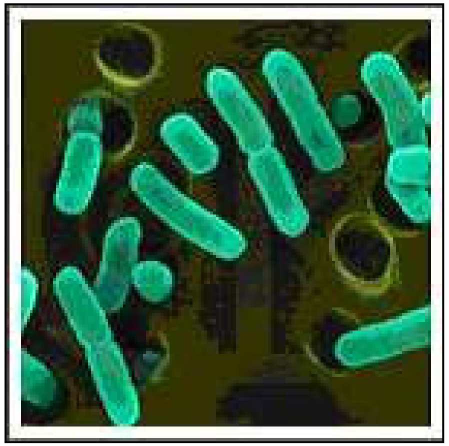
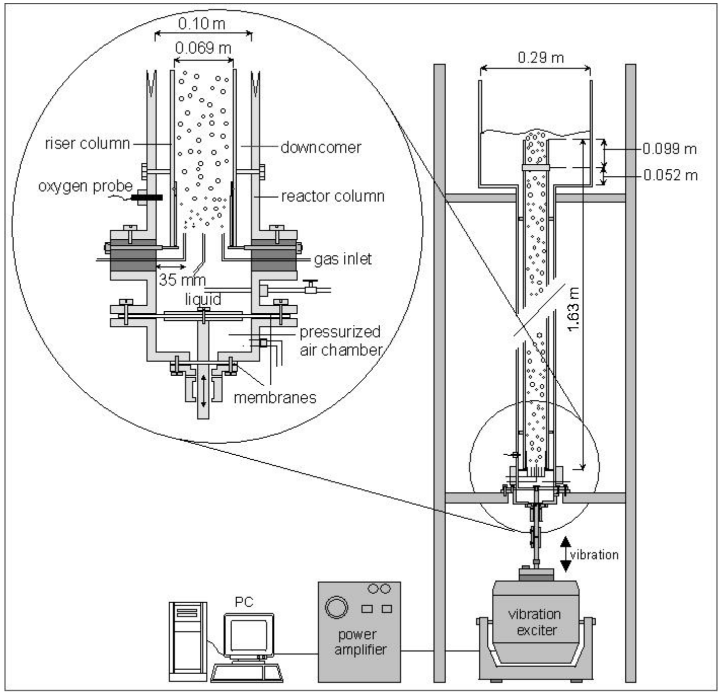
Einführung in die Bioverfahrenstechnik 1

Grundlagen der Bioverfahrenstechnik





20.und 27.11. 2024

FRANKFURT UNIVERSITY OF APPLIED SCIENCES

Prof. Dr. Axel Blokesch - erreichbar: blokesch@fb2.fra-uas.de
Büro: Gebäude 8, Raum 212 a Tel. z.Z. nicht angeschlossen.

Als Prüfungsausschussvorsitzender auch Ansprechpartner für die Anerkennung von Leistungen aus einem vorhergehenden Studium und Planung eines Auslandsaufenthaltes

Vorlesungen: Donnerstag, den 20.11. und 27.11.2024
von 10:00 bis 11:30 in HC 113 (Hungener Str.)

Exkursion: Montag, den 16.12.2024
Treffpunkt Tor Ost des Industrieparks Höchst um 11:15
Besuch der Lantus-Produktionsanlage (Langzeit-Insulinanalogon) um 12:30

Tasks of the process development:

- choice of a reactor type
- optimization of the process
- scale-up

Possible demands of bioreactors:

- sufficient residence time
- high oxygen transfer (in case of aerobic fermentation), good carbon dioxide transfer and removal of excess oxygen in case of oxygenic photosynthetic organisms, e.g. green algae, cyanobacteria
- in the case of photosynthetic organisms also efficient illumination
- no sedimentation of biomass
- good mix of the compounds
- no accumulation of inhibitors
- small mechanical stress
- compliance with temperature, pH
- prevention of foam formation (→ *mechan*.: foam breaker; *chemic*.: anti-foam)
- Sterilization and simple cleaning (→ “Sterilization in Place”, “Cleaning in Place”)
- flexible application

Basic Differences between Bioreactors

1. **Distribution of cells**

   surface culture, submerged culture

2. **Different forms of reactors**

   stirred tank ~, bubble column ~, air lift reactor

3. **Operational mode**

   batch, fed-batch, continuous

4. **Energy supply**

   nutrient media,

   in case of photosynthetic cellular systems: irradiation with light

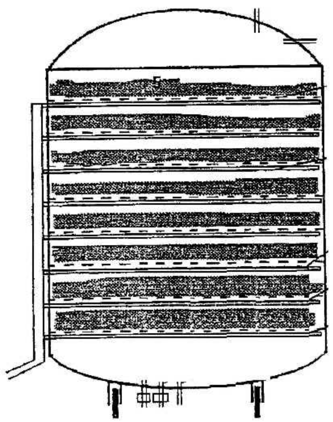
Basic Differences between Bioreactors

1. Distribution of cells

surface culture

- Substrate on trays
- Inoculation with microorganisms (preferably on substrate surface)
- Realization preferably under aerobe conditions

Prophyta, US Patent 6620614



submerged culture

- Equal distribution of micro-organisms within the substrate by stirring or flow
- Oxygen supply (preferably from the bottom of the culture medium
- Realization either under anaerobe and aerobe conditions

Growing Microorganisms

Surface Cultivation **on solid nutrients**	**Submerged Fermentation** **in liquid culture**
on a lab scale:	
• agar plates • slant agar tubes	• shaker flasks
large scale production	
• Solid Surface Fermentation (SSF)	• **S**tirred **T**ank **R**eactor • **A**ir **L**ift **R**eactor

Growing Microorganisms

Surface Cultivation	Submerged Fermentation
on solid nutrients	in liquid culture

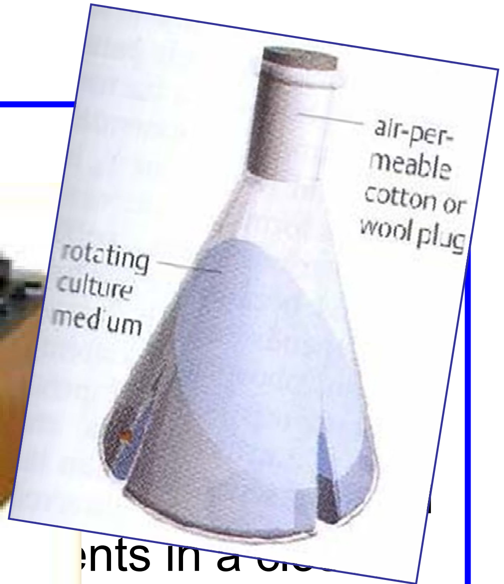
on a lab scale:

- agar plates

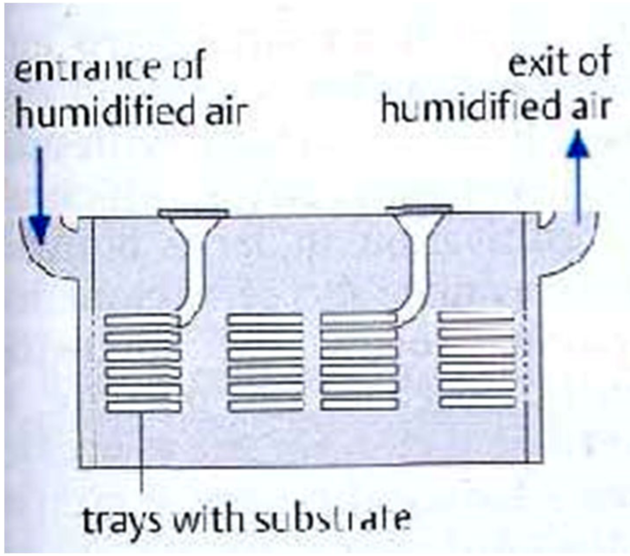
- slant agar tubes

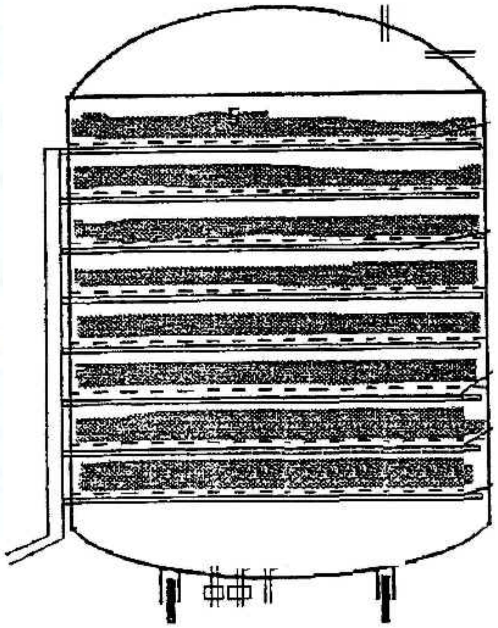
- shaker flasks

G25 New Brunswick Floor Model Incubator



Surface Bioreactor

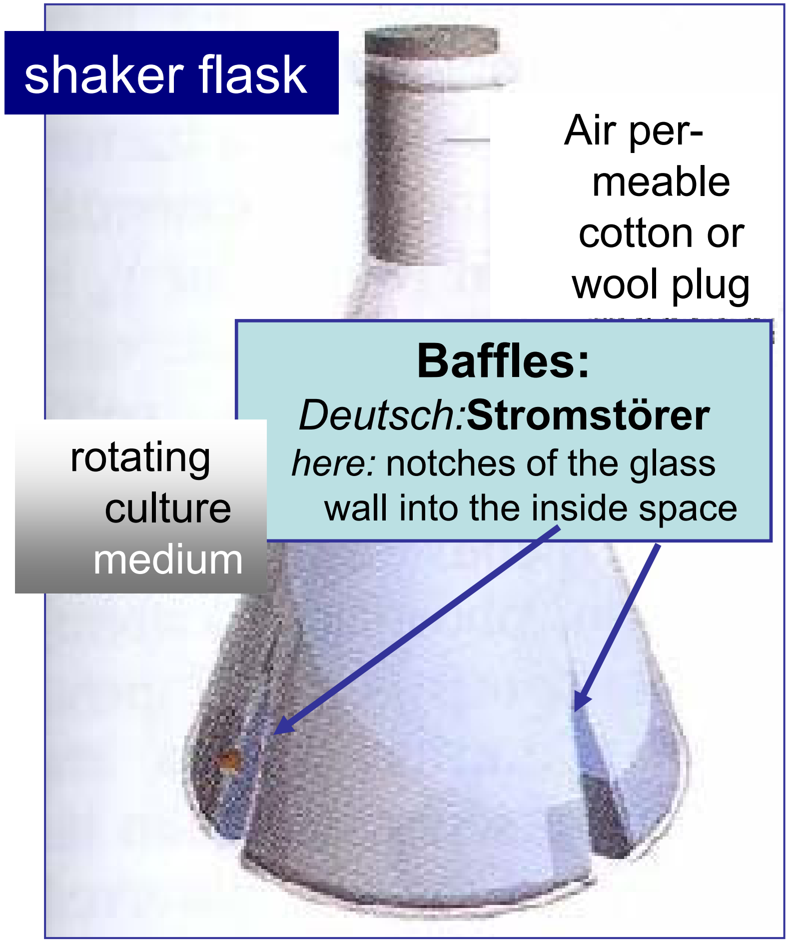




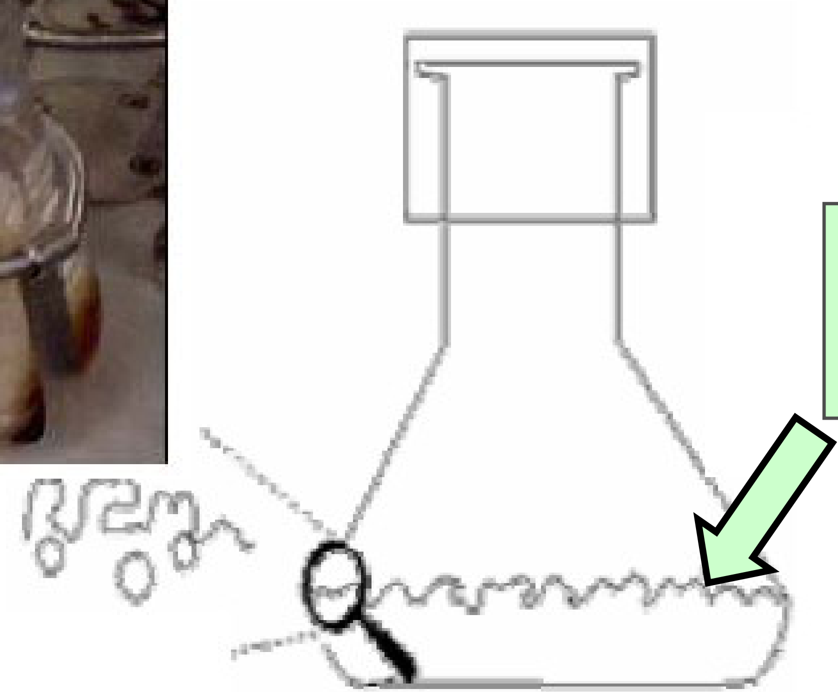
Prophyta, US Patent 6620614

- \+ simple to operate
- \+ space-time-yield rather low
- − often unequal distribution of microorganisms
- applications:
  - production of citric acid with *Aspergillus niger* - *previously*
  - aerobic waste water treatment - *frequently*
  - enzyme production – *occasionally, frequently in SE Asia*

