

Fed-batch process development for monoclonal antibody production with cellferm-pro[®]

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1 Introduction:

Cost-effective large-scale production of monoclonal antibodies (mAb) is creating a strong demand for reliable and prompt development of highly productive, scalable processes. One of them is fed-batch, which is widely used for production of recombinant proteins, due to its operational simplicity, reliability, and flexibility in multipurpose implementation. The major advantage of fed-batch, comparing to batch, is the ability to increase maximum viable cell concentration, prolong culture lifetime, and allow product accumulation to a higher concentration.

The maximisation of final product concentration in a hybridoma fed-batch process is a function of the Integral of Viable Cells Concentration (*IVCC*). It follows, that an increase of this variable through feeding strategy optimisation will boost the final product titer.

Pre-defined feeding protocols, which are based on nutrient requirement estimations, e.g. stepwise or a sigmoid-based addition of nutrient concentrates are unlikely to meet the nutritional demands of cells growing in batch culture which vary with time and environmental conditions. It often leads to nutrient depletion or accumulation of substrates or metabolites to inhibitory levels (Zhou *et al.*, 1996). A much better way for nutrient supply would be the online monitoring of the cell culture's metabolic activity, and a real time control of nutrient feeding based on this parameter.

The novel feeding protocol discussed here is based on the oxygen uptake rate (*OUR*) on-line estimation. *OUR* is a very important indicator for the metabolic activity of the biological system. In cell cultures it correlates with the glucose or glutamine consumption rate. In our experiments *OUR* was used as the control parameter for adapting the feed rate according to the cell culture's requirements in real time. The experiments were performed using the novel cellferm-pro[®] system.

2 cellferm-pro[®] system

2.1 System's assembly:

The cellferm-pro[®] system (DASGIP AG, Juelich, Germany) consists of five modules as shown in Fig.1.

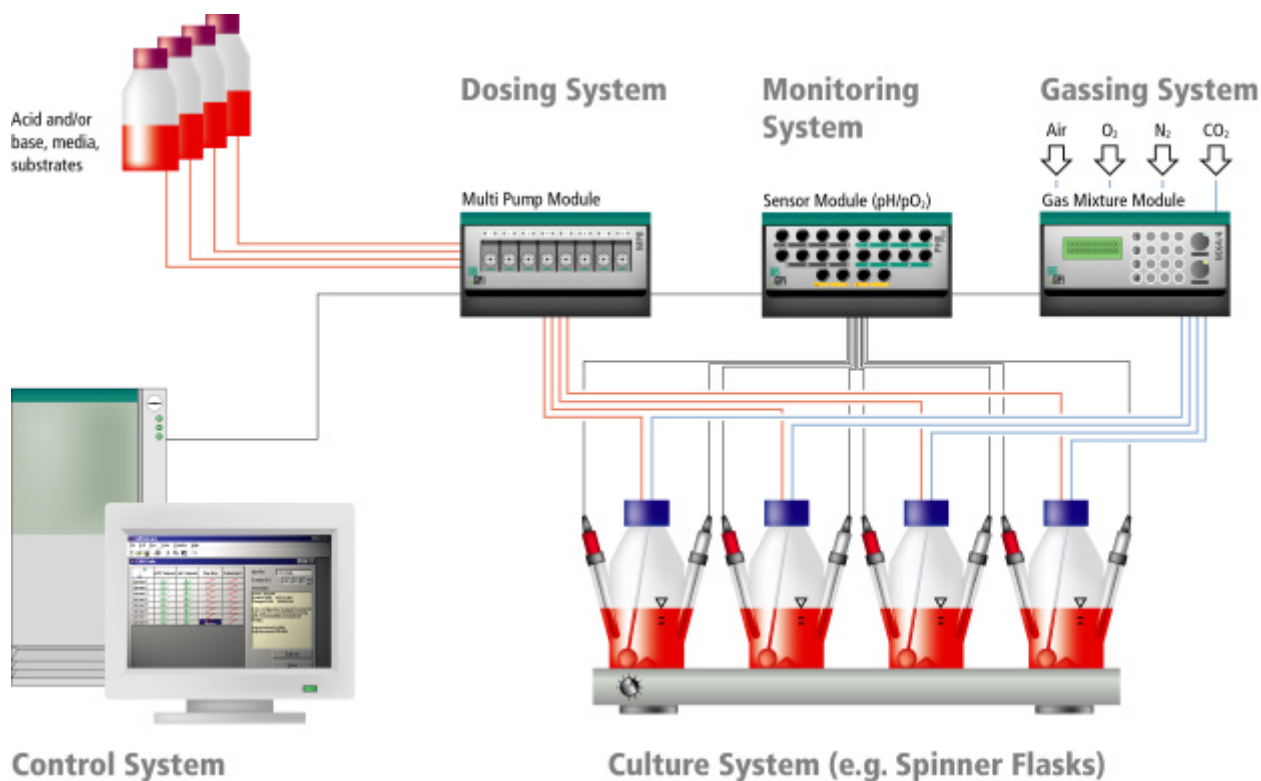


Figure 1: cellferm-pro[®], a parallel and fully controlled cultivation system (DASGIP AG, Juelich, Germany)

