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MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY

University of Chemistry and Technology Prague
Faculty of Food and Biochemical technology

Engineering aspects of biotechnological processes

What is Biotechnology?

Károly Ereky invented the word "biotechnology" in Hungary during 1917 to describe a technology based on biological conversion of raw materials into a more useful product.

Biotechnology can be described as "the commercial application of living organisms or their products, which involves the deliberate manipulation of their DNA molecules".

Other definitions of biotechnology

- The integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological (industrial) application capabilities of microorganisms, culture tissue cells and their parts.
- Really no more than a name given to a set of techniques and processes.
- The use of living organisms and their components in agriculture, food production and other industrial processes.

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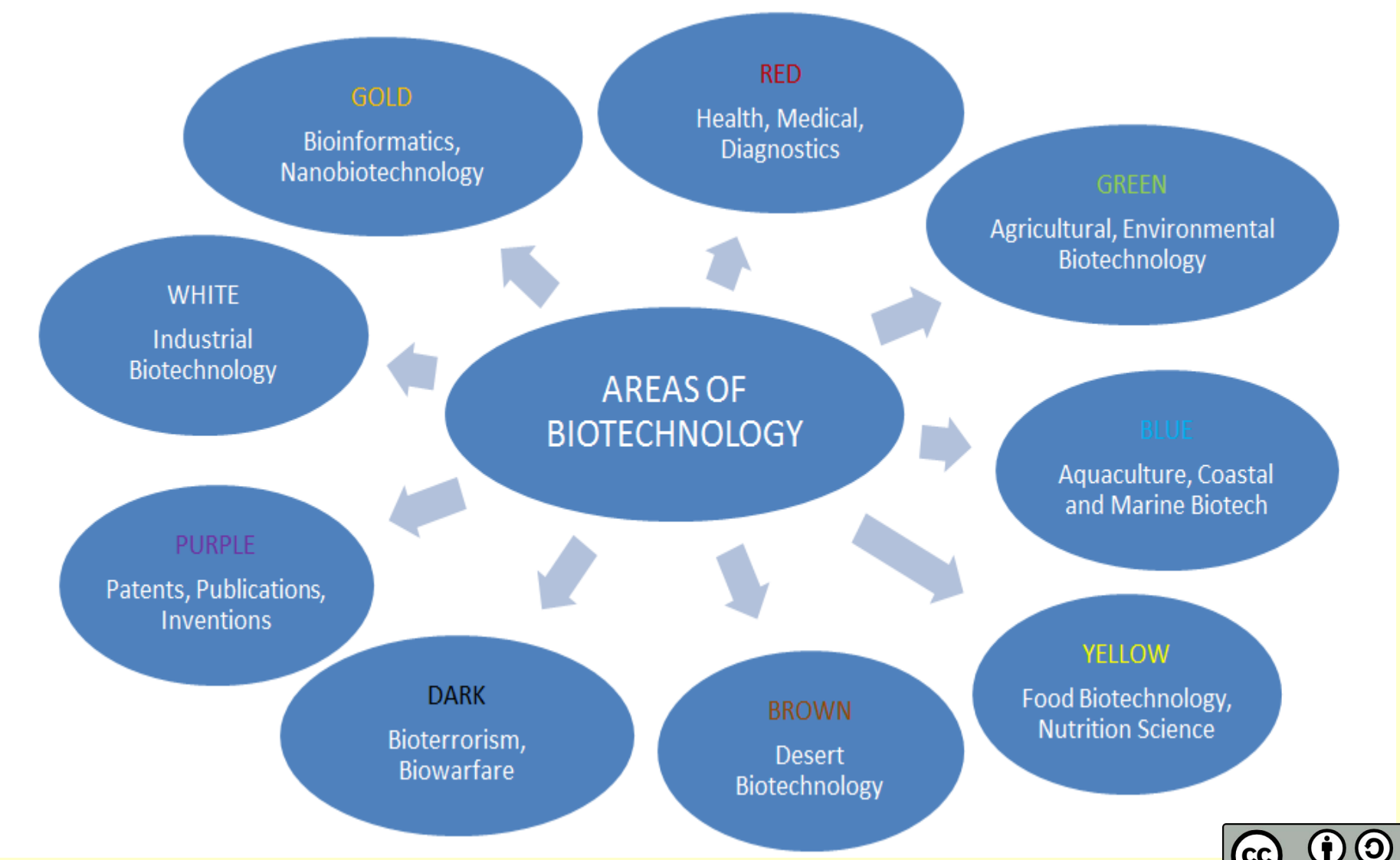
Introduction



Classification of Biotechnologies

Nowadays, biotechnology is a cross-sectoral technology that has been successfully applied in many industrial branches.

Red Biotechology - is applied to medical processes.

Today, 20 % of marketed medicines, 50 % of those in clinical trials, and 80 % in early development are biotech-based products. Typical products of red biotechnology are recombinant vaccines, antibodies, blood clotting agents, and hormones.

White Biotechology - biotechnological processes in industrial production.

It is the application of biotechnology for industrial purposes, including manufacturing, alternative energy (or "bioenergy"), and biomaterials. It includes the practice of using cells or components of cells like enzymes to generate industrially useful products.

Green Biotechnology – is applied to agricultural processes.

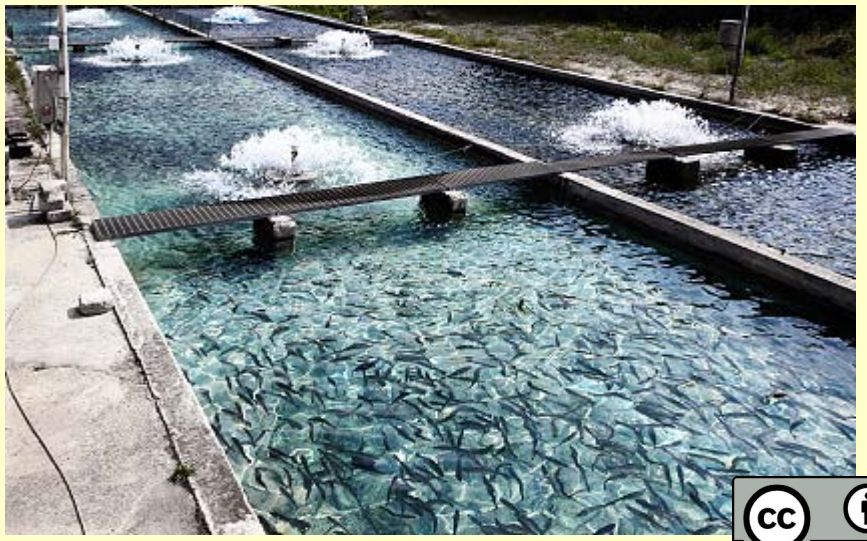
This area of biotechnology involves the introduction of **foreign genes** into economically important plant species, resulting **in crop improvement and** the production of **novel products in plants**.

Green biotechnology might also produce **more environmentally friendly** solutions **than traditional** industrial **agriculture**. An example of this is the **engineering of a plant to express a pesticide**, thereby eliminating the need for external application of pesticides.

Blue Biotechnology - is a term that has been used to describe the marine and aquatic applications of biotechnology

Not all blue ideas, however, are ready for practical and industrial applications.

Biomass from fishing or aquaculture industry is, in fact, complex, geographically and seasonally dependent.



Interdisciplinary character

Biotechnology draws on the pure biological sciences (genetics, microbiology, animal cell culture, molecular biology, biochemistry, embryology, cell biology) and in many instances is also dependent on knowledge and methods from outside the sphere of biology (chemical engineering, bioprocess engineering, information technology, biorobotics).

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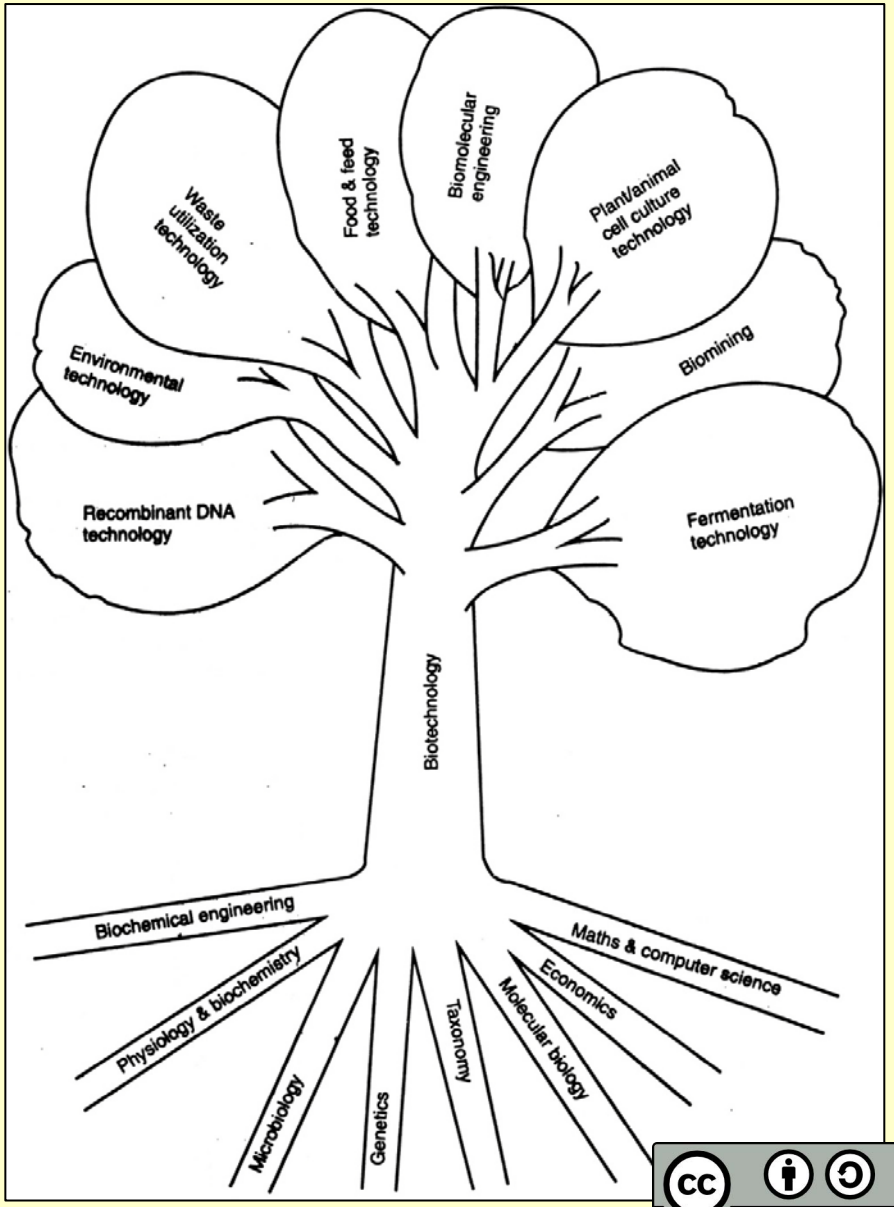
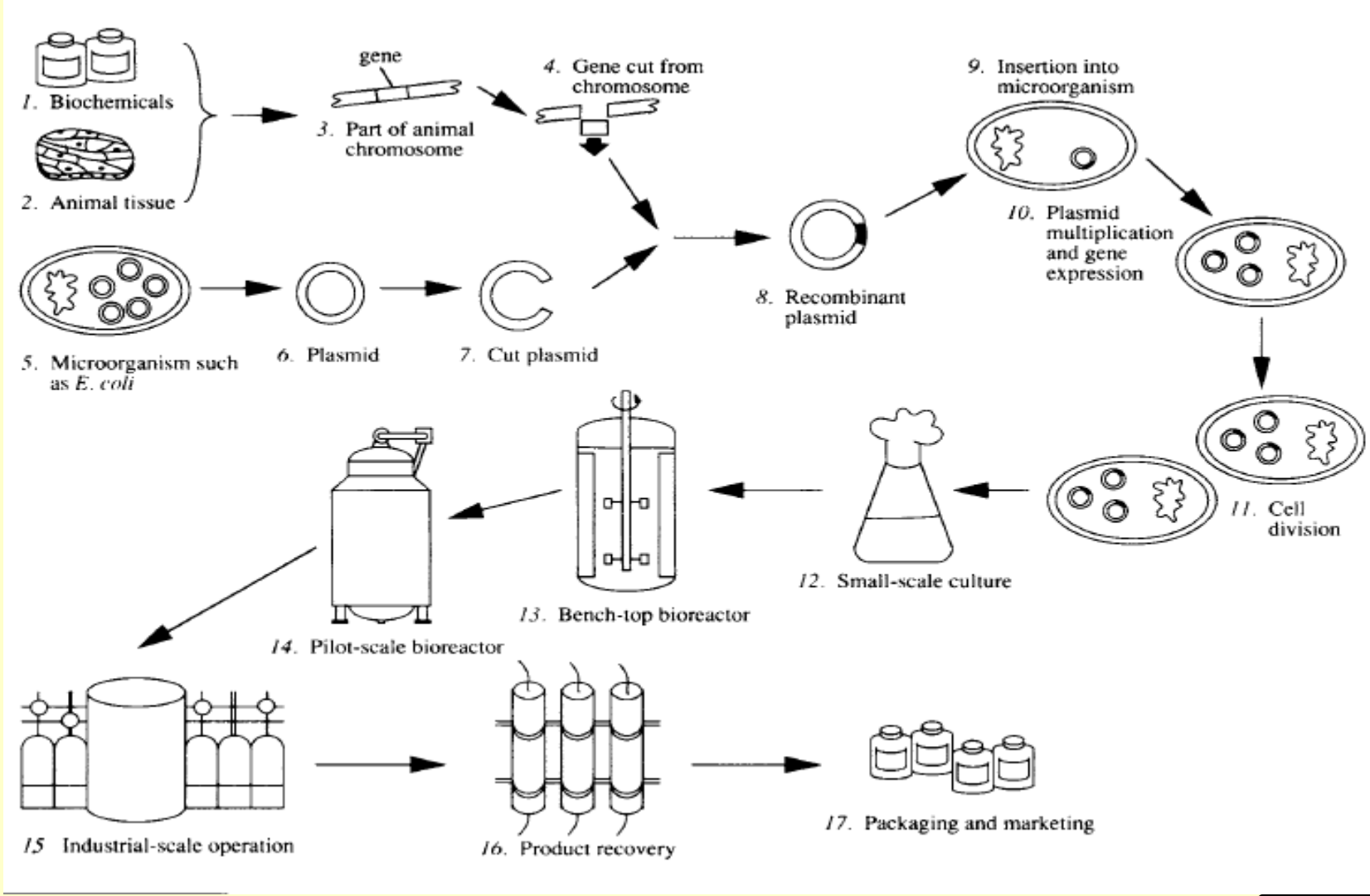
Introduction



Biotechnologies are tightly connected with engineering areas

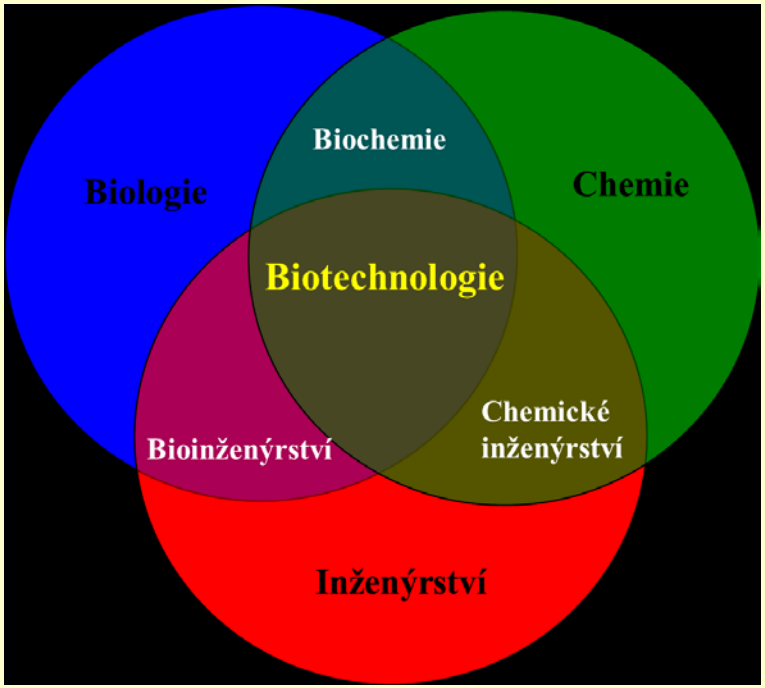
The connection exists at all stages of process development, but the importance of engineering aspects grows especially in the stage of scale-up from the flask to the laboratory fermenter and, of course, further into the industrial bioreactor!


1. Biochemicals
2. Animal tissue
gene
3. Part of animal chromosome
4. Gene cut from chromosome
5. Microorganism such as E. coli
6. Plasmid
7. Cut plasmid
8. Recombinant plasmid
9. Insertion into microorganism
10. Plasmid multiplication and gene expression
11. Cell division
12. Small-scale culture
13. Bench-top bioreactor
14. Pilot-scale bioreactor
15 Industrial-scale operation
16. Product recovery
17. Packaging and marketing

Bioengineering as regarded in this subject

- Kinetics of processes
- Modeling and prediction
- Material and energy balance
- Scale-up
- Up and down-stream processes
- Multiphase flow
- Construction and building
- Measurement and regulation
- Process design

Simplified view



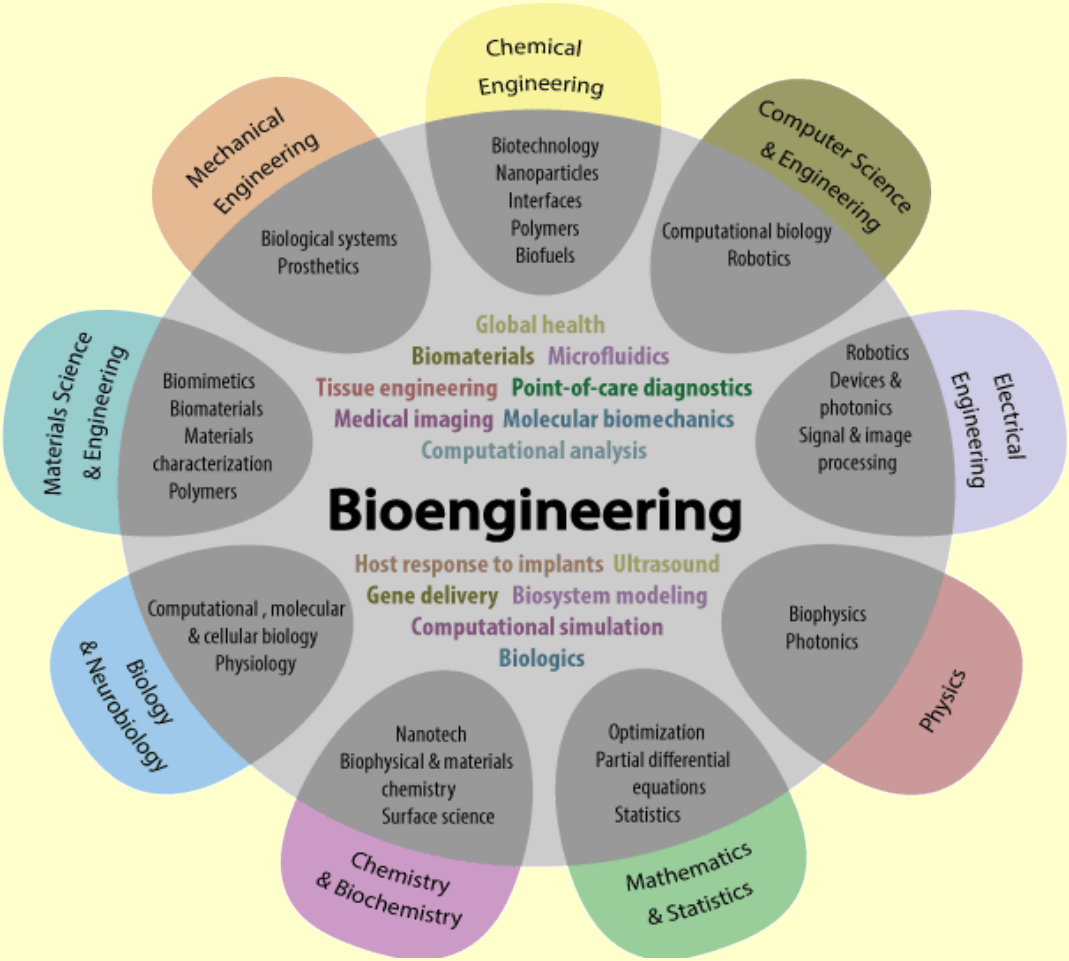
Bioengineering is a scientific discipline dealing with the quantitative description and interpretation of events taking place in the biochemical industry systems and facilities.

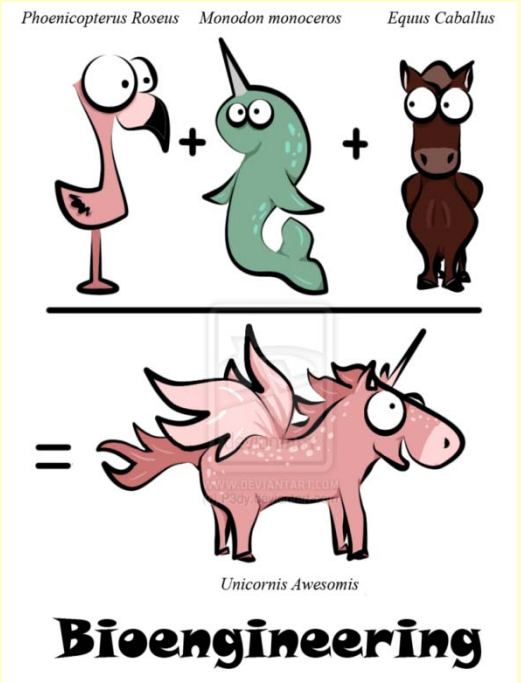
Bioengineering builds on science disciplines such as biochemistry, chemistry, physics, mathematics and biology.

Bioengineering methods allow:

- Rationally design and operate equipment for the preparation of raw materials, production and separation of the product
- Optimize operating mode to minimize environmental impact

Another view of "**bioengineering**" is much wider and more interdisciplinary.





Bioengineers are engineers who use the principles of biology and engineering tools to create usable, tangible and economically viable products.

There is a wide variety of biotechnological processes

According to claims for asepticity, they can be divided into:

- Open processes (non-aseptic, sewage treatment, some microalgae)
- Closed processes (aseptic, food)
- Closed processes (high-aseptic, pharmaceutical)

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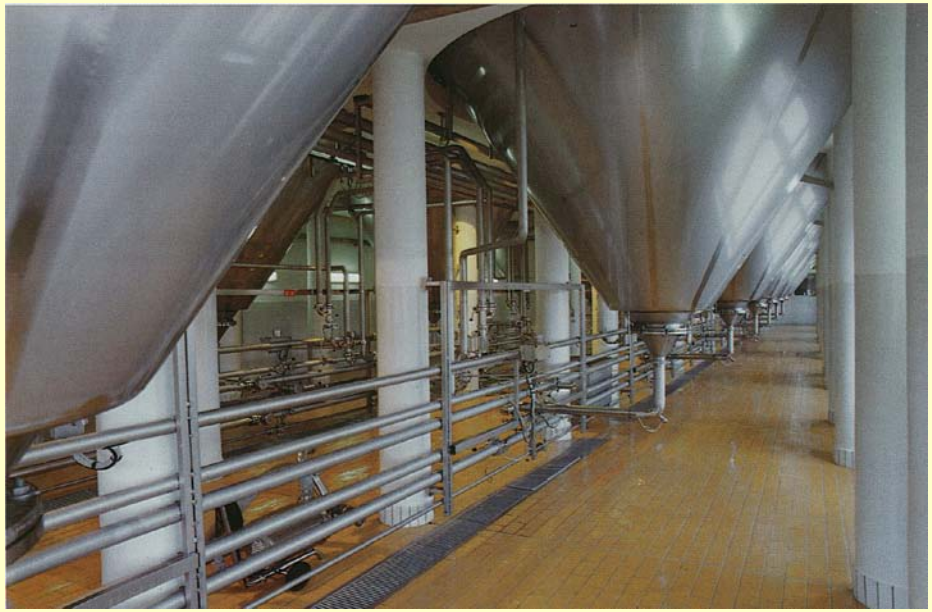
Introduction



When applying bioengineering methods in biotechnology, it is necessary to keep in mind:

1. Generalizing knowledge when using enzymes, micro-organisms or tissue cultures can be only limited. Changes in engineering parameters may have different responses, depending on the physiological state and the type of biological agent in the process.
2. The process must be monitored as a dynamic (variable in time). Changes in engineering parameters may trigger irreversible changes in the quality and quantity of response from the biologic factor and the initiation of new metabolic processes.

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History, presence and future of industrial microbial biotechnologies

Breakthrough in industrial application of microbial technologies was possible thanks to development in two areas:

- technologies of sterile bioprocesses (clean rooms, sterile unit operations)
- gene engineering (horizontal gene transfer)

Annual turnover of industrial scale suspension cultures is 260 bilion USD.

The most important market segments are: pharmaceuticals, cosmetics, industrial enzymes, food and nutrition supplements, feed.

History

1893 Production of lactic acid (Boehringer Ingelheim)

1916 Acetone-butanol-ethanol (ABE), *Clostridium sp*., fuel, I. World war (UK)

1923 Production of citric acid (Pfizer), beverages

1934 Vitamin C biooxidation of glucose (Hoffmann-La Roche)

1943 Peniciline by fermentation (Merck, Pfizer, USA), since 1948 also in Europe

1956 L-glutamic acid (Japan), in 1983 production of AA by fermentation represented 4% GDP of Japan

Since 80. of 20th century – Enzymes (technical and industrial applications), dominant role of Europe (Novozyme, Gist Brocades → DSM, Genencor).

Single cell proteins – *Candida sp*., largest sterile reactor ever built (1500 m^3), ICI UK, economic failure

1982 First recombinant insuline

Since 80. of 20th century – Biofuels, controversial topic

1994 Recombinant bovine growth hormone (Monsanto)

2012 First drug from suspension culture of plant cells approved by US FDA (Elelyso, company Protalix)

Present

In 1986 USA and Japan were indisputable leaders in biotechnologies

In 2010 almost half of overall biotechnological production comes from China and India

China is leader in traditional processes (vitamins, antibiotics atd.) due to technology transfer – cheap labour

In the field of high technology USA and Europe are still at the forefront

The period of major blockbusters reaching a large number of consumers is probably gone

Future

Personalized healthcare (therapy) will lead to further fragmentation of market. There will be increasing need for single use (disposable) production systems with flexible modular design.

Scal-up of stem cell culture in suspension is the largest challenge of current bioengineering.

The least exploited source of biodiversity with enormous biotechnological potential are oceans!

Only 1% of all known microorganisms are culturable in laboratories as axenic cultures.

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Present

Area	Colour	Products	Market size	No. companies
Pharmaceutical biotechnology	Red	Monoclonal antibodies and other therapeutic proteins, vaccines, insulin, DNA	>170 billion USD (12% ann. growth)	>6000
Industrial biotechnology	White	Small pharma molecules, bulk chemicals, flavour and aroma comp., industrial enzymes	>60 billion USD without biofuels (6% ann. growth)	>4000
Agro biotechnology	Green	Transgenic a genetically modified seed and plants	15 billion USD (11% ann. growth)	100
Environmental biotechnology	Gray	Biotechnologie s and services for environment, bioremediations, waste waters	<1 mlrd. USD (5-10% meziroční růst)	<50
Marine biotechnology	Blue	Products made by species from from marine environment	2 billion USD (4% ann. growth)	<50
Total			>250 billion USD (11% ann- growth)	10600

- Industrial biotechnologies (white) have 350 000m^3 fermentation capacities (60 billion USD)
- Pharmaceutical biotechnologies (red) have 5000m^3 fermentation capacities (170 billion USD)

Pharmaceutical biotechnologies (market aspects)

The total pharma market in 2012 was 990 billion USD, of that 820 billion USD were small molecules.

Pharma products made by sterile suspension cultures in bioreactors have a market value of ca. 200 billion USD

Biopharmaceuticlas (large molecules, red biotech.): market share (170 billion USD): 28% monoclonal antibodies, 24% vaccines, 18% proteins and peptides, 14% glycoproteins a glycopeptids, 8% insulin (annual growth 12% mainly due to consumption in China and India), 4% therapeutic enzymes, 3% stem cells

Small molecules (white biotech.) made by sterile suspension cultures in bioreactors: mainly secondary metabolites (antibiotics, cytostatics, imunosupressants, antiparasitics atd.). Often produced by chemical synthesis in connection with biotransformation.

Personal care products (market aspects)

Market volume produced by biotechnologies is ca. 1 billion USD.

Skin care applications are dominant: enzymes (lightening, anti-aging), proteins (moisturizing, skin protection), peptides (antimicrobial, dental hygiene), saccharides (moisturizing, skin protection, tanning), lipids (biosurfactants, moisturizing), vitamins etc.

Novel identified compounds are often of natural origin. Not possible to extract them in a sufficient amount from natural resources:

(i) Production by recombinant expression systems in suspension cultures

(ii) genetic manipulation of the original host engineered towards increased productivity.

Chemicals, industrial and technical enzymes (market aspects)

It is expected that in 2020 20% of global chemicals will be derived from biotechnologies.

Enzymyes– detergents (proteases, lipases, amylases, cellulases), textile and leather industry, organic synthesis (large potential for development).

Biopolymers – fuels, lubricants, biodegradable plastics

Fine chemicals and bulk chemicals (succinate, inositol, dihydroxyaceton atd.)

Fragrances, biosurfactants, live microorganisms (insecticides, biotransformations, bioremediations)

Food, Dietary supplements, Feed (market aspects)

Functinal foods and nutraceuticals in 2010, market size 7 billion USD.

Vitamins (partial or complete biosynthesis), ascirbic acid, carnitine, menachinon, biotin, folic acid (B9), B12, B2, E etc.

Amino acids, polyunsaturated fatty acids

Enzymes and proteins withou catalytic activity (stabilizers, sweeteners, probiotics, antimycotics, bactericidal compounds)

Polysaccharides (stabilizers, viscosifier), oligosaccharidyes (prebiotics), flavour compounds, colorants, provitamins, organic´acids

Live microorganisms (probiotics, preservants)

Present

First modern microbial fermentations are as old as the first electric programmable computer Colossus (1943) → the potential of microbial biotechnologies is far to be fully exploited.

Recombinant *Escherichia coli* and Chinese Hamster Ovary (CHO) cells are the main expression platforms of the pharma biotech industry. However, new expression platforms based on microalgae or transgenic plant cell can be expected. These will need a different bioengineering approach.

To produce profit in red biotechnology is still the easiest. In other areas the business margin is smaller.

The bright future of microbial biotechnologies is unquestionable. Massive development can be expected in the area of stem cells, bulk/feedstock chemicals and single use bioreactors.

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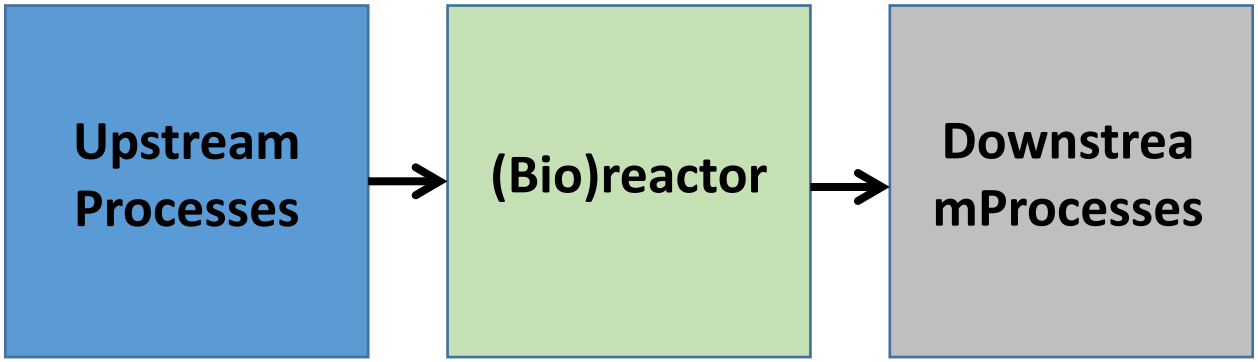
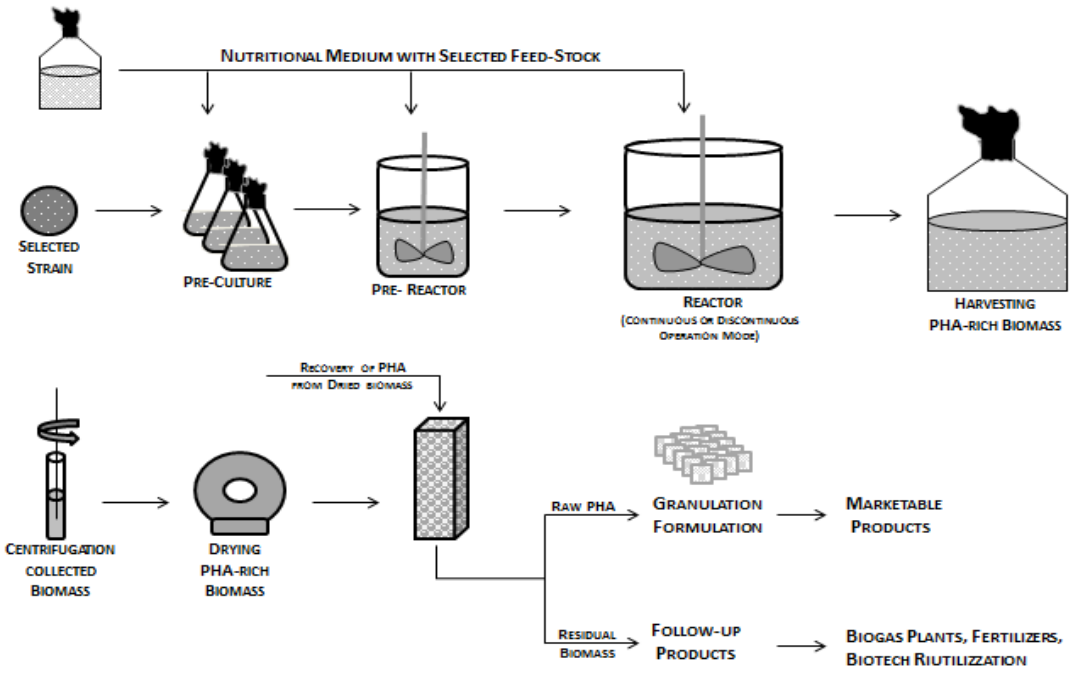
Structure of bioprocesses, overview of unit operations

Fundamental blocks of biotechnological processes



All steps require the knowledge and experience in bioengineering (chemical engineering) – unit operations, transport phenomena, enzyme and microbial kinetics, theorie of reactors, system engineering as well as knowledge in process regulation and control.


Nutritional Medium with Selected Feed-Stock
Selected Strain
Pre-Culture
Pre- Reactor
Reactor
(Continuous or Discontinuous Operation Mode)
Harvesting
PHA-rich Biomass
Recovery of PHA from Dried biomass
Centrifugation collected Biomass
Drying PHA-rich Biomass
Raw PHA
Granulation Formulation
Marketable Products
Residual Biomass
Follow-up Products
Biogas Plants, Fertilizers, Biotech Riutilizzation

Overview of unit operations used in food engineering

- Not all operations from Tab. 2.2 are used in all areas of biotechnologies
- In Tab. 2.2 are missing mainly the processes of preparative chromatography and formation and maintanance of clean roomsr

Table 2.2 Classification of Unit Operations of Food Processing

Group of Operations	Typical Food Processing Operations
Mechanical Transport	Pumping of Fluids Pneumatic Conveying Hydraulic Conveying Mechanical Conveying
Mechanical Processing	Peeling, Cutting, Slicing Size Reduction Sorting, Grading Mixing, Emulsification Agglomeration Extrusion, Forming
Mechanical Separations	Screening Cleaning, Washing Filtration Mechanical Expression Centrifugation
Heat Transfer Operations	Heating, Blanching Cooking, Frying Pasteurization Sterilization Evaporation Cooling, Freezing, Thawing
Mass Transfer Operations	Drying Extraction, Distillation Absorption, Adsorption Crystallization from Solution Ion Exchange
Membrane Separations	Ultrafiltration Reverse Osmosis
Non-Thermal Preservation	Irradiation High Pressure Pulsed Electric Fields
Packaging	Filling, Closing Metallic, Plastic Packages Aseptic Packaging

Up-stream operations

The economic success of the industrial fermentation process (or, in general, the biotechnology process) depends on the production strain and the optimization of the culture conditions in the bioreactor.

Creation and optimization of the culture medium

Cultivation medium is particularly important as it affects cell growth and finishing operations (isolation, purification).

It is necessary to define an objective way of evaluating the optimization (improvement) of the media. Most often, this is the amount of product, but it can also be the biomass concentration or specific productivity.

It is also important that the medium is as cheap as possible.

Creation and optimization of the culture medium

It is based on existing information about the microorganism and the medium is "fine-tuned" or must start from the beginning.

Design of Experiments (DOE) is used.

Complete optimization process:

1. Identification of the most important media components (screening)
2. Identification of optimal concentration ranges (narrowing)
3. Identification of optimal composition
4. Verification of optimization

Optimization platforms: flasks, microtiter plates, parallel minibiorectors

Sterilization

The goal is to remove all microorganisms present.
Sterilization can be done in several ways:

1. Heat
2. Filtration
3. Chemically (e.g., ethylene oxide, NO2, O3, NaClO, glutaraldehyde)
4. Irradiation (UV, X-rays, cathode radiation)

Heat sterilization and filtration are the most widely used in industry.

Biotechnology requires sterilization of:

1. Culture media, raw materials, products
2. Devices
3. Air

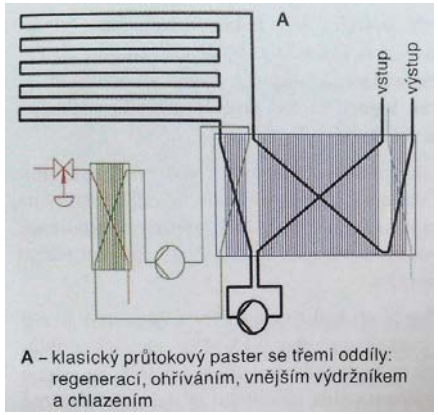
Heat sterilization

Batch process

For normal preparation of small volumes of nutrient media, 121°C (15 min), 126°C (10 min), 134°C (3 min) are used.

Operating reactors are sterilized in situ indirectly (steam, oil) or directly steamed into the media.

Suitable for media free of thermolabile compounds.



A – klasický průtokový paster se třemi oddíly: regenerací, ohříváním, vnějším výdržníkem a chlazením

Continuous process

Saving steam (135°C) and shortening the process (5-8 min).
Heating either by direct steam injection into the medium or by the exchanger.
It is preferred for solid-phase media containing thermolabile components.
This method requires bioreactor sterilization (steam, hot air, chemically)

Sterilization by filtration

Suitable for media containing thermolabile materials

For removal of bacteria and spore - filter with a porosity of 0.2 μm

Construction: membrane filters and candle filters

Prior to sterilization of the liquid (gas), it is necessary to filter the filter itself

Filter Sterilization: autoclave (121°C, 15 min), dry heat (180°C, 2h), ethylene oxide or formaldehyde (2%, 24h), UV radiation (50 cm from the surface, 20-30 min) nylon membrane filters, e.g. 145°C, 10h).

After each sterilization, filter testing is required.

Air sterilization

Due to limited air solubility, it is necessary to supply large amounts of air into aerobic processes, which increases the risk of contamination.

Air sterilization can be carried out in a variety of ways, but filtration is economically feasible for aerating the bioreactor**.**

Air filtration mechanism

Depth filtration - material layer (cm)

Mechanism: direct capture of large particles, slow motion and subsequent capture (ca 1 μm), weak physical interaction of particles (below 1 μm) with filter material.

Membrane filtration
Mechanism: direct capture due to particle size, particle adhesion due to van der Waals forces and electrostatic interactions.

Filtration materials: glass fiber (5-18 µm diameter), nitrate cellulose, teflon, polyamide, polyacrylate

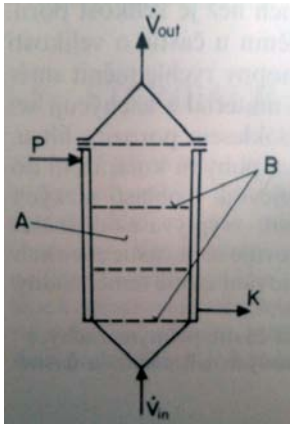
Air Preconditioning: Air is on an industrial scale compressed by turbochargers and compressors, the compressed air then passes through an oil separator and is guided into the pre-filter (removes the oil aerosol).

Filters for air sterilization

Filtration filter: uniform glass fiber filling, depth filtration, direct steam sterilization and subsequent drying using a duplicator jacket.

Advantages: Higher flow rates, less requirement for pre-filtration.

Disadvantages: loss of efficacy after wetting, energy demands on air heating (above dew point due to moisture), laborious sterilization and replacement.

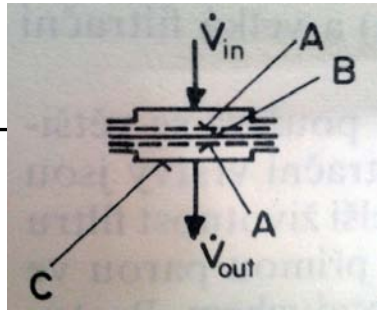


Depth filter:
A - fiberglass filling,
B - sieves, P - steam inlet,
K - condensate

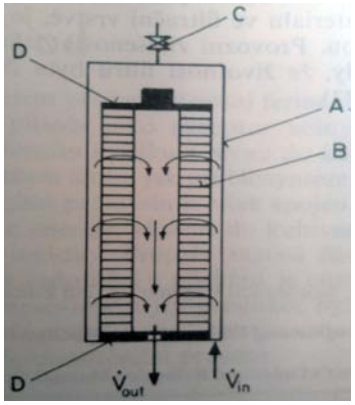
Filters for air sterilization

Membrane filters

- Steam sterilization in the direction of air flow
- Hydrophobic membranes - moisture does not affect filtration efficiency
- Disc filters have a small filter surface - faster fouling - only laboratory use
- Candle filters - larger filtration area (folded diaphragm) - suitable for large flows (industrial reactors)
- Simple and fast maintenance, long service life



Disk membrane filter:
A - porous support plate,
B - filter membrane,
C - filter jacket



Candle filter:
A - filter jacket, B – filter candles, C - vent valve, D - candle sealing

Bioreactors

They can be considered as key elements of the production line of biotechnological processes.

Classification of bioreactors can be done according to various criteria:

Based on the type and form of the catalyst used

1. Free cell culture reactors
2. Reactors with immobilized anzymes or cells

Based on biomass balance

1. Open systems (cells leave with culture medium)
2. Closed systems (only culture medium is released, cells / enzymes are retained in the system, or returned after separation e.g. membrane reactors)

Based on medium feed startegy

1. Batch - no nutrients are added to the system during cultivation, cultivation takes place until they are depleted.
2. The fed-batch fermentor is initially filled with a certain amount of culture medium, and the remainder of the substrate then flows gradually or at a constant rate, eliminating the substrate inhibition
3. Flow-through or continuous cultivation - Continuous inflow of nutrients into the system with simultaneous removal of the culture medium, advantageous because it allows long-term exponential growth of microorganisms. An alternative version is a so-called semi-continuous cultivation.

By use of microorganisms

1. Microorganisms cultivated for biomass (production of bacterial proteins, yeast)
2. Cultivation is a means of continuous chemical transformation of nutrients into products of metabolism (antibiotics, citric acid, proteins, degradation of pollutants, etc.)

According to the technical layout

1. Homogeneous (ideally stirred reactors, the environment does not change over time → the physiological state of the cells does not change over time)
2. Heterogeneous (cells at different locations in the system are in different states)

By degree of asepticity

1. Non-Aseptic Reactors (Sewage Treatment Plant)
2. Aseptic reactors (food, small amounts of non-pathogenic contamination do not mean product degradation)
3. Strict aseptic reactors (pharmaceutical production)

By mixing

1. Mechanically stirred reactors
2. Pneumatically stirred reactors
3. Hydraulically stirred reactors
4. Unmixed Reactors (packed-bed) *

According to the scale

1. Laboratory (up to 30 L)
2. Semi-pilot (30-100 L)
3. Pilot (100 L-5 m^3)
4. Industrial (over 5 m^3)

Mechanically stirred reactors

They use different agitators that perform homogenizing and dispersing functions.

90% of industrial biotechnological processes (microbial, enzymatic) are carried out in these types of reactors (batch, fed-batch).

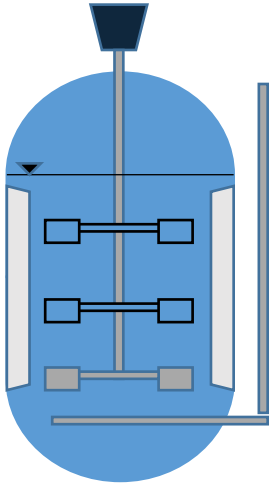
The dimensions of the STR (stirred tank reactor) range within a wide range.

Aerated ones usually have a higher H / D ratio.

A large number of structurally different mixers can be used.

They are usually filled to 75-80% of volume.

Additional equipment: heating, cooling, temperature sensors, pH, dissolved oxygen, pH control, antifoam, inoculation.



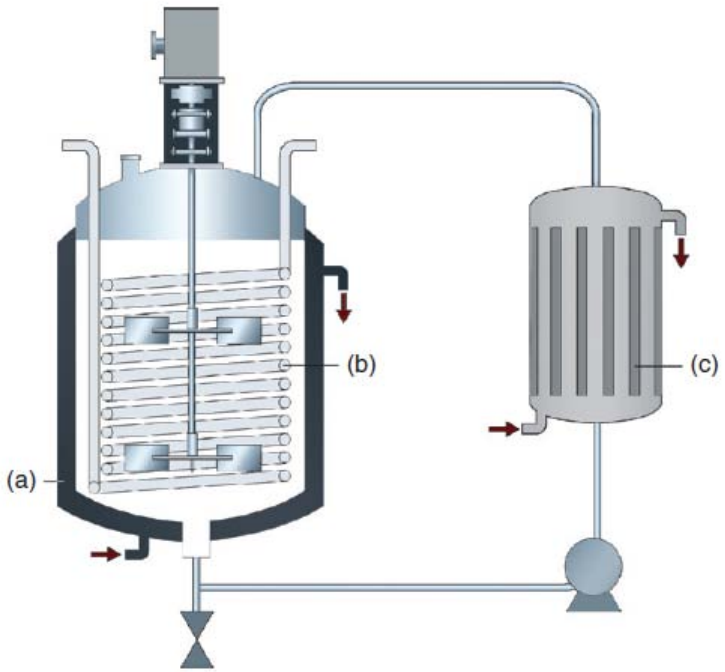
Mechanically stirred reactors

Biological processes require strict temperature regulation.

Typical physiological temperatures are ca. 30 ° C, but also considerably higher can occur (thermophilic micro-organisms / enzymes)

Temperature regulation by means of a heat exchange surface in the form:

- Jackets
- Coils
- External heat exchanger



Construction of mechanically stirred bioreactors

Materials

Lab. and semi-pilot scale reactors: glass, lids, agitator, baffles stainless steel

Industral scale reactors: stainless steel (316 and 316L), glass sight glass

Cylindrical shape, laboratory have flat bottoms, larger reactors have arched bottom (greater mechanical strength and without dead zones), smoothness Ra≤0.6µm.

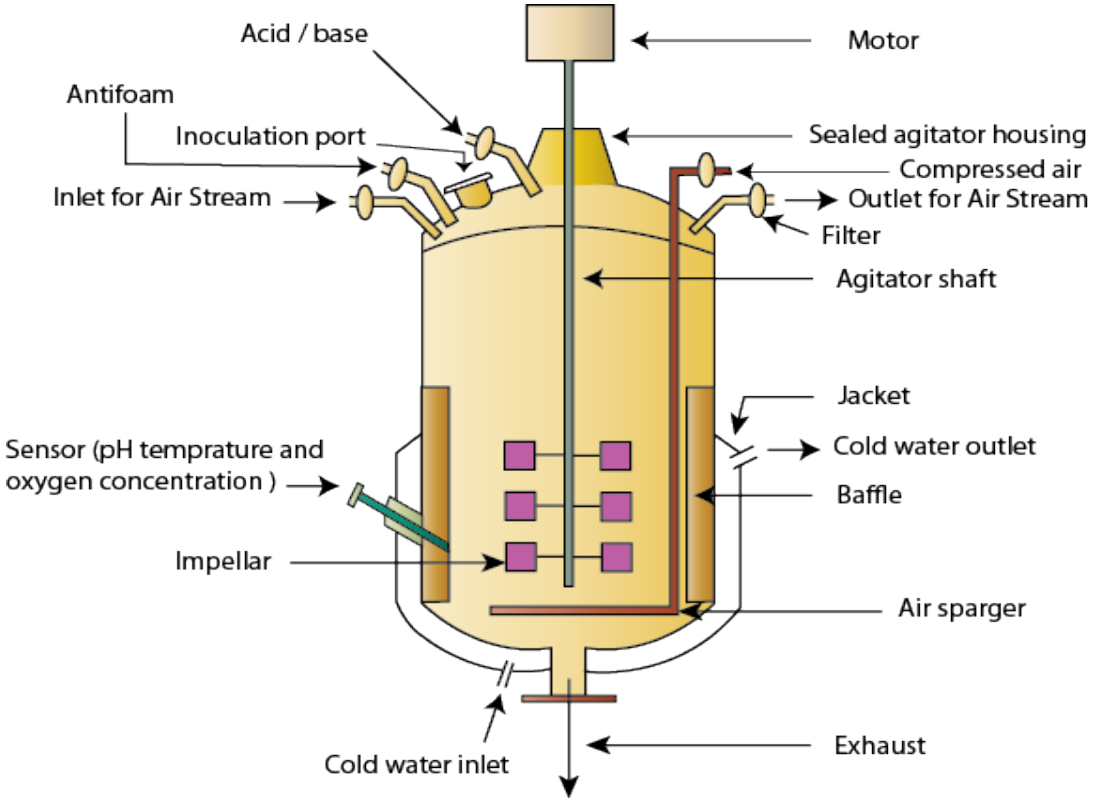
Agitators: usually turbine (more specifically in the lecture on mixing)

Gas distributor: Nozzle or aerator ring with holes down (1-2 mm for micro-organisms, 3-5 mm for filamentous microorganisms).

Drive of the agitators: from the top or bottom, the shaft is sealed by the seal at the inlet, a magnetic coupling is used for strictly aseptic conditions.

The measuring sensors are placed in 1/3 of the reactor height.

Cleaning and washing of the reactor: combination of mechanical and hydraulic cleaning, spray ball.


Acid / base
Motor
Antifoam
Inoculation port
Sealed agitator housing
Compressed air
Inlet for Air Stream
Outlet for Air Stream
Filter
Agitator shaft
Jacket
Sensor (pH temprature and
oxygen concentration)
Cold water outlet
Baffle
Impellar
Air sparger
Cold water inlet
Exhaust

Pneumatically stirred reactors

Bubble columns - no built-in, chaotic fluid flow.

Gaslift Reactors - Circulator inbuilts, directed fluis flow.

The gas (air, oxygen, CO_2) has a simultaneous homogenization and transport (aeration) effect.

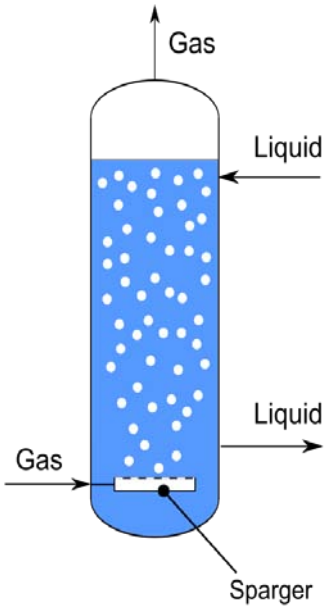
Lower shear forces than STR, but also a lower efficiency of mixing for higher viscosity media.

With high gas flow, foam problems may occur.

Easier process of scale up/down.

High ratio of mass transfer to power input.

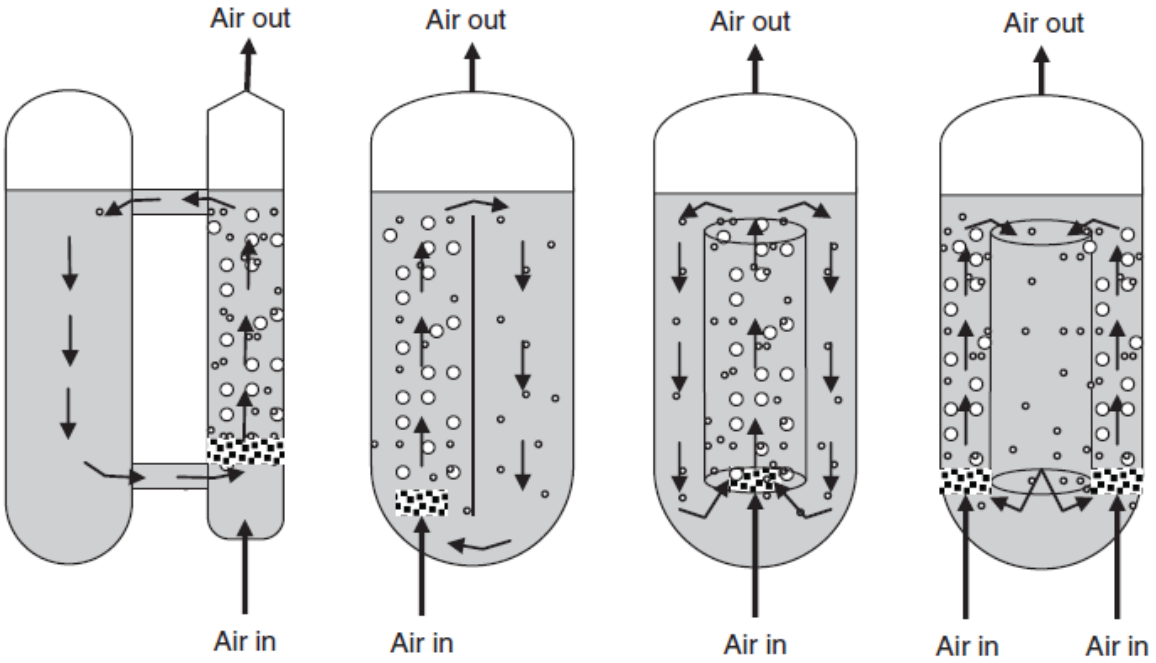
Usage: animal and plant cells sensitive to mechanical stress, wastewater treatment – low energy consuming reactors



Gaslift (airlift) reactors

From left to right:

1. Airlift reactor with external circulation
2. Airlift reactor with partition
3. Airlift reactor with internal circulation
4. Airlift reactor with internal circulation (reverse flow)



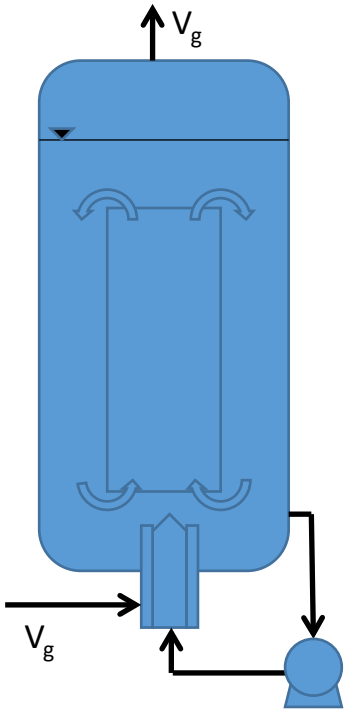
Hydraulically stirred reactors

The principle of mixing is to bring circular fluid flow through the action of pump.

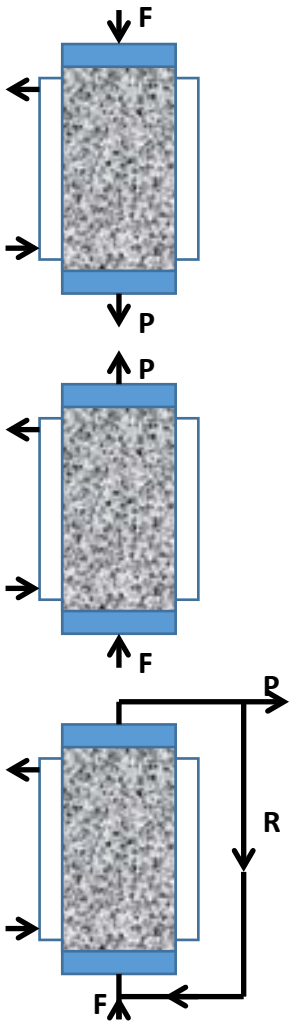
The task of the nozzle below the surface is to disperse the gas and cause the circulation of the liquid.

The disadvantage of this arrangement is the strong foaming.

There are variants of the submerged nozzle with a downward flow (reverse flow). An advantage is the prolongation of bubble residence time in the reactor.



Reactor connection
F - Substrate inflow
P - Product outflow
R - recycle



Packed-bed reactors

The function of package is the attraction of microorganisms (enzymes).

The system behaves as a tubular reactor with a biocatalyst on the surface of an inert carrier.

Use limited due to:

- Abilities of microorganisms to adhere
- Need to regulate the amount of biomass (risk of clogging)
- Fluctuating amounts of biomass - fluctuations in reactor efficiency
- Difficult to reuse immobilized biomass/enzymes

Advantages: easy operation, low cost, easy scale-up

Disadvantages: Pressure loss, biomass growth and depletion, limited mixing and due to less oxygen transfer, difficult regulation by means of sensors (non-homogeneous system)

Uses: chips, biological filter (fragments of granite, bricks)

Disposable Bioreactors

Pre-sterilized plastic reactors for all cell types.

Advantages:

- Do not require validation of cleaning procedures (less paperwork, lower costs)
- They do not require CIP / SIP piping and other equipment (lower investment costs)
- Reduced reactor preparation time
- Shorten the transition time to another product
- Reduction of personal and investment costs

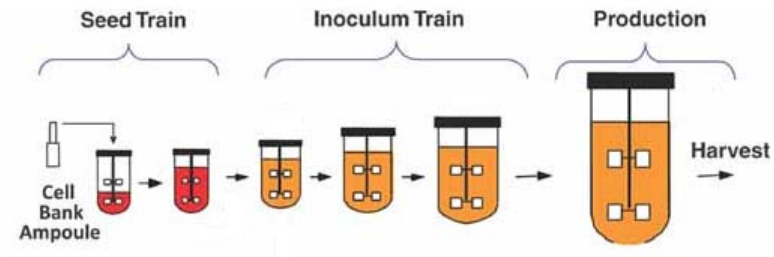
Disadvantages:

- Lower oxygen transfer due to lower agitator power (O_2 can be used)
- Lower heat transfer coefficient of plastics
- Previous parameters are further reduced by the total reactor volume
- Cannot achieve high biomass concentrations (HCD systems)

Disposable Bioreactors

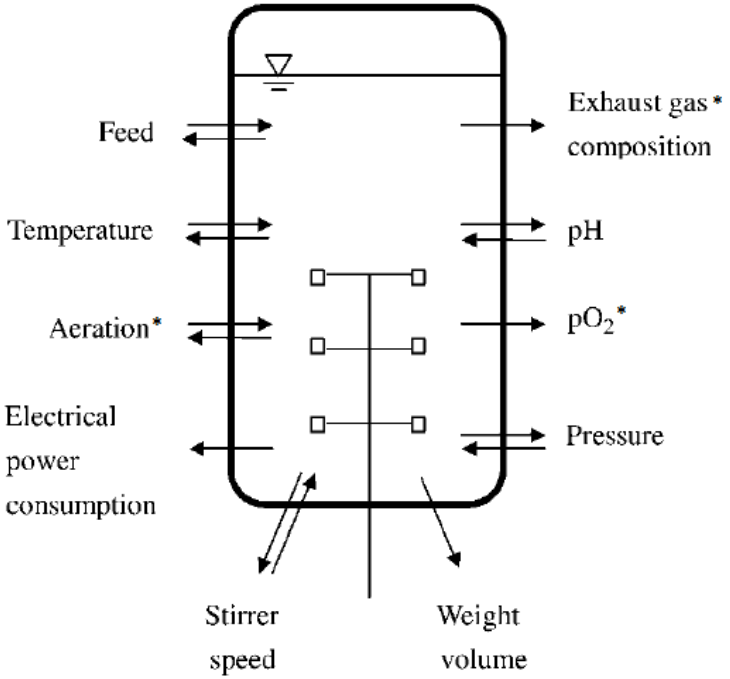
Possible use:

- In the case of products required in small quantities
- In production with frequent change of products
- In manufacturing for personalized medicine
- Cultivation of anaerobic microorganisms
- Seed train (initial phase of the production line)



Measured and controlled parameters

- Input and Output Media
- Temperature
- Aeration
- Electrical power consumption
- Agitator speed
- Batch weight
- Pressure
- Dissolved oxygen conc.
- pH
- Composition of the exhaust gases



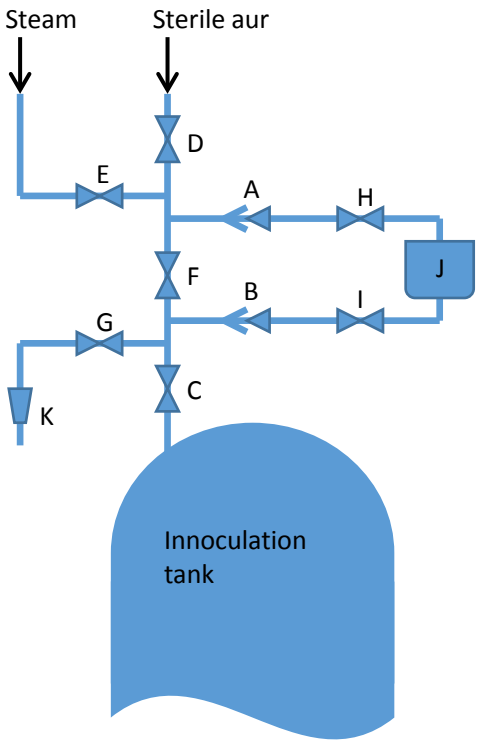
Common measurement instruments and control units of bioreactors as generally accepted as routine equipment (→ measurement only, ⇆ measurement and open- or closed-loop control)

Aseptic inoculation

Pipes required for aseptic transfer of spore suspension into innoculation tank.

Procedure

1. The container J and its piping are sterilized. Spores are introduced into J and the system is connected to A and B to the innoculation tank.
2. Valves E, F and G are opened and the connections at A and B are released. The steam escapes at points A and B. After 20 minutes (120 ° C), E and G are closed and A and B tightened.
3. After cooling the pipeline (can be helped by sterile air) F is closed, the H, I and C valves are opened and the spores are transferred into the tank with sterile air.
4. The valves D, C, H and I are then closed and J is disconnected at point A and B.



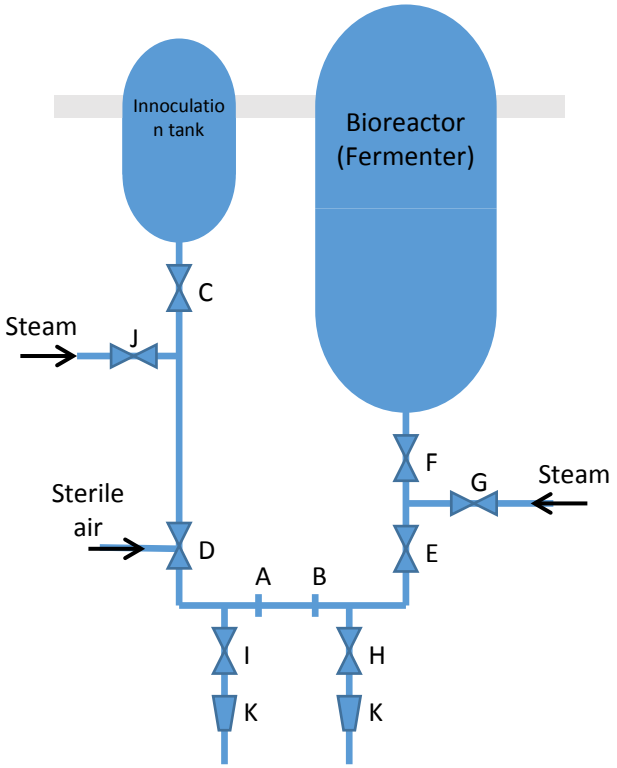
K – condensation pot

Aseptic inoculation

Pipes required for aseptic transfer of spore suspension into bioreactor.

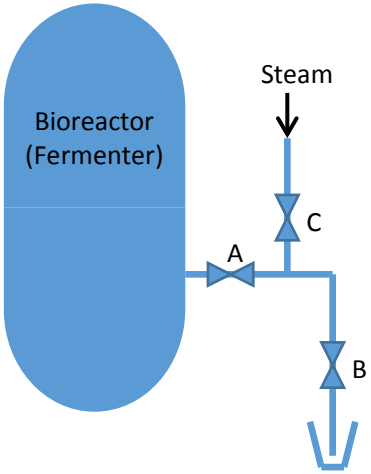
Procedure

1. The containers are connected by a flexible tube at the A and B points.
2. Sterilization of the medium in the fermenter: the steam from the valves J and G passes through D, E and F. The valve C is closed and H and I are slightly open in order to escape the steam and accumulated condensate.
3. After sterilization, G, J, H and I are closed while F, E and D are open. Sterile air cools the medium. Then the air supply is closed.
4. The valves C are opened and the innoculum by gravity / pressure difference flows into the bioreactor. Then C and F are closed and the pipeline is sterilized again before disconnecting in A and B



Aseptic sampling

Samples are often sampled during sterilization and cultivation. The sampler must be sterilizable by steam.



Procedure

1. Normally, valves A, B and C are closed and the end of the sampling tube is inserted, for example, into 40% formaldehyde solution.
2. When sampling the disinfectant container is disconnected, valves B and C open and the steam escapes long enough to dry the batch.
3. Then B and C are partially sealed to escape a little steam and condensate from B. The valve A then opens slightly, a little media escapes to the drain and cools the pipes.
4. The valve C is then closed and the sample is taken into a sterile bottle. Then A is closed and section C to B is again sterilized by steam.

Downstream operations

They are often underestimated bioprocess sequences.

Key questions for choosing finishing operations in biotechnology:

- What is the price of the product?
- What is the acceptable quality (purity) of the product?
- Where does the processed fraction contain the product?
- What are the impurities in the fractions?
- What are the physicochemical properties of the product?
- Which are the main impurities (contaminants) of the product?
-What is the economic balance of the individual segregation alternatives?

The most important techniques of separation and purification:

- Membrane processes (ultrafiltration, reverse osmosis)
- Chromatographic processes (preparative chromium)

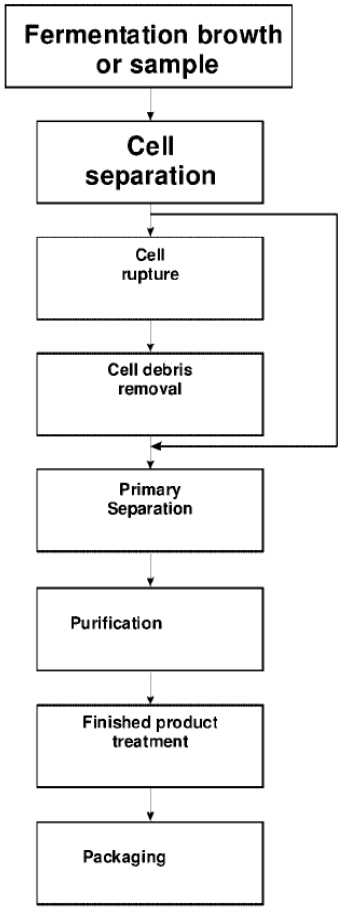


Figure 26. General downstream scheme in biotechnology

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University of Chemistry and Technology Prague
Faculty of Food and Biochemical technology

Methods of optimization and modeling of biological processes

EUROPEAN UNION
European Structural and Investing Funds
Operational Programme Research,
Development and Education

MINISTRY OF EDUCATION,
YOUTH AND SPORTS

Design of experiment

In order to optimize bioprocesses, products etc.

Design of Experiments, DOE

The development of methodologies from the 20s of the 20th century

Usage grew only in the 1990s

The methods are based on a series of tests in which we carry out sophisticated changes in input parameters (variables) of the process and we observe the appropriate responses in order to obtain information to improve the process.

What is DOE good for?

In the case of processes and products, the output characteristic (Y) is affected by a number of input variables (factors) X.

We are interested in this:

- Which variables (X) most influence Y and how (f)?
- How to optimize X to get the best Y?

$$Y = f(X_1 \ldots X_N)$$

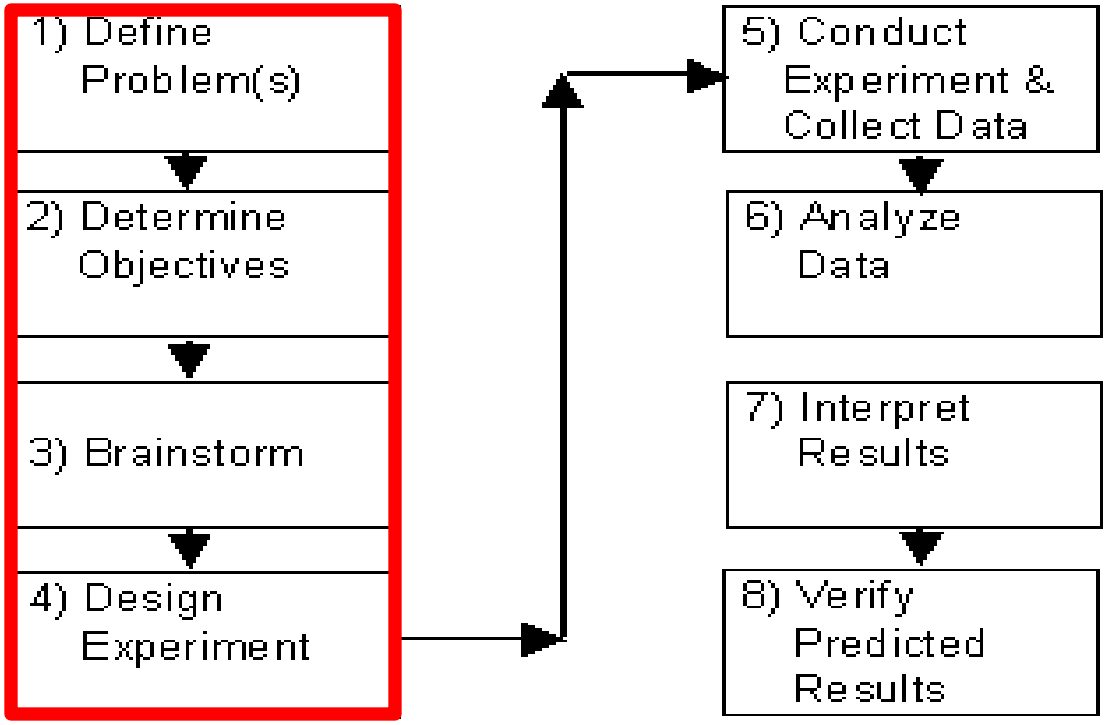
DOE is used both in research and in industrial practice to :

- design and development of processes and products
- improving processes
- testing and validation
- study interactions between variables
- screening the meaning of variables
- solving quality problems

The major obstacles to the use of DOE methods:

- The process we want to optimize is unstable. An unstable process means a process in which from time to time there are unpredictable and undesirable effects
- The actual output of the process is totally unlike the output required - the process is inherently misconceived.

Experimental Design Process



Plánování experimentu

The right process leading to DOE and its implementation requires: Experiment Planning, Quantitative Measurement of Outputs, Repetition to Diminish the Effects of Noise Factors, Randomizing Experiments, Excluding known variability sources, Performing experiments in sequences, Investigating critical findings

Design of experiments (Planning)

This stage is the most important stage of experiment design.
When planning, the objectives of the experiment must be clearly defined.
Selection of factors (variables) that the experiment will significantly influence and factors that are insignificant.
Incorrect selection of factors may cause the following problems:

- Exploring too many or too few factors
- Measuring incorrect outputs
- Obtaining conclusions that are already known

Quantitative response measurement

Many errors occurring during the design of the experiment arise because outputs can not be measured quantitatively. A classic example is visual quality control.

Repetition to mitigate the impact of noise factors

Repetition increases the accuracy of experiments and the chance of detecting a statistically significant phenomenon that is hidden by natural process variability (noise). In some processes, the signal literally "drowns" in noise.

Random order of experiments

It is advisable that the order in which experiments are performed is random in order to avoid the influence of random sources of variability, such as tool wear, ambient temperature, etc. These changes, mostly time-dependent, may significantly affect the response to input variables (factors).

Exclusion of known sources of variability (Blocking)

This is blocking the noise caused by known sources of variability, such as the difference between batching, differences between machines, etc. By dividing the individual iterations of experiments into homogeneous groups and subsequently arithmetically removing the differences, we can greatly increase the effectiveness of the DOE method.

Performing experiments in sequences

This procedure will allow the results obtained by one experiment to be used in the following experiment. This means that it is preferable not to do experiment with a huge number of factors at a time, but rather start with less experiment and then use the results.

Important terms

Factors (Variables)

Variable affecting the measurement, the level of which is determined by the experimentator. In this case, we talk about manageable factors.
Uninfluenced (noise) factors whose values can not influence the operator and should have minimal influence onthe process. These factors cause the so-called **experimental errors.**

A factor can be anything that affects process outputs:

- Machines or devices
- Different technology or production methods
- Input material used
- Operators or shifts
- Ambient conditions (temperature, humidity, pH, concentration ...)

By **level of the factor** we mean the specific value we set in the process. Of course, you can set the level only for manageable factors.

Response

The quantity we use to express the results of the experiment and to monitor it to improve the process or satisfy the customer.

Response is a dependent variable on the input variables (factors) and may contain one or more quality characteristics.

Factorial designs

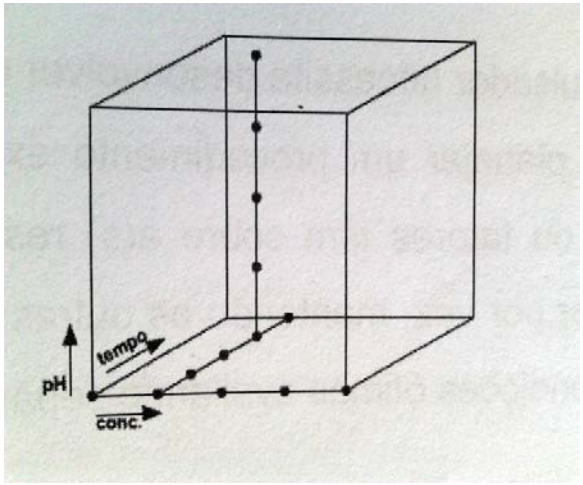
If our experiment has factors that have just two levels, we can make a linear transformation so that both levels of all factors have values -1 and +1. Such factors are called standard factors.

Using two-level factor coding, these are **first-order plans**. This is suitable for cases where the dependence between factors is linear.

For supposed non-linear dependencies between factors, more than two-level factor encoding must be selected.

One factor at time plan

For each test, one factor is changed, others are constant.
By this method, however, we do not get information on how the change of factor causes changes in other factors.
This plan is also known as "One factor at time".

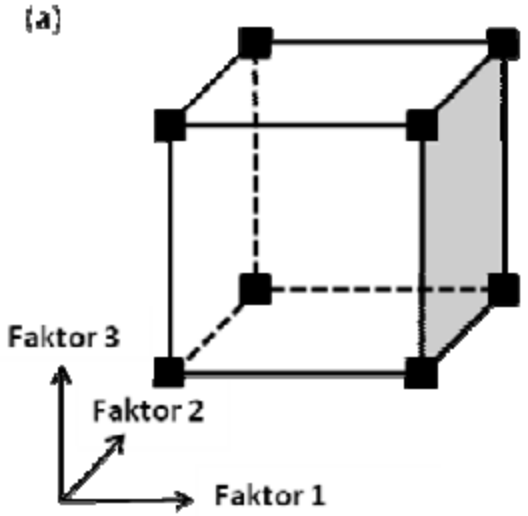


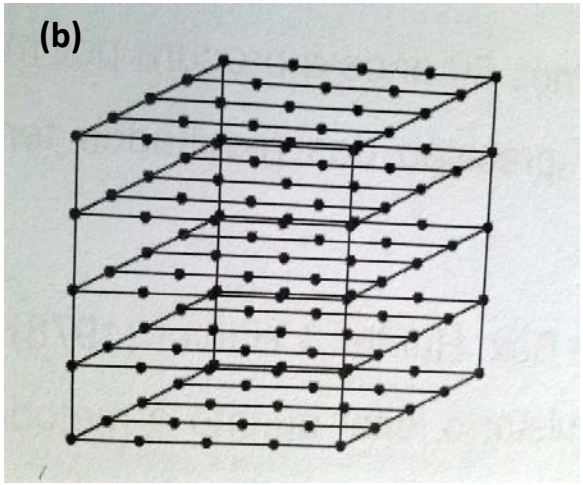
Factors: pH, time, concentration
Number of levels: 5

- Requires at least 13 attempts (5 + 4 + 4)
- The experimental space of 3 factors (5 levels) is limited
- The procedure will not allow to find effects of mutual influence of several factors
- The result is not neccessarily at an optimum value

Full factorial design

In this case, all possibilities are captured.
The disadvantage is the high test costs caused by a large number of tests.
Experimental plans are N^P type, where N is the number of factor levels and P is the number of factors.





Factors: pH, time, concentration
Number of levels: 5

- (a) Requires $2^3 = 8$ experiments
- (b) Requires $5^3 = 125$ experiments
- Unnecessarily high number of attempts
- Due to experimental difficulty, experiments are usually not repeated → statistical methods can not be used to express experimental errors

Full factorial design

It is usually used only for 2 level factors (linear)
More complex tasks with nonlinear relationships require the use of a factor plan with three (or more) levels.
These plans are called RSM (response surface methodology) and allow the drawing of curved surfaces. For 3 levels of factors, the number of trials 3^P and the number of individual points increases the number of trials performed.

Počet faktorů (P)	úplný faktorový plán (N=2)	RSM (N=3)	CCD (N=3)
2	$2^2 = 4$	$3^2 = 9$	$2^2 + 2x2 + 1 = 9$
3	$2^3 = 8$	$3^3 = 27$	$2^3 + 2x3 + 1 = 15$
4	$2^4 = 16$	$3^4 = 81$	$2^4 + 2x4 + 1 = 25$

Fractional factorial design

It may happen that we have too many variables and it is not possible to look for all the links between the variables.
In industry, so-called incomplete factor plans are the most commonly used.

The formula for calculating the number of experiments is $2^{(P-K)}$, where P is the number of factors and $1/2^K$ is the remainder of the complete 2^P factor plan

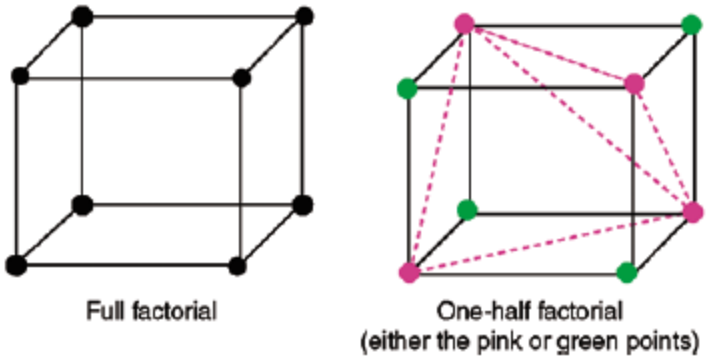


Figure 1. Full factorial and one-half factorial in three dimensions.

One of the most important incomplete factor plans are Plackett-Bruman's plan.

Fractional factorial design

Since the strength of interactions and the effects of influencing factors before performing the test on many assigned tasks are very difficult to estimate, these test plans may mostly lead to incorrect results.

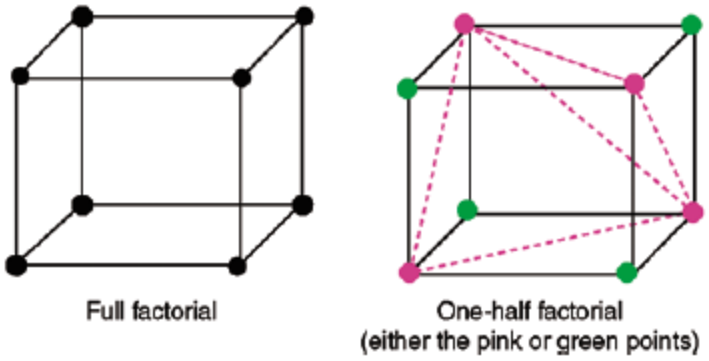


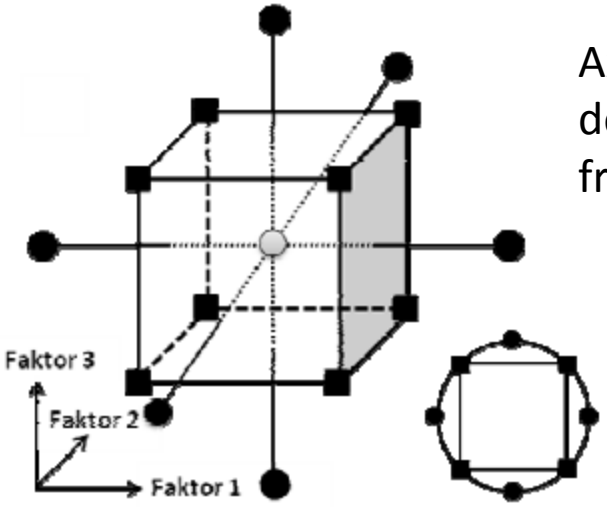
Figure 1. Full factorial and one-half factorial in three dimensions.

On the other hand, if we can identify some of the most serious interactions, we can achieve a much simpler plan to get similar results to using a complicated full factor plan.

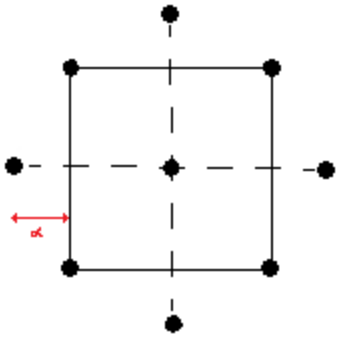
Incomplete factor plans are easier to implement, but some of the available information is lost.

Central composite design, CCD

They are one of the variants of shortened factor plans.
By adding other dots to the center of the square (for 2 factors) or by the center of the cube wall through the distance of the sphere's surface, an expanded model with a lower number of attempts is created: $2^P + 2P +$ 1 (or more for reproducibility)



Axial points in a centrally composed design are always at a distance of a from the central value (0).



P (faktory)	2	3	4	5
α	±1,4142	±1,6818	±2,000	±2,8284

Central composite design, CCD

The advantage of CCD is the magnification of the studied experimental area with fewer attempts.
It enables the creation of a mathematical model, its statistical validation and subsequently the Response surface.

(10^6 cells/mL)

Computer programs

Design Expert Software

JMP statistical discovery software from SAS

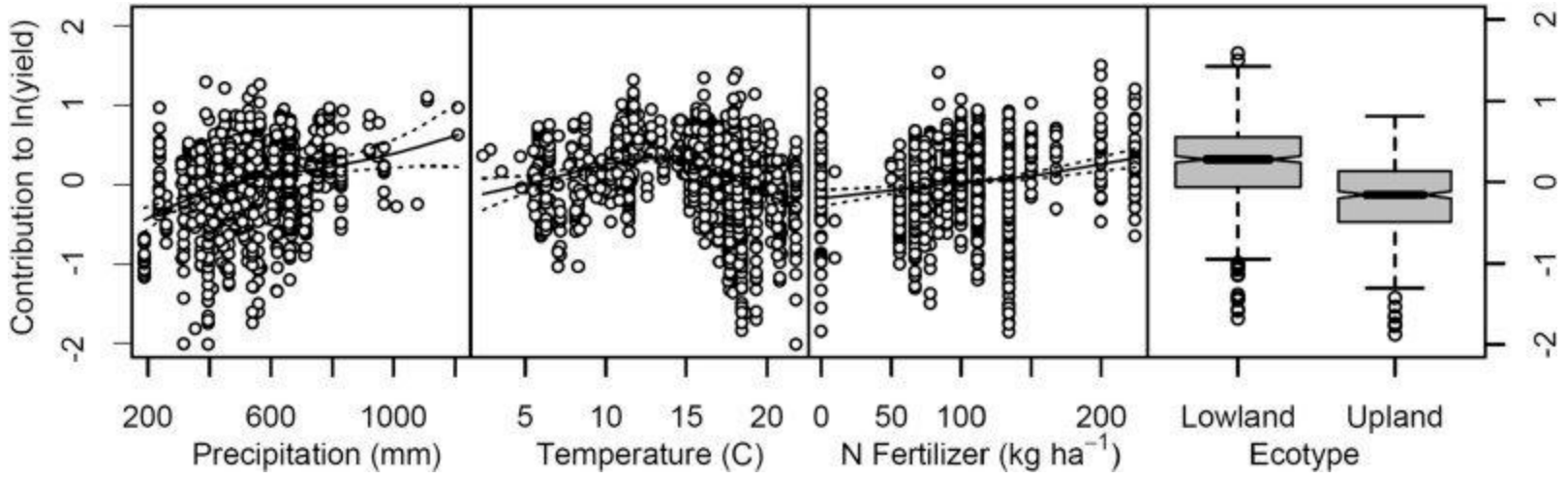
Statistica StatSoft, Inc.

Minitab® 17

Basics of modeling

Using models to understand, design and optimize bioprocesses (bioreactors)

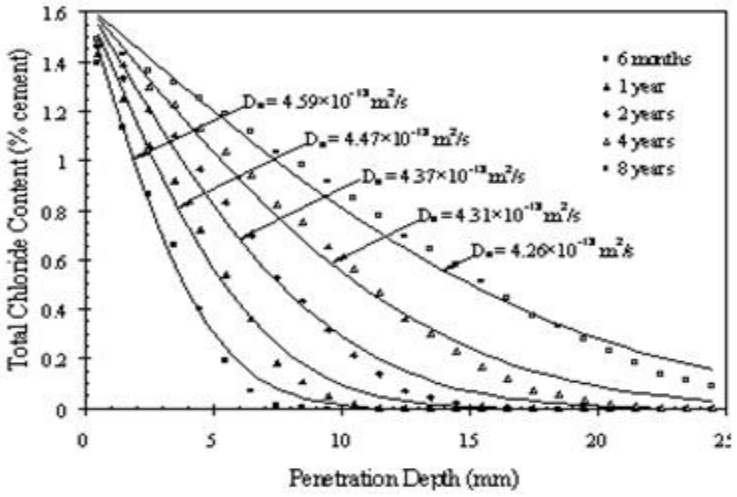
Empirical approach: it is necessary to perform experiments in all combinations of operating conditions and to make correlations from them.
Advantage: there is no need to think too much
Disadvantage: large number of experiments



Modeling approach: compile a mathematical model and suggest attempts to determine the model parameters. Compare the model behavior with the experiment and use the model to design, manage, and optimize the process.

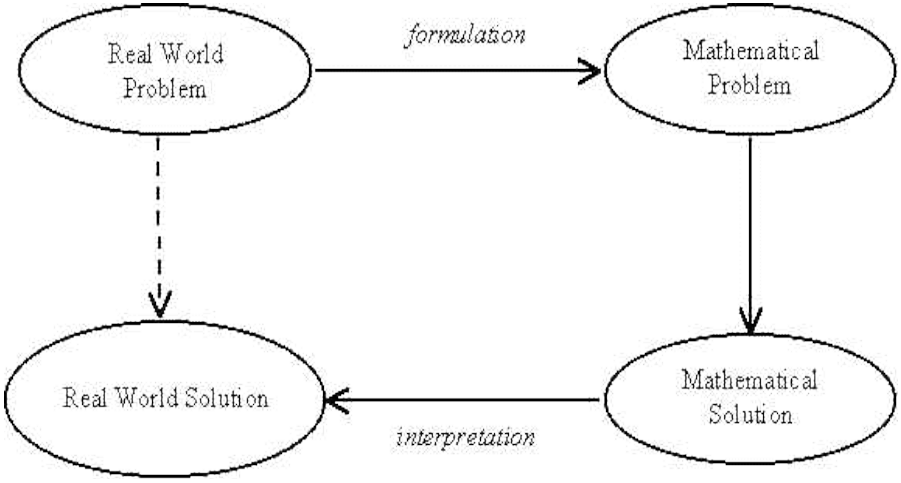
Advantage：a small number of experiments, understanding the process

Disadvantage : sometimes it requires difficult thinking



The mathematical model is a system description using mathematical concepts.

Mathematical models are used in the natural sciences (physics, biology, meteorology) and engineering (computer science, artificial intelligence), but also in social sciences (economics, psychology, sociology and political science).



Classification of mathematical models

Mathematical models are usually composed of variables and their mutual relationships. Relationships can be described by operators such as algebraic operators, functions, differential operators, etc. Variables are abstractions of significant system parameters that can be quantified.

Linear vs. nonlinear: If all operators in the mathematical model show linearity, the resulting mathematical model is defined as linear. Otherwise, the model is considered non-linear. The definition of linearity and non-linearity is context-dependent, and linear models can contain non-linear expressions.

Classification of mathematical models

Statical vs. dynamic: The dynamic model represents and expresses a time-dependent change in the state of the system, while the static (or steady state) model is computing the system in equilibrium, and is therefore time-invariant. Dynamic models are typically expressed by a set of differential equations.

Discrete vs. continuous: A discrete model handles objects as separate, such as particles in a molecular model or states in a statistical model; while the continuous model represents the objects in a continuous manner, such as the fluid velocity field in the pipeline, the solid phase temperature, and the voltage in the electric field.

Classification of mathematical models

Deterministic vs. Stochastic (probability): the deterministic model is one in which each set of variable states is uniquely determined by the model parameters and by a set of previous states of these variables. Therefore, deterministic models always produce the same output for a set of initial conditions. On the contrary, in the stochastic model, randomness is present, and states of variables are not described by a single value, but rather by the distribution of probability.

Deductive vs. inductive: deductive model is a logical structure based on theory. The inductive model is based on empirical findings and generalizations from them.

