Advanced Course in Fermentation Technology

Applied systems biology for analysis of bioprocesses

11. -15. February 2019
Chiang Mai University, Thailand

Prof. Dr.-Ing. Peter Götz
Beuth University of Applied Sciences Berlin

Preface

The workshop “Advanced course in Fermentation Technology” is the renewal of a workshop which has been conducted at Khon Kaen University in September 2003, then organized by Assist. Prof. Dr. Vichai Leelavatcharamas, Dr. Naruemol Noisommit-Rizzi, the late Dr. Paiboon Danvirutai and Prof. Peter Götz.

The workshop is planned to demonstrate the integration of experimental work and theoretical approaches to analyze biological processes on a molecular, intracellular and bioreactor level.

The workshop is to be held at the Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand from February 11-15. It is supported by the Core-to-core Program on "Establishment of an international research core for new biological fields with microbes from tropical area" , allowing a joint event organized and conducted by Thai and German professors and lecturers.

I want to thank everybody in our Core-to-core network to make this cooperation possible, especially our host for the workshop at Chiang Mai University, Assist. Prof. Dr. Chartchai Khanongnuch. Furthermore I want to thank the National Research Council of Thailand (NRCT) as well as the Japan Society for the Promotion of Science JSPS for financial support and my colleagues Prof. Dr. Mamoru Yamada (Yamaguchi University) and Assoc. Prof. Dr. Gunjana Theeragool (Kasetsart University, Bangkok) for organizing and collaborating.

Prof. Dr.-Ing. Peter Götz
February 2019

PROGRAMME
Advanced Course in Fermentation Technology Workshop
on "APPLIED SYSTEMS BIOLOGY FOR ANALYSIS OF BIOPROCESSES"
11 –15 February 2019
at Division of Biotechnology, Faculty of Agro-Industry, Chiang Mai University, Thailand

DATE / TIME	DESCRIPTION
11 February 2019	**Monday**
8:00-9:00	Registration
9:00-9:30	**Opening Ceremony & Group Photo** ***- MC: Introduce our Workshop and Invite 3 speeches respectively***
9:00-9:05	**Welcome address** by Assist. Prof. Dr. Sujinda Sriwattana Dean, Faculty of Agro-Industry, Chiang Mai University
9:05-9:10	**Opening address** by Prof. Dr. Mamoru Yamada CCP Japanese Coordinator, Yamaguchi University
9:10-9:15	**Opening address** by Assoc. Prof. Dr. Gunjana Theeragool CCP Thai Coordinator, Faculty of Science, Kasetsart University
9:15-9:30	**Group Photo** ***(All person) at In front of faculty of Agro-Industry***
9:30	***Brief Introduce and Invite*** ***Prof.Dr. -Ing. Peter Goetz, German Coordinator and Mr. Thorsten Jamrath***
9:30-10:30	Lecture Ia: Balance equations and reaction kinetics By Prof. Dr.-Ing. Peter Goetz, German Coordinator, Beuth University of Applied Sciences
10:30-10:45	**Coffee Break**
10:45-12:30	Lecture Ib: Balance equations and reaction kinetics by Prof. Dr.-Ing. Peter Goetz
12:30-13:30	**Lunch**
13:30-15:00	Experiment Ia: Introduction into lab equipment (bioreactors), preparation of inoculum, preparation of bioreactors and media by Dipl.-Ing. Thorsten Jamrath and laboratory assistant
15:00-15:15	**Coffee Break**
15:15-17:00	Exercise I: Computer simulation of bioprocesses by Prof. Dr.-Ing. Peter Goetz
17:00	**- Depart from Faculty of Agro-Industry by Van** **- Go to Salatham (Main Campus) and Move to Electric car for Main Campus Tour**
18:30 -20:30	**Reception party** at Salatham, Chiang Mai University (Main campus)

12 February 2019	**Tuesday**
9:00-10:30	Experiment Ib: Fed-batch cultivation by Dipl.-Ing. Thorsten Jamrath, Prof. Dr.-Ing. Peter Goetz and laboratory assistant
10:30-10:45	**Coffee Break**
10:45-12:30	Experiment Ic: Fed-batch cultivation by Dipl.-Ing. Thorsten Jamrath, Prof. Dr.-Ing. Peter Goetz and laboratory assistant
12:30-13:30	**Lunch**
13:30-15:00	Experiment Id: Fed-batch cultivation by Dipl.-Ing. Thorsten Jamrath, Prof. Dr.-Ing. Peter Goetz and laboratory assistant
15:00-15:15	**Coffee Break**
15:15-17:00	Experiment Ie: Fed-batch cultivation, Evaluation, Interpretation of Results by Prof. Dr.-Ing. Peter Goetz, Dipl.-Ing. Thorsten Jamrath and laboratory assistant
13 February 2019	**Wednesday**
9:00-10:30	Lecture II: Balances for intracellular reactions, pathways and networks by Prof. Dr.-Ing. Peter Goetz
10:30-10:45	**Coffee Break**
10:45-12:30	Lecture III: *lac* operon model by Prof. Dr.-Ing. Peter Goetz
12:30-13:30	**Lunch**
13:30-17:00	Experiment IIa: Preparation of inoculum, preparation of bioreactors and media by Dipl.-Ing. Thorsten Jamrath and laboratory assistant
15:00-15:15	**Coffee Break**
15:15-17:00	Exercise II: Computer simulation – *lac* operon model / diauxic growth by Prof. Dr.-Ing. Peter Goetz
14 February 2019	**Thursday**
9:00-10:30	Experiment IIb: Batch cultivation (Diauxic growth) by Dipl.-Ing. Thorsten Jamrath, Prof. Dr.-Ing. Peter Goetz and laboratory assistant
10:30-10:45	**Coffee Break**
10:45-12:30	Experiment IIc: Batch cultivation (Diauxic growth) by Dipl.-Ing. Thorsten Jamrath, Prof. Dr.-Ing. Peter Goetz and laboratory assistant
12:30-13:30	**Lunch**
13:30-17:00	Experiment IId: Batch cultivation (Diauxic growth) by Dipl.-Ing. Thorsten Jamrath, Prof. Dr.-Ing. Peter Goetz and laboratory assistant
15:00-15:15	**Coffee Break**
15:15-17:00	Experiment IIe: Batch cultivation, Evaluation, Interpretation of Results by Prof. Dr.-Ing. Peter Goetz, Dipl.-Ing. Thorsten Jamrath and laboratory assistant

15 February 2019	**Friday**
9:00-10:30	Test and Participant Assessment by Prof. Dr.-Ing. Peter Goetz
10:30-10:45	**Coffee Break**
10:45-11:45	Certificate and Conferment by Prof. Dr.-Ing. Peter Goetz
11:45-12:00	**Closing Ceremony** by Assist.Prof. Dr. Chartchai Khanongnuch Chairman of Organizing Committee, Chiang Mai University
12:00-12:30	**Lunch (Box set)**
12:30-16:00	**Excursion**

Part I

Balance equations and reaction kinetics

Advanced Course in Fermentation Technology
Applied systems biology for analysis of bioprocesses

P. Götz, Slide 3

The workshop is planned to demonstrate the integration of
experimental work and
theoretical approaches
to analyze biological processes on a molecular, intracellular and bioreactor level.

$$\frac{dc_X}{dt} = \mu \cdot c_X$$

$$\frac{dc_{Glc}}{dt} = -\frac{1}{Y_{X/Glc}} \cdot \mu_{max,Glc} \cdot \frac{c_{Glc}}{K_{S,Glc} + c_{Glc}} \cdot c_X$$

$$\frac{dc_{Lac}}{dt} = -\frac{1}{Y_{X/Lac}} \cdot (v_{E,Lac} \cdot X_{E,Lac}) \cdot \frac{c_{Lac}}{K_{S,Lac} + c_{Lac}} \cdot c_X$$

$$\frac{dX_{E,Lac}}{dt} = k_E \cdot X_{m-RNA,E} - \mu \cdot X_{E,Lac}$$

$$\frac{dX_{m-RNA,E}}{dt} = r_{m-RNA,E,max} \cdot f(\mu) \cdot \varphi_{ind} \cdot \varphi_{KR} - k_D \cdot X_{m-RNA,E} - \mu \cdot X_{m-RNA,E}$$

Balance equations

P. Götz, Slide 4

Quantification of a process requires evaluation of measured variables and parameters over time. Balance equations provide the respective theoretical framework.

Balance equations can be set up only for extensive quantities (amount of substance, mass, energy, momentum, volume, entropy, …). Equations for description of measured variables (concentration, temperature, velocity, …) are then derived from these balance equations.

Balance equations are set up for macroscopic (homogeneous) systems (**Integral** balances) or for differential description of spatial profiles (**Differential** balances).

Balance equations

P. Götz, Slide 5

Balancing should be performed systematically:

1. Choose the process variable to be described.
2. Select the respective balance quantity.
3. Prepare a sketch of your system, including the system boundary and the variables / fluxes / parameters of your system.
4. Assign the respective phenomena (transport fluxes Φ and conversions R) to the general equation for the balanced quantity:

   Accumulation in the system = Transport across system boundary + Conversion within the system

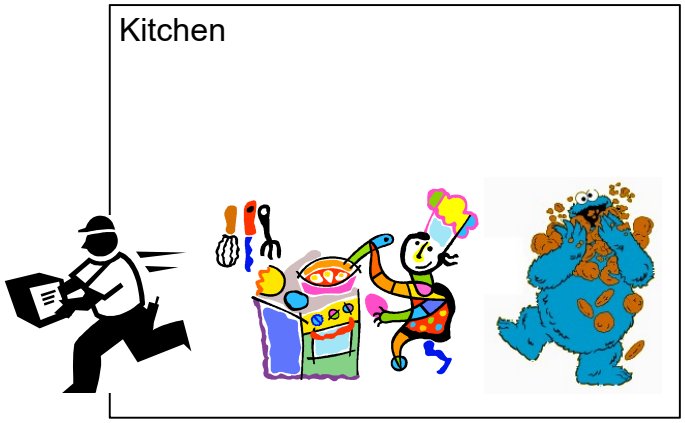
5. Rearrange equation to get desired process variable description.

Balance equations

P. Götz, Slide 6

Integral balances

1st Example: Balancing of cookies in a kitchen



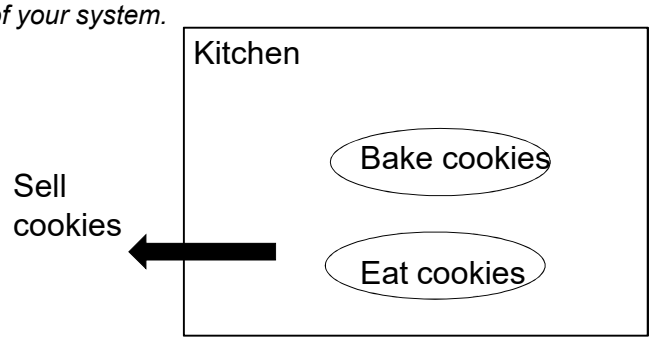
Balance equations

P. Götz, Slide 7

1. Choose the process variable to be described.
Number of cookies in kitchen

2. Select the respective balance quantity.
Number of cookies is balanceable

3. Prepare a sketch of your system.



Balance equations

P. Götz, Slide 8

4. Assign the respective phenomena to the general equation for the balanced quantity:

Accumulation of cookies in the kitchen =
Transport of cookies out of kitchen for sales +
Baking of cookies (conversion) –
Eating of cookies (conversion)

$$\frac{d(Cookies)}{dt} = -\Phi_{Transport\ to\ sales} + R_{Baking} - R_{Eating}$$

5. Rearrange equation to get desired process variable description.
Not necessary (process variable = balanced quantity)

Balance equations

P. Götz, Slide 9

You sell 100 cookies per day, bake 160 cookies and 40 cookies are eaten:

$$\frac{d(Cookies)}{dt} = -100\frac{Cookies}{d} + 160\frac{Cookies}{d} - 40\frac{Cookies}{d}$$

$$\frac{d(Cookies)}{dt} = 20\frac{Cookies}{d}$$

Accumulation of cookies in the kitchen will be 20 cookies per day
Now you want to acheive a steady state in your kitchen, i.e.:

$$\frac{d(Cookies)}{dt} = 0$$

How many cookies have to be eaten for a steady state?

Balance equations

P. Götz, Slide 10

$$\frac{d(Cookies)}{dt} = 0 = -\Phi_{Transport\ to\ sales} + R_{Baking} - R_{Eating}$$

$$R_{Eating} = -\Phi_{Transport\ to\ sales} + R_{Baking}$$

$$R_{Eating} = -100\frac{Cookies}{d} + 160\frac{Cookies}{d} = 60\frac{Cookies}{d}$$

For a steady state, 60 cookies have to be eaten per day.

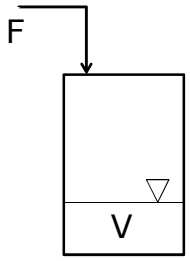
Balance equations

P. Götz, Slide 11

2nd Example (integral balance):
Set up a balance for liquid in a 100 Liter (working volume) tank which is filled with a flow rate F of 2 Liter/min (starting volume 10 Liter). How long does it take to fill the tank?

Process variable is volume V, quantity volume is balanceable.

$$\frac{dV}{dt} = F \qquad \int_{V=0}^{V} dV = \int_{t=0}^{t} F\,dt$$



$$V\Big|_{V_{t=0}}^{V} = F \cdot t\Big|_{0}^{t} \qquad V = V_{t=0} + F \cdot t$$

$$t = \frac{V - V_{t=0}}{F} = \frac{100\,l - 10\,l}{2\,\frac{l}{\min}} = 45\ \min$$

Nomenclature

P. Götz, Slide 12

A	surface/interfacial area	[m²]			
a	spec. area	[m²/m³]	R	reaction rate	[g/h]
c	concentration	[g/L] or [mol/L]	r	reaction rate per volume	[g/(Lh)]
D	diffusion coefficient	[m²/s]	V	volume	[L]
F	flow rate	[m³/s]	w	velocity	[m/s]
k_L	mass transfer coefficient	[m/s]	x,y,z	coordinates	[m]
M	mass	[g]	y	molar fraction in gas	[-]
M_A	molar mass	[g/mol]	ρ	(partial) density	[g/L]
N	amount of molecules	[mol]	η	dynamic viscosity	[Pas]
p	pressure	[bar]			

Balance equations
Transport phenomena

P. Götz, Slide 13

The general mass balance is given (for a component i) by

ACCUMULATION OF MASS IN THE SYSTEM =
TRANSPORT OF MASS ACROSS SYSTEM BOUNDARY +
+ CONVERSION OF MASS WITHIN SYSTEM

As an equation it may be written:

$$\frac{dm_i}{dt} = \Phi_{TRANSPORT,IN} - \Phi_{TRANSPORT,OUT} + R_{PRODUCTION} - R_{CONSUMPTION}$$

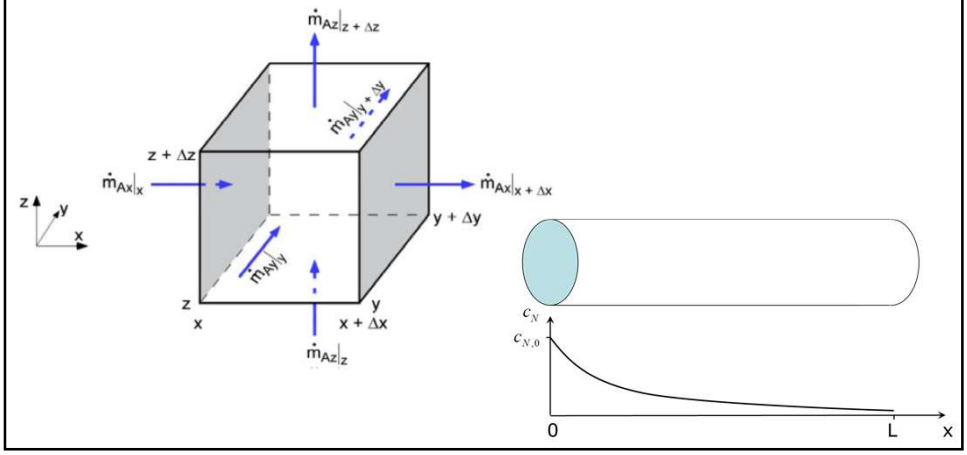
Other important quantities for balances in bioprocess engineering are energy and momentum.

The term „transport phenomena“ in (bio-)chemical engineering is usually related to **mass, energy and momentum**.

Differential balances

P. Götz, Slide 14

Performing balances on differentially small volume elements is used to describe space dependent entities. A differential mass balance for example will allow decription of concentration profiles in a tubular reactor.



