Biology, Biotechnology
2. Lecture

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Content:

2.Lecture: Cell-biology

- Overview of microbiology, and of microbial physiology
- Type of industrial microbes,
- Main biochemical characteristics: aerobs and anaerobs,
- Basic microbial metabolism

Biochemistry for Chemical Engs.

According to the subject description, the 3. lecture is about an overview of microbiology and microbial physiology, furthermore types of industrial microbes and their main biochemical characteristics as well as basic metabolism.

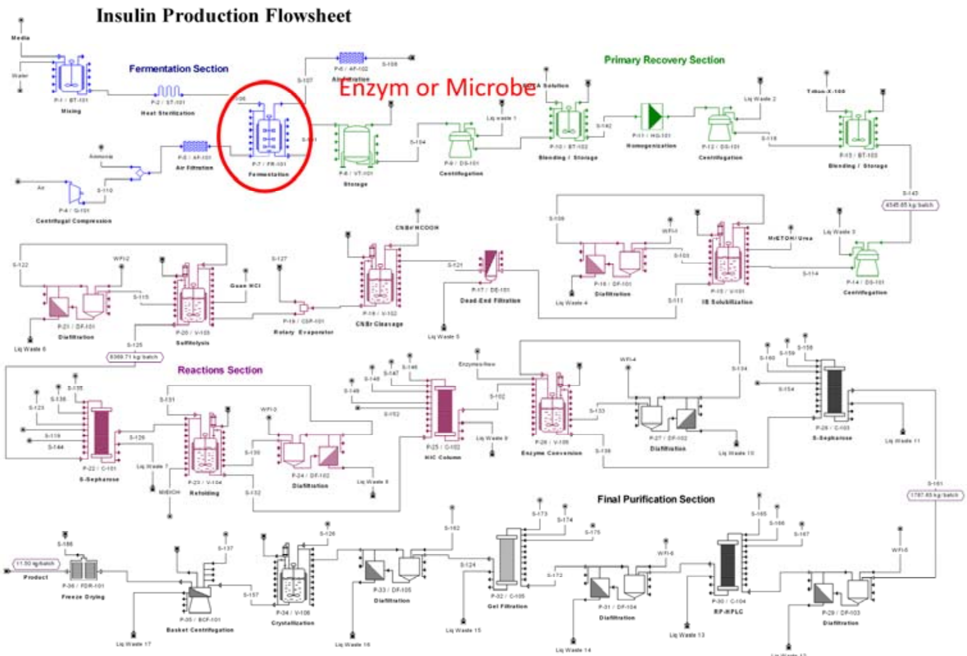
Since the 2. lecture was about Cell biology including physiology (like energy production, „cell-organs” etc.) and metabolic pathway were considered detailed in subject of „Biochemistry for chemical engineers, the focuse of this presentation is on microbiology, especially on industrial microbiology.

Introduction

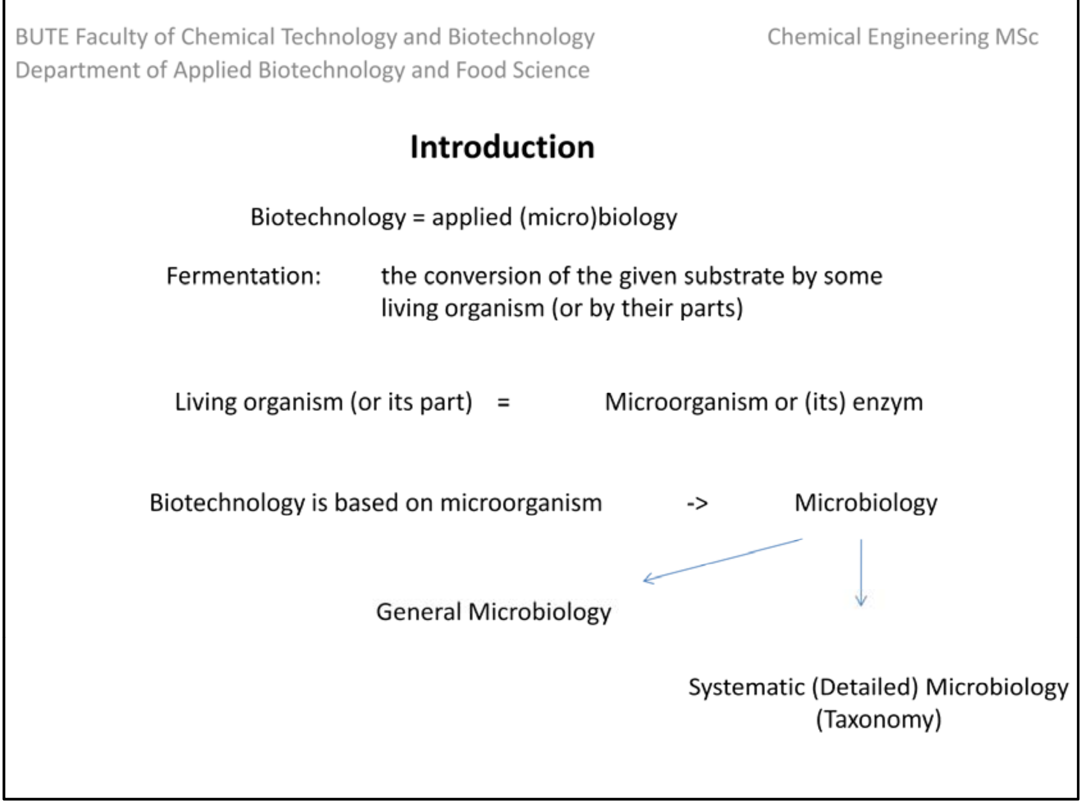
Biotechnology = applied (micro)biology

Biotechnology can be considered as the industrial application of different areas of Biology. There exist plant biotechnology (for example improvement of crops with genemodification), but in the chemical industry mainly microbiology is applied. A bio-based factory is usually divieded into two parts: the main production and its supporting operations together called =upstream processes, and product purification and isolation is called =downstream section.

