Technologizing ATMP Processes – Efficient methods to enhance NK functionality

Christoph Herwig

Oscar Garcia

Reseach Area Biochemical Engineering, Vienna University of Technology

Obstacles to lymphocyte expansion

High input variability

- High material complexity
- Very high source variations
- Significant protocol differences

Low process underst.

- Low characterization of CPPs
- No defined design spaces
- Very low data integration
- Highly sensitive cells

Mainly manual

- Mostly performed in flasks
- Prone to contamination
- High personnel interv.

Inefficient materials use

- Non-structured feeding strategies
- Very high costs in manufacture
- Sub-optimal medium management

Little process tracking

- Missing non-invasive principles
- Poor outlet control
- Mostly off-line analysis

High failure rate

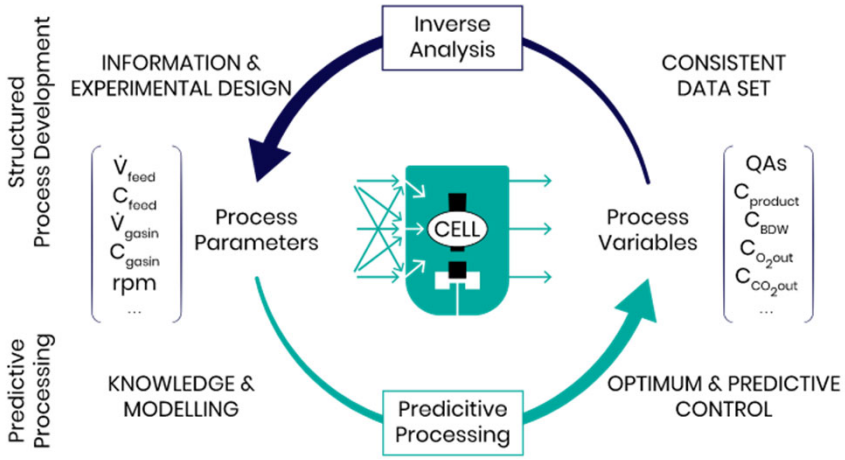
- No point of harvest prediction
- Variable functionality
- Not unified CQAs
- High process failure rate

Research Motivation

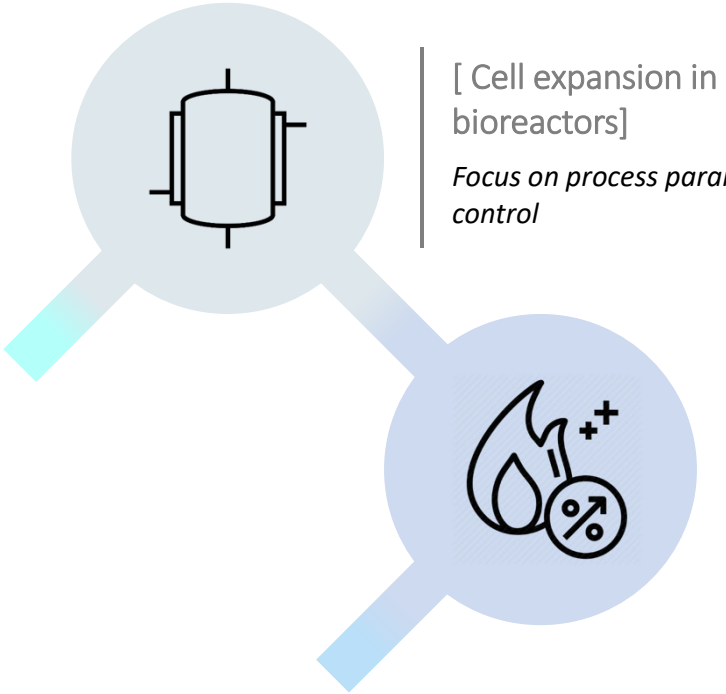
NK cell expansion is still mainly a **hospital technology** and not industrial but it is gaining importance.

To increase in scale and patient availability **NK cell expansion has to be technologized and engineered** just like regular CHO cell culture.

Our research approaches use **systematic engineering and state-of-the-art analytical tools** to characterize NK cell expansion therefore **improve in yield** and enable more robust processes. That results in a better product and eventually lower production costs therefore a higher revenue.



Main research directions



[Cell expansion in bioreactors]

Focus on process parameters and control

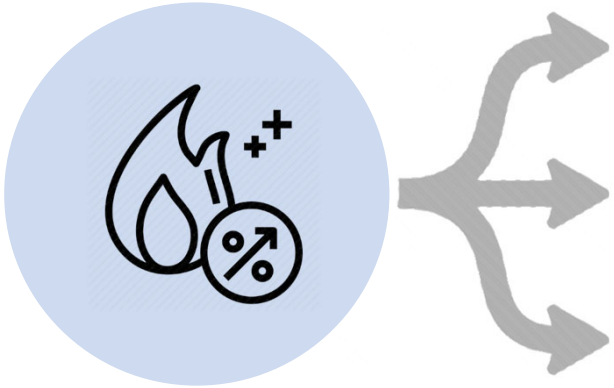
[Raw material characterization]

Focus on raw material's variability and their influence on culture

Raw Material Characterization

Our hypothesis: **the expansion process** through process parameters **has to adapt to** the high **raw material variability**, such as different donors or serum batches.

However, to optimize the relevant process parameters the effect of different raw materials on the expansion process itself has to be understood first.



Metabolic profiles of the expansion process correlated with culture parameters show the important factors for cultivation performance.

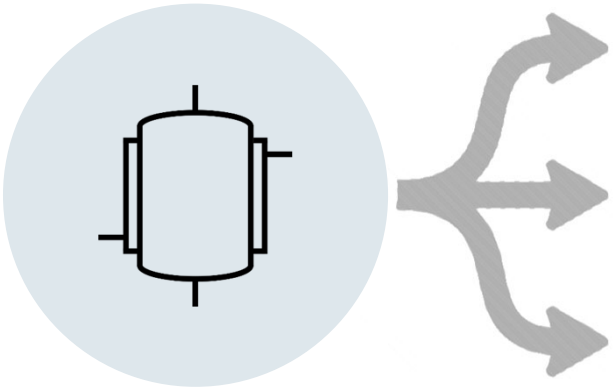
Optimal and cost effective **feeding strategies** can be set up by the analysis of amino acid consumption and the monitoring of interleukin concentration.

Media complexity could be reduced based on the metabolic profiles allowing tighter process control and consistent product quality.

Cell expansion in Bioreactors

Our hypothesis: NK cells should be cultured in a ***well-controlled*** **bioreactor environment**.

These cultures can achieve similar expansion levels to current clinical practice or even higher if the culturing process is adjusted to accommodate raw material variabilities. Which is possible in bioreactors.

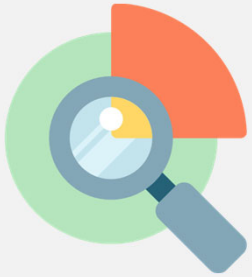


In **bioreactors**, consistent expansion can be achieved due to high level of process parameter control.

Culture conditions such as **temperature, dissolved oxygen, carbon dioxide, pH,** etc. can be optimized to different raw material variabilities.

Balanced **dynamic/static** culturing conditions can provide the necessary stimulation that is optimal to lymphocytes.

