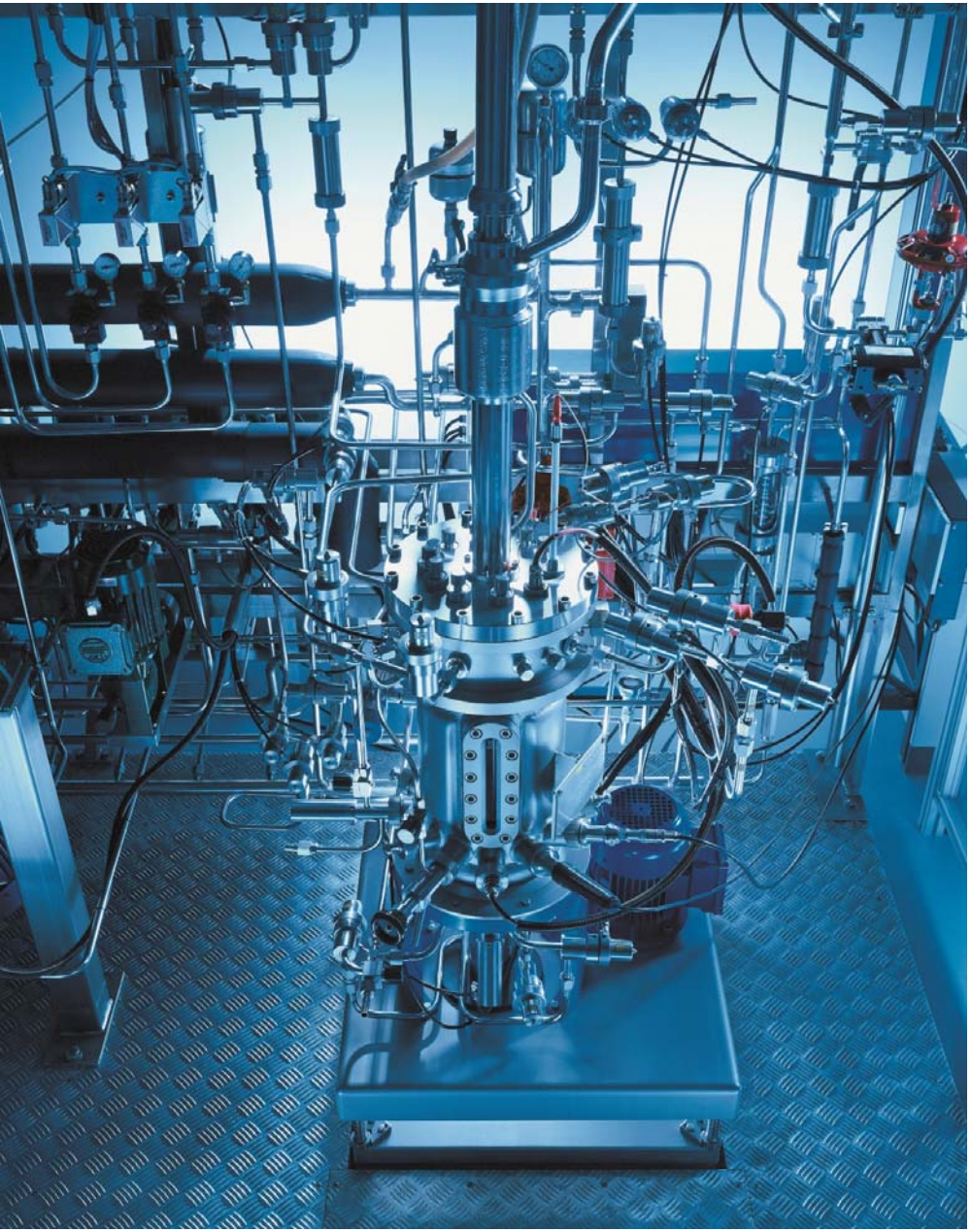


Advanced Bioreactors

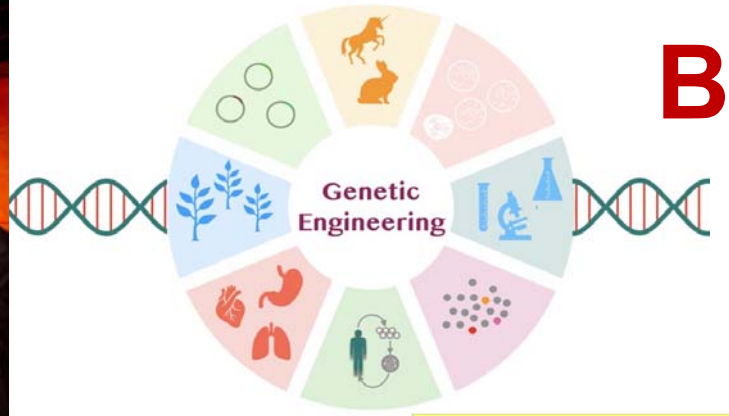
Biochemical Engineering
ChE-311

Manfred Zinn

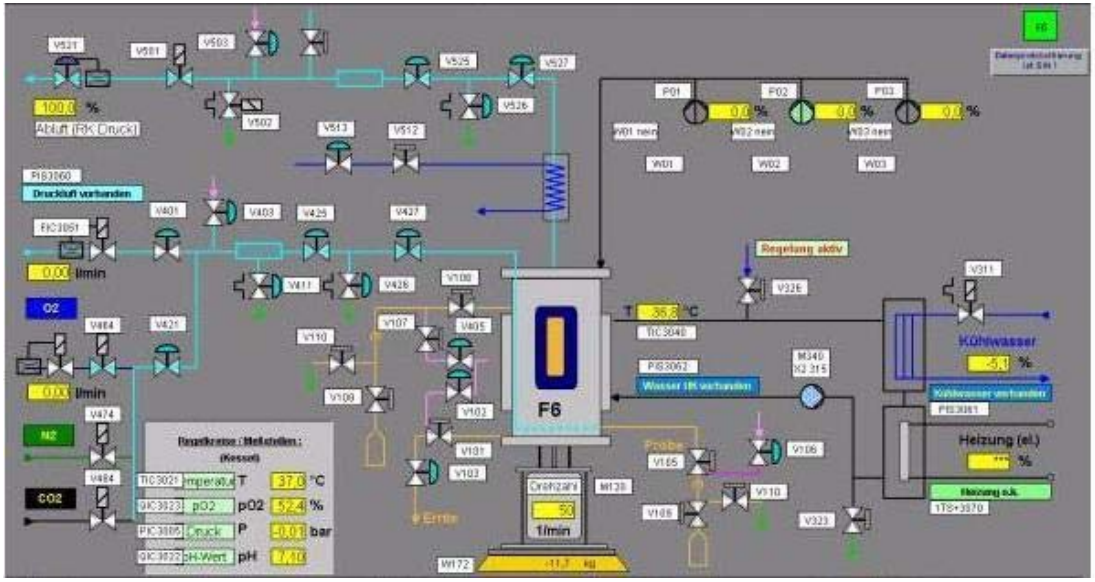
manfred.zinn@epfl.ch

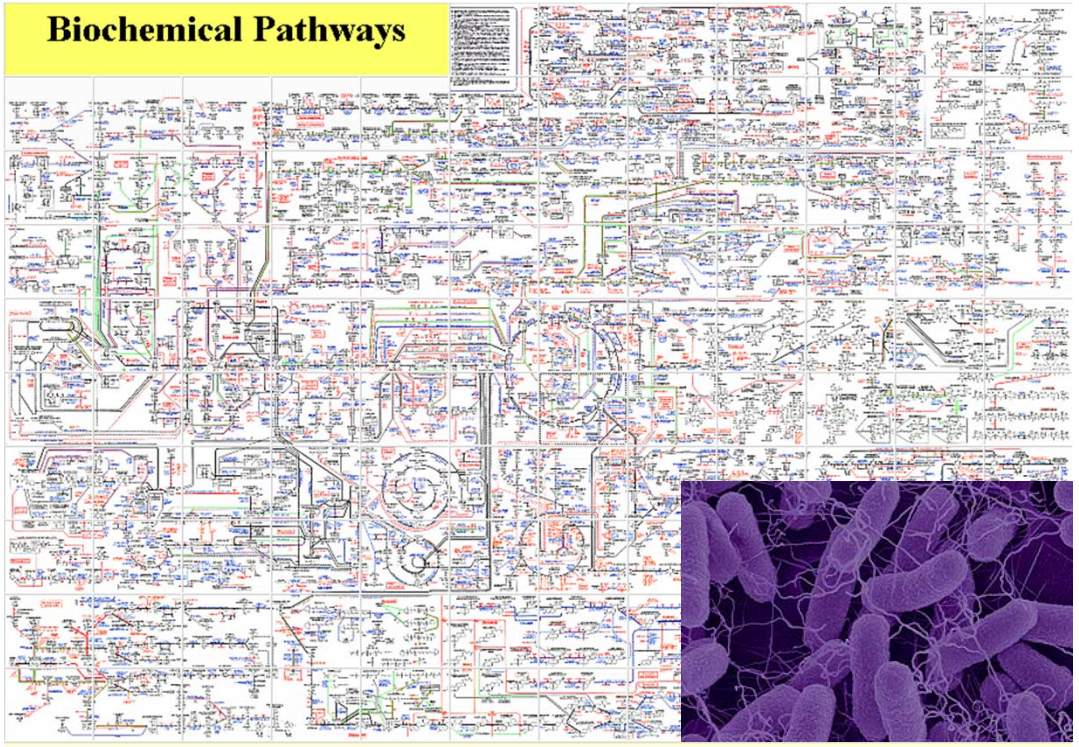
Agenda

- Control strategy of bioprocesses
- Bioprocess automation and QbD/PAT
- Upstream processing: Sterilization of bioreactors
- Design of sterilization processes
- Exercises on sterilization



Bioprocess Engineering





General sequence of a bioprocess

- Cleaning in place (CIP)
- Sterilization in place (SIP)
- Preparation of medium
- Calibration
- Inoculum preparation

- Biomass and product formation

- Separation of biomass
- Product isolation and purification

Note: Generally « fermentation » is considered being part of upstream processing.

Reasons for increasing need for automation and use of computers in bioprocessing

There is a need for accelerated process development and increased efficiency and **economics** of production processes!

Process level

- Process regimes became far more complex and sophisticated e.g. (batch ➔ fed batch)
- Continuous increase of variables to be monitored
- Increasing complexity of variables

Level of quality management

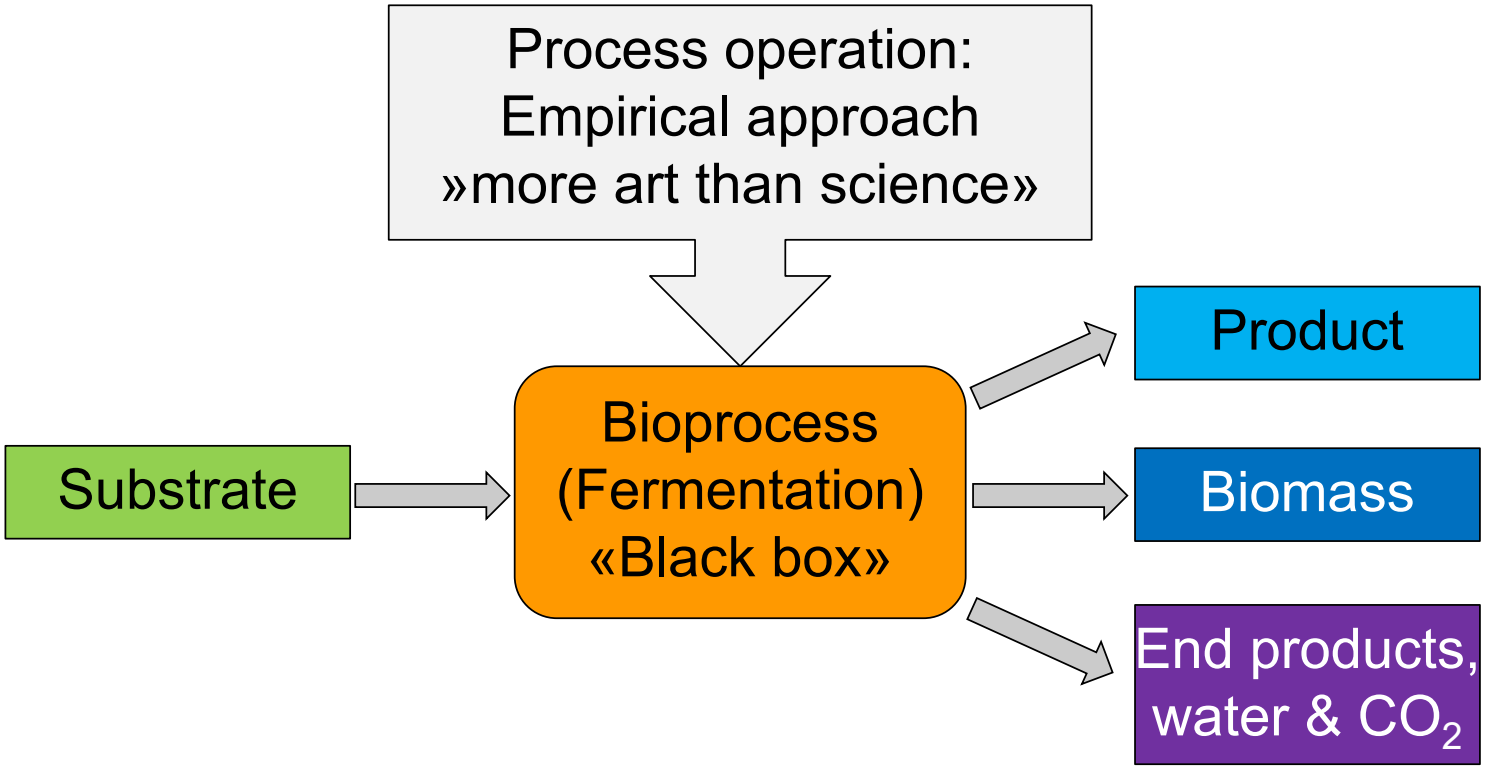
- Demands related to quality management increased dramatically (hidden plant, makes approx. 35% (up to 70%) of costs)
- Increased demands on documentation (**GMP**)

Bioprocess control

It is of greatest importance in biotechnological processes to analyse **and** control the biological, chemical and physical surroundings *continuously*.

There is a strong need for stable sensors and systems for the monitoring, control and regulation of complex processes = process control systems (process computer) in combination with a process information management system (PIMS).

Bioprocess Operation



There is a strong need for automation!

Process control and automation

Goal: optimal utilisation of biological systems

- Biosynthesis
- Transformation
- Degradation

Impact of process control on bioprocessing

Efficiency (yield) of a bioprocess is determined by

- Performance of the cell factory
- Environmental parameters

Main tasks:

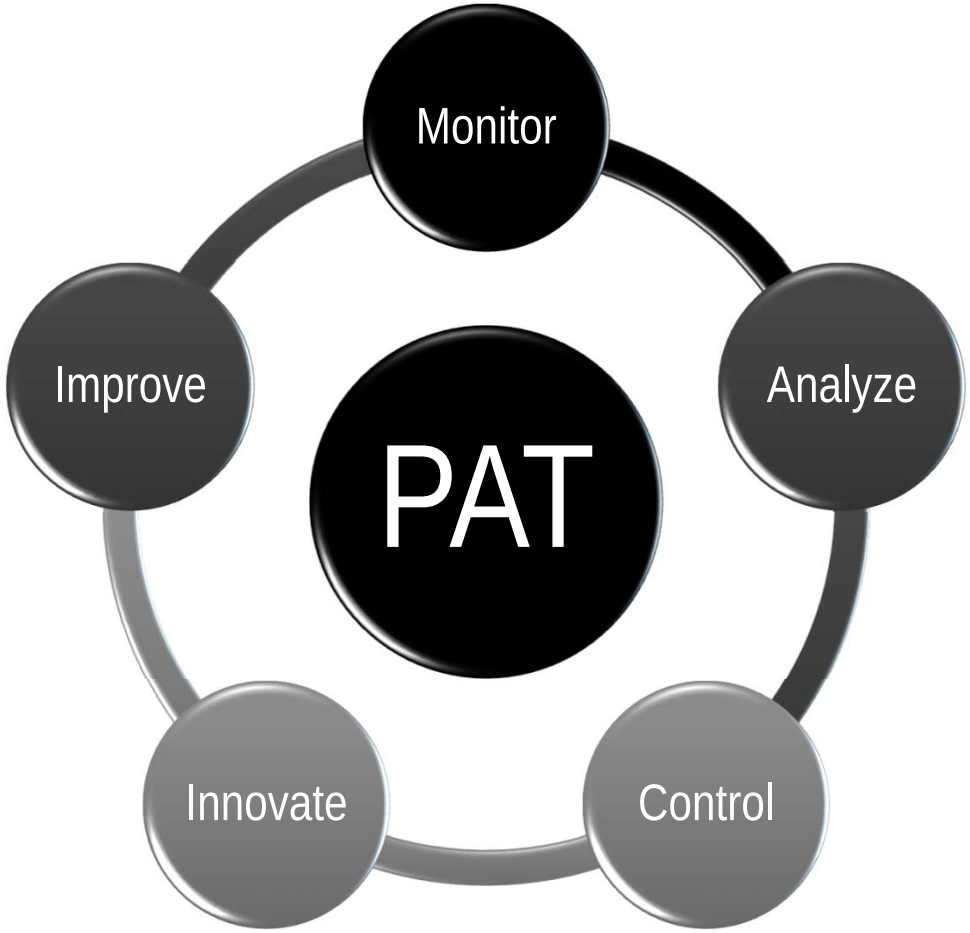
- Maintenance of process conditions by monitoring and control
- Documentation

Process automation

A few definitions:

- **Process**
  Integrated system by which the transformation and/or transport of materials, energy and/or information is accomplished.
- **Process control**
  Automatic control of a process, in which a computer system is used to regulate the usually continuous operations or processes.
- **Process monitoring**
  Acquisition of key variables.
- **Automation**
  Sequence of operations running without the interaction of man but following adapted algorithms. Advanced level of automation is robotics.

Process Analytical Technology (PAT)



A few abbreviations in PAT

API:	Active pharmaceutical ingredient
COG:	Cost of goods
COGM:	Costs of goods manufactured
CQA:	Critical quality attribute
DAQ:	Data acquisition
DCS:	Distributed control system
DoE:	Design of experiment
FMEA:	Failure mode and effects analysis
IT:	Information technology
MES:	Manufacturing execution system
MSA :	Measurement system analysis
MSPC:	Multiple setpoint control
MTBF:	Mean - time between failures
MVDA:	Multivariate data analysis
PA :	Process analytics
PAI:	Preapproval inspection
PAT:	Process analytical technology
PIMS:	Process information management system
PoC:	Proof of concept
PUC:	Process understanding and control
PV:	Process validation
QA/QC:	Quality assurance/Quality control
QbD:	Quality by design
RTA:	Real - time assurance
RTM:	Real - time (process) monitoring
SPC:	Setpoint control

How everything started…

- In the mid 1980s process validation (PV) was viewed as a new regulatory requirement to be performed sometime in phase 3 of the clinical development cycle. However, final **product quality** was the dominant aspect.
- PV reports were issued but ended up on book shelf or in a drawer. Only when changes to the process were made, they were reconsidered.
- Establishing PV reports were not adding to the value but rather a check-the-box value!

