Bioprocess Engineering 22 (2000) 275–279 © Springer-Verlag 2000

A novel fiber optic probe for on-line monitoring of biomass concentrations

G. Janelt, N. Gerbsch, R. Buchholz

Abstract A fiber optic biomass probe for on-line measurement of biomass concentration was designed. Results of biomass concentration monitoring experiments with suspended cells of baker's yeast as well as an experimental cultivation of *S. cerevisiae* are presented. The device was able to observe biomass concentrations of 14 g l^{-1} *S. cerevisiae*. By means of correlations the capability of estimating the biomass concentration from the probe signal is demonstrated.

List of symbols

μ	h^{-1}	maximum growth rate
b_0–b_4		regression parameters
n	rpm	stirrer speed
r^2		correlation coefficient
t	min, h	time
$\dot{v}$	l min^{-1}	aeration rate
x_{max}	g l^{-1}, counts s^{-1}	maximum value
x_0	g l^{-1}, counts s^{-1}	start value
y	g l^{-1}, counts s^{-1}	addend for offset

1 Introduction

The knowledge of relevant physical and chemical parameters is the basis of optimal process control. With the growing market of food industry and biotechnology the demand for measurement and control of biological parameters increases. In bioprocesses involving cultivations the biomass concentration is one of the most important parameters in biotechnology [1] for both product synthesis and degradation of e.g. harmful substances. The importance of bioprocess engineering involving microorganisms and cell cultures becomes clear from a view on a great variety of applications [2], for example production of pharmacological substances such as vaccines, monoclonal antibodies, insulin, narcotics, antibiotics and anti-cancer agents, as well as classical biotechnology, like the production of beer, wine and vinegar. To control such bioprocesses it is important to obtain on-line and real-time information about the concentration of the microorganisms. The standard off-line methods of biomass dry weight and cell number measurement cannot provide these on-line data. Present and mostly used on-line methods for biomass determination are nephelometric measurements based on scattering, transmission and absorption. In biotechnological applications these methods are restricted for several reasons: the soiling of the sensor by biofouling and media particles, measurement interferences by bubbles and in the case of transmission measurement (optical density) the measuring range [3, 4]. Here, the incident light of a single wavelength passes the turbid sample and is, due to the number and characteristics of the particles, affected by absorption, scattering and refraction. This results in a decreasing intensity of the signal with increasing cell density. Suspensions of higher concentrations can be measured only by diluting the sample or reducing the light path through the sample respectively [4]. The aim of this work was therefore the development and construction of a fiber optic probe for on-line and in-situ monitoring of biomass concentrations in bioprocesses which overcomes these problems. The probe measurement is based on back scattering and diffuse reflection which results in an increasing signal with higher biomass concentration. This paper demonstrates the results of biomass concentration monitoring of experiments for example of suspended yeast cells of *S. cerevisiae* as well as an experimental cultivation of *S. cerevisiae*.

Received: 14 April 1999

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The authors are grateful to the Stiftung Industrieforschung (IF), Köln, Germany for a grant to carry out this work. For financial and material support we gratefully acknowledge the Fonds der Chemischen Industrie (FCI), Frankfurt, Germany, FiberTech GmbH, Berlin, Germany and Euroferm GmbH, Berlin, Germany.

2 Materials and methods

2.1 Biomass probe

The fiber optic biomass probe was designed and realised [5] by using conventional light guides as transmitter (EL200 Lumenyte, Costa Mesa, USA and FBSF 70–1.0, FiberTech, Berlin, Germany) for halogen light to illuminate the measurement area and a special detection fiber (SL600/7, FiberTech), which receives the responding light signal of back scattering and diffuse reflection caused by the presence of particles (microorganisms) through the

whole cylindrical surface. Figure 1B (enlarged detail) clarifies this measurement principle. In Fig. 1A the basic set-up of the biomass probe and its peripheral equipment is shown. The enlarged detail of Fig. 1C presents a technical outline of the arrangement of the fibers. The measured light signal is guided by a transmission fiber (PCS600 IRT, FiberTech) to the spectrophotometer (SD1000, World Precision Instruments, Sarasota, USA). An A/D-board (PC+, National Instruments, München, Germany) converts the data for computing. The used measurement control application was programmed with the graphical development software LabVIEW 4.01™ (National Instruments). A reference fiber (PCS600 IRT, FiberTech) was applied to observe the intensity of the light source to correct the measurement data. A fluorinated ethylene propylene copolymer coating of the detection fiber prevents soiling and biofouling.