Obstacles to lymphocyte expansion



We need process understanding

Target 1

Identify metabolic patterns during culture

Target 2

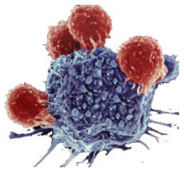
Explore and characterize process

Identify mechanistic links inherent to lymphocyte expansion and develop robust digital twins

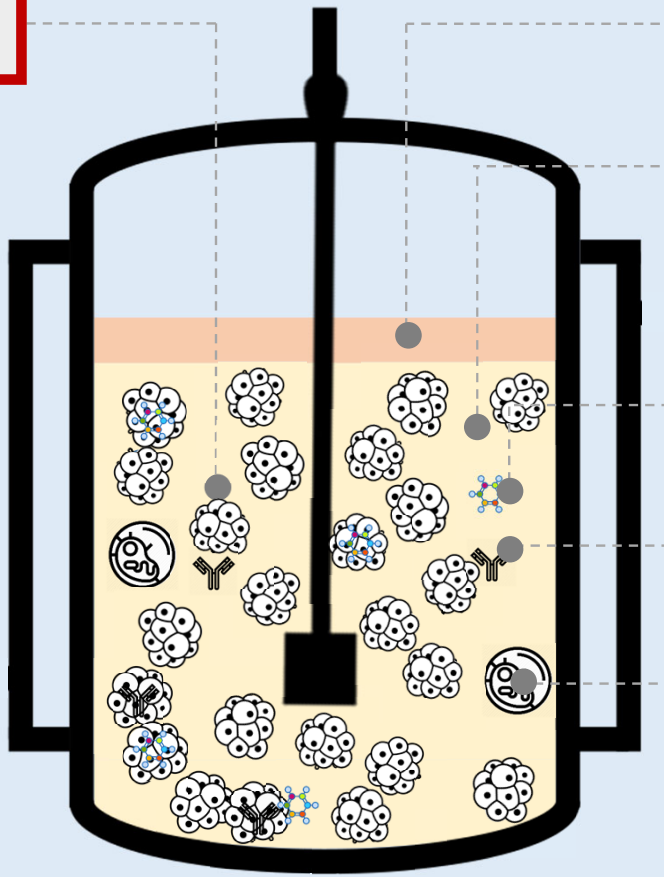
Experimental objectives and methods

Cells (Apheresis/Line)
Mixture, UCB possible
1 x 10^8 seed; USD 500 cryo

Cell line **NK-92**



It is a well known natural killer cell line, already tested clinically and successfuly used for CAR development



Serum (Animal/Human)
Reduce!

Brand specialized medium
Test and reduce!

Cytokines [IL2; IL15; IL17; IL21…]
IL2 & IL15

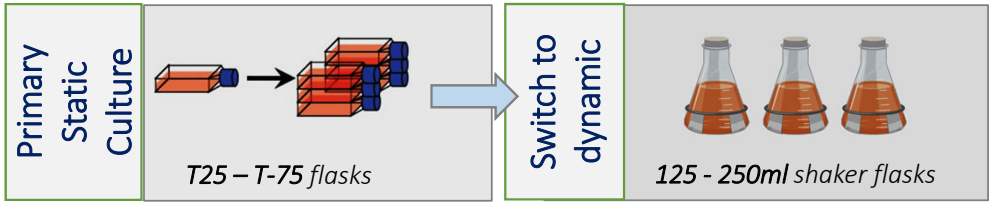
Activators [Anti-CD]
Avoid

Feeder cells
Avoid

Experimental objectives and methods

Objectives

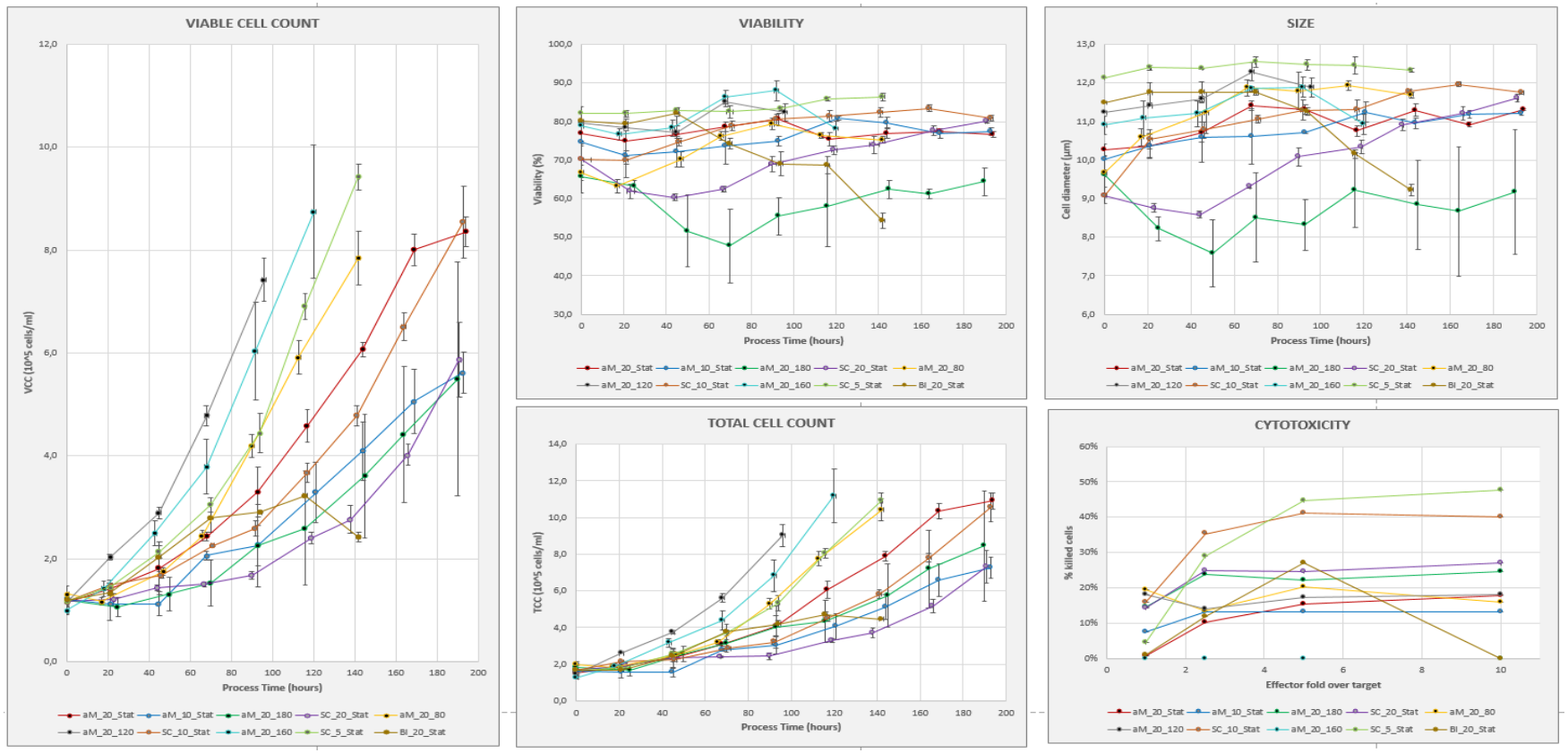
	Identify optimal culturing conditions in a dynamic system and under hypoxic conditions
	Evaluate the effects of scaling up in the context of costs reduction
	Test the effects of different serum concentrations on growth and functionality
	Generate a metabolic palette that enables correlations with proliferation and function
	Identify promising metabolites for further development of soft sensors



Methods

- VCC, TCC, viability, aggregation rate and the average cell diameter were measured with a Cedex HiRes device and trypan blue staining
- Enzymatic photometric assay for Glucose, Lactate, Acetate, Ammonia, Glutamine and Glutamate were performed on a Cedex BioHT device.
- LDH release based method was used for measuring cytotoxicity; K-562 cells were used as target.
- Amino acid concentrations were determined with an Agilent Eclipse AAA 3,5 µm 3x150 mm column using an in-needle derivatization method

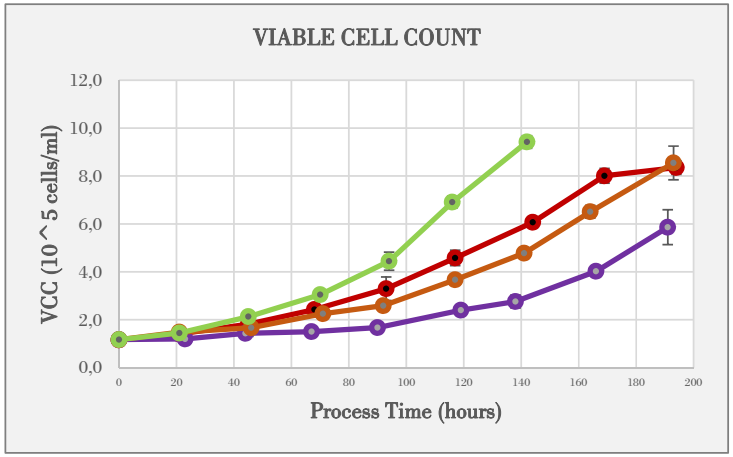
Creating a palette of proliferative/functional profiles