Biotechnology = applied (micro)biology



The production is carried out usually either by microbial *de novo* fermentation or bioconversion ,or by enzymatic bioconversion. For the latter one, the enzyms must be prepeared also exclusively by fermentation. This conclude, that there is no biotechnology without fermentation, which plays a key-role (=key step). We call fermentation that processes, in which living organism (or their part) help to convert the given substrate into products. These living organism are most commonly microorganism (ie microbes).



This conclude, that there is no biotechnology without fermentation, which plays a key-role (=key step). We call fermentation that processes, in which living organism (or their part) help to convert the given substrate into valuable products. These living organism are most commonly microorganism (ie microbes). Thus finally biotechnology relies on microbs, and the Discipline dealing with microbes is Microbiolgy. This is usually discussed in two parts: 1) General microbiology, 2) taxonomy. We will see little samples for tasting –with industrial aspects.

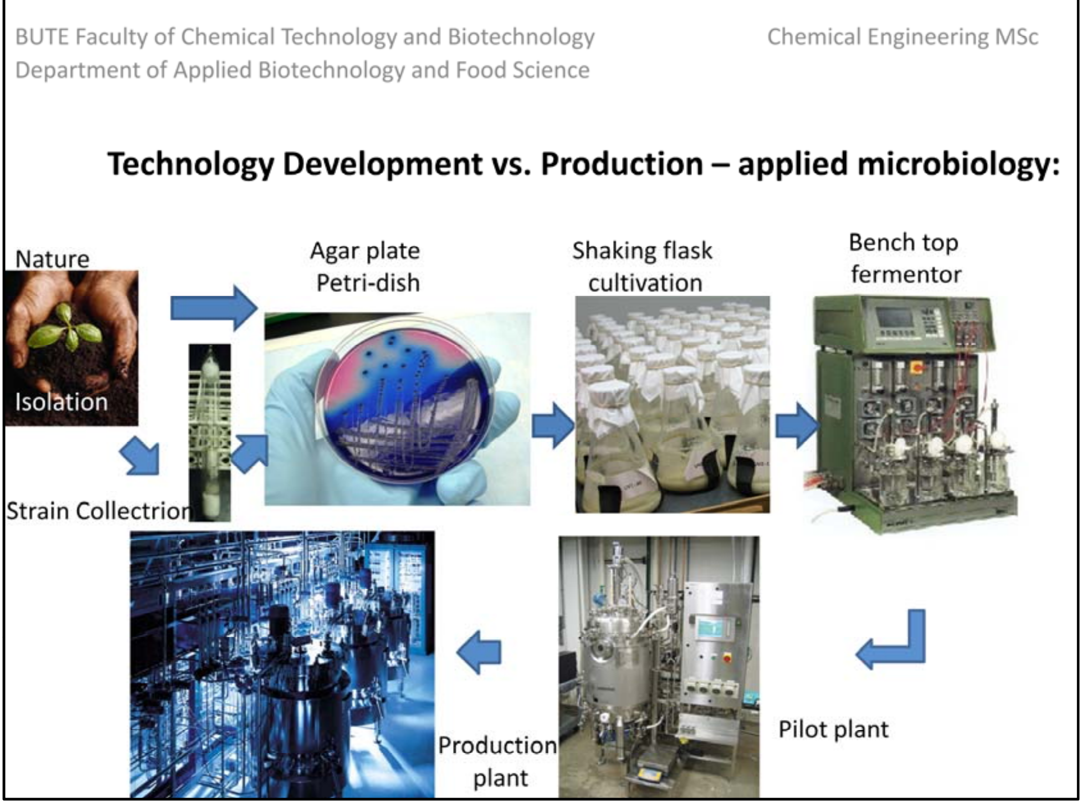
Microorganism : microscopic living organism, which is unvisible by eyes

Motto : „ It might look that I am doing nothing, but at a cellular level I am really quite busy."

Comments:

1. There's no „stupid" microbes.
2. Microbes only want to live.
3. But we selfish serve and utilize them.
4. They give us in change profit and salary.
5. BUT: unvisible, thus often their accompany processes are also unvisible
   (for example: contamination)

But before, here are some remarks to give an attitude on how to think and how to handle microbes.



During development of a bio-based technology the first step is to find or create the production strain. For this, usually microbes are isolated from soil in a petri dish, or if this was already done by a Strain collection, then have to place an order from them, and microbes will be delivered in liophylised form, and during revive we got again to the petri dish. This latter one is made from glass containing media for cultivation. On the surface of the media colonies or strips will be formed, but sticked [szúrt] cultivation also exist. The goal on the Petri-dish is to isolate individual, clean clones (colonies), which will go under screening experiments to find the best producer (or best candidate). For these screening either microtitet plates or shaking flasks are used. The cultivation of the choosed isolate will be then optimized, then scaled up, if the microbe tolerate it, since microbes are often sensitive agiainst scale up.

Production has the same process, since every batch of an already developed and existing fermentation plant is always started from either a petri-dish or an ampull, and through continous scale up they reach the production scale. The reasons are: 1.: microbes are sensitive against changing a scale, because the hydrostatic preasure will increase, which also change the oxygen solubility, and usually the mixing rate is also differnt with different shear-force, etc. 2.: every operaton must be carried out under sterile conditions to avoid the sidereaction of contaminating microbes (ie. Substrate decrease and byproduct formation), however sterilization can only be considered as a statistical operation: if the criterium is 10^{-3}=0,001 it means, that from every 1000 sterilization one will be lost. If we propagate a huge reactor with cells from a petri dish, that cells will be so much diluted, that very long fermentation time will be needed (cells should fill up the media with binary cell division!), and the potentially existing survived contaminations could be in the same magnitude than inocula, which resulted a questionable competition.

