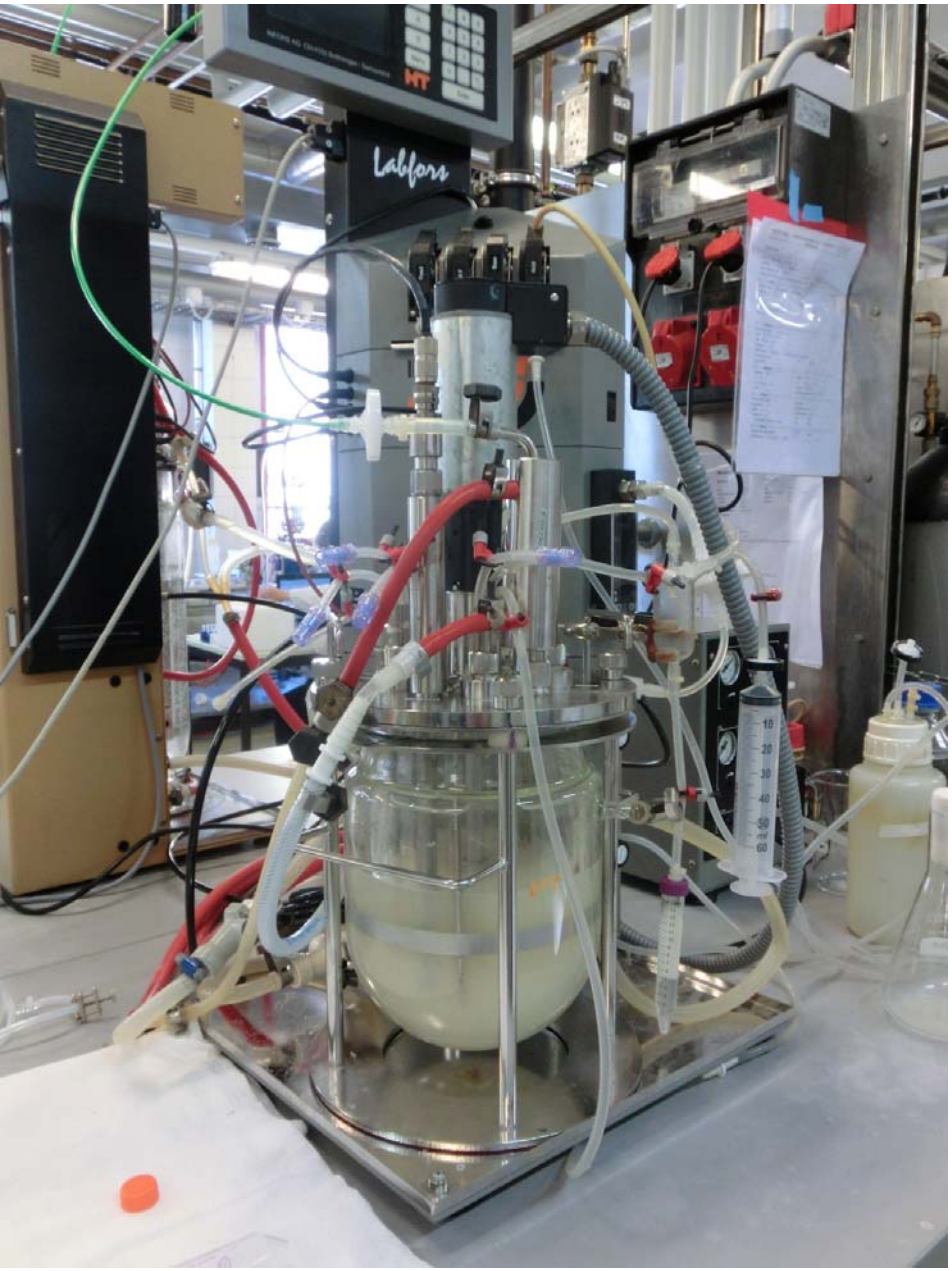

Performance of bioreactors

Biochemical Engineering

ChE-311

Manfred Zinn

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Agenda

- Definition and performance of a bioreactor
- Different types of bioreactors:
  - Non-stirred and non-aerated systems
  - Non-stirred and aerated systems
  - Stirred and aerated systems
- Special bioreactors
- Mass transfer
  - Oxygen transfer rate
- Summary

Definition of a bioreactor

- An apparatus for growing organisms (yeast, bacteria, or animal cells) under controlled and repeatable conditions.
- Used in industrial processes to produce pharmaceuticals, synthons, vaccines, antibodies, or biocatalysts.
- Also used to convert raw materials into useful byproducts such as in the bioconversion of hemicellulose into ethanol.

Materials used for bioreactors

Not only metal is used for bioreactors:

- Borosilicate glass: Sight glasses
- Silicone rubbers and ethylene propylene diene monomer (EPDM): Gaskets
- Polytetrafluorethylene (PTFE): Sleeves and support of bearings
- Silicone carbide (ceramic): Mechanical seal
- Graphite: Seals and rupture discs

However, all materials need to be certified in order to be used in bioreactors (*e.g.,* food contact materials).

Metal used for bioreactors

Stainless steel

Stainless steel refers to various alloys of primarily iron, nickel and chromium. Molybdenum may also be added to increase the resistance of the steel to corrosion.

Stainless steels come in different grades. The commonly encountered grades are designated by standard codes, for example:

- 302
- 304
- 316
- 318

In general, the higher the number, then the greater the resistance of the steel. The grade of stainless steel most widely used in the construction of bioreactors is ***316L***. The "L" indicates the steel has a low carbon content.

Stainless steels used in bioreactors are often polished to a mirror finish. This finish makes cleaning and sterilization easier. Stainless steel components used in the construction of bioreactors are joined in an oxygen-free environment using a special technique known as TIG welding.TIG stands for Total Inert Gas and the technique involves the use of argon to displace the air. The presence of oxygen in the welds can cause corrosion at the weld. In addition, hydrochloric acid as pH control acid should be avoided because of corrosion. Suitable acids are 2M H_3PO_4 or 4M H_2SO_4.

Same metals used for chemical reactors!

Bioreactor/Fermenter vs. Chemical Reactor

a) The **specific density** of the cells are almost identical to the medium and sedimentation is slow. In contrast, in chemistry often chemical reactions are carried out with heavy metal catalysts.

b) The **cells are much smaller** than chemical catalysts. Big Reynolds numbers cannot be reached.

c) Polysaccharides and/or mycelial structures influence the flow performance of the culture medium significantly. Consequently, the design of new bioreactors for such bioprocesses represents a significant challenge.

d) The **transfer of nutrients** is strongly limited by diffusion in large cell agglomerates (e.g. pellets of mycels or clumps) and can hardly be influenced neither by mixing nor agitation.

Bioreactor/Fermenter vs. Chemical Reactor

e) The **CO_2** that is produced by the cells can influence the cell metabolism (*e.g.*, growth inhibition) and therefore needs to be removed.

f) Many **parameters** (*e.g.*, pH, T) **have to be controlled continuously** and accurately in order to avoid irreproducible results or even irreversible damage to the cells.

g) The **concentrations of substrates** and products are usually quite small in bioreactions and therefore the gradients that boost the mass transfer are also small.

h) The **reaction speeds for microbial processes are smaller** than for chemical processes. That's why **bioreactors are larger** than chemical reactors. In continuous processes the residence time needs to be larger.

A new trend: Single-use bioreactors

https://www.sartorius.com/en/products/fermentation-bioreactors/single-use-bioreactors

Criteria for selecting a bioreactor

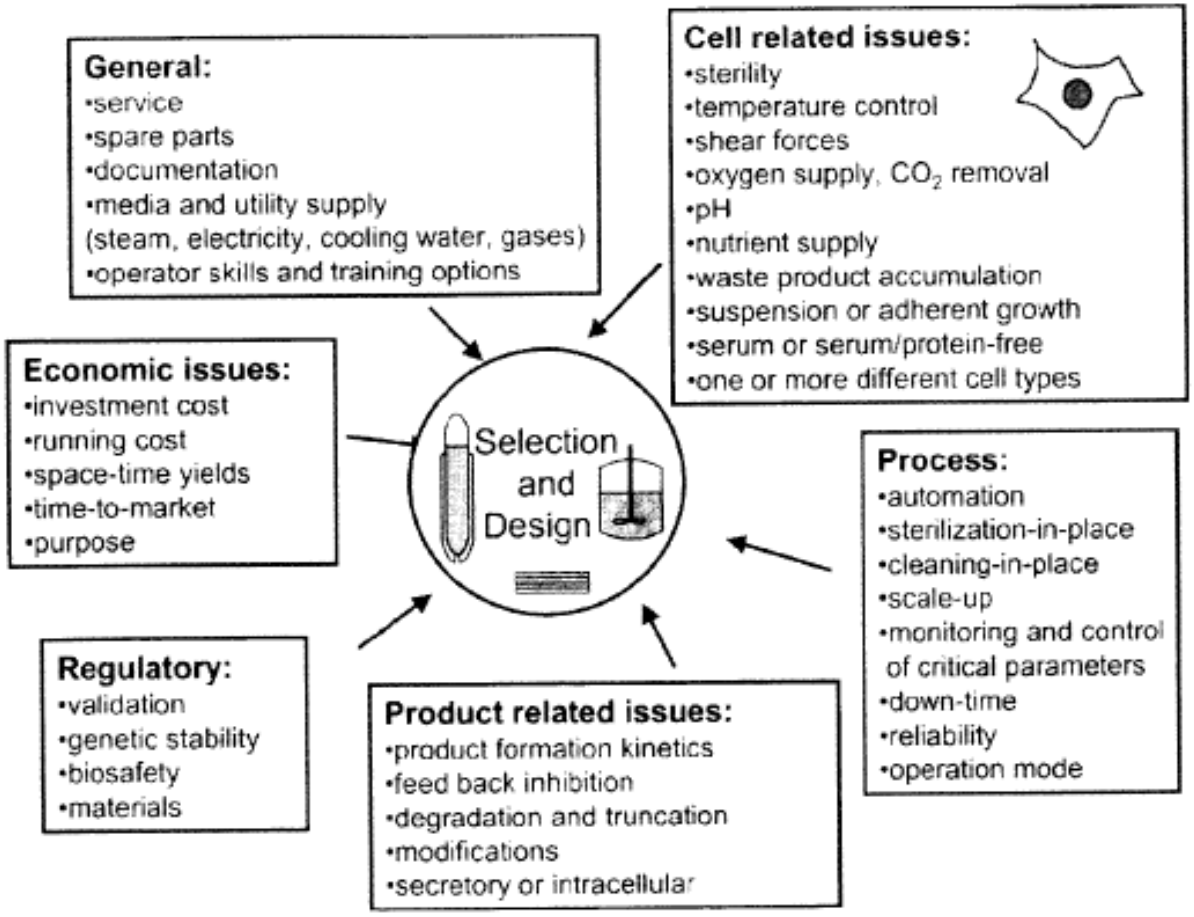


Figure 13 Factors affecting the design and selection of bioreactors.

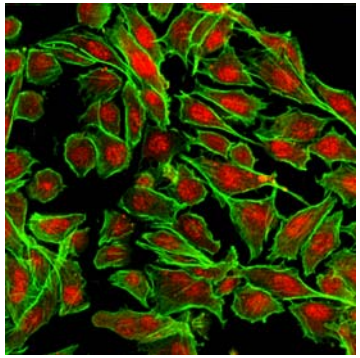
What is the best bioreactor for my process?

There are three groups of reactors currently in use for industrial production:

1. Non-stirred, non-aerated system: about 70%
2. Non-stirred, aerated systems: about 10%
3. Stirred and aerated systems: about 20%

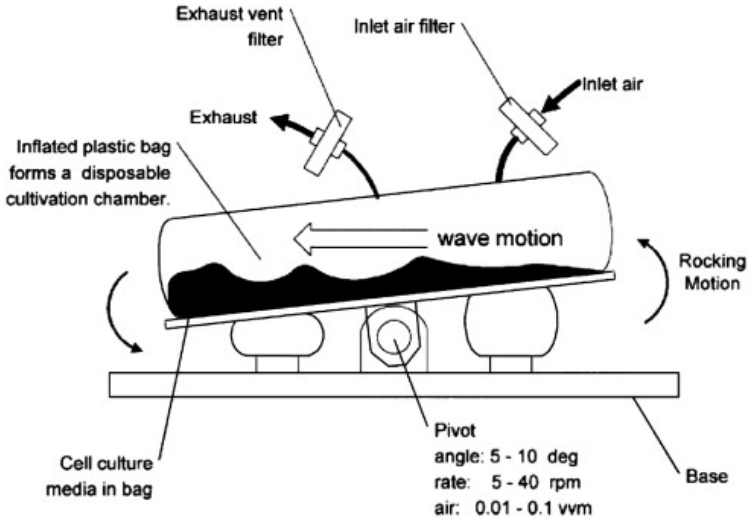
1. Non-stirred, non-aerated systems

T-flasks



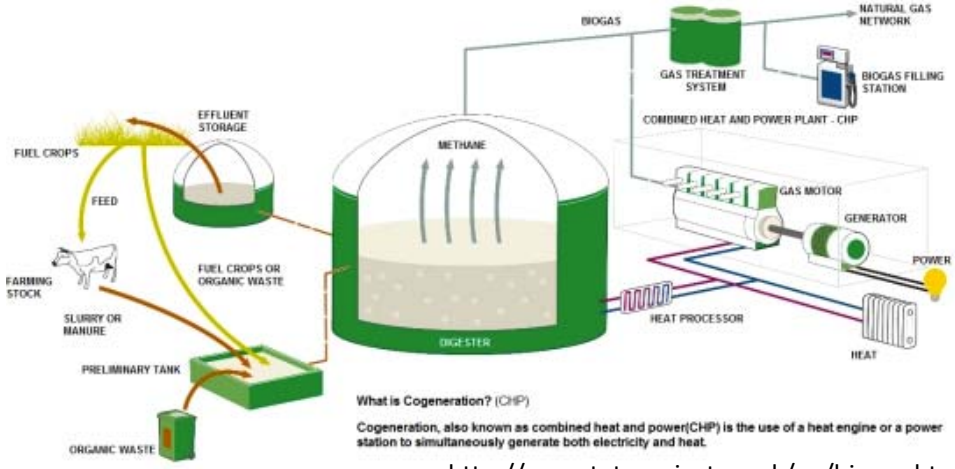
CHO cells

Animal cell cultures are very sensitive to shear forces caused by gas bubbles. A gentle mixing is therefore of utmost importance.



