POLITECNICO DI MILANO

School of Industrial and Information Engineering

Course 096125 (095857)

Introduction to Green and Sustainable Chemistry





Enzymatic Catalysis/Bioprocesses in Green Chemistry.

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https://iscamapweb.chem.polimi.it/citterio/it/education/course-topics/

Definitions.

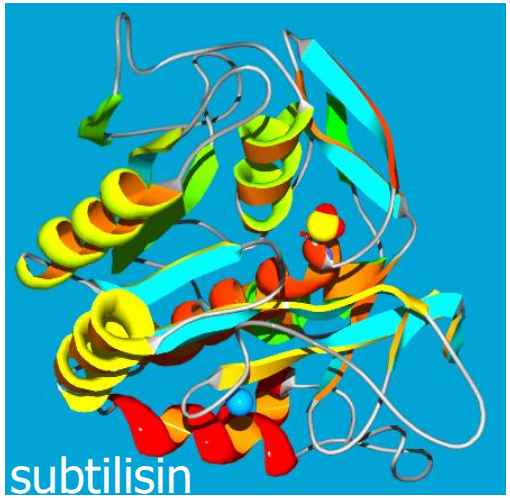
- **Biochemistry**
  - **The study of the chemistry of living systems**
  - **The study of biological molecules**
    1. How they function
    2. Their 3D structures
    3. How their functions combine to produce a living system.
- **Bioengineering**
  - **A broad title and would include electrical, mechanical, industrial, environmental, and chemical engineers that work on medical and agricultural systems** (Biological engineering means the same)**.**
- **Biomedical engineering**
  - **As biochemical engineering usually applies to medical applications.**

Definitions (2).

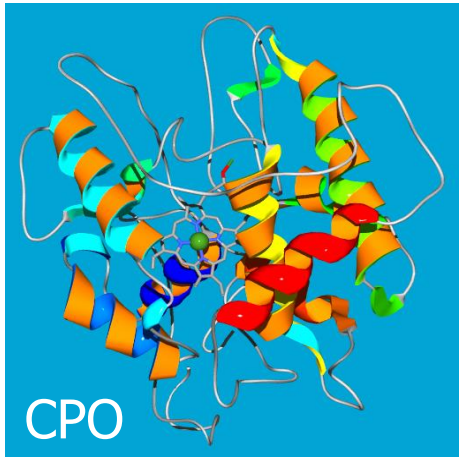
- **Biochemical Engineering**
  - The use of living organisms or the products of biological systems for practical purposes.
  - Engineering of processes using biocatalysts, bio-organic feedstock, and/or bio-adsorbents using the principles of chemical engineering.
- **Biotechnology**
  - Any technique that uses living organisms or substances from organisms to make or modify a product, to improve plants or animals, or to develop micro-organisms for specific uses.
  - Usually implies the use or development of methods of direct genetic manipulation for desirable goals (genetic engineering or recombinant DNA technology).
  - The use of microbes, animal, and plant cells or components to produce useful substances or processes.

Definitions (3).

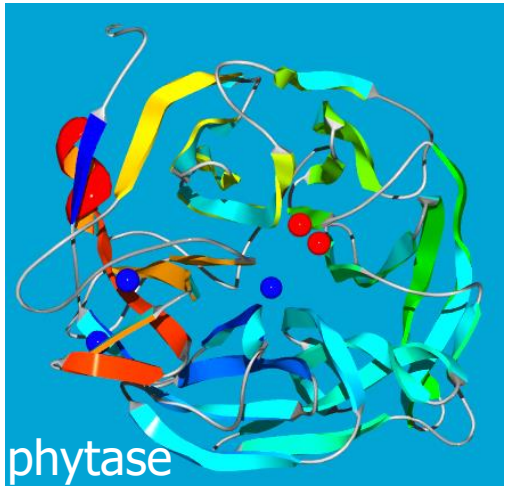
- **Enzymes,**
  - Produced by living organisms, are compounds of proteic nature with catalytic proprieties. These catalysts are both efficient and highly specific for an individual chemical reaction which involves the synthesis, degradation or alteration of a compound. In these reactions, where molecules are reduced, oxidized, transposed, or assembled, cofactors are frequently involved. Some enzymes are modified covalently by phosphorylation, glycosylation, and other processes.



Promotes the proteolysis of a peptide bond. .



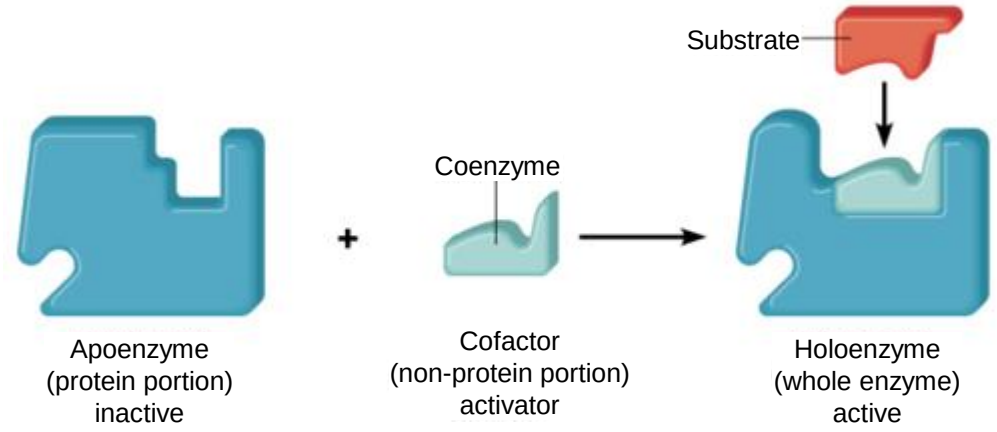
Chloroperoxidase catalyzes several oxidations of organic substrates.



Catalyzes the hydrolysis of phytic acid.

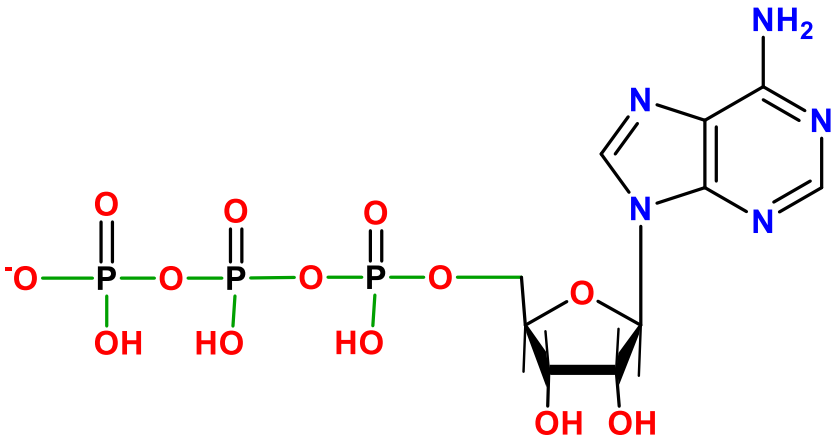
Definitions (4).

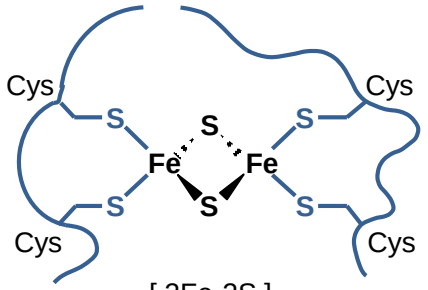
- **Co-factors**
  - A non-protein chemical compound that is bound to a protein and is required for the protein's biological activity. Cofactors can be considered "helper molecules" that assist in biochemical transformations.
  - Cofactors are either organic or inorganic. They can also be classified depending on how tightly they bind to an enzyme, with loosely-bound cofactors termed coenzymes and tightly-bound cofactors termed prosthetic groups. Some sources also limit the use of the term "cofactor" to inorganic substances. An inactive enzyme, without the cofactor is called an apoenzyme, while the complete enzyme with cofactor is the holoenzyme.
  - Coenzymes serve as transient carriers of specific functional groups.
  - They often come from vitamins (organic nutrients required in small amounts in the diet).



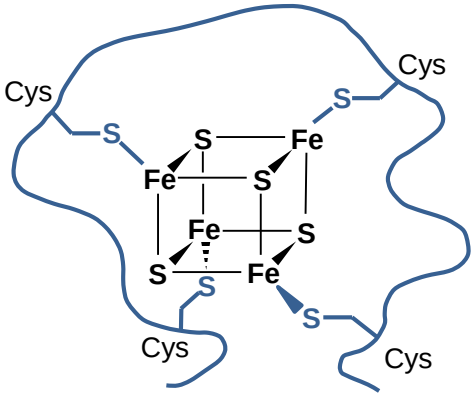
Cofactors and Coenzymes.

- Cofactors are necessary for some enzymes. More often metal ions.
- Coenzymes
  - Organic molecules
  - Soluble
  - Prosthetic groups
    - Apoenzyme vs. Holoenzymes.



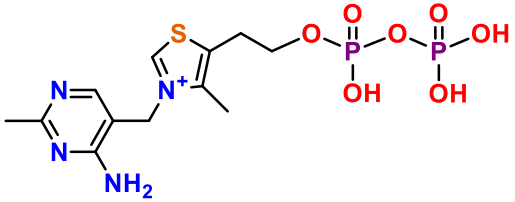


[2Fe-2S]

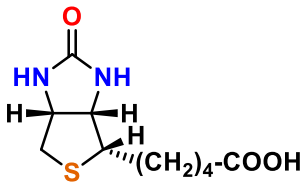


[4Fe-4S]

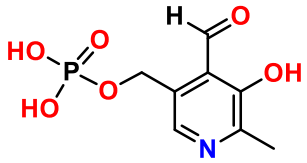
Some Cofactors.



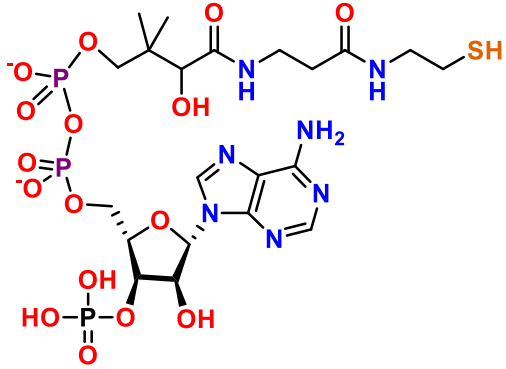
Thiamine Diphosphate



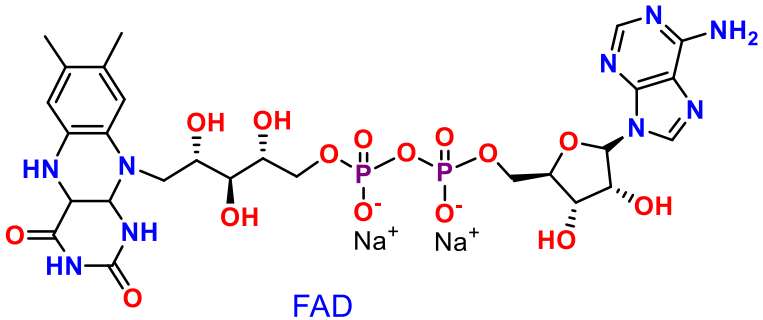
Biotin



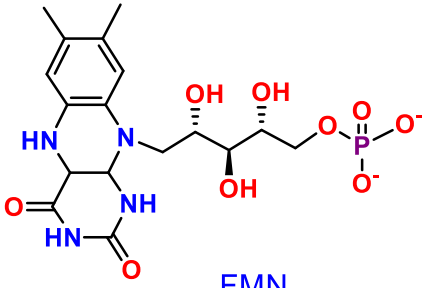
Pyridoxal Phosphate



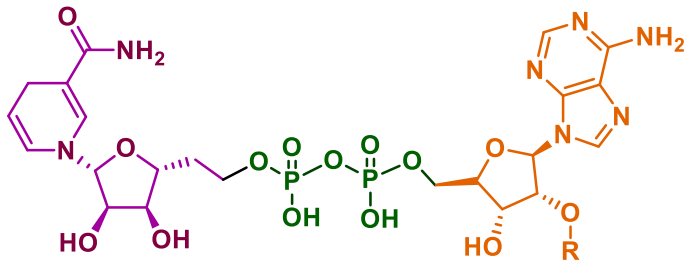
Coenzyme A (CoA)



FAD

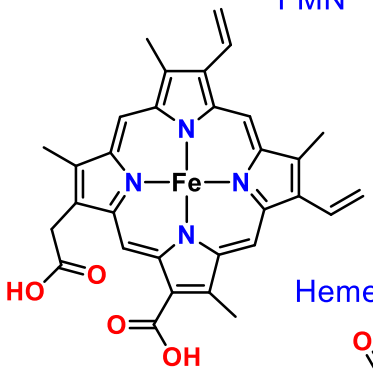


FMN

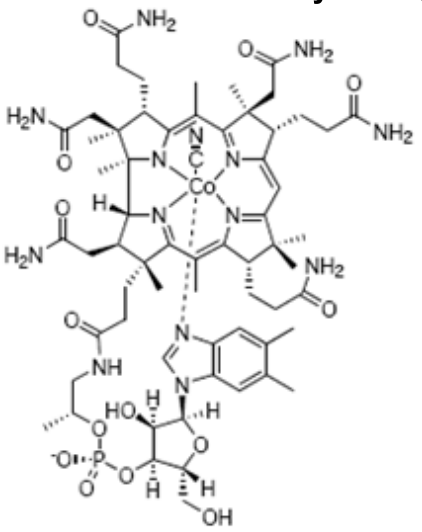


R = H NADH

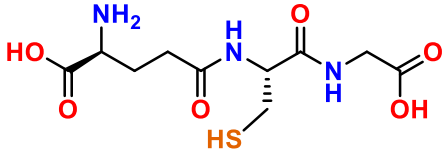
R = -PO_3H_2 NADPH



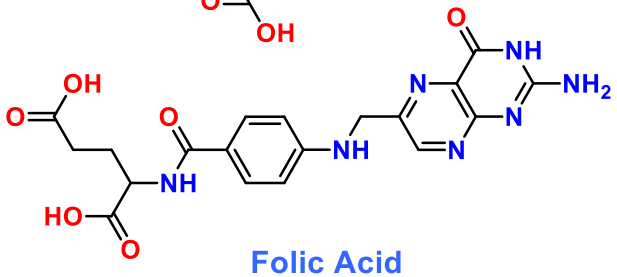
Heme



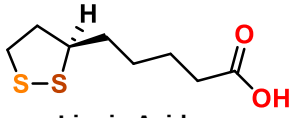
Vitamin B12



Glutathione



Folic Acid



Lipoic Acid

Characteristics of Common Coenzymes.

Coenzyme	Reaction mediated	Vitamin Source	Human Deficiency Disease
Biocytin	Carboxylation	Biotin	a
Coenzyme A	Acyl transfer	Pantothenate	a
Cobalamin coenzymes	Alkylation	Cobalamin (B_{12})	Pernicious anemia
Flavin coenzymes	Oxidation-reduction	Riboflavin (B_2)	a
Lipoic acid	Acyl-transfer	-	a
Nicotinamide coenzymes	Oxidation-reduction	Nicotinamide (niacin)	Pellagra
Pyridoxal phosphate	Amino group transfer	Pyridoxine (B_6)	a
Tetrahydrofolate	One-carbon group transfer	Folic acid	Megaloblastic anemia
Thiamine pyrophosphate	Aldehyde transfer	Thiamine (B_1)	Beriberi

[a]No specific name: deficiency in human is rare or unobserved.

Biotechnology.

- Manipulation of genes is called genetic engineering or recombinant DNA technology.
- Genetic engineering involves taking one or more genes from a location in one organism and either
  - Transferring them to another organism
  - Putting them back into the original organism in different combinations.

- **Involved Sectors:**
  - cell and molecular biology
  - microbiology
  - genetics
  - anatomy and physiology
  - biochemistry
  - engineering
  - computer science

- **Types of biotechnology**
  - Recombinant, R protein, R DNA
  - Genetically Modified Organism (GMO)
  - Antibody (monoclonal antibody)
  - Transgenic
  - Gene therapy, Immunotherapy

- **Risks and advantages of biotech**

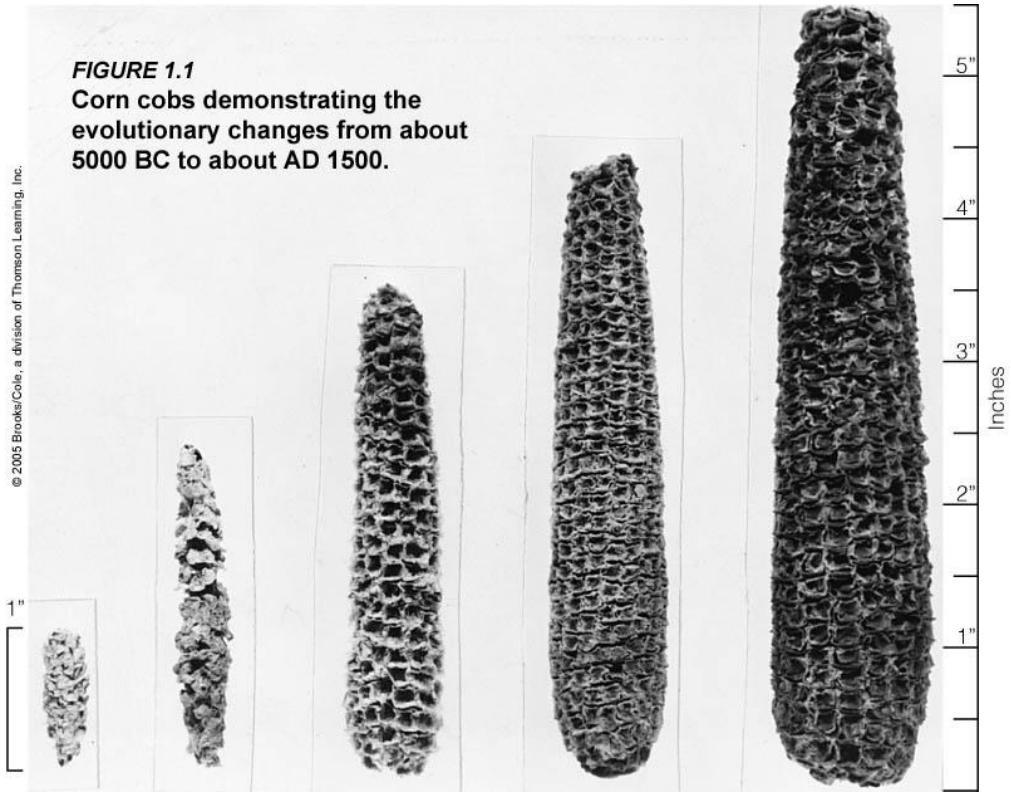
Applications of Biotechnology.

- Virus-resistant crop plants and livestock
- Diagnostics for detecting genetic diseases and acquired diseases
- Therapies that use genes to cure diseases
- Recombinant vaccines to prevent disease.

- simple or complex chemical compounds (i.e. proteins) via gene over-expression.

- biotechnology can also aid the environment.

evolving corn!



Goals of Biotechnology.

- To understand more about the processes of inheritance and gene expression.
- To provide better understanding and treatment of various diseases, particularly genetic disorders.
- To generate economic benefits, including improved plants and animals for agriculture and efficient production of valuable biological molecules.

Examples:

- Vitamin A fortified engineered rice
- Engineered corn resisting to fungal attacks
- Engineered drought resistant plants
- Bioleaching process that recovers metals from ores which are not suitable for direct smelting because of their low content.

Biotechnology Development.

- **Ancient biotechnology** - history related to food-shelter; Includes domestication
  - Paleolithic peoples began to settle and develop agrarian societies about 10,000 y ago (ancient farming sites in Americas, Far East, and Europe);
  - Early farmers in the Near East cultivated wheat, barley, and possibly rye;
  - 7,000 years ago, pastoralists roamed the Sahara region with sheep, goats cattle, and also hunted and used grinding stones in food preparation;
  - Early farmers arrived in Egypt 6,000 years ago with cattle, sheep, goats, and crops such as barley, emmer, and chick-pea;
  - Fermented food, 1500 BC (Yeast - fruit juice, wine, brewing beer, baking bread, alcohol, Egyptians used yeast in 1500 BC, 1915-20 Baker's Yeast).
- **Classical biotechnology** - built on ancient biotechnology; Fermentation promoted food production, and medicine.
- **Modern biotechnology** - manipulates genetic information in organism; Genetic engineering.

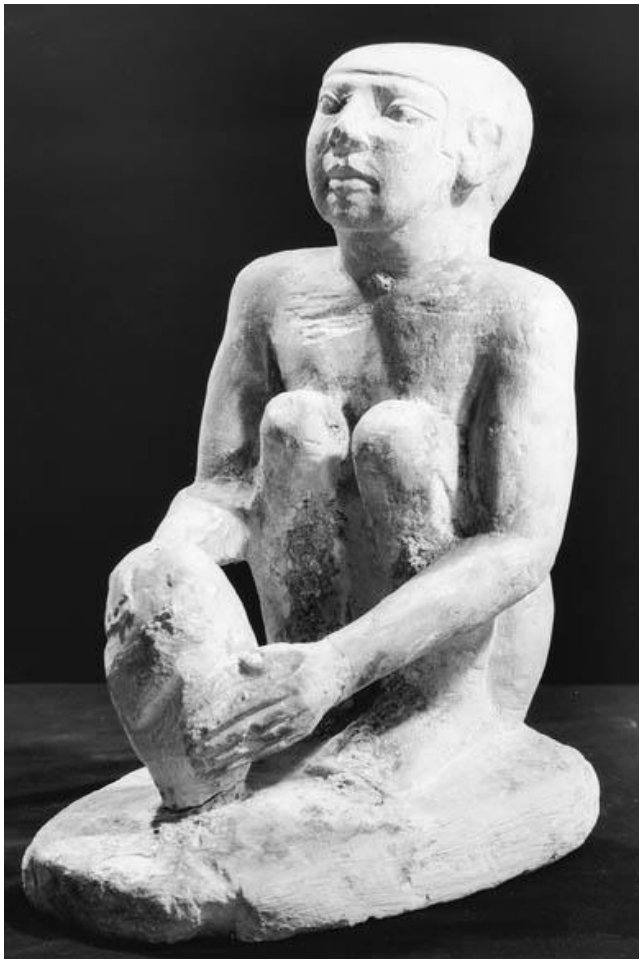
Fermentation.

Microbial process in which enzymatically controlled transformations of organic compounds occur.

- Fermentation has been practiced for years and has resulted in foods such as bread, wine, and beer
- 4000 - 9000 B.C. - Drawing of cow being milked Yogurt
- 5000-9000 B.C. - Chinese Cheese curd from milk
- Fermented dough was discovered by accident when dough was not baked immediately.
- Modern cheese manufacturing involves:
  - inoculating milk with lactic acid bacteria
  - adding enzymes such as rennet to curdle casein
  - heating
  - separating curd from whey
  - draining the whey
  - salting
  - pressing the curd
  - ripening.

Fermented Beverages.

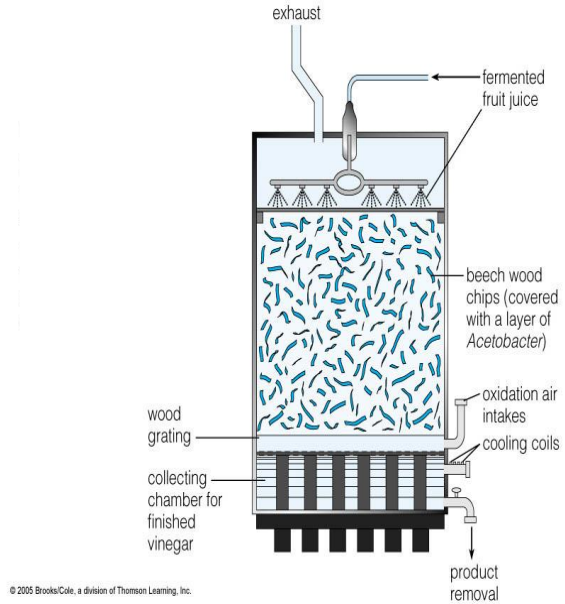
- Beer making began as early as 6000-5000 B.C.
- Egypt ~5000 B.C made wine from grapes
- Barley malt – earthenware
  Yeast found in ancient beer urns
- Monasteries - major brewers
- 1680 - Leeuwenhoek observed yeast under microscope
- Between 1866 and 1876 - Pasteur established that yeast and other microbes were responsible for fermentation.



Classical Biotech.

Describes the development that fermentation has taken place from ancient times to the present.

- **Top fermentation** - developed first, yeast rise to top;
- 1833 - **Bottom fermentation** - yeast remain on bottom;
- 1886 – Brewing equipment made by E.C. Hansen and still used today;
- World War I – fermentation of organic solvents for explosives (glycerol)
- World War II – bioreactor or fermenter:
  - Antibiotics
  - Cholesterol – Steroids
  - Amino acids
  - Large quantities of vinegar are produced by *Acetobacter* on a substrate of wood chips.



Classical Biotech (2).

- In the 1950's, cholesterol was converted to cortisol and sex hormones by reactions such as microbial hydroxylation (addition of -OH group);
- By the mid-1950's, amino acids and other **primary metabolites** (needed for cell growth) were produced, as well as enzymes and vitamins;
- By the 1960's, microbes were being used as sources of protein and other molecules - **secondary metabolites** (not needed for cell growth).

Today many things are produced:

- Pharmaceutical compounds such as antibiotics
- Amino Acids
- Many chemicals, hormones, and pigments
- Enzymes with a large variety of uses
- Biomass for commercial and animal consumption (such as single-cell protein).

Old Biotech Meets New.

- Fermentation and genetic engineering have been used in food production since the 1980s;
- Genetically engineered organisms are cultured in fermenters and are modified to produce large quantities of desirable enzymes, which are extracted and purified;
- Enzymes are used in the production of milk, cheese, beer, wine, candy, vitamins, and mineral supplements
- Genetic engineering has been used to increase the amount and purity of enzymes, to improve an enzyme's function, and to provide a more cost-efficient method to produce enzymes:
  - Chymosin, used in cheese production, was one of the first produced.

1590 - Zacharias Janssen - First two lens microscope (30×);

1665 - Robert Hooke - Cork "Cellulae" (Small Chambers);

1676 - Anthony van Leeuwenhoek – (200×) animalcules (in pond water);

1684 – "" protozoa/fungi;

Foundations of Modern Biotechnology.

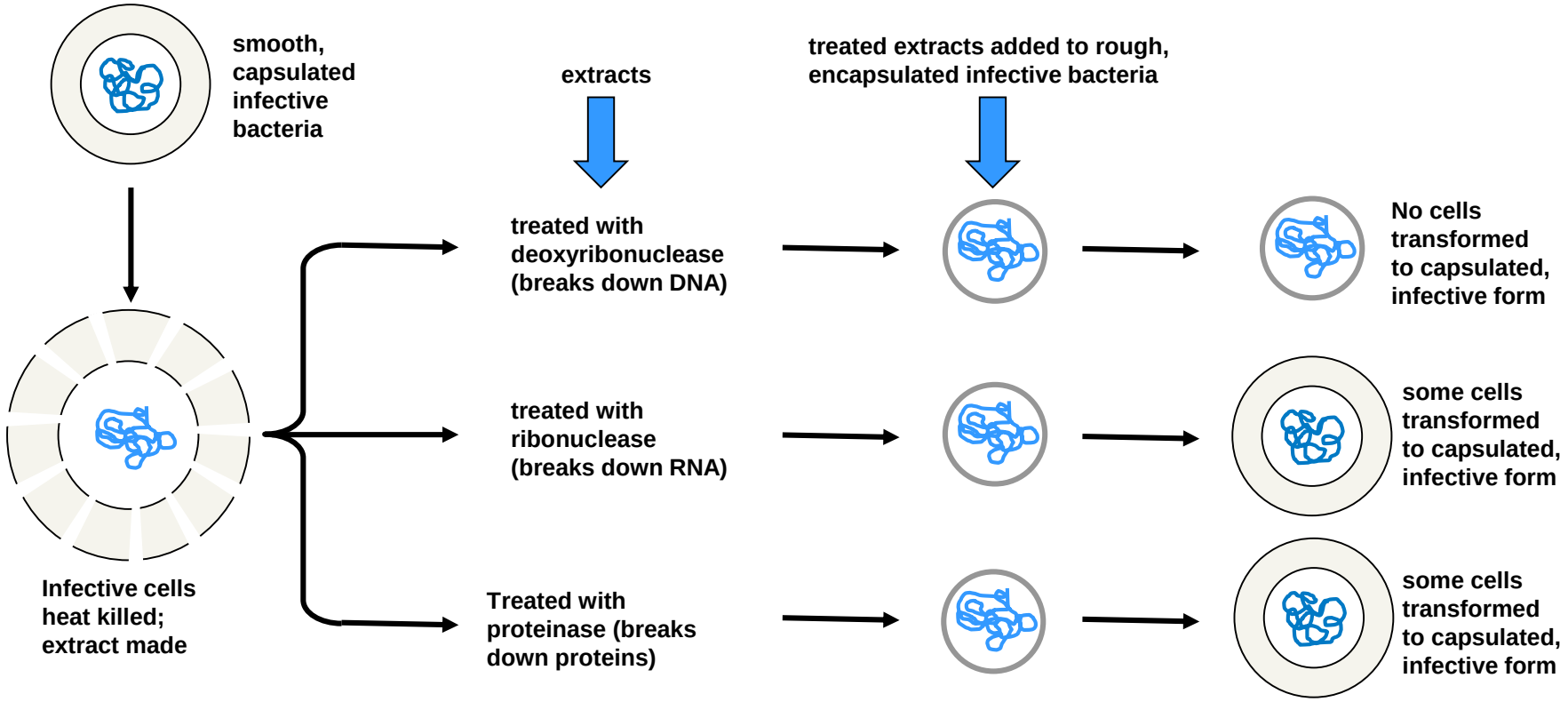
- 1838, Matthias Schleiden, determined that all plant tissue was composed of cells and that each plant arose from a single cell;
- 1839, Theodor Schwann, came to a similar determination as Schleiden, for animals;
- 1858, Rudolf Virchow, concluded that all cells arise from cells and the cell is the basic unit of life;
- Before cell theory the main belief was **vitalism**: whole organism, not individual parts, possess life;
- By the early 1880s, microscopes, tissue preservation technology, and stains allowed scientists to better understand cell structure and function;
- 1928 - Fred Griffith performed experiments using *Streptococcus pneumonia*

  Two strains: Smooth (S) - Virulent (gel coat) Rough (R) - Less Virulent

  Injected R and heat-killed S - mice died and contained S bacteria

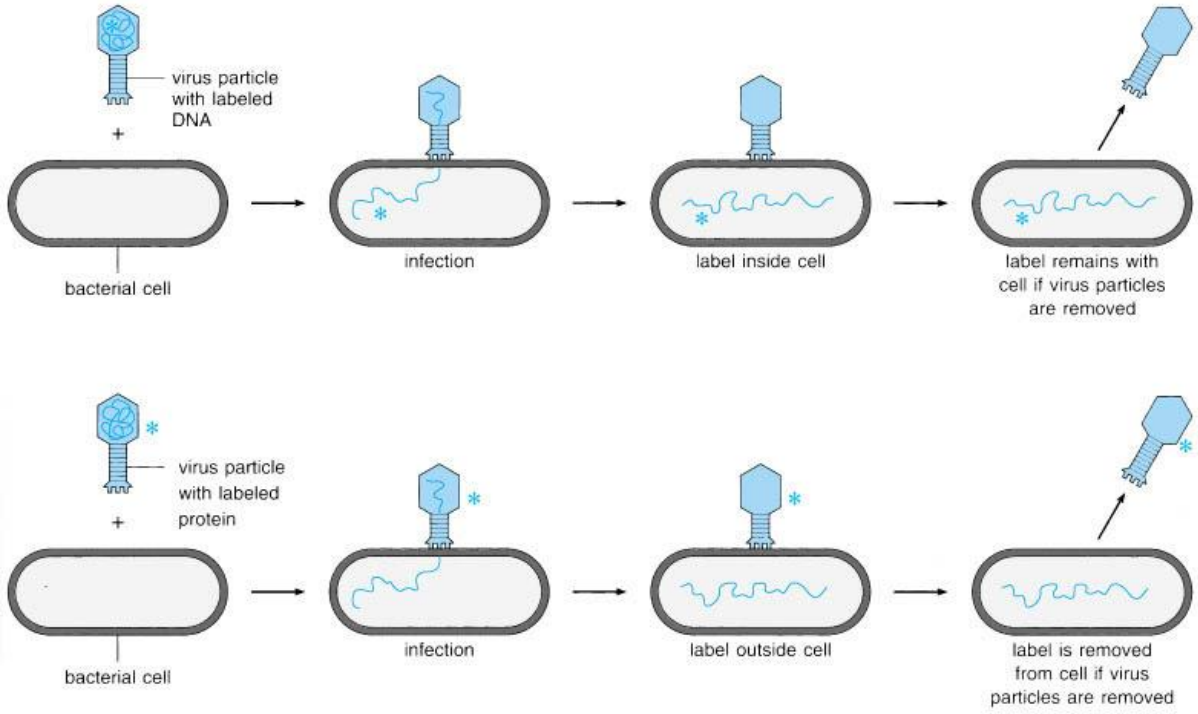
  Unsure of what changed R to S, which he called the “Transforming principle”.

Transforming Principle.



1952 – Alfred Hershey and Martha Chase.

- Used T2 bacteriophage, a virus that infects bacteria;
- Radiolabeled the bacteriophage with S35 (Protein) and P32 (DNA);
- Bacterial cells were infected and put in a blender to remove phage particles;
- Analysis showed labeled DNA inside the bacteria and was the genetic material.



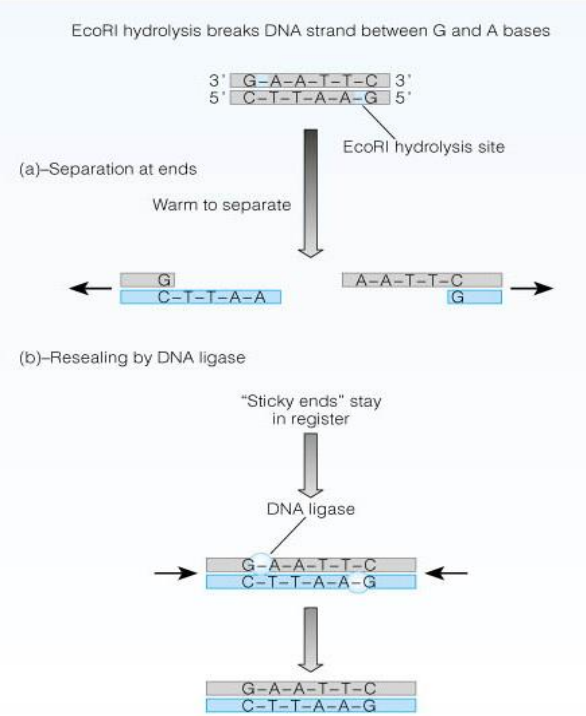
1953 Watson and Crick.

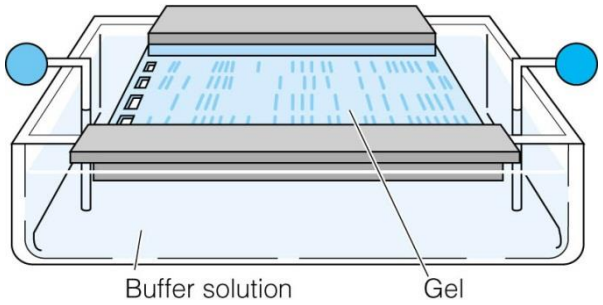
Determined the structure of DNA

- Rosalind Franklin and Maurice Wilkins provided X-ray diffraction data;
- Erwin Chargaff determined the ratios of nitrogen bases in DNA;
- DNA replication model – 1953;
- DNA bases made up of purine and pyrimidine:
  - **A** pairs with **T** and **G** pairs with **C**
- Nobel Prize – 1962.
- Not clear the relation between DNA and RNA.

First Recombinant DNA Experiments.

- 1971 scientists manipulated DNA and placed them into bacteria;
- 1972 scientists joined two DNA molecules from different sources using the **endonuclease** EcoRI (to cut) and DNA **ligase** (to reseal);
- H. Boyer later went to Cold Spring Harbor Laboratories and discovered a new technique called **gel electrophoresis** to separate DNA fragments:
  - A current is applied so that the negative charged DNA molecules migrate towards the positive electrode and is separated by fragment size.





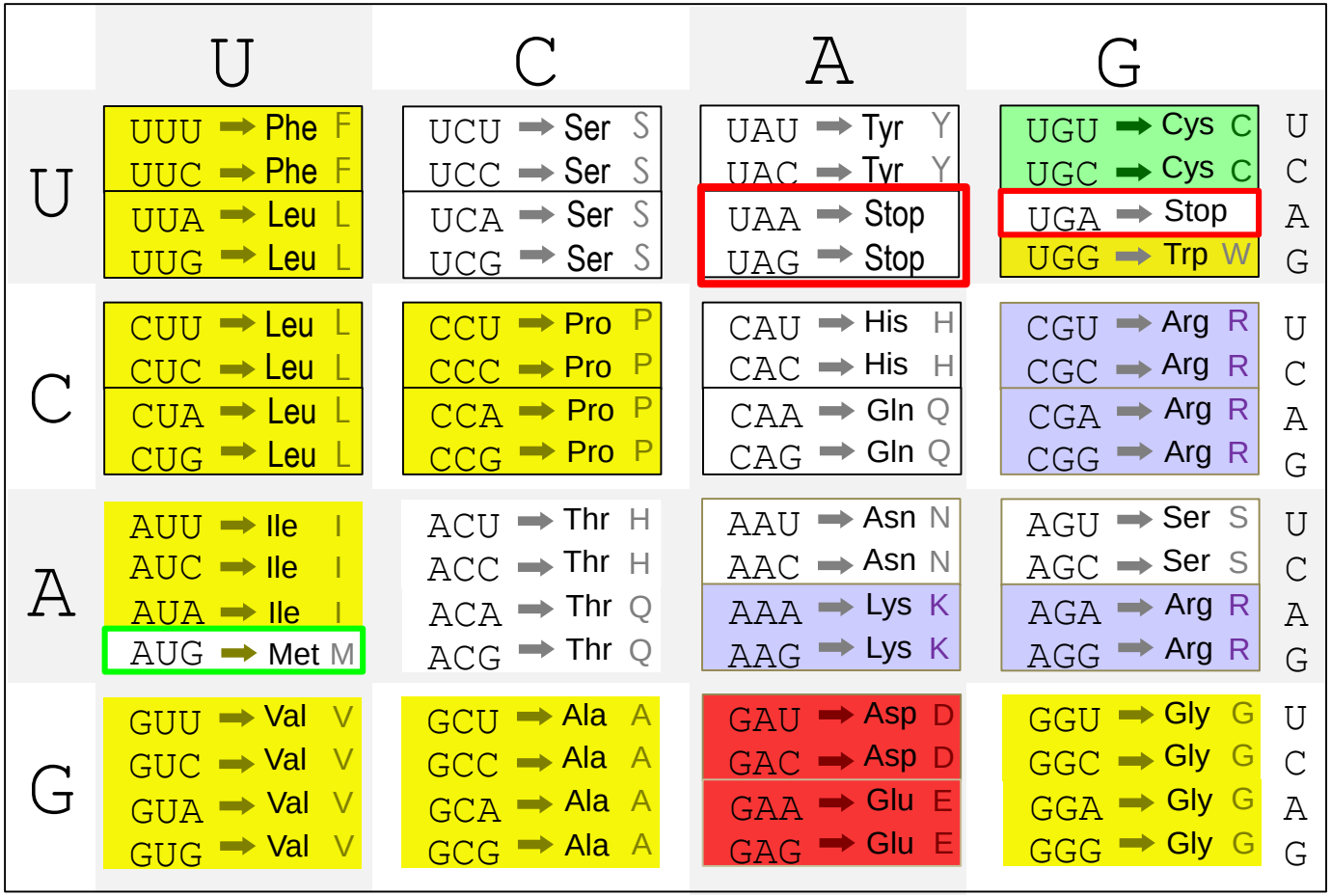
Biotech Revolution: Cracking the Code.

- 1961, Nirenberg and Mattei made the first attempt to break the genetic code, using synthetic **messenger RNA** (mRNA).

- Nirenberg and Leder developed a binding assay that allowed them to determine which triplet codons specified which amino acids by using RNA sequences of specific codons.

