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Exometabolome characterization of high cell density culture perfusion and optimization of the cell specific perfusion rate

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SHORT VERSION (i.e. removed several OH's)
Exometabolome characterisation of high cell density culture perfusion and optimization of the cell specific perfusion rate

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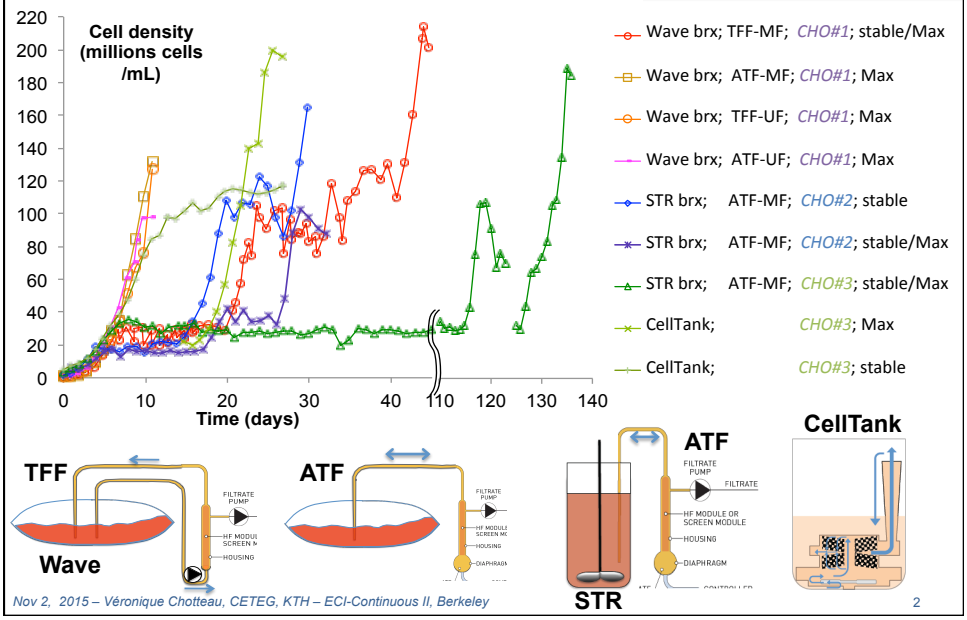
Very high cell density perfusion
Four systems studied at CETEG (Cell Technology Group, KTH)
Three different CHO cell lines, all producing MAb



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Outline

- Exometabolome in perfusion culture
- Optimization of perfusion rate
- Conclusions

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Exometabolome in perfusion culture

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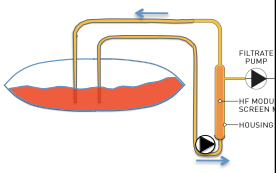
Study in WAVE Bioreactor™ in perfusion with TFF

CHO #1

Goal

- Study of perfusion in Wave Bioreactor™
- Study of the limits of the system

TFF



System

- Two types of cell separation based on hollow fiber filtration:
  - Tangential flow filtration – TFF
- IgG production production in CHO cell system, CHO#1
- Application of cryopreservation / cell banking
- Working volume 4L
- Base medium supplemented by feed concentrate (Irvine Scientific, USA)

Clincke et al 2013a, Clincke et al 2013b

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Perfusion in Wave™ Bioreactor using TFF at very high cell densities

