Bioprocesses and Downstream Processing

Transfer phenomena

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Basic mass transfer

The rate of mass transfer of a gas into a liquid can be represented by the basic relationship

Rate = driving force/resistance

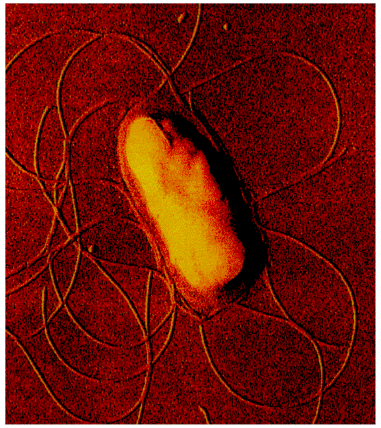
$C_6H_{12}O_6$
C-Source

NH_4^+
N-Source

O_2 in solution

Air

Additives



$CH_{1.6}O_{0.5}N_{0.15}$
Biomass

Products of metabolism,
I-, II-Metabolite

CO_2 in solution

H_2O

Gas

HEAT

Tasks of a bioreactor

Mixing:
Stirrer
Static mixer
Movement guidance (Loops)

Dividing:
Static:
- Ring nozzle
- Hole plate
- Sinter stones

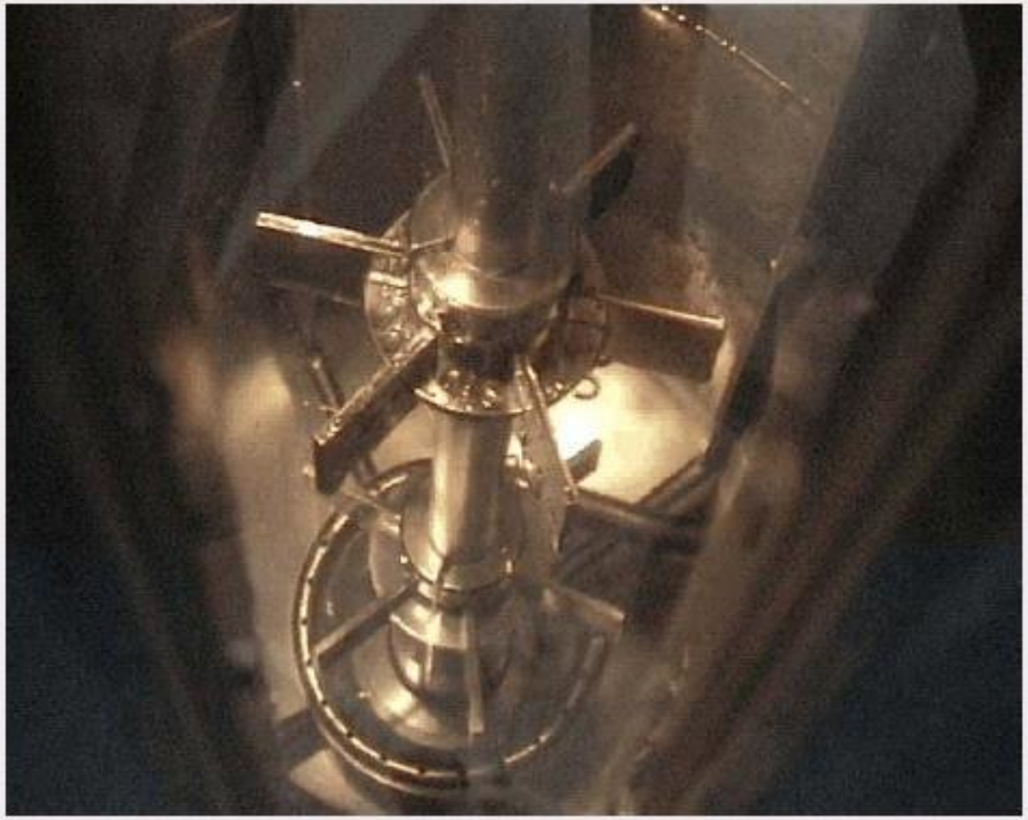
Dynamic:
- Jets
- Stirrer

Heat Transport:
Double coat
Heating / Cooling system
externe heat exchangers

Energy Transport
mechanical
pneumatical
hydrodynamical
Combined

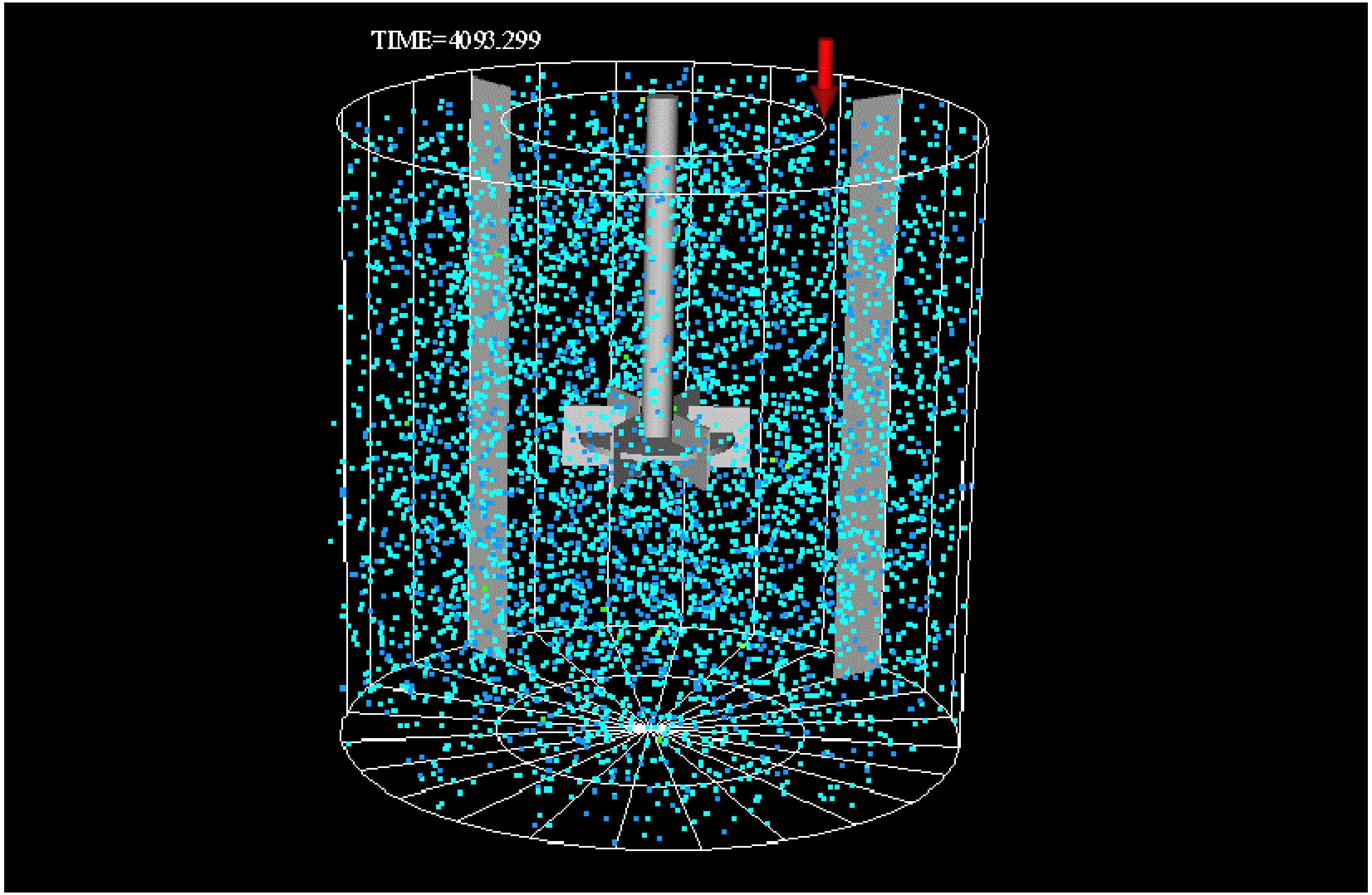
Tasks of a bioreactor

Rühren und Begasen...



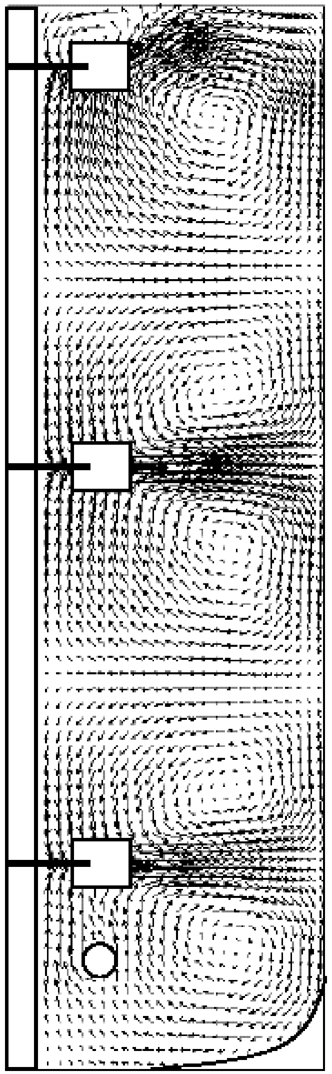
Tasks of a bioreactor

\Vorlesung\Turbulenz.avi



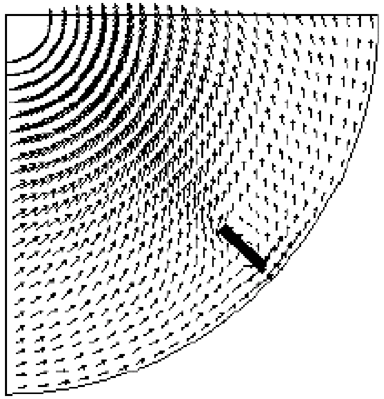
Strömungsfeld: Zeitpunkt 1

V = 300 l



zwischen den
Strombrechern

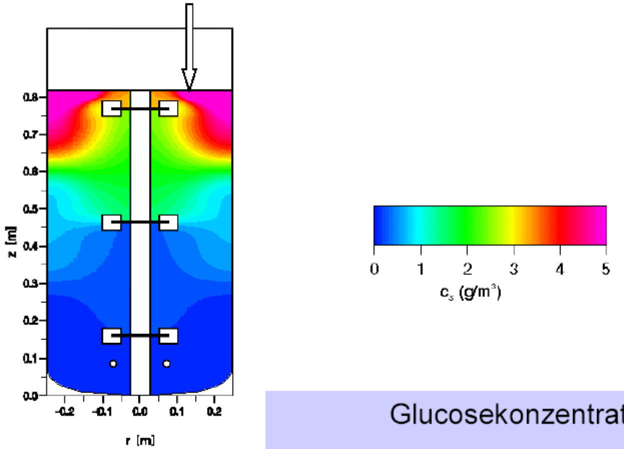
z = 0.572 m



Strömung im 300 l-Reaktor
bei 150 l Inhalt

Substratverteilung Zeitpunkt 1

V = 300 l

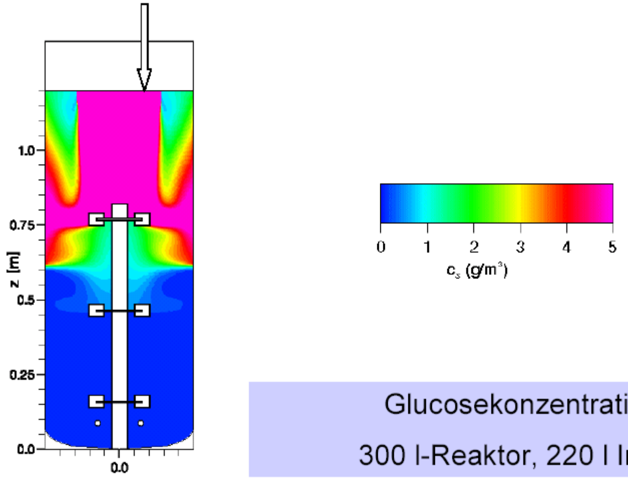


Glucosekonzentration

300 l Tank, 150 l Inhalt

Substratverteilung Zeitpunkt 2

V = 300 l

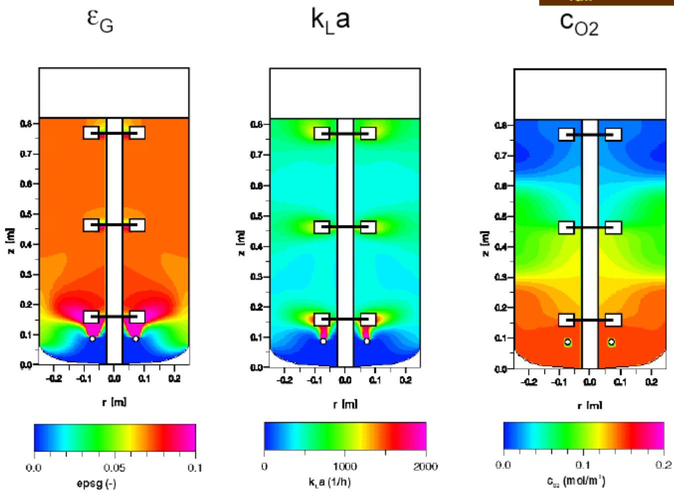


Glucosekonzentration

300 l-Reaktor, 220 l Inhalt

Parameter: Zeitpunkt 1

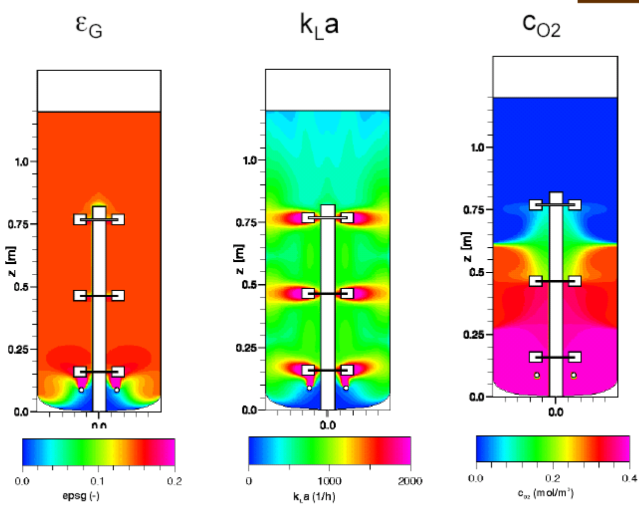
V = 300 l
$V_{füll}$=150 l



Video Mixing

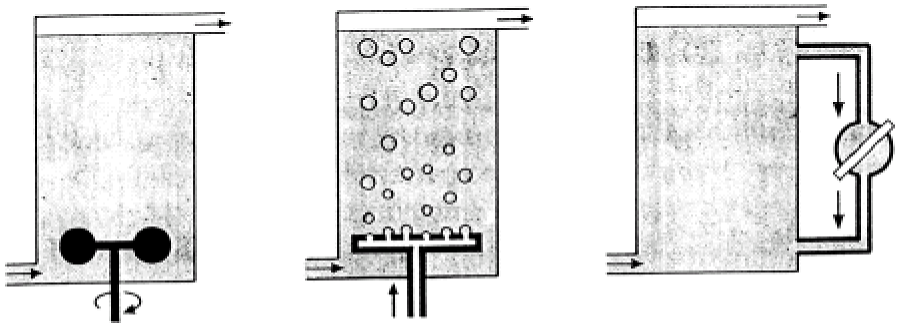
Parameter: Zeitpunkt 2

V = 300 l



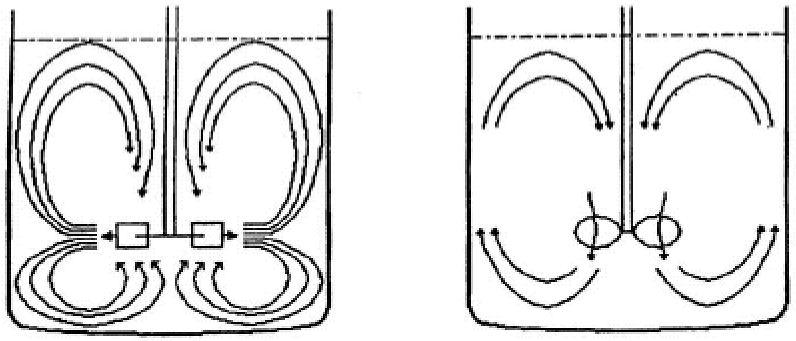
300 l-Reaktor, 220 l Inhalt

Tasks of a bioreactor



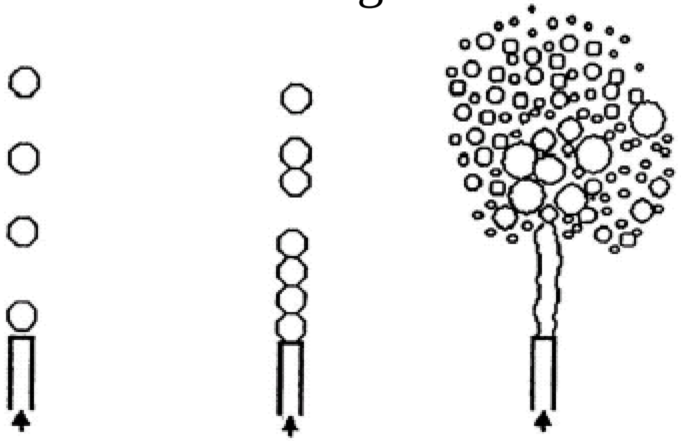
1. Mechanische 2. Pneumatische 3. Hydrodynamisch

Energieeintragung



Turbinenrührer Propellerrührer

Dividing



Einzelblasen Ketten Jet

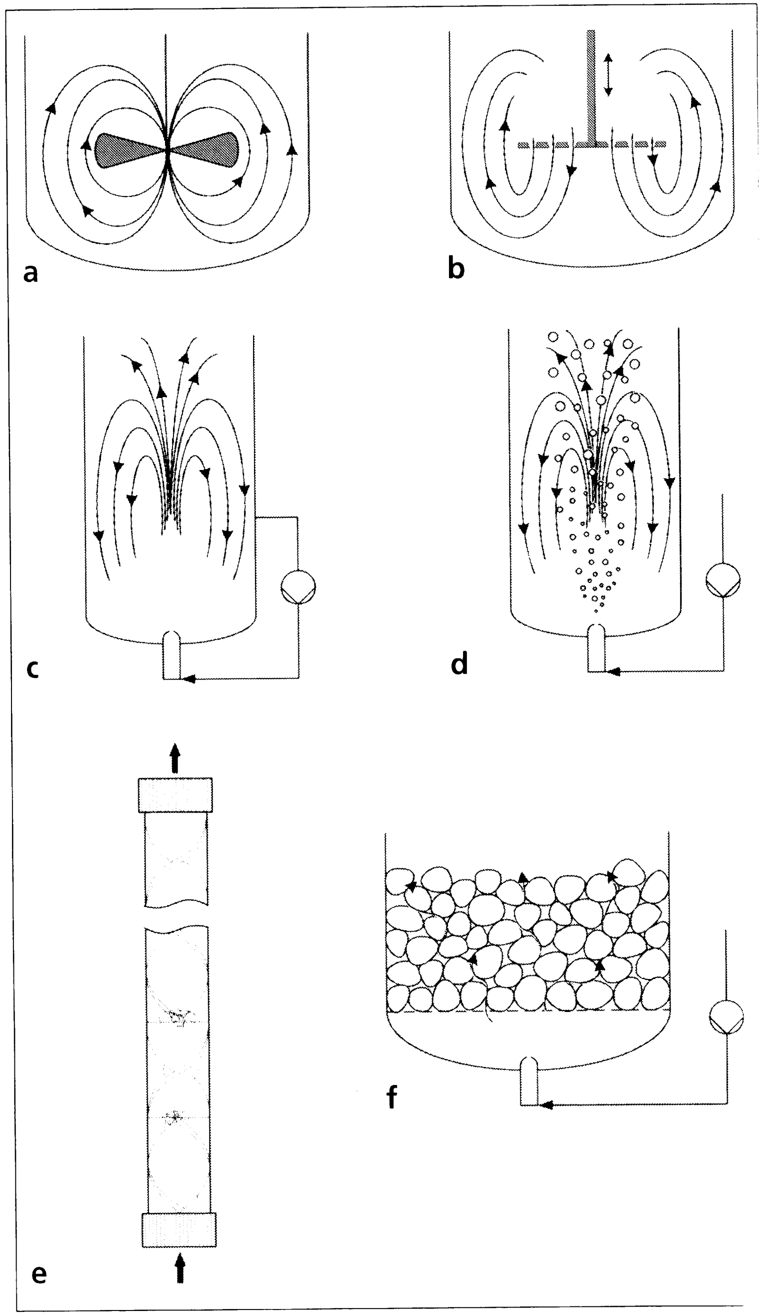
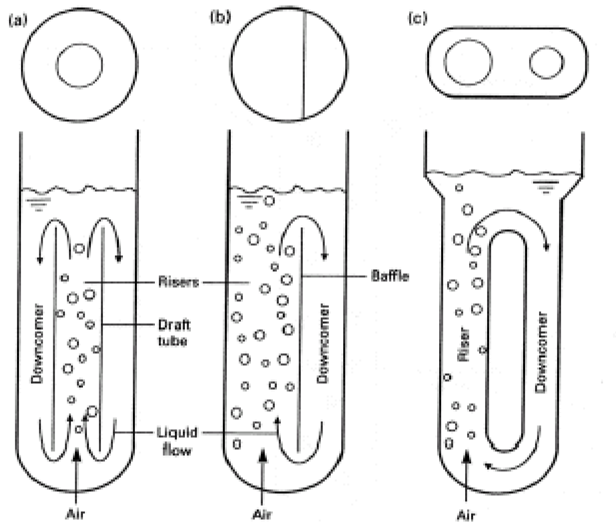


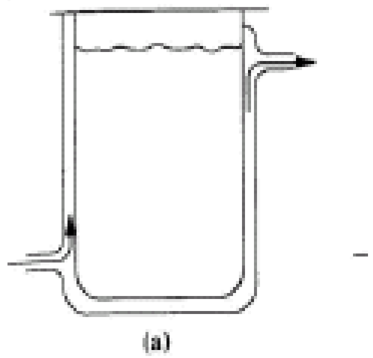
Abb. 7.1 Einteilung der Mischer nach Energieeintrag und Strömungsführung
a) rotierende Welle, b) Siebplatte, c) Freistrahl, d) Gaseintrag, e) statisch, f) Festbett

Tasks of a bioreactor

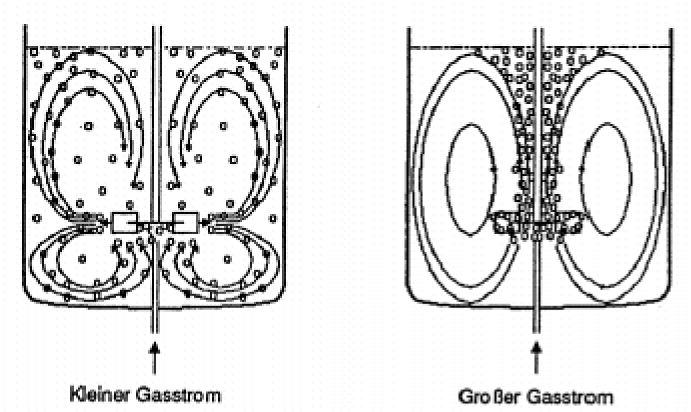
Movement guidance (loops)



Heat Transport: Double coat



Mixing Dynamics



Bioprocess mass transfer

3 phase systems:

gas-liquid
liquid-liquid
Liquid-solid
(gas-solid)

Mass transfer across phase boundaries important, requiring efficient:

Mixing/ agitation
Aeration/ hold- up
Shear
Heat transfer

Mass transfer: gas-liquid

Critical factors:

OTR- oxygen transfer rate
CER- carbon dioxide evolution rate

Example of CO_2 toxicity:

pCO_2 in Inlet air (%)	Relative product yield
0	100
1	66
2	15
3	0
4	0

CO_2 mass transfer

Example of a dissolved gas which can undergo liquid phase reactions

$$C_t = [CO_2] + [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}]$$

Concentration of each species depends on pH:

pH	**Major species**
<5	CO_2
7-9	Bicarbonate
>11	Carbonate

Oxygen Transfer

Important because:

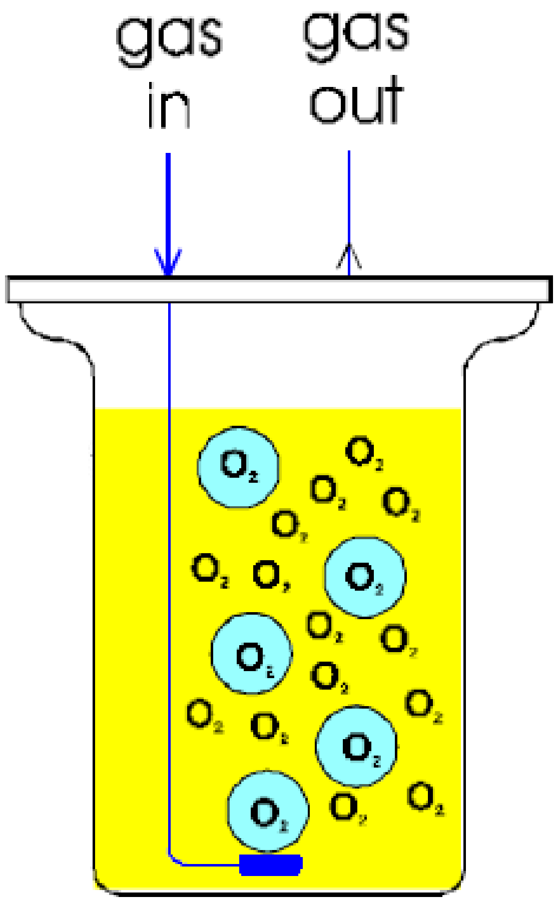
Non-reacting gas in aqueous solution
A major substrate for aerobic processes
Poorly soluble in aqueous culture media
Frequently growth limiting
Often dictates bioreactor configuration

Solubility of O_2 in 1 litre H_2O at 20°C:

0.3 mM = 9 ppm = mg l^{-1}

But:
solubility decreases with temperature and salt concentration

Gas / Liquid mass transfer

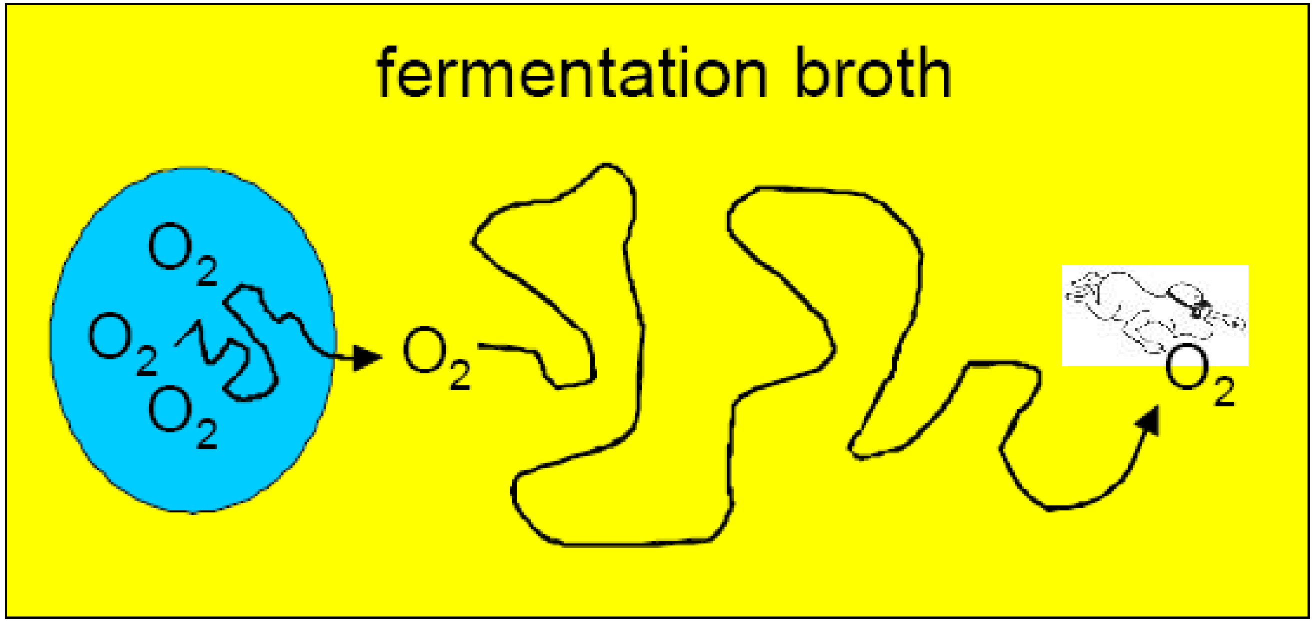


- Microorganisms can take up only dissolved gaseous substrates from aqueous media. Therefore an oxygen molecule must escape from its gas bubble and dissolve into the fermentation broth before it can be metabolised.
- This step is known more generally as gas/liquid mass transfer.

