Industrial microbiology

pp. **166-173,** 1032-1038,
1039-1045,1046-1050

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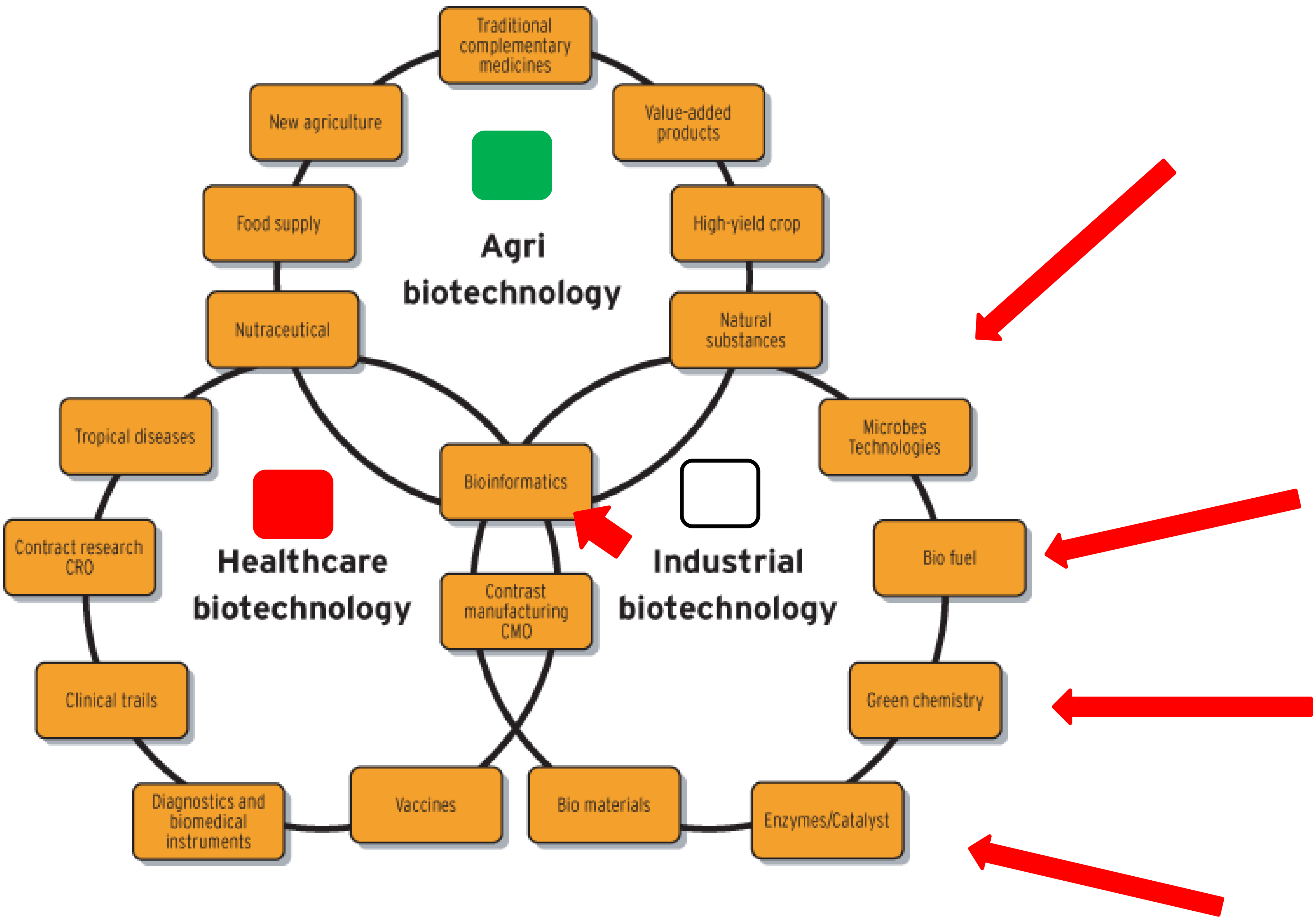
We are here

- **Industrial microbiology → biotechnology**
- Why the increased interest
- Microbiological versus chemical processes
- Type of ‘fermentation’ processes
- The fermentation itself
- Microbial growth & product formation
- Metabolic engineering


Traditional complementary medicines
New agriculture
Value-added products
Food supply
High-yield crop
Agri biotechnology
Nutraceutical
Natural substances
Tropical diseases
Microbes Technologies
Bioinformatics
Contract research CRO
Healthcare biotechnology
Industrial biotechnology
Bio fuel
Contrast manufacturing CMO
Clinical trails
Green chemistry
Diagnostics and biomedical instruments
Vaccines
Bio materials
Enzymes/Catalyst

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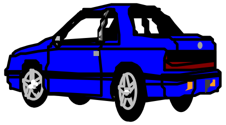
Petroleum-based to Bio-based chemicals to….

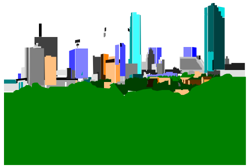
….***reduce*** **global warming!**

….***provide*** **energy for**



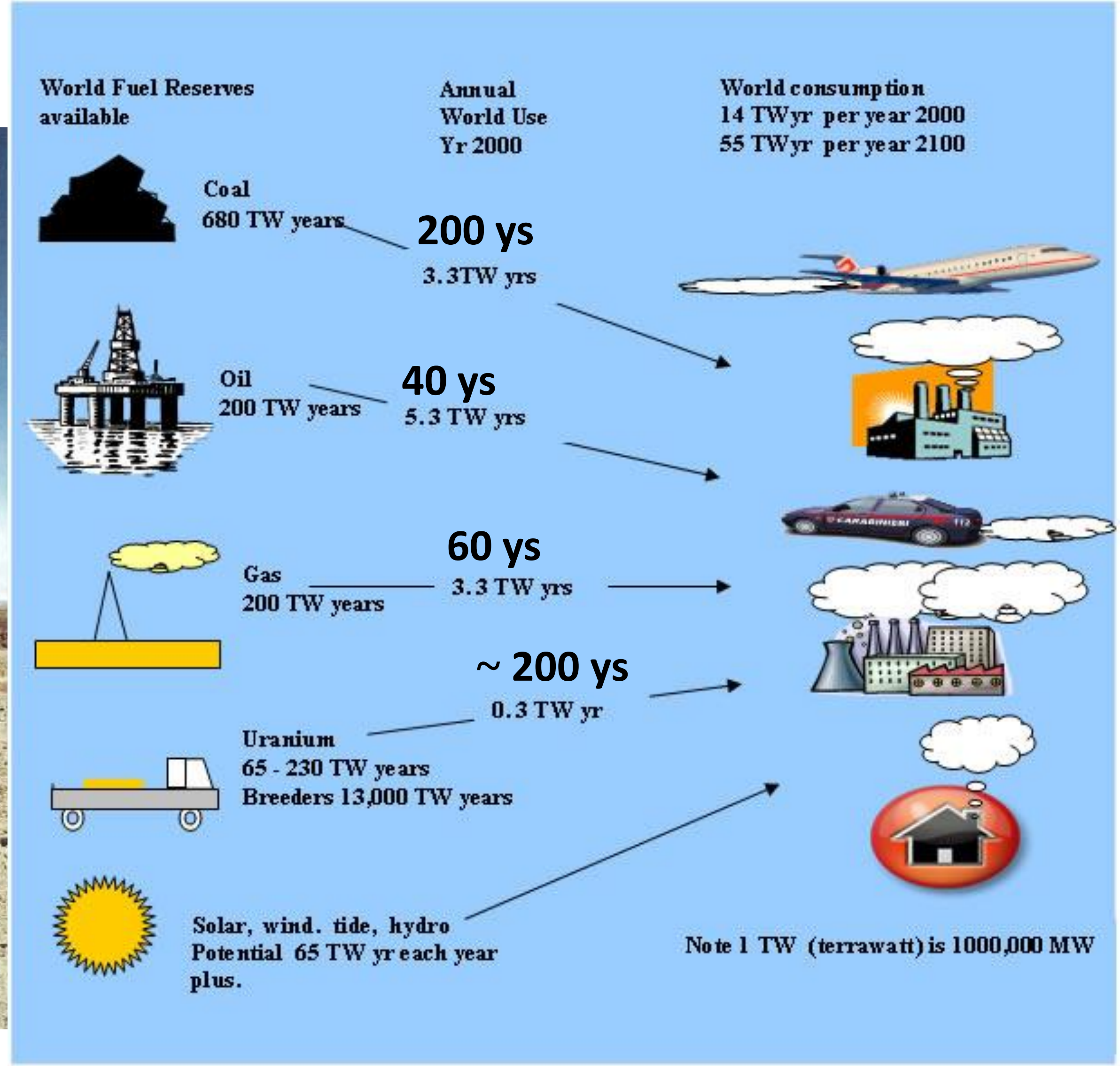
transportation

district heating



electricity

Secondly:



Search for alternatives

-Sustainable (ecological balance)

-Renewable (regenerate the sources)

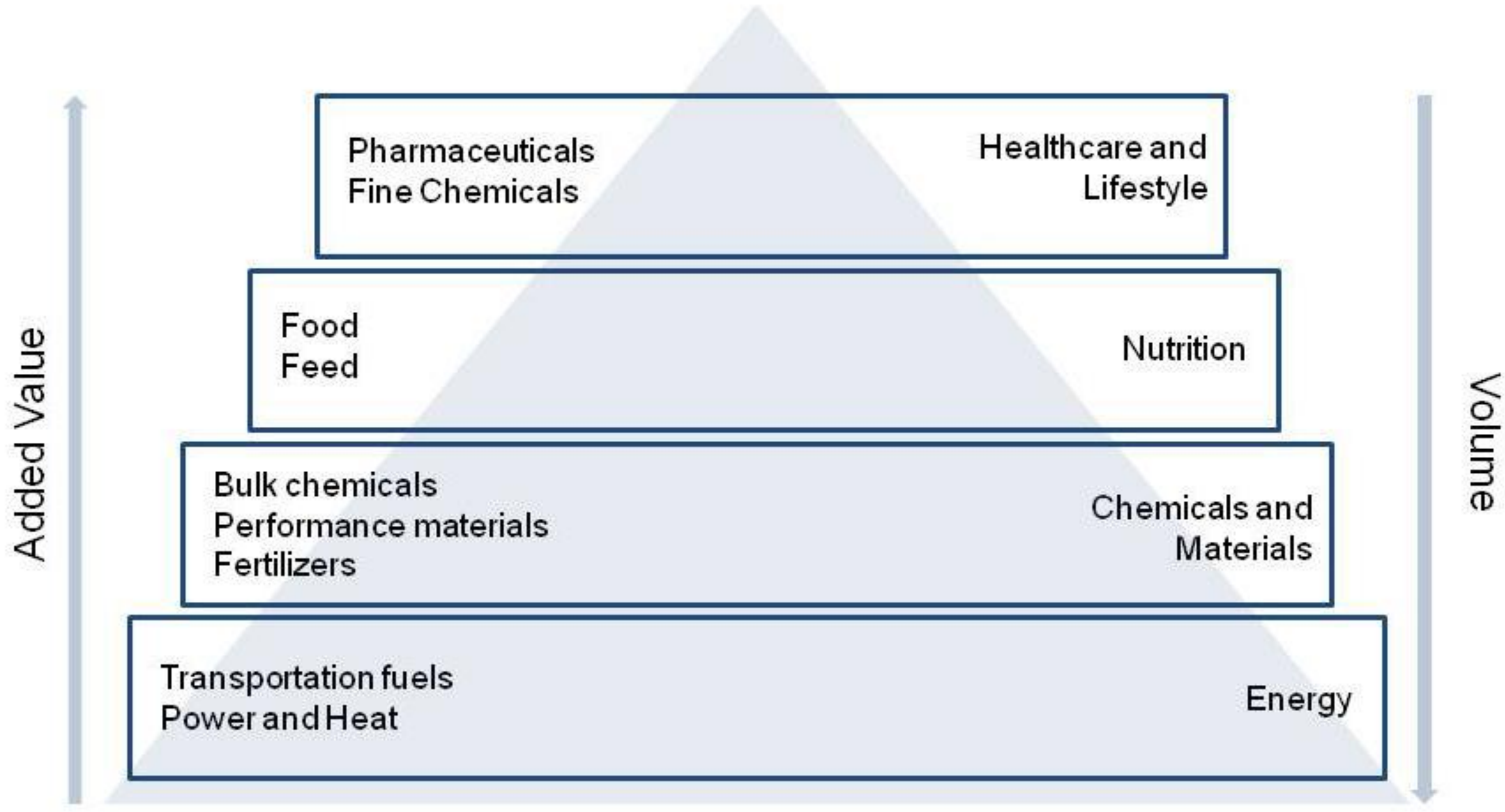
Biomass as carbon & energy source

- **Organic wastes (agriculture, domestic, industry)**
- **Energy crops (but: fertilization)**
- **Forest**
- **Algae & waterplants**

