Aspects and Implications of Physiological Process Control

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Agenda

- Motivation
- State of the Art – Technical control
- Physiological Control
  - Which variable to control?
  - Online BM estimation
- Applications
- Summary

Motivation

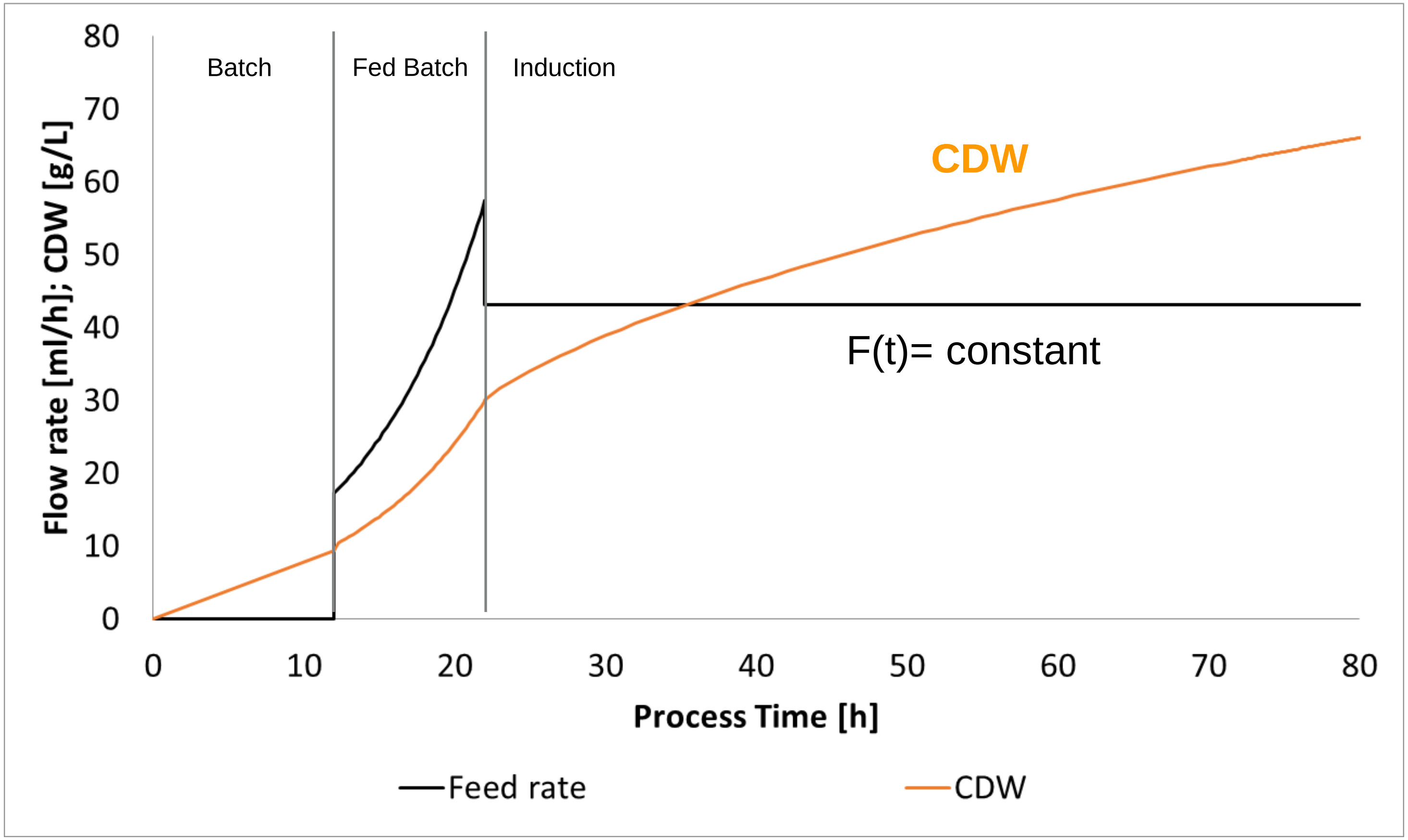
Process Development

- Max. Productivity
- Constant product quality
- Robustness/Stable scale up
- Transferability of gained knowledge
  - Time to market

State of the Art

Technical Control

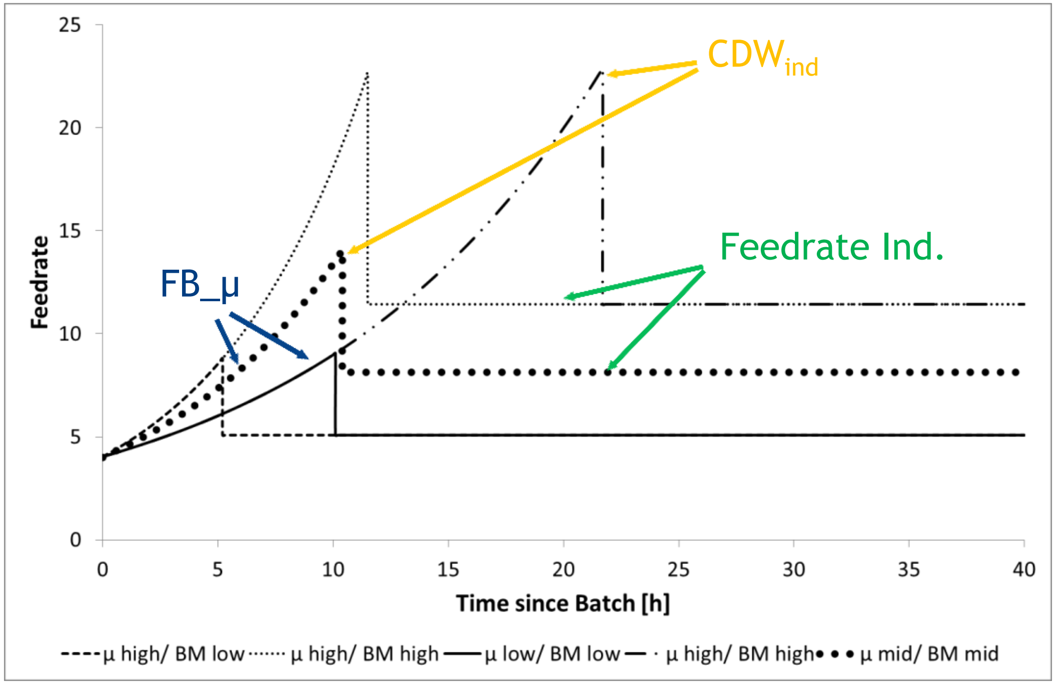
Current driver: Simplicity – constant feed rate