The culture system comprises of a temperature controlled incubator with 4 or 8 vessels, placed on a magnetic stirrer platform, equipped with pH and pO₂ electrodes, and feed and air supply/removal connections.

The gassing system provides an individual mix of up to four gases to each culture vessel (e.g. compressed air, oxygen, nitrogen and carbon dioxide). O₂ and CO₂ gas concentration are controlled by feedback from pO₂ and pH electrodes. There is an electronic mass flow control and a gas totalizer function for each vessel.

The monitoring system simultaneously processes the signals from pH and pO₂ electrodes, regulates pH and pO₂ in the medium through adjustment of CO₂ and O₂ in the gas mixture to maintain the set-points, and determines the *OUR* online.

The dosing system offers an individual and regulated delivery of a feed medium. The dosing proceeds according to user defined profiles or fully automated based on the online determined *OUR* (Metabolic Activity based feeding).

The control system is based on Microsoft Windows. Here pH, pO₂, gassing and dosing with user defined profiles are configured and the calibration of the electrodes and dosing system performed. The analyses of logged data are done using DASGIP's ChartWizard for MS Excel[®].

2.2 Metabolic Activity Tool:

A sophisticated algorithm computes the *OUR* online for each culture vessel of the cellferm-pro[®] system. Online *OUR*, supplemented with known concentration of one important feed medium component and pre-estimated ratio between substrate supply rate and oxygen uptake rate, allows an individual and automated addition of liquid media according to the actual metabolic activity of the cells.

3 Experimental

Fed-batch processes were performed with the cellferm-pro[®] system using the Metabolic Activity tool, with an *a priori* determined ratio between the substrate supply rate and the oxygen uptake rate (Y_S/Y_{O_2}). The criteria for the choice of an optimum Y_S/Y_{O_2} ratio were: optimum cell growth (maximum *IVCC*), maximum final product titer and minimum lactate produced per glucose consumed, since lactate is supposed to be one of the main inhibitors in hybridoma cell cultures.

As a reference for the Metabolic Activity based fed-batch processes served a standard batch process, also performed in cellferm-pro[®].

3.1 Culture conditions:

A recombinant mAb secreting GS-NS0 cell line has been used in the present work as a model system for suspension cell culture. All cultures were carried out in a proprietary serum-free, glutamine-free medium based on Iscove's Modified Dulbecco's Medium (IMDM, Amimed). For the automated Metabolic Activity based feeding a 10-fold concentrated basal medium was used. Additionally, 20-fold concentrated Iscove's amino acids solution (IMDM/AA, Amimed) was dosed manually once a day during four subsequent days after the cell density reached 1×10^6 cells/mL. In the first cultivation days NH_4HCO_3 was dosed to the culture to maintain NH_4^+ concentrations at about 0,5 mM.

The culture vessels used in the cellferm-pro[®] system were 1 L Spinner flasks, equipped with glass ball agitator, pH and pO₂ sensors and sampling ports, with a minimum start volume of 300 mL and a maximum working volume of 600 mL.

3.2 Analytic:

The concentration of viable cells and viability were automatically determined using the Cedex[®] system (Innovatis GmbH, Bielefeld, Germany). The *IVCC* was calculated as described by Sauer *et al.* (2000).

The concentration of the medium's main components such as Glucose, Glutamine, Glutamate and Lactate, four ions (Na^+ , K^+ , Ca^{++} , NH_4^+), together with pH, pO_2 , pCO_2 and osmolarity were determined using a Nova Bioprofile™ 200 Analyzer (Nova Biomedical Corp., Waltham, MA, USA)

4 Results and discussion

4.1 Choice of an appropriate substrate supply/oxygen uptake ratio

Glucose, one of the most important energy sources for mammalian cell cultures, was chosen as the medium component on which the ratio of substrate supply rate to oxygen uptake rate (Y_S/Y_{O_2}) was based. The theoretical ratio between substrate and oxygen uptake rates is 0.17 mol/mol, as 1 mole glucose requires 6 mole oxygen for complete oxidation. Since several essential substrates, except glucose, could also be limiting, four different Y_S/Y_{O_2} ratios were tested around the theoretical one in the range 0.1-0.5 mol/mol. The results are presented in Fig.2.