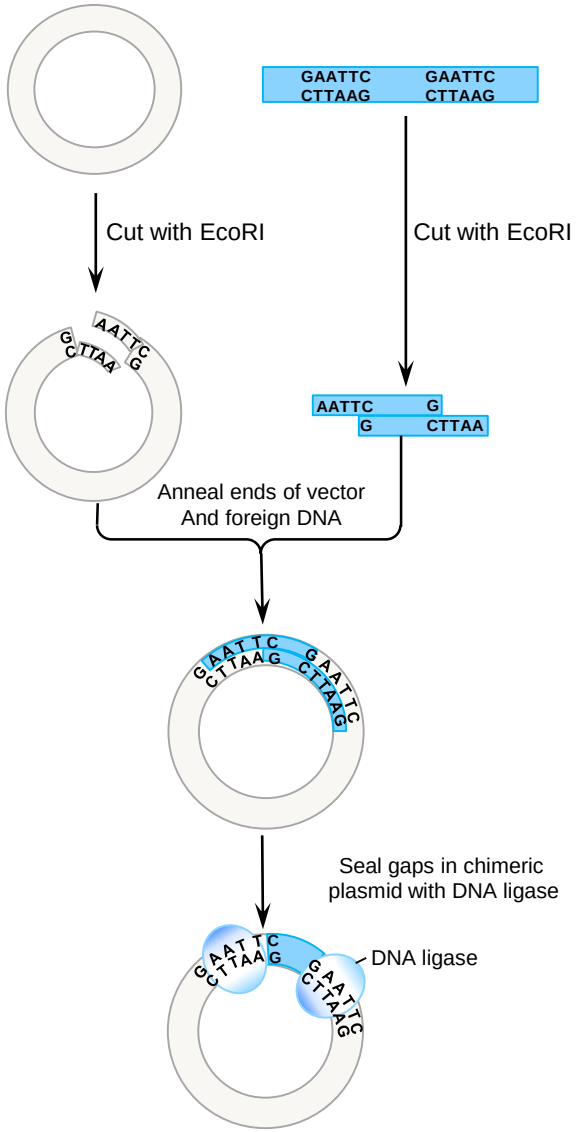
First Base	Second Base				Third Base
	U	C	A	G	
U	phenylalanine	serine	tyrosine	cysteine	U
	phenylalanine	serine	tyrosine	cysteine	C
	leucine	serine	stop	stop	A
	leucine	serine	stop	**tryptophan**	G
C	leucine	proline	histidine	arginine	U
	leucine	proline	histidine	arginine	C
	leucine	proline	histidine	arginine	A
	leucine	proline	histidine	arginine	G
A	isoleucine	threonine	asparagine	serine	U
	isoleucine	threonine	asparagine	serine	C
	isoleucine	threonine	asparagine	arginine	A
	(start) **methionine**	threonine	asparagine	arginine	G
G	valine	alanine	aspartate	glycine	U
	valine	alanine	aspartate	glycine	C
	valine	alanine	aspartate	glycine	A
	valine	alanine	aspartate	glycine	G

The Standard Genetic Code.



First DNA Cloning.

- Boyer, Helling Cohen, and Chang joined DNA fragments in a vector, and transformed an *E. coli* cell;
- Cohen and Chang found they could place bacterial DNA into an unrelated bacterial species:
- In 1980 Boyer and Cohen received a patent for the basic methods of DNA cloning and transformation:
  - Recombinant DNA technology sparked debates more than 30 years ago among scientists, ethicists, the media, lawyers, and others;
  - In the 1980's it was concluded that the technology had not caused any disasters and does not pose treats to human health and to the environment;
  - 1997 cloned sheep – "Dolly" in Edinburgh.



Industrial Development of Biotech.

- First biotech companies formed:

  1976 - Genentech
  1978 - Biogen
  1980 - Amgen
  1981 - Immunex
  1981 - Chiron
  1981 – Genzyme

- More than 325 million people worldwide have been helped by 160 approved biotech drugs and vaccines.
- >350 more biotech drugs and vaccines now in clinical trials targeting more than 200 diseases.
- Biotechnology is responsible for hundreds of diagnostic tests, including HIV tests and home pregnancy tests, DNA fingerprinting…

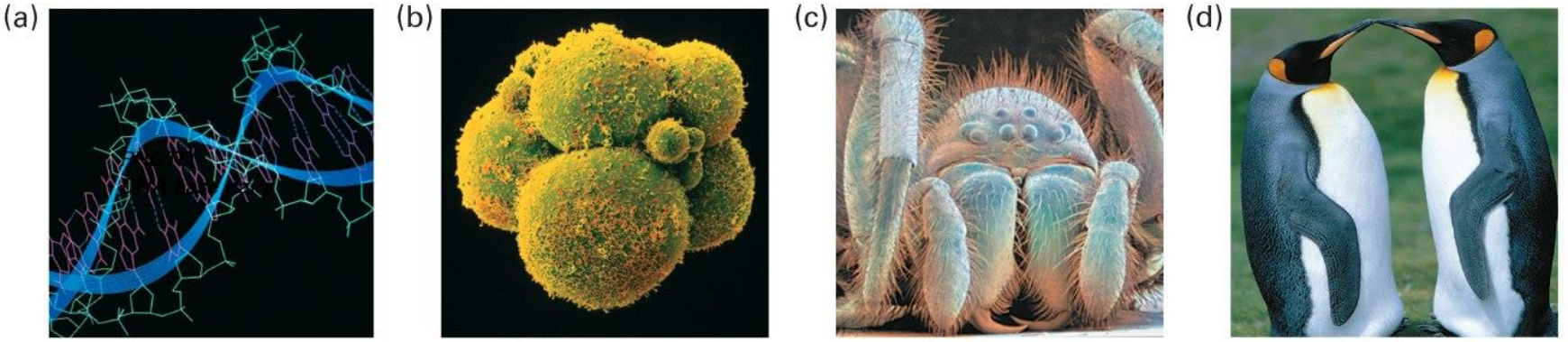
Progress Continues.

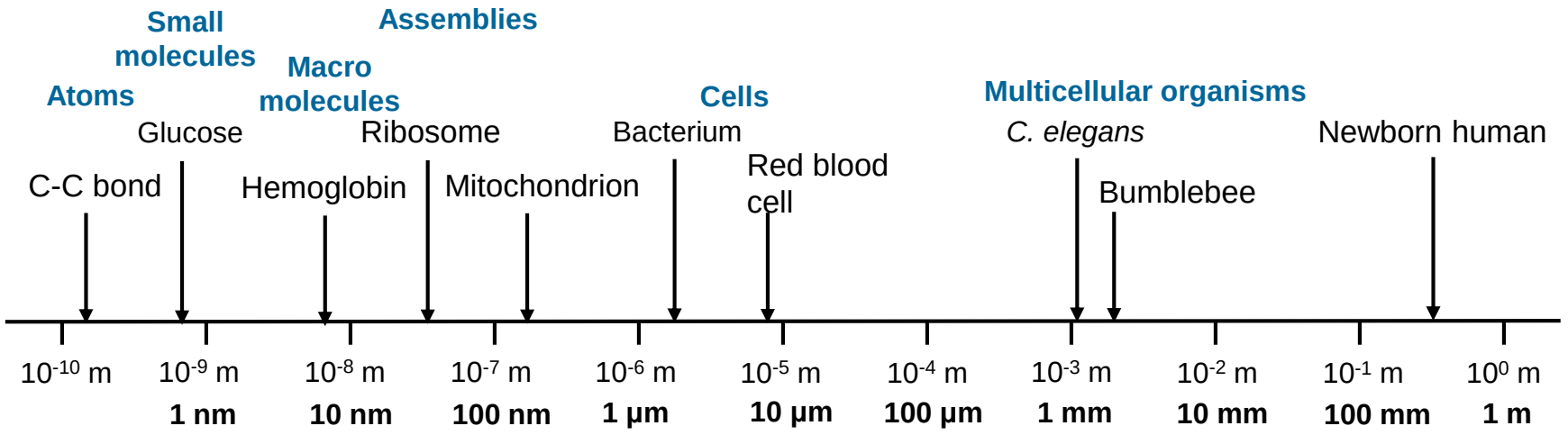
However, concerns have focused on both applications and ethical implications:

- Gene therapy experiments have raised the question of eugenics (artificial human selection) as well as testing for diseases currently without a cure;
- Animal clones have been developed, and fears have been expressed that this may lead to human cloning (in 2018 firstly reported in China);
- In agriculture, there is concern about gene containment and the creation of "super weeds" (herbicide and/or pesticide resistant weeds);
- Today, fears have focused on genetically engineered foods in the marketplace and has resulted in the rapid growth of the organic food industry.

- Many genetically modified disease, pest, and herbicide-resistant plants are awaiting approval for commercialization.
- Genes involved in disease are being identified.
- New medical treatments are being developed.
- Molecular "pharming," where plants are being used to produce pharmaceuticals (**biopharmaceuticals)**, is being developed.

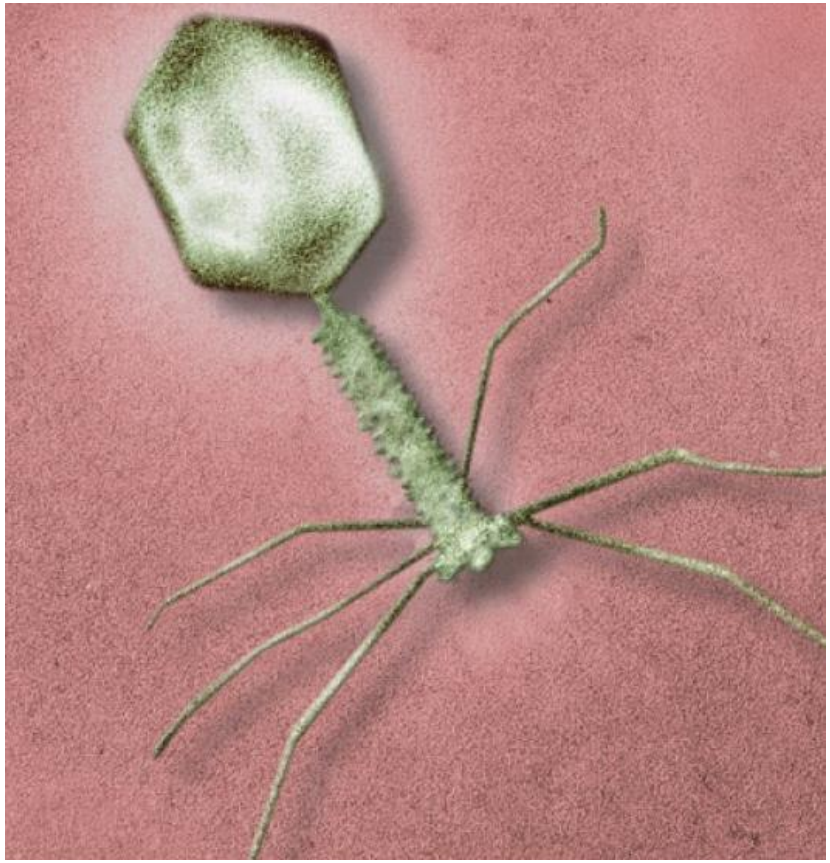
Sizes in Biotechnology.





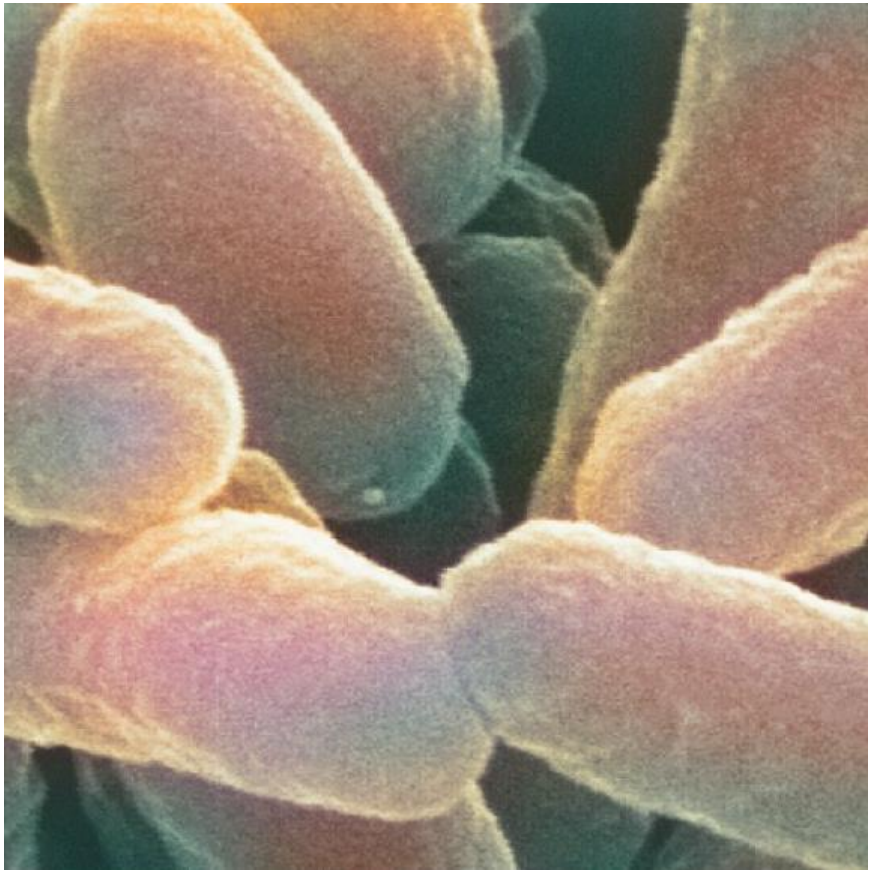
Viruses.

- Proteins involved in DNA, RNA, protein synthesis;
- Gene regulation;
- Cancer and control of cell proliferation;
- Transport of proteins and organelles inside cells;
- Infection and immunity;
- Possible gene therapy approaches.



Bacteria.

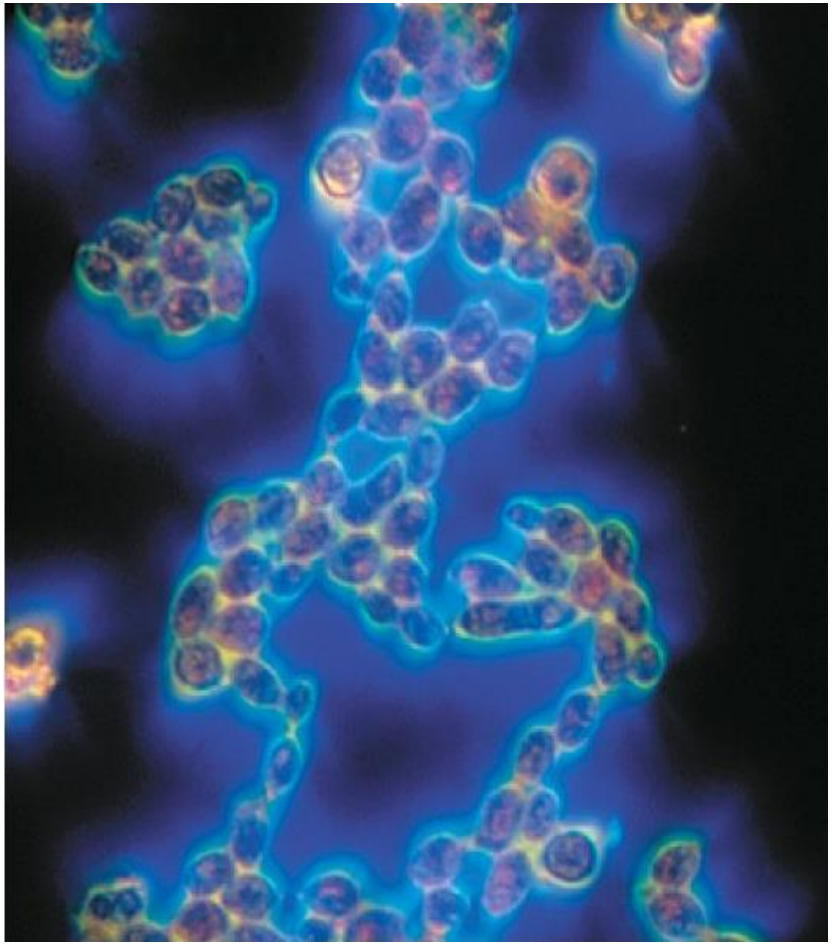
- Proteins involved in DNA, RNA, protein synthesis, metabolism;
- Gene regulation;
- Targets for new antibiotics;
- Cell cycle;
- Signaling.



Yeast.

E.g. - Saccharomyces cerevisiae

- Control of cell cycle and cell division;
- Protein secretion and membrane biogenesis;
- Function of the cytoskeleton;
- Cell differentiation;
- Aging;
- Gene regulation and chromosome structure.



Roundworm.

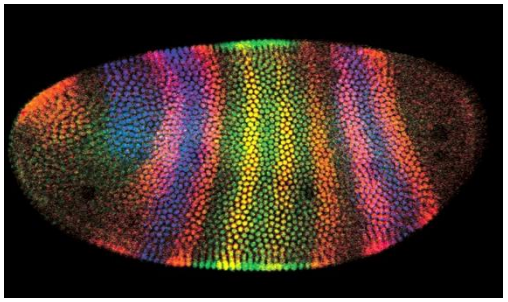
E.g. - Caenorhabditis elegans

- Development of the body plane;
- Cell lineage;
- Formation and function of the nervous system;
- Control of programmed cell death;
- Cell proliferation and cancer genes;
- Aging;
- Behaviour;
- Gene regulation and chromosome structure.

Fruit Fly.

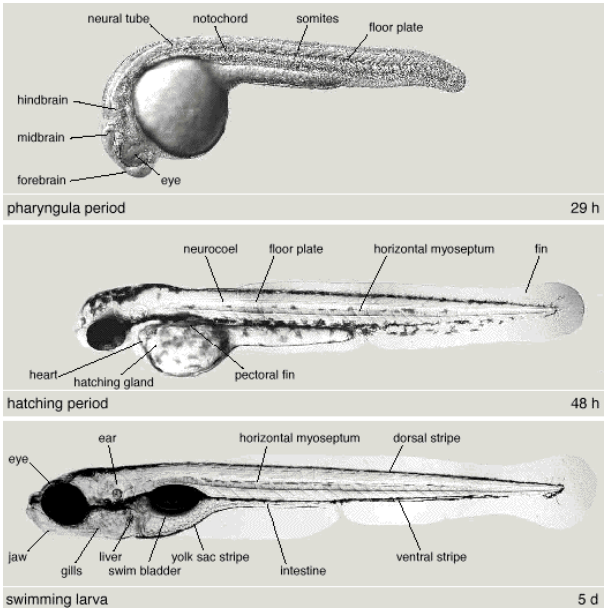
E.g. - Drosophila melanogaster

- Development of the body plan;
- Generation of differentiated cell lineages;
- Formation of the nervous system, heart and musculature;
- Programmed cell death;
- Genetic control of behaviour;
- Cancer genes and control of cell proliferation;
- Control of cell polarisation;
- Effect of drugs, alcohol and pesticides.



Fish, e.g. Zebrafish.

- Development of vertebrate body tissue;
- Formation and function of brain and nervous system;
- Birth defect;
- Cancer.

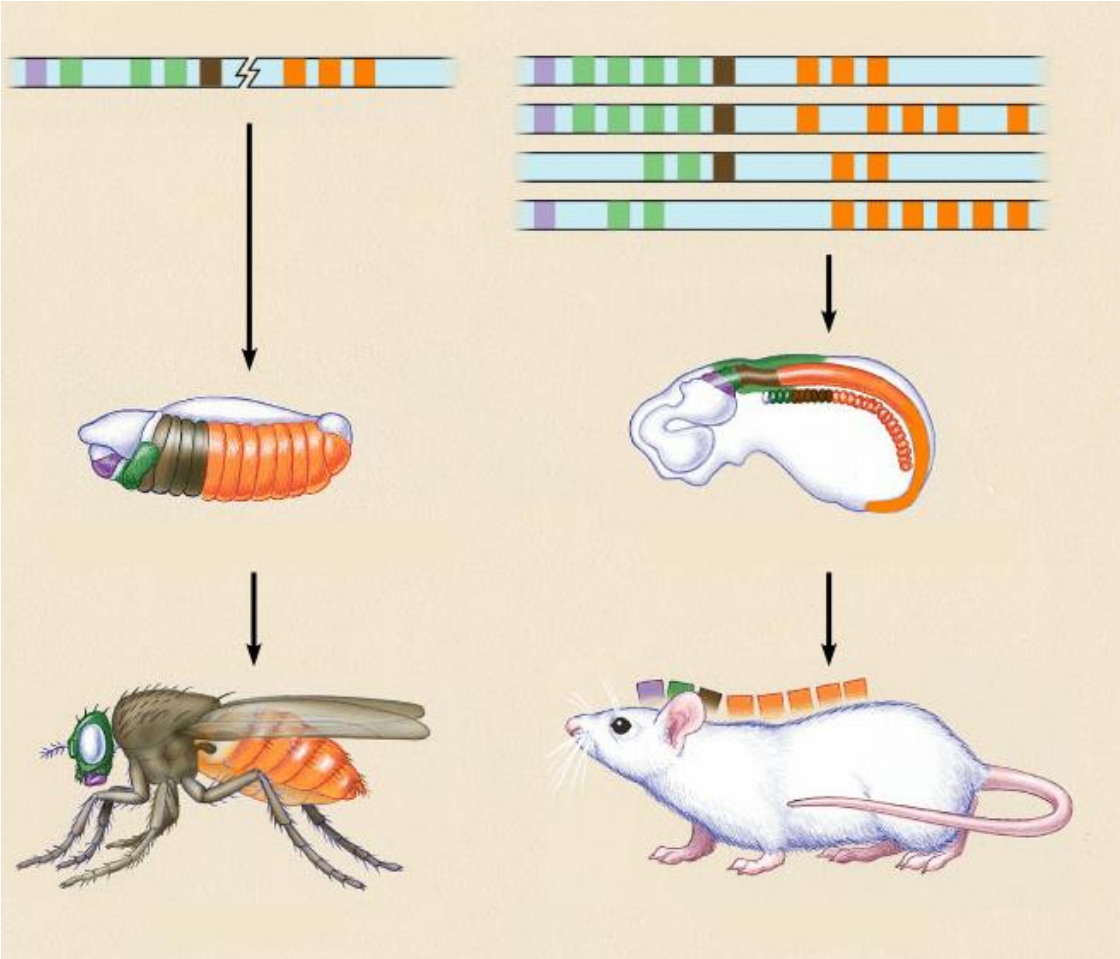


Mouse

- Development of body tissues;
- Function of mammalian immune system;
- Formation and function of brain and nervous system.
- Models of cancer and other human diseases;
- Gene regulation and inheritance;
- Infectious disease.

Homeotic Genes.

- The order of homeotic genes is the same;
- The gene order corresponds to analogous body regions.



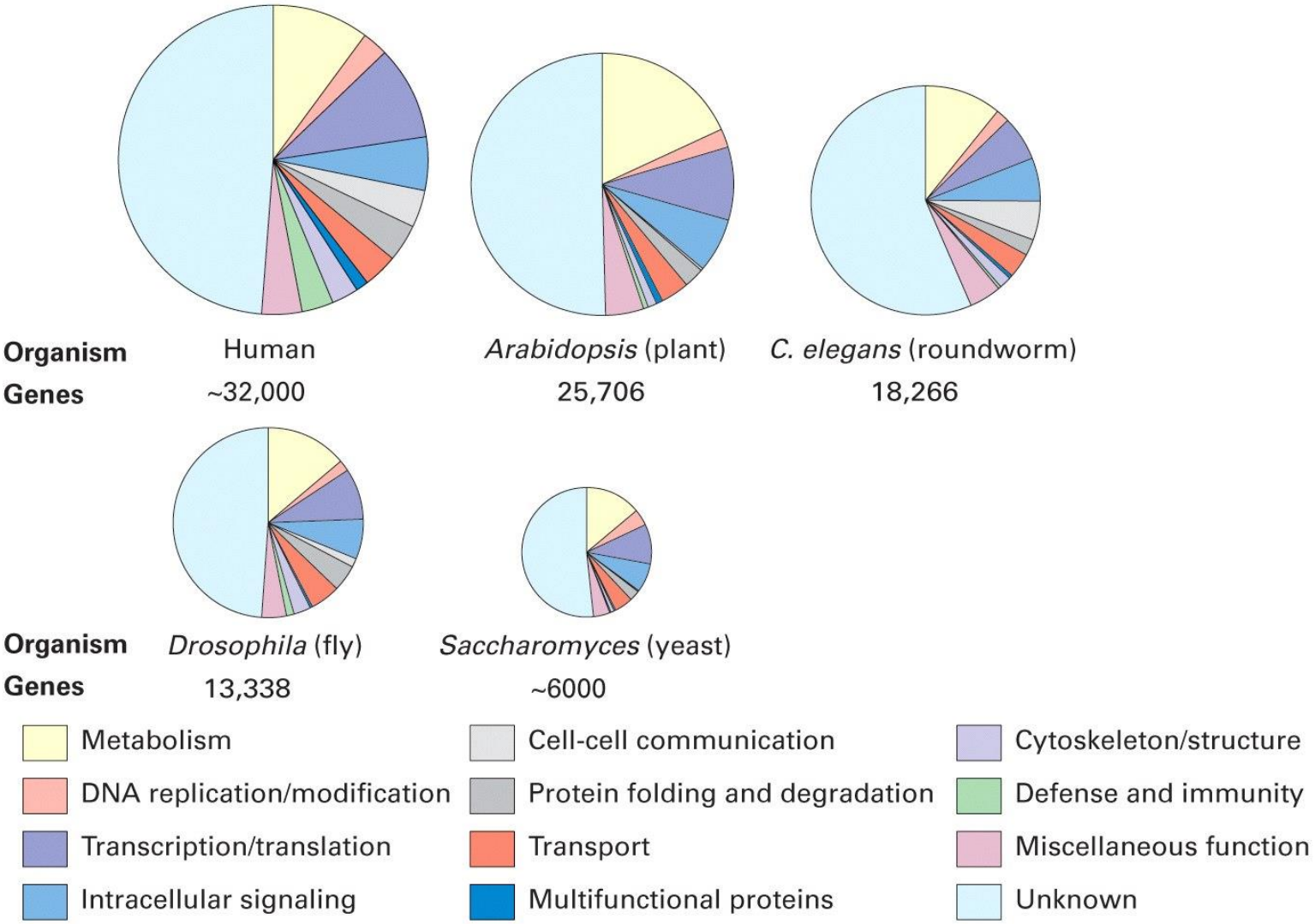
Plants.

- Development and patterning of tissues;
- Genetics of cell biology;
- Agricultural applications;
- Physiology;
- Gene regulation;
- Immunity;
- infectious disease.

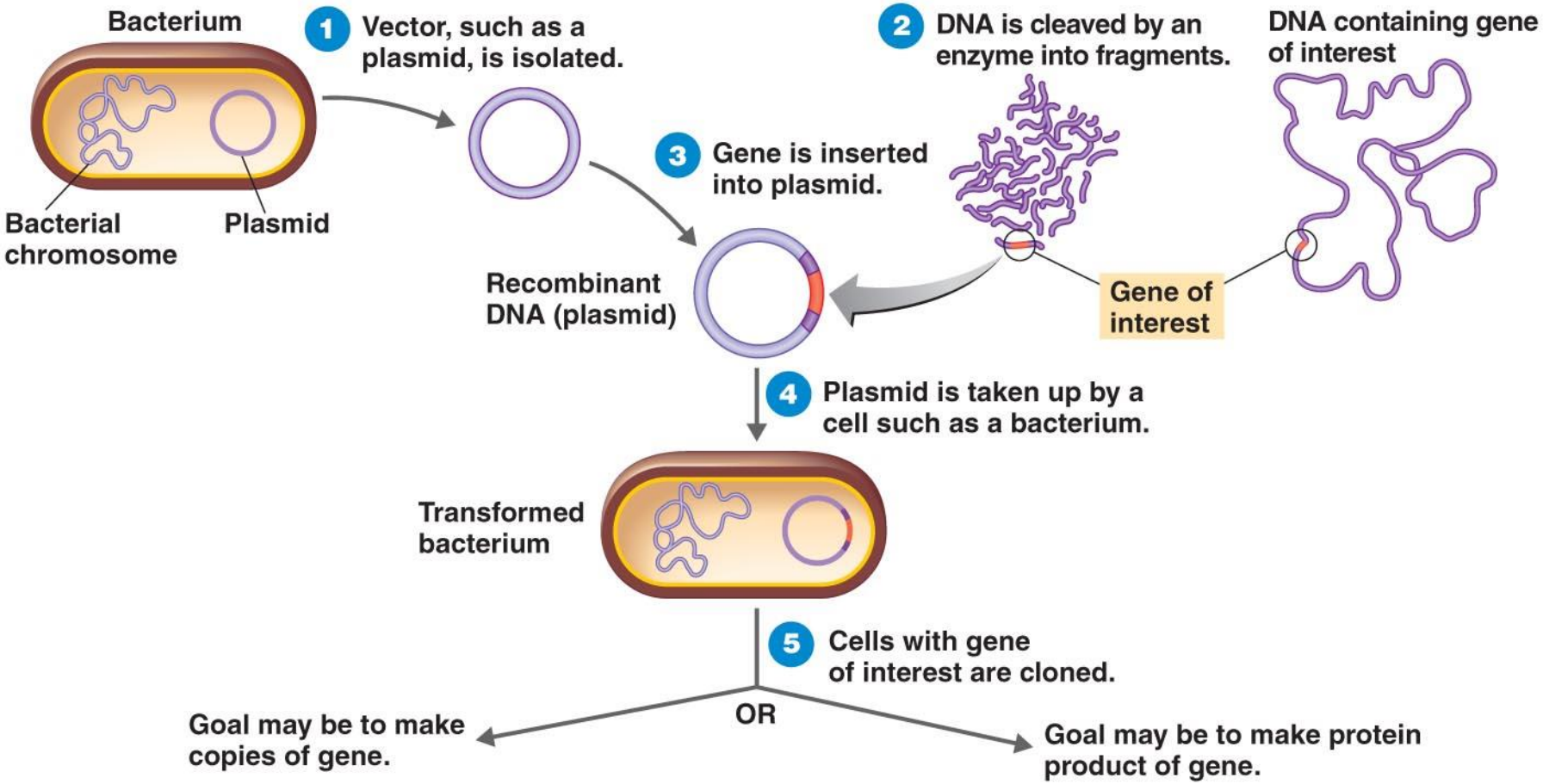
Genome Specification.

Organism	Type	Chromo-some #	Gene # (bp)	Genoma Size
Hepatitus B	virus	1	4	3215
E. coli	bacteria	1	4,394	4,639,221
S. cerevisiae	yeast	16	6,183	12,000,000
D. melanogaster	fruit fly	4	14,000	140,000,000
C. elegans	nematode	6	19,000	90,000,000
A. thaliana	plant	5	25,000	125,000,000
M. musculus	mouse	20	35,000	3,000,000,000
H. sapiens	human	23	35,000	3,000,000,000

Genome Specification (2).



A Typical Genetic Modification.



Type of Expression Systems for Genetic Modified Organisms (GMO).

- ✓ Bacterial
- ✓ Insect
- ✓ Yeast
- ✓ Mammalian cell lines
- ✓ Transgenic
  - Animal
  - Plant

Bacteria.

Advantages

1. Simple and well characterized genetics;
2. Rapid cell growth (doubles in 20-30 min);
3. Easy to grow in inexpensive culture media;
4. Easy to scale up fermentation;
5. High expression levels.

Disadvantages

1. Lack of glycosylation and other post-translational modifications;
2. Cell disruption gives more complex purification problems;
3. Inclusion body formation; solubilization and refolding required;
4. Presence of endotoxin and host cell proteins.

Yeast (e.g. *S. cerevisiae, P. pastoris).*

Advantages

- Well known genetics;
- Rapid cell growth (doubles in - 90 min);
- Inexpensive culture media;
- Provides and facilitates disulfide bond formation;
- Relatively few purification problems.

Disadvantages

- Protein may be incorrectly glycosylated and folded;
- Overglycosylation is a risk;
- Limited other posttranslational modifications;
- Generally lower expression levels than in bacterial systems.

Insect cells (*Baculovirus vector*).

Advantages

- Secretion systems available;
- Enable post translational modifications required for higher eukaryotic proteins;
- High expression;
- Baculovirus vectors are nonpathogenic to humans.

Disadvantages

- Slow cell growth;
- Expensive culture media;
- Possibility of post-translational modifications not identical to higher systems;
- Sensitive to shear forces.

Mammalian Cells.

Advantages

- Glycosylation of the complex type;
- Other post-translational modifications;
- Secretion systems available.

Disadvantages

- Slow cell growth (doubles in 18-24 hours);
- Low final cell density;
- Expensive culture media;
- Sensitive to shear forces.

Production of vaccines, enzymes, hormones, monoclonal antibody, native or modified proteins, fusion proteins

CHO (Chinese hamster ovary)

Human cells – concern: potential disease carrier

Different Types of Cells.

Prokaryotes

- No true nucleus;
- No membrane bound organelles;
- Small size;
- Circular chromosome;
- Single cells.

Eukaryotes

- Membrane bound nucleus;
- Intracellular organelles;
- May contain multiple linear chromosomes;
- Generally larger cells;
- May be organized into multicellular organisms.

Prokaryotic Cells.

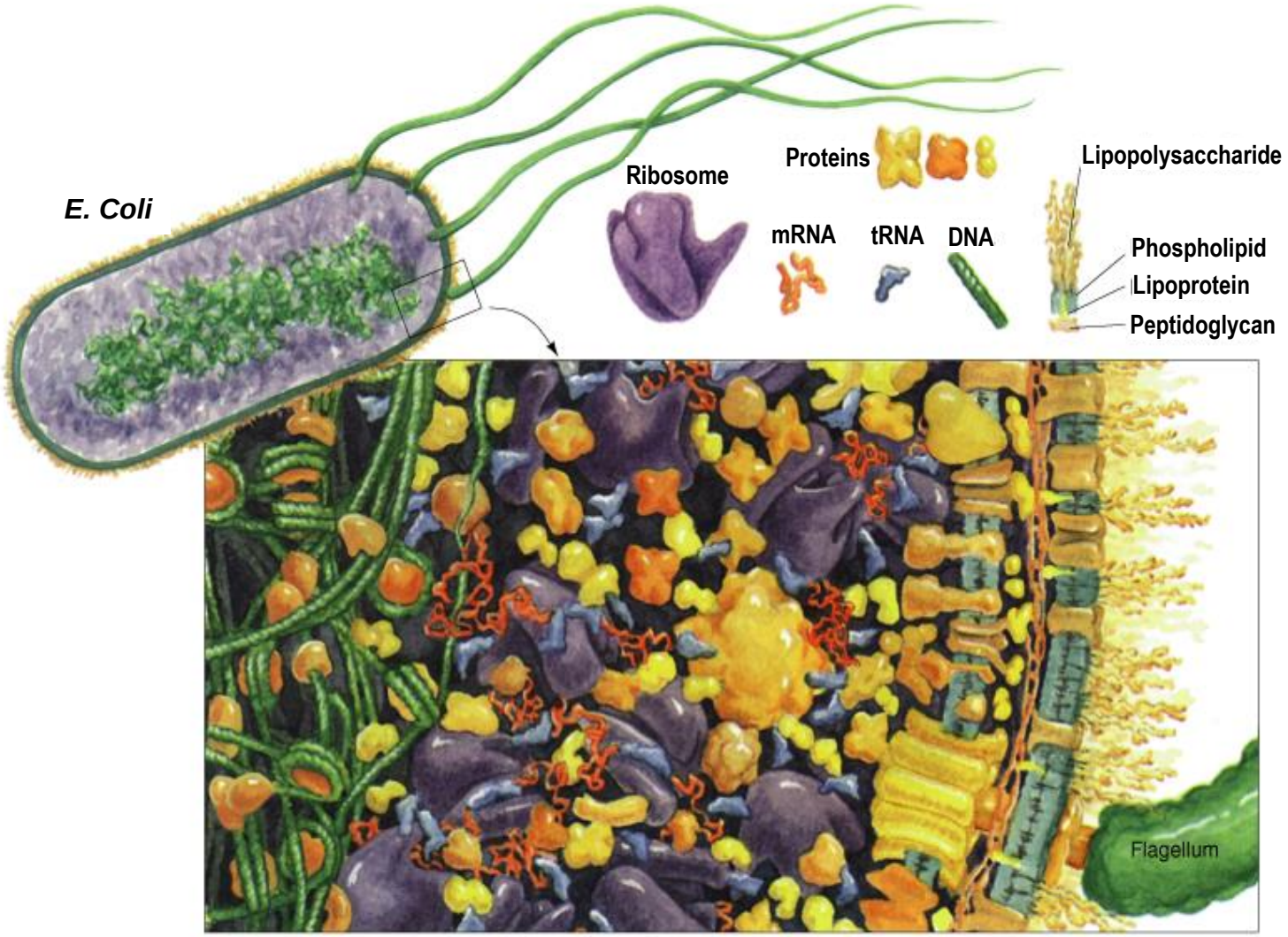
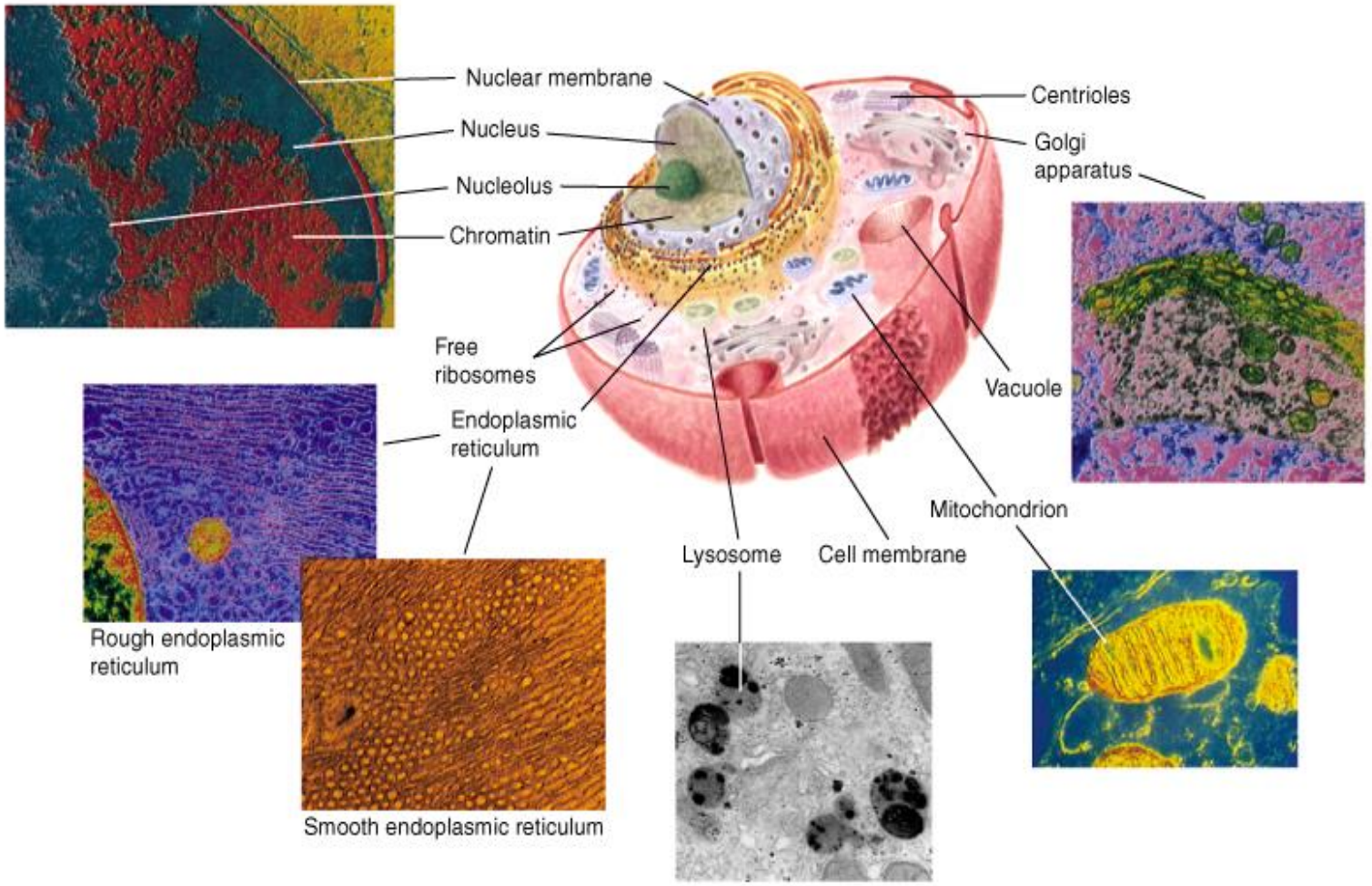
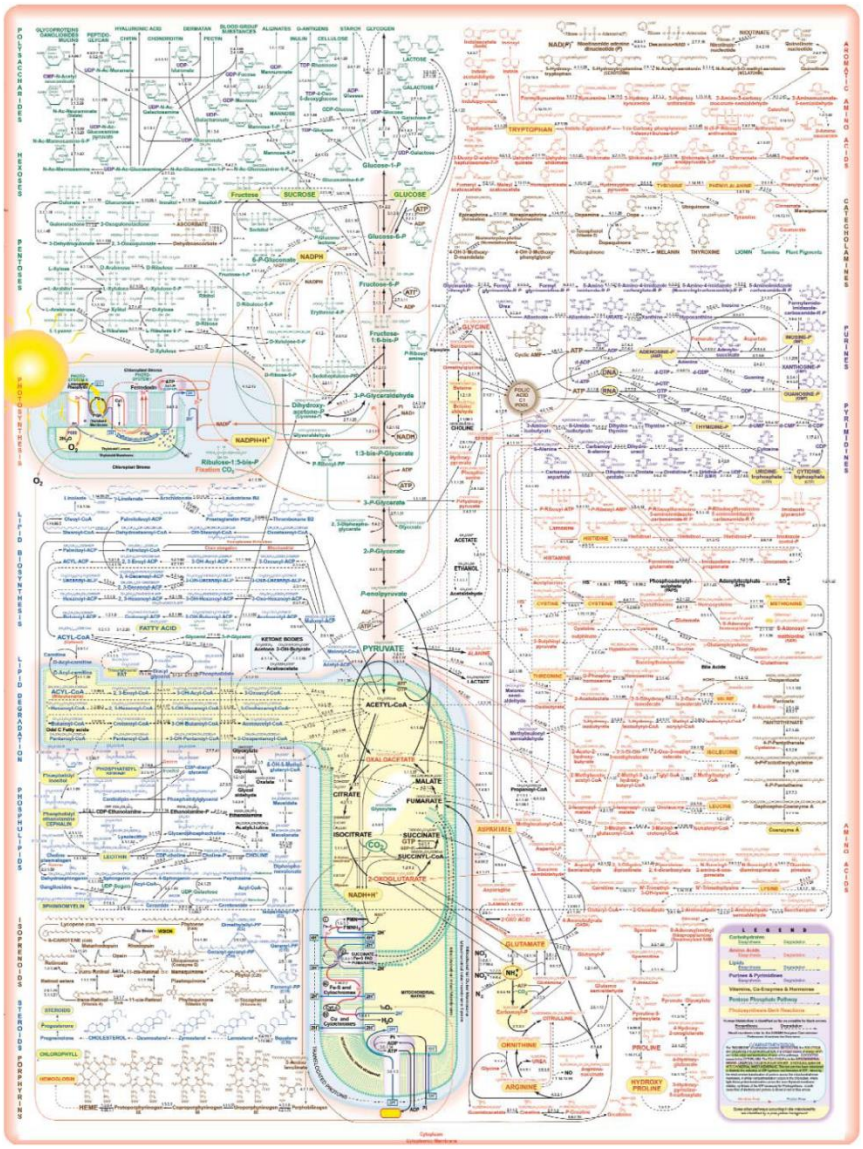


