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Continuous turbidometric measurements of microbial cell density in bioreactors using a light-emitting diode and a photodiode

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Summary

A simple device for optical measurements of the concentration of microbial cells in a bioreactor is described. It consists of a green or red light-emitting diode as light source and a photodiode as light detector. The device can be used for continuous measurements on cell suspensions circulated through a measuring chamber comprised of a tube or vial. The diodes can also be mounted in glass tubes inside a bioreactor for continuous in situ measurements. The use of a biexponential calibration function allows the diode signal to be used to measure cell concentration over a range of more than a factor of 100.

Key words: Biomass measurement; Fermenter; Optical measurement

Introduction

Measurements of the cell concentration in bioreactors are necessary in many studies of microbial growth physiology and fermentation technology. Continuous measurements have obvious advantages and of the various possible approaches [reviewed 1–3] optical measurements of culture turbidity remain the most immediately accessible approach. In contrast to measurements using impedance or ultrasound, optical measurements are conceptually straightforward and on-line results can be directly related to the off-line measurements of culture turbidity which have been used in microbiological laboratories for many years [4].

We describe here the construction and calibration of a simple and inexpensive device for continuous measurements of culture turbidity and its use in monitoring the growth of several types of microorganism. The central idea of our approach lies in the use

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of diodes as both light source and light detector. Since both light-emitting diodes (LEDs) and photodiodes are small (typically a cylinder 5 mm in diameter and 5–8 mm in length), they can be mounted to provide optimal utilisation of the relatively weak illumination involved. Biomass-measuring systems using two diodes have been previously reported by Maxwell et al. [5] who described a device for making measurements of light scattering in large culture flasks, and by Filho and Ledingham [6] who described a system for making in situ turbidity measurements in laboratory-scale bioreactors.

Materials and Methods

Components and electronic circuit

The LEDs used were from RS Components, Corby, UK. These are available in several colours and intensities. We have used a high-intensity green diode (No. 587–838) and high-intensity and ultrabright red diodes (Nos. 587–822, 588–263). The PIN photodiode used was type BPX 65 (No. 304–356), also from RS Components. The two diodes were connected to a box containing the circuit shown in Fig. 1. The output signal depends on the intensity of the light received by the photodiode and could be connected to a chart recorder or input to a computer. The magnitude of the output signal depends on the type of LED and the distance between the diodes and was typically in the range 100–1000 mV.

Turbidity measurements using external flow loops

One approach to continuous measurements of culture turbidity involves circulation of the cell suspension through an external measuring chamber. This chamber can consist either of a length of glass tubing or a cylindrical glass vial. The system using a flow tube is shown in Fig. 2. The two diodes are mounted in the two halves of a cylinder of black polyacetal (Delrin) which is split lengthwise and contains a central hole machined to fit tightly around a length of glass tubing 8.0 mm od, 6.0 mm id. The two ends of the glass tubing are closed with rubber septa (sleeve stoppers, Aldrich Z10,072–2) which are pierced by hollow needles attached to the tubing used to circulate the cell suspension.

When it was necessary to stir the sample in the measuring chamber to maintain the culture in suspension, a small flat-bottomed glass vial (id 12 mm) fitted with a septum stopper was used as measuring chamber. This contained a Teflon-coated stirring bar.

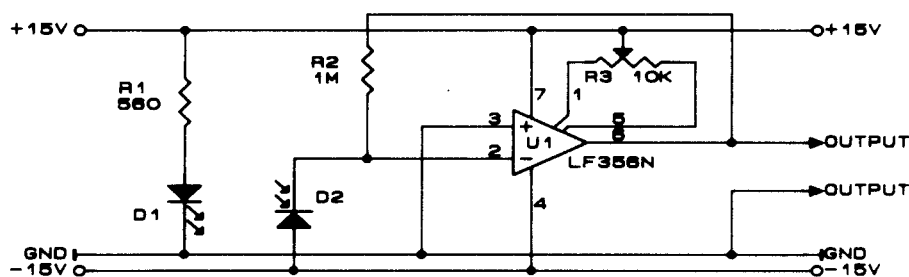


Fig. 1. Circuit diagram of electronic part of system, providing a power supply to LED and a voltage output proportional to signal from photodiode.

