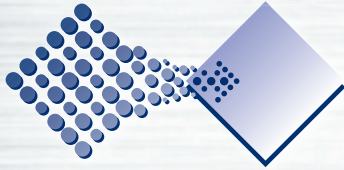


Viable Cell Growth Monitor

Model 53TF Fluorometer



CST offers a low cost fiber optic based Fluorescence transmitter and insitu probe for monitoring the fluorescence of NADH production from viable bacterial, yeast, or mammalian cells during fermentation. The system incorporates state of the art electronics connected to a fiber optic based insitu probe and produces a control signal proportional to the Ex/Em of NADH. The probe is a (patent pending) front surface design which minimizes by the inner-filter effect and scattering affect from highly turbid samples.

Defining the Problem

Product yields may be greatly increased if monitoring of cell viability is managed properly. There are many factors affecting the maximum growth of cells in a batch reactor such as: balance of dissolved nutrients, pH, temperature, pO_2 , pCO_2 etc. Continuously monitoring the rate of NADH can provide a record that the cells have reached their maximum density within a give time frame.

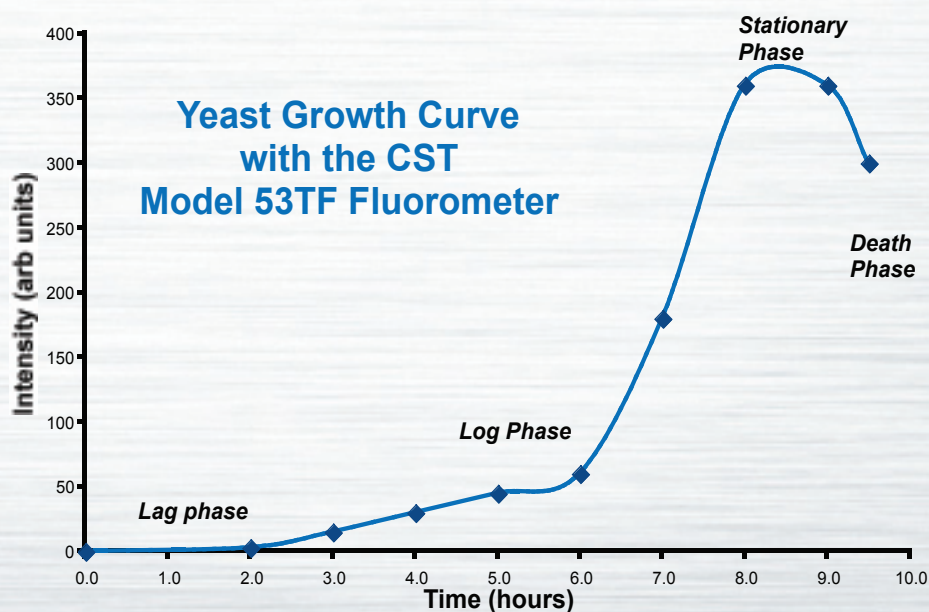
Monitoring cell growth traditionally has been done with scatter or turbidity type instruments that measure the optical density (OD_{630}). Although this approach is an indicator of cell density it also measure the TOTAL amount of light scattered which is the sum of the living cells, dead cells, cell debris, and possible reabsorption by the growth media. OD measurements do not indicate all phases of growth, i.e., does not indicate the *Death Phase* of growth because it cannot distinguish this from total cell density. Only fluorescence will indicate if cells are viable or not, (see graph below).

The CST Solution

Numerous bioanalytical assays are based on the fact that the coenzyme NADH is fluorescent, while NAD^+ is not. As a result, all enzymatic reactions based on NAD/NADH are amenable to fluorescence analysis. The CST Viable Cell Growth Monitor, delivers excitation energy from a pulsed UV lamp and monitors the amount of fluorescent NADH in the cell. One very important parameter for the assessment of bioprocesses is the metabolic state of the organisms. This parameter yields information about the metabolic activity of the growing cells, the biomass development, and changes in the composition of the nutrition medium.

Endogenous fluorescence signals attributed to the amount of reduced nicotinamide adenine dinucleotide (NADH) in the cell have been correlated with;

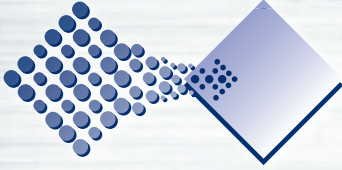
| Viable Cell Activity | Nutritional Uptake |
|----------------------|------------------------------|
| Growth of Organism | Anerobic/aerobic respiration |
| End of Growth Phase | Beginning of Death Phase |



Data provided by
UNC-Pembroke

Viable Cell Growth Monitor

Continued...



Sample Interface

The CST front surface probe is designed to withstand CIP, SIP, and autoclave conditions. Our insitu probe fit the standard Ingold type fittings and 12mm diameter bodies are supplied with PG 13.5 nuts.



Insitu Front Surface Fluorescence Probe, Patent Pending

The Photometric Transmitter

The Custom Sensors & Technology photometric transmitter displays a relative signal change and retransmits a signal that is proportional to the amount of fluorescent energy captured in the fermentation reaction vessels. This reading is based on the amount of fluorescence from the viable cell suspension in the fermentor as compared to a reference signal.

A pulsed Xenon UV lamp delivers the excitation energy to the fiber optic probe and the emitted energy is measured with surface mounted photomultiplier tubes. A ratio of the fluorescence intensity to excitation intensity are scaled over a 4-20mA output. Monitoring the relative signal change and not total intensity reduces background interferences.



Model 53TF shown with insitu fluorescence probe

Applications for the Fluorometer

- Fermentation monitoring of cell vitality and viability
- Automation of pitching and cropping in breweries
- Bioprocess validation
- Mammalian cell
- Custom fermentation
- Yeast Plants
- Upstream processing
- Downstream processing

Note: CST manufactures several different types of sample interface extractive flow cells, insitu probes and accessories, please consult factory for any other sample accessory you might have a need for.

Product Specifications

Transmitter

| | |
|------------------------|---|
| Measured parameter | Cell viability based on NADH Fluorescence |
| Temperature Range | -10°C to +55°C |
| Response time | < 60 sec to 90% of step change |
| Maximum Zero shift | <0.5% of full scale |
| Long term output drift | <2%/month |
| Repeatability | 1% of range |
| Source | Xenon Flash Lamp, typical life is 3 years |

User Display & control

| | |
|--------------------------|--|
| Type of display | LED display |
| Display numerical format | 3-1/2 digits in user defined engineering units |

Electrical

| | |
|------------------------|-------------------------|
| Power requirement | 24VDC (10-36VDC) |
| Power consumption | 400mA @24V |
| Analog outputs | 4-20mA isolated |
| Analog loop resistance | 500 Ohms, maximum @ 24V |

Mechanical

| | |
|------------------------|---|
| Analyzer weight | 1.5 lbs |
| Enclosure construction | Extruded Aluminum, Nema 4X, Optional 8" x 3-7/8" x 1.5" (HWD") |

Front Surface Fluorescence Probe

| | |
|--------------------|---|
| Materials | 316SS, other material available, please consult factory |
| Temperature rating | 315.5C (600°F) |
| Diameter | 12mm with PG 13.5 nut |
| Length | 120, 200, 325mm; please specify |
| Pressure rating | up to 5,000 psig |

Custom Sensors & Technology is a full service provider. We also supply photometric transmitters, fiber optic probes & flow cells, O₂ transmitters, sample handling systems, and services including: application engineering, commissioning & start-ups, product validation, factory acceptance testing, process stream GAP Analysis, and in-house repair.