Wave bioreactors

Biogas fermentors



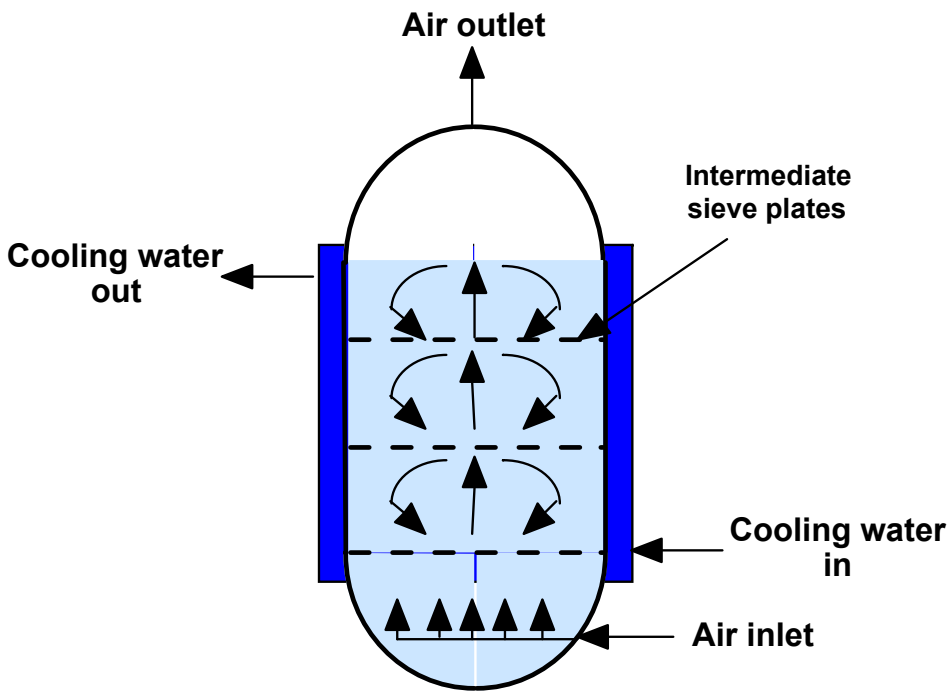
http://www.tetaproject.co.uk/en/biogas.html

Sedimentation tanks

Membrane bioreactors

2. Non-stirred, aerated systems

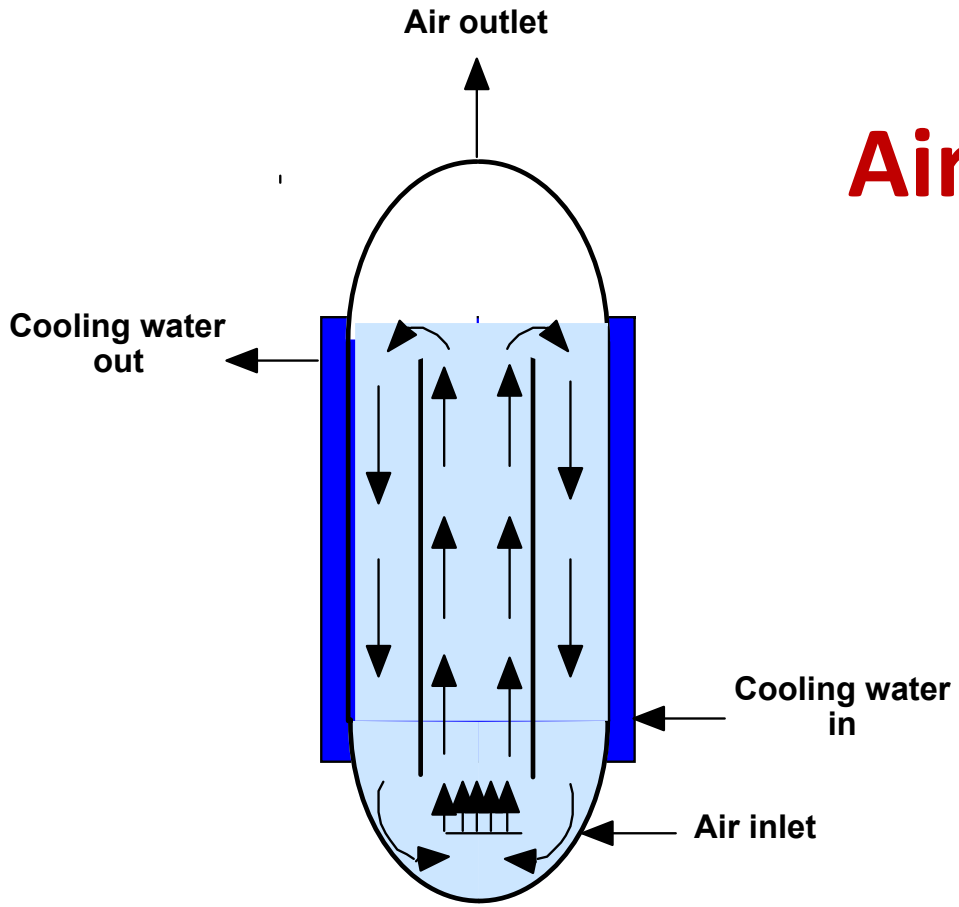
Bubble column reactor



BUBBLE COLUMN REACTOR

In general all column reactors have a high aspect ratio of about 8 to 20.

Air-lift reactor

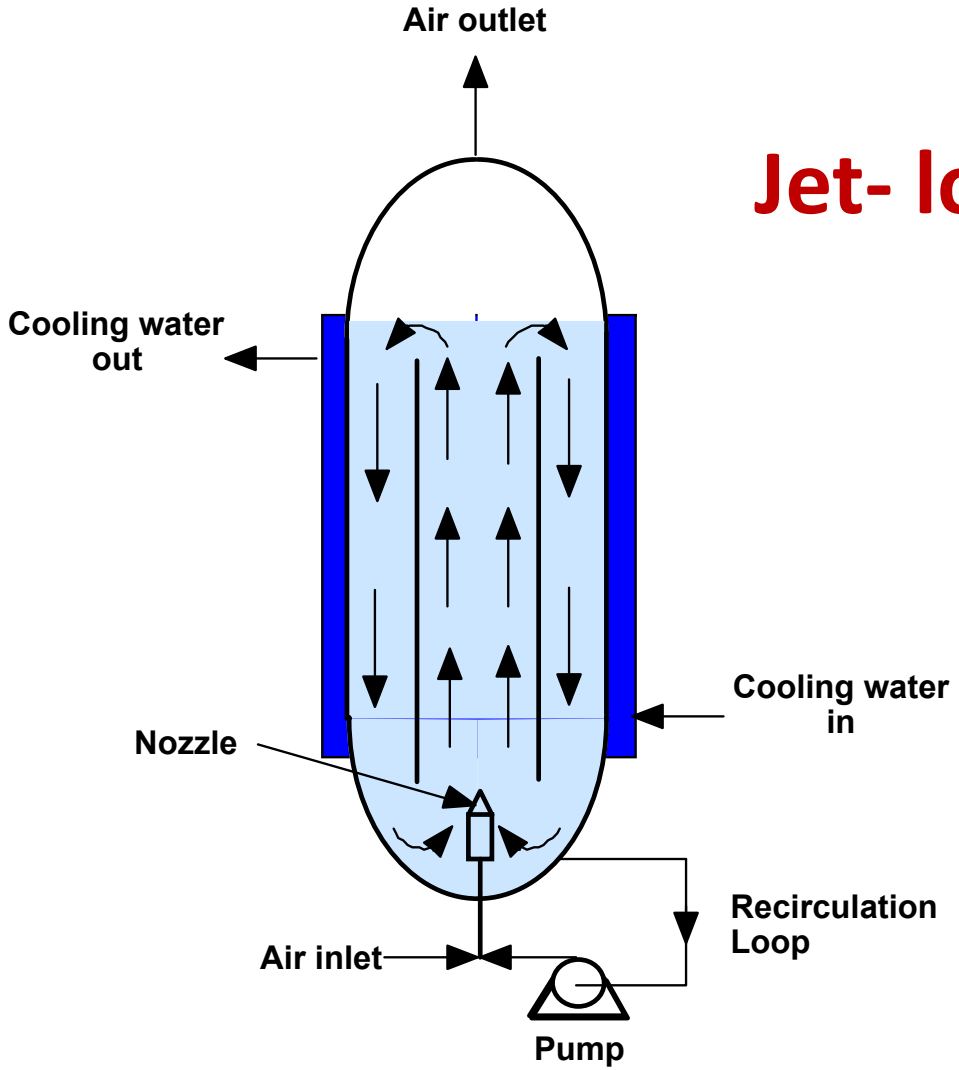


AIR LIFT REACTOR
with CENTRAL DRAFT TUBE

An air-lift fermenter differs from a bubble column bioreactor by the presence of a draft tube.

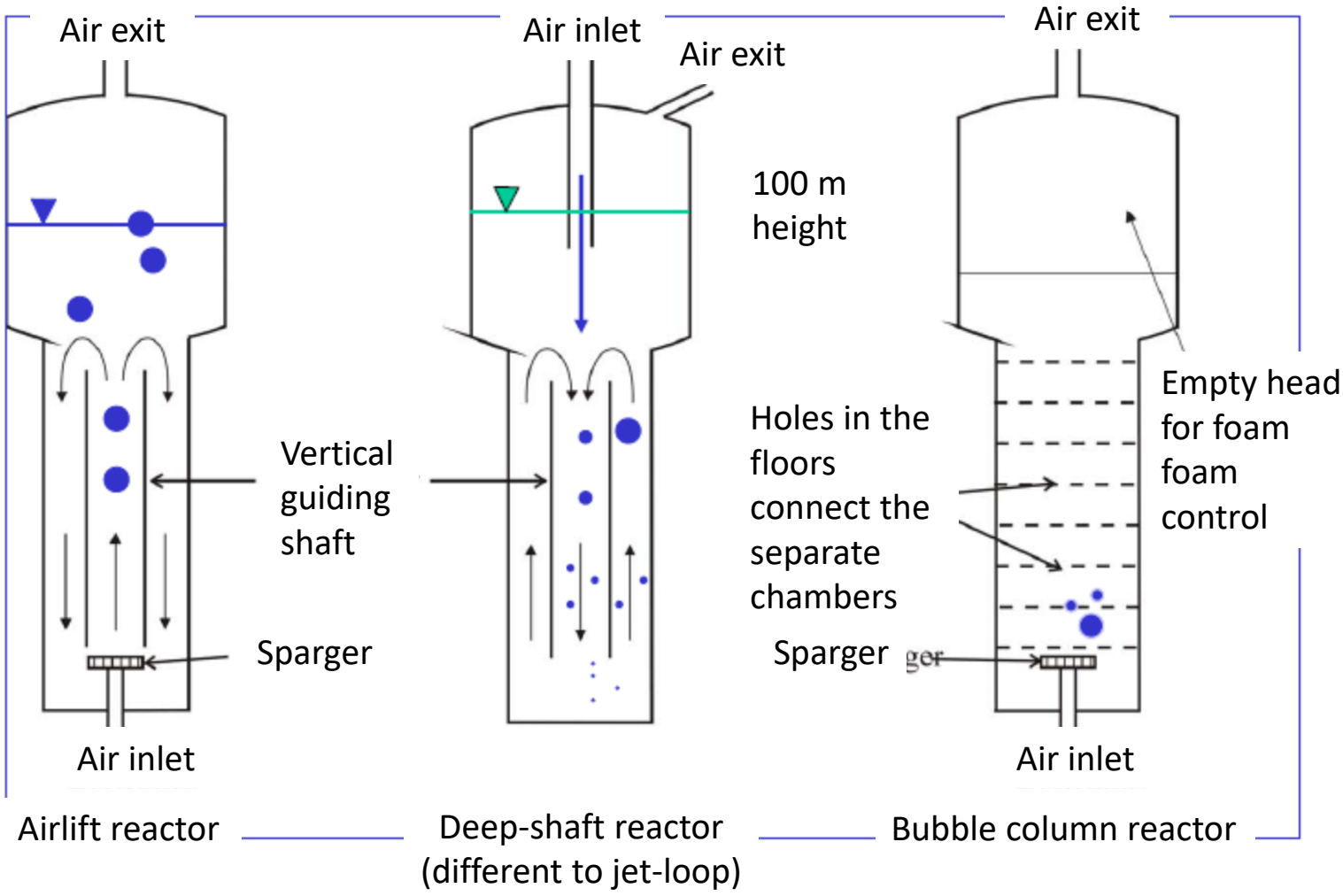
Note: Aspect ratio for column reactors minimum 6:1

Jet- loop reactor



Jet loop reactor
(example of internal loop reactor)

Bubble column reactors



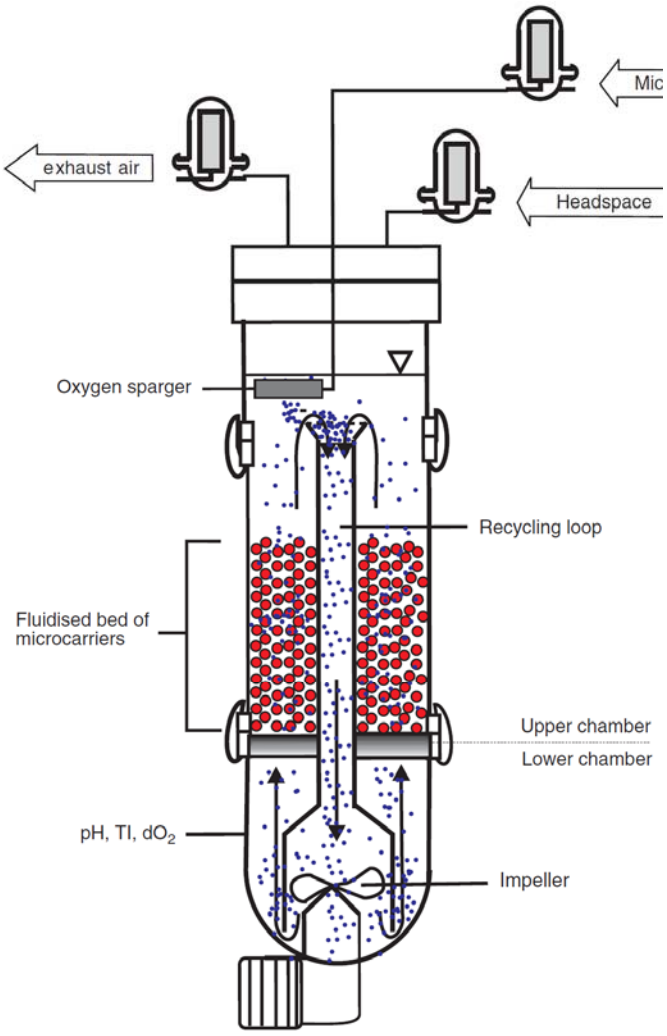
Fluidized-bed reactor for research

Lab scale: Siran beads

2 L culture volume,
100-500 mL microcarriers

Cytopilot (Vogelbusch)