General Microbiology

Detailed Microbiology

1. Microbiology knowledge:

A. Structure of a microbial cell
B. Microscopic observations →
C. Microecology

Taxonomic system:
-Phenotype based
-Genotype based

2. Microbiology operations:

A. Media preaparation
B. Isolation
C. Screening
D. Identification →
E. Preservation and maintenance
F. Subcultivation (transfering)
G. Strain imprvements

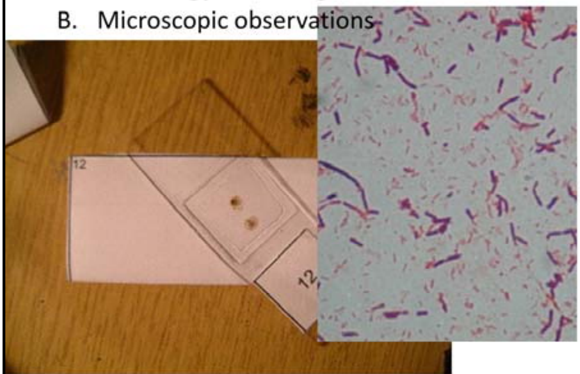
BUTE Faculty of Chemical Technology and Biotechnology
Department of Applied Biotechnology and Food Science

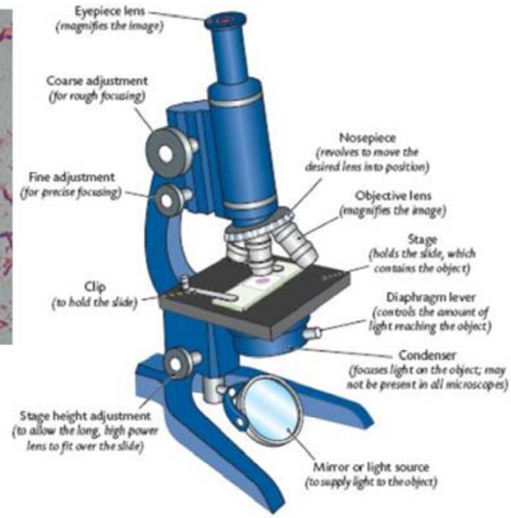
Chemical Engineering MSc

General Microbiology

1. Microbiology knowledge:

B. Microscopic observations





Bacteria: 1-2 μm 1000x

Yeasts: 10 μm 400x

Molds: 10 μm-1mm 100x

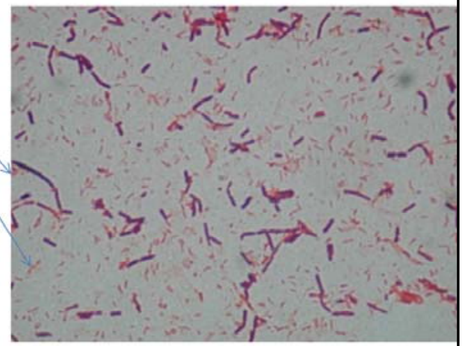
For microscopic observations broth containing microbes will be dropped onto a glass subjectplate (slide), and will be covered by another glassplate, and will be put onto the stage. We set up the correct distance between the sample and the objective with the rough and fine adjustment, and through the eyepiece lens we make observations. The way of light: lamp (and/or mirror), condenser, slide, objective, tubular, eyepiece lens, eyes. By multiplicating the magnification of objective and eyepiece lens we receive the nominal enlargement. The size of different microbes can differ even in more orders of magnitude, for this different objectives can be applied. To see the smaller microbes (bacteria) so called oil-immersion should be used, because the oil droplets between the sample and the objective has the same refractive index then the glass, thus the light will not be broken on the surface of asample-air-objective, and results larger magnification.

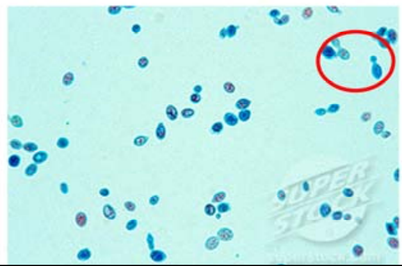
General Microbiology

Microscope:

Bacteria:	1-2 μm	1000x
Yeasts:	10 μm	400x
Molds:	10 μm-1mm	100x

Gram+
Gram -





Our different „friends” look like these images in microscope.

General Microbiology

Measurements of microbes:

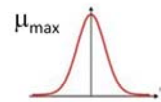
1. OD-optical density (UV-Vis photometer, 600-660nm)
2. Turbidimetria (online)
3. Mikcroscope – cellcounting with Buerker chamber (10^6 pieces/ml)
4. Automatic Cellcounter (10^6 pieces/ml)
5. Cell Dry Weight (1-10 g/L)
6. Diluting-dispersing method (CFU/ml)

The measurment of microbes is important to follow their growth. With different methods different unit of their amount can be obtained. CFU=colony forming unit

General Microbiology

1. Microbiology knowledge:

C. Mikroecology



Livingplaces: air, water, soil
Life-kind: saprophyta, symbionic, commensalism, parasitism,
Life-conditions: temperature tolerancy (psychrophil, mesophilic, thermophylic)
pH tolerancy (acidophilic, neutrophilic, alkalophilic)
halophyilic (osmotolerancy)
Role of Microbes in biosphere: photosynthesis: fixing CO2, produce (bind) Energy
Degrading: C, N, P recycling

General Microbiology

2. Microbiology operations:

A. Prepearing media

Fluid media <-> Solid media (+agar, or gelrite)

In both: C-source: carbohydrates

N-Sources: proteins, aminoacids, oligopeptides, ammonium-salts

P-sources: phosphatides

+salts, vitamines, precursors etc.

Anaerobs: reducing components (DTT, NaSH)+O_2 indicator)

Bact.: organic N-source (protein), C-source also incl., sugar is not always neccessary

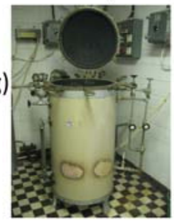
Yeasts+molds: N-source can be inorganic salt => much easier downstream, cheaper up and downstream

Media sterilization:

Physical methods (filtration, irradiation, **thermal handling**)

Chemical methods (decontaminating agents)

Biological methods (cellwall degrading enzymes)



Not all components is compatible, sometimes should them separate

The media contains the important materials for microbes: alwasy include C-source (sugars), N-source (proteins or ammonium-salts), P-sources (phosphatides), salts (for appropriate osmotic condions), spec. Materials (vitamines, precursors etc) + in case of petri dishes agar-agar, which form a gel from the media->solid media.

