Real-Life CHO Cultivation in Single Use Bags

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A novel fibre optic conversion model which connects single-use sensors to conventional controllers could provide new readings using electrochemical signals. How will the device fare after the application of an endurance test?

In a recent study, a prototype fibre optic device has been applied for monitoring cultivation parameters in suspension-adapted Chinese hamster ovary (CHO) cell culture. This device converts the reading of chemical optical sensors integrated in the cultivation bag into electrochemical signals, which are transferred to a conventional controller. CHO cells were successfully cultivated for five days in this set-up. Although the culture showed untypical growth, correct functioning of the prototype device throughout the whole cultivation period was verified, even though operational errors occurred.

Most controllers are designed to work with electrochemical sensors for oxygen and pH monitoring. A novel device now allows scientists to convert the reading of chemical optical sensors into an electrochemical signal (ECS), which can be transferred to the controller. In this way, the functionality of conventional controllers can be expanded to work with optical sensors for measurement of the important culture parameters' oxygen and pH.

Chemical optical sensors – so-called optrodes – are very advantageous for cultivation monitoring: measurements can be taken non-invasively, which reduces the risk of contamination. Furthermore, these sensors can be integrated in single-use bioreactor systems.

Keywords

Chinese hamster ovary (CHO) cells
Electrochemical signal (ECS)
Dissolved oxygen (DO)

In the following tests, a prototype of the device was evaluated for its functionality in combination with the ez-control (Applikon). Chemical optical sensors integrated in a cultivation bag were read out, and their signal successfully converted and transferred to the controller.

The combination of the systems from different manufacturers worked accurately, and allowed online oxygen and pH monitoring in the CHO cell culture.

Set-Up for Cultivation Control with Optrodes

The Optrode Dual prototype was connected to the ez-control, which was linked via Ethernet cable to a PC for data collection (see Figure 1). Data recording for dissolved oxygen and pH was realised with the software BioXpert (version 2.93.122b2) with a sampling rate of one minute. The optical sensors integrated in a 20L cultivation bag were pre-calibrated. The Optrode Dual prototype allows sensor calibration via barcode scan, so a specific barcode was generated with the software QR Code Generator in manual mode, entering the pre-calibration data, and then scanning it with the fibre optic device.

The cultivation bag was placed on a BioWave 20 SPS platform (Wave Biotech). Suspension-adapted CHO cells (CHO XM111-10, obtained from Fussenegger *et al*, ETH University) were used for cultivation, which have a tetracycline regulated promotor for secreted alkaline phosphatase (SEAP) expression. However, the study only involved a growth experiment without product formation, where tetracycline is added to suppress the SEAP expression.

The cultivation bag was filled three hours prior to inoculation with 1.5L chemically defined medium (CHO Master HP1, Cell Culture Technologies) containing tetracycline (2.5mg L^{-1}) and Pluronic F68 (2mg L^{-1}). The medium was conditioned to 37°C and aerated until it was saturated. The bioreactor was inoculated with 0.5 x 10^6 cells mL^{-1} with a starting volume of 2.4L.

Approximately 48 hours and 72 hours after inoculation 3L, respectively 5L of fresh culture medium were added to reach the maximum working volume recommended by the bag manufacturer. To ensure optimal mixing, the angle and speed of the rocking motion of the cultivation bag were adapted to the filling volume of the bioreactor. It is well known that the oxygen transfer rate and the mixing time depend on the motion parameters and the filling level.

Samples were taken of approximately 5mL volume four times a day. Cell count and determination of cell viability were done automatically with NucleoCounter NC-100 (ChemoMetec). Substrate and metabolite concentrations were determined with the analytic system Bioprofile Bplus (Laborsysteme Fluekiger); samples were centrifuged at 3,500g for three minutes and measurements taken in the supernatant. In addition, pH was measured offline with a pH meter (Mettler Toledo) to verify pH reading of the chemical optical sensors.

Monitoring CHO Cell Culture

This set-up was used to test the functionality of the prototype device during cultivation of suspension-adapted CHO. Figure 2 shows the change of cell density and viability throughout the entire cultivation period.

