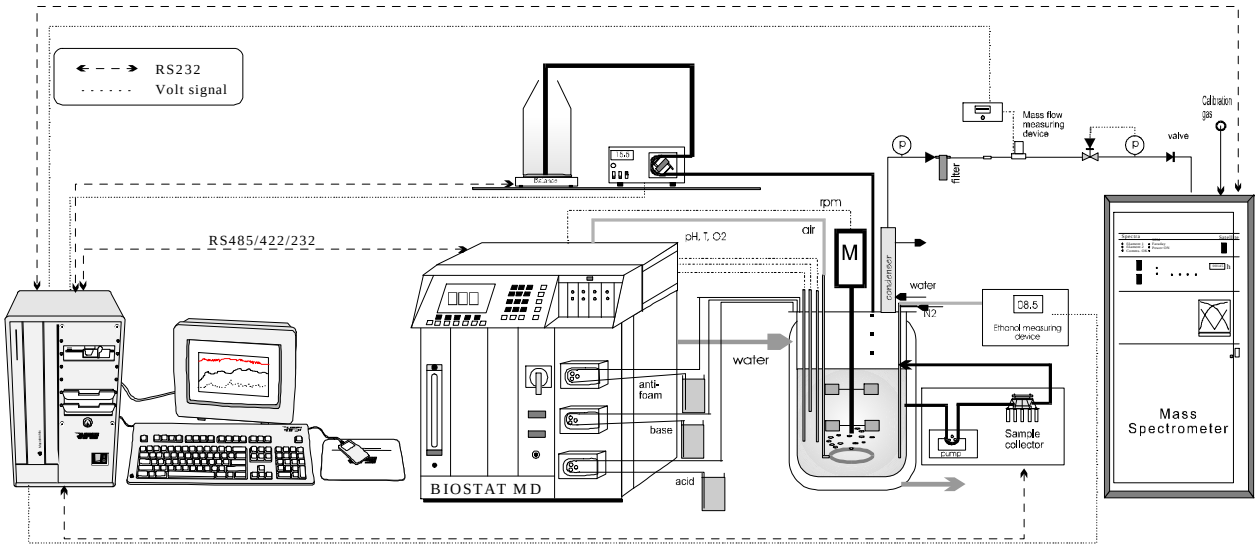

Fig. 1. Macro experimental rig

The knowledge of liquid phase composition, in terms of state variables, is obtained by measuring the following variables: biomass, glucose, dissolved ethanol, oxygen and carbon dioxide.

The ethanol is measured on-line through a sensor placed in the fermenter and linked to its own electronic device. The dissolved oxygen is measure on-line with a polarographic electrode linked to DCU. Carbon dioxide concentration is inferred from the exhaust gas composition. Biomass and glucose concentration are determined off-line. Periodically, an automatic sampler collects, in a sterile way, a small culture volume in order to analyse biomass and glucose. The biomass concentration is analysed by optical absorbance in a spectrophotometer; this property is correlated to biomass concentration in suspension (dry weight), which characterizes the biological phase. The glucose concentration is obtained through liquid chromatography (HPLC-High Performance Liquid Chromatography).

The on-line gas analysis, in terms of produced gas mass flow and its composition (percentage of nitrogen, oxygen and carbon dioxide), is done by a mass spectrometer.

A computer is linked to the DCU and other hardware in order to implement data acquisition, monitoring and open and closed control loop strategies (in open loop to impose constant or variable feed flow and in closed loop to test PID laws and adaptive control algorithms). In order to overcome the difficulties of off-line measures of biomass and glucose, the supervisor computer also determines their concentration by on-line estimation techniques.

III. BIOREACTOR-ON-A-CHIP

The microchip-based technology resembles microelectronic computer chip. The microchips can be produced using photolithography and chemical etching techniques that are quite similar to those used in the manufacture of integrated circuits [5].

Microchannels are etched into the substrate to carry fluids: chemical reagents and sample solutions. The substrate can be glass, quartz or silicon.

A general schematic view of the bioreactor-on-a-chip is presented in Fig. 2. Microsystems like this one are usually referred as Micro Total Analysis Systems (μTAS). They allow a variety of analytical chemistry methods or process control of simple mixtures, with the resolving powers of today's macro analytical systems [6].

The microfluidic device is fabricated in a glass wafer. Glass was chosen for its transparency and because it is an electrical insulator [7]. Therefore, electrophoretic flow principle can be used to move fluids through the microchannels, which avoids mechanical pumps and valves. Thus, fluid movement results from electrokinetic's forces derived from small voltages that are applied to specific regions in the microsystem [8].

Wet-etching techniques applied to the wafer glass allows the fabrication of microchannels, mixerchannel and detection chamber. The silicon wafer has an integrated photodiode and readout electronics with a light-to-frequency converter. All these functions are done in CMOS (Complementary Metal Oxide Semiconductor) technology in a standard process.

An optical filter can be included on the top of glass wafer.

The biomass detection system consists in color analysis based on optical absorption. A monochromatic light beam must be directed into the detection chamber, where the light is absorbed or reflected. The intensity of the transmitted light when measured by the photodetector can therefore give information about the biomass concentration.

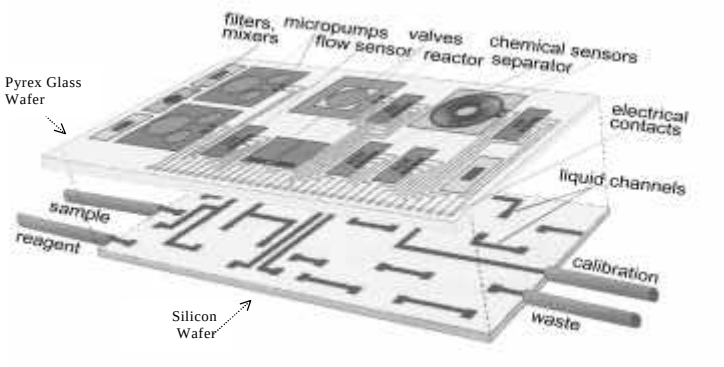

Fig. 2. A Micro-Total Analysis System (μTAS) [9]

IV. "MACRO" RESULTS

The results were obtained in the laboratory scale rig presented. These results will be used to validate the microanalysis system, running in open and close loop.

