Performance of a Commercially Available Biomass Sensor for On-line Monitoring of High Density *Escherichia coli*

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Presented at the Annual Meeting of the Society for Industrial Microbiology, Toronto, Canada, August, 1993.

ABSTRACT

Bacterial cell mass monitoring has typically relied on an off-line measurement of optical absorbance or dry cell weight. Optical fiber technology has allowed improvements in the design of a reliable, accurate and in-situ sterilizable sensor for monitoring bacterial cell mass on-line during a fermentation process.

A commercially available biomass sensor has been developed for on-line monitoring of bacterial cell mass. Light transmitted from a laser diode through a debubbled culture broth provides an optical transmittance to detect turbidity. Algorithms were developed to linearize and convert the transmitted light values to absorbance and dry cell weight. A growth profile correlating light transmittance to optical density and dry cell weight was developed from a single 10L fermentation of *Escherichia coli.* This correlation profile was successfully evaluated for accuracy and reproducibility during successive fermentations at 10 liter scale and during scale-up to 100 liters. The effects of medium components and growth conditions on the sensor's performance will be discussed.

INTRODUCTION

Determination of bacterial cell mass concentration is an important measurement in fermentation scale-up and production control. The ability to monitor cell mass concentration is critical to the optimization of the fermentation process. The most routine method for measuring bacterial cell mass concentration has typically relied on off-line, intermittent measurements of optical density with the use of an external spectrophotometer. Recently, a novel on-line optical density sensor using a laser as the source of illumination has been introduced by Cerex Corp. (Ijamsville, MD).

The performance of Cerex's MAX^{tm} cell sensor system was evaluated with a recombinant *Escherichia coli* for: 1. accuracy and reproducibility during successive 10L fermentations, 2. media interferences associated with different medium formulations, 3. high biomass 10L fermentation (> 120 O.D. units, 40 g/L DCW) and 4. process scale-up to 100L.

ADVANTAGES OF ON-LINE BIOMASS MEASUREMENT

1. Enables Process Automation and Fingerprinting
2. Decreases Risk of Contamination
3. Improves Time, Cost, and Labor Efficiency
4. Avoids Dilution Technique Errors Associated with Off-Line Measurement
5. Avoids Volume Loss During Off-Line Sampling
6. Eliminates Aerosols Produced During Sampling

MAX SENSOR PRINCIPLE OF OPERATION

Figure 1A shows the design of the MAX sensor. Light from a laser diode mounted in the proximal end of the sensor is transmitted through an optical fiber to an axicon reflector ring (Figure 1B). The axicon reflector ring deflects the light at a precise 90° angle through the sample chamber to the opposite reflector and back through a second optical fiber to the photo-amplifier detector.

During operation:
1. The solenoid opens
2. Culture broth enters the chamber
3. The valve closes to debubble the sample through the riser port
4. The sample is then read by the optical detection electronics

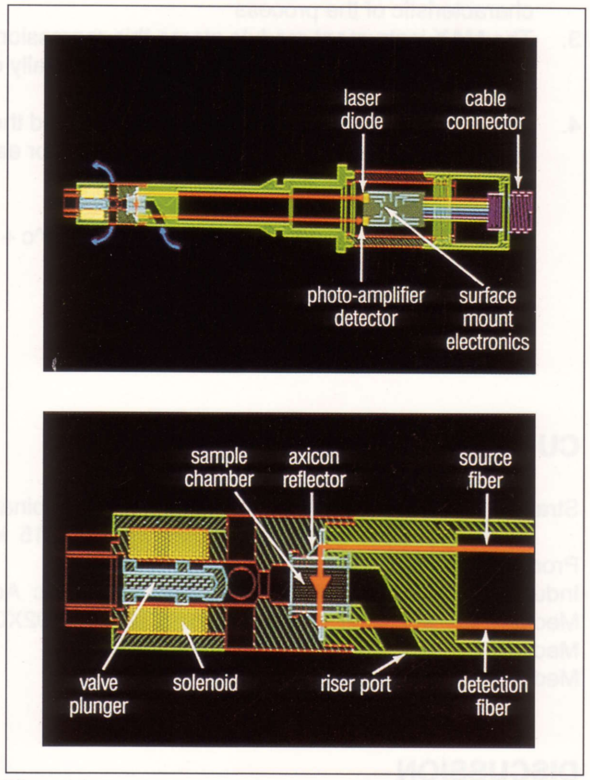


Figure 1a.

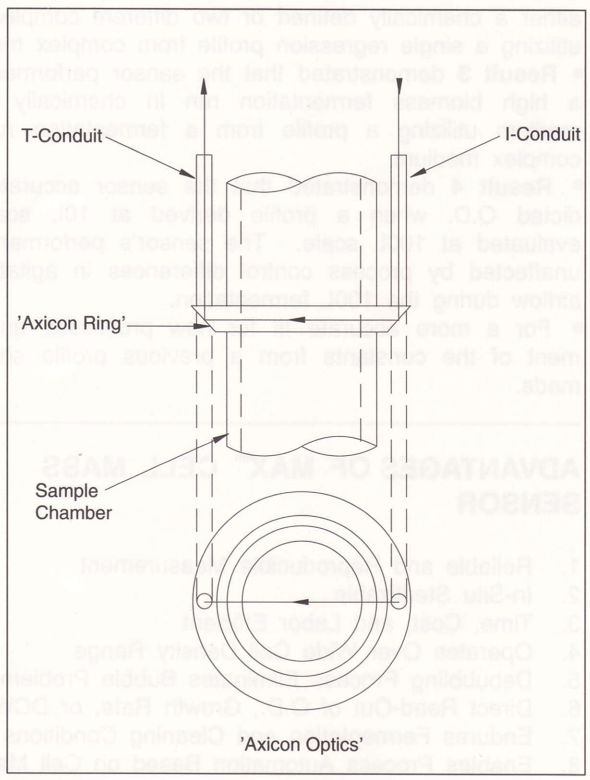


Figure 1b.