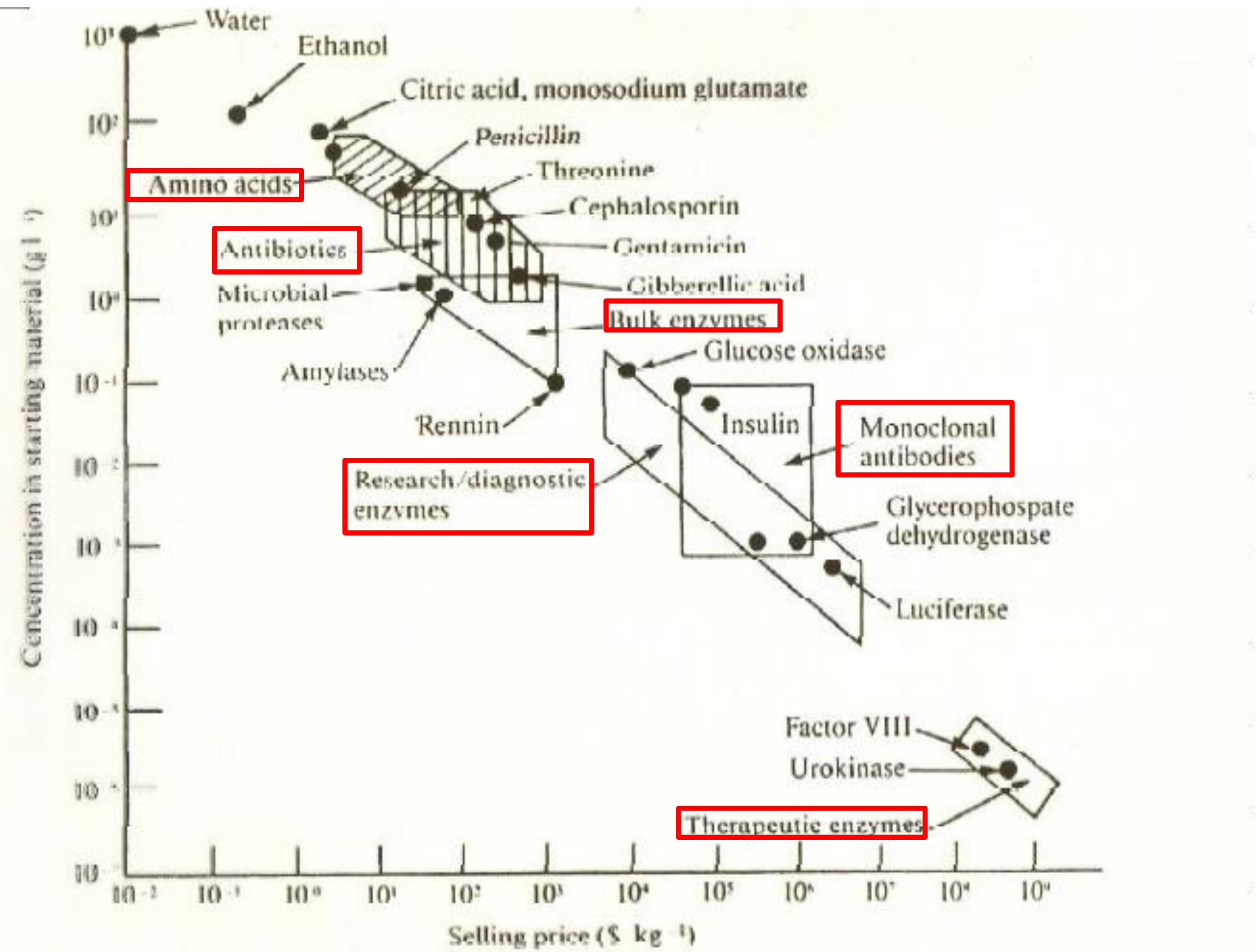
Lignocellulosic biomass:
- **Majority of biomass**
- **No competition with food or feed**




Biomass value-pyramid

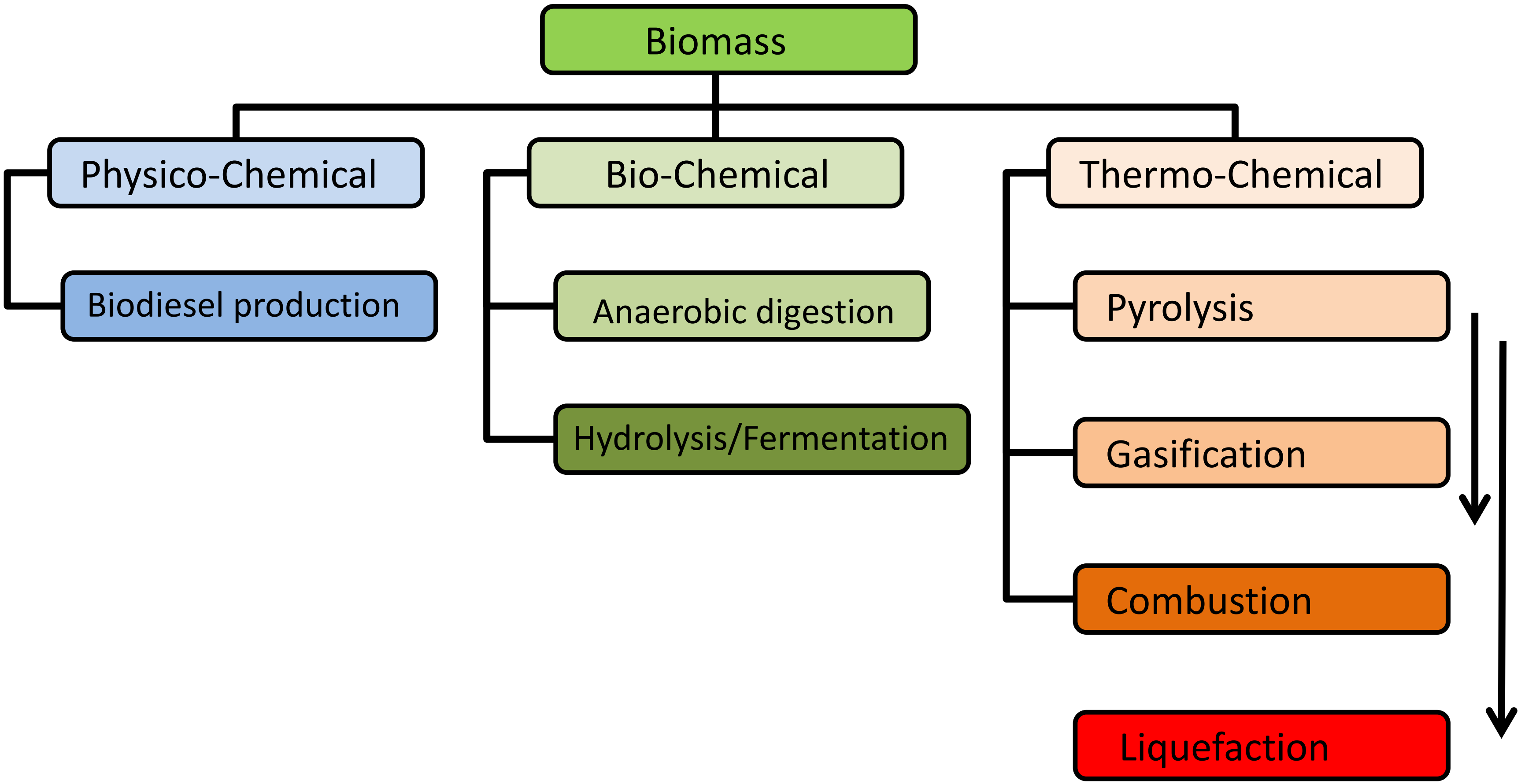


Selling prices versus Concentration



Relationship between product concentration in the broth or medium and final selling price of the product (Dwyer, 1984).

Biomass conversion technologies



For fermentations…

Sugar/starch <u>must</u> be replaced by **Lignocellulose**

Glucose/Maltose/Sucrose

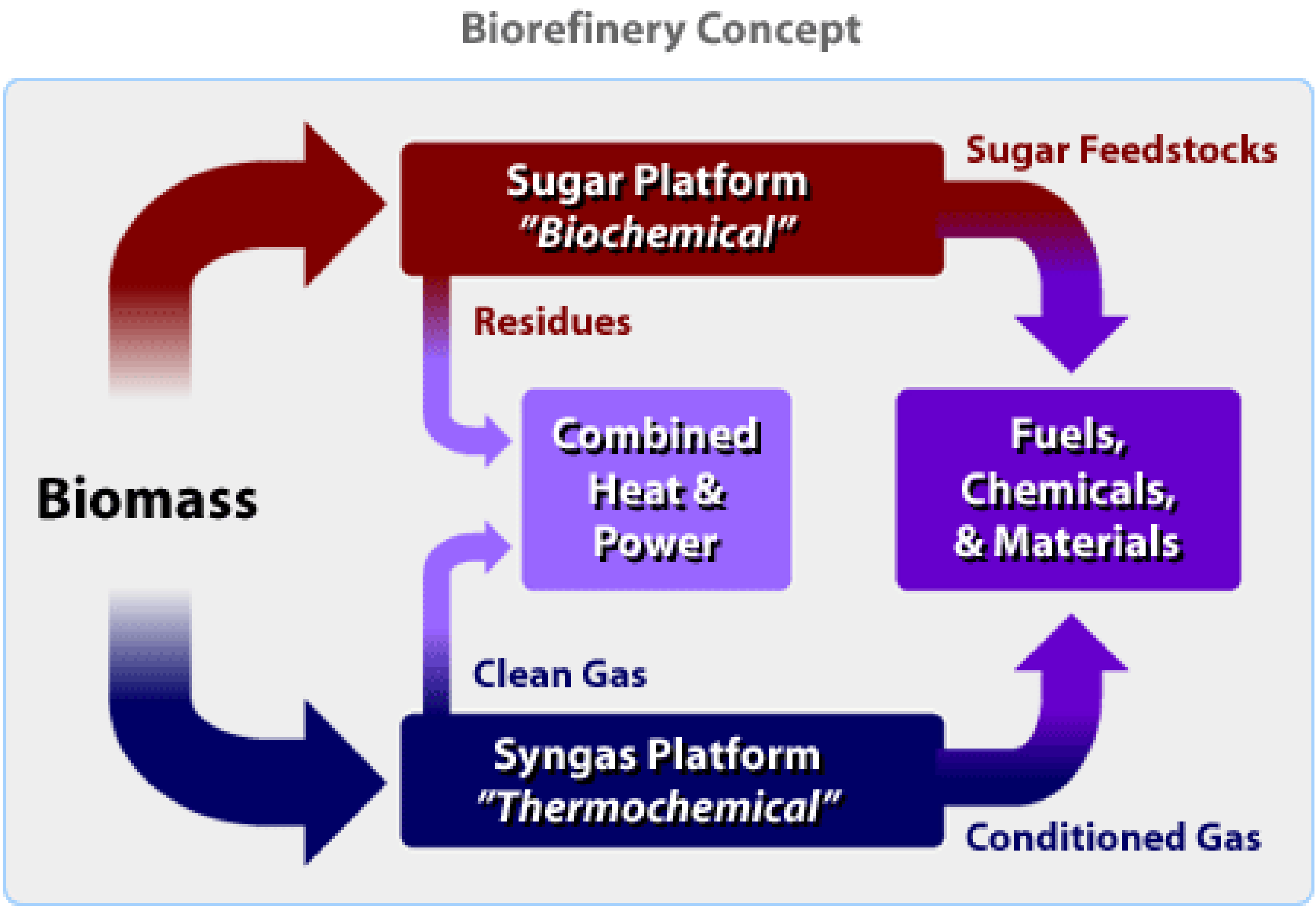
Glucose/Mannose/Galactose
Xylose/Arabinose