Different forms of bioreactors for submerged fermentation

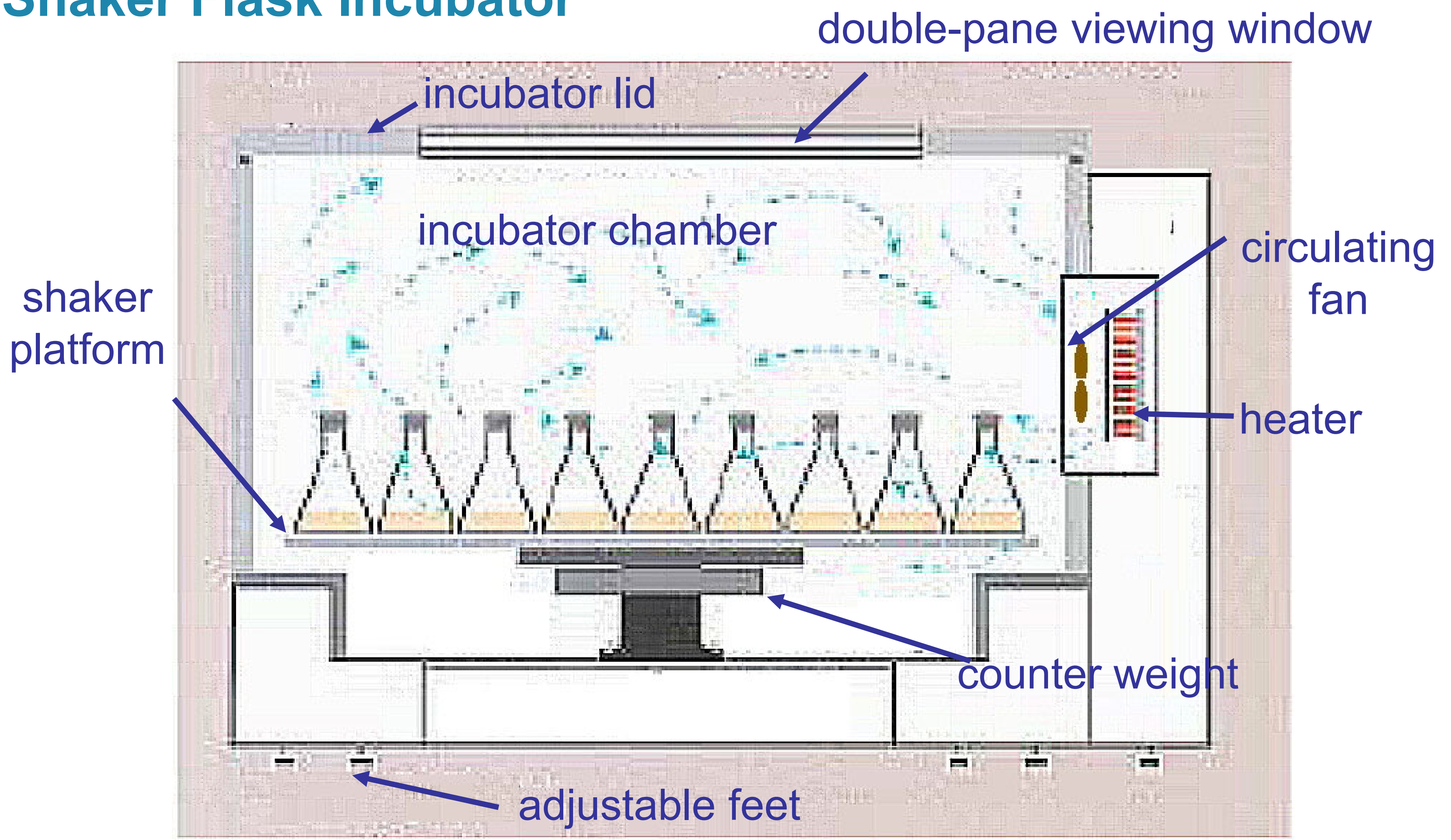


Shaker Flask Incubator



Agitation increases the surface area for oxygen transfer

Shaker Flask Incubator



Schüttelkultur-Inkubator

Modell von New Brunswick, bis März 2024 im Bio-Labor in Gebäude 7, 2. OG

Bis 2023 wurde im Rahmen von Projekten und Abschlussarbeiten die Grünalge *Chlorella vulgaris* kultiviert.

Submerged Fermentation

Stirred **T**ank **R**eactor

Braun Fermenter at DSM Biopract GmbH

aeration by mechanical agitation

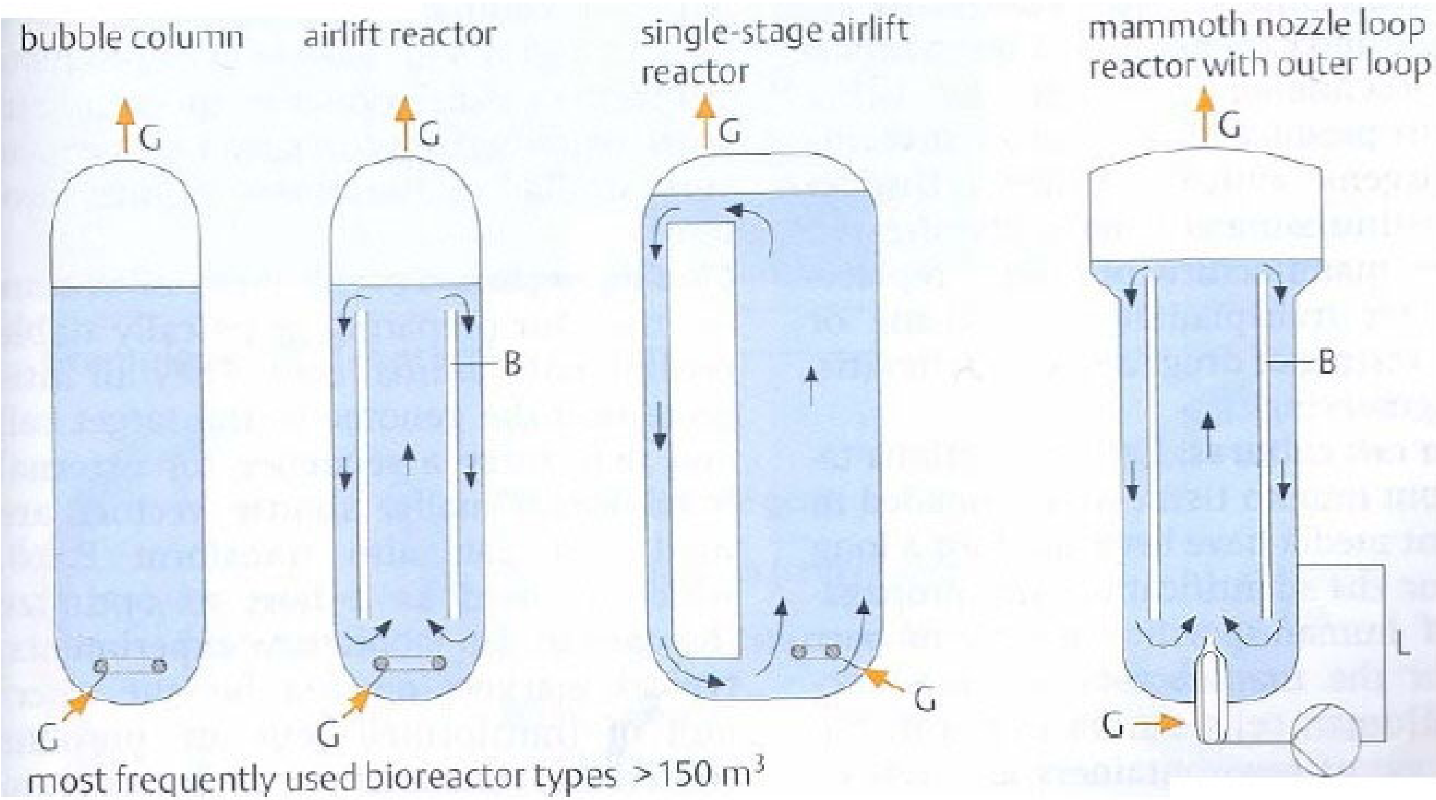
Bubble Column Reactor

bubbling air into media for aeration and for mixing of the reaction media

Submerged Fermentation

Bubble Column Reactor
Deutsch: **Blasensäule**

Air Lift Reactors

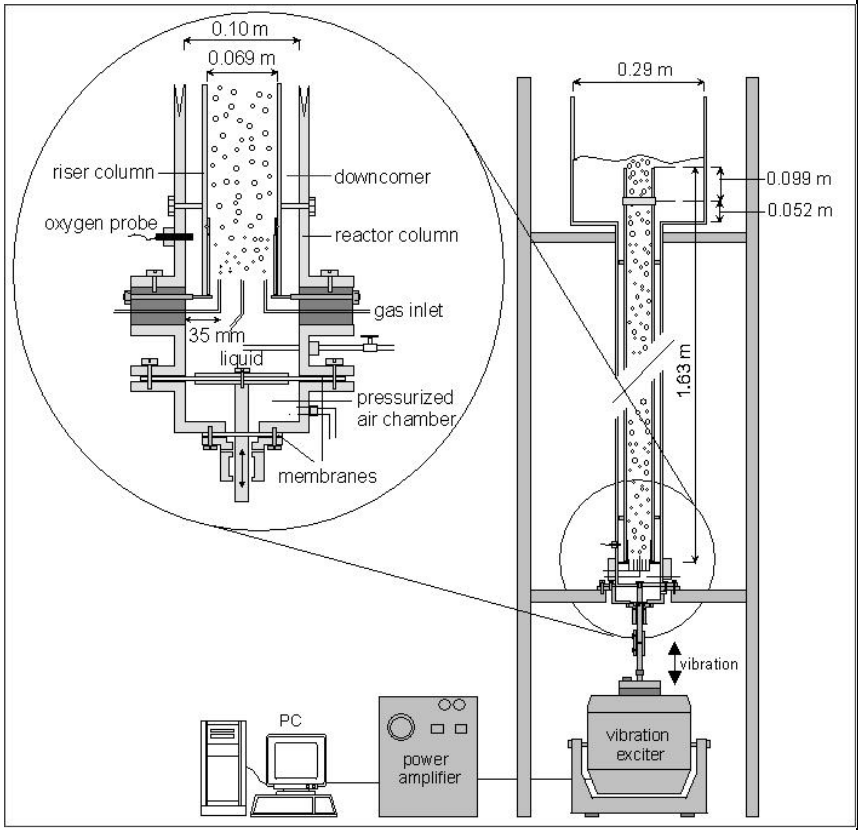


20.und 27.11. 2024

Submerged Fermentation

Air Lift Reactor

Aeration from the bottom → Air cycles back from the top to the bottom through an internal or external loop.



also possible:

Loop reactors combining bubbles rising in a draft tube enhanced with

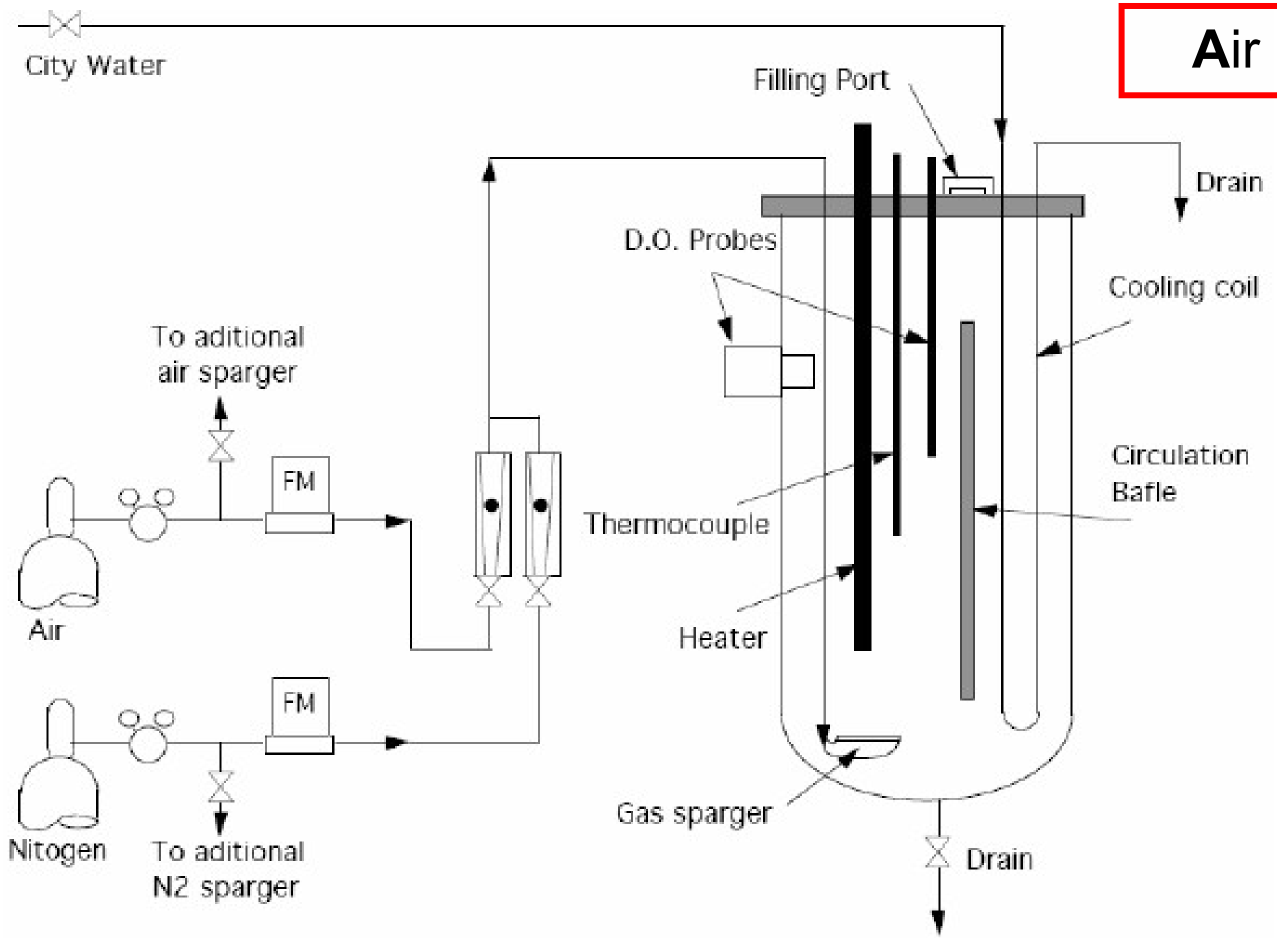
a) stirring by an impeller

b) Circulation of the liquid medium by a pump

Faculteit der Natuurwetenschappeen, Universiteit van Amsterdam
http://www.science.uva.nl/research/cr/AirliftVib/experimental_setup.html

