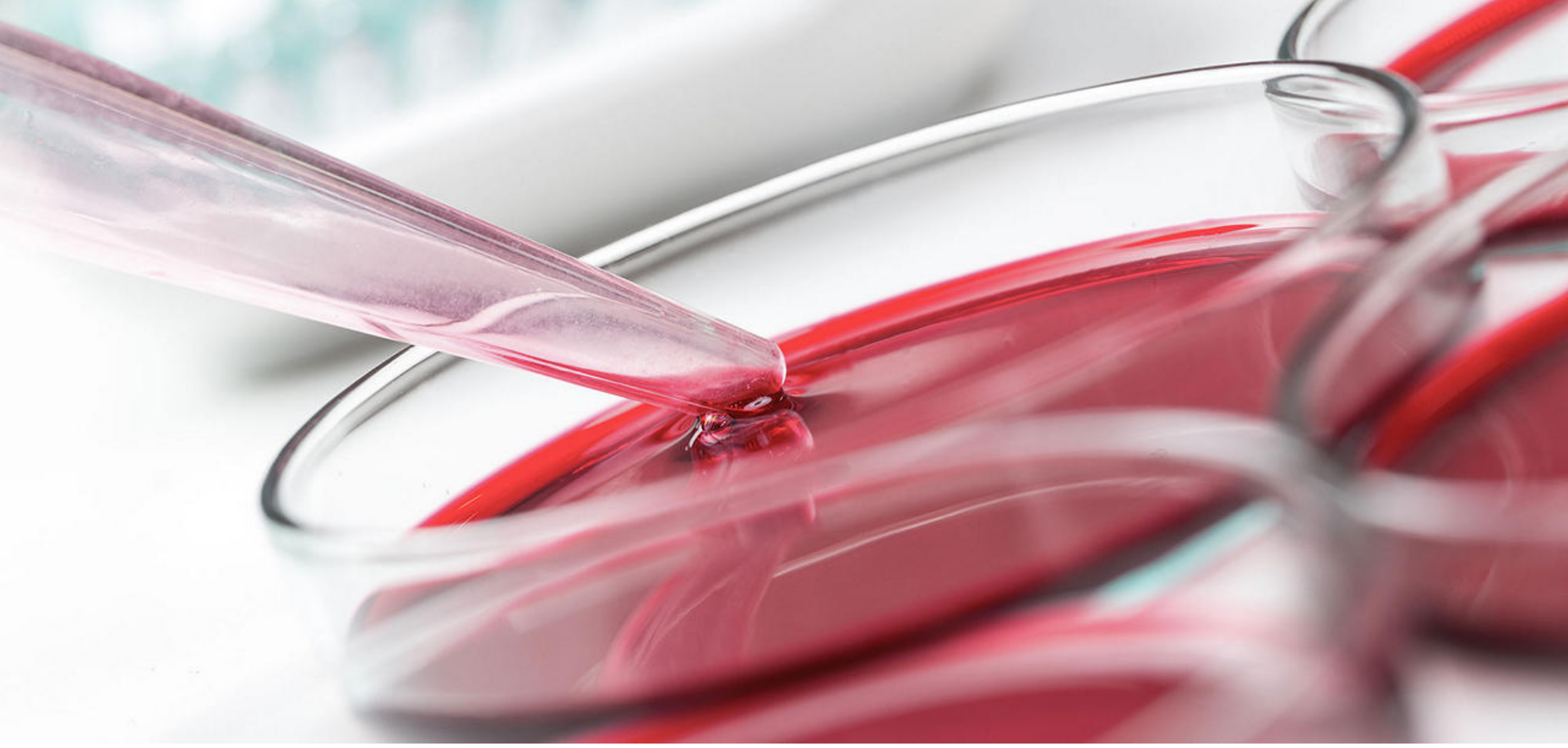

Application Note AN-PAN-1065

Inline monitoring of cell cultures with Raman spectroscopy

Cell culture is a key part of the biopharmaceutical industry to monitor the health of cells. Tight control of this process is necessary to overcome unwanted side reactions or poor yields.

Glucose is essential for cell energy, but its conversion to energy through glycolysis produces lactate. Lactate accumulation induces acidity, causing cellular stress and reduced cell growth rates.

Manual laboratory methods can be quite cumbersome and can introduce bias depending on the analyst, resulting in inaccurate results and sample contamination. To address these issues, inline Raman spectroscopy is a very well-suited analysis technique for this industry as it does not require any reagents and the sample remains unaltered.

This Process Application Note presents a method to accurately monitor cell growth inside a bioreactor in «real-time» with the 2060 Raman Analyzer from Metrohm Process Analytics. In this case, an inline process analyzer is the preferred solution to guarantee high product quality and avoid contamination from measurement.

INTRODUCTION

Cell culture is an essential step in the production of a wide range of biopharmaceuticals, including complex therapeutic proteins, monoclonal antibodies, and vaccines [1]. This process involves growing cells in a controlled environment, typically in a bioreactor, to produce the desired product.