Lignocellulose composition

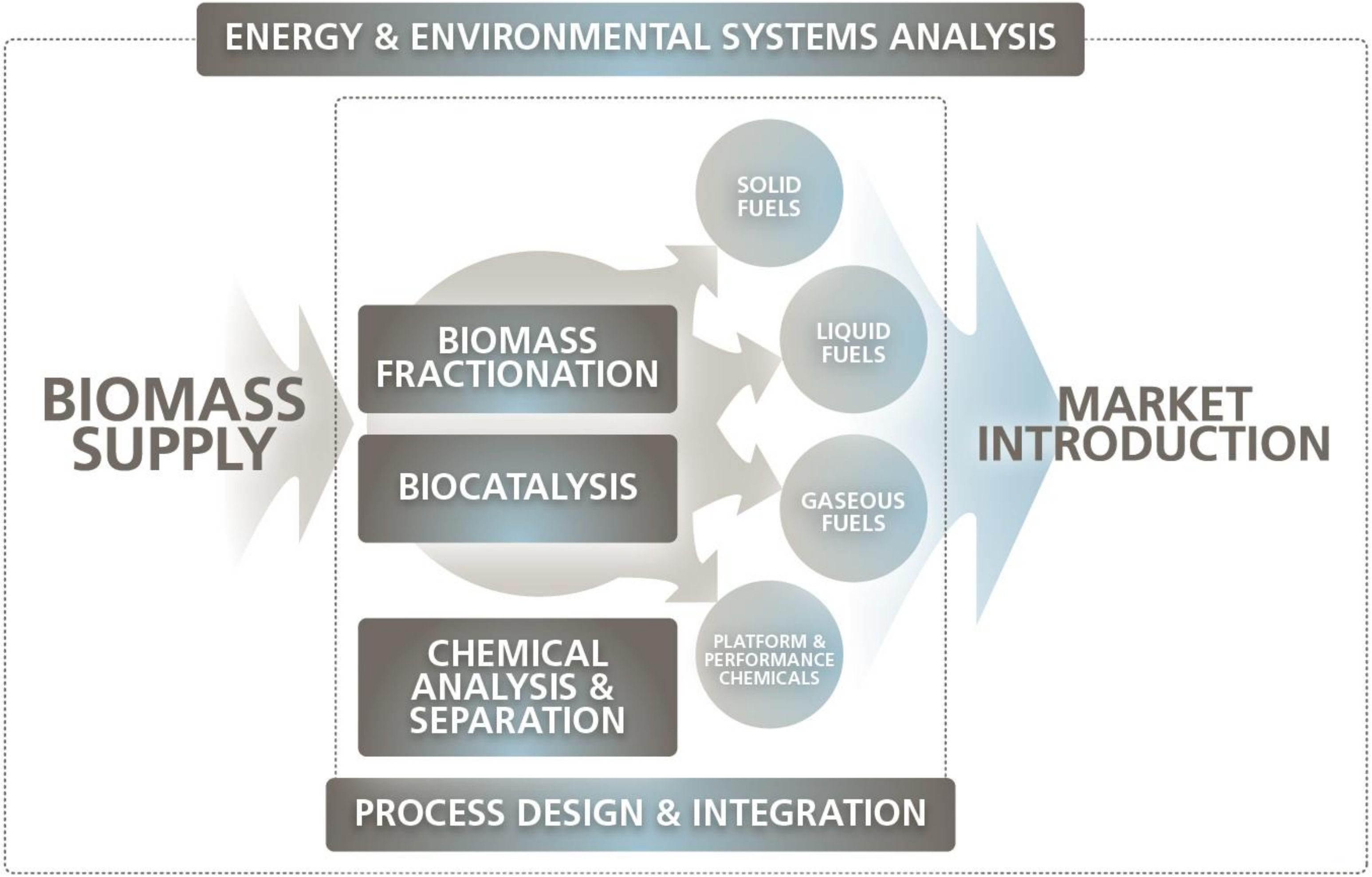
	"Sugars"	(hemi)cellulose	lignin (%)
• Brazil: sugarcane	47	34	9
• US: corn	25	65	7
• Sweden: wood	0	69	28

Raw material	Xylan (Xylose) (%)	Arabinan (Arabinose) (%)
Birch	19	-
Spruce	6	1
Wheat bran	19	15
Corn stover	19	3
Corn cob hulls	20	14
Grass	16	5

Hayn et al. (1993) In: Bioconversion of forest and agricultural plant residues. pp 33-72.


Biorefinery Concept
Biomass
Sugar Platform
"Biochemical"
Sugar Feedstocks
Residues
Combined Heat & Power
Fuels, Chemicals, & Materials
Clean Gas
Syngas Platform
"Thermochemical"
Conditioned Gas

LU Biofuels concept



http://www.lth.se/lu_biofuels/

http://www.lth.se/fileadmin/energiportalen/Energy_Portal/Files/Bioenergy_School_Book__hyperlinks_version_.pdf

Biorefinery in practice:

Depends on:
-What biomass source
-Where is it growing (location)

Net energy = Energy(out) – Energy(in)

For Sweden

But little bit of microbiology sofar...

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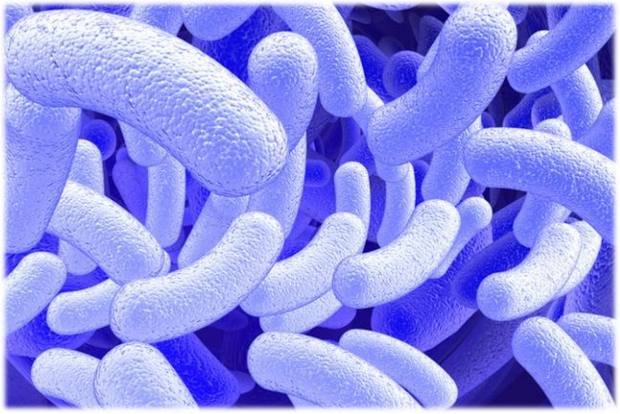
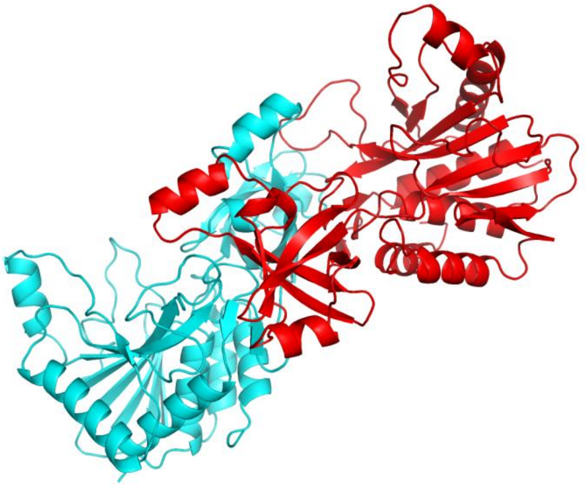
Biotechnology in process industry





Biotechnological processes

- Renewable raw materials
- Specific reactions

- "Neutral" pH
- "Low" temperatures
- "Low" ion strength (osmolarity)
- No toxic solvents
- HUGE quantities of H_2O (dilute streams)

Type of (micro-)organisms & products

Cells

- **Bacteria**
- **Yeasts**

Filamentous fungi
Plant cells
Mammalian cells
Insect cells

Products

Amino acids
Organic acids
Nucleotides
Vitamins
Polysaccharides
Antibiotics
Insulin & other hormones
Biosurfactants
Enzymes

Platform chemicals
(for chemical industry):

Succinate
1,3-Propanediol
Itaconic acid

Fuels (EtOH, butanol, CH_4, H_2)

Type of ‘fermentation’ processes

- **Biotransformation** (Chemical industry)
- **Biomass & enzyme production**
- **Food fermentations** (e.g. Beer/Vinegar/Dairy)
- **Fine chemicals** (e.g. Pharmacy)
- **Bulk chemicals** (e.g. Lactic acid/Ethanol)

Example of a biotransformation

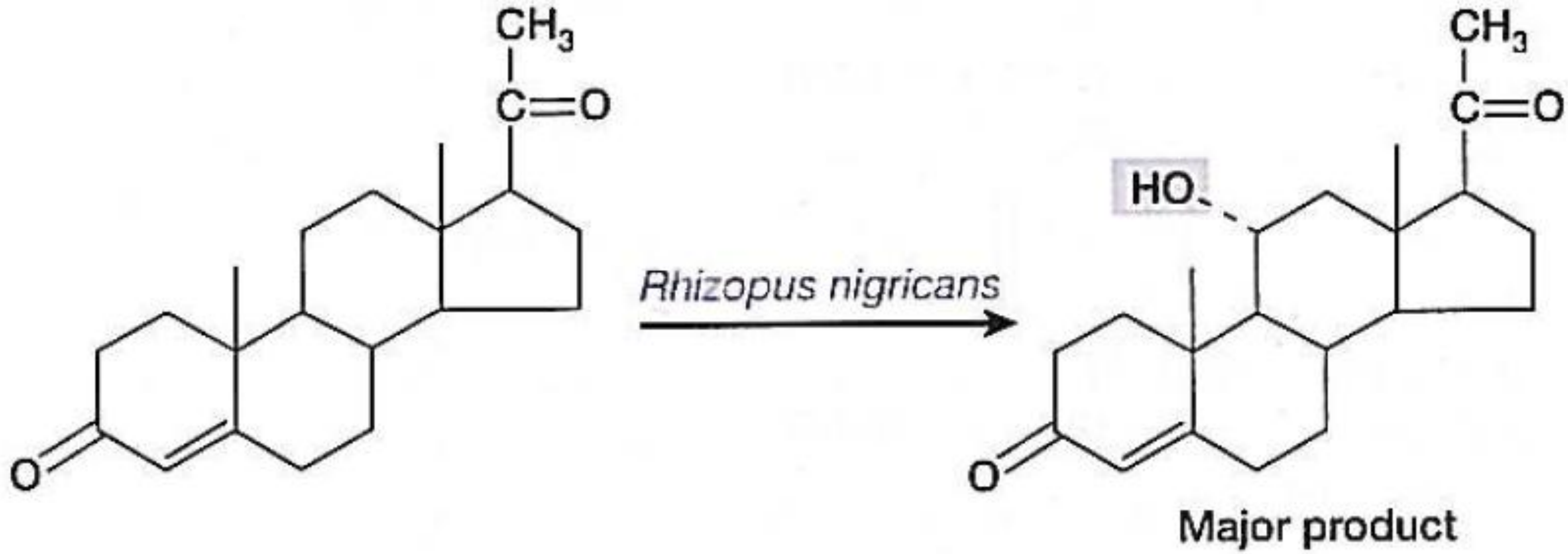


Figure 41.20 Biotransformation to Modify a Steroid. Hydroxylation of progesterone in the 11α position by *Rhizopus nigricans*. The steroid is dissolved in acetone before addition to the pregrown fungal culture.

Stereo-specific - no toxic solvents - no by-products - 1 step

Microbial biomass production

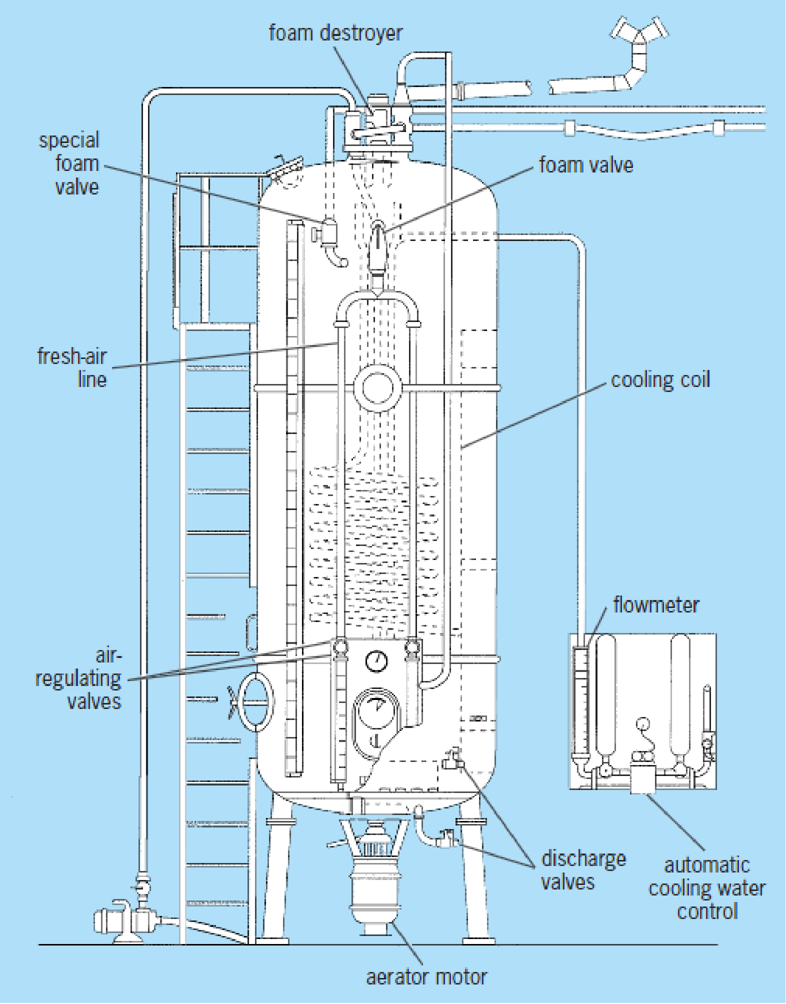
Yeast

Quorn

☞ How will you grow these organisms, if biomass is the product?