Results from differential balances to describe transport phenomena in space and time

P. Götz, Slide 15

Mass

Component Balance:

$$\frac{\partial \rho_A}{\partial t} + w_x \frac{\partial \rho_A}{\partial x} + w_y \frac{\partial \rho_A}{\partial y} + w_z \frac{\partial \rho_A}{\partial z} = D_{AB}\left(\frac{\partial^2 \rho_A}{\partial x^2} + \frac{\partial^2 \rho_A}{\partial y^2} + \frac{\partial^2 \rho_A}{\partial z^2}\right) + \dot{r}_A \tilde{M}_A$$

Total Mass Balance (Continuity Equation):

$$\frac{\partial \rho}{\partial t} + \frac{\partial}{\partial x}(\rho w_x) + \frac{\partial}{\partial y}(\rho w_y) + \frac{\partial}{\partial z}(\rho w_z) = 0$$

Momentum

Navier-Stokes-Equations:

$$\rho\left(\frac{\partial w_x}{\partial t} + w_x \frac{\partial w_x}{\partial x} + w_y \frac{\partial w_x}{\partial y} + w_z \frac{\partial w_x}{\partial z}\right) = -\frac{\partial p}{\partial x} + \eta\left(\frac{\partial^2 w_x}{\partial x^2} + \frac{\partial^2 w_x}{\partial y^2} + \frac{\partial^2 w_x}{\partial z^2}\right) + \rho g_x$$

$$\rho\left(\frac{\partial w_y}{\partial t} + w_x \frac{\partial w_y}{\partial x} + w_y \frac{\partial w_y}{\partial y} + w_z \frac{\partial w_y}{\partial z}\right) = -\frac{\partial p}{\partial y} + \eta\left(\frac{\partial^2 w_y}{\partial x^2} + \frac{\partial^2 w_y}{\partial y^2} + \frac{\partial^2 w_y}{\partial z^2}\right) + \rho g_y$$

$$\rho\left(\frac{\partial w_z}{\partial t} + w_x \frac{\partial w_z}{\partial x} + w_y \frac{\partial w_z}{\partial y} + w_z \frac{\partial w_z}{\partial z}\right) = -\frac{\partial p}{\partial z} + \eta\left(\frac{\partial^2 w_z}{\partial x^2} + \frac{\partial^2 w_z}{\partial y^2} + \frac{\partial^2 w_z}{\partial z^2}\right) + \rho g_z$$

Heat

$$\rho c_p\left(\frac{\partial T}{\partial t} + w_x \frac{\partial T}{\partial x} + w_y \frac{\partial T}{\partial y} + w_z \frac{\partial T}{\partial z}\right) = \lambda\left(\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2}\right) - \dot{r}_A \Delta h_R \tilde{M}_A$$

incl. dissipation (temperature increase due to friction in viscous flows):

$$+2\eta\left\{\left(\frac{\partial w_x}{\partial x}\right)^2 + \left(\frac{\partial w_y}{\partial y}\right)^2 + \left(\frac{\partial w_z}{\partial z}\right)^2\right\} + \eta\left\{\left(\frac{\partial w_x}{\partial y} + \frac{\partial w_y}{\partial x}\right)^2 + \left(\frac{\partial w_x}{\partial z} + \frac{\partial w_z}{\partial x}\right)^2 + \left(\frac{\partial w_y}{\partial z} + \frac{\partial w_z}{\partial y}\right)^2\right\}$$

Mathematical modeling in biotechnology: Purpose, complexity and input

P. Götz, Slide 16

MODEL TYPE		UNDERLYING DATA	PURPOSE
Macroscopic level			
1. Yield coefficients (static)		Endpoint substrate / product concentrations	Calculation of process economics
2. Mass balances (dynamic, unstructured)		Time series substrate/ product concentrations	Description and simple prediction of process
3. Mass balances (dynamic, structured)		Time series key components, energy/redox state, etc.	Detailed prediction of process
Molecular level			
stoichiometric relations	4. Carbon balance	Metabolic network structure	Prediction of theoretical yield coeffcients
	5. Elementary modes		
metabolism (steady state)	6. Stationary flux analysis	Substrate and product fluxes at (quasi-) steady state	Description of fluxes, bottleneck identification
metabolism (dynamic)	7. Instationary flux analysis	Metabolome time series	Description of changing fluxes (metabolite dynamics)
	8. Metabolic control analysis	+ Proteome, enzyme kinetics	Prediction of metabolite dynamics after perturbation
	9. Model of regulation	+ Genome, Transcriptome	Prediction of cell dynamics
10. *In silico* cell		+ Environome	Multi-scale system prediction

Balance equations:
Bioreactor (Simple case: Biomass and substrate in batch reactor)

P. Götz, Slide 17

Set up integral mass balances for biomass and substrate in an ideally mixed batch reactor. The reactor working volume V is constant

We consider growth of biomass by consumption of substrate.

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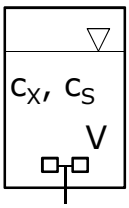


$$\frac{dm_X}{dt} = R_X \qquad \frac{dm_S}{dt} = -R_S$$

Transform equations to describe the observable concentrations via dividing by constant Volume V:

$$\frac{dc_X}{dt} = r_X \qquad \frac{dc_S}{dt} = -r_S$$

To complete the model, kinetic equations for the reaction rates must be introduced.

Reaction rates: The law of mass action

P. Götz, Slide 18

The rate of reaction v is directly proportional to the **concentrations** of the reactants. The rate constant k indicates how frequently the raction occurs (reaction probability).

First order

$$A \xrightarrow{k} P \quad (1)$$

$$v = -\frac{dc_A}{dt} = kc_A \quad (2)$$

Second order

$$A + B \xrightarrow{k} P(+Q +\ldots) \quad (3)$$

$$v = -\frac{dc_A}{dt} = -\frac{dc_B}{dt} = \frac{dc_P}{dt} = kc_A c_B \quad (4)$$

$$A + A \xrightarrow{k} P$$

$$v = -\frac{dc_A}{dt} = kc_A^2 \quad (5)$$

Reaction rates: The law of mass action

P. Götz, Slide 19

When n molecules interact in a reaction, the rate equation for the n-th order reaction contains n concentration terms.
Reactions with order greater than two are rare, they would require ternary collisions, which have very low probabilities.

General case for n-th order reaction

$$qA + rB + \ldots \xrightarrow{k} products \quad (6)$$

$$v = -\frac{dc_A}{dt} = kc_A^q c_B^r \ldots \quad (7)$$

$$n = q + r + \ldots \quad (8)$$

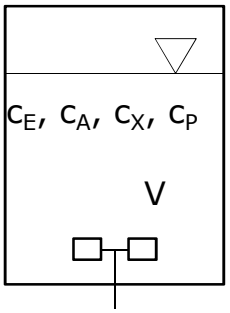
The units of the reaction rate constant are (concentration^{n-1} · time^{-1})

Michaelis Menten enzyme kinetics (1913), derivation according to Briggs and Haldane (1925)

P. Götz, Slide 20

For an enzymatic (catalytic) reaction, kinetics can be derived from balances for the reaction partners in a closed system. Considering substrate A, enzyme E, enzyme/substrate complex X and product P, the reaction scheme is:

$$E + A \underset{k_2}{\overset{k_1}{\rightleftarrows}} X \xrightarrow{k_3} P + E$$



Setting up

$$\frac{dm_A}{dt} = -r_1 \cdot V + r_2 \cdot V$$

$$\frac{dc_A}{dt} = -k_1 \cdot c_A \cdot c_E + k_2 \cdot c_X$$

Michaelis Menten enzyme kinetics (1913), derivation according to Briggs and Haldane (1925)

P. Götz, Slide 21

Accordingly, the following set of equations can be derived:

$$\frac{dc_A}{dt} = -k_1 \cdot c_A \cdot c_E + k_2 \cdot c_X$$

$$\frac{dc_X}{dt} = k_1 \cdot c_A \cdot c_E - k_2 \cdot c_X - k_3 \cdot c_X$$

$$\frac{dc_E}{dt} = k_2 \cdot c_X + k_3 \cdot c_X - k_1 \cdot c_A \cdot c_E$$

$$\frac{dc_P}{dt} = k_3 \cdot c_X$$

Additionally, we have the conservation equation for the total amount of enzyme (the biocatalyst): $c_{E,total} = c_E + c_X$

Be careful, this equation is only valid for molar concentrations!

Michaelis Menten enzyme kinetics (1913), derivation according to Briggs and Haldane (1925)

P. Götz, Slide 22

The central assumption now is the steady state for the enzyme substrate complex X: $\frac{dc_X}{dt} \approx 0$

For the second equation in our system of equations then follows:

$$k_1 \cdot c_A \cdot c_E = (k_2 + k_3) \cdot c_X$$

Substituting $c_E = c_{E,total} - c_X$ we get: $c_X = \frac{k_1 \cdot c_A \cdot c_{E,total}}{k_1 \cdot c_A + k_2 + k_3}$

Putting this into the rate of product formation $r_P = k_3 \cdot c_X$

we get for the rate of product formation:

$$r_P = k_3 \cdot \frac{k_1 \cdot c_A \cdot c_{E,total}}{k_1 \cdot c_A + k_2 + k_3}$$

Michaelis Menten enzyme kinetics (1913), derivation according to Briggs and Haldane (1925)

P. Götz, Slide 23

With the definitions $r_{P,\max} = k_3 \cdot c_{E,total}$

and $K_A = \frac{k_2 + k_3}{k_1}$

we finally get: $\boxed{r_P = r_{P,\max} \cdot \frac{c_A}{K_A + c_A}}$

Bioreactor models: Batch

P. Götz, Slide 24

Unstructured bioreactor models (Integral balances)
When modeling bioreactors, the most simple approach is an unstructured model, treating biomass and substrate as homogeneous and ideally mixed quantities in the liquid phase.

For a **batch** cultivation, from the respective mass balances the well known model equations can be derived :

$$\frac{dc_X}{dt} = \mu \cdot c_X$$

$$\frac{dc_S}{dt} = -\frac{1}{Y_{X/S}} \cdot \mu \cdot c_X$$

The specific growth rate can be described by Monod-kinetics: $\mu = \mu_{\max} \cdot \frac{c_S}{K_S + c_S}$

Modes of bioreactor operation

P. Götz, Slide 25

Batch cultivation:
Closed system with constant volume
(Exceptions: Aeration, pH control, antifoam agent addition, evaporation).
At t=0 all substrates are present in excess.

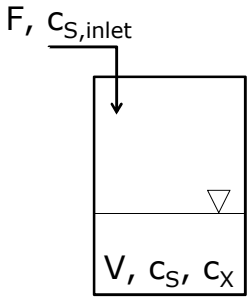
Fed-batch cultivation:
Substrate component(s) are fed into the reactor.
Volume is increasing and biomass is increasing according to substrate feed.

Continuous cultivation:
Medium is fed to the reactor and broth is removed from the reactor at the same flow rate to keep the volume constant. Control options are e.g.
- chemostat (constant dilution rate)
- turbidostat (constant biomass concentration)
- nutristat (constant substrate concentration).

Bioreactor models: Fed-batch

P. Götz, Slide 26

For a **fed-batch** cultivation, the respective model equations are:



$$\frac{dc_X}{dt} = \mu \cdot c_X - \frac{F}{V} \cdot c_X$$

$$\frac{dc_S}{dt} = (c_{S,inlet} - c_S) \cdot \frac{F}{V} - \frac{1}{Y_{X/S}} \cdot \mu \cdot c_X$$

$$\frac{dV}{dt} = F$$

Note the third equation due to variable volume.
The second expression on the right hand side of the top equation is neither derived from transport or conversion expressions, it describes the dilution of biomass concentration by feeding biomass-free substrate solution.

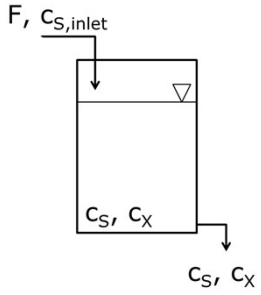
Bioreactor models: Continuous cultivation

P. Götz, Slide 27

For a **continuous** cultivation (V=const.), the model equations are:

$$\frac{dc_X}{dt} = (\mu - D) \cdot c_X \qquad D = \frac{F}{V}$$

$$\frac{dc_S}{dt} = (c_{S,inlet} - c_S) \cdot D - \frac{1}{Y_{X/S}} \cdot \mu \cdot c_X$$



For this type of cultivation (open system), steady states can be defined, leading to operating points:

$$0 = (\mu - D) \cdot c_X$$

$$0 = (c_{S,inlet} - c_S) \cdot D - \frac{1}{Y_{X/S}} \cdot \mu \cdot c_X$$

Operating point 1: $c_X = 0$ $\qquad c_S = c_{S,inlet}$

Operating point 2: $\mu = D$ $\qquad c_X = Y_{X/S} \cdot (c_{s,inlet} - c_S)$

From reactor balances to balances of intracellular compounds and biological networks

P. Götz, Slide 28

The concepts of balancing can be extended to living systems (cells) and their sub-systems (pathways, metabolic networks,…). These systems are open systems with influx (substrate) and eventually flux(es) out (product, CO_2, …).

The respective operating point of these systems corresponds e.g. to the term homeostasis in higher organisms.

In order to describe the dynamics of intracellular events in a model which is structured on a molecular level, balances can be set up for metabolites and other intracellular compounds.

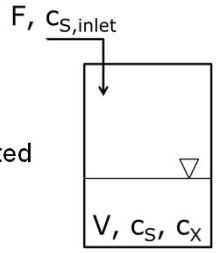
Exercise: Feed strategies for fed-batch cultivation

P. Götz, Slide 29

To develop a feed strategy for a **fed-batch** cultivation, model equations can be used. A common goal is reaching a high cell density while avoiding inhibitory side products. This usually requires feeding a concentrated substrate solution while limiting the growth rate to prevent overflow metabolism.

Starting with the biomass balance:

$$\frac{dm_X}{dt} = \mu \cdot m_X$$



we set the growth rate to a constant value for limited growth, so for μ=const. we get the solution:

$$m_X = m_{X,(t=0)} \cdot e^{\mu \cdot t}$$

Exercise: Feed strategies for fed-batch cultivation

P. Götz, Slide 30

Assuming Monod-kinetics, a constant growth rate corresponds to a constante substrate concentration in the environment of the microorganisms:

$$\frac{dc_S}{dt} = 0$$

Using this in the substrate equation for the fed-batch reactor, we get:

$$0 = (c_{S,inlet} - c_S) \cdot \frac{F}{V} - \frac{1}{Y_{X/S}} \cdot \mu \cdot c_X$$

Rearranging for F yields:

$$F = \frac{\mu \cdot c_X \cdot V}{(c_{S,inlet} - c_S) \cdot Y_{X/S}}$$

Exercise: Feed strategies for fed-batch cultivation

P. Götz, Slide 31

Introducing the concentration into the equation for biomass, we get:

$$c_X \cdot V = c_{X,(t=0)} \cdot V_{(t=0)} \cdot e^{\mu \cdot t}$$

Inserting this into the equation for the feed rate F:

$$F = \frac{\mu \cdot c_{X,(t=0)} \cdot V_{(t=0)} \cdot e^{\mu \cdot t}}{(c_{S,inlet} - c_S) \cdot Y_{X/S}}$$

Since the feed concentration is much higher than the limiting substrate concentration in the bioreactor, we can neglect the latter:

$$F = \frac{\mu \cdot c_{X,(t=0)} \cdot V_{(t=0)} \cdot e^{\mu \cdot t}}{c_{S,inlet} \cdot Y_{X/S}}$$

Part II

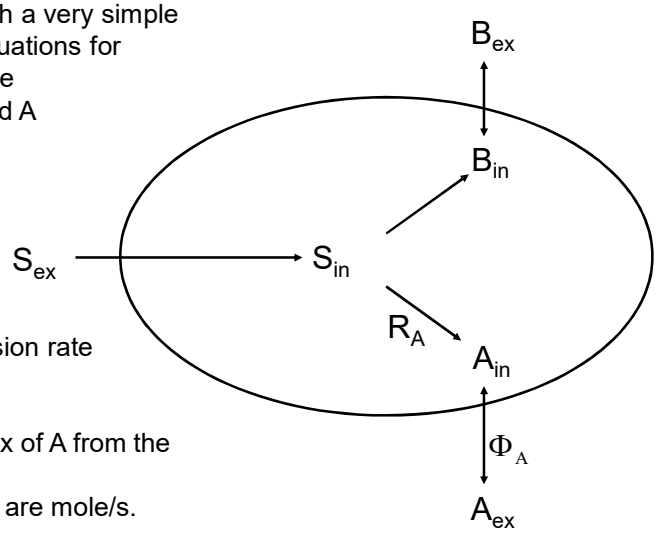
Balancing intracellular and extracellular compounds

Balancing intracellular and extracellular compounds

P. Götz, Slide 2

Starting from a cell with a very simple metabolic network, equations for the intracellular and the extracellular compound A will be derived from integral balances.

System boundary is the cell membrane



R_A is the total conversion rate of A in the system.

Φ_A is the transport flux of A from the system.

Units are mole/s.

Balancing intracellular and extracellular compounds: Relations between variables

P. Götz, Slide 3

In a batch bioreactor, the constant reaction volume V_R (the biosuspension) is segregated into

- "Biovolume" of cells V_C
- liquid supernatant V_L

$$V_R = V_C + V_L = const.$$

Definitions:

m_X: Total biomass within reactor, $[m_X]=g_{DW}$

ρ_X: Density of biomass pellet, $[\rho_X]=g_{DW}/Liter_{cells}$

c_X: Biomass concentration in reactor, $[c_X]=g_{DW}/Liter_{suspension}$

$$\rho_X = \frac{m_X}{V_C} = \frac{V_R \cdot c_X}{V_C} \qquad V_L = V_R - V_C = V_R \cdot \left(1 - \frac{c_X}{\rho_X}\right)$$

Goal is the derivation of equations for modeling the time course of intracellular and extracellular concentration of substance A.

