Study of a perfusion process of Chinese Hamster Ovary cells by ATF filtration in bioreactor

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Layout

- Objectives

- Introduction
  - CHO cells
  - Modes of operation
  - Perfusion by **A**lternating **T**angential **F**low (ATF)

- Results
  1. The first three perfusion runs
  2. Effect of surfactant and antifoam on oxygen transfer rate in a bioreactor perfused with ATF
  3. Improvements of the cultivation system and the four next perfusion runs

- Conclusions and future orientations

Objectives

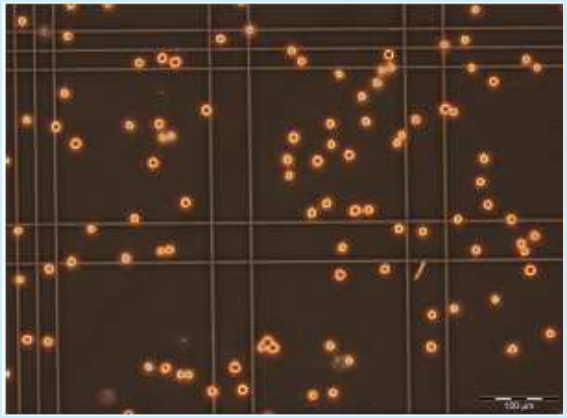
- To gain knowledge of the ATF-system as a retention device for perfusion cultures, e.g. study of
  - ATF ‘behavior’
  - ATF settings
  - Pressure in ATF system
  - Cell size in perfused culture
- To achieve high cell densities
- To study total medium exchange

Introduction

CHO cells

- Chinese Hamster Ovary cells
- Used in genetic, toxicologic screening, genes expression
- Industrial applications
  - Robust
  - Extensive knowledge
  - High security
  - Culture in suspension
  - Expression of large amounts of target products

Cell line most used in the bioprocess field



Microscopic observation of CHO cells (Bürker's chamber)

Operation modes in bioprocesses used in industry

- Batch
  - Cell inoculation in the final volume of culture medium
  - Cells grown until lack of nutrients
  - Waste products accumulation: Negative impact on cell growth
  - Product might be degraded
  - Target product harvested and separated from the cells by filtration or centrifugation at the end of the culture

- Fed Batch
  - Culture medium added at fixed intervals
  - Higher yield than with a batch process
  - Waste products accumulation: Negative impact on cell growth
  - Product might be degraded
  - Target product harvested and separated from the cells by filtration or centrifugation at the end of the culture

Fed batch process is the industry favourite for stable products

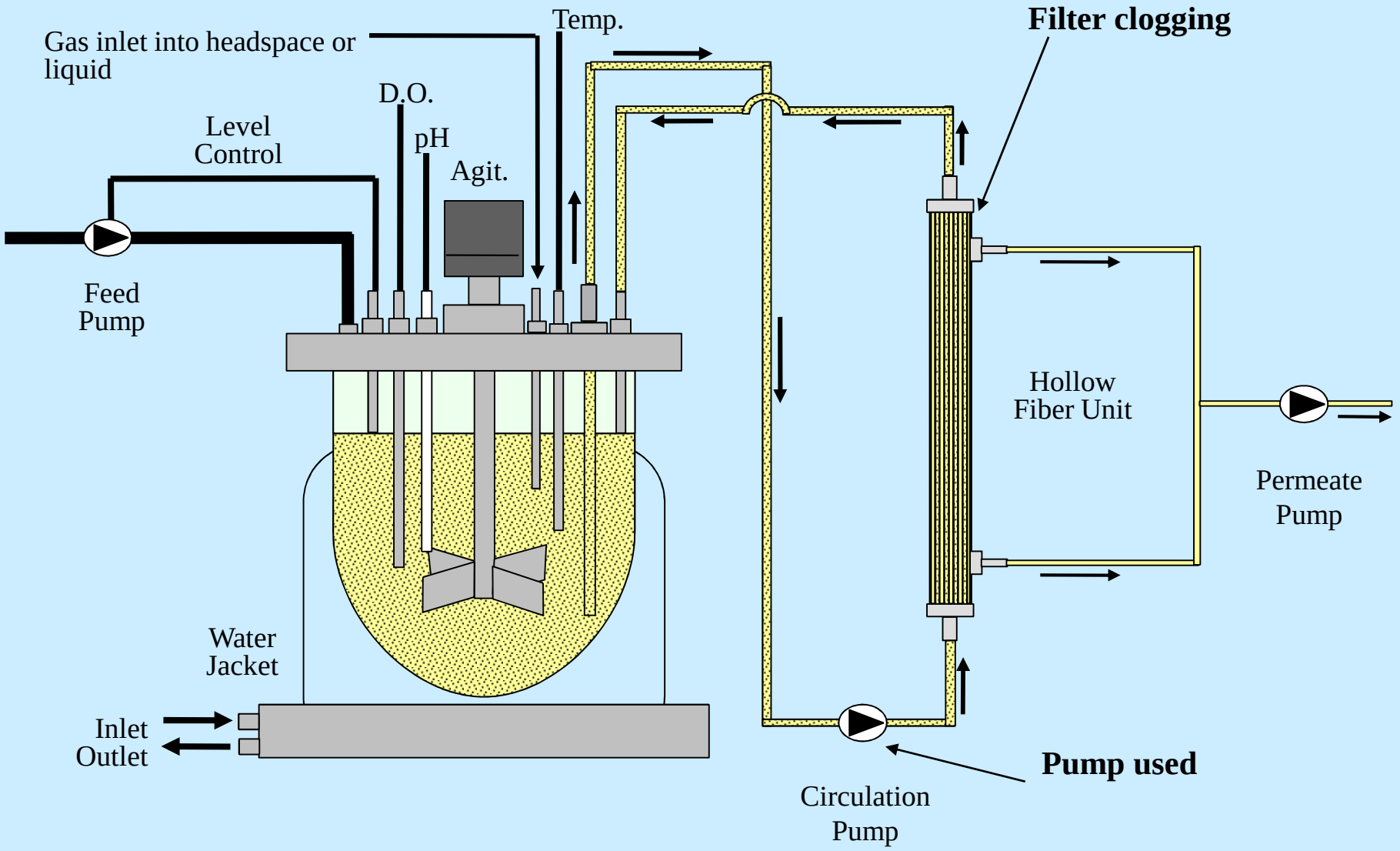
Operation modes in bioprocesses used in industry (cont')

- Perfusion
  - Continuous elimination of waste products
  - Continuous addition of new medium → constant nutrient concentrations
  - Cells retention system (filter)
  - Continuous harvest of target product → possibility to cold down the product

- Perfusion advantages
  - High cell density
  - Potentially high productivity
  - Removal of toxic by-products and proteases
  - Decreased risk of lost product

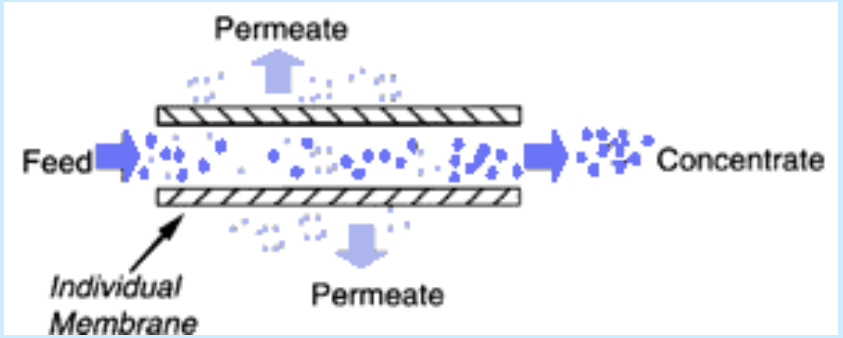
- Less used in industry
  - Retention devices non reliable (e.g. fouling, cell damage)
  - High density cells → more challenging
  - Technical issues more challenging (e.g. mechanics, sterility)

Perfusion by recirculation method



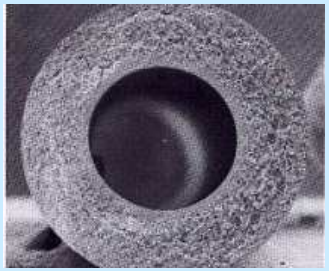
Perfusion by Alternating Tangential Flow (ATF) system

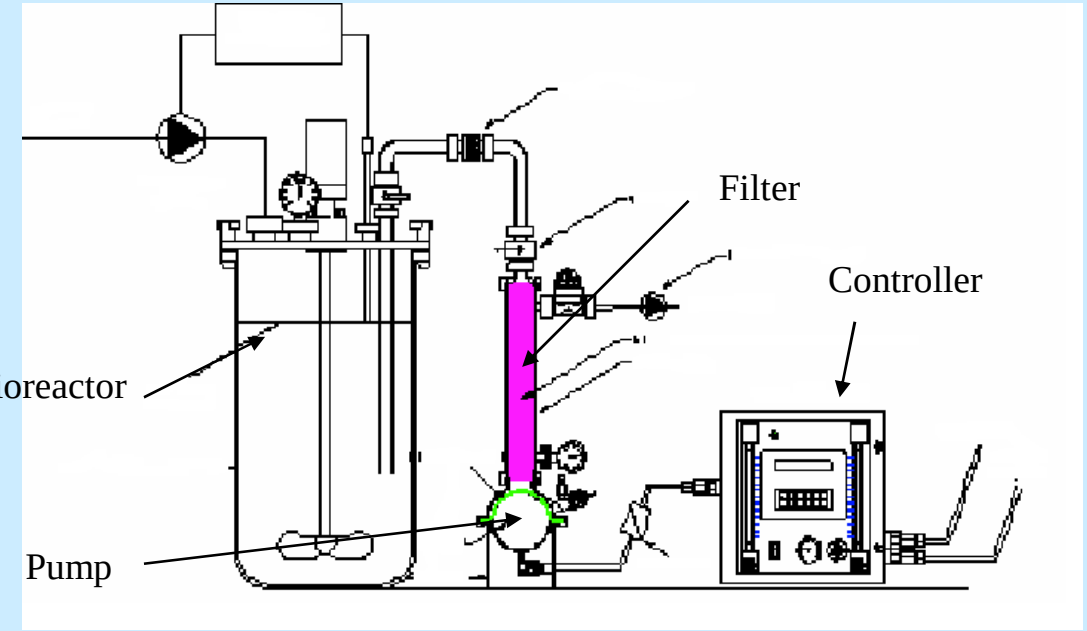
- Principle
  - Tangential filtration
  - Alternating Tangential Flow