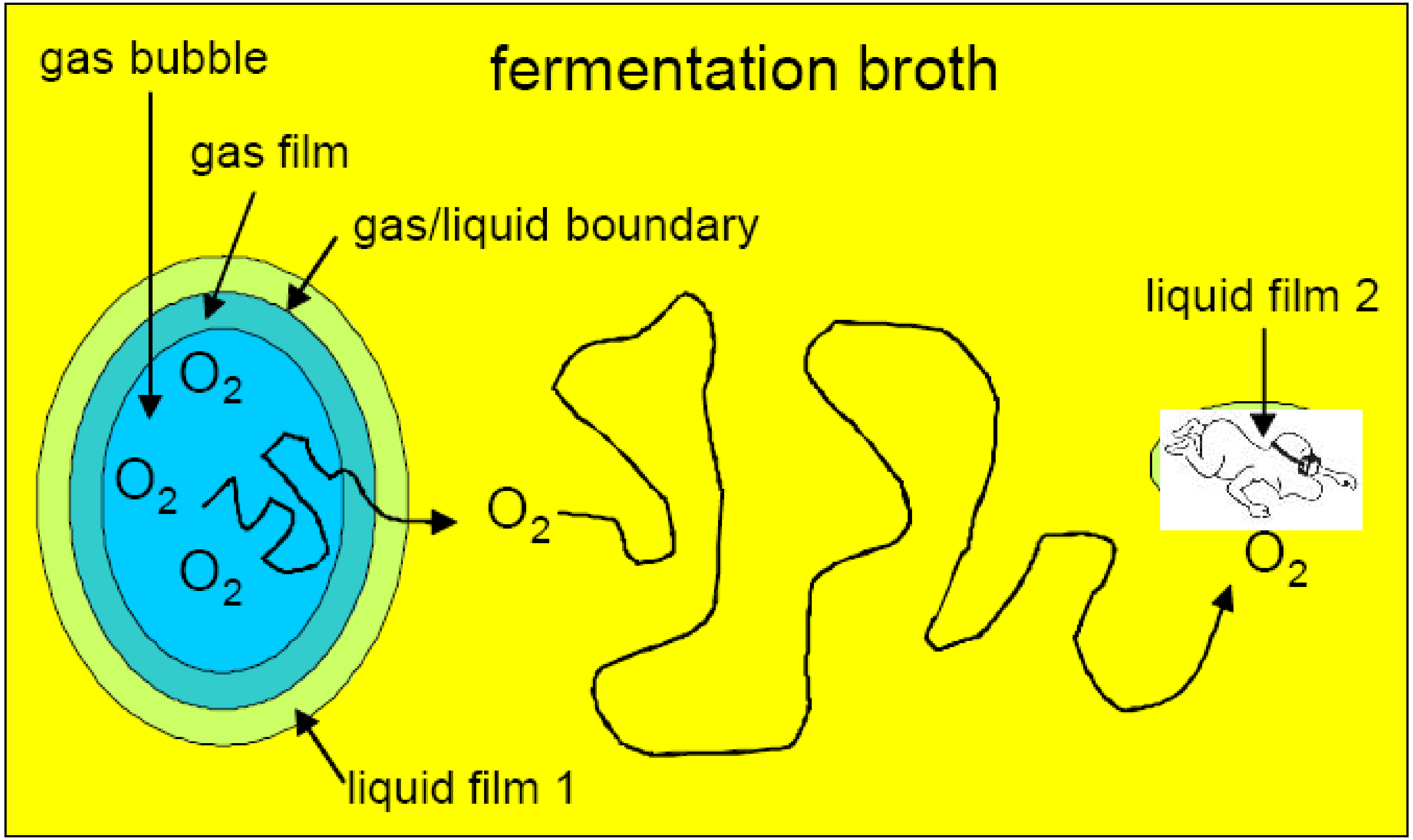
Video: prinicples of oxygen transfer

Gas / Liquid mass transfer

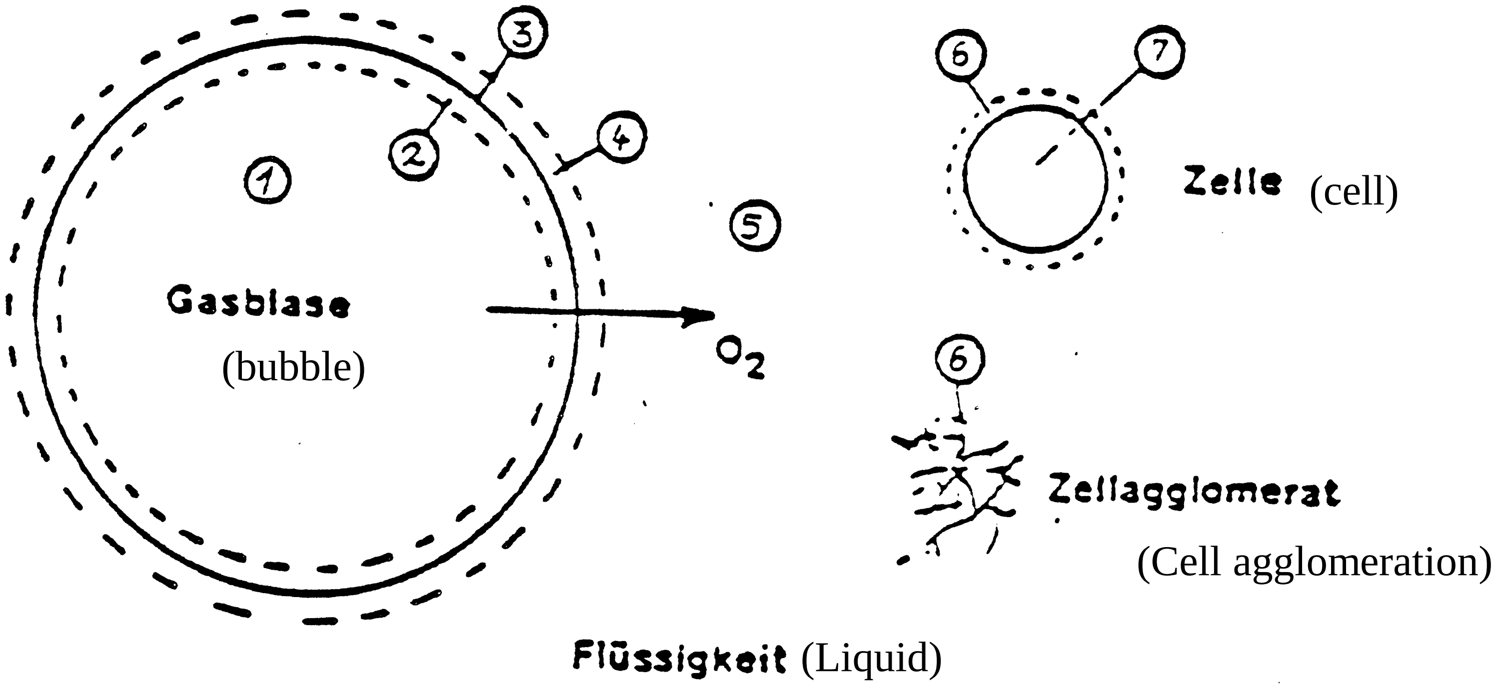
In order to describe the oxygen mass transfer in a bioreactor, a more detailed understanding of the transport procedures of oxygen molecules from gas bubbles →bulk liquid →microorganisms is necessary.



Two film model (2)



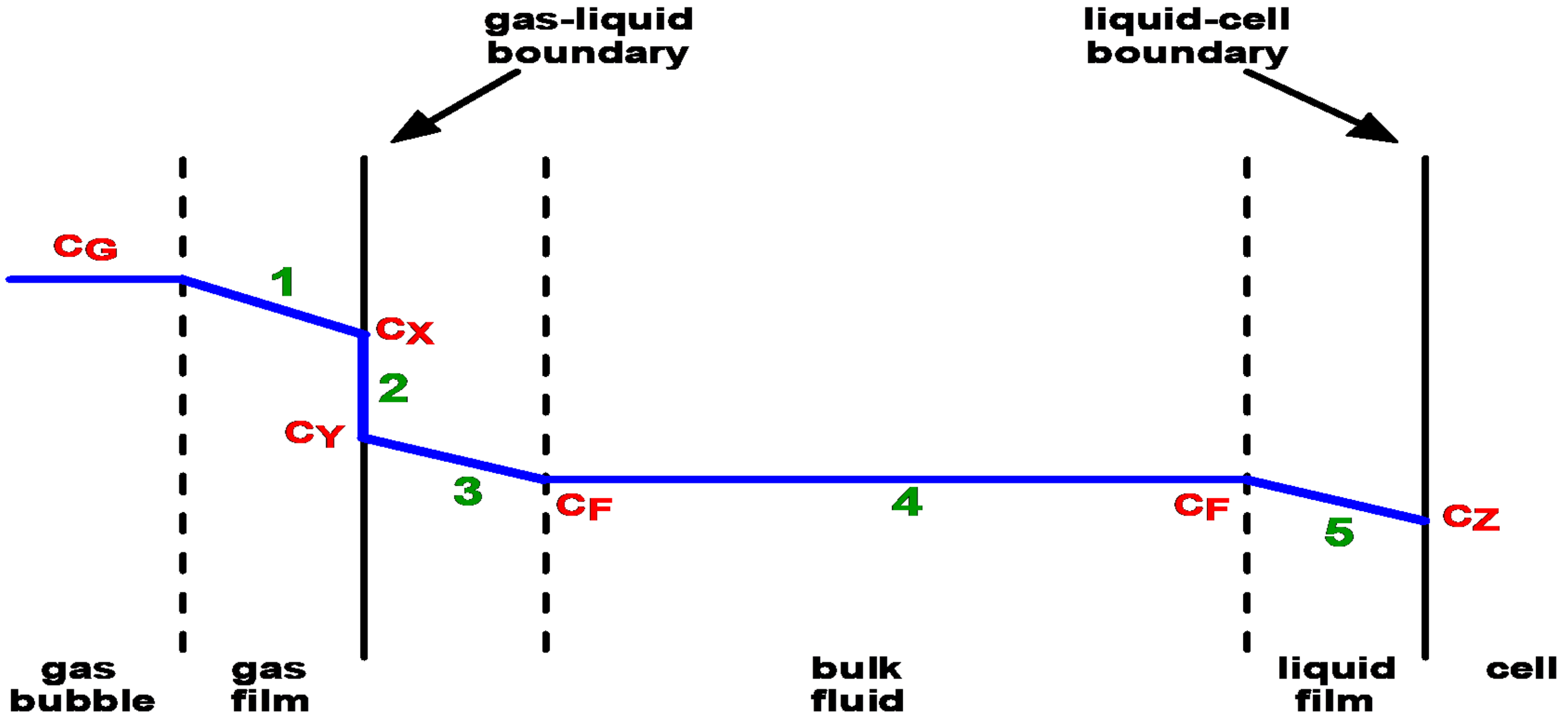
Resistance to oxygen transfer from air bubble to microbial cell



1. Convection
2. Diffusion
4. Diffusion

5. Convection
6. Diffusion
7. uptake

Resistance to oxygen transfer from air bubble to microbial cell



Oxygen Transfer Rate OTR

If the concentration difference Δc is replaced by the difference of the oxygen saturation concentration c_{O2}^* and the actual measured concentration of oxygen c_{O2}, following equation for the oxygen transfer rate OTR results:

$$OTR = k_L a \cdot (c_{O_2}^* - c_{O_2})$$

OTR	[mg/(l·h)]	oxygen transfer rate
$k_L a$	[1/h]	volumetric mass transfer coefficient
c_{O2}^*	[mg/l]	(liquid phase) oxygen saturation concentration
c_{O2}	[mg/l]	actual measured (liquid phase) oxygen concentration

Volumetric mass transfer coefficient k_La

- The unit of k_L results from a further definition for k_L:

$$\mathbf{k_L = D_G/d}$$

k_L [1/h] liquid film coefficient
D_G [m²/h] diffusional coefficient
d [m] liquid film width

- Multiplied by the unit of the specific surface area a [m²/m³] the unit of k_La results as:

$$\left[\ \frac{m^2}{h} \cdot \frac{1}{m} \cdot \frac{m^2}{m^3} = \frac{1}{h}\ \right]$$

- Typical k_La-values in bioreactors are in the range of **100 to 1000 1/h**.

How is $k_L a$ affected? (1)

The value of the volumetric mass-transfer coefficient $k_L a$ depends among other factors:

- On the **medium viscosity** (k_L and a):
  - Increase of the viscosity leads to thicker liquid films (higher d) **=> $k_L a$ decreases.**

  - Increase of the viscosity leads to bubble coalescence (smaller a) => **$k_L a$ decreases.**

- On the degree of **mixing** (k_L):
  - Increase of mixing leads to an increase of the relative velocity between gas bubble and fluid phase **=> $k_L a$ increases**.

How is k_La affected? (2)

The value of the volumetric mass-transfer coefficient k_La depends among other factors:

- On the employment of **surface-active substances** (k_L and a):
  - Antifoam agents **decrease k_La substantially.**

- On the **salt concentration** (a):
  - An increase of the salt concentration reduces the gas bubble size **=> k_La increases.**

  (**But:** An increase of the salt concentration decreases the oxygen solubility!)

How can OTR be increased? (1)

The following methods are frequently used to increase the oxygen transfer rate OTR:

- Increase of the **stirrer speed** = increase of the specific power input:
  - Shear stress reduces the gas bubble size.
  - Relative velocity between gas bubbles and fluid phase increases.

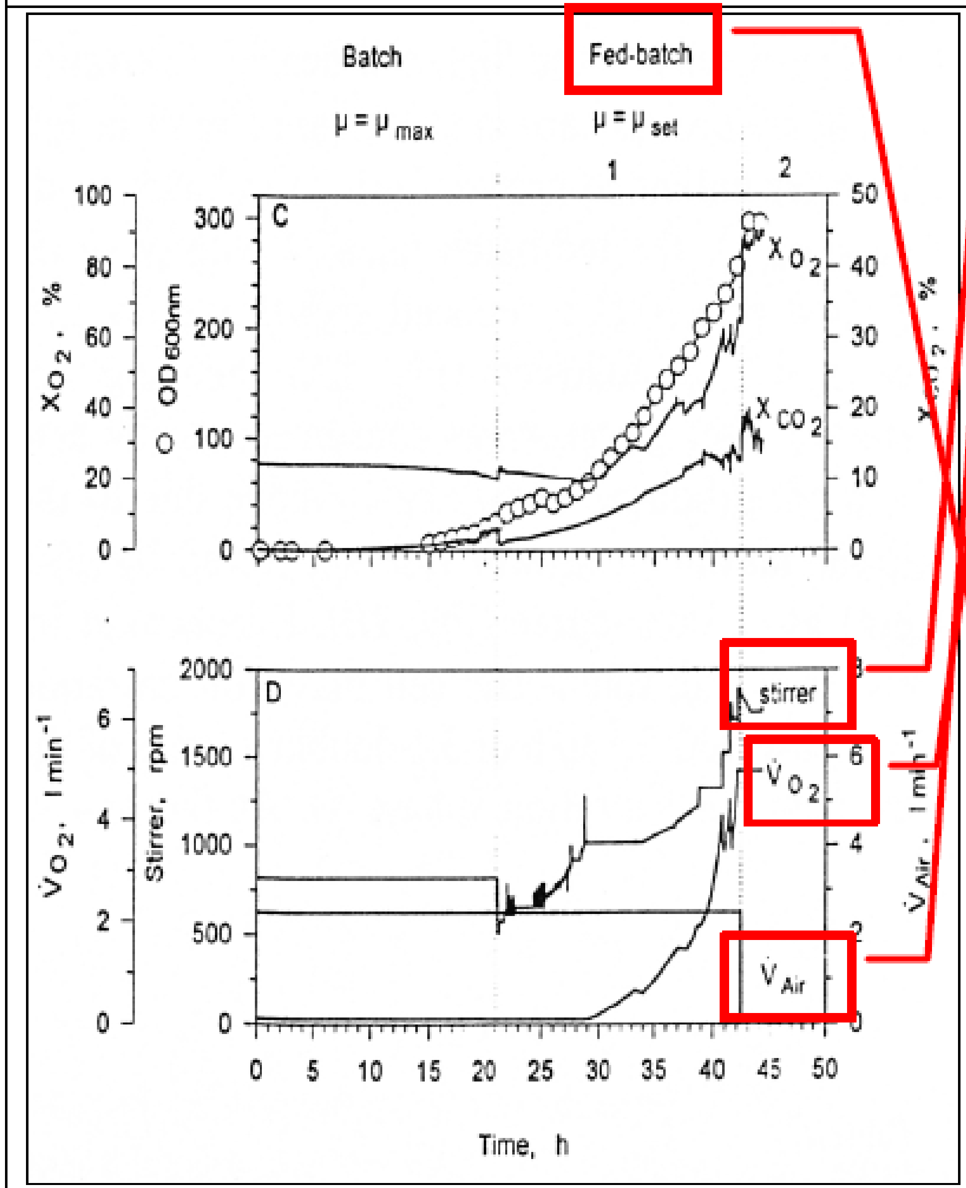
- Increase of the **aeration rate** (Caution: High aeration rates could lead to impeller flooding!)
  - Number of gas bubbles per unit volume increases.
  - Reduction of O_2-depletion in the gas bubbles.

How can OTR be increased? (2)

The following methods are frequently used to increase the oxygen transfer rate OTR:

- Increase of the **reactor pressure** to 2-3 bar:
  - c_{O2}^* and therefore ? c increases.
- Enrichment of aeration air with **pure O_2** (expensive!)
  - c_{O2}^* and therefore ? c increases.
- Using of a fermentation protocol with a **low process temperature**:
  - c_{O2}^* and therefore ? c increases.

Measures to avoid O_2-limitations during HCDC:

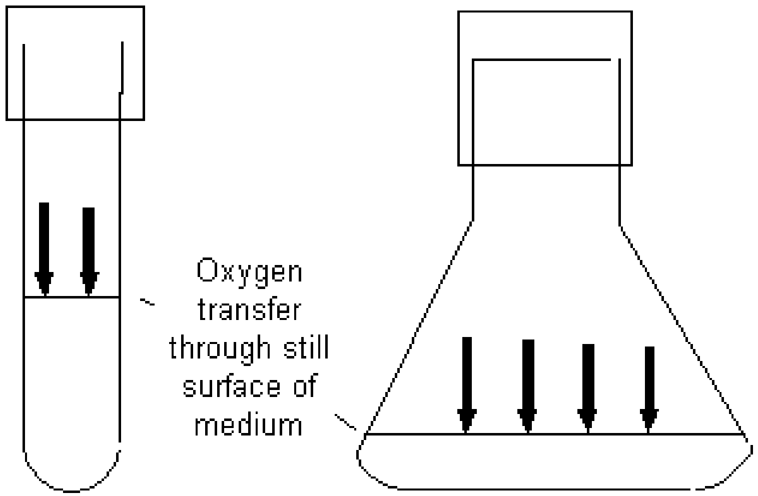


- Increase of the **stirrer speed**
  - Caution: Heat development
- Increase of the **aeration rate**
  - Caution: Impeller flooding
- Using of **pure oxygen** enrichment
  - Caution: Enrichment of CO_2
- Using of a **fed batch strategy**
- Avoid excess amount of **antifoam agent**
- Increase of **reactor pressure**
  - Caution: Enrichment of CO_2

Oxygen Transfer

Standing cultures

In standing cultures, little or no power is used for aeration. Aeration is dependent on the transfer of oxygen through the still surface of the culture.



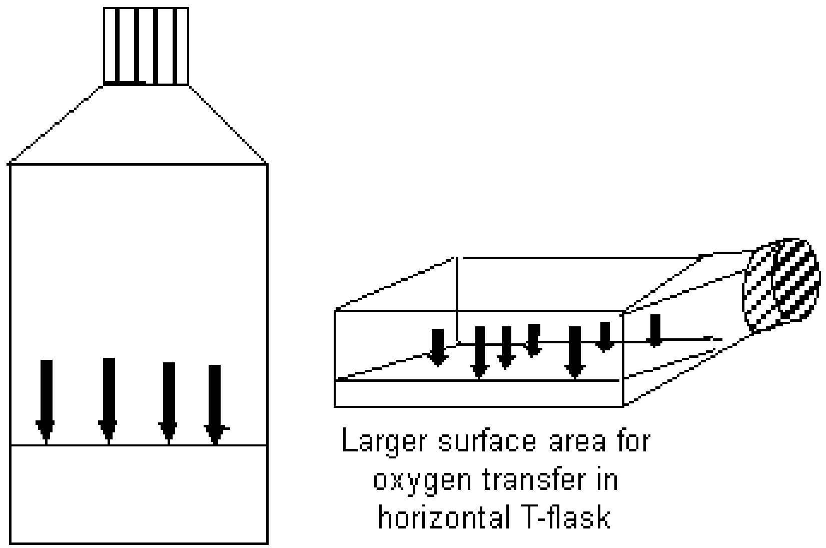
The rate of oxygen transfer will be poor due to the small surface area for transfer.

Standing cultures are commonly used in small scale laboratory systems in which oxygen supply is not critical. For example, biochemical tests used for the identification of bacteria are often performed in test-tubes containing between 5-10 ml of media.

Oxygen Transfer

Standing cultures - T flasks

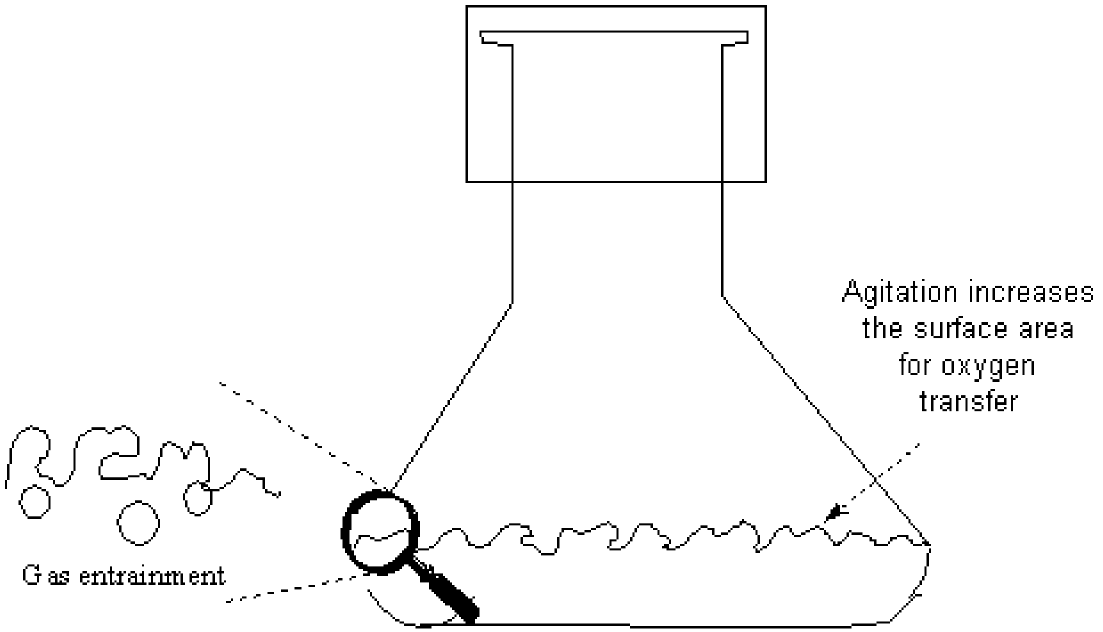
T-flasks used in the small scale culture of animal cells are another example of a standing culture. T-flasks are normally incubated horizontally to increase the surface area for oxygen transfer.



The surface aeration rate in standing cultures can be increased by using large volume flasks.

Oxygen Transfer

Shake flasks



Shake flasks are commonly used for small scale cell cultivation. Through continuous shaking of the culture fluid, higher oxygen transfer rates can be achieved as compared to standing cultures. Shaking continually breaks the liquid surface and thus provides a greater surface area for oxygen transfer. Increased rates of oxygen transfer are also achieved by entrainment of oxygen bubbles at the surface of the liquid.

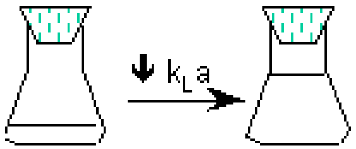
Although higher oxygen transfer rates can be achieved with shake flasks than with standing cultures, oxygen transfer limitations will still be unavoidable particularly when trying to achieve high cell densities

Oxygen Transfer

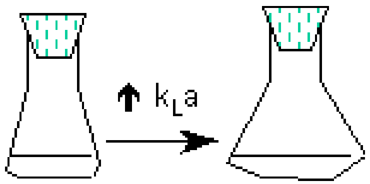
Shake flasks- factors affecting $k_L a$

The rate of oxygen transfer in shake flasks is dependent on the

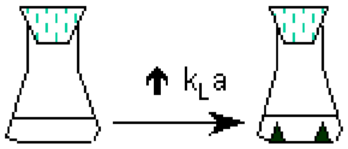
- shaking speed
- the liquid volume
- shake flask design.



$k_L a$ decreases with liquid volume



$k_L a$ increases with liquid surface area



$k_L a$ is higher when baffles are present

The $k_L a$ will increase with the shaking speed.
At high shaking speeds, bubbles become entrained into the medium to further increases the oxygen transfer rate. The appropriate liquid volume is determined by the flask volume. For example, for a standard 250ml flask, the liquid volume should not exceed 70 ml while for a 1 litre flask, the liquid volume should be less than 200 ml. Larger liquid volumes can be used with wide based flasks.