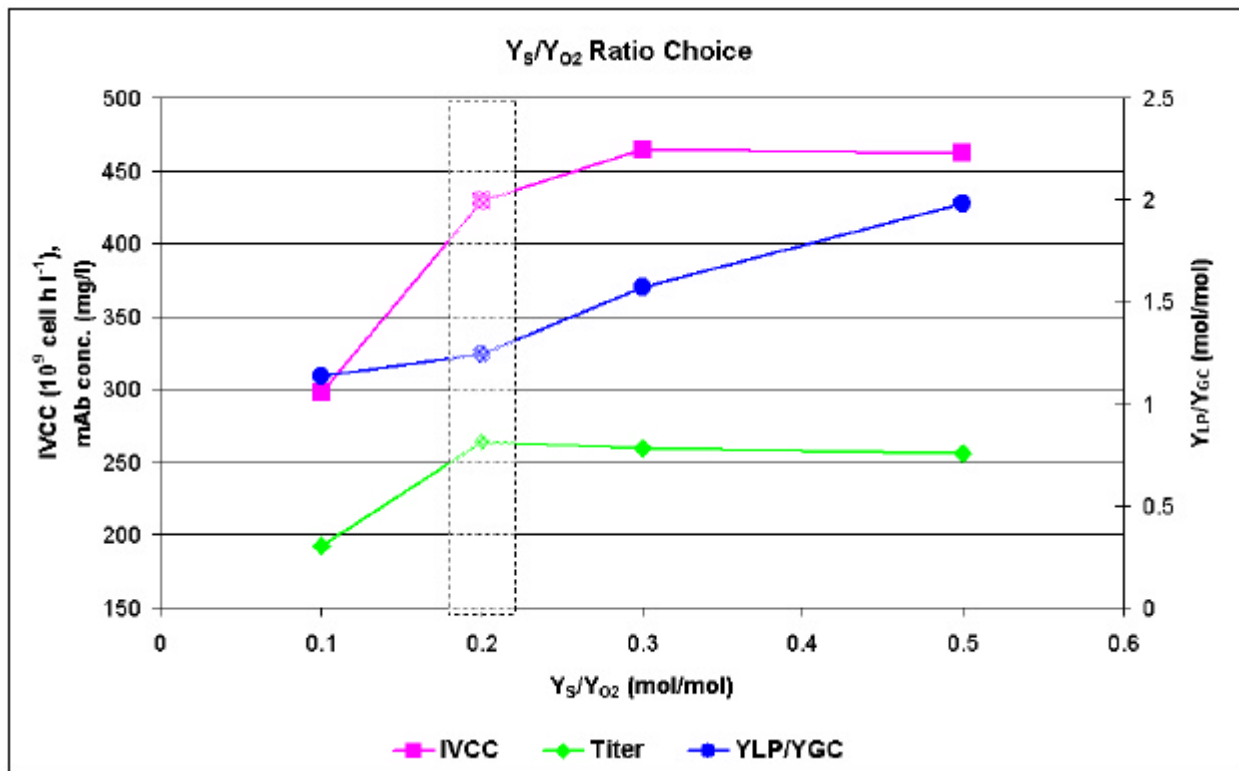


Figure 2: Choice of the ratio between substrate supply rate and OUR (Y_S/Y_{O_2}), based on the three criteria: $IVCC$, final product titer and minimal ratio of lactate produced per glucose consumed (Y_{LP}/Y_{GC}).

For the Y_S/Y_{O_2} of 0.1 mol/mol the smallest $IVCC$ was observed as well as a significantly lower titer. In this case cell growth ceased before the maximal working volume could be reached, assuming cell starvation (data not shown). The highest product titer of 260 mg/l was observed at $Y_S/Y_{O_2} = 0.2$. The Y_{LP}/Y_{GC} ratio increased with increasing Y_S/Y_{O_2} . The most excessive

lactate accumulation was observed for the ratio $Y_S/Y_{O_2} = 0.5$ mol/mol, representing in this case a theoretical maximum. Based on these observations, the Y_S/Y_{O_2} ratio of 0.2 mol/mol was chosen for the *OUR*-based nutrient feeding. This ratio is close to the theoretical one of 0.17 mol/mol, indicating the balanced concentration of nutrients in the feed medium.