Principle of tangential filtration

- 3 major components
  - Filter connected to the bioreactor
  - Diaphragm pump
  - ATF controller

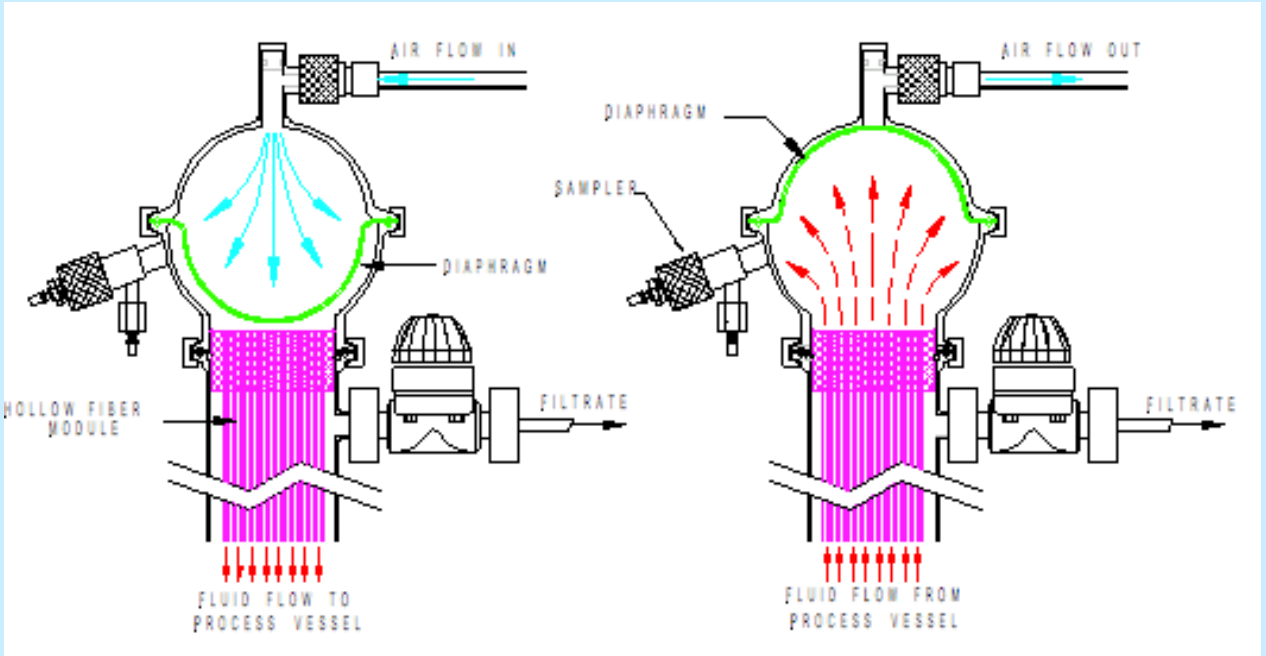




Perfusion by Alternating Tangential Flow (ATF) system (cont')

- Alternating tangential flow generated by pump through the hollow fibers
  - Back and forth movement
  - Liquid reversed through the filter
  - Cleaning of filter surface
  - Prevent clogging

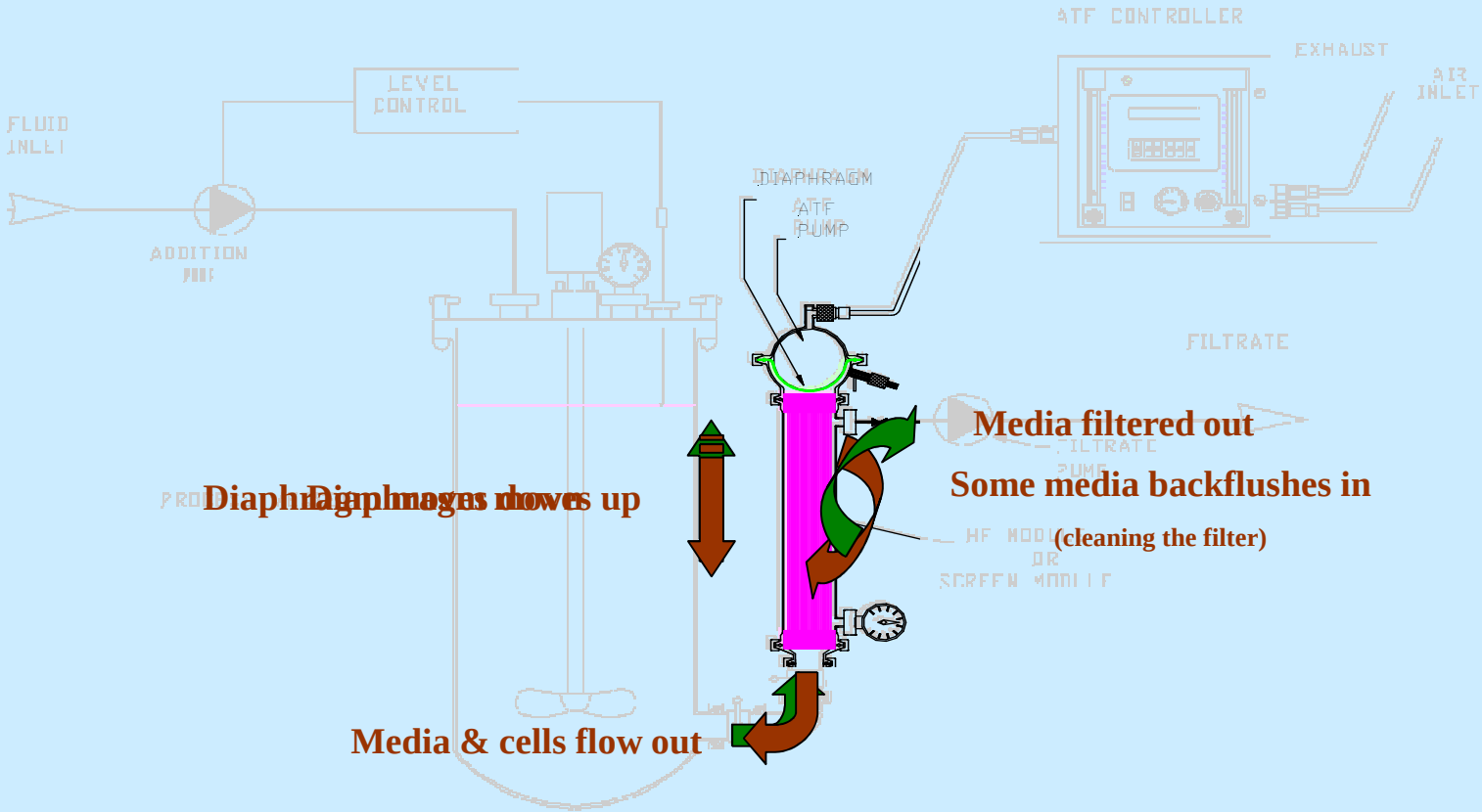
- Two pump cycles
  - Pressurization cycle
  - Exhaust cycle



Pump cycles

Perfusion by Alternating Tangential Flow (ATF) system (cont')

- Low shear laminar flow
- Filtrate harvested with peristaltic pump
- Biofilm formation decreased

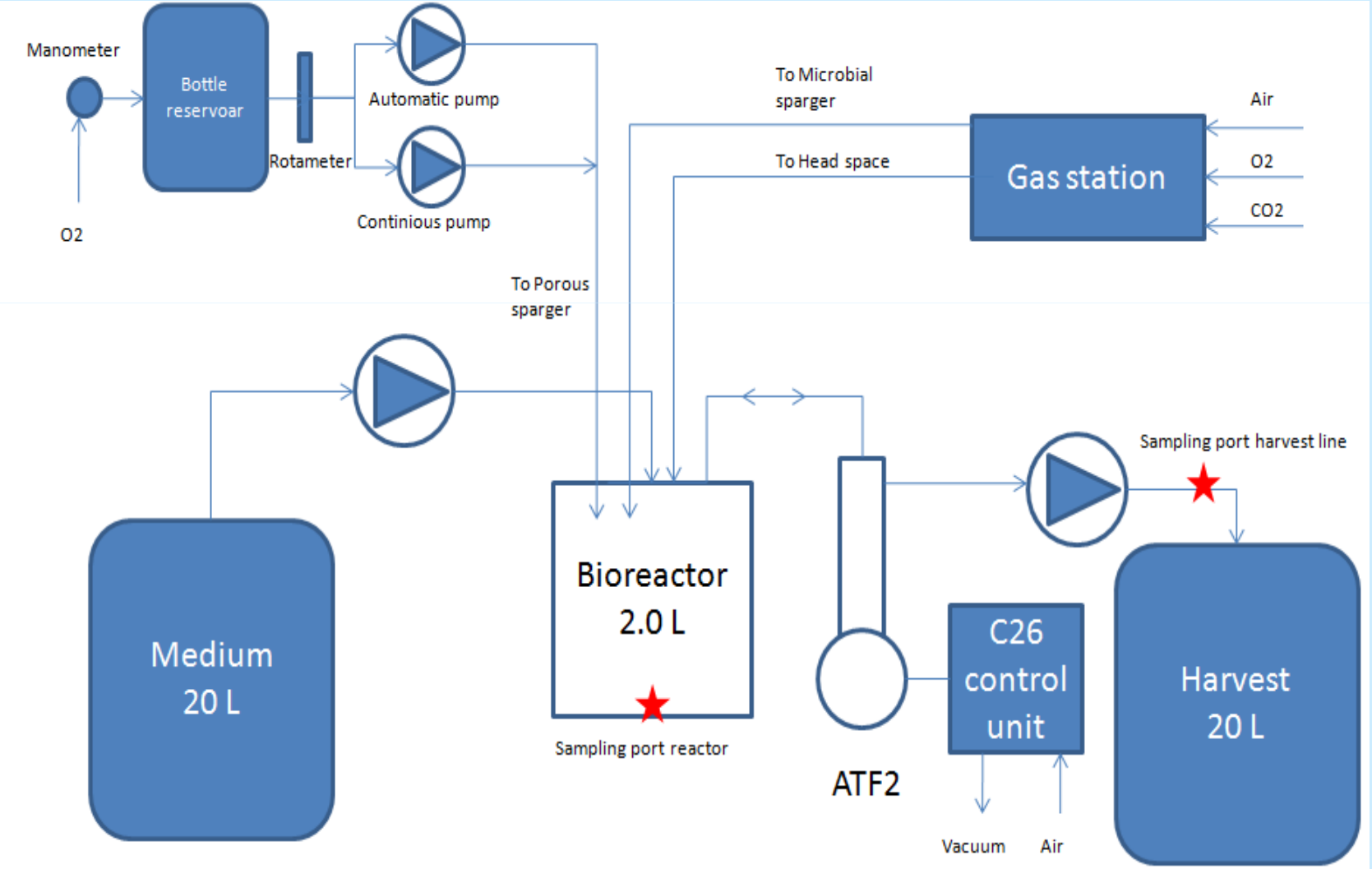


Results

1. The first three perfusion runs

General setup

- IgG producing CHO-K1 cell line (low production level)
- 5L glass bioreactor, 2L working volume
- ATF2 with 460 cm^2 filter
- Aeration: - microbial (large bubbles)
  - porous (small bubbles) for higher cell densities

