Optimizing Bioprocessing With Continuous Cell Density Monitoring

Cell cultures and microorganisms are foundational to modern pharmaceutical development and biotechnology. Because these systems are highly sensitive to environmental changes, precise control and monitoring of cultivation conditions is essential throughout the growth process. Amongst key parameters such as temperature, pH, and dissolved oxygen must be carefully managed, cell density stands out as one of the most critical process indicators. It can be conveniently assessed through measurement of the optical density (OD) of the culture medium, or through backscatter techniques at very high cell densities.

UPSTREAM PROCESSING

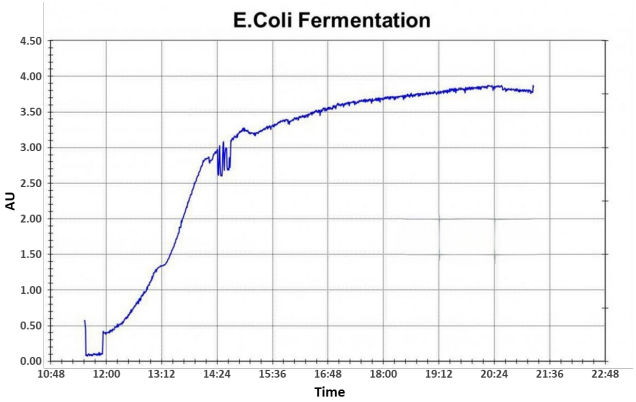
Biotechnological production methods are employed when complex active pharmaceutical ingredients (APIs) cannot be synthesized chemically. Monoclonal antibodies, recombinant proteins, and similar biologics are produced using genetically engineered organisms (bacteria, yeast, or mammalian cell lines) selected based on the post-translational modifications and protein folding characteristics required for the target molecule. The structural complexity of these APIs is directly correlated with process sensitivity and risk: even minor deviations in culture conditions can significantly impact product properties and therapeutic efficacy. For this reason, biopharmaceutical manufacturing ranks among the most demanding production environments in the industry.

The upstream process where cells are cultivated in bioreactors using batch, fed-batch, or perfusion strategies is particularly critical. To maximize both yield and product quality, bioreactor conditions must be carefully optimized and maintained. The most important parameters include temperature, pH, agitation rate, culture medium composition, and dissolved oxygen concentration.

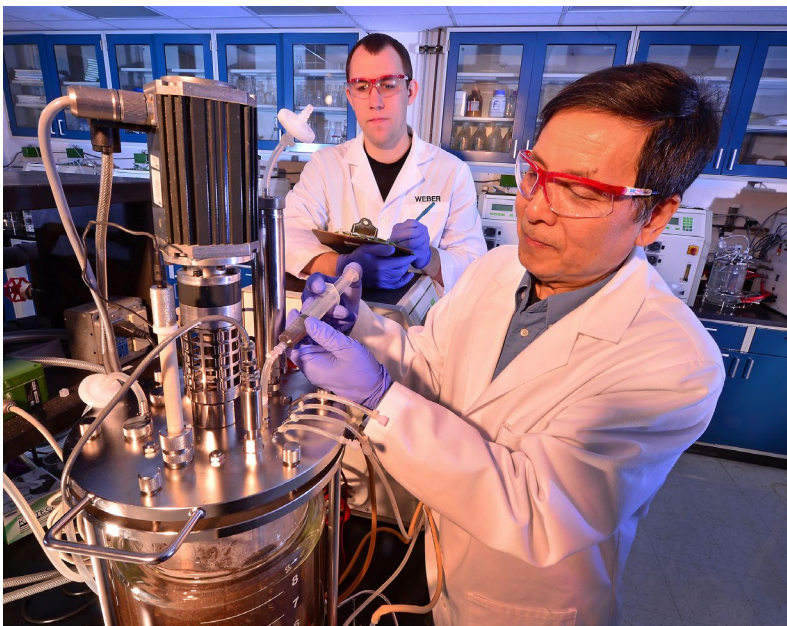
OPTICAL DENSITY AND BACKSCATTER: COMPLEMENTARY MEASUREMENT TECHNIQUES

Optical density measurement, typically performed via absorption photometry, provides a reliable, real-time indicator of cell culture growth. In this method, a beam of light is directed through the culture medium, and a detector measures the resulting attenuation. The degree of light reduction correlates directly with cell density, a critical metric because the quantity of target protein produced is often proportional to the viable cell population. OD data is used to determine optimal timing for nutrient feeds, carbon source switching, induction events, and harvest.

OD measurement is also used to calculate dilution rates. To achieve absorption readings appropriate for most cell types and to minimize interference from medium color, sensors operating in the near-infrared (NIR) wavelength range are preferred. NIR sensors detect all light-scattering and light-absorbing particles including live cells, dead cells, and cellular debris. Despite this limitation, OD measurement has proven highly effective for monitoring the growth phase due to the speed and simplicity of in-line implementation. An additional advantage is the ability to detect process deviations early, as reduced cell growth is often one of the first indicators of an emerging problem.



At the very high cell densities commonly encountered in high-performance fed-batch or perfusion processes, traditional transmission-based OD measurement can become unreliable due to detector saturation. In these applications, backscatter measurement offers a practical alternative.