Glucose is an important component of cell culture, as it provides cells with the energy they need to grow, divide, and form the final product [2]. Figure 1 illustrates the fundamental process of converting glucose into energy within a cell.

The conversion of glucose to energy (i.e., glycolysis) results in the production of lactate, the ionized form of lactic acid. Accumulation of lactate in cell culture media can adversely impact cell growth and productivity. As lactate functions as an acid, its buildup in the culture medium can lower the pH, inducing cellular stress and diminishing growth rates [4].

To prevent lactate accumulation, manufacturers must monitor and adjust cell culture conditions to maintain lactate levels within an acceptable range. This may involve continuously measuring the glucose concentration in the culture medium, ensuring precise adjustments to prevent over- or underdosing.

Typically, analysts perform such measurements manually (offline). However, contamination is common in this setup, and the data fails to reflect the true state of the cell culture process.

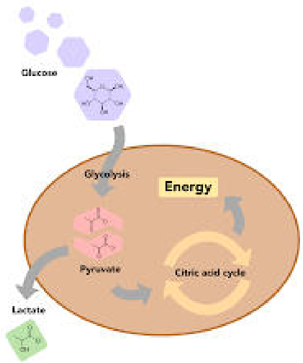

Figure 1. Overview of glucose mechanism in human cells. Extracted from [3].

A safer, more efficient, and faster way to monitor multiple parameters simultaneously in bioreactors is via inline analysis with reagent-free Raman spectroscopy (Figure 2). This is a nondestructive and non-contact technique – thus it is ideal for inline analysis directly at the bioreactor (Figure 2, left side).

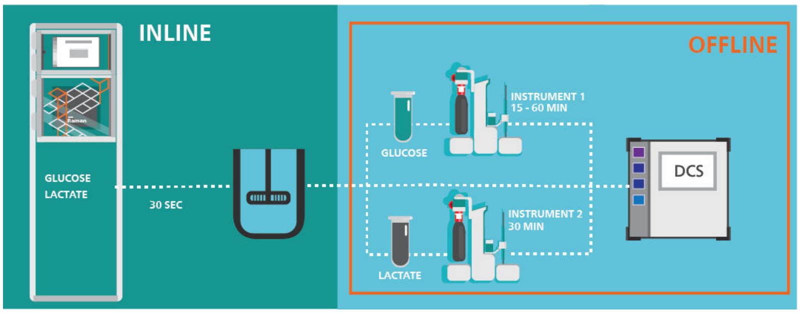

Figure 2. Steps to measure glucose and lactate in a bioreactor by implementing inline (left) or offline (right) analysis.

The 2060 Raman Analyzer by Metrohm Process Analytics enables the comparison of «real-time» spectral data from the process to a reference method (e.g., titration, ion chromatography, HPLC) to create a simple, yet indispensable calibration model for monitoring glucose and lactate in the cell culture bioreactor.

«Real-time» analysis of the state of the culture keeps the glycolysis reaction within control. Additionally, inline analysis can provide information about the current state of different processes (e.g., substrate consumption and kinetics).

APPLICATION

Laser used: 785 nm. Inline analysis is possible using an autoclavable immersion probe designed by Metrohm Process Analytics.

Spectral data was collected with a 2060 Raman Analyzer (Figure 3) by using both IMPACT and Vision software from Metrohm. Measurements were collected every minute.

Figure 3. 2060 Raman Analyzer for quantitative analysis of lactate and glucose in a cell culture bioreactor.

Table 1. Prediction accuracy of the two measured parameters in a cell culture bioreactor as analyzed by the 2060 Raman Analyzer (Figure 3).

	Glucose	Lactate
Minimum concentration	0.1 g/L	0.0 g/L
Maximum concentration	40 g/L	5.0 g/L
Bias	-0.1349	-0.0849
SEP	0.2009	0.1166

CONCLUSION

Raman spectroscopy is used for many different applications and industries where high-quality data is essential. The 2060 Raman Analyzer is a high-performance Raman system designed for operations like cell culture monitoring, which require non-contact analysis. Together with Metrohm's Vision and IMPACT software, the 2060 Raman Analyzer can be used to acquire near real-time results, supporting time-critical processes such as pharmaceutical development.

RELATED DOCUMENTS

TA-045 Real-time monitoring of cell density and growth potential in bioreactors using near-infrared spectroscopy

BENEFITS FOR RAMAN IN PROCESS

- **Early detection of batch failures** during cell culture.
- **Multiple parameters** from a single measurement.
- Minimize risks of sample contamination by being a **non-contact technique**.
- Unique Raman spectra that serve as specific fingerprints for **material identification**.
- **Low signals from water** or moisture which complements other spectroscopy techniques.
- **Nondestructive** technique, thus ideal for inline analysis.

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