General Microbiology

2. Microbiology operations:

B. (Strain) Isolation

•Solid→ most frequent (soil), because largest diversity
-sample->onto petri dish
-sample->suspension in water->onto petri dish
-from surface of sample with steril cotton-wool
} Incubation
•Fluid →more dilute-> concentrate: preincubation or filtration+agar+incubation
•Air

Petris dishes with agar for isolation:
mixed culture in soil->selective preasure
additives to media for exm.: antibiotics->only funghi can grow
antifunghi compouds-> bact. grow,
little acidification: yeast

General Microbiology

2. Mcrobiology operations:

C. Screening

Two goals: 1) to find a producer strain among the isolates
2a) to find a better producer for an existing technology
2b) strain improvement (see later)

For these: examination of microbes (isolates) in large numbers
(100-1000 or even more) – lot of sample=>automatisation
BUT
Every case is unique (the key parameters, methode of detection etc.)->manual

↓

Combination

General Microbiology

2. Microbial operations:

C. Screening

-HTS : High Througput System

-manually: additions to agarplates: usually turbid/cleaned zone around the colony

For example.: $CaCO_3$-> acid producers (cleaned zone)

szensitive microbe->antibiotic producer (cleaned z.)

oil emulsion+Ca->lipase producers (turbid zone)

protein aggregate->protease producers (cleaned z.)

Lipase producers: from oil lipase will produce lipoic acid which forms with Ca present insoluble Ca-lipoate=turbidity

General Microbiology

2. Microbiology operations:

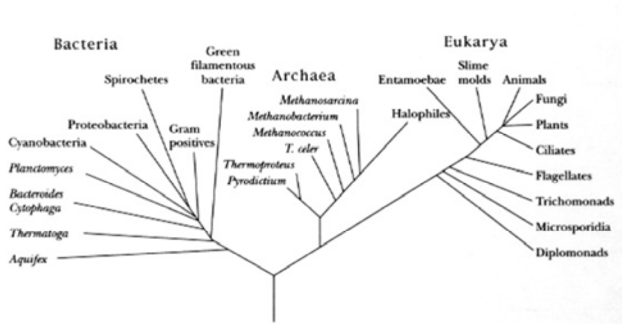
D. Identification

Goal: to identify, and rank into taxonomic system

-HTS system: automatised on both pheno/genotype

phenotype based: ~manual (see next)

genotype based: conserved geneom ->relationship (phylogenetic tree)



More detailed: in Detailed Microbiology

General Microbiology

2. Mikrobiológiai műveletek:

D. Identification

-manual: on the basis of phenotype and biochemical charachteristaion, for example:

macroscopic: color, smell, shape of the colony

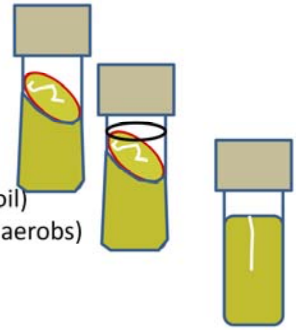
microscopic: cell shape, groupping of cells, motion-organs,
cellcorn, cellwall (Gram staining, other staining)

Biochemical tests: oxydase probe, aerob/anaerob dextróz consumption,
urease, hydrogensulphide etc

Genearl Microbiology

2. Microbiology opeartions:

E. Storage and maintenance



Maintenance of isolates:

-in active forms:

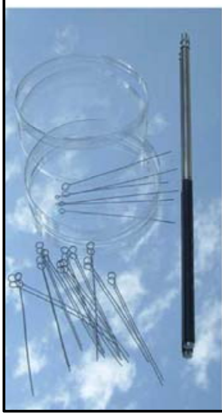
-freezdried in an ampul (lyophylised)

-slowed down in a fridge

-on agarslant in a testtube(+oil)

-punctured (sticked) agar (anaerobs)

-agar in petridish



-in inactive form: spores

General Microbiology

Microbiology operations:

F. Transfer- subcultivations

-daily usage-> maintenance in active form->but become aged->transfer to fresh
-starting an inoculum from maintenance culture (from solid to fluid)

Laminar box<> sterile box

Accessories:

-steril place
-steril loop
-bunsen flaming
-autoclave for sterilization
-sterile pipetta tip or glass-pipette
-sterile water (for suspension)

Sub-transfer: the new culture will be incubated, then stored in fridge. Next time wil be used.

Fülke bekapcsolása (UV+ légáram ~15min), oltókacs leégetése (vörös izzás), steril térben lehűlés, agaros kémcső leégetése, kupak nyitás, mintavétel, zárás, üres agar nyitás, beoltás, zárás kacs égetés-> átoltott inkubálása
Mikrobák veszélyességi osztájai a WHO ajánlása alapján BSc Level 1-4 (BioSafety Class):
1: mindennapos, akár humán mikroflóra – 4:legveszélyesebb.