FDA comes into action

- In 1990s FDA wanted to have all production processes validated (**also retroactive validation**) and triggered a hectic activity in pharmaceutical industry.
- A large sum of money was spent just to keep products on the market and companies out of regulatory trouble.
- There still was **no benefit for the process development**!

The benefit of PV to industry

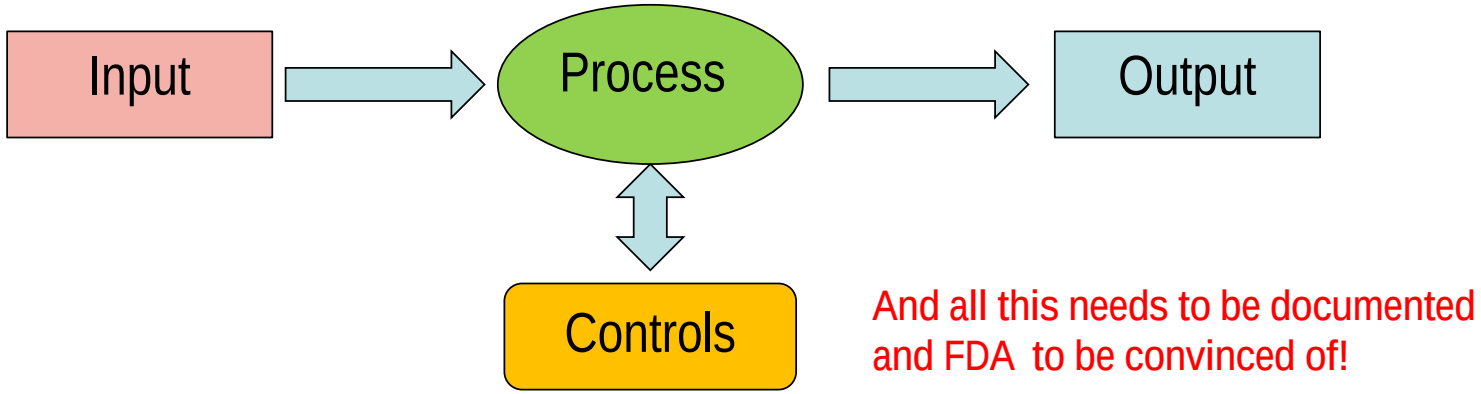
- Over time companies realized that they can profit from a profound PV.
- **Ruggedness** and **reproducibility** became a key issue and were proudly presented by companies in order to demonstrate the quality of their process.
- Modifications of the PV protocol became important and were assessed in 3 PV runs for identifying worse-case settings and parameter importance.
- There was a gain for the process but it was related to a lot of work.

The new initiative by FDA

- FDA was viewed by many pharmaceutical companies as a **highly conservative agency**. Modifications of a process resulted in a huge amount of paper work and… binding ressources at FDA!
- Consequently FDA pushed a new concept called **Q**uality **b**y **D**esign (QbD) and was part of their « GMP in the 21st Century » initiative.
- FDA created a new visionnary approach where **product and process understanding** became key issues.

QbD principles

- **Process development** is the key: companies need to make sure that they have understood their process and own capabilities.
- Different starting conditions (e.g., source material) may have an influence on the process!



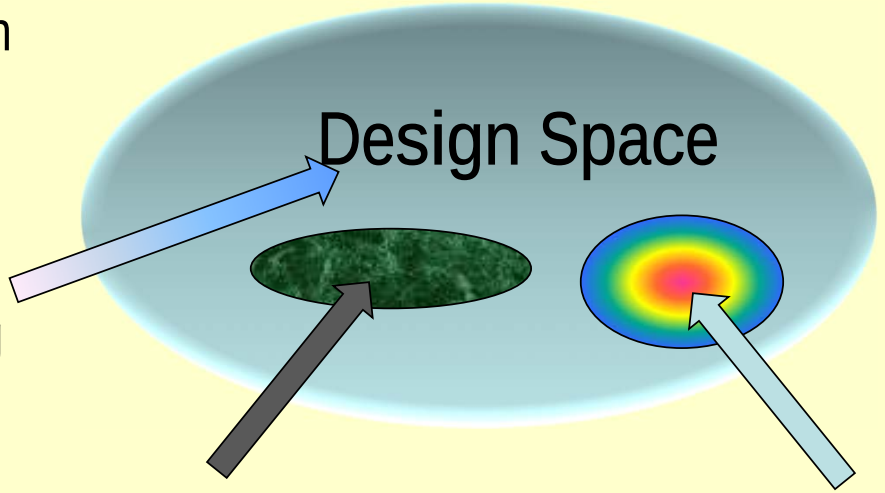
Advantages of doing a good job for FDA

- Successful convincing FDA of the production process may result in avoiding **preapproval inspections** (PAI).
- Further, a **design space** can be established to enable the company to freely act without regulatory oversight. This space will not stay static but is forever being refined. It is the area within which a company can operate with predictable outcomes to make acceptable products every time.
- Outside the design space regular change control is required.

Design space

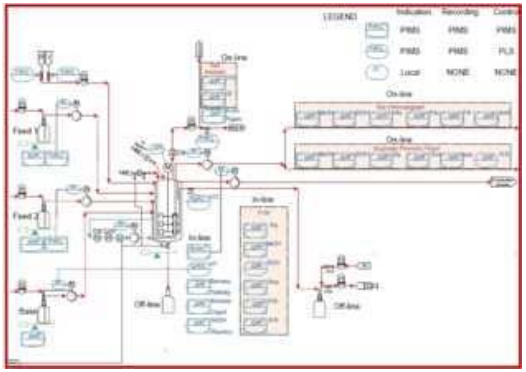
Nondesign Space
(failure area; areas with regulatory submission required to operate)

Operation within **design space** using internal change control.

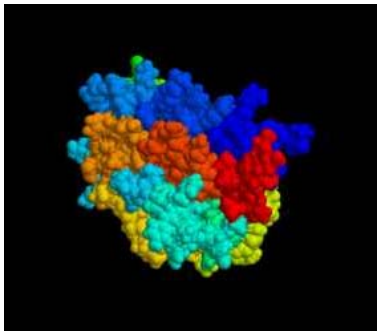


Alternative operation range
(requires no agency submission)

Normal operating range



How to elaborate this highly complex relationship ???



Process Parameters
PPs

- Temperature
- Stirrer speed
- pH
- Dissolved oxygen
- Air flow
- Pressure
- Feedrate
- Nutrient concentrations
- Biomass concentration
- . . .

Quality Attributes
QAs

- Protein folding
- Yield coefficients
- Glycosylation pattern
- Stability
- Impurities
- Batch-to-batch variability
- Ease of further processing (Downstream)
- . . .

Elaborate

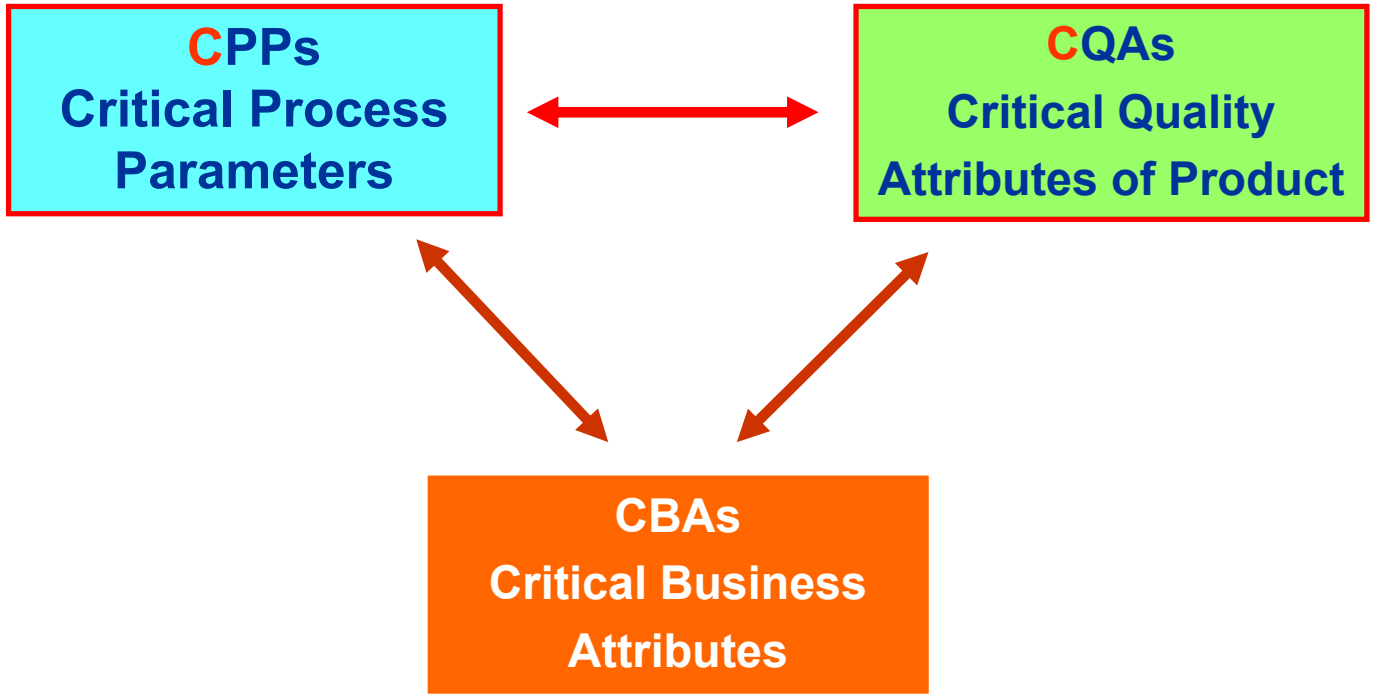
Critical Quality Attributes
CQAs

of the product, which are then considered with respect to the entity of Process Parameters.

Identify those Process Parameters which have an impact on CQAs, this subset of all process parameters is considered as

Critical Process Parameters
CPPs

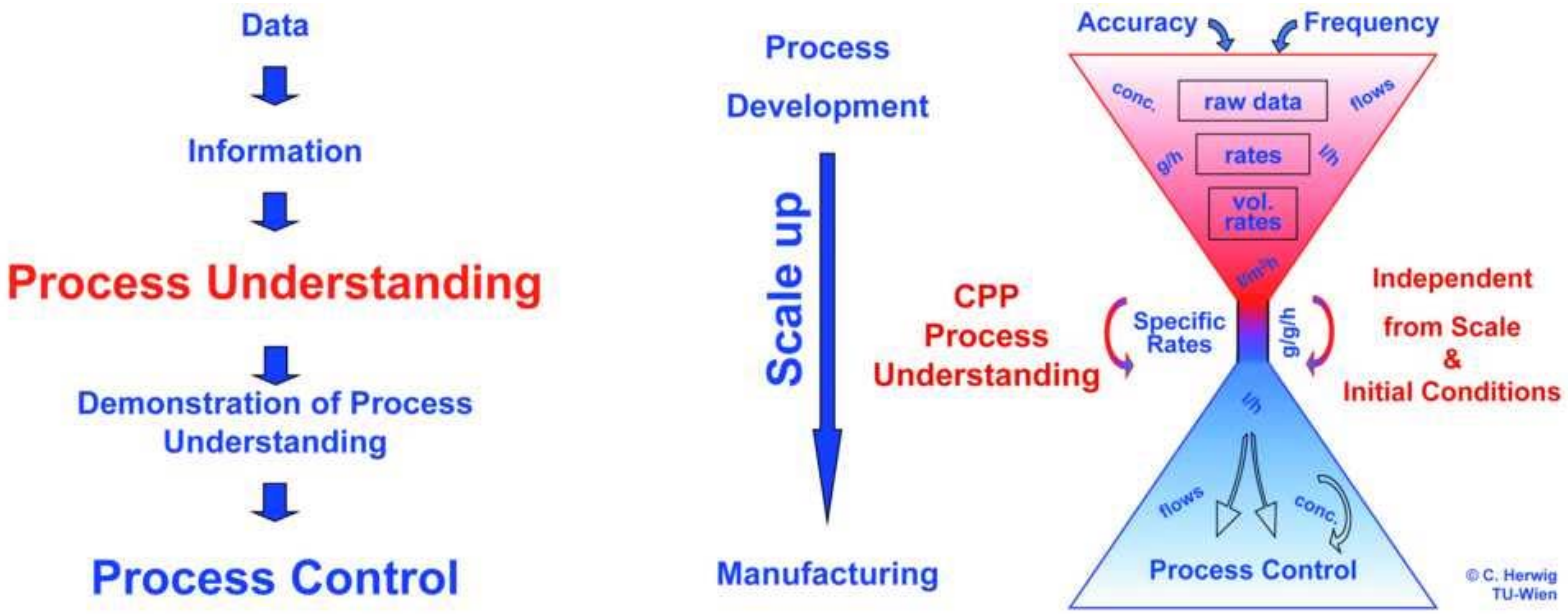
***Note*: The terminology of parameters used in the Process Analytical Technology (PAT) context does not reflect the original definition from engineering sciences!**



Critical Business Attributes are NOT an integral part of PAT and Quality by Design (QbD)!

However, they are important economic driving forces.

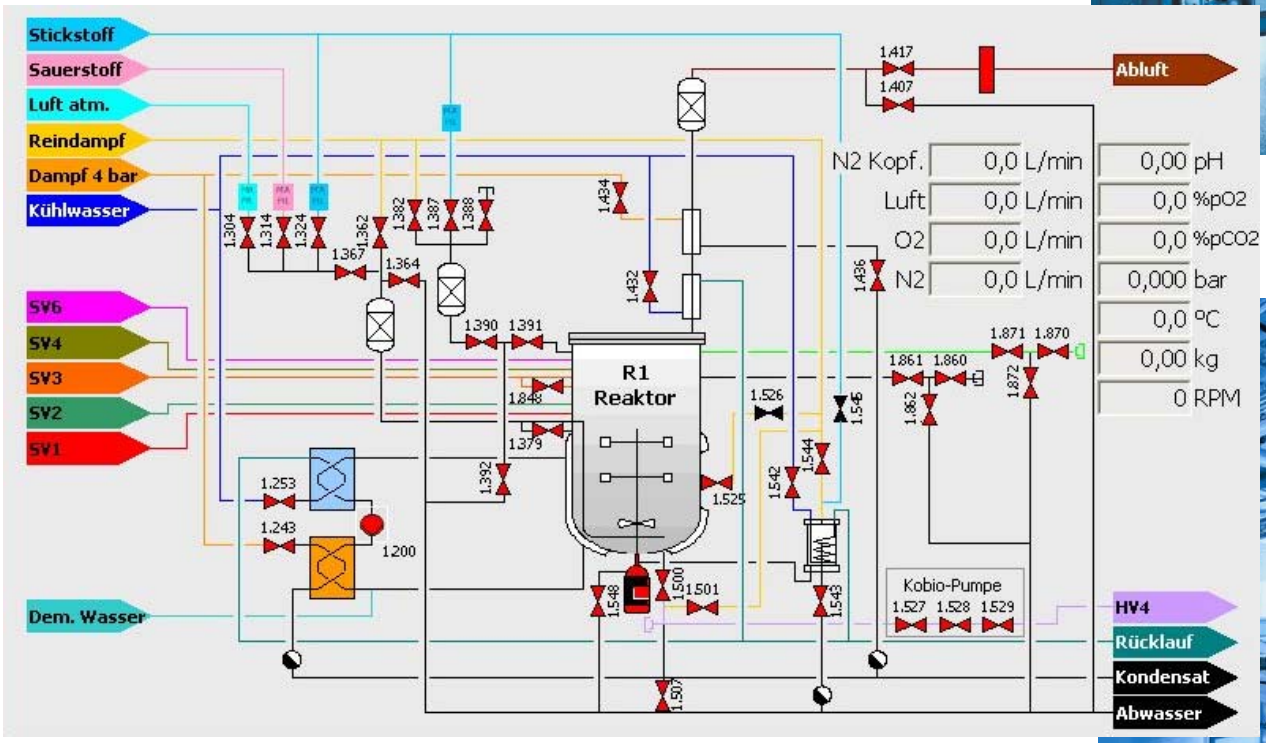
PAT compliant generation of Process Understanding, and its transfer from Process Development to Scale-up and Production . . .



. . . reflects an integral approach for transformation of raw data into process understanding and its use in process control.
The concept of determination of specific rates allows a *scale-independent transfer* of a developed process into industrial manufacturing.

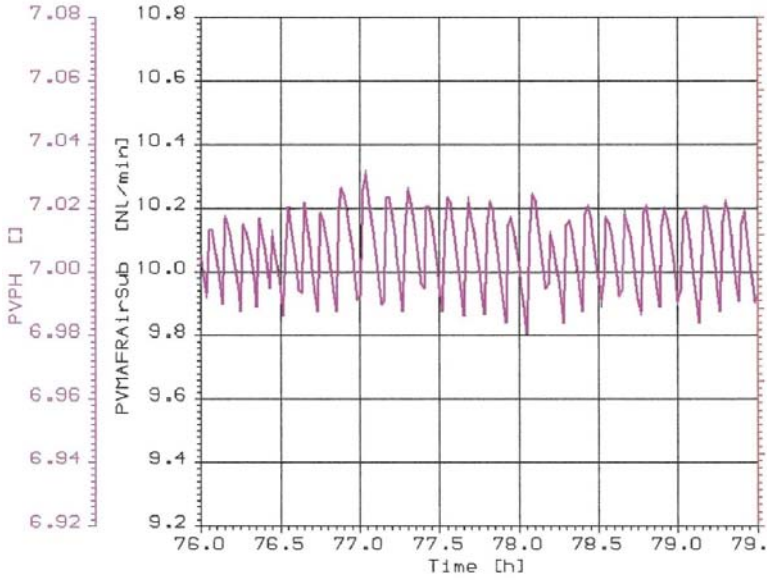
High pressure bioreactor

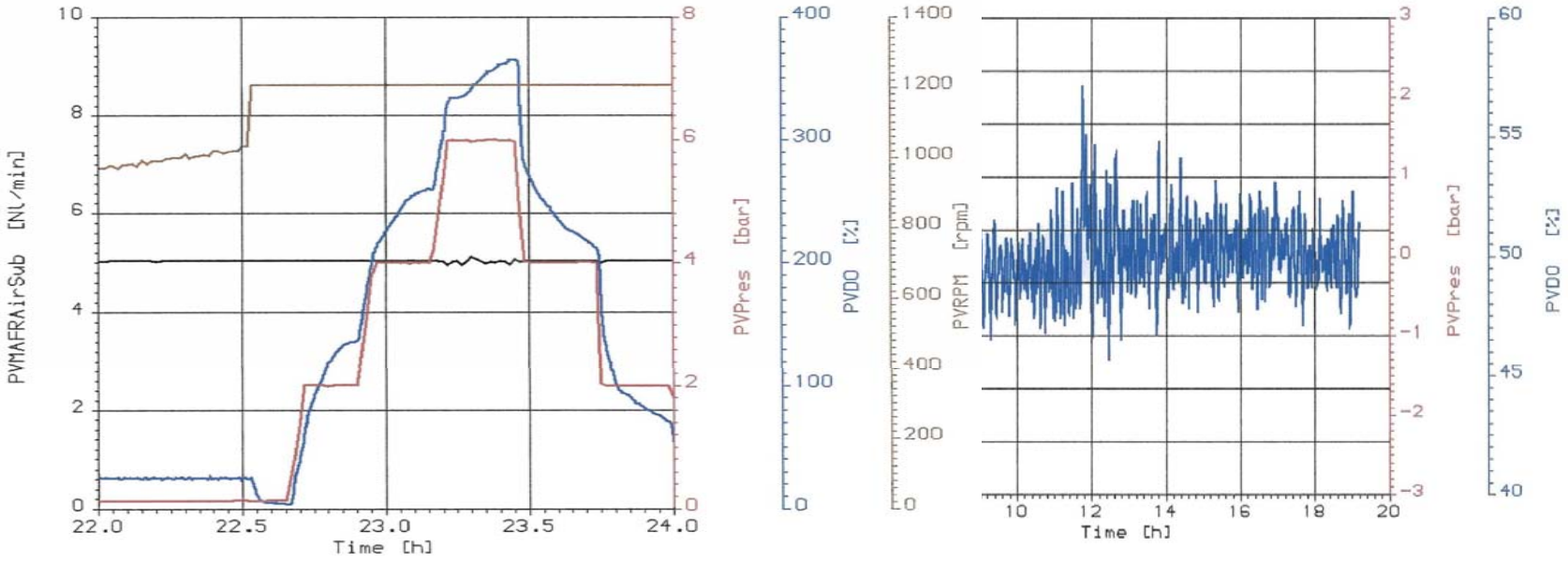
More than 144 valves to be controlled!



