

Novel single-use sensors for online measurement of glucose – use in combination with online DO and pH monitoring

Dipl. Ing. FH Irina Bauer

University of Applied Science, Grönten, CH-8820 Wädenswil

John Gernot

PreSens GmbH, ,



Fig. 1: Cap for shaker flask with CITSens Bio Glucose sensor incorporated

Introduction

According to the FDA-guidance for industry , Process Analytical Tools (PAT) “are intended to support innovation and efficiency in pharmaceutical development” [1]). The agency encourages manufacturers to use the PAT framework to develop and implement effective innovative approaches in development, manufacturing and quality assurance. The sensors described below are one possible response to the requirement of systems, which are able to analyze and control critical cultivation parameters by real-time measurements during the process.

Working principle of the bio sensors



Fig. 2: Sensors in shaker flask connected to the CITSens Bio Beamer (blue). In the background the receiver ZOMOFI to receive the wireless transmitted signal

Glucose was measured using the sensor CITSens Bio (C-CIT, Wädenswil, Switzerland). It makes use of an enzymatic oxidation process and direct electron transfer from glucose to the electrode (anode) by a chemical wiring process. In contrast to other enzymatic glucose sensors it is working without cross-sensitivity to oxygen. In order to separate the sensor from the cultivation medium, a dialysis membrane is cast over the sensing head. This sensor head is delivered ready-to use, i.e. gamma-irradiated and integrated into the ventilation cap of the shake flasks. The signal provided by the biosensor is read-out by the connected water tight beamer (Bio Beamer (Fig. 2 blue) and wireless transmitted to the receiver (Fig. 2, ZOMOFI®). Each sensor and Bio Beamer is identified by a specific tag. One receiver ZOMOFI® is able to acquire the response of up to 1000 sensors (Cell culture vessels). Each sensor and Bio Beamer is identified by a specific tag to ensure correct data assignment. The receiver is connected to the PC, where data processing is performed.

The SFR Shake Flask Reader (PreSens, Regensburg, Germany) was used in all experiments to monitor pH and dissolved oxygen concentration. The system applies chemical optical sensors attached to the bottom of the shake flasks. These chemical-optical sensors change their fluorescent due to changes in pH and oxygen. The SFR tray is mounted in the shaker and transmits the data wireless to the PC or laptop.



Fig. 3: SFR Shake Flask Reader – non-invasive, online monitoring of pH and DO in shake flask culture.

Experiments, biocompatibility and specifications

Three gamma irradiated glucose sensors from C-CIT AG were mounted on three 125ml shake flasks with integrated pO₂ and pH sensor spots. The flasks were filled with cell culture media (FM 111) and inoculated with CHO cells. The cell density at the beginning was $0.64 \cdot 10^5$ at 97 % viability while glucose concentration was 3.59 g/l measured with BioProfile and 4.05 measured with HPLC. For practical reasons the glucose sensors were calibrated according to BioProfile at 3.6 g/l. The calibration of the glucose sensors is a simple one point calibration which is made just after adding the cells by inserting the start concentration of glucose in the software interface of CITSens Bio. During the whole experiments samples were analyzed offline with BioProfile and HPLC for glucose and BioProfile for pH. During the first 4 days cells were incubated at 36°C and cell mass was produced. On day 4 cell culture media was exchanged and temperature was reduced to 30°C to shift to protein production for the next 6 days.

Results and discussion

At the beginning glucose was measured offline with HPLC and BioProfile. The start concentration measured by HPLC was 4.05 g/l the start concentration measured by BioProfile was 3.59 g/l which is a difference of 12.5% between the two methods. This clearly indicates some problems of the off-line methods such as sample preparation and others. The in-line glucose sensors were calibrated according to the values of the BioProfile and therefore show 3.6 g/l at the beginning of the process (see Fig. 4). All three sensor types (glucose, pH and DO₂) clearly indicate the end of the exponential growth phase on day 3 followed by the