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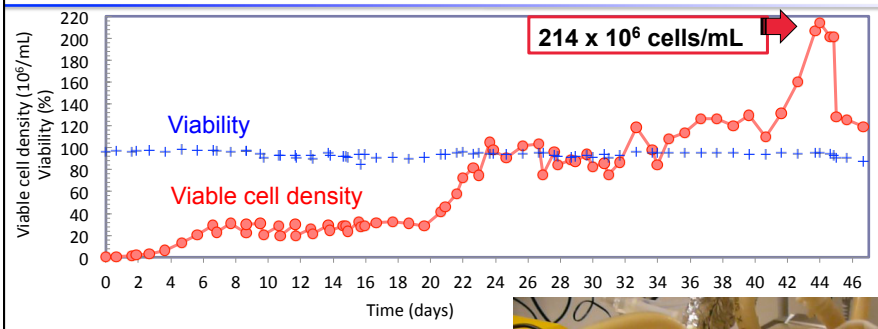
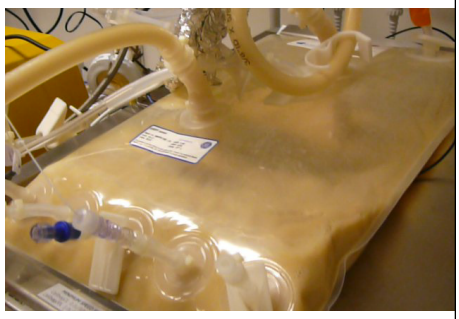


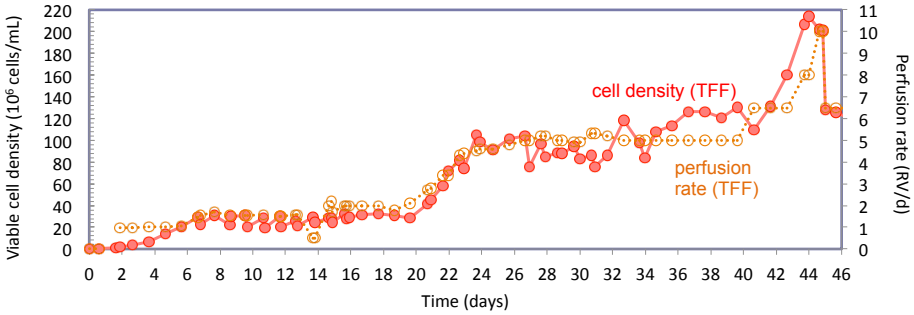
CHO cells at 200 x 10⁶ cells/mL



Clincke et al 2013a, Clincke et al 2013b

Perfusion in Wave™ Bioreactor using TFF at very high cell densities

CHO #1



Perfusion rate proportional to the cell density

Cell **S**pecific **P**erfusion **R**ate where

$$\textbf{CSPR} = \frac{\text{perfusion rate}}{\text{cell density}}$$

CSPR = 50-60 pL/cell/day

Exometabolome characterisation - Metabolomics in the culture supernatant in perfusion vs. fed-batch

Leila Zamani

- Purpose = Screening
- < 10 kDa fractions
- LC-MS: U-HPLC system (C18 column), coupled to an ESI-QTOF MS
- ≈ 10,000 peaks (intensity≥1,000) detected/sample

➔ Data analyses

- Principal Component Analysis (PCA)

→ Visualize the complex data set

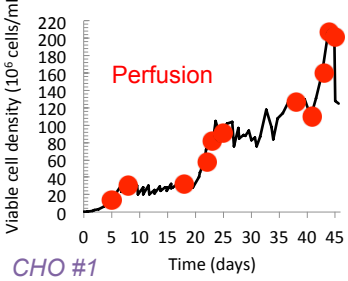
→ Identify similarities /differences between samples

- Partial Least Squares (PLS) regression

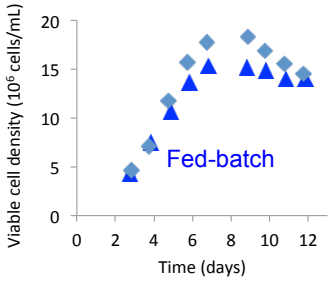
→ Identify relationships between the LC-MS data and culture parameters cell density and cell specific rates