General assumptions for building a bioengineering model

Assembling the mass and energy balance of the process

- Add kinetic equations eg growth rate, substrate consumption and product formation rate, mass and heat transfer rate
- Add equilibrium relationships, change of state, solubility, control steps

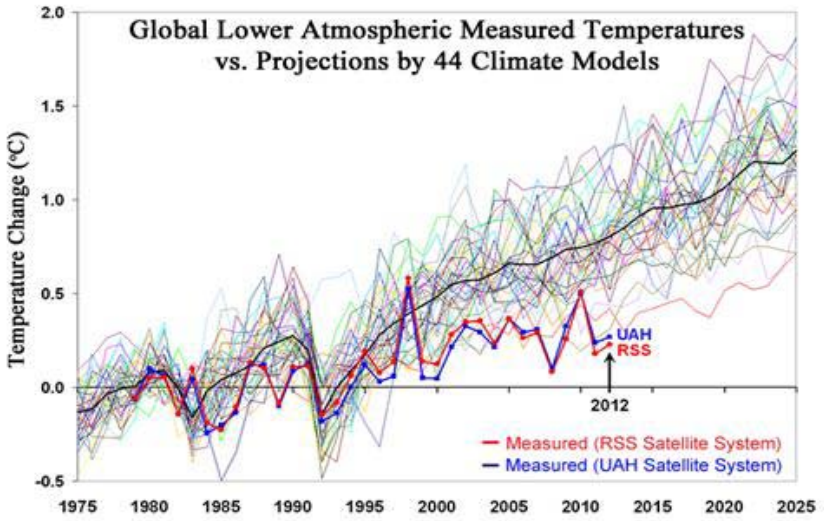
Models can be simple (useful for determining basic parameters) or very complex (dependence and correlation for e.g. pH, compartments etc. are added)

Complexity of models

- Structured kinetic models can be very complex
- Complexity increases the inhomogeneity of reactor environments - the need to divide the reactor into sections
- Comlex model - highly demanding identification of model parameters
- ***One of the most important skills of modeling is the ability to derive the simplest model that is already reliably describing the system.***

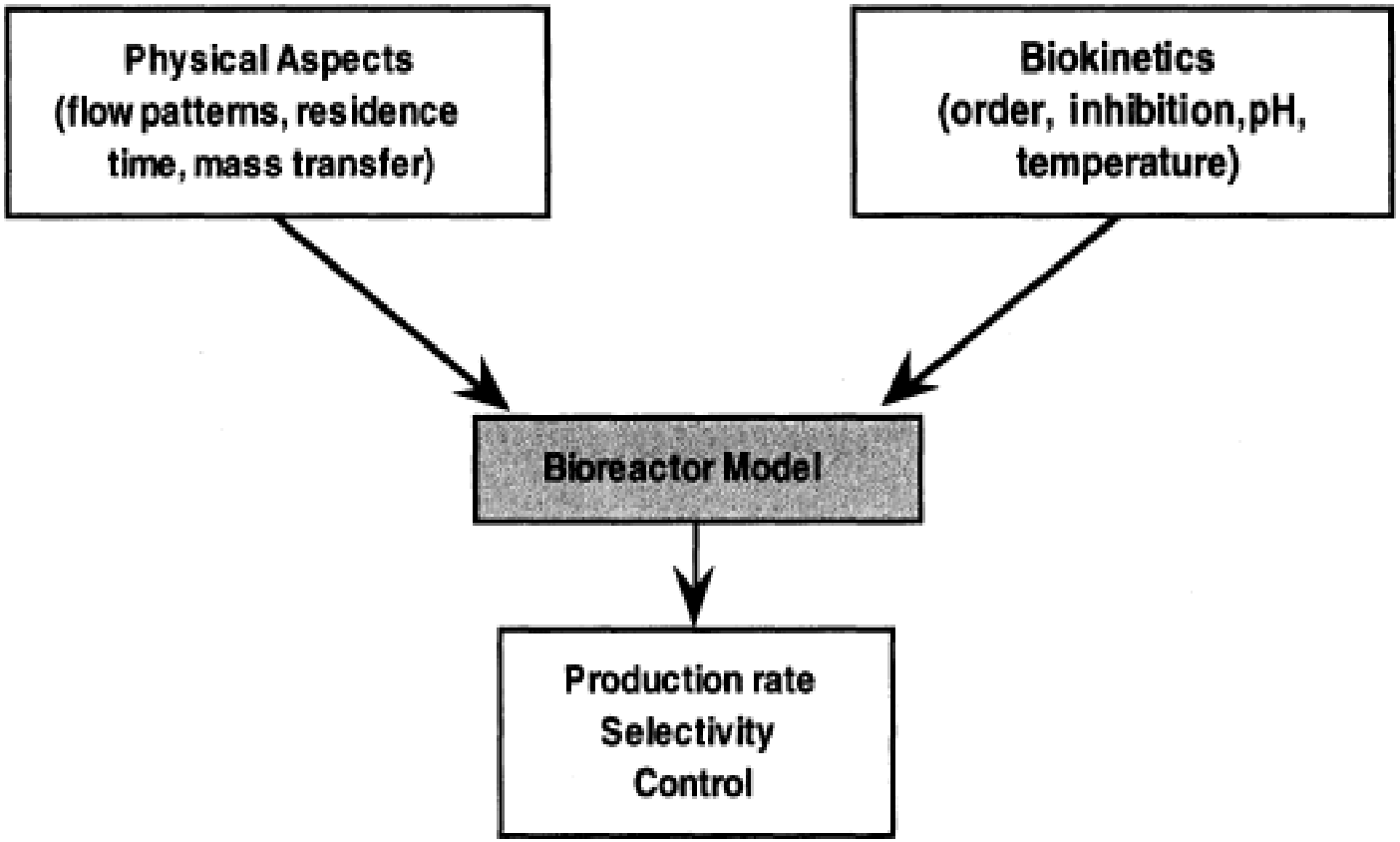
Using models and their simulations has advantages:

- Modeling allows understanding of processes
- Models help designing experiments
- Models can be used for prediction in process design and control
- Use in training and teaching
- Models can be used for process optimization



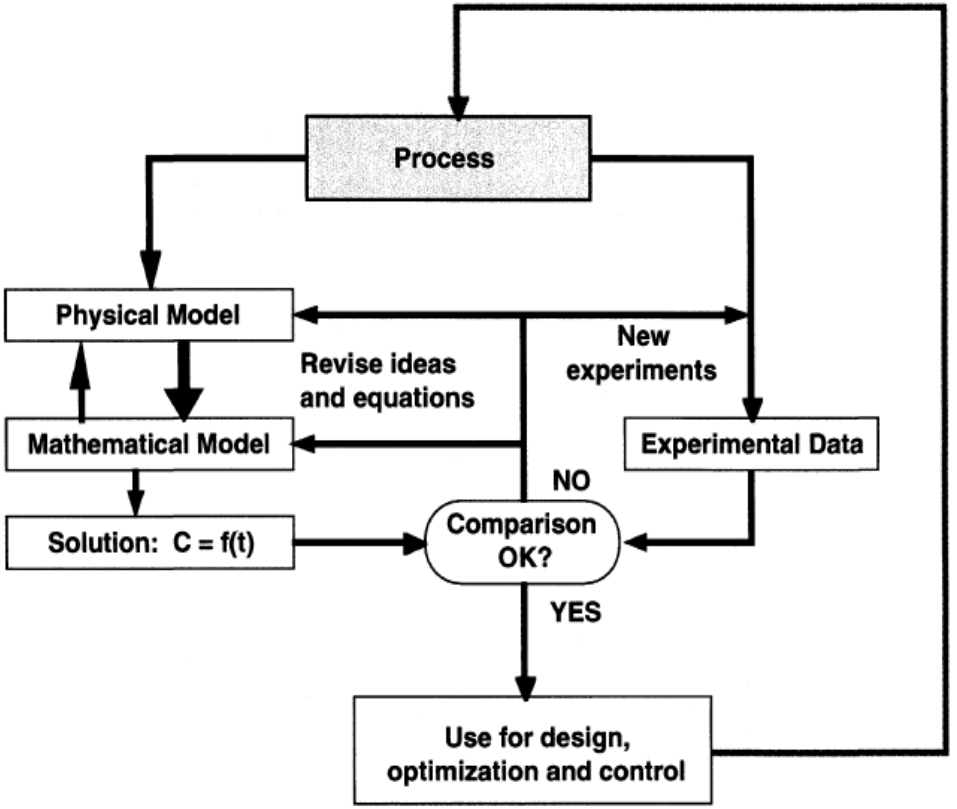
Spencer and Christy (2013)

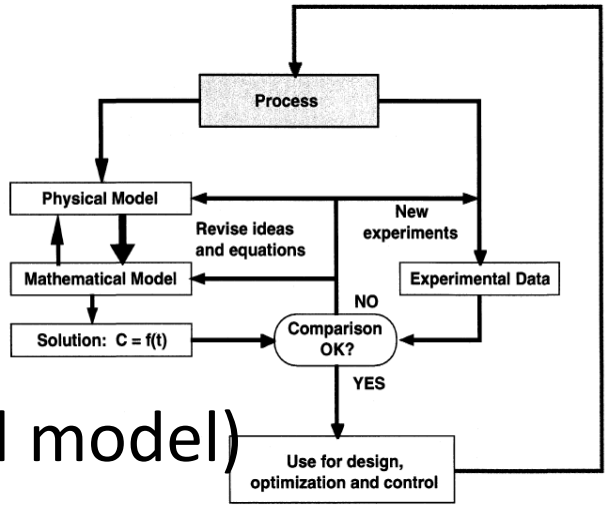
Optimization and modeling



Modeling procedure - in general

- Frequent modeling requirement is to revise the physical model of the process and its mathematical expression in order to achieve consistency with experimental data

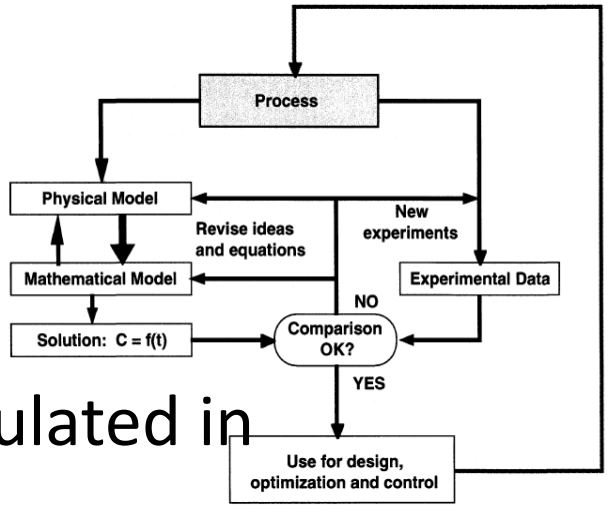




Modeling procedure - in general

(i) Definition of the problem(physical model) and its goals

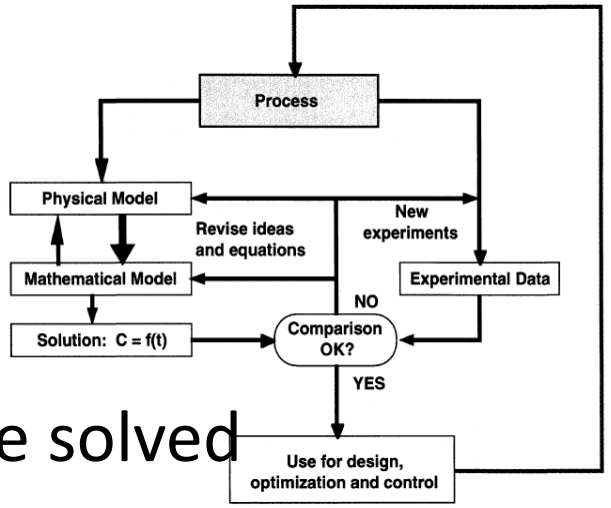
- It is necessary to evaluate all available theories about the process experience
- It is also advisable to choose an alternative physical model
- It is better to start with a simple model and gradually increase its complexity, but only when needed!



Modeling procedure - in general

(ii) The created model must be formulated in mathematical terms (equations)

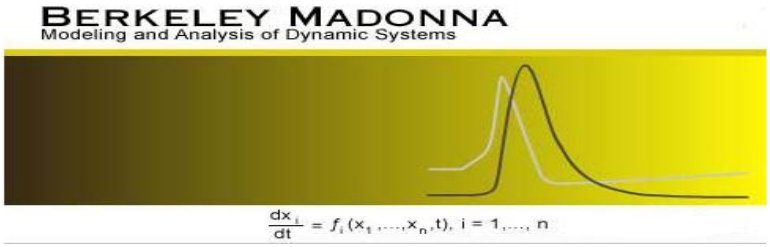
- Biological processes are characterized by a large number of variables (cell, substrate, product, growth rate, consumption and production rates), many of which are time-dependent.
  - Therefore, the mathematical model can be a set of significant number of differential equations
  - Mathematical models are based on balance (mass, energy, momentum)

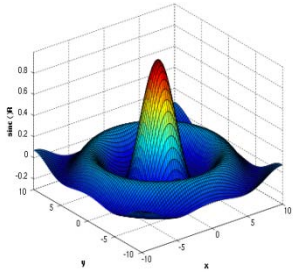


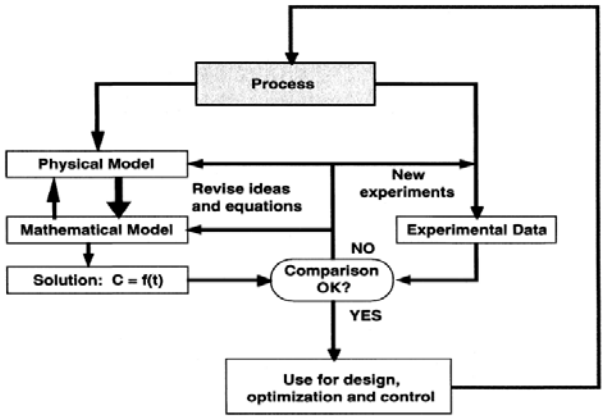
Modeling procedure - in general

(iii) The mathematical model must be solved

- Mathematical models of bioreactors are complex and often nonlinear and therefore their analytical solution is complex.
- Numerical integration with computer programs (Matlab, Berkeley Madonna, etc.) is most often used







Modeling procedure - in general

(iv) The validity of the simulation prediction using the model must be verified, and steps i-iii often repeated

- The validity of the model depends on the correct choice of the physical and mathematical model, the ability to correctly determine the model parameters and the sufficient accuracy of the numerical solution of the model.
- Biological systems are complex, interactive and therefore difficult to accurately describe - simplification is often required - this requires experience.

BASIC PART OF THE MODEL - MASS BALANCE

Types of mass balance

Steady state system

$$\begin{pmatrix}\text{Rate of mass flow}\\ \text{into the system}\end{pmatrix} = \begin{pmatrix}\text{Rate of mass flow}\\ \text{out of the system}\end{pmatrix}$$

Dynamic system

$$\begin{pmatrix}\text{Rate of accumulation of}\\ \text{mass in the system}\end{pmatrix} = \begin{pmatrix}\text{Rate of}\\ \text{mass flow in}\end{pmatrix} - \begin{pmatrix}\text{Rate of}\\ \text{mass flow out}\end{pmatrix}$$

$$\begin{pmatrix}\text{Rate of}\\ \text{accumulation of mass}\\ \text{of component}\\ \text{in the system}\end{pmatrix} = \begin{pmatrix}\text{Mass flow of}\\ \text{the component}\\ \text{into the system}\end{pmatrix} - \begin{pmatrix}\text{Mass flow of}\\ \text{the component out}\\ \text{of the system}\end{pmatrix}$$

MASS BALANCE

Types of mass balance

Dynamic systém with reaction

$$\begin{pmatrix}\text{Rate of}\\ \text{accumulation}\\ \text{of mass}\\ \text{of component}\\ \text{in the system}\end{pmatrix} = \begin{pmatrix}\text{Mass flow}\\ \text{of the}\\ \text{component}\\ \text{into}\\ \text{the system}\end{pmatrix} - \begin{pmatrix}\text{Mass flow}\\ \text{of the}\\ \text{component}\\ \text{out of}\\ \text{the system}\end{pmatrix} + \begin{pmatrix}\textbf{Rate of}\\ \textbf{production}\\ \textbf{of the}\\ \textbf{component}\\ \textbf{by the reaction}\end{pmatrix}$$

MASS BALANCE

Types of mass balance

Input + Source = Output + Accumulation

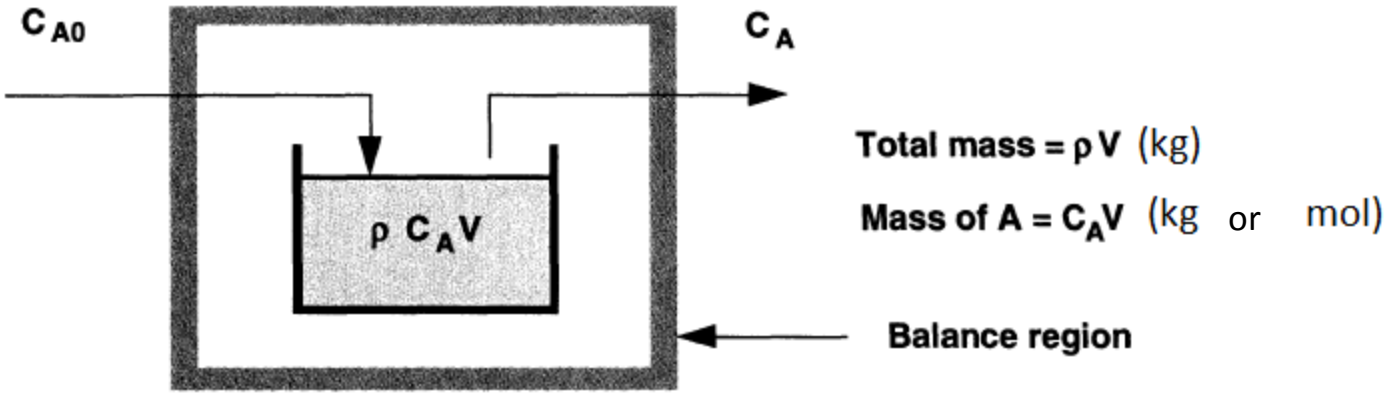
Steady state	X	–	X	–
Dynamic system	X	–	X	X
Dynamic systém with reaction	X	X	X	X

Elemental balance (C, H, O, N) - the source member (reaction) can not be used because the elements in the reaction do not rise.

PROCESS OF MASS BALANCE FORMATION

I. Choose a balanced system and determine its boundaries
   - Balanced system: reactor, part of the reactor, one phase reactor, cell, organelle
   - It is preferred that the system is homogeneous

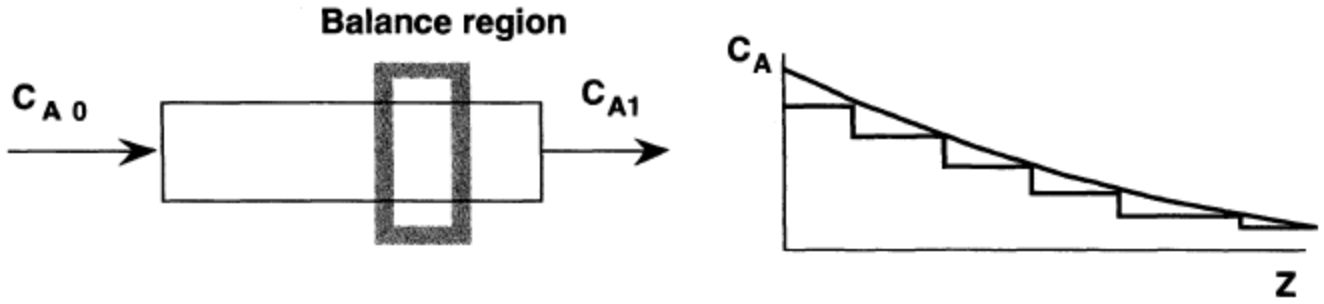
Example: Continuous (ideally) mixed reactor



PROCESS OF MASS BALANCE FORMATION

I. Choose a balanced system and determine its boundaries

Example: Tubular reactor (piston flow)

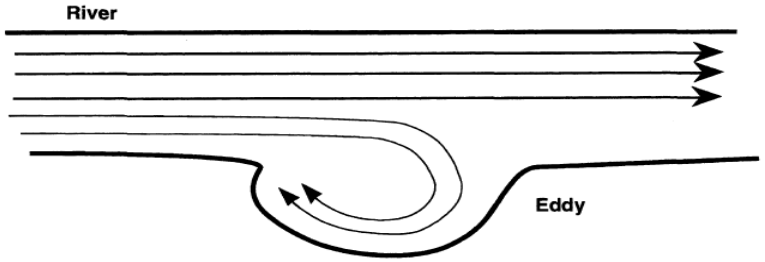


- The composition of the reactor varies along the flow even in the steady state
- A sufficiently small balance area is chosen to be homogeneous within the composition
- The system will consist of many ideally stirred reactors linked *in series*

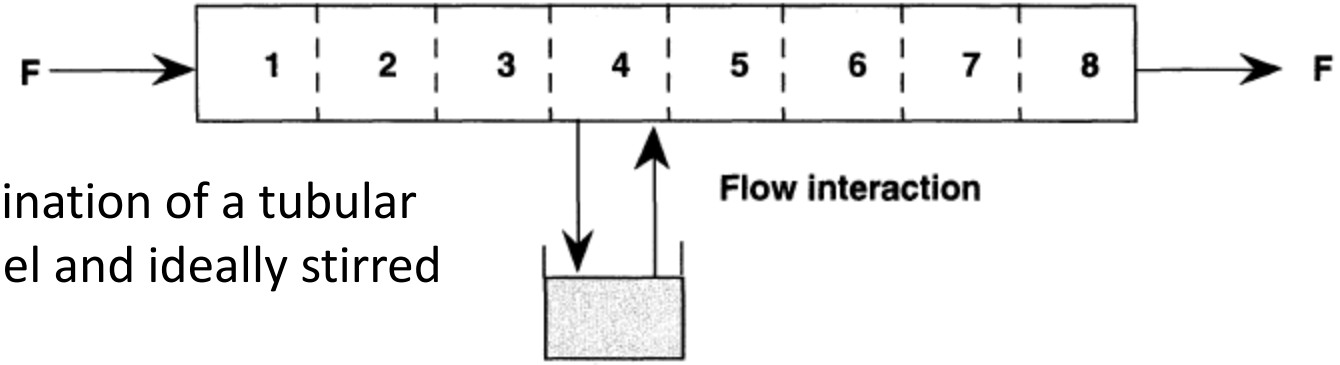
PROCESS OF MASS BALANCE FORMATION

I. Choose a balanced system and determine its boundaries

Example: River with a vortex (eddy)



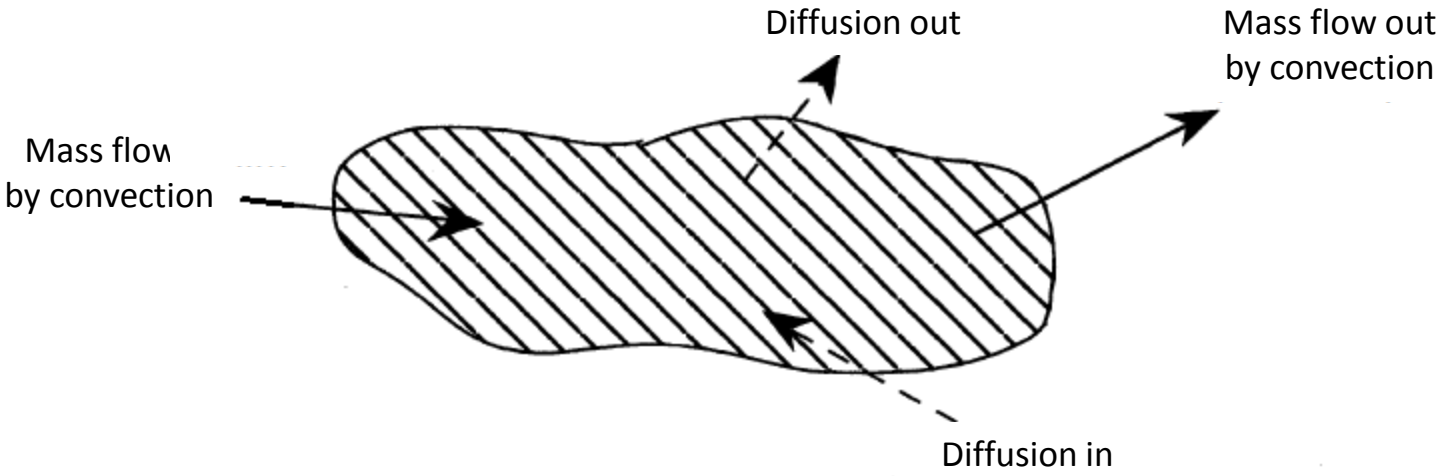
- All 9 subsystems must be balanced
- In this way we can describe e.g. the degradation of pollutant in the river



- It is a combination of a tubular reactor model and ideally stirred

PROCESS OF MASS BALANCE FORMATION

II. Identify mass flows across system boundaries



System inputs and outputs: convection, diffusion, interfacial transport.
It is important to identify the flow direction. The direction can be reversed in the model by changing the sign.

PROCESS OF MASS BALANCE FORMATION

III. Define the mass balance verbally

$$\begin{pmatrix}\text{Rate of}\\ \text{accumulation}\\ \text{of mass}\\ \text{of component}\\ \text{in the system}\end{pmatrix} = \begin{pmatrix}\text{Mass flow}\\ \text{of the}\\ \text{component}\\ \text{into}\\ \text{the system}\end{pmatrix} - \begin{pmatrix}\text{Mass flow}\\ \text{of the}\\ \text{component}\\ \text{out of}\\ \text{the system}\end{pmatrix} + \begin{pmatrix}\text{Rate of}\\ \text{production}\\ \text{of the}\\ \text{component by}\\ \text{the reaction}\end{pmatrix}$$

An important step leading to the fact that mathematical equations will have a physical meaning.

Getting started with mathematical equations is a source of frequent errors.

PROCESS OF MASS BALANCE FORMATION

IV. Express each member of the balance mathematically

Accumulation

The rate of mass accumulation (components i) $= \left(\frac{dM_i}{dt}\right)$ (kg/h, kg/s, mol/h, mol/s)

$$\frac{dM_i}{dt} = \frac{d(C_i V)}{dt}$$ Ci (mol/m3, kg/m3)

Quantities suitable for mass balance are: volume, concentration, partial pressure

$$p_i V = n_i R T \qquad C_i = \frac{n_i}{V} = \frac{p_i}{RT} = \frac{y_i p}{RT}$$

y_i – molar fraction of komponent i
p-total pressure

Gaseous phase accumulation member

$$\frac{dM_i}{dt} = \frac{d(C_i V)}{dt} = \frac{d\left(\frac{p_i V}{RT}\right)}{dt} = \frac{d\left(\frac{y_i p V}{RT}\right)}{dt}$$

$$\frac{dM}{dt} = \frac{d(\rho V)}{dt}$$

$$\frac{kg}{s} = \frac{kg}{m^3}\frac{m^3}{s}$$

Total mass accumulation

PROCESS OF MASS BALANCE FORMATION

IV. Express each member of the balance mathematically

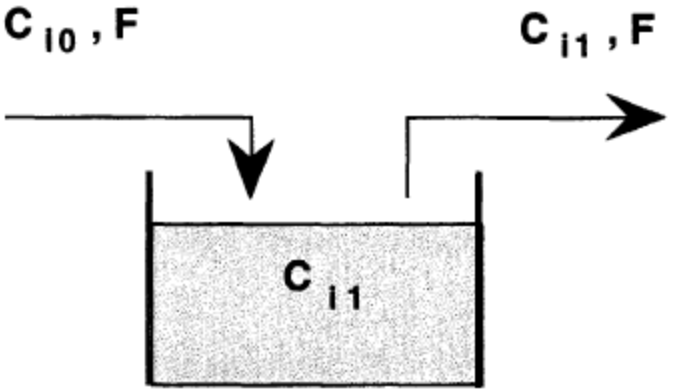
Mass flow by convection

Total mass flow rate and mass flow rate of component i

$$\dot{M} = F \rho$$

$$\dot{M}_i = F C_i$$

$$\frac{kg}{s} = \frac{m^3}{s} \frac{kg}{m^3}$$



Mass flow in an ideally stirred reactor

Simplifying assumptions: In an ideally stirred reactor, the concentration of the component in the reactor is the same as in the output stream

PROCESS OF MASS BALANCE FORMATION

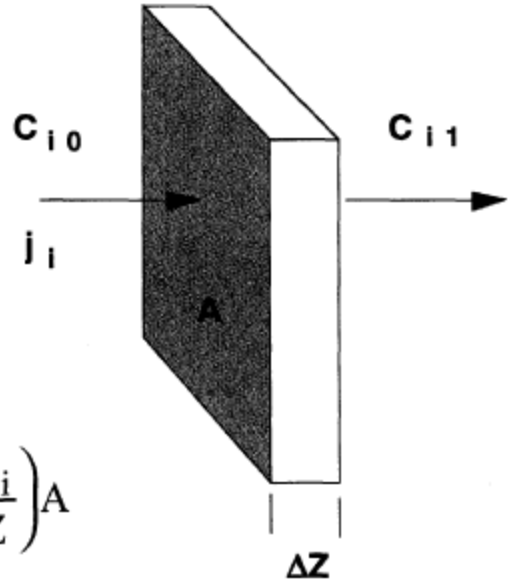
IV. Express each member of the balance mathematically

Mass flow by molecular diffusion

Fick's law describes the diffusion flow of the substance even in a steady state

$$j_i = -D_i \frac{dC_i}{dZ}$$

J_i (mol/m²h, kg/m²h), D_i (m²/h), dC_i/dZ (mol/m⁴)



Mass flow ba duffusion driven by conc. gradient $(C_{i0} - C_{i1})/\Delta Z$ through area A

$$\begin{pmatrix}\text{Massrate of component i}\end{pmatrix} = \begin{pmatrix}\text{Diffusivity of component i}\end{pmatrix}\begin{pmatrix}\text{Concentration gradient of i}\end{pmatrix}\begin{pmatrix}\text{Area perpendicular to transport}\end{pmatrix} = -D_i\left(\frac{dC_i}{dZ}\right)A$$

$$j_i A = -D_i\left(\frac{\Delta C_i}{\Delta Z}\right) A \qquad \frac{kg}{s\ m^2}\ m^2 = \frac{m^2}{s}\frac{kg}{m^4}\ m^2 = \frac{kg}{s}$$

PROCESS OF MASS BALANCE FORMATION

IV. Express each member of the balance mathematically

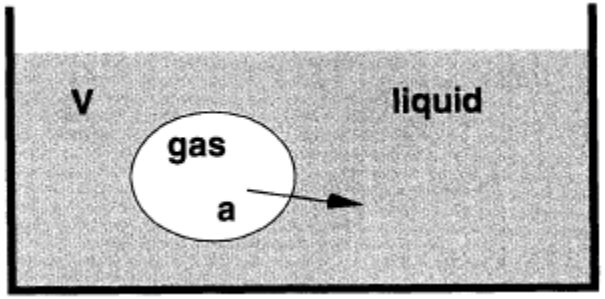
Interfacial mass flow

$$\begin{pmatrix}\text{Rate of}\\ \text{mass transfer}\end{pmatrix} = \begin{pmatrix}\text{Mass}\\ \text{transport}\\ \text{coefficient}\end{pmatrix}\begin{pmatrix}\text{Area per}\\ \text{volume}\end{pmatrix}\begin{pmatrix}\text{Concentration}\\ \text{driving force}\end{pmatrix}\begin{pmatrix}\text{System}\\ \text{volume}\end{pmatrix}$$

Oxygen transfer rate

$$OTR = K_L a\, \Delta C\, V$$

$$\frac{kg}{s} = \frac{l}{s}\,\frac{kg}{m^3}\,m^3$$



Oxygen transfer via interface surface (specific area a, volume V)

Specific interphase surface $a = A/V \ (m^2/m^3)$

Concentration difference $\Delta C \ (mol/m^3, kg/m^3)$

Total mass transfer coefficient $K_L \ (m/s)$

PROCESS OF MASS BALANCE FORMATION

IV. Express each member of the balance mathematically

Rate of product formation

This member expresses the formation or consumption of the component in a (bio) chemical reaction

$$\begin{pmatrix}\text{Rate of accumulation of mass of component in the system}\end{pmatrix} = \begin{pmatrix}\text{Mass flow of the component into the system}\end{pmatrix} - \begin{pmatrix}\text{Mass flow of the component out of the system}\end{pmatrix} + \begin{pmatrix}\textbf{Rate of production of the component by the reaction}\end{pmatrix}$$

$$\begin{pmatrix}\text{Mass rate production of component A}\end{pmatrix} = \begin{pmatrix}\text{Reaction rate per volume}\end{pmatrix} (\text{Volume of system}) = r_A V$$

$$\frac{kg}{s} = \frac{kg}{s\ m^3}\ m^3$$

Balances can also be expressed in moles instead of kg!

Volume creation / consumption rate: r_A is positive (creation), negative (consumption)

PROCESS OF MASS BALANCE FORMATION

IV. Express each member of the balance mathematically

Rate of product formation and substrate consumption

$$\begin{pmatrix}\text{Mass rate of}\\ \text{biomass production}\end{pmatrix} = r_X V = \begin{pmatrix}\text{Growth rate}\\ \text{per volume}\end{pmatrix} (\text{Volume of system})$$

$$\frac{kg}{s} = \frac{kg}{s\ m^3}\ m^3$$

Volumetric rate of biomass formation: r_X

$Y_{X/S}=\Delta X/\Delta S$

$$\begin{pmatrix}\text{Mass rate}\\ \text{of substrate}\\ \text{consumption}\end{pmatrix} = \begin{pmatrix}\text{Growth rate}\\ \text{per volume}\end{pmatrix}\left(\frac{1}{\text{Biomass-substrate yield}}\right)(\text{Volume})$$

$$-r_S V = \frac{r_X}{Y_{X/S}} V$$

$$\frac{kg\ substrate}{s\ m^3}\ m^3 = \frac{kg\ biomass}{s\ m^3}\ \frac{kg\ substrate}{kg\ biomass}\ m^3$$

PROCESS OF MASS BALANCE FORMATION

Define additional equations

Equations defining mass flow are key to the balance, but are rarely sufficient.

The number of equations must be the same as the number of dependent (unknown) variables.

Additionally, you can add:

- Kinetic reaction rate equations as a function of T, pH
- Stoichiometric ratios and yields
- Equation of ideal gas
- Correlation of physical properties
- Changes in pressure as a function of fluid flow
- Equilibrium relationships
- $K_L a$ changes, gas holdups, etc. as a function of physical system properties, mixing and flow rates, etc.

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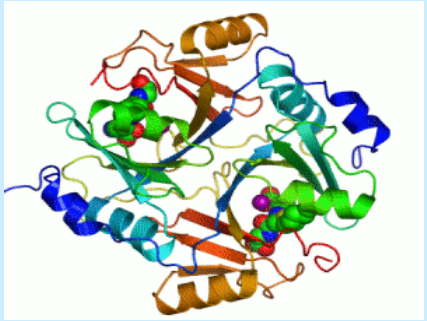
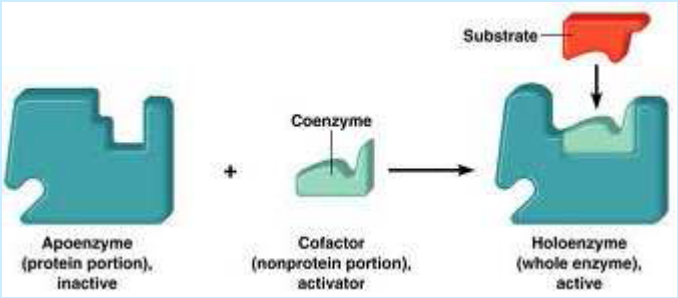
Enzyme kinetics

Tomáš Brányik



Enzymes are biological molecules that catalyze (i.e., increase the rates of) chemical reactions.



In enzymatic reactions, the molecules at the beginning of the process, called substrates, are converted into different molecules, called products.

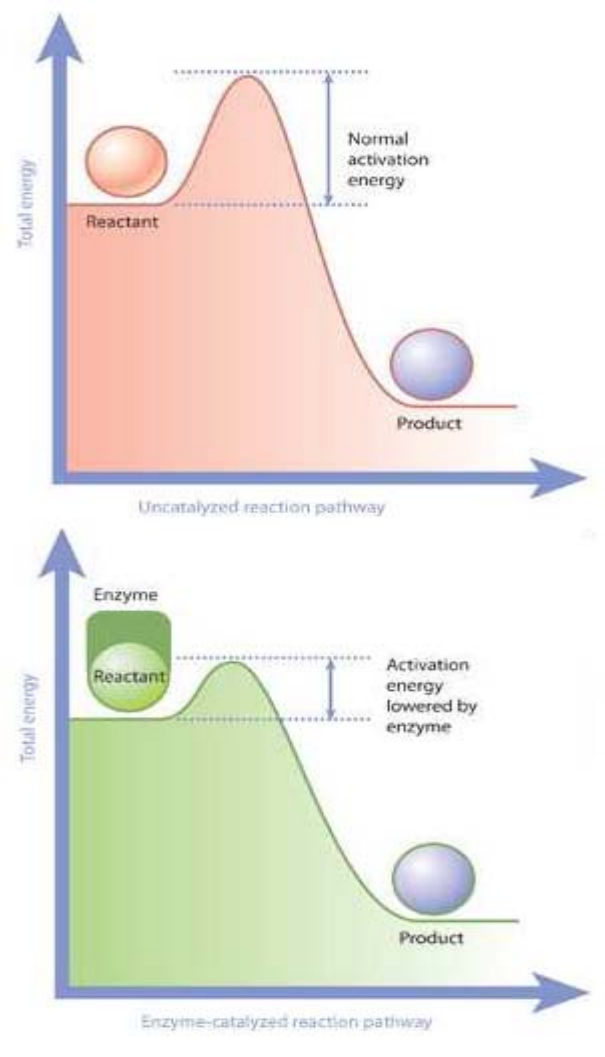
Enzymes are selective for their substrates and speed up only a few reactions from among many possibilities.

Enzymes are in general globular proteins. The activities of enzymes are determined by their three-dimensional structure.
The region that contains these catalytic residues, binds the substrate, and then carries out the reaction is known as the active site. Enzymes can also contain sites that bind cofactors/coenzymes, which are needed for catalysis.

Enzymes are known to catalyze about 4,000 biochemical reactions.

Enzymes

- Globular proteins that catalyze the reactions (Catalyst = Increases the reaction rate but does not affect the reaction equilibrium)
- Reduce the activation energy of the reaction
- Greek "en zyme = in yeast" (from where they were first isolated)
- formerly called ferments



Enzyme

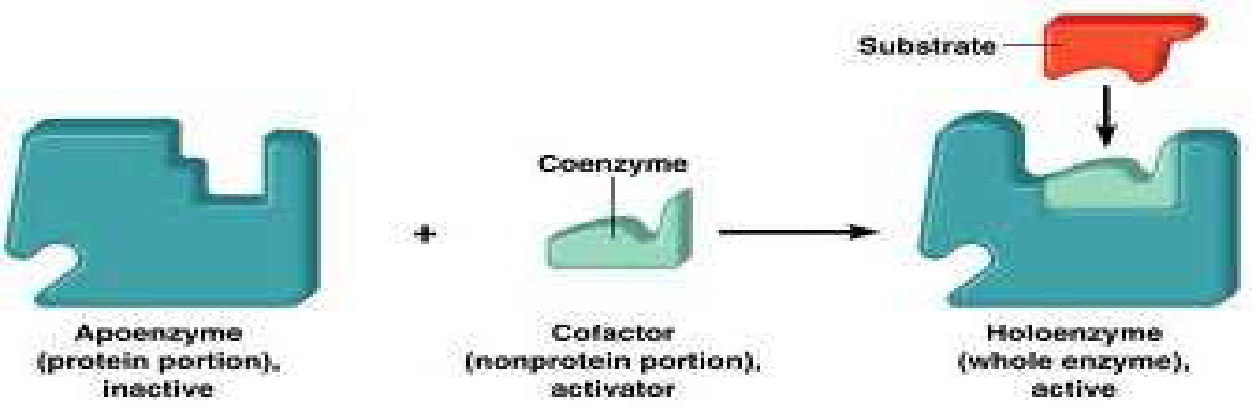
- cofactor (non-protein enzyme component)

- metal ions (Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, Na^{+}, Zn^{2+})

- coenzyme (complex organic molecule) (NAD, FAD, CoA, ATP)

- apoenzyme (inactive protein)

- holoenzyme = enzyme = cofactor + apoenzyme

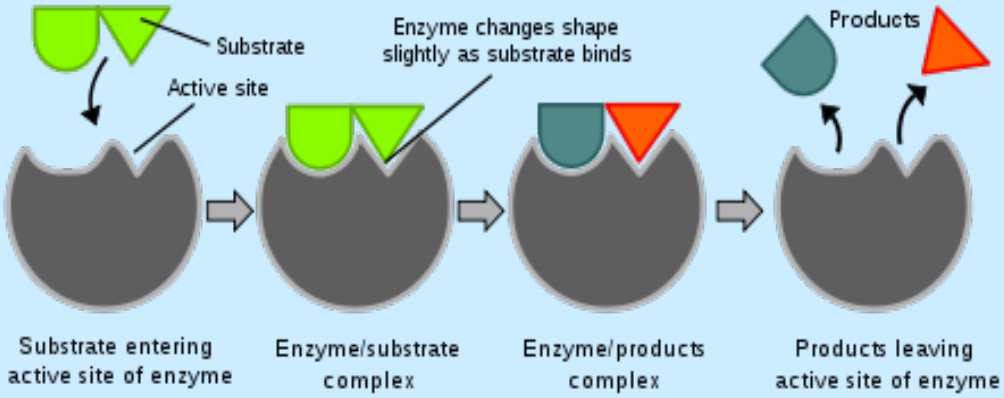


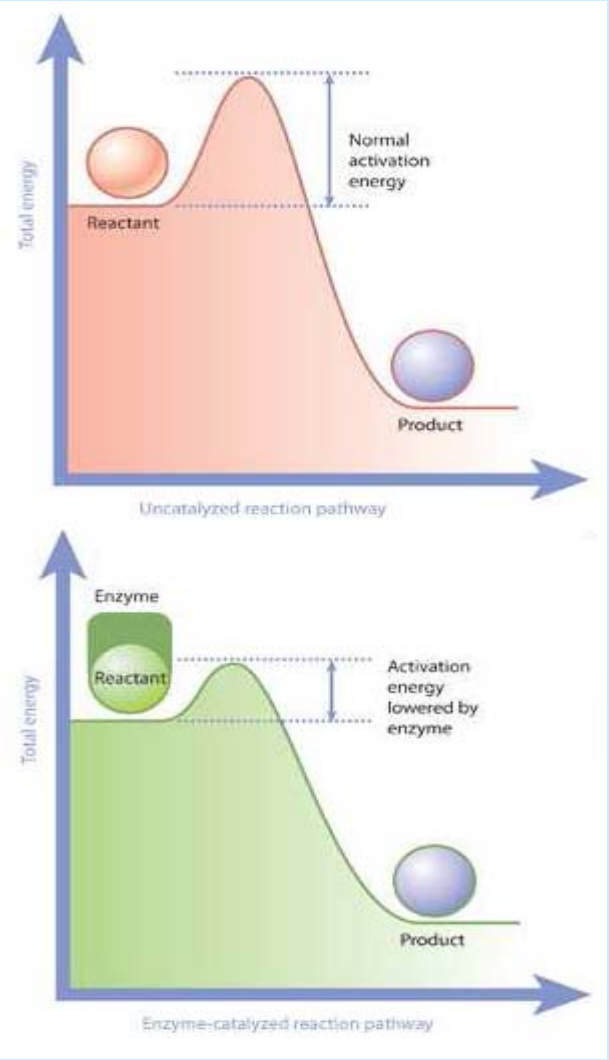
Department of Biotechnology

Like all catalysts, enzymes work by lowering the activation energy ($E_a^{\ddagger}$) for a reaction, thus dramatically increasing the rate of the reaction.

As with all catalysts, enzymes are not consumed by the reactions they catalyze, nor do they alter the equilibrium of these reactions.

$$k = Ae^{-E_a/RT}$$





"Lock and key" model

The active site is continuously reshaped by interactions with the substrate as the substrate interacts with the enzyme.

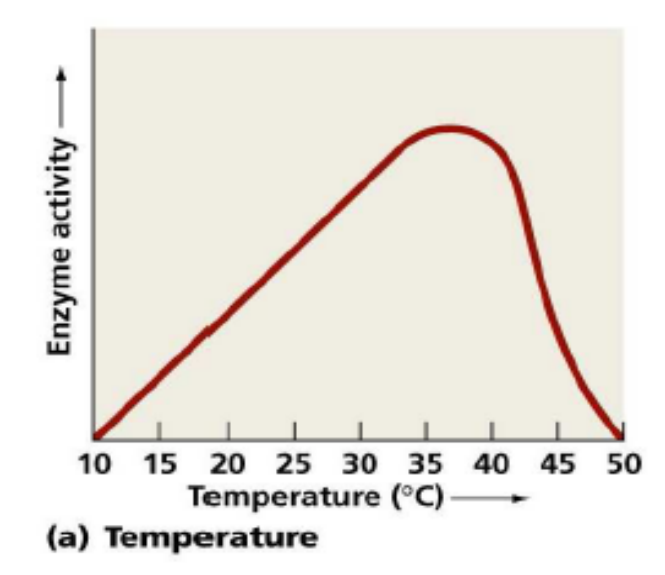
The effect of temperature on enzyme activity

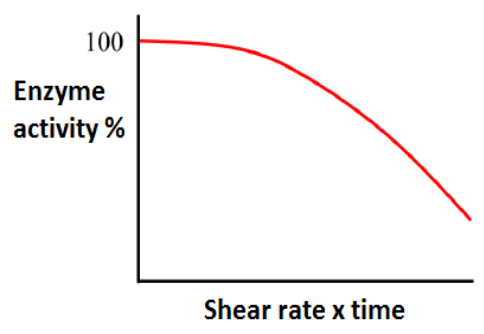
Temperature has na effect on terciary structure of enzymes (active site)

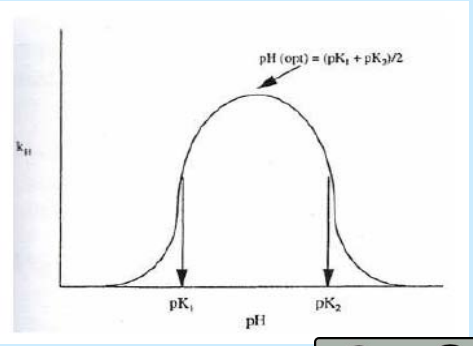
- Effects the weak physicochemical interactions defining the shape of protein molecule

$$k = A e^{-E_a/RT}$$

Arrhenius equation
k is the rate constant
T is the absolute temperature (in kelvins)
A is the pre-exponential factor, a constant for each chemical reaction. According to collision theory, A is the frequency of collisions in the correct orientation
E_a is the activation energy for the reaction
R is the universal gas constant







Chemical reaction kinetics - rate equation

For a chemical reaction n A + m B → C , the rate equation or rate law is a mathematical expression used in chemical kinetics to link the rate of a reaction to the concentration of each reactant. It is of the kind:

$$\frac{d[C]}{dt} = k(T)[A]^n\,[B]^m$$

In this equation k(T) is the *reaction rate coefficient* or *rate constant.*

The exponents *n* and *m* are called reaction orders and depend on the reaction mechanism.

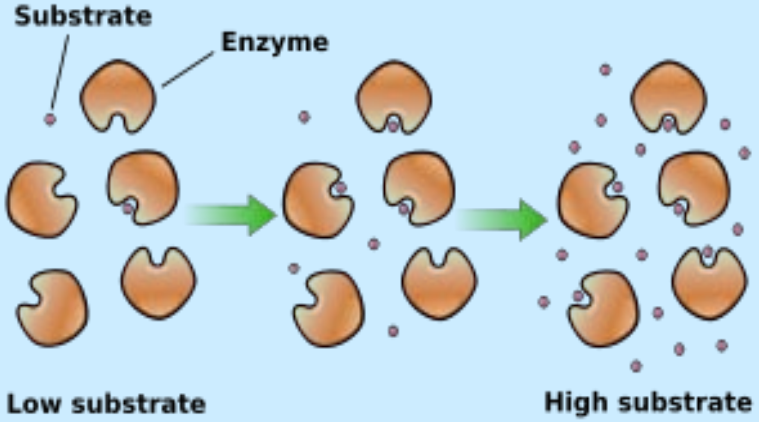
Enzyme kinetics

Enzyme kinetics is the study of the chemical reactions that are catalysed by enzymes.

In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction investigated.

Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of this enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme.

Like other catalysts, enzymes do not alter the position of equilibrium between substrates and products. However, unlike uncatalysed chemical reactions, enzyme-catalysed reactions display saturation kinetics.



Enzyme kinetics

Studies the time course of the enzyme reaction under different reaction conditions and deals with factors that affect the rate of reactions of catalyzed enzymes

The rate of enzyme reaction depends on:

- Concentration of substrates
- Concentration of the enzyme
- Physical chemical properties of the environment (ionic strength, pH, temperature ...)
- Presence of activators and inhibitors

Why study enzyme kinetics?

- Characterization of substrate enzyme preference
- Identification and study of inhibitors

Enzyme activity („turnover number“)

•Expressing the number of molecules reacting with the active site of the catalyst per time unit

•Enzymes have several times higher turnover numbers than synthetic catalysts at the same temperatures

•Synthetic catalysts increase their activity with increasing temperature

•Enzymes are active only in a narrow temperature range

•Katal (1 kat = 1 mol S converted by the enzyme per 1 s)

Enzyme	Turnover number (per second)
Carbonic anhydrase	600,000
3-Ketosteroid isomerase	280,000
Acetylcholinesterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Tryptophan synthetase	2
Lysozyme	0.5

Types of enzymatic reactions

One-substrate reactions

One substrate → one product (isomerase)
One substrate → two products (lyases)
One substrate and water → two products (hydrolases)

Two-substrate reactions

Two substrates → two products (oxidoreductases, transferases)
Two substrates → one product (lyases)

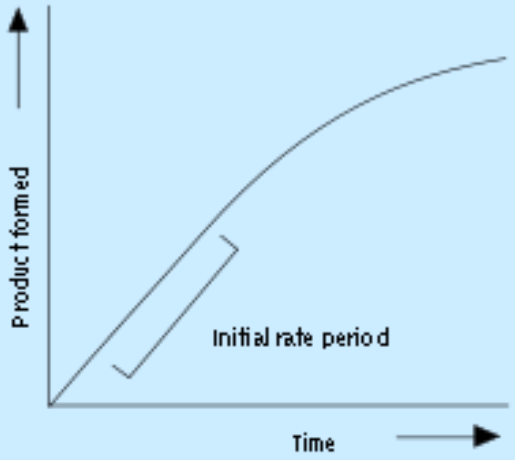
Three-substrate reactions

Two substrates and ATP → one major product and two ATP products (ligases)

Enzyme assays (How to measure enzyme kinetics?)

Enzyme assays are laboratory procedures that measure the rate of enzyme reactions.

Enzyme assays usually follow changes in the concentration of either substrates or products to measure the rate of reaction. There are many methods of measurement: spectrophotometric, radio isotopes, mass spectrometry, fluorescence, electrode and polarimetric methods etc.

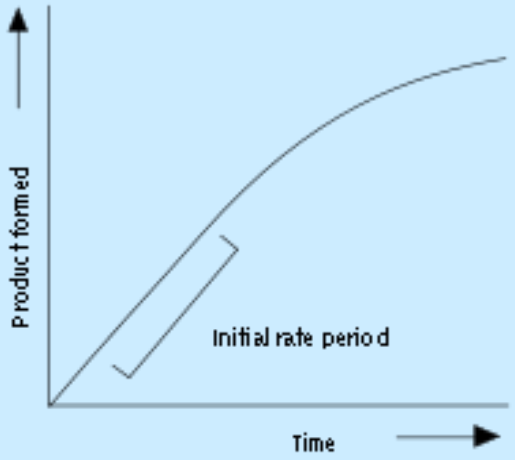


The enzyme produces product at an initial rate that is approximately linear for a short period after the start of the reaction.

Most enzyme kinetics studies concentrate on this initial, approximately linear part of enzyme reactions. However, it is also possible to measure the complete reaction curve and fit this data to a non-linear rate equation.

Enzyme assays (How to measure enzyme kinetics?)

Initial reaction rate is such at which the degree of conversion α does not exceed 0.05.



It is defined by the ratio of the amount of reagent $(nA)_{reac}$ of the chosen component of mixture A, which has been converted to reaction products at equilibrium, to the amount $(nA)_0$ of component A contained in the initial reaction mixture. The quantity of substance $(nA)_{reac}$ that has been converted is given by the difference between the initial substance $(nA)_0$ and the equilibrium amount of the component nA. For the reaction of substances in the solution (V = const) the degree of conversion **α** can be expressed also by concentration.

$$\alpha = \frac{(n_A)_{reac}}{(n_A)_0} = \frac{(n_A)_0 - n_A}{(n_A)_0} = \frac{[A]_0 - [A]}{[A]_0}$$

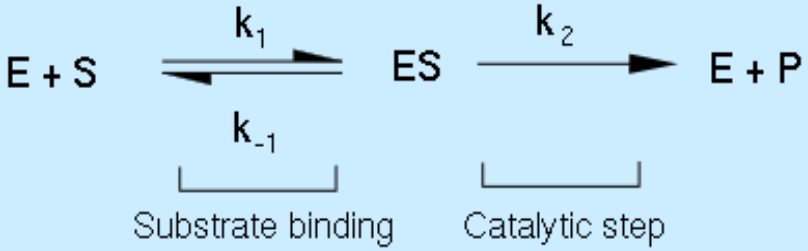
Enzyme kinetics

Leonor Michaelis

Maud Menten

Enzyme kinetics

Objective: To develop an equation expressing the rate of catalysed reaction

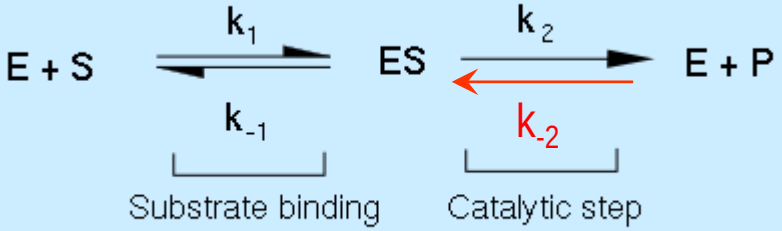


The rate of reaction is characterized by the rate of increase of product P
The reaction rate is directly proportional to the substrate concentration [S].

$$\frac{dP}{dt} = v = k[S]$$

Michaelis–Menten kinetics

Biochemical reactions involving a single substrate are often assumed to follow Michaelis–Menten kinetics, without regard to the model's underlying assumptions.



1. Assumption

k_{-2} the rate of this reaction is low, especially at low conc. of P (initial period)

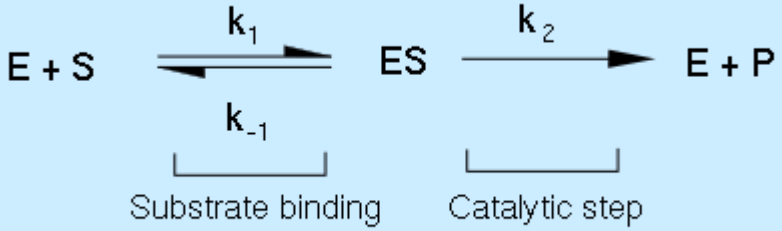
2. Assumption

The substrate is in instantaneous chemical equilibrium with the complex:

$$K_M = \frac{k_{-1}}{k_1} = \frac{[S][E]}{[ES]} \Rightarrow K_M = \frac{([E_0]-[ES])[S]}{[ES]} \Rightarrow [ES] = \frac{[E_0][S]}{[S]+K_M}$$

$$[E_0] = [E] + [ES]$$ Total concentration of enzyme

E – enzyme, S – substrate, P- product, ES- enzyme/substrate complex



Michaelis–Menten kinetics

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$$[E_0] = [E] + [ES]$$ Total concentration of enzyme

3. Assumption

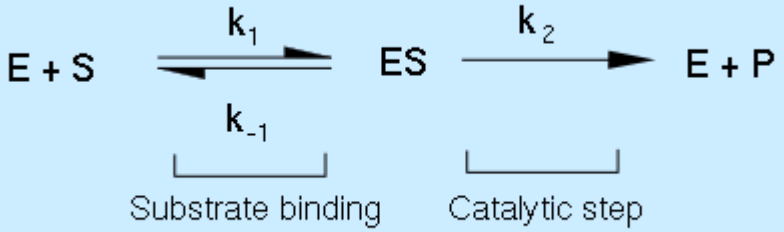
The velocity of the reaction – the rate at which P is formed is:

$$v = \frac{dp}{dt} = k_2[ES] \Rightarrow v = \frac{k_2[E_0][S]}{[S]+K_M}$$

$$V_{max} = k_2 E_0 \Rightarrow v = \frac{V_{max} S}{K_M + S}$$

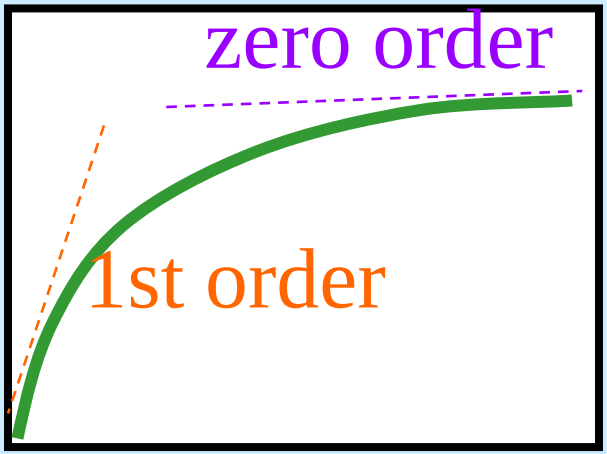
Michaelis–Menten kinetics

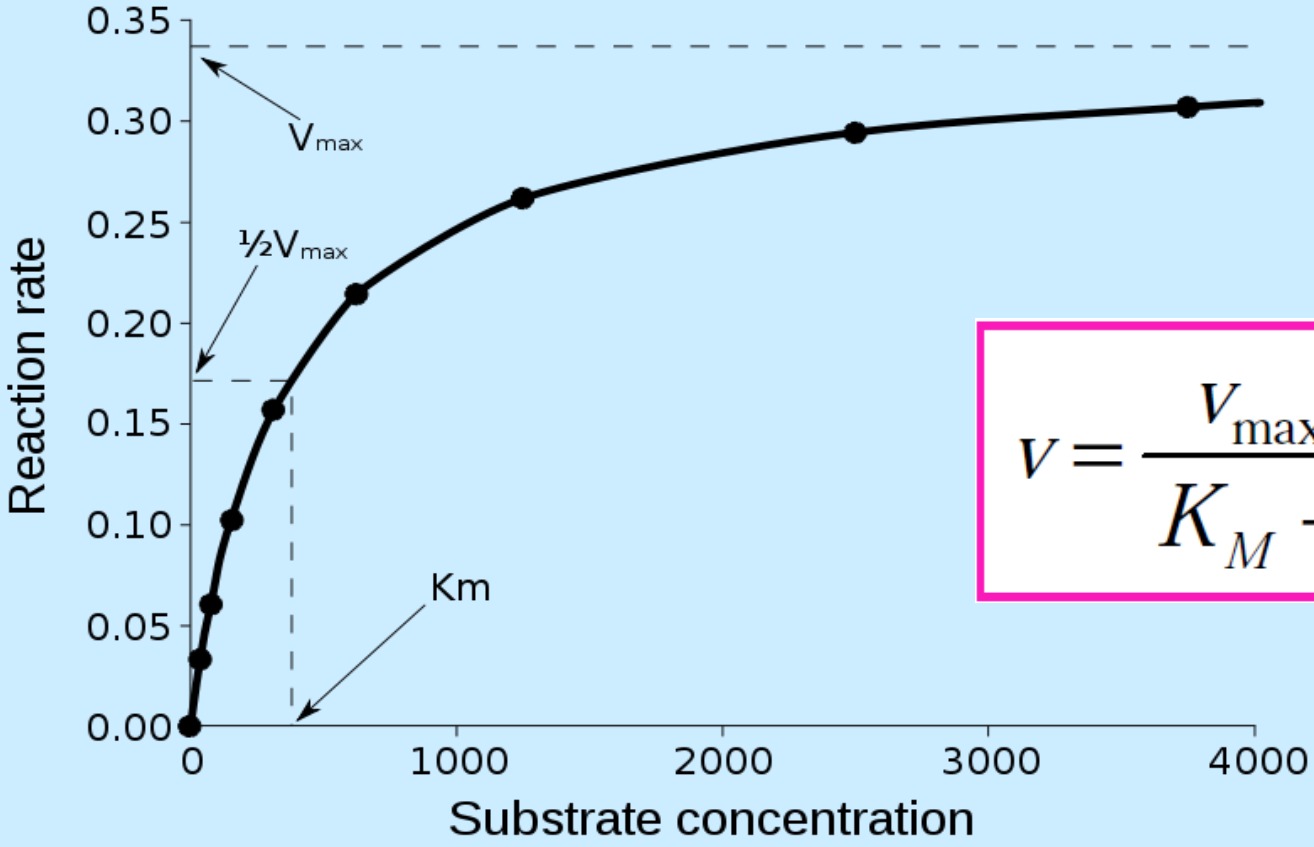
$$v = \frac{v_{max} S}{K_M + S}$$



For low [S], $1 << \frac{K_S}{[S]}$ a $v \rightarrow \frac{v_{max}}{K_S}[S]$

For high [S], $1 >> \frac{K_S}{[S]}$ and $v \rightarrow v_{max}$





$$v = \frac{v_{\max} S}{K_M + S}$$

Michaelis–Menten kinetics

The Michaelis constant K_M is experimentally defined as the concentration at which the rate of the enzyme reaction is half V_{max}

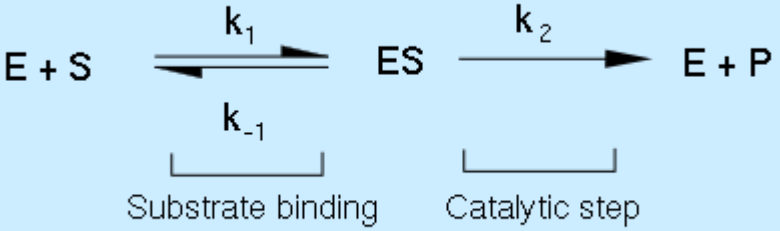
K_M- dissociation constant for the enzyme-substrate complex

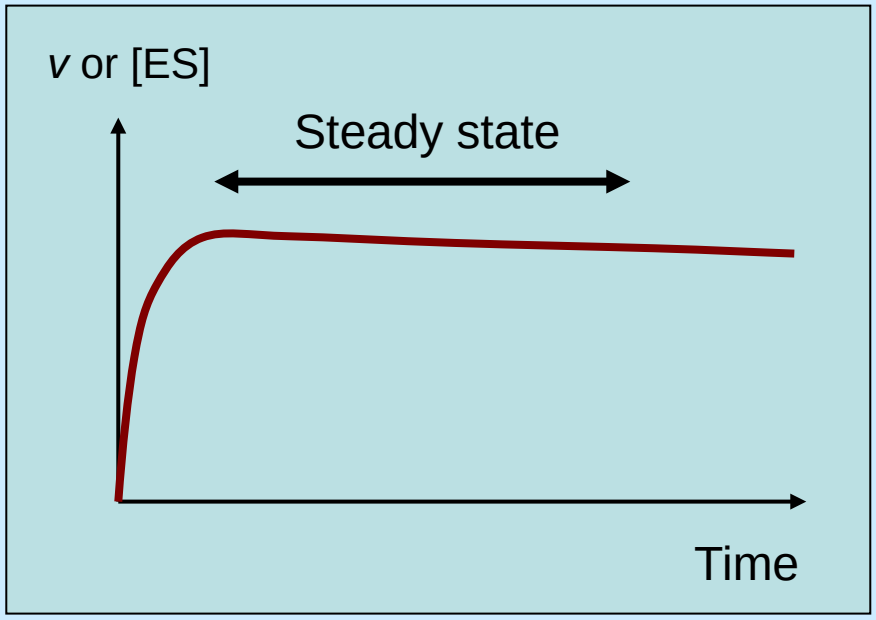
Briggs-Haldane kinetics

We can not assume that k_1 and k_{-1} are always greater than k_2

We consider a steady-state formation. We assume that (for $[S]_o >> [E]_{tot}$) the reaction rapidly reaches the state during which the concentration [ES] is constant.

$$\frac{d[ES]}{dt} = 0$$





$$v_o = \frac{V_{max}[S]}{K_m + [S]}$$

Practical significance of kinetic constants

The study of enzyme kinetics is important for two basic reasons:

• it helps explain how enzymes work

• it helps predict how enzymes behave in living organisms. The kinetic constants defined above, K_M and V_{max}, are critical to attempts to understand how enzymes work together to control metabolism.

How to obtain them?

The plot of *v* versus [S] above is not linear; although initially linear at low [S], it bends over to saturate at high [S].

This nonlinearity could make it difficult to estimate K_M and V_{max} accurately.

Therefore, several researchers developed linearisations of the Michaelis–Menten equation.

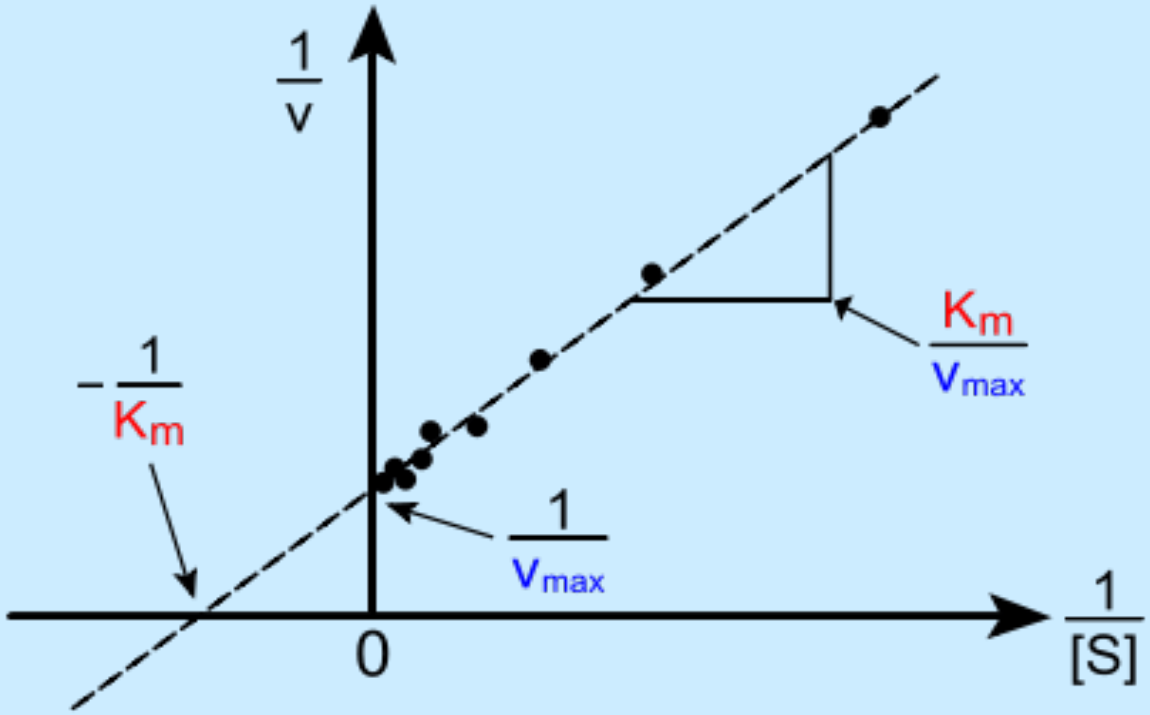
Linear plots of the Michaelis–Menten equation

Lineweaver–Burk plot (or double reciprocal plot)

$$v = \frac{V_{\max} S}{K_M + S}$$

$$\frac{1}{V} = \frac{K_m + [S]}{V_{\max}[S]}$$

$$\frac{1}{V} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}} \frac{1}{S}$$



$$\frac{1}{v} = 0 \Rightarrow 0 = \frac{1}{v_{\max}} + \frac{K_M}{v_{\max}} \frac{1}{S} \Rightarrow -\frac{1}{v_{\max}} = \frac{K_M}{v_{\max}} \frac{1}{S}$$

* Naturally, no experimental values can be taken at negative 1/[S]

Linear plots of the Michaelis–Menten equation

There are other linearization methods:

Eadie plot

$$v = V_{\max} - K_M \times \frac{v}{S}$$

Hanes plot

$$\frac{S}{v} = \frac{K_M}{V_{\max}} + \frac{1}{V_{\max}} \times S$$

The importance of K_M and V_{max}

- **Michaelis constant**:

  - Depending on the type of substrate and conditions such as pH, temperature and ionic strength of the solution. It is independent of enzyme concentration.

  - The concentration of the substrate to achieve the limit velocity is about $100 \times K_M$

- **Two basic meanings K_M:**

  - Concentration of the substrate in which half of the active sites of the enzyme are occupied by the substrate. It corresponds to the substrate concentration, at which the initial reaction rate is equal to half the limit reaction rate

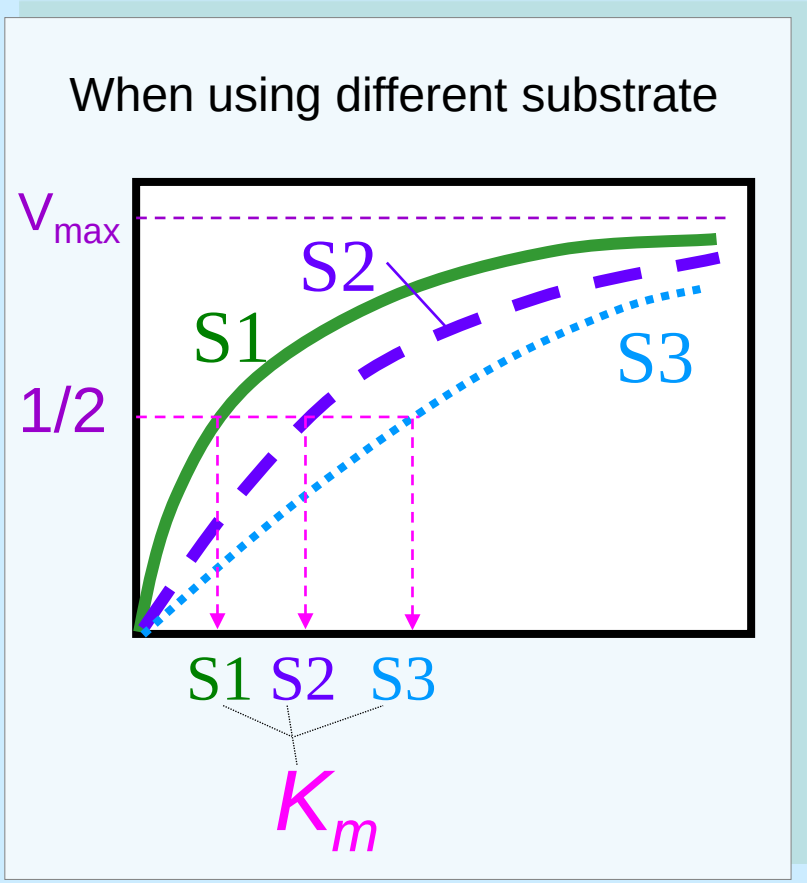
  - $K_M = (k_{-1} + k_2) / k_1$ is the relationship between K_M and the velocities of the enzyme reaction in the sense of the Michaelis and Menten equations.

The importance of K_M and V_{max}

- If k_{-1} is much larger than k_2 it means that the ES complex dissociates to E and S much faster than the product is formed.
- The relationship is simplified to $K_M = k_{-1} / k_1$. The dissociation constant of the EC complex is:

$$K_{ES} = [E]\,[S] / [ES] = k_{-1} / k_1$$

- In other words, K_M is in this case equal to the dissociation constant of the ES complex.
- High K_M values indicate a low affinity of the substrate for the enzyme and low affinity for high affinity

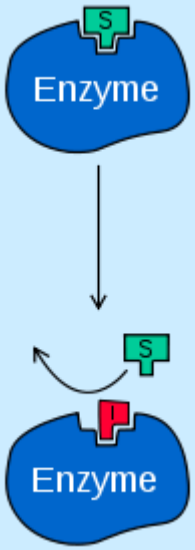


Enzyme inhibition

An **enzyme inhibitor** is a molecule that binds to enzymes and decreases their activity.

Since blocking an enzyme's activity can kill a pathogen or correct a metabolic imbalance, many drugs are enzyme inhibitors. They are also used as herbicides and pesticides.

Enzyme inhibitors also occur naturally and are involved in the regulation of metabolism. For example, enzymes in a metabolic pathway can be inhibited by downstream products. This type of negative feedback slows flux through a pathway when the products begin to build up and is an important way to maintain homeostasis in a cell.

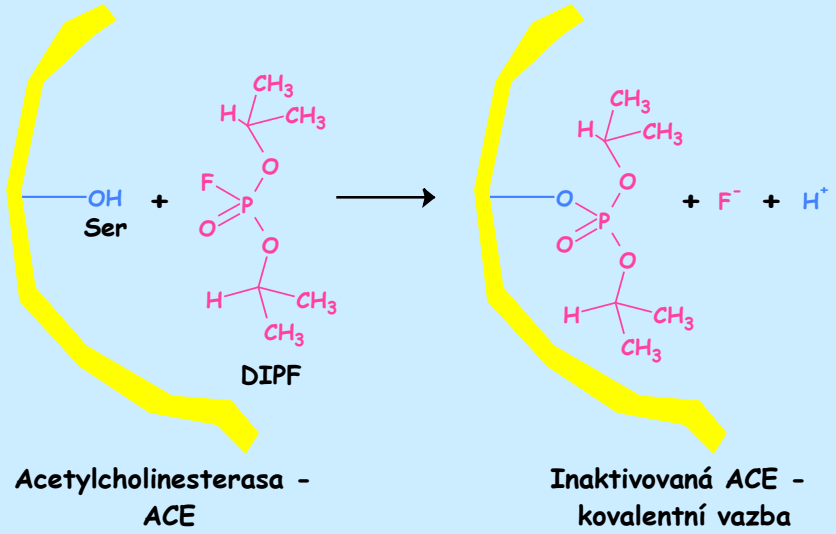


Competitive inhibition: substrate (S) and inhibitor (I) compete for the active site

Enzyme inhibition

Enzyme inhibition can be either ***reversible*** (i.e., removal of the inhibitor restores enzyme activity) or ***irreversible*** (i.e., the inhibitor permanently inactivates the enzyme).

Reversible inhibitors bind to enzymes with non-covalent interactions such as hydrogen bonds, hydrophobic interactions and ionic bonds. Multiple weak bonds between the inhibitor and the active site combine to produce strong and specific binding.



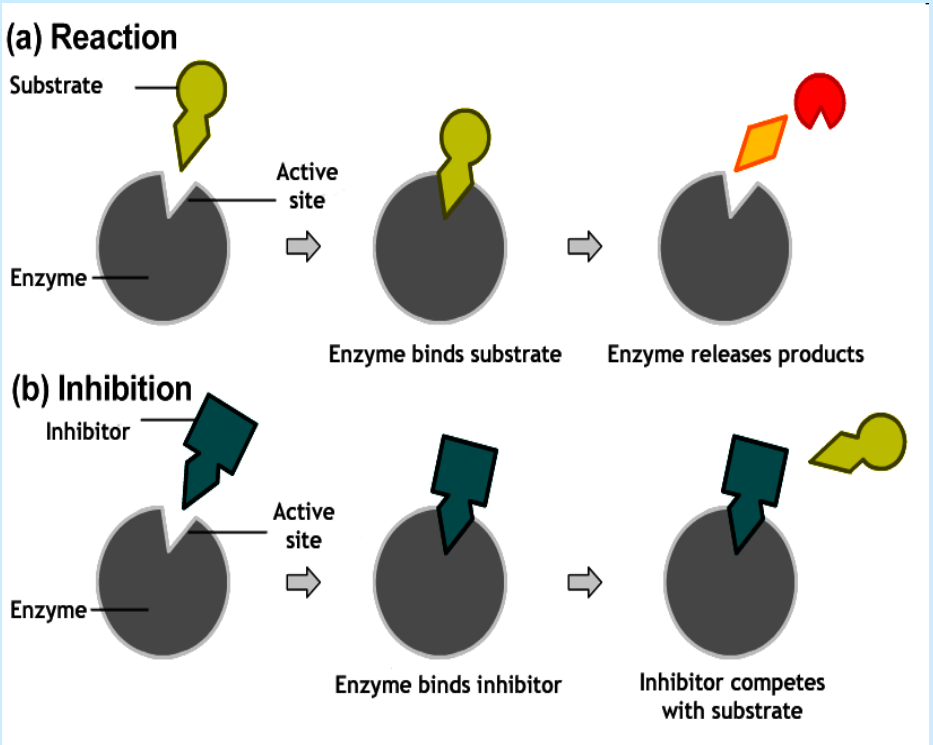
In contrast to substrates and irreversible inhibitors, reversible inhibitors generally do not undergo chemical reactions when bound to the enzyme and can be easily removed by dilution or dialysis.

Types of reversible inhibitors

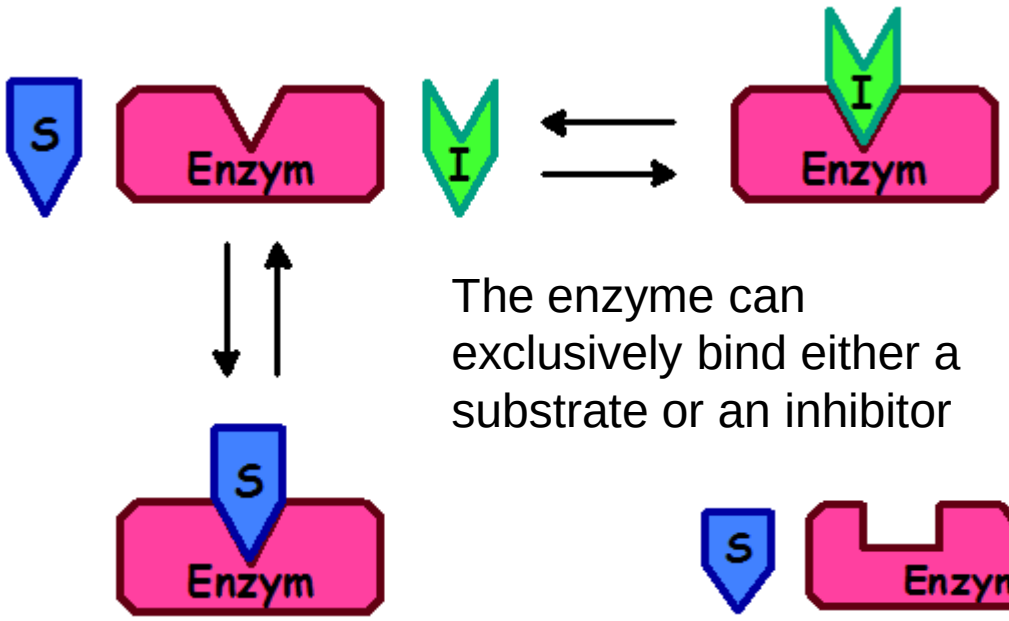
There are four kinds of reversible enzyme inhibitors. They are classified according to the effect of varying the concentration of the enzyme's substrate on the inhibitor.

Competitive inhibition

- the substrate and inhibitor cannot bind to the enzyme at the same time.
- the substrate and inhibitor *compete* for access to the enzyme's active site
- this type of inhibition can be overcome by sufficiently high concentrations of substrate (Vmax remains constant), i.e., by out-competing the inhibitor.
- competitive inhibitors are often similar in structure to the real substrate.

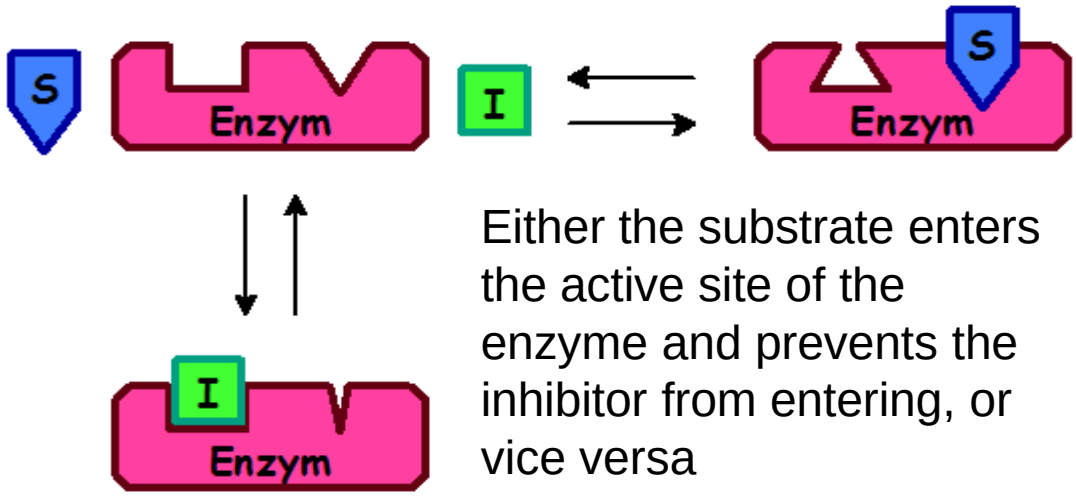


Classical competitive inhibition



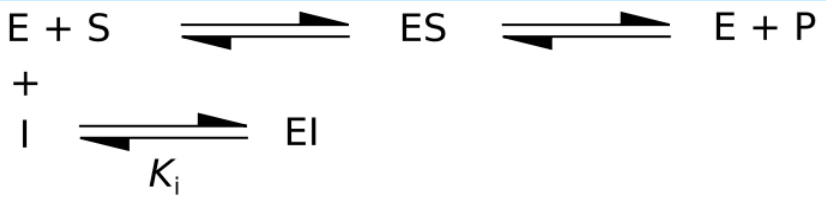
The enzyme can exclusively bind either a substrate or an inhibitor

Non-classical competitive inhibition



Either the substrate enters the active site of the enzyme and prevents the inhibitor from entering, or vice versa

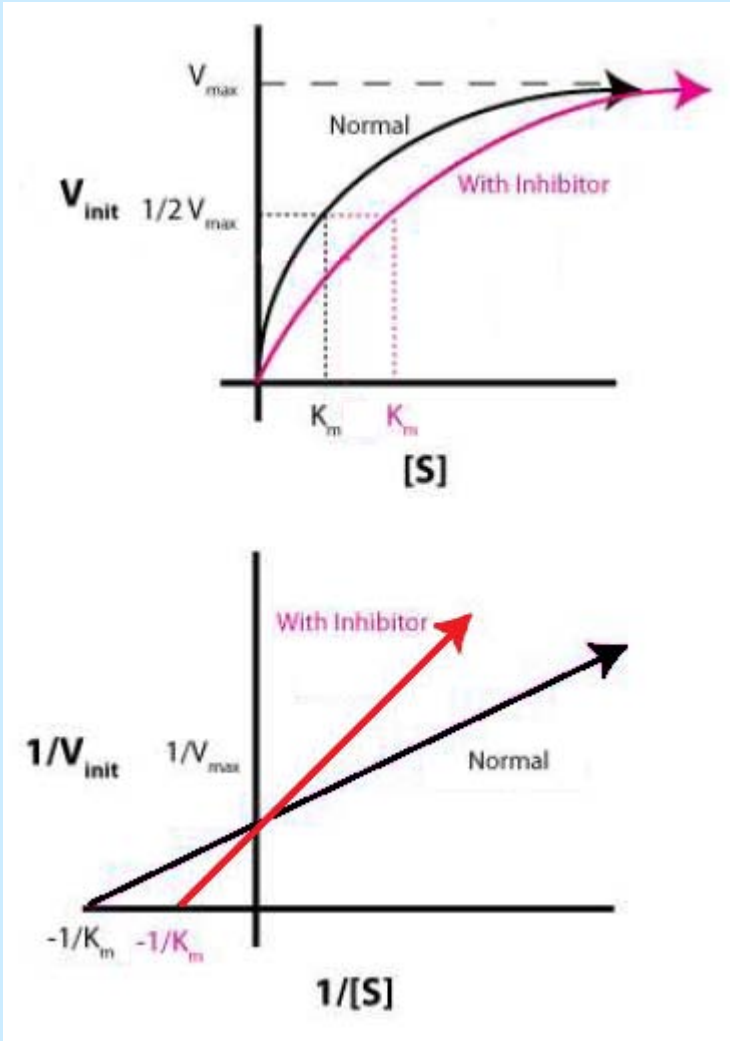
Competitive inhibition



K_i is the inhibitor's dissociation constant and [I] is the inhibitor concentration

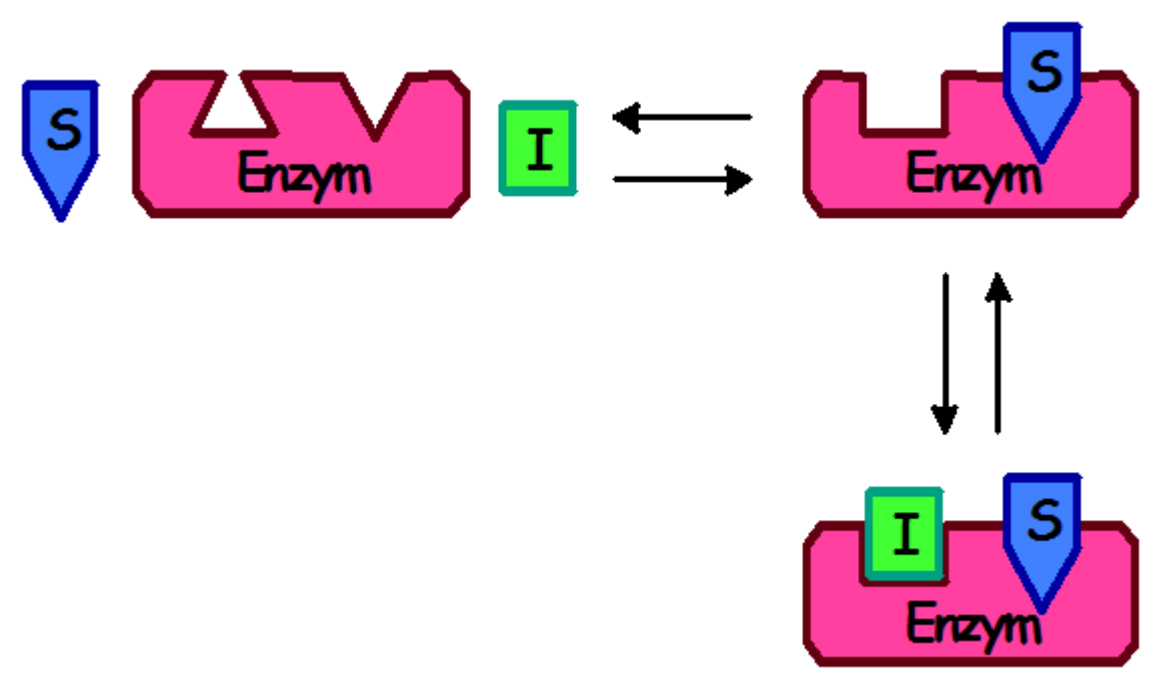
$$K_I = \frac{[E][I]}{[EI]}$$

$$v = \frac{k_2[E_0][S]}{S + K_M\left(1 + \frac{[I]}{K_I}\right)}$$



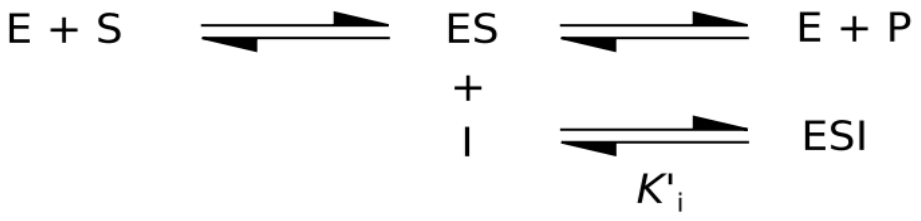
Uncompetitive inhibition

An inhibitor binding condition is substrate binding. The inhibitor reversibly binds to the ES complex, and the resulting ESI complex can not be converted to product.



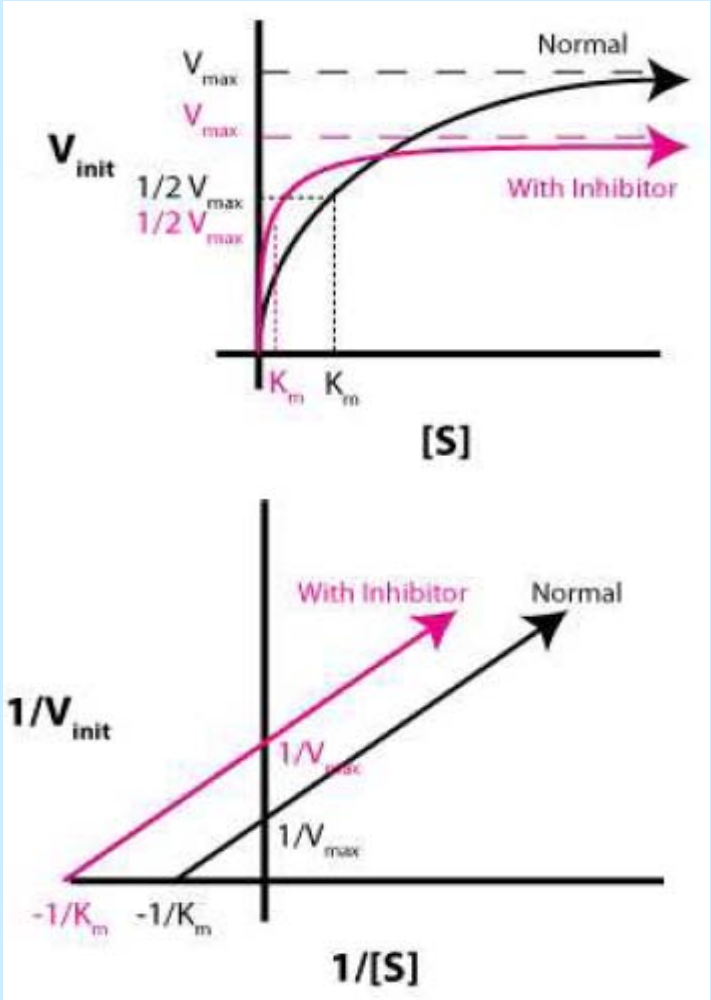
Uncompetitive inhibition

- the inhibitor binds only to the substrate-enzyme complex
- this type of inhibition causes Vmax to decrease and Km to decrease



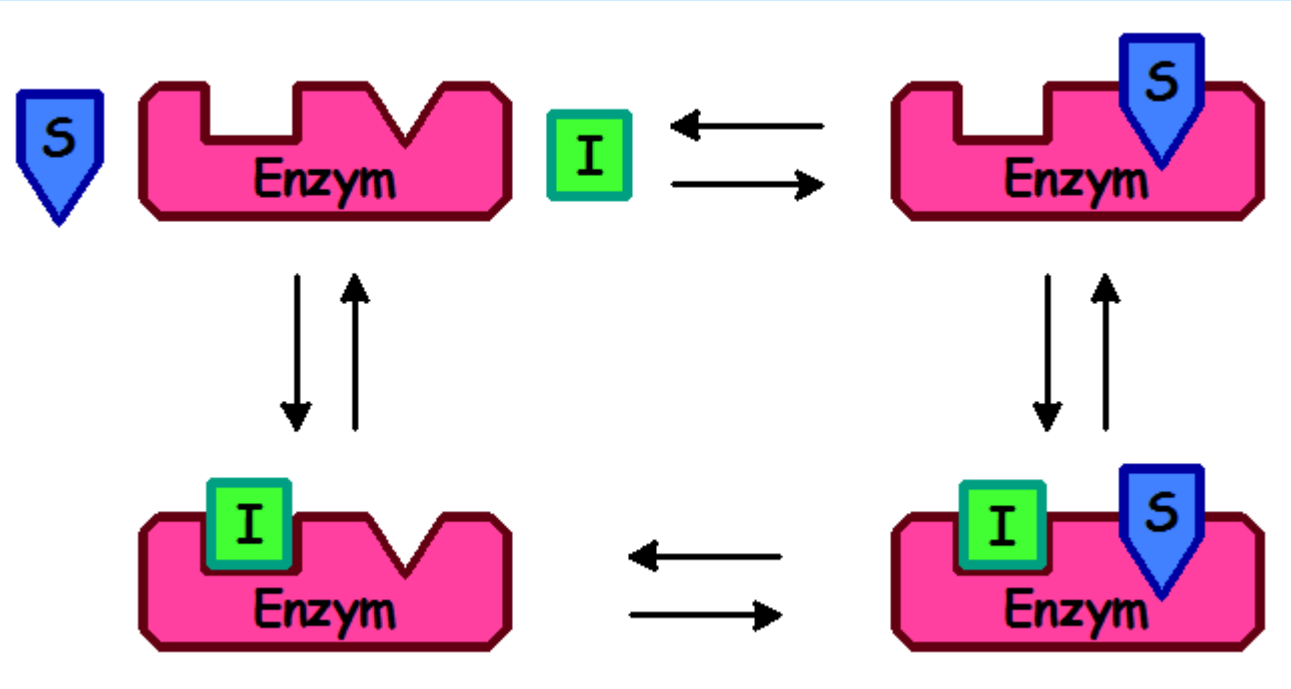
$$K_I = \frac{[ES][I]}{[ESI]}$$

$$v = \frac{S.\frac{V_{max}}{1+\frac{[I]}{K_I}}}{S+\frac{K_M}{1+\frac{[I]}{K_I}}}$$



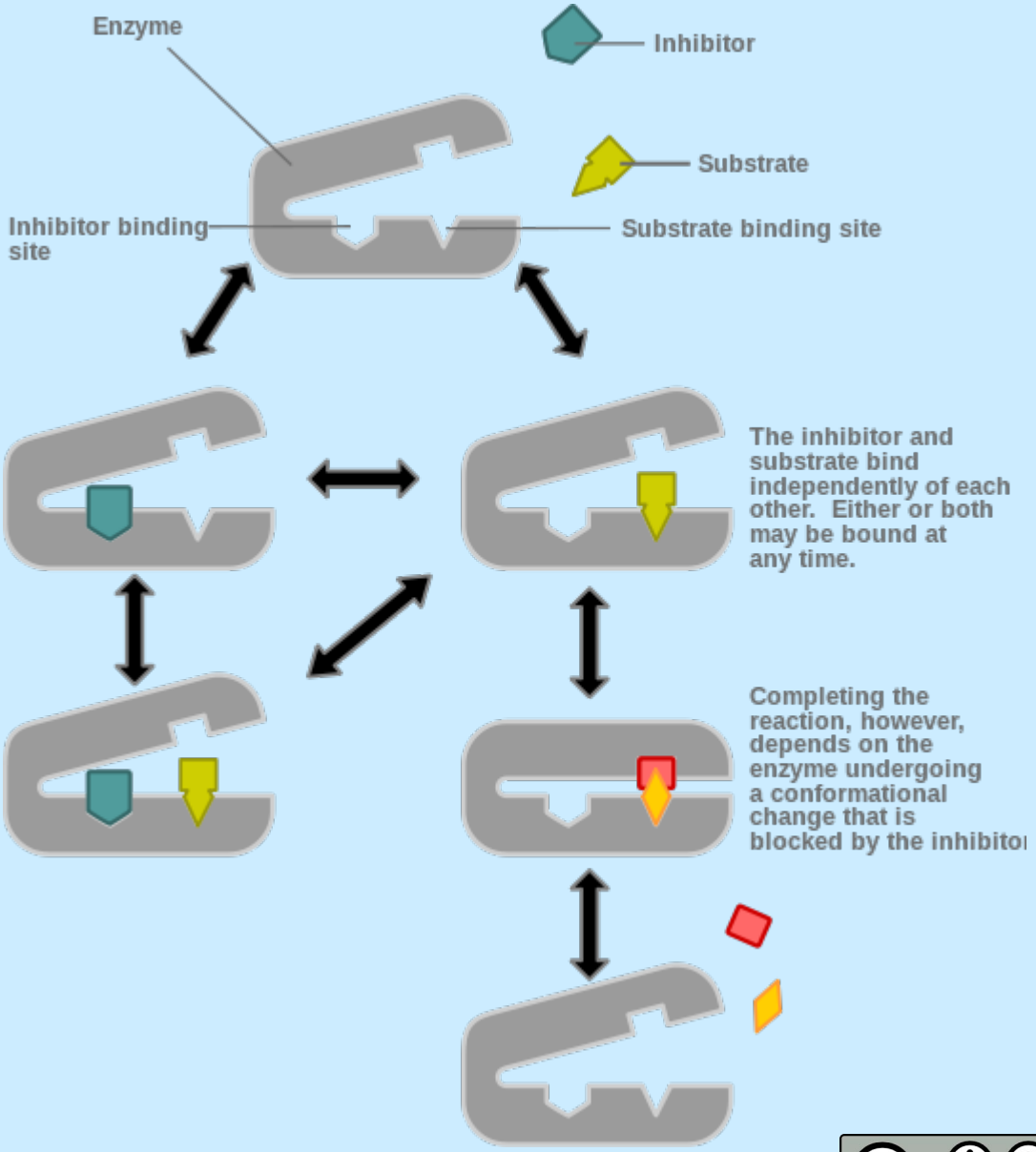
Noncompetitive inhibition

The S and I bonds to the enzyme are reversible, mutually independent and fast, so the slowest, controlling process is the disintegration of the ES complex into the product. The enzyme must contain binding centers for S and I that do not interact with each other.



Non-competitive inhibition

- Inhibition where the binding of the inhibitor to the enzyme reduces its activity but does not affect the binding of substrate (inhibitor does not bind at the active site).
- As a result, the extent of inhibition depends only on the concentration of the inhibitor.
- Vmax will decrease due to the inability for the reaction to proceed as efficiently
- Km will remain the same as the actual binding of the substrate, by definition, will still function properly.



