What have we learned during first part?

1.What are drug products.mp4

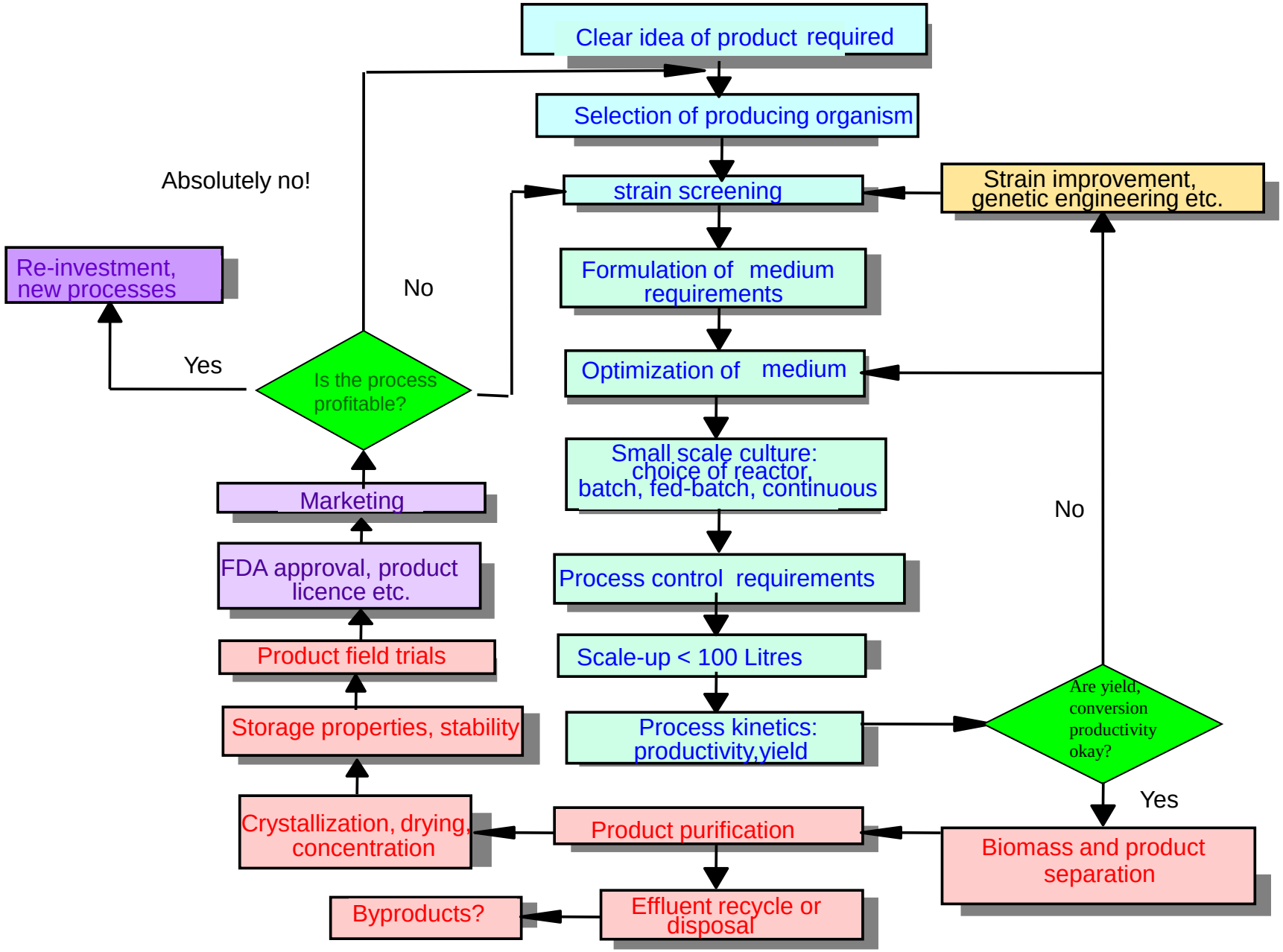
2.History of Modern Biomanufacturing.mp4

3.Using cells to manufacture chemicals.mp4

4.USING CELLS TO produce Penicillin and vaccines.mp4

5. the birth of modern biomanufacturing.mp4

DEVELOPMENT OF AN INDUSTRIAL BIOLOGICAL PROCESS



Design criteria

- Concentration
- Productivity (volumetric, specific)
- Yield/ conversion
- Quality
  - Purity
  - Sequence
  - Glycosylation
  - Activity (in vitro, in vivo)

Design criteria for pharmaceutical product

Order of importance

- Quality
- Concentration
- Productivity
- Yield/ Conversion

Design criteria for bulk product e.g. ethanol

Order of importance

- Concentration
- Productivity
- Yield/ Conversion
- Quality

For new process

- Clearly define product, quality and activity
- Market analysis to determine application and market share
- Estimation of dose and frequency of dose (mg/dose and mg/treatment)
- Estimation of desired productivity (g/year)
- Estimation of scale of production based on known kinetics
- Estimation of production costs and sale price

Novel processes: Gene & Cell Therapy

Gene therapy is a novel approach for the treatment of ***inherited*** and ***non-inherited*** genetic disorders by the ***delivery of therapeutic genes*** to specific organs or tissues

Cell therapy is another novel approach where cells from the patient (autologous cell therapy) or from a donor, are introduced into the patient to replace damaged cells e.g. stem cell therapy skin grafts etc.

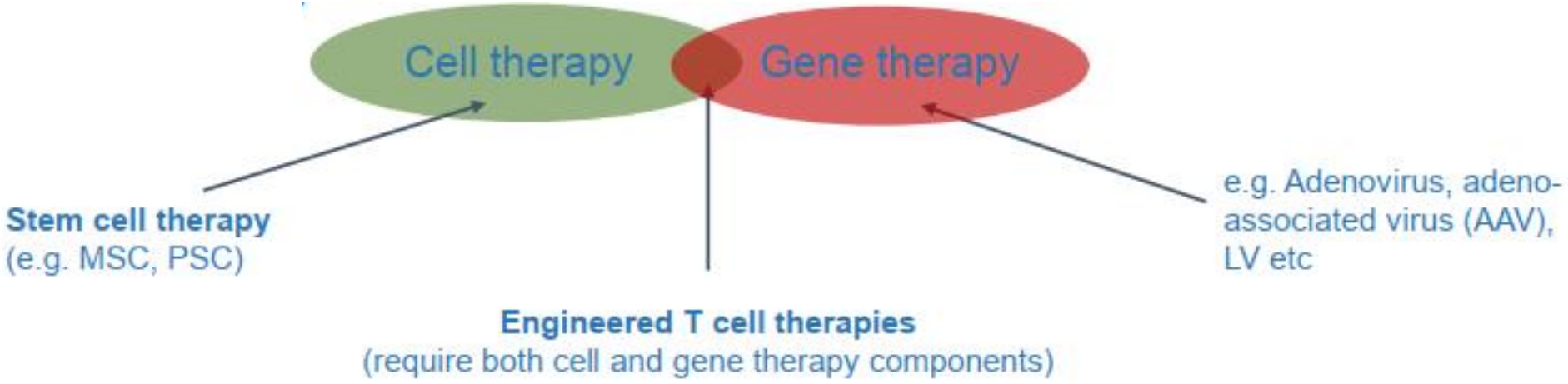
The cells may be genetically modified before implantation e.g. CAR-T therapies

In general these ***new therapies are cures***, ie. the effects are long- lasting

These cures are forms of ***personalised medecine*** and are usually called ***regenerative medicine (ATMP)***

ATMP - Advanced therapy medicinal products

Products and Processes?



