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UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

Biotechnology in Food Industry

Lecture I

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Biotechnology in Food Industry

Introduction to the topic – common principles of microbial technologies I

Content

- Feedstock and its adjustment

- common and future feedstock

- Sterilization, cleaning

Cultivation of microorganisms composition of cultivation medium

- Carbon – starch, saccharose
- Nitrogen – ammonium ionts
- Phosphorus - salts
- Macroelements– K, S, Ca, Mg, Na
- Microelements (trace elements) – Fe, Zn, Mn, Cu, Co, Se
- Complex sources – yeast autolysate, whey..

Feedstock of first generation

a) saccharose containing

molasses (waste stream from sugar industry), sugar beet, sugar cane

b) **starch containing**

corn, maize, potatoes, cassava

c) **Other** – jerusalem artichokes (inulin)

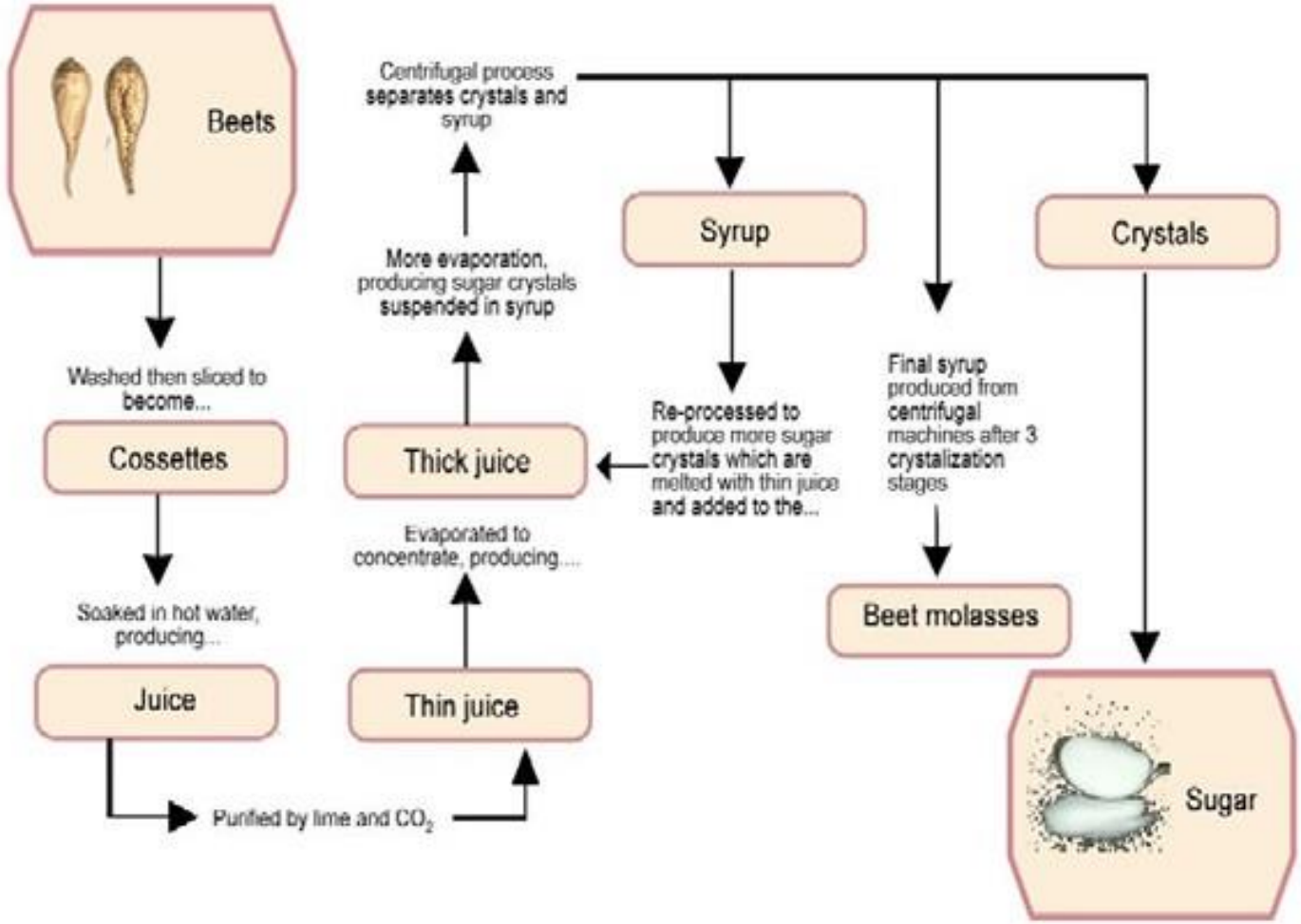
Mollasses (sugar beet juice)

- Sucrose content around 50%, utilized. N 0.18-0.65, inorganic salts, organic N-compounds, colorants, colloids
- Buffering capability - alkaline pH
- Microbial pollution
- Variable quality - fermentation test

Mollasses cultivation medium

- Clarification - acidic or alkaline
- Centrifuging - sludge separators
- Pasteurization, sterilization
- Nutrient supplementation - NH_4OH, $(NH_4)_2SO_4$, K_2HPO_4, $MgSO_4$, biotin ...
- Acidification
- Adjustment of concentration

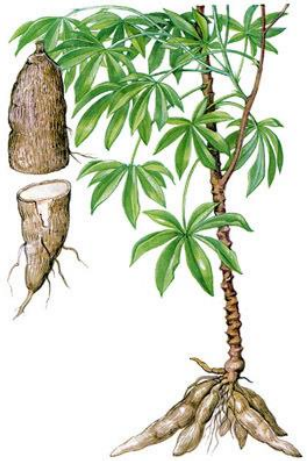
Feedstock pre-treatment



Corn, maize – milling, two step enzyme hydrolysis of starch (liquefaction- thermostable α-amylase + saccharification - glucoamylase) (in past acid hydrolysis)

triticale

Cassava – peeling, cutting, two step enzyme hydrolysis of starch (liquefaction + saccharification)

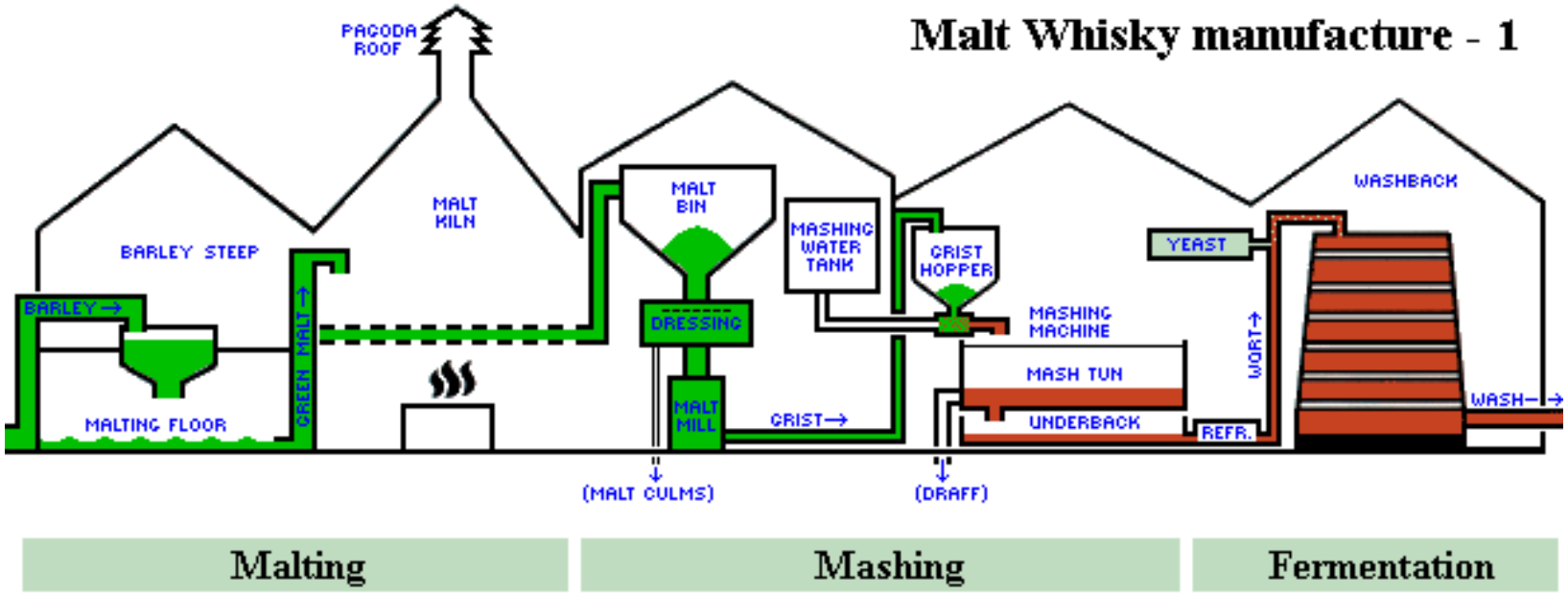


Manihot esculenta Cassava, manioc, tapioca

Jerusalem artichokes – washing, cutting, water extraction of inulin, inulinase treatment or acid hydrolysis

Helianthus tuberosus, topinambur

Malt whisky manufacturing



http://www.dcs.ed.ac.uk/home/jhb/whisky/glossary.html

Other sources of carbon

- Whey
- Sulfite extracts
- Cellulose
- Lignocellulose waste hydrolysate
- Waste paper and bark
- Distilleries slops
- Wastes from fruit processing

Feedstock of second generation (non-food biomass) and its pre-treatment

Agricultural waste – wheat and rice straw, corn stover, bagasse…

Municipal waste, paper

Fast growing trees (poplar, willow), *Miscanthus*, sawdust, twigs…

Feedstock availability and price

Wheat straw

–multiple uses – animal bedding, fertilizer, construction material, production of biogas, pellets for burning, shoes, packiging material

- Collection area (transport cost)

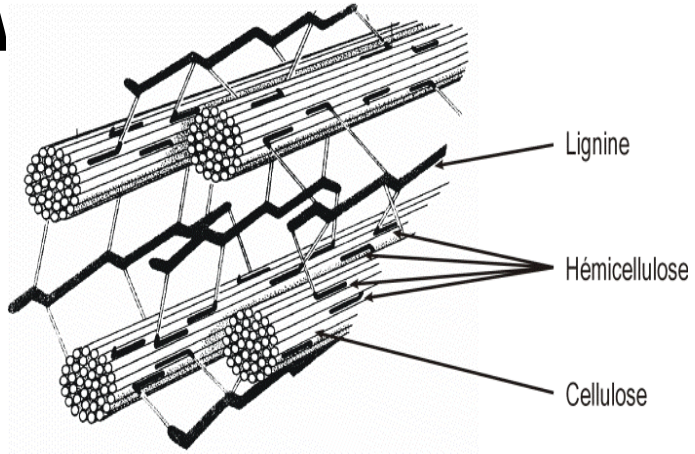
Wood

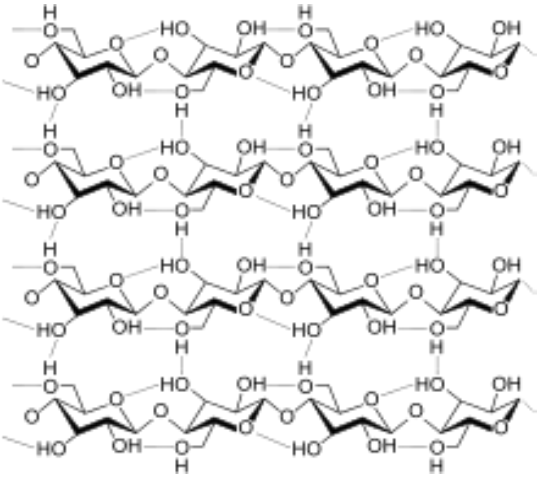
- Construction material, wood chips and pelets…
- High price

MSW (municipal waste)

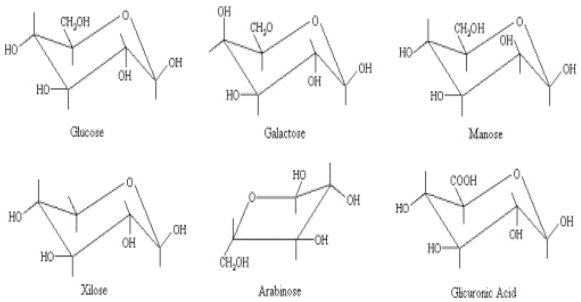
- Necessary sorting
- Can have **negative cost** (tax on landfills)

Wheat straw

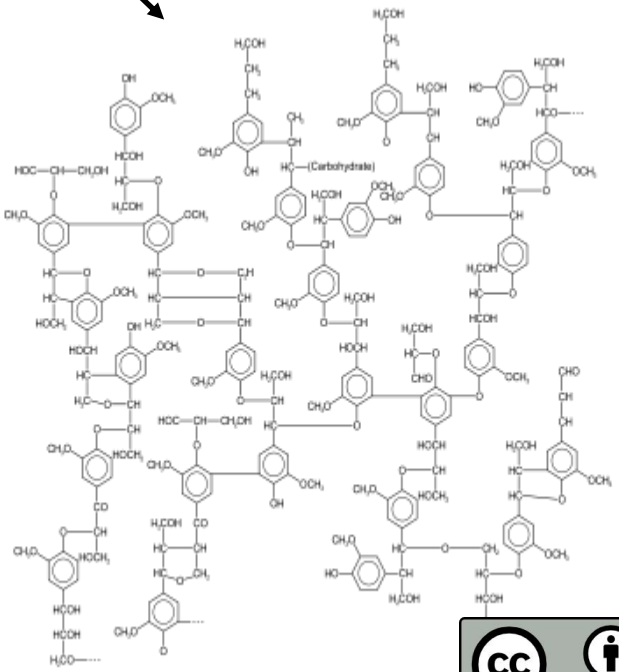




cellulose



hemicellulose sugars



Different lignocellulose materials

Wheat straw

36 % cellulose
16 % hemicellulose
27% lignin
6% ash

Miscanthus

39 % cellulose
15 % hemicellulose
26 % lignin
3 % ash

Poplar wood

31 % cellulose
15 % hemicellulose
35 % lignin
2 % ash

Dry poppyheads

15% cellulose
12% hemicellulose
30% extractives
20% lignin
10% ash

Usual current pre-treatment approach

1. Mechanical – cutting, milling

2. Physico-chemical – acid/alkaline hydrolysis, ammonium expansion (AFEX), steam explosion…

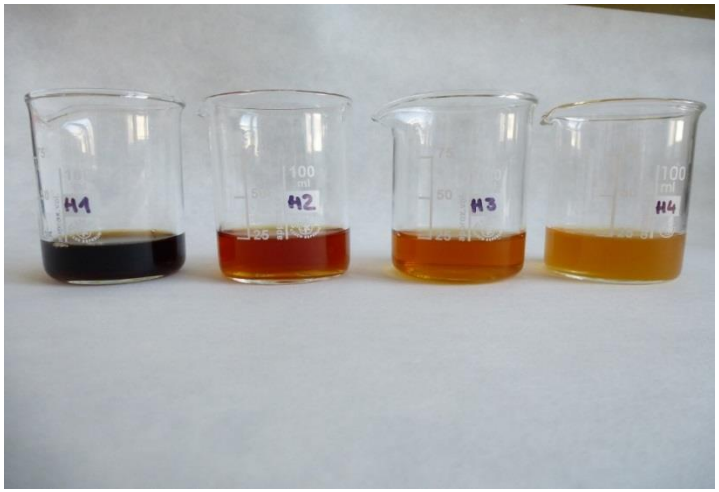
3. Enzyme decomposition - endocellulase, Exocellulase (cellohydrobiolase), cellobiase (beta-glukanase) Novozymes, Genencor – enzymes obtained from fungi especially *Trichoderma reesei*

Types of physico-chemical pre-treatment

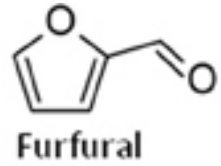
1. **Dilute acid** – increased accesibility of cellulose X low lignin removal, inhibitors
2. **Dilute alkali** – decreased crystalinity of cellulose, lignin removal, no inhibitors X solubilization of hemi cellulose
3. **Steam explosion** – cellulose digestibility, lignin redistribution X inhibitors, incomplete decomposition, high energy
4. **Organosolv** – biomass fractionalization, less energy X explosion hazard, environemtal concern, loss of hemi cellulose
5. **AFEX (ammonia fiber explosion)** – lignin removal, decreased crystalinity of cellulose, low inhibitors X environmental concern, price

Paulova et al. (2013) Production of 2nd Generation of Liquid Biofuels http://dx.doi.org/10.5772/53492

Process selection

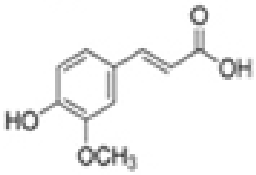


Different hydrolysates

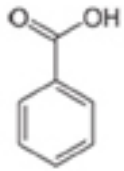


Furfural

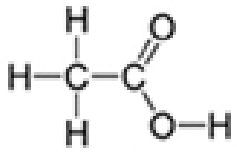
HMF



Ferulic acid



Benzoic acid



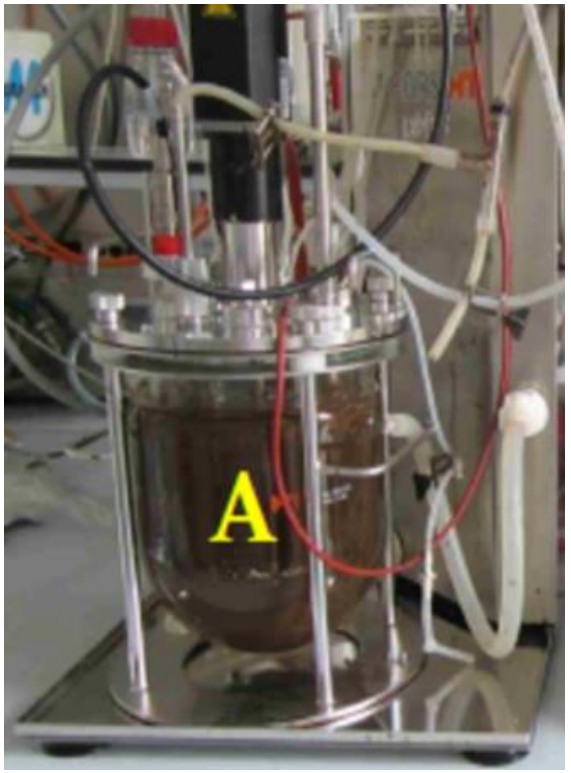
Acetic acid

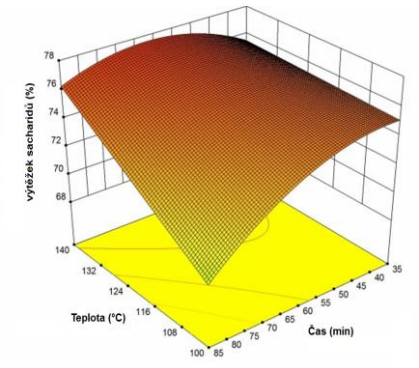
Selection criteria:

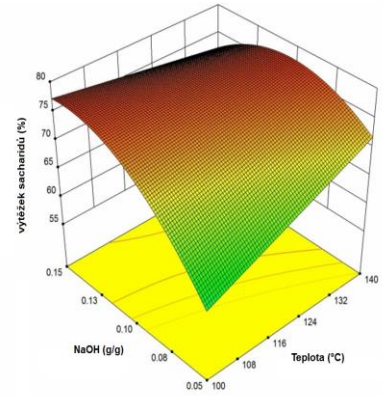
- Feedstock
- Minimization of inhibitors formation
- Economic demands (chemicals, heat, equipment..)
- Environmental concerns (waste..)

Paulova et al., Biotechnol. Adv. 2015, doi:10.1016/biotechadv.2014.12.002

RSM optimization of alkali pre-treatment of wheat straw







Optimal conditions : temperature 80 °C, 40 min, dosing 0.18 kg NaOH + 0.06 kg $Ca(OH)_2$ per 1 kg of straw

Jaisamut et al., PSE 59 (2013)

Main bottlenecks of lignocellulose utilization

- High hydromodule – low concentration of sugars (~4%)
- Mixed substrate - hexoses and pentoses
- Inhibitors formation (mainly 2-furaldehyde, hydroxymethyl-2-furaldehyde) – problems in fermentation and enzyme decomposition
- Demanding pre-treatment
- High cost and high dosing of cellulolytic enzymes
- Low concentration of product

Combined approaches toward substrate pre-treatment and fermentation

SHF (separate hydrolysis and fermentation) – optimal conditions for both hydrolysis and fermentation X time consuming

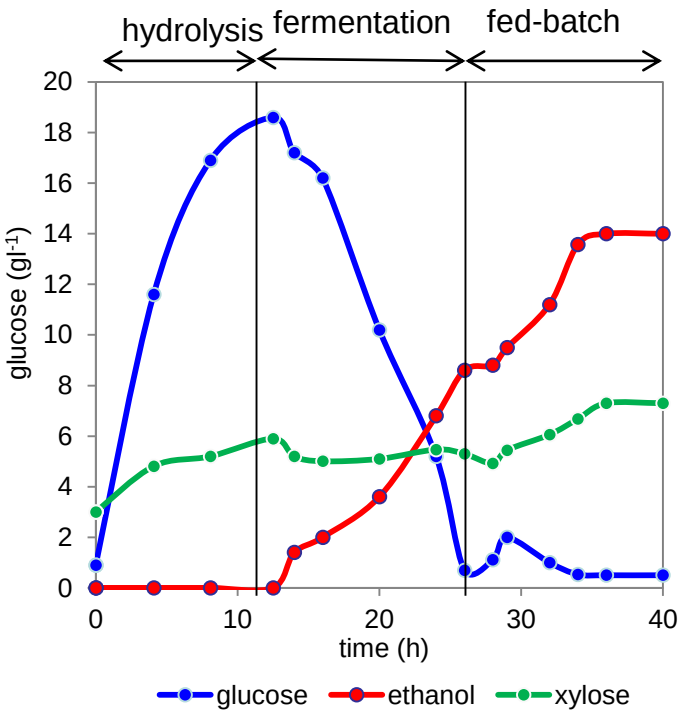
SSF (simultaneous saccharification and fermentation) – simultaneous application of enzymes and microorganisms X compromised conditions for both processes

CBP (consolidated bio-processing) - use of microorganism or microbial consortium able to decompose cellulose and produce required metabolites

Fed-batch delayed SSF (dSSF)

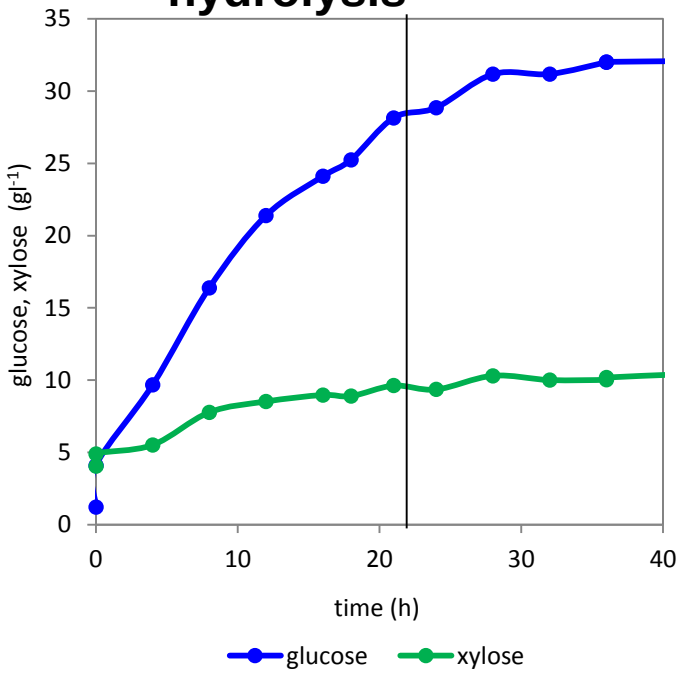
FED-BATCH PROCESS – increase of sugar concentration by feeding new (concentrated) substrate

Bioreactor A -fermentation



feeding

Bioreactor B - hydrolysis



yield from cellulose 0.37 g/g, ethanol concentration 18 % v/v

Paulova et al., Fuel 122, (2014)

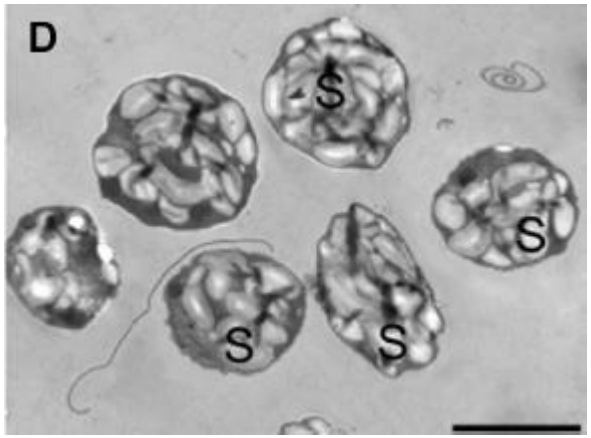
Comparison of efficiency of selected CBP processes

Category I – cellulase producers, category II – ethanol producers

microorganism	feedstock	Ethanol (g/l)	Yield (%)	Productivity (g/l/h)
Category I				
Clostridium thermocellum	cellulose (unspecified)	23.0	40	0.02
Fusarium oxysporum	Cellulose (unspecified)	14.5	53	0.1
Category II				
Engineered *S.cerevisiae*	Acid pre-treated straw	26	63	0.27
consortium				
C.thermocellum Thermoanaerobacterium saccharolyticum	**Avicel**	38	**90**	**0.3**
S.cerevisiae	**Corn, sugar beet**	**100-140**	**95-98**	**2.8-4.0**

Feedstock of third generation - algae

Fresh water micro algae – *Chlorella* (starch accumulation in cells – up to 60% of dried weight)

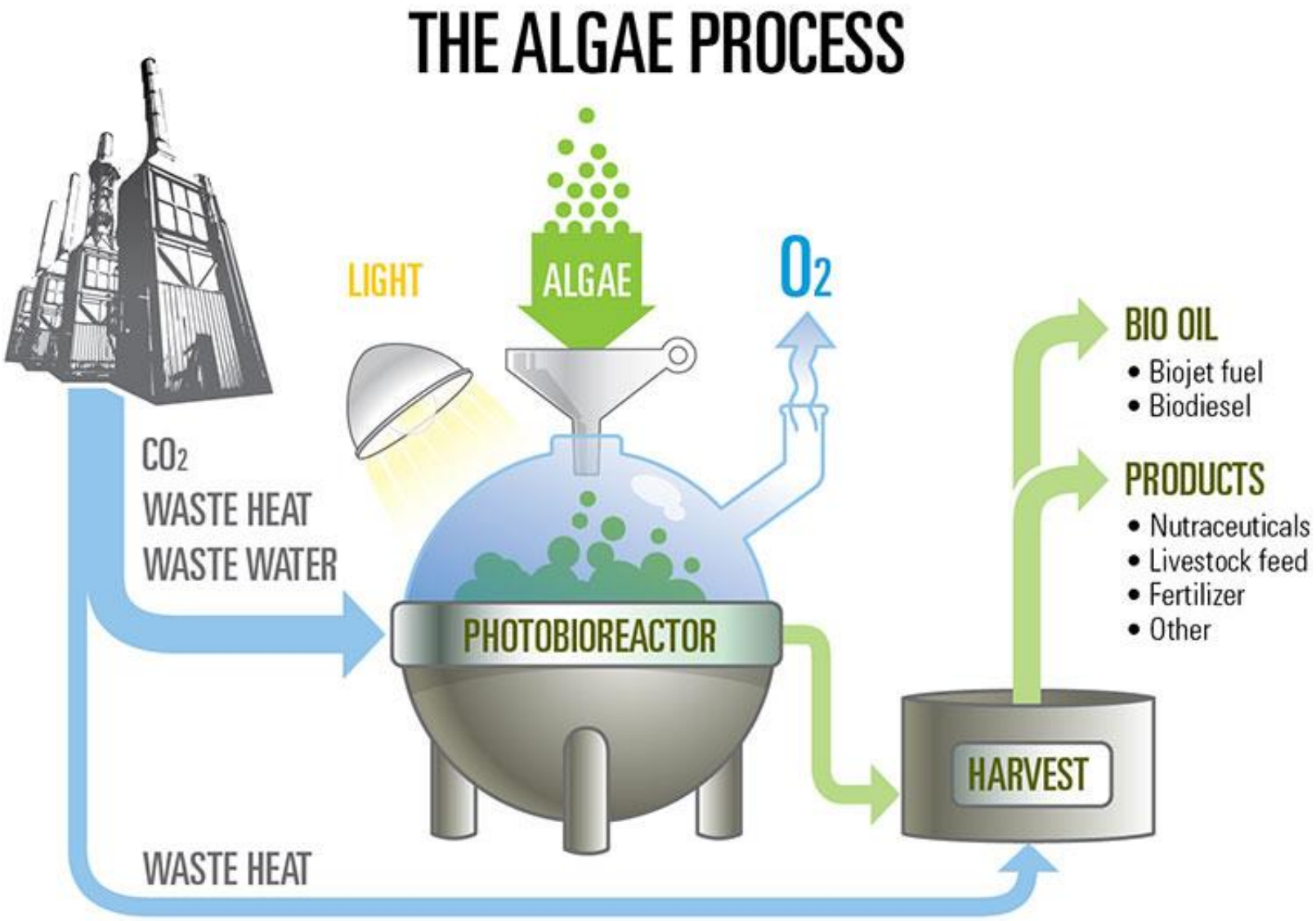


Brányiková et al., 2009

Sea weeds – macro algae *Ulva, Gracillaria*… (starch accumulation in cells)

Pretreatment

Harvesting (sedimentation, centrifugation..), drying, desintegration of cells, enzyme hydrolysis (possible use of both starch and cellulose wall)



https://www.cosia.ca/the-algae-project

Outdoor algae cultivation in convenient conditions

Cyanotech's facility on the Big Island of Hawaii

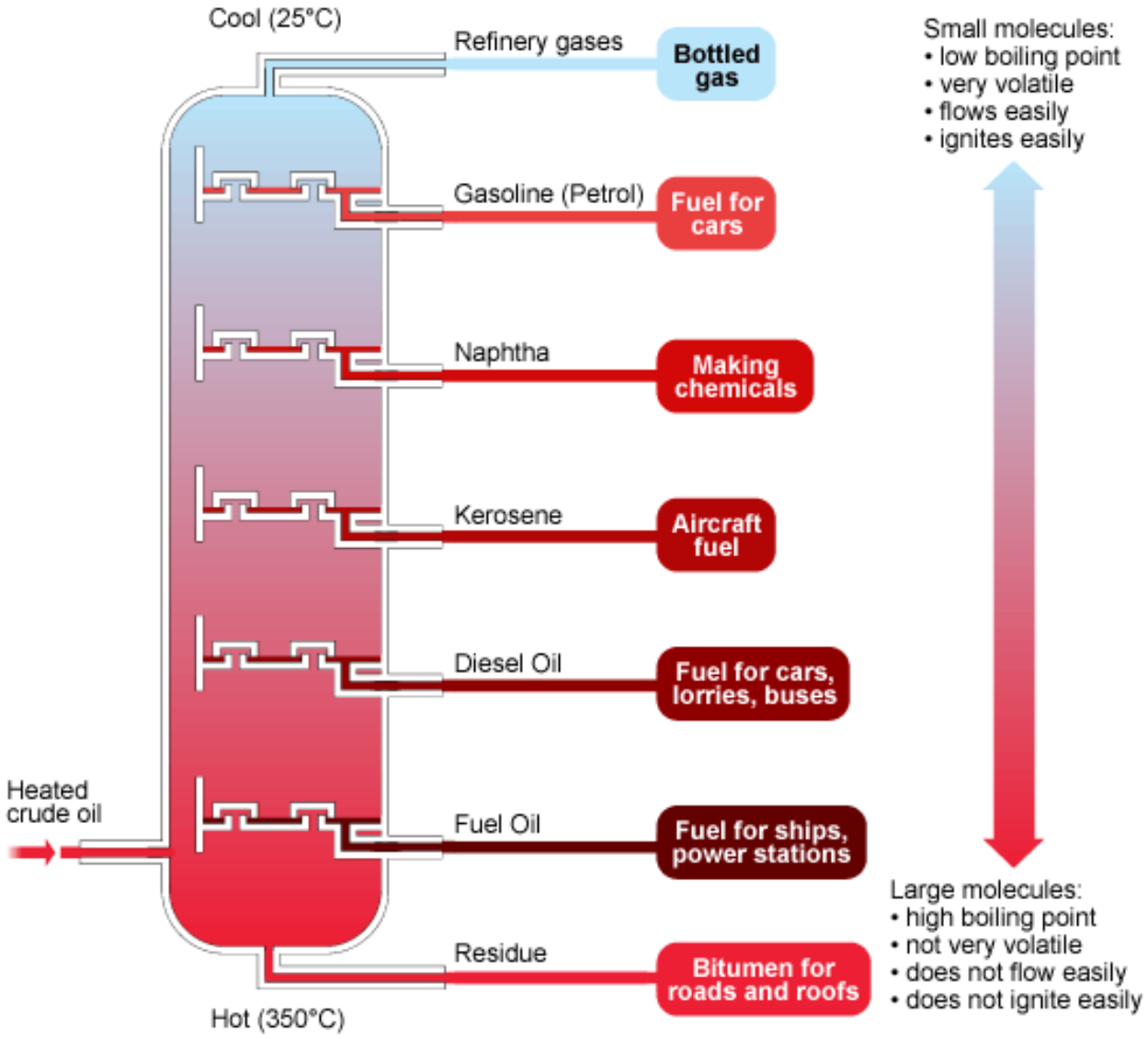
Biorefinery

A biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, power, and chemicals from biomass. The biorefinery concept is analogous to today's petroleum refineries, which produce multiple fuels and products from petroleum.

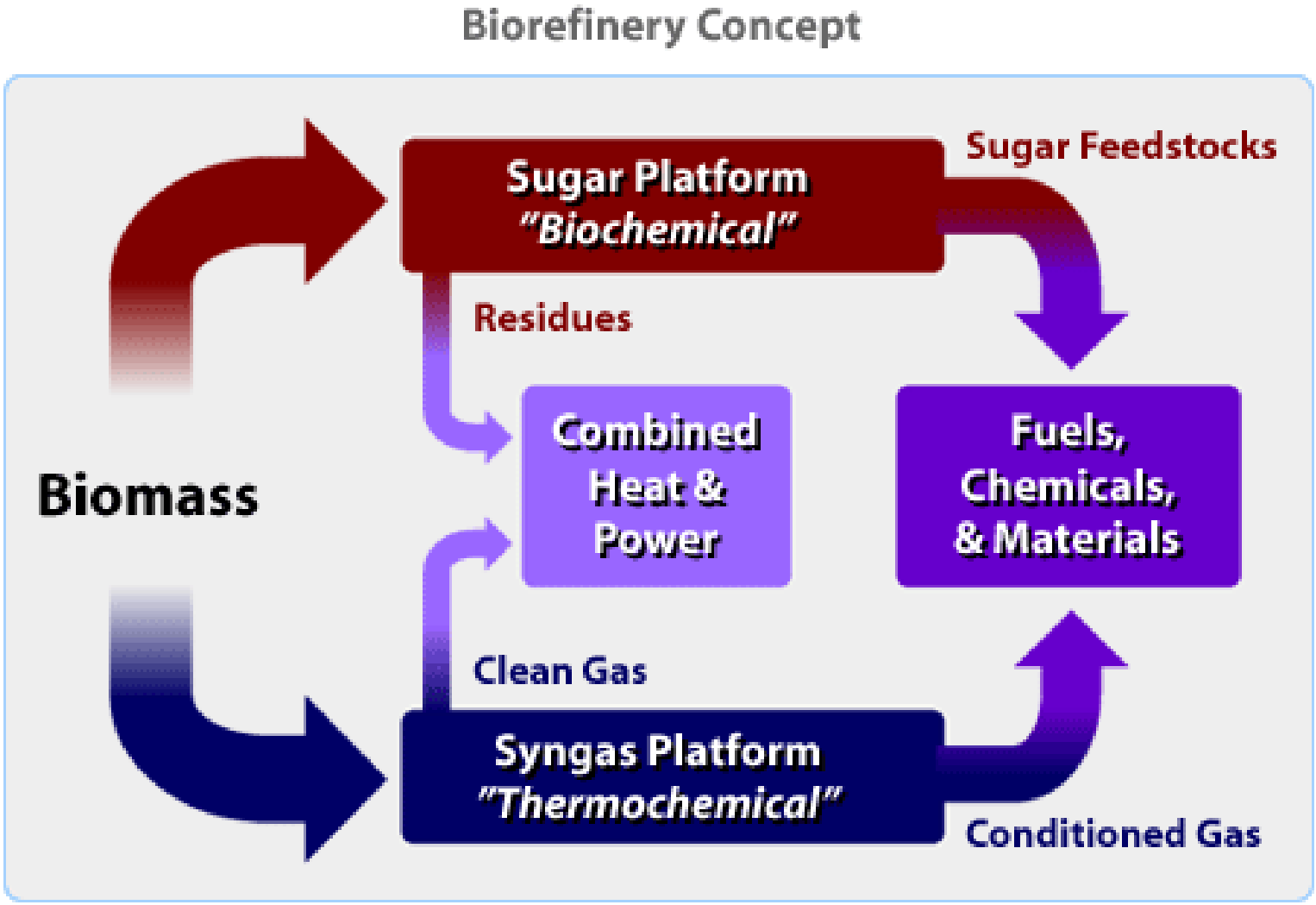
http://www.nrel.gov/biomass/biorefinery.html

NREL – National Renewable Energy Laboratory (USA)

Petroleum refinery

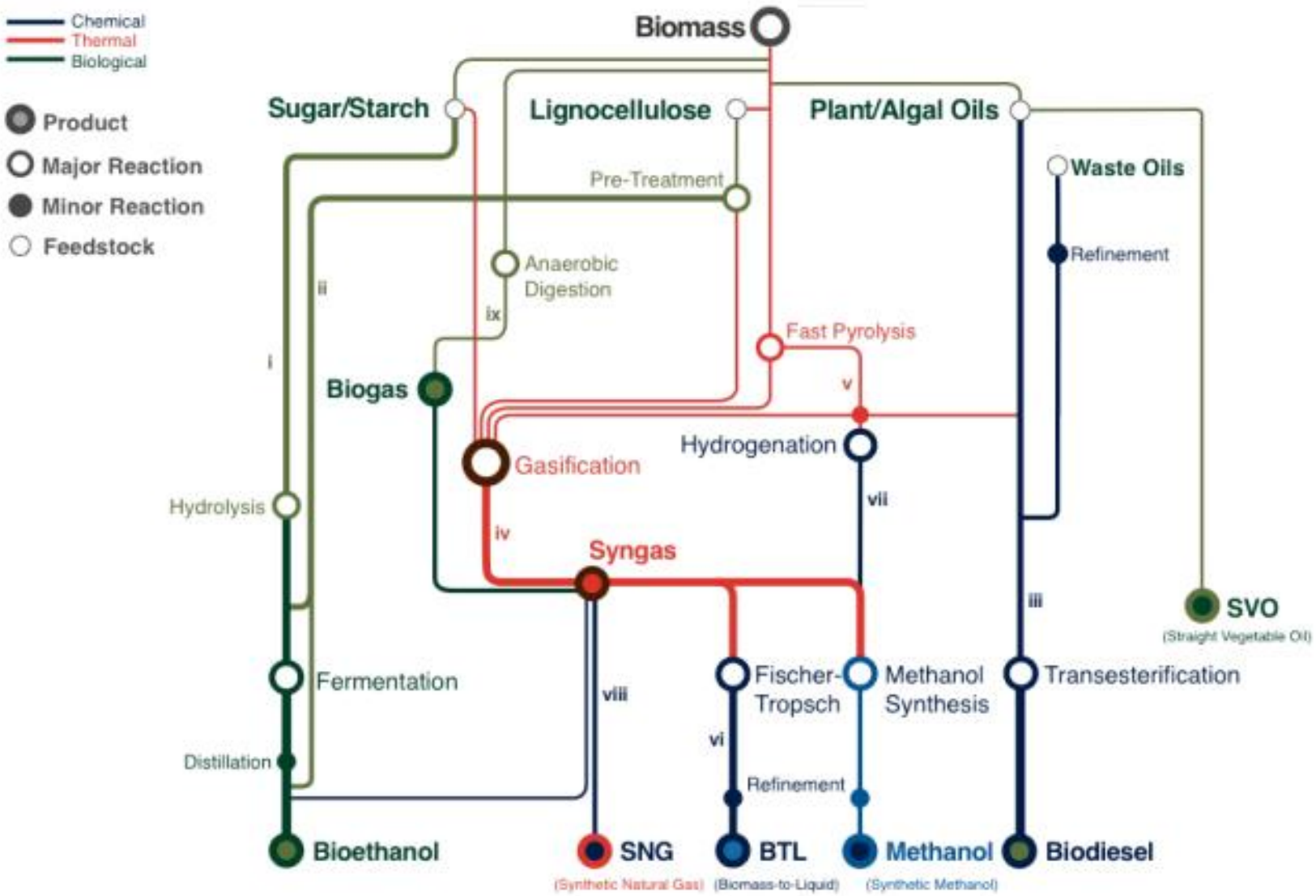


http://www.energybc.ca/profiles/oil.html



http://www.nrel.gov/biomass/biorefinery.html

Biofuel flow (whole plants)



http://www3.weforum.org/docs/WEF_FutureIndustrialBiorefineries_Repo
df

Nitrogen source

1) Basic resources

ammonium salts

nitrates

urea

amino acids

2) Natural sources - autolysates, peptones, yeast extract, whey ...

Phosphorus sources

- Phosphates
- Complex resources

- Macro- and microelements
- Salts - sulphates, chlorides or complex raw materials

Defoaming agents

Oils and fats, alcohols, sorbitan derivatives, polyethers, silicones ..

Influence of the viscosity and surface tension of the culture medium
X toxicity to cells

Water

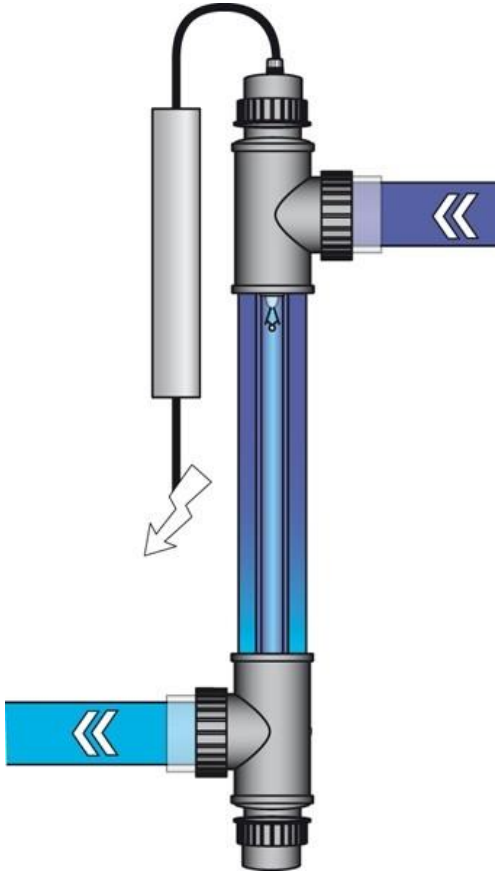
Hygienic requirements

Technology requirements - salt content

Water treatment - distillation, deionization

Sterilization

- Thermal
- Filtration (for substances sensitive to heat)
- UV irradiation (water, space)
- Chemical (Petri dishes ...)



Sterilization in situ X ex situ

- In situ –in bioreactor
- Flow sterilization (pasteurisation) of the culture medium
- CIP and SIP for a bioreactor

CIP –cleaning-in-place (also SIP – sterilization in place and WIP – washing in place)

- Closed piping system connected by tight valves with a production line
- Suitable for beverage industry, dairy, syrup production, oil ...
- Suitable where classic cleaning requires the whole line to be dismantled
- Contains dispensing pump, detergent source, ...

Cycle of CIP- example

1. 3x rinse with water (length 20s, interval 40s)
2. Add detergent to water, steam spraying to achieve the desired temperature
3. Circulation of heated washing solution (10-12min.)
4. 2x rinse with water (length 20s, interval 40s)
5. Rinse with acidic solution (pH 4.5-5, 3min.)
6. Draining of washing water, drying

Safe deposit of microbial strains

1. Freezing with cryoprotectives

   10% glycerol, 5% DMSO

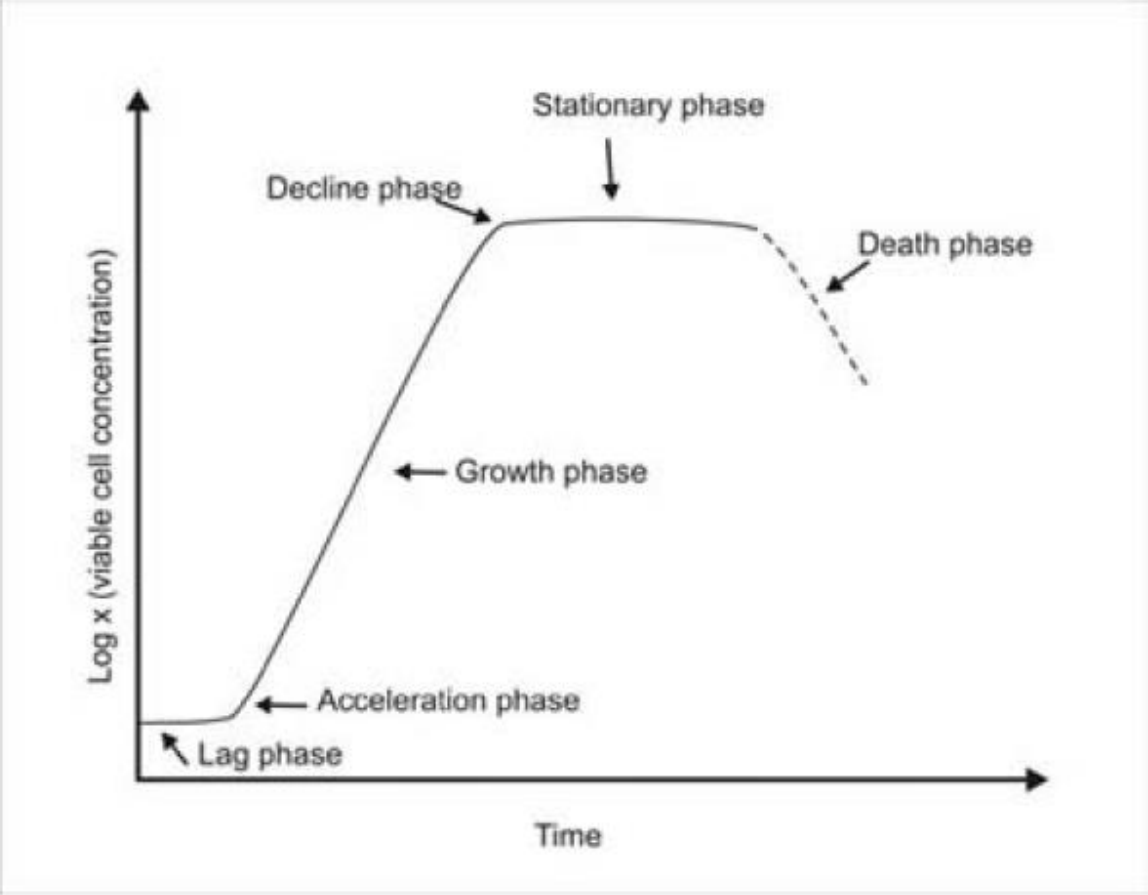
2. Lyophilization

   12% saccharose, dried milk

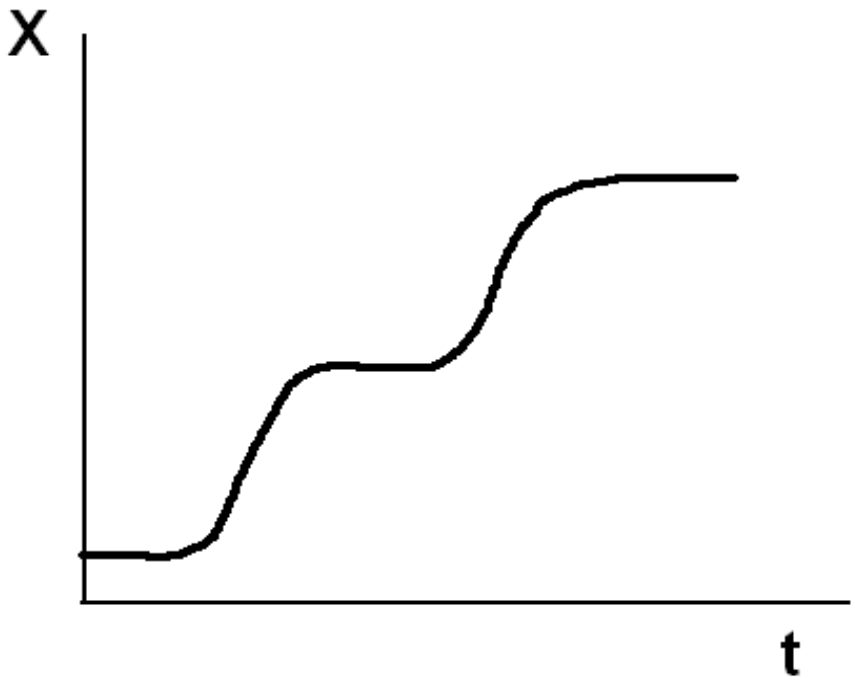
3. Other ways

BATCH CULTIVATION

- TRANSIENT SYSTEM
- CONCENTRATIONS CHANGING IN TIME PERIOD



Diauxia



SPECIFIC GROWTH RATE

- GROWTH RATE – proportional of concentration of biomass

$$\frac{dX}{dt} = \mu X \Rightarrow \mu = \frac{1}{X}\frac{dX}{dt}$$

- SPECIFIC GROWTH RATE - growth rate related to one unit of biomass
- Amount of biomass originated from 1 g of biomass per one hour
- UNIT:

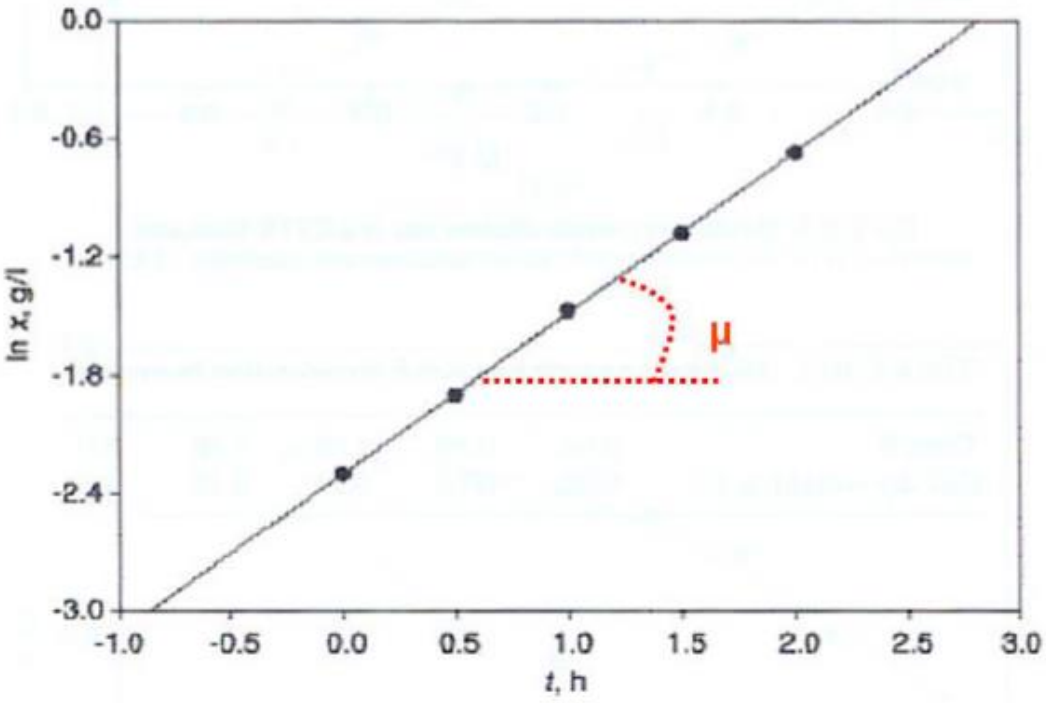
$$\left(\frac{g}{l.h}\right).\left(\frac{l}{g}\right) = (h^{-1})$$

- WHAT MEANS μ = 0.2 h^{-1} ?

CALCULATION OF μ FROM EXPERIMENTAL DATA

$$X = X_0 e^{\mu t}$$ **LINE EQUATION:** $$\ln X = \ln X_0 + \mu t$$

SEMILOGARITMIC PLOT
SLOPE OF THE LINE - μ



YIELD

CONVERSION OF SUBSTRATE TO PRODUCT, BIOMASS,….

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

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

- **UNIT: $g.g^{-1}$**

PRODUCTIVITY

- AMOUNT OF PRODUCT PER TIME PERIOD PER VOLUME

$$p_{\max} = \frac{c_{\max} - c_0}{t_0 - t_k}$$

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Biotechnology in Food Industry

Lecture II

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Biotechnology in Food Industry

Introduction to the topic – common principles of microbial technologies II

Content

- Type of cultivation process
- Bioreactor equipment
- Product separation
- Summary of the bioprocess

Laboratory/Process Microbial Cultivation

In liquid medium

- composition of the medium
- Cultivation in shaken flasks

On a solid substrate

- Substrate composition
- Cultivation on agar plates

Cultivation in bioreactor

Type of cultivation (batch,fed-batch, continuous)

Monitoring process parameters

Solid substrate cultivation

- Water activity
- Particle size
- pH of the substrate
- Aeration and mixing
- Heat removal

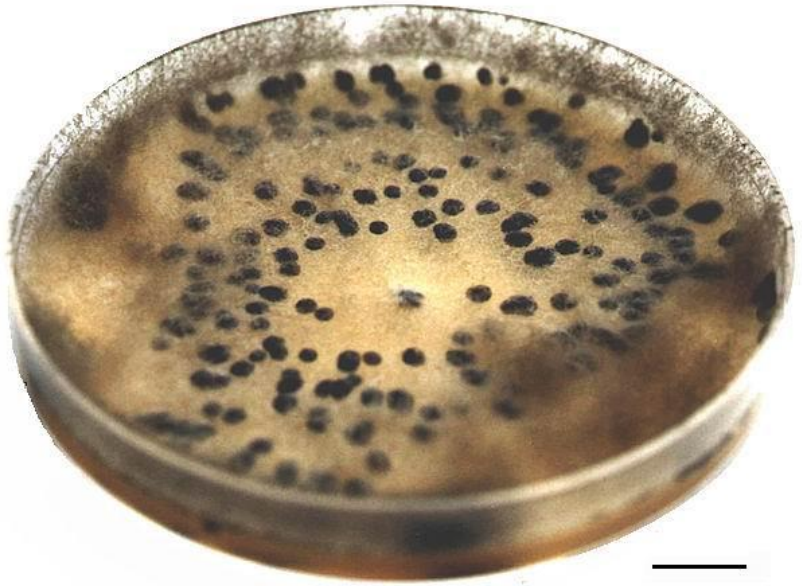


Fig.3 Rice grains after 7 days cultivation of *M.purpureus* at 30 ^{O}C

DIFFERENCES SSC X SLC?

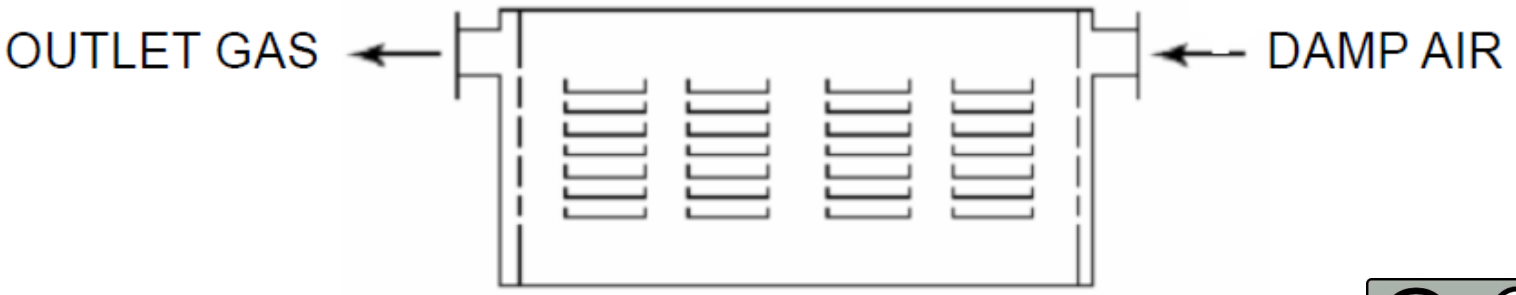
CULTIVATION ON SOLID SUBSTRATE	CULTIVATION WITH LIQUID MEDIUM
MEDIUM– SOLID	MEDIUM- LIQUID
LOW LAYER OF SUBSTRATE	HIGH DEPTH OF MEDIUM
CELLS GROW ON SUBSTRATE SURFACE	CELLS GROW IN MEDIUM
GRADIENT OF NUTRIENTS, HEAT	NUTRIENTS DISSOLVED HOMOGENOUSLY
TRANSPORT OF NUTRIENTS, HEAT AND OXYGEN LIMITED	TRANSPORT PROCESSES ARE NOT LIMITED
OXYGEN MAINLY IN GAS PHASE	OXYGEN DISSOLVED IN LIQUID
COOLING REALISED using gas phase	COOLING REALISED USING external cooling (cold water in double wall)
MONITORING AND CONTROL COMPLICATED	MONITORING AND CONTROL IS COMMONLY USED
PRODUCT CONCENTRATED	PRODUCT DISSOLVED IN LI

SOLID STATE BIOREACTORS (SSB)

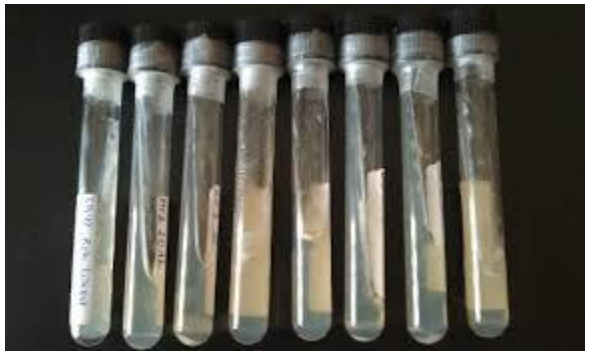
- LOW WATER ACTIVITY
- NON-HOMOGENIC
- GRADIENT OF NUTRIENTS, pH, HEAT…
- CULTIVATION OF MOLDS
- DIFFERENT TYPE AND TECHNOLOGICAL LEVEL

SSB – TRAY BIOREACTOR

- SEVERAL TRAYS IN TEMPERATED CHAMBER
- PERFORATED BOTTOM
- LOW DEPTH OF SUBSTRATE
- AERATION
- NON-ASEPTIC
- MANUAL MIXING
- TEMPEH, KOJI



Laboratory propagation



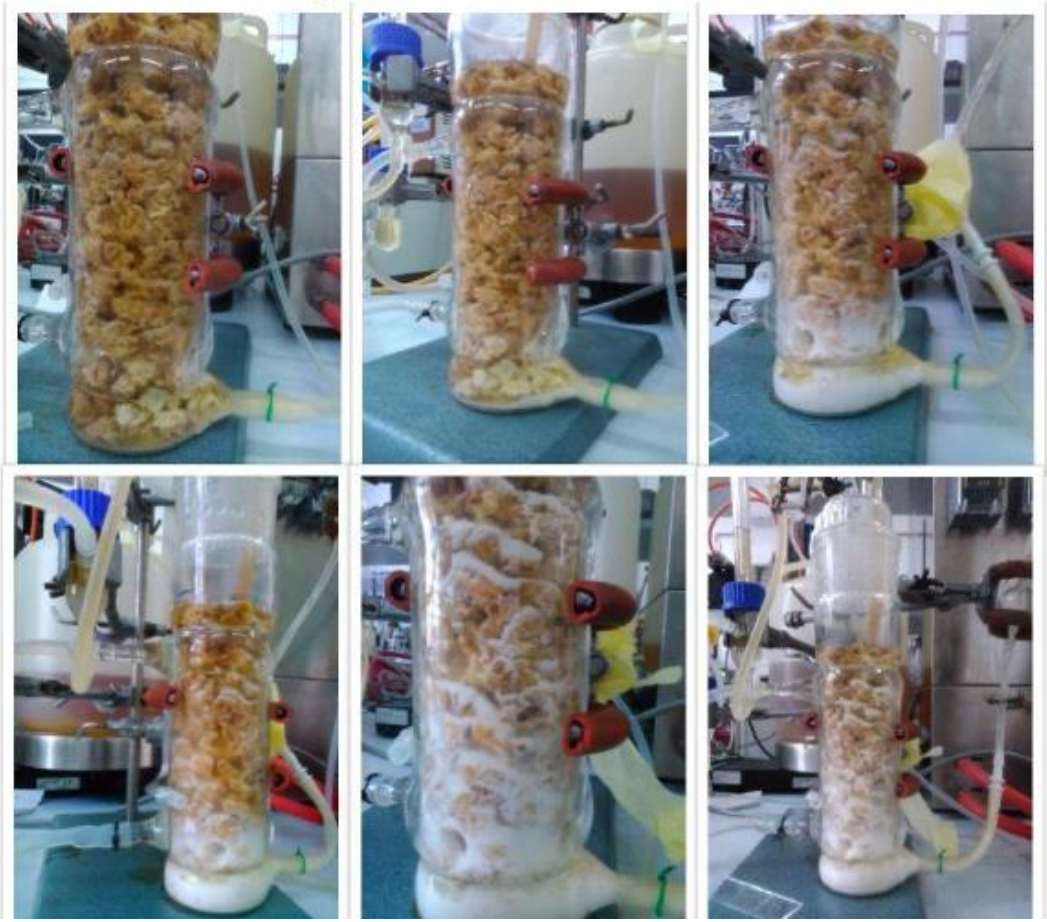
Cultivation (fermentation) process

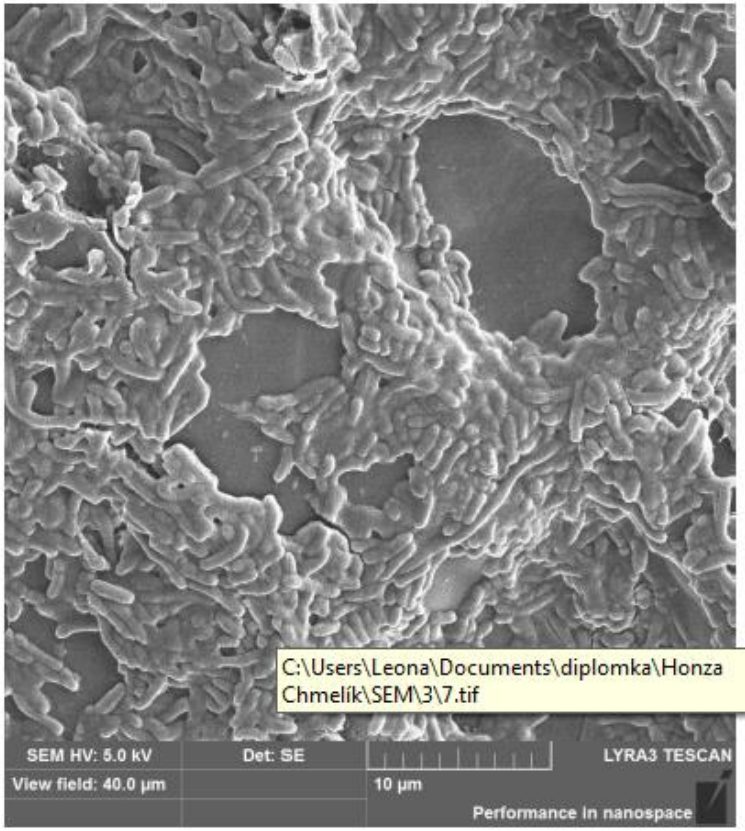
Bioreactor

- Preparation of the culture medium (broth)
- Preparation of the inoculum (microorganism)
- (sterilization)
- Fermentation (cultivation)
- Product acquisition (isolation and purification)

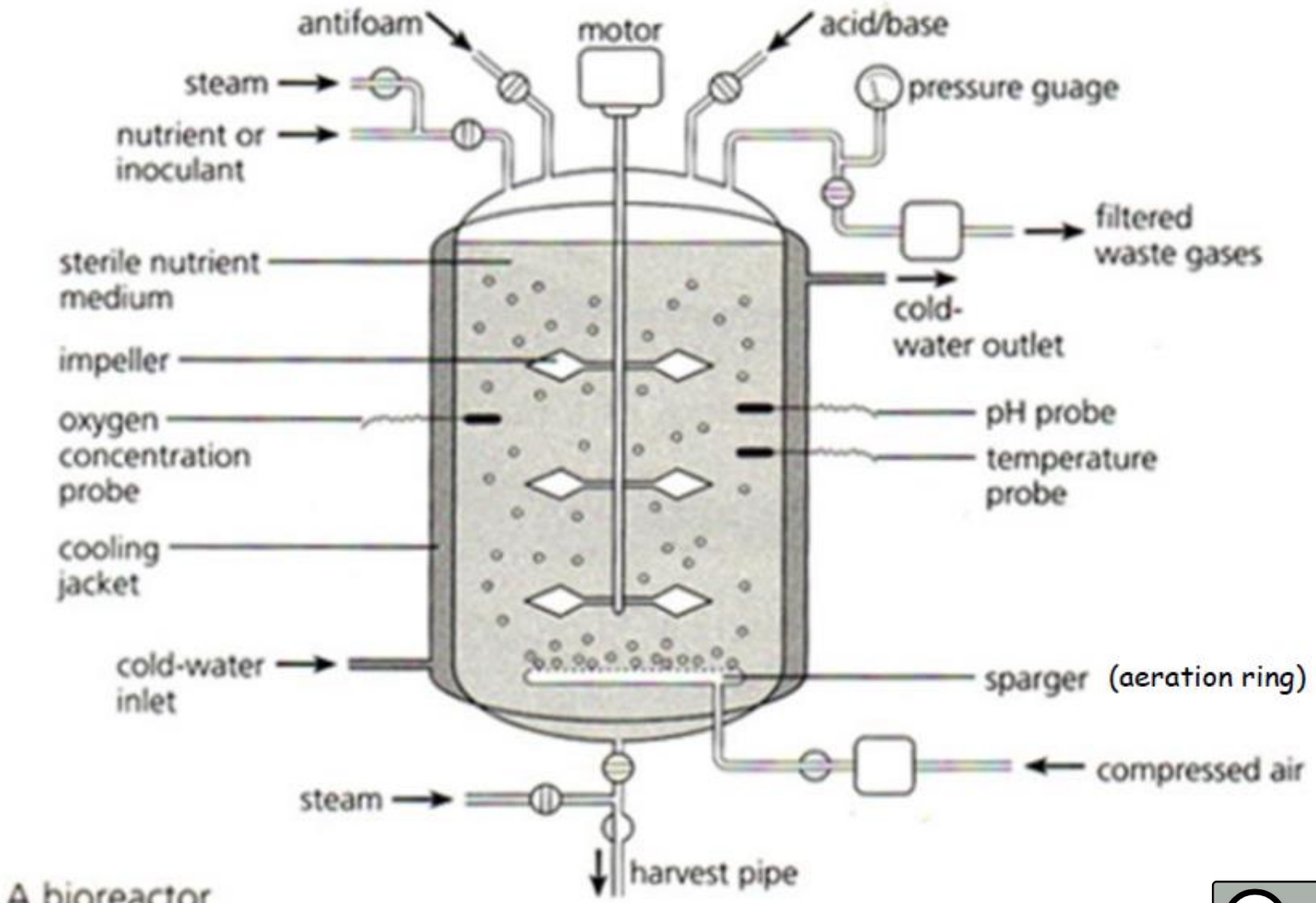
TYPES OF BIOREACTORS

FREE CELLS, IMMOBILIZED CELLS





STIRRED BIOREACTOR - PARTS



A bioreactor

Bioreactor equipment

Mixing

Mechanical

Pneumatic

Hydraulic

Mixing function - Homogenization of air content and dispersion

X shear force

Bioreactor equipment

Aeration

Aerating wreath, nozzle

Sometimes associated with mixing!

Probes

pH, temperature, O_2,

defoaming ...

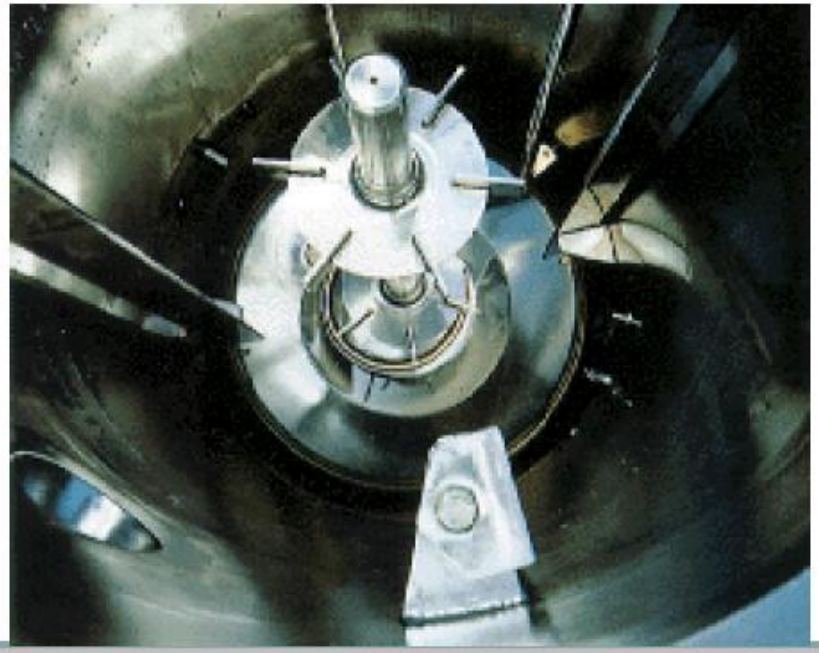
STIRRED BIOREACTOR - STIRRER

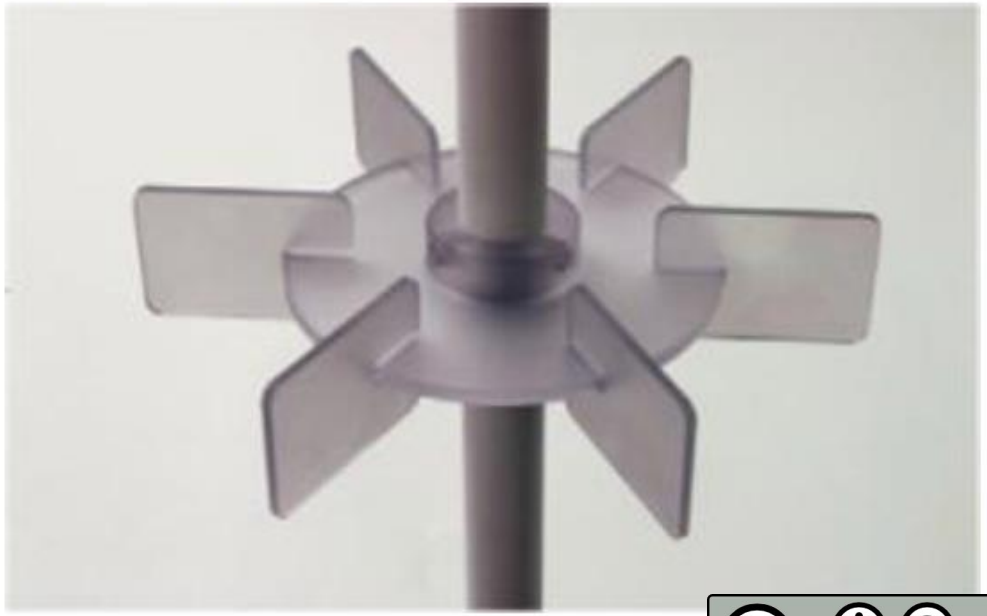
- **HOMOGENIZATION EFFECT**

  TRANSFER OF NUTRIENTS, ENERGY, HEAT, OXYGEN, METABOLITES

- **DISPERGATION EFFECT**

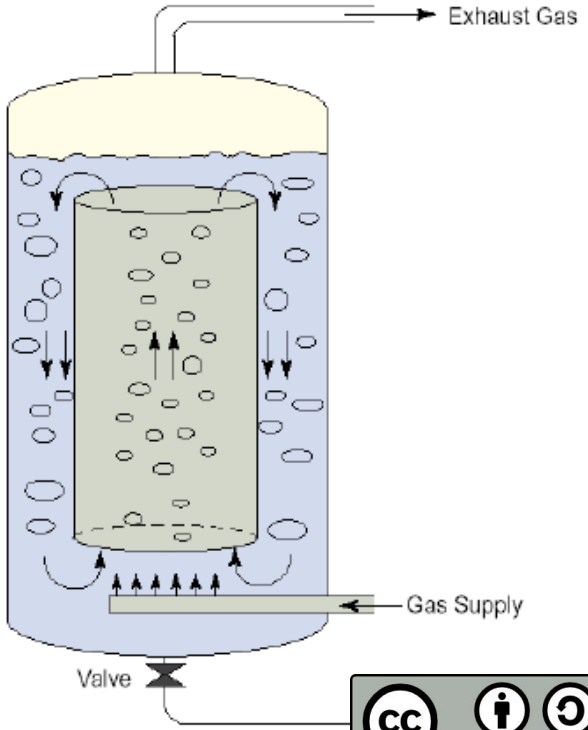
  OXYGEN TRANSFER FROM GAS TO LIQUID





PNEUMATICALLY AGITATED BIOREACTORS

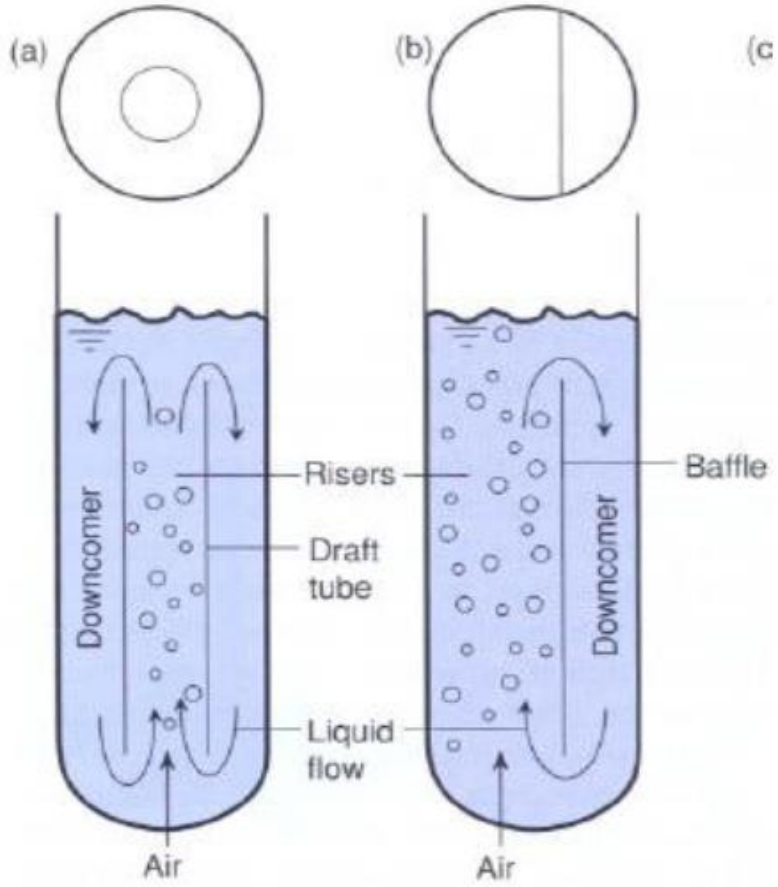
- AIR-OPERATED MIXING
- SHEAR STRESS SENSITIVE CELLS
- AEROBIC CULTIVATION
- LOW OXYGEN TRANSFER RATE



AIR-LIFT BIOREACTORS

- **HIGH h/d**
- **CIRCULATING TUBE or**

FITTING – increased dispergation, increased holding time



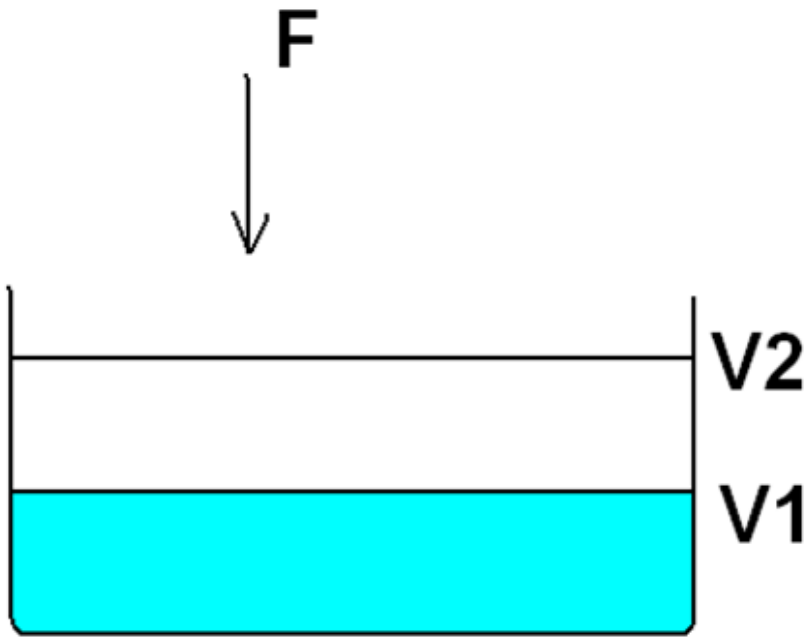
Types of microbial cultivation

1. Batch
2. Fed-batch
3. Continuous

Batch cultivation

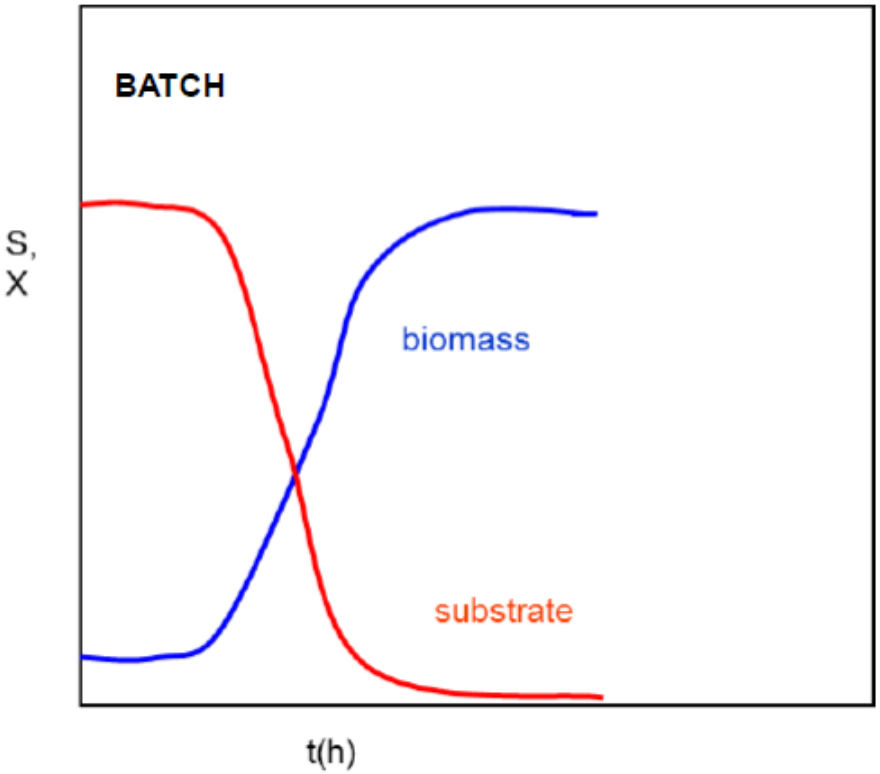
Constant volume

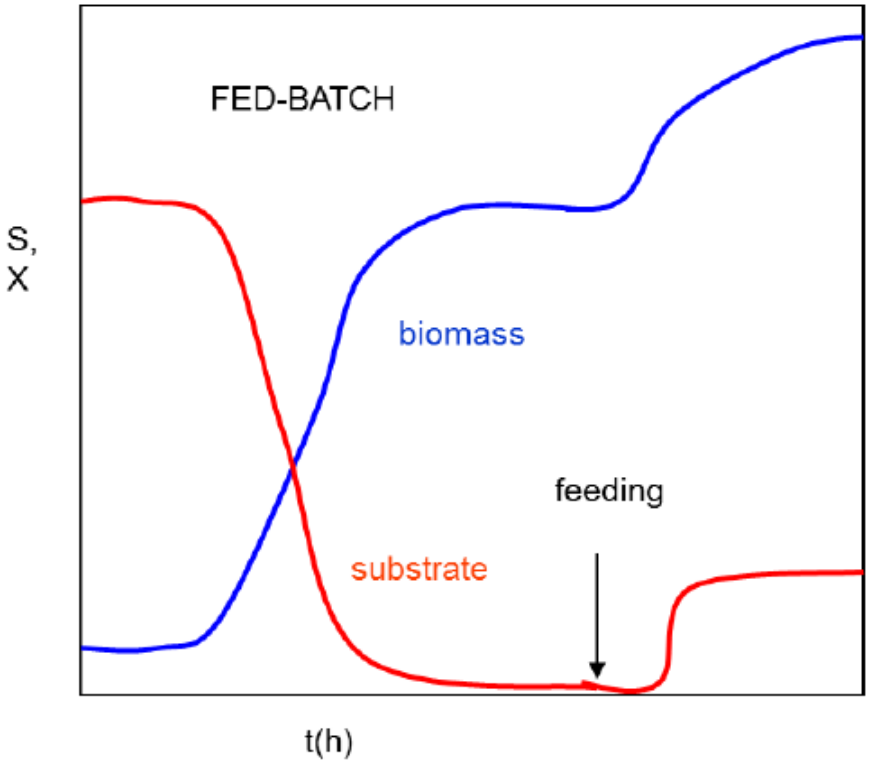
Fed-batch cultivation



Reduction of substrate inhibition

GROWTH IN FED-BATCH





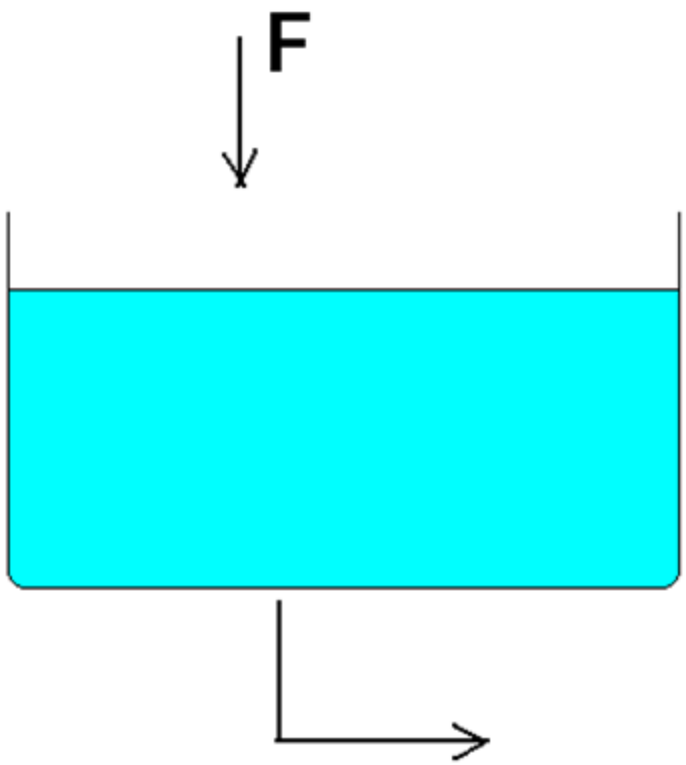
FED-BATCH IS USED FOR:

- INCREASED PRODUCTIVITY – more substrate addition -prolonged cultivation time
- SUBSTRATE INHIBITION – growth is inhibited by high concentration of substrate (ethanol, methanol..)
- CRABTREE EFFECT – high concentration of glucose – ethanol production at aerobic condition
- HIGH CELL DENSITY CULTURES – growth is supported by exponential substrate addition (SCP, recombinant proteins,….)
- VISCOSE MEDIA – microbial prolymers (dextran, xanthan) – dilution of media (mixing, aeration)

FEEDING RÉGIME

- **BASED ON EXPERIENCE**
- **ACCORDING TO MODEL (based on cultivation data) – continuous, discontinuous, constant, linear, exponential**
- **DIRECT REGULATION – based on actual concentration of substrate**
- **FEEDBACK REGULATION – based on on-line measured signal of parameter connected with cell metabolism** (pH, O_2, CO_2)

Continuous cultivation

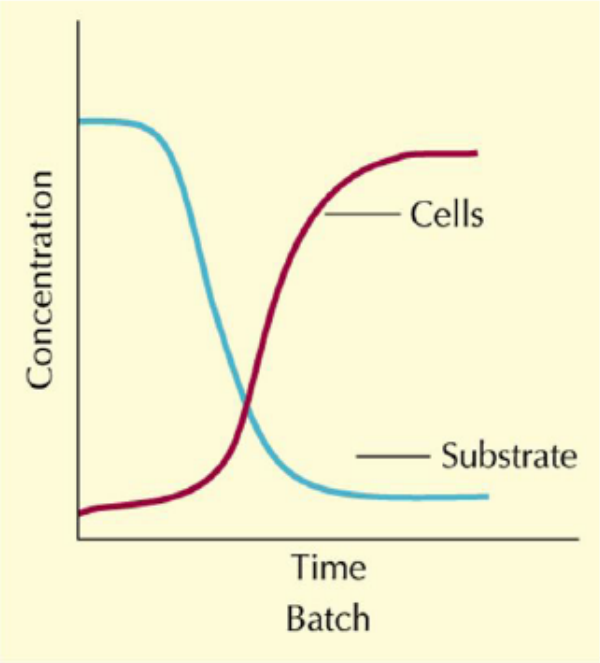


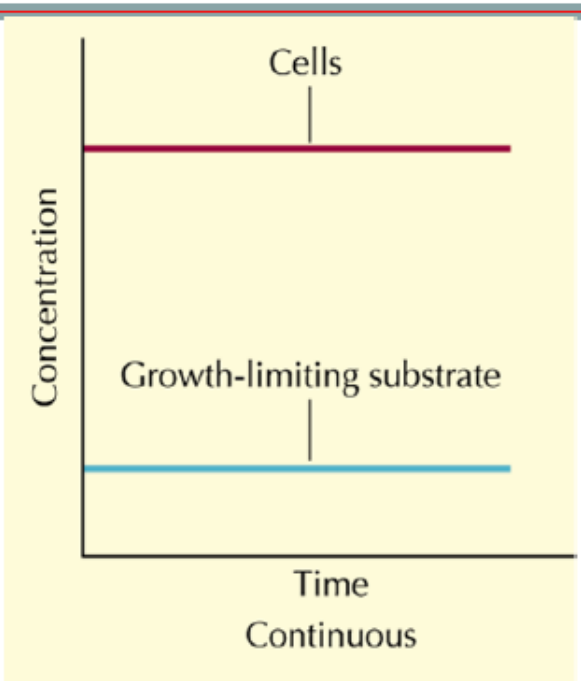
Dilution rate:
$D=F/V$

$D<\mu$ (if not gradual flooding of microorganisms from the systém)