Example of enzyme application: Soap

Vinegar: food "fermentation"



$$C_2H_5OH + O_2 \rightarrow CH_3COOH + H_2O$$

***Acetobacter* & *Gluconobacter* species**

Frings Acetator: high productivity

Various definitions of “Fermentation”

1. Any process involving the mass culture of microorganisms (aerobic or anaerobic)
2. Any biological process that occurs in the absence of O_2
3. Food spoilage
4. The production of alcoholic beverages
5. Use of an organic substrate as the electron donor and acceptor
6. Use of an organic substrate as an electron donor,

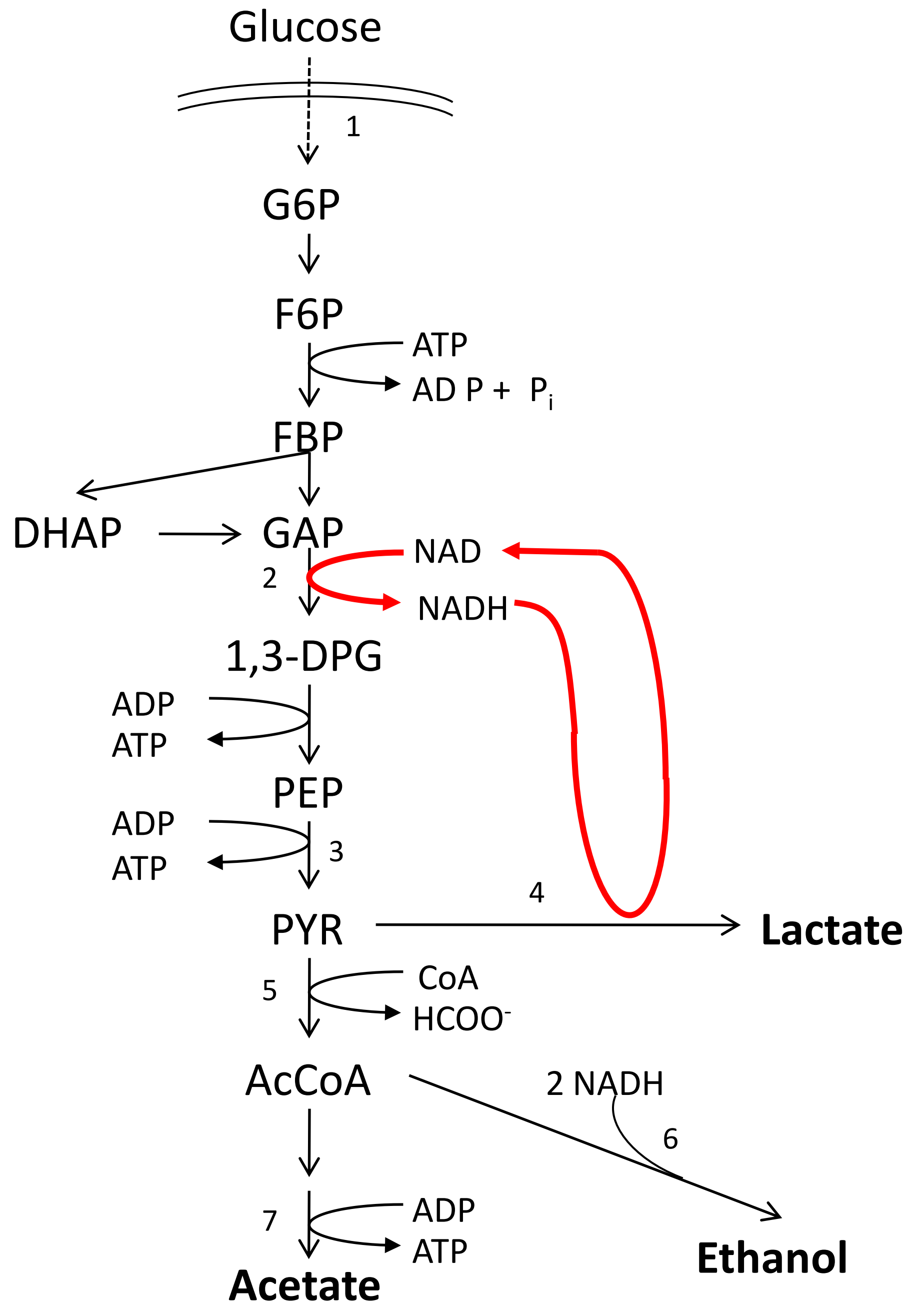
   and of the same partially degraded organic substrate as an electron acceptor
7. Growth dependent on substrate-level phosphorylation

Physiologist view on fermentation definition

For instance Lactococcus lactis:

Lactic acid production (Primary product)

Definitions 5 & 6 in previous slide



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Industrial fermentation media

Source	Raw Material
Carbon and energy	Molasses
	Whey
	Grains
	Agricultural wastes (corncobs)
Nitrogen	Corn-steep liquor
	Soybean meal
	Stick liquor (slaughterhouse products)
	Ammonia and ammonium salts
	Nitrates
	Distiller's solubles
Vitamins	Crude preparations of plant and animal products
Iron, trace salts	Crude inorganic chemicals
Buffers	Chalk or crude carbonates
	Fertilizer-grade phosphates
Antifoam agents	Higher alcohols
	Silicones
	Natural esters
	Lard and vegetable oils

Influences cost price of product!

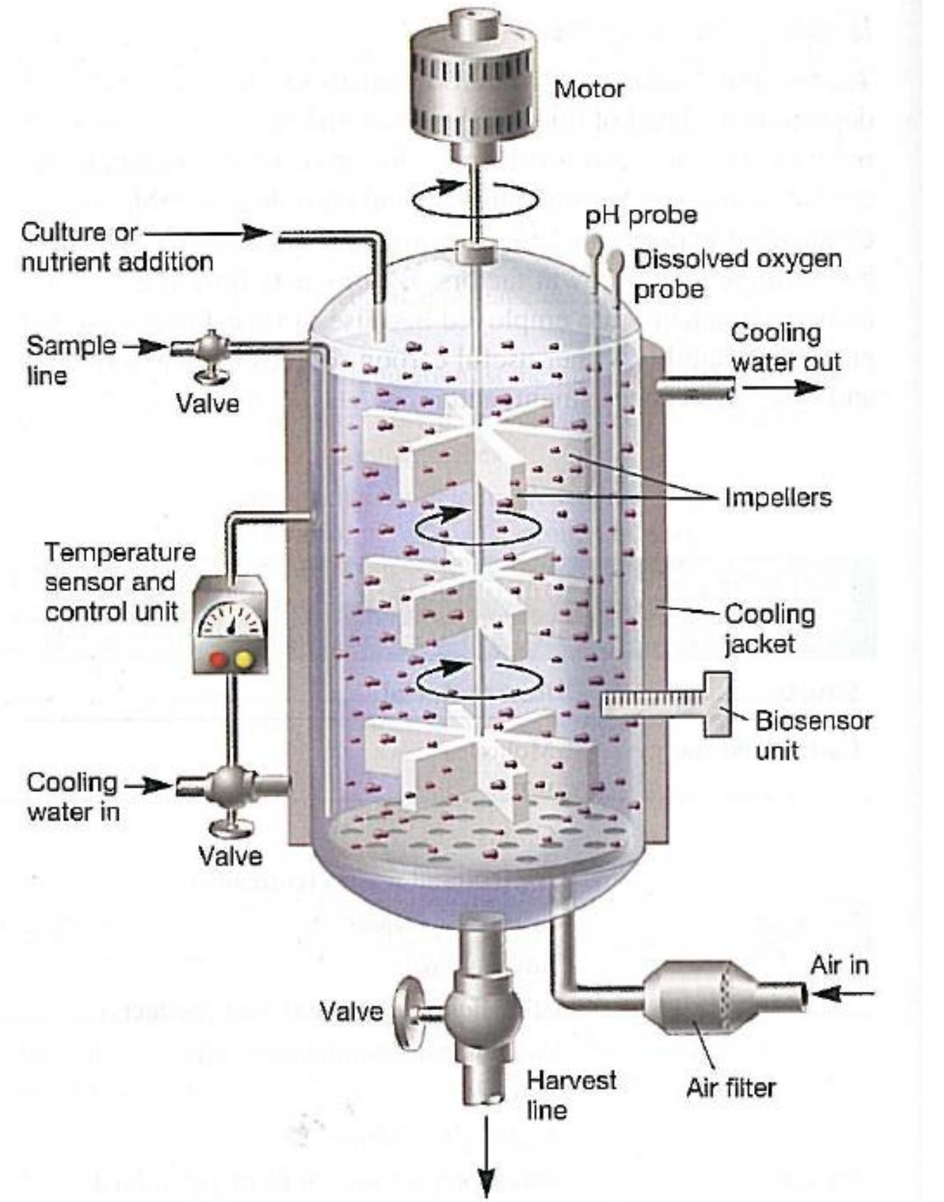
What about Yeast Extract?

Conventional industrial bioreactor:

Continuous stirred tank reactor

(CSTR)

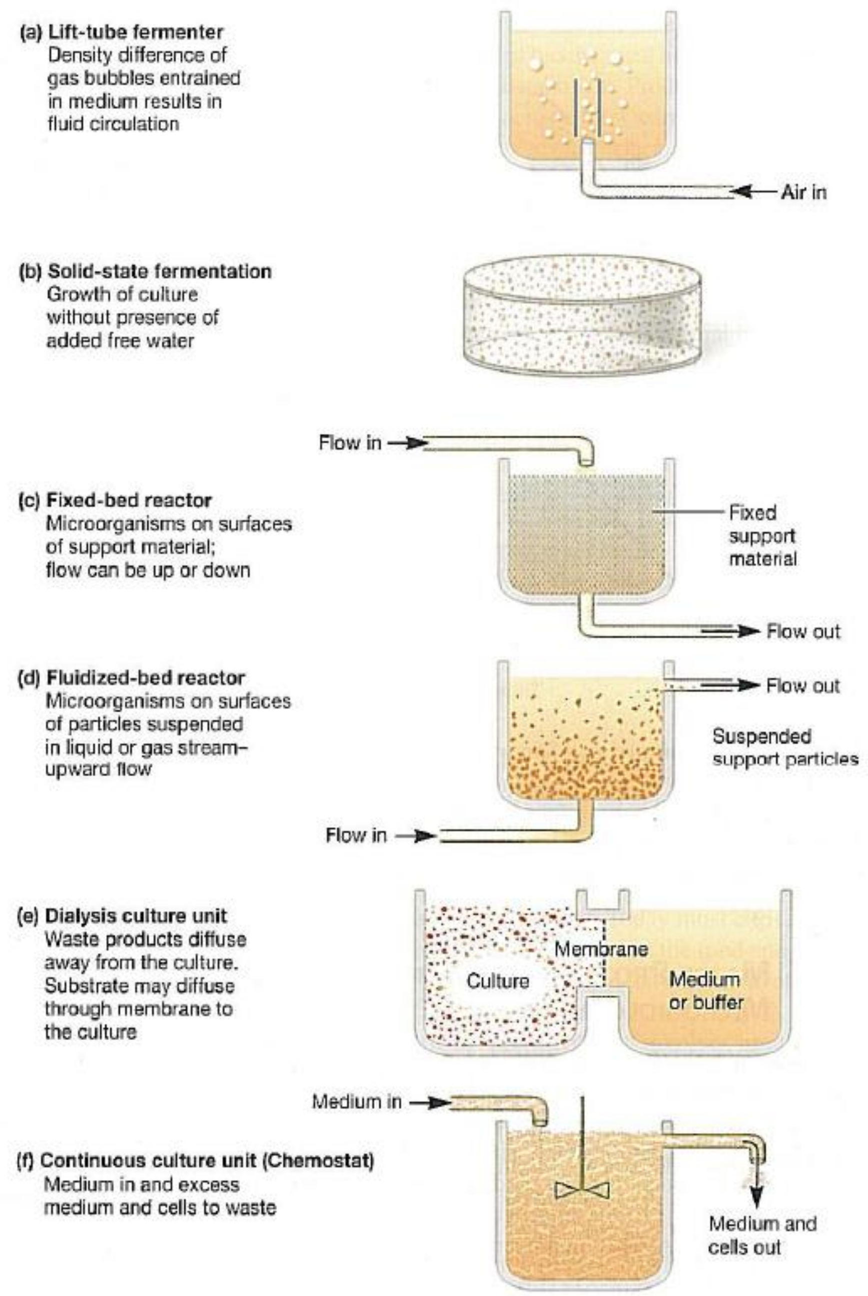
-Oxic & anoxic conditions
-Nutrient additions
-Sample taking
-Fermentation monitoring