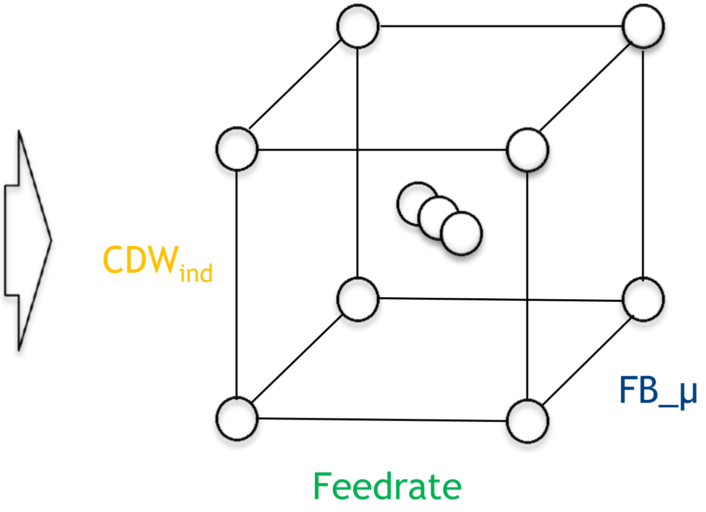


State of the Art

Technical Control

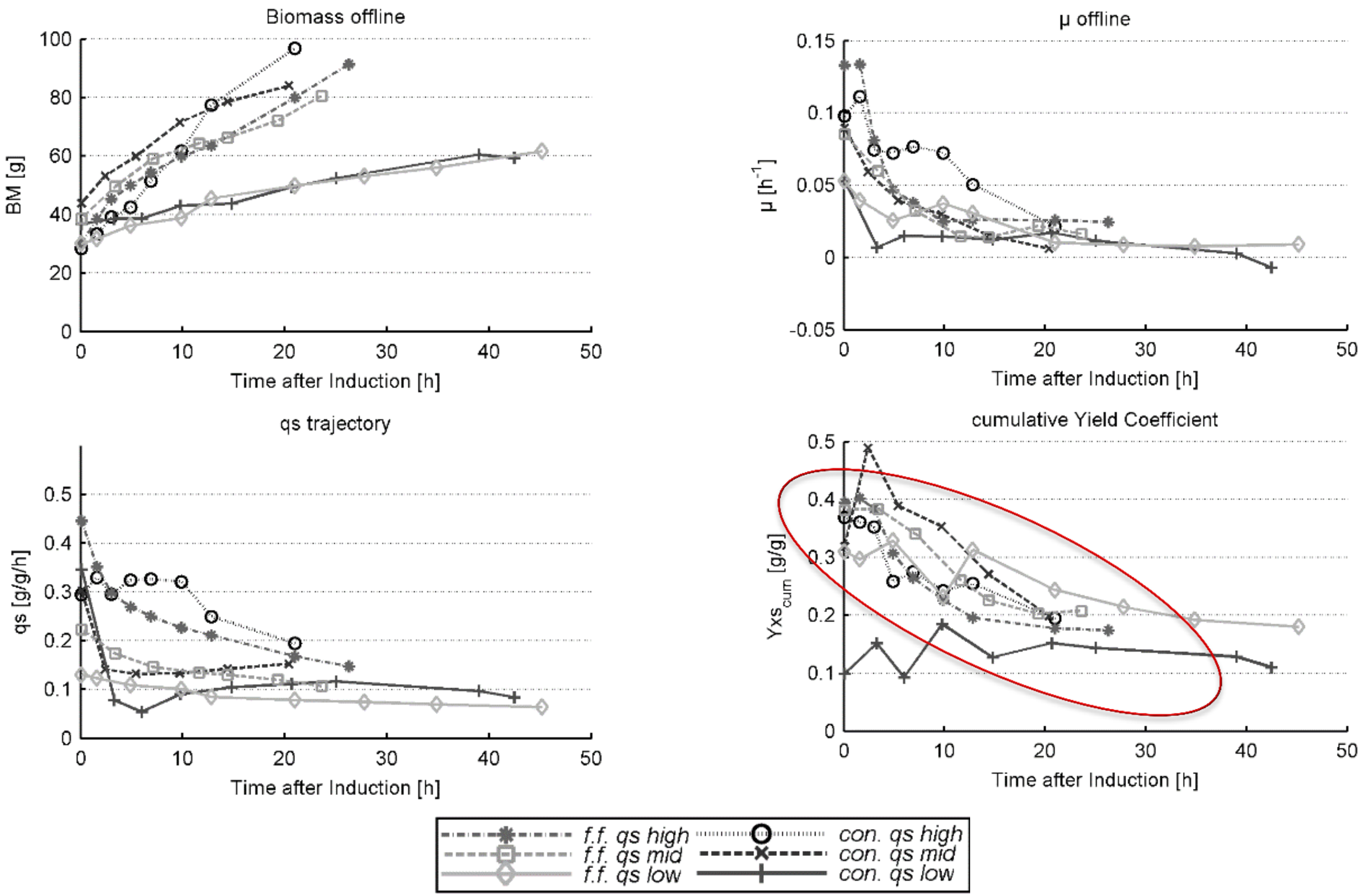
Exemplary process development routine





State of the Art

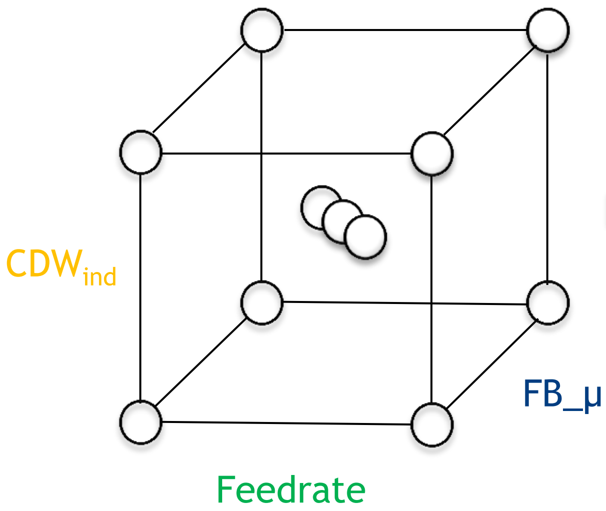
Technical Control



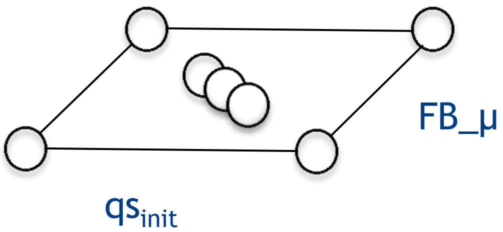
State of the Art

Technical Control

- From technical to physiological <u>factors</u>
  - Combination of feedrate and CDW_{ind} to qs_{init}