Constant volume, reduction of product inhibition

CONTINUOUS CULTIVATION





NUTRIENTS DEPLETION – GROWTH STOPPED

CONTINUOUS NUTRIENTS ADDITION – CONSTANT GROWTH AND PRODUCTION, HOMOGENOUS CULTURE, STEADY STATE

CONTINUOUS CULTIVATION

- DILUTION RATE: $D = \frac{F}{V}$
- UNIT: h^{-1}
- STEADY STATE – concentration of biomass, metabolites and nutrients in bioreactor is constant and independent on time (dependent only on cultivation condition)
- CHEMOSTAT – constant dilution rate, grow is limited by availability of one nutrient (source of carbon)

CHEMOSTAT

- F=const, V= consts, $D = \frac{F}{V}$, D=const
- GROWTH RATE CONTROLLED BY NUTRIENT AVAILABILITY

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

- STEADY STATE:

  COCENTRATION OF NUTRIENTS = const

  SPECIFIC GROWTH RATE = const and equal to DILUTION RATE μ=D in the interval $0<D<\mu_{max}$

STEADY STATE

- F=const, V= consts, $D = \frac{F}{V}$, D=const
- GROWTH RATE CONTROLLED BY NUTRIENT AVAILABILITY

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

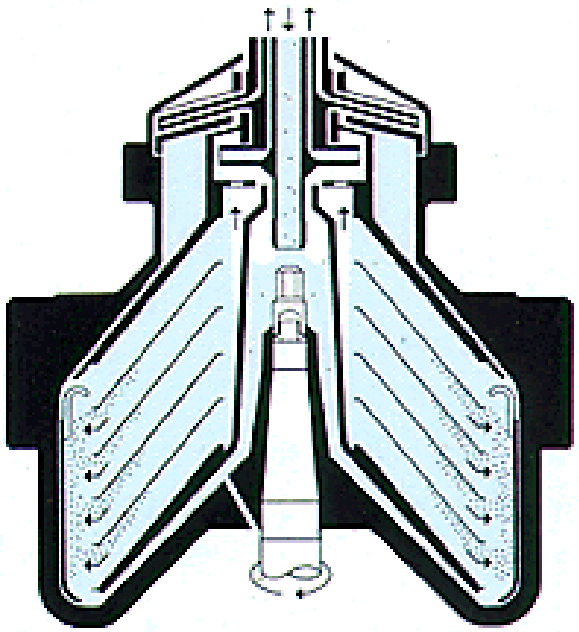
- STEADY STATE:
  1. CONCENTRATION OF NUTRIENTS = const
  2. SPECIFIC GROWTH RATE = const and equal to DILUTION RATE μ=D in the interval $0<D<\mu_{max}$
  3. concentration of biomass, product, metabolites = const

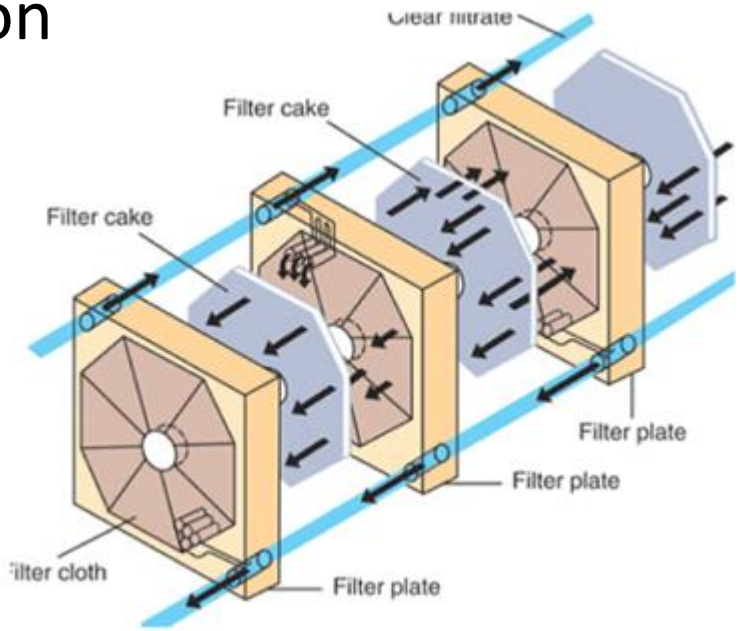
STEADY STATE

- IN A STEADY STATE ALL PARAMETERS ARE NOT DEPENDENT ON TIME BUT ONLY ON CULTIVATION CONDITION
- PHYSIOLOGICAL STUDIES
- OPTIMIZATION EXPERIMENTS – relation between concentration of nutrients, cell growth, production, yield and productivity

Separation from microorganisms from cultivation medium

- Centrifuging
- Filtration
- Sedimentation
- Flotation





Isolation of metabolites from fermentation medium

- Distillation
- Precipitation and crystalization
- Extraction by solvents
- Adsorbtion and elution
- Membrane separation

Basic process scheme

- Feedstock and its adjustment, supplementation
- Microorganism and its requirements
- Metabolism – aerobic/anaerobic, production of metabolites
- Process arrangement – batch/ fed-batch/ continuous
- What is product? Biomass or metabolite
- Product separation, purification and final adjustment

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Biotechnology in Food Industry

Lecture III

Petra Patáková
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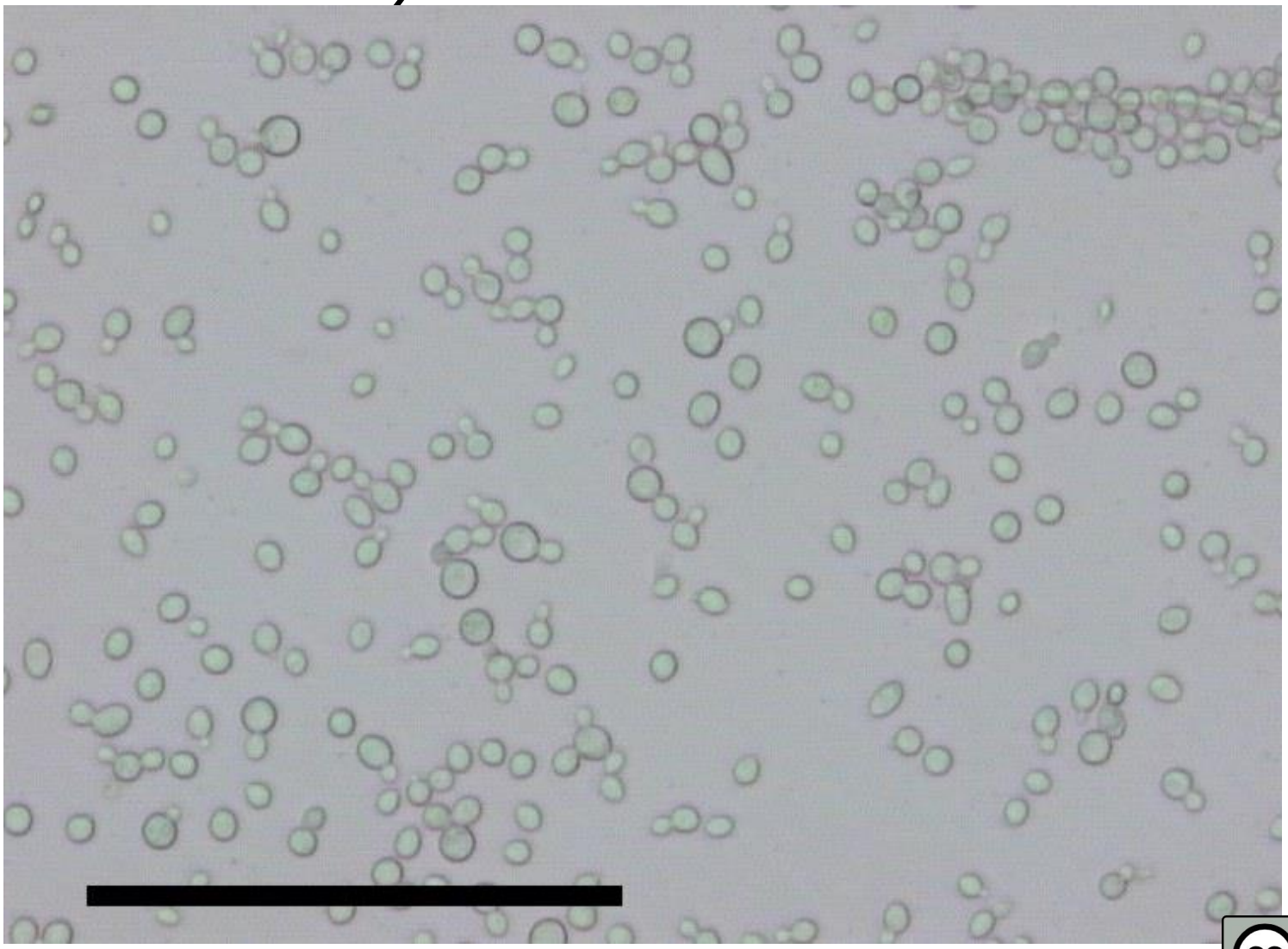
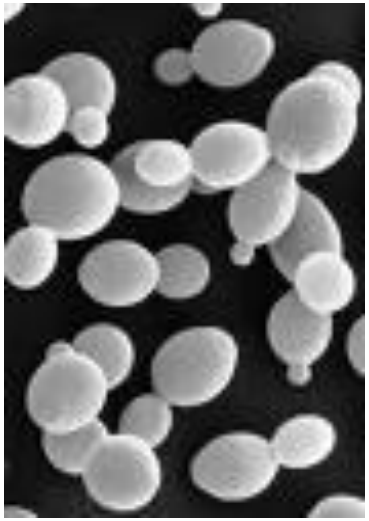
Baker`s yeast production and production of other types of biomass

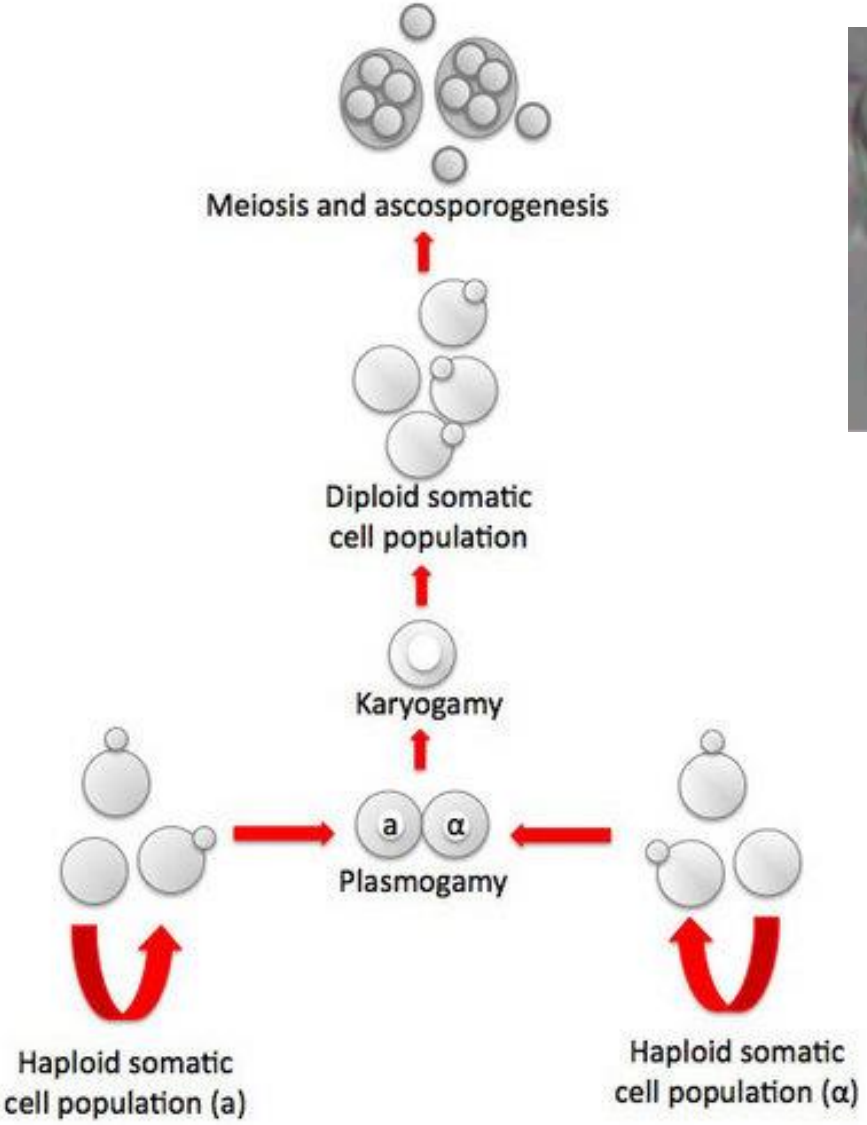
1. Microorganism
2. Metabolism
3. Molasses glow
4. Technological stages of yeast production
5. Active dried yeast
6. Evaluation of quality indicators of bakery yeast

Saccharomyces cerevisiae



Life cycle of *S.cerevisiae*





Metabolism of saccharides

Yeast *S.cerevisiae* – facultatively anaerobic

Anaerobic (fermentation)

Glycolysis leading to ethanol formation

HMP pathway

Biomass yield 10%

Gain of Energy – 2 mol ATP per 1mol glucose

Substrate phosporylation

Aerobic (respiration)

Glycolysis + TCA cycle

HMP pathway

Biomass yield 50%

Gain of Energy – 38 mol ATP per 1mol glucose

Oxidative phosporylation

- Inhibition of respiration by glucose -
- Pasteur effect
- Oxygen limitation X hyperoxia
- Effect of ethanol

- Optimal – fed-batch cultivation
- Feed - continuous or discontinuous

Technological steps in bakery yeast production

1. Laboratory propagation
2. Preparation of cultivation medium
3. Process propagation of yeast
4. Pre-fermentation
5. Production of generation yeast
6. Production of expedition yeast

Laboratory propagation

Goal- 0.5-1kg yeast

- Pure culture
- Propagation ratio 1:5
- Anaerobic cultivation
- Rich cultivation medium
- Cultivation time 24-48h
- cultivation in flasks

Preparation of molasses medium

- Clarification – acid or alkali
- Centrifugation –sludge separators
- Pasteration, sterilization
- Supplementation – NH_4OH, $(NH_4)_2SO_4$, K_2HPO_4, $MgSO_4$, biotin…
- Acidification
- Adjustment of concentration

Process propagation of yeast

Goal – increase of yeast amount

- 2-4 steps
- In bioreactors
- aeration
- Fermentation control
- Content of proteins in cells 5 fermentation ability, low shelf-life

Pre-fermentation

Goal –scale up, change of cultivation conditions

- Molasses medium
- Fed-batch cultivation
- Aeration 0.1 VVM (air **V**olume per bioreactor **V**olume per **M**inute)
- Ethanol max. 4.5%

Generation yeast I

Goal- to prepare enough active yeast for the last production step

- 2 generation
- Fed-batch cultivation
- Decrease of N and P compounds→ decrease of protein amount in cells
- Aeration up to 1 VVM
- Lower cultivation temperature

Generation yeast II

- Centrifugation
- Washing – acid bath (0.1-0.4% H_2SO_4) –yeast de-colorization, activity increase
- Cultivation time 8-15h

Expedition yeast

- Fed-batch
- Concentration of ethanol max. 0.5%
- Low cultivation temperature
- Acid bath before cultivation
- Cells – cca 42% proteins, leavening in the dough 50-90 min, shelf life up to 250h at t=30^{o}C

Yeast separation from the medium

Centrifugation – 4000-6000 rpm

Washing

↓

Yeast milk

↓

Cooling and filtration

↓

Compressed yeast – D_{27}

Adjustment and expedition of compressed yeast

Formulation and Packaging

(mixing with water and

Lecithin)

Production of dried yeast

- Increase of saccharides concentration (trehalose, glycogen) and lipids in cells
- Additivation (ascorbic acid, mono- and di-glycerides)
- Granulation
- Drying – hot air and vacuum driers
- Packaging

Evaluation of bakery yeast

Senzorical evaluation

Smell

Colour

Appearance

Consistence

Taste

deliquescence

Chemical analysis

Biochemical tests

- Leavening
- Shelf life

Microbiological test

Wild yeast 15%, dead to 0.5%, max. 5%of budding cells

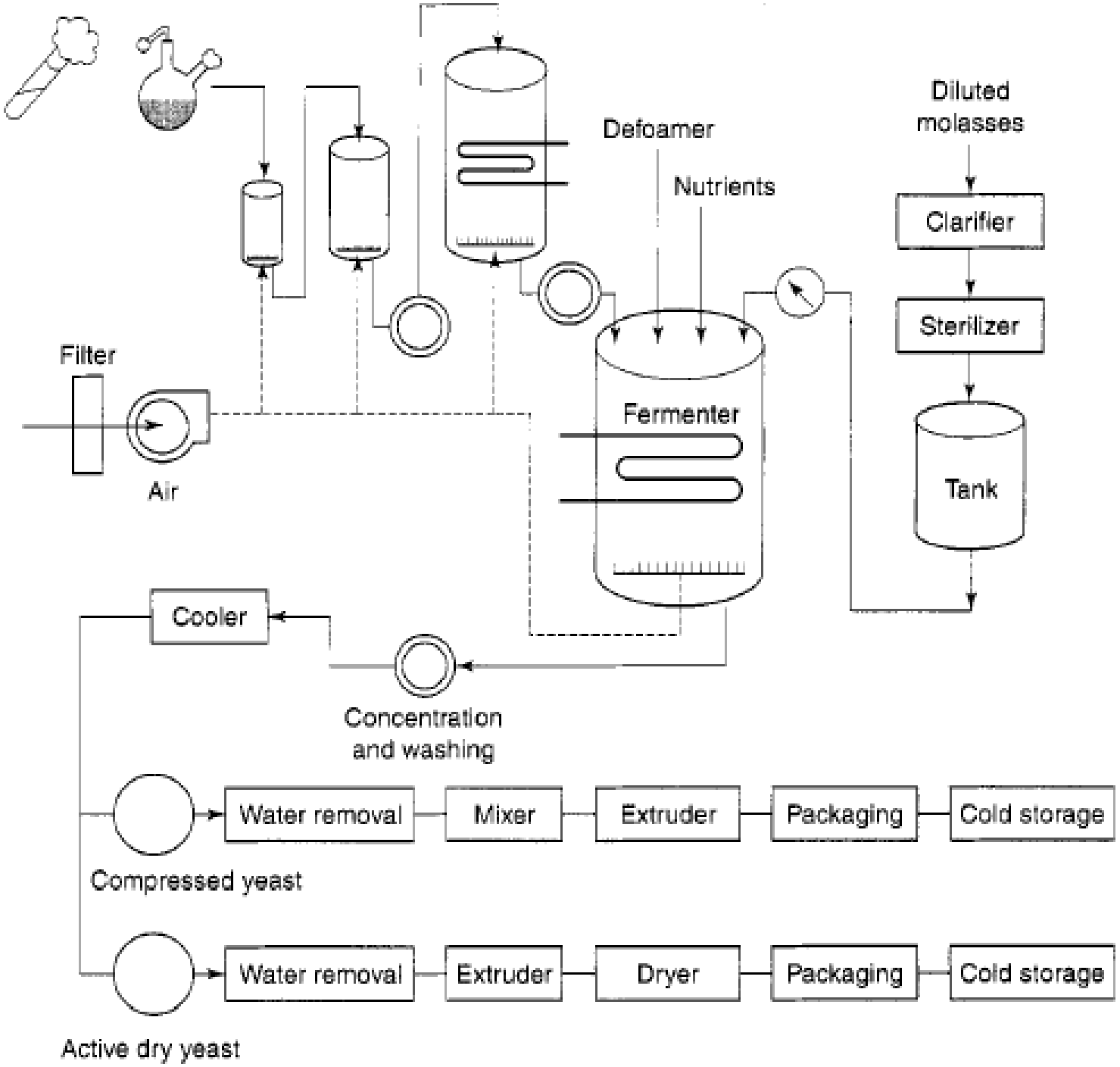
Contaminating microorganisms

Source of contamination – molasses, water, air

Aerofilic yeast – *Candida*..

Fungi – *Aspergillus, Penicillium*..

Bacteria – from molasses (*Clostridium*), water (*Pseudomonas*) and air (acetic acid bacteria)


Defoamer
Nutrients
Diluted molasses
Clarifier
Sterilizer
Tank
Filter
Air
Fermenter
Cooler
Concentration and washing
Water removal
Mixer
Extruder
Packaging
Cold storage
Compressed yeast
Water removal
Extruder
Dryer
Packaging
Cold storage
Active dry yeast

Biomass for animal feeding

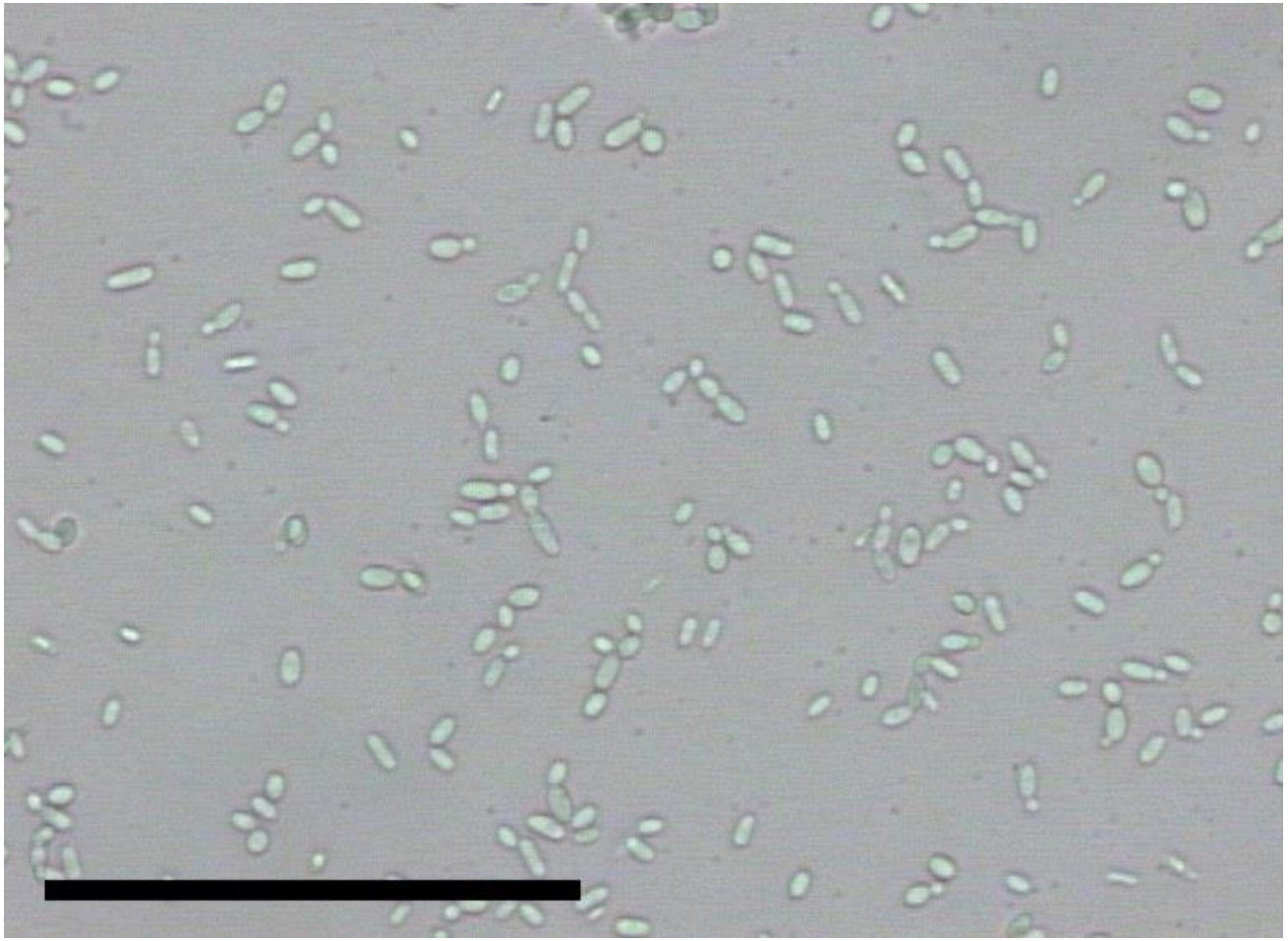
1. Propagation of microorganisms
2. Cultivation
3. Separation of cells from media
4. Thermolysis
5. Evaporation of water and drying

Survey of used microorganisms

Bacteria	Carbon source
Methylophilus	methanol
Methylococcus	methane
yeast	
Candida lipolytica	n-alcanes
Candida utilis	Sulphite extracts
Kluyveromyces fragilis	whey
Saccharomyces cerevisiae	mollasses

Fungi	
Chaetomium	Lignocellulose hydrolysates
Cephalosporium	starch
Rhizopus oryzae	starch
Algae	
Scenedesmus	CO_2, light
Spirulina	CO_2, light

Production of microbial biomass from from sulphite extracts



Candida utilis

Sulphite extracts

- composition depends on the type of wood and cooking conditions
- Max. 5% carbohydrates - glucose, mannose, xylose, galactose, arabinose
- Lactonic acids, lignosulfonic acid, methanol, formic acidCa^{2+}, Mg^{2+} salts
- **SO_2**

Treatment of sulphite extracts

1. **Decrease in SO_2** (max. conc. 400mg/l)

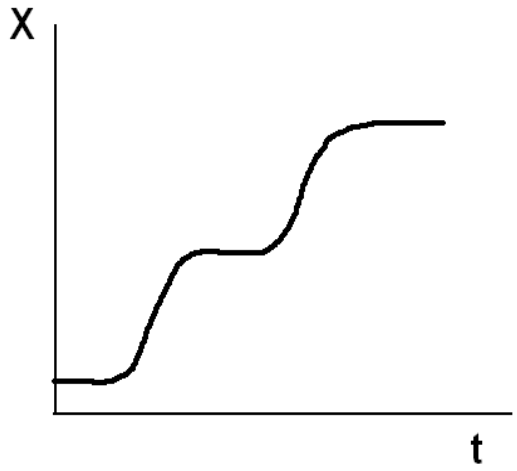
↓

Steam spraying, thickening, aeration, precipitation of sulphates

2. Neutralization - pH 5.2 (precipitation of calcium sulphate)
3. Cooling
4. Nutrient supplementation - N, P, K - salts

Propagation of yeast

- Using sulphite extract
- gradual adaptation to SO_2,
- Batch
- polyauxia



Cultivation

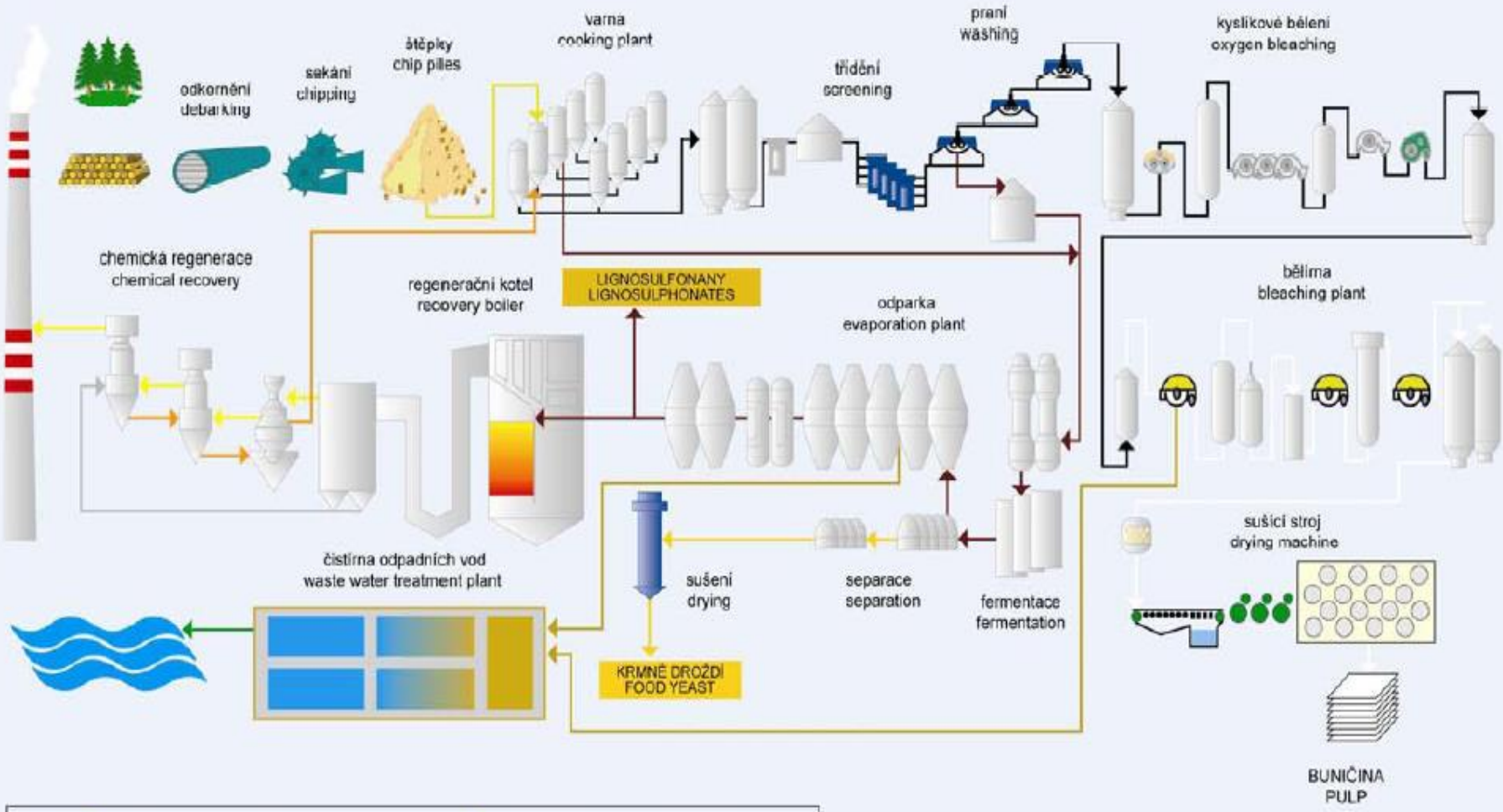
- Continuous, dilution rate 0,25 l/l/h
- Regulation pH
- Defoaming
- Aeration
- Yeast should not form pseudomycelium

Separation and washing

- Continuous centrifugation
- 3 degrees
- Countercurrent

- Thermolysis
- at t = 70°C
- thickening
- Drying (93% w / w dry matter)

Technologické schéma výroby buničiny a krmného droždí
Technological scheme of pulp and food yeast production



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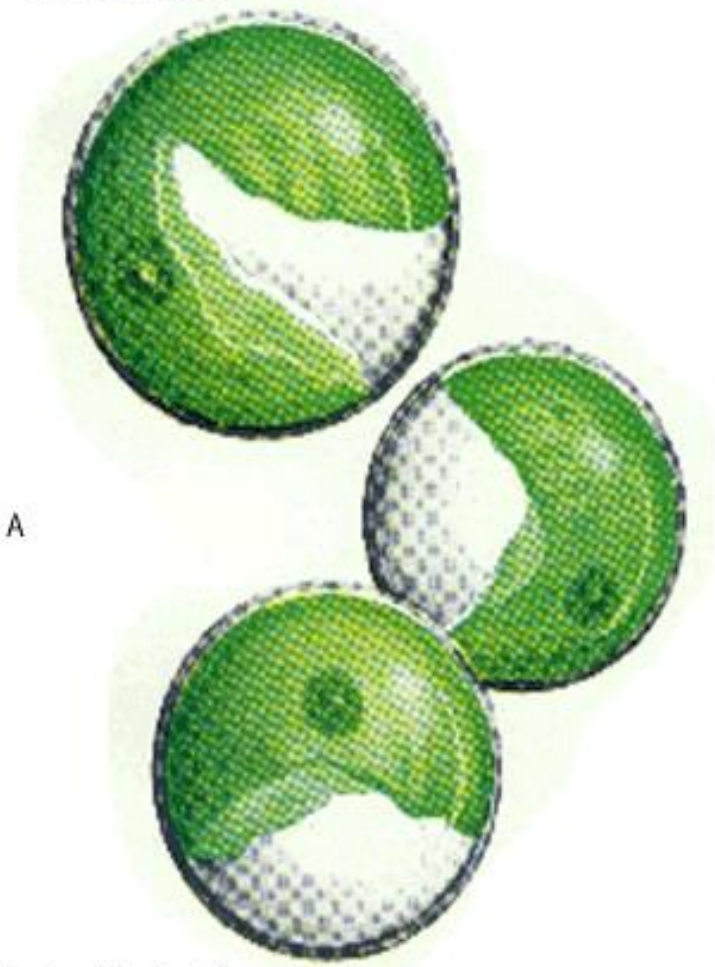
http://www.biocel.cz/c_html/index.htm

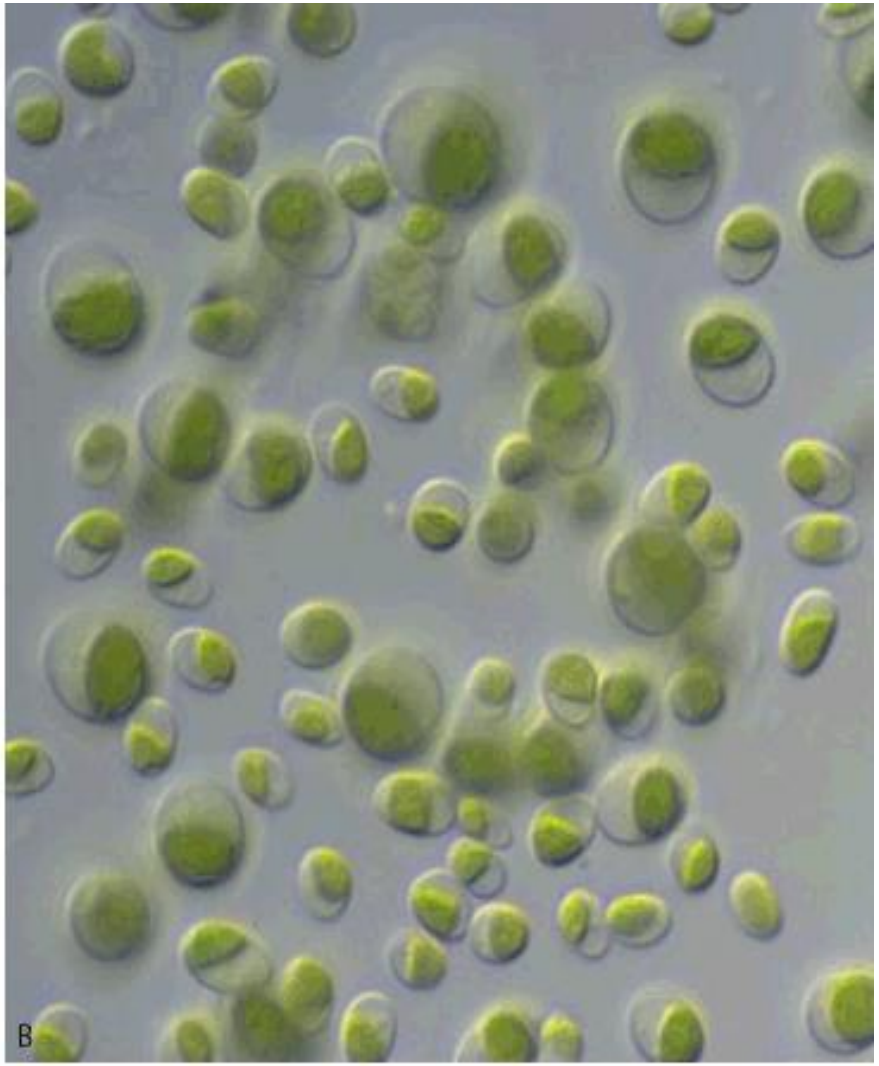
Algae

- Plant kingdom, lower plants
- Photoautotrophic organisms, aquatic environment
- Body-insole - uni- or multicellular – micro- and macro-algae
- Reproduction of unicellular algae by dividing
- Creation of sexual spores
- Trivial classification by content of photosynthetic dyes – green, brown, red, green-blue

Chlorella



A



B

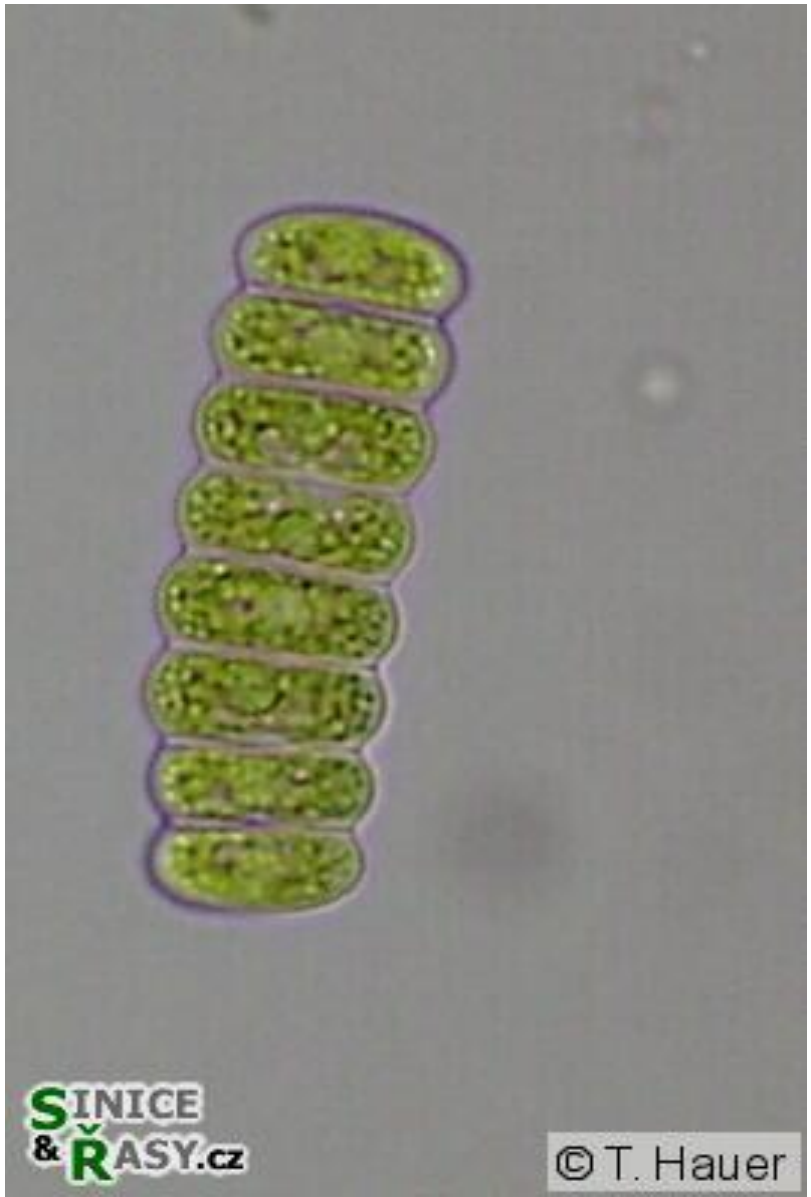
A after Mervin Palmer (1962)

B © National Institute for Environmental Studies, see http://www.nies.go.jp/biology/mcc/home.htm

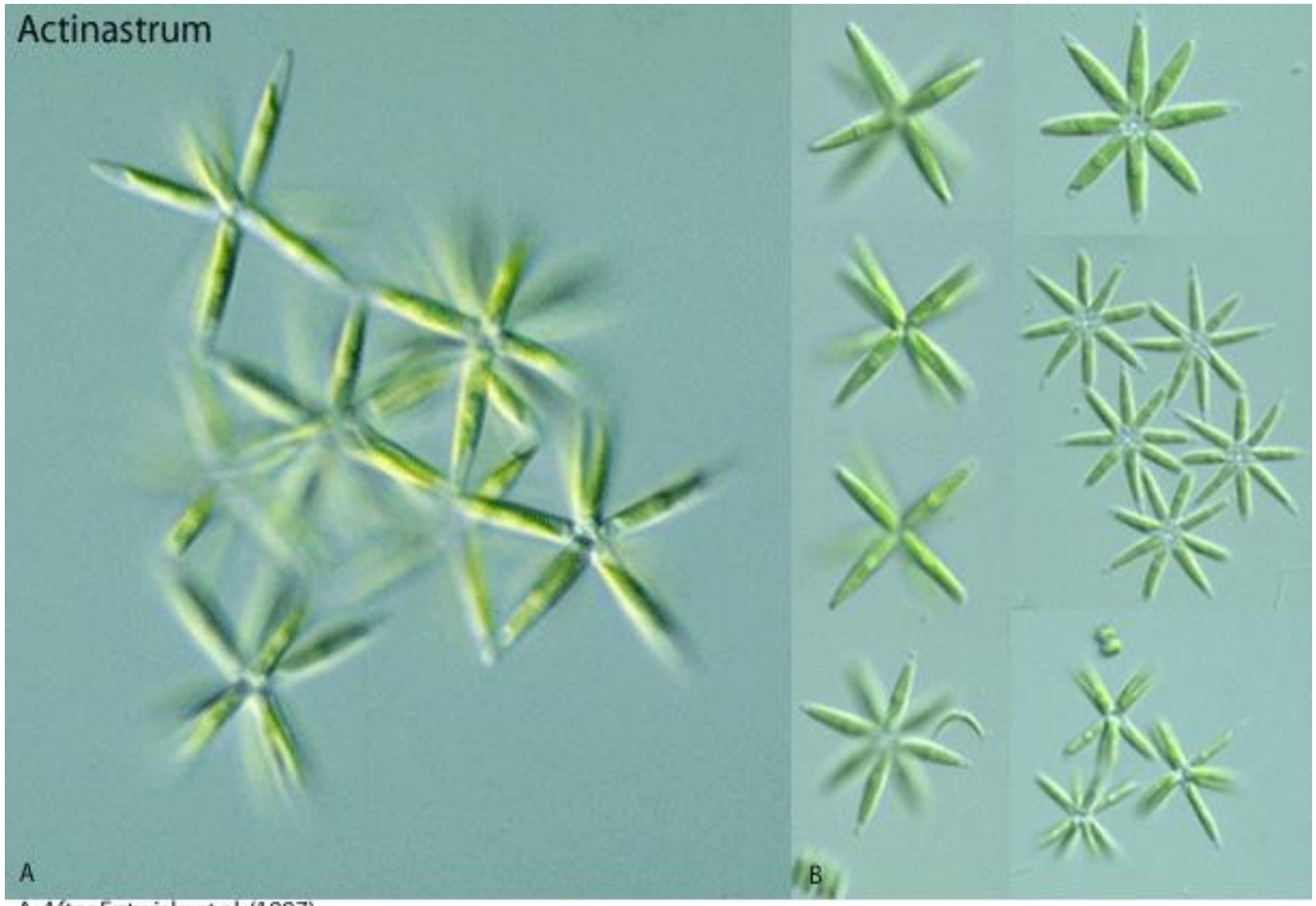
Micractinium

Microspora

Scenedesmus



Actinastrum



A. After Entwisle et al. (1997)
B. © Y. Tsukii, see http://protist.i.hosei.ac.jp/Protist_menuE.html

Ulva

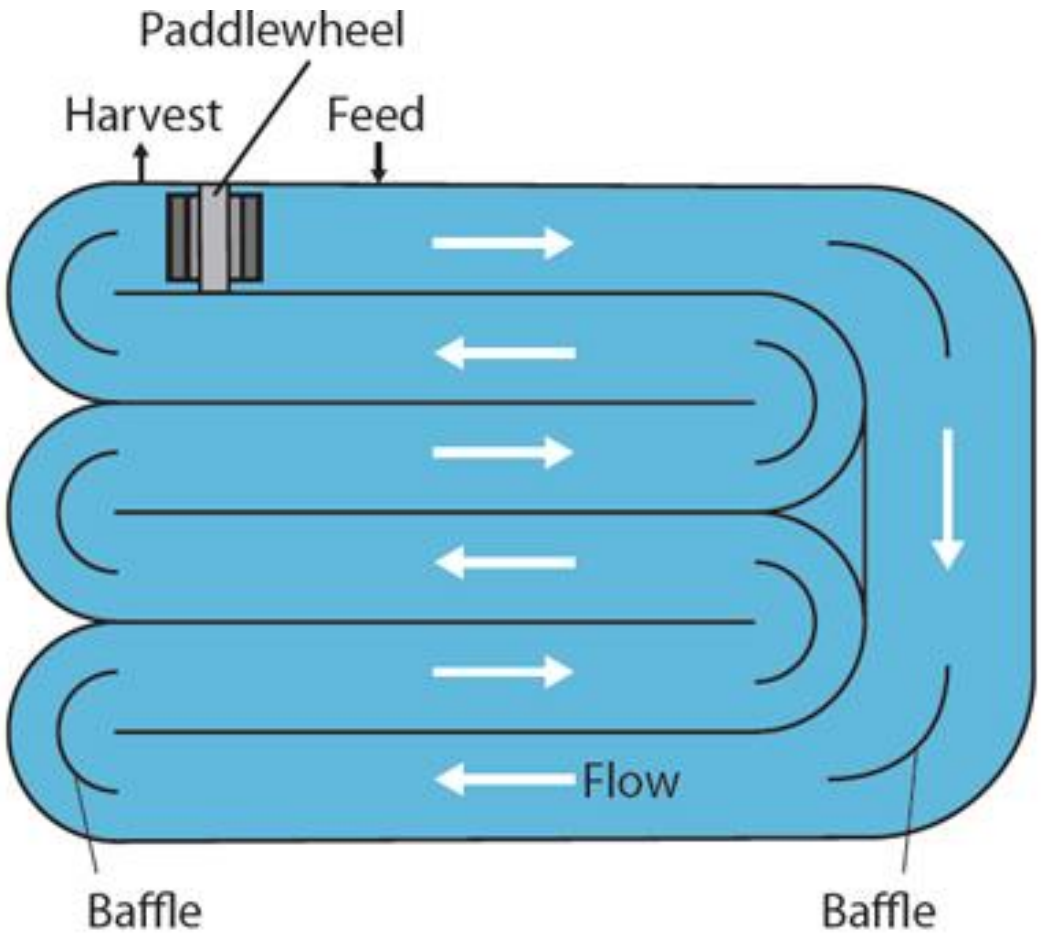
Algae culture- algaculture

- Tanks, fermenters
- Batch or continuous cultivation
- Access to light
- Gentle mixing
- CO_2 content
- risk of contamination

Chlorella cultivation

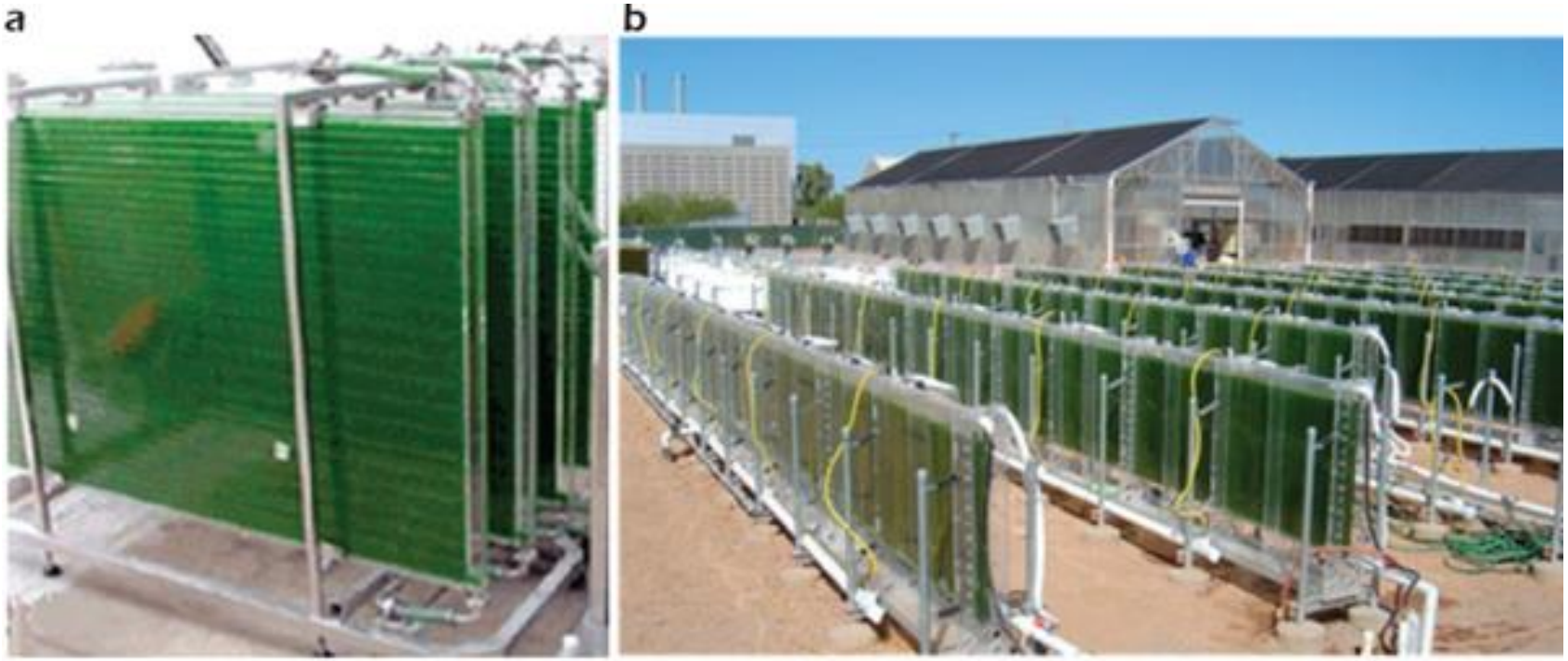
- Round pond with rotating shoulder
- Raceway pond, bubbling with CO_2 and stirring with a paddle stirrer
- thin layer culture mixed by pumping
- Cultivation season (outdoor) – here 150 days, in Africa - 300-320 days

Open-pond design



Raceway pond

Flat-plate photobioreactors



Photobioreactors

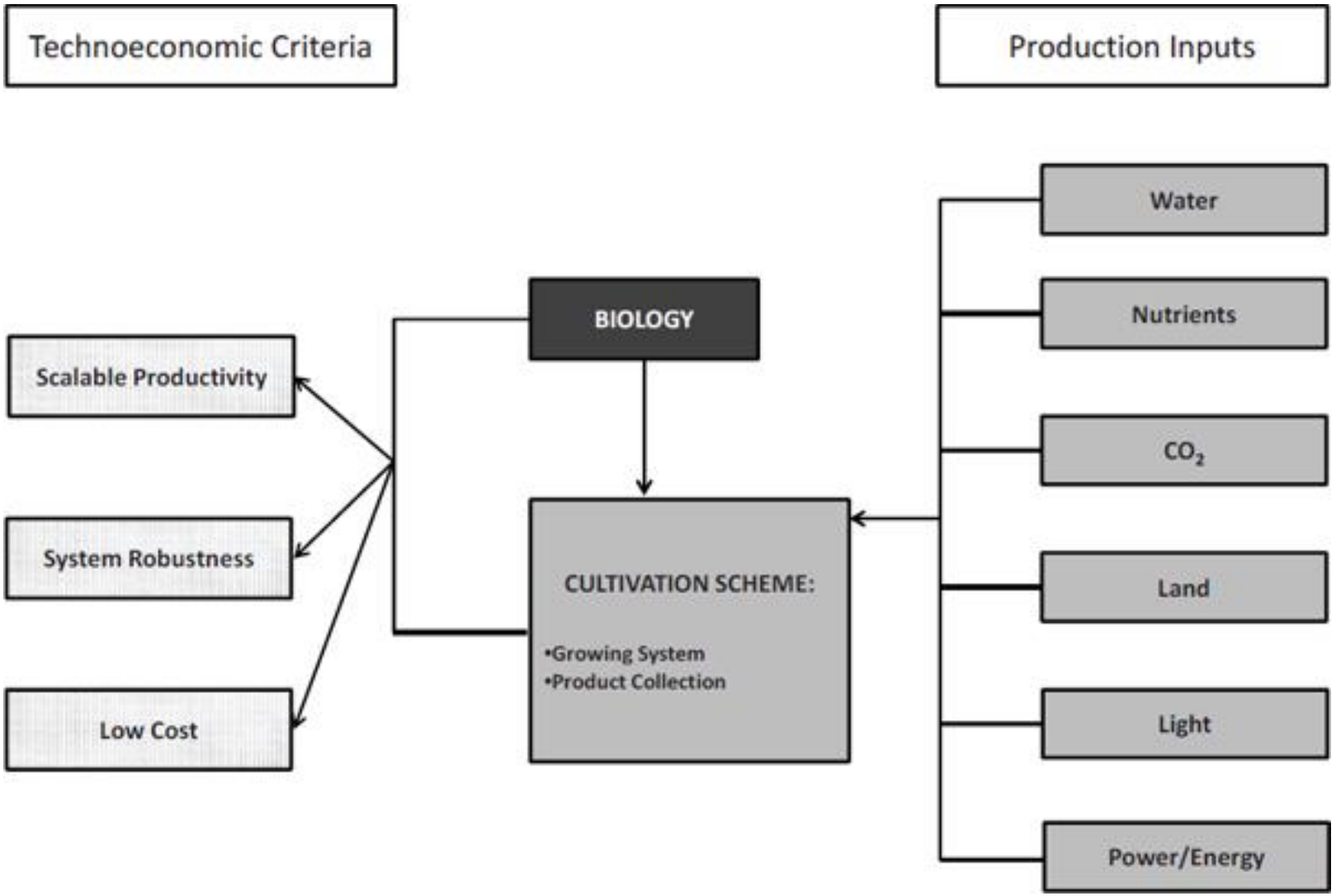
- Bubble columns
- Air-lift bioreactors
- Adjustable light source
- Delivery of CO_2
- stirring

Photobioreactor

Harvest and processing algae

- Harvest - sedimentation, filtration, centrifugation
- Cell disintegration - crushing
- Drying - heat, solar radiation
- The yield - the effectiveness of photosynthesis– max. 60g/m^2/day

Material and energy outputs for algae cultivation



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Biotechnology in Food Industry

Lecture IV

Petra Patáková
petra.patakova@vscht.cz

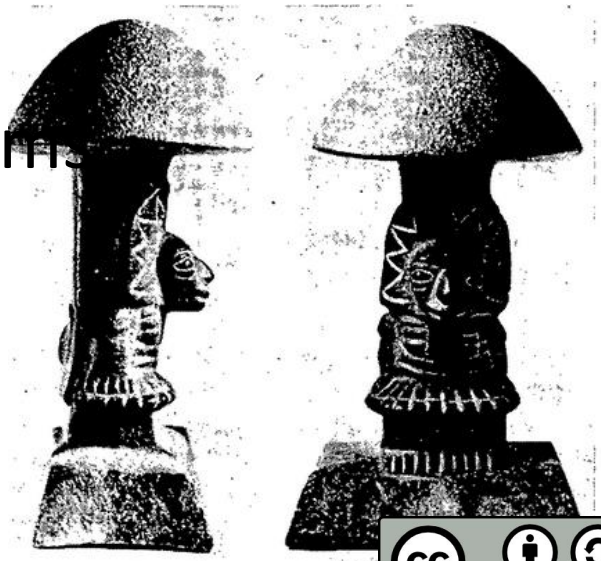
Mushroom cultivation - macromycetes

Content

- Incorporation into the system and history of mycology
- Morphological structure and reproduction
- Ecological groups
- mushrooms (saprophytic, symbiotic,
- parasitic)
- Cultivation of edible mushrooms (*Agaricus, Pleurotus*)

History of mycology

- Supernatural explanations of hub growth
- An effort to assign mushrooms to plants
- The popularity of mushrooms - ancient times, modern times
- Geographic extension of mushroom picking
- The cult of hallucinogenic mushrooms
- Fossil fungi- paleozoikum



Main differences of fungi

	Fungi	Plants	Animals
plastides	**-**	**+**	**-**
Nutrition	**heterotrophic**	**auxotrophic**	**heterotrophic**
Reserve saccharide	glycogen	starch	glycogen
movement	**- (+)**	**-**	**+**
Cell wall	Chitin, manane, cellulosa	cellulosa	chitin

Reproduction

Both asexual and sexual– teleomorphs

Only asexual– anamorphs

↓

auxiliary subdivision

DEUTEROMYCOTINA

(up to 30% fungi)

DEUTEROMYCOTINA

1. Auxiliary class BLASTOMYCETES

Candida, Rhodotorula, Cryptococcus

2. Auxiliary class HYPHOMYCETES

Aspergillus, Penicillium, Oidium, Trichoderma, Alternaria, Botrytis

3. Auxiliary class COELOMYCETES

Phoma

4. Auxiliary class AGONOMYCETES

Sclerotium

Taxonomy of fungi

Kingdom– Fungi

Division– Eumycota

Subdivision – Zygomycotina

Class Zygomycetes

Order Mucorales (*Rhizopus, Mucor*..)

Family, Genus, species

Subdivision Ascomycotina

Class Hemiascomycetes

Order Saccharomycetales,
Schizosaccharomycetales

Class Ascomycetes

Order Pezizales (*Tuber,*
Morchella, Verpa..)

Eurotiales (Aspergillus,
Penicillium…)

….

Verpa bohemica

Subdivision Basidiomycotina

Class
- Homobasidiomycetes

Order Cantharellales

Polyporales, Boletales, Agaricales, Lycoperdales, Russulales

Boletus edulis

Morphology of filamentous fungi

Thalus (body) is formed by hyphae

/ \

uni-cellular (without septa)

multicellular (with septa)

mycelium

Reproduction

Vegetative (asexual)

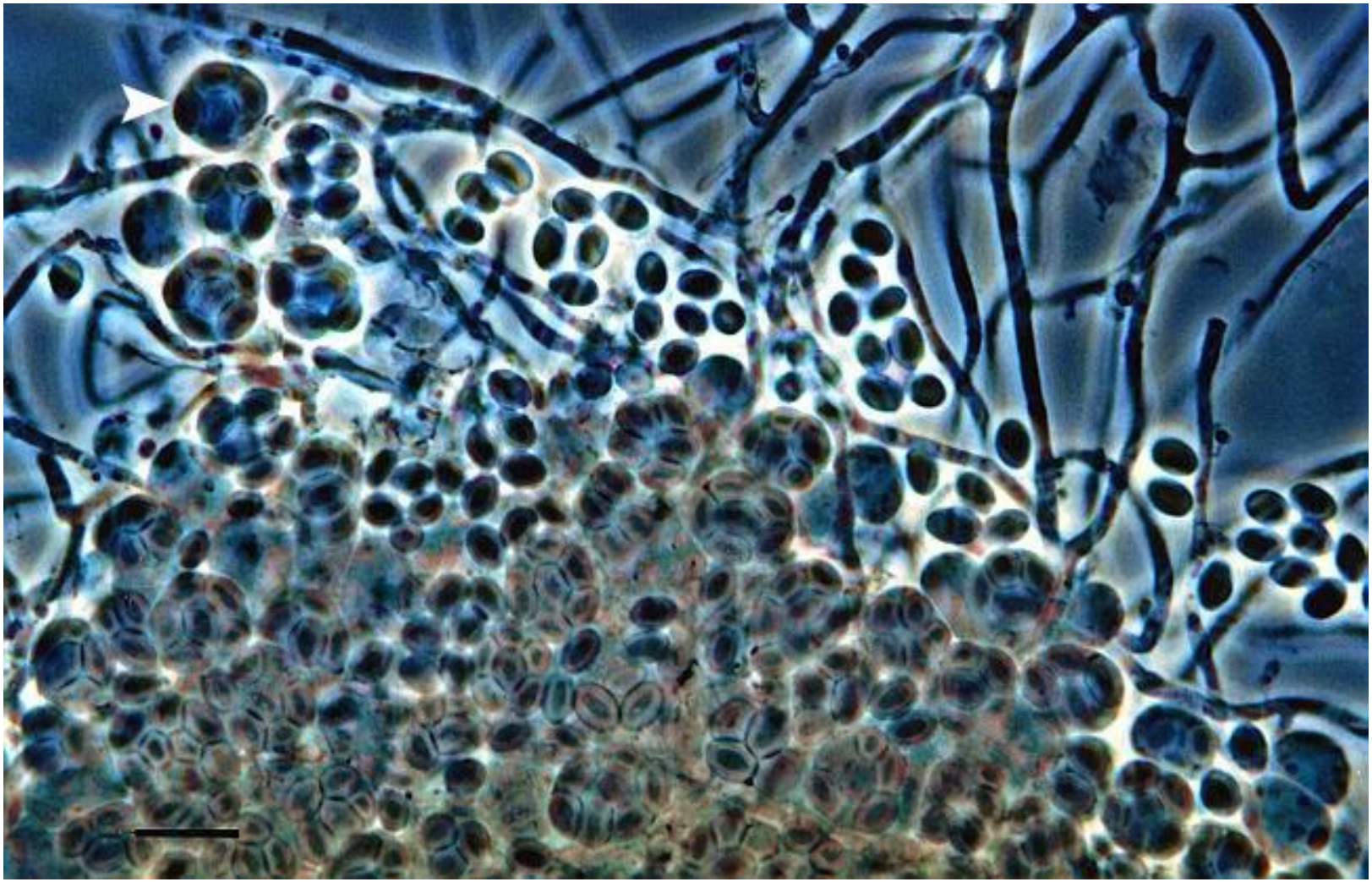
- Nucleus division - mitosis
- Creation of asexual spores - conidia on vegetative hyphae or in fructification organs
- Parasexual reproduction

Sexual

- Mitosis, meiosis, mitosis
- Conjugation of two cells
- Homothalic and heterothalic fungi
- Formation of sexual spores

Conidiophor – *Penicillium chrysogenum*

Ascospores in ascus – *Byssochlamys nivea*



Reproduction

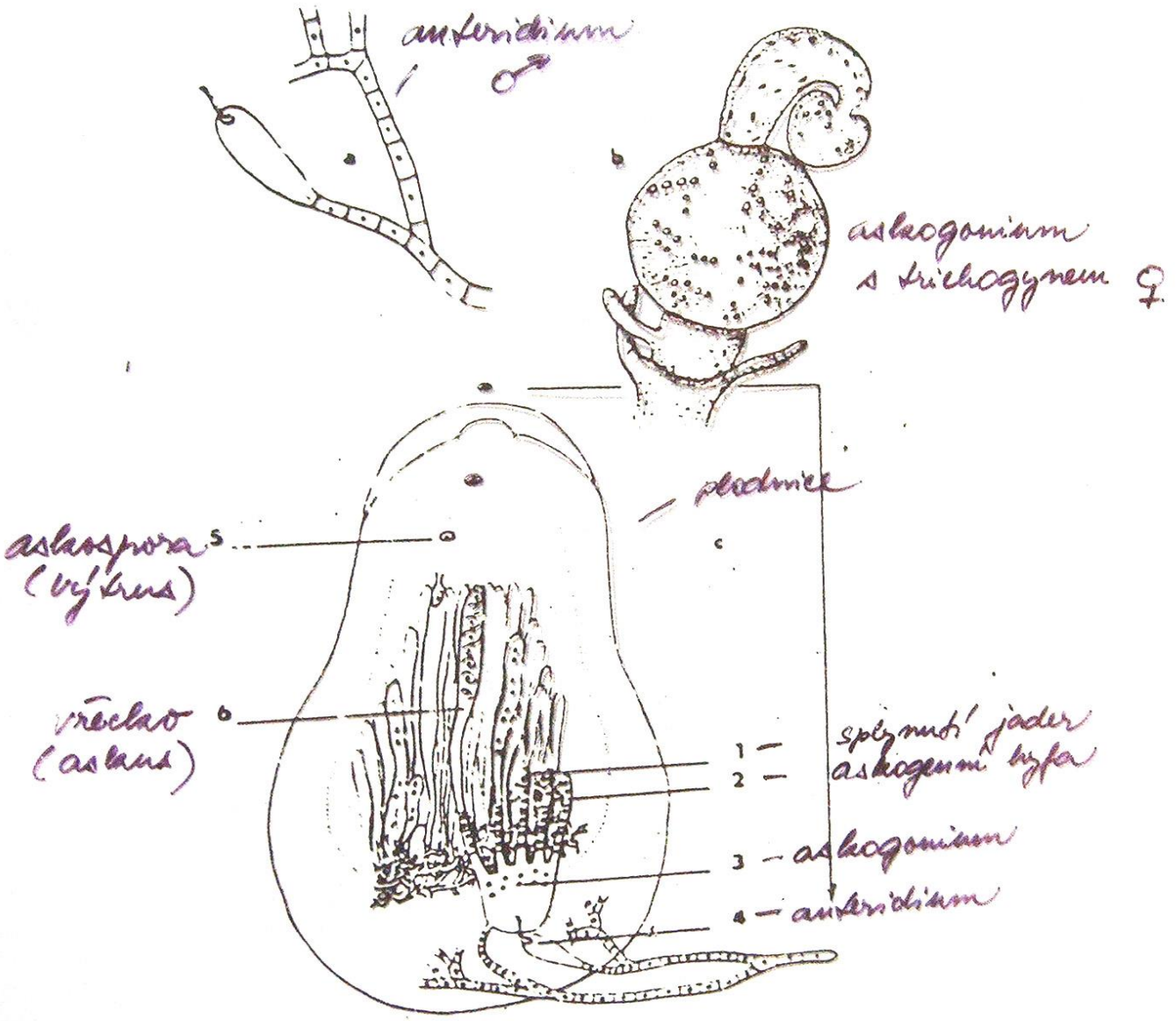
Ascomycetes

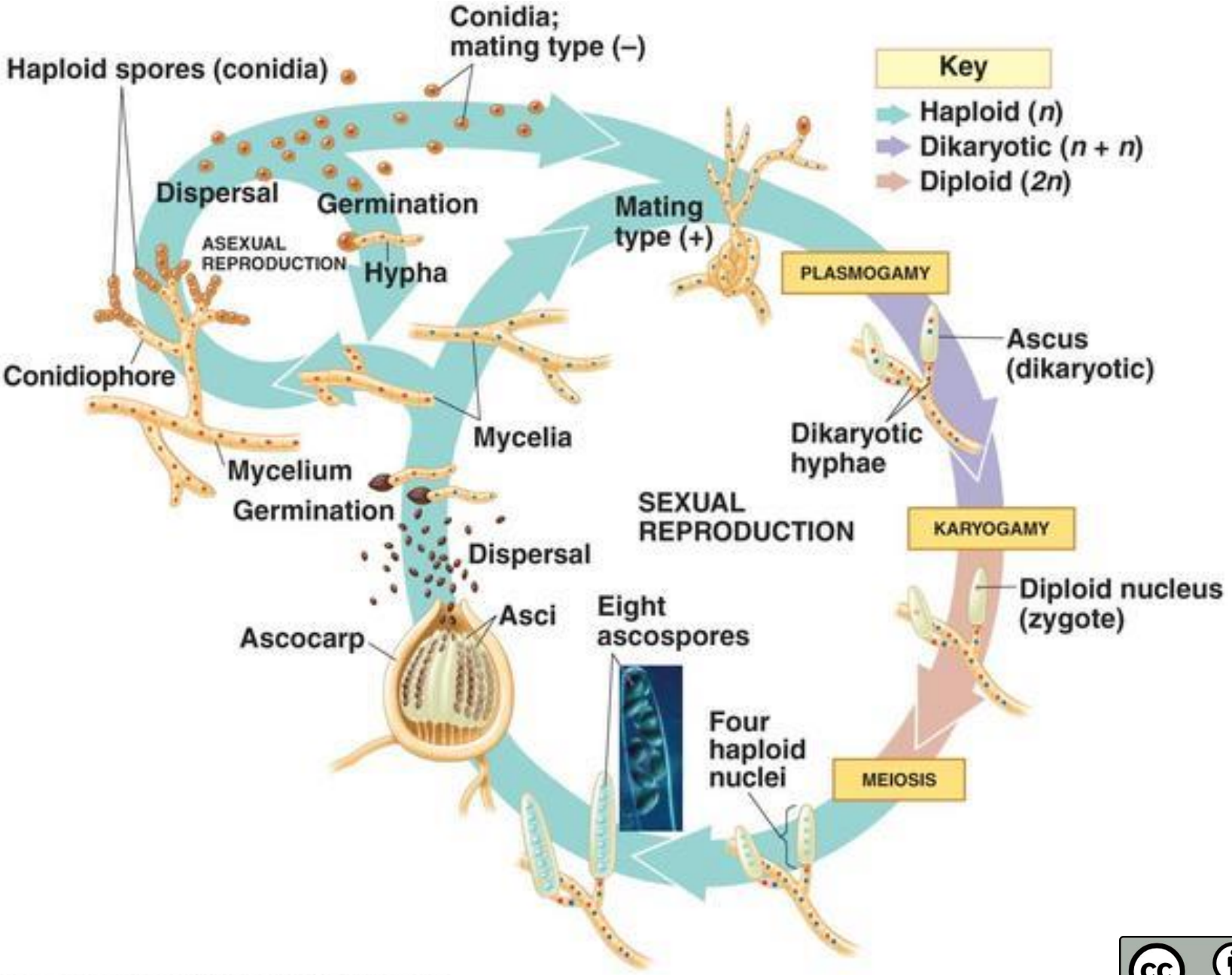
- Creation of asexual spores- abundant
- Differentiated sexual organs (anteridium (male), askogonium(female))

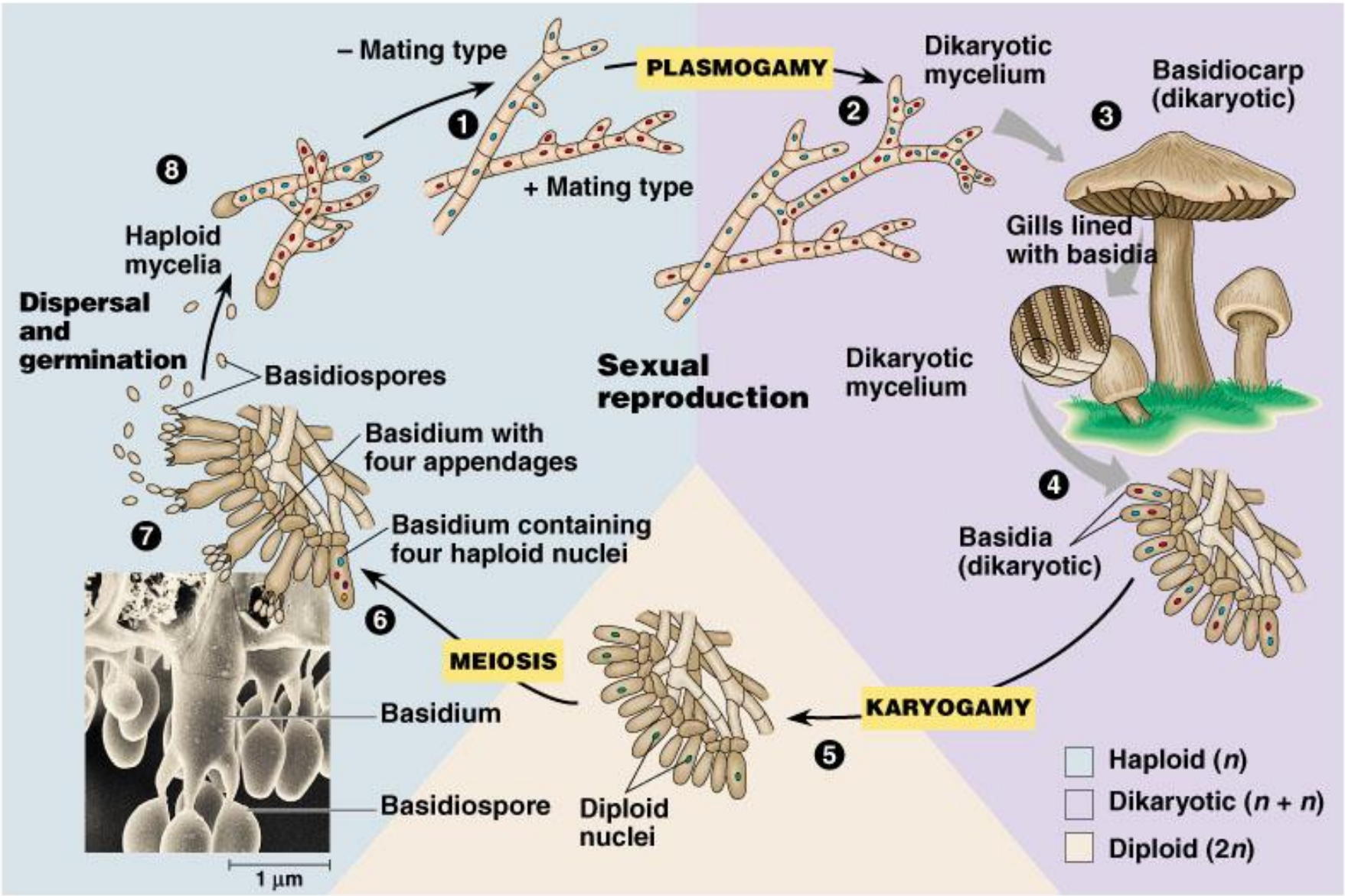
Bazidiomycetes

- Asexual spores–seldom
- Sexual organs are not differentiated
- Binuclear mycelium
- Merging nuclei in basidia

POHLAVNÍ ROZMNOŽOVÁNÍ VŘECKOVÝTRUSÝCH HUB







Fruiting body of mushroom(sporocarp, carpophor)

- It serves predominantly for the formation of sexual spores
- It consists of intertwined hyphae - generative, ligational, skeletal
- The most important part of fruiting body is the hymenophor, it carries a hymenium – there are spores (various shapes)
- 1 fruiting body –millions of spores

Development of fruiting body

It depends on: the development stage of the fungus, the composition of the substrate, temperature, light, air flow, surrounding communities, humidity ...

I.) initiation - germ creation - primordia

II.) Differentiation – formation of fruiting body

Growth of hyphae - apical in mycelium X omnidirectional growthin fruiting bodies

Hymenium(during fruiting body development)

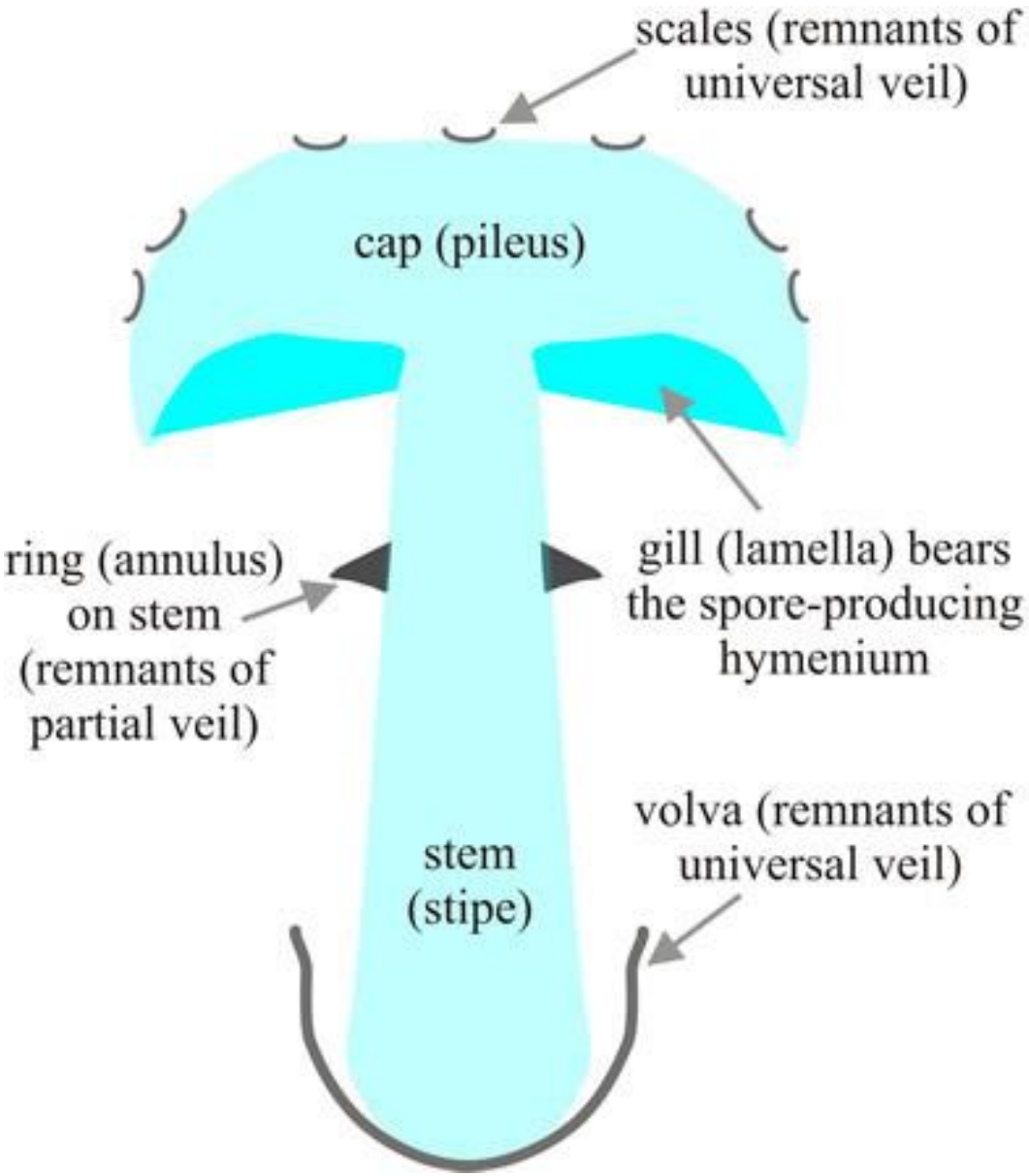
Angiocarpic development

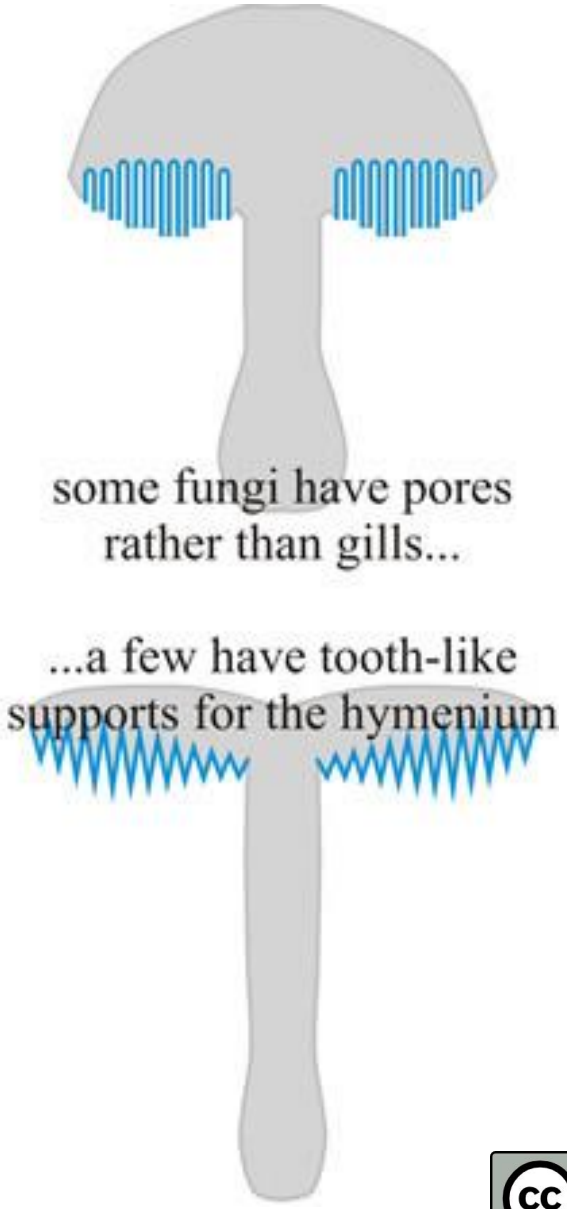
in velum

Gymnocarpic development

open

Boletales, toadstools–in youth in –
hemiangiocarpic development


scales (remnants of universal veil)
cap (pileus)
ring (annulus) on stem (remnants of partial veil)
gill (lamella) bears the spore-producing hymenium
stem (stipe)
volva (remnants of universal veil)


some fungi have pores rather than gills...
...a few have tooth-like supports for the hymenium

Release and germination of spores

- Release: passive and active
- Germination: After a rest period - dormancy (constitutional or exogenous)
- Swelling
- Fat digestion
- Synthesis of the cell wall
- Burst of spore in thin spots – germination pores

Saprophytic mushrooms

Obtaining nutrients by enzymatic decomposition of organic matter

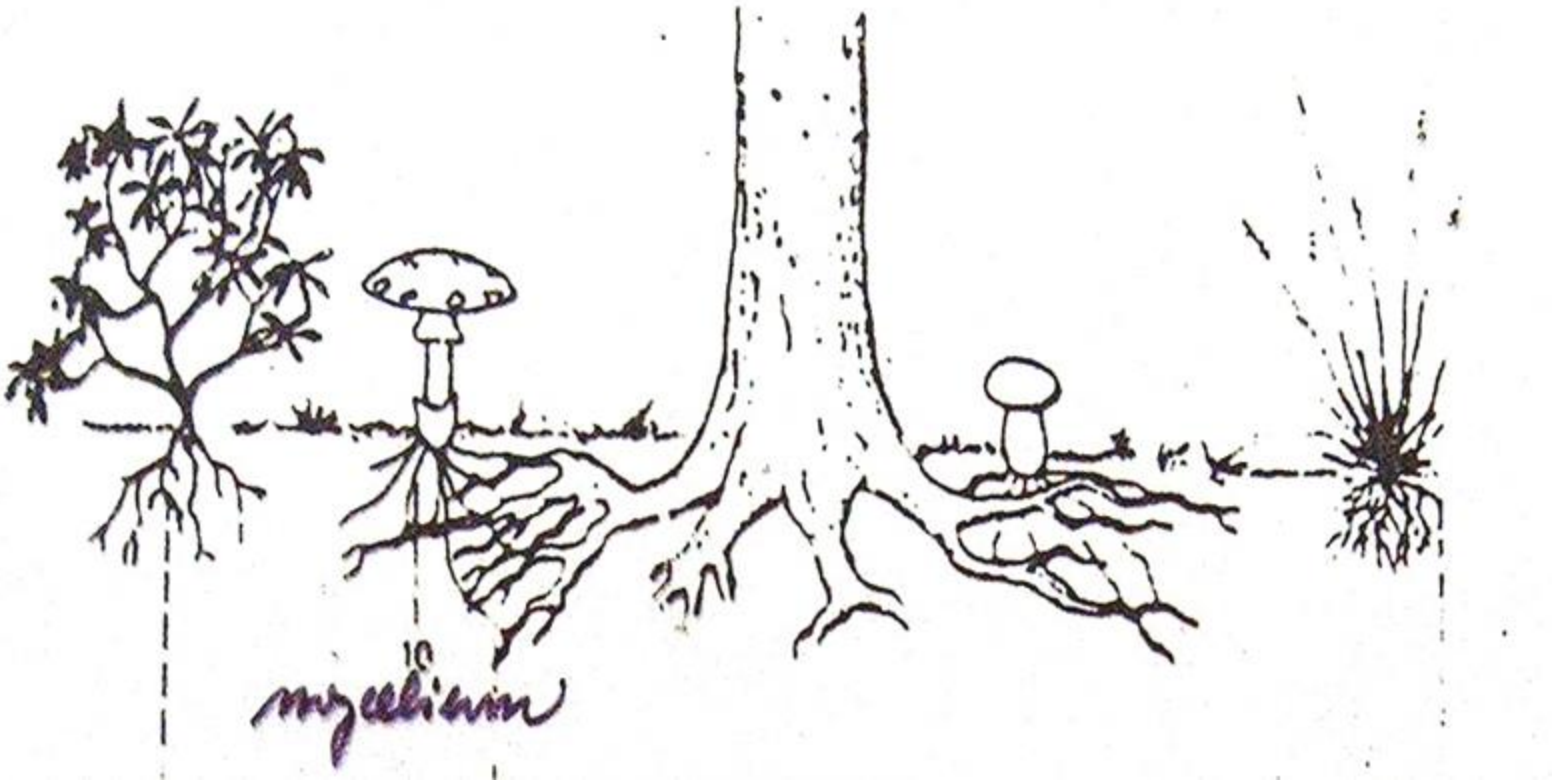
In the soil, in the upper humus layer

In wood

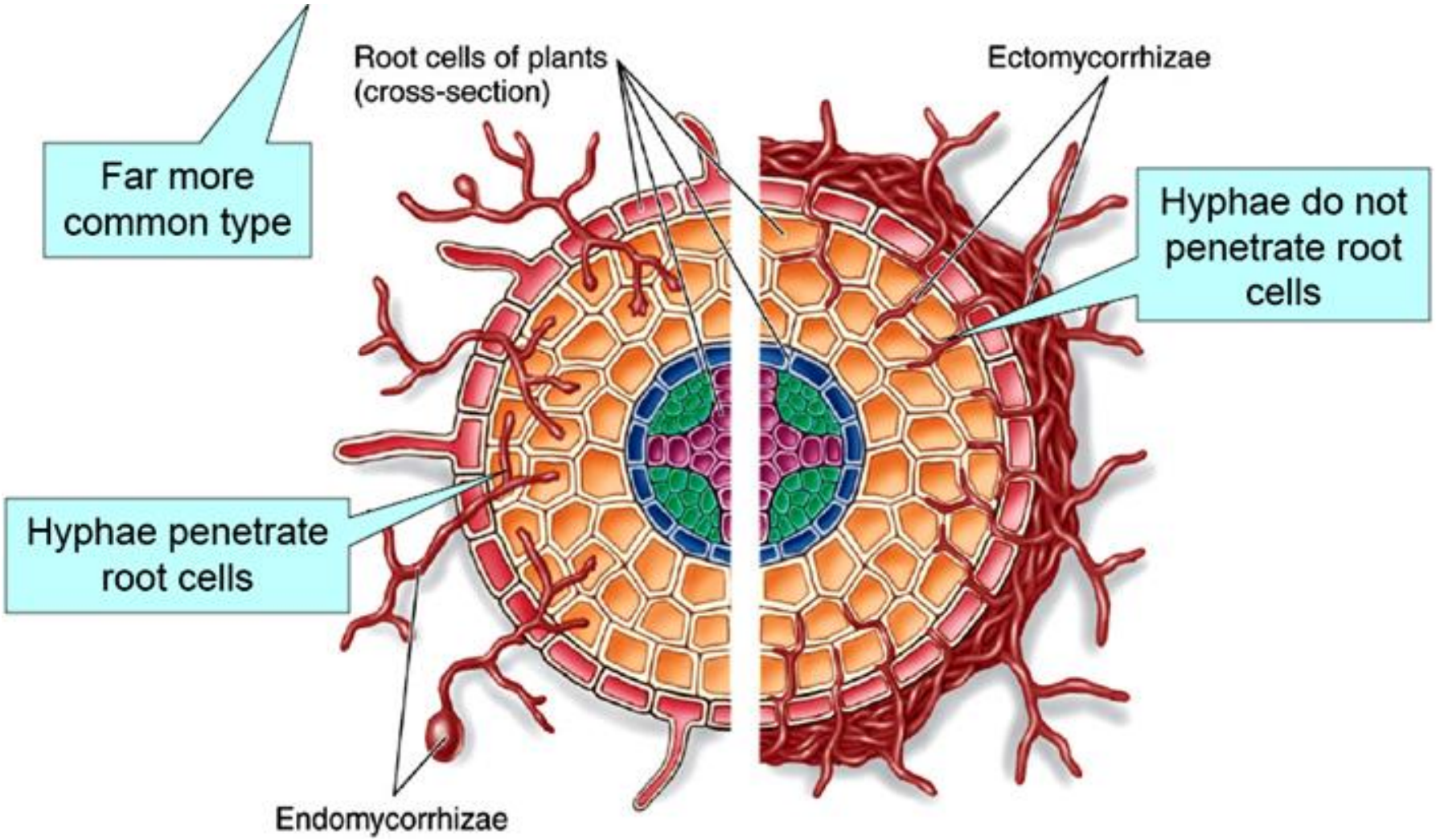
Symbiotic mushrooms

The most important - symbiosis with plants - mycorrhiza
The mushroom needs carbohydrates
Plants need water and minerals
Ectomycorrhiza - the surface of the roots is covered with hyphae - the Hartig net
Endomycorrhiza - hyphae penetrate host cells

On the ground



Root section



Types of Mushrooms

Oyster Mushroom

Straw Mushroom

Reishi Mushroom

Enokitake Mushroom

Shitake Mushroom

Wood Ear Mushroom

Benefits of growing edible mushrooms

- Use of waste products
- Cellulose decomposition
- Fruiting body- easily separable, generally acceptable
- Cultivation simple
- Low acquisition costs
- Suitable technology for developing countries

Agaricus bisporus
white button mushroom

Preparation of the mushroom substrate

Main Components:

horse manure, straw, poultry litter, pig slurry, gypsum....