1. Cell therapy: Use of cells, tissues or organs to repair, replace or correct damage in the body

2. **Gene therapy: Use of gene-modifying technology to repair, replace or correct damage in the body. The genetic modification can take place *in vivo* or *in vitro***

Bei uns in der Produktion geht's ganz schön rund, was?
CZICHOS

Purposes of Bioreactors

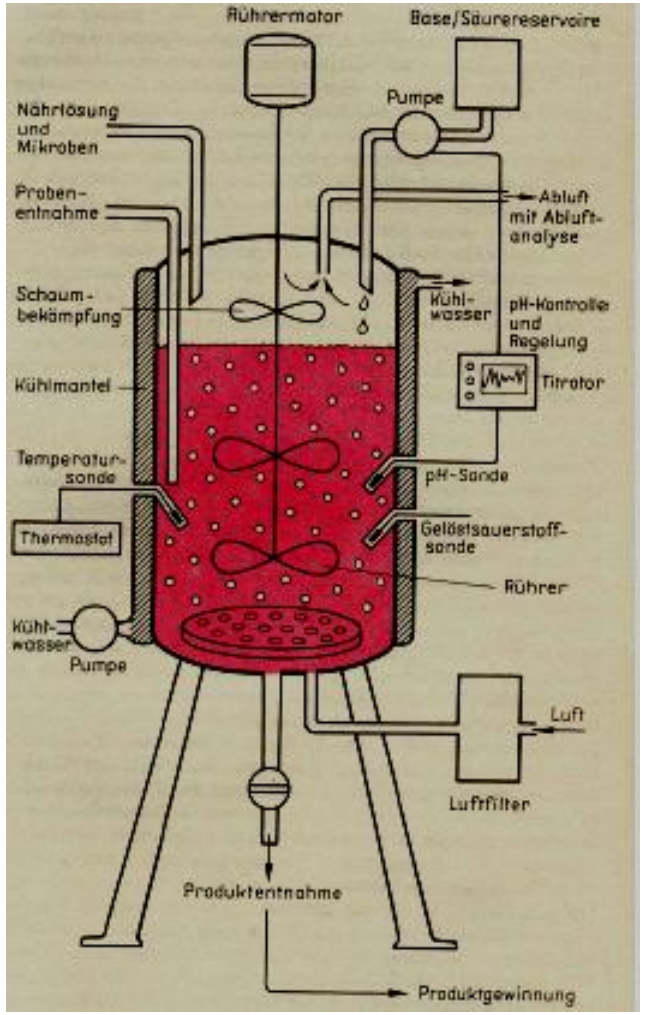
- Cultivation of microorganisms for the production of biological products, e.g. antibiotics and other pharmaceuticals, bulkbiochemicals, fine chemicals

- Cultivation of microorganisms for the study of their physiological characteristics

- Screening of organisms, e.g. for selecting optimal enzyme from a clone library, for physiological characterization of mutants

- Incubation of enzymes for biotransformation

Bioreactor ≠ Fermenter



Equipment / Components

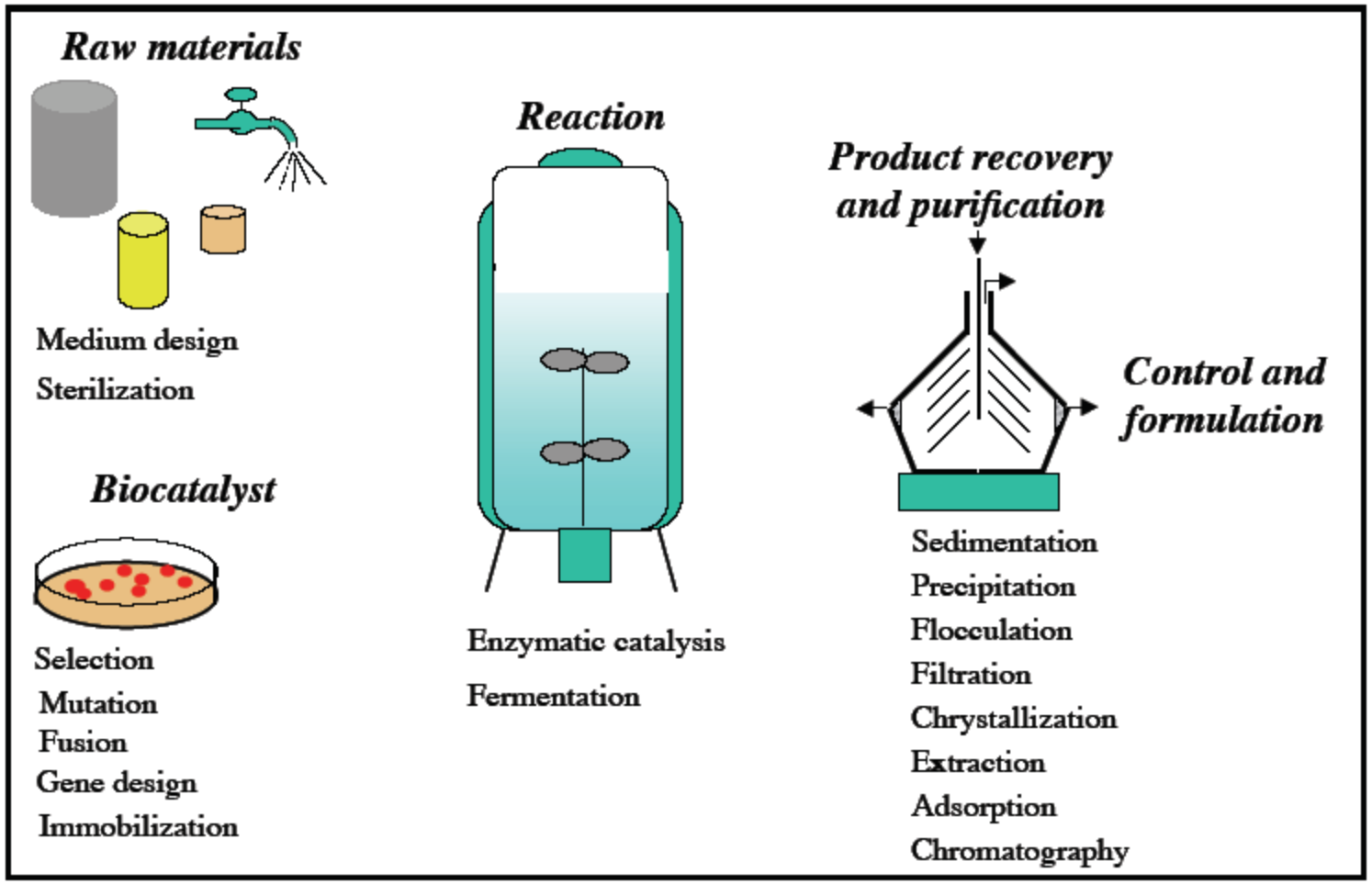
- *Filling / Empty (Valves-soupape)*
- *pressure resistant (sterilisation)*
- *Mixing*
- *Temperature control (cooling / heating)*
- *Gassing (air / oxygen)*
  - Air in (Mass flow meter/Filter)
  - Distribution (Nozzle, injector..)
  - Air out (Filter)
- *Supervision:*
  - pH
  - pO_2
  - T
  - ….
- *Size / Volume:*
  - *laboratory: 1-20 L*
  - *industry: 10 – 400 m^3*

Bioreactor Design Criteria

Design issue	Purpose	Design means	Parameters
Gas transfer in submerged culture	Ensure high growth rate, avoiding oxygen starvation	Reactor geometry Sparger design Baffles Overpressure Impeller geometry	Aspect ratios $K_L a$ *OTR* *OUR* *CER*
Mixing efficiency	Avoiding gradients of heat, nutrients and additives, stress Reduce power	Impeller geometry Baffles Mixing analysis CFD	Aspect ratios Mixing time t Power number
Nutrient supply and addition	Efficient transfer to bioreactor volume	Feeding regime Multiple ports	Linear and exponential profile
Liquid–solid transfer	Enhance reaction rate Reduce gradients	Flow distributors Porous support	Thiele modulus
Heat transfer	Efficient removal of metabolic heat	Internal coils Recycling of media Jacket Cooling media	Dimensionless numbers

Sterility	Ensure whole unit is devoid of foreign microorganisms to avoid infection	Sterilization procedure Overpressure Barriers Containment Microfilters	Sterilization time and temperature
Strain selection	Finding strain with properties adapted to media and reactor constraints	Microbial analysis Omics	Specific rates (μ, q_P, q_S) Inhibition constants
Scale-up procedure	Ensuring same conditions at large scale	Design geometry of vessels and impellers Range of mixing	Aspect ratio Scale-up rule parameters Dimensionless numbers
Rheology		Additives affecting viscosity CFD	Reynold's number CFD data
Homogeneity of culture	Avoiding gradients for ideal reactor conditions	CFD	Zonal analysis data
Media composition	Balanced culture media	Factorial analysis Omics methods	Model fit parameters