General Microbiology

2. Microbiology operations:

G. Strain improvement

The charachterisation of a living organism is determined by genom =>
for improvement the genom should be changed=mutation

Phyisical mutagens:
- -radiations (UV, gamma)

Chemical mutagens:
- -DNA modifing compounds (carcinogen)

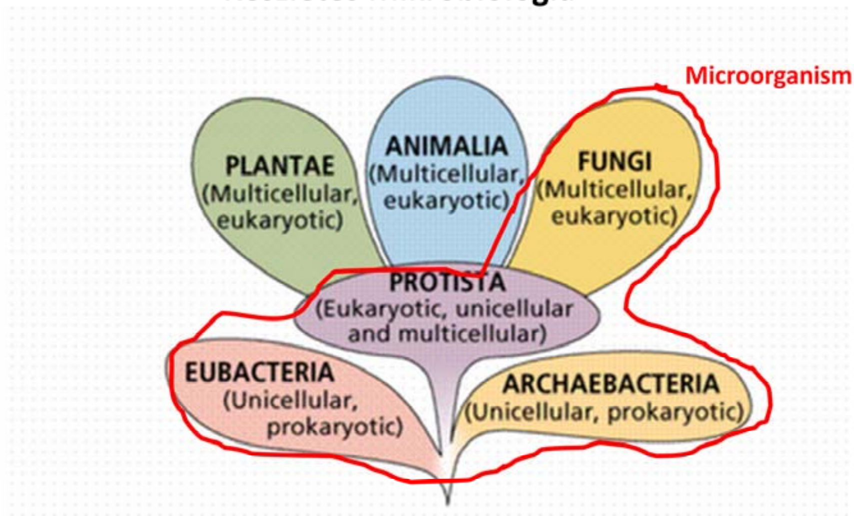
Dose:
- λ and intensity of radiation and time
- concentration+time

1. Mutatio->2.cultivations of mutants (isolation)->3.screening of mutants (which is better)->

4.Little better-> re-mutation

Significance of dosage: if too big, than lot of mutant/mutation, but little survivers; too little dosage: lot of survival, but only a few mutant (cells have DNA repair!)
Here also have to examine great number of cultures, so a specific and sensitive method is nesseccery for detection of main charachterization, which is suitable for measurment series in short time.

Részletes Mikrobiológia



Tree of Life = az élet fája

Detailed Microbiology

A) Procaryotes

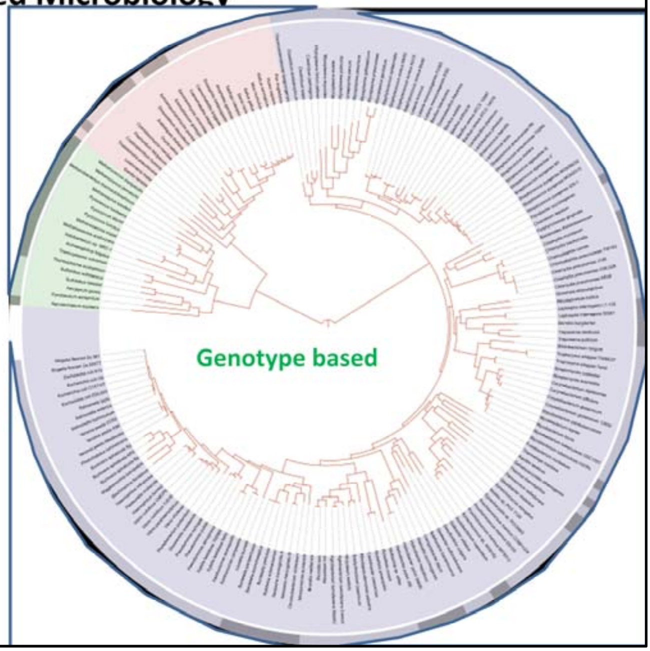
Bergey I. edition (years of 1980-90
4 division

I. Gram (-) bacteria
II. Gram (+) bacteria
III. Bacteria without hard cellwall
IV. Archeabacteria

Phenotype+Biochemistry based

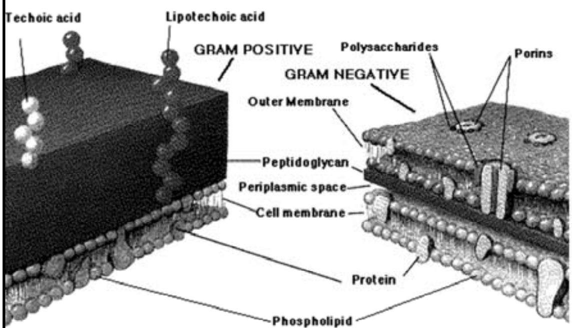
Bergey II. edition (from 2001)
2 domains 25 phylum

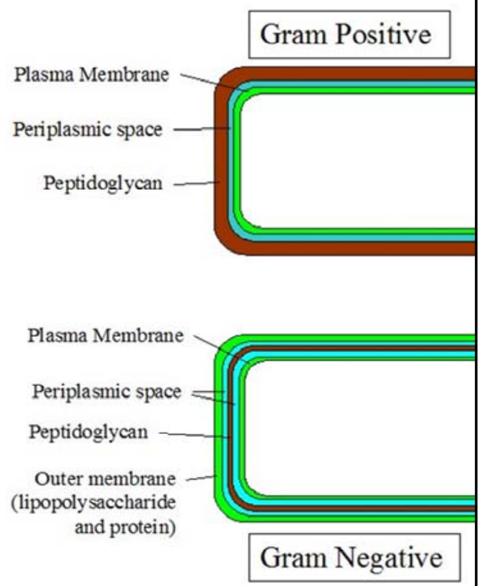
I. Archea
 1. A1 phylum
 2. A2 phylum
II. Eubacteria
 1. B1 phylum
 ...
 23.B23 phylum



Tree of Life = az élet fája

Detailed Microbiology





On the basis of cellwall:
1. Archea: no mureinlayer
   or pseudomurein
2. Gram (-) bacteria
3. Gram (+) bacteria

On the basis of cellshape
1. Sphere(Coccus)
2. Rod shaped (bacillus)
3. Twisted (spirohaeta)