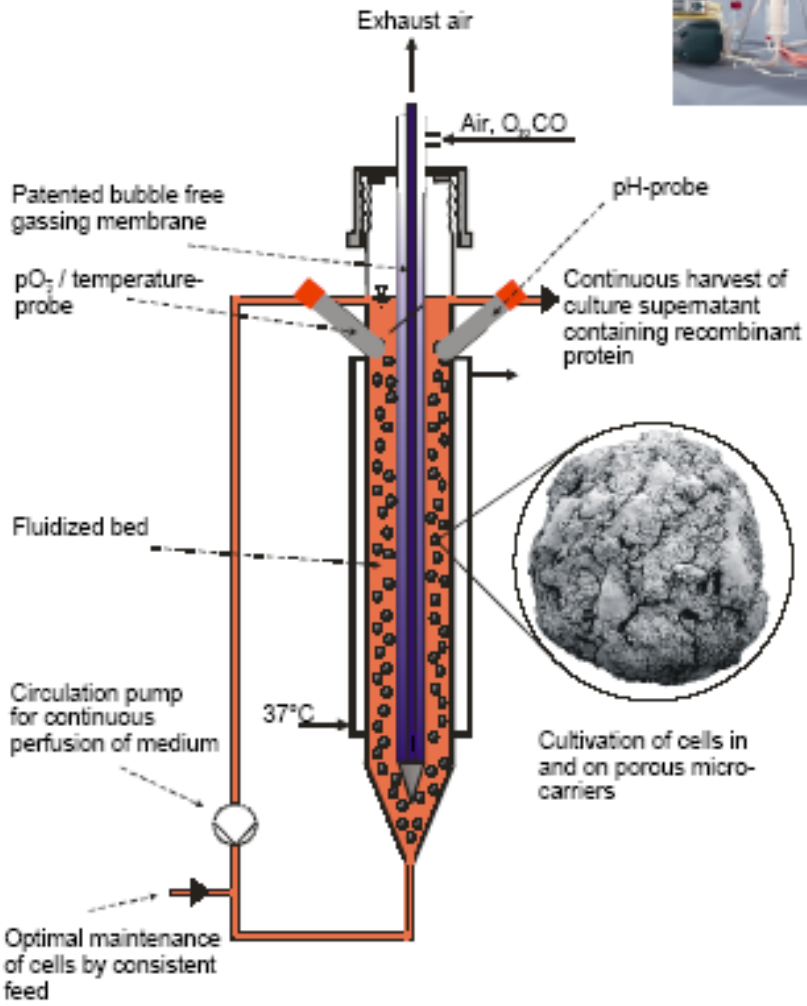
80 liter internal loop reactor



Cytopilot

Tools for Cell Cultivation

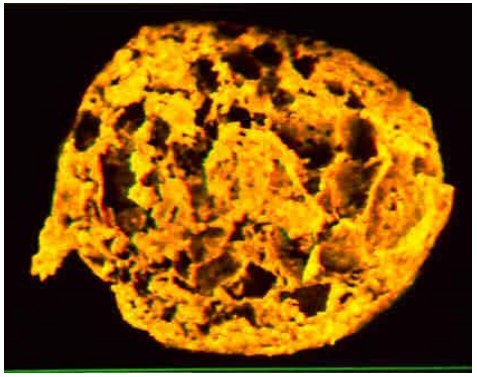
Fluidized Bed Reactor (schematic)



Cell Culture Bioreactors

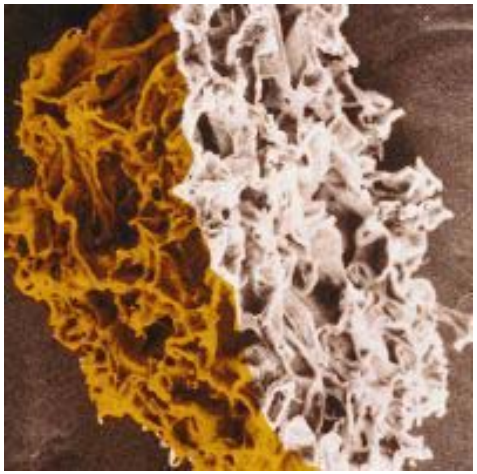
Macroporous support matrices

Cytoline

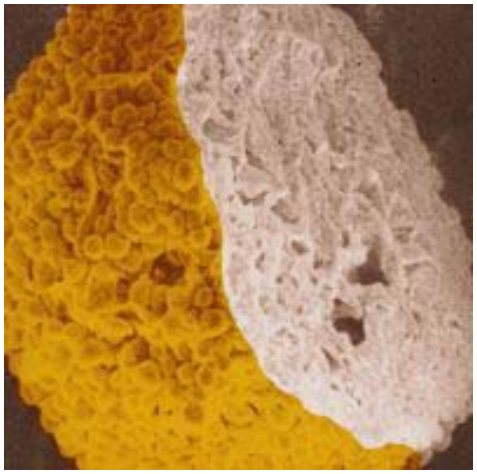


Cytoline + cells

Cytopore



Cytopore + cells



1.1 m^2/g dry weight

Pore size: 30 µm

Various types of cell support

- Adsorption on solid particles or on surfaces, e.g. glass or polymer beads, T- flask, roller bottle.
- Adsorption within macroporous supports, e.g. Cytolines, Cytopores, Cultisphere, Siran glass beads or HDPE structures (for waste water treatment plants)
- Membranes, e.g., hollow fiber reactors
- Encapsulation in agar beads

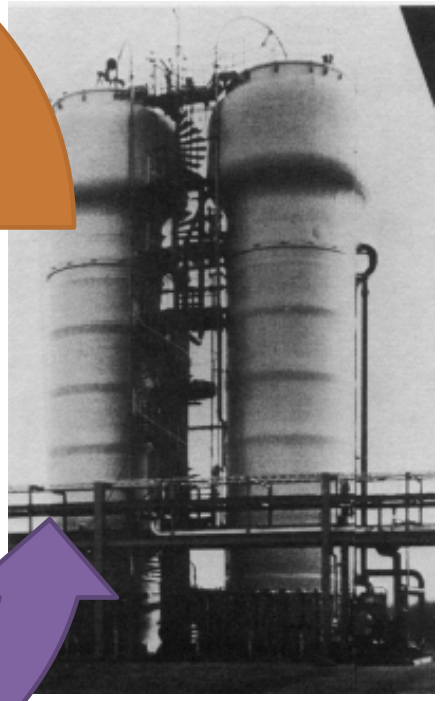
Siddique, N.A et al. 2006. J. Plant Sci., 1: 106-118.

Fluidized-bed reactor for industry

?

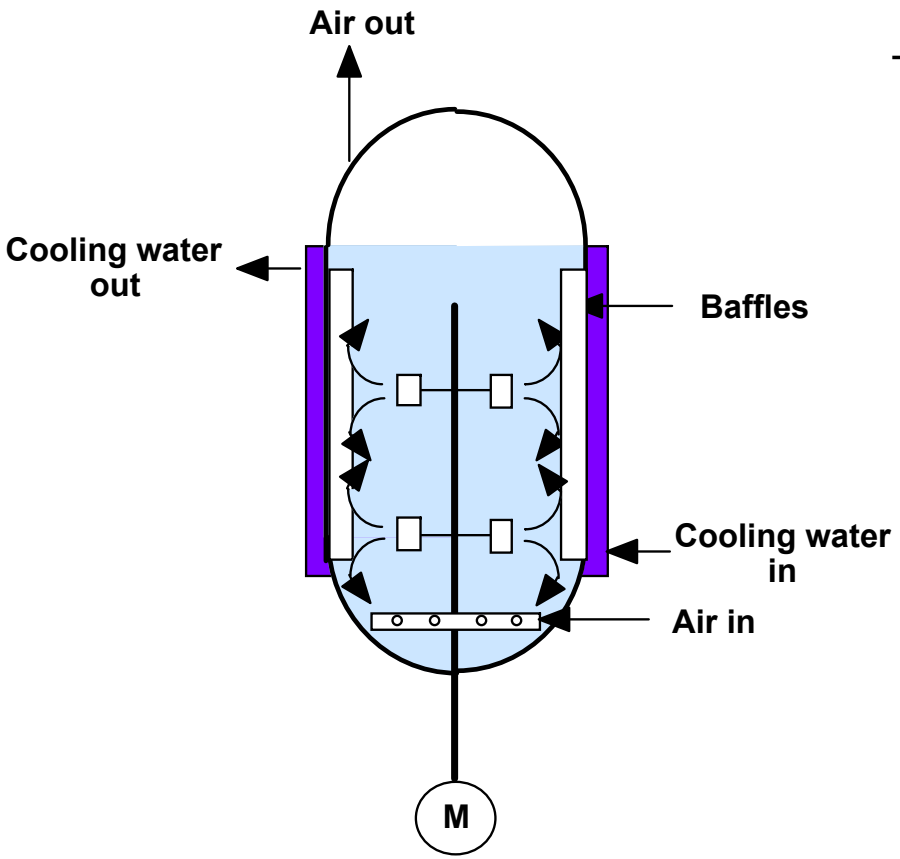
Scale-up and scale-down are difficult tasks.

?



3. Stirred and aerated systems

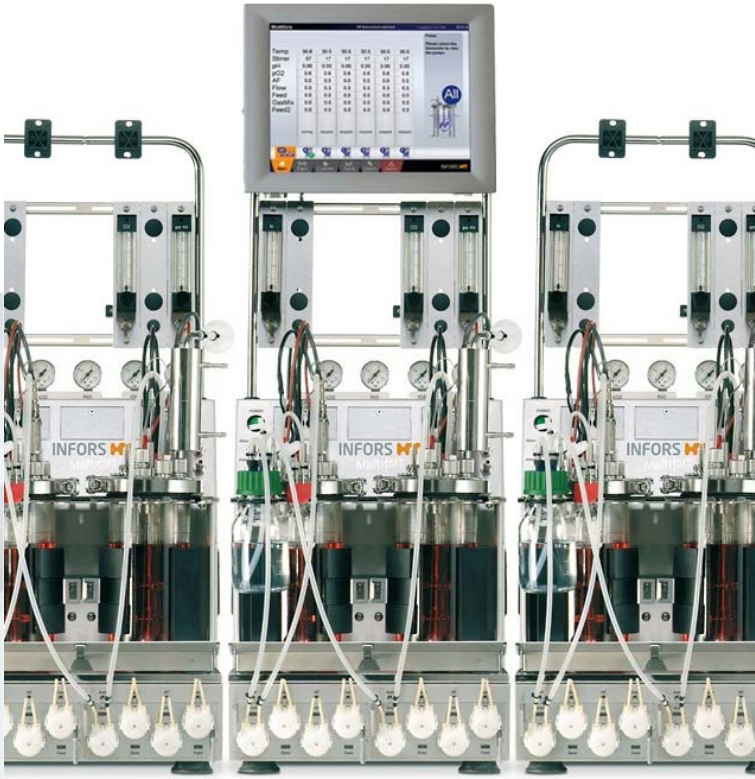
STR: Stirred Tank Reactor



Typically, a STR is equipped with:

- An agitator system
- An oxygen delivery system
- A foam control system
- A temperature control system
- A pH control system
- Sampling ports
- A cleaning and sterilization system.
- A sump and dump line for emptying of the reactor.

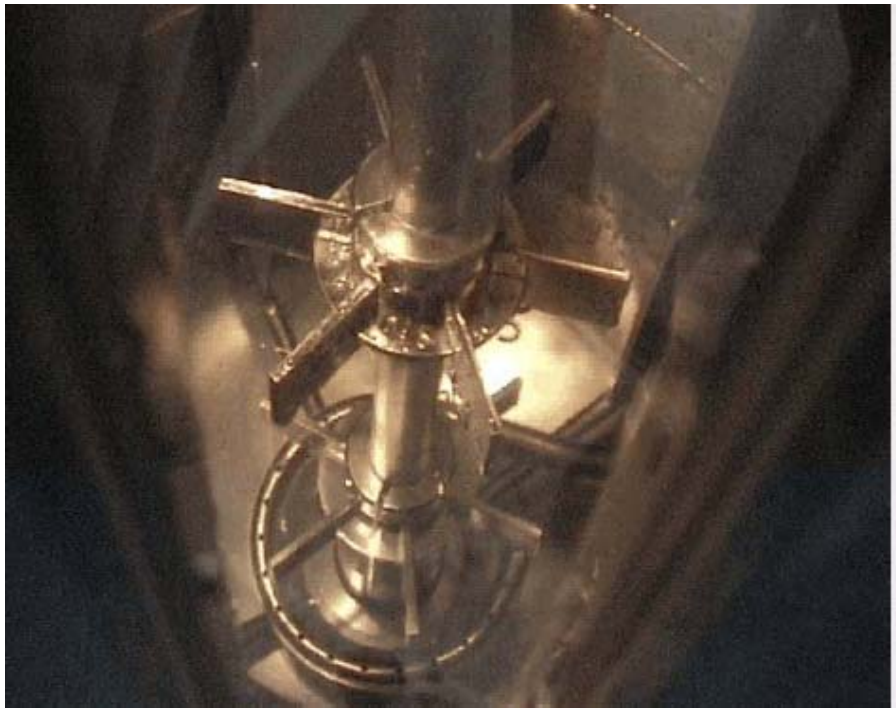
Note: Laboratory scale bioreactors with liquid volumes of less than 10 liters are constructed out of Pyrex glass. For larger reactors, stainless steel (V4A, 316L) is used.



