DOI 10.5162/11dss2013/L2

Online monitoring of biomass concentration with the biO$_2$mass sensor technology

I. Stempin [1], Z. Kovács [1,2], P. Czermak [1,3,4]
[1] Institute of Bioprocess Engineering and Pharmaceutical Technology, University of Applied Sciences Mittelhessen, Wiesenstrasse 14, 35390 Giessen, Germany
[2] Department of Food Engineering, Corvinus University of Budapest, Ménesi st 44, H-1118 Budapest, Hungary
[3] Department of Chemical Engineering, Kansas State University, Durland Hall 1005, Manhattan, KS, 66506-5102, USA
[4] Faculty of Biology and Chemistry, Justus Liebig University, Giessen, Germany

Abstract

In situ measurement of biomass is one of the most critical analytical procedures in biotechnological processes. In our laboratory, we have developed a sensor, called biO2mass sensor (Patent No DE 10 2013 006 972.6), for measuring biomass concentration of aerobic cultures during fermentation. The principle of the measurement is based on periodically creating a small, enclosed sampling room in the fermenter in which the rate of decay in dissolved oxygen correlates with the actual biomass concentration. The applicability of the proposed technique is proven by experimental investigations on fermentation processes using various strains such as *Pichia pastoris* and *Escherichia coli* K 12. The obtained data from the biO2mass sensor shows excellent correlation with dry cell weight and optical density measurements. This simple procedure allows a rapid, sensitive, cheap, in situ measurement of the concentration of living aerobic cells.

Key words: Bioprocess monitoring, Biomass, In Situ, On-line, Sensors, Optode

Introduction

To meet the requirements and goals of the PAT-Initiative and the quality-guidelines according to GMP, the importance of the documentation of process-information gained during the running process increases. *In-situ*-procedures are especially beneficial; they enable a contemporary measuring of a respective medium, and contamination-risks that might occur during the critical point of measuring are reduced to a minimum.

The biomass represents an important process parameter, regardless of its secondary importance of cultivation in most industrial production processes. The knowledge of the biomass-concentration is essential for the determination of the kinetics and stoichiometry of microbial growth. The cell-growth is a defining parameter for the quality and efficiency of the cultivation-process. The achievement of online process-information in-situ, without major mechanical effort is becoming more and more important, so that the result is available for timely control action.

A huge number of different applications have been realized and are in use. The methods used in the quantification of biomass concentration in situ are mainly dielectric spectroscopy and various optical methods [1]. Optical measuring methods, which are based on the interaction between electromagnetic radiation and substance, are becoming more and more important. Depending on the method of measuring the absorption- or reflection-effects play a decisive role. The in-situ-microscopy is about a photographical, optical measuring procedure, which makes it possible to draw conclusions about the biomass-concentration. Additionally one can determine the cell-viability and morphology in-situ [2|. The methods used in the quantification of biomass concentration can be classified in two categories, depending on whether biomass is being determined direct or via the conclusions drawn from the interactions of physical or chemical quantities, like substrate-, product- or metabolite concentration. The state of the art of *in-situ* sensors have been reviewed in [3].

In our laboratory, we have developed a sensor, called biO2mass sensor, for measuring biomass concentration of aerobic cultures during

fermentation. Patent application describing the proposed technology is submitted (No. DE 10 2013 006 972.6). The principle of the measurement is based on periodically creating a small, enclosed sampling room in the fermenter in which the rate of decay in dissolved oxygen correlates with the actual biomass concentration. This simple procedure allows a rapid, sensitive, cheap, in situ measurement of the concentration of living aerobic cells.

The biO$_2$mass method

The biO2mass method is a combination of a system of optical oxygen-measurement and an in-situ probe which makes the oxygen determination inside a self-contained room possible. For the determination of the dissolved oxygen level, we use the *Fibox* 3 system manufactured by *PreSens GmbH*, Germany (see Figure 1).