Conversion rates and transport fluxes for compound A

P. Götz, Slide 4

Volumetric rates:

Intracellular rate

$$r_{\mathrm{A,in}} = \frac{R_A}{V_C}$$

Reactor related rate

$$r_{\mathrm{A}} = \frac{R_A}{V_R} = r_{A,in} \cdot \frac{V_C}{V_R} = r_{A,in} \cdot \frac{c_X}{\rho_X}$$

Biomass related rate:

$$q_{\mathrm{A}} = \frac{r_A}{c_X} = \frac{r_{A,in}}{\rho_X}$$

$r_{A,in}$ allows use of enzyme kinetics related to intracellular concentrations.

Reactor volume related flux across total cell membrane area:

$$\varphi_{\mathrm{A}} = \frac{\Phi_A}{V_R}$$

e.g.: $\varphi_{\mathrm{A}} = P \cdot a \cdot \Delta c_{\mathrm{A}}$

(Permeability P, [P]=m/s
volumetric membrane area a , [a]=1/m)

Intracellular molar component balance

P. Götz, Slide 5

Balance for amount of intracellular component A in the sub-system „cell volume V_C" in a batch reactor:

$$\frac{dn_{A,in}}{dt} = R_A - \Phi_{\mathrm{A}} \qquad n_{A,in} = c_{A,in} \cdot V_C$$

$$\frac{d\left(c_{A,in} \cdot V_C\right)}{dt} = R_A - \Phi_{\mathrm{A}}$$

$$\frac{d\left(c_{A,in} \cdot V_R \cdot \frac{c_X}{\rho_X}\right)}{dt} = R_A - \Phi_{\mathrm{A}}$$

$$c_{A,in} \cdot \frac{d\left(\frac{c_X}{\rho_X}\right)}{dt} + \left(\frac{c_X}{\rho_X}\right) \cdot \frac{dc_{A,in}}{dt} = \frac{V_C}{V_R} \cdot r_{A,in} - \varphi_{\mathrm{A}}$$

Intracellular molar component balance

P. Götz, Slide 6

With ρ_X = const.:

$$\left(\frac{c_X}{\rho_X}\right)\cdot\frac{dc_{A,in}}{dt}=\frac{V_C}{V_R}\cdot r_{A,in}-\varphi_A-c_{A,in}\cdot\frac{1}{\rho_X}\cdot\frac{dc_X}{dt}$$

$$\frac{c_X}{\rho_X}=\frac{V_C}{V_R}$$

$$\frac{dc_{A,in}}{dt}=r_{A,in}-\frac{\rho_X}{c_X}\cdot\varphi_A-c_{A,in}\cdot\frac{1}{c_X}\cdot\frac{dc_X}{dt}$$

Equation describing biomass growth in a batch reactor:

$$\frac{dc_X}{dt}=\mu\cdot c_X$$

The final equation for intracellular concentration of A is then:

$$\frac{dc_{A,in}}{dt}=r_{A,in}-\frac{\rho_X}{c_X}\cdot\varphi_A-\mu\cdot c_{A,in}$$ **or** $$\frac{dc_{A,in}}{dt}=\frac{\rho_X}{c_X}\cdot\left(r_A-\varphi_A\right)-\mu\cdot c_{A,in}$$

The choice of conversion rate depends on the experimental method of measurement.

Intracellular molar component balance

P. Götz, Slide 7

$$\frac{dc_{A,in}}{dt}=r_{A,in}-\frac{\rho_X}{c_X}\cdot\varphi_A-\mu\cdot c_{A,in}$$ **or** $$\frac{dc_{A,in}}{dt}=\frac{\rho_X}{c_X}\cdot\left(r_A-\varphi_A\right)-\mu\cdot c_{A,in}$$

$r_{A,in}$ is the intracellular reaction rate, which is e.g. caused by an intracellular enzyme under intracellularconditions. Kinetics of this intracellular enzyme reaction could be e.g. Michaelis-Menten type kinetics.

$$r_{A,in}=r_{A,in,\max}\frac{c_{A,in}}{K_{M,A,in}+c_{A,in}}$$

r_A is the observed reaction rate in the reactor, the conversion within the reactor.

This equation can be used for evaluating experiments.

Extracellular molar component balance

P. Götz, Slide 8

Balance for amount of extracellular component A in the sub-system „liquid supernatant V_L" in a batch reactor:

$$\frac{dn_{A,ex}}{dt} = \Phi_A \qquad n_{A,ex} = c_{A,ex} \cdot V_L$$

$$\frac{d\left(c_{A,ex} \cdot V_L\right)}{dt} = \Phi_A$$

$$\frac{d\left(c_{A,ex} \cdot V_R \cdot \left(1 - \frac{c_X}{\rho_X}\right)\right)}{dt} = \Phi_A$$

$$c_{A,ex} \cdot \frac{d\left(1 - \frac{c_X}{\rho_X}\right)}{dt} + \left(1 - \frac{c_X}{\rho_X}\right) \cdot \frac{dc_{A,ex}}{dt} = \varphi_A$$

Extracellular molar component balance

P. Götz, Slide 9

$$-\frac{c_{A,ex}}{\rho_X} \cdot \frac{dc_X}{dt} + \left(1 - \frac{c_X}{\rho_X}\right) \cdot \frac{dc_{A,ex}}{dt} = \varphi_A$$

$$\frac{dc_{A,ex}}{dt} = \frac{\frac{\mu \cdot c_X}{\rho_X} \cdot c_{A,ex} + \varphi_A}{\left(1 - \frac{c_X}{\rho_X}\right)}$$

Interpretation of RHS terms:

$\frac{\mu \cdot c_X}{\rho_X} \cdot c_{A,ex}$ Increase of extracellular concentration by reduction of supernatant volume by cell growth.

$\left(1 - \frac{c_X}{\rho_X}\right)$ Correction factor for V_R based flux in an equation based on supernatant volume V_L.

Definitions of specific reaction rates

P. Götz, Slide 10

Reaction rates of metabolic biochemical reactions can be related to different systems, reflected in the units:

Related to reactor working volume (Productivity of the reactor):

$$[r_A] = \frac{mole_{metabolite\ A}}{l_{reactor\ volume} \cdot h}$$

Related to cell volume (Productivity of cells, possible transfer of enzyme kinetics from *in vitro* to *in vivo*):

$$[r_{A,in}] = \frac{mole_{metabolite\ A}}{l_{cell\ volume} \cdot h}$$

Related to dry weight of biomass (Productivity of cells):

$$[r_{A,X}] = \frac{mole_{metabolite\ A}}{g_{biomass\ DW} \cdot h}$$

Example

P. Götz, Slide 11

During a cultivation (c_X=30 g/Liter, μ=0,2 h^{-1}), cells are in quasi-steady-state, metabolizing glucose with a reactor related rate of 12 g/(Liter h) to glucose-6-phosphate (substance A).

$Glucose_{extracellular} \rightarrow$ Gluc.-6-phosph. (A) $\rightarrow$ Fructose-6-phosph. (B)

$$\frac{dc_{A,in}}{dt} = \frac{\rho_X}{c_X} \cdot r_A - \frac{\rho_X}{c_X} \cdot r_B - \mu \cdot c_{A,in}$$

Calculate the rate of formation for glucose-6-phosphate r_A !
What is the maximum rate of formation for pyruvate?

Calculate and compare the rate of formation for A with the last RHS term!
The intracellular concentration of glucose-6-phosphate is around 1mmole/Liter.
Which simplification follows?
(ρ_X=300 g/Liter, $M_{glucose}$=180g/mole)

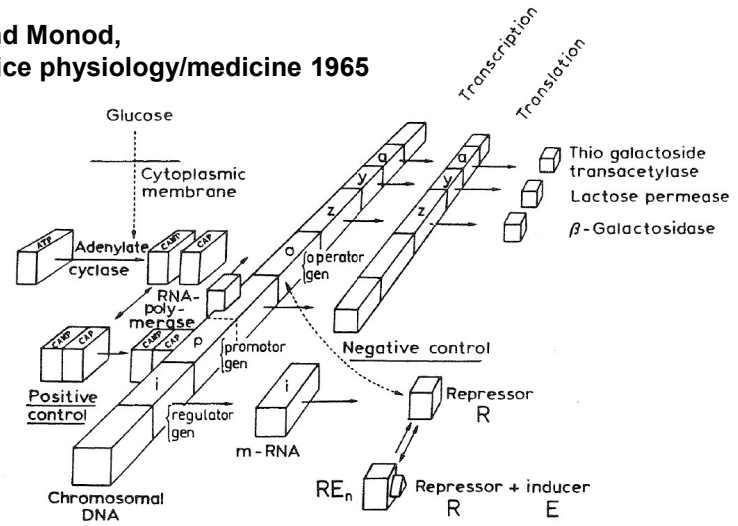
Part III

Modeling regulation: lac operon

Lac-Operon - Induction of enzymes for lactose utilization: Catabolite repression and induction by substrate

P. Götz, slide 2

Jacob and Monod,
Nobel price physiology/medicine 1965



Mathematical modelling of diauxic behavior: Example glucose and lactose as substrates

P. Götz, slide 3

Biomass growth on glucose and lactose
- Glucose constitutive
- Lactose inducible

Synthesis of lactose utilizing enzymes by m-RNA translation

Induction of transcription of m-RNA coding for enzymatic lactose onversion

Mathematical modelling of diauxic behavior: Example glucose and lactose as substrates

P. Götz, slide 4

Consumption rates $r_{S,Glc}$ and $r_{S,Lac}$ are defined for substrates glucose and lactose.
- Glucose consumption is constitutive.
- Lactose is only utilized if no glucose is present.

Biomass growth occurs on both substrates

$$r_X = \mu \cdot c_X \qquad \mu = \mu_{Glc} + \mu_{Lac}$$

$$r_X = Y_{X/Glc} \cdot r_{S,Glc} + Y_{X/Lac} \cdot r_{S,Lac}$$

Mathematical modelling of diauxic behavior: Example glucose and lactose as substrates

P. Götz, slide 5

For glucose, Monod-kinetics are chosen as simple approach:

$$\mu_{Glc} = \mu_{\max,Glc} \cdot \frac{c_{Glc}}{K_{S,Glc} + c_{Glc}} \qquad r_{S,Glc} = r_{S,Glc,\max} \cdot \frac{c_{Glc}}{K_{S,Glc} + c_{Glc}} \cdot c_X$$

Lactose is only utilized, if the corresponding enzymes, represented by a key enzyme E, are present. The enzyme is quantified by its fraction of biomass $X_{E,Lac}$ in the cell ($[X_{E,Lac}] = g_E/g_X$).
Maximum velocity of lactose conversion depends on the amount of this enzyme:

$$r_{S,Lac} = (r_{E,Lac,\max} \cdot X_{E,Lac}) \cdot \frac{c_{Lac}}{K_{S,Lac} + c_{Lac}} \cdot c_X$$

$$\mu_{Lac} = (v_{E,Lac} \cdot X_{E,Lac}) \cdot \frac{c_{Lac}}{K_{S,Lac} + c_{Lac}}$$

Mathematical modelling of diauxic behavior: Translation of the lactose utilizing enzyme

P. Götz, slide 6

Synthesis of enzyme E will be described by its translation. For rate of enzyme production, we assume dependence on the cellular content of the respective m-RNA $X_{m\text{-}RNA,E}$:

$$\frac{d(X_E \cdot c_X)}{dt} = k_E \cdot X_{m-RNA,E} \cdot c_X$$

$$c_X \cdot \frac{dX_E}{dt} + X_E \cdot \frac{dc_X}{dt} = k_E \cdot X_{m-RNA,E} \cdot c_X$$

$$\frac{dX_E}{dt} = k_E \cdot X_{m-RNA,E} - \mu \cdot X_E$$

Mathematical modelling of diauxic behavior: Transcription of m-RNA for lactose-utilizing enzyme

P. Götz, slide 7

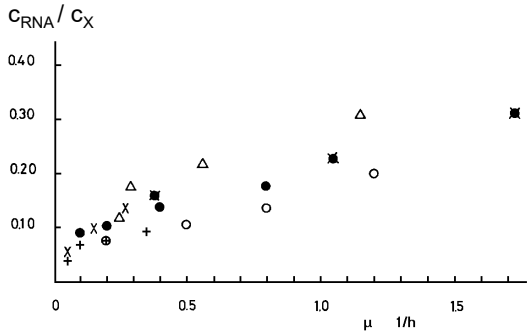
Synthesis of m-RNA in turn depends on processes on a DANN level, described by the following functions:

- Status of induction $0 \leq \Phi_{ind} \leq 1$
- Catabolite repression $0 \leq \Phi_{kr} \leq 1$
- Cellular capacity for RNA-synthesis $0 < f(\mu) < 1$

Capacity of protein synthesis in cells is represented by cellular RNA-content

P. Götz, slide 8

The expression f(μ) can be quantified based on the observation, that RNA content of cells depends linearly from their specific growth rate μ.



$c_{RNA} / c_X = a + b \cdot \mu$

$a = 0{,}042\ g_{RNA} \cdot g_{TS}^{-1}$
$b = 0{,}146\ h \cdot g_{RNA} \cdot g_{TS}^{-1}$

after: Roels, J.A., (1983), Energetics and Kinetics in Biotechnology, Elsevier, Amsterdam

Ansatz zur mathematischen Modellierung von Diauxie: Beispiel Glucose und Lactose

P. Götz, slide 9

A dimensionless formulation of the relation $c_{RNA} / c_X = a + b \cdot \mu$ yields:

$$f(\mu) = \frac{a + b \cdot \mu}{a + b \cdot \mu_{\max}}$$

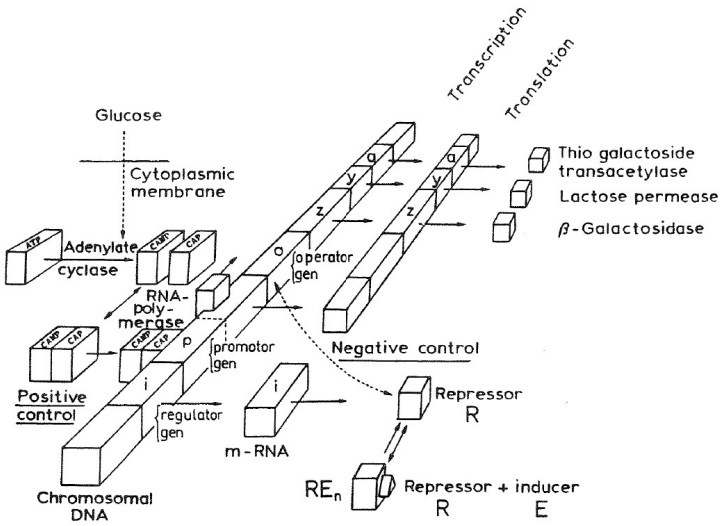
Considering intracellular m-RNA degradation by a first order reaction, we get for m-RNA:

$$\frac{d(X_{m-RNA,E} \cdot c_X)}{dt} = r_{m-RNA,E,\max} \cdot f(\mu) \cdot \phi_{ind} \cdot \phi_{KR} \cdot c_X - k_D \cdot X_{m-RNA,E} \cdot c_X$$

Expressions for status of inductions and catabolite repression are required, preferably depending on observable quantities.