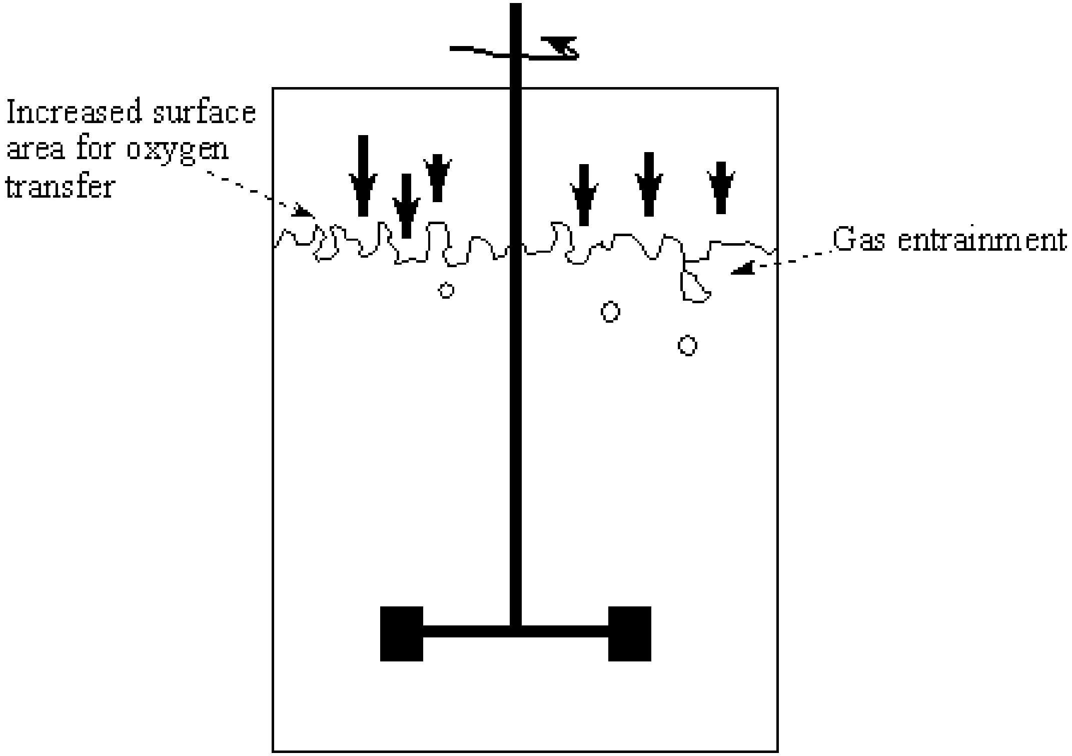
Oxygen Transfer

The presence of baffles in the flasks will further increase the oxygen transfer efficiency, particularly for orbital shakers. The following photographs show how baffles increase the level of gas entrainmentment in a shake flask being shaken in an orbital shaker at 150 rpm

Note the high level of foam formation in the baffled flask due to the higher level of gas entrainment.
The same improvement in oxygen transfer is not as evident with horizontal reciprocating shakers.

Oxygen Transfer

Mechanically stirred bioreactors



For aeration of liquid volumes greater than 200 ml, various options are available. Non-sparged mechanically agitated bioreactors can supply sufficient aeration for microbial fermentations with liquid volumes up to 3 litres. However, stirring speeds of up to 600 rpm may be required before the culture is not oxygen limited.

In non-sparged reactors, oxygen is transferred from the head-space above the fermenter liquid. Agitation continually breaks the liquid surface and increases the surface area for oxygen transfer.

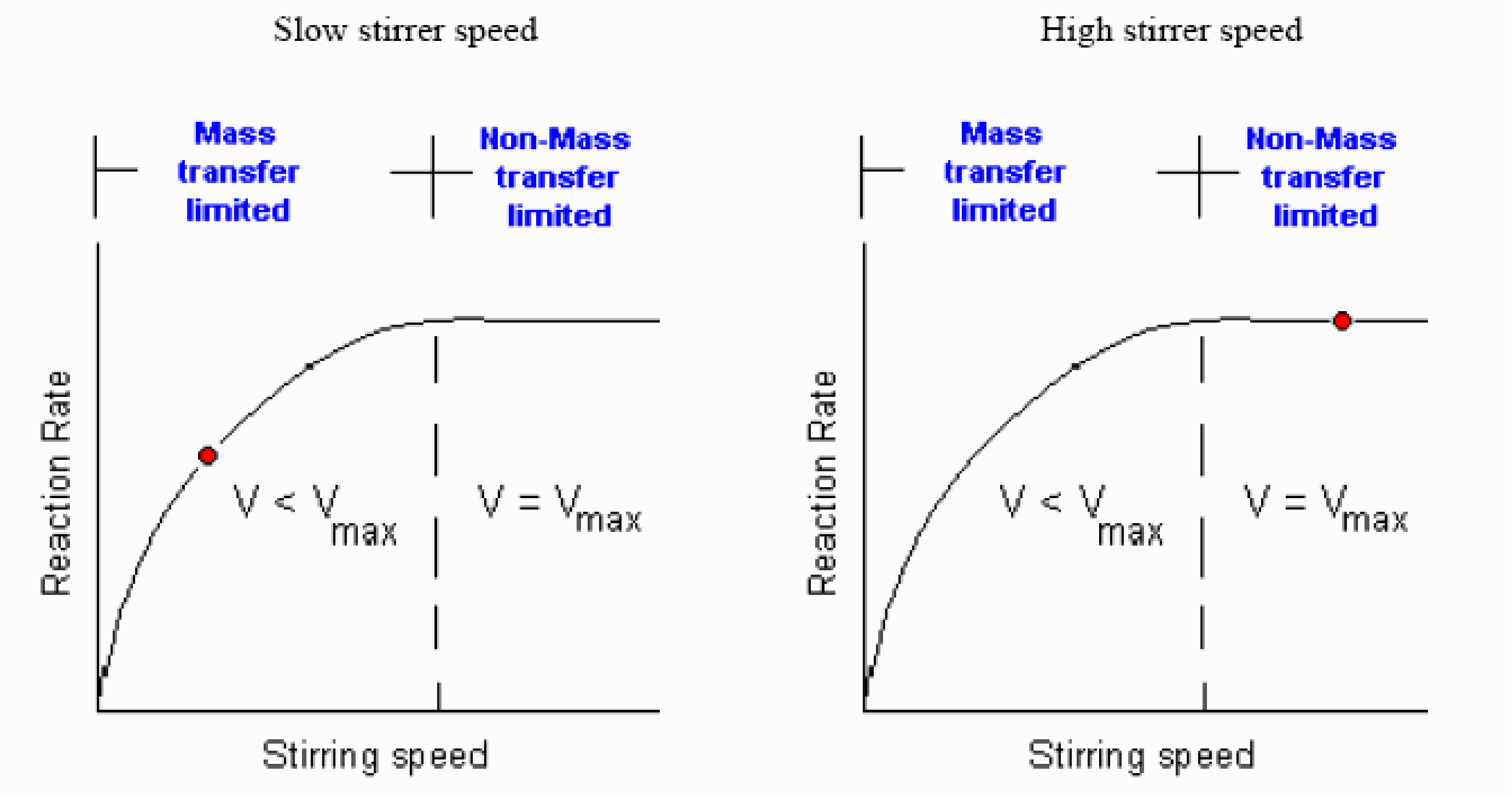
Oxygen Transfer

Sparged stirred tank bioreactors - Exercise

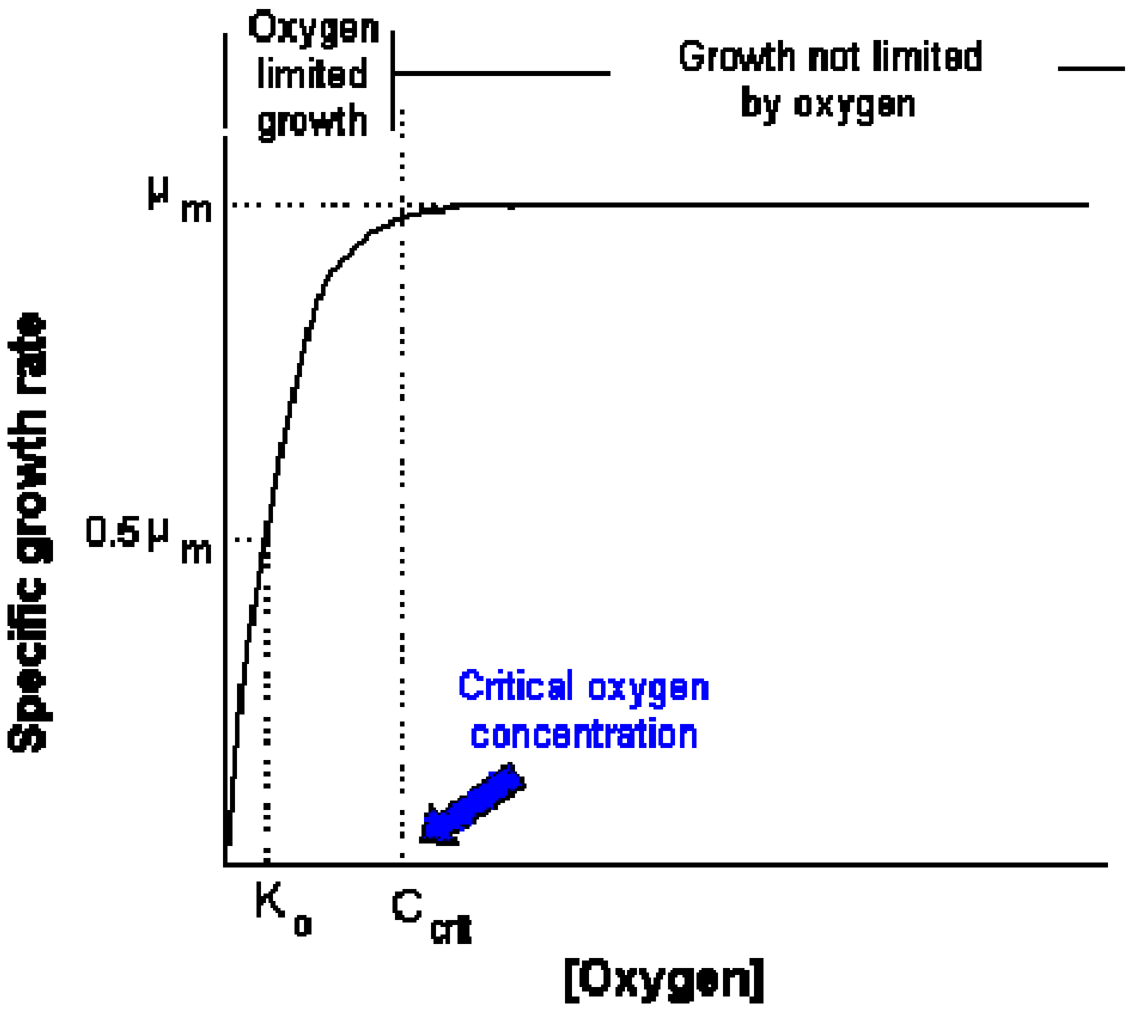
Which of the following would have the highest oxygen transfer rate characteristics?

a) A sparged stirred tank bioreactor being stirred at 200 rpm
b) A non-sparged stirred tank bioreactor being stirred at 200 rpm
c) A shake flask being mixed at 200 rpm
d) All of the above would have equivalent oxygen transfer rate characteristics.

Specific oxygen requirements for a range of cells



Oxygen as growth limiting nutrient



Specific oxygen requirements for a range of cells

Organism	q_{O2} (mmol O_2 g^{-1} h^{-1})
Aspergillus niger	3.0
Streptococcus griseus	3.0
Penicillium chrysogenum	3.9
Klebsiella aerogenes	4.0
Saccharomyces cerevisiae	8.0
Escherichia coli	10.8
Diploid embryo WI-38	0.15 mmol $O_2 10^{-6}$ cells h^{-1}
HeLa	0.4 mmol $O_2 10^{-6}$ cells h^{-1}

Specific oxygen requirements for a range of cells

Kritische Sauerstoffkonzentration $C_{O2,\ krit}$ für Organismen

Mikroorganismus	C_{krit} mg/l
Escherichia coli	0,1 - 0,3
Pseudomonas denitrificans	0,3
Penicillium chrysogenum	0,3 - 0,7
Saccharomyces cerevisiae	0,6
Aspergillus oryzae	0,6
Pseudomonas ovalis	1,1
Azotobacter vinelandii	1,1
Torulopsis utilis	2,0

Maximale spezifische Sauerstoffaufnahme $q_{O2,\ max}$ für Organismen

Mikroorganismus	$q_{O2,\ max}$ $mgO_2/(gTS\ h)$
Escherichia coli	346
Saccharomyces cerevisiae	256
Klebsiella aerogenes	128
Penicillium chrysogenum	125
Streptomyces griseus	96
Aspergillus niger	96

$$q_{O_2} = q_{O_2,\max} \cdot \frac{C_{O_2}}{K_O + C_{O_2}}$$

OUR (Oxygen Uptake rate)

Yeast Culture (at 10 g/L DW):	ca. 26.77 mmol O_2 L^{-1} h^{-1}
E. coli	max. 120-150 mmol O_2 L^{-1} h^{-1}

Values from literature:

E. coli	*0.346 kg O_2 kg^{-1}Biomasse h^{-1}*
Saccharomyces cerevisiae	*0.256 kg O_2 kg^{-1}Biomasse h^{-1}*
Aspergillus niger	*0.096 kg O_2 kg^{-1}Biomasse h^{-1}*
Penicillium chrysogenum	*0.125 kg O_2 kg^{-1}Biomasse h^{-1}*

Oxygen as a substrate

Like any substrate given by Monod equation:

$$\mu = \mu_m \frac{S}{K_S + S}$$

For oxygen this is given by:

$$OUR = q_{O_2} = q_{O_{2m}} \frac{C_L}{K_{O_2} + C_L}$$

Where qO_2 = specific oxygen uptake rate (mmolO_2 g^{-1} h^{-1})
qO_{2m} = maximum specific OUR (mmol O_2 g^{-1} h^{-1})
K_{O2} = saturation constant for O_2 (mM)

At steady state:

$$\boxed{\mathbf{OUR = X \cdot qO_{2m} = k_L a\,(C^* - C_L) = OTR}}$$

Basic form of k_La-correlations

- Zahlreiche Autoren haben aus experimentelle Daten k_La-Korrelationen aufgestellt. Die unterschiedlichen Angaben in der Literatur weichen jedoch deutlich voneinander ab.
- k_La-Korrelationen haben häufig die Form einer einfachen Potenzbeziehung:

$$k_L a = C \cdot \left(\frac{P}{V_L}\right)^a \cdot u_G{}^b$$

$k_L a$	[1/s]	volumetrischer Stoffübergangskoeffizent
C	[-]	empirisch ermittelte Konstante
a, b	[-]	empirisch ermittelte Exponenten
P	[W]	Leistungseintrag
V_L	[m^3]	Flüssigkeitsvolumen (Arbeitsvolumen)
u_G	[m/s]	Gassleerrohrgeschwindigkeit

$k_L a$-Korrelation von Vant´t Riet

- Vant´t Riet (1979) hat für Rührkesselreaktoren mit koaleszierenden niederviskosen Medien folgende Korrelation aufgestellt:

$$k_L a = 0{,}42 \cdot \left(\frac{P}{V_L}\right)^{0{,}4} \cdot u_G^{\;0{,}5}$$

$k_L a$	[1/s]	volumetrischer Stoffübergangskoeffizent
P	[W]	Leistungseintrag
V_L	[l]	Flüssigkeitsvolumen (Arbeitsvolumen)
u_G	[m/s]	Gassleerrohrgeschwindigkeit

P/V_L wird oft auch als lokale Energiedissipation e bezeichnet.

$k_L a$-Korrelation von Schlüter

- Basierend auf einem Konzept von Zlokarnik (1978) hat Schlüter (1991) eine Korrelation speziell für die Anwendung im Bereich Biotechnologie entwickelt:

$$k_L a = C \cdot \left(\frac{P / V_L}{\rho \cdot \left(\nu \cdot g^4 \right)^{1/3}} \right)^a \cdot \left[\frac{\dot{V}_G}{V_L} \cdot \left(\frac{\nu}{g^2} \right)^{1/3} \right]^b \cdot \left(\frac{g^2}{\nu} \right)^{1/3}$$

$k_L a$	[1/s]	volumetrischer Stoffübergangskoeffizent
P	[W]	Leistungseintrag
V_L	[l]	Flüssigkeitsvolumen (Arbeitsvolumen)
ρ	[kg/m³]	Dichte
ν	[m²/s]	kinematische Viskosität
$\dot{V}_G$	[m³/s]	Begasungsrate

Scheibenrührer empirisch	
a	0,62
b	0,23
C	7,94E-04


Scheibenrührer

Methods for measuring $k_L a$

1) Non-steady state method
2) Steady state method, Dynamic method
3) Chemical reaction methods e.g. sulphite

1) Non-steady state method

- Fill reactor with water or medium
- Determine C*- solubility under operating conditions (temperature, pH, pressure gas composition, medium composition etc.)
- Sparge with N_2 to calibrate pO_2 electrode to 0%
- Sparge with air or O_2 to calibrate pO_2 electrode for 100% saturation
- Sparge with N_2 to give pO_2 of 0%
- Sparge with air or O_2 and measure slope of increase in pO_2 with time:

$$\frac{dC_L}{dt} = k_L a\left(C^* - C_L\right)$$

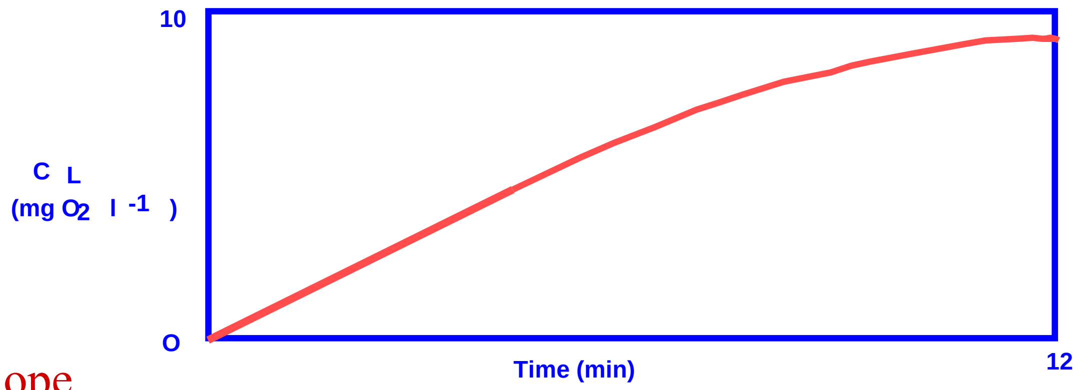
or

$$\frac{-d\left(C^* - C_L\right)/dt}{\left(C^* - C_L\right)} = k_L a$$

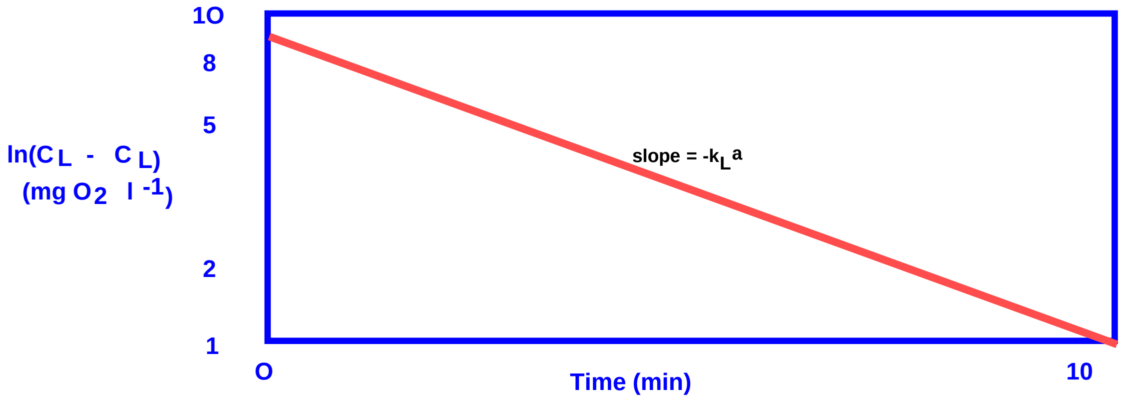
Non-steady state method for k_La determination

Or: **$\mathrm{Ln}(C^* - C_L) = -k_L at$**

Note the form: $y = ax + b$



Plot of $\ln(C^*-C_L)$ versus time gives slope $= k_La$



2) Dynamic method

$$\frac{d\left(V \cdot C_{O_2}\right)}{dt} = k_L a \cdot \left(C^*_{O_2} - C_{O_2}\right) \cdot V - q_{O_2} \cdot X \cdot V$$

$$\frac{dC_{O_2}}{dt} = \underbrace{k_L a \cdot \left(C^*_{O_2} - C_{O_2}\right)}_{\text{Zufuhr, OTR}} - \underbrace{q_{O_2} \cdot X}_{\text{Verbrauch, OUR}}$$

$k_L a$: Stofftransportkoeffizient für laminaren Flüssigkeitsfilm * spez. Austauschfläche h^{-1}

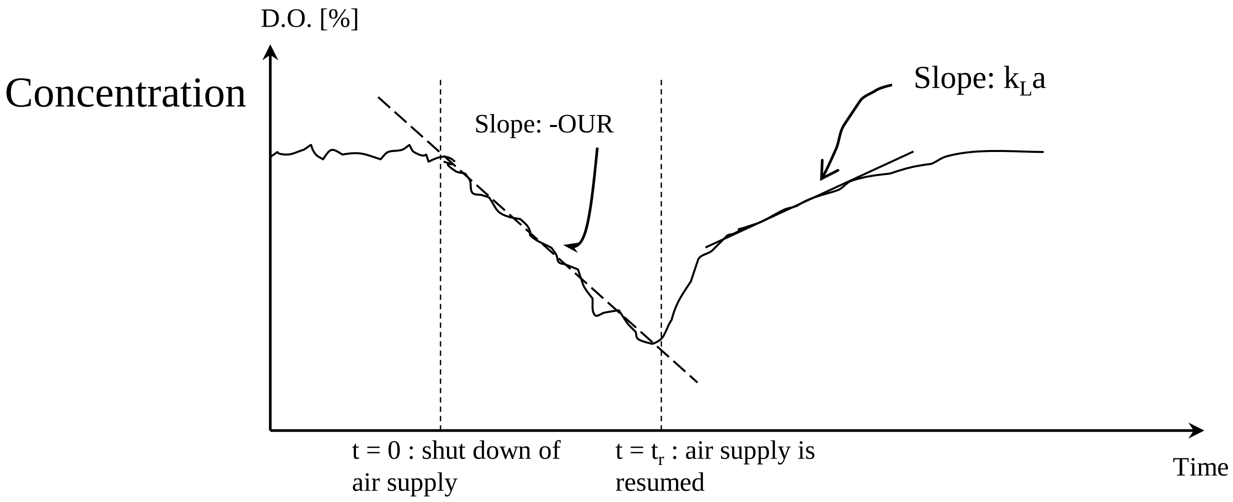
$C^*_{O_2}$: Sättigungslöslichkeit für Sauerstoff an der Grenzfläche mg l^{-1}(s.Tabelle)

$q_{O_2} = \frac{1}{Y_{X/O}} \cdot \mu$: spezifische Sauerstoffverbrauchsgeschwindigkeit h^{-1}

Dynamic method

Uses fermenter with active cells.

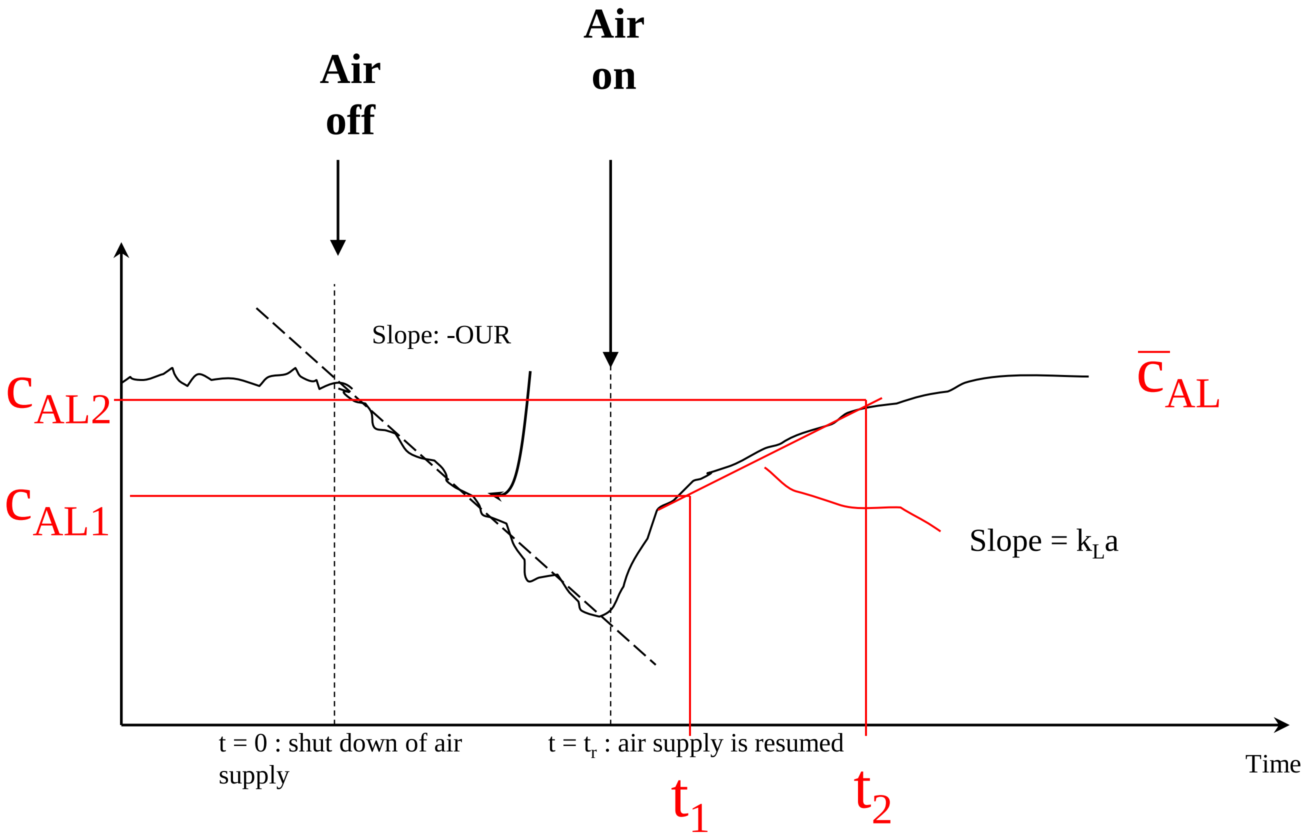
$$\frac{dC_L}{dt} = k_L a \cdot \left(C_L^* - C_L\right) - X \cdot q_{O_2} = OTR - OUR$$



$$\underline{\underline{\ln \frac{\left[C_L^{t=\infty} - C_L^{t=0}\right]}{\left[C_L^{t=\infty} - C_L^{t}\right]} = k_L a \cdot t}}$$

Schematic DO profile against time. At t=0, air supply is shut down (dashed line). Afterwards, the DO decreases until the air supply is resumed. The slope of the decay gives the OUR. The profile after t_r can lead to the $k_l a$.

Variation of oxygen tension for dynamic measurement of $k_L a$



$$\frac{dC_{AL}}{dt} = k_L a\,(C^*_{AL} - C_{AL}) - q_{O_2}\,x$$

Rate of oxygen consumption

If $dC_{AL}/dt = 0$ and $C_{AL} = \overline{C}_{AL}$

Then: $q_{O_2}\,x = k_L a\,(C^*_{AL} - \overline{C}_{AL})$

$$\frac{dC_{AL}}{dt} = k_L a\,(\overline{C}_{AL} - C_{AL})$$ $k_L a$ = const. / then → Integration

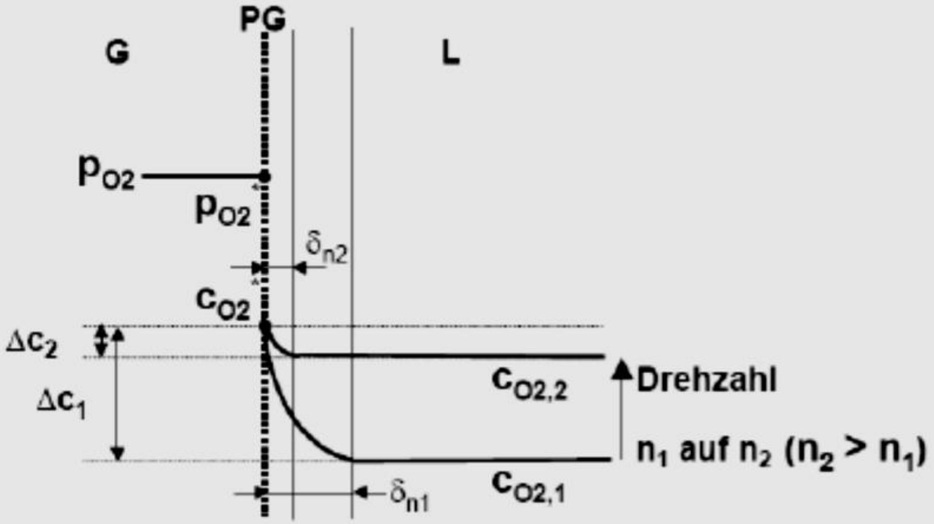
$$k_L a = \frac{\mathrm{Ln}\left[\dfrac{\overline{C}_{AL} - C_{AL1}}{\overline{C}_{AL} - C_{AL2}}\right]}{t_2 - t_1}$$

$$\frac{dC_{O_2}}{dt} = k_L a \cdot \left(C^*_{O_2} - C_{O_2}\right) - q_{O_2} \cdot X$$

Bilanzgleichung zeigt die Möglichkeiten auf, den Stoffaustausch zu beeinflussen.