4.2 Metabolic Activity based fed-batch

Fig.3 shows a Metabolic Activity based feeding profile with the corresponding cell density and mAb titer over the process time. No lag phase in cell growth was observed. Exponential cell growth was observed for the first four days followed by a quasi-stationary phase of about 5 days. After the maximal working volume was reached on day 9 Metabolic Activity based feeding stopped and was replaced by stepwise feeding. In this phase the system continued evaluating the volume to be dosed, however, was only able to feed when a sample volume was taken and registered in the system as a negative value which could be replaced. In this manner, the cells obtained about 6 mL of feed once a day. Under these conditions the cells were kept alive at a cell density above $1,0 \times 10^6$ cells/mL for a period of 12 days. After day 15 the cells died and mAb accumulation stopped.

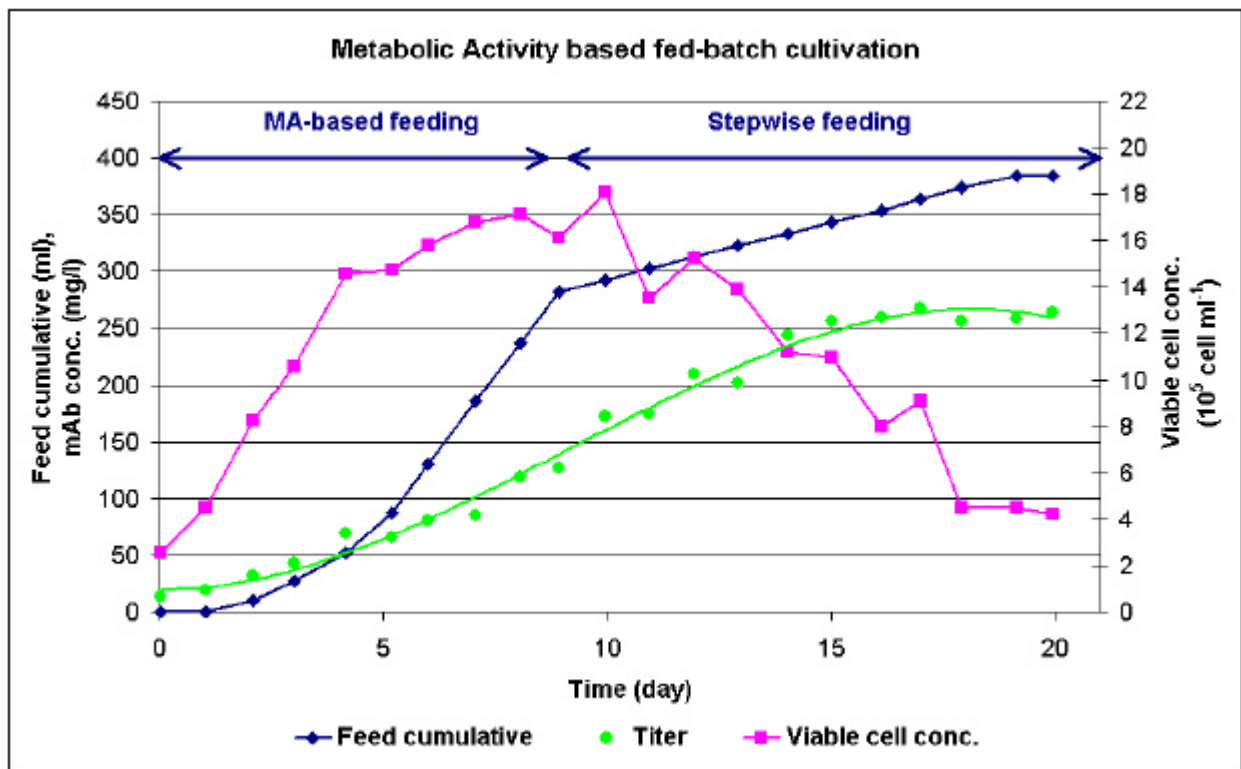


Figure 3: Metabolic Activity based fed-batch cultivation: feeding profile, cell density and mAb concentration.

4.3 Comparison to standard batch culture

In a reference batch experiment, also performed with cellferm-pro[®] (data not shown) the process duration was only 7 days, during which no quasi-stationary phase was observed. A comparison of the standard batch process to the Metabolic Activity based fed-batch process is presented in Fig.4. As a result of improved and balanced nutrient supply the process duration could not only be increased by 196 %, more importantly the *IVCC* was increased by 285%, and the final product titer was increased by 209%. As the culture volume after feeding was doubled (600 mL) against the standard batch volume (300 mL) the total amount of mAb obtained was increased by 519%.