(b)

Other ways of 'fermentation'

- May have lower operating costs
- Special growth conditions for maximized product formation



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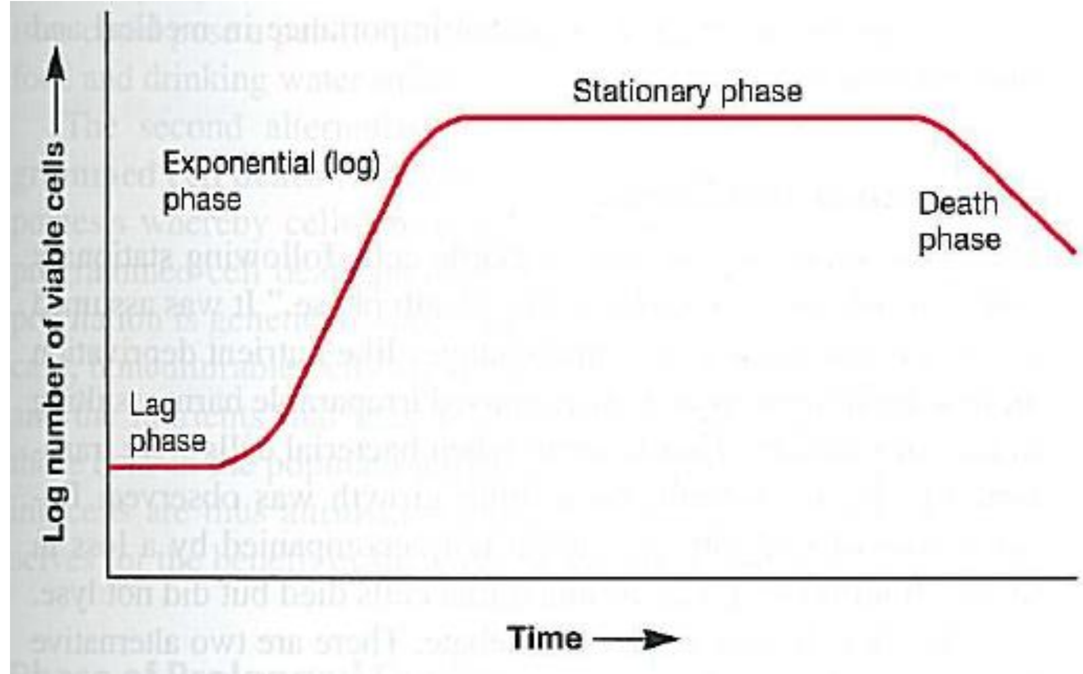
Microbial growth curve in batch mode



What happens in each phase?

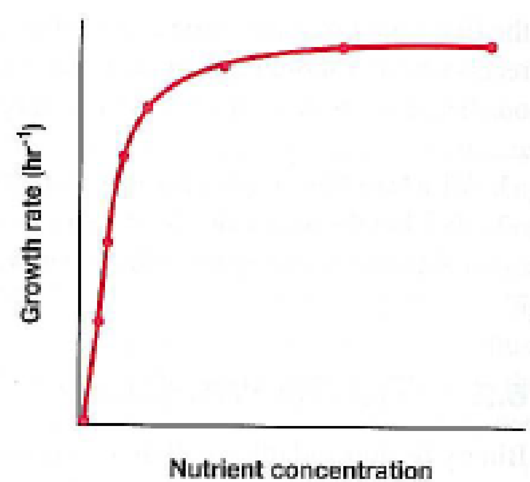
- Growth-limiting nutrient

- Maximum specific growth rate (μ_{MAX})

$$\mu = \frac{\mu_{max}[S]}{Ks + [S]}$$

Where,

μ	=	Specific growth rate
μ_{max}	=	Maximum specific growth rate
S	=	Substrate concentration
Ks	=	Specific substrate removal coefficient.



Exponential growth or Logarithmic growth

N_t = population at time t
N_0 = initial population
n = number of generations at time t

$$N_t = N_0 \cdot 2^n$$

$$\log N_t = \log N_0 + n \cdot \log 2$$

$$n = (\log N_t - \log N_0) / \log 2$$

k = n/t (number of generations per unit time)

If t = 1 h; then k = number of generations/h

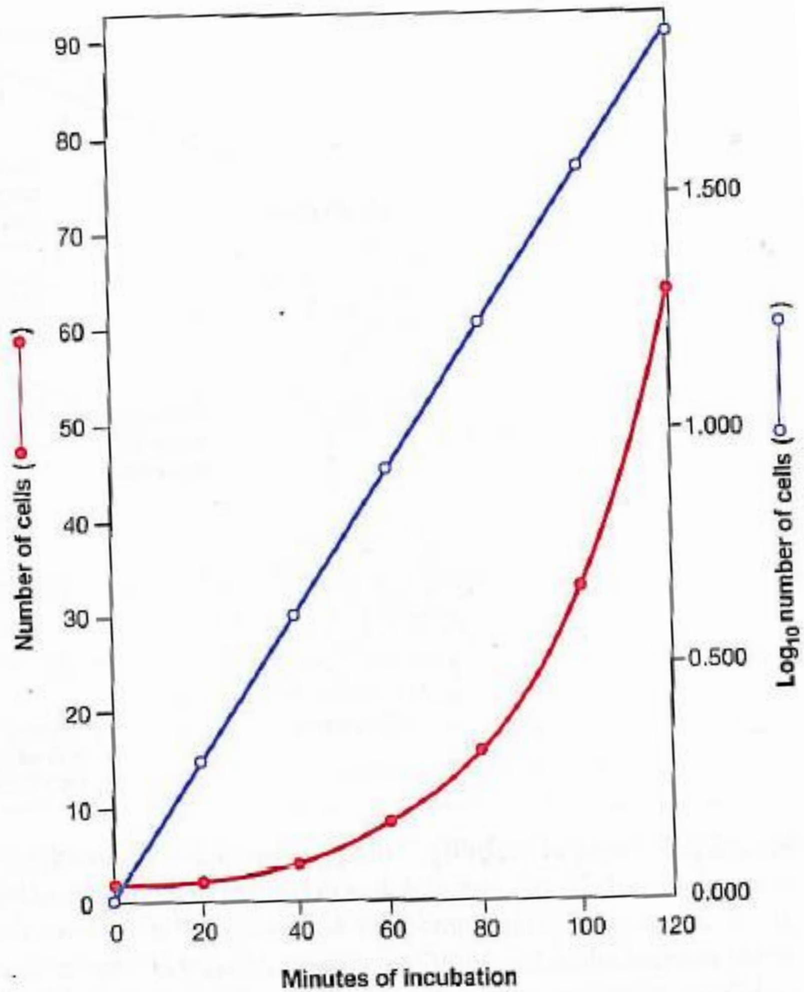
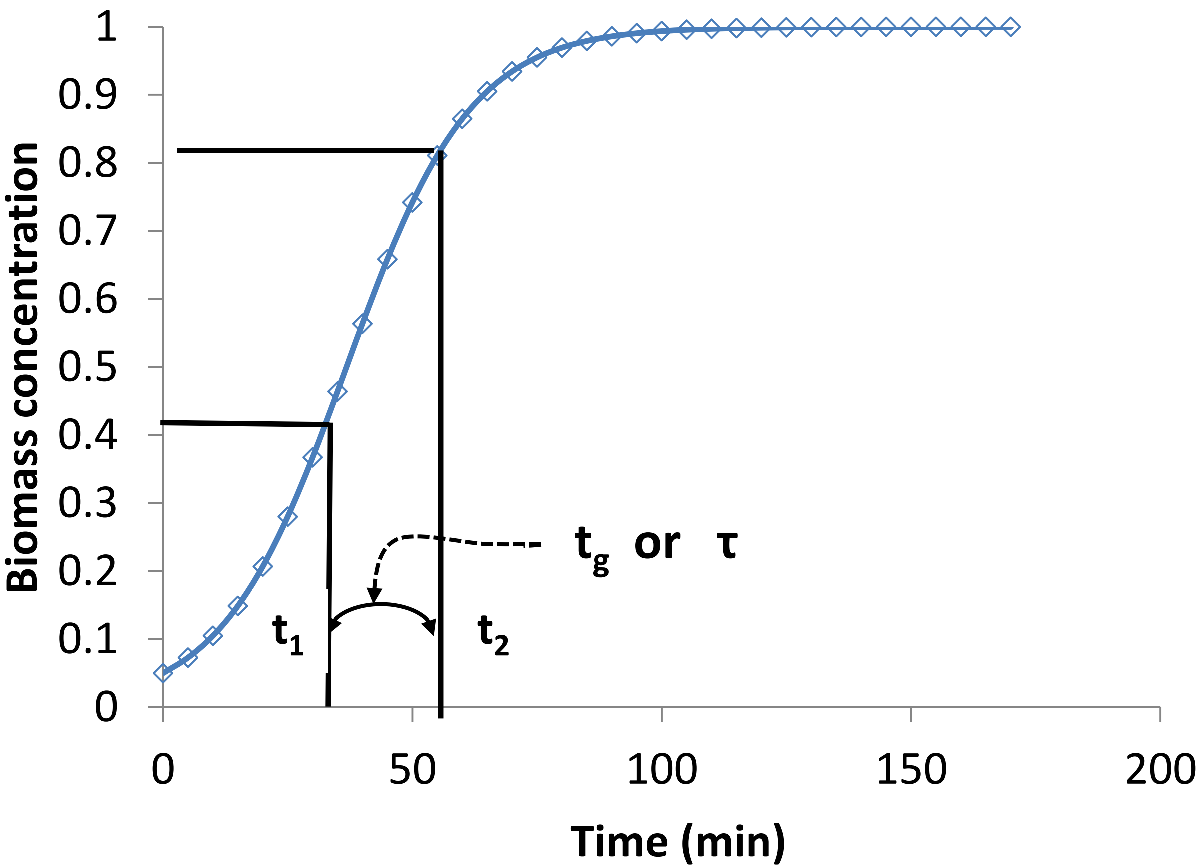


Figure 6.10 **Exponential Microbial Growth.** The data from table 6.1 for six generations of growth are plotted directly (•—•) and in the logarithmic form (∘—∘). The growth curve is exponential as shown by the linearity of the log plot.