The initial density of 0.5 x 10^6 cells mL^{-1} increased to a value of 2.11 x 10^6 cells mL^{-1} within 48 hours, showing a growth rate of 0.03h^{-1} corresponding to a doubling time of 23 hours. This is slightly higher than typical doubling times routinely obtained in the laboratory.

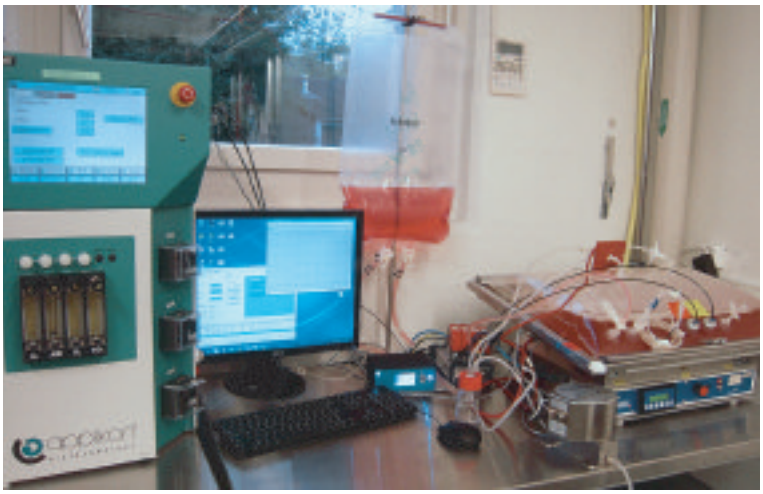


Figure 1: Measurement set-up: the Optrode Dual connected to the ez-control

Source: Institute of Biotechnology, Zurich University

About 25 hours after inoculation, cell growth had begun to stagnate.

Although no oxygen limitation was detected and mixing was expected to be sufficient, the movement rate of the bioreactor was raised from 15rpm to 19rpm and the gassing rate of 0.2slpm (standard litre per minute) adjusted to 0.4slpm (0.1vvm to 0.2vvm). This reestablished the growth in the culture, and the cell density further increased. One explanation for the growth stagnation could therefore be seen in sedimentation of cells in the small pockets inside the cultivation bag, where the optical sensors are fixed. This is in agreement to previous observations.

Decreasing Cell Density

Due to cell growth, the concentrations of the substrates glutamine and glucose decreased to

Figure 2: Cell density and viability during CHO cell cultivation in a 20L cultivation bag. The arrows indicate addition of HP-1 cell culture medium

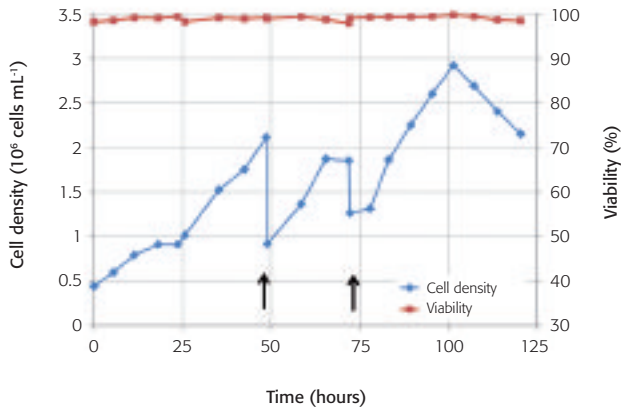
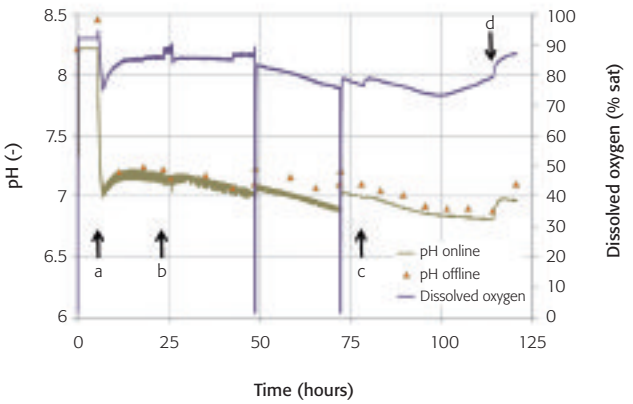


Figure 3: Online measurement data for dissolved oxygen and pH; offline measured pH values are also shown for comparison. The arrows indicate finalising the recalibration of the optical pH sensor (a), the increase in supply rate (b) and (c), and the termination of CO_2 supply (d)



0mmol L^{-1} and 1.5g L^{-1}, while ammonium and lactate levels rose to 1.4mmol L^{-1} and 1.9g L^{-1} respectively.