$k_L a$: Drehzahl n des Rührers; Rührergeometrie

$C^*_{O_2}$: Gesamtdruck; Partialdruck O_2; Gasdurchsatz



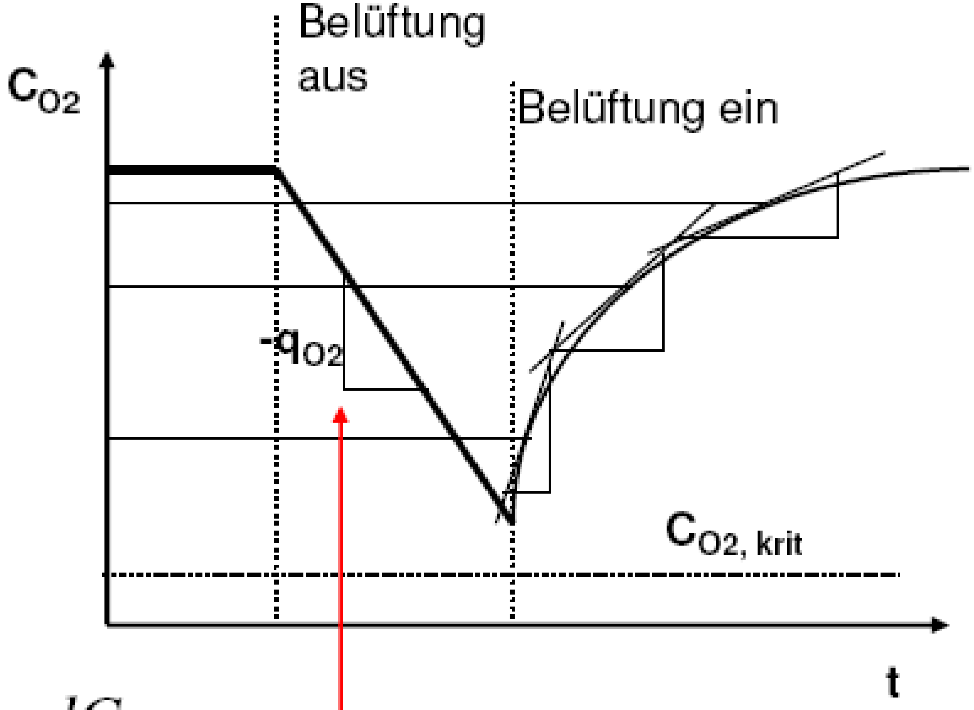
C_{O_2} : möglichst niedrige Konzentration, aber $C_{O2} > C_{O2,\,krit}$

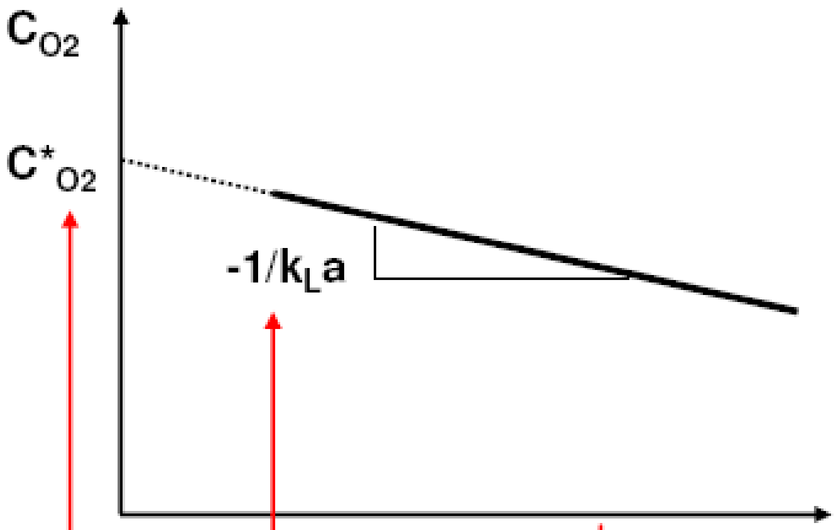
Bestimmung der drei Parameter durch dynamische Methode während einer Fermentation:

$$\frac{dC_{O_2}}{dt} = \mathbf{k_L a} \cdot \left(\mathbf{C^*_{O_2}} - C_{O_2}\right) - \mathbf{q_{O_2}} \cdot X$$

Im Wiederanstieg werden an mehreren Stellen Steigungen und die zugehörigen C_{O2} bestimmt.

Problem: Sondendynamik!





$$\frac{dC_{O_2}}{dt} = -\mathbf{q_{O_2}} \cdot X$$

$$C_{O_2} = \mathbf{C^*_{O_2}} - \frac{1}{\mathbf{k_L a}} \cdot \left(\overbrace{\frac{dC_{O_2}}{dt} + \mathbf{q_{O_2}} \cdot X}\right)$$

Summary: Dynamic determination of k_La-value

1. Luftzufuhr geschlossen

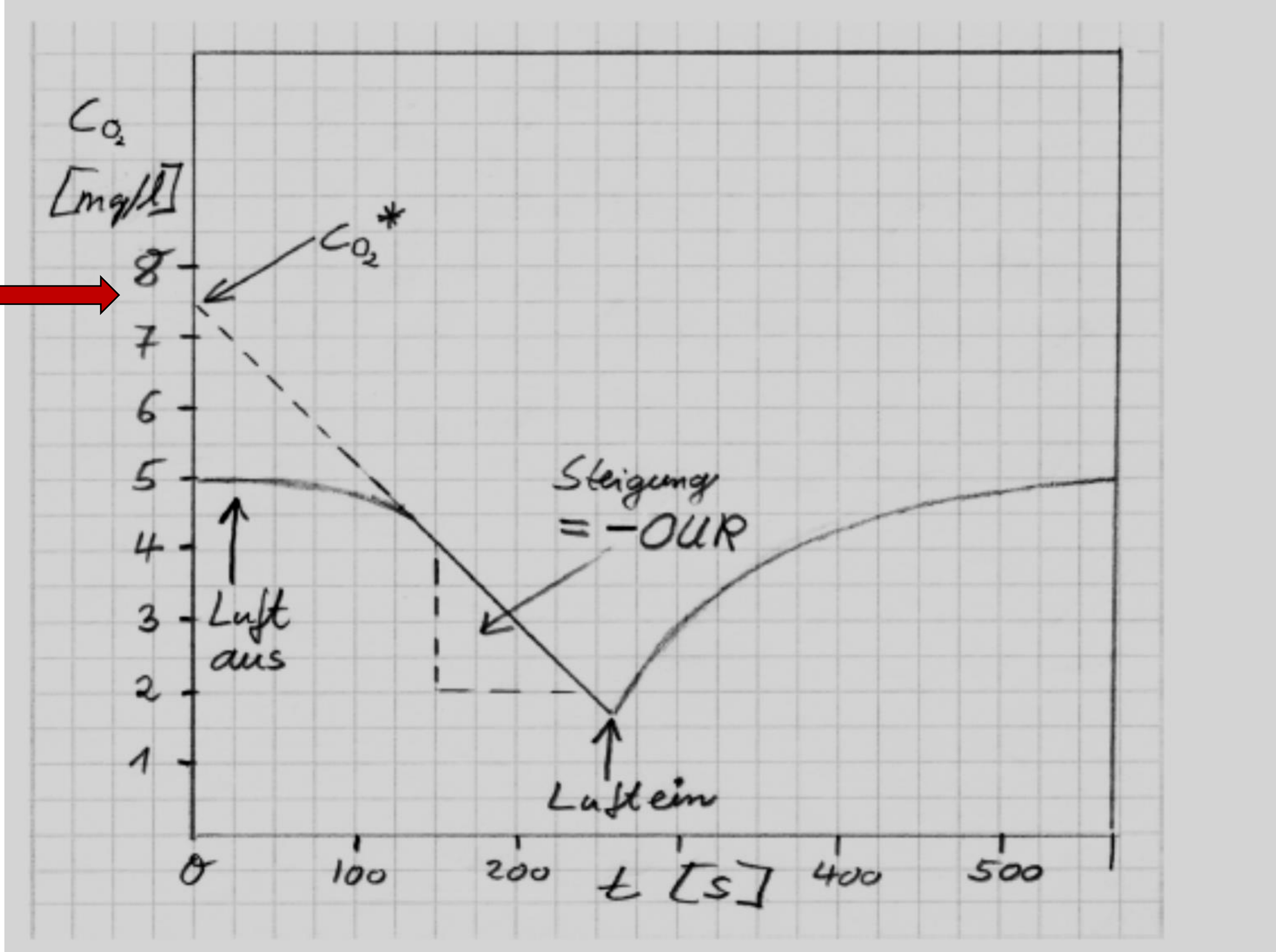
Wird die Belüftung geschlossen fällt der O_2-Eintrags-Term aus der Bilanzgleichung weg. Übrig bleibt nur noch:

$$\frac{dc_{O_2}}{dt} = -OUR$$

Wird die Gleichung integriert ergibt sich die Gerade:

$$c_{O_2} = -OUR \cdot t + c^*_{O_2}$$

Die Steigung einer Auftragung von c_{O2} über t ist daher -OUR.



c_{O_2} [mg/l]
$c_{O_2}^*$
Steigung = −OUR
Luft aus
Luft ein
t [s]
0
100
200
400
500
1
2
3
4
5
6
7
8

Example: Determination of - OUR

Stamm: *Diaporthe carbinicola*

Produkt: Labenzym

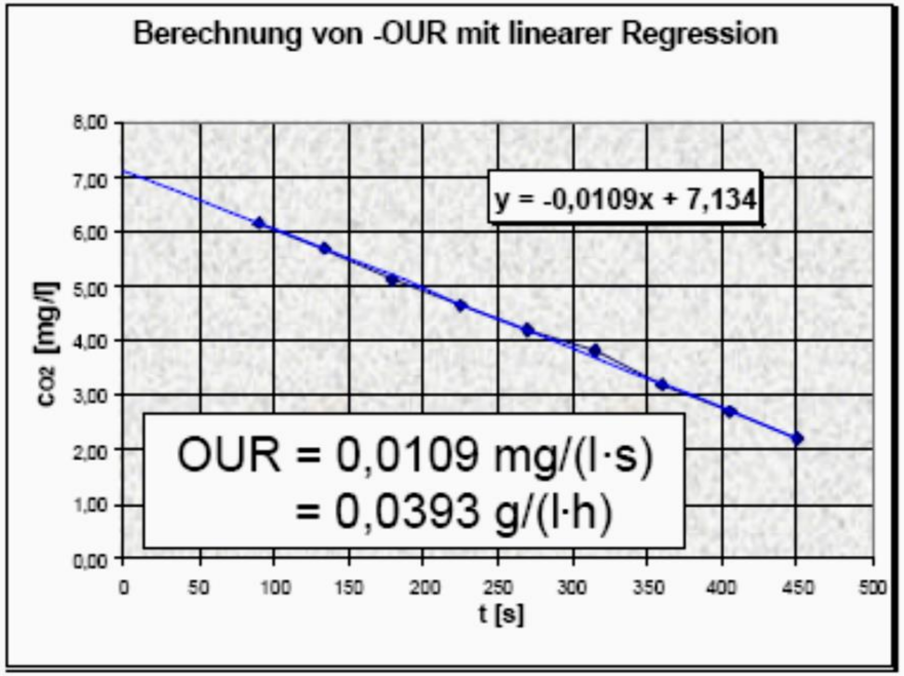
Bioreaktor: 200 l

Temp.: 30°C

Begasung: 0,375 vvm

Drehzahl: 150 min^{-1}

Begasung	t	pO_2	C_{O2}
	[s]	[%]	[mg/l]
aus	0		
	~~45~~	~~82,2~~	~~6,20~~
	90	81,6	6,15
	135	75,6	5,70
	180	67,6	5,09
	225	61,7	4,65
	270	55,7	4,20
	315	50,4	3,80
	360	42,4	3,20
	405	35,8	2,70
	450	29,1	2,19



2. Luftzufuhr wieder geöffnet

Wird die Luftzufuhr geöffnet, ergibt sich:

$$\frac{d_{c_L}}{dt} = k_L a \cdot (c^*_{O_2} - c_{O_2}) - OUR$$

Die Gleichung kann umgestellt werden zu:

$$c_{O_2} = -\frac{1}{k_L a} \cdot \left(\frac{dc_{O_2}}{dt} + OUR\right) + c^*_{O_2}$$

Die Steigung einer Auftragung von c_{O2} über $\left(\frac{dc_{O_2}}{dt} + OUR\right)$

ist daher $-\frac{1}{k_L a}$

$$\frac{dc_{O_2}}{dt} = k_La \cdot (c_{O_2}^* - c_{O_2}) - OUR \qquad \Big| + OUR$$

$$\frac{dc_{O_2}}{dt} + OUR = k_La \cdot (c_{O_2}^* - c_{O_2}) \qquad \Big| \cdot \frac{1}{k_La}$$

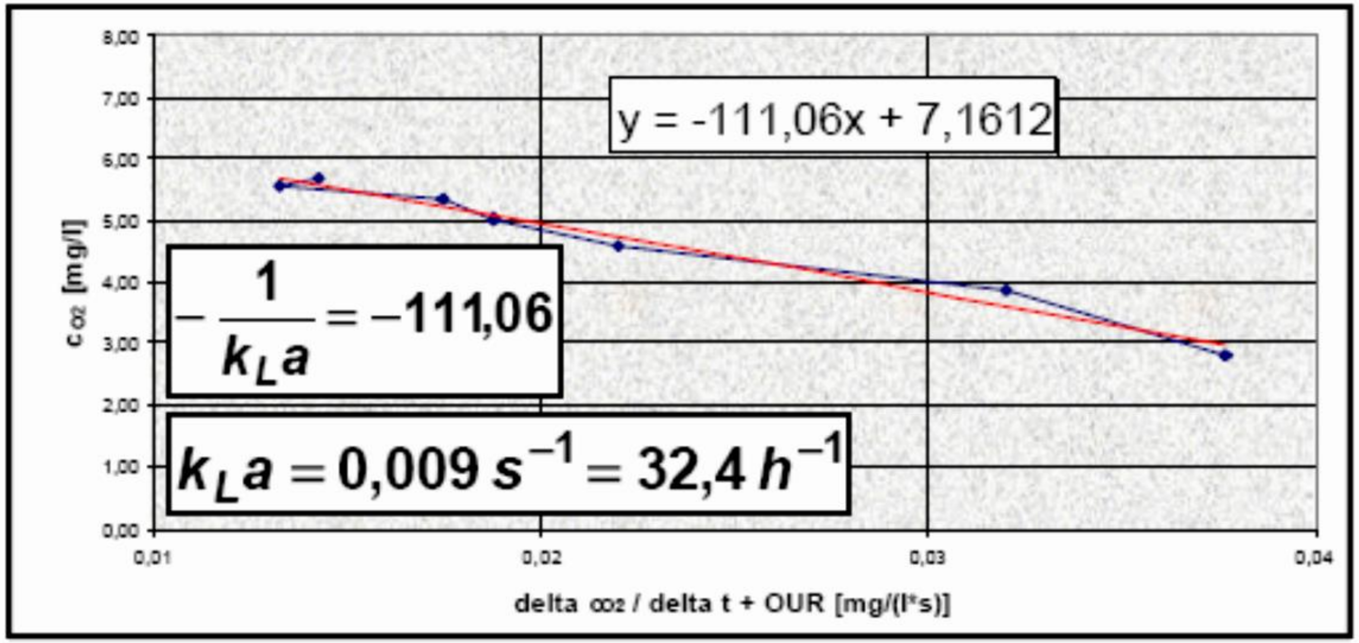
$$\frac{1}{k_La} \cdot \left(\frac{dc_{O_2}}{dt} + OUR\right) = c_{O_2}^* - c_{O_2} \qquad \Big| - c_{O_2}^*$$

$$\frac{1}{k_La} \cdot \left(\frac{dc_{O_2}}{dt} + OUR\right) - c_{O_2}^* = -c_{O_2} \qquad \Big| \cdot -1$$

$$\boxed{c_{O_2} = -\frac{1}{k_La} \cdot \left(\frac{dc_{O_2}}{dt} + OUR\right) + c_{O_2}^*}$$

Begasung	t	pO_2	c_{O2}	delta c_{O2}	delta t	dc_{O2} / d t	dc_{O2}/dt + OUR	Mittel c_{O2}
	[s]	[%]	[mg/l]	[mg/l]	[s]	[mg/(l*s)]	[mg/(l*s)]	[mg/l]
an	~~450~~	~~29,1~~	~~2,19~~					
	495	29,1	2,19					
	540	45,1	3,40	1,2059	45	0,026798	0,037715	2,80
	585	57,7	4,35	0,9496	45	0,021103	0,032020	3,87
	630	64,3	4,85	0,4974	45	0,011054	0,021971	4,60
	675	69,0	5,20	0,3542	45	0,007872	0,018789	5,02
	720	72,9	5,49	0,2939	45	0,006532	0,017449	5,35
	765	74,3	5,60	0,1055	45	0,002345	0,013262	5,55
	810	76,3	5,75	0,1507	45	0,003350	0,014267	5,68

Beispiel: Praktische Bestimmung von $k_L a$



Example: Estimating $k_L a$ using the dynamic method

A 20 L stirred tank fermenter containing a *Bacillus thuringiensis* culture at 30°C is used for the production of microbial insecticide. $k_L a$ is determined using the dynamic method. Air flow is shut off for a few minutes and then the dissolved-oxygen level drops; the air supply is then re-connected. When steady-state is established, the dissolved-oxygen tension is 78% air saturation. The following results are obtained:

Time (s)	5	15
% air saturation	50	66

a) Estimate $k_L a$

b) An error is made determining the steady-state oxygen level which, instead of 78%, is taken 70%. What is the percentage error in $k_L a$ resulting from this 10% error in C_{AL}

Solution

3) Sulphite method

Based on zero order reactions where SO_3^{2-} oxidized to SO_4^{2-} in presence of catalyst e.g. Co^{2+} or Cu^{2+} (very rapid):

$$\frac{1}{2}\frac{dC_{SO_4^{2-}}}{dt} = k_L a C^*$$

1/2 since 0.5 mole O_2 used per mole SO_4^{2-} formed (so must measure in moles), therefore:

$$k_L a = \frac{1}{2}\frac{dC_{SO_4^{2-}}/dt}{C^*}$$

This usually overestimates k_La due to enhancement of chemical reaction rate in liquid film around bubbles

Units of k_La = h^{-1} or min^{-1} e.g. 11.5 h^{-1}

Frontier of oxygen transport

$$\frac{dC_{O_2}}{dt} = 0 = k_L a \cdot \left(C^*_{O_2} - C_{O_2,krit} \right) - q_{O_2} \cdot X^e_{\max}$$

Maximale Konzentration an Biomasse, die mit maximalem $k_L a$ des Reaktors ausreichend mit Sauerstoff versorgt werden kann, wenn die O_2-Konzentration gerade den kritischen Wert annimmt.

$$X^e_{\max} = \frac{k_L a}{q_{O_2}} \cdot \left(C^*_{O_2} - C_{O_2,krit} \right)$$

$$S^0_{\max} = \frac{1}{Y_{X/S}} \cdot X^e_{\max}$$

Daraus lässt sich über den Ausbeutekoeffizienten ganz einfach die maximale Substratkonzentration S^0 zu Beginn der Batch-Reaktion bestimmen.

2) Steady state method

Uses bioreactor as respirometer

Measure O_2 in inlet and outlet gas streams and value of C_L

Therefore:

$$k_L a = \frac{OUR}{C^* - C_L}$$

Require gas analyzer e.g. paramagnetic oxygen analyzer and pO_2 electrode

Calculation of OUR / CPR with help of gas-analysis

O$_2$-Aufnahmerate [g/(l·h)]

Begasungs-Volumenstrom [l/h]

O$_2$-Molanteil in der Zuluft [-]

O$_2$-Molanteil in der Abluft [-]

$$OUR = \frac{\dot{V}_G^E}{22{,}4} \cdot 32 \cdot (X_{O_2}^E - X_{O_2}^A) \cdot \frac{1}{V_L}$$

Molvolumen [l/mol]

Molmasse O$_2$ [g/mol]

Flüssigkeits-volumen [l]

Basic informations

For calculation of OUR:

- O_2-concentration at outlet
- gas volume flow at inlet and / or outlet
- and liquid volume of fermenter (working volume) must be known

Gas flow in = gas flow out

But gas flow out can be calculated – if gas composition at outlet is known

Standard Air composition:

20,930 % O_2
0,033 % CO_2

Calculation of gas volume flow at outlet (N_2–balance)

$$\dot{V}_G^E \cdot x_{N_2}^E = \dot{V}_G^A \cdot x_{N_2}^A$$

$$\dot{V}_G^A = \dot{V}_G^E \cdot \frac{x_{N_2}^E}{x_{N_2}^A}$$

$$1 = x_{O_2}^A + x_{CO_2}^A + x_{N_2}^A$$

$$x_{N_2}^A = 1 - x_{O_2}^A - x_{CO_2}^A$$

$$\dot{V}_G^A = \dot{V}_G^E \cdot \frac{x_{N_2}^E}{1 - x_{O_2}^A - x_{CO_2}^A}$$

$$OUR = \left[\frac{32}{22{,}4}\cdot\dot{V}_G^E\cdot X_{O_2}^E - \frac{32}{22{,}4}\cdot\boxed{\dot{V}_G^A}\cdot X_{O_2}^A\right]\cdot\frac{1}{V_L}$$

$$\boxed{\dot{V}_G^E\cdot\frac{X_{N_2}^E}{1-X_{O_2}^A-X_{CO_2}^A}}$$

$$OUR = \left[\boxed{\frac{32}{22{,}4}\cdot\dot{V}_G^E}\cdot X_{O_2}^E - \boxed{\frac{32}{22{,}4}\cdot\dot{V}_G^E}\cdot\boxed{\dot{V}_G^E\cdot\frac{X_{N_2}^E}{1-X_{O_2}^A-X_{CO_2}^A}}\cdot X_{O_2}^A\right]\cdot\frac{1}{V_L}$$

$$OUR = \left[\boxed{\frac{32}{22{,}4}\cdot\dot{V}_G^E}\cdot\left(X_{O_2}^E - \frac{X_{N_2}^E}{1-X_{O_2}^A-X_{CO_2}^A}\cdot X_{O_2}^A\right)\right]\cdot\frac{1}{V_L}$$

CPR / CER

$$CPR = \left[\frac{44{,}01}{22{,}4} \cdot \dot{V}_G^E \cdot \left(-X_{CO_2}^E + \frac{X_{N_2}^E}{1 - X_{O_2}^A - X_{CO_2}^A} \cdot X_{O_2}^A\right)\right] \cdot \frac{1}{V_L}$$

RQ (Respiratory Quotient)

$$RQ = \frac{(CPR \cdot 32)}{(44{,}01 \cdot OUR)}$$

Aerobic: RQ ~ 1
Anaerobic: RQ >> 1

RQ Table

Respiratory substrate	RQ
Carbohydrate	1.0
Lipid	0.7
Protein	0.9

Oxygen transfer rate (OTR)

$$\boxed{N_A = k_L a(C^* - C_L) = OTR}$$

N_A =	volumetric mass transfer rate ($mMO_2 l^{-1} h^{-1}$)
k_L =	mass transfer coefficient at phase boundary (ms^{-1})
a =	volumetric mass transfer area ($m^2 m^{-3} = m^{-1}$)
C* =	dissolved gas concentration in phase boundary ($mM\ l^{-1}$)
C_L =	dissolved oxygen concentration ($mM\ l^{-1}$)
OTR =	oxygen transfer rate ($mM\ l^{-1}\ h^{-1}$)

Oxygen transfer rates (OTR) in bioreactors / fermenters

Reactor Volume (m^3)	Impellor	Assay method	OTR mM $O_2 L^{-1} h^{-1}$
0.1	Turbine	Sulphite	100-223
0.8	Turbine	Sulphite	94
1.2	Turbine	Sulphite	64
5.0	Turbine	Sulphite	45-72
47.7	Turbine	Sulphite	42
34.2	Waldhof	Yeast	16-22
58.5	Vogelbusch	Yeast	26-43

k_La-Werte für verschiedene Reaktor-systeme, die in der Biotechnologie zum Einsatz kommen:

Reaktortyp	k_La h-1
Schüttelkolben	8…200
Rührkesselreaktor	325…2650
Blasensäule	140
Druckschlaufenreaktor	400
Tropfkörper	350
Air-Lift-Schlaufenreaktor	350
Siebbodenreaktor	< 1000
Paddelrad-Reaktor	1000

Oxygen transfer capacity in bioreactors

	OTR (mmol L^{-1} h^{-1})	K_La (h^{-1})
Rotary shakers:		
flasks without baffles	15-30	60-120
flasks with baffles	< 150	< 600
Fermenters (microbial):		
lab scale	200-400	800-1600
production scale (20-300m^3)	< 100	< 400
Animal cell bioreactors	< 1	< 4

Example 1: Cell concentration in aerobic culture

A strain of *Azotobacter vinelandii* is cultured in a 15m^3 stirred Fermenter for alginate production. Under current operating conditions k_La is 0.17 s^{-1}. Oxygen solubility in the broth is approx. 8 x 10^{-3} kg m^{-3}.

a) The specific rate of oxygen uptake is 12.5 mmol g^{-1} h^{-1}. What is the maximum possible cell concentration?

b) The bacteria suffer growth inhibition after copper sulphate is accidently added to the fermentation broth. This causes a reduction in oxygen uptake rate to 3 mmol g^{-1} h^{-1}. What maximum cell concentration can now be supported by the fermenter?

Example 2: Specific oxygen uptake in *E.coli* culture

It is assumed, that the specific oxygen uptake rate (qO_2) of *E. coli* is 5.0 mmol g^{-1} h^{-1}. Which cell concentration X can be reached in a laboratory reactor with a k_La of 25 h^{-1}. When C_L = 10 % C*. and for the medium at 37 °C is C* = 0.17 mmol L^{-1}

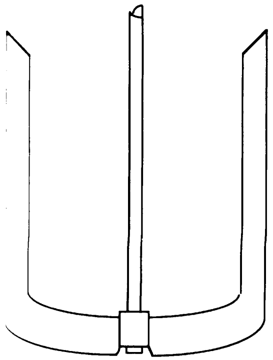
Example 3:

1. Estimate how fast the dissolved oxygen concentration is consumed in a bioreactor with KLa 1000 h-1, containing a 10 g/L culture growing with $\mu = 0.5\ h^{-1}$ if the aeration is interrupted.