There is a need for accurate control of CPPs!

…but also know the limits of your system!





Fermentation process

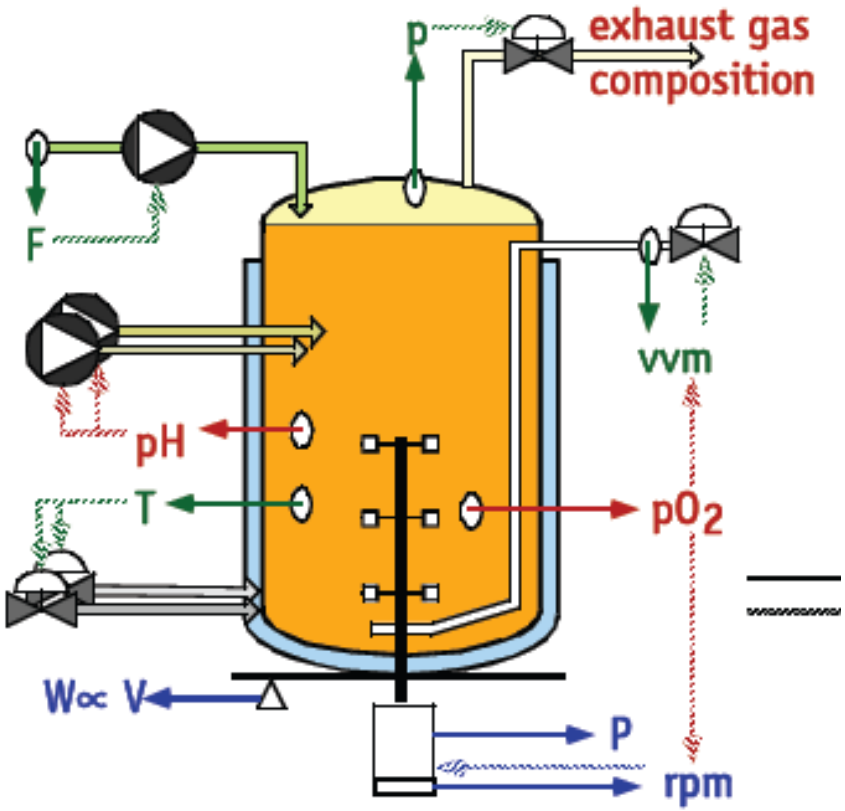
Main characteristics:

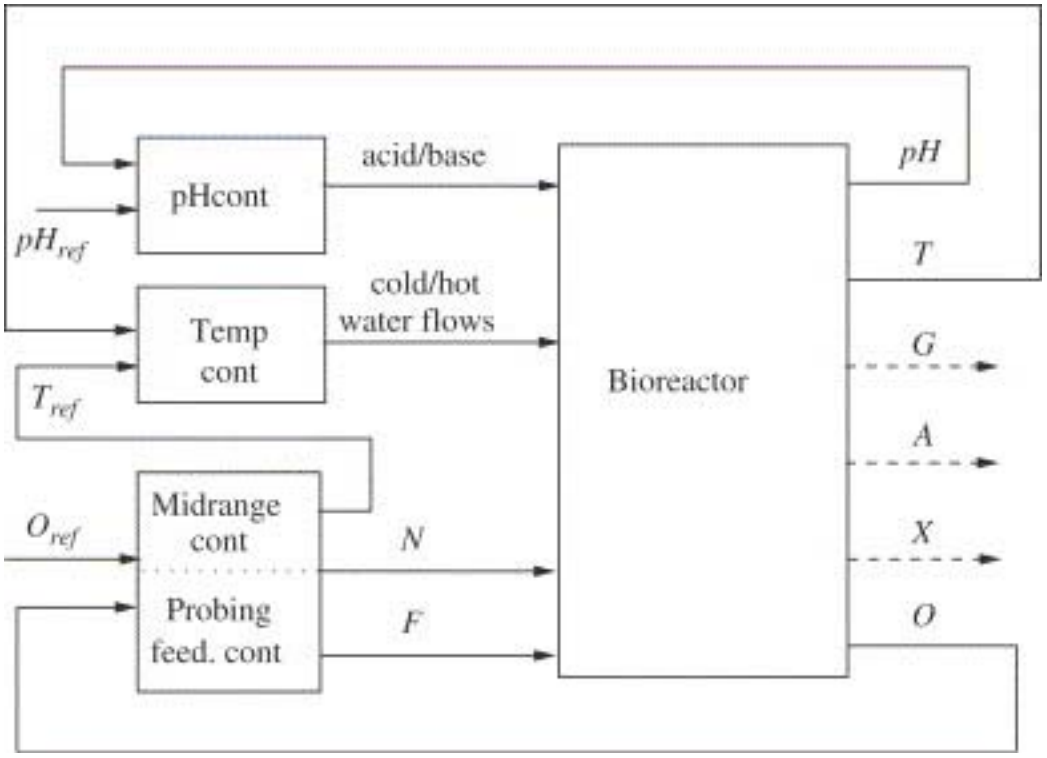
- complex
- highly interactive
- parallel
- non linear

Role of automation

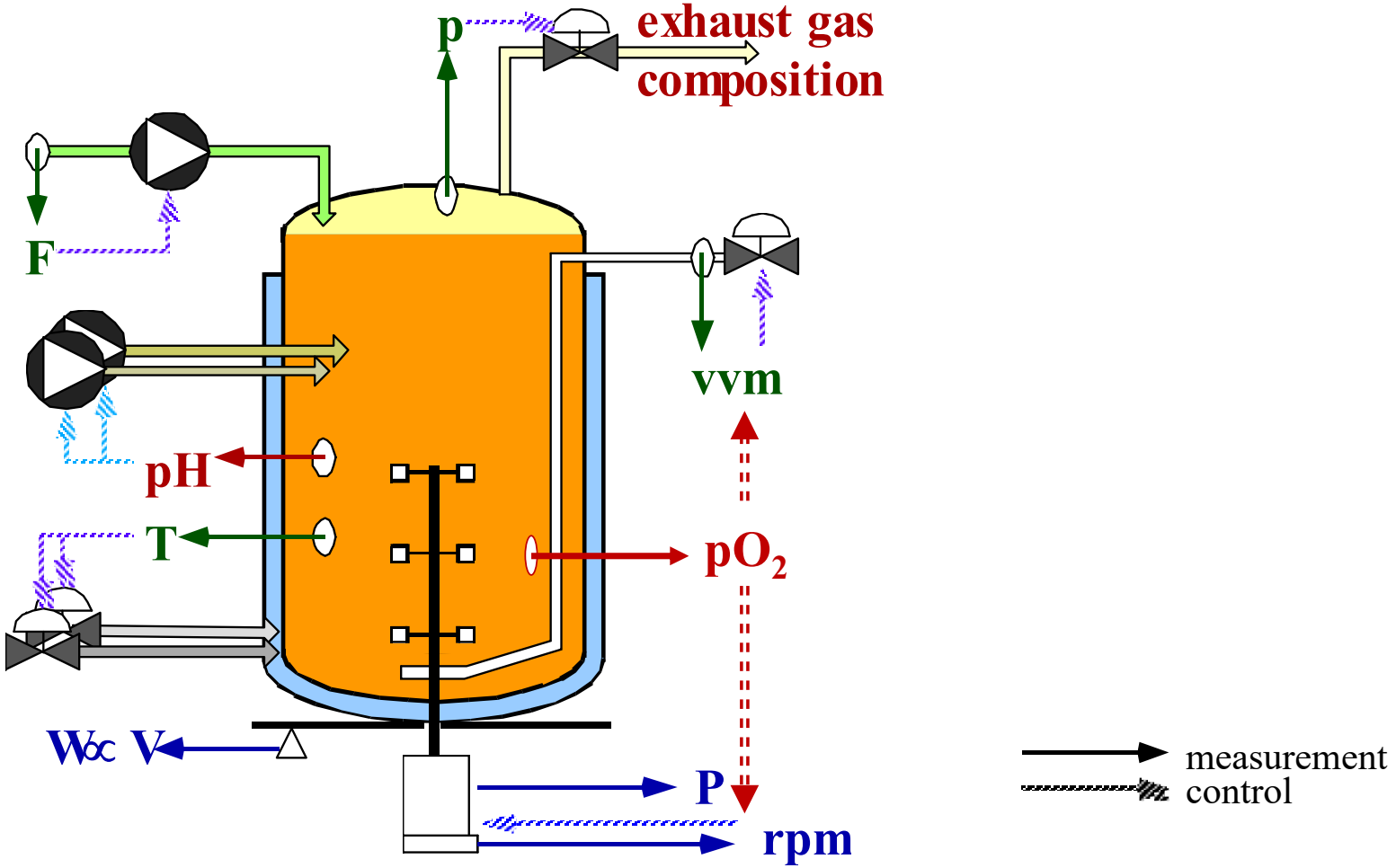
- Control of state variables
  - Temperature, pH, pO_2, rpm, conductivity, pressure, etc.
- Control of procedures
  - sterilization
  - sampling
  - feed (substrate, inducer, precursor,...)
  - harvest
- Documentation

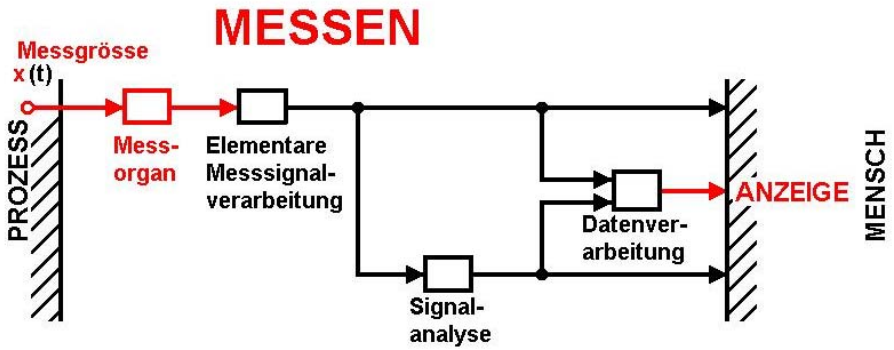
Bioprocess automation



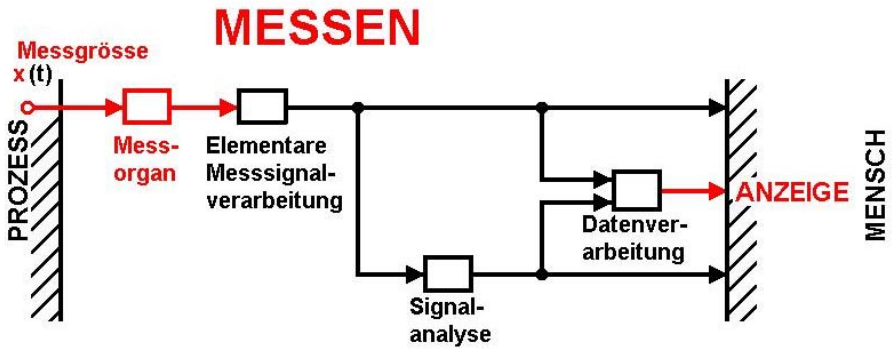


Accepted standard of M&C in biochemical engineering





Measure/Monitor



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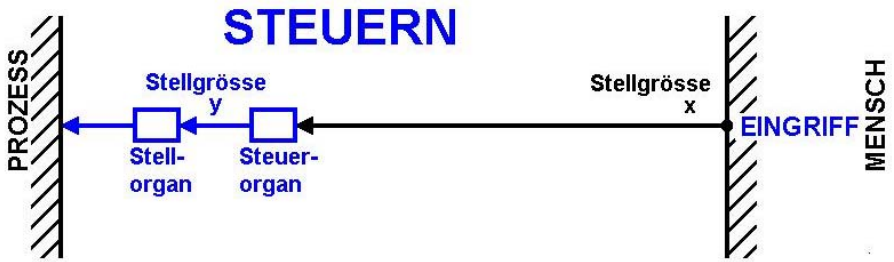
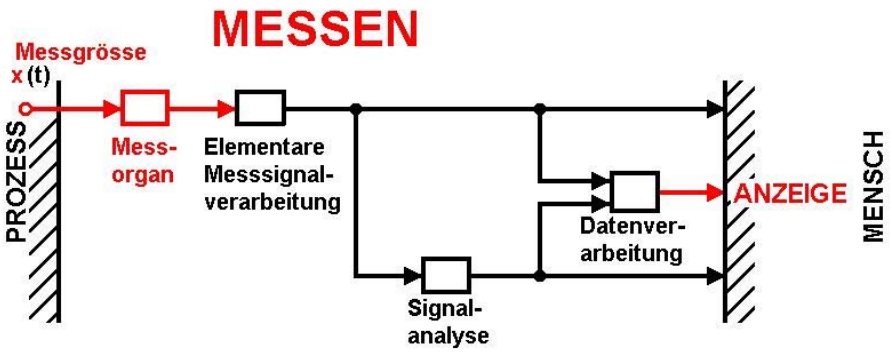
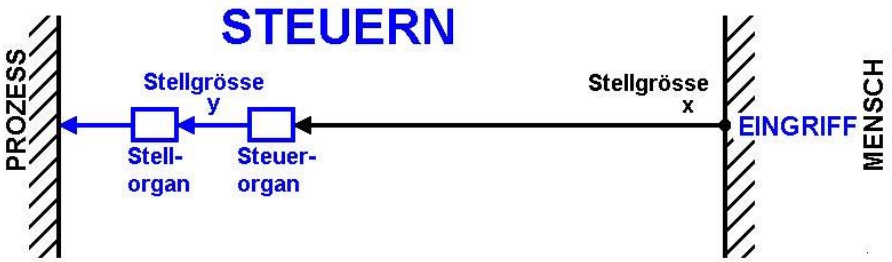


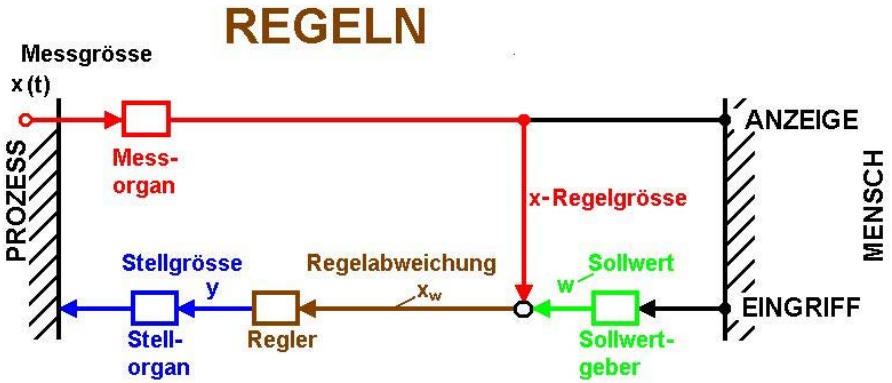
Control (open loop)



Measure/Monitor



Control (open loop)



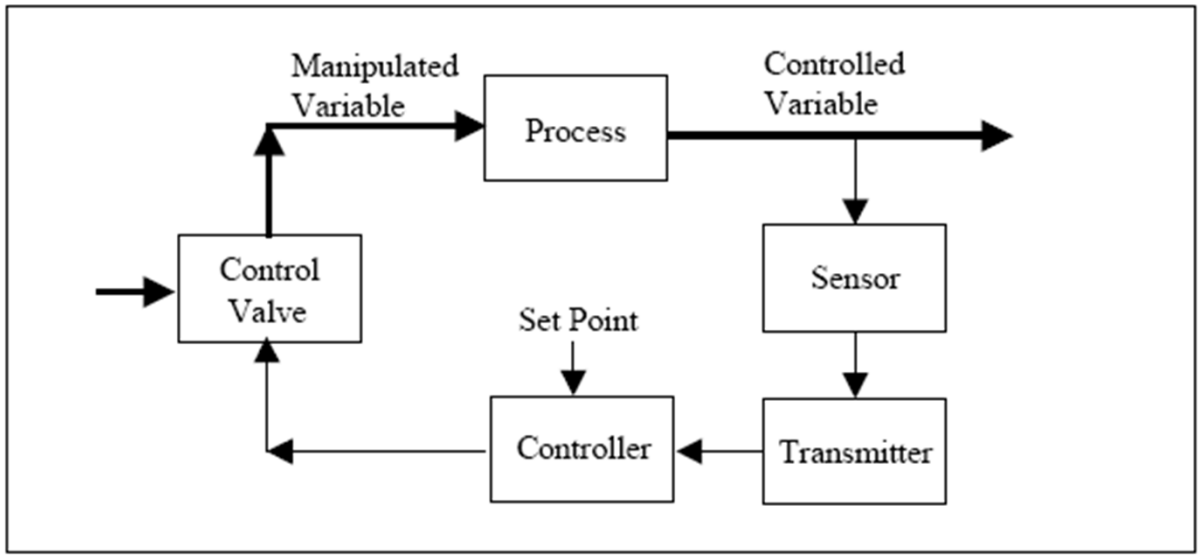
PID regulation

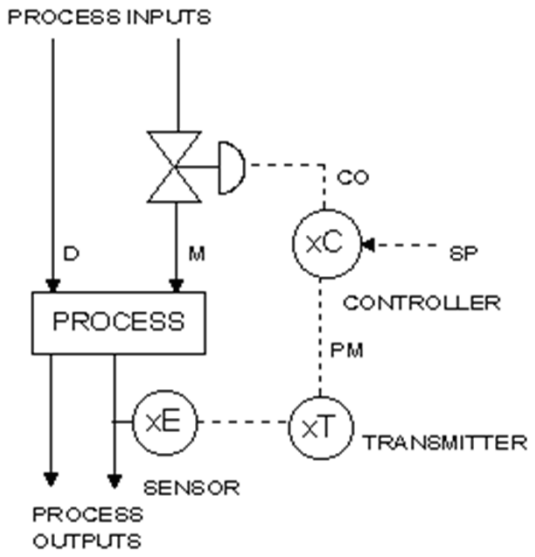
P ➡ $u = K_p . e$

PD ➡ $u = K_p . \left(e + T_d . \frac{de}{dt} \right)$

PID ➡ $u = K_p . \left(e + T_d . \frac{de}{dt} + \frac{1}{T_i} \int_0^t e(\tau) d\tau \right)$