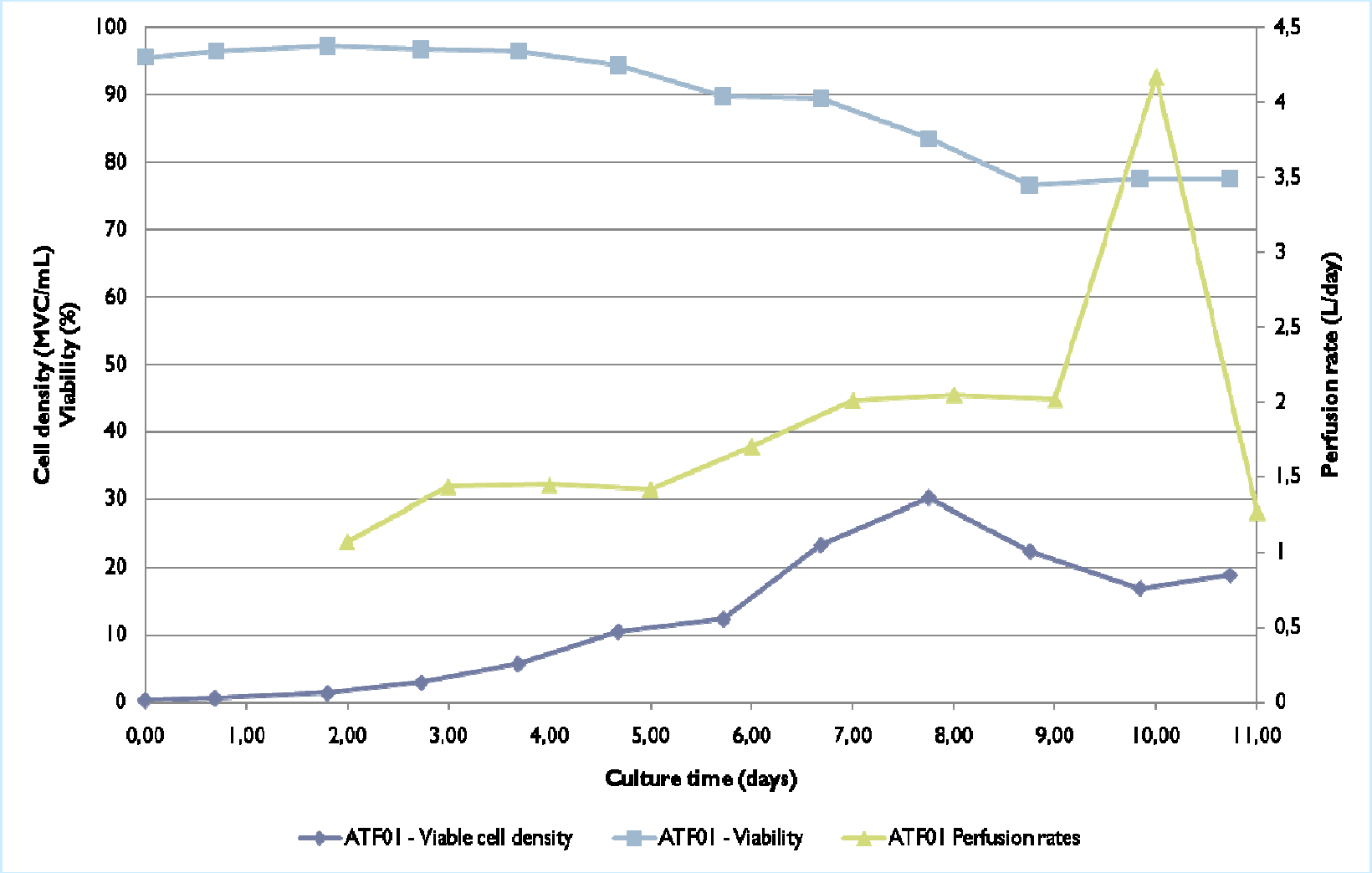


Perfusion cell culture with the ATF system

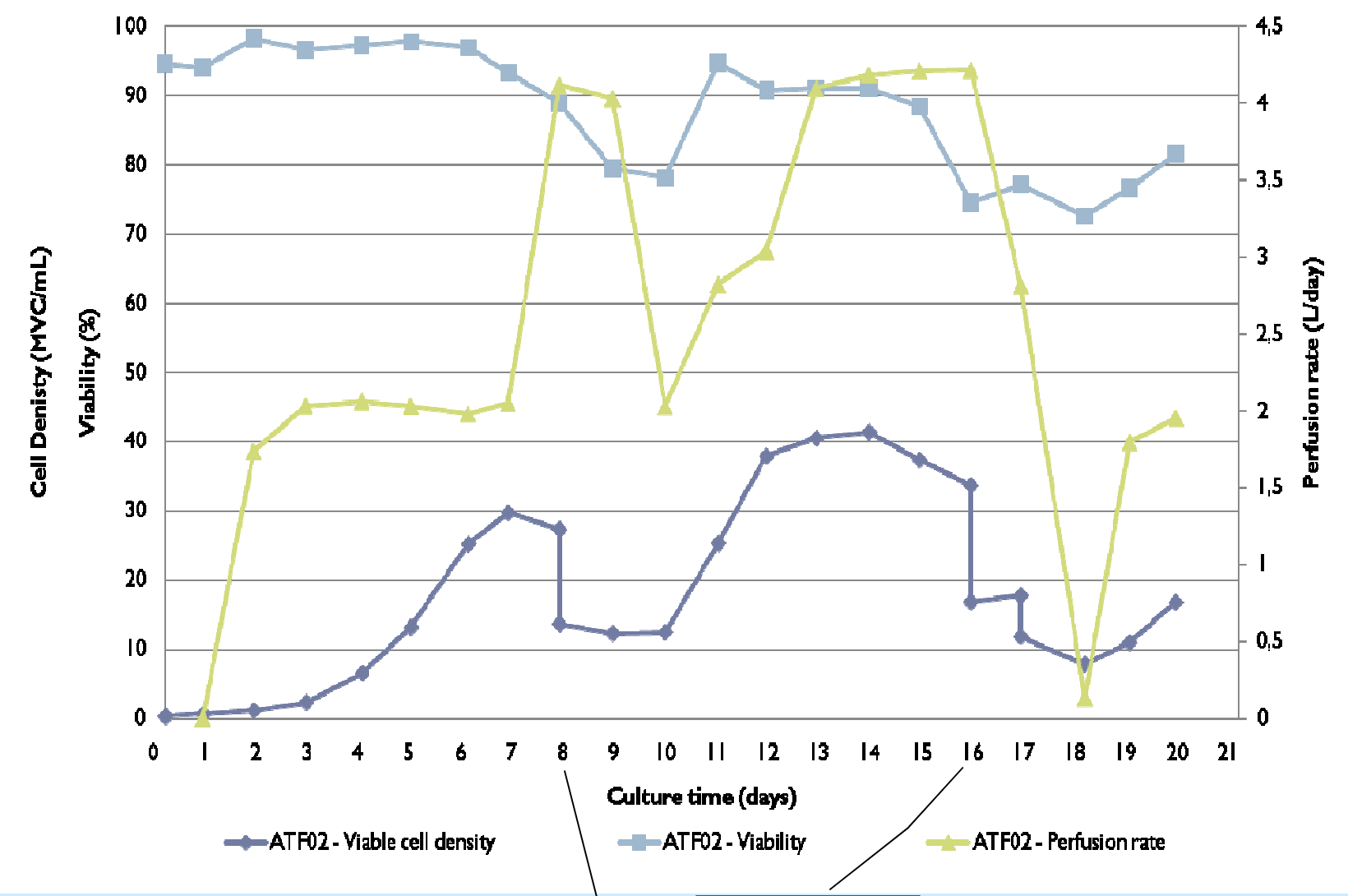
Experimental design of the three first perfusion runs

- ATF01
  - ”Lab-run-through”
  - Total medium exchange
- ATF02
  - Adjustment of aeration technique
  - Cell diameter comparison with semi-perfused shake flasks
- ATF03
  - Improvement of aeration control
  - Focus shifted towards reaching and obtaining a stable medium-high viable cell density.