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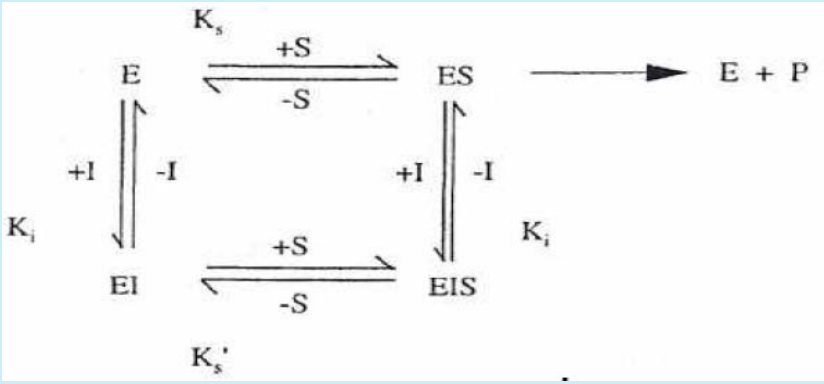

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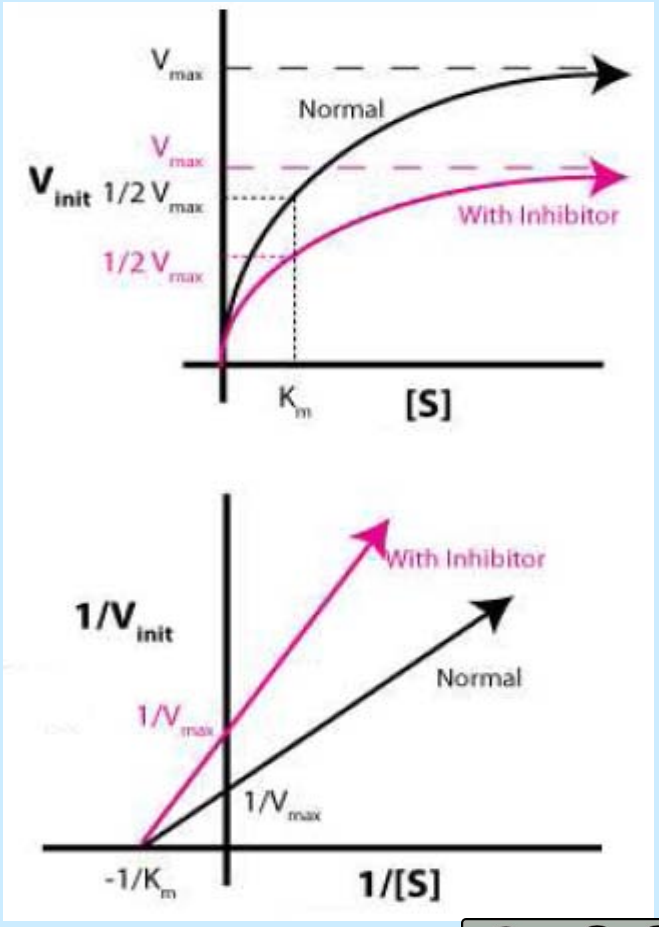
- Km will remain the same as the actual binding of the substrate, by definition, will still function properly.

$$K_I = \frac{[E][I]}{[EI]} = \frac{[ES][I]}{[ESI]}$$

$$V = \frac{V_{max}}{1 + \frac{I}{K_I}} \cdot \frac{S}{S + K_S}$$



Mixed inhibition

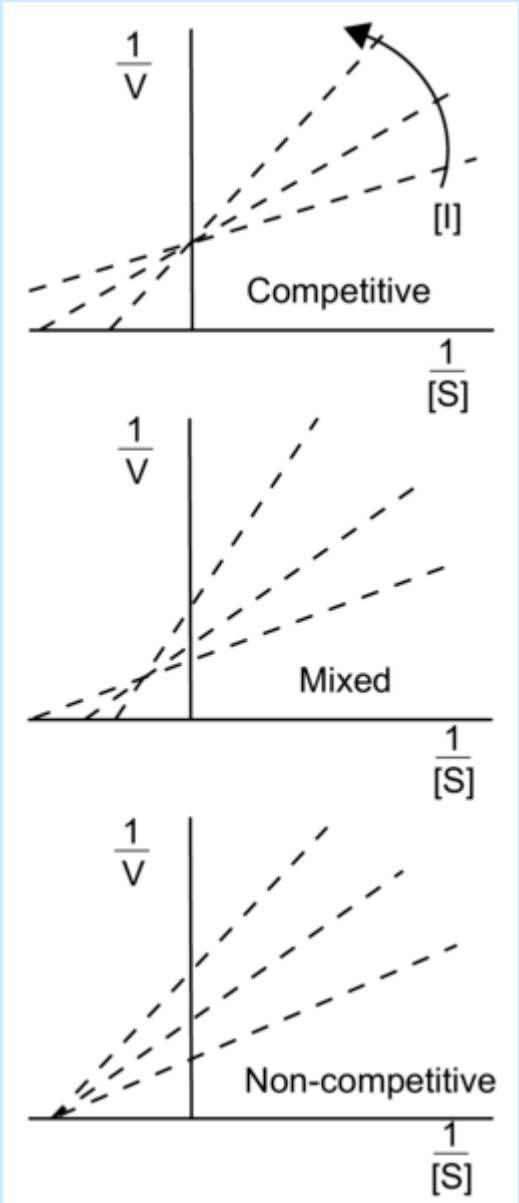
•the inhibitor can bind to the enzyme at the same time as the enzyme's substrate.

•the binding of the inhibitor affects the binding of the substrate, and vice versa.

•This type of inhibition can be reduced, but not overcome by increasing concentrations of substrate.

•Inhibitor changes the conformation (i.e., tertiary structure or three-dimensional shape) of the enzyme so that the affinity of the substrate for the active site is reduced.

•Mixed inhibition results in a decrease in the apparent affinity of the enzyme for the substrate and a decrease in the apparent maximum enzyme reaction rate.



Lineweaver–Burk plots of different types of reversible enzyme inhibitors. The arrow shows the effect of increasing concentrations of inhibitor

Other types of enzyme inhibition

•Substrate inhibition - at very high substrate concentration - Attempting to bind more substrate molecules to the active enzyme center => Conversion is not possible.

•Product inhibition - if the product is not removed, it stops releasing from the enzyme after being formed and blocks it (significant mechanism of enzyme activityregulation).

•Allosteric inhibition - the inhibitor binds to another site on the enzyme, so-called allosteric center, causing a change in the conformation of the active center => inability to bind the substrate.

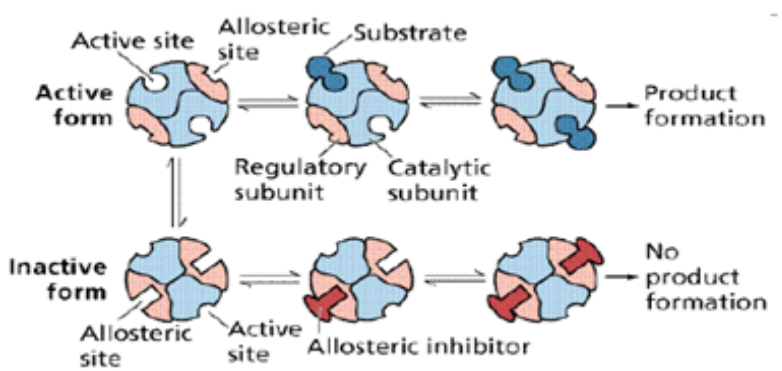
Department of Biotechnology

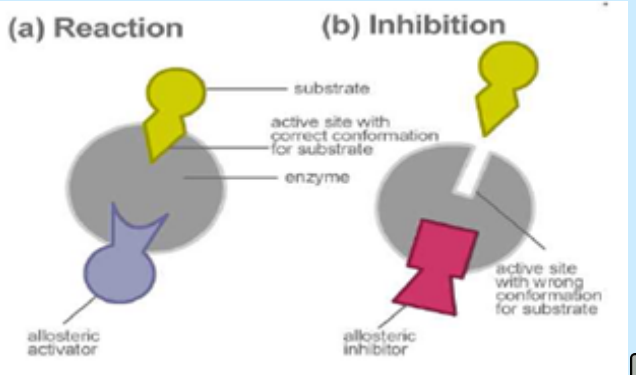
Allosteric enzymes

Allosteric enzymes are enzymes that change their conformational ensemble upon binding of an effector, which results in change in binding affinity at a different ligand binding site.

The site to which the effector binds is *allosteric site*. Effectors that enhance the protein's activity are referred to as *allosteric activators*, whereas those that decrease the protein's activity are called *allosteric inhibitors*.

Allosteric regulations are a natural example of control loops, such as feedback from downstream products or feedforward from upstream substrates. Allosteric regulation is also particularly important in the cell's ability to adjust enzyme activity.



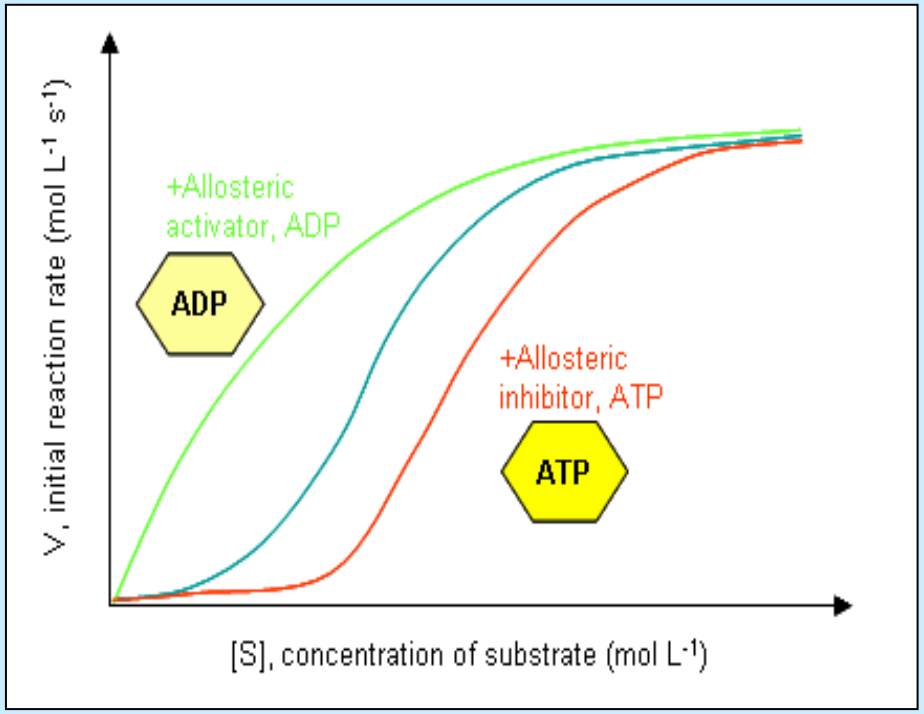


Allosteric enzymes

Hill kinetics

- sigmoidal character

$$v = -\frac{dS}{dt} = \frac{v_{max} S^n}{K_M^n + S^n}$$



Phosphofructokinase

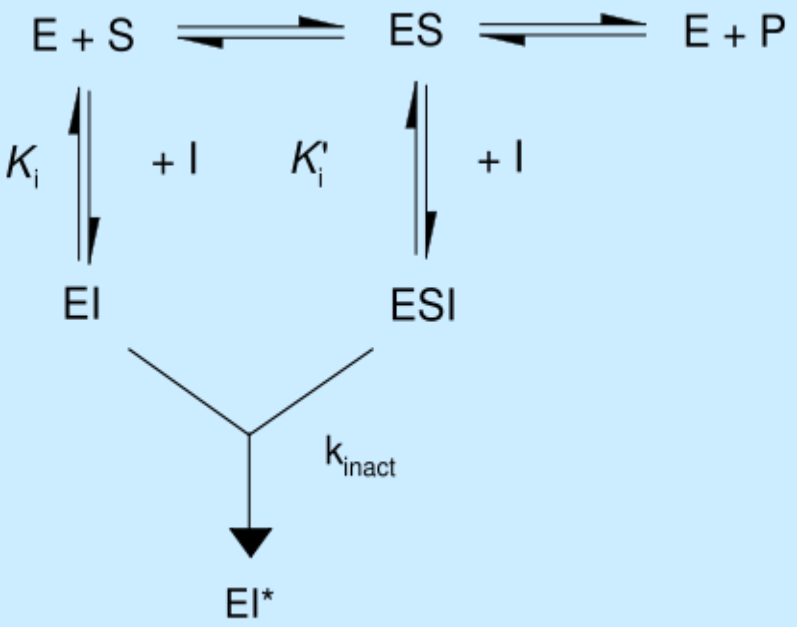
When a multi-subunit enzyme is fully in the active form, it approximates Michaelis-Menten kinetics (hyperbolic curve)

Irreversible inhibition

•the irreversible inhibitors form a reversible non-covalent complex with the enzyme (EI or ESI) and this then reacts to produce the covalently modified "dead-end complex" EI*.

•The rate at which EI* is formed is called the inactivation rate or k_{inact}.

•Since formation of EI may compete with ES, binding of irreversible inhibitors can be prevented by competition either with substrate or with a second, reversible inhibitor. This protection effect is good evidence of a specific reaction of the irreversible inhibitor with the active site.



Multi-substrate reactions

Multi-substrate reactions follow complex rate equations that describe how the substrates bind and in what sequence.

•The analysis of these reactions is much simpler if the concentration of substrate A is kept constant and substrate B varied.

•Under these conditions, the enzyme behaves just like a single-substrate enzyme and a plot of *v* by *[S]* gives appa*rent Km* and *Vmax* constants for substrate B.

•For an enzyme that takes two substrates A and B and turns them into two products P and Q, there are two types of mechanism:

1. Sequential (random, ordered)
2. Ping–pong.

Multi-substrate reactions – Sequential mechanism

The first important type of bi-bi reaction is known as **sequential**, which means that all substrates must add to the enzyme before any reaction takes place

The sequential bi-bi can be

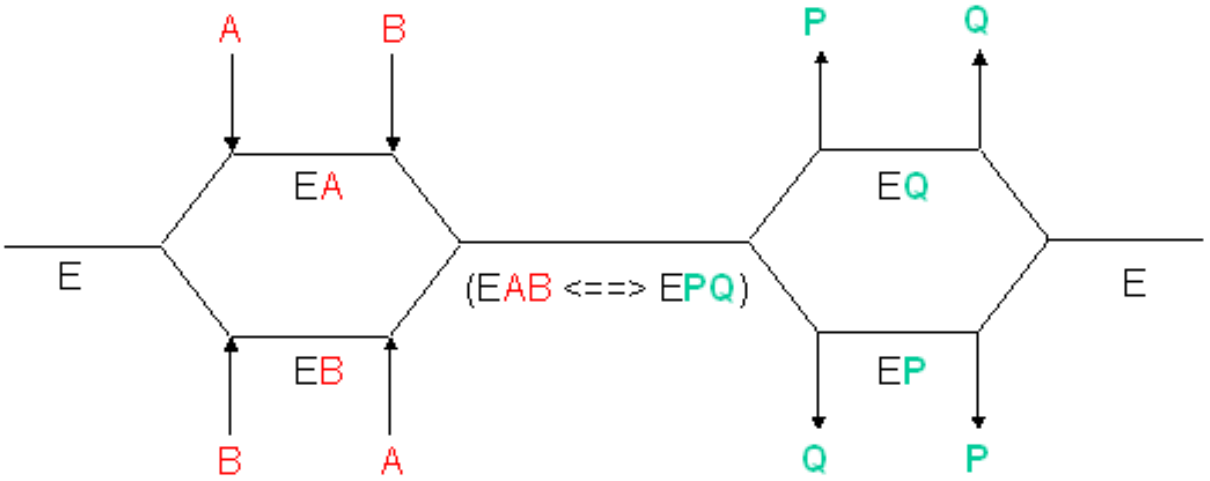
random, any substrate can bind first to the enzyme and any product can leave first

ordered, meaning that the substrates add to and products leave the enzyme in a specific order

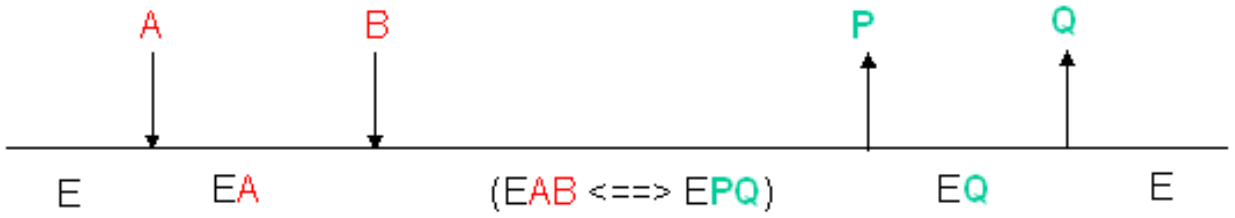
A ternary complex (E + both substrates) is formed in both cases

Multi-substrate reactions – Sequential mechanism

A. **Random Sequential**: random order of reactants binding and products leaving

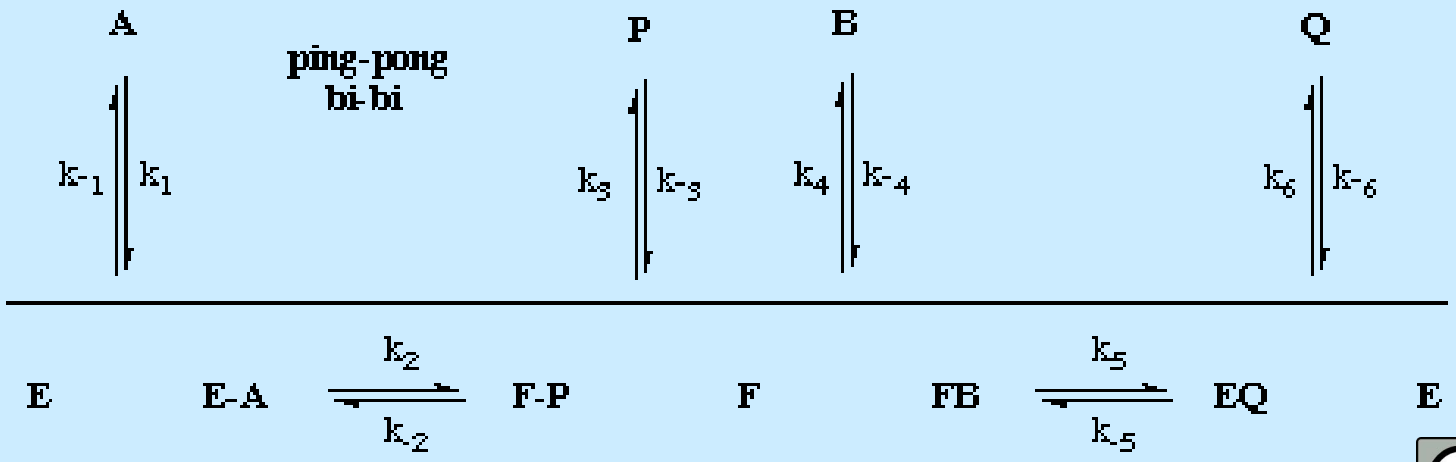


B. **Ordered Sequential**: specific order of reactants binding and products leaving



Multi-substrate reactions – Ping-pong mechanism

- One substrate bind first to the enzyme followed by product P release
- Typically, product P is a fragment of the original substrate A
- The rest of the substrate is covalently attached to the enzyme E, which we now designate as F
- Now the second reactant, B, binds and reacts with the enzyme to form a covalent adduct with the covalent fragment of A still attached to the enzyme to form product Q
- This is now released and the enzyme is restored to its initial form, E



COMMENTS

•Enzymes with ping–pong mechanisms include some oxidoreductases such as thioredoxin peroxidase, transferases such as acylneuraminate cytydilyltransferase and serine proteases such as trypsinand chymotrypsin.

•Serine proteases are a very common and diverse family of enzymes, including digestive enzymes (trypsin, chymotrypsin, and elastase), several enzymes of the blood clotting cascade and many others.

•Sequential kinetics can be distinguished from ping-pong kinetics by initial rate studies.

Literature

L. Bartovská, P. Chuchvalec: Kinetika a katalýza (příklady a úlohy), VŠCHT, 1991

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University of Chemistry and Technology Prague
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Principles of microbial growth, batch cultivation

Tomáš Brányik

Growth conditions of microbes

Ideal conditions:

- the growth is not limited by substrate concentration
- the growth is not limited by product
- the medium is inoculated by one single cell

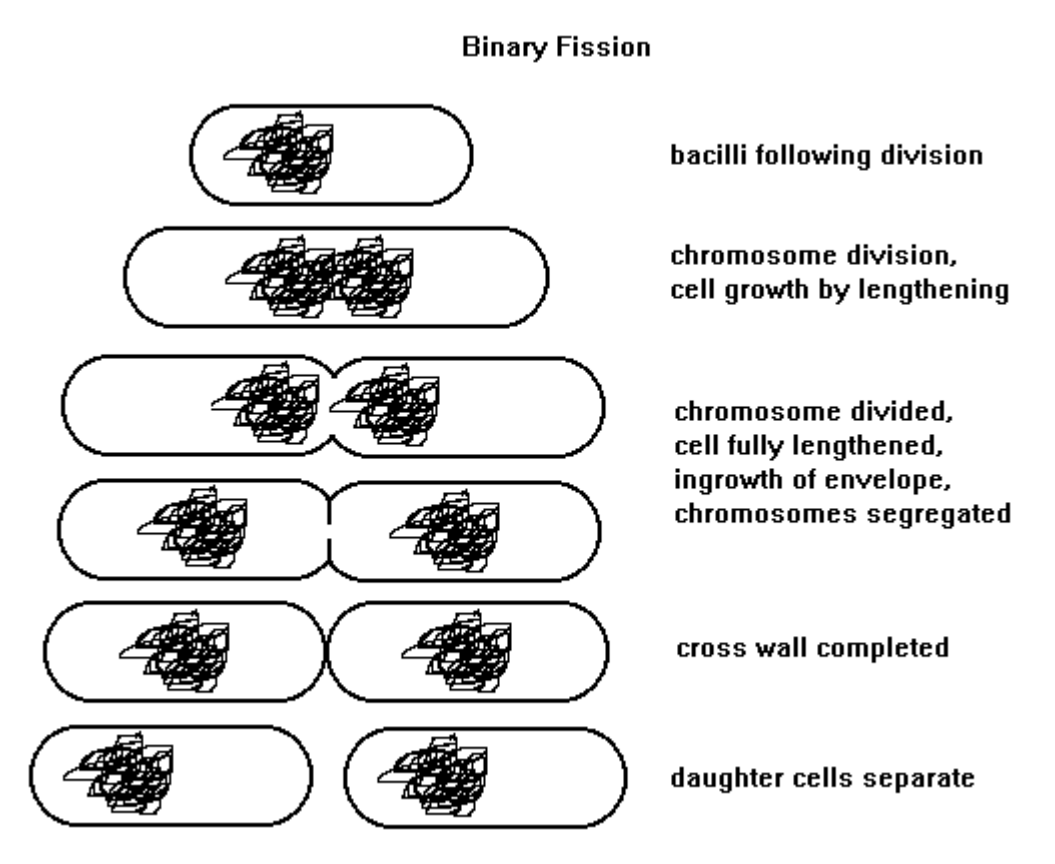
Real conditions:

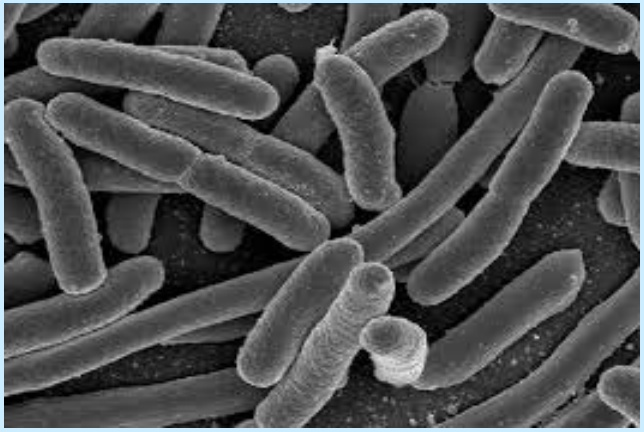
- influnce of a wide range of environmental factors: character and concentration of substrate, trace elements, temperature, humidity etc.
- accumulation of metabolites which may inhibit the growth

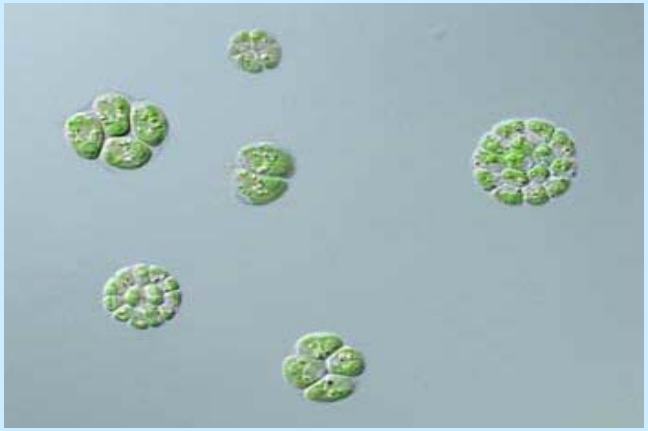
Nutritional requirements of microbes

Energy source	Oxidizing donor source	Carbon source	Name	Examples
Sun Light *Photo-*	Organic *-organo-*	Organic *-heterotroph*	Photoorganoheterotroph	Some bacteria (*Rhodobacter*)
		Carbon dioxide *-autotroph*	Photoorganoautotroph	
	Inorganic *-litho-**	Organic *-heterotroph*	Photolithoheterotroph	
		Carbon dioxide *-autotroph*	Photolithoautotroph	Some bacteria (blue green algae), some eukaryotes (eukaryotic algae, land plants). Photosynthesis.
Breaking Chemical Compounds *Chemo-*	Organic *-organo-*	Organic *-heterotroph*	Chemoorganoheterotroph	Some eukaryotes (heterotrophic protists, fungi, animals)
		Carbon dioxide *-autotroph*	Chemoorganoautotroph	Some archaea (anaerobic methanotrophic archaea).[8] Chemosynthesis.
	Inorganic *-litho-**	Organic *-heterotroph*	Chemolithoheterotroph	Some bacteria (*Oceanithermus profundus*)[9]
		Carbon dioxide *-autotroph*	Chemolithoautotroph	Some bacteria (*Nitrobacter*, Methanobacteria). Chemosynthesis.

Growth conditions of microbes

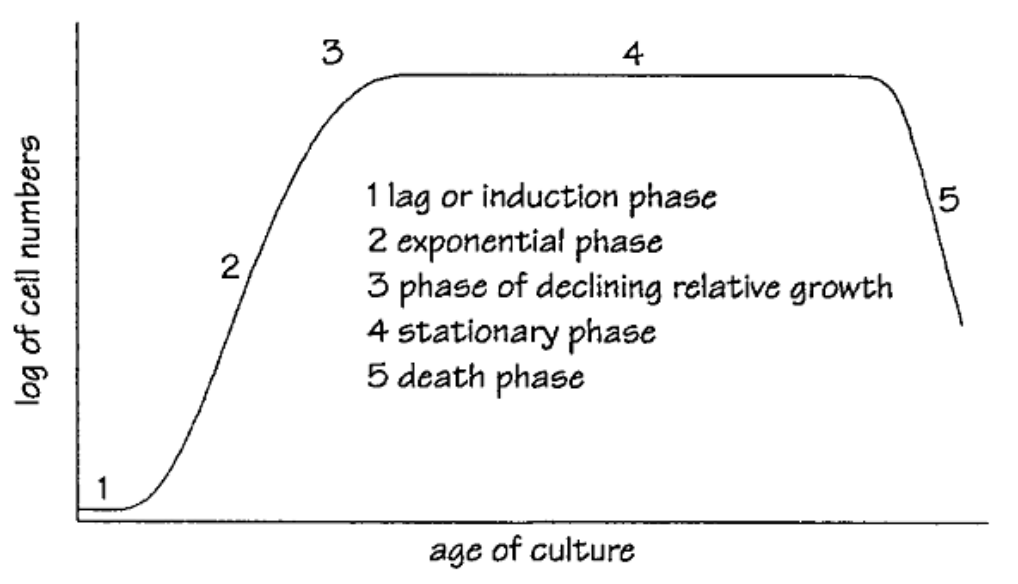






Growth of microbes

„Growth curve“



1. Lag or induction phase
This phase during which little increase in cell density occurs is relatively long when an algal culture is transferred from a plate to liquid culture.

2. Log or exponential phase: the cell density increases as a function of time according to a logarithmic function.

3. Phase of declining growth rate; cell division slows down when nutrients, light, pH, carbon dioxide or other physical and chemical factors begin to limit growth.

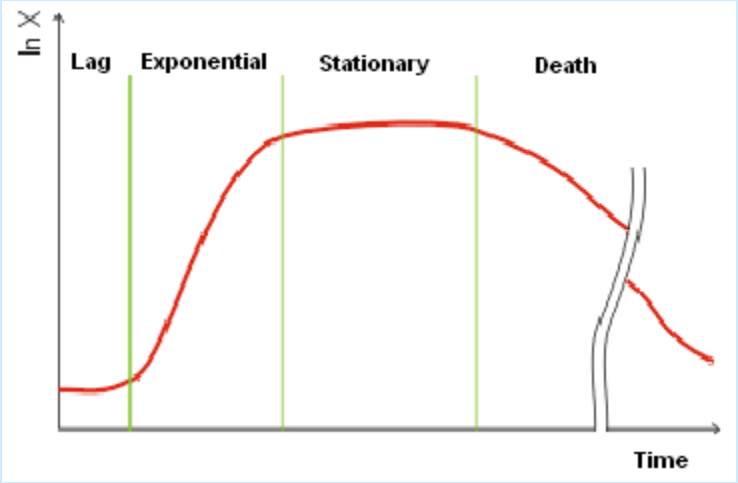
4. Stationary plateau phase: the limiting factors and the growth rate are balanced, which results in constant cell density. There is either no growth or growth = death

5. Death or "crash" phase: water quality deteriorates and nutrients are depleted to a level incapable of sustaining growth. Cell density decreases rapidly and the culture eventually collapses.

Types of growth models

1. Unstructured Growth Models
   - Assume constant cell composition - balanced growth
   - Does not reflect a change in internal cell processes under changing environmental conditions
   - Fast response of cells to environmental perturbation - pseudo-balanced growth
2. Non-Segregated Growth Models
   - Cell population is considered as a single phase interacting with the surrounding environment
3. *Structured Growth Models*
   - *Cell as a set of internal components*
   - *Includes individual reactions or systems of reactions occurring in cells*
4. *Segregated Growth Models*
   - *Cells are considered at different stages in the life cycle*

Microbial growth model - unlimited



Microbes are dividing in geometric order

Time of observation

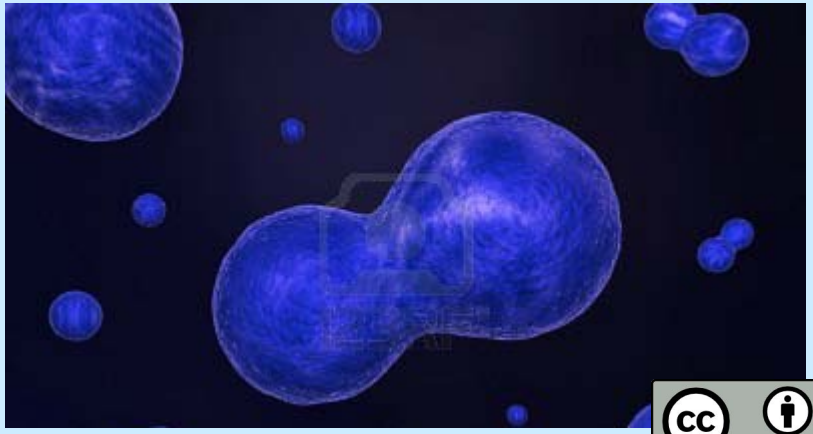
Duration of observation	No. of cells
$t_0 = 0$	$X_0 = X_0\ 2^0$
$t_1 = 1T$ (T-doubling time)	$2X_0 = X_0\ 2^1$
$t_2 = 2T$	$4X_0 = X_0\ 2^2$
$t_3 = 3T$	$8X_0 = X_0\ 2^3$
:	:
$t = nT$	$X = X_0\ 2^n$

$T = t/n$; $1/T = n/t = c$ (rate constant)

$$X = X_0\ 2^{ct} \quad (1)$$

Exponencial function (1) can be transformed by
$y = a^x = e^{bx}$ (where $b = \ln a$) to
$X = X_0 e^{tc\ln 2}$ (where $c\ln 2 = \mu$)

$$\mathbf{X = X_0 e^{\mu t}}$$



$X = X_0 2^{ct}$ (1)

Microbial growth model - unlimited

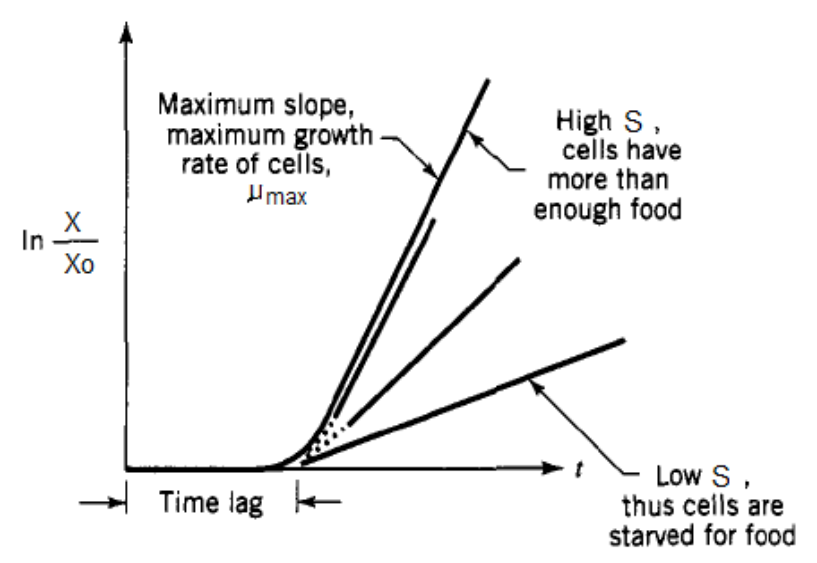
$$X = X_0 e^{\mu t}$$

Instant growth rate can be calculated by temporal derivation of equation (1)

$f(x): a^x \rightarrow f'(x): a^x \ln a$

$$dX/dt = (X_0 2^{ct} \ln 2)c$$

$$X = X_0 2^{ct} ; c \ln 2 = \mu$$



$dX/dt = \mu X$

The growth rate is proportional to actual cell concentration, autocatalytic process.

Specific growth rate

$\mu = dX/dt \times 1/X$ [h^{-1}]

Unabridged dimension [$g.g^{-1}.h^{-1}$] or [$n.n^{-1}.h^{-1}$]

T – doubling time = time necessary to double to population (cell number)

$$T = 1/c = \frac{\ln 2}{\mu} = \frac{0.693}{\mu}$$

G – Generation time = the time from the birth of cell to its reproduction (duration of cell cycle)

Microbial growth model - limited

However, specific growth rate (μ) is constant only during unlimited growth

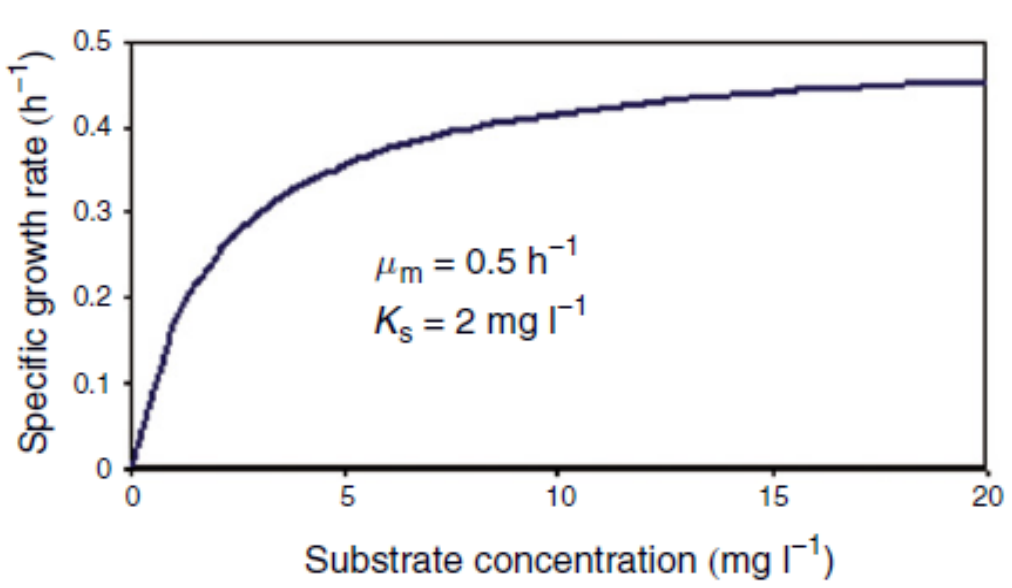
In real world, μ is a function of concentration of substrate, type of C and N source, presence of products, inhibitors, temperature, pH, concentration of oxygen etc.

MONOD (1942)

$$\mu = \mu_{max} \cdot \frac{S}{K_S + S}$$

parameters: μ_{max} , $S \rightarrow \infty$

K_s , S at which $\mu = \mu_{max}/2$



Jacques Lucien Monod (9.2. 1910 – 31.5. 1976), a French biochemist, won the Nobel Prize in Physiology or Medicine in 1965

Stationary phase

$$\frac{dX}{dt} = \mu X - kX = 0$$

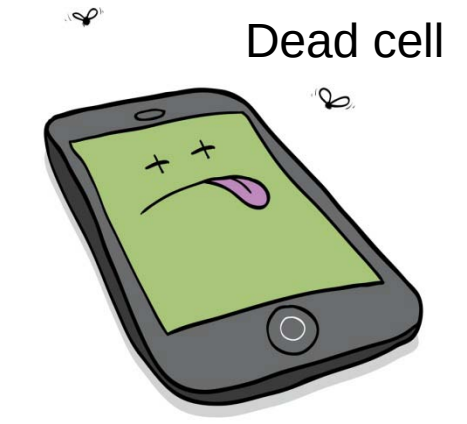
k - specific rate of cell death
(cell loss rate per unit of cells)

Reasons leading to stationary phase: consumed substrate (source of C and energy, other nutrients), accumulation of metabolites including products (direct toxicity, indirect toxicity through pH and redox, high cell concentration).

Characteristics of the cell population in stationary phase:
- cell concentration is constant
- μ is approaching 0
- rebuilding cells from growing to resting
- accumulation of storage of substances (glycogen, poly-β-hydroxybutyric acid, polyphosphates...)
- there may be preparation for sporulation

Death phase

$$\frac{dX}{dt} = -kX$$



The action of destructive physical and chemical forces on chemical bonds in macrololecular and supramolecular structures prevails over repair mechanisms.

Specific case is autodestruction (low pH, alcohols, antibiotics).

Cell death can be accompanied by autolysis (cryptic growth).

$$k = \frac{-dX}{dt} \times \frac{1}{X}$$

k - specific rate of cell death (cell loss rate per unit of cells)

Microbial growth model - limited

Monod model is a semi-empirical and non-strucured.

Suitable for many cases when growth is cell suspension is diluted. It counts only with one limiting substrate.

If we have <u>more than one limiting substrate</u>
- Special case is limiting by oxygen and C source

Multiplicative kinetics

$$\mu = \mu_{\max}\left(\frac{S_1}{K_1 + S_1}\cdot\frac{S_2}{K_2 + S_2}\right) \Rightarrow \mu = \mu_{\max}\prod_{i=1}^{n}\left(\frac{S_i}{K_{S_i} + S_i}\right)$$

Additive kinetics

$$\mu = \frac{\mu_{\max} S_1}{K_1 + S_1} + \frac{\mu_{\max} S_2}{K_2 + S_2} \Rightarrow \mu = \sum_{i=1}^{n}\left(\frac{\mu_{\max S_i}}{K_{S_i} + S_i}\right)$$

Growth models in the presence of inhibitors

Monod equation describes the substrate dependent growth but does not include inhibition.

What can an inhibitor cause? Change the chemical potential of the substrate, intermediates or products. Change permeability of the cell membrane. Change enzyme activity. Affect the synthesis of enzymes….

Types of inhibition:

- Substrate inhibition
- Product inhibition

Inhibitory constants in kinetic equations have physiological meaning only if growth is controlled by only one enzyme reaction. Otherwise they have the meaning of formal numerical constants, determined by regression methods.

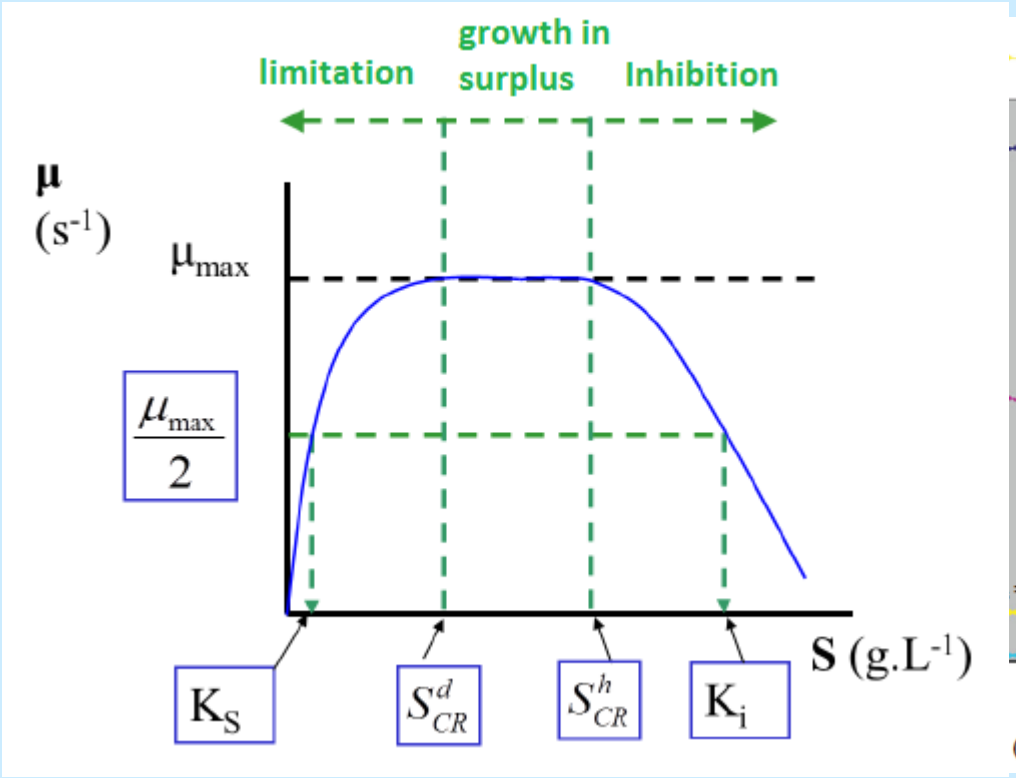
Analogy to enzyme kinetics

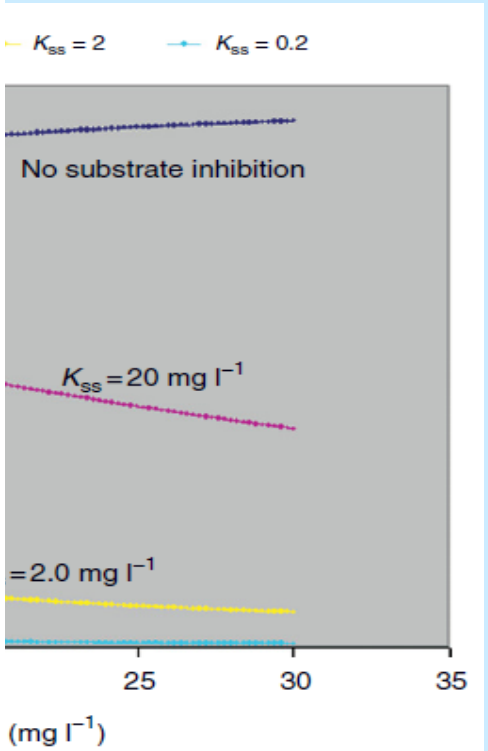
$$X + S \overset{K_S}{\Leftrightarrow} XS \overset{k}{\rightarrow} \text{new cells}$$

$$XS + S \overset{K_I}{\Leftrightarrow} XS_2$$

Nonreactive complex

$$K_S = \frac{[X][S]}{[XS]} \qquad K_i = \frac{[XS][S]}{[XS_2]}$$



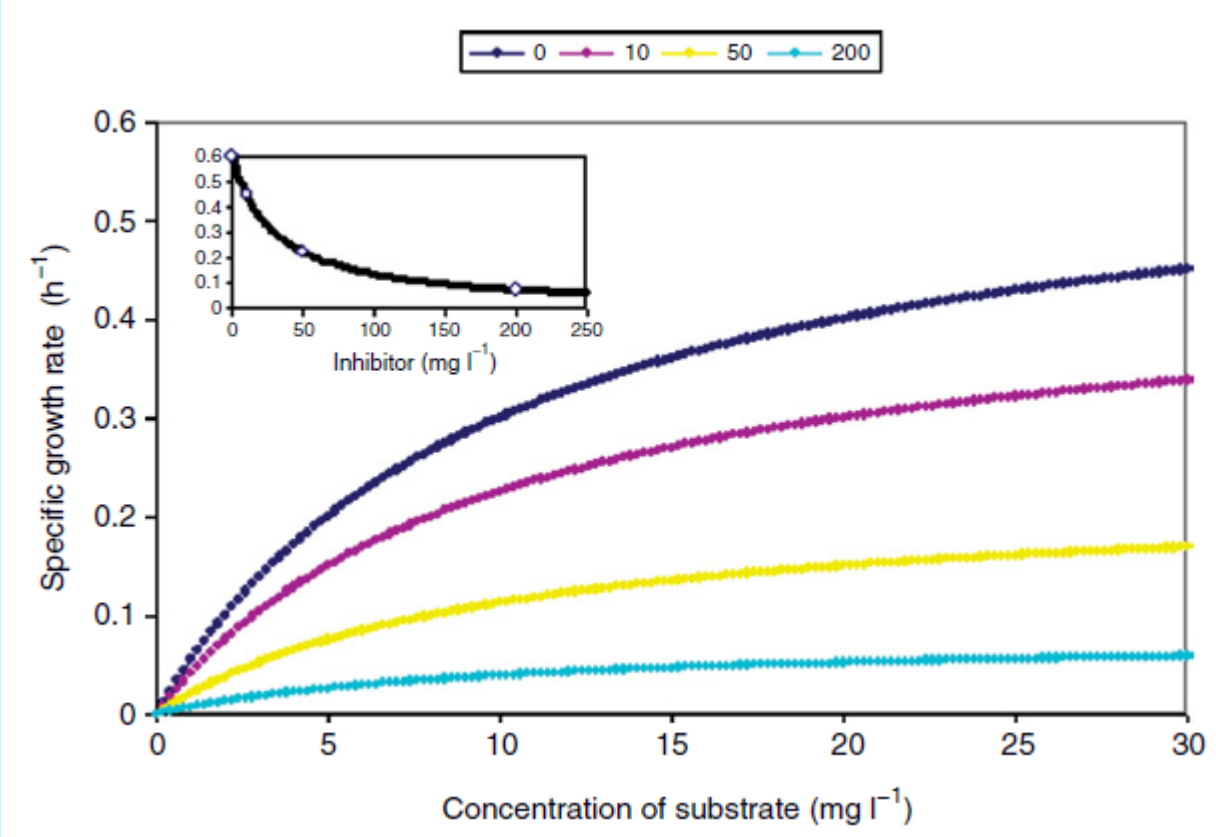


- **inhibition by substrate**

$$\mu = \mu_{max} \cdot \frac{S}{\left(K_S + S + \frac{S^2}{K_I}\right)}$$

K_I = inhibition constant

There are many different models to describe the growth of cell under product inhibition



- **inhibition by product**

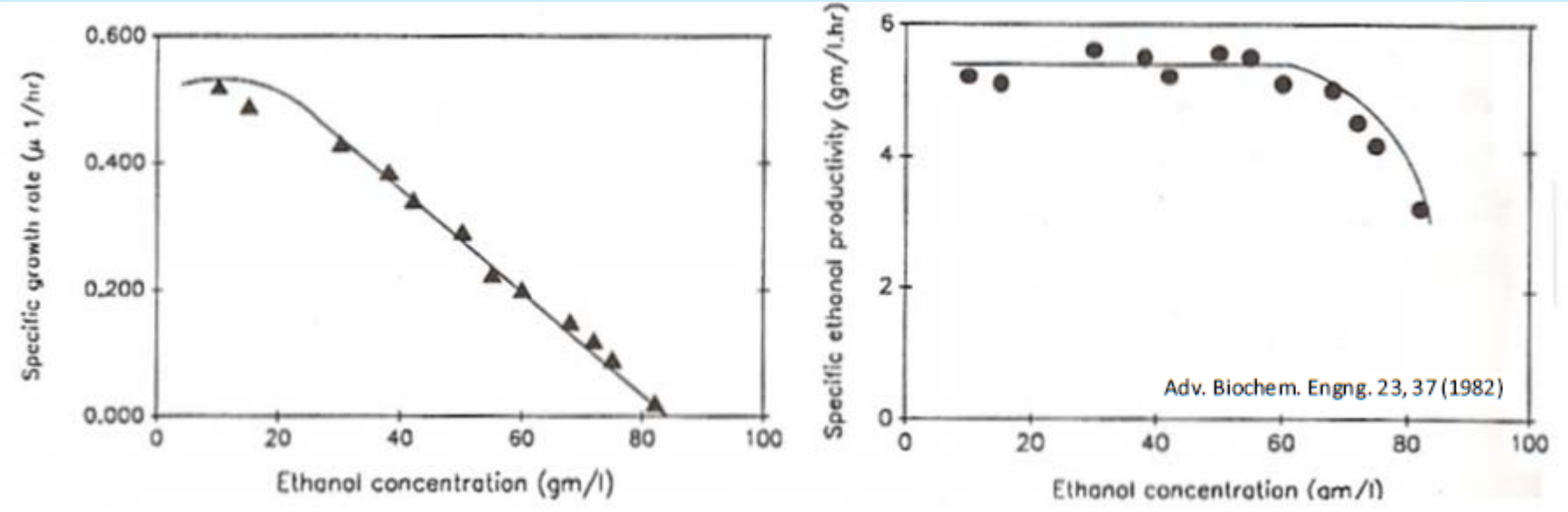
Analogy with non-competitive enzyme inhibition

$$\mu = \mu_m \frac{s}{K_s + s} \times \frac{K_p}{K_p + p}$$

K_P = inhibition constant

P = Product conc.

Growth inhibition by product



Effect of ethanol concentration on specific growth rate and specific rate of ethanol production for *Zymomonas mobilis*. Linear part of curve μ vs. P is simulated by a model:

$$\mu = \mu_{max}\left(1-\frac{P}{P_m}\right)\left(\frac{S}{K_S+S}\right)$$

where μ_{max} = 0.66 h^{-1}, Pm = 82.5 g/L, Pm – concentration of product, at which the growth stops.

Growth yields (Y)

The amount of biomass is proportional to the amount of consumed substrate

X = Y×S, where X[e.g. g/l, mol/l] S[e.g. g,/l, mol/l]

The proportionality factor is called yield coefficient Y = X/S, [e.g. g/g, mol/mol, g/mol]

$$Y_{X/S} = \frac{dX}{-dS}$$

The growth rate is proportional to substrate consumption rate

$$\frac{dX}{dt} = Y_{X/S}\frac{-dS}{dt}$$

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

The actual values of Y and q inform us about the alterations in cell physiology caused by e.g. changed growth conditions. They are important for controlling cell cultivation.

$$\frac{-dS}{dt} = \frac{1}{Y_{X/S}}\frac{dX}{dt} = \frac{\mu}{Y_{X/S}}X$$

Growth yields (Y)

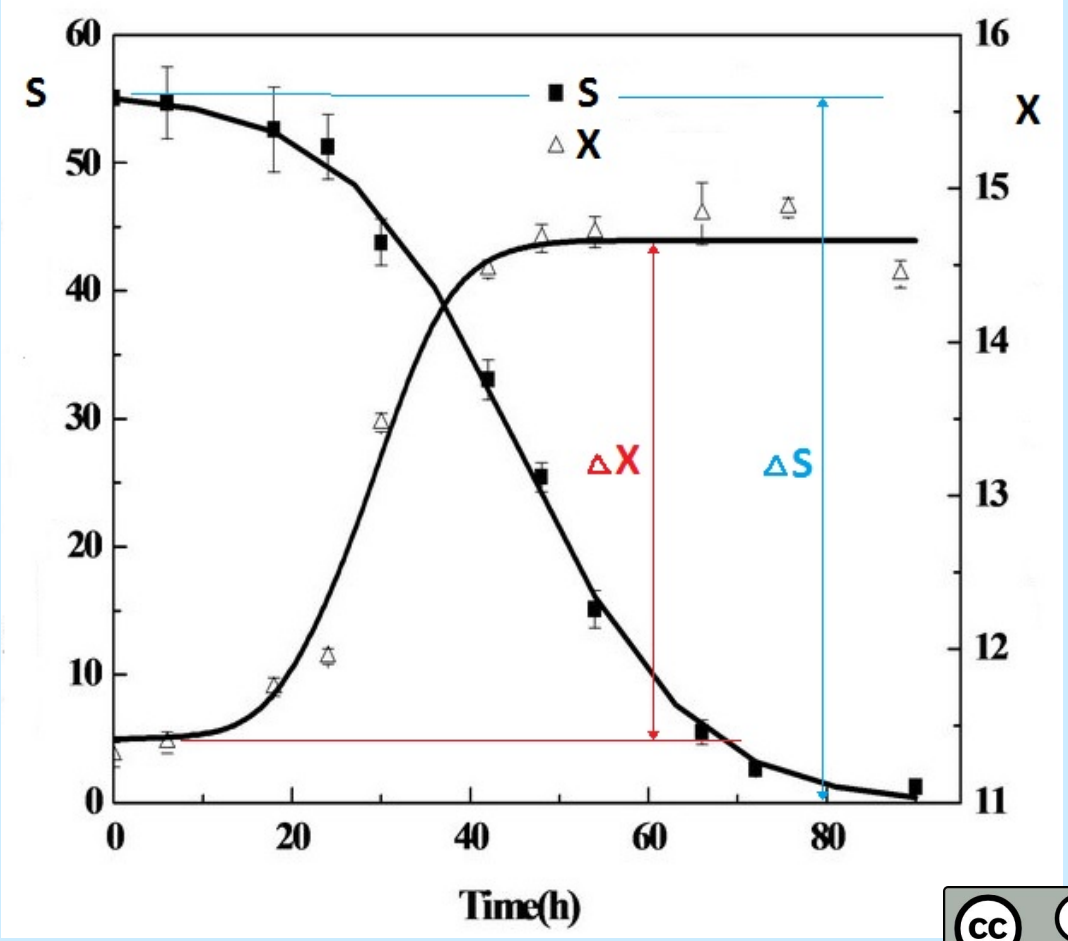
$Y_{X/S}$ = X/S, [e.g. g/g, mol/mol, g/mol]

partial total

$$Y_{X/S} = \frac{dX}{dS} = \frac{\Delta X}{\Delta S}$$

For most yeast and bacteria growing aerobically on glucose $Y_{X/S}$ is typically 0.4 – 0.6 g/g.

Anaerobic growth is much less efficient.

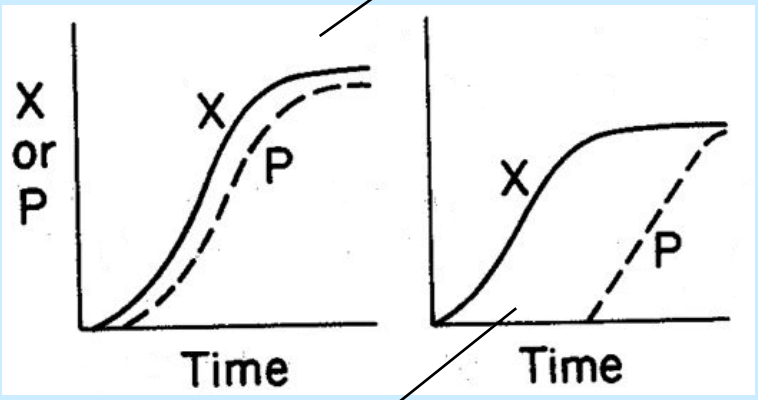


Growth yields (Y)

For products:

$$Y_{P/S} = -\frac{dP}{dS} \qquad \frac{dP}{dt} = -Y_{P/S}\frac{dS}{dt}$$

$$Y_{P/X} = \frac{dP}{dX} \qquad \frac{dP}{dt} = Y_{P/X}\frac{dX}{dt}$$



$$\frac{dP}{dt} = \beta X$$

For oxygen:

$$Y_{X/O_2} = \frac{dX}{-dC_{O2}}$$

For most yeast and bacteria growing aerobically $Y_{X/O}$ is typically 0.9 – 1.4 g/g.

For highly reduced substrates (e.g. CH_4) $Y_{X/O}$ is lower.

$$Y_{P/S} = Y_{X/S} \times Y_{P/X}$$

Mutual relationship of yield coefficients

Growth yields (Y)

For ATP:

$$Y_{ATP} = \frac{\Delta X}{1 molATP} = 5 - 32 g_{dry} / molATP$$

Often Y_{ATP} is **10.5 $g_{dry\ weight}$/mol ATP**. Conditions for that:

1. Complex medium where C source is used as energy source and only 3% as C source.
2. Tight coupling between catabolism and growth. In uncoupled catabolim and growth the C source can be used for synthesis of secondary metabolite, mechanical movement, synthesis of reserve materials or just hydrolysis for heat generation.
3. Constant maintenance energy.
4. Canstant catabolic metabolism.

Metabolic coefficients

$$q_S = \frac{\mu}{Y_{X/S}} = \frac{-dS}{dt}\frac{1}{X}$$

The specific rate of substrate consumption [mol(g).g^{-1}.h^{-1}]

$$q_{O_2} = \frac{\mu}{Y_{X/O_2}} = \frac{-dC_{O_2}}{dt}\frac{1}{X}$$

The specific rate of oxygen consumption [mol(g).g^{-1}.h^{-1}]

$$q_P = \mu.Y_{P/X} = \frac{dP}{dt}\frac{1}{X}$$

The specific rate of product formation [mol(g).g^{-1}.h^{-1}]

$$RQ = \frac{vol.CO_2}{vol.O_2}$$

Respiratory quotient [volume (mol) of produced CO_2/ volume (mol) of consumed O_2]

Maintenance energy

Monod equation describes the substrate dependent growth but does not include the so called maintenance energy, which requires to modify the Monod equation:

$$\underset{\delta S_E}{\text{Total energy source consumed}} = \underset{\delta S_G}{\text{Consumption for cell growth}} + \underset{\delta S_M}{\text{Consumption for maintenance}}$$

It is na energy needed to maintain the homeostasis (internal environment) of cells. Any living cell represents a system where decomposition processes are still running, which requires endogenous metabolism to spend maintenance energy.

The maintenance energy is almost constant. However, it is influenced, for example, by the O_2 concentration, limitation by nutrient other than E-source.

Non-ideal external environment requires to release more energy to maintain an optimal internal environment.

m_S - indicates the amount of substrate (g) that is required to deliver to 1g of cells for maintenance purposes per hour (for oxygen m_O, for ATP m_{ATP})

Organism	*Substrate*	*Maintenance coefficient for substrate m_s*
Aerobacter aerogenes	Glucose	5.4
Aerobacter aerogenes	Glycerol	7.6
Saccharomyces cerevisiae	Glucose	1.8
Escherichia coli	Glucose	5.4
Methane bacteria	Methane	2.0
Penicillium chrysogenum	Glucose	2.2
Aerobacter aerogenes	Citrate	5.8

m_s (x 10^2)(gm/gm dry cell weight-hr)

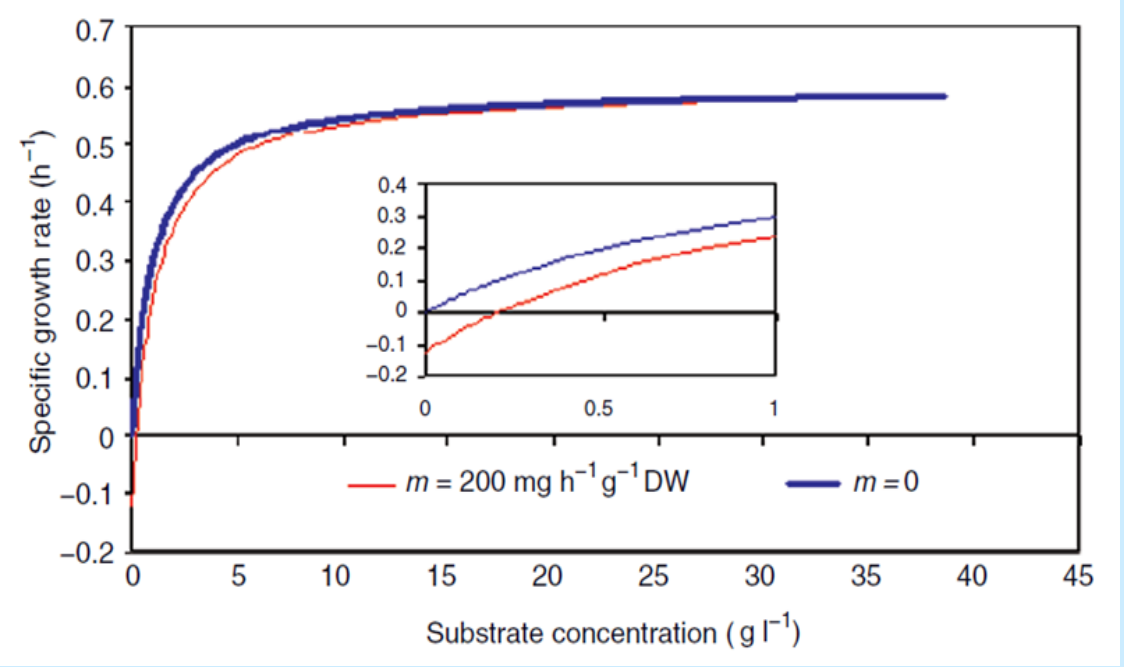
Maintenance energy

-Maintenance of potentials across membranes

-Replacement of denaturated (hydrolysed) molecules

-Maintenance of intracellular pH

-Turnover of AT throug alternative pathways



$$q_S = \frac{\mu}{Y_{X/S}}$$

m_s - indicates the amount of substrate (g) that is required to deliver to 1g of cells for maintenance purposes per hour

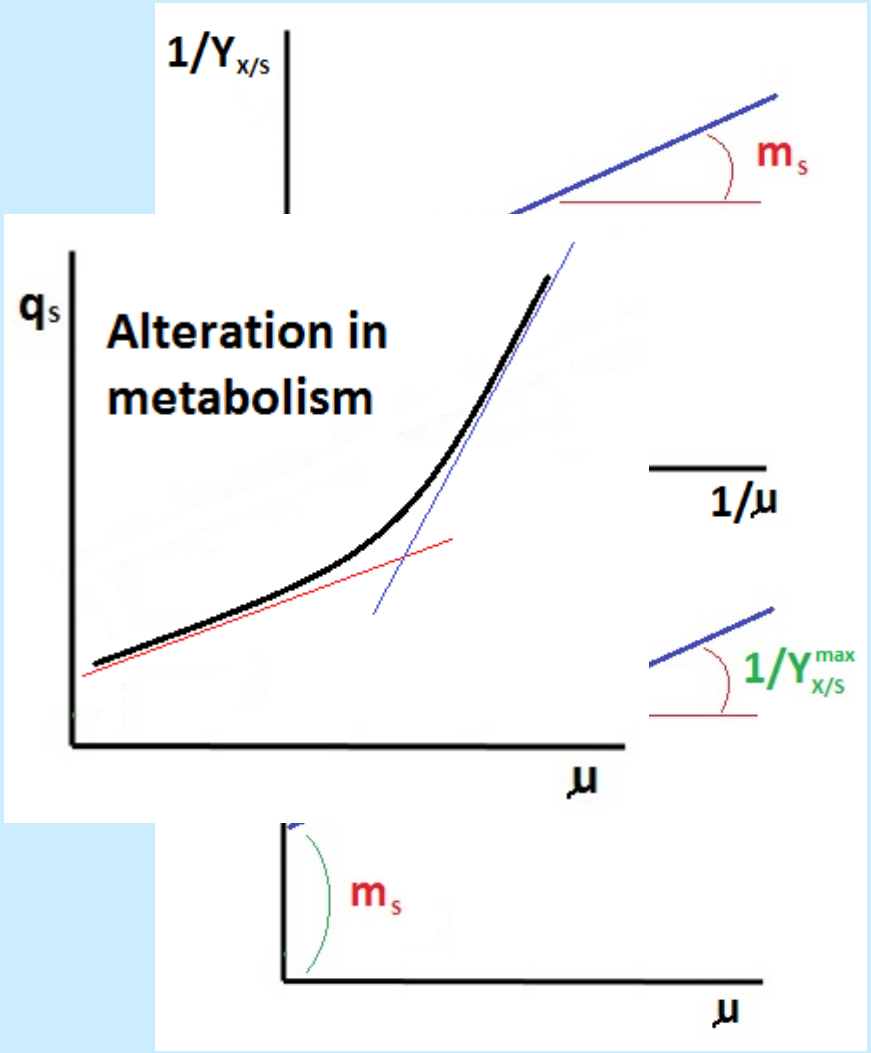
$$\frac{dS}{dt} = \left(\frac{dS}{dt}\right)_g + \left(\frac{dS}{dt}\right)_m$$

$$\frac{dS}{dt} = \frac{\mu X}{Y_{X/S}^{max}} + m_S X$$

$$\frac{\mu X}{Y_{X/S}} = \frac{\mu X}{Y_{X/S}^{max}} + m_S X$$

$$\frac{\mu}{Y_{X/S}} = \frac{\mu}{Y_{X/S}^{max}} + m_S$$

$$\frac{1}{Y_{X/S}} = \frac{1}{Y_{X/S}^{max}} + \frac{m_S}{\mu}$$



m_E - indicates the amount of ATP (mol) that is required to deliver to 1g of cells for maintenance purposes per hour

$$q_{ATP} = \frac{\mu}{Y_{ATP}} = \frac{\mu}{Y_{ATP}^{max}} + m_E$$

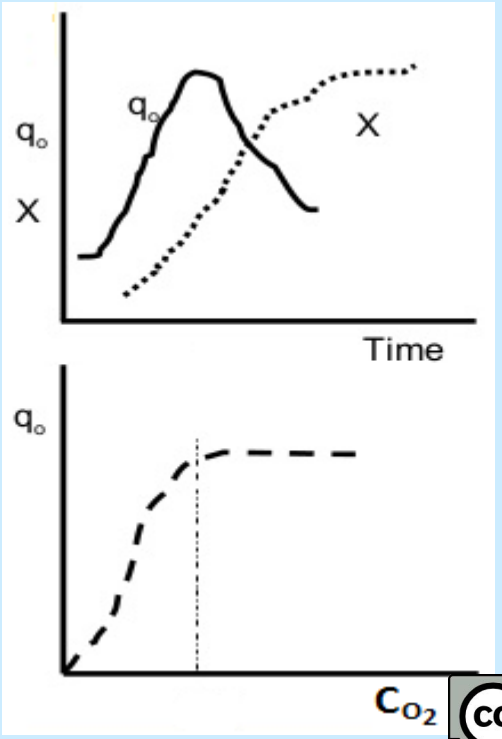
m_E values are in range 0.5-3.0 (mmol ATP/ g.h), extreme values can be up to 200 mmol ATP/g.h)

It is influenced by ionic strength, pH, T, DOT, medium composition etc.

m_O - indicates the amount of oxygen (mol) that is required to deliver to 1g of cells for maintenance purposes per hour

$$q_{O_2} = \frac{\mu}{Y_{X/O_2}} = \frac{\mu}{Y_{X/O_2}^{max}} + m_O$$

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



Stoichiometry of biomass growth

Application for design of medium composition, estimation of oxygen consumption, estimation of yield etc.

$$C_wH_xO_yN_z + aO_2 + bH_gO_hN_i \longrightarrow cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O$$

1 mol substrate — Biomass

C:	w		= c	+	d
H:	x+	bg =	$c\alpha$	+	e
O_2:	y + 2a +	bh =	$c\beta$	+ 2d +	e
N_2:	z+	bi =	$c\delta$		

Four equations with 5 unknown variables

$$RQ = \frac{vol.CO_2}{vol.O_2} = \frac{d}{a}$$

Respiratory quotient [volume (mol) of produced CO_2/ volume (mol) of consumed O_2]

Microorganism	*Limiting Nutrient*	$\mu(hr^{-1})$	*% C*	*% H*	*% N*	*% O*	*% P*	*% S*	*% ash*	*Empirical chemical formula*	*Formula molecular weight*
Bacteria			53.0	7.3	12.0	19.0			8	$CH_{1.66}N_{0.20}O_{0.27}$	20.7
Bacteria			47.1	7.8	13.7	31.3				$CH_2N_{0.25}O_{0.5}$	25.5
E. aerogenes			48.7	7.3	13.9	21.1			8.9	$CH_{1.78}N_{0.24}O_{0.33}$	22.5
K. aerogenes	glycerol	0.1	50.6	7.3	13.0	29.0				$CH_{1.74}N_{0.22}O_{0.43}$	23.7
K. aerogenes	glycerol	0.85	50.1	7.3	14.0	28.7				$CH_{1.73}N_{0.24}O_{0.43}$	24.0
Yeast			47.0	6.5	7.5	31.0			8	$CH_{1.66}N_{0.13}O_{0.40}$	23.5
Yeast			50.3	7.4	8.8	33.5				$CH_{1.75}N_{0.15}O_{0.5}$	23.9
Yeast			44.7	6.2	8.5	31.2	1.08	0.6		$CH_{1.64}N_{0.16}O_{0.52}P_{0.01}S_{0.005}$	26.9
C. utilis	glucose	0.08	50.0	7.6	11.1	31.3				$CH_{1.826}N_{0.19}O_{0.47}$	24.0
C. utilis	glucose	0.45	46.9	7.2	10.9	35.0				$CH_{1.84}N_{0.20}O_{0.56}$	25.6
C. utilis	ethanol	0.06	50.3	7.7	11.0	30.8				$CH_{1.82}N_{0.19}O_{0.46}$	23.9
C. utilis	ethanol	0.43	47.2	7.3	11.0	34.6				$CH_{1.84}N_{0.20}O_{0.55}$	25.5

Elemental composition of selected microorganisms, in percent by weight. [From B. Atkinson and F. Mavintuna, Biochemical Engineering and Biotechnology Handbook, Nature Press, (1983)].

Literature

F. Kaštánek: Bioinženýrství, Academia, 2001

G. Najafpour: Biochemical Engineering and Biotechnology, Elsevier, 2006

Department of Biotechnology

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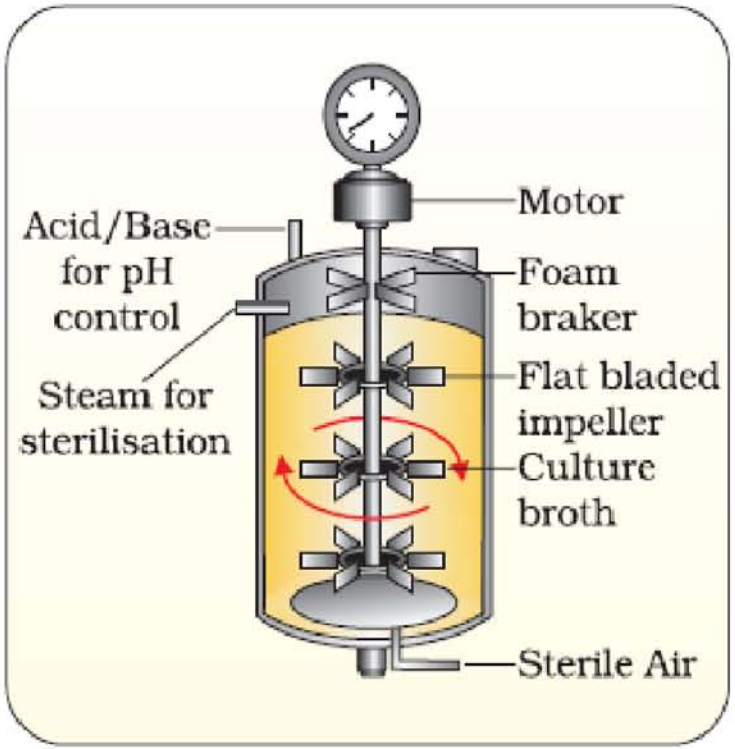
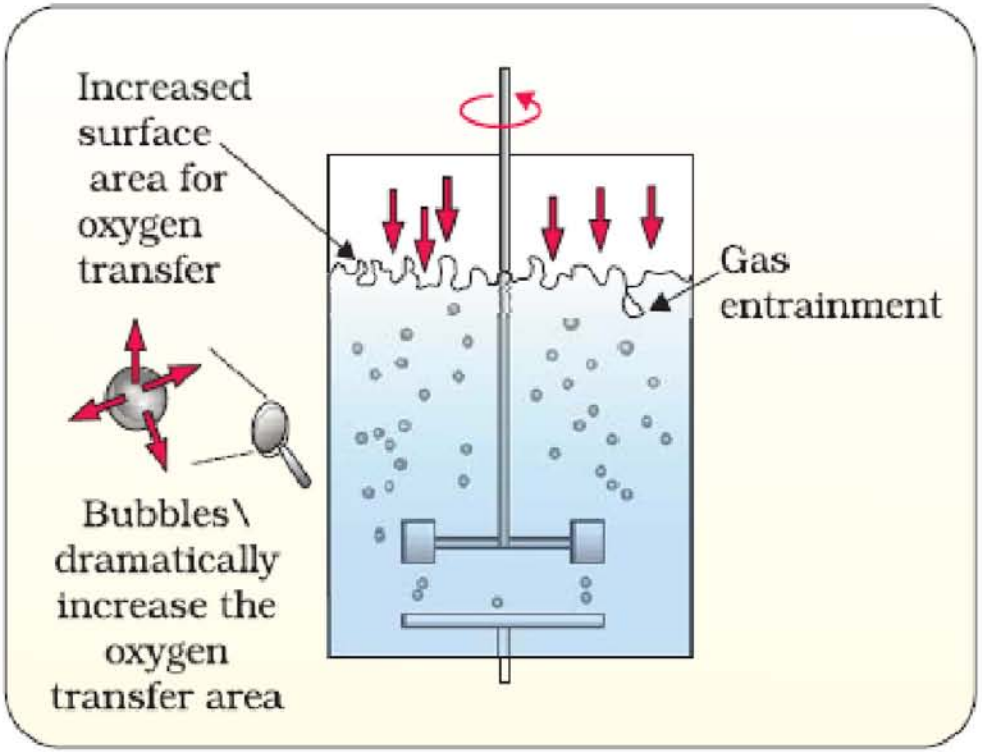
MINISTRY OF EDUCATION,
YOUTH AND SPORTS

Microbial cultivation - batch, fed-batch and continuous

Scheme of the mixed tank bioreactor



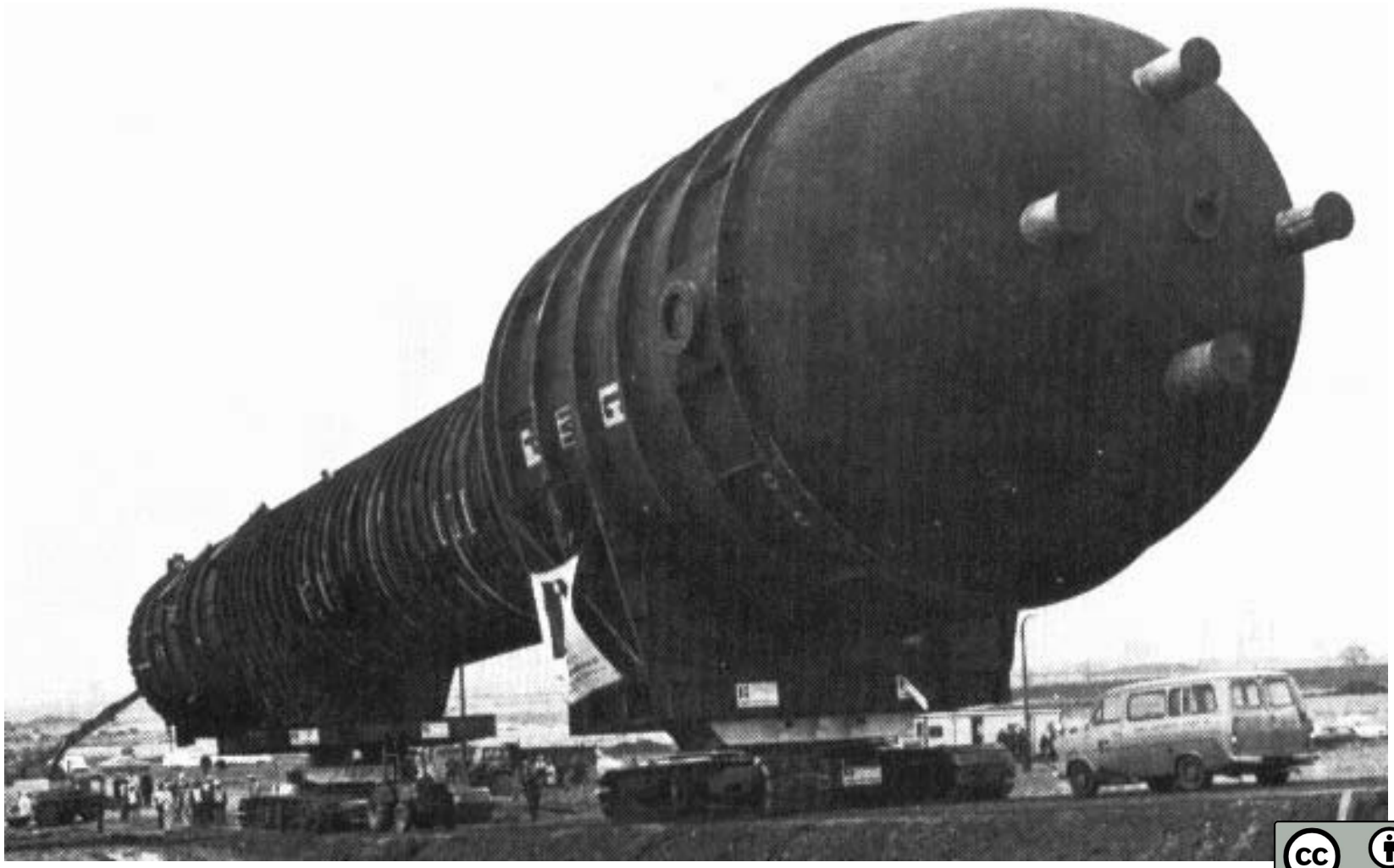


Mixed tank bioreactors

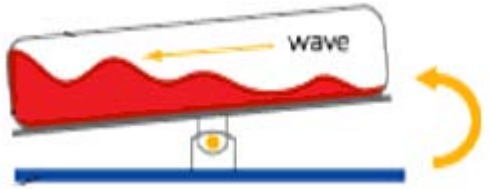
Pneumatically mixed reactors

Laboratory –scale air-lift reactor (with external loop) for mammalian cells cultivation

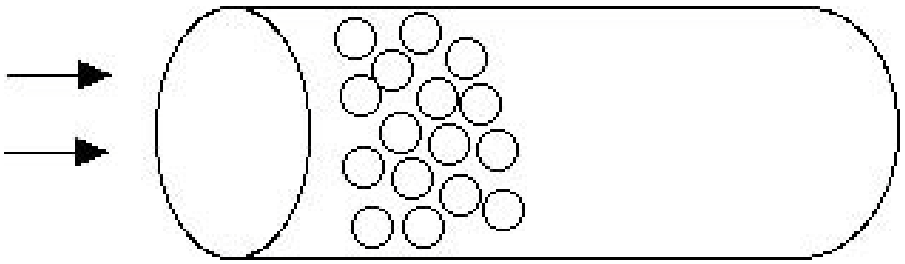
Air-lift fermenter

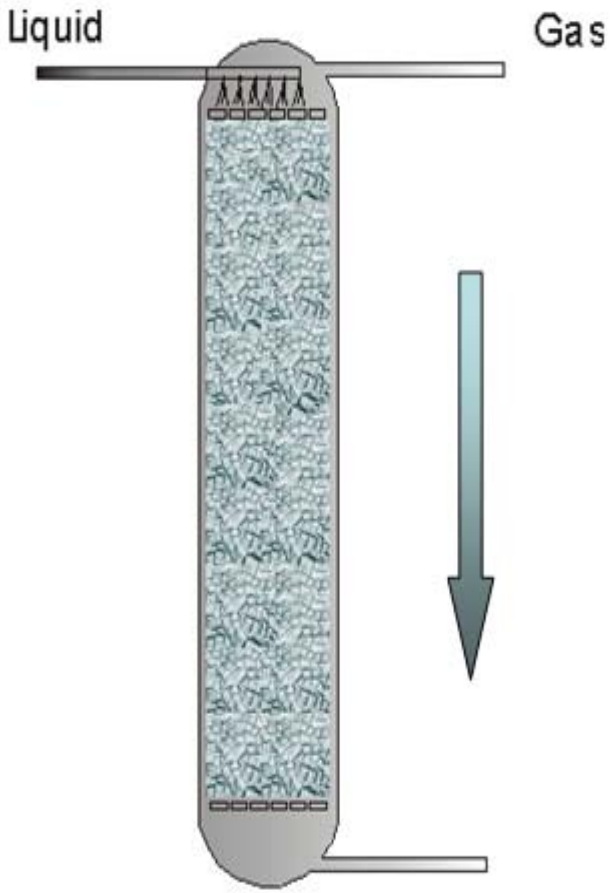


Bioreactors with rocking motion



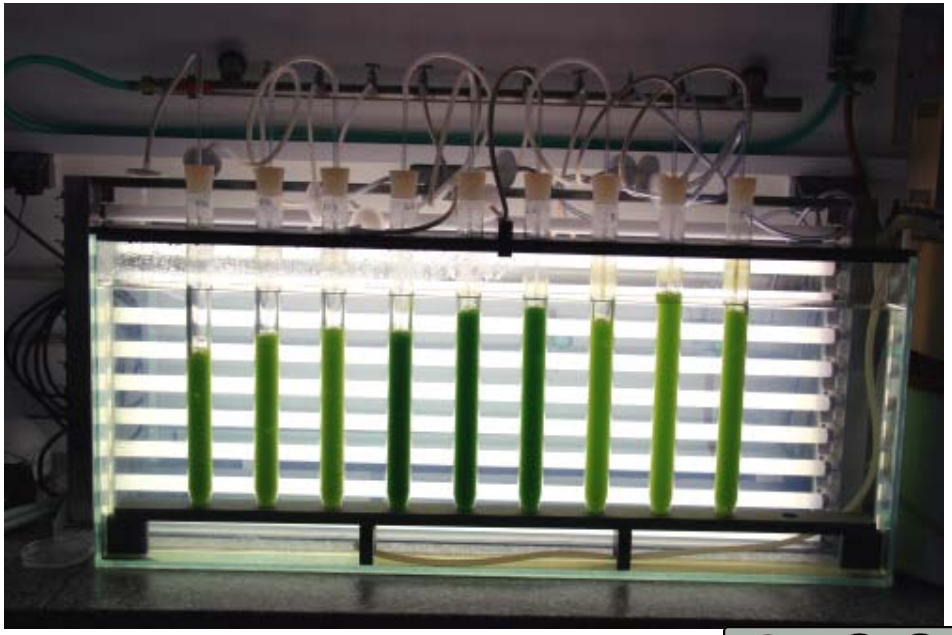
Bioreactors with immobilized enzymes and cells





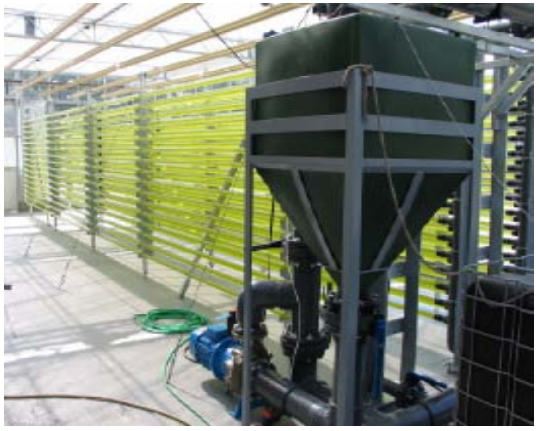
Scheme of the horizontal and vertical bioreactor with immobilized enzymes

Photobioreactors - experimental

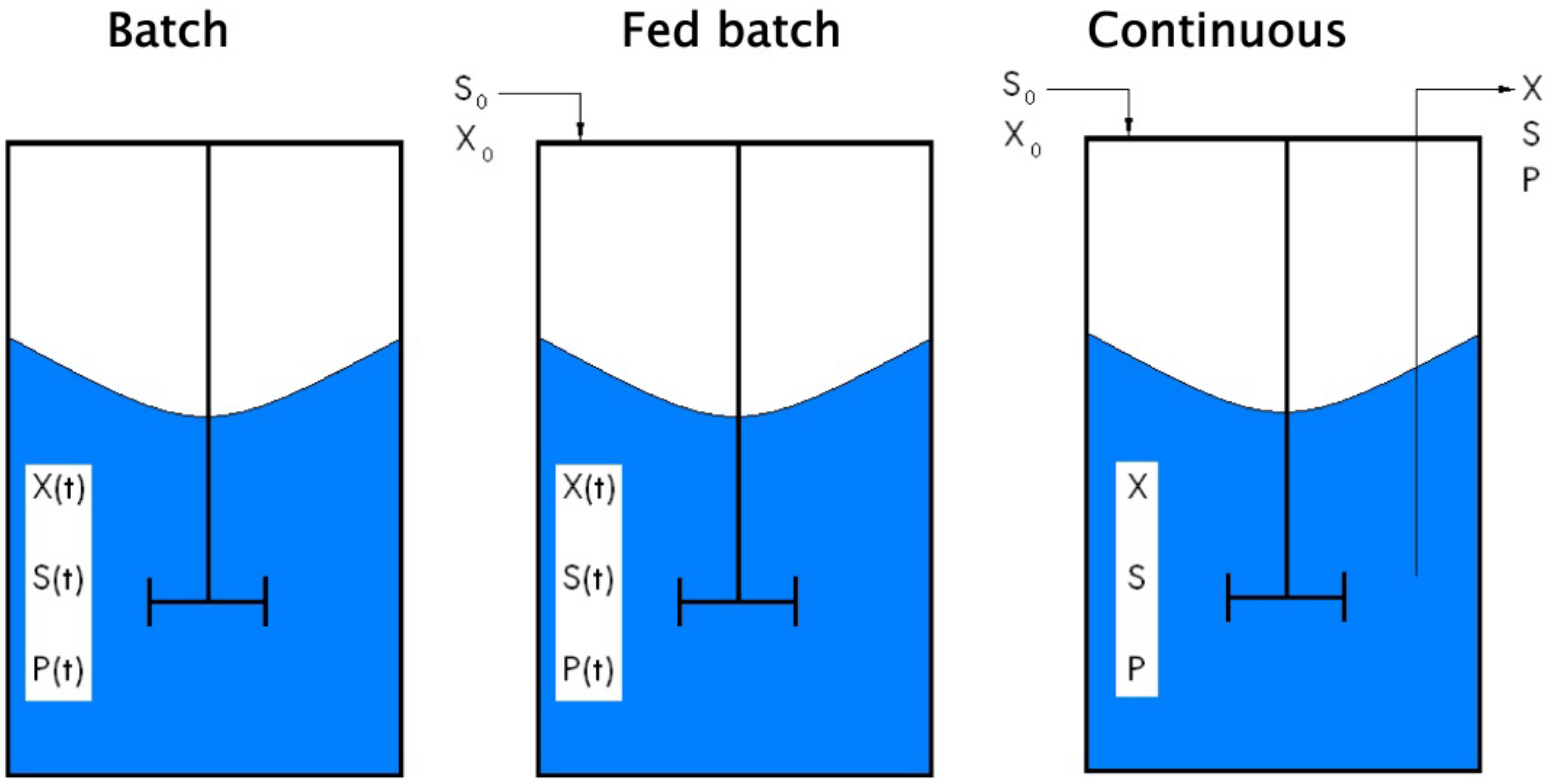


Research photobioreactors,
EcoFuel Labs.

Tubular photobioreactors

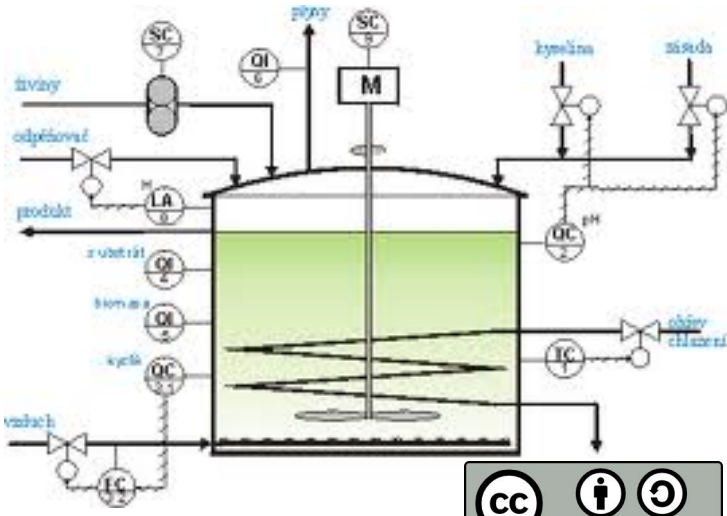


Microbial fermentation
batch, continuous and fed-batch

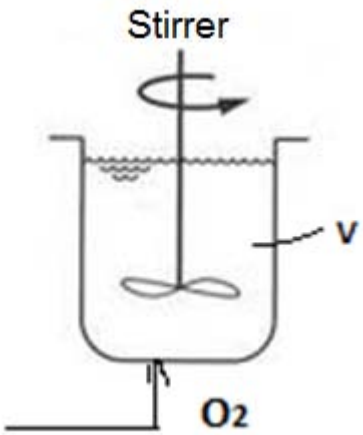


Batch fermenter

- The fermentation broth, the inoculum (aeration is on) is inserted into the reactor and, after reaching the desired parameters, the reactor is emptied
- Advantages:
  - Easy sterilization of medium and reactor
  - Limitation of dangerous mutations
  - Easy change of reaction conditions for individual batches
  - Possible separation of individual phases of growth
  - Low consumption of media and inoculum
  - Zero concentration of extracellular products at the beginning
- Disadvantages:
  - Time losses between indiviual batches
  - Time losses due to repeated lag phases at each batch
  - Low productivity
  - Loads by cyclic washing and sterilizations
  - Not suitable in case of substrate and product inhibition



1) Batch fermentation



- **Simplifications and assumptions:**
  - Perfectly stirred reactor
  - Balance period from inoculation (0) till the end of cultivation
  - Negletion of water vaporisation, addition of acids and bases for pH regulation

- **Dry cells balance:**

INPUT + SOURCE (REACTION) = OUTPUT + ACUMULATION

- The rate of cell growth is proportional to the cell concentration
- Coefficient of proportionality is the specific growth rate μ (h^{-1}), $r = \mu X$

$$0 + \mu X = 0 + \frac{dX}{dt}$$

$$\frac{dX}{dt} = \mu X \quad (1)$$

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

- Initial conditions: $t = 0,\ X = X_0,\ s = s_0,\ p = 0$

$$\int_{X_0}^{X} \frac{dX}{X} = \int_0^t \mu dt$$

- But $\mu = f(s)$ a $s = f(t)$, therefore μ varies with time and integration would be complex
- In case of Monod kinetics $\mu = \mu_{max} \frac{s}{K_S + s}$, K_S (g/l) is saturation constant
- Simplifying condition (valid for long time of fermentation): $\boldsymbol{s \gg K_S}$; $\mu = \mu_{max}$

$$ln\left(\frac{X}{X_0}\right) = \mu_{max} t$$

$$\frac{X}{X_0} = e^{\mu_{max} t}$$

$$\boldsymbol{X = X_0 e^{\mu_{max} t}}$$

1) Batch fermentation

- **Substrate balance:**

INPUT = OUTPUT + LOAD OF REACTIONS + ACCUMULATION

- The substrate is consumed for cell formation, product formation and cell maintenance

$$0 = 0 + \frac{1}{Y_{X/S}}\frac{dx}{dt} + \frac{1}{Y_{P/S}}\frac{dp}{dt} + m_S\, x + \frac{ds}{dt}$$

$$-\frac{\boldsymbol{ds}}{\boldsymbol{dt}} = \frac{\mathbf{1}}{\boldsymbol{Y_{X/S}}}\frac{\boldsymbol{dx}}{\boldsymbol{dt}} + \frac{\mathbf{1}}{\boldsymbol{Y_{P/S}}}\frac{\boldsymbol{dp}}{\boldsymbol{dt}} + \boldsymbol{m_S\, x} \quad (\mathbf{2})$$

- $\frac{1}{Y_{X/S}}\frac{dx}{dt}$ expresses the amount of substrate (in grams per liter per hour) consumed for cell growth with a constant of proportionality $Y_{X/S}$, $m_S x$ expresses the amount of substrate associated with cell maintenance
- $Y_{X/S}$ is the yield coefficient (stoichiometric coefficient indicating how many g of cells are produced from 1 g substrate),
- m_S (h^{-1}) is the maintenance coefficient, indicates how many g of substrate is needed for 1 g of cells to survive per hour

Dimensional analysis:

$$-\frac{ds}{dt} = \frac{1}{Y_{X/S}}\frac{dx}{dt} + m_S x \qquad \left[\frac{g}{l.h}\right] = \left[\frac{1}{\frac{g}{g}}\right].\left[\frac{g}{l.h}\right] + \left[\frac{1}{h}\right].\left[\frac{g}{l}\right]$$

$Y_{X/S}$ expresses $\frac{g\ (cells)}{g\ (substrate)}$, that means $\frac{1}{Y_{X/S}}$ expresses $\frac{g\ (substrate)}{g\ (cells)}$, then dimensionally everything fits:

$$\frac{ds}{dt} \equiv \left[\frac{g\ (substrate)}{l.h}\right],$$

$$\frac{1}{Y_{X/S}}\frac{dx}{dt} \equiv \left[\frac{g\ (substrate)}{g\ (cells)}\right].\left[\frac{g\ (cells)}{l.h}\right]$$

$$m_S x \equiv \left[\frac{g\ (substrate)}{l.h}\right]$$

Solution – equation for the substrate

$$-\frac{ds}{dt} = \frac{1}{Y_{X/S}}\frac{dx}{dt} + m_S x$$

Complex numeric solution, S and X is changing with time. We are interested in analytical solution, so we introduce the approximation (simplification): we consider that for a long period of fermentation $K_S \ll s$ is valid. Therefore:

$$\frac{dx}{dt} = \mu_{max} x$$

And so: $-\frac{ds}{dt} = \frac{1}{Y_{X/S}}\mu_{max} x + m_S x = x\left(\frac{1}{Y_{X/S}}\mu_{max} + m_S\right)$

I have already resolved that when $K_S \ll s$, for $X = X_0 e^{\mu_{max} t}$

After replacement:

$$-\frac{ds}{dt} = X_0 e^{\mu_{max} t}\left(\frac{1}{Y_{X/S}}\mu_{max} + m_S\right)$$

So I solve the differential equation

$$-ds = X_0 e^{\mu_{max} t} \left(\frac{1}{Y_{X/S}} \mu_{max} + m_S \right) . dt$$

Where $X_0, \mu_{max}, Y_{X/S}, m_S$ are constants

Initial conditions: $t = 0$, $s = s_0$

After integration:

$$\boldsymbol{s = s_0 - \left(\frac{1}{Y_{X/S}} + \frac{m_S}{\mu_{max}} \right) . (X_0 e^{\mu_{max} t} - 1)}$$

It is the simplest approximate relationship, we do not consider cell death, inhibition.