Unit operations in bioprocessing

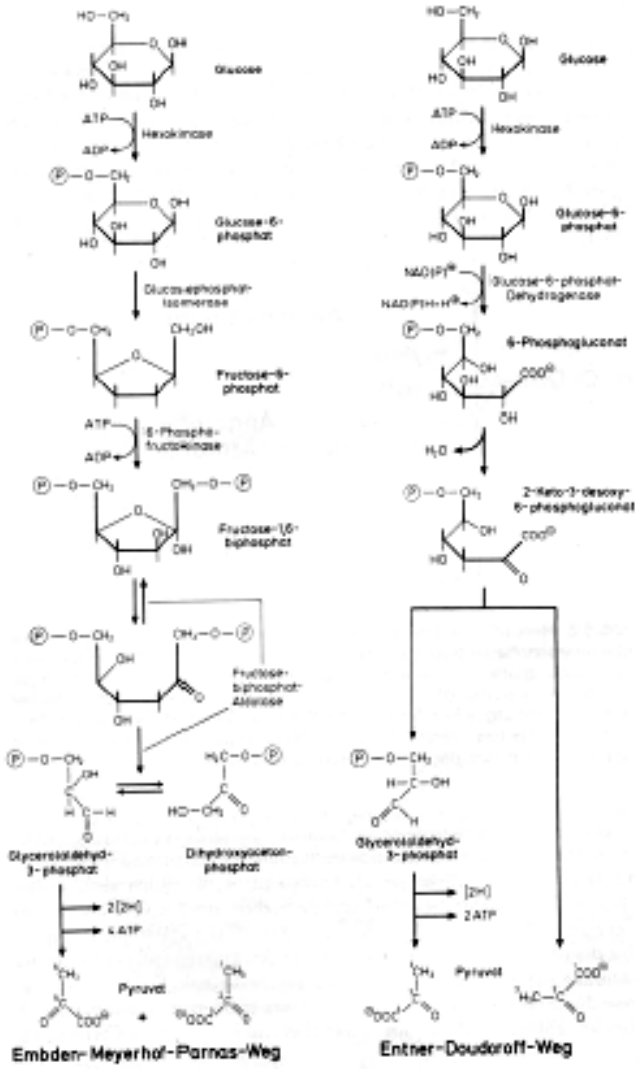


Link: [Produktionsverfahren.ppt]

Nutrients (Substrate)

Criteria	**Source**	
Energy source	light oxidation of chemical substances	Photosynthetic Chemotroph
C-source	CO_2 org. compounds	Autotroph Heterotroph
H_2-donor	anorg. compounds org. compounds	Litotroph organotroph

Nutrients (Substrate)



Biomass Yield:

$Yx/s\ [g\ X/g\ S] = \Delta X/\Delta S$

Depending on:

- Kind of substrates (KH, RH, ROH, RCOOH)
- Substrate concentration (Substrate inhibition)
- Form of metabolisms:
  - Glucose: fermentation (2 ATP); respiration (36 ATP)
- Way of metabolism (Diversity of Microorganisms)
  - Glycolyse (EMP-way)
  - Pentose-phosphate-way
  - Entner-Doudroff-Way
- Growth rate (maintenance metabolism)

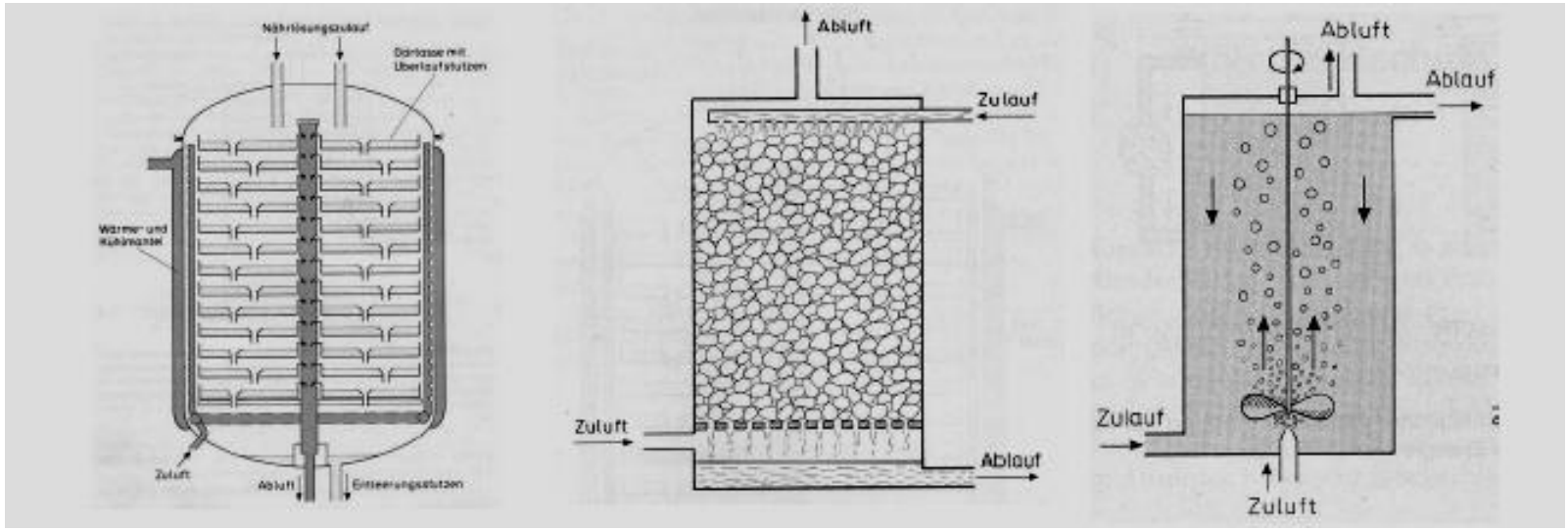
Substrate	Y_s (g cell/g substrates)
Acetate	0.36
Glucose	0.51
Maleate	0.34
Ethanol	0.68
Methanol	0.40
Isopropanol	0.43
n-Alkanes (C_{12}–C_{18})	1.00
Methane	0.62

Upstream process - raw materials

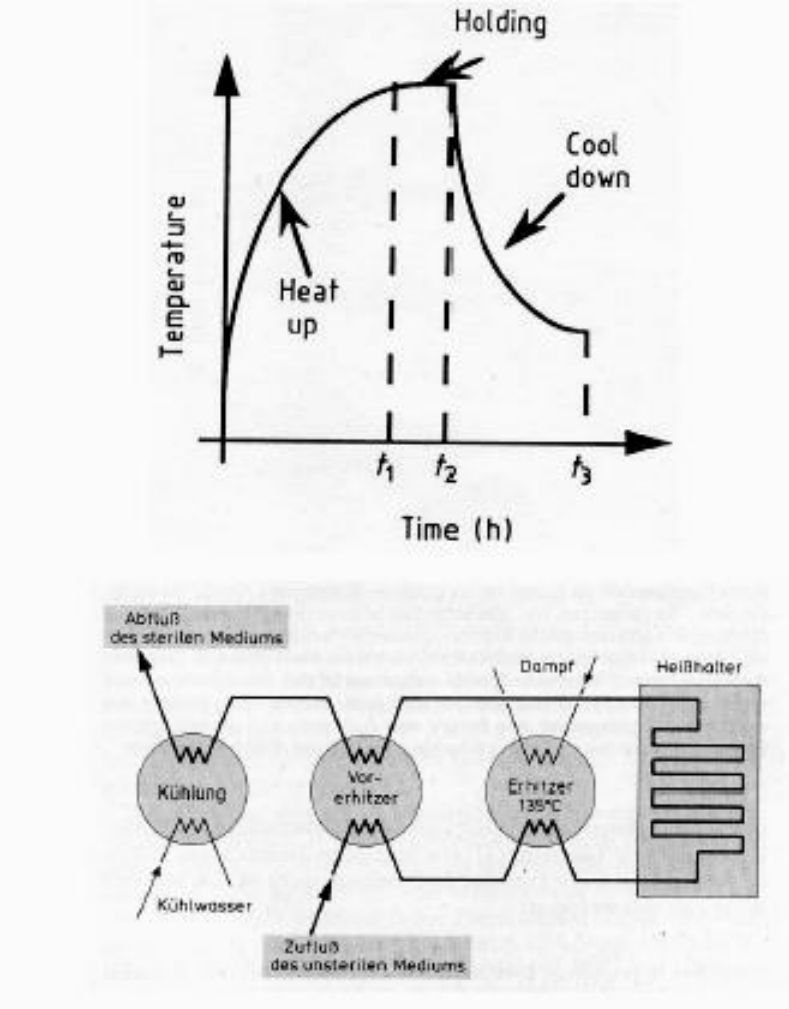
Oxygen as substrate

Oxygen demand
Oxygen Uptake Rate (OUR)
Oxygen Transfer Rate (OTR)