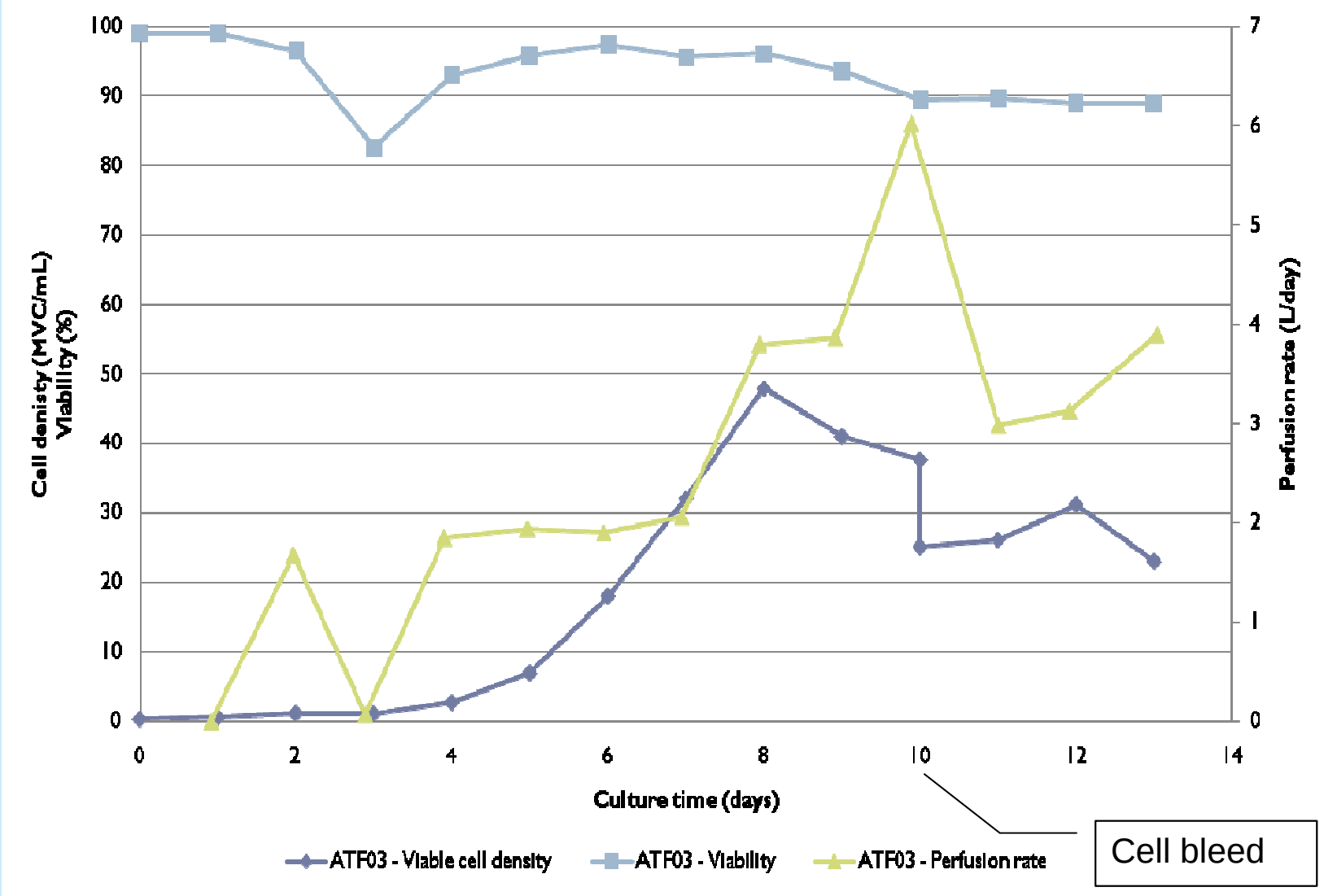
Cell density during ATF01 run



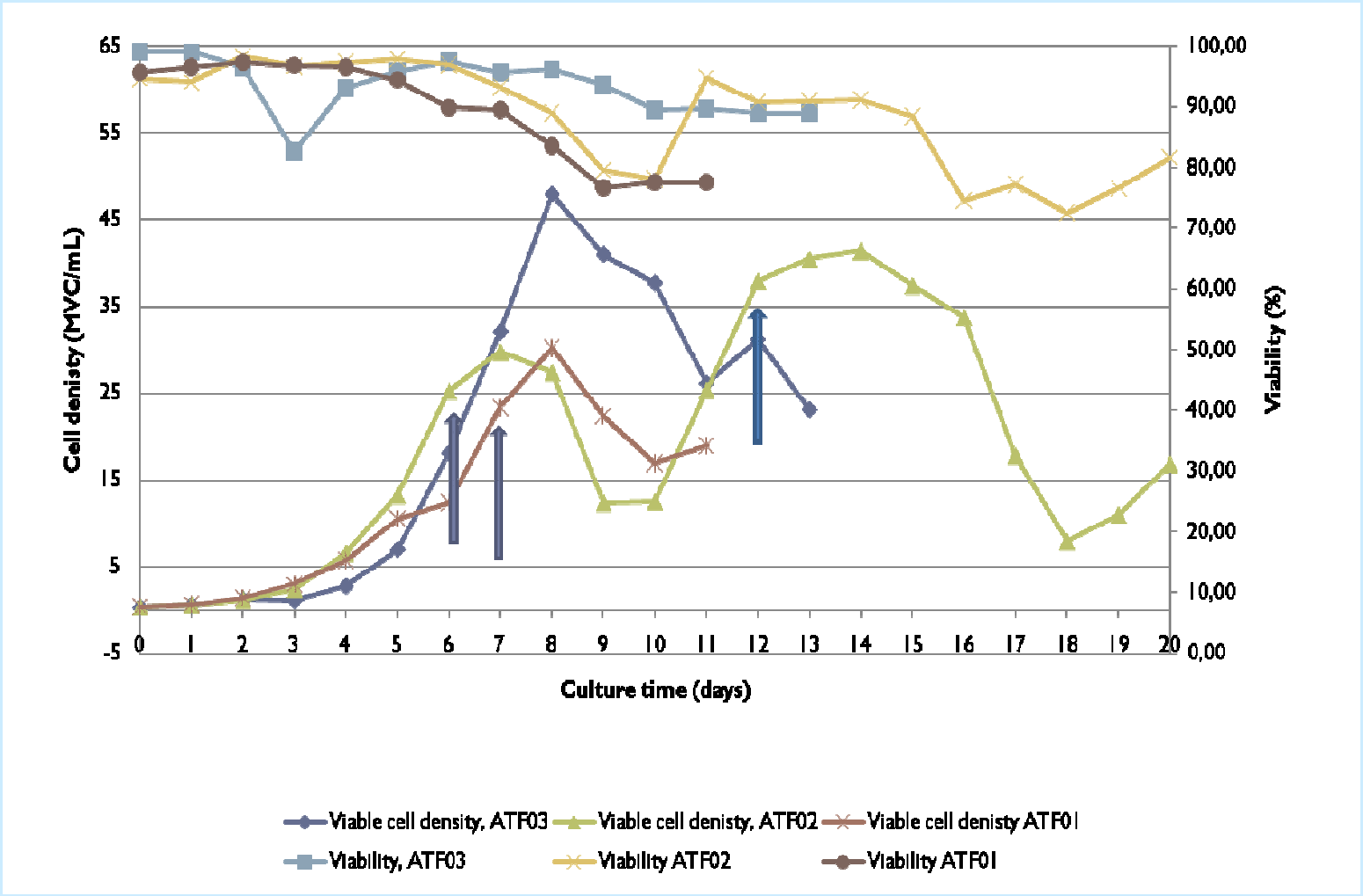
Cell density during ATF02 run



Cell density during ATF03 run



Runs ATF01, ATF02 and ATF03

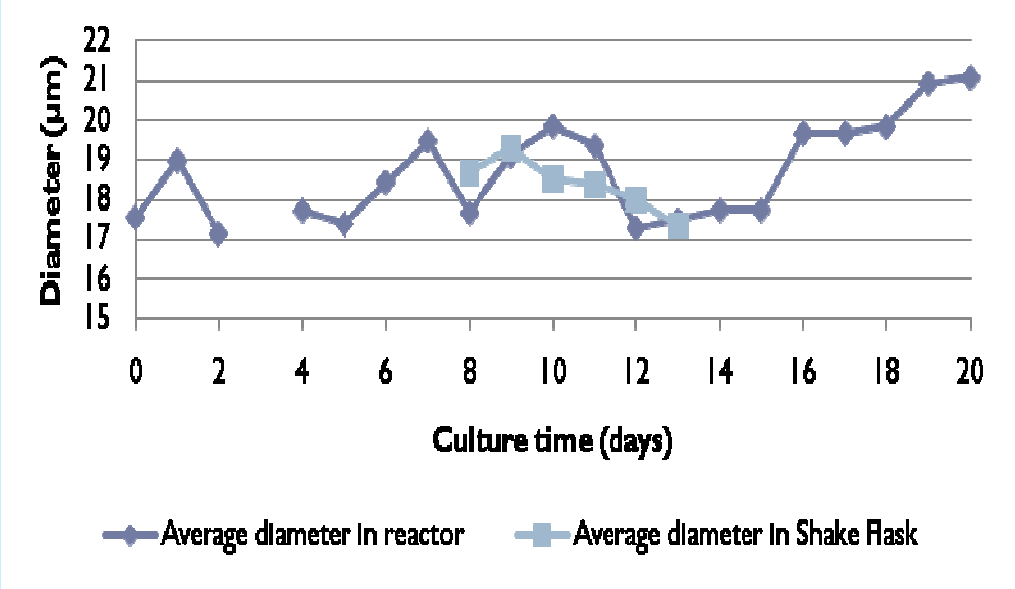


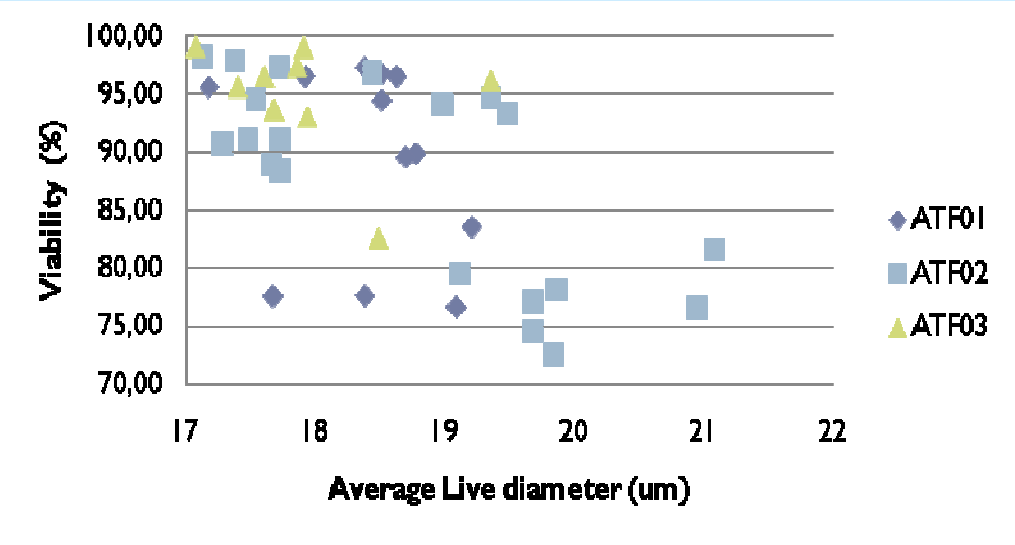
Arrows indicate use of porous sparger

Runs ATF01, ATF02 and ATF03 (cont')