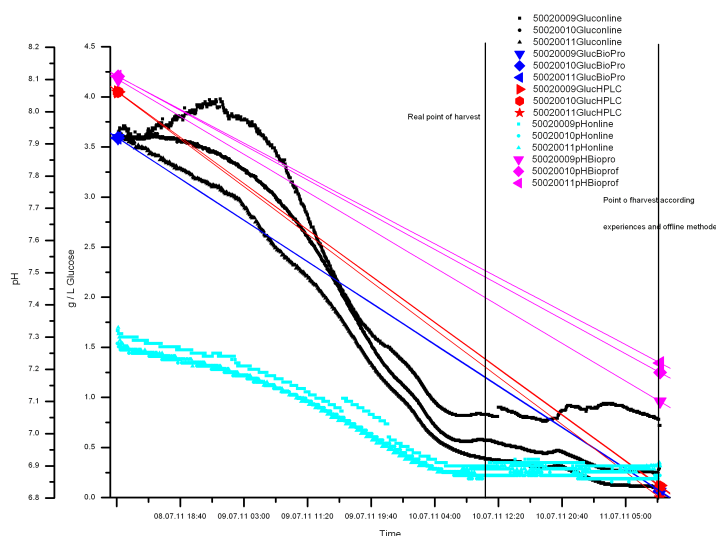


Fig. 4: Four days of cell cultivation of CHO-cells in shaker flasks. Shown are in-line pH measurement (cyan), in-line glucose (black) and off-line glucose (BioProfile and HPLC). Both in-line sensors indicate the end of the exponential phase on day three. On day four glucose sensors show same concentrations as off-line methods. The sensors were only calibrated at the beginning of the process.

stationary phase until day four (see Fig. 4 and Fig. 5). The three glucose sensors do show the same glucose consumption and on day four they show same values as off-line methods. The sensors were not calibrated during the process.

The pH was measured with the offline method BioProfile as well as with the pH sensor spots from PreSens. The pH measured with BioProfile was 8.1 and the pH measured with the pH sensor spots from PreSens was 7.2 – 7.3 (see Fig. 7). The pH difference results not from a wrong measuring method but from different samples which were measured. The incubator atmosphere is set to a CO₂ concentration of x % v/v. Therefore, CO₂ needs to equilibrate in the media and the final pH is only reached after having the cell culture media in the incubator for a couple of

minutes. Due to the fact that the sample measured with BioProfile was drawn from fresh media the pH was not adjusted to the incubator atmosphere. During cultivation off-line pH values will therefore always be higher than the pH measured with in-line sensors. Even at the end of the four days off-line pH was around 7.2 – 7.3 while pH measured with in line sensors was 6.7 – 6.8. But the pH measured with in-line sensors always reflects the real situation while off-line methods are not measured under real condition.

During the first four days all three sensor types (pH, pO₂ and glucose) showed the three typical grow phases (lag-phase, exponential phase and stationary phase) of the cell cultures (see Fig. 5). Due to former experiences and external sample analysis it was thought that the cell culture needs four days (Friday till Monday morning) to reach maximal cell density at a high level of living cells. As it can be seen in Fig. 5 it is not the real situation. The online sensors showed the optimal point of harvest on day 3 instead of day 4. This is also indicated by the rather small numbers of living cells which already decreased during the stationary phase (see Fig. 6). If the cells were harvested on day 3 the living cell number would be higher. By calculating the glucose consumption per

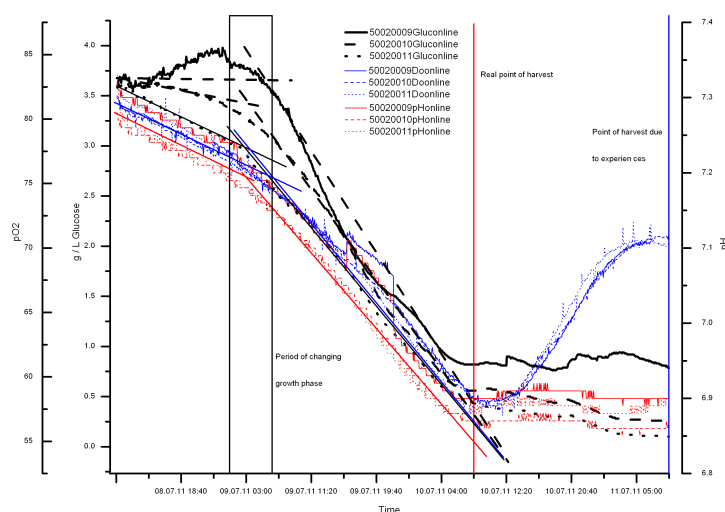


Fig. 5: During the cell cultivation the three growth phases are clearly indicated by all three sensor types. By calculating the hourly glucose consumption the growth phases can be shown in real time. The optimal point of harvest can be predicted for each flask and without manual work.

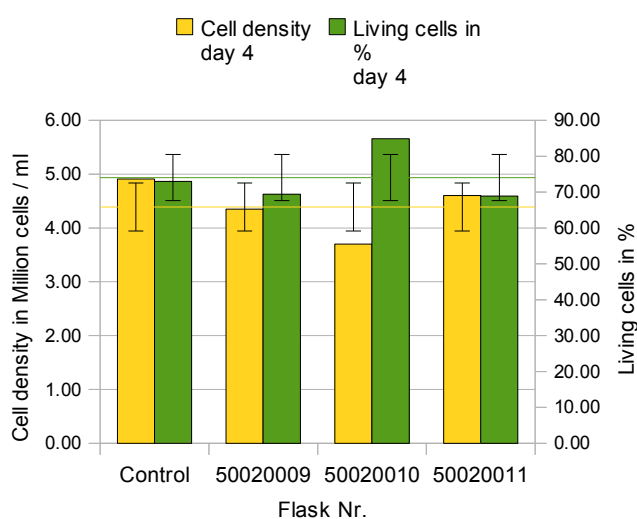


Fig. 6: Cell density and living cell numbers in % on day 4. Shown are also the mean value and the y-error for both the living cells and the cell density