Lac-Operon - Induction of enzymes for lactose utilization: Catabolite repression and induction by substrate

P. Götz, slide 10



Catabolite repression: In absence of glucose, cAMP is produced, activating the promotor

P. Götz, slide 11

„Hunger-signal“

cAMP-Receptor-protein (CRP) + m cAMP $\rightleftharpoons$ Receptor protein - $(cAMP)_m$ – complex

Promotor is activated

Receptor protein - $(cAMP)_m$ - complex + Promotor $\rightleftharpoons$ Promotor-Receptor protein - $(cAMP)_m$ - complex

RNA-Polymerase may bind at the activated promotor
for transcription of genes
for synthesis of lactose utilizing enzymes

Derivation of expression for catabolite repression

P. Götz, slide 12

$$CRP + m \cdot cAMP \xleftrightarrow{k5} CRP\_(cAMP)_m$$

$$P + CRP\_(cAMP)_m \xleftrightarrow{k6} P\_CRP\_(cAMP)_m$$

Concentrations are represented by their biomass related fractions X . The equilibrium constants are:

$$k5 = \frac{X_{CRP\_(cAMP)_m}}{X_{CRP} \cdot (X_{cAMP})^m} \qquad k6 = \frac{X_{P\_CRP\_(cAMP)_m}}{X_P \cdot X_{CRP\_(cAMP)_m}}$$

it follows:

$$X_{CRP} = \frac{X_{CRP\_(cAMP)_m}}{k5 \cdot (X_{cAMP})^m} \qquad X_P = \frac{X_{P\_CRP\_(cAMP)_m}}{k6 \cdot X_{CRP\_(cAMP)_m}}$$

Derivation of expression for catabolite repression

P. Götz, slide 13

For the catabolite-repression function, we postulate **0** $\leq \Phi_{KR} \leq$ **1** (repression between **100%** and **0%**). It is described by the ratio of activated promotor genes to total number of promotor genes:

$$\phi_{KR} = \frac{X_{P\_CRP\_(cAMP)_m}}{X_{P,total}}$$

A funcion shall be derived which is dependent on observable quantities, e.g. concentrations of proteins or cAMP.

cAMP	signal molecule dependent on glucose
CRP	free protein
$CRP\_(cAMP)_m$	protein - cAMP complex
P	available binding sites at DNA (promotor genes)
$P\_CRP\_(cAMP)_m$	complex bound to DNA
CRP_{total}	total receptor protein in cell

Derivation of expression for catabolite repression
Conservation of CRP

P. Götz, slide 14

The total fraction of CRP in the cell is the sum of free CRP, complexed CRP and complexed CRP bound to DNA. Since there are only a few binding sites P at the DNA which only bind a few molecules of CRP, we neglect the last term:

$$X_{CRP,total} = X_{CRP} + X_{CRP\_(cAMP)_m} \quad (+X_{P\_CRP\_(cAMP)_m})$$

Rearranging and replacing X_{CRP} yields:

$$X_{CRP\_(cAMP)_m} = X_{CRP,total} - \frac{X_{CRP\_(cAMP)_m}}{k5 \cdot (X_{cAMP})^m}$$

or

$$X_{CRP\_(cAMP)_m} = \frac{X_{CRP,total}}{1 + 1/(k5 \cdot (X_{cAMP})^m)}$$

Derivation of expression for catabolite repression
Balance for promotor genes (binding sites)

P. Götz, slide 15

The total number of binding sites is:

$$X_{P,total} = X_P + X_{P\_CRP\_(cAMP)_m}$$

Rearranging and replacing X_P results in:

$$X_{P\_CRP\_(cAMP)_m} = X_{P,total} - \frac{X_{P\_CRP\_(cAMP)_m}}{k6 \cdot X_{CRP\_(cAMP)_m}}$$

This can be rearranged to an expression for Φ_{KR}

$$\frac{X_{P\_CRP\_(cAMP)_m}}{X_{P,total}} = \frac{1}{1 + 1/(k6 \cdot X_{CRP\_(cAMP)_m})}$$

Derivation of expression for catabolite repression

P. Götz, slide 16

After combining the results of the CRP balance and the promotor balance, we get:

$$\phi_{KR} = \frac{X_{P\_CRP\_(cAMP)_m}}{X_{P,total}} = \frac{k5 \cdot k6 \cdot (X_{cAMP})^m \cdot X_{CRP,total}}{1 + k5 \cdot (X_{cAMP})^m + k5 \cdot k6 \cdot (X_{cAMP})^m \cdot X_{CRP,total}}$$

In this function, catabolite repression only depends from concentrations of free cAMP and total CRP.

Induction: In presence of lactose, the repressor of the operator gene is bound, RNA-polymerase may start transcription

P. Götz, slide 17

Available substrate

Repressor + n Lactose $\rightleftharpoons$ Repressor – n Lactose - complex

free

Operator gene + Repressor $\rightleftharpoons$ **transcription blocked** Operator gene – Repressor - complex

RNA-polymerase may pass the operator gene for transcription of genes for synthesis of enzymes to utilize lactose.

Derivation of an expression for induction

P. Götz, slide 18

$$R + n \cdot Lac \xleftrightarrow{k3} RLac_n$$

$$O + R \xleftrightarrow{k4} OR$$

Again, concentrations are quantified by biomass related fractions X of the components, the equilibrium constants are:

$$k3 = \frac{X_{RLac_n}}{X_R \cdot (X_{Lac})^n} \qquad k4 = \frac{X_{OR}}{X_O \cdot X_R}$$

it follows:

$$X_{RLac_n} = k3 \cdot X_R \cdot (X_{Lac})^n \qquad X_{OR} = k4 \cdot X_O \cdot X_R$$

Derivation of an expression for induction

P. Götz, slide 19

The function representing the induction status $0 \leq \Phi_{ind} \leq 1$ (Induction between 0% and 100%) is the ratio of activated operator genes to total operator genes:

$$\phi_{ind} = \frac{X_O}{X_{O,total}}$$

A respective funcion shall be derived which is dependent on observable quantities, e.g. concentrations of lactose (inducer) or repressor protein.

O	available binding sites at DNA (operator genes)
R	free repressor protein
OR	operator gene – repressor protein complex
Lac	precursor for inducer (monomer)
R_$(Lac)_n$	repressor protein – inducer complex
O_{total}	total operator genes (binding sites) in cell

Derivation of an expression for induction
Conservation of repressor protein R

P. Götz, slide 20

The total fraction of Repressor R in the cell is the sum of free R, complexed R and R bound to DNA. Since there are only a few binding sites O at the DNA which only bind a few molecules of R, we again neglect the last term:

$$X_{R,total} = X_R + X_{RLac_n} \quad (+X_{OR})$$

Rearranging and introducing X_{REn} yields:

$$X_{R,total} = X_R + k3 \cdot X_R \cdot (X_{Lac})^n$$

or

$$X_R = \frac{X_{R,total}}{1 + k3 \cdot (X_{Lac})^n}$$

Derivation of an expression for induction
Balance for operator genes (binding sites)

P. Götz, slide 21

For the binding sites follows:

$$X_{O,total} = X_O + X_{OR}$$

Introducing X_{OR} and rearranging for X_O yields:

$$X_O = \frac{X_{O,total}}{1 + k4 \cdot X_R}$$

Introducing the result from the repressor conservation, we get:

$$X_O = \frac{X_{O,total}}{1 + \frac{k4 \cdot X_{R,total}}{1 + k3 \cdot (X_{Lac})^n}}$$

Derivation of an expression for induction

P. Götz, slide 22

Rearranging for an expression for Φ_{ind} yields:

$$\phi_{ind} = \frac{X_O}{X_{O,total}} = \frac{1 + k3 \cdot (X_{Lac})^n}{1 + k3 \cdot (X_{Lac})^n + k4 \cdot X_{R,total}}$$

Induction depends on lactose concentration in the cell and on total amount of represor molecules.

For the intracellular cAMP-concentration we choose an empirical relation to the extracellular glucose concentration:

$$X_{cAMP} = \frac{K_{KR}}{K_{KR} + c_{Glc}}$$

The intracellular fraction of lactose is assumed to be proportional to the extracellular concentration:

$$X_{Lac} = K_{Lac} \cdot c_{Lac}$$

System of equations: Differential equations

P. Götz, slide 23

$$\frac{dc_X}{dt} = \mu \cdot c_X$$ Biomasse growth

$$\frac{dc_{Glc}}{dt} = -\frac{1}{Y_{X/Glc}} \cdot \mu_{max,Glc} \cdot \frac{c_{Glc}}{K_{S,Glc} + c_{Glc}} \cdot c_X$$ Glucose consumption

$$\frac{dc_{Lac}}{dt} = -\frac{1}{Y_{X/Lac}} \cdot (v_{E,Lac} \cdot X_{E,Lac}) \cdot \frac{c_{Lac}}{K_{S,Lac} + c_{Lac}} \cdot c_X$$ Lactose consumpt.

$$\frac{dX_{E,Lac}}{dt} = k_E \cdot X_{m-RNA,E} - \mu \cdot X_{E,Lac}$$ Translation

Transcription

$$\frac{dX_{m-RNA,E}}{dt} = r_{m-RNA,E,max} \cdot f(\mu) \cdot \varphi_{ind} \cdot \varphi_{KR} - k_D \cdot X_{m-RNA,E} - \mu \cdot X_{m-RNA,E}$$

System of equations: Algebraic equations

P. Götz, slide 24

$$\mu = \mu_{Glc} + \mu_{Lac}$$ spec. growth rate

$$X_{cAMP} = \frac{K_{KR}}{K_{KR} + c_{Glc}}$$ Hunger-signal-relation

$$f(\mu) = \frac{a + b \cdot \mu}{a + b \cdot \mu_{max}} \quad (>0!)$$ RNA-synthesis capacity

$$X_{Lac} = K_{Lac} \cdot c_{Lac}$$ Lactose in/ex

$$\phi_{KR} = \frac{k5 \cdot k6 \cdot (X_{cAMP})^m \cdot X_{CRP,total}}{1 + k5 \cdot (X_{cAMP})^m + k5 \cdot k6 \cdot (X_{cAMP})^m \cdot X_{CRP,total}}$$ Catabolite repression

$$\phi_{ind} = \frac{1 + k3 \cdot (X_{Lac})^n}{1 + k3 \cdot (X_{Lac})^n + k4 \cdot X_{R,total}}$$ Induction

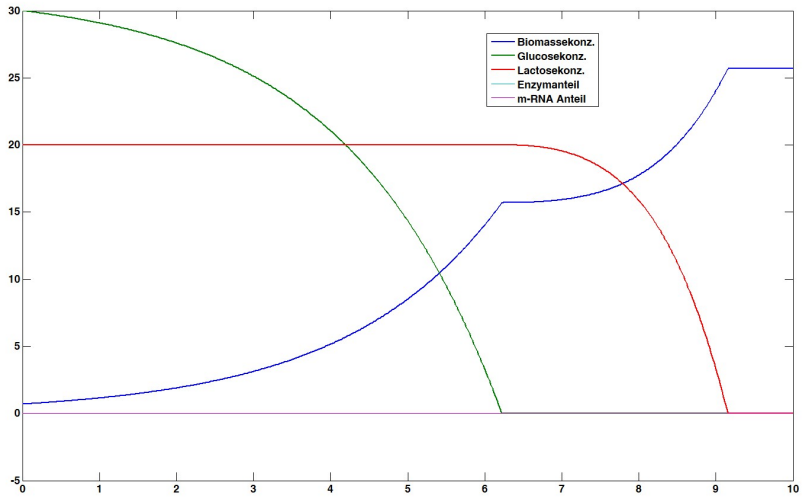
Code for model in MATLAB (Model parameters freely estimated)

P. Götz, slide 25

```
% Modellparameter
muemaxGluc=0.5;
nuemaxLac=4000;
KsGluc=0.001;
KsLac=0.001;
YxGluc=0.5;
YxLac=0.5;
Kkr=0.01;
k3=10;
k4=0.1;
k5=10;
k6=0.1;
XCRPtot=0.001;
XRtot=0.001;
KLac=1;
kE=2;
rmRNAmax=15;
kDmRNA=10;
expm=3;
expn=3;

% Monodkinetiken
  mueGluc=muemaxGluc*y(2)/(KsGluc+y(2));
  mueLac=nuemaxLac*y(4)*y(3)/(KsLac+y(3));
  mue=mueGluc+mueLac;
  muemax=muemaxGluc+nuemaxLac*y(4);

% Zusammenhang cGluc und XcAMP, unten eingesetzt
% XcAMP=(Kkr/(Kkr+y(2)));

% Zusammenhang cLac und XLac, unten eingesetzt
% XLac=(KLac*y(3));

% Proteinsynthesekapazität
Fmue=0.042+0.146*mue/(0.042+0.146*muemax);

% Repressionsstatus
Fkr=k5*k6*((Kkr/(Kkr+y(2)))^expm)*XCRPtot/(1+k5*((Kkr/(Kkr+y(
2)))^expm)+k5*k6*((Kkr/(Kkr+y(2)))^expm)*XCRPtot);

% Induktionsstatus
Find=(1+k3*((KLac*y(3))^expn))/(1+k3*((KLac*y(3))^expn)+k4*XR
tot);

% Differentialgleichungen für cX  cGluc cLac  cE  cmRNA  in
Vektorschreibweise
dydt = [mue*y(1);
        -mueGluc*y(1)/YxGluc;
        -mueLac*y(1)/YxLac;
        kE*y(5)-mue*y(4);
        rmRNAmax*Fmue*Find*Fkr-kDmRNA*y(5)-mue*y(5)
      ];
```

Simulation result for model equations (MATLAB): Diauxic behavoir -> Model verification

P. Götz, slide 26



Part IV

Technical aspects

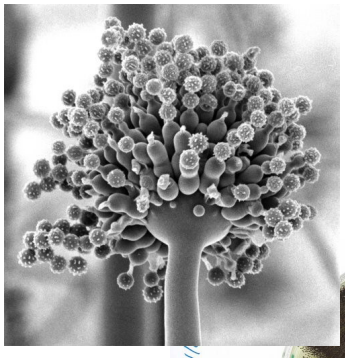
Fermentation (Upstream Processing)

- *Fermentation* has the aim to cultivate microorganisms (bacteria, yeasts or fungi) or cells (plant, insect, fish, mammalian) in reaction vessels (bioreactors) to turn substrates (derived from renewable sources) into products.
- Most fermentation take place under aerobic conditions.
- A subcategory of fermentation is *biocatalysis*, here a substrate is turned into a product by biocatalytic enzymes.

de.wikipedia.org/wiki/Biokatalyse

2

Is citic acid produced with lemons?



Aspergillus niger

3

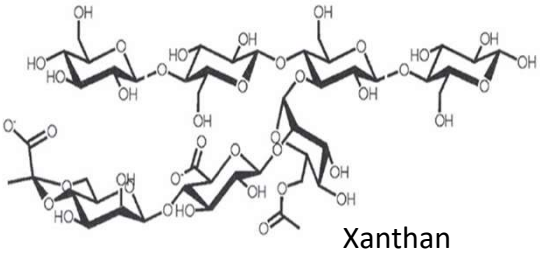
Use of citric acid

- In grocery as acidifier (E330)
  - Flavour (lemonade)
  - Preservative
  - Peptisator (prohibits flocculation) → sauce
- Cleaning agent
  - Anti-liming agent
  - Water softener (e. g. in fabric softener)
  - Rust remover
- Medicine
  - Anticoagulant in blood bottles
  - Energency medication against heavy metal intoxication

4

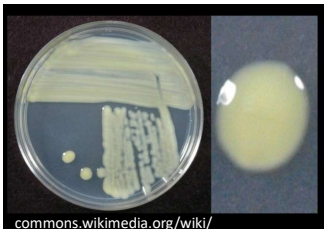
Why do herbs float in salad dressing?

c2.staticflickr.com/4/3885/15197763405_e9a7d93d26_b.jpg



Xanthan

Xanthomonas campestris



commons.wikimedia.org/wiki/File:Xanthomonas_Culture.jpg

5

Usage of xanthan gum

- Production approx. 50.000 t/a
- Food industry (E415)
  - Thickening agent, emulsifier, stabilisator
  - Sauces, jam, dairy products
- Crude oil production
  - Enhanced oil recovery by flooding oil fields with polymer solution
- Other technical applications
  - Emulsifier in printing ink
  - Emuslsifier in cosmetics

de.wikipedia.org/wiki/Pudding

www.flickr.com/photos/johnjoh/4398034471

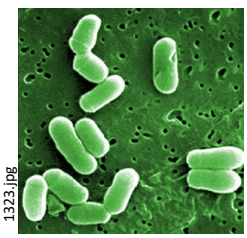
Application of amino acids

- Flavor enhancer (glutamate)
- Animal feed upgrade
- Dietary supplement
- Synthesis of artifical sweetener (aspartam = aspartic acid + phenylalanine)

de.wikipedia.org/wiki/Kartoffelchi

de.wikipedia.org/wiki/Kaugumm

c1.staticflickr.com/1/190/5106766658_425afb1323.jpg



Corynebacterium glutamicum

de.wikipedia.org/wiki/Cola

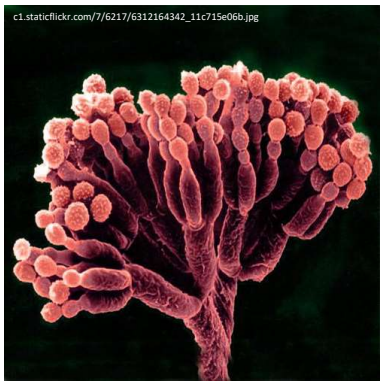
7

Laundry detergent

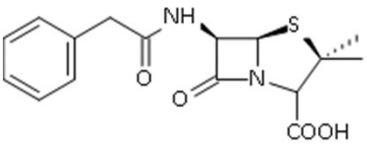
- Beyond tensides, washing agents also contain different enzymes to remove stains from organic sources, e.g.:
  - Food (amylases, proteases)
  - Blood (proteases)
  - Oil (Lipases)
  - Grass (Cellulases)
- For washing programms >40°C often thermostable enzymes are used.