Collaboration: Stockholm Univ., Karolinska Institutet

Sampling

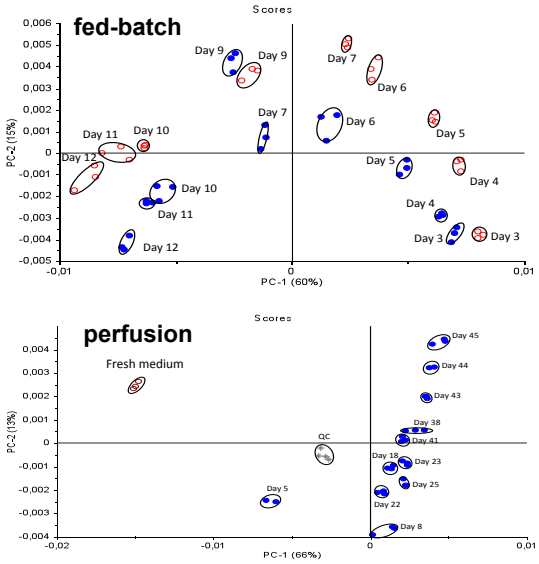


CHO #1



Foot printing - Score plot from data generated by Principal Component Analysis

CHO #1



PCA score plots based on positive and negative ion data for samples from individual days

Fed-batch

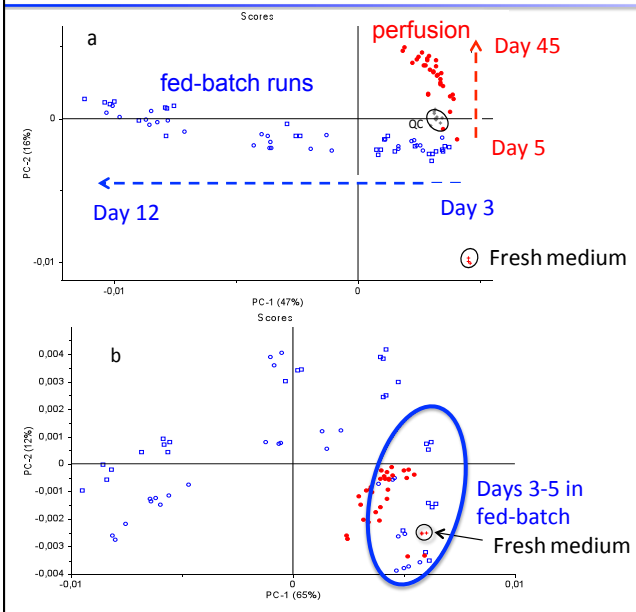
- Samples distributed according to time
- Longer distances between the samples

Perfusion

- Samples not distributed according to time
- Shorter distances between the samples

Score plot from data generated by PCA Modeling using fed-batch and perfusion data

CHO #1



Model #A

calibrated by data from fed-batch and perfusion runs

Model #B

calibrated by data from fed-batch runs **only**

→ Perfusion and fresh medium predicted by the model

→ clustering with days 3 to 5 of fed-batch

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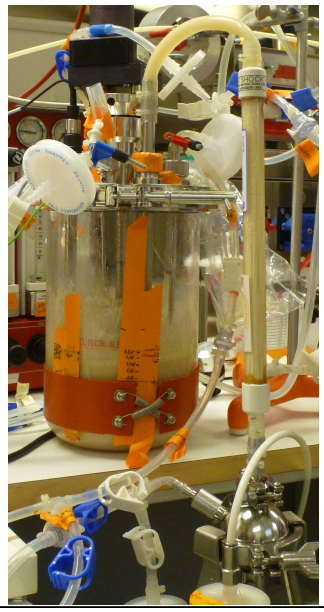
CHO #2 CHO#3

Perfusion using ATF in stirred tank bioreactor

Optimization of the perfusion rate



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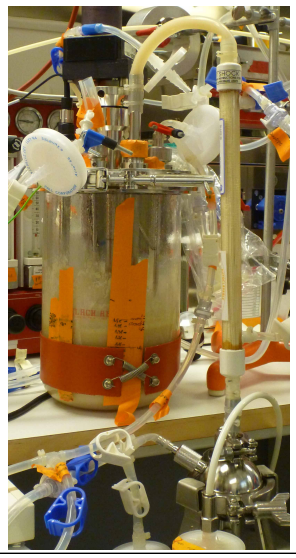
Perfusion in stirred tank bioreactor with ATF