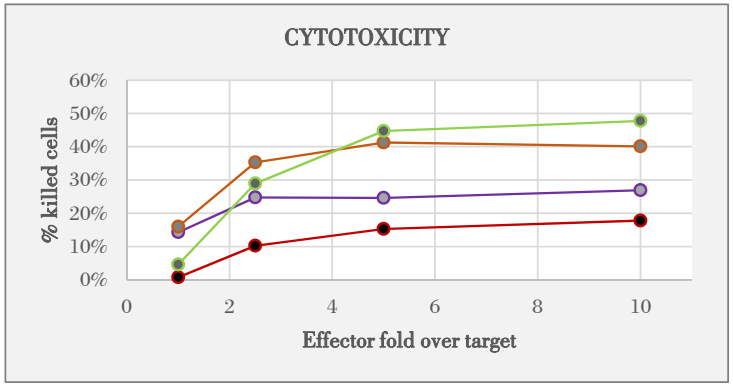


A series of cultures performed under different **dynamic, medium and serum conditions** allowed us to develop a palette of growth, functionality, viability and size behaviors

Effect of Serum and Shear on Metabolism and Functionality

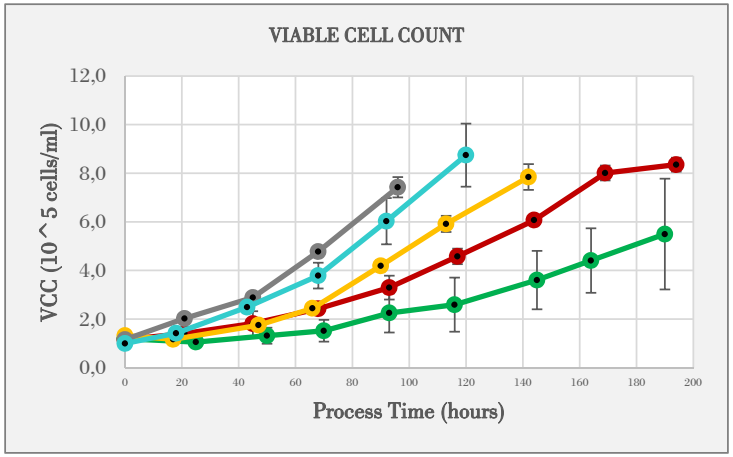
Decrease serum concentration to affect NK cells functionality and growth

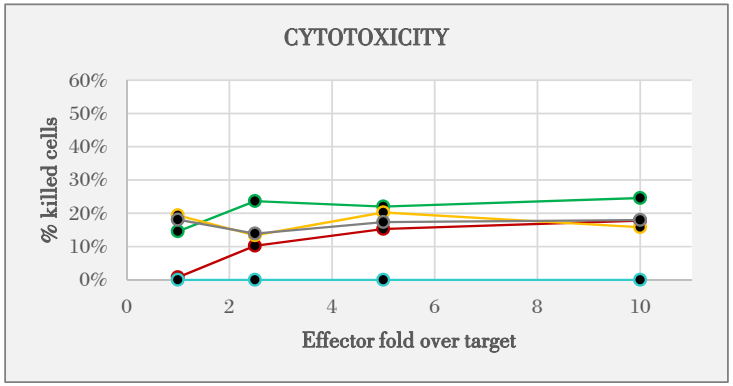




Supplemented serum: 5%, 10%, 20%, aMEM control

Increasing shear to affect growth and keep cell cytotoxicity

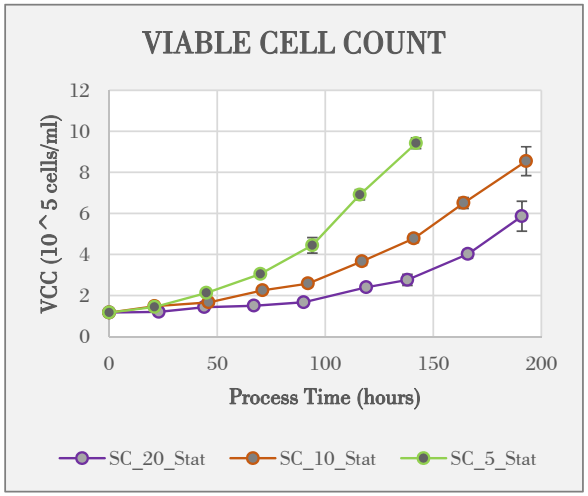


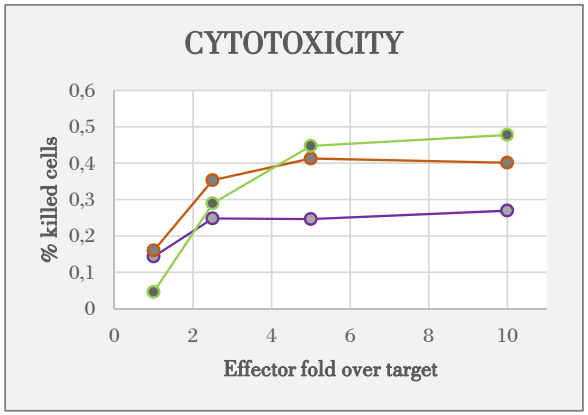


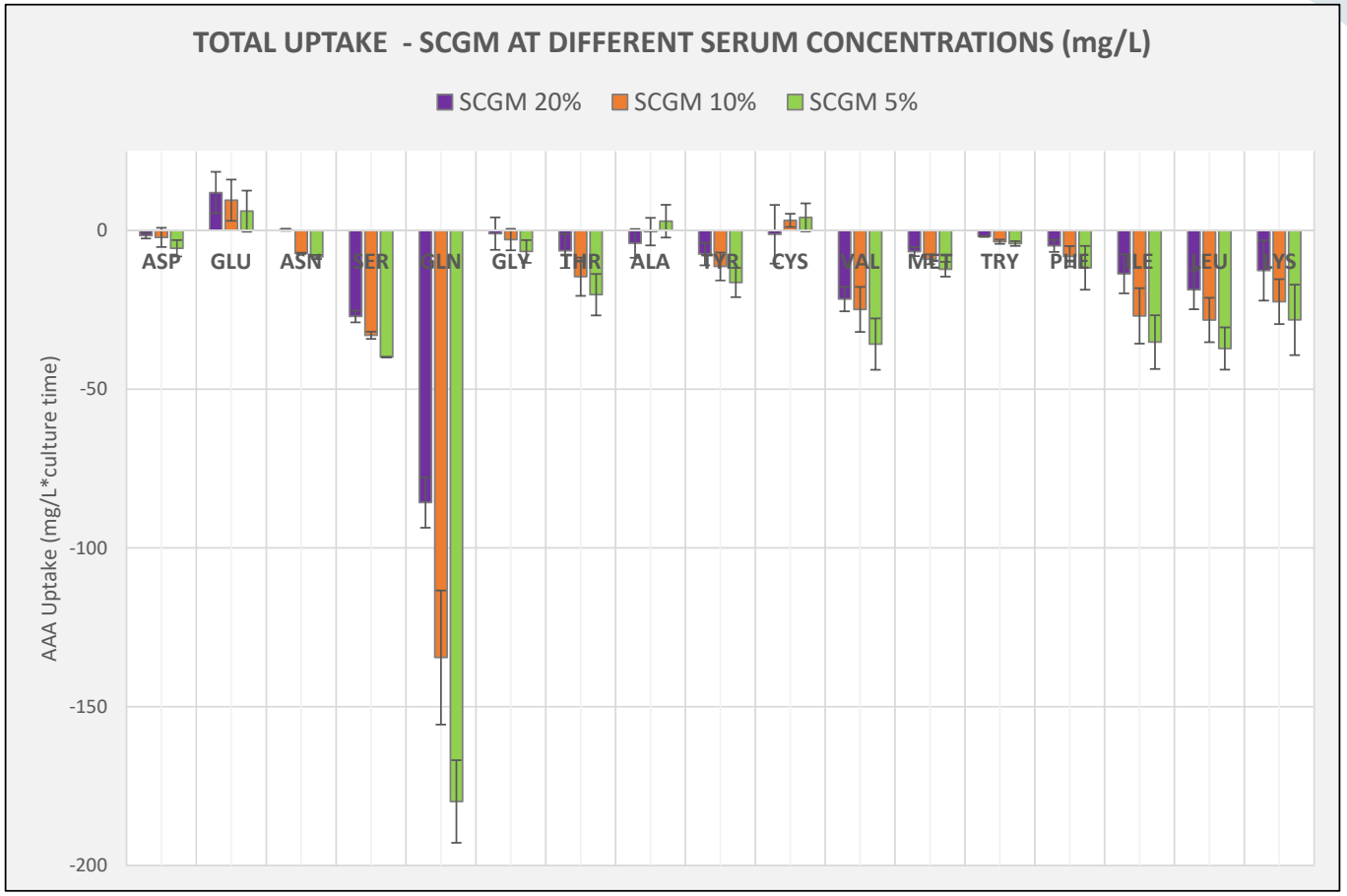
Shaking speed (rpm): 80, 120, 160, 200, static control

The concentration of serum affects both growth and cytotoxicity.
Shear affects the proliferation of the NK cells, but it has little effect on functionality.
Combining these two effects can induce a desired behavior.

