REAL-TIME MONITORING OF CHO CULTURE WITH AN IN-LINE MICROSCOPE

Priyal Patel, Jean-François P. Hamel (Contact: Dr. Hamel, jhamel@mit.edu)
Massachusetts Institute of Technology, Department of Chemical Engineering,
Cambridge, MA 02139

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ABSTRACT:

To advance biopharmaceutical research and development, new analytical tools have been integrated in the process to collect and analyze data in real time. For example, real-time *in-situ* monitoring of cell culture parameters can be used to control substrate concentrations, cell growth and desirable product quality. Data are presented for a batch CHO cell culture, in which cell attributes and the cell environment were monitored continuously with an in-line microscope and an integrated on-line automatic sampling system-biochemical analyzer. The culture was started at 0.3 million cells/mL, and cell concentration increased by one order of magnitude during the twelve-day long run. The in-line microscope uses differential digital holographic microscopy[1], a novel technology which can discriminate between viable and dead cells. In this way, we could determine cell concentration and cell viability in real time, without using a label. Off-line analysis from samples withdrawn manually was carried out, for comparison of the on-line data set.

INTRODUCTION:

A well-engineered bioprocess can lead to successful industrial level production of desired products. For the production of many biopharmaceutical products, CHO cells are often cultured in batch or fed-batch mode, in which key analytes and target products are measured off-line. Off-line measurements constrain the analysis for fast feedback control of the process. Continuous, *in-situ* real-time monitoring technology thus holds the prospect of providing data that can be used quickly for controlling the process and in turn, controlling the quality of the product. In this experiment, a batch culture was performed in a 2 liter bench-top glass bioreactor. An in-line microscope, which uses differential digital holographic imaging technology, performed an image capture every 30 minutes of sample constantly flowing through sample loop and recycling it back to the vessel. In this way, the sample remained in a closed system, reducing the risk of contamination and also gave real-time information about the cell density and viability of the culture. This set up is an example of Process Analytical Technology (PAT) which can help in improving process performance for an overall increase in efficiency and productivity.

OBJECTIVES:

- Monitor cell density and viability during a batch CHO cell culture using an in-line microscope
- Compare data from the in-line microscope with an off-line biochemical analyzer

METHODS:

- **Initial Cell Culture**
  - CHO-DG44 cells were used for the experiment. The initial culture was prepared in a spinner flask using CD OptiCHO Medium (Thermo-Fisher, MA) at 37°C until cell density of 2-3 x106 cells/mL was achieved. The bioreactor was seeded at a cell density of 0.3 x 106 cells/mL.

- **Bioreactor Set-Up**
  - A bench top 2L Bioreactor (Sartorius Stedim A plus- Sartorius, Germany) was set up containing 1750 mL of culture. The head plate contained the ports for air sparging, pH probe, auto sampler, inline microscope, medium addition, inoculation, condenser, temperature probe and- the sampling port.
  - A fluidic system (BioConnect -Ovizio Imaging System, Belgium) was setup with the reactor using a 12mm compression port which delivered the samples to the in-line microscope (Iline F-Ovizio Imaging Systems, Belgium), which performed Digital Holographic Imaging and displayed real time data on the monitor. The sample travelled a distance of 1.7 meters for about 120 seconds in the fluidic system (BioConnect -Ovizio Imaging System, Belgium) and was recycled back to the reactor.

- **Batch Mode**
  - The CHO-DG44 cells were maintained in a working volume of ~1.8 L for 12 days, at 37°C with a pH of 7. A mixture of air and CO2 was supplied continuously through out the culture. The agitation rate was set at 200 RPM.

Figure 1. Bioreactor Set Up – To the extreme left is the in-line microscope (ILine F-Ovizio Imaging Systems, Belgium) connected to the reactor through a fluidic system (BioConnect - Ovizio Imaging Systems, Belgium). To the extreme right is the control tower (Sartorius Stedim, Germany) of the bioreactor.

- **Sampling**
  - Samples were taken daily and analyzed on the off-line bioanalyzers, FLEX 1 and FLEX 2(Nova Biomedical,MA). The in-line microscope (ILine F- Ovizio Imaging Systems, Belgium) took online samples every 30 minutes. During the experiment, the monitor of the In-line microscope displayed the trend and current readings of the cell density and the viability.

