Online monitoring of growth and cell shape of CHO cells in stirred bioreactors

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Abstract

Monitoring of suspension cell cultures often relies on sampling followed by a staining procedure. Estimations of the cell count and cell viability are performed once a day using trypan-blue cell exclusion as a method of choice. This involves manual operations and weekend work

Quantitative phase imaging (QPI) is a new technique that allows cell counting as well as cell viability monitoring in a continuous, label-free set-up. No need for sampling, staining and waiting for results generated by an off-line counter: results are available in nearly real-time over the whole run.

Additionally, QPI offers the collection of quantitative phase information, covering the shape and density of an object.

In this study, we compared the results generated by two iLine F with two reference methods: an automated trypan-blue staining with the Cedex (Roche Diagnostics) and a fluorescence based staining with acridine orange with the NucleoCounter NC-200 (chemometec). The iLine F was set to generate 2 cell count cycles per hour and the culture was left to grow in batch mode for a total of seven days.

Materials & Methods

- Two **iLine F** microscopes
- **BioConnect**: autoclavable and disposable closed-loop interface with the bioreactor.
- **Reusable pump engine** with automated flushing (in case air bubbles or large aggregates are detected, patent pending).
- **OsOne Acquisition & Analysis software** (developed in-house by OVIZIO), version 6.3.
- **Applikon 3 liters glass bioreactor** controlled by ez-Control.
- **CHO XM 111-10** cells (CCOS 837) inoculated at $0.5 \cdot 10^6$ viable cells mL^{-1} in ChoMaster® HP1 medium (CCT), batch culture.

Fig. 1: Applikon 3 L bioreactor connected with an iLine F

Results

- Two successful batch cultivations, resulting in maximum viable cell densities of $2.5 \cdot 10^6$ cells mL^{-1} are presented.
- A high comparability between both iLine F for all cell density and cell shape measurements could be achieved.
- Good qualitatively correlation of the iLine F with the Cedex and the NucleoCounter cell density measurements for the growth and stationary phase are visible.
- The viable cell density measurements using the QPI during the death phase resulted in higher values compared to the offline devices.
- The monitoring of the cell diameter decrease during the cultivation is highly reproducible.
- Real time online monitoring of the circularity and aggregation rate shift while cells entering the stationary phase ease the detection of process events.