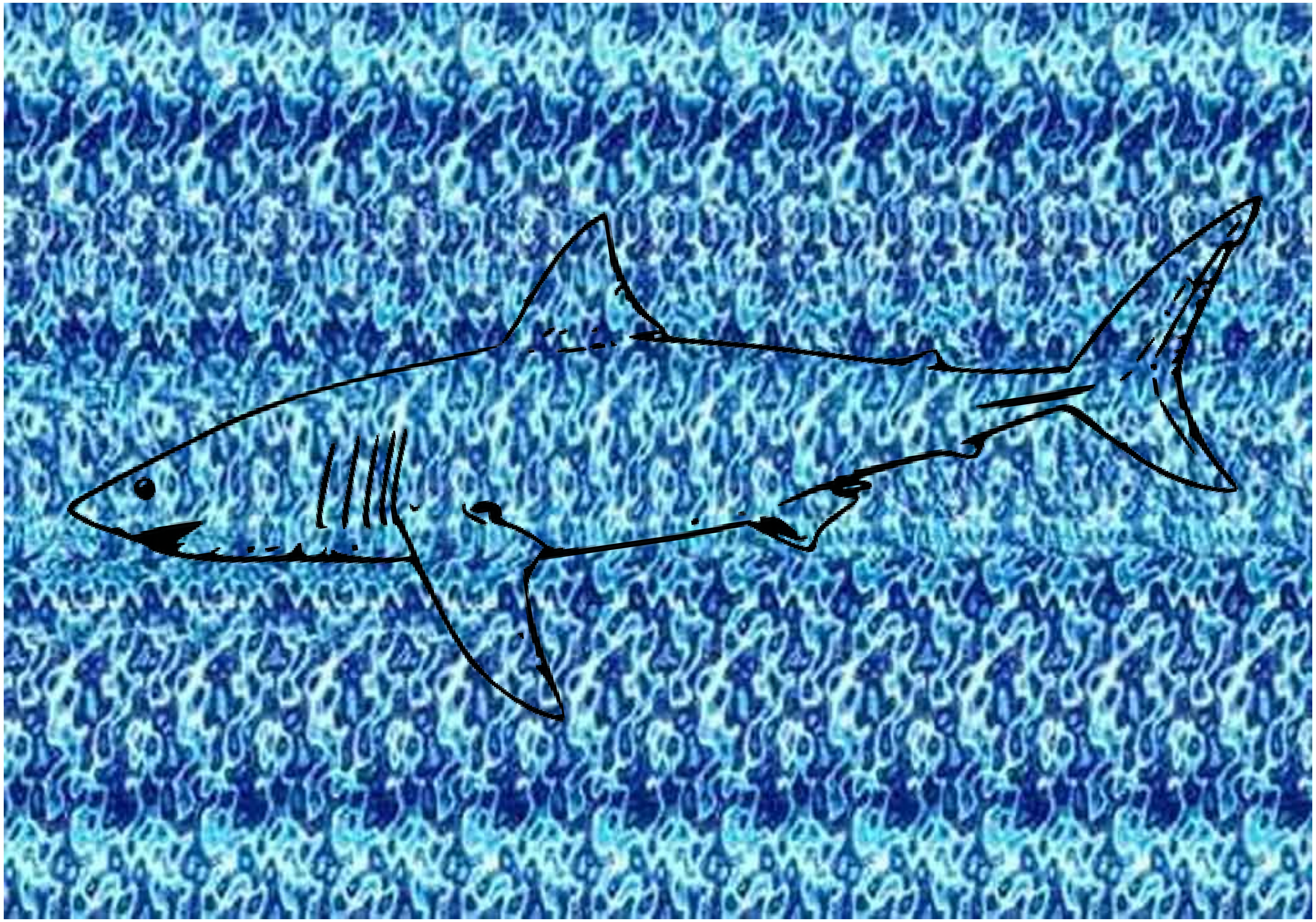


$$qs_{init} = \frac{Feedrate\ [\frac{g}{h}]}{CDW\ [g]}$$



Wechselberger et al 2012: Efficient feeding profile optimization for recombinant protein production using physiological information

All Good?