The measuring principle of the optical oxygen sensor is based on fluorescence quenching of a fluorescence dye in the excited state by oxygen. The fluorescence dye is typically an organometallic dye of the phenathroline-type. The fluorescence dye molecules are being excited by light of a defined wavelength and emit red-shifted fluorescent light of longer wavelengths. The collision between molecular oxygen and fluorescent dye molecules in the excited state cause to a decrease of fluorescence. The *Fibox 3 system* uses the resulting decrease of the phase angle Φ as the information carrier for oxygen correlation. [4]

We have developed an in-situ probe that consists of a solid outer tube, and an inner tube, which is vertically movable. At the bottom of the inner tube, a sensor spot is glued to a glass surface, and an opening enables the direct contact with the fluid. The in-situ probe is 360 mm long and has a diameter of 12 mm designed for 12 mm standard ports.

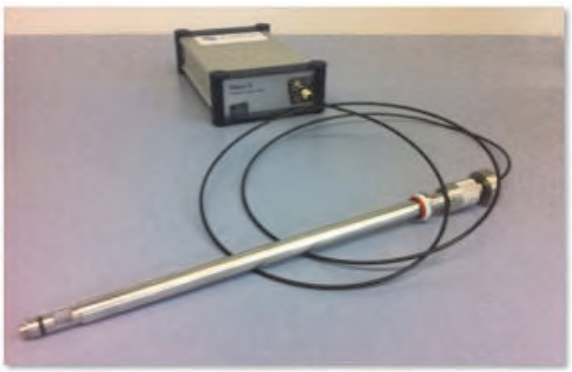

Fig. 1. The Fibox 3 transmitter (PreSense GmbH), polymer optical fiber (PreSense GmbH) in the background and the biO2 mass probe

The Functional Principle

The functional principle is based on periodically creating a small, enclosed sampling room with the in-situ probe. The probe has two measuring modes. Figure 2 shows the *open initial position*, where the inner tube is completely pushed out, so that the medium can flow through the opening (*measuring zone)* of the probe. This way the dissolved oxygen can be measured in the entire bioreactor throughout the fermentation process.

By pulling the inner tube inside the outer tube the measurement zone is closed, so there is no more flow of fluid. In the *closed measuring position,* the decrease of the oxygen concentration, which is triggered by its consumption through the enclosed cells, can be measured (see Figure 3). The sealings (not shown) enable an air- and gas-tight containment of the fluid, which makes the biomass determination possible.

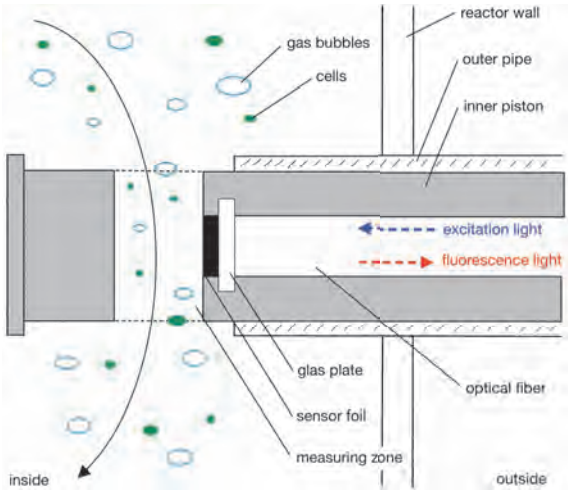

Fig. 2. Scheme of the in-situ probe in the open initial position

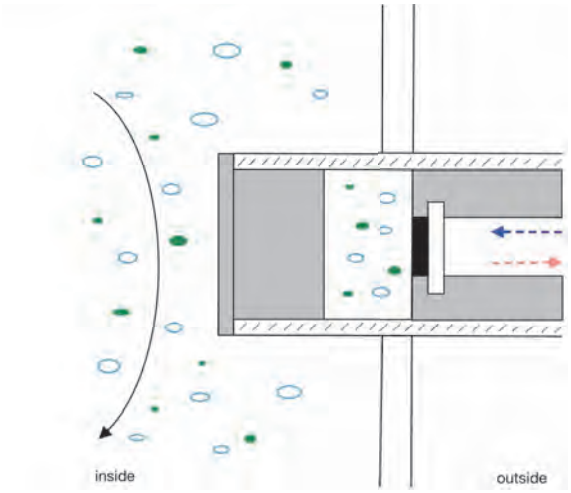

Fig. 3. Scheme of the in-situ probe in the closed measuring position

Method of Determination

The determination of the biomass concentration is based on dissolved oxygen determination after closing the measuring zone. The resulting progressions of the oxygen saturation at increasing cell concentrations during a batch fermentation of *Pichia pastoris* are shown in Figure 4. A measuring time of 10 minutes was applied for each sampling. The following characteristics regarding the progression of oxygen concentration was observed:

