Study of perfusion process of CHO cells with CellTank 2202 prototype, bench-top Single-Use-Bioreactors (SUB) with 150 cm^3 CellCore matrix

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Introduction

- Major advantages of perfusion are high cell numbers and high total production in a relatively small size bioreactor. Moreover, perfusion is optimal when the product of interest is unstable or if the cell line product yield is low. On the other hand, disadvantages are for example technical challenges originating from non-robust cell separation devices as well as sterility concerns from the more complex set-up needed.

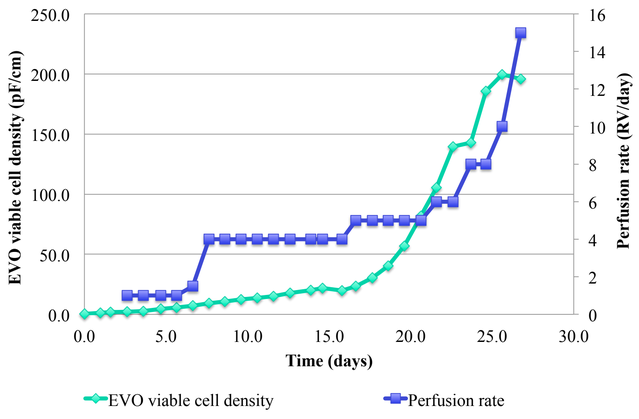
- Recently, CerCell® (Denmark) has developed a perfusion integrated Single-Use-Bioreactor named CellTank that operate on magnetic-stirrer-tables. A revolutionary matrix for cell densities beyond 100 million cells/ml is integrated in the CellTank and the cells stay harboured inside the matrix.
- The EVO 200 iBiomass System (FOGALE nanotech) uses the dielectric properties of living cells and is capable of measuring the live cell density independently of cell size variations.
- In the present work, CellTank 2202 prototype, bench-top Single-Use-Bioreactor (SUB) with 150cm^3 CellCore matrix(CerCell®) was investigated. Perfusion cultivations were performed using a recombinant CHO cell line producing a monoclonal antibody as a model system.

Conclusions

- Very high viable cell density of **200 x 10^6 viable cells/ml** achieved at perfusion rate 10RV/day (1RV=150ml).
- Very high viable cell density of 130 x 10^6 viable cells/ml maintained for 11 days at perfusion rate 8-10RV/day with temperature lowered gradually from 37°C to 29°C.
- The use of a single-use-bioreactor equipped with a real-time cell mass sensor offers a solution alleviating technical and sterility challenges occurring in perfusion processes. Operation using CellTank SUB much easier and handy compared with traditional perfusion technologies with robust integrated perfusion device.
  The CellTank very lightweight, easy to transfer into laminar flow cabinet if sterile operation is required.
- IgG accumulated nicely with time and increasing cell density with a cell specific productivity comparable or higher to batch culture. No retention of IgG in the polymer matrix.

Results – Cell density, viability and perfusion rate

1st Run

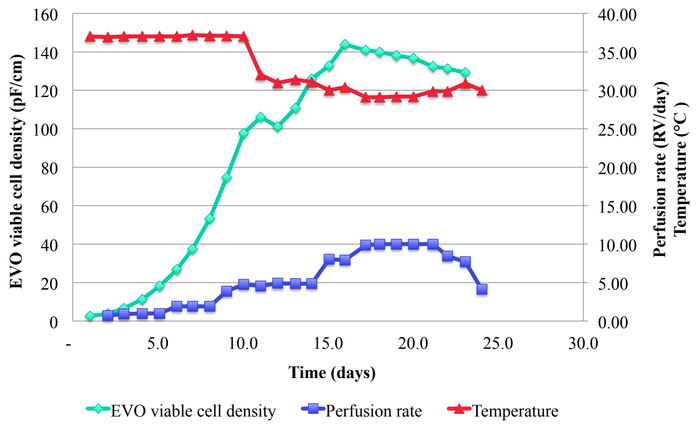


One pF/cm read on the EVO biomass sensor system is equivalent with 1x10^6 viable cell/ml