Ye Zhang Caijuan Zhan

Goal

- Better knowledge of the perfusion process → **optimization**



System

- IgG production in CHO cell system, CHO #2 (IgG#2), CHO#3 (IgG#3)
- Glass bioreactor - working volume 1L
- ATF2 (Refine)
- Control of perfusion by CytoSys (Iprabio) – 1 run
- Base medium supplemented by feed concentrate (Irvine Scientific, USA)

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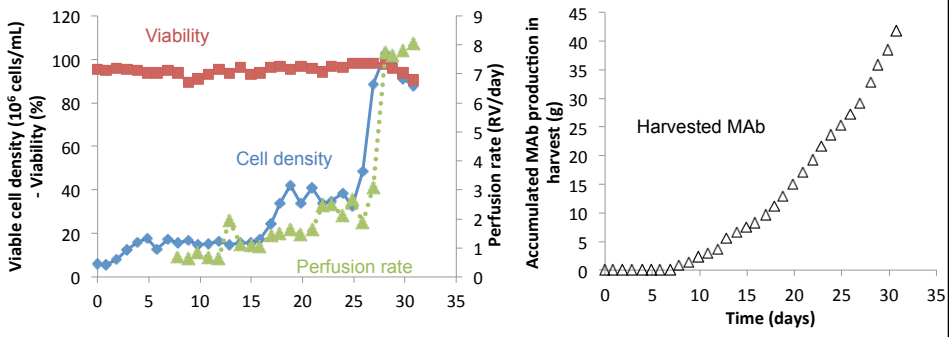
Perfusion in stirred tank bioreactor with ATF – Run #1

CHO #2



- High cell density with high viability
- Study of effect of CSPR

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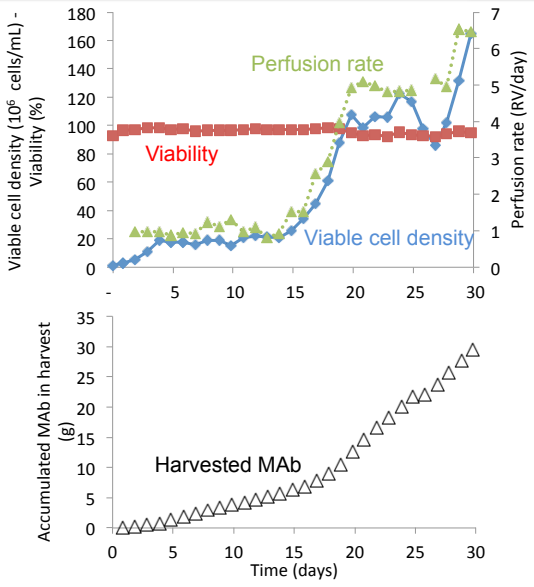
Perfusion in stirred tank bioreactor – Run #2

CHO #2



- Achievement of very high cell density with very high viability (> 92 %)

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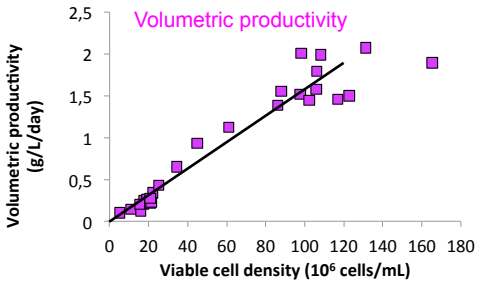
Perfusion in stirred tank bioreactor – Run #2

CHO #2



- Volumetric productivity linear with cell density
- Volumetric productivity of 2 g/L/day at ≈ 110 x 10^6 cells/mL vs. ≈ 2g/L obtained in a fed-batch for this cell line
- By-product levels not high