- A pronounced decrease in oxygen concentration as cell concentration rises
- An approximately linear progress down to 40% oxygen saturation
- Afterwards the curves flatten out

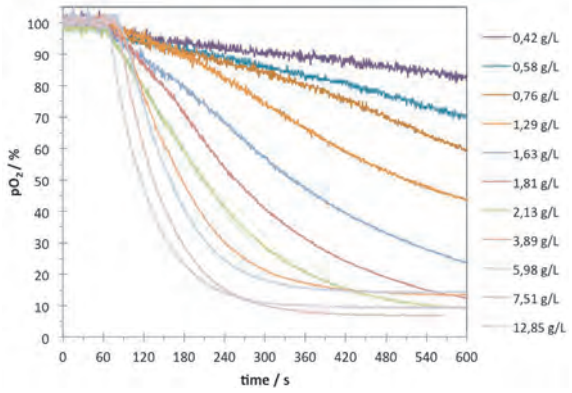

Fig. 4. Different oxygen trends by increasing consumer concentration during the fermentation with Pichia pastoris (GMO).

The decrease of oxygen is due to the consumption of active, aerobic cells. The cells in that closed space consume only the oxygen enclosed in that space.

The decrease of oxygen saturation dpO_2 in this space can be seen as proportional to the cell concentration x:

$$x \sim \frac{dpO_2}{dt} \quad (1)$$

One possibility of the correlation of the biomass can be mathematically determined with the measuring of the slope m at the beginning of the linear section.

$$m = \frac{\Delta pO_2}{\Delta t} \quad (2)$$

A measuring-period Δt of 30 seconds is sufficient to determine the correlation. Through the determination of the slope of all the curves in Figure 4 we can compare the biO_2mass method with the *dry cell weight (DCW)* (see Figure 5). Additionally to our determination method we take samples in regular intervals and determine externally the DCW. The course of the curve of the biO2mass data corresponds well with the offline measuring method, especially down to low cell concentrations of 2 g L^{-1}. At the start of the exponential phase the calculated growth shows a steeper increase than the DCW curves.

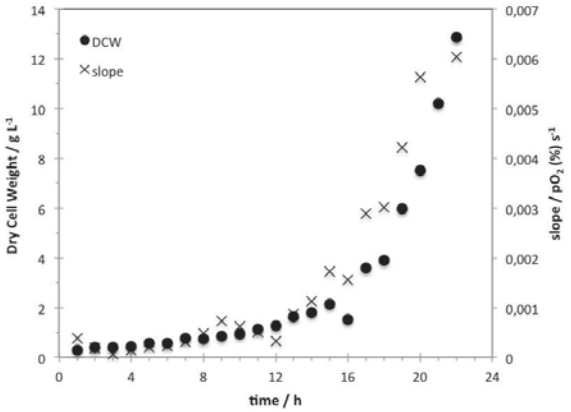

Fig. 5. Biomass determination of Pichia pastoris by dry cell weight and biO_2mass techniques

Through application of the slope over the DCW one receives the correlation-curve:

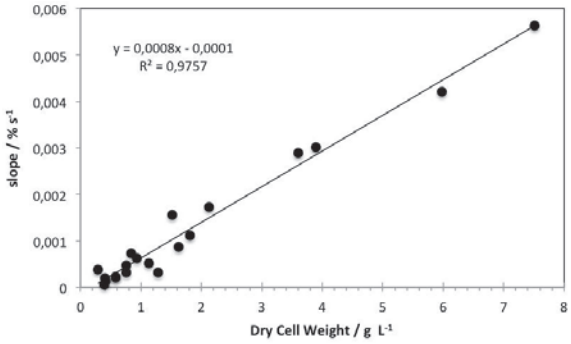

Fig. 6. Correlation between biO_2mass and dry cell weight

Figure 6 shows a strong linear relationship between dry cell weight and the determined slopes up to a DCW of 8 g L^{-1}. It is possible to correlate the biomass concentration by using the correlation curve illustrated in Figure 7.