Submerged Fermentation

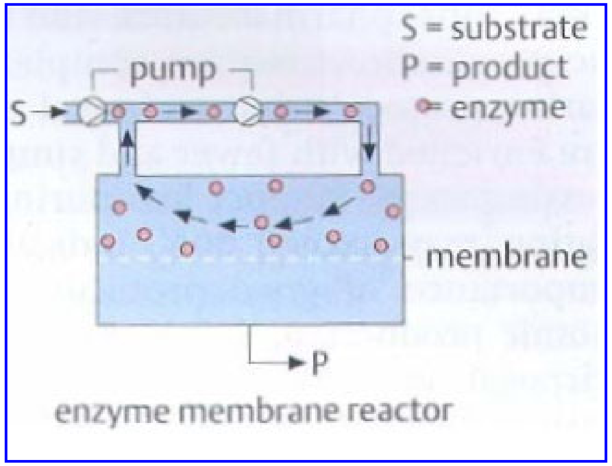
Air Lift Reactor

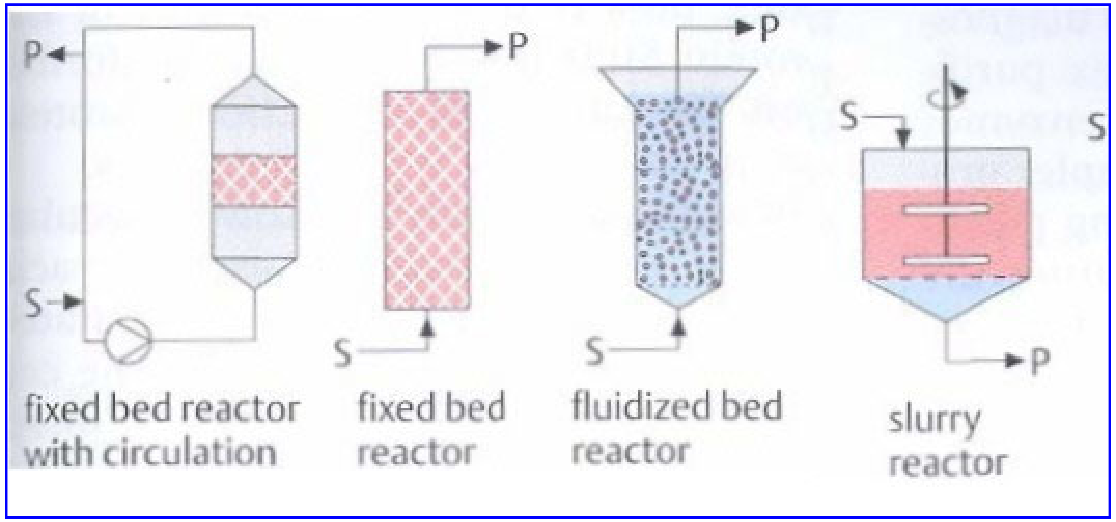


Submerged Fermentation

others

- **membrane bioreactors**
- **bed reactors**



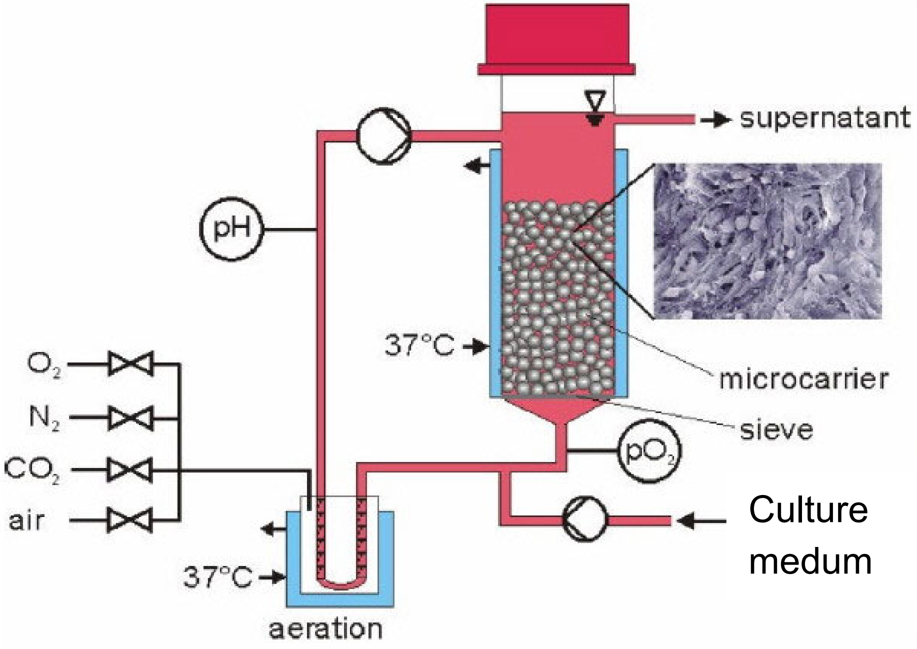


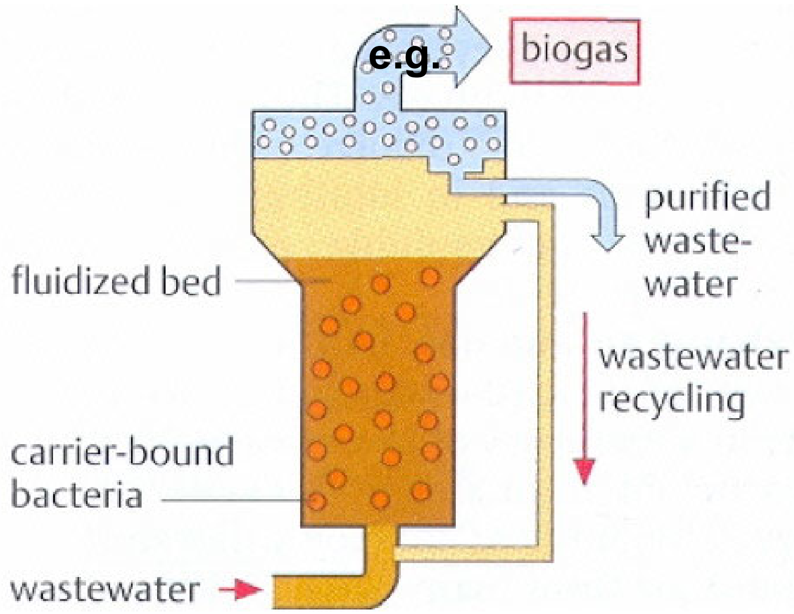
20.und 27.11. 2024

Submerged Fermentation

others

- **fixed bed reactors** **fluidized bed reactors**

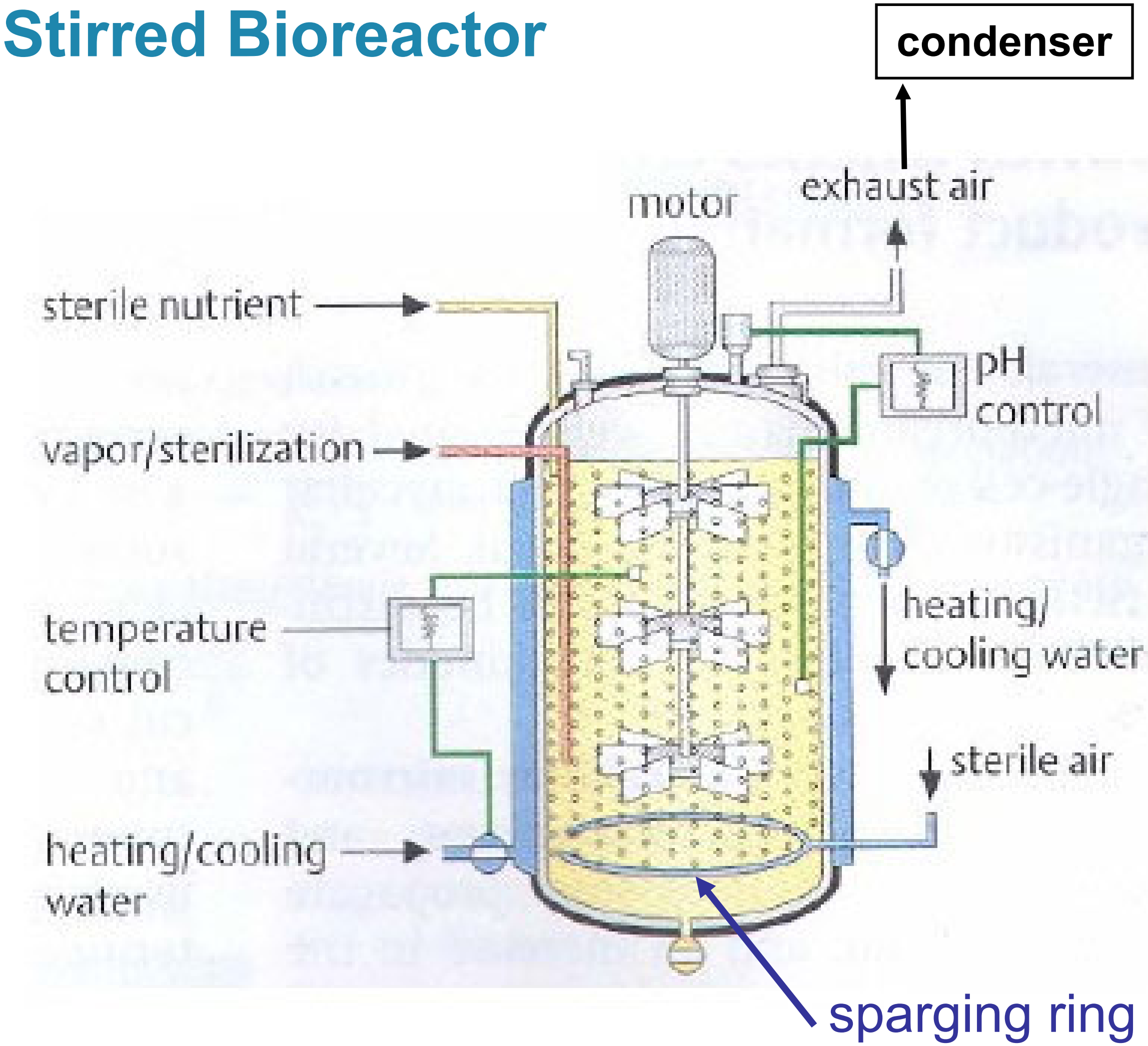




20.und 27.11. 2024

FRANKFURT UNIVERSITY OF APPLIED SCIENCES

Stirred Bioreactor



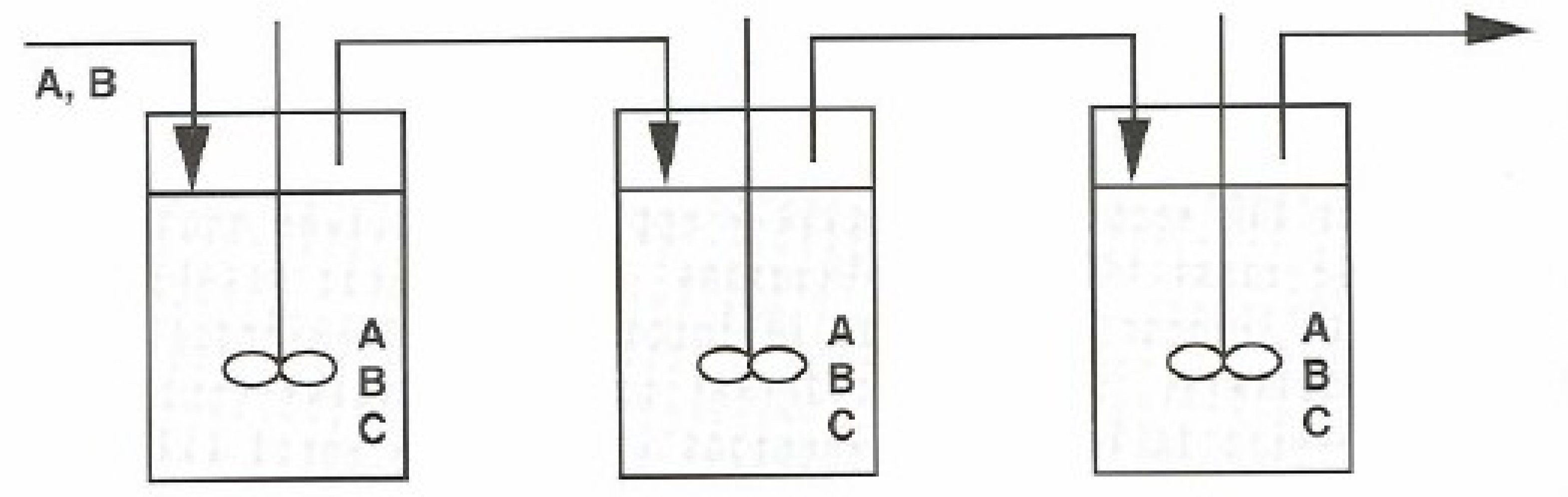
Additional equipment

- feed addition port
- inoculum port
- sampling port
- pressure transducer
- agitator drive shaft bearing and seal
- foam breaker drive shaft bearing and seal

Monitoring

- thermometer / temperature sensors
- pH electrode
- oxygen electrode
- possibly other electrodes/sensors
- foam sensor(s)