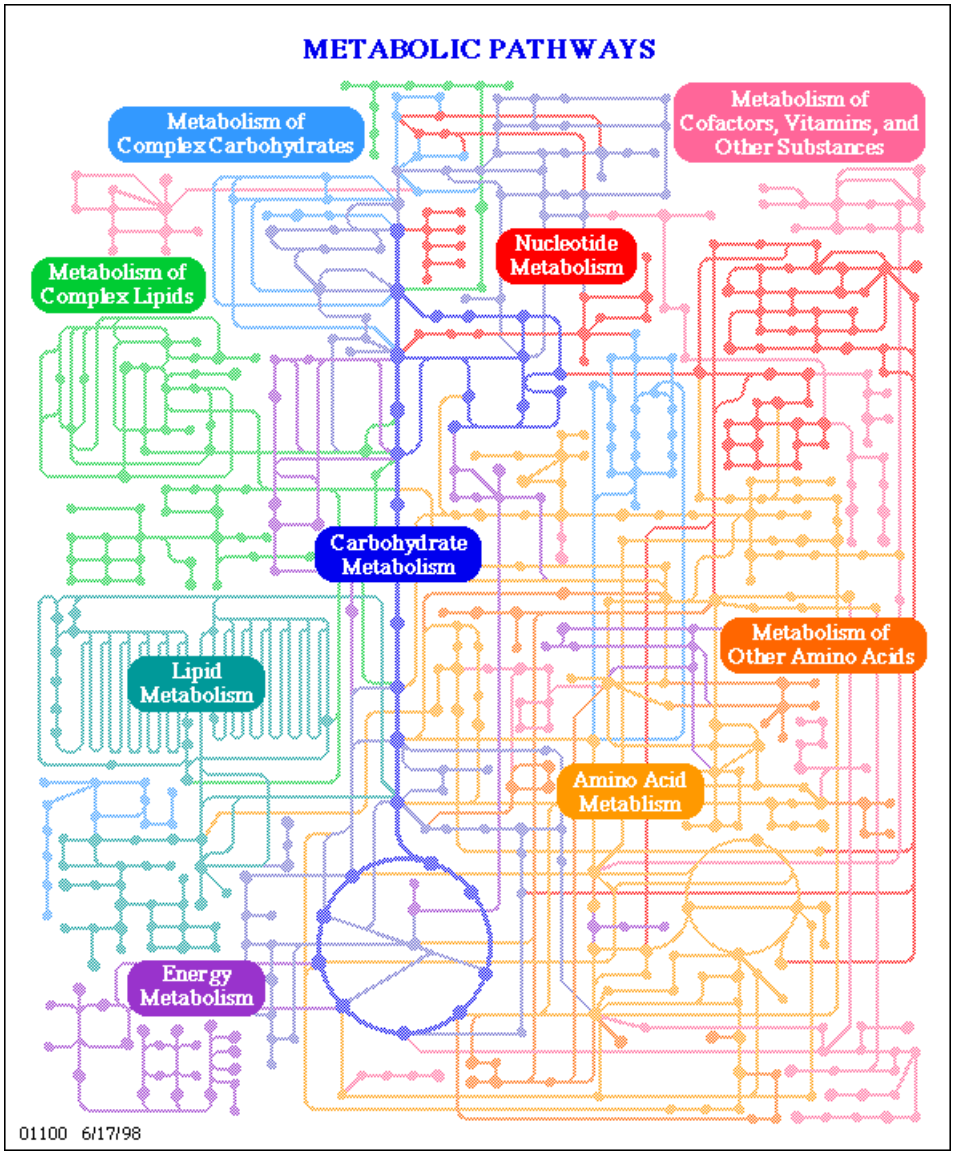
Figure 1-6. Cross section of an *E. coli* cell. [After a drawing by David Goodsell, UCLA.]

Eukaryotic Cells.



Cell Metabolism.



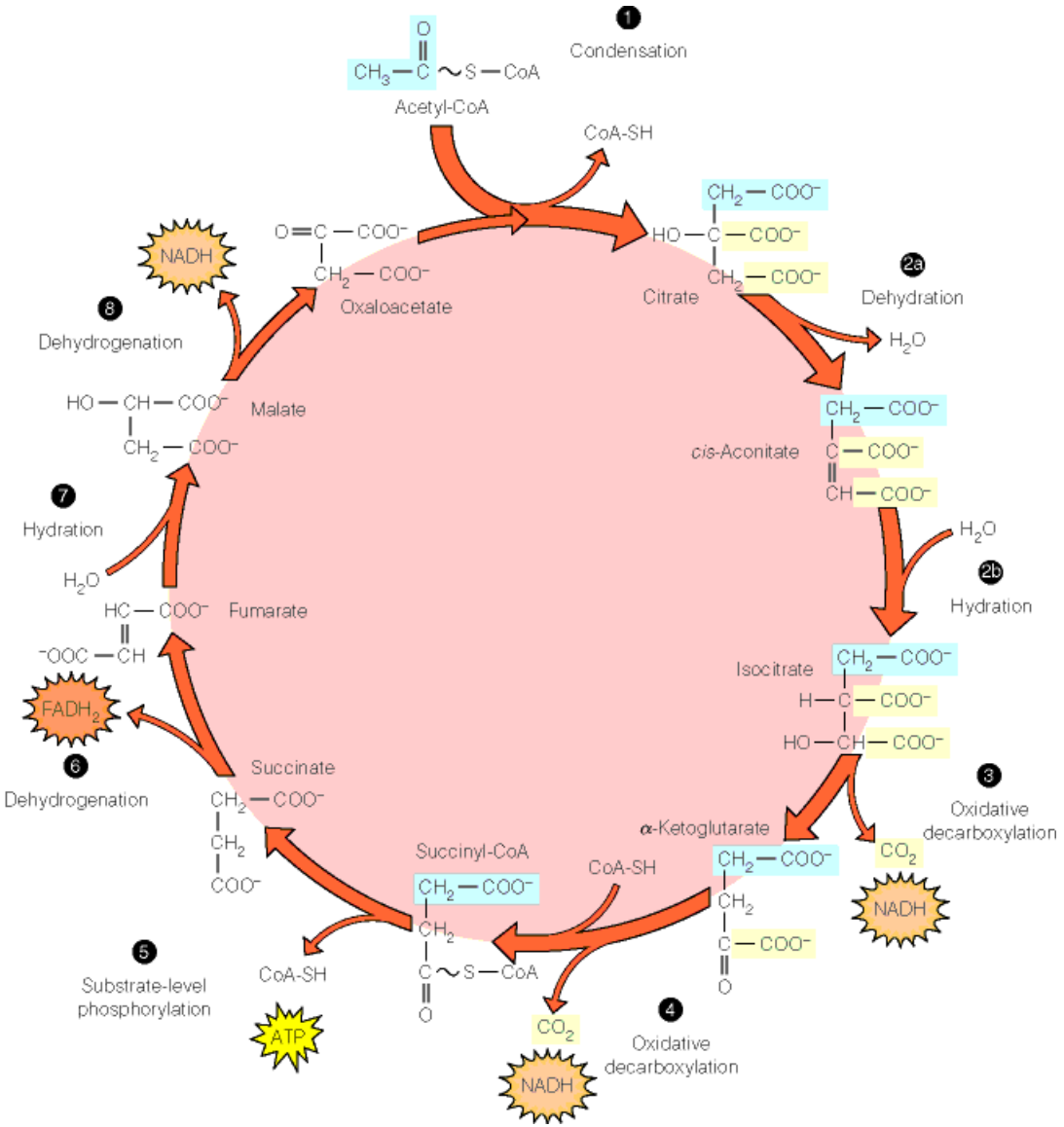


Cell Metabolism (Citric Acid, Cycle - CTA).

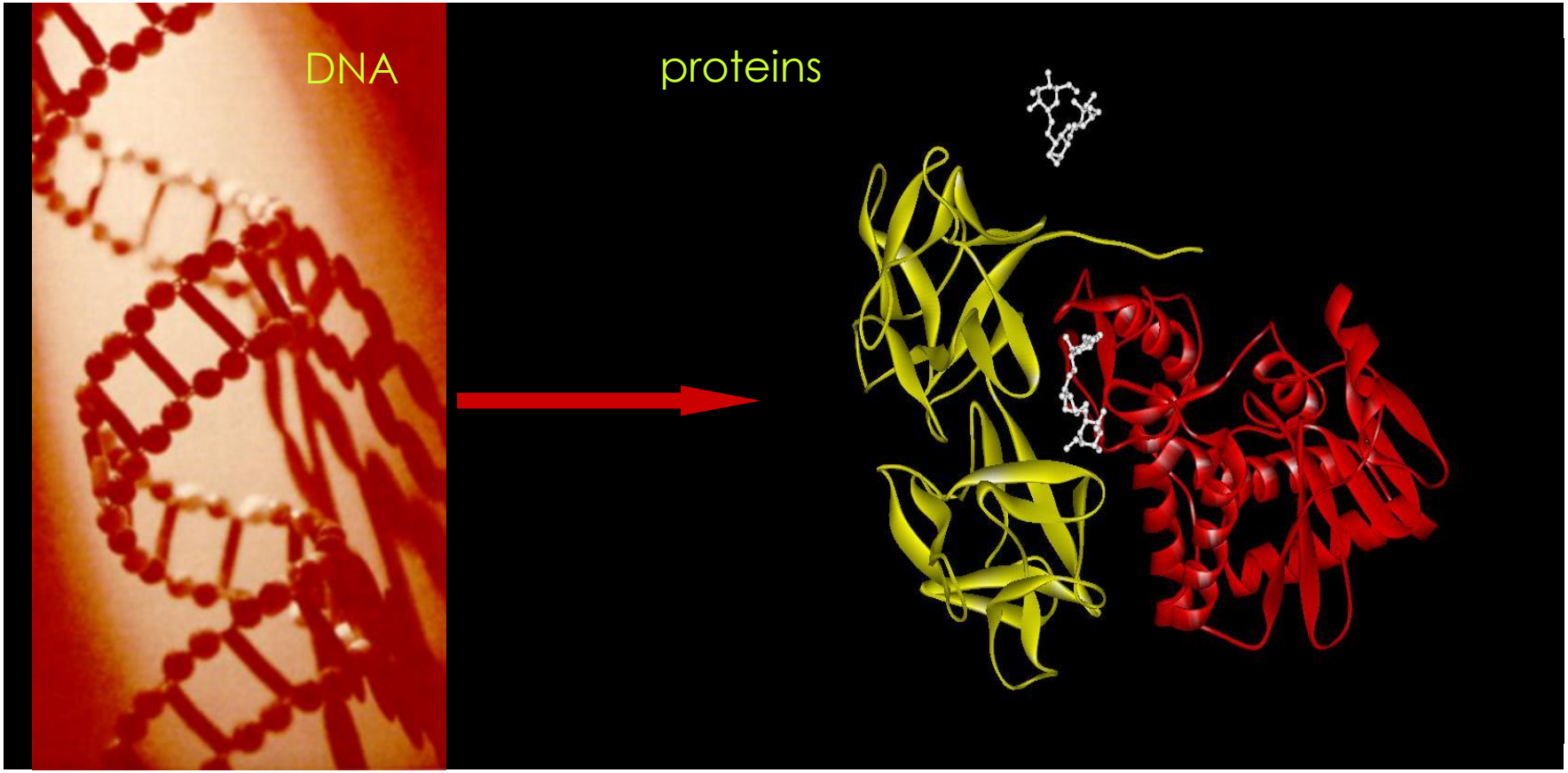
The metabolic oxidation of substrates occurs as a 3 step process:

- carbon is incorporated into acetyl-CoA;
- carbon is then oxidized to CO_2, reduced electron transfer agents and a small amount of ATP;
- the reduced electron transfer agents are reoxidized producing energy for the synthesis of further ATP (oxidative phosphorylation).

The activity of TCA cycle is favored by low ratio of $NADH/NAD^+$.



The Promise of Biotech.



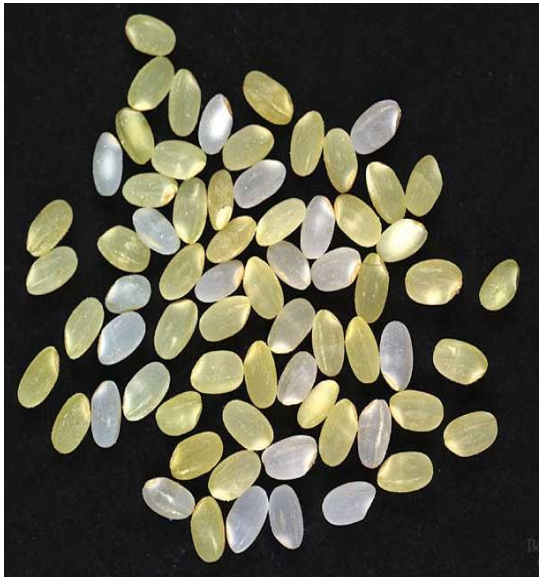
Drugs are so complex they can only be synthesized in a living system.

Biotech Results in the Vegetable Field.

- **Golden Rice**

Worldwide, 7% of children suffer vitamin A deficiency, many of them living in regions in which rice is a staple of the diet.

Golden rice (yellow) with standard rice (white).

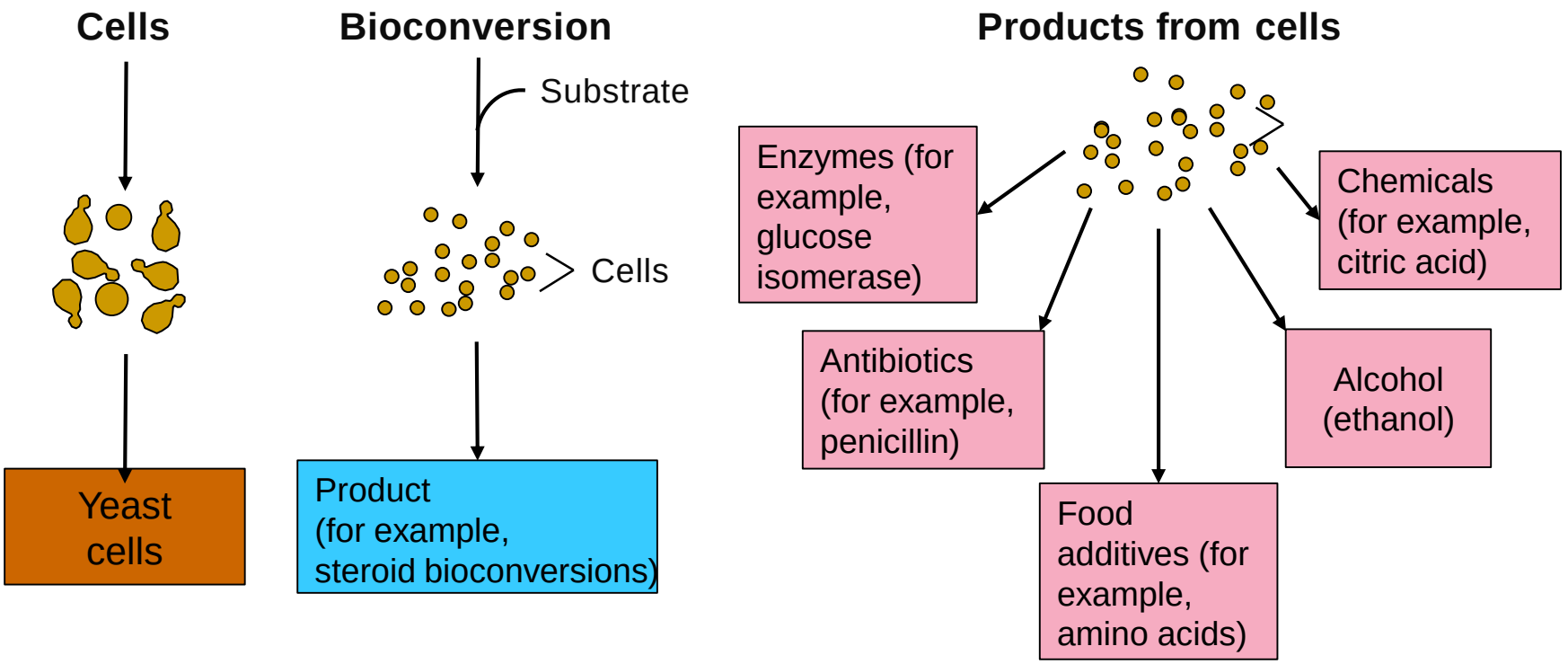


- **Mine Detection**

Mine detected

- Patented transgene added to plants
- When metal from mine is detected
  - ***Plant turns from green to red***
- Technology developed by ***Aresa Biodetection***

Products of Microbiology.



Industrial Products and the Microorganisms That Make Them.

- Properties of a useful industrial microbe include:
  - Produces spores or can be easily inoculated;
  - Grows rapidly on a large scale in inexpensive medium;
  - Produces desired product quickly;
  - Should not be pathogenic;
  - Amenable to genetic manipulation.
- Microbial products of industrial interest include:
  - Microbial cells;
  - Enzymes;
  - Antibiotics, steroids, alkaloids;
  - Food additives:
  - *Commodity chemicals:*
    - Inexpensive chemicals produced in bulk
    - Include ethanol, citric acid, and many others.

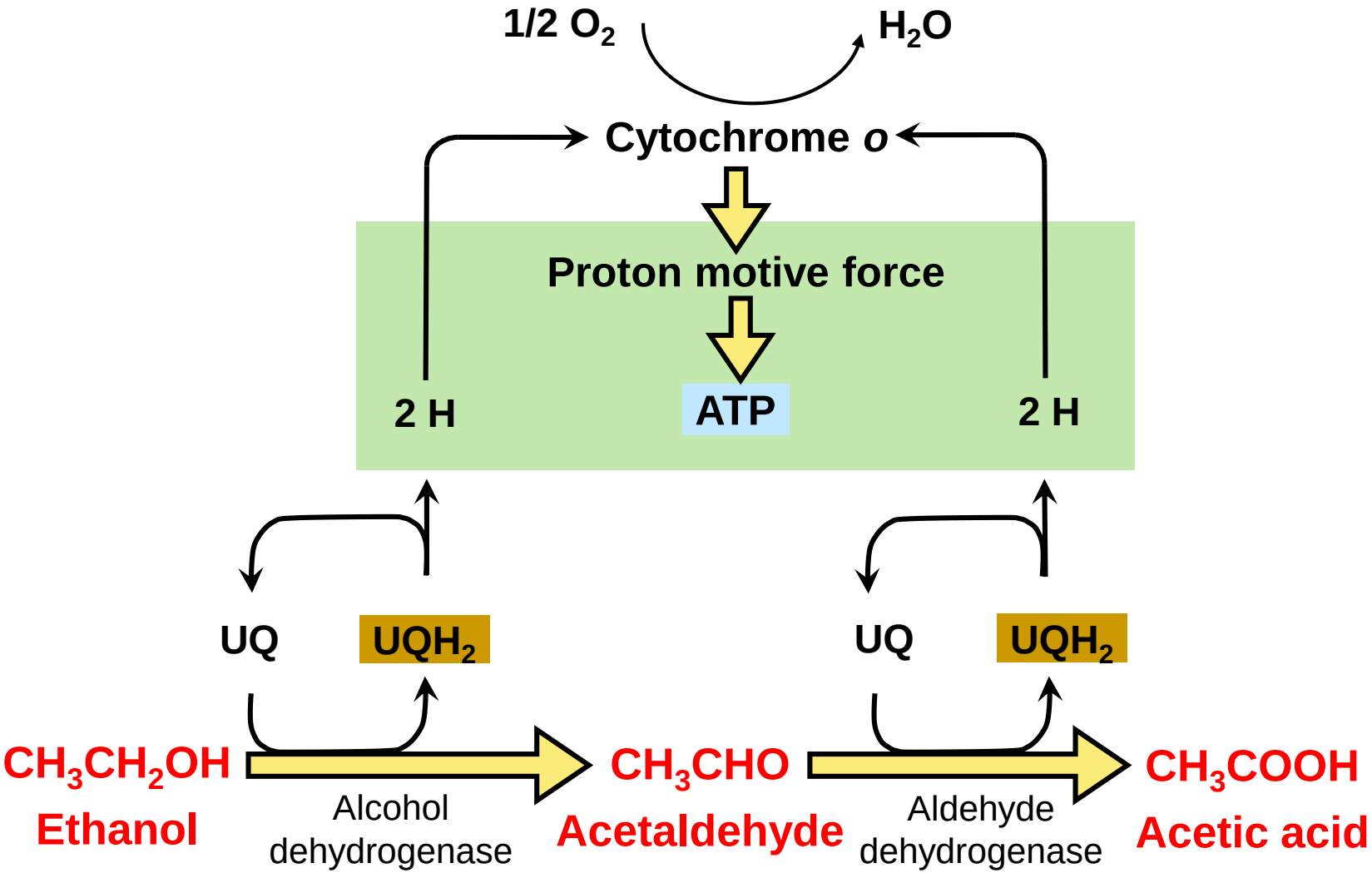
Production and Scale.

- *Fermenter* is where the microbiology process takes place;
- Any large-scale reaction is referred to as a fermentation:
  - Most are aerobic processes;
- Fermenters size: 5 - 500,000 liters:
  - Aerobic / anaerobic fermenters;
- Large-scale fermenters are almost always stainless steel:

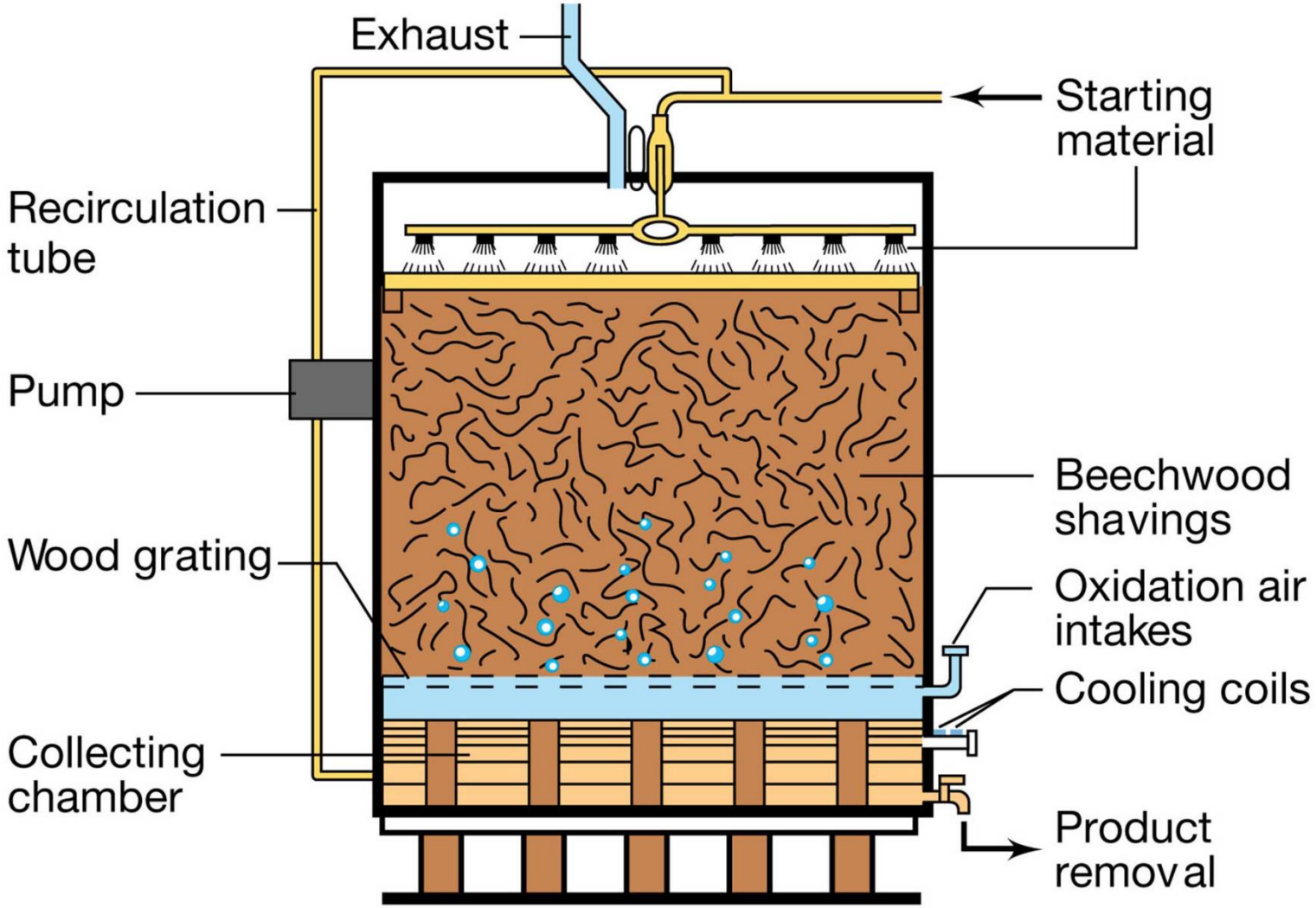
  - Impellers and spargers supply O_2.

(a)

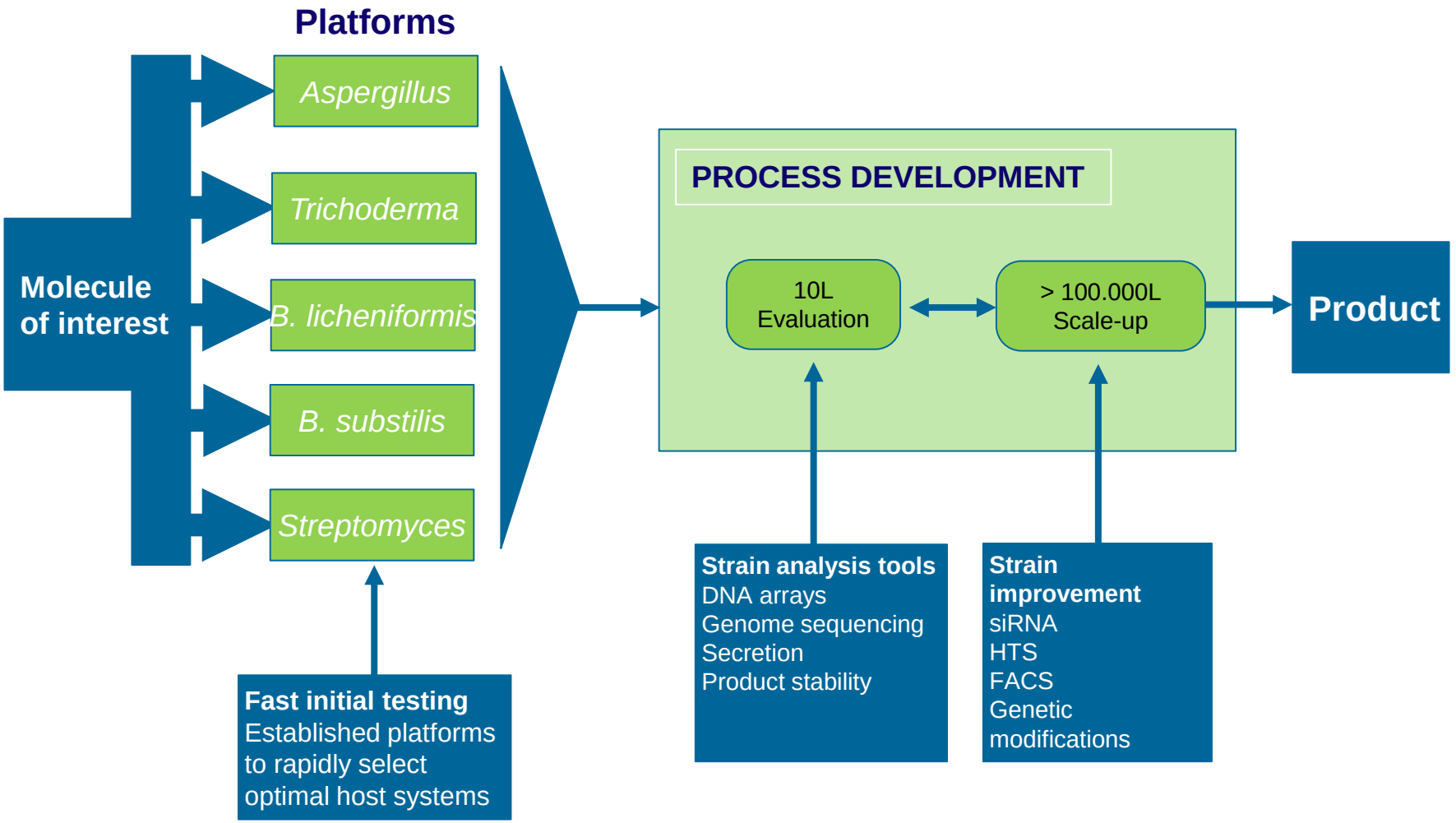
Production of Vinegar.



Production of Vinegar (2).

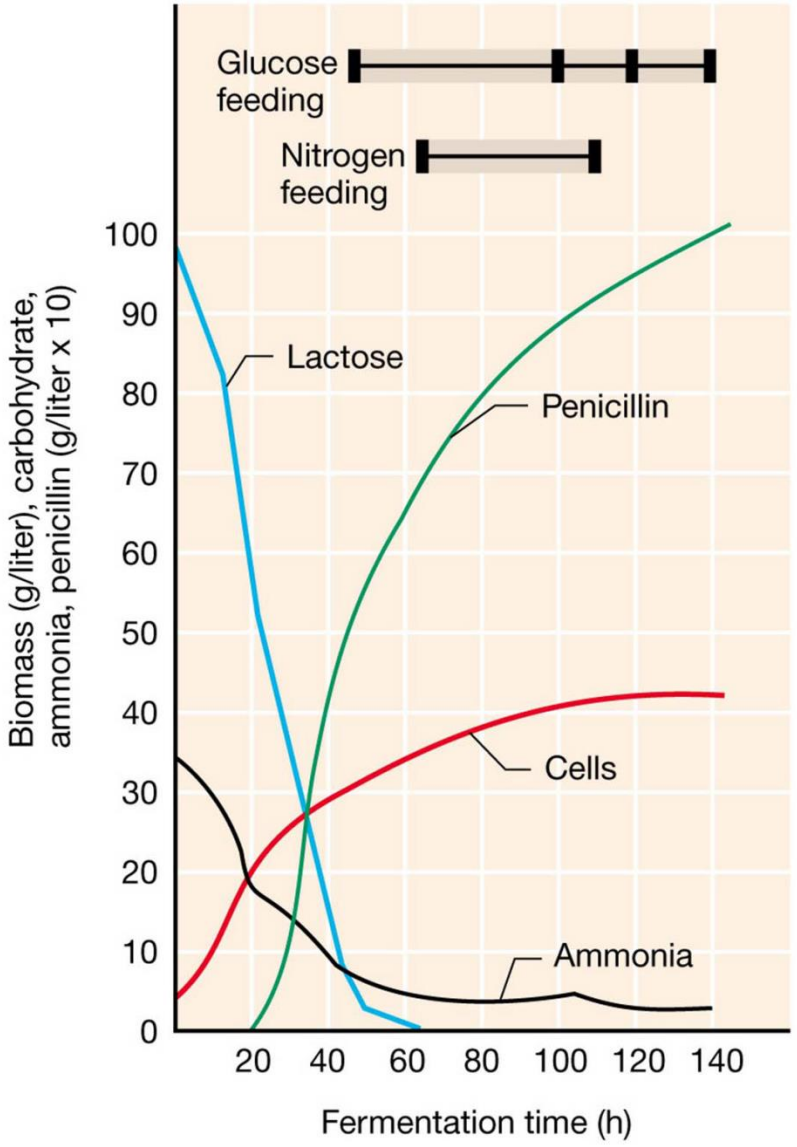


Innovation in Enzyme Production Systems.

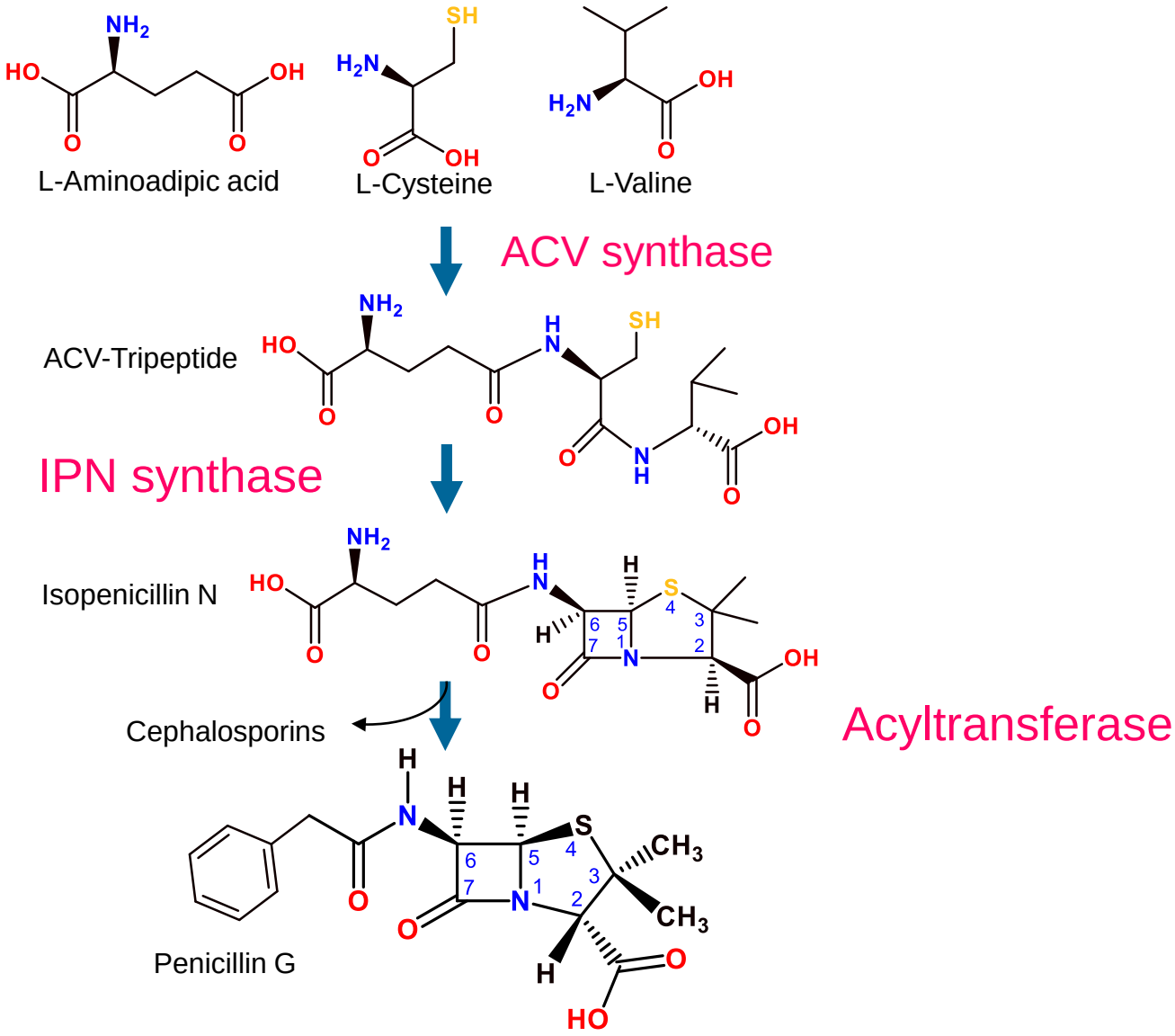


Antibiotics.

- Requires precise control of nutrients;
- Final product can be modified to yield a variety of semisynthetic penicillins.



Biosynthetic Pathway of Penicillin.



Industrial Production of Penicillins.

Penicillin fermentation

Biosynthetic penicillin I

Add precursor I

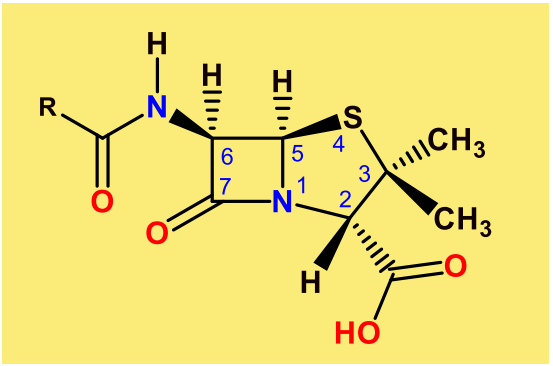
Add precursor II

Biosynthetic penicillin II

Add precursor III

Biosynthetic penicillin III

Chemical or enzymatic treatment of penicillin G



6-Aminopenicillanic acid

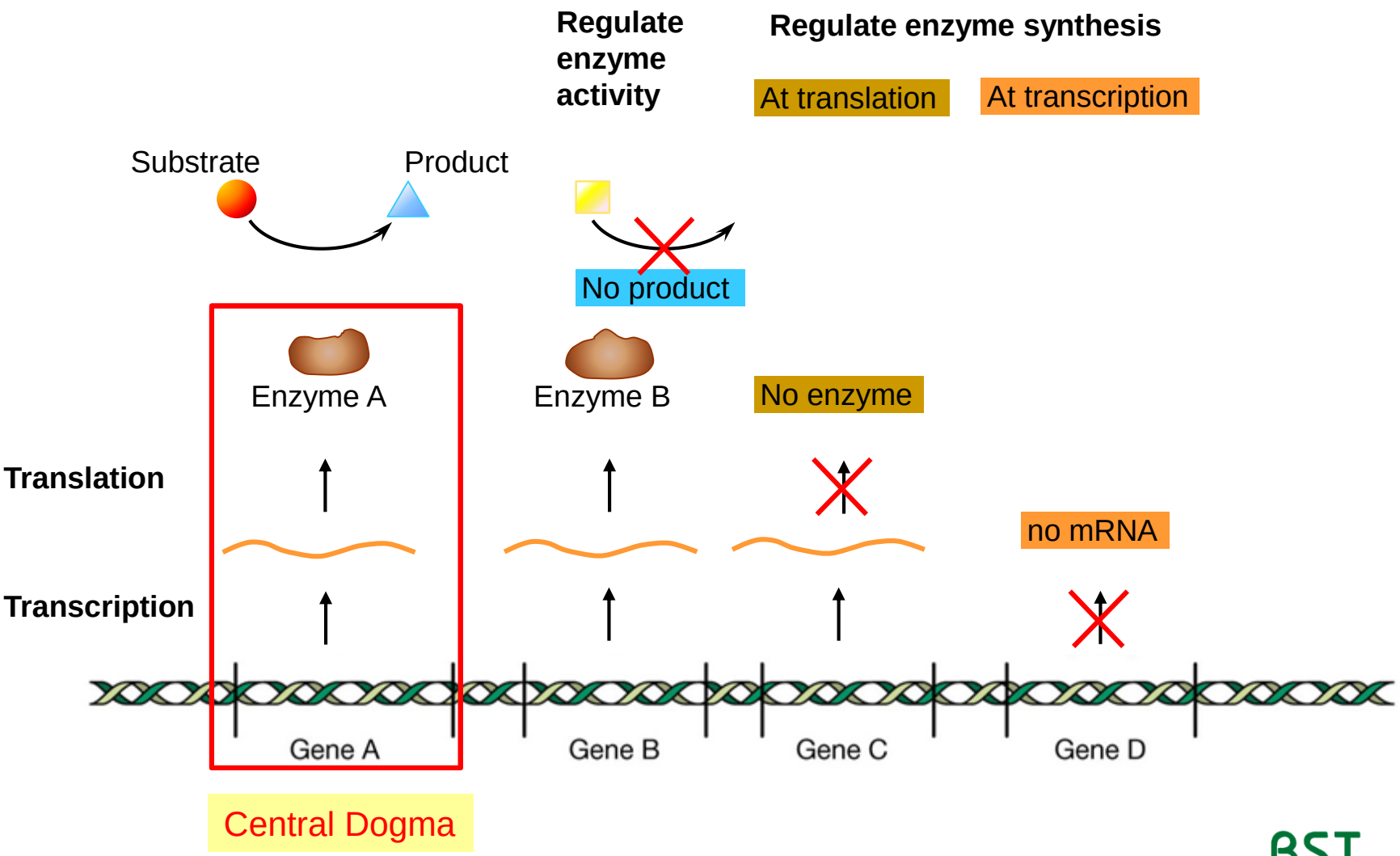
Add side chains chemically

Semisynthetic penicillins (for example, ampicillin, amoxycillin, methicillin)

Natural penicillins (for example, penicillin G)

BST

Regulation Mechanisms.