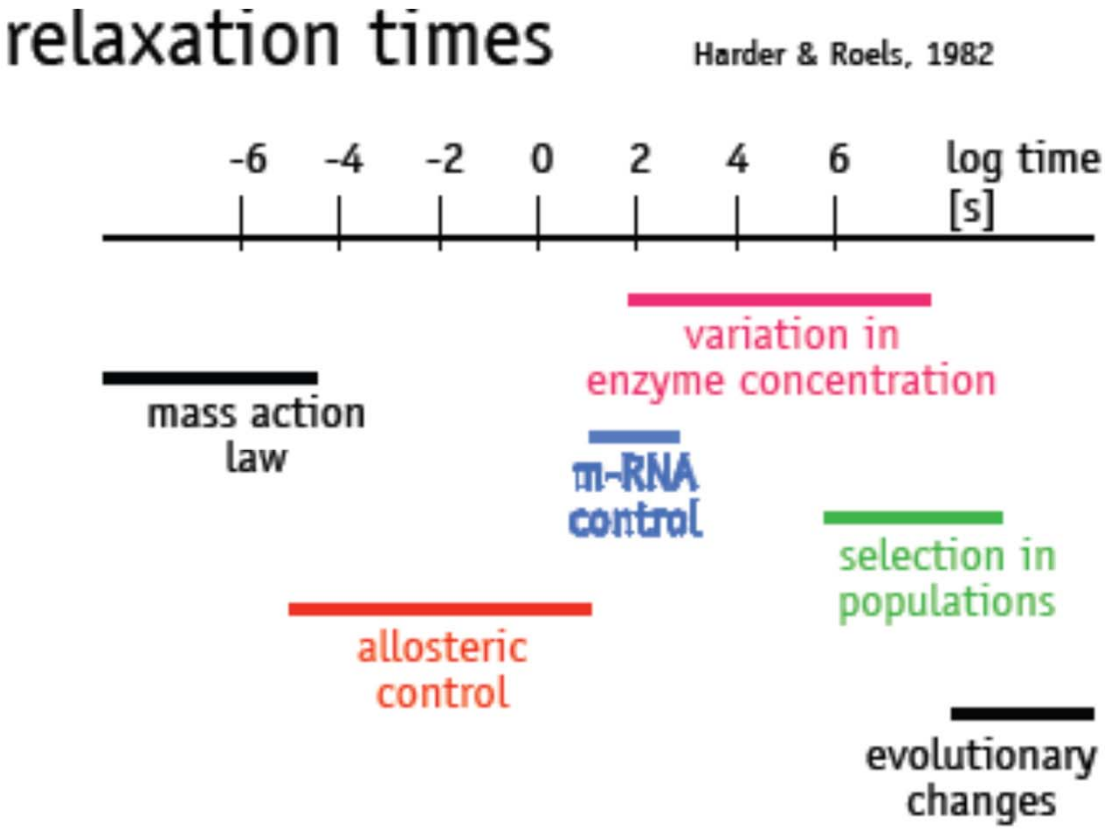
Feed-back control loop





…more problematic are biological signals.

Bioprocess automation: What are the limitations?



Definitions

on-line: measurement can be registrated directly. e.g., pH measurent

at line: sample taken carried to a different environment and information used to modify process conditions

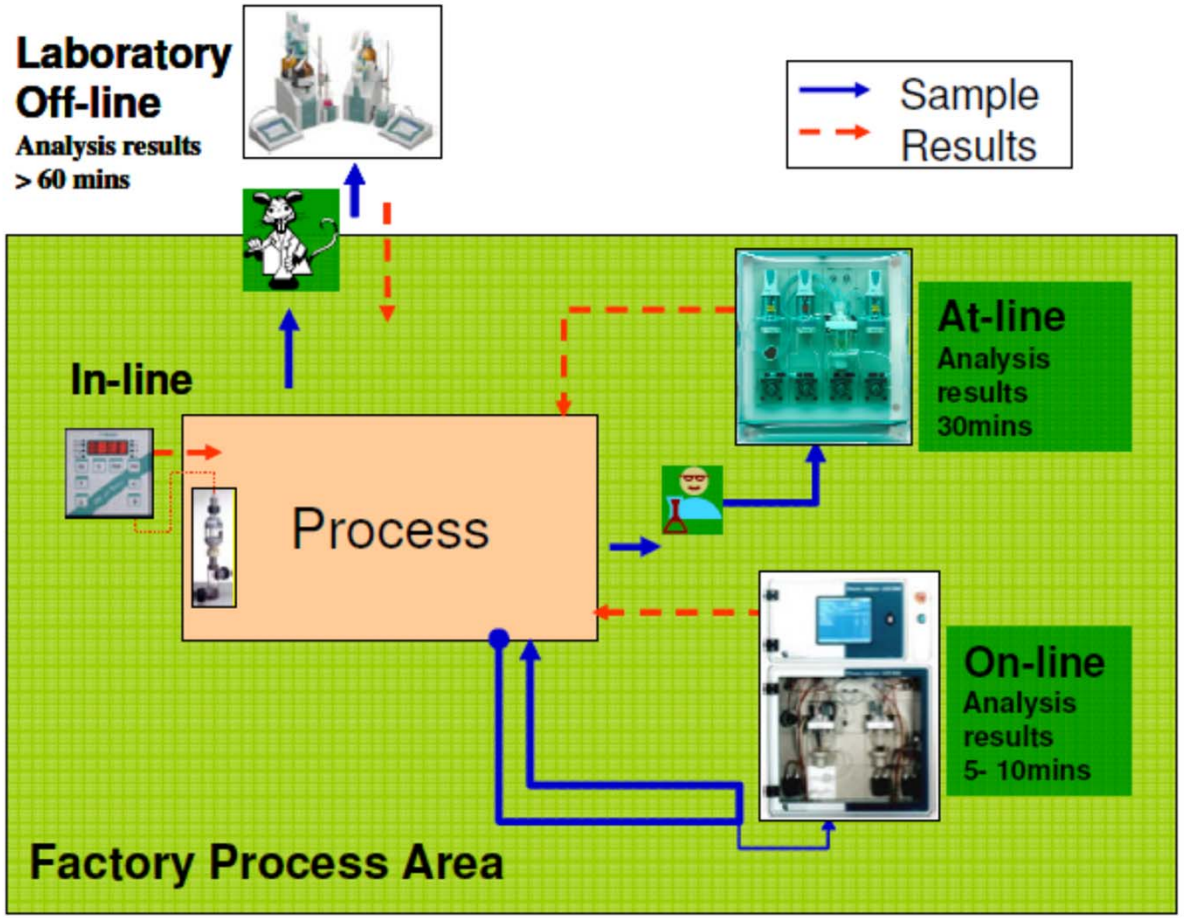
off-line: sample taken and analysed extern / preparation of samples
⇨delay

in-situ: sensor inside bioreactor

ex-situ: sample transferred to an external device for analysis

Wish: on-line, in-situ

Bioprocess automation: What are the limitations?

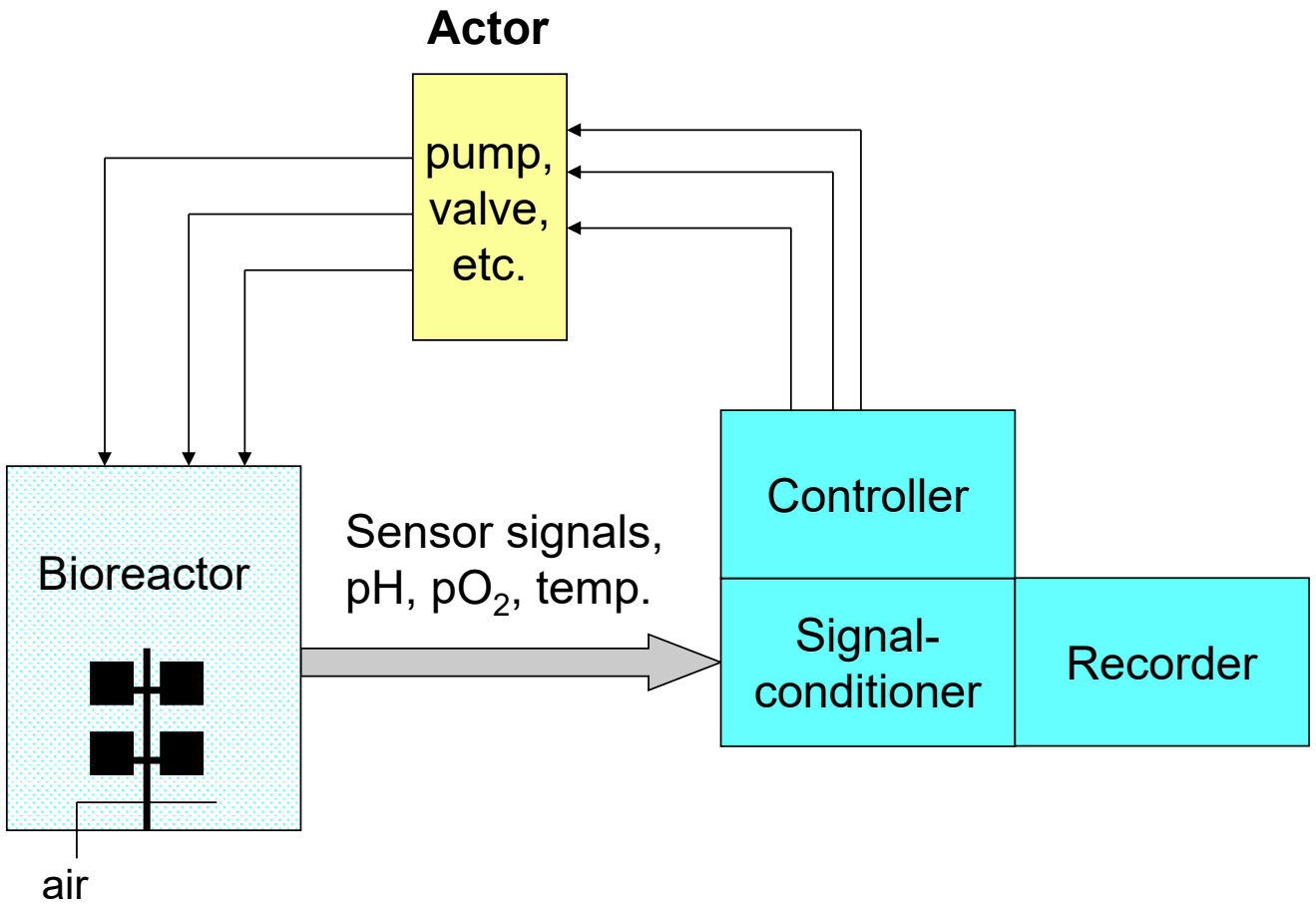


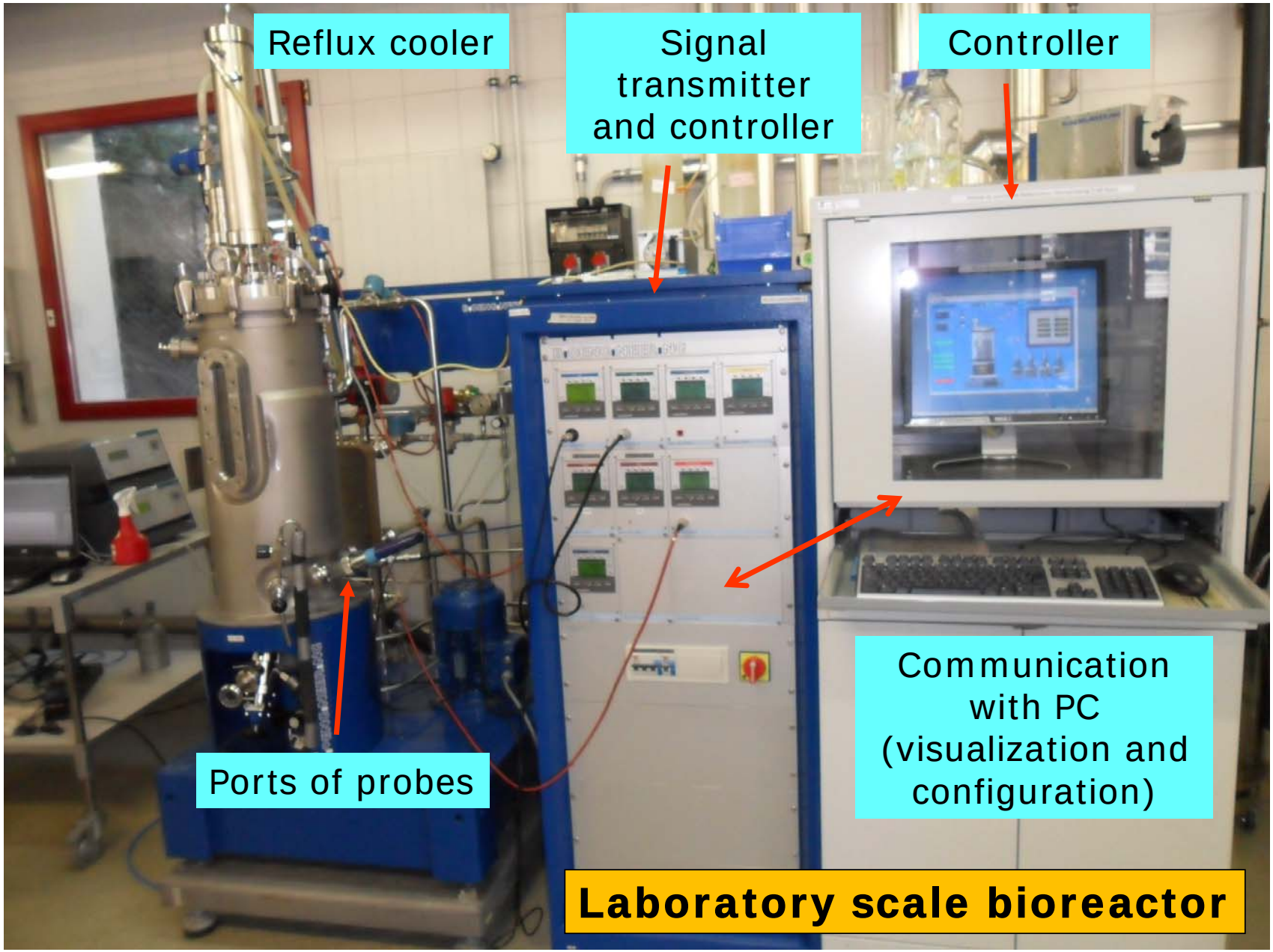
What we can dream of measuring

physical variables	chemical variables	biological variables	...
T, p, F, V, w, rpm, P/V, λ, ...	pH, rH, pO_2, $\%O_2$, (pCO_2), $\%CO_2$, (Fluores), glucose?	OD, RQ, μ, heat production, bugs?	in situ on line continuous
	extracellulars: bio- & ionselective sensors & devices		in bypass on line continuous
rheology	extracellulars: FIA, GC, LC, CE, ELISA?, MS?	biomass: number, size & morphology, composition?	in bypass on line discontinuous
	intracellulars: lab analyses	biomass, enzymes, plasmids, genes, ...: lab analyses	off line discontinuous

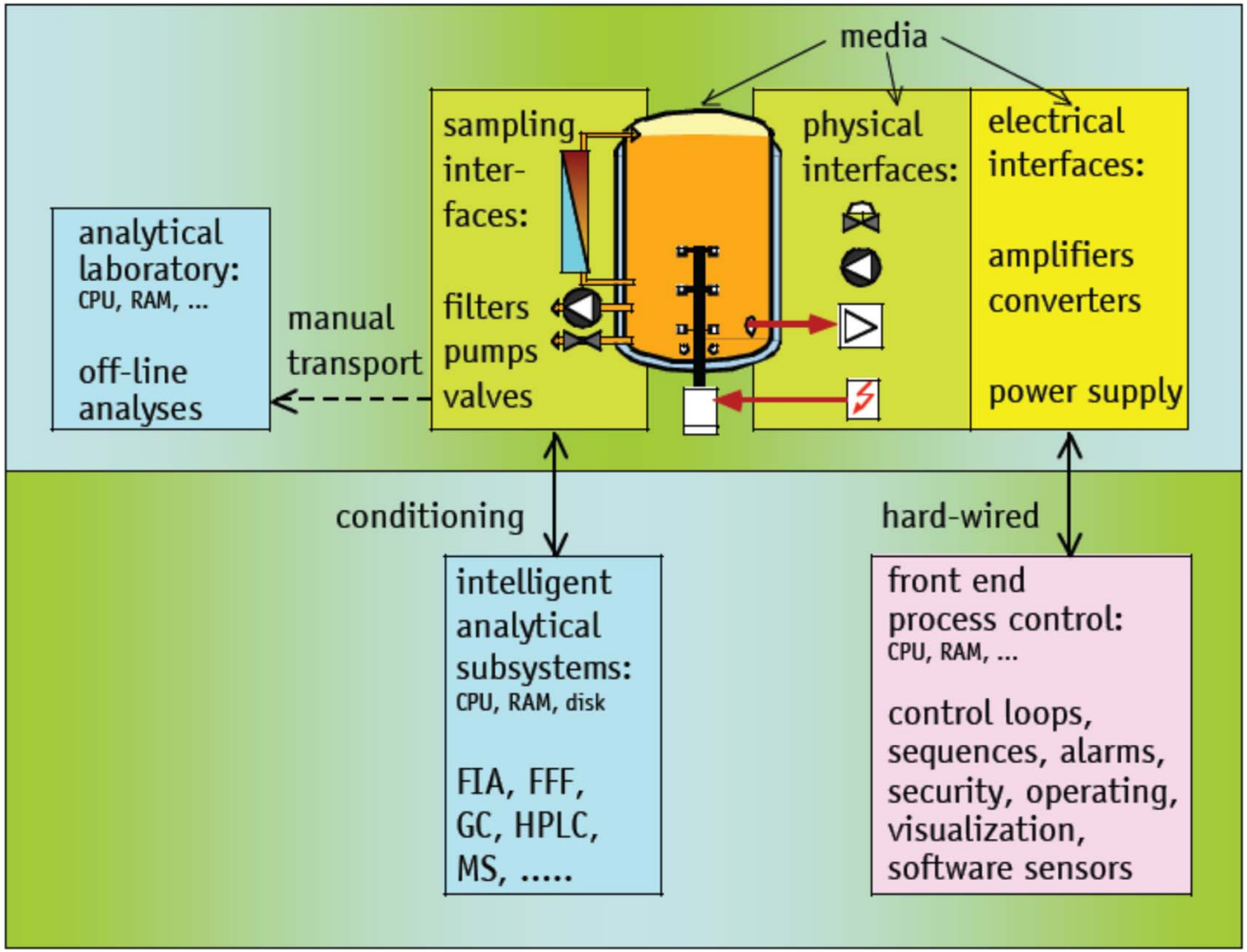
Classical control scheme of fermentation process