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RESULTS:

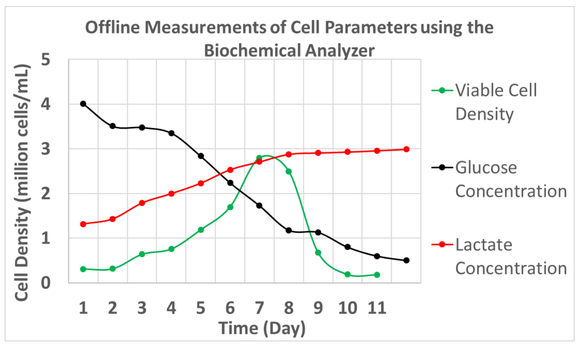

Figure 2. Offline Monitoring of Cell Density and Viability in Batch CHO Culture-The graph displays the cell density of the culture during the 12 day long run. The measurements were taken using the off-line bioanalyzer. As noted the peak cell density was achieved on the 7th day of the run. The graph reveals a decline in the cell density towards the end of the run (Days 9, 10, 11, 12) which was a result of the decrease in the glucose concentration an the increase in the lactate and other metabolite concentration. The lactate and glucose concentration readings were taken off-line using the Bioanalyzer.

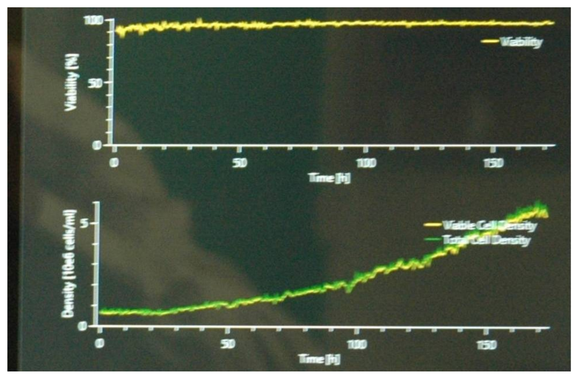

Figure 3. Trends of Viability and Cell Density as recorded by the In-line Microscope's Software, OSOne-The graph displays the trends of viability and cell density of the culture. The graph reveals a gradual increase in the cell density and an almost constant trend in the viability of the cells. The capture was taken on the seventh day of the run, just before the cells went to death phase of their lifecycle due to a depletion of glucose and nutrients in the medium.

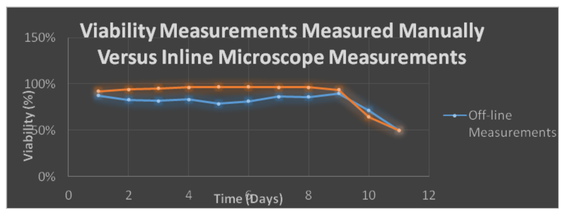

Figure 4. In-line and Off-line Viability Measurements- Viability measurements showing similar trends between the off-line (Blue) and in-line (Orange) measurements. The in-line measurements were recorded from the monitor of the in-line microscope at the same the time when the off-line measurements were analyzed using the Bioanalyzer.

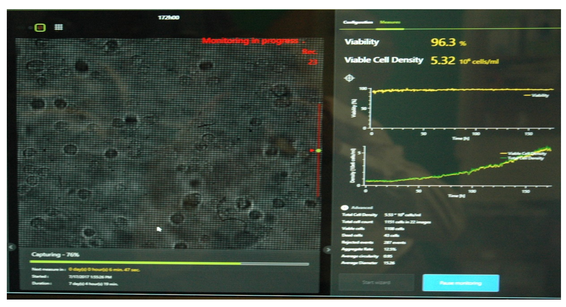

Figure 5. Screen capture from the In-line Microscope on seventh day of the run- The picture is a screen capture from the monitor of the computer attached with the in-line microscope, in the center of the screen is the images of the cells as viewed by the in-line microscope and towards the right are the cell density and the viability trends observed by the software along with additional information on cell size and culture parameters.

CONCLUSIONS AND FUTURE WORKS:

- Off-line and in-line data were in agreement with each other, both displaying a sharp decrease in viability at day 9.
- The in-line microscope monitored the cell culture 24 hours a day, while manual sampling was possible only during the working hours.
- The viable cell density measurements from the in-line microscope throughout the run assisted in determining the stage of cell growth, which helped in assessing which biochemical assays were needed (for example, glucose, lactate, pH, carbon dioxide)
- The results of this study are a prelude to future developments of real time monitoring of the process and controlling the cell culture environment.

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