Growth rate determination - alternative

In practice:



Generation or doubling time = t_g or τ

Theoretical:

$dX/dt = \mu X$

$\int 1/X \, dX = \int \mu \cdot dt$

$\ln (X - X_0) = \mu \cdot (t - t_0)$ with $t_0 = 0$

$\ln (X/X_0) = \mu \cdot t$ (Eq. 1)

$X/X_0 = \exp(\mu \cdot t)$

$X = X_0 \cdot \exp(\mu \cdot t)$

Generation time (t_g) when:

$X = 2 \cdot X_0$ add this to Eq. 1:

$\ln (2 \cdot X_0 / X_0) = \mu \cdot t_g$

$\ln 2 = \mu \cdot t_g$ or:

$t_g = \ln 2/\mu$

Continuous culture

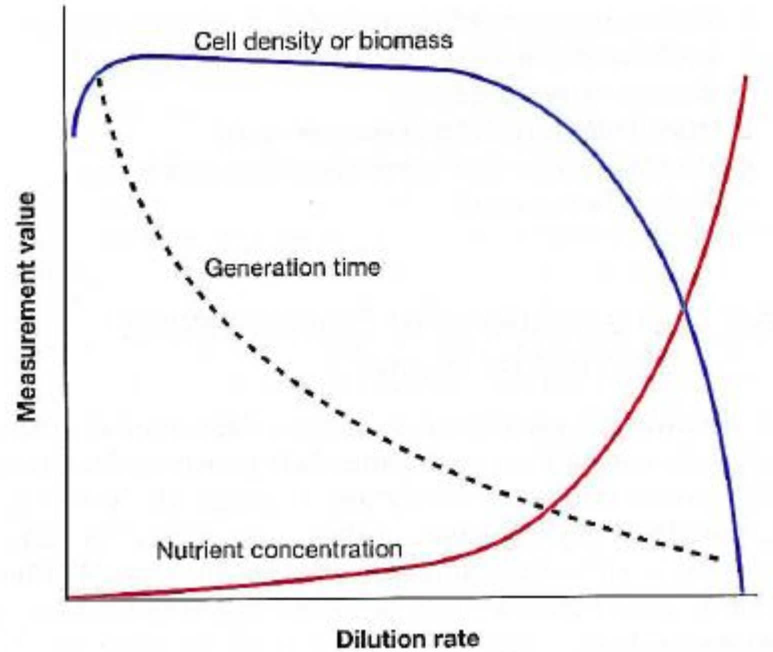
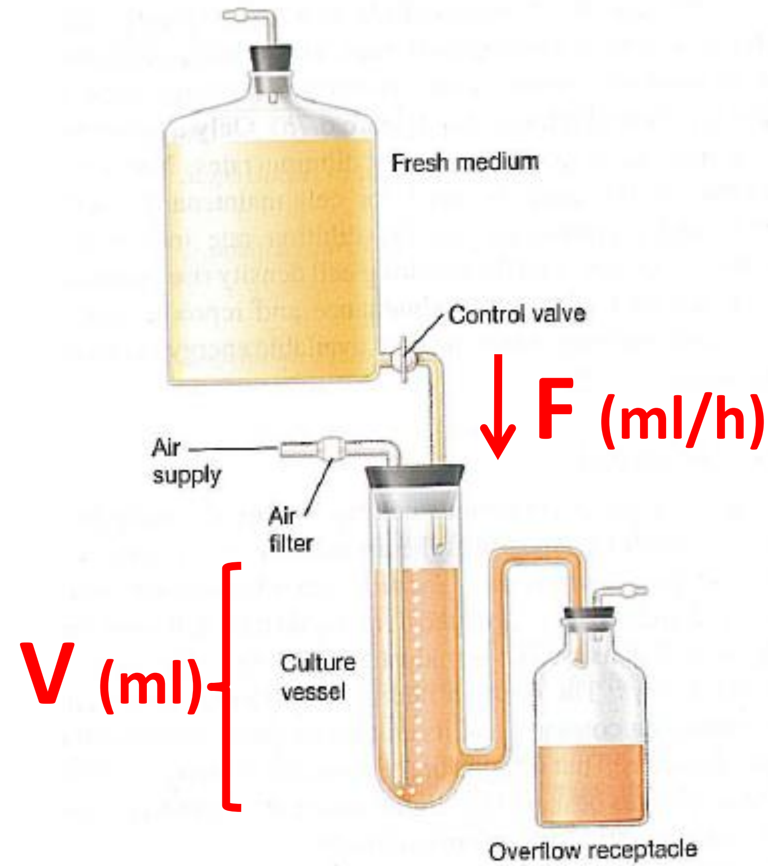


Figure 6.17 Chemostat Dilution Rate and Microbial Growth. The effects of changing the dilution rate in a chemostat.



Change = In + Accumulation - Out

D= f/V = dilution rate (1/h)

$dX/dt = 0 + \mu X - DX$

$dX/dt = 0 = (\mu - D)X$

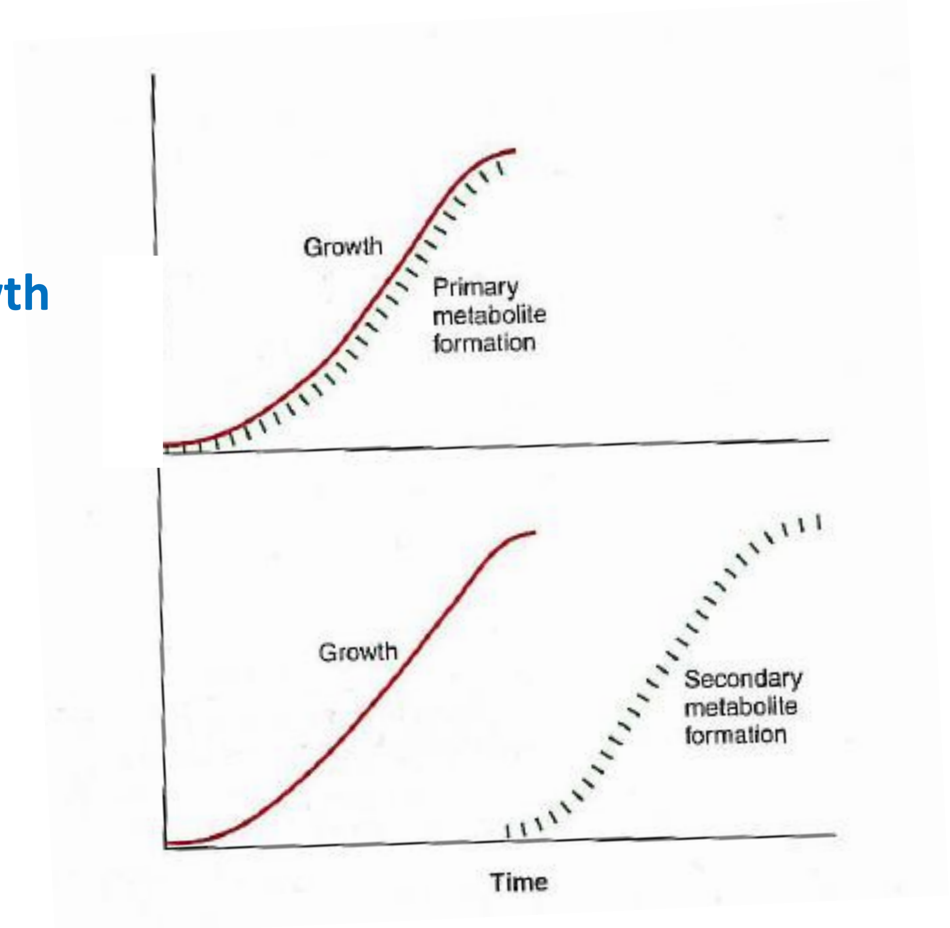
Steady state: Change = 0

Thus $\mu = D$

Primary & Secondary metabolites

Primary metabolite:
Produced during growth

Secondary metabolite:
Produced after completion of growth
Or after growth limitation



Depends on:
- Organism
- Desired product

Secondary metabolite production (Example 1)

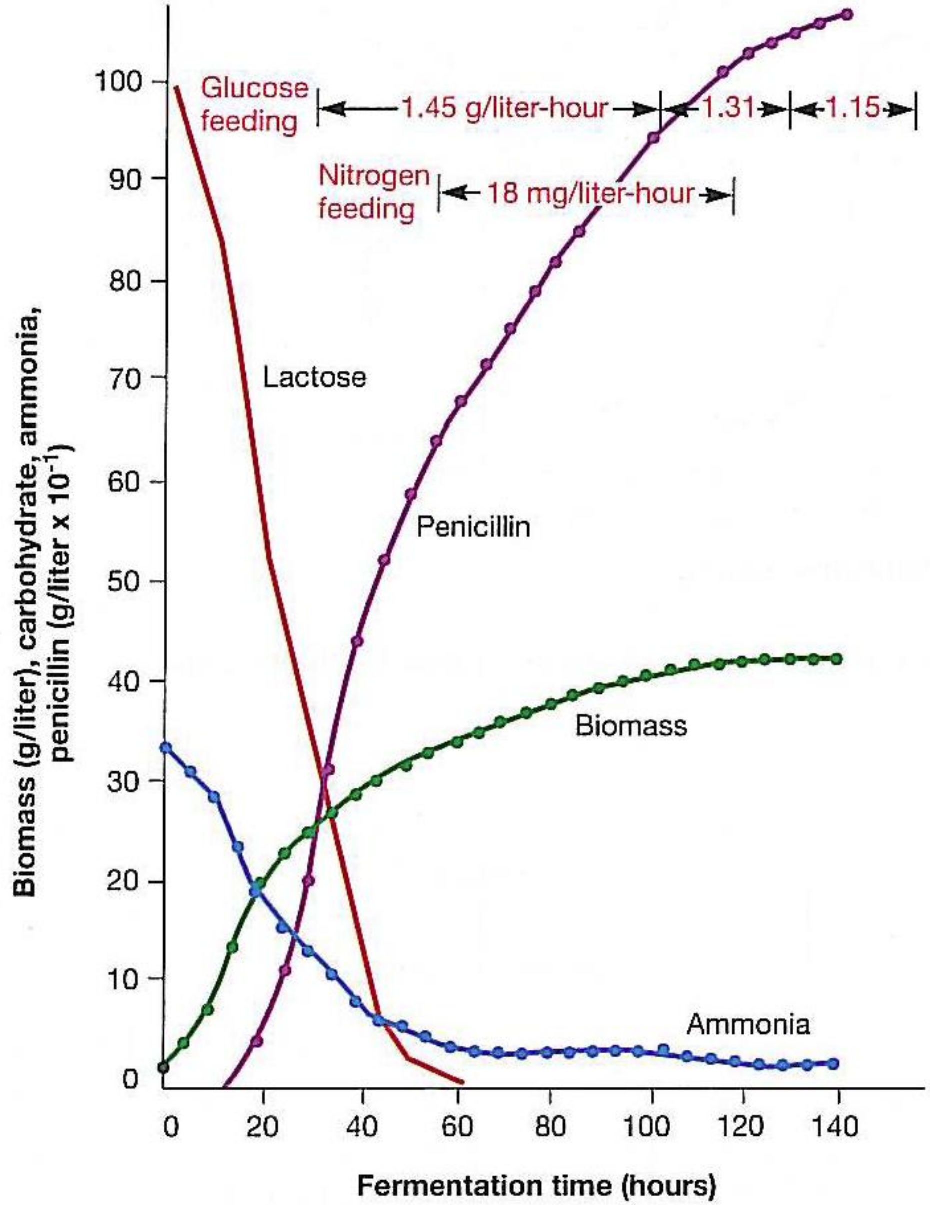
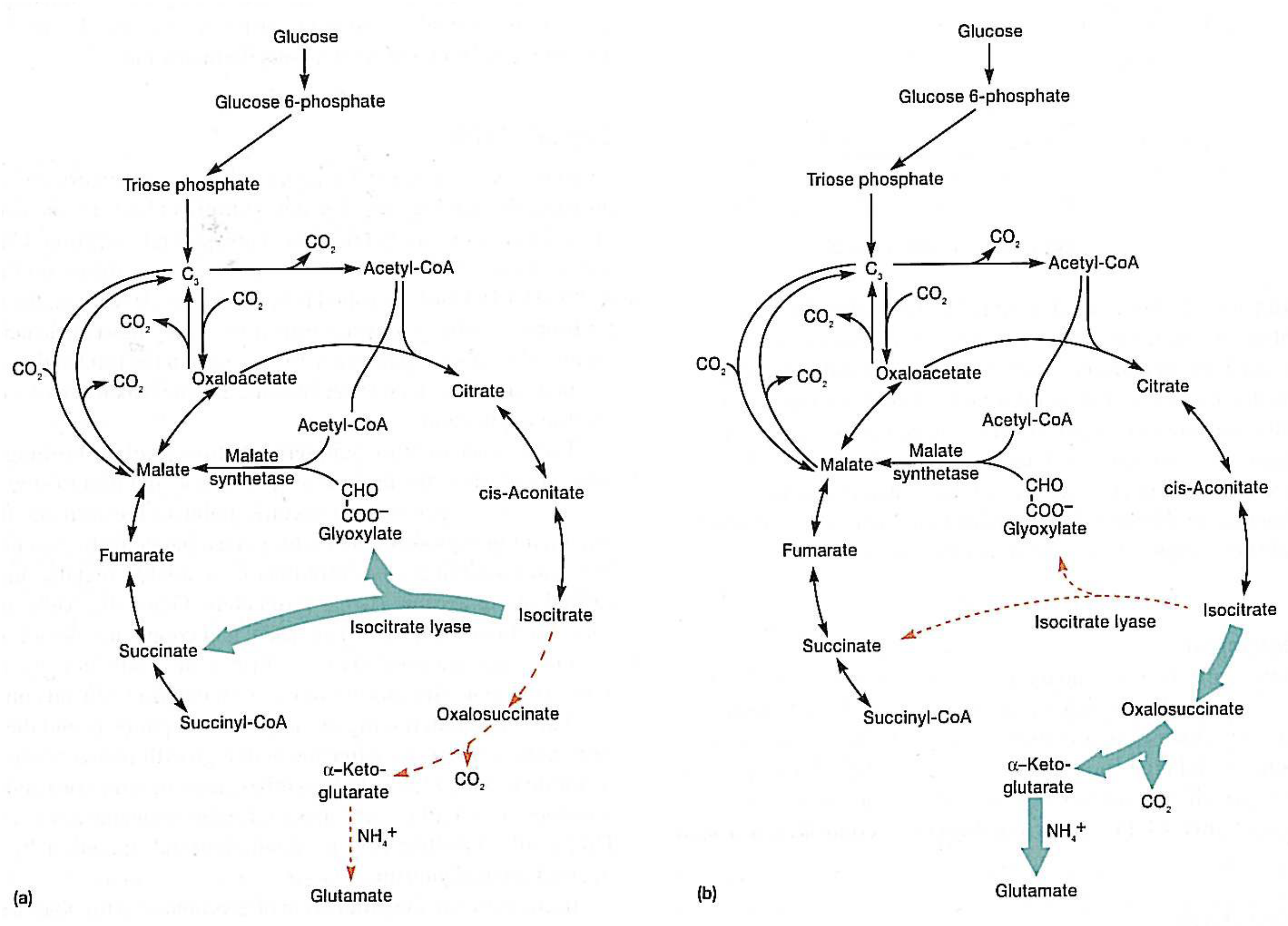


