Real-Time Total Cell Density Measurement of Yeast Fermentations

Bioprocess Performance Optimization by In-line Monitoring

Industry: Bioprocesses Research and Development
Application field: Scale-up and scale-down studies of *Saccharomyces cerevisiae* fermentations
Hamilton products: Dencytee, Arc View Controller

In-line Measurement of Key Performance Indicators

Manufacturing products such as biopharmaceuticals is a complex process, requiring continuous optimization of the culture conditions to improve the reproducibility of the bioprocesses involved and maintain a constant product yield. Scale-up and scale-down studies are commonly used to identify the optimal conditions; bioprocesses are performed on a small scale and then implemented on a larger scale. Data is collected from multiple sources for statistical bioprocess optimization, in compliance with pharmaceutical industry innovation initiatives, such as Process Analytical Technologies (PAT). Key Performance Indicators (KPIs) are monitored during the studies to control the status of the culture during the tests.

Total Cell Density (TCD) is one of the most important KPIs in fermentation studies, and can be correlated to the production yield. TCD is commonly measured offline in the laboratory by reference methods such as optical density at a specific wavelength (OD 600 nm), wet cell weight mass/volume (WCW g/L) and dry cell weight mass/volume (DCW g/L). However, off-line measurement technologies have some disadvantages; they cannot be used to collect continuous real-time data to obtain full information about the process, and require a sterile sampling step, increasing the risk of contamination occurring.

Today, these issues have been resolved by new technologies that make it possible to measure TCD directly in the bioreactor in real-time. Most innovative biopharma companies have implemented these technologies, which use in-line or in-situ sensors, in compliance with PAT guidelines.

Benefits of the Dencytee Sensor

- Accurate determination of yeast growth in real-time
- Good correlation to all the off-line reference methods tested: OD, WCW, DCW
- Easy installation and use

Figure 1: 5 L Electrolab Biotech Ltd bioreactor used for scale-up and scale-down studies.

Ingenza, a biotech company based in Edinburgh, UK, operates GMP-compliant laboratories for the construction, optimization and application of engineered microbial strains. This application note discusses the evaluation of an in-line sensor for TCD measurement of *S. cerevisiae* cultures in a laboratory scale bioreactor to enable continuous process control, with the long-term goal of implementing the system in a pilot-scale fermenter.

Total Cell Density Monitoring of *S. cerevisiae*

The in-line sensor was evaluated by fed-batch cultivation of an *S. cerevisiae* strain, modified by Ingenza, in a 5 liter Electrolab Biotech Ltd bioreactor (figure 1). Data was collected over a 50 hour period, covering the log phase of the reaction. In-line TCD measurements were compared with measurements obtained by off-line reference methods (OD, WCW and DCW), collecting samples regularly between 0 and 15h, 25 and 35h, and at 50h.

Real-time, in-line (or in-situ) measurement of TCD was performed throughout the experiment using a Dencytee sensor (Hamilton Bonaduz AG). This optical density-based technology measures the turbidity of the cell suspension, detecting all particles and molecules – including live and dead cells, and cell debris – that absorb or scatter light at 880 nm in the near-infrared (NIR) spectrum.

Evaluating In-line TCD Performance

The real-time (in-line) and off-line TCD measurements obtained are shown in figure 2. In-line values were calculated using regression algorithms, and separate algorithms were developed for each of the reference methods (OD, WCW and DCW).

Real-time measurement of TCD showed a good correlation to the off-line reference methods. This demonstrates that in-line turbidity sensors can be successfully employed to monitor TCD in yeast in real-time and, therefore, to study the optimization of fermentation conditions.

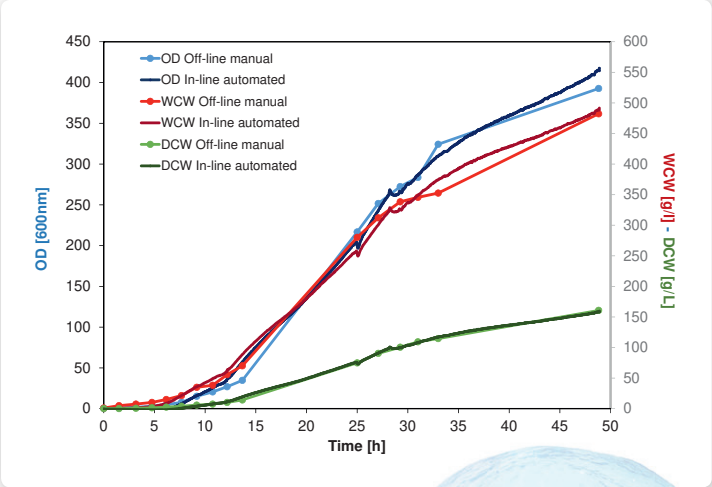

Figure 2: In-line turbidity versus off-line cell density measurements. The darker lines without dots represent the Dencytee in-line automated measurements. The lighter lines with dots represent TCD measurements from manual off-line reference methods.

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