Stirred-Tank Reactors in a Series



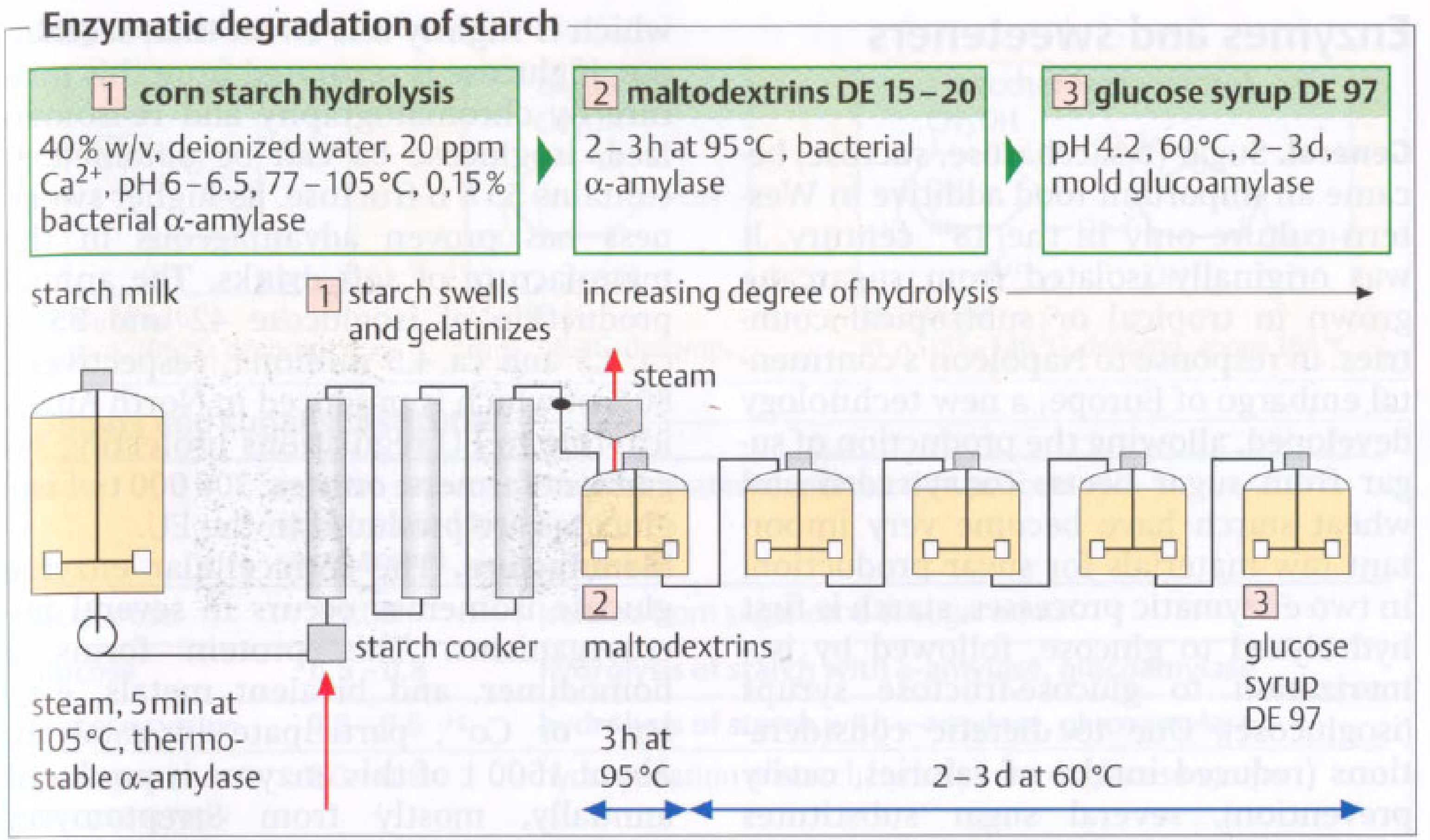
Stirred-tank reactors in series

- **cascade to produce the same product (C) in increasing concentrations (*see figure*)**

  or

- **production of consecutive products, e.g. hydrolyses of starch, a polysaccaride**

Enzymatischer Stärkeabbau in einer Rührkesselkaskade



R.D. Schmid; Pocket Guide to Biotechnology and Genetic Engineering, Wiley-VCH (2003); page 83

Batch reactor with a single external cooling jacket

Kühlmantel, besser Temperiermantel, *Heat transfer jacket* (bei Kultivierung von Säugerzellen dient der Mantel zum Temperieren auf 36-37 °C, beim Sterilisieren auf Temperaturen über 100 °C

- **outer jacket surrounding the vessel**
- **injection of heat transfer fluid at high velocity through nozzles, flow around the jacket**
- **Temperature in the vessel is regulated by changing the temperature of heat transfer fluid.**

Bildquelle: Wikipedia, Creative Commons

Batch reactor with half coil jacket

Reaktor für Satzbetrieb mit Halbrohrschlange (zum Temperieren)

- made by welding a half pipe around the outside of the vessel
- → semi circular flow channel, heat transfer fluid passes in a plug-flow fashion
- for larger reactors: possibility to use several coils
- like in the case of the single external cooling jacket: regulation of vessel temperature by changing the temperature of heat transfer fluid

Bildquelle: Wikipedia, Creative Commons

Batch reactor with constant flux jacket (COFLUX)

A recent development

- **series of >20 small jacket element**
- **temperature control by opening/closing valves to these jacket elements**
- **Temperature of the heat transfer fluid is kept constant.**

Bildquelle: Wikipedia, Creative Commons

Measurement and Control in Bioreactor Technology

Physical parameters	Chemical parameters	Biological parameters
• temperature	• pH value	• enzyme activity
• pressure	• dissolved O_2	• ATP content
• power input	• O_2 and CO_2 in exhaust gas	• NADH content
• viscosity	• redox potential	• protein content
• flow rate of air	• substrate concentration	
• nutrient feed	• product concentration	
• turbidity	• concentration of ions	
• bioreactor weight		

	measured parameters	regulated parameters set values
Deutsch:	Messgrößen	Stellgrößen

On-line monitoring

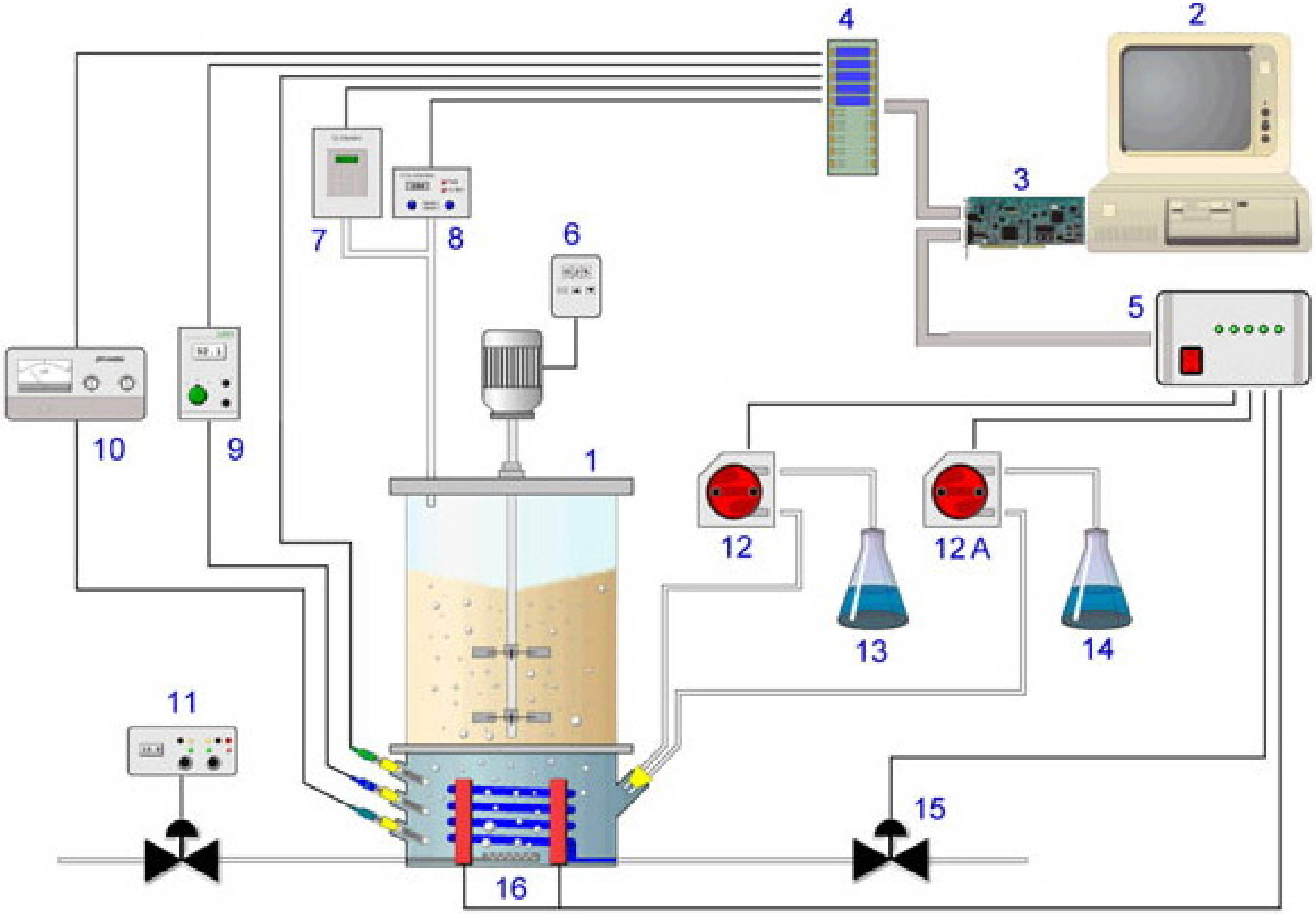
Fermentations require constant control and adjustment:

- nutrient concentrations
- bacteria concentrations
- pH
- temperature
- contaminant concentrations (inlet and outlet)
- vapour emissions
- removal of solids

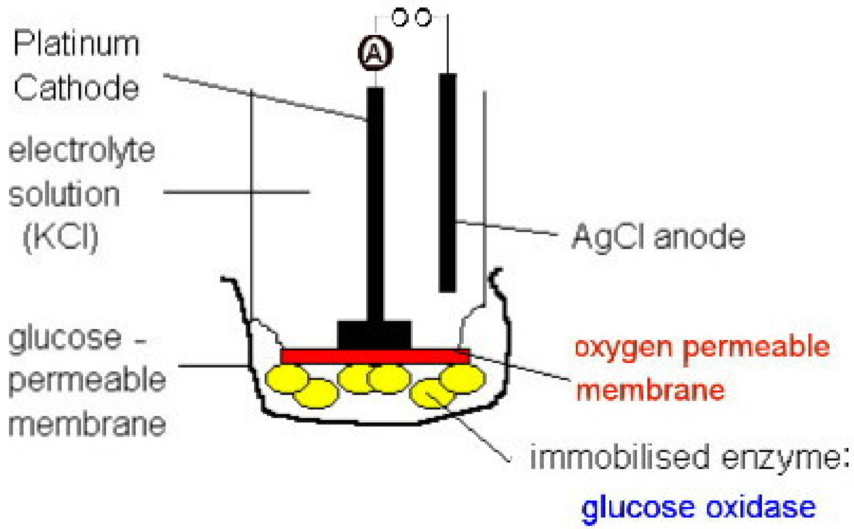
➢ **in case of aerobic fermentation:**

- oxygen levels & transfer efficiency

On-line monitoring



On-line monitoring of Substrate Concentration



example: Glucose Sensor
by Amperometric Detection:

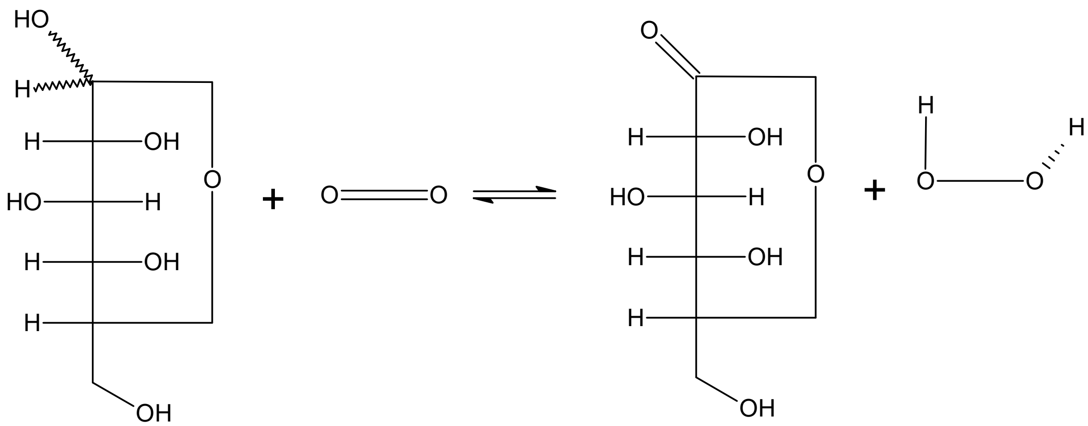
either oxygene reduction at a Pt cathode

→ signal weakened in the presence of glucose

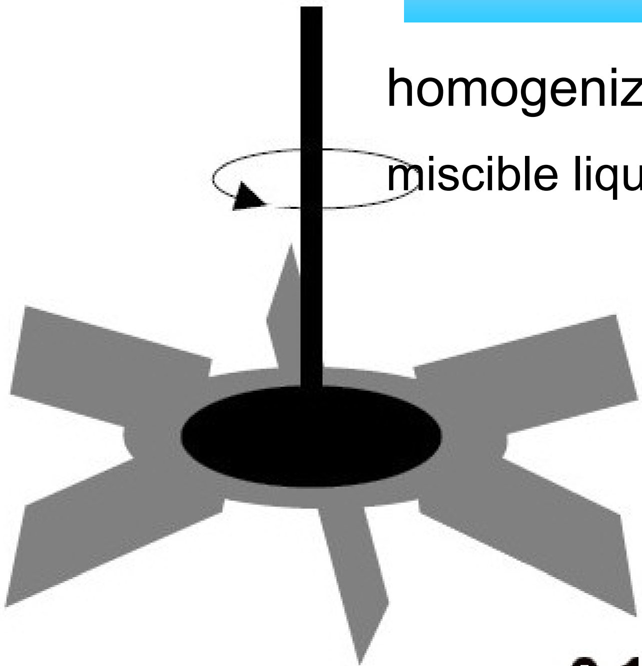
or H_2O_2 oxydation to oxygen at a AgCl anode

→ signal enhanced in the presence of glucose

Teeside University, http://sst-web.tees.ac.uk/external/u0003076/BIOSENSORS_WEB/amperometric/clarketc.htm, mod. Blokesch