Figure 41.16 Penicillin Fermentation Involves Precise Control of Nutrients. The synthesis of penicillin begins when nitrogen from ammonia becomes limiting. After most of the lactose (a slowly catabolized disaccharide) has been degraded, glucose (a rapidly used monosaccharide) is added along with a low level of nitrogen. This stimulates maximum transformation of the carbon sources to penicillin. The scale factor is presented using the convention recommended by the ASM. That is, a number on the axis should be multiplied by 0.10 to obtain the true value.

Secondary metabolite: Glutamic acid (Example 2)



Growth: using glyoxylate bypass (Biosynthesis precursors)

After growth: production of glutamate

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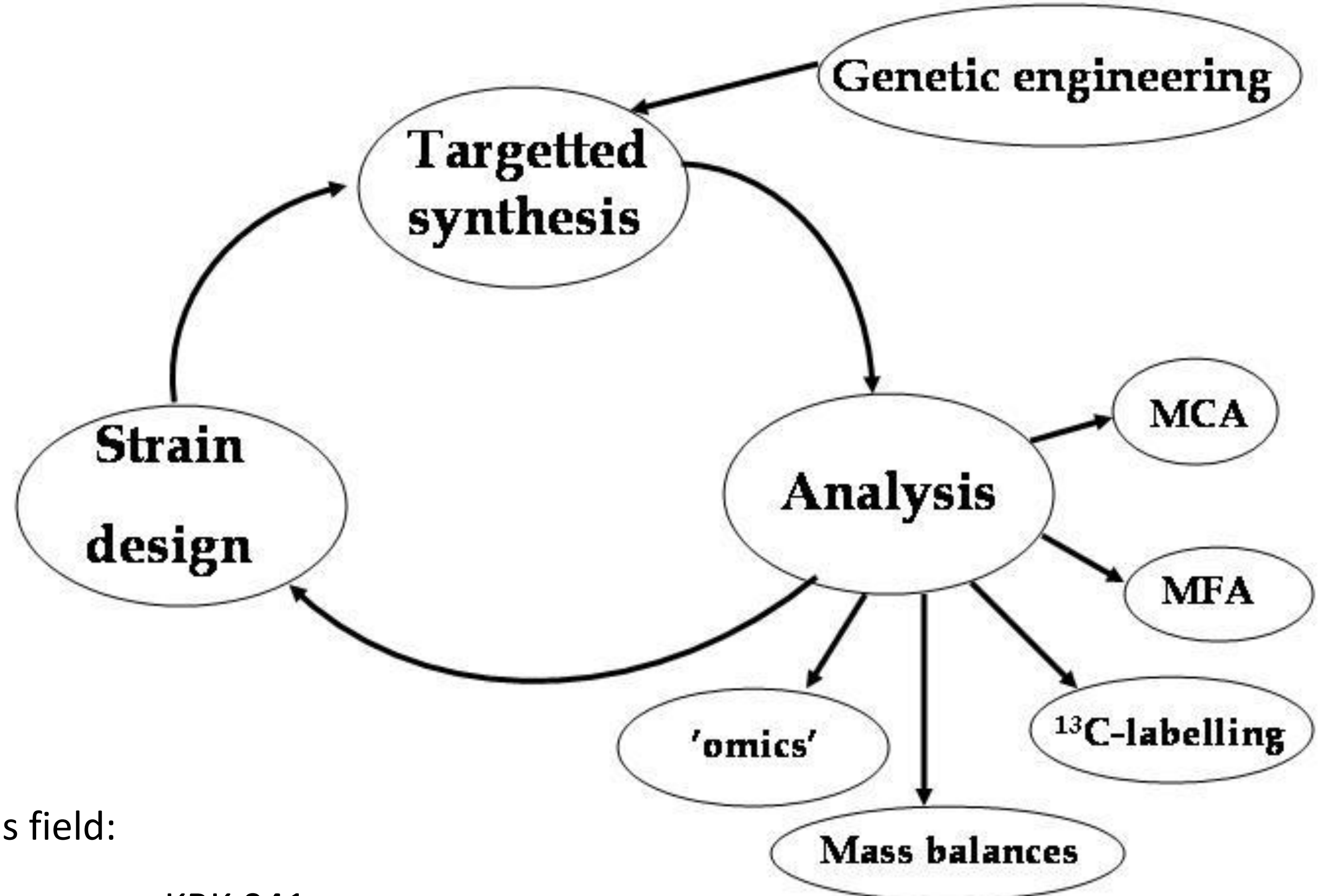
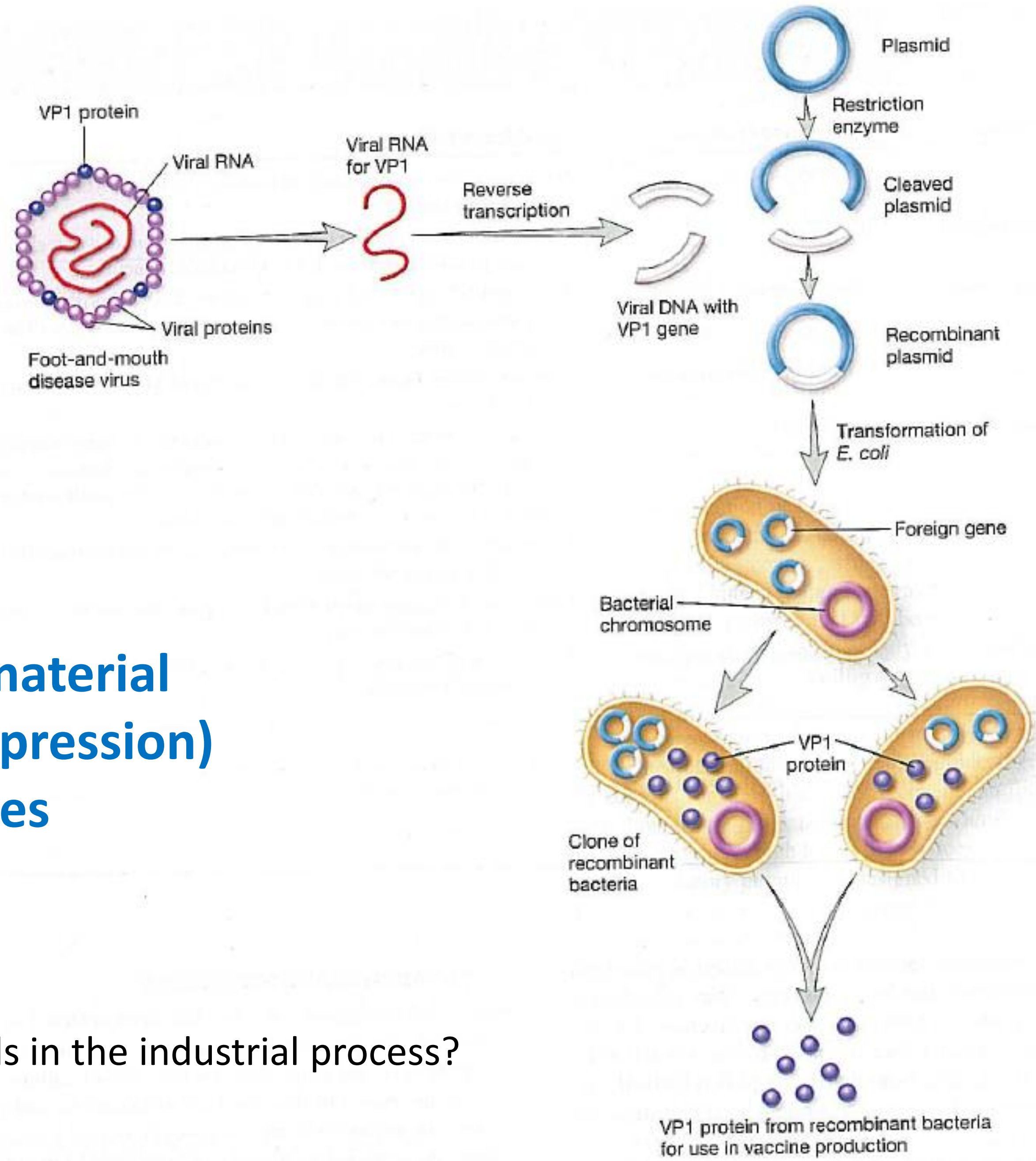
Principle of Metabolic Engineering



Courses in this field:

Gene technology, KBK 041
Metabolic Engineering, KMB 040

Principle of genetic engineering



- Bring in foreign DNA material (heterologous gene expression)
- Overexpress own genes
- Knock out genes

Why is it not wise to use plasmids in the industrial process?

