Study of perfusion process of CHO cells with CellTank 2202 prototype, bench-top Single-Use-Bioreactors (SUB) with 150 cm³ CellCore matrix

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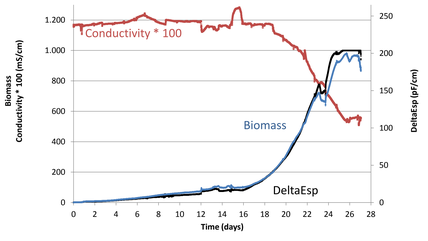
Introduction

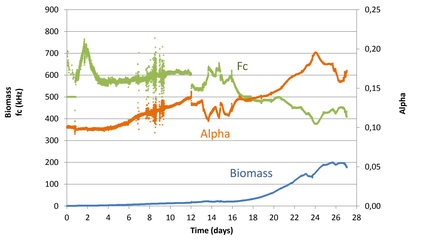
- Major advantages of perfusion are high cell numbers and high total production in a relatively small size bioreactor. Moreover, perfusion is optimal when the product of interest is unstable or if the cell line product yield is low. On the other hand, disadvantages are for example technical challenges 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 operated by magnetic stirring. The cells stay harboured inside a revolutionary matrix for cell densities beyond 100 million cells/ml is integrated in the CellTank.
- The EVO200 Biomass System (FOGALE nanotech) uses the dielectric properties of living cells and is capable to measure the live cell density independently of cell size variations.
- In the present work, CellTank 2202 prototype, bench-top Single-Use-Bioreactor (SUB) with 150cm³ 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 10⁶ viable cells/ml** achieved at perfusion rate 10 RV/day.
- Very high viable cell density of 130 x 10⁶ viable cells/ml maintained for 11 days at perfusion rate 8-10 RV/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.
- IgG accumulated nicely with time and increasing cell density with a cell specific productivity comparable or higher than batch culture. No retention of IgG in the polymer matrix.
- Similar concentrations of metabolites were measured in the bioreactor and harvest line indicating a very good fluid homogeneity in the CellTank (data not shown).

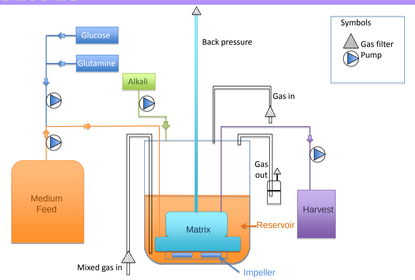
Results – Fogale iBiomass sensor EVO 200 signals for the 1st Run





➔**Fc**: Fc is an indicator of average cell size. Between day 16 and 21, Fc was stable; it was then decreasing indicating a larger cell diameter. After day 24, it decreases again, coinciding with a smaller diameter.
➔**Alpha**: Alpha is an indicator of the size homogeneity. During the run, alpha was increasing, indicating an increasing inhomogeneity of the cell sizes (or different radii if the cells were not spherical anymore).
➔**DeltaEps**: Due to the very high viable cell density (200 x 10⁶ viable cells/mL), the limit for DeltaEps signal measured by the Fogale EVO 200 system was reached – it seems to be 1000 pF/cm.
➔**Conductivity**: From day 19 the conductivity was dropping to almost half value at the end, which might be due to the fact that some ions have been consumed while not fully replaced by the perfusion medium.
➔There is a very nice correlation between biomass (2 frequencies) and DeltaEps (all frequencies)