Tree of Life = az élet fája

Detailed Microbiology

A) Procaryotes

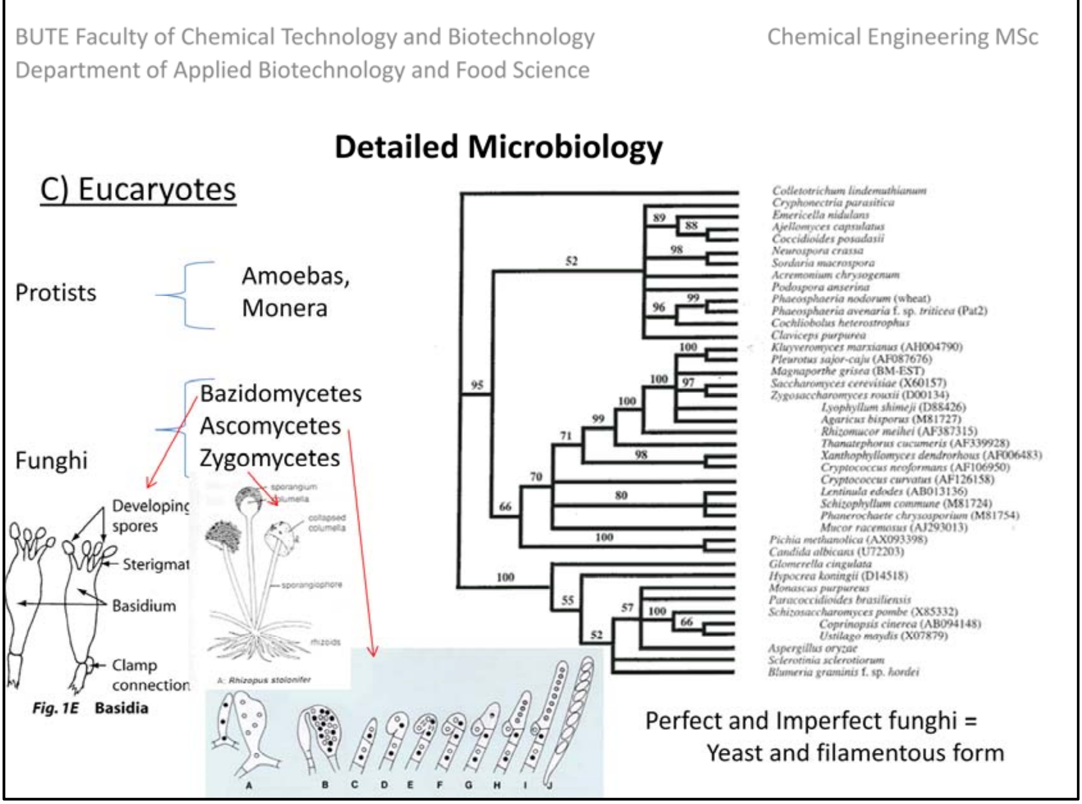
Archea

Gram (-)
- Coccus
- Rod shaped:
  - -aerob — Pseudomonas, Brucella, Legionella
  - -facultative anaerobs — Enterok :Escherichia, Klebsiella, Salmonella
  - -anaerob

Gram (+)
- Coccus — Staphylococcus, Streptococcus
- Rod shaped:
  - -endospore forming — Bacillus (aerob), Clostridium (anaerob)
  - -without spores — Lactobacillus
  - - unshaped without spores — Corynebacterium, Streptomyces, Actinomyces

B) Parabiotes (viruses, prions)

Tree of Life = az élet fája



Ascomycetes: *Aspergillus, Penicillium* strains (organic acids, antibiotics)
Zygomycetes: Rhizopus, Mucor strains are usefull

Potential of Biotechnology

What can be produced by microbes?

1) Primery metabolites:
   produced under normal living conditions of microbes

2) Secondary metabiolites:
   metabolites of microbes not coupled to life (antibiotics)

3) Bioconversions product

4) (recombinant) proteins, enzymes

Potential of Biotechnology

What can be produced by microbes?

1) Primary metabolites:

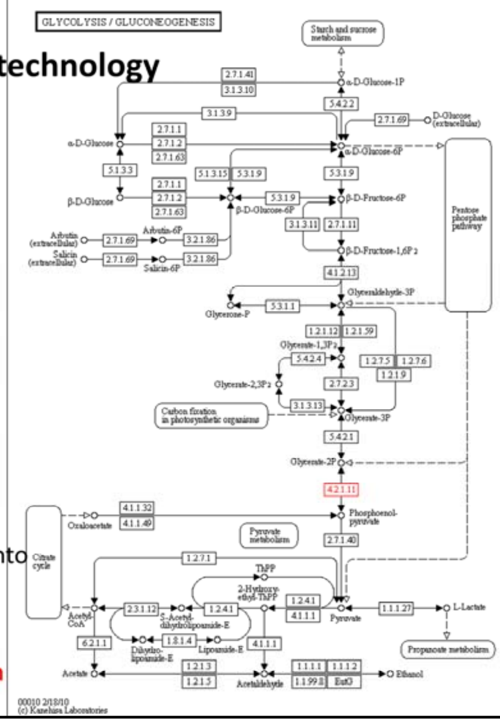
Energy production: **glycolisis+TCA**
glucose/fructose/other sugars

Chemical energy: ATP v. $NADH_2$

Direct Energy utilization

Converted into ATP and regenerated

Terminal oxydation



Potential of Biotechnology

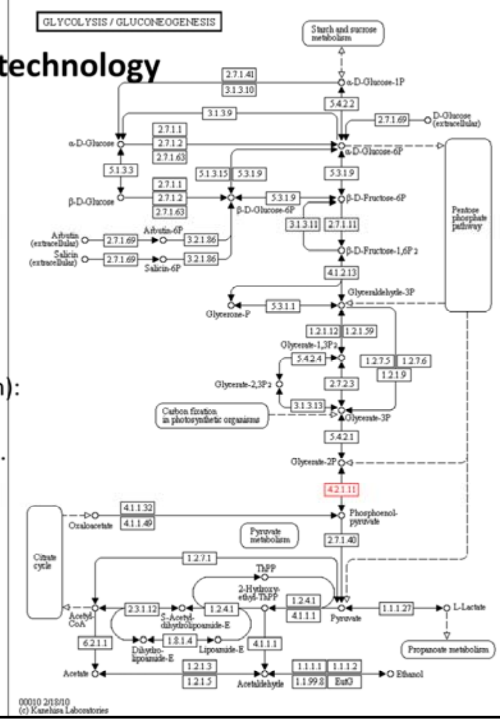
What can be produced by microbes?