single, separated loops without networking of variables



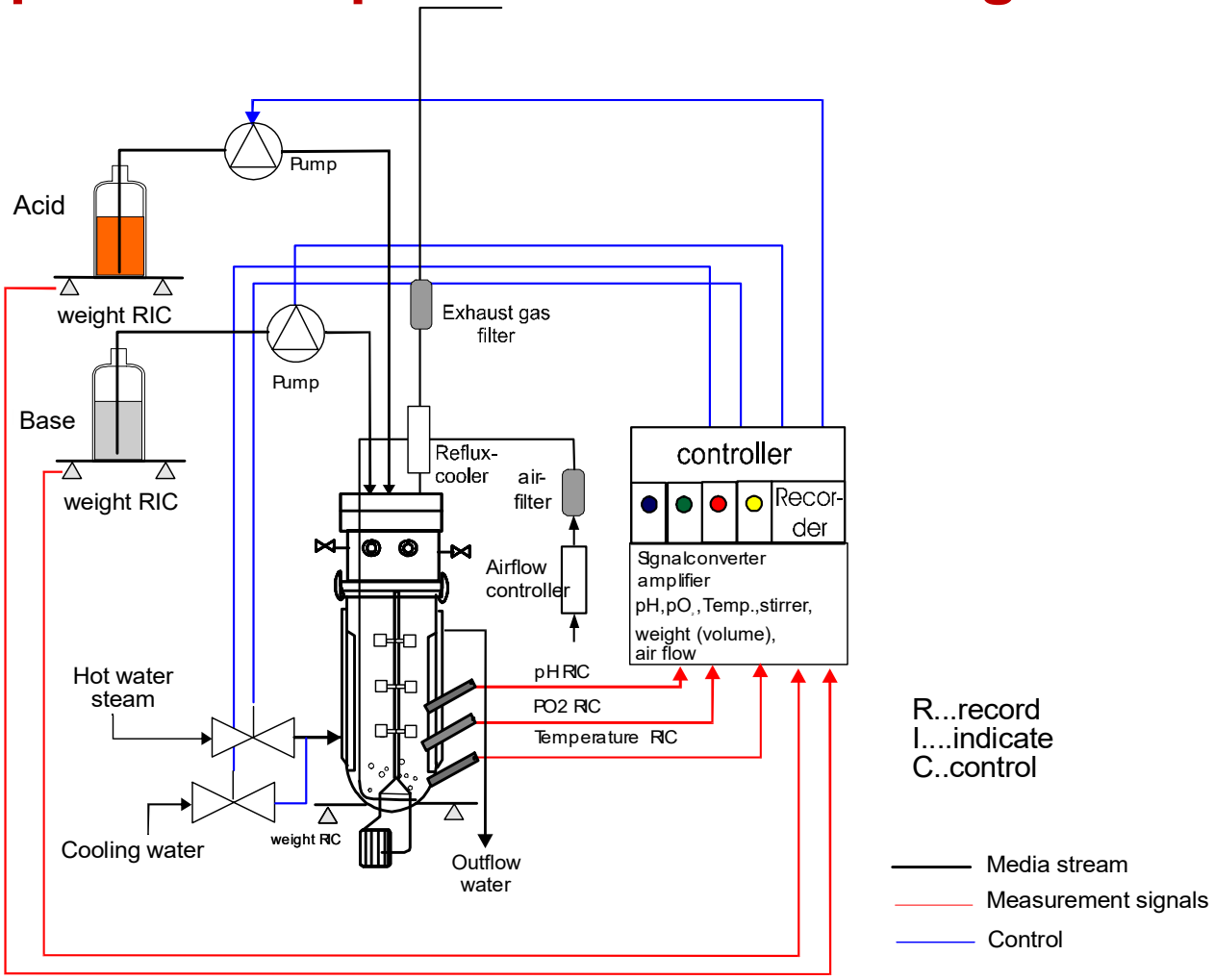

Reflux cooler
Signal transmitter and controller
Controller
Ports of probes
Communication with PC (visualization and configuration)
Laboratory scale bioreactor

concept for hierarchical bioprocess automation

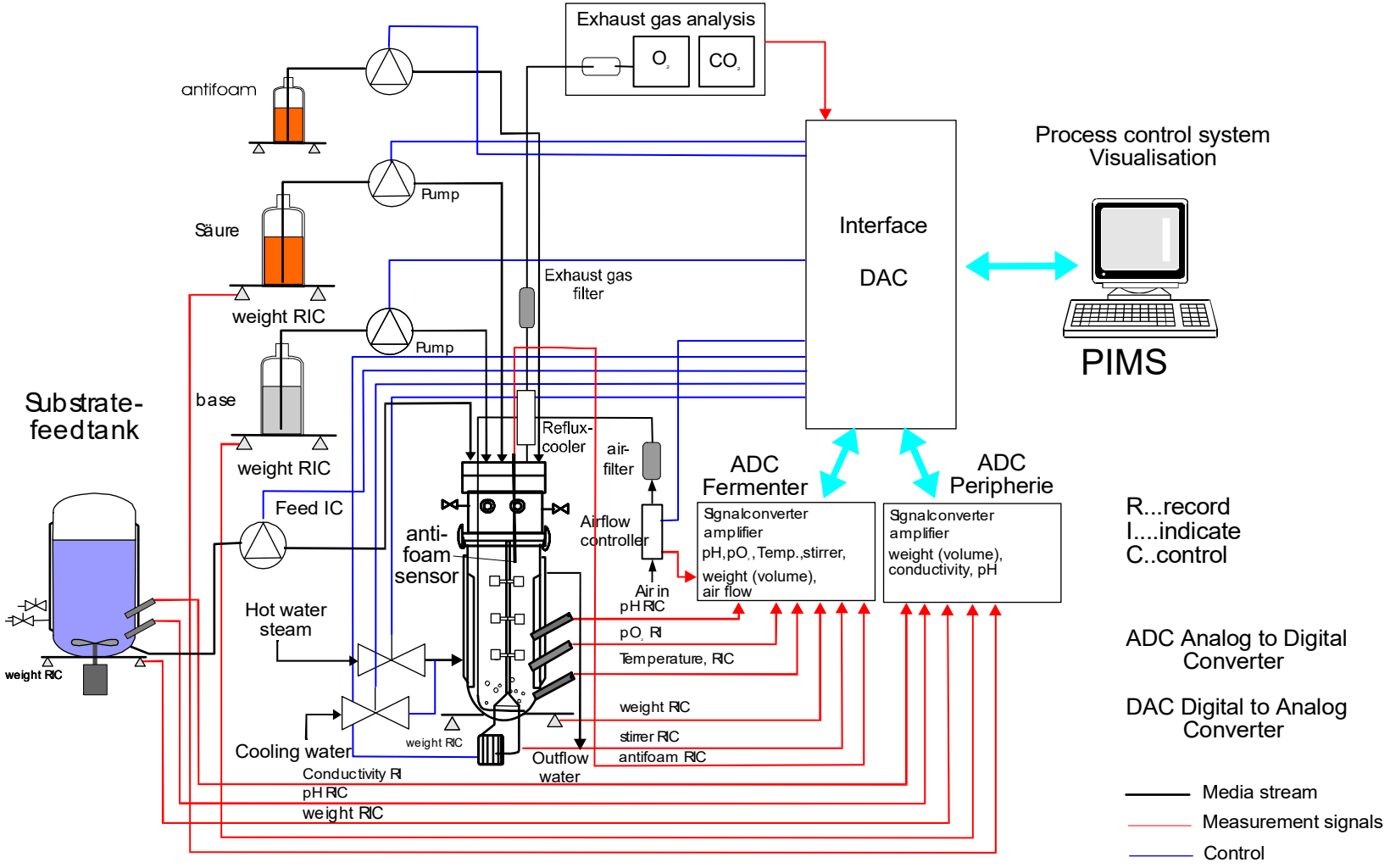


Classical control scheme of fermentation process

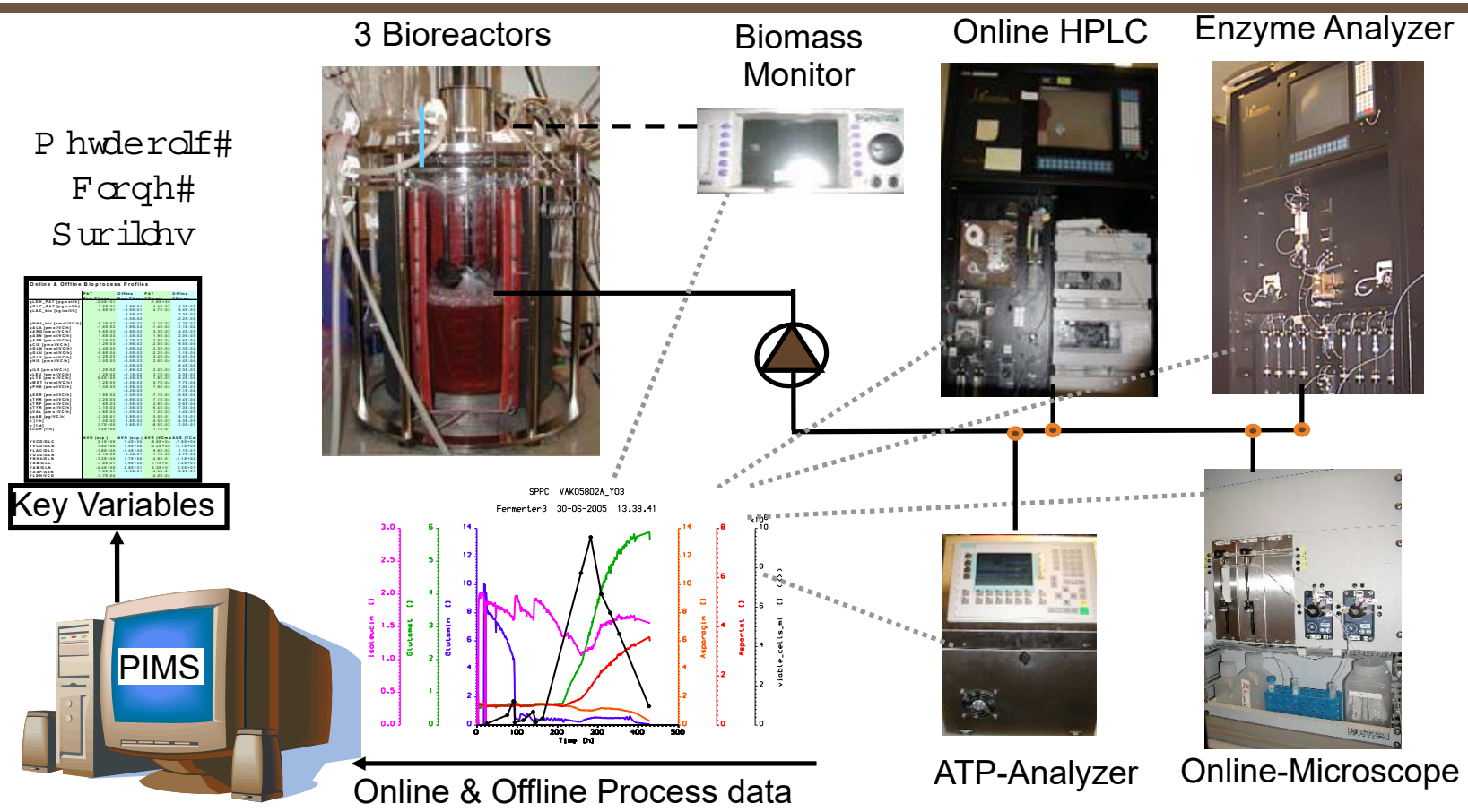
single, separated loops without networking of variables



Bioprocess monitoring and control

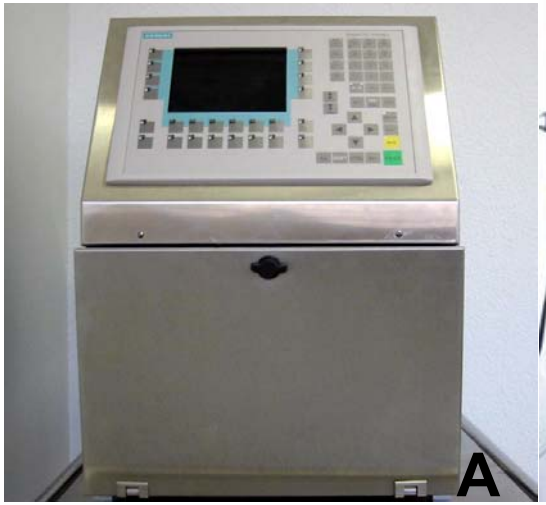


QbD compliant tool “PAT”:
PAT-Workstation for Metabolic Clone Profiling

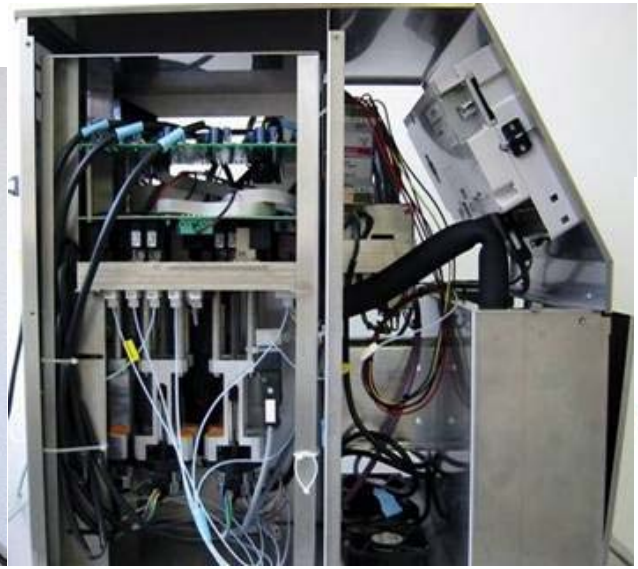


PIMS = Process Information Management System

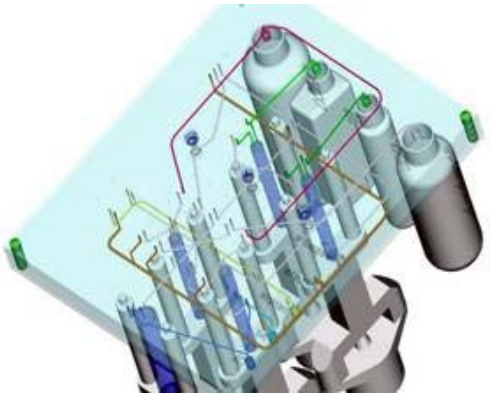
ATP MASTER *PAT System for the Cellular Energy Charge*



Front view



Lateral view with open frame



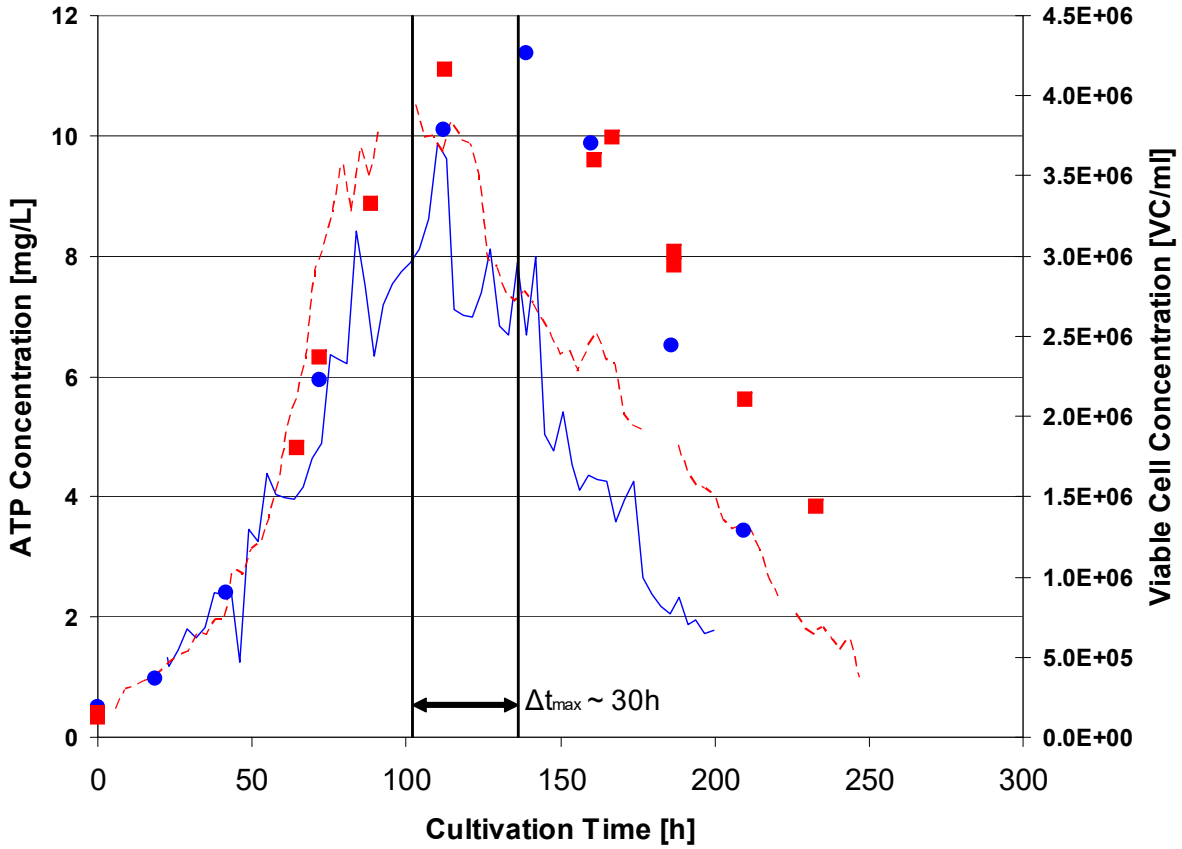
Novel multilayer fluid technology

$$ATP + D-Luciferin + O_2 \xrightarrow{Luciferase} Oxyluciferin + AMP + PPi + h\nu$$

(Cambrex; LT07-221)