Small stirred tank reactors for research: Volume ca. 0.5 L

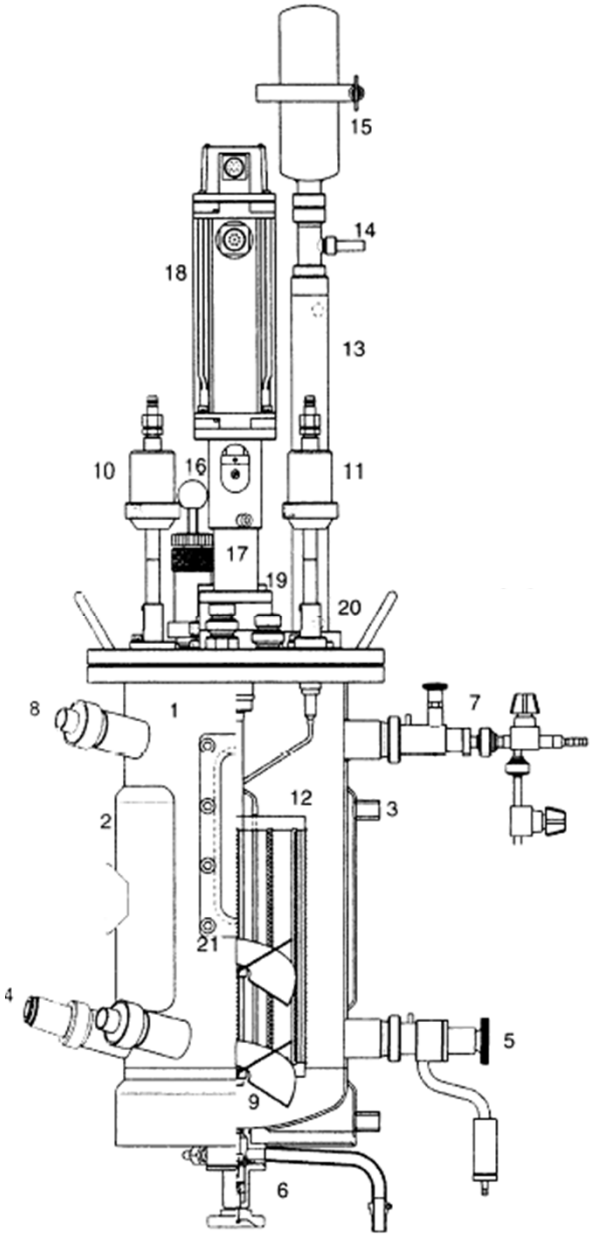
STR: Stirred-tank reactor

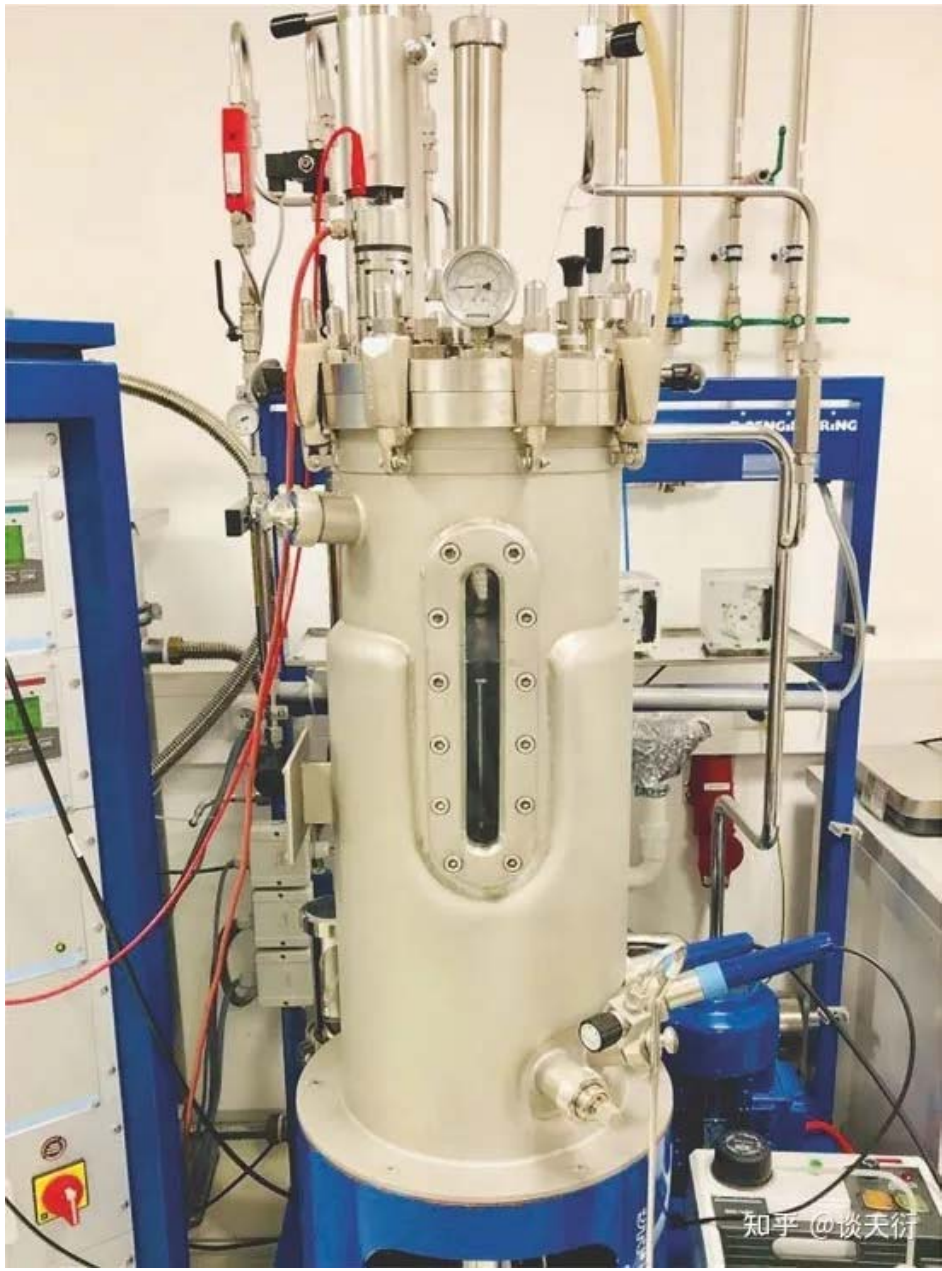
20 liter STR



The stirred tank bioreactor (STR)

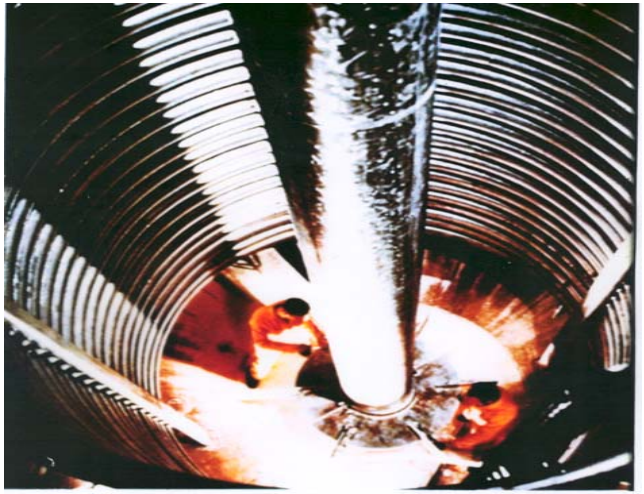
1 reactor vessel
2 jacket
3 jacket connections
4 ports for pH, temp., pC_2 elek.
5 sample valve with steam connection
6 harvest valve with steam connection
7 inoculum valve array with steam connection
8 connection for acid, base, antifoam
9 3 blade segment impeller
10 air inlet filter of aeration basket
11 air outlet filter of aeration basket
12 aeration basket
13 exhaust cooler
14 high foam alarm
15 exhaust filter
16 relief valve
17 mechanical seal
18 motor
19 sensor port
20 several sight glass with light (not shown)
21 lateral sight glass





STR in industry

20 m^3 STR



Large production bioreactors are installed in special buildings over several floors. They are in use for more than 340 days per year.

Advantages of STR

- Polyvalent: It may be used for multiple products
- Low installation costs
- Ease of operation and maintenance
- Suitable for suspension, immobilized and encapsulated cells
- May be operated in batch, fed- batch or continuous modes (CSTR)
- May be combined with cell recycle and perfusion systems to increase productivity
- Ease of validation, lot-to-lot variation reduced, robust

Disadvantages of STR

- Mass transfer limited- requires sparging (low O_2 solubility)
- Shear sensitivity of cells
- Scale-up difficult to maintain transfer rates constant
- High labour costs
- Integration with DSP requires suitable scaling
- Large energy input results in high cooling costs

Standard geometry of STR's

- A stirred tank reactor will either be approximately cylindrical or have a curved base. A curved base assists in the mixing of the reactor contents.

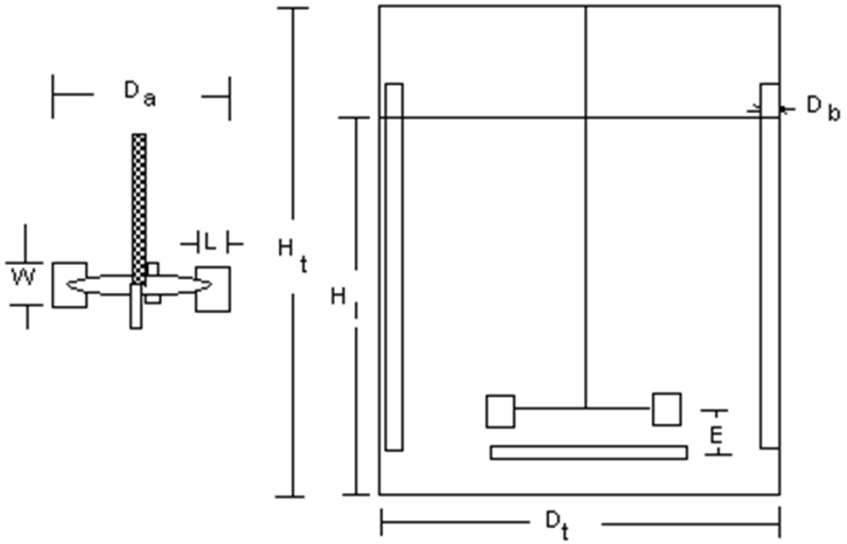
- Stirred tank bioreactors are generally constructed to standard dimensions.

- That is, they are constructed according to recognized standards such as those published by the International Standards Organisation and the British Standards Institution.

- These dimensions take into account both mixing effectiveness and structural considerations.

The design of STR's

Standard geometry

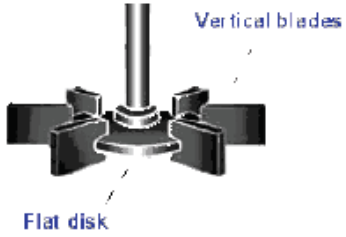


Specific

A mechanically stirred tank bioreactor fitted with

- a sparger and
- a Rushton turbine

will typically have the following relative dimensions:



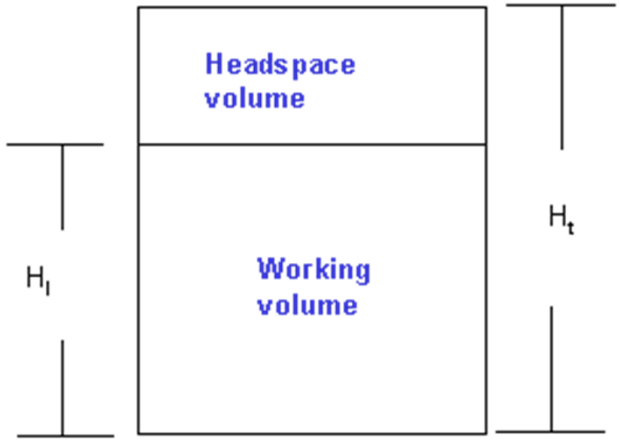
Ratio		Typical values	Remarks
Height of liquid in reactor to height of reactor	H_L/H_t	~0.7-0.8	Depends on the level of foaming produced during the fermentation
Height of reactor to diameter of tank	H_t/D_t	~1 - 3	Bioreactors for animal cell cultures have aspect ratios of 1, for microbial cells in STR about 3. In general, European reactors tend to be taller than those designed in the USA
Diameter of impeller to diameter of tank	D_a/D_t	1/3 - 1/2	Rushton Turbine reactors are generally 1/3 of the tank diameter. Axial flow impellers are larger.
Diameter of baffles to diameter of tank	D_b/D_t	~0.08 - 0.1	
Impeller blade height to diameter of impeller	W/D_a	0.2	
Impeller blade width to diameter of impeller	L/D_a	0.25	
Distance between middle of impeller blade and impeller blade height	E/W	1	

Note: A tank's height:diameter ratio is often referred to as its aspect ratio.

The stirred tank bioreactor (STR)

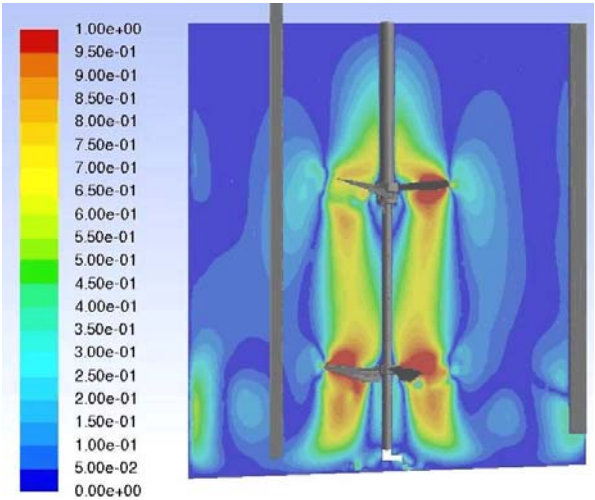
Headspace volume

A bioreactor is divided in a working volume and a head-space volume. The working volume is the fraction of the total volume taken up by the medium, microbes, and gas bubbles. The remaining volume is called the headspace.



Typically, the working volume will be **70-80%** of the total fermenter volume.
This value will however depend on the rate of foam formation during the reactor. If the medium or the fermentation has a tendency to foam, then a larger headspace and smaller working volume will need to be used.

Mixing in a stirred tank reactor



Mixing in a stirred tank bioreactor (STR)

Agitation system

The function of the agitation system is to

- provide **good mixing** and thus increase mass transfer rates through the bulk liquid and bubble boundary layers.
- provide the **appropriate shear conditions** required for the breaking up of bubbles.

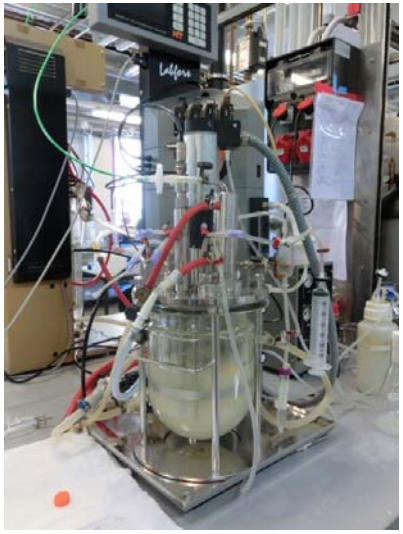
The agitation system consists of the agitator and the baffles.
The baffles are used to break the liquid flow to increase turbulence and mixing efficiency.