Table 41.7 Combinatorial Biology in Biotechnology: The Expression of Genes in Other Organisms to Improve Processes and Products

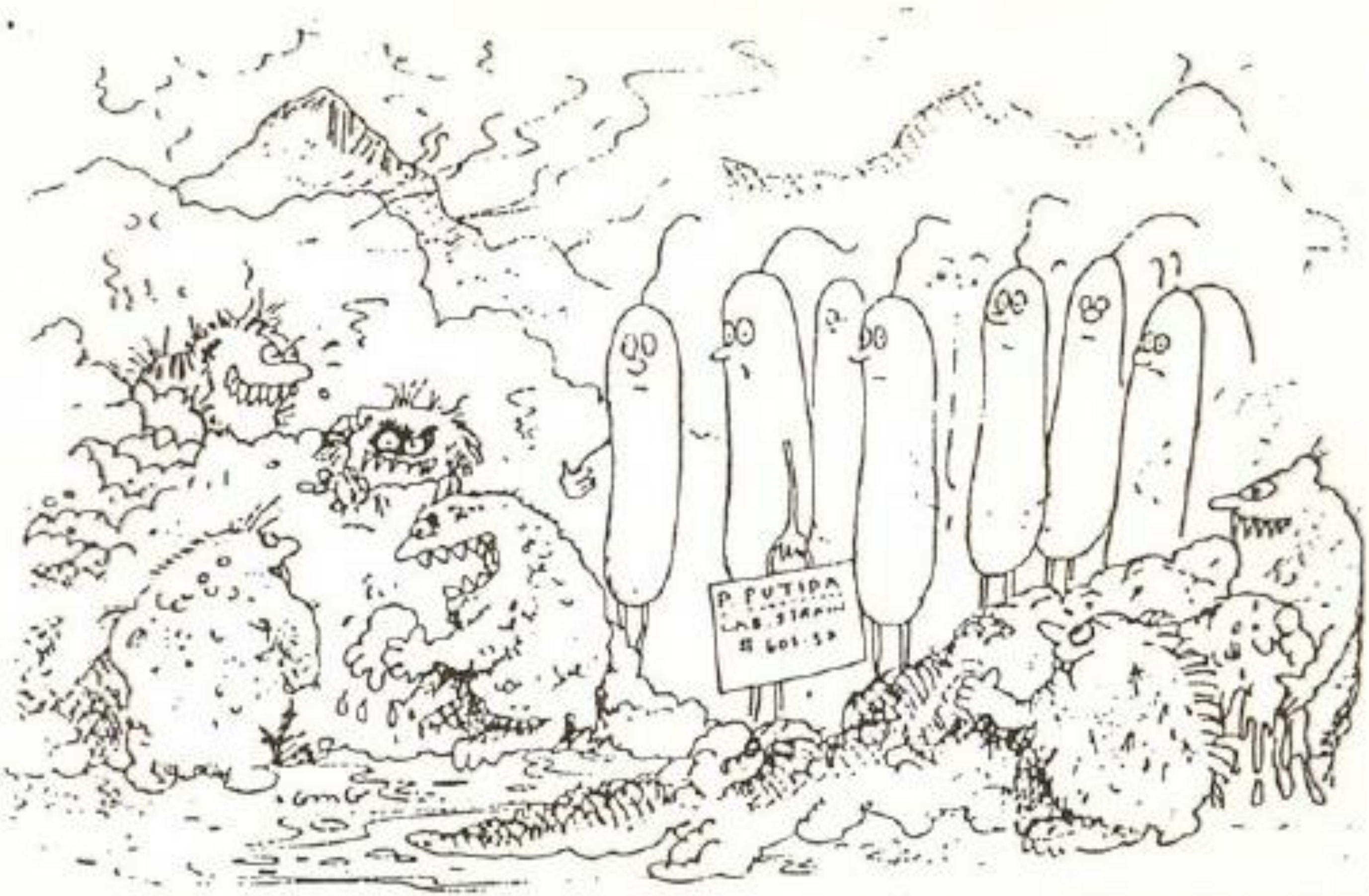
Property or Product Transferred	Microorganism Used	Combinatorial Process
Ethanol production	*Escherichia coli*	Integration of pyruvate decarboxylase and alcohol dehydrogenase II from *Zymomonas mobilis.*
1,3-Propanediol production	*E. coli*	Introduction of genes from the *Klebsiella pneumoniae dha* region into *E. coli* makes possible anaerobic 1,3-propanediol production.
Cephalosporin precursor synthesis	*Penicillium chrysogenum*	Production of 7-ADC and 7-ADCA[a] precursors by incorporation of the expandase gene of *Cephalosoporin acremonium* into *Penicillium* by transformation.
Lactic acid production	*Saccharomyces cerevisiae*	A muscle bovine lactate dehydrogenase gene (LDH-A) expressed in *S. cerevisiae.*
Xylitol production	*S. cerevisiae*	95% xylitol conversion from xylose was obtained by transforming the *XYLI* gene of *Pichia stipitis* encoding a xylose reductase into *S. cerevisiae,* making this organism an efficient organism for the production of xylitol, which serves as a sweetener in the food industry.
Creatininase[b]	*E. coli*	Expression of the creatininase gene from *Pseudomonas putida* R565. Gene inserted in a plasmid vector.
Pediocin[c]	*S. cerevisiae*	Expression of bacteriocin from *Pediococcus acidilactici* in *S. cerevisiae* to inhibit wine contaminants.
Acetone and butanol production	*Clostridium acetobutylicum*	Introduction of a shuttle vector into *C. acetobutylicum* results in acetone and butanol formation.

[a]7-ACA = 7-aminocephalosporanic acid; 7-ADCA = 7-aminodecacetoxycephalosporonic acid.

[b]T.-Y. Tang; C.-J. Wen; and W.-H. Liu. 2000. Expression of the creatininase gene from *Pseudomonas putida* RS65 in *Escherichia coli. J. Ind. Microbiol. Biotechnol.* 24:2–6.

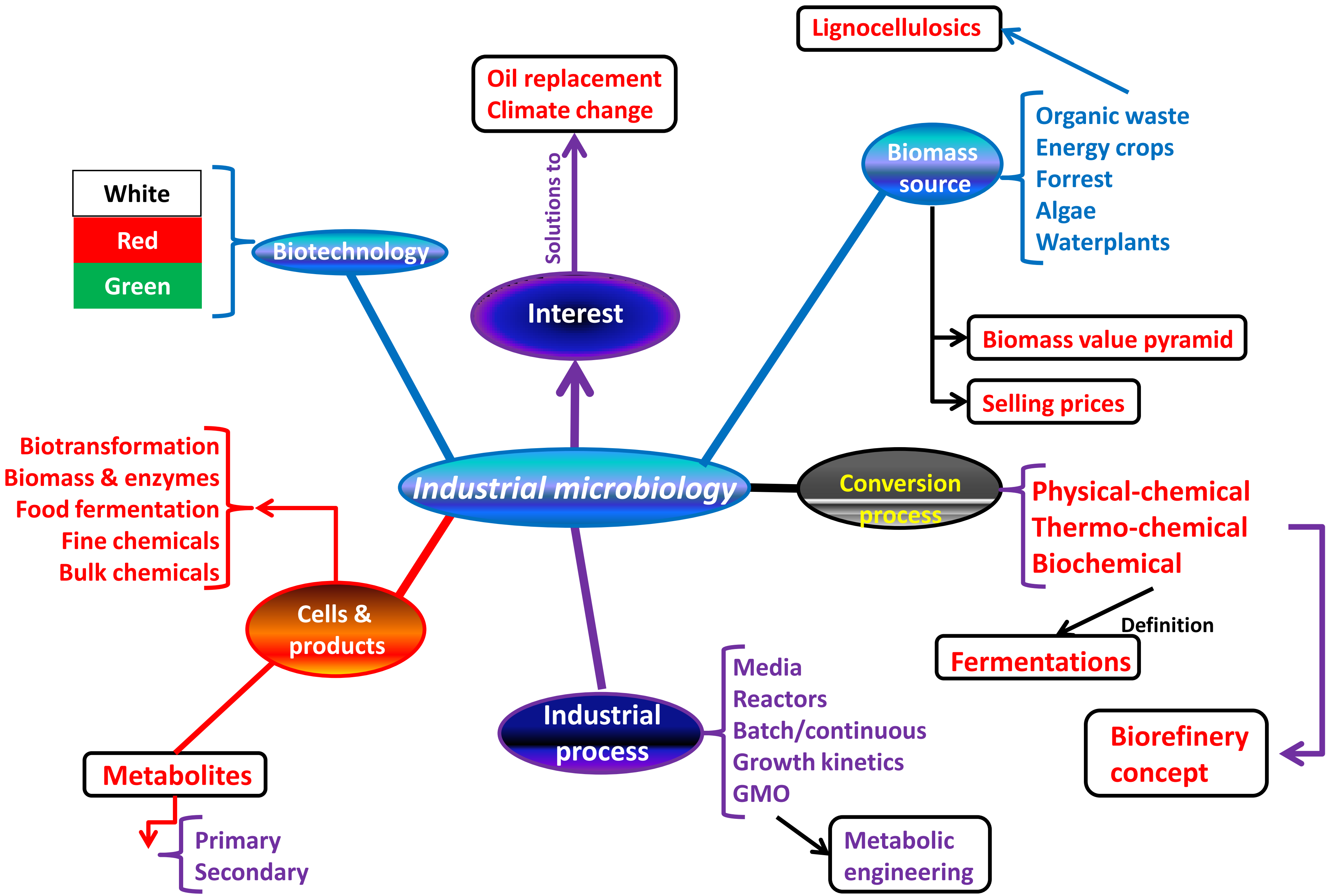
[c]H. Schoeman; M. A. Vivier; M. DuToit; L. M. Y. Dicks; and I. S. Pretorius. 1999. The development of bactericidal yeast strains by expressing the *Pediococcus acidilactici* pediocin gene (pedA) in *Saccharomyces cerevisiae. Yeast* 15:647–656.

Adapted from S. Ostergaard; L. Olsson; and J. Nielson. 2000. Metabolic engineering of *Saccharomyces cerevisiae.* Microbiol. Mol. Biol. Rev. 64(1):34–50.



"Oh dear! I didn't realize 'in the field' would be like this! We should have stayed in the laboratory."

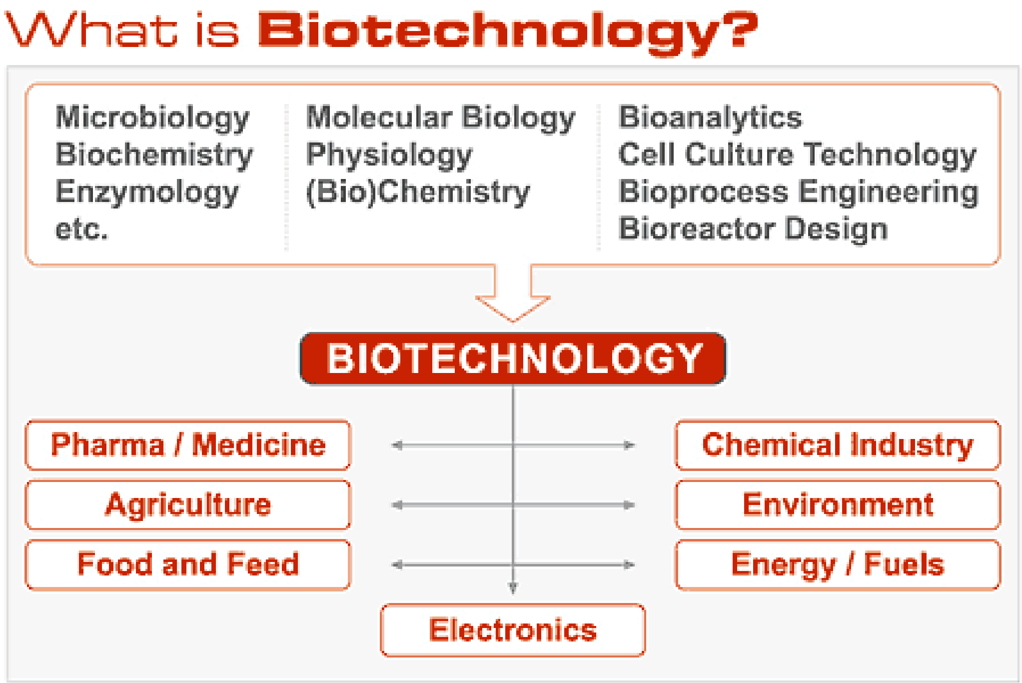
Mindmap



Lignocellulosics
Oil replacement
Climate change
Solutions to
Organic waste
Energy crops
Forrest
Algae
Waterplants
Biomass source
White
Red
Green
Biotechnology
Interest
Biomass value pyramid
Selling prices
Biotransformation
Biomass & enzymes
Food fermentation
Fine chemicals
Bulk chemicals
Industrial microbiology
Conversion process
Physical-chemical
Thermo-chemical
Biochemical
Definition
Fermentations
Cells & products
Media
Reactors
Batch/continuous
Growth kinetics
GMO
Industrial process
Biorefinery concept
Metabolites
Primary
Secondary
Metabolic engineering

Extra info

Industrial microbiology ➢ biotechnology



White Biotechnology – Industrial Biotechnology
http://www.europabio.org/Industrial_biotech/

Red Biotechnology – Health Care Biotechnology
http://www.europabio.org/Healthcare/

Green Biotechnology – Agricultural Biotechnology
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