Ratio C: N - 30-35: 1 - at the beginning of fermentation

Water addition (70-75%) and gypsum (pH adjustment)

Fermentation of the mushroom substrate

1. Mixing and humidification - fermentation pile
2. Phase I fermentation - decomposition of nitrogenous substances - bacteria - t = 20-30^{o}C
3. Phase II of fermentation - thermophilic bacteria, fungi Humicola sp., Chemical reactions - formation of lignohumus complex, ventilation

   Temp. = 40-60^{o}C

Substrate - 65-68%, ash 20-35% dry matter, 0.4% $NH4^{+}$, 1.6-2.1% org.N

Covering soil components:

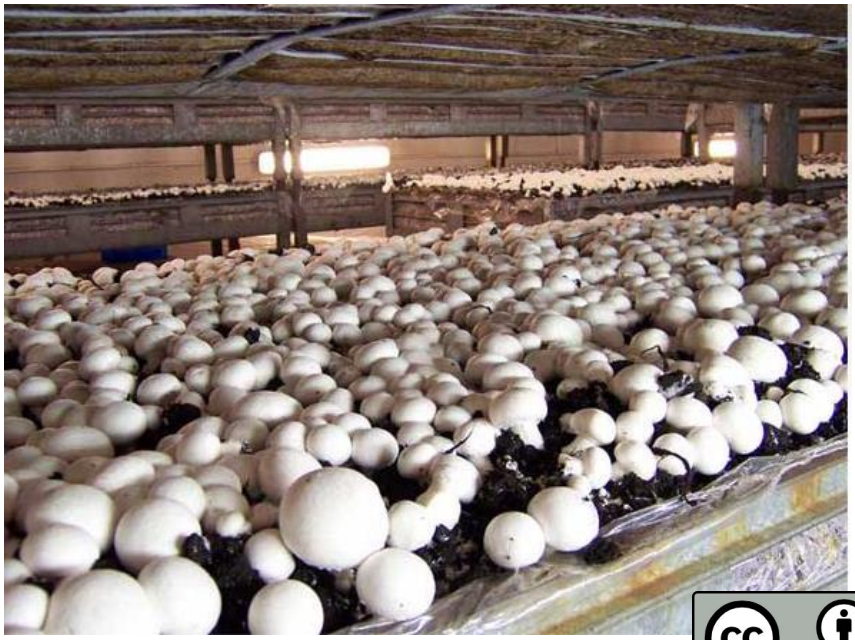
Peat, lime milk, bentonite

Mushroom seedlings:

Grain seed, inoculation - 0.6% seed / substrate

Cultivation

- Propagation of seedlings in substrate
- The substrate is covered with cover earth
- Creation of primordia, then fruiting bodies
- Harvest waves



Run of Agaricus cultivation

Seeded substrate	1 day
Covering with bentonite	2 weeks
Raking (aeration)	3 weeks
Ventilation	25.day
First harvest wave	5-6 weeks
Second harvest wave	7 weeks
Third harvest wave	9 weeks
Waste disposal	61.day
disinfection	62.day

Growing systems: shelving, box, bag ...

End of culture:

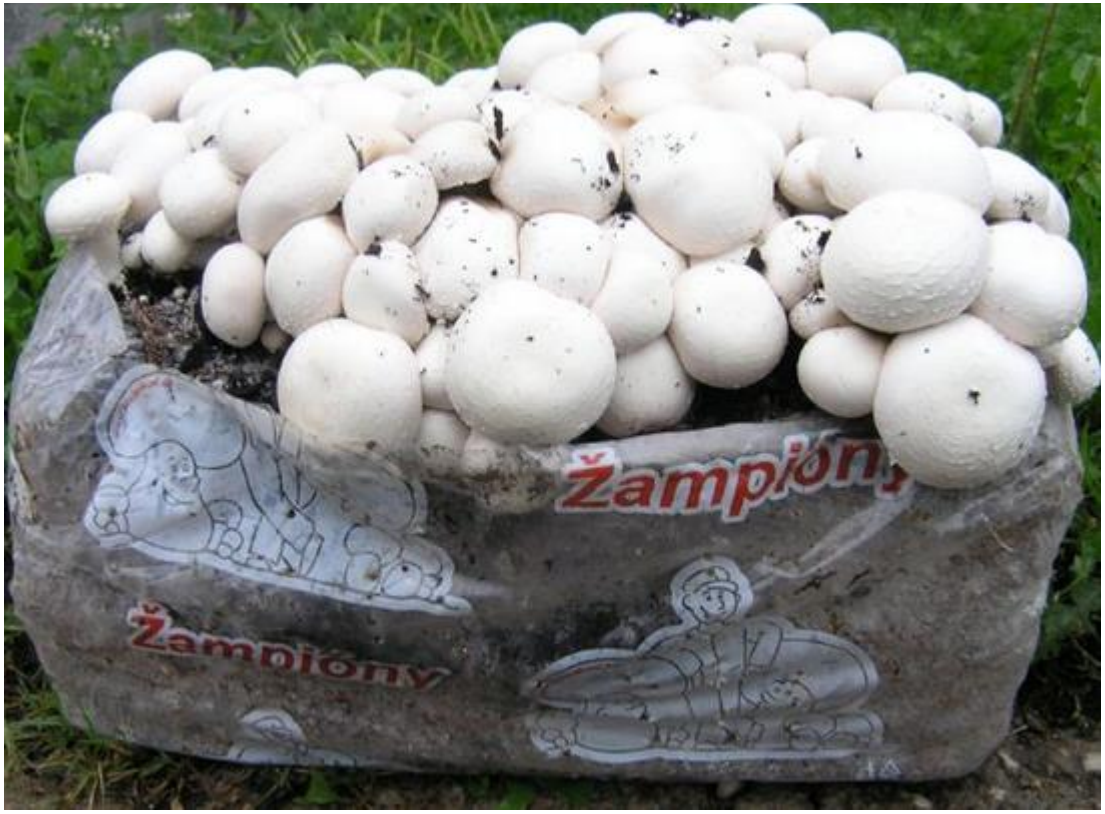
Warm up the entire area at 75°C for 12h

Chemical treatment - formaldehyde

Excavated substrate - fertilizer, cover earth

Shelf (Racks)

Bag system



Diseases and pests of mushroom culture

Bacterioses

Viroses

Fungal diseases

Competitive mushrooms

Nematods

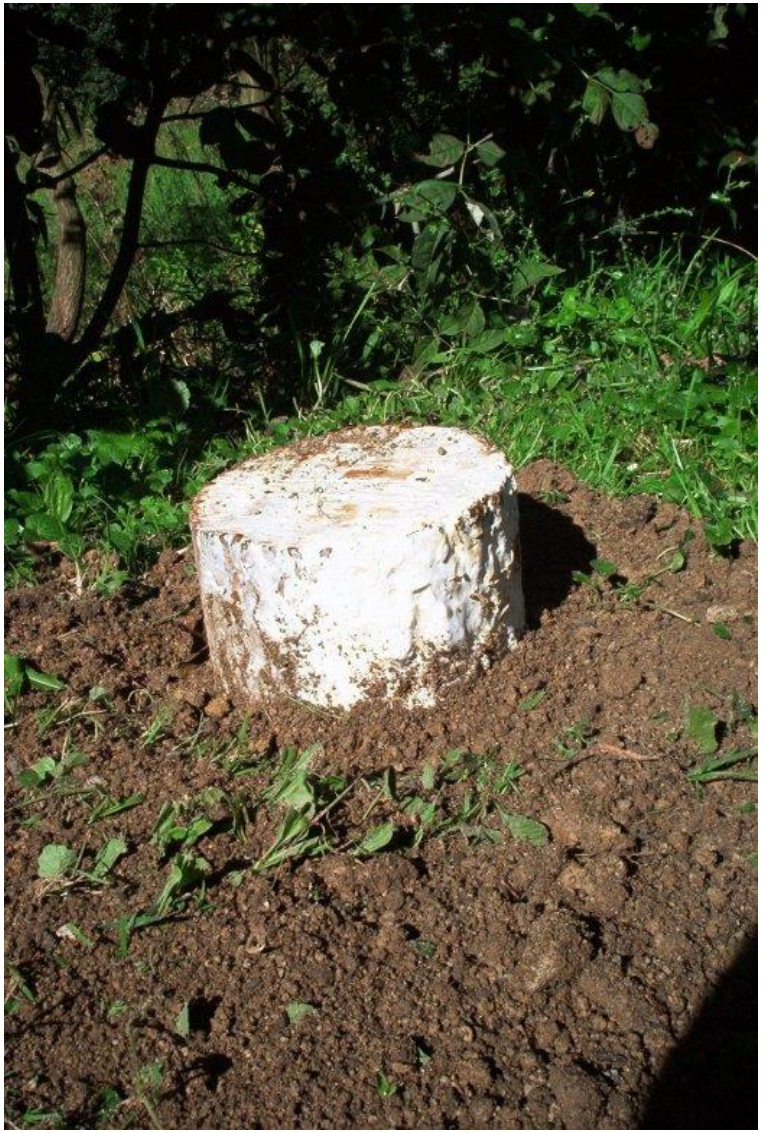
Flies

Mites

Pleurotus ostreatus
Oyster mushroom

In nature – oyster mushroom on wood

On logs or stumps



Intensive cultivation of Pleurotus

Substrate preparation

Straw, corn spindles ..

Moistening and **heat treatment (sterilization)**

Substrate seeding

Mixing with grained seed (2-5%)

Proliferation of mycelium

14 days, 25-27°C, without aeration (anaerobic) X intensive cooling

Oyster mushroom

Initiation of fruiting bodies formation

20-22°C, ventilation with humid air

Harvest in waves

Spore release - Required respirator

Exfoliated substrate - Feeding for pigs

Diseases and pests - molds, flies

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Biotechnology in Food Industry

Lecture IX

Petra Patáková
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Fermented foods from soya and rice

Common features of Orient fermentations

- Solid substrate cultivation (SSC)
- Mixed (undefined) microbial culture
- Sequential, multi-step fermentation
- Semi-aseptic conditions
- Use of „koji“ as enzyme source
- Substrate – soya, rice, (wheat, barley…)
- Microbial production of vitamins

Soya

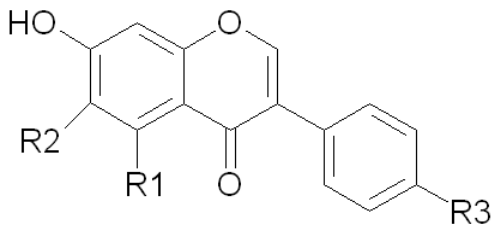
- High protein content (45% in dry matter.), fats (20%.), saccharides (35%.) and fiber
- Consumption – benefitial effects on health
- Isoflavones – antioxidants, plant hormons

X antinutritional factors – inhibitors of proteases, antivitamins, non-digestible oligosaccharides

- GM soya?

http://bioinfo03.ibi.unicamp.br/soja/

Isoflavony v sóje



daidzein R1=H, R2=H, R3=OH

genistein R1=OH, R2=H, R3=OH

Fermentation of cooked soya

- Digestion of proteins to amino acids and peptides → atractive smell and taste
- Digestion of oligosaccharides (stachyose, raffinose and melibiose)
- Increase of amount of free isoflavones
- Production of riboflavin,nicotinic acid, nicotinamide and vitamin B_6
- Higher shelf-life

Fermented products:

1. Spicy and flavouring compounds– **soy sauce**, soy paste, soy nuggets, **sufu**
2. Soy foods – **tempeh, natto**, soy jogurt

Koji („bloom of mould“)

- Source of proteolytic, peptidolytic, amylolytic, (hemi)celulolytic enzymes
- Use of nnon-toxinogenic fungi – *Aspergillus oryzae*, *Aspergillus sojae…*
- SSC (dishes with perforated bottoms, periodic mixing)
- Inocultaion with spores or seed cultures
- Different types of koji –based on substrate (cooked) and intended use

http://thesakechronicles.word

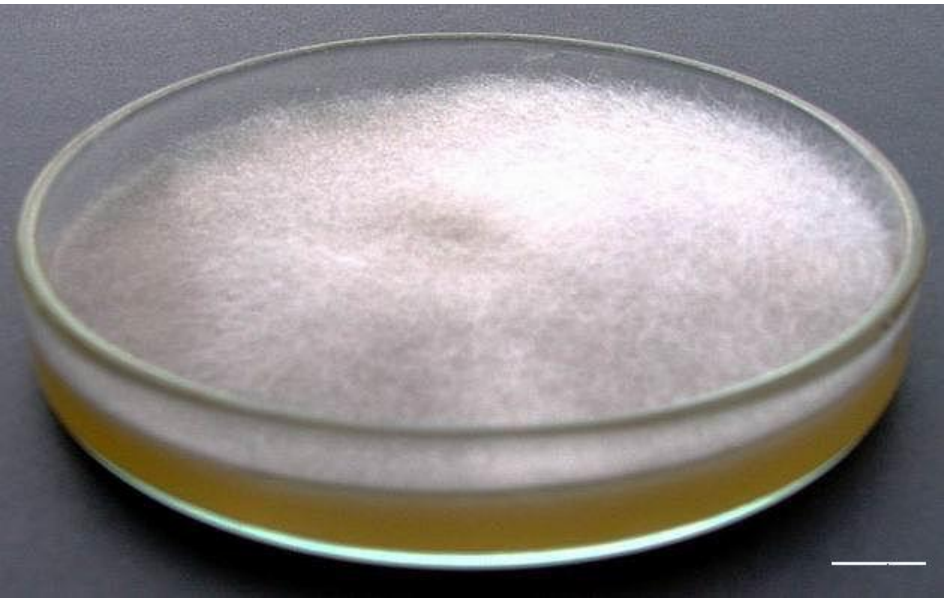
Used microorganisms

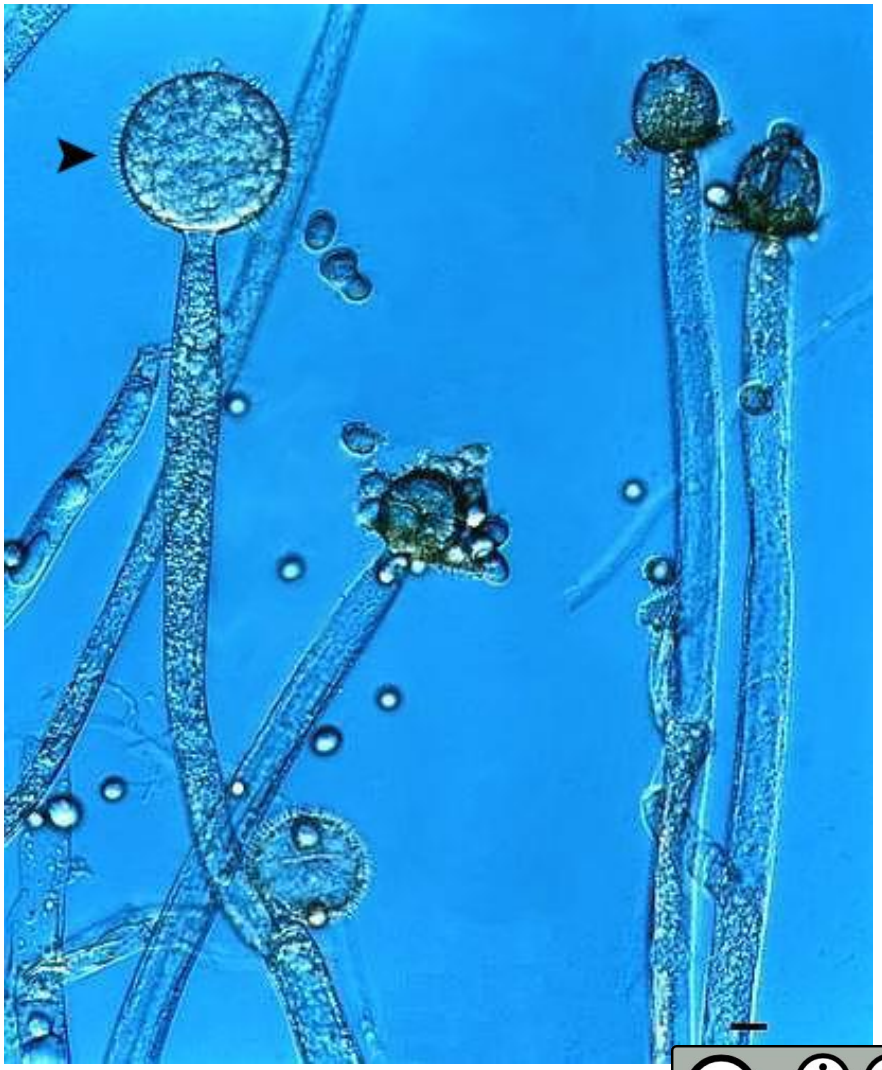
1) Fungi – *Actinomucor*, *Mucor*, *Rhizopus*, *Aspergillus*

2) Lactic acid bacteriy

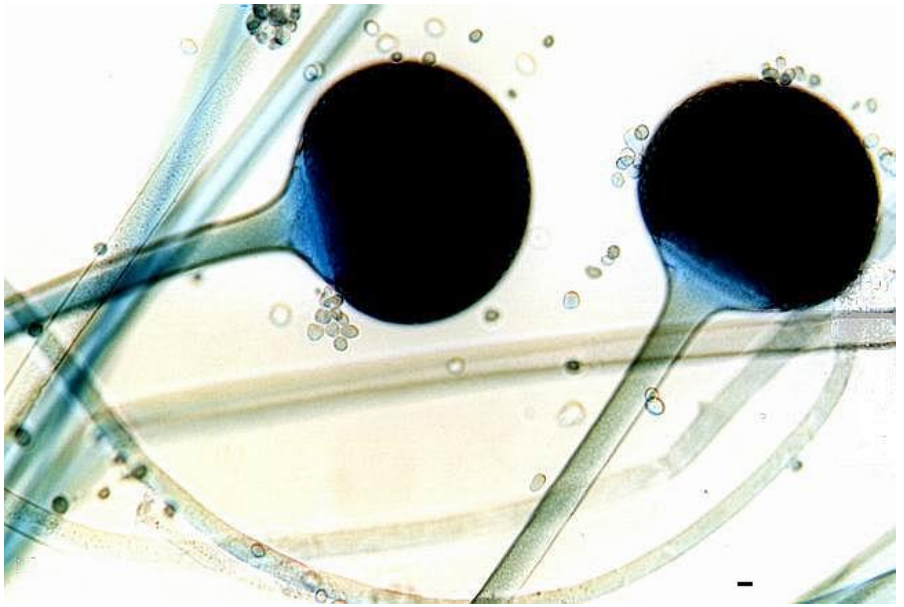
3) Halofilic yeast

Mucor





Rhizopus



Traditional way

Soya beans

wheat

steaming

roasting

A.oryzae + *A.sojae*

koji

brine

moromi

hydrolysis and fermentation

6-8 months

lactic and alcoholic fermentation

maturing

filtration

Bioreactor production

Defatted Soya beans

wheat

steaming

roasting

Hydrolysis (40-50°C, 3 days)

Continuos koji production

moromi

filtration

salt

Immobilized glutaminase

Imob. LAB *P.halophilus*

imob. yeast *Z.rouxii, C.versatilis*

2 days

pasteration

Soy sauce

Wheat is imported from overseas countries.

The wheat is roasted and crushed.

For more informations on the ingredients and the fermentation process, please click here.

Kikkoman's *Aspergillus* (mold) plays a vital role in determining the flavour of Kikkoman Soy Sauce. Mold is added to the wheat and soybeans. The resulting mixture is then cultured for three days to form a dry mash called *Koji*.

For more informations on the production of Koji, Mixing and aging, please click here.

Soybeans are imported from overseas countries.

The soybeans are steamed to cook.

Salt is dissolved in water.

Koji is combined with a salt water solution to form *moromi*. The *moromi* is then fermented in tanks for about half a year.

Moromi is poured onto cloths, folded and pressed. The raw soy sauce is squeezed out. Soy sauce pressed cake and oil are the by-products.

The Raw soy sauce is then refined and pasteurized.

For more informations on the pressing nad rrefining, please click here.

Samples of the refined soy sauce are sent to the laboratory, where they are carefully analyed to ensure of it's finest quality.

For more informations on the bottling, inspection and shipment, please click here.

Differences between soy sauces

fermented soy sauce

• enzyme hydrolysis and fermentation under ambient temperature
•Flavour – mild, pleasant
•Essential amino acids, „shoyu“ polysaccharides
•biogenic amines – „syndrom of China restaurant“?

Non-fermented soy sauce

• acid hydrolysis under high temperature
• flavour – rough, unpleasant
•Other seasonings, conservants
• high content of chlorpropanoles (3-MCPD) (EC limit 0,02mg/kg)

Indicator of falsification –tryptofan, levulic acid

Soya beans

peeling, soaking, cooking

de-watering

inoculation — *Rhizopus oligosporus, R.oryzae*

packaging

incubation

tempeh

http://cs.wikipedia.org/wiki/Tempeh

http://www.tempeh.info/

Soya beans

soaking, milling, warming

filtration — okara

Soya milk

coagulation — $CaSO_4$, $MgSO_4$

pressing — Soya whey

tofu

inoculation — *Aspergillus, Mucor, Rhizopus (Monascus)*

fermentation

pehtze

marinade

salting — maturing — **Fermented tofu (sufu)**

http://en.wikipedia.org/wiki/Fermented_bean_curd

http://www.absolutechinatours.com/china-travel/Shanghai/Stinky-tofu.html

http://redcook.net/2009/10/24/cooking-sustainably-with-mussels/

Soya beans

soaking, steaming

Steamed soya beans

rice

Aspergillus

koji

salt

green miso

Z.rouxii, Tetragenococcus halophilus, Torulopsis, Halophilus, Streptococcus faecalis

anaerobic fermentation, 25-30°C, 1 week-1 year

Mixing, pressing, pasteration, packaging

miso

http://www.mindbodygreen.com/0-5563/3-Secret-Healing-Benefits-of-Miso.html

http://www.8miso.co.jp/english.html

http://www.asianfoodgrocer.com/product/honzuku miso-paste-35-2-oz?green=A8F0FE

Soya beans

washing, steaming

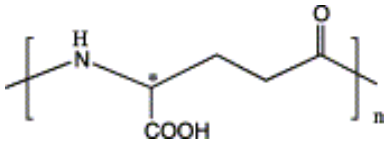
Steamed soya beans

Bacillus subtilis natto

incubation, 40°C, 24h

cooling, maturing, packaging

natto



γ-PGA

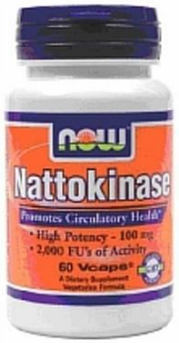
http://www.pref.ibaraki.jp/bukyoku/seikan/kokuko/e-ibaraki-report/0603natto.html

http://nakazora.wordpress.com/2010/07/10/ferment-ii-natto/

Influence of natto on health

- **„natto kinase“** (serine protease, subtilisin) – reduces thrombs by fibrinolysis and by inhibition of plazminogen activator; anti Alzheimer disease
- **vitamin K_2** (menachinon) –anti osteoporose
- **antioxidant PQQ** (pyrochinolin chinon) – antioxidant effect
- **probiotic effect of *B.subtilis* (**up to 10^8 cells (spores)/g, strain R0179- GRAS – January 2012; FOSHU status in Japan)
- **isoflavones from soya**
- **Other effects–**cholesterol reduction, anti obesity,improvement of digestion

http://www.magister.sk/produkt/detail/produkty-od-a-po-z/natto-nkcp-



http://www.jamiesonvitamins.sk/tag/starostlivost-o-klby-a-artritida/

The summer's rice crop
Feeds winter
fermentation
In spring, celebrate
Haiku of Daimon-san

http://www.alkoholweb.cz/

http://web-japan.org/nipponia/nipponia44/en/feature/feature01.html

rice

polishing, soaking, steaming

steamed rice

Aspergillus oryzae

Saccharomyces saké

koji

moto

moromi

filtration

raw saké

filtration, pasterization, maturation, mixing, bottling

pasterization

saké

http://kyotofoodie.com/hanamizake-sake-and-sakura-blossoms/

Saké

http://www.esake.com/Store/us-lineup.html

- Usual ethanol conc. 15%
- Addition of ethanol
- Sake - Japanese term for alcohol
- Different types of saké –differences in polishing rice, filtration and addition of ethanol
- Saké serving– cold, hot, at room temp.

Mikrobial production of glycerol

Glycerol
(1,2,3- propantriol)

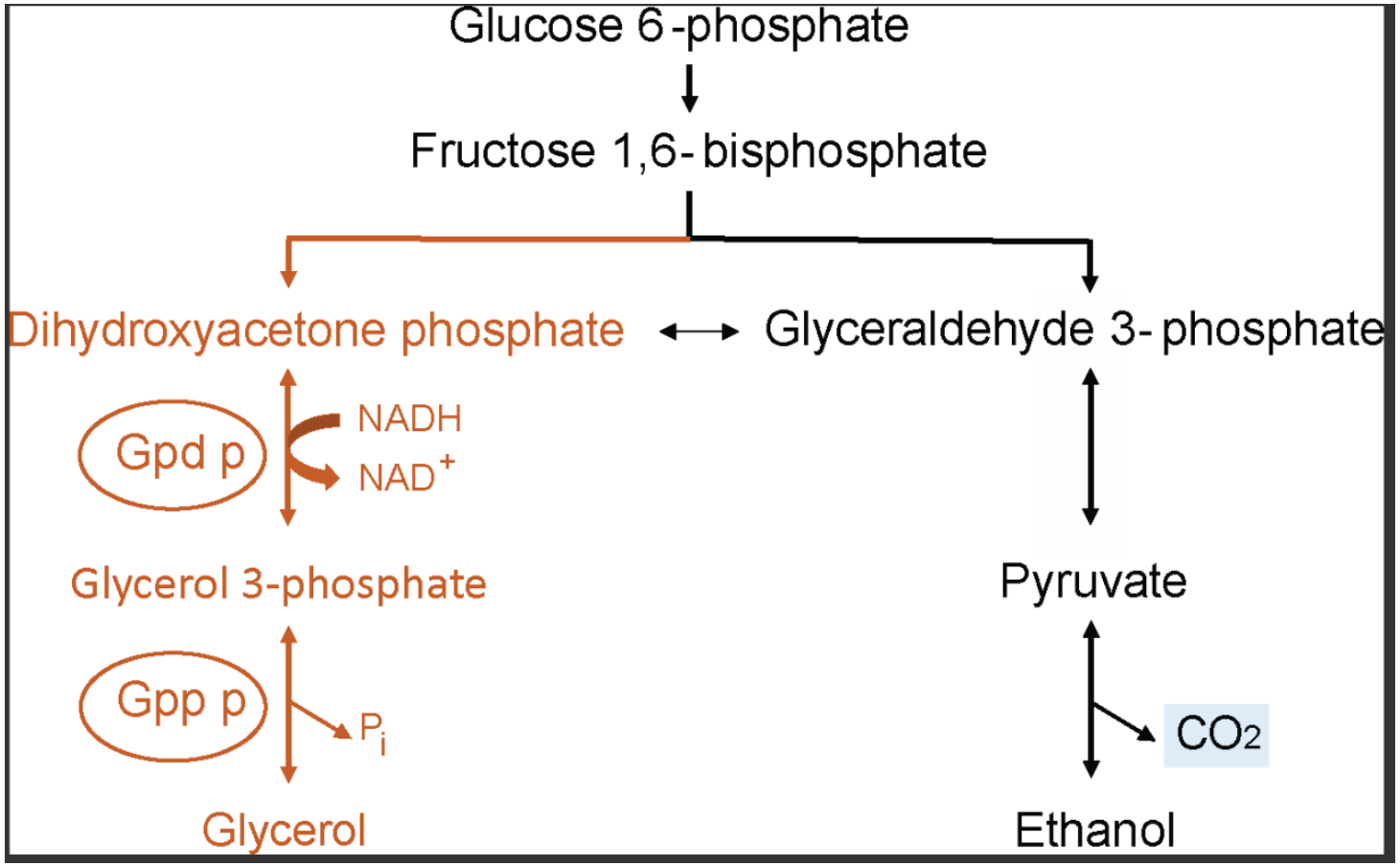
Uses - cosmetics, painting, food, pharmacy, leather and textile industry

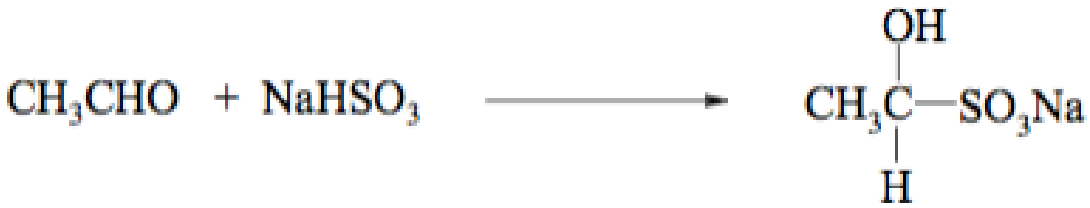
Chemical production - fat hydrolysis

Microbial production - by yeast:

- Sulphite way $NaHSO_3$
- Alkaline way

Acetaldehyde is blocked by sulphite





Sulphite way

1. Blocking acetaldehyde by sulphite

Hexose + SO_2→ acetaldehyde-sulphite + CO_2 + H_2O + **glycerol**

2. Adaptation of wine yeast *S. cerevisiae* to sulphite
3. Anaerobic fermentation – 30h, temperature 30-32^oC
4. Saccharide concentration– 15%, yield 51%

Alkaline way

2hexose→ 2**glycerol** + ethanol + acetate + $2CO_2$ + 2 H_2O

Saccharide concentration up to 17%

Addition of sodium, magnesium or calcium carbonate– pH > 7

Process 4-5 days

Production by osmotolerant yeast

- *Zygosaccharomyces acidifaciens*
- Aerobic process, formation of ethanol and acids
- High concentration of saccharides
- Up to 20% glycerol

Production of glycerol by osmotolerant algae

- *Dunaliella*

Glycerol production - response to stress caused by increased NaCl concentration – however mainly intracellular glycerol is produced

Glycerol isolation

- Yeast and sludge centrifugation
- Distillation of ethanol
- Cleaning
- Concentration by evaporation
- Vacuum distillation of glycerol X polymerization

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Biotechnology in Food Industry

Lecture V

Petra Patáková
petra.patakova@vscht.cz

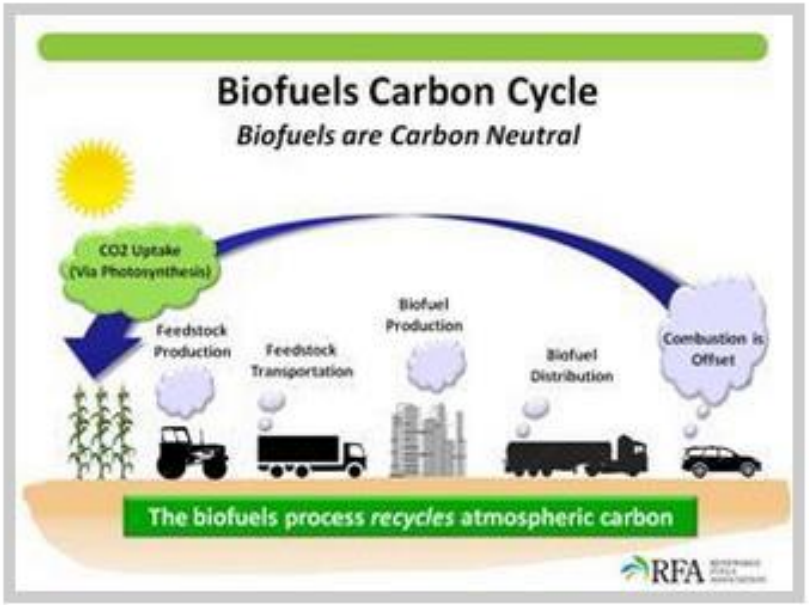
Ethanol production

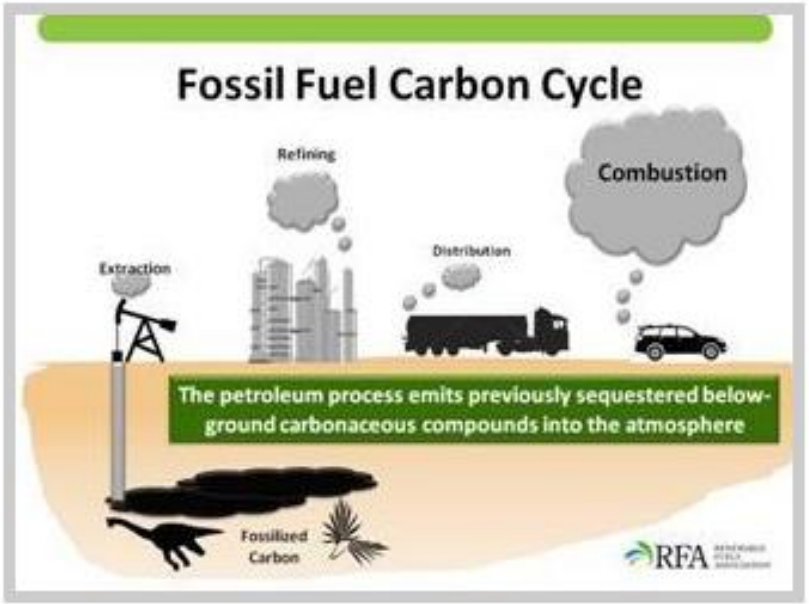
Content of the presentation

I
Global politics regarding biofuels production
Feedstock and its pre-treatment
Processing of lignocelulosic materials
Ethanol fermentation
distillation
and final adjustment

Why to produce biofuels?

- to reduce oil dependance
- to alternate part of fuel consumed in transport with renewable one
- to reduce emissions
- to support sustainable agriculture
- to create new jobs





http://www.ethanolrfa.org/news/entry/rfa-carbon-a
should-be-equitable-based-on-science/

Types of biofuel

- **Biochemical conversion of biomass**
  ethanol, butanol, biogas, biohydrogen

- Thermochemical conversion
  BTL (biomass to liquid), gassification (syngas), methanol, hydrocarbons, biohydrogen

- Oleochemical conversion
  FAME (biodiesel), HVO/HEFA

Definitions

Biofuels of 1st generation - from food crops (e.g. corn, sugar beet, sugar cane)

Biofuels of 2nd generation – from non-food crops or waste mostly lignocellulosic biomass

Biofuels of 3rd generation – from algae biomass

Advanced biofuels:

- produced from lignocellulosic feedstocks
- having low CO_2 emission or high GHG reduction
- reaching zero or low ILUC impact

ILUC – indirect land use change

FOOD OR FUEL?

Nearly a billion people will go hungry tonight, yet this year the U.S. will turn nearly 5 billion bushels of corn into ethanol. That's enough food to feed 412 million people for an entire year.

8 BUSHELS OF CORN = **21.6** GALLONS OF ETHANOL FUEL OR ENOUGH FOOD TO FEED A PERSON FOR A WHOLE YEAR

DOING THE MATH...

5 billion bushels / 8 bushels of corn (enough calories to feed a person for a year) = sufficient calories to support 625 million people, minus one-third to account for distiller's grain (DDG) = 412 million

8 bushels of corn (feeds a person for a year)
X 2.7 gallons of ethanol per bushel
= 21.6 gallons of ethanol per bushel

SOURCES

450 pounds of corn supplies enough calories for one person for a year
(http://www.foreignaffairs.com/articles/62609/c-ford-runge-and-benjamin-senauer/how-biofuels-could-starve-the-poor)

About 5 billion bushels of U.S. corn production is slated for ethanol production
(http://www.usda.gov/oce/commodity/wasde/latest.pdf)

One bushel of corn produces 2.7 gallons of ethanol
(Purdue Extension, "How Fuel Ethanol is Made From Corn," http://www.extension.purdue.edu/extmedia/ID/ID-328.pdf)

resourcemedia

Legislation

In EU

-directives 2003/30/EC and **RED (Renewable Energy Directive) 2009/28/EC**

- compulsory blending of gasoline with min. 4.1% v/v of ethanol and diesel with 6% v/v with biodiesel

-**strategic goal 20-20-20** (20% reduction in greenhouse gas emissions, 20% reduction in primary energy consumption, 20% of total energy from renewable sources in comparison with year 1990 by the year 2020)

-**April 28, 2015 – new iLUC directive**

In U.S.A.

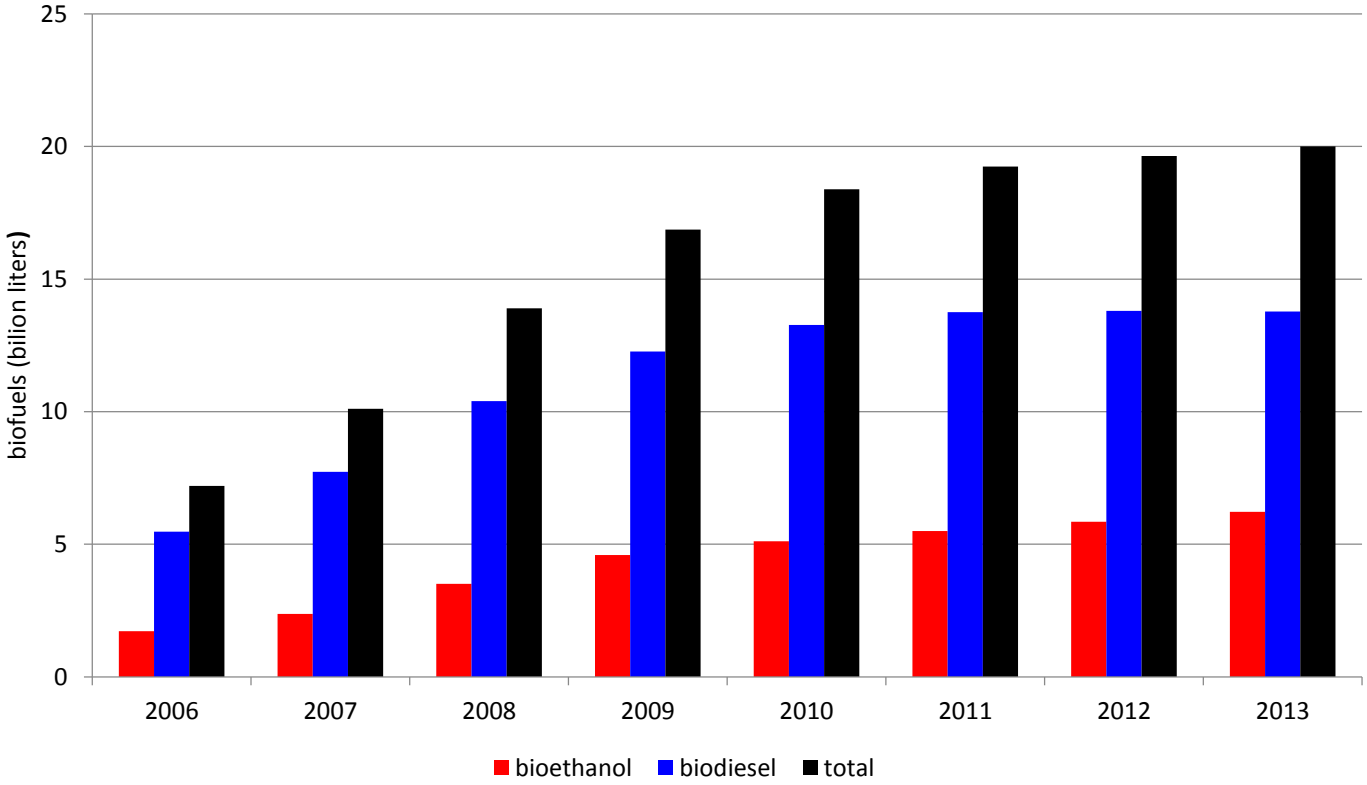
- Energy Independent and Security Act of 2007 (EISA)

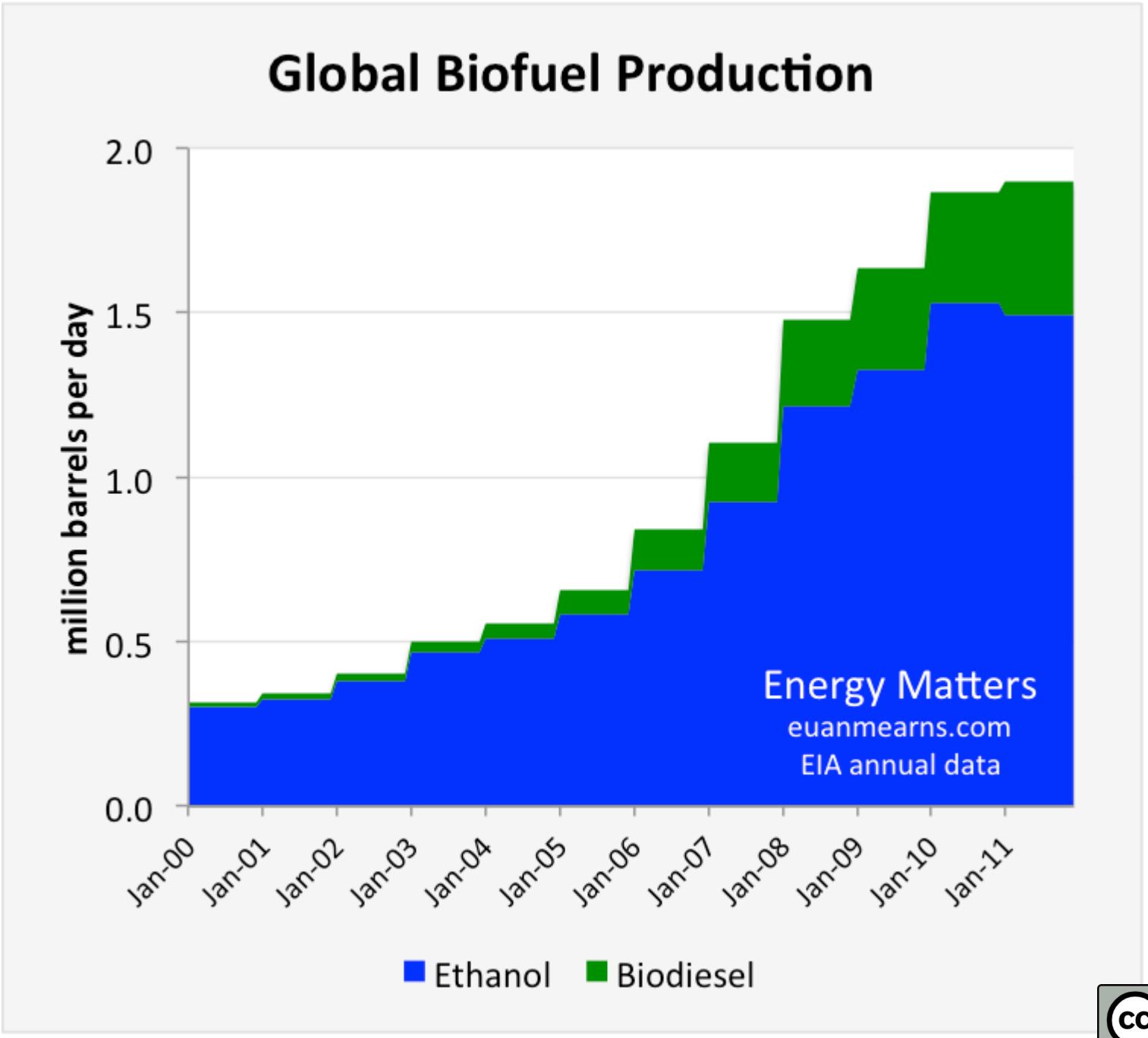
-RFS – renewable fuel standard – allows to blend gasoline with up to 10% v/v ethanol or 16 % v/v of 1-butanol

-strategic goal - by 2022 to use 136 mil. L of renewable fuels in transport

EU transport fuel consumption

- **2012 - bioethanol 28% market, biodiesel 72% market**




Global Biofuel Production
million barrels per day
2.0
1.5
1.0
0.5
0.0
Jan-00
Jan-01
Jan-02
Jan-03
Jan-04
Jan-05
Jan-06
Jan-07
Jan-08
Jan-09
Jan-10
Jan-11
Energy Matters
euanmearns.com
EIA annual data
Ethanol
Biodiesel

Asian countries
Motivation – reduction of dependance on oil, reduction of GHG

Country	Current Feedstock		Ethanol (millions of liters)		Biodiesel Â (millions of liters)	
	Ethanol	Biodiesel	2008	2012	2008	2012
China	Corn, wheat, cassava	Waste vegetable oil	2,002	2,433	355	568
India	Molasses	Jatropha, pongamia	2,150	2,171	317	300
Indonesia	Molasses, cassava	Palm oil	212	300	753	1,300
Malaysia	None	Palm oil	70	N/A	443	178
Philippines	Sugarcane	Coconut oil	105	245	211	393
Thailand	Molasses, cassava	Palm oil, waste cooking oil	408	1,120	48	860
Vietnam	Molasses, cassava	Animal fat	164	N/A	0	N/A
TOTAL			5,111	6,269	1,772	3,59

Feedstock of first generation

a) saccharose containing

molasses (waste stream from sugar industry), sugar beet, sugar cane

b) **starch containing**

corn, maize, potatoes, cassava

c) **Other** – jerusalem artichokes (inulin)

Feedstock pre-treatment

Molasses – clarification, sludge separation, acidification, addition of necessary nutrients (ammonium and phosporous salts…), (sterilization)

Sugar beet, sugar cane – washing, cutting, water extraction →juice production (clarification, concentration, addition of nutrients)

Corn, maize – milling, two step enzyme hydrolysis of starch (liquefaction + saccharification) (in past acid hydrolysis)

Cassava – peeling, cutting, two step enzyme hydrolysis of starch (liquefaction + saccharification)

Jerusalem artichokes – washing, cutting, water extraction of inulin, inulinase treatment or acid hydrolysis

Feedstock of second generation (non-food biomass) and its pre-treatment

Agricultural waste – wheat and rice straw, corn stover, bagasse…
Municipal waste, paper
Fast growing trees (poplar, willow), sawdust, twigs…

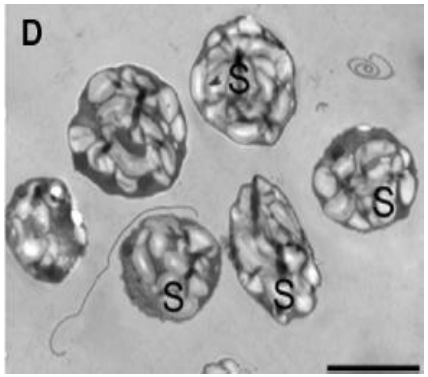
http://energyfromwasteandwood.weebly.com/generations-of-biofuels.html

Pre-treatment

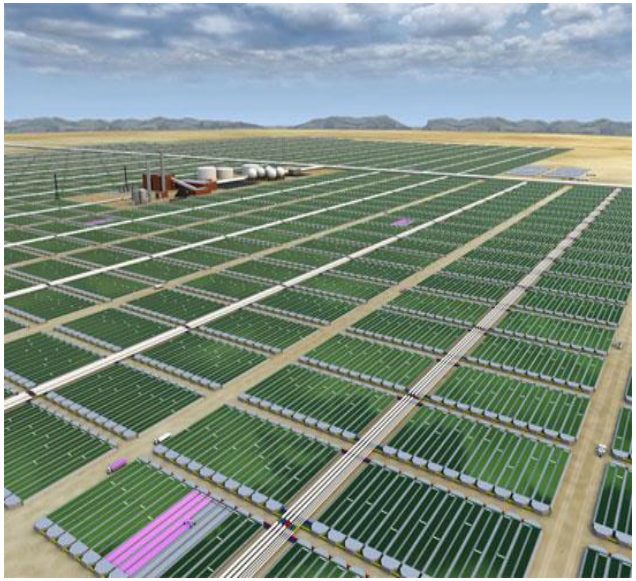
1. Mechanical – cutting, milling
2. Physico-chemical – acid/alkaline hydrolysis, ammonium expansion (AFEX), steam explosion…
3. Enzyme decomposition

Feedstock of third generation - algae

Fresh water micro algae – *Chlorella* (starch accumulation in cells – up to 60% of dried weight)



Brányiková et al., 2009



http://www.energyrant.com/algae-biodiesel-the-real-green-fuel/

Sea weeds – macro algae *Ulva, Gracillaria*… (starch accumulation in cells)

Pretreatment

Harvesting (sedimentation, centrifugation..), drying, desintegration of cells, enzyme hydrolysis (possible use of both starch and cellulose in cell wall)

First generation ethanol - bioethanol

In EU – 4,6 bilion litres of sugar beet and corn ethanol in 2010
In USA – 45 bilion litres of corn starch ethanol in 2010

Ethanol fermentation by *Saccharomyces cerevisiae*

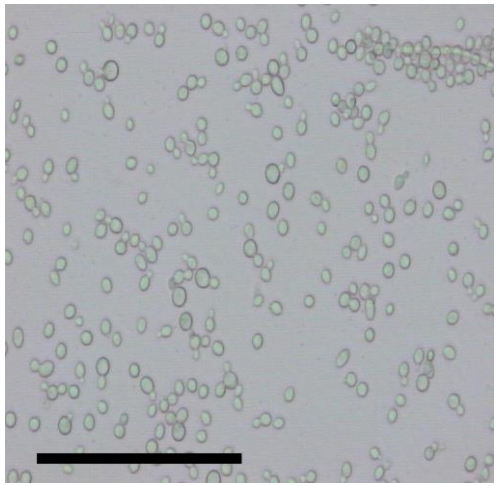
$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$

Theoretical yield:

100% glucose → 51.14% EtOH + 48.6% CO_2

Reality – 6% of by-products + yeast maintenance

glycerol, higher alcohols, aldehydes, acetoin, organic acids and their esters (generated mainly in glycolysis and from present amino acids; depends on used feedstock and nitrogen source)



http://www.vscht.cz/main/soucasti/fakulty/fpbt/ostatni/miniatlas/images/kvasink

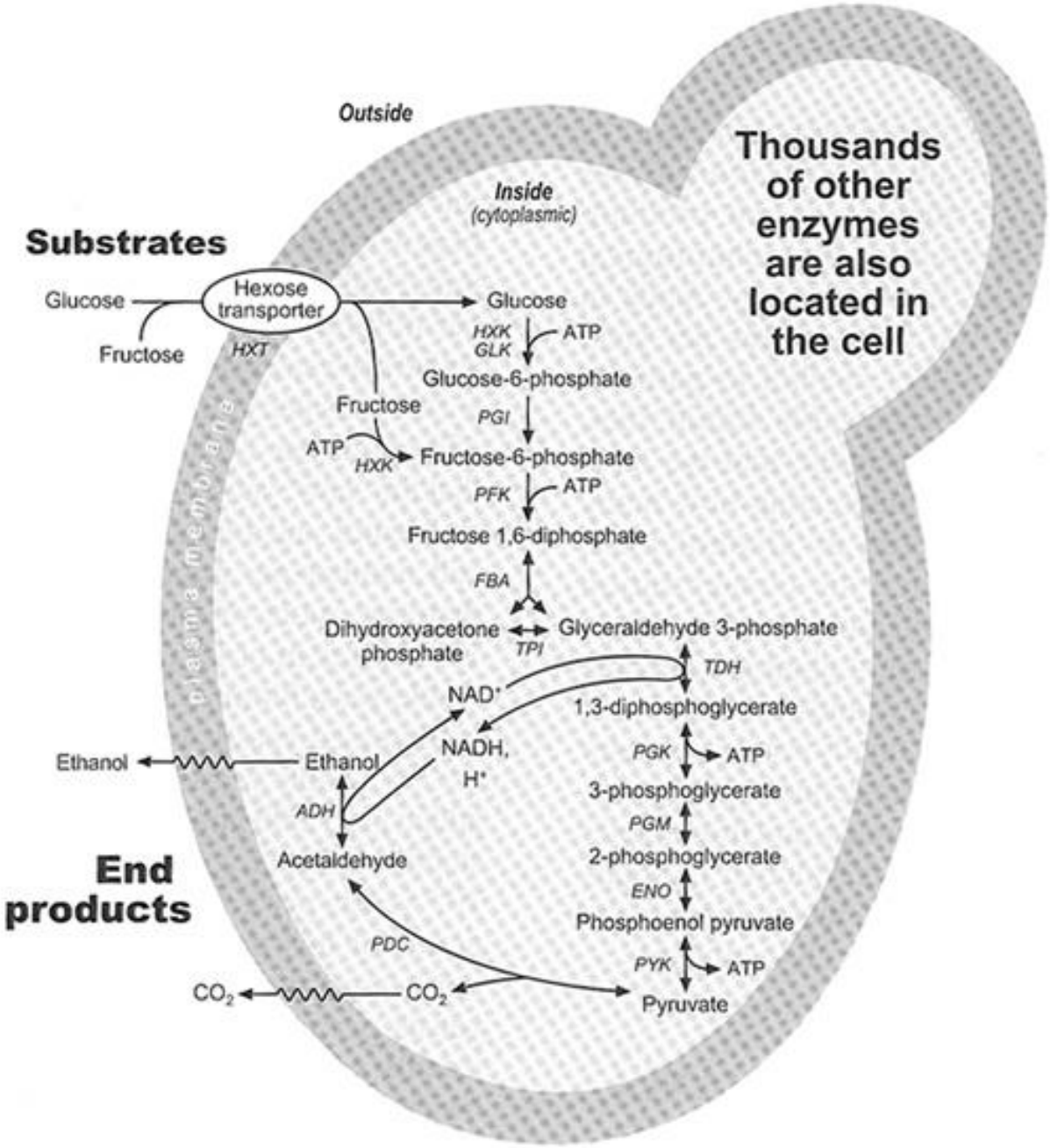
By-products of ethanol fermentation I

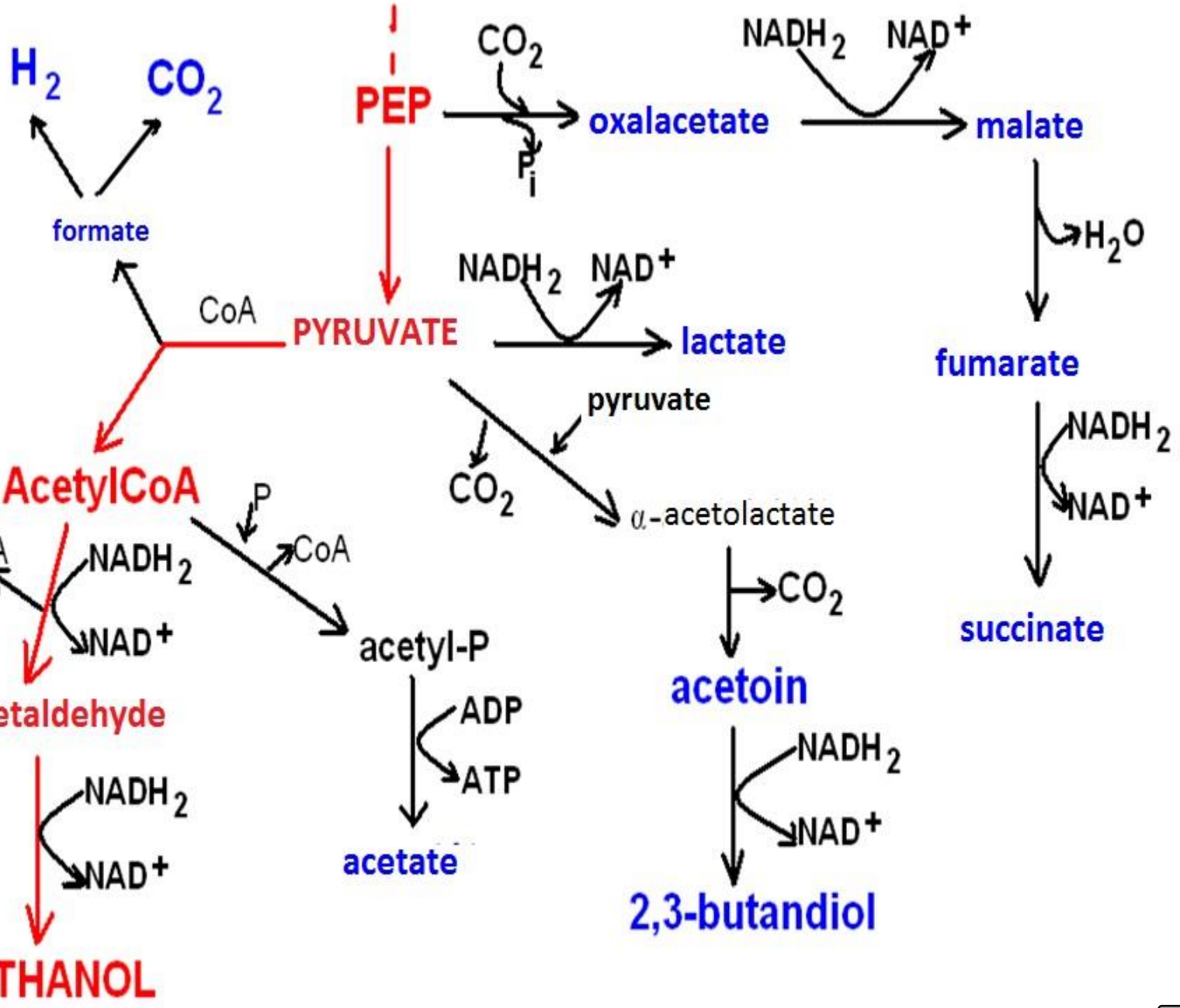
- Glycerol - at the start of fermentation in the absence of acetaldehyde (in glycolysis)
- Higher alcohols - a part of the so-called fusel - amylacohols, isobutyl alcohol (deamination of amino acids)
- Aldehydes - fusel- Isovaleraldehyde (from Leucine), Acetaldehyde (Glycolysis)
- Acetoin, 2,3-butanediol (from glycolysis)

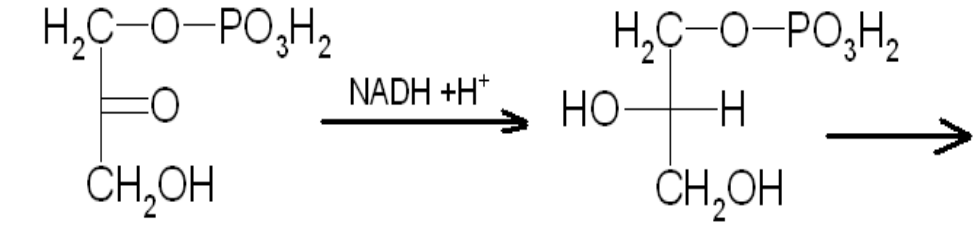
By-products of ethanol fermentation II

- Organic acids - acetic acid, succinic acid ... (both in glycolysis and otherwise)
- Esters of organic acids
- Methanol - (from pectin and pulp)
- Hydrogen cyanide and benzaldehyde - (from fruit stones)

Compounds produced by the activity of contaminating micro-organisms

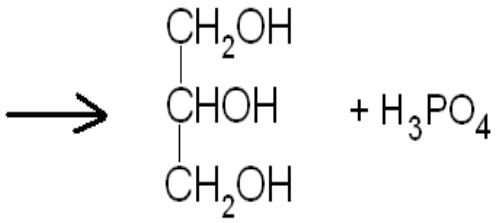

Outside
Inside
(cytoplasmic)
Thousands of other enzymes are also located in the cell
Substrates
Glucose
Fructose
Hexose transporter
HXT
Glucose
HXK
GLK
ATP
Glucose-6-phosphate
PGI
Fructose
ATP
HXK
Fructose-6-phosphate
PFK
ATP
Fructose 1,6-diphosphate
FBA
Dihydroxyacetone phosphate
TPI
Glyceraldehyde 3-phosphate
TDH
NAD+
1,3-diphosphoglycerate
NADH, H+
PGK
ATP
3-phosphoglycerate
PGM
2-phosphoglycerate
ENO
Phosphoenol pyruvate
PYK
ATP
Pyruvate
Ethanol
Ethanol
ADH
Acetaldehyde
PDC
CO2
CO2
End products
plasma membrane


H2
CO2
formate
PEP
CO2
Pi
oxalacetate
NADH2
NAD+
malate
H2O
fumarate
NADH2
NAD+
succinate
CoA
PYRUVATE
NADH2
NAD+
lactate
pyruvate
CO2
α-acetolactate
CO2
acetoin
NADH2
NAD+
2,3-butandiol
AcetylCoA
P
CoA
CoA
NADH2
NAD+
acetyl-P
ADP
ATP
acetate
acetaldehyde
NADH2
NAD+
ETHANOL



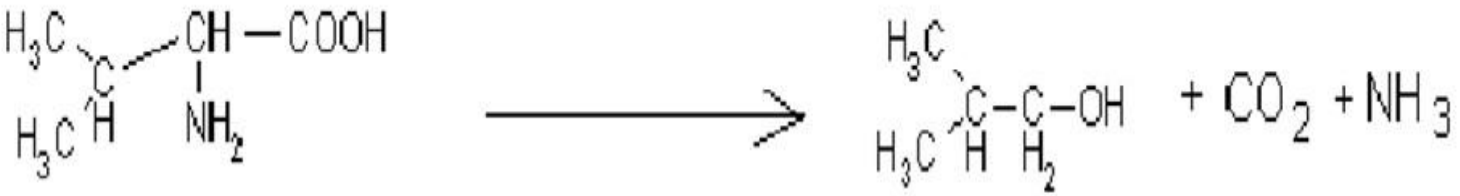
dihydroxyacetonfosfát

glycerolfosfát



Glycerol

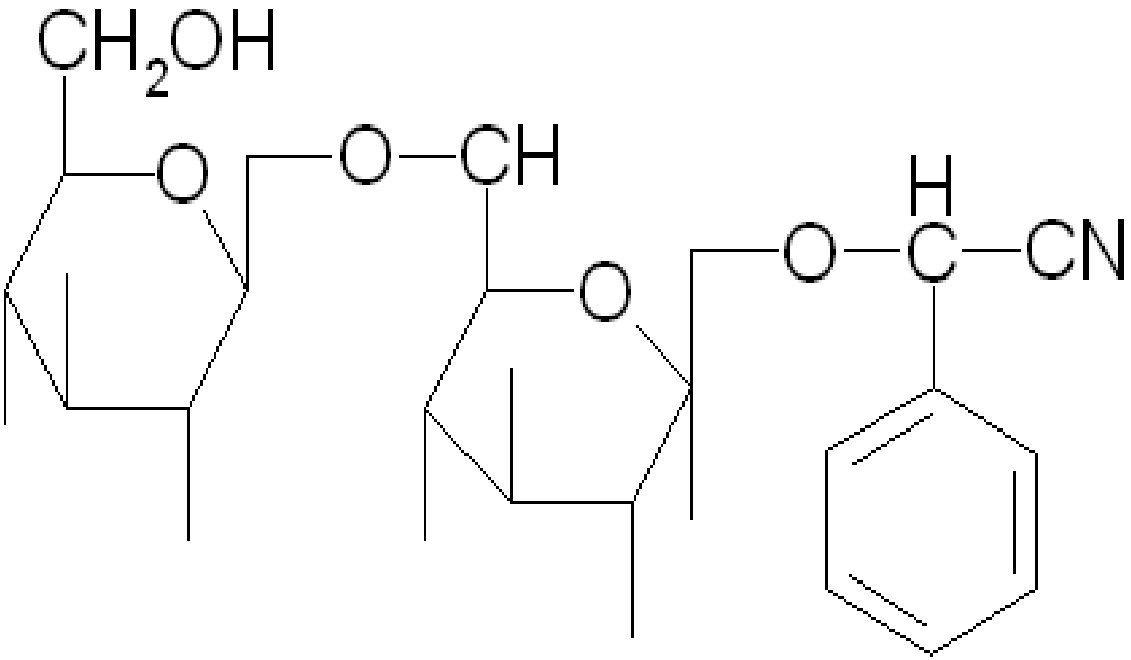
Formation of higher alcohols from amino acids



valine

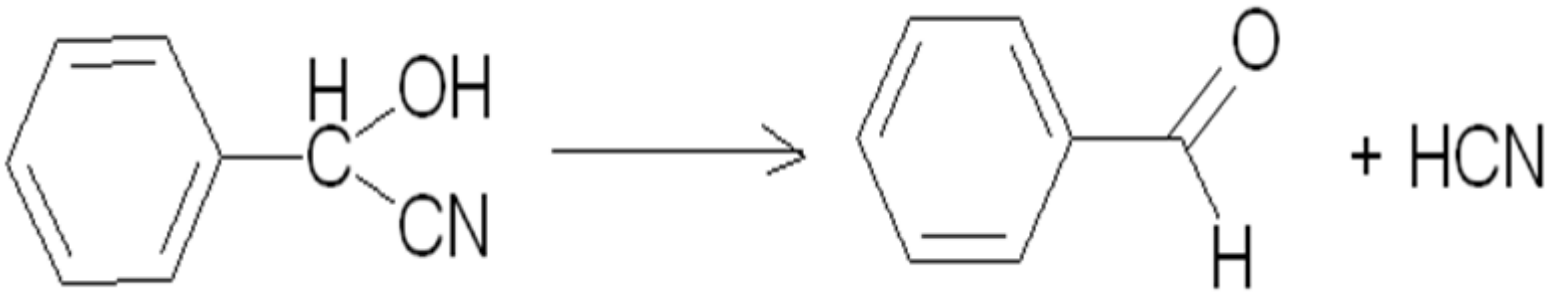
isobuthyl alcohol

AMYGDALIN



gencibiosa **benzaldehydkyanhydrin**

Formation of benzaldehyde and hydrogen cyanide

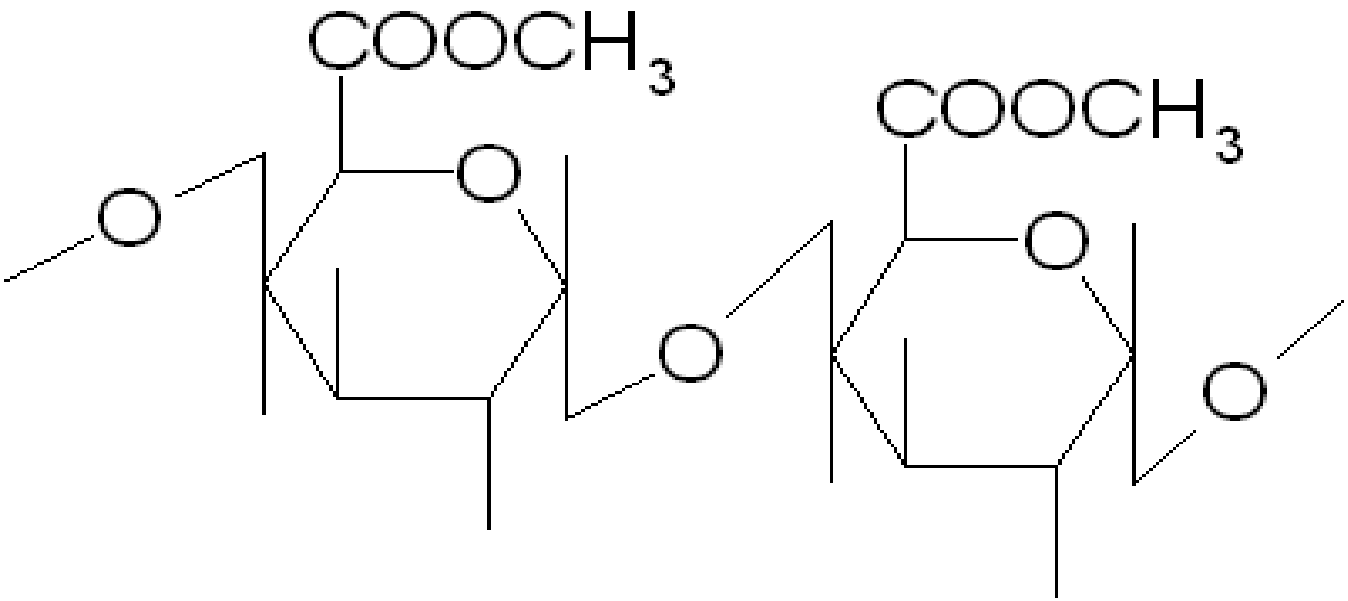


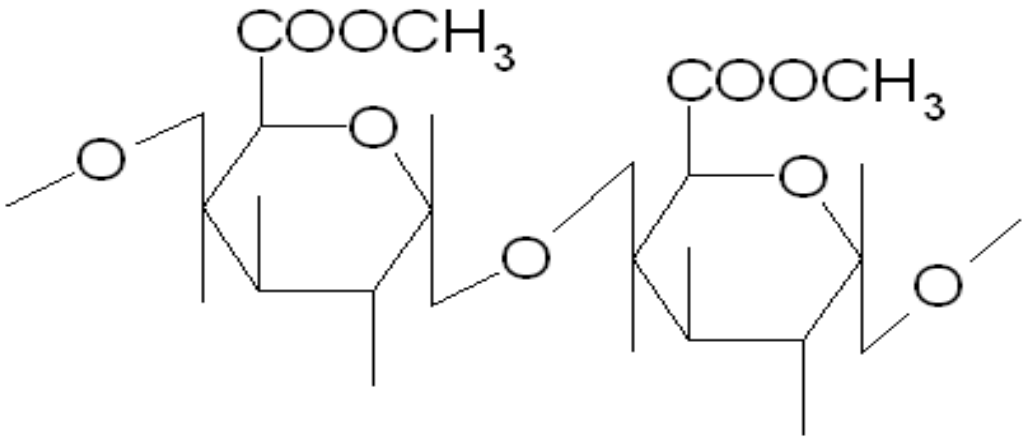
Formation of methanol

Protopectin – complex of pectin with cellulose, arabanes and galactanes

↓ protopectinase

Pectin





Methylester of poly-D-galacturonic acid

↓pekcinesterase, polygalacturonidase

CH_3OH + monogalacturonic acids

Ethanol fermentation

1. **Medium preparation** – feedstock pre-treatment, (nutrients addition, sterilization)
   Nitrogen source – NH_4^+ →increase of glycerol production
   - amino acids →increase of fusel alcohols and aldehydes
   -nitrogen limitation – low fermentation rate
2. **Yeast propagation –** high inoculum concentration, usually aerobic process – high growth rate
   (in some distilleries – use of dried yeasts without propagation or yeast recirculation)
3. **Fermentation** – temp. 30°C, pH 4.5-5, 16-48 h (depends on the process arrangement), 10-14 % v/v final ethanol concentration, yield 90-95 % of max. theoretical yield
4. Fermentation control – microbiological, analytical (saccharide and ethanol concentrations)

Process arrangement – ethanol fermentation

1. Batch – only in small distilleries for alcohol beverages
2. **Fed-batch** – traditional, proven solution – with or without yeast recirculation
3. **Continuous and semi-continuous processes** – nowadays preferred solution, usually battery of bioreactors working up to 21 days

Pros

- no inoculum preparation
- reduction of operation times necessary for cleaning and sterilization

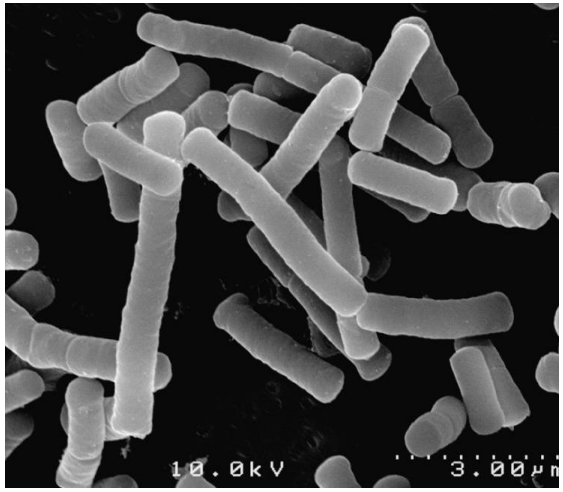
cons

- **risk of contamination***
- lower yield and final ethanol concentration

** Only 16% of fuel ethanol in USA is currently produced in continuous systems due to contamination problems.*

Fermentation contaminants

1. **Lactic acid bacteria** - group of different bacteria, the most dangerous *Lactobacillus fermentans* – living on distilleries equipment, forming biofilm
2. Anaerobic bacteria (*Clostridium* sp.)
3. Mycotoxins from rotten feedstock



Lactobacillus sp.
http://www.institut-rosell-lallemand.com/page.php?idPage=33&idLangue=2

By-products of fermentation

Dried Yeast - Feed Compound X Losses of EtOH

CO_2 - mixing

- production of dry ice

Distillation

-different arrangement of distillation columns e.g. mash + rectification column followed by rafination column

- **formation of azeotroph** ethanol/water – 98.3% v/v ethanol /2.7% v/v water with lower boiling point than pure ethanol ⇒ impossible to gain pure ethanol using distillation under atmospheric pressure

http://www.cukrovaryttd.cz/vyroba/zavody/agroetanol-ttd-lihovar-dobrovice/

Different fate of ethanol – superfine ethanol for alcoholic beverages of anhydrous ethanol ?

Distillation

Boiling point EtOH 78.32 ^{o}C

Rectification = Repeated distillation

Azeotropic mixture - EtOH: water

97.3% v / v: 2.7% v / v, boiling point 78.15 ° C

Azeotropic point - the volatility of both components is the same

↓

Reduction of column pressure - shift of azeotropic point → p = 9.3 kPa - anhydrous alcohol

Destillation

Deflegmation - fractional condensation of the steam mixture - condensation of the water condenses (condensation of the alcohol content in vapors) - reflux - reflux

Backflow ratio - R = L / D

L - moles (amount) of returned distillate

D - moles of the distillate removed

Destillation

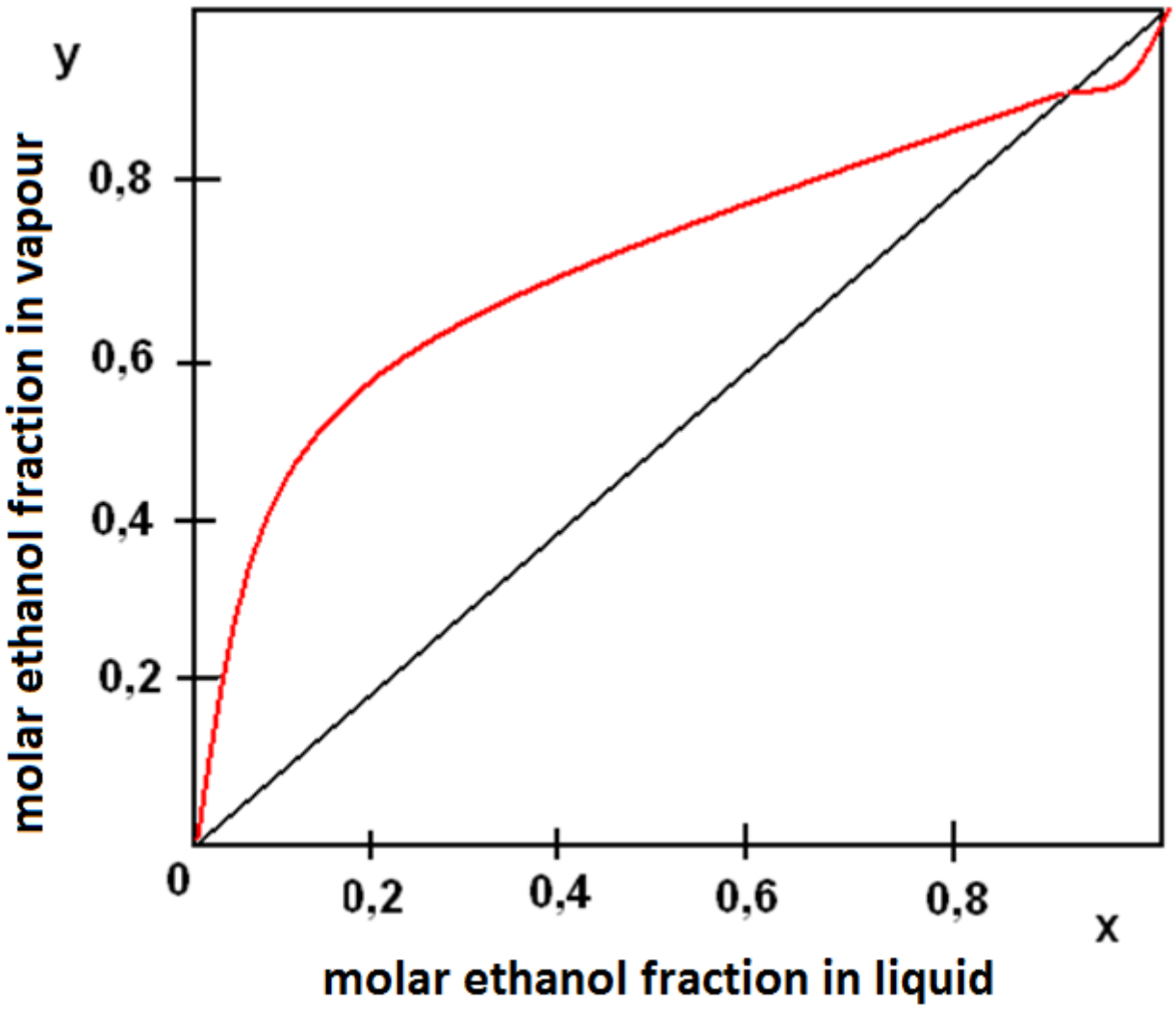
Refining coefficient

The ratio between the impurities in the vapor and the liquid

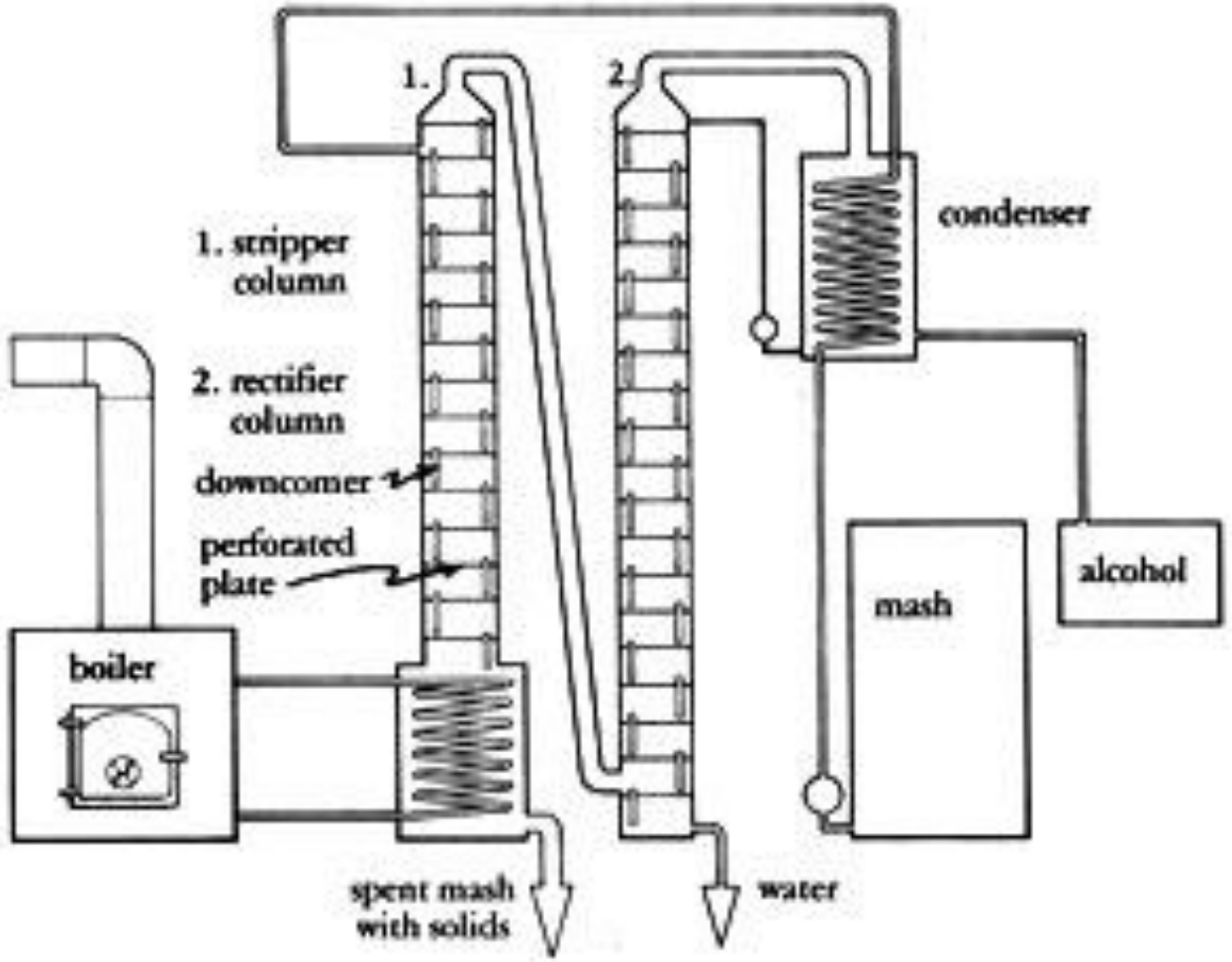
> 1 ....head of boil, compounds more volatile then ethanol

<1 ....bottom of boil, compounds less volatile than ethanol

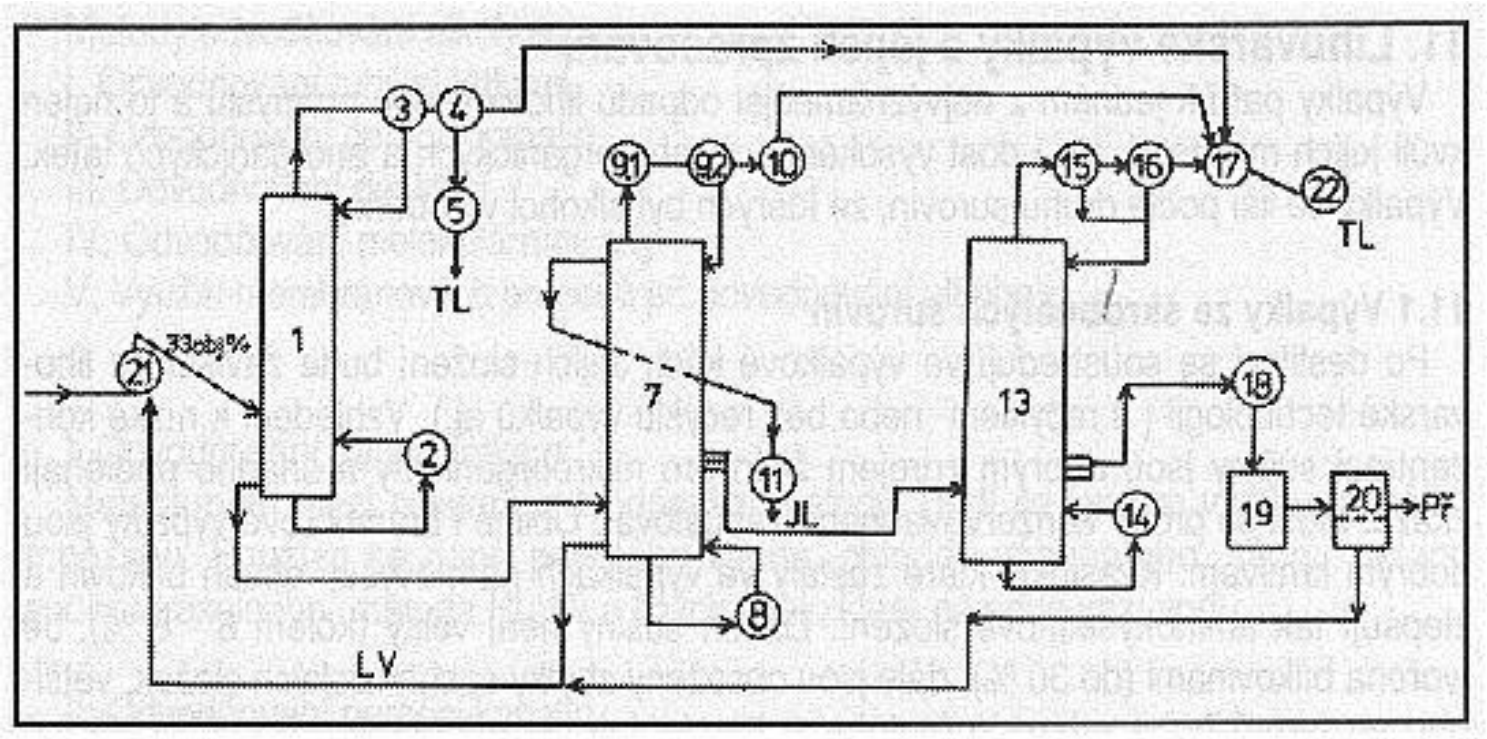
Dependence between EtOH in vapour and in liquid during equilibrium distillation



Two columns mash distillation



Barbet refining device



1- dropping column, 2,8,4 - cooker, 3, 9,1; 9.2; 15 and 16 - deflector; 4, 10, 17 - condensator;
5, 11, 18, 22 - cooler; 7 - a refining column with a luter column; 13 – bottom boil column;
19 – fusel washing machine; 20 - a decanter to separate the brim;
21 - mixing of raw alcohol with luter water (LV); JL - refined alcohol;
TL - technical alcohol; Př- fusel; Do -fusel

Anhydrous ethanol

1. **Molecular sieves (zeolites)** in column arrangement – separation of ethanol/water based on size of molecules
2. Azeotropic distillation – increase of partial pressure of water by addition of benzen
3. Extractive distillation – addition of hygroscopic liquid (glycerol)
4. Distillation under decreased pressure

Zeolites structures

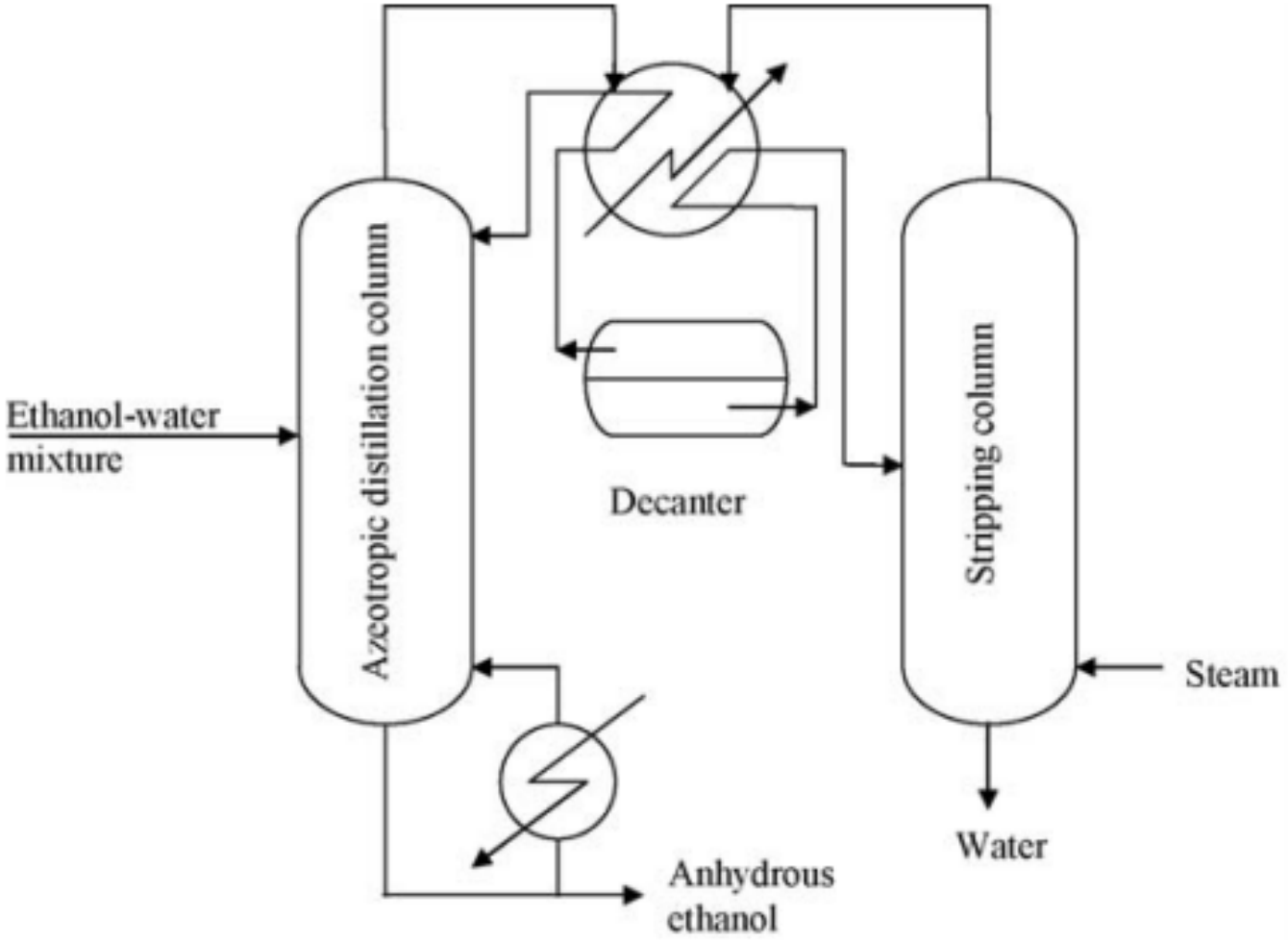


Fig. 2. Heterogeneous azeotropic distillation of ethanol–water mixtures.