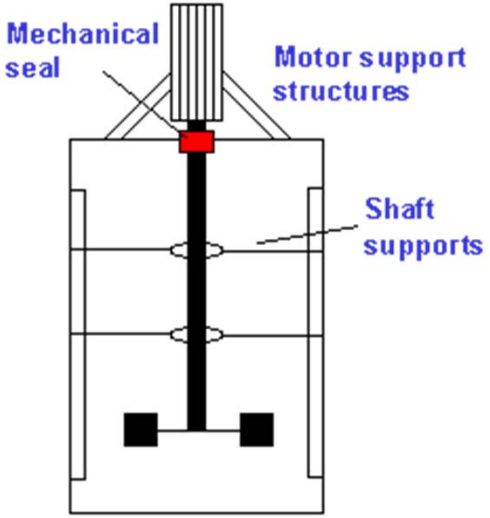
The **number of impellers** will depend on the height of the liquid in the reactor. Each impeller will have between 2 and 6 blades. Most microbial fermentations use a **Rushton turbine impeller**.

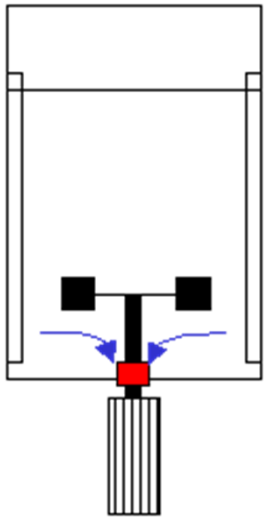
A single phase (i.e. 240 V) drive motor can be used with small reactors. However, for large reactors, a 3 phase motor (i.e. 430 V) should be used. The latter will tend to require less current and therefore will generate less heat.

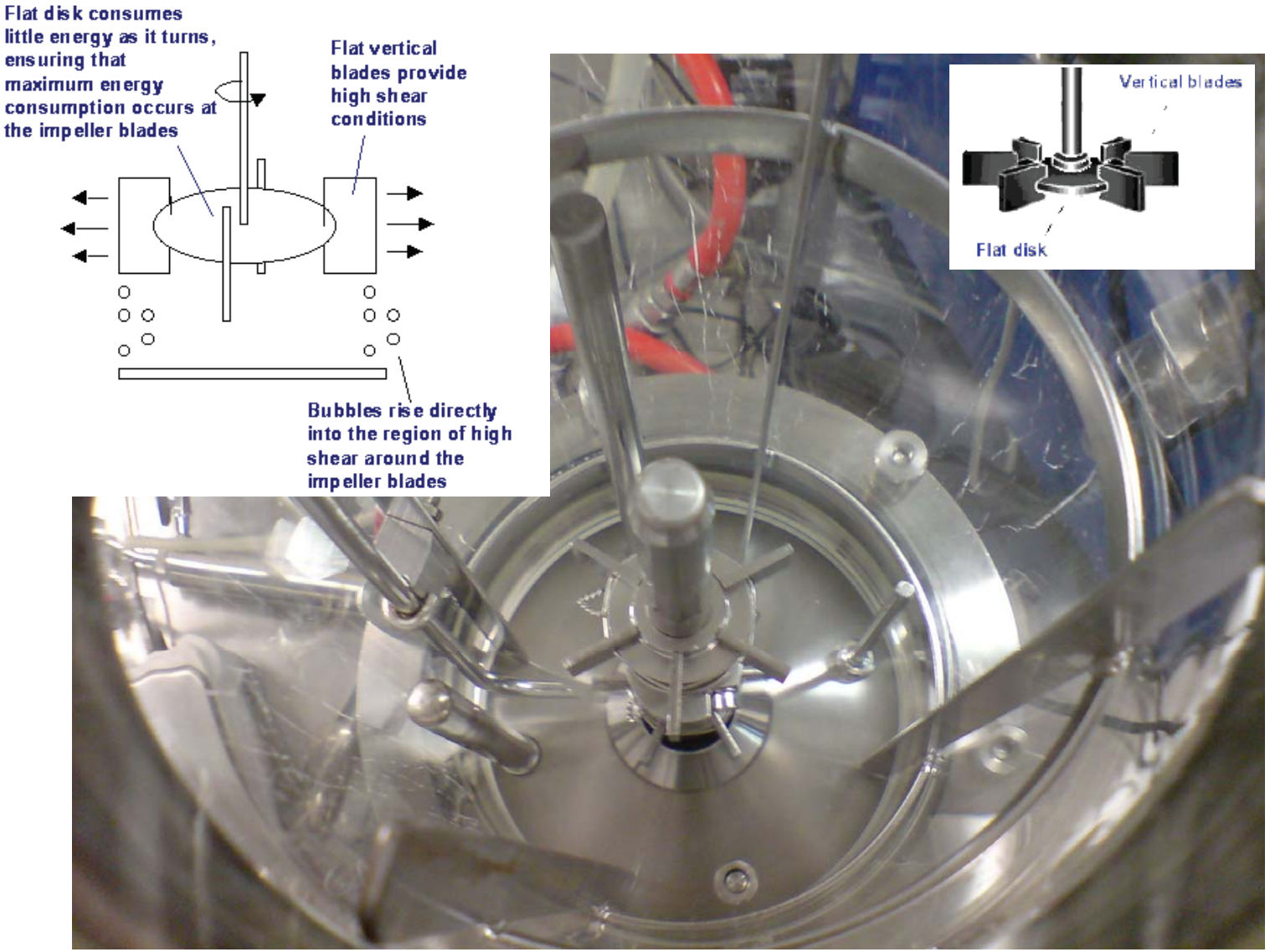
The stirred tank bioreactor (STR)

Agitation system - Top entry and bottom entry impellers





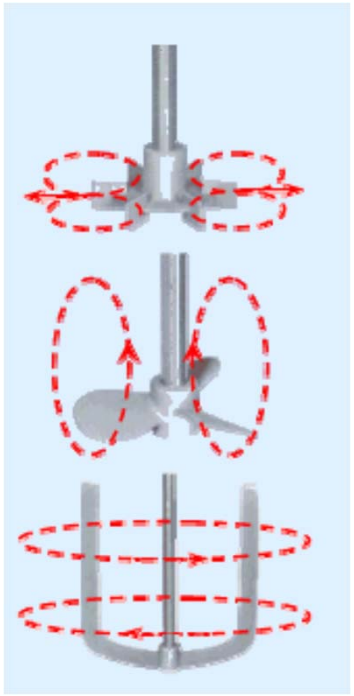




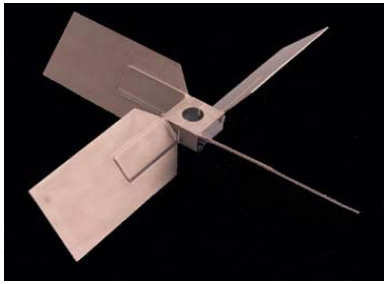
Bioengineering KLF 2000

The stirred tank bioreactor (STR)

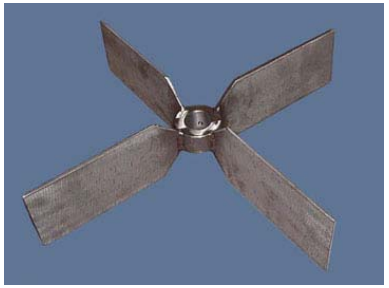
Standard geometry: stirrer

Disk stirrer		Radial mixing
Propeller stirrer		Axial mixing
Anchor stirrer		Tangential stirring

Pitched-blade



Flat-blade radial



Propeller

Helic impeller

Rushton turbine with three blades

Flat-blade disc turbine

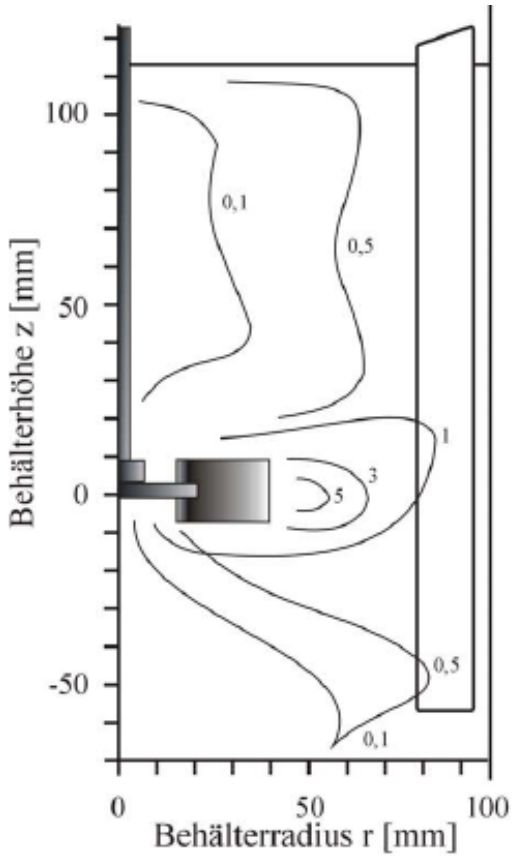
Ribbon

Gate anchor

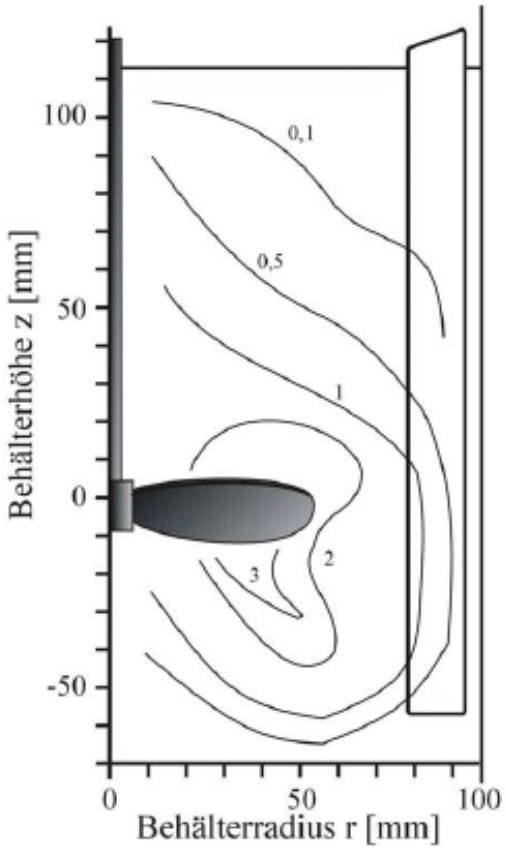
Helical screw

- All stirrers are made of stainless steel.
- Most of them can be fixed at different positions of the stirrer shaft.

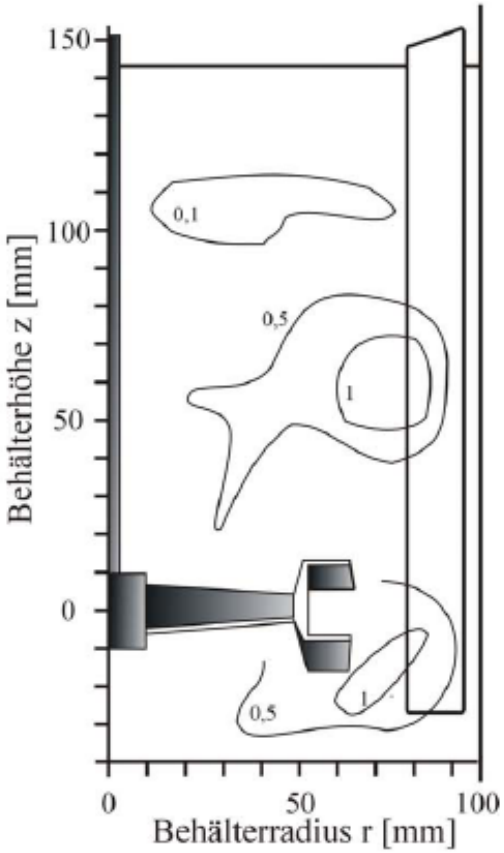
The stirred tank bioreactor (STR) : Power Input (W/kg)



Rushton with 6 blades



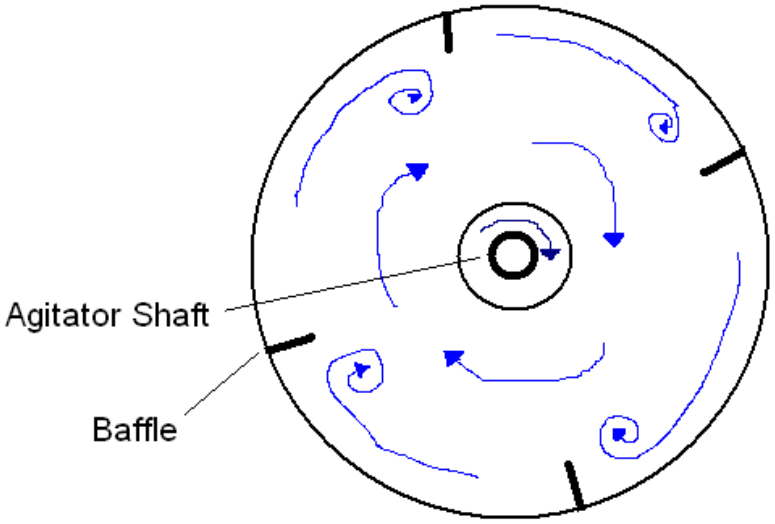
Propeller with 3 blades

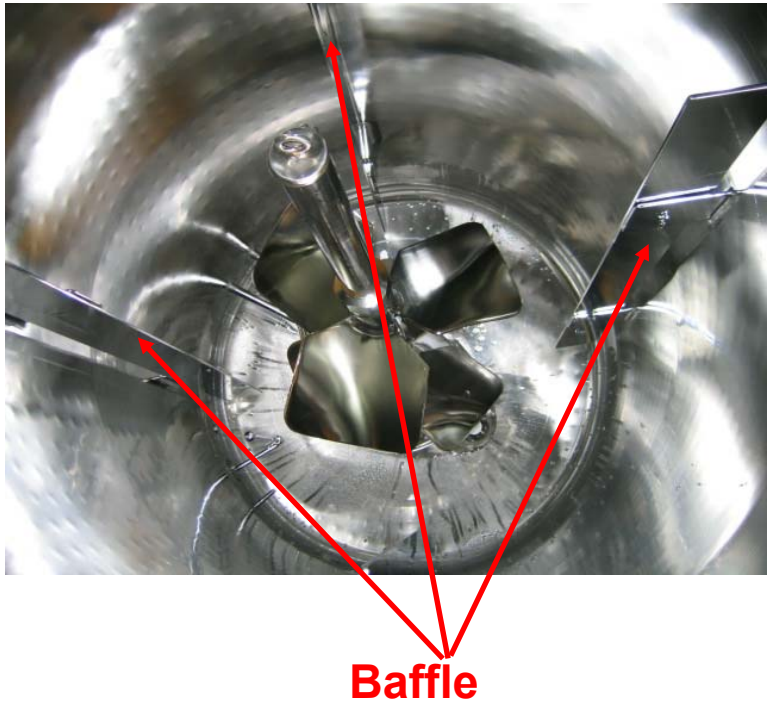


2x INTERMIG stirrer

Bioreactor - Baffles

- **Baffles** are obstructions on the side of the vessel that generate turbulence in the flow of the culture.
- Baffles are made out of stainless steel and are welded to the inside of the vessel.
- Baffles help to mix the culture by creating a more turbulent flow.



