On-line Monitoring of Bacterial Mass Using *In-situ* Steam Sterilizable Sensor

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SUMMARY

The on-line sensor is a very useful tool for following concentration changes in submerged culture of microorganisms. The instrument demonstrated here is a sterilizable, in-place sensor that reliably follows the changes in the bacterial concentration. The instrument output can be either a direct reading of the transmittance or a calculated OD. In either case, the information reflects the bacterial biomass. The instrument output can be interfaced to a data acquisition and control system to interact directly in the fermentation process.

Figure 1. The Turbidity Instrument

INTRODUCTION

Following bacterial growth by measuring the culture turbidity is the method of choice for tracking changes in the bacterial culture. Even though several other methods are routinely being implemented to follow bacterial growth (CO_2 production rate, fluorescence, oxygen consumption rate, dry weight, etc.), the turbidity measurement is the most common.

The major disadvantage in following the culture turbidity using a spectrophotometer is that it is an off-line measurement, and in addition to the inconvenience, it is impossible to incorporate to an automated data acquisition system. On-line measurement of bacterial growth is available using methods which track different metabolic activities of the microorganism and, therefore, are an indirect measurement of bacterial biomass. Measurement of CO_2 production rate, oxygen consumption rate or base consumption are reliable methods to follow the bacterial growth, but the off-line measurement of bacterial biomass (by optical density or other means) is not replaced. In practice, dependable measurement of bacterial growth requires following the bacterial metabolic activity (e.g. glucose consumption, CO_2 production) as well as the bacterial biomass.

The present report contains results on following *E. coli* growth using an on-line sterilizable sensor capable of following the changes in the culture turbidity. The instrument is based on light emission from a laser diode and provides good correlation to the actual off-line turbidity measurements. After initial calibration, the turbidity probe follows reliably the bacterial biomass concentration until an OD of 50 at 600mu.