1) Primary metabolites:

A) Glycolisis+Terminal oxydation

It needs O_2 – aerob metabolism

Without oxigyen(anaerobic metabolism):
Alternative NADH regeneration,
Like: pyruvate->LA reduction NAD form.
or Ac-CoA->AcOH



Potential of Biotechnology

What can be produced by microbes?

1) Primary metabolites:

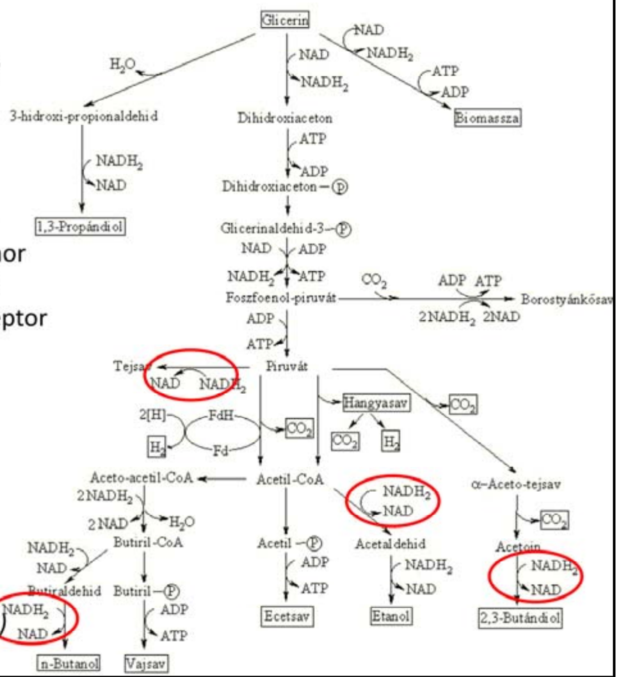
Respiration <> Fermentation

organic e^-donor organic e^-donor

Aerob/anaerobic Anaerobic

INorganice^-acceptor organic e^-acceptor

Several products,
Different fermentaion:
-lactic (*Lactobacillusok*)
-acetic (*Acetobacterek*)
-alcoholic (Yeasts: *S. cerevisiae)*
-butyric (*Clostridium butyricum)*
-aceton-butanol ferm. (*C. acetobutylicum)*



Potential of Biotechnology

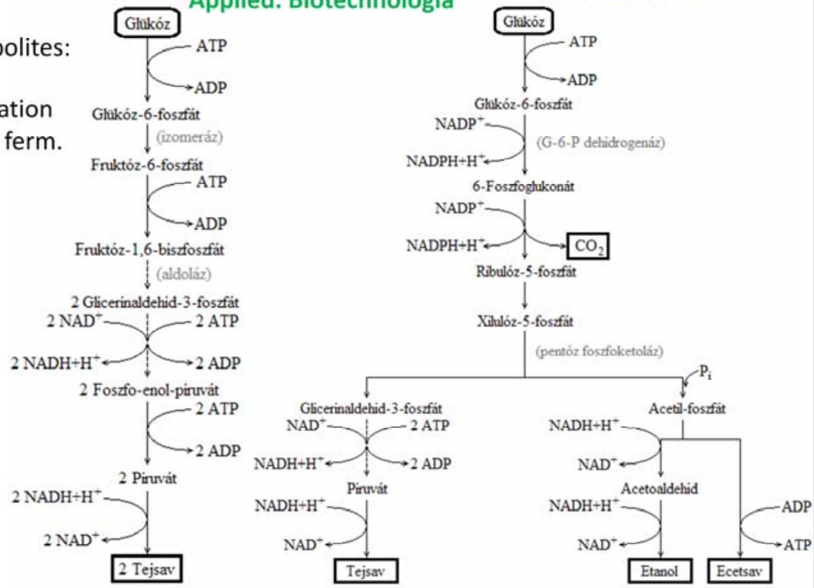
What can be produced by microbes?

Applied. Biotechnológia

Food Industry

1) Primary metabolites:

Like: Lactic fermentation
Homo/hetero lactic ferm.

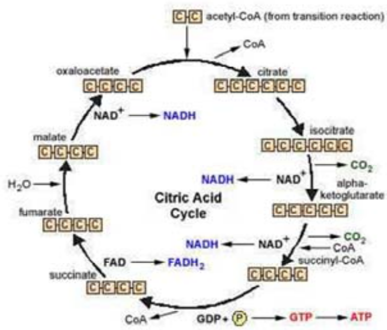


Potential of Biotechnology

What can be produced by microbes?

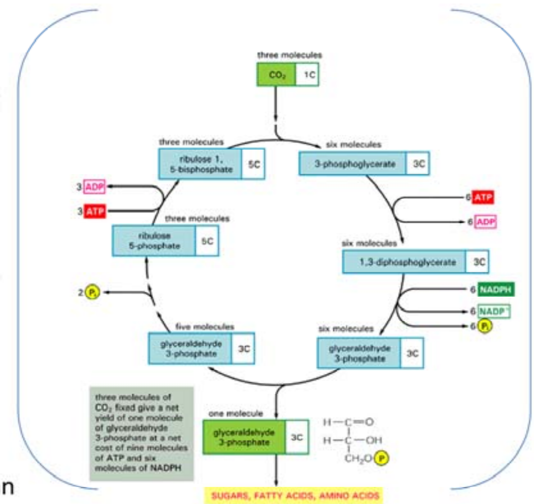
1) Primary metabolites:

B) TCA-cycle (mitocondria)



MIKROBES-degradation

Calvin ciklus - kloroplaszt



Breaking the cycle, its compounds can be produced if the strain have anaplerotic pathways

PLANTS-synthesis

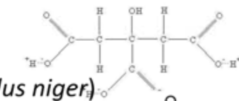
According to oppinions of some researchers, in the first generation of White biotechnology microbes degraded the biomass synthetised by plants, and in the future (next generation) is to utilize the plant potential, because there are a lot of „upstreaming” (synthetic) pathways (certanly production will be not carried out by plants, but plant pathways should be expressed in microbes).