Effect of Serum: Differences in amino acid uptake

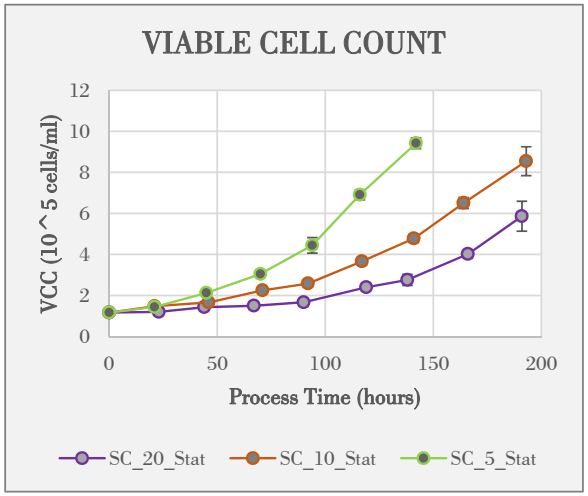


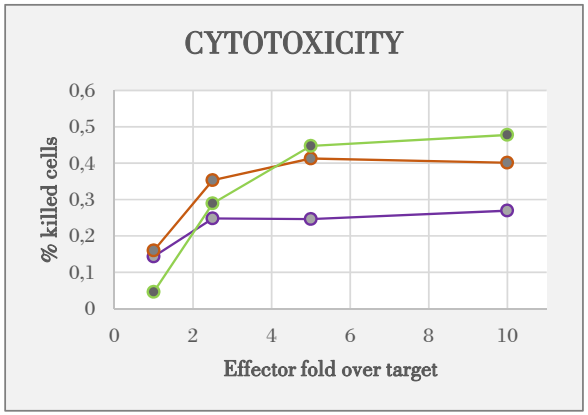


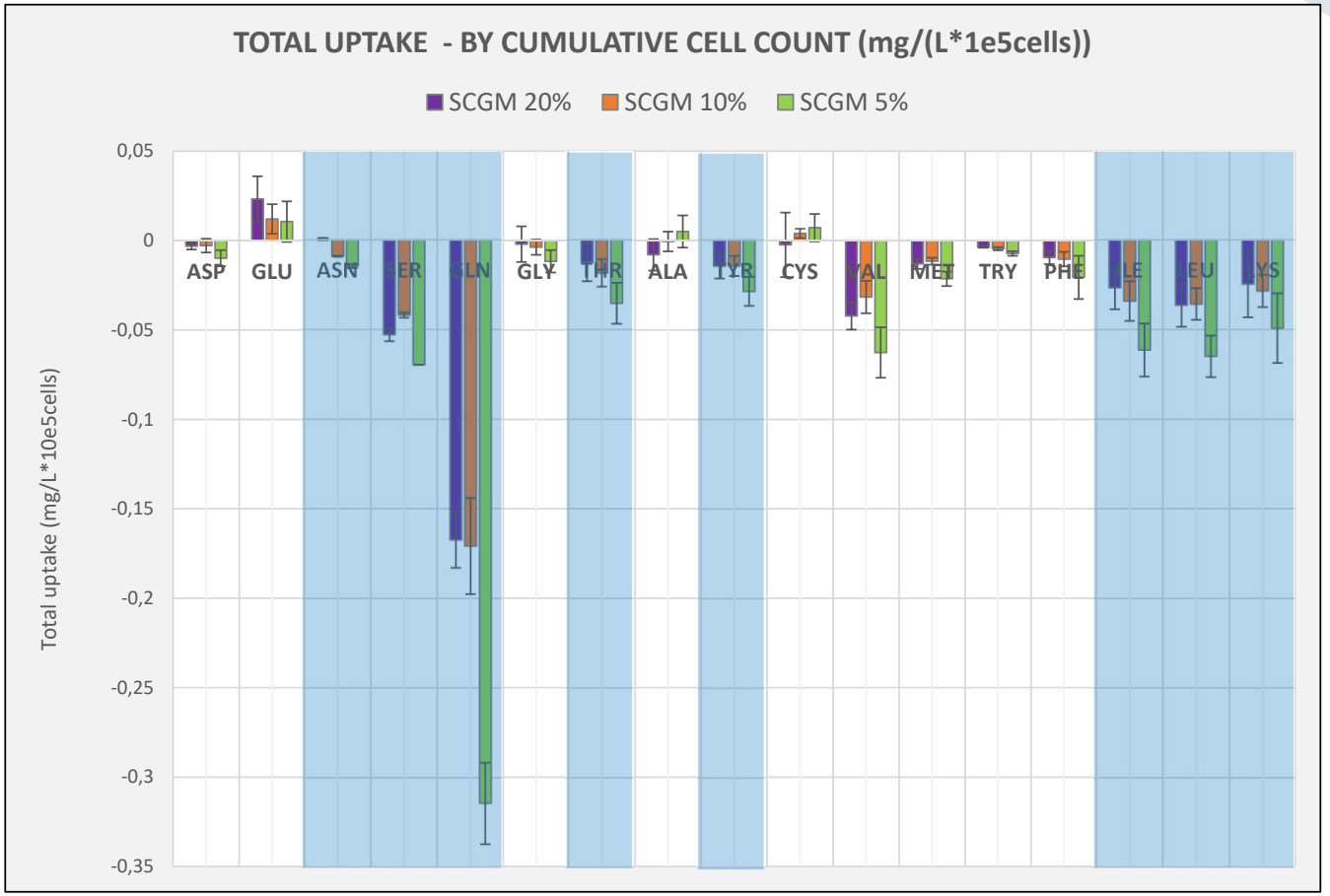


The total uptake of amino acids also exhibit differences that can be related to the cytotoxicity of the cultured cells.