- Nutrients and Metabolites
  - High glucose medium – high lactate levels
  - Trends followed perfusion rates and changes in cell numbers
- Performance of the ATF-device
  - Only small adjustments in settings
- Slow growing infections
  - Detected in ATF02 & ATF03
  - No or little effect on cell growth and viability

Comparison of cell diameter during ATF perfusion and in shake flask (ATF02)





Cells
→ taken from the bioreactor

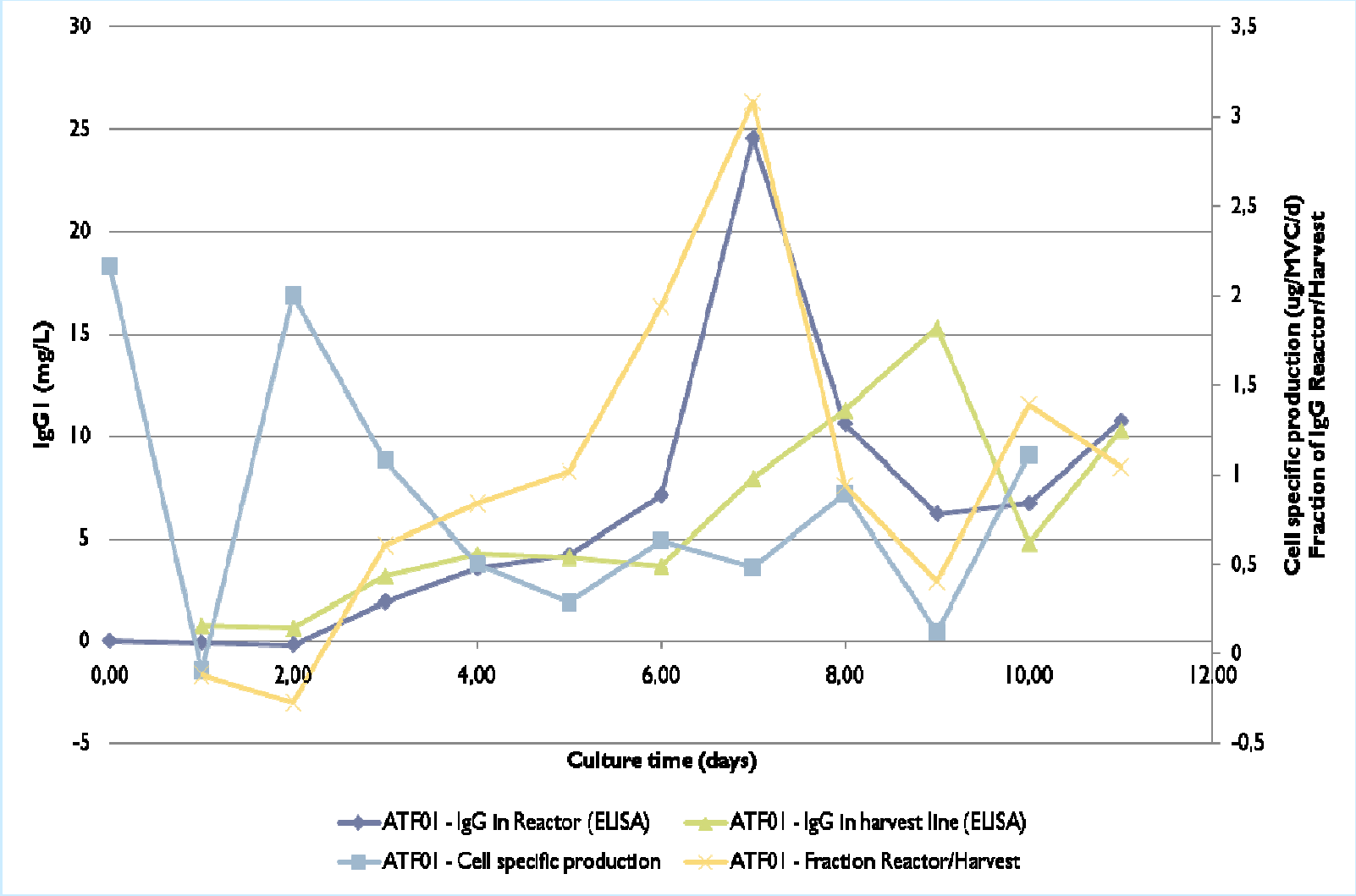
→ grown in semi-perfused mode in shake flask

→ in parallel with bioreactor

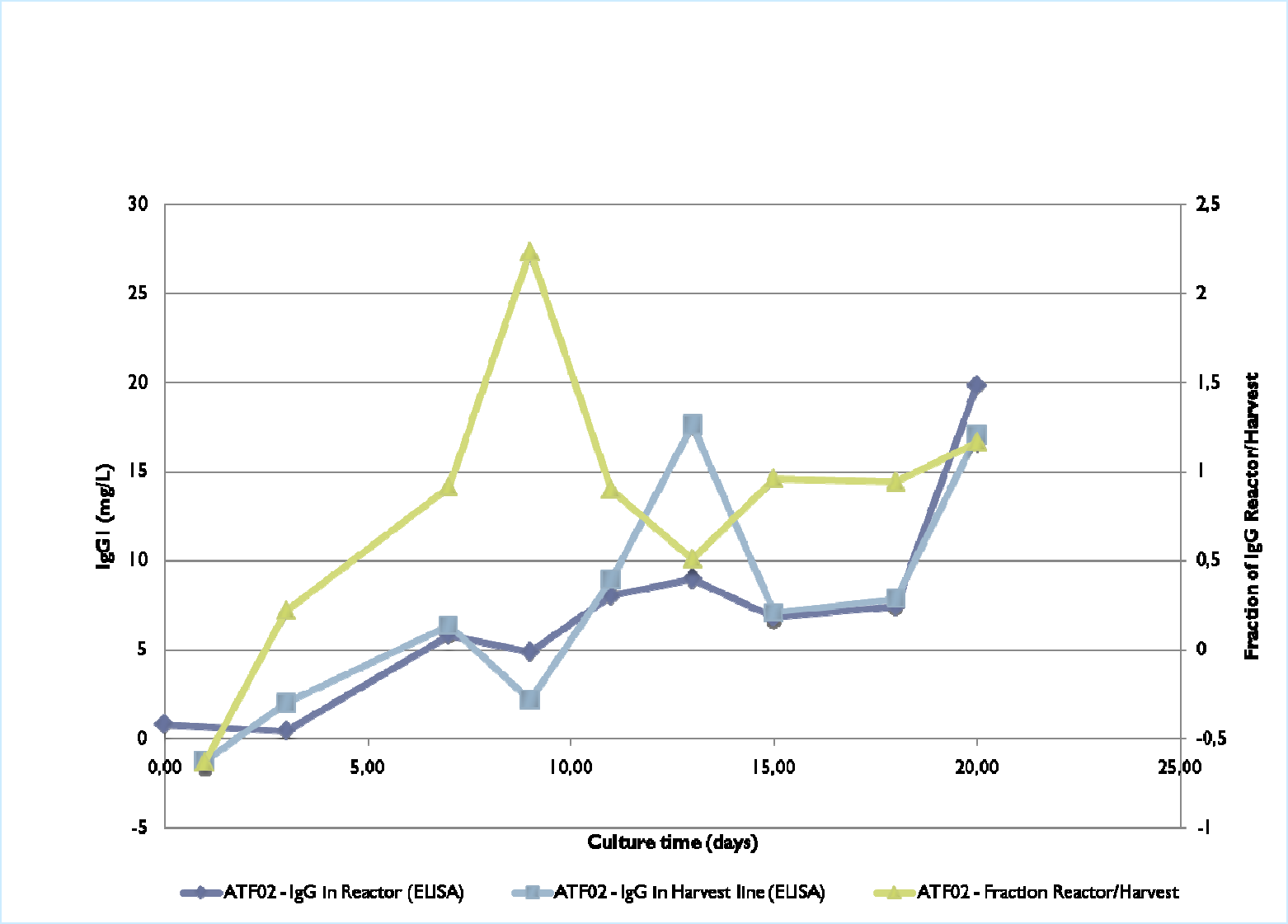
Production of IgG

- Determined by sandwich ELISA
- Little or no retention of product in the ATF filter
- Unreliable results with ELISA protocol
- Production within expected ranges for low producing cell line
- Decrease in production after time?