Purpose of Stirring



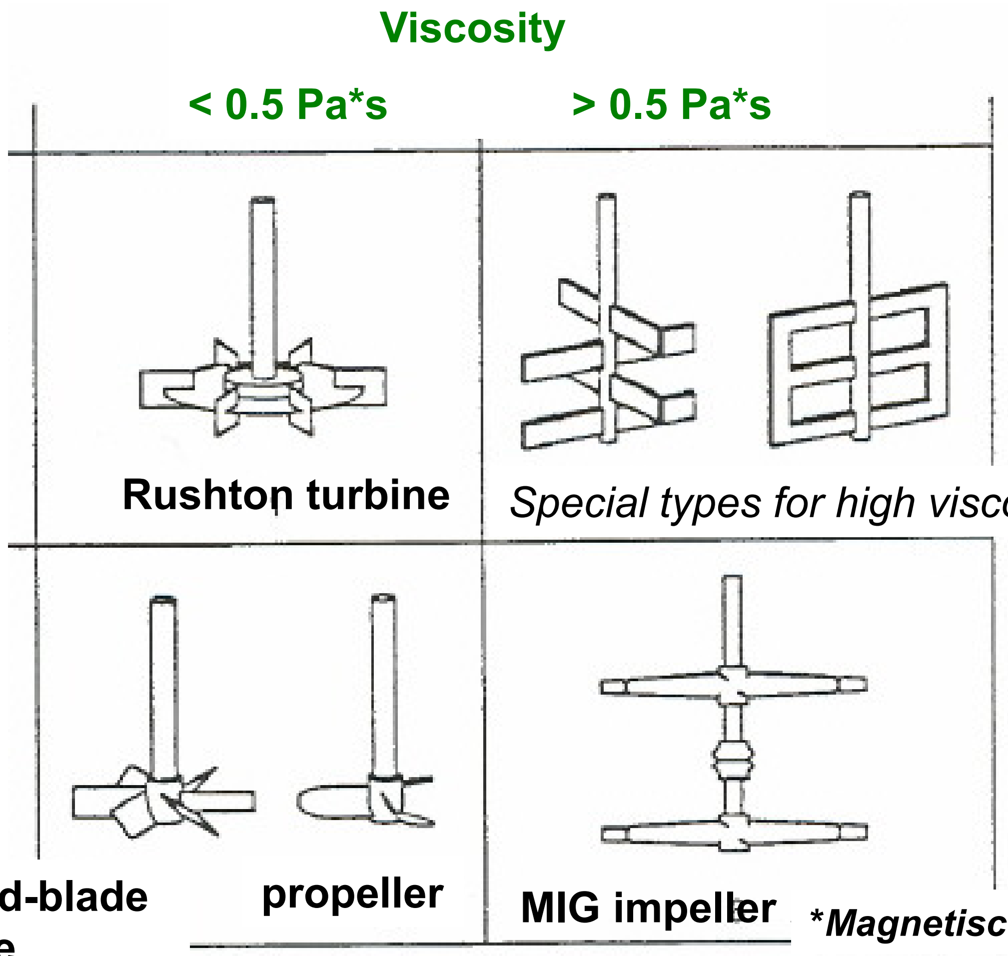
heat transfer

Mass transfer

homogenize	suspend	disperse
miscible liquids	solids in liquid	e.g. gas in liquid: O_2 for **aerobic** microorganisms CO_2 for microalgae solids in liquid
	larger particles	smaller particles

...0.1...............0.5...................5.0...kW/m^3

Types of Common Impellers



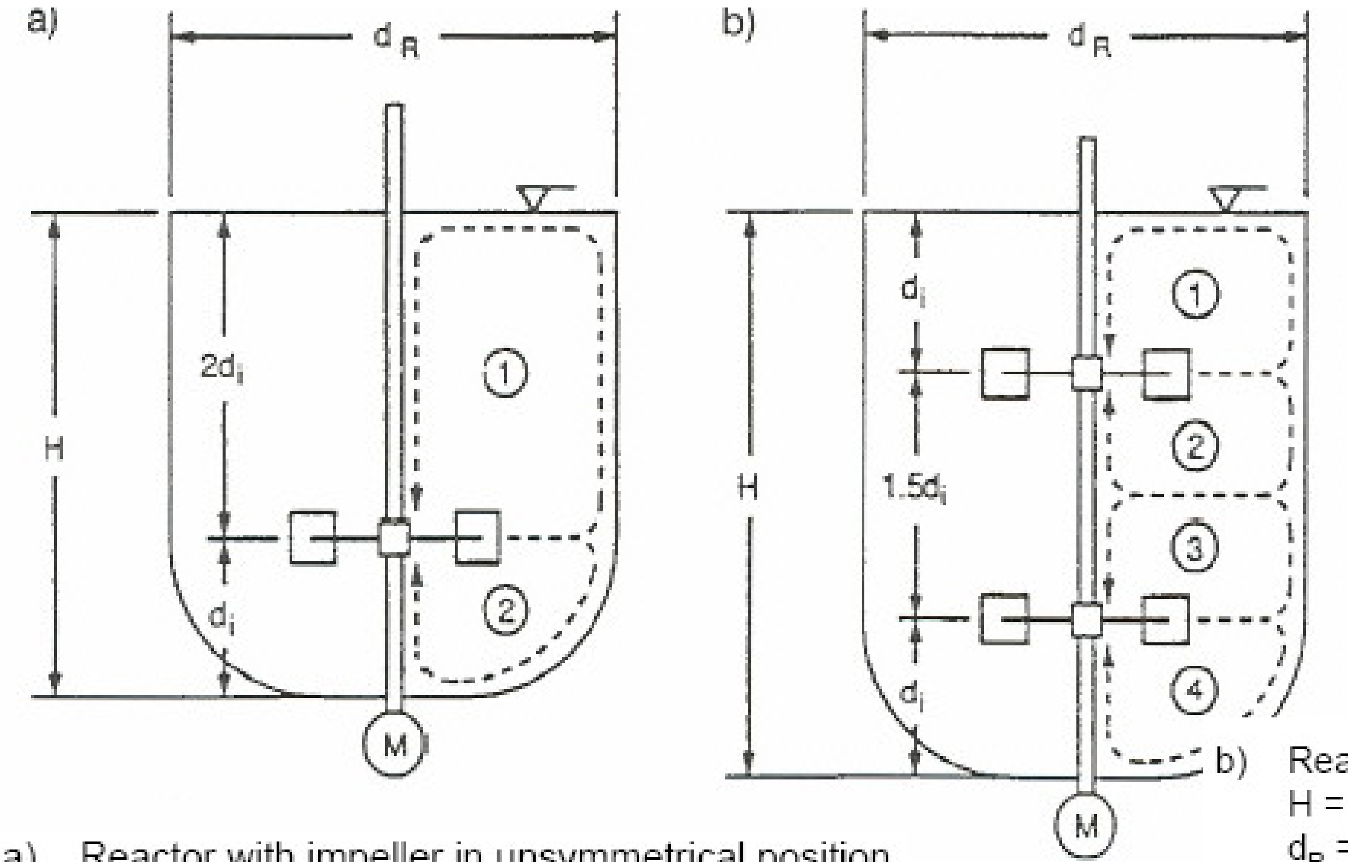
Fuid flow:

radial to tangential

axial

***Magnetischer Impulsgeber** rotary encoder

Flow pattern with circulation flow in stirred-tank reactors



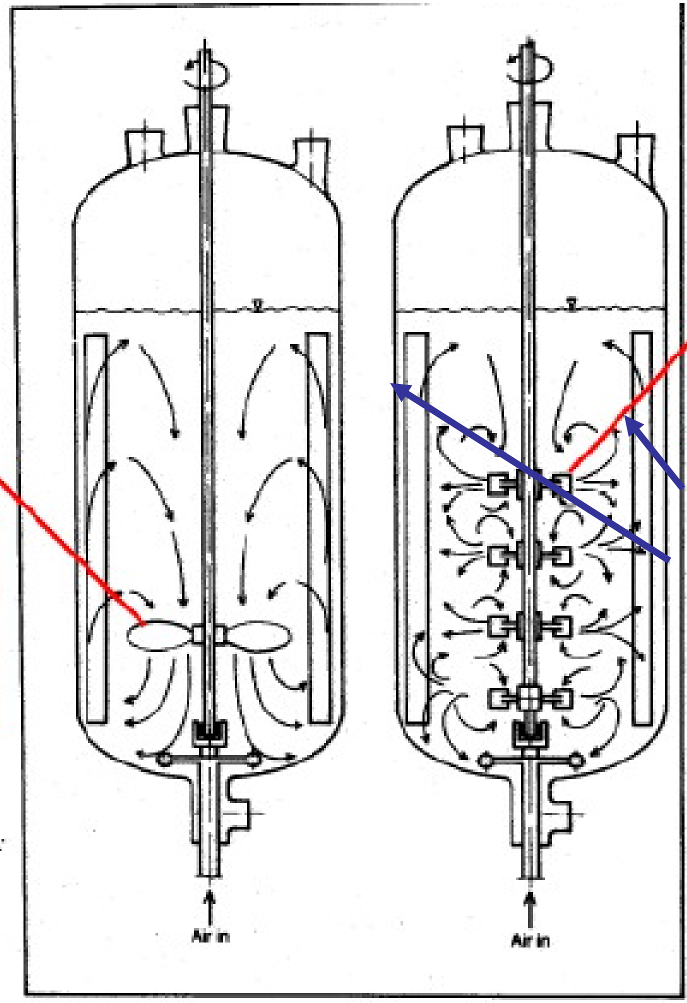
a) Reactor with impeller in unsymmetrical position

b) Reactor with two impellers
H = height
d_R = reactor diameter
d_i = impeller diameter
1 to 4 = circulation pathways

Flow pattern with circulation flow in stirred-tank reactors

Axial flow hydrofoil Impeller

- lower energy demand
- comparable gas transfer
- superior axial mixing
- lower shear stress



Rushton Impeller (Disk-type)

Baffles:
panels inserted to provoke eddies

"Bioprocess Engineering: Basic Concepts" Shuler and Kargi, Prentice Hall, 2002

20.und 27.11. 2024

Power Transferred to the Medium by the Impeller

$$P_R = N_P \, \rho \, n^3 \, d_R{}^5$$

P_R power released, i.e. transferred from impeller to medium

N_P power number

in German: Ne Newton-Zahl, allgemein Verhältnis von Fließwiderstands- zur Trägheitskraft einer Strömung, hier Anteil der Leistung des Rührwerks, die tatsächlich als hydraulische Leistung im Kessel zur Verfügung steht. Eine weitere wichtige Kennzahl ist die Reynoldszahl Re, das Verhältnis von Trägheits- zu Zähigkeitskräften. Im Rührkessel ist bei Re > 10000 der Übergang von laminarer zu turbulenter Strömung.

r medium density

n number of revolutions per time unit (usually minutes)

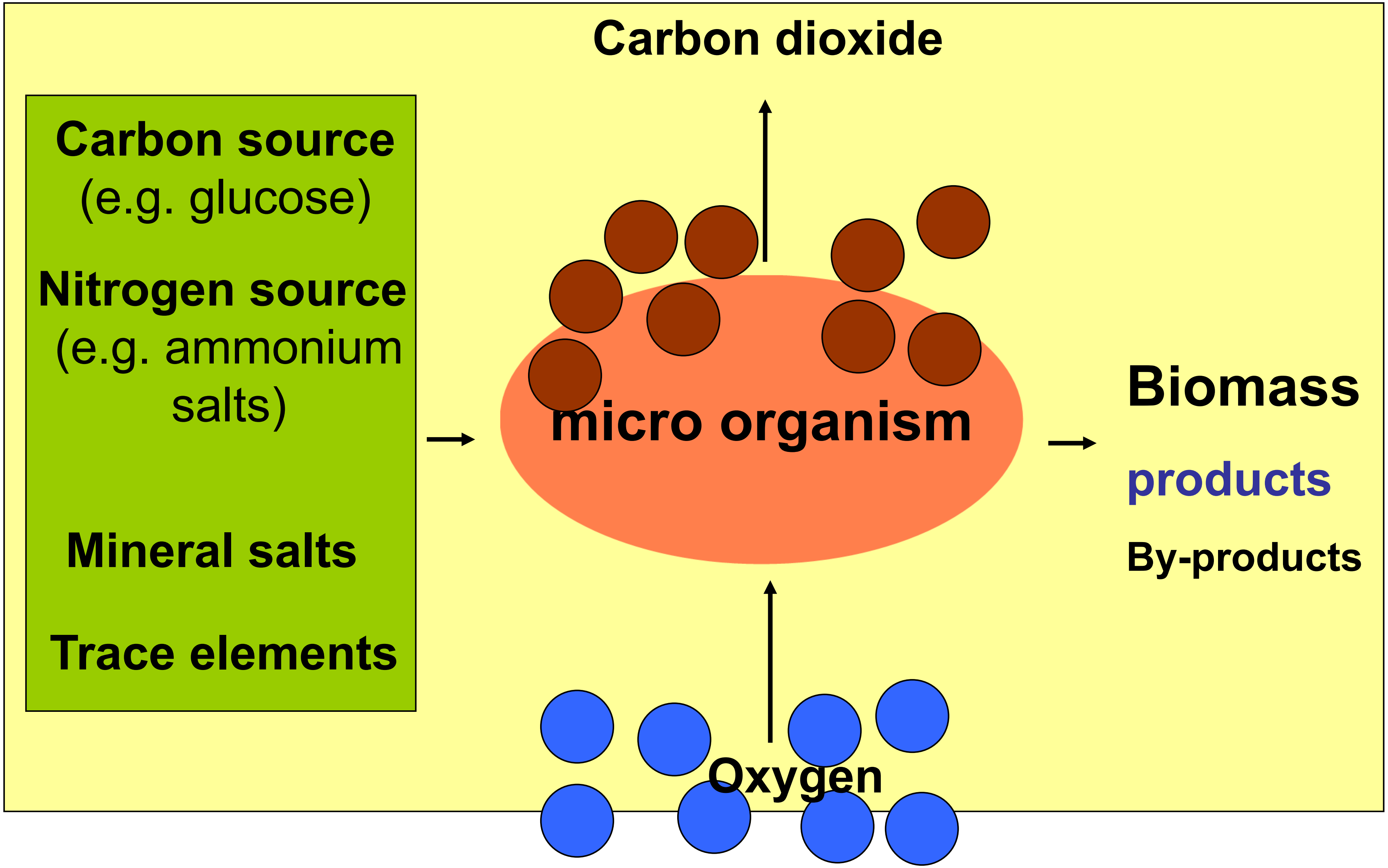
d_R (inner) diameter of the rotor blades