Effect of Serum:
Differences in amino acid uptake

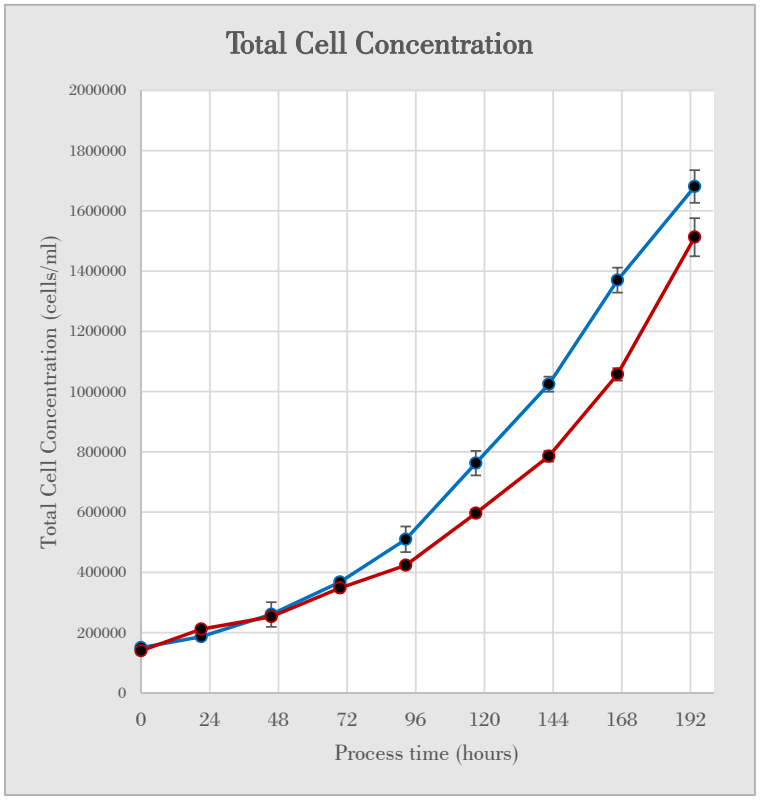


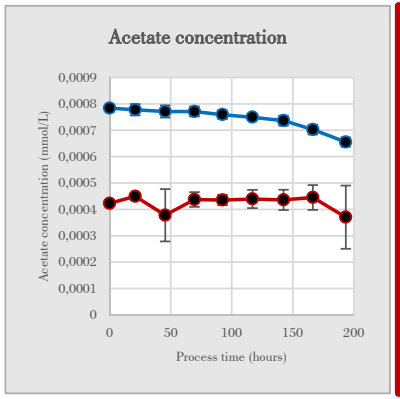


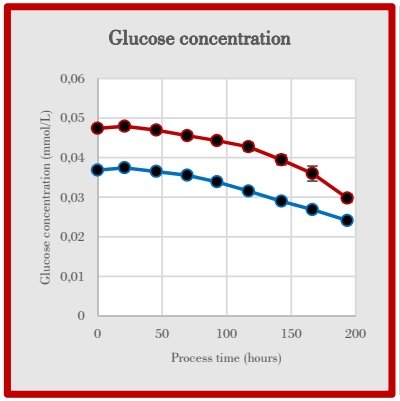


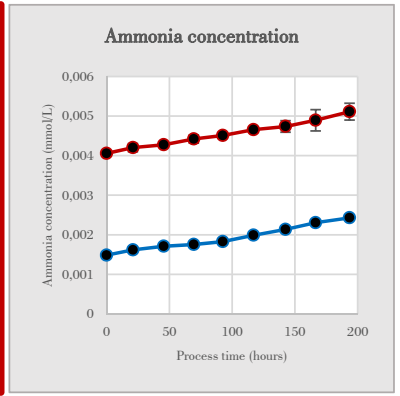
The total uptake of asparagine, serine, threonine, tyrosine, isoleucine, leucine and lysine are probable good predictors for functionality

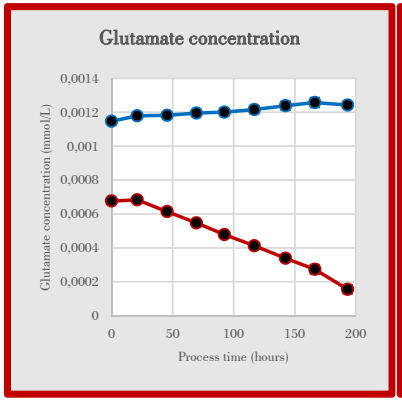
Combining Serum Amount and Shear: Concentration Profiles

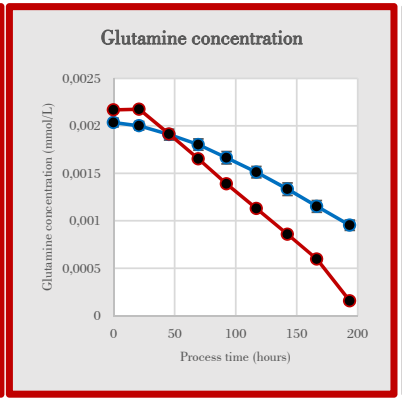


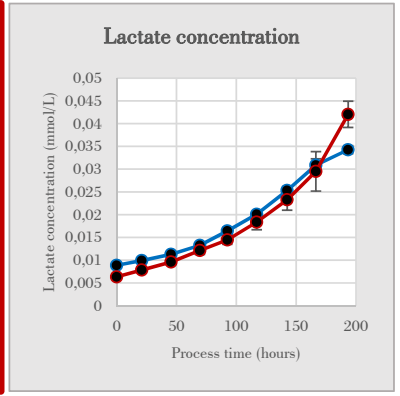










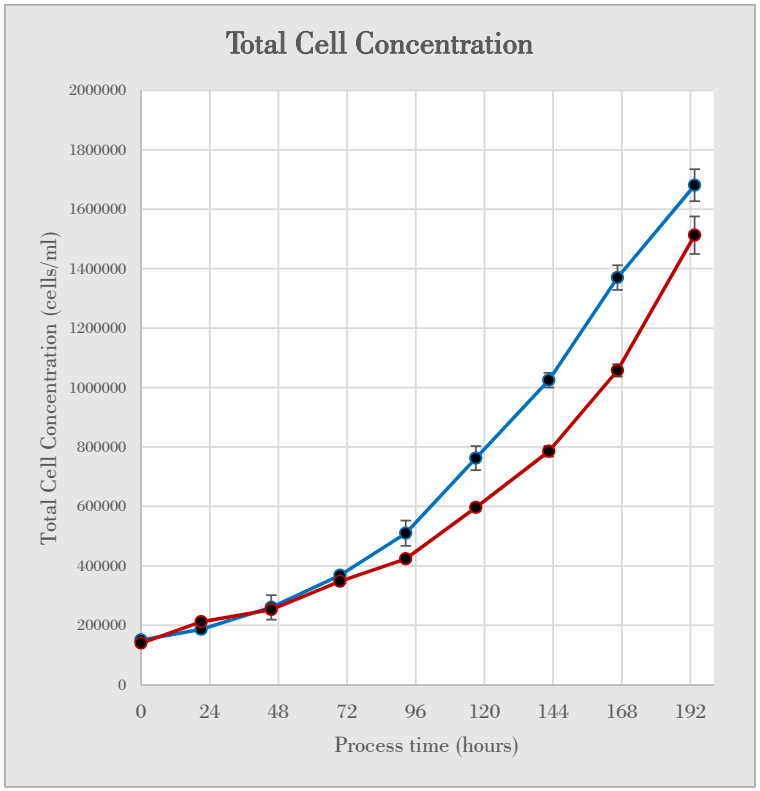


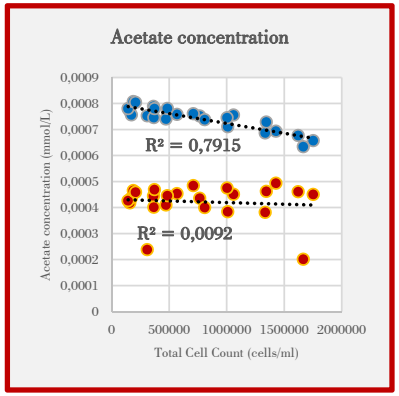
80 rpm + 20% serum Vs. Static + 10% serum

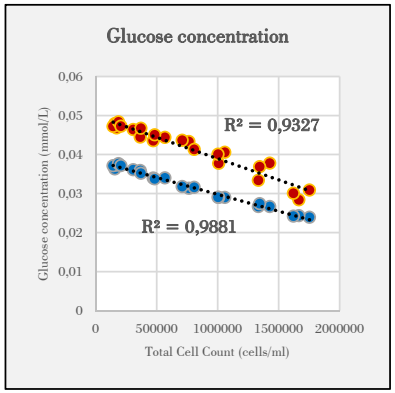
PROTOCOL-A: 18% cytotoxicity - **PROTOCOL-B: 45%** cytotoxicity

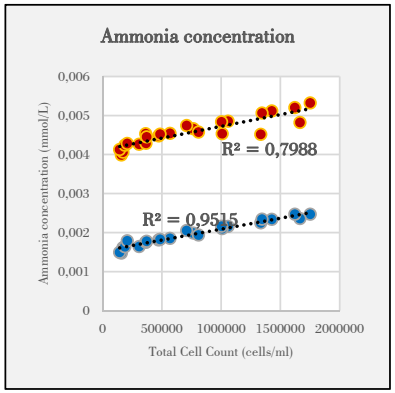
Two cultures exhibiting similar growth patterns but different functional profiles and also differences in the overall consumption of glucose, glutamine and glutamate.

