Evaluation of a Scalable Disposable Bioreactor System for Manufacturing of Mammalian Cell Based Biopharmaceuticals

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Abstract: The use of disposable equipment in biopharmaceutical up- and downstream processes has increased in the last few years. This results in higher process flexibility and safety at reduced CIP and SIP efforts. The lack of robust scaleable disposable bioreactors initiated our own engagement in this area.

A new generation of membrane-based bioreactor was designed. This family of reactors starts with a small scale device, where eight bioreactors operate simultaneously for focused multiple process parameter optimisation. The up scaling concept includes a pilot scale bioreactor for the production of clinical trial materials in flexible pilot manufacturing plants; and a commercial scale unit for market supply. To demonstrate early feasibility at moderate costs, a lab scale prototype was specially designed for this purpose.

This new generation of bioreactors works with an efficient oxygen supply to support high cell densities in a continuous perfusion process. The cell retention is integrated in the bioreactor, thereby ensuring continuous cell-free harvesting over several months.

The development of a mathematical model describing the background of efficient nutrient supply is in progress, as well as a financial model for the operation of these new disposable bioreactors in manufacturing plants. Results of the biocompatibility tests of the disposable system components, as well as long term performance studies on the membrane behaviour are summarized. Test runs were carried out by the cultivation of a CHO suspension cell line expressing a recombinant protein and the feasibility data will be discussed.

Key words: disposable, scalable, bioreactor, membrane based, perfusion, biopharmaceuticals

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1. INTRODUCTION

The continuing specification of indications as well as an individualization of medicine request more flexibility in manufacturing of biopharmaceuticals. Further more the increasing efficiency of biopharmaceuticals causes smaller batch amounts. To meet these requirements with future manufacturing of biopharmaceuticals, a new kind of bioreactor is necessary which allows a high flexibility in a multi product facility with moderate COG's. The solution would be a robust, mobile, disposable and scalable bioreactor applicable to multi product facilities.

2. WORKING PRINCIPLE OF THE BIOREACTOR

The bioreactor consists of two parts, one disposable unit including all cell touching parts and one stationary unit with pumps, measurement technique, control devices and so on. The heart of the disposable part is a disposable module which contains a number of identical membrane surrounded micro compartments. The sum of all inner volumes of the micro compartments forms the cell culture volume. The cylindrical module (vessel) itself is half filled with media and will continuously be flown through by media and gas.

Caused by the rotation of the module every cell containing micro compartment is periodically exposed either to media or to gas. So nutrient feed as well as oxygen supply is ensured.

The membrane of the micro compartments is permeable for nutrients and oxygen but also for the produced protein. By this way the protein can be harvested continuously and cell-free. A ultra filtration unit will be installed in the outflow line, to concentrate the protein solution directly.

3. MODELING OF CELL YIELD DEPENDENT ON MEMBRANE DESIGN

By equation of the Fick's law for the oxygen flow through the membrane and the oxygen uptake of the cells the relation between cell concentration in the culture volume and membrane thickness can be calculated.

$$n = D_{50} \cdot A \cdot \frac{\Delta c}{\Delta x} \qquad n = q_{O2} \cdot X \cdot V_{CV} \qquad \Delta x = \frac{4 \cdot D_{50} \cdot \Delta c}{q_{O2} \cdot X \cdot d_i}$$

n-mass transfer, D_{50}-diffusion coefficient, A-inner membrane surface, Δc-oxygen concentration gradient over the membrane, Δx-thickness of the membrane wall, q_{O2}-specific oxygen uptake rate, X-cell concentration, V_{CV}-culture volume, d_i-inner diameter of the cylindrical compartments

Assumptions:
- oxygen transport through the membrane exclusively by diffusion
- diffusion coefficient D_{50} over the membrane is 0,05cm^2/h (50% of pure water)
- specific oxygen uptake rate q_{O2}: low 1,6*10^{-9}mg/c*h; high 6,4*10^{-9}mg/c*h

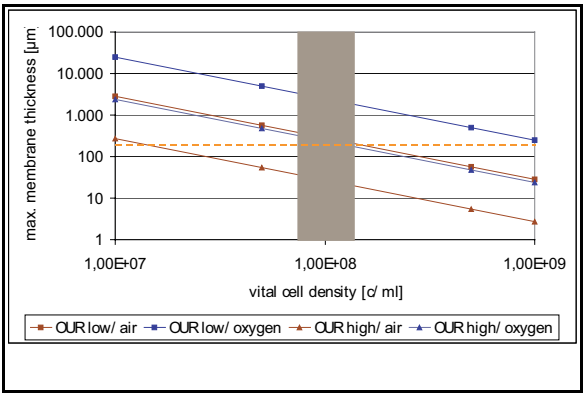

Figure 1. Allowed membrane thickness dependent on the cell concentration for unlimited oxygen supply.

The gray area in Fig. 1 marks the management target for vital cell concentration. The dotted line marks the thickness of the used membrane. With the 200µm membrane the target could be reached with cells of a low oxygen uptake rate. A gasing with pure oxygen would increase the vital cell concentration tenfold.

4. METHODS AND RESULTS

For feasibility testing two kinds of prototypes were designed. The Test Scale with ~4 ml Culture Volume and 100 ml vessel volume and the lab scale with 110 ml culture volume and 15 L vessel volume. The vessel volume of 15 L could supply the tenfold culture volume but the amount of cells should stay limited during the test phase.

For the tests a CHO line producing a recombinant protein was used. The daily protein concentration in outflow was measured by a specific ELISA, the productivity was calculated based on the amount of purified protein.