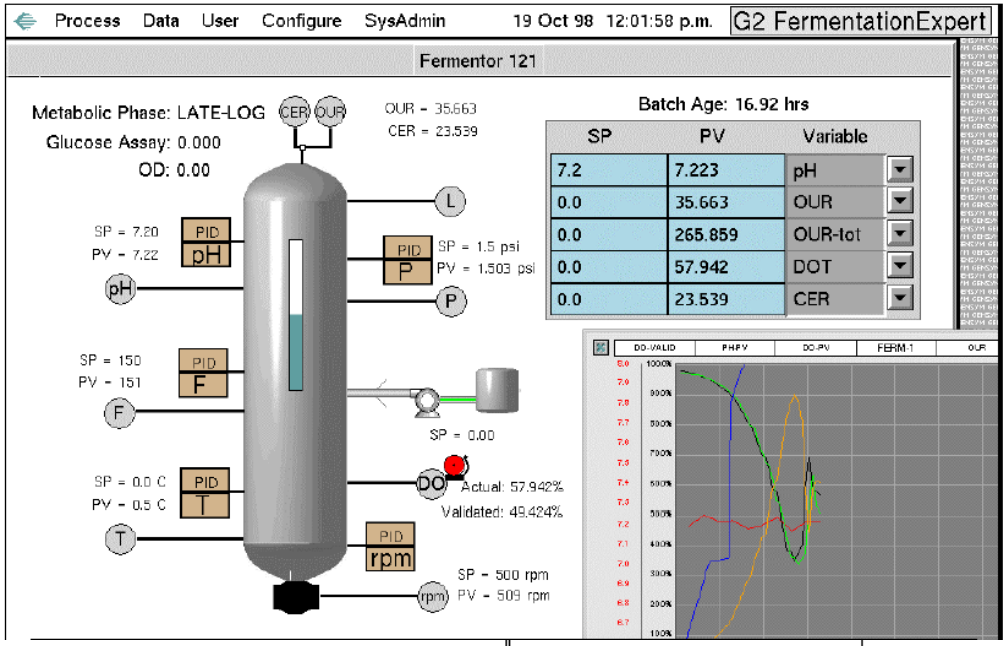
BST

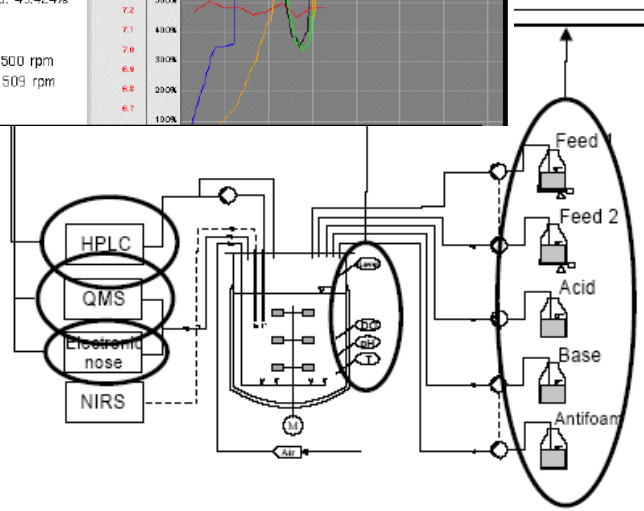
Modification of Gene Expression.

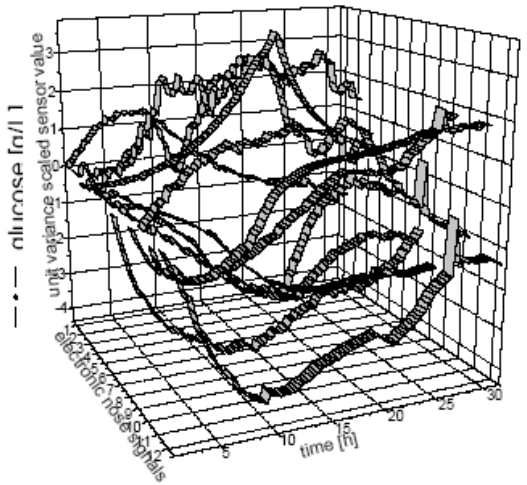
Allows for overproduction of a product, production of more than one product by the same organism, or synthesis of modified products:

- Pathway architecture
  - analysis, design, and modification of biochemical pathways to increase process efficiency.
- Metabolic pathway engineering
  - intentional alteration of metabolic pathway by inactivation of specific genes.
- Metabolic control engineering
  - alteration of control mechanisms of specific genes.

Expert Systems in Biotechnology.



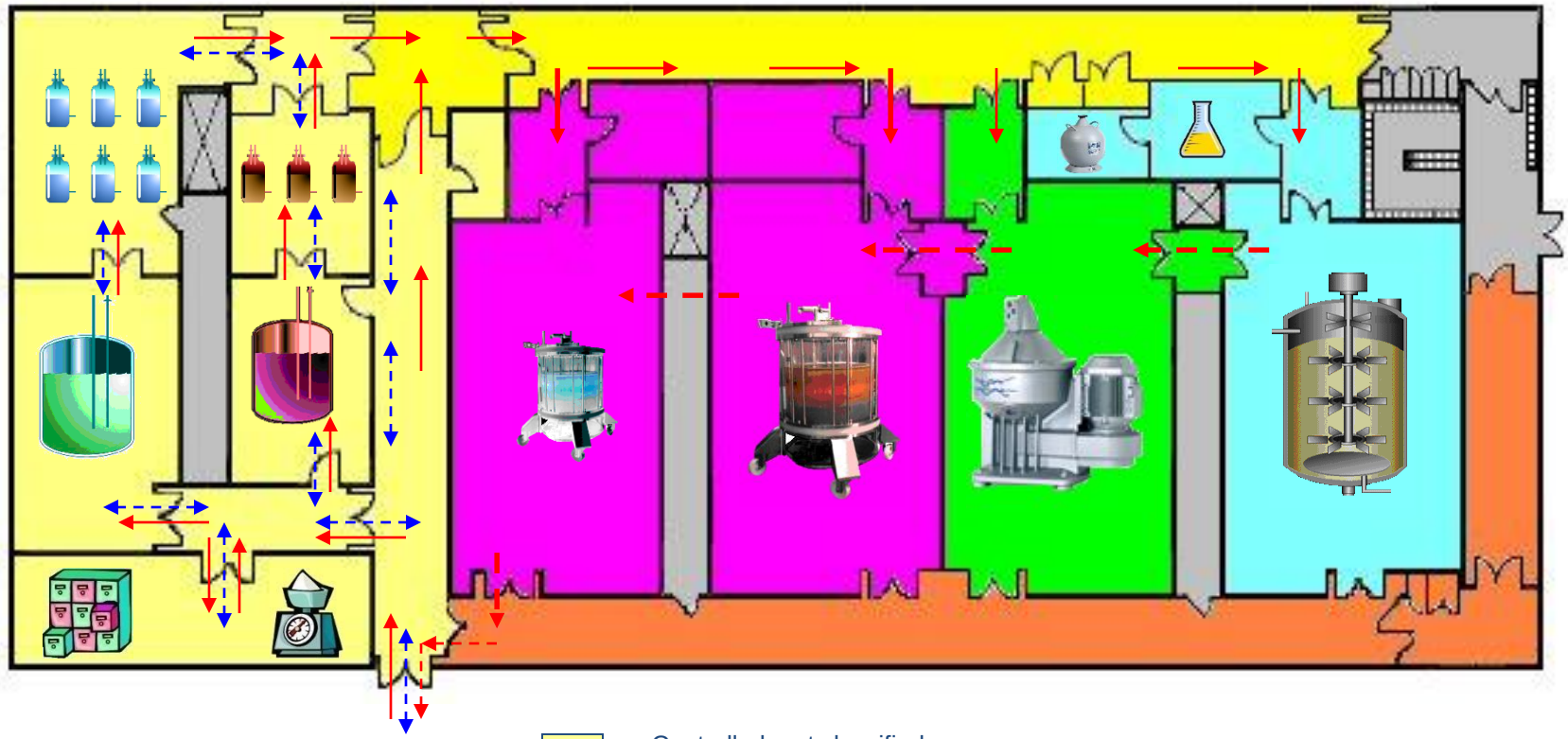




On line controls

Information flow

Production Structure on Biotechnological Pilot Scale.



Controlled, not classified

Classified areas
(Clean rooms)

Exit (“dirty”) corridor

Utility (“grey”) spaces

Bioprocessing personnel

Central Services personnel

Raw materials

Intermediates and Bulk product

POLITECNICO DI MILANO

School of Industrial and Information Engineering

Course 096125 (095857)

Introduction to Green and Sustainable Chemistry



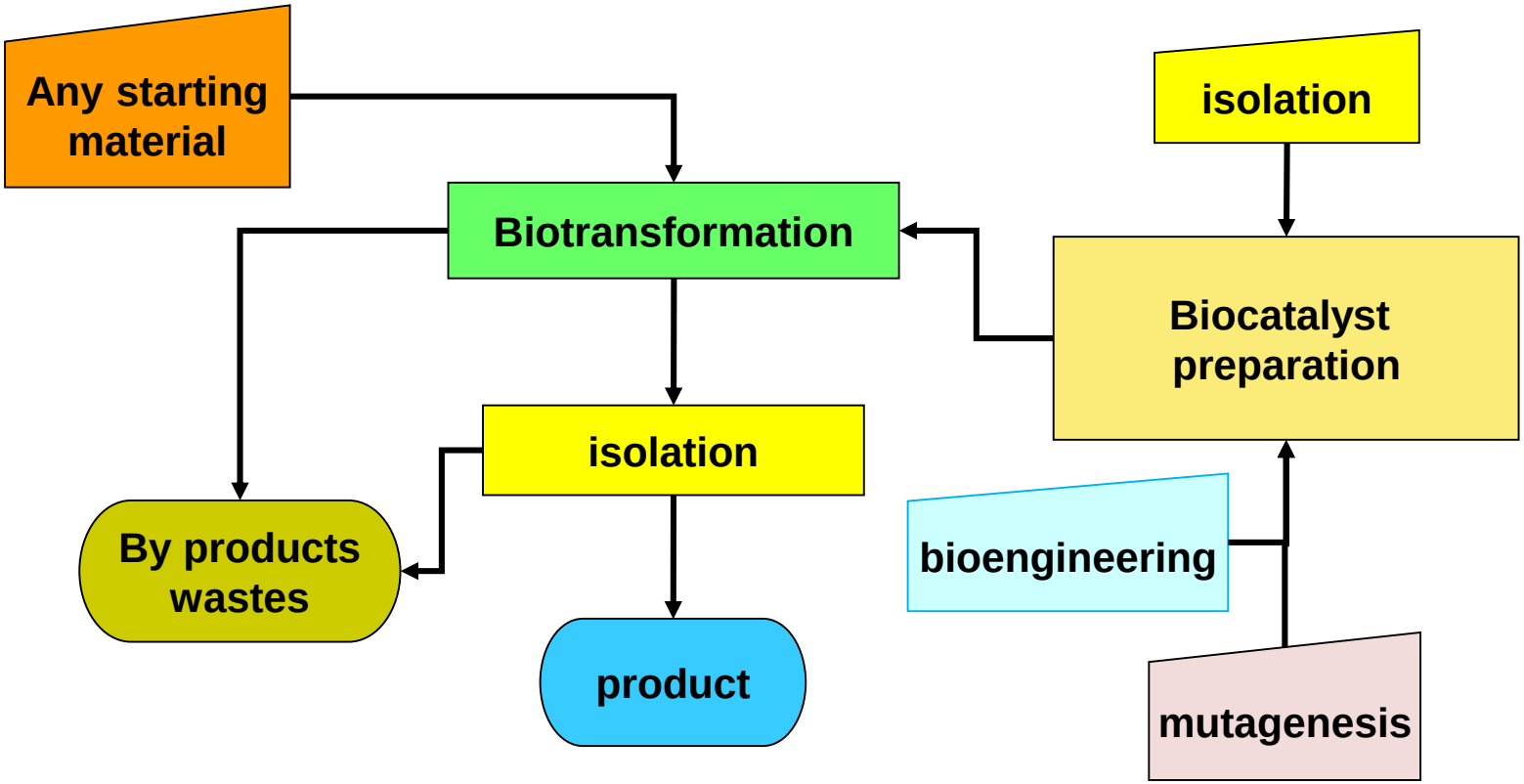


Bioconversions.

(transformation of one chemical into another using a biocatalyst)

Biotransformations and Bioprocesses.

"the process whereby a material is converted into another using biological agents (e.g. microorganism, plant or animal cell), a vital extract from such cells, or a (partly) purified enzyme. This field combines chemical engineering, microbiology and biochemistry."



Enzymes: Sources and Uses.

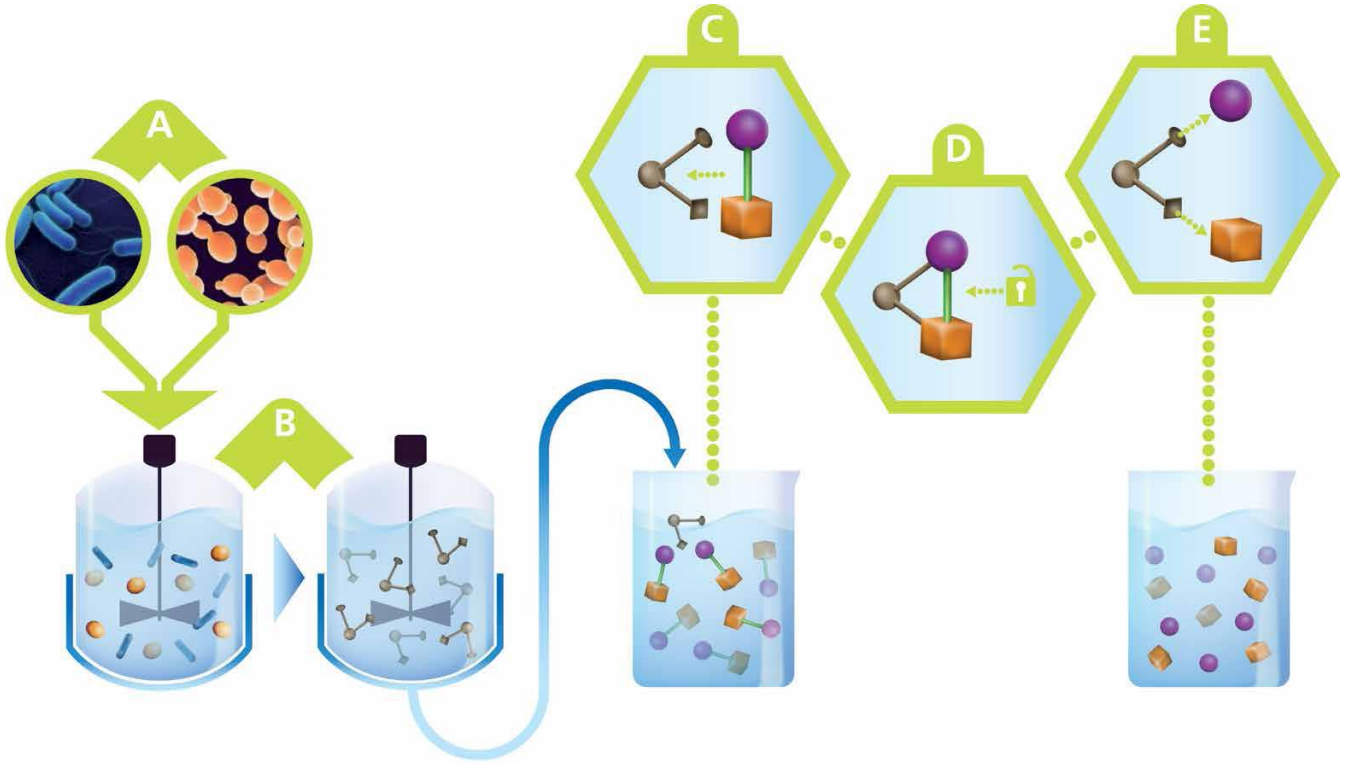
A. Microorganisms can be used to produce natural catalysts such as enzymes;

B. The enzymes are purified from the microorganism for industrial use;

C. An enzyme attracts specific substrates to its active site;

D. It catalyzes the chemical reaction by which products are formed;

E. It then allows the products to separate from the enzyme surface.



Catalytic Power of Enzymes.

Enzyme	Nonenzymatic Reaction Rate (s^{-1})	Enzymatic reaction Rate (s^{-1})	Rate enhancement
Carbonic anhydrase	1.3×10^{-1}	1×10^{6}	7.7×10^{6}
Chorismate mutase	2.6×10^{-5}	50	1.9×10^{6}
Triose phosphate isomerase	4.3×10^{-6}	4300	1.0×10^{9}
Carboxypeptidase	3.0×10^{-9}	578	1.9×10^{11}
AMP nucleosidase	1.0×10^{-11}	60	6.0×10^{12}
Staphylococcal nuclease	1.7×10^{-13}	93	5.6×10^{14}

Source: Radzicka, A.; Wolenden R. Science, 267, 91 (1995).

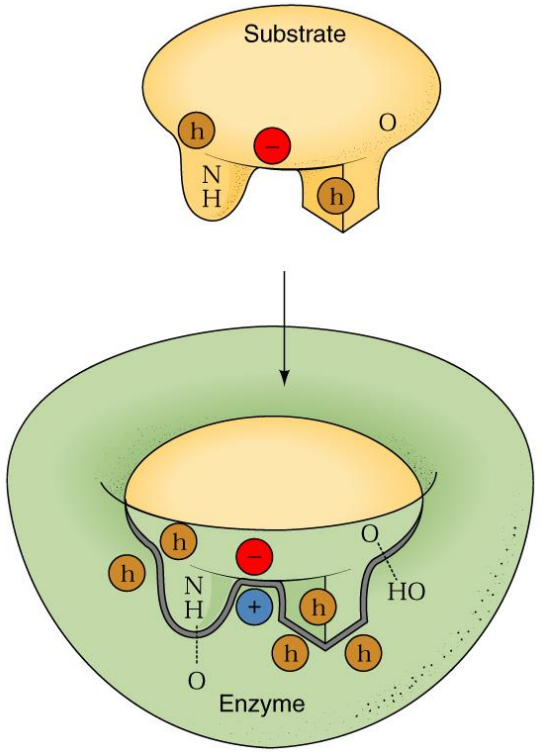
Purification and Enzyme Functions.

Purification:

- Certainly a good option for several enzymes;
- Are expensive;
- Seldom unpractical if cofactors are required (i.e. redox reactions).

Function :

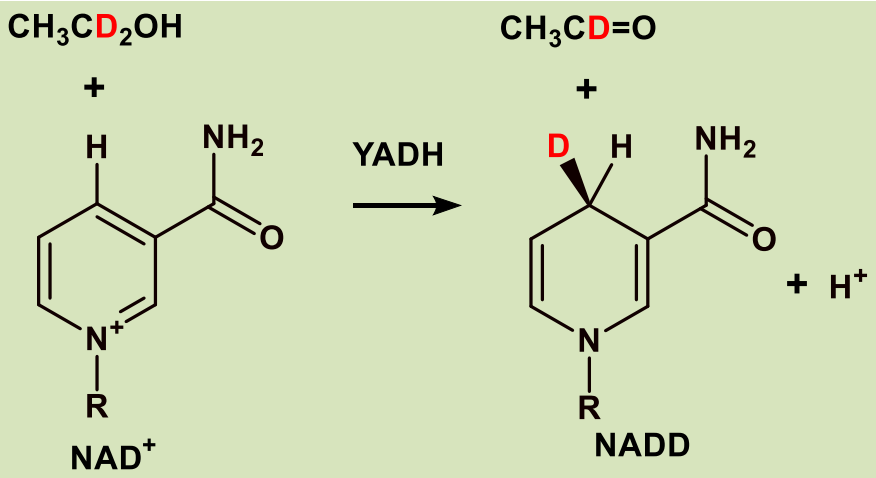
- Substrate recognition;
- Catalysis (decrease of E_{att} or S_{att});
- Selectivity.

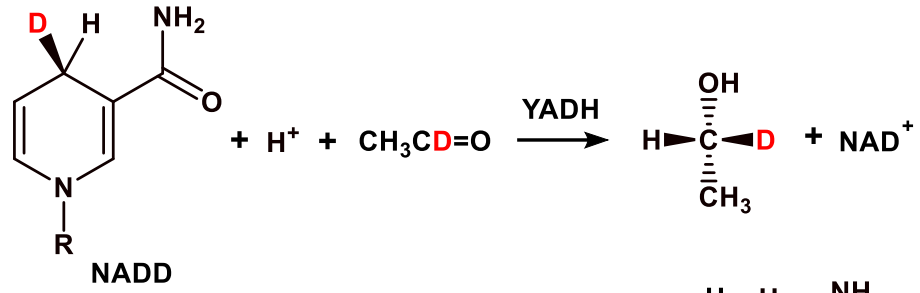


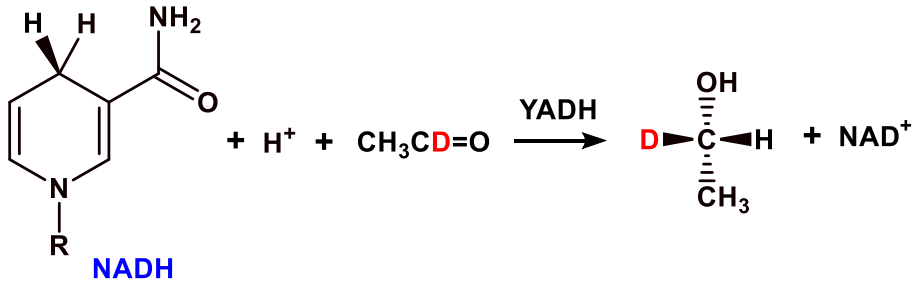
<u>Substrate specificity</u>:

- Geometric complementarity;
- Electronic complementarity;
- Induced fit.

Stereo-Specificity.







Where Obtain Enzymes: From Complex Animals?

- Society in general is less frighten from macroscopic forms of life than members of microscopic world;
- People have been using animals to for biotrasformations for a long time;
- Many ethical and environmental concerns associated;
- Probably expensive.

… and on Plant Use?

- Great diversity and metabolic capacity;
- **Less** ethical and environmental concerns associated;
- Probably inexpensive;
- Most old and new reports refer to suspended plant cells;
- That is just the same us using microorganisms but actually more complicated:
  - Slow growing;
  - Frequent contamination;
  - Special equipment required;
  - Incredible expensive.

Plant Parts as Chemical Reactions.

The problems can be overcome if you use the “plant itself” (or a part of it) instead of working with a cell culture:

- Cells are already grown;
- No special equipment;
- No contamination;
- Cofactors are there;
- Little environmental concerns;
- Very small budget.

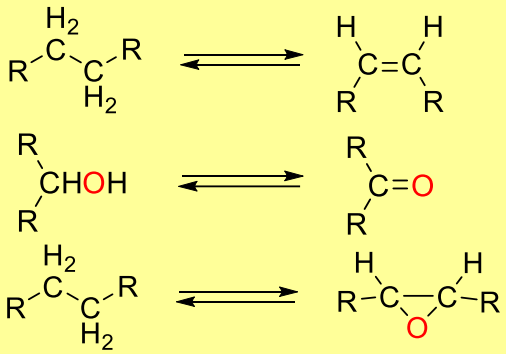
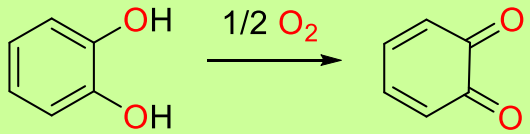
The IUB Number and Classification of Enzymes.

Main Classes and Subclasses	Main Classes and Subclasses
1: Oxidoreductase 1.1: acts on the CH-OH group of donors 1.2: acts on the aldehyde or keto group of donors 1.3: acts on the CH-CH group of donors 1.4: acts on the CH-NH_2 group of donors 1.5: acts on the C-NH group of donors 1.6: acts on (reduced) NADH or NADPH as a donor of H- 1.7: acts on other nitrogenous compounds as donor 1.8: acts on sulphur groups as donor 1.9: acts on heme groups as donor 1.10: acts on diphenols and related substances as donor 1.11: acts on H_2O_2 as electron acceptor 1.12: acts on H_2 as donor 1.13: acts on single donors with incorporation of oxygen (oxygenases) 1.14: acts on paired donors with incorporation of oxygen into one donor (hydrolase). **2: Transferase** 2.1: transfers one-carbon group 2.2: transfers aldehyde or ketone 2.3: acyltransferase 2.4: glycosyltransferase 2.5: transfers other alkyl groups 2.6: transfers nitrogenous groups 2.7: transfers phosphorous-containing groups 2.8: transfers Sulphur-containing groups	**3: Hydrolase** 3.1: hydrolysis of the ester bond 3.2: hydrolysis of the glycosylic bond 3.3: hydrolysis of the ether bond 3.4: hydrolysis of the peptide bond 3.5: hydrolysis of C-N bond other than the peptide bond 3.6: hydrolysis of the acid-anhydride bond 3.7: hydrolysis of C-C bond 3.8: hydrolysis of the C-halide bond 3.9: hydrolysis of the P-N bond **4: Lyase** 4.1: lysis of C-C bond 4.2: lysis of C-O bond 4.3: lysis of C-N bond 4.4: lysis of C-S bond 4.5: lysis of C-halide bond 4.99: others **5: Isomerase** 5.1: racemization and epimerization 5.2: *cis-trans* isomerization 5.3: intramolecular oxidoreduction, e.g. aldehyde-ketone, keto-enol, double bond migration 5.4: intramolecular group transfers 5.99: other isomerizations **6: Ligase** 6.1: formation of C-O bond 6.2: formation of C-S bond 6.3: formation of C-N bond 6.4: formation of C-C bond

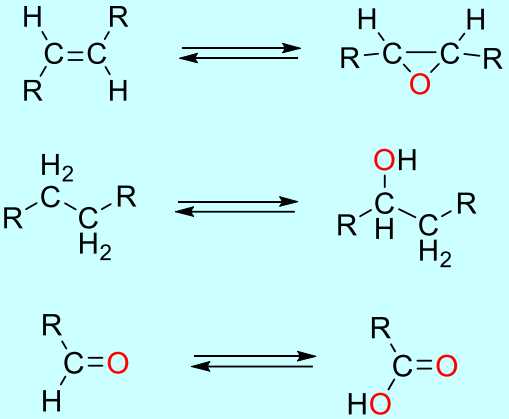
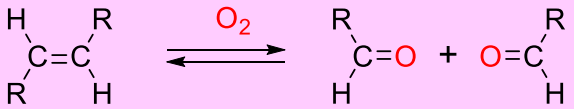
Enzyme Classification According to Reaction Type.

CLASSIFICATION	Type of CATALYZED REACTION
1. **Oxidoriductases**	oxidation-reduction: transfer of e^- from a donor which is oxidized to an acceptor which is reduced
2. **Transferases**	transfer of functional groups
3. **Hydrolases**	hydrolysis, for example, of ester or amide groups, or esterification
4. **Lyases**	elimination of a group of adjacent groups of atoms to form a double bond, or addition of a group of atoms to a double bond
5. **Isomerases**	conversion of a compound into its Isomer
6. **Ligases**	Bond formation coupled with ATP hydrolysis; also known as synthases

The Four Major Types of Biological Oxidation Reactions Catalyzed by Oxidoreductases.

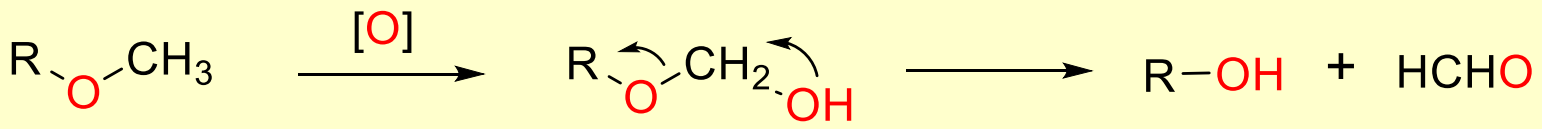
Type of Oxidation	Description	Schematic Reaction and Examples
Dehydrogenase	Removes of two H atoms from the substrate, and transfers this to another organic compound. The H-acceptor, **A**, is a coenzyme.	**S**H_2 + **A** ⇄ **S** + **A**H_2 
Oxidase	Removes two H atoms from the substrate and utilizes O_2 or H_2O_2 as the H-acceptor.	**S**H2 + ½O_2 ⇄ **S** + H_2O **S**H_2 + H_2O_2 ⇄ **S** + 2H_2O 

The Four Major Types of Biological Oxidation Reactions Catalyzed by Oxidoreductases.

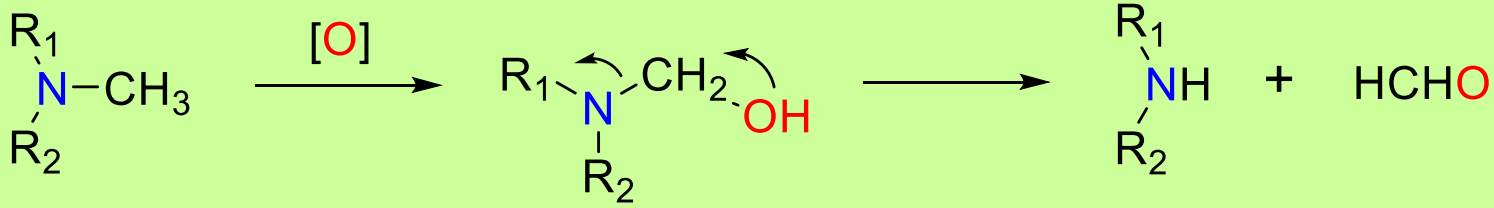
Type of Oxidation	Description	Schematic Reaction and Examples
Monooxygenase	Adds one O atom to the substrate. **A** is a coenzyme.	**S** + **A**H_2 + O_2 ⇄ **S**O + **A** + H_2O 
Dioxygenase	Adds two O atoms to the substrate	**S** + O_2 ⇄ SO_2 

Elimination and Rearrangement Reactions Following Oxidation.

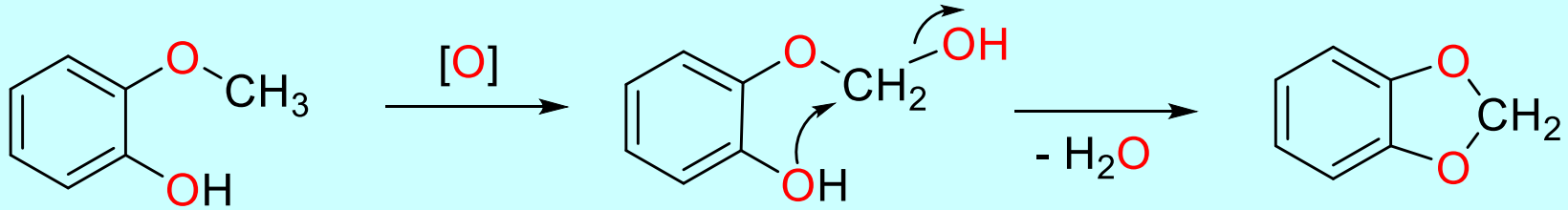
A. Demethylation: Methyl ether to alcohol



B. Demethylation: Methyl amine to amine

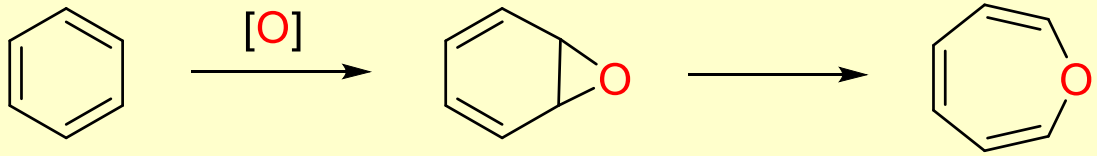


C. Formation of phenyl methylendioxy ring

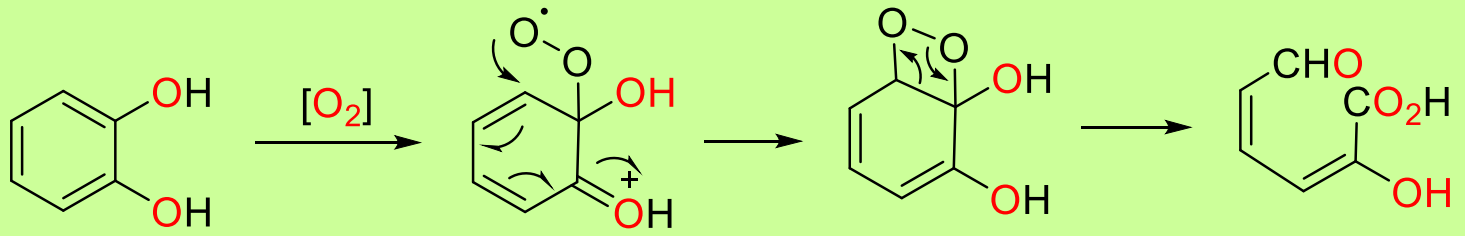


Elimination and Rearrangement Reactions Following Oxidation (2).

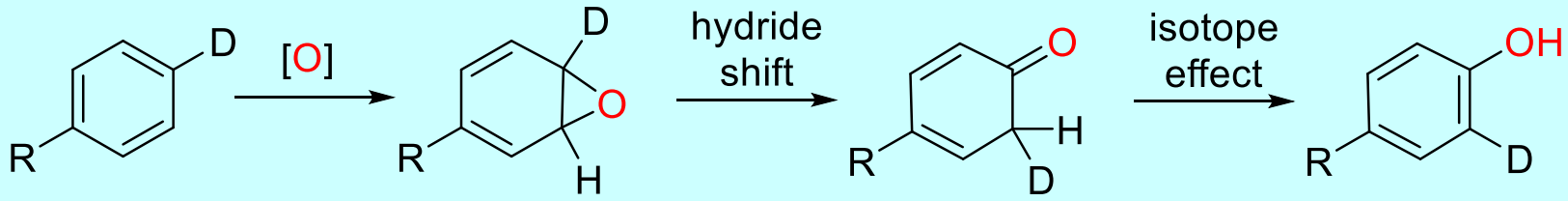
D. Aromatic ring opening reaction (mono-oxygenase)



E. Aromatic ring opening reaction (di-oxygenase)

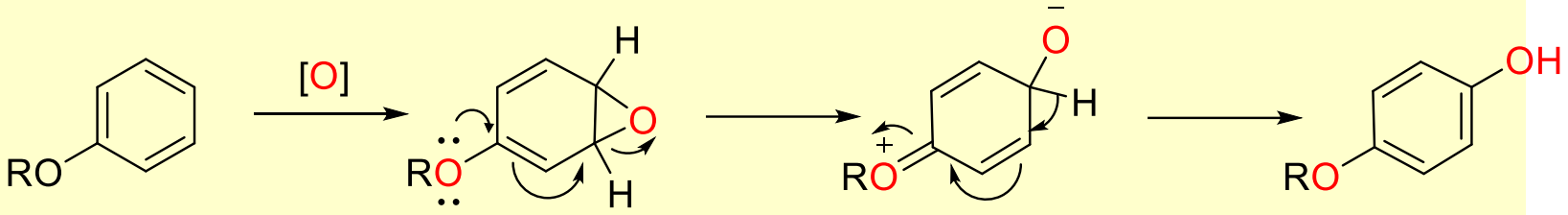


F. Oxidation of aromatic ring: NIH shift (hydride shift; R = alkyl group)

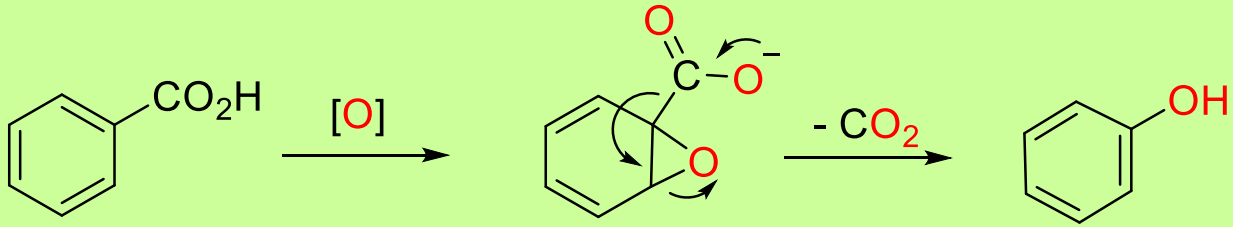


Elimination and Rearrangement Reactions Following Oxidation (3).

G. Para oxidation of aromatic ring.

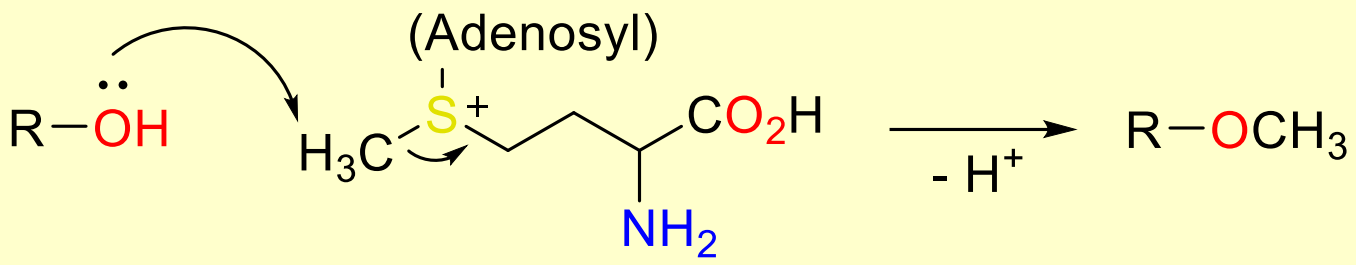


H. Oxidative decarboxylation of aromatic carboxylic acid.

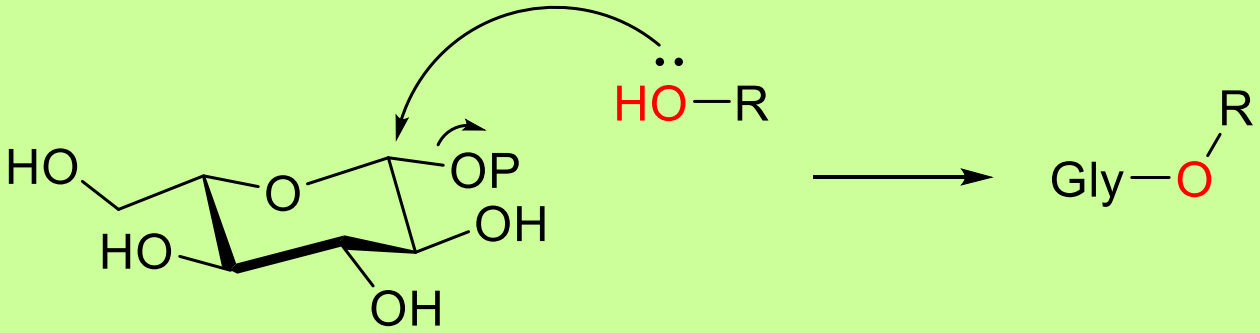


C-C bond Formation by SN2 Displacement of a Stable Nucleophile on an Electrophilic Alkylating Agent (2).