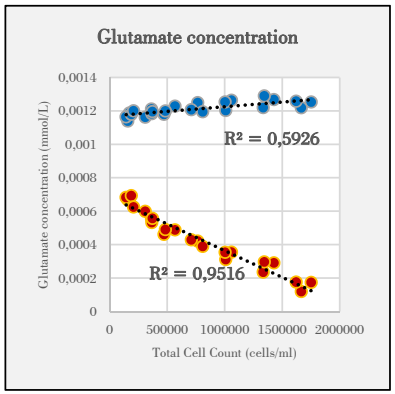
Combining Serum Amount and Shear: Time Resolved Stoichiometric Analysis

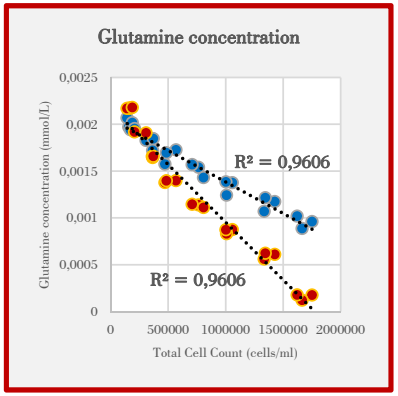


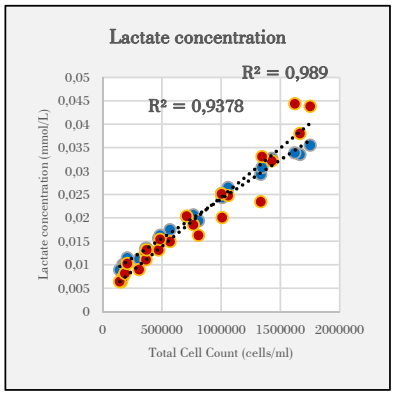










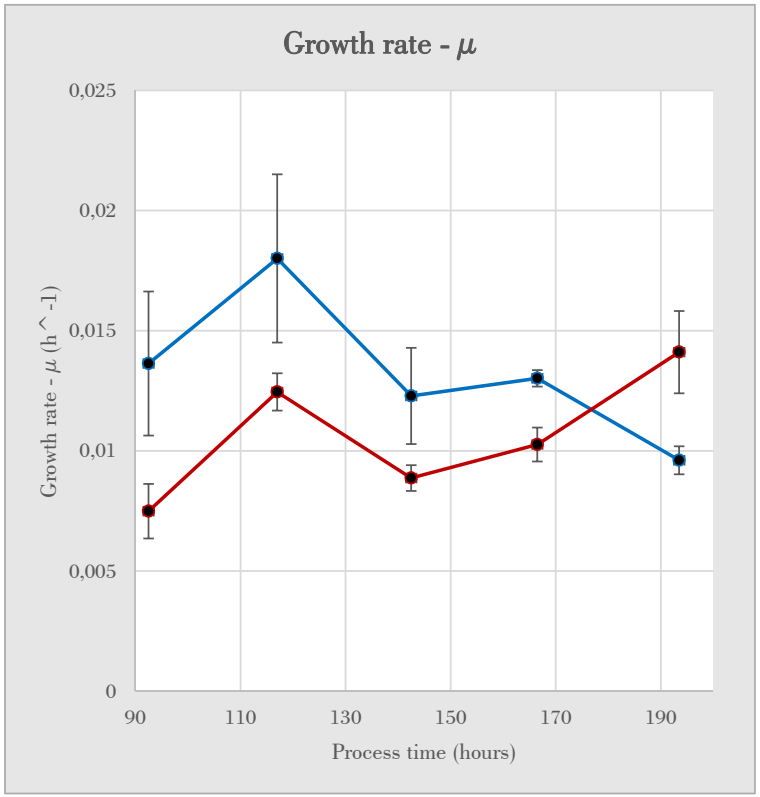


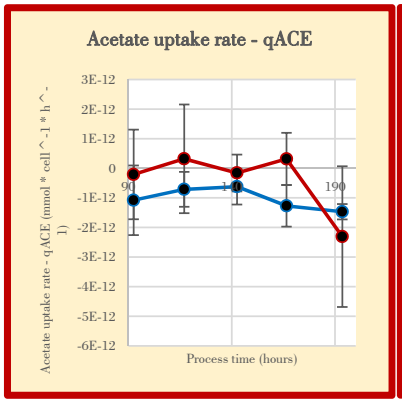
80 rpm + 20% serum Vs. Static + 10% serum

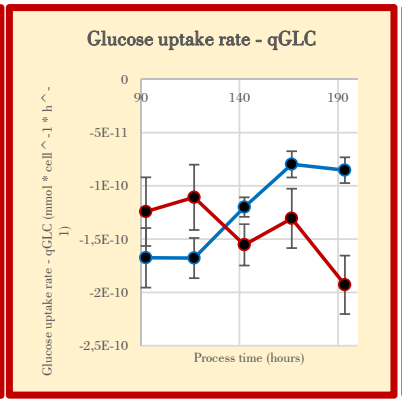
PROTOCOL-A: 18% cytotoxicity - **PROTOCOL-B: 45%** cytotoxicity

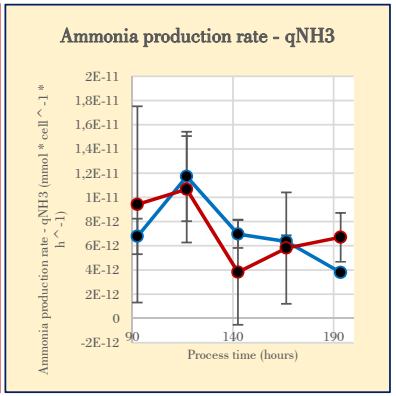
Differences can be highlighted by comparing the correlations the cell concentration and the metabolite of interest = Yields. Acetate and Glutamine can be clear markers of differences in functionality.

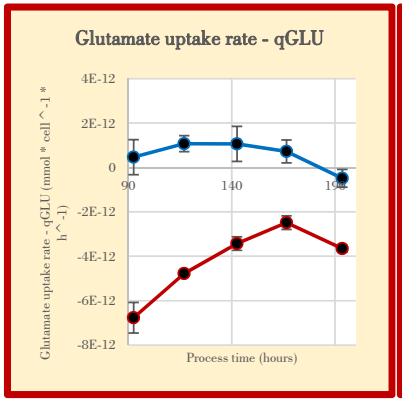
Combining Medium Change and Shear: Analysis of Specific Metabolic Rates

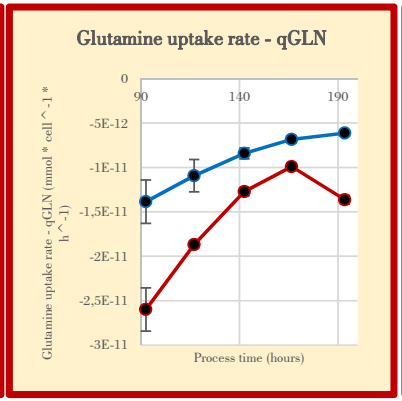


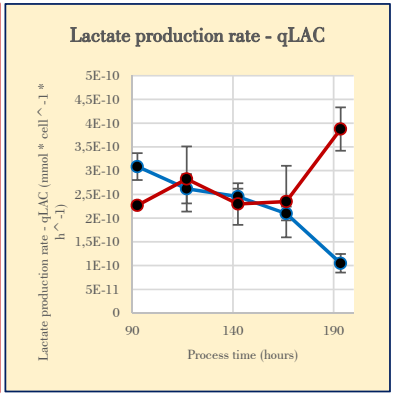












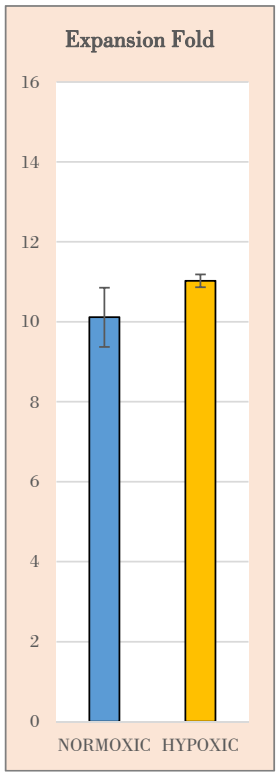
80 rpm + 20% serum Vs. Static + 10% serum

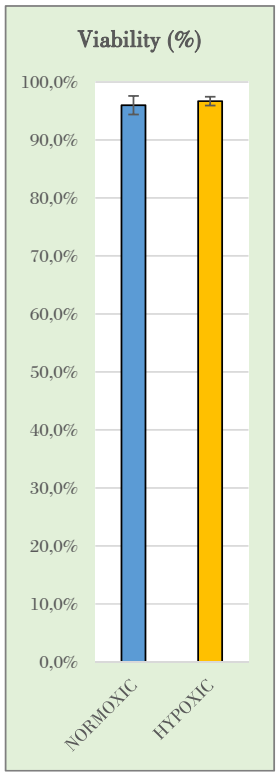
PROTOCOL-A: 18% cytotoxicity - **PROTOCOL-B: 45%** cytotoxicity