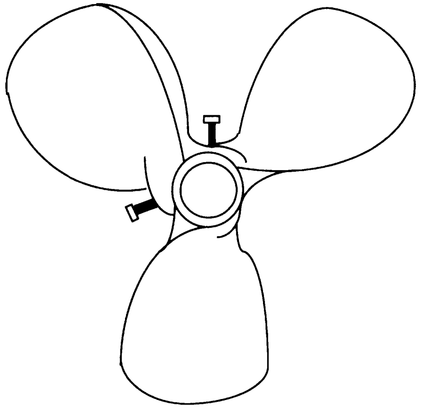
First calculate the quasi-steady state oxygen concentration. Assume $Y_{X/O} = 1$ g/g and the oxygen solubility in the medium equilibrium with air $C^* = 7$ mg/L

Mixing /Mixing equipment

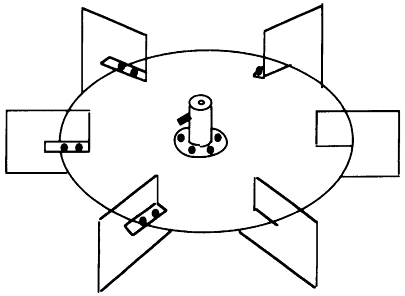
Video principles of mechanical agitation



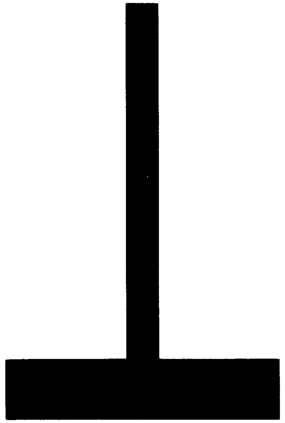
Anchor



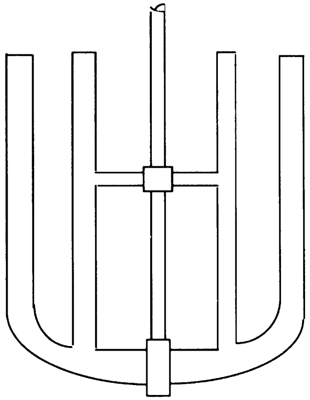
Propeller



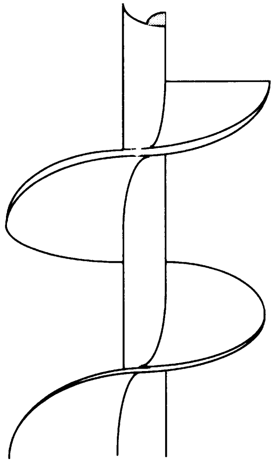
6-flat-blade disc-turbine



Paddle

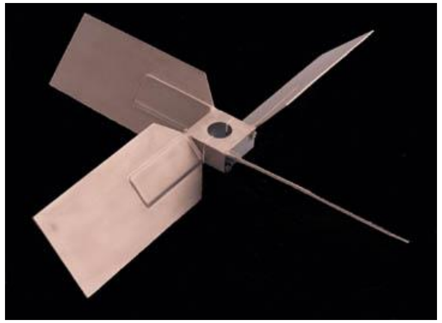


Gate anchor



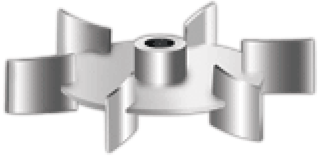
Helical screw

Pitched-blade



Flat-blade radial

Propeller



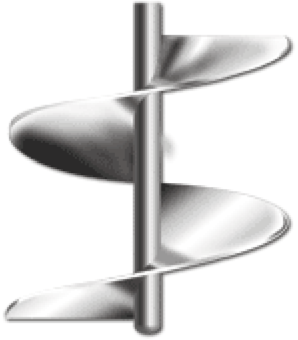
Flat-blade disc turbine

Helic impeller

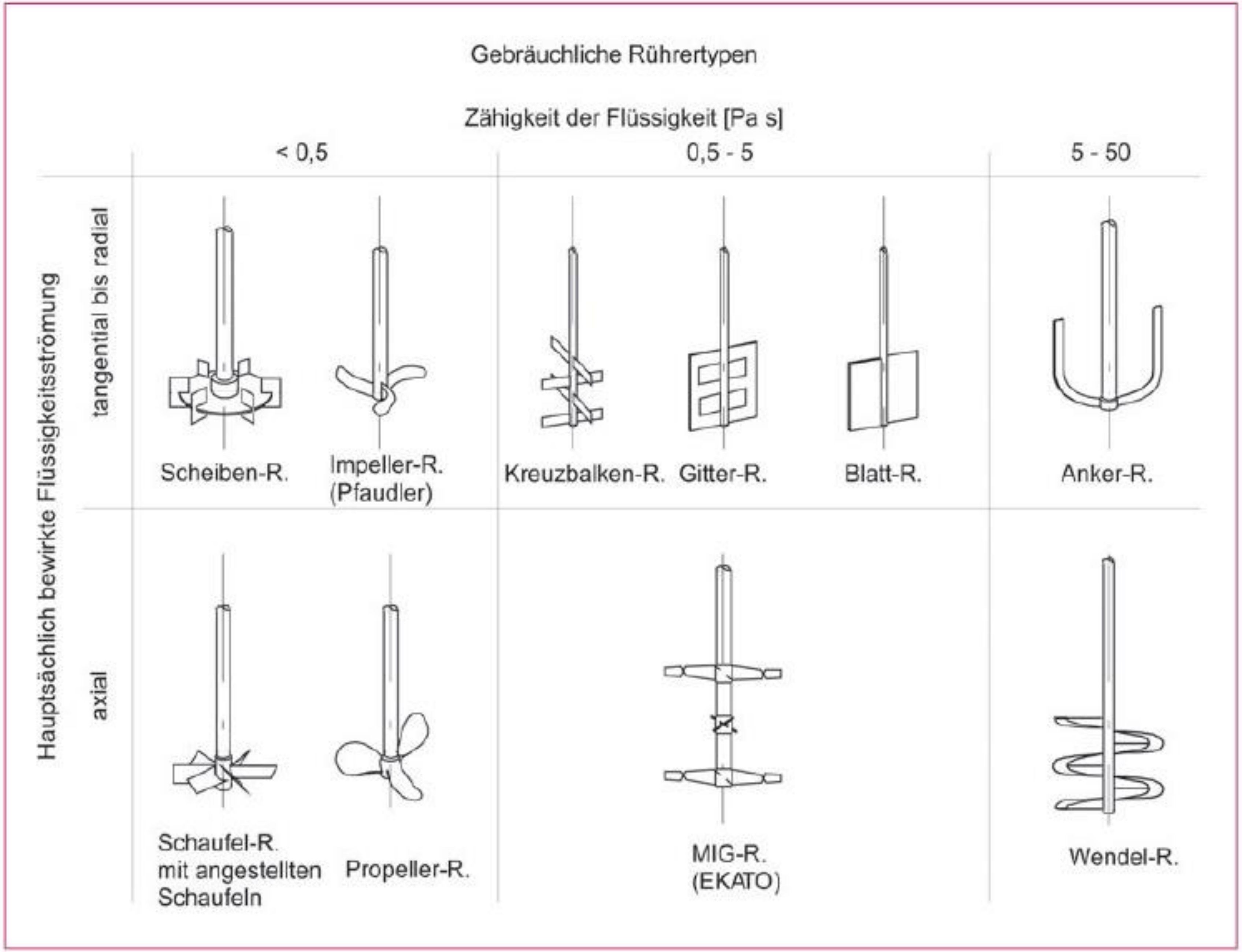
Rushton turbine
with three blades.

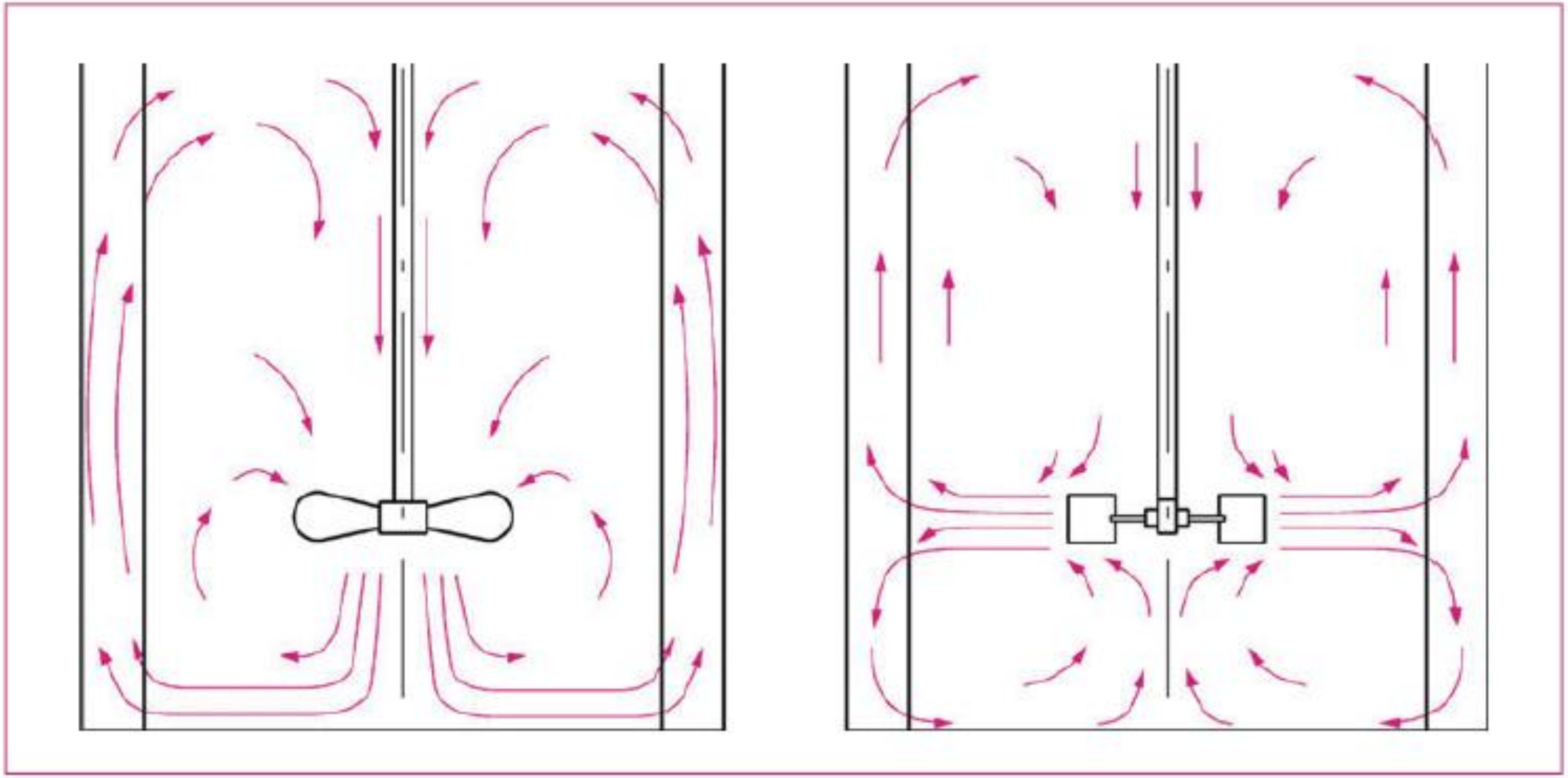
ribbon

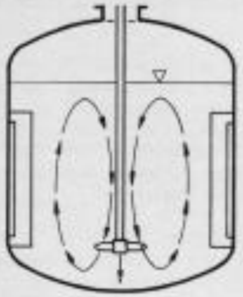
Gate anchor



Helical screw

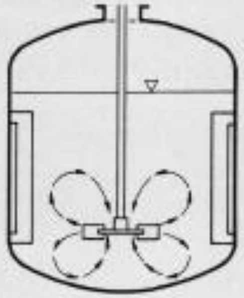

Gebräuchliche Rührertypen
Zähigkeit der Flüssigkeit [Pa s]
< 0,5
0,5 - 5
5 - 50
Hauptsächlich bewirkte Flüssigkeitsströmung
tangential bis radial
axial
Scheiben-R.
Impeller-R. (Pfaudler)
Kreuzbalken-R.
Gitter-R.
Blatt-R.
Anker-R.
Schaufel-R. mit angestellten Schaufeln
Propeller-R.
MIG-R. (EKATO)
Wendel-R.





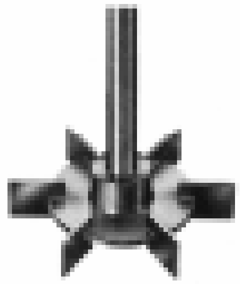
d_2/d_1	u [m/s]	η_{max} [m Pa s]	Strömungsbereich
0,1 ÷ 0,5	2 ÷ 15	10 000	turbulent

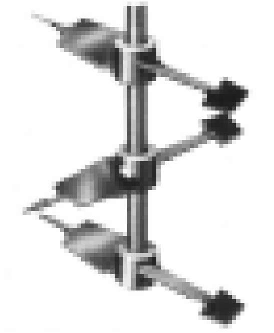
Propeller

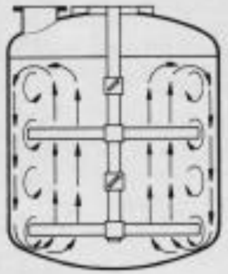


d_2/d_1	u [m/s]	η_{max} [m Pa s]	Strömungsbereich
0,2 ÷ 0,5	3 ÷ 7	10 000	turbulent

Scheibenrührer

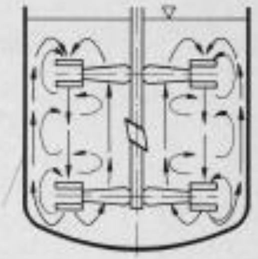






d_2/d_1	u [m/s]	η_{max} [m Pa s]	Strömungsbereich
0,6 ÷ 0,9	2 ÷ 6	50 000	turbulent/laminar

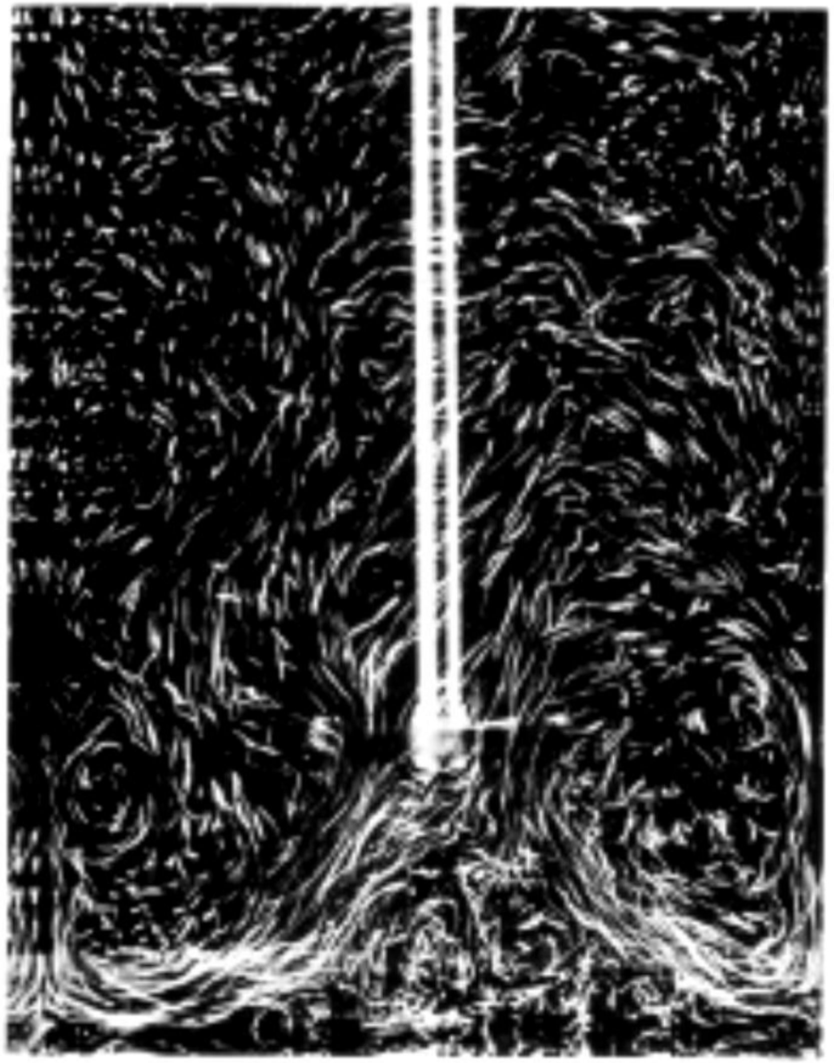
Kreuzbalkenrührer ($\alpha = 45°$)



d_2/d_1	u [m/s]	η_{max} [m Pa s]	Strömungsbereich
0,5 ÷ 0,95	1 ÷ 9	40 000	turbulent/laminar

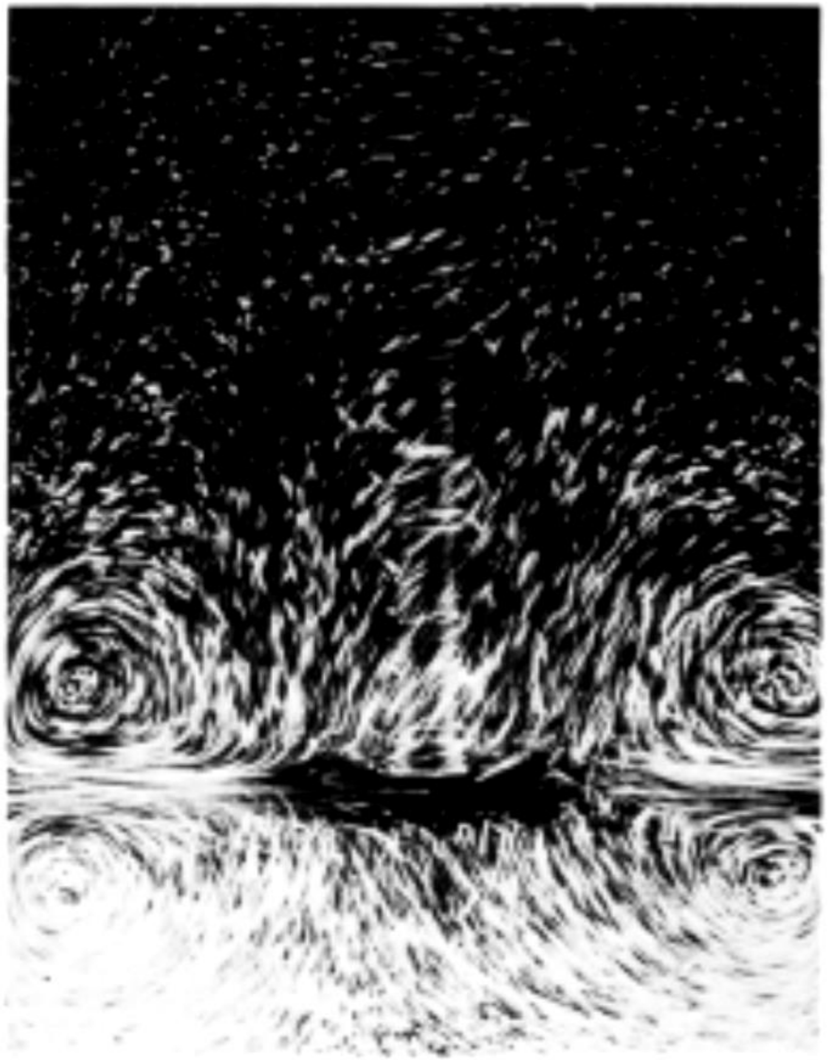
INTERMIG®

Propeller



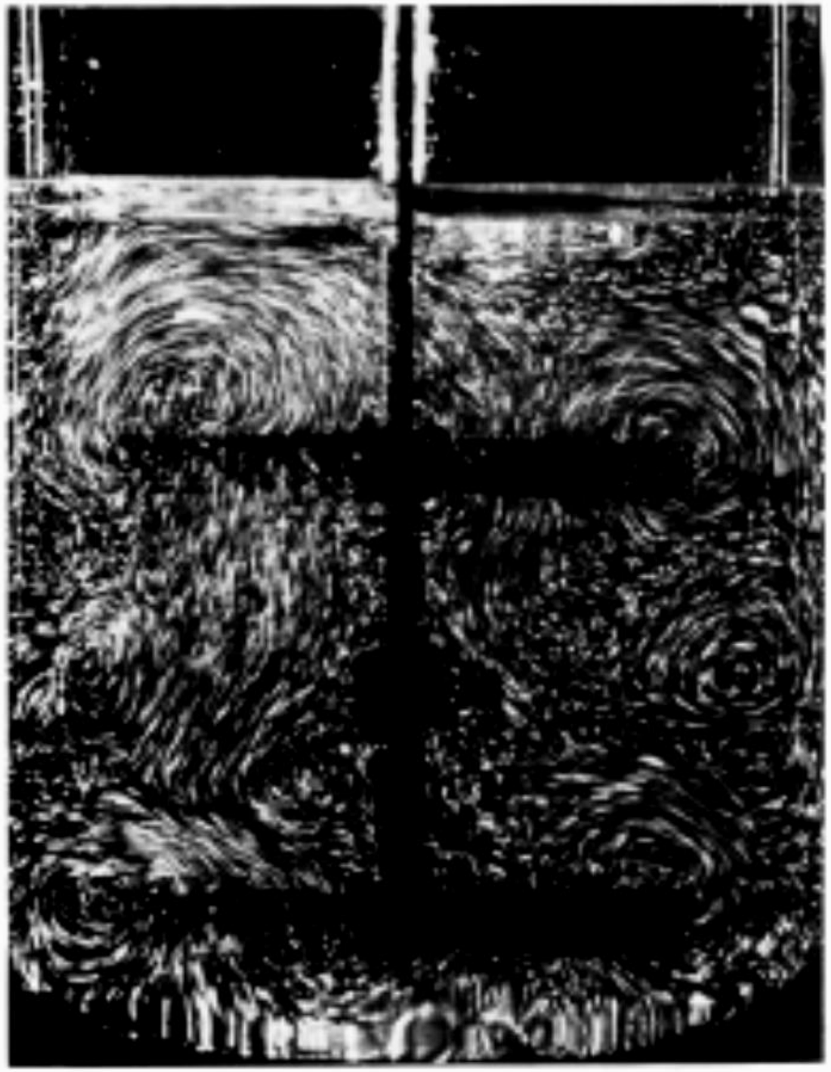
$Re = 5 \cdot 10^4$ *(turbulent)*

Scheibenrührer



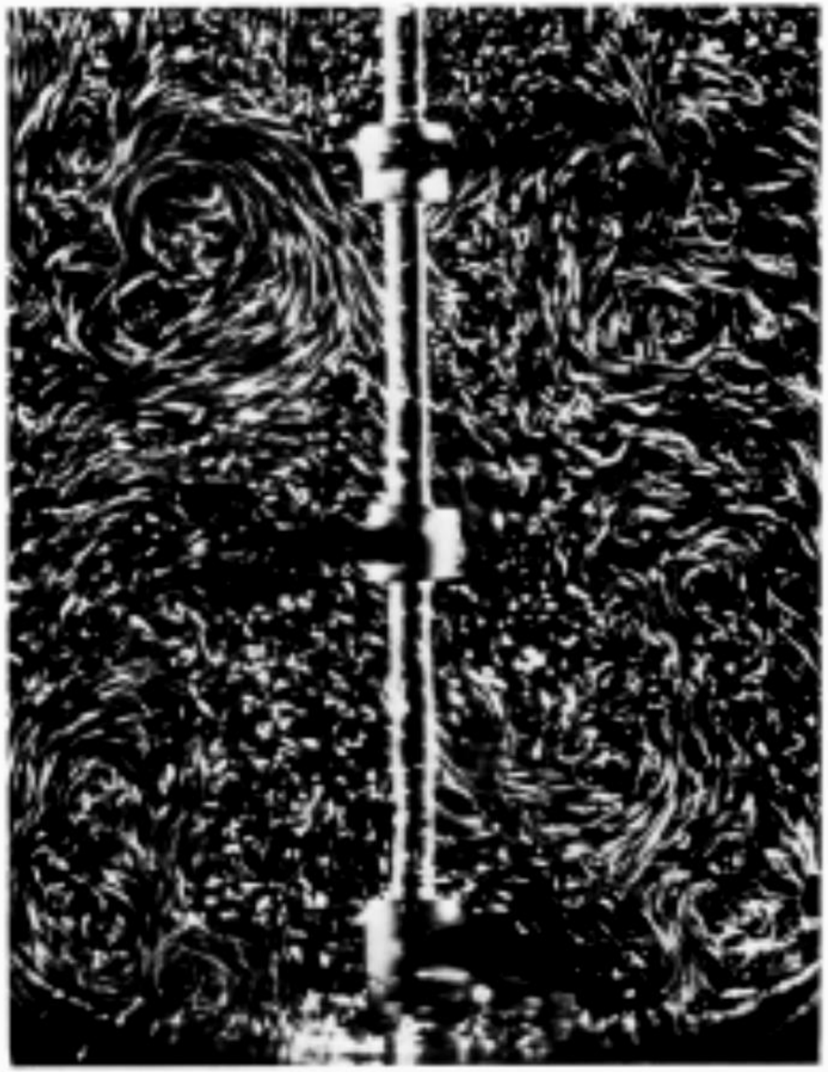
$Re = 5 \cdot 10^4$ *(turbulent)*

Kreuzbalkenrührer ($\alpha = 45°$)



Re = 300 (laminar)

INTERMIG®

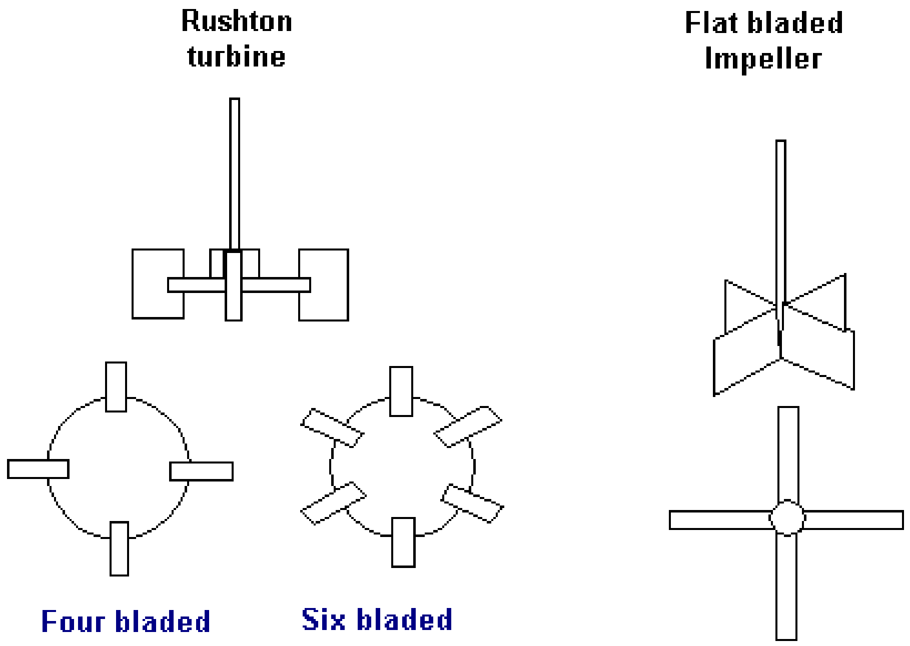


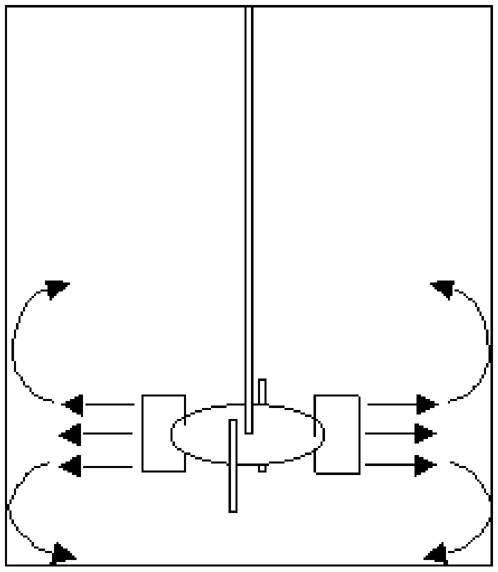
Re = $5 \cdot 10^4$ (turbulent)

Agitator design and operation

Radial flow impellers

Radial flow impellers contain two or more impeller blades which are set at a vertical pitch:





With radial flow impellers, the liquid is pushed towards the wall of the tank; that is, along the radius of the reactor

With radial flow impellers, vertical (or axial) mixing is achieved with the use of baffles.