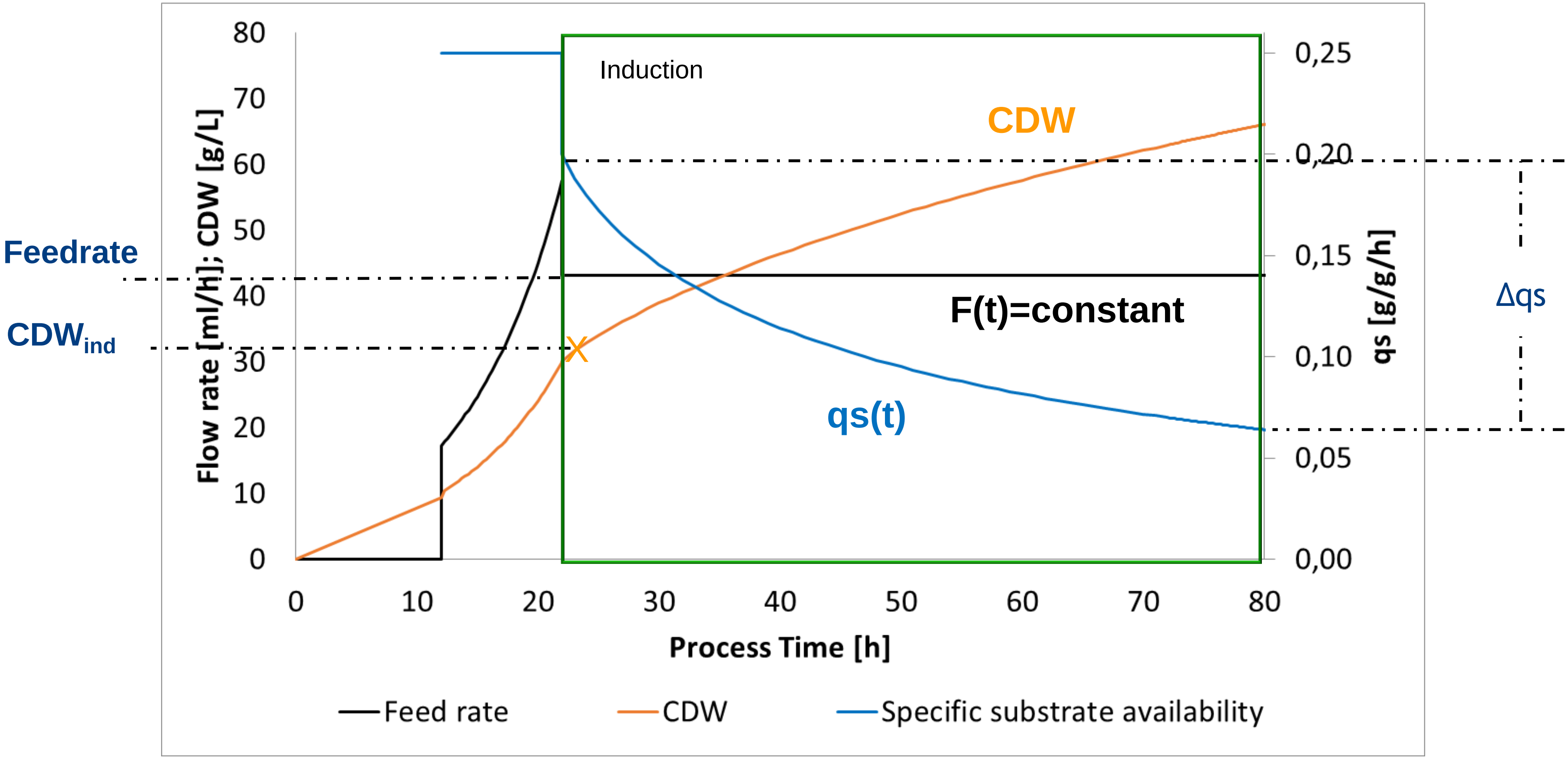


Simply Water? - See the shark? (Magic eye image)

State of the Art

Technical Control – Hidden factors

Current Driver: Simplicity – constant Feed Rate

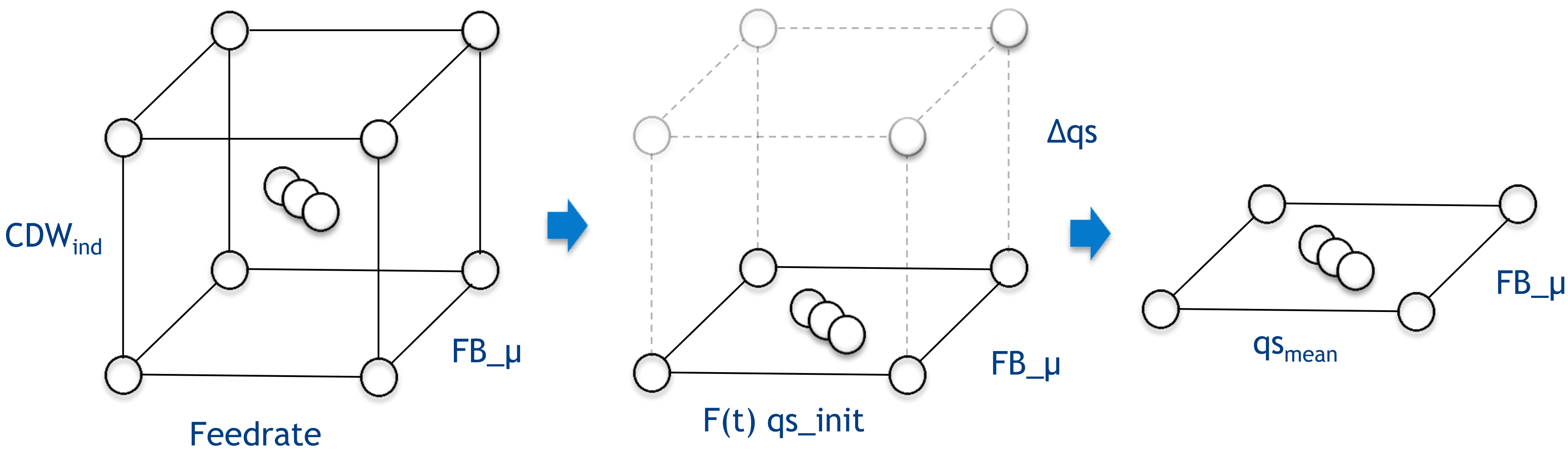


Result: UNCONTROLLED decrease of qs - Δqs

Physiological Process Control

addressing "hidden" factors

- From physiological factors to physiological descriptors
  - Physiological CONTROL necessary



Motivation

Revisited

- Max. Productivity
- Constant product quality
- Robustness/Stable scale up
- Transferability of gained knowledge
  - Time to market

- Problem statement of state of the art
  - Uncontrolled/undefined physiological status of the culture
    - Hidden factors
  - Focus of feeding strategy is “the reactor”

➢ Put the cells in the center of focus

Technical vs. Physiologic Control

Physiologically uncontrolled

- E.g. volumetric constant feed
- Neglects cell physiology
- Poor process knowledge, unknown effect of physiological changes

Physiologically controlled

- Dynamically adapted feed rate
- Active control of cell physiology (μ; qs; ….)
- Enhanced process knowledge
- Scale Up/Transferability!

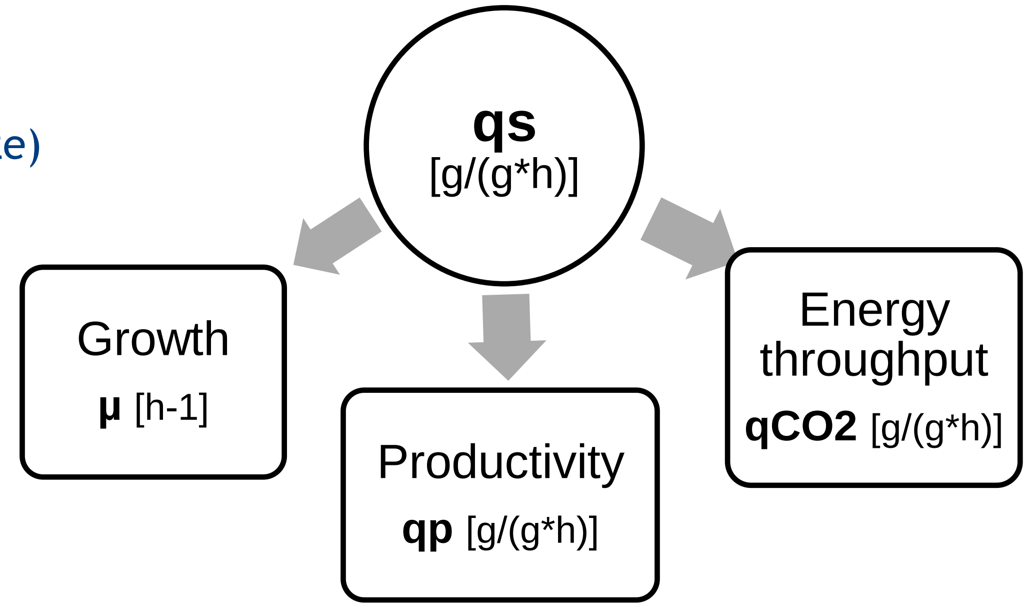
» Which physiological variable to control?