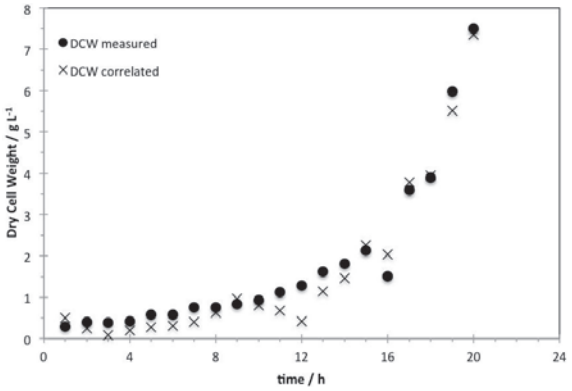

Fig. 7. Comparison between the measured dry cell weight and the correlated dry cell weight

It is important to note, that the proposed method is not restricted to one particular organism, but also applicable to other organisms, like *Escherichia coli* (see Figure 8).

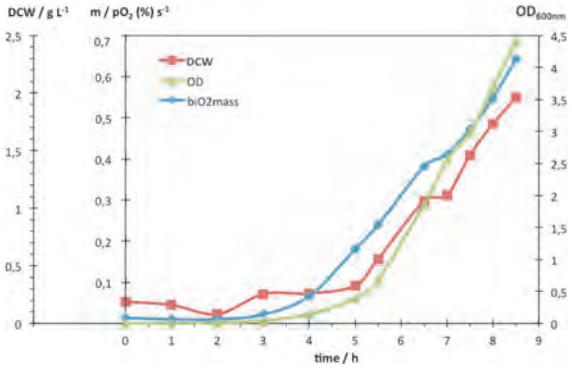

Fig. 8 Biomass determination of Escherichia coli K 12 (GMO) by Dry Cell Weight (DCW), Optical Density at 600 nm (OD_{600nm}) and biO_2mass techniques

Summary

The bio2mass sensor technology enables an in-situ biomass determination based on the decline of dissolved oxygen in a self-contained space that corresponds with the actual biomass concentration in the fermenter. The biomass determination can be achieved online during the running process. The biO2mass method is essentially an indirect measuring method, but it reveals the biomass as measuring value with a relatively simple measuring principle. With a minor mechanical effort, an exact measurement is possible. The necessary measuring-volume is very small, therefore its influence on the entire system is negligible. The probe can be specifically used for the determination of aerobe aerobic living cells. Based on the simple modular construction, the probe can be applied in-situ, ex-situ or as a bypass-probe, for laboratory- and industrial-applications, as well as a mobile device.

Our further research will focus on specifying the upper- and lower limits of the biO2mas sensor technology regarding to biomass concentration. Note that the flattening-out-effect can possibly be microorganism-specific. Thus, our method may be used as an alarm system for side-fermentations. The application of the biO2mass sensor for adherent cells on microcarriers is another topic for further R&D work.

Acknowledgements

The authors thank the Hessen State Ministry of Higher Education, Research and the Arts for the financial support within the Hessen initiative for scientific and economic excellence (LOEWE-Program). The second author is grateful for the Marie Curie FP7 Integration Grant provided by the 7th European Union Framework Programme (PCIG11-GA-2012-322219).

References

[1] Kiviharju, K., Salonen, K., Moilanen, U., Eerikäinen, T.: Biomass measurement online: the performances of in situ measurements and software sensors. In: Journal of Industrial Microbiology and Biotechnology. 35 (2008): 675-665; doi: 10.1007/s10295-008-0346-5

[2] Rudolph, G., Brückerhoff, T., Bluma, A., Korb, G., Scheper, T.: Optische Inline-Verfahren zur Zellzahl- und Zellgrößenbestimmung in der Bioprozesstechnik. In: CIT: Chemie Ingenieur Technik. 79 (2007): 42-51; doi: 10.1002/cite.200600110

[3] Vojinovic, V., Cabral, J.M.S., Fonseca, L.P.: Real-time-bioprocess monitoring Part 1: In-situ sensors. In: Sensors and Actuators B: Chemical. 114 (2006): 1083-1091; doi: 0.1016/j.snb.2005.07.059

[4] Huber, C., Krause, C.: Instruction Manual Fibox3, Software Version 5.32. PreSens GmbH, 2006