A. Methylation of alcohol or amine with S-adenosyl-L-methionine as alkylating agent

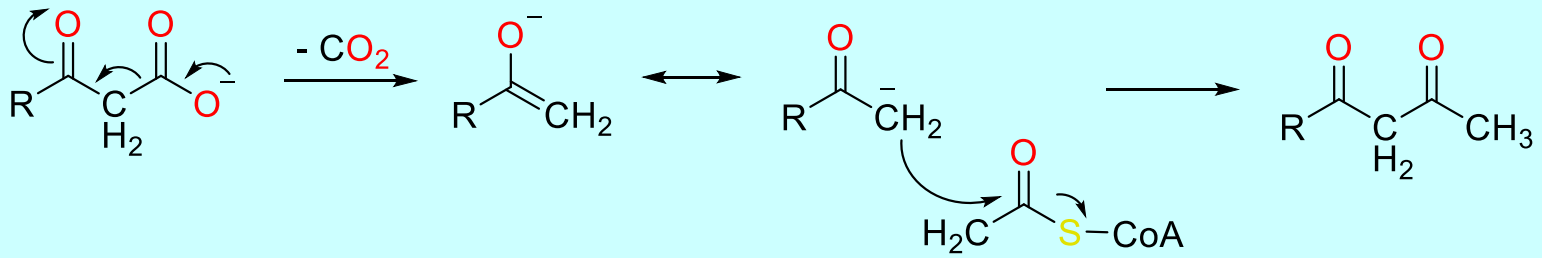


B. Glycosylation of an alcohol with glycosyl phosphate as alkylating agent

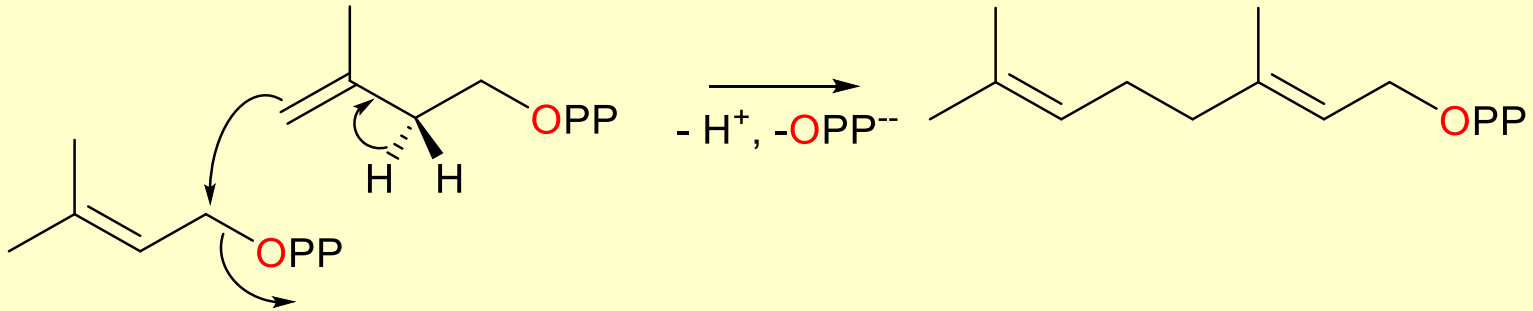


C-C bond Formation by S_N2 Displacement of a Stable Nucleophile on an Electrophilic Alkylating Agent.

C. Alkylation of a stabilized carbanion with acetyl-coA as alkylating agent



D. S_N2 displacement of pyrophosphate.



Note: One common series of reactions for S_N2 displacement is:

- phosphorylation of R-OH group → R-OPP^-, followed by
- S_N2 displacement of OPP^- by a nucleophile.

Enzymes in Biotechnology.

Enzymes in food and beverage production:

- Dairy industry
- Beer industry
- Wine and juice industry
- Alcohol industry
- Protein industry
- Meat industry
- Baking industry
- Fat and Oil industry

Enzymes as industrial catalysts:

- Starch processing industry
- Antibiotic industry
- Fine Chemicals industry

Enzymes in Biotechnology (2).

Enzymes as final products:

- Detergent industry
- Cleaning agent industry
- Pharmaceutical industry
- Animal feed industry
- Analytical applications

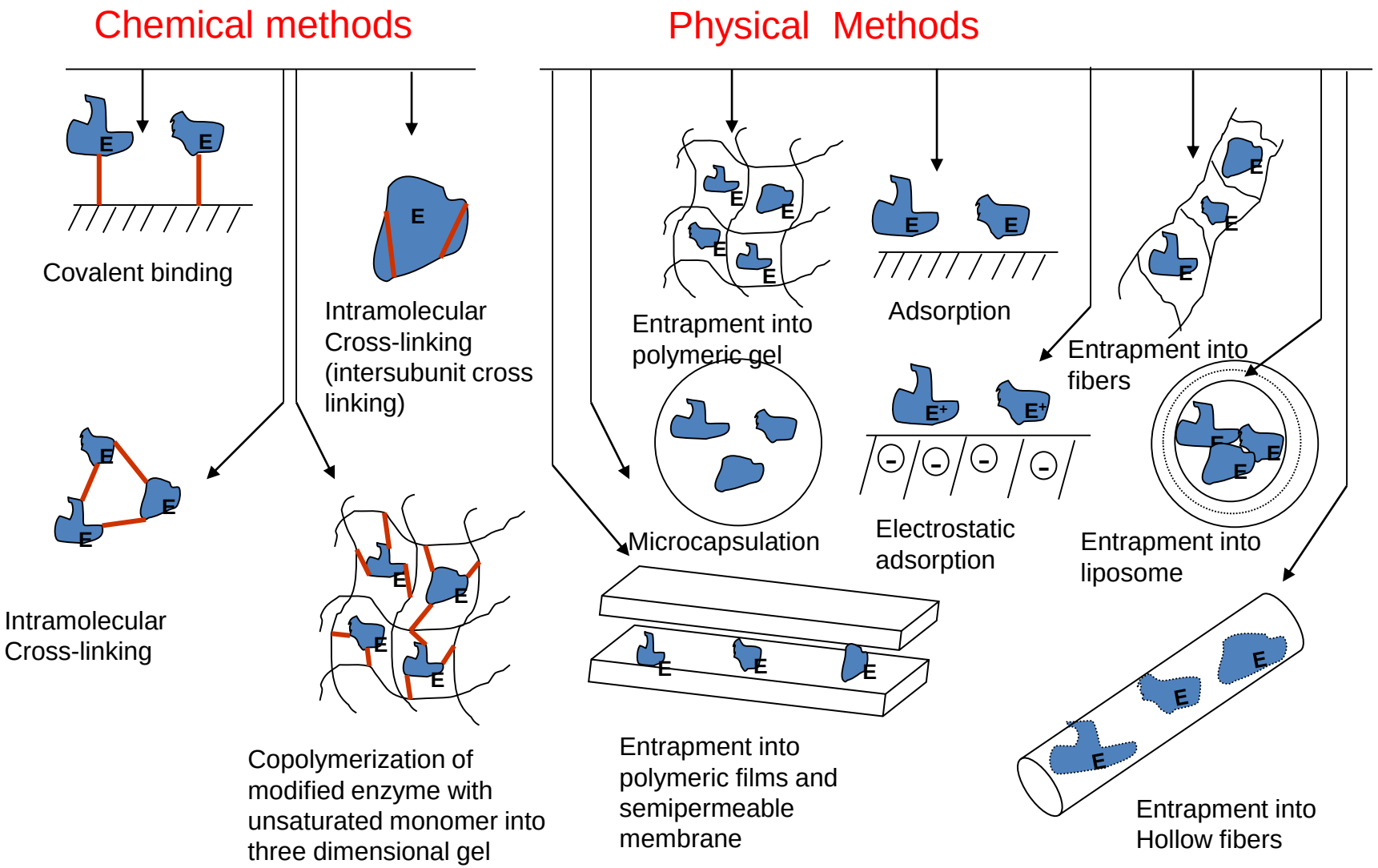
Enzymes as processing aids:

- Textile industry
- Leather industry
- Paper and pulp industry
- Sugar industry
- Coffee industry

Important Factors in Using Enzymes.

- Reactions possible that are not possible using normal chemical transformations;
- Specificity of reaction including substrate specificity, positional specificity, stereo specificity;
- Allows milder process conditions, e.g. temperature, pressure, pH, sterility, etc.;
- Reduces number of process steps required;
- Eliminates the need to use organic solvents in processing;
- Immobilization of enzyme to allow its reuse or continuous use;
- Use of enzymes in combination with other separate chemical steps;
- Genetic engineering to improve enzymes.

Methods of Immobilization of Enzymes.



Industrial World Market of Enzymes.

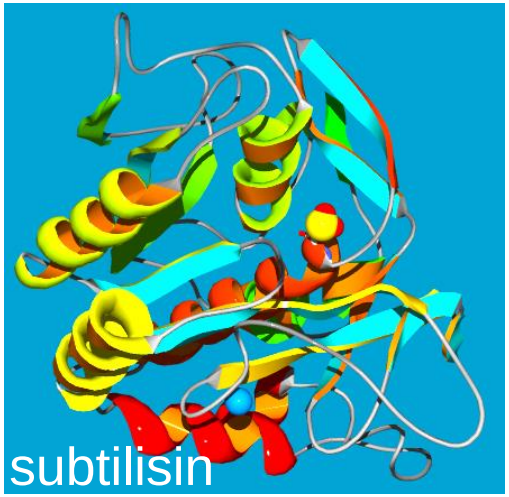
Annual Sales: $ 1.6 billion.

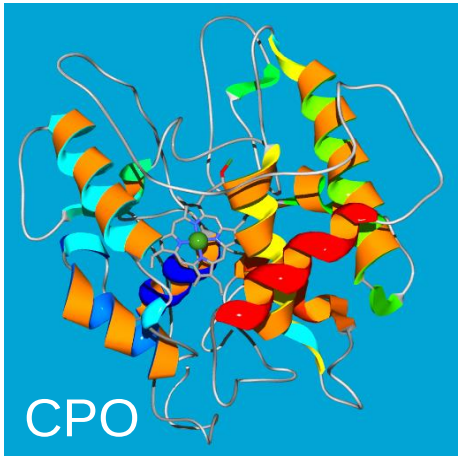
Food and starch processing:	45%
Detergents:	34%
Textiles:	11%
Leather:	3%
Pulp and paper:	1.2%

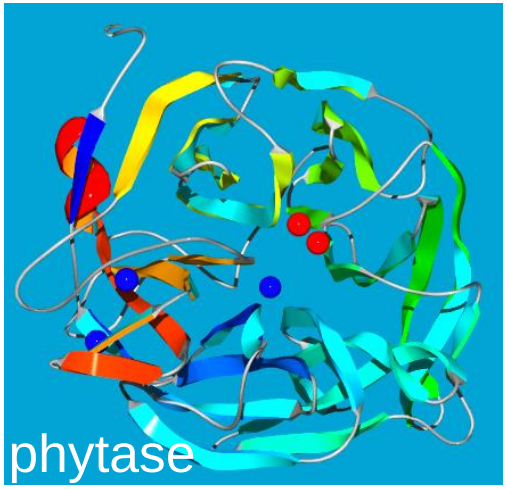
Products of Enzymatic Reactions:

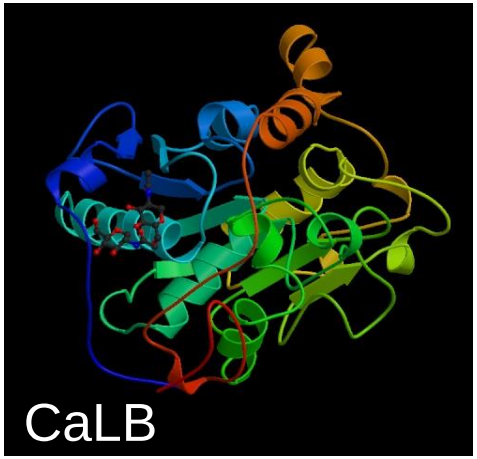
High fructose corn syrup	$ 1 billion
Aspartame	$ 850 million
Acrylamide	$ 300 million

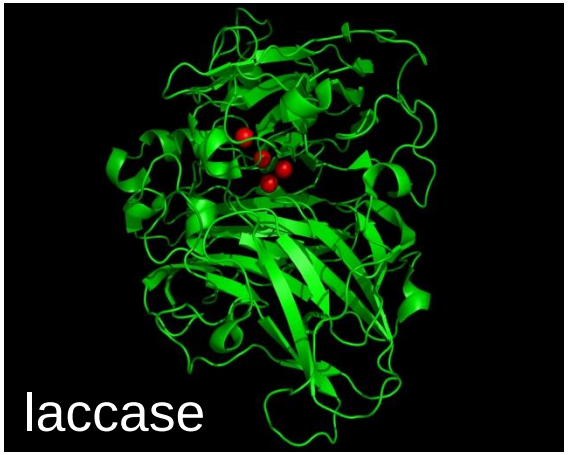
New Enzymes & Enzymatic Reactions.

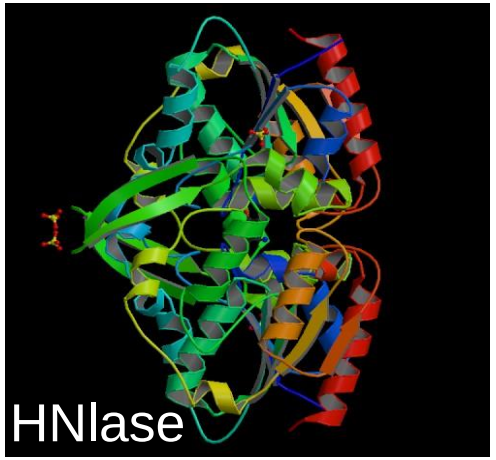












Hydrolases: Production of Glucose from Starch.

Liquefaction	Saccharification	DE	Glucose
Acid	Acid	92	85
Acid	Glucoamylase	95	91
Acid/α-amylase	Glucoamylase	96	92
α-Amylase/High pressure cooking/ α-amylase	Glucoamylase	97	93
α-Amylase (thermostable)	Glucoamylase	97	94
α-Amylase (thermostable)	Glucoamylase	97-98.5	95-97.5

Hydrolases: Production of High Fructose Corn Syrups from Starch.

Corn Starch Slurry (30-35% DS, pH 6.0-6.5, Ca^{2+} 50 ppm)

Liquefaction
- **Thermostable α-Amylase**
- **Gelatinization (105°C, 5 min)**
- **Dextrinization (95°C, 2h)**

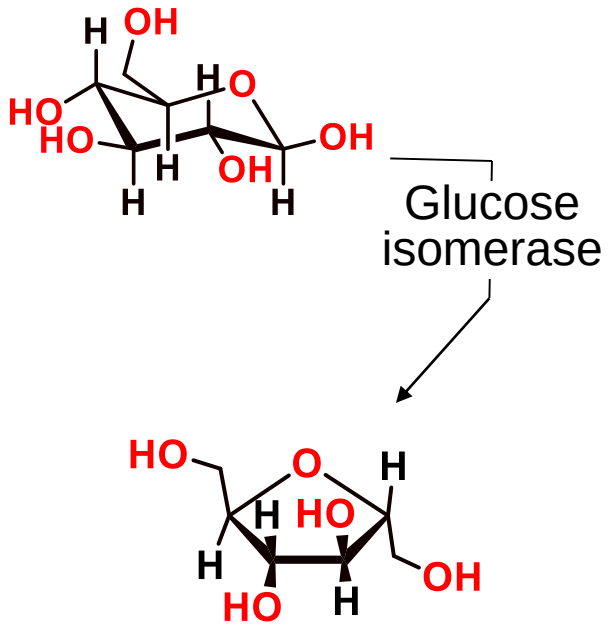
Liquefied Starch DE 10-15

Saccharification
- **Glucoamylase**
- **(60°C, pH 4.0-4.5, 24-72 h)**

Glucose Syrups DE 95-96

Isomerization
- **Glucose isomerase**
- **(pH 7.5-8.0, 55-60°C, 5 mM Mg^{2+})**

High Fructose Corn Syrups (42% fructose)

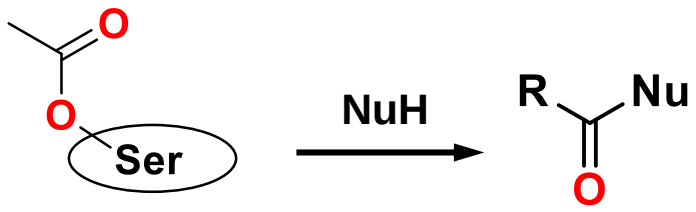


Advantages of Using Pullulanase in Starch Saccharification Processes.

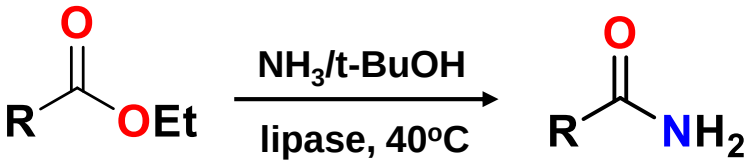
- Increases the glucose yield (about 2%) with glucoamylase
- Increases the maltose yield (about 20-25%) with β-amylase
- Reduces the saccharification time (to 48 h)
- Allows an increase in substrate concentration (to 40%, DS)
- Allows a reduction in the use of glucoamylase (up to 50%)

Ammoniolysis of Esters.

Enzymatic Ammoniolysis

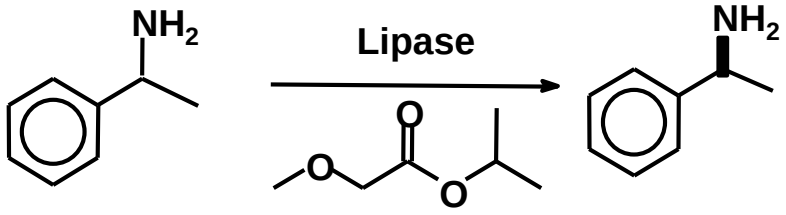


Nu = OH, OR, NH_2 , RNH, OOH, etc.

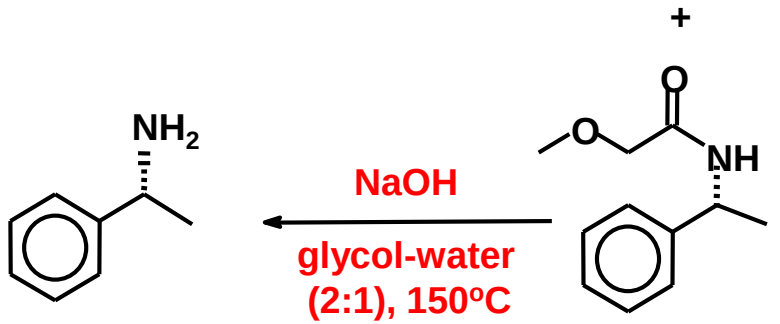


- **Green amide synthesis**
- **Enantioselective with amino acid esters**

BASF Process



ee > 99% (*S*)



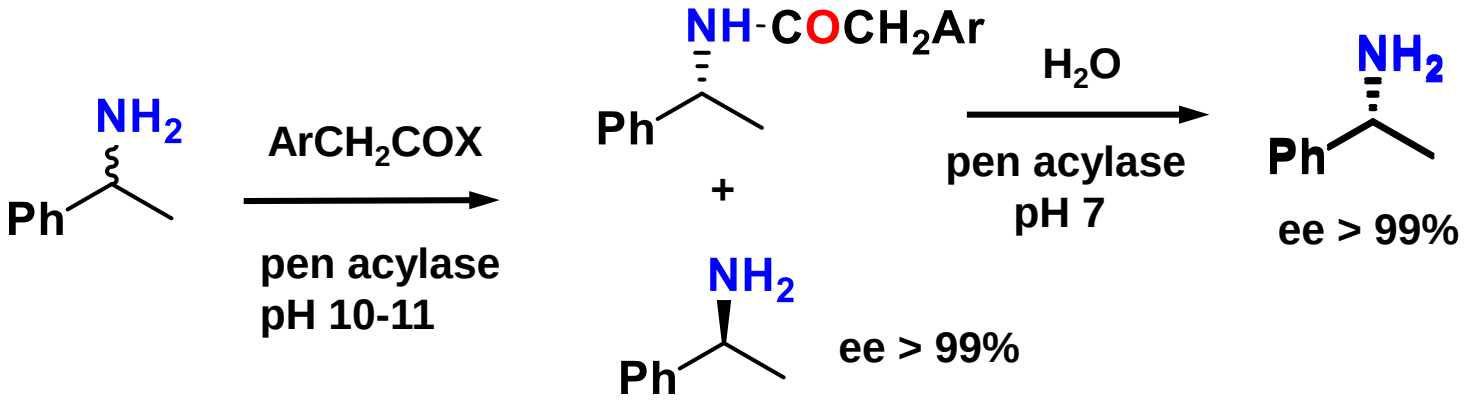
ee > 99% (*S*)

ee > 99% (*R*)

> 3000 t/year

Steverink (1995), Hacking (1999), Wegman (2001)

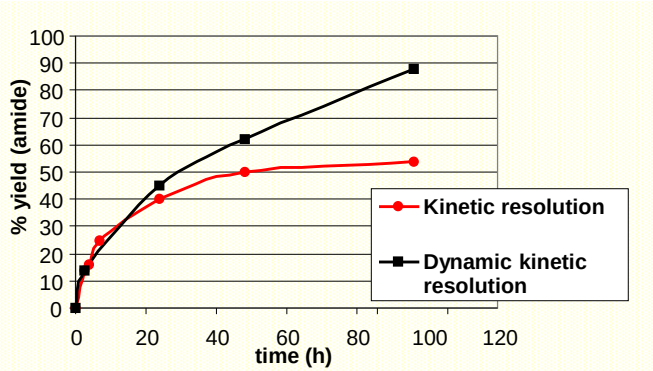
Easy-on-Easy-off Resolution.



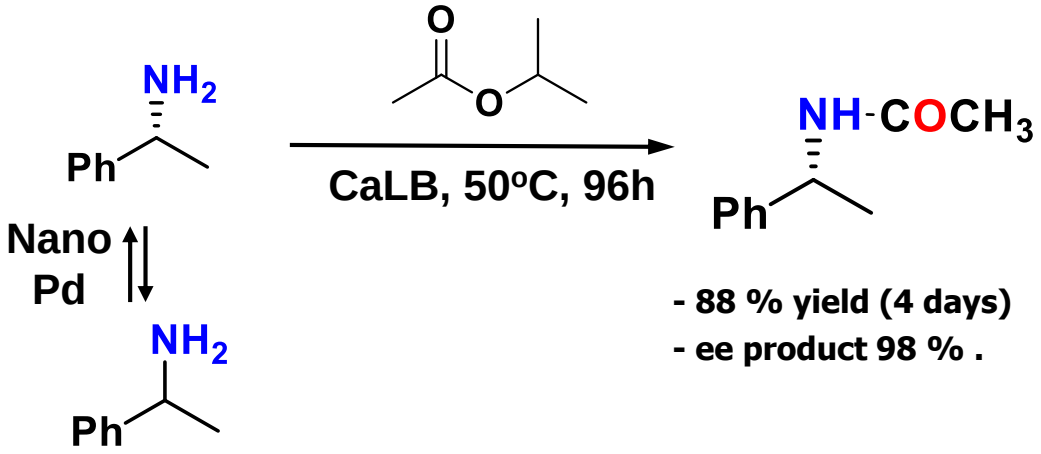
V. Svedas

L.van Langen(2001), R.Madeira Lau(2003), H.Ismail(2007)

Dynamic Kinetic Resolution



H. Ismail(2007)



- 88 % yield (4 days)
- ee product 98 % .

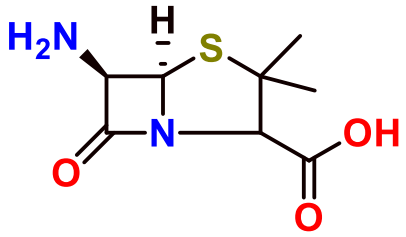
Enzymatic Synthesis of Penicillins: 6-Aminopenicillanic Acid (6-APA).

Penicillin:

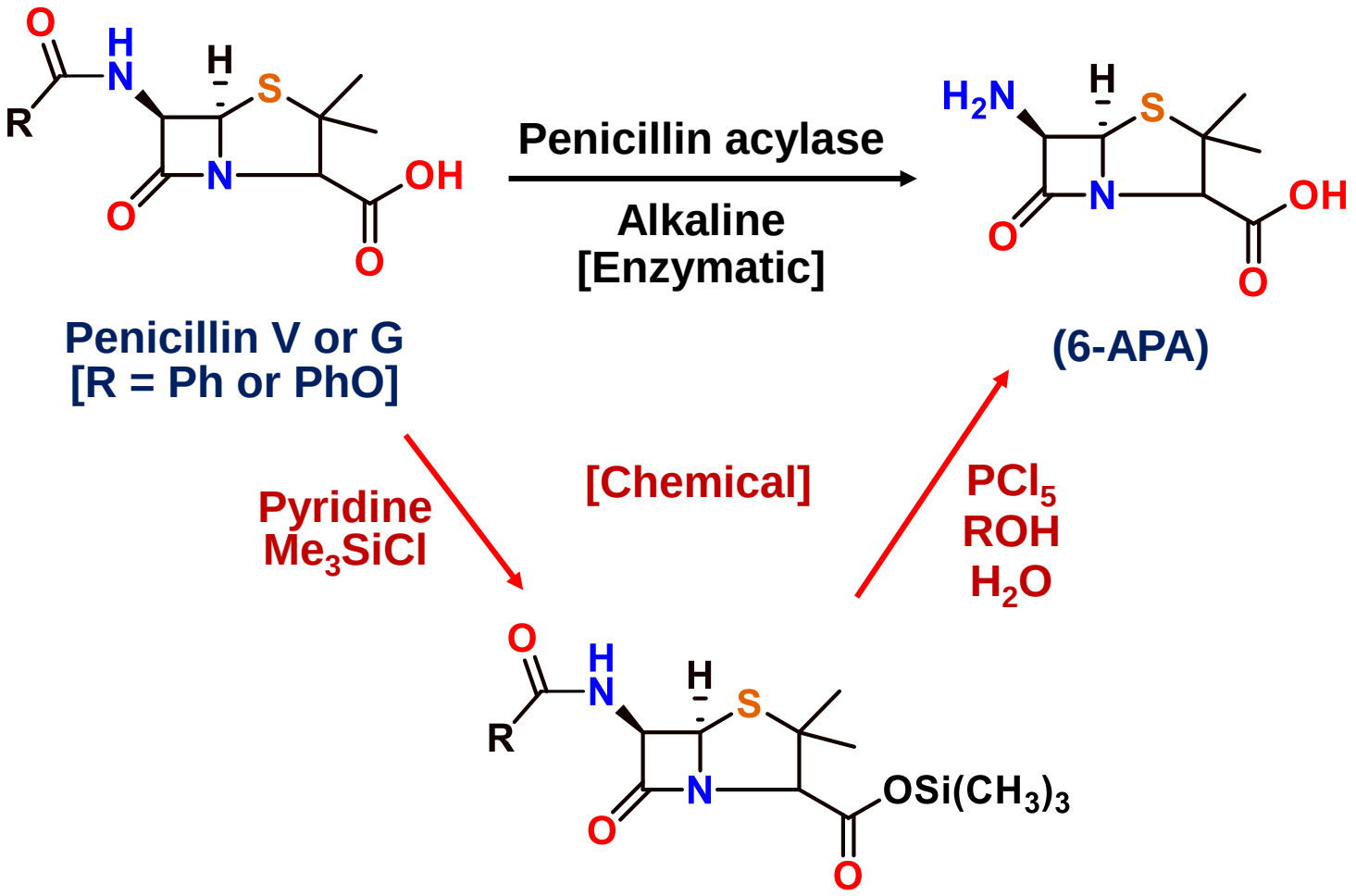
- First discovered by Fleming in 1932
- 19% of worldwide antibiotic market.
- Superior inhibitory action on bacterial cell wall synthesis
- Broad spectrum of antibacterial activity
- Low toxicity
- Outstanding efficacy against various bacterial strains
- Excessive use has led to development of resistant pathogens.

6-APA:

- Raw material for production of new semisynthetic penicillins (amoxicillin and ampicillin)
- Fewer side effects
- Diminished toxicity
- Greater selectivity against pathogens
- Broader antimicrobial range
- Improved pharmacological properties.



Chemical and Enzymatic Deacylation of Penicillins to 6-APA.



6-Aminopenicillanic Acid (6-APA).

Chemical method:

- **Use of hazardous chemicals - pyridine, phosphorous pentachloride, nitrosyl chloride.**

Enzymatic method:

- **Regio- and stereo-specific**
- **Mild reaction conditions (pH 7.5, 37 °C**
- **Enzymatatic process is cheaper by 10%.**

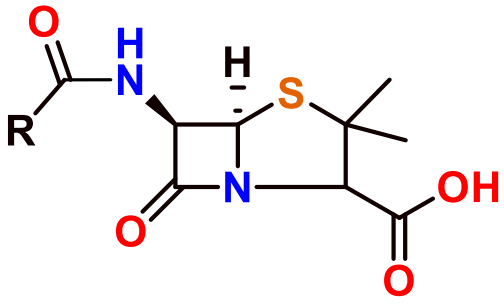
Enzymes:

- **Penicillin G acylase (PGA) - *Escherichia coli, Bacillus megaterium, Streptomyces lavendulae***
- **Penicillin V acylases (PVA) - *Beijerinckia indica var. Penicillium, Fusarium sp., Pseudomonas acidovorans***

Immobilized Enzyme:

Life, 500-2880 hours.

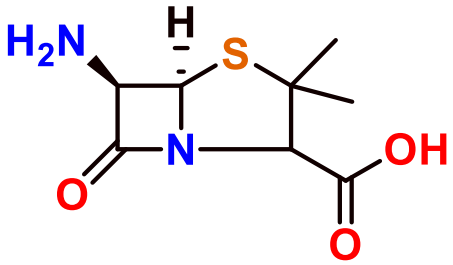
Enzymatic Modification of Penicillins to 6-APA and Semisynthetic Penicillins.



Penicillin V or G

Penicillin acylase

Alkaline
[Deacylation]



(6-APA)

Penicillin acylase
[Acylation]

Acidic

Semisynthetic
Penicillins

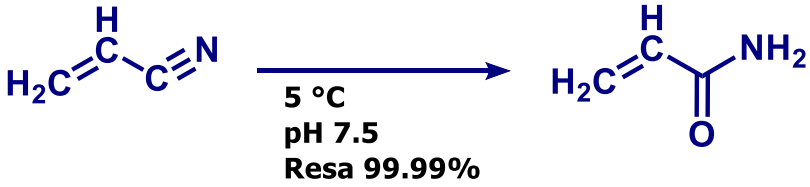
Enzymatic Synthesis of Acrylamide.

- **Monomeric raw material for the manufacture of polymers and synthetic polymers**
- **Obtained by hydration of the cyanide function of acrylonitrile**
- **World market, 200,000 tpa.**

Chemical Process:

- **Reaction of acrylonitrile with water in the presence of**
- **H_2SO_4 (90°C) or a metal catalyst (80-140°C)**
- **Formation of toxic waste (HCN)**
- **The reaction must be stopped to prevent the acrylamide itself being converted to acrylic acid.**

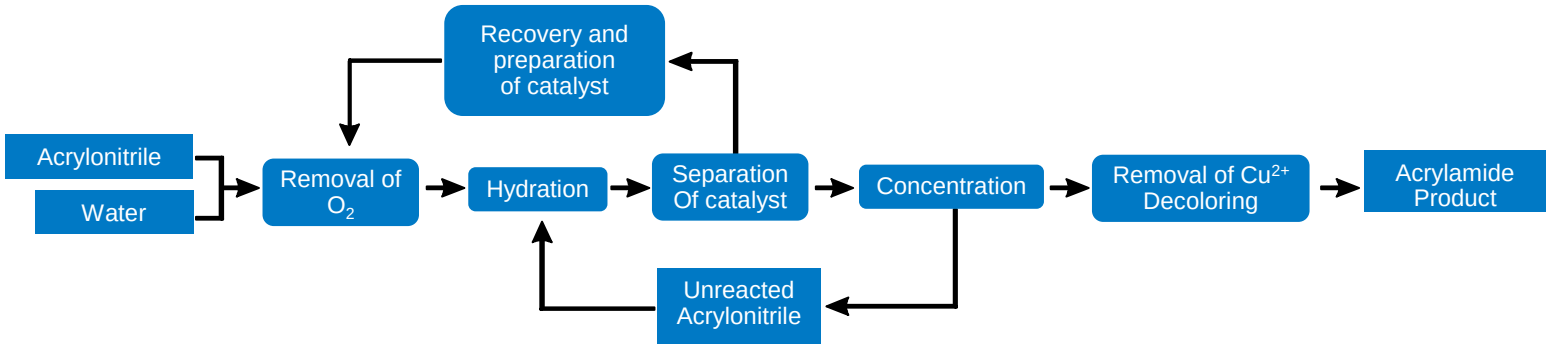
Enzymatic Process:



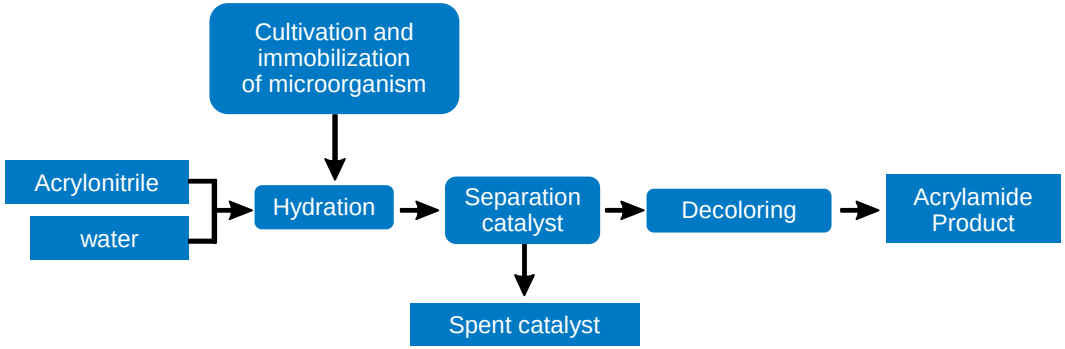
- **99.9% yield**
- **Kg quantity product / g cells**
- **Acrylic acid is not produced**
- **Fewer process steps are involved**
- **Much more environmental friendly**
- **Nitto Chemical Industry: 6,000 tons annually.** The active enzyme is nitrile hydrolase present in intact cells of *Rhodococcus rhodochrous*, immobilized on a poly(propenamide) gel.

Process Comparison Between Chemical and Biochemical Synthesis of Acrylamide.

Copper-catalyzed process

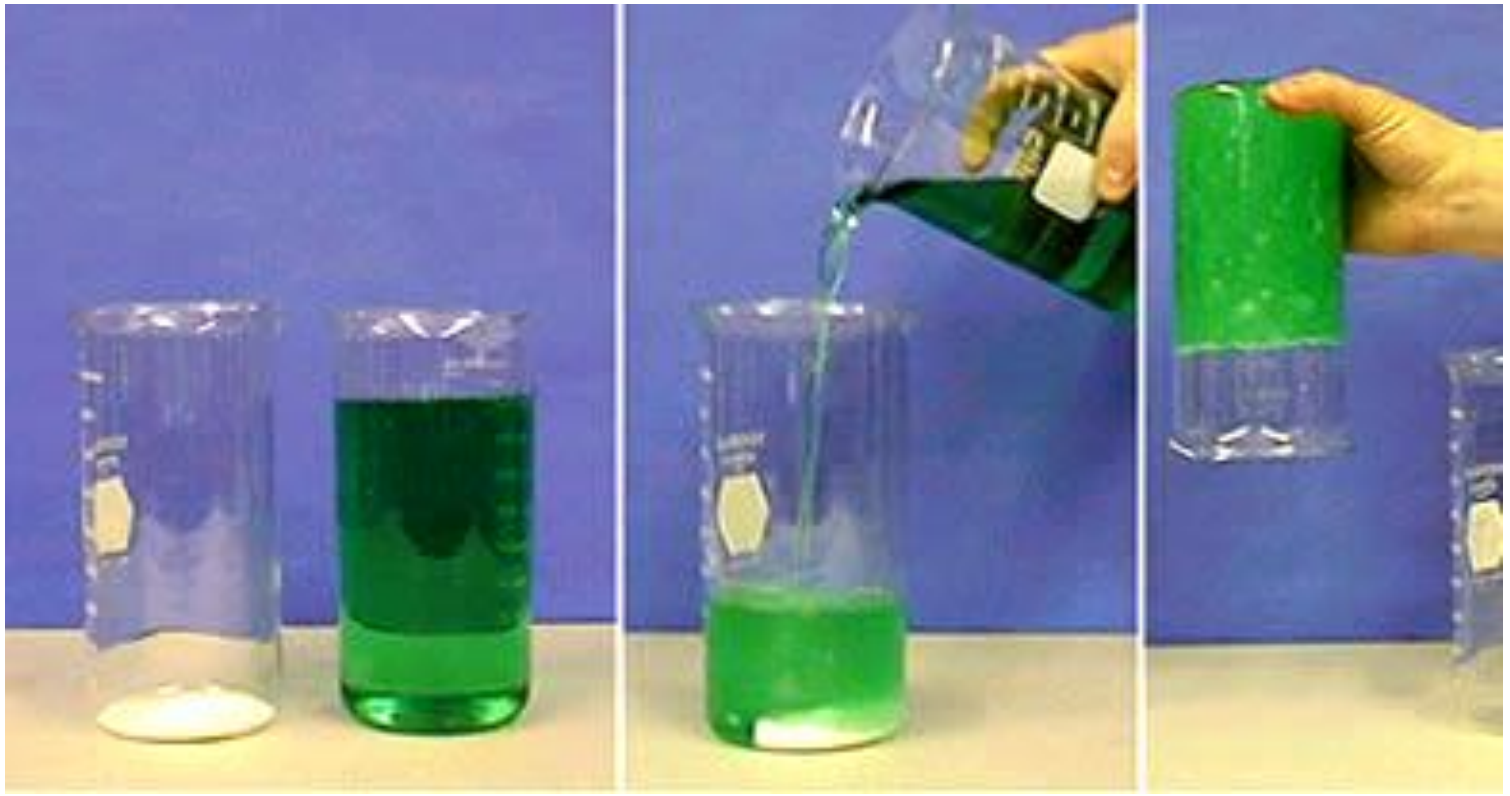


Microbial process



Nitrile hydratase and amidase reactions

Biocatalysis and Acrylamide.



Poly(propenamide)
Polyacrylamide

water with green dye

Mixing

Resulting gel

Matrix for separation of biological macromolecules.

Synthesis of Aspartame (L-Asp-L-Phe-Methyl Ester).

Aspartame is dipeptide sweetener formed by linking the methyl ester of phenylalanine with aspartic acid:

- Extensively used in food and beverages;
- 200 times as sweet as sucrose;
- Annual sale: 200 million Ibs, $ 850 million;
- Nutrasweet Corp. retains 75% of the US market.

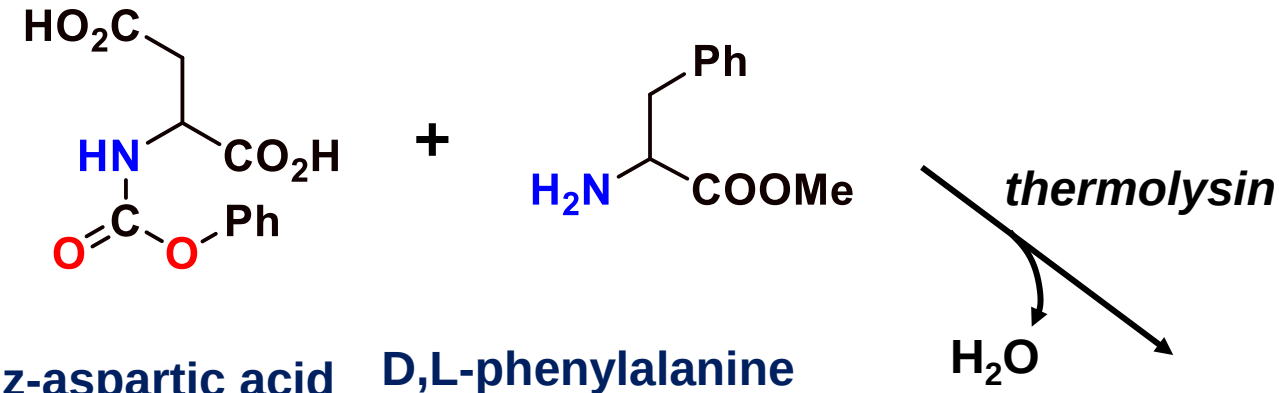
Chemical method:

- The amino group of aspartic acid needs to be protected to prevent its reacting with another molecule to give unwanted by-products;
- The correct single enantiomer of each of the reactants must be used to give the required stereochemistry (beta-aspartame is bitter tasting).