Technical solutions:



Upstream process - sterilisation



Removal of natural biolog. contamination

Heat: $N = No\ e^{-k*t}$

Liquids: (Charge/Flow):

- Pasteurisation (72°C; 30 min)
- vegetat. cells
- UHT (130°C, 3-5 s)
- Sterilisation: (121°C;15-20 min, 1,2 atü)

Solids: 180°C; 2 –4 h

By-products:

- colorisation; Lost of vitamins

Filtration:

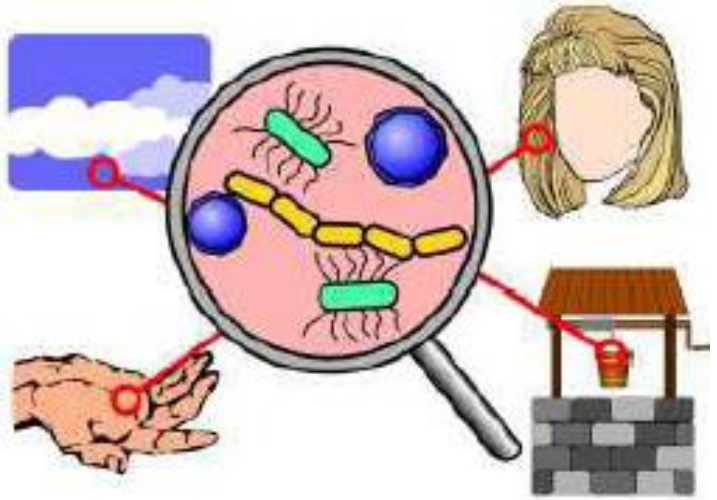
- membrane filtration (pores < 0,5 µm) (gas /liquid)
- depth filter [adsorption] (gas)

Chemicals:

- oxidation compounds (ozone, Iodines, chlorine)
- alcohols (ethanol, phenol)
- formaline
- ethylenoxide

Radiation: X-; γ-beams

Biological material



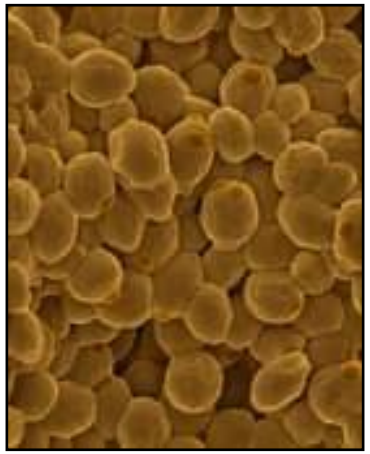
Technically used microorganisms
– Bacteria, yeast, molds
– origin and isolation

Growth
– Influence factors (Kinetic, Temperature, pH)
– media / components / yield

Strain improvement (gene technology)
– mutation / selection
– DNA recombination
– cell fusion / hybride cells

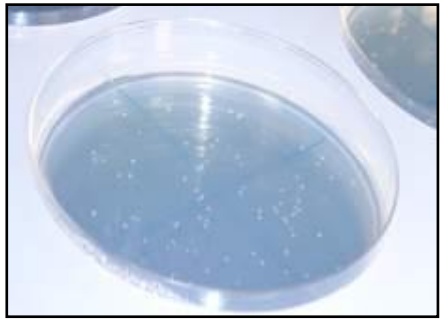
Video

Current Choices of Host Cells in Biotech



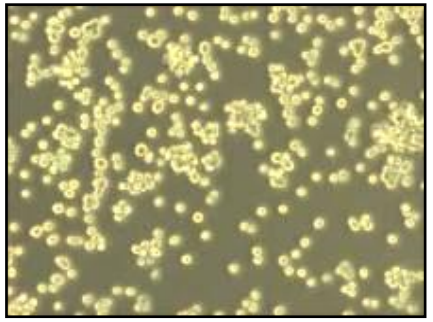
Yeast

Transgenic Animals



Bacteria Cells

Transgenic Plants



Animal Cells

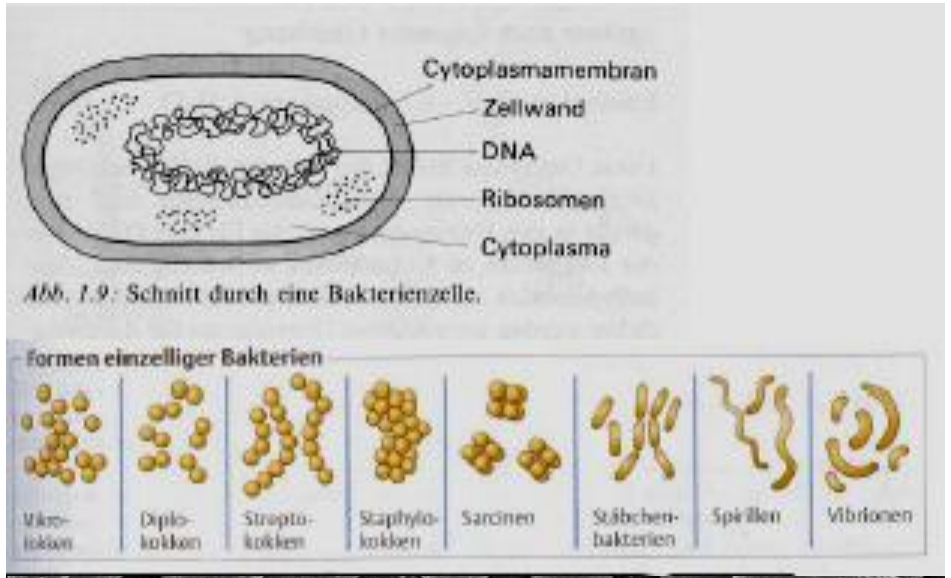
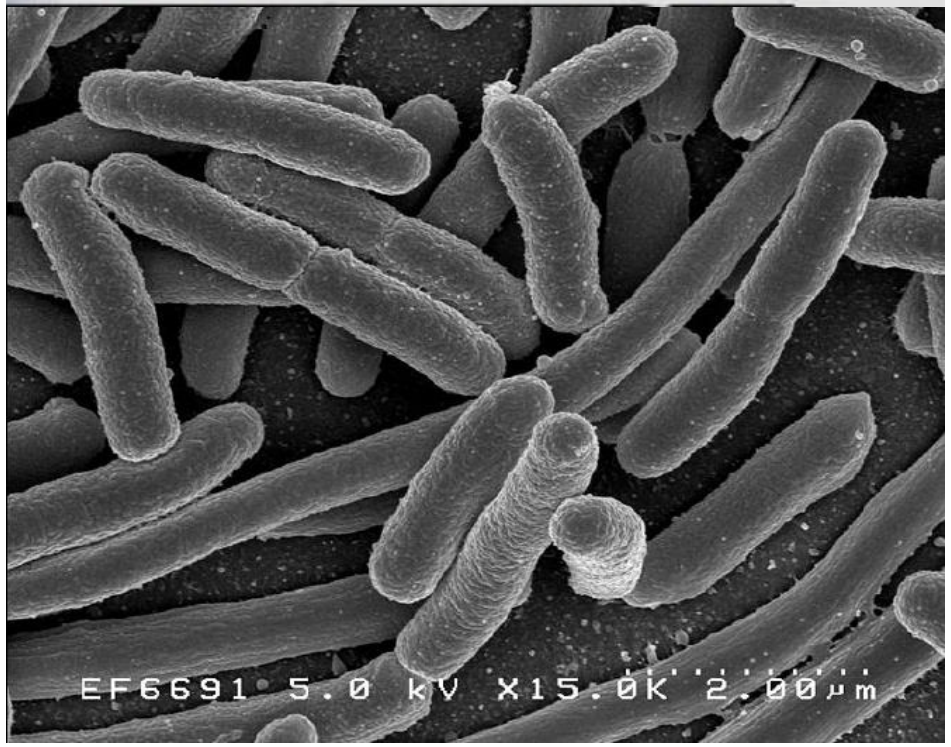
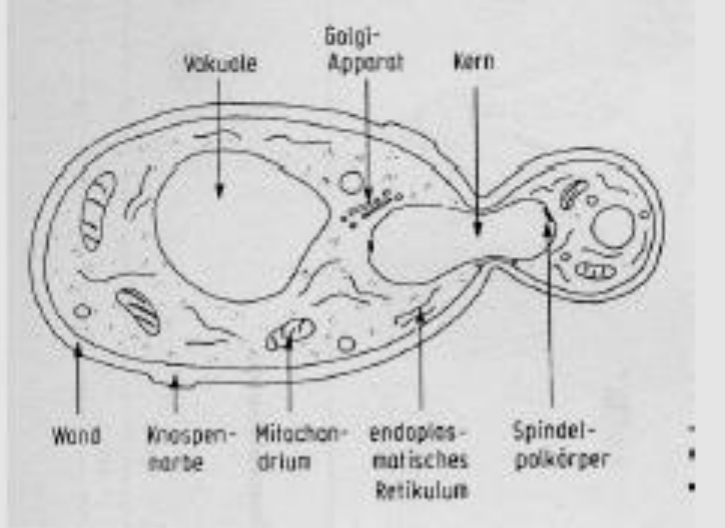