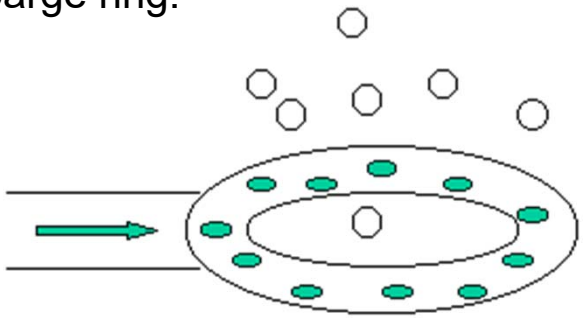


Aeration of stirred tank bioreactors

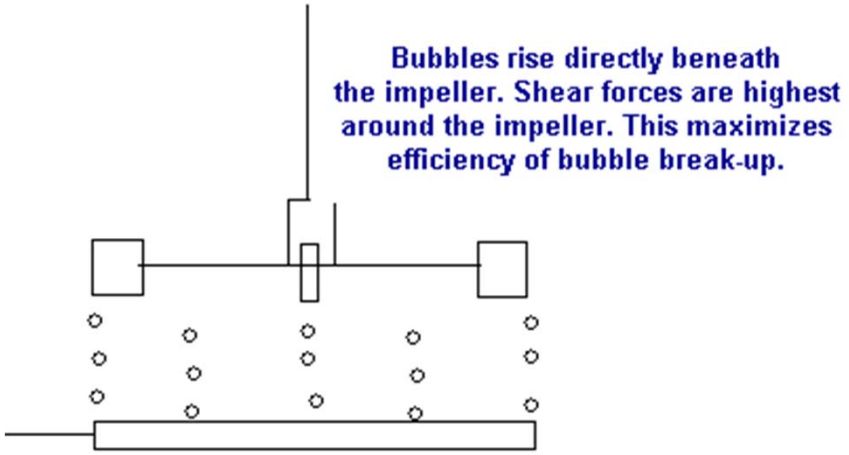
The stirred tank bioreactor (STR)

Oxygen delivery system - Sparger

The air sparger breaks the incoming air into small bubbles.
Various designs can be used such as porous materials made of glass or metal.
However, the most commonly used type of sparger used in modern bioreactors is the sparge ring:



The sparge ring must be located below the agitator and be approximately the same diameter as the impeller.



Thus, the bubbles rise into the impeller blades, facilitating bubble break up.

The stirred tank bioreactor (STR)

Oxygen delivery system - Sparger

Micro sparger

Ring sparger

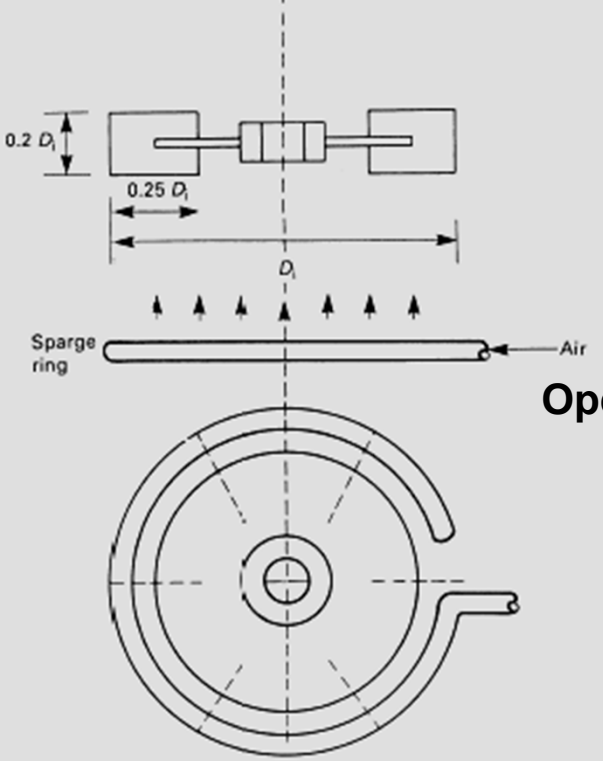
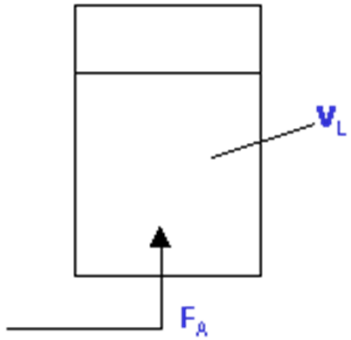


Fig. 4.5 Flat-blade turbine and sparge ring.

Openings on ring sparger

Oxygen delivery system - Air flow rates

Air flow rates are typically reported in terms of
Volume air per volume liquid per minute
or
vvm



$$vvm = \frac{F_A\,[L\,min^{-1}]}{V_L\,[L]}$$

Oxygen delivery system – Foam control

Foam control is an essential element of the operation of a sparged bioreactor. The following picture shows the accumulation of foam in a 2 liter laboratory reactor.

Excessive foam formation can lead to blocked air exit filters and to pressure build up in the reactor. The latter can lead to a loss of medium, damage to the reactor and even injury to operating personnel.

Foam is typically controlled with aid of **antifoaming agents** based on silicone or on vegetable oils. Excessive antifoam addition can however result in poor oxygen transfer rates.

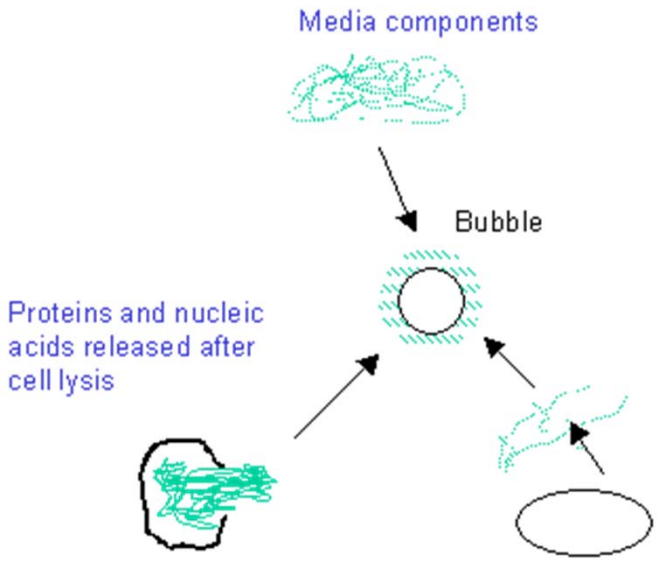
Factors affecting antifoam requirements

The following factors affect the foam formation and the requirement for antifoam addition:

- the fermentation medium
- products produced during the fermentation
- the aeration rate and stirrer speed.
- the use of mechanical foam breakers
- the head space volume
- condenser temperature

Factors affecting antifoam requirements – medium and cells

Media **rich in proteins** will tend to foam more readily than simple media. For example, the use of whey powder and corn steep liquor, two common nitrogen sources will contribute significantly to rate of foam formation and the antifoam requirement.



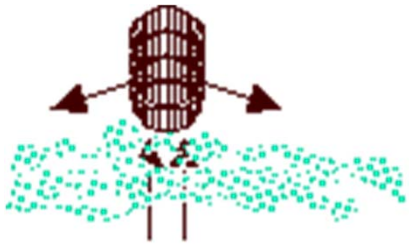
Many cells also produce **detergent-like molecules**.
These molecules can be nucleic acids and proteins released upon the death of the cells or proteins and lipid compounds produced during the growth of the cells.

The stirred tank bioreactor (STR)

Factors affecting antifoam requirements - Mechanical foam breakers

Mechanical foam breakers can eliminate or at least reduce the antifoam requirement.

These devices generate sit above the liquid and generate high shear forces which break the bubbles in the foam. High shear agitators and nozzles connected to high shear pumps are often used.
For small scale reactor systems such as those used in the culture of animal cells, ultrasonic foam breakers are sometimes used. These generate high frequency vibrations which break the bubbles in the foam.



The foam is sucked into a high shear device and in the process is broken up.

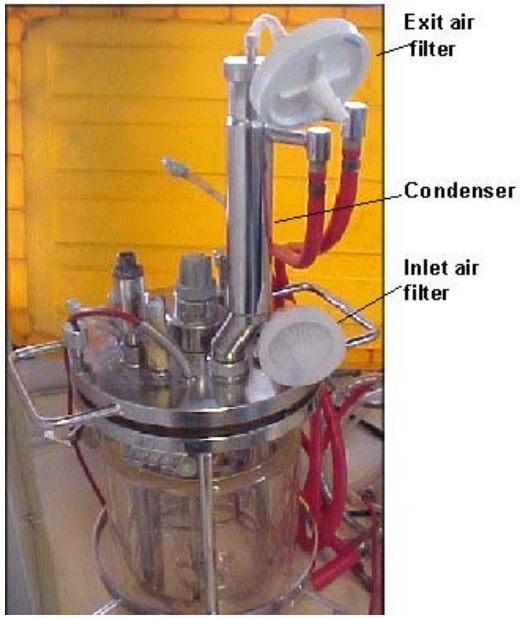
For small scale reactor systems such as those used in the culture of animal cells, ultrasonic foam breakers are sometimes used. These generate high frequency vibrations which break the bubbles in the foam.

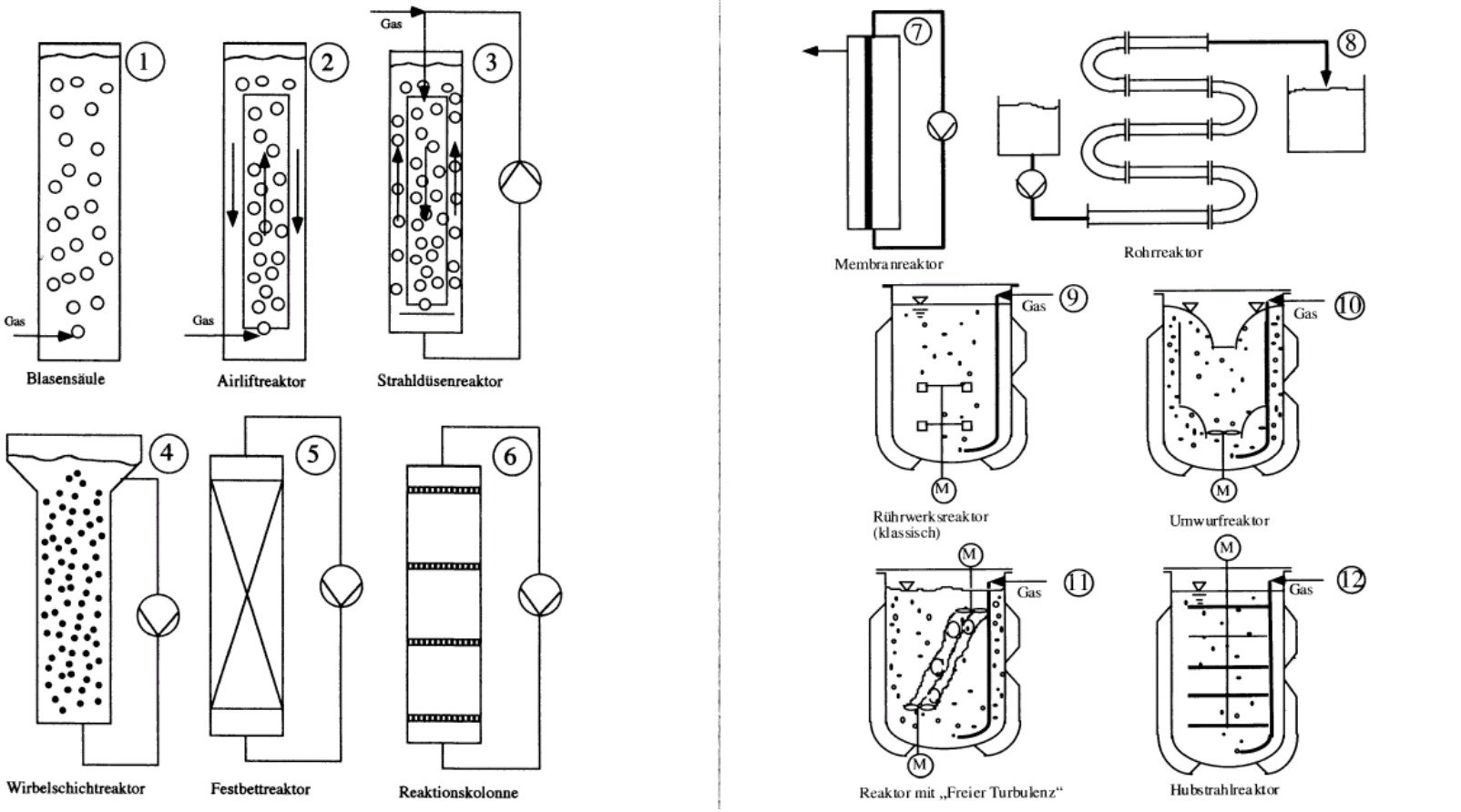
The stirred tank bioreactor (STR)

Oxygen delivery system – Foam control

Headspace volume

In laboratory scale reactors, a cold **condenser temperature** can help to control the foam. The density of the foam increases when it moves from the warm headspace volume to the cold condenser region. This causes the foam to collapse.