Bacillus subtilis

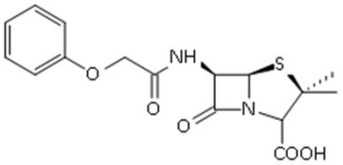
Antibiotics, Penicillin



Penicillium

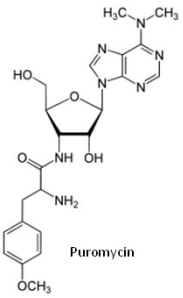


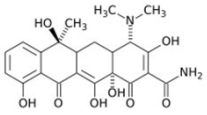
Penicillin G

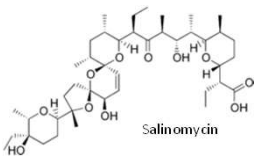


Penicillin V

9

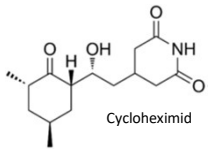

Puromycin

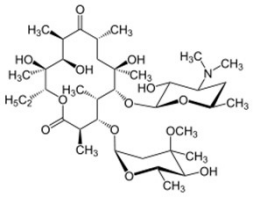

Tetracyclin

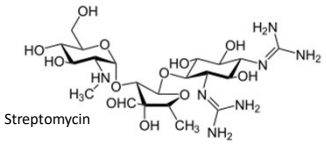

Salinomycin

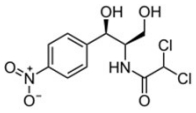
Antibiotics from

Streptomyces


Cycloheximid

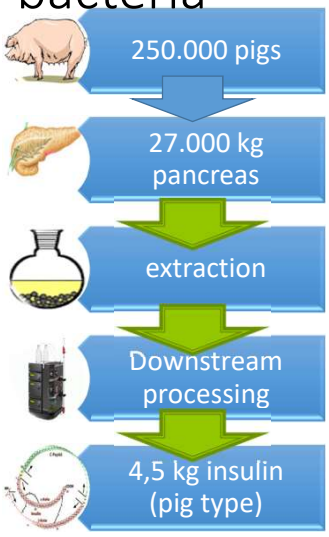

Erythromycin

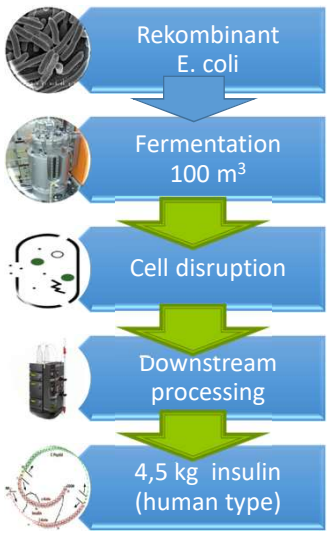

Streptomycin


Chloramphenicol

10

Comparison of the insulin production using pigs or bacteria

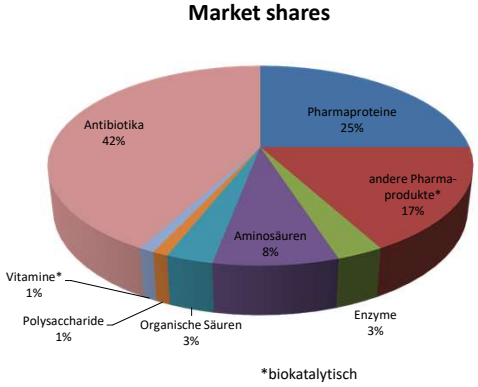




11

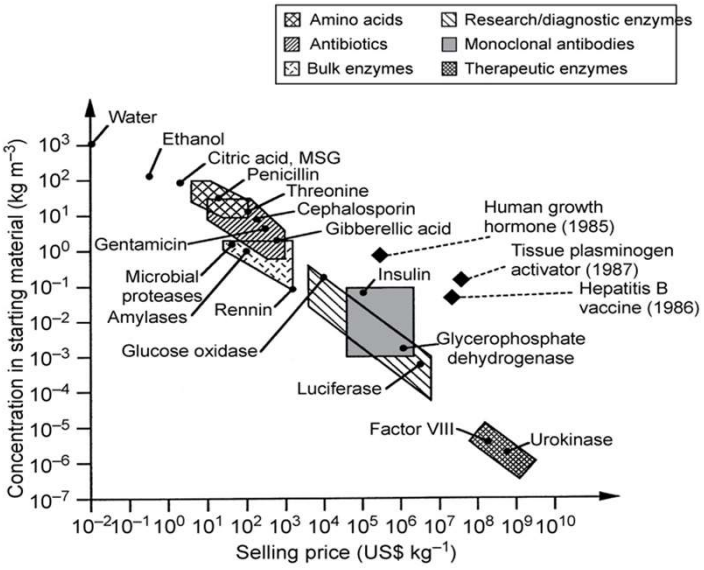
Products in biotechnology

- Antibiotics
- Pharma proteins
- Amino acids
- Enzymes
- Organic acids
- Biopolymers/Polysaccharids
- Solvents



12

Products in biotechnology



13

Products of the „white biotechnology“

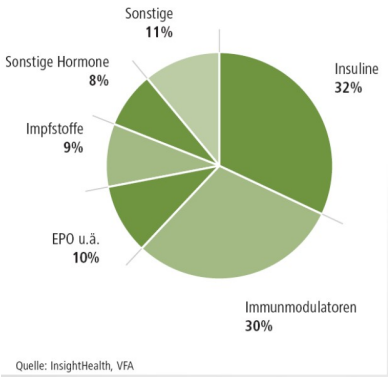
Produkte der Weißen Biotechnologie, die im Tonnenmaßstab hergestellt werden.

Produkt	Weltjahres-produktion (t/a)	Anwendung	Produkt	Weltjahres-produktion (t/a)	Anwendung
Säuren			**Antibiotika**		
Zitronensäure	1.000.000	Lebensmittel, Waschmittel	Penicilline	45.000	Medizin
Essigsäure	190.000	Lebensmittel	Cephalosporine	30.000	Medizin
Gluconsäure	100.000	Lebensmittel, Textil, Metall	Tetracycline	5.000	Medizin
Itaconsäure	15.000	Kunststoff, Papier, Klebstoff	**Biopolymere**		
L-Apfelsäure*	100	Säuerungsmittel	Polymilchsäure	140.000	Verpackung
			Xanthan	40.000	Erdölförderung, Lebensmittel
Aminosäuren¹			Dextran (-derivate)	2.600	Blutersatzstoff
L-Glutamat	2.500.000	Geschmacksverstärker	**Vitamine**		
L-Lysin	1.500.000	Futtermittelzusatz	Ascorbinsäure (Vit. C)	100.000	Pharma, Lebensmittel
L-Threonin	230.000	Futtermittelzusatz	L-Sorbose	50.000	Pharma, Lebensmittel
L-Methionin	600.000	Futtermittelzusatz	Riboflavin (B_2)	30.000	Wirkstoff, Futterzusatz
L-Phenylalanin	80.000	Aspartam, Medizin			
L-Tryptophan	50.000	Ernährung, Futtermittel	**Kohlenhydrate**		
L-Arginin	10.000	Medizin, Kosmetik	Glucose*	20.000.000	Flüssigzucker
L-Valin	5.000	Infusionslösungen	High Fructose Syrup*	8.000.000	Getränke, Ernährung
			Fructooligosaccharide*	10.500	Präbiotikum
Lösungsmittel			Cyclodextrine*	5.000	Kosmetik, Pharma,
Bioethanol	18.500.000	Energieträger, Lösungsmittel			Lebensmittel

(* enzymatisch hergestellt), Daten nach Becker & Wittmann, 2012 (1) und DECHEMA, 2004.

14

Umsatz 2005: 2,06 Mrd. Euro

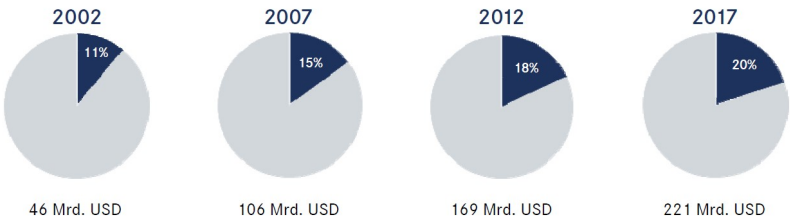


Quelle: InsightHealth, VFA

Red Biotechnology

German market shares of pharmaceutical products produced by genetic engineering

Sales of biotechnological products within the pharmaceutical market (worldwide)

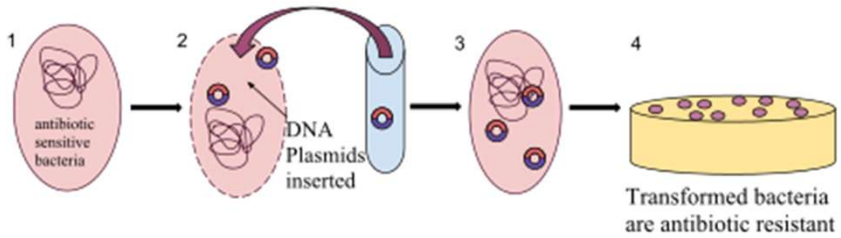


Quelle: IMS Health

15

Fermentative product manufacturing

- Classic: Production strains are bred by selection from wild strains
- Recombinant: Hetrologous genes are expressed in a host organism (genetically engineered microorganism)



en.wikipedia.org/wiki/Transformation_(genetics)#/media/File:Artificial_Bacterial_Transformation.svg

16

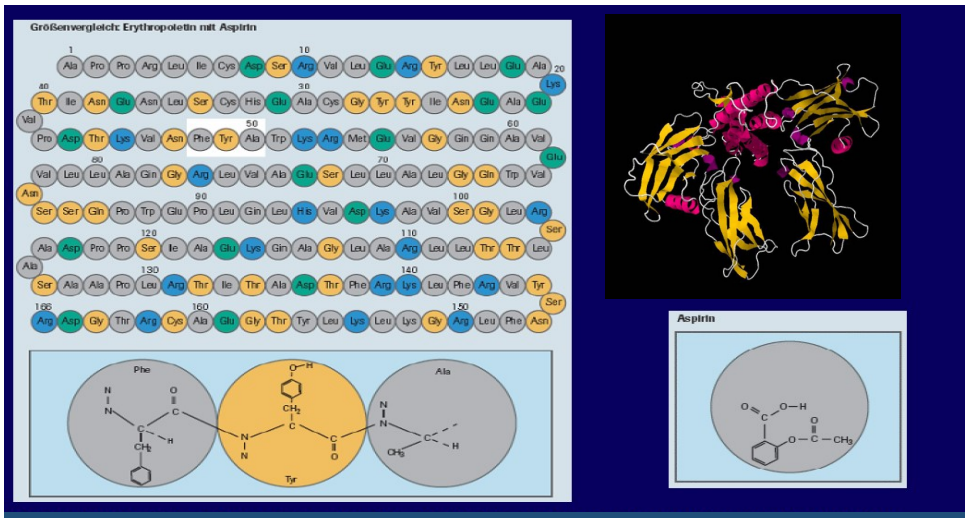
Products in biotechnology

- Biotechnological production by:
  - **Fermentation** of microorganims or cell culture
  - Enzymatical substrate conversion (**Biocatalysis**)

1. New products that are only possible due to biotechnology
2. Alternative to chemical production-/ synthesis processes, often the biotechnolgical process is combined with other chemical synthesis steps

17

Rekombinant pharmaceuticals



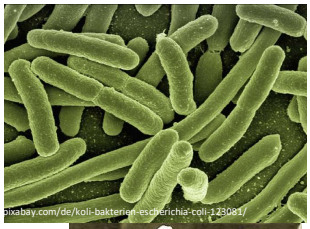
Size comperance erythropoietin (EPO) with acetylsalicylic acid

Source: Roche Arzneimittel aus dem Fermenter

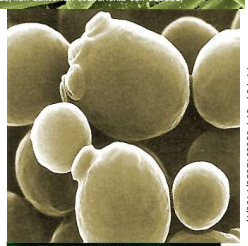
18

Production strains

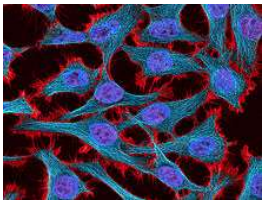
- Bacteria
- Yeasts
- Funghi
- Cell cultures (plant or animal sources)



pixabay.com/de/koli-bakterien-escherichia-coli-123081/



c2.staticflickr.com/4/3314/3507133086_b40acb2da1.jpg



de.wikipedia.org/wiki/HeLa-Zellen#/media/File:HeLa-II.jpg

c1.staticflickr.com/7/6217/6312164342_11c715e66b.jpg

19

Production strains (bacteria)

Escherichia coli	Bacillus subtilis	Coryne-bakterium glutamicum	Clostridium aceto-butylicum	Xanthomonas campestris	Ralstonia eutropha
Popular strain for recombinant expression: **insulin**, somatropine , interferone, plasminogenactiv ator , also enzymes	Produces extracellular enzymes (amylases, proteases, lipases) → washing agent-**enzyme**	Metabolic mutant with overexpression of the amino acid **L-Glutamate**	Anaerob, Solvents: **butanole**, acetone, ethanol; Acids:: acetic acid, butyric acid	Plant-pathogen produces the exopolysaccharide **xanthan gum**	Soil bacerium., can sythesis PHA (Polyhydroxy-alkanoate) und **PHB** (Poly-hydroxy butyric acid) = degradeable plastic

www.intechopen.com/books/polyester/whey-lactose-as-a-raw-material-for-microbial-production-of-biodegradable-polyesters

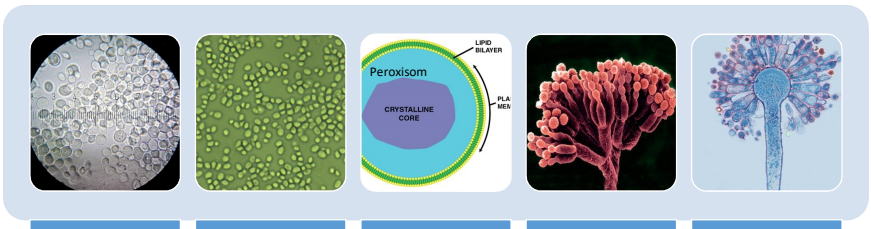
en.wikipedia.org/wiki/Escherichia_coli#/media/File:EscherichiaColi_NIAID.jpg
en.wikipedia.org/wiki/Bacillus_subtilis#/media/File:Bacillus_subtilis_Gram.jpg
c1.staticflickr.com/1/190/510676658_425afb1323.jpg
en.wikipedia.org/wiki/Clostridium_difficile_(bacteria)#/media/File:Clostridium_difficile_01.jpg
F. García-Ochoa et al. / Biotechnology Advances 18 (2000) 549±579

size 0,1 – 3 µm

Recombinant expression in bacteria

+	Simple, cheap media
+	Fast growth (t_D approx. 20 min)
+	Cultivation to high cell densities (biomass > 100 g/l)
+	Well characterized / explored
+	Simple cultivation in the fermenter
+	many different expression vectors
-	Products often in inclusion bodies
-	Proteins oft not or wrongly folded
-	Active products often toxic for the host
-	no post-translational protein modifications

Production strains (yeast/fungi)



Saccharomyces cerevisiae	Candida utilis	Pichia pastoris	Penicillium notatum	Aspergillus niger
•Baker's yeast •Brewer's yeast •Compressed- and dry yeast	•Accepts many C-sources (also Xylose) •Single Cell Proteins •Animal feed •Kefir	•Popular strain for recombinant expression, because methylotrophe (Peroxisoms very active) •e.g. Vaccin against Hepatitis B, differnt lipases and amylases	*Pinselschimmel* - Begin of the production of antibiotics - Discovery of **Penicillin** (1928)	*Gießkannen-schimmel* - Large scale production of citric acid

de.wikipedia.org/wiki/Backhefe#/media/File:20100911_232323_Yeast_Live.jpg
de.wikipedia.org/wiki/Candida_glabrata
en.wikipedia.org/wiki/Peroxisome#/media/File:Peroxisome.svg
c1.staticflickr.com/7/6217/6312164342_11c715e06b.jpg
c1.staticflickr.com/7/6016/5987578301_2a2cf8a0f7_z.jpg

size 3 – 10 µm

22

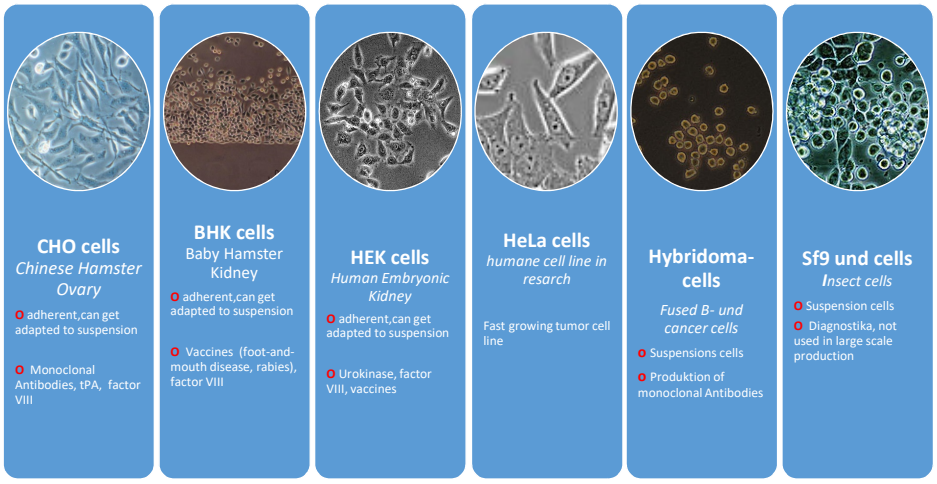
Rekombinant expression in yeasts/fungi

+ moderate media cost
+ well explored
+ Proteins partially with correct folding
+ Different posttranslational modifications possible
+ Products sometimes extracellular
+ Easy cultivation in fermenter

- Partly degradation of the product proteins
- Slower growth (t_D approx. 80 min)
- Some undesired modifications possible (Hyperglykosylation)

23

Established cell lines



CHO cells
Chinese Hamster Ovary
- adherent,can get adapted to suspension
- Monoclonal Antibodies, tPA, factor VIII