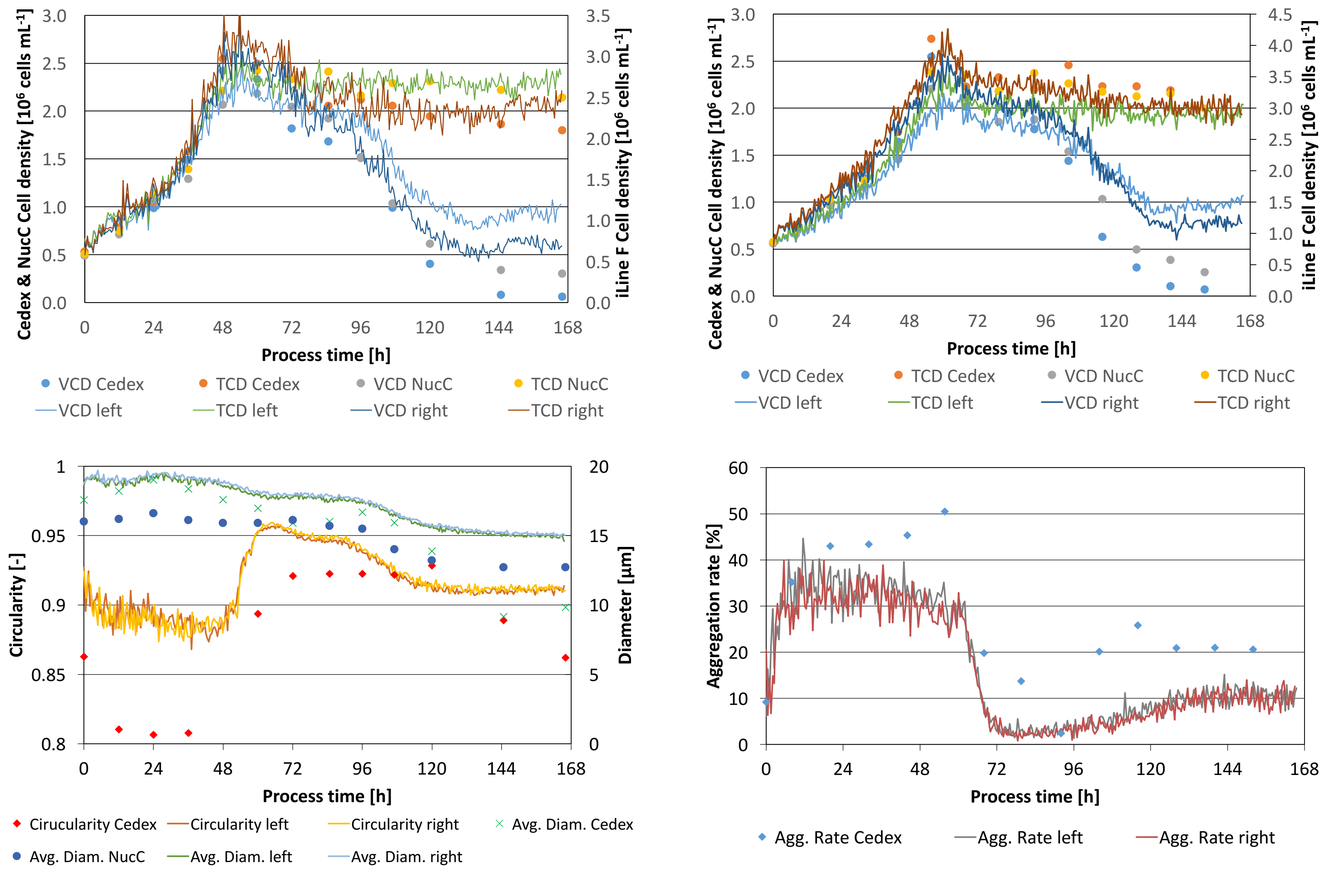

Fig. 2: Comparison of the viable cell density (VCD),total cell density (TCD) and the average circularity, diameter and aggregation rate measurements of two iLine F with the measurements of a Cedex and NucleoCounter (NucC). Left: measurements from the first run, right: measurements from the second run

Conclusion

The online monitoring of cell density and morphology utilizing the iLine F was successfully compared to established offline methods. All devices produced comparable growth curves. Furthermore, the reproducibility of the iLine F was proven. However, there are deviations in the absolute values. Changes in cell morphology (indication a shift in cell metabolism) can be tracked in nearly real-time, allowing a faster response and adaptation.

Further application

A potential application is the monitoring of cell sedimentation, e.g. during the change from growth to production media. It can be seen that both iLine F measurements are similar and give a real-time prognosis of the cell density at their level. Thus, the ideal moment for media exchange can be found easily.

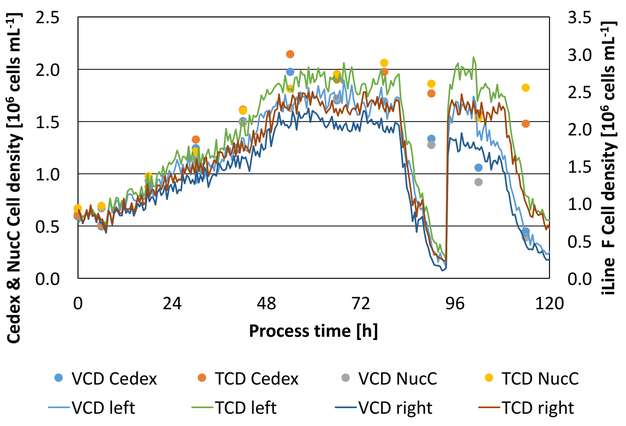

Fig. 3: Monitoring of cell sedimentation after 81 and 109 h

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