

Development of a Partly Controllable System at Shake Flask Scale

Enhanced Data Quality Obtained From Fed-Batch Shake Flask Cultivation by Minimizing Disturbance Variables Monitored by the SFR

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The main objective of this study was to develop an easy to handle and partly controllable process cultivation system at shake flask scale. First, it was investigated how disturbances, like taking the flasks out of the incubator for feeding and sampling, affect culture conditions and results. An adapter was constructed that allowed automated feed in the shake flasks. Online monitoring of oxygen partial pressure (pO_2) and pH were performed with the SFR Shake Flask Reader by PreSens, so limitations could be detected and adjustments made in time. This "semi-controlled" system clearly reduced the workload and minimized the disturbance variables, thus more reliable and representative data could be obtained for further scale-up processes.

In bioprocess development small scale systems are used to pick appropriate cell lines, media, and feeds before applying more expensive, controlled cultivation systems at larger scale. Comparability is still an issue as process developments still have to rely on data generated in uncontrolled small scale cultivation systems.

A commonly used small scale format for mammalian suspension cell cultures are shake flasks. Incubators providing a suitable environment by carbon dioxide (CO_2) supply and humidity control are used to ensure sufficient oxygen transfer and homogenization of cell suspension by appropriate mixing. In order to match scale-up parameters power inputs are adjustable by adaptation of the shaking frequency and eccentricity of the shaking movement, or the working volume of the shake flasks. As no reliable system for automated feeding and sampling at shake flask scale is available so far, it is necessary to take the flasks out of the incubator. These disturbances might have an effect on metabolism and overall performance of the cultivated cells. Every disturbance might possibly decrease comparability to controlled systems, where those interferences are excluded.

This is why in this study the influence of different disturbances on the cultures as well as the culture environment in the incubator was tested. To avoid the necessity of removing the shake flasks from the incubator and thus minimize the amount of disturbance variables a prototype adapter was developed. It allows automated feed in the flasks by continuous pump or syringe based dosing systems. Moreover online monitoring of pH and pO_2 was conducted with the SFR Shake Flask Reader by PreSens. This system allows the non-invasive measurement of the two parameters in several shake flasks in parallel without taking them out of the incubator.



Fig. 1: SFR Shake Flask Reader

The Partly Controllable System at Shake Flask Scale

Cultivation of CHO antibody producing cell lines was performed in ISF1-X incubators (Kühner AG). The shake flasks used for pO_2 and pH monitoring were each equipped with a chemical optical oxygen and pH sensor spot at the inside bottom wall. These sensors were read out non-invasively with the SFR. The system is connected to a PC via Bluetooth, so online monitoring could be conducted without taking cultures out of the incubator or interrupting shaking movements. If necessary adjustments were made using incubator settings for CO_2 and shaking frequency. The newly developed autoclavable shake flask adapter has an internal screw thread for the shake flask itself and an external screw thread for the vented cap. A modification of the shake flask itself is not necessary. Addition and removal of liquids can be performed via three ports. In this experiment continuous substrate supply to the cell lines via the adapter was performed using pumps.

Shake Flask Adapter



Fig. 2: Adapter prototype w/o (left) and w/ shake flask (right).