- First run (medium and setting adjustments days 0 to 14) followed by exponential growth

➔Cell density up to **200x10^6 viable cells/ml** after 25 days of cultivation at a perfusion rate 10RV/day

2nd Run



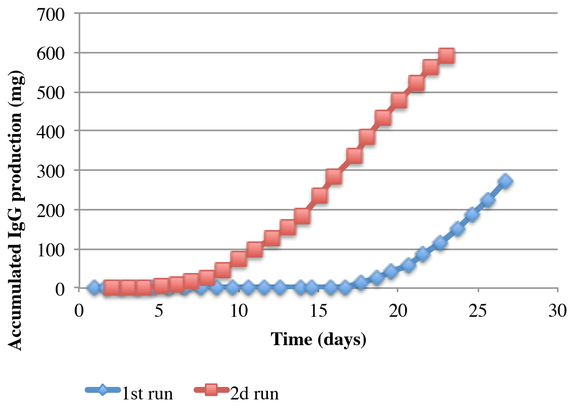
➔Cell density kept ≈ **130x10^6 viable cells/ml** at perfusion rate of 8/10 RV/day for over 10 days

➔Temperature lowered from 37ºC to 32ºC on day 10, to 31ºC on day 11 and to 30ºC on day 14, resulting in only partial growth arrest.

➔ Temperature decreased from 29ºC on day 16, resulting in complete cell growth arrest. The cell density was then maintained at a stable level for the following days.

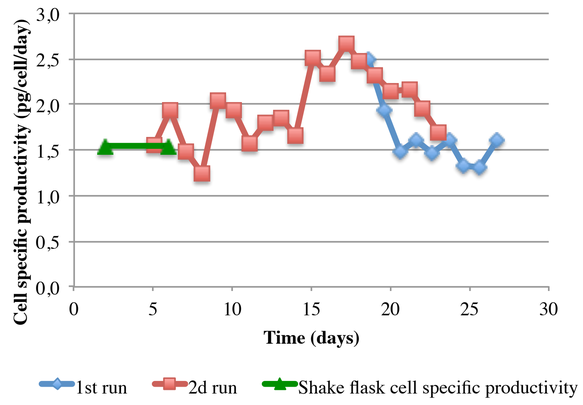
Results – Total mAb production

Acculumated IgG production



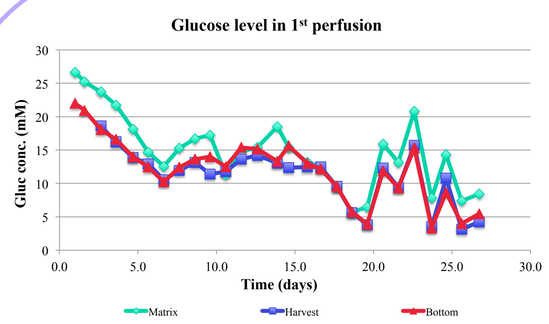
Product accumulated nicely with time and increasing cell density (first after day 14 for 1st run)