2.2 Microorganism and cultivation conditions

The yeast *Saccharomyces cerevisiae* (*Vital Gold*, apiece 42 g, Deutsche Hefewerke, Hamburg, Germany) and the yeast *Saccharomyces cerevisiae* (*IfG 0745*, Strain collection of the Institut für Gärungsgewerbe und Biotechnologie, Berlin, Germany) were used in experiments. Experiments with *S. cerevisiae Vital Gold* were carried out in physiologic sodium chloride solution (9.0 g l^{-1}) to prevent growth and bursting of the cells. For the cultivation of *S. cerevisiae IfG 0745* a complete medium was employed which contained 64.35 g l^{-1} glucose as a carbon source. The pH was adjusted to 4.5 and to prevent foaming 1.0 ml PPG was added to the medium. The cultivations were stepped up from Erlenmeyer flasks and carried out as well as the other experiments in a bioreactor (Biostat E, B. Braun, Melsungen, Germany) with 11 l reaction volume. The inoculation of the cultivation medium was 12% at a growth temperature of 30 °C. The O_2 supply was adjusted manually by increasing stirrer speed and volumetric gas rate respectively.

2.3 Experimental set-up

The measurement device was designed as an immersion probe which is inserted into the reactor lid shown in Fig. 2 as a cross sectional outline. The light source is evaluated before each run and the value is set to 100% for further data correcting. During experiment, every 15 minutes an average of 50 wavelength scans was saved as on-line measurement.

To determine the biomass concentration in experiments with purchased baker's yeast (*S. cerevisiae Vital Gold*), the cells were weighed and then suspended. In preliminary experiments it could be observed that 1 g dry weight corresponds with 3.7 g wet weight. To prevent sedimentation of cells, the agitation speed was 500 rpm and the volumetric air flow was constant at 5 l min^{-1}. Cultivation experiments were started with an agitation speed of 250 rpm.

For data comparison between probe measurements and the classical determinations of biomass concentration during cultivations, samples were taken every 60 min. The optical density and the dry weight were analysed off-line as well as the concentration of glucose to verify the state of its

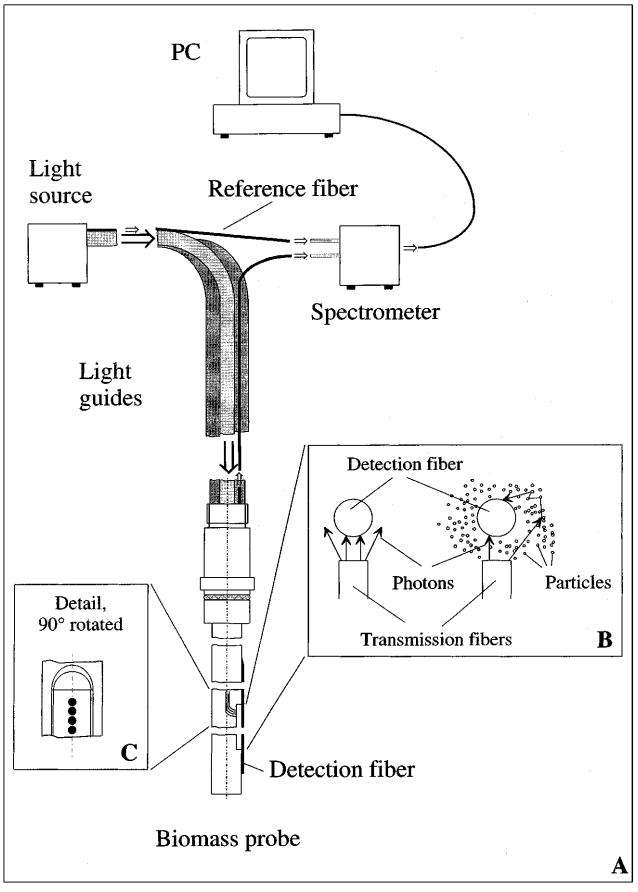

Fig. 1. Outline of the basic set-up of the biomass probe and its peripheral equipment. The enlarged detail 1B presents an outline of the measurement principle. Detail 1C presents a technical outline of the fiber arrangement