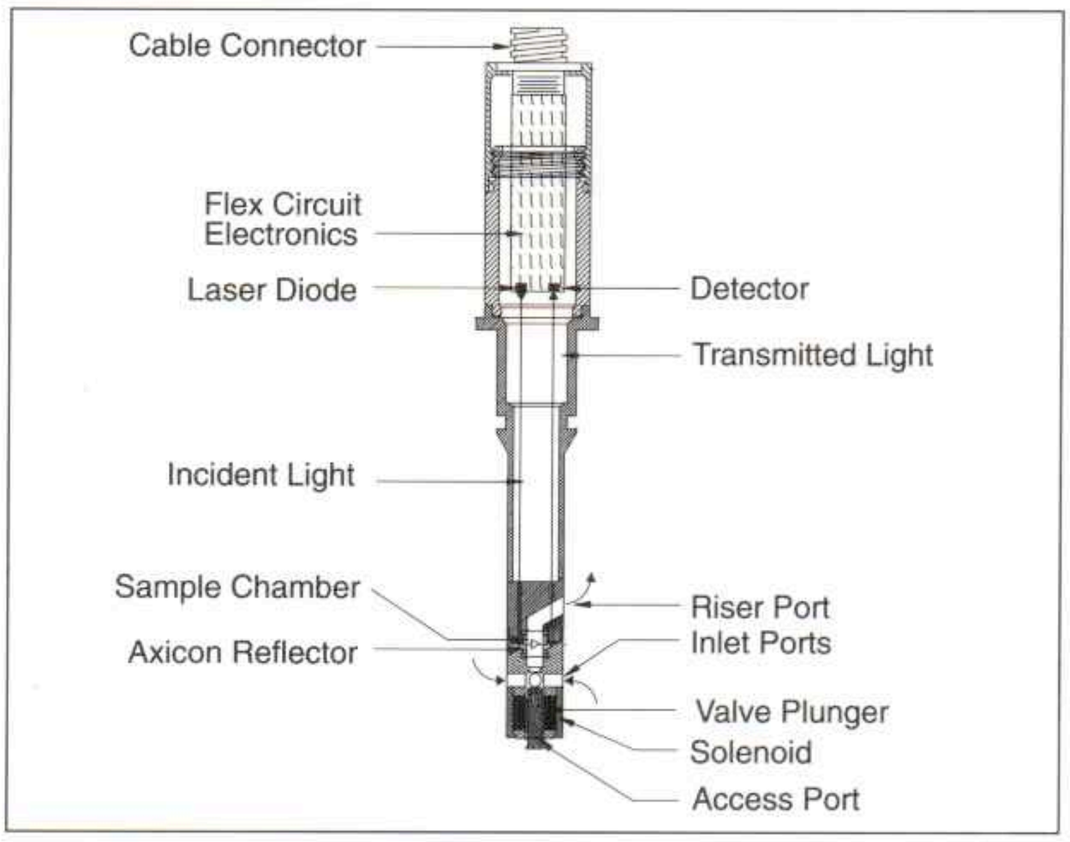

Figure 2. Cross Section View of the Optical Probe

MATERIALS AND METHODS

a. Turbidity Probe

The turbidity probe measurement system was built by Cerex Corporation (Ijamsville, MD) and consists of a sensor and an instrument console (Figure 1) capable of analyzing, correlating and sorting the data from the sensor. The system can be interfaced with a supervisory control system by either analog signals or through an RS232 connection. The principle of the instrument is shown in Figures 2 and 3. Light emanating from a laser diode mounted in the upper housing of the sensor is transmitted down an optical conduit to the axicon reflector. The light is reflected at a precise 90 degree angle through the sample chamber to the opposite reflector and back through a second optical conduit to a photoamplifier detector. During operation, a teflon plunger with an embedded magnet moves up and down as a result of alternating magnetic field. The plunger movement allows culture to flow into the optical chamber and prevents new culture from entering the chamber by blocking the side ports. Air bubbles which may have been present in the culture continue to rise out of the chamber. Typical plunger cycles are one minute open and one minute closed, providing a new optical density reading every two minutes.

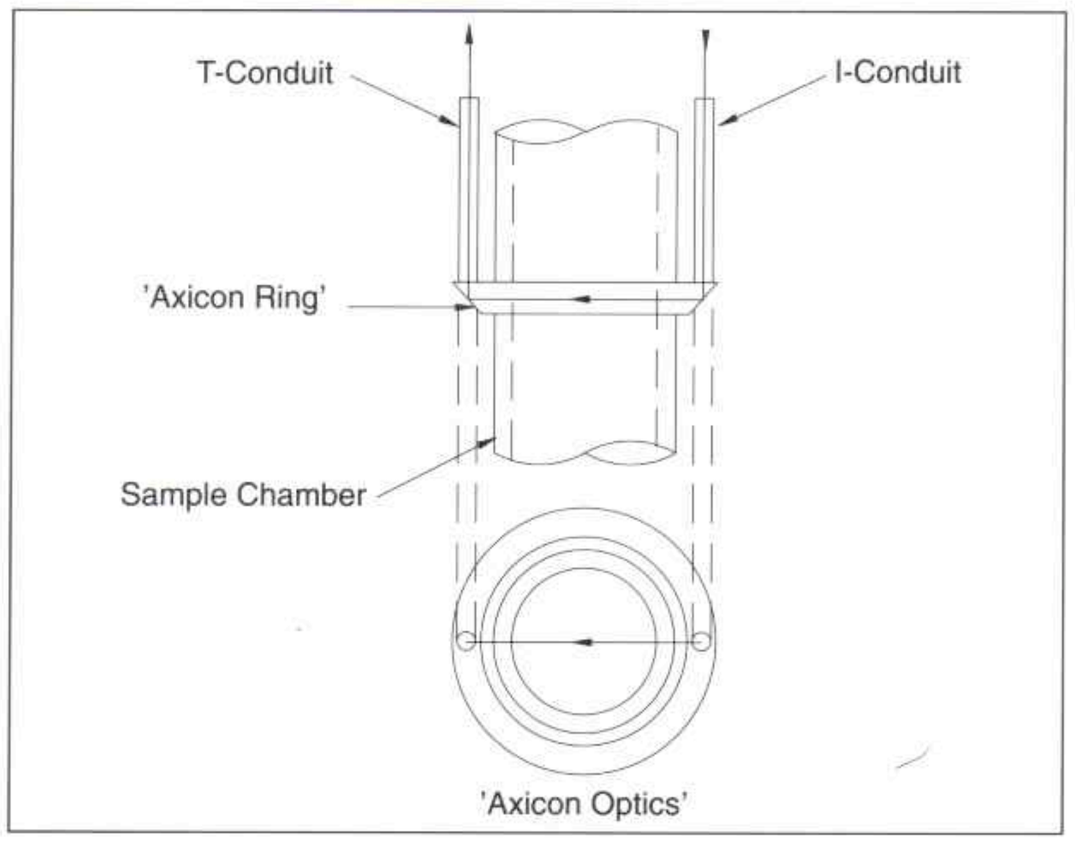

Figure 3. Optical Path Schematic of the Optical Probe

b. Optical Density Measurement and Calculations

The sensor measures the optical density according to the Beer-Lambert Law (equation 1):