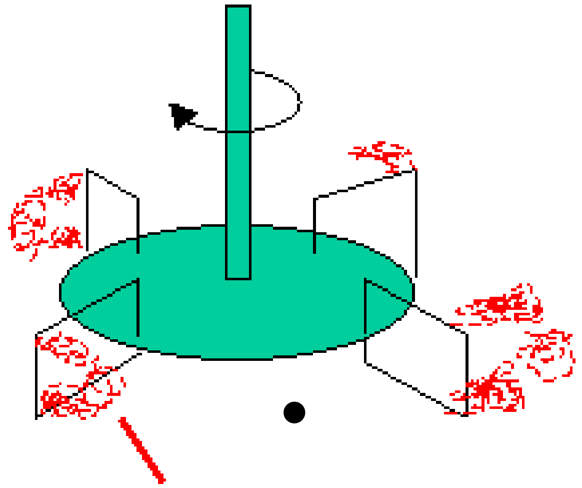
Radial flow mixing is not as efficient as axial flow mixing for radial flow impellers, a much higher input of energy input is required to generate a given level of flow as compared to axial flow impellers.

Radial flow impellers - Shear characteristics

Radial flow impellers do and are designed to, generate high shear conditions. This is achieved by the formation of vortices in the wake of the impeller:

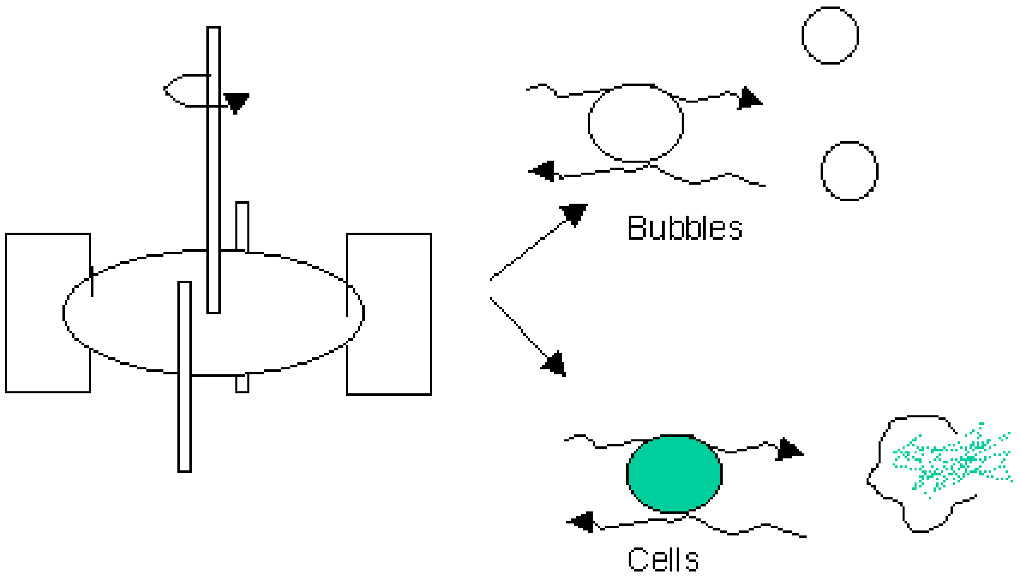
The high shear is effective at breaking up bubbles. For this reason, radial flow impellers are used for the culture of aerobic bacteria.

High shear can also damage shear sensitive materials such as crystals and precipitates and shear sensitive cells such as filamentous fungi and animal cells



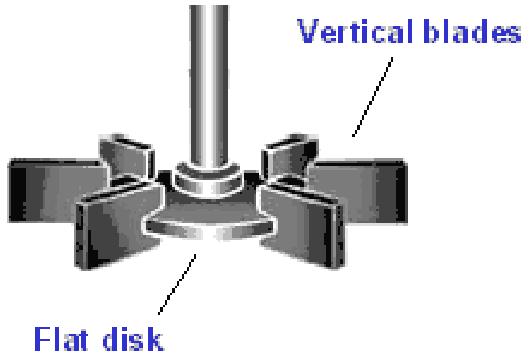
Eddies form in the wake of the impeller blades and generate a high shear environment

Radial flow impellers are effective at generating high shear conditions. This aids in breaking up bubbles but can also lead to cell damage



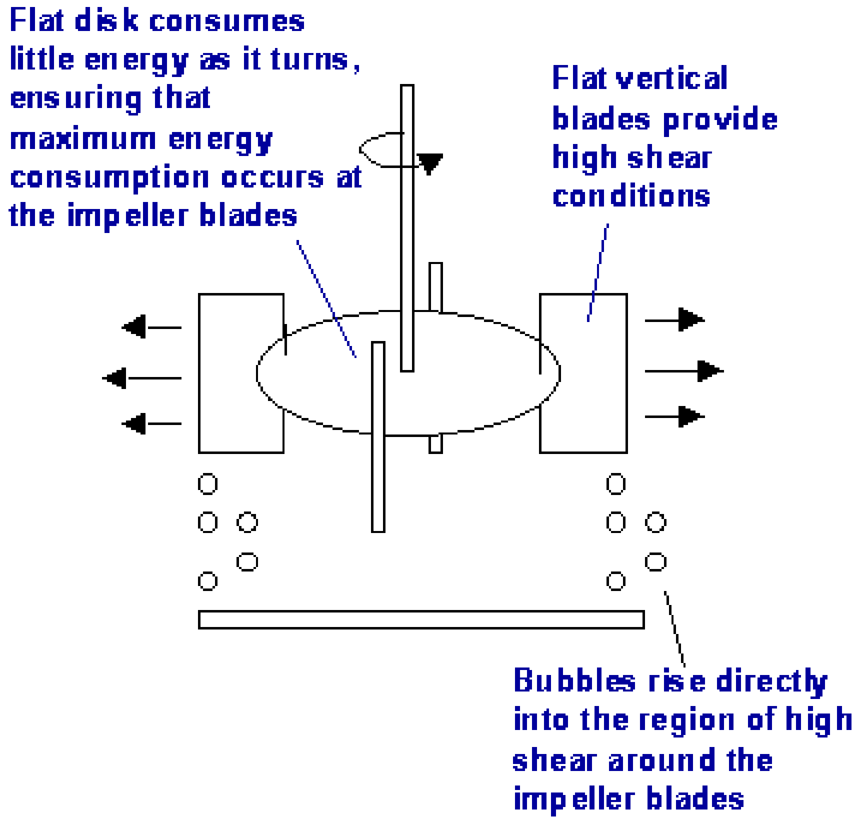
Radial flow impellers - Rushton turbine

The most commonly used agitator in microbial fermentations is the Rushton turbine.
Like all radial flow impellers, the Rushton turbine is designed to provide the high shear conditions required for breaking bubbles and thus increasing the oxygen transfer rate.
The Rushton turbine has a 4 or 6 blades which are fixed onto a disk. The diameter of the Rushton turbine should be 1/3 of the tank diameter

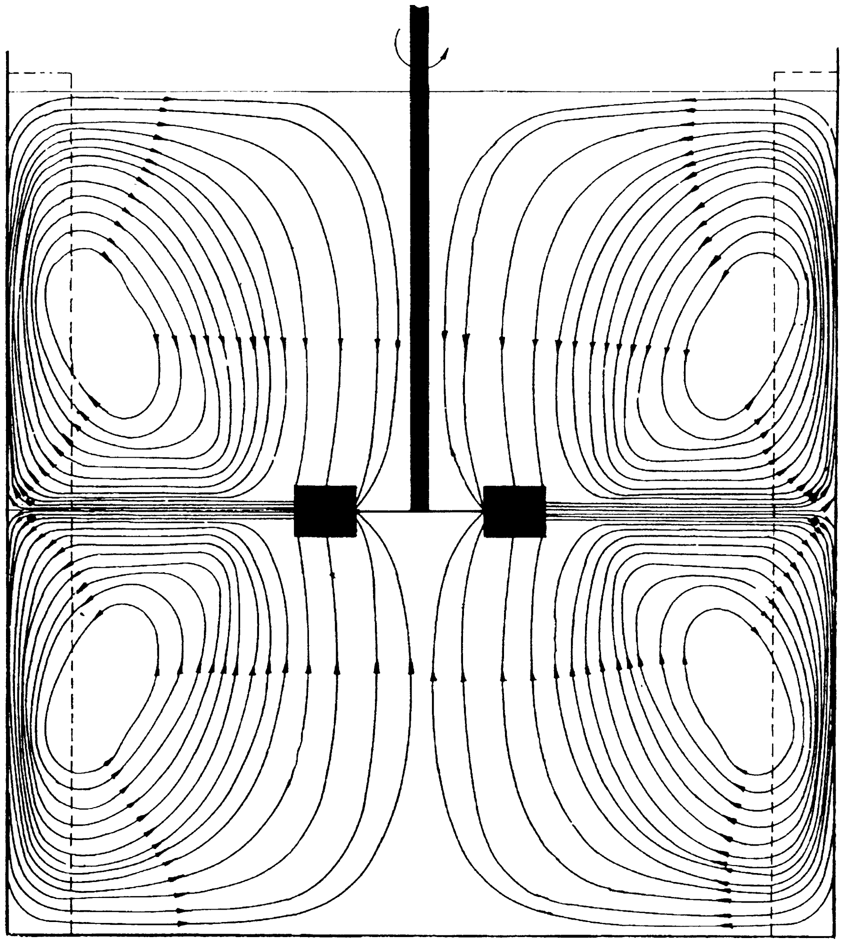


A Rushton turbine is often referred to as a disk turbine.

The disk design ensures that most of the motor power is consumed at the tips of the agitator and thus maximizing the energy used for bubble shearing.



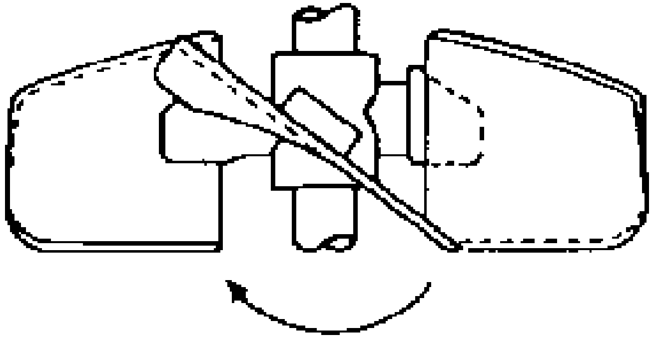
Radial flow impellers - Rushton turbine

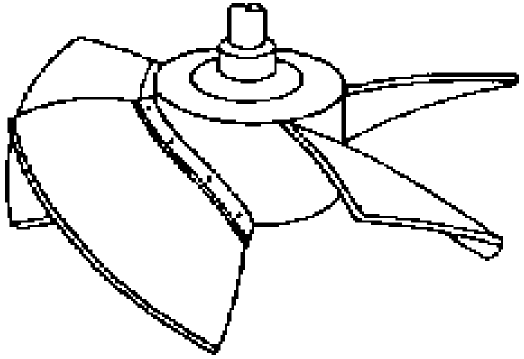


Flow pattern developed by a centrally-positioned radial-flow impeller

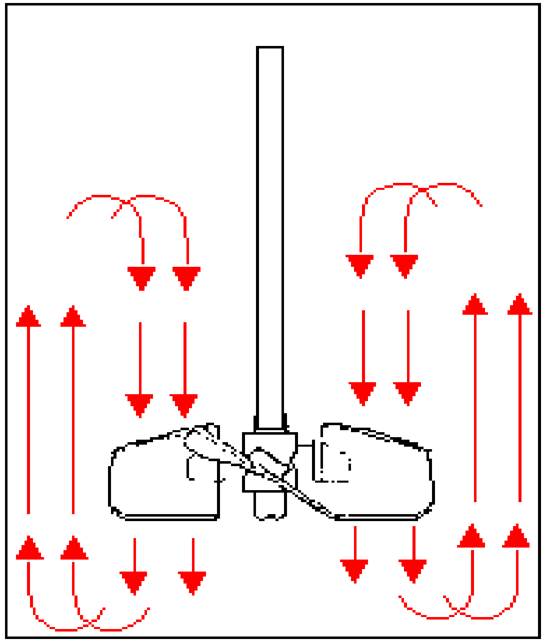
Axial flow impellers

Axial flow impeller blades are pitched at an angle and thus direct the liquid flow towards the base of the tank.
Examples of axial flow impellers are marine impellers and hydrofoil impellers.





The resultant flow pattern is thus predominantly vertical; ie. along the tank axis

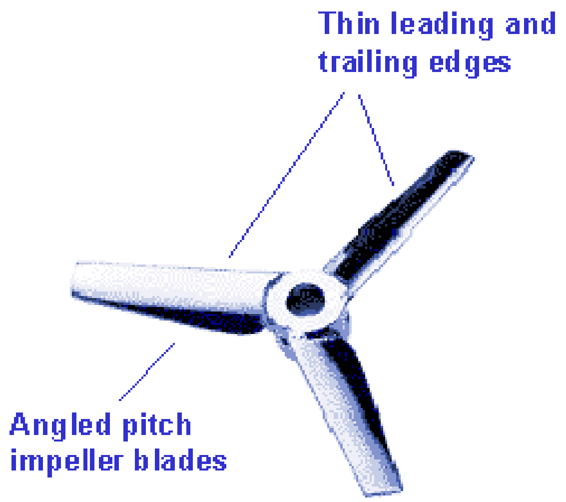


With axial impellers, the liquid is pushed in a downward direction; that is, along the axis of the reactor.

Axial flow mixing is considerably more energy efficient than radial flow mixing.

Axial flow impellers

They are also more effective at lifting solids from the base of the tank. Axial flow impellers **have low shear properties**. The angled pitch of the agitators coupled with the thin trailing edges of the impeller blades reduces formation of eddies in the wake of the moving blades.



Low shear conditions are achieved by pitching the impeller blades at an angle and by making the edges of the impeller blades thing and smooth.

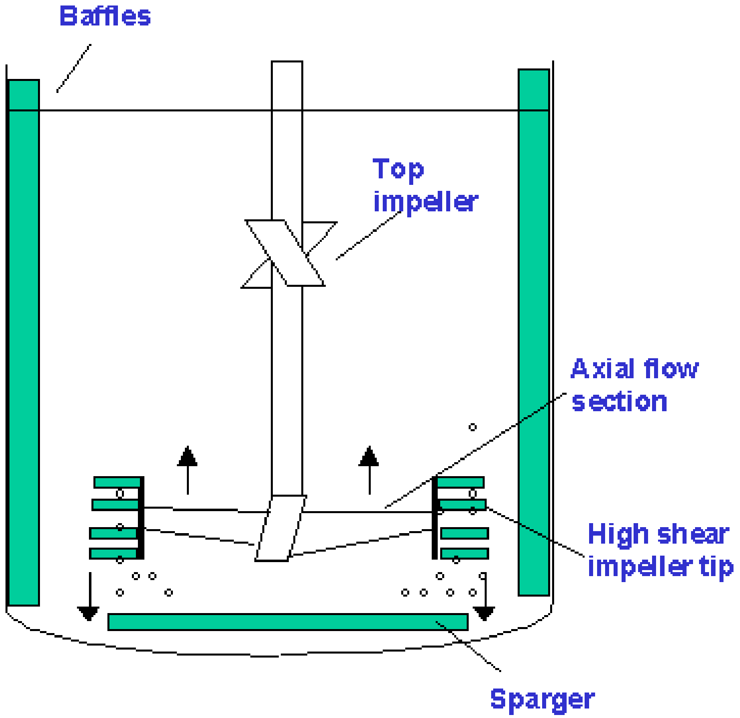
Axial flow impellers are used for mixing shear sensitive processes such as crystallization and precipitation reactions. They are also used widely in the culture of animal cells.

Their low shear characteristics generally makes them ineffective at breaking up bubbles and thus unsuitable for use in aeration of bacterial fermentations

Axial flow impellers

Intermig Impeller

The Intermig impeller is a axial flow which is used for microbial fermentations. The impeller is shown in the following diagram:



The agitation system has two impellers. The bottom impeller has a large axial flow section. The tips of the impeller contain finger like extensions which create a turbulent wake for breaking bubbles. As the high shear region exists only at the tip, the overall shear conditions in the reactor are lower than would be generated by a radial flow impeller such as a Rushton Turbine. Intermig impellers are used widely for agitation and aeration in **fungal fermentations.**

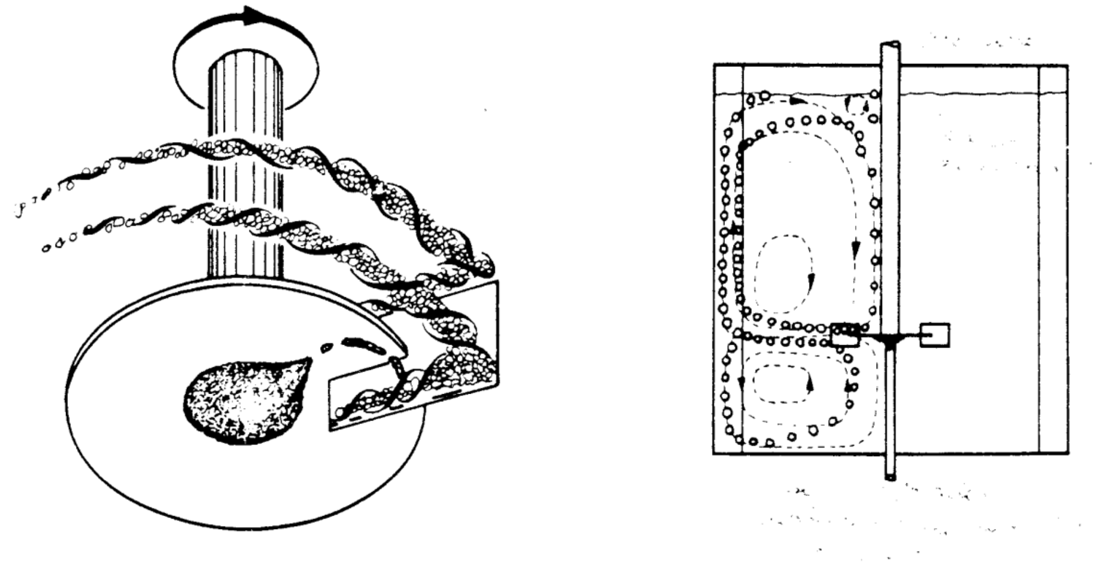


Abb. 18: Dispergierung der Luft und Blasenstroemung bei kleinem Gasdurchsatz, Scheibenruehrer [9]

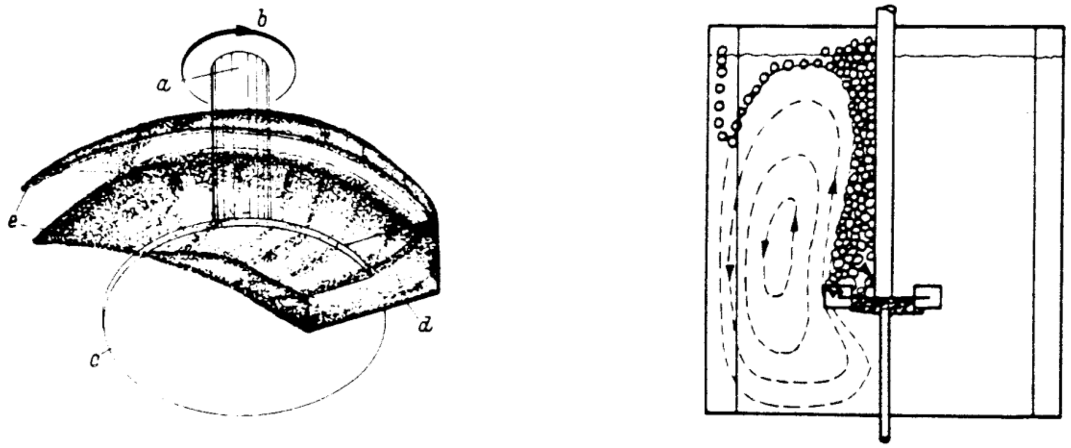
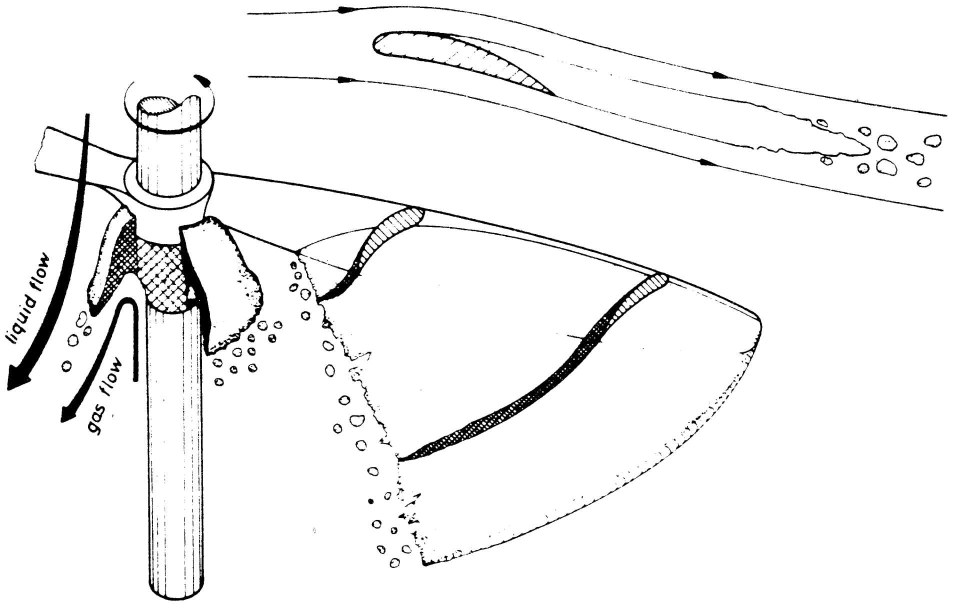


Abb. 19: Dispergierung der Luft und Blasenstroemung bei grossem Gasdurchsatz, Scheibenruehrer [9]



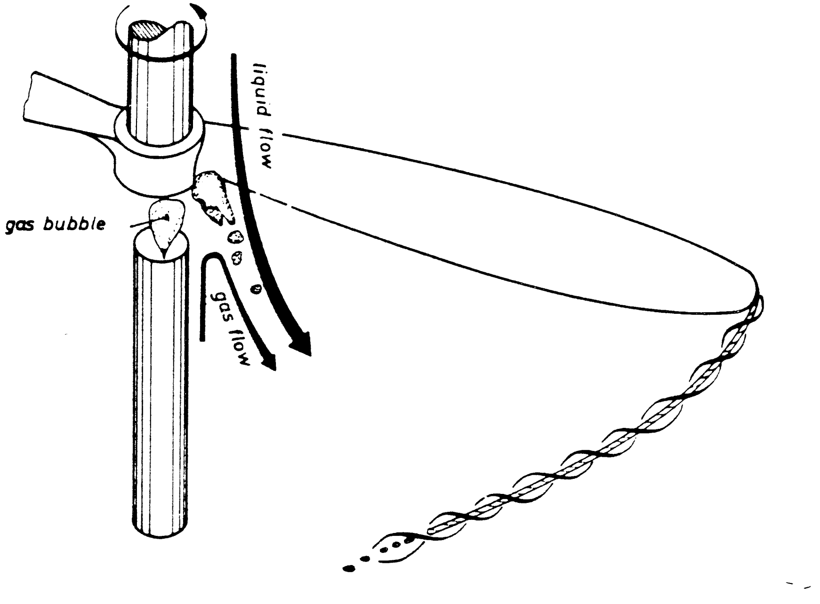
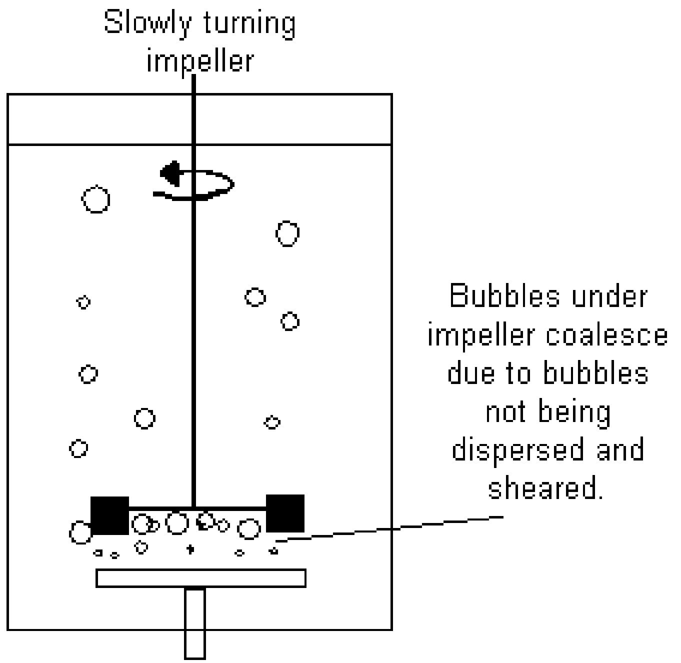


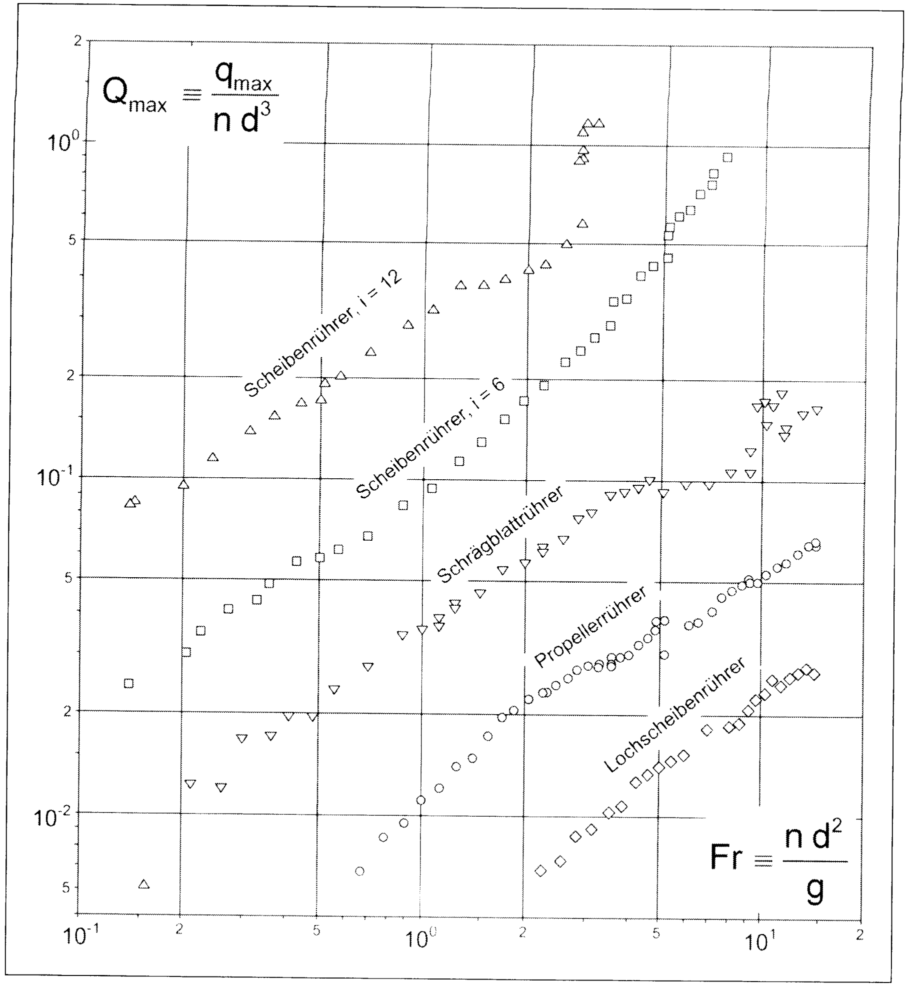
Abb. 20: Dispergierung der Luft durch einen Propellerruehrer bei grossem (oben) und bei kleinem Gasdurchsatz (rechts) [9]

Flooded impeller

If the agitation speed is too low or the air flow rate is too high, then a phenomenom known as a **flooded impeller** will occur.