Typical constant values:

- s_0~ 15-20 g/l
- μ_{max} ~ 0,3 h^{-1}
- m_S~0,03 h^{-1}
- $Y_{X/S}$~0,5
- t is given in hours

1) Batch fermentation

- **Product balance:**

INPUT + SOURCE (REACTION) = OUTPUT + ACCUMULATION

$$0 + r_P = 0 + \frac{dp}{dt}$$

Product is accumulating iin the reactor. For solving the equation, we need to introduce product-speeding patterns:

Associative model of product creation

The rate of formation of the product is proportional (coefficient of proportionality α) of the cell mass inkrement:

$$\boldsymbol{\frac{dp}{dt} = \alpha \frac{dX}{dt}}$$

It follows from equation (1) $\frac{dX}{dt} = \mu X$, resp. $\frac{dX}{dt}\frac{1}{X} = \mu$, therefore:

$$\frac{dp}{dt} = \alpha \mu X$$

$$\frac{dp}{dt}\frac{1}{X} = \alpha \mu = q$$

Where q (h^{-1}) is the specific production factor (indicates the amount of product produced per unit of time per unit mass of microorganisms).

1) Batch fermentation

Non-associative model

The rate of product formation is proportional (coefficient of proportionality β) the amount (concentration) of cells in the system:

$$\frac{dp}{dt} = \beta X$$

$$\frac{dp}{dt}\frac{1}{X} = \beta\ (konst)$$

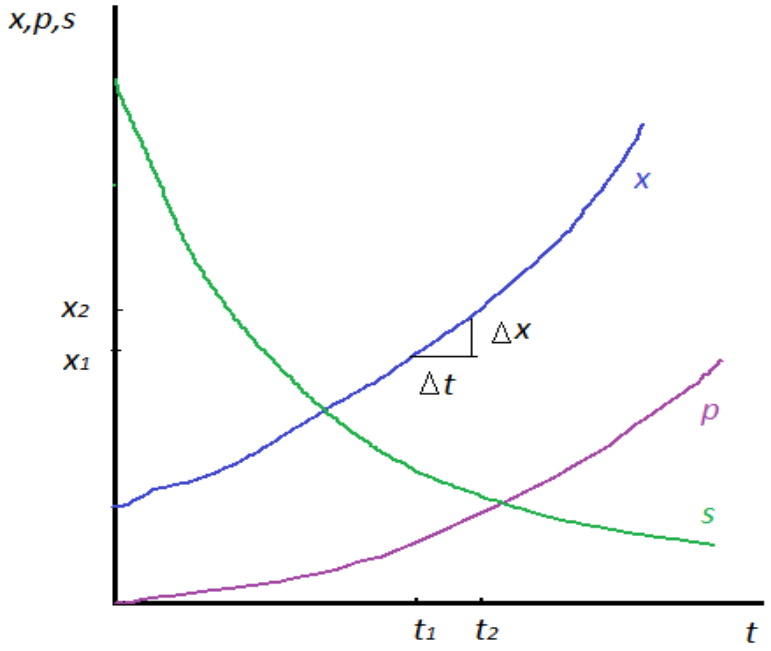
Combining both models:

$$\frac{dp}{dt} = \alpha\frac{dX}{dt} + \beta X\ \ (3)$$

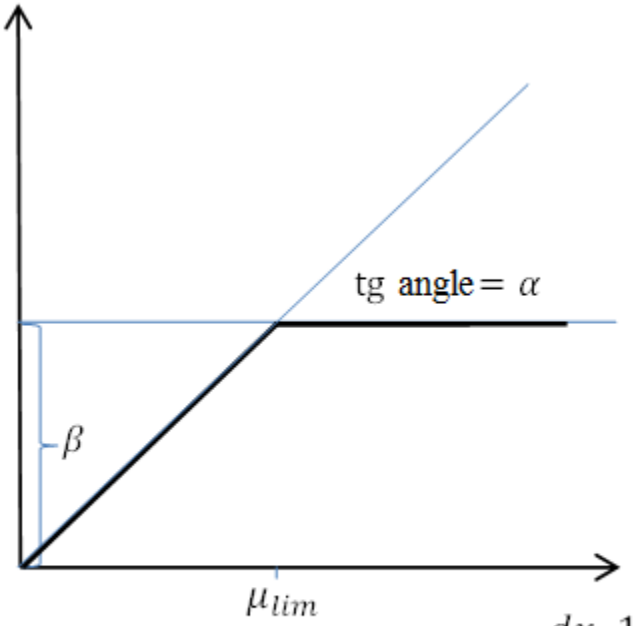
α, β are determined from the experimental measurement X, P in dependence on time t over the time segments t1, t2, etc.

Derivation is expressed as $\frac{\Delta x}{\Delta t}$ or $\frac{\Delta p}{\Delta t}$ respectively. For example at point t_1 from the chart: $\frac{dX}{dt} = \frac{t_2 - t_1}{x_2 - x_1}$ etc. and draw on the chart $\frac{dp}{dt}\frac{1}{X}$ against $\frac{dX}{dt}\frac{1}{X}$

α, β from experimental measurement



$$q \equiv \frac{dp}{dt} \cdot \frac{1}{x}$$



$$\mu = \frac{dx}{dt} \cdot \frac{1}{x}$$

1) Batch fermentation

- Conclusion: The batch fermentor is solved as a set of 3 differential equations for unknown ***X***, ***P*** and ***S*** (or 4 in the case of oxygen inclusion). All parameters are numerically enumerated, optimized or got out from experiments.

$$\frac{dX}{dt} = \mu X \quad (1)$$

$$-\frac{ds}{dt} = \frac{1}{Y_{X/S}}\frac{dx}{dt} + \frac{1}{Y_{P/S}}\frac{dp}{dt} + m_S\, x \quad (2)$$

$$\frac{dp}{dt} = \alpha\frac{dX}{dt} + \beta X \quad (3)$$

$$k_L a(O^* - O) = \frac{1}{Y_{X/O}}\frac{dx}{dt} + m_O x + \frac{1}{Y_{P/O}}\frac{dp}{dt} + \frac{dO}{dt} \quad (4)$$

Conclusion – balance of batch fermentor

- From literature – when the only product is a substrate the equation is sometimes given in the form

$$\frac{dS}{dt} = -\left(\frac{1}{Y_{X/S}}\mu + m_S\right)x$$

$$\frac{dx}{dt} = \mu x$$

$$\frac{dO}{dt} = k_L a(O^* - O) - \left(\frac{1}{Y_{X/O}}\mu + m_O\right)x$$

- In the equations, considering the numerous possibilities of microbial growth kinetics, specific growth rate $\boldsymbol{\mu}$ we put appropriate approximations and mostly solve numerically

Conclusion – balance of batch fermentor

- Examples of different models and typical values of their parameters

Model	Form of equation	Parameter values
Monod´s	$\mu = \frac{\mu_{max} s}{K_S + s}$	$\mu_{max} = 0{,}4\ h^{-1}$, $K_S = 0{,}05\ g.L^{-1}$, $Y_{X/S} = 0{,}4\ g.g^{-1}$ For **s** in g.L^{-1}
Substrate inhibition	$\mu = \frac{\mu_{max} s}{K_S + s + \left({}^{s^2}/_{K_1}\right)}$	$\mu_{max} = 0{,}53\ h^{-1}$, $K_S = 0{,}12\ wt.\%$, $K_1 = 2{,}2\ wt.\%$, $Y_{X/S} = 0{,}4\ g.g^{-1}$, $m = 0{,}01\ h^{-1}$ For **s** in wt. %
Substrate inhibition with variable $Y_{X/S}$	$\mu = \frac{\mu_{max} s(1 - as)}{K_S + s + \left({}^{s^2}/_{K_1}\right)}$	$\mu_{max} = 0{,}504\ h^{-1}$, $a = 0{,}204\ wt.\%$, $K_S = 8{,}5 \times 10^{-4}\ wt.\%$, $K_1 = 2{,}46\ wt.\%$
	$Y_{X/S} = \frac{Y_{X/_{S,O}}(1 - as)}{1 + bs + cs^2}$	$Y_{X/_{S,O}} = 0{,}383\ g.g^{-1}$, $b = 2{,}98\ wt.\%$, $c = -0{,}5\ wt.\%$ For **s** in wt. %

Conclusion – balance of batch fermentor

- For example – more general case involving substrate inhibition and two substrate kinetics (the second substrate is dissolved O_2)

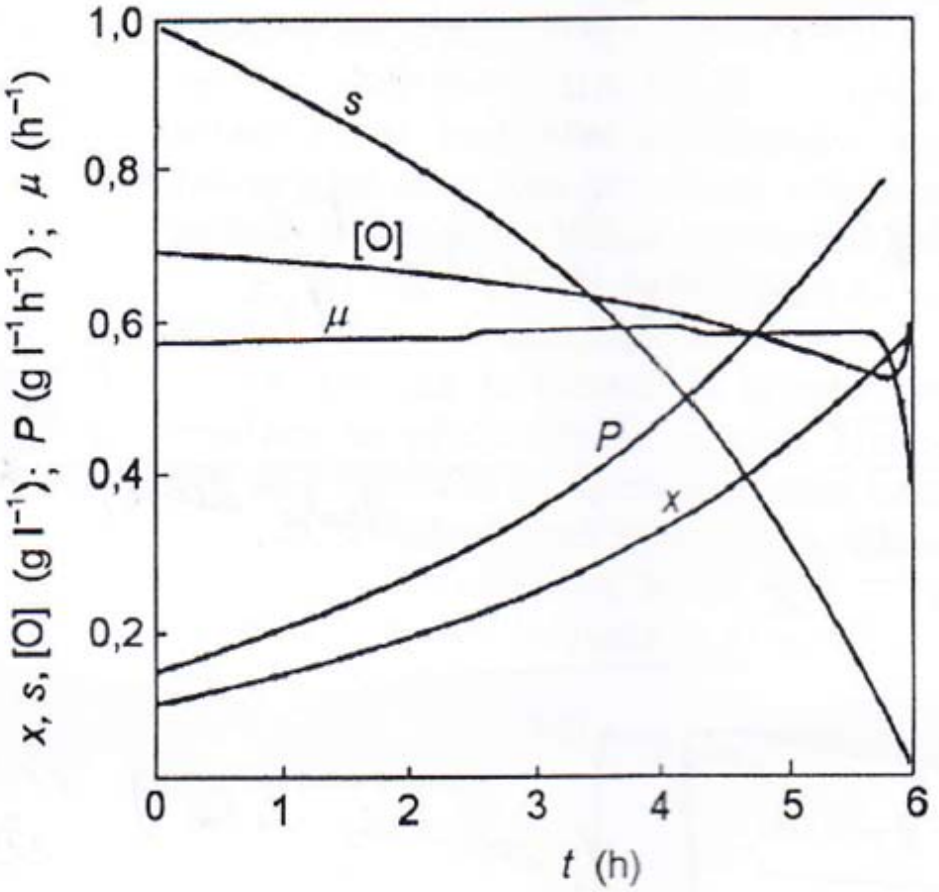
$$\frac{ds}{dt} = -\left(\frac{1}{Y_{X/S}}\frac{\mu_{max}s}{K_s + s + \frac{s^2}{K_I}}\frac{O}{K_O + O} + m_S\right)x$$

$$\frac{dx}{dt} = \left(\frac{\mu_{max}s}{K_s + s + \frac{s^2}{K_I}}\frac{O}{K_O + O}\right)x$$

$$\frac{dO}{dt} = k_L a(O^* - O) - \left(\frac{1}{Y_{X/O}}\frac{\mu_{max}s}{K_s + s + \frac{s^2}{K_I}}\frac{O}{K_O + O} + m_O\right)x$$

- In case when $K_I \to \infty, K_O \to 0$ the system switches to Monod's kinetics. Otherwise, this simple set already contains 7 parameters, the determination of which is very difficult.

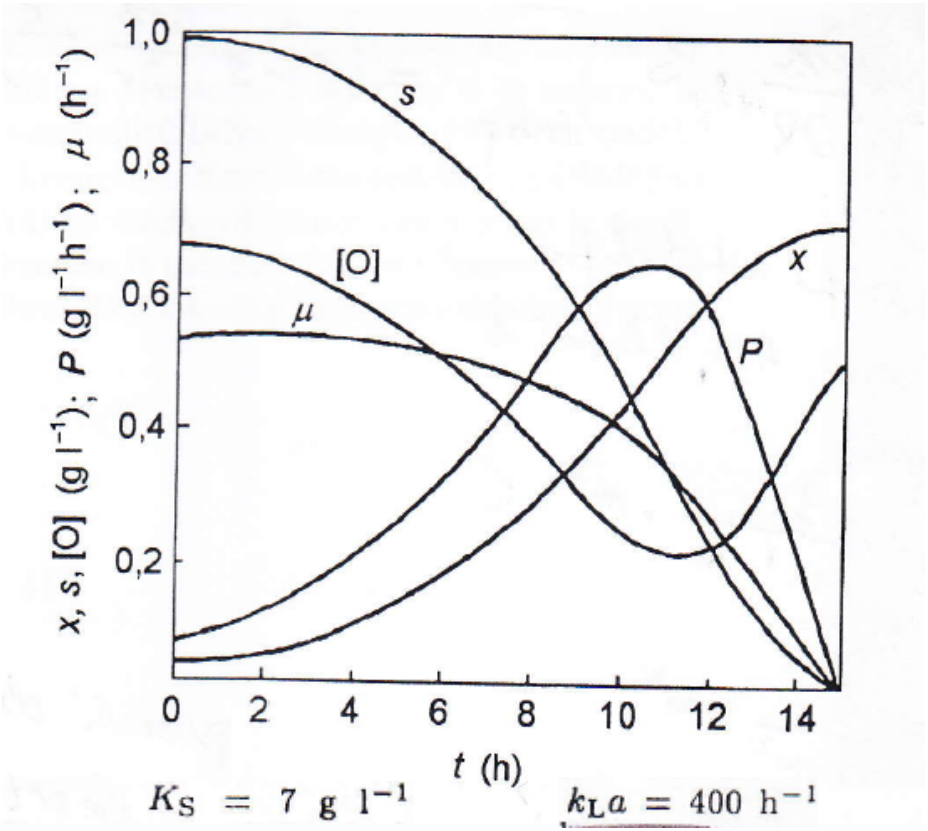
The result of numerical simulation with Monod´s kinetics without substrate inhibition and intensive aeration

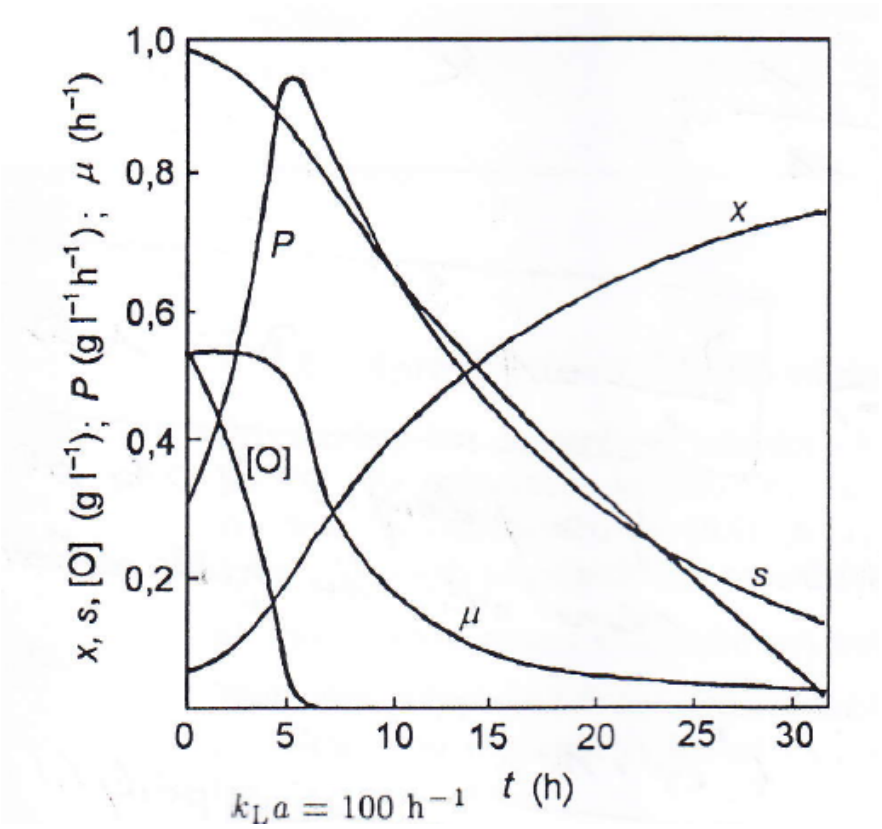


Just before depletion of the substrate there is a decrease in the specific growth rate ***μ***. The oxygen concentration is almost constant throughout the fermentation time and does not affect the growth rate.

Concentration profiles of simulated batch fermentation $s_0 = 10\ \text{g l}^{-1}$, $x_0 = 1\ \text{g l}^{-1}$, $K_S = 0{,}02\ \text{g l}^{-1}$, $K_O = 0{,}1 \cdot 10^{-3}\ \text{g l}^{-1}$, $\mu_{max} = 0{,}3\ \text{h}^{-1}$, $Y_{X/O} = 0{,}886$, $Y_{X/S} = 0{,}5$, $m_S = 0{,}03\ \text{h}^{-1}$, $m_O = 0{,}063\ \text{h}^{-1}$, $k_La = 1000\ \text{h}^{-1}$, $s_{max} = 10\ \text{g l}^{-1}$, $x_{max} = 10\ \text{g l}^{-1}$, $[O]_{max} = 0{,}01\ \text{g l}^{-1}$, $P_{max} = 2\ \text{g l}^{-1}\ \text{h}^{-1}$

Monod´s kinetics, strong inhibition (high K_S) for different intesities of aeration (high or low value of $k_L a$)





Growth is inhibited by inhibition; for the concentration of biomass, the existence of an inflection point is typical.

Lack of aeration → low $k_L a$ → low concentration of dissolved oxygen and its rapid exhaustion → a rapid decrease of the specific growth rate ***μ***

General mass balance in transient state

INPUT + SOURCE = OUTPUT + ACCUMULATION

Resp.

INPUT = OUTPUT + CONSUMPTION + ACCUMULATION

Into the reactor

output from the reactor

cell growth, product creation, and preservation of functions

Oxygen:

INPUT: $k_L aO^*$

OUTPUT: $k_L aO$

CONSUMPTION: $\frac{1}{Y_{X/O}}\frac{dx}{dt} + m_O + \frac{1}{Y_{P/O}}\frac{dp}{dt}$

ACCUMULATION: $\frac{dO}{dt}$

Substrate:

INPUT: 0

OUTPUT: 0

CONSUMPTION: $\frac{1}{Y_{X/S}}\frac{dx}{dt} + m_S x \left(\text{ev.} + \frac{1}{Y_{P/S}}\frac{dp}{dt}\right)$

ACCUMULATION: $-\frac{ds}{dt}$ (decrease of substrate)

Cells:

INPUT: 0
SOURCE: μx
OUTPUT: 0

ACCUMULATION: $\frac{dx}{dt}$

Example

- Determine the yeast and glucose (dry matter) concentration after 5h fermentation in the batch fermentor if:

- Input glucose concentration is 10 $g.L^{-1}$
- Inoculum 1 $g.L^{-1}$
- K_S = 0,02 $g.h^{-1}$
- μ_{max} = 0,3 h^{-1}
- $Y_{X/S}$ = 0,5
- m_S = 0,03 h^{-1}

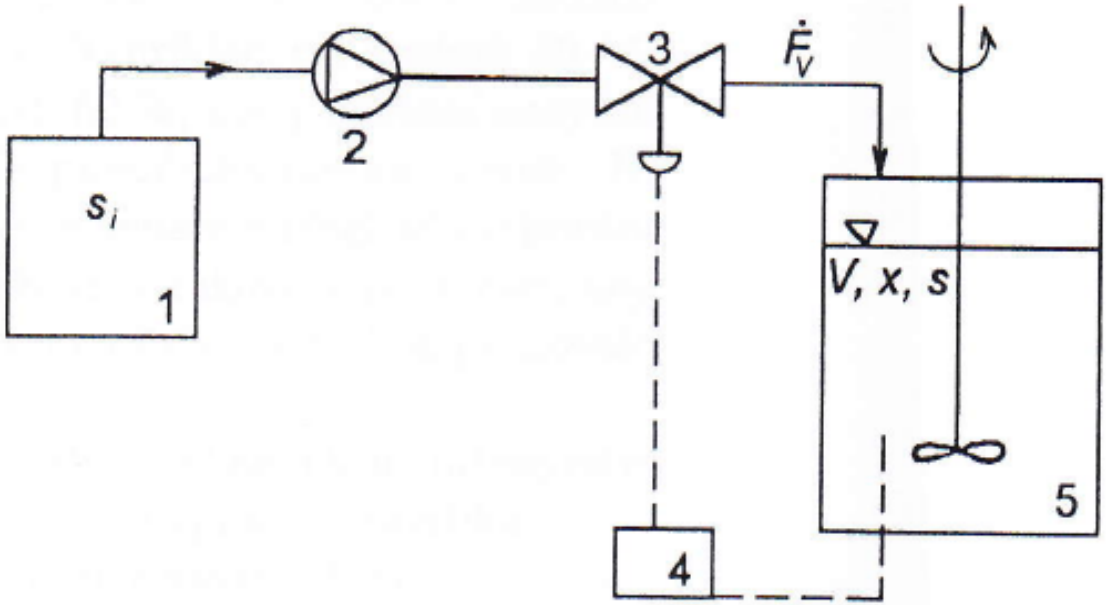
- Assumption – $S >> K_S$

$$X = X_0 e^{\mu_{max} t} = 1e^{0,3.5} = \mathbf{4,48\ g}$$

$$s = s_0 - \left(\frac{1}{Y_{X/S}} + \frac{m_S}{\mu_{max}}\right).(X_0 e^{\mu_{max} t} - 1) = 10 - \left(\frac{1}{0,5} + \frac{0,03}{0,3}\right).(1e^{0,3.5} - 1) = \mathbf{2,7\ g}$$

3) Fed-batch fermentation

- A fermentation technique where one or more nutrient components are dosed into the fermenter during cultivation and the product remains in the fermenter until the end of the operation
- The nutrients can be dosed intermittently (pulsed) or continuously (maintaining a constant substrate level or changing the substrate concentration according to the chosen algorithm – e.g. exponential growth)
- The nutrient intake is mostly controlled on the basis of the signal of the composition of the fermentation mixture in the reactor



1 – nutrient reservoir
2 – pump
3 – control valve
4 – detector and control unit
5 – reactor

3) Fed-batch fermentation

- Advantage – controlled nutrient concentration can affect the yield or productivity of the desired product/metabolite

- Suitable for:

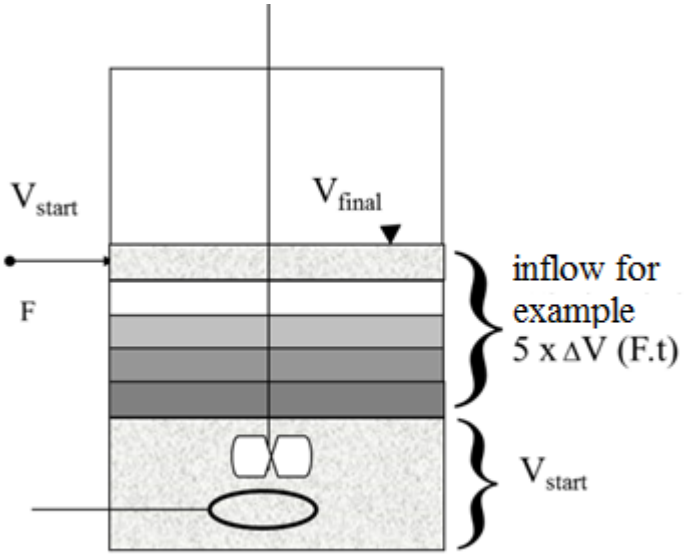
    - Substrate inhibition
    - High final cell concentration – requires high substrate concentration but has inhibition effect
    - Optimizing of metabolite production – selected "starvation" level of the cells, with maximum production of metabolite
    - Prolonging the production period of the formation of secondary metabolites
    - Replacement of water lost by evaporation
    - Special cases – glucose effect, catabolic represion, etc.

3) Fed-batch fermentation

One-time culture with a gradual feeding, so called fed-batch is characterized:

- Starts as a batch cultivation but only with a partially filled reactor (e.g. ¼ of total volume)
- Concentration of extracellular products is zero at the beginning

- **Media feed speed**
  - The goal is to ensure optimal nutrition. High concentration of substrate causes inhibition, on the contrary, low concentration causes limitation
  - Constant
  - Exponencial
    - increasing the feed rate of the medium with the time of cultivation to ensure exponential growth
    - the inflow value is calculated for $\mu=\mu_{max}$ (not only exponential but also maximum growth)
  - Variable (regulated)
    - the media feed value based on current process requirements

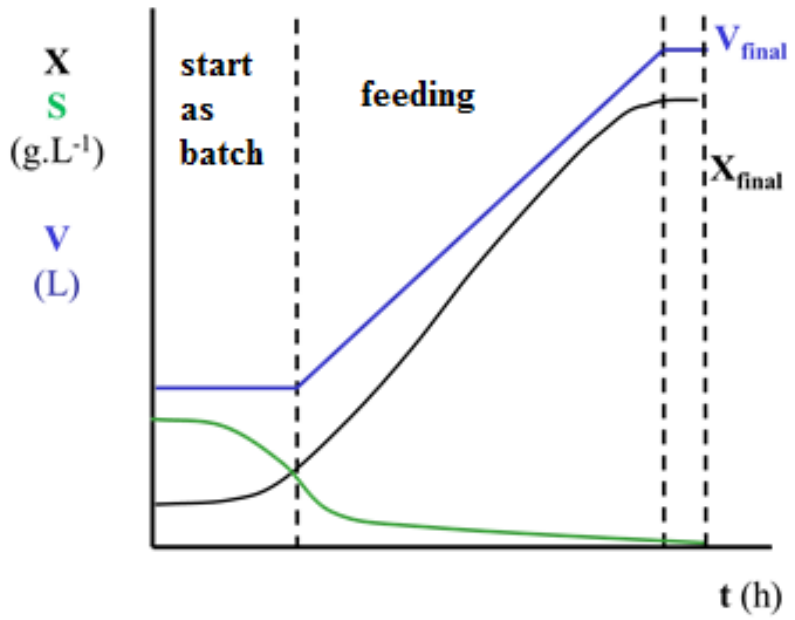


(See M. Halecký: Basics of Bioengineering)

3) Fed-batch fermentation

- ***Constant feed of media***
  - The simplest design
  - Gradual continuous increase of bioreactor volume → dilution of the produced metabolite
  - Suitable for cultivations where the product concentration has a negative effect on metabolic activity

  However, there are disanvantages
  - The least efficient feed of the media ($\mu<\mu_{max}$)
  - Does not respond to the current state of the cells
  - The consumption of the substrate increases with increasing cell concentration, but the delivery is constant



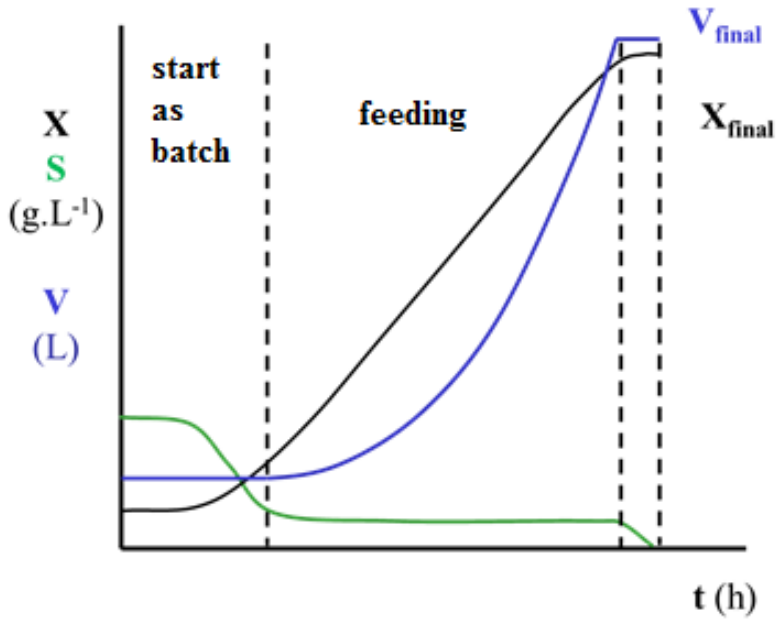
(See M. Halecký: Basics of Bioengineering)

3) Fed-batch fermentation

- ***Exponential feed of media***
  - the feed of the limiting substrate increases proportionally to the rate of exponential growth
  - Constant **μ**, constant **S**, exponential feed
  - The ability to maintain a high growth rate for a long time
  - obtaining the maximum amount of cells in the shortest time in systems with substrate inhibition

Disadvantages:

- does not respond to the current state and needs of cells – it is based on <u>assumed</u> cell behavior

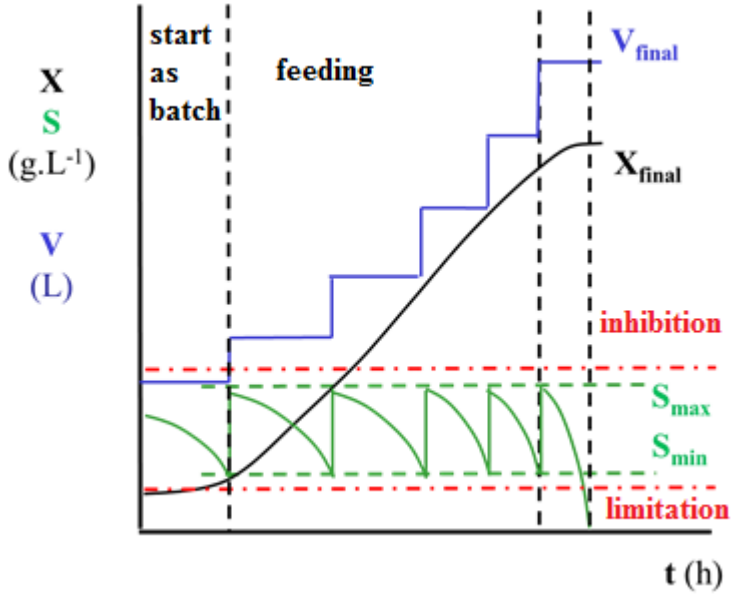


(See M. Halecký: Basics of Bioengineering)

3) Fed-batch fermentation

Variable speed of media feed

- Media feed changes during cultivation as required by microorganisms
- The goal is to optimize the process – growth rate, metabolite yield, process productivity based on actual and current (not calculated or predicted) microorganism requirements
- Use for special products – enzymes, antibiotics, aminoacids, recombinant proteins



Disadvantages:

- Costly regulation (investment and operational)

It is necessary to select a suitable variable for measurement and use for subsequent flow control

- Direct determination of substrate concentration
- Indirect determination – parameters closely related to cell growth and metabolism, and optimally easy and on-line measurable (CO_2, gas measurement, dissolved oxygen measurement, oxygen measurement in gasses, pH measurement

(See M. Halecký: Basics of Bioengineering)

3) Fed-batch fermentation

Cell balance in the Fed-batch system

Batch

$\frac{dx}{dt} = \mu X$ (accumulation of cells)

Continuous

$\mu XV = FX$ (what grows in volume, it leaks)

resp. in volume **V**: $\mu XV = \frac{dx}{dt}V$

Fed-batch – the volume is not constant, but it increases → the accumulation in increasing volume is equal to what grows in the increasing volume (observe derivative of the compound function):

$$\frac{d(VX)}{dt} = \mu XV$$

$$\frac{dV}{dt}X + \frac{dX}{dt}V = \mu XV$$

3) Fed-batch fermentation

Volume balance

- the increase in volume is equal to the flow times the change of time

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

→ into equation of cell balance

$$FX + \frac{dX}{dt}V = \mu XV$$

$$\boldsymbol{\frac{dX}{dt} = \left(\mu - \frac{F}{V}\right)X = (\mu - D)X} \quad (7)$$

In the continuous process, the **dilution speed D=F/V** is constant, in fed-batch **V** is changing → **D** is not constant

3) Fed-batch fermentation

Substrate balance during continuous feeding

The amount of substrate flowing in ($g.h^{-1}$) of concentration s_i (initial) is equal to the consumption for cell growth, cell retention and accumulation

$$Fs_i = \frac{1}{Y_{X/S}} \frac{d(VX)}{dt} + m_S XV + \frac{d(Vs)}{dt}$$

Derivations:

$$Fs_i = \frac{1}{Y_{X/S}} \left(\frac{dV}{dt} X + \frac{dX}{dt} V \right) + m_S XV + \frac{dV}{dt} s + \frac{ds}{dt} V$$

3) Fed-batch fermentation

$$Fs_i = \frac{1}{Y_{X/S}}\left(\frac{dV}{dt}X + \frac{dX}{dt}V\right) + m_S XV + \frac{dV}{dt}s + \frac{ds}{dt}V$$

From volume and cell balances $\frac{dV}{dt} = F$ and $\frac{dX}{dt} = (\mu - D)X$, divide ***V*** and set ***F/V=D***

$$Ds_i = \frac{1}{Y_{X/S}}(DX + (\mu - D)X) + m_S X + Ds + \frac{ds}{dt}$$

$$D(s_i - s) = \frac{1}{Y_{X/S}}\mu X + m_S X + \frac{ds}{dt}$$

$$\boldsymbol{\frac{ds}{dt} = D(s_i - s) - \left(\frac{\mu}{Y_{X/S}} + m_S\right)X} \quad (8)$$

3) Fed-batch fermentation

I deal with a set of differential equations 7, 8 and 9, where **μ** is from a suitable kinetic equation

$$\frac{dX}{dt} = \left(\mu - \frac{F}{V}\right)X = (\mu - D)X \quad (7)$$

$$\frac{ds}{dt} = D(s_i - s) - \left(\frac{\mu}{Y_{X/S}} + m_S\right)X \quad (8)$$

$$\frac{dO}{dt} = k_L a(O^* - O) + D(O_0 - O) - \left(\frac{\mu}{Y_{X/O}} + m_O\right)X \quad (9)$$

3) Fed-batch fermentation

- Approximative solution for Monod´s kinetics and $\boldsymbol{s} \gg \boldsymbol{K_S}$: $\mu = \mu_{max}$

Cell concentration solution

$$\frac{dX}{dt} = \left(\mu_{max} - \frac{F}{V}\right) X$$

$$\frac{dX}{x} = \mu_{max} dt - \frac{F}{V} dt$$

Assumption – the volume ***V*** at time ***t*** will equal the volume at the beginning V_0 + what flows in during the time ***t*** with the volume flow ***F***

$$V = V_0 + Ft$$

Integration from 0 to ***t***

$$\int_{X_0}^{X} \frac{dX}{x} = \mu_{max} \int_0^t dt - \int_0^t \frac{F}{V_0 + Ft} dt \quad (10)$$

3) Fed-batch fermentation

$$\int_{X_0}^{X} \frac{dX}{x} = \mu_{max} \int_0^t dt - \int_0^t \frac{F}{V_0 + Ft} dt \quad (10)$$

Substitution $V_0 + Ft = u \rightarrow$ derivation $Fdt = du \rightarrow$ set into the integral

$$\int \frac{F}{V_0 + Ft} dt = \int \frac{F}{u} \frac{du}{F} = ln(u) = ln(V_0 + Ft)$$

$$\int_0^t \frac{F}{V_0 + Ft} dt = [ln(V_0 + Ft)]_0^t = ln \frac{V_0 + Ft}{V_0}$$

Set back into (10) and after integration

$$ln \frac{X}{X_0} = \mu_{max} t - ln \frac{V_0 + Ft}{V_0}$$

3) Fed-batch fermentation

$$ln\frac{X}{X_0} = \mu_{max}t - ln\frac{V_0 + Ft}{V_0}$$

$$ln\frac{X}{X_0} + ln\frac{V_0 + Ft}{V_0} = \mu_{max}t$$

$$ln\left(\frac{X}{X_0}.\frac{V_0 + Ft}{V_0}\right) = \mu_{max}t$$

$$\boldsymbol{X = \frac{X_0 V_0}{V_0 + Ft} e^{\mu_{max} t}} \quad \boldsymbol{(11)}$$

3) Fed-batch fermentation

Solution of substrate concentration – approximative balance

„The amount of substrate in grams that flows into the systém in time ***t*** is partially utilized for biomass formation and partly remains in the reactor“

(Product creation and cell maintenance is not cosidered)

$$\boldsymbol{F s_i t = \frac{1}{Y_{X/S}} (XV - X_0 V_0) + sV - s_0 V_0} \quad \boldsymbol{(12)}$$

$sV - s_0 V_0$ – amount of substrate (g) that remains in reactor (sV total amount of substrate minus $s_0 V_0$ amout of substate at the beginning)

$XV - X_0 V_0$ – actual amount of cells (g) minus amount of cells at the beginning

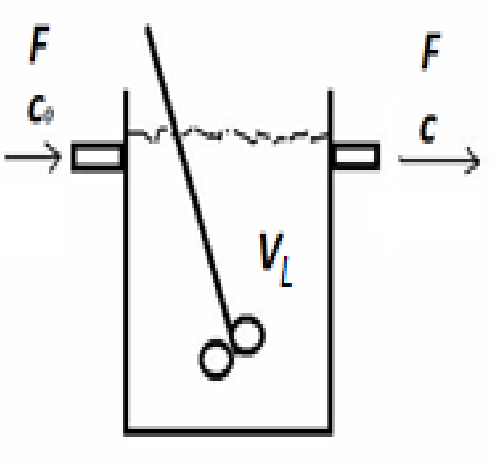
In equation (12) ***X*** is replaced from relation (11) and get ***s***.

The oxygen balance solution is very complex, numerical, so in practice we prefer, if possible, to vigorously aerate so the oxygen is not limiting.

2) Continuous fermentation

Advantages

- Eliminates idle times for repeated filling, emptying, cleaning and sterilization of equipment
- The number of cells and the product composition can be influenced by choosing the average residence time of the liquid in the reactor (i.e., maintaining the selected flow, the product has a constant composition)
- The process is suitable for the production of biomass and primary metabolites, but is not suitable for the production of secondary metabolites (which usually require different conditions for the growth and production phases)



Most industrial continuous fermenters approach a model of ideally stirred reactor where individual "particles" of liquid in the reactor have different "ages", theoretically from 0 to ∞. The average age of the particles then provides a mean delay time given by the ratio of the volume of liquid in the reactor V_L to its volume flow F.
Due to the fact that some microorganisms may remain in the reactor for longer time than the average residence time, uncontrolled growth of pathogenic microorganisms or mutations in the production strain may occur. Growth can also negatively affect the presence of improperly mixed reactor portions.

2) Continuous fermentation

Dry cells balance

INPUT = OUTPUT + LOSS OF REACTION + ACCUMULATION

- The accumulation is zero in the flow system in the stationary state
- In the balance, I assume that the system is already running (i.e., initially grow the sufficient cells in batch fermentation, then the controlled flow continuously adds the substrate)

$$0 = xF - \mu x V_L + 0$$

Cells in the reactor grow with speed $r = \mu x$, i.e., „loss of reactions“ in the volume of liquid in the reactor V_L is $-\mu x V_L \left[\frac{1}{h}\frac{g}{l}l = \frac{g}{h}\right]$ and flow out $xF \left[\frac{g}{l}\frac{l}{h} = \frac{g}{h}\right]$

$$\rightarrow \quad \mu x V_L = xF$$

2) Continuous fermentation

The reversed value of the mean delay time is the so-called **dilution speed**

$$D = \frac{F}{V_L}\ (h^{-1})$$

$$\mu x = xD$$

$$\boldsymbol{\mu = D}$$

But also $\boldsymbol{\mu \leq \mu_{max}}$ → the dilution velocity can not be higher than μ_{max} value since the cells would not grow enough and would be leaking from the reactor

There is a critical dilution speed at which microorganisms are already washed out.

Always applies $\boldsymbol{D_{krit} < \mu_{max}}$

For „wash out“ or „cell leaching“ from the system applies:

If $D = D_{krit}$ then $s = s_0,\ x = 0$ and for D_{krit} applies

$$\boldsymbol{D_{krit} = \mu_{max} \frac{s_0}{K_S + s_0}}$$

If $K_S \ll s_0 \rightarrow D_{krit}$ and μ_{max} are almost equal

2) Continuous fermentation

Substrate balance

INPUT = OUTPUT + LOSS OF REACTION + ACCUMULATION

The substrate is consumed for cell formation and cell maintenance (or for product formation), accumulation is zero in the flow system

$$Fs_0 = Fs + \frac{\mathbf{1}}{\boldsymbol{Y_{X/S}}}\mu X V_L + m_S X V_L \left(+\frac{\mathbf{1}}{\boldsymbol{Y_{P/S}}}\beta x V_L\right) + 0$$

$$\left[\frac{l}{h}\frac{g}{l} = \frac{l}{h}\frac{g}{l} + \frac{1}{h}\frac{g}{l}l + \frac{1}{h}\frac{g}{l}l\,(+\frac{1}{h}\frac{g}{l}l)\right]$$

I do not consider product formation, /:F

$$\boldsymbol{Ds_0 = Ds + \frac{1}{Y_{X/S}}\mu X + m_S X} \quad (5)$$

2) Continuous fermentation

I need to calculate the concentration on the reactor output.

From cell balance: $\mu = D$

$$X = \frac{s_0 - s}{\frac{1}{Y_{X/S}} + \frac{m_S}{D}}$$

Therefore Monod´s kinetics can be modified

$$\mu = \mu_{max} \frac{s}{K_s + s} = D \qquad (6)$$

$$s = \frac{DK_s}{\mu_{max} - D}$$

Assuming an ideal mixer, so the concentration inside the reactor is equal to the concentration at the outlet ***S***

!!! The concentration at the outlet does not depend on the input concentration at all, but on the dilution speeds.

2) Continuous fermentation

Calculation

- Calculate D_{crit}
- Choose $D < D_{crit}$
- Knowing kinetics ($K_s, Y_{x/s}, \mu_{max}$) – resp. approximate the values found from the batch experiment and choose the substrate concentration at the input, from equation (6) calculate ***S***
- From equation (5) calculate cell amount on the output ***X***

In continuous fermentation, I mostly want to produce as many cells as possible

Cell productivity $P_X = X.D \left[\frac{g}{l}\frac{1}{h}\right]$ – amount of cells formed in 1 hour in 1 liter of reactor

High ***D*** → low ***S***, low ***X***

Low ***D*** → lot of time for cell growth but small flow → few cells will actually go out

Goal: high cell productivity, ie optimize function *X.D=fce (D)*

2) Continuous fermentation

Practical ways to operate (manage) continuous fermentation:

- Chemostat – the flow of the substrate (and nutrients) into the reactor is constant
- Turbidistat – the input parameters (usually the dilution rate) are controlled according to the biomass concentration, which is kept constant
- Nutristat – the concentration of the leaving substrate is kept constant

Literature

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G. Najafpour: Biochemical Engineering and Biotechnology, Elsevier, 2006

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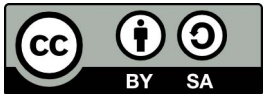

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Liquid flow characteristic - residence time distribution, flow models

Flow characteristics

- The nature of the flow affects either the required reactor size or the concentration of the component at the reactor outlet, the cell growth, the formation of intermediates, etc.

- Conversion:

$$X = \frac{c_0 - c}{c_0}$$

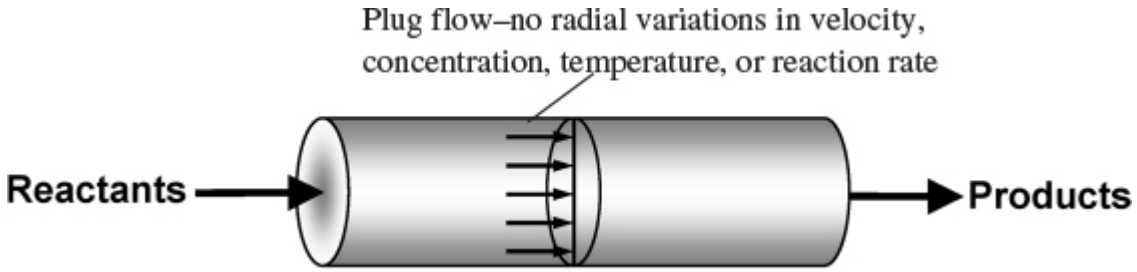
Where c_0 is the concentration at the reactor inflow, c concentration at reactor outflow.

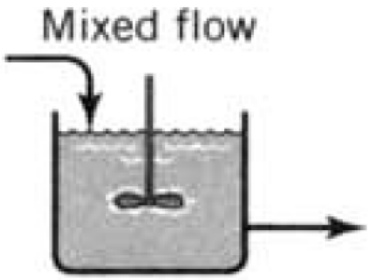
If $c = c_0$ => $X = 0$ (no reaction)
If $c = 0$ => $X = 1$ (100% reaction)

- Assessing the nature of the flow by the method of monitoring the age of the labeled "particles" of the fluid, whereby a small volume of fluid is considered to be a particle. Molecules do not leave or enter the particles, ie the particle acts as a miniature batch reactor.
- We can not label cells as particles directly because they multiply. We will assume that cells move at the same speed as the surrounding medium. Cells can grow in the "particle", but they do not leave it during their stay in the reactor.

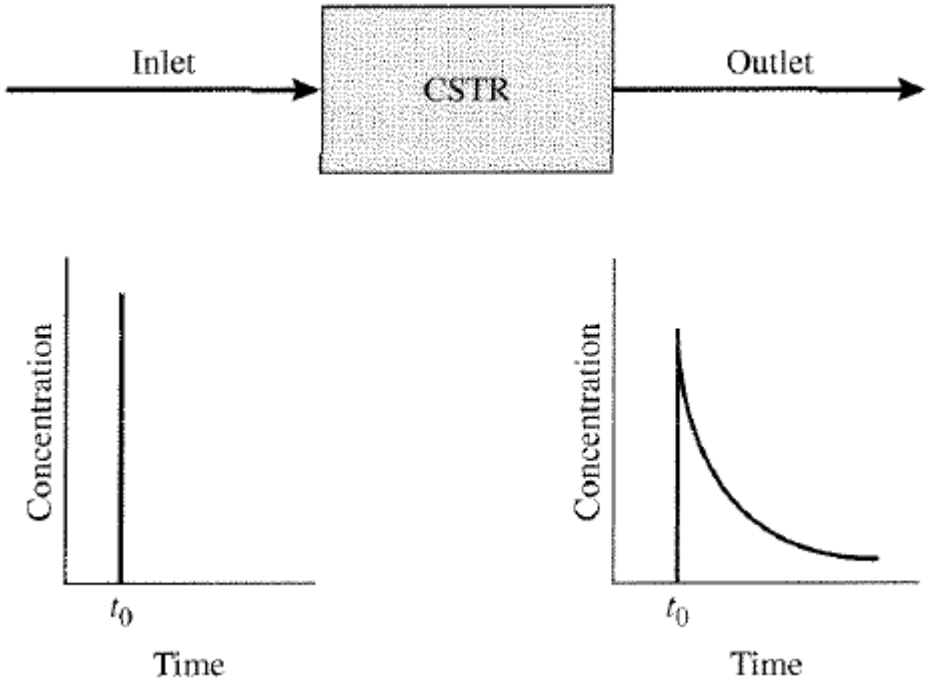
Flow characteristics

- The nature of the flow is determined experimentally by a tracer substance (eg color) to enter the reactor and monitor the response (dye concentration) at the outlet.
- After a short time, the detector on the output records the fraction of the least aged part, ie the shortest delay time, most of the particles with the age around the mean delay time, then it will decrease again.
- The age distribution of the particles will be in line with the hydrodynamic conditions in the reactor
- There are 2 extreme types of flow: ideally stirred flow and plug flow

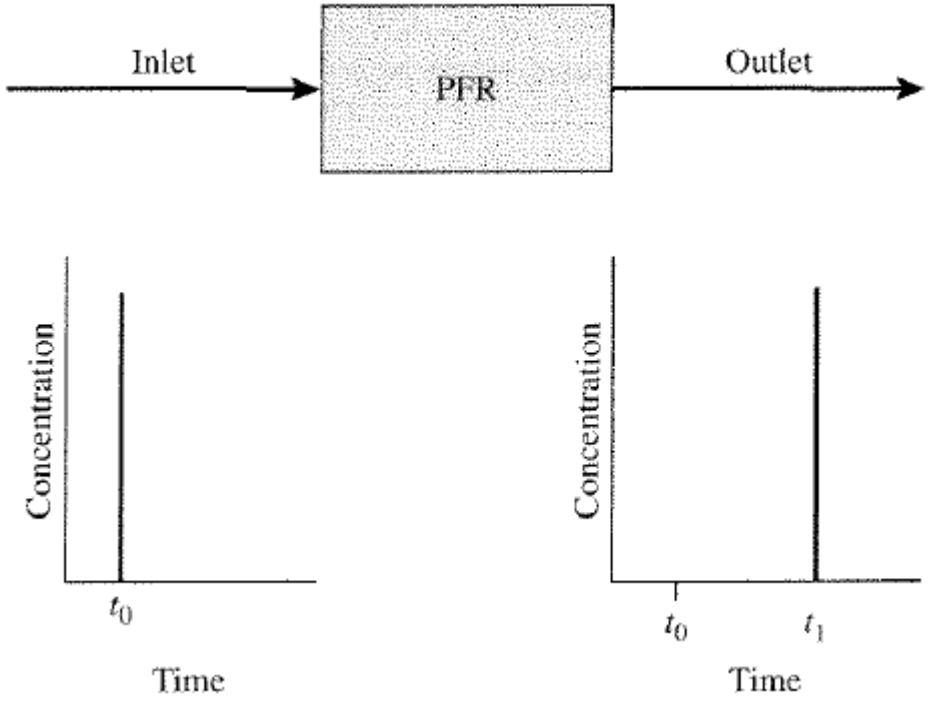




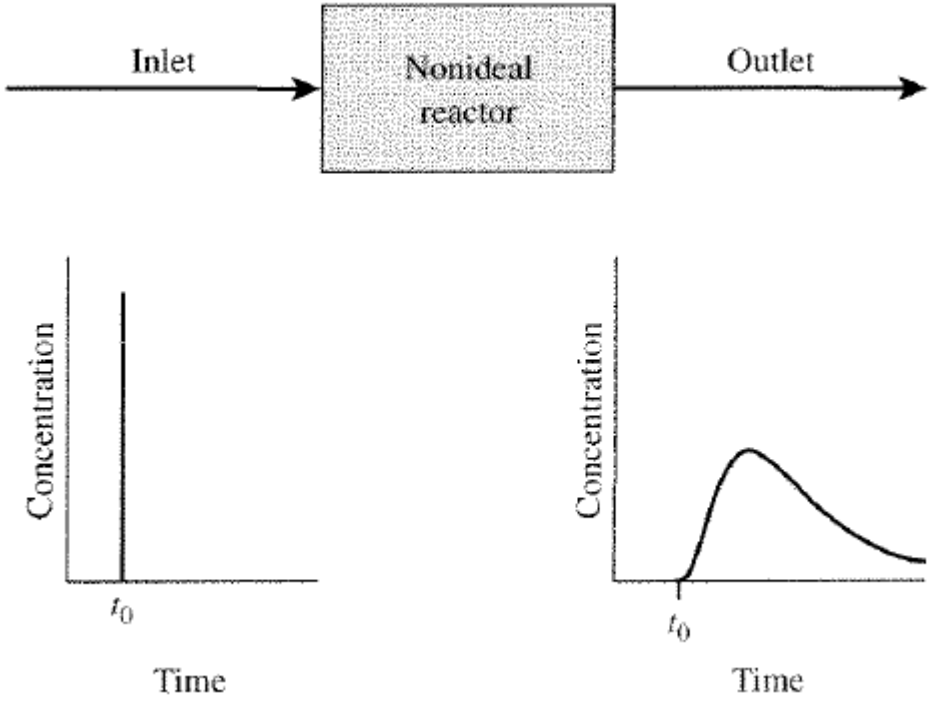
Continuous stirred tank reactor



Plug flow reactor



Nonideal reactor



Residence time distribution

We will observe the age of the parts that run out of the system. The average age of the particles will be equal to the mean residence time in the reactor. The mean residence time of the fluid in the reactor can be expressed as the volume of fluid in the reactor V divided by the volumetric flow of the fluid F in this reactor:

$$\bar{t} = \frac{V}{\dot{\mathrm{F}}_v} \qquad \text{(h)}$$

It is useful to define so-called dimensionless time as a fraction of actual time and mean residence time:

$$\Theta = \frac{t}{\bar{t}}$$

We measure the age from the fluid inlet into the system. We define the so-called E-particle distribution in such a way that (E d Θ) is the fraction of the fluid particles in the output stream, their age lies in the range of Θ to (Θ + d Θ). Since the sum of all parts of the fluid particles must be equal to one, it is valid:

$$\int_0^\infty E \, d\,\Theta = 1$$

Residence time distribution

Deviations from the ideal flow in the plants arise due to short-circuits, recycling, or the formation of stagnation zones within the plant. In all types of equipment (reactor, exchanger, charge absorbers, strippers, etc.), this type of imperfect flow is a reduction in power.

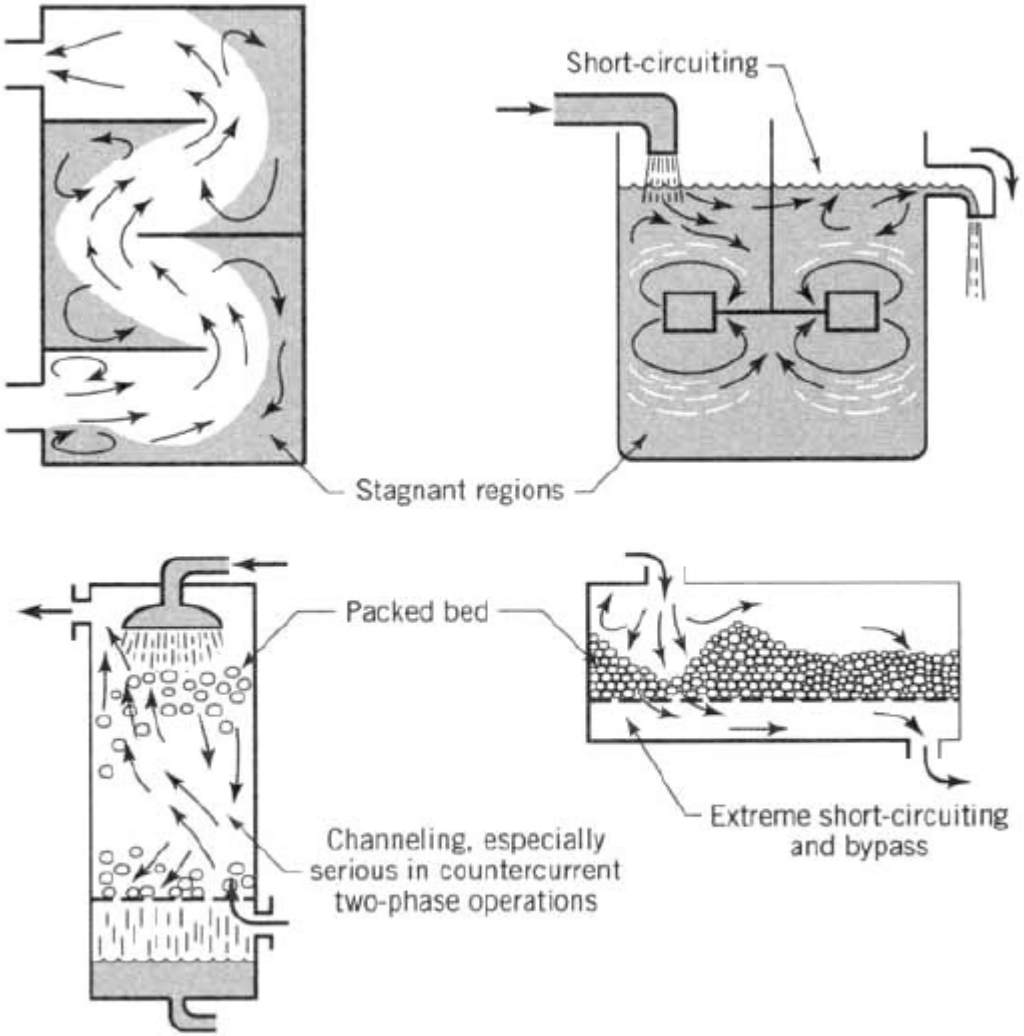


Figure 11.1 Nonideal flow patterns which may exist in process equipment.

E-distribution of fluid

Particles entering the device will drive the different paths to the moment of output from the device, and therefore spend a considerable amount of time on it. The distribution of these times is called E-particle age distribution (E has unit 1 / time). A preferred method of recording this particle age distribution is such that the area under the E-curve is unitary. The condition is that each particle enters and exits the reactor only once:

$$\int_0^\infty E\,dt = 1$$

Fraction of particles younger than t1
(spends less than t1 in the device)

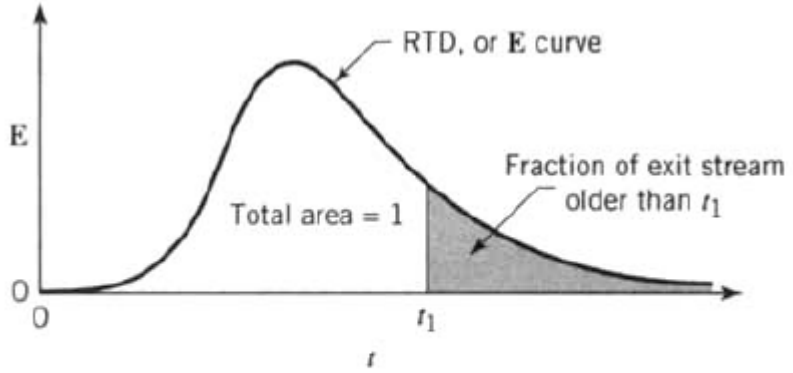


Figure 11.6 The exit age distribution curve **E** for fluid flowing through a vessel; also called the residence time distribution, or RTD.

Experimental methods for finding E

The easiest way to find the E curve is to trigger a signal (noise) at the input and to monitor the response at the output.

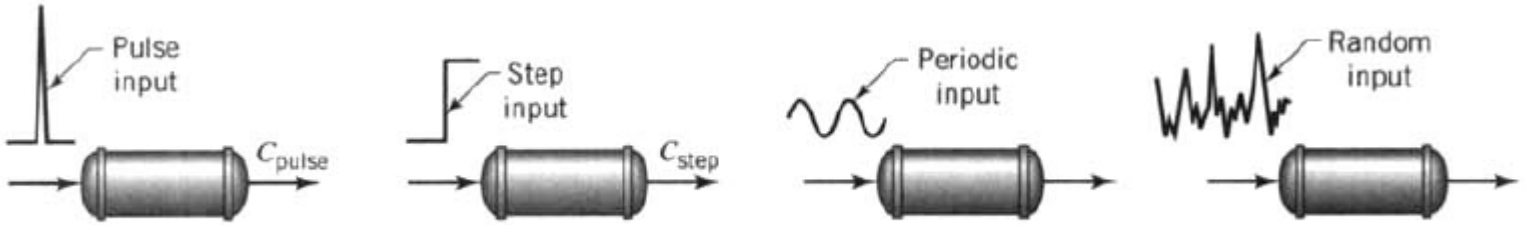


Figure 11.7 Various ways of studying the flow pattern in vessels.

Signal can be triggered by: coloured compounds, pH change, salt (conductivity), heat, radioactive tracer etc.

The pulze experiment

From the response curve record (Figure 4) information on the hydrodynamic behavior of the studied system can be obtained. The most important variables: V - volume of the reactor (m3), v - volume flow (m3 / s), M - tracer quantity (mol, kg), C (or Cpulse) concentration of tracer leaving the reactor, A-area under the Cpulse curve.

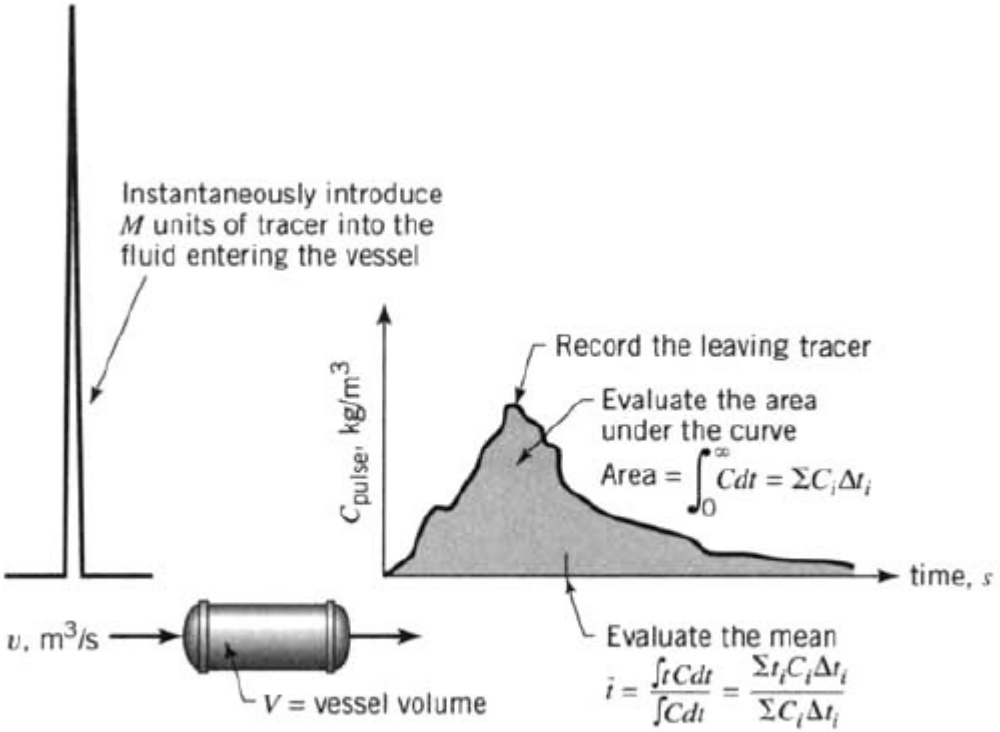


Figure 11.8 The useful information obtainable from the pulse trace experiment.

The pulze experiment

$$\left(\begin{matrix}\text{Area under the}\\ C_{\text{pulse}}\text{ curve}\end{matrix}\right): \quad \mathrm{A} = \int_0^\infty C\,dt \cong \sum_i C_i \Delta t_i = \frac{M}{v} \qquad \left[\frac{\text{kg}\cdot\text{s}}{\text{m}^3}\right]$$

$$\left(\begin{matrix}\text{Mean of the}\\ C_{\text{pulse}}\text{ curve}\end{matrix}\right): \qquad \bar{t} = \frac{\int_0^\infty tC\,dt}{\int_0^\infty C\,dt} \cong \frac{\sum_i t_i C_i \Delta t_i}{\sum_i C_i \Delta t_i} = \frac{V}{v} \quad [\text{s}]$$

Convert the Cpulse curve to E according to the following formula:

$$E = \frac{C_{\text{pulse}}}{M/v}$$

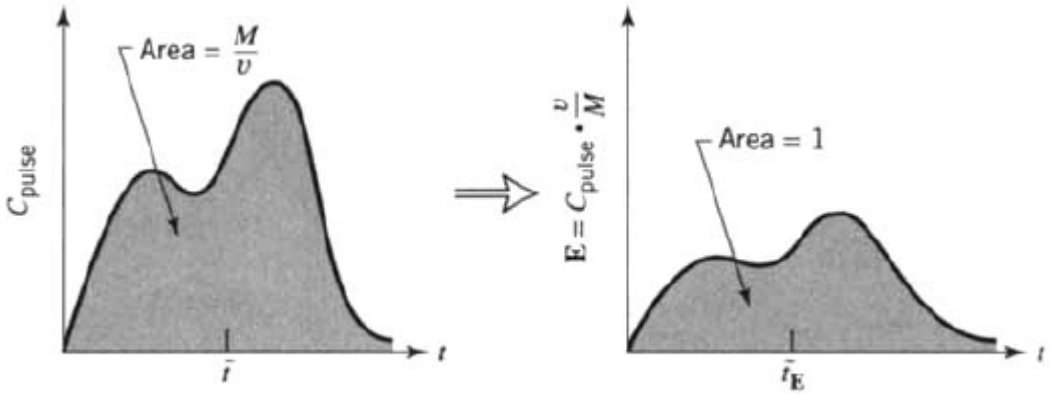


Figure 11.9 Transforming an experimental C_{pulse} curve into an **E** curve.

The pulze experiment

Another possibility to express the delay time distribution is the E_Θ curve, where instead of time, a dimensionless time Θ is used.

$$E_\Theta = \bar{t}E = \frac{V}{v} \cdot \frac{C_{pulse}}{M/v} = \frac{V}{M} C_{pulse}$$

The mean residence time can also be calculated as the area under the curve t * E (t) vs. t:

$$\langle t \rangle = \frac{\int_0^\infty \bar{t}E(\bar{t})d\bar{t}}{\int_0^\infty E(\bar{t})d\bar{t}} = \int_0^\infty \bar{t}E(\bar{t})d\bar{t}$$

Dispersion model

One of the models describing the ideal flow, or the deviation of the system from the ideal one, is the dispersion model. We always compare the response curve that we get from the model with the experimental response curve, and we look for consensus.

In the case of the ideal pulse signal injection and the dispersion of this injected liquid along the flow, the ideal impulse will "dissipate" over time. Observed quantities: u - flow rate (m / s), L - flow length (m), D - dispersion coefficient (m2 / s). Be careful not to confuse it with diffusion coefficient! Although, in the process of dispersion, the diffusion plays an important role.

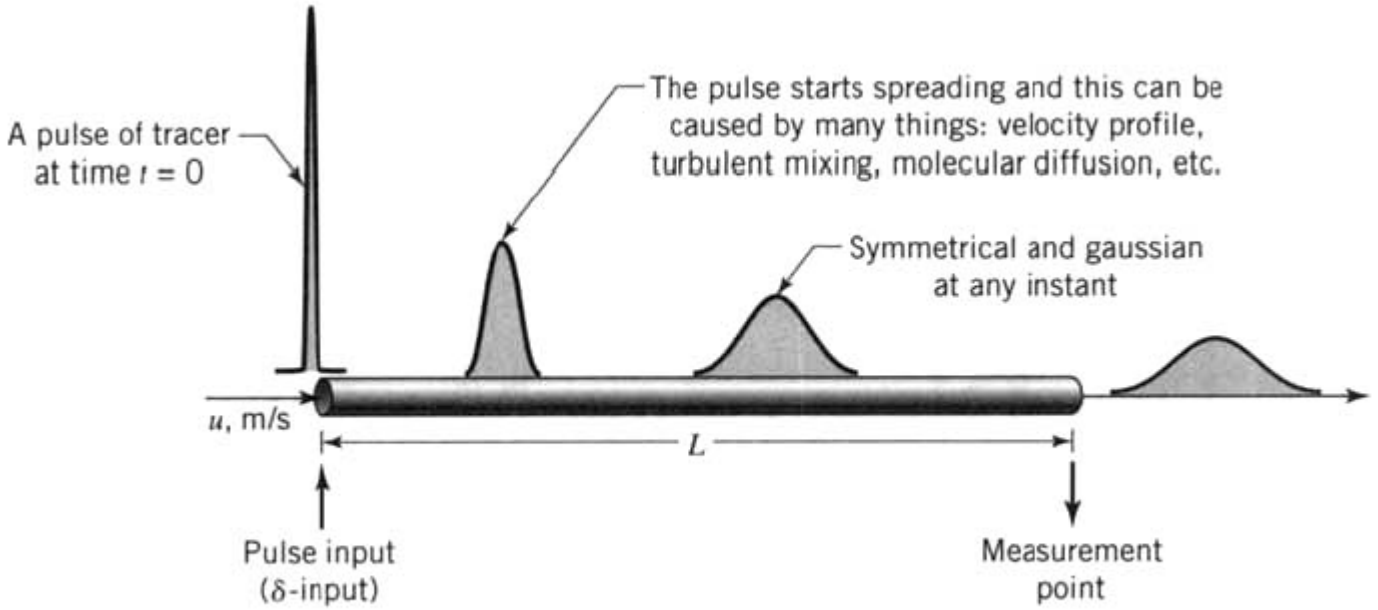


Figure 13.1 The spreading of tracer according to the dispersion model.

Dispersion model

The dispersion rate is characterized by the dimensionless D / uL criterion, which is 1 / Pe (Pe - Peckle number). An ideal piston flow and a flow with a dispersion at the leading edge. Of course, in reality there will be dispersions both on the front and the back edge of the hypothetically limited piston.

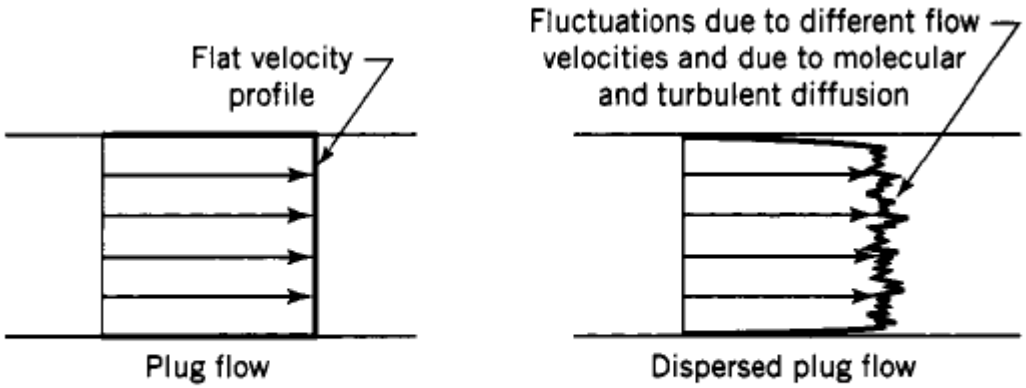


Figure 13.3 Representation of the dispersion (dispersed plug flow) model.

Dispersion model

Negligible dispersion, plug flow:

$$\frac{D}{\mu L} \to 0$$

High dispersion, mixed flow:

$$\frac{D}{\mu L} \to \infty$$

In the dispersion model, E_Θ curve vs. dimensional time Θ expressed as follows

$$\varepsilon(\theta) = \frac{1}{2}\sqrt{\frac{Pe}{\pi}} \cdot \exp\left(\frac{-(1-\theta)^2 \cdot Pe}{4 \cdot \theta}\right), \text{ where } Pe = \frac{u \cdot L}{D}; \theta = \frac{t}{\tau}$$

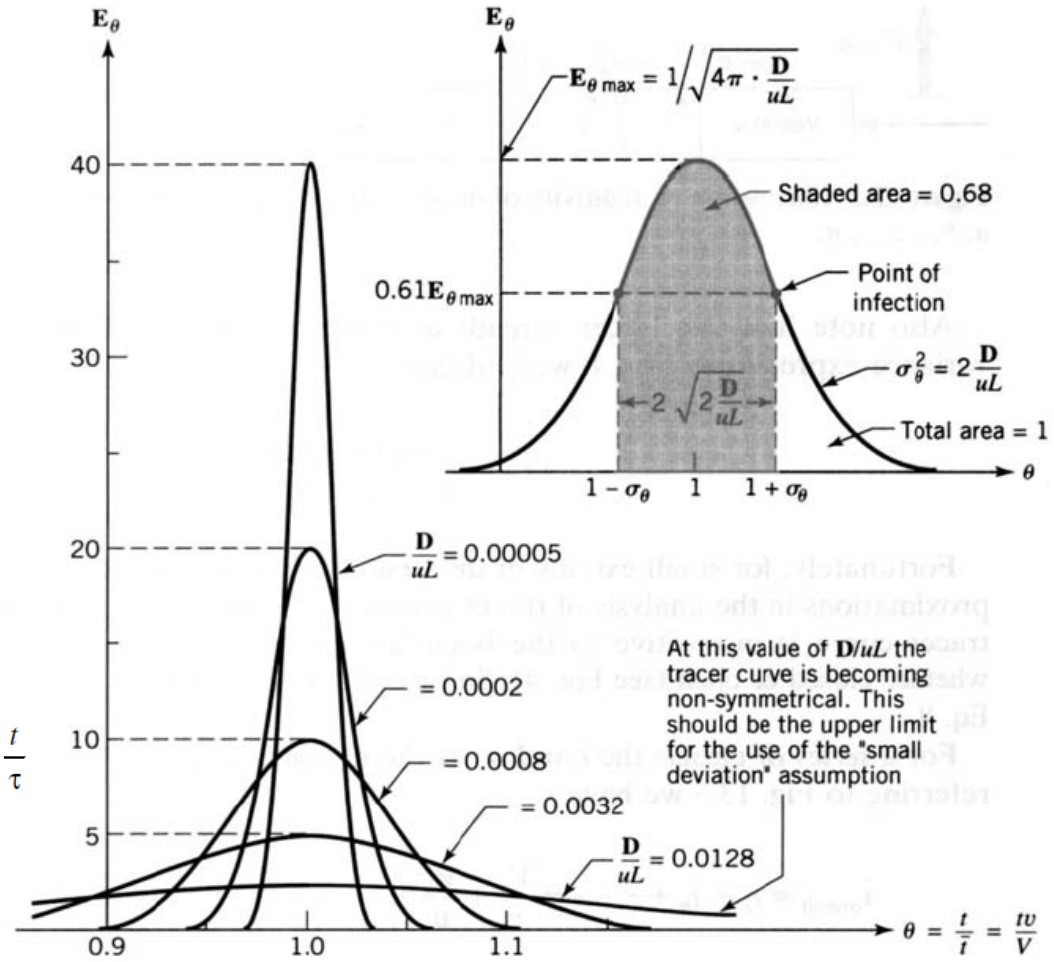


Figure 13.4 Relationship between D/uL and the dimensionless E_θ curve for small extents of dispersion, Eq. 7.

Tank-In-Series model

Another model describing the neideal flow is the cascade model of ideal mixers.

By increasing the number of ideal mixers (N) connected in series, simulation of the transition from ideally stirred vessel to nonideal flow with piston flow characteristics.

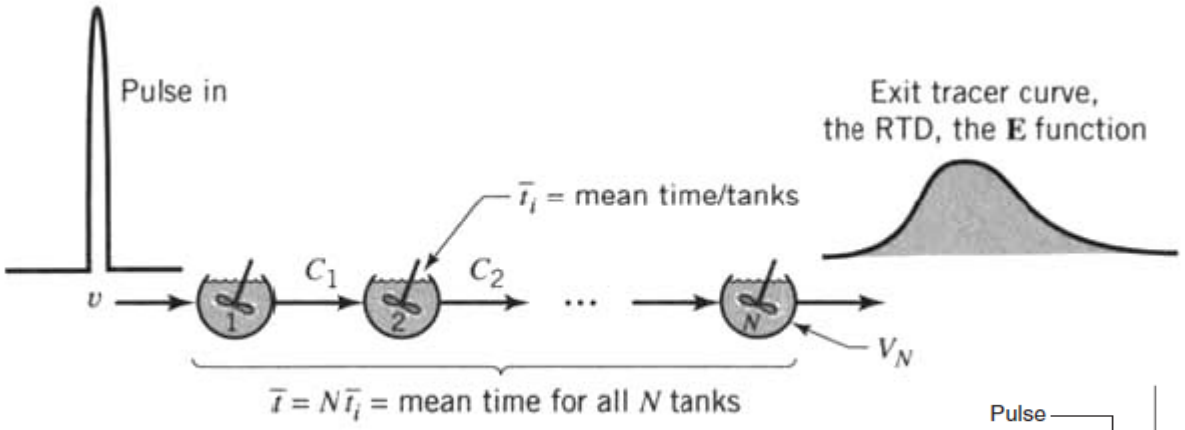
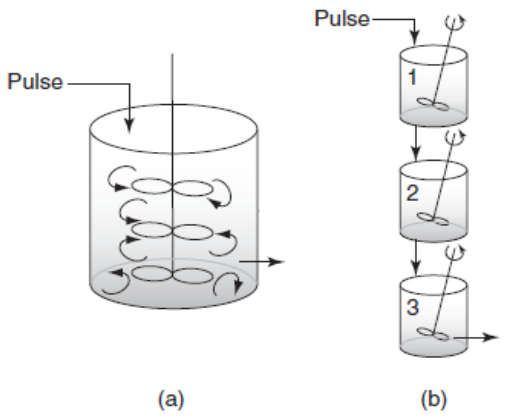


Figure 14.1 The tanks-in-series model.



-2 Tanks in series: (a) real system, (b) model system.

Tank-In-Series model

In the cascade of ideally mixed tanks model, the E_Θ curve vs. dimensional time Θ expressed as follows:

$$\mathbf{E}_\theta = (N\bar{t}_i)\,\mathbf{E} = N\frac{(N\theta)^{N-1}}{(N-1)!}e^{-N\theta}$$

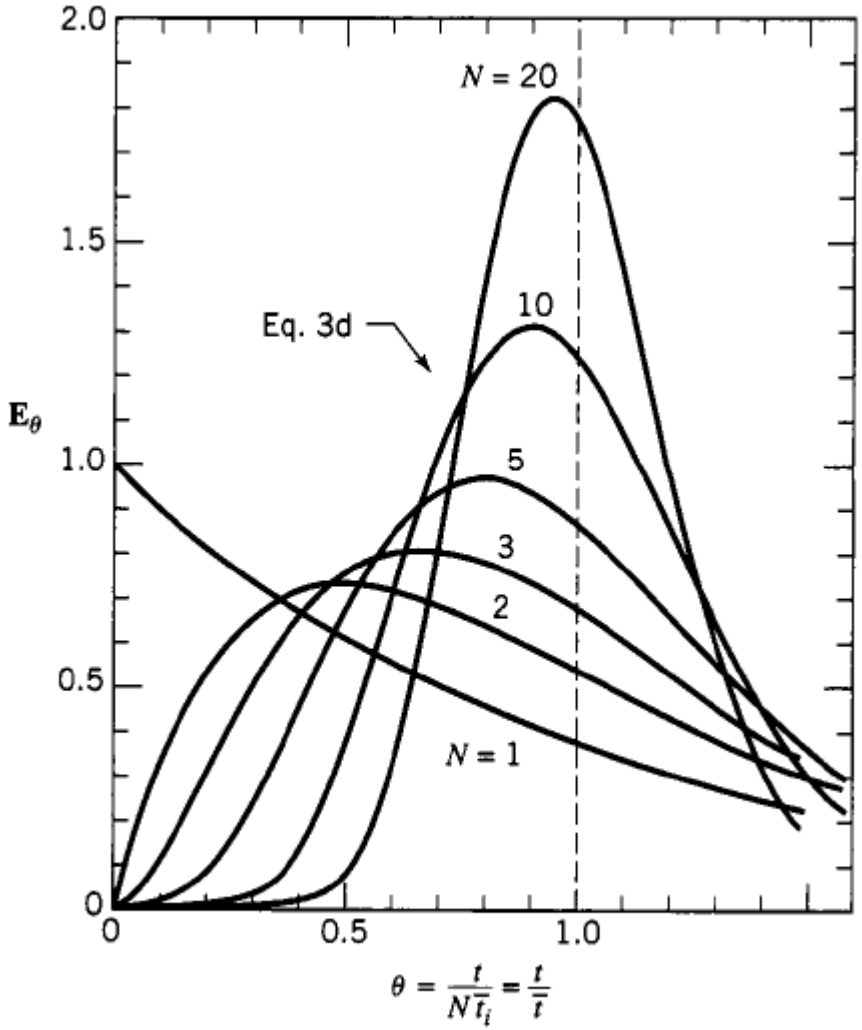
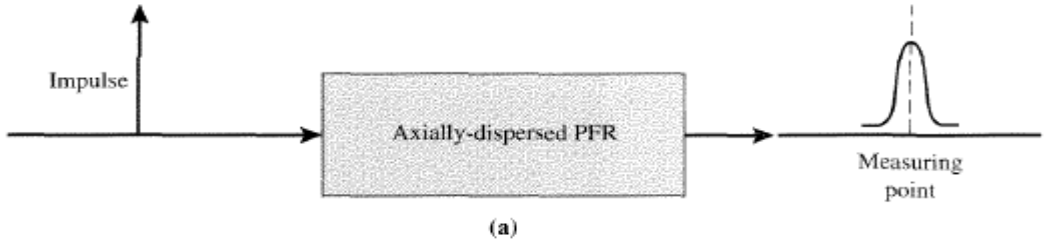
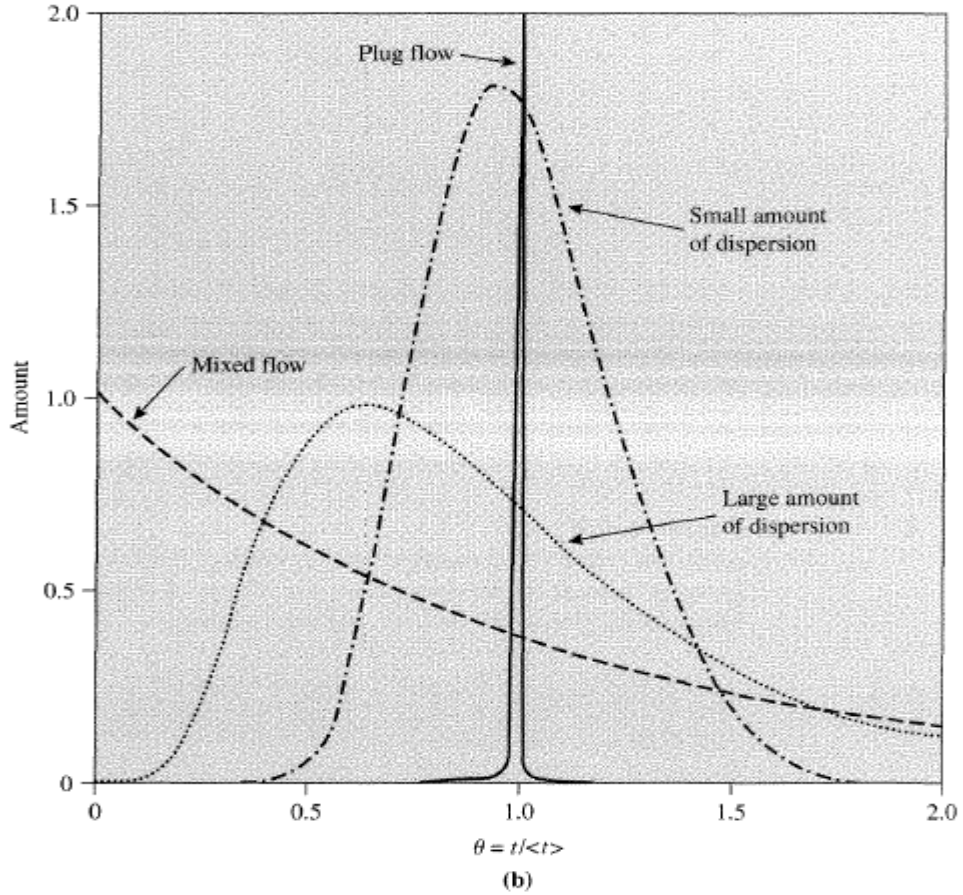


Figure 14.2 RTD curves for the tanks-in-series model,



(a)



(b)

Figure 8.4.2 |
(a) Configuration illustrating pulse input to an axially-dispersed PFR. (b) Results observed at measuring point.

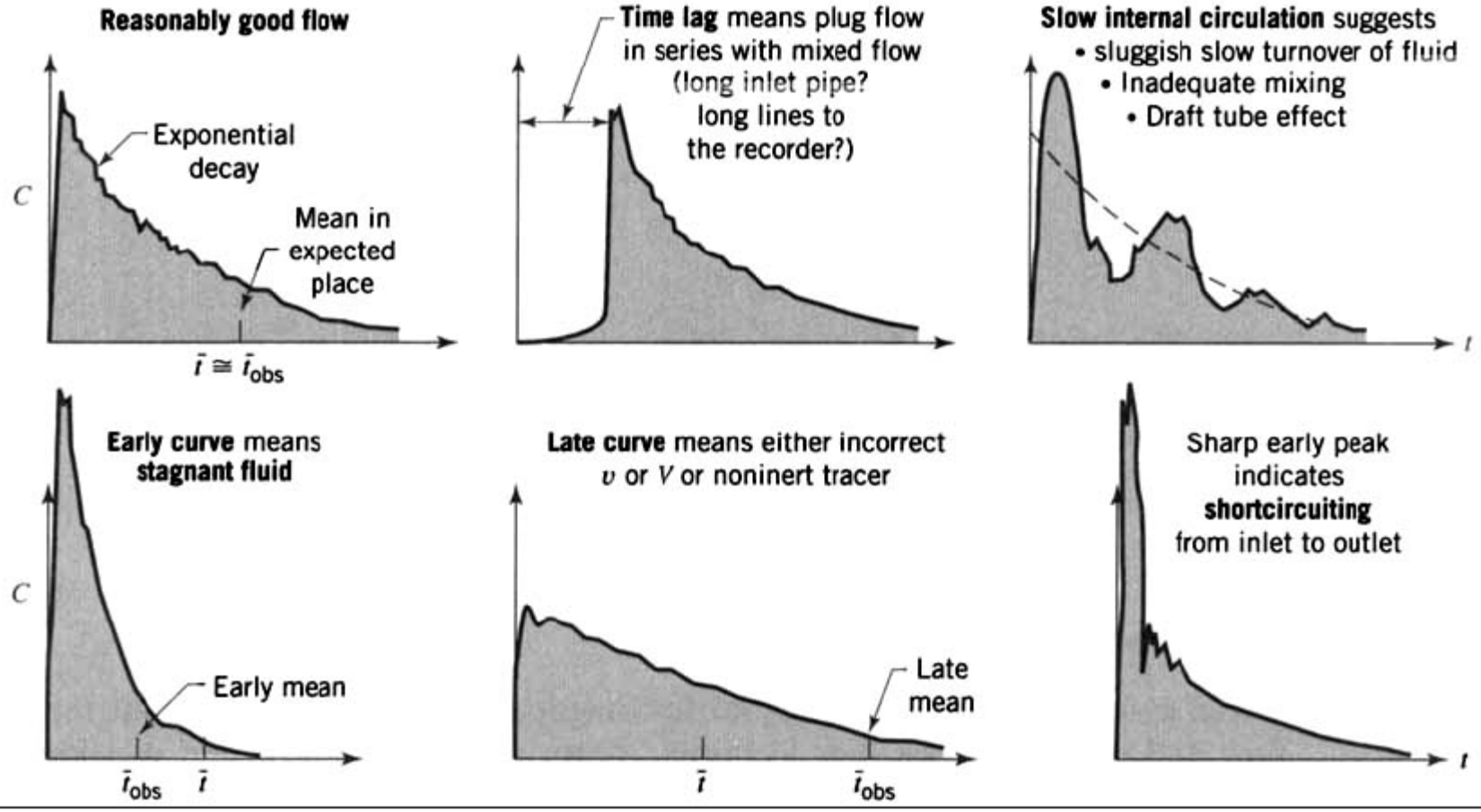


Figure 12.4 Misbehaving mixed flow reactors.

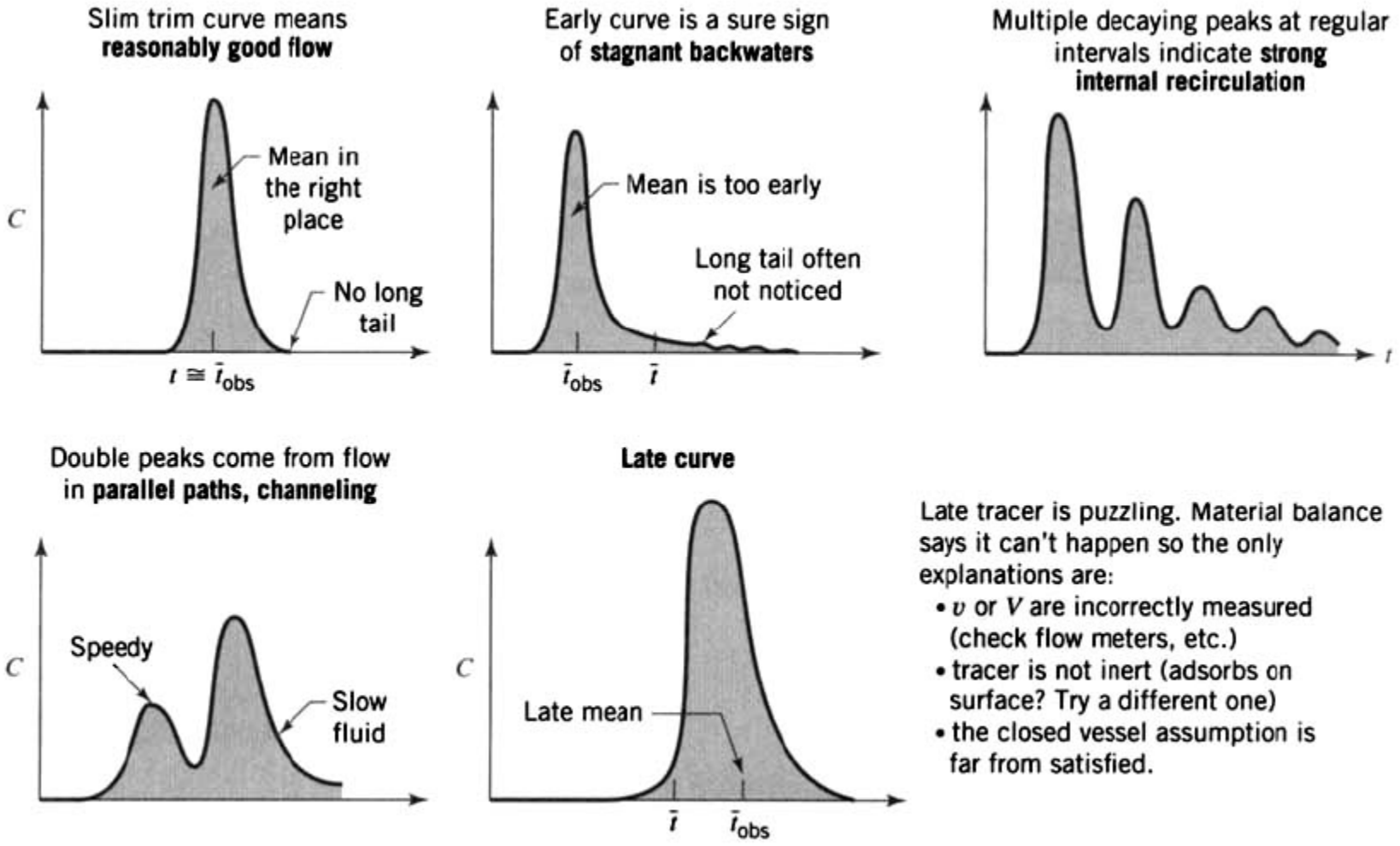


Figure 12.3 Misbehaving plug flow reactors.

Literature

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F. Kaštánek: Bioinženýrství, Academia, 2001

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Mass transfer, aeration and $k_L a$

Stoichiometry of respiration

To consider the Stoichiometry of respiration the oxidation of glucose may be represented as;

$$C_6H_{12}O_6 + 6O_2 = 6H_2O + 6CO_2$$

Atomic weight of	Carbon	12
	Hydrogen	1
	Oxygen	16

Molecular weight of glucose is 180

How many grams of oxygen are required to oxidise 180g of glucose?

Answer 192g

Solubility of Oxygen

- Both components oxygen and glucose must be in solution before they become available to microorganisms
- Oxygen is 6000 times less soluble in water than glucose
- A saturated oxygen solution contains only10mg dm^{-3} of oxygen
- Impossible to add enough oxygen to a microbial culture to satisfy needs for complete respiration
- Oxygen must be added during growth at a sufficient rate to satisfy requirements

Reasoning

Solubility of air oxygen in water is at 25°C and

1 atm pressure cca. 10 mg/L

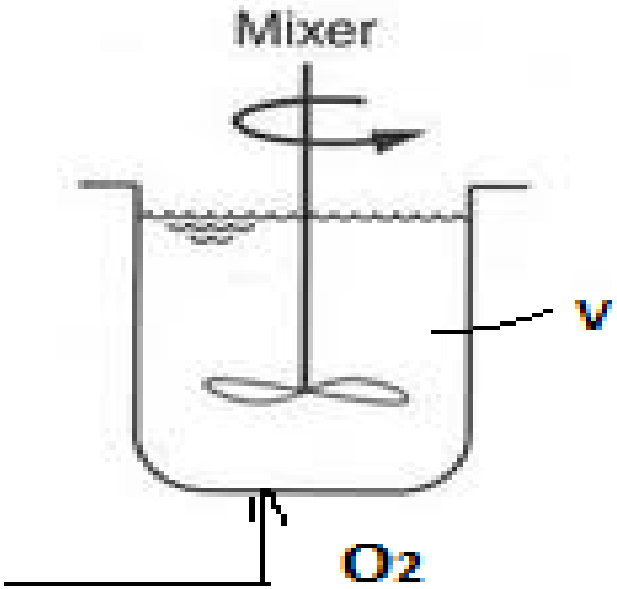
Aerobically growing yeast need 0,3 g $O_2/g_{DCW}h$

0.3 g $O_2/g_{DCW}h \times 20\ g_{DCW}/L$ = **6 g O_2/Lh**

Aerobically metabolizing yeast need per hour 600 timrs more oxygen than is its saturation concentration

Aeration is an important and a challenging problem!

