timegate
ahead of time

How to increase product yields in biopharmaceutical manufacturing? Introduction to Timegated® Raman monitoring approch

AUTHORS:

Timegate Instruments Oy, Finland:
Mari Tenhunen
Lauri Kurki
Jyrki Savela
Jouni Takalo

University of Helsinki, Finland:
Martin Kögler

Biopharmaceuticals have very high medical and economical importance and they bring numerous social benefits. However, there are operational and technological challenges to produce them. Reproducing large molecules reliably at an industrial scale requires manufacturing capabilities of a previously unknown sophistication. This sophistication comes at a great cost. Biopharmaceutical facilities are costly to build and run. Bioprocesses require expensive raw materials, long process durations and result in low product yields and, not least, they need for a team of highly skilled experts to operate them. Strong demand for newly developed biopharmaceuticals has driven significant profits, despite the high cost of goods sold. However, as biopharma moves from the scientific frontier to the business mainstream, the industry will increasingly be forced to confront the same challenges faced by other businesses: maintaining competitiveness by ensuring affordability, quality, and delivery performance. Therefore, there exists a real need in biopharmaceutical industry to improve the process efficiency through improved yields and decreased processing times.

Inside a bioreactor, many thousands of different chemical reactions take place and the cells simultaneously consume and produce many nutrients and metabolites. Most importantly, the therapeutic itself is highly complex and is either accumulated inside the cell or excreted in a matrix with many other proteins. Therefore, an in-line cell growth monitoring system for biopharmaceuticals is much more challenging, and at the same time more of a necessity. The cell culture processing conditions (such as dissolved oxygen, nutrients, osmolality), the mode of operation (batch, fed- batch or perfusion) and the downstream processing influence on protein quality. Even slight changes in the overall production process may translate into significant differences in overall protein structure or unwanted post-translational modifications. For this reason continuous monitoring of these process conditions is essential to ensure that the main characteristics of the product are maintained throughout the process within acceptable ranges. Therefore, in-line complex analysis of (critical) amino acids would be a very valuable tool in process development and process control. In this application note, we focus on the monitoring needs and solutions in the seed and productions reactors.

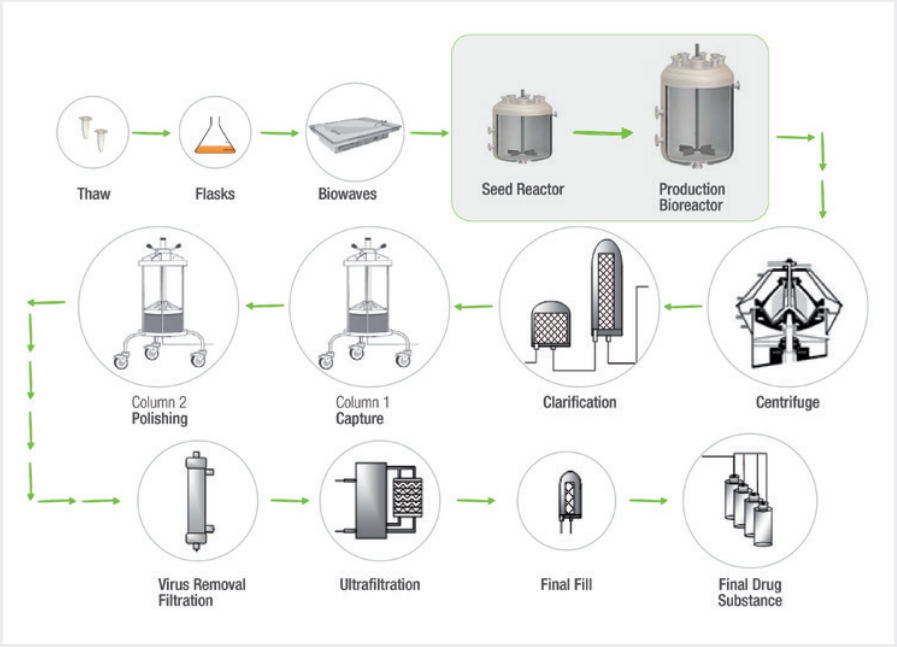

Figure 1:
On-line monitoring can bring huge advantage to the production efficiency and reliability in seed and production reactors.