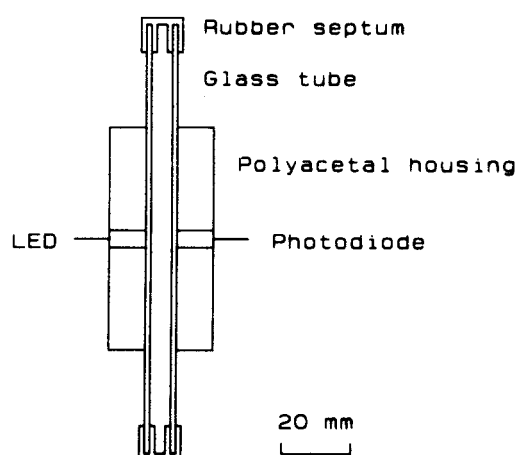


Fig. 2. Diagram of measuring system with a flow tube.

The vial fits inside a black polyacetal cylinder containing the two diametrically opposed diodes. The septum is pierced by two needles; the influent needle is placed lower than the effluent needle but above the light path.

Both types of chamber could be easily replaced using sterile technique if cells became attached to the walls. The sampling inlet inside the bioreactor was curved so that the opening faced upwards, greatly decreasing the chance of transferring a bubble into the measuring circuit.

In situ measurements in bioreactors

Measurements in situ in laboratory-scale bioreactors were made by modifying the headplate to allow the insertion of closed glass tubes (9.0 mm id, 12 mm od). A diode was mounted on an aluminium rod (8.5 mm diameter) and inserted into the glass tube after the bioreactor had been autoclaved. Absorbance was measured either between an internally mounted diode and one mounted close to the wall of a glass vessel or between two internally mounted diodes. Interference from ambient light was avoided by surrounding the reactor with a cover made from black cloth.

Results

Calibration

The optical properties of the measuring system mean that a linear relation between diode signal and cell density is only observed at values $> 85\%$ of the signal from medium alone. In this range, it was possible to follow the increase in cell concentration directly by measuring the signal from the photodiode with a chart recorder (results not shown).

To make measurements over a wider range of cell densities, it was necessary to record the signal with a microcomputer-based system and calculate the cell density using a calibration function. Over a wide range of cell densities, we found a good empirical fit to the sum of two exponentials, reduced to a three-parameter function by normalising to the signal obtained with water.

$$d/d_0 = a \times \exp(-b_1c) + (1-a) \times \exp(-b_2c)$$

where d/d_0 is the ratio between the photodiode signal and that in the absence of cells, a , b_1 and b_2 are the empirical parameters and c is the cell density. The best fit to this function was found by nonlinear regression using the program of Duggleby [7]. It should be emphasised that in many cases a number of combinations of parameters will give an almost equally good fit to the equation above but this is immaterial if the only aim is to find a continuous function relating the photodiode signal to cell density.

Calibration curves for several microorganisms are shown in Fig. 3. It is noteworthy that the curves for *Paracoccus* and *Lactobacillus* in the same measuring system are very similar. The calibration curves are usable over a cell-density range of at least a 100-fold. The use of different optical paths enables the system to be used over different ranges of cell density as demonstrated by the two curves for *Paracoccus*. In the case of phototrophs, it is possible to make measurements with LEDs which correspond to pigment absorbance. The improved sensitivity provided by this is demonstrated by the calibration curve for the cyanobacterium using a red LED corresponding to chlorophyll absorbance, compared to the biomass which is given in units of absorbance at a wavelength where the contribution from pigments is minimal.

Performance

A continuous turbidometric monitor can be used in a variety of ways in studies of microbial growth. A relatively undemanding application is in monitoring the stability