Rather than measuring transmitted light, backscatter probes detect light reflected back from particles in the medium. This geometry remains effective across a much wider range of cell densities and does not require sample dilution, making it well suited for dense culture monitoring throughout the full production cycle.

For transmission-based OD measurement, the Exner EXcell absorbance probe is a well-established instrument that delivers continuous, drift-free in-line readings in biopharmaceutical applications. For high-density cultures where backscatter measurement is preferred, the Kemtrak TC007 is a proven fiber-optic photometric probe capable of operating across a broad dynamic range. Both instruments are designed for in-situ use and meet the material and hygienic standards required for biopharmaceutical production environments.

12mm Kemtrak backscatter probe

FROM LAB-SCALE DEVELOPMENT TO PRODUCTION

To fully leverage OD and backscatter measurement, correlations to meaningful units such as total cell count and dry cell weight must be established during the development phase using complementary analytical methods. Dielectric spectroscopy is another useful technique worth considering in this context. By measuring permittivity (or biocapacitance), this method exploits the ability of intact, living cells to store electrical charge. Changes in permittivity can therefore be used as a complementary measurement to distinguish viable cell volume from damaged or dead cells, information that neither OD nor backscatter measurement alone can deliver.

In many development laboratories, and even in some production environments, OD is still measured "offline": an operator manually collects samples at predefined intervals for analysis outside the bioreactor. This approach has significant drawbacks. Data gaps occur between sampling events and during unattended periods such as overnight runs. Furthermore, removing samples from the process introduces the risk of contamination.

Continuous, uninterrupted monitoring of all critical quality and process parameters is a cornerstone of robust bioprocess development and operation. Only through consistent environmental control can an optimal growth environment be established and maintained. Increasingly, inline sensors and instruments capable of continuous in-situ data acquisition are being adopted. These tools that can be used seamlessly from early development through commercial production. This approach significantly improves process reproducibility and yield, and eliminates the need for complex cross-calibration between laboratory and production measurement systems.

PROBE DESIGN CONSIDERATIONS

Given the sensitivity of biopharmaceutical processes, all components, including the bioreactor, agitators, and measurement probes, must meet stringent quality requirements. Material selection is paramount; stainless steel is the standard, with carefully controlled surface finishes. Electropolished surfaces with very low roughness values are preferred to minimize microbial adhesion and facilitate cleaning.

Probe geometry should minimize dead zones and crevices to prevent medium contamination and facilitate effective cleaning. All recesses and undercuts required by the design, especially those in contact with the process fluid, must be fully accessible for cleaning in place. CIP/SIP compatibility and autoclavability are now considered baseline requirements for any measurement device used in biopharmaceutical processing.

An in-line OD or backscatter probe meeting these design standards provides continuous, real-time monitoring of cell growth without the need for sample preparation or dilution, eliminating a common source of analytical error. The probe requires no recalibration during the process and delivers a stable, drift-free signal that is straightforward to interpret without additional data processing. It is important to note, however, that neither OD nor backscatter probes distinguish between live and dead cells, and therefore cannot directly replace offline methods such as viable cell counts or dry cell weight measurements. When designed to the specifications described above, the probe can be installed and qualified together with the bioreactor as part of a fully integrated measurement system.

KEY TAKEAWAYS

- Cell density is one of the most critical parameters in biopharmaceutical upstream processing, directly influencing product yield and quality.
- Optical density (OD) measurement uses NIR light absorption through the culture medium to estimate cell density in real time. NIR wavelengths are preferred to minimize interference from medium color.
- At the very high cell densities common in fed-batch and perfusion processes, transmission-based OD measurement can saturate. Backscatter measurement, which detects light reflected from the cells rather than absorbed by them, remains effective across a much wider density range and is the preferred technique in these applications.
- The Exner EXcell absorbance probe is a proven instrument for in-line transmission OD measurement in biopharmaceutical processes, delivering continuous, drift-free readings without sample preparation.
- The Kemtrak TC007 fiber-optic photometric probe is a well-established solution for backscatter-based biomass measurement, offering a broad dynamic range suited to high-density culture monitoring.
- Dielectric spectroscopy complements both OD and backscatter measurement by using permittivity to distinguish living cells from dead ones, providing a more complete picture of culture health.
- The Offline sampling still common in many labs creates data gaps, introduces contamination risk, and cannot support continuous process oversight.
- Continuous in-line monitoring enables real-time detection of process deviations and better-informed decisions on feeding, induction, and harvest timing.
- Using the same sensors from development through production improves reproducibility and eliminates the need for cross-calibration and correlation between lab and manufacturing environments.
- All probes must meet biopharmaceutical standards: stainless steel construction, electropolished surfaces, minimal dead volume, and full CIP/SIP and autoclave compatibility.
- Once correlated to offline reference measurements during development, in-line biomass probes can effectively replace routine offline sampling, dramatically reducing manual intervention while maintaining continuous, quantitative process insight.

- Exner EXcell probes are available in 12mm and DN25 formats, and fit most benchtop, pilot, and production scale bioreactors..
- Optical Pathlengths can be selected for the best resolution of measurement depending upon cell density

GET IN TOUCH

SOUTH FORK INSTRUMENTS
info@southforkinstruments.com

3845 Buffalo Road
Auburn, CA 95602

CALL US AT: (925) 461-5059