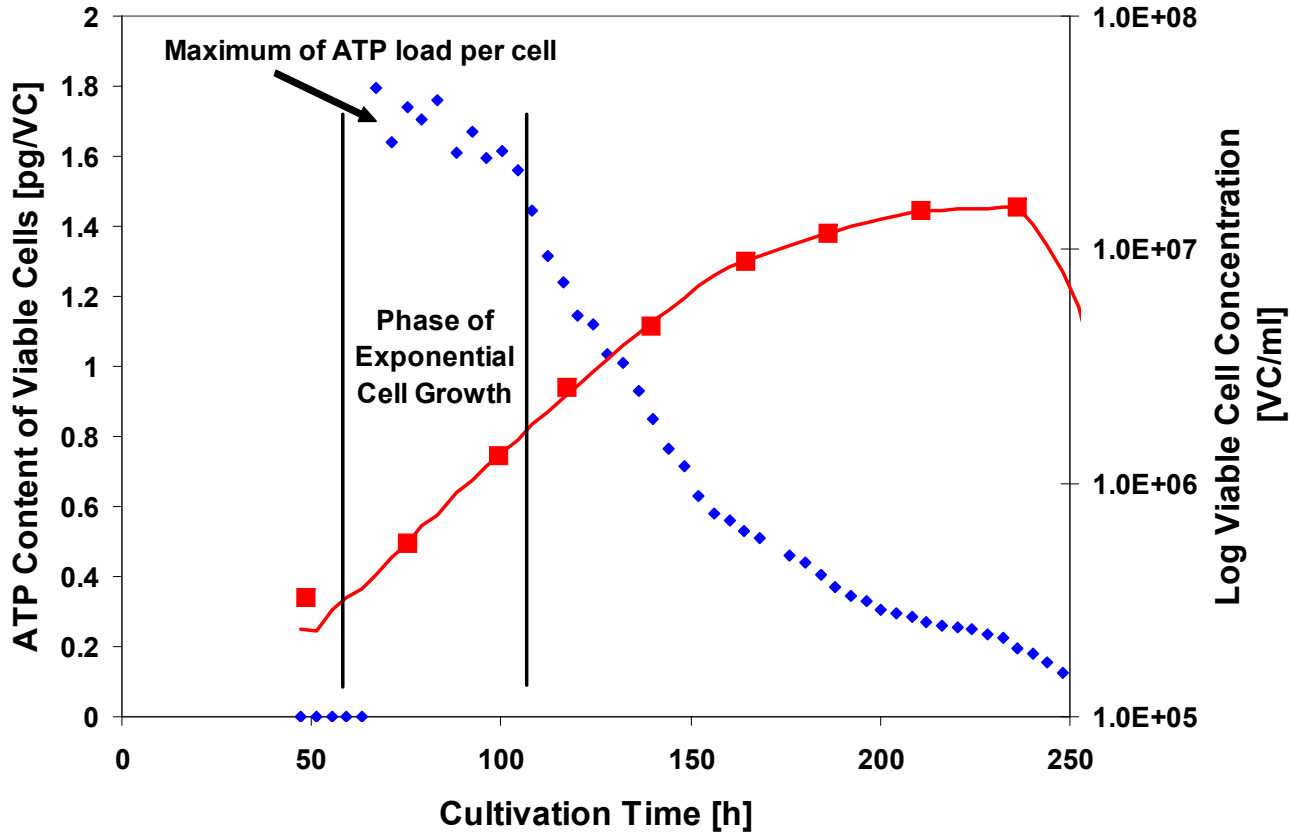
A novel online ATP measurement device was successfully developed within a CTI project. The new system established cell internal ATP measurement methods based on commercialized luciferin-luciferase bioluminescent assays with a new cutting edge analytical and fluidic technology for metabolic control

ATP Analyzer Indicates Early Stationary Phase of SP2/0



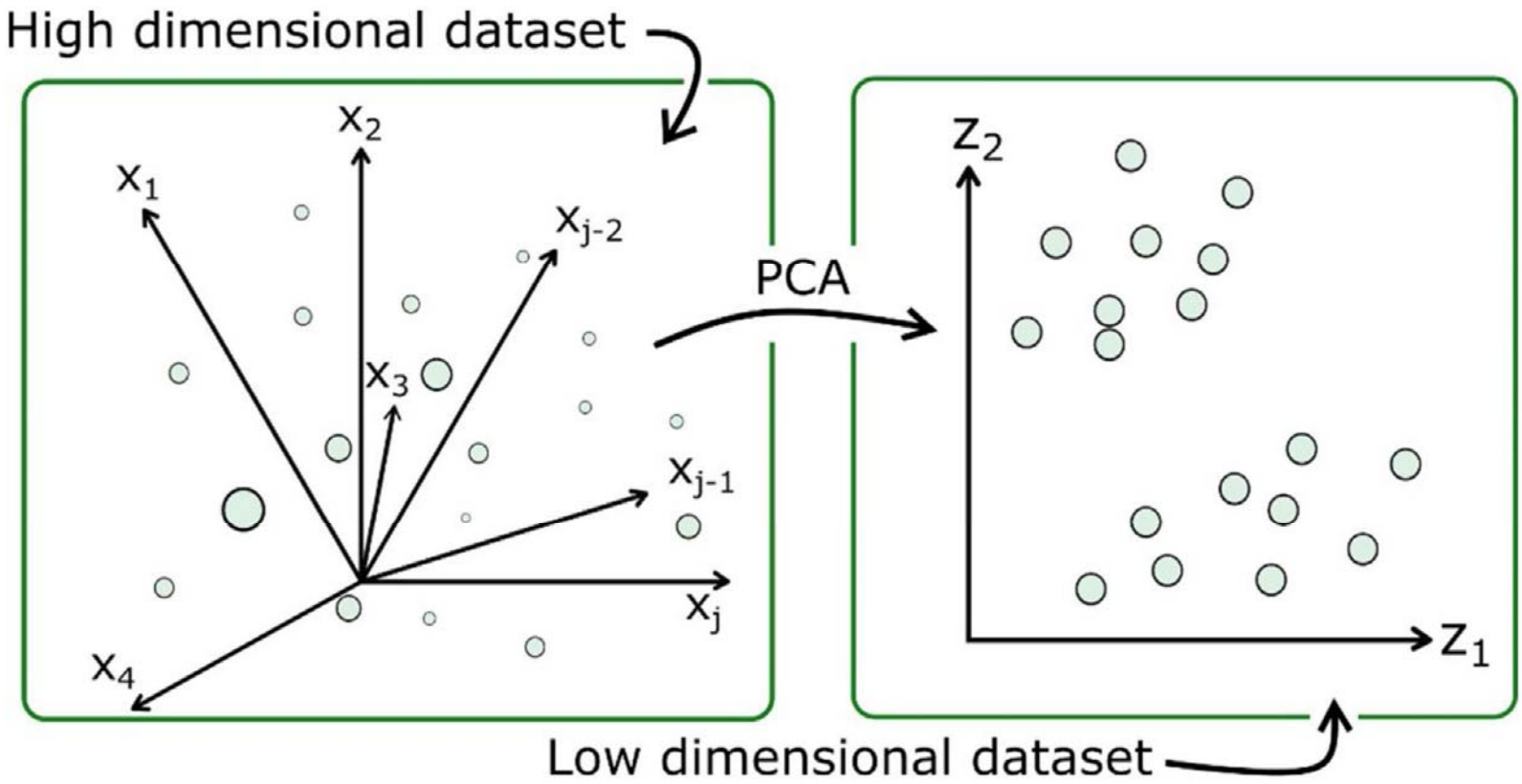
Viable cell density and ATP content of two fed-batch cultivations of SP2/0 cells. Typically, an exponential rise in ATP content is correlated with the fist days of cultivation until a sudden drop indicates a drift in the central metabolism of the production cells to another energy state .

ATP Analyzer Indicates Early Stationary Phase of CHO



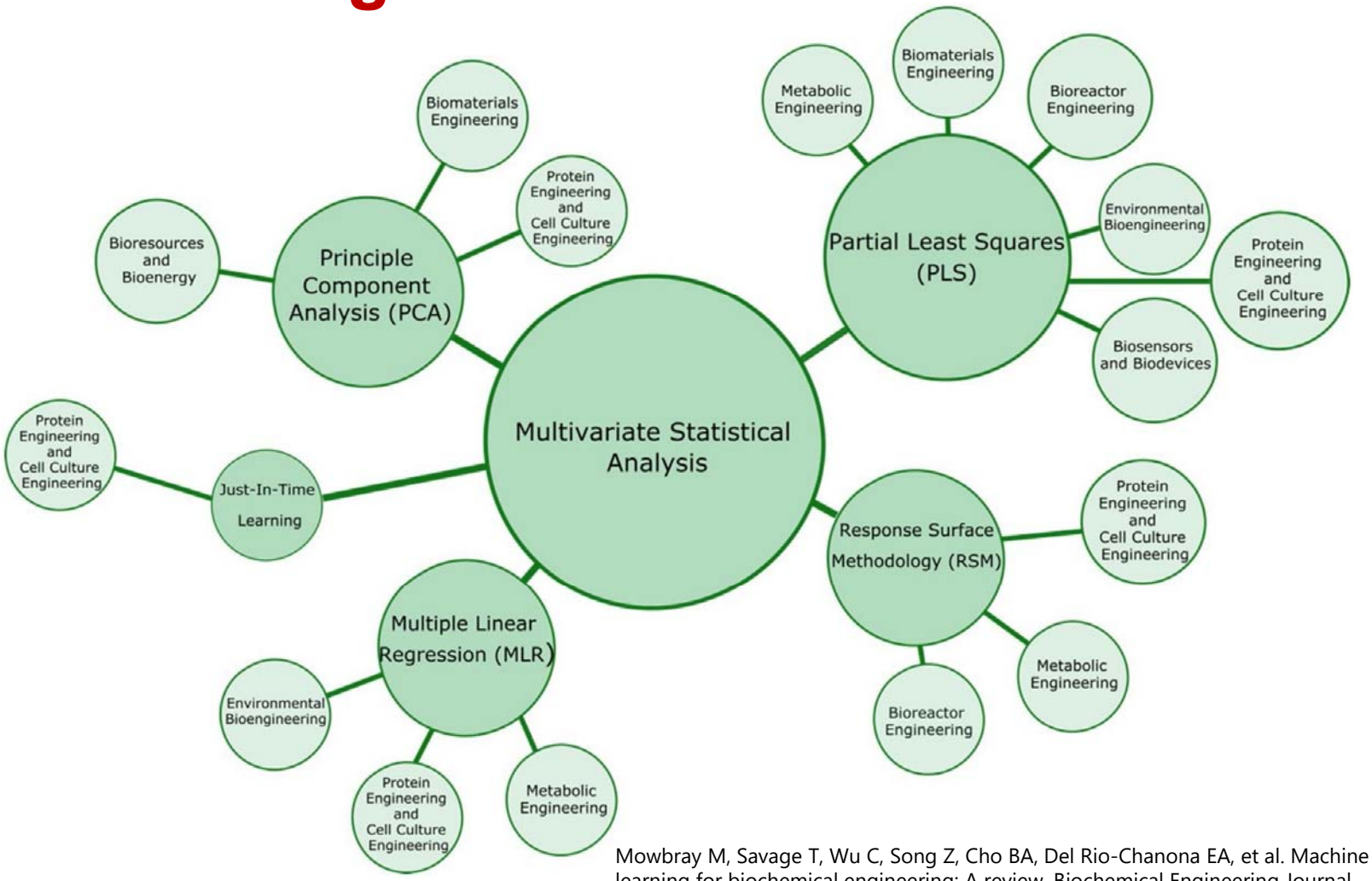
When the ATP pool inside the CHO-cells starts decreasing, the cells changes from exponential to linear growth.

Big data analysis



Mowbray M, Savage T, Wu C, Song Z, Cho BA, Del Rio-Chanona EA, et al. Machine learning for biochemical engineering: A review. Biochemical Engineering Journal. 2021;172:108054.

Machine learning



Mowbray M, Savage T, Wu C, Song Z, Cho BA, Del Rio-Chanona EA, et al. Machine learning for biochemical engineering: A review. Biochemical Engineering Journal. 2021;172:108054.

Tired of cleaning your bioreactor?

Use CIP and SIP!

Bioprocess sterilization

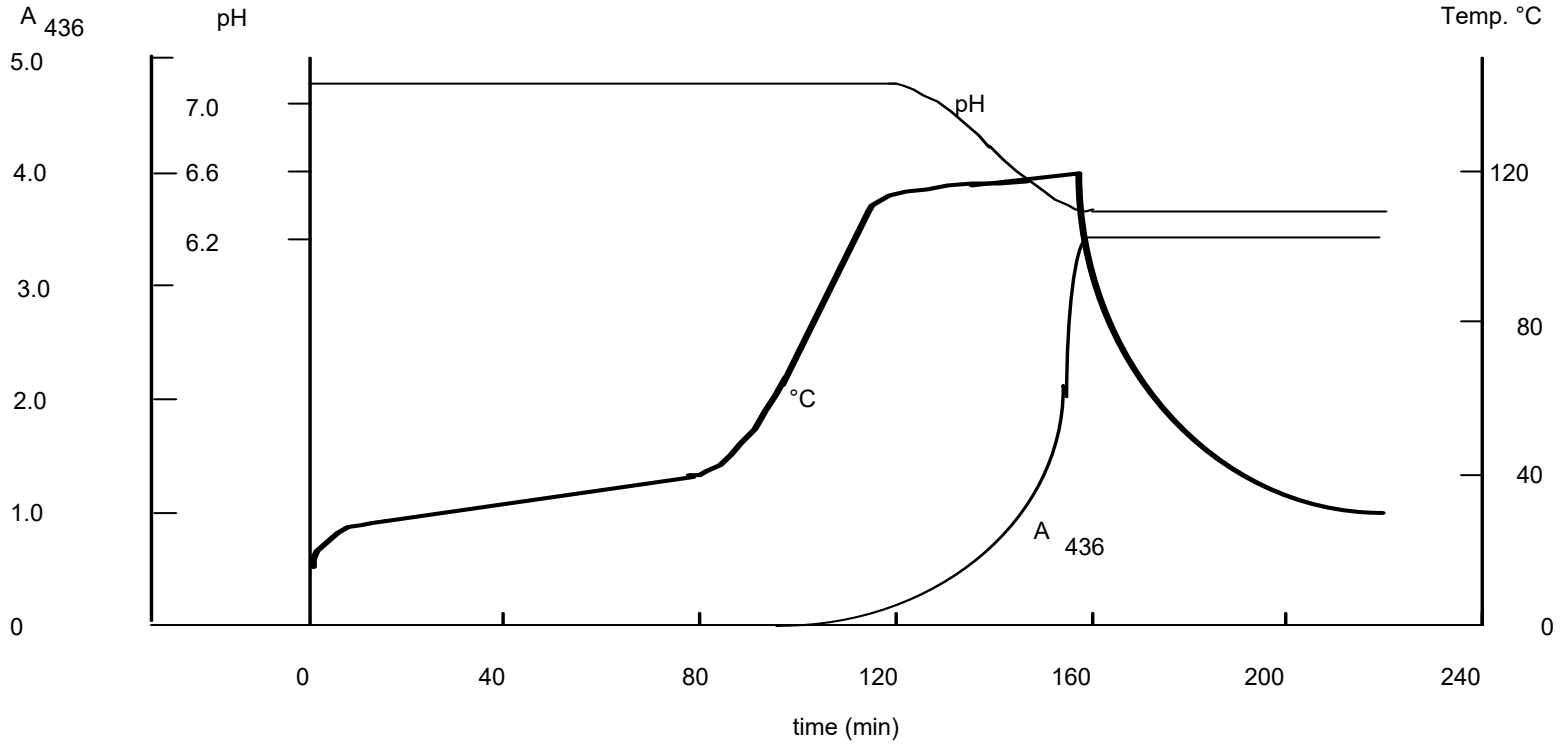
- Cell culture media contains heat labile components: sugar, amino acids, hormones and growth factors and sterilized by microfiltration.
- Reactor and peripherals (acid/ base, medium reservoirs and piping) are generally heat (vapor) sterilized.
- DSP equipment usually sterilized chemically (0.1-2 M NaOH or acid).
- All production equipment requires CIP and SIP protocols and analytical methods for validation.

What are the methods of sterilization?

- Thermal
- Chemical
- Irradiation
- Barrier (filtration)

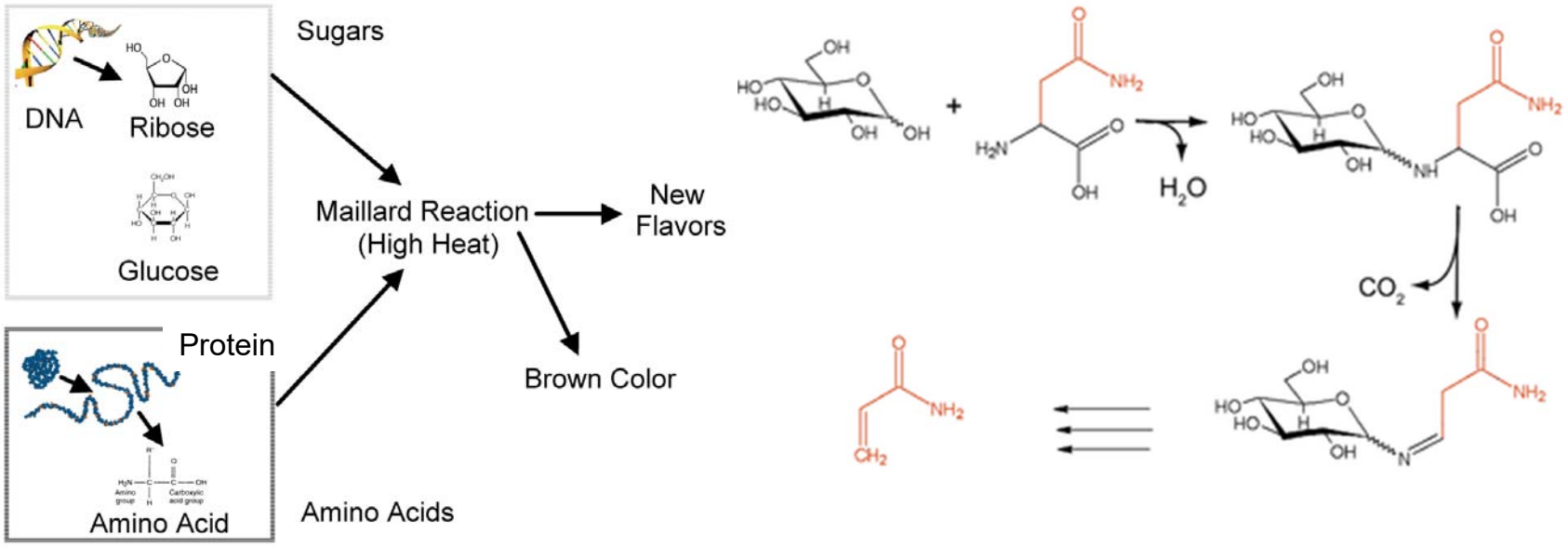
Thermal sterilization is preferred, providing that materials and solutions can withstand elevated temperatures for a sufficient period of time.