Oxygen balance during aerobic fermentation

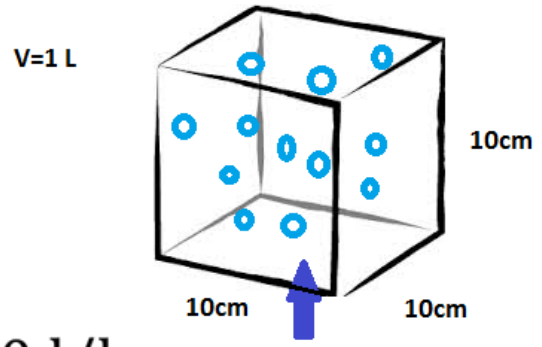


I am interested in: How much oxygen will flow from the bubbles into the fermenter?

It indicates the **oxygen flux J** ($g.s^{-1}.l^{-1}$) or ($ml.min^{-1}.l^{-1}$)

Example: How much oxygen (g) is absorbed in 1L liquid (cubic shape, bubble column) if the linear bubble rate is w = 1 cm / s and we use enriched mixture with 30% oxygen at T = 300 K

$V = A.h = 100.10 = 1000 cm^3$



Linear bubble rate: $w = \frac{G}{A}$

G … air flow (cm^3/s, l/h etc.)

Then $G = w.A = 1.100 = 100\ ^{cm^3}/_s = 360$ l/h

That is, I know the flow of air (and therefore also oxygen). I am interested in not the volume, but the weight of absorbed O_2.

Conversion of gas volume to mass => state gas equation :

$$pV = nRT, \qquad resp.\ pV = \frac{m}{M}RT$$

Where p is the total pressure, V gas volume, M molecular weight gas, R gas constant and T temperature in Kelvin (R = 0.082, when expressed in K, g, l, atm).

Enriched mixture: M = 0,3*32 (M_{O_2}) + 0,7*28 (M_{N_2}) = 29,2 g/mol

T=300 K

V=1 L

$m = \frac{pVM}{RT}$ = 1,186 g (thus 1L of air with 30%O_2 weights 1,186g.)

Thus, air with 30% O2 at the cube inlet has a mass concentration of oxygen:

$O_{2,input}$ = 0,3*1,186 = 0,3558 g/l

Oxygen balance in cube (without reaction):

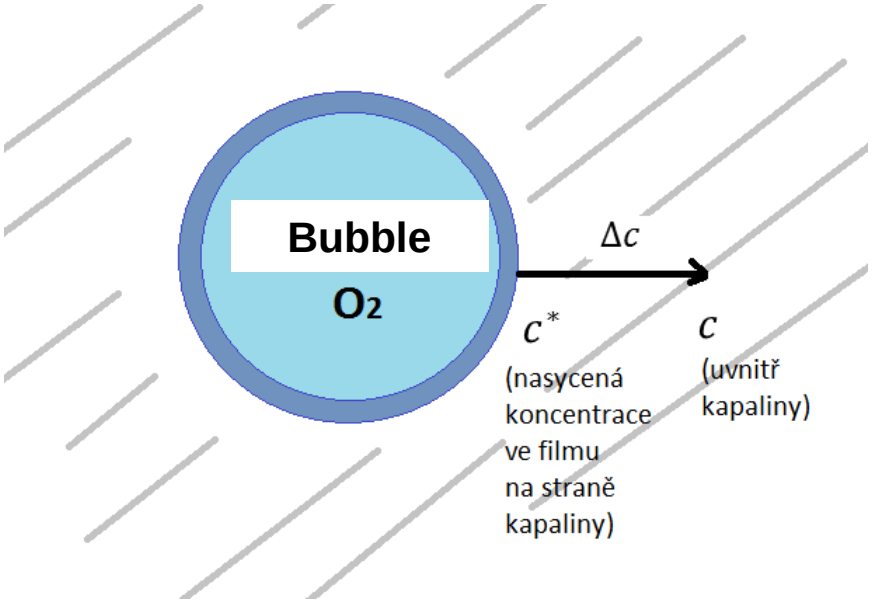
"Amount of oxygen (e.g. in g/h) at input = amount of oxygen at the outlet + quantity absorbed"

$$G.O_{2,input} = G.O_{2,output} + k_L a(c^* - c).V$$

Oxygen flow: $J = k_L a(c^* - c)$

(g/h.l) (1/h) (g/l) $\Delta c = (c^* - c)$ driving force

$$G.O_{2,input} = G.O_{2,output} + k_L a(c^* - c).V$$



In water: $c^*_{20°C,water} \cong 10\ mg/l$ (=0,01g/l), thus the balance:

360*0,356 = 360*$\boldsymbol{O_{2,output}} + k_L a(0{,}01 - \boldsymbol{c}).1$

Next to kLa we have 2 unknown in equation: $\boldsymbol{O_{2,output}}$ $\boldsymbol{and\ c}$

Complication: batch reactor is in non-steady state. Lets suppose that there is no oxygen at the beginning (e.g. Bubbling by N2). During bubbling, the oxygen concentration is increasing, but as the **c** increases, the value of the term $k_L a(0{,}01 - \boldsymbol{c})$ decreases and it influences tha value of $\boldsymbol{O_{2,output}}$

$$360*0{,}356 = 360*\boldsymbol{O_{2,output}} + k_L a(0{,}01 - \boldsymbol{c}).1$$

Experimentally, I will measure with oxygen electrode c, if I know kLa, I can calculate $\boldsymbol{O_{2,výstup}}$. In the batch reactor c changes, so the calculation is relatively difficult.

If in the reactor there will be something that consumes the dissolved oxygen immediately (very quickly), then ***c = 0 .*** If we know $k_L a$, we can easily calculate $\boldsymbol{O_{2,output}}$ without measuring ***c***.

$\boldsymbol{k_L a}$ *... volumetric mass transfer rate.*

With it, I can count not only the transfer of oxygen but, for example, CO_2 transfer during algae cultivation, H_2 transfer to oil, etc.

But how can be $k_L a$ calculated?

Determining Value $k_L a$

$k_L a$ will depend on whether or not we just bubble or stir, on the properties of the medium (density, surface tension, viscosity, ...), what kind of agitator we use, etc. All of this will affect the state of the heterogeneous bubbling layer.

In the literature there are dozens of correlation equations between kLa and selected parameters!

Experimental determination of $k_L a$ (gassing out method)

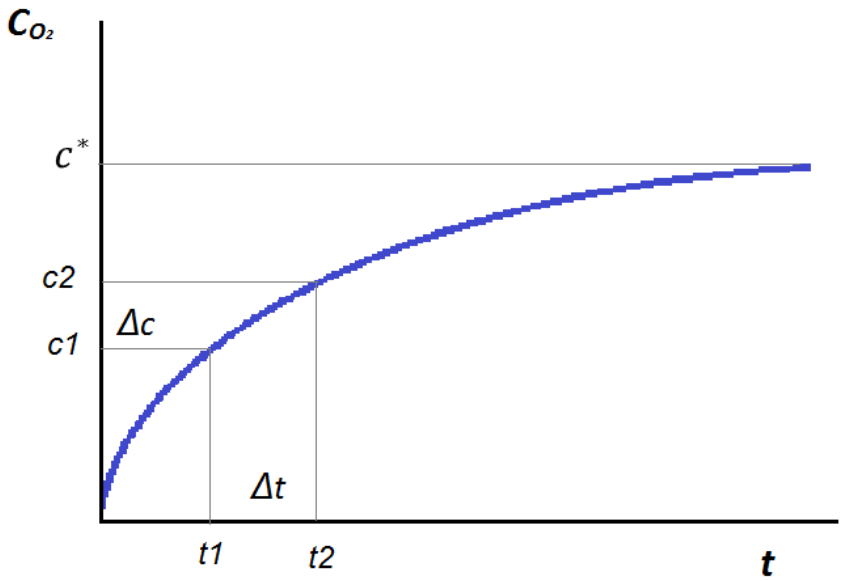
1. First, I take the nutrient solution but without micro-organisms and put it in the batch fermentor
2. I get rid of the oxygen solution (by bubbling with N2 or CO2)
3. I place a dissolved oxygen probe that has no delay (i.e., I assume an immediate response)
4. I'll start bubbling through G flow rate
5. I measure the concentration of dissolved oxygen over time and draw a chart

Experimental determination of $k_L a$

I measure the concentration curve dissolved oxygen over time.

I want to express this curve now by Mathematical Relationship.

The balance applies to the change of oxygen concentration:



oxygen input to the system - oxygen output of system = $k_L a(c^* - c)$

If I express this for a short (differential time), then:

$$\frac{dc}{dt} = k_L a(c^* - c)$$

$$\frac{dc}{dt} = k_L a(c^* - c)$$

Integration:

$$\int_0^c \frac{dc}{c^* - c} = k_L a \int_0^t dt$$

Substitution:

$$c^* - c = \mathrm{u}$$

Derivation:

$$0 - dc = \mathrm{du}$$

$$-\int \frac{du}{u} = -lnu = -[\ln(c^* - c)]_0^c = -ln\frac{c^* - c}{c^*}$$

$$\boldsymbol{-ln\frac{c^* - c}{c^*} = k_L a.t}$$

c is measured. For different ***c*** and ***t*** we calculate $k_L a$. We plot various values of the equation and calculate the slope $k_L a$.

This experimentally detected $k_L a$ will then be used in cell or enzyme calculations, neglecting that my cells change properties (viscosity, …) of the medium.

Sulfite determination method $k_L a$

We bubble the solution of sodium sulfite by air, which slowly oxidizes to the sulfate according to the equation :

$$Na_2SO_3 + 0{,}5O_2 \rightarrow Na_2SO_4$$

The rate of the oxidation reaction will be affected by the size of the bubbles, the mixing, the size of the interfacial area, the diffusivity coefficient and the kinetics of the chemical reaction (order of reaction). The systém is simultaneously under the influence of **difusion** and **kinetics.**

To simplify the model, I add Cu^{2+} salt as catalyst that speeds up the reaction (once the oxygen molecule reaches the solution, it reacts immediately) to eliminate the kinetic effect, thus diffusion is the rate control step:

The reaction rate will then be proportional to the rate of transfer of oxygen from the bubbles to the solution

Amount of O_2 that goes to solution (oxygen flow in $g.h^{-1}l^{-1}$):

$$k_L a(c^* - c) = k_L a(c^* - 0)$$

c=0 since it all reacts immediately

c^* cannot be determined (because the saturated oxygen concentration in the sulfite solution when

Given the equation $Na_2SO_3 + 0{,}5O_2 \rightarrow Na_2SO_4$, I can determine the theoretical oxygen consumption from the stoichiometric equation, with the concentration of the sulfate determined by gravimetry after precipitation with barium salt. The amount of BaSO4 is then proportional to the oxygen consumption and this is equal to the amount of oxygen that I had to deliver to the solution, $\boldsymbol{k_L a . c^*}$

If I knew $\boldsymbol{c^*}$, I could calculate $\boldsymbol{k_L a}$.

I assume the assumption that "the saturated concentration of O2 in sodium sulphite is roughly the same as the saturated O2 concentration in sodium sulphate". I can measure it or find it in the literature.

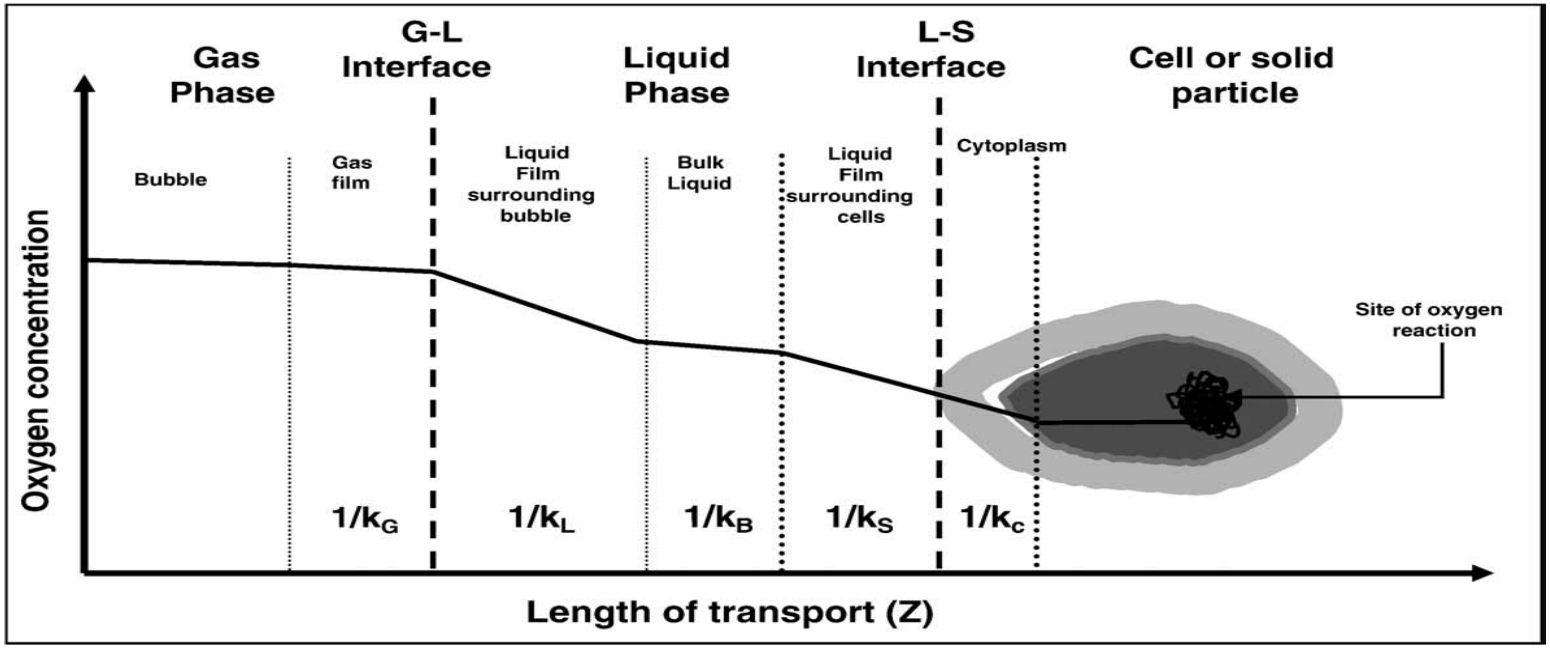
$\boldsymbol{k_L}$ ... **mass transfer coefficient from <u>film theory</u>**

It shows how fast the oxygen passes into the water, the velocity cm / s.

Water "does not like" absorbs oxygen, according to the film theory, the resistance against the transfer is concentrated in a film of the thickness δ (delta) surrounding the bubble.

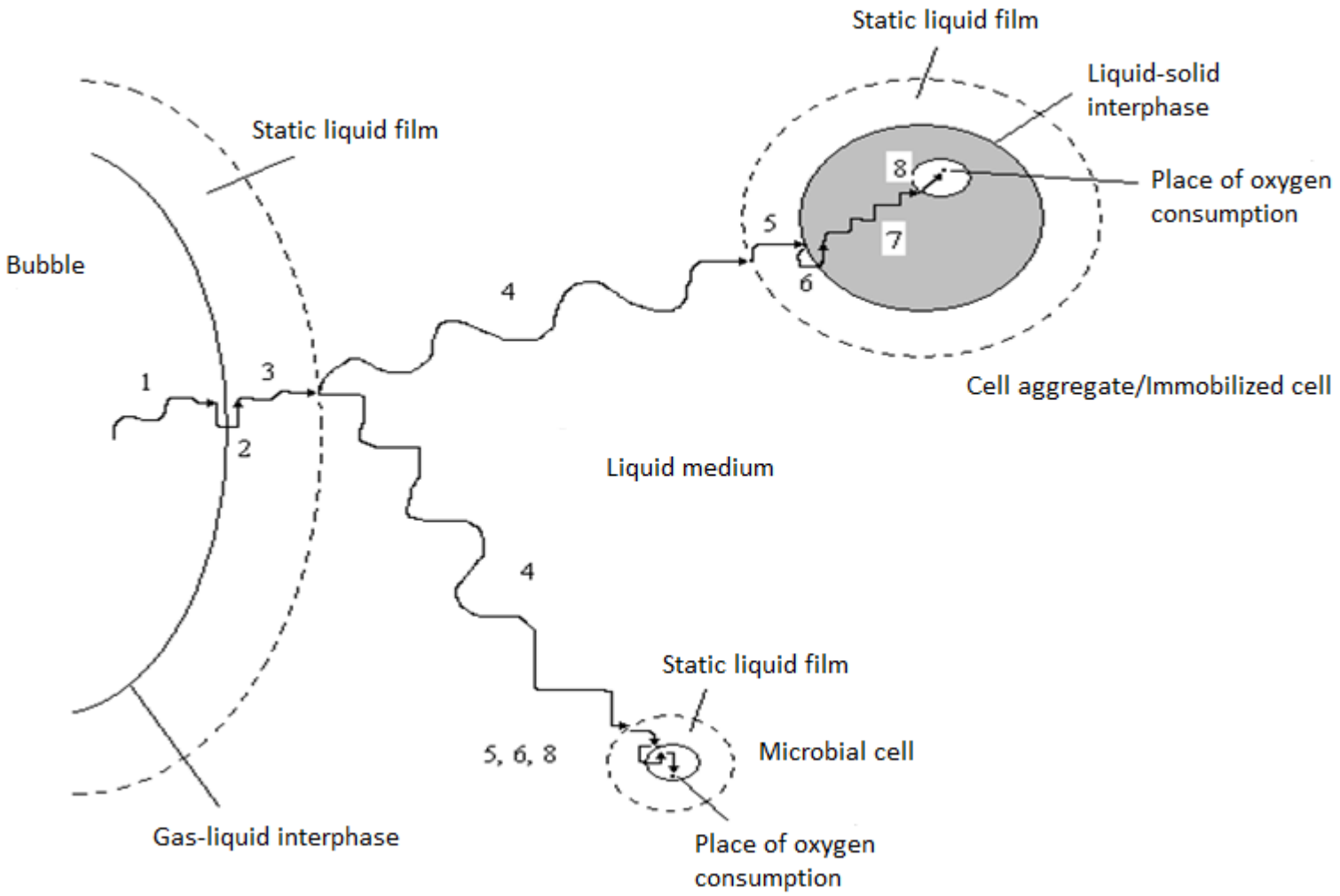
$\boldsymbol{k_L = \frac{D}{\delta}}$ where D is diffusion coeficient (m^2/s)

Film theory of oxygen transfer



- Film theory: molecule of oxygen passes through:
  - A gas film placed on the surface of a bubble (so-called film theory). The transfer rate is characterized by the parameter k_G (speed velocity m/s, specifying the rate at which the molecule passes through the brake film) - the mass transfer coefficient in the gas phase, resp. transfer resistance $1/k_G$.
  - A static film of fluid surrounding the bubble, characterized by the coefficient k_L (m / s), the mass transfer coefficient in the liquid phase in a static film.
  - "Bulk", ie the volume of liquid with coef. k_B (this change is rapid due to liquid mixing and turbulence in liquid)
  - Static film around the cell, koef k_S
  - Cytoplasm within the cell, k_C.

Partial steps of oxygen transfer from gas phase to the cells



- The relative magnitude of the resistance to oxygen transfer influences: the composition and rheological properties of the liquid, the mixing intensity, the size of the bubbles, the size of the cell clusters, the interphase adsorption
- A static film around the gas bubble exhibits the greatest resistance to oxygen transfer (3)
- In viscous media, the liquid medium can exhibit considerable convection resistance
- A static film around microbial clusters may exhibit considerable diffusion resistance
- Cell aggregates, immobilized biocatalyst (gels, etc.) may exhibit considerable internal diffusion resistance

Mass transfer

The driving force of mass transfer is the difference in the concentration of the transported component or the concentration gradient.

1. Molecular diffusion - depends on the material properties of the environment, conc. gradient, temperature

2. Convection - influenced by fluid flow

In fact, these two basic mechanisms intersect and complement each other. One of them can be considered predominant.

Molecular diffusion

$$J_{A,dif} = \frac{N_A}{a} = -D_A \frac{\partial C_A}{\partial y}$$

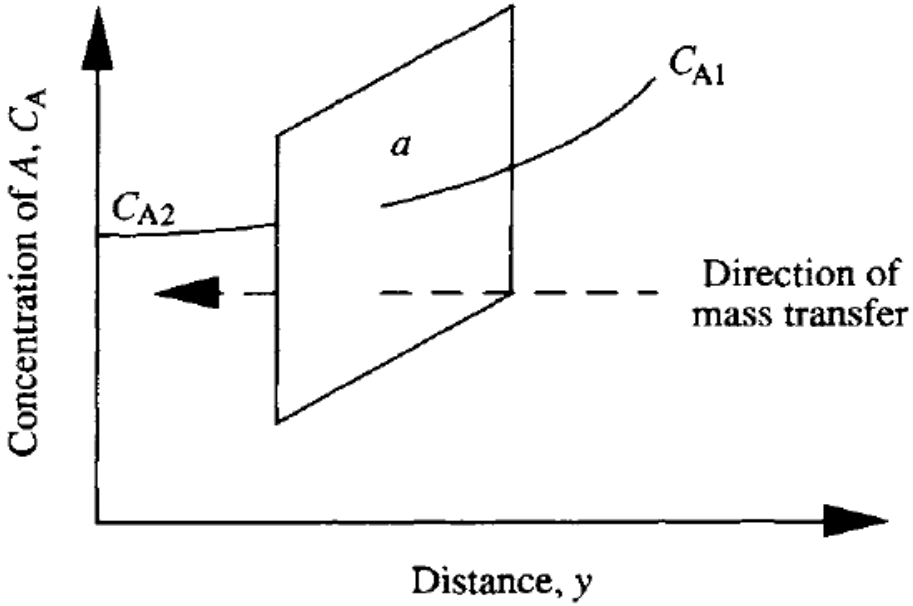
$J_{A,dif}$ – intensity of molar mass flow ba diffusion (mol/m^{2}s)

N_A – mass flow rate (mol/s)

a - area (m^2)

D_A - diffusion coefficient (m^2/s)

C_A – molar concentration (mol/m^3)



Diffusion rate can be increased:
- by increasing the area available for mass transfer
- by increasing the concentration gradient
- increasing the diffusion coefficient

Diffusion coefficient: the material property (tables), depending on both components of the mixture, is a function of temperature, pressure (gases) and conc. (liquids), in liquids (10^{-9} m^{2}s^{-1}) is by order of magnitude lower than in gases (10^{-5} m^{2}s^{-1})

Analogy between matter, heat and momentum

$$J_{A,dif} = -D_A \frac{\partial C_A}{\partial y}$$

$$\dot{q} = -k \frac{dT}{dy}$$

$$\tau = -\mu \frac{dv}{dy}$$

All these processes are the result of molecular movement, but at the molecular level they vary considerably.

There are similarities:

Intensity of flow = constant x driving force

Constant – material property

The analogy for mass transfer, heat transfer and momentum transfer applies to the processes of movement and vibration of molecules.

In the case of turbulent flow the analogy applies to heat and mass, while in the case of momentum it is more complex.

The role of diffusion in bioprocesses

1. Mixing has an effect (convection mass transfer) at the size of the device corresponding to the smallest vortex. In smaller devices, diffusion prevails.
2. Presence of solid phase (clusters of cells, biofilm, immobilized biocatalyst). Within the solid phase there is no transfer of matter by convention and the prevalence of molecular diffusion. This applies to both substrates and products.
3. Mass transfer through the phase interface on which stagnant films are created (film theory).

Mass transfer at flow - convection

Mass transfer in the presence of fluid flow consists of diffusion and convection. The size of the contribution of individual mechanisms is a function of mixing intensity.

$$J_{A,konv} = c_A \frac{d\dot{V}}{dA} = c_A v$$

$$J_A = J_{A,konv} + J_{A,dif} = c_A v - D_A \frac{\partial c_A}{\partial y}$$

General expression of mass transfer equation :

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

$N_A = ka\Delta C_A = ka(C_{Ao} - C_{Ai})$ while $J_A = N_A/a$

k - mass transfer coefficient (empirical magnitude, material (viscosity, diffusivity) and hydrodynamic (flow velocity, system geometry) property)

C_{Ao} - conc. of A in bulk, C_{Ai} - conc. of A at interphase

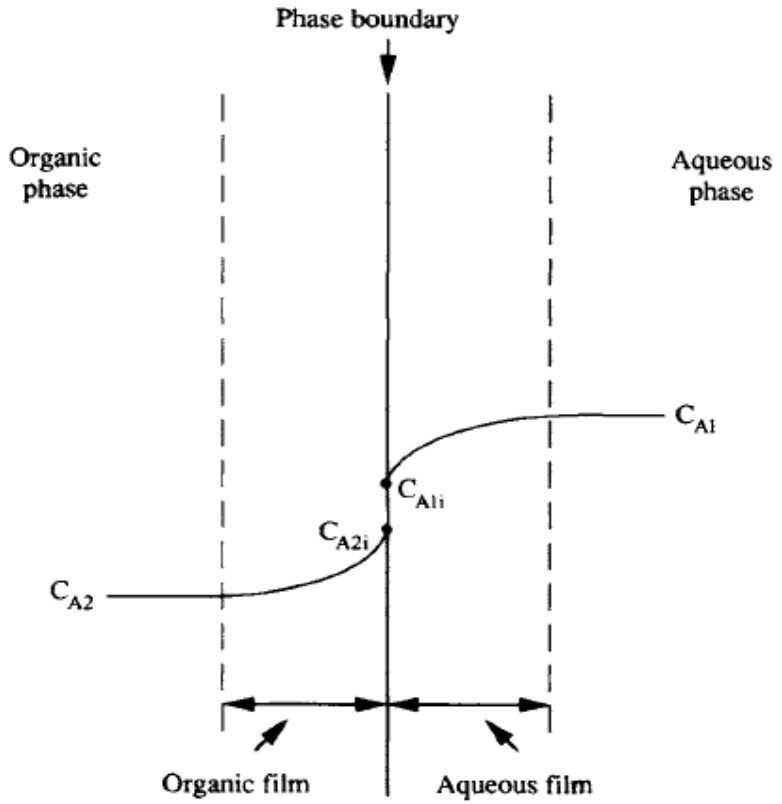
Mass transfer from one phase to another

The mass transfer describes transporting matter from the core of the fluid to the interface, but not beyond the phase interface

Film theory - the turbulence of the liquid extinguishes near the interfacial interface

These assumptions are required:

- there is no accumulation on the phase interface
- the equilibrium is at the phase interface (the concentration is affected by solubility)



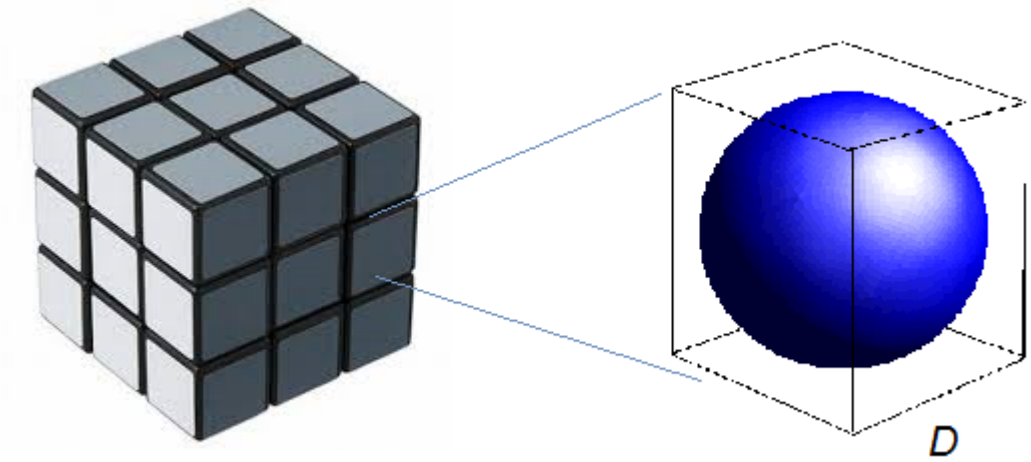
a … specific interfacial area (m^{-1}) in $k_L a$

Indicates the surface area of all bubbles in 1 m3 of the bubbled layer (i.e. $\frac{m^2}{m^3}$)

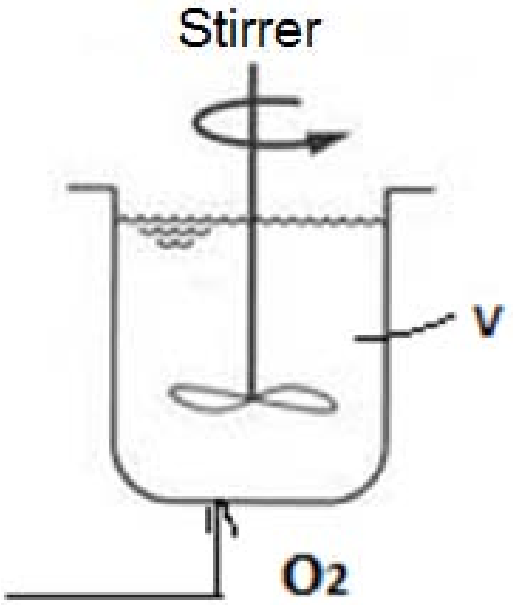
It is very difficult to measure the size of the interfacial area (photos of bubbled layer, 3D photos and models).

Finding the interfacial area from a model idea:

- Bubbles are round and are arranged in regular cubes, in each cube there is one bubble



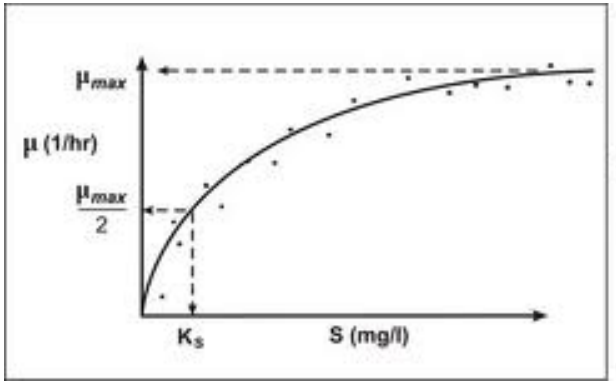
Oxygen balance in aerobic batch fermentation



Attention: even if it is batch cultivation, oxygen is fed continuously

Influence of oxygen

- Aeration is costly operation → important
- The rate of cell formation is inhibited if the concentration of dissolved O2 in the medium falls below the critical value (between 0 and 20% of the saturated concentration at a given temperature)
- Simplified: if the flow of O2 from the gas through the phase interface to the liquid is higher than the theoretical consumption by the microorganism, then the growth of the cell number corresponds to the Monod exponential kinetics
- When a large number of cells grow and the O2 supply is not sufficient, kinetics do not apply and cell growth is inhibited
- For some microorganisms, short-term stopping O2 leads to total collapse
- Algae – vice versa – inhibition by oxygen



$$\mu = \mu_{max}\left(\frac{S}{S+K_S}\right) \quad (5)$$

Interphase Oxygen Flow

- Influenced by 3 critical parameters
  - Microturbulence charakteristics expressed by mass transfer coefficient $\mathbf{k_L}$
    - Depends on the nature of the interfacial contact (film-film, bubble-turbulent liquid,...) and on the physico-chemical properties of both phases
  - The size of the interfacial area **a** through which the contact is made
    - depends on the physicochemical properties of both phases and the amount of dissipated energy to form the interfacial interface - by mixing, spraying, gas flow, etc.
  - A concentration gradient of dissolved oxygen **(o*-o)** where **o*** is the saturated concentration on the liquid-side interfacial interface and **o** is the local concentration in the core of the liquid (given for example by the rate of cell respiration)

Oxygen balance in aerobic batch fermentation

- The resistance in the liquid film of the bubble k_L is crucial
- Parameter oxygen flow J ($g.s^{-1}.l^{-1}$) or ($ml.min^{-1}.l^{-1}$)
  - How much oxygen passes from bubbles to the fermenter
- Considering just the crucial action $k_L \rightarrow$ then the flow of oxygen J is proportional to the rate at which it passes through the static liquid film (k_L), the specific surface area of the bubbles a (m^{-1}), and the driving force of the action, which is the difference of the saturated concentration of O_2 in the liquid right at the interfacial interface – O^* ($g.l^{-1}$) and concentration within the liquid O ($g.l^{-1}$)

$$J = k_L a(O^* - O)$$

- $k_L a$ (s^{-1}) is referred as the volume transfer coefficient of oxygen – depends on the type of agitator, speed, composition and viscosity of the medium, reactor design, etc. In biological systems, $k_L a$ can change unpredictably during the process itself
- a indicates the area of the bubbles (m^2) relative to the reactor volume (m^3) – or can be based on the volume flow (volume of liquid + bubble volume, typically 20% liquid)
- Typically O^* is about 10 $mg.l^{-1}$, i.e. about a saturated oxygen concentration in water at 20 ° C.

1) Batch fermentation

- **Oxygen balance:**

„The amount of oxygen that has passed from the bubbles to the reactor is consumed for cell growth, cell maintenance, product formation, and accumulation in the system“

$$k_L a(O^* - O) = \frac{1}{Y_{X/O}} \frac{dx}{dt} + m_O x + \frac{1}{Y_{P/O}} \frac{dp}{dt} + \frac{dO}{dt}$$

- $Y_{X/O}$ - a yield factor of how many g of cells are produced from 1 g of oxygen
- $Y_{P/O}$ - a yield factor of product
- m_O (h^{-1}) – maintenance coefficient relative to oxygen, i.e., the amount of oxygen in grams required to maintain 1 g of cells per hour

3) Fed-batch fermentation

Oxygen balance

„Oxygen input by bubbling + oxygen input in media = cell consumption + maintenance consumption + accumulation“

$$k_L a(O^* - O)V + FO_0 = \frac{1}{Y_{X/O}} \frac{d(VX)}{dt} + m_O XV + \frac{d(VO)}{dt}$$

O^* – oxygen concentration at the interfacial bubble interface, O_0 – concentration in the inlet medium

Derivations

$$k_L a(O^* - O)V + FO_0 = \frac{1}{Y_{X/O}} \left(\frac{dV}{dt} X + \frac{dX}{dt} V \right) + m_O XV + \frac{dV}{dt} O + \frac{dO}{dt} V$$

3) Fed-batch fermentation

$$k_L a(O^* - O)V + FO_0 = \frac{1}{Y_{X/O}}\left(\frac{dV}{dt}X + \frac{dX}{dt}V\right) + m_O XV + \frac{dV}{dt}O + \frac{dO}{dt}V$$

From cell and volume balances $\frac{dV}{dt} = F$ and $\frac{dX}{dt} = (\mu - D)X$, divide ***V*** and set ***F/V=D***

$$k_L a(O^* - O) + D(O_0 - O) = \frac{1}{Y_{X/O}}\mu X + m_O X + \frac{dO}{dt}$$

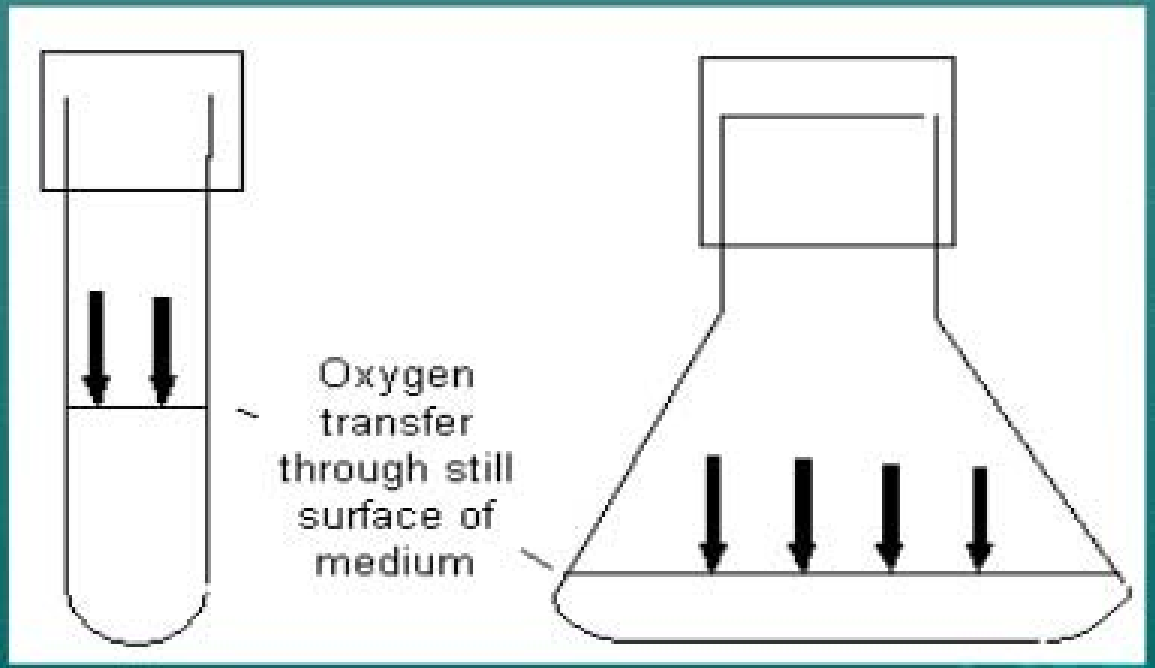
$$\boldsymbol{\frac{dO}{dt} = k_L a(O^* - O) + D(O_0 - O) - \left(\frac{\mu}{Y_{X/O}} + m_O\right)X} \quad (9)$$

Methods of Aeration

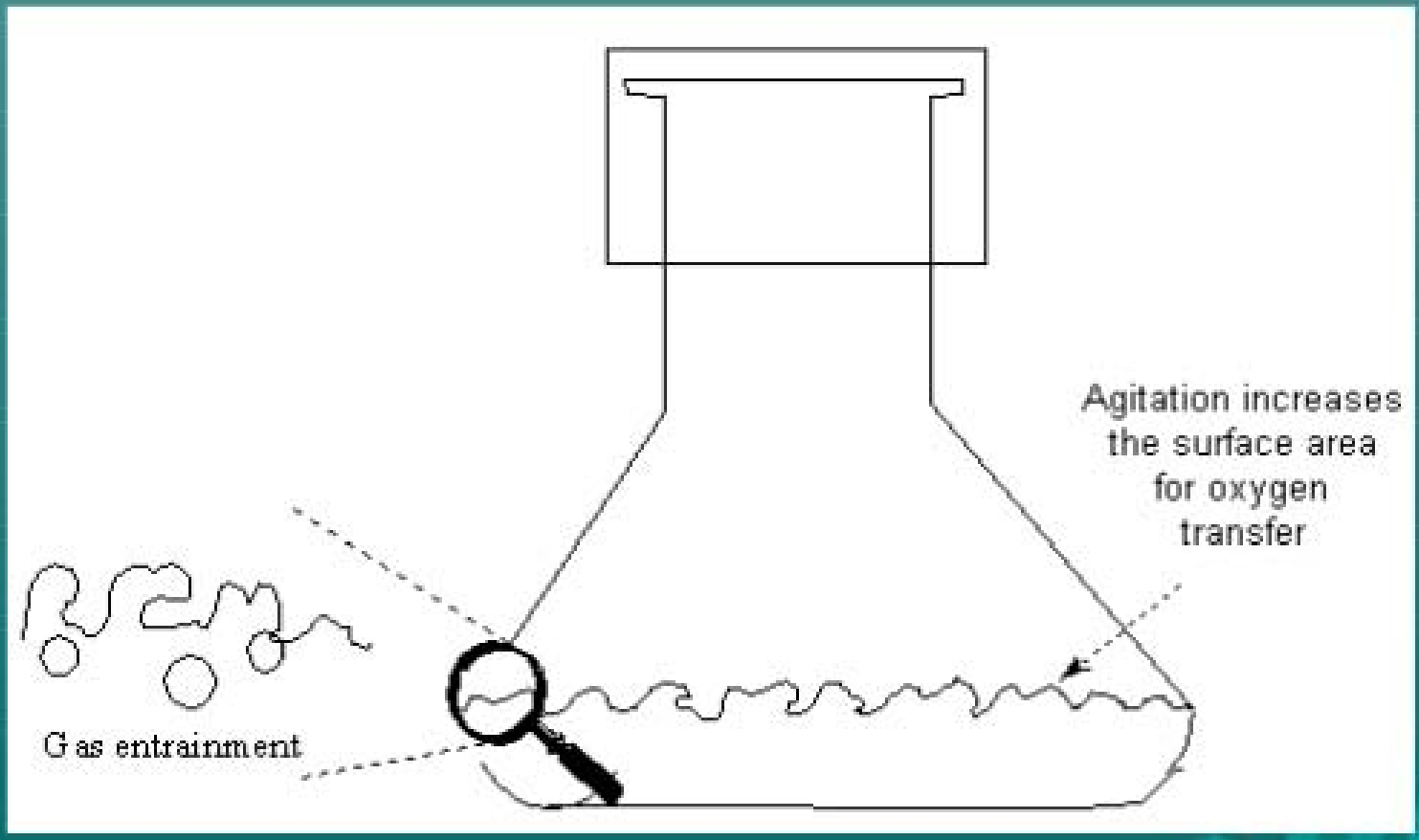
- A bioreactor is a reactor system used for the culture of microorganisms. They vary in size and complexity from a 10 ml volume in a test tube to computer controlled fermenters with liquid volumes greater than 100 m^3. They similarly vary in cost from dollars to a few million dollars.
- In the following sections we will compare the following reactors
  - Standing cultures
  - Shake flasks
  - Stirred tank reactors
  - Bubble column and airlift reactors
  - Fluidized bed reactors

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.

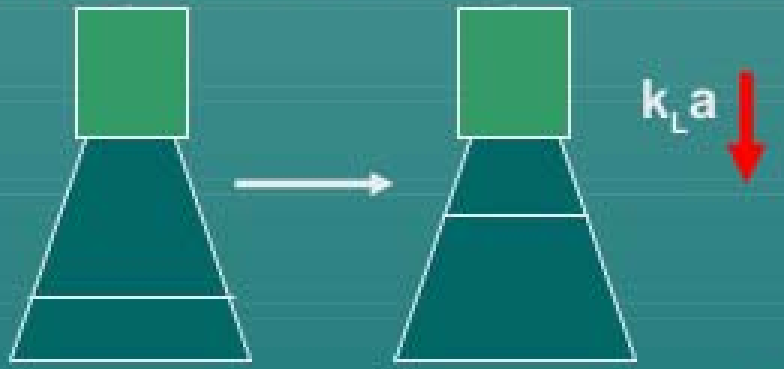


Shake flasks

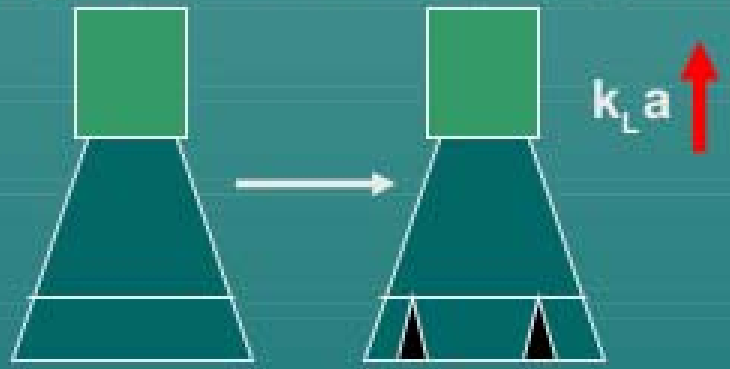


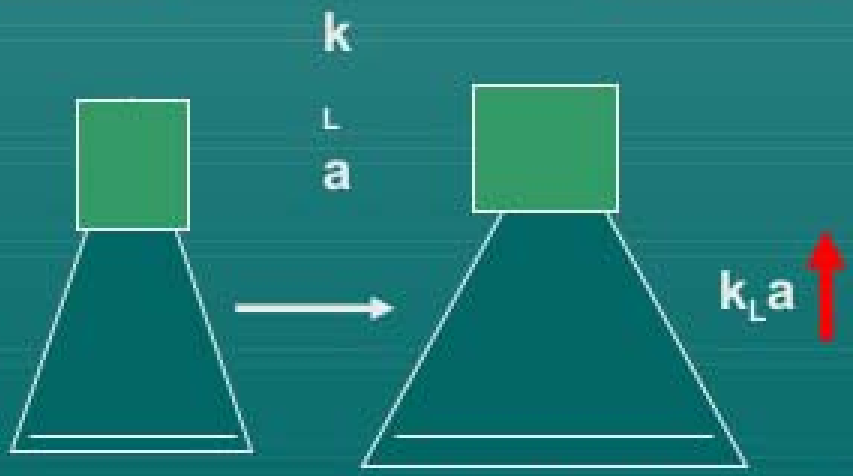
Shake flasks O_2 Transfer

$k_L a$ decreases with liquid volume



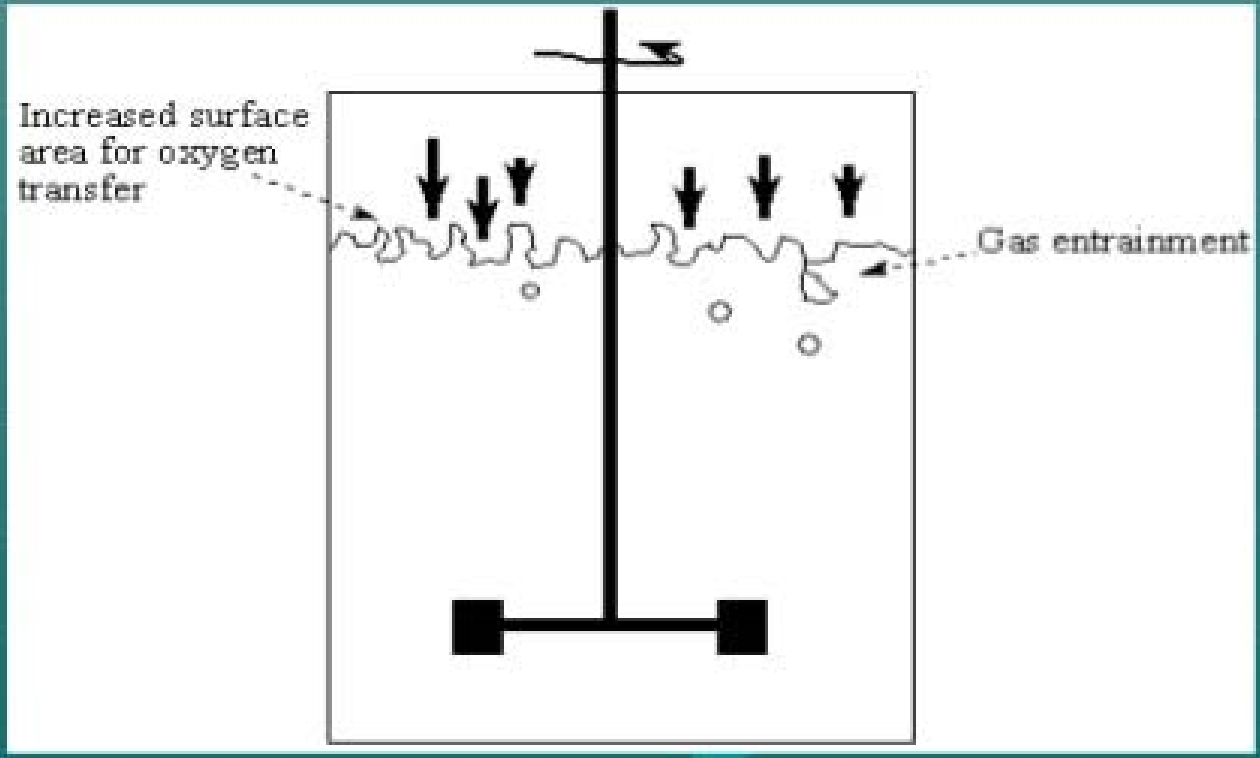
$k_L a$ is higher when baffles are present





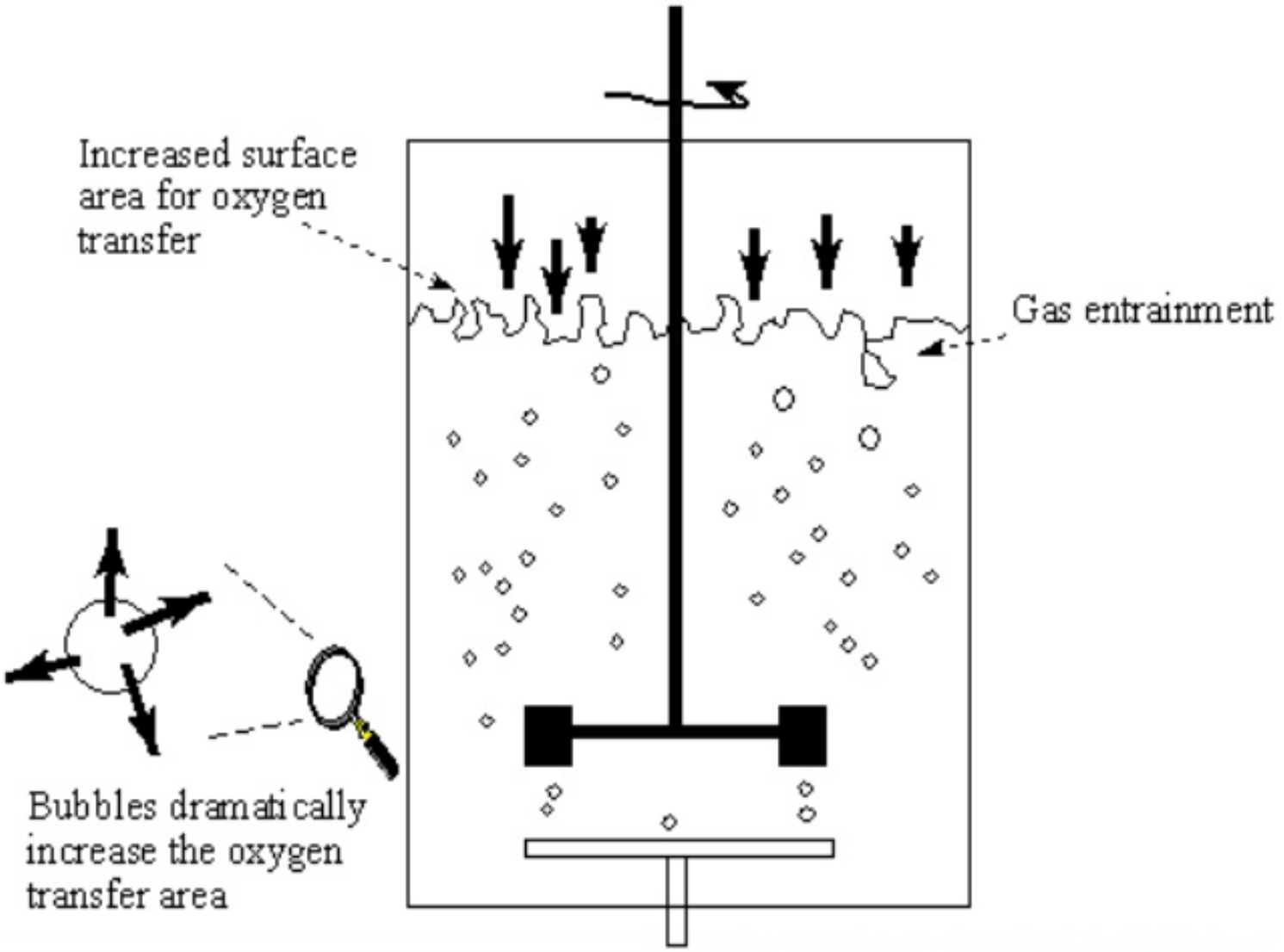
$k_L a$ increases with liquid surface area

Mechanically stirred bioreactors

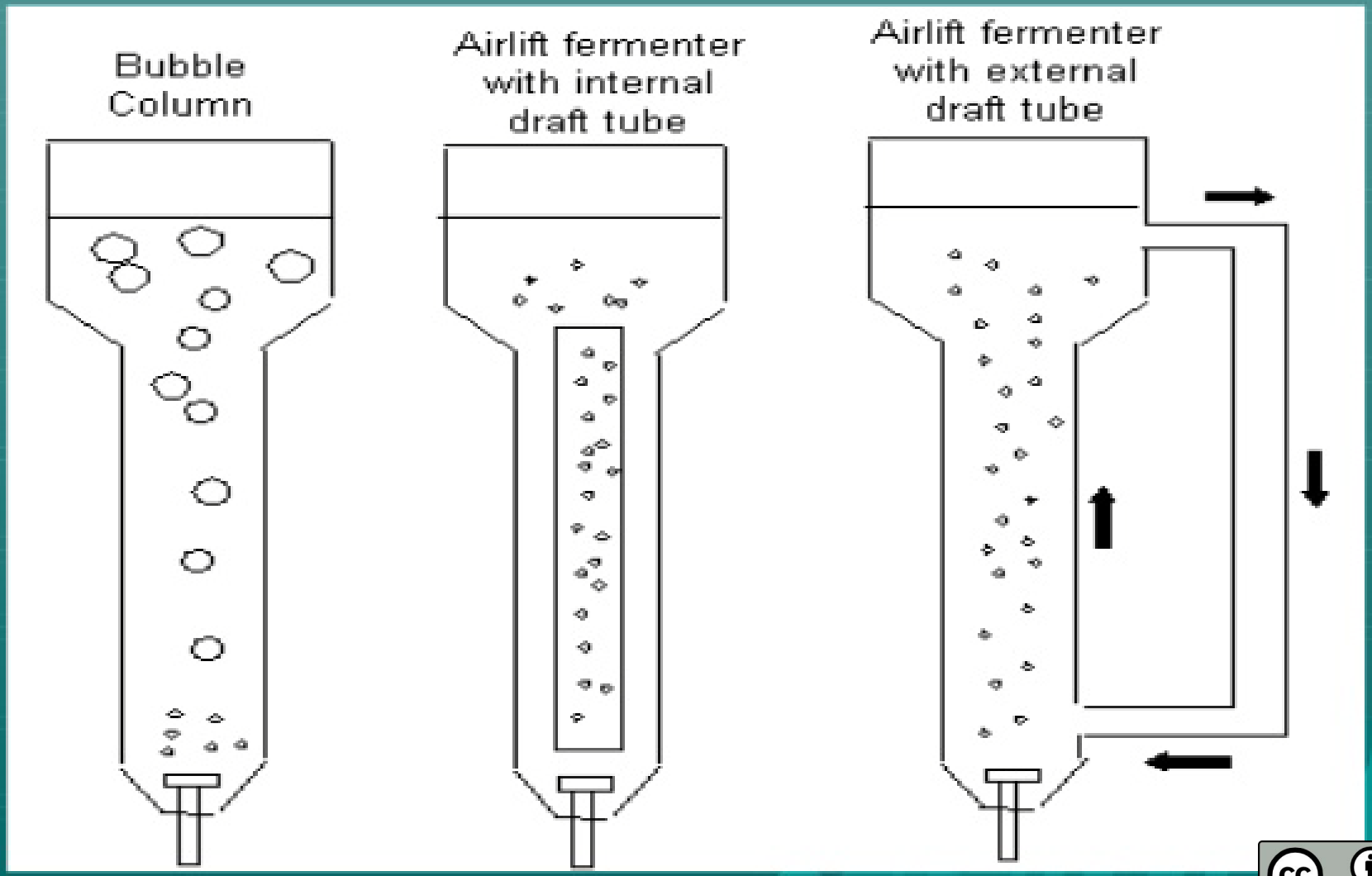


Mechanically stirred reactors - Sparged stirred tank bioreactors

- For liquid volumes greater than 3 litres, air sparging is required for effective oxygen transfer.
- The introduction of bubbles into the culture fluid by sparging, leads to a dramatic increase in the oxygen transfer area.
- Agitation is used to break up bubbles and thus further increase k_La.
- Sparged fermenters required significantly lower agitation speeds for aeration efficiencies comparable to those achieved in non-sparged fermenters.
- Air-sparged fermenters can have liquid volumes greater than 500,000 litres.


Increased surface area for oxygen transfer
Gas entrainment
Bubbles dramatically increase the oxygen transfer area

Bubble driven bioreactors

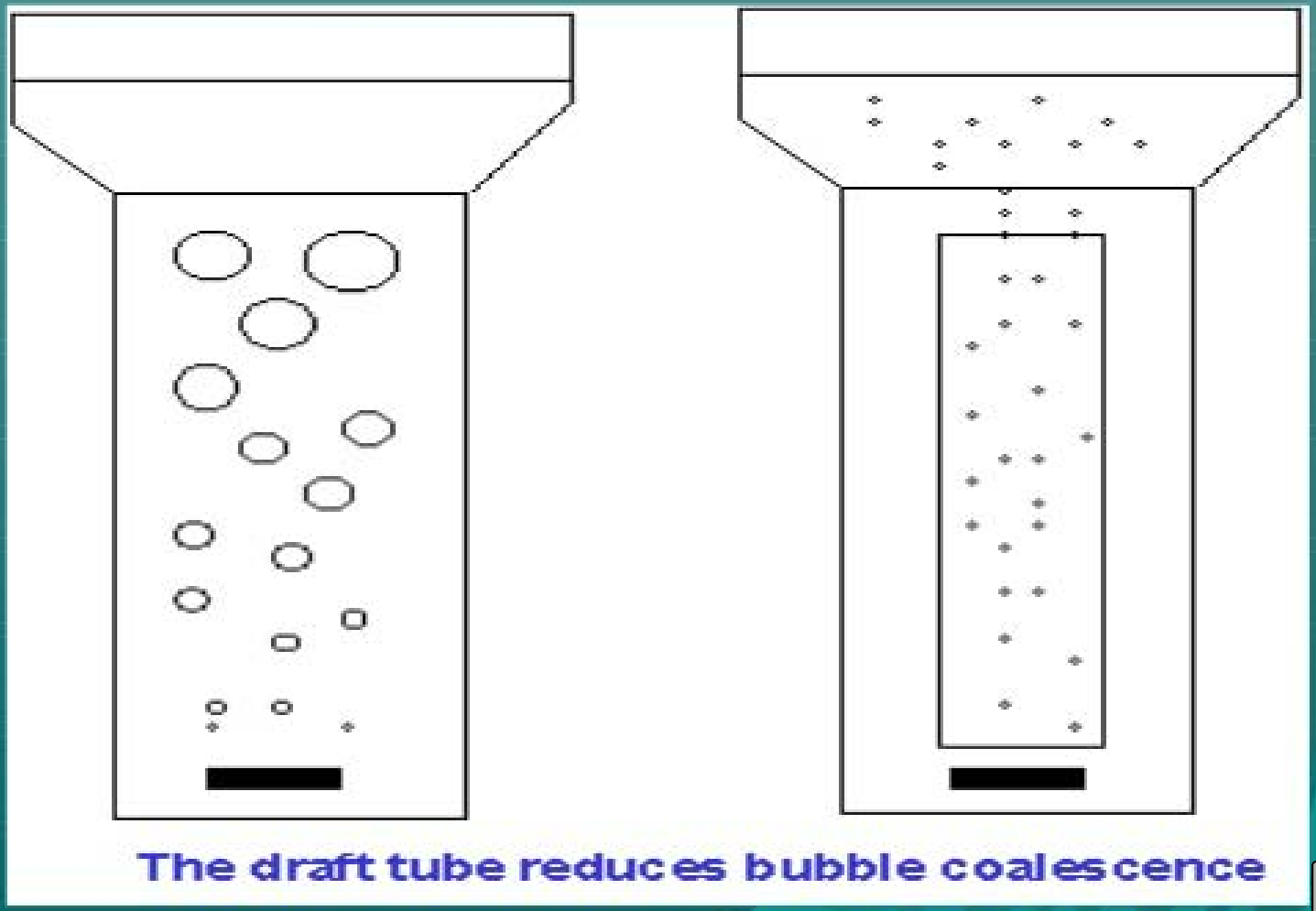


Bubble driven bioreactors

- An airlift fermenter differs from bubble column bioreactors by the presence of a draft tube which provides
  - better mass and heat transfer efficiencies
  - more uniform shear conditions.
- Bubble driven fermenters are generally tall with liquid height to base ratios of between 8:1 and 20:1.
- The tall design of these fermenters leads to high gas hold-ups, long bubble residence times and a region of high hydrostatic pressure near the sparger at the base of the fermenter.
- These factors lead to high values of k_La and C_o^* thus enhanced oxygen transfer rates

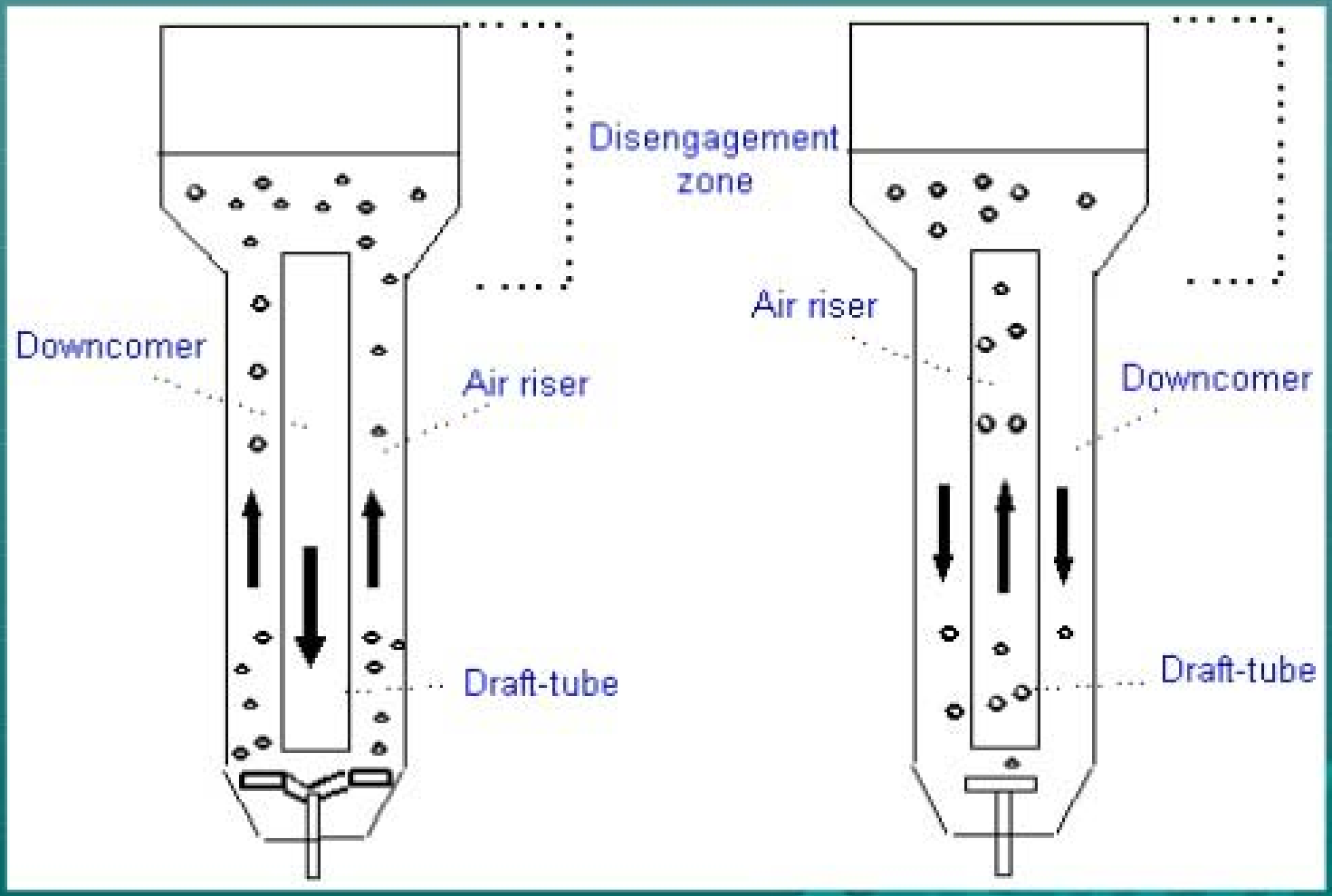
Airlift bioreactors

Small bubbles lead to an increased surface area for oxygen transfer.



The draft tube reduces bubble coalescence

Airlift bioreactor



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F. Kaštánek: Bioinženýrství, Academia, 2001

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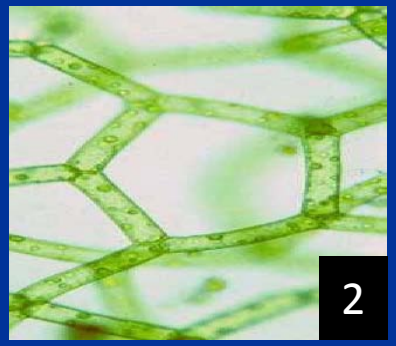
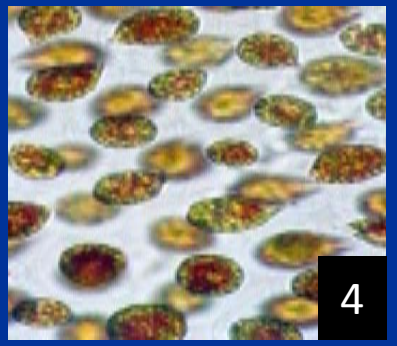
University of Chemistry and Technology Prague
Faculty of Food and Biochemical Technology

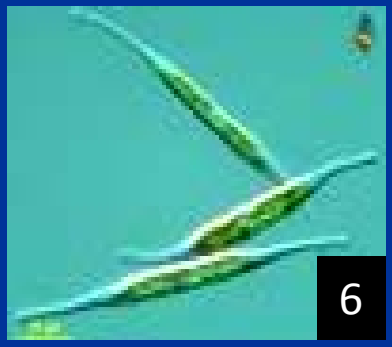
Specific aspects of microalgal cultivation

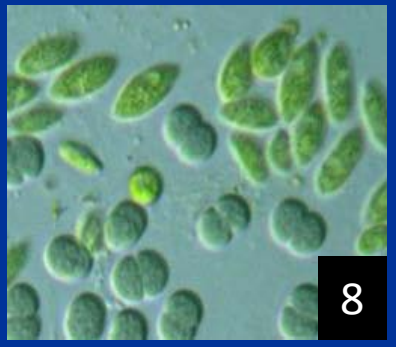
Tomáš Brányik

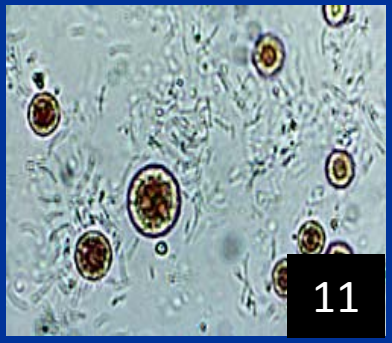
Examples of industrially promissing species

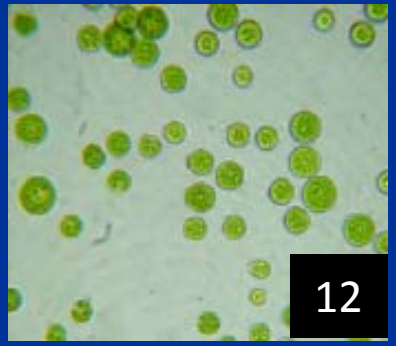


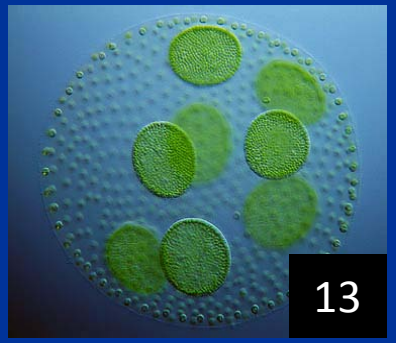


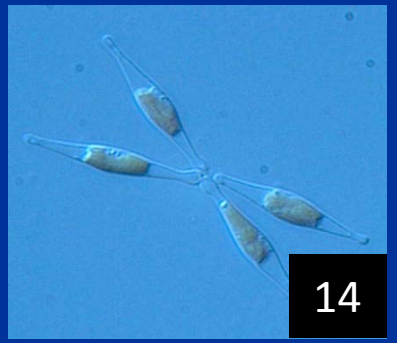








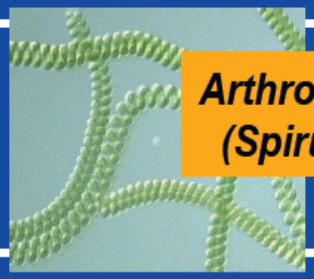




1, Spirulina; 2, Hidrodiction; 3, Dunaliella; 4, Haematococcus; 5, Nostoc;
6, Cylindrotheca; 7, Anabaena; 8, Synechocistis; 9, Chlamydomonas: 10, Scenedesmus;
11, Porphyridium; 12, Haematococcus; 13, Botryococcus; 14, Phaeodactylumy 15, Thalassiosira

What are microalgae/cyanobacteria?

- Primitive photosynthetic plants
- ~45,000 species
- Marine, freshwater, brackish water



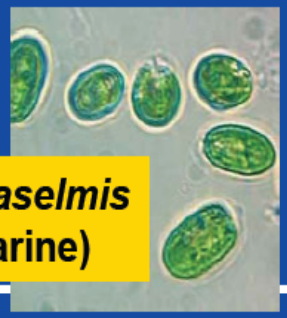
Arthrospira (Spirulina)

- Prokaryotes
- No nucleus

Five major groups

1. **Cyanobacteria**
2. **Green algae** (Chlorophyta)
3. **Chromista**
   - Diatoms
   - Brown algae
   - Calcifying algae
   - Macroalgae

***Scenedesmus* (freshwater/brackish)**



***Tetraselmis* (marine)**

Phaeodactylum tricornutum

Macroalgae - seaweeds

Microalgae/cyanobacteria…

Five major groups…

Water salinity based on dissolved salts in parts per thousand (ppt)			
Fresh water	Brackish water	Saline water	Brine
<0.5	0.5–30	30–50	>50

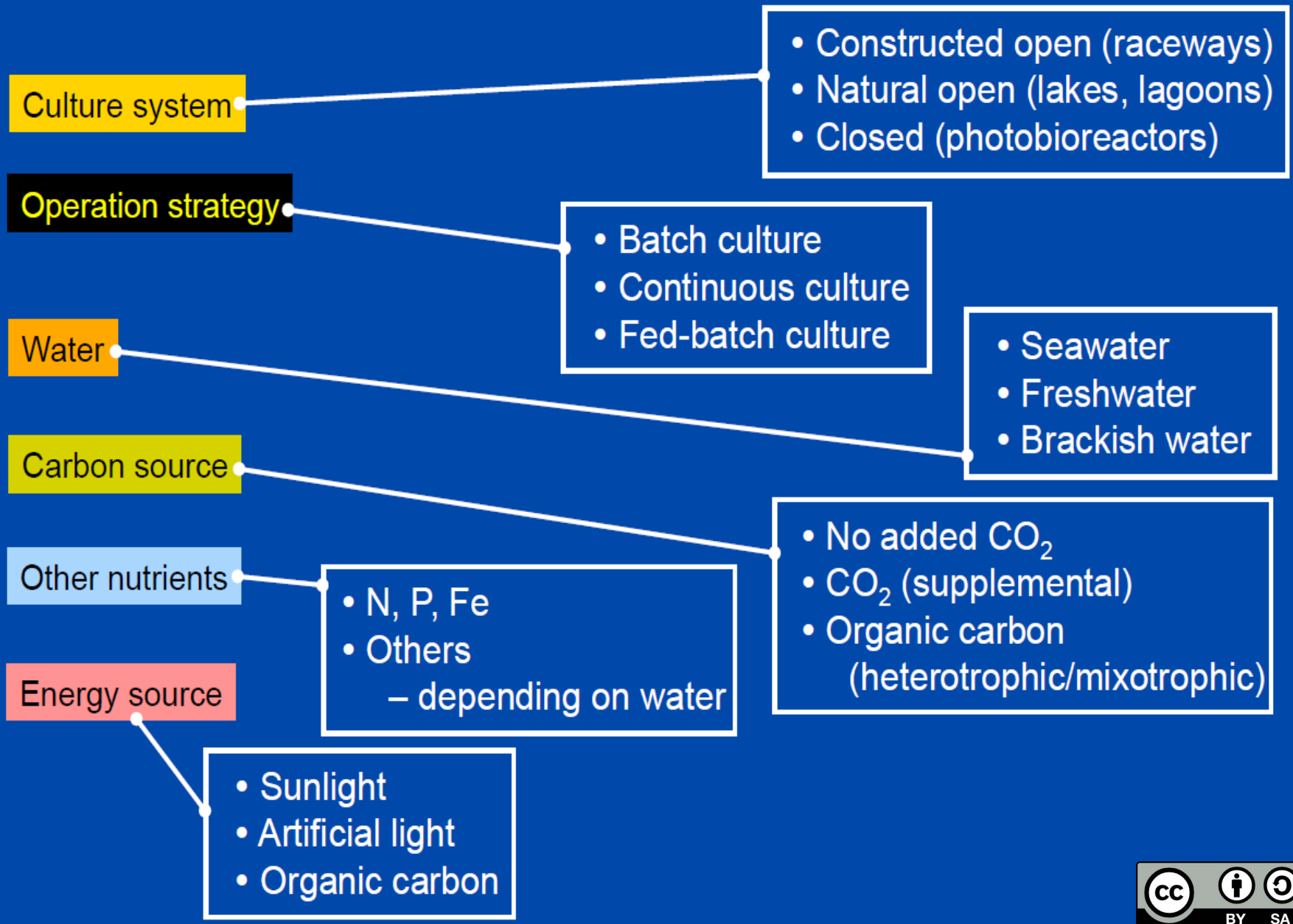
4. Red algae (Rhodophyta)

5. Euglenophyta (motile/phagocytosis)

A large group of flagellate protozoa. Many euglenophyta possess chloroplasts and so obtain energy through photosynthesis. These chloroplasts are surrounded by three membranes and contain chlorophylls *A* and *C*, along with other pigments, so are probably derived from a captured green alga.

A Euglena bloom in a freshwater pond

Algae culture requirements/options



Comment on water

Water usage is another important parameter. For the production of 1 liter of biofuel from fuel crops, approximately 10,000 liters of water are needed. Microalgae need much less water. For photosynthesis alone, ~0.75 liter of water is needed per kg of biomass produced. Per liter of biofuel, assuming a lipid content of 50%, 1.5 liters of water are required. In practice, water use in production systems is much larger because water is also used for cooling closed systems, and fresh water needs to be added to open ponds to compensate for evaporation.

Wijffels and Barbosa, *Science 329, 796 (2010)*

Algae culture requirements – major nutrients

For each metric ton of dry biomass

Carbon dioxide

- 0.507 tons of C (or 1.86 tons of CO_2)

Nitrogen

- 0.065 tons of N (or 0.813 t of NH_4NO_3)
- Significant energy input (~1.5% of global energy consumption)

Phosphorous

- 13.1 kg of P (or 65.5 kg of phosphate rock at 20% P in rock)

Comment on carbon dioxide

1.3 billion tons of CO_2 would be required for the production of 0.4 billion m3 of biodiesel to supply the European transportation market. The European Union produces about 4 billion tons of CO_2.

So production of microalgae could go some way toward relieving this CO_2 excess. However, the distance across which CO_2 may need to be transported in this context is a matter of concern.

Wijffels and Barbosa, *Science 329, 796 (2010)*

Comment on nutrients

The nutrients needed for the production of microalgae are nitrogen and phosphorus. The biomass of the algae consists of 7% nitrogen and 1% phosphorus. Consequently, for the European biofuel market ~25 million tons of nitrogen and 4 million tons of phosphorus are needed. This is about twice the amount that is presently produced as fertilizer in Europe.

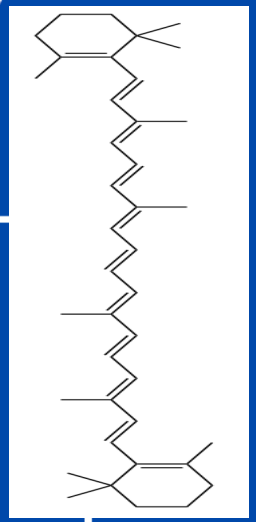
For sustainable production of biodiesel from microalgae, it will be important to make use of residual nutrient sources (about 8 million tons of nitrogen in Europe) and to recycle nutrients as much as possible.

Wijffels and Barbosa, *Science 329, 796 (2010)*

Microalgal products

Microalgae and cyanobacteria

Aquaculture



Agriculture

- Biofertilizers
- Soil inoculants
- Animal feed

Speciality chemicals & materials

Nutraceuticals/pharmaceuticals

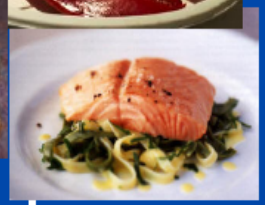
- PUFAs
- β-carotene

Pigments and colorants

- Astaxanthin

Others

- Insecticides
- Materials



Environment & energy

- Wastewater
- Biofuels

Energy options from algae

Microalgae

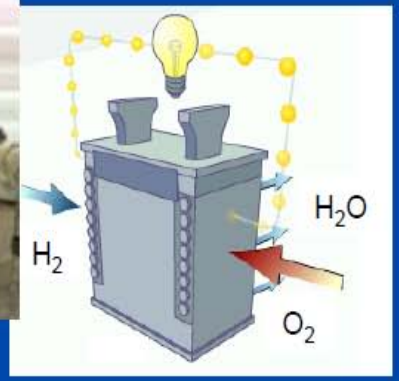
Biogas – methane
- Anaerobic digestion

Bioethanol
- Fermentation

www.algenolbiofuels.com

Biodiesel – microalgal oils
- Liquid hydrocarbon fuels
- Jet fuel

Biohydrogen
- H_2 fuel cells



Microalgae – an only option for biofuels?

The case of biodiesel

United States biodiesel needs = 0.53 billion m^3
(to replace all transport fuel – 2007 data)

Not feasible

Crop	Oil yield (L/ha)	Land area needed (M ha)	Percent of existing US cropping area
Corn	172	3,080	1,692
Soybean	446	1,188	652
Canola	1,190	446	244
Jatropha	1,892	280	154
Coconut	2,689	198	108
Oil palm	5,950	90	48
Microalgae	35,202	15.2	8
Microalgae	70,405	7.6	4

20% w/w oil in biomass

40% w/w oil in biomass

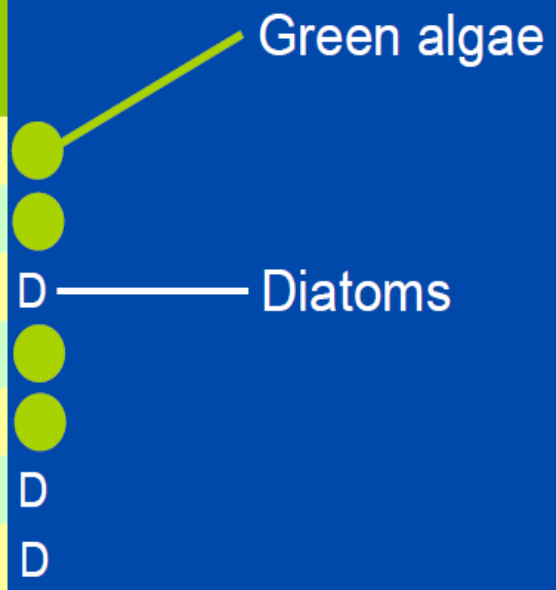
Proved productivity

1.535 kg m^{-3} day^{-1}

(~158 tons ha^{-1} $year^{-1}$)

Oil content of some microalgae

Microalga	Oil content (% dry wt)	
Botryococcus braunii	25–75	
Chlorella sp.	28–32	
Cylindrotheca sp.	16–37	D
Nannochloropsis sp.	31–68	
Neochloris oleoabundans	35–54	
Nitzschia sp.	45–47	D
Phaeodactylum tricornutum	20–30	D



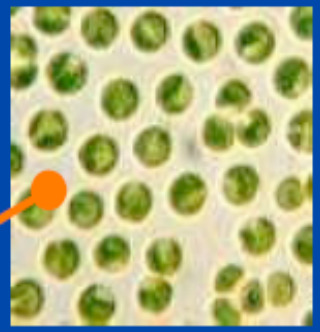
Oil productivity

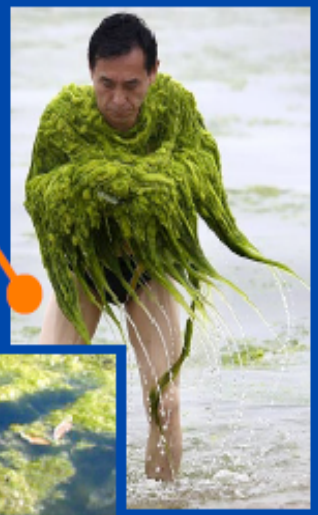
Oil productivity = dilution rate × biomass concentration × oil content

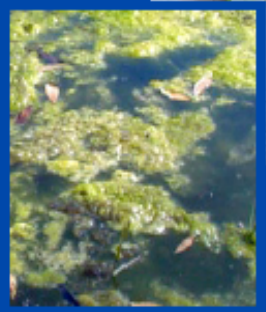
Attributes of a commercial microalga

Desired characteristics…

- High **oil productivity** during **growth**
- **Morphology** – **large, freely suspended** cells
  – no **films, filaments, surface growth**
- **Temperature tolerance** – both high and low
- **Robustness** – low sensitivity to shear force
- High value of **light saturation constant**
- **Open culture** – tolerant of **high/variable salinity**
  – good **resistance to competitors**





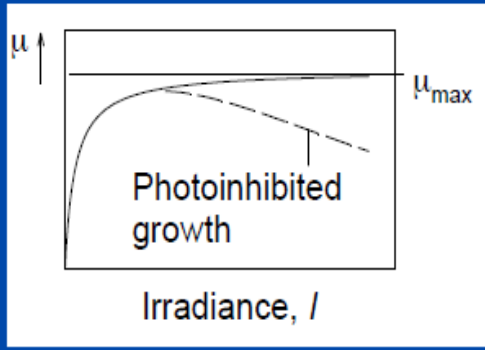


Improving the energy prospects of algae

Genetic and metabolic engineering issues

1. Increase **photosynthetic efficiency** and **biomass yield**
2. Biochemistry of **oil synthesis** - increase oil content in biomass
3. Improve **temperature tolerance** of algae
4. Reduce/eliminate **photoinhibition**
5. Incorporate **age-dependent cell lysis** to simplify **oil recovery**

Photoinhibition



Improving the energy prospects of algae…

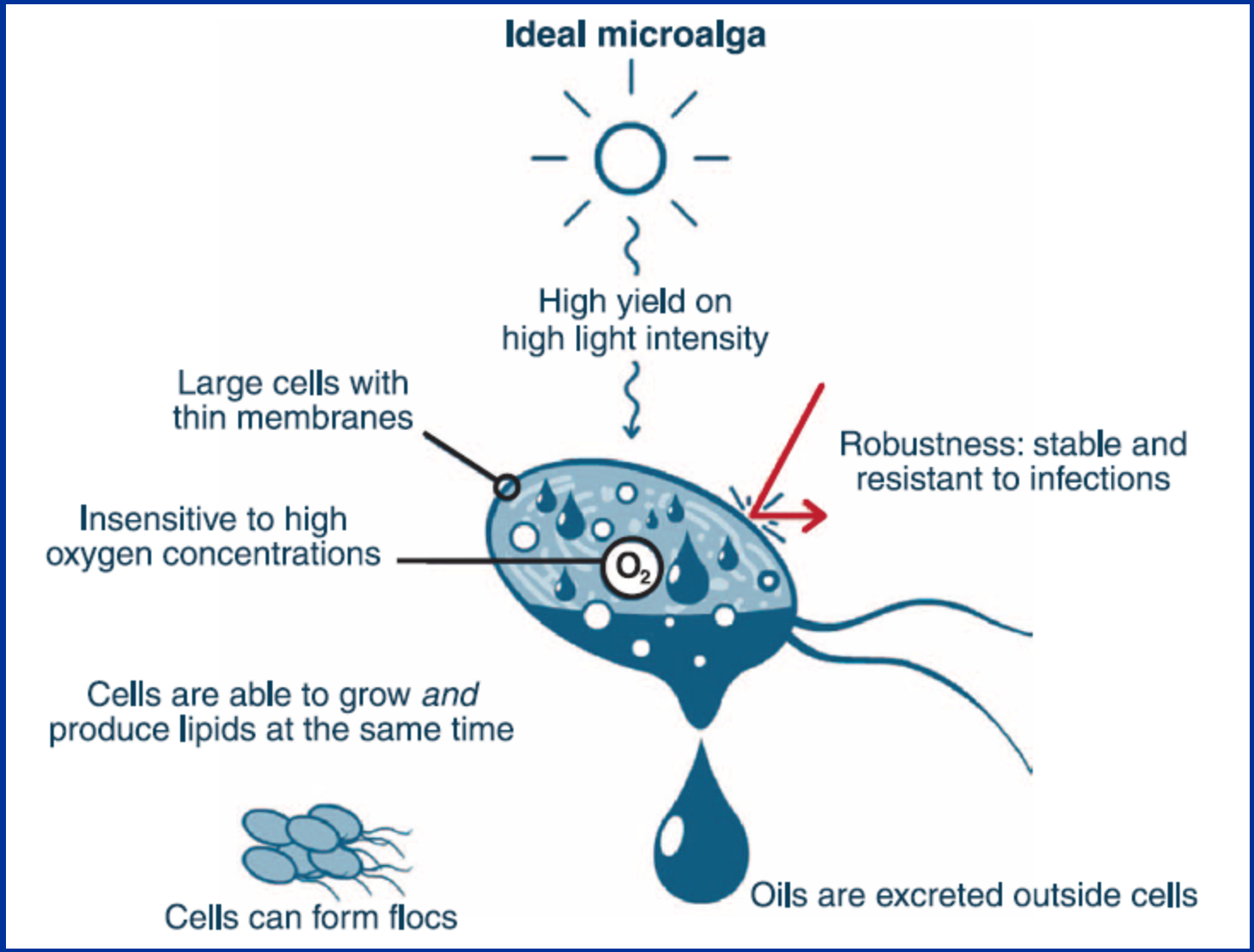
Bioprocess engineering

1. Low-cost **photobioreactors**

2. Low-cost and efficient **biomass recovery methods**
   - froth flotation
   - flocculation
   - rapid filtration/ultrasound

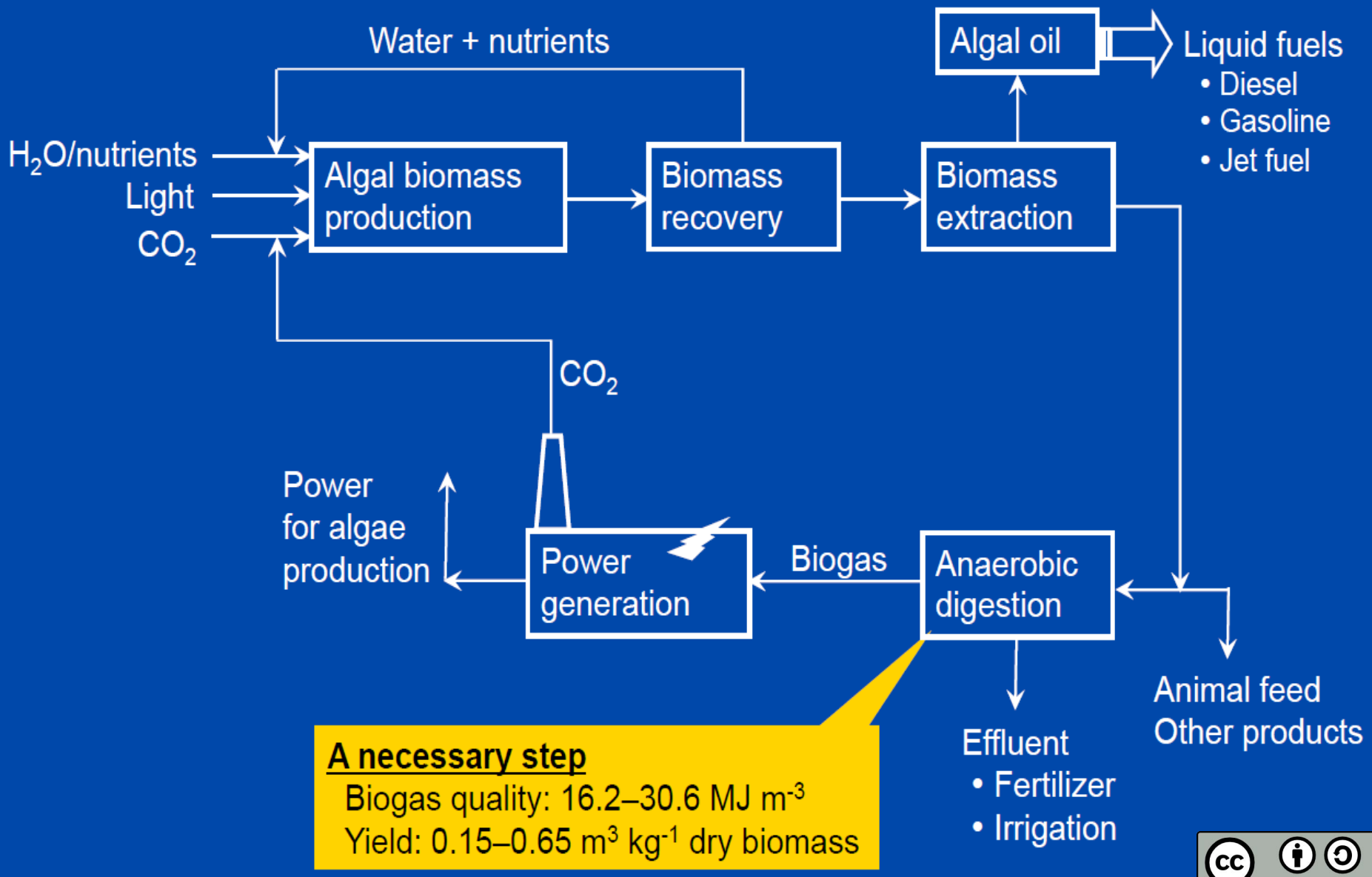
3. Inexpensive methods for **recovering lipids**