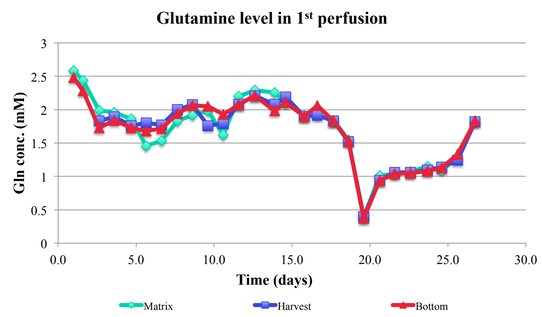
Cell specific productivity

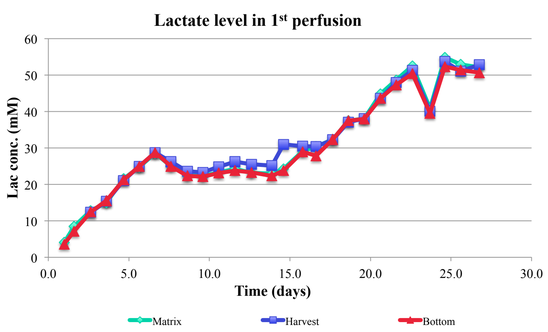


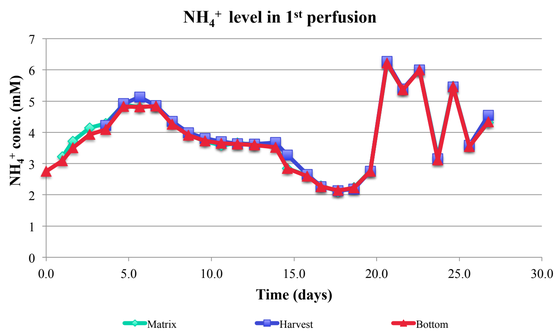
Cell specific productivity in perfusion mode comparable to shake flask productivity except at 30˚C where it was ≈ 40 % higher

Results – Comparison of different sampling locations









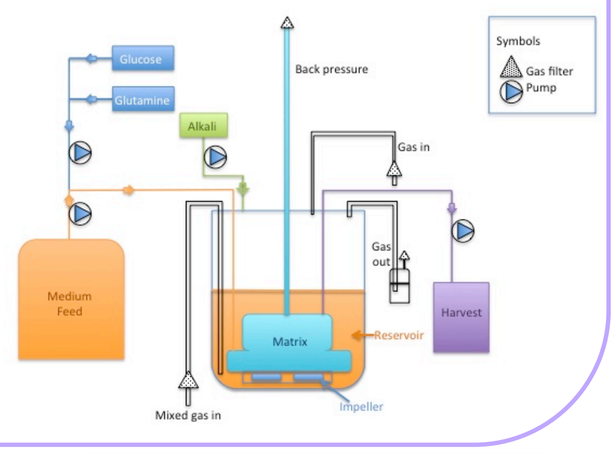
→ Samples from matrix sample port, harvest sample port and bottom (reservoir) sample port

➔ **There are no obvious difference among different sample locations**
➔ **Glucose and glutamine concentrations slightly higher in matrix samples**
➔ **Lactate and NH_4^+ concentrations slightly higher in harvest samples**
➔ **In agreement with the fact that the fresh medium goes into the CellCore directly**

Materials and Methods

CHO DP-12 clone 1934 (ATCC) producing monoclonal antibody was used for this study. A prototype CellTank 2202 single-use-bioreactor with 150 cm^3 CellCore matrix was equipped with EVO 200 (Fogale nanotech) sensor. The cultivation system was controlled by Belach phantom control system.
Watson-Marlow 120U pumps were used intermittently for fresh medium feeding, harvest and supplementary glucose or glutamine additions. The set-points of DO, pH and temperature were 40%, 7 and 37°C respectively. The agitation rate targeted to have 1.6L/min re-circulation flow. The actual stirring speed was adjusted daily to obtain the target re-circulation flow due to the increasing back pressure. During the 1st experiment, the air, O_2,CO_2 and N_2 were mixed before adding into the reactor through the open tube sparger. A general setup for the system is given here. N_2 was continuously added in to avoid too high DO in the culture. During the 2nd experiment, only O_2 was blown through the open tube sparger, while the addition of the other gases were performed to the headspace.

The cells were cultivated in animal-component free IS CHO CD XP medium (Irvine Scientific, USA) with hydrolysate, supplemented with 3% of IS-CHO Feed-CD XP (Irvine Scientific, USA) and 2mM glutamine. Supplementations of glucose or glutamine were performed according to the cell need. The pH was controlled by adding 0.5M Na_2CO_3 or pulsing CO_2 into the mixed gas inlet (Exp.1) or headspace (Exp.2). The viable cell density was measured by EVO 200 biomass sensor (Fogale nanotech) mounted with measuring electrodes inside the matrix. pH, pCO_2, concentrations of glucose, lactate, glutamine, glutamate, ammonia and osmolality were measured by Bioprofile FLEX (Nova Biomedical). The mAb concentrations were analyzed by protein A HPLC.



Acknowledgement

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