» Requires online BM estimation

Physiological Process Control

Which physiological variable to control?

- Accessible specific rates:
  - qs (specific substrate uptake rate)
  - μ (specific growth rate)
  - qCO2 (specific carbon evolution rate)
- Common denominator:
  - Energy supply (feed)
- qs has the hierarchically highest position
  - Providing energy, growth independently
  - Online estimation and prediction of productivity possible



Physiological Process Control

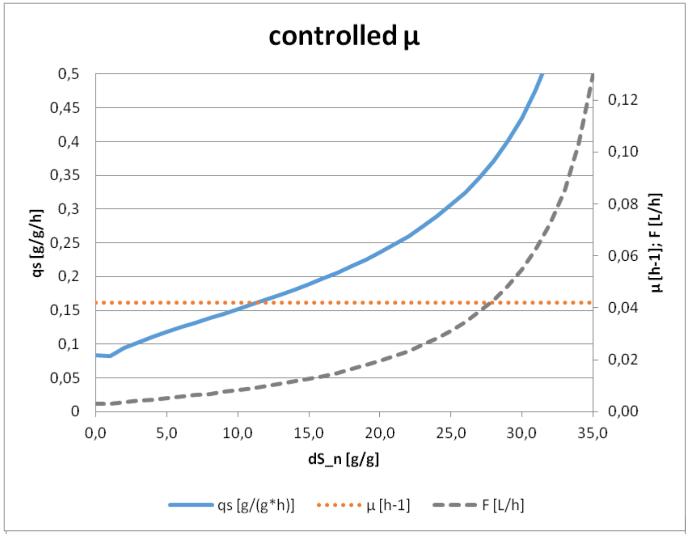
Which physiological variable to control?

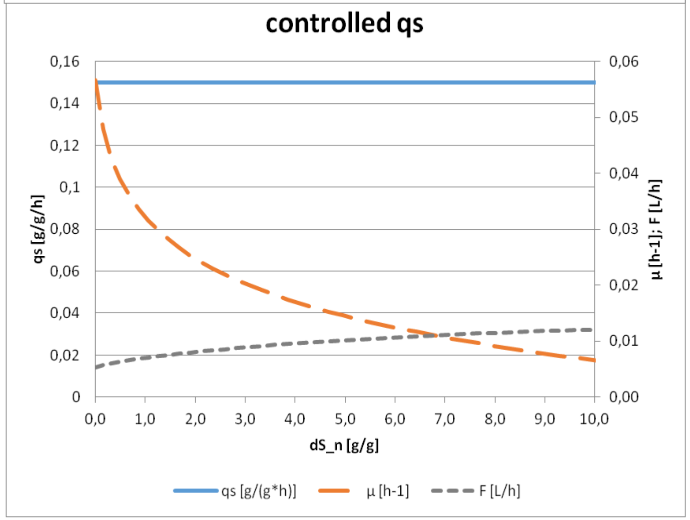
- Induction phase
  - Recombinant protein production competes with growth
  - qs/µ = Yxs(t) (variable!)

- µ Control during induction $\mu[\frac{1}{h}] = \frac{r_x\left[\frac{g}{h}\right]}{X[\mathrm{g}]}$
  - Error prone BM estimation is used twice
  - qs - is boosted
  - Feedrates – technically not feasible!

- qs Control during induction $qs[\frac{g}{g*h}] = \frac{r_s\left[\frac{g}{h}\right]}{X[\mathrm{g}]}$
  - Error prone BM estimation is used once
  - Steady µ decrease
  - Feedrate increase of low dynamics

➢ qs control appears technically more feasible

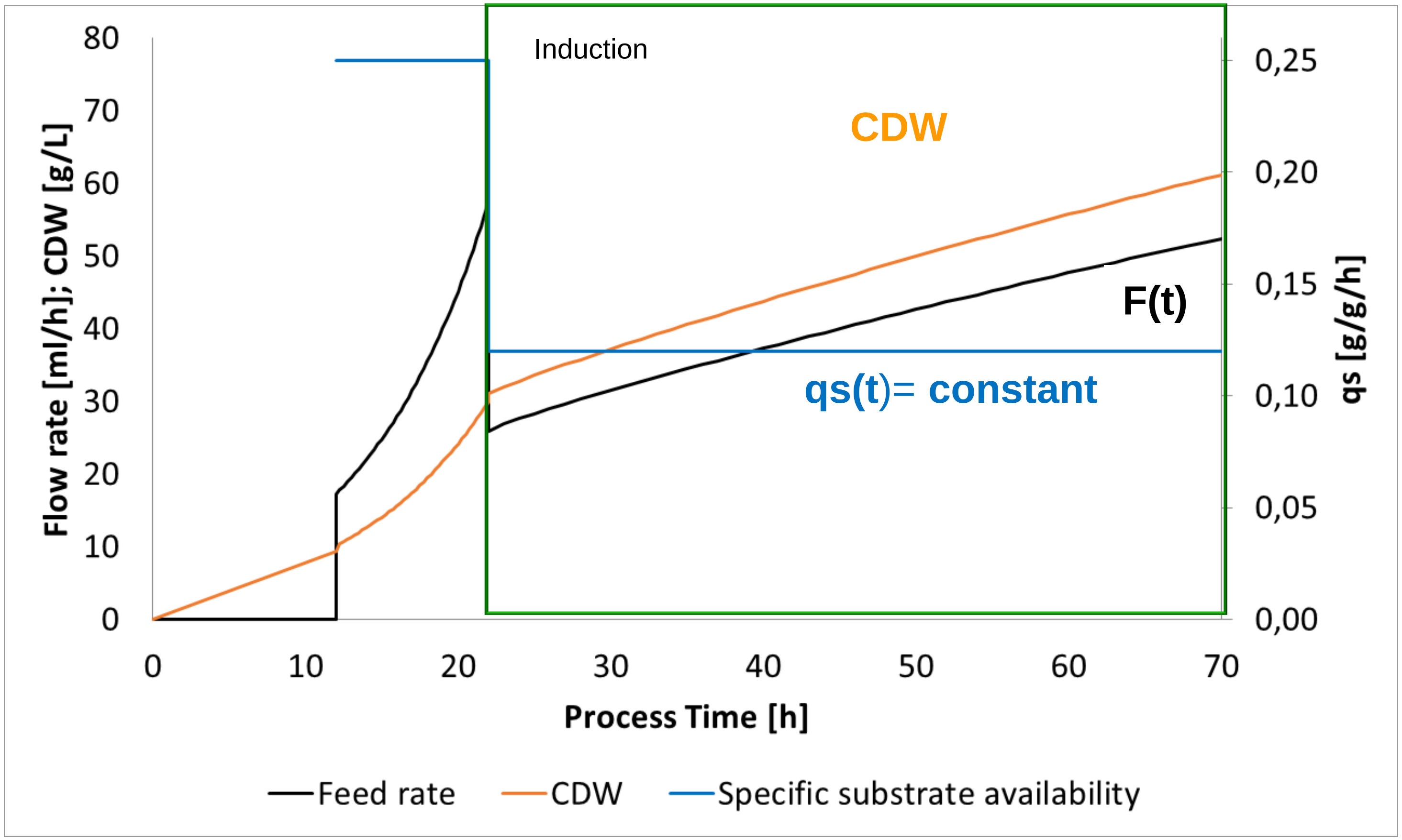




Physiological Process Control

Which physiological variable to control?