4. Improved productivity of **anaerobic digestion**

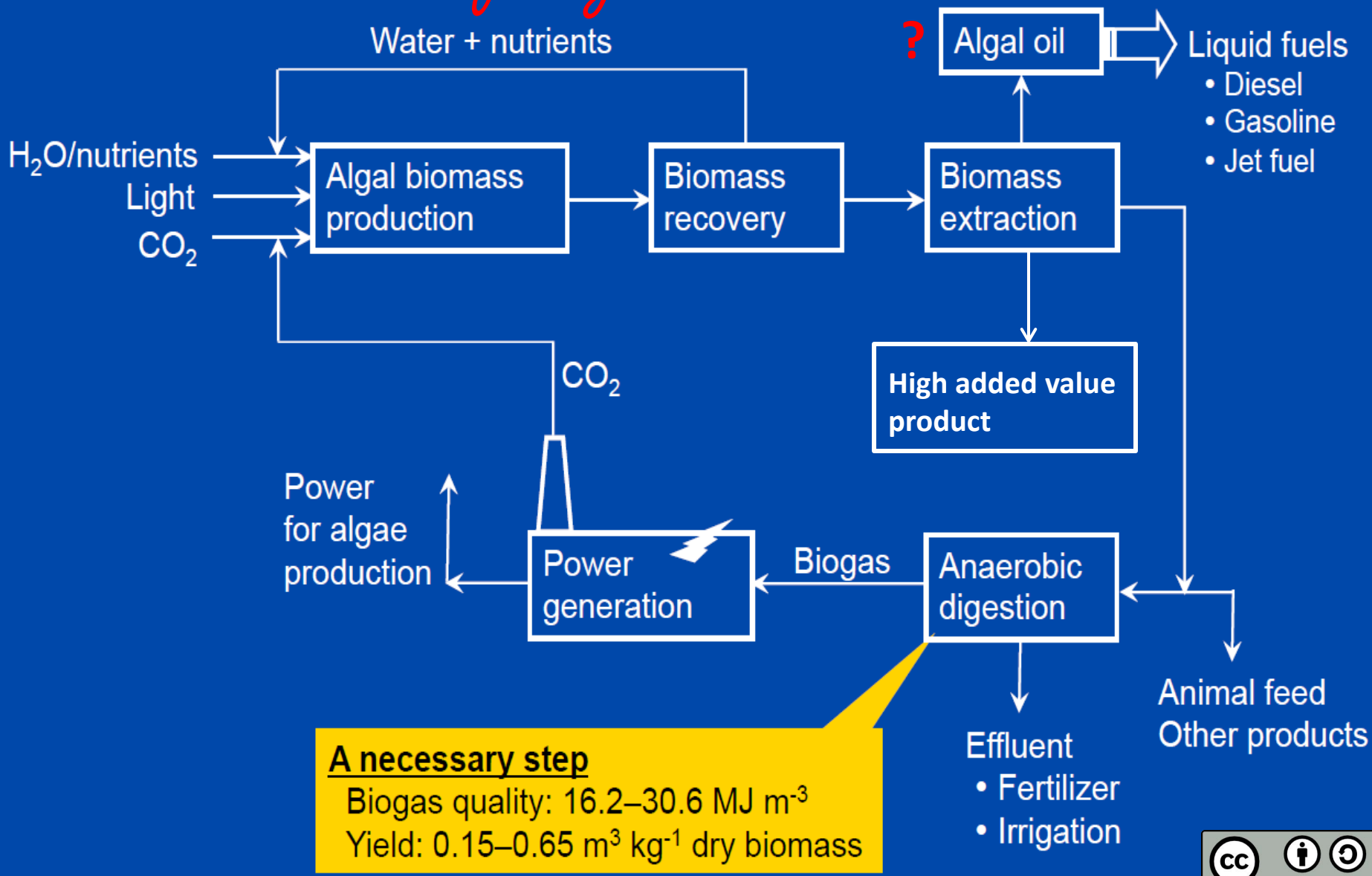


Wijffels and Barbosa, *Science 329, 796 (2010)*

Microalgal fuels process concept



Microalgal ~~fuels process~~ biorefinery concept



Algae cultivation systems: Ponds and Photobioreactors

Classification of photobioreactors

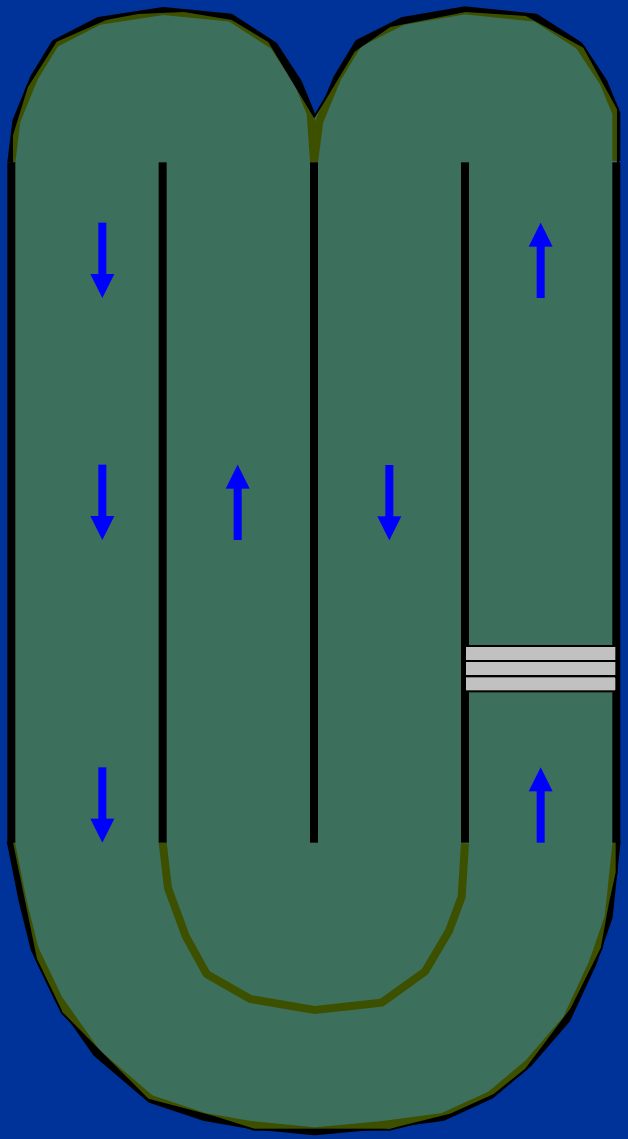
– based on construction

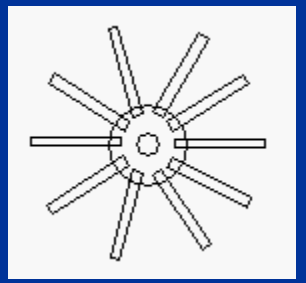
Open

Closed

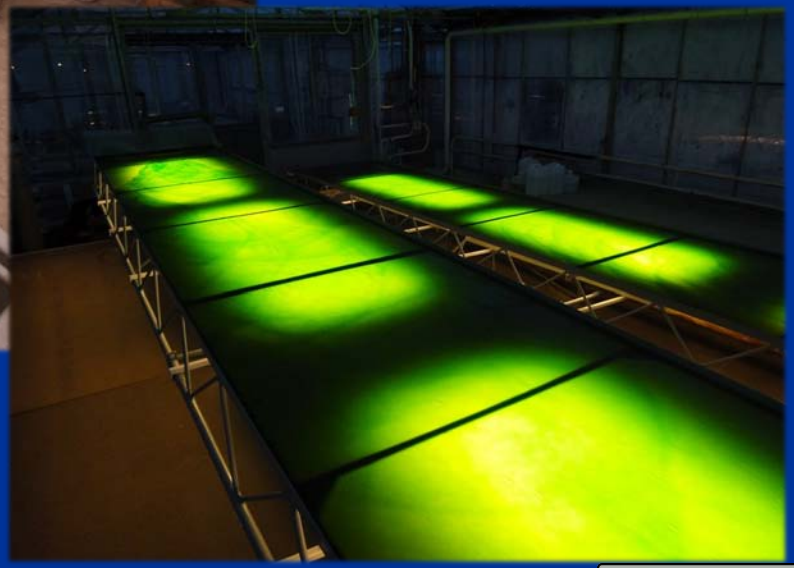
Raceway pond – lab scale

Raceway pond – large scale



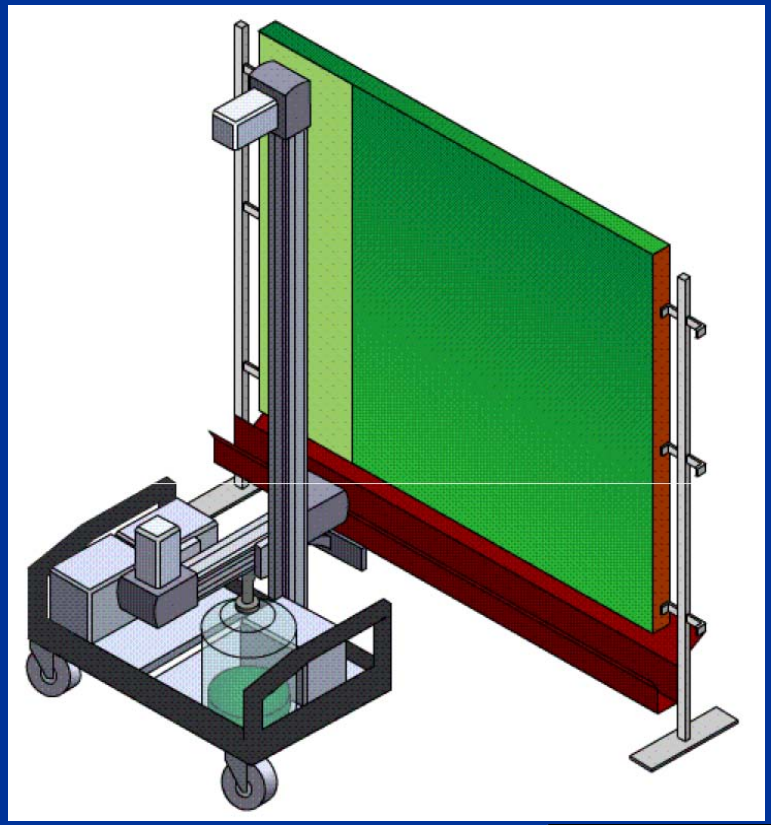


Thin layer photobioreactors

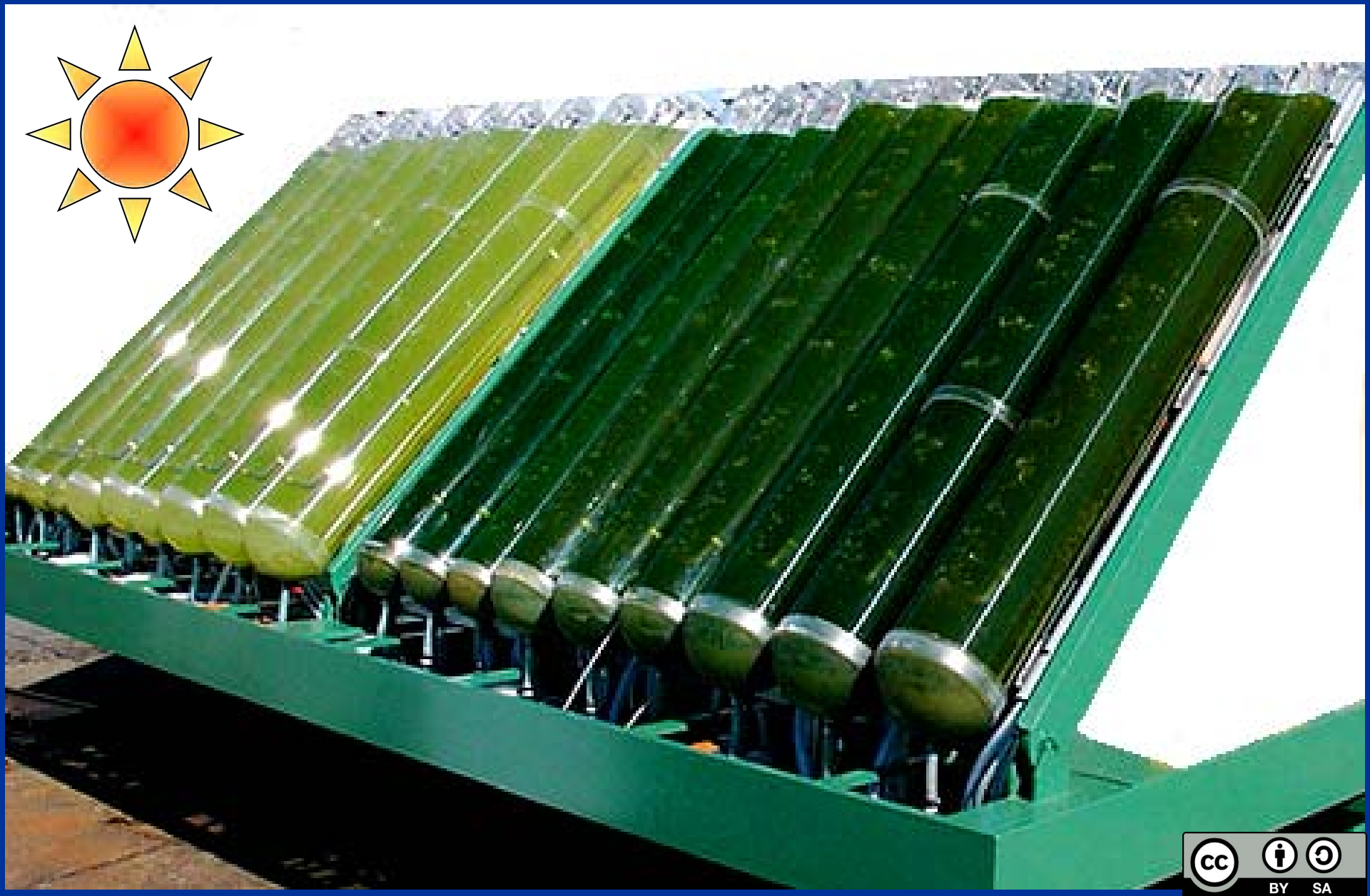


Biofilm like photobioreactors

Authomatic harvesting of algal biofilm



Closed photobioreactors

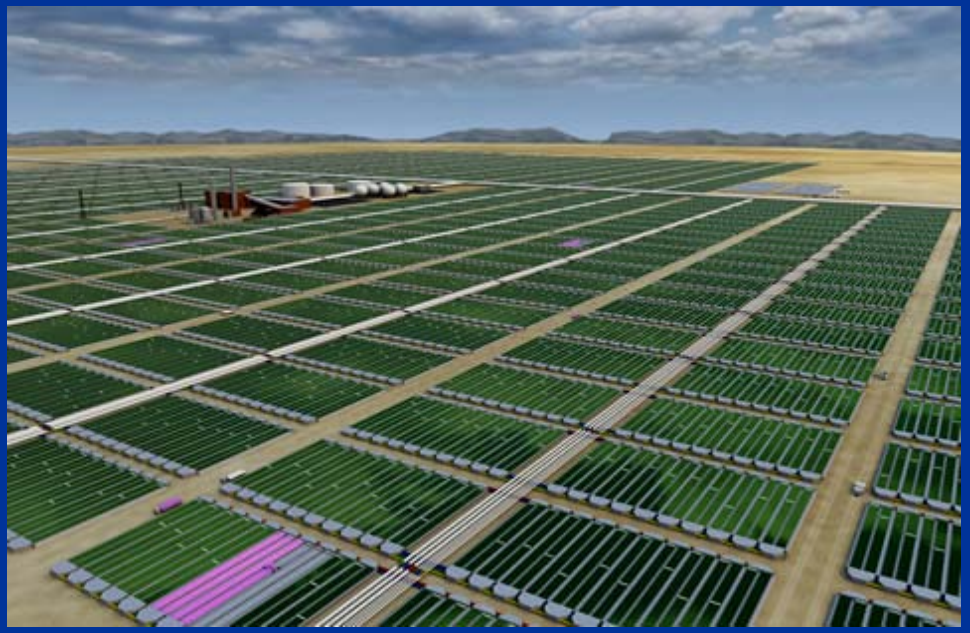


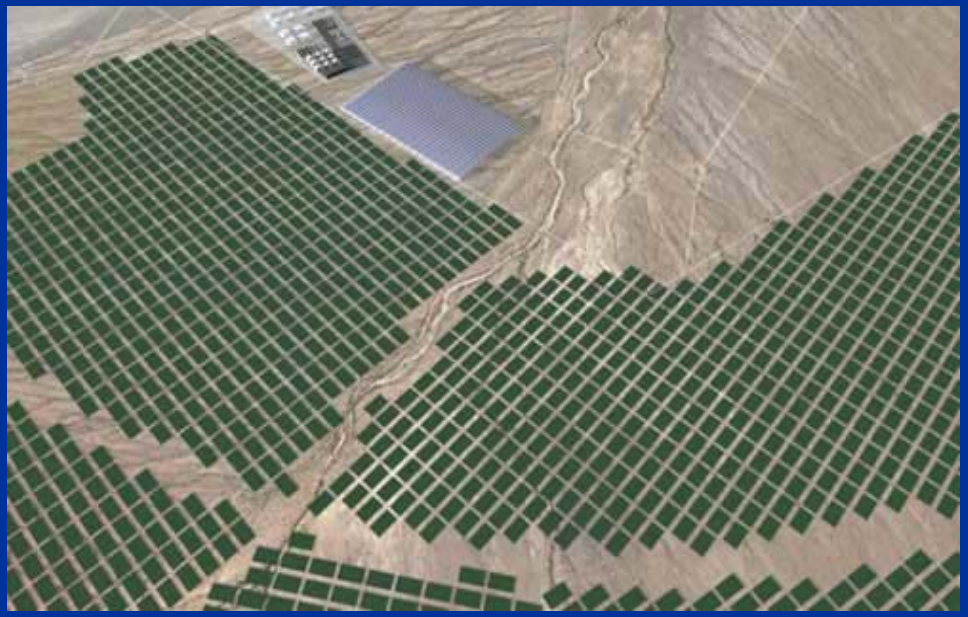
Closed photobioreactors (PhBR)

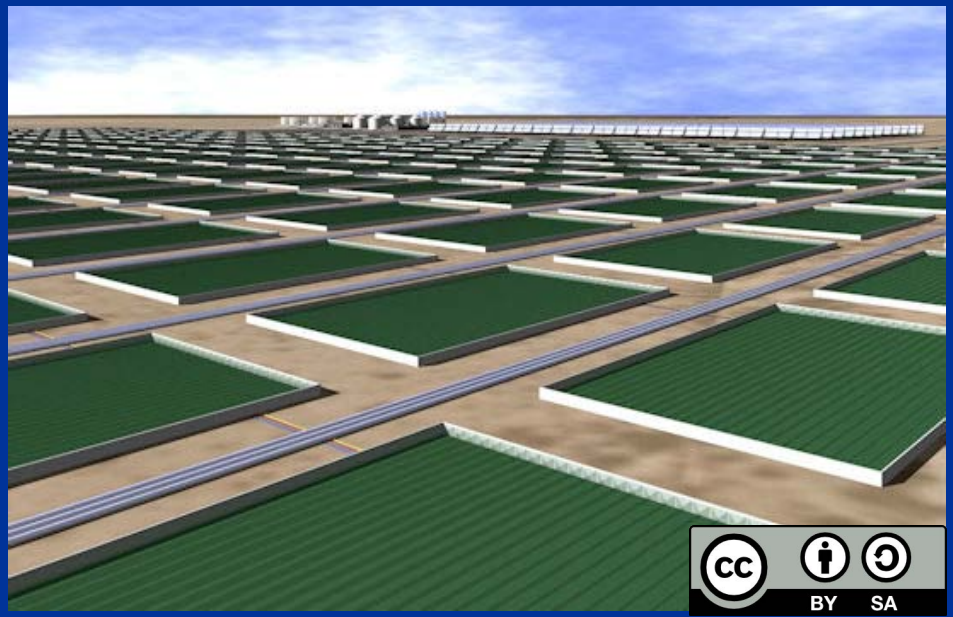
Classification based on geometry

- **Tubular**
  - **Vertical tubular PhBR**
  - **Horizontal tubular PhBR**
  - **Spiral tubular PhBR**
  - **„Triangle“ PhBR**
- **Panel PhBR**
- **Column PhBR (fermenters)**
- **Plastic bags**

Large scale cultivation







Large scale cultivation

Large scale cultivation

Need for cheap and available:

-Water

-Nutrients (N, P)

-CO_2

-Sunlight

Biomass price

Type	Production method	Cost ($\$ kg^{-1}$)	Notes/Assumptions
Cost	Open pond	5	*Spirulina* production
Cost	Open pond	3.60[a]	*Dunaliella* production
Estimate	Attached culture	0.70–0.97	Using dairy wastewater
Estimate	Open pond	6.93[a]	Netherlands location
Estimate	Closed PBR	5.81[a]	Netherlands location
Estimate	Open pond	3.80	Free CO_2
Estimate	Closed PBR	2.95	Free CO_2
Forward-looking estimate	Open pond	1.79[a]	Free CO_2 and growth media, 60% improved photosynthetic efficiency, Dutch Antilles location
Forward-looking estimate	Closed PBR	0.98[a]	Free CO_2 and growth media, 60% improved photosynthetic efficiency, Dutch Antilles location
Forward-looking estimate	Open pond	0.60	100× increased production for better economy of scale
Forward-looking estimate	Closed PBR	0.47	100× increased production for better economy of scale

[a] Calculated using a conversion factor of 1.4 dollars per euro.

Fuel price USA:
Gasoline 0.45-0.68 USD/L
Diesel 0.63-0.76 USD/L

Fuel price CZ:
Gasoline 1.09 Euro/L
Diesel 1.055 Euro/L

Product price

Microalgae	Annual production	Producer country	Application and product	Price (€)
Spirulina	3000 tonnes dry weight	China, India, USA, Myanmar, Japan	Human nutrition Animal nutrition Cosmetics Phycobiliproteins	36 kg^{-1} 11 mg^{-1}
Chlorella	2000 tonnes dry weight	Taiwan, Germany, Japan	Human nutrition Cosmetics Aquaculture	36 kg^{-1} 50 l^{-1}
Dunaliella salina	1200 tonnes dry weight	Australia, Israel, USA, Japan	Human nutrition Cosmetics B-carotene	 215–2150 kg^{-1}
Aphanizomenon flos-aquae	500 tonnes dry weight	USA	Human nutrition	
Haematococcus pluvialis	300 tonnes dry weight	USA, India, Israel	Aquaculture Astaxanthin	50 l^{-1} 7150 kg^{-1}
Crypthecodinium cohnii	240 tonnes DHA oil	USA	DHA oil	43 g^{-1}
Shizochytrium	10 tonnes DHA oil	USA	DHA oil	43 g^{-1}

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Downstream processing - Algal biomass recovery and dewatering

Algal cells and broth

Algal **cell density**: 1080–1110 kg m^{-3}

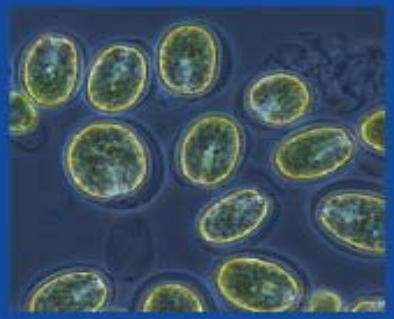
Density of **seawater**: 1020–1030 kg m^{-3}

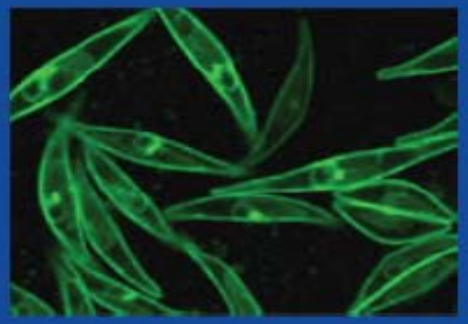
Density of **freshwater**: 1000 kg m^{-3}

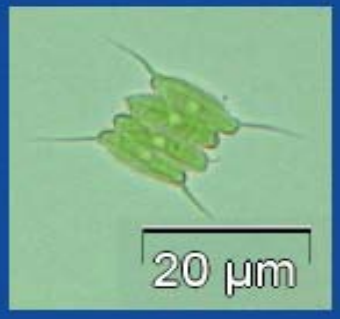
Algal **cell diameter**: 3–30 μm

Biomass concentration in broth: 0.5–5 g L^{-1}

Diverse **morphologies**:



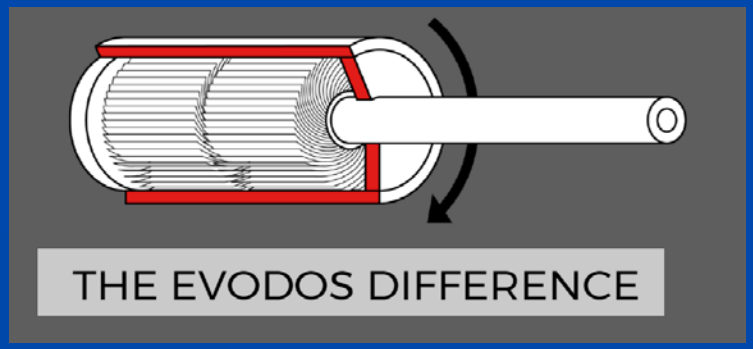




Centrifugation

- Separation of **biomass** from **water**
  - Single cells
  - Flocs of cells

- Sedimentation tank with **enhanced gravitational field**
  - For example: 3000–15 000 × g



Using centrifuges

Expensive

- Capital investment
- Operating cost

High energy consumption

- 0.9 – 8 kW h (per m3)

Algae concentration in paste

- 15–22% (by dry wt)
  (78–85% water)

Flocculation

Algae cells in broth

Flocculant

Floc

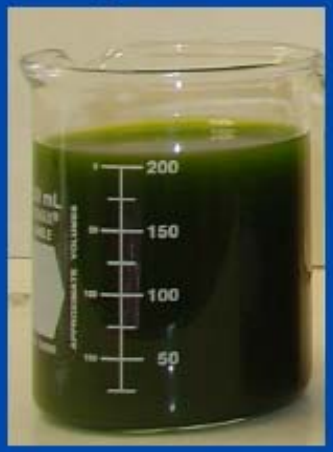
Flocculants

- Multivalent cations: Al^{3+}, Fe^{3+}
- Cationic polymers (polyelectrolytes)
- Acid (H^+)

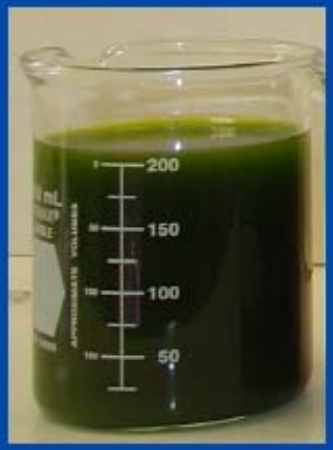
Flocculation – the process

Three steps…

Flocculant



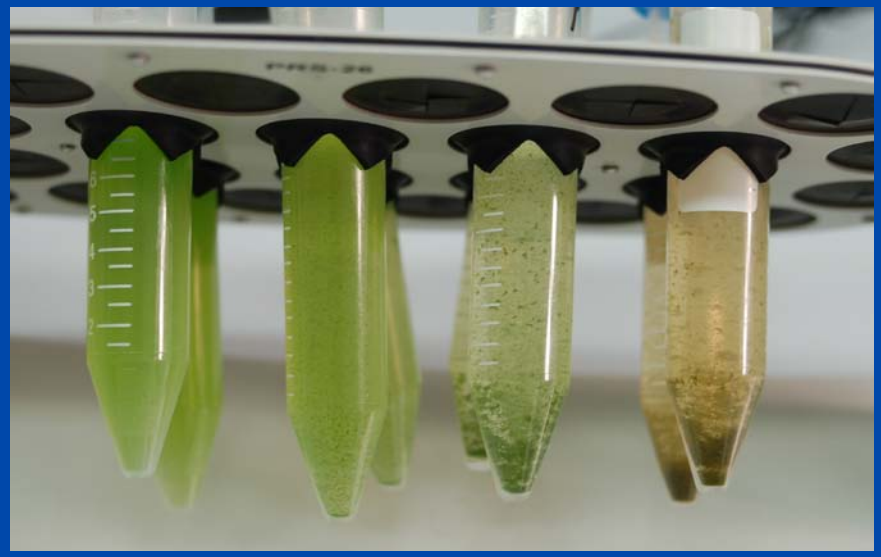
Add flocculant and mix (rapid mixing)



Flocculation (gentle mixing)

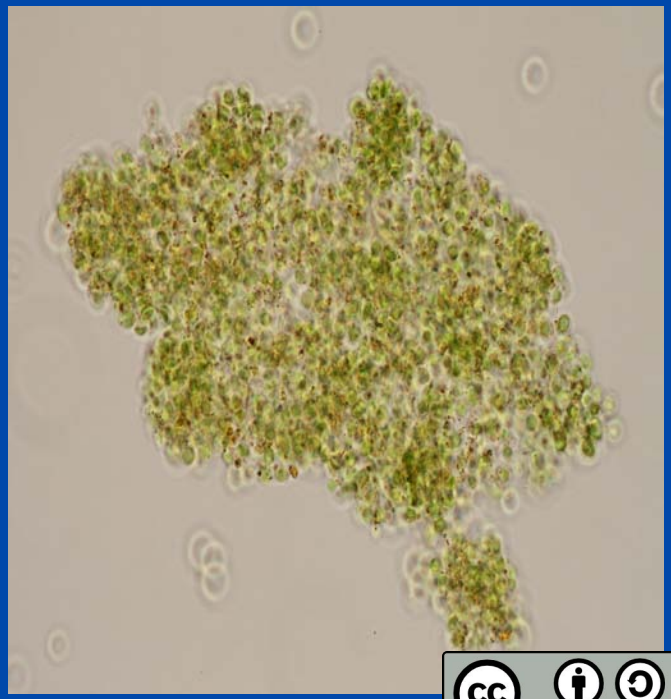
Sedimentation (no mixing)

Suitable flocculants

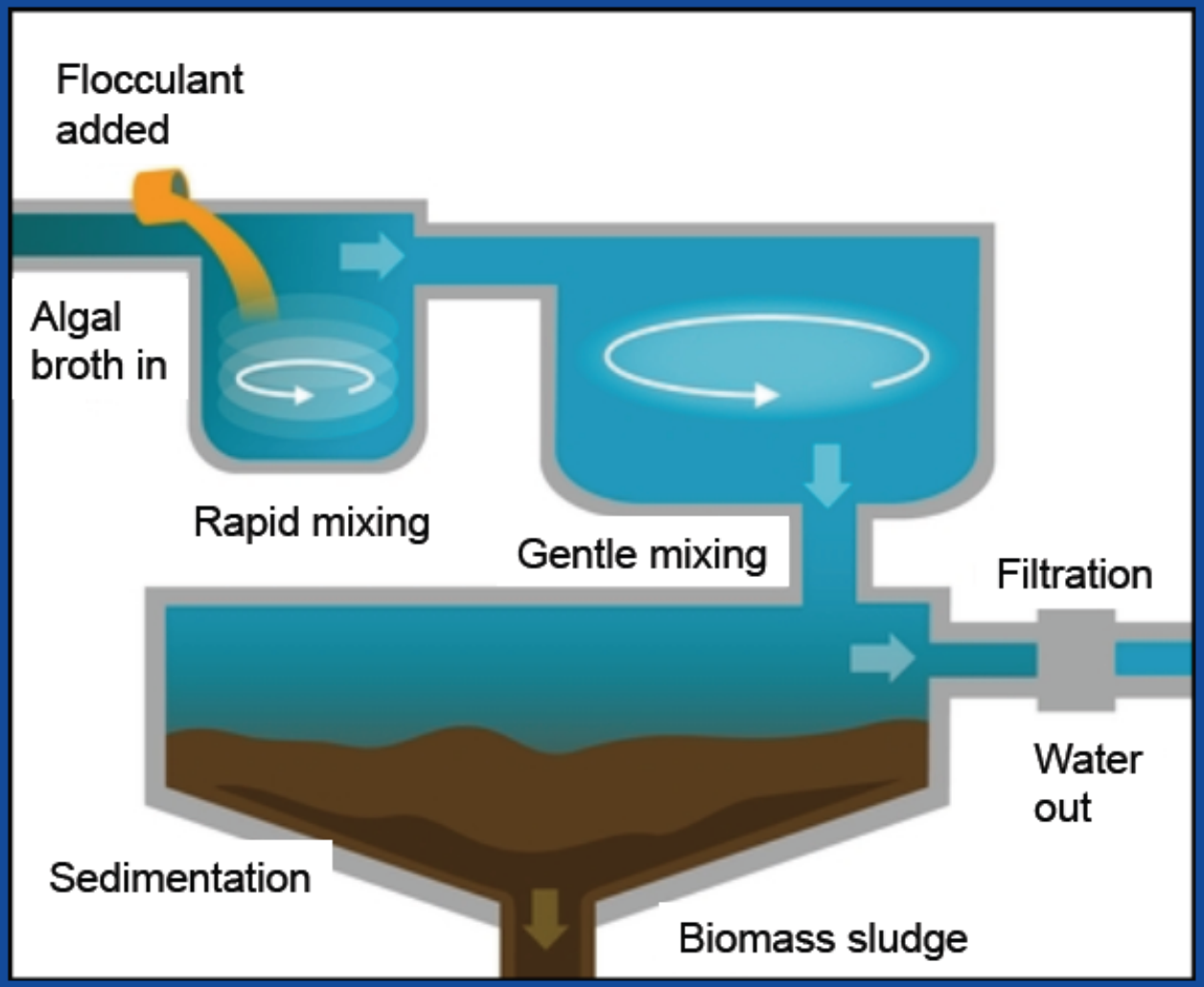


1. Must be **cheap**
2. **Effective**
3. Available in **quantity**
4. **Safe** – negligible impact on environment

- Aluminium sulfate ($Al_2(SO_4)_3$)
- Flocculants based on magnetic particles
- Flocculants based on waste materials
- Polymeric flocculants



Flocculation – industrial implementation

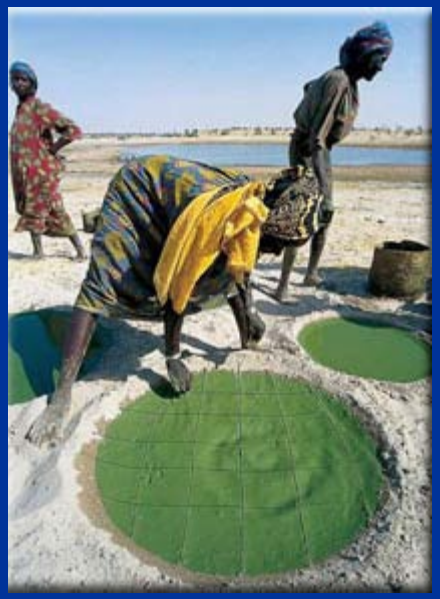


Extraction and purification of microalgal biomass

Dehydration processes

The harvested biomass slurry (typical 5–15% dry solid content) must be processed rapidly after harvest

- sun drying (slow)
- low-pressure shelf drying
- spray drying (expensive)
- drum drying
- fluidized bed drying
- freeze drying (expensive, additional cell disruption)



Extraction and purification of microalgal biomass

Extraction and purification of biofuels

• Dry extraction of biofuels with solvents - it is important to establish a balance between the drying efficiency and cost-effectiveness of extraction to keep the maximum net energy output of the fuels.

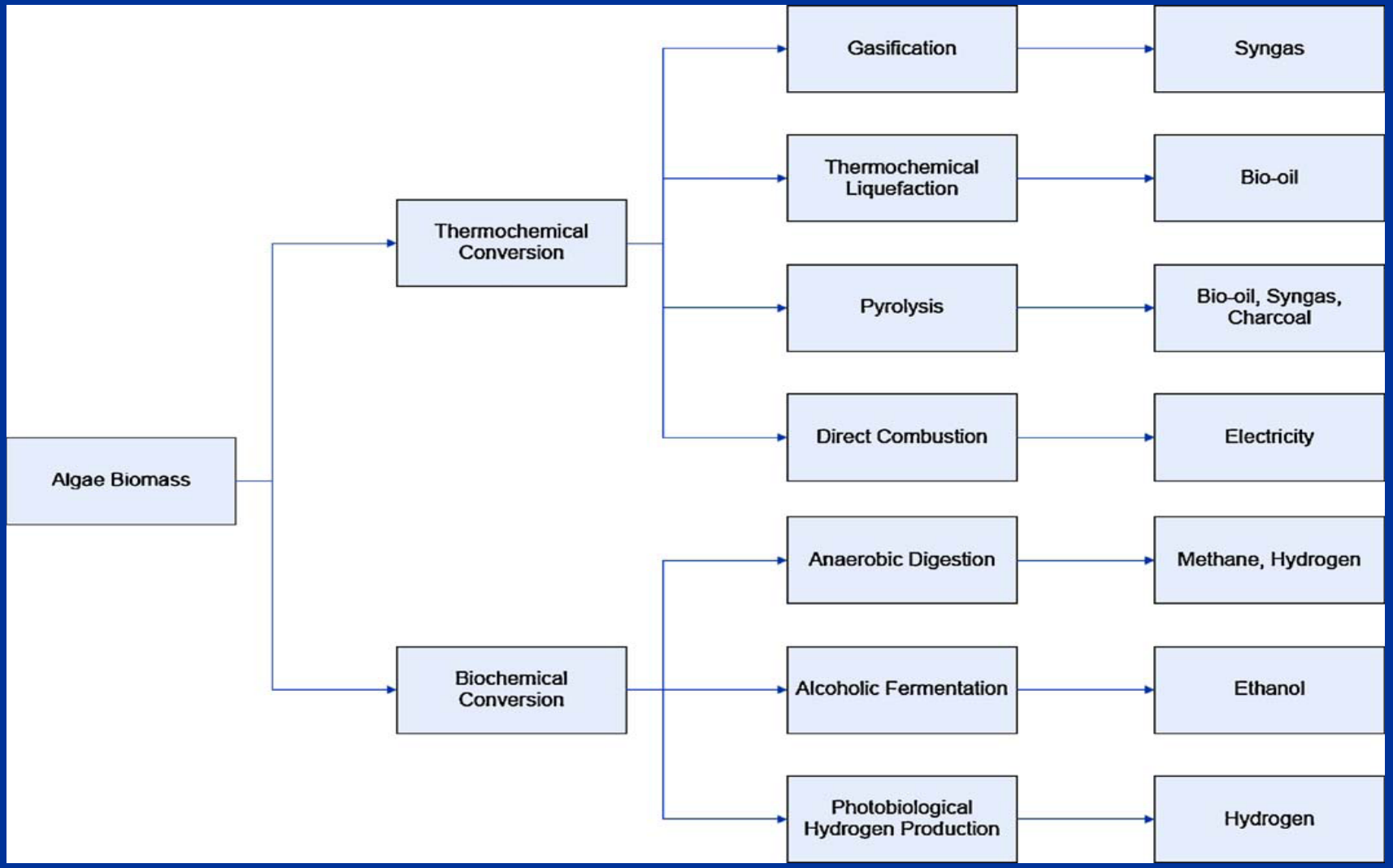
• Wet extraction process that uses enzymes or combines ultrasound and electromagnetic pulse induction to break the algae cell walls. Alternative is mechanical oil press.

Extraction and purification of microalgal biomass

Extraction and purification for algal metabolites

- Cell disruption is often required for recovering intracellular products from microalgae.
- Cell disruption methods include: high-pressure homogenisers, enzymatic hydrolysis, autoclaving, acidic or alkaline lysis.
- Solvents are widely used to extract metabolites such as astaxanthin, β-carotene and fatty acids from algal biomass.

Potential algal biomass conversion processes



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C. Posten, S.C. Feng (Edts.): Microalgae Biotechnology, Springer, 2016

University of Chemistry and Technology Prague
Faculty of Food and Biochemical Technology

EUROPEAN UNION
European Structural and Investing Funds
Operational Programme Research,
Development and Education

MINISTRY OF EDUCATION,
YOUTH AND SPORTS

Industrial cultivation of insect cells

Introduction

- Production of recombinant proteins
- universal platform – insect cells infected with baculovirus (**IC/BEVS** = **insect cell/baculovirus expression vector system**)

- First cultivation of insect cells – 1915 (Goldschmidt, moth *Samia cecropia*)
- First recombinant baculovirus – 80. of 20. century

- IC/BEVS
  - Relatively cheap
  - safe
  - baculovirus – high capacity
  - an effective alternative to human and animal cell lines
  - easy scale-up (insect cells – robust growth)

Biotechnological use of IC/BEVS

- Recombinant proteins
  - prokaryotic, eukaryotic, intracellular, extracellular, membrane
  - multimeric protein complexes
  - veterinary and human vaccines
- Gene therapy (modified baculovirus, AAV)
- Fundamental research
  - interactions between the host cell and the pathogen
  - metabolism and cell physiology
  - cellular immunity
  - insecticides

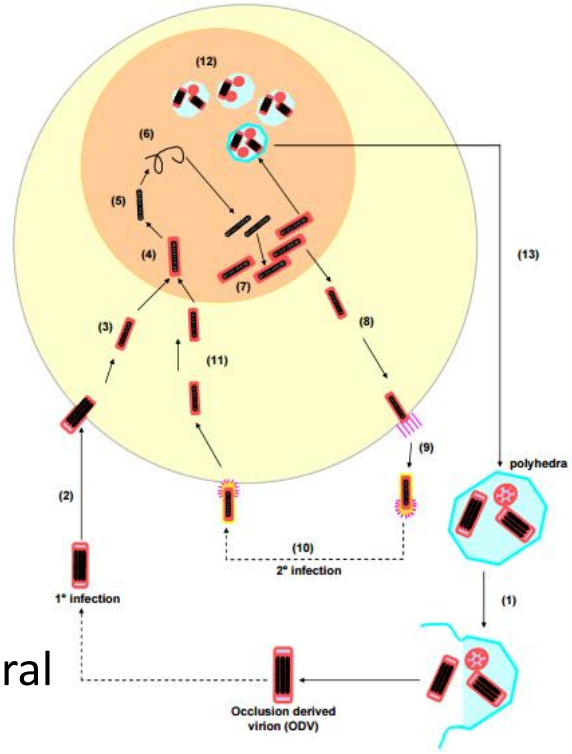
Charakteristics of IC/BEVS

- baculovirus
  - AcMNPV = *Autographa californica* multiple nuclear polyhedrosis virus
  - pathogen of insect larvae (arthropods)
  - large, wrapped, dsDNA

- Insect cell lines (tody over 600)
  - order Lepidoptera (moles and butterflies)
  - *Spodoptera frugiperda* (***Sf*-9**, *Sf*-21)
    - *Trichoplusia ni* (***Tn*-5**)
    - *Bombyx mori* (*Bm*-5, *Bm*N)
  - order Diptera (flies and mosquitoes)
    - *Drosophila* Schneider 2 S2

Construction of recombinant baculovirus

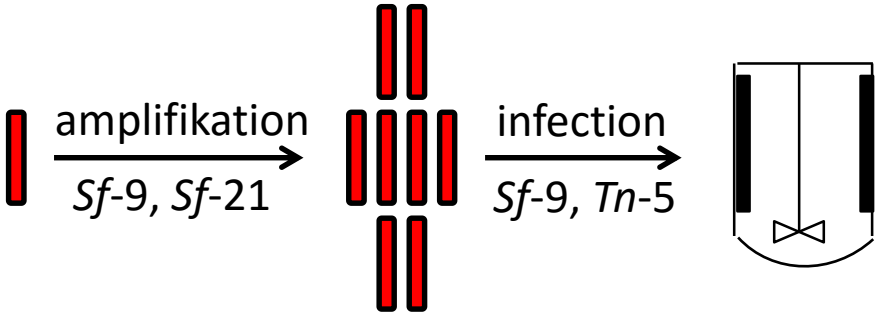
- Life cycle of baculovirus
  - entry into the host cell (endocytosis)
  - transport to the nucleus
  - release of viral DNA
  - replication → origin of EV, OV
  - infection of other cells, lysis
- Replication cycle – 2 step
  - 2 phenotyps of virus
  - extracellular viral particles (EV) - transmission of viral DNA between cells
  - enveloped viruses (OV) - polyhedrin capsule, virus survival
- recombinant baculovirus
  - polyhedrin promotor (*polh*) + recombinant gene



Haines F., Possee R., King L. (2007) Baculovirus Expression Vectors. http://www.expressiontechnologies.com/pdf/BEV%20Paper.pdf

Construction of IC/BEVS

- process



- disadvantage - lytic character → cell lysis (→ release of proteas → negative effect on protein quantity and quality, complications of purification)
- today - can vector be transfected into chromosomal DNA insect cells → non-genetic character → high yields, rapid expression

Design of bioprocess

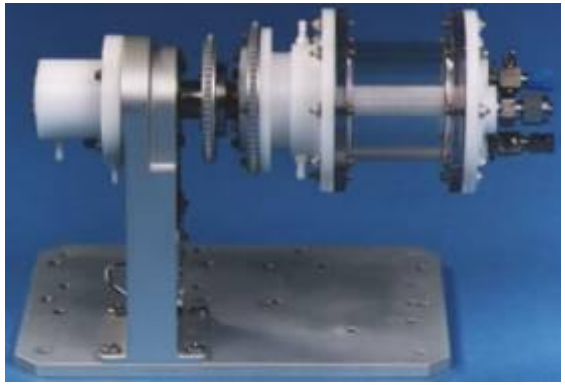
- temperature – opt. 27 °C (CDW = 0.6 – 1.8 g l^{-1})
- pH – opt. 6,0 – 6,4
- Medium composition
  - Organic compounds, amino acids, salts
  - serum – no need
  - C source – glucose
  - N source – amino acids (Asp, Asn, Glu, Gln, Ser, Ala)
  - lipids, cholesterol, vitamins
- oxygen – at least OTR = OUR
- Shear forces – cell sensitivity

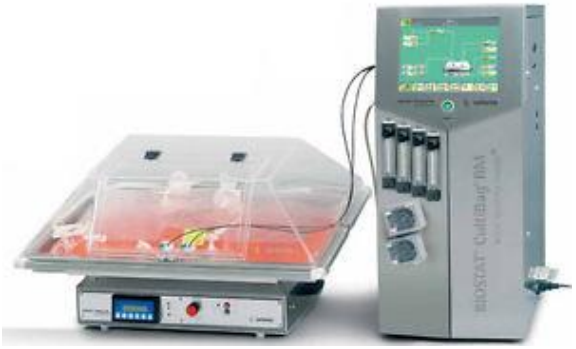
Cultivation

- Adhered layers of cells
- Suspension
  - Shaken flasks
  - Agitated flasks (spinners)
  - rollers
  - bioreactors
    - Batch
    - Fed-batch
    - Perfusion
    - Continuous

Types of bioreactors

- Mechanically agitated
  - agitators – radial, axial
- Rotating bioreactors
- Wave agitation bioreactors
- Single use bioreactors





- aeration
  - surface
    - Oxygen above surface
    - Minimum stress
    - Requires high S:V
  - Gas sparging
    - Bubble size

Key parameters of bioprocess

- multiplicity of infection = **MOI**
  - number of viral particles per cell
  - commercial production of recombinant proteins – MOI > 1 (→ 100 % synchronized infection)
- time of harvest = **TOH**
  - Opt. TOH – specific for given protein (protease sensitivity)

Downstream processes

- techniques - easy scale-up
- microfiltration, chromatography
- removal of dirt
  - derived from the product (other conformation → other biological activity)
  - "Process" impurities (proteins, DNA, viruses, proteases)
- Quality controll
  - SDS-PAGE
  - ELISA
  - microscopy (TEM or SEM)
  - N-/C-terminal sequencing og protein
  - Mass spectrometry
  - HPLC
  - qPCR
  - …

Bioreactors for animal and plant cells

Bioreactors for Animal and Plant Cells Growth („single use“ bioreactors)

- In bioreactors where the goal is to grow cells or tissues for therapeutic purposes, the design is significantly different from industrial bioreactors.
- Cells are grown either in suspension or in adherent cultures.
- Many cells and tissues, especially mammalian ones, must have a structural support in order to grow and agitated environments are often destructive to these cell types and tissues.Higher organisms also need more complex growth media.

Bioreactors for Animal and Plant Cells

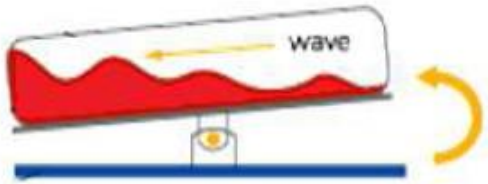
- **Growth of animal cells in a suspension**
  - The CELLine bioreactor is a disposable, two-compartment cultivation device suitable for many cell culture applications, e.g. the production of monoclonal antibodies on a laboratory scale. Efficient cell cultivation is dependent on an optimal supply of oxygen and nutrients.
  - The two-compartment bioreactor is designed by dividing the bioreactor into a medium compartment and a cell compartment. A semi-permeable membrane between the compartments allows small molecules to diffuse from one compartment to the other. Higher molecular weight molecules secreted by the proliferating cells are retained within the cell compartment.
  - The CELLine is perfectly suited for a wide range of applications involving suspension cell culture, like monoclonal antibody production or long-term continuous culture maintenance.

Bioreactors for Animal and Plant Cells

Bioreactors for Animal and Plant Cells

- **Bioreactors with rocking motion**
  - Disposable (plastic) are suitable for microbial and mammalian cells (*in suspension or adherent*): Cell culture medium and cells contact only a pre-sterile, disposable chamber, the cell bag, that is placed on a *special rocking platform*. The rocking motion of this platform induces waves in the cell culture fluid. These waves provide mixing and oxygen transport, resulting in a perfect environment for cell growth. These cell culture devices are suitable for application in animal, virus, insect and plant cell cultures in suspension or on micro carriers, disposable cell culture systems for 0.1 –500 liters of the volumes.

Bioreactors with rocking motion



Bioreactors for Animal and Plant Cells

- **Single use bubble-less bioreactors**

- Its main feature is a *membrane aeration stirrer* which enables controlled and gentle mixing, bubble free aeration while avoiding foam generation. The gassing membrane ensures higher oxygen transfer and thus optimal growth conditions and higher cell densities compared to standard spinner flasks.

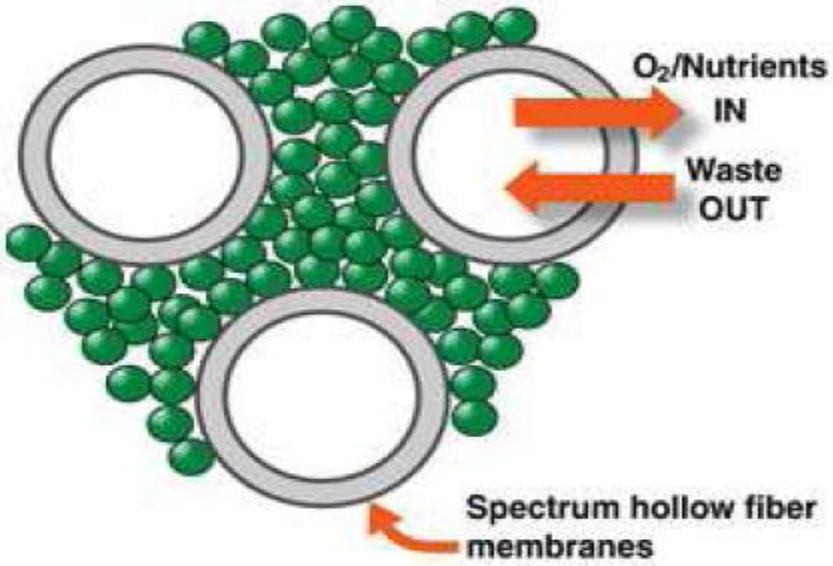
Industrial-size single use bioreactors for animal cell cultivation

PadReactor system provides enhanced gas exchange, due to its moving sparger. It also achieves low shear mixing due to the vertices of the square tank, which act as natural baffles. These advantages, plus the system's scalability, make it ideal for the production of vaccines, monoclonal antibodies and other secreted proteins

Bioreactors for Animal and Plant Cells

- **Single use membrane bioreactors**
  - Growth of a hybridoma culture, along with production of monoclonal antibody, was demonstrated over extended periods in polysulfone hollow fiber membrane modules.
  - Hollow fibers are tubular membranes with pore sizes ranging from 10 kD to 0.3µm. Cells grow on and around the large surface area provided by the network of hollow fibers. When perfused with culture media, the hollow fibers allow oxygen and nutrients to be supplied to the cells while metabolic waste products are eliminated.

Single use membrane bioreactors



Hollow fiber module and scheme of the live- mammalian cells cultivation

Literature

H.P. Meyer, D.R. Schmidhalter (eds.): Industrial scale suspension cultures of living cells. Wiley Blackwell, 2014

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Operational Programme Research,
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MINISTRY OF EDUCATION,
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Agitation and Mixing of Liquids

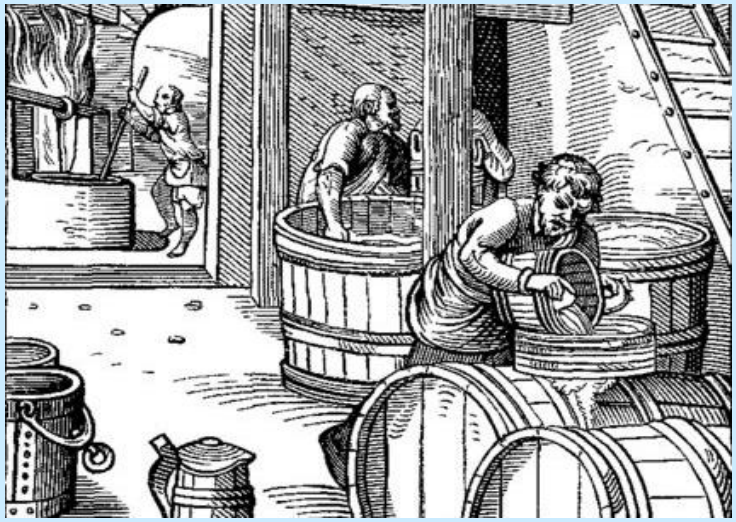
Agitation

Induced motion of a material in a specific way, e.g. in a circulatory pattern inside of container.

Mixing

Random distribution of two or more initially separate phases. The degree of homogeneity can be very different.

Historical agitation



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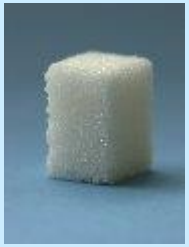
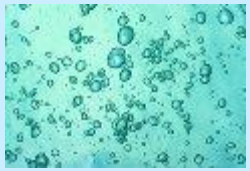


→ Will be the sugar dissolution rate and even tea sweetness affected by agitation?

\+

→ Will sugar consistency affect the rate of dissolution?



→ <u>Analogy with sugar</u>
Will mixing affect bubble size and consequently the rate of oxygen dissolution?

&

→ Will agitation of tea / soup affect heat transfer rate?

Purposes of agitation:

- Suspending solid particles
- Blending miscible liquids
- Dispersing gas through the liquid in the form of small bubbles
- Dispersing second liquid, immiscible with the first, to form an emulsion
- Promoting heat transfer between the liquid and a coil of jacket

---

- *To achieve concentration homogeneity*
- *To achieve heat homogeneity*
- *To improve heat and mass transfer*

Engineering problem

One type of agitator does not meet all requirements!

Solution

1. Defining requirements – choice of the agitator is affected by the aspect most important for the process
2. Design of the mixer is done with regard to operational requirements and economic considerations

Outline of lecture

1. Definition of terms
2. Division of mixing methods
3. View of mixing under different magnification
3. Classification of impellers
4. Power Definition and description of Power Correlations
5. Method of selecting the stirrer by power + calculations
6. Influence of agitator geometry and liquid properties on power
7. Mixing time
8. Dispersing effect of agitator
9. What You Should Know

Methods of agitation – creation of forced flow

Mechanical: usually rotary, in small volumes also vibratory stirrers.

Pneumatic: The gas is dispersed in the liquid by the distributor at the bottom of the vessel. The rising gas drives the liquid to move.

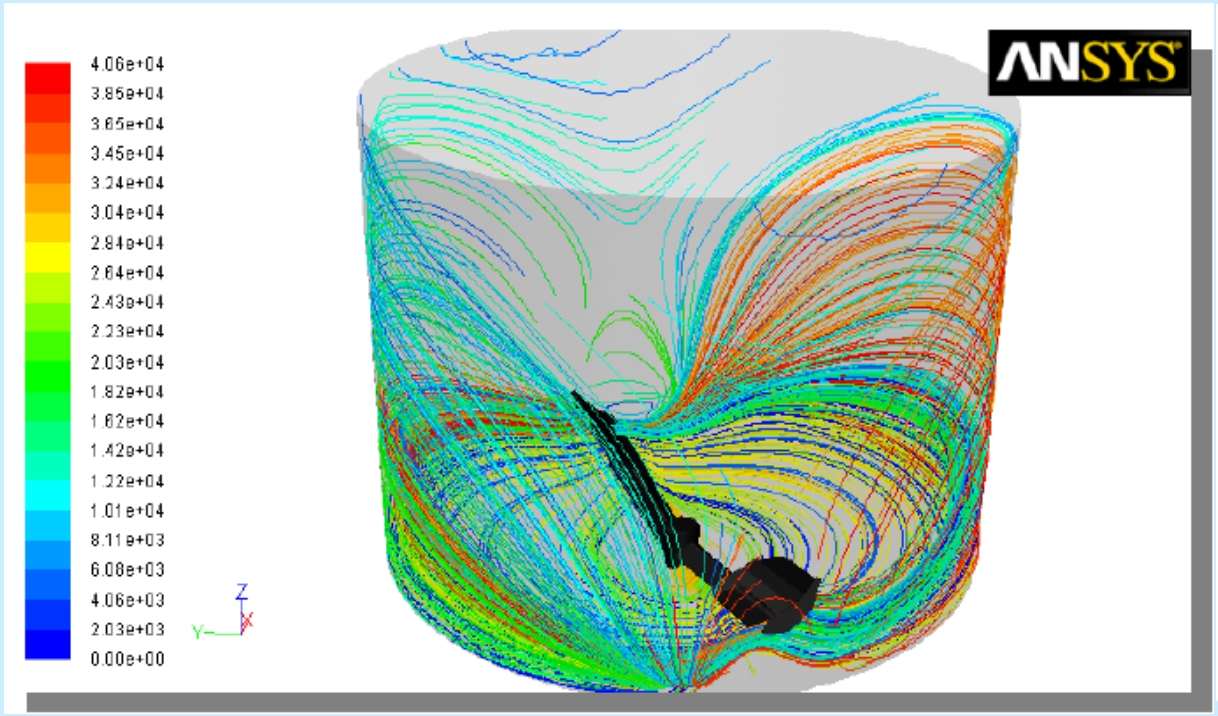
Hydraulic: a conical-shaped liquid emerges from the submerged nozzle, pulls and pushes the liquid, and forms a swirl at the edge of the stream.

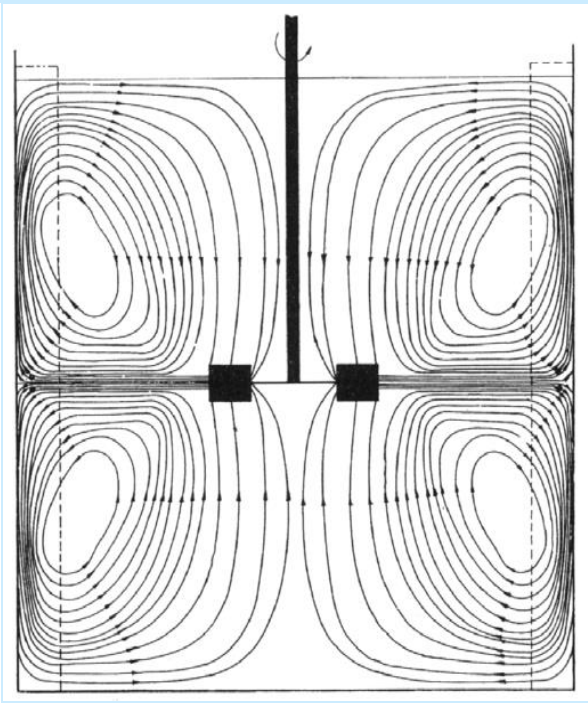
Static: When flowing in the pipeline, mixing of different layers of liquid in the radial direction is created.

Mixing with mechanical rotating agitators (impellers)

View of mixing under the magnifying glass

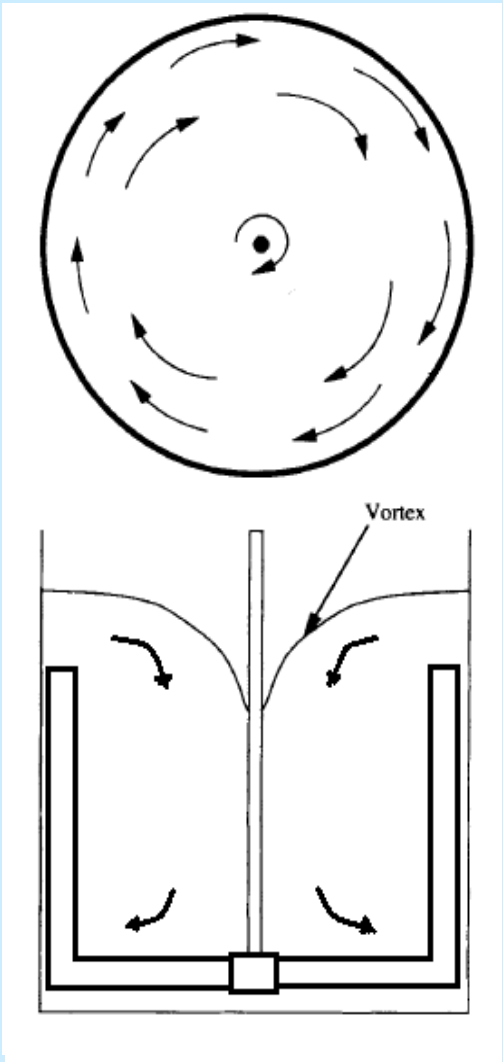
Distribution (macromixing), along streamlines, tracking material, long track, slowest step in large reactors



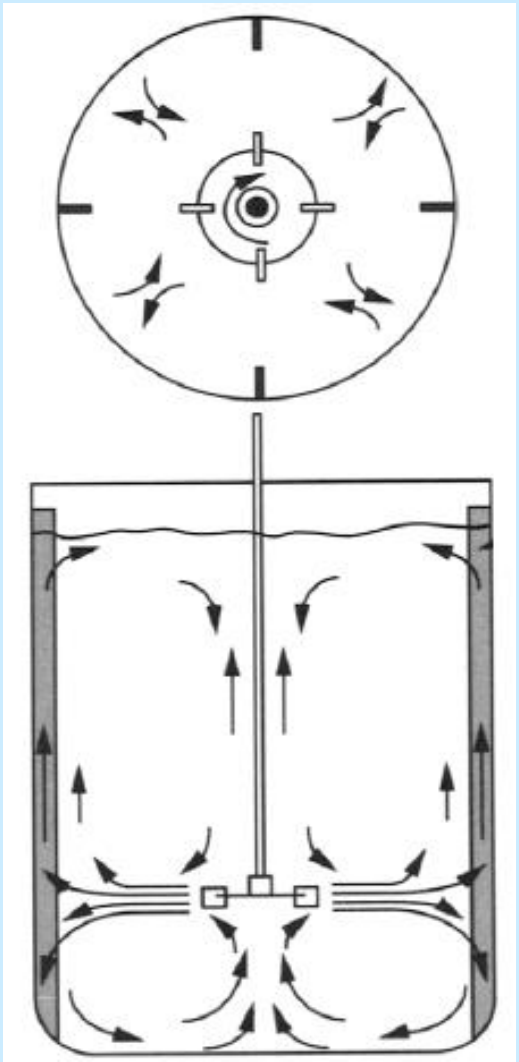


Flow patterns in mixed vessels (macromixing)

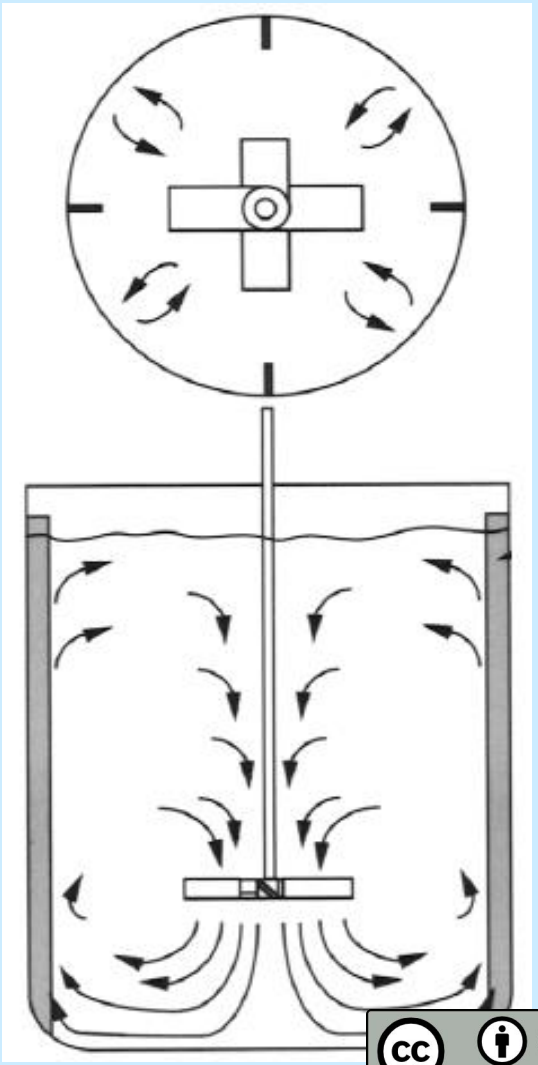
Swirling flow



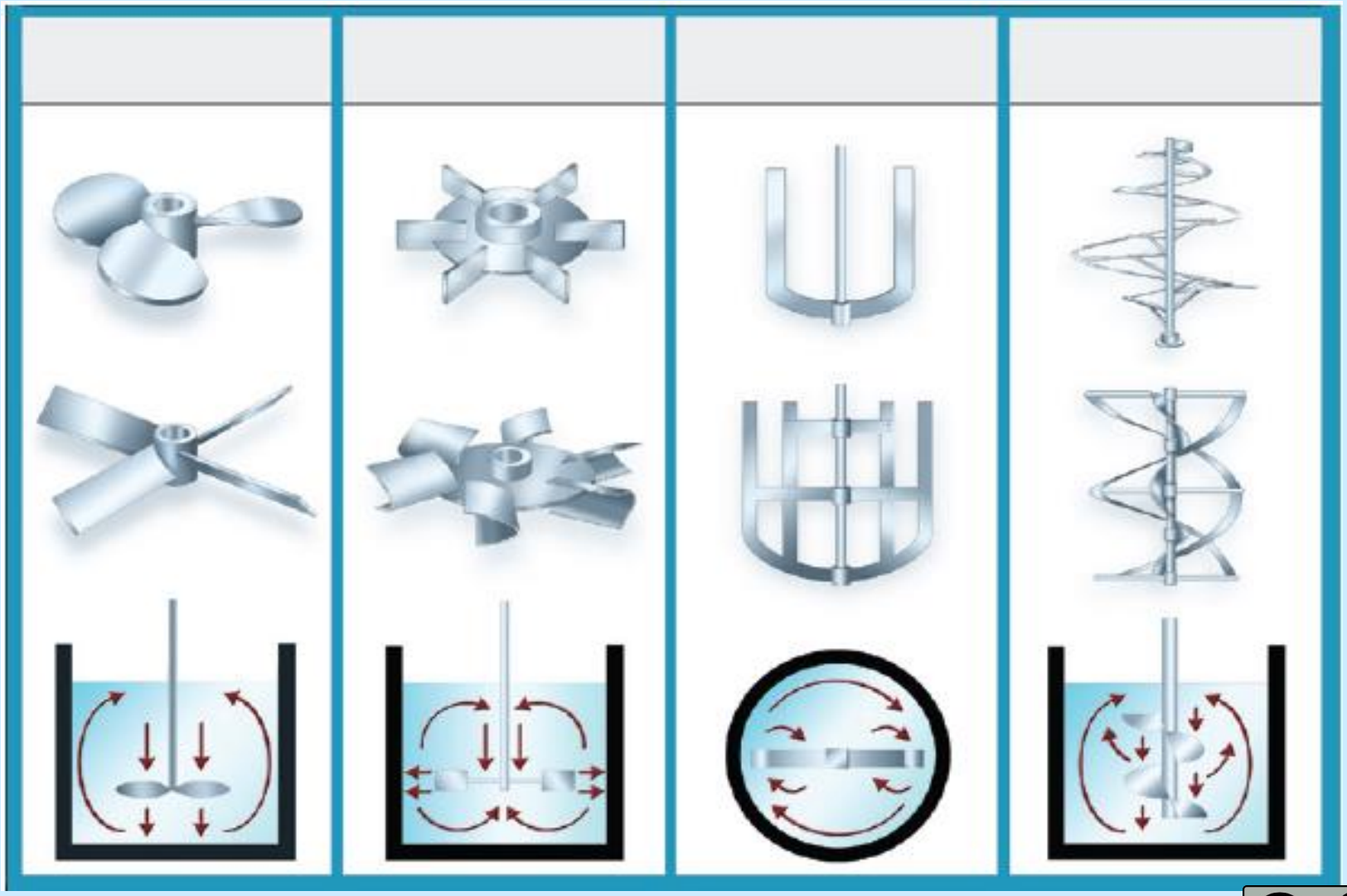
Radial flow



Axial flow



Flow patterns in mixed vessels (macromixing)

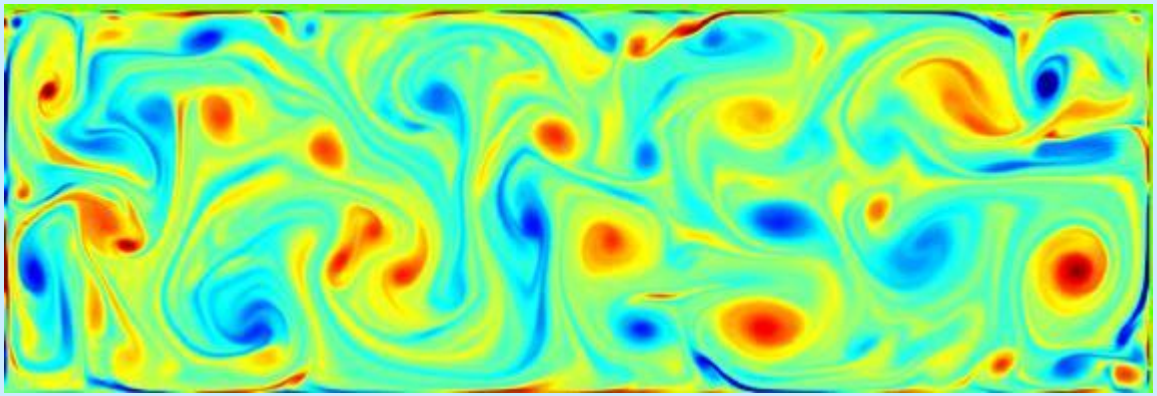


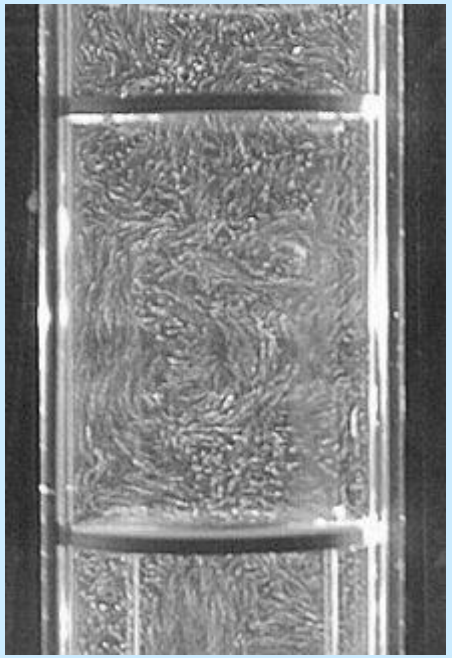
View of mixing under the magnifying glass

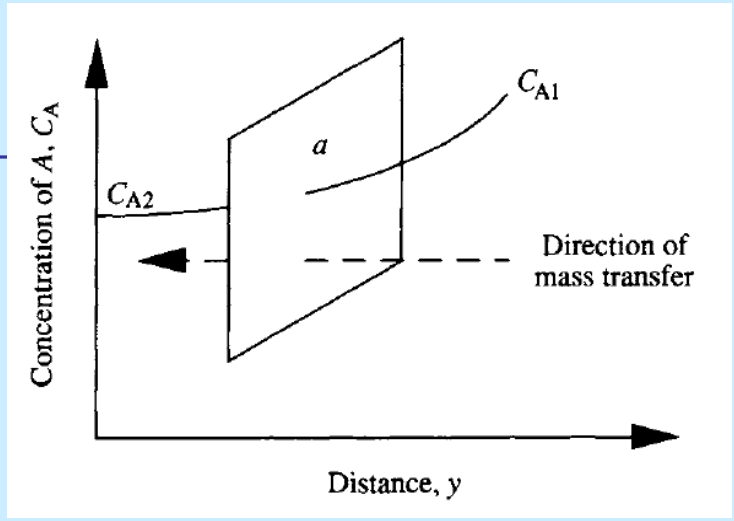
2. Dispersion (macro / micromixing), in turbulent mode, there are large eddies from the blades, which are gradually decaying (energy dissipation), the size of the smallest vortex (Kolmogorov)

$$\lambda = \left(\frac{\nu^3}{P/(\rho V)} \right)^{1/4}$$

ν - kinematic viscosity
λ - for water ca. 30-100μm
ρ - density







Mixing under the magnifying glass

3.Diffusion (micromixing), inside smallest vortices is laminar flow and therefore molecular diffusion must be applied

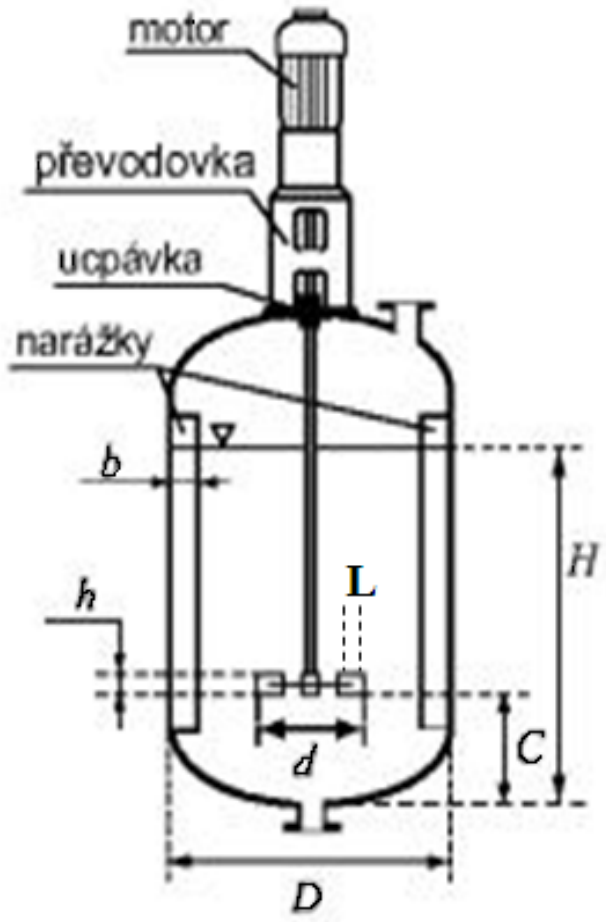
$$J_{A,dif} = \frac{N_A}{a} = -D_A \frac{\partial C_A}{\partial y}$$

$J_{A,dif}$ – intensty of molar mass flow by diffusion (mol/m^{2}s)
N_A – rate of mass flow (mol/s)
a - area (m^2)
D_A – diffusion coefficient (m^2/s)
C_A – molar concentration (mol/m^3)

Rate of diffusion can be increased by:
- increasing the area available for mass transfer
- increasing the concentration gradient
- increasing the diffusion coefficient

Diffusion coefficient: material property (databases), depends on both components, function of temperature, pressure (gases) and concentration (liquids), in liquids (10^{-9} m^{2}s^{-1}) is by orders lower than in gases (10^{-5} m^{2}s^{-1})

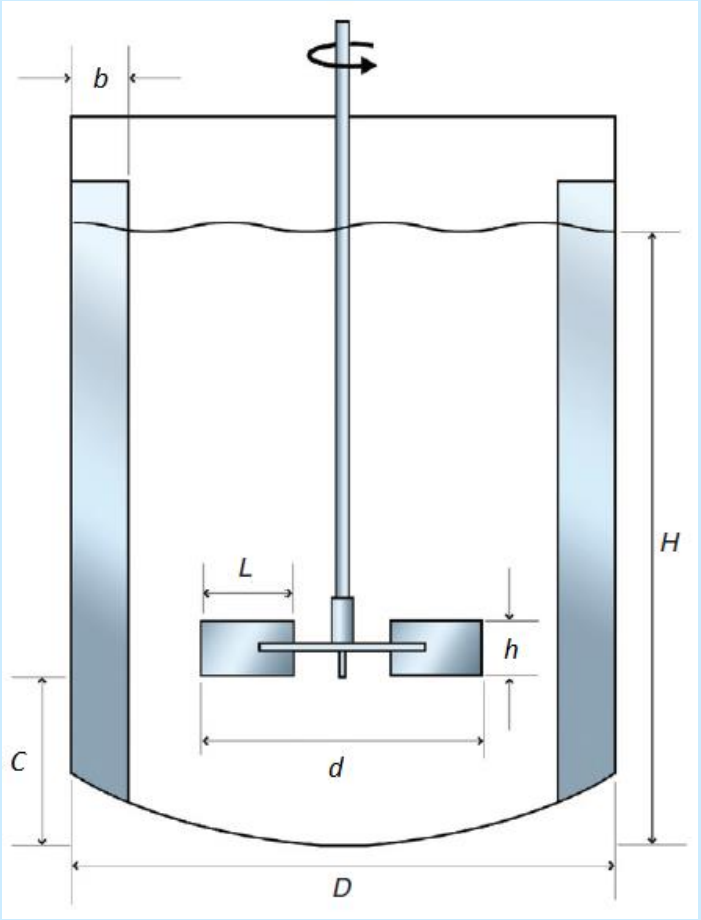
Diagram of the vessel equipped with a turbine and baffles



b - šířka narážek
C - vzdálenost míchadla nad dnem nádoby
d - průměr míchadla
D - vnitřní průměr nádoby
h - výška lopatek míchadla
H - výška plnění vsádky

Standardně užívané poměry
b = 0,1D
C = 0,2 - 0,5D
d = 0,25 - 0,5D
h = 0,2d
H=D
L = 0,25d

Diagram of the vessel equipped with a turbine and baffles



The container bottom is often rounded to avoid dead zones without circulation.

The location of the stirrer: C is approximately equal to d.

When the engine is at the bottom, sealing is even more demanding.

As far as the fermentor is concerned, there are no sensors and additional equipment in the drawing.

There are endless variations in the device size.

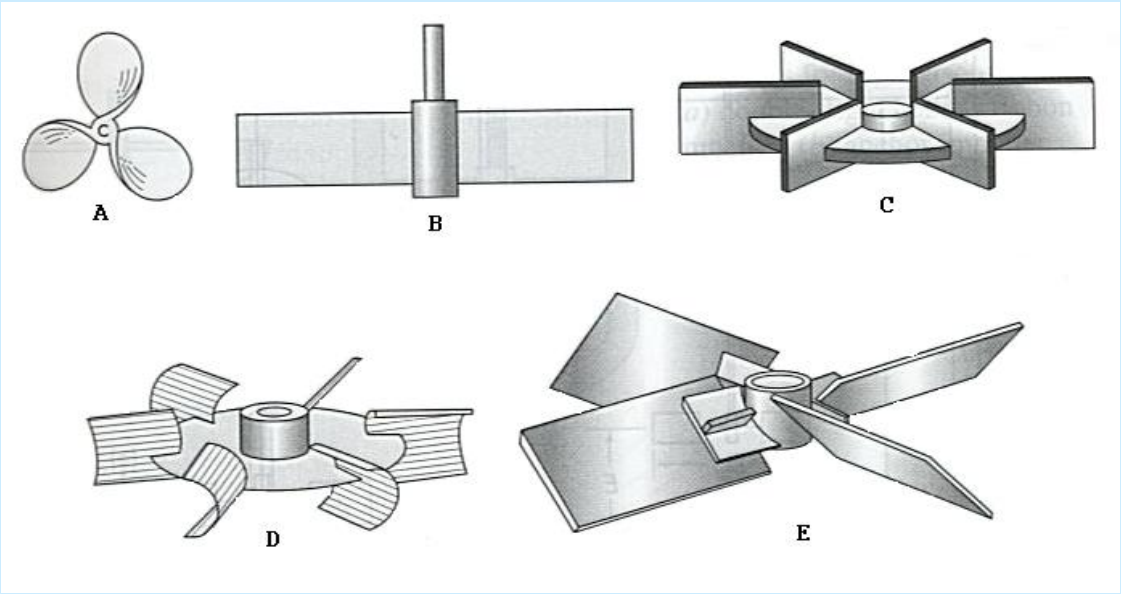
Number of blades on disk turbine is 4-16, but most often 6-8.

Four baffles are usually placed in the vessel, but at larger volumes there can be 6.

Impellers

Types of impellers: most commonly they are divided by the rotation frequency

• **high speed**: for low to moderate viscosity liquids, d/D = 0.3-0.5, baffles b = 0.1D



A – three-blade marine propeller

B – simple straight blade turbine

C – disk turbine (Rushton)

D – concave-blade impeller

E –pitched-blade turbine

High speed impellers

Disk turbine

- create high shear forces, are suitable for dispersing gases
- d/D usually 1/3-1/4, frequency n=120-1200 min^{-1}, blade tip velocity $u=\pi dn$ = 3-9 ms^{-1}
- Pitching the blade turbines increases axial flow (desirable when mixing slurries so that they do not sediment)

Marine propellers

- suitable for intensive circulation in large containers with low energy consumption, smaller propellers up to 2000 min^{-1}, larger 400-800 min^{-1}
- deep vessels can be fitted with more stirrers per shaft, their diameter rarely exceeds 50 cm
- production costs are greater than others (casting but can also be screwed), the important parameter is the climb of the sheet (larger climb causes center vortex)

High speed impellers

Blade turbine

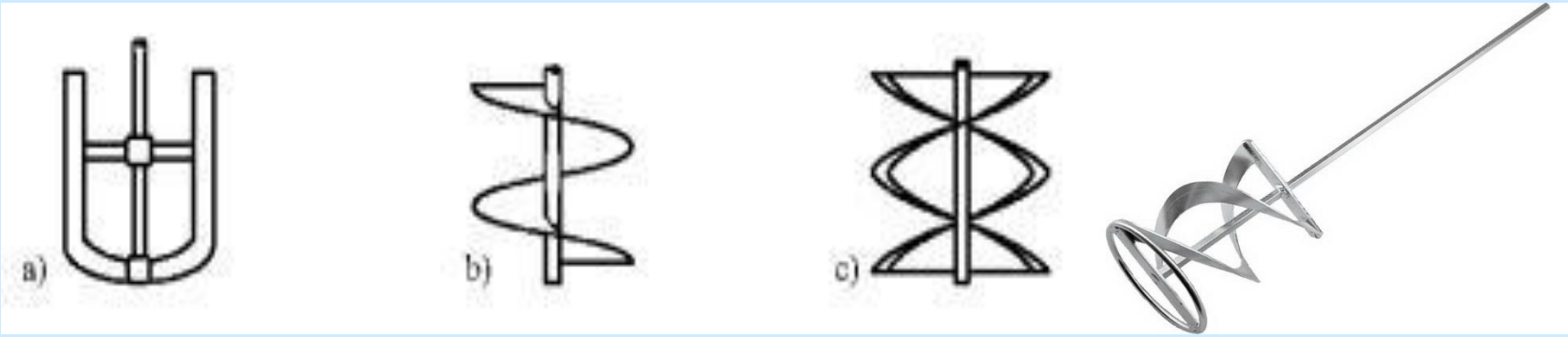
- almost does not give the fluid axial flow, the frequency n = 20-150 min^{-1}, generates predominantly a tangential flow
- Design simple, inexpensive, their distinction from turbines is sometimes difficult, they are also used near the bottom where turbines would no longer have pumping efficiency

Pitched blade turbine

- six-blade, good homogenization, good phase strength
  three-blade, requires less power, can be used at higher speeds, often replaced by propeller mixers

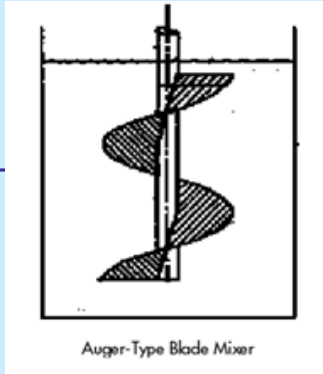
Tooth stirrer

- suitable for solid phase dispersion in liquids
  high shear effects, small flow through stirrer (depends on tooth height)



• **Low-speed**: for viscous liquids (0.1-100 Pa.s, circumferential speed up to 1.5 m s^{-1}, d / D ÷ 0.5

- From viscosity values above 20 Pa.s are most commonly used slow-speed agitators
- The diameter of the mixers is close to the diameter of the vessel (stirring at the wall)
- Anchor stirrer, mixing less efficient but good transfer of heat from the tank to the filling



Low-speed impellers

Anchor and grid impeller

- Frequency 20-60 min^{-1}, speed 0.5-1.5 ms^{-1}, for very viscous non-Newtonian liquids up to 100 Pa.s
- It forms a tangential flow, suitable for intensification of heat transfer

Auger-type blade mixer

- Frequency 60-240 rpm, for highly viscous non-Newtonian liquids up to 100 Pa.s
- for the same mixing requires less energy than the propeller, typically it delivers liquid from the bottom up

Open helical ribbon

- for particularly viscous liquids up to 1000 Pa.s, good for heat transfer

Cross beam

- less expensive than propeller, good batch circulation

Agitators can also be divided according to the nature of the flow (the flow is never quite unambiguous but one is predominant):

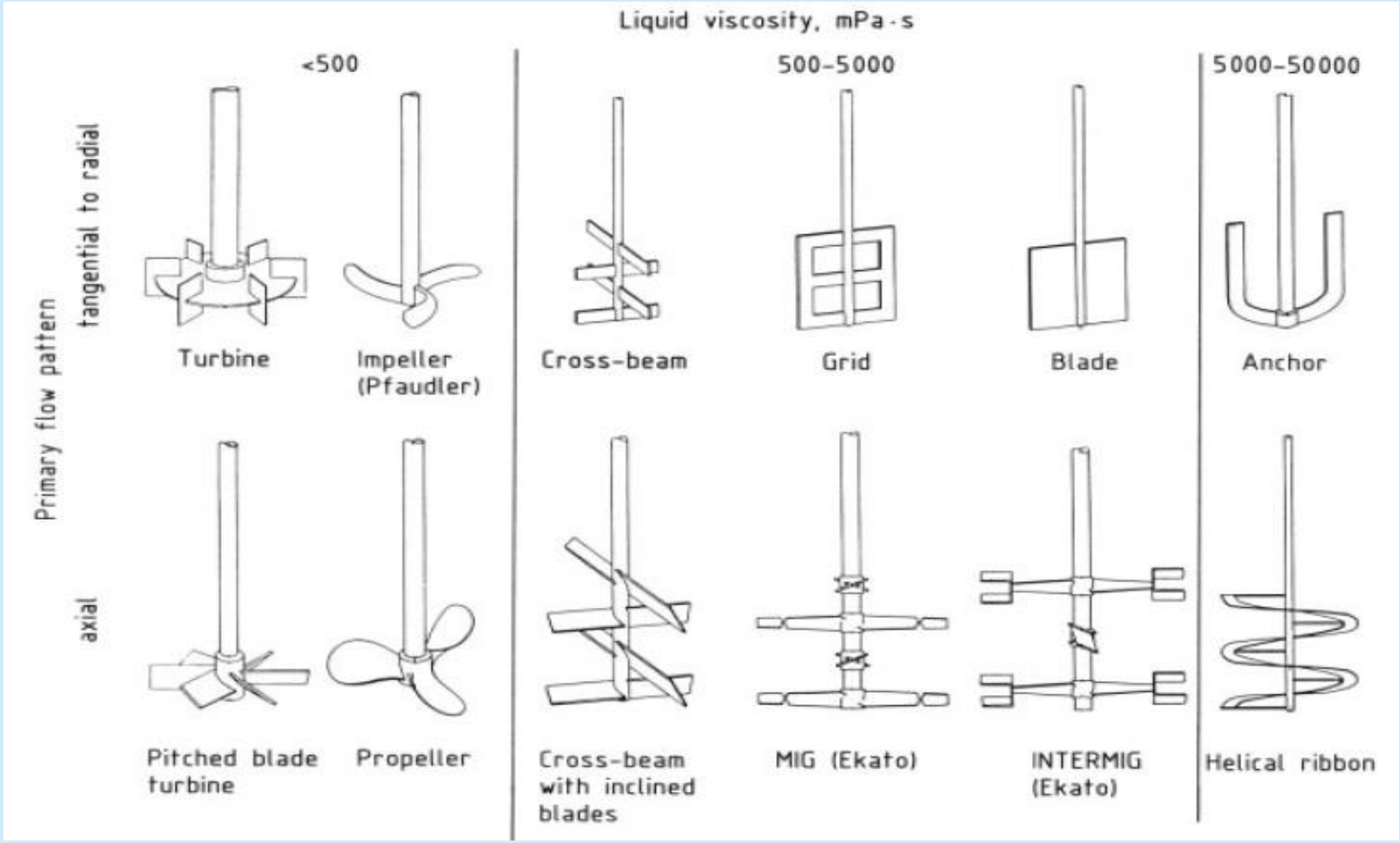
1-axial agitators, liquid flow is predominantly parallel to the shaft axis (propeller, pitched blades, screw, belt), this flow is supported by circulation tubes

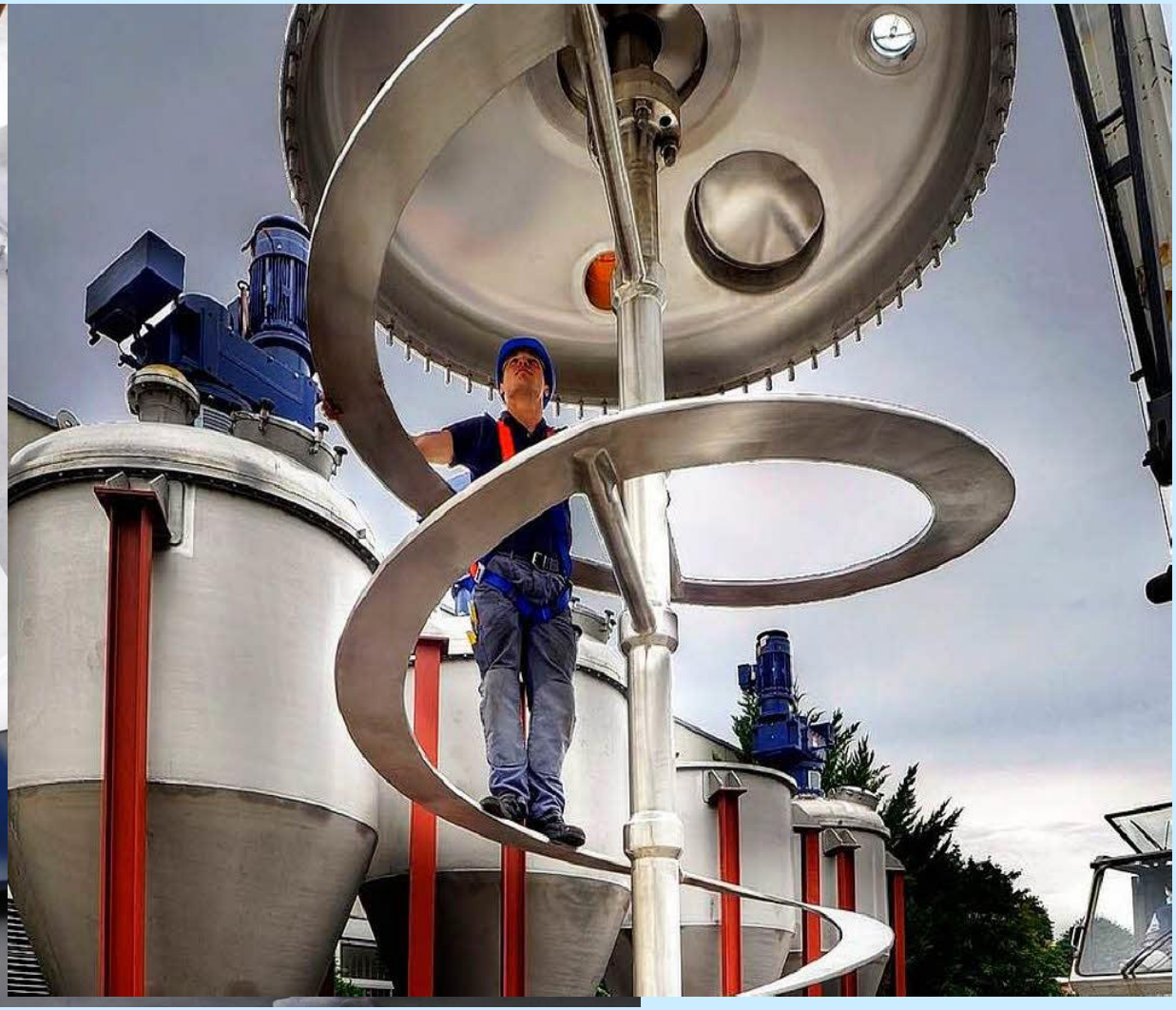
2 - radial agitators, the main liquid stream leaving the agitator space is perpendicular to the axis of the shaft (turbine, blade with perpendicular blades)

3 - tangential agitators (most commonly slow-speed blades and anchors, or high-speed without baffles). The central vortex can also be prevented by the eccentric placement of the agitator.