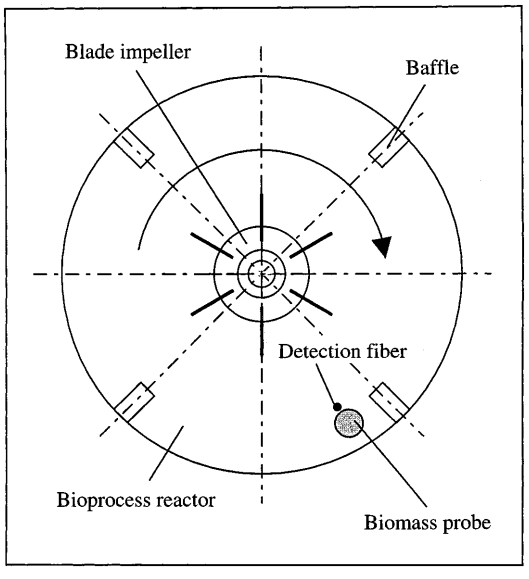

Fig. 2. Top view on reactor vessel with biomass probe position

consumption. The processes were stopped after reaching the stationary phase and complete utilization of Glucose.

2.4
Glucose, cell density and biomass dry weight measurements

The concentration of glucose in the cell free supernatant was determined photometrically at 505 nm by an enzymatic reaction (Sigma Diagnostics, Deisenhofen, Germany). The cell density was observed as optical density at 590 nm with a flow-through Zeiss-Spectrophotometer. For dry weight measurements of the biomass a sample of the microorganisms was filtrated (filter Ø 50 mm, 0.45 µm, Sartorius, Göttingen, Germany) and the filter dried to a constant weight (24 h at 105 °C).

2.5
Data analysis

First for data analysis the signals were corrected on-line by the reference signal which observes the light source. Due to the fact that growth of microorganisms is slow and the necessity to compensate changes of measurement signal which depend on changes in agitation speed or aeration rate, it is possible to use the signal before and after changes for correction by subtraction. The output signals of the spectrometer (from A/D-converter) are counts per second per wavelength. For analysis and comparison of measurement data, the probe signal is used which is the integral of the signals per wavelength in the interval 400–700 nm with an increment of 1 nm (301 values) in counts per second. This probe signal is corrected for the drift in light intensity and if required, for changes due to agitation speed and volumetric air flow variation, respectively.

3
Results

3.1
Experiments with *S. cerevisiae*

These experiments were carried out to verify the range and performance of the probe measurement. Figure 3 shows the probe signal corrected for changes in light intensity versus dry weight of the baker's yeast. The probe signal increases with increasing suspended yeast cells up to a maximum value which is reached at a concentration of approx. 14 g l^{-1}. A further increase of the amount of cells resulted in a decreasing signal. The experiments processed qualitatively equal. They differed in light intensity of the light source and therefore in absolute signal but their maximal difference results in approx. 2.3%.

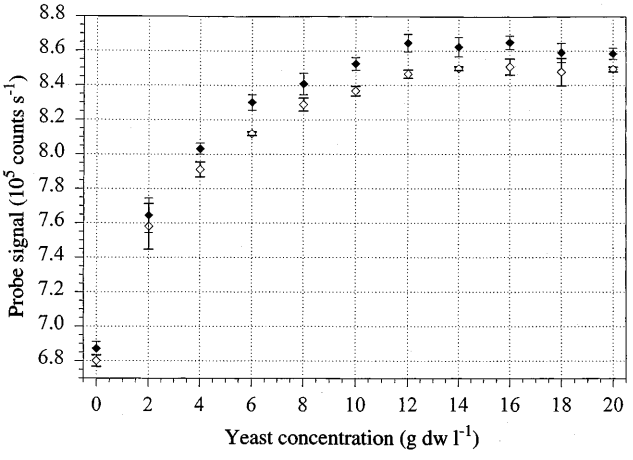

Fig. 3. Probe signal corrected for changes in light intensity vs. dry weight of the baker's yeast *S. cerevisiae* in experiment 1 (◇) and experiment 2 (◆)

3.2
Cultivation experiment with *S. cerevisiae*

Figure 4 presents the data of the probe signal during cultivation of *S. cerevisiae* where agitation speed was increased from 250 to 450 rpm after 255 min. Due to that higher agitation speed an increase of the signal of $0.37 \cdot 10^5$counts s^{-1} is clearly noticeable. Therefore, it had to be corrected to prevent false interpretation by an addend of −0.37. For a comparison of measurement data to classical methods of biomass determination additional analysis was carried out with the data corresponding to the sampling (black circles).