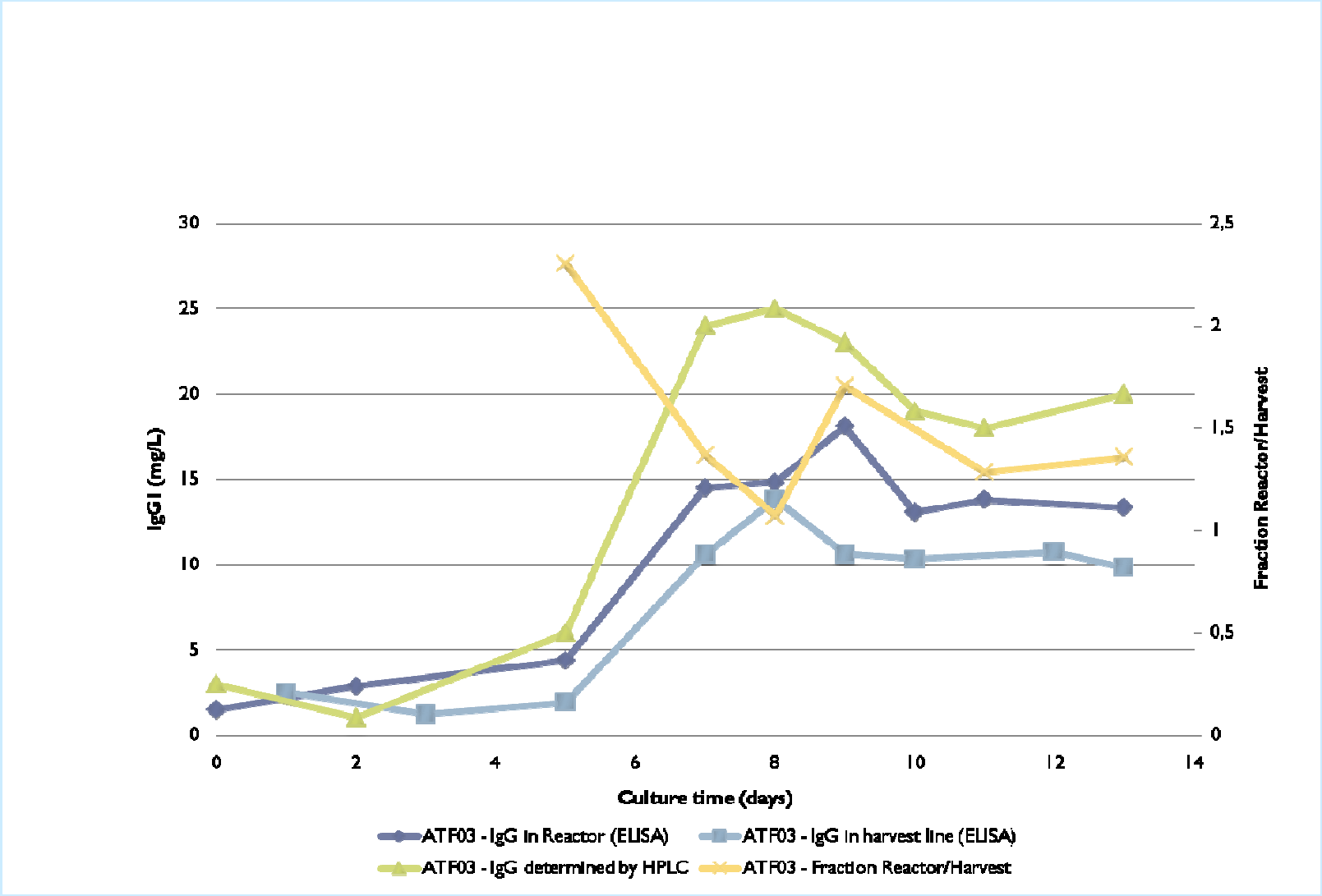
IgG production during ATF01 run



IgG production during ATF02 run



IgG production during ATF03 run



Discussion and conclusions of the first three perfusion runs

- High viable cell densities reached but not sustained

- Small bubbles (porous sparger)

  → foam formation and poor DO control

  → negative effect on viability and cell growth

- Prevention of nutrient depletion

  → perfusion to be based on expected cell density

- It could not be shown that the ATF-system had an effect on average cell size

- A correlation of decreasing viability with increasing cell size could be seen

Next step in the study

- Solve cell death problem consecutive to switch to small bubble aeration

- Addition of surfactant and antifoam during the cultivation
  - Surfactant pluronic F68 → to protect the cells
  - Antifoam C → to suppress the foam

- Experimental plan
  - Effect of surfactant and antifoam on oxygen transfer rate ($K_L a$) in a bioreactor perfused with ATF

  - Cell cultivations by perfusion with the ATF system
    - Find an optimal oxygenation system avoiding cell death (ATF04 & ATF05)

    - Develop addition of pluronic and antifoam C (ATF06 & ATF07)

    - Reach the highest cell concentration possible (ATF07)

Results

2. Effect of surfactant and antifoam on oxygen transfer rate (KLa) in a bioreactor perfused with ATF

Introduction: Effect of surfactant and antifoam in cell culture

- Cell protection from bubbles and foam formation

- Pluronic F68
  - Protective agent against hydrodynamic stress
  - Creation of a microenvironment between bubbles et cells
  - Avoid cell attachment to bubbles
  - High foaming propensity

- Antifoam C
  - Suppress foam formation
  - Hydrophobic silicone emulsion
  - Decrease foam stability
  - Interaction with cells at high concentration resulting in easy transport to the surface of the liquid (Bubble bursting region)

- Few studies about their impact on oxygen transfer rate in bioreactors

Study design: Impact of surfactant and antifoam on K_La

- Studied parameters
  - Pluronic concentration
  - Antifoam concentration
  - ATF impact

Porous sparger

- Oxygenation mode
  - Headspace
  - Microbial sparger (open tube) → ‘big’ bubbles
  - Porous sparger → ‘small’ bubbles

- Experimental conditions
  - 5 L bioreactor
  - 2 L saline solution (7 g/L NaCl)
  - Temperature 37℃
  - Stirring speed 150 rpm

- K_La determination by a non chemical technique (gassing out)

Kla determination for the 3 aeration modes studied

Experiment	Media			Aeration		Results	
	Saline solution (7g/L)	Pluronic	Antifoam	Type	Flow rate	Kla (h^{-1})	R^2
1.1	X			Open tube	Constant *(150ml/min)*	3,258	0,9844
1.3	X			Open tube		3,384	0,9877
1.2	X			Porous sparger		11,520	0,8979
8	X			Head space		0,796	0,9838

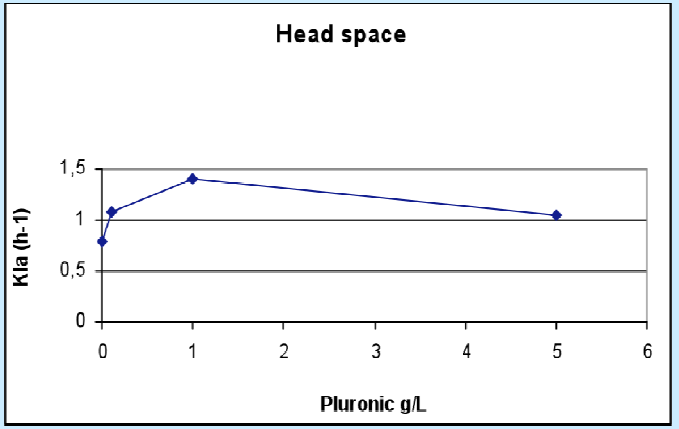
K_La without pluronic and antifoam for the three aeration modes

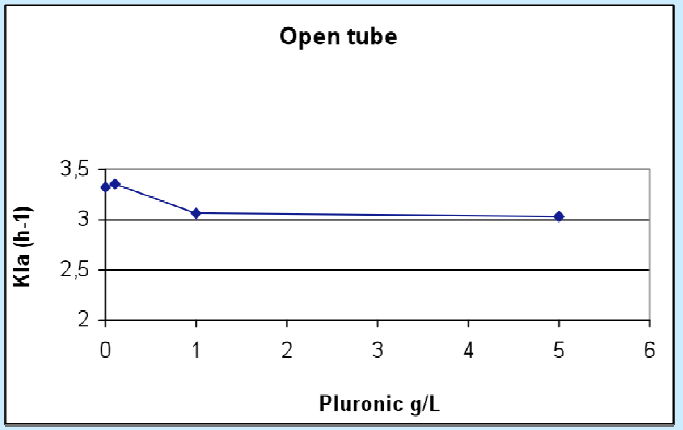
- **Optimal oxygen transfert with small bubbles (K_La 3 times higher with the porous sparger)**

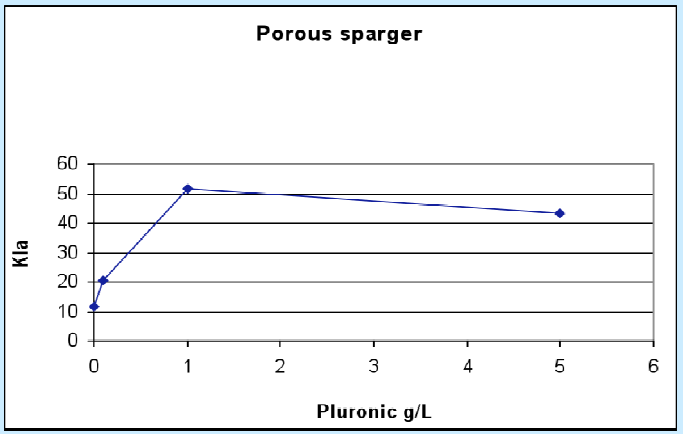
Pluronic impact

- 4 concentrations studied [0 - 0.1 - 1 - 5] g/L
- Increase of $K_L a$ between 0 and 1g/L for headspace aeration (+40%) and porous sparger (+80%)
- Decrease of $K_L a$ between 0 and 1g/L for open tube aeration
- $K_L a$ stable between 1g/L and 5g/L