Temperature profile for a 3 m^3 reactor containing carbohydrate in medium



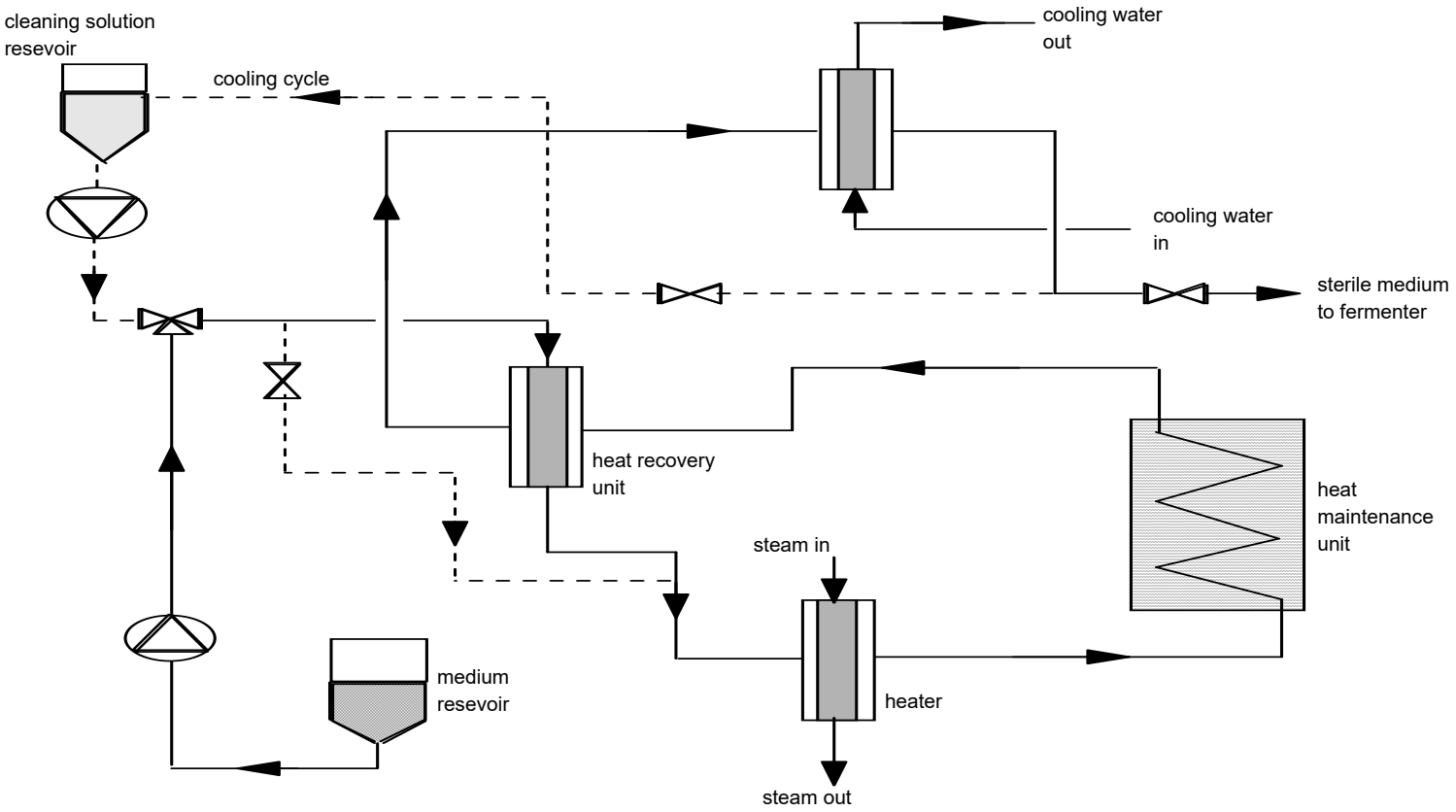
Note: pH falls and A_{436} rises- indicative of thermal degradation of medium

Maillard reaction



Reaction of sugars upon heating, especially in presence of salts, ammonia or proteins.

Schematic representation of a continuous sterilization system



A few definitions

- Inactivation: complete destruction of all biological activity (living cells, virus, prions, plasmids).
- Sterilization: complete destruction of all microorganisms (including spores).
- Desinfection: Reduction of germs (pathogenic cells) below the contamination limit.
- Contamination: Introduction and increase of biological activity.

Method of sterilization

- Thermal (flame, dry air, saturated vapor)
- Chemical (oxidation agents, radicals, alcohols, formaldehyde, EtO)
- Filtration
- High energy rays (gamma, UV)

Desinfection methods

Thermal

- Pasteurization: 62°C, 30 min
- Short temperature increase: 72-74°C, 20 sec
- Ultra high heating: 85°C, 5 sec
- Tyndallization 100°C, 30 min; 30°C, 4 h (2x repetition)

Chemical

- EtOH (70% flammable kills vegetative cells)
- Sodium hypochlorite (NaClO) (pH 4-6.5, kills bacteria, spores and virus)
- Peracetic acid (pH 2-3.5; kills bacteria, spores, virus and fungi)

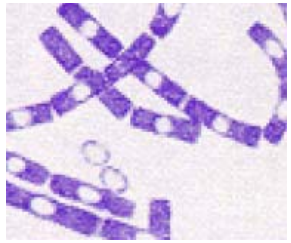
acetic acid hydrogen peroxide peroxyacetic acid water

Sterilization

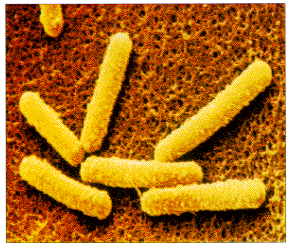
Mechanisms of heat inactivation of microorganisms

Two classes with respect to heat sensitivity

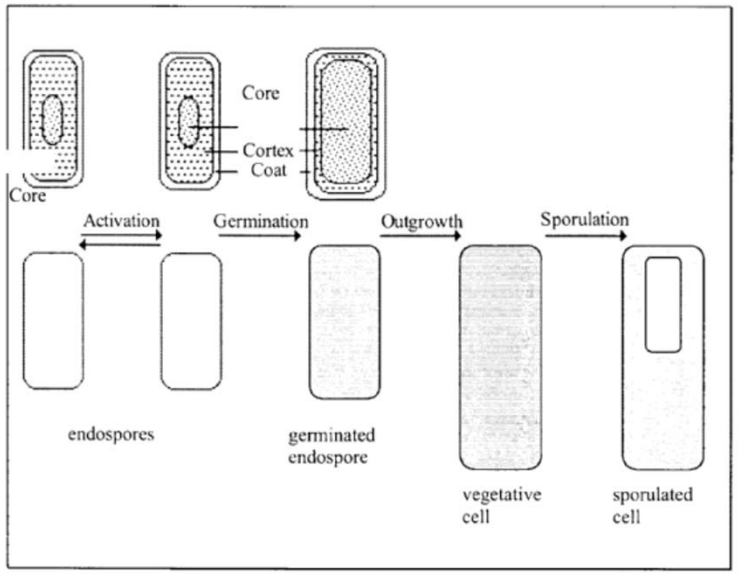
Bacterial endospores (*Bacillus, Clostridium*)



Vegetative cells and spores of other types: fungal spores

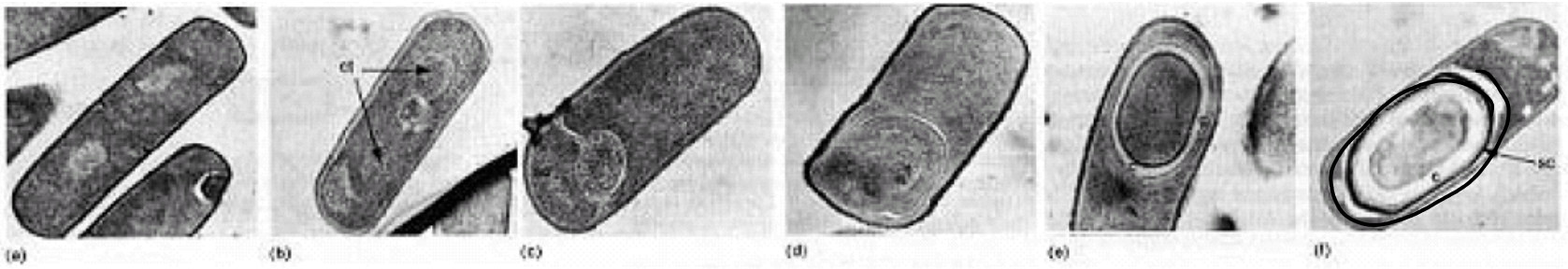


Germination of bacterial endospores

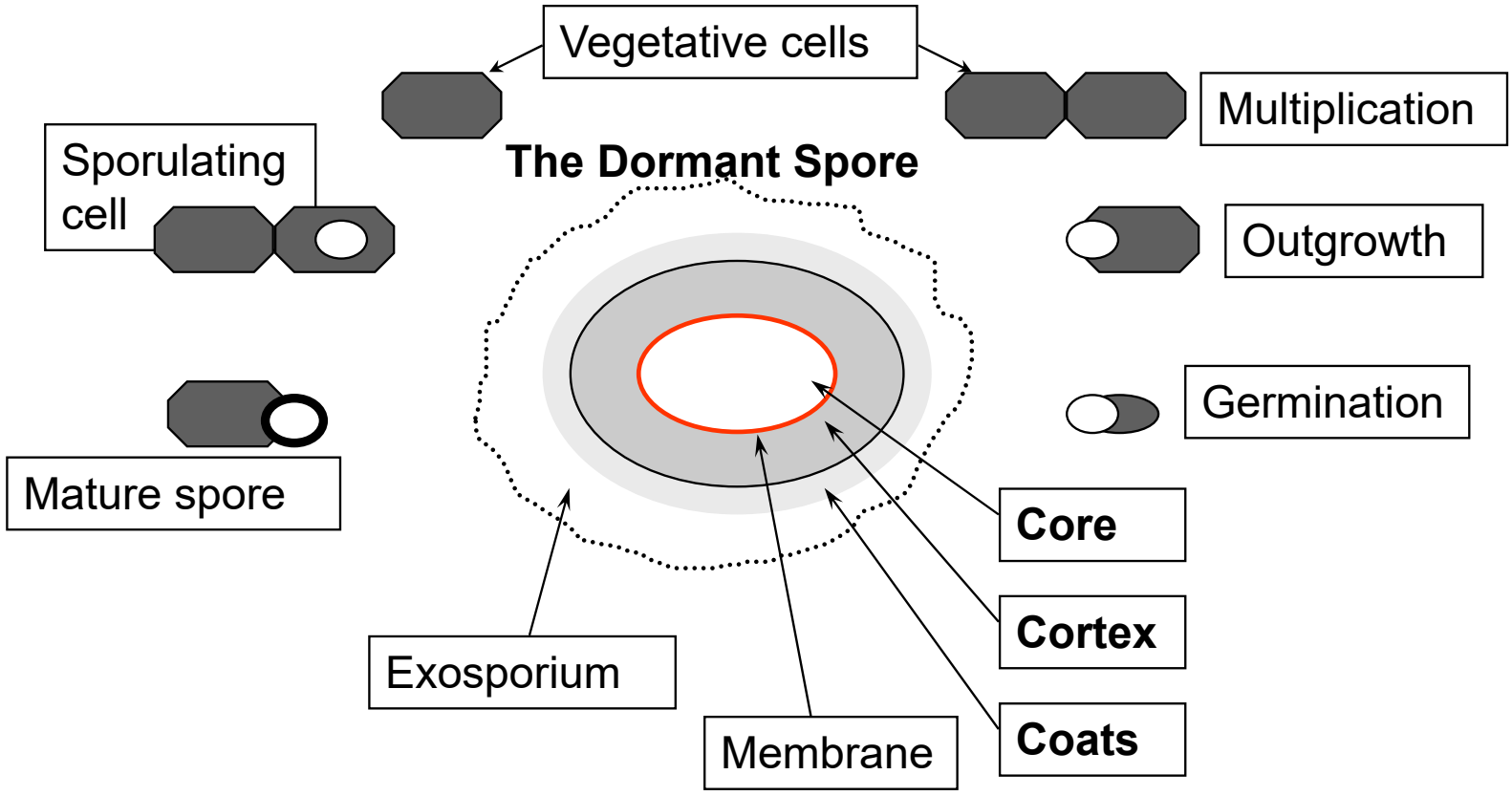


Observations by SEM

Observations using phase contrast microscopy



Bacterial spores



Kinetics of heat sterilization

$$\frac{-dN}{dt} = k * N \quad [1]$$

k (min^{-1}) specific heat inactivation constant, also known as death rate constant

Integration: $$\ln\left(\frac{N}{N_0}\right) = -k*t \quad [2]$$

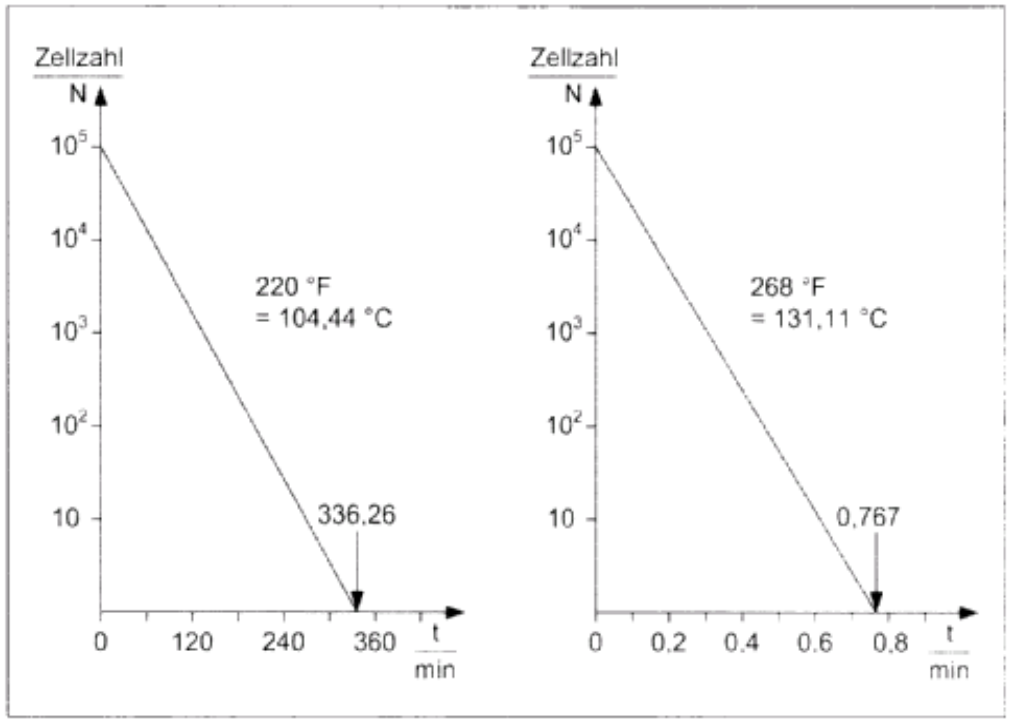
which can be rearranged to

$$\ln(N) = \ln(N_0) - kt \quad [3a]$$

or $$N = N_0 \, e^{-kt} \quad [3b]$$

k: characteristic value for a strain but depends also on physiological state, environmental conditions (pH, solids in medium, temperature, etc.).

Kinetics of heat sterilization



Inactivation of *B. stearothermophilus* at two different temperatures.

Kinetics of heat sterilization

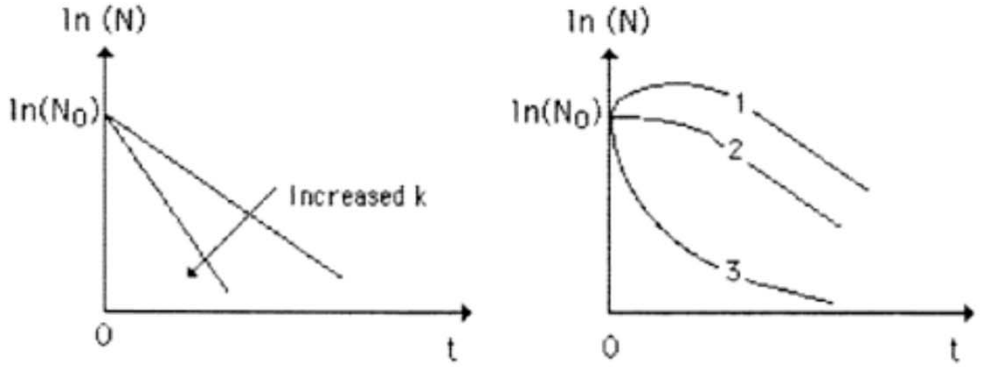


Fig 10.2 Heat inactivation curves. The left hand figure shows two inactivation curves with different death rate constants. The right hand figure shows some deviations from the model:

1. This form may be caused by super-dormant spores, which are activated by the first heat treatment and do not germinate unless they get this treatment;
2. This may be caused by delayed heat transfer in the experiment or it may be observed in samples that contain aggregates of cells, since analysis is made by viable count that gives number of colony forming units rather than number of cells. The viable count does then not decline until the last cell in an aggregate is killed;
3. Non-uniform heat resistance in the population, e.g. when the sample contains species with different thermal sensitivity.

Kinetics of heat sterilization

The heat inactivation constant depends on temperature like most rate constants of chemical reactions. This is usually described by Arrhenius equation:

$$k = A * e^{-\Delta E/(R*T)} \qquad [4]$$

A (min^{-1}); E ($Jmol^{-1}$), R (~8.314 J mol^{-1} K^{-1}), T (K)

$$\rightarrow \ln k = -\frac{\Delta E}{R} * \frac{1}{T} + \ln A \qquad [5]$$

Table 10.1 Examples of ΔE values for heat inactivation of spores and some chemical reactions.

Inactivation of	$-\Delta E$ (kJ mol^{-1})
B. subtilis spores	318
B. stearothermophilus spores	283
Cl. botulinum spores	343
Folic acid	70
d-panthotenyl alcohol	88
Cyanocobalamin	97
Thiamine HCl	92
Maillard reactions	≈125

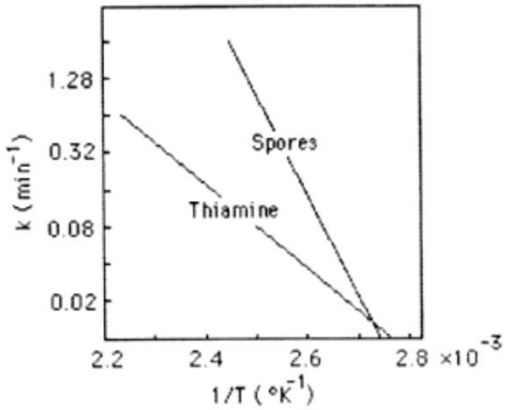


Fig 10.3 Arrhenius plots of inactivation of *B. stearothermophilus* spores and thiamine. Note that a temperature increase has a larger effect on the spore inactivation rate than on the vitamin inactivation rate.

Calculation of sterilization time

According to the model for heat inactivation $\ln(N) = \ln(N_0) - kt$
it is not possible to calculate the time needed to reach sterility.

Thus, a sterility criterion, ∇, has to be defined:

$$\nabla = \ln\left(\frac{N_0}{N_f}\right) \qquad [6]$$

N_f: final number of organisms
∇: is also called ***Del factor*** or the ***design criterion often also mentioned as S_L***

Estimation of sterilization time, F, using eq. [2]

$$F_T = \frac{\nabla}{k} \qquad [7a]$$

This sterilization time depends also on the temperature applied since k is a function of temperature.