Controlling Physiology



Physiological Process Control

Required BM Estimation

- Problem statement
  - qs control requires BM estimation for dynamic feed rate adaption

$$F(t) = \frac{qs \; * X\,(t) * \varphi}{c}$$

$F(t)$...	*Flow rate [g/h]*
$X\,(t)$ ...	*Biomass [g]*
c...	*Feed concentration [g/L]*
φ...	*Density Feed [g/L]*
qs...	*specific substrate rate [g/g/h]*

- Output:
  - Substrate feed rate F(t) [g/h]
  - Feeding rate needs to be adjusted for changes in BM(t)
  - Calculation cycle for F(t) every 5 min

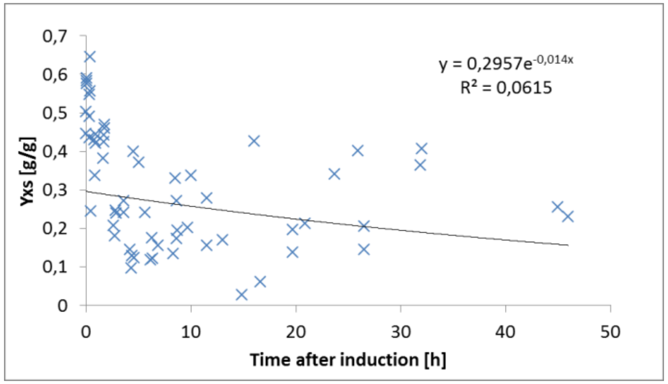
- Required Inputs:
  - Accurate biomass estimation is required -> BM(t)
  - Reactor content monitoring (Biomass reduction; sampling)

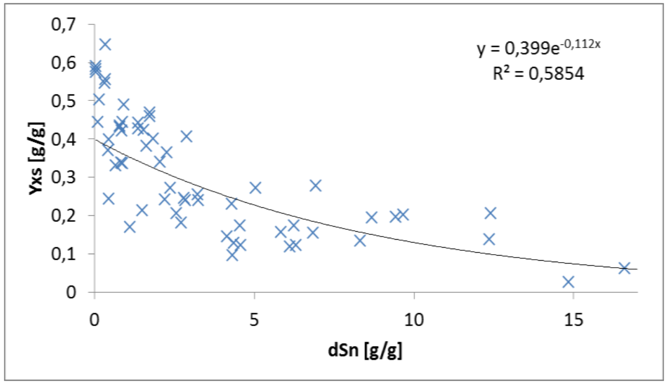
Physiological Process Control

Required BM Estimation

<u>Yx/s trajectory</u> based control principle:

- Yx/s trajectory function based on historical data
- Yx/s as a function of the fed substrate (dSn)
  - Fed substrate is normalized on BM @ induction ->**dSn**
  - a dimension to quantify the cell age/experienced metabolic stress
- Open loop controller (no feed back loop)
  - beforehand feed profile calculation



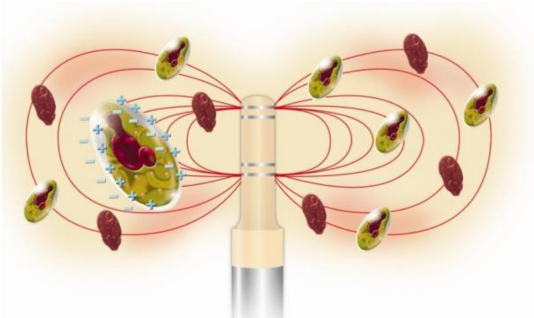


Physiological Process Control

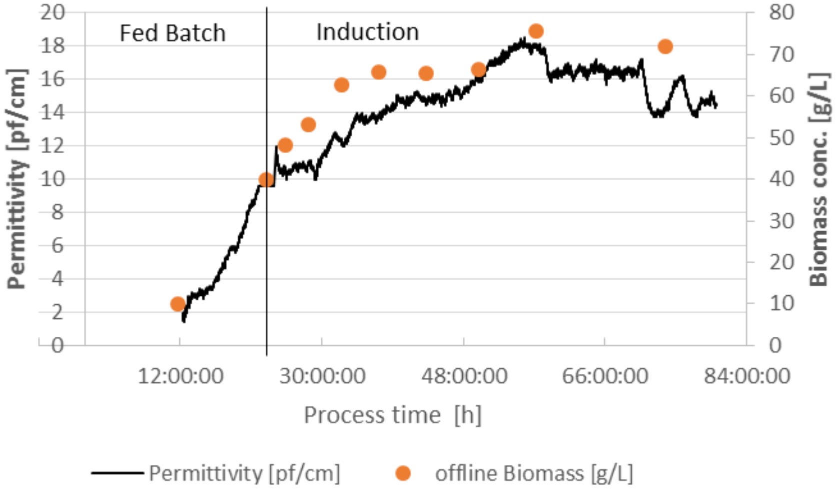
Required BM Estimation

BM estimation via permittivity measurement

- Measurement principle:
  - Permittivity ~ capacitance of liquids [pf/cm]
    - Resistance to a spreading electric field
  - Cells have a transmembrane potential dependent on the fitness of the cell
    - Integer cells act as condensers (Energy retention)
- Biomass estimation:
  - Permittivity signal correlated to viable Bio-volume
  - Bio-volume = Biomass dry-weight
    - Assuming a constant bio-density
- Obligatory Inputs
  - Permittivity signal
  - Correlation of permittivity and BM dry weight
    - Requires a calibration