Today, the process attributes typically measured continuously in seed and production reactors are pH, temperature and dissolved oxygen . The most of other process parameters are measured only daily or twice a day through manual sampling. During the last decades a huge amount of R&D work has already been done to develop the real-time, in-line, non-contact and non-destructive sensing solutions for the bioprocess monitoring purposes. Different optical, spectroscopic technologies have been among the most potential candidates for this task, because they can provide highly specific chemical information about the cell culture components. They are fast, non-destructive and easy to integrate to process monitoring tasks. One of the most promising technology which has been in the core of process monitoring and automation development, is Raman spectroscopy. Raman has been used for the in-line monitoring of the changes in the cell growth media. However, the Achilles' heel of Raman spectroscopy is the fluorescence emission that can in the worse case totally overlap the weak Raman scattering signal and prevent the use of this technology or at least the fluorescence background impairs the measurement sensitivity significantly. It has been estimated that far less than half of the current cell cultures can benefit from this technology. In all others, the fluorescence makes the use of Raman spectroscopy unreasonable for the process monitoring purposes.

Special features of Timegated® Raman technology in continuous monitoring of bioreactors

Timegated® Raman technology provides three major advances compared to the current state-of art, conventional Raman technology in the cell culture monitoring. First of all, it will provide real fluorescence rejected Raman spectra and enlarge the applicability of Raman technology to the cultures where Raman has not be applied before due the masking of fluorescence signal. Timegated® Raman technology is based on the differentiation of Raman and fluorescence signals in time domain. This measurement approach has been known for fluorescence suppression for decades, however, the high cost and complexity of the earlier time domain based technologies has prevented the commercialization and wider adaption of this approach. The new, patented CMOS-SPAD (Single Photon Avalanche Diode) electrically gated detection offers an affordable solution with decreased cost and complexity, and makes this technology applicable to numerous different monitoring tasks, where conventional CW Raman cannot be used due to the high fluorescence interference.

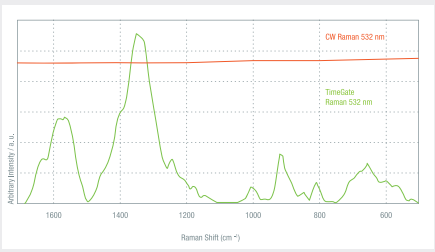

Figure 2:
Real fluorescence rejection with Timegated® Raman technology. Comparison of CW (Continuous Wave) and Timegated® Raman measurements of E. Coli cell cultures. With CW measurement, no Raman signal was detected behind the fluorescence background.

Secondly, this technology provides the separation of Cell and Media Signals by Hardware. The use of pulsed laser excitation and extremely fast, 100 picoseconds, detection provides totally new type of measurement approach.

The intensity of elastically signal after each laser pulse depends on the optical properties of the material where the laser pulse hits, and therefore each cell gives different signal intensity than growth media. This means that after each laser pulse, the detected Raman scattering can be classified into the two different categories and separate Raman spectra based on the backscattered light intensity - one coming from the cells and the other coming from the growth media. This makes the data processing and process modeling much easier and more reliable by avoiding overlapping spectral bands of cells and media.

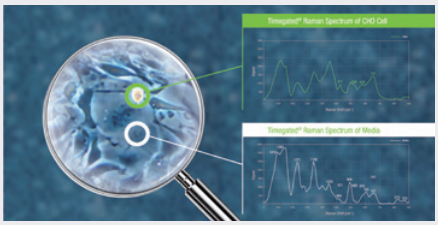

Figure 3:
Two different signals – one from cells and the other from media – separated by hardware.

Thirdly, Timegated® Raman technology also allows monitoring the changes in cell density. The pulse-by pulse detection and the separation of signal origin give also information about changes in cell density. If we compare the ratio of laser hits to cells and to media in a set time frame, we get information about changes in the relative number of cells. This information can be used for cell density calibrations.

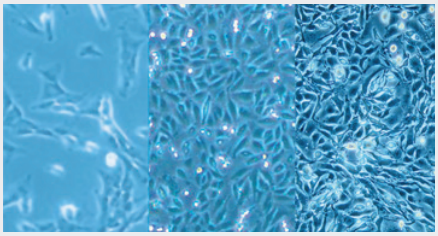

Figure 4:
Timegated® data can also be used to give information about changes in the cell densities during the bioprocessing.

Summary

Timegated®Raman Approach offers a totally new in-line monitoring solution to many current bioprocessing challenges. Benefits that can be achieved include:

01

No need for sampling and no risk for process contamination

02

Real time data of growth media composition, cell density and produced proteins

03

Improved SNR (Signal to Noise Ratio) for signal from cells

04

In-line control of nutrient feeds to bioreactors

timegate

ahead of time

Visiting address:

Timegate Instruments Oy
Teknologiantie 5B
90590 Oulu
Finland

Info@timegate.fi