$OD = e*L*\log_{10}(I_0/I)$

where:

e = extinction factor (fluid composition, wavelength)
L = path length of cell
I_0 = incident light intensity
I = transmitted light intensity

When measuring bacterial density, the Beer Lambert law only applies to concentration for optical density below 0.7 OD units. To measure optical densities above this range, samples are diluted, and the resulting measurements are multiplied by the dilution factor. The sensor utilizes an intense laser diode (higher I_0) and 5mm light path to extend the range above that of a spectrophotometer. To allow the sensor to be utilized beyond the linear range at high cell densities, simple algorithms are employed to convert the sensor transmittance measurements to the optical density measurements obtained by off-line diluted samples measured in a spectrophotometer. The following algorithm was employed in these experiments (Equation 2):

$OD = a+b(1/T-1/T_0)+c(1/T-1/T_0)^2$

where:

a, b, and c = adjustable constants
T = instrument transmittance
T_0 = the initial transmittance reading

The adjustable constants were determined by the sensor's instrument console after off-line measurements corresponding to the instrument reading were programmed into the unit. This correlation was then stored in memory and utilized for all subsequent fermentations.

RESULTS

a. Determination of the Correlation Equation Constants

As was explained in the experimental section, the instrument measures only the transmittance values. The determination of the actual OD values, which are the accepted numbers for evaluating bacterial biomass, from the transmittance values requires a specific correlation equation.

In order to determine the correlation equation and its constants several fermentation runs were conducted in which the turbidity probe response, as well as off-line measurement of the OD values at 600mu of the culture, was followed. The algorithm described in the methods section was employed, and the suitable correlation between the transmittance values and the OD values was determined. In order to achieve greater accuracy two separate ranges were calibrated, 0-8 OD and 8-50 OD. The two equations were stored in memory and utilized later for all subsequent fermentations.

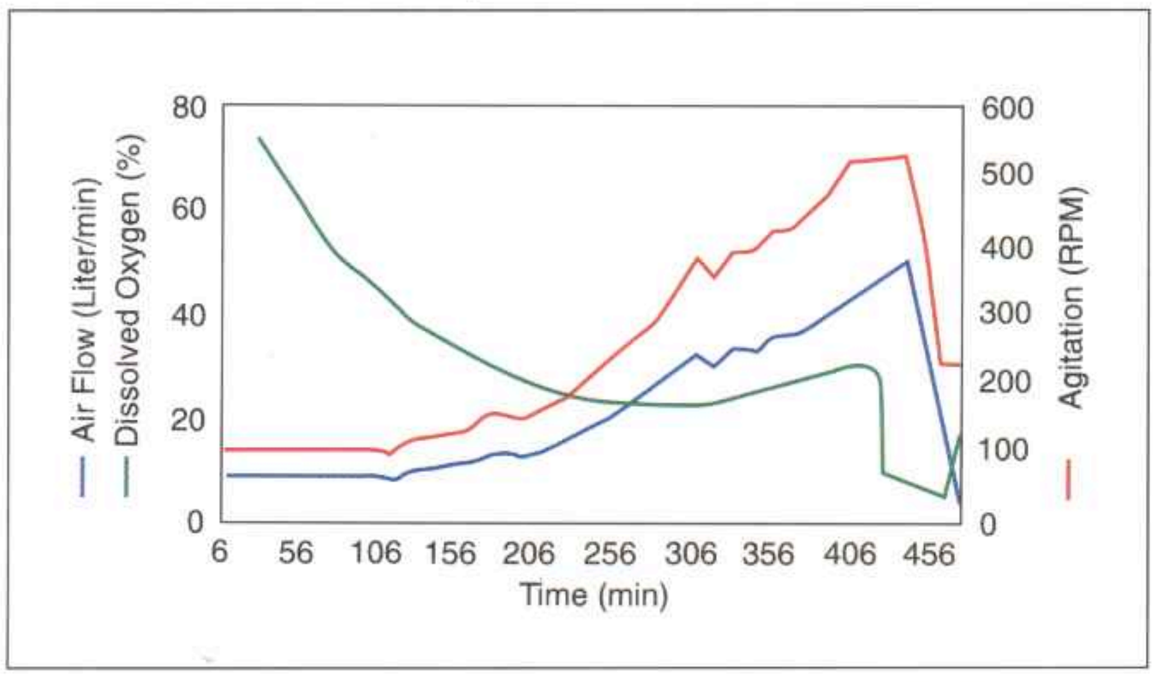

Figure 4a. Agitation, Aeration and Dissolved Oxygen

Equation 2 was used for the correlation:

$OD = a+b(1/T-1/T_0)+c(1/T-1/T_0)^2$

Where a = -0.380, b = 8.2720, c = -1.9803 for the lower OD range, and a = 1.8805, b = 3,2100, c = -0.1022 for the higher OD range.

b. Fermentation

Typical *E. coli* fermentation for the production of recombinant protein under the T7 promotor is seen in Figure 4a. The bacteria, *E. coli* BL21 DE3 containing a plasmid encoding Pseudomonas aeruginosa exotoxin A, grew exponentially until the induction point when IPTG was added to the culture. At this point the growth slowed down as indicated by the aeration and agitation levels.