Enzymatic method:

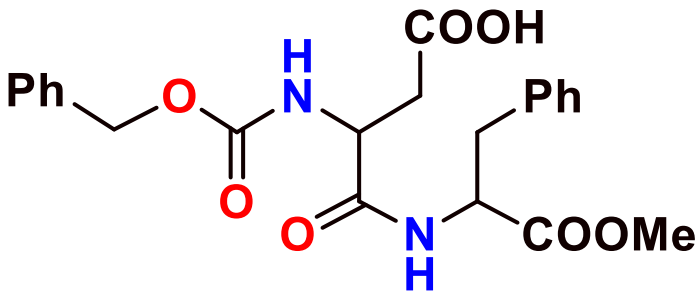
- Thermolysin promotes reaction only at the alpha-functionality;
- Mild condition, pH 6-8, 40°C;
- Cbz. = benzyloxycarbonyl.

Biocatalytic Production of Aspartame.



N-Cbz-aspartic acid

D,L-phenylalanine methyl ester



Cbz-aspartame

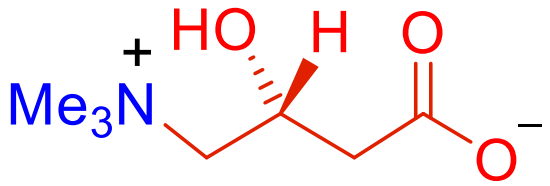
Cbz = benzyloxycarbonyl
($PhCH_2OCO$-)

L-Carnitine.

- Thyroid inhibitor
- Slimming agent
- Dietary supplement for athletes
- Only one enantiomer of the compound is used

Two biocatalytic routes are available to make L-carnitine:

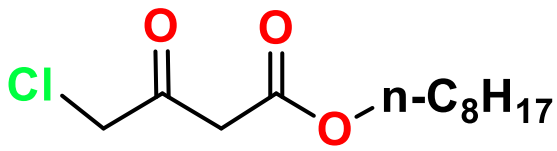
- *Saccharomyces cerevisiae*
- *Rhizobiaceae*



L-Carnitine

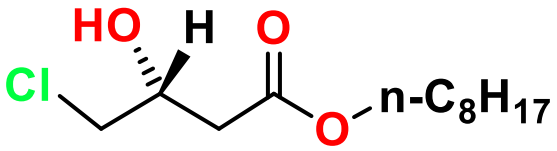
Synthesis of L-Carnitine.

(1)

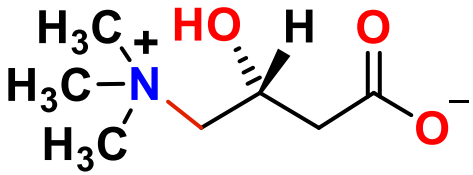


γ-chloroacetoacetic acid octyl ester

reductase

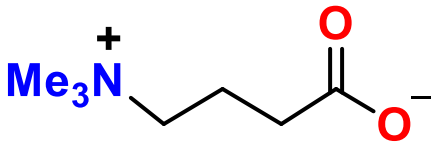


(R)-γ-chloro-b-hydroxybutanoic acid octyl ester



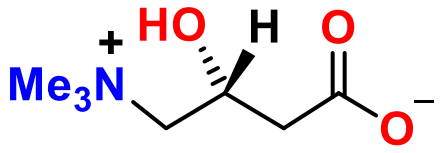
L-carnitine

(2)



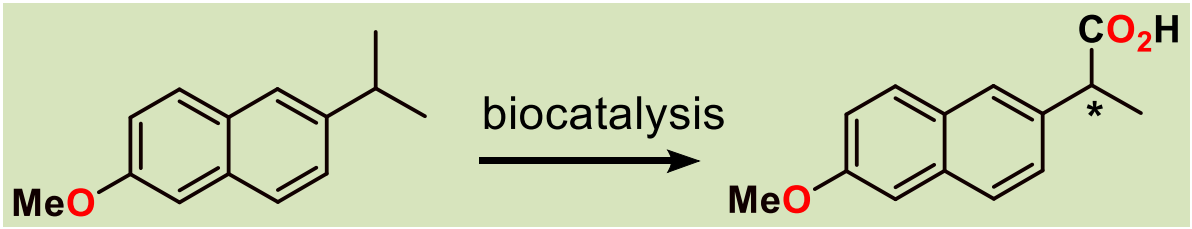
γ-butyrobetaine

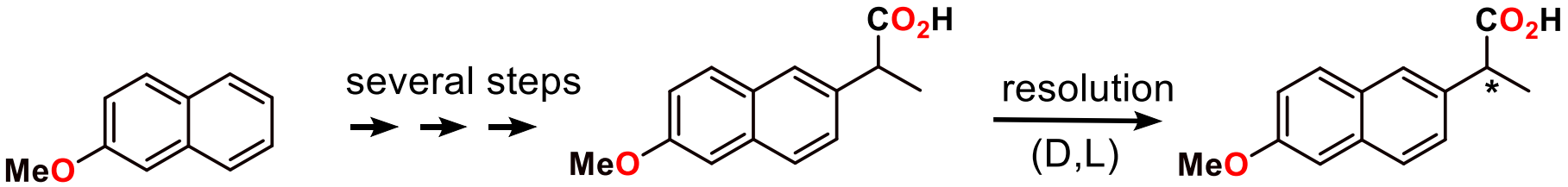
hydroxylase

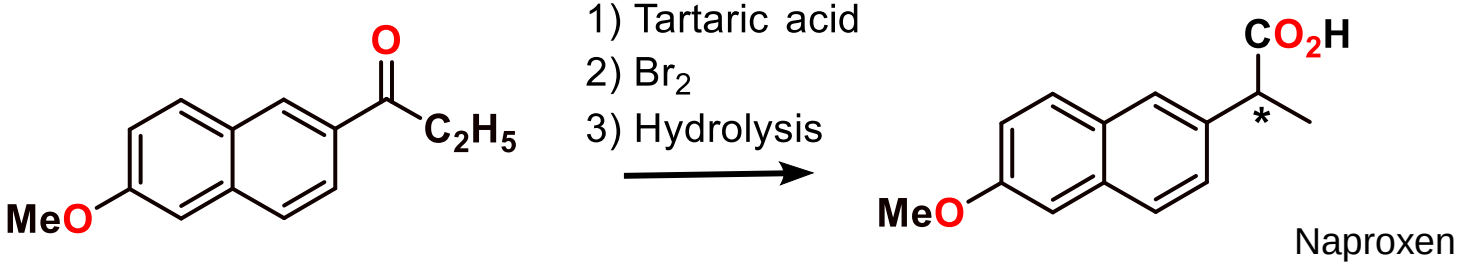


L-carnitine

Synthesis of Naproxen.

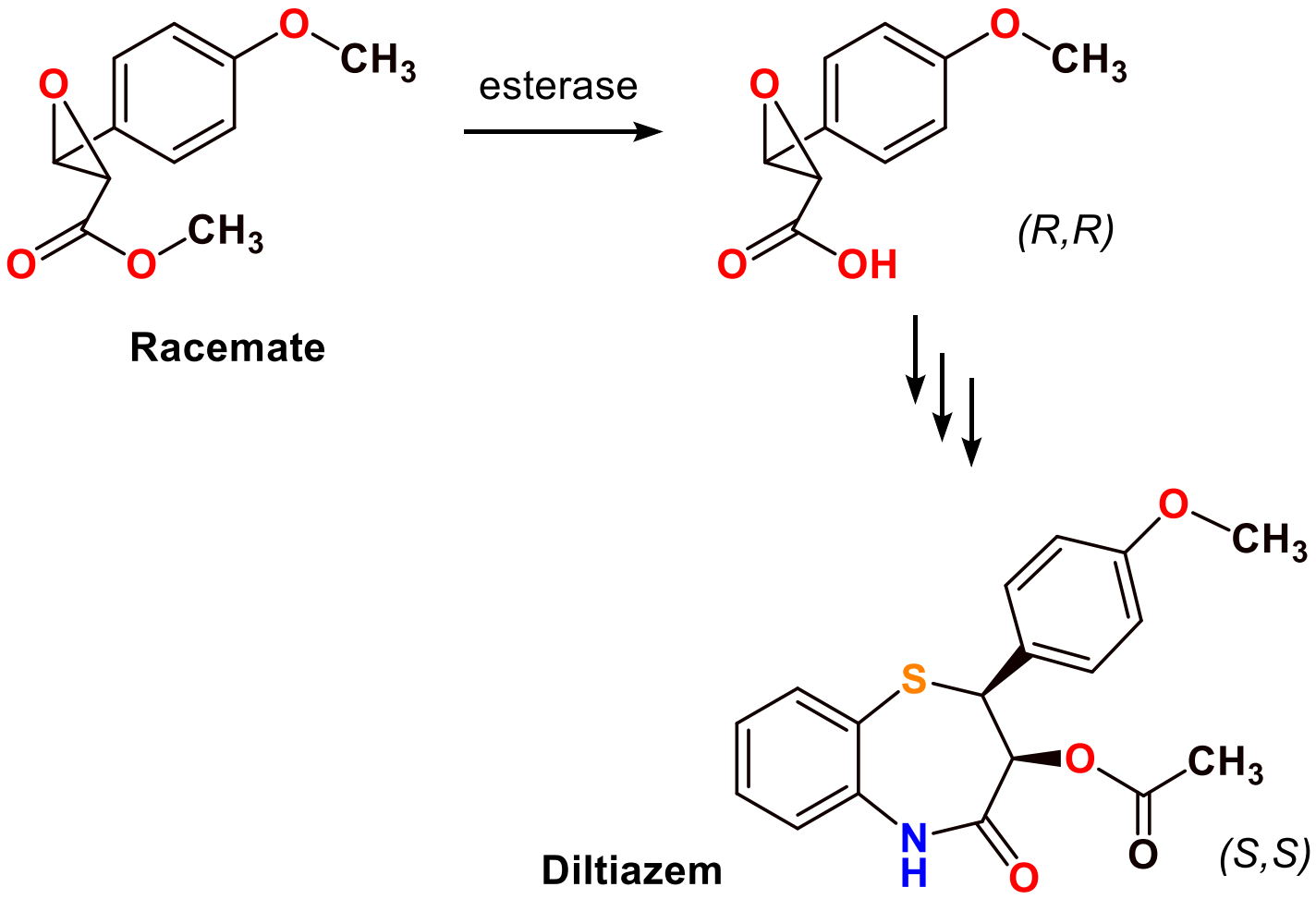




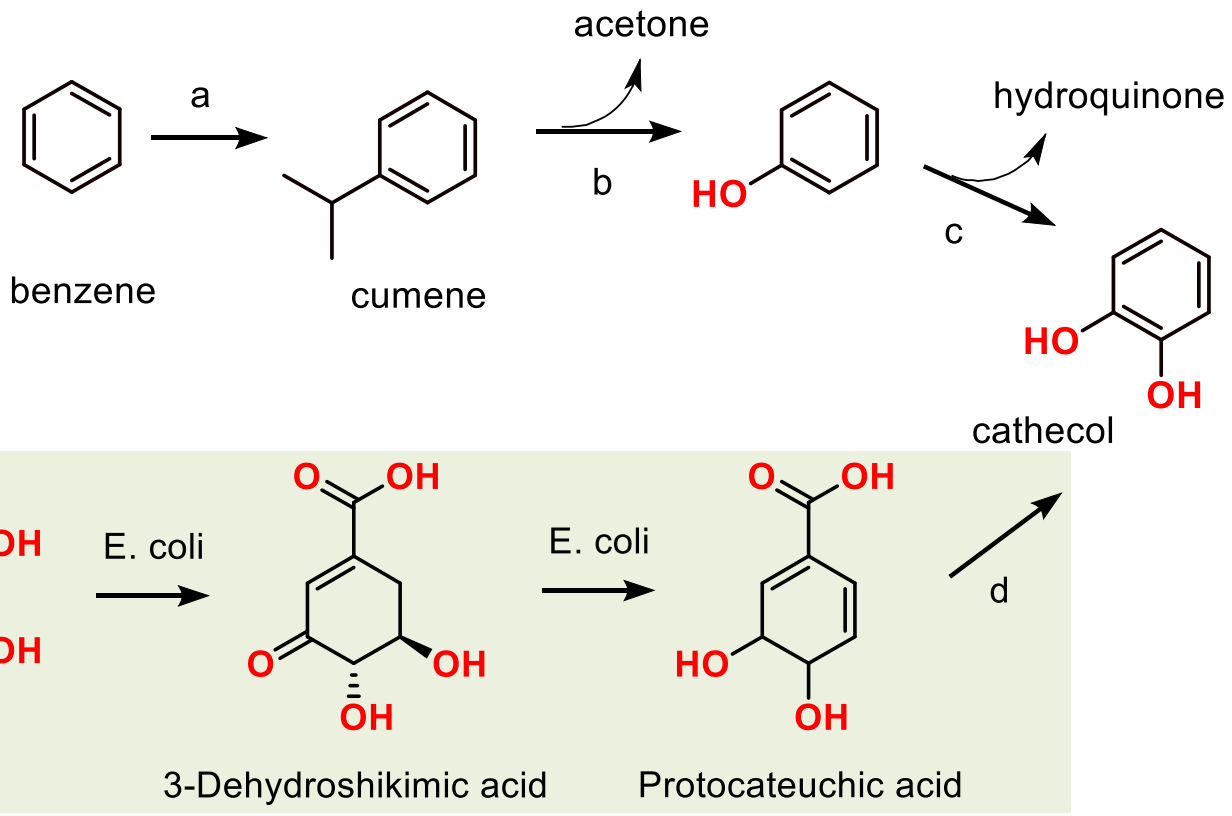


Naproxen

Synthesis of Calcium Antagonist Diltiazem.



Environmentally Compatible Synthesis of Catechol from Glucose.



(a) propylene, solid catalyst H_3PO_4, 200-260°C, 400-600 psi.
(b) O_2, 80-130°C then SO_2, 60-100°C.
(c) Ti-Silicalite, 70-80°C
(d) *E. coli* AB2834/pKD136/pKD9.069A, 37°C.

Draths and Frost, 1995

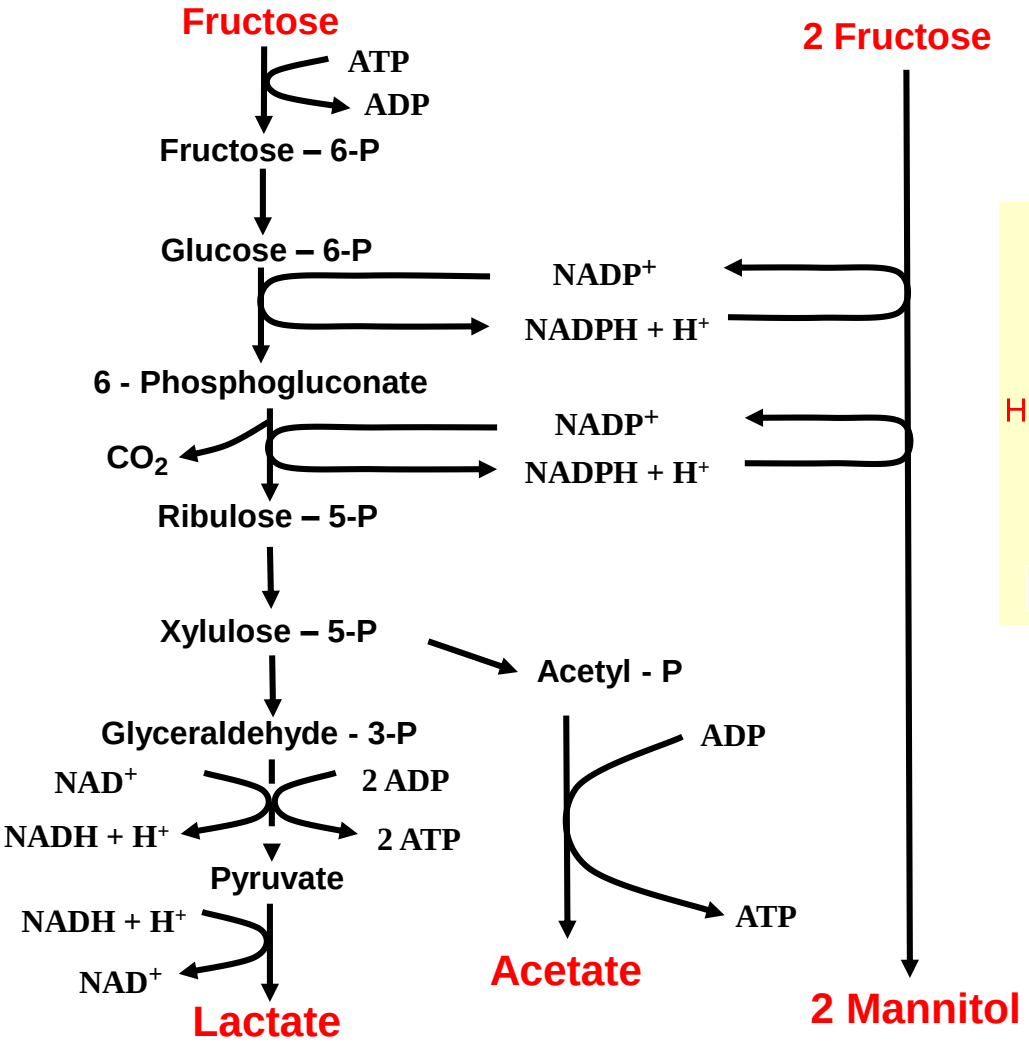
Debittering of Protein Hydrolysates.

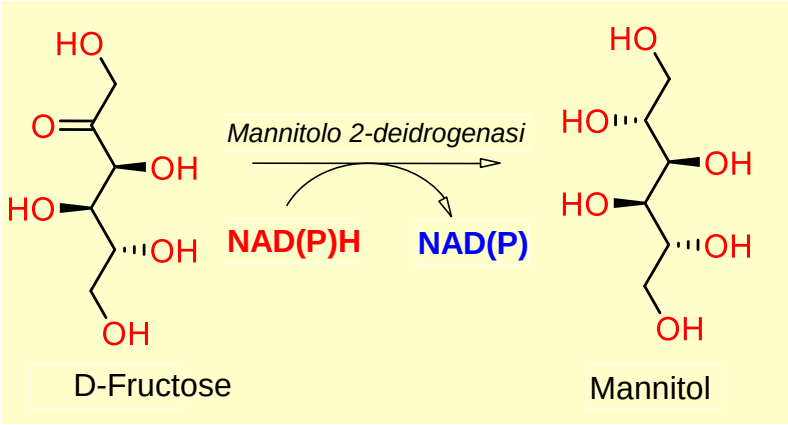
- Treatment with activated carbon
- Extraction with alcohol
- Isoelectric precipitation
- Chromatographic separation
- Masking of bitter taste
- Enzymatic hydrolysis of bitter peptides
  - with aminopeptidase
  - with alkaline/neutral protease
  - with carboxypeptidase
- Condensation reactions using protease.

Mannitol.

- Food additive;
- Reduces the crystallization tendency of sugars and is used as such to increase the shelf-life of foodstuffs;
- Used in chewing gum;
- Pharmaceutical formulation of chewable tablets and granulated powders;
- Prevents moisture adsorption from the air, exhibits excellent mechanical compressing properties, does not interact with the active components, and its sweet cool taste masks the unpleasant taste of many drugs;
- Mannitol hexanitrate is a well-known vasodilator, used in the treatment of hypertension;
- The complex of boric acid with mannitol is used in the production of dry electrolytic capacitors;
- It is an extensively used polyol for the production of resins and surfactants;
- It has low solubility in water of only 18% (w/w) at 25°C;
- In alkaline solutions, it is a powerful sequestrant of metallic ions;
- It is about half as sweet as sucrose.

Heterofermentative Conversion Pathway of Fructose into Mannitol.



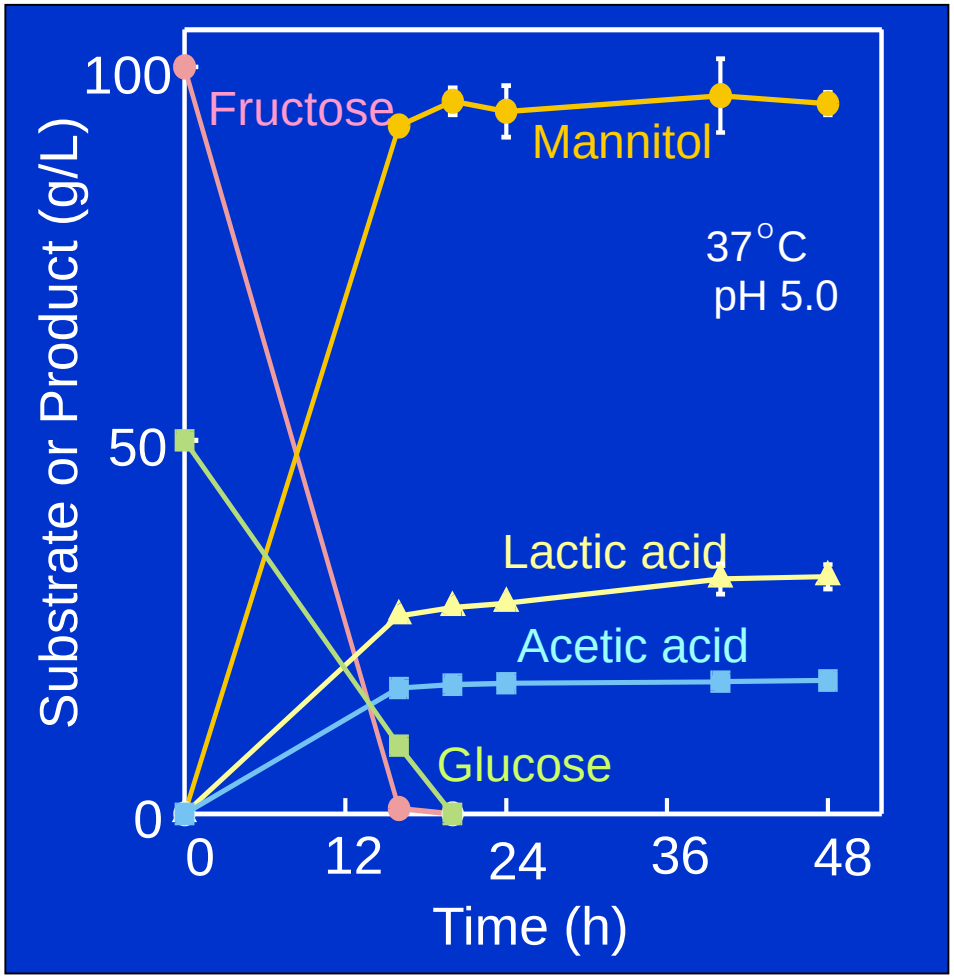


Mannitol Production from Fructose in pH-Controlled Batch Fermentation.

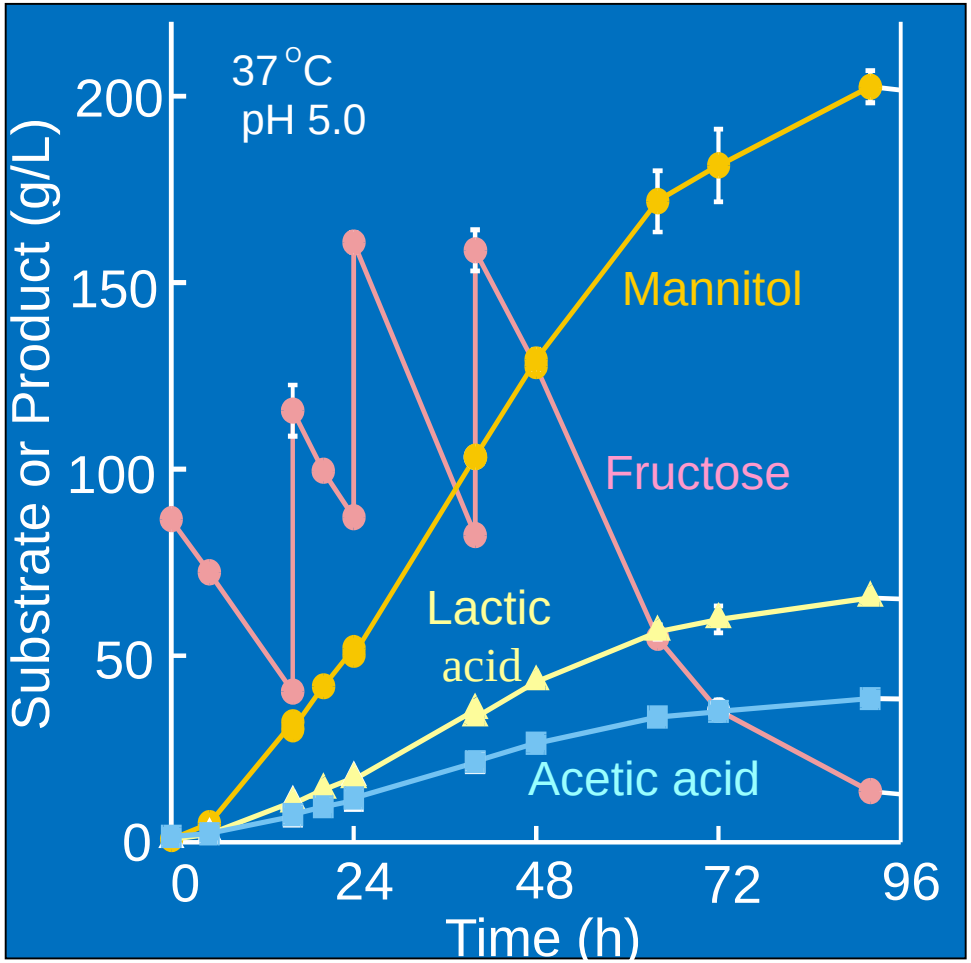
Fructose (g/L)	Time (h)	Mannitol (g/g)	Lactic acid (g/g)	Acetic acid (g/g)
150	15	0.72±0.00	0.17±0.00	0.12±0.00
200	40	0.69±0.03	0.17±0.00	0.13±0.00
250	64	0.70±0.02	0.16±0.00	0.12±0.00
300	136	0.66±0.03	0.15±0.01	0.11±0.00

At 37ºC, 130 rpm, Initial pH 6.5, pH controlled at 5.0, 500 ml fleaker with 300 ml medium.

Fructose and Glucose (2:1) Co-Utilization and Mannitol Production.



Mannitol Production in pH-Controlled Fed-Batch Fermentation.



Fructose used: 300 g/L (final concentration)

Comparison between Fermentation and Catalytic Hydrogenation.

FERMENTATION

- All fructose converted to mannitol
- Co-product: lactic acid and acetic acid one half of mannitol
- Glucose is hydrogen source in hydrogenation
- Nitrogen source essential for growth
- Electrodialysis for removing organic acids
- Use of less pure substrates poses no problem

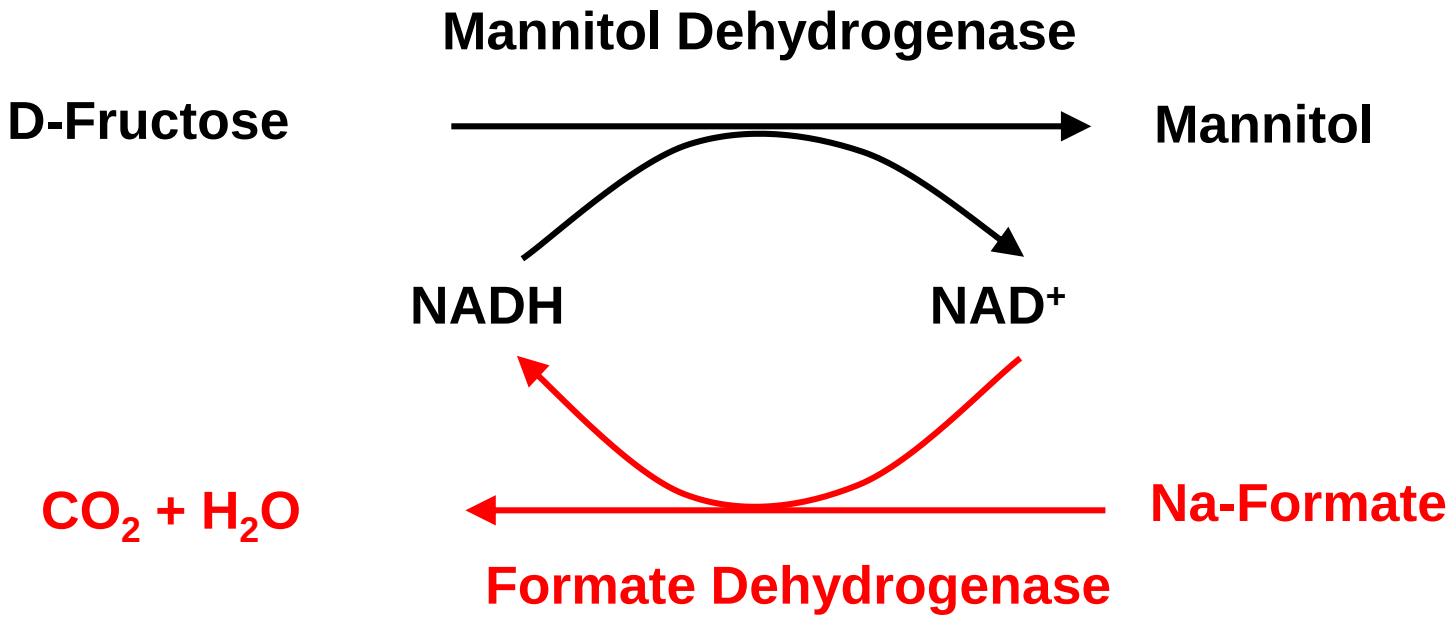
HYDROGENATION

- Only half of fructose converted to mannitol
- Co-product: sorbitol in large excess (3)
- Highly pure hydrogen gas necessary
- Nickel catalyst essential
- Ion exchanger for nickel ions removal
- Highly pure substrates necessary to avoid catalyst inactivation.

Cofactor Regeneration.

- Chemical
- Photochemical
- Electrochemical
- Biological
- Enzymatic

Enzymatic Conversion of Fructose to Mannitol with Simultaneous Cofactor Regeneration.

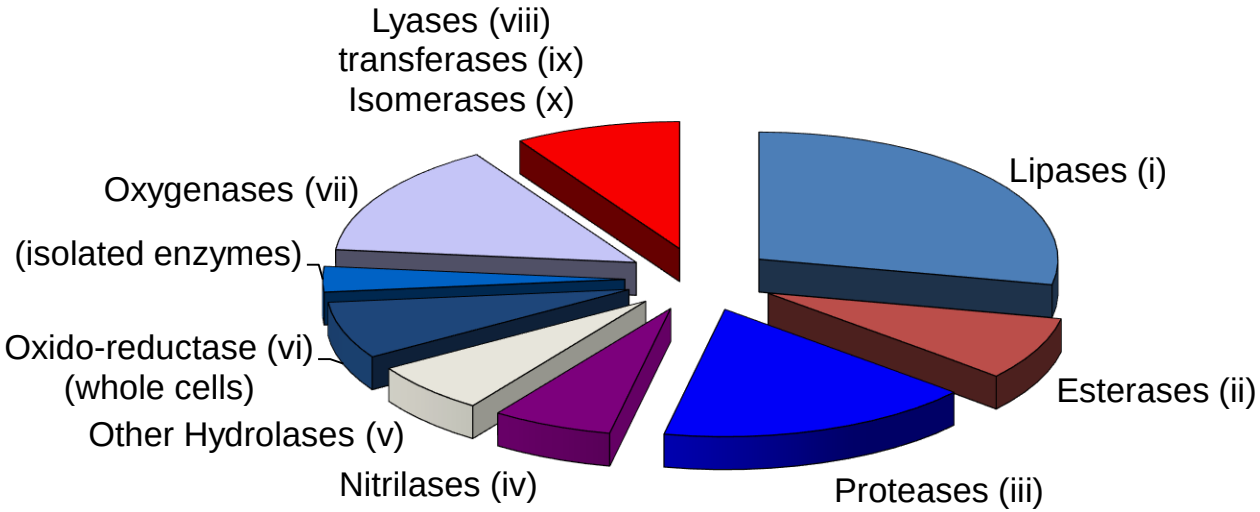


or

Gluconic acid ← Glucose + H_2O

Glucose Dehydrogenase

Biocatalytic Oxidation Reactions Available in Organic Synthesis.



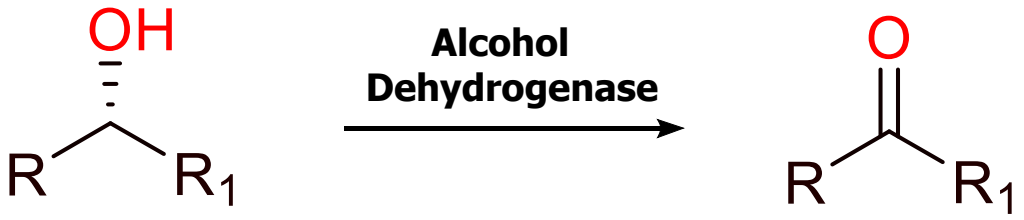
Biological Oxidation processes are not as widely exploited as e.g. hydrolases:

1. lack of commercial availability and
2. perceived complexity(e.g. microbiological facilities/expertise required for implementation, non-enzymatic 'cofactors' and essential redox proteins).

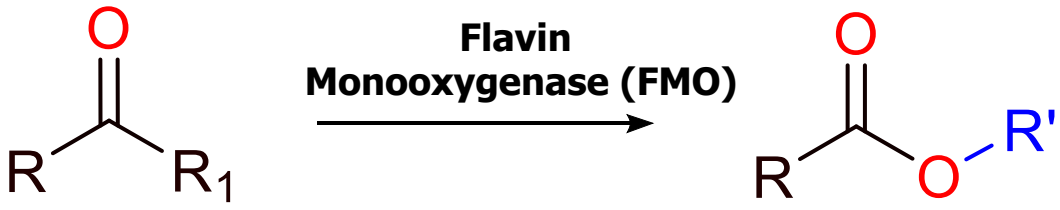
Biocatalytic Oxidation Reactions Available in Organic Synthesis.

Hydroxylation

Alcohol oxidation

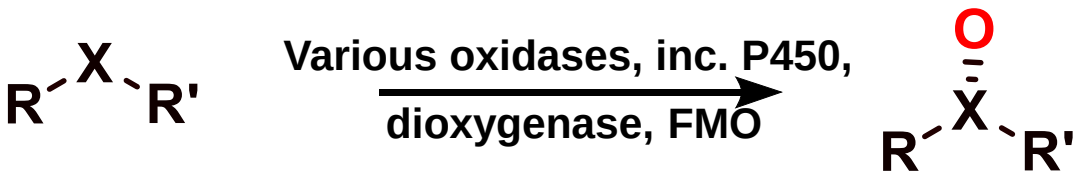


Baeyer-Villiger Oxidation



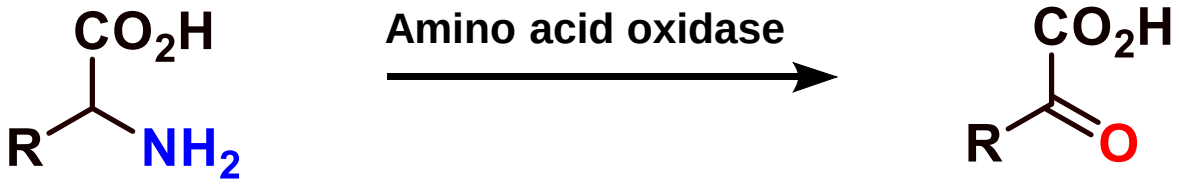
Biocatalytic Oxidation Reactions Available in Organic Synthesis (2).

Heteroatom oxidation

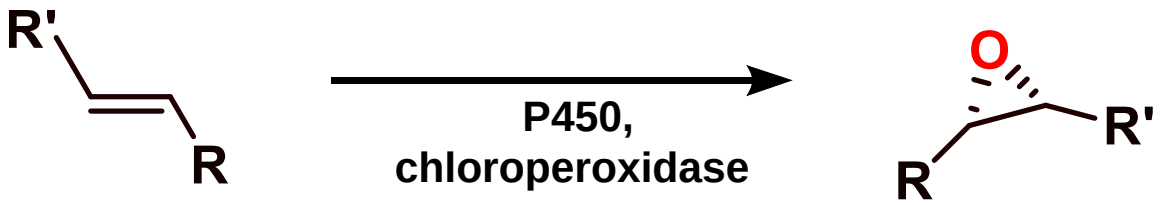


X = S, Se, N

Amino acid oxidation

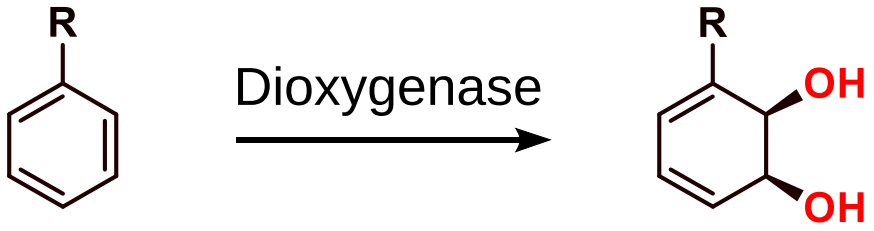


Epoxidation

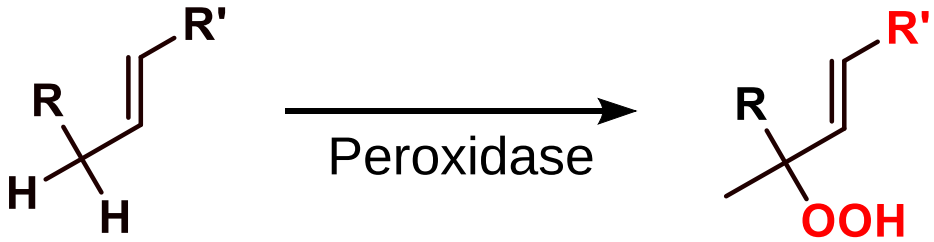


Biocatalytic Oxidation Reactions Available in Organic Synthesis (3).

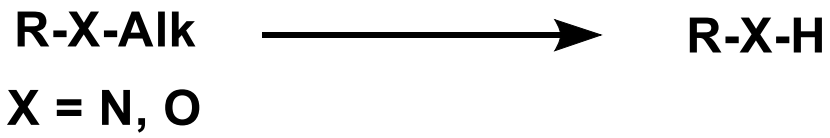
Dihydroxylation



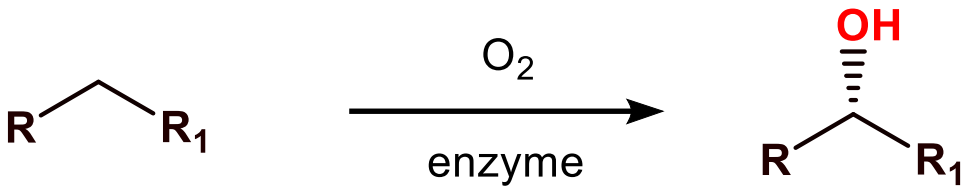
Formation of peroxides



Dealkylation

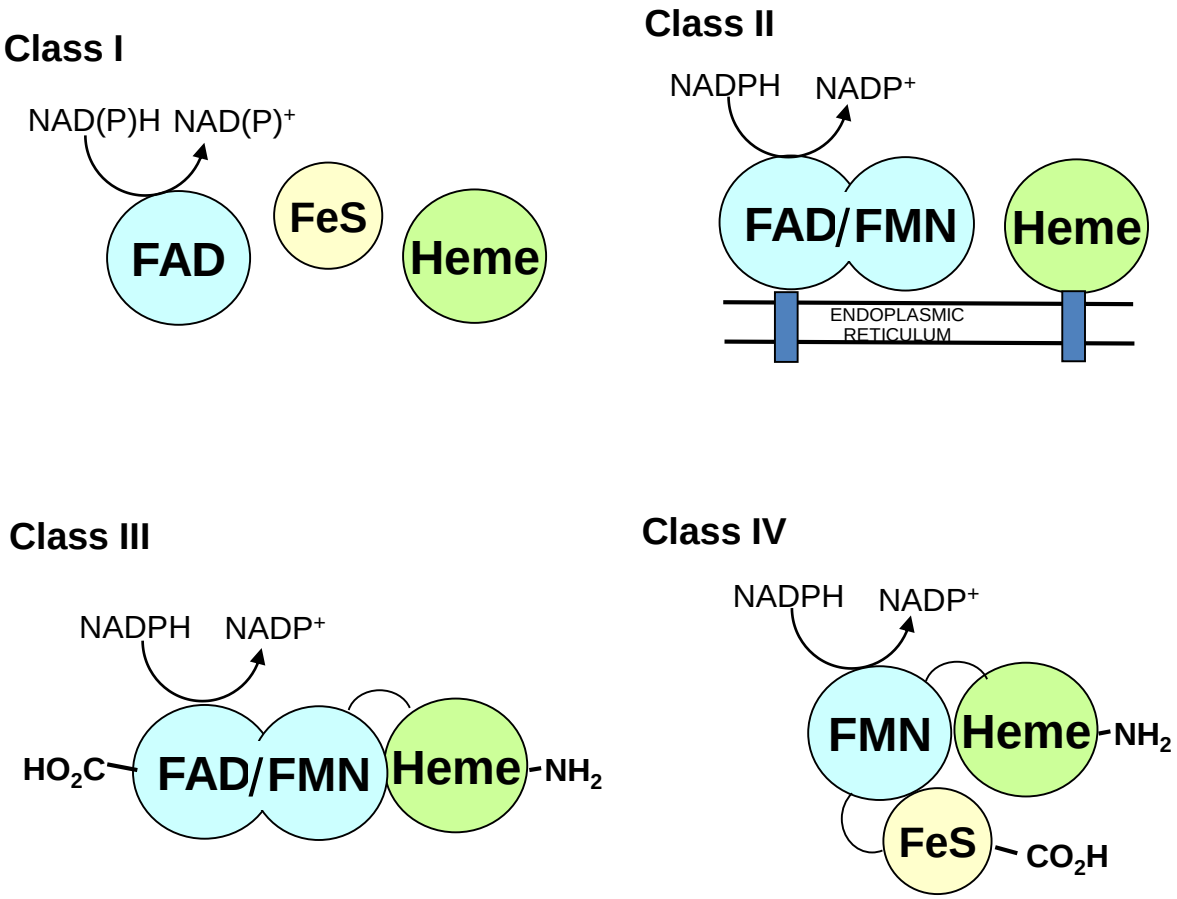


Hydroxylation.