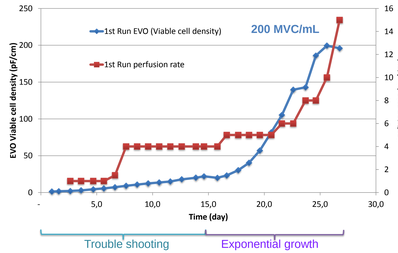
Materials and Methods



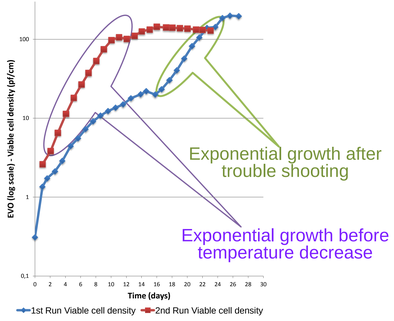
Parameters	1st Run	2nd Run
Cell line	mAb producing DHFR- CHO (DP12)	
Inoculation viable cell density	10 MVC/mL (= 1.7 10⁶ viable cells/mL)	
DO	45 %	40 %
pH	7.1	7.0
Temperature A → shifted to B)	37°C	A) 37°C → B) 32°C at 100 MVC/mL → C) further decreased to 29°C progressively
Recirculation flow rate	1.0 L/min	1.6 L/min
Cell density specific perfusion rate	≥ 0.05 nL/cell/day (for 1 Reactor Volume/day or 20 MVC/mL)	
Perfusion rate	≥ 1 RV/day (= Reactor volume/day)	
Bioreactor and separation device	CellTank with matrix	
Real culture working volume	150 mL	
Volume of reservoir	1280 mL	
Cultivation medium	animal-component free ISCHOCDXP medium with hydrolysate (Irvine Scientific) + 3.5 % of IS-CHO Feed-CDXP (Irvine Scientific)	
Alkali	0.5 M Na_2CO_3	
Analyses by NovaBioprofile FLEX	cell density, viability, cell diameter, pH, pCO2, osmolality, concentrations of glucose, glutamine, lactate and ammonia	
Analysis of mAb concentration	protein A HPLC	

Results – Cell density, viability and perfusion rate

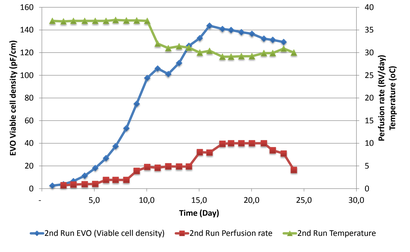
1st Run



➔ One pF/cm read on the EVO biomass sensor system is equivalent with 1x10⁶ viable cell/ml
➔ First run (medium and setting adjustments days 0 to 14) followed by exponential growth
➔ Cell density up to **200x10⁶ viable cells/ml** after 25 days of cultivation at a perfusion rate 10RV/day



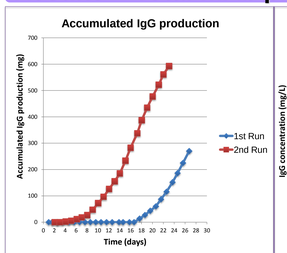
2nd Run



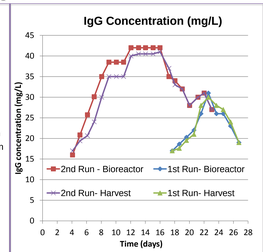
➔Cell density kept ≈ **130x10⁶ viable cells/ml** at perfusion rate of 8/10 RV/day for over 10 days
➔Temperature lowered from 37ºC to 32ºC on day 10, to 31ºC on day 11 and to 30ºC on day 14, resulting in only partial growth arrest.
➔ Temperature decreased to 29ºC on day 16, resulting in complete cell growth arrest.
Cell density maintained then at a stable level for the following days.

➔The accumulated viable cell number lost in harvest was analyzed by daily off-line samples in the harvest line.
➔The viability of the cultivations and the dead cell number generated daily in the harvest line were calculated based on LDH measurement (Lactate Dehydrogenase).
➔The viability were high (>95%) through out the whole cultivations for both runs.

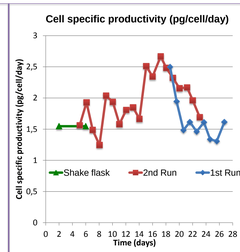
Results – Total mAb production



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

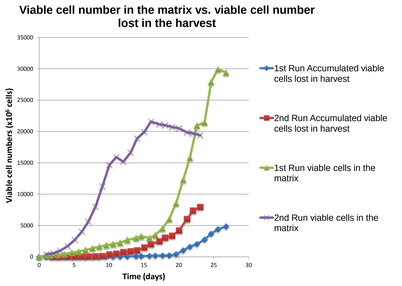


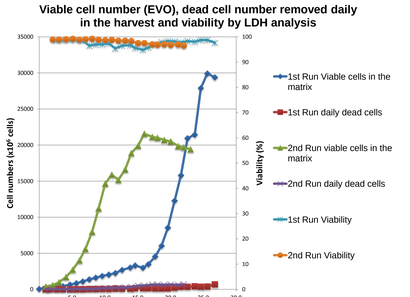
➔No retention of IgG in the polymer matrix.



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

Results – Off-line cell measurements





Acknowledgements

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