Potential of Biotechnology

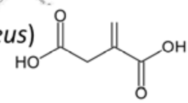
What can be produced by microbes?

1) Primary metabolites:

B) TCA-cycle (mitocondria)



1. Citric acid production with filamentous funghi (*Aspergillus niger*)
2. Itaconic acid production with filamentous funghi (*Aspergillus terreus*)
3. Amino acids production: Lys, Glu (*Corynebacterium*)

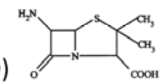


2) Secondary metabolites:

-penicilins (Penicillin G, 6APA, *Penicillium crysogenum*)

-cephalosporins (Cephalosporin C, *Cephalosporium acremonium*)

- Fumagilins (*Aspergillus fumigatus*)

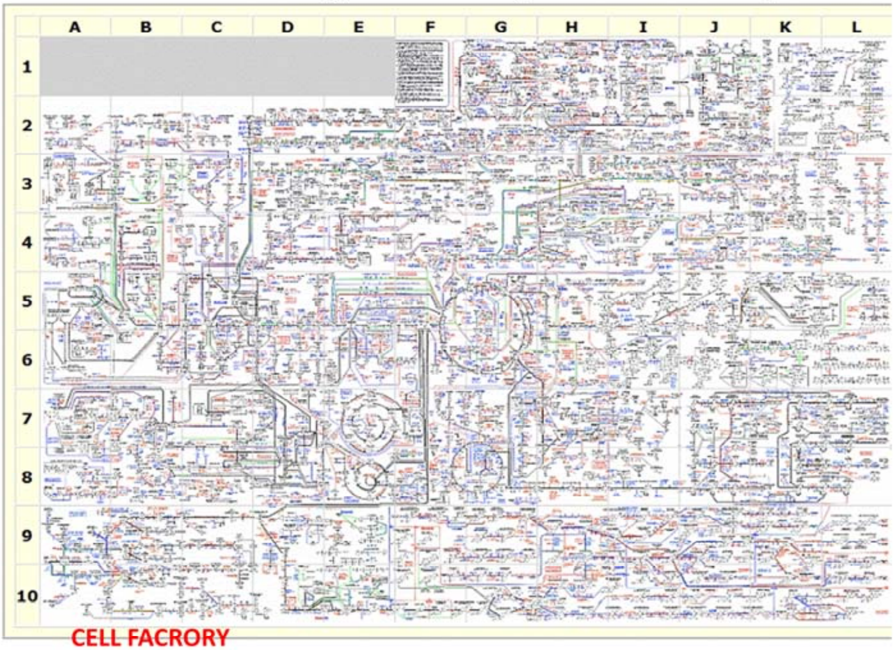


The goal with Sec. Met. Prod of microbes:

to surpress under limiting conditions the competitors (=bacteria)

Potential of Biotechnology

Boeringher Metabolic Pathway



CELL FACRORY

Potential of Biotechnology

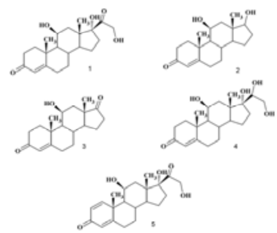
What can be produced by microbes?

3) Bioconversion products

Previous: *de novo* fermentations = synthesis
(mostly natural reactions)

This:one point reaction in chemical synthesis routes:
- -because of good yield
- -stereospecific
- -more simple to change a spec. functional group then totalsynthesis

Figure 1. Chemical structure of substrate hydrocortisone (**1**) and the biotransformed products 11β,17β-dihydroxyandrost-4-en-3-one (**2**), 11β-hydroxyandrost-4-en-3,17-dione (**3**), 11β,17α,20β,21-tetrahydroxypregn-4-en-3-one (**4**) and prednisolone (**5**).



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Article

Characterization of Hydrocortisone Biometabolites and 18S rRNA Gene in *Chlamydomonas reinhardtii* Cultures

Younes Ghasemi [1,*], Sara Rasoul-Amini [2], Mohammad Hossein Morowvat [1], Mohammad Javad Raee [1], Mohammad Bagher Ghoshoon [1], Fatemeh Nouri [1], Narges Negintaji [1], Rezvan Parvizi [1] and Seyed Bagher Mosavi-Azam [1]

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Abstract: A unicellular microalga, *Chlamydomonas reinhardtii*, was isolated from rice paddy-field soil and water samples and used in the biotransformation of hydrocortisone (**1**). This strain has not been previously tested for steroid bioconversion. Fermentation was carried out in BG-11 medium supplemented with 0.05% substrate at 25°C for 14 days of incubation. The products obtained were chromatographically purified and characterized using spectroscopic methods. 11β,17β-Dihydroxyandrost-4-en-3-one (**2**), 11β-hydroxyandrost-4-en-3,17-dione (**3**), 11β,17α,20β,21-tetrahydroxypregn-4-en-3-one (**4**) and prednisolone (**5**) were the main products of the bioconversion. The observed bioreaction features were the side chain degradation of the substrate to give compounds **2** and **3** and the 20-ketone reduction and 1,2-dehydrogenation affording compounds **4** and **5**, respectively. A time course study showed the accumulation of product **2** from the second day of the fermentation and of compounds **3**, **4** and **5** from the third day. All the metabolites reached their maximum concentration in seven days. Microalgal 18S rRNA gene was also amplified by PCR. PCR products were sequenced to confirm their authenticity as 18S rRNA gene of microalgae. The result of PCR blasted with other sequenced microalgae in NCBI showed 100% homology to the 18S small subunit rRNA of

Potential of Biotechnology

What can be produced by microbes?

4) Proteins – enzyms (recombinant)
   - -for bioconversion
   - -for detergents in washing
   - -for food industry
   - -for therapies
   - -for organic catalysis (in hetero phase system)
   - -for agriculture: endotoxin of *B. thueringhinesis*

Extra<>Intracellulare