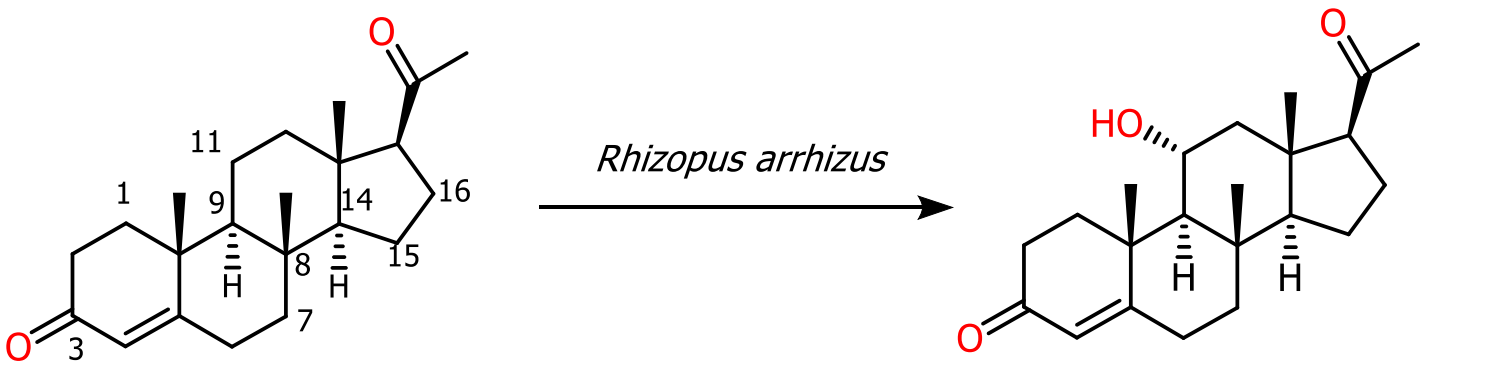
- Catalysed by Cytochromes P450 (Heme containing oxidases involved cellular detoxification processes).
- Whole cells are most frequently used as :
  1. P450s tend to be bound to the cell membrane (therefore intractable);
  2. Activity is dependent on non-protein ‘cofactors’ AND usually auxiliary redox proteins.

Hydroxylation (2).



Types of cytochrome P450 (from Roberts G.A., Grogan, G., Greter, A. Flitsch, S.L. e Turner N.J. *J. Bacteriol.* (2002) **184**, 3898-3908.

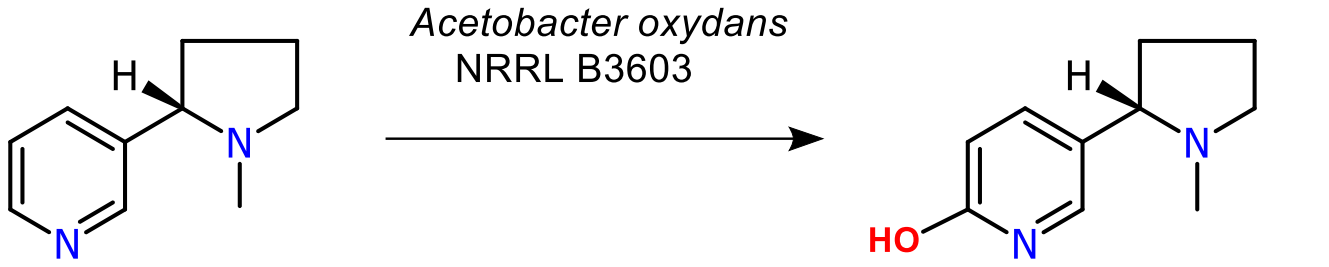
Hydroxylation (3).



Progesterone **11α-Hydroxy-Progesterone**

- Hydroxylation of steroids [e.g. Peterson et al. J. Am. Chem. Soc. 74, 5933-5936 (1952)]
- Commercial application of 11a hydroxylation of progesterone removed half of the steps to the synthesis of hydrocortisone
- A biocatalyst exists for the selective hydroxylation of EVERY position on the steroid nucleus
- No abiotic equivalent demonstrates the same selectivity.

Hydroxylation (4).

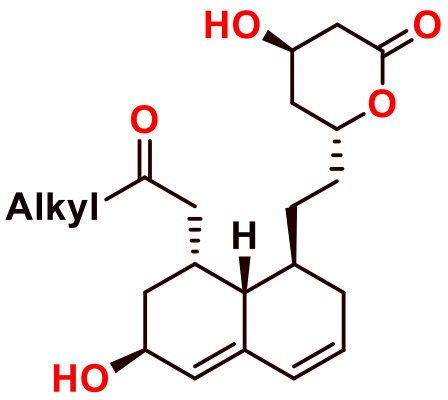


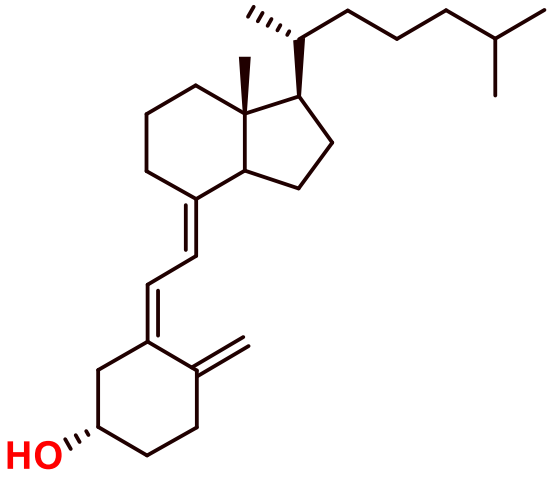
(*S*)-nicotine **6-Hydroxy-(*S*)-nicotine**

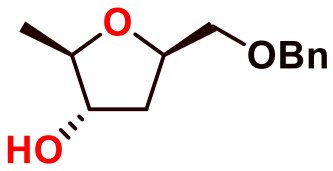
Hydroxylation of (*S*)-nicotine by *A. oxydans* operated by Lonza for the production of epibatidine.

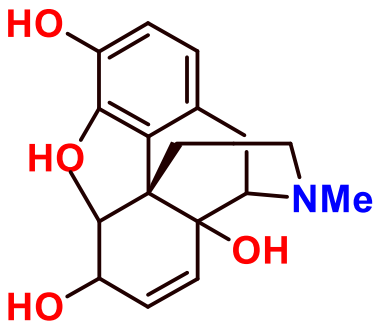
Schmid, A., Dordick, J.S., Hauer, B., Kiener, A., Wubbolts, M., Witholt, B., *Nature* (2001) **409**, 258-268.

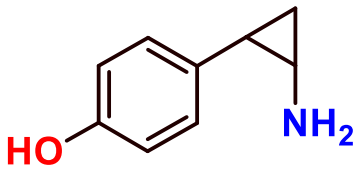
Hydroxylation (5).

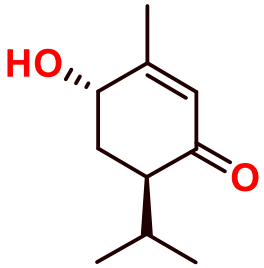




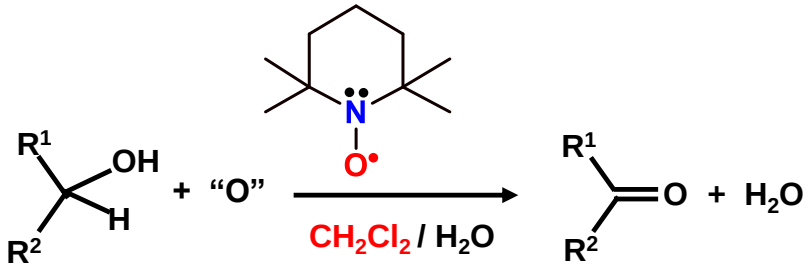






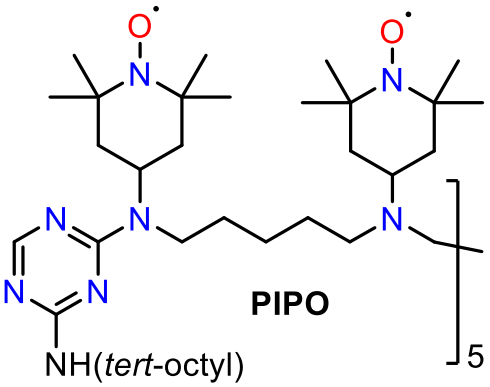


Alcohol Oxidations.



"O" = NaOCl, m-CPBA, oxone (+ Br -)

van Bekkum et al , Synthesis, 1996, 1153

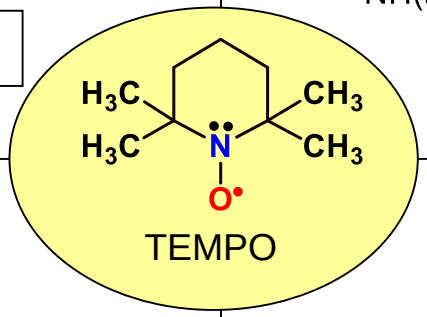


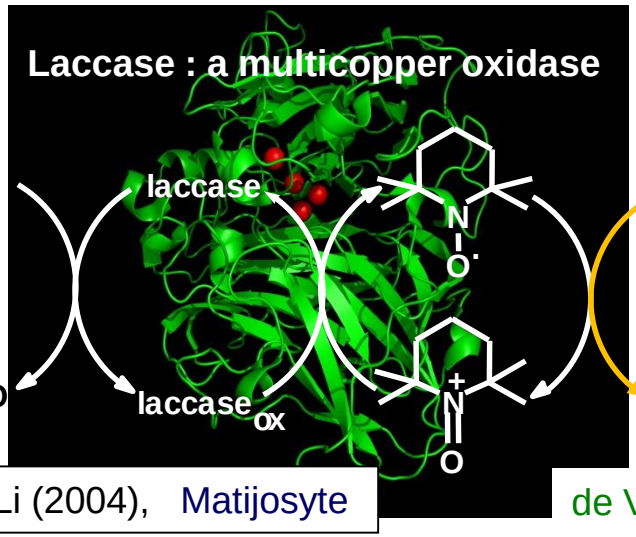
Dijksman (2001)

- No solvent
- No Br^-
- **NaOCl**
- Recyclable
- Cheap raw material

Chimassorb 944

Cu(II) / PIPO / O_2



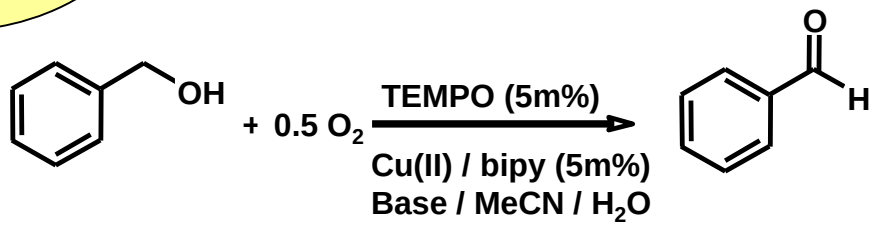


RCH$_2$OH

RCHO

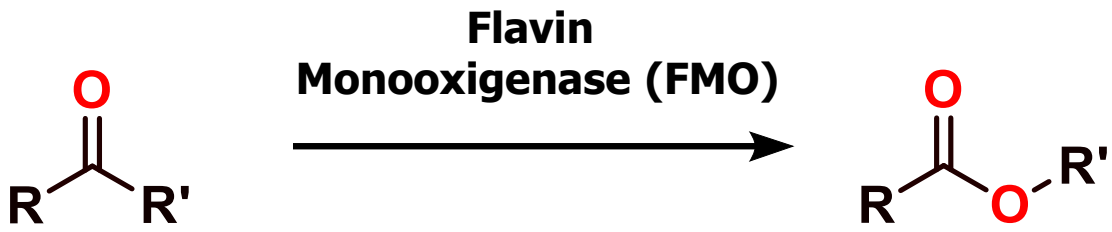
Li (2004), Matijosyte

de Vries/Hagen



Gamez

Baeyer-Villiger Oxidation.

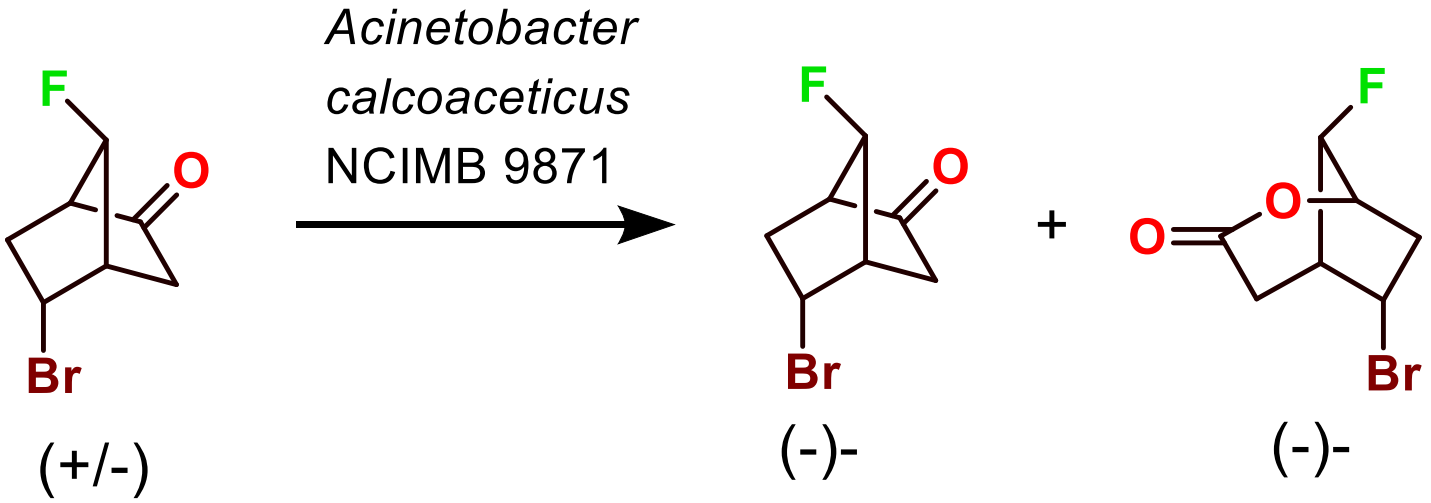


Insertion of an oxygen atom adjacent to a carbonyl group catalysed by (Baeyer-Villiger Monooxygenases (BVMOs).

BVMOs require

- A flavin cofactor
- A nicotinamide nucleotide cofactor
- O_2

Baeyer-Villiger Oxidation (2).

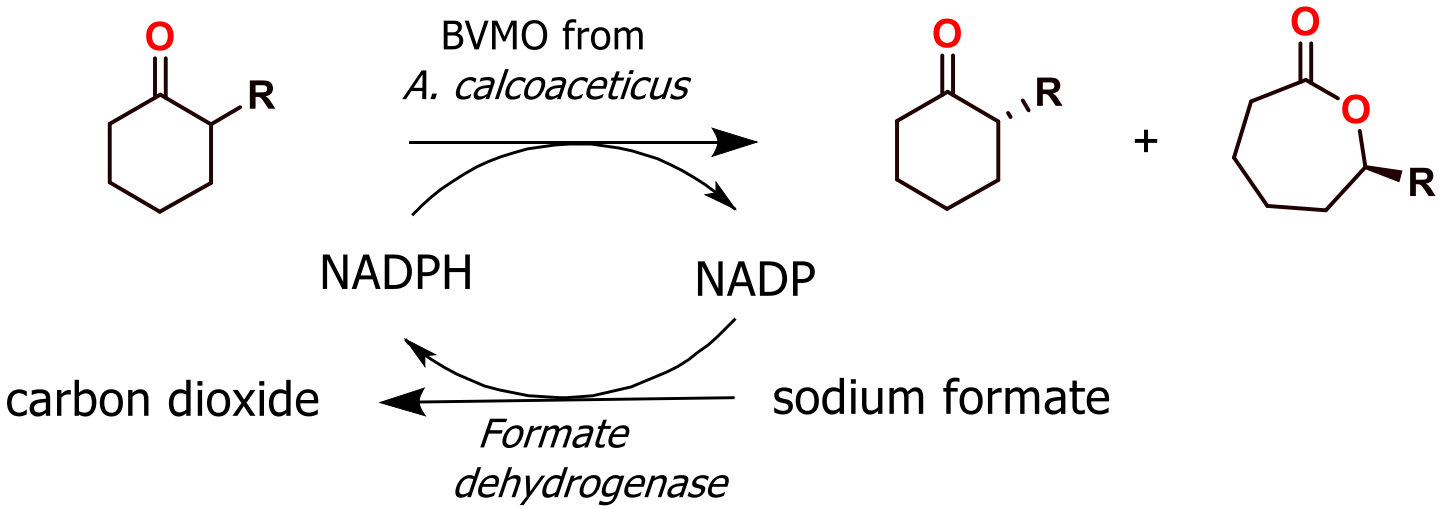


Levitt, M.S., Newton, R.F., Roberts, S.M. and Willetts, A.J., *J. Chem. Soc. Chem. Commun.*, (1990) 619-620.

Unfortunately, *A. calcoaceticus* is an ACDP di Class II pathogen.

Baeyer-Villiger Oxidation (3).

1. Use isolated enzyme

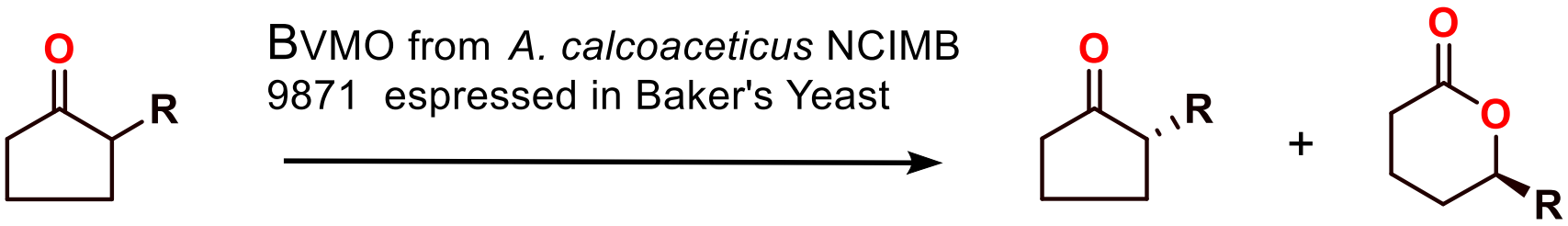


Seelbach K., Riebel B., Hummel W., Kula M.R., Tishkov V.I., Egorov A.M., Wandrey C., Kragl U., *Tetrahedron Lett.*, (1996) **37**, 1377-1380.

This is expensive (NADPH Sigma Catalogue £ 500/g!)

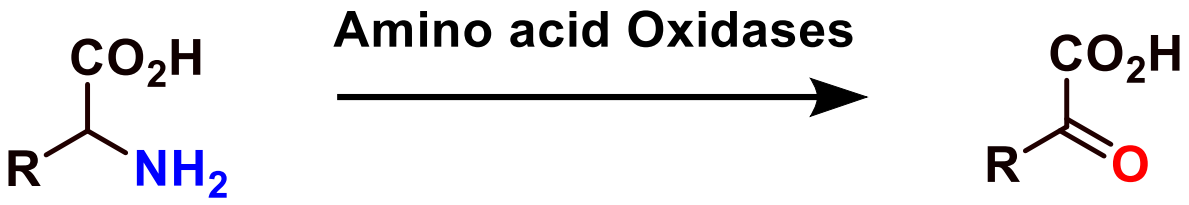
Baeyer-Villiger Oxidation (3).

2. Use engineered organism; BVMO expressed in ‘designer’ yeast or Class I *Escherichia coli*



Wang, S., Chen, G. Kayser, M.M., Iwaki, H., Lau, P.C.K. and Hasagawa, Y. *Can. J. Chem.*, (2002) **80**, 613-621.

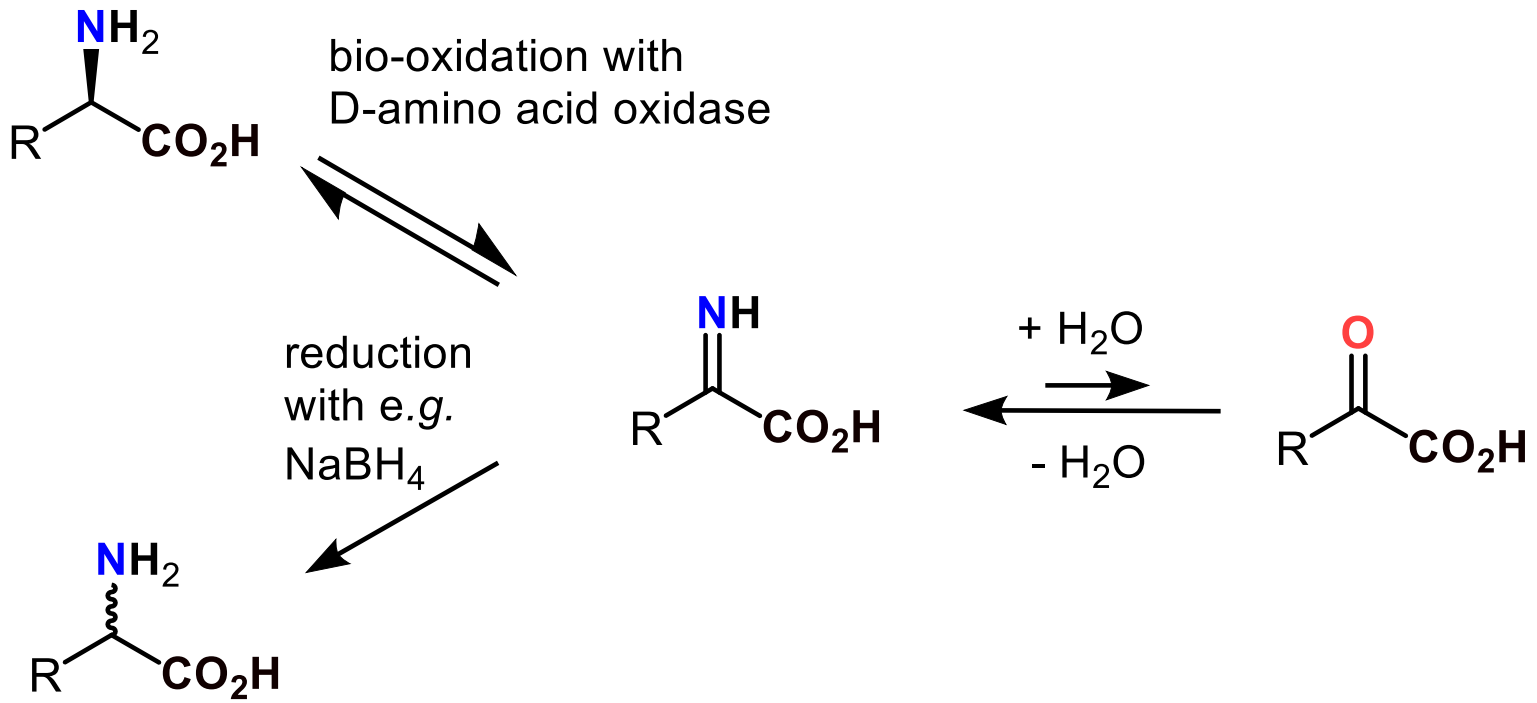
Amino acid Oxidases – Deracemisation of Amino Acids.



Amino acid oxidases

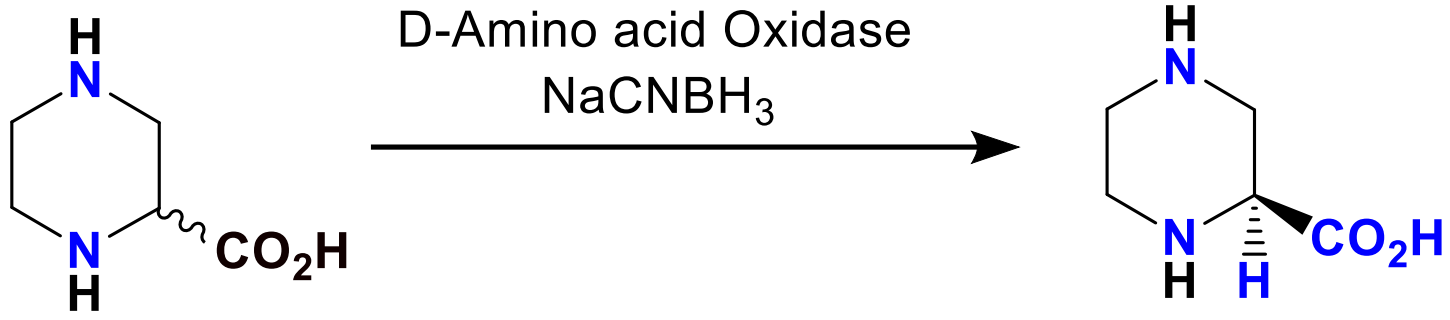
- Catalyse the oxidative deamination of amino acids to α-keto acids
- Require molecular oxygen and a flavin as cofactor
- Are commercially available with both ‘D’ and ‘L’ selectivity
- Can be used as both isolated enzymes, or expressed in whole cell systems.

Amino acid Oxidases – Deracemisation of Amino Acids.



Chemoenzymatic deracemisation of amino acids. After Hafner, E.W. and Wellner, D., *Proc. Natl. Acad. Sci.*, 1971, **68**, 987.

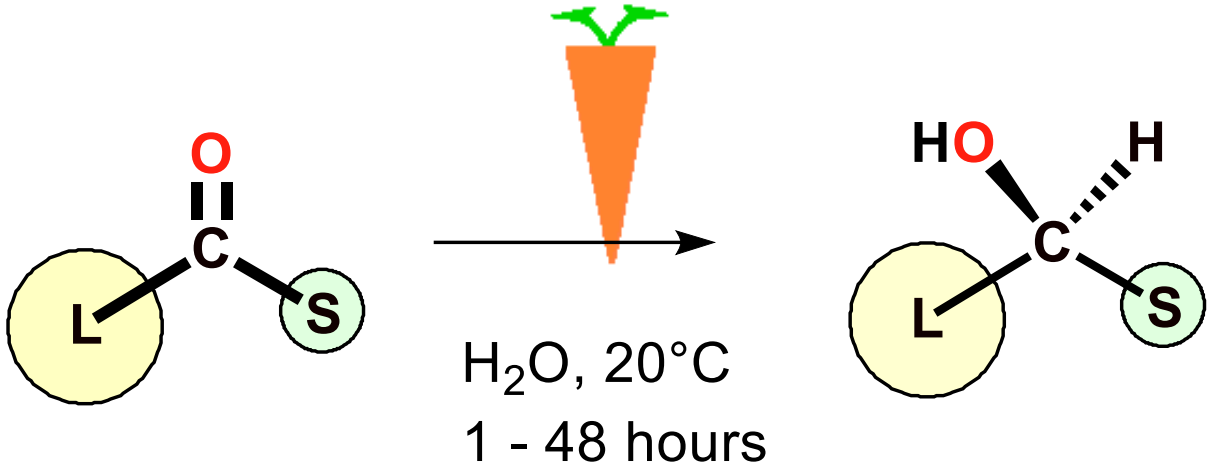
Amino Acid Oxidases.



Deracemisation of D,L-piperazine-2-carboxylic acid
(a component of the HIV protease inhibitor Crixivan).

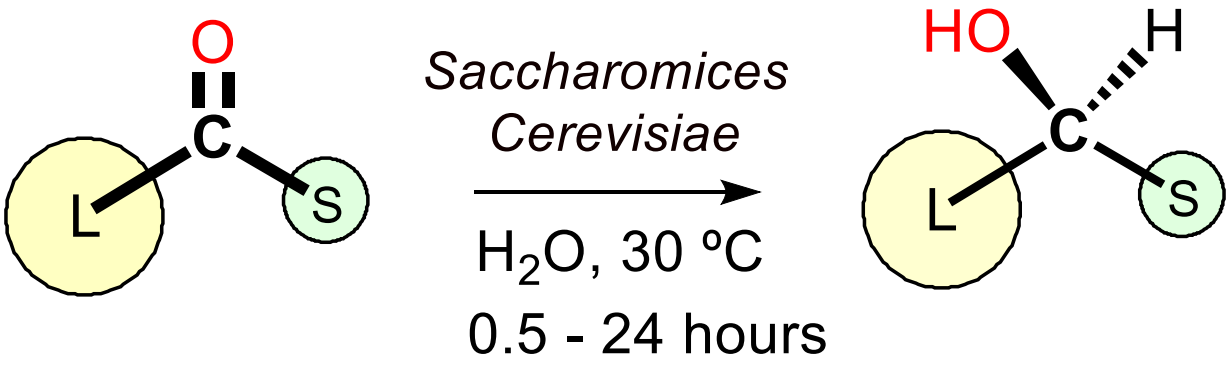
Beard T.M. e Turner N. J., *J. Chem. Soc. Chem. Commun*. (2002) 246-247.

Enzymatic Hydrogenation: *"Daucus carota"* Reductions.



- Yadav, J. S.; Nanda, S.; Thirupathi Reddy, P.; Bhaskar Rao, A. *J. Org. Chem.* **2002**, 67, 3900.
- Maczka, W. K.; Mironowicz, A. *Tetrahedron: Asymmetry* **2002**, 13, 2299.
- Bruni, R.; Fantin, g.; Medici, A.; Pedrini, P.; Sachetti, G. *Tetrahedron Lett.* **2002**, 43, 3377.
- Baldassarre, F.; Bertoni, G.; Chiappe, C.; Marioni, F. *J. Mol. Cat. B: Enzymatic* **2000**, 11, 55.
- Chadha, A.; Manohar, M.; Soundararajan, T.; Lokeswari, T. S. *Tetrahedron: Asymmetry* **1996**, 7, 1571.

Baker's Yeast Reductions.



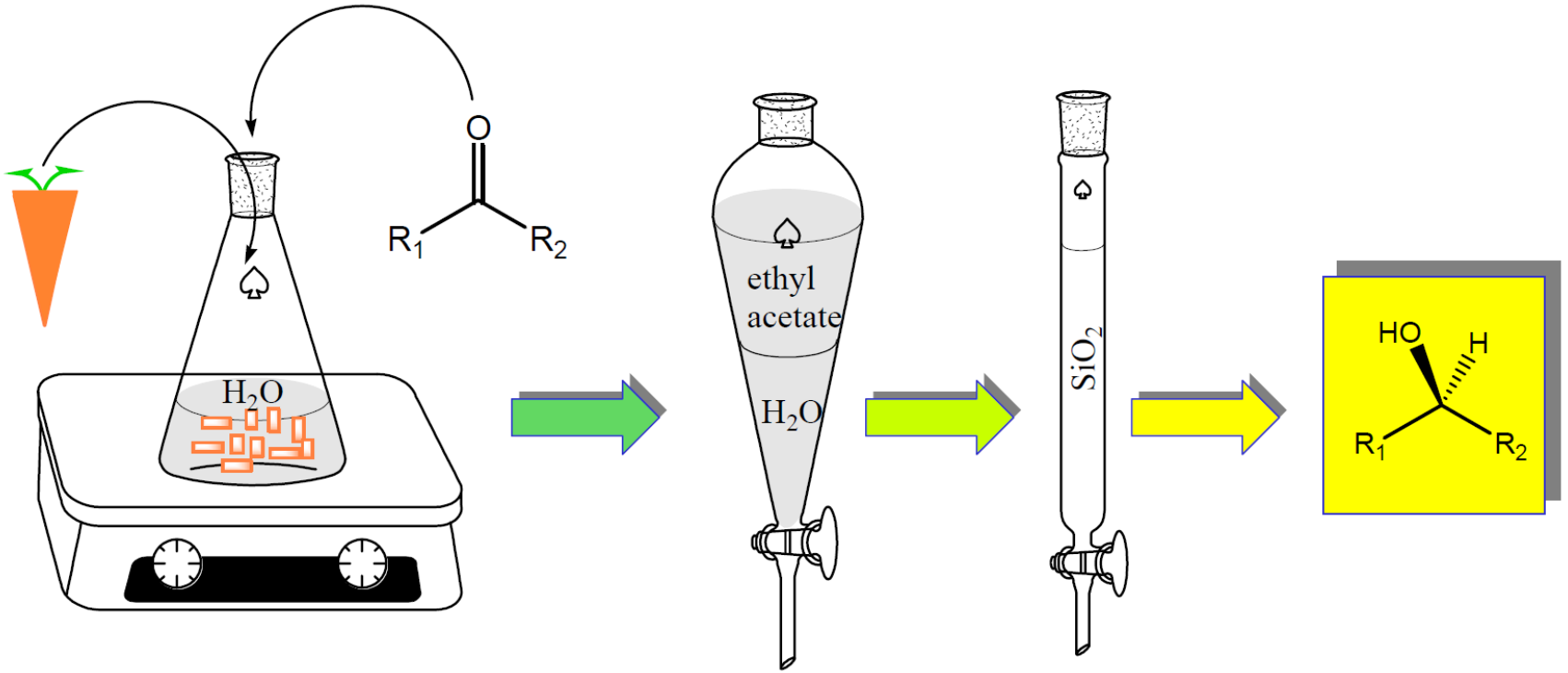
- stereoselective
- green
- easy to perform
- reasonably priced

- "Baker's Yeast" is the only microorganism that can buy at the grocery store.
- Does not need aseptic conditions nor a microbiology lab.
- Does not need a microbiologist in the team.
- Actually do not need to know any microbiology at all.

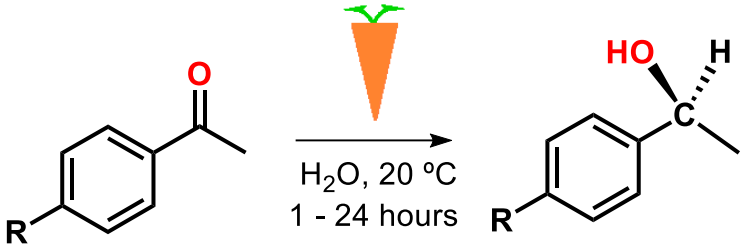
Drawbacks in the BY Use.

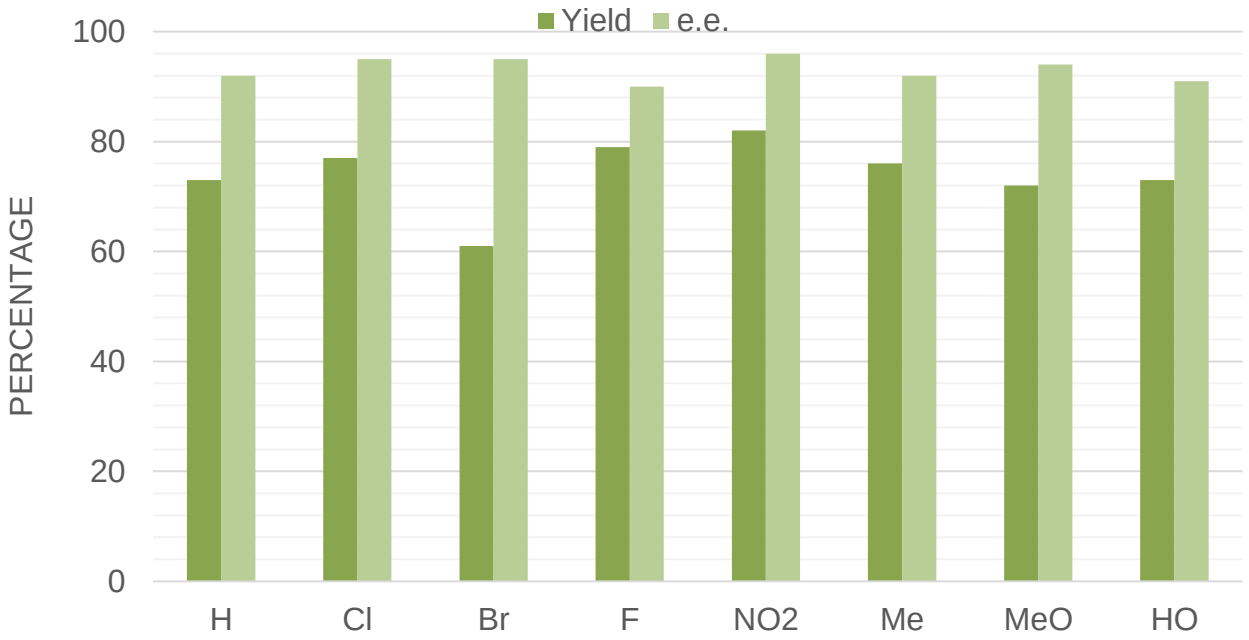
- There are many different enzymes in the system therefore side reactions cannot be ruled out;
- Commercially BY is not pure and frequently affords unexpected results;
- Isolation of the product is sometimes complicated;
- There might be an environmental impact involved in BY preparation.

A Simple Procedure.



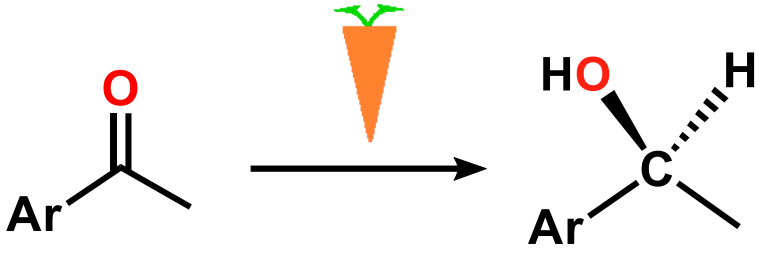
Substituent Effect.

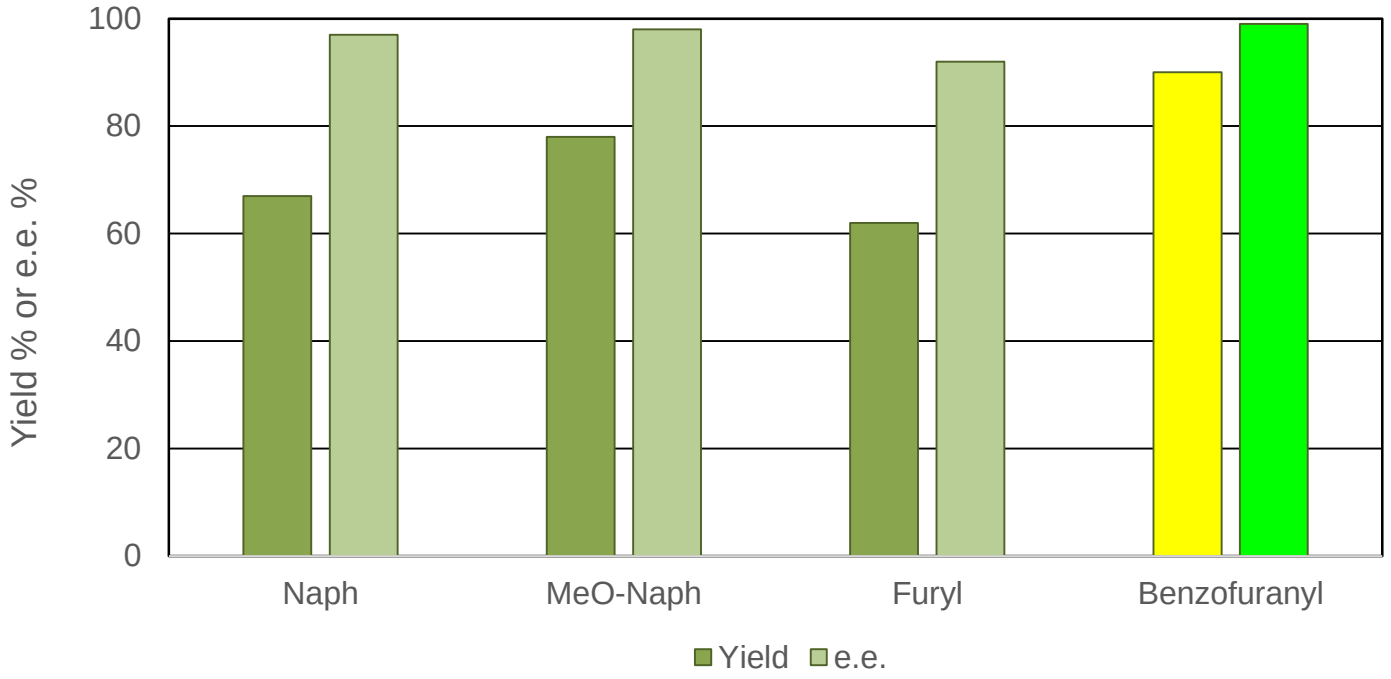




Yadav, J. S.; Nanda, S.; Thirupathi Reddy, P.; Bhaskar Rao, A. *J. Org. Chem*. **2002**, 67, 3900.