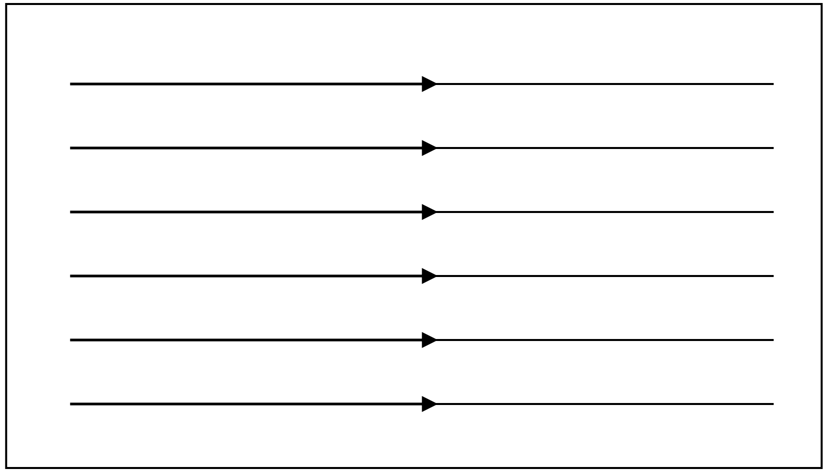


When the impeller is flooded, bubbles will accumulate underneath the impeller and coalesce. This leads to the formation of large bubbles and poor oxygen transfer efficiencies.



Characteristic of flooding for different impellers
D/d = 5; H/D = 1, i = amount of blades for impeller

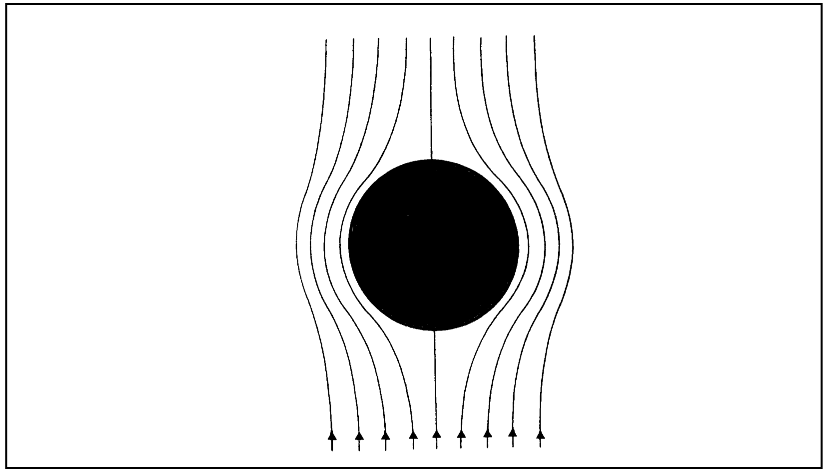
Reynolds-Number



Constant fluid velocity

$$Re = \frac{D_i \bullet v \bullet \rho}{\eta}$$

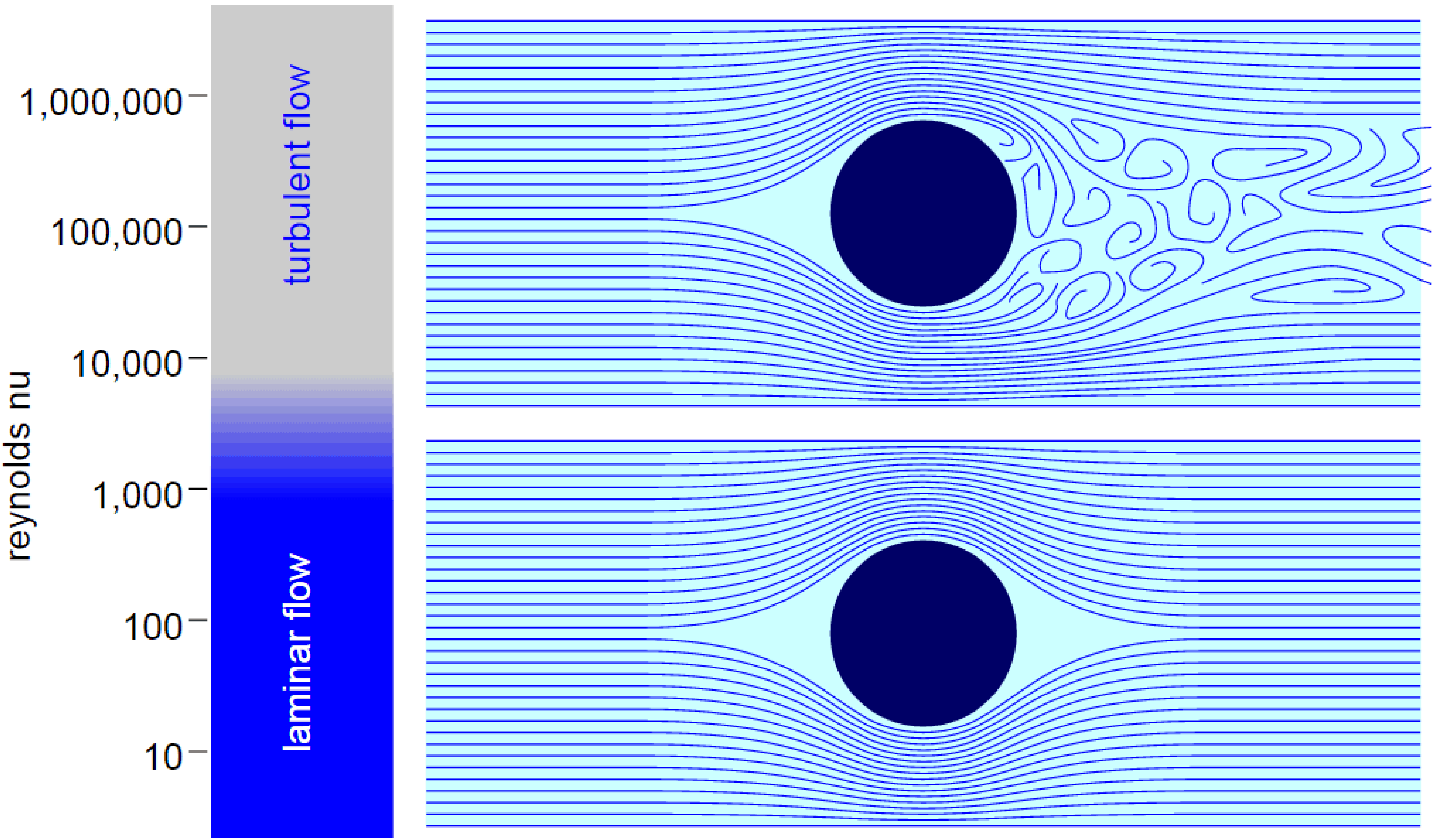
D: Pipe-Diameter; *v:* average linear velocity
ρ: fluid density; η: fluid viscosity



Steady flow over a submerged object

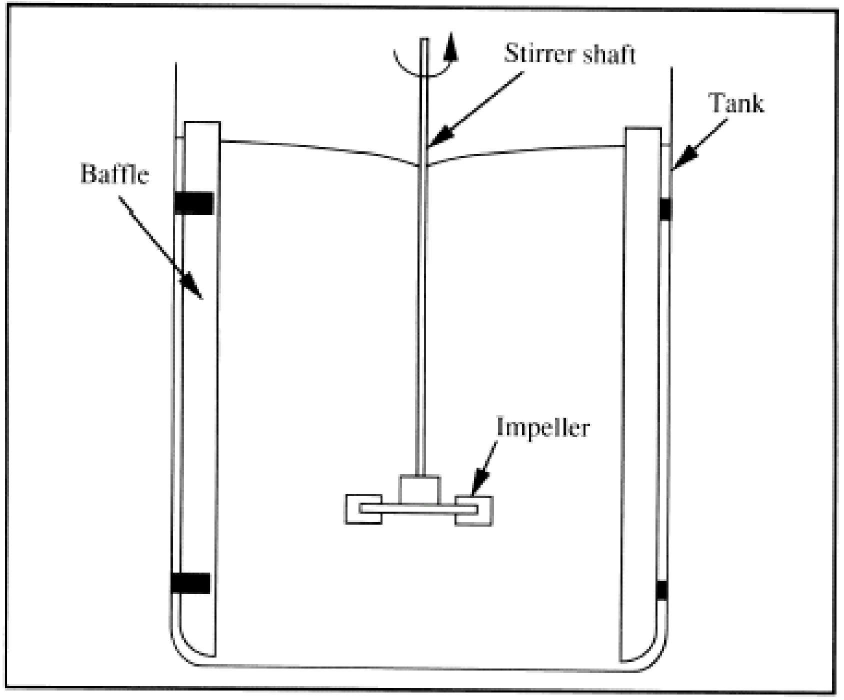
$$Re = \frac{D_i^2 \bullet N_i \bullet \rho}{\eta}$$

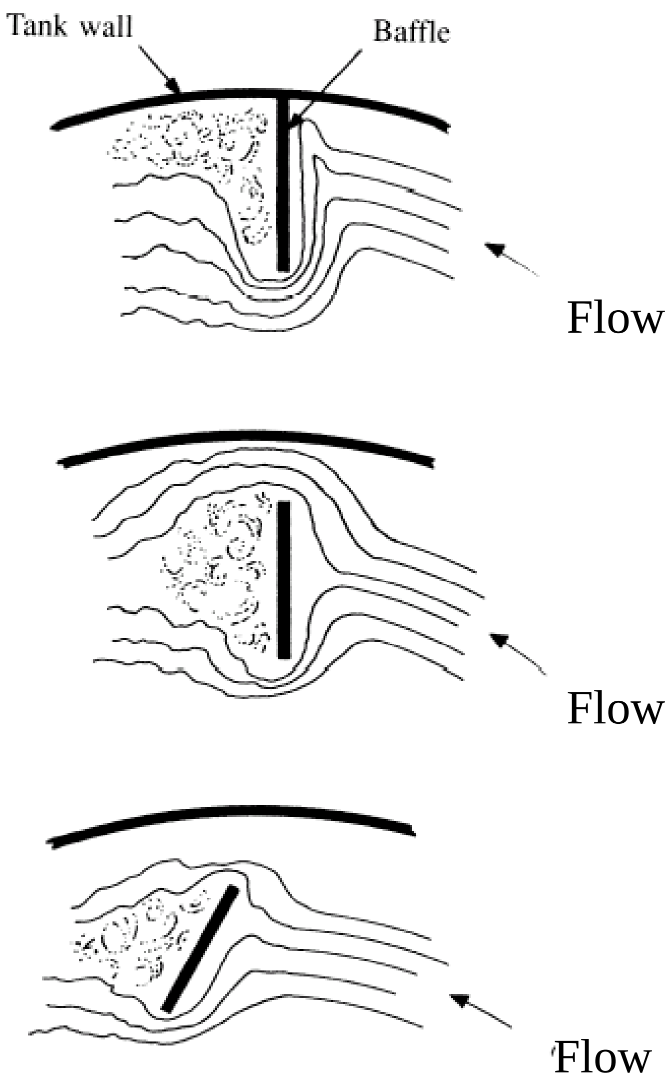
D: Impeller-Diameter; N_i: stirrer speed
ρ: fluid density; η: fluid viscosity



1,000,000
100,000
10,000
1,000
100
10
reynolds nu
turbulent flow
laminar flow

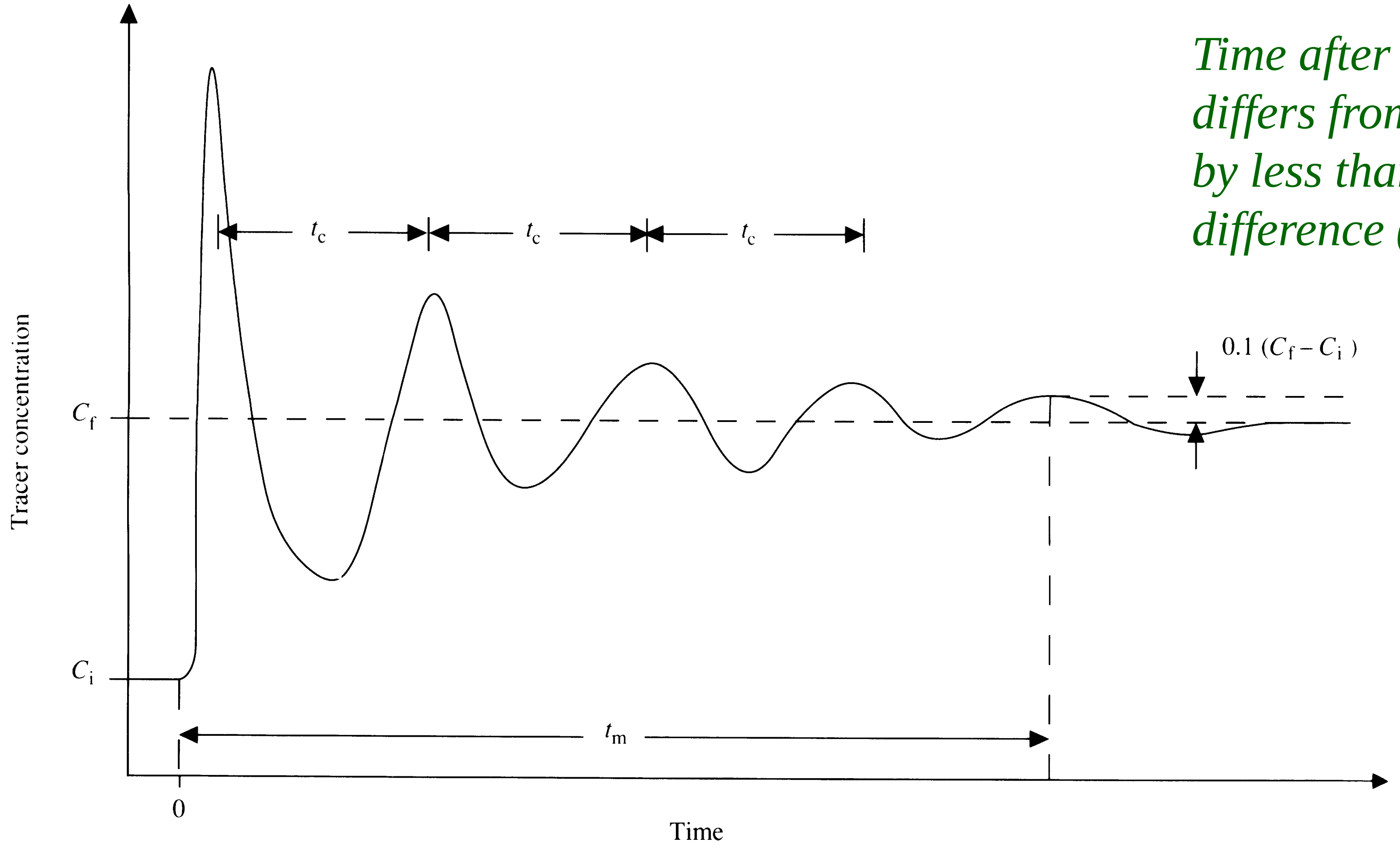
Mixing /Mixing equipment





Mechanism of mixing:
- distribution (macromixing)
- dispersion
- diffusion (micromixing)

Mixing: assessing mixing effectiveness

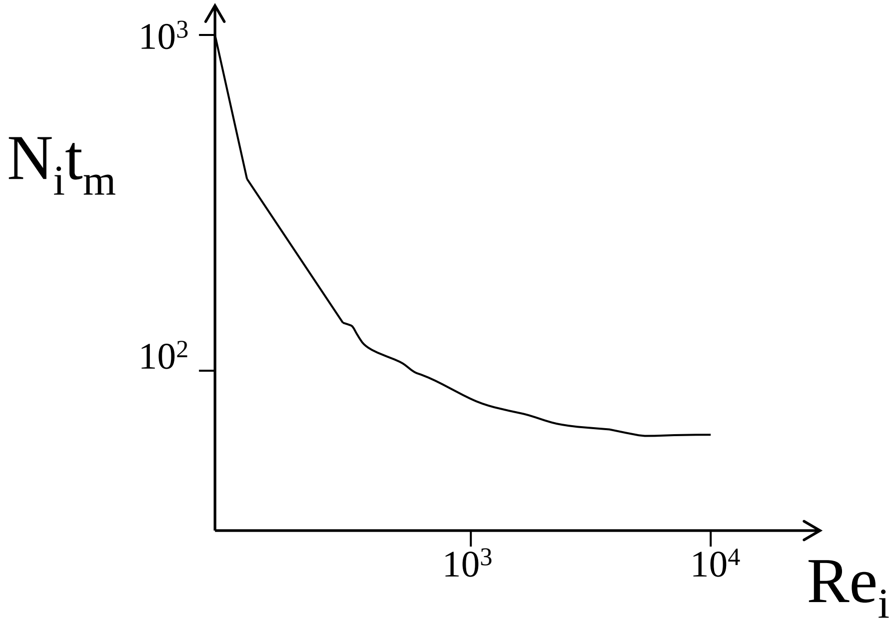


Time after which the concentration of tracer differs from the final concentration C_f by less than 10% of the total concentration difference ($C_f - C_i$).

For a single-phase liquid in a stirred tank with several baffles and small impeller: $t_m = 4\ t_c$ t_c: circulation time

Industrial scale (1-100 m^3): 30 – 120 s mixing times

Mixing: assessing mixing effectiveness



For Rusthon turbines:

$$N_i t_m = \frac{1.54\ V}{D_i^3}$$

(At high Re_i)

Ni: speed of impeller t_m: mixing time
V: liquid Volume D_i: impeller diameter

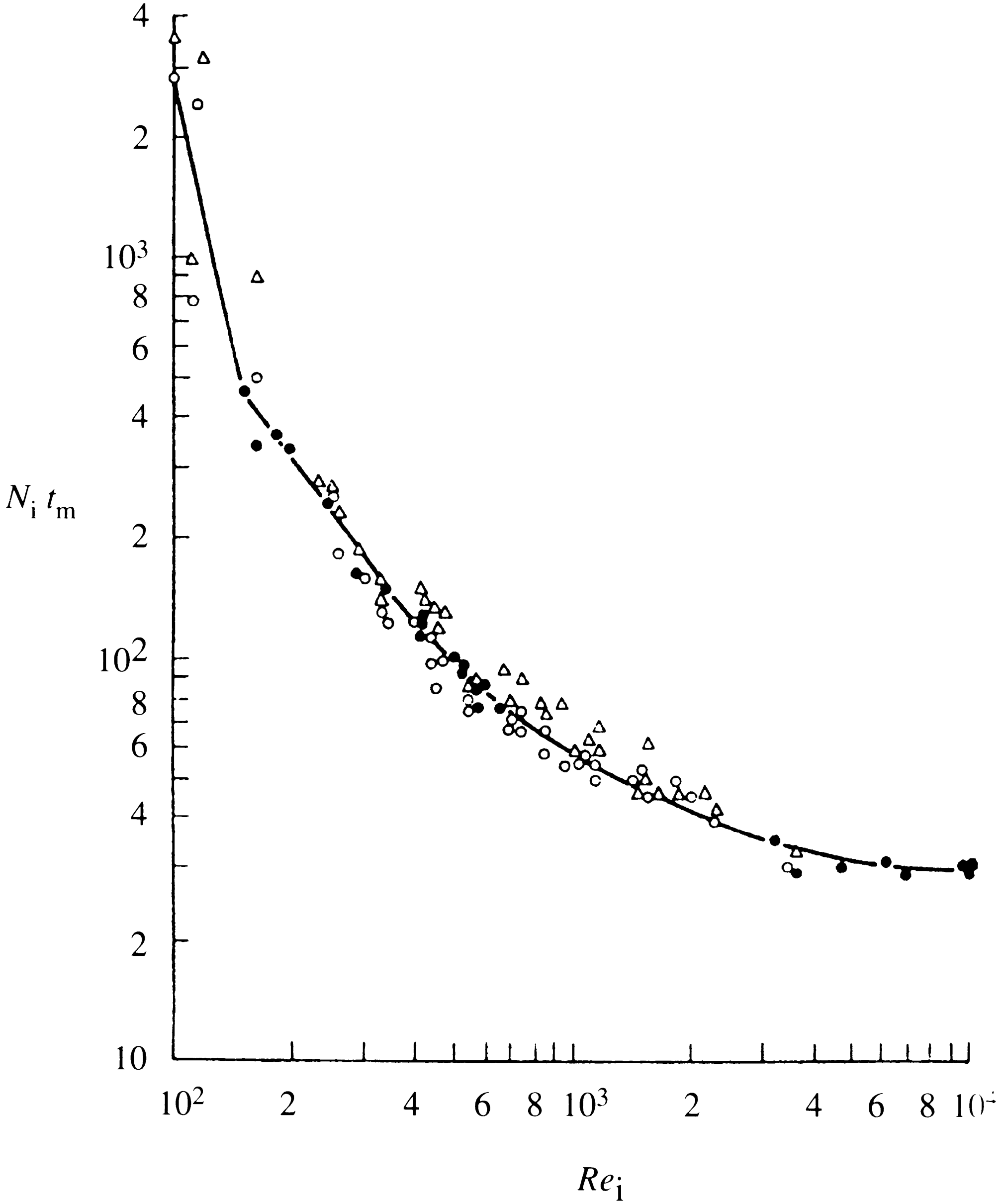
At low Reynold, $N_i t_m$ increases significantly with decreasing Re_i

$Re_i > 5 \times 10^3$, $N_i t_m$ approaches a constant value which persists at high Re_i
$N_i t_m$ at high Reynolds-numbers depends only on the size of the tank and stirrer

$N_i t_m$: represents the number of stirrer rotations required to homogenise the liquid

Mixing: assessing mixing effectiveness

Variation of mixing time with Reynolds number for a six-blade Rushton turbine in a baffled tank. The impeller is located one-third the tank diameter off the floor of the vessel; the impeller diameter is one-third the tank diameter. The liquid height is equal to the tank diameter; the tank has four baffles of width one-tenth the tank diameter. Several measurement techniques and tank sizes were used: (●) thermal method, 1.8-m diameter vessel; (○) thermal method, 0.24-m vessel; (△) decoloration method, 0.24-m vessel. (Reprinted from C.J. Hoogendoorn and A.P. den Hartog, Model studies on mixers in the viscous flow region, *Chem. Eng. Sci.* 22, 1689–1699. Copyright 1967, with permission from Pergamon Press Ltd, Oxford.)



Mixing and agitation

$$\mathrm{Re} = N_{\mathrm{Re}} = \frac{D_i^2 \bullet N \bullet \rho}{\eta} \quad [-]$$

where:
D_i = stirrer diameter
N = agitation speed (s^{-1})
ρ = density ($g\ cm^{-3}$)
η = dynamic viscosity ($g\ cm^{-1}\ s^{-1}$)

For most bioreactors the relative velocity between the nutrient solution and individual cells should be approximately **0.5 ms^{-1}** (i.e. highly turbulent)

	Small fermenter	**Large fermenter**
Water (η = 10^{-2} g cm^{-1} s^{-1} = 1 centi Poise)	**4×10^5**	**6.9×10^6**
Culture medium (η = 5 g $cm^{-1}s^{-1}$ = 500 centi Poise)	**$8 * 10^2$**	**$1.4 * 10^2$**

Reynold 's numbers for typical bioreactors

Example : Estimation of Mixing time

A fermentation broth with viscosity 10^{-2} Pa s and a density 1000 kg m^{-3} is agitated in a 2.7 m^3 baffled tank using a Rushton turbine with diameter 0.5 m and a stirrer speed 1 s^{-1}. Estimate the mixing time.

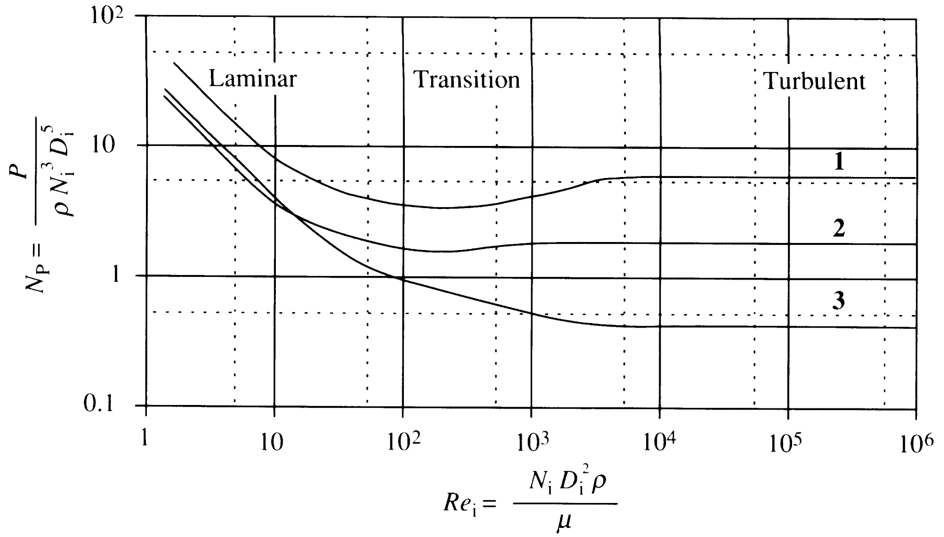
1 Pa·s = **1 kg/(m·s)**

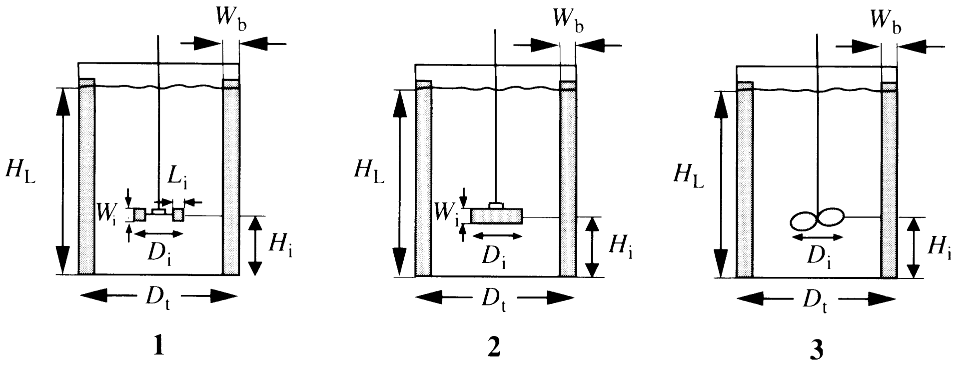
Power number

$$N_P = \frac{\text{imposed force}}{\text{inertial force}}$$

$$\boxed{N_P = \frac{P_0}{N^3 D_i^5 \rho}}$$

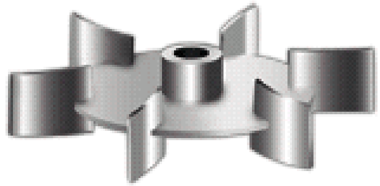
P_0 = stirring power (kW)
N = stirrer speed (s^{-1})
D_i = stirrer diameter (cm)
ρ = medium density ($g\ cm^{-3}$)



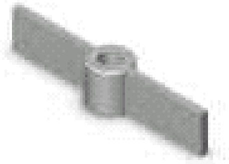


Impeller	D_t/D_i	H_L/D_i	H_i/D_i	Baffles	
				W_b/D_t	Number
1. Rushton turbine $W_i/D_i = 0.2$, $L_i/D_i = 0.25$	3	3	1	0.1	4
2. Paddle $W_i/D_i = 0.25$	3	3	1	0.1	4
3. Marine propeller Pitch = D_i	3	3	1	0.1	4

Correlation between power number and Reynolds number for Rusthon turbine, paddle and marine propeller without sparging

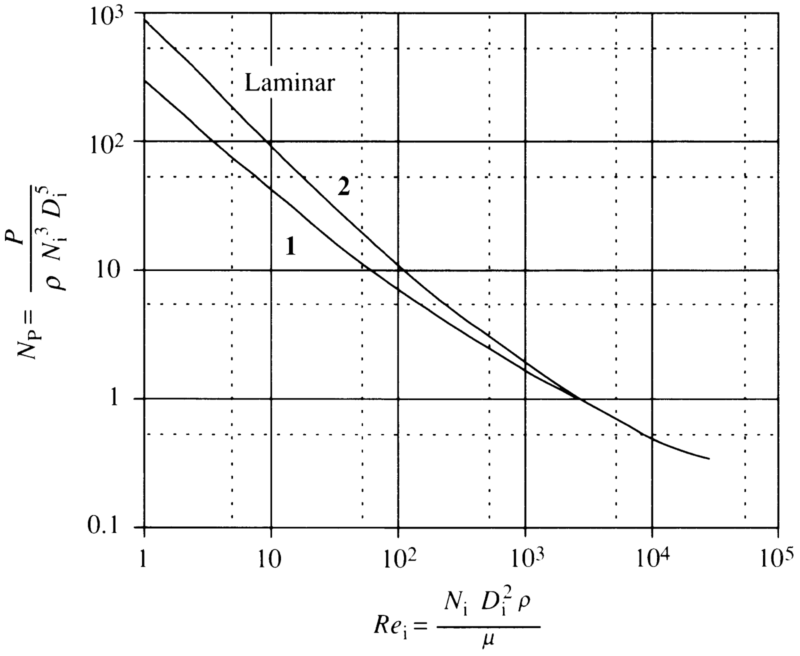


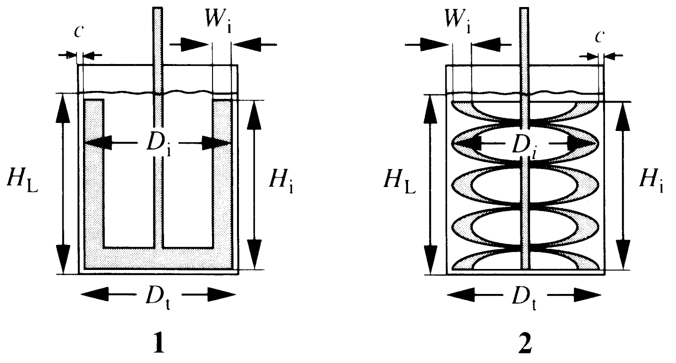
1.