The test scale was supplied with regular media exchange and gasing, there was no continuous perfusion. In the test scale the cultivation of different cell lines was tested. Therefore the always used CHO line as well as a second CHO clone and a Hybridom line, all producing the same recombinant protein were cultivated. The experiment lasted 5 days. The processes were not optimized in any way. The goal was only to hold the cells vital. This was reached in all cases. The Hybridom culture even increased its vitality (Fig. 2).

The lab scale was continuously flown through by media and gas and run 10 to 23 days. In experiments with single micro compartments the good permeability of the membrane for the recombinant protein was proved. The productivity of the lab scale outlined in Fig. 3 was calculated by the amount of purified protein out of the continuous harvest. The horizontal bar marks the management target for feasibility proof. This goal was surpassed in two processes.

Figure 4 shows that the protein was produced constantly. In spite of the continuous media perfusion the outflow protein concentration stayed constant.

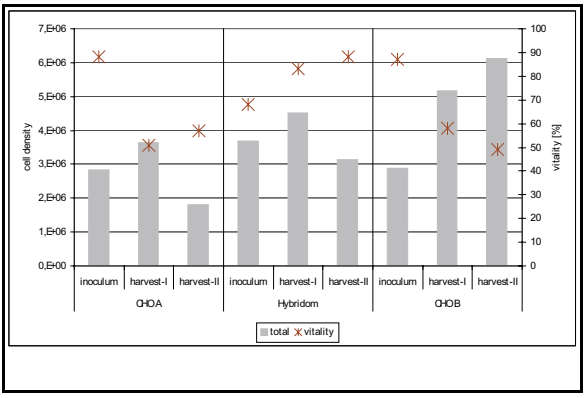

Figure 2. total cell density and vitality of different cell lines cultivated over five days in test scale.

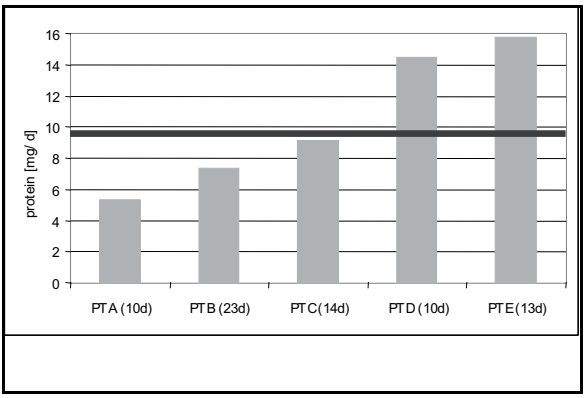

Figure 3. Produced protein per day in different lab scale runs.

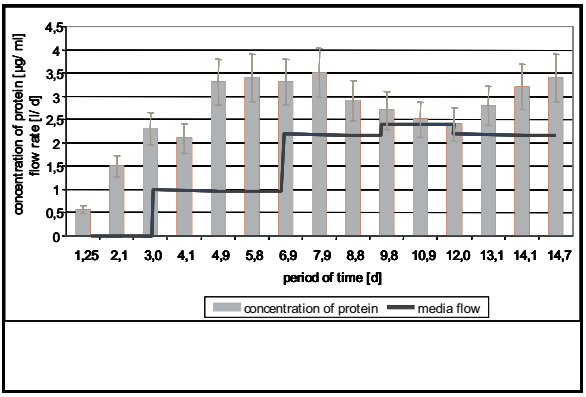

Figure 4. protein concentration in the outflow media of one lab scale run.

5. ISSUES OF SCALABILITY AND FACILITY CONCEPT

An important request on future protein manufacturing systems is the scalability from units for process optimization to reactors for market supply. Fig. 5 shows the design of the bioreactor family. With the Process Development Device it is possible to run eight processes in parallel with different parameters. The pilot scale can be used for manufacturing of material for toxicity studies and pre clinical phase to phase II. The commercial scale will be used for market supply and will work with the same stationary hardware unit as the pilot scale. This increases the flexibility of a facility very much.

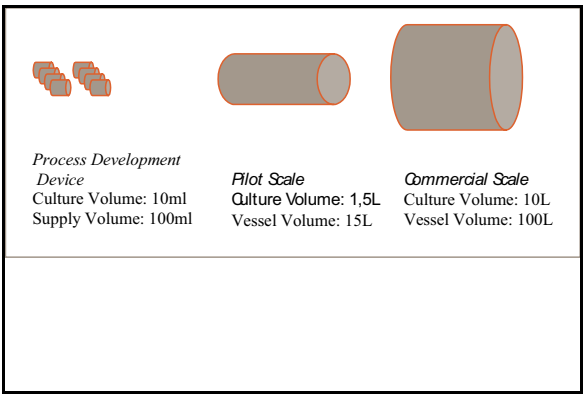

Figure 5. Design of the reactor family.

All the modules including all tubes are disposable devices. The pH and oxygen control will be based on an optical measurement technique. At this the media touching sensor parts are also disposable and included into the pre-sterilized module.

A manufacturing facility based on the presented bioreactor technique is divided in Single Manufacturing Units (SMU's). Every SMU forms an independent, closed manufacturing area and consists of four dedicated rooms. One each for pre culture, up stream, down stream and final filtration. The whole facility is based on disposable material, there is no steam installation but stacking ground for media and buffer bags. The construction of the facility is modular so it can consist of one, two or many SMU's.

6. SUMMARY

The developed bioreactor is a continuous system with integrated membrane based cell retention system. This causes short residence times for the produced protein in the system. Furthermore the bioreactor technique is scalable and disposable.

Pilot and commercial scale bioreactors support the construction of flexible multi product facilities with very low capital expenditures and moderate COG's.