Neither stirring nor pressurized aeration needed:

- traditional anaerobic fermentation processes in food production (e.g. beer, wine, dairy products)
- often large vats (= vessels)
- long-time fermentation, low growth rate

Stirring and/or pressurized aeration:

- most industrial fermentations (aerobic) for the production of biotechnological products


Carbon dioxide
Carbon source
(e.g. glucose)
Nitrogen source
(e.g. ammonium salts)
Mineral salts
Trace elements
micro organism
Biomass
products
By-products
Oxygen

The Respiratory Chain

O_2 g (gaseous)

Lung ↓

O_2 d (dissolved)

Blood ↓

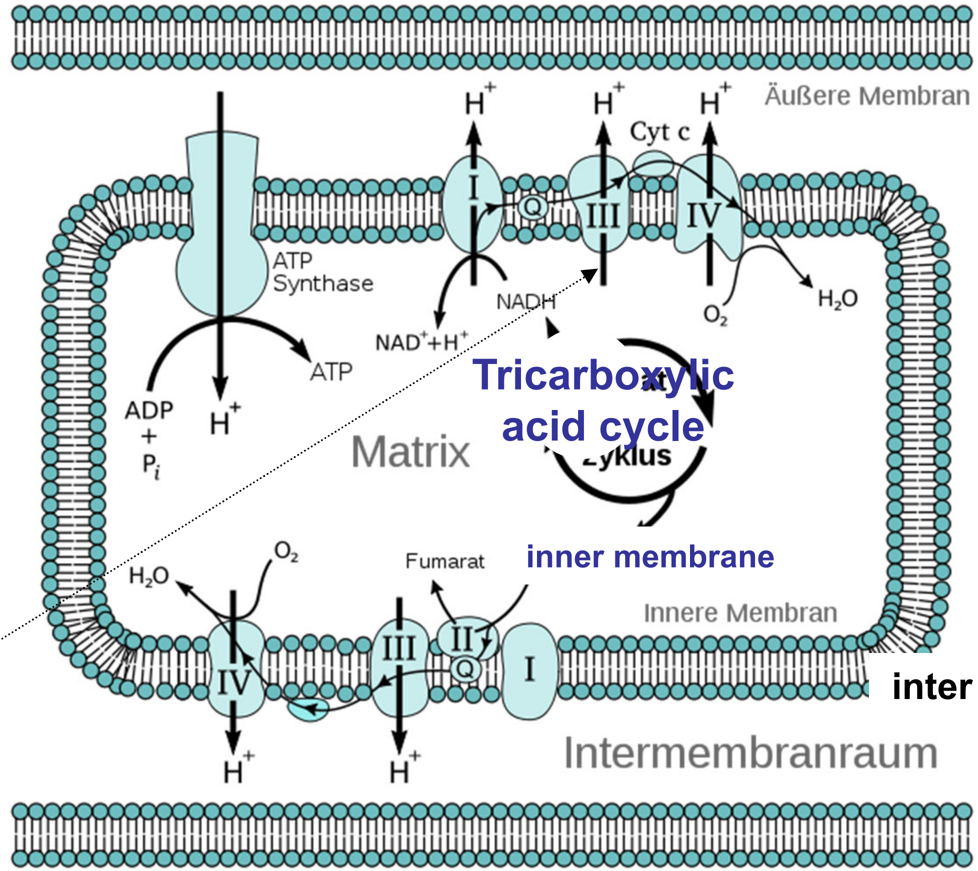
Oxyhemoglobin

tissue ↓

Oxymyoglobin

↓

O_2 d



outer membrane

In eucaryots: inner compartment (cristae) of mitochondria

inter membrane space

Source: Klaus Hoffmeier, Übersetzung des engl. Originals von Fvasconcellos 22:35, 9 September 2007 (UTC)

Critical Oxygen Concentration

Below that concentration, biomass growth and product formation of aerobicly growing microorganisms decreases rapidly due to oxygen limitation.

Organism	C_{crit} [mg/l]	C_{crit} [ml/l]
Escherichia coli	**0.26**	**0.20**
Penicillium chrysogenum	**0.40**	**0.30**
Saccharomyces cerevisiae	**0.60**	**0.46**
Pseudomonas ovalis	1.10	0.84
Torulopsis utilis	2.00	1.52

Crueger, W., Crueger, A.: Biotechnologie – Lehrbuch der angewandten Mikrobiologie, Oldenbourg Verlag GmbH, Munich, Vienna, 1989.

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$$

➡ **stoichiometric molar ratio**

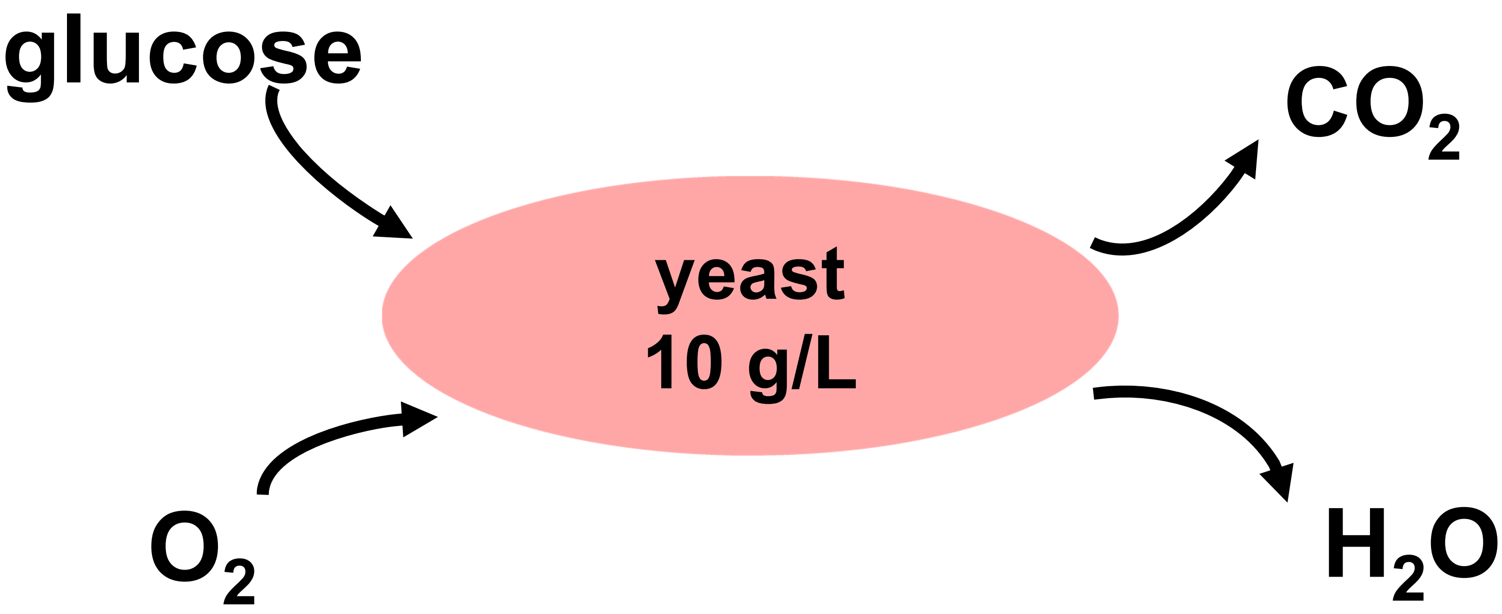
Glucose : Oxygen

1 : 6

real molar ratio in saturated solutions

$$1 : 5 \cdot 10^{-5}$$

Model Fermentation – Necessity of Oxygen Supply

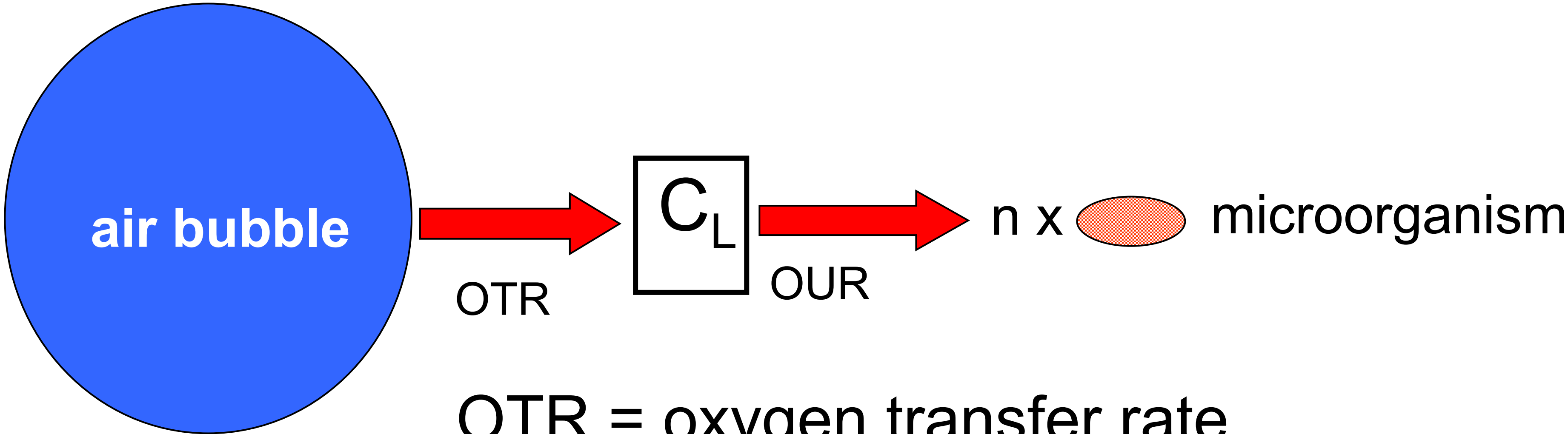


Oxygen demand: 10 ml L^{-1} min^{-1}.

Maximal soluble concentration in medium: 4 ml L^{-1}

Dissolved oxygen expires within 24 seconds

Parameters Influencing O_2 Concentration in the Reactor



OTR = oxygen transfer rate

OUR = oxygen uptake rate

$$\frac{dC_L}{dt} = OTR - OUR$$

Oxygen Supply

Supplying sufficient oxygen represents one of the predominant and most challenging tasks for the operators of bioreactors.

OTR = oxygen transfer rate $\leq$ 3000 mL L^{-1} h^{-1}

gas → liquid medium

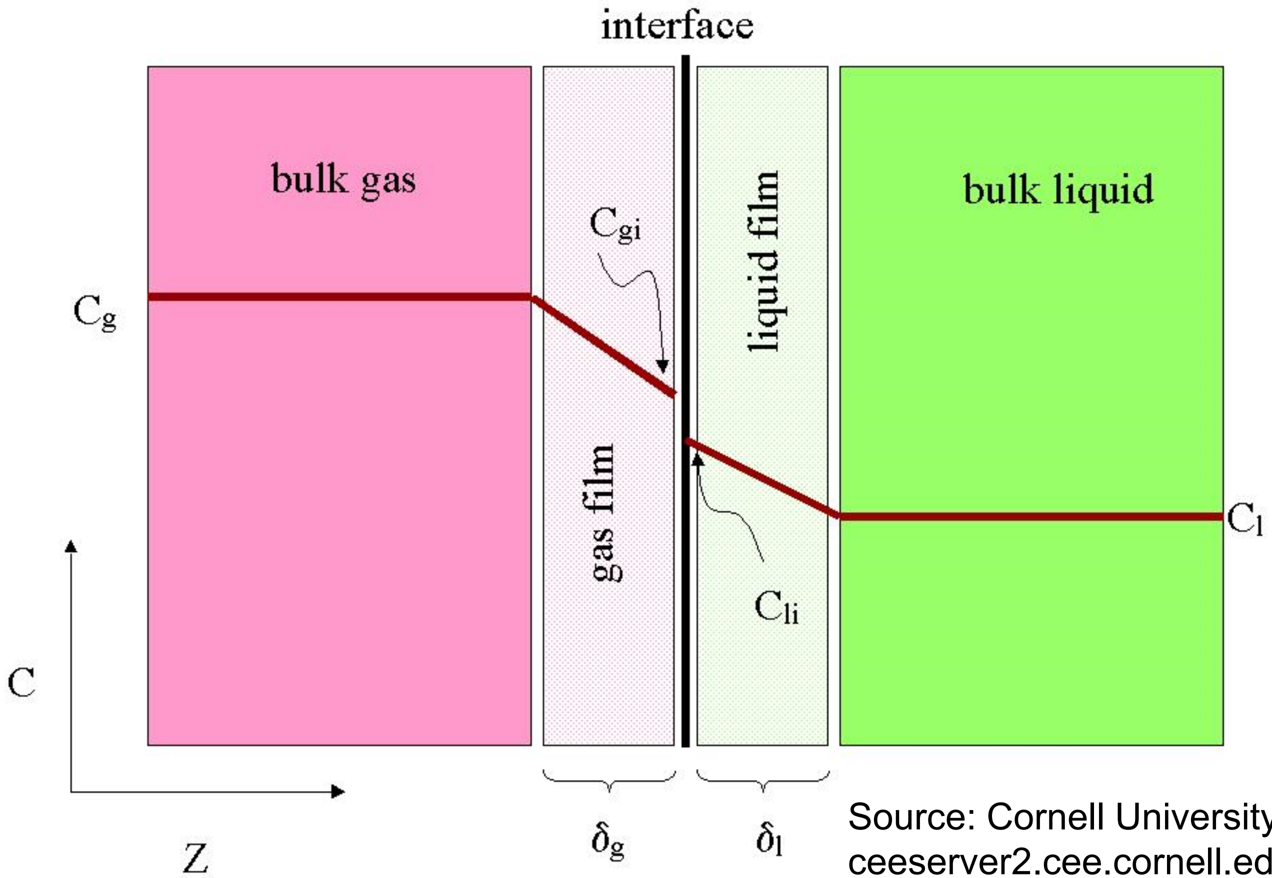
OUR = oxygen uptake rate $\leq$ 4000 mL g^{-1} h^{-1}

liquid medium → microorganism

Please consider that biomasses formed during fermentation are often exceeding 1 g L^{-1}.