2.

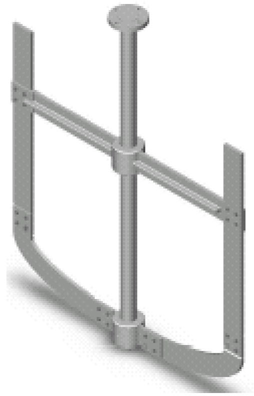
3.



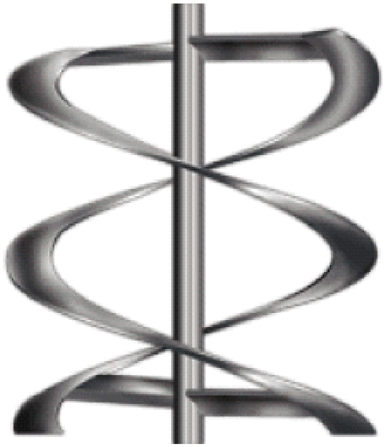


Impeller	D_t/D_i	c/D_i	H_i/D_i	W_i/D_i
1. Anchor	1.02	0.01	1	0.1
2. Helical ribbon	1.02	0.01	1	0.1

Correlation between power number and Reynolds number for anchor and ,helical-ribbon impellers without sparging



1.



2.

Correlation between power number and Reynold's number

In the laminar flow region of mixing speeds (N_{Re} <10)

$$\mathbf{N_P = K_1 (N_{Re})^{-m}}$$

where:

K_1 = a constant, independent of reactor size but dependent on reactor geometry and impellor shape/ size

m = 1

The power required for agitation is **independent** of culture **density** but correlated with viscosity:

$$\boxed{\mathbf{P_0 = K_1 \cdot N^2 \cdot D_i^3 \cdot \eta}}$$

Correlation between power number and Reynold’s number

In the turbulent flow range of mixing speeds (N_{Re}>10^4) the power number (N_P) is **constant and independent of the Reynold ’s number:**

$$\mathbf{N_P = K_2 = constant \ m = 0}$$

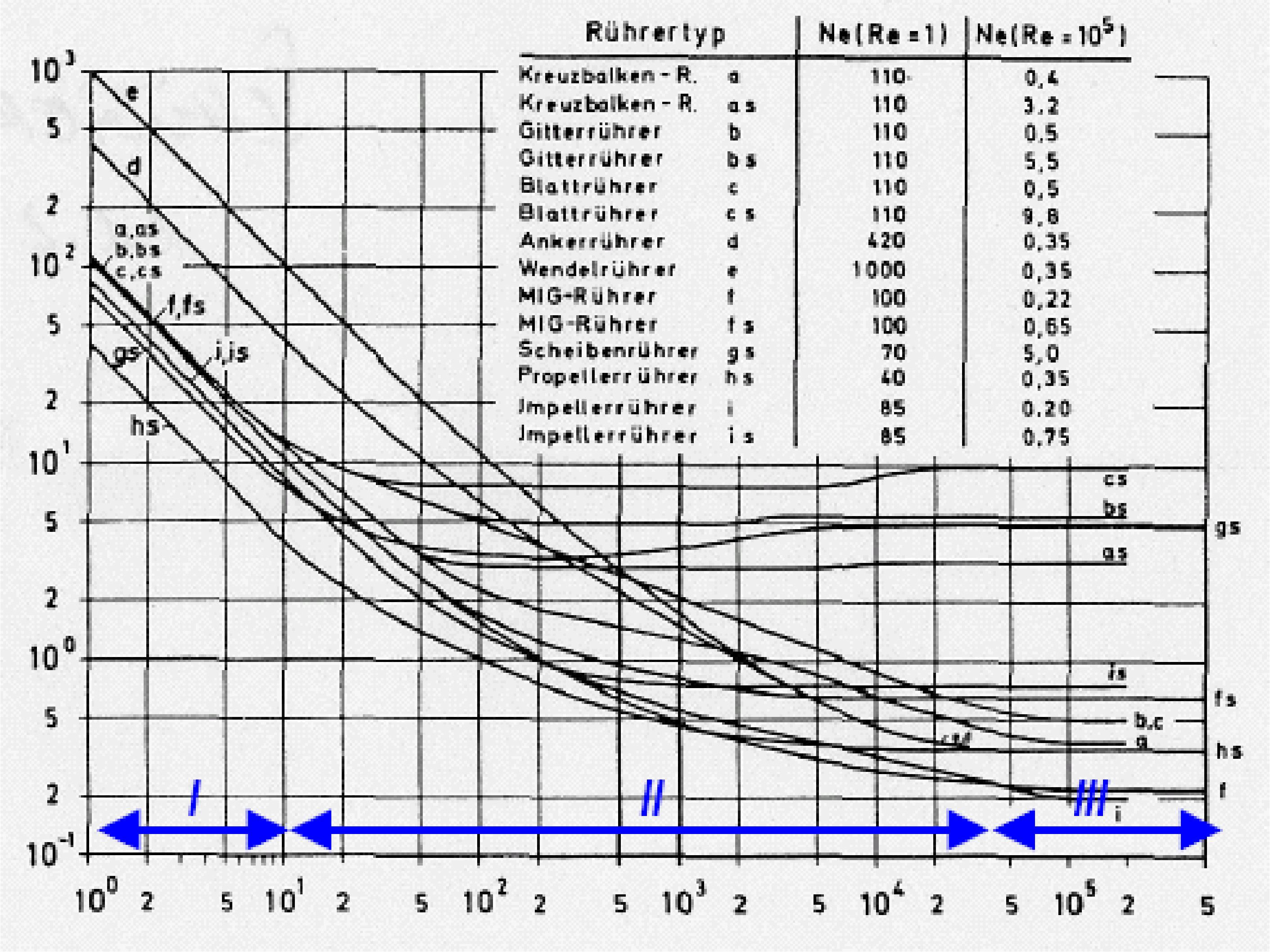
Under these conditions the power number is independent of viscosity:

$$\boxed{\mathbf{P_0 = K_2 \cdot N^3 \cdot D_i^{5} \cdot \rho}}$$

In the transient range of mixing speeds (N_{Re} = 10-10^4) there is **<u>no</u>** simple correlation between N_P and N_{Re}

Correlation between power number and Reynold's number

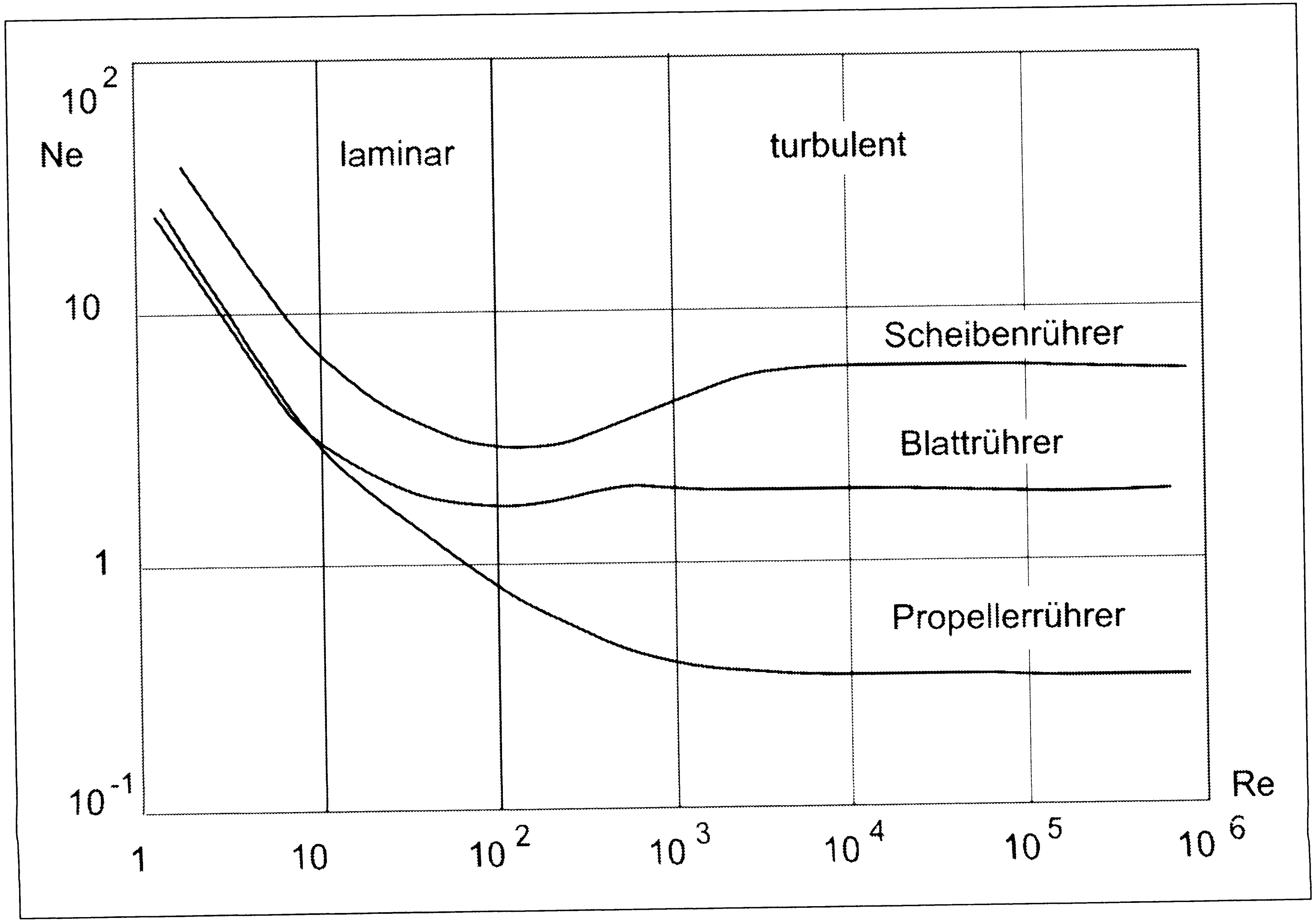
Impeller type	K_1 ($N_{Re} = 1$)	K_2 ($N_{Re} = 10^5$)
Rusthon turbine	70	5-6
Paddle	35	2
Marine Impeller	40	0.35
Anchor	420	0.35
Helical ribbon	1000	0.35



$c_P = K_2$

Rührertyp	c_P-Wert
Blattrührer	9,8
Scheibenrührer (12 Blatt)	8,0
Gitterrührer	5,5
Scheibenrührer (6 Blatt)	5,0
Kreuzbalkenrührer	3,2
Impellerrührer	0,75
Propellerrührer	0,35

(s: mit Strombrecher)



Ne
10^2
10
1
10^-1
laminar
turbulent
Scheibenrührer
Blattrührer
Propellerrührer
Re
1
10
10^2
10^3
10^4
10^5
10^6

Example: Calculation of power requirements

A fermentation broth with viscosity 10^{-2} Pa s and a density 1000 kg m^{-3} is agitated in a 50 m^3 baffled tank using a marine propeller 1.3 m in diameter. The tank geometry is:

Calculate the power required for a stirrer speed of 4 s^{-1}.

1 W = 1 kg m^2 s^{-3} 1 Pa·s = 1 kg/(m·s)

Effect of viscosity

For **Newtonian fluids** the dynamic viscosity is constant at constant temperature and is dependent on the ratio of the shear stress to the rate of shear as described by Newton 's law of friction:

$$\eta = \frac{T}{\gamma} = \text{constant (for Newtonian fluid)}$$

$$[\eta] = \frac{\mathrm{kg}}{\mathrm{m} \cdot \mathrm{s}} = \mathrm{Pa} \cdot \mathrm{s} = \frac{\mathrm{Ns}}{\mathrm{m}^2}$$

$1\ Ns/m^2 = 1\ Pa{\cdot}s = 10$ Poise
1 Centipoise = 1 cP = $10^{-3} Ns/m^2$.

where: T = Shear stress ($kg\ m^{-1}$)
γ = Shear rate (s^{-1})

For non- Newtonian cultures the dynamic viscosity is dependent on temperature and rate of shear. Such fluids include:

Pseudoplastic- apparent viscosity decreases with increasing shear rate
Dilatant- apparent viscosity increases with increasing shear rate
Bingham plastic- will not flow unless a stress, T_0 is imposed as given by:

$$\frac{T - T_0}{\gamma} = \eta = \text{constant}$$

Common non-Newtonian fluids

Fluid type	*Examples*
Newtonian	all gases, water, disperions of gas in water, low-molecular-weight liquids, aquous solutions of low-molecular-weight compounds
Non-newtonian	
Pseudoplastic	rubber solutions, adhesives, polymer solutions, some greases, starch suspensions, cellulase acetate, mayonaisse, some soap and detergent slurries, some paper pulps, paints, wallpaper paste, biological fluids
Dilatantsome	cornflour and sugar solutions, starch, quciksand, wet beach sand, iron powder dispersed in low-viscosity liquids, wet cement a aggregates
Bingham	some plastc melt, margarine, cooking fats, some greases, toothpaste, some soap and detergent slurries, some paper pulps
Casson plastic	Blood, tomato sauce, orange juice, melted chocolate, printing ink

Culture	*Shear rate* (s^{-1})	*Viscometer*	*Comments*
Saccharomyces cerevisiae (pressed cake diluted with water)	2–100	rotating spindle	Newtonian below 10% solids (μ < 4–5 cP); pseudoplastic above 10% solids
Aspergillus niger (washed cells in buffer)	0–21.6	rotating spindle (guard removed)	pseudoplastic
Penicillium chrysogenum (whole broth)	1–15	turbine impeller	Casson plastic
Penicillium chrysogenum (whole broth)	not given	coaxial cylinder	Bingham plastic
Penicillium chrysogenum (whole broth)	not given	coaxial cylinder	pseudoplastic; *K* and *n* vary with CO_2 content of inlet gas
Endomyces sp. (whole broth)	not given	coaxial cylinder	pseudoplastic; *K* and *n* vary over course of batch culture
Streptomyces noursei (whole broth)	4–28	rotating spindle (guard removed)	Newtonian in batch culture; viscosity 40 cP after 96 h
Streptomyces aureofaciens (whole broth)	2–58	rotating spindle/ coaxial cylinder	initially Bingham plastic due to high starch concentration in medium; changes to Newtonian as starch is broken down; increasingly pseudoplastic as mycelium concentration increases

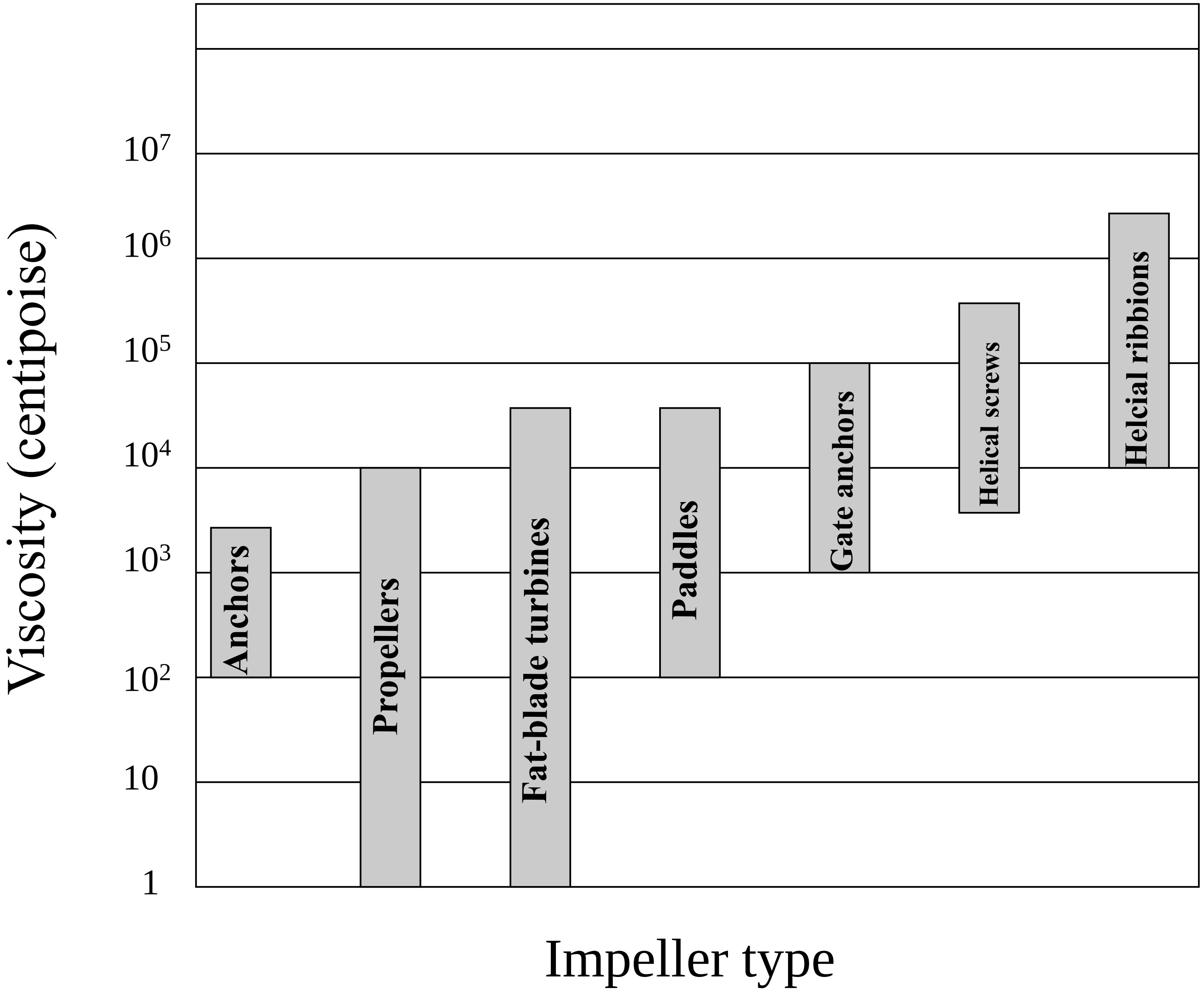
Streptomyces aureofaciens (whole broth)	2–58	rotating spindle/ coaxial cylinder	initially Bingham plastic due to high starch concentration in medium; changes to Newtonian as starch is broken down; increasingly pseudoplastic as mycelium concentration increases
Aureobasidium pullulans (whole broth)	10.2–1020	coaxial cylinder	Newtonian at the beginning of culture; increasingly pseudoplastic as concentration of product (exopolysaccharide) increases
Xanthomonas campestris	0.0035–100	cone-and-plate	pseudoplastic; K increases continually; n levels off when xanthan concentration reaches 0.5%; cell mass (max 0.6%) has relatively little effect on viscosity

Viscosity of filamentous fermentations

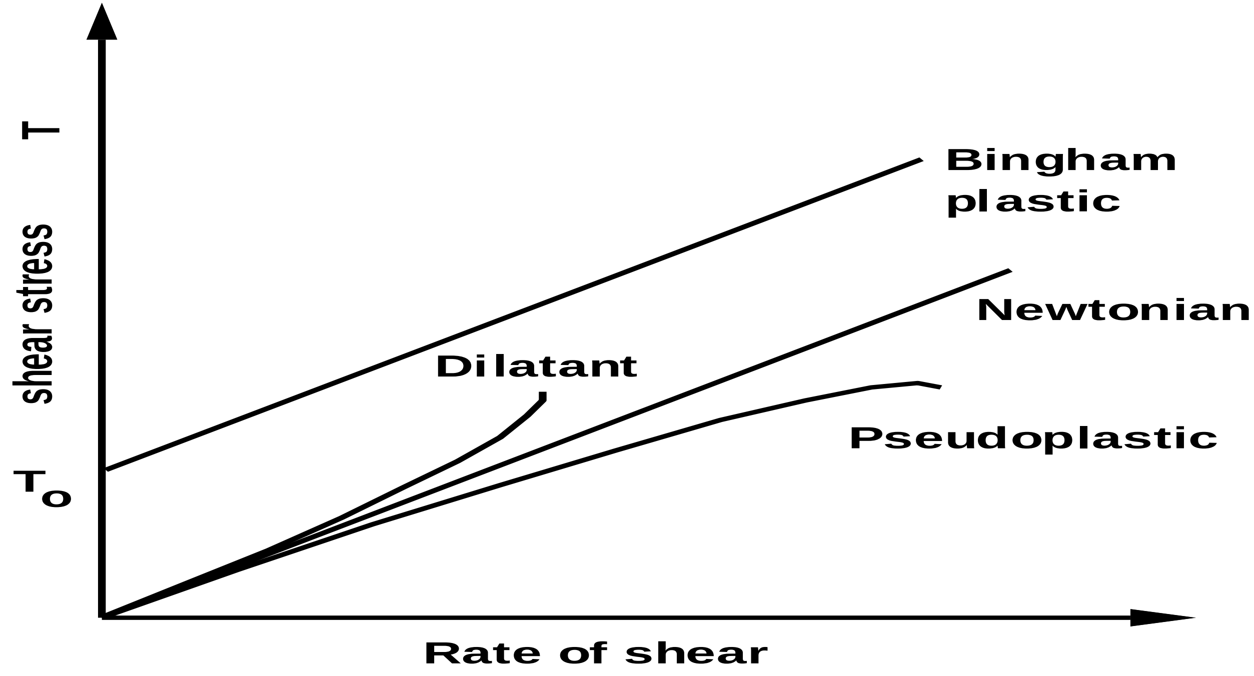
Microorganism	Application	Viscosity
P.chrysogenum	penicillin	Pseudoplastic
Coniothyrium hellbori	steroid hydroxylation	Bingham
Streptomyces noursei	nystatin	Newtonian
A. niger		Bingham
Streptomyces niveus	novobiocin	Bingham
Streptomyces griseus	streptomycin	Bingham
Streptomyces sp.		Newtonian and Pseudoplastic
Endomyces sp.	glucoamylase	Pseudoplastic

Note: animal cell fermentations Newtonian

Viscosity ranges for different impellers



Shear stress



Correlation between shear rate and shear stress in culture media with Newtonian and non- Newtonian properties

Heat Transfer

- In large reactors, the two main limitations on size are the abilities of the design to provide an adequate supply of oxygen and to remove metabolic heat efficiently

- Large reactors use either internal coils or a jacketed vessel for heat removal
  - Internal coils provide advantages over cooling jackets, as they provide a larger surface area for heat transfer
  - In some systems the coils can rapidly become fouled by microbial growth, decreasing heat transfer and often adversely affecting mixing

Heat Transfer

Cell metabolism us usually the largest source of heat in fermenters, the capacity of the system for heat removal can be linked directly to the maximum cell concentration in the reactor.

$$-\Delta H_{rxn} - M_v \Delta h_c - Q + W_s = \frac{dE}{dt}$$

Rate of heat flow

Rate of heat absorption or
Evolution due to metabolic
Reaction
Neg: exothermic reaction
Pos: endothermic reaction

Mass flow rate of
evaporated liquid leaving
the system

Rate of shaft work done on
System (e.g by stirring)

Latent heat of
Evaporation

$$\boxed{\text{At steady state: } \frac{dE}{dt} = 0 \Rightarrow Q = -\Delta H_{rxn} - M_v \Delta h_c + W_s}$$

Assuming that heat dissapated from the stirrer and the cooling effects of evaporation are negligible with the heat of reaction:

$$Q = -\Delta H_{rxn}$$

Outlined earlier, approx. 460 kJ heat is released for each mole oxygen consumed.
If Q_{O2}: rate of oxygen uptake per unit volume (gmol m^{-3} s^{-1})

$$\Delta H_{rxn} = (-460 \text{ kJ gmol}^{-1})\, Q_{O2}\, V$$

Overall heat-transfer coefficient
(Wm^{-2} K^{-1})

$$\boxed{Q = -\Delta H_{rxn} = (460 \text{ kj gmol}^{-1})\, Q_{O2}\, V = qO_2 \times V = UA\,\Delta T}$$

Surface area

Fastest rate of heat transfer: if **ΔT maximum**

(hypothetically , this occurs when the cooling water remains at its inlet T: **$\Delta T = T_F - T_{ci}$ (T_F : fermenter T, T_{ci}: water inlet T)**

$$Q = -\Delta H_{rxn} = (460\ kJ\ gmol^{-1})\ Q_{O2}\, V = qO_2\, x\ V = UA\,\Delta T$$

$$\rightarrow\ X_{max} = \frac{UA(T_F - T_{ci})}{(460\ kJ\ gmol^{-1})\ q_{O2}\, V}$$

Consequences:

if max. cell concentration is lower than that desired -> improvement of heat-transfer facilities.

For example: area A could be increased by installing a longer coiling coil, or U could be improved by increasing the stirrer speed