hour the different growth phases are clearly visible and can be shown in real time. In the first 10 hours there is a clear lag-phase in which the slope of all three sensors is different to the slope followed for the next 36 – 42 hours during the exponential growth phase. In the stationary phase followed glucose and pH doesn't change anymore and O₂ is increasing due to less O₂ consumption at same rotation speed.

After the first four days the cell culture media was exchanged and the cells were used to produce protein. The temperature in the incubator was decreased to 30°C and the glucose sensors were calibrated again. In the next 6 days samples were drawn daily and were measured with BioProfile and HPLC. After feeding the cells on day four the glucose sensors were

recalibrated. On day 7 again fresh media was feed which is clearly indicated by the in-line glucose sensors. The sensors were not recalibrated. During the hole experiment the cell density and living cells were counted. During the hole experiment no difference in cell density and amount of living cells was observed compared to the cell cultures without sensors. Due to the fact that at the beginning only one sample for all flasks was used for cell counting and living cell analysis one can not see differences between the flasks after inoculation. One is assuming that the cell density and amounts of living cells are in each flask the same. This might be wrong and should be controlled in further experiments. So the small differences between the flasks may be due to differences at the beginning. Also there was only one control flask.

Summary

As stipulated by FDA in their guidance for industry to PAT (process analysis), the examples above demonstrate the effectiveness of continuous monitoring to control the quality of bioprocesses. In-line sensors offer several advantages such as:

- no sampling
- reduced risk of contamination's
- no reagents costs compared to batch analyzers

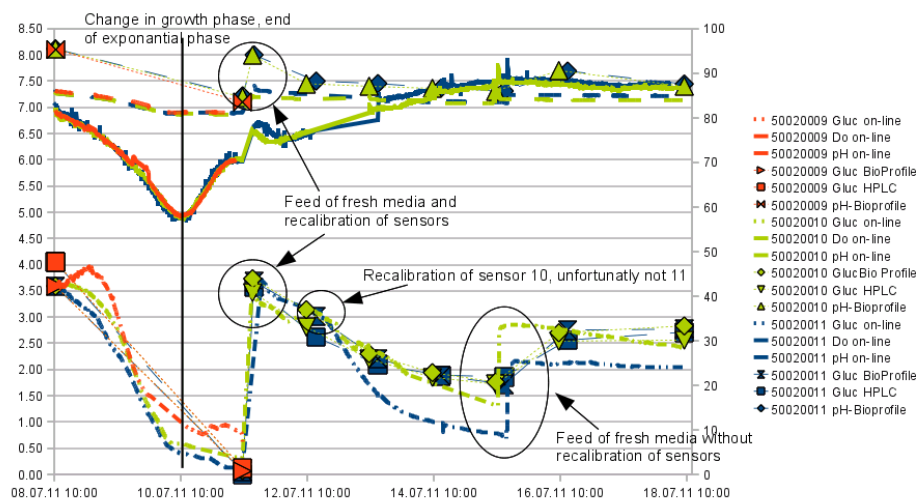


Fig. 7: Cell cultivation over 10 days. First four days cell growth next 6 days producing protein. During the hole process in-line sensors for glucose, pH and O₂ were used to control the three parameters. Glucose and pH were measured also by BioProfile and glucose by HPLC two. In the first four days only on day four a sample was taken. During the next 6 days every day a sample was taken. The in-line glucose sensors were calibrated at the beginning and on day 4 and 5.



Fig. 8: Group of bioreactors used with glucose sensor CITSens Bio

- chemical analysis is executed near the process, process control is centralized.
- immediate detection of any process change.
- For all different disposable bioreactors available

It was shown that without sampling a process can be controlled by in-line sensors. The combination of the three parameters pH, DO₂ and glucose allows full process control at low costs and no manual work. Working with in-line sensors may give you even a better understanding of your process and may give you new information saving extra money.

If you work with new in-line sensors you should always consider:

- Be careful by comparing in-line and off-line data's the conditions measured the values are not the same
- Be careful of what you compare
- Be open for new information about your bioprocess even you think you know your cells.
- during introduction of in-line sensors in your process work closely together with the inventors of the technology
- Be careful by interpreting the in-line data
- in-line sensors are tools to help you to understand and control your bioprocess they are not highly precise measuring tools such as HPLC or AAS.