BHK cells
Baby Hamster Kidney
- adherent,can get adapted to suspension
- Vaccines (foot-and-mouth disease, rabies), factor VIII

HEK cells
Human Embryonic Kidney
- adherent,can get adapted to suspension
- Urokinase, factor VIII, vaccines

HeLa cells
humane cell line in resarch

Fast growing tumor cell line

Hybridoma-cells
Fused B- und cancer cells
- Suspensions cells
- Produktion of monoclonal Antibodies

Sf9 und cells
Insect cells
- Suspension cells
- Diagnostika, not used in large scale production

size 10-100 µm

de.wikipedia.org/wiki/CHO-Zellen#/media/File:Cho_cells_adherend2.jpg
commons.wikimedia.org/wiki/File:Pfc_bhk21.jpg
de.wikipedia.org/wiki/HEK-Zellen
de.wikipedia.org/wiki/Datei:HELA-Zellen.JPG
en.wikipedia.org/wiki/Hybridoma_technology
en.wikipedia.org/wiki/Sf21

24

Rekombinant expression in cell cultures
(animal- and insect- cells)

+ Completely folded proteins
+ Multitude of posttranslational modifications
+ Often extracellular products
+ Cell disruption is easy

- Very slow growth (t_D = 24 h)
- Special requirements for nutrient media -> expensive
- Expression is often only moderate
- High risk for contamination

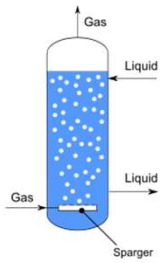
25

Expression systems

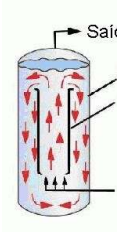
Charakteristik	Bakterien	Hefe	Insektenkultur	Säugetierkultur
Zellwachstum	schnell	schnell	langsam	langsam
Komplexität des Kulturmediums	minimal	minimal	komplex	komplex
Kosten der Kulturmedien	niedrig	niedrig	hoch	hoch
Expressions-Niveau	hoch	niedrig bis hoch	niedrig bis hoch	niedrig bis mäßig
Extrazelluläre Expression	Sekretion ins Periplasma	Sekretion ins Medium	Sekretion ins Medium	Sekretion ins Medium
Posttranslationale Modifikation:				
Proteinfaltung	Nachfaltung erforderlich	Nachfaltung gegebenenfalls erforderlich	vollständige Faltung	vollständige Faltung
N-Glykosylierung	keine	hoch (Mannose)	einfach	komplex
O-Glykosylierung	nein	ja	ja	ja
Phosphorylierung	nein	ja	ja	ja
Acetylierung	nein	ja	ja	ja
Acylierung	nein	ja	ja	ja
γ-Carboxilierung	nein	nein	nein	ja

26

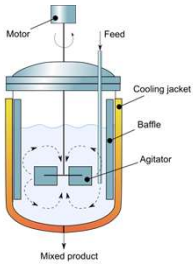
Bioreactor for microbial fermentations



Bubble column



Air lift reactor



Stirred tank reactor

Tall, slim design mostly D/H=3

- Prolonged retention time of the gas bubbles within the liquid phase
- Larger surface for heat exchange

en.wikipedia.org/wiki/Bubble_column_reactor
commons.wikimedia.org/wiki/File:Airlift_bioreactor.jpg
commons.wikimedia.org/wiki/File:Agitated_vessel.svg

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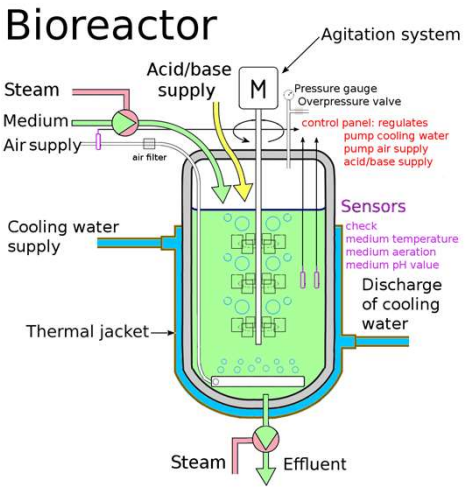
Stirred Tank Reactor

Tasks

- Control the essential parameters
  pH value, temperature, substrate
- Mixing
  Distribute nutrients and oxygen homgeneously within the vessel
- Heating and Cooling
  Heating at the beginning of a fermentation process, later cooling to remove heat produced by metabolism
- Gassing
  O2 supply, CO_2 removal
- Sterility
  Protect the production strain from external germs, confines the microorganisms inside the Vessel



upload.wikimedia.org/wikipedia/commons/b/bd/Real_life_bioreactor.png

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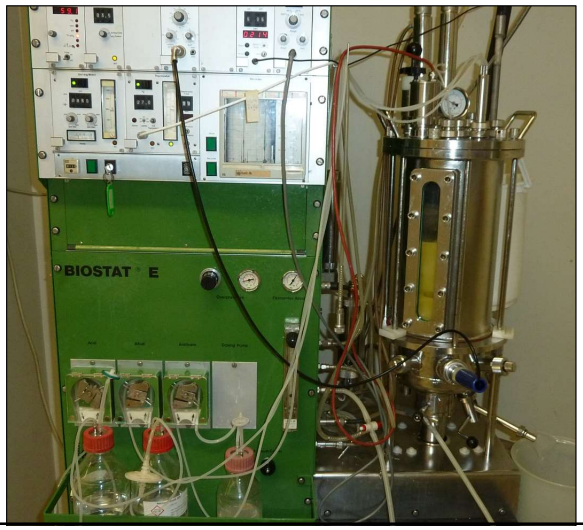
Stirred Tank Reactors

stainless steel (working volume approx. 15 L)



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Stirred Tank Reactors,

glass vessels in the range from 1 to 10 L

Scale up

en.wikipedia.org/wiki/Petri_dish#/media/File:Agar_plate_with_colonies.jpg

Strain collection

Shake flask

c2.staticflickr.com/8/7024/6464583867_16c8ccdc9a_b.jpg

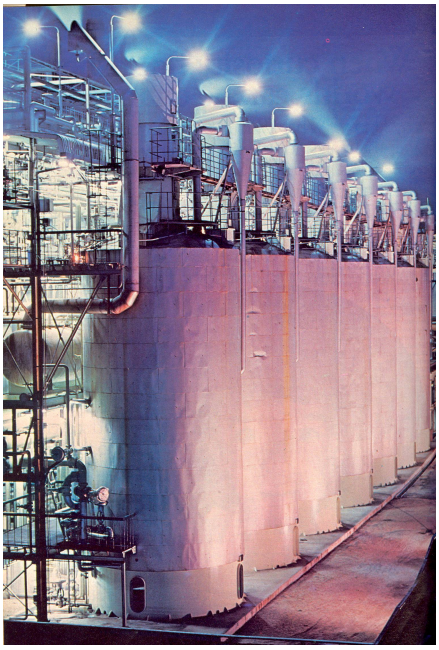
Fermenter

commons.wikimedia.org/wiki/File:Jninternational4.jpg

Fermentation tanks used for microbiological production of glutamate and lysine, Hofu, Japan

Tanks are 100ft high and contain 63,420 gallons

= 30 m
240 m^3



en.wikipedia.org/wiki/Bio_Base_Europe#/media/File:BBEPP_pilot_scale_fermenters.jpg

en.wikipedia.org/wiki/Chemical_reactor#/media/File:Chemical_reactor_CSTR_AISI_316.JPG

Overview

Bioreactor system

consisting of

- Vessel
- Measure and control unit
- Supply unit
- Pumps for correcting agents

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Vessel

- Cover
- Jacket
- Connection branches
- Shaft feedthrough for the agitator

Material:
Exposed to labratory: **Cr-Ni-steel** DIN 1.4301 (V2A)
Exposed to fermentation broth: **Cr-Ni-steel with titanium und molybdenum** DIN 1.4571 (V4A)
Glass: **borosilicate**

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Vessel lid

- Lamp
- Manometer
- Safety relief valve
- Air supply filter
- Condenser
- Foam detection probe

Septum, socket and dummy plug

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Inoculation in labratory scale

- Sterile flasks are filled with inoculum under sterile conditions e.g. laminar flow hood
- The fermenter is inoculated with a needle which is connected using an open flame

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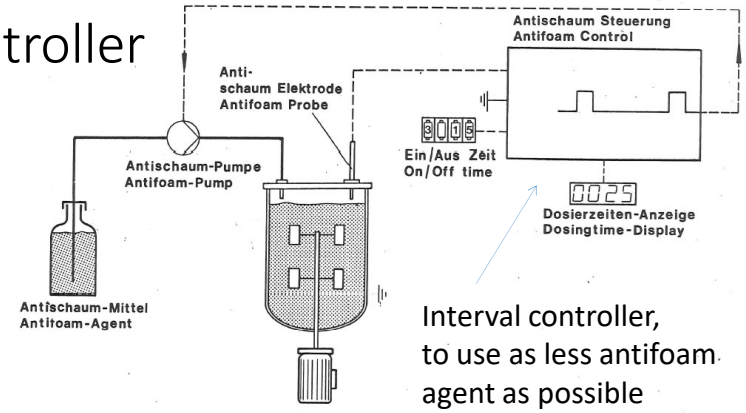
Antifoam controller



Interval controller,
to use as less antifoam agent as possible

Foam can clog the exhaust air filter
→Pressure rises in fermenter due to gassing
→Security valve opens
→Fermentation broth will leak, sterile barrier is broken

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Manometer and safety relief valve

- Measures pressure above atmospheric pressure
- Fermentation: 0 bar
- Sterilization: 1 bar

- Spring loaded valve

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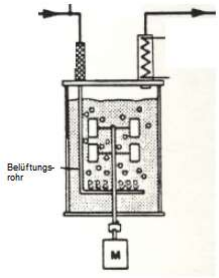
Supply air filter

Sterile filter, to remove microorganisms from the air supply flow
e.g. depth-type filter or membrane filter (cut off 0,2 µm)



During autoclaving, the filter has to be seperated from the sparger!
Otherwise medium can enter the filter.

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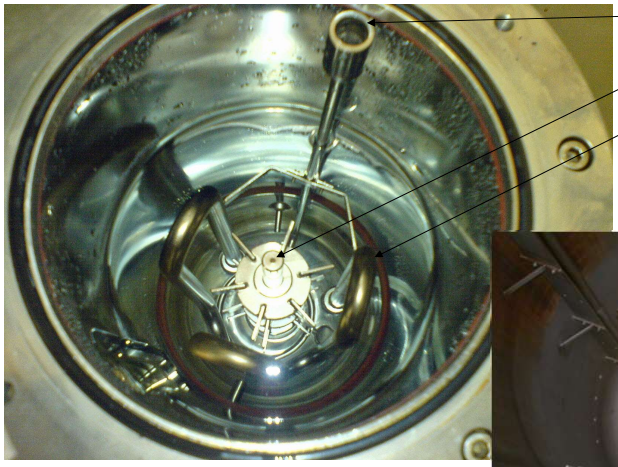
Inside the vessel



Gas pipe

Stirrer on shaft

Heat exchangers

Flow breaker

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Exhaust air line

← Condenser with control windows

Exhaust air filter →

Dries the exhaust air and recycles condensate
→No concentration of the medium
→Exhaust filter stays dry

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Lower ports

Connections for short probes, e.g.:

- DO probe
- pH probe
- Thermo-sensor (Pt-100)
- Redox probe
- pCO_2 probe
- OD-/Biomass probe

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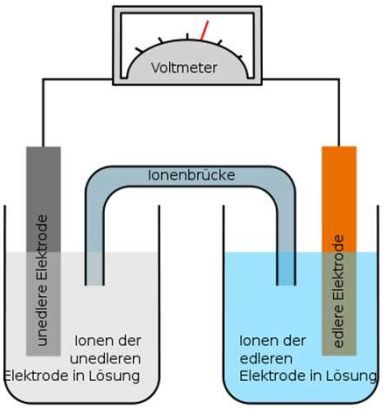
Amperometric oxygen measurement

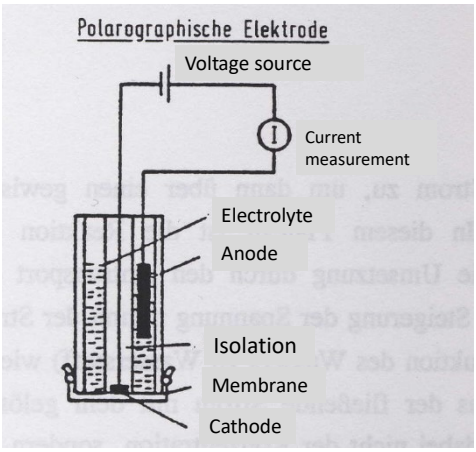


1. Base electrode is connected to an
2. Ion bridge to the
3. Precious metal electrode

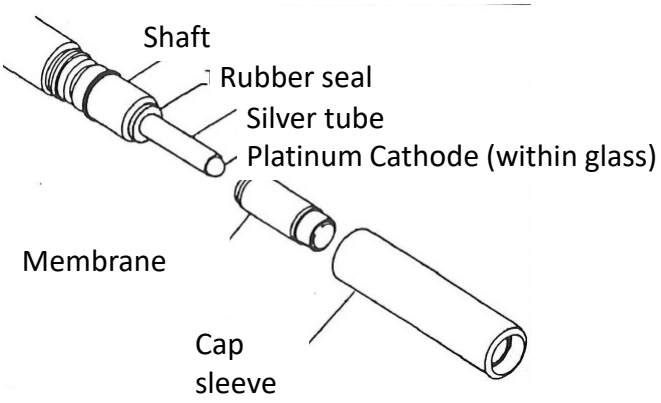
Polarographical oxygen probe

- Membrane: PTFE oder silicone
- Only gas can pass
- Cathode consists of gold oder platinum
- Anode made of silver

→Polarisation-voltage between anode und cathode is necessary



DO probe



DO probe equation

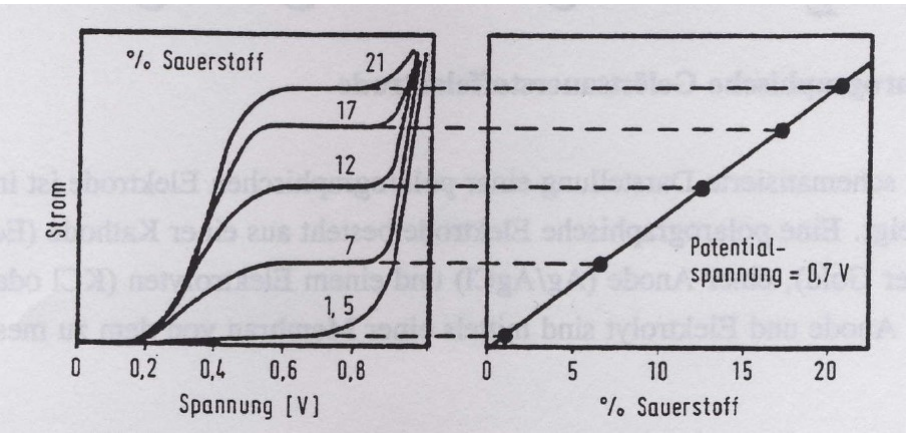
Kathode: $O_2 + 2H_2O + 2e^- \rightarrow H_2O_2 + 2OH^-$

$H_2O_2 + 2e^- \rightarrow 2OH^-$

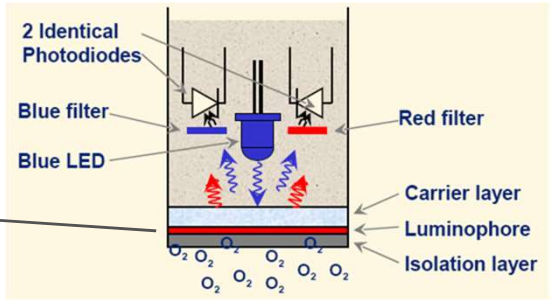
Anode: $4OH^- + 4KCl \rightarrow 4KOH + 4Cl^-$

$4Cl^- + 4Ag \rightarrow 4AgCl + 4e^-$

Polarogram



Optical DO probe



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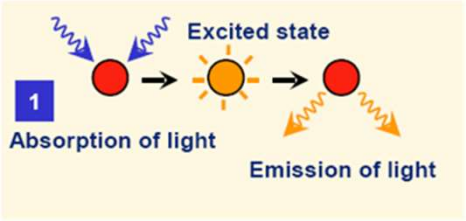
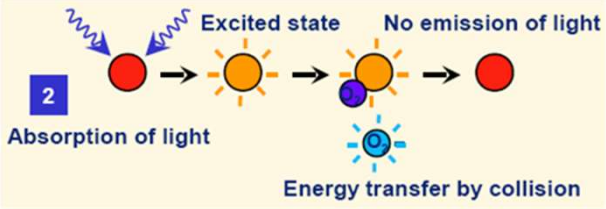
Dynamic luminescence quenching

