A Bioreactor Based on Optical Measurements for Baker's Yeast Fermentation

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SUMMARY

This paper presents a bioreactor for baker's yeast fermentation. Small volumes are used in the bioreactor (< 100 ml) comparing with the macro setup (5 l). Biomass, ethanol and pH measurements are carried out by optical spectral analysis: the first two by optical absorption method and the last one is determined by fluorescence. HPTS (8 Hydroxypyrene-1,3,6-trisulfonic acid Trisodium salt) is chosen for pH measurement due to be the most suitable for precise and stable pH analysis (aqueous acid/base, biomass, ethanol and glucose solutions). A resolution of 0.2 in pH scale was achieved. Known biomass concentrations are measured with a stable pH=4 (no interference between the two measurements). Design and implementation of a bioreactor-on-a-chip for baker's yeast fermentation is the final goal of this study.

Keywords: bioreactor**,** baker's yeast, spectral analysis.

Subject category: Chemical sensors, Applications.

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.

Baker's yeast production is a fed-batch fermentation with an inoculum of *Saccharomyces cerevisiae* (ATCC 32167) culture at a constant pH 4. 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 setup for studies of modeling and control of fermentation processes is expensive and complex. Moreover, on-line measures of state variables are, still nowadays, difficult to handle, expensive and not very reliable. This situation makes more difficult to implement new control laws based on state variable profiles. Usually, the analysis of some state variable is made off-line with a large response time [2].

A 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 small quantities of reagents and samples are needed [3].

This paper presents a bioreactor applied to baker's yeast fermentation, which includes optical detection of biomass, ethanol and pH determination by spectral analysis.

DESIGN

Figure 1 presents a schematic for the experimental measurements of three variables, biomass, ethanol, and pH values. Biomass, ethanol and pH measurements are carried out by optical spectral analysis: the first two by optical absorption method and pH is determined by fluorescence.

The intensity of the transmitted light when measured by the photodetector can give information about the ethanol and biomass concentrations. The biomass detection system (optical absorption) is processed at λ_{Abs} 620 nm. The ethanol detection is also based on optical absorption method by using a specific membrane (λ_{Abs} 305 nm).

When the sample is excited with a wavelenght of 403 nm, other photodetector measures the emission light, that gives information about pH-value [4]. The excitation wavelength employed is λ_{Ex} 403 nm and the emission wavelenght is λ_{Em} 510 nm.

EXPERIMENTAL RESULTS

As baker's yeast production should run at a constant pH 4, a fine calibration curve is then needed for control purposes, in that neighborhood. Several acid/base aqueous sample solutions were prepared in the pH range from 3 to 5. Nevertheless, samples were also prepared for a large pH scale: from 1 to 11.

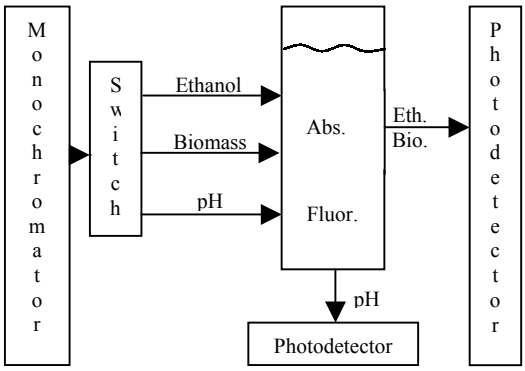

Fig. 1: *Experimental measurements for ethanol, biomass and pH by absorption and fluorescence*

Two pH indicators were tested: DHPDS (6,8-Dihydroxypyrene-1,3-disulfonic acid Disodium salt) and HPTS (8 Hydroxypyrene-1,3,6-trisulfonic acid Trisodium salt). In the application, HPTS is the most stable.

All absorption analysis were performed in a Jasco V-560 spectrophotometer; pH measurement was processed by fluorescence methods in a Jasco FP 6200 system.

Figure 2 shows that the fluorescence of HPTS decreases with pH increases when excited at 403 nm in acid/basic sample water solutions. The reverse can be true when exciting at another wavelenght, e.g., in the case of HPTS, at 460 nm [4]. A resolution of 0.2 in pH-scale was attained.

Ethanol and glucose were added to the sample solutions to test any interference with the HPTS. The fluorescence intensity was not lost compared with aqueous free ethanol/glucose samples.

A 100 ml batch fermentation was run and samples were analyzed each hour for biomass concentration and pH. The results obtained point out that neither HPTS interferers with biomass absorption analysis nor biomass interferes with pH measurement by fluorescence.

CONCLUSIONS

A bioreactor based on optical measurements of biomass, ethanol and pH variables was presented. Optical absorption method (biomass and ethanol) and fluorescence method (pH) are used. Amongst the pH indicators studied, HPTS is the most suitable for baker's yeast sample solutions. A resolution of 0.2 in pH-scale was achieved.

The design and implementation of a bioreactor-on-a-chip, the final goal of this project, has the potential to automate the sample preparation procedures and the fed-batch fermentation itself, drastically reducing costs and state variable time analysis and improving safety associated with macro experiments.

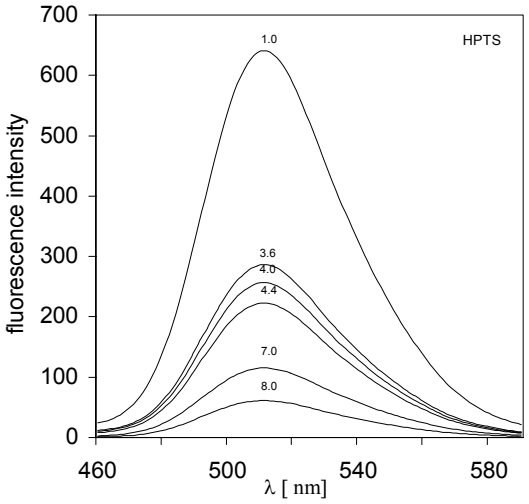

Fig. 2: *Fluorescence spectrum of HPTS at various pH-values. Excitation wavelength 403 nm,conc.4.1μM*

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