Aber Instruments Ltd, Aberystwyth, Wales



Physiological Process Control

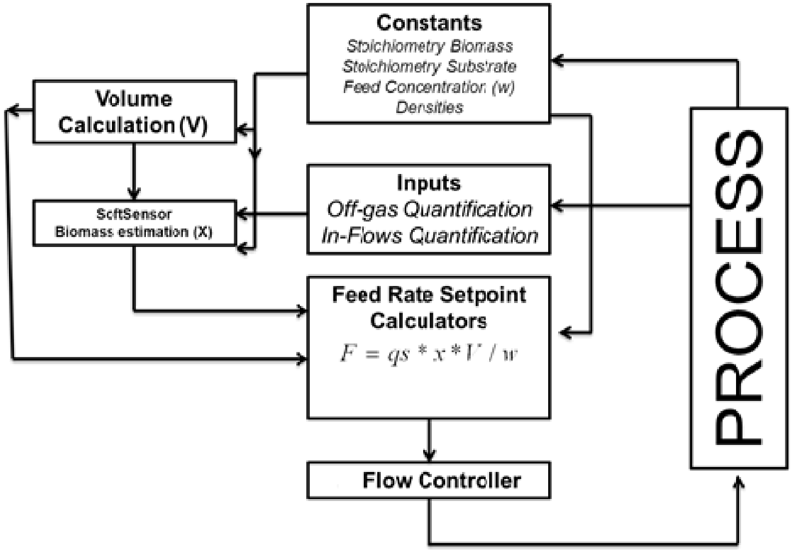
Required BM Estimation

Biomass estimation via Balancing (Softsensor)

- Measurement principle:
  - Real time rX estimation in time increments (dt) based on balances;
    - Cin – Cout = Cbound (elementary balance)
    - DoRin – DoRout = DoRreactor (electron balance)

- Biomass estimation:
  - Integration of rX over dt yields incremental BM production
  - Cumulation of increments for total BM estimation

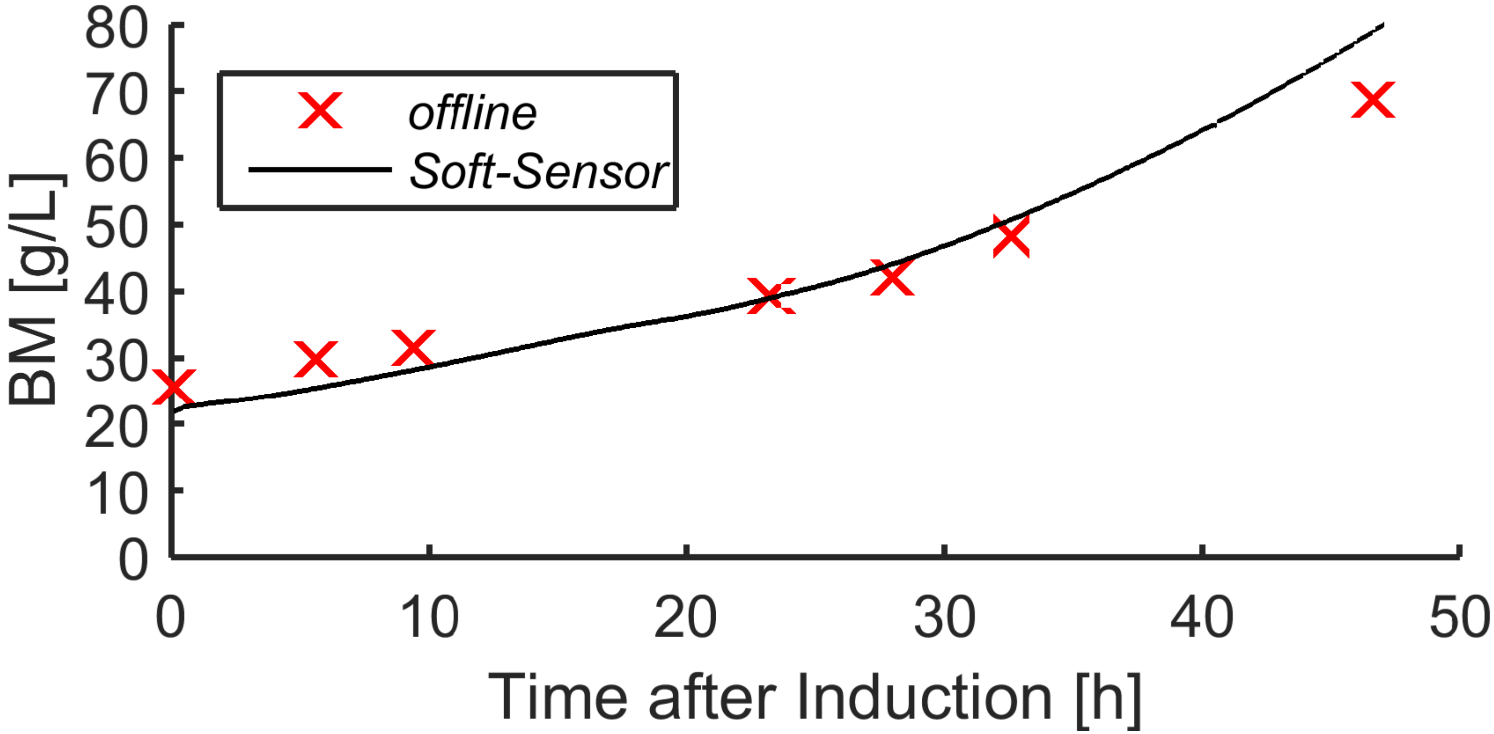
- Obligatory Inputs
  - Feed conc./ density
  - Stoichiometry of BM + BM at softsensor start
  - Measured variables
    - CO2/O2 concentration Offgas
    - Flow IN (Feed/Gas)/Flow OUT (Gas/Sample)



Sagmeister et. al. soft sensor assisted dynamic bioprocess control: Efficient tools for bioprocess development (2013)