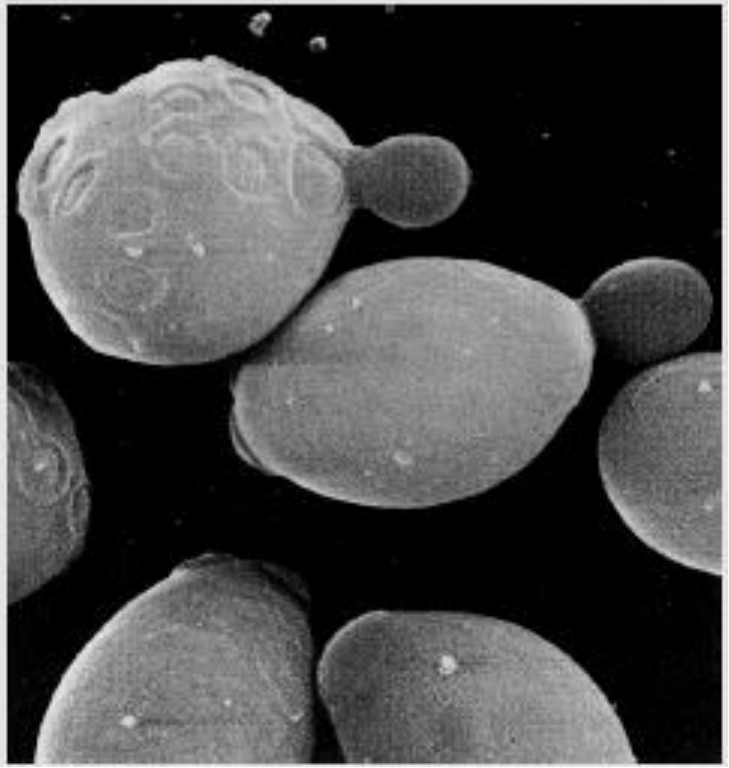
Abb. 1.9: Schnitt durch eine Bakterienzelle.



Bacteria

- No compartments
- Size: 1-5 µm
- Difference:
  - shape
  - mobility
  - spores
  - cell structure
  - utilisation of substrate
  - anaerob / aerob
- growth: t_d= 0.2 –1 h
- low growth material demand
- DNA: circular (3-9 mio Bp), plasmids
- high metabolic activity
- simple cultivation
- big metabolic diversity
- GRAS

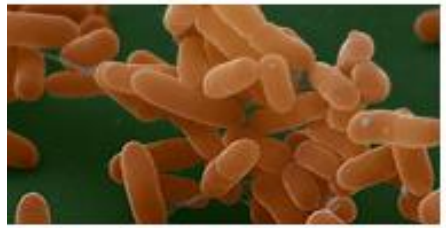




Yeasts

- compartments (eucaryont)
- Size: 10 µm
- growth: t_d= 2-10 h
- asco-spores (sexual cycle; dipoid/haploid)
- Genome: 4-16 Chromosoms; 10-20 Mio Bp; Plasmids
- cell wall: Mannan/ß-Glucan/Chitine
- heterotroph (pH 3,5 –5)
- simple cultivation
- low growth factor demand
- GRAS

Different Hosts for Different Products

Escherichia coli	*Pichia pastoris*	*Bacillus subtilis*
		
– Expresses >80 % of commercial microbial products – Expresses simple to moderately complex proteins including Fabs – Soluble expression simplifies recovery and downstream processing – Produces aglycosylated proteins – Ideal for plasmid DNA production	– Expresses larger complex proteins and small polypeptides – Solubility issues in *E. coli* may be solved with *Pichia* – Secretes for easier recovery & downstream processing – Lacks endotoxin	– Expresses simple monomeric proteins – Soluble expression simplifies recovery and downstream processing – Lacks endotoxin – Produces aglycosylated protein

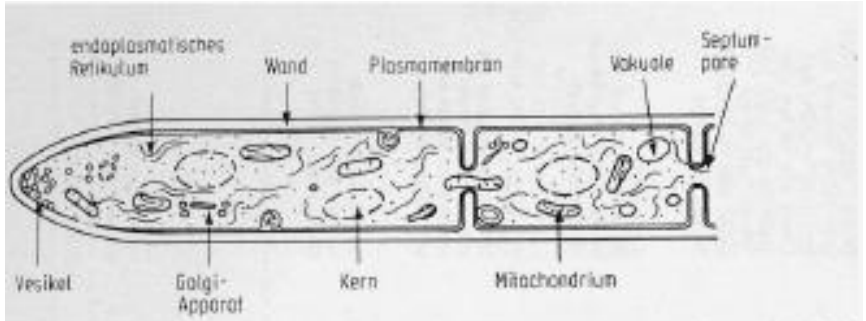
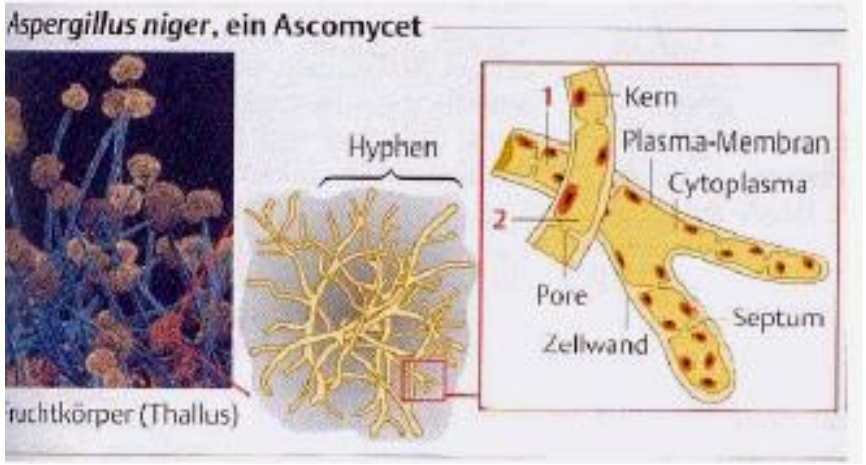
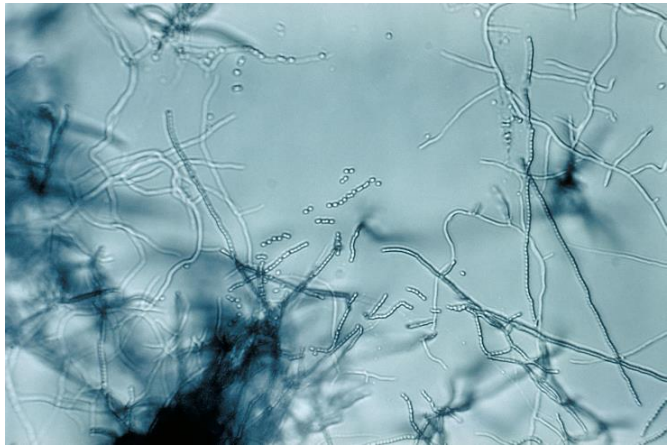
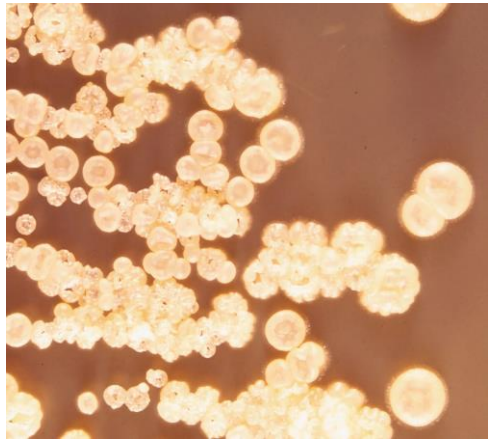


Abb. 2.4. Pilzhyphe (mit freundlicher Genehmigung entnommen aus Deacon, 1984).