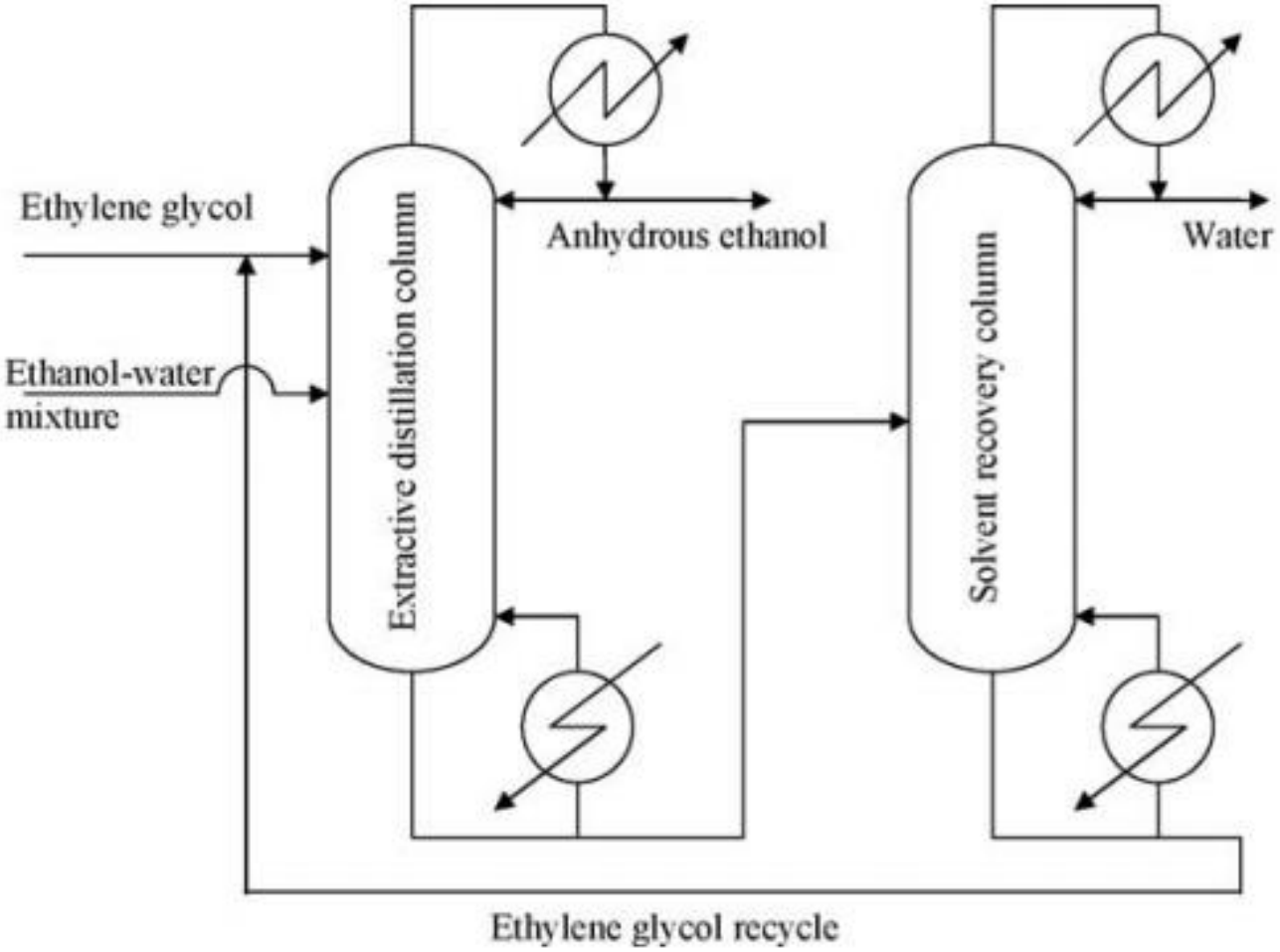


Fig. 3. Extractive distillation of ethanol–water mixture with ethylene glycol.

Production of alcohol

Duties of the distillery:

Alcohol production on the device guarantees reliable detection of the amount of alcohol produced

Measuring the amount of alcohol produced by approved meters

Customs supervision (excise duty)

Storage of alcohol

Working with Class I flammables

Room ventilation - max. 1 g / m^3

Flash point 12^oC for 100% alcohol

Explosion limits:

2.8% to 13.7% vapor in air

50 to 260 g / m^3

Prohibition of open flame handling

Denaturation of ethanol

Alcohol for burning, heating, technical purposes

Denaturant - a mixture of 3-4 substances

Unpleasant taste and smell

It can not be easily removed from alcohol

1-2%

Denaturation for fuel purposes - mixture of naphtha, kerosene and rock-oil

By-products of ethanol fermentation

1. Biomass
2. Stillage, destillation slops–feed, fertilizer, biogas production
3. CO_2
4. Fusel

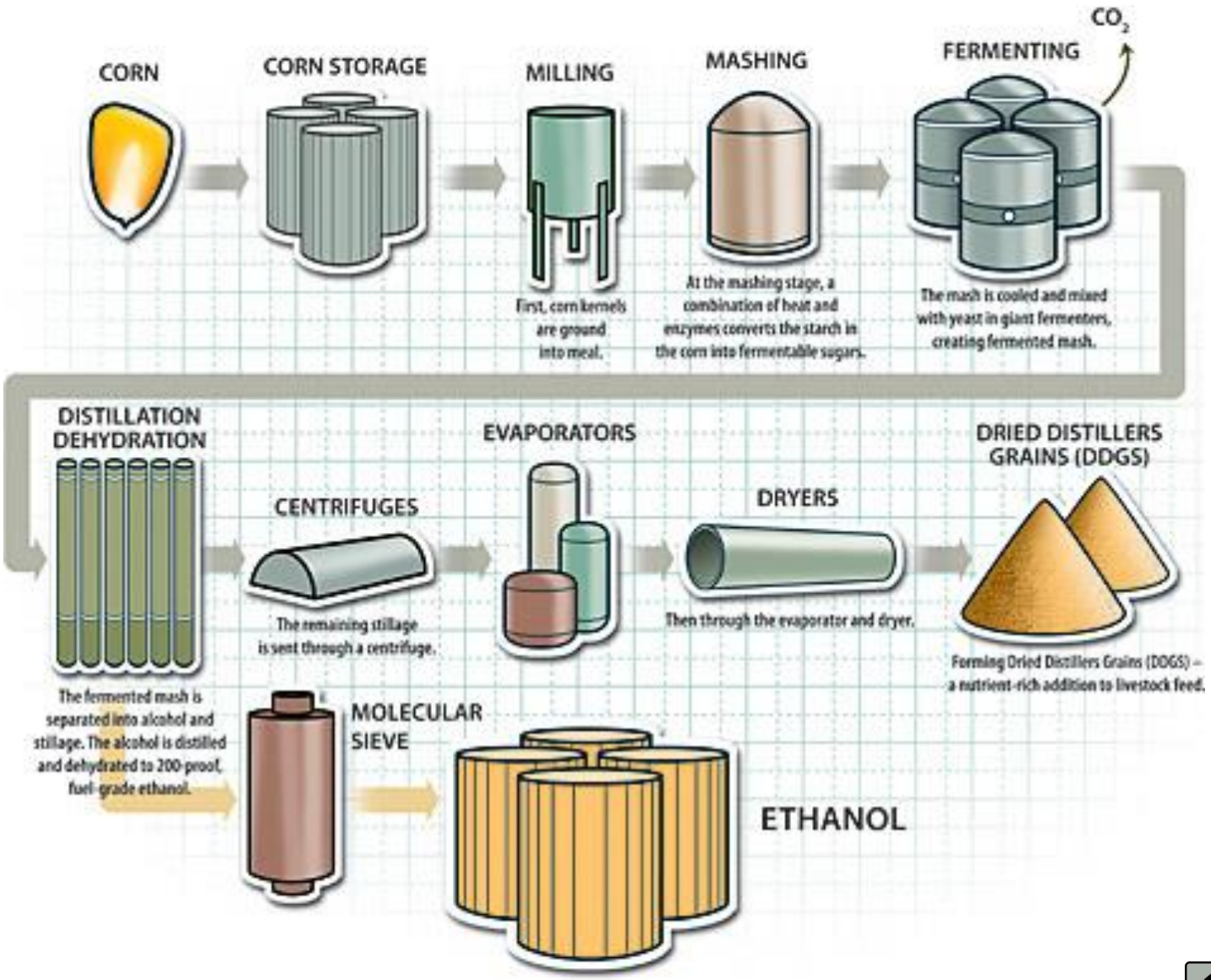
Use of anhydrous ethanol - mixing with petrol

EU - 5.75% mandatory ethanol content in petrol fuels
RED - renewable energy directive - 2020 targets

Other blends - E10, E85, E75 .... (FFV -flexi fuel vehicles- engine modification)

USA - RFS (renewable Fuel standard)
Mixing up to 10%

Fuel ethanol from corn (maize)



http://me1065.wikidot.com/ethanol-as-a-fuel

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

MINISTRY OF EDUCATION,
YOUTH AND SPORTS

UNIVERSITY OF
CHEMISTRY AND TECHNOLOGY
PRAGUE

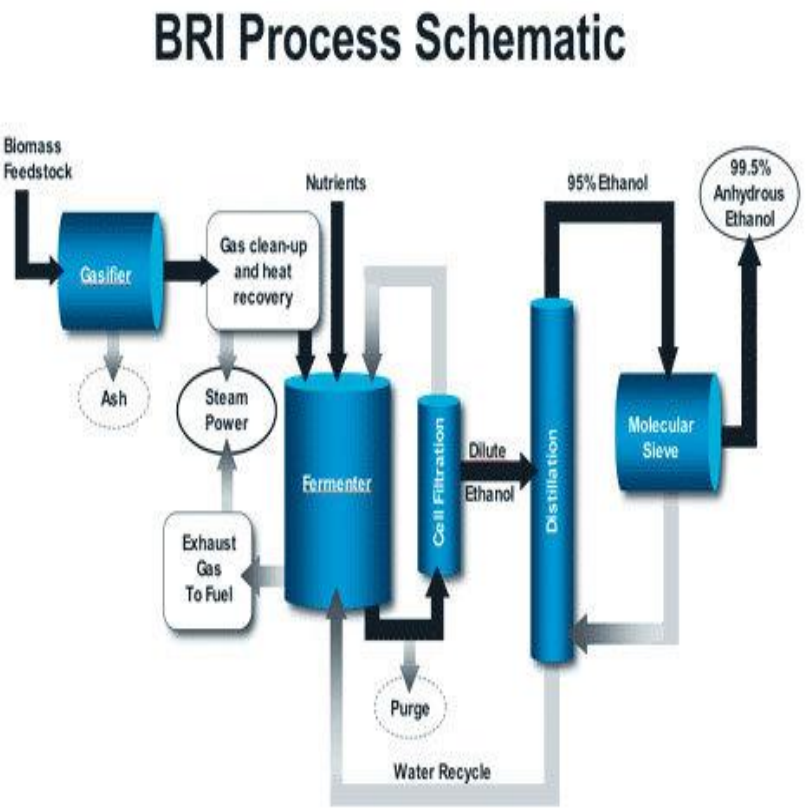
Biotechnology in Food Industry

Lecture VI

Petra Patáková
petra.patakova@vscht.cz

Gas fermentation to ethanol

1. Biomass gassification
2. Wood-Ljungdahl metabolic pathway, acetogens
1. Bioreactor design, medium composition
2. Process parameters
3. Cell separation, product recovery
4. Commercialization
5. Conclusion

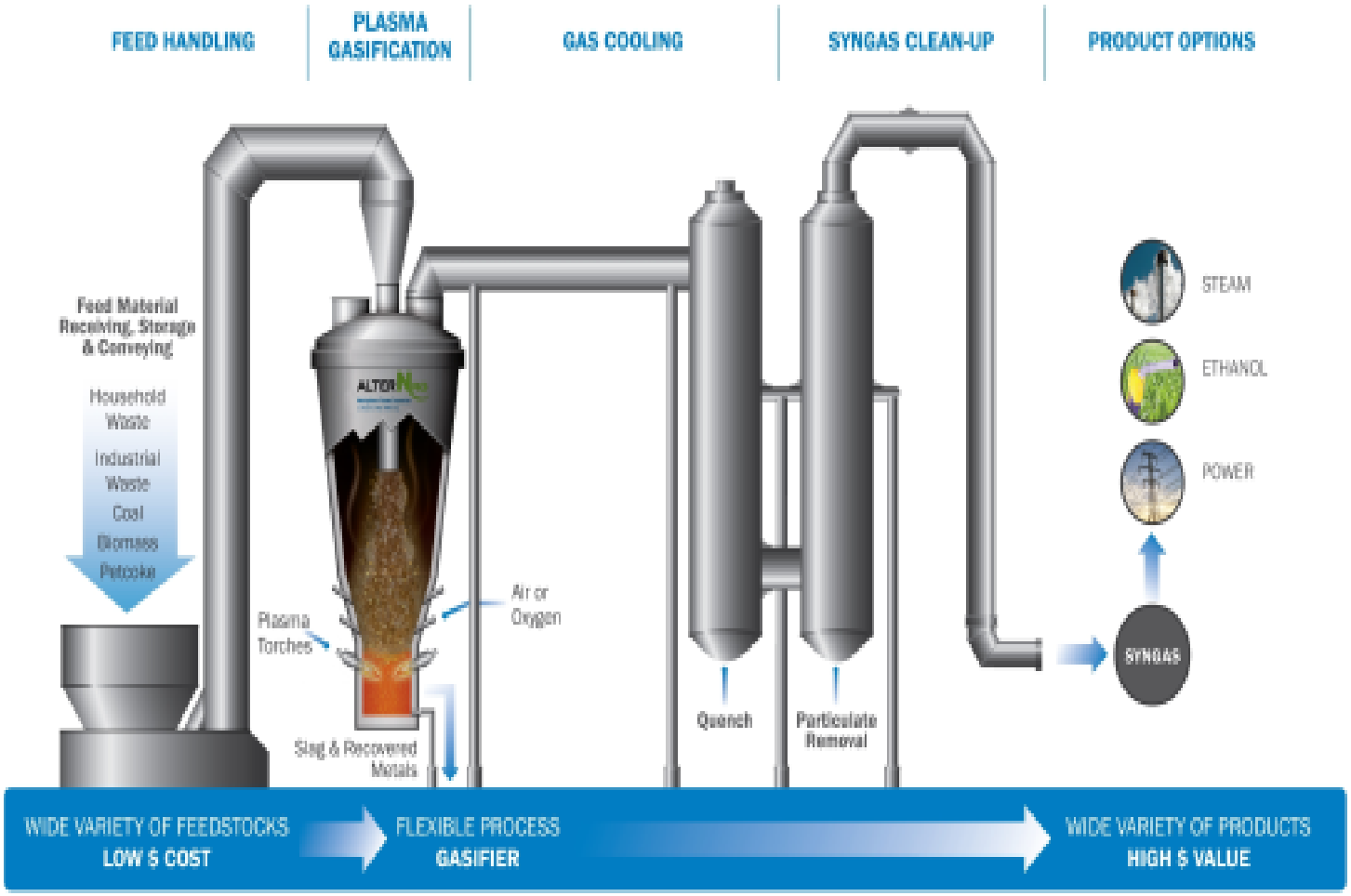


http://thefraserdomain.typepad.com/energy/2005/12/synthesis_gas_f.html

Gas fermentation to ethanol

- Combination of thermochemical and biochemical processing of biomass
- Gassification using high temperature (up to 1500°C) and oxidizing agent (air, oxygen)-drying, pyrolysis, oxidation, reduction
- Processing mixture of gases – CO, CO_2, H_2, N_2, CH_4….
- Acetogenic bacteria, anaerobic, mesophilic or thermophilic
- Reductive acetyl-CoA pathway, Wood-Ljungdahl pathway
- Main products – acetate, ethanol, butyrate, lactate, butanol…

Biomass gassification

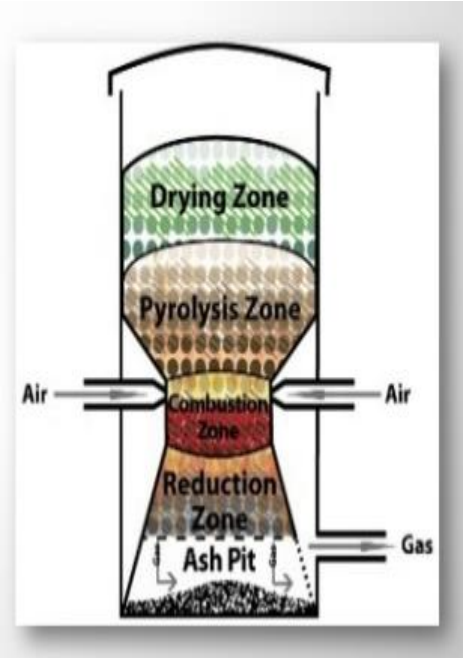


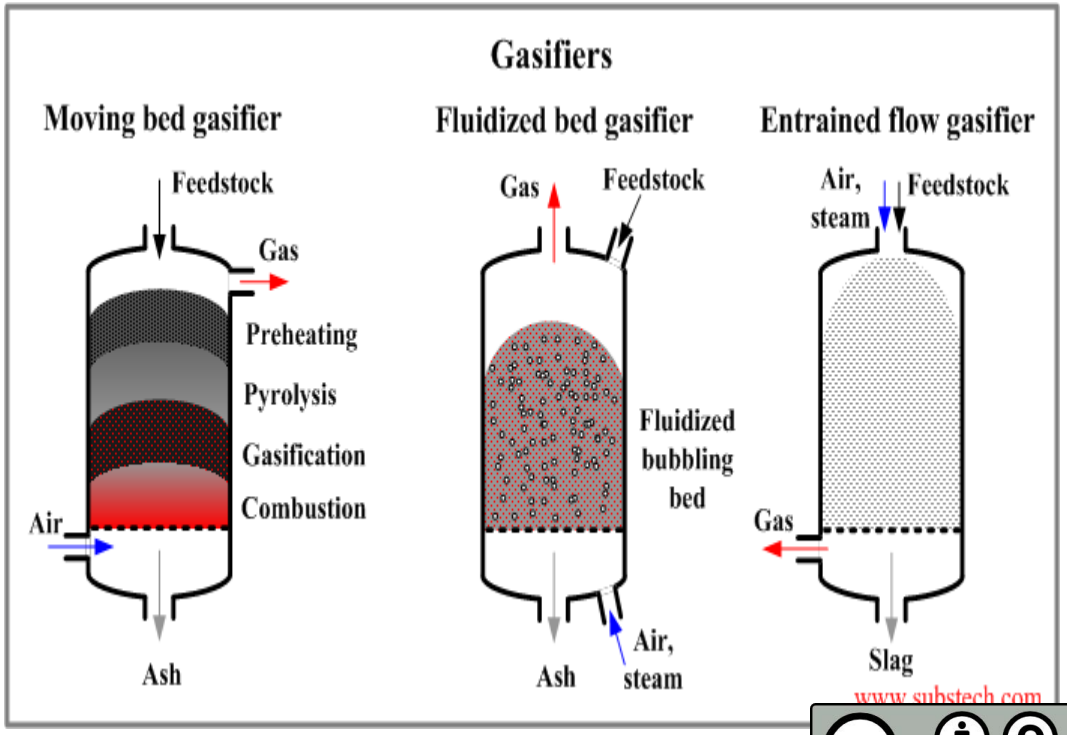
http://wppenergy.com/biomass-gasification.html

Biomass gassification

- **Upstream operations** – drying, size reduction, pyrolysis, fractionation, leaching
- Gassification temperature 600-1000°C
- Oxidizing agent – air, steam, oxygen

Fixed bed gassifier





https://www.slideshare.net/agpoliveira/downdraft-biomass-gasification-experimental-investigation-and-aspen-plus-simulation

Syngas composition and requirements for microbial utilization

- Biomass derived syngas composition:

CO (8-15 %vol.), **CO_2** (15-17 % vol.), **H_2** (3-18 % vol.), **N_2** (56-65 % vol.), **CH_4** (0,4-6 % vol.), **other** (0,4-3 % vol.)

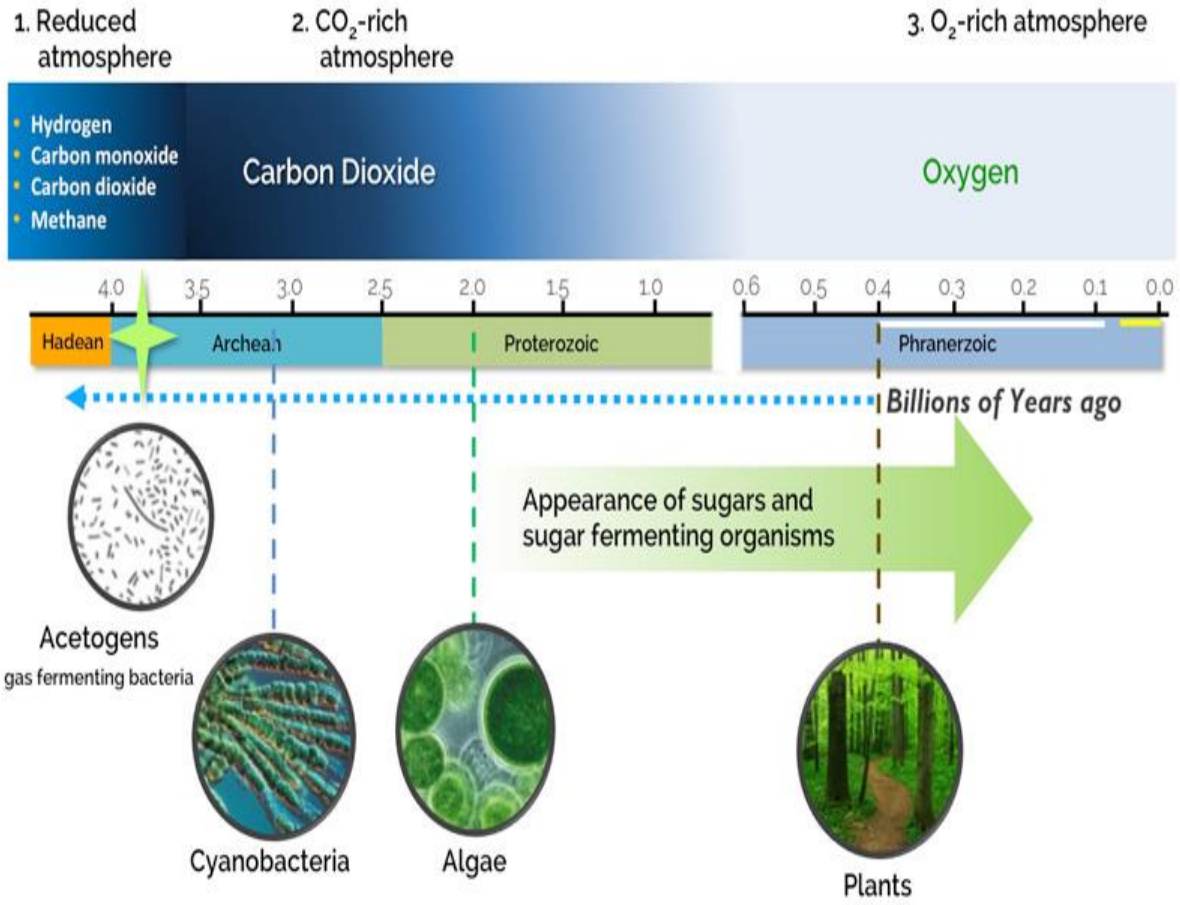
Increase of syngas partial pressure can increase cell density!

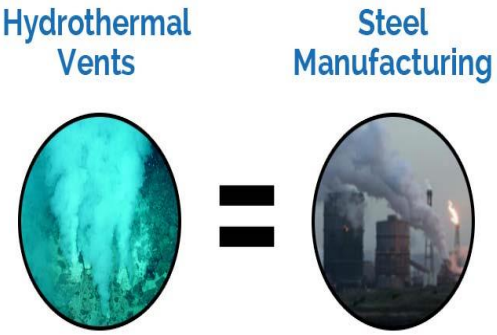
Common impurities:

Tar, ash, char, ethan, ethylene, acetylene, H_2S, NH_3, NO, CN^-

The impurities may cause inhibition/stimulation of syngas utilization in dependance on the particular microorganism.

Our Rock Star: *Clostridium autoethanogenum*



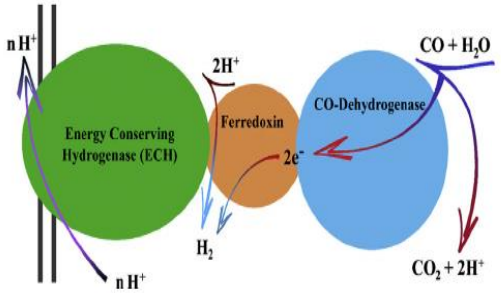


Gases were the only carbon and energy source used by the first life forms.

http://www.lanzatech.com/innovation/te

Wood-Ljungdahl metabolic pathway

water-gas shift reaction



Hosseini et al. 2015

$$CO + H_2O \longrightarrow CO_2 + 2H$$

Acetogens

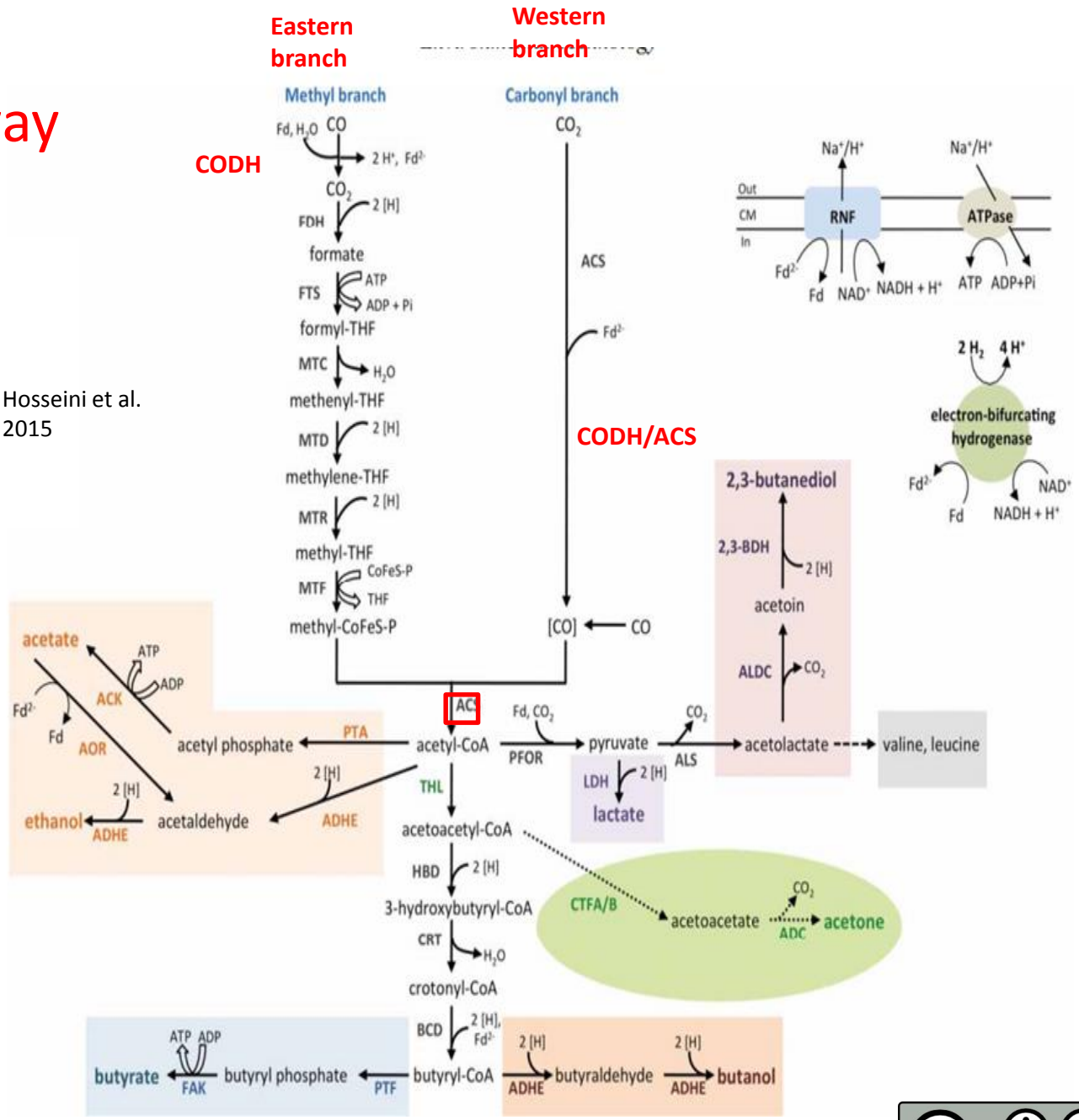
Anaerobic, autotrophic bacteria

Different species – different products

sole end product – acetate

Acetate + ethanol – *Clostridium ljungdahlii*

CODH/ACS –carbon monooxide dehydrogenase/acetyl CoA synthase

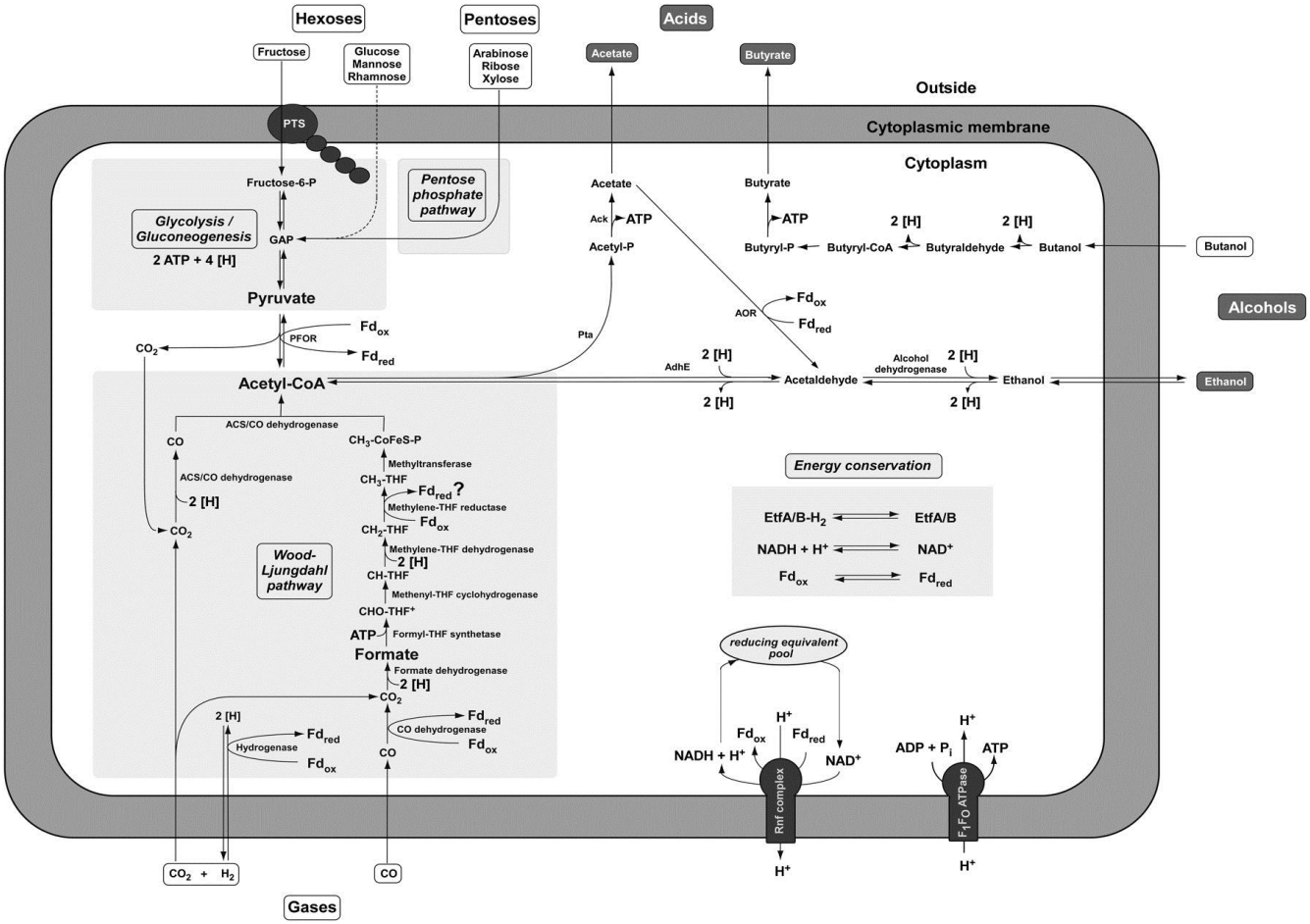


Example of mesophilic acetogens

Species	Substrate	Product(s)	T_{opt} (°C)	pH_{opt}	Genome Status
Mesophilic Microorganisms					
Acetobacterium woodii	H_2/CO_2, CO	Acetate	30	6.8	Available
Acetonema longum	H_2/CO_2	Acetate, butyrate	30-33	7.8	
Alkalibaculum bacchi	H_2/CO_2, CO	Acetate, ethanol	37	8.0-8.5	
Blautia producta	H_2/CO_2, CO	Acetate	37	7	
Butyribacterium methylotrophicum	H_2/CO_2, CO	Acetate, ethanol, butyrate, butanol	37	6	
Clostridium aceticum	H_2/CO_2, CO	Acetate	30	8.3	Under construction
Clostridium autoethanogenum	H_2/CO_2, CO	Acetate, ethanol, 2,3-butanediol, lactate	37	5.8-6.0	
Clostridium carboxidivorans or "P7"	H_2/CO_2, CO	Acetate, ethanol, butyrate, butanol, lactate	38	6.2	Draft
Clostridium drakei	H_2/CO_2, CO	Acetate, ethanol, butyrate	25-30	5.8- 6.9	
Clostridium formicoaceticum	CO	Acetate, formate	37	NR	
Clostridium glycolicum	H_2/CO_2	Acetate	37-40	7.0-7.5	
Clostridium ljungdahlii	H_2/CO_2, CO	Acetate, ethanol, 2,3-butanediol, lactate	37	6	Available
Clostridium magnum	H_2/CO_2	Acetate	30-32	7.0	

http://cdn.intechopen.com/pdfs-wm/43690.pdf

Scheme of basic metabolic pathways and energy conservation in *C. ljungdahlii* when growing heterotrophically on sugars (hexoses or pentoses) or autotrophically on gases (CO or CO2 + H2).

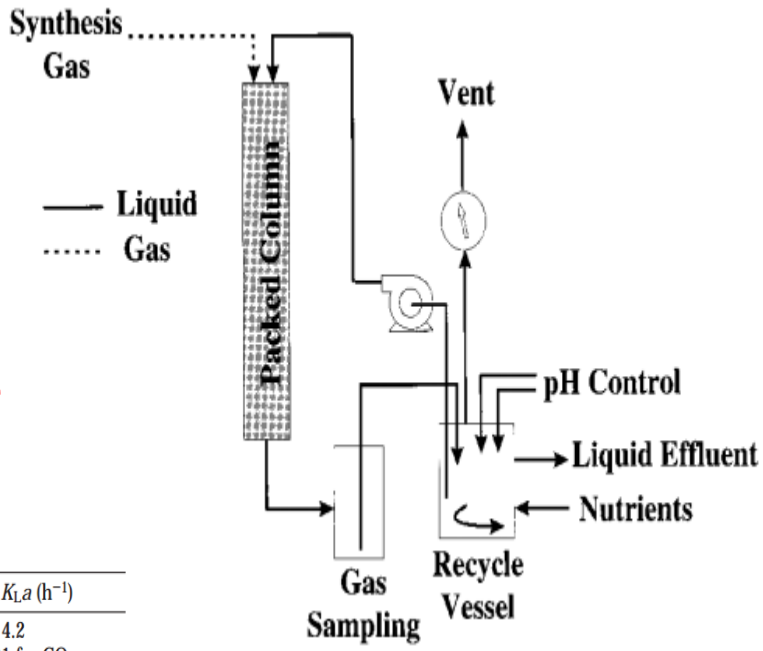


Michael Köpke et al. PNAS 2010;107:13087-13092

Bioreactor design

- Continuous stirred tank reactor (CSTR)
- Bubble column reactor
- Immobilized cell column reactor
- Trickle bed reactor

Gas to liquid/solid mass transfer is rate limiting step



Trickle bed reactor

Table 2. Mass-Transfer Coefficients from Synthesis-Gas Fermentations

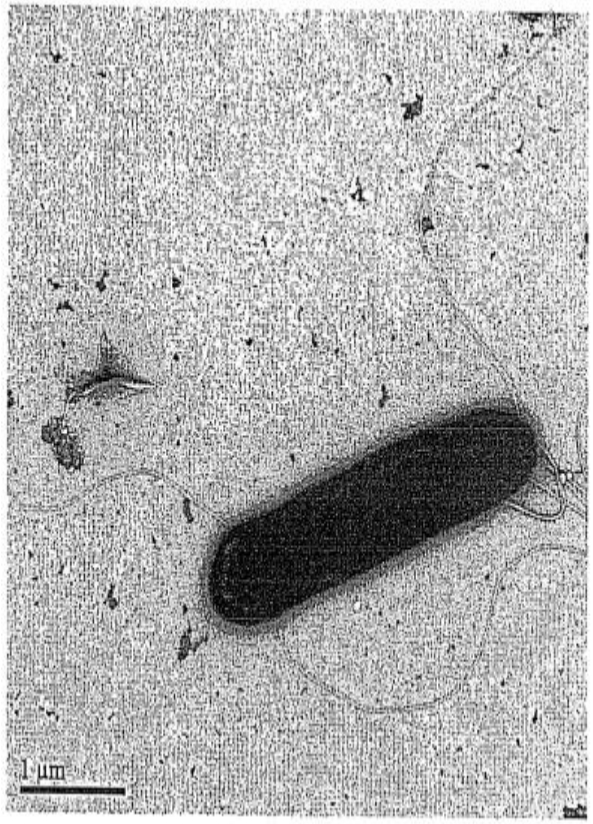
reactor type	biological system	feedstock	K_La (h^{-1})
stirred tank - 200 rpm	*B. methylotrophicum*	CO	14.2
stirred tank - 300 rpm	SRB mixed culture	synthesis-gas mix	31 for CO 75 for H_2
stirred tank - 300 rpm	*C. ljungdahlii*	synthesis-gas mix	35 for CO
stirred tank - 300 rpm	*R. rubrum, M. formicum, M. barkeri*	synthesis-gas mix	28.1 for CO
stirred tank - 450 rpm	*R. rubrum, M. formicum, M. barkeri*	synthesis-gas mix	101 for CO
stirred tank - 200 rpm microbubble sparging	*B. methylotrophicum*	CO	90.6 for CO
stirred tank - 300 rpm microbubble sparging	SRB mixed culture	synthesis-gas mix	104 for CO 190 for H_2
packed-bubble column	*R. rubrum, M. formicum, M. barkeri*	synthesis-gas mix	2.1
trickle bed	*R. rubrum, M. formicum, M. barkeri*	synthesis-gas mix	55.5
trickle bed	SRB mixed culture	synthesis-gas mix	121 for CO 335 for H_2
trickle bed	*C. ljungdahlii*	synthesis-gas mix	137 for CO

Bredwell et al., 1999

Medium formulation

- Syngas quality and partial pressure
- Addition of other medium components – vitamins, trace metals, nitrogen source…- balanced – good for bacterial growth X limitation for solventogenesis
- Reducing agents – low redox potential necessary for strict anaerobes – sodium thioglycolate, ascorbic acid, methyl viologen…
- pH 5-6
- Temperature – 30-40°C for mesophilic, 55-60°C thermophilic

- **Growth can be bi-phasic**
- **Can form spores**
- Many acetogens can grow heterotrophically (fructose, glycerol..)
- Can be adapted for biofilm formation (NaCl stress)



Clostridium coskatii

https://www.google.com/
110229947

Yield, concentration, productivity

Biocatalysts	Reactor/gas composition [a]	Products (g/L)	Yield from CO [b] (%)	Productivity [c] (mg/L·h)
Clostridium ljungdahlii	CSTR with cell recycle (55% CO, 20% H_2, 10% CO_2 and 15% Ar)	Ethanol: 48 Acetate: 3.0	70.2	168.0
	CSTR without cell recycle (55% CO, 20% H2, 10% CO2 and 15% Ar)	Ethanol: 6.50 Acetate: 5.43	38.9	48.8
	Two stage CSTR & bubble column with cell recycle (60%CO, 35% H_2 and 5% CO_2)	Ethanol: 19.7 Acetate: 8.6	100	306.4
Clostridium carboxidivorans	Bubble column reactor without cell recycle (25% CO, 15% CO_2, 60% N_2)	Ethanol: 1.6 Acetate: 0.4 Butanol: 0.6	39.6	42.7
	HFR (20% CO, 15% CO_2, 5% H_2, 60% N_2)	Ethanol: 24.0 Acetate: 5.0	72.0	112.5
	Bubble column reactor (20% CO, 15% CO_2, 5% H_2 60% N_2)	Ethanol: 3.2 Acetate: 2.35	51.0	64.1
	MBR (20% CO, 15% CO_2, 5% H_2, 60% N_2)	Ethanol: 4.9 Acetate: 3.1	51.0	97.9
	Serum bottles (70% CO, 20% H_2, 10% CO_2)	Ethanol: 3.0 Acetate: 0.5 Butanol: 1.0 Hexanol: 0.9	ND [d]	21.4
Clostridium ragsdalei	CSTR (20% CO, 15% CO_2, 5% H_2, 60% N_2)	Ethanol: 9.6 Acetate: 3.4	60.0	26.7
Mixed culture *of Alkalibaculum bacchi & C. propionicum*	CSTR without cell recycle (28% CO, 60% H_2, 12% N_2)	Ethanol: 8.0 Acetate: 1.1 Propanol: 6.0 Butanol: 1.1	30.6	40.0
Clostridium autoethanogenum	CSTR without cell recycle (100% CO)	Ethanol: 0.9 Acetate: 0.9	ND	4.5

[a] CSTR: continuous stirred tank reactor; HFR: hollow fiber membrane reactor; MBR: monolithic biofilm reactor
[b] Ethanol yield = (mol EtOH consumed/mol CO consumed)*100%/(1 mol EtOH/6 mol CO)
[c] Ethanol productivity
[d] ND: not determined

S.cerevisiae **corn, sugar beet** **ethanol** **120** **95-98** **2800-4000**

Misleading information

http://www.ethanolproducer.com/articles/10343/a-waste-filled-proposition

Yield calculation:

Acetogens

$6CO + 3H_2O \rightarrow C_2H_5OH + 4CO_2$

S.cerevisiae

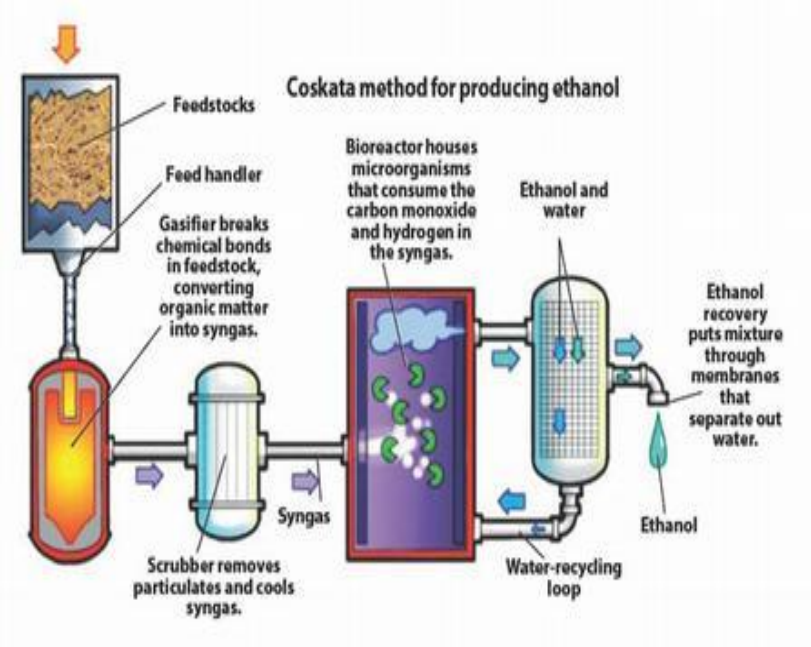
$C_6H_{12}O_6 \quad 2C_2H_5OH + 2CO_2$

Devarapalli and Atiyeh / Biofuel R (2015) 268-280

Cell separation and product recovery

Cell separation – ultra filtration units, spiral wound filtration system, hollow fibres, cell recycling membranes, centrifugation

Product recovery – low product concentration, inhibition of producing strain
Before/ in addition to distillation – liquid-liquid extraction, perstraction, pervaporation, gas stripping…



http://machinedesign.com/news/scanning-ideas-better-way-make-ethanol

Commercialization – Lanza Tech

Pilot plant in China

Mobile pilot in USA

http://www.lanzatech.com/facilities

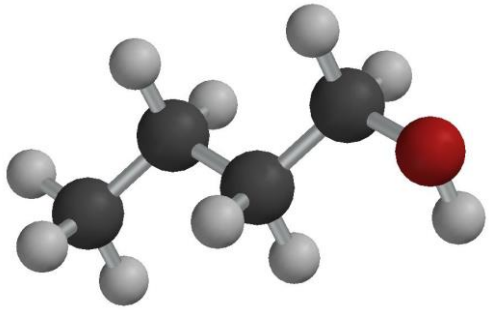
Conclusion for syngas to ethanol process

- from microbiological point of view extremely interesting
- from bioengineering point of view demanding
- from biotechnological point of view challenging
- from industrial point of view underdeveloped

Overall evaluation - promissing

Biobutanol production - Why biobutanol?

As Fuel extender



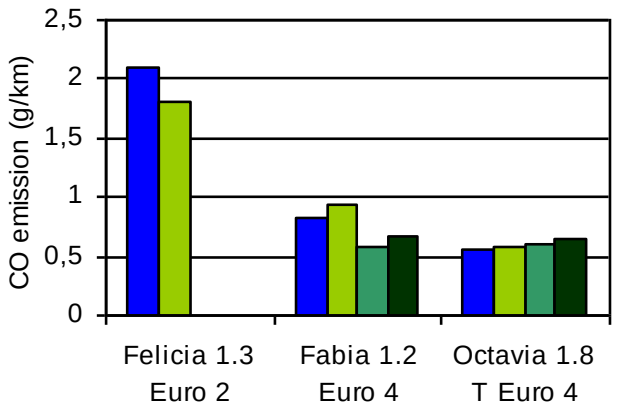
1-butanol

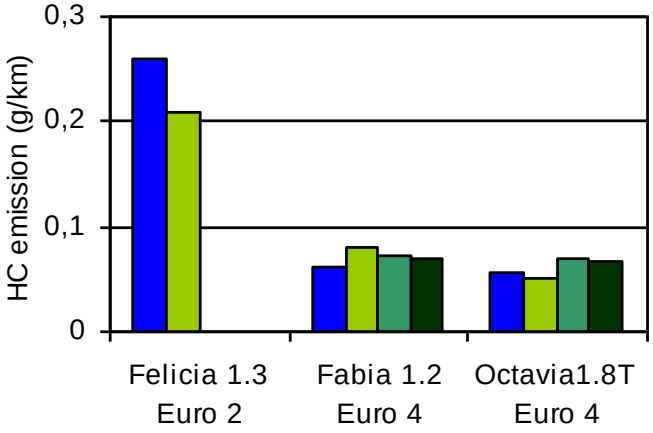
In comparison with ethanol:

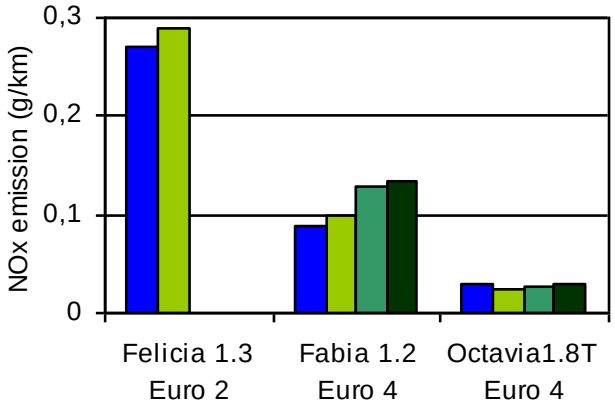
- Increased engine power
- Lower corrosivity
- Hydrofobicity
- Lower volatility
- Up to 30% v/v in gasoline no need of engine modification – **butanol /gasoline blend demonstrated by BP in BMW cars on London Olympic Games**
- Possible to use same transportation system as in case of gasoline

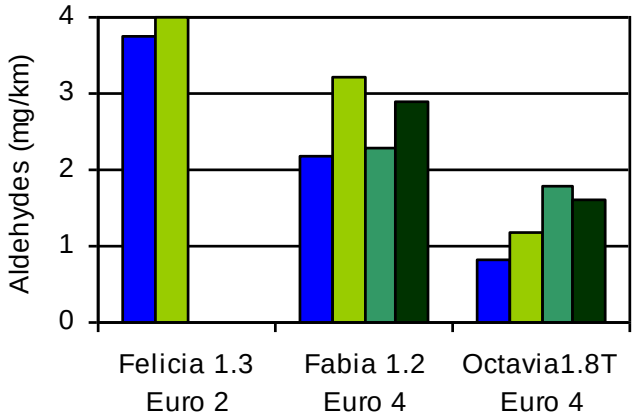
Clostridium bacteria – ability to ferment glucose, saccharose, starch, lactose, inulin and pentoses present in lignocellulosic hydrolysates

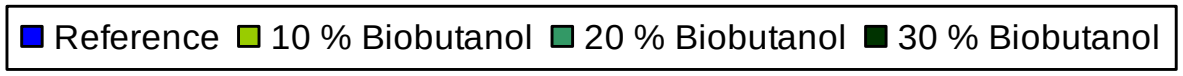
Effect of butanol in gasoline fuel on emissions of regulated pollutants (CO, HC, NO_x) and aldehydes in ECE 83.03 emission test











Biofuels/book 4, (2011), InTech Open Access

History of industrial acetone-butanol-ethanol (ABE) fermentation

World

- Ch.Weizmann – strains isolation, technology development
- WWI- first industrial ABE fermentation in England, then in Canada
- Terra Haute plant in USA
- After 1936 –plant all over the world
- WWII – Bromborough semi-cont. fermentation
- In South Africa – till 1983
- China, the Soviet Union – till 1980s

Czechoslovakia

- isolation of wild strains from soil – before WWII
- research of ABE fermentation – Prof. J. Dyr (ICT Prague) – 1950s
- 1952-1965 butanol plant in Czechoslovakia (feedstock – potatoes, rye, molasses)

Present situation regarding industrial ABE fermentation

about 100 000 t of acetone and butanol per year produced by fermentation in China ???, small plant (8000 t per year) in Brazil - 2010

In the rest of the world – race for 1-butanol production plant in Europe, U.S.A., Asian countries…
Butamax, Green Biologics, Cobalt Technologies, Butalco …

shift of interest from biofuel to pure chemical

Ethanol X Butanol

- ✓ Single microorganism – yeast *S.cerevisiae*
- ✓ ethanol –primary metabolite, its formation associated with growth
- ✓ Relatively low demands on sterilization
- ✓ *S.cerevisiae*- facultatively anaerobic

- ✓ Different species of *Clostridium*
- ✓ Butanol – stationary phase metabolite; probably associated with sporulation onset
- ✓ Sterilization necessary – high risk of contamination or bacteriophage attack
- ✓ Clostridia – strictly anaerobic

Fermentation schemes

Ethanol

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

Butanol

$$C_6H_{12}O_6 \longrightarrow 1{,}35\ H_2 + 2{,}21\ CO_2 + 0{,}56\ C_4H_9OH + 0{,}22\ C_3H_5O + 0{,}07\ C_2H_5OH + 0{,}14\ C_2H_2O_2 + 0{,}04\ C_4H_8O_2$$

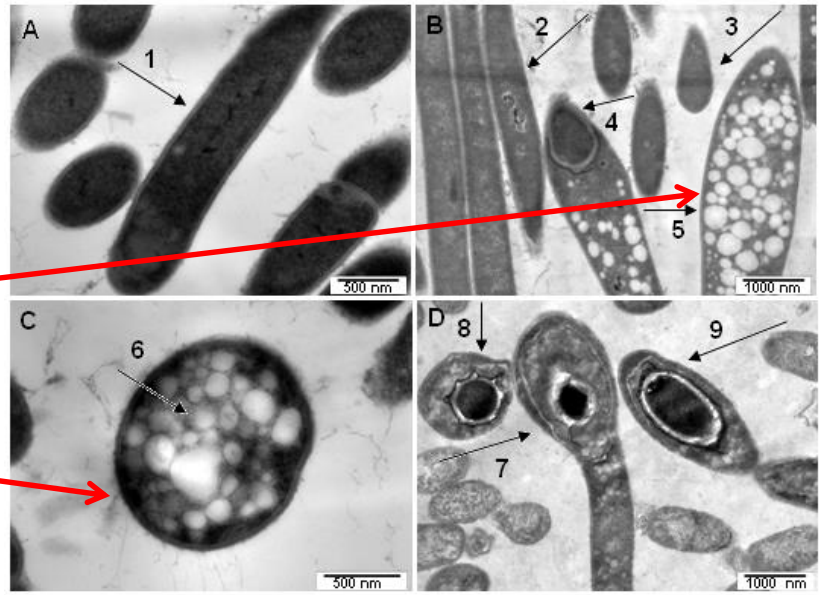
(Jones and Woods, 1986)

butanol yield – max. about 30-35%, solvent (sum ABE) yield – max. 40-45%

Production microorganisms

Clostridium bacteria: *C.acetobutylicum, C.beijerinckii, C.saccharoperbutylacetonicum…*

- G^+ type of cell wall
- anaerobic
- Sporulating (accumulation of stock polysaccharide granulose prior sporulation)
- heterofermentative

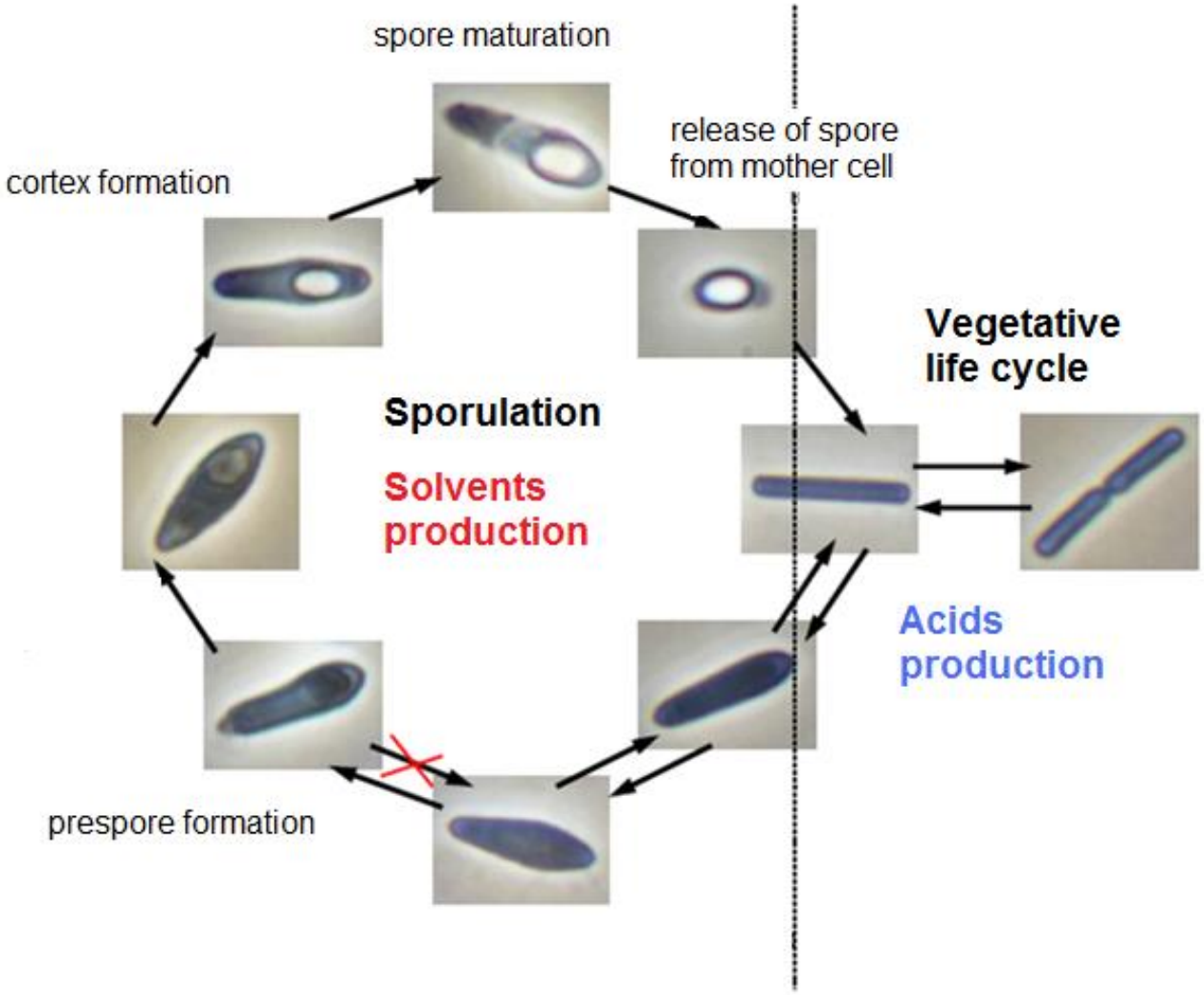


C.pasteurianum
1-3 vegetative cells, 4-7 sporulating cells, 8,9 mature spores

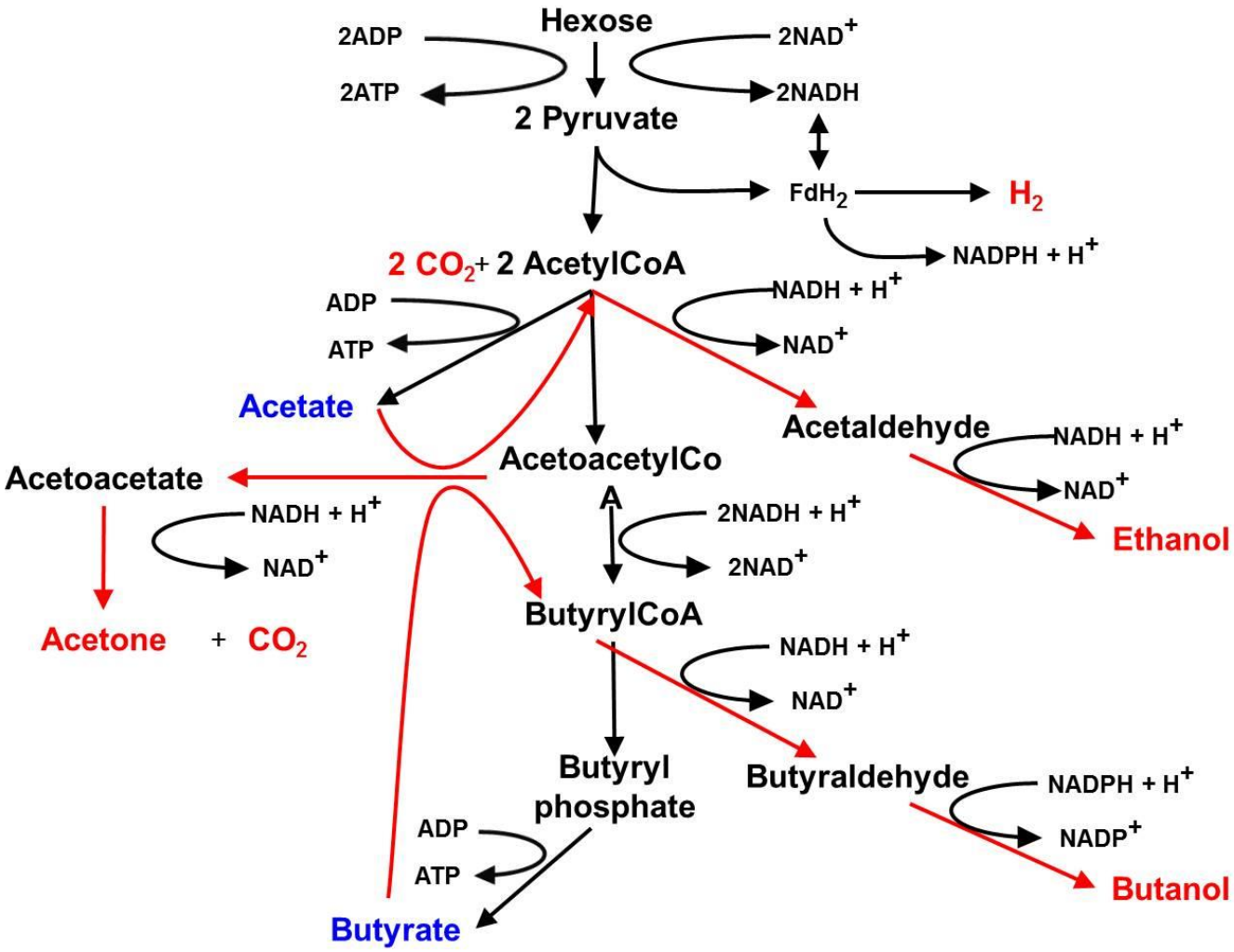
+ GM non clostridial microorganisms - efficient 1-butanol production not demonstrated

(*for survey see Kharkwal et. al. (2009) Rec.Pat. Biotechnol. 3, 202)*

Clostridial life cycle versus metabolites formation



Simplified metabolic scheme of ABE fermentation

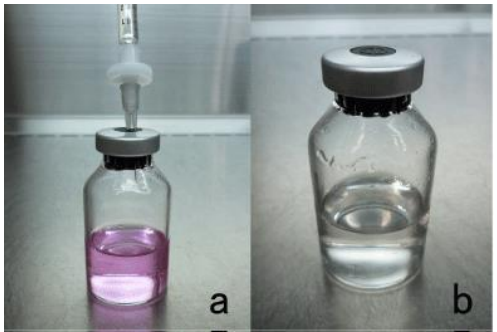


Handling anaerobic bacteria

- Storage – freezing, **spore suspensions**, lyophilization
- **Cultivation medium** – removal of oxygen, addition of reducing agents to the medium **(sodium thioglycolate, cysteine.HCl…)**

Small scale cultivation:

- Hungate technique tubes
- Anaerostats
- Anaerobic chamber (glove box)



J. Petrol. Env. Biotechnol. 2011

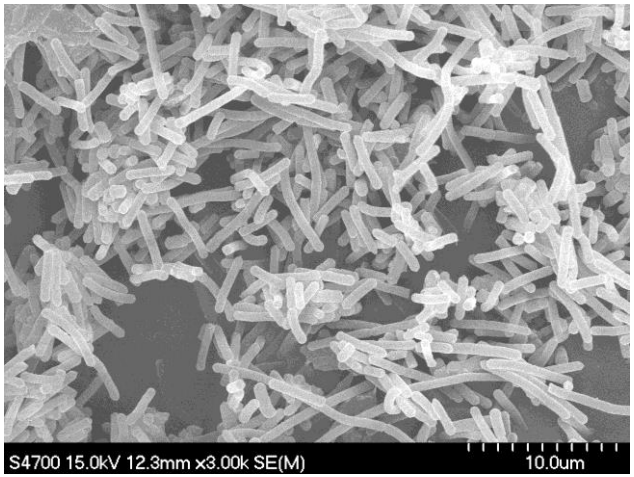
Anaerostat, anaerobic chamber

Purging with nitrogen, CO_2 or gas mixture

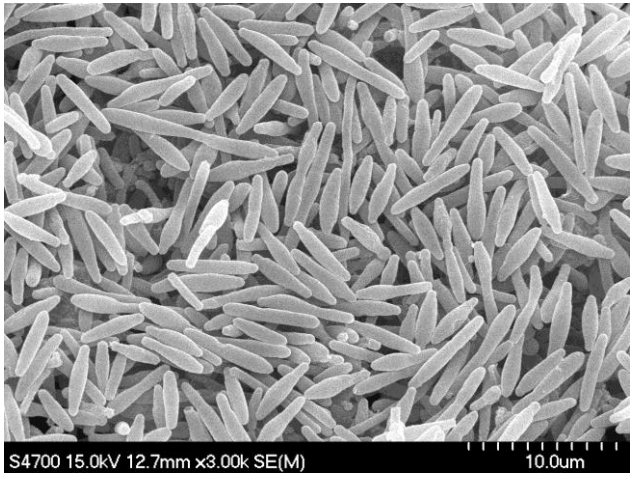
Gas exchange using cylinders with oxygen free gases, small airlock chamber for input and output of material, work with bare hands, thermostat

ABE fermentation

1. **Medium preparation** - feedstock (same as for ethanol); starch hydrolysis not necessary, addition of complex nitrogen sources (clostridia are often auxotrophs)
2. Bacteria propagation – under strict anaerobic condition
3. **Fermentation** – 37°C, pH 7, 36-72h, biphasic process- growth associated with acids generation, stationary phase (sporulation) associated with solvents formation; max. butanol/ABE final concentrations – 12-20/20-30 g/l



rod shaped vegetative cells of *C.beijerinckii*



„clostridium“ shaped cells accumulating

Fermentation arrangement

Batch – simple, max. final butanol concentration
Fed-batch – reduction of lag growth phase ⟶ slight productivity increase
Continuous – in one bioreactor – oscillatory behaviour due to life cycle; in more bioreactors

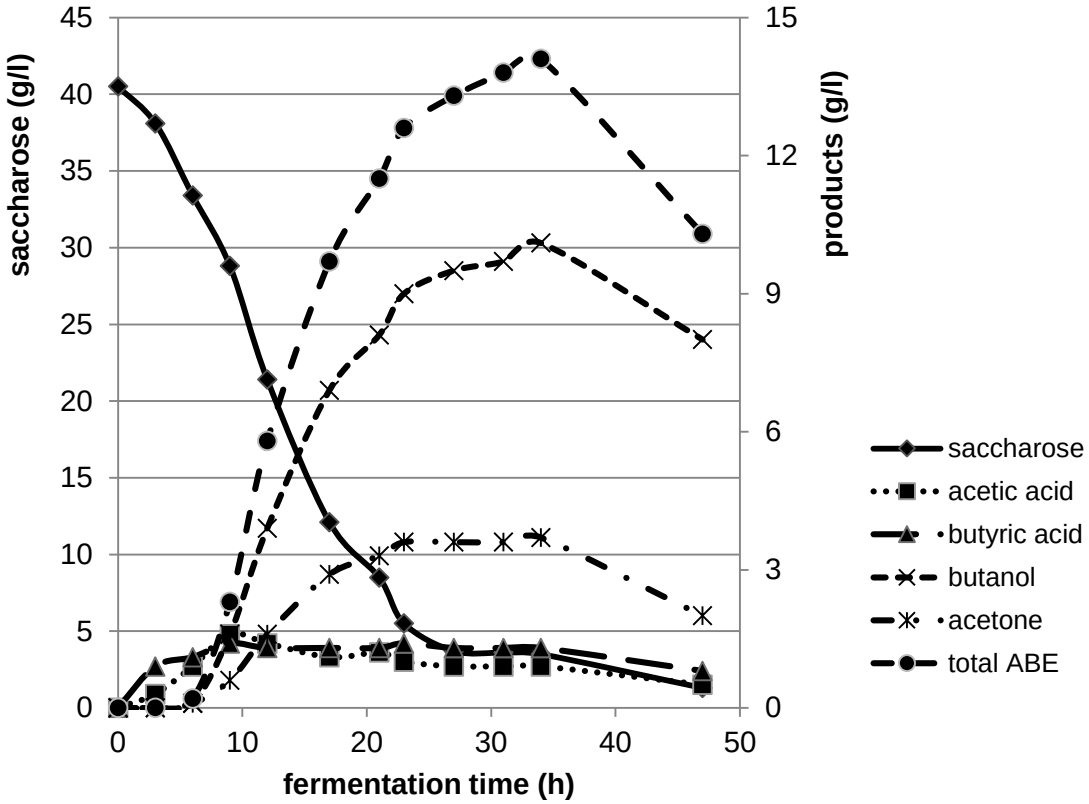
pros

- no inoculum preparation
- reduction of operation times necessary for cleaning and sterilization
- increase of productivity and yield ?

cons

- danger of cells wash-out
- pressure on cells growth - risk of strain degeneration
- stringently aseptic conditions
- lower end product concentrations
- sterilization of bigger medium volume – higher amount of waste

1-butanol from sugar beet juice by *C.beijerinckii*



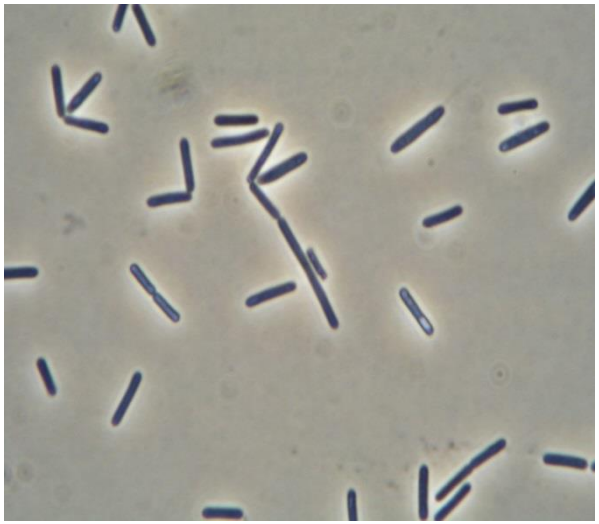
1.00 $C_{12}H_{22}O_{11}$ ⟶ **1.22** $C_4H_{10}O$ + **0.60** C_3H_6O + **0.04** C_2H_6O + **0.25** $C_2H_4O_2$ +

0.20 $C_4H_8O_2$ + **1.60** CO_2 + **0.80** H_2

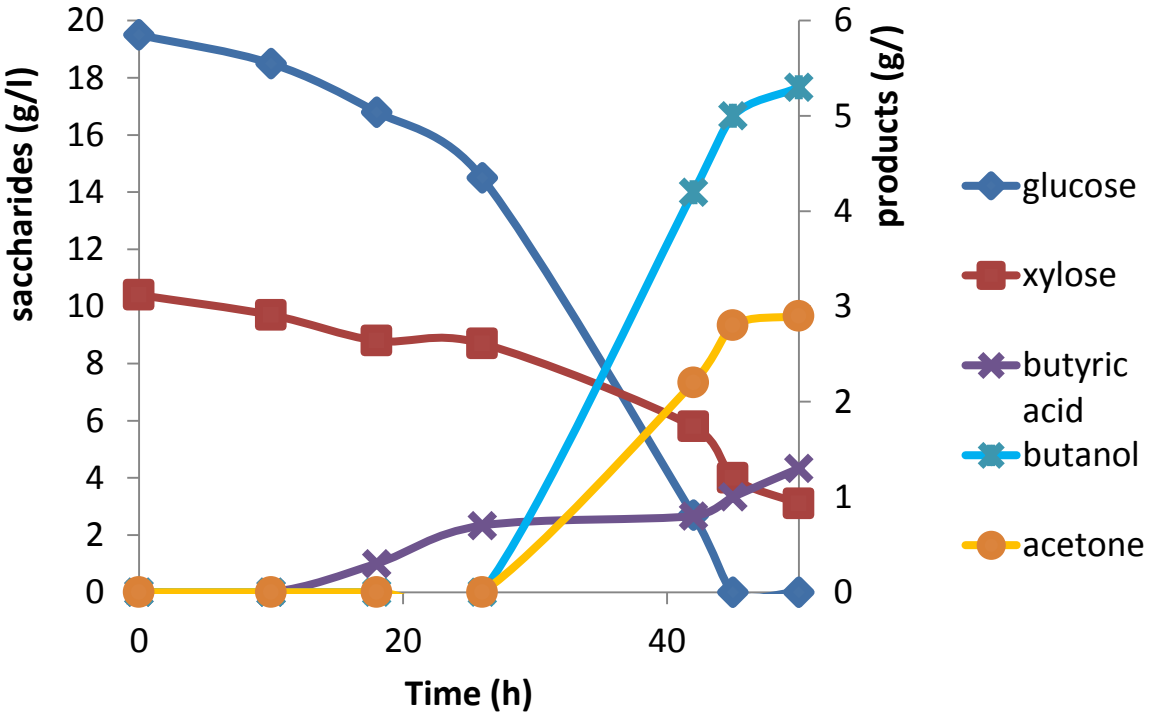
Patakova et al. (2011) Sugar and Sugar Beet

1-butanol production from wheat straw hydrolysate

Hydrolysate composition (in g/l): glucose 20, xylose 10, arabinose 2
-necessary to supplement source of nitrogen and other components



Clostridium pasteurianum



Yield of solvents 0.46 g/g cellulose

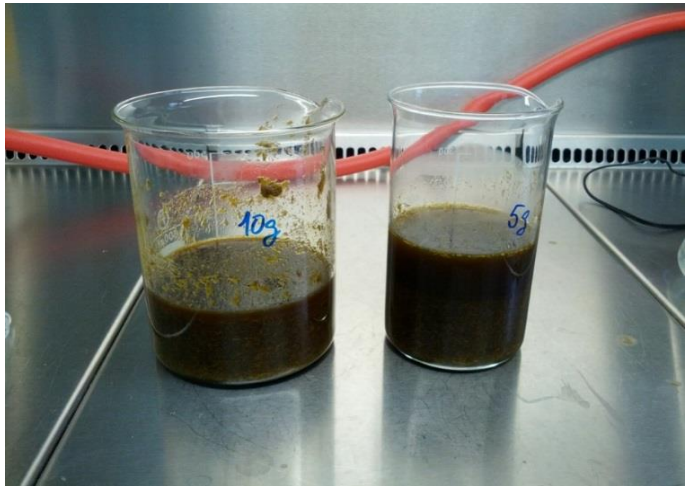
Processing of waste feather

Mixed hydrolysate
straw + feather
Preparation step

Feather peptone

Joint hydrolysis of straw and feather

Wet poultry feather



Joint hydrolysate
Different dosing of material

Distillation – final separation step for 1-butanol

• in past – mash destillation – high consumption of energy (high volumes)

• azeotroph butanol – water (b.p. 93˚C, 55% butanol) - extractive distillation or distillation with decantation

• irreplacable method for final butanol separation

Currently possible pre-concentration before distilation or integration of separation with fermentation (alleviation of product toxicity)

Membrane techniques

Pervaporation
Perstraction
Reverse osmosis

Non- membrane techniques

Gas stripping
Extraction liquid-liquid
Sorption

+ final distillation

Main bottleneck – Toxicity of 1-butanol

Wild strains– max. 20 g/l solvents

Hyperproducing mutants of *Clostridium*

- ***C.beijerinckii* BA101** – 30 g/l solvents

GM microorganisms

X 1-butanol tolerance by microorganisms is approx. same

How to succeed with biobutanol ?