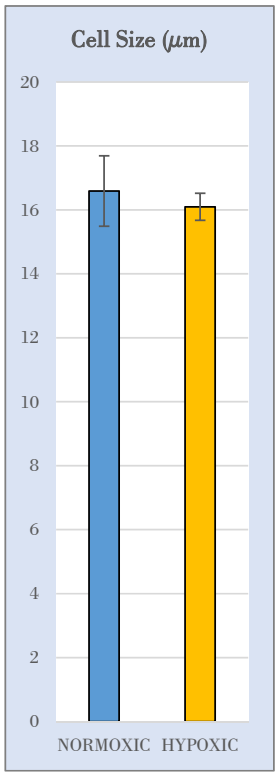
Analysis of growth, uptake and production rates: Almost every metabolite is found to have a very specific profile depending of the process parameters

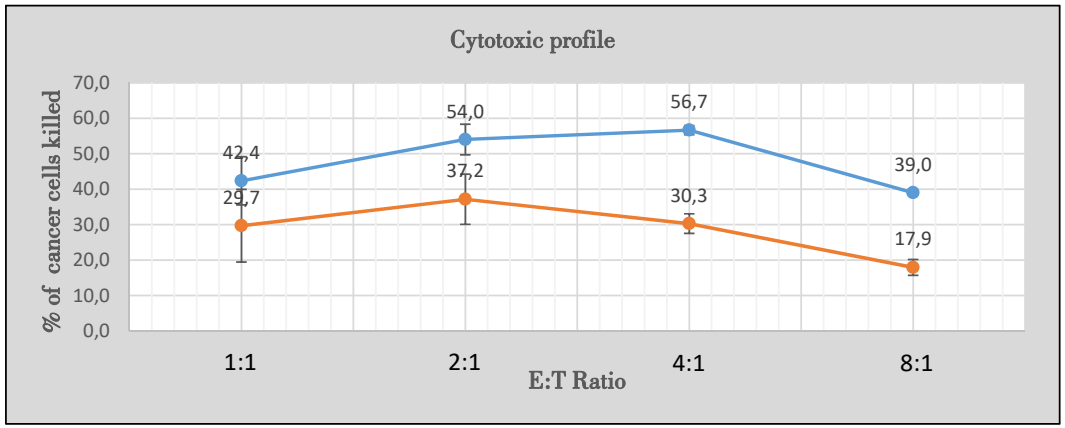
Hypoxic Conditions

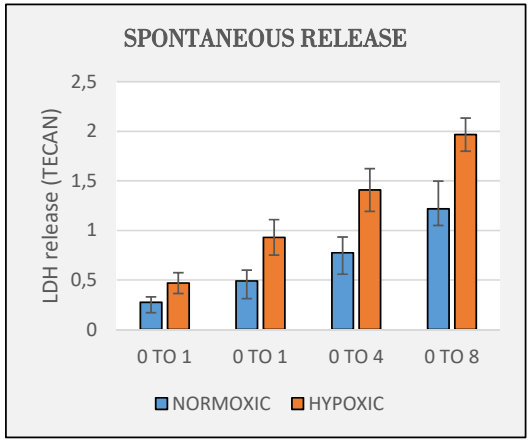
Normoxic Hypoxic











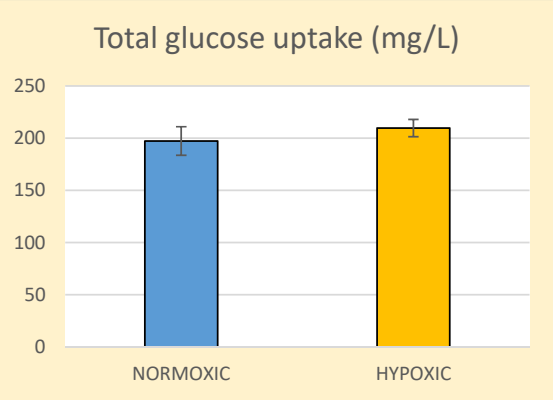
Normoxic cells have a better cytotoxic profile at every Effector / Targert (E:T) ratio

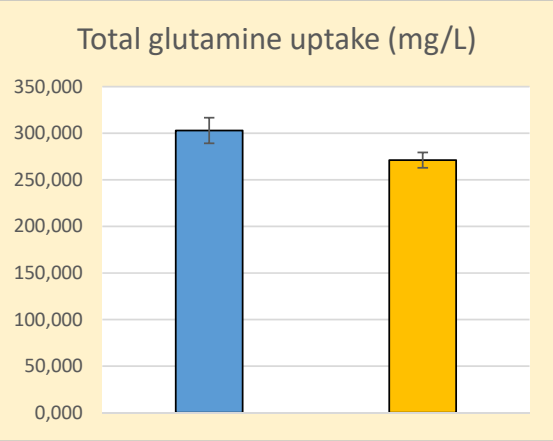
The decrease of cytotoxicity at high E:T ratio might indicate a saturation.

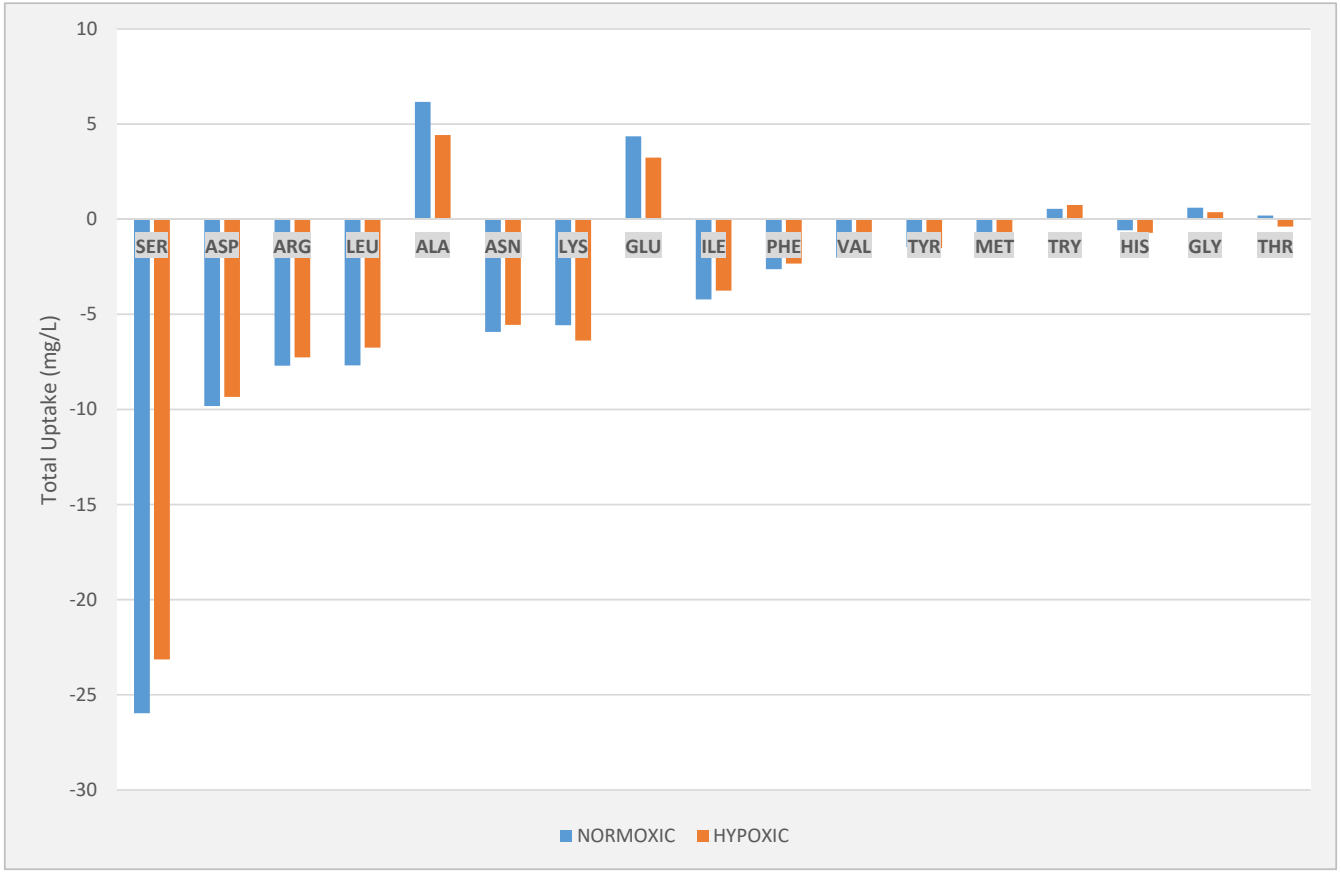
The higher LDH release during the spontaneous control for hypoxic NK cells might indicate a cell death.