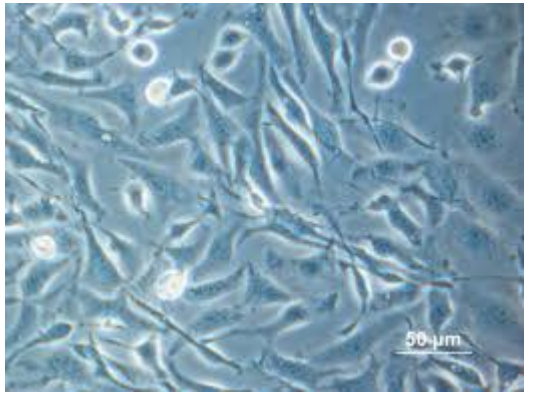




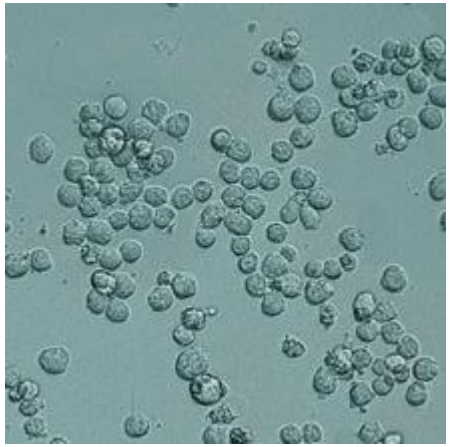
Moulds / Molds

- compartments (eucaryont)
- Size: up to 100 µm, several cells
- branched, Hyphe, mycelia
- cell wall: Chitin/ ß-Glucane
- a sexual spore building
- Sexual fruit body building
- GRAS
- aerobic metabolism
- easy cultivation

Basic Animal Cell Structures



- Complexity of cells: highly differentiated
- No cell walls
- Complex cell membranes, with transport functions, receptors and ion channels
- Complex highly differentiated intracellular cell membrane structures e.g. nucleus, endoplasmic reticulum, Golgi apparatus, mitochondria, lysosomes etc.
- Specialized protein secretion mechanisms (exocytosis and endocytosis)



Important Differences Between Microbial and Animal cells

Microbial cells are generally:

- unicellular, simple structure
- capable of living as individuals within an environment
- have little, if any cell-to-cell contact or communication between cells
- are adapted to growth in a range of environments
- are capable of synthesizing most cell components from simple substrates
- usually have rigid cell walls- shear and osmotic stress resistant
- Support wide pH and temperature ranges

Important Differences Between Microbial and Animal cells

Animal cells are generally:

- of complex structure, highly differentiated forming part of specialized tissues or organs
- not capable of living as individuals within an environment
- have important cell-to-cell contact and therefore communication between cells (e.g. have receptors and need hormones or other signalling molecules
- are adapted to growth in specialized environments
- are not capable of synthesizing all cell components from simple substrates
- Shear and osmotic stress sensitive

Trends: fewer production hosts for rec. proteins

Host	*Advantage*	*Disadvantage*	*Product type*
E. coli	genetics, medium	secret. glycosyl.	small proteins
B. subtilis	GRAS, secretion	medium, protease	bulk proteins
Yeasts	genetics, GRAS	medium	food addit.
Asp. oryzae	secretion, GRAS	rheology	bulk proteins
Animal cells	postranslational modifications	expensive	large complex proteins
Plants	cheap	purification	bulk proteins
Mammals milk	cheap	etics? safety?	pharma prod.
Hen's egg	cheap, established		pharma. prod.

Table 1. Characteristics of production platforms for recombinant therapeutic proteins[a)]

Expression system	Classification	Development of system	Disulfide bonds	Glycosylation	Secretion	Cost of fermentation	Use of antibiotics	Safety costs	Processes developed	Product on market
E. coli	Gram-negative bacterium	Completely developed	(Yes) in the periplasm	No	Periplasmic secretion	Promoter-dependent low to moderate	Typically required	Low costs	Industrial scale	Yes
Saccharomyces cerevisiae	Budding yeast	Completely developed	Yes	Yes; high mannose	Possible	Low	Not required	Low costs	Industrial scale	Yes
Pichia psatoris	Methylo-trophic yeast	Completely developed	Yes	Yes; no terminal α1,3 mannose	Possible	Low	Not required	Low costs	Industrial scale	Yes
Hansenulat polymorpha	Methylo-trophic yeast	Completely developed	Yes	Yes; no terminal α1,3 mannose	Possible	Low	Not required	Low costs	Industrial scale	Yes
Yarrowia lipolytica	Dimorphic yeast	Early stage	Yes	Yes; exact features jet unknown	Possible	Low	Not required	Low costs expected	Lab scale	No
Plant cells	Higher eukaryote	Completely developed	Yes	Yes; terminal fucose	Possible; size-restrictions	Moderate	Not required	Low costs	Pilot scale; production scale	Yes (Cuba)
Mammalian cells (e.g., CHO)	Higher eukaryote	Completely developed	Yes	Yes; typically human-like	Usually	High	Not required	High costs	Industrial scale	Yes
Animals	Mammals	Completely developed	Yes	Yes; typically human-like	Usually	Farming; moderate costs	Not required	High costs	Industrial scale	Yes

a) Modified from [6].

Short summary

1. Overview 1
2. Overview 2
3. Bioprocessing Cell Culture Overview Video (1).mp4
4. Biomanufacturing is the vehicle to deliever biologics.mp4
5. Principles of cultivation.mp4

Definitions:

European Federation of Biotechnology(EFB)

1981: ***European Federation of Biotechnology***

[Biotechnology is] "the integrated use of biochemistry, microbiology, and engineering sciences in order to achieve technological (industrial) application of the capabilities of micro-organisms, cultured tissue cells, and parts thereof" (3).

1989:

*Biotechnology is the integration of natural sciences and engineering sciences in order to achieve the **application** of organisms, cells, parts thereof and molecular analogues for products and services*

Definitions:

Biotechnology is the integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological (industrial) application of the capabilities of microorganisms, cultured tissue cells and parts thereof. EFB, 1981

Biotechnology is the integration of natural sciences and engineering sciences in order to achieve the application of organisms, cells, parts thereof and molecular analogues for products and services. EFB, 1989

Biotechnology is the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services. OECD, 1982

The application of science and technology to living organisms, as well as parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services. OECD, 2001

Alle Verfahren, die lebende Zellen oder Enzyme zur Stoffumwandlung und Stoffproduktion nutzen. http://www.science-live.de/info/glossar.html

Biotechnologie ist die gezielte Anwendung von Mikroorganismen, Pflanzen, Zellkulturen oder isolierten Enzymen um chemische, landwirtschaftliche und pharmazeutische Produkte herzustellen. http://www.corporate.basf.com/de/produkte/biotech/glossar.html

Eine Gruppe von Technologien, mit denen lebende Organismen so verändert werden, dass sie chemische Prozesse ausführen oder Stoffe wie Enzyme, Hormone oder Antibiotika produzieren. http://www.archiv.hoechst.de/deutsch/publikationen/future/298/art10.html

Definitions:

Further definitions (often used in media)