Calculation of sterilization time

The **sterilization time (F_T)** required to satisfy the same sterility criterion at another temperature (T in K) than the reference temperature (T_{ref} in K) at which the sterilization time F_{ref} once has been determined:

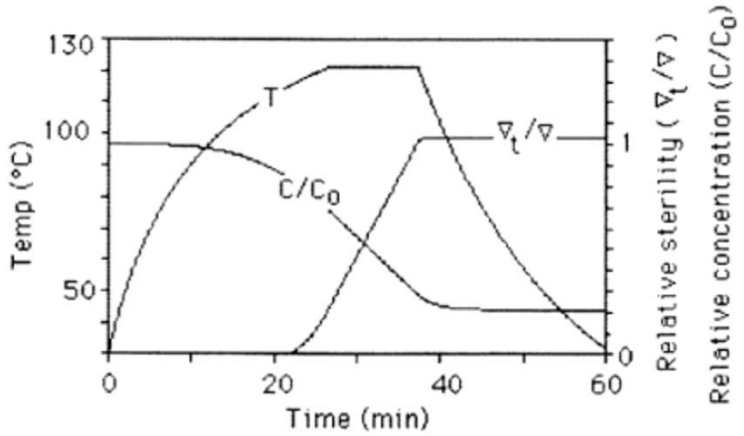
$$\nabla = F_T\, k_T = F_{ref}\, k_{ref} \qquad [7b]$$

using Arrhenius:

$$F_T = F_{ref}\, e^{-\frac{\Delta E}{R}\left(\frac{1}{Tref} - \frac{1}{T}\right)} \qquad [8]$$

Batch sterilization

If the death rate k is known and constant during the sterilization, equation $F = \nabla/k$ gives the answer for the sterilization time required to satisfy the sterility criterion. But the normal profile is different:



Since k depends on temperature, this effect should be introduced in the calculation. Therefore, a time dependent relative sterilization dose $\nabla_t = \ln (N_0/N_t)$ will be introduced. ***The sterility criterion is then satisfied when*** $\nabla_t = \nabla$

Batch sterilization

Combining eq. [6] with eqs. [2] and [4] then gives an expression that shows how the sterilization dose depends on time:

$$\nabla_t = t\,k = t\,A\,e^{-\Delta E/RT} \qquad [9]$$

Since the temperature varies with time during batch sterilization, the time dependent sterilization dose is obtained as the integral of eq. [9]

$$\nabla_t = A \int (e^{-\Delta E/RT})\,dt \qquad [10]$$

Sterility is reached when $\nabla t = \nabla$. This can be calculated without knowing constant A in eq. [10] by using eq. [4] and eq. [7]:

$$\nabla = F_{ref} A\,e^{-\Delta E/RTref} \qquad [11]$$

Then the ratio between ∇t and ∇ gives:

$$\frac{\nabla_t}{\nabla} = \frac{\int (e^{-\Delta E/RT})\,dt}{F_{ref}\,e^{-\Delta E/RTref}\,dt} \qquad [12]$$

Comparison of two different volumes

$$N_{0(1)} / N_{0(2)} = V_1 / V_2$$

$$N_{0(1)} / N = (N_{0(2)} / N) \cdot (V_1/V_2)$$

$$\ln (N_{0(1)}/N) = \ln (N_{0(2)}/N) + \ln(V_1/V_2)$$

Using eq. [6]

$$\nabla_1 = \nabla_2 + \ln(V_1/V_2)$$

Or $$\nabla_1 = \nabla_2 + 2.3\log(V_1/V_2)$$

V: volume; 1: bigger reactor, 2: smaller reactor

Heat sterilization – short summary

- **Criterium for sterilization**: $S_L = \ln(N/N_0)$; $N_o = \sim 10^6$ cells/ml;
  N = 1 germ / 1000 sterilizations (= 99.9 % security) – engineering
  N = 1 germ / 1.000.000 sterilizations – hygienic aspects

  $S_L = \ln[V * 10^6 * 10^3 / 10^{-3}] = \ln(V * 10^{12})$ [e.g: Reactor with volume V]
  Change in volume: $SL_2 = SL_1 + \ln(V_2/V_1)$
  $S_L = D * \log(N_o/N) = 1/k * \ln(N_o/N)$ at T = const.
  $S_L = \int k(T)\, dt$ [heating phase, holding phase, cooling phase]

- Resistance [No= 10^6; N = 10^{-6}]

I	pathogenic Streptococci, Listeria, Polio-virus	61,5ºC/30 min
II	Vegetat. bacteria,yeasts, moulds, virus	80ºC/30 min
III	Hepatitis-B-virus, spores of moulds	100ºC/5-30 min
IV	Bacillus anthracis-spores	105ºC/5 min
V	Bacillus stearothermophilus-Clostridium-Spores	121ºC/15 min
VI	Prions	132ºC/60 min

Note: S_L and ∇ are synonyms and both used in literature.

Killing kinetics

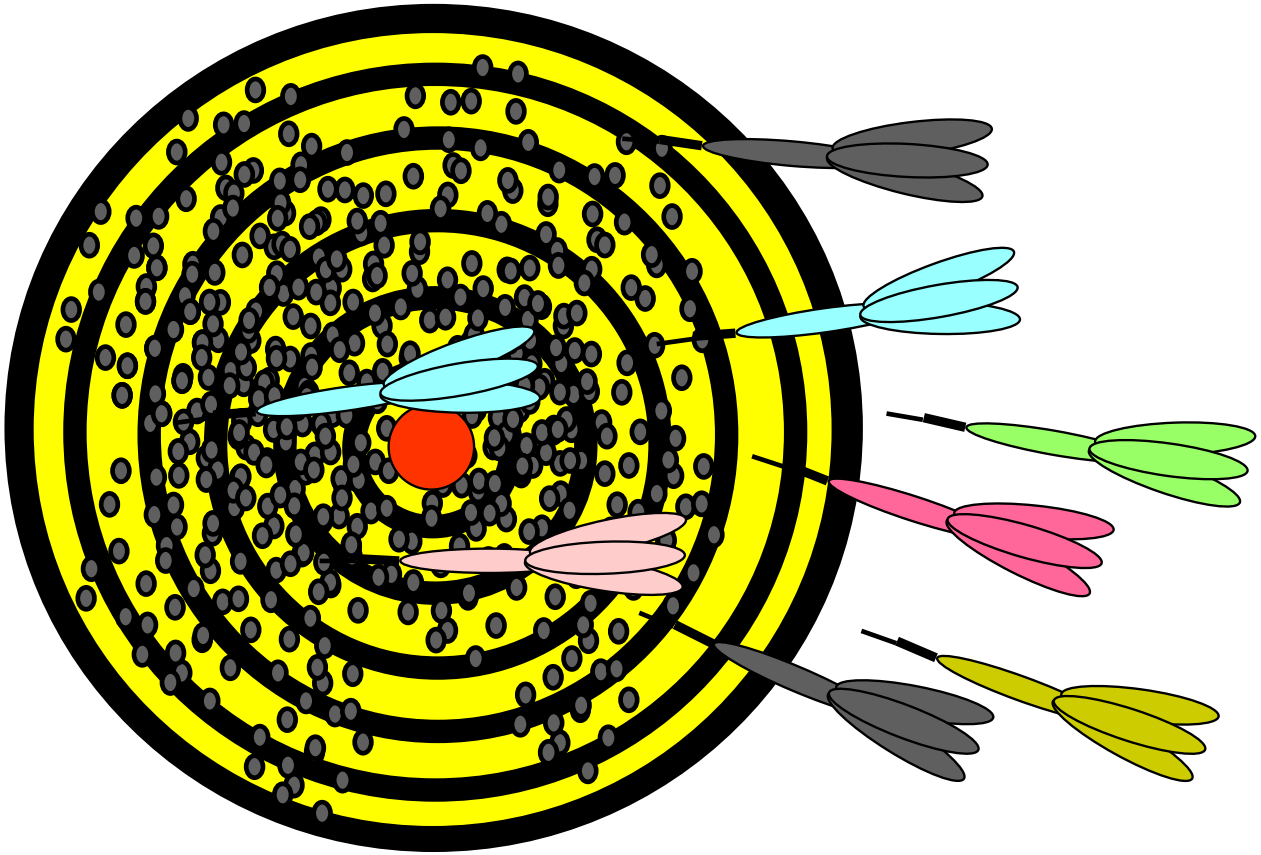
$$\ln \frac{1}{10} = -k_d \cdot t$$

$$-2{,}303 = -k_d \cdot t$$

$$\boxed{t = D = \frac{2{,}303}{k_d}}$$

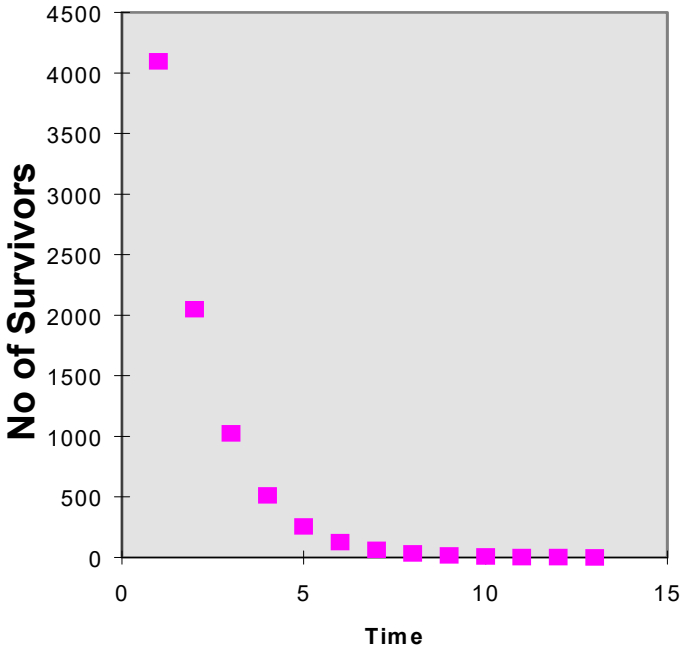
Time	Survivors	Death per unit time		Total death	Log Red
0	1 000 000	0		0	0
1	100 000	900 000	= 90%	900 000	1
2	10 000	90 000	= 90%	990 000	2
3	1 000	9 000	= 90%	999 000	3
4	100	900	= 90%	999 900	4
7	0,1	0,9	= 90%	999 999,90	7
8	0,01	0,09	= 90%	999 999,99	8

Killing Hypothesis

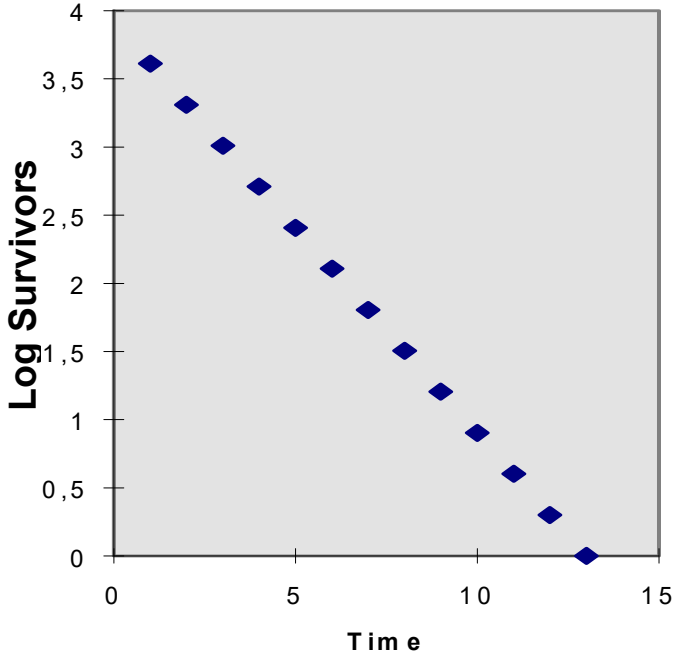


Graphs of survival curves

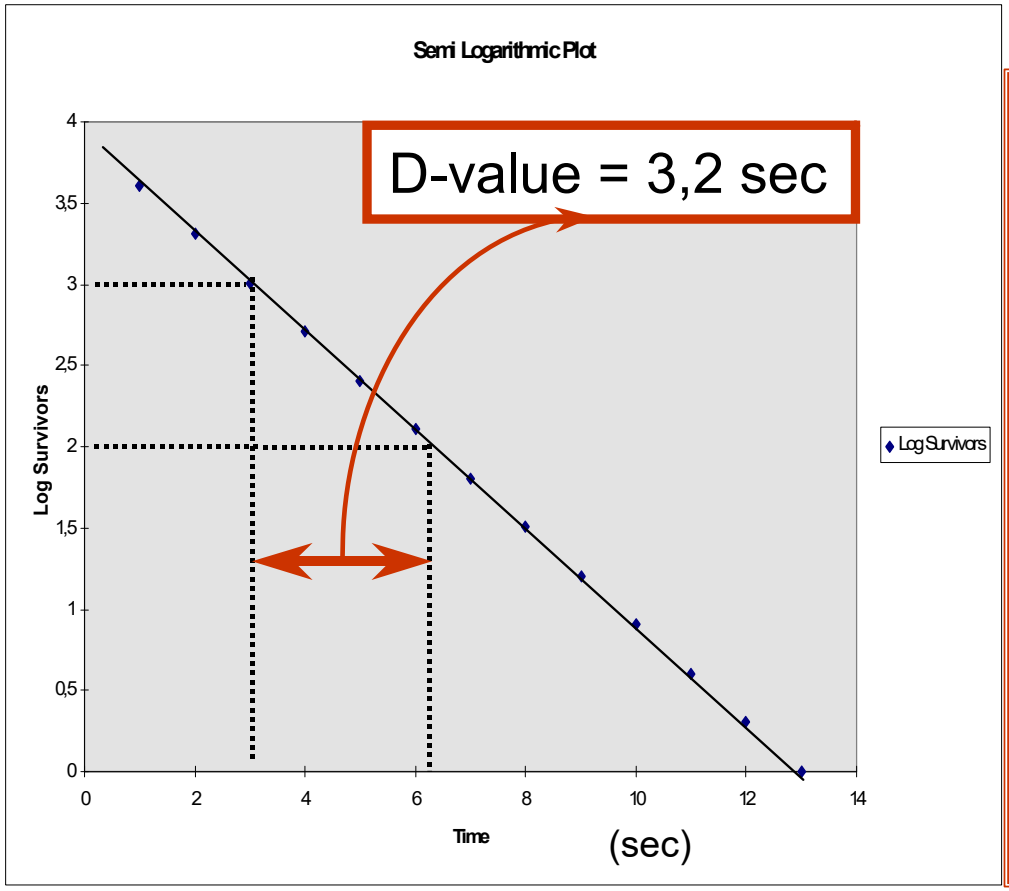
Arithmetic



Semilogarithmic



Decimal Reduction Value



D-value:

is the time required for a process to reduce the number of a specific organism by 90 % or with

1 Log Red or

1 D

Explanation of D-value

The expression D_{65} = 1 min
means that the number of organisms is reduced with 1 D when treated one minute at a processing temperature of 65 ˚C.

Two (2) minutes exposure results in 2D.

1D = 90	% reduction = 1 Log Red
2D = 99	% reduction = 2 Log Red
3D = 99,9	% reduction = 3 Log Red
4D = 99,99	% reduction = 4 Log Red

Typical killing rate data for some microorganisms

Group of organism	*D-value (min)*	*Temp. (^{0}C)*	*Reduction by 12D*
Vegetative Bacteria (heat sensitive)	1	65	75 ^{0}C / 7 sec
Vegetative Bacteria (heat resistant)	10	65	75 ^{0}C / 70 sec
Bacterial spores	0,2	121	121 ^{0}C / 2,5 min
Yeast and Mould	1	65	75 ^{0}C / 7 sec
Mould / ascospores	3	90	121 ^{0}C / 2 sec

Heat resistance of bacteria

Bacteria	D_{90}-value **(hrs)**	D_{121}-value **(sec)**
L. monocytogenes	0,01 sec	"0"
B. cereus	1	1,4
B. subtilis	30	60
B. stearothermophilus	>100	330
C. botulinum, strains B/E	< 0,02	<0,048
C. botulinum, strains A	3,3	12

Thermal sterilization

Basic principles also apply to sterilization by radiation e.g. gamma or gas e.g. ethylene oxide

D- value

The time required for a one- log reduction in the microbial population

(i.e. time required for 90 % reduction in microbial population)

i.e. determines *rate* of killing of organisms

Sterilization efficiency

The Sterilization Efficiency of a process is the number of Log Red that it gives on the most resistant microorganism.

z-value

z-value is the change in temperature in ˚C required to cause a 10-fold change in D-value

$$Z = \frac{T_1 - T_2}{\text{Log } D_{T_2} - \text{Log } D_{T_1}}$$

where

Z is temperature in ^0C and

D_{T_1} and D_{T_2} are measured D-values at temperatures (˚C) T_1 and T_2 respectively.

How to use the z-value

A certain microorganism is characterized by a z-value of 10 ˚C and a D_{65} = 50 sec.

That means that D_{75} = 5 sec

D_{85} = 0.5 sec

D_{95} = 0.05 sec

Relationship between z- and F- values

The accepted z-value is:

10°C for *B. stearothermophilus* spores under steam sterilization and

20°C for same spores under dry heat

From z-value the effective time exposure at a desired temperature necessary for sterilization can be calculated-

i.e. the F-value

F-value determination

The F value therefore doesn't measure real time but equivalent time at which the temperature was held e.g.

at 121°C

$$F = \Delta t \sum 10^{(T - T_0)/Z}$$

delta$_t$ = time interval for measurement of product temperature T, T$_0$ = reference temperature e.g 121°C

F_0 expression

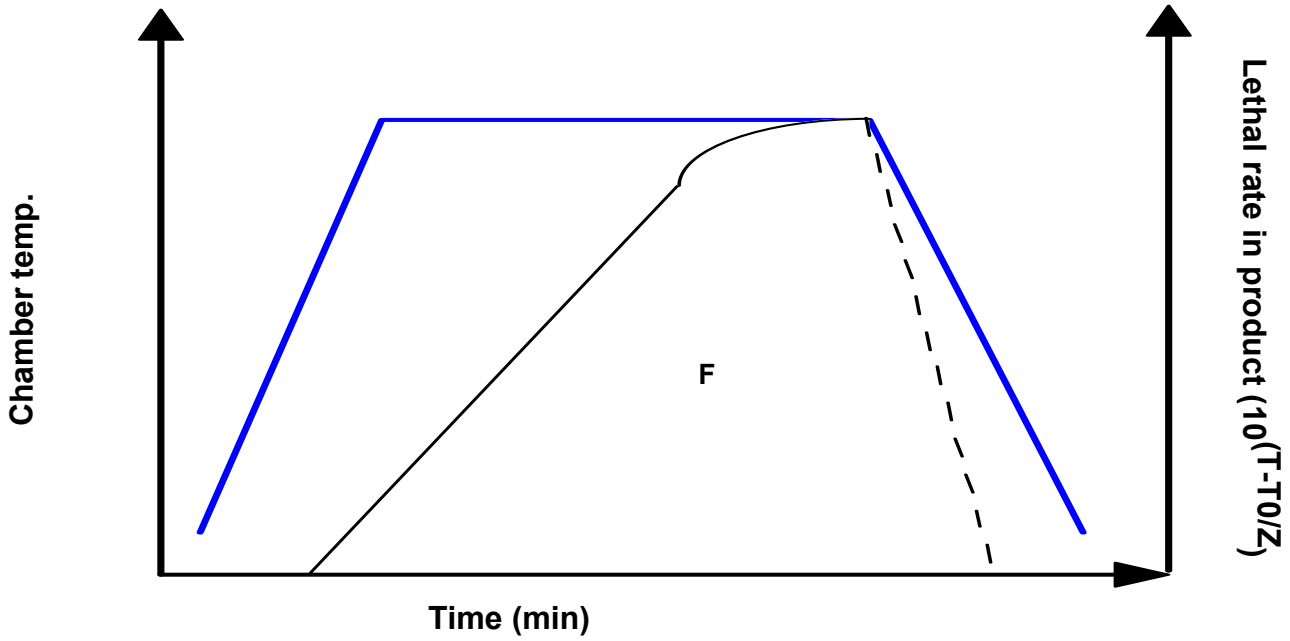
The more usual form is F_0 equation specific for a Z-value of 10°C and a T_0 of 121°C:

$$F_0 = \Delta t \sum 10^{(T-121)/10}$$

cGMPs suggest that steam sterilization must be sufficient to produce an F_0 value of at least 8 min,

i.e. the coolest part of the sterilizer must be exposed to the equivalent of 8 min at 121°C.

Importance of F value



Summary

- Upstream processing is also engineering and can significantly affect the outcome of bioprocesses (e.g. sterility aspects).
- Besides GMP and other norms, new regulations at FDA require tight control and monitoring of bioprocess (process analytical technology, PAT).
- Scale-up of bioprocesses can be simplified by PAT.
- Sterilized equipment is an essential base for successful bioprocesses.