Biorefinery approach necessary

Biofuels by-products

Distillation stillage – biogas production, fodder component (*Clostridium* can acumulate riboflavin), fertilizer (DDGS)

Gaseous products:
CO_2 – dry ice, liquid CO_2
H_2 (in ABE process) – heat generation

Acetone, ethanol (from ABE) - chemicals
Water – must be recycled
Heat – must be recovered

UCT PRAGUE

Thank you very much for your attention

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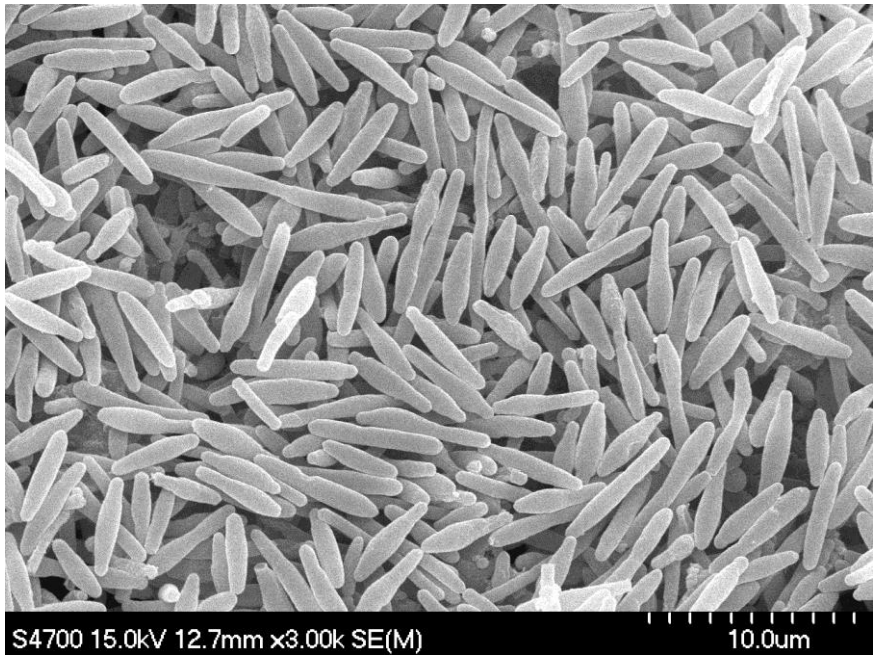

Clostridium beijerinckii cells accumulating granulose

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Biotechnology in Food Industry

Lecture VII

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Fruit brandies and spirits

Fermented spirits

Typical feedstock
Seed or indigenous microbial culture
Batch fermentation
Pot-still or patent still distillation
(Maturation in casks)

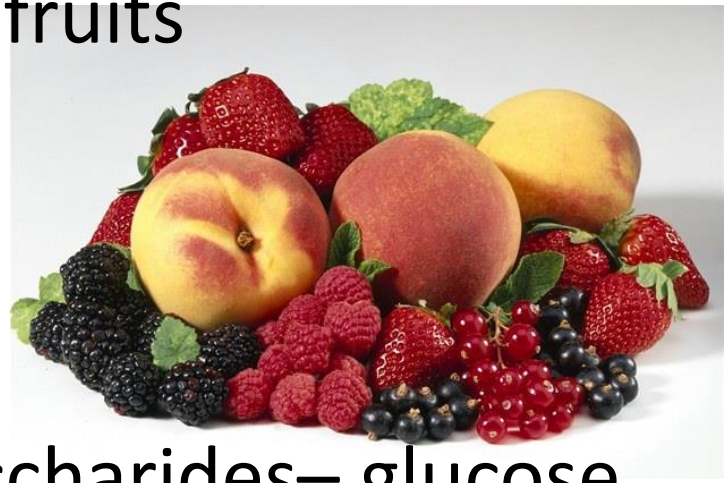
Spirits produced by „cold way“

Main feedstock – superfine ethanol
+ water
+ sugar syrup
+ herbs and their water or ethanol leaches or extracts
+ pigments
+ bonificators
+ egg yolks + milk + coffee…(emulsion spirits e.g. Baileys)

Main problem – clouds, precipitates

Fermentation of fruits

1) Chemical composition of fruits



a) Saccharides

berries ….3-19%

stone fruit….. 6-25%

Apples, pears….. 5-15%

Main components –monosaccharides– glucose, fructose

Saccharose

Polysaccharides – starch, dextrins, cellulose, hemicellulose, inulin

b)Other nitrogen-free compounds

Organic acids:

volatile– formic, acetic

Non-volatile – malic, tartaric, citric, lactic, benzoic, salicylic

waxes –esthers of fatty acids with higher alcohols

Fragrant compounds – esthers of organic acids, aldehydes, ketones, higher alcohols

c) Nitrogen compounds

proteins, amino acids

d) Mineral componds (ash)

K,Na, Ca, Mg, P, Cl, Mn, Cu, Fe

Water – 72-93%

Spontanneous ethanol fermentation

Yeast

1.Ascomycetes yeast

Saccharomyces cerevisiae –usually upper fermentation at higher temperature

Other yeast: *S.cerevisiae carlsbengensis* (bottom), *S.pasterianus, S. fragilis, S.melis*

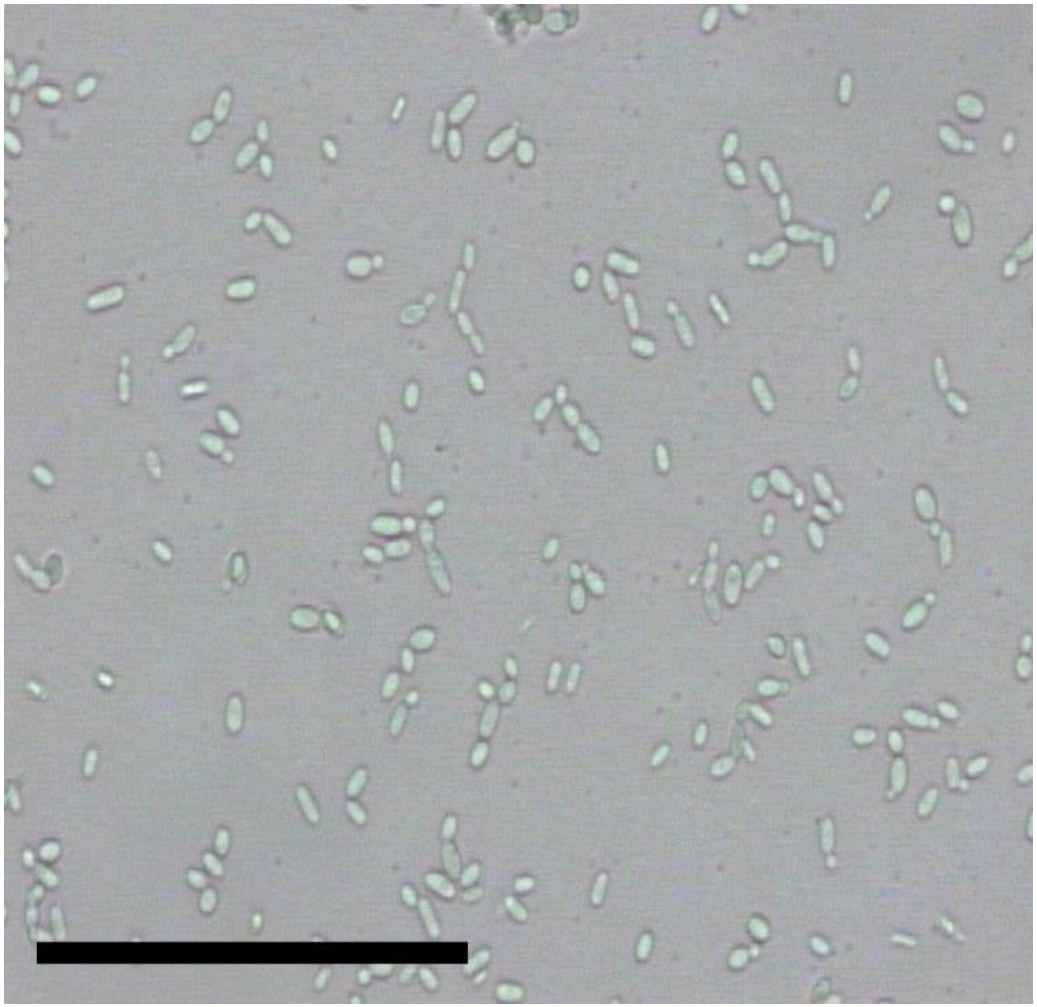
Schizosaccharomyces pombe

*Hansenula anomala, Pichia (*oxidation of EtOH to acetic acid*)*

Kluyveromyces marxianus

2. Family Cryptococcaceae

Candida –oxidation of ethanol up to CO_2, formation of pseudomycelium



Bacteria in mash

1. **Lactic acid bacteria**

Lacobacillus delbrueckii, L.buchneri, L.plantarum

Streptococcus, Leuconostoc

lactic acid formation

2. Acetic acid bacteria

Acetobacter pasterianum, A.xylinum, A.aceti, A.suboxydans

Oxidation of ethanol to acetic acid or to CO_2, oxidation of glucose to gluconic acid,on the surface of fermentation mash, short rods

3. Butyric and propionic acid bacteria

Clostridium butyricum, C.sporogenes, Granulobacter

Sporeforming bacteria

Anaerobic and facultatively anaerobic

Formation of butyric, propionic, formic, acetic and lactic acids and higher alcohols

4. **Pectin degrading bacteria**

Methanol formation

Sporeforming

Plectridium pectinovorum, Granulobacter pectinovorum

5. **Rotting bacteria**

Degradation of proteins, formation of hydrogen sulphide, ammonia, aerobic and anaerobic *Pseudomonas, Serratia, Proteus*

C)Filamentous fungi in mash

Strictly aerobic

On the walls, vats, on the surface of the yeast, together with the aerobic yeasts form the so-called blanket

Production of organic acids from carbohydrates

On fruit– *Aspergillus (A.glaucus, A.oryzae, A.niger)*

Blanket– *Penicillium* – fungal odour

Botrytis –tannin oxidation

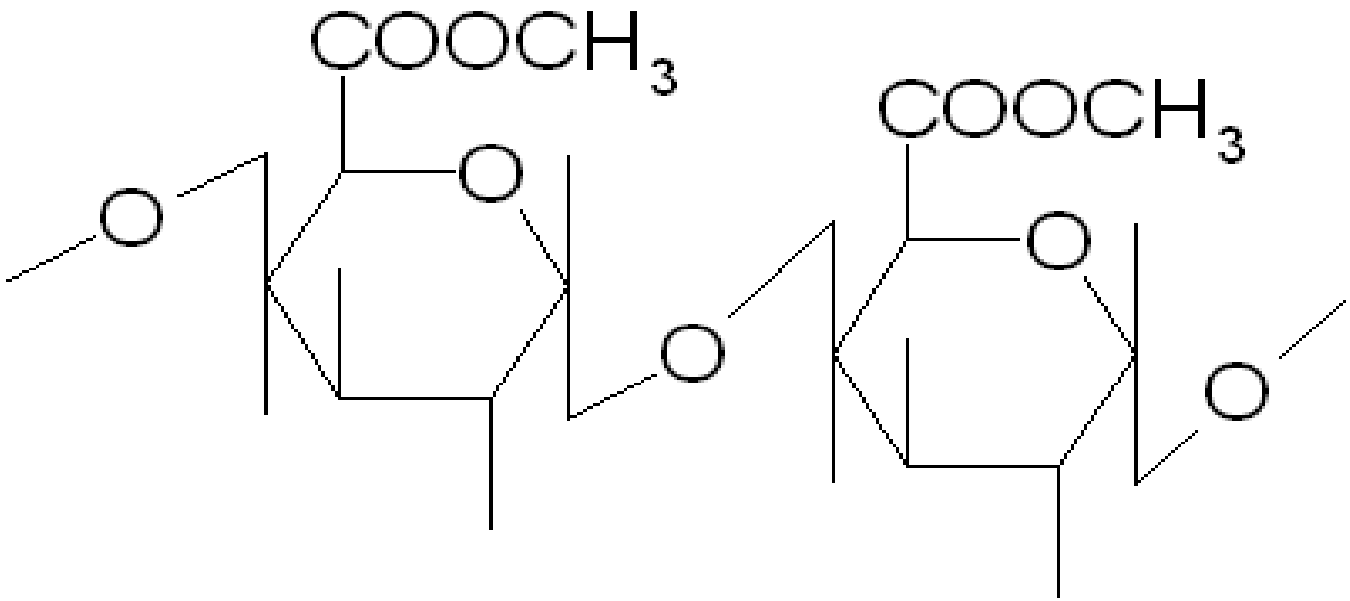
Rhizopus, Mucor, Oospora, Fusarium, Cladosporium

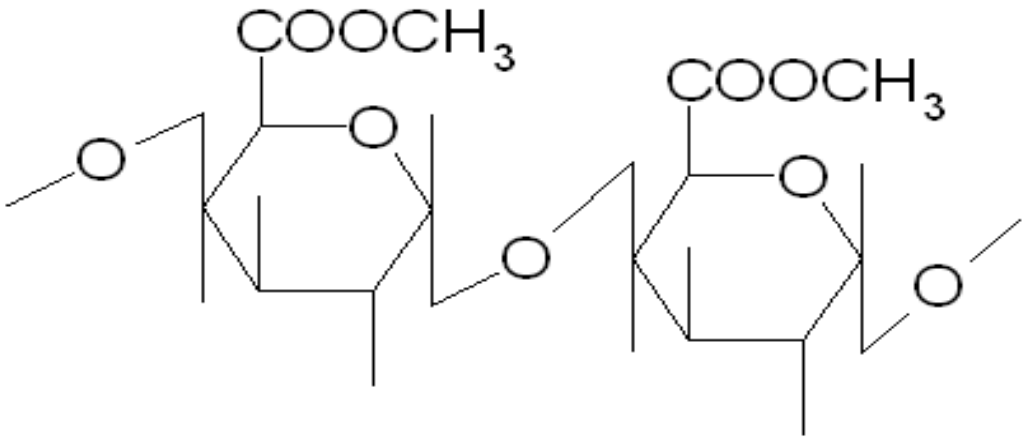
Formation of methanol

Protopectin – complex of pectin with cellulose, arabanes and galactanes

↓ protopectinase

Pectin



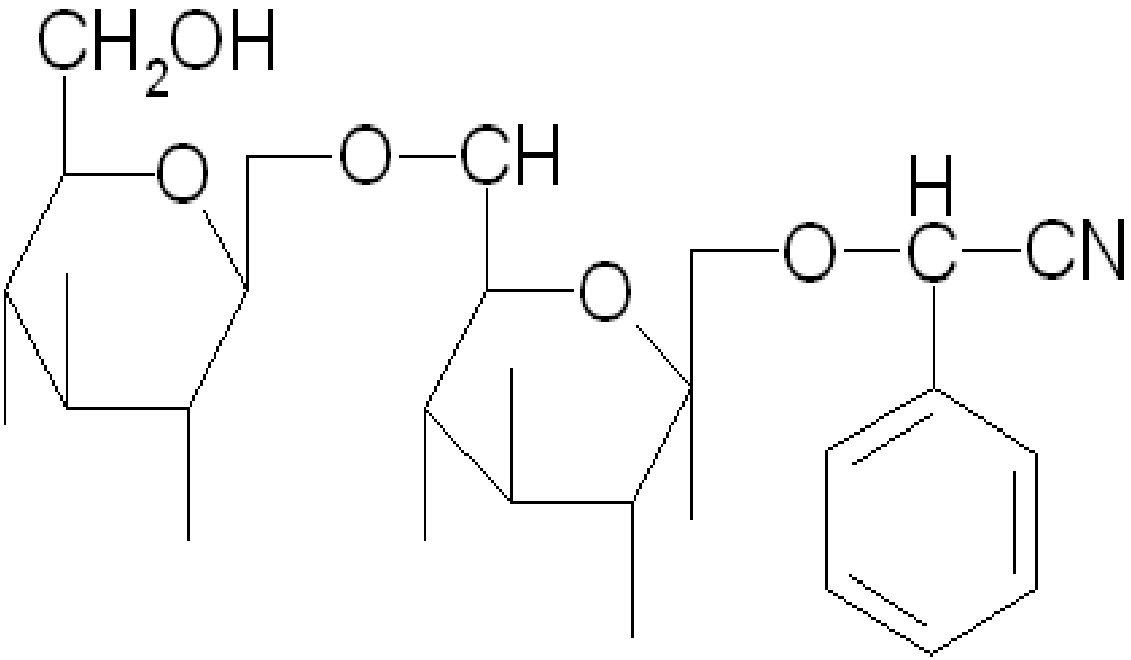


Methyl esther of poly-D-galacturonic acid

↓pectinesterase, polygalacturonase

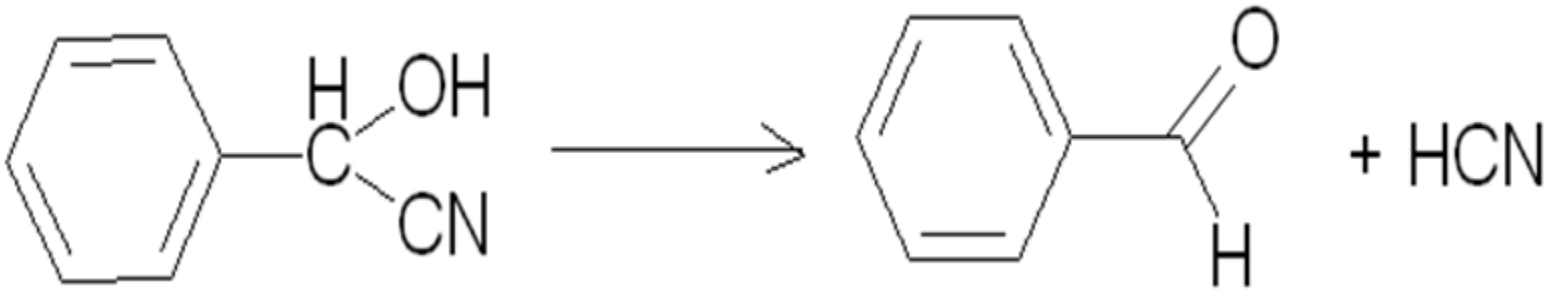
CH_3OH + mono galacturonic acids

AMYGDALIN



gencibiosa **benzaldehydkyanhydrin**

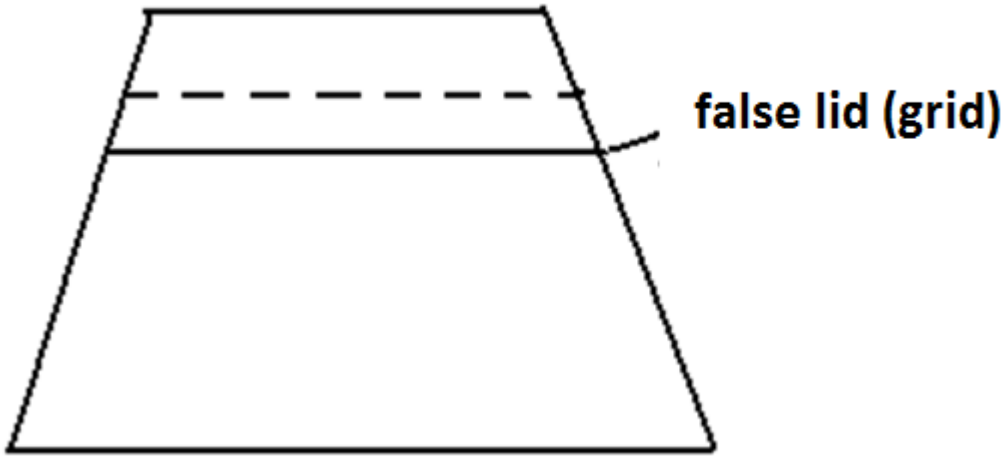
Formation of benzaldehyde and hydrogen cyanide



Fermentation

Fermentation room – closed building, sewarage, drainage, aeration

Fermentation vessels – wood, ferroconcrete, stainless steel



Fermentation

Course of fermentation

- Beginning of fermentation
- Stormy fermentation –CO_2, decrease of sugar concentration, increase of EtOH
- Slow fermentation
- End of fermentation – no CO_2, sinking fermentation cover

Distillation and rectification

- Simple distillation
- Cupreous vessels
- Heating of distillation boiler
- Quality of fermentation!!!
- (pH)
- Homogenization
- Filling the boiler -1/2- 2/3
- Destillation slow – spillage, substance, end

Distillation room

- Rectification gentle
- Dilution of distillates – distilled or de-carbonized water
- Removal of clouds – filtration, clarification

storage of distillates

– maturation –

esterification- bottling

Plum brandy

- Different kinds of plums
- Saccharides content > 8%,
- Shaking down into canvas
- Fallen fruit –special treatment

washing, inoculum necessary

Without pressing, with stones– indigenous fermentation by cold way (September– December)

Slow distillation, substance immediately bottled

Whisky

Scotch, American, Irish

1. Scotch whisky

malt

Exclusively malt

Destillation „pot still“

blended whisky

Highland, Lowland

grain

Malt + barley

Destillation

„patent still“

Factors affecting quality

- water
- Destillation device
- wood
- climate
- people!
- kilning the malt over a peat fire
- Just 2 destillation
- Maturation in casks

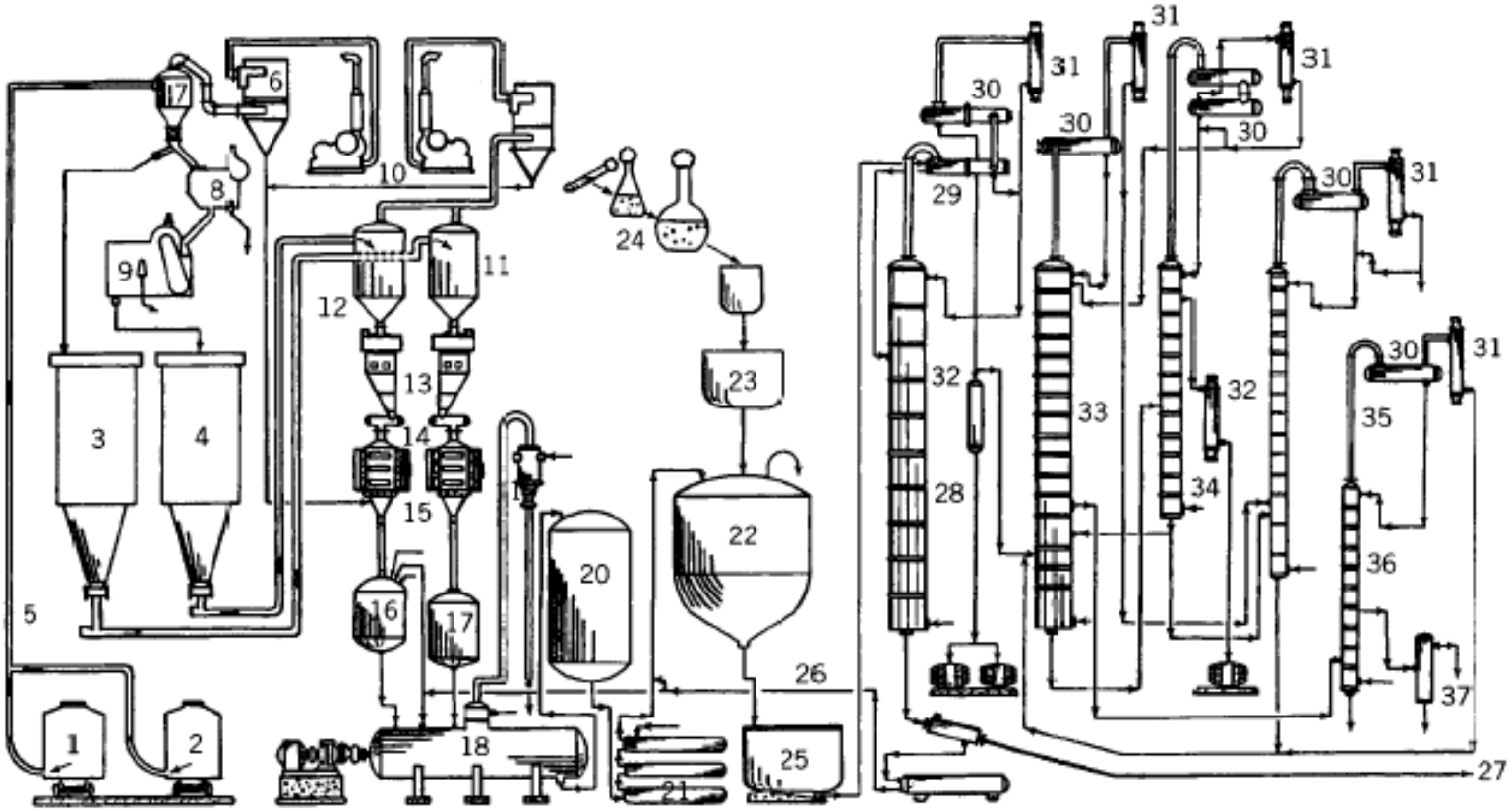
2. American whisky (grain)

Maize – bourbon whisky

Rye – rye whisky

Destillation „patent still“

Fermentation: sweet mash –yeast inoculation sour mash – bourbon – inoculation from previous fermentation– alcohol + lactic fermentation



1. Cereal grains
2. Malt
3. Malt bin
4. Cereal grain bin
5. Unloading elevator
6. Dust filter
7. Collector
8. Scalperator
9. Millerator
10. Reclaiming exhauster
11. Malt receiver
12. Cereal grain receiver
13. Automatic scale
14. Mill feeder
15. Roller mills
16. Precooker
17. Malt infusion
18. Cooker
19. Barometric condenser
20. Converter
21. Mash coolers
22. Fermenter
23. Final yeast propagator
24. Yeast culture and intermediate yeast propagator
25. Fermented-mash holding vessel
26. Stillage return system
27. Stillage flow to recovery system
28. Whisky separating column
29. Heat exchanger
30. Dephlegamtor
31. Vent condenser
32. Product cooler
33. Selective distillation column
34. Product concentrating column
35. Aldehyde concentrating column
36. Fusel oil concentrating column
37. Fusel oil decanter

Figure 2. Material process flow, modern beverage spirits plant.

Rum

- Sugar cane juice and foam, mollases
- Destillatio + rectification
- Maturation in casks up to 10 years
- 3 brands according to esther number
- **X artificial rum – alcohol +**

essence + pigment + sweetener

Brandy

- Feedstock – wine – destillation immediately after fermentation together with yeast
- Maturation in oak casks
- Cognac –only specific area in France

Feedstock for non-fermented spirits

- Fine ethanol
- water
- sugar – syrups

„normal syrup“ – 100 kg sugar/42l distilled water, boiling + 75 g citric acid, 10 min, removing foam

starch syrup – glucose, maltose, dextrines

- Lactose syrup

- Drugs (plants):

- Extremely bitter – sage-brush, aloe…
- Very bitter – gentian, centaury…
- Aromatic, mildly bitter –hop, orange slice……
- Aromatic, non-bitter –chamomile, mint….
- Mildly aromatic, non-bitter – lavender, thyme..
- faintly aromatic – ruta…
- Aromatic, sharply spice– pepper…
- Aromatic with vanilla flavour – vanilla..
- Aromatic with sweet taste – annise, fennel…
- Aromatic, bitter – almonds..
- Spice – cloves
- Plant and animal pigments – elderberries, cochineal..

- Extracts, mixture of extracts

Extraction, distillation, pressing, maceration, mixing extracts

- Pigments

a) **Natural – fruit juices (blackberries,** raspberries, cherries, black current, elderberries),

pigment plants (turmeric, mallow, marigold, saffron..), caramel

b) artificial pigments

- Bonificators

Rounding taste, flavour enrichment

- wines–malaga, sherry, port
- Aromatic resins
- Aromatic oils

Production of liqueurs and non-sweet spirits

- Basic operation step – **mixing**– mechanical or pneumatic
- Addition of individual components gradually
- Ethanol addition –by weigh
- Maturation (short)
- Bottling

Emulsion spirits

Feedstock – yolks (10-16/l), sugar, fine ethanol, destillate, (milk, cacao, coffee..)

Production procedure:

Whipping yolks with water (+ eggwhite), straining

Emulgation, addition of syrup, heating to 70°C

Cooling to 35°C, addition of ethanol + distillate

Mixing, cooling

maturation

bottling

Fruit spirits (e.g. Kirsch)

Fruit juices– clarification, pectolysis, filtration

Maturation X oxidation, clouds

Herbal liquers

Maceration of plants, filtration, distillation

Mixing, removal of clouds

consolidation

maturation

bottling

Vodka

Feedstock – 2x rectified ethanol of any origin

Production of „sortirovka“ –mixing ethanol with water

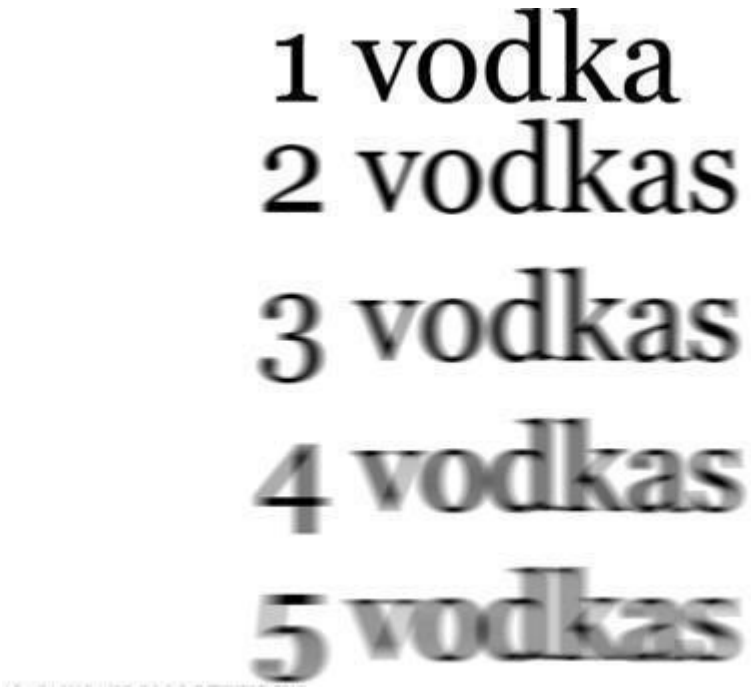
1. Sedimentation of precipitates
2. Addition $NaHCO_3$ + sodium acetate
3. Filtration using active carbon

Formation of clouds

- Salts of anorganic and organic acids – from water - Cu, Mg, Fe, Al, Zn (acetates, carbonates)
- Tannins – colour clouds – coloid solutions
- Organic compounds – oil clouds, opalescence, etheric oils, terpenes, resins

Legislative framework for the production of distillates, spirits and ethanol

- The Act on alcohol No. 61/1997 Coll.
- Food law
- Small fruit distilleries - up to 30 l EtOH / household / year
- Not allowed to distill ethanol at home - Criminal Code No. 392/1992 Coll. § 194a illicit alcohol production



Thank you for your attention

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Biotechnology in Food Industry

Lecture VIII

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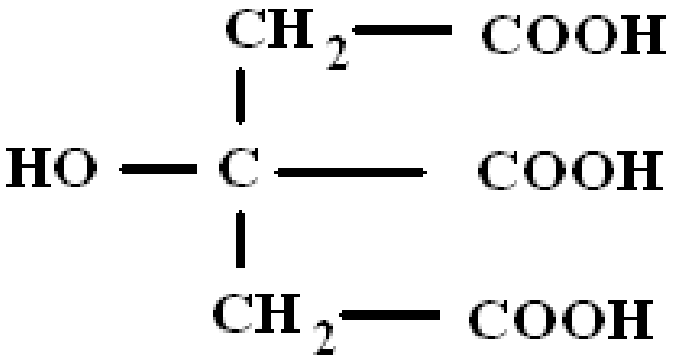
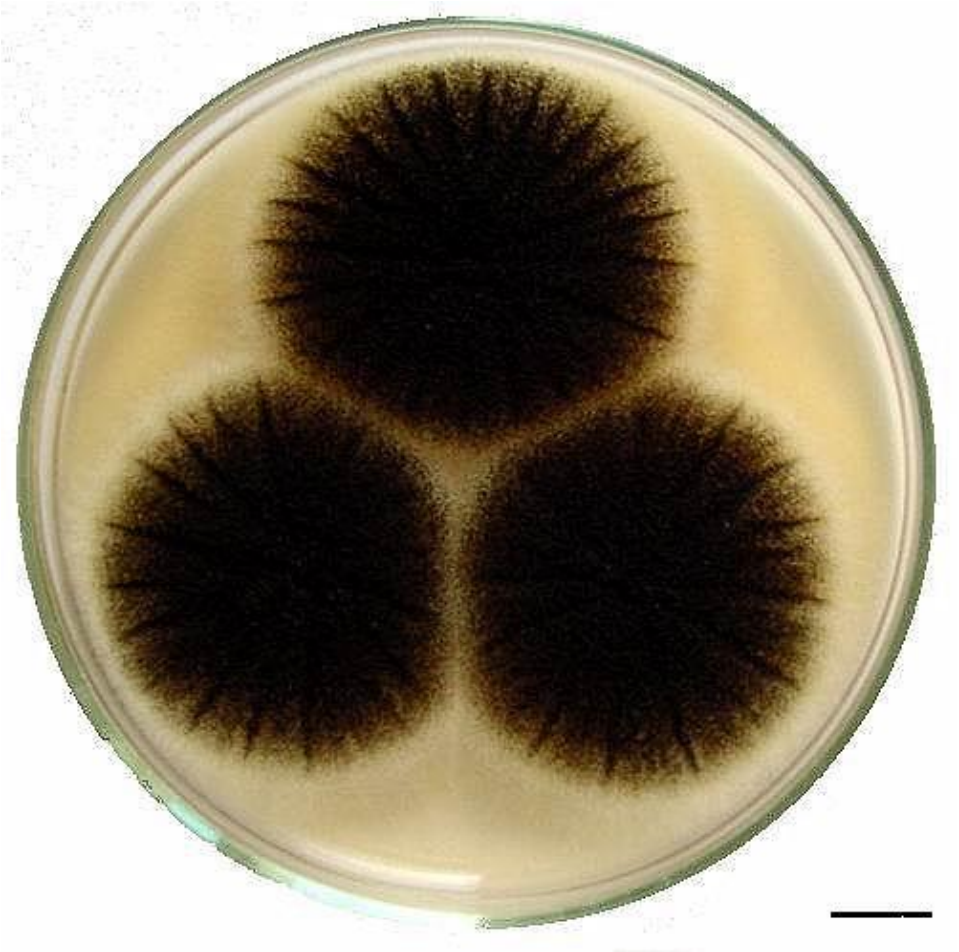
Citric acid

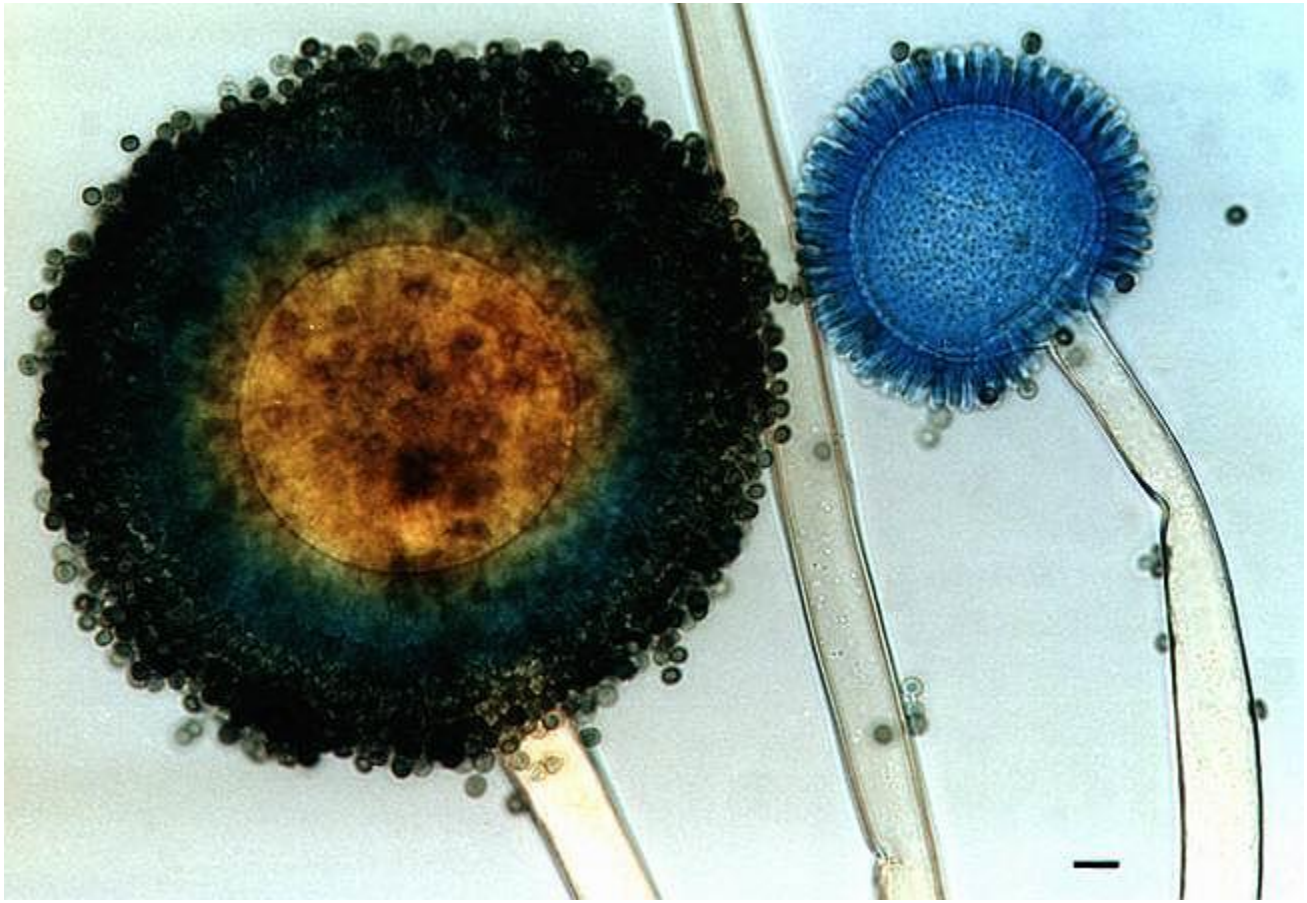


Use in foods: acidifying agent, pH regulator, production of jams and marmelades…

Main producent- *Aspergillus niger*



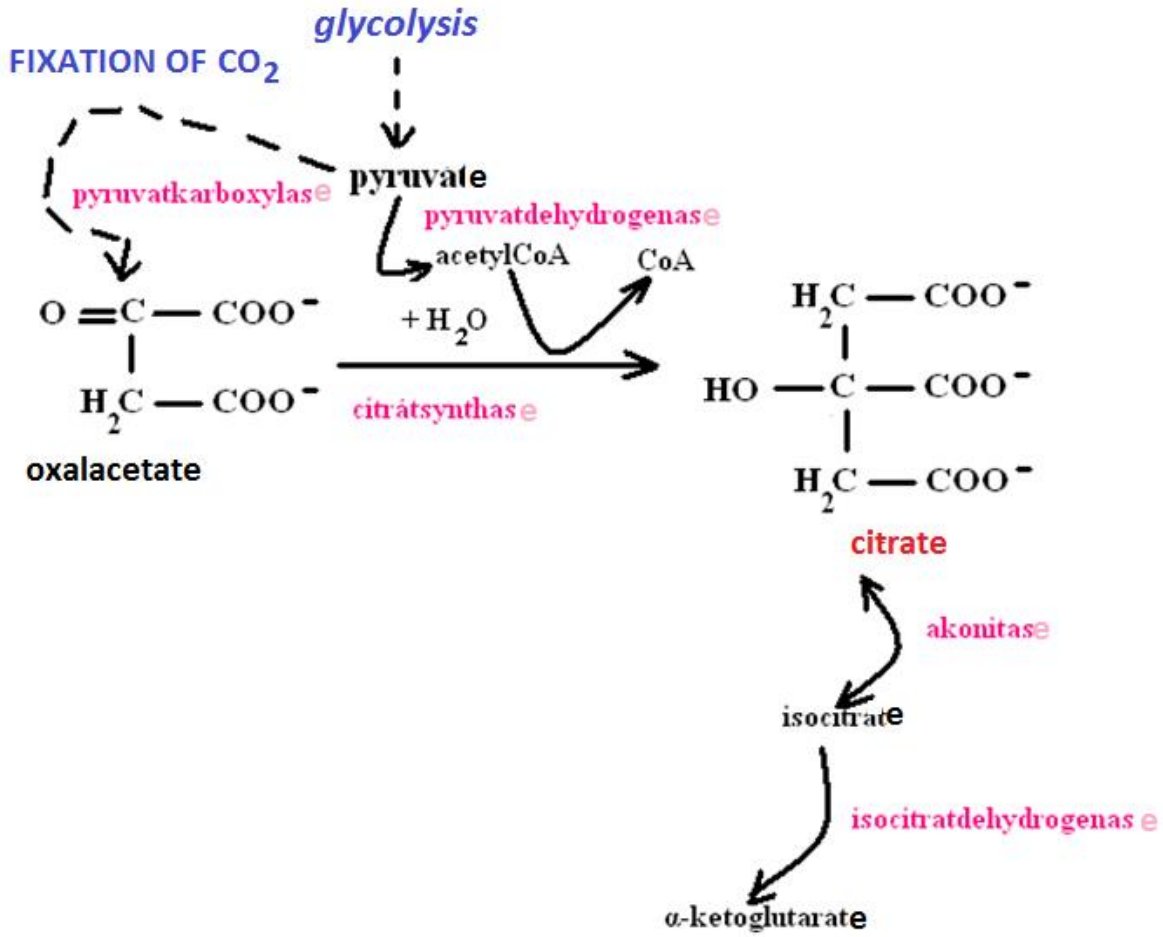
Aspergillus niger



Preparation of production strain

- Selection – testing colonies
- Mutagenesis and selection
- Conservation of spores in dried state
- lyofilization

Formation of citric acid



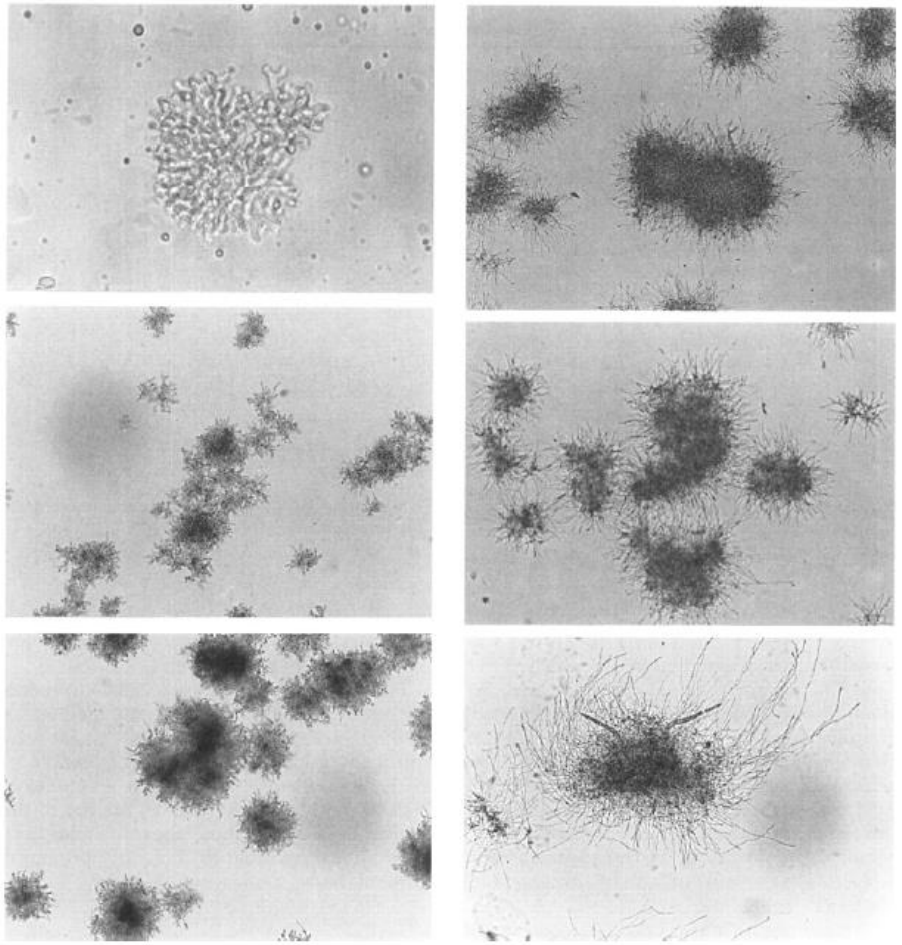
Factors affecting citric acid formation I

- Trace elements – Fe, Zn, Mn, Cu
- pH: spores germination 4 - 5, production 1,4 - 3,5, at higher pH gluconic and ocalic acids
- Aeration (too quick affects fixation of CO_2 in surface production X submese production)
- Temperature 28 – 34^{o}C

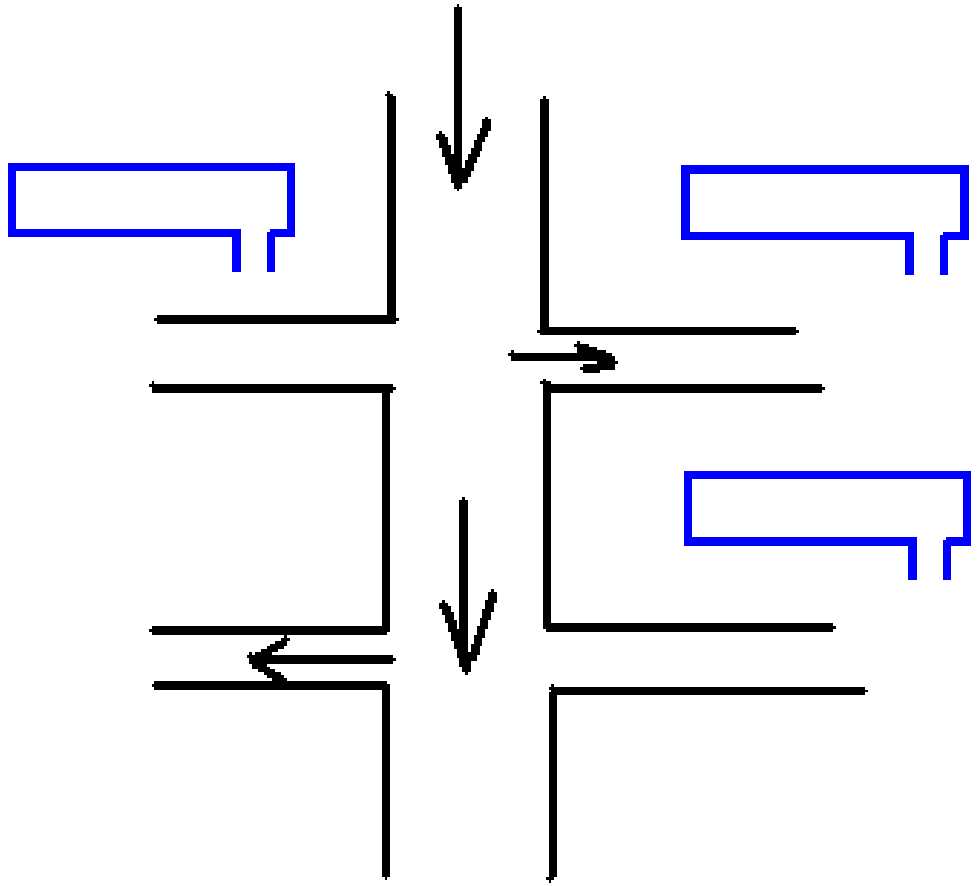
Factors affecting citric acid formation II

- Lipids –antifoaming agents–yield increase
- Inoculum –surface way– spores,

  submerse way spores or mycelium



Surface way of production



Surface way of production

Surface way of production

cultivation medium – molasses – 15%

Filling medium

Inoculum – spores

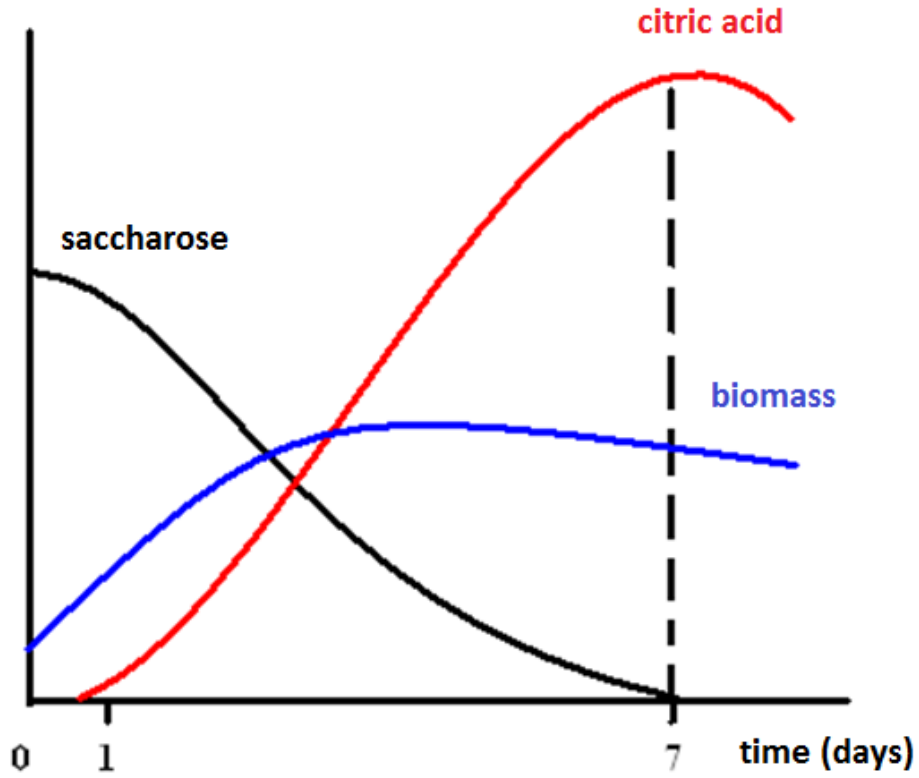
Cycle of sterile temperated

Non-diving mycelium

8-9 days, 10% citric acid

Desinfection – steam, formaldehyde

Submerse way of production



Submerse production

Substrate preparation – flow sterilazation, Inoculum –seed bioreactor

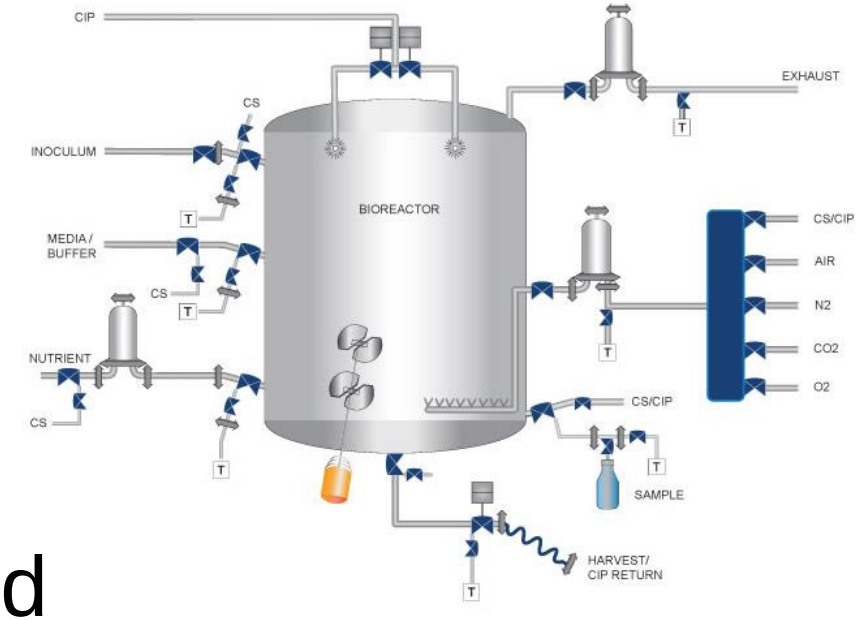
Cultivation – mixing,

cooling, aeration,

anti-foaming, pH control,

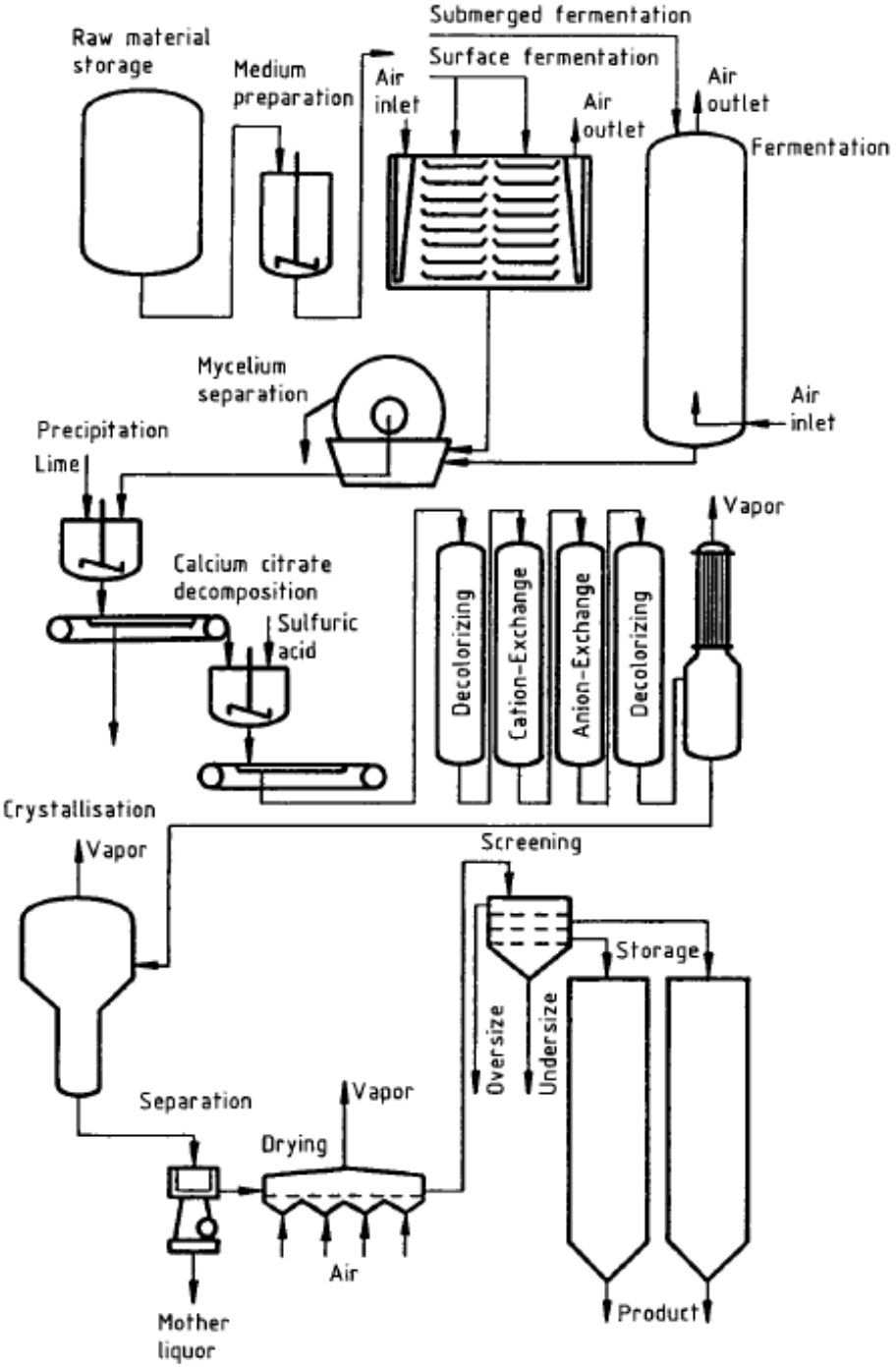
4-7 days

In-time endingX lysis, acid consumption



Isolation of citric acid

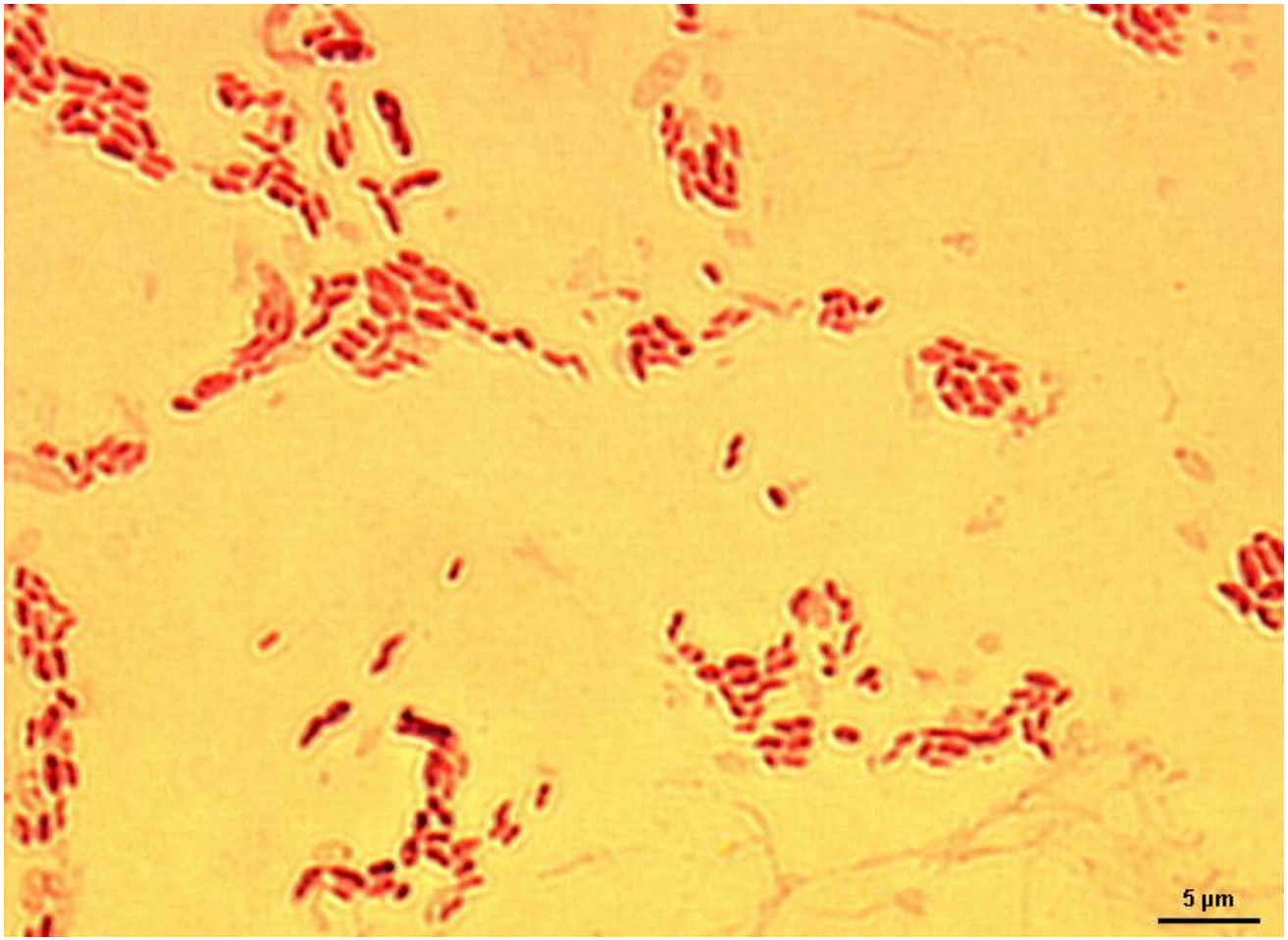
- Filtration of mycelium, draining of dishes
- Hot precipitaion at pH 5,8 with $CaCO_3$
- Hot filtration– insoluble calcium citrate
- Decomposition with sulphuric acid, filtration of gypsum ($CaSO_4$)
- Purification– active carbon, ion-exchanging columns
- Concentration
- Crystalisation in form of mono hydrate


Raw material storage
Medium preparation
Air inlet
Submerged fermentation
Surface fermentation
Air outlet
Air outlet
Fermentation
Air inlet
Mycelium separation
Precipitation
Lime
Calcium citrate decomposition
Sulfuric acid
Decolorizing
Cation-Exchange
Anion-Exchange
Decolorizing
Vapor
Crystallisation
Vapor
Screening
Storage
Oversize
Undersize
Separation
Drying
Vapor
Air
Mother liquor
Product

Acetic acid

CH_3COOH

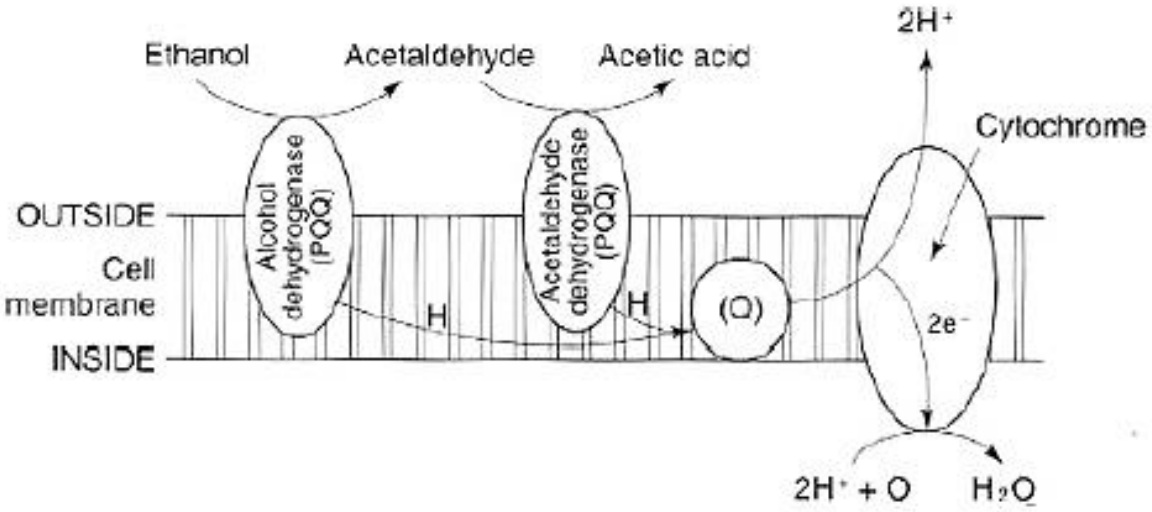
Acetobacter aceti



Oxidation of ethanol to acetic acid

$$2\,CH_3CH_2OH + O_2 \longrightarrow 2\,CH_3CHO + 2\,H_2O$$

$$2\,CH_3CHO + O_2 \longrightarrow 2\,CH_3COOH$$



Over-acidification

Contamination with *Acetobacter peroxydans*

Failure in technology –lack of etanol

Factors affecting production and quality of vinegar

- Oxygen
- Concentration of ethanol – optimum 2.5 – 3%,

  below 0.3% over oxidation

Temperature

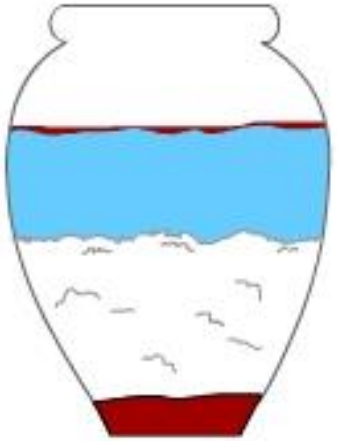
- Nutrients – N,P salts, trace elements
- Feedstock
- Way of production

Production by Orleans method

- Slow acidification
- Surface way – formation of kris (pellicle) in open vessel (cask)
- Acidification– 1-3 months
- Temperature 20^{o}C
- Remaining ethanol 0.5%, acetic acid 6%

Tsubo Vinegar

Tsubo Vinegar is a production method which makes vinegar at one time in a jar.



Koji, the steamed rice, and water are put in from bottom up, and koji is sprinkled from over.

The koji on the water surface serves as a lid, and saccharification and alcoholic fermentation is happen.

When acetic acid fermentation progresses, the koji on the water surface sinks.

It progresses maturing and vinegar is made. Unrefined sake is precipitated at a bottom.

Acetaia San Giacomo

How the Artisanal Traditional Balsamic is made

LAMBRUSCO grapes
TREBBIANO grapes

FERMENTATION
sugar --> yeast ---> alcohol
35% only 15% transf. in alcohol

OXIDATION
COOKED MUST VINEGAR
alcohol ---> a.a. bacteria -->acetic acid
a lot of sugar and a bit of acid acetic

MATURATION
No more bacteria activity because too thik and dense for them.

AGEING
Enzymatic activity
Complex
Balanced

COOKED MUST
by direct fire
= sugar carameliz.
= reduction/concent.

220 lt

70 lt | 60 lt | 50 lt | 40 lt | 30 lt | 20 lt | 10 lt

BATTERIES
different woods (oak, mulberry, chestnut, cherry, juniper, etc...) - different capacity

SABA
Reduced to 1/3

AGRO DI MOSTO

CONDIMENTO SAN GIACOMO

ESSENZA

CONSORTIUM for TASTING

ACETO BALSAMICO *TRADIZIONALE*
DI REGGIO EMILIA D.o.p.
1 consortium about 15 producers about 37.000 bottles
or
ACETO BALSAMICO *TRADIZIONALE*
DI MODENA D.o.p.
2 consortium about 150 producers i about 115.000 bottles

- **aged at least 12 years**
- **only cooked must**
- **only official 100 ml bottle same 4 all producers and different btw Reggio Emilia an Modena**

Rapid acidification

1. Preparation of substrate– 25-30% ethanol+ nutrients, dilution to 2.5-3% ethanol
2. Packed-bed bioreactor with immobilized acetic acid bacteria –packing is trickled with substrate using spraying
3. Periodic system – substrate circulates with the bioreactor (7-8 days), aeration with counter air

4. End of one batch – 11% acetic acid, 0.3% ethanol
5. Supressing over oxidation–flooding bioreactor with vinegar– 13%, cooling, stopping aeration
6. Life time of package -20-30 years, regeneration with hot and col water

Beech shavings

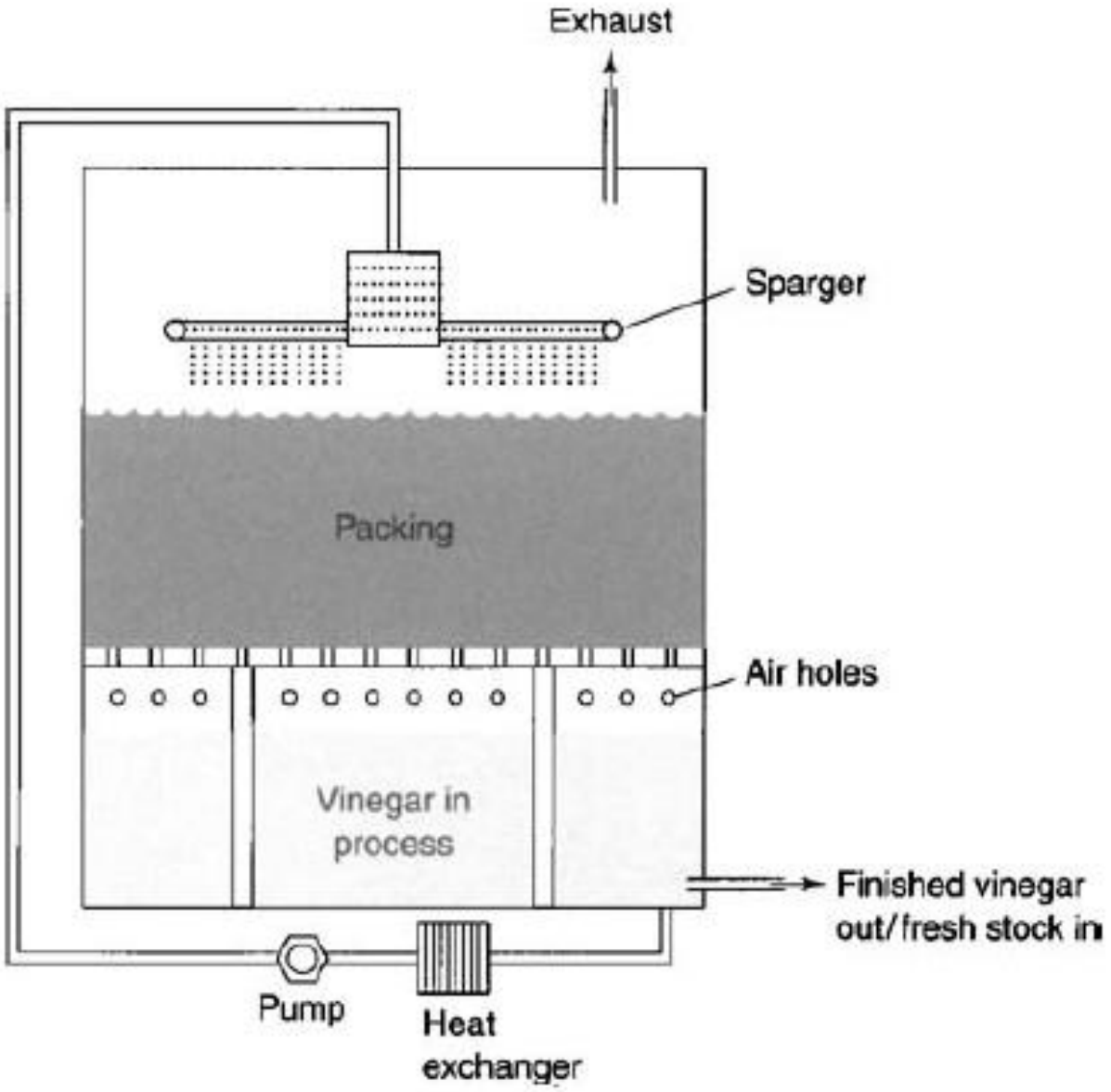


Figure 3 The quick vinegar process.

Submerse production of vinegar

- in bioreactor (acetator) with special mixing (self-priming turbine)
- Batch cultivation 48-72 h
- Substrate 11-12% ethanol + nutrients
- Product 11-12% acetic acid
- Filtration of bacteria

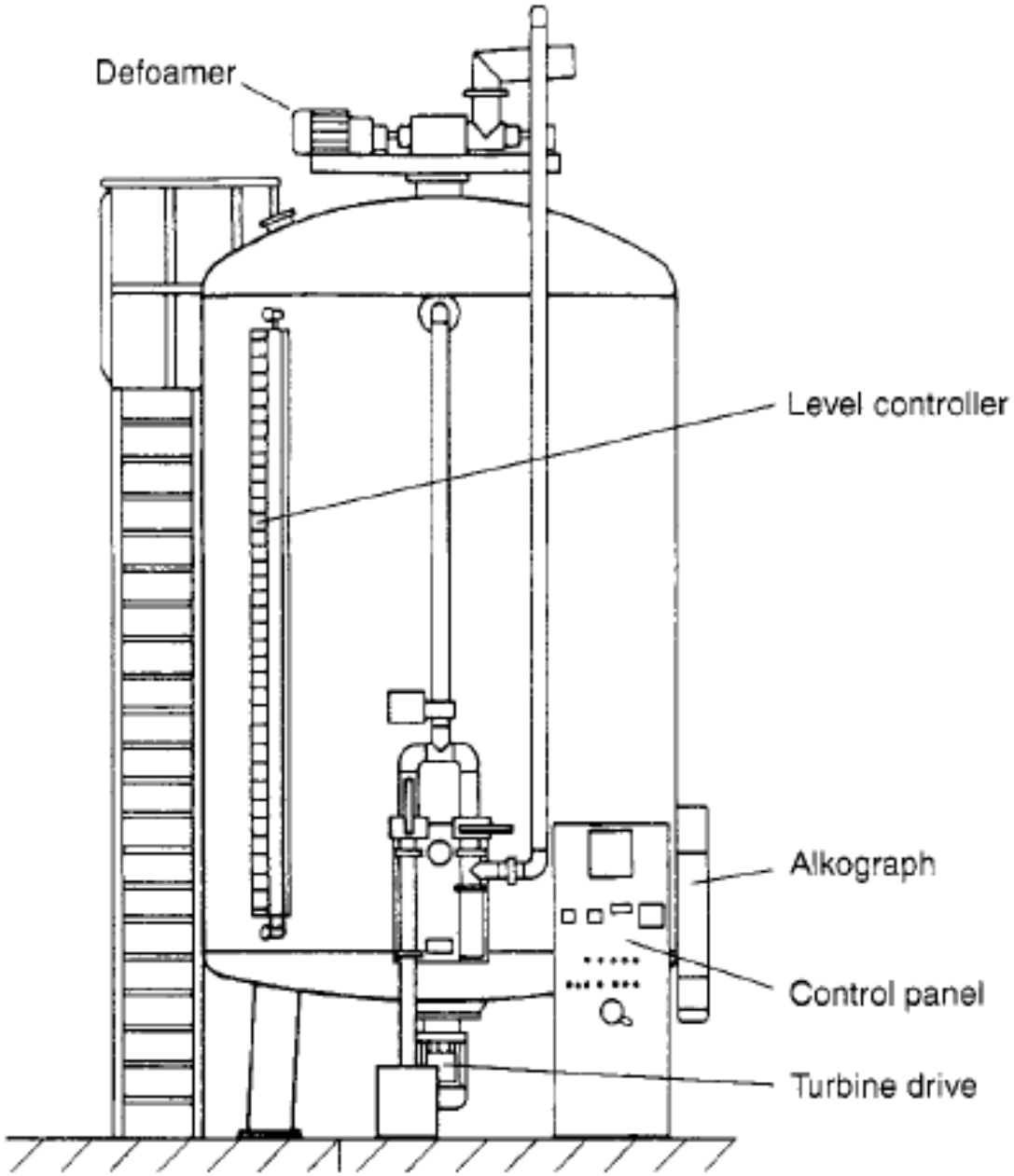


Figure 4 The Frings Acetator.

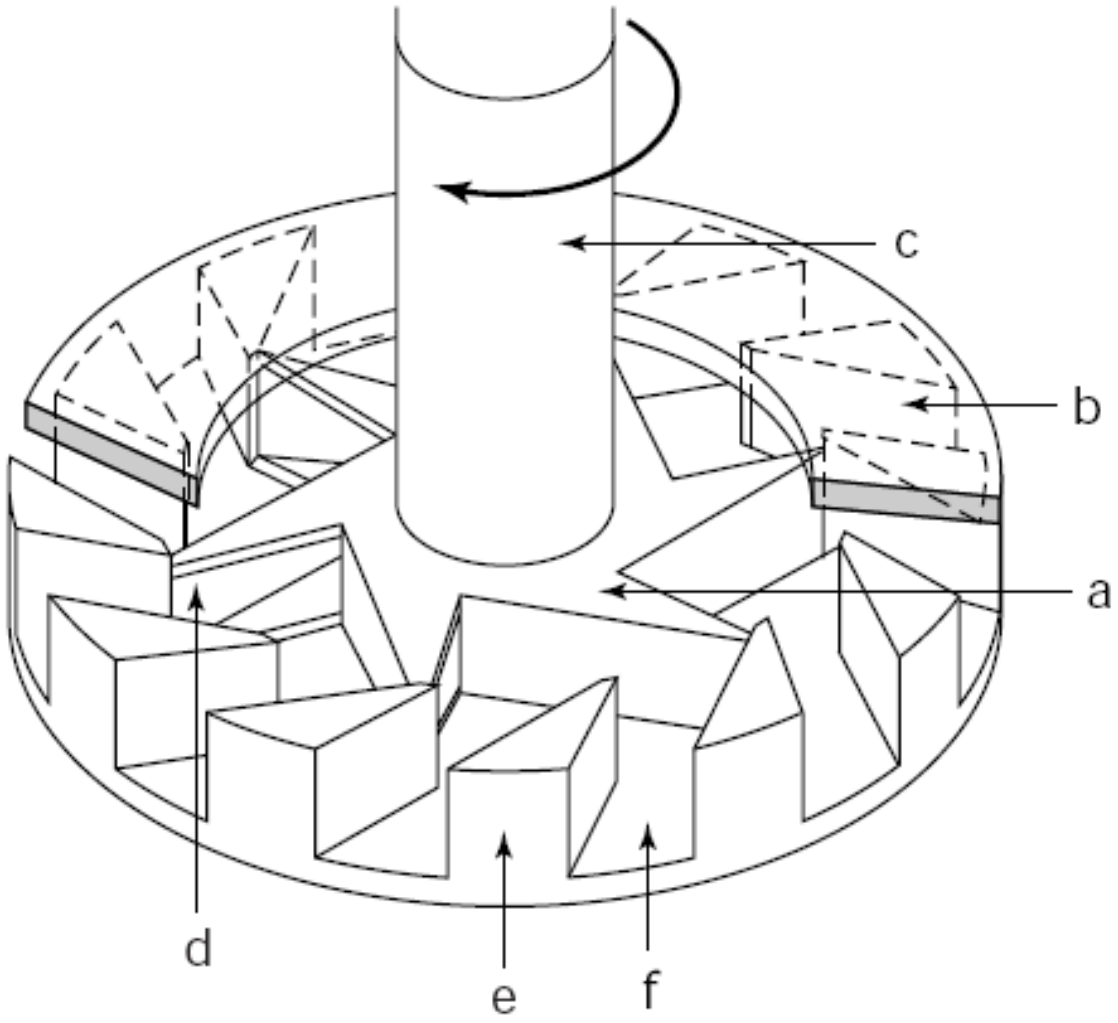


Figure 1. The Frings Aerator. a = hollow rotor; b = stator; c = air suction pipe; d = openings for air exit; e = wedges to form the channels; f = channels to form the beams of air–liquid emulsion.

Frings acetator

Final adjustments

- Dilution to 8%
- Clarification, filtration, maturation, pasterization, filling
- aromatization

Defects of vinegar

- Nematode
- Mite
- fly

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PRAGUE

Biotechnology in Food Industry

Lecture X

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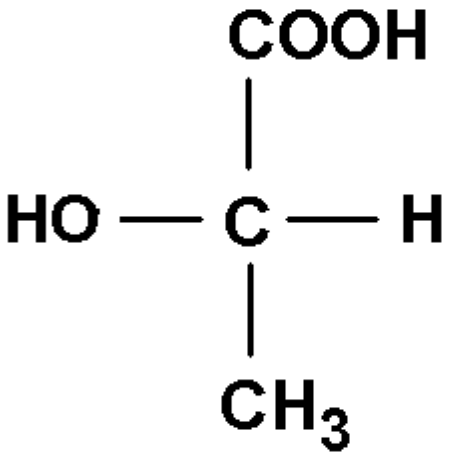
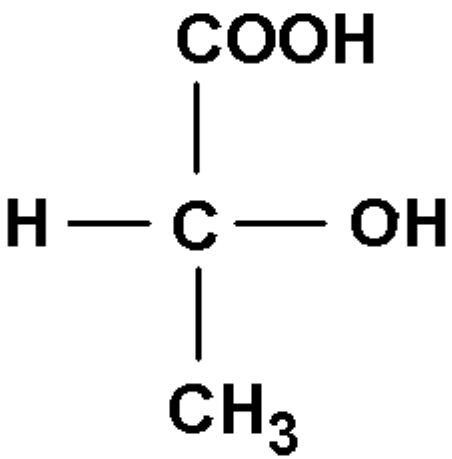
Lactic acid

95% produced by fermentation



L(+)- lactic acid



D(-)- lactic acid

The first industrial fermentation process in the USA in 1881

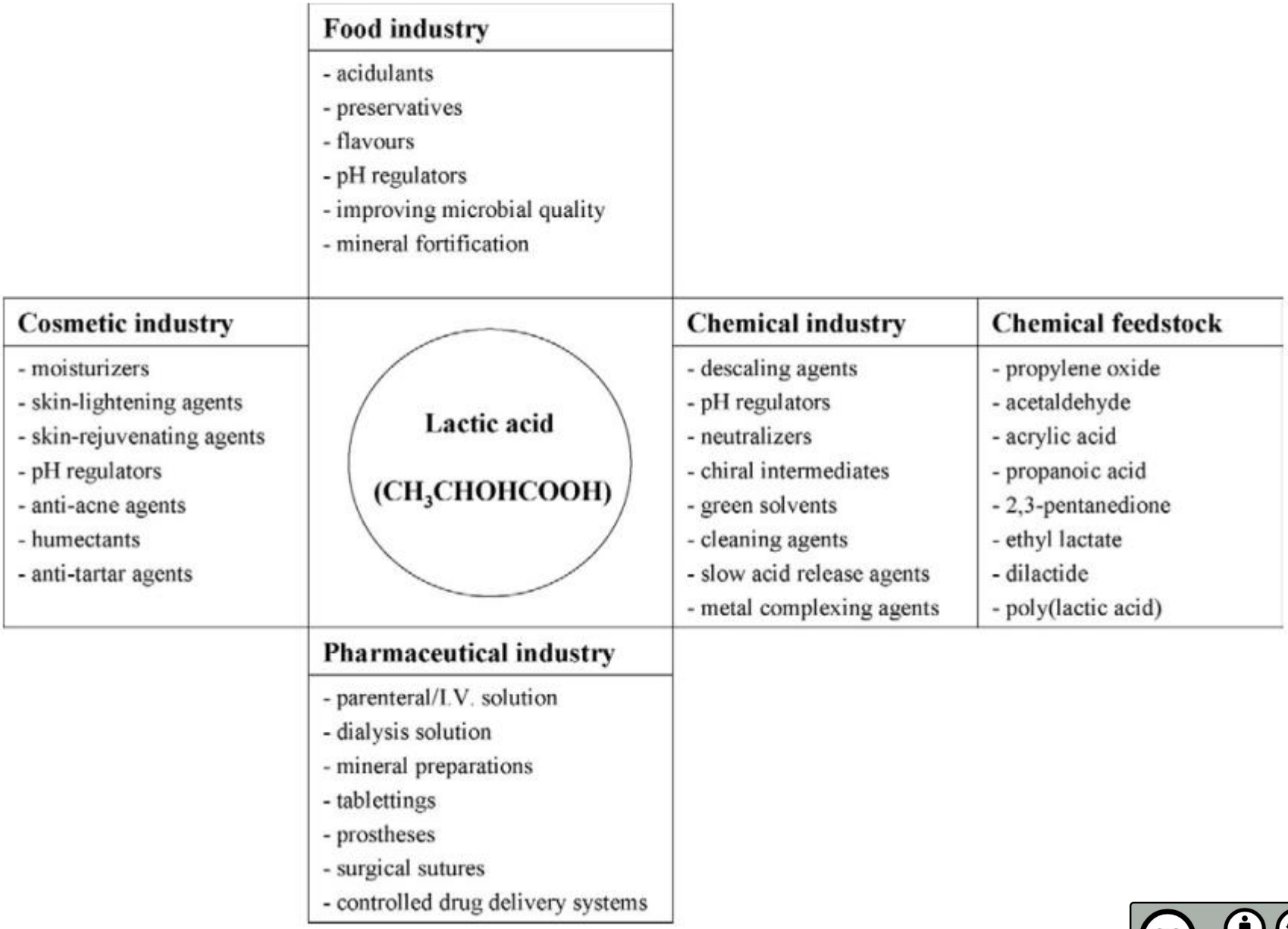
Use of lactic acid

- Preservative
- GRAS food additive
- Leaching skins
- Pharmacy
- Biodegradable Plastics (PLA)

1.3 kg CO2 eq./kg polymer and 42 MJ/kg polymer

versus PET 3.2 kg CO2 eq./kg polymer and 80 MJ/kg polymer,

130-150 thousands tons annualy


Food industry
- acidulants
- preservatives
- flavours
- pH regulators
- improving microbial quality
- mineral fortification
Cosmetic industry
- moisturizers
- skin-lightening agents
- skin-rejuvenating agents
- pH regulators
- anti-acne agents
- humectants
- anti-tartar agents
Lactic acid
(CH3CHOHCOOH)
Chemical industry
- descaling agents
- pH regulators
- neutralizers
- chiral intermediates
- green solvents
- cleaning agents
- slow acid release agents
- metal complexing agents
Chemical feedstock
- propylene oxide
- acetaldehyde
- acrylic acid
- propanoic acid
- 2,3-pentanedione
- ethyl lactate
- dilactide
- poly(lactic acid)
Pharmaceutical industry
- parenteral/I.V. solution
- dialysis solution
- mineral preparations
- tablettings
- prostheses
- surgical sutures
- controlled drug delivery systems

Chemical production

(a) Addition of Hydrogen CyanideCatalyst

$$\underset{\text{Acetaldehyde}}{CH_3CHO} + \underset{\text{Hydrogen cyanide}}{HCN} \xrightarrow{\text{Catalyst}} \underset{\text{Lactonitrile}}{CH_3CHOHCN}$$

(b) Hydrolysis by H_2SO_4

$$\underset{\text{Lactonitrile}}{CH_3CHOHCN} + H_2O + 1/2 \underset{\text{Sulphuric acid}}{H_2SO_4} \rightarrow \underset{\text{Lactic acid}}{CH_3CHOHCOOH} + \tfrac{1}{2}\,(NH_4)2SO_4$$

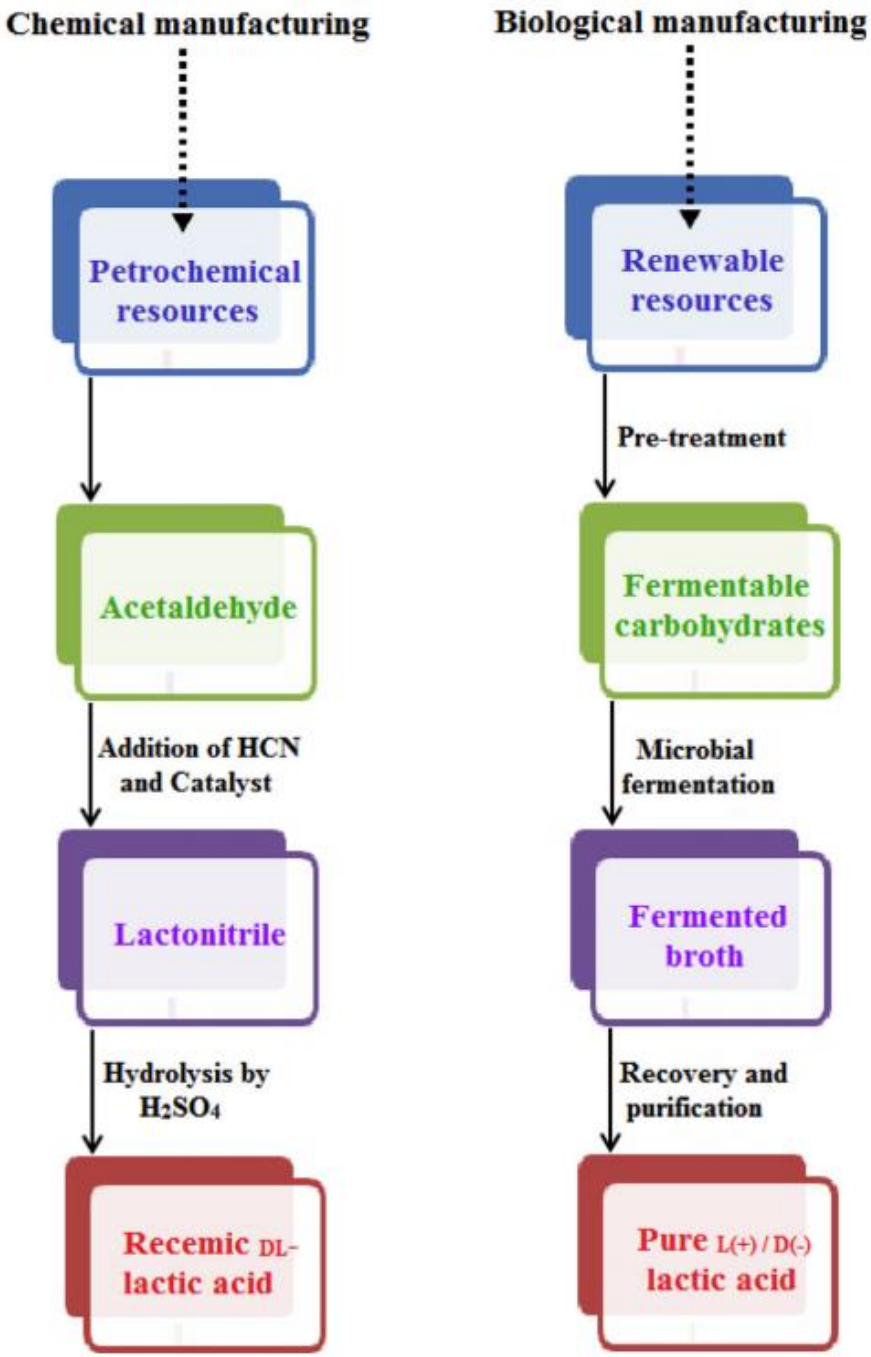
Ammonium salt

(c) Esterification

$$\underset{\text{Lactic acid}}{CH_3CHOHCOOH} + \underset{\text{Methanol}}{CH_3OH}\,CH_3 \rightarrow \underset{\text{Methyl lactate}}{CHOHCOOCH_3} + H_2O$$

(d) Hydrolysis by H_2O

$$\underset{\text{Methyl lactate}}{CH_3CHOHCOOCH_3} + H_2O \rightarrow \underset{\text{Lactic acid}}{CH_3CHOHCOOH} + \underset{\text{Methanol}}{CH_3OH}$$


Chemical manufacturing
Petrochemical resources
Acetaldehyde
Addition of HCN and Catalyst
Lactonitrile
Hydrolysis by H_2SO_4
Recemic DL-lactic acid
Biological manufacturing
Renewable resources
Pre-treatment
Fermentable carbohydrates
Microbial fermentation
Fermented broth
Recovery and purification
Pure L(+) / D(-) lactic acid

Production microorganisms

- Homofermentativní G^+ mléčné bakterie:

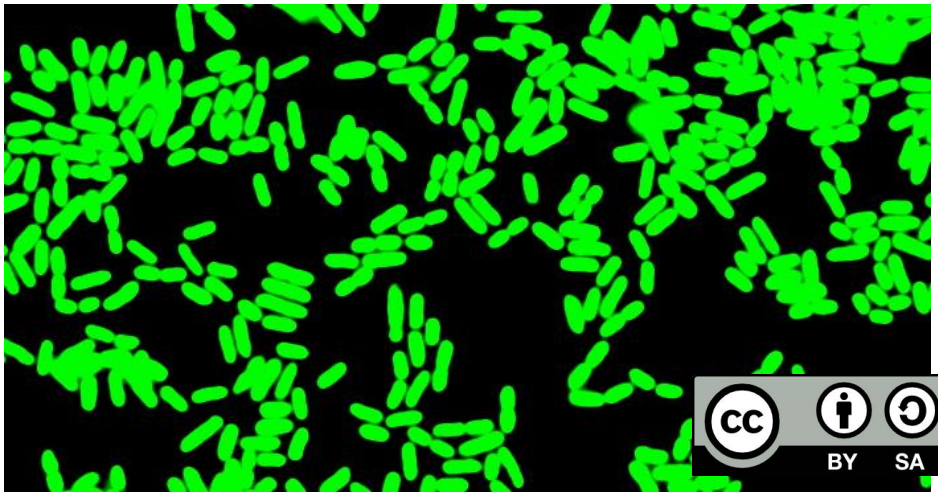
  Lactobacillus, Streptococcus

  L. delbrueckii ssp. delbrueckii, L.delbrueckii ssp. Bulgaricus

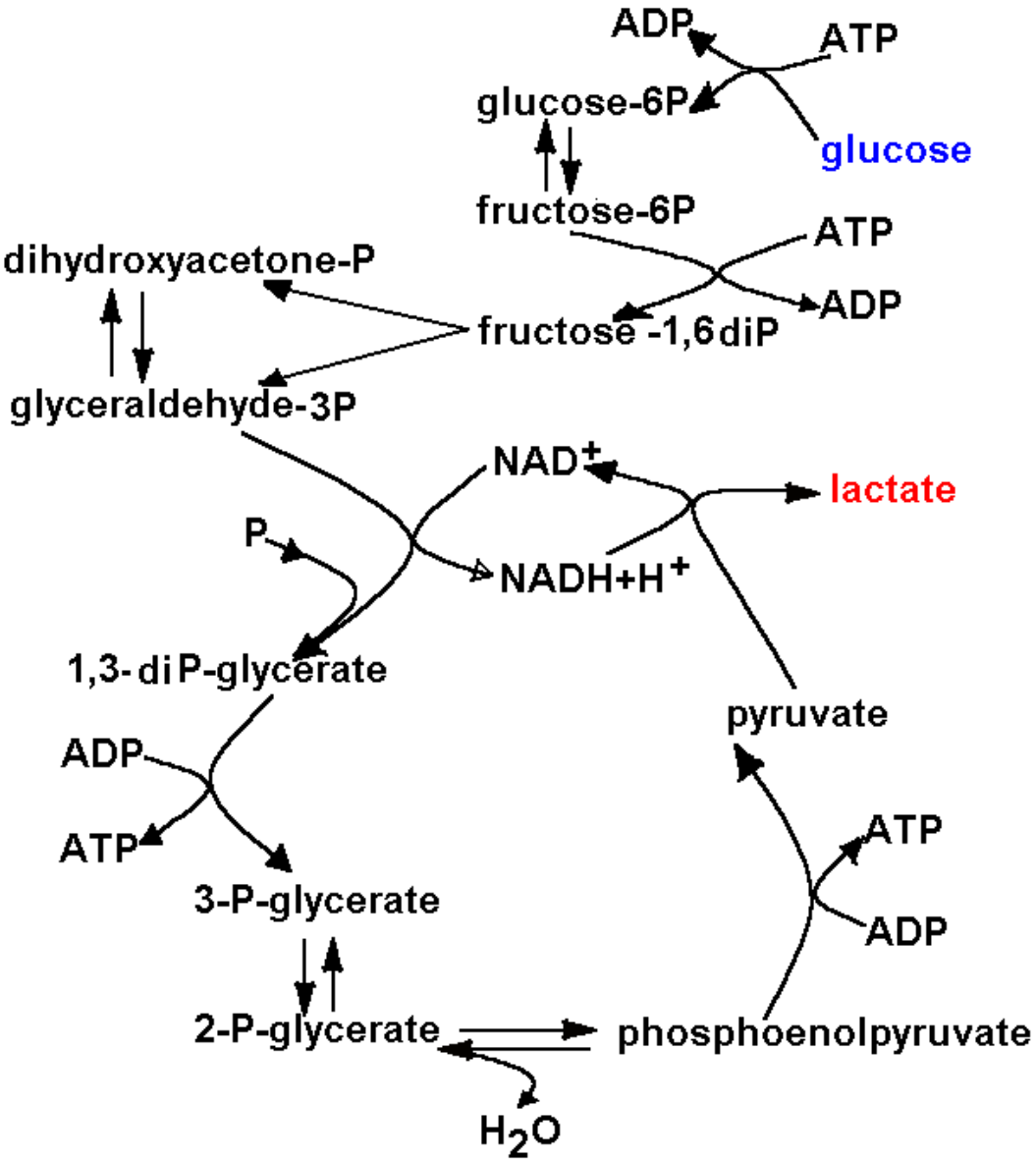
Lactobacillus plantarum

- Plísně:

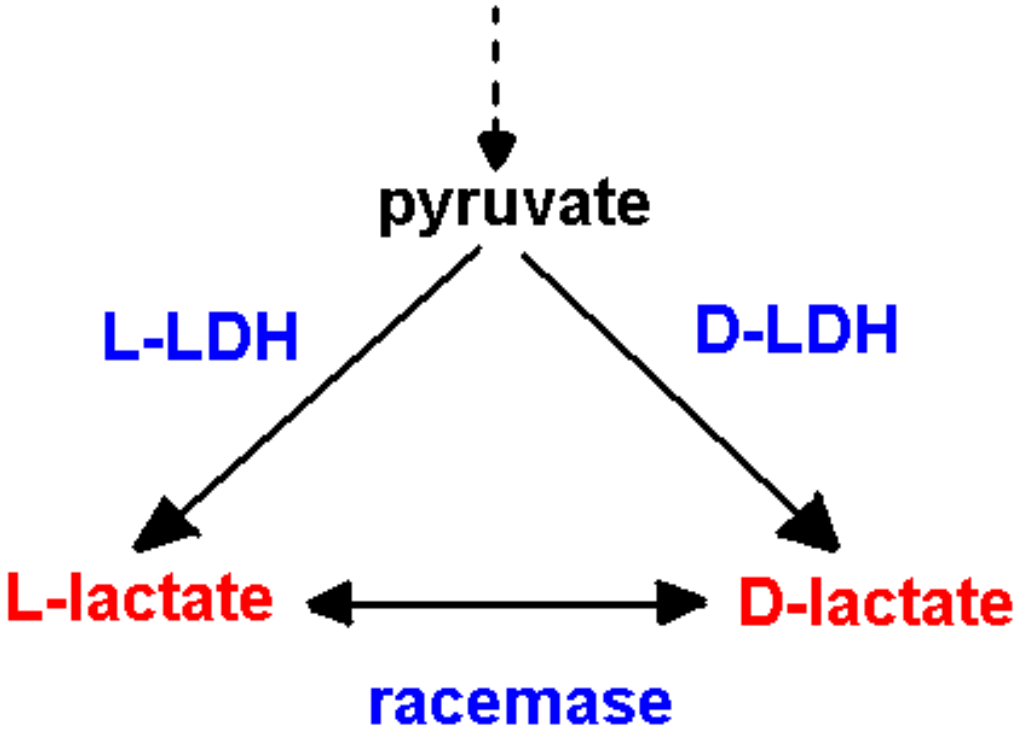
Rhizopus oryzae



Metabolic pathway



Formation of racemic mixture



Properties of lactic acid bacteria (LAB)

- Relation to oxygen: anaerobic, microaerophilic, aerotolerant
- Often auxotrophic - amino acids, vitamins, peptides
- Genetically Unstable - Plasmid Inheritance, Conjugation, Transduction, Transposition
- Susceptible to bacteriophage attack
- Mesophilic or thermophilic
- Creation of bacteriocins
- Sensitive to pH drop
- They use glucose, fructose, maltose, lactose, sucrose

Cultivation of LAB

Substrate: lactose (whey), glucose, sucrose, starch + nutrients (complex sources of amino acids)

Newly - hydrolysates of lignocellulosic materials

Storage of the strain - frozen

The need to regulate pH during cultivation

Types of cultivation: Batch, fed-batch, continuous, immobilized systems

Possible use of agricultural waste

Process 1 – use of calcium lactate

- Concentration of sugar source 120-180 g / L, complex sources of nitrogen
- Concrete bioreactors with a special surface
- Cultivation temperature> 45^{o}C
- The addition of $CaCO_3$ or $Ca(OH)_2$ (maintaining the pH between 5.5 and 6)
- Inoculum - 10% volume
- Decomposition of sulfuric acid lactate, formation of gypsum

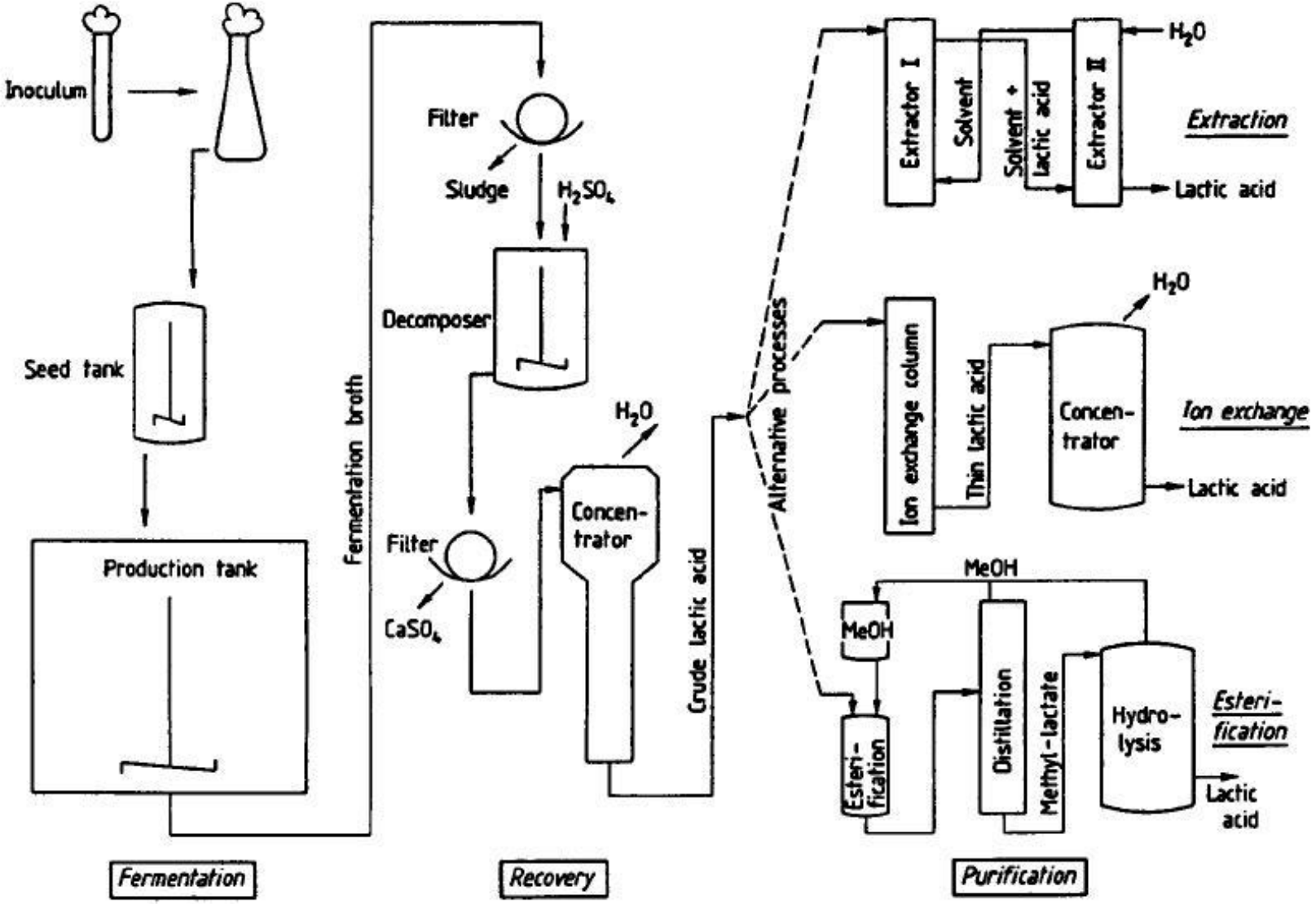


Fig. 2. Industrial production of lactic acid (BUCHTA, 1983; with permission from VCH).