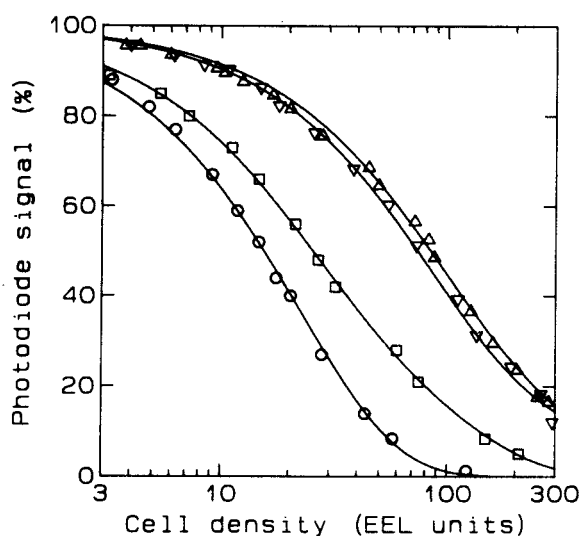


Fig. 3. Calibration curves for a variety of microorganisms showing fit to empirical calibration function shown by continuous lines. Cell density is given in EEL units, corresponding to amount of cell material giving appropriate apparent absorbance at 550 nm in a 1.0-cm pathlength cuvette in an EEL (Evans ElectroSelenium) filter-photometer after dilution to linear range. Scale of EEL photometer is such that 100 corresponds to an absorbance value of 1.0. Δ , *Paracoccus denitrificans*, green LED, 6 mm pathlength; ∇ , *Lactobacillus plantarum*, green LED, 6 mm pathlength; \square , *Paracoccus denitrificans*, red LED, 12 mm pathlength; \circ , *Synechococcus* sp., (cyanobacterium), red LED, 12 mm pathlength. 1 EEL unit corresponded to $5.3 \text{ mg} \cdot \text{l}^{-1}$ cell C for *P. denitrificans*, $3.2 \text{ mg} \cdot \text{l}^{-1}$ cell C for *L. plantarum* and $3.7 \text{ mg} \cdot \text{l}^{-1}$ cell C and $0.25 \text{ mg} \cdot \text{l}^{-1}$ chlorophyll for *Synechococcus* sp.

of a continuous culture or determining whether a new steady state has been reached following a change in conditions. In this case, output to a chart recorder is adequate and the main requirement placed on the system is long-term stability.

We have successfully used the LED-photodiode system with an external measuring loop as a routine monitor of the stability of continuous cultures of a variety of microorganisms including cyanobacteria, purple phototrophic bacteria and nonpigmented heterotrophs. The stability of the system during a typical experiment with *Paracoccus denitrificans* in a nitrate-limited chemostat is shown in Fig. 4 which shows the variation of the signal around the average value using an expanded scale. Apart from a short excursion at 20 h, the variation of the signal was only $\pm 2\%$. This corresponds to a variation of $\pm 6\%$ in the cell concentration determined from calibration curves of the type described above. At least some of this variability results from instability in the chemostat rather than in the measuring system.

Measurements of growth kinetics in batch culture require calibration and processing of the raw data using a computer. Figure 5 shows two examples of such measurements in which the results from the calibrated diode system are compared with measurements on samples removed from the culture. In one experiment, we measured growth of *Paracoccus denitrificans* under anaerobic conditions in a medium containing succinate and nitrate. In this case, the cell suspension was continuously circulated through a flow tube and returned to the culture. The agreement between the two sets of values is good, apart from a period at the end of the exponential phase where the photodiode measurements were probably influenced by the formation of bubbles as gases came out of solution in this vigorously denitrifying culture. In another experiment, we measured the growth of *Lactobacillus plantarum* using a photodiode mounted inside the bioreactor. In this case, a red LED was used to minimise the contribution to the measurements from the coloured growth medium containing tryptone and yeast extract.