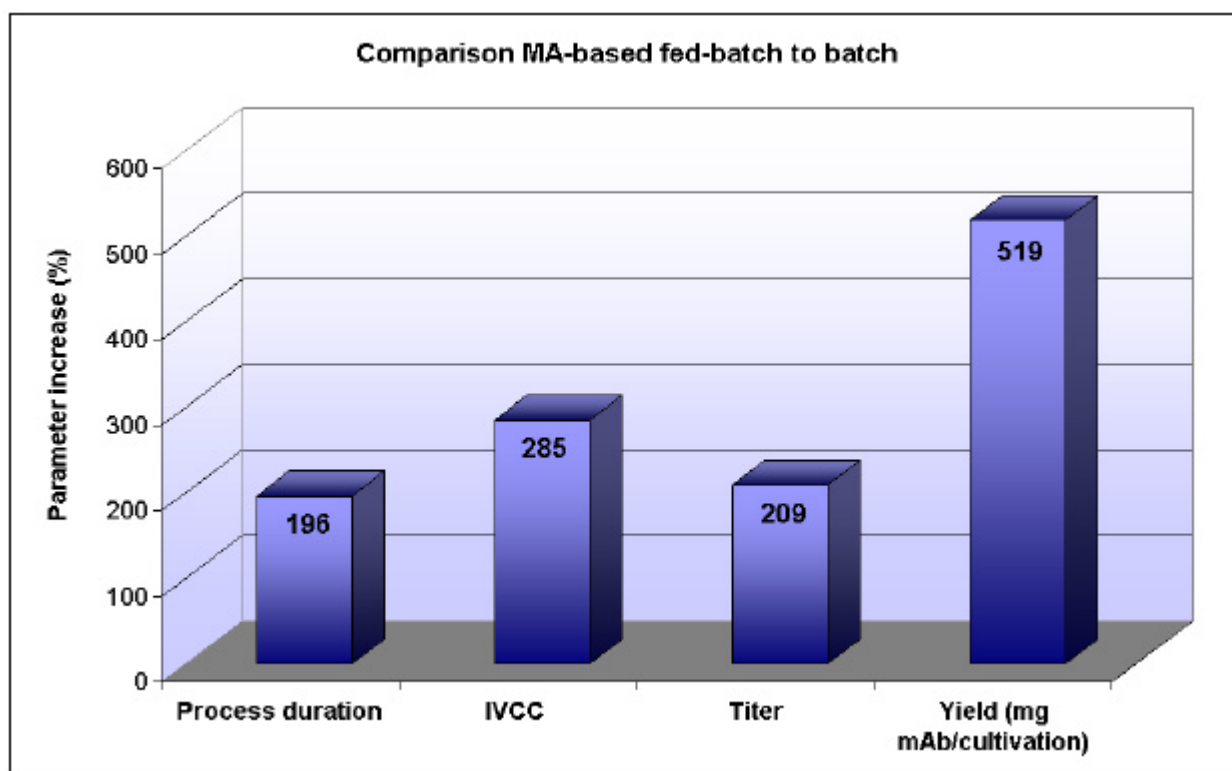


Figure 4: Comparison of process duration, *IVCC*, titer and yield of Metabolic Activity based fed-batch with batch process.

5 Conclusions

The ideal method to adapt nutrient feeding in real time to the changing requirements of a cell culture would be online determination of a key substrate, e.g. glucose, and to use this parameter as input variable for feeding control. Online glucose determination in a sterile environment, however, is still not feasible for industrial applications. Lately a new instrument, the cellferm-pro[®] system, and the related Metabolic Activity software tool was introduced which implemented the *OUR* online determination as described by Ruffieux et al. (1998) and Ducommun et al. (2000) as a measure for glucose consumption. Using this instrument *OUR* can be used as the input variable for substrate feeding control. We have demonstrated that with the cellferm-pro[®]

system and the Metabolic Activity tool a balanced and controlled nutrient supply to the cell culture is possible and that significantly improved mAb titer and productivity can be achieved. The cellferm-pro[®] system allowed a high experimental throughput, due to the possibility of running up to 8 experiments in parallel under the same environmental conditions. Additionally, we could use the data acquired from the cellferm-pro[®] process for easy scale-up of the fed-batch process to larger cell culture systems such as a 20-L Wave[™] bioreactor. The cellferm-pro[®] system is thus a powerful tool for process development, optimization and validation.

6 References:

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