Process 2 – use of ammonium lactate

Neutralization of the formed acid with ammonia

Esterification of ammonium lactate, release of ammonium ion

Distillation

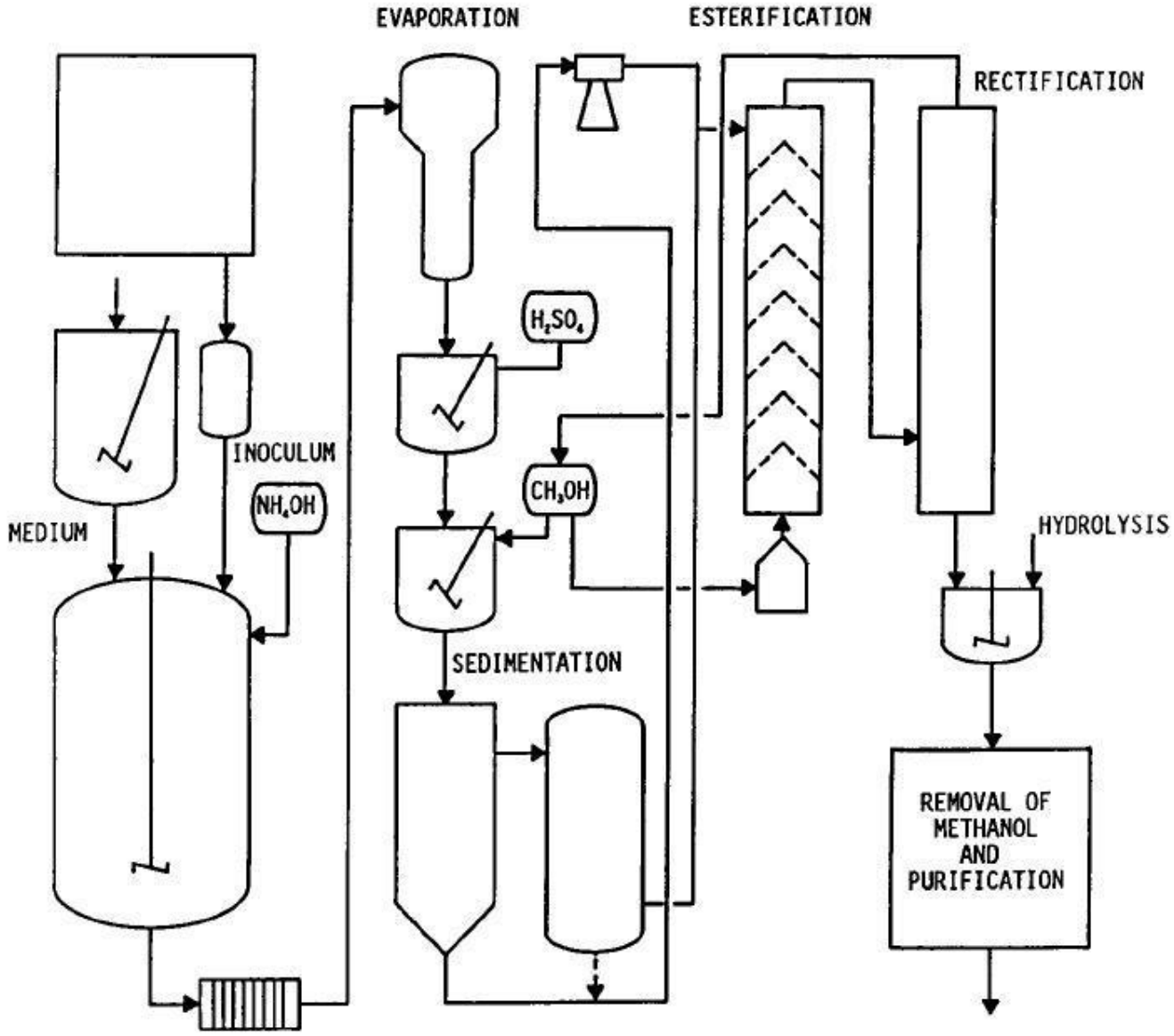
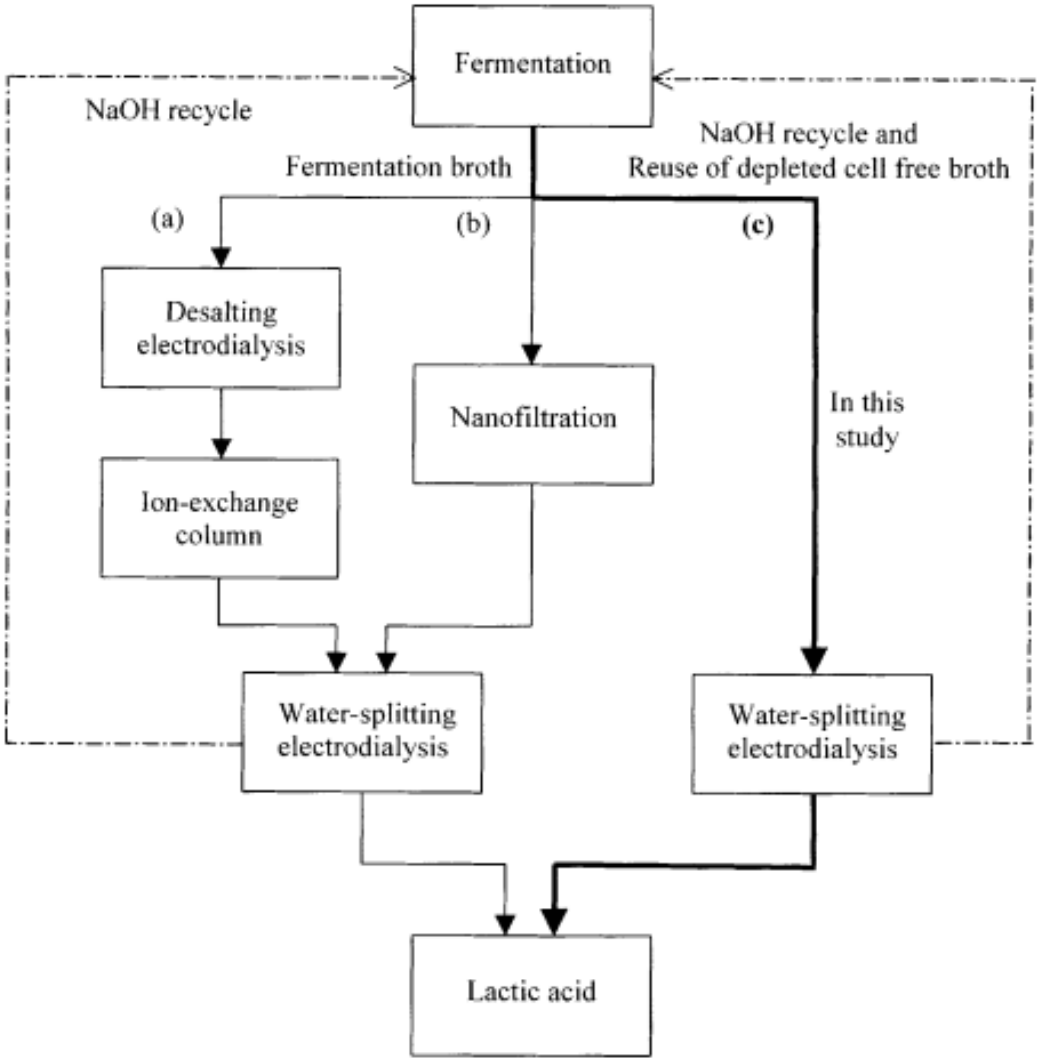


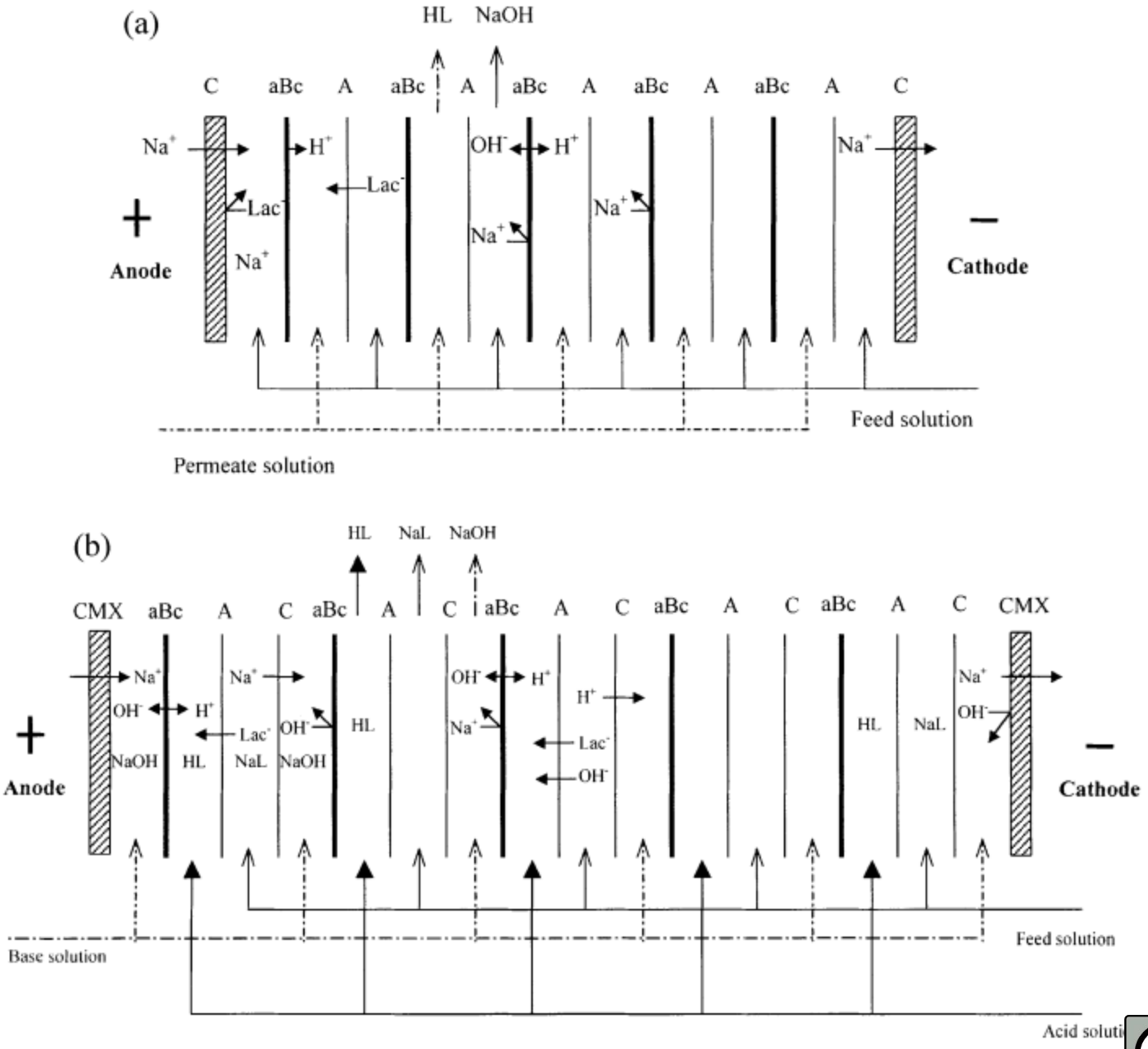
Fig. 3. Industrial production of lactic acid using the ammonium lactate process.

Izolation of lactic acid

- Calcium lactate precipitation, filtration, release from salt by increasing T and pH, again precipitation and thickening
- Solvent extraction with isopropyl ether, isobutanol, re-extraction into water, precipitation
- Sorbtion on ionexes
- Electrodialysis
- Distillation after esterification with methanol

Use of electrodialysis for the purification of lactic acid

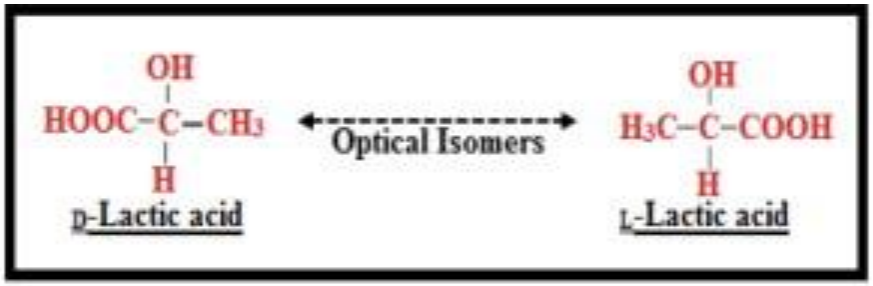


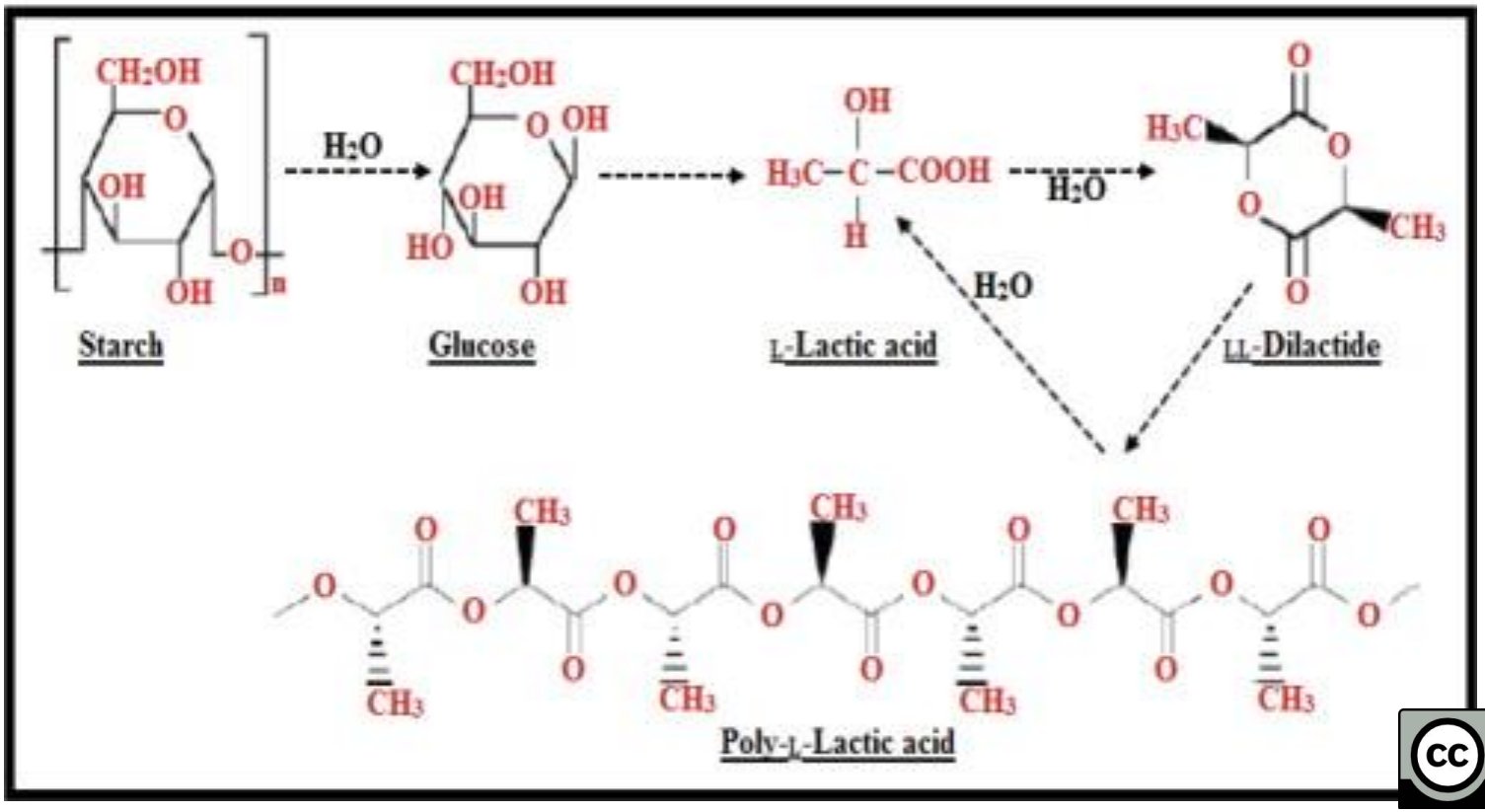

(a)
HL
NaOH
C
aBc
A
aBc
A
aBc
A
aBc
A
aBc
A
C
Na+
H+
Lac-
Lac-
Na+
OH-
H+
Na+
Na+
Na+
+
Anode
−
Cathode
Feed solution
Permeate solution
(b)
HL
NaL
NaOH
CMX
aBc
A
C
aBc
A
C
aBc
A
C
aBc
A
C
aBc
A
C
CMX
Na+
OH-
NaOH
H+
HL
Na+
Lac-
NaL
OH-
NaOH
HL
OH-
H+
Na+
H+
Lac-
OH-
HL
NaL
Na+
OH-
+
Anode
−
Cathode
Base solution
Feed solution
Acid soluti

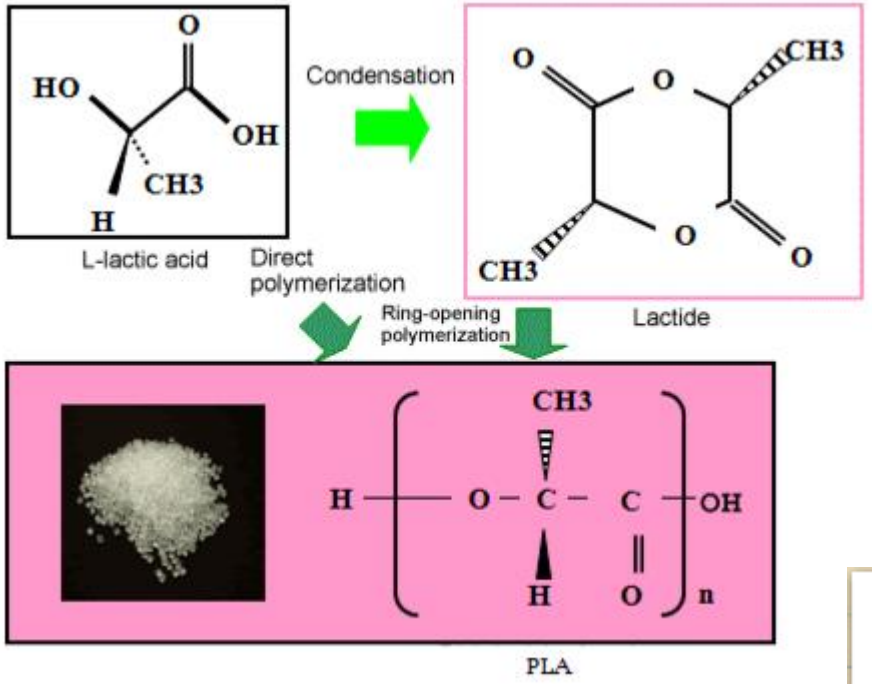
Purification of lactic acid

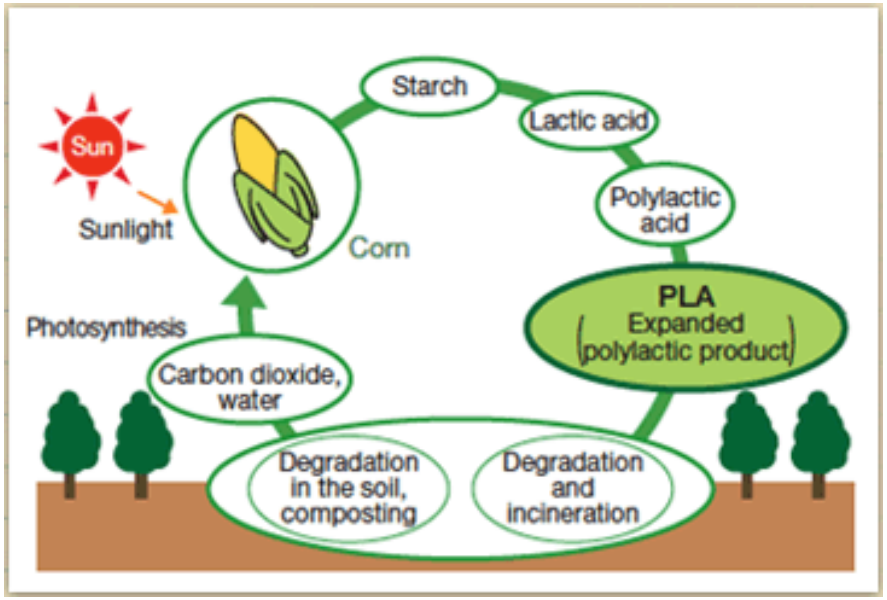
- Active carbon
- precipitation of heavy metals
- Thickening up to 80%
- Ionex resins

Production of PLA






HO
O
OH
CH3
H
L-lactic acid
Condensation
O
O
CH3
O
O
CH3
O
Lactide
Direct polymerization
Ring-opening polymerization
CH3
H
O
C
C
OH
H
O
n
PLA


Sun
Sunlight
Starch
Lactic acid
Polylactic acid
Corn
PLA
Expanded
polylactic product
Photosynthesis
Carbon dioxide, water
Degradation in the soil, composting
Degradation and incineration

Production of lactic acid using Rhizopus mold

Advantages and disadvantages compared to lactic acid bacteria:

Using defined media

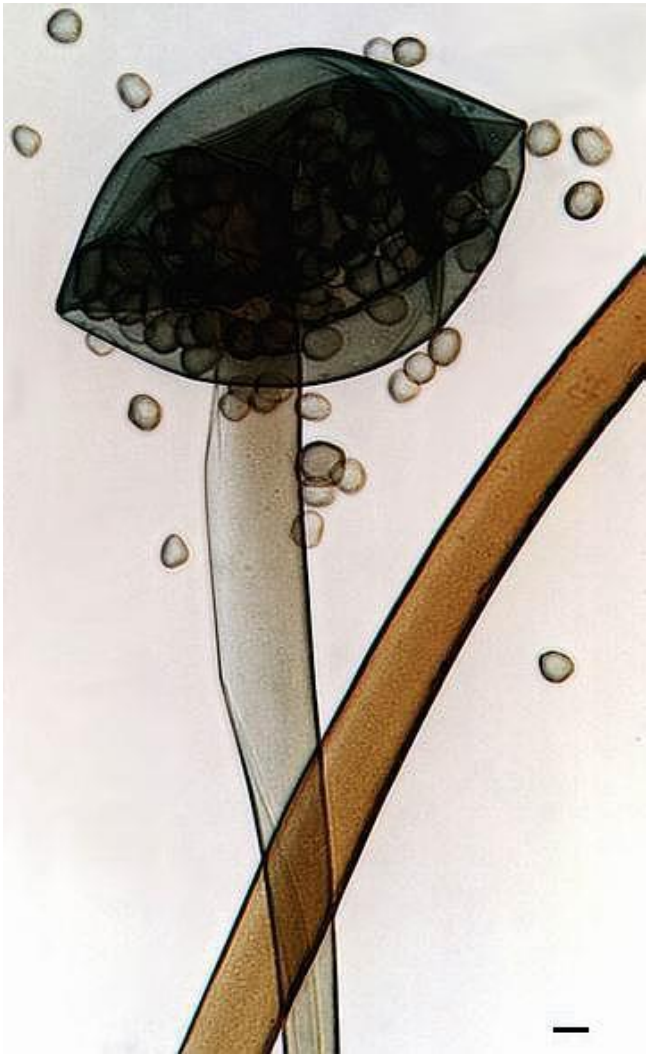
Inorganic nitrogen source

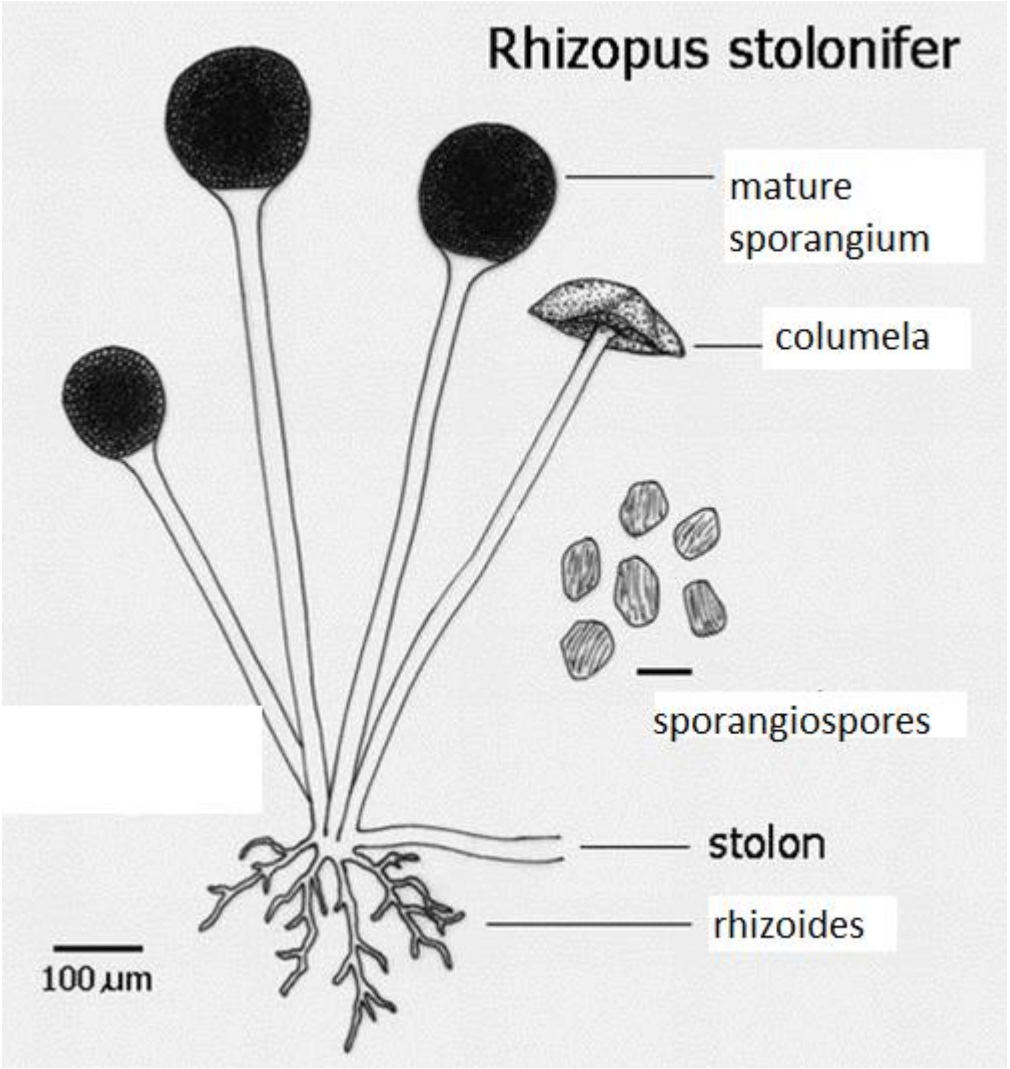
High initial sugar concentrations

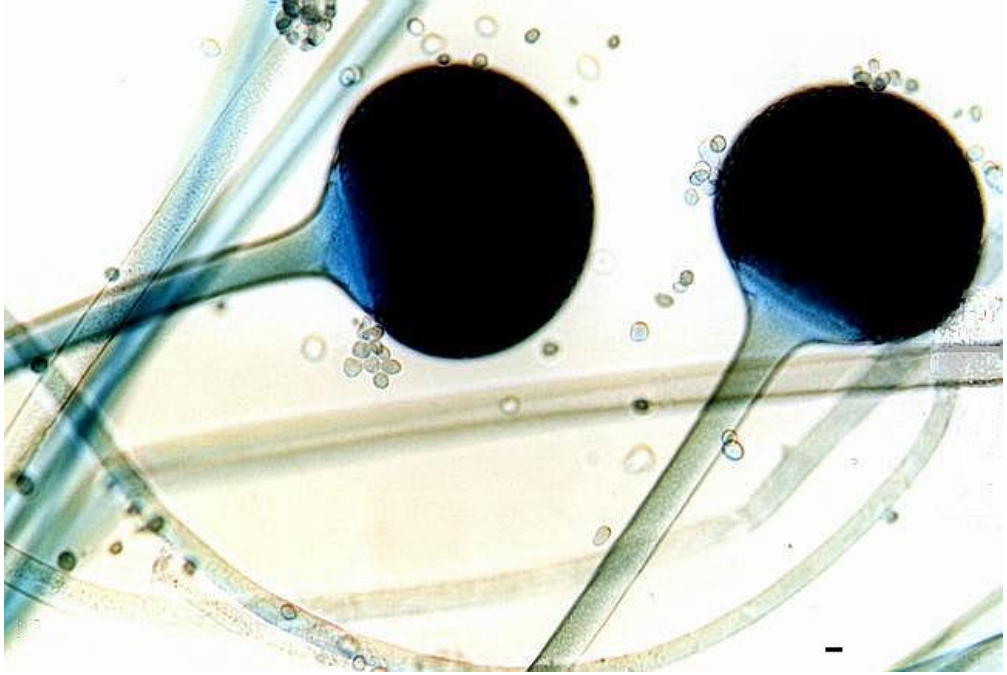
Production of exclusively L-lactic acid

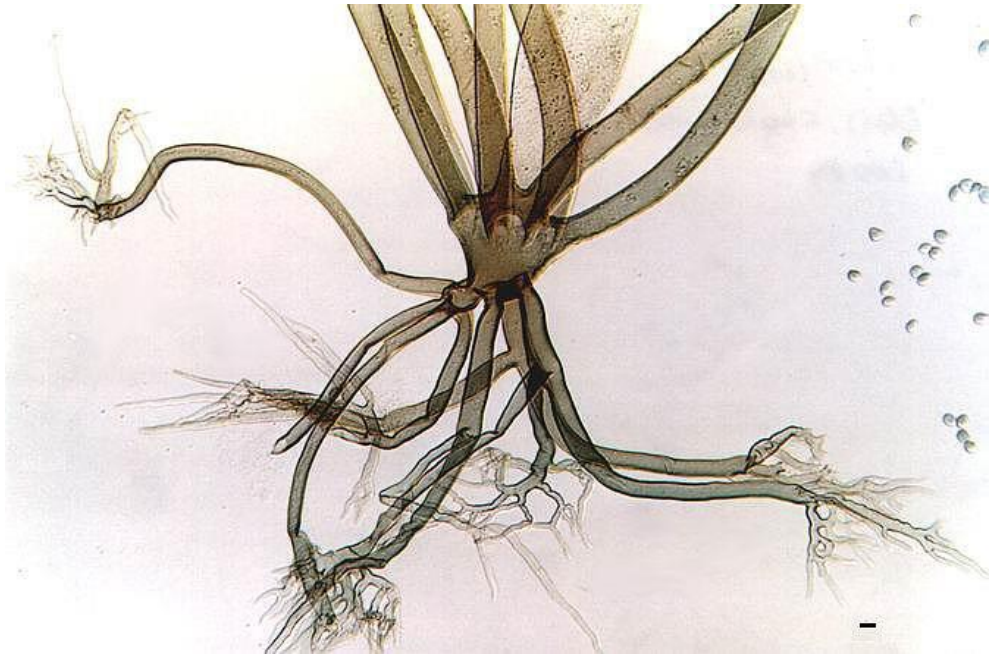
X side production - ethanol, fumaric acid ...

Rhizopus









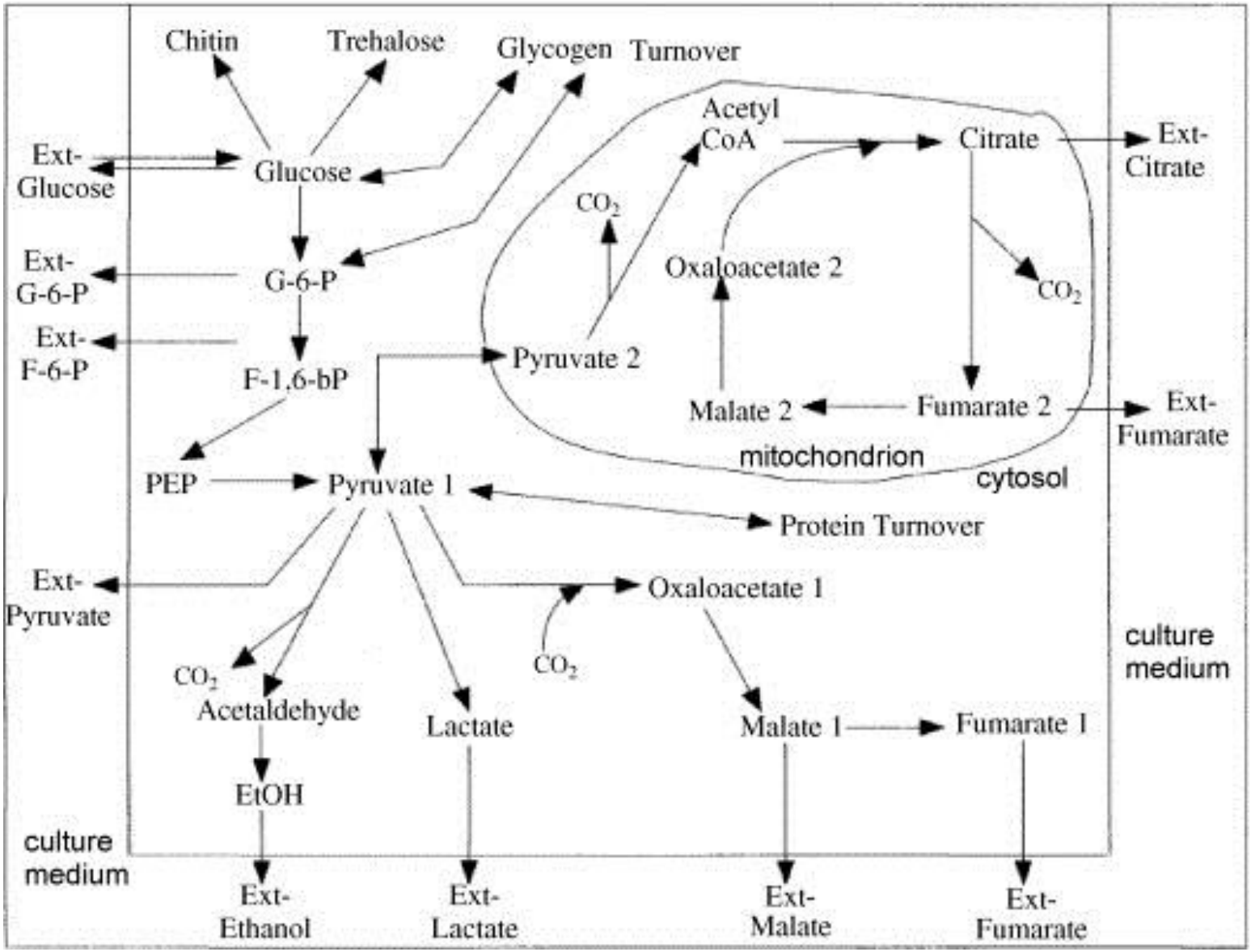
Rhizopus oryzae

Taxonomic classification : Zygomycota, Mucorales

Reproduction: sexual zygosporangia, asexual sporangiospores. Heterothalic species.

Formation of lactic acid

- GlycolysisUnder Aerobic Conditions - More Lactate
- Under anaerobic conditions - more ethanol


Chitin
Trehalose
Glycogen
Turnover
Ext-
Glucose
Glucose
Acetyl
CoA
Citrate
Ext-
Citrate
CO_2
Ext-
G-6-P
G-6-P
Oxaloacetate 2
CO_2
Ext-
F-6-P
F-1,6-bP
Pyruvate 2
Malate 2
Fumarate 2
Ext-
Fumarate
mitochondrion
cytosol
PEP
Pyruvate 1
Protein Turnover
Ext-
Pyruvate
Oxaloacetate 1
culture
medium
CO_2
CO_2
Acetaldehyde
Lactate
Malate 1
Fumarate 1
EtOH
culture
medium
Ext-
Ethanol
Ext-
Lactate
Ext-
Malate
Ext-
Fumarate

Silage production

Target:

- preserving feed by means of lactic fermentation
- prevention of toxin production
- aerobic stability
- no butyric acid, low acetic acid content

Factors influencing the ensiling process

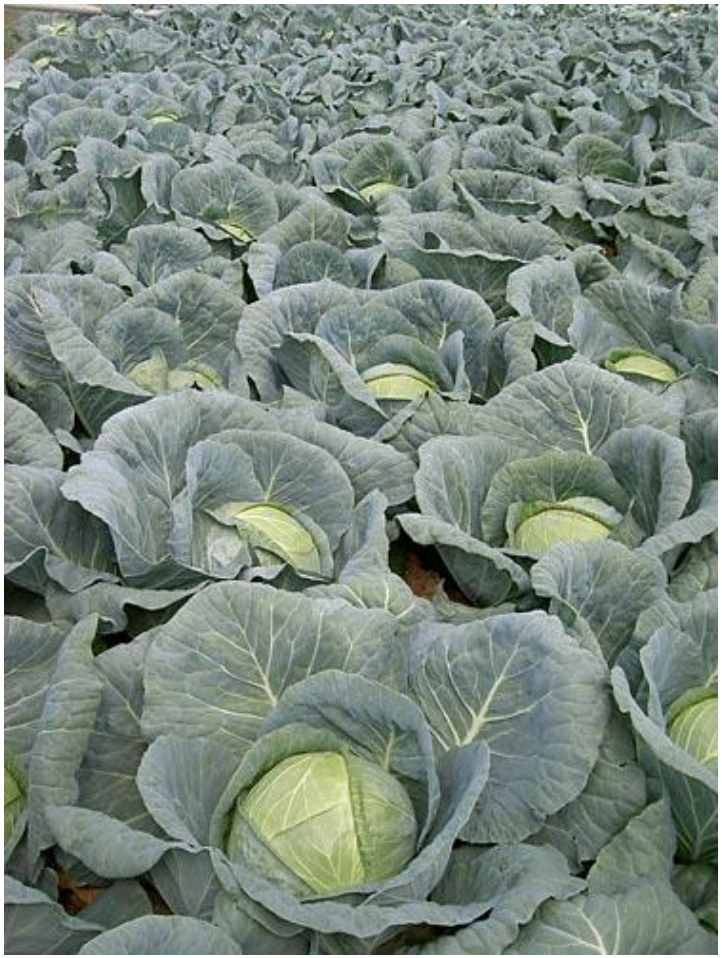
- Substrate composition: grass, corn ... (content of usable carbohydrates, starch ...)
- Inoculating silage preparation (homofermentative or heterofermentative mixed culture of lactic bacteria)
- Sufficient suction, airtight pit closure (anaerobic process)

The most common problems

- Molding of silage (wet silage) - prevention - homofermentative bacteria
- Poor aerobic stability - prevention - Heterofermentative bacteria
- Secondary heating - prevention - acetic acid production - prevention of yeast growth

Fermented vegetable

Sauerkraut



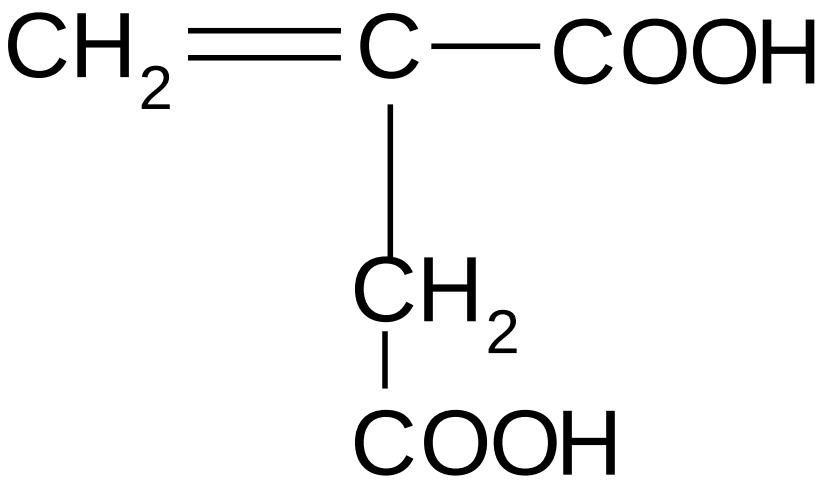
Processing

1. Quality of raw material
2. Post-harvest crop sorting
3. Grinding, cutting
4. Sitting in a vat
5. Spice addition - salt, caraway (sugar)
6. Inoculation
7. Own fermentation
8. Packaging
9. (Pasterization)

Fermentation

- Anaerobic
- homo- or heterofermentative lactic acid bacteria
- **Undesirable processes**: formation of biogenic amines, butyric acid, secondary alcoholic fermentation, contamination of the fermenting liquid with aerobic microorganisms

Itaconic acid



Use:

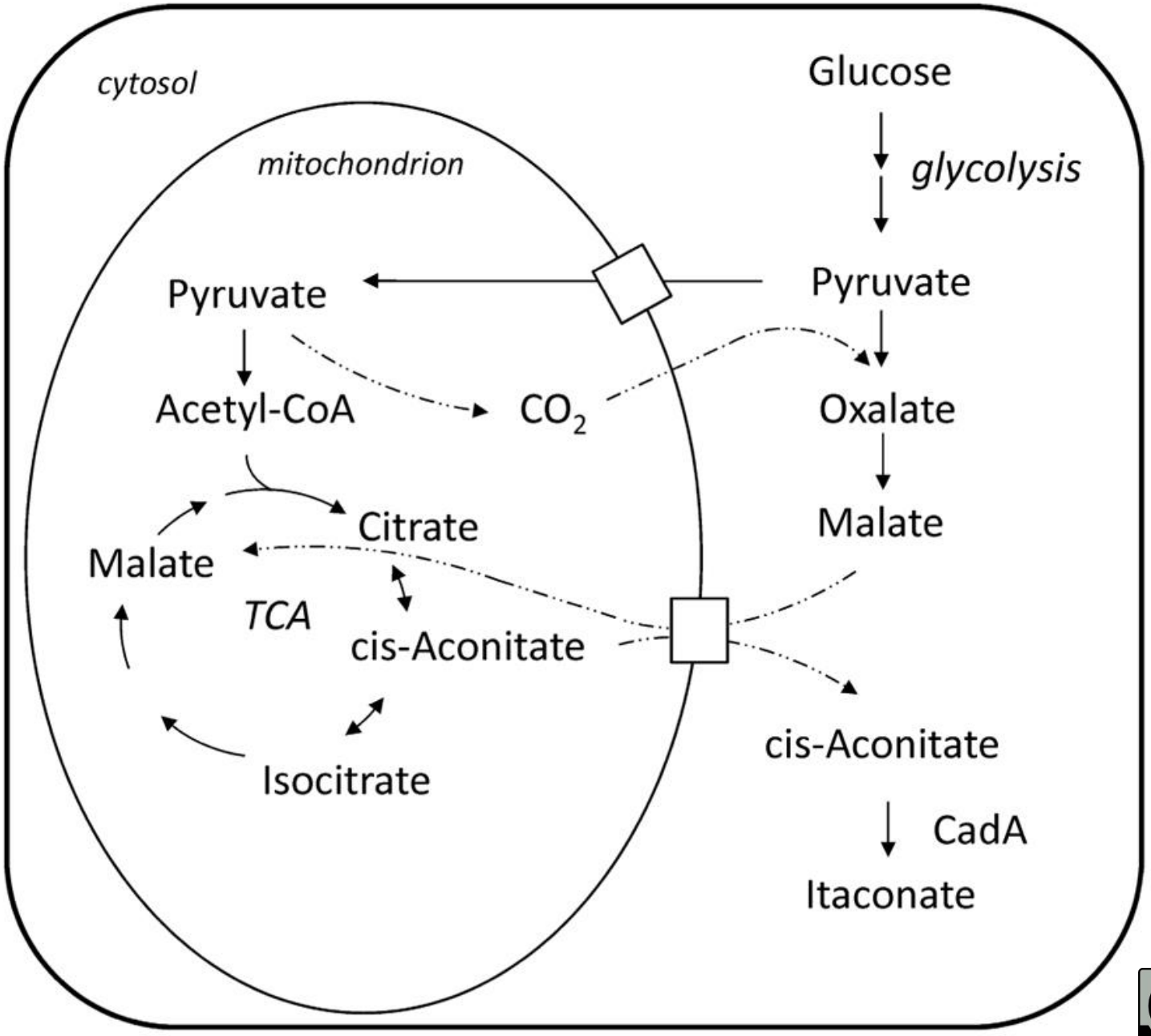
Manufacture of plastics

Production of printing ink

Production of acrylics

Producer of itaconic acid :

Aspergillus terrreus, Aspergillus itaconicus


cytosol
mitochondrion
Glucose
glycolysis
Pyruvate
Pyruvate
Acetyl-CoA
CO_2
Oxalate
Malate
Citrate
Malate
TCA
cis-Aconitate
cis-Aconitate
Isocitrate
CadA
Itaconate

Production of itaconic acid

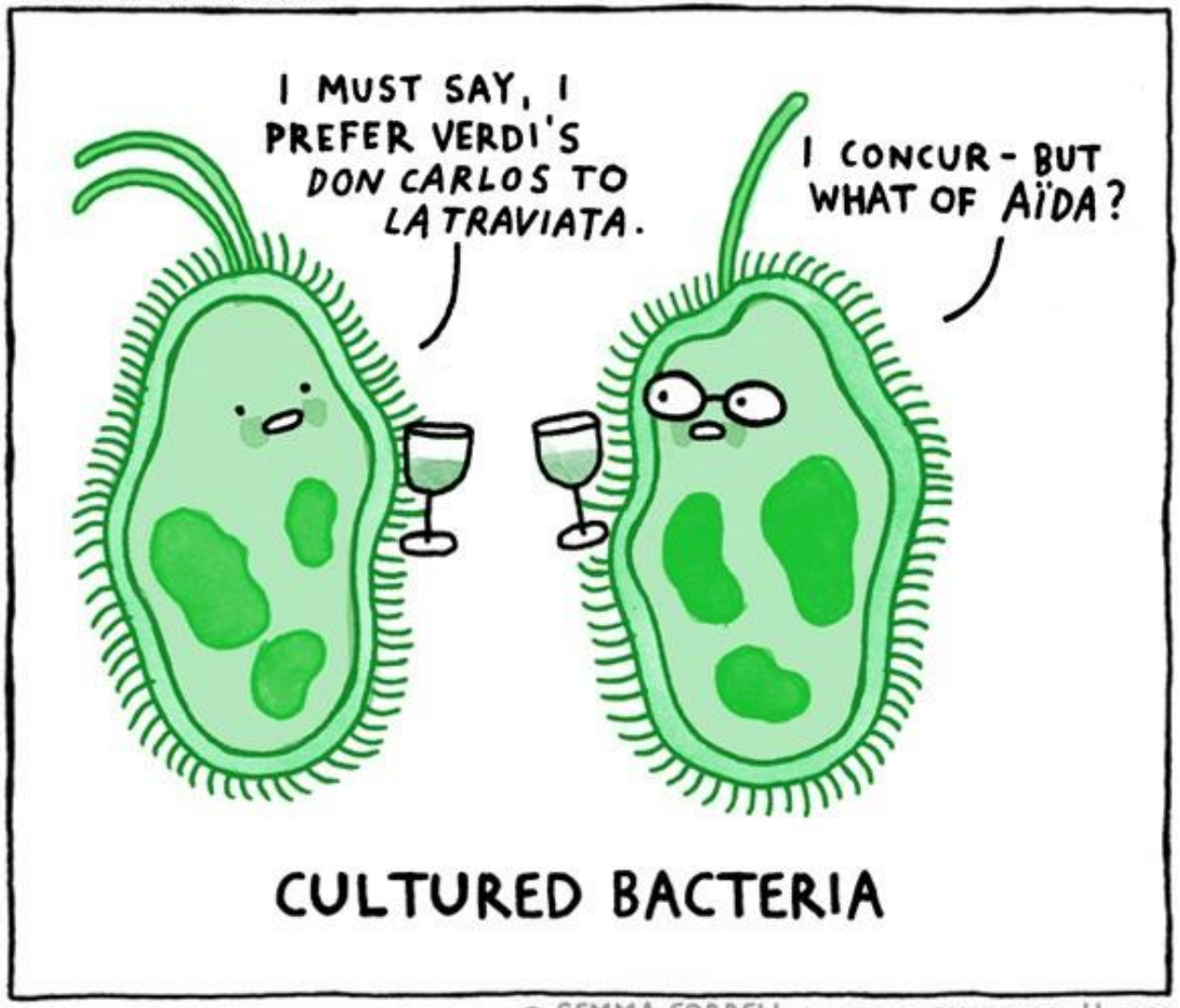
- Molasses medium (15% sugar)
- Continuous aeration
- Production temperature 45 ° C
- Neutralization of pH using calcium or ammonium hydroxide
- Influence of morphology on acid production

Izolation and purification

- Molasses medium (15% sugar)
- Continuous aeration
- Production temperature 45 ° C
- Neutralization of pH using calcium or ammonium hydroxide
- Influence of morphology on acid production

MONDAY PUNDAY

BY GEMMA CORRELL
WWW.GEMMACORRELL.COM



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UNIVERSITY OF
CHEMISTRY AND TECHNOLOGY
PRAGUE

Biotechnology in Food Industry

Lecture XI

Petra Patáková
petra.patakova@vscht.cz

Mikrobial production of amino acids

Use of amino acids

- Food industry - MSG (sodium glutamate) - umami taste
- L-aspartyl-L-phenylalanine (aspartame)
- glycine, L-cysteine
- Feed industry - Dietary supplements for D, L-methionine, L-lysine and L-threonine (L-tryptophan)
- Pharmaceutical industry - intravenous nutrition - infusion solutions, drug production
- Cosmetics - cysteine ...

Amino acids essential for humans

- L-isoleucine
- L-leucine
- L-lysine
- L-methionine
- L-fenylalanine
- L-treonine
- L-tryptohan
- L-valine

Industrial production of amino acids

- Chemical synthesis glycine, D, L-methinonine
- Extraction from hydrolysates
- Fermentation - MSG, L-lysine, L-threonine, L-phenylalanine, L-tryptophan
- Enzymatic catalysis

Tab. 2. Production of Amino Acids, Methods and Volume

Type	Amino Acid	Preferred Production Method
I	L-Alanine	enzymatic catalysis
I	L-Asparagine	extraction
I	L-Glutamine	fermentation, extraction
I	L-Histidine	fermentation, extraction
I	L-Isoleucine	fermentation, extraction
I	L-Leucine	fermentation, extraction
I	L-Methionine	enzymatic catalysis
I	L-Proline	fermentation, extraction
I	L-Serine	fermentation, extraction
I	L-Tyrosine	extraction
I	L-Valine	enzymatic catalysis, fermentation
II	L-Arginine	fermentation, extraction
II	L-Cysteine	reduction of L-cystine (extraction)
II	L-Tryptophan	fermentation
III	Glycine	chemical synthesis
III	L-Aspartic acid	enzymatic catalysis
III	L-Phenylalanine	fermentation
III	L-Threonine	fermentation
IV	D,L-Methionine	chemical synthesis
IV	L-Glutamic acid	fermentation
IV	L-Lysine	fermentation

Type I	100–1000 t a^{-1}
Type II	1000–8000 t a^{-1}
Type III	8000–100000 t a^{-1}
Type IV	100000–800000 t a^{-1}

Microbial amino acid production

Food industry

L-glutamic acid.
L-asparagic a.
L-phenylalanine

In foods 0.1-0.8% MSG (sodium glutamate)

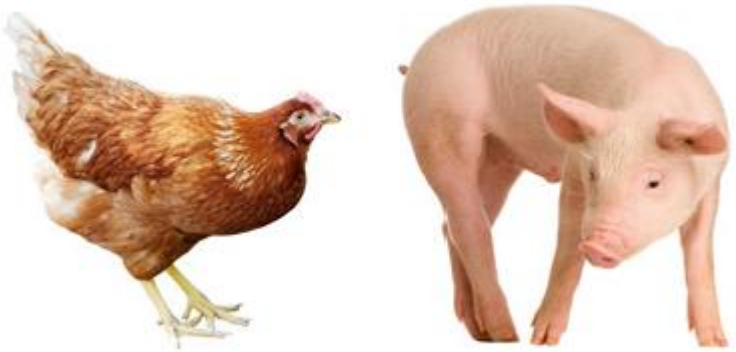
Feed

D, L-methionine

L-lysine

L-threonine

L-tryptophan



Pharmacy

L-alanine

L-arginine

L-glutamine

L-histidine

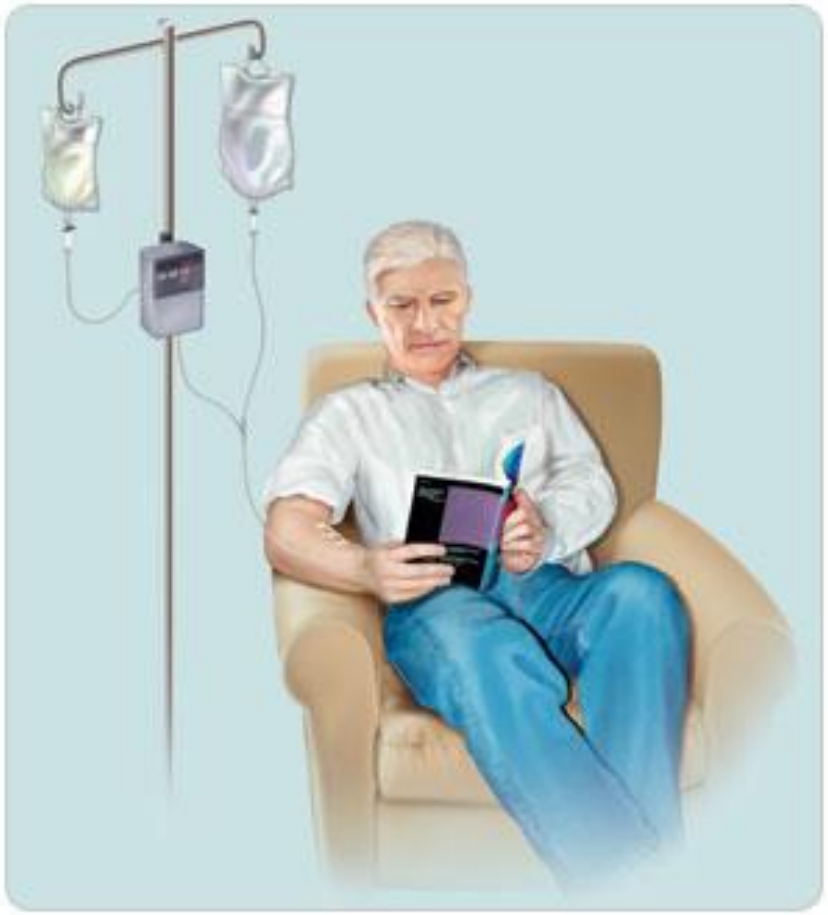
L-isoleucine

L-leucine, L-valine

L-methionine

L-proline

L-serine



A taste of amino acids

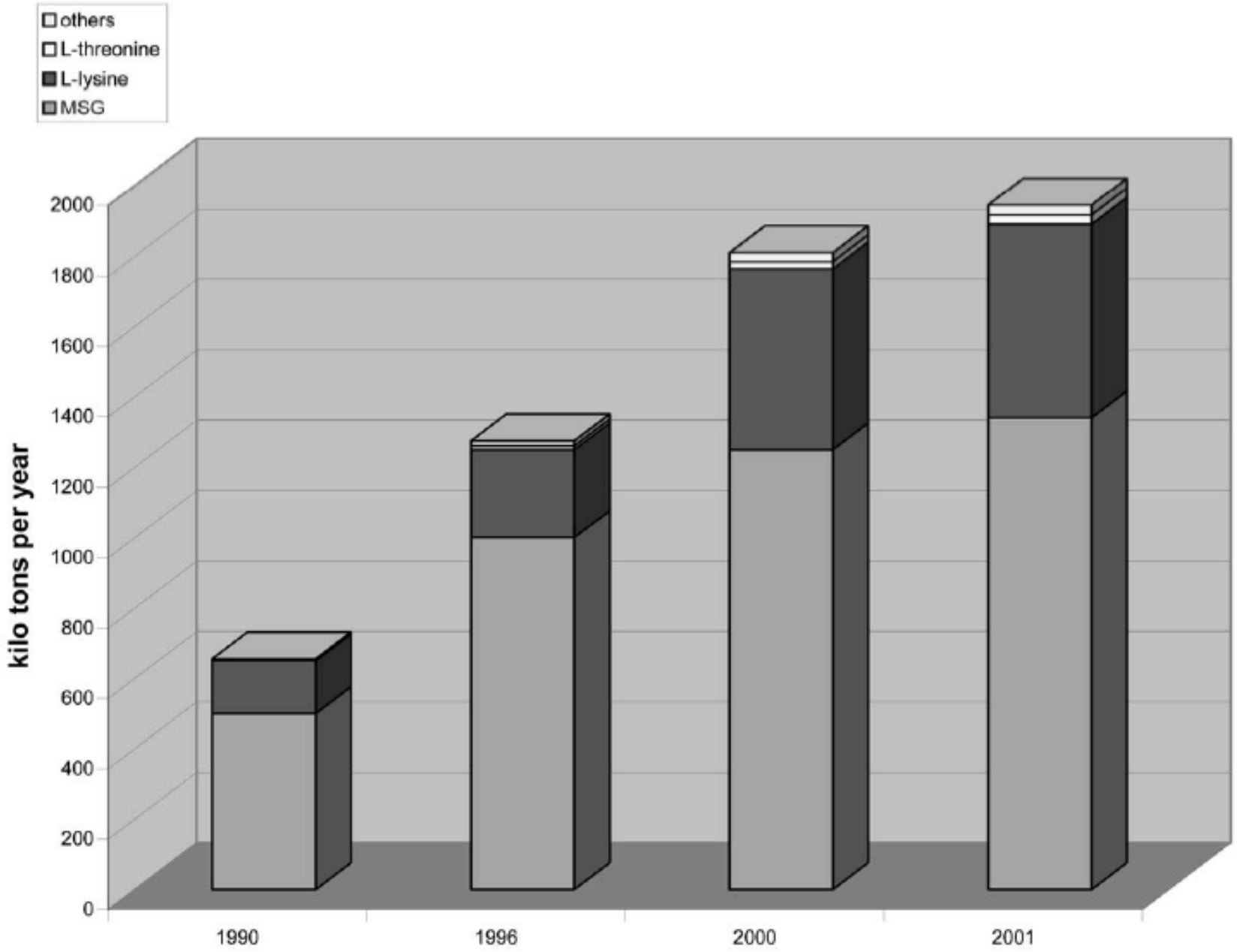


Fig. 1. Market development of biotechnological produced amino acids.

2

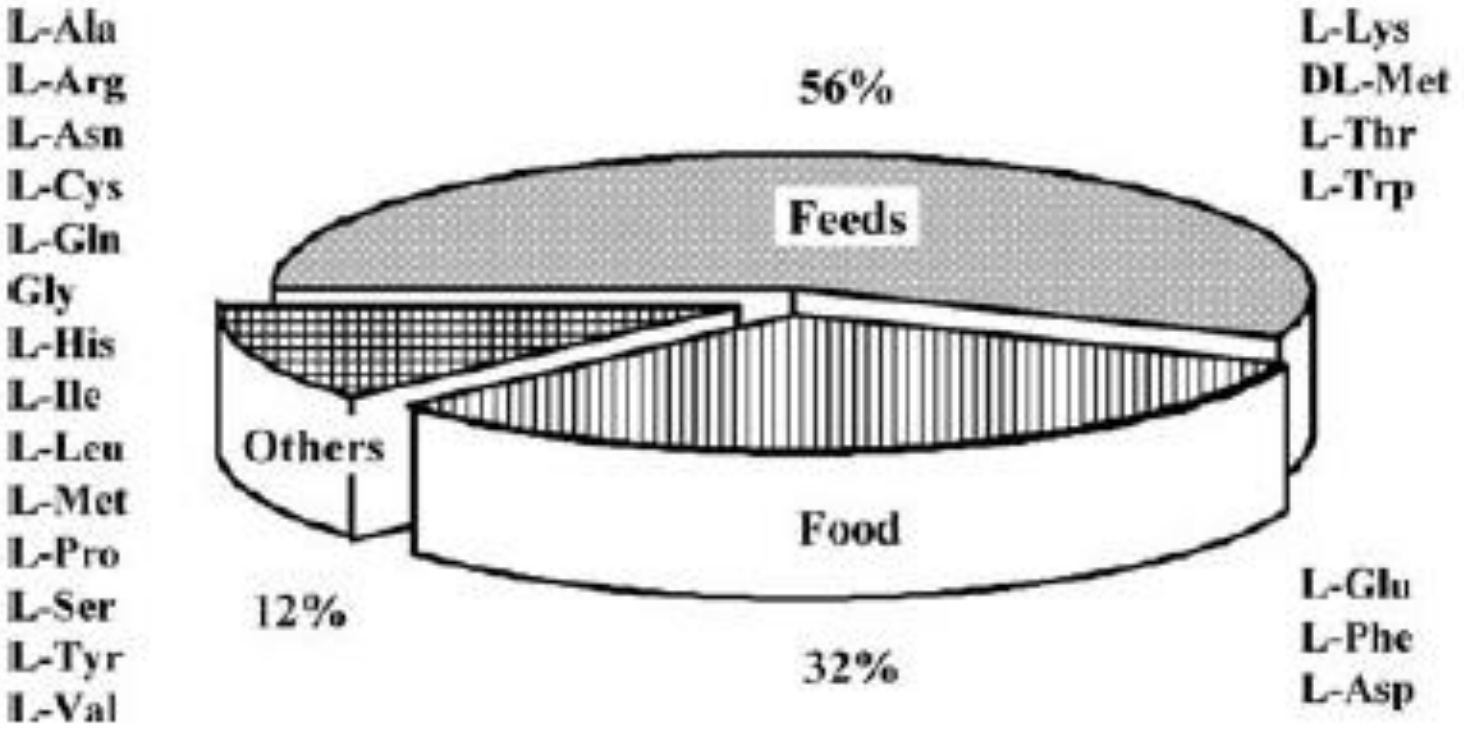
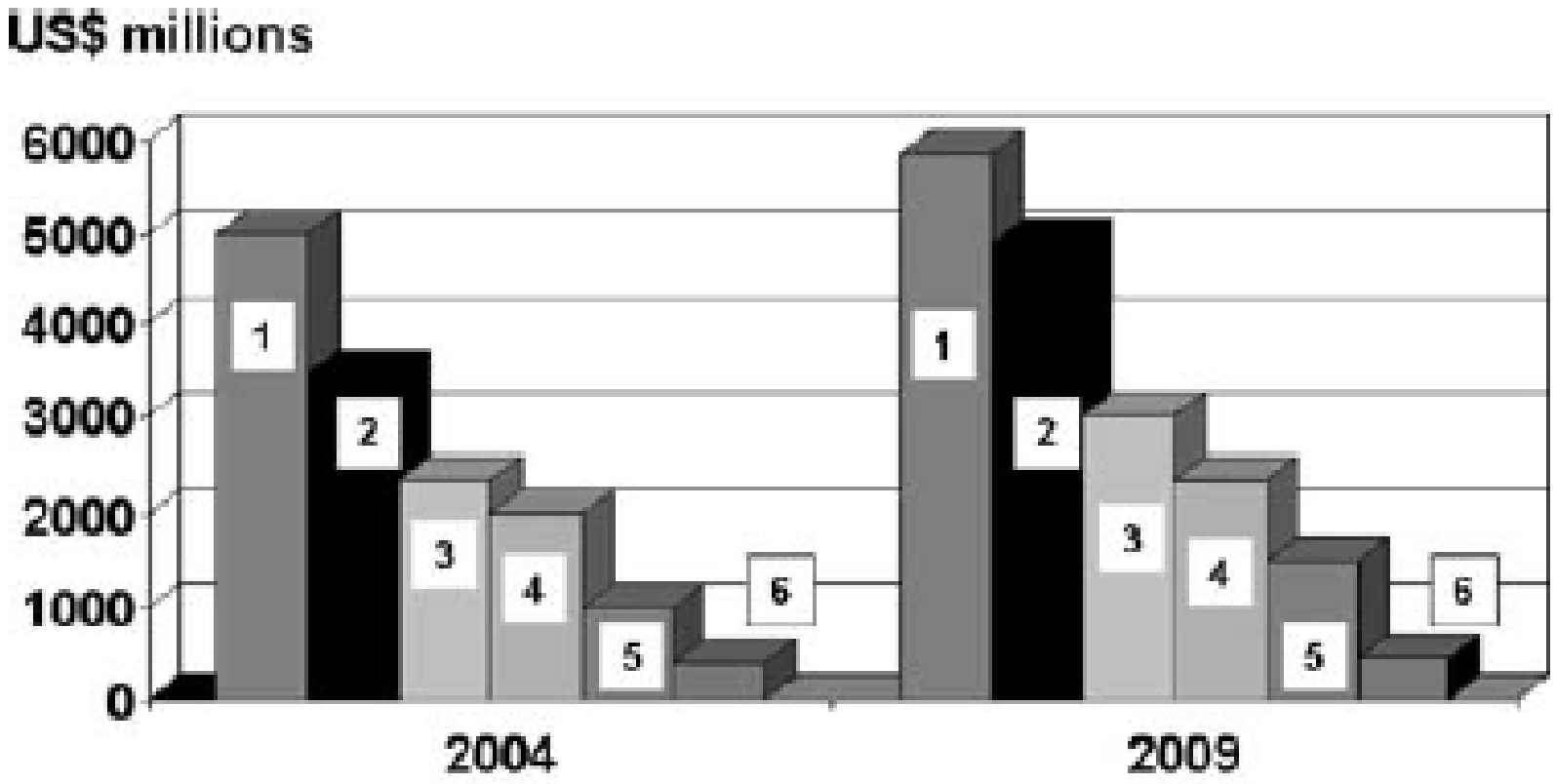


Fig. 1 Global market amino acids, 2004: US $4.5 billion



1 Crude antibiotics 2 Amino acids 3 Organic acids

4 Enzymes 5 Vitamins 6 Xanthan

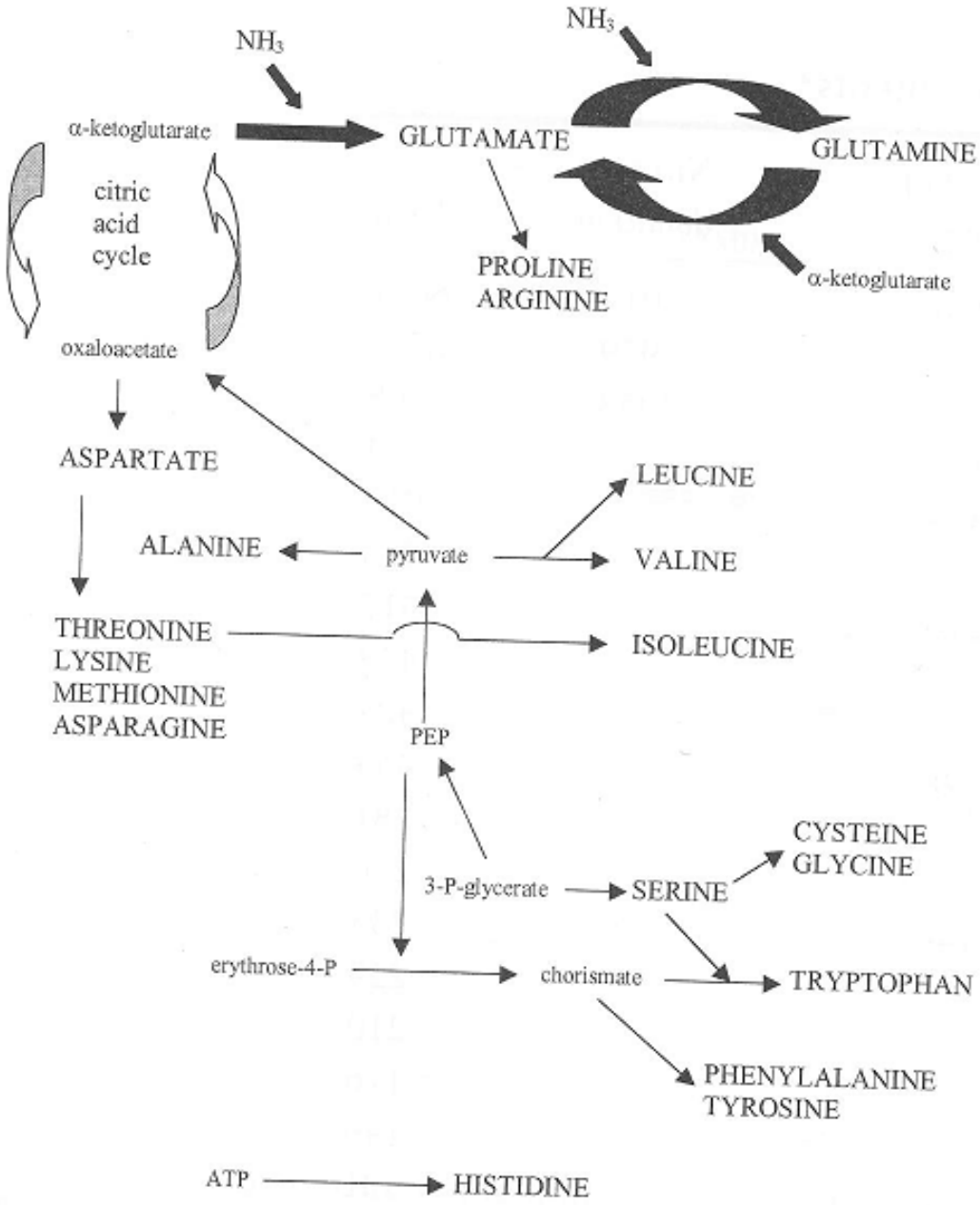
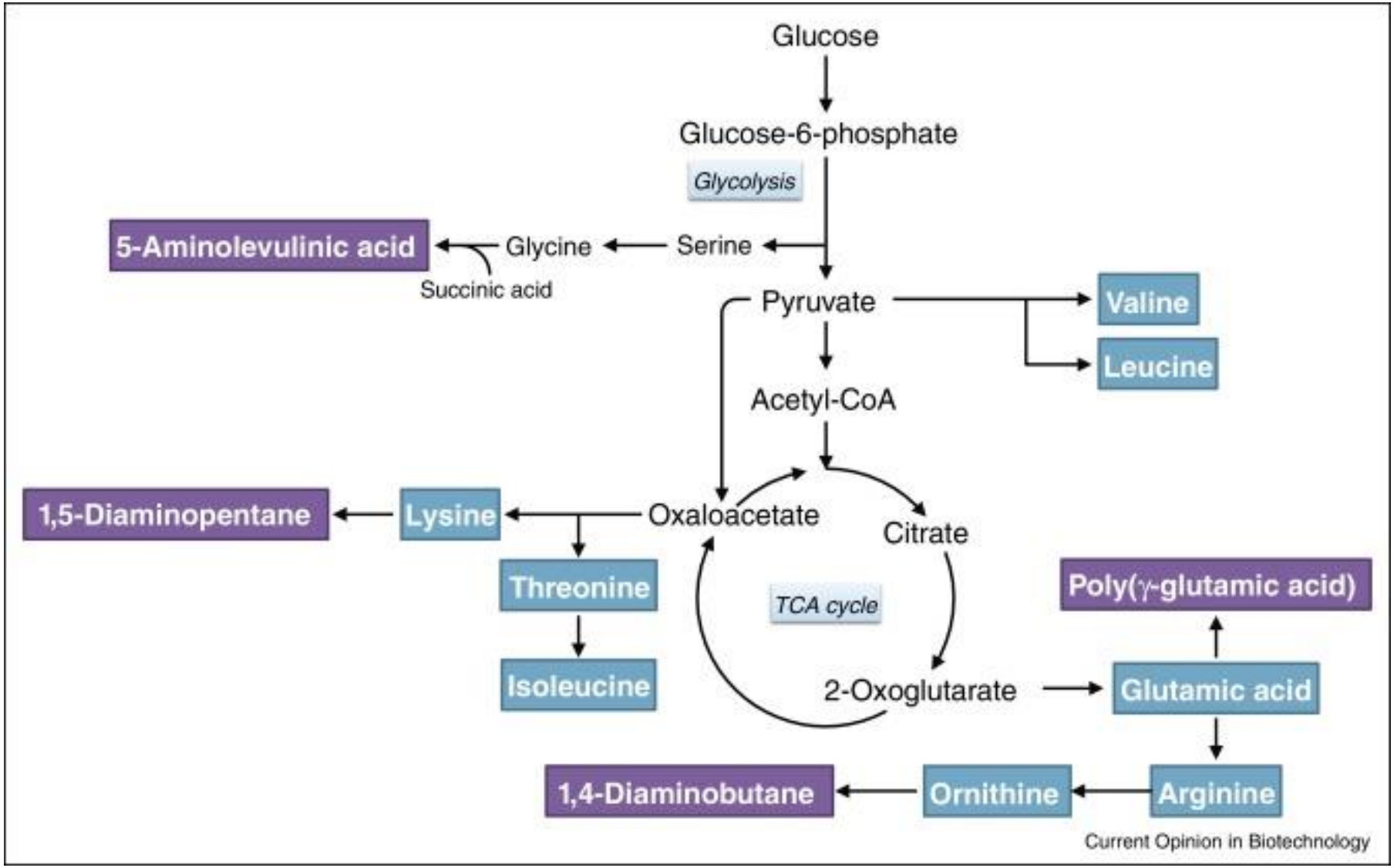


Fig. 1. Amino acid precursors and relationships between amino acids.


Glucose
Glucose-6-phosphate
Glycolysis
5-Aminolevulinic acid
Glycine
Serine
Succinic acid
Pyruvate
Valine
Leucine
Acetyl-CoA
1,5-Diaminopentane
Lysine
Oxaloacetate
Citrate
Threonine
TCA cycle
Poly(γ-glutamic acid)
Isoleucine
2-Oxoglutarate
Glutamic acid
1,4-Diaminobutane
Ornithine
Arginine
Current Opinion in Biotechnology

Microbial strains for amino acid production

1. Wild strains - *Corynebacterium glutamicum* and *Brevibacterium flavum* for the production of L-glutamic acid.
2. Mutant strains:
   - auxotrophic
   - resistant to amino acid analogues

   example - production of L-lysine and L-tryptophan
3. Recombinant strains - *E. coli*

   example - produces L-threonine

Amino acid	Strain/mutant	Titer (g/l)	Estimated yield (g/100 g sucrose)
L-Lysine HCl	*C. glutamicum* B-6	100	40–50
L-Threonine	*E. coli* KY 10935	100	40–50
L-Tryptophan	*C. glutamicum* KY9218/pIK9960	58	20–25
L-Tryptophan	*E. coli*	45	20–25
L-Phenylalanine	*E. coli* MWPWJ304/pMW16	51	20–25
L-Arginine	*Brevibacterium flavum* AJ12429	36	30–40
L-Histidine	*C. glutamicum* F81/pCH99	23	15–20
L-Isoleucine	*E. coli* H-8461	30	20–30
L-Serine	*Methylobacterium* sp. MN43	65	30–35
L-Valine	*C. glutamicum* VR 3	99	30–40

Culture medium for amino acid production

- Carbon source - molasses, glucose syrup, (methanol, n-alkanes ..)
- Nitrogen source - in small amounts, regulation of pH with ammonia
- Source of phosphorus, other minerals
- Supply of oxygen and / or CO_2

Bioprocess management

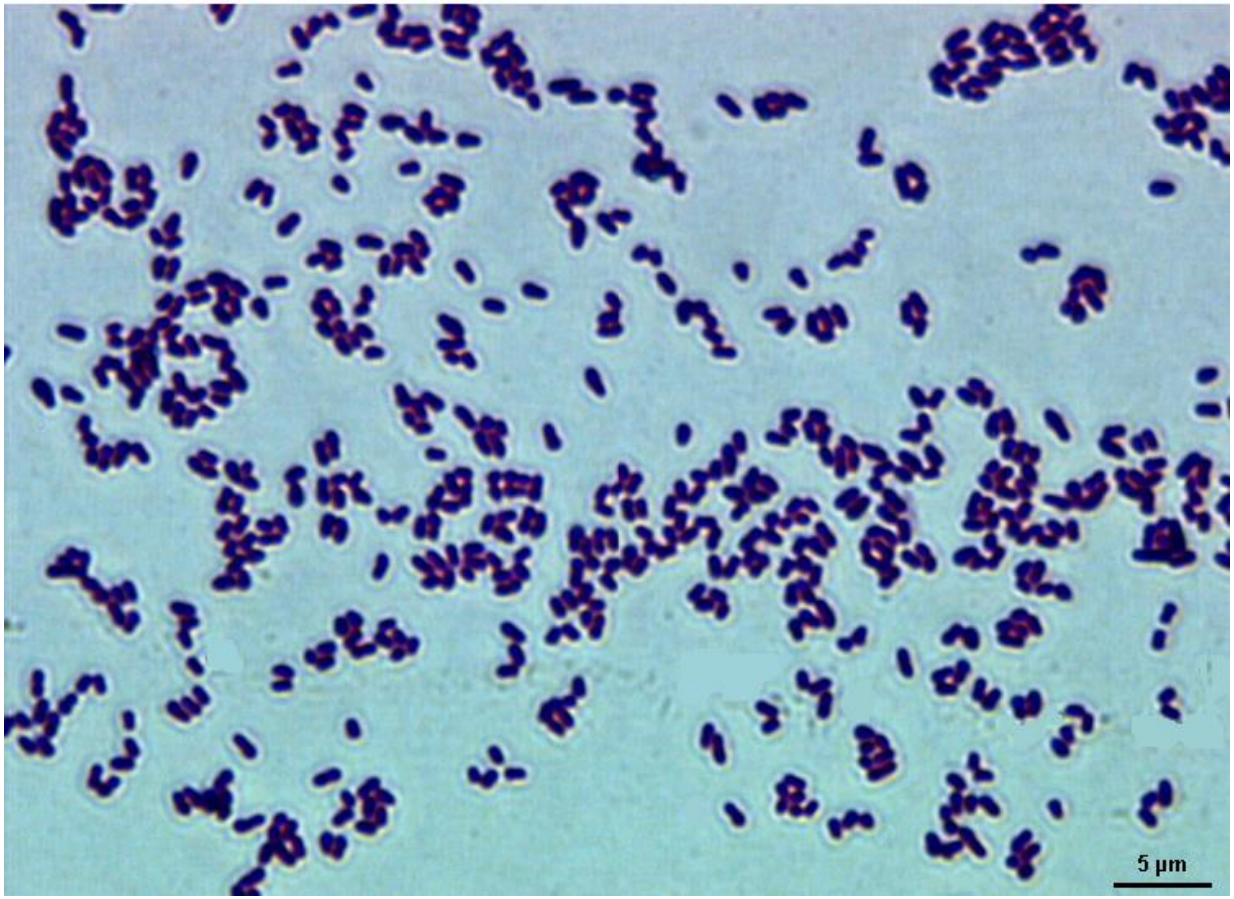
- Inoculation 1:10
- Usual size of production bioreactors 50-500 m^3
- Batch or fed-batch process
- Repeated fed-batch process (part of the old medium with cells is used as an inoculum)
- Continuous process

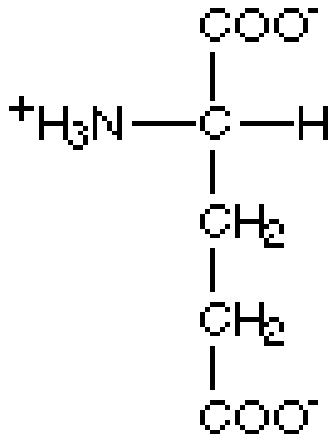
Isolation and separation of amino acids

- (Removal of cells - centrifugation, decantation, filtration), not always for feed lysine
- Ionex
- Concentration and crystallization X requirements for wastewater treatment

L-glutamic acid

Corynebacterium glutamicum (discovery in 1957)
G^+, nonsporulating, immobile, requiring biotin
Both wild, mutant and recombinant strains are used





Key factors influencing the fermentation of L-glutamic acid

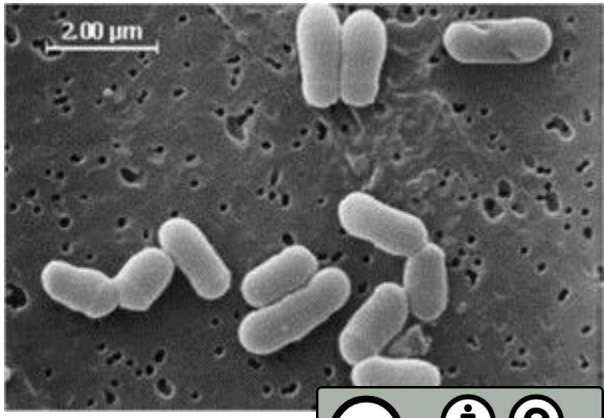
Presence of biotin for growth optimal for production at suboptimal concentration

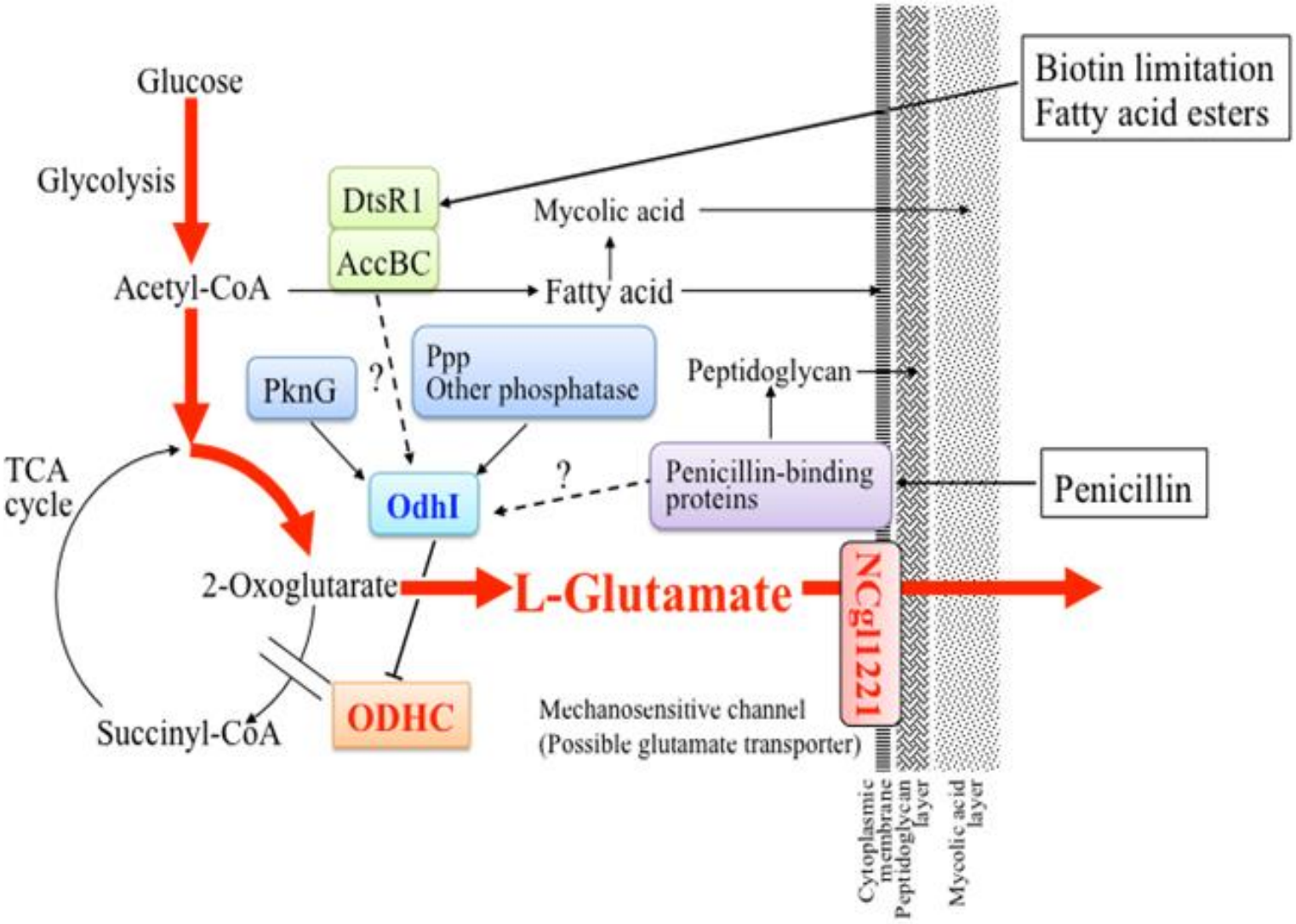
Adding penicillin or tween or cultivation of strains requiring oleic acids under limiting conditions - allowing acid transport from cells

Supply of oxygen

Carbon sources used: molasses, cereal hydrolysates

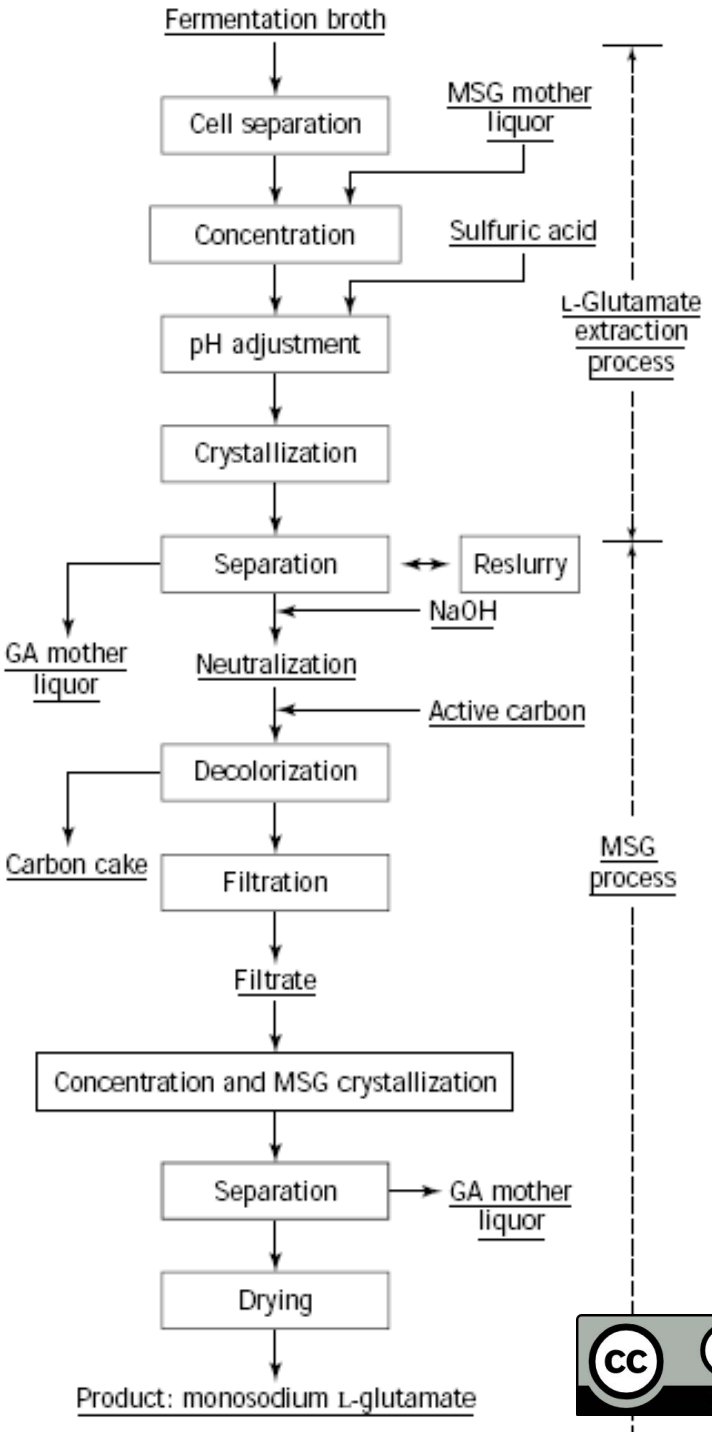
Multi-step inoculation, fed-batch cultivation, cultivation in air-lift bioreactors, cultivation time - 40-60h, 150g / l glutamate .