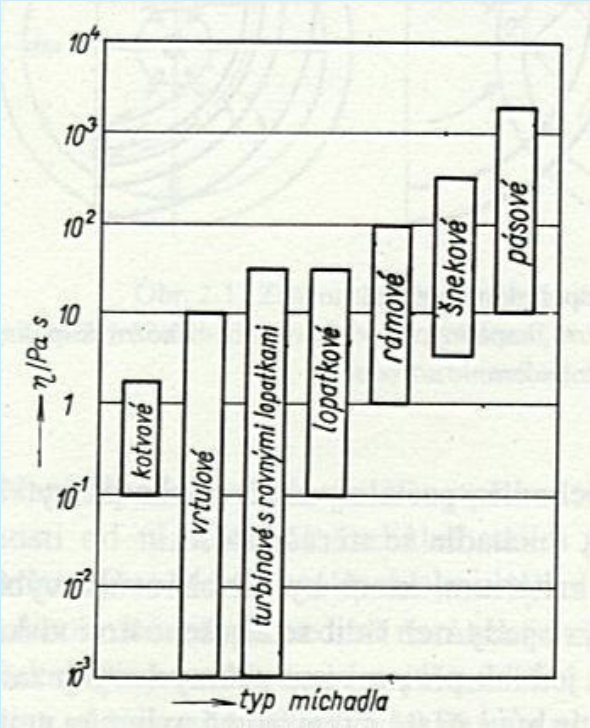
The main types of mechanical agitators are standardized in ČSN 69 1002 Mixing equipment, ON 69 013 - Types of rotary liquid mixers

Impellers



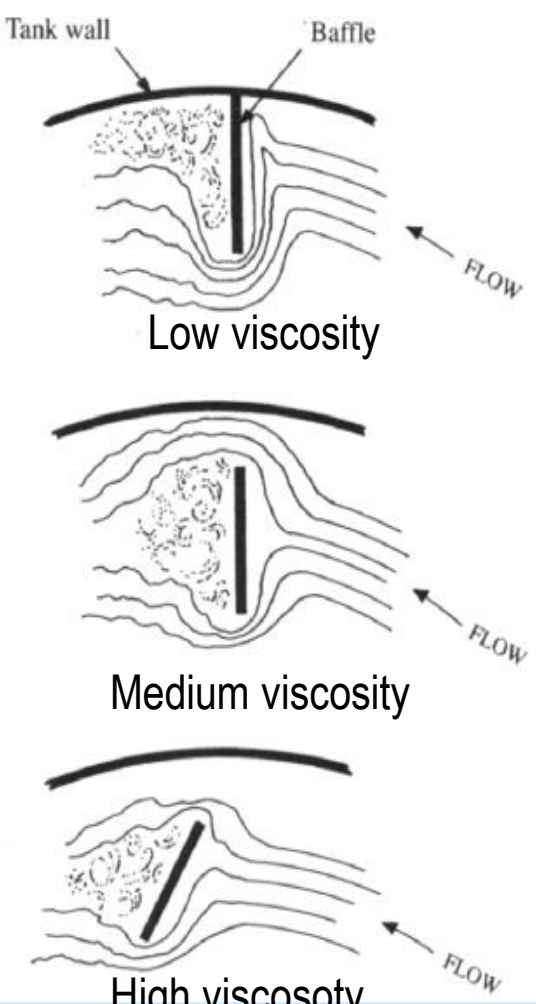


The choice of agitator influences mainly the physical parameters of the mixed batch (viscosity), but also the tradition of technology



Operace	Druh míchadla	Rozsah použití*)	Veličina limitující operaci	Geometrické parametry míchacího zařízení D/d	H/D	Počet míchadel na hřídeli a jejich umístění
míchání	turbínové vrtulové lopatkové objem	4000 m³	a) objemová cirkulace	3–6	libovolné	jedno nebo několik
disperze vzájemně nemísitelných kapalin	turbínové vrtulové lopatkové průtok	4 m³ min⁻¹	a) průměr kapiček b) recirkulace	3–3,5	1–0,5	$H_2/H \leqq 1/2$
chemické reakce v roztocích	turbínové vrtulové lopatkové objem	80 m³	a) intenzita míchání b) objemová cirkulace	2,5–3,5	1–3	jedno nebo několik
rozpouštění částic tuhé fáze	turbínové vrtulové lopatkové objem	40 m³	a) střižná napětí b) objemová cirkulace	1,6–3,2	0,5–2	$H_2/H \leqq 1/2$
tvorba suspenzí	turbínové vrtulové lopatkové koncentrace částic	100 %	a) cirkulace b) rychlost	2–3,5	1–0,5	závisí na průměru částic a) $H_2 = d$ b) těsně nade dnem
míchání systémů plyn–kapalina	turbínové vrtulové lopatkové průtok plynu	150 m³ min⁻¹	a) střižné napětí b) cirkulace c) velká rychlost	2,5–4	4–1	a) několik, nejnižší ve výši $H_2 = d$ b) samonasávací, těsně pod hladinou kapaliny
míchání velmi viskózních kapalin	turbínové vrtulové lopatkové viskozita	10^6 mPa s	a) objemová cirkulace b) malá rychlost	1,5–2,5	0,5–2	jedno nebo několik
přestup tepla	turbínové vrtulové lopatkové objem	80 m³	a) objemová cirkulace b) velká rychlost podél výměnné plochy	–	–	jedno nebo několik
krystalizace	turbínové vrtulové lopatkové objem	80 m³	a) cirkulace b) malá rychlost c) střižné napětí	2–3,2	2–1	jedno $H_2/H \leqq 1/2$

Baffles



Baffles with gap are also used for low viscosity liquids, as they are easier to clean, replaceable

Selection of impeller by power

Impeller power

The power of the impeller PM (W) means the energy transferred by the stirrer of the mixed batch per unit of time (work performed by the agitator per unit of time)

Power consumption by the engine PP (W) is usually higher due to energy losses in mechanical gears and sealings

$$P_P = \frac{P_M}{\eta_p \eta_u}$$

η_p - mechanical efficiency of gears

η_u - mechanical efficiency of sealings

The low-power mixer achieves homogenization for a long time
The high-power mixer reaches the target quickly but at the expense of energy loss
A suitable combination of agitator, drive, transmission and sealing is important both in technology and energy terms

Selection of the mixer by power

We determine the power input of the impeller from the experimentally determined power curve: $P_O = f\left(Re_M, Fr_M, d/D, C/D.....\right)$

P_o – power number (modified Euler number for agitation)

$$Eu_M = P_O = \frac{P_M}{\rho n^3 d^5}$$

P_M – impeller power (W)

ρ - density (kg m^{-3})

n – impeller frequency (s^{-1})

d – impeller diameter (m)

Re_M – Reynolds number for agitation

$$Re = \frac{vl\rho}{\eta} \rightarrow Re_M = \frac{nd^2\rho}{\eta}$$

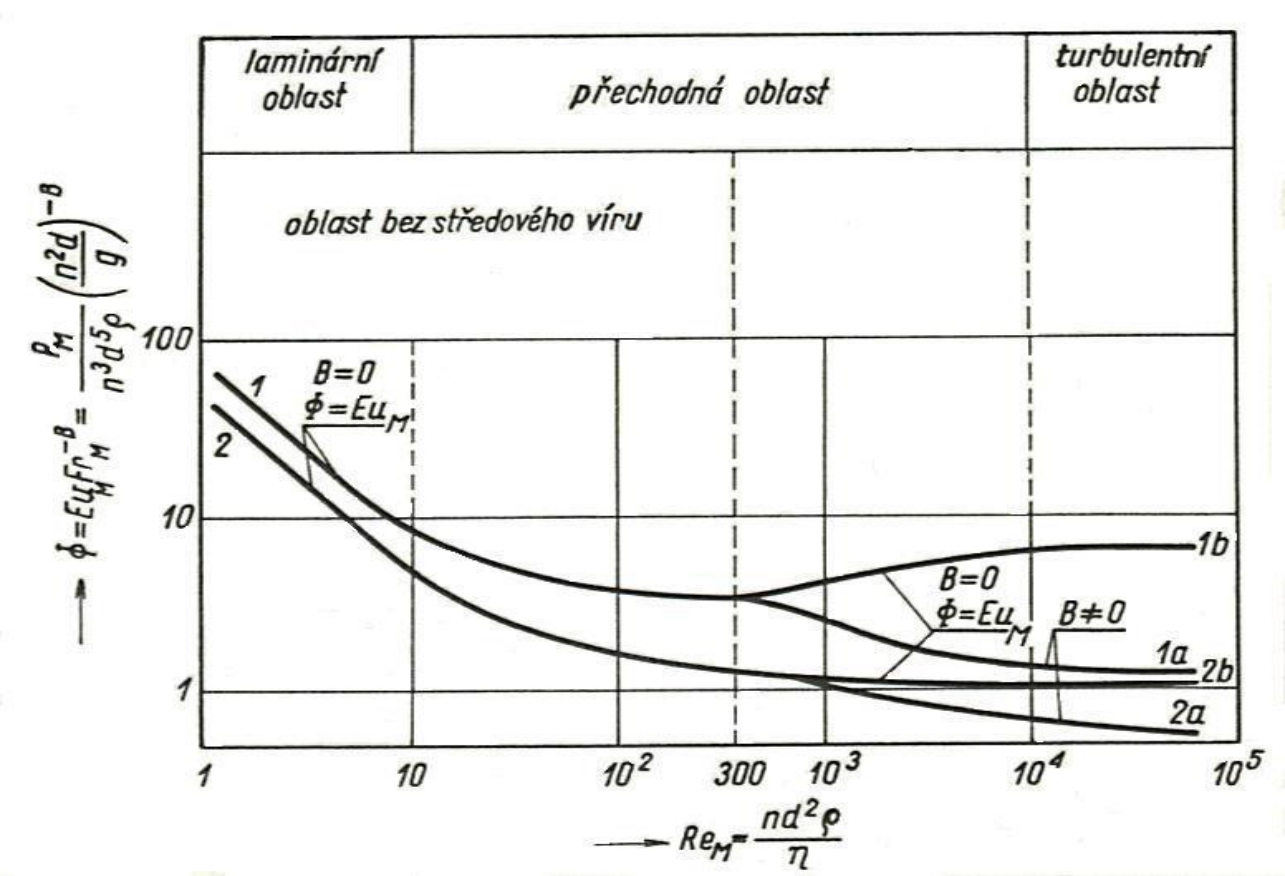
η - dynamic viscosity (Pa s)

Characteristic speed is replaced by peripheral speed of blade tips $v = \pi dn$

Characteristic length replaced by impeller diameter $l = d$

$\phi = P_o$ when B=0

Power correlation



When B=0

$$Eu_M = f\left(Re_M\right)$$

When B≠0

$$\Phi = \frac{Eu_M}{Fr_M^B} = f\left(Re_M\right)$$

1a-turbine impeller without baffles, 1b-turbine impeller with baffles, 2a-propeller without baffles, 2b-propellers with baffles

Specific power input

High-speed impellers: 150 – 600 W.m^{-3}

$$\varepsilon = P_M / V$$

Low-speed impellers: 600 – 1500 W.m^{-3}

From the power-specific values, power can be determined for a given volume of the mixed liquid and that can be used to calculate the desired stirrer rotation frequency.

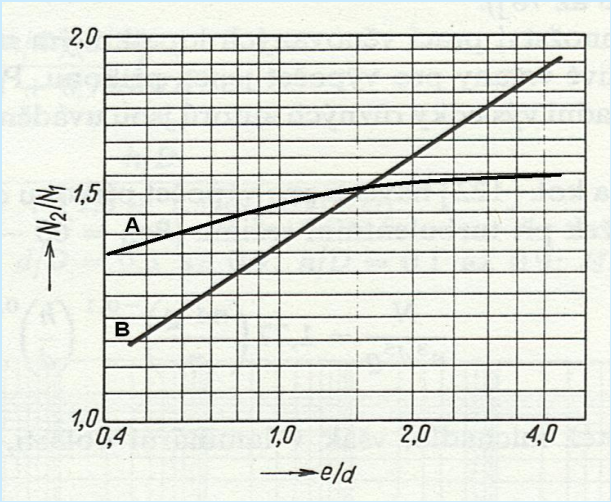
To verify the accuracy of the calculated parameters, it is advisable to check the circumferential speed of the agitator with respect to the risk of cavitation

Impeller	Circ. speed (m.s^{-1})
Turbine	3 - 6
Propeller	5 – 15
Anchor	0,2 – 2

Influence of geometric parameters on impeller power

Shifting or sliding of the agitator shaft: partially replaces baffles and increases power, suitable for propeller mixers

Influence of two impellers on one shaft:
A - turbine, B - propeller, N2 power
two impellers, N1-power of one impeller,
e-mutual distance of the impellers, d-diameter of impellers



Impact of the blades on the turbine impeller: The number of blades from 3 to 6 will increase the power consumption by 75%, further increasing the number of blades will no longer have a more significant effect.

Influence of turbine blade shape: Blade curvature reduces power in turbulent region

Pumping and homogenizing effect of rotary impellers

Homogenization is achieved in the following ways:

1. Due to the recirculation flow where mixing is caused by the unevenly rapid movement of the individual particles of the fluid on uneven lengths (convection)
2. Due to turbulent flow where mixing causes turbulent vortices (turbulence)
3. Due to molecular diffusion

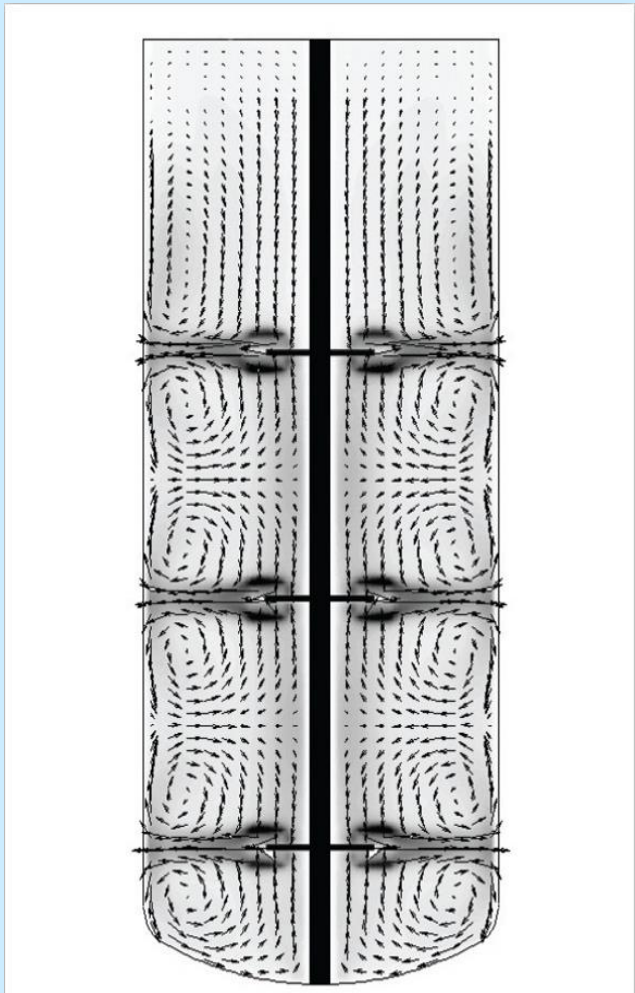


Figure 1. CDF simulation of the liquid flow pattern in a stirred tank equipped with three Rushton turbines.

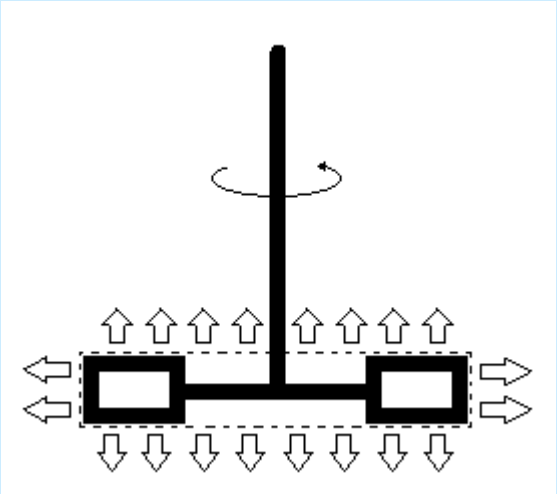
Volumetric flow rate of impellers (Pumping efficiency)

The amount of liquid flowing out of the impeller rotor area

The results of the experimental determination of the volumetric flow of the mixers can be generalized

$$V = Cnd^3$$

V- volumetric flow out of impeller *(m^3/s)*
C- *constant, n-frequency, d-diameter*



The volume flow rate of the agitator depends on the type of agitator and on the geometric parameters of the system

C (turbine) ~ 0,75

C (marine propeller) ~ 0,5

Conclusions regarding the volumetric flow rate of impellers:

- Radial agitators - The height of the blade h increases the constant C
- Axial agitators - Higher pitch ratio of propeller increases C
- Installing baffles into the container increases the pumping efficiency
- By increasing the slope of flat blades we increase pumping efficiency
- The dividing disk does not affect the pump efficiency of turbines
- Increasing the d / D ratio increases the pump efficiency

Mixing time

Time period required to achieve homogeneity of batch

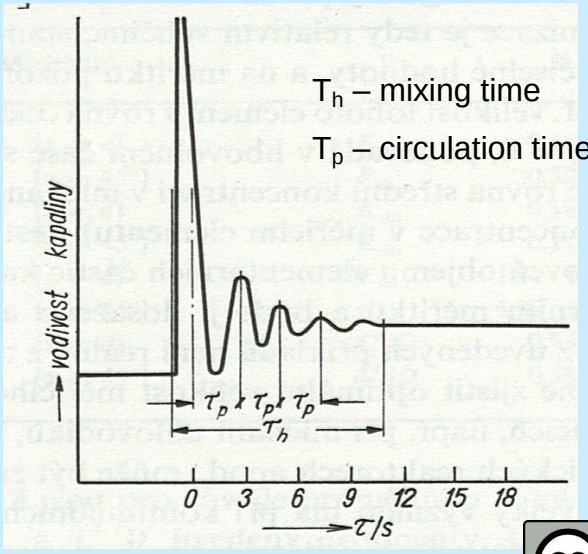
The homogenizing effects of mixing equipment are crucial for some processes (e.g. homogenization of temperatures, pH, nutrients, etc.)

The degree of homogeneity is the ratio of actual mixing to the ideal (ca. 0.95)

The homogenization time (τ) can serve as a benchmark for assessing the intensity of mixing in different types of mixing devices

Measurement of mixing time:

- thermal methods (thermal impulse in the form of a small amount of hot liquid)
- chemical methods (impulse in the form of acid, bases, salts, etc.)
- optical methods

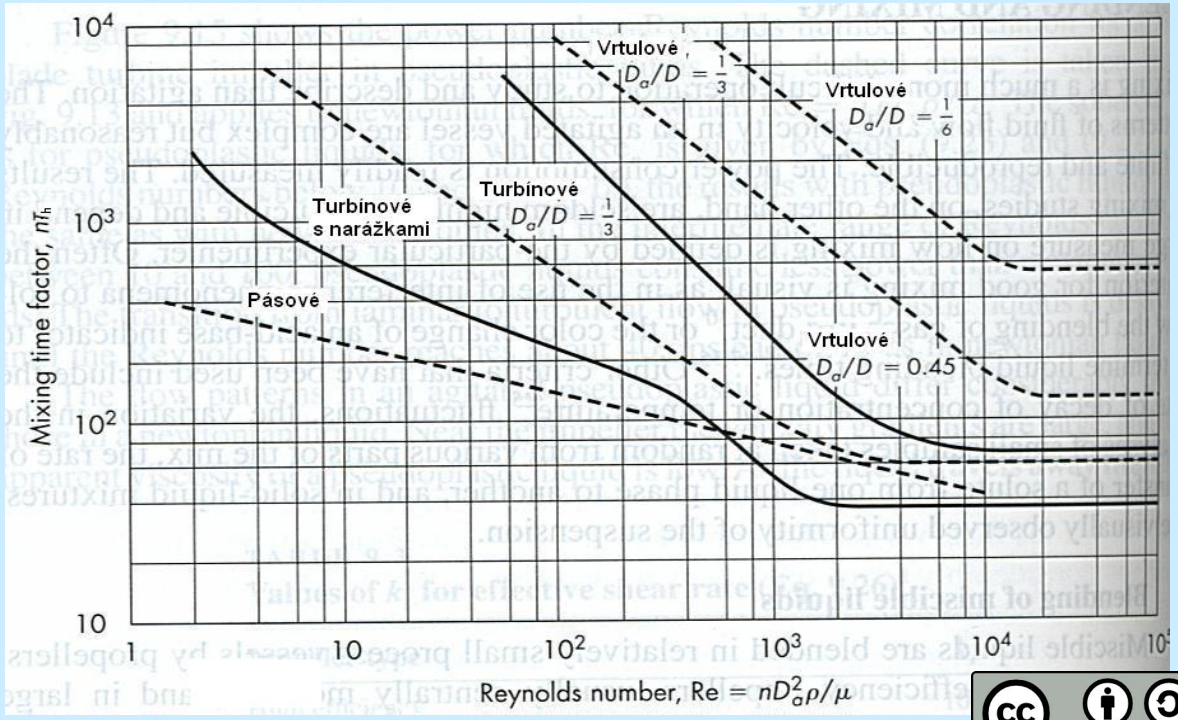


Mixing time

Usually in a dimensionless correlation ⇒ $nT_h = f(Re)$

For high-speed impellers (C=D/3, H=D, 4 baffles, homogeneity 95%) ⇒ $T_{h(0,95)} = \frac{5{,}3}{P_O^{1/3} n}\left(\frac{D}{d}\right)^2$

Dimensionless criteria nT_h vs. Reynolds number for some imellers



For turbine at high $Re > 5.10^3$

$$nT_h = \frac{1{,}54V}{d^3}$$

V – volume of reactor, d – impeller diameter

Mixing heterogeneous systems

Heterogeneous system

a) liquid – gas

b) liquid – liquid (immiscible)

c) liquid – solid

The purpose of mixing these systems is to intensify the transfer of matter.

$$\dot{m} = \beta.S.\Delta\rho_A$$

m – mass flow of the component, β - mass transfer coefficient, S –interfacial area, $\Delta\rho_A$ – concentration difference of the component in the liquid and at the interface

It follows from the equation that the main purpose of mixing these systems will be to achieve the greatest interfacial area and high mass transfer coefficient.

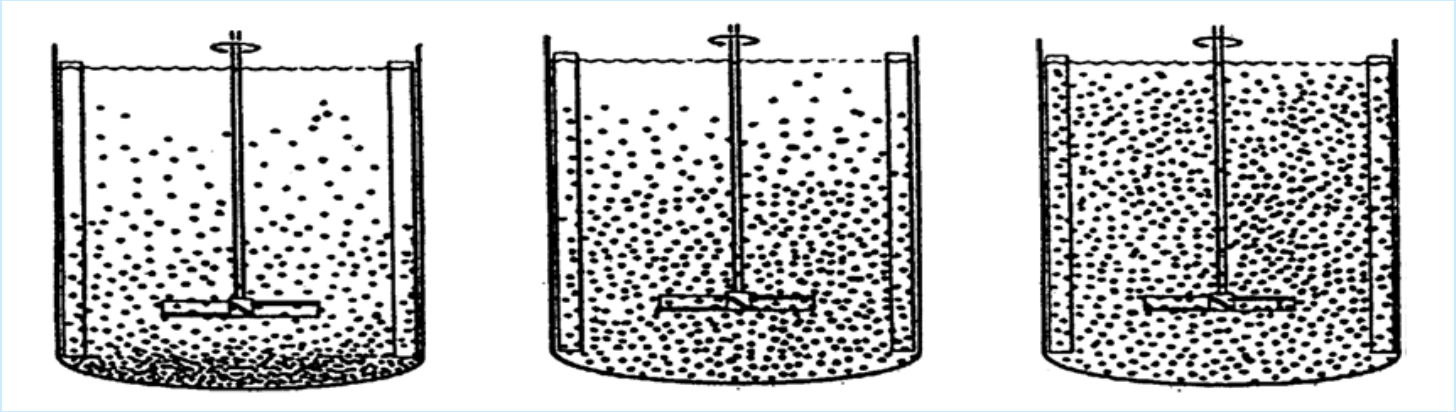
Two-phase liquid-solid systems

The aim is to achieve an uniform solid phase distribution in the liquid.

Uniform distribution is a state where all solid particles are dispersed and surrounded by liquid. No particle is on the bottom of the vessel.

For dilute suspensions, the power consumption relationships are the same (need to use the resulting density and viscosity of the suspension).

Concentrated slurries (sludge) behave differently (sometimes they can be considered as non-Newtonian liquids).



$$\mathrm{Re}_M = \frac{nd^2\rho}{\eta} \qquad P_O = \frac{P_M}{\rho n^3 d^5} \qquad T_{h(0,95)} = \frac{5{,}3}{P_O^{1/3} n}\left(\frac{D}{d}\right)^2$$

Example 3

Mixing of keiselguhr, dose 20 kg/hl water,

D=1m, H=1m

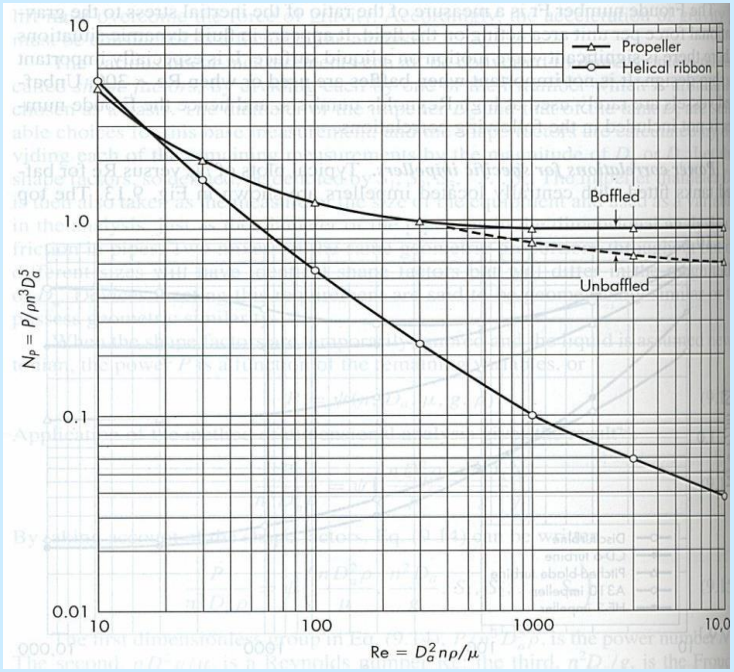
$\eta \cong 1{,}9\times10^{-3}$ Pas, $\rho \cong 1200 kg/m^3$

$\eta_s = \eta_l(1+4{,}5\Phi_r) = 0{,}001(1+4{,}5.0{,}2) = 0{,}0019$ Pas

Propeller+baffles, d1=20cm, n1=60min^{-1}

$Re_M = (1\times0{,}2^2\times1200)/0{,}0019 = 25263$

$P_O = 0{,}9 = P_M/(1200\times1^3\times0{,}2^5)$



P_M=0,3456 W, Mixing time: $T_h = 5{,}3/(0{,}9^{1/3}.1)(1/0{,}2)^2 = 137$s

We increase the tank diameter to D(H)=1,5m, while want to keep T_h const.

Have to keep ratio D/d=1/0,2=5 i.e. 1,5/d2=5 → d2=0,3m or increase frequenca from n1=60min^{-1} to n2=135min^{-1}

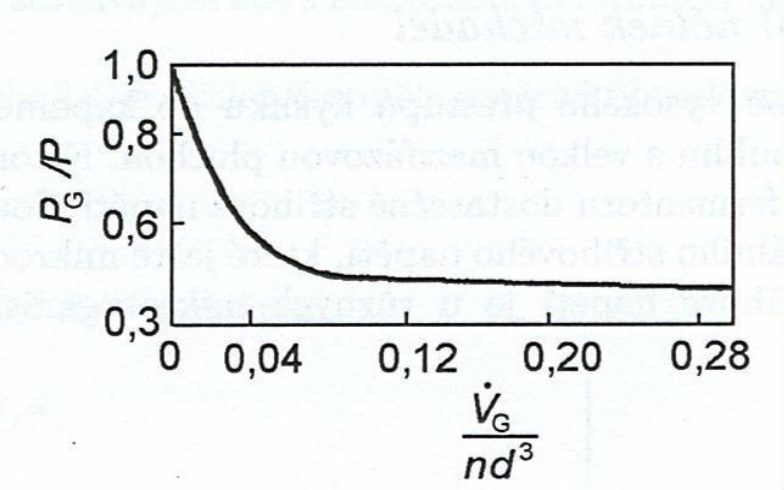
Increased diameter: P_M=2,62W Increased frequency: P_M=3,93W

Two-phase system gas-liquid

Frequent cases in fermenters and biological sewage treatment plants

power in the bubbling system will be always lower when mixing only liquid

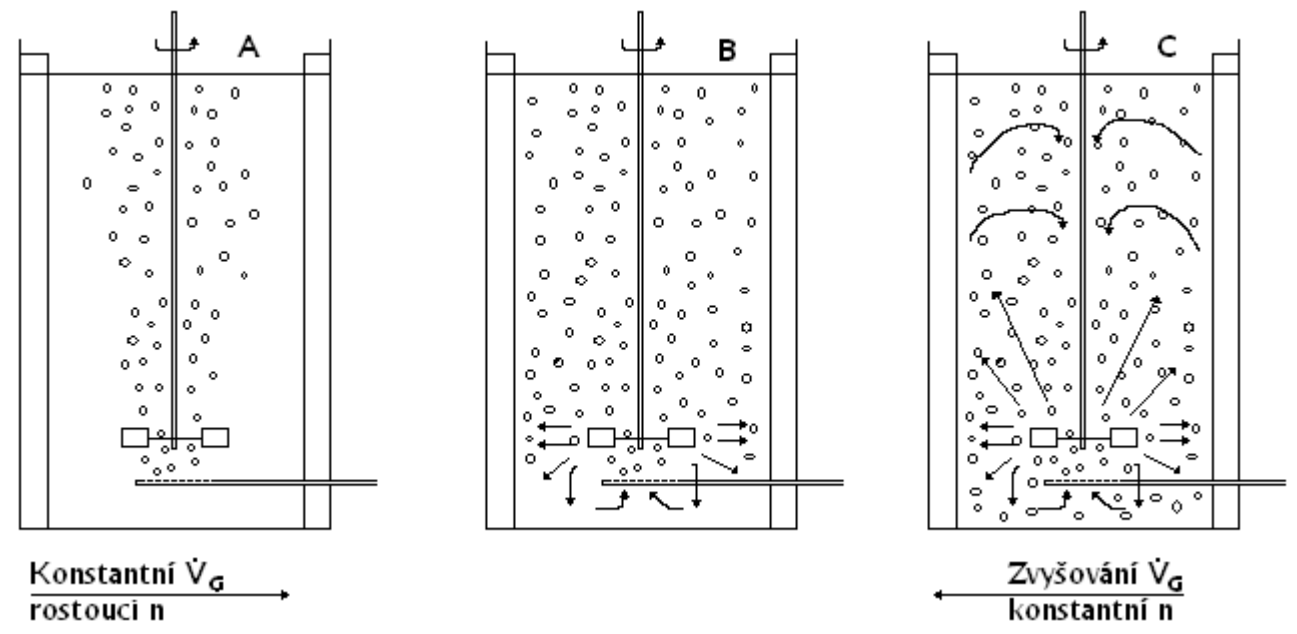
$$\frac{P_G}{P} = f\left(\frac{\dot{V}_G}{nd^3}\right)$$



•For a turbine located above the circular gas distributor the PG / P ratio is approximately constant for higher volumetric gas flow rate (Vg)

•When designing impellers, it is necessary to consider that the power of the agitator increases significantly when the gas supply fails. Therefore the impeller has to be designed to mix the clean liquid.

Basic hydrodynamic regimes for dispersing gas in the fermenter



A: The agitator is overwhelmed with gas. The gas rises vertically to the surface

B: Overcrowding is overcome. Bubbles are radially dispersed

C: Intensive dispersion of gas below the mixer.

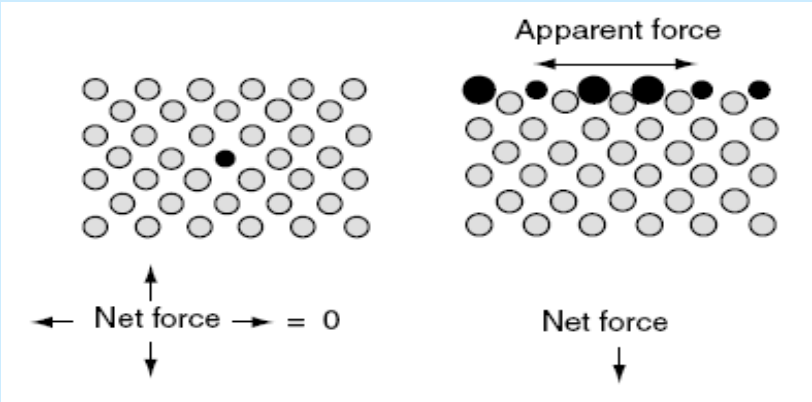
$$\frac{\dot{V}_G}{nd^3} = 30\frac{n^2 d}{g}\left(\frac{d}{D}\right)^{3,5}$$

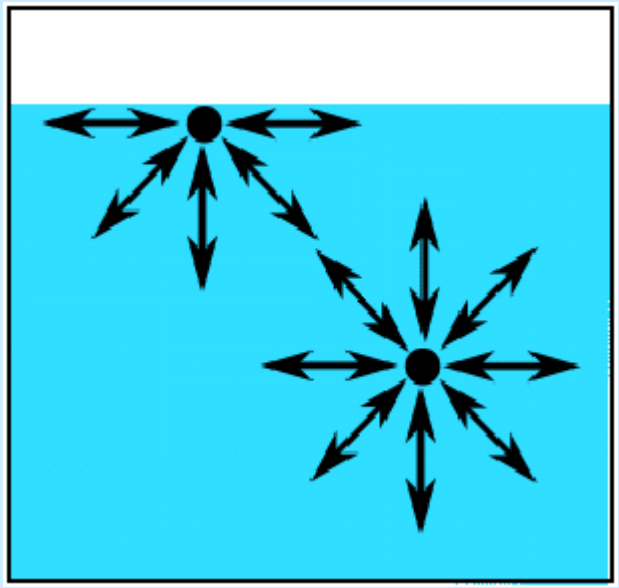
Calculation of critical gas flow V_G (turbidity at given frequency) for turbine impeller

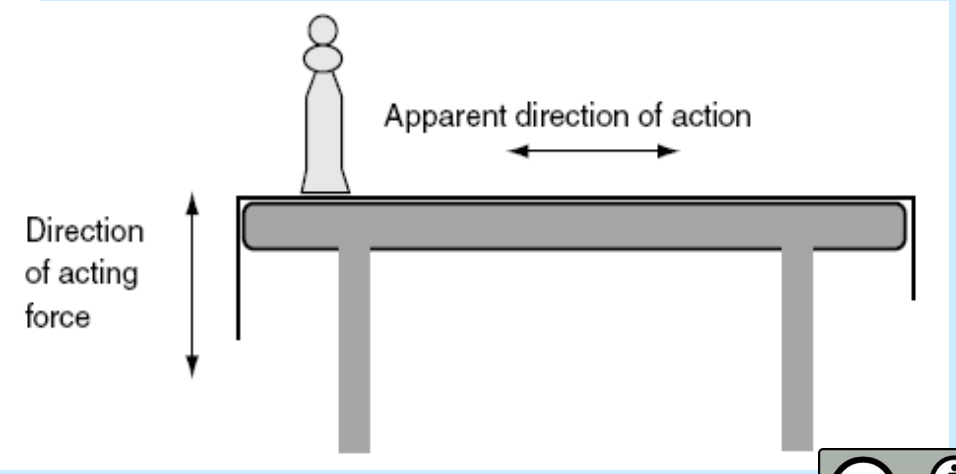
Dispersing effect of impeller

Surface and interphase tension

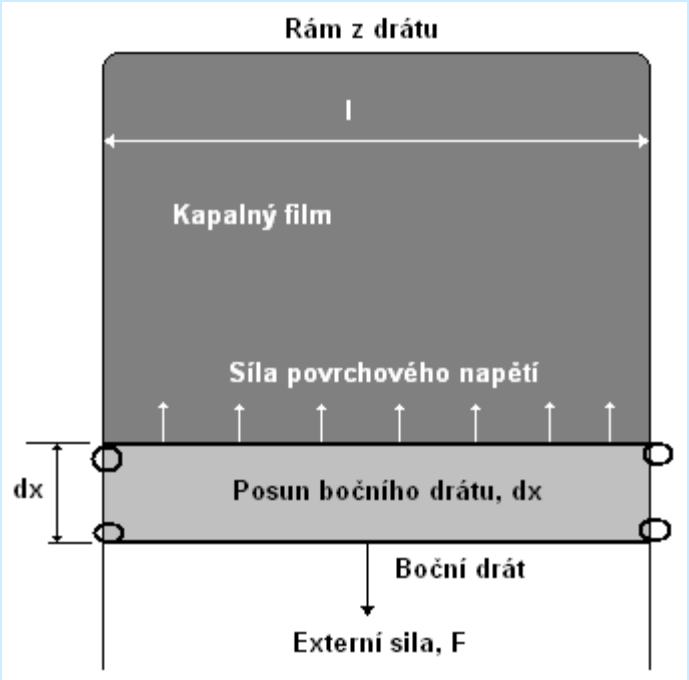
Surface tension - force pulling molecules on the surface inside







Surface tension



The forces act along the length of l, the film has two sides (2×l)

The surface tension (γ) of the liquid film attracts the side wire until it is aligned with the external force F

γ = F/2l (mN/m) - force per unit length

When applying greater than the equilibrium force F, the lateral wire is displaced by dx to increase the surface of the film by dA = 2ldx

The work done, is equal to the force times the distance :

$\Delta w = Fdx = \gamma 2ldx = \gamma dA$ (mJ/m^2) - work (energy) needed to create a new surface

COMMENTS: In fact, we measure the interfacial tension e.g. water-air. For mixtures γ is not the medium of the γ of pure components.

Interfacial tension and curved surface

Curved surface causes increased pressure on the concave side (inside the drops, bubbles)

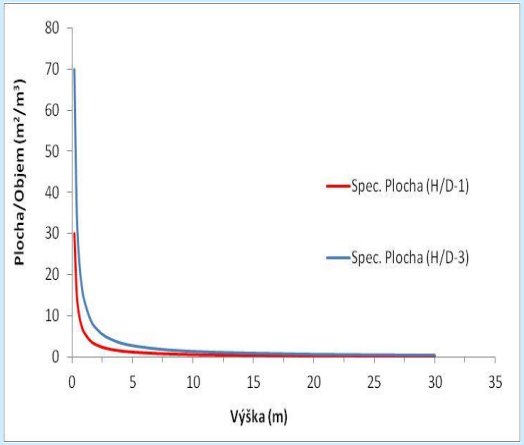
$\Delta p = 2\gamma / r$ (Young-Laplace)

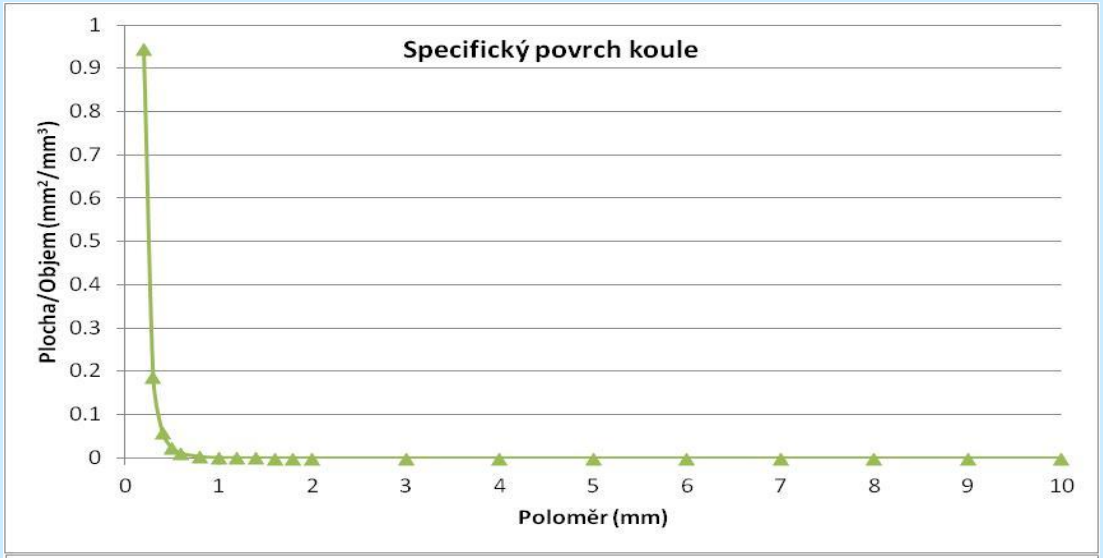
In order for the bubble to break, it is necessary to deform it - it is done there by the velocity / pressure gradients (vortices) created by the impeller paddle and they must be of the order of magnitude as Laplace's pressure.

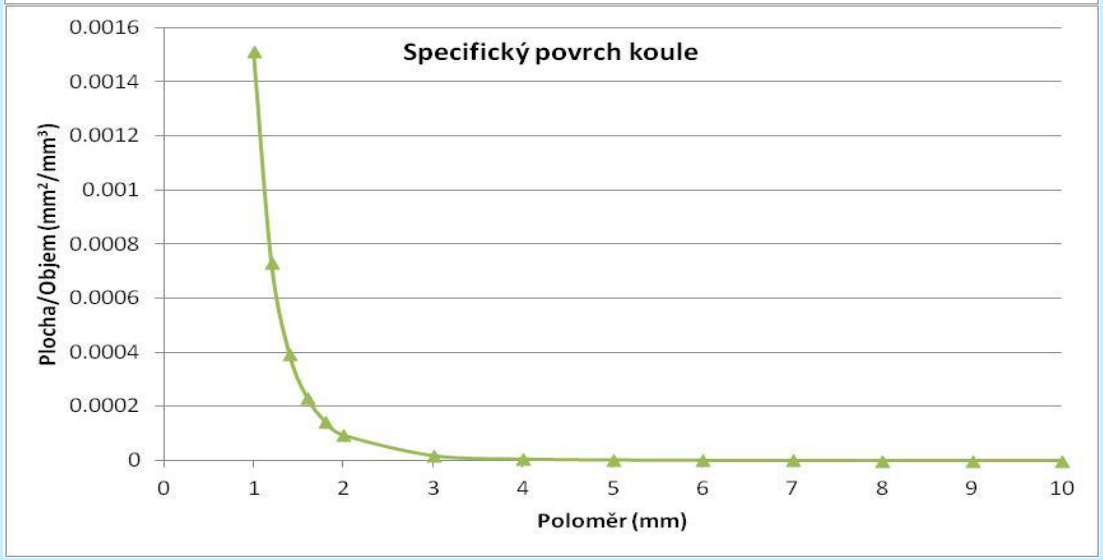
Laplace's pressure acts against the deformation of bubbles:

Δ*P* for air bubbles in water				
Bubble radius	1 mm	0.1 mm	1 μm	10 nm
Δ*P* (atm)	0.0014	0.0144	1.436	143.6

For example: bubble 1 mm has Δp = 144 Pa
Velocity gradient = $\Delta p/\mu = 144\ (Pa)/1.10^{-3}\ (Pa.s) = 1.4\times10^{5}\ s^{-1}$
Extremely high value!!!
Although there are local extremes of speed gradients, it is very difficult to produce bubbles less than a tenth of a mm by mechanical stirring.


Plocha/Objem (m²/m³)
Spec. Plocha (H/D-1)
Spec. Plocha (H/D-3)
Výška (m)


Specifický povrch koule
Plocha/Objem (mm²/mm³)
Poloměr (mm)


Specifický povrch koule
Plocha/Objem (mm²/mm³)
Poloměr (mm)

Viscosity

-represents fluid resistance to motion
-liquid is a substance subject to deformation at
-the action of shear forces
-the gradient speed (dv/dy) is proportional to F

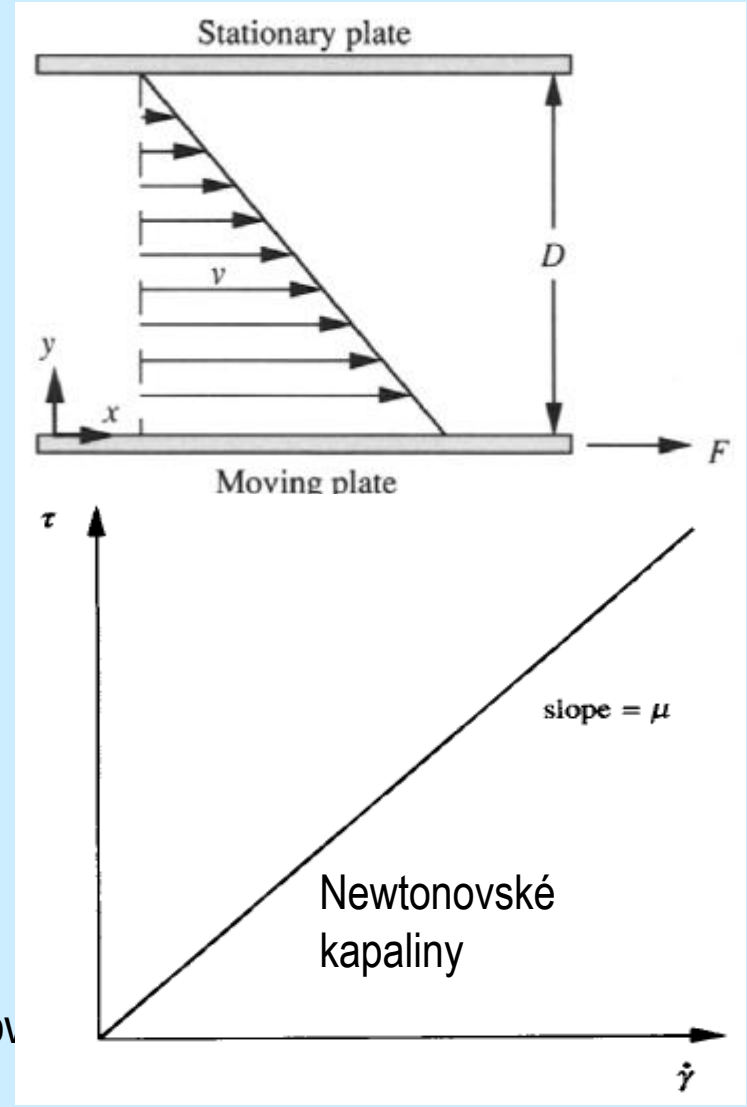
-The shear stress is the shear force acting on the surface

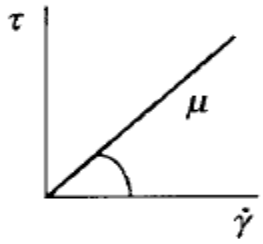
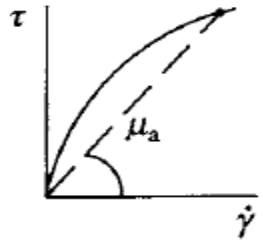
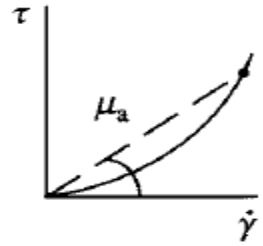
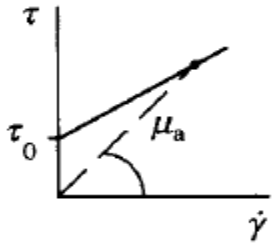
$$\tau = \frac{F}{A}$$ Analogy with pressure! (N/m^2)

$$\tau = -\mu \frac{dv}{dy}$$

$$\dot{\gamma} = \frac{dv}{dy}$$ shear rate

The shear stress (τ) can be understood as the flo
of momentum (x) in the y direction



Fluid	Flow curve	Equation	Apparent viscosity μ_a
Newtonian		$\tau = \mu\dot{\gamma}$	Constant. $\mu_a = \mu$
Pseudoplastic (power law)		$\tau = K\dot{\gamma}^n$ $n < 1$	Decreases with increasing shear rate. $\mu_a = K\dot{\gamma}^{n-1}$
Dilatant (power law)		$\tau = K\dot{\gamma}^n$ $n > 1$	Increases with increasing shear rate. $\mu_a = K\dot{\gamma}^{n-1}$
Bingham plastic		$\tau = \tau_0 + K_p\dot{\gamma}$	Decreases with increasing shear rate when yield stress τ_0 is exceeded. $\mu_a = \frac{\tau_0}{\dot{\gamma}} + K_p$

Shear stress

It is necessary to create sufficient shear stress, but do not damage the cells!

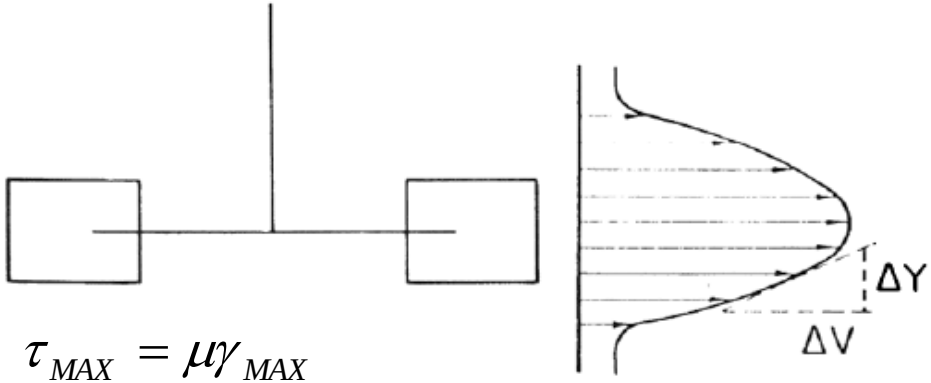
$$\tau = -\mu \frac{dv}{dy}$$

1: $\gamma_{AV} = \kappa N$

2: $\gamma_{AV} = \sqrt{\frac{P}{V \times \mu}}$

κ - geometric const.
N – peripheral speed
μ – dynamic viscosity

Distribution of velocities on the outflow of fluid from the blade



$\tau_{MAX} = \mu \gamma_{MAX}$

$\tau_{AV} = \mu \gamma_{AV}$

$\gamma = \frac{\Delta V}{\Delta Y}$

If $\gamma_{max} = dv_{max}/dy$ up to 300 $s^{-1} \rightarrow \tau$ (1: 0-0,3 Pa, 2: 0-1,5 Pa)

The shear stress (τ) can be expressed in Newtonian fluid as proportional to the shear rate (γ), the dynamic viscosity of the liquid (μ)

For rotating agitators, the shear stress is proportional to the agitator peripheral velocity

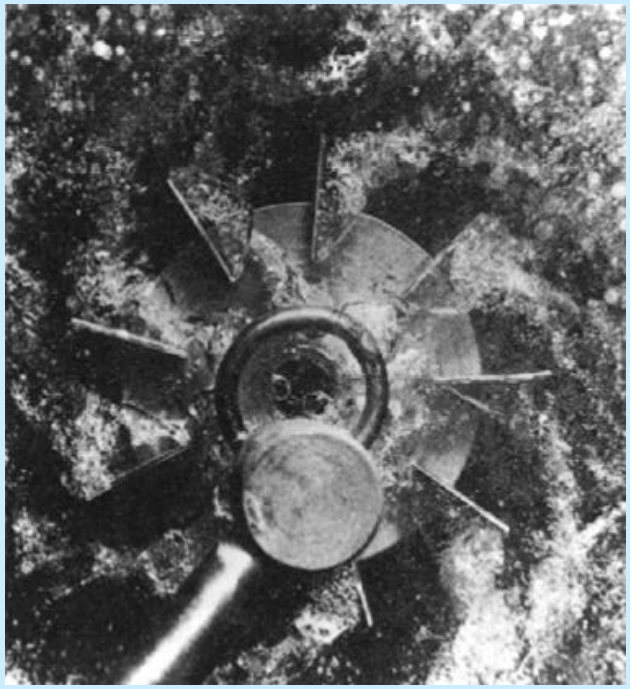
$$\tau \sim nd$$

Impeller type	*k*
Rushton turbine	10–13
Paddle	10–13
Curved-blade paddle	7.1
Propeller	10
Anchor	20–25
Helical ribbon	30

Dispersing effect of impeller

Due to sufficient oxygen transfer, it is necessary to disperse gas into bubbles with high interfacial area

Small cells have a lower ascending rate - a longer time for oxygen dissolution

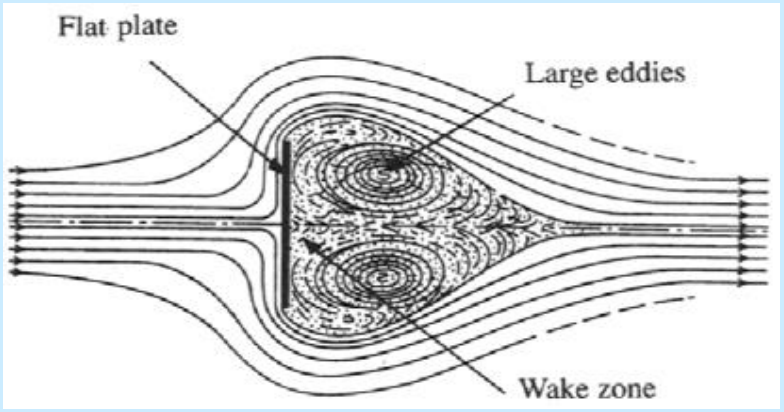


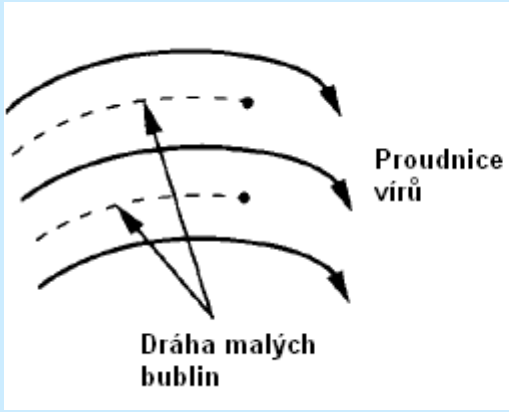
$$\varepsilon = \frac{V_G}{V_L + V_G}$$

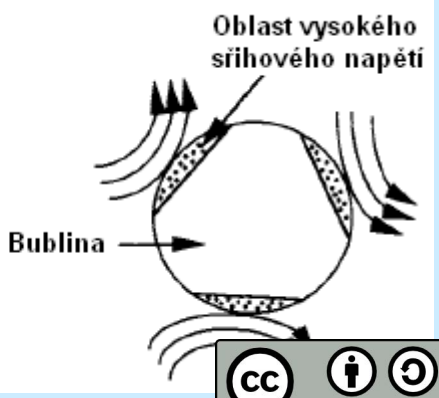
Gas holdup, V_L –liquid volume, V_G-gas volume

$$\lambda = \left(\frac{\nu^3}{P/(\rho V)} \right)^{1/4}$$

Kolmogorov size of vortices







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University of Chemistry and Technology Prague
Faculty of Food and Biochemical technology

Heat transfer, bioreactor heat balance, sterilization by heat

Tomáš Brányik

Basic concepts and mechanisms of heat transfer

Heat is the form of energy, the result of translational, rotational and vibratory movement of particles (molecules).

The measure of this energy is the temperature.

The exchange of energy in matter is called heat transfer.

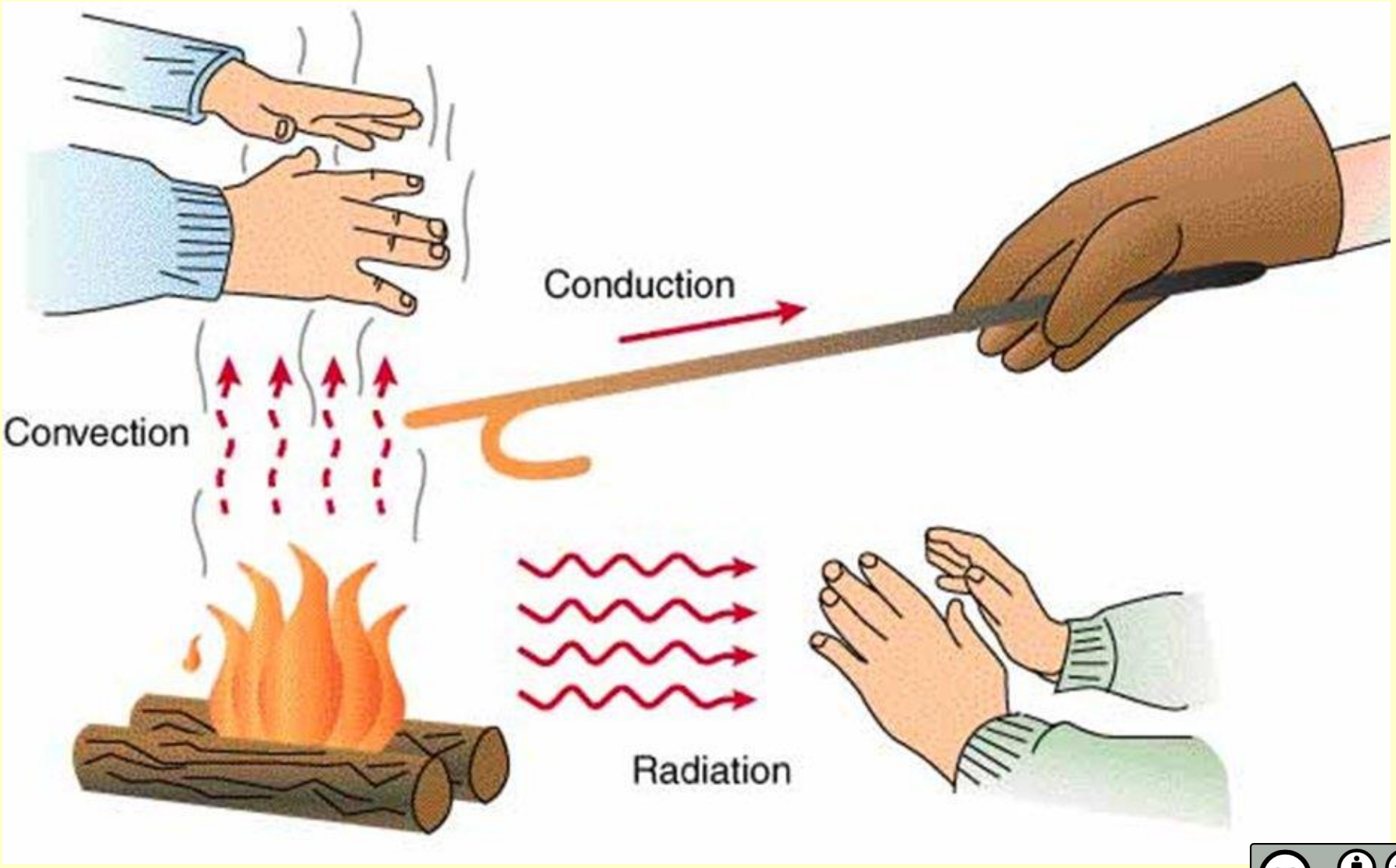
The driving force of heat transfer is the difference in energy of particle motion at different points of the system (temperature difference).

Heat flow $\dot{Q}$ is the amount of heat Q that passes through a given area per unit of time in a direction perpendicular to that area.

$$\dot{Q} = \frac{dQ}{d\tau}$$

Units: heat 1 joule (J), heat flow 1 $J.s^{-1}$ = 1 watt (W)

Heat transfer.....



Mechanisms of heat transfer

- **Conduction**: Mutual collisions between molecules with different energy. Driving force - Different temperatures in different places of matter.

- **Convection**(flow): When fluid flows, the mass moves and hence transfers (also shares) heat. Streaming can be spontaneous or forced.

- **Radiation**: Energy is transmitted in the form of electromagnetic radiation.

- In practice, we often encounter a combination of these mechanisms for which we will use the terms heat transfer or heat flow.

Conduction

$$\frac{\partial T}{\partial x}, \frac{\partial T}{\partial y}, \frac{\partial T}{\partial z}$$

The temperature gradient is a vector whose direction indicates the direction of growth, and its absolute magnitude indicates how fast it grows.

Fourier's Law for Heat Conduction

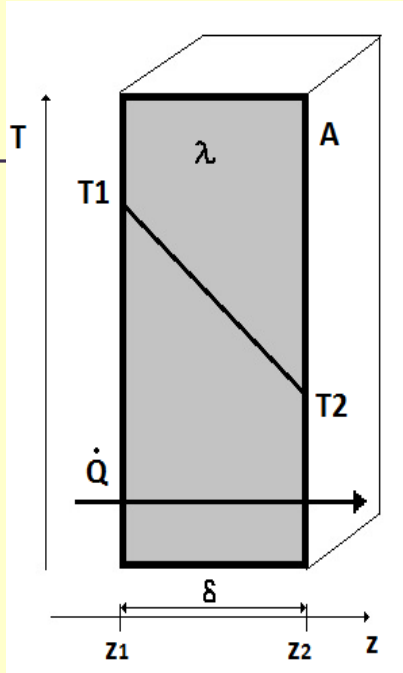
The heat flow through surface A (perpendicular to the z axis) in the z-axis direction is proportional to the surface area and the negative temperature derivation according to the z coordinate.

$$\dot{Q} = -A\lambda \frac{\partial T}{\partial z}$$

Thermal conductivity λ is a material property but is also dependent on temperature and pressure (for gases) [$Wm^{-1}K^{-1}$]

Intensity of heat flow

$$q_z = \frac{\dot{Q}}{A} = -\lambda \frac{\partial T}{\partial z}$$



Heat conduction through a flat wall

Simplifying concept: steady unidirectional heat flow, constant heat flow, constant λ

Result: temperature T is a linear function of z

$\dot{Q} = -A\lambda \frac{dT}{dz}$ arrange $\frac{\dot{Q}}{\lambda A} dz = -dT$

Integration in range $T(z_1)=T_1$ and $T(z_2)=T_2$ gives

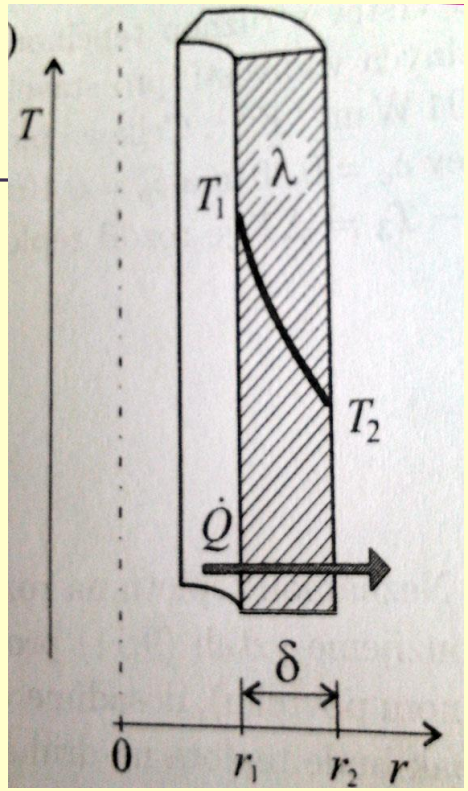
$\frac{\dot{Q}}{\lambda A}(z_2 - z_1) = T_1 - T_2$ where $\delta = z_2 - z_1$ and $R = \frac{1}{A}\frac{\delta}{\lambda}$

R – heat transfer resistance (thermal resistance)

Heat conduction through a flat wall → $\dot{Q} = A\lambda \frac{T_1 - T_2}{\delta}$

Heat conduction through complex wall → $\dot{Q} = \frac{\sum_j \Delta_j T}{\sum_j R_j}$ $R = \sum_j R_j = \frac{1}{A}\sum_j \frac{\delta_j}{\lambda_j}$

Heat conduction through a cylindrical wall



Heat flow in steady state $$\dot{Q} = Aq = -2\pi r L \lambda \frac{dT}{dr}$$

After integration we obtain $$\dot{Q} = 2\pi L \lambda \frac{T_1 - T_2}{\ln \frac{r_2}{r_1}}$$

where $\dot{Q} = \frac{\Delta T}{R}$ and $R = \frac{1}{2\pi L}\frac{1}{\lambda}\ln\frac{r_2}{r_1}$

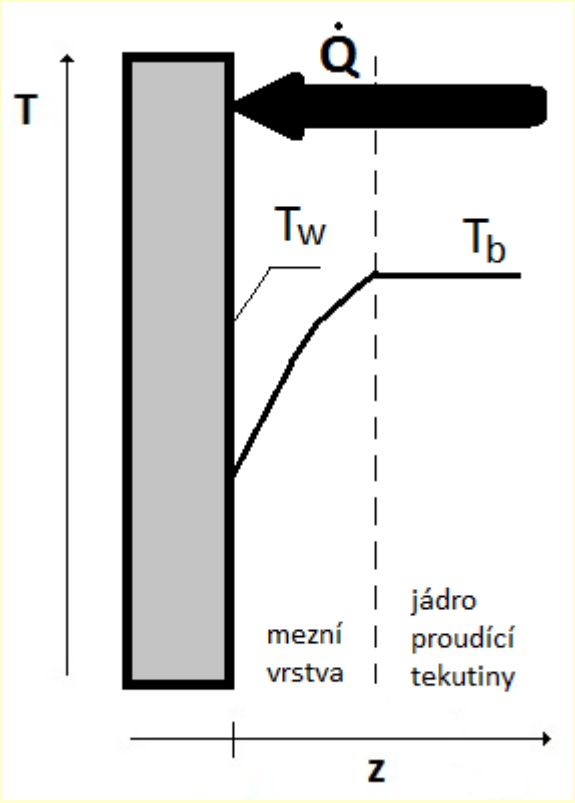
r – radius of cylinder
L – lenght
λ - thermal cunductivity

Heat conduction through complex cylindrical wall

$$\dot{Q} = \frac{\Delta T}{R} \qquad R = \sum R_j = \frac{1}{2\pi L}\sum_j \frac{1}{\lambda_j}\ln\frac{r_{j+1}}{r_j}$$

Heat flow in fluids

In the flowing fluids, the heat is transmitted by both conduction and convection.



Empirical equation

$$\dot{Q} = \alpha A(T_b - T_w)$$ where $\dot{Q} = \frac{\Delta T}{R}$ and $R = \frac{1}{A\alpha}$

α- heat transfer coefficient [$Wm^{-2}K^{-1}$]

This law does not outline the heat transfer mechanism nor the proportion of convection and conduction

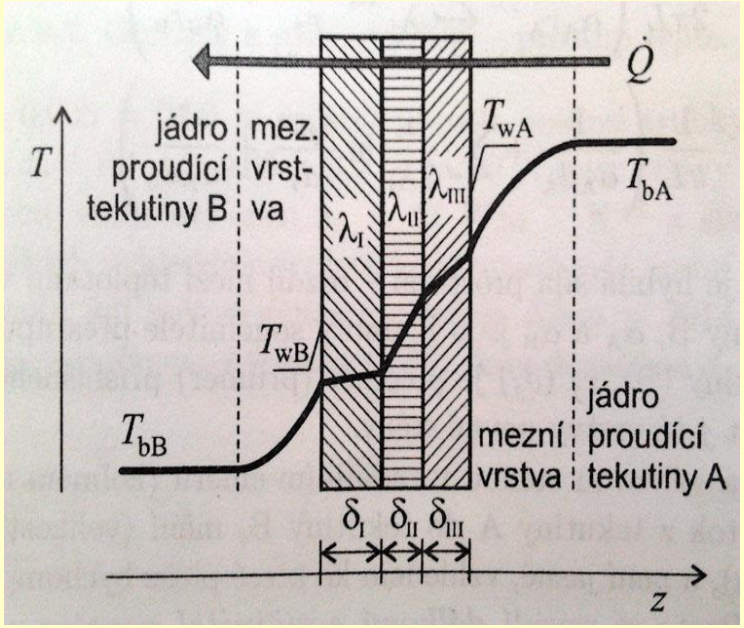
But describes the essence, i.e. the driving force is ΔT and simplifies the problem of finding the value of the heat transfer coefficient α.

Calculation of α is not easy. See Chem. Engineering I., natural / forced flow, dimensionless criteria Nu, Pe, Fo ...

Combined heat transfer

In the flowing liquid separated by the wall, e.g. heat exchangers

- Heat transfer from the core of the warmer fluid A to the surface of the wall.
- Heat conduction by wall (e.g. composite)
- Transfer of heat from wall surface to liquid core B.

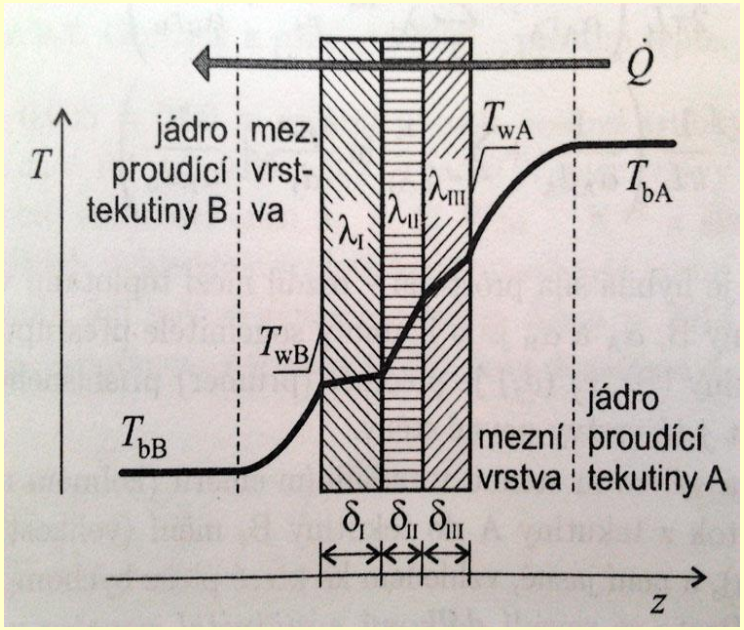


Heat transfer resistance: the sum of the resistances of all partial processes.

Driving force: difference between mean temperature T_{bA} and T_{bB}.

In practical terms, this heat transfer formulation is very important because temperatures can be easily measured.

Combined heat transfer (flat wall)



Total heat flow:

$$\dot{Q} = \frac{\Delta T}{R} \quad \text{where} \quad R = \sum_j R_j = \frac{1}{A}\left(\frac{1}{\alpha_A} + \sum_j \frac{\delta_j}{\lambda_j} + \frac{1}{\alpha_B}\right)$$

$$\Delta T = T_{bA} - T_{bB} \quad \text{driving force}$$

α- heat transfer coeff. In liquid A and B

δ_j – thickness of j-layer, λ_j – thermal conductivity of j-layer

Heat transfer coefficient per unit area

$$K = \frac{1}{AR} = \frac{1}{\frac{1}{\alpha_A} + \sum_j \frac{\delta_j}{\lambda_j} + \frac{1}{\alpha_B}}$$

Total heat flow can be written:

$$\dot{Q} = KA\Delta T$$

Combined heat transfer (cylindrical wall)

Total heat flow :

$$\dot{Q} = \frac{\Delta T}{R} \quad \text{where} \quad R = \sum_j R_j = \frac{1}{2\pi L}\left(\frac{1}{\alpha_A r_A} + \sum_j \frac{1}{\lambda_j} \ln \frac{r_{j+1}}{r_j} + \frac{1}{\alpha_B r_B} \right)$$

$$\Delta T = T_{bA} - T_{bB} \quad \text{driving force}$$

α- heat transfer coeff. In liquid A and B

r_j – radius of j-layer, λ_j – thermal conductivity of j-layer

Heat transfer coefficient per unit area

$$K_L = \frac{1}{LR}$$

Total heat flow can be written : $\dot{Q} = K_L L \Delta T$

Heat balance of bioreactor

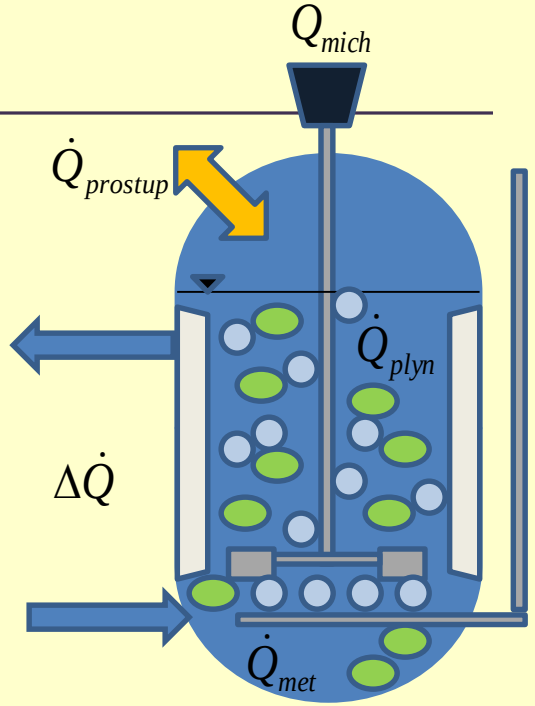
Bioreactors they are not adiabatic systems, so they share heat with the environment.

- The biological reaction generates heat that must be removed from the system. Cooling is demanding due to a small heat gradient (fermentation takes place at 25-40 ° C). Cooling is a problem especially for high density cell cultures, reduced substrates and high viscosity media.

- The biological reaction does not generate enough heat to be delivered to the system (laboratory reactors with a large specific surface, anaerobic digestion, thermophilic microorganisms, thermostable enzymes)

- Sterilization of media *in situ* (heating / cooling)

Heat balance of bioreactoru



Macroscopic energy balance:

$$\frac{dQ}{dt} = \dot{Q}_{met} + \Delta\dot{Q} + \dot{Q}_{mich} + \dot{Q}_{plyn} + \dot{Q}_{prostup}$$

$\frac{dQ}{dt}$ Accumulation of heat in the system

$\dot{Q}_{met}$ Generation of heat by metabolism (W)

$\Delta\dot{Q}$ Changes in system enthalpy caused by difference between input and output(W)

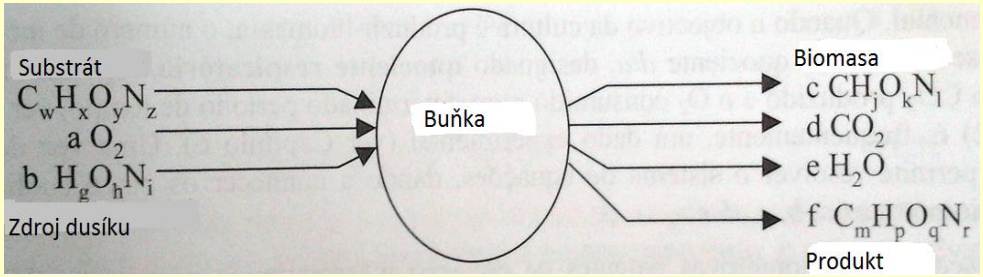
$\dot{Q}_{mich}$ Heat input of agitation (W)

$\dot{Q}_{plyn}$ Heat input by gas flow (W)

$\dot{Q}_{prostup}$ Heat transfer with through walls (W)

Heat formation by microbial metabolism

In the growth phase when the substrate is used as a source of energy as well as carbon, 50-60% of the enthalpy of the substrate is released in the form of heat.



Example 1: Stochiometry of cell growth is known

$$C_wH_xO_yN_z + aO_2 + bH_gO_hN_i \rightarrow cCH_jO_kN_l + dCO_2 + eH_2O + fC_mH_pO_qN_r$$

Usually not available!

E.g. Growth of *Saccharomyces cerevisiae* on glocose

$$C_6H_{12}O_6 + 3{,}918O_2 + 0{,}316NH_3 \rightarrow 1{,}929CH_{1{,}703}O_{0{,}459}N_{0{,}171} + 4{,}098CO_2 + 4{,}813H_2O$$

Heat formation by microbial metabolism

Generic reaction record

$$a_1S_1 + a_2S_2 + ... + a_nS_n \rightarrow b_1P_1 + b_2P_2 + ... + b_mP_m$$

$$\dot{Q}_{met} = r\Delta H = r\left(\sum_{i=1}^{m} b_i H_{P,i} - \sum_{j=1}^{n} a_j H_{S,j}\right)$$

r – reaction rate (mol s^{-1})

ΔH – entalpy of reaction (J mol^{-1}), sometimes it can be found in the literature

$H_{P/S,i}$ – standard merging enthalpy products / substrates (J mol^{-1}), can be found in the literature or determined from combustion heat.

$$\dot{Q}_{met} = r\Delta H = -\frac{dc}{dt} M\Delta H$$

c – conc. of sugar [mol/kg], ΔH_r – reaction heat [kJ/mol], M [kg] – weight of batch, Conversion of 1 mole of glucose to ethanol develops 113 kJ of heat *

*Rehm H.I.: Technische Mikrobiologie, Springer Verlag Berlin, 1969

Heat formation by microbial metabolism

Example 2: it is possible to quantify the O_2 consumption (aerobic processes).

The rate of heat generation correlates with the rate of oxygen consumption and biomass productivity.

$$\dot{H}_{met} = 460 \times 10^3 OUR \cdot V$$

OUR - oxygen uptake rate (mol m^{-3} s^{-1})
V – reactor working volume (m^3)

This correlation is generally valid for a number of aerobic processes including most of the microbial cultivations in which product is formed.

There is no general correlation for anaerobic processes. Usually, the released heat correlates with the consumption rate of the substrate that is the carbon source.

Heat formation by microbial metabolism

Mikroorganism	Substrate	$Y_{X/S}$	Oxygen consumption ($g_{O2}/g_{Biomass}$)	Formed heat ($kJ/g_{Biomass}$)
Bacteria	n-Alkanes	1,0	1,72	32,66
Yeast	Saccharides	0,5	0,67	15,91
Yeast	n-Alkanes	1,0	1,97	33,45

Yeast Candida sp., continuous reactor, $D=0,1h^{-1}$, $X=15\ kg/m^3$

Substrate	$Y_{X/S}$	Formed heat ($kJ/g_{Biomass}$)	Heat formation rate (J/s)
Metane	0,6	75,5	34,7
n-Alkanes (C12-C18)	1,0	32,66	13,9
Saccharose	0,5	15,9	6,9

Changes in enthalpy caused by the difference between input and output

$$\Delta\dot{Q} = \dot{N}_G^{in} H_G^{in} + \dot{N}_L^{in} H_L^{in} - \dot{N}_G^{out} H_G^{out} - \dot{N}_L^{out} H_L^{out}$$

H – entalpy
Ṅ - molar flow

Influence of enthalpy difference between liquid (L) and gaseous (G) at the inlet and outlet

The difference between the enthalpies of the L and G inlet and outlet due to the temperature change of these streams can often be neglected under bioreactor conditions.

Intense aeration can, however, cause a large amount of liquid to evaporate (10-20% by volume).

$$\Delta\dot{Q} \cong \dot{Q}_{vyp} = F\Delta\Psi h_{vyp}$$

F – volumitric flow of dry air (m^3/s)

$\Delta\Psi$ - humidity difference between air inlet and outlet (mol_{H2O}/m^3)

h_{vyp} – molar boiling heat (evaporation) of water (J/mol)

Changes in enthalpy caused by the difference between input and output

$$\Delta\dot{Q} \cong \dot{Q}_{vyp} = F\Delta\Psi h_{vyp}$$

The calculation of $\Delta\Psi$ is simplified if the inlet air can be assumed to be dry and outlet air saturated.

Otherwise, mass balance is needed.

In some cases, it is necessary to calculate the dissolution (solid components of the medium) and the dilution heat (concentrated liquids, e.g. pH adjustment) for correct enthalpy balance.

Thermal energy delivered by agitation

The power of impeller (W) means the energy transferred by the stirrer of the mixed batch per unit of time (work performed by the agitator per unit of time)

This energy is dissipated by the mechanical friction of mass particles in heat $\dot{Q}_{mich}$ $\rightarrow$ heat generation by mixing

What is needed to calculate the agitator power? See. Lecture "Mixing„

The volumetric power of agitators of conventional fermenters is in the range of **0,1-2 kW/m³**

Thermal energy delivered by aeration

The energy transmitted by aeration can be estimated from gas expansion (simplifying assumptions - ideal gas, isothermal process):

$$\dot{Q}_{plyn} = \dot{N}RT \ln \frac{p_i}{p_o}$$

Ṅ - molar gas flow (mol/s)

p_i – gas pressure at inlet

p_o - gas pressure above liquid level

Heat transfer (heating/cooling/losses)

Microbial bioreactors typically work at higher than ambient temperatures

Small laboratory bioreactors - Large heat loss through the wall, heating required

When increasing the scale, the volume of the rector rises with the 3rd power of dimension, while the surface only with square. Large bioreactors are generally needed to cool.

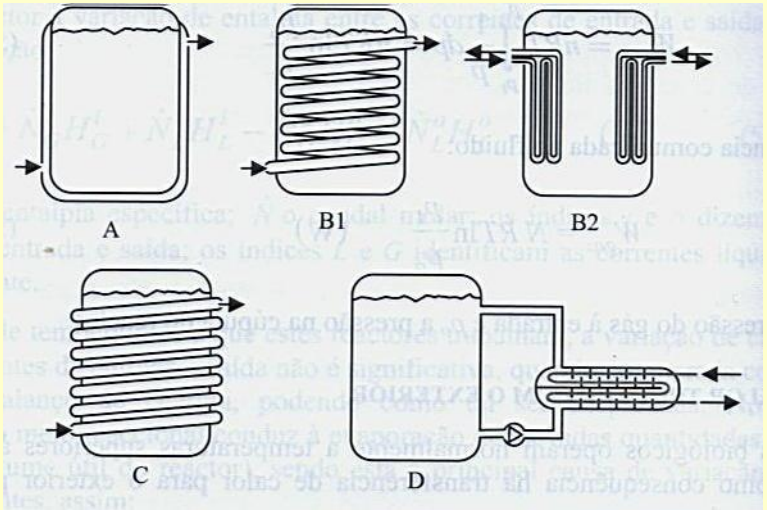
Therefore, internal / external auxiliary exchangers are built in.

$$\dot{Q}_{prostup} = KA\Delta T$$ (W)

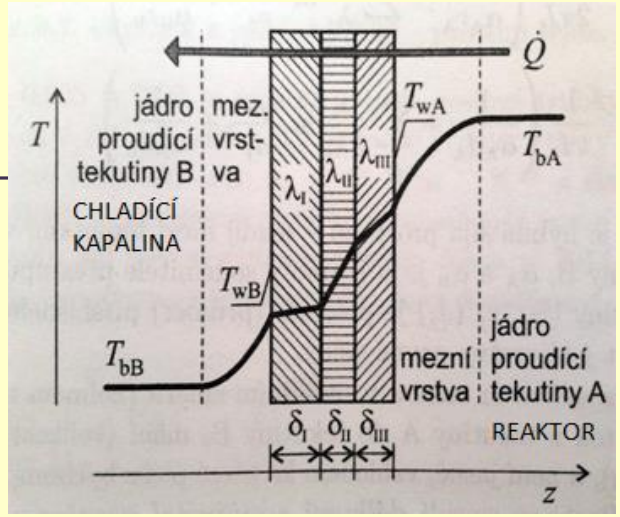
K – total hear transfer coefficient (W/m^2K)

A – heat transfer area (m^2)

ΔT – temperature difference between batch and cooling liquid



A – cooling/heating jacket, B – cooling/heating coil internal, C – coil external, D – heat exchanger

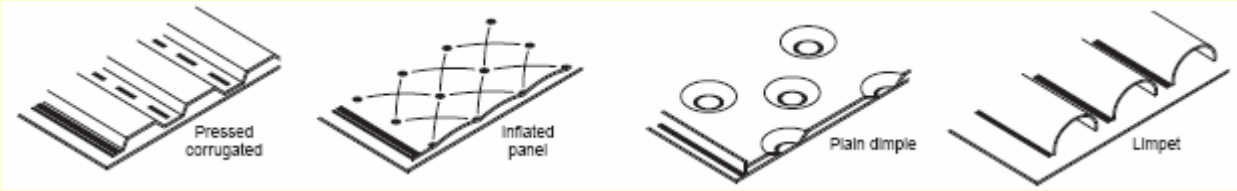


Heat transfer (heating/cooling/losses)

$$\dot{Q}_{prostup} = KA\Delta T$$

$$K = \frac{1}{AR} = \frac{1}{\frac{1}{\alpha_A} + \sum_j \frac{\delta_j}{\lambda_j} + \frac{1}{\alpha_B}} = \frac{1}{\frac{1}{\alpha_A} + \frac{\delta_{deposit}}{\lambda_{depodit}} + \frac{\delta_{ocel}}{\lambda_{ocel}} + \frac{\delta_{biofilm}}{\lambda_{biofilm}} + \frac{1}{\alpha_B}}$$

α- heat transfer coeff. In liquid A a B [$Wm^{-2}K^{-1}$]
δ_j – thickness of j-layer
λ_j – thermal conductivity of j-layer [$Wm^{-1}K^{-1}$]
Vrstva I – deposit of salts from cooling water
Vrstva II – stainless steel of reactor walls
Vrstva III – biofilm of microorganisms



Heat transfer (heating/cooling/losses)

	α [$Wm^{-2}K^{-1}$]
Water*	1700-11000
Gases*	20-300
Solvents*	350-3000
Oils*	60-700
Ammonia (at evaporation)	100-2300

* without changing the state

Deposit	δ/λ [$m^2K(kW)^{-1}$]
From water	0,09-0,58
From steam	0,052-0,18
From air	0,25-0,50
From solvent	0,14
Brine	0,27
Tar	2,0

In addition, there are a number of correlations (usually dimensionless) that express the dependence of heat transfer coefficients on the geometric arrangement of the cooling system, the type of reactor, the type of coolant, etc. They have limited validity!

Sterilization by heat

Sterilization is a set of activities aimed at removing or killing cells (vegetative and spores) in a given environment (solid surface, liquid, gas).

For thermal sterilization, it is necessary to take into account the thermal resistance of bioreactor components (seals, visors, etc.).

Typically, liquid media is heat-sterilized.

The exception is the thermolabile components of the medium - sterilization by filtration and subsequent aseptic mixing with the heat-treated components of the medium.

Thermal destruction of microorganisms

Microorganisms have a range of temperatures in which they grow.

E.g. *Escherichia coli* does not grow below 10°C and above 45°C. Above 50°C its spec. growth rate (μ) is much lower than spec. deactivation rate (k_d).

$$\frac{dN}{dt} = -k_d N \quad \text{or} \quad N = N_0 e^{-k_d t}$$

N_0=conc. living cells at t=0
N=conc. living cells at t
t=time of sterilization
k_d=spec. deactivation (dead) rate of cells

Constant k_d depends on temperature (Arrhenius equation)

$$k_d = A e^{\frac{-E}{RT}}$$

A=pre-exponential factor (const. for reaction)
E=activation energy of thermal death
R,T=gas const., temperature

The number of surviving microorganisms can be determined:

$$\frac{dN}{dt} = -A e^{\frac{-E}{RT}} N \quad \text{after integration} \quad \ln \frac{N_0}{N} = \int_0^t A e^{\frac{-E}{RT}} dt = A t e^{\frac{-E}{RT}}$$

$$\frac{dN}{dt} = -k_d N$$

Thermal destruction of microorganisms

$$\frac{d[C]}{dt} = k(T)[A]^n [B]^m$$

The activation energy values of thermal cell death (E) are in the range of 50-150 kcal mol $^{-1}$

Thermal deactivation of some media components takes place through kinetics of the first order.

$$k_d = Ae^{\frac{-E}{RT}}$$

Medium components /Bacterial spores	Activation energy (kcal mol^{-1})
Folic acid	16,8
Thiamine(vitam. B1)	22,0
Cobalamin (vitam. B12)	23,1
Clostridium botulinum	82,0
Bacillus subtilis	76,0
Bacillus stearothermophilus	67,7

The rate of thermal destruction of the cells is higher than the rate of denaturation of the media components.

It is worth using thermal sterilization even if a little more thermolabile components of the medium are added to the medium. The surplus during the sterilization is denatured.

Thermal destruction of microorganisms

$$\ln\frac{N_0}{N} = Ate^{\frac{-E}{RT}} = k_d t$$

$$\ln\frac{N_0}{N} = \ln\frac{10}{1} = k_d t = k_d D_{10}$$

The time (t) required for the thermal destruction of 90% of the cells is referred to as D_{10}.

$$D_{10} = \frac{\ln 10}{k_d}$$

Relative resistance to humid heat	
Mikroorganism	Relative resistance
Vegetative bacteria and yeast	1
Bacterial spores	3×10^6
Spores of fungi	2-10
Viruses	1-5

Spores *Bacillus stearothermophilus*	
Temperature (°C)	k_d (min^{-1})
100	0,02
110	0,21
120	2,1
130	17,5
140	136

Heat transfer.....

Table 5.8. Selected values of the activation energy for nutrients, enzymes and cells.

Compound or reaction		Activation energy (kJ. g mol^{-1})
spores	*B. stearothermophilus*	287.2
	B. subtilis	318.0
	Cl. botulinum	343.1
nutrients	vitamin B_{12}	96.6
	thiamine.HCl (B_6)	92.0
	riboflavin (B_2)	98.7
	folic acid	70.2
	d-pantothenyl alcohol	87.8
enzymes	trypsin	170.5
	peroxidase	98.7
	pancreatic lipase	192.3
reactions	Maillard (browning)	130.5

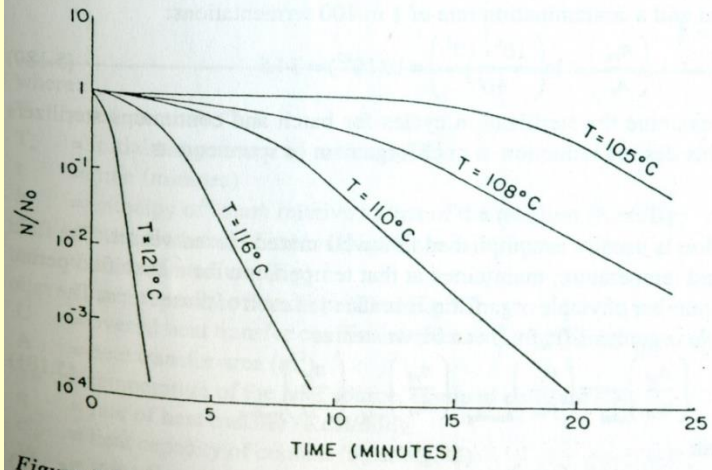


Figure 5.26. Typical death rate data for B. stearothermophilus spores.

Typical values of k for B. stearothermophilus spores.

Temperature (°C)	k (min^{-1})
100	0.02
110	0.21
120	2.0
130	17.5
140	136
150	956

$$\frac{dN}{dt} = -k_d N$$

Thermal destruction of microorganisms

The effect of temperature causes an exponential decrease in the number of living cells.
Therefore, the complete killing of all cells takes a long time.
Industrial fermentation counts with a certain risk - contamination of 1 of 10^3-10^4 fermentation due to lack of sterilization is acceptable.
In the case of human food preservation, this is 1 out of 10^{12}.

In fact, sterilization (media, surfaces) of mixed culture takes place.

Sterilization time is calculated for the species with lowest kd (if known).

If the species composition of contaminants is unknown, sterilization is proposed with a large margin $\rightarrow$ considered contamination by 10^5-10^6 spores/mL.

For each microorganism there is an infinite number of time-temperature combinations capable of leading to the desired sterilization.

Because of nutrient denaturation - "high temperature-short time„ is used.

Thermal destruction of microbes

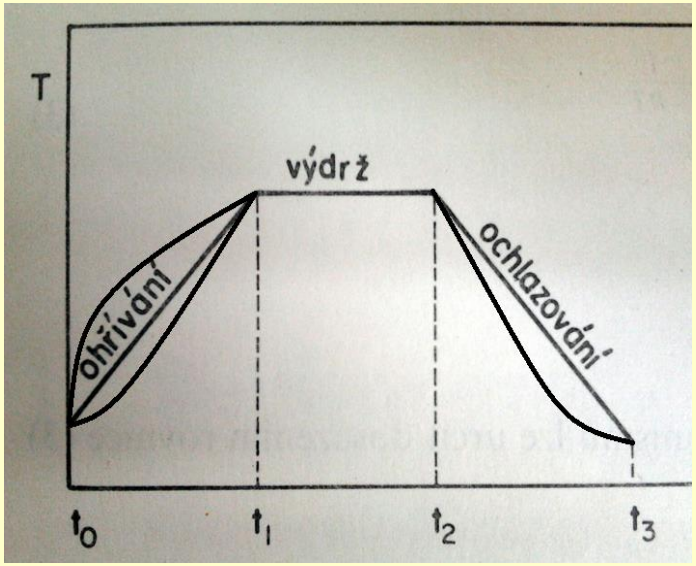
Sterilization - batch

For common preparation of small volumes of nutrient media: 121°C (15 min), 126°C (10 min), 134°C (3 min).

Industrial reactors are sterilized *in situ* indirectly (steam, oil, electricity) or directly steamed into the media. Suitable for media without thermolabile components.

When sterilizing large volumes, the heating and cooling phases are long. This has an effect on the thermal degradation of the components of the medium and cell death.

The overall sterilization effect is calculated :



Heating profile:
Direct steam injection – hyperbola
Electric heating – linear
Duplicator – exponential (for cooling too)

$$\ln\frac{N_0}{N} = \int_{t_0}^{t_1} k_d\,dt + \int_{t_1}^{t_2} k_d\,dt + \int_{t_2}^{t_3} k_d\,dt$$

Thermal destruction of microorganisms

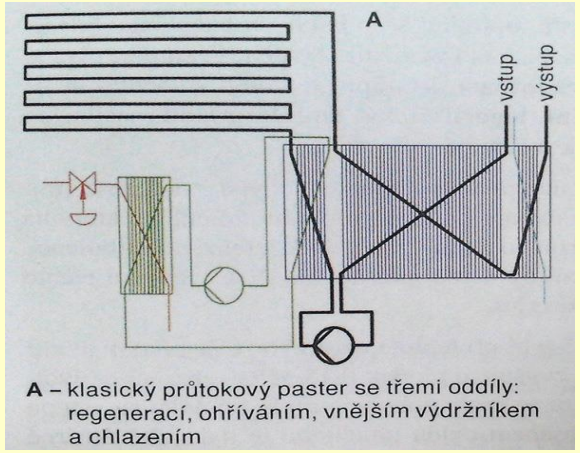
Sterilization - continuous

Saving steam (135°C) and shortening process (3-5 min), especially heating and cooling phases

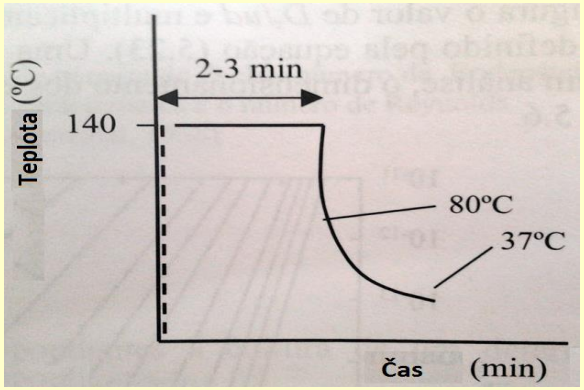
Heating either by direct steam injection into the medium or by heat exchanger.

Preferred for solid-free media (the particles do not rapidly heat up) or containing thermolabile components.

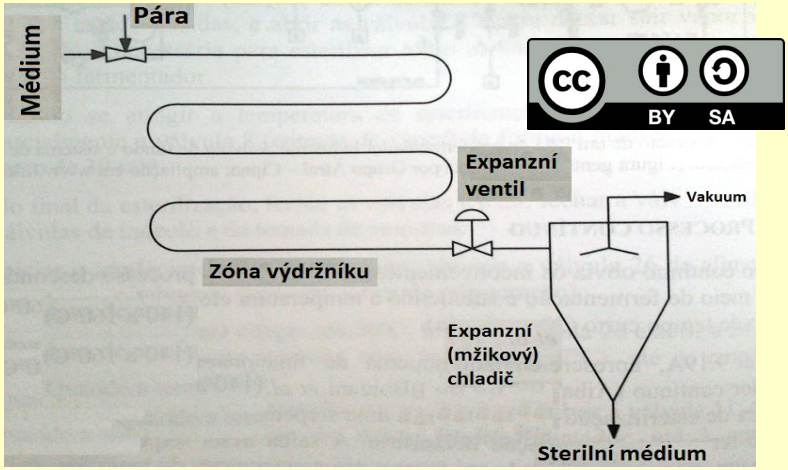
This method requires bioreactor sterilization (steam, hot air, chemically)



A – klasický průtokový paster se třemi oddíly: regenerací, ohříváním, vnějším výdržníkem a chlazením



Direct steam injection and expansion cooling



Thermal destruction of microorganisms

Sterilization - batch

Another disadvantage: Sterilization cycle on an industrial scale lasts 8-12h, which represents a considerable part of the production capacity of the fermenter.

Sterilization - Continuous

Another disadvantage: Accumulation of deposits on the internal surface of the exchangers (reduced heat flow).

Another disadvantage: Direct steam injection is less susceptible to deposit creation, but there is a risk of foam formation.

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Scale-up of bioprocesses

Scale-up

"Got a few problems going from lab scale to full-scale commercial."

What is Scale-up (Scale up)

Convert process from smaller scale to larger

laboratory → semi-pilot → pilot → industrial*

Up to 30 L 30-100 L 100-5000 L >5000 L

Alternatively

Microbiological volume (up to 1L) – Erlenmeyer flasks, thermostats - temperature and shake control, media optimization

Biotechnological (laboratory) volume (1-5L) – fermenters with temperature control, pH, DO2, mixing, foaming

Pilot scale (50-1000L) – efforts to maintain lab geometry, fermenters and conditions that led to the best results. It is necessary to determine the parameters characterizing the transfer of matter and heat, sp. oxygen consumption, agitator power.

Industrial scale (up to 1000 m^3) – require extensive auxiliary operations (compressed air, steam, sterilization, media and inoculation preparation, process control)

* V. Krumphanzl, Mikrobiální technologie, Academia Praha 1988

Importance of scale-up: time and money!

Successful scale-up means shortening industrial process implementation, market advantage, cost savings.

The size and complexity of the process increases!

Successful scale-up requires knowledge:

1. **Transport phenomena (mass, heat)**
2. **Effect of shear forces on microorganisms**
3. **Genetic stability of the biological agent**
4. **Construction materials**
5. **Isolation and purification methods**
6. **Bioprocess control and management mechanisms**

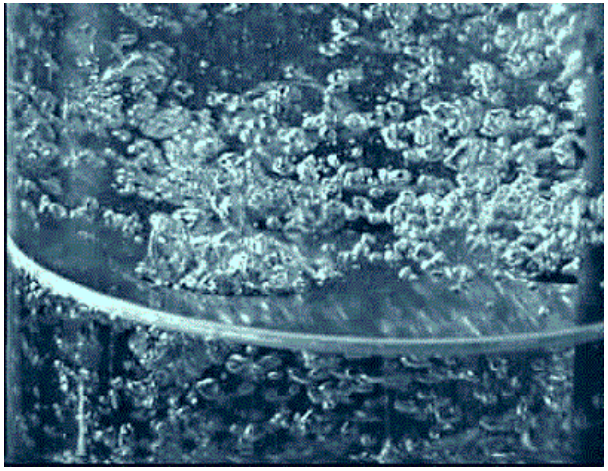
The more complex the knowledge of individual areas, the more likely a successful scale-up is.