Figure 4b shows the turbidity probe response. The blue line is the transmittance values, and the red line is the calculated optical density based on the correlation equation. The off-line measurements of the turbidity are shown on the green line.

DISCUSSION

Evaluation of bacterial biomass by measuring the turbidity of the bacterial suspension is the acceptable method for following bacterial growth. In most cases the measurement of the culture's optical density is done off-line using a spectrophotometer in the 600mu range. The culture suspension is diluted so the reading does not go over 0.8 OD. On-line methods to measure the bacterial optical density would obviate this inconvenience and permit automated data acquisition.

Unlike direct bacterial biomass measurements like wet weight or dry weight, the optical density measurement is indirect and, therefore, serves as a relative value, which should be correlated to cell mass values. The important property of the on-line turbidity sensor is that its response is corresponding to the changes in the biomass concentration regardless of its numerical values.

The results show very good correlation between the off-line turbidity measurements (OD at 600mu) and the on-line calculated values up to an OD value of 50. This fact allowed us to connect the turbidity meter to our data acquisition and control system and to activate a pump to add the needed IPTG at the desired optical density in the case of the T7 promoter, or to change the temperature in the case when the expression was under the control of the PL promotor.

The ability to follow on-line bacterial biomass concentration, together with metabolic parameters like CO_2 production, oxygen consumption, acid or base addition can provide better understanding of the bacterial culture and, therefore, a reliable decision concerning changes in the fermentation pathway.

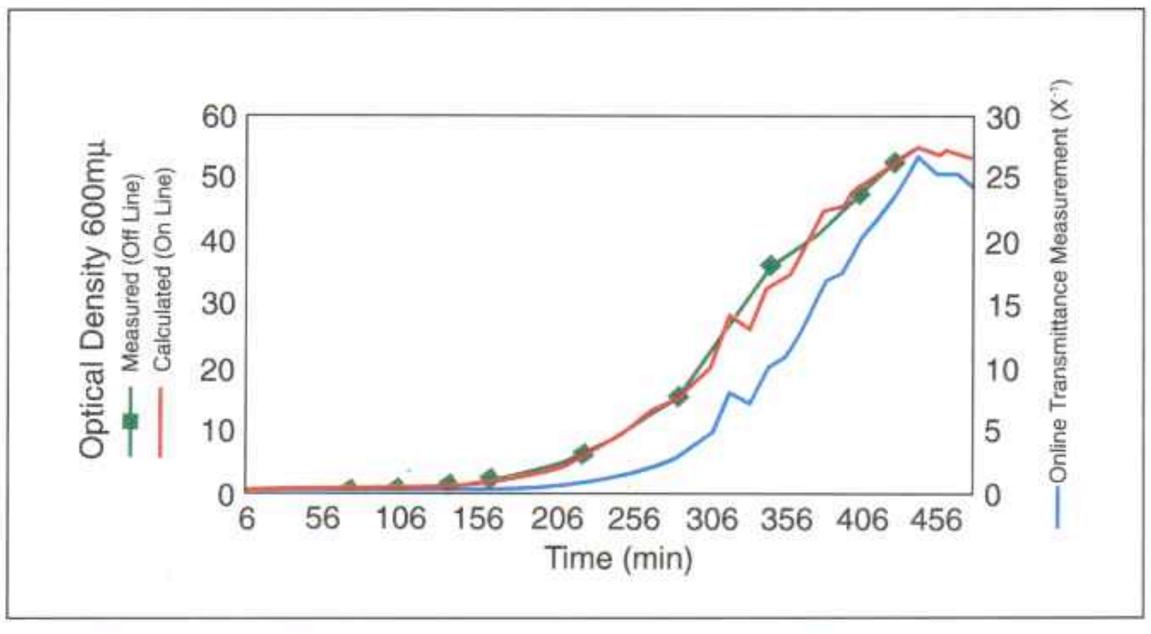

Figure 4b. Off-lin Optical Density, On-line Transmittance Measurement and On-line Calculated Optical Density Values