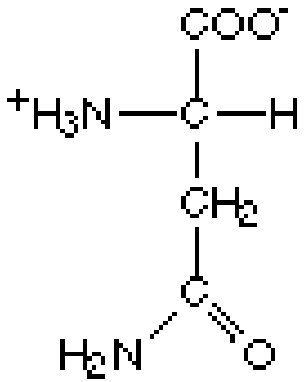

Glucose
Glycolysis
Acetyl-CoA
TCA cycle
2-Oxoglutarate
Succinyl-CoA
DtsR1
AccBC
PknG
?
Ppp
Other phosphatase
OdhI
ODHC
Biotin limitation
Fatty acid esters
Mycolic acid
Fatty acid
Peptidoglycan
?
Penicillin-binding proteins
Penicillin
L-Glutamate
NCgl1221
Mechanosensitive channel
(Possible glutamate transporter)
Cytoplasmic membrane
Peptidoglycan layer
Mycolic acid layer

Isolation of L-glutamic acid

- Centrifugation or filtration of biomass
- Concentrating the rest of the medium
- Crystallization
- Filtration
- Drying
- Yield 65-70% in isolation, remainder - use as fertilizer

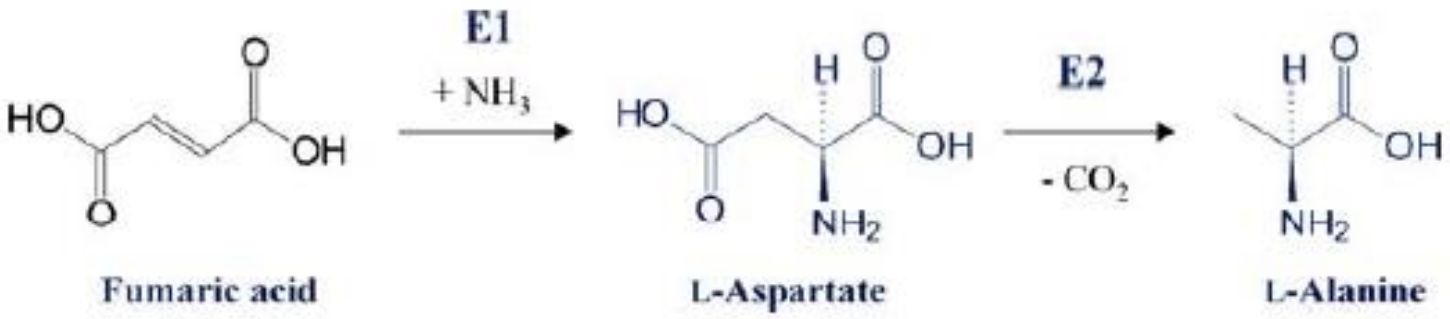


L-aspartic acid



Enzyme catalysis - L-aspartate-ammonium lysate

Fumar acid + NH_3 → L-aspartic acid



Enzyme from *E. coli,* immobilized, sometimes whole *E. coli* cells immobilized

in the polyacrylamide gel

Product Separation - Crystallization after acidification

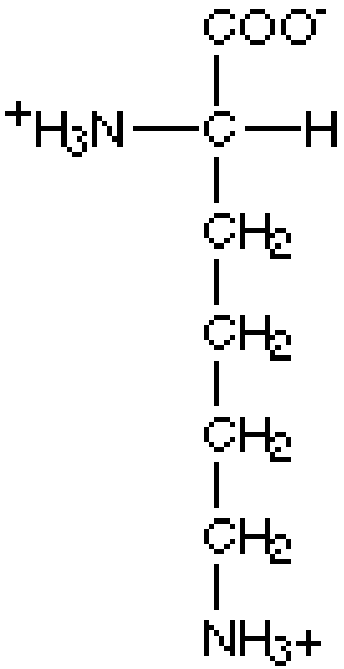
L-lysine

C. glutamicum mutants:

a) Auxotrophic mutants - auxotrophic to an amino acid with the same metabolic pathway as L-lysine (Thr, Leu, Met, Hse) - limitation of feedback inhibition

b) Regulatory mutants - resistant to toxic L-lysine analogues - high concentrations of lysine do not inhibit the enzyme aspartate kinase

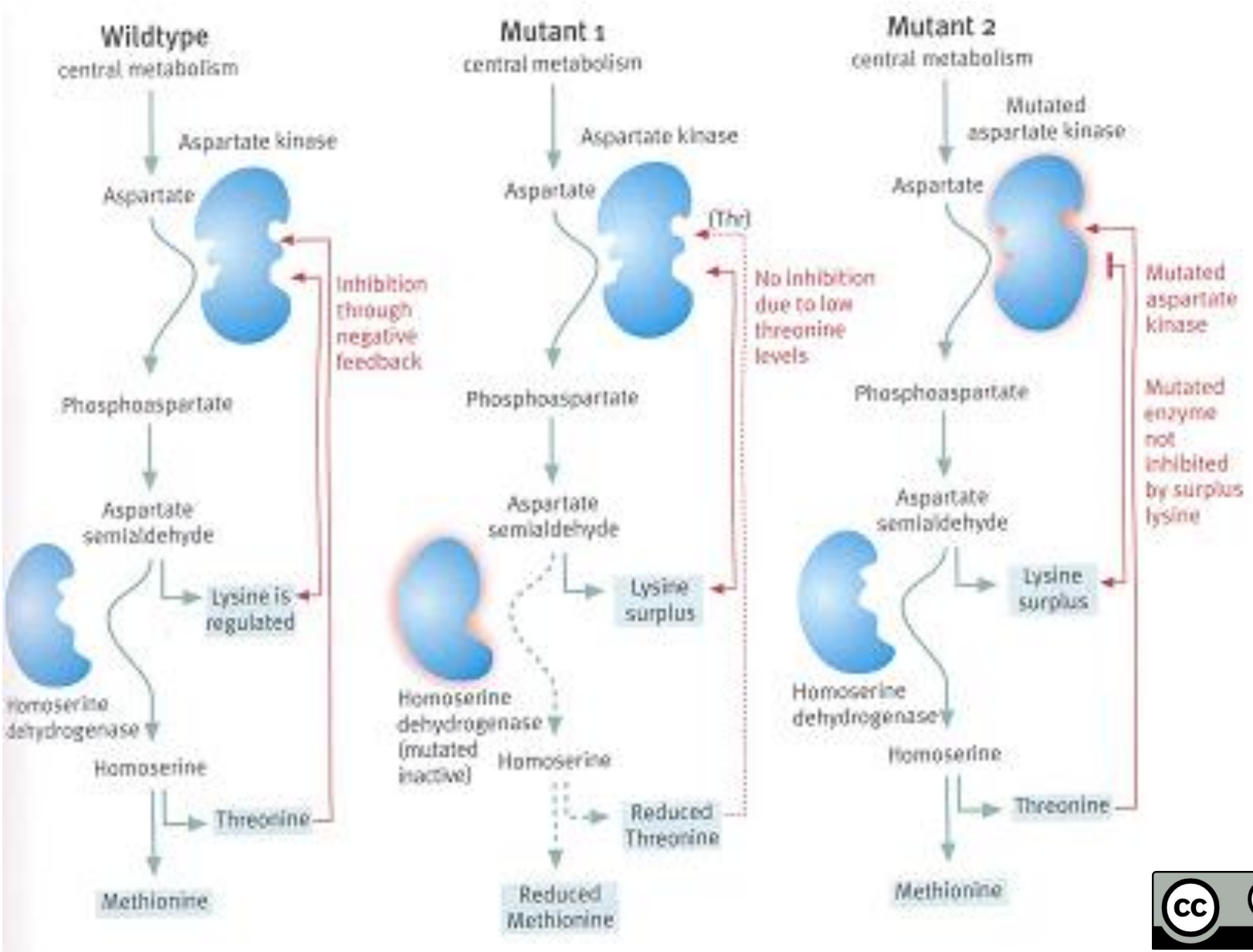
c) Combined mutations



Lysine
(lys)

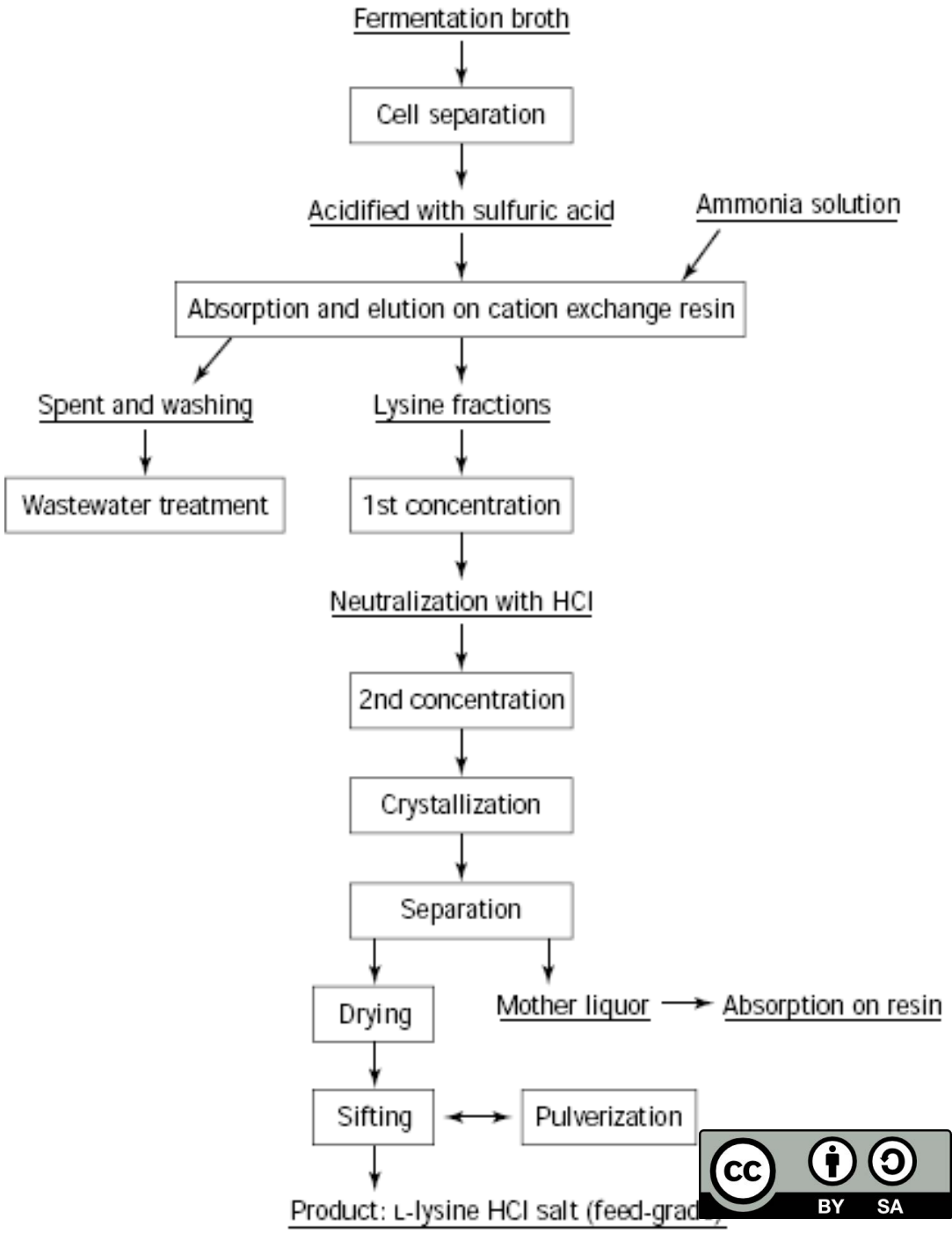
Auxotrophic mutant

Regulation mutant

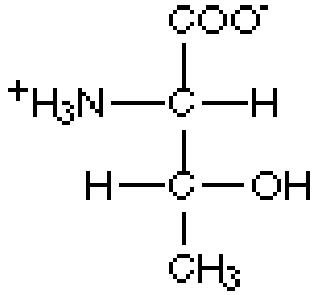


Cultivation

- Fed-batch, addition of ammonium sulphate to feed, carbon source limitation, 60h, final end. lysine 95 g / l
- Product isolation - cell removal, absorption of lysine to ion exchanger, crystallization or spray drying as L-lysine hydrochloride



L-threonine

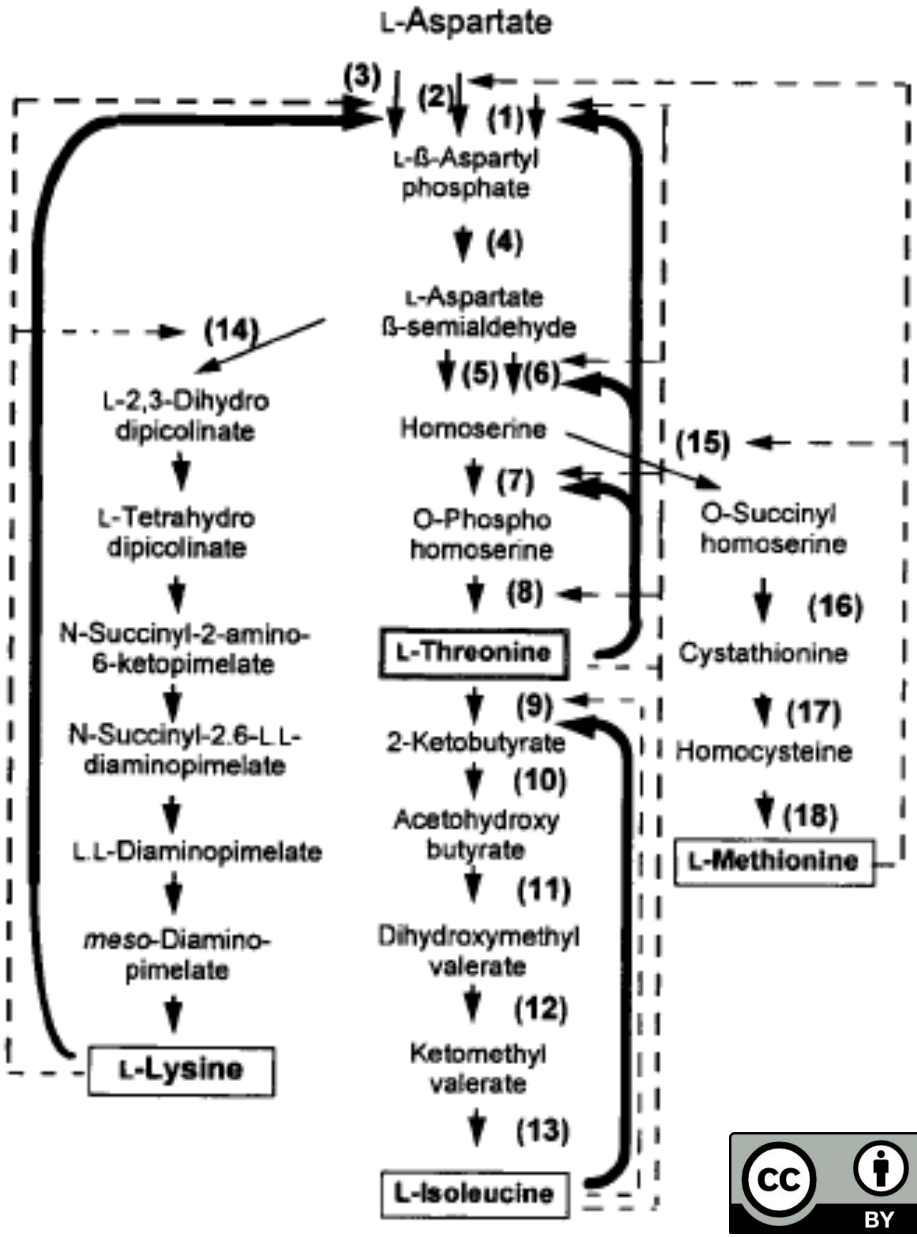


Threonine
(thr)

Recombinant *E. coli* strains

The most successful *E. coli* K12 with the plasmid carrying the thr operon

Isolation of Thr from the filtrate after thickening, discoloration - crystallization



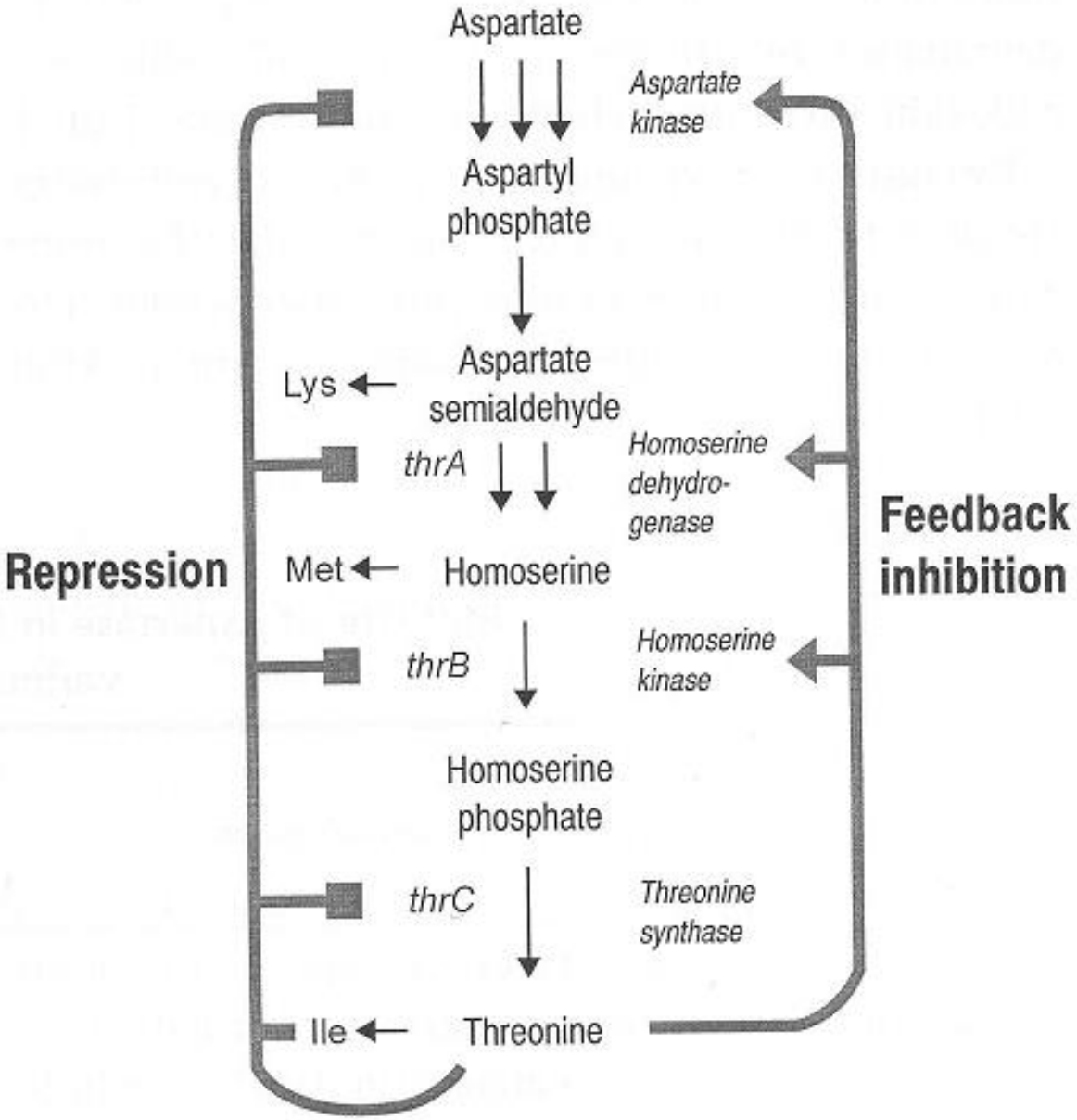
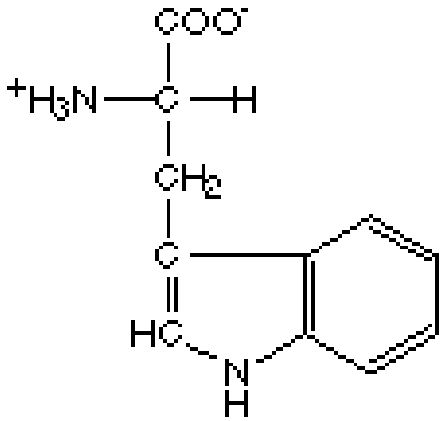


Fig. 5. Regulation of L-threonine biosynthesis in *Escherichia coli.* Only the regulation by L-threonine and L-isoleucine is shown.

L-tryptophan

- Enzymatic production of indole and L-serine under the effect of tryptophanase enzyme
- Fermentation using precursors - indole *(C.glutamicum)* or anthranilic acid *(B.subtilis, B.amyloliquefaciens) X* toxic by-products
- Direct fermentation from sugar substrates using auxotrophic or regulatory mutants *(Brevibacterium flavum, C. glutamicum, B. subtilis)*



Tryptophan
(trp)

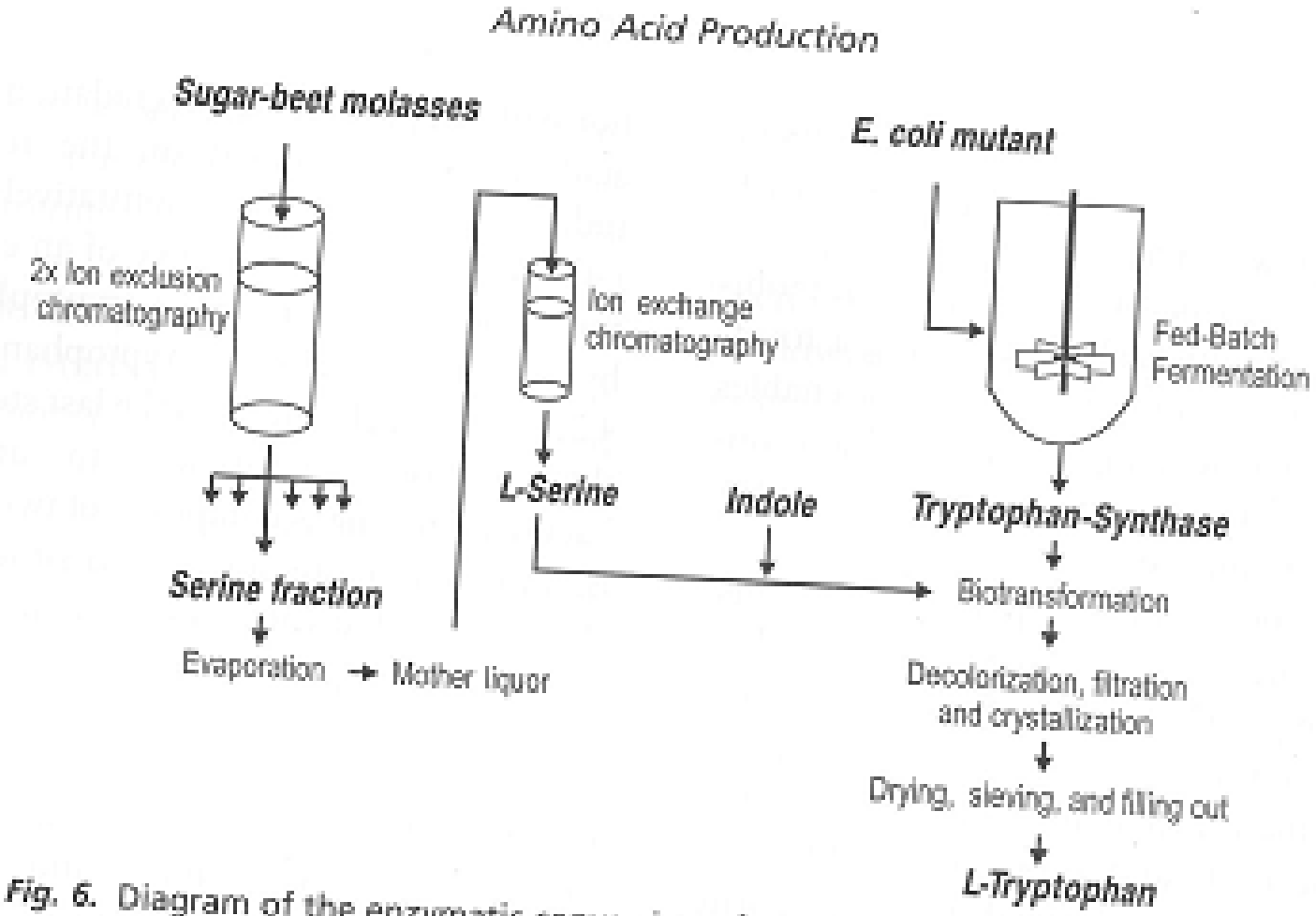


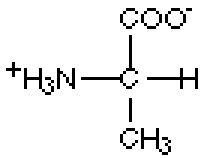
Fig. 6. Diagram of the enzymatic conversion of L-serine (obtained from molasses) with indole to L-tryptophan.

Production of amino acids for the pharmaceutical and cosmetic industries

- Protein hydrolysates
- From chemically defined precursors using enzymes
- Fermentation from sugar substrates

L-alanine

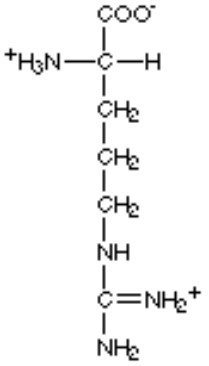
From L-aspartic acid using immobilized *Pseudomonas dacunhae* cells



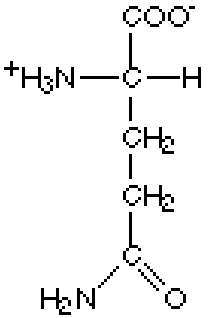
Alanine
(ala)

L-arginine

Fermentation of sugar substrates by regulatory mutant *C. glutamicum* and *B.subtilis*



Arginine
(arg)



Glutamine
(gln)

L-glutamine

Using wild strains of Corynebacteria

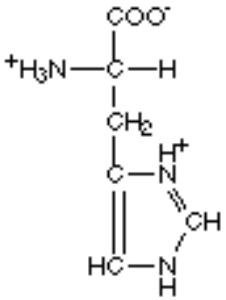
L-histidine

C. glutamicum and *S.marcescens* - regulatory mutants

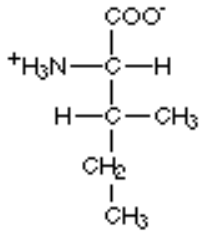
L-isoleucine

Using *C.glutamicum* with a precursor of 2-ketobutyrate

Fermentation of saccharides by recombinant strains of *C.glutamicum, S.marcescens* and *E.coli*



Histidine
(his)

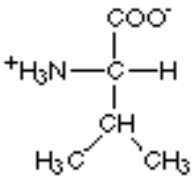


Isoleucine
(ile)

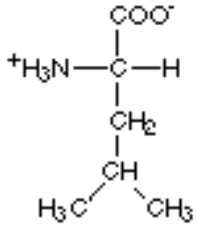
L-leucine and L-valine

Production of L-leucine from 2-ketoisocapronate under the effect of *C.glutamicum* transaminase enzyme

Direct fermentation using *S. mutationscens* and corynebacteria control mutants



Valine
(val)

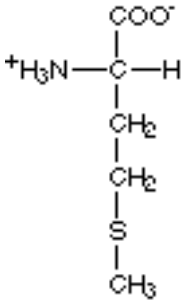


Leucine
(leu)

L-methionine

Enzymatic conversion of N-acetylmethionine using acylase from *Aspergillus oryzae*

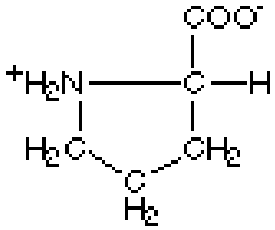
Conversion of D, L-5- (2-methylthioethyl) hydantoin by the growth of *Pseudomonas*



Methionine
(met)

L-proline

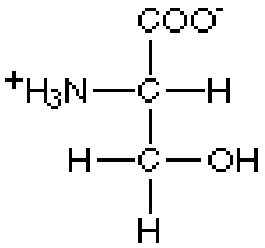
Control and combined mutants of *B.flavum, S. marcescens* and corynebacteria



Proline
(pro)

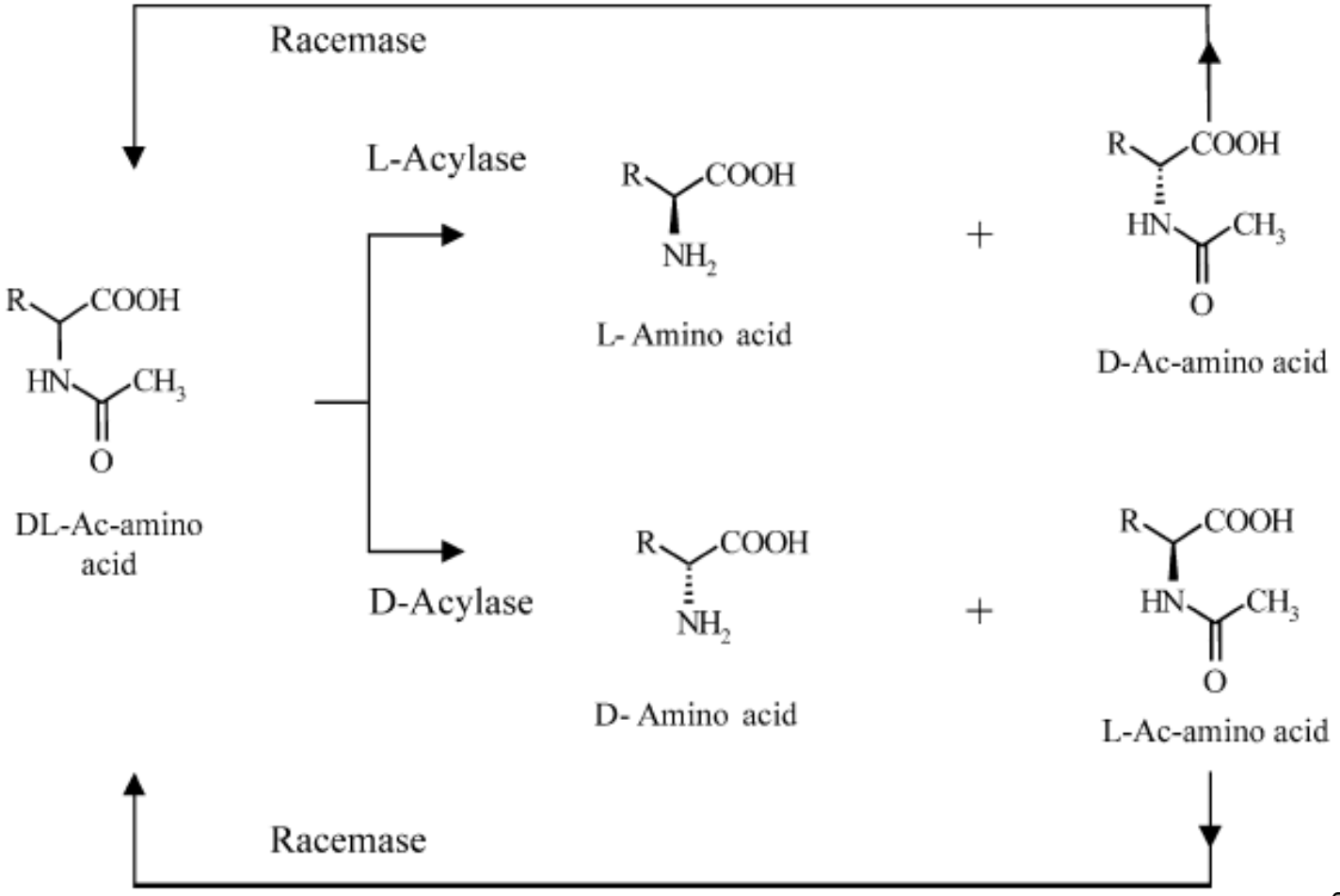
L-serine

From glycine and formaldehyde with serine hydroxymethyltransferase from *Klebsiella aerogenes*



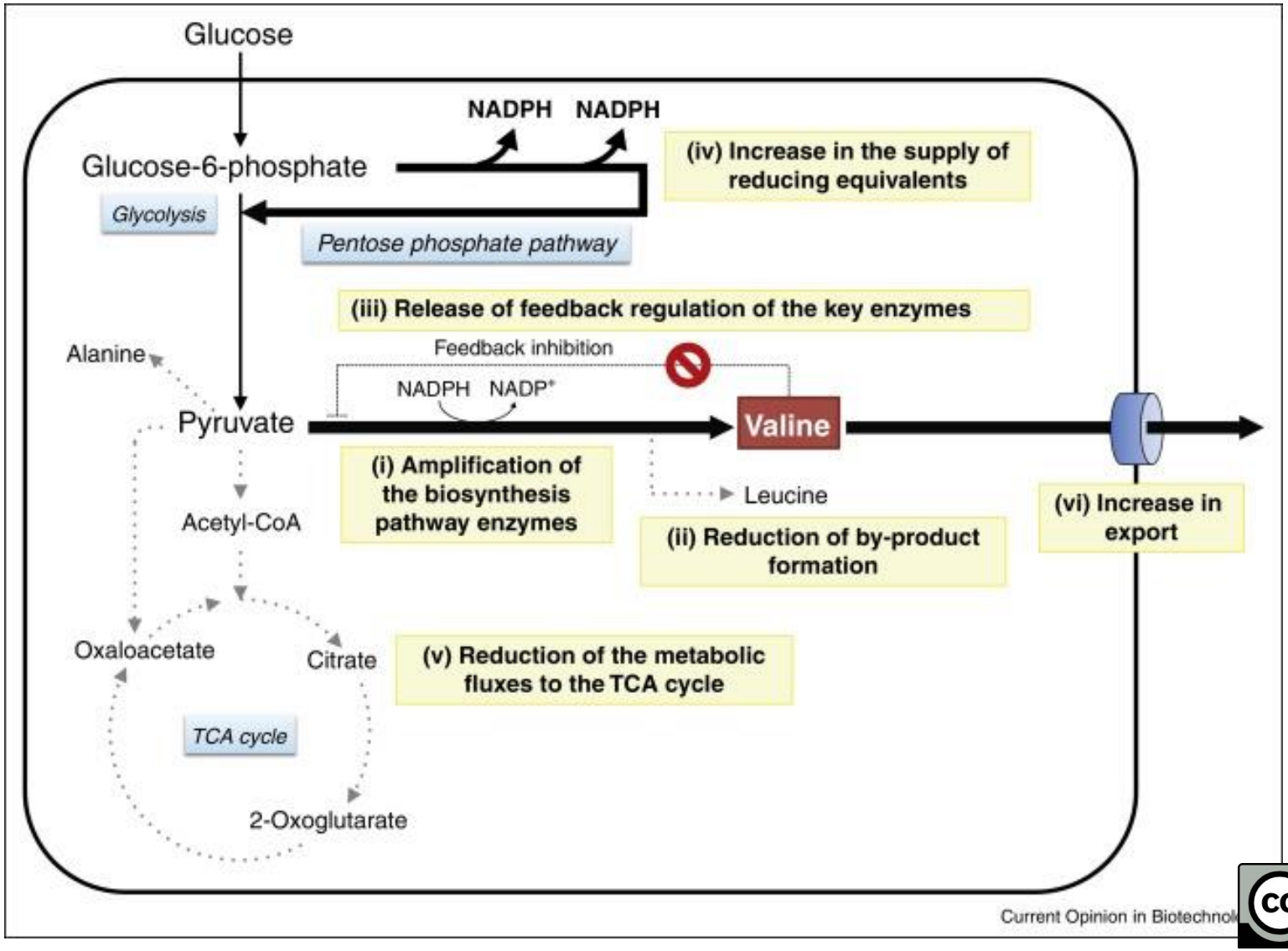
Serine
(ser)

Enzymatic production of L-methionine and L-valine with acylases

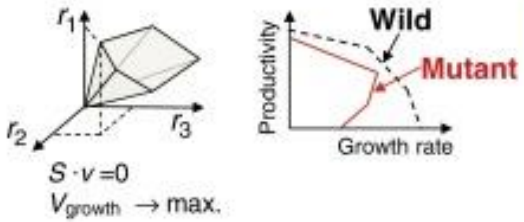


Current strategies to boost amino acid production

Biosynthetic pathway enhancement, reduction of secondary metabolites, prevention of feedback inhibition, enhancement of NADPH production, reduction of secondary metabolic pathways, enhancement of cellular export

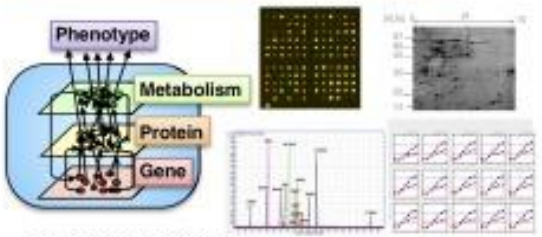


1. Metabolic pathway design



- Estimation of gene knockout strategies for optimized production

2. Genome-wide analyses

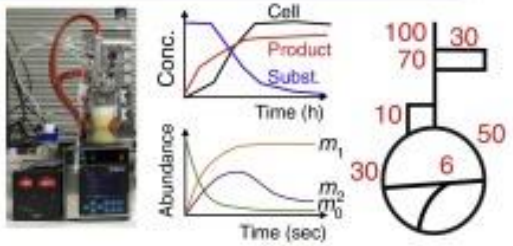


- Systems biology
- Transcriptome, proteome, metabolome, phenome, etc.

3. Breeding of host microbial cells

- Recombinant DNA techniques: gene overexpression and deletion
- Control of the gene expression networks

4. Evaluation of the created cells



- Culture experiments
- Metabolic flux analysis

Current Opinion in Biotechnology

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PRAGUE

Biotechnology in Food Industry

Lecture XII

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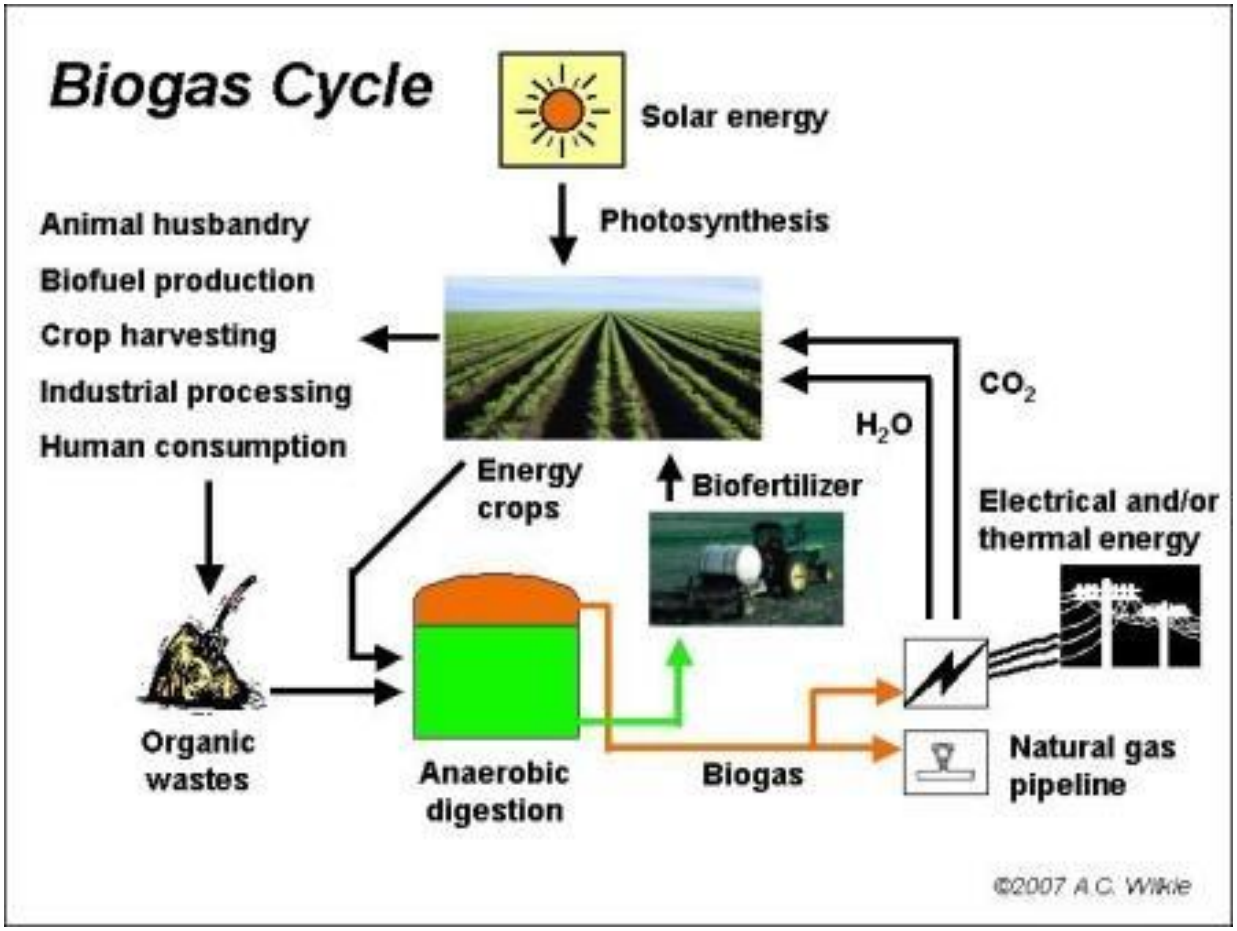
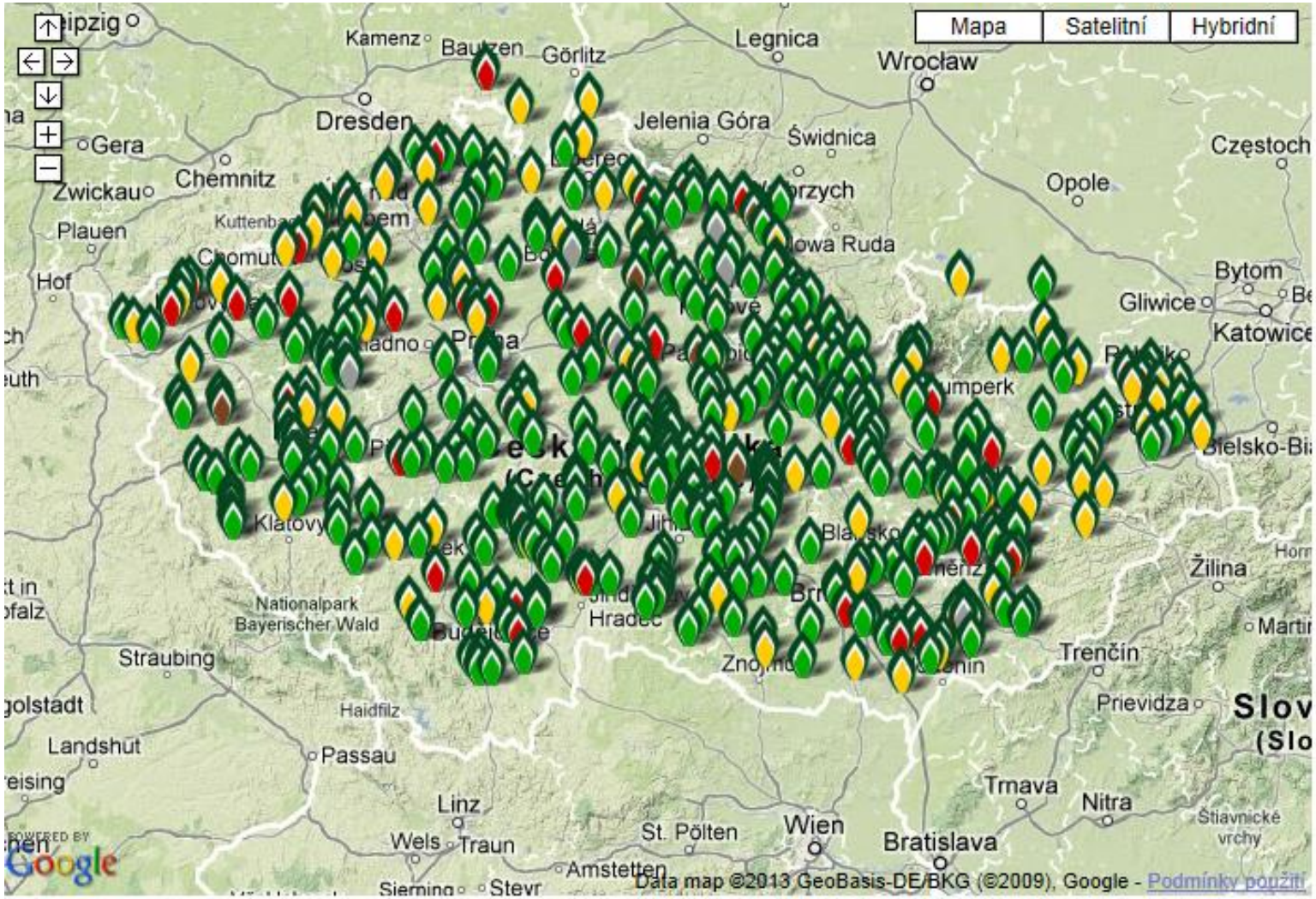
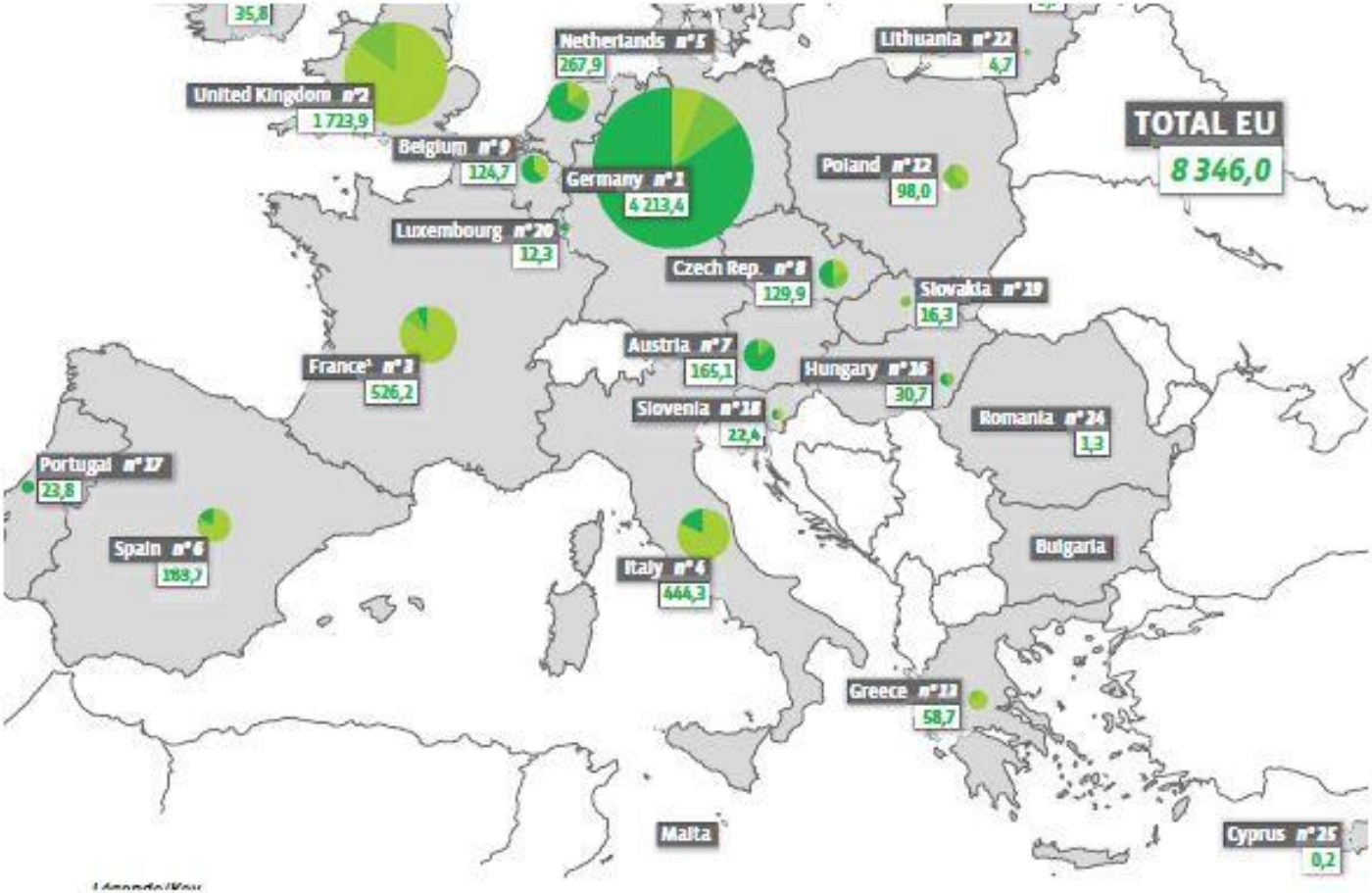
Biogas





http://www.czba.cz/mapa-bioplynovy



4 231,4 *Les chiffres en vert indiquent la production totale en ktep.* **Green figures show total production in ktoe.**

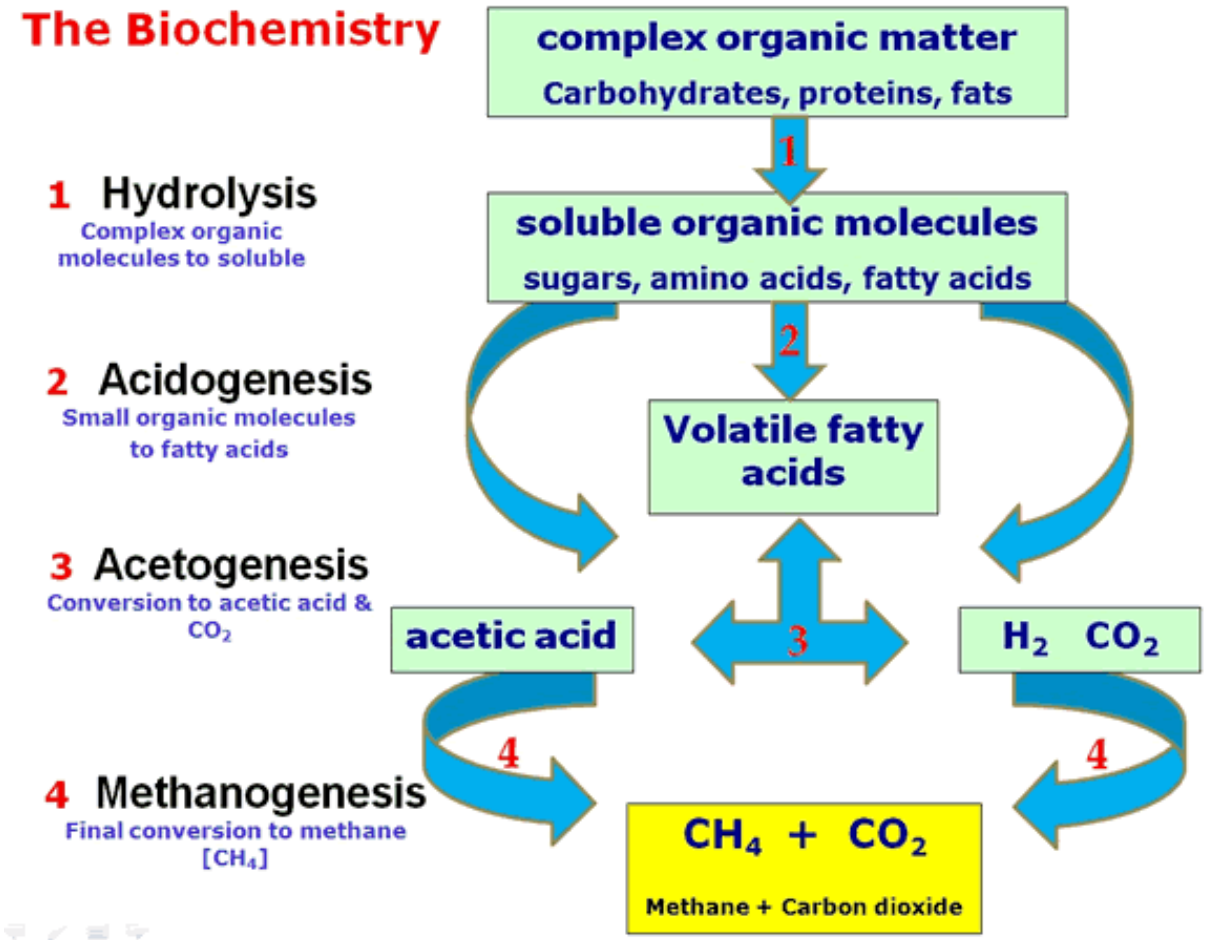
Biogaz de décharges. **Landfill gas.**

Station d'épuration urbaine et industrielle. **Urban sewage and industrial effluent sludge gas.**

Autres biogaz. **Other biogas.**
Unité décentralisée de biogaz agricole, unité de méthanisation des déchets municipaux solides, unité centralisée de codigestion.
Decentralised agricultural plant, municipal solid waste methanisation plant, centralised co-digestion plant.

** Estimation.*
1 – DOM non inclus. **French overseas departments excluded.**
Source: EurObserv'ER 2010.

Anaerobic digestion of organic matter



Types of substrate

- substrate - agricultural crop and animal waste, maize, communal waste, food and agricultural waste
- mixed culture of anaerobic microorganisms
- process - hydrolysis, acidogenesis, acetogenesis, methanogenesis
- the resulting product - biogas (55-70% CH_4 + CO_2 + hydrogen sulphide, ammonia, hydrogen ...)
- biogas treatment - removal of water, CO_2, hydrogen sulfide ...
- by-product - digestate (fertilizer)
- use of biogas - heat production, heat production + electricity (cogeneration)

Important parameters of anaerobic digestion

- Oxidation Reduction Potential:

-100 to 100 mV for non-methanogens, -400 to -150 mV for methanogens

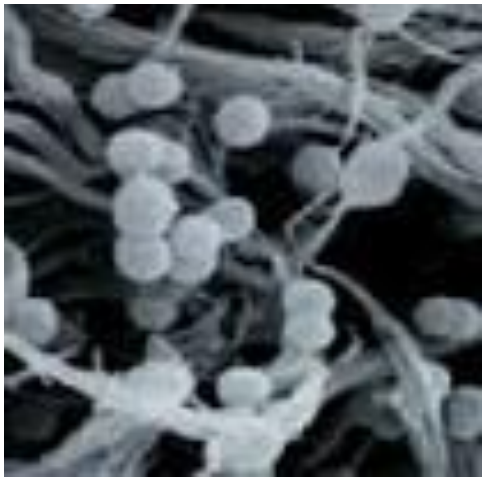
- Temperature (mesophilic or thermophilic digestion)
- Substrate
- pH
- C / N ratio
- Inhibitors
- Inoculum
- stirring

Microbiology of anaerobic digestion

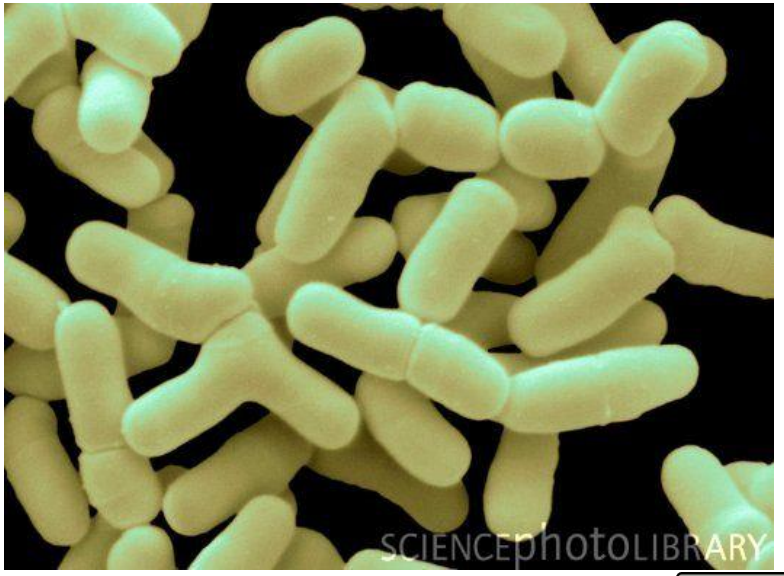
I. Hydrolysis and acidogenesis

Clostridium, Butyrivibrio, Ruminococcus, Bacteroides – hydrolysis, fermenting bacteria

Propionibacterium, Acetivibrio, Bifidobacterium, Eubacterium, Lactobacillus, Selenomonas, Streptococcus… - hydrogen producing acetogens



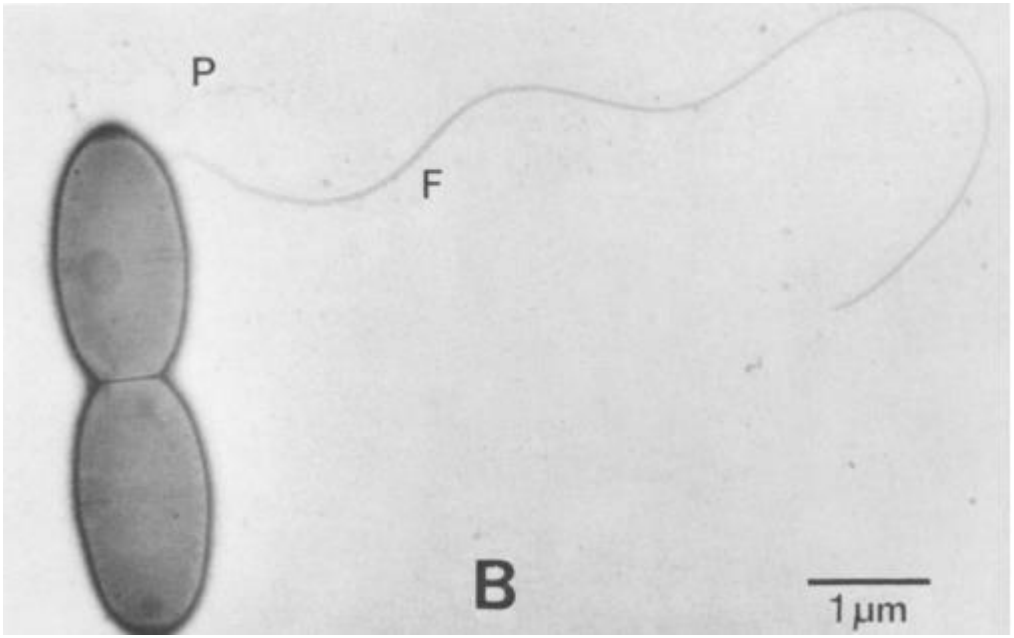
Ruminococcus



Bifidobacterium

II. Acetogenesis

Acetobacterium, Acetoanaerobium, Acetogenium, Butyribacterium, Clostridium, Eubacterium, Pelobacter

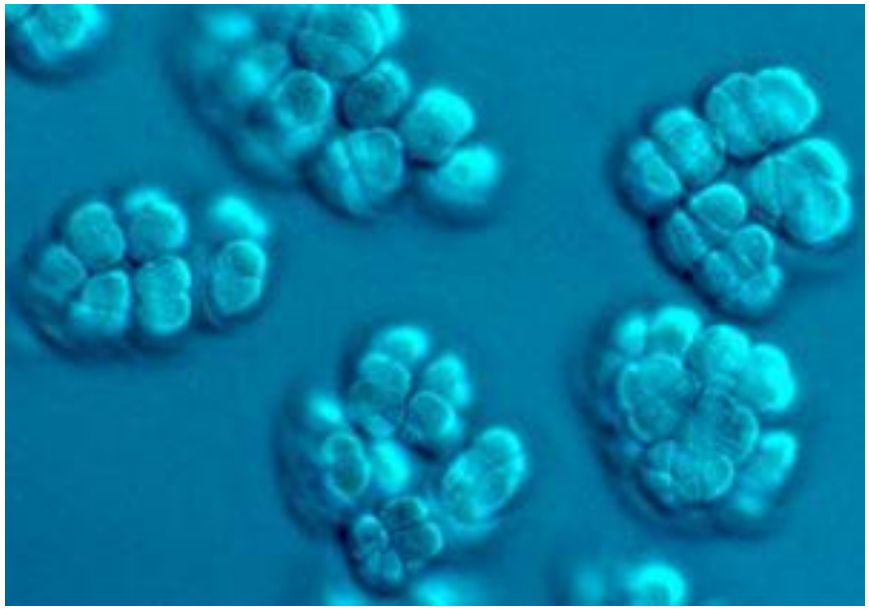


Acetobacterium

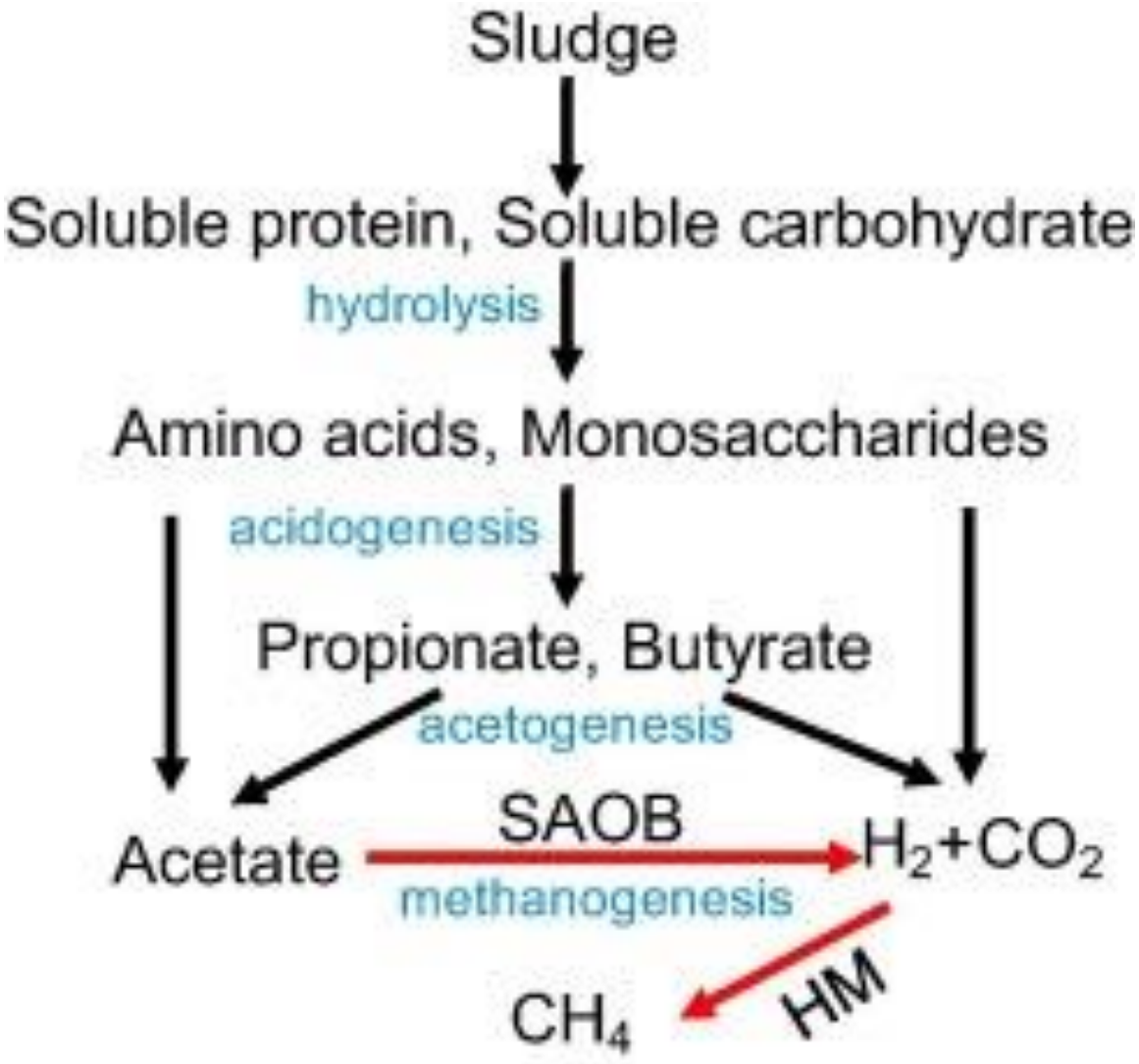
homoacetogens

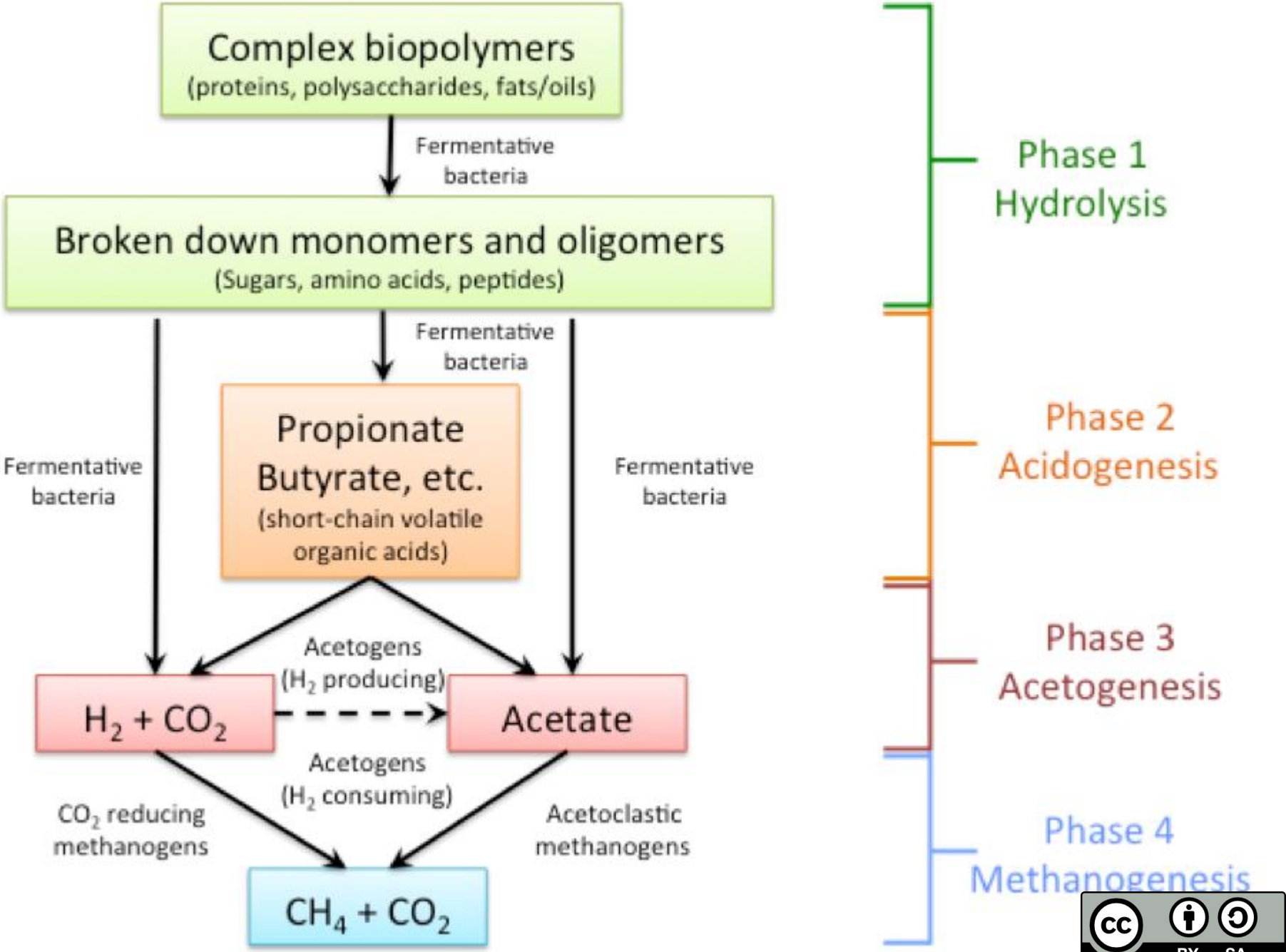
III. Methanogenesis

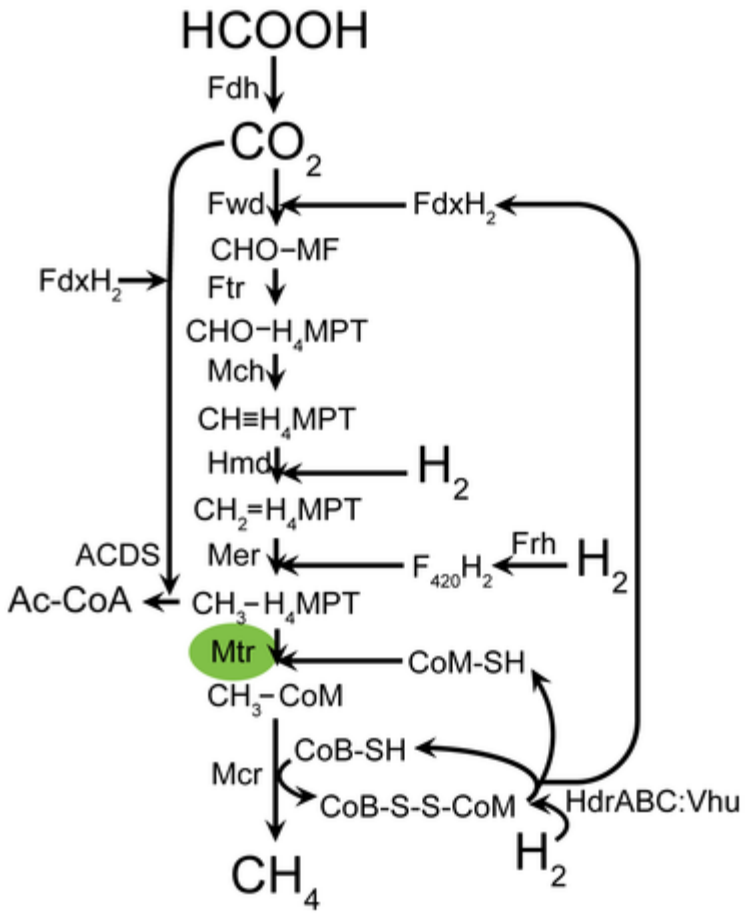
Methanosarcina, Methanothrix

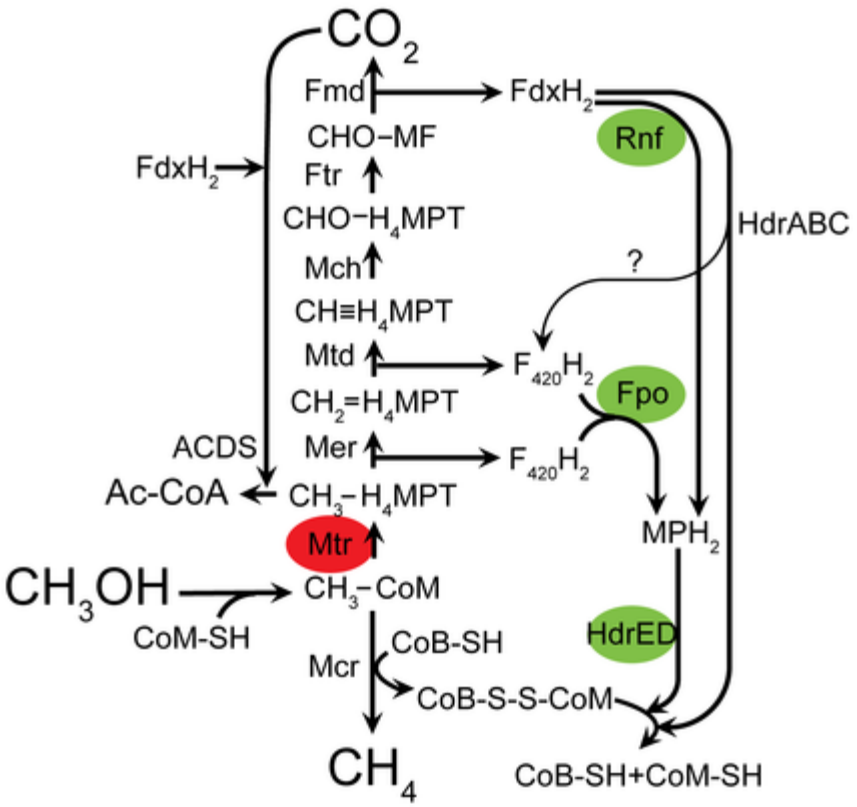


Methanosarcina


Sludge
Soluble protein, Soluble carbohydrate
hydrolysis
Amino acids, Monosaccharides
acidogenesis
Propionate, Butyrate
acetogenesis
Acetate
SAOB
H_2+CO_2
methanogenesis
CH_4
HM


Complex biopolymers
(proteins, polysaccharides, fats/oils)
Fermentative bacteria
Broken down monomers and oligomers
(Sugars, amino acids, peptides)
Fermentative bacteria
Fermentative bacteria
Propionate
Butyrate, etc.
(short-chain volatile organic acids)
Fermentative bacteria
Acetogens
(H_2 producing)
$H_2 + CO_2$
Acetate
Acetogens
(H_2 consuming)
CO_2 reducing methanogens
Acetoclastic methanogens
$CH_4 + CO_2$
Phase 1
Hydrolysis
Phase 2
Acidogenesis
Phase 3
Acetogenesis
Phase 4
Methanogenesis


A Methanococcus
HCOOH
Fdh
CO2
Fwd
FdxH2
CHO−MF
FdxH2
Ftr
CHO−H4MPT
Mch
CH≡H4MPT
Hmd
H2
CH2=H4MPT
ACDS
Mer
F420H2
Frh
H2
Ac-CoA
CH3−H4MPT
Mtr
CoM-SH
CH3−CoM
Mcr
CoB-SH
CoB-S-S-CoM
HdrABC:Vhu
H2
CH4

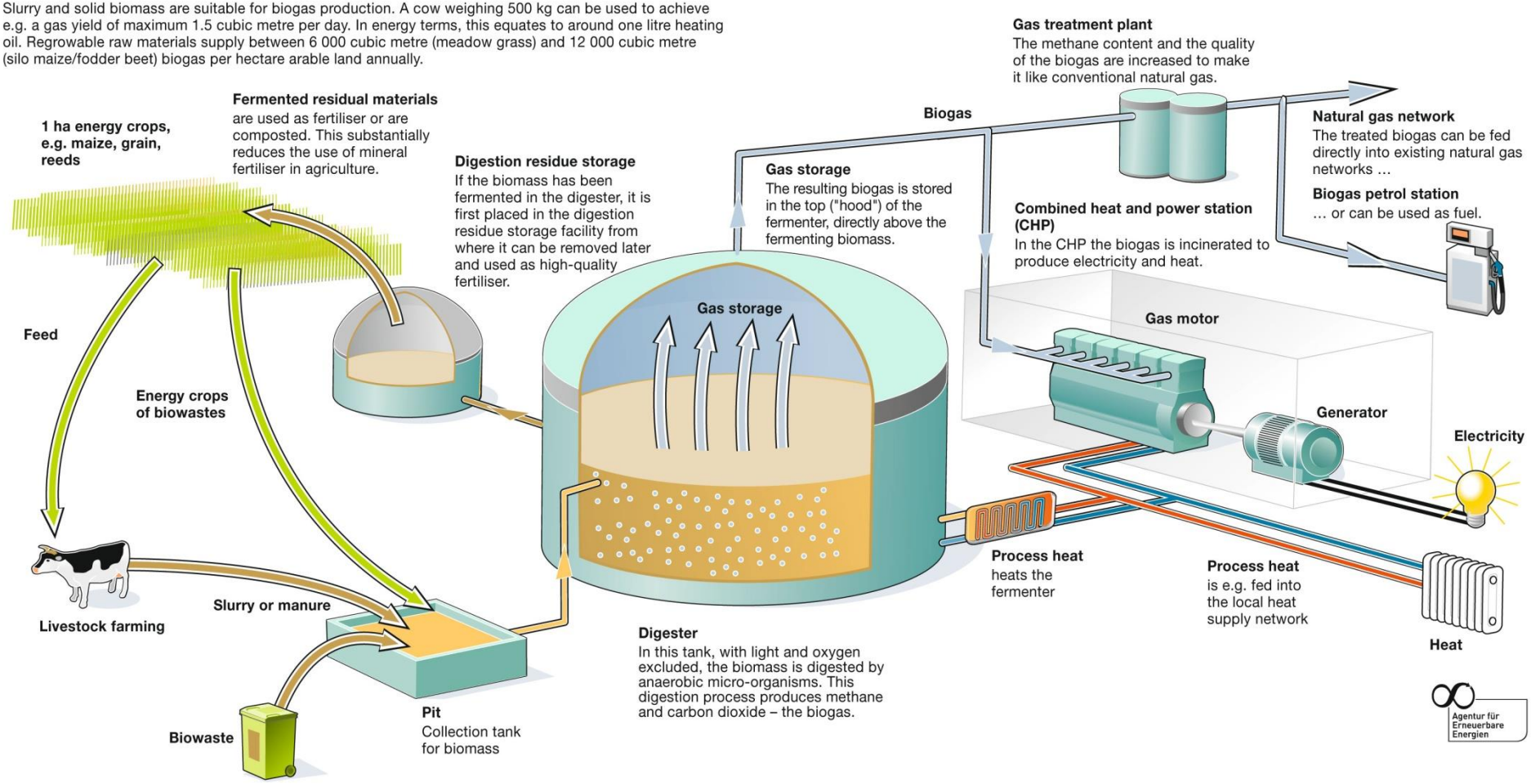

B Methanosarcina
CO2
Fmd
FdxH2
Rnf
CHO−MF
FdxH2
Ftr
CHO−H4MPT
HdrABC
?
Mch
CH≡H4MPT
Mtd
F420H2
Fpo
CH2=H4MPT
ACDS
Mer
F420H2
Ac-CoA
CH3−H4MPT
MPH2
Mtr
CH3OH
CH3−CoM
CoM-SH
CoB-SH
HdrED
Mcr
CoB-S-S-CoM
CH4
CoB-SH+CoM-SH

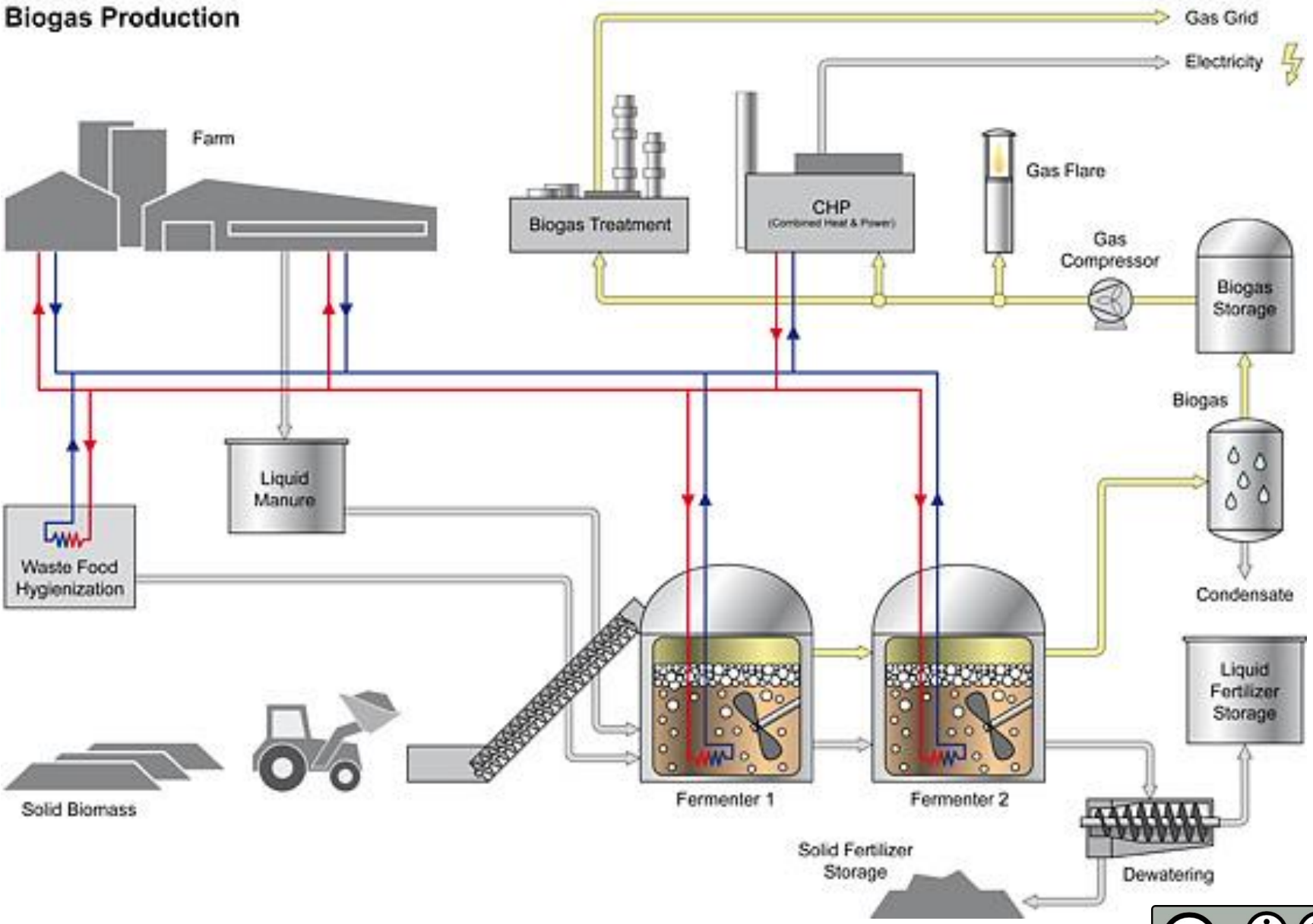
Biogas production

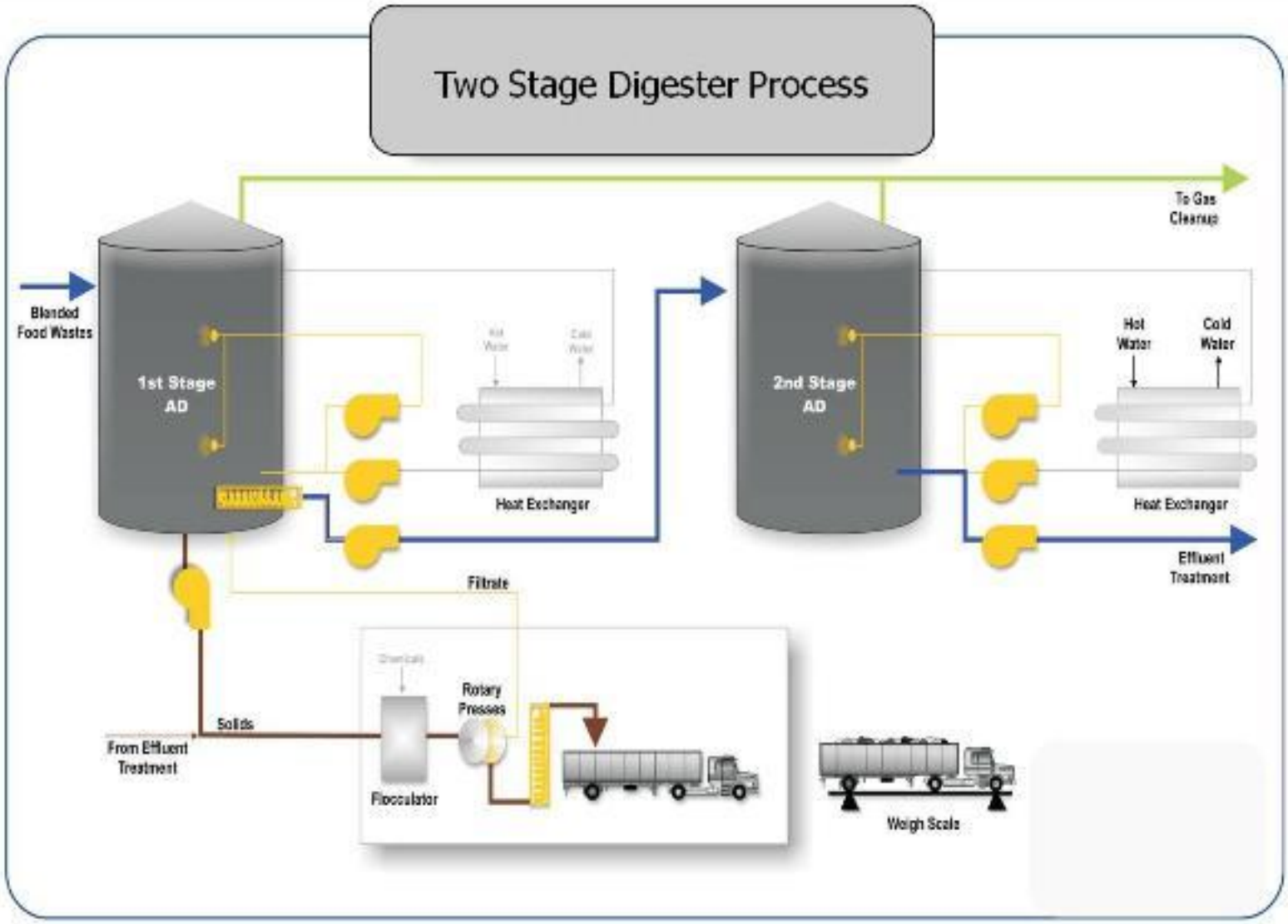
- substrate – agricultural, plant, animal waste, maize, municipal waste
- Mixed culture of anaerobic microorganisms
- process – hydrolysis, acidogenesis, acetogenesis, methanogenesis
- Resulting product– biogas (55-70% CH_4 + CO_2 + H_2S, ammonia,hydrogen..)
- Biogas adjustement–dewatering, removal of CO_2, H_2S …
- by-product – digestate (fertilizer)
- use of biogas – heat generation, cogeneration (production of heat and electricity)

Biogas system

Slurry and solid biomass are suitable for biogas production. A cow weighing 500 kg can be used to achieve e.g. a gas yield of maximum 1.5 cubic metre per day. In energy terms, this equates to around one litre heating oil. Regrowable raw materials supply between 6 000 cubic metre (meadow grass) and 12 000 cubic metre (silo maize/fodder beet) biogas per hectare arable land annually.