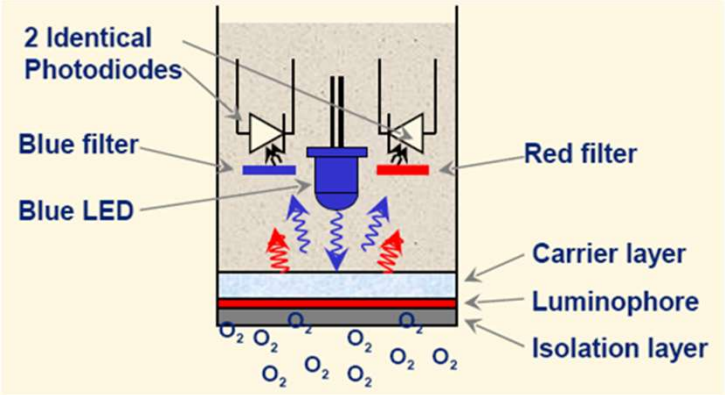
- Absence of oxygen



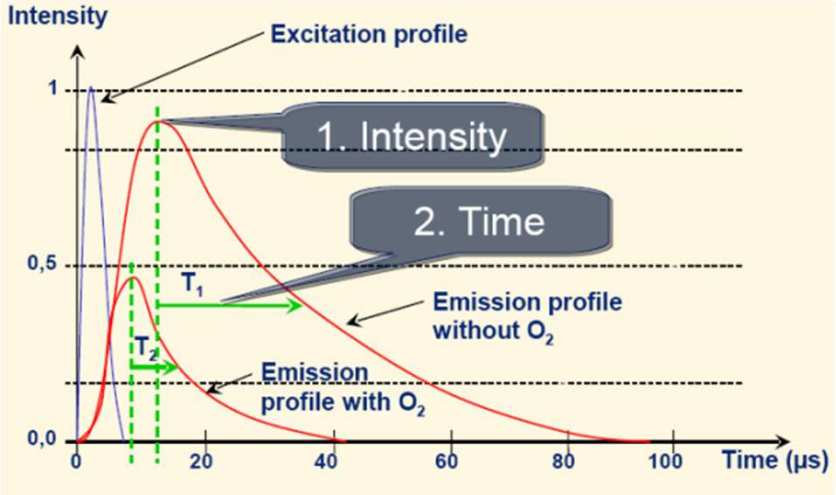
- Presence of oxygen



Sensor head

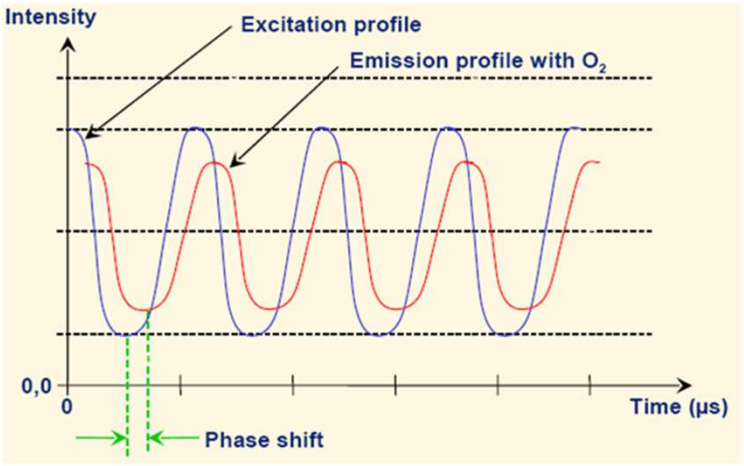


Calculation of the DO concentration



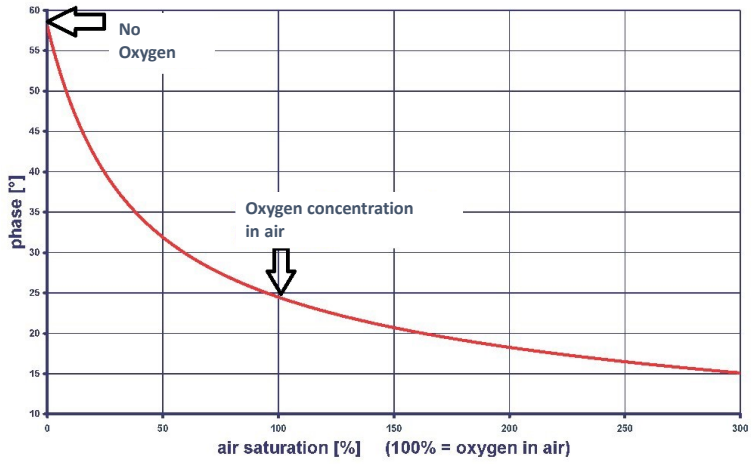
Calculation of the DO concentration

- Phase shift



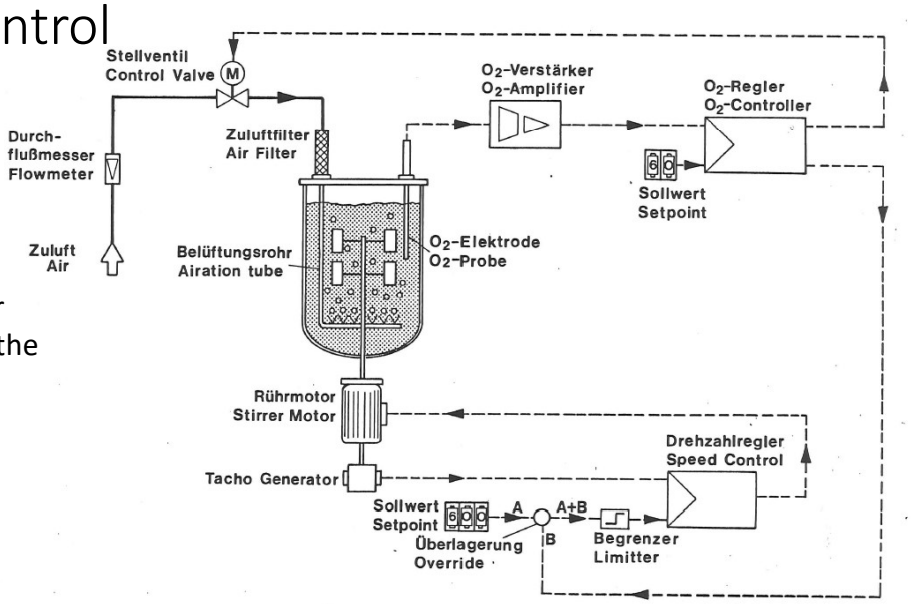
Phasenwinkel (deg) $\varphi^\circ = 360^\circ \cdot f \cdot \Delta t$

Calibration curve



pO_2-Control

- Input setpoint
- DO probe delivers actual value
- Depending on the deviation, the master controller calculates the response
- Slave controllers regulate:
  - Airflow
  - Stirrer speed
  - Pressure in fermenter head space



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Calibration of DO probes

- Measuring the oxygen concentration in % of saturation partial pressure
- 2-point-calibration
  - 0% via gassing (and stirring) with nitrogen
  - 100% via gassing (and stirring) with air

100% pO_2 means the maximum of the solveable oxygen possible by gassing with air at atmospheric pressure and fermentation temperature.

When gassing with pure oxygen (expensive), pO_2 may be beyond 100%

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pH probes for fermentation

Needs to be autoclaveable

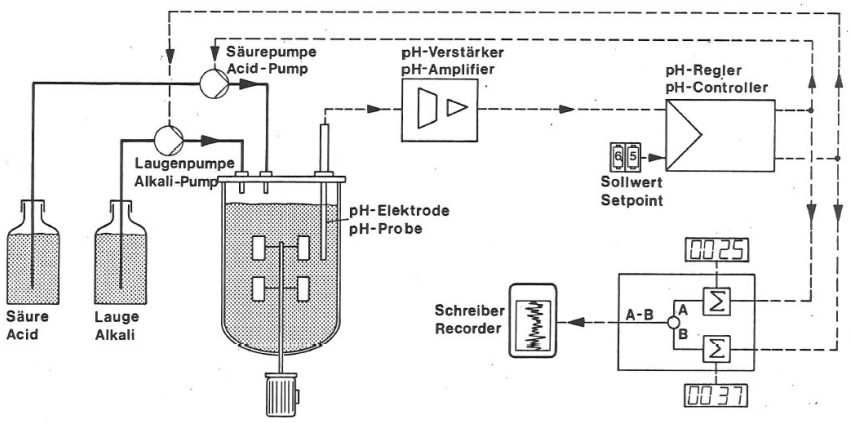
Electrodes filled with a gel

- Pressurized
- Gel is not exchangeable
- Precision of measurementsis lower
- Shorter operating time
- Higher response time

Stainless steel mounting

pH control

- Enter setpoint
- pH probe delivers actual value
- Depending on the deviation, the controller calculates the response either for the acid or the base pump
- Correction agents: e.g. phosphoric acid / ammonia solution



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Pumps for correction agents

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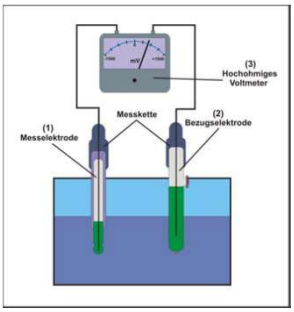
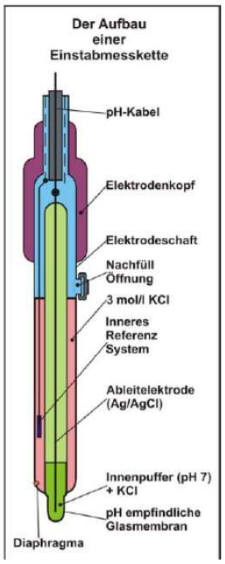
pH-measurement in bioreactor



1. pH-sensitive glass electrode (measuring electrode)
2. Reference electrode (always pH 7)
3. Measuring amplifier

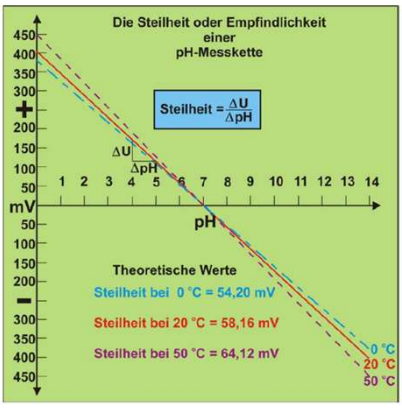


Between measuring and reference electrode a electric potential builds up.
To calculate pH-values, calibration has to be preformed.

Calibration of a pH-probe

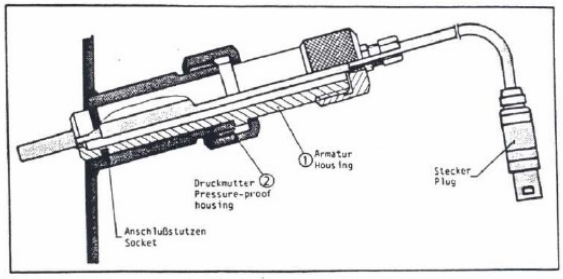
- Two-Point-Calibration:
  - Use of reference buffers
  - First with pH 7 = 0 mV
  - Afterwards with pH 4 = Slope

Slope=ΔU/ΔpH = 58,16 mV at 20°C



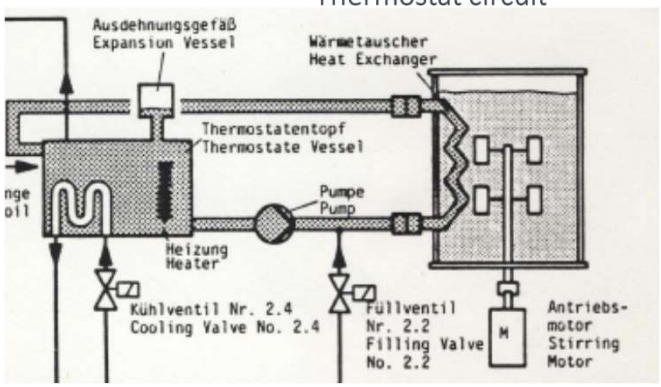
Temperature measurement

- Commonly a Pt-100 resistance thermometer is used
- Measuring principle is the dependence of the electric resistance from temperature
- Signal generation by Wheatstone -Bridge
- Temperature range from -250 – 1000°C



Temperature control

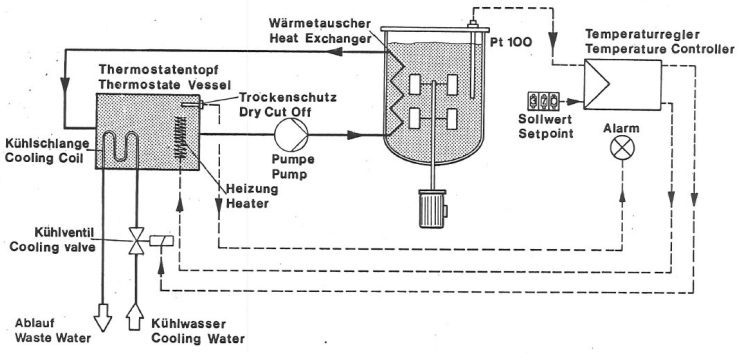
Thermostat circuit



Manuell über Kippschalter „Fill Thermostat“ zu öffnen

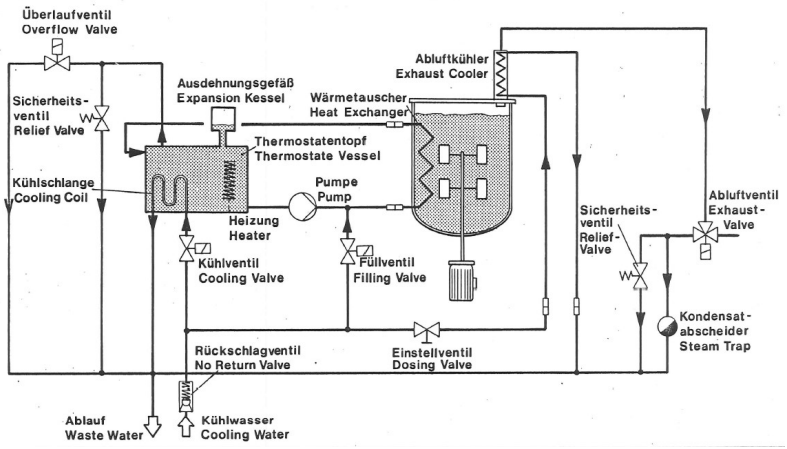
- Components: Heat Exchangers (or double jacket), electric heater or steam, cooling system and pump

Temperature control

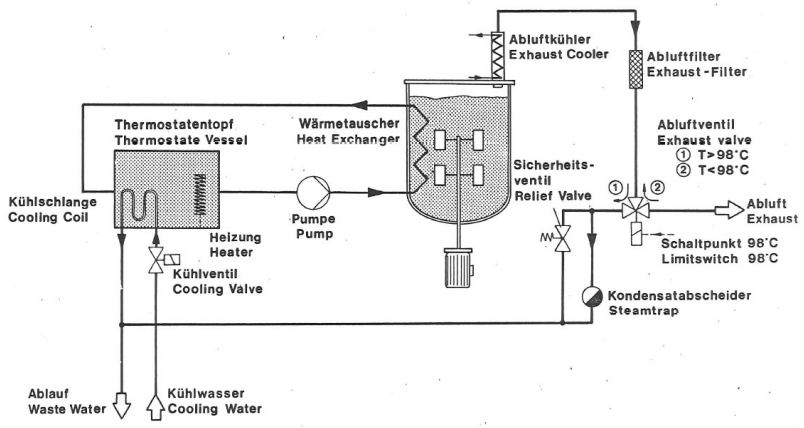


- Components: Heat Exchangers (or double jacket), electric heater or steam, cooling system and pump

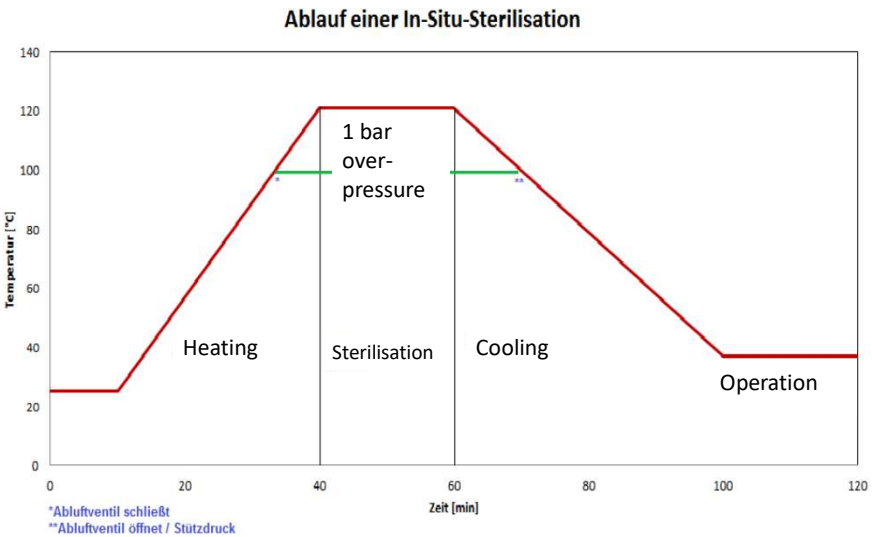
Thermostat System



Sterilization system



Phases of the sterilization process



In-Situ-Sterilization of a fermenter:

1. Heating phase: 98°C Inlet- and Outlet-valves are closed (-> overpressure)
2. Sterilisation phase: 121°C, 1 bar, 20 min
3. Cooling phase: 98°C Inlet- and Outlet-valves are opening again (pressure relief with support pressure)