Effect of Disturbances and Performance of the System

As shown in Fig. 3, recovery of the CO₂ content in the incubator air can take up to 10 minutes. Having many shake flasks and consecutively removing and putting them back for sampling or feeding might lead to persistently low CO₂ levels inside the incubator. pH in the shake flasks might be influenced by that. Temperature of cultures in 125 mL and 250 mL shake flasks dropped over 3 °C when removed from the incubator and kept at room temperature for 10 minutes. These are just two examples of how manual feeding and sampling outside the incubator are a cause of inconsistency as they are subject to many factors that are impossible to reproduce every single time. Another significant source of error is sampling itself, for volume and viable cells are removed from the culture. In contrary to larger scales, e. g. a 2 L bioreactor system, where the removal of 1 mL sample would only be 0.2 % of volume, the removal of the same amount in a shake flask with 50 mL working volume would be 2 % - this is a factor of 10 comparing the two values. If feed rates are not adjusted after every sampling to the new suspension volumes, performance may be altered (see Fig. 4). Figure 5 shows online data for pO₂ and pH collected with the SFR in the shake flask cultures. Limitations could be detected, because immediate drops in overall oxygen uptake lead to increasing pO₂ in cell suspension. Appropriate steps could be taken to avoid limitations. The SFR helped establishing a partly controlled system. The new adapter did not limit oxygen transfer into the shake flasks and automated feeding - causing fewer disturbances - was proven successful. Together with the SFR that allowed assessment of the cultivation and partly controlling parameters, the disturbance variables could be decreased. Necessary frontloading by preparing and connecting adapters to the shake flasks were overcompensated by automated feeding and decrease of sampling.

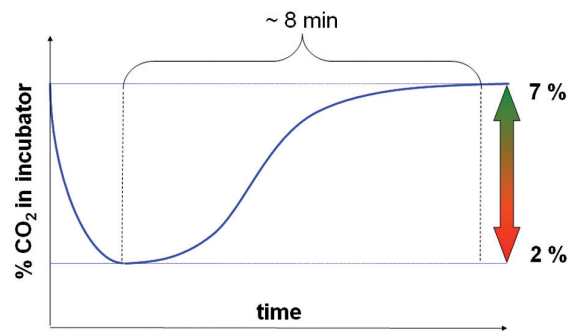


Fig. 3: Recovery back to 7 % CO₂ after the incubator door was opened for 10 seconds once (Incubator ISF1-X, Kühner AG, CO₂ measurement by GM70, Vaisala).

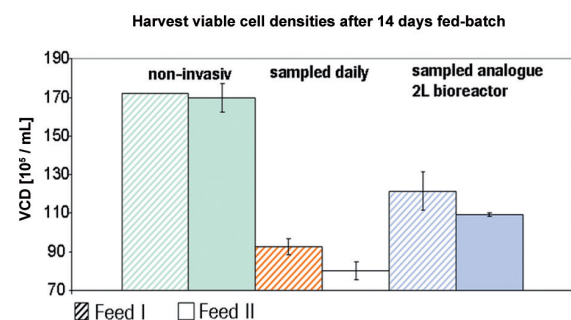


Fig. 4: Harvest viable cell densities after 14 days of shake flask fed-batch cultivation decrease with increasing overall sampling volumes if feed volumes are constant.

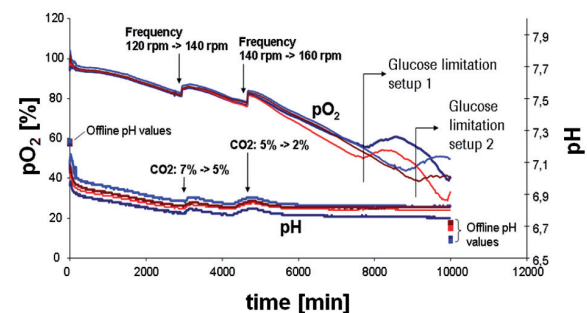


Fig. 5: pO₂ and pH measurement performed with the SFR. Monitoring efficiency of pO₂ and pH can be increased for disturbances like opening incubator doors are no longer necessary, moreover limitations can be detected.

Conclusion

Monitoring pO₂ and pH online with the SFR is a further step to enhance the efficiency of process development at shake flask scale. The adapter prototype enabled automated feed, thus minimizing disturbances, which can have significant influence on the cultures as shown in this study. Using both devices together it is possible to decrease overall workload per shake flask, enabling higher throughput, and ensuring more stable culture performance. Therefore more reliable and comparable data will be obtained for further scale-up processes.

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