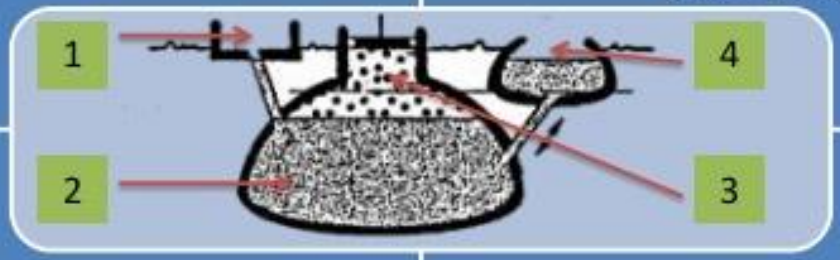

Biogas Production
Gas Grid
Electricity
Farm
Biogas Treatment
CHP
(Combined Heat & Power)
Gas Flare
Gas Compressor
Biogas Storage
Biogas
Liquid Manure
Waste Food Hygienization
Condensate
Liquid Fertilizer Storage
Solid Biomass
Fermenter 1
Fermenter 2
Solid Fertilizer Storage
Dewatering


Two Stage Digester Process
To Gas Cleanup
Blended Food Wastes
1st Stage AD
2nd Stage AD
Hot Water
Cold Water
Heat Exchanger
Heat Exchanger
Effluent Treatment
Filtrate
Rotary Presses
Solids
From Effluent Treatment
Flocculator
Weigh Scale

Basic parts of Biogas plant

1. INFLUENT COLLECTING TANK
(Mixing tank) :
- To homogenize various substrates & to setup required consistency.

4. DISPLACEMENT PIT
(SLURRY STORAGE)
- The fully Digested slurry leaves the Digester through the Outlet pipe into it.



2. DIGESTER
- The bacteria multiply & produce Biogas into the Digester.
- Digester provides the required conditions for this process

3. GAS HOLDER & GAS PIPE
- The Biogas is stored until consumption into the Gasholder
- The gas pipe carries the Gas to the Desired place of Consumption.

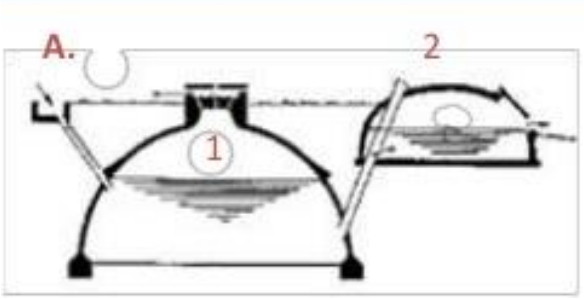
*Depending on the plant type & requirements Variations & Additions in components are possible

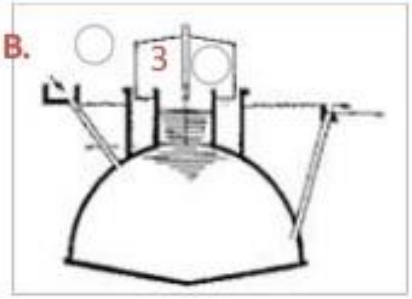
Sketch/Photo source: gtz/GATE

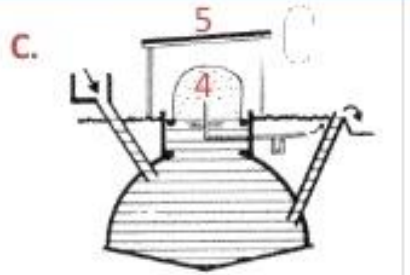
Types of Biogas Plants

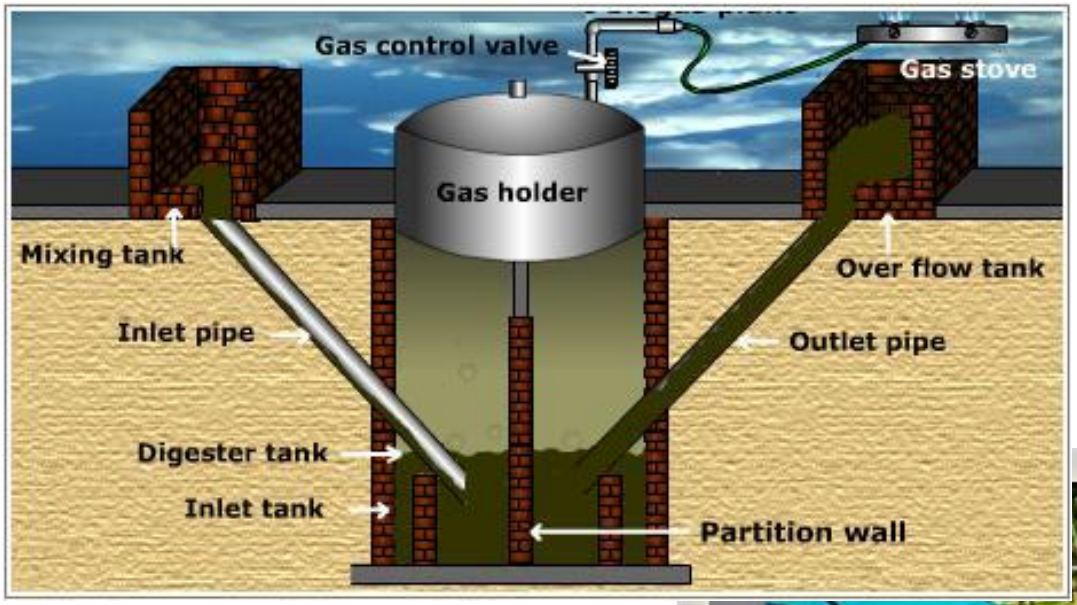
On the Basis of the type of construction the 3 well performing & Mature Designs suitable for Farming Households are:

A. **FIXED-DOME PLANT :**	B. **FLOATING-DRUM PLANT :**	C. **PLASTIC COVERED / BALLON PLANT:**
- Relatively low cost - Long life (20yrs or more) - Least Maintenance. - Simple Mechanism - No day/night temp Fluctuations so better Bacteriological processes - Labor intensive – Employment - Great care-design& workmanship.	- Which rises according to the volume of gas production - Easy to understand & operate - Provide Gas at constant pressure - Stored Gas-volume recognizable - Expensive , Maintenance Intensive (painting, cleaning) - Drum life is Short(abt 10years)	- Standardized prefabrication - Suitable for high ground water table - high digester temp - warm climate - Uncomplicated Cleaning, Emptying & Maintenance - Low gas pressure, short life, damage, not repaired locally



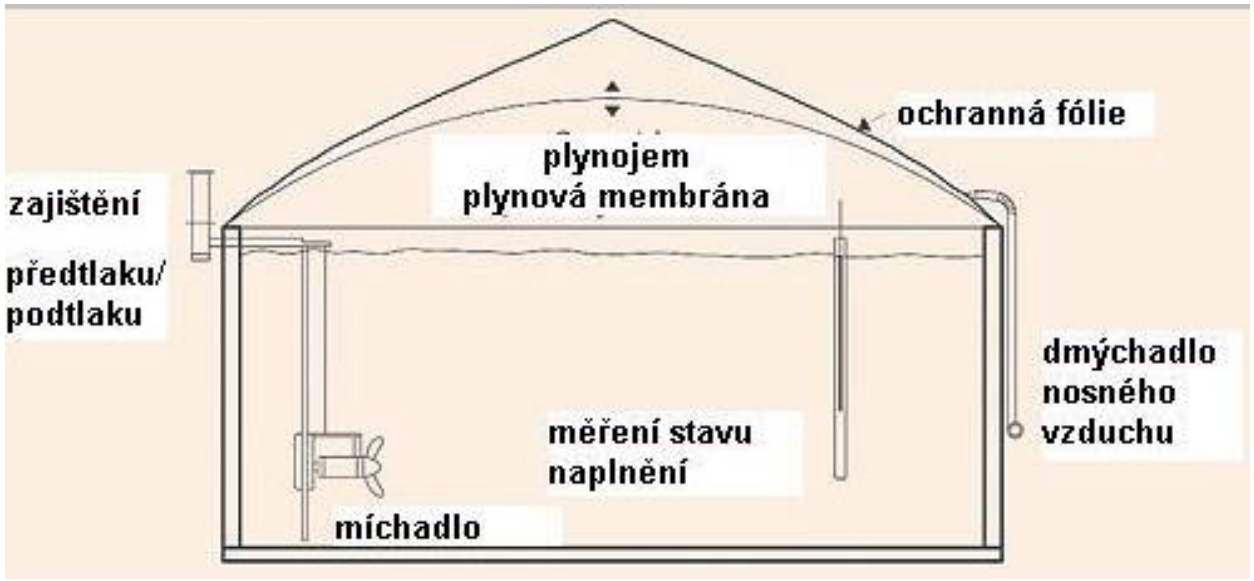





Gas control valve
Gas stove
Gas holder
Mixing tank
Over flow tank
Inlet pipe
Outlet pipe
Digester tank
Inlet tank
Partition wall

Gas containers for biogas plants

1. Internal (part of bioreactor)
2. External – for storage of biogas double- membranes for pressure balancing



Adjustment of biogas quality

Raw biogas –saturated with water, containing H_2S, CO_2, other volatile compounds

- De-watering– condensation (cooling), adsorbtion on silicagel
- Removal of sulphur – activated coal (obtaining sulphur), biological way – *Thiobacillus* (aerobic – cca 2%, immobilization of bacteria)
- Removal of CO_2 – absorbtion in $Ca(OH)_2$, organic solvents, active coal, zeolites, membrane separation…

Safety of biogas stations

Burner for combustion of bioagas excess–accident prevention,
Maintenance of cogeneration unit …

Burner

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Biotechnology in Food Industry

Lecture XIII

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Waste disposal in food/biotechnological industry

Degradation of waste compounds

- Aerobic systems – activated sludge or biofilm
- Anaerobic systems - consortium of microorganisms including methanogens
- Solid waste - composting

Aerobic or anaerobic waste disposal?

Aerobic

Activated sludge + water

More biomass

Convenient for mild poluted water

Low investment but high operation cost

Nitrification processes

Second stage for phosphorus removal

Anaerobic

Biogas + sludge + water

Heavily poluted water

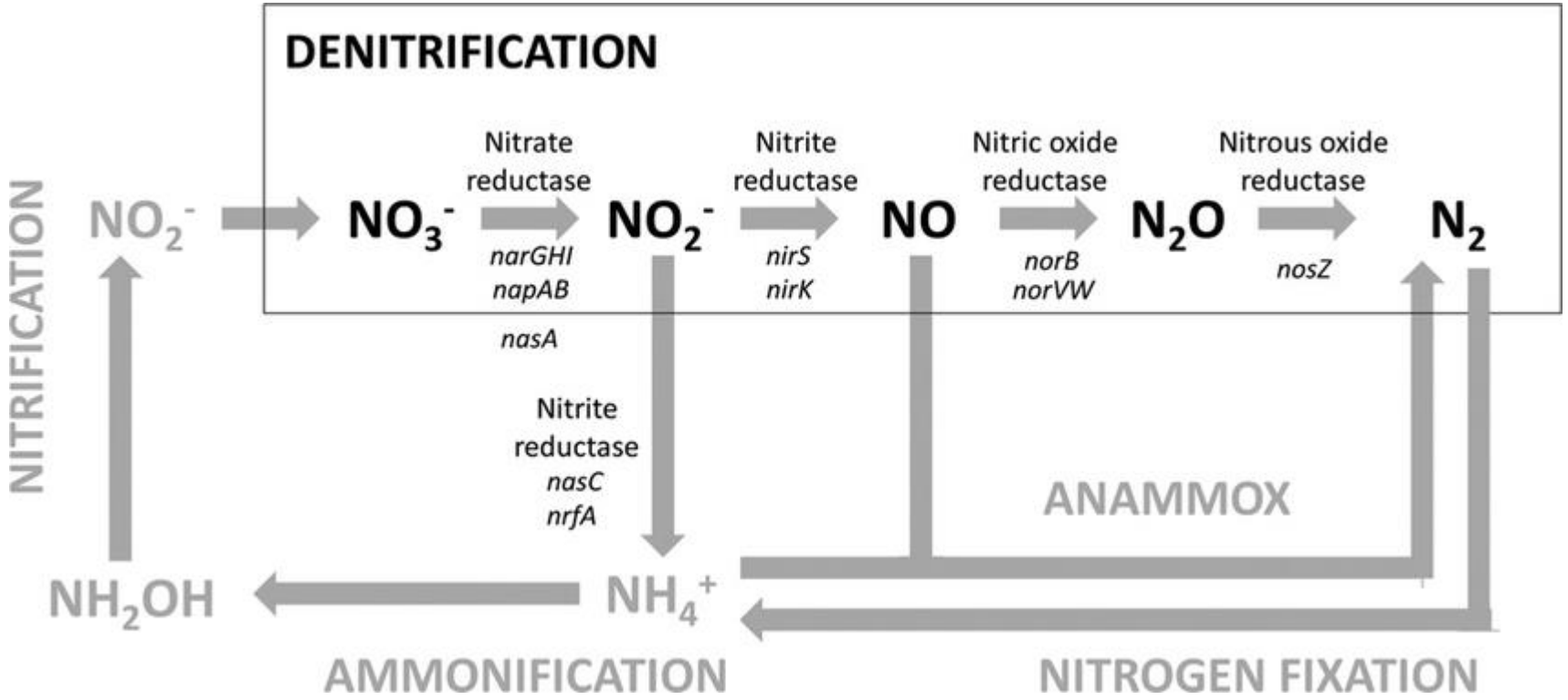
Can be combined wit aerobic treatment as first step

High investment but low operation cost

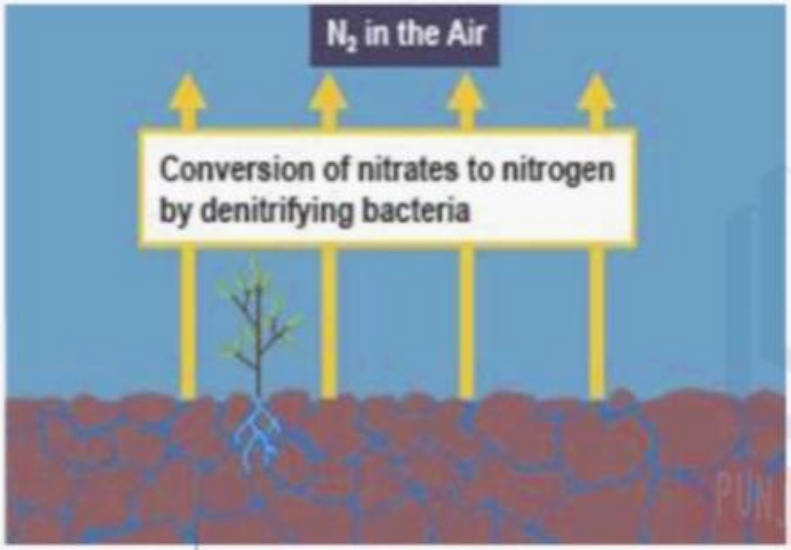
Denitrification processes

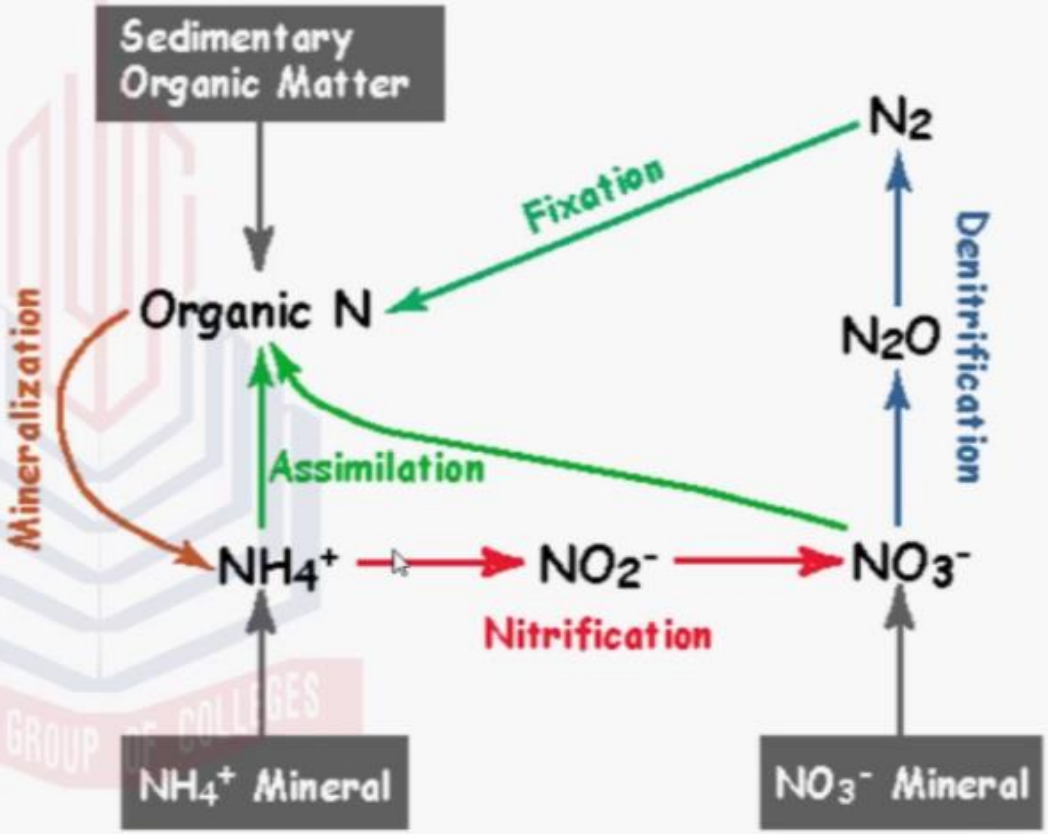
First stage for phosphorus removal

Denitrification - process by which nitrites or nitrates are reduced to nitrogen gas

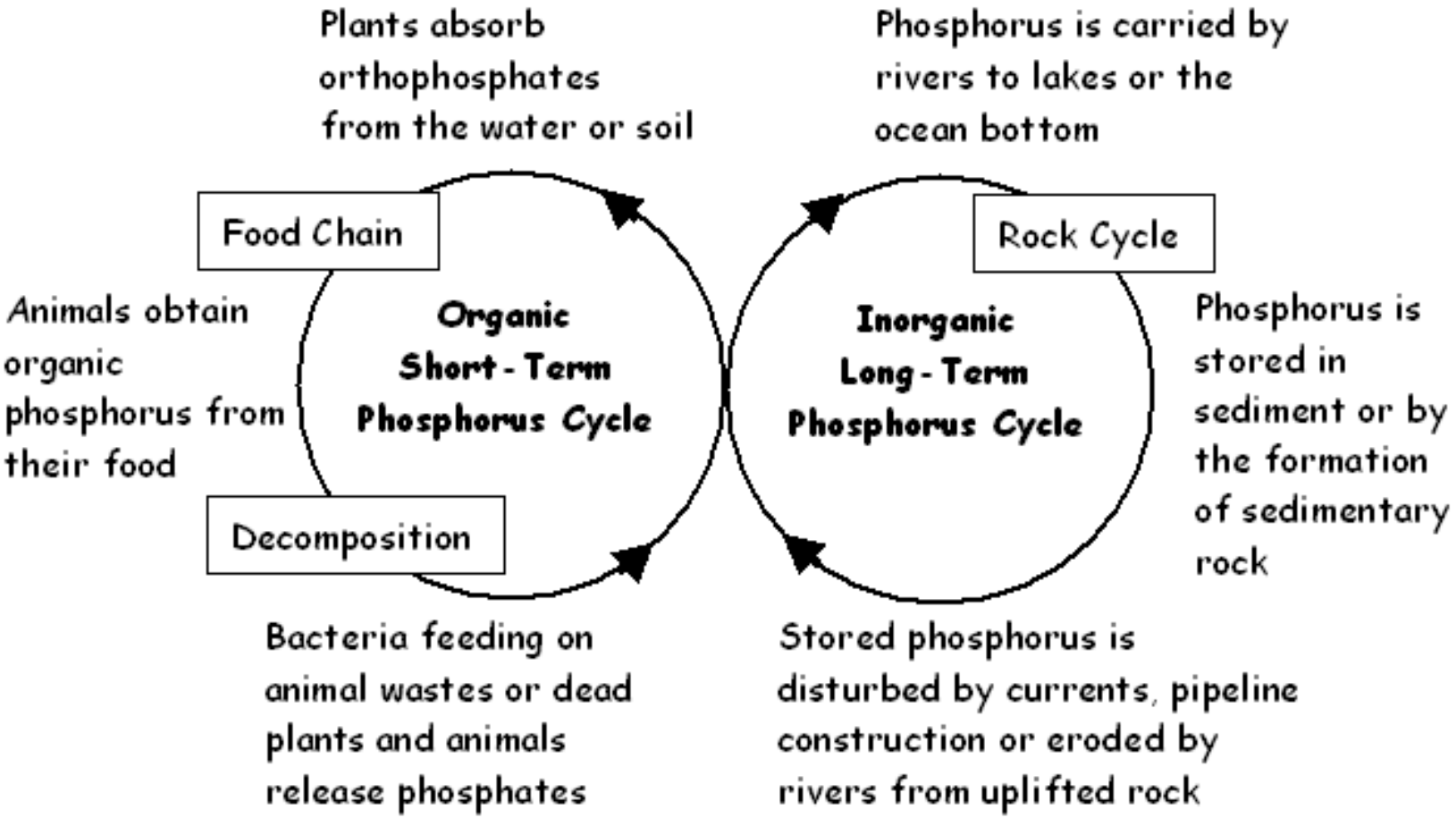


Denitrification

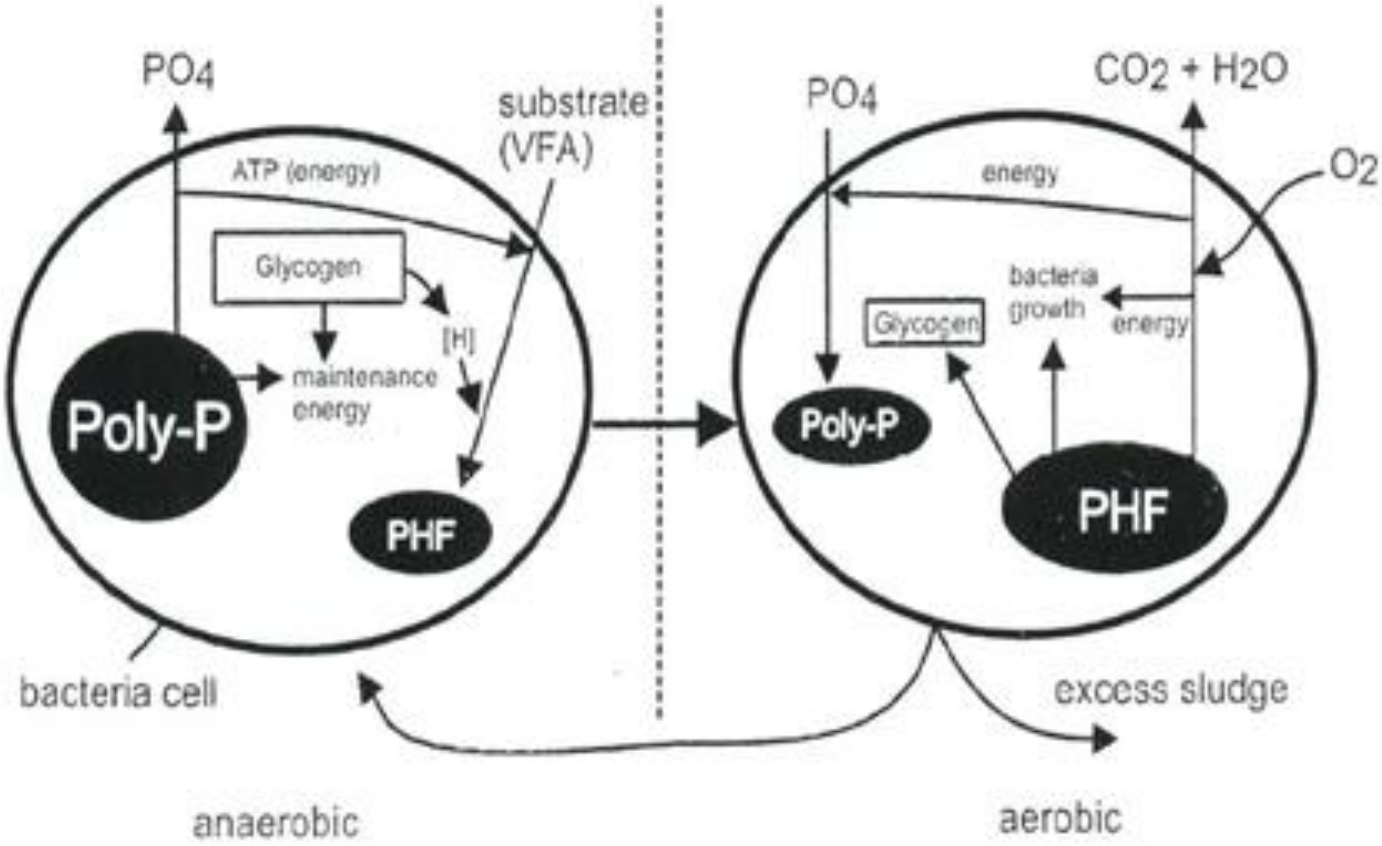




Phosphorus cycle



Two-step biological reduction of phosphates

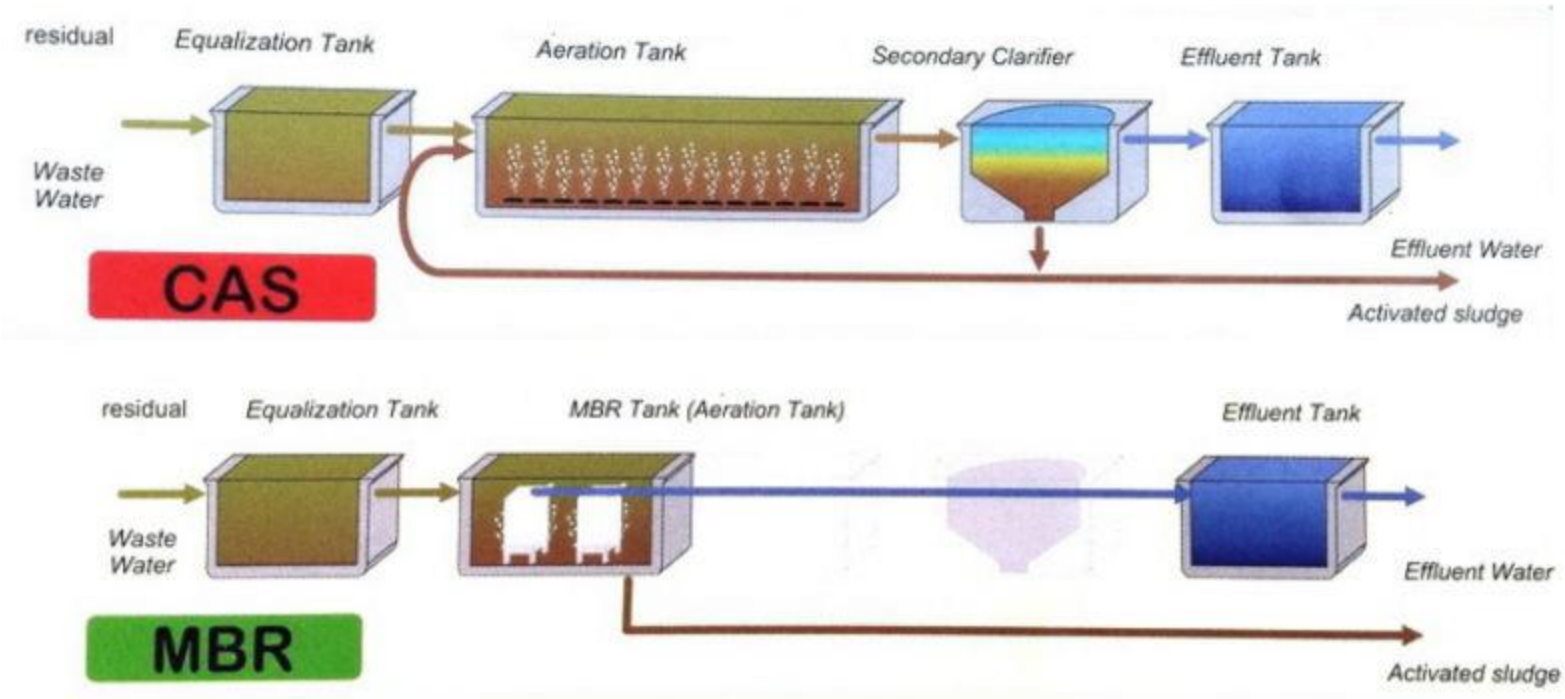


Aerobic treatment of waste water

- Activated sludge formation (growth of biomass under aerobic conditions)
- Settling tank– separation of biomass from water by sedimentation

Wastewater treatment

Mixing, aeration, decantation, recyclation of sludge



Prague wastewater treatment – formation of activated sludge

Prague waste water treatment – settling tank

Activated Sludge Process

Screens

Grit Removal

Primary clarifier

Primary sludge

Aeration section

Final clarifier

Ultra Violet Disinfection

Return Activated Sludge

Waste Sludge

Sludge storage tanks

Belt Press

Incineration

Bioset

Trucked to the Landfill

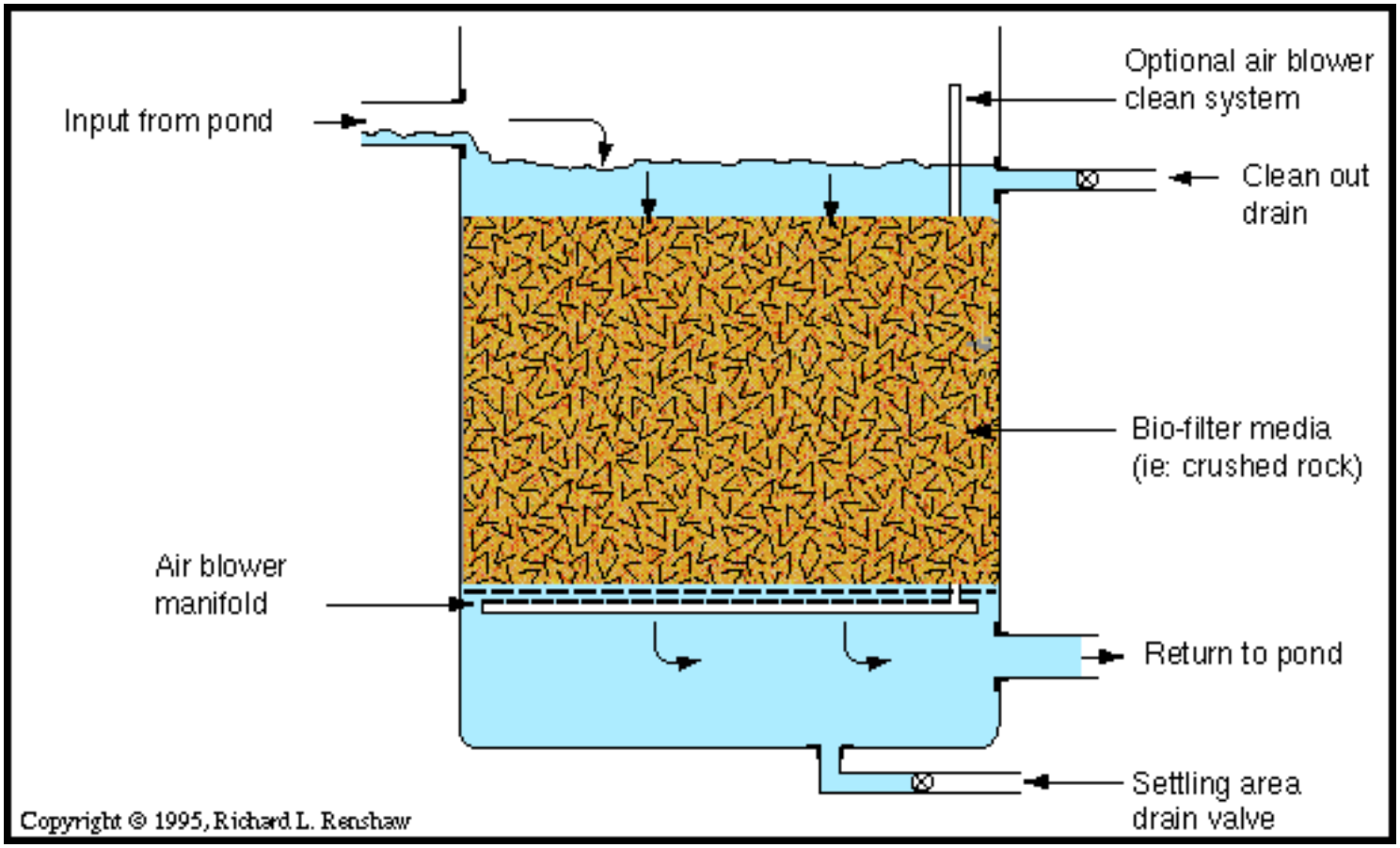
Biofilm formation

- Adhesion to surface of convenient material
- Carrier with microbial population – rotation or trickling bed

Moving bed biofilter

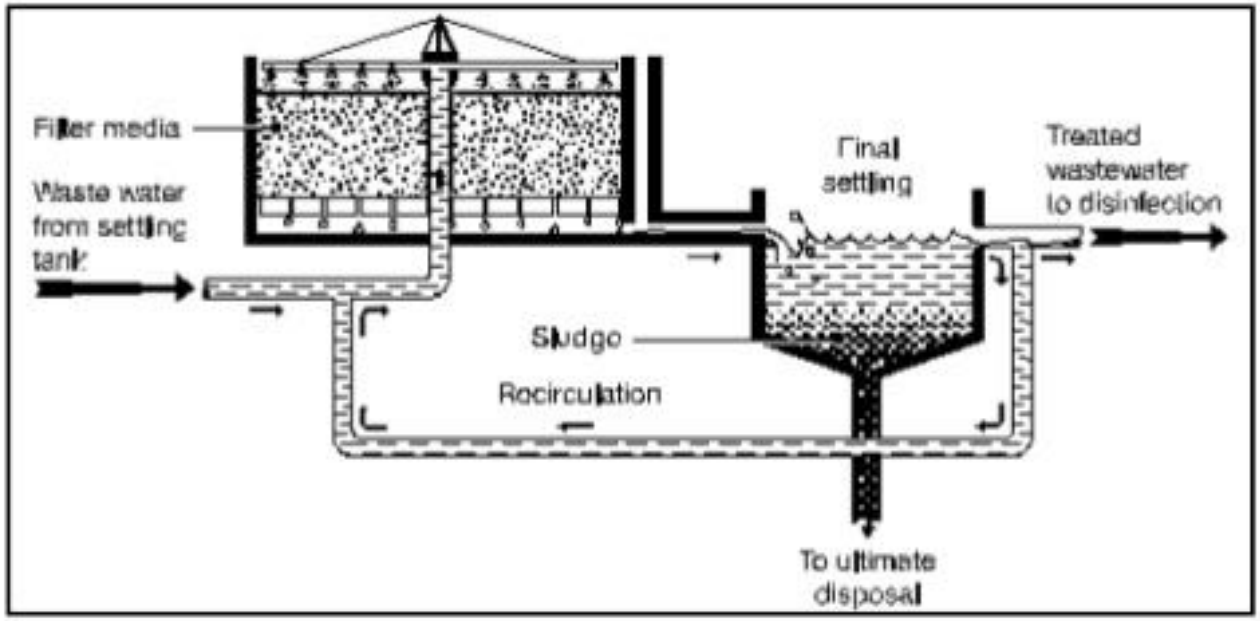
carrier material

Biofilter



Trickling Filters

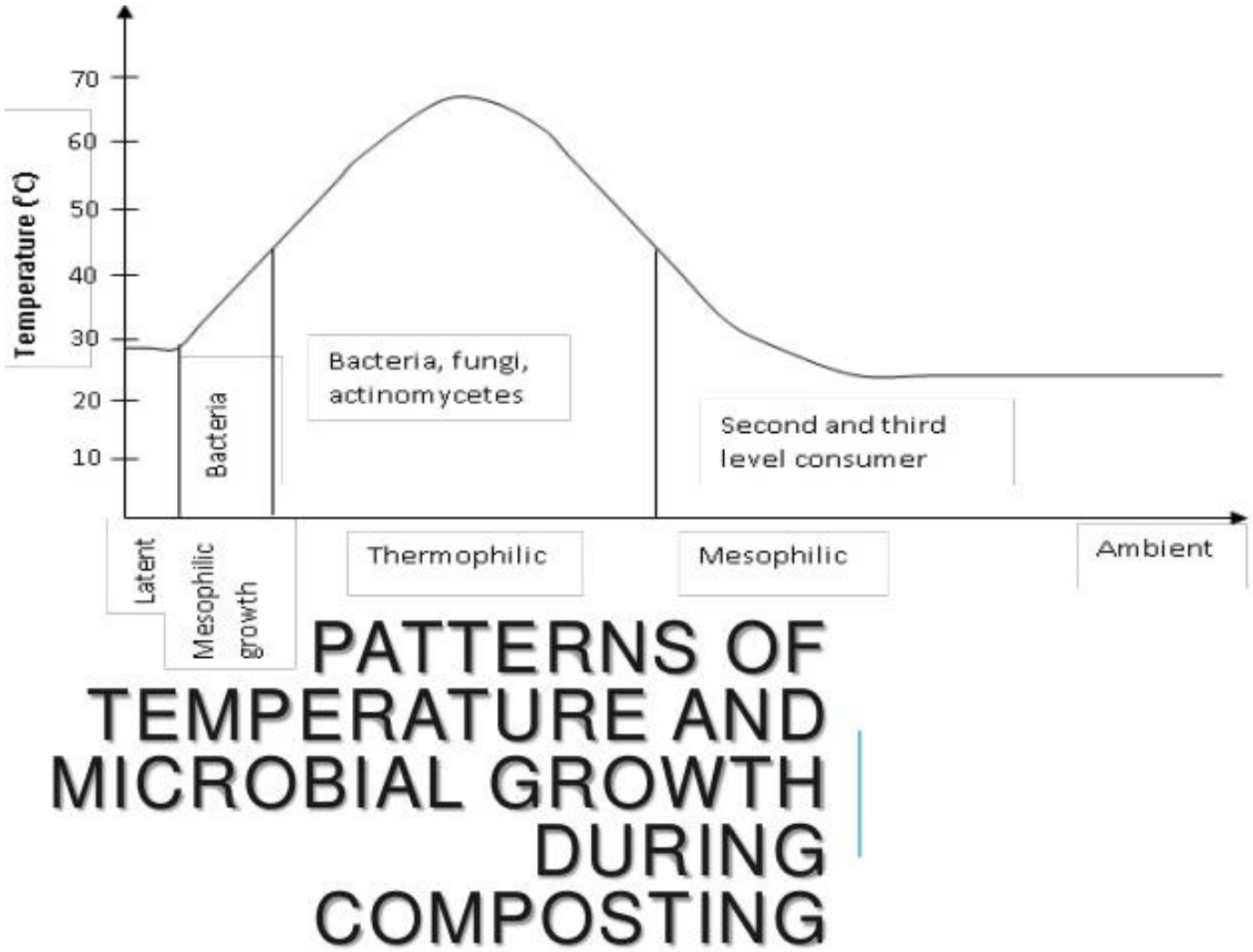
- The trickling filter or biofilter consists of a bed of permeable medium of either rock or plastic
- Microorganisms become attached to the media and form a biological layer or fixed film. Organic matter in the wastewater diffuses into the film, where it is metabolized. Periodically, portions of the film slough off the media



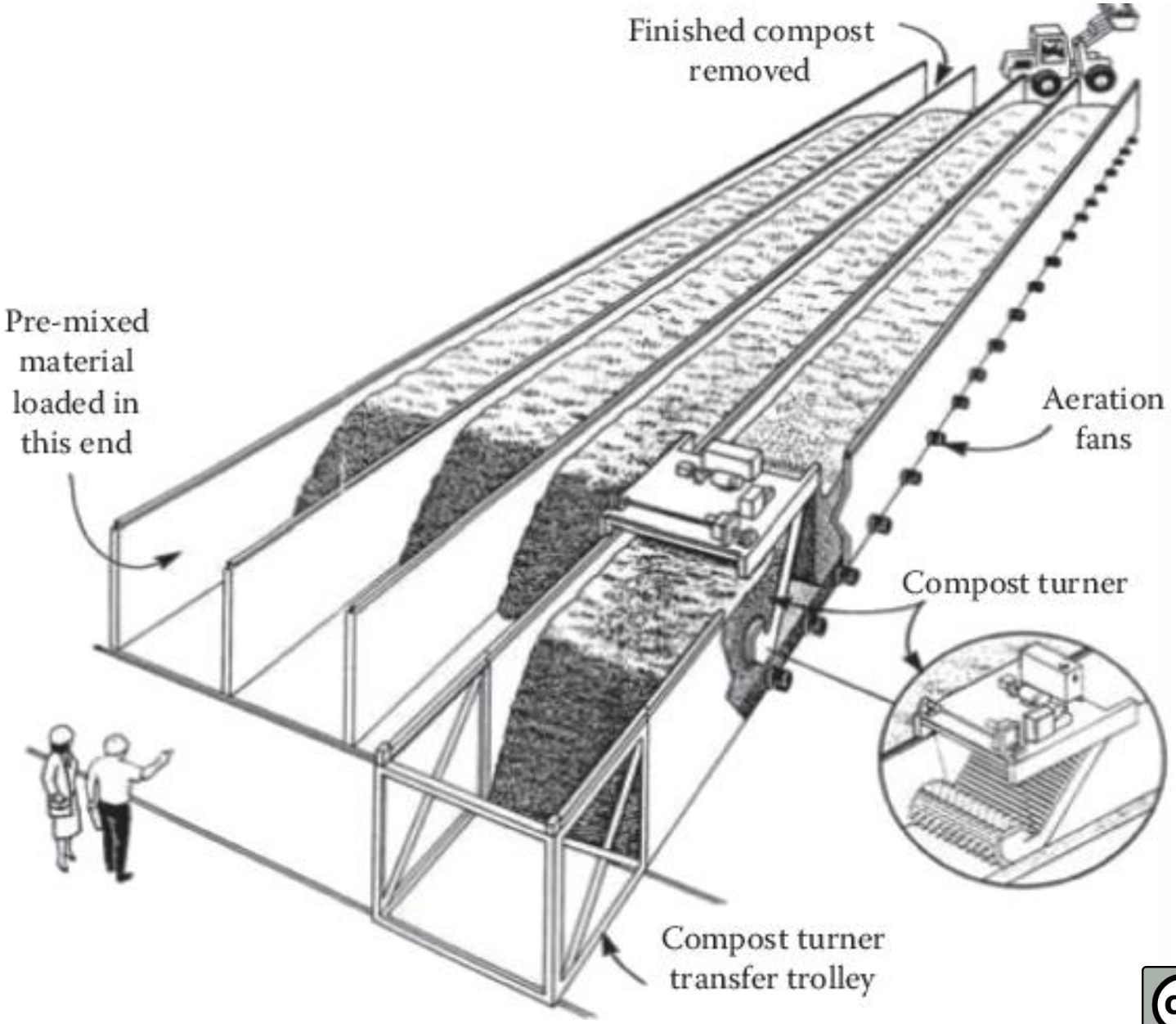
Composting

- Aerobic process for solid waste disposal
- Municipal waste, activated sludge, biological waste
- Degradation of dangerous compounds
- Volume reduction
- Drying
- Increase of temperature during composting – destroying pathogenic microorganisms

Composting phases

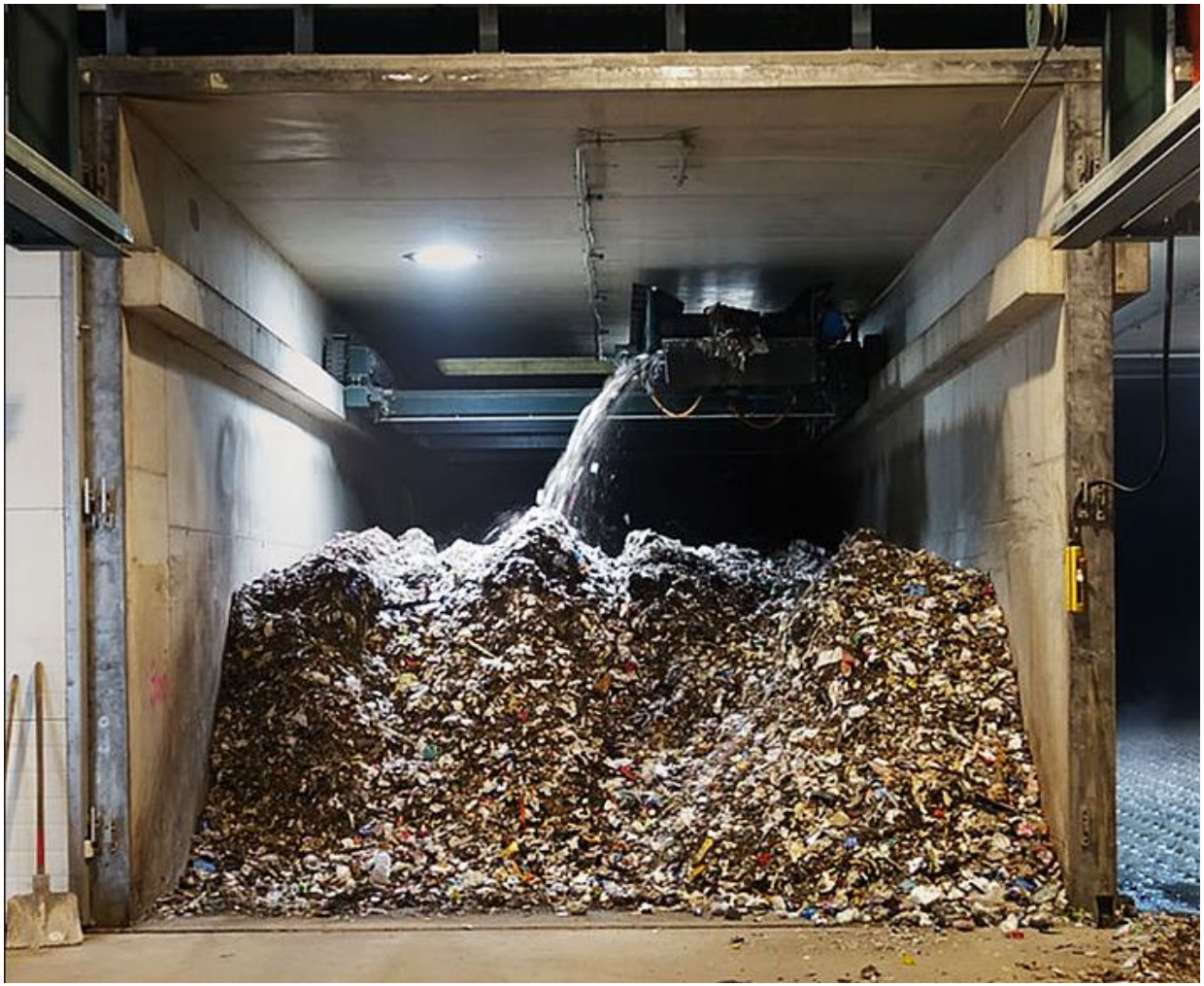


PATTERNS OF TEMPERATURE AND MICROBIAL GROWTH DURING COMPOSTING


Finished compost removed
Pre-mixed material loaded in this end
Aeration fans
Compost turner
Compost turner transfer trolley

Tedders of compost piles - aeration, mixing

Tunnel composter



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CHEMISTRY AND TECHNOLOGY
PRAGUE

Biotechnology in Food Industry

Lecture XIV

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Winemaking

Winemaking-vinification-eonology

Viniculture – planting, growing and harvesting vine grapes
Wine-making – production of wine from grapes

Vitis vinifera – species of genus *Vitis*

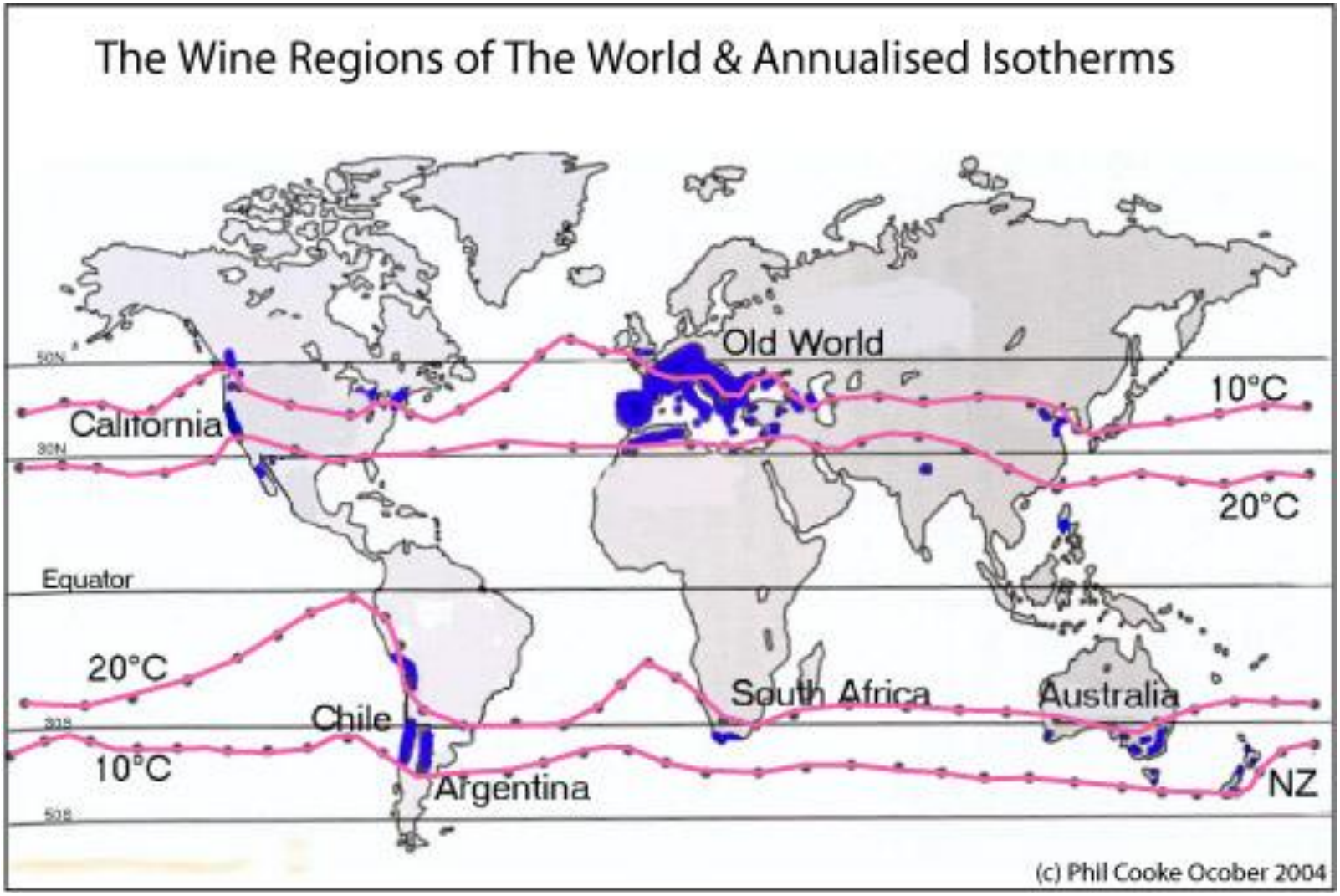
- thousands of different varieties of *Vitis vinifera* grapes
- liana
- the fruit is a berry, known as a grape

Varieties: wine x table grape

Vinyards: hot and sunny climates

- **to fully ripen**
- **develop a balance between acids and sugars**
- **latitude 30-50°**
- important factors are climate, slope and soil

flatter terrain x slope - greater intensity of the sun's rays
Favorable soil conditions include: aeration, loose texture, good drainage and moderate fertility
- climates vary from country to country, selecting the best strain is an important decision in grape cultivation



http://www.wineappreciation.co.uk/BWAC%20Tastings/Around%20TW.html


World Wine Production, 2017
Top 10 shown
Italy
France
Spain
United States
Australia
Argentina
China
South Africa
Chile
Germany
Rest of world

Volume in thousands of hectoliters; e.g., global production is around 247 million hl

	2013	2014	2015	2016	2017
Italy	**54,000**	**44,200**	**50,000**	**50,900**	**39,300**
France	42,100	46,500	47,000	45,200	36,700
Spain	45,300	39,500	37,700	39,300	33,500
United States	24,400	23,100	21,700	23,600	23,300
Australia	12,300	11,900	11,900	13,100	13,900
Argentina	15,000	15,200	13,400	9,400	11,800
China	11,800	11,600	11,500	11,400	11,400
South Africa	11,000	11,500	11,200	10,500	10,800
Chile	12,800	9,900	12,900	10,100	9,500
Germany	8,400	9,200	8,900	9,000	8,100
Portugal	6,200	6,200	7,000	6,000	6,600
Russia	5,300	4,900	5,600	5,600	5,600
Romania	5,100	3,700	3,600	3,300	5,300
Brazil	2,700	2,600	2,700	1,300	3,400
Hungary	2,600	2,400	2,800	2,800	2,900
Rest of World	*31,000*	*27,100*	*26,800*	*27,300*	*24,600*
World	**290,100**	**269,500**	**274,700**	**268,800**	**246,700**

...from grapes to wine!

WHITE GRAPES

Albariño
Aligoté
Arneis
Bourboulenc
Chardonnay
Chenin Blanc
Clairette Blanche
Colombard
Fiano
Garganega
Gewürztraminer
Grenache Blanc
Grenache Gris
Grüner Veltliner
Kerner
Maccabéo
Marsanne
Melon
Morio-Muskat
Müller-Thurgau
Muscadelle
Muscat
Neuburger
Picpoul Blanc
Pinot Blanc
Pinot Gris
Riesling
Rousanne
Sauvignon Blanc
Scheurebe
Semillon
Terret Blanc
Terret Gris
Torrontes
Ugni Blanc/Trebbiano
Verdelho
Verdicchio
Vernaccia
Viognier

BLACK (RED) GRAPES

Alicante Bouschet
Aramon
Barbera
Black Corinth
Cabernet Franc
Cabernet Sauvignon
Carignan
Carmenère
Carnelian
Charbono
Cinsault
Corvina
Counoise
Duriff
Gamay Noir
Grenache
Grignolino
Gros Verdot
Malbec
Merlot
Meunier
Mission
Mourvédre/Mataro
Muscardin
Negrette
Nebbiolo
Petit Verdot
Petite Sirah
Picpoul
Pinot Noir
Pinot Meunier
Pinot St. George
Pinotage
Primitivo
Rubired
Ruby Cabernet
St. Laurent
Sangiovese
Souzão
Syrah/Shiraz
Tempranillo
Terret Noir
Tinta Barroca
Tinta Cão
Tinta Negra Mole
Vaccarese/Camarese
Valdepeñas
Valdiguié
Zinfandel

http://www.rosettawines.com/main.asp?request=ARTICLES&article=7

Table grapes

- primarily intended for human consumption
- bigger beries, big stem, rigid and firm skin
- lower sugar and acids content

Grapes for wine production

- Grapes suitable for must or juice production
- Black grapes – manufacturing of red wines
- White grapes – manufacturing of white wines

Grapevine growing cycle

Influenced by: weather, Vitis varieties, vineyard locality

- **Bud break – bud burst – shoots grow – flowering**
- **green berry – rapid increase in volume - production org. acids and precursor phenolic and aromatic substances**
- **Slow berry growth - change colour, softening – sugar accumulation, fenol. and arom. substances – acidity decreasing**
- **Final ripening – sugar and anthocyanes accumulation – acidity and tanines decrease**
- **over-ripening of grapes and the shrinkage of the berry – gradual drying of stems, flow of water and nutrients stops,water evaporation,increase in sugar content...**

Harvesting

picking of grape clusters from the grapevines

- **Term dependent on: variety, locality, climate, grapes health, desired product (white grapes earlier to prevent looses in acidity, black latter – phenolic maturity)**

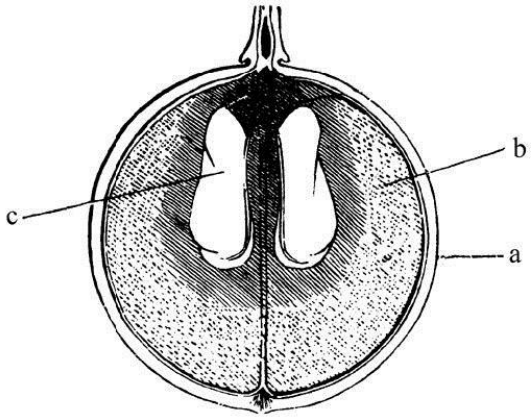
- **Optimum maturity:**
  - determination of sugar, polyphenol and acid concentration

Grape bunch morphology

Cluster - stem and berry
Berries - 95 – 98 % from total cluster mass.

Berry morphology

Skin, pulp, seeds



Bobule na průřezu

a) slupka; b) dužnina; c) pecičky;

Berry composition

Skin (6-12% hm.)

- Wax layer on a berry surface (protective fce)
- celulose, hemicelulose, pectine rigidity and flexibility
- tanines (more in black varietes)
- Aromatic substances and pigments (flavones a anhtocyanes dyes)

Seeds (2-5% hm.)

- In average 2-3 seeds in 1 berry
- oil
- tanines

Pulp (83-92% hm.)

- Sugar and acids – concentrated in the middle

Harvest
Destemming
Crushing

Pressing

Maceration
Pressing

Alcohol fermentation

Malo-Lactic fermentation

Racking to remove the lees

Clarification/Stabilisation/Preservation
Maturation
Filtration
Botteling
Ageing

Harvest

Hand picking (gentler handling, bunch selection**) x Mechanical harvest**

Video - https://www.youtube.com/watch?v=yCLoXZssTe4

Transportation from vineyard to winery

Partialy desintegrated berries -risk of oxidation, contamination

- **Temperature at harvest (as cool as possible, active cooling in hot region)**
- **As quick as possible**
- **First addition of SO_2**

Destemming - crushing

Destemming – removal of stems
Crushing –release of juice

Stems – harsh and astringent polyphenols – mainly catechins and leucoanthocyanins

Crushing with stems – extraction of sensoricaly unwanted substances

Destemming and crushing in one step

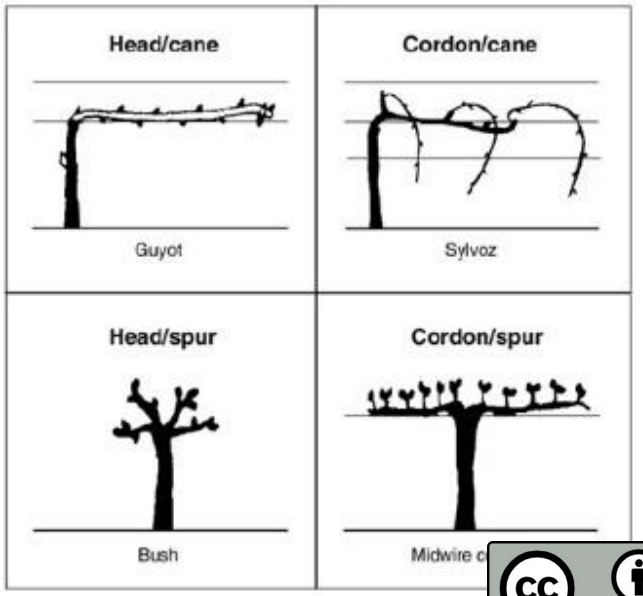
Specialized tools – destemmer - https://www.youtube.com/watch?v=KPygrlCeRRQ

Addition of of SO2 into crushed grapes – suppresion of natural MO, increase in pigments solubility

Grape must composition

Factors with highes impact:

- ❑ **Vitis variety, vineyard location, soil type + composition**
- ❑ **weather (rain falls, temperature)**
- ❑ **mechanization, grape vine training**
- ❑ **grapes processing (desteming, crushing, pressing...)**



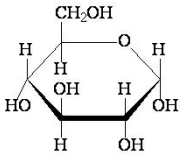
Složení hroznového moštu

- sugars
- Organic acids
- N-substances
- phenols
- aromatic substances
- mineral substances
- pectines
- vitamins
- Natural pigments
- fat, oil, wax

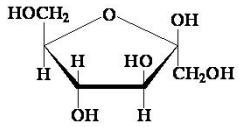
Chemistry of grape must

Sugars - carbohydrates

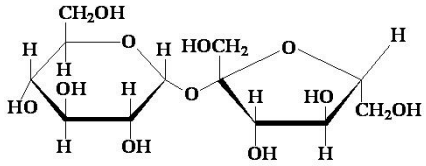
- **sucrose – minority – sugar transporter**
- **Glucose and fructose – main sugars, fermentable**
- **Pentoses** – non-fermentable **(arabinose, xylose), low content**
- **polysacharides – fermentable after enzymatic cleavage**



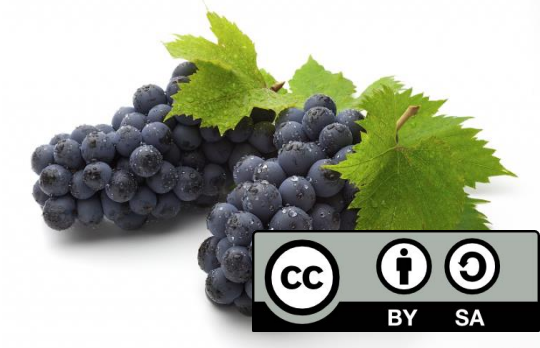
glucose



fructose



sucrose



Sugar content – must density

- Hydrometer scales to measure density
- 4 different scales are used internationally

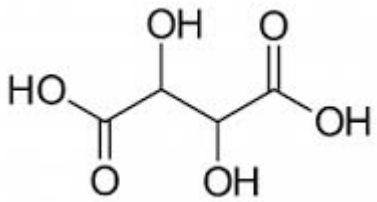
°Brix - Antoine Brix
°Balling – Karl Balling
°Baumé - Antoine Baumé – used in Australia
°Oechsle - Ferdinand Oechsle – used in Germany

- historicaly, different reference teperatures used for settings
- nowadays – Brix and Balling are identical setted for 20°C
- 1° on the Baume´ Scale is equivalent to 1.81Brix (or Balling)
- Oeschle - difference in weight of one liter of must compared to one liter of water

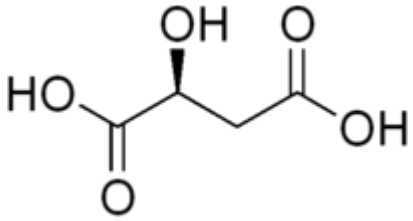
Chemistry of grape must

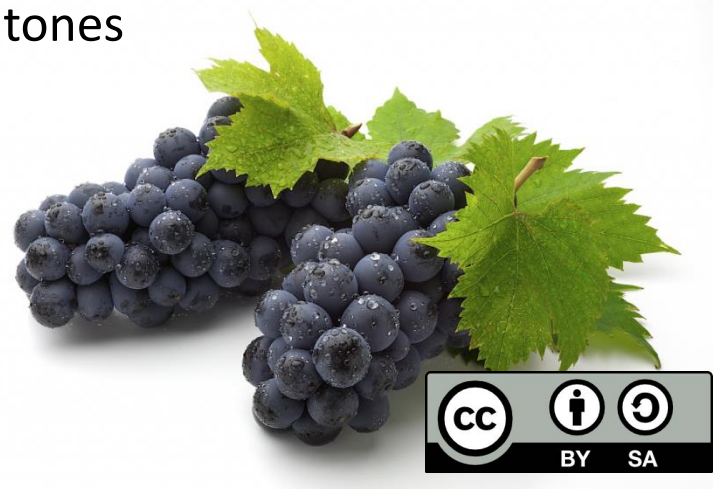
Organic acids

- **Decrease during ripening to 6-12 g/l**
- **70-90 % - tartaric and malic acids – major acids in grape must**
- **Lower content: citric acid,succinic acid, lactic, formic and acetic acid**
- **Tartaric acid –** responsible for majority of acidic taste of must and wine



- **Malic acid -** sharp, harsh and immature taste tones





Chemistry of grape must

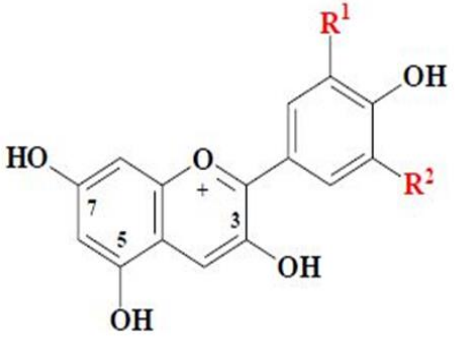
Nitrogen substances

- **240-1600 mg/L total amount of nitrogen**
- **aminoacids** – nutrients for yeasts + precursor of aromatic substances
- **peptides** – aromatic compounds **+** wine body (light to full)
- **proteins** - enzymes + wine stability
- **ammonium inos, amines, nitrates, nitrites**

Chemistry of grape must

Fenolic substances

- **Big difference in content in black and white grapes**
- **Highest amount: stems, seeds, skin**
- **Responsible for colour, bitterness, astringency, antioxidative properties**

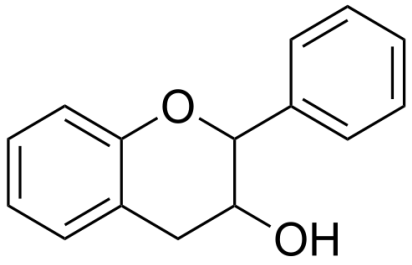


→ antocyanes

- Pigments in red and rose wines
- **Mostly in a form of glycosides**

→ tanines

- **flavan-3-oly: katechin, epikatechin, prokyanidines**

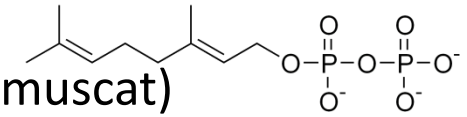


Chemistry of grape must

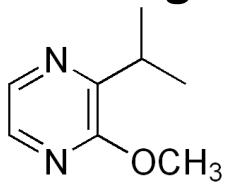
Aroma and flavour

- **Varietal aroma (odrůdové aroma), grape-derived**
- Free form of aromatic substances – may be lost or decreased during fermentation
- **Chemically bound aromatic substances** – aroma developes during fermentation and wine aging

- **Esters** - reaction of acids and alcohol in the wine
- **monoterpenes** – two isoprene units - floral aroma (muscat)



- **norisoprenoides** - C_{13}-norisoprenoids - Carotenoid derived aromatic compounds, spice notes **(Chardonnay)**
- **Methoxypyrazines** **–** mostly undesirable, green flavour of **Sauvignon Blanc**



- **volatile phenols** –unplesant aroma

❑ Inorganic constituents

soil (geografy), weather, nutients addition -fertilizers

kationty: K^+, Mg^{2+}, Ca^{2+}, Na^+

anionty: CO_3^{2-}, PO_4^{3-}, SO_4^{2-}, Cl^-

❑ Pectins

Polysacharide rich in galacturonic acid

Decreases exctraction efficiency

Complicate sedimentation and filtration

Possible adition of pectolytic enzymes

❑ Vitamins

C (60-80 mg/l), tiamin (B1, 150-300 mg/l), panthotenic acid (B5), biotin,folic acid , cobalamin (B12)

❑ Natural pigments

white grapes → chloroplasts (chlorophyl) and chromoplasts (carotenoids, xanthophyles)

black grapes → **anthocyanes**

Crushed grapes:

White wine ➡ pressing

Red wine ➡ maceration, start fermentation

Pressing - separation of liquid part from solid grape residues (grape pomace)

❑ <u>factors influencing the process</u>:

Pressure pressing – efficiency of pressing , the lower the better
type of press (mechanical, hydraulic, pneumatic)
Degree of crushing grapes
speed of pressing – prolonged for high quality wine
vine variety,
grape maturity
presses: mechanical, hydraulic, pneumatic

Must yield = approx. 70%

Whole bunch pressing - white wine from red grapes
- juicy, low phenolic, fine wines

Treatment/adjustment of must composition

Settling/clarification

Juice from the press is drained into tanks, where skin, stalks and pip fragments settle down.

- ❑ Carried out under decreased temperature
- ❑ Approx 24h
- ❑ Rate of grape solids remained depends on desired product
- ❑ Modern clarification: filtration or centrifugation

Adjustment of must composition

Adjustment - take into consideration legislation of particular state/region

Sugar content

- Addition of saccharose
- Addition of concentrated must: **vacuum evaporation 70°C, lyophilization, reverse osmosis**
- **Dependent on the climate of particular region:**
  - **warm – high sugar content**
  - **cool – low sugar content**

Adjustment of must acidity

Target: to reach harmonic balance between acidity and sweetnes

- **optimal acidity 6-7 g/l (for tartaric acid) - pH 3,1-3,4**
- **Higher pH → risk of contamination – needs more SO_2, low colour stability, wines without freshness**
- **Changes in acidity:**

fermentation based production of organic acids
precipitation of tartarate
malolactic fermentation
Blending/coupage – mixing diferent musts with distinct acidity
Tartaric acid addition
$CaCO_3$ – precipitation of calcium tartarate

Must adjustment

Application of sulfur dioxide SO2

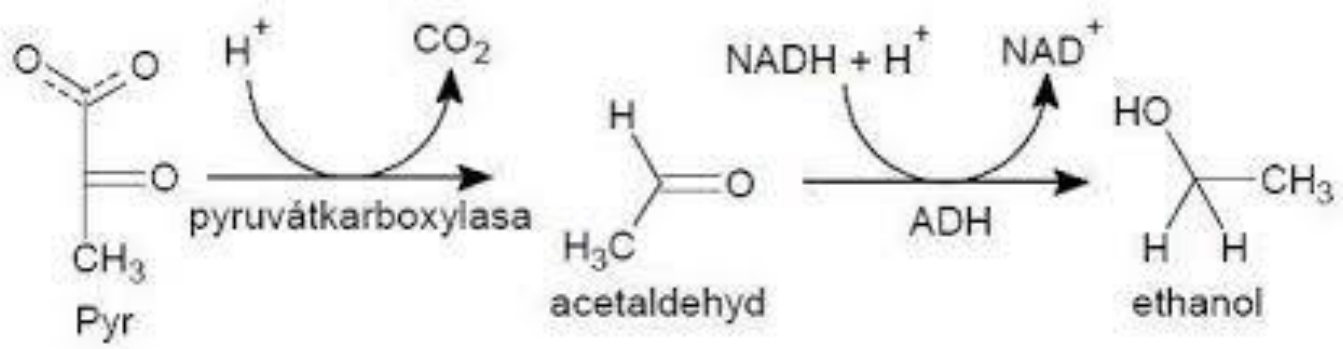
- **Preventive action aggainst contaminant MO (bacteria generaly more sensitive than yeast)**
- **stabilization** – suport proteins coagulation
- **antioxidant** – prevets oxidation by reaction with **O2**
- **antienzymatic effect – inhibition of oxidative enzymes**
- Amount in wine given by legislation – higher amount: negative health impact, negatively influences wine taste and aroma

Ways of SO2 application

- **Burning of sulfur plates**
- **Potassium metabisulfite K2S2O5 (powder) – decreases asidity via tartarate precipitation**
- **gaseous / liquid SO2 from pressure bottle**

Fermentation

$C_6H_{12}O_6$	→	$2\ C_2H_5OH$	+	$2\ CO_2$
glukosa		ethanol		oxid uhličitý
100 g		*51,14 g*		*48,86 g*



Alkoholové kvašení

- theoretic yield EtOH: 51% w/w – derived from ethanol fermentation equation!
- 5-8% sugar utilized for cell growth and side products
- Side fermentation products produced directly by MO metabolism:
  - Glycerol
  - Lactic acid
  - Acetic acid
  - Succinic acid
  - Citric acid

Secondary products of fermentation process

Highly impacted by producing MO used

- **Higher alcohols** – n-propanol, amylalcohol, isobutanol (fusel alcohol)
  - **From pyruvate or by aminoacids deamination**
  - **pozitive as well as negative impact on aroma**
  - **Discusion about its cause of hangover syptoms**

- **Esters –** reaction of alcoholes with organic acids
  -Fruity tones

- **aldehydes, ketones, acetoin, diacetyl (buttery flavour), etc.**

Producing microorganisms

- ***Saccharomyces cerevisiae, Saccharomyces bayanus***

- **More than 15 yeast genus (eg. *Brettanomyces, Candida, Saccharomycodes*)**
- **Industrial strains are selected for wine production:**
  - **according to fermentation rate**
  - **yeast activity**
  - **ethanol tolerance**
  - **fermentation under decreased temperature**
  - **production of sensorically active compounds**

Producing MO

Different varieties selected for distinct wine types

Pure strains x mixture of more strains

cultured stock yeast x wild yeasts

Cultured stock yeast are stored in form of „**active dry yeast**“ - need to be activated befor use.

Factors affecting alcoholic fermentation

- Temperature (optimum 20-27°C)
- Nutrients
- Sugar content – higher concentration slows down proliferation – higher osmotic preasure
- Ethanol concentration
  - Wild type yeasts – up to 4% v/v
  - *S. cerevisiae* – up to 12-13% v/v
  - *S. bayanus* – up to 15-16% v/v
- CO_2 preasure (partial ventilation during fermentation)
- activatores (vitamines, minerals) x inhibitores (pesticides, tanines...)
- Presence of O_2

Industrial fermentation

carried out in **Controled vessels**

Stainless steel

- **Temperature regulation – double coat or internal cooler**
- **CO2 preasure regulation – active exhaustion**
- **Regular regime for „cap“ immersion into liquid - to ensure contact between skins and wine**

Fermentation

Fermentation timing vary from 2 days to 3 weeks – according to wine style

After fermentation is completed:

- white wine – settling down the leeds (Gross lees) – racking (draw off)

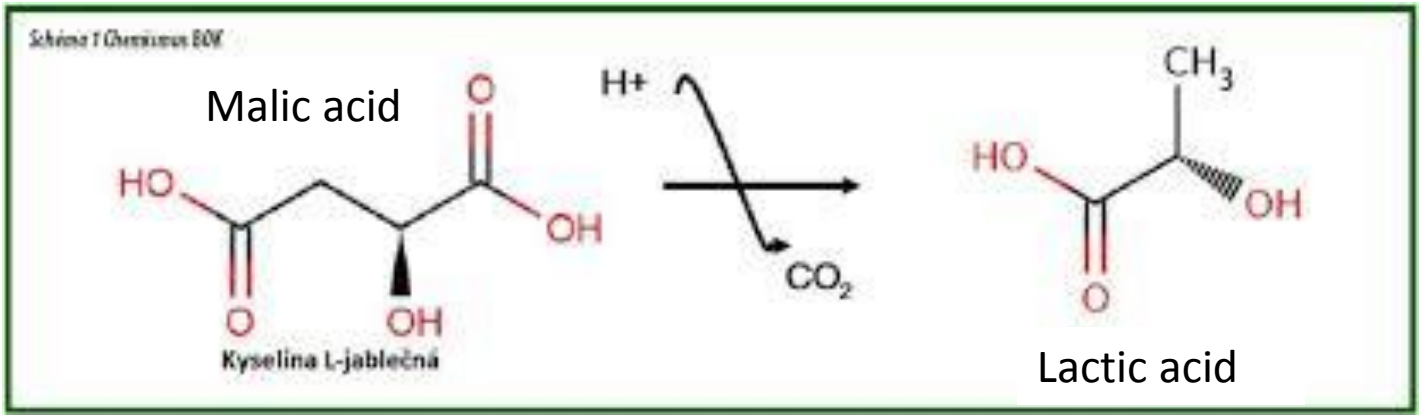
- red wine – free run wine removed from vessel – pressing the residual grape mass (pomace)

„extended maceration“ – prolonged contact with seeds, skins, yeast after fermentation) – eg. Bordeaux wines

Malo-Lactic fermentation

Biotransformation of malic acid to lactic acid

Lactic acid bacteria fermentation – spontaneous x controled



- **spontaneous process** – can cause negative sensorical profile
  - starts spontaneously under higrer temperatures and lower SO2 content

- **controled process** - **inoculation by selected strains, mostly** ***Oenococcus oeni***

Malo-Lactic fermentation

Lactic acid – more pleasant taste than malic acid (imature, sharp tones)

- **decrease in acidity, harmonization of acids, sugar and EtOH**
- **improves fullnes of wine body**
- **positive effect only for some types of wine (preferably red)**
- warm regions – spontaneous process - undesirable –decreasing acidity + **increased danger of contamination owing to pH**

Clarification, Stabilization and Preservation

Clarification – wine clarity – consumers recquirement

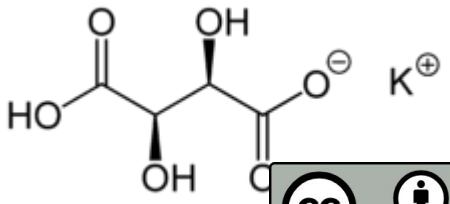
Two fractions to give rise turbidity:

1) gross particles – yeast, debris, grape residues
2) macromolecules – e.g. proteins, tanines, pectines, gums, metalo- colloids

Stabilization process

– to stabilize substances diluted in wine for a reasonable shelf-live (a minimum 1 year)

- if left for very long period – it would stabilize itself
- an effort to expedite stabilization employing specialized procedures
- when carried out improperly – can lower wine quality



Clarification, Stabilization and Preservation

gross particles

– yeast, debris, grape residues

- removal by filtration or sedimentation

- filtration serves to clarify – not to stabilize!

macromolecules – tends to increase turbidity via reactions in bottle

Clarification/fining – complex process – the main principle is based on electrostatic attraction

- fining agent carry particular charge and react with oppositely charged wine constituent to form precipitate

- chemical reaction as well as absorption process can take place

Clarification, Stabilization and Preservation

Fining procedure:

Mix fining agent in wine → suspenzion →sedimentation → racking → filtration

Clarification, Stabilization and Preservation

Fining agents:

1) Proteins such as gelatine, casein, albumen etc.
2) Earth such as bentonite, kaolin
3) Polysaccharides alginates, arabic-gum
4) Carbons
5) Sythetic polymers – nylon, polyvinyl polypyrrolidone (PVPP)
6) Silicone dioxide gel
7) Tannines
8) Miscellaneous, eg. Metal chelators, enzymes, …

Fining agents

Proteinaceous fining agents

-adjust polyphenol content of wine by hydorgen bonding between the phenolic hydroxyl and carbonyl oxygen of peptide bond

- Decreases bitter and astringent substances

Casein

- **Heterogenous phosphoprotein from milk**
- **Obtained by coagulating milk**

Egg white

- **One of the oldest fining agent**
- **active substance – egg albumin**

Gelatin

Produced by hydrolysis of multi-stranded collagen

Fining agents - continuing

Agar

- **polysacharide from algae (linear polymer of galactose)**
- **negative charge – removing of proteine based turbidity**

Active coal

- **big surface – colour adjustment, defect of flavour**

Polyvinyl polypyrolidone

- **Synthetic, insoluble plastic**
- **Remove low molecular weight phenolics**

Bentonite

- **Natural, clay-like material of volcanic origin**
- **negative charge –** proteineous cloudiness
- **diferent types: Ca-bentonit, Na-bentonit, Ca-Na-bentonit**

Not recomended for red wines- removes colour

Tartaric acid

Precipitate of salt of taratric acid – represents major non-biological instability

- the biggest complaint in wine trade
- Can not be fully prevented
- Cryslats are harmless

Stabilisation:

Static cold stabilisation: wine is chilled down to freezing point (approx. -4°C) and stored insulated for 7-14 days

Contact cold stabilisation: addition of KHT (potassium bitartarate) crystals to promote crystalization via increase in nucleation sites

Ion-exchange stabilisation – most frequent replacing K+ with Na+

Wine preservation

Ethanol and tannines

– natural preservatives

- do not ensure 100% protection

- residual sugar serves as an energy source

- Lactic acid bacteria, acetic acid producing bacteria

Most frequent is application of SO_2

Another possibilities (prior botteling):

sorbic acid – prevents yeast proliferation

sterile filtration prior botteling

pasteration (65 – 70°C)

All the procedures must be carried out - to preserve the quality of wine while maintaining product stability

Wine maturation and ageing

Wine maturation and ageing

Family of interlinked chemical changes

Maturation – bulk storage period

Ageing – smaller container storage period (bottles)

„mature“ wine is „ripe“ to bottel, in which it „age“

Wine maturation and ageing

Maturation

Mostly: oxidative reactions
extraction during maturation in wooden barrels
non-oxidative polymerisation

White wines - kept mostly under reducing atmosphere

Red wine - prospects of micro-oxidation (occures naturally when aged in wooden vat)
- maturation in oak barrel: month – years
- extraction of sensoricaly active compounds (mostly phenols and aldehydes)

barrique: at least 3 month in oak barrel of defined volume (210 – 250L) used not more than 36 month

Mikro-oxygenation

Relatively new (1991)

Widely popular - oxygenation of fully fermented wine

A small dose of oxygen is supplied into wine as microscopic bubbles

Action of oxygen is complicated and influenced by manny factors

the main effect – modification of phenolic compounds resulting in:

„smoother“ and „softer“ wine

Harvest
Destemming
Crushing

Pressing

Maceration
Pressing

Alcohol fermentation

Malo-Lactic fermentation

Racking to remove the lees

Clarification/Stabilisation/Preservation
Maturation
Filtration
Botteling
Ageing

White wine technology

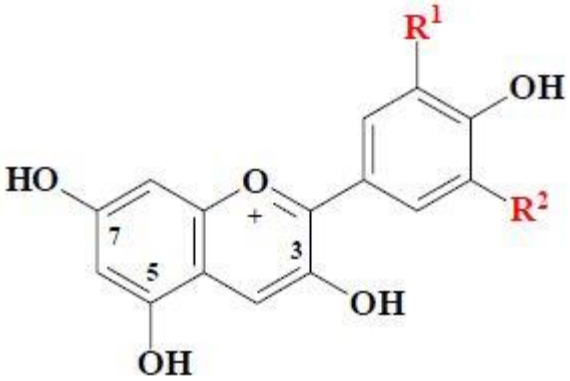
- Grape ripenes given by aromatic ripenes and sugar and acids contents
- Destemming
- Crushing
- Pressing
- Short maceration – if recquired, lower temperature
- Sedimentation (filtration)
- Must adjustement
- Fermentation: optimal temp: 15-18°C (fresh 13-18°C)
- Malo-Lactic fermentation mostly undesirable
- Reductive atmosphere
- …..

Red wine technology

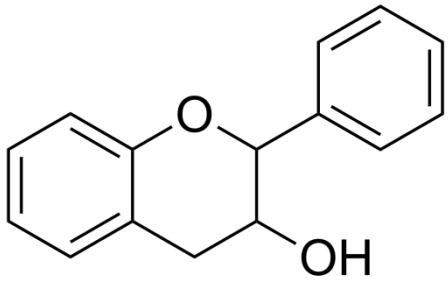
Key role – „fenolic ripenes of grapes“

Phenolic compounds more important than aromatic ones

Pigments – ANTHOCYANES



Taste – TANNINES (flavan-3-oly)



Red wine technology

- Destemming
- Crushing
- Must composition adjustement
- Fermentation and maceration
- Maceration – higher temperature (28-30°C), if recquired – „extended maceration“
- Pressing
- Malo-Lactic fermentation
- Micro-oxidative atmosphere
- Maturation in oak barrels
- …..

Rose wine technology

Rose wine is produced from black grapes using white wine technology

- **Lower content of anthocyanes and tannines**
- **Short maceration under low temperature**
- **Colour rose, pink, salmon-like**
- **Malo-Lactic fermentation applied according to specific recquirement**