Fig. 3 shows the biomass, glucose and dissolved ethanol measurements obtained in a twenty hours running fed-batch fermentation (points). It is also drawn on the graphic the off-line simulation results (continuous line) obtained with the mathematical of baker's yeast fed-batch fermentation. The experiment runs in open loop, at constant feed rate (0.05 l/h), with state variables initial concentrations equal to: biomass 0.23 g/l, glucose 3.1 g/l, ethanol 0.72 g/l, oxygen 0.0066 g/l and carbon dioxide 0.008 g/l. The simulation results adjust correctly the experimental values.

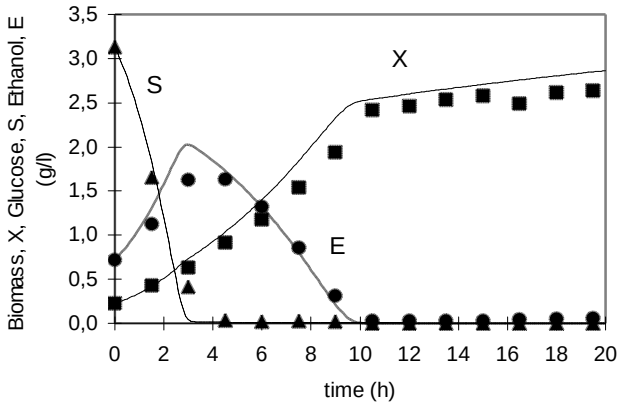

Fig. 3. Open loop experiment: Biomass (¦), glucose (?) and ethanol (?) concentrations experimental values (points) and simulation results (lines), with feed flow equals to 0.05 l/h

Fig. 4 shows a closed loop experiment. An adaptive control law is applied to maintain ethanol concentration at 0.5 g/l during the first 12 h. At this time a set-point change is introduced: new ethanol reference value is 0.75 g/l. State variables initial concentrations are: biomass 0.72 g/l, glucose 0.54 g/l, ethanol 2.37 g/l, oxygen 0.0066 g/l and carbon dioxide 0.008 g/l.

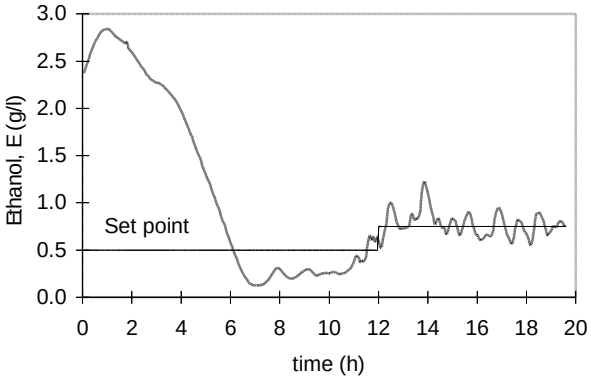

Fig. 4. Closed loop experiment: adaptive control algorithm to impose an ethanol concentration at 0.5 g/l during the first 12 h and 0.75 g/l till the end

Fig. 5 presents the corresponding values of the manipulated variable, the feed flow rate (F), and the volume of fermenter (V).

Analyzing both figures we can see that the control law is inactive for the first 6 h as ethanol concentration is higher than the reference value. After that time, the control algorithm starts to calculate and actualizes the manipulated variable. In spite of noise, the controller manages to handle ethanol set point.

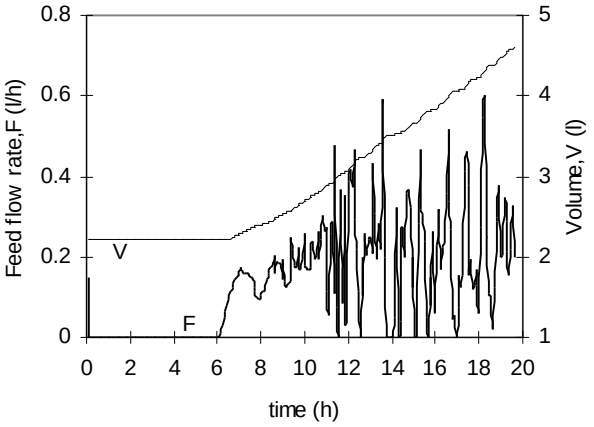

Fig. 5. Closed loop experiment: feed flow, F, and volume of fermenter, V, corresponding to ethanol adaptive loop of Fig. 4

V. CONCLUSIONS

The macro laboratory set-up is already assembled and tested. PID and adaptive control strategies of ethanol on-line regulation are implemented and compared. The measurement of biomass and glucose concentrations are done off-line and compared with on-line observation methods.

The bioreactor-on-a-chip has the potential to automate the sample preparation procedures and the fed-batch fermentation itself, drastically reducing costs and fermentation time (from a 20 hours experiment to a few minutes) and improving safety associated with macro experiments. The technology has the potential of lowering the prices of the normally bulky analyzers by large factors. Shrinking the bioreactor dimensions reduces dramatically: the analysis time and the price per analysis. Reliable on-line measurements (specially important for biomass and glucose) are achieved. Moreover, since low quantities of hazardous chemical reagents are needed, the resultant environmental pollution starts to be no longer a problem.

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