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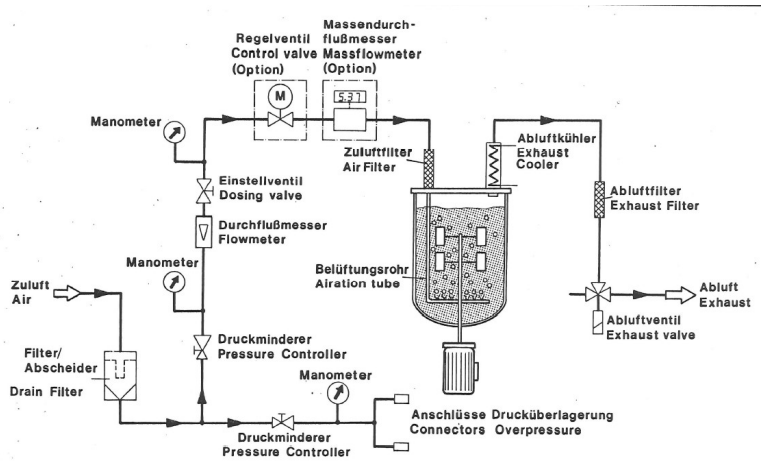
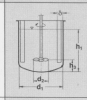
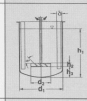
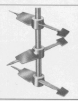
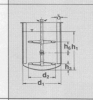
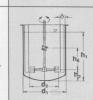
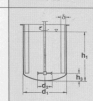
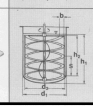
Gas system



Stirrer types

Tasks

- Dispersing the inlet air
  -> Achieve a higher interface between air/liquid
  for a better mass transfer
- Esablish homogeneous conditions in the whole liquid phase (substrate, DO, pH, T...) by mixing
- Improve the heat transfer

Benennung Kurzzeichen	Bauweise	Bevorzugte geometrische Abmessungen und Anordnungen	Einbauverhältnisse	Primärströmungsrichtung
Propeller-Rührer PR		 $d_2/d_1 = 0{,}33$; $h_2/d_1 = 0{,}3$; $\alpha = 25°$; $h_1/d_1 = 1$; $b/d_1 = 0{,}1$	zentr. 3 Stromstörer; exzentr., schräg u. seitlich o. Stromstörer u. im Leitrohr	axial
Schrägblatt-Rührer SBR		 $d_2/d_1 = 0{,}33$; $h_2/d_2 = 0{,}125$; $h_3/d_1 = 0{,}3$; $h_1/d_1 = 1$; $b/d_1 = 0{,}1$	zentr. 2–4 Stromstörer bzw. exzentr. o. Stromstörer	axial-radial
Scheiben-Rührer SR		 $d_2/d_1 = 0{,}33$; $h_2/d_2 = 0{,}2$; $b/d_2 = 0{,}25$; $h_3/d_1 = 0{,}3$; $h_1/d_1 = 1$; $b/d_1 = 0{,}1$	zentrisch 2–4 Stromstörer	radial
Mehrstufen-Impuls-Gegenstrom-Rührer MIG®		 $d_2/d_1 = 0{,}7$; $h_2/d_1 = 0{,}16$; $h_3/d_1 = 0{,}28$; $h_1/d_1 = 1$; $b/d_1 = 0{,}1$	zentrisch 2–4 Stromstörer ($d_2/d_1 < 0{,}7$) bzw. o. Stromstörer ($d_2/d_1 > 0{,}7$)	axial-radial
Interferenz-Mehrstufen-Impuls-Gegenstrom-Rührer INTERMIG®		 $d_2/d_1 = 0{,}7$; $h_2/d_1 = 0{,}22$; $h_3/d_1 = 0{,}5$; $h_1/d_1 = 1$; $b/d_1 = 0{,}1$	zentrisch 2–4 Stromstörer ($d_2/d_1 < 0{,}7$) bzw. o. Stromstörer ($d_2/d_1 > 0{,}7$)	axial-radial
EKATO-MIZERSCHEIBE® EM		 $d_2/d_1 = 0{,}33$; $h_2/d_1 = 0{,}3$; $h_1/d_1 = 1$; $b/d_1 = 0{,}1$	zentrisch oder exzentrisch mit und ohne Stromstörer	radial
Wendel-Rührer WR		 $d_2/d_1 = 0{,}90$; $b/d_2 = 0{,}1$; $s/d_2 = 0{,}5$; $h_2/d_2 = 1$; $h_1/d_1 = 1$	zentrisch ohne Stromstörer 1- oder 2-gängig mit Innenschnecke	axial zwangsfördernd

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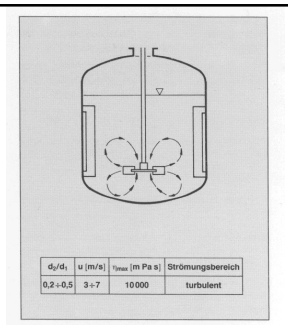
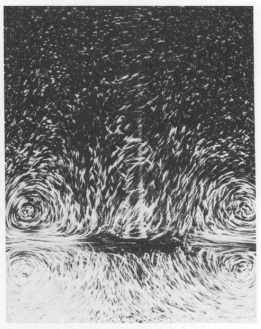
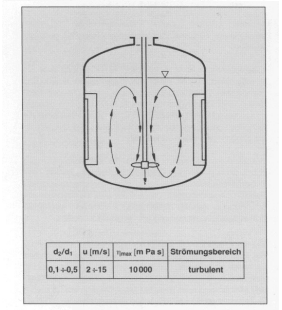


d_S/d_1	u [m/s]	η_{max} [m Pa s]	Strömungsbereich
0,2 ÷ 0,5	3 ÷ 7	10 000	turbulent

Scheibenrührer

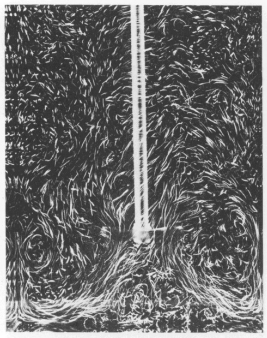


Re = 5 · 10^4 (turbulent)



d_S/d_1	u [m/s]	η_{max} [m Pa s]	Strömungsbereich
0,1 ÷ 0,5	2 ÷ 15	10 000	turbulent

Propeller



Re = 5 · 10^4 (turbulent)

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Stirrers

Disk stirrer (6-blade-disk-stirrer , Rushton turbine)

- Standard in many bioreactors
- For microbial fermentation
- radial liquid transport
- Good gas dispersion
- High local shear forces

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6-Blade-Disk-Stirrer in action

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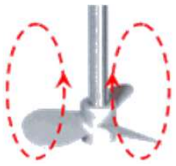
Stirrers

Propeller stirrer

- Axial liquid flow
- Lower gas dispersion
- Lower local shear forces
- Flow breaker can be necessary
- Use in cell culture with low stirrer speed



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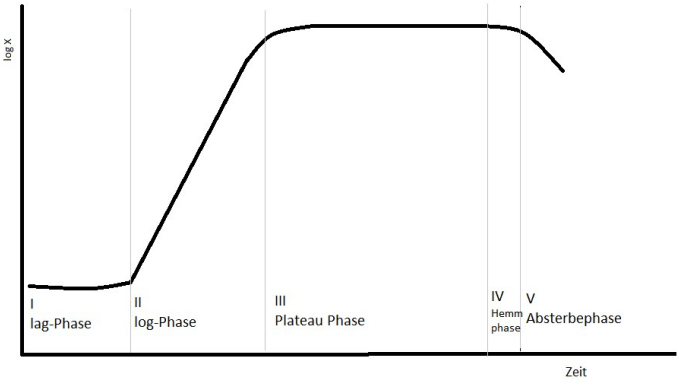
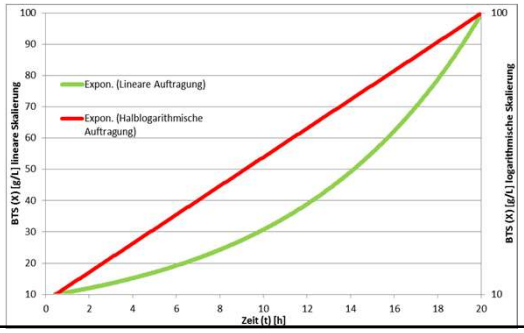
Microbial growth





I: lag-Phase (adaptation to the medium),

II: log-Phase (exponential growth)

III: Stationary phase (substrate is exhausted)

IV: Accumulation of inhibitors

V: Death/Lytic Phase

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Measurement of the biomass concentration c(X) during fermentation

- Direct
  - Centrifuge 10 mL sample, discard supernatant
  - Resuspend in 0,9% NaCl
  - Centrifuge again, discard supernatant
  - Dry pellet at approx. 80°C over night
  - Weigh dry pellet
- Photometrical (Optical density)
  - Photometrical turbidity measurement at 600 nm
  - Sample has to be deluted to 0,2 – 0,8 AU
  - For exact result a calibration curve is needed → Conversion factor
    e.g. for E. coli: c(X)/OD = ca. 0,35
    Expensive inline probes for online-measurement are available

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Calculation of specific growth rate µ

- log-phase:

$$\frac{dX}{dt} = \mu X$$

$$X_1 = X_0 \, e^{\mu \Delta t}$$

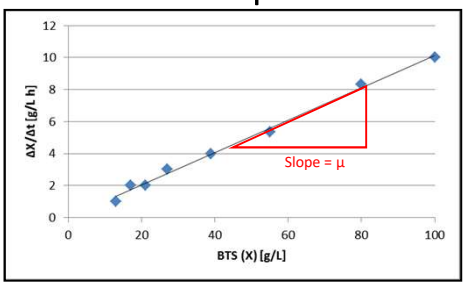
- Specific growth rate:

$$\mu = \frac{\ln(\frac{X_1}{X_0})}{\Delta t} = \frac{\ln(X_1) - \ln(X_0)}{\Delta t}$$

- Doubling time:

$$2 X_o = X_0 \, e^{\mu t_D}$$

$$t_D = \frac{ln2}{\mu}$$



X_t – biomass concentration, dry at t=1 [g/L]
X_0 – biomass conc. at t=0 [g/L]
µ - specific growth rate [h^{-1}]
t – time [h]
Δt – time difference between X_0 und X_1 [h]
t_D – doubling time [h]

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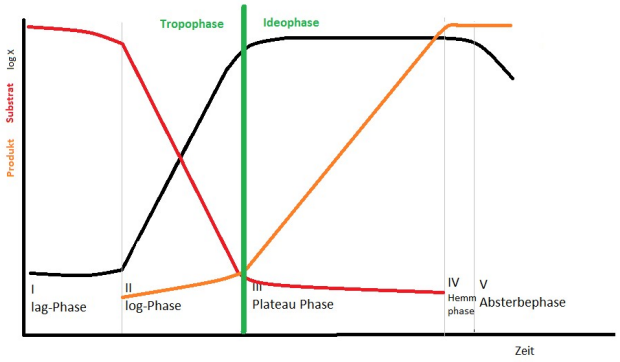
Secondary metabolites



Tropophase = growth phase

Idiophase = Product forming phase

Induction of secundary metabolites

- Targeted shortage of one nutrient source → product formation is a physiological reaction (e.g. xanthan)
- Change of a nutrient source

→ e.g. change from glucose to starch leads to formation of enzymes

- Genetically engineered microorganisms carry plasmids with an induceable promotor
  - Change of the C-source
  - Chemical induction (IPTG, Arabinose)
  - Thermal induction (increase of the fermentation temperature)

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Fermentation process modes

- Batch
  - No Feeding
  - No continuous harvesting
- Fed Batch
  - With Feeding
  - No continuous harvesting
- Continuous
  - Chemostat (constant feeding- and harvesting-rate)
  - Turbidostat (Feeding-/harvest-rate depends on optical density, which is kept constant)

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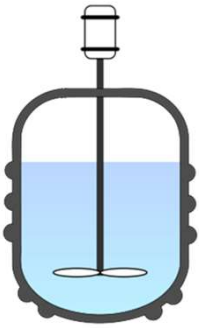
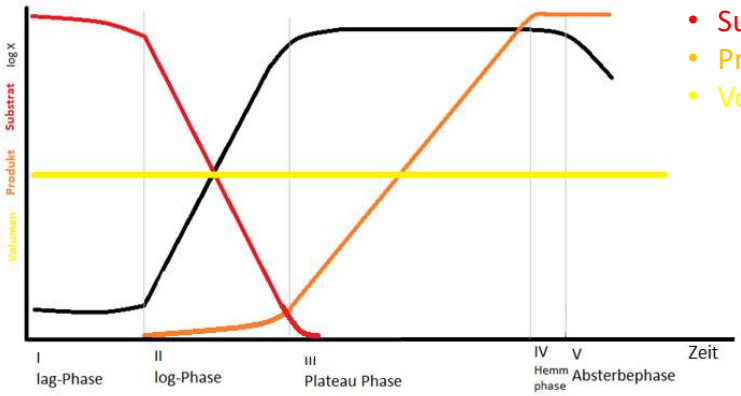
Batch-Fermentation





- Biomass
- Substrate
- Product
- Volume

- No feed or harvest during the fermentation

→ Volume stays constant

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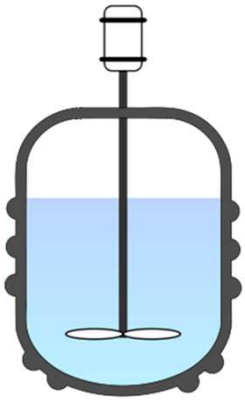
Batch-Fermentation



\+ No additional equipment needed
\+ Low costs
\+ Harvest of defined batches
\+ Risk of contamination is low

\- Fewer options for optimization
\- Lower productivity
\- Pathways with substrate excess overflow metabolism

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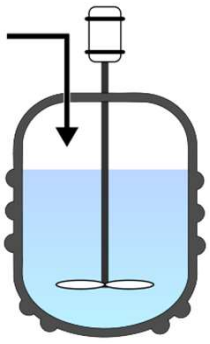
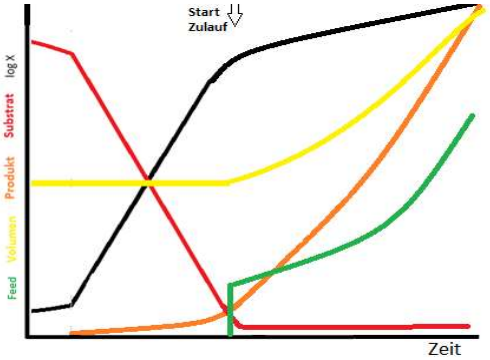
Fed-Batch-Fermentation





- Biomass
- Substrate
- Product
- Volume
- Feed

Adjustable parameters:
- substrate concentration in feed
- Feed start after batch
- Feed rate and strategy

E.g.: Penicillin production
Baker‘s yeast production

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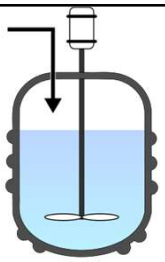
Advantages using Fed-Batch

- High cell- and product concentrations (High-Density-Fermentation)
- High productivity (space-time-yield)
- Efficient media conversion
- Avoid pathways with overflow metabolism (Crabtree, Glucose Overflow)

Problems



- High cell density → High demand for nutrients and O_2 (risk of O_2-limitation)
- Feed media are highly concentrated – in production scale: risk of overflow metabolism
- More effort on controllers
- Higher risk of contamination

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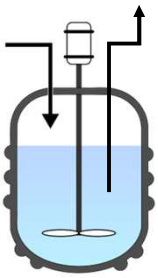
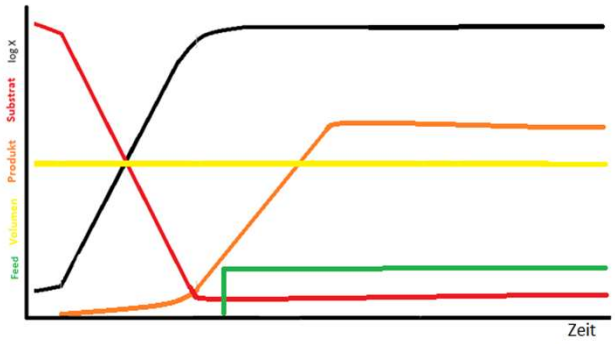
Fed-Batch strategies

- Feed start: immediately or delayed, e.g. after batch fermentaion
- Feed rate
- Feed mode
  - Linear constant
  - Linear increase
  - Logarithimic increase
  - …..

Continuous fermentation





- Bio mass
- Substrate
- Product
- Volume
- Feed

- Feed and harvest during the fermentation with the same flow rate

→ Volume is constant

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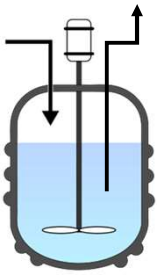
Continuous fermentation

Continuous cultivation (Chemostat)



+ growth rate is adjustable
+ long running time
+ fewer preparation periods

- Risk of mutation, loss of plasmide is possible
- Risk of contanimation
- Production-batch definition necessary
- Biomass concentration is not much higher than in fed-batch
- Higher effort

E.g.: Insulin (Novo Nordisk)
Ethanol (*S. cerevisiae*)
waste water treatment