REGRESSION ANALYSIS STRATEGY OF MAX SENSOR

1. Run fermentation under normal conditions
2. Enter corresponding off-line optical density or other concentration units to the sensor's transmittance values at arbitrary time points to generate a regression analysis characteristic of the process
3. The MAX instrument module stores this regression profile and constants can be stored and manually entered for each distinct process
4. The regression equation in this evaluation used the same constants from the first 10L fermentation run for each additional and distinct process:

   On-Line O.D. =
   $A*\log10(R/T) + B*\log_{10}(R/T)\text{^}b + C*\log_{10}(R/T)\text{^}c + D$

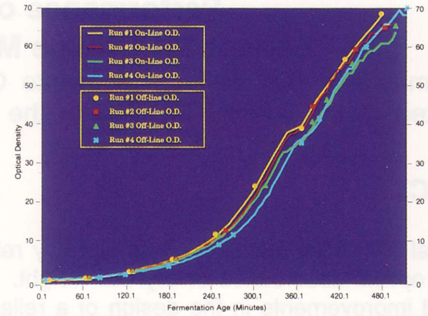
   A,B,C,b,c = empirically derived constants
   D = $O.D._{-1} - A*\log_{10}(R/T1)$
   T_1, $O.D._{-1}$ = first values post inoculation
   R = the T-value in deionized water

CULTURE AND MEDIA

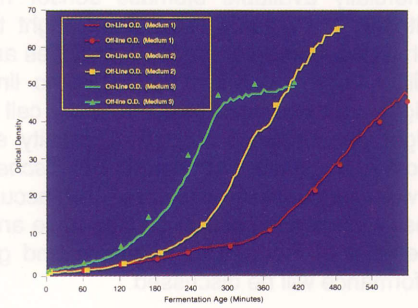
Strain:	*E. coli* W3110 expressing a recombinant protein with a molecular weight < 15 kD
Promoter:	RecA
Induction:	$O.D._{550nm}$ =15 with 50 ug/ml Nalidixic Acid
Medium 1:	M9 salts + 2X casamino acids (M92XCAA)
Medium 2:	Chemically defined medium (CDM)
Medium 3:	Brain Heart Infusion (BHI)

DISCUSSION

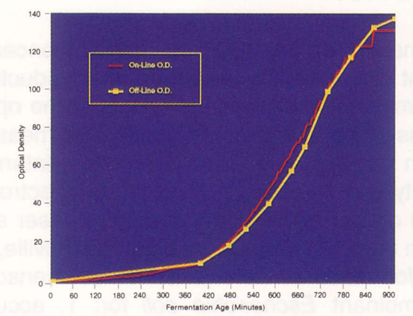
- **Result 1** demonstrated that the MAX cell mass sensor correlated on-line O.D. measurements to within 5% of off-line O.D. measurements and was consistent in four successive 10L fermentations.
- **Result 2** demonstrated that the sensor performed well in either a chemically defined or two different complex media utilizing a single regression profile from complex medium.
- **Result 3** demonstrated that the sensor performed well in a high biomass fermentation run in chemically defined medium utilizing a profile from a fermentation run in a complex medium.
- **Result 4** demonstrated that the sensor accurately predicted O.D. when a profile derived at 10L scale was evaluated at 100L scale. The sensor's performance was unaffected by process control differences in agitation and airflow during the 100L fermentation.
- For a more accurate fit for new processes an adjustment of the constants from a previous profile should be made.



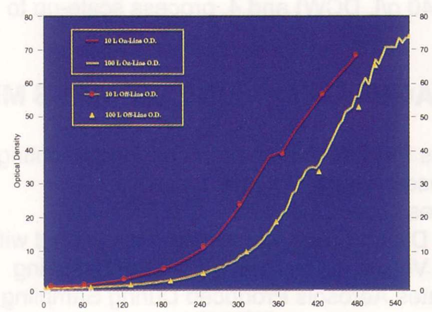
Result 1.
Sensor Reproducibility in Successive 10 Liter Fermentations



Result 2.
Sensor Performance in 10 Liter Fermentations with Different Media



Result 3.
Sensor Performance in 10 Liter High Cell Density Formation



Result 4.
Sensor Performance During Scale-up from 10 Liter to 100 Liter Fermentations

ADVANTAGES OF MAX™ CELL MASS SENSOR

1. Reliable and Reproducible Measurement
2. In-Situ Sterilizable
3. Time, Cost, and Labor Efficient
4. Operates Over Wide Cell Density Range
5. Debubbling Process Eliminates Bubble Problems
6. Direct Read-Out of O.D., Growth Rate, or DCW
7. Endures Fermentation and Cleaning Conditions
8. Enables Process Automation Based on Cell Mass

SUMMARY

- Use of the Cerex MAX cell mass sensor allows for continuous and reliable measurement of cell density in recombinant *E. coli* fermentations.
- The sensor was very accurate and reproducibly performed with the same process run four successive times.
- The sensor performed well during process scale-up.
- The sensor performed well over a broad range of optical density during high cell density fermentation.
- The sensor performed well in different fermentation media.