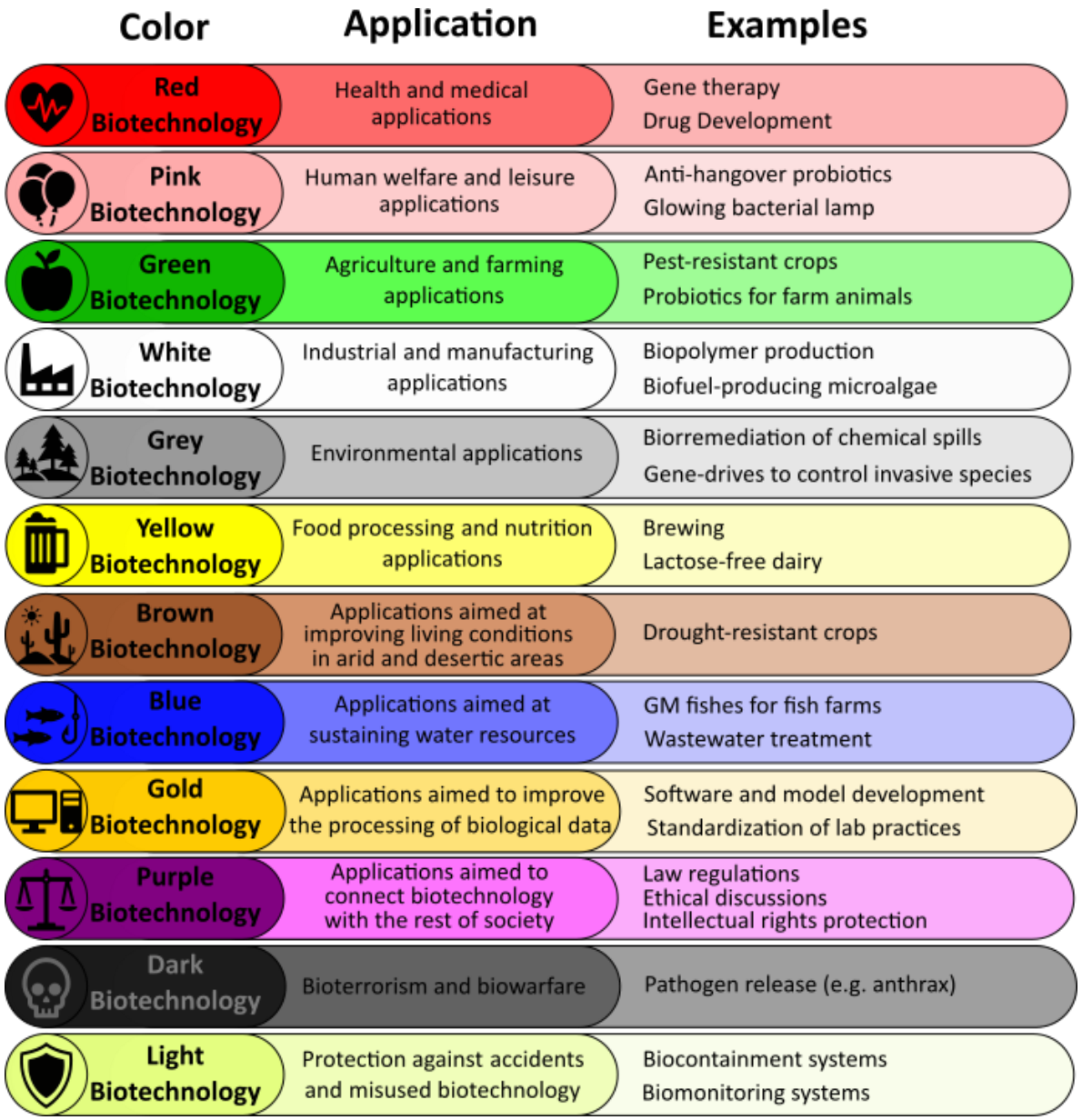
- *use of all biological techniques*

- *use of cell biological methods*

- *use of all molecular-biology methods*

- *Parts of modern medical diagnostics and therapy (somatic gene therapy, reproduction medicine, etc)*

Definitions:



Introduction

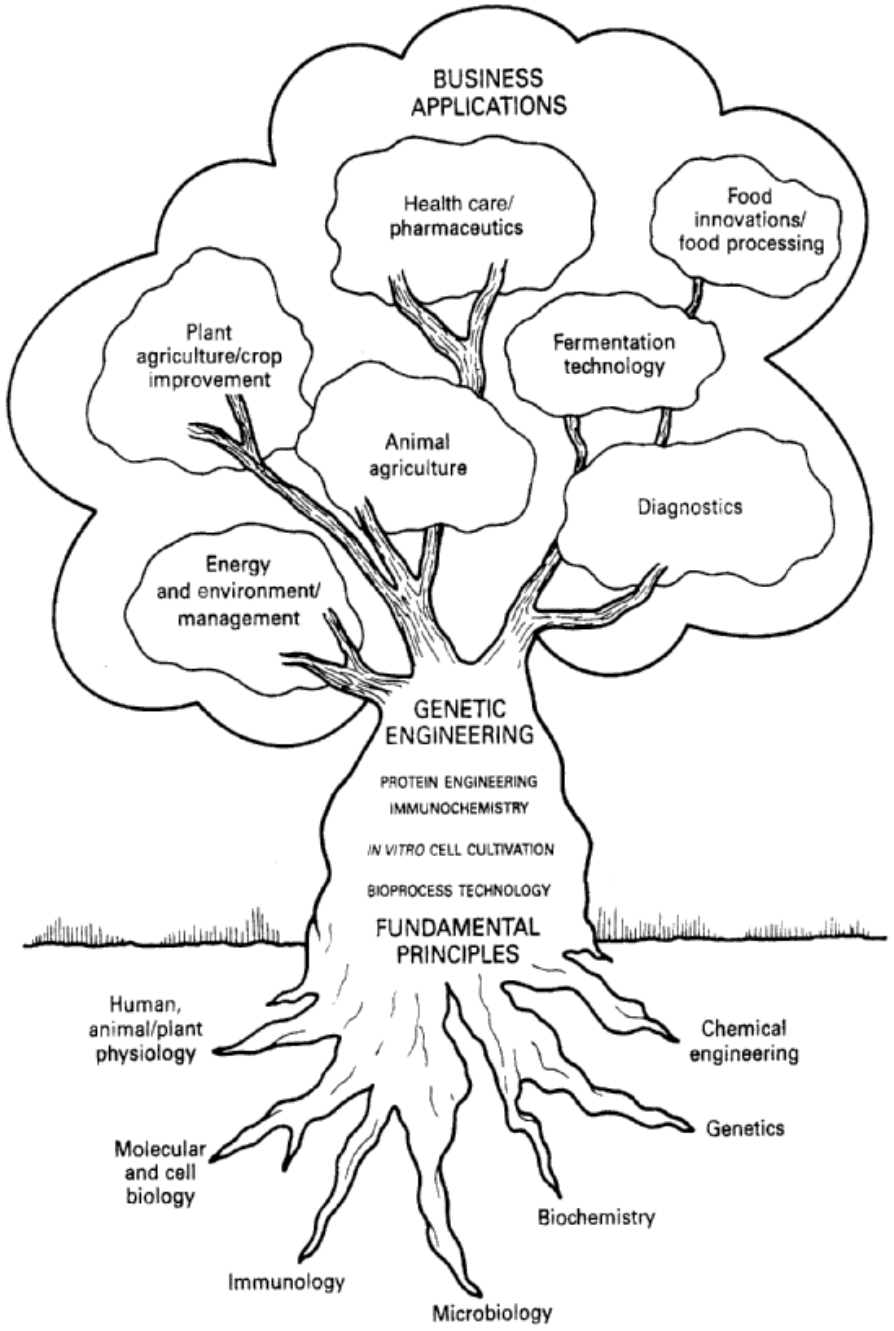


Fig. 1.3 The biotechnology tree.

Table 1.6. The main areas of application of biotechnology

Bioprocess technology
Historically, the most important area of biotechnology, namely brewing, antibiotics, mammalian cell culture, etc.; extensive development in progress with new products envisaged, namely polysaccharides, medically important drugs, solvents, protein-enhanced foods. Novel fermenter designs to optimize productivity.

Enzyme technology
Used for the catalysis of extremely specific chemical reactions; immobilization of enzymes; to create specific molecular converters (bioreactors). Products formed include L-amino acids, high fructose syrup, semi-synthetic penicillins, starch and cellulose hydrolysis, etc. Enzyme probes for bioassays.

Waste technology
Long historical importance but more emphasis now being made to couple these processes with the conservation and recycling of resources; foods and fertilizers, biological fuels.

Environmental technology
Great scope exists for the application of biotechnological concepts for solving many environmental problems – pollution control, removing toxic wastes; recovery of metals from mining wastes and low-grade ores.