Those high metabolite concentrations are known to affect the cell growth and, therefore, 3L of fresh medium were added to the bioreactor after 48 hours of cultivation, which resulted in the dilution of metabolites and the cells. Afterwards, another period of exponential growth followed, again with an average doubling time of 23 hours. Some 72 hours after inoculation, another 5L of medium were added to the bioreactor, again followed by a growth phase.

The culture reached maximum cell density of 2.88 x 10^6 cells mL^{-1} after 100 hours, which is about 40 per cent lower than typical values routinely found in the laboratory for the used cell line. Glutamine was completely consumed by then, and glucose concentration reached a level of 0.35g L^{-1} while lactate and ammonium concentrations of 1.92g L^{-1} and 1.35mmol L^{-1} were detected. In the last 24 hours, the cell density was decreasing and reached 2.15 x 10^6 cells mL^{-1} when the cultivation was terminated, but with 98 per cent viability.

Online recorded data for dissolved oxygen (DO) and pH are shown in Figure 3, page 29. During the first six hours of cultivation, a constant pH value of 8.22 was measured, because the calibration of the sensor was not conducted correctly due to an operational error. The barcode was read by the fibre optic device, but measurement was not restarted. Furthermore, the CO_2 supply was not functioning correctly, so the pH value could only be adjusted to 7.2 after 10 hours.

Because of the increased streaming of CO_2 into the bag, the dissolved oxygen level decreased from 92 per cent to 77 per cent, and was rising again after gassing was correctly adjusted to 0.2slpm with 10 per cent CO_2. A further increase in the DO level was recorded after 24 hours when gassing and movement of the bioreactor were changed. Due to the increased mixing of the bag content, the oxygen transfer exceeded the oxygen consumption of the cells in this period.

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Increasing DO Measurements

To determine the weight of the bioreactor during medium addition, it was taken off the controlling system. This is why DO reading stopped at 48 hours and 72 hours. At the same time, the pH value rose with adding the slightly basic medium for 0.1pH and 0.3pH units, respectively. After 77 hours of cultivation, another increase in DO could be investigated, caused by the second increase in gassing to 0.5slpm. From hour 100 on, DO increased from 75 per cent to 80 per cent due to decreasing cell growth. The last increase in DO was recorded after 110 hours of cultivation time, when the CO_2 supply was turned off. The pH was measured offline to verify the online sensor reading. During the whole cultivation, acceptable differences of below 0.2pH units could be detected with offline determined values always being higher. This was probably caused by the time difference between sampling and measurement, during which pH-regulating CO_2 might have escaped the medium and caused pH to increase.

Conclusion

The test described here was designed to evaluate correct functioning of a prototype fibre optic device that converts the reading of chemical optical sensors into an ECS, which is then transferred to a controller. Although the prototype of the Optrode Dual was being used for the first time in the laboratory, and the CHO cell culture showed a rather untypical growth, accurate measurement data was obtained by connecting the device to the optical sensors in the cultivation bag.

During the tests, no functional and technical errors occurred for the whole five-day period. The Optrode Dual proved to be a reliable tool for measuring culture parameters with optical sensors and transferring data to the controller. This was verified by subsequent experiments with pH and DO shifts using different mixtures of CO_2, oxygen and nitrogen in cell-free culture media.

Furthermore, it can be concluded that the Optrode Dual is time-saving, as it can be easily connected to the electrochemical inputs of the controller and no further changes of controller settings are necessary. With a fibre optic device like this, it is possible to save costs as the purchase of new process analysis tools for reading optical sensors might not be necessary, and non-invasive monitoring of pH and oxygen can be conducted.