It is not possible to maintain the same physical and chemical parameters of the scale-up environment and therefore there will be differences between the behavior of microorganisms.

Success of scale-up depends on keeping process speed, yield and product purity!

Since it is not possible to maintain all physical and chemical parameters at scale-up constant, it is important to know the limits in which to move and at least the basic tendencies of the influence of the parameters on the process

Example: Different combinations of mixing and aeration can lead to the same result.
However, by varying one or both parameters (independently variable), a number of dependent variable parameters will change: circulation time, investment and operating costs, foaming, shear forces, interface size, etc.



There is no general plan for scale-up of microbial processes. Groups of micro-organisms have their own specifics.

Characteristics of Microbial and Cell Culture Systems

	Bacteria	Mold/fungi	Animal/plant cells
Doubling time	Shortest	Intermediate	Slowest (days)
Viscosity	Normally low	Often a problem	Low
Medium cost	Low	Low to intermediate	High
Shear resistance	High	Often high	Often low
Cost of downstream processing	Modest	Modest	High
Culturing	Suspension	Suspension	Substrate or suspension
Product concentration	High	Intermediate	Can be very low
Aggregation	Nil to low	Low to intermediate	May be a problem
Product value	Low to intermediate	Low to high	High to very high
Cell density	Can be very high	Intermediate to high	Usually low
Clean steam/WFI	Almost never	Almost never	Very often
Contamination	Nil to low	Nil to low	Often a problem
Genetic stability	Usually stable*	Occasional problems*	Sometimes a problem

* If genetically modified, plasmid stability is often a problem.

Bioprocess scale-up

„Checklist“ potenciálních problémů

•Not all areas are problematic each time, but assuming that problems do not occur without sufficient verification (information) is a bad choice

Scale-Up Considerations (Potential Concerns)

	Potential Concerns	
Strain selection	Purity	Aggregation
	Stability	Viscosity
	Mutation	Degeneration
	Byproducts	Phage resistance
Raw materials	Purity (vendor audit)	Availability
	Standards	Cost
	Uniformity	Substrate concentration
	Interactions	Defoamer
Inoculum development	Transfer time	Storage parameters
	Hold time	Number of vessels
	Number of generations	Quality control
Sterilization	Total heat input	Maillard reaction
	Method used	Hold time
	Fouling	
Selection of parameters	Mixing (turnover)	Power and shear
	DO_2 level	CO_2 level
	Homogeneity	Oxygen transfer rate
	pH (ORP)	Pressure
	Gradients	Temperature
Materials of construction and cleaning	Ionic contaminants	Surfactants
	Corrosion/erosion	Agents used
	Release agents	Surface and weld quality
Monitoring	Sensor stability	Quality of control
	Response time	Alarms (and response)
	Sampling	
Harvest	Transfer time	Cell degradation
	Hold time (stability)	Asepsis
Isolation	Stability	Impurities
	Cost (quality of control)	Recycle streams
	Regeneration	

It is advisable to seek advice when troubleshooting

Sterilization

- Sterile laboratory environment can not be achieved in the same way in industry (laminar box is not the same as clean room)
- Techniques of aseptic formation may affect the behavior of the biological agent
- Sterilization 1 L → 5000 L, total and specific heat load changes, influence on thermolabile components of the medium
- Time
- Inoculation in the laboratory in 30 seconds takes place in the industry in 30 minutes
- With these time scale changes, it is necessary to ensure quality and composition (inoculum, media components and products, etc.)
- The predictions of these changes are complex, but underestimate them does not have to pay off

Cleaning

- The problem of sediment creation in flask can be solved by throwing it out ≠ Bioreactor?
- Washing and visual inspection in the laboratory is ok ≠ Bioreactor?
- Washing and sanitizing products can leave gaseous / liquid residues in the bioreactor → Strict testing of cleaning agents is necessary

Specific large-scale problems appear again when the new production strain is integrated into the production process

Example: adaptation of cultivation medium in the industrial process (cheaper / more suitable)

- Does this create new by-products?
- Is product cleanliness preserved?
- Is product stability maintained?

Example: new production strain

- What mixing does it require?
- Does it require a different medium or other substrate dosage?
- Strain sensitivity to DO2 or DCO2
- Strain sensitivity to ionic composition and ionic strength

Strategies applicable to scale-up:

- **Fundamental approach**
- **Principle of similarity**
- **Dimensional analysis**
- **Rules of thumb**
- **Scale-down/Mode analysis**
- **Trial and error (heuristic)**

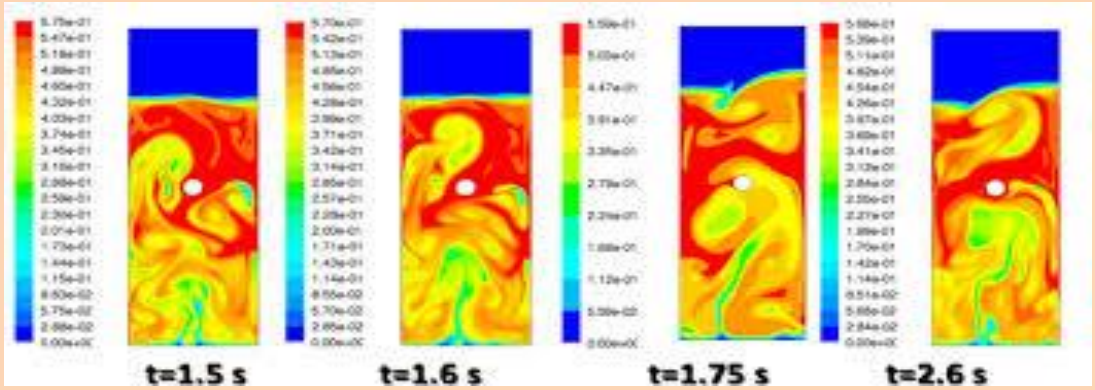
Heuristic (Greek – find, discover) is any approach to problem solving, learning, or discovery that employs a practical method not guaranteed to be optimal or perfect, but sufficient for the immediate goals. Where finding an optimal solution is impossible or impractical, heuristic methods can be used to speed up the process of finding a satisfactory solution. Heuristics can be mental shortcuts that ease the cognitive load of making a decision. Examples of this method include using a rule of thumb, an educated guess, an intuitive judgment, guesstimate, stereotyping, profiling, or common sense.

Fundamental approach

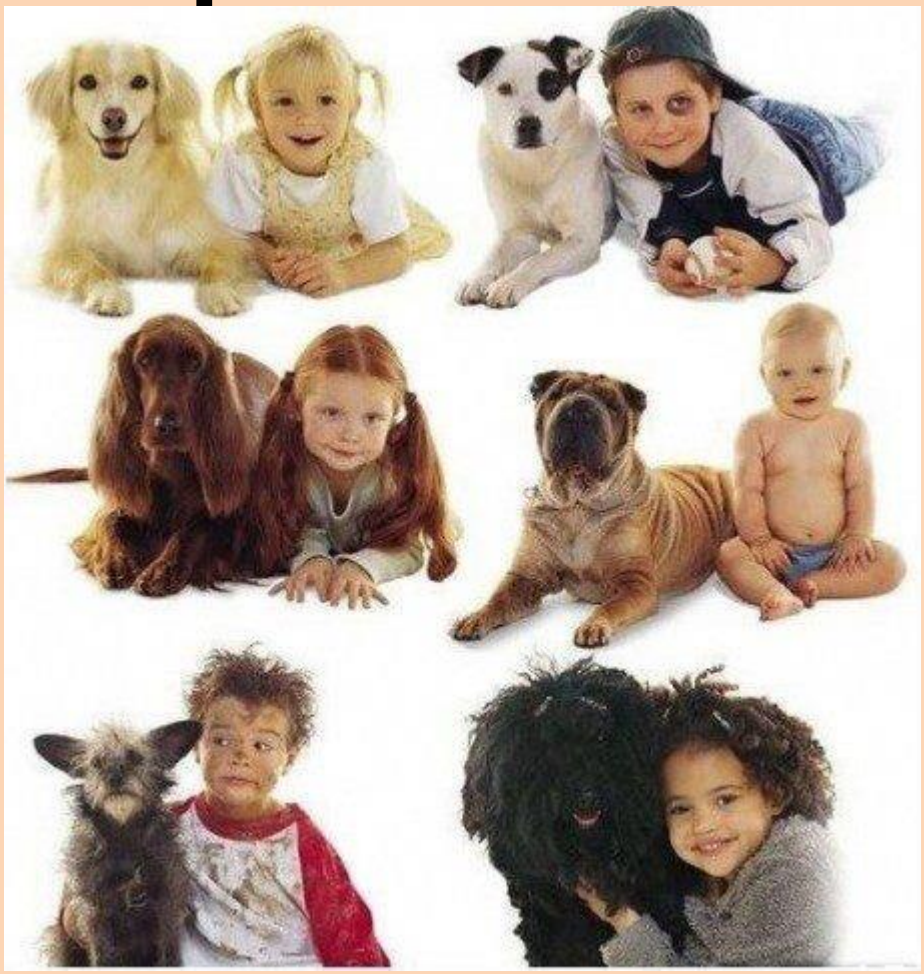
Basic research in hydrodynamics and its interaction with biological process factor.

It uses modeling and prediction methods (e.g. CFD).

The demanding area is sometimes too time-consuming and complicated for practical purposes, but the development of computer technology is growing.



Principle of similarity



Principle of similarity

1. **Geometric** – similarity between the ratio of linear dimensions of systems
2. **Kinetic** – similarity between the ratio of rates
3. **Dynamic** - similarity between the ratio of forces (especially momentum and energy) acting in two systems

Systémys can be similar in 1, 1+2, or 1+2+3 (but we need more info each time)

How to proceed?

1. **Traditional approach -** when we know the geometric, kinetic and dynamic characteristics of the system. They are often used in dimensionless form.
2. **Dimensional Analysis -** When 1 is unknown, we need to make a dimensional analysis and get a dimensionless equation characterizing the system

Traditional approach – identify the parameter most affecting the biological process and maintain its absolute value at all scales..

The most common problem of bioreactor scale-up is the formation of velocity and subsequently concentration gradients of nutrients and products.

Preservation of geometric similarity

Geometric similarity - characteristic dimensions (H, D, blade diameter, stirrer placement, etc.) are in the same ratio in the model and industrial device.

H_L/D_T = const. (1-3 mech. Agitated react.)

H_L- liquid height, D_T – reactoru diameter

D_T/D_A = const. (2-4 mech. Agitated react.)

D_A – impeller diameter

Preservation of kinetic and dynamic similarities

Scale-up of mechanically agitated fermenters

- If we want to maintain the same maximum shear rate values, we select a constant circumferential blade speed ($v=\pi nd$)

  nd = const. n – impeller frequency

  $(\pi dn)_{ind} = (\pi dn)_{lab}$

  $n_{ind} = n_{lab} (d_{lab}/d_{ind})$

  Typical circumferential impeller speeds are 2.5 – 7 m/s

- If the mixing time is decisive, we select a constant frequency (n = const.) – to maintain the specific pumping efficiency of the impeller

  Pumping efficiency: $V = Cnd^3$

  Specific pumping efficiency: $V/Vr = Cn \sim n$

  Mixing time: 10-50L→1-5s, 1000-2000L→20-30s, 100000L→2min

$$P_O = \frac{P_M}{\rho n^3 d^5}$$

Scale-up of mechanically agitated fermenters

- If it is critical to maintain the same dispersion conditions and volumetric mass transfer coefficient (kLa) at scale-up, we choose a constant specific power input

P_M/V is in range 0,5-1,5 kW/m^3 (microbial technologies)

ca. 0,01 kW/m^3 (tissue cultures)

It is often necessary to maintain constant kLa (especially aerobic processes associated with biomass formation)

$k_La \sim (P_M/V)^{0,5} u_G^{0,4}$ u_G – gas velocity (m/s)

The constant kLa can be achieved by adjusting the specific power input (beware, the reduction will affect the homogenization time) or the gas velocity

Increased gas flow shortens homogenization time

Bioprocess scale-up

Scale-up criterium	description	Reactor 80L	Industrial reactor 10000L			
Impeller frequency	n	1	0,34	1,0	0,2	0,04
Impeller diameter	d	1	5,0	5,0	5,0	5,0
Impeller power input	P_M (n^3d^5)	1	125	3125	25	0,2
Specific power input	P_M/V_r	1	1,0	25	0,2	0,016
Pumping efficiency	$V=Cnd^3$	1	42,5	125	25	5,0
Specific pumping efficiency	V/V_r	1	0,34	1,0	0,2	0,04
Circumferential speed	$\nu=\pi nd$	1	1,7	5,0	1,0	0,2
Reynolds number	$Re=nd^2\rho/\eta$	1	8,5	25	5,0	1,0

Increasing impeller diameter 5 x

Dimensional analysis

Mach number	$Mc = V/V_{sound}$
Marangoni number*	$Ma = \Delta\sigma/\mu V$
Morton number	$Mo = g\mu^4\,\Delta\rho/\rho^2\sigma^3$ $(=We^3/Fr\,Re^4)$
Nusselt number*	$Nu = k_{heat}/(\lambda/L)$
Peclet number*	$Pe = LV/\kappa$ (heat)
Peclet number*	$Pe = LV/D$ (mass)
Prandtl number	$Pr = Pe_{heat}/Re = \nu/\kappa$
Rayleigh number	$Ra = \alpha\,\Delta\Theta\,gL^3/\nu\kappa$ (heat) $(=Gr\,Pr)$
Rayleigh number	$Ra = \beta\,\Delta C\,gL^3/\nu D$ (mass) $(=Gr\,Sc)$
Rayleigh number	$Ra = g'eL^3/\nu_{mix}D_{hydro}$ (dispersion)
Reynolds number*	$Re = \rho LV/\mu = LV/\nu$
Richardson number	$Ri = (\Delta\rho/\rho)(gL/V^2)$ $(=(\Delta\rho/\rho)/Fr)$
Rossby number	$Ro = V/\Omega L$
Schmidt number	$Sc = \nu/D$ $(=Pe_{mass}/Re)$
Sherwood number*	$Sh = k_{mass}/(L/D)$
Stanton number	$Sn = (k_{heat}/V)(\kappa/\lambda)$ (heat) $(=Nu/Pe_{heat})$
Stanton number	$Sn = k_{mass}/V$ (mass) $(=Sh/Pe_{mass})$
Stokes number	$St = \rho_p LV/\mu$
Strouhal number*	$Sr = L/TV$
Šebestová number	$Še = 1/Mc$
Thiele number	$Th = L(k_{reac}/D)^{1/2}$
Weber number*	$We = (\Delta)\rho LV^2/\sigma$

Dimensional analysis

The mathematical expression of a (physically) technological problem is of general validity only assuming that the equation is dimensionally homogeneous (this is dimensionless) and thus applies to a system of any size.

Table 1 Base Quantities, Their Dimensions, and Their Units According to SI

Base quantity	Base dimension	Base unit
Length	L	m (meter)
Mass	M	kg (kilogram)
Time	T	sec (second)
Thermodynamic temperature	Θ	K (Kelvin)
Amount of substance	N	mol (mole)
Electric current	I	A (ampere)
Luminous intensity	I_v	cd (candela)

Table 2 Often-Used Physical Quantities and Their Dimensions According to the Currently Used SI in Mechanical and Thermal Problems

Physical quantity	Dimension
Angular velocity	T^{-1}
Shear rate, frequency	
Mass transfer coefficient	
Velocity	
Acceleration	
Kinematic viscosity	
Diffusion coefficient	
Thermal diffusivity	
Density	
Surface tension	
Dynamic viscosity	
Momentum	
Force	$M L T^{-2}$
Pressure, stress	$M L^{-1} T^{-2}$
Angular momentum	$M L^2 T^{-1}$
Energy, work, torque	$M L^2 T^{-2}$
Power	$M L^2 T^{-3}$
Heat capacity	$L^2 T^{-2} \Theta^{-1}$
Thermal conductivity	$M L T^{-3} \Theta^{-1}$
Heat transfer coefficient	$M T^{-3} \Theta^{-1}$

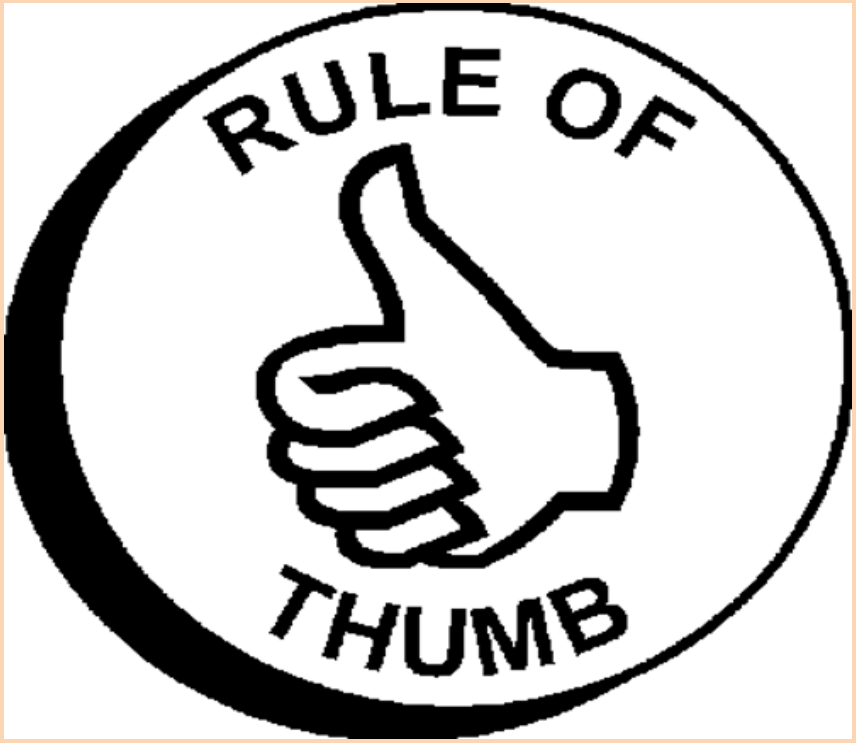
Table 3 Important Secondary Measuring Units in Mechanics, Named After Famous Researchers

Secondary quantity	Dimension	Measuring unit	Abbreviation for:
Force	$M L T^{-2}$	kg m sec^{-2} (N)	Newton
Pressure	$M L^{-1} T^{-2}$	kg m^{-1} sec^{-2} (Pa)	Pascal
Energy	$M L^2 T^{-2}$	kg m^2 sec^{-2} (J)	Joule
Power	$M L^2 T^{-3}$	kg m^2 sec^{-3} (W)	Watt

The aim of the dimensional analysis is to verify whether the system (problem) can be described in a dimensionally homogeneous (dimensionless) shape
This requires two steps :

1. Choose one dependent variable and identify all important variable parameters that must be independent of each other. The choice is highly subjective and requires a profound knowledge of the subject. It can only be recommended not to forget the system geometry, material properties, kinetic and dynamic aspects, external conditions).
2. Conversion to dimensionless form

Qualified guess, or "experience„

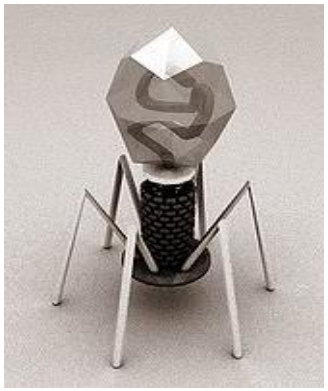


Good judgment comes from experience,
and a lot of that comes from bad judgment.

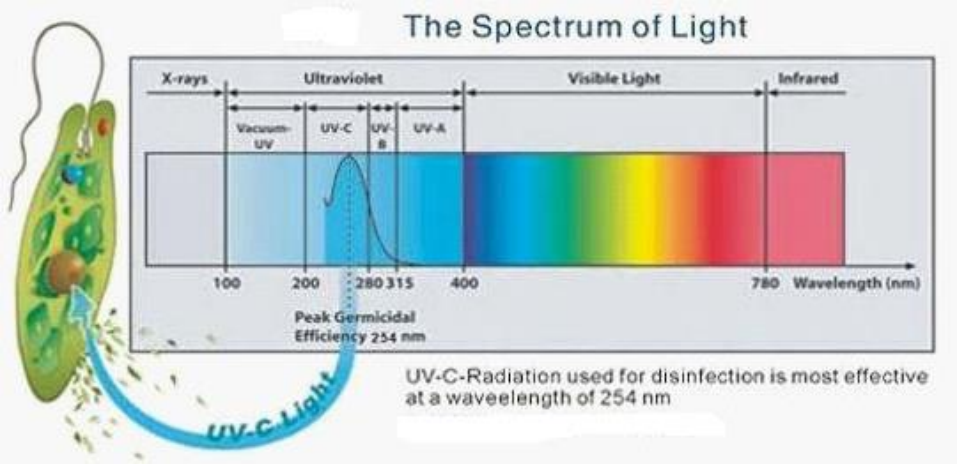
- Will Rogers

In addition to mixing-aeration issues, scale-up of fermentation processes may also influence some bio-factors:

- genetic stability of the micro-organism
- resistance to microbial / phage infection
- cell and product resistance to shear forces
- susceptibility to pellet formation
- heat generation
- minimum DO2 tolerance
- maximum DCO2 tolerance



Other factors affecting the success of fermentation processes after scale-up:



- water quality
- Sterilization
- steam quality
- raw materials
- repeated application of the microorganism
- volume of inoculum
- detergent and chemicals for pH control

Selected areas of bioprocess scale-up

Fermentation process

Media preparation

Preparation of the inoculum

Process control

Harvest (Separation)

Medium preparation (medium components)

Reliability of the vendor - vendors of chemicals do not inform (or even know) that the composition of the ingredient has changed. Companies merge, buy or crash - there must be a replacement solution!

Guarantee of supplies - laws and regulations are changed, the carrier changes, the exchange rate, the fluctuation of prices. Is there enough storage, under what conditions, who controls it?

Quality assurance - It must be clear who performs quality control and when. If the component does not pass quality control, it must be clear from the contract what is going to happen. Storage conditions must be pre-defined and maintained.

Medium preparation (medium components)

Component testing - Regular testing for both composition and fermentation is required. New media components even more important!

Disruption of supply - not only in terms of flow of material but also conditions during delivery (container cleanliness, temperature, humidity)

The components of tissue culture media (hormones, growth factors) are more sensitive and usually require a higher degree of purity.

Another risk is the presence of contamination - eg endotoxins

Preparation of the inoculum

Preparation of the inoculum is given the same attention as fermentation.
The areas that should be addressed are:
Age of inoculum - necessary to set quantitative criteria for scale-up inoculation at the right stage of growth
Keeping the inoculum - how long can it be stored without changing the quality and under what conditions? Terms of "expulsion" (destruction) of inoculum?
Stability of the inoculum - some cells (recombinant, special) can quickly change quality. It is necessary to introduce parameters that adequately describe the quality of the inoculum.

Preparation of the inoculum

Cross-Inoculation - In the case of loss of inoculum, it is possible to use culture from a secondary fermenter as an inoculum? Economically convenient!
Inoculation time - Increases with volume. It is necessary to set limits that already have a negative impact on quality.
Inoculum handling - Any inoculation is a potential risk. It is advisable to reduce growth and pumping times (better 2% than 10%). Lyophilized (frozen) cells are a better variant of the inoculum if they give the same result. Using semiconductor technology is more beneficial than fresh inoculum vaccination - not every process allows it.

Process control

Bioprocesses require strict control and maintaining parameters within the required range:
Sterility - Need to sterilize sensors, most are not contactless
Stability - Need to calibrate sensors after cleaning and sterilization
Excessive measurement - due to the failure of key sensors, it is necessary to install a duplicate number of sensors or just a portable meter?
Centralization of management - must local intervention be possible?
Environment - the need to ensure, maintain and validate the cleanliness of production areas (particle number, overpressure, T, humidity)

Harvesting (separation)

Bioprocesses require strict control and maintaining parameters within the required range:

Harvest time - The fermenter usually does not serve to keep the batch until it enters the process of finishing operations. The key is harvest time and residence time before product isolation.

Batch storage tank - may require special conditions (T, inert gas, mixing, additives, cleaning and sterilization requirements)

Destruction of Batch - It is necessary to define the conditions and the course of the destruction of the unsuccessful batch

Selected areas of bioprocess scale-up

Sterilization, cleaning and aseptic design

Sterilization methods: wet/dry heat, irradiation, filtration, chemical agents, cold plasma

Problems related to sterilization and scale-up:

1. Media often contain thermostable components. Autoclaving 50 ml of medium does not pose a problem (fast heating, 30 min, rapid cooling). Sterilization 500 hl is not the same! In particular, heating and cooling are longer. Heating sometimes requires direct steam injection. Sterilization of large volumes - High thermal load of the medium (degradation of labile substances, production of products). An alternative is continuous sterilization.

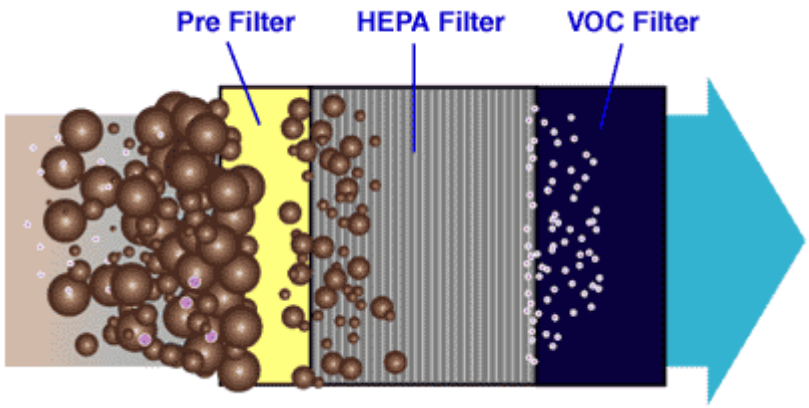
Problems related to sterilization and scale-up:

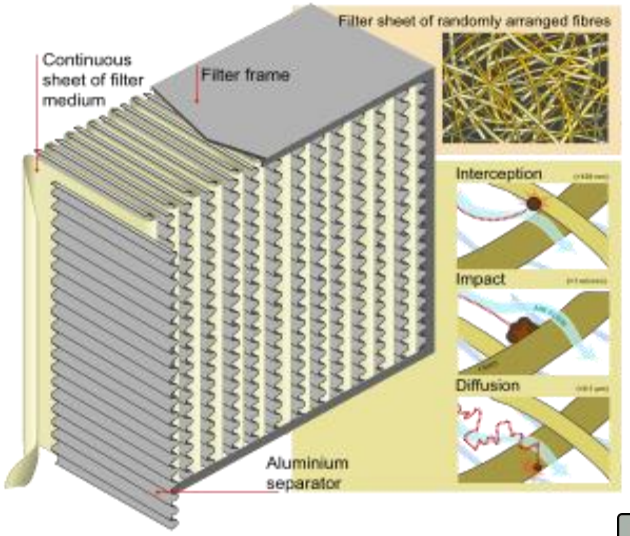
2. On lab. scale, the medium may be thrown out when sterilizing problems. On ind. scale, extreme scenarios must be verified during scale-up studies. Repeated sterilization can suppress contamination but can damage the process.
3. Changing the type of filter used in the lab can be smooth. In industry, new filters must be validated! Type, size, manufacturer - cytotoxicity in combination with medium yes / no?
4. The autoclave, the radiation chamber, and the driers are tested under increased load conditions. In the autoclave, the temperature of the liquid is in the middle of the container, not on its surface!

Problems related to sterilization and scale-up:

5. Membrane air filtration - neccessary careful testing, consideration of pre-filtering, condensation undesirable - Air temperature must be above dew point.

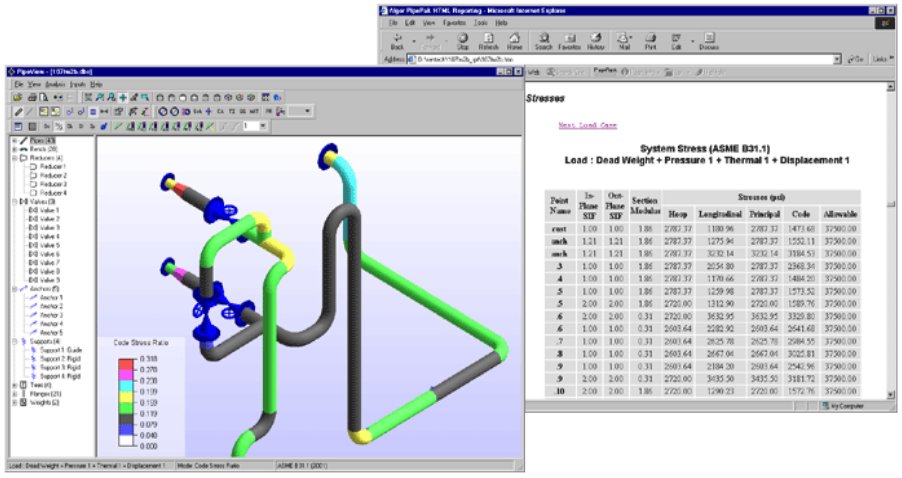
6. In the case of chemical sterilization, complete elimination or degradation of the agent - absence in the product - must be guaranteed.



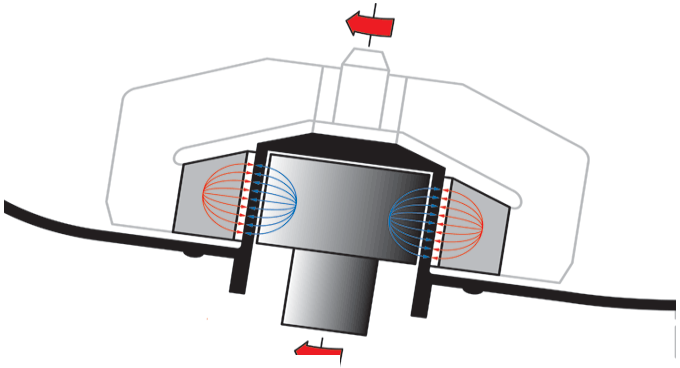


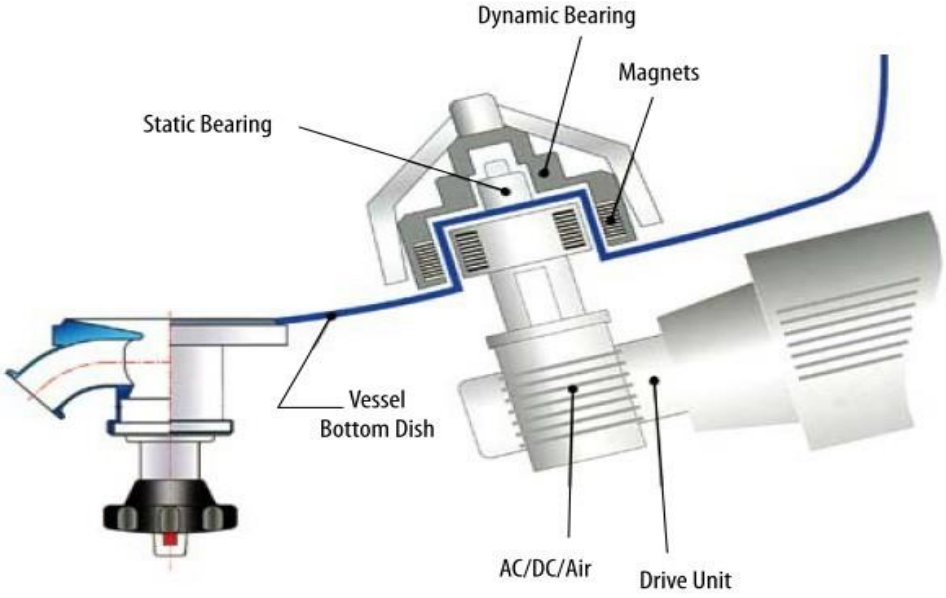
Problems related to aseptic design and scale-up:

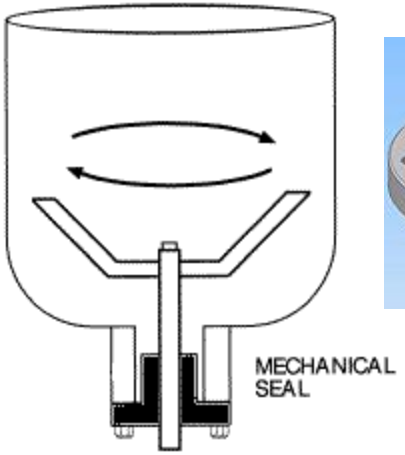
1. All pipelines must be designed so that the fluid flows out spontaneously.
2. Pipes leading to and from the fermenter should have a steam closure, i.e. the possibility of keeping it under steam pressure. Beware of the need for cooling before use.

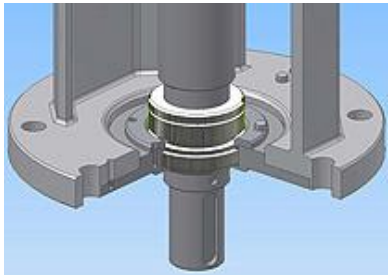


3. Use magnetic stirring if possible. If this is not the case, use a double mechanical seal that resists high temperatures (lubricant too). If there is a risk of leakage, it is still necessary to use a safety seal (to prevent leakage and aerosol formation).



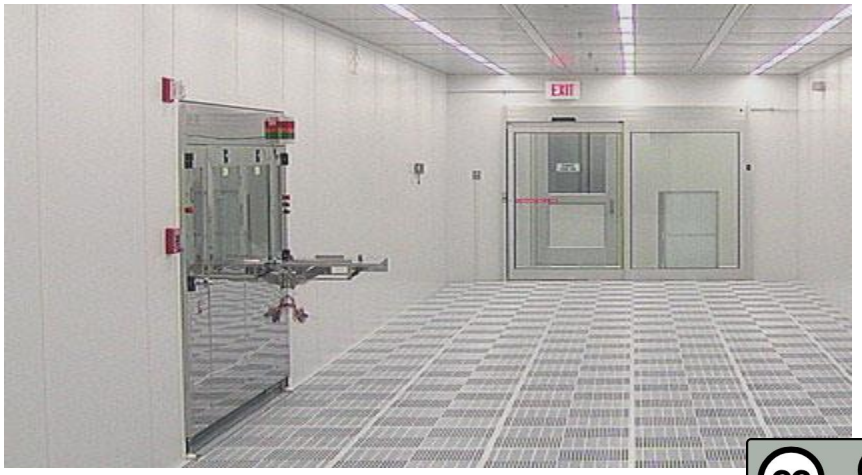






Problems related to aseptic design and scale-up:

4. If necessary, the gases leaving the reactor must be neutralized by filtration or combustion.
5. Clean rooms - The design must match the required degree of cleanliness (filters, flow, overpressure, clothing, entry chambers, etc.). Testing, validation, monitoring and staff training are extremely important.



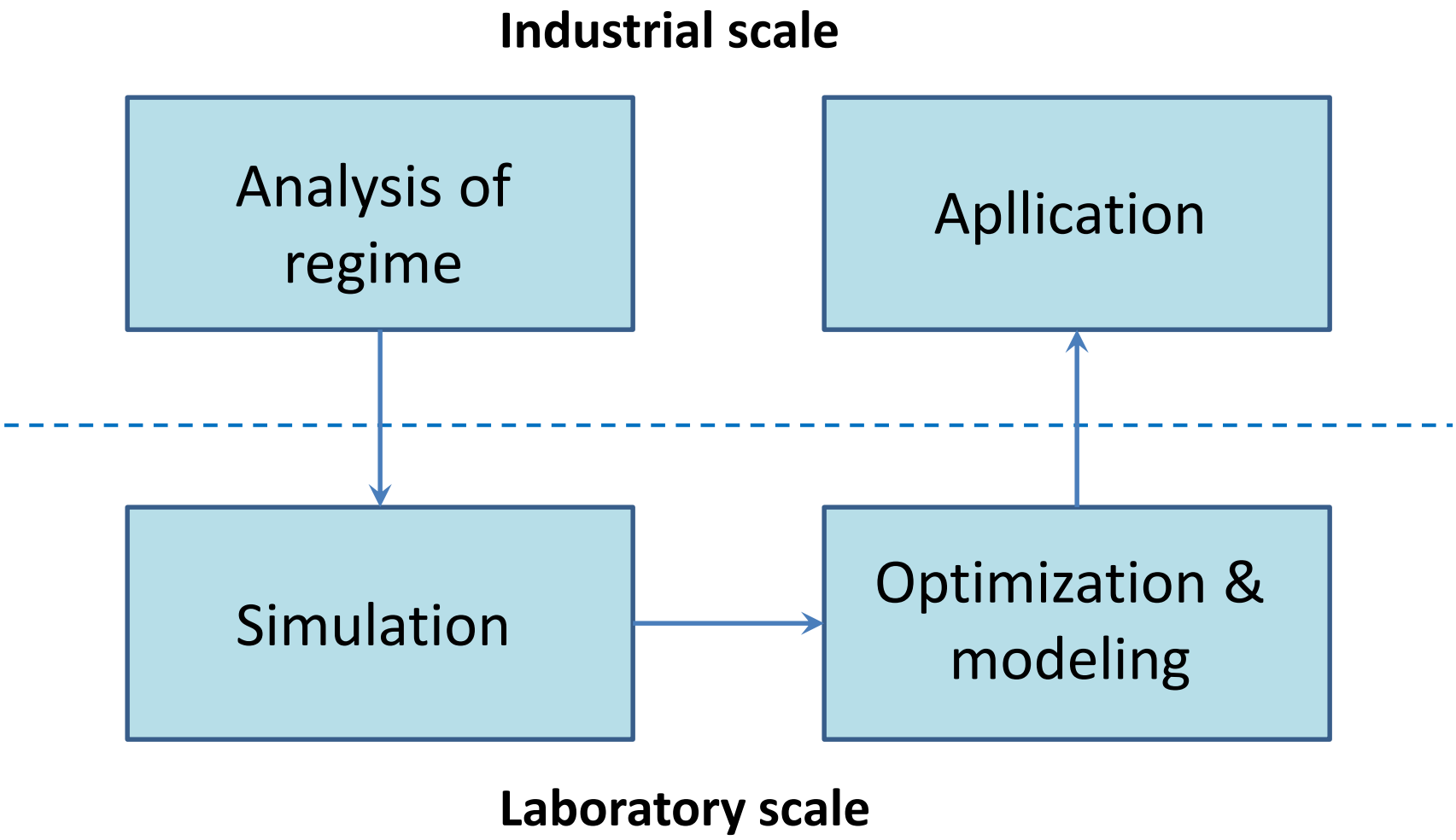
Problems related to aseptic design and scale-up:

6. Contact between the sterile and non-sterile parts of production must be limited to the maximum.
7. In sterile parts of plant, overpressure must be present.
8. Sterile parts of the plant must be designed to allow easy cleaning and inspection. The schedule of inspections must be specified.

Scale-down

What is it good for?

- It allows you to investigate on a laboratory scale various aspects of the operation of an already existing industrial reactor. In particular, this concerns operational failures.
- It allows the testing of new processes or production strains, testing of which would be highly-risky/costly.
- Testing the two previous cases on a small scale is cheaper and faster.


Industrial scale
Analysis of regime
Apllication
Simulation
Optimization & modeling
Laboratory scale

Analysis of regime: has the task of identifying the key process. Is it one (homogeneous mode) or more (heterogeneous mode) processes? This key process (e.g. limitation, fluctuation) is then to be simulated on a small scale.

Tools of regime analysis: dimensional analysis, principle of similarity, estimation of characteristic times.

Dimensional analysis is very demanding for biological processes, especially in non-steady state.

In the process of enlarging the scale, it is never possible to preserve all the characteristic times and dimensionless criteria, it is necessary to identify and preserve the key ones!

Simulation: small scale test conditions should be representative of the industrial scale.

Optimization/modeling: not all optimization results can be converted into a large scale.

Application: transfer of models and knowledge to industry. Success depends on the appropriate design of experiments on a laboratory scale.

Process productivity on an industrial scale is often lower than in laboratory experiments.

→Therefore, it is sometimes advantageous to simulate small-scale industrial processes.

Examples:

1. The mechanically stirred reactor has a homogeneous and well-oxygen supplied impeller area, while the distant reactor zone not.

   → the situation can be simulated in 2 parallel laboratory reactors with varying mixing and aeration intensities

   → the situation can be simulated by changing impellers, so the homogenization time can be varied in a wide range

Examples:

2. When dosing the substrate in a fed-batch mode, glucose concentrations may reach up to 10,000 times the concentration at the dosing site than the reactor diameter. The same is true for maintaining the pH. It has a negative effect on the physiology of the cell population (especially dangerous for tissue culture).

   → the situation can be simulated in laboratory reactors by oscillation of the substrate concentration (oxygen, pH)

   → the situation can be simulated in laboratory reactors connected in series (in series)

   → the situation can be simulated in laboratory reactors with the so-called circular loop, they can also have a piston flow

Disadvantages of small scale experiments

- They do not allow the training of operators on industrial facilities

- Small-scale experiments are limited in terms of the available sample volume, do not allow the installation of all types of industrial sensors (problem of size and location), and it is difficult to simulate the effect of hydrostatic pressure

The decision whether to use small-scale experiments or to proceed to scale up to pilot scale is based on economic considerations.

CONCLUSIONS

Planning of scale-up

- Build a multi-disciplinary team
- All team members should be involved in the project from the start
- Clearly define the scope of work, the key criteria and the objectives of the project
- Define the flexibility of each criteria and the alternative path
- Set the budget
- Define ways of communication (both formal and informal)

Bioprocess scale-up

In scale-up, it has to be remembered that achieving optimal speed and yield of production at the cost of high production costs is not an appropriate solution.

Product price is a key factor in choosing criteria.

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Construction materials and corrosion

Construction materials

The choice of construction material can affect the efficiency, safety and economy of operation

Basic requirements for materials in chemical operations:

1. Good workability
2. Strength and shape stability at high and low temperatures
3. Mechanical stability
4. Resistance to the chemical environment (corrosion)
5. Resistance to mechanical wear (erosion)
6. Possibility to produce large enough pieces
7. Thermal conductivity or insulation properties
8. Reasonable price and availability

Construction materials for biotechnology plant machinery and equipment are selected taking into account the specific requirements of the entire manufacturing process.

1. Inertness of surfaces in contact with foodstuffs and pharmaceutical preparations

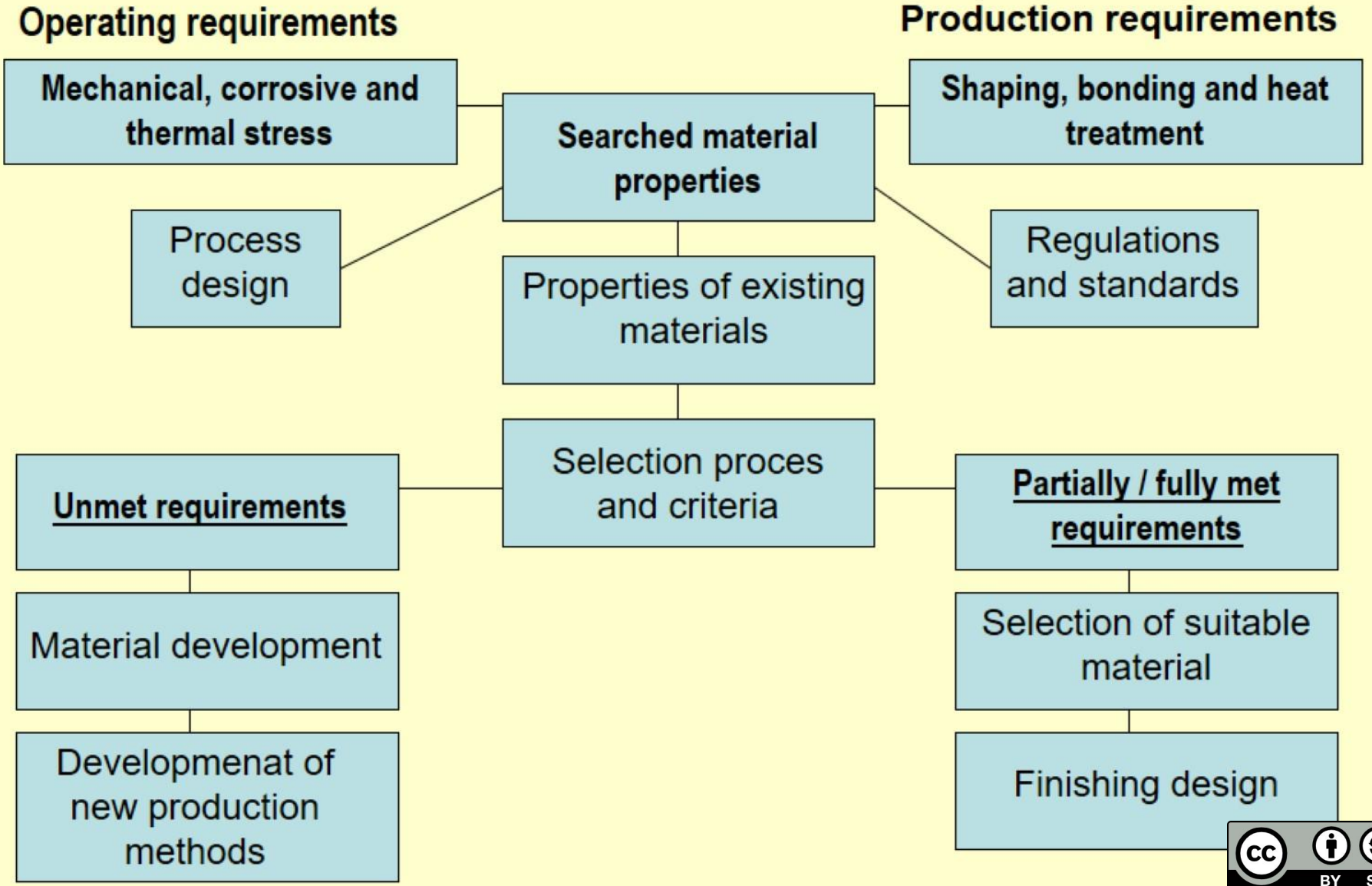
2.No odor and influence on taste and smell

3.Smooth surface

4.The absence of "dead spaces„

5.Sterilizability

Basic Material Selection Procedure



Classification of construction materials

Metals and their alloys:

- Cast iron
- Non-alloy steels (carbon steel)
- Alloy steels
- Stainless steels and alloys
- Other metals and their alloys

Non-metallic materials:

Inorganic

- Glass
- Ceramics

Organic (polymers)

- Plastics
- Rubbers
- Wood

Composite materials

Metals

The main components are metallic elements (most commonly used Fe, Al, Cu, Pb, Zn)

All metallic materials are alloys

•Deliberate addition of elements – alloying

Some elements improve materiál properties (strength, hardness etc.)

- Steel (alloys of Fe with C, C increases strength and hardness)

Metallic materials

Non-ferrous metals and their alloys

Light metals (Al, Mg, Ti)

-Metals with low melting points (Pb, Sn, Zn)

-Metals with medium melting points (Cu, Ni)

-Metals with high melting points (W, Mo)

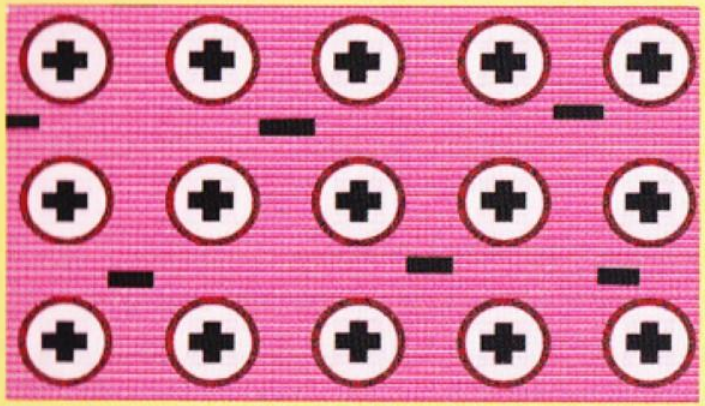
-Noble metals (Ag, Au, Pt)

Structure of metallic materials

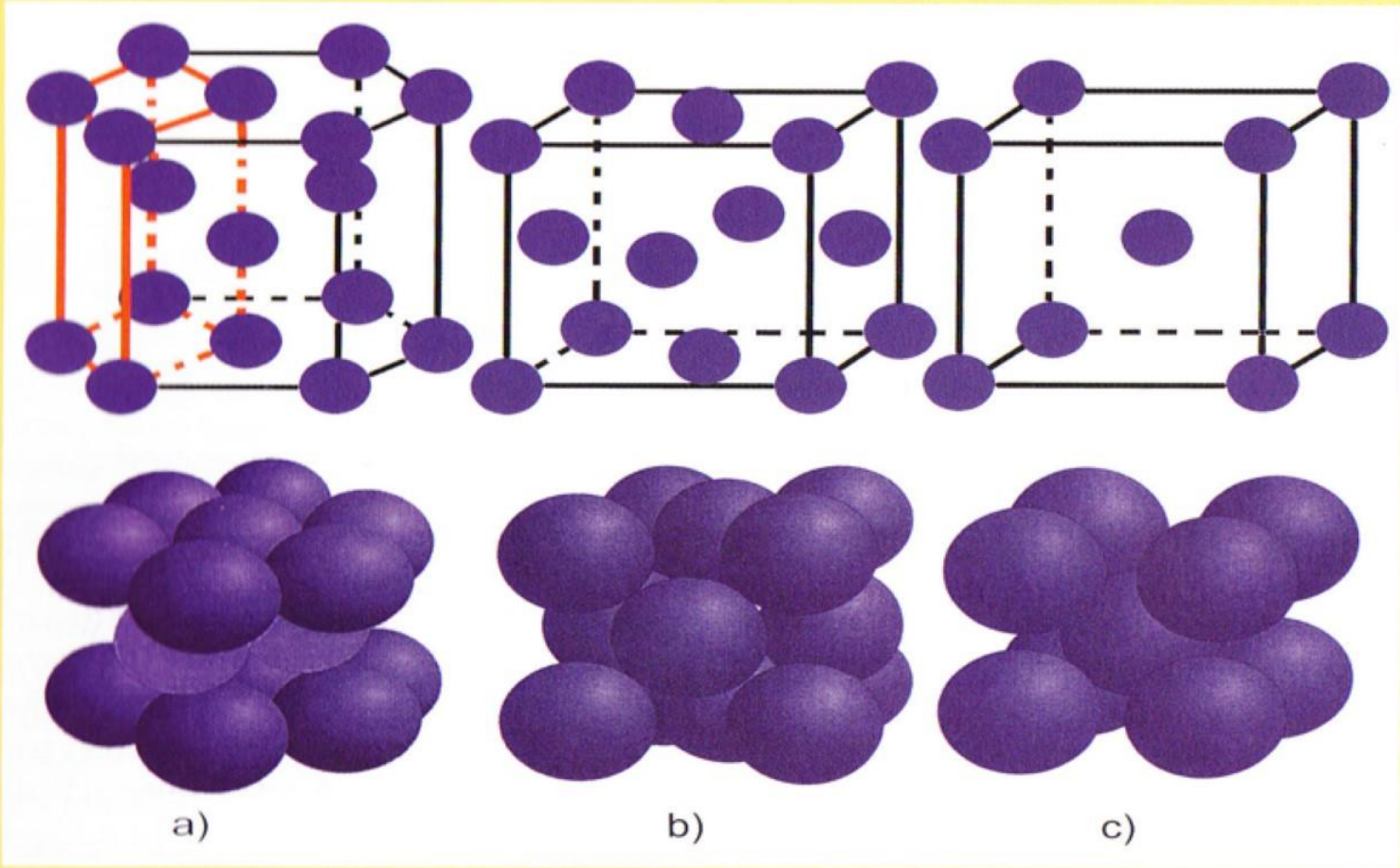
In metals metallic bonds dominate.
It is a set of positive cations that are shielded by delocalized electrons (so-called electron gas)

Metals have crystal structure.

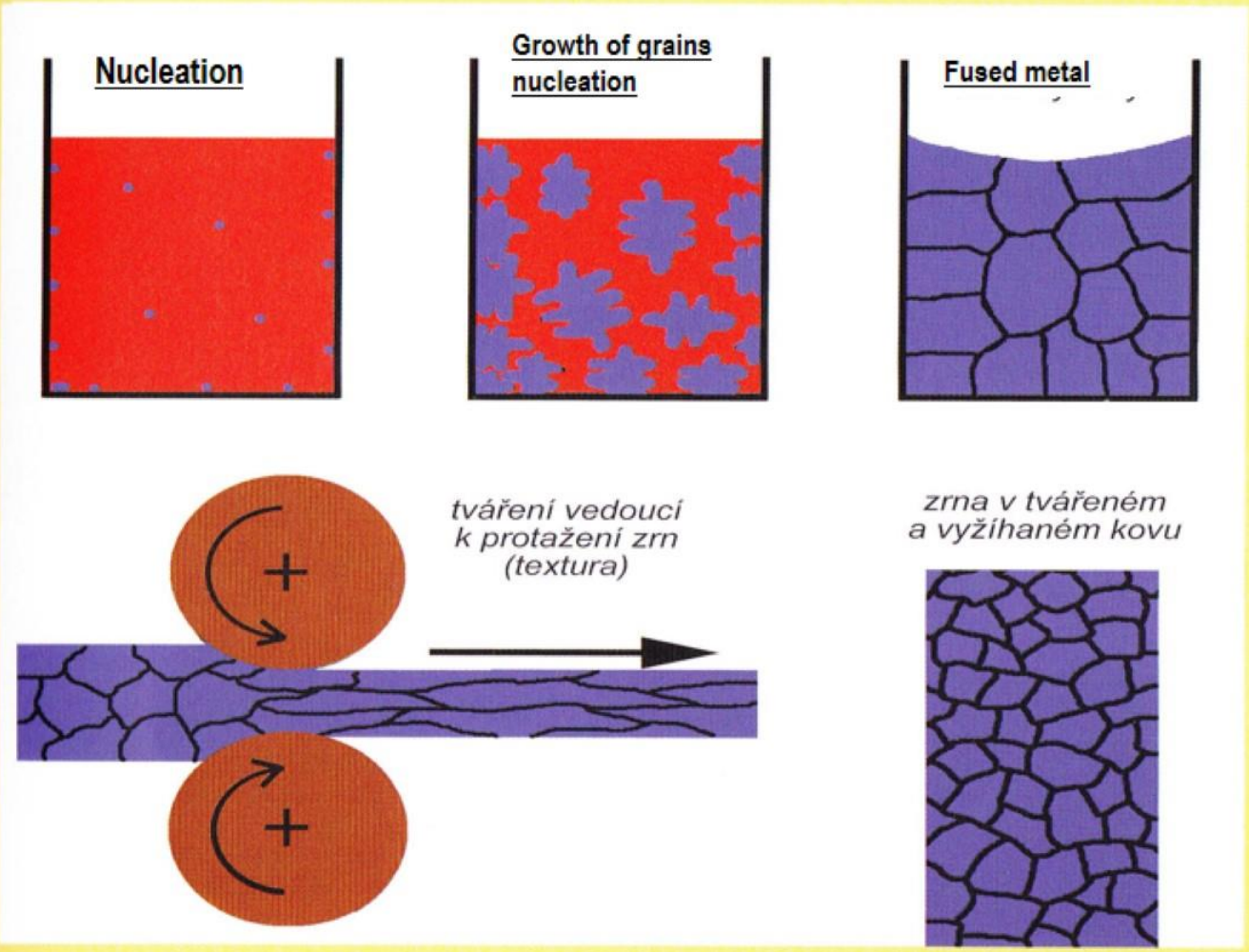
Described by a crystal lattice:
There is an elementary cell that repeats itself regularly.



Obr. 2.1: Model kovové vazby. Vazba vzniká v důsledku elektrostatické interakce mezi kladně nabitými kationty a záporně nabitým „elektronovým plynem“



Examples of crystal structure in metals.



Obr. 2.4: Schematické znázornění vzniku zrn při tuhnutí kovu a jejich změny při tváření a následném žíhání

Production of iron and steel

Important iron ores:

Fe_2O_3– hematite, Fe_3O_4 – magnetite

FeO(OH) – limonite, $FeCO_3$ – siderite

Production procedure:

1. Reduction of ore to metal (raw iron) in blast furnaces

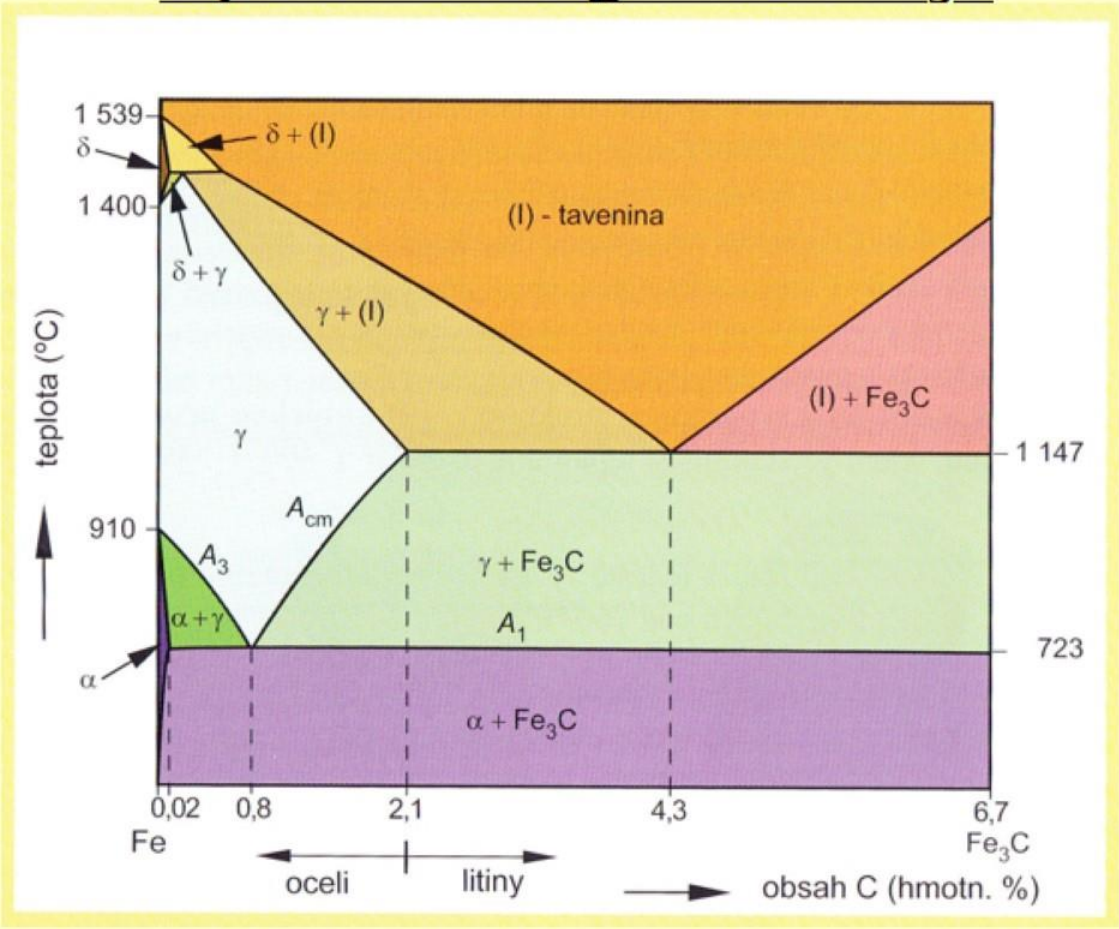
$3CO + Fe_2O_3 = 2Fe + 2CO_2 + 5.7$ kcal

Calcium is added to remove silicates - slag

2. The operation, i.e. the removal of interfering impurities or, addition of valuable additives (alloying) in electric furnaces

- it is the process of oxidation of iron impurities (Si, P, C)
- alkaline (typical) $3Fe + 2O_2 = Fe_3O_4$
- acidic $Fe_3O_4 + 4Fe_3C = 15Fe + 4CO$

Eqilibrium diagram Fe-Fe_3C



Solid lines show the existence of different phases

Technical alloys of iron - steel

ČSN EN 1 0020 defines steel as materials whose iron content is greater than of any other element and which generally have **less than 2.1%** C and contains also other elements.

- Max. solubility of carbon in austenite is 2,1% at 1147°C
- Most of the steel produced has a carbon content below 0.3%
- The low carbon content (i.e. cementite) causes the steels to be malleable, i.e. they can be processed by rolling, forging

Metals and their alloys: Cast Iron (Crude Iron)

Ferrous alloys with carbon (2-5% by weight), silicon (0.8-3% by weight) and other additive elements (Mn, P, S)

- Cast alloy contains Al, Ni, Cr, Cu (5% production), unalloyed cast iron is not used in the food industry

Metals and their alloys : Carbon steel

Iron-carbon alloys and a number of additives and alloying elements that do not affect their properties

-Soft steel (0.1-0.25%)
-Medium carbon content (0.25-0.5%)
-High carbon content (0.5-0.95%)
-Very high carbon content (do 2.1%)

Carbon steel is used in the chemical industry:

- For construction of tanks and pipes not exposed to strong corrosion and as structural / building steel. **NOT for food, not in contact with product!**

Metals and their alloys:

Alloy steels

Iron alloys with carbon and amounts of alloying elements that affect their properties.

Austenitic steels are very expensive and therefore only used in areas subject to high temperatures. Besides these, low-alloy steels are used.

Alloy elements and their influence on structure, mechanical, corrosion and other properties

Cr - the most important ingredient, ensures passivity and thus resistance to oxidation in oxidizing agents

Ni - stabilizes austenite, increases corrosion resistance in reducing acids

Mn - over 3% contributes to suppression of weld cracking

C - increases strength properties, generates intergranular corrosion

N - increases strength, increases resistance to point and slot corrosion

Mo - increases corrosion resistance, except boiling HNO_3 solutions

Cu - increases resistance to H_2SO_4, 3-4% improves workability

Ti, Nb, Al - increase fire resistance

S, Se, P, Pb - increase workability, reduce corrosion resistance

Distribution of steels according to use

Stainless steels

Highly alloyed steels containing 12-30% Cr, up to 30% Ni, up to 24% Mn and smaller quantities of Mo, Cu, Ti, Nb, W, Si and N (elements modify mechanical and structural properties)

Cr - facilitates the formation of chromium (Cr_2O_3) - passivation

Corrosion resistance also increases Mo and reduces the C content to below 0.03%

Expensive Ni is often replaced by N (up to 0.3%)

Use: chemical, food and pharmaceutical industries, energy, construction (pipelines, pumps, tanks, exchangers, implants, dishes)

Stainless steels families and their main characteristics

Within stainless steels, there are four families

Austenitic stainless steel (include the so-called super-austenitics)

When nickel is added, the austenite structure of iron is stabilized. This crystal structure makes such steels virtually non-magnetic and less brittle at low temperatures.Significant quantities of manganese have been used in many stainless steel compositions. Manganese preserves an austenitic structure in the steel, similar to nickel, but at a lower cost.

Ferritic stainless steel

hey generally have better engineering properties than austenitic grades, but have reduced corrosion resistance, because of the lower chromium and nickel content. They are also usually less expensive.

Martensitic stainless steel

For greater hardness and strength, more carbon is added. With proper heat treatment, these steels are used for such products as razor blades, cutlery, and tools. Duplex stainless steel (also called austenitic- ferritic stainless steels)

Duplex stainless steel (also called austenitic-ferritic stainless steels)

Surface treatment of stainless steels

Cleaning: washing with soap, water (<250 ppm Cl^-), detergents, solvents → removing fats, oils (degreasing)

Pickling: chemical, thermochemical and electrolytic removal of the layers of oxides formed during the heat treatment

Mechanical grinding and polishing: it is carried out due to increased corrosion resistance, improved appearance and ease of cleaning, food industry (contamination), Milling - 0.6-0.8 μm, Grinding - 0.4-0.5 μm

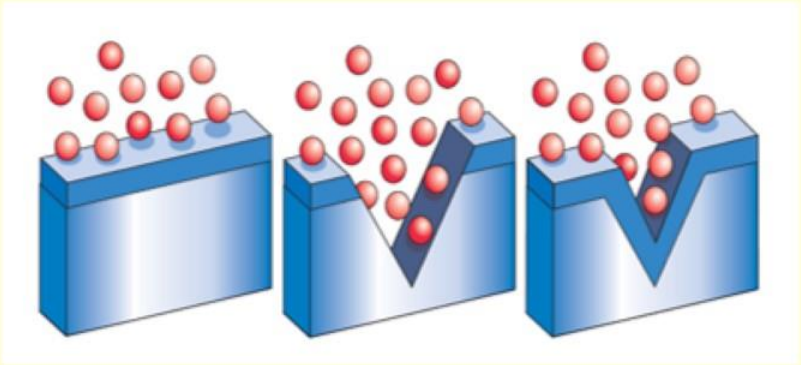
Electrolytic polishing: for outstanding smoothness, polished surface in polishing electrolyte (anode), insoluble stainless steel (cathode), 0.3-0.4 μm

-preserves the mechanical and chemical structure of the material

-Ra, roughness average

Surface treatment of stainless steels

PASSIVATION



-HNO_3 (20-50%), 50-70°C, 10-30 min

or

-HNO_3 (20-50%), 20-38°C, 30-60 min

Followed by

Wash with water and then 10% NaOH + 4% KMnO4 (potassium permanganate)

- Other procedures for matte and glossy surfaces
- Modern is passivation with citric acid, less ecological risk
- The composition of passivating solutions is not known (company know-how)

Metals and their alloys: Aluminium

Aluminium is a light metal (ρ=2,7 g/cm^3)

It is soft and ductile, small strength limits constructional applications

It can be alloyed with Zn, Mg, Cu, Mn, Si reinforcing elements

It is an excellent electrical and thermal conductor

Use:

- packaging (foils, cans)
- airframe, engines, bodywork
- roofing, window frames, doors
- el. current wiring
- consumer goods

Obr. 2.13: Výrobky z hliníku a jeho slitin

Metals and their alloys: Aluminium

Good corrosion resistance - protective passivation layer Al_2O_3, insoluble at pH 4.5 - 8.8

Use:

Very pure (99.99%) - food and pharmaceutical industry (not fermentors), covering material

Purity over 99.5% - chemical industry (ethane and acetylene cylinders), refrigeration components, shipbuilding, benzene tanks)

Attention!

Not resistant to alkaline environment (pitting corrosion)
Lower electrochemical nobleness and therefore, in combination with a more resistant metal, galvanic corrosion
Not resistant to concentrated acids
Relatively soft and therefore unsuitable for underpressure applications (tank collapse)

Metals and their alloys: Copper

Good corrosion resistance in water and steam, in air and diluted acids - protective passivation layer Cu_2O and CuO, not suitable for NH_3

Use: Brewhouse, distillery boilers, food-generally-not used, water pipes, condensers, cooling circuits, heat exchangers

Copper alloys - additions Zn, Al, Ni and Sn increase strength

Cu-Al (Al do 7.8%) resist sea water

Cu-Sn+(Zn/Pb) screws, pipes, springs, bearings, screw and gear, pumps

Cu-Ni-Fe increased corrosion resistance including dilute NH3, marine technology, desalination of sea water, coolers, condensers

Metals and their alloys: Copper

Obr. 2.16: Výrobky z mědi a jejích slitin

Brass Cu, up to 45% Zn + alloying Si, Pb, Al, Mn, Ni; screws, pressure valves, cartridges, musical instruments, shaped tubes

Titanium It is not subjected to pitting or slit corrosion in the presence of chloride ions. Resistant to acetic acid, milk and lemon. Because of the high price, use is exceptional.

Tin Purity requirements 98-99.9%. Passive oxide layer is poorly soluble → good corrosion resistance to food, beverages, org. acids, **used for surface treatment of equipment, tanks and cans in the food industry (non-toxic).**

Cadmium, Zinc, Lead toxic, can not be used in the food and pharmaceutical industry in contact with the product

Inorganic non-metallic materials

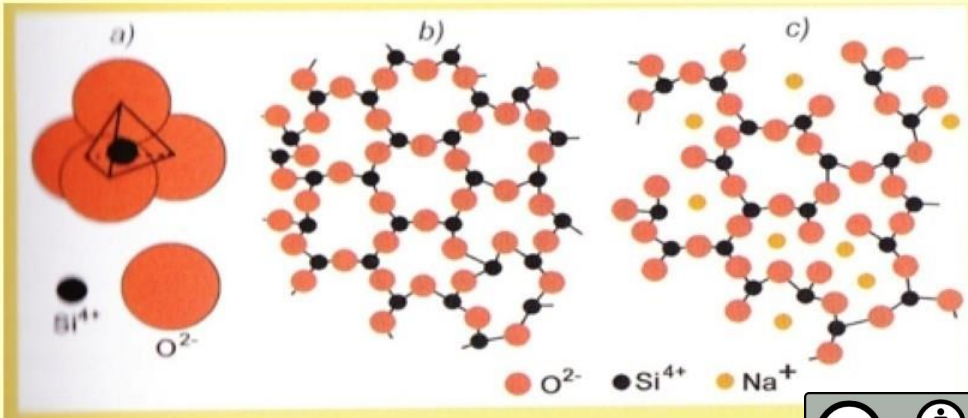
Glass

Non-crystalline homogeneous materials - transparent

Relatively hard, but brittle and less firm when bended

Most glasses are SiO_2-based, but Na_2O, CaO, B2O3, Al_2O_3, etc. can also be present.

The glass structure is non-crystalline (amorphous)



Obr. 3.2: Struktura oxidových skel:
a) základní strukturní jednotka – tetraedr $(SiO_4)^{4-}$,
b) trojrozměrná síť tetraedrů $(SiO_4)^{4-}$ v amorfním oxidu křemičitém (pro jednoduchost znázorněno v rovině),
c) vliv kationtů alkalických kovů a kovů alkalických zemin na strukturu oxidových skel (pro jednoduchost znázorněno v rovině)

Significant types of glass

Borosilicate glass (Pyrex) is highly resistant to corrosion up to 200 °C (except HF and strong alkalis).

Above 200 °C dissolves in acids, especially in H3PO4

Advantages: smooth surface, transparency, sterility, catalytic inertness, nontoxic, low price, possibility to combine with other material (plastics, metals)

Disadvantages: fragility, low tensile strength, difficult sterilization in situ

Use: fermenters, distillation units, gas absorbers and washers, pipelines, HNO_3 thickeners, heat exchangers

Other glass types: Quartz glass (> 99.5% SiO_2), Sodium-calcium glass

Inorganic non-metallic materials

Ceramic materials

They often have a heterogeneous polyphase (crystalline, non-crystalline) pore structure

High hardness, brittleness, good resistance to high temperatures, good chemical resistance, light-proof, electric. insulators

They are made from powdered raw materials in a solid state (does not melt)

Chemically: complexly bound oxides of SiO_2 and Al_2O_3, plus Fe_2O_3, CaO, MgO, TiO_2, Na_2O etc.

Significant types of ceramics

Traditional ceramics

It is made from natural clay materials

-Porcelain (kitchen utensils, art objects) and stoneware

-Building ceramics (bricks, roofing, tiles, tiles)

-Sanitary ceramics (wash basins ...)

-Technical ceramics (shaft seals in fermenters, bearing components)

Inorganic non-metallic materials

Polymeric materials

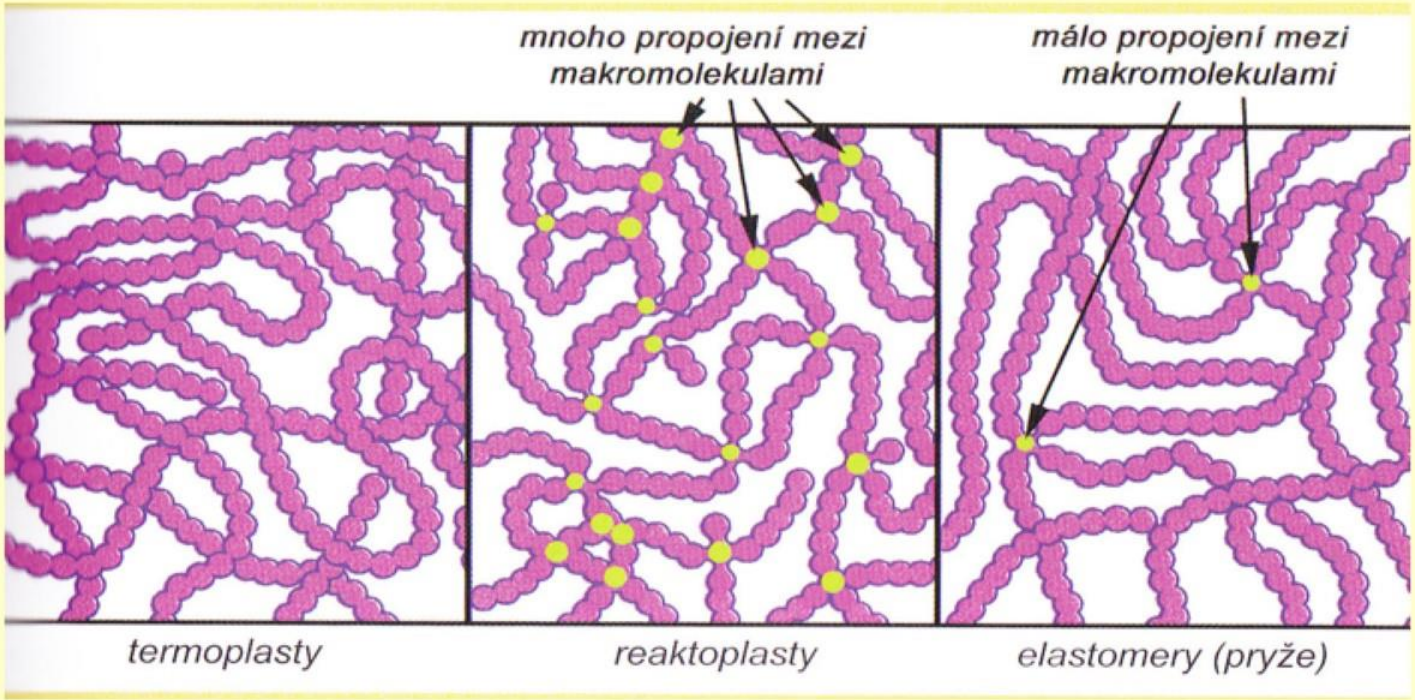
The most common are carbon and hydrogen compounds and also O, N, S, Cl

They usually have very low density and hardness, low tensile strength and heat resistance, electrical insulators, low energy-intensive production.

1. Thermoplastics
2. Reactoplasts (thermosets, resins)
3. Elastomers (rubbers)

Structure of polymeric materials

Macromolecules in which atoms are covalently bonded



Composite materials

Heterogeneous materials more than 2 phases, with different properties:

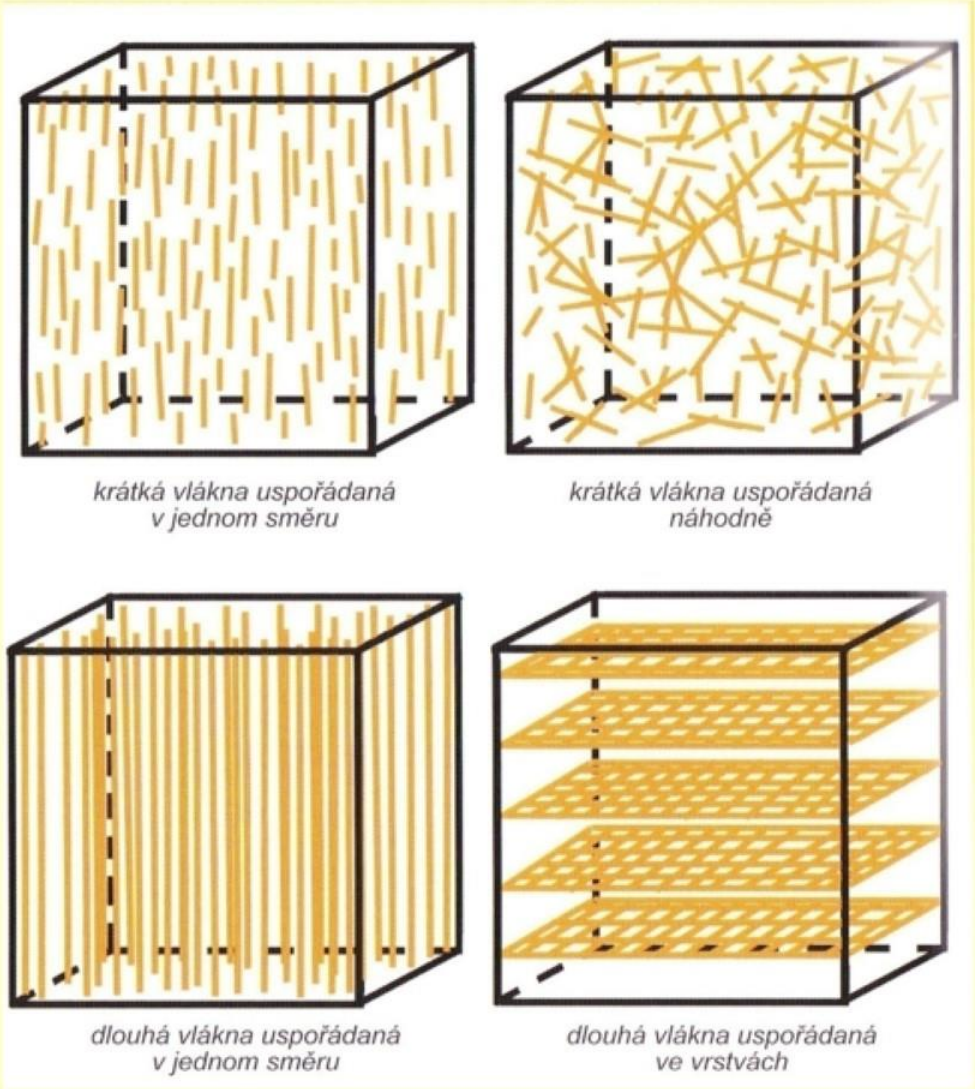
1. Matrix (continuous phase)
2. Reinforcement (phase discontinuous)

Synergism – the properties of the composite are better than the sum of the properties of each component

Depending on the type of reinforcement, they are divided into :

1. Fibers (boron fibers, glass fibers, silicon carbide fibers, carbon fibers)
2. Particulate (SiC, Al_2O_3, SiO_2, ZrO_2, Y_2O_3, WC)

Mast typical matrices: epoxy and polyester resins, polyamide, metal alloys Al, Cu, Ni, Co



Geometric arrangement of reinforcement fibres

Wood

From the point of view of cleaning and sanitation it is not a perfect material

Traditional construction material of some traditional products:

-Brewing and fermentation of malt mash for the production of Scotch whiskey (oak or larch vats, up to 150 hl, necessity to clean and treat the surface each year)

-Scotch whiskey maturing in oak barrels (wood affects organoleptic quality → can not be replaced)

-Winery (ripening wine in wooden barrels → perfect character)

Being replaced with stainless steel devices.

Corrosion

Damage and destruction of the material through its interaction with the environment

Dry - oxidation at high temperatures

Wet - water environment of electrolytes

Corrosion

Corrosion presents chemical, electrochemical, biological and physical material violations.

Corrosion is a broad concept relating to metals, plastics, building materials etc.

Most metals and alloys are essentially unstable and spontaneously switch to an oxidized state with varying degrees of transition.

Basic principle of corrosion

Most corrosive processes are oxidation - the metal ion emerges from the grid and forms: oxide, hydroxide, salt, and so on.

Basic principle of metal corrosion - oxidation of metals and reduction of oxidant released by electrons.

The principle is a common for both chemical and electrochemical corrosion.

Both processes can take place simultaneously.

Basic principle of corrosion

There are two simultaneous processes:

Anodic process: oxidation (dissolution) of metal

$M \rightarrow M^{n+} + ne^-$

Cathodic process: reduction of oxygen acceptor most often oxygen (other acceptors: sulfur, nitrogen, chlorine, hydrogen, chromate, nitrite ions)

For instance $O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$

(dissolved O_2 and electrons from anodic reaction)

Overall reaction: $2Fe + O_2 + 2H_2O \rightarrow Fe^{++} + 4OH^-$

Electrochemical corrosion

The separately occurring oxidative (anodic) and reductive (cathodic) reactions are connected to the circuit with electrolyte.

Electrochemical corrosion is most often explained by the theory of macro and microcells (depending on the size of the circuits)

Macrocell: joining of two different metals, different environments at two locations of the same metal (aeration, conc. of electrolyte)

Microcell: slits, crevices, deposits, pockets, etc.

Passivity

The corrosion resistance of steels and alloys lies primarily in their ability to passivate.

The most famous theory of passivity : metal or alloy are in passive state covered with a very thin invisible oxide coating formed by reaction with the surrounding environment.

Passivity significantly reduces the chemical reactivity of metals and alloys, and they behave almost as immune.

The oxide film acts as a barrier with a negligible dissolution rate between the metal and the environment.

The passive layer is usually not visible on the surface.

Influence of alloying elements and environment on passivity

Fe-Cr: is much easier to passivate, and iron alloying with chromium makes passivity considerably easier

Fe-Cr-Ni: easier passivation and lower corrosion speed

+Mo: easy to passivate

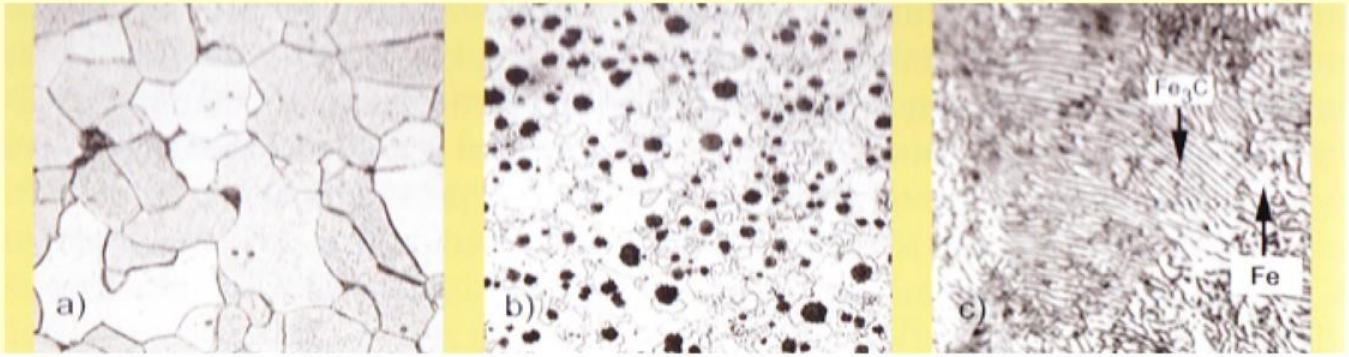
Compared to the steel composition, the film is enriched with chromium, molybdenum, etc. This layer is more easily converted into an oxidized form, and their films have the proper composition.

Adverse environmental impact on passivity

Local defects: slits, non-homogeneous matrices, mechanical stresses, deposits of microorganisms

They weaken the stability of the film especially where the matrix surface is inhomogeneous

Chlorides: reduce the stability of films by diminishing the passive area



a) pure iron, b) cast iron with 3.8% wt. carbon, c) steel with 0.8% wt. carbon (iron carbide precipitates)

Types of corrosion

- **Total corrosion**
- **Uneven types of corrosion**
  - **Galvanic corrosion**
  - **Pitting corrosion**
  - **Crack corrosion**
  - **Cross Crystal Corrosion**

  } without mechanical stress

  - **Tension corrosion (vibration, cavitation)**- with mechanical stress
- **Biological corrosion**

Total corrosion

With total corrosion, the surface of the steel exposed to the electrolyte corrodes evenly.

Happens to carbon steel (rarely), roughening the surface.

In food processing (material, temperature) there is usually no risk.

At rate over 1 mm per year, the material selection is not suitable.

At rate less than 0.1 mm per year, the resistance of the materials is suitable.

Unven types of corrosion

In terms of structural strength reduction, they are the most dangerous

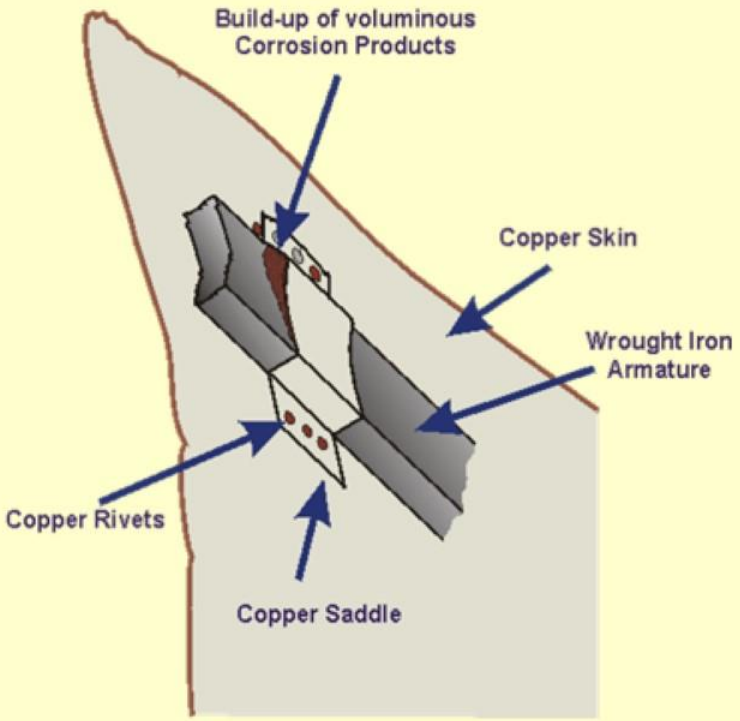
It significantly influences the mechanical characteristics without changing the appearance of the surface of the corroded part, and eliminates the amount of material

They occur in materials resistant to corrosion in their passive state

Dangers: Aggressive depassivating anions (especially Cl^-)

Galvanic corrosion

I arises at joining two metals with different electrochemical potential, in particlar at presence of electrolyte.

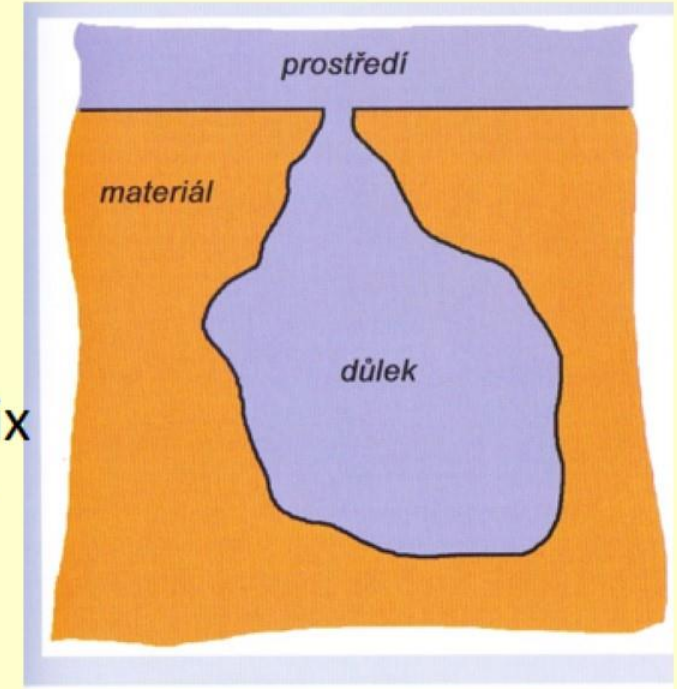


Pitting corrosion

Halogen ions (chlorine, fluorine, bromine, iodine) penetrate easily with protective passive film → local anode dissolution in active state

Local disturbance of the passive film :

- at defects of the material reaching the surface
- at sites of non-metallic inclusions (MnS, FeS, CaS)

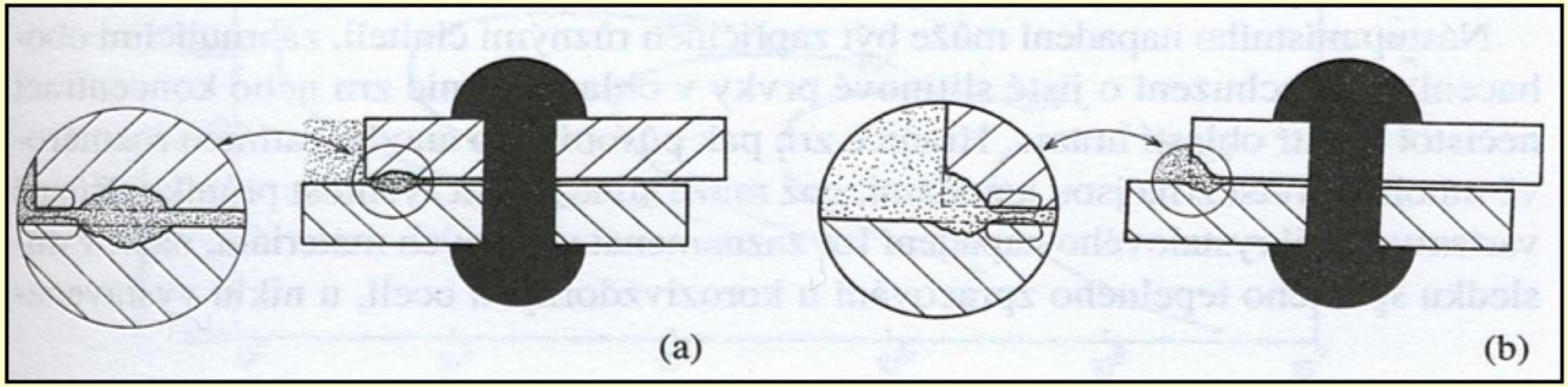


Material resistance determines: metal matrix composition + composition, shape, number and distribution of inclusions or other inhomogeneities

Crevice corrosion

Occurrence: places with poor circulation (eg two sheets joined by rivets, spot welds, under seals)

Different concentrations of the electrolyte or oxygen ions in the crevice and at the orifice form a concentration cell



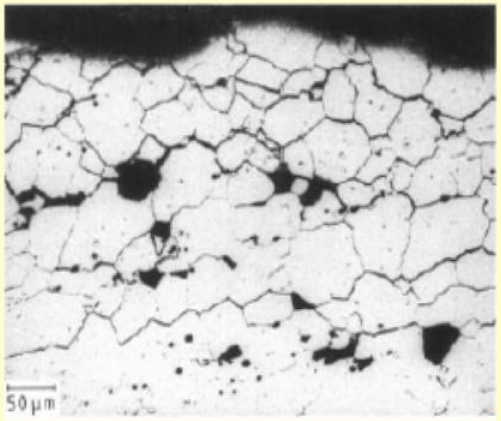
Crevice corrosion can be avoided by the quality of welds, appropriate design and frequent inspections.

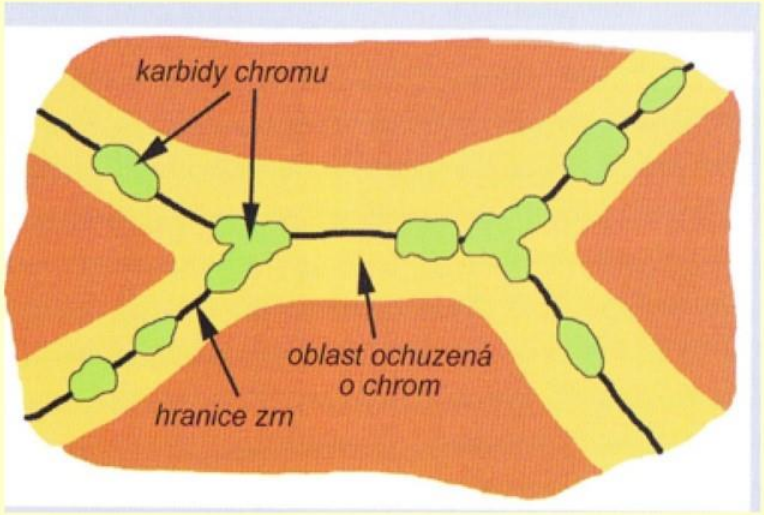
Intercrystalline corrosion

It preferably takes place between crystals (grains) along their interfaces

Occurrence: structural changes at grain boundaries during aging, heating, heat treatment (e.g. welding)

Threatens: stainless steels, Ni alloys, Al alloys





Biological corrosion

The process of biological corrosion leading to local corrosion can be very rapid, depending on the conditions.

E.g. after the pressure test in the tank the remaining microbiologically unsuitable water. At a suitable temperature ca. 40-45 ° C may result in pitting corrosion caused by a biological agent within a few hours.

Microbial corrosion involves : aerobic bacteria

Aerobic sulphur bacteria: oxidize reduced forms of sulfur compounds up to sulfuric acid

Aerobic iron bacteria: oxidize the Fe^{2+} and Mn^{2+} ions to trivalent and they after reaction with chlorides, increase the corrosion potential

Corrosion of non-metallic construction materials

The mechanisms of these corrosive processes differ completely from metals

Plastic material: change of structure due to environment (cross-linking, degradation), leaching of components, swelling

Inorganic materials: chemical dissolution (enamel coatings, ceramics, glass)

Concretes: cracking and peeling due to corrosion, aggressive chemicals, frost, carbonation, sulphation etc.

Methods of protection against corrosion

1. Choice of the most suitable construction material

- metals with high corrosion resistance are expensive and their mechanical properties less advantageous

- an attempt to use these materials in the form of cladding layers or tiles

2. Changing the aggressiveness of the environment

- modifying the operating mode can ensure passivation of the material

- little used - detailed practical and theoretical knowledge is required

3. Restriction of causes of intense corrosion (structural interventions) restriction of pockets, crevices, inappropriate seals

4. Electrical protection (without surface protection layer)

- Cathodic protection (change of potential to negative values)

- Anodic protection (increase of passivity potential)

Cathodic protection

-**galvanic anodes** (internal parts of tanks and boilers, metal structures of ships, pipelines etc.)

- Zinc is often sacrificed

Anodic protection

- requires careful laboratory testing

- protective current must be potentiometrically controlled

- **Use**: unalloyed steel in acidic and basic medium and salt solution, NaOH evaporator, in the production of sulfuric acid.

5.Surface protection

Metal coatings

Sheet metal welding: the material is stainless steel, nickel alloys, copper, titanium, etc. The anticorrosion property is close to pure material. Efficiency proportional to thickness, required coat integrity

Coating by immersion in molten metal: zinc, lead, tin, sometimes aluminum, layers 80-100 μm.

Metal spraying: zinc, aluminum, lead (hygienically hazardous technique), layers 0.3-0.5 mm

Inorganic non-metal coatings

-acid-resistant enamels

-refractory oxides (Al_2O_3, ZrO_2)

Organic coatings

- plastics, foil gluing, plastic spraying

- rubber, insertion of rubber plates and additional vulcanization

- protective coatings, only for less aggressive environment (atmospheric corrosion), PVC copolymers, different resins, etc., layer 0.2-0.25 mm

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