❖ **No critical $K_L a$ decrease for a concentration range of [1-5] g/L**

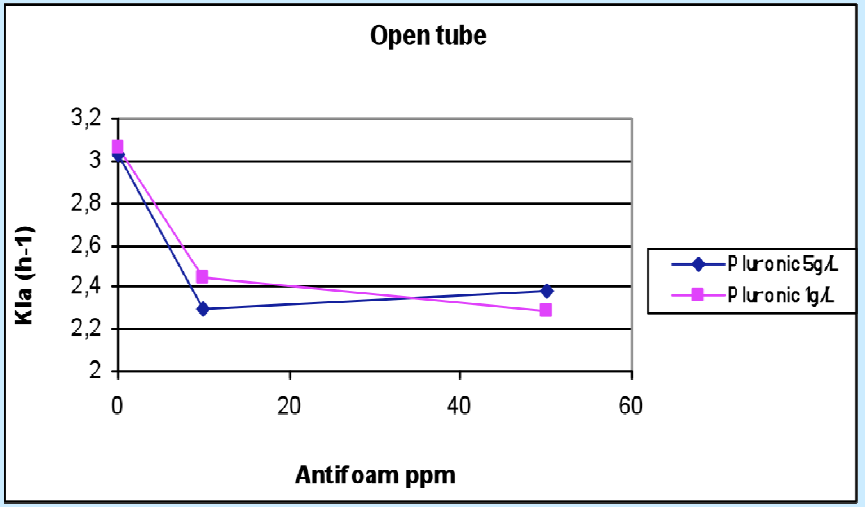


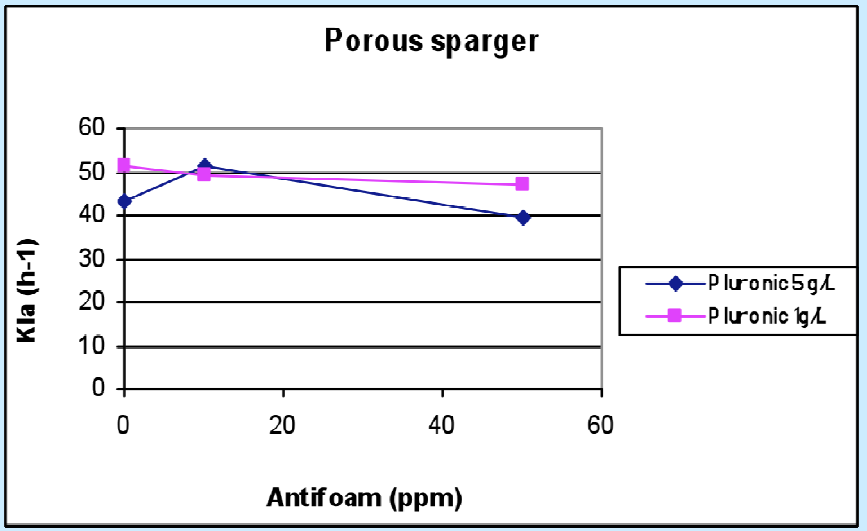




Pluronic and antifoam impact

- 3 antifoam concentrations studied 0–10–50 ppm
- 2 pluronic concentration studied 1-5 g/L
- Open tube aeration
  - Decrease of $K_L a$ between 0 and 10ppm
  - $K_L a$ stable between 10 and 50ppm
- Porous sparger aeration
  - K_L a stable between 0 and 50ppm
- Similar curves for 1g/L and 5g/L of pluronic
  - Pluronic does not impact antifoam effect on $K_L a$





Conclusions of the K_La study

- Higher oxygen transfer rate with porous sparger
  - Used for high cell concentrations
- Pluronic does not impact K_La in the concentration range studied (1-5g/L)
- Antifoam does not impact K_La when porous sparger is used
- Pluronic does not impact antifoam effect
- Complementary trials
  - ATF does not impact K_La
  - Same conclusions with a lower air flow rate

❖ **No critical decrease of K_La with pluronic or antifoam addition**

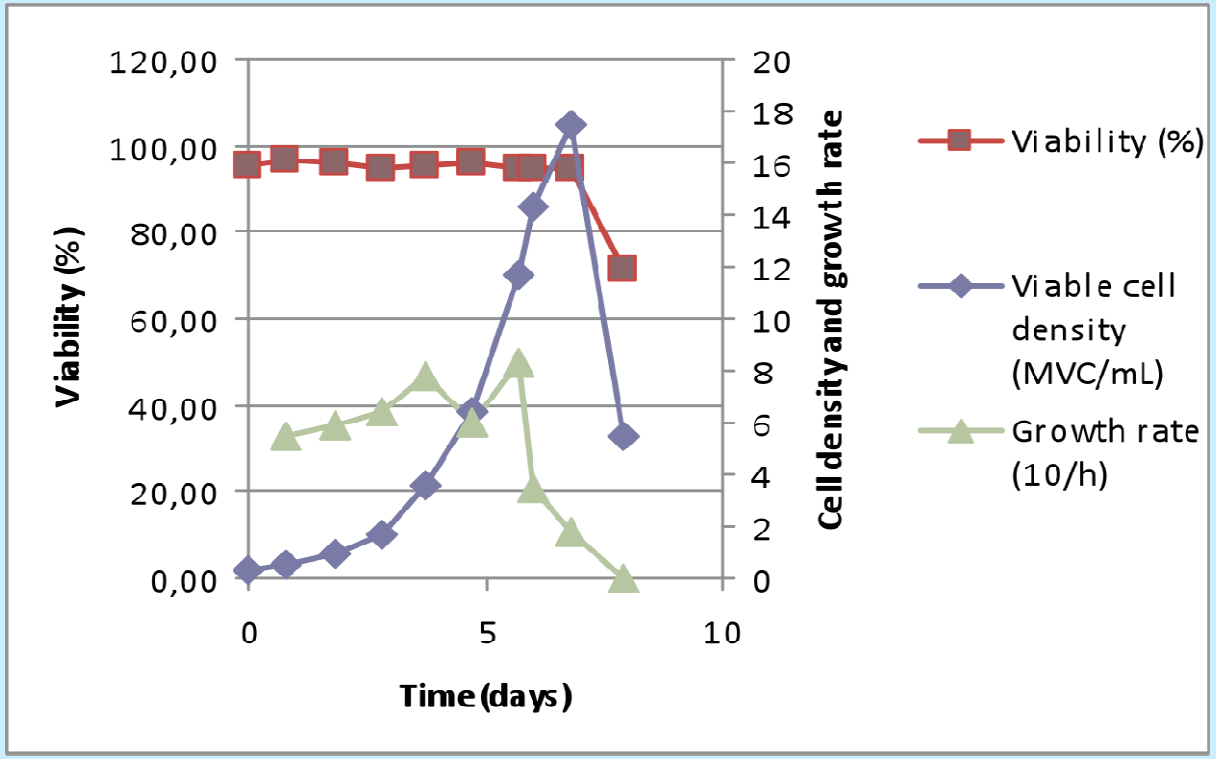
➔ **Pluronic and antifoam to be continuously added during cultivation**

Results

3. Improvements of the cultivation system and the four next perfusion runs

Run ATF04

- 5L bioreactor
- Working volume 2L
- 9 days cultivation
- Maximal cell concentration $17.5x10^6$ cells/mL
- Cultivation ended due to an infection
- No pertinent conclusions



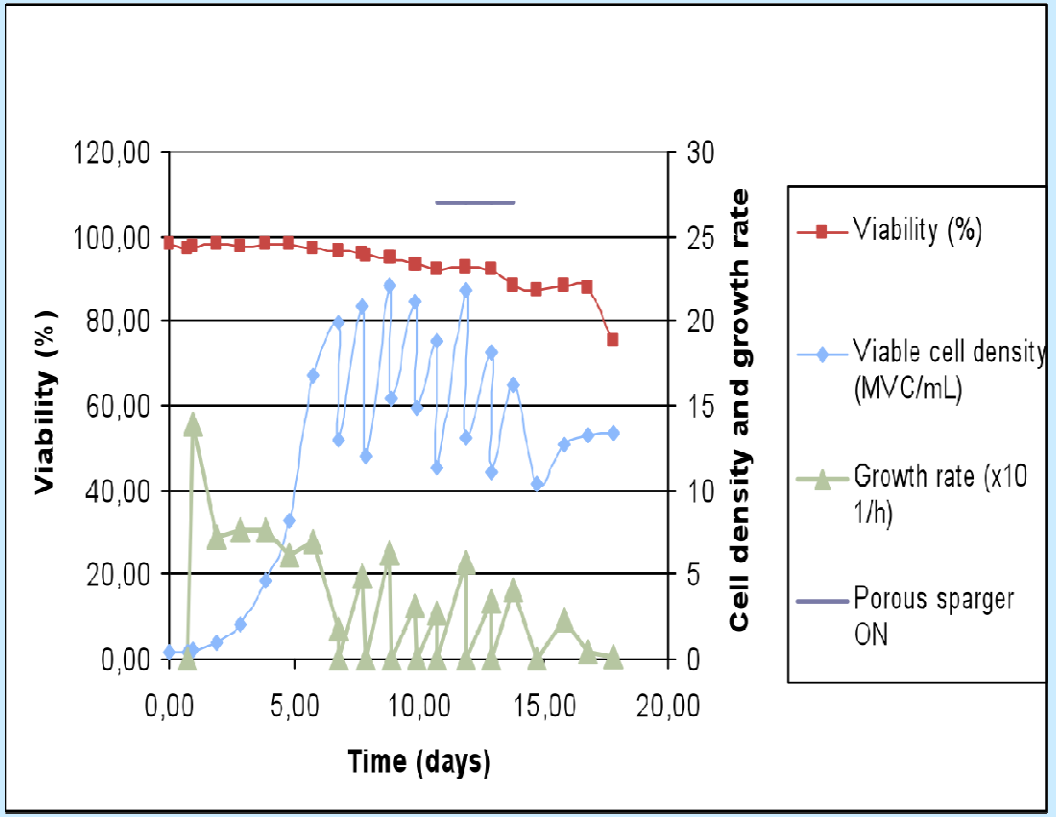
ATF04 culture profile

❖ Improvements

- **Change of bioreactor model**
- **Addition of antibiotics in culture medium**

Run ATF05

- Goal
  - Oxygenation study
- Strategy
  - Cell concentration maintained at $20x10^6$ cells/mL by cell bleed
- 18 days cultivation
- Maximum cell density $22x10^6$ cells/mL
- Perfusion rate 1.5 - 2 bioreactor volume per day
- Porous sparger used from day 12 to day 14 of cultivation



ATF05 culture profile

- **Decrease in cell viability before the use of the porous sparger**
- **No correlation between small bubbles and cell death**
- **Foam suspected to have a negative impact on cells**

Foam control and cell protection strategy

- Continuous addition of pluronic and antifoam in the bioreactor

- Pluronic
  - Increase of the concentration in the culture medium related to the cell density
  - Compensation of pluronic uptake by cells