Hypoxia can also be used as a process parameter to induce specific growth/functional behaviors.

Differences in Uptake under Hypoxic Conditions





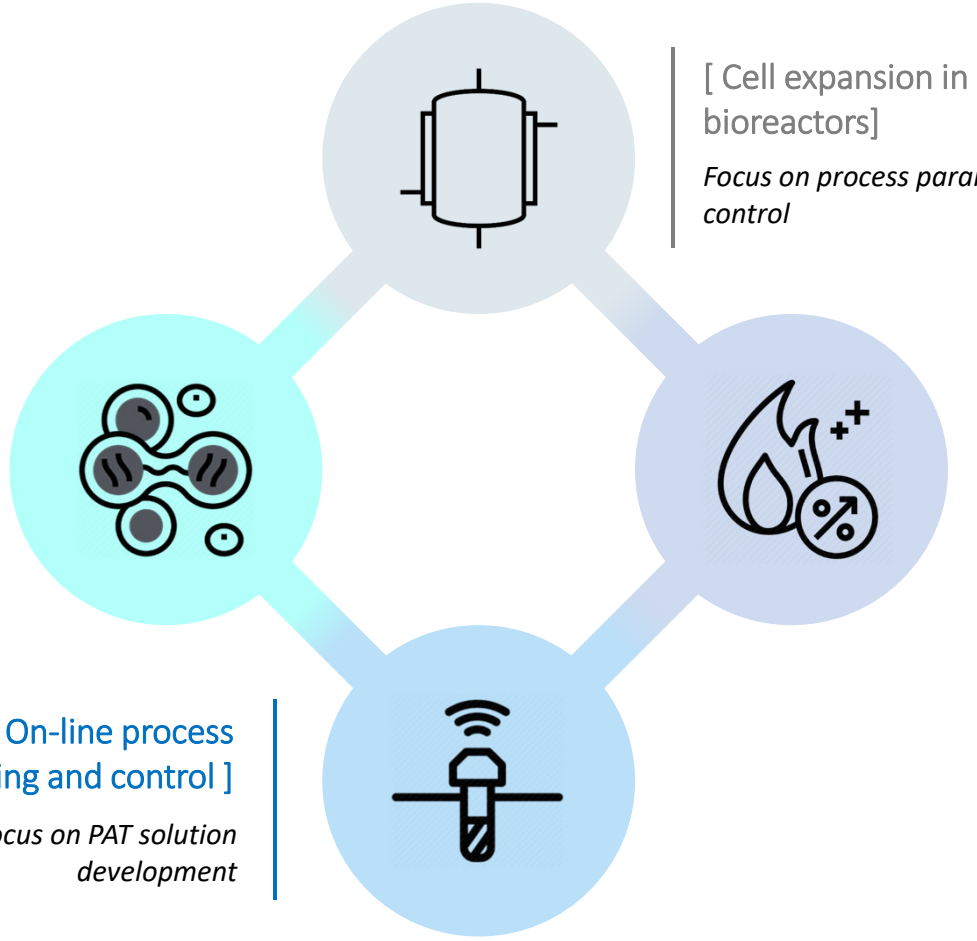


The pattern of amino acid uptake pattern is different from the one induced by different serum concentrations. Hypoxia may have a independent effect on cell cytotoxicity.

Main research directions

[Cell expansion in bioreactors]

Focus on process parameters and control



[NK cell growth modeling]

Focus on expansion kinetics and point of harvest determination

[Raw material characterization]

Focus on raw material's variability and their influence on culture

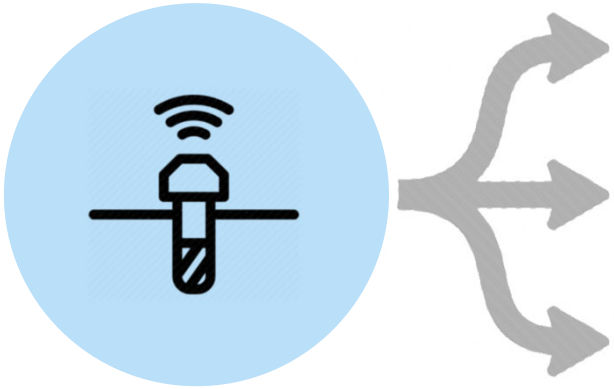
[On-line process monitoring and control]

Focus on PAT solution development

On-line process monitoring and control

Our hypothesis: the **on-line monitoring and control** of the expansion process in bioreactors allow to control the cell proliferation according to the cells' needs providing more successful batches with consistent product quality.

Developing such control techniques requires the combination of bioengineering and data science approaches.



Arsenal of different bioreactor **sensors** can measure CPPs on-line, non-invasive but relevant to CQAs.

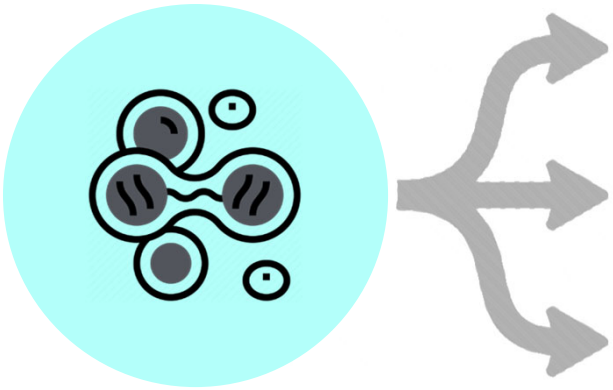
On-line sensors would help to determine the **optimal point of harvest** and assure the highest possible expansion fold.

On-line data can be used to generate a process **"fingerprint"** that can be used for batch-to-batch comparison.

NK cell expansion modeling

Our hypothesis: a process model based approach for process optimization can bring in shorter time the desired robust and stable expansion process with higher folds.

Mechanistic understanding of the expansion process helps to set up targeted experiments on relevant process parameters.



A **model** on the basis of **cell metabolism** would be beneficially used for improvements in expansion kinetics and process control.

A **digital twin** of the expansion process that is based on cell growth could help in the tighter control of process parameters.

Model could be used to **adjust** the **process parameters according** to raw material variability.

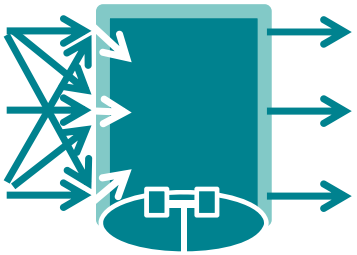
Research rationale: methodological approach

Scalable Process Understanding

Parameters & Materials

Capture & Implement Digital Twins

Inverse Analysis



Predictive Processing

Process Analytical Technology

Quality & Performance

Optimized & Predictive Control

Conclusions

- We achieve process understanding of NK expansion processes by using our established bioprocess technological methods.
  - Dynamic culture conditions can be beneficial for culturing the NK-92 cell line.
  - Uptake and production rates show different patterns during culture and relate to the functionality of the cells.
  - Combining oxygen concentration, shear stress and serum supplementation can be used to decouple functionality and metabolism.
- Process Understanding is of central importance for developing mechanistic models able to integrate functionality and growth and control despite raw material variability.
- Next steps: dynamic bioreactor systems; PAT, digital twins!

Thank you
for your attention!

Univ.Prof. Dr. Christoph Herwig
Vienna University of Technology
Institute of Chemical Engineering
Research Division Biochemical Engineering
Gumpendorferstrasse 1a/ 166 - 4
A-1060 Wien, Austria
emailto: christoph.herwig@tuwien.ac.at
Tel (Office): +43 1 58801 166400
Tel (Mobile): +43 676 47 37 217
URL : http://institute.tuwien.ac.at/chemical_engineering/bioprocess_engineering/EN/