Two film model

Developed Lewis-Whitman in 1923 – improving the previous film model proposed by Nernst in 1904 (with just one stagnant film at the interface)



Source: Cornell University, ceeserver2.cee.cornell.edu/jjb2/cee6560/5-gas%20transfer.ppt

Penetration and Surface Renewal Models

More realistic models of the process have been proposed by Higbie (1935, penetration model) and by Danckwerts (1951, surface renewal model).

In these models bulk fluid packets (eddies) work their way to the interface from the bulk solution. While at the interface they attempt to equilibrate with the other phase under non-steady state conditions. No film concepts need be invoked. The concentration profile in each eddy (packet) is determined by the molecular diffusion …

Source: Cornell University,
ceeserver2.cee.cornell.edu/jjb2/cee6560/5-gas%20transfer.ppt

A simplified **Two film model** is used to explain oxygen transfer from the air bubble to the liquid medium because it is the easiest model for calculation.

Since oxygen transport within the gas film (*figure: left side*) is much faster than in the liquid film (*figure: right side*), that is $k_g >> k_l$, only the transport in the liquid film is considered.

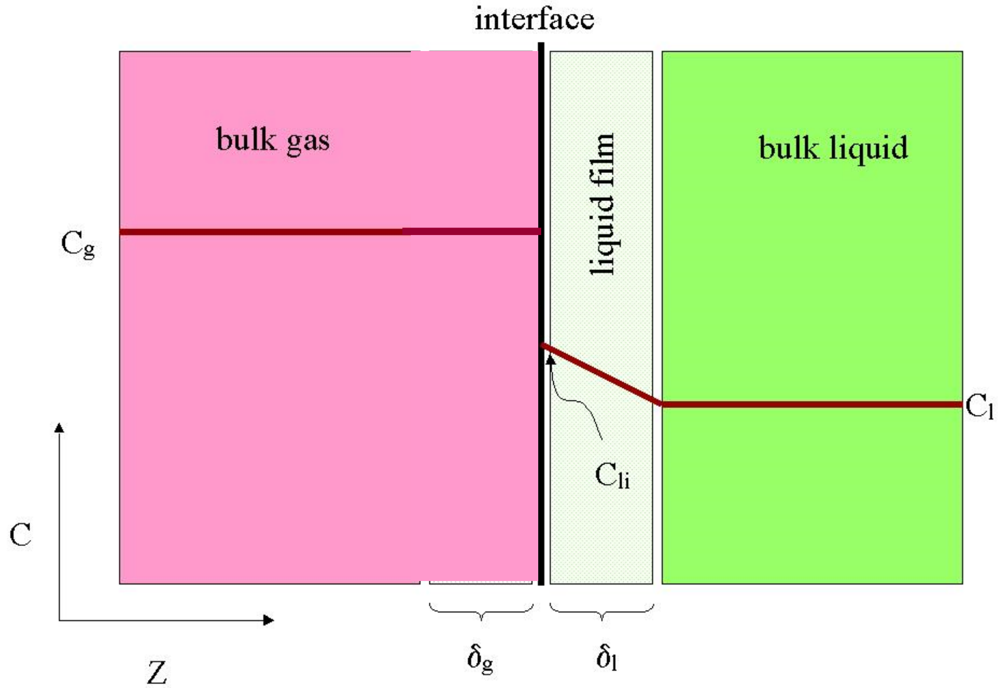


Figure: Cornell University, ceeserver2.cee.cornell.edu/jjb2/cee6560/5-gas%20transfer.ppt

The time for oxygen transport from the liquid medium to the living cell is also neglected.

The equilibrium of the partition of oxygen at the interface of gaseous and liquid phase is expressed by the law of Henry:

$$H * c_{li} = c_{gi}$$

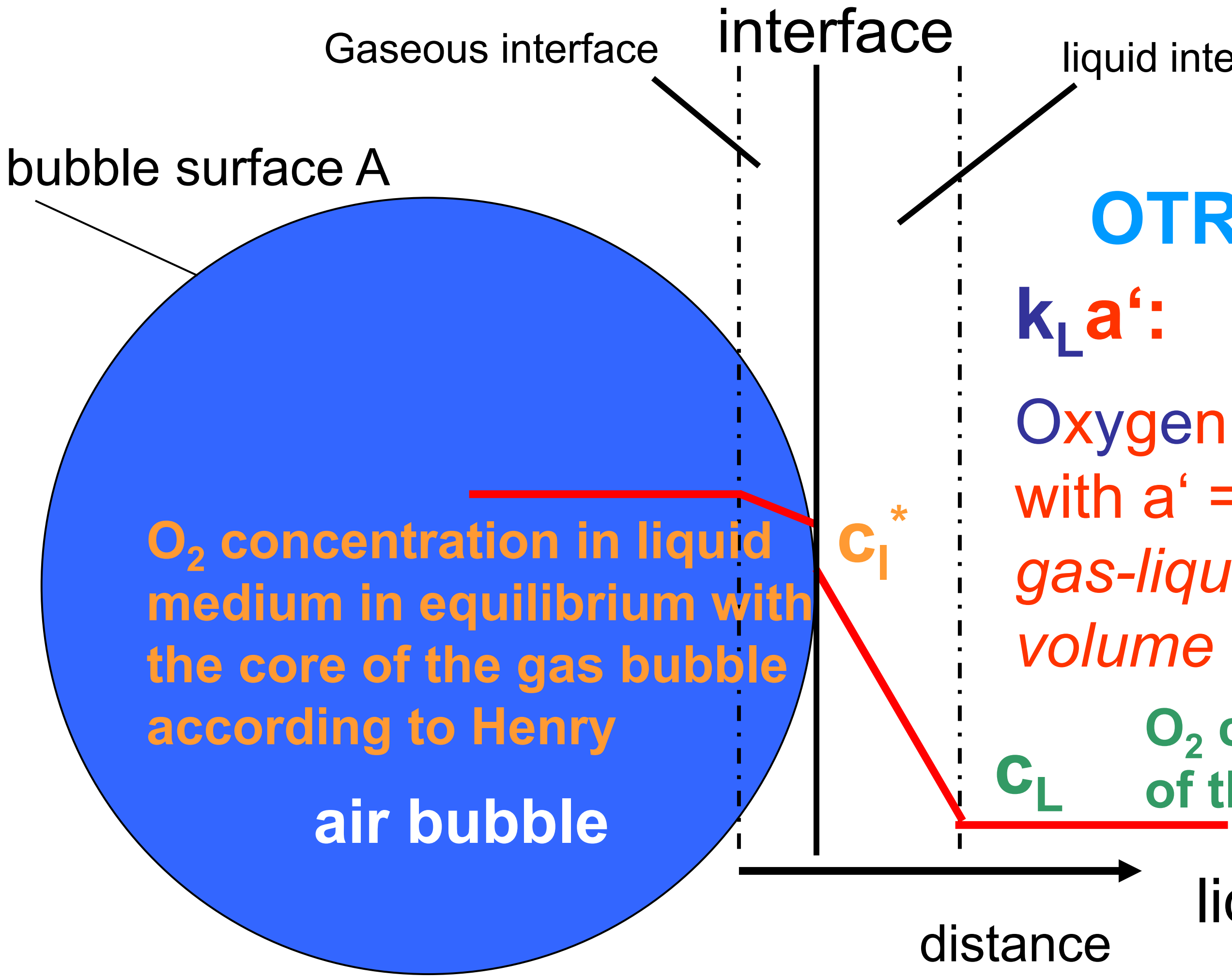
Depends on:
- air pressure
- temperature
- salts and other compounds in the liquid media

	H	c_{li}
p_{air} ↑	=	↑
T ↑	↑	↓
c_{salt} ↑	↑	↓

Oxygen Concentration in water at 20°C and 10^5 Pa air pressure, : $2 * 10^4$ Pa partial pressure of O_2 :

0.275 mmol / l **< 9 mg / l** **< 7 ml / l**

O_2 Concentration Gradient at the Interface of Gas to Liquid

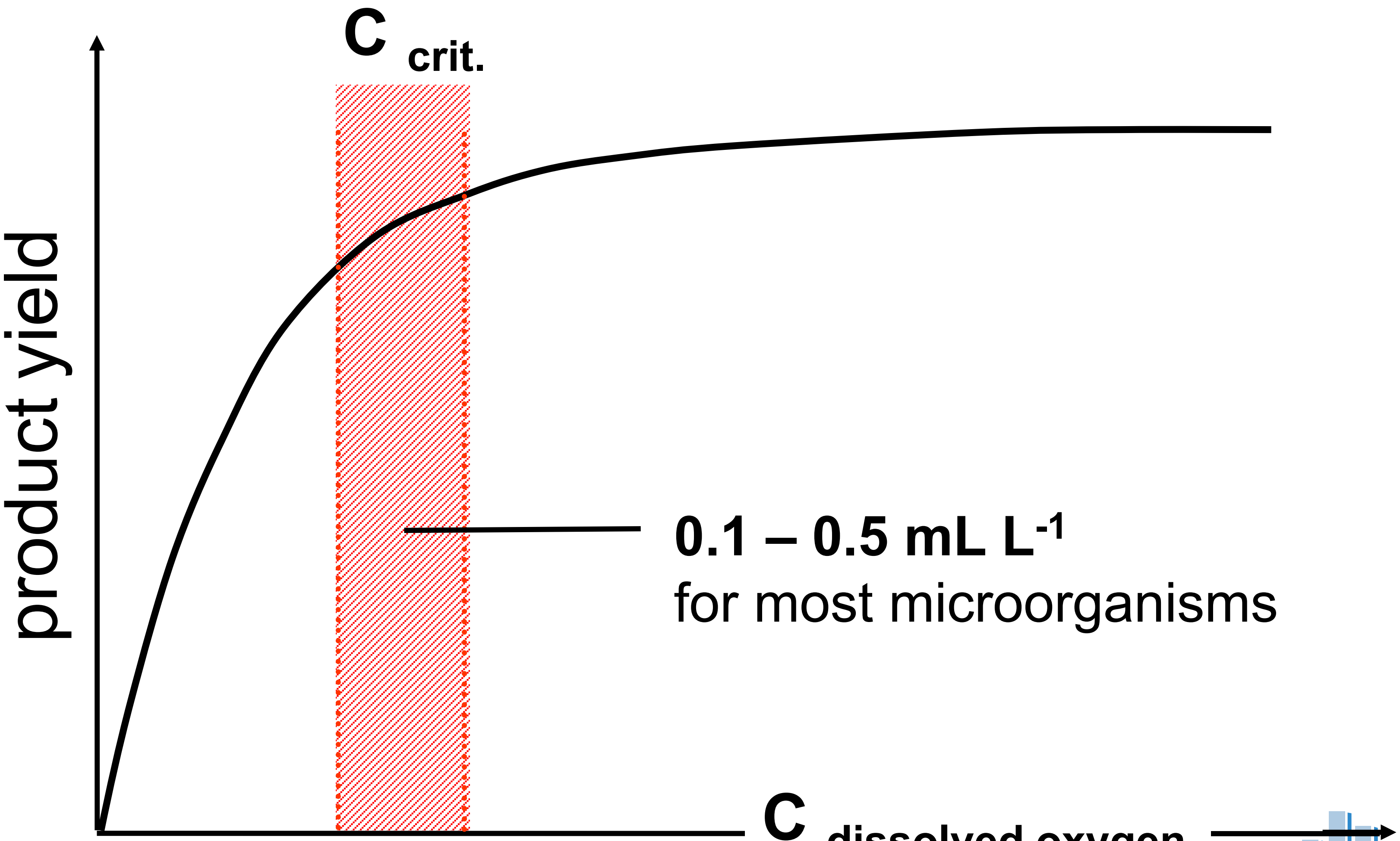


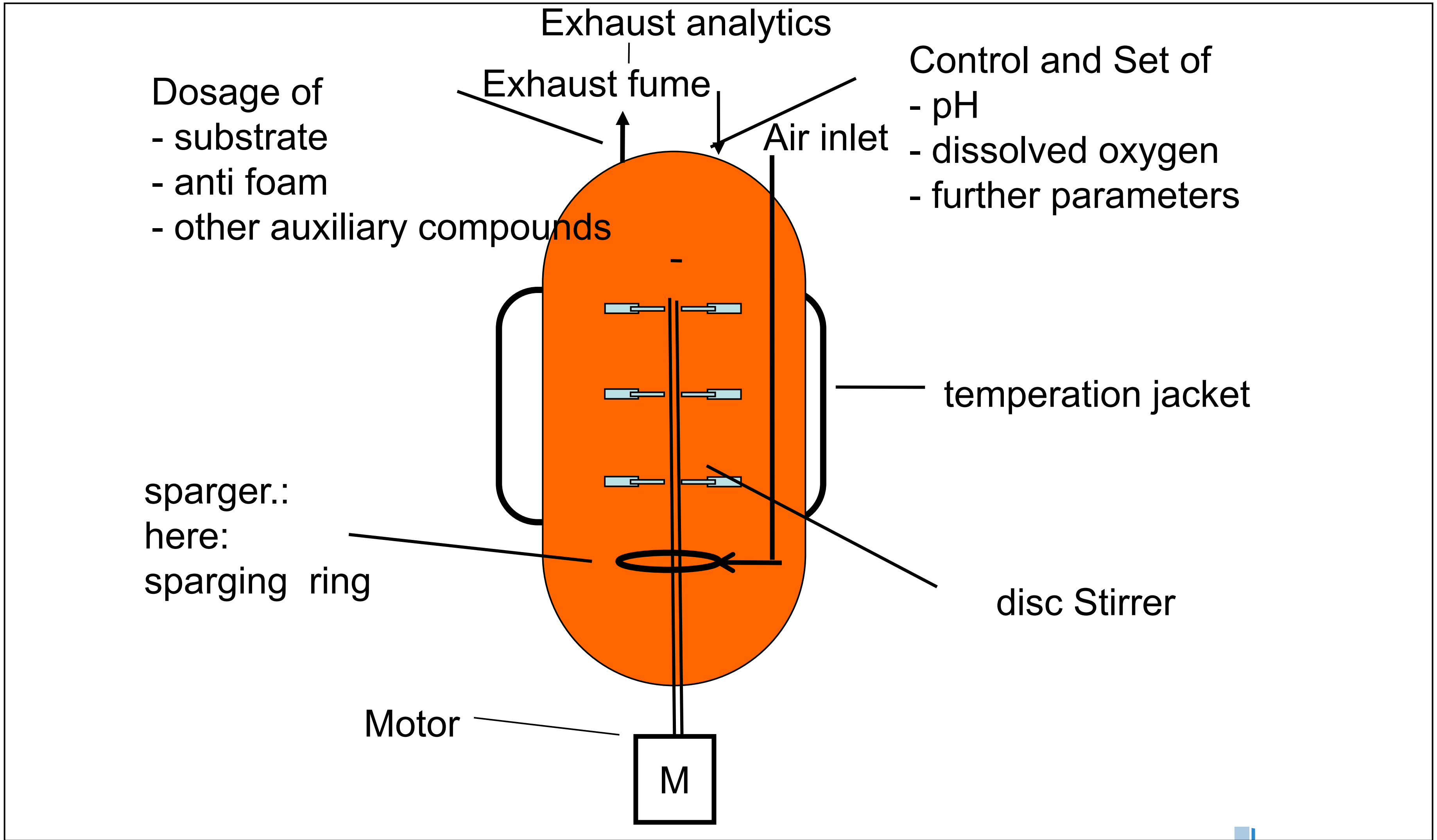
$$OTR = k_l\, a' \,(c_l^* - c_l)$$

$k_L a'$:

Oxygen transport coefficient with $a' = A / V$, *gas-liquid interface A per volume unit V*

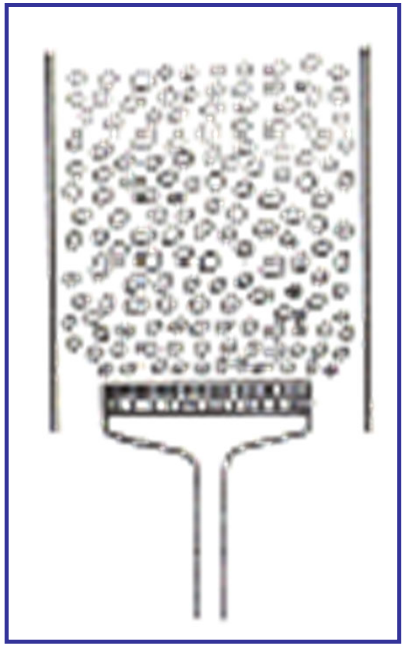
O_2 Concentration and Product Yield



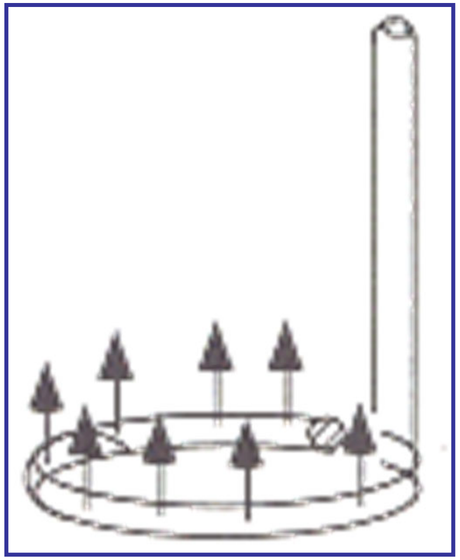

Exhaust analytics
Exhaust fume
Control and Set of
- pH
- dissolved oxygen
- further parameters
Air inlet
Dosage of
- substrate
- anti foam
- other auxiliary compounds
temperation jacket
sparger.:
here:
sparging ring
disc Stirrer
Motor
M

Aeration

- sparger
- sparging frit



- sparging ring



Machine Engineering Faculty of the Dresden University of Technology (TU)
http://mlu.mw.tu-dresden.de/module/m018/Kap8/kap8_2/kap8_2_4/gasverteiler.html
Accessed on 19-4-2010

Aeration

- sparger
- sparging frit
- sparging ring
- combined with stirring

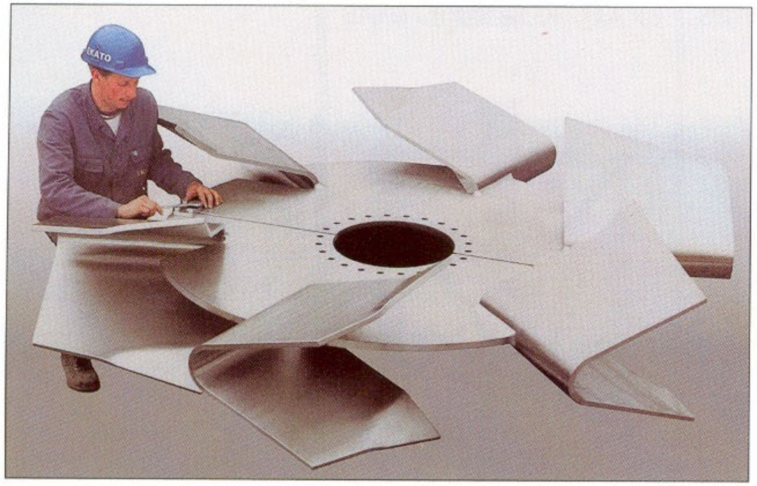


Abb. 9 EKATO PHASEJET

gasjet propeller
EKATO phasejet

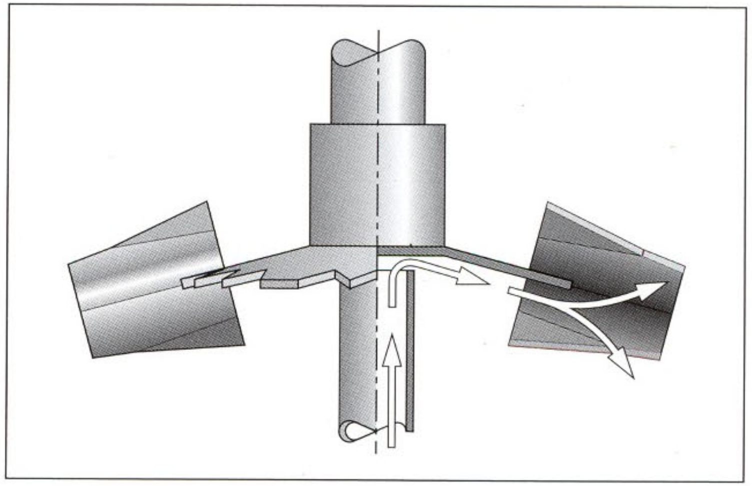


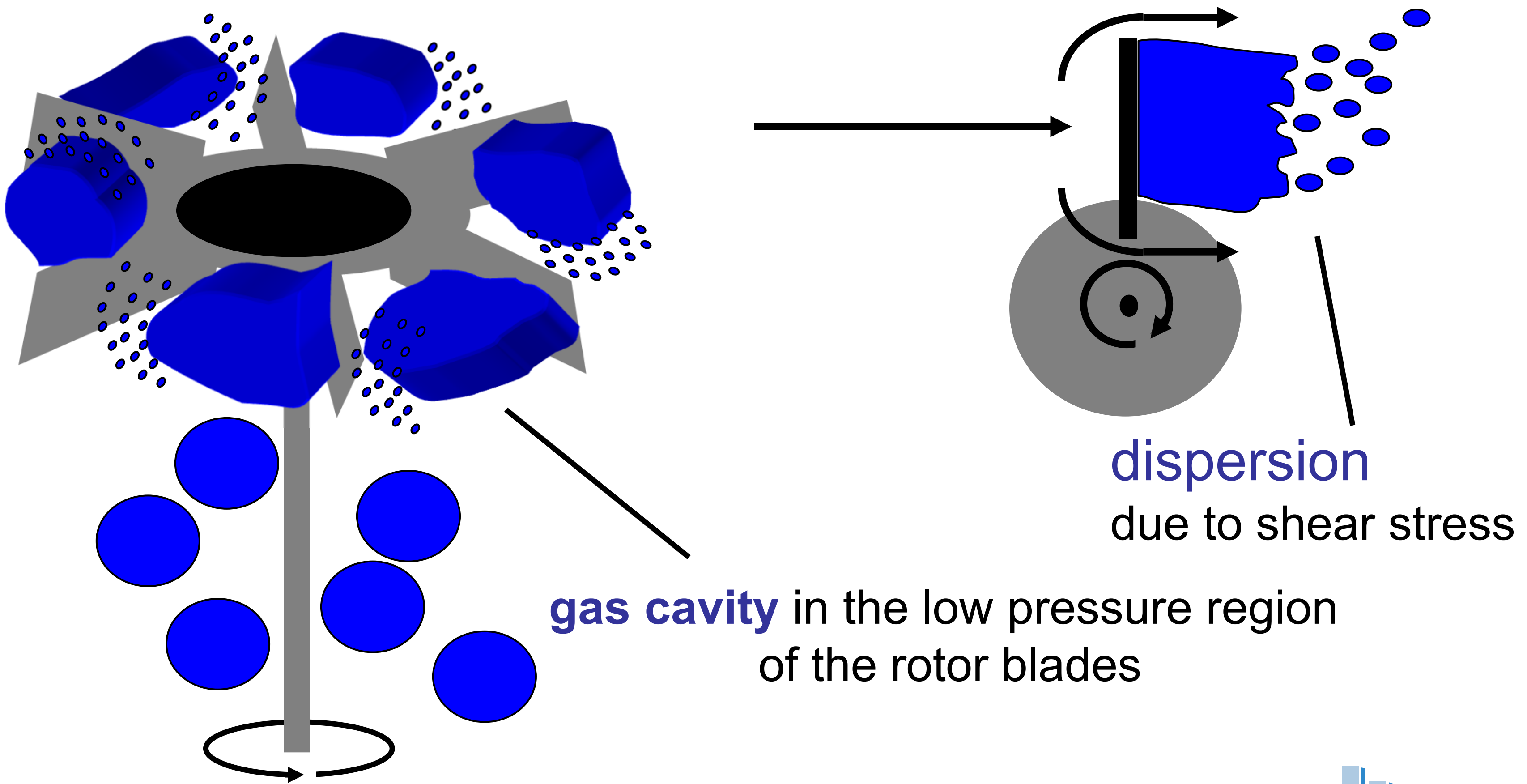
Abb. 10 Schnittbild des rotierenden Gasverteilers

rotating gas sparger

Aeration

Oxygen Transfer Rate (OTR)

Increased by Air Bubble Formation at the Impeller



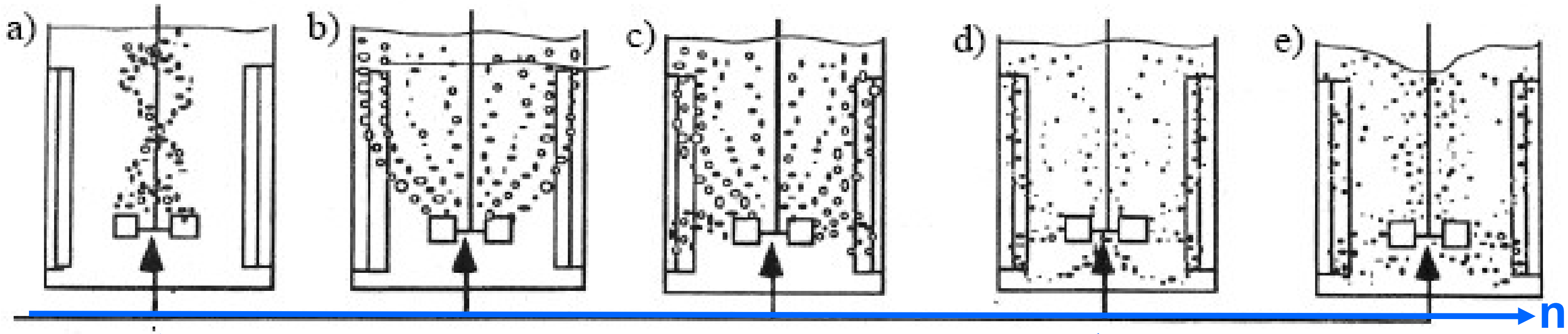
Aeration – *an empirical formula*

$$k_L a \sim (P_{Rg} / V)^{0,7-0,8} \, \mathrm{V_S}^{0,6}$$

P_{Rg}	performance released by impeller in aerated state
V	volume of liquid in the reactor vessel
V_S	speed of gas flow in empty tube

Gassing Modes

with a constant gas flow and increasing stirring speed n



$n < n_f$ | $n > n_f$ | $n >> n_f$ | $n < n_b$ | $n > n_b$

Flooding

Plug Flow

Gas Backmixing **in the lower part**

complete

Suction from the Surface → **Vortex Formation**

Source: Storhas, W.: Bioreaktoren und periphäre Einrichtungen, Vieweg, Braunschweig, Wiesbaden 1994, transl. A. Blokesch

Reactor Geometry and Layout

- Height to diameter ration of ~ 3 : 2
- sterile air inlet and sparger
- impellers and baffle plates
- cooling coils
- foam breakers
- working volume (liquid capacity) □ 0.75 vessel volume)

Preparation of the Fermenter and Fermentation

A) time of preparation

1. cleaning of the reactor
2. preparation of the substrate
3. sterilisation of the substrate (thermic or filter)
4. sterilisation of the reactor (cleaning in place)
5. calibration of the instruments of measurement (pH, pO_2, pCO_2, O_2, CO_2)
6. inoculation (monoculture or defined mixed culture)

B) time of reaction (growth time)

7. fermentation (batch, fed-batch, repeated fed-Batch)
8. aeration
9. mixing (mechanical, pneumatical or hydrodynamical)
10. parameter control (with regulation, control, documentation)
11. sampling (manually or automatically)

C) time of harvest

12. harvest
13. extraction of products
14. cleaning of the reactor

Explanations

baffle	inserted panels close to the reactor vessel wall (in shaker flasks: notches of the glass wall into the inside space) conceived to disturb flow of fluid, provoke turbulence Stromstörer
nozzle	narrow end of a tube (or hose), where fluid or gas jet issues (at high speed) Sprühdüse
sparger	tube for moistening (= sprinkling), especially in brewery → *sparging ring, sparging frit* Begasungsring, Begasungsfritte
eddie, vortex, whirlpool	fast circling motion of fuid or gas Wirbel
plug-flow	flow in a tube in one direction and equal velocity along the tube diameter Pfropfenströmung
hamster	small rodent (same term as in German)