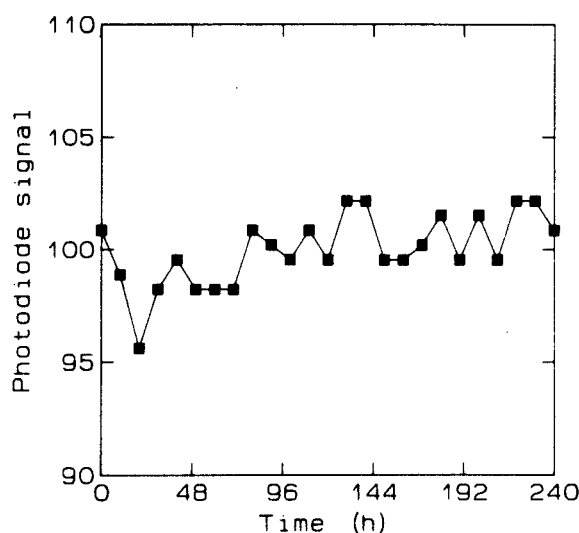


Fig. 4. Stability of LED-photodiode system during 10 days of monitoring density of *Paracoccus denitrificans* in a nitrate-limited chemostat. Bacteria were grown on a mineral salts medium containing 25 mM succinate and 25 mM nitrate, under an atmosphere of N_2 . Dilution rate was $0.2 \cdot h^{-1}$. Measurements were made using a green LED and a flow tube (id 6.0 mm). Points shown are replotted from a continuous chart recorder trace, with mean value normalised to 100. Cell density corresponded to $\approx 500 \text{ mg} \cdot l^{-1}$ cell C.

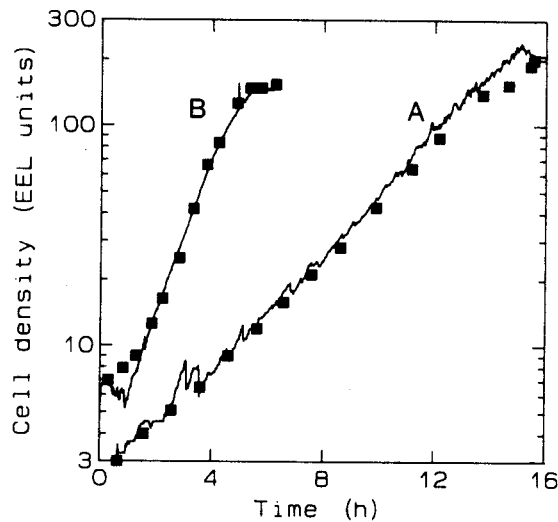


Fig. 5. Comparisons of cell density measured on samples removed from bioreactor and cell density calculated from diode signal using a calibration function as described in text. Trace A shows measurements during batch growth of *Paracoccus denitrificans* on medium described in legend to Fig. 4. Measurements were made using a green photodiode and a flow tube (id 6.0 mm). Trace B shows measurements during batch growth of *Lactobacillus plantarum* in MRS medium minus meat extract and with only $5 \text{ g} \cdot \text{l}^{-1}$ glucose. Glucose was autoclaved separately to prevent formation of coloured breakdown products. Measurements were made using a photodiode mounted inside bioreactor and an ultrabright red photodiode mounted outside (minimum pathlength through bacterial culture 13 mm). EEL units are defined in legend to Fig. 3. For *P. denitrificans*, measurements were made at 550 nm and 1 EEL unit corresponds to $5.3 \text{ mg} \cdot \text{l}^{-1}$ cell C. For *L. plantarum*, measurements were made at 650 nm and 1 EEL unit corresponds to $3.8 \text{ mg} \cdot \text{l}^{-1}$ cell C.

Discussion

The general problems associated with on-line turbidometric measurements of reactor biomass [1–3] are (1) nonlinearity, (2) interference from coloured substances, particles and bubbles and (3) the growth of organisms on surfaces. Nonlinearity is not a real problem if a calibration function can be obtained and a microcomputer is used for data collection as is increasingly the case nowadays. Interference from coloured substances can be minimised if a red illumination source is used since typical components of growth media, such as yeast extract, have absorbance maxima in the ultraviolet and decreasing absorbance as wavelength increases from the blue to the red regions of the visible spectra. Even greater improvement could be obtained by using near-infrared diode pairs.

The problems caused by bubbles and wall growth are difficult to avoid during in situ measurements but are less of a problem if an external measuring chamber is used, particularly if it is designed to be readily replaceable whilst avoiding contamination as is the case with the device described here. The main limitation to the use of an external measuring chamber is that cells are subjected to conditions different to those found in the reactor. The extent to which this is acceptable will depend on the particular growth conditions involved. In the case of continuous culture, the problem is avoided if measurements are confined to a portion of the effluent stream which is pumped though the measuring device from below the surface of the culture, the remainder leav-

ing the bioreactor in the usual way. It is also possible to use an external flow system to monitor batch cultures in a 'sample and discard' mode, if the total flow through the measuring chamber is small compared to the reactor volume.

Our experience with a variety of types of bacterium over more than twelve months suggests that turbidity measurements using the device described here are a simple, inexpensive and flexible approach to monitoring microbial growth in bioreactors.

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