The complete set of measured parameters like optical density, dry weight and concentration of glucose as well as the corrected probe signal during cultivation are shown in Fig. 5. The observed maximum optical density reached up to approx. 10.0 and a biomass concentration of approx. 10.0 g l^{-1} maximum dry weight respectively. According to expected results, the graphs of optical density and dry weight are equal. All graphs indicate the typical logistic growth by sigmoidic shapes.

3.3
Data fitting according to a model of logistic growth

To determinate correlations of probe signal and optical density or dry weight respectively, the cultivation data

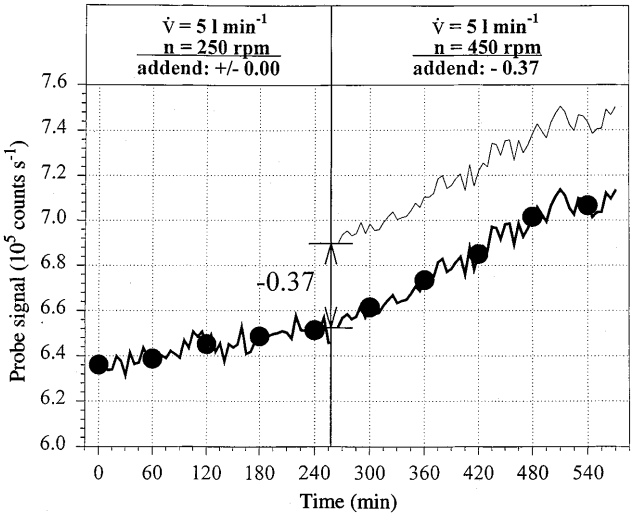

Fig. 4. Probe signals vs. cultivation time for batch cultivation of *S. cerevisiae*. The original measured signal (—), the addend corrected signal for changes in agitation speed and aeration rate respectively (—) and the corrected signal at times of sampling (●) is shown

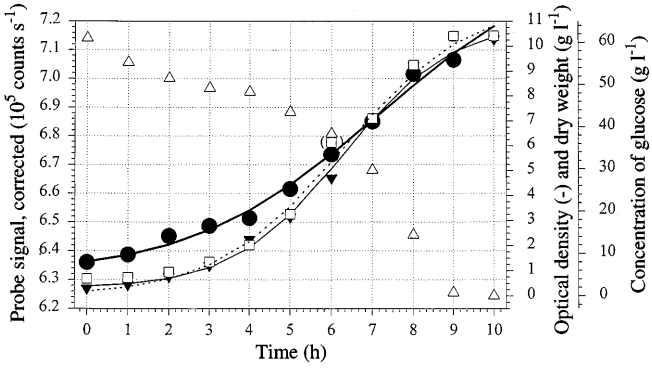

Fig. 5. Corrected probe signal at times of sampling (●), optical density (□), dry weight (▼) and concentration of glucose (△) vs. cultivation time of *S. cerevisiae* and a logistic data fit for the corrected probe signal (—), optical density (····) and dry weight (—)

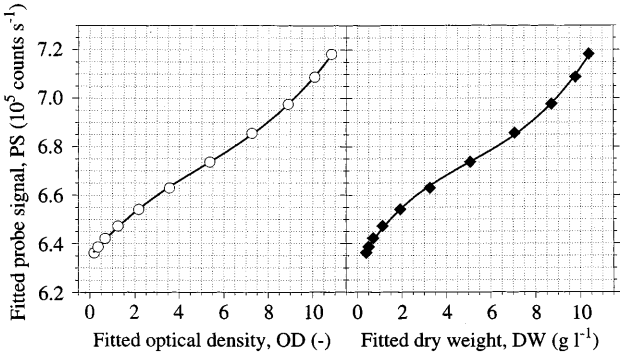

Fig. 6. Correlations of fitted data of corrected probe signal vs. optical density (○) and vs. dry weight (◆) for *S. cerevisiae*. Lines (—) represent 4th order regressions in accordance with Table 2