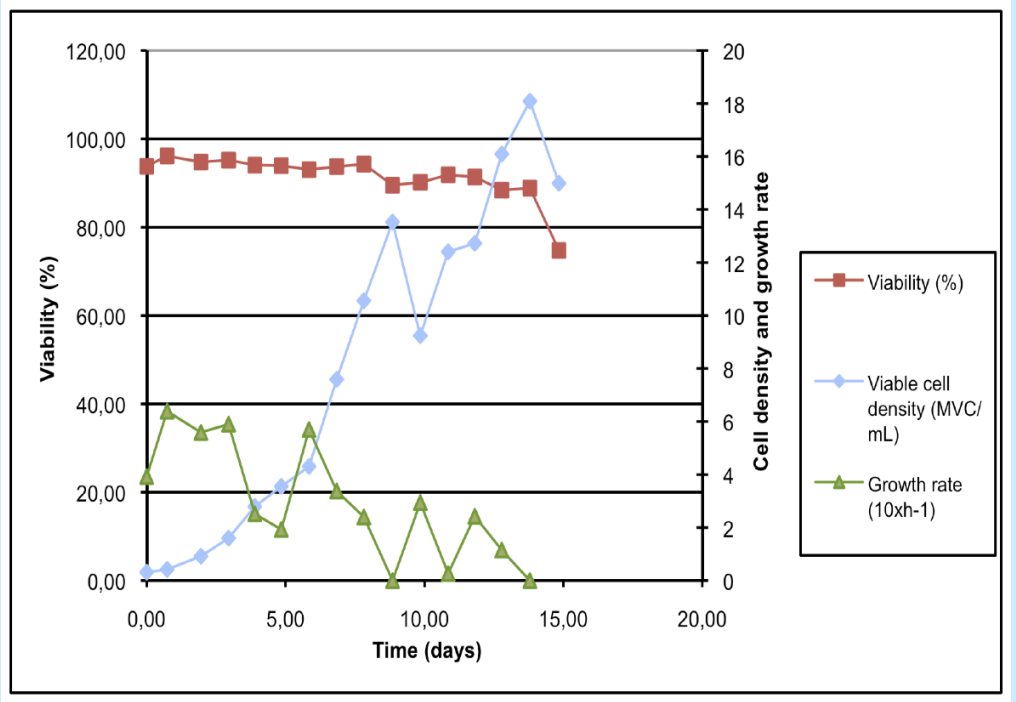
Cell concentration (MVC/mL)	Pluronic concentration	Pluronic uptake	Compensation (adding of a 1g/L pluronic stock solution)
0-10	1g/L	6%/18h	0,111 mL/min
10-20	2g/L	12%/18h	0,222 mL/min
20-30	3g/L	18%/18h	0,333 ml/min
30-40	4g/L	24%/18h	0,444 mL/min

Strategy of pluronic addition during cultivation

- Antifoam
  - Addition correlated to the perfusion rate
  - Visual control

Run ATF06

- Improvements
  - Additional oxygenation system without bubbles (Gore-Tex tube)
  - Addition of a second impeller to improve stirring and reduce foam
- 15 days of cultivation
- Maximum cell density 18x10^{6}cells/mL



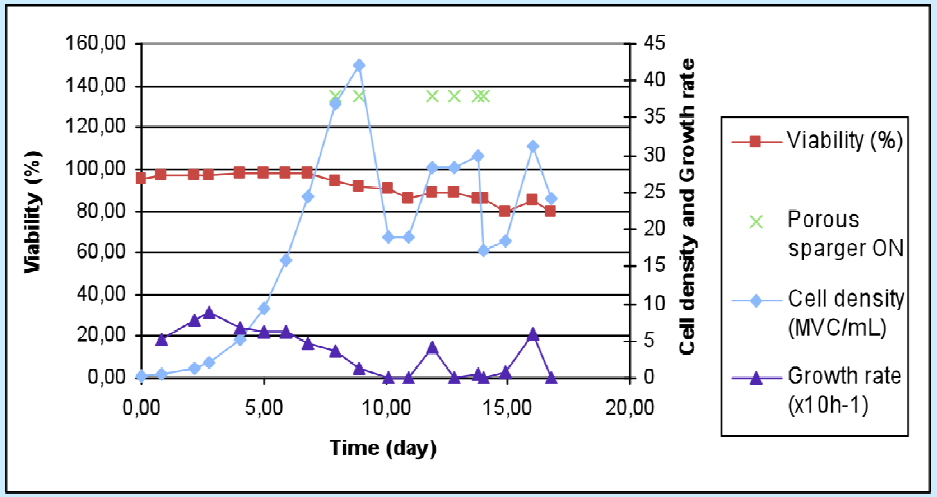
ATF06 culture profile

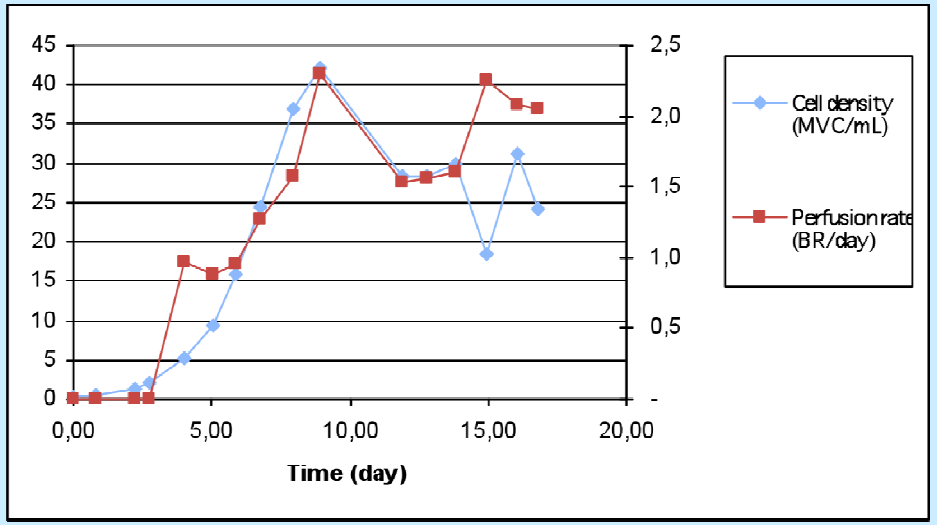
- **Poor cell growth**
- **Gore-tex tube suspected to induce a bad DO regulation**
- **No interesting informations concerning the pluronic and antifoam addition system used**

Stirring device

Run ATF07

- Goal
  - Assess pluronic and antifoam addition system efficiency
- 17 days of cultivation
- Maximum cell density 42x10^6 cells/mL
- Perfusion rate increased related to cell concentration
- Porous sparger used two times
- Decrease of cell viability (day 7)





ATF07 culture profile

- **Correlation small bubbles – cell death not 100% confirmed**
- **Porous sparger or insufficient perfusion rate ??**
- **Foam controlled efficiently**
- **Pluronic addition not sufficient for total cell protection**

Conclusions of the last four runs

- Pluronic and antifoam do not seem to have an impact on the $K_L a$
  - Continuous addition in the medium culture

- Many improvements done on the equipments
  - Change of the bioreactor glass vessel
  - Modification of the stirring device
  - Improvement of the oxygen flow control
  - Implementation of automatic additions of pluronic and antifoam

- Maximum cell density not improved

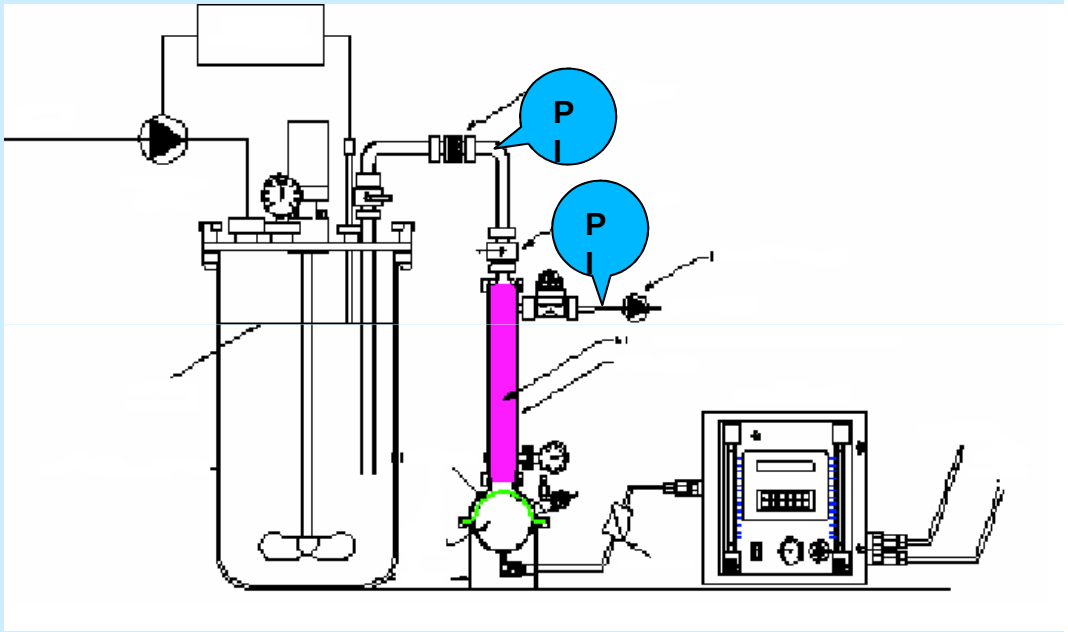
- Foam formation problem solved

Hypothesis

- Bubbles from the porous sparger damaging the cells
- Culture conditions in the filter not optimal (Very low DO)
- Cell stress

Pressure before and after the ATF device

- With used settings
- Flow rate (Pressure and Exhaust) used between 0.7 and 1.1 L/min
- Pressure **before** ATF → low and similar at high and low cell density
- Pressure **after** ATF → low, similar at high and low cell density and similar at different perfusion rates



Addition of pressure gauges before and after the ATF device

Total medium change

- Purpose of total medium change
  - Replacement of the conditioned (used) medium with fresh medium at fast rate

- Example of application
  - Medium change before viral infection

- Results
  - 2 - 0.5 L → 45 min
  - Limitation in harvest rate
  - Refill of fresh medium limited by the heating capacity to avoid over-heating

Conclusions

Conclusions

- Perfusion system
  - A perfusion system using ATF device has been developed, including automatic addition of pluronic and antifoam (to prevent foam formation)
  - Aeration is performed by large and small bubbles of oxygen
  - No critical decrease of $K_L a$ is observed with pluronic and / or antifoam addition
  - Cell densities higher than 30 x 10^6 cells/mL require a higher oxygen transfer rate → porous sparger (small bubbles)

- Perfusion performances
  - Maximum cell densities of 40 to 48 x 10^6 cells/mL have been obtained several times
  - Cell viability decrease is associated with high cell density
  - Cell viability decrease is not due to foam
  - Maintaining a cell density of 20 x 10^6 cells/mL with cell bleeds is ‘easy’

- ATF device
  - Easy to adjust the settings
  - No clogging

Futures orientations

- Usage of small bubbles of a larger diameter
- Study cell behaviour in the ATF filter
  - Frequency of one cell passage in the filter
  - Residence time
  - DO in the filter
- Adapt ATF parameters
  - Avoid filter clogging
  - Reduce the frequency of cell passage in the filter
- Increase the surface area of the filter

Questions?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IMED


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