Physiological Process Control

Required BM Estimation

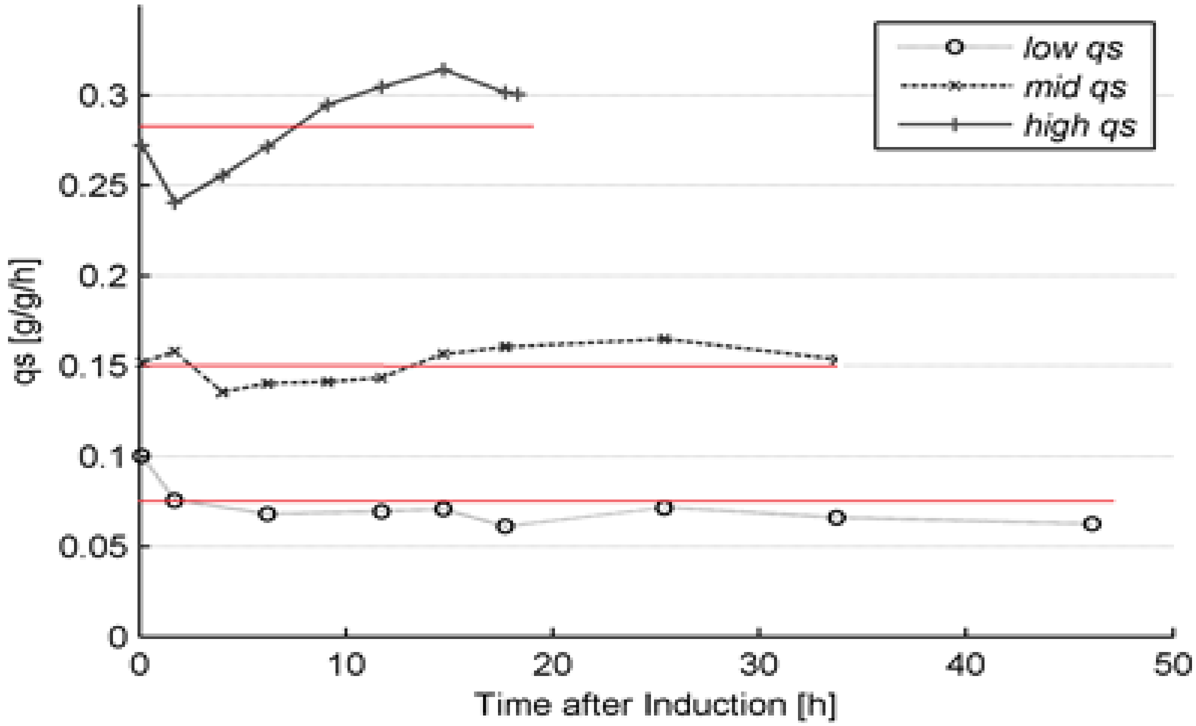


Transferability! → Data from Transfer to Industrial environment!

Physiological Process Control

Soft-sensor: real qs trajectories

- qs closely controlled
- qs-control is compromised when approaching qs_{max}



Physiological Process Control

Required BM Estimation

	Yxs trajectory	Soft sensor	Permittivity
Principle	Biomass estimation through $Y_{x/s}$ trajectory	Elemental balance based, non- invasive, real time measurements	Biomass - permittivity signal correlation
Implementation effort	Low costs No additional equipment/ Software	Low cost Software integration + Off gas analyzer	High cost hard type sensors needed (exclusive suppliers)
Accuracy	Sufficient with high process knowledge	Good Dependent on C-balance BM start point!	Good Calibration quality
Robustness	Low (open loop)	High, consistency check possible	Mid (closed loop) Signal highly sensitive
Transferability	Historical data required (Scale dependent?)	Highly transferable	Calibration not transferable

Physiological Process Control

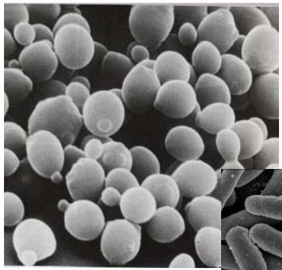
Applications

Softsensor based control principle

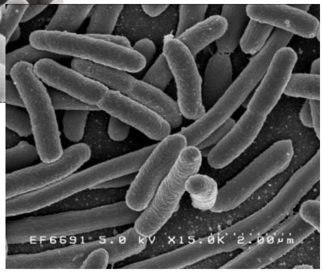
- Transferability
  - Organisms (Tested for *E. coli*/*P. pastoris*)
  - Process Modes Batch/Fed Batch
- Usage for estimation of different physiological variables
  - Implement N-Balance for metabolite accumulation

Additional degree of freedom for process development

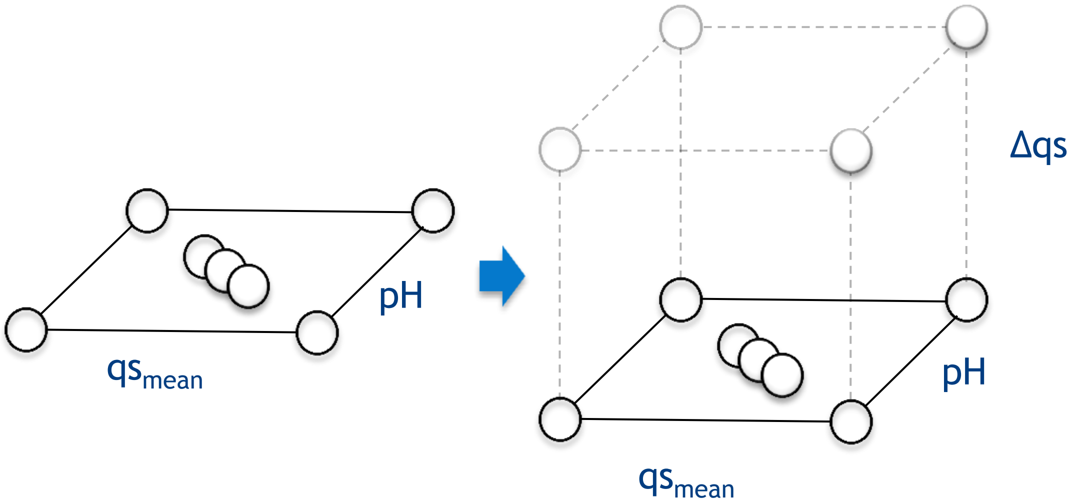
- Used CONTROLLED dynamics
- Dynamic qs trajectory (ramps/oscillations)

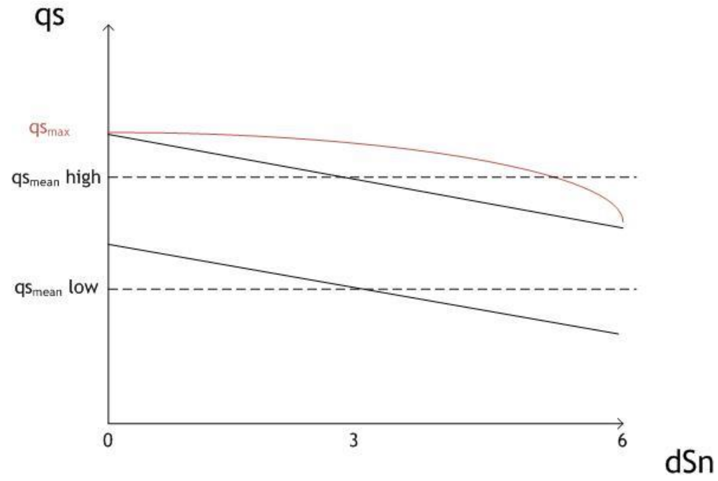


P. pastoris



E. coli





Physiological Process Control

Summary

- Controlled and defined culture state
  - Physiological state maintained during the process
  - Transferable concept
    - To industrial environment
    - Various physiological factors (μ/qs/qCO2)

- Ease bioprocess development
  - Additional degree of freedom for DoE`s
    - Clearly defined factors
    - High dynamic range
  - Exclusion/Elucidation of hidden effects
  - Controlled dynamics – benefit of physiological process control
    - Positive/ negative ramps
    - Mixed feed profiles