Table 1. Parameters for data fitting according to the model of logistic growth for *S. cerevisiae*, Eq. (1)

	x_{max}	x_0	μ	y
Probe signal	3.4839	1.1166	0.4367	6.3151
Dry weight	50.7719	10.6751	0.7579	0.3
Optical density	48.3411	11.7917	0.6527	0.0

were fitted according to a model of logistic growth by the following equation:

$$f = \frac{x_{max}}{1 + e^{\left(\frac{x_0}{x_{max}} - \mu t\right)}} + y \quad . \tag{1}$$

In accordance with Eq. (1) the data of corrected probe signal, optical density and dry weight were fitted. The used parameters are shown in Table 1. Referring to Fig. 5 the parenthesized point of optical density was not used for fitting. The results for *S. cerevisiae* are presented as different lines in Fig. 5. As mentioned, the graphs of optical density and dry weight are equal. The fitted probe signal increases less than the other graphs and its sigmoidic shape is less distinctive.

3.4 Correlations of both probe signal and optical density and dry weight

Figure 6 presents the correlations of the fitted data between corrected probe signal and optical density and dry weight respectively. The coefficients of the 4th order regressions are shown in Table 2. Hence the general equation for x = OD and DW is:

$$PS = b_0 + b_1 x + b_2 x^2 + b_3 x^3 + b_4 x^4 \quad . \tag{2}$$

4 Discussion

The experiments were realised with purchased baker's yeast. It represents a biological model with heterogeneous particle sizes in contrast to i.e. cornstarch. Cell growth and lysis as well as cell division was avoided by employing physiological sodium chloride solution. The increase of the probe signal in Fig. 3 is due to an increasing amount of light that scatters back or reflects diffusely. The fact of a decreasing signal beyond 14 g l^{-1} dry weight reflects the increasing amount of absorbed and scattered light in the light path between illuminating fibers and detection fiber. One option to avoid these losses is to decrease the light path to a minimum depending on the numerical aperture of the illuminating fibers. The numerical aperture has to be large enough to ensure that photons can pass the detection fiber (Fig. 1B). However, the results show the possibility of measuring a signal that corresponds with the biomass concentration with low variance. Therefore, the capability of this biomass probe for measurements in biomass concentration was verified in a cultivation experiment.

This cultivation experiment was carried out with a yeast strain of *S. cerevisiae* which is used for the production of alcohol. Consequently, the strain shows a tolerance to ethanol, and furthermore the possibility to process the cultivation in batch mode. In Fig. 4 the effect of a change in agitation speed is clearly noticeable as an offset in signal height of the probe measurement. In experiments with *E. coli* it was analogous. The fluctuations of the signal are mainly caused by fluctuations of the power supply of the light source. However, the data set of 'probe signals' at the times of sampling is satisfactory for the representation of the offset corrected graph and hence provides the possibility of comparison with other data. Figure 5 reflects the typical curves of logistic growth for optical density and dry weight as expected as well as for the probe signal. The fits of the curves (lines) according to the model of logistic growth, Eq. (1), represent the data sets quite satisfactory.

The regressions of 4th order of probe signal (PS) and optical density (OD) as well as probe signal and dry weight (DW) as shown in Fig. 6 reflect their correlations with correlation coefficients $r^2 > 0.999$ highly satisfactory. Hence the values of probe signal can be calculated by Eq. (2) (and vice versa) using the parameters presented in Table 2.

In conclusion it can be stated that the probe signal is capable to be used for calculations of optical density and dry weight respectively. Therefore, the developed probe is likewise capable for estimations of biomass concentration. On-line and in-situ measurements during cultivation

Table 2. Parameters of 4th order regression for correlation of the fitted data for *S. cerevisiae*, Eq. (2)

	b_0	b_1	b_2	b_3	b_4	r^2
Fitted probe signal vs. fitted optical density	6.346754	0.111308	−0.011645	$8.76e^{-4}$	$-8.75e^{-6}$	0.9998
Fitted probe signal vs. fitted dry weight	6.316423	0.153627	−0.022244	$1.77e^{-3}$	$-2.75e^{-5}$	0.9994

processes can be realised using the complete visible spectrum of light. Even without further changes, the device is able to observe a biomass concentration of *S. cerevisiae* up to approx. 14 g l^{-1}. In further experiments the measurement performance of the probe with modified design of the light path will be determined.

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