1. Bubble column
2. Airlift-reactor
3. Jet-reactor (Jet nozzle reactor)
4. Fluidized bed reactor
5. Solid-bed reactor
6. Reaction column reactor
7. Membrane reactor
8. Plug flow reactor / Tube
10. Stirred loop reactor
12. reciprocating-jet-bioreactor

Kriterium	Einheit	Zuordnung der Reaktoren zum Kriterium					
Viscosity	in Pa · s	> 2	< 2		< 0,4		< 0,1
	Reaktor →	9/11/12	10		3/4/6/7/8		1/2/5
Ease of pumping medium		s. schlecht	schlecht		gut		sehr gut
	Reaktor →	9/11/12	10/1		2/4/7/8		3/5/6
Stability of m.o.		nicht		etwas		gut	
	Reaktor →	1/2		4/6/9/10		3/5/7/8/11/12	
Max. size of reactor	in m^3	> 500	< 400	< 300	< 100	< 10	< 5
	Reaktor →	1	9/4/12	2/11	3/5/6/10	7	8
Content of solid particles		sehr hoch →					sehr gering
	Reaktor →	9 11 12	10 4	2	1	8	3 6 5 7
Tendency for foam formation		sehr groß →					sehr gering
	Reaktor →	10 3 9	7 11	2	1	8	12 4 5 6
Ease of homogenization		sehr schlecht →					sehr gut
	Reaktor →	10 9 11	12 8	3	6	5	4 7 2 1
Heat and mass transfer		sehr groß →					sehr klein
	Reaktor →	3 9 10	11 12	8	7	6	2 4 1 5
Sterility requirements		sehr groß →					sehr klein
	Reaktor →	1 2 3	4 8	9	10	11	7 12 6 5
Biological safety		sehr groß →					sehr klein
	Reaktor →	1 2 3	4 8	9	10	11	7 12 6 5

Very high Very low

Mass transfer

Mass transfer in practice

What happens in reality…….

- The kinetic characteristics of a biological catalyst (cellular or enzymatic) do not always limit the rate of the overall reaction.

- Mixing is not always close to perfect and neither are the catalyst nor the substrates always in aqueous forms.

- The transfer of molecules between phases e.g.
  - from gas to liquid
  - non aqueous to aqueous
    - solid to liquid or liquid to solid

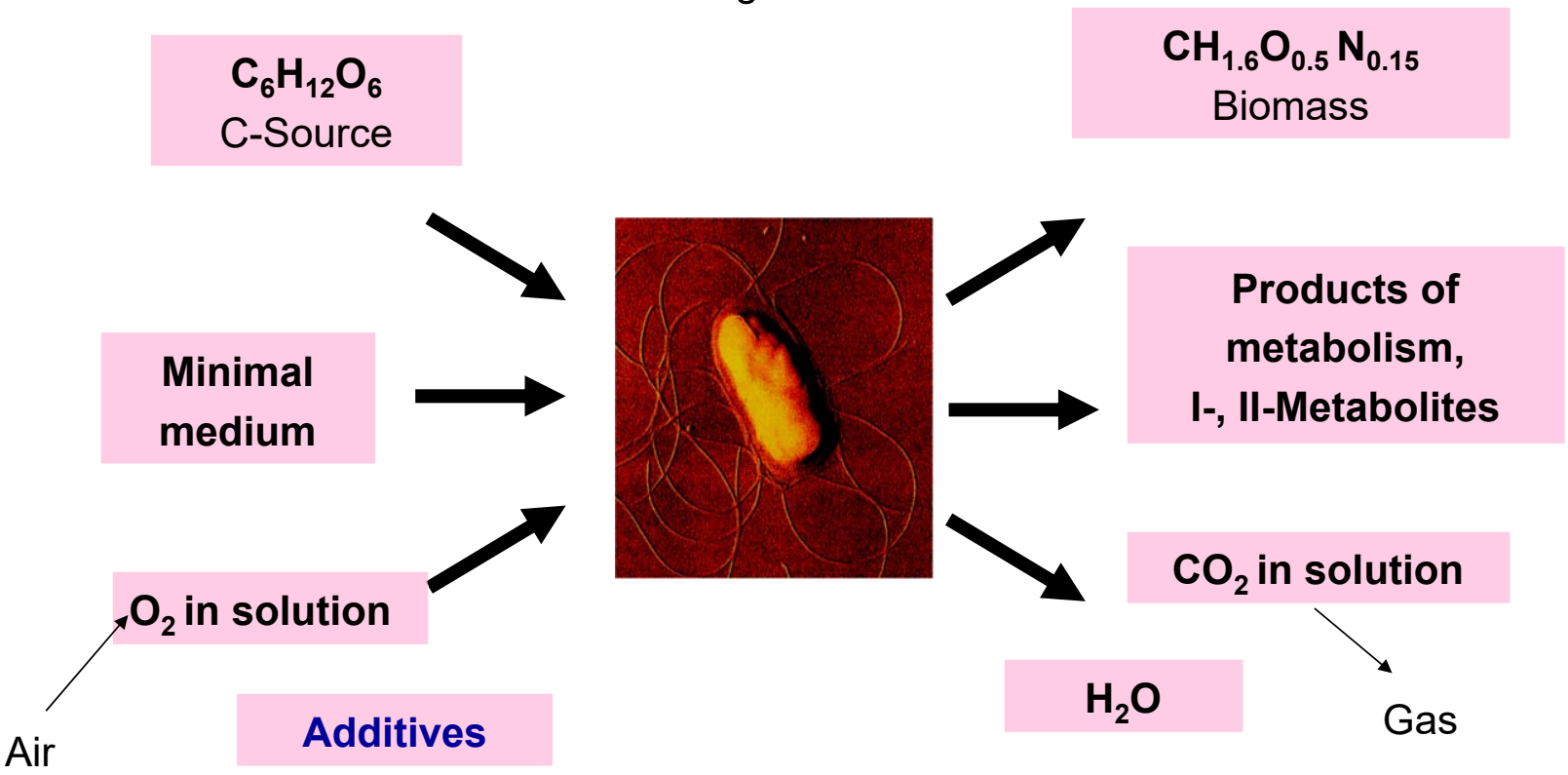
  is often the rate limiting step in a biological reaction!

Important:
In any reaction, mass must move and if the rate of movement of mass is slow, then the reaction rate will also be slow.

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



The Requirements for Growth: Chemical Requirements

Oxygen (O_2)

TABLE 6.1 The Effect of Oxygen on the Growth of Various Types of Bacteria

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Micro-aerophiles
Effect of Oxygen on Growth	Only aerobic growth; oxygen required.	Both aerobic and anaerobic growth; greater growth in presence of oxygen.	Only anaerobic growth; ceases in presence of oxygen.	Only anaerobic growth; but continues in presence of oxygen.	Only aerobic growth; oxygen required in low concentration.
Bacterial Growth in Tube of Solid Growth Medium					
Explanation of Growth Patterns	Growth occurs only where high concentrations of oxygen have diffused into the medium.	Growth is best where most oxygen is present, but occurs throughout tube.	Growth occurs only where there is no oxygen.	Growth occurs evenly; oxygen has no effect.	Growth occurs only where a low concentration of oxygen has diffused into medium.
Explanation of Oxygen's Effects	Presence of enzymes catalase and superoxide dismutase (SOD) allows toxic forms of oxygen to be neutralized; can use oxygen.	Presence of enzymes catalase and SOD allows toxic forms of oxygen to be neutralized; can use oxygen.	Lacks enzymes to neutralize harmful forms of oxygen; cannot tolerate oxygen.	Presence of one enzyme, SOD, allows harmful forms of oxygen to be partially neutralized; tolerates oxygen.	Produce lethal amounts of toxic forms of oxygen if exposed to normal atmospheric oxygen.

Oxygen Transfer

Oxygen is very 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 liter H_2O at 20°C:

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

But:
Solubility decreases with increase of temperature and salt concentration!

Bacteria and oxygen consumption

- Microorganisms have a much larger oxygen demand per unit of mass than plant and animal cells.

Bacteria
1 - 4 g O_2/(g·h)

Fish
0.03 g O_2/(g ·h)

Yeast
0.1 g O_2/(g·h)

Human (working)
0.01 g O_2/(g·h)

- Therefore, the aeration of bacterial cells is a substantially larger problem than e.g. the aeration of a fish aquarium.

Oxygen Limitation

- A too small oxygen concentration in the growth medium leads to an **oxygen limitation**. Under such growth condition cells could use more oxygen than if made available per unit of time. Consequently, the entire production process is affected:
  - In the growth phase only a constant quantity of biomass can be formed per unit of time. Growth changes from **exponential to linear** (μ decreases continuously).
  - In the stationary phase energy production decreases and the formation of product per unit of time is reduced.
- Products of the **anaerobic metabolism** (*e.g.,* acohols or organic acids) can be formed, which negatively affect the production process.

Oxygen solubility

- In microbial cultures, an oxygen limitation develops if the dissolved oxygen concentration drops below 2-7% of the saturation concentration
- The saturation concentration of any gas can be calculated by using the Henry's law (which states that the solubility of a gas in a liquid at a given temperature is almost directly proportional to the partial pressure of that gas):

$$c_G^* = H_G \cdot p_G$$

c_G^*	[mol/L]	Saturation concentration of gas G in liquid phase
H_G	[mol/(LPa)	Henry coefficient for gas G in liquid phase
p_G	[Pa]	Partial pressure of gas G in gas phase

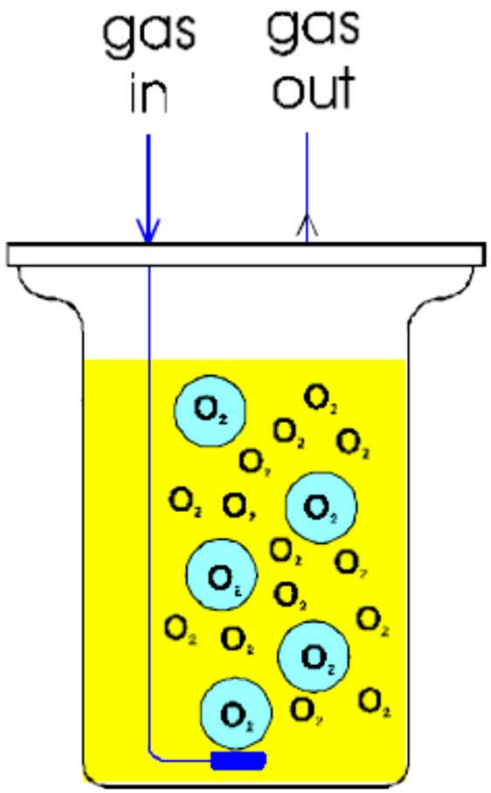
Correlation of Truesdale

- The Henry coefficient is temperature-dependent. Since the temperature of fermentations may vary, the Henry coefficient must be adjusted accordingly.
- For oxygen, the empirical correlation of Truesdale (1955) is helpful.

$$c^*_{O_2}(T) = \frac{p_{O_2}}{p_{atm}} \cdot (67.75 - 1.887\,°\mathrm{C}^{-1} \cdot T + 0.0369\,°\mathrm{C}^{-2} \cdot T^2 - 0.000309\,°\mathrm{C}^{-3} \cdot T^3)\,\frac{\mathrm{mg}}{\mathrm{L}}$$

$c^*_{O_2}$	[mg/L]	Saturation concentration of oxygen in liquid phase
p_{atm}	[Pa]	Standard normal pressure
p_{O_2}	[Pa]	Partial pressure of oxygen in gas phase
T	[°C]	Temperature

Gas/liquid mass transfer



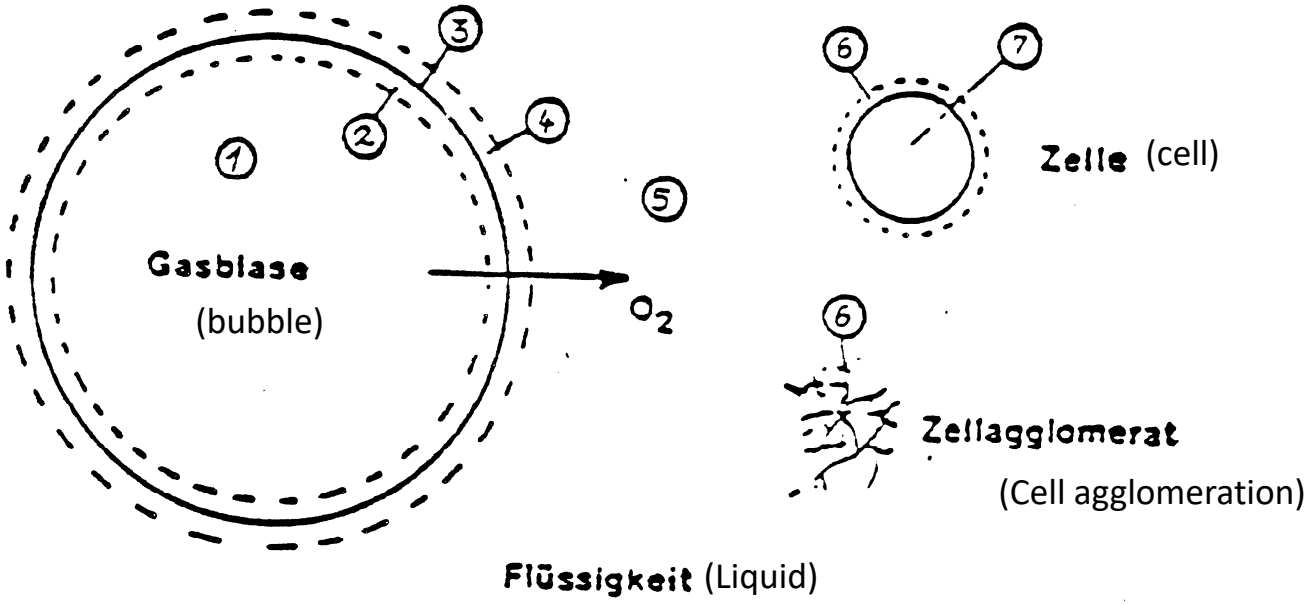
- Microorganisms can take up only dissolved gaseous substrates from aqueous media. Therefore an oxygen molecule must escape from its gas bubble and dissolve into the fermentation broth before it can be metabolised.
- This step is known more generally as gas/liquid mass transfer.

Rate limiting steps

- ✓ All physical mass transfer steps occur in series. The **slowest** step therefore will be the **rate controlling step.**
- ✓ Diffusion of oxygen in air is **10.000 times faster** than compared to the diffusional process in water. The oxygen transfer rate in the **gas film** therefore can be neglected.
- ✓ If the bulk liquid is well mixed, the convective transport of oxygen through the bulk liquid is **much faster** than compared to the diffusion in the liquid films.
- ✓ The **rate limiting steps** therefore are the oxygen diffusion through the **liquid films** (gas phase and microorganisms).