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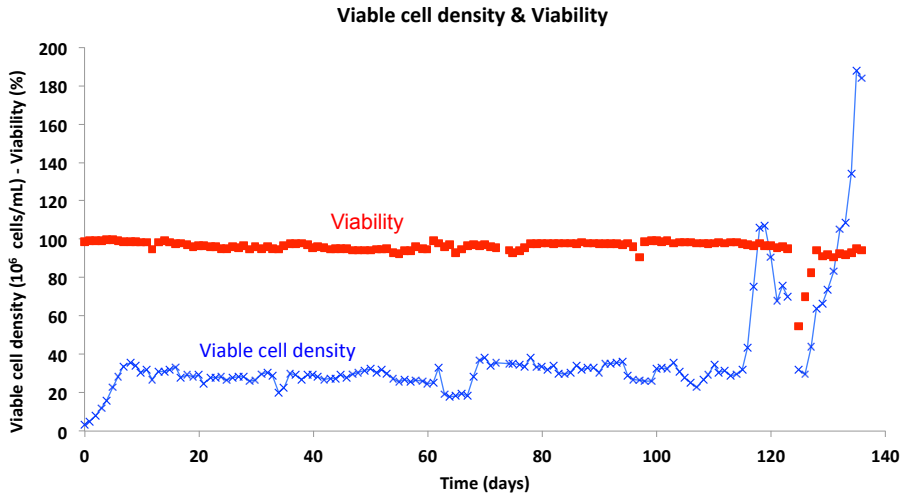
Study of perfusion in stirred tank bioreactor using ATF – Run #3

CHO#3



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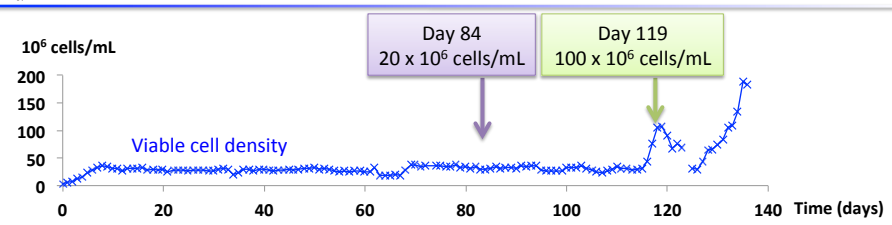
Cell thaw from long-term perfusion Productivity test

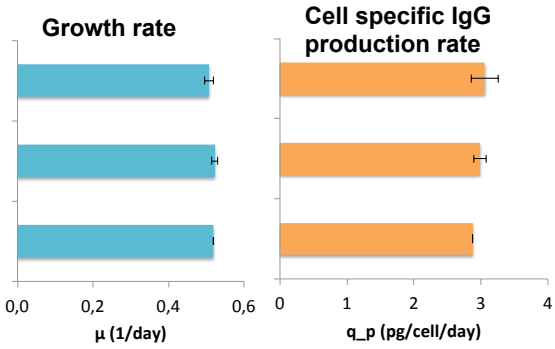
CHO#3



From day 119,
cell density = 100 x 10^6 cells/mL, DMSO directly added into cell broth (N=2)

From day 84,
cell density = 20 x 10^6 cells/mL (N=2)

Backup in shake flask (N=1)



Preliminary data

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Conclusions

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Conclusions

- Exometabolome foot printing in perfusion showing much higher consistency of the process from cell densities 10 to 200 x 10^6 cells/mL compared to fed-batch
- Observation of current metabolites confirmed by wide MS-based exometabolomics
- Identification of potential biomarkers for the cell density indicated glutathione metabolism modification towards higher cell densities
- Very high cell densities obtained in STR + ATF and other systems using CSPR
- IgG glycosylation not affected by high cell density up to 165 x 10^6 cells/mL and by low CSPR – Importance of CSPR for glycosylation
- CSPR minimized without affecting the cell health but lower IgG productivity

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Acknowledgements

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