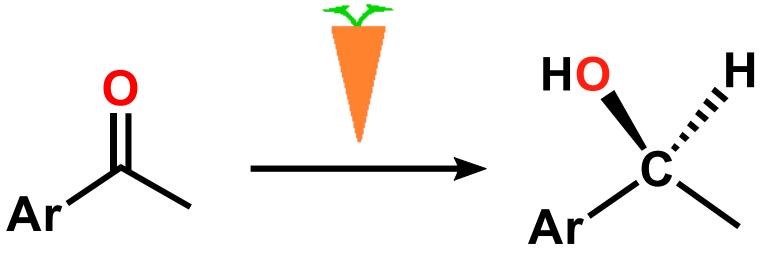
Aromatic Ring Effect.

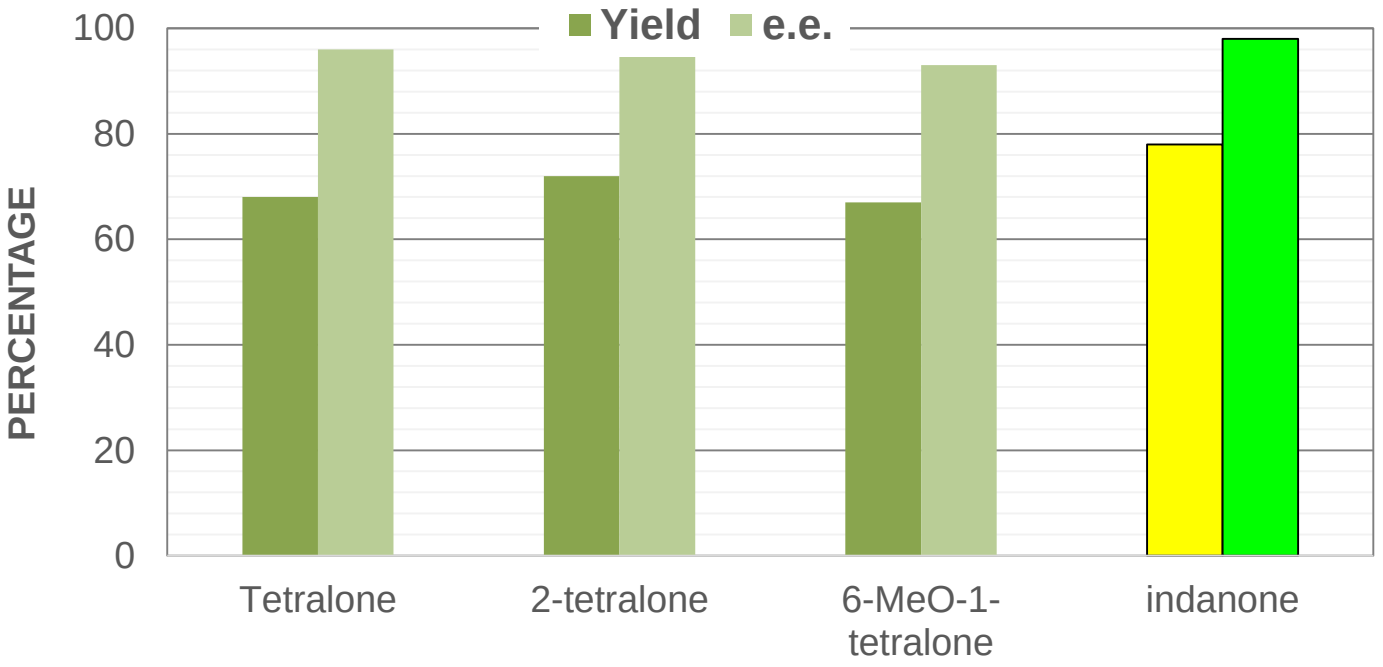




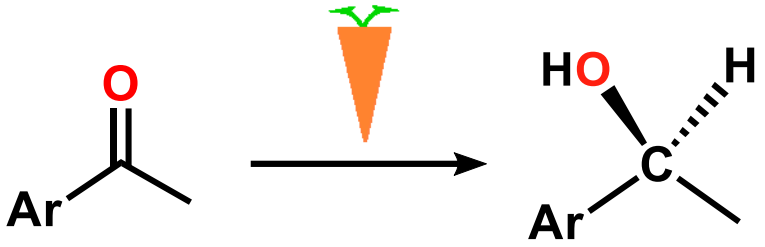
Yadav, J. S.; Nanda, S.; Thirupathi Reddy, P.; Bhaskar Rao, A. J. Org. Chem. **2002**, 67, 3900.

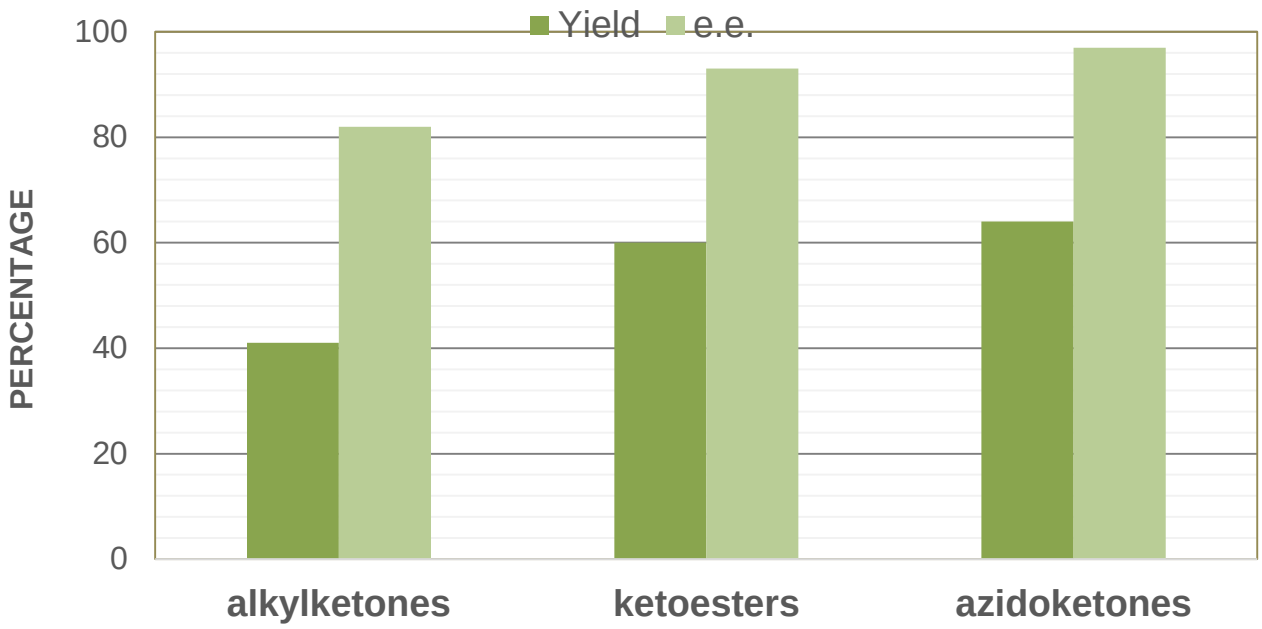
Aromatic Ring Effect (2).





Other Carbonyl Compounds.

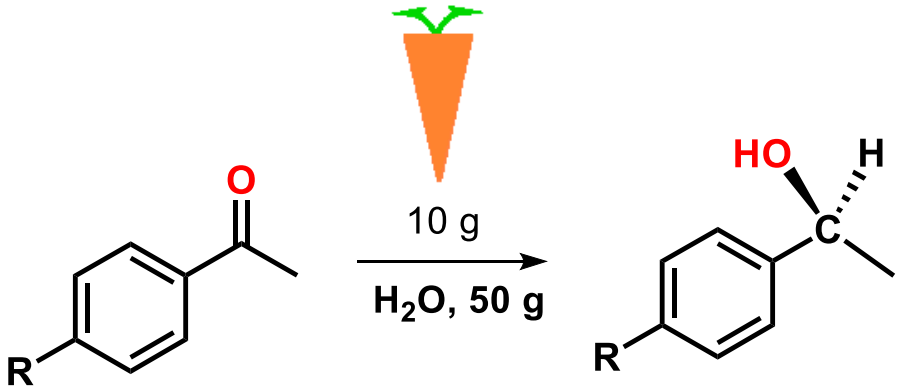




Efficiency: E Factor and EMY in Biotrasformations.

E Factor = $\frac{\text{Kg waste mass}}{\text{Kg desired Product}}$

EMY Factor = $\frac{\text{Kg Product mass}}{\text{Kg Nbio waste*}}$



100 mg

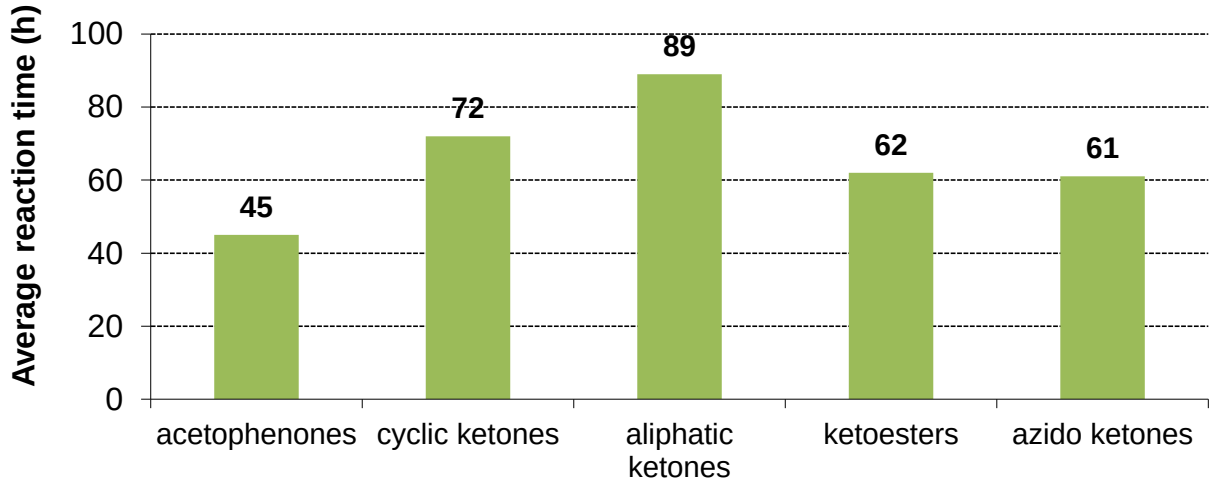
70 mg

E = 10 g / 70 × 10^{-3} g = 143

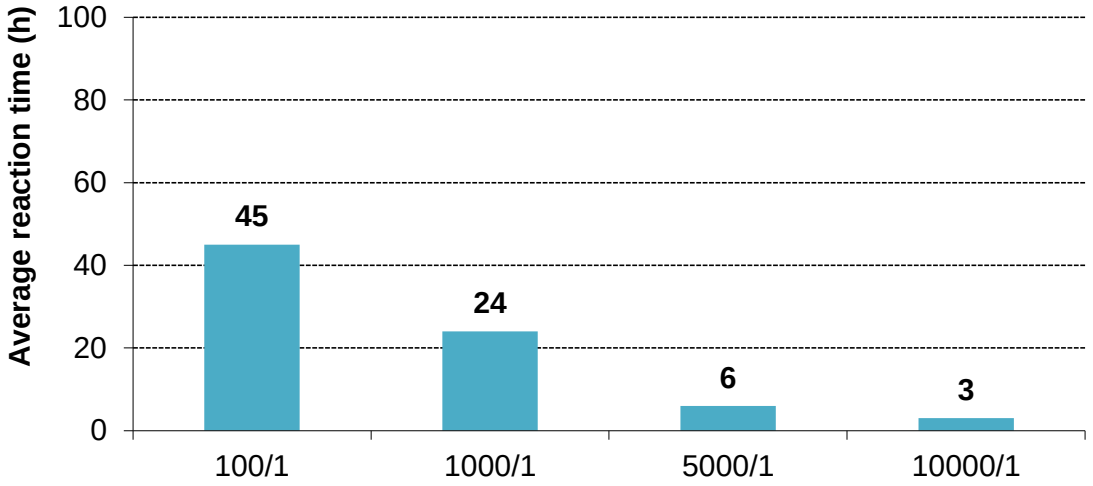
EMY = 70 × 10^{-3}g / 70 × 10^{-3} (+10)g = 1 to 0.007

* NBio = total mass that can not be disposed or recycled safely

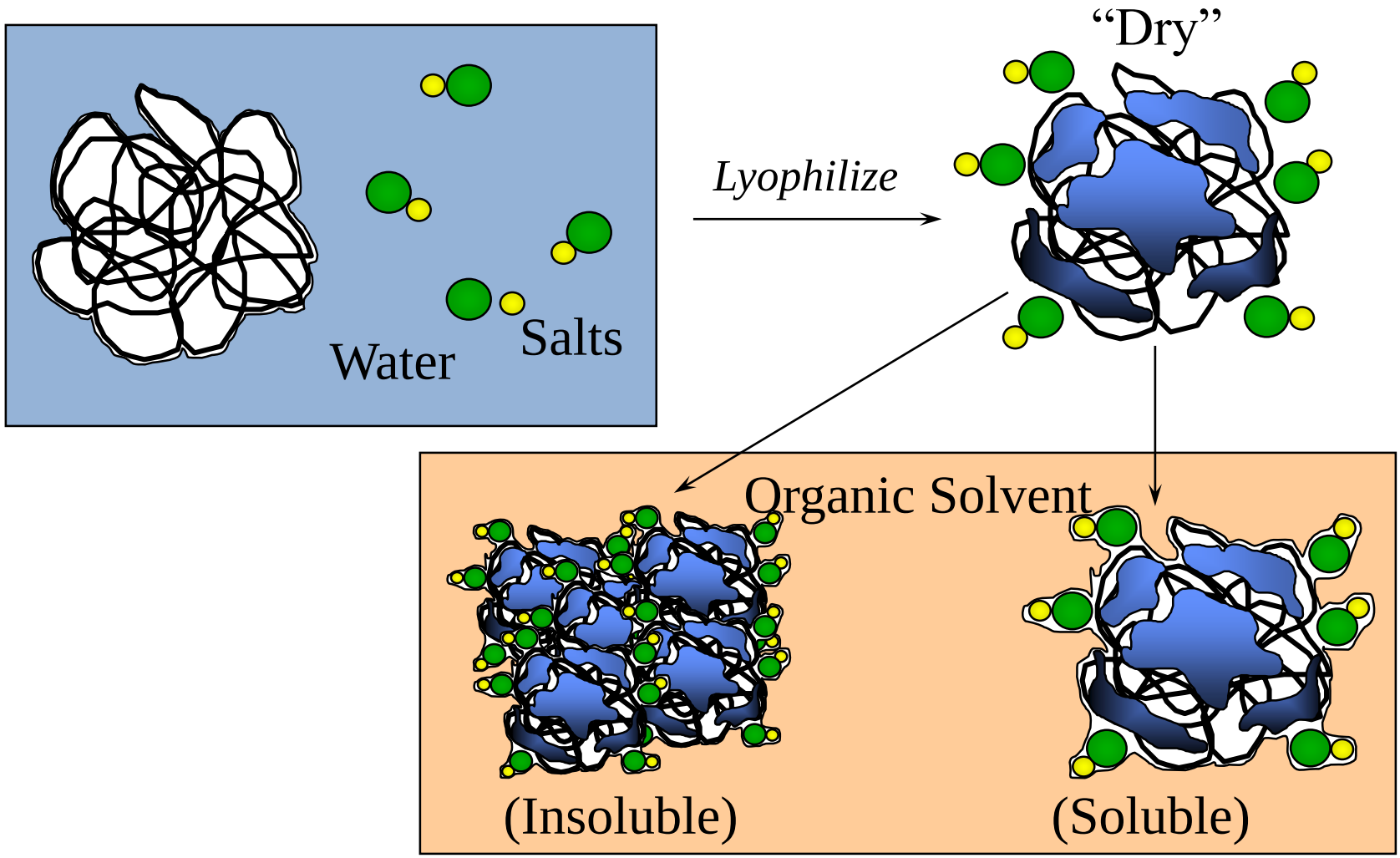
Reaction Time.



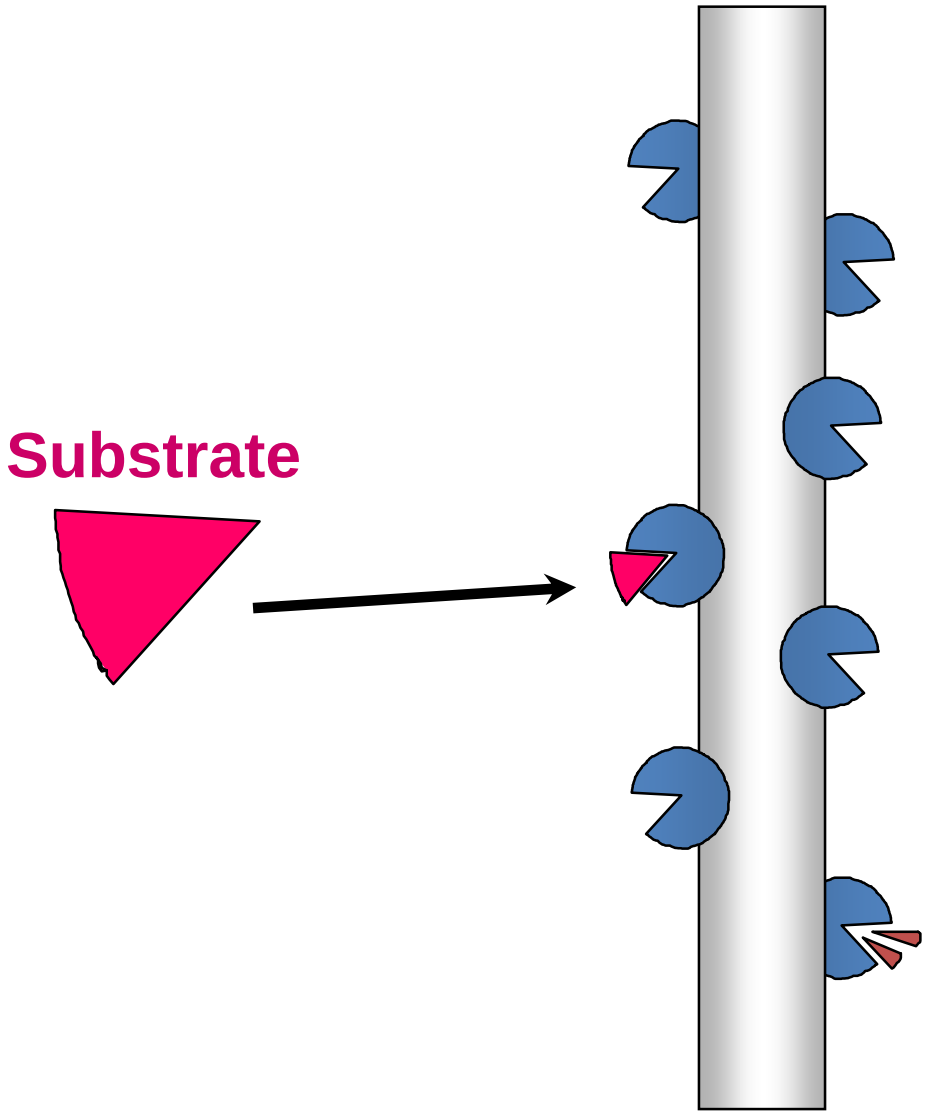
Carrot/Substrate ratio



Unconventional Uses of Enzymes in a Nonaqueous World.

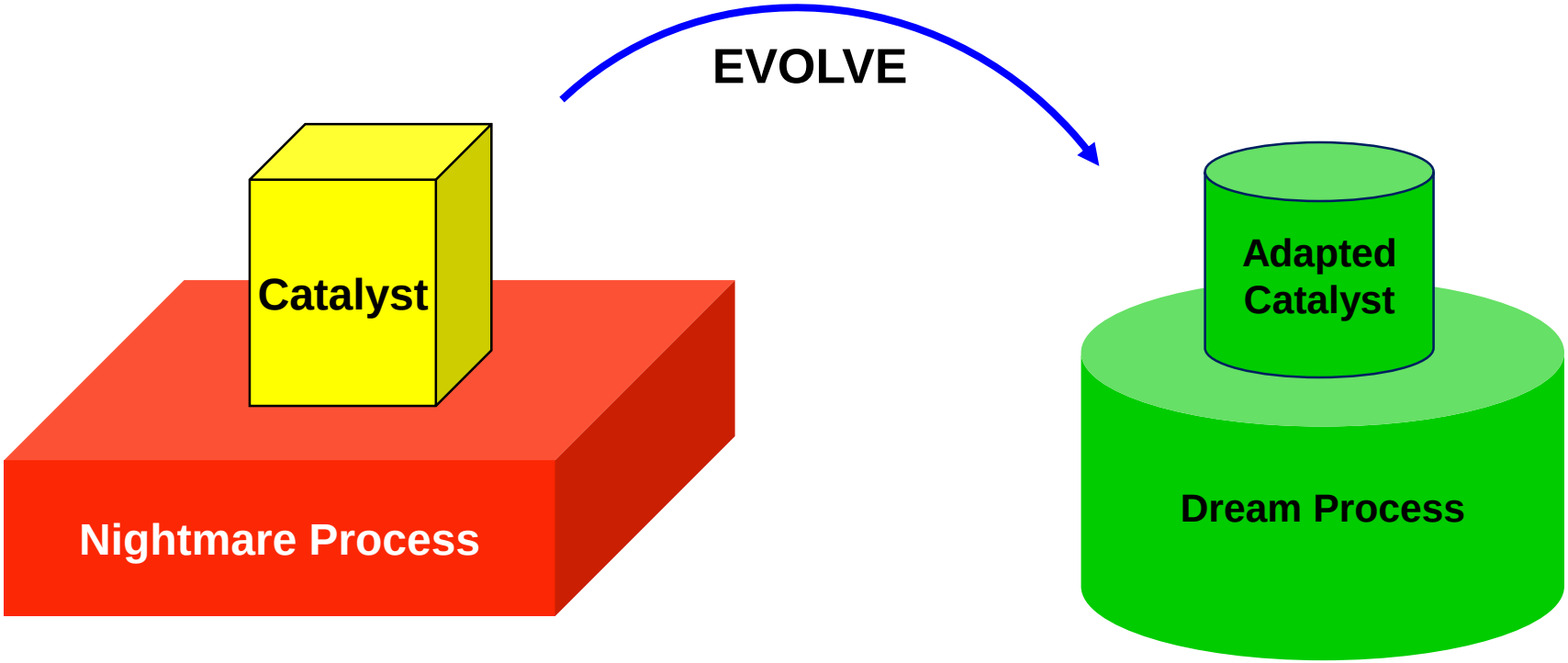


Unconventional Use of Enzymes: Dry-State.



- Higher temperatures allowed
- Higher concentration of vapors
- Higher stability/durability
- <u>Kinetically limited,</u> not interphase transport limited
- Proven for esterification, testing CWA simulant

Adapt Enzyme Catalyst to fit Ideal Process.



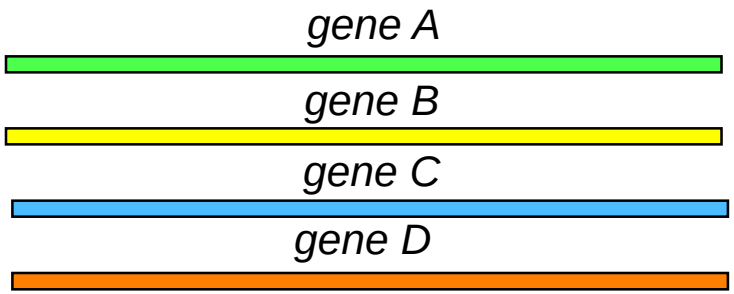
Compromise process to accommodate catalyst

Adapt catalyst to optimum process

Directed Evolution

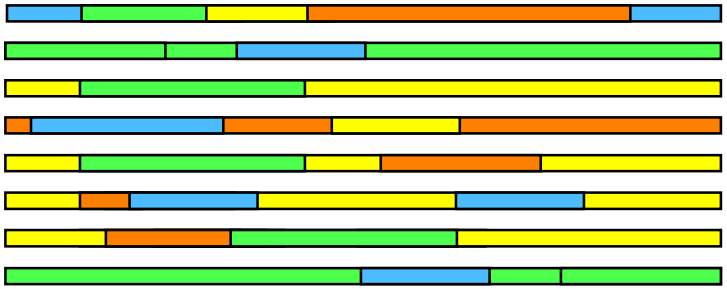
DNA Shuffling : Evolution in the Fast Lane.

Genes From Nature

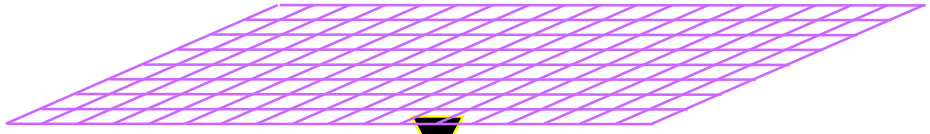


DNA Shuffling™

Library of Novel Genes



HTP Screening

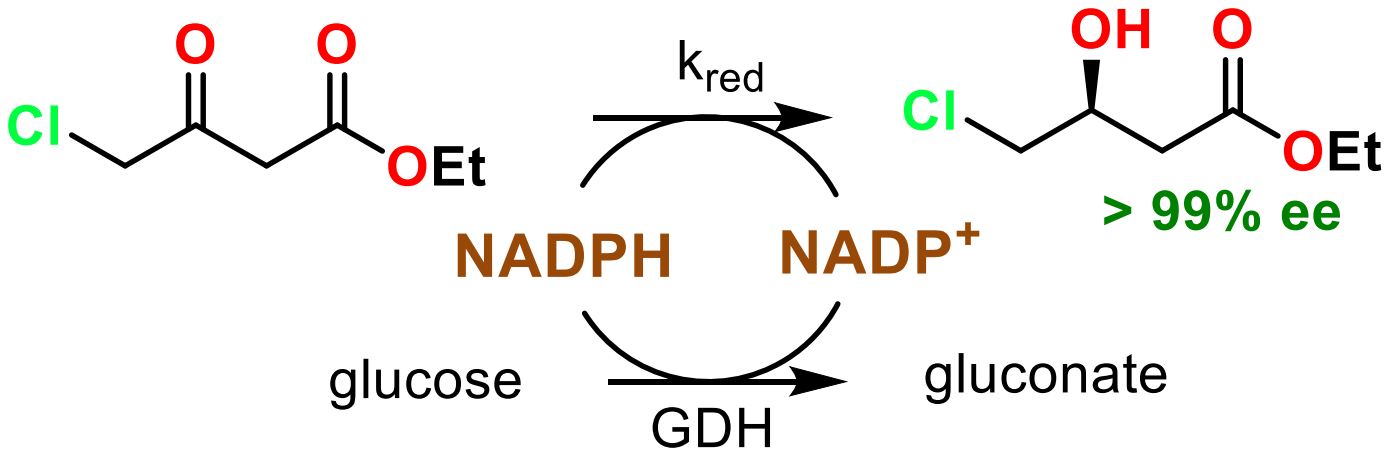


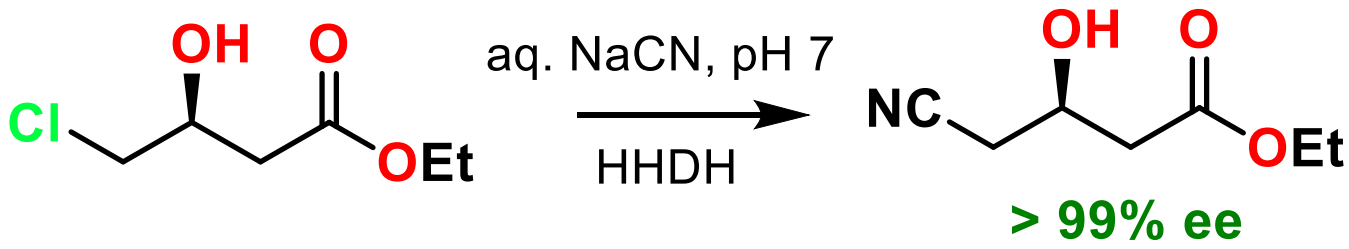
Novel Genes

Repeat

Green Synthesis of Lipitor Intermediate (Codexis).

Presidential Green Chemistry Challenge Award 2006





KRED = keto reductase ; GDH = glucose dehydrogenase
HHDH = halohydrin dehalogenase (non-natural nucleophile)

Nature Biotechnol. 2007, 25, 338-334

Improving Performance by Directed Evolution: test tube to commercial process with gene shuffling.

1. KRED + GDH

Parameter	Process Design	Initial Performance	Final Performance
Substrate loading	160 g/L	80 g/L	180 g/L
Reaction time	<16 hrs	24 hrs	8 hrs
Enzyme loading	<1 g/L	10 g/L	0.7 g/L
Isolated yield	>90%	~80%	97%
Phase separation time		>1 hr	~1 min.
Volumetric Productivity	>240 g/L.day	80 g/L.day	540 g/L.day

2. HHDH

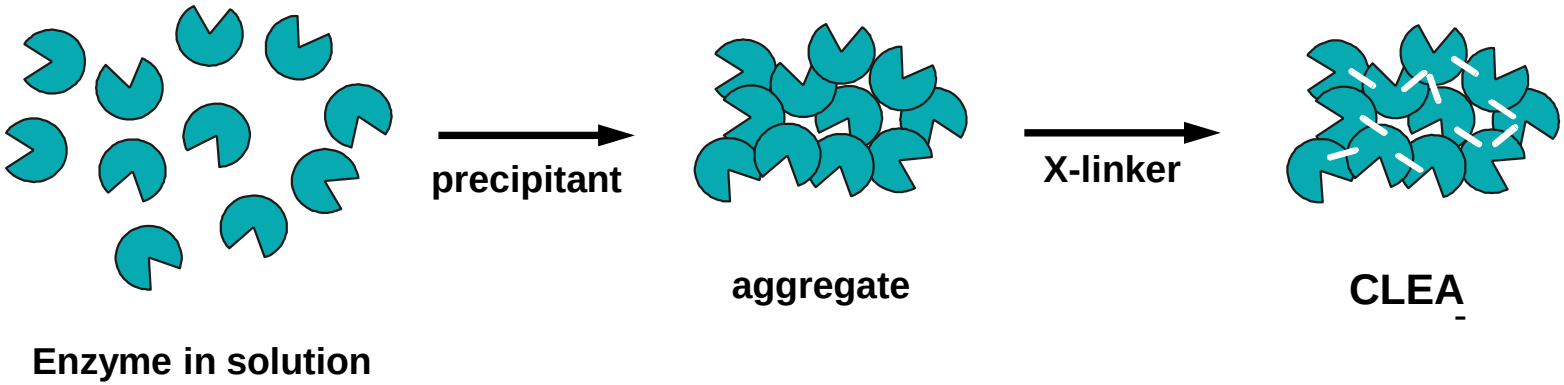
er	Process Design	Initial Performance	Final Performance
Substrate loading	120 g/L	20 g/L	140 g/L
Reaction time	<16 hrs	72 hrs	5 hrs
Enzyme loading	<1.2 g/L	130 g/L	1.2 g/L
Isolated yield	>90%	~60%	92%
Volumetric Productivity	>180 g/L.day	7 g/L.day	670 g/L.day

Disadvantages of Enzymes.

- Low operational stability & shelf-life
- Cumbersome recovery & re-use
  (batch vs. continuous operation)
- Product contamination

Solution :
Immobilization!

Cross-Linked Enzyme Aggregates (CLEAs).



glutaraldehyde or dextran polyaldehyde as X-linker

- **Enables recycling via filtration**
- **Higher productivity**
- **No need for highly pure enzyme**
- **Simple procedure / widely applicable**
- **Stability towards denaturation**

CLEAS active in:

- $scCO_2$ (M. Poliakoff)
- ILs (Sheldon)

Sorgedrager (2006), Janssen (2006)

Examples of Successful CLEAtion.

Hydrolases

- Pen. acylases (2)
- Lipases (7)
- Esterases (3)
- Proteases (3)
- Nitrilases (2)
- Aminoacylase
- Phytase
- Galactosidase
- OPH

Oxidoreductases

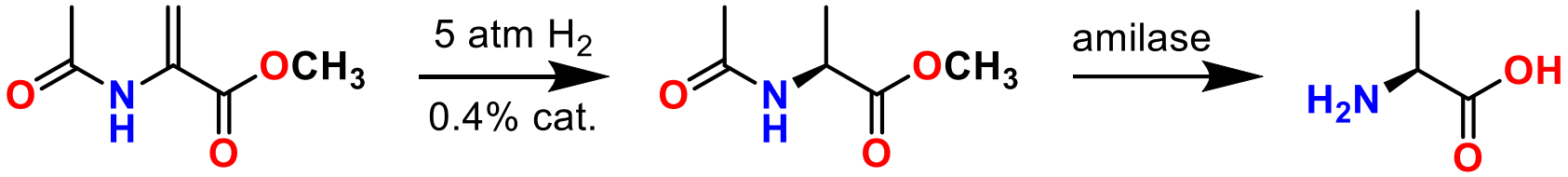
- ADH
- FDH
- Glucose oxidase
- Galactose oxidase
- Laccase
- Catalase
- Chloroperoxidase

Lyases

- *R*- & *S*- HnLases
- PDC
- DERA
- Nitrile hydratase

Cao, Lopez-Serrano, Mateo, Perez, van Langen, Sorgedrager Janssen, Bode, van Pelt, Chmura, Matijosyte, Aksu-Kanbak,

Catalytic Cascade Processes.

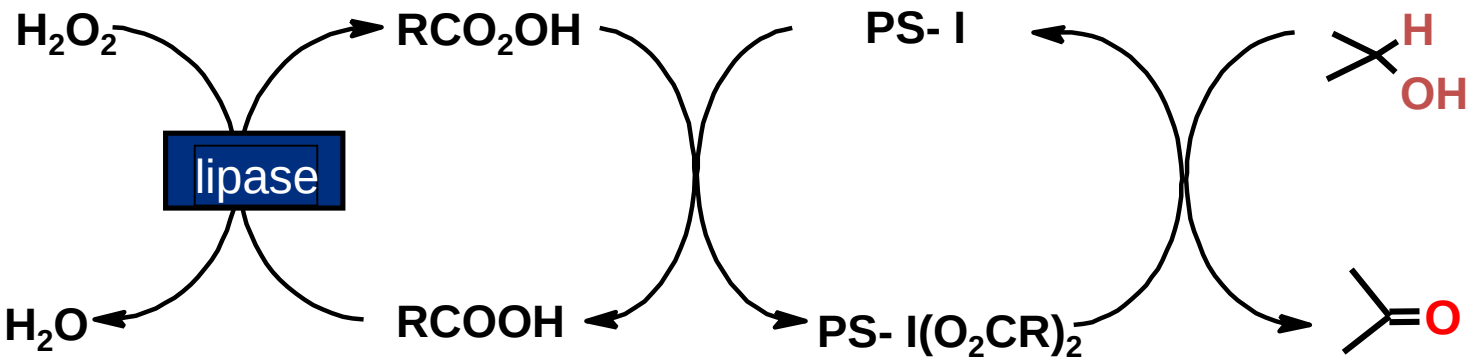


Catalyst :
Rh(monophos) on TUD-1

99% yield / 95% ee

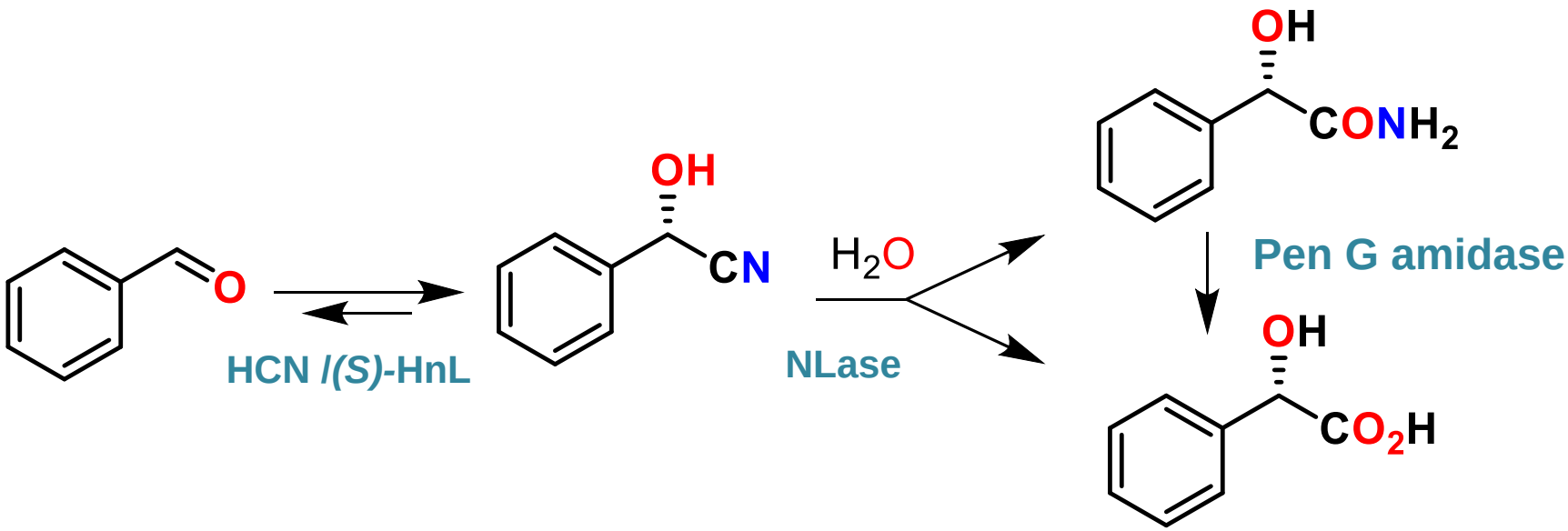
97% yield / > 99% ee

Simons (2007)



Kotlewska

Trienzymatic Cascade with a Triple-Decker combi CLEA.



Conv. 96% / ee >99%

Chmura, Stolz

Conclusions.

- **Biocatalytic reactions continue to be studied with great interest as:**
  - In many cases, no equivalent ‘abiotic’ reactions of comparable selectivity exist;
  - General characteristics of Biocatalysed Reactions lend them to be acknowledged as ‘greener’ routes to many compounds.

- **Obstacles**
  - Perceptions of the use of microbial/ biochemical systems by the organic community;
  - Perceptions of the use of GM organisms/reagents in the production of material for human consumption.

Bioprocessing Research Areas.

- Production of fuels and chemicals
- Bioprocessing of fossil fuels
- Biotreatment & bioremediation
- Applied Biology
- Organic Synthesis.

Capabilities

- BioChem. Engr. - novel reactors, separations, modeling, & system integration
- Multi-phase & nonaqueous biocatalysis
- Microbial strain development & bioprospecting
- Bioprocessing Research User Facility.

Related areas - Separations (electrically driven)
- Biomimetics (sorbents, catalysis, materials).

Biotreatment & Bioremediation of Wastes.

- Biofiltration and Biosolubility of VOCs (alkanes, NOx, TCE);
- Chem-Bio-Agents;
- Nonaqueous (‘dry’) biocatalysis for hazardous vapors (CWA, VOCs);
- Biosorption of heavy metals (U, Cd) with biopolymers;
- Mercury removal and treatment;
- Bioremediation using nonaqueous thermophilic enzymes (Chlorinated solvents);
- PCB Biodegradation;
- BTEX and fuels biodegradation;
- Microbial over expression of degradative enzymes and GEM production;
- Pesticide biodegradation.

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