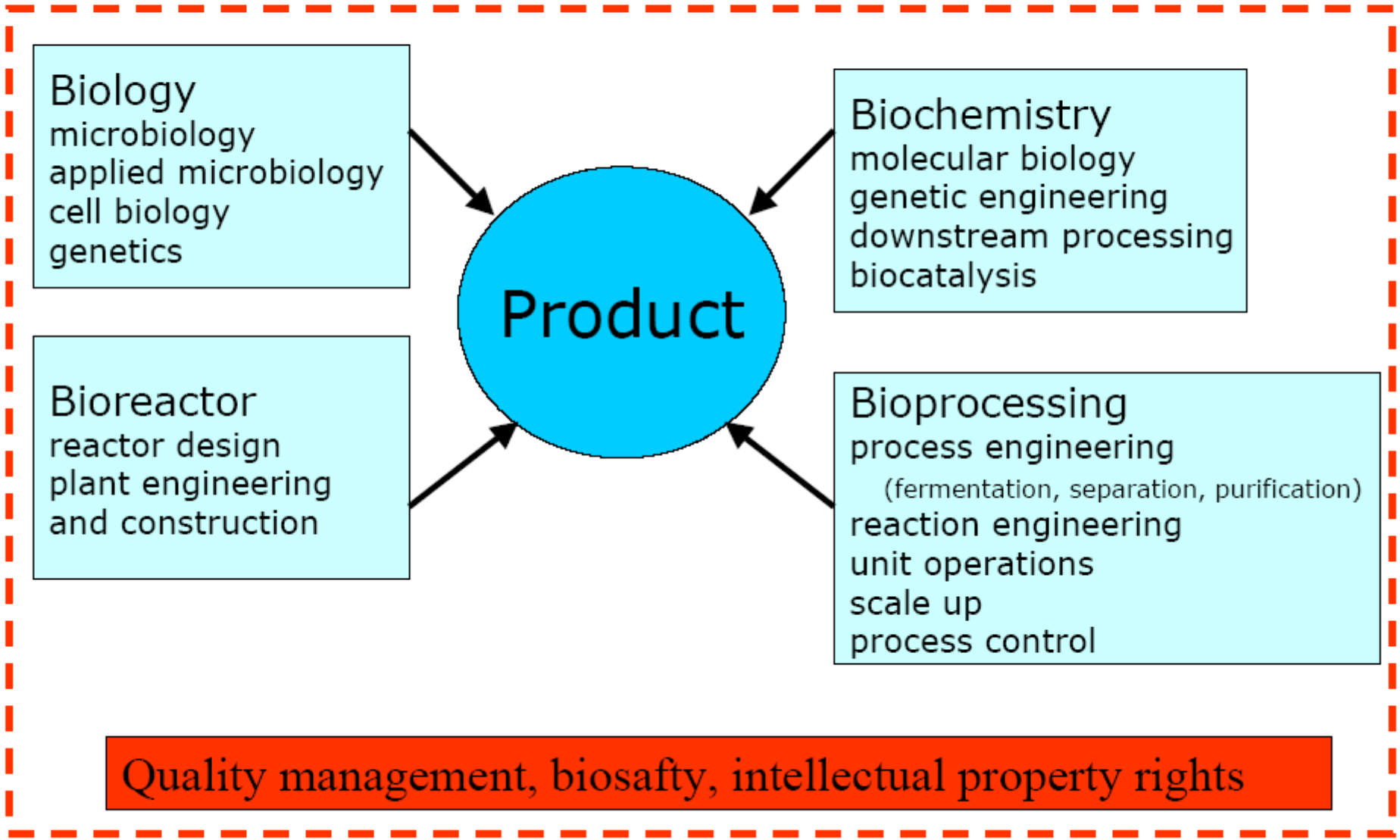
Renewable resources technology
The use of renewable energy sources, in particular lignocellulose, to generate new sources of chemical raw materials and energy – ethanol, methane and hydrogen. Total utilisation of plant and animal material. Clean technology, sustainable technology.

Plant and animal agriculture
Genetically engineered plants for improved nutrition, disease resistance, keeping quality; improved yields and stress tolerance will become increasingly commercially available. Improved productivity, etc., for animal farming. Improved food quality, flavour, taste and microbial safety.

Health care
New drugs and better treatment for delivering medicines to diseased parts. Improved disease diagnosis, understanding of the human genome – genomics and proteomics, information technology.

Key elements of biotechnology

„integrated approach from science to technology“



Death

The irreversible loss of the ability to multiply

Sterilization

The destruction or removal of all microorganisms

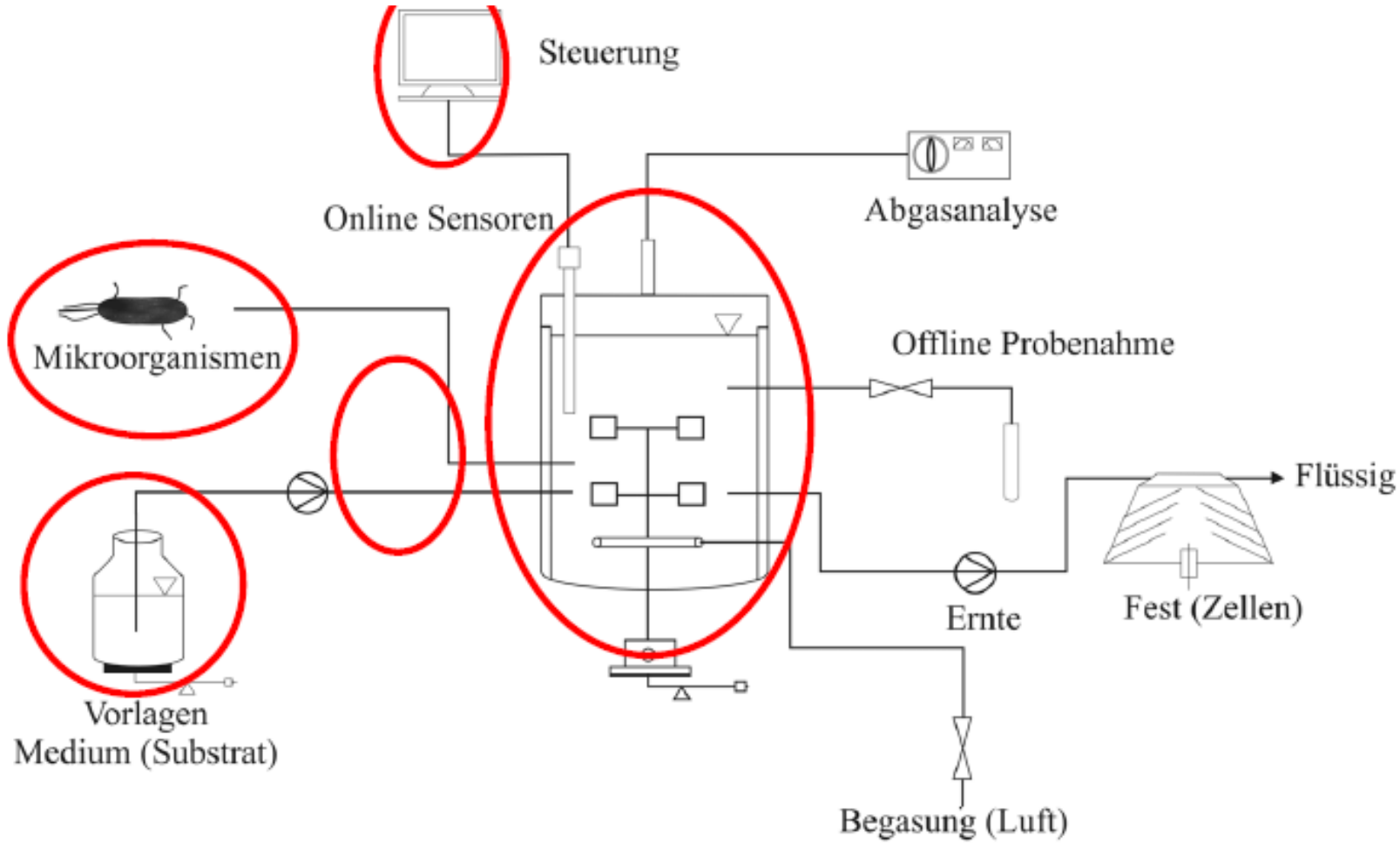
Sterility

Sterility means that no living microorganism is present

To reach sterility is a question about

PROBABILITY

Rührkessel Fermenter
Stirred Tank Reactor


Steuerung
Online Sensoren
Abgasanalyse
Mikroorganismen
Offline Probenahme
Flüssig
Ernte
Fest (Zellen)
Vorlagen
Medium (Substrat)
Begasung (Luft)