Diffusional coefficent of O_2 in gas approx.:	10^{-4}	m^2/s
Diffusional coefficent of O_2 in liquid approx.:	10^{-9}	m^2/s

Two liquid film model

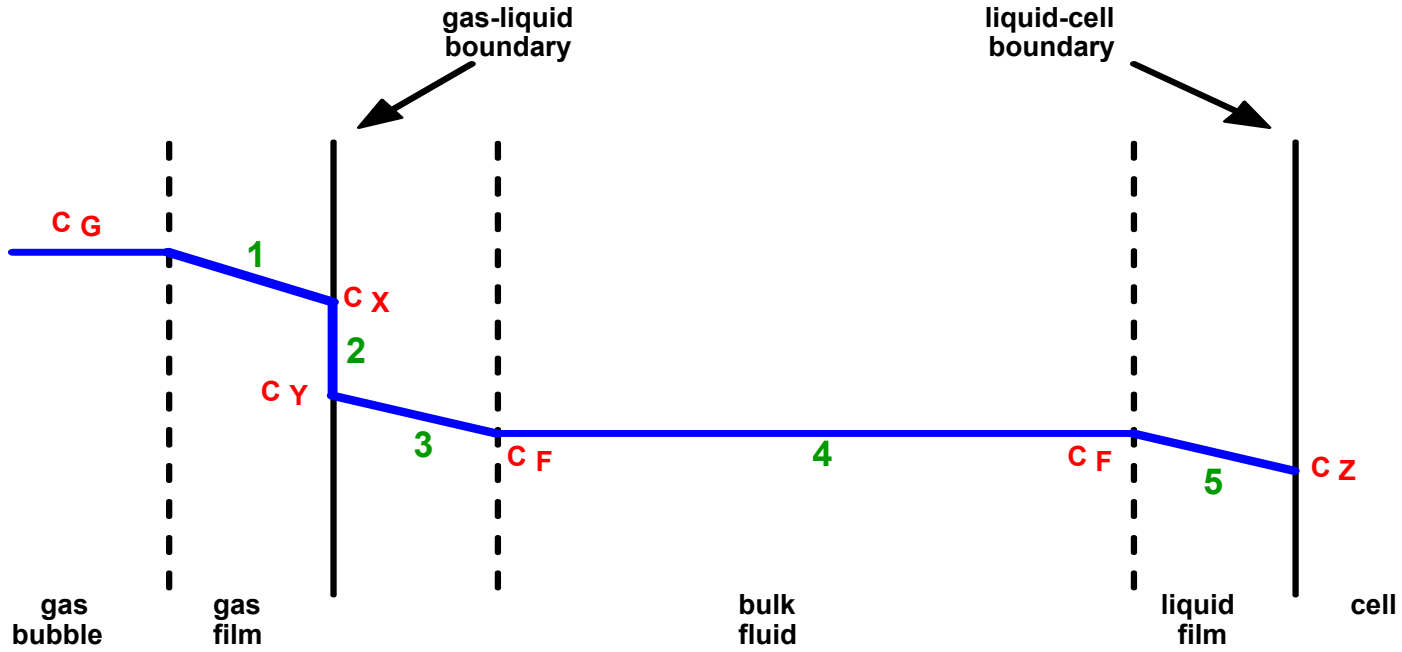


A combination of the following transfer resistances are typically found for O_2:

1. Diffusion from bulk gas to gas-liquid interface
2. Passage through gas-liquid interface
3. Diffusion through the poorly mixed liquid film to well mixed bulk liquid
4. Transport through the bulk liquid to liquid film (poorly mixed) surrounded cell
5. Transport through liquid film at cell interface
6. Diffusion into flocs, cell aggregates, mycelia or immobilisation matrix
7. Transport across cell envelope into cell and internal diffusion to reactive site e.g. mitochondria

Remember: The diffusion of O_2 in air is about 100'000x faster than in H_2O!

Resistance to oxygen transfer from air bubble to microbial cell



Note: Antifoam and protective agents (e.g. Pluronic™ F-68) increase O_2 solubility but also increase mass transfer resistance.

Oxygen transfer rate OTR (1)

- To quantify the rate (speed) of oxygen transferred from the gaseous phase to the microorganisms the following relation is used:

$$OTR \approx \frac{\text{driving force of concentration difference}}{\text{diffusional resistence}}$$

$$\approx \frac{\Delta c}{R}$$

- The driving force Δ c in this relation is the concentration difference of oxygen at both sides of a film or barrier.

- The main transport residence R is the mass transfer through the gas and liquid films, the boundary layers and the bulk medium.

Liquid films: k_L and A

The mass transfer rate in liquid films can be described by:

- The size of the **boundary surface area A** (thus the surface area of the gas bubbles or the surface of the microorganisms).

- The **mass transfer coefficient k_L** ,which is determined by:
  - The rate of molecular **oxygen diffusion** through the liquid films.
  - The **thickness d** of the liquid films.

The **diffusional resistance R** of the liquid films therefore can be expressed as:

$$R = \frac{1}{k_L \cdot A}$$

Oxygen transfer rate OTR (2)

By introducing the term: $$R = \frac{1}{k_L \cdot A}$$

in the relation: $$OTR \approx \frac{1}{R} \cdot \Delta c$$

reformulation gives: $$OTR \approx k_L \cdot A \cdot \Delta c$$

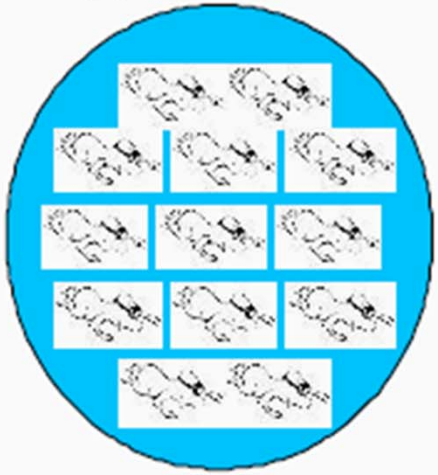
Specific surface area a

- For practical reasons it is advantageous to introduce the specific (referred to the volume) surface area a:

$$R = \frac{1}{k_L \cdot a}$$

- The specific surface area of the gas bubbles is substantially smaller than compared to the specific surface of the small bacteria. Therefore the diffusional procedures within the liquid film and the boundary layer of the microorganisms can be neglected.

ø gas bubble
approx. **1 mm**



ø bacteria
approx. **1 µm**

Convective mass transfer

Rate of mass transfer is directly proportional to the driving force for transfer, and the area available for the transfer process to take place. This can be expressed as:

Transfer rate $\propto$ (transfer area) x (driving force)

This proportionality coefficient in this equation is called the ***mass transfer coefficient,*** so that:

Transfer rate = (mass transfer coefficient) x (transfer area) x (driving force)

$$N_A = k\, a\, \Delta C_A = k\, a\, (C_{Ao} - C_{Ai})$$

N_A : rate of mass transfer of component A
k: mass transfer coefficient
a : area available for mass transfer

k: mass transfer coefficient
C_{Ao}: bulk concentration of component A away from phase boundary
$C_{Ai:}$ concentration of A at interface

Oxygen transfer rate OTR

If the concentration difference Δ c 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:

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

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 $k_L a$

- 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	[m^2/h]	diffusional coefficient
d	[m]	liquid film width

- Multiplied by the unit of the specific surface area a [m^2/m^3] the unit of $k_L a$ results as:

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

- Typical $k_L a$-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 $k_L a$ affected? (2)

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

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

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

- Enrichment of aeration air with **pure O_2** (expensive!)
  - c_{O2}^* and therefore Δ c increases.

- Using of a fermentation protocol with a **low process temperature**:
  - c_{O2}^* and therefore Δ c increases.

Oxygen Transfer

Problem:

- HCDC = High cell densities of exponentially growing *E. coli* lead to an exponentially increased oxygen consumption.

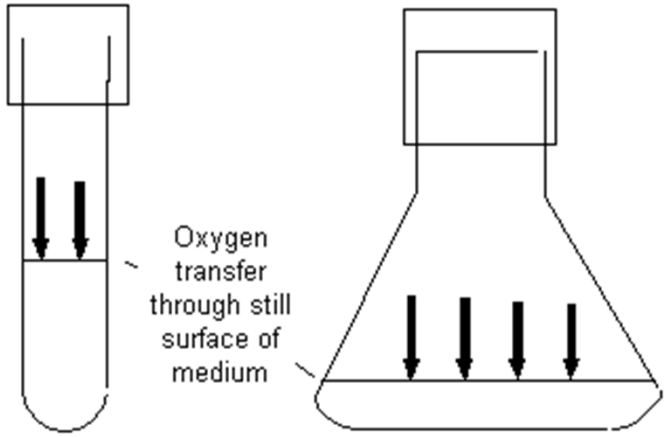
$$OUR = \frac{\dot{m}_{O_2}}{V_L} = \frac{1}{y_{X/O_2}} \cdot \mu \cdot X$$

OUR	Oxygen uptake rate	[g O_2/(l·h)]
m_{O2}	Mass flow of O_2	[g O_2/h]
V_L	Liquid (suspension) volume	[l]
$y_{X/O2}$	Biomass substrate yield coefficient (for O_2 as substrate)	[g Biomass/ g O_2]
μ	Specific growth rate	[h^{-1}]
X	Biomass concentration	[g/l]

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.



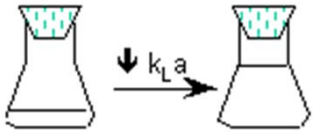
The rate of oxygen transfer will be poor due to the small surface area for transfer. Standing cultures are commonly used in small scale laboratory systems in which oxygen supply is not critical. For example, biochemical tests used for the identification of bacteria are often performed in test-tubes containing between 5-10 mL of media.

Oxygen Transfer

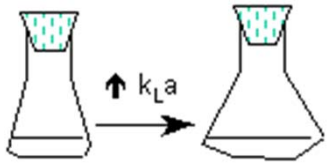
Shake flasks- factors affecting k_La

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

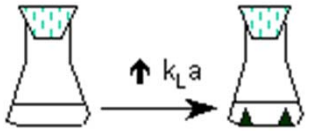
- shaking speed
- the liquid volume
- shake flask design.



k_La decreases with liquid volume



k_La increases with liquid surface area



k_La is higher when baffles are present

- The k_La 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 250 ml 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 entrainment 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.

CO_2 mass transfer

Example of a dissolved gas which can undergo liquid phase reactions

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

$$CO_2 + H_2O <-> H_2CO_3 <-> H^+ + HCO_3^- <-> 2H^+ CO_3^{2-}$$

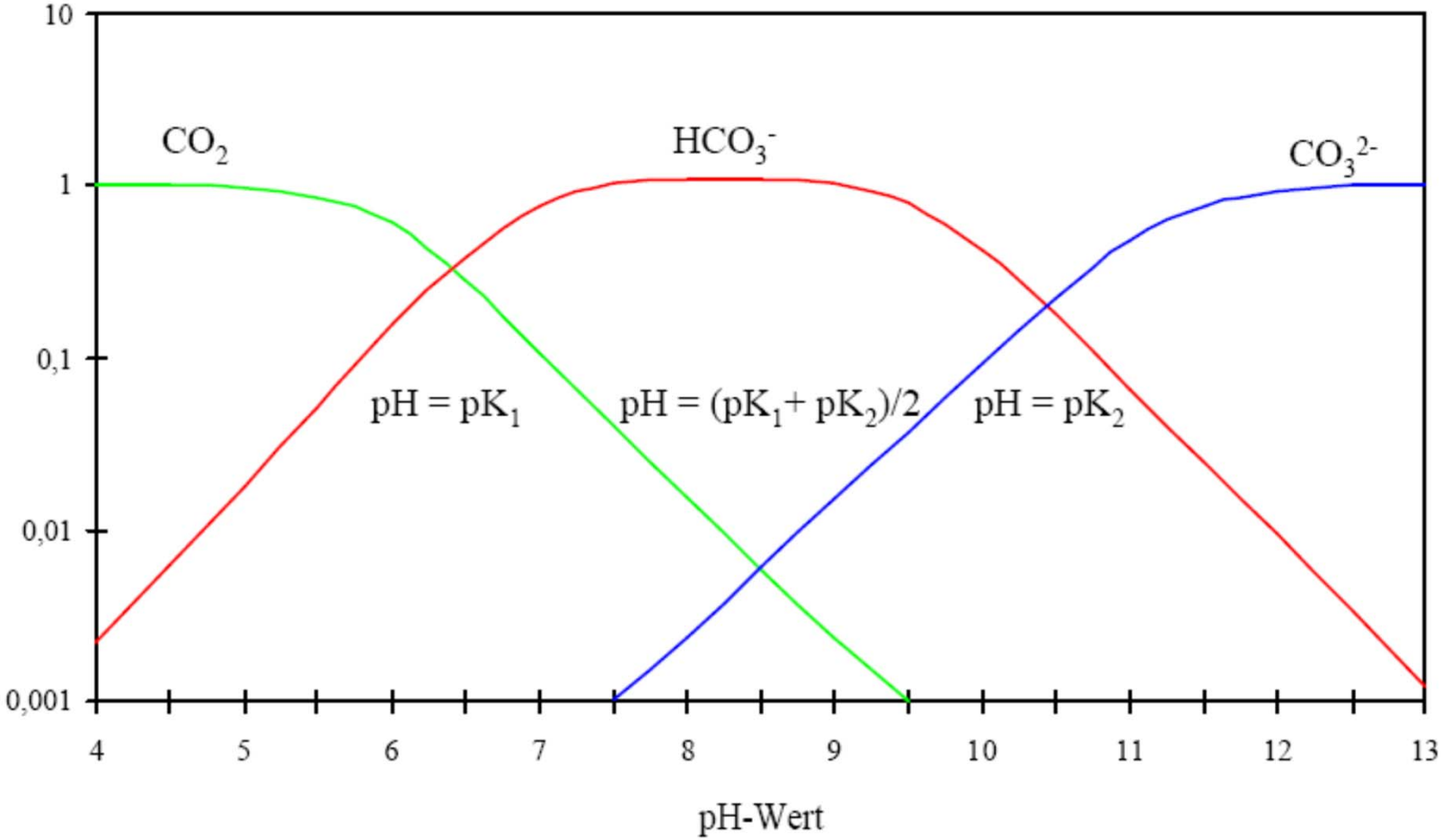
Concentration of each species depends on pH:

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

Note: Carbonic acid H_2CO_3 is not stable and reacts directly to bicarbonate HCO_3^-

CO_2 addition is used to control the pH of animal cell cultures.

CO_2 mass transfer



Summary: General requirements for bioreactors / fermenters

- Materials should not influence the metabolism of microbes
- Big oxygen transfer (OTR) and kLa values
- Homogeneous distribution of all reactants in the bioreactor
- Avoidance of badly mixed zones
- Efficient heat removal
- Minimization of foam formation
- Robust equipment and uncomplicated to handle
- Sterile process guidance must be guaranteed
- System must be suitable for scale-up
- Energy consumption of the agitation system should be low
- System should be flexible for different process requirements

…and finally the 1 million $ question:

What's wrong on the picture?

http://marine-vectors.eu