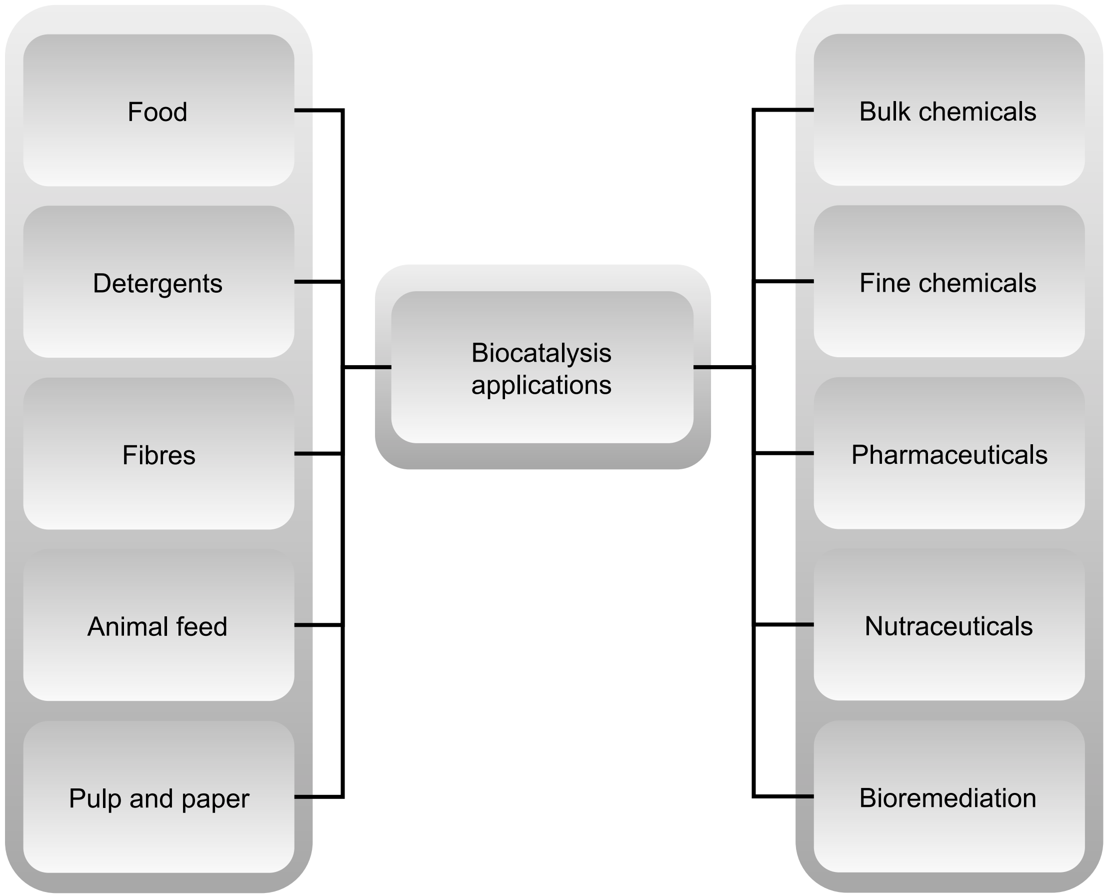
13. BIOCATALYSIS

BIOCATALYSIS: GENERAL FEATURES



Catalysis/ Rothenberg, ISBN
978-3-527-31824-7.
www.catalysisbook.org

BIOCATALYSIS: GENERAL FEATURES

- Enzymes are the most proficient catalysts, offering much more competitive processes compared to chemical catalysts. The number of industrial applications for enzymes has exploded in recent years, mainly owing to advances in protein engineering technology and environmental and economic necessities.

- Applications of enzymes and whole cell biocatalysis for producing diverse types of chemical and biological substances have become a proven technology in chemical and pharmaceutical industries because enzyme-based processes usually lead to a **reduction in the process time, number of reaction steps, and amount of waste**

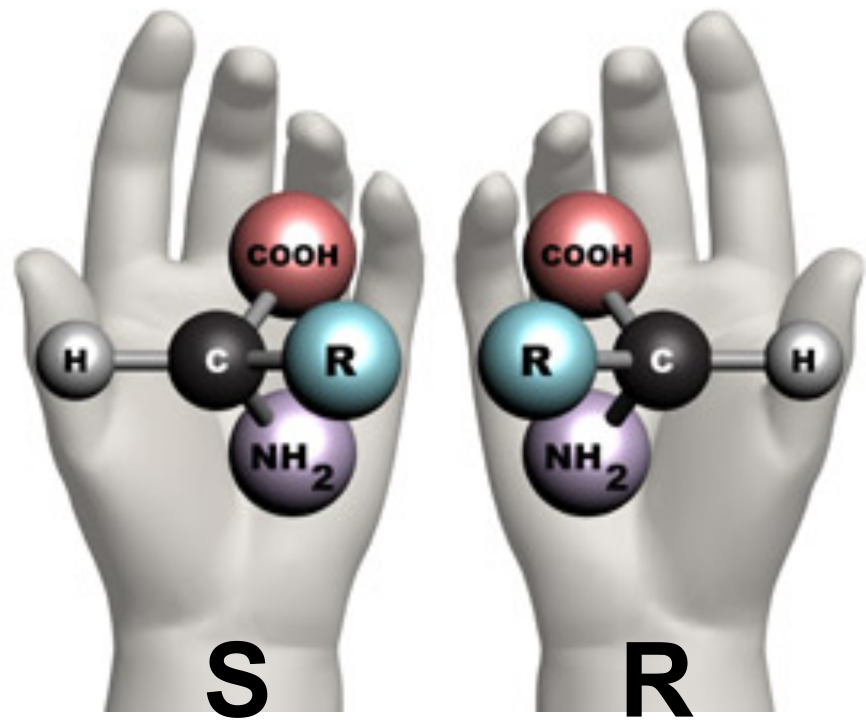
- In particular, enzymes provide a more powerful way of producing enantiomerical pure compounds mainly through high **chemoselectivity, regioselectivity, and streoselectivity**

WHY BIOCATALYSIS

- Sometimes it is the most efficient route to a target molecule

- Generally, it can be difficult to synthesize a single chiral center, thus enzymes are excellent at generating a single enantiomer

- Industrial Friendly
- Green Chemistry



Stereoisomers

WHY BIOCATALYSIS

- The key word for organic synthesis is selectivity which is necessary to obtain a high yield of a specific product.
- There are a large range of selective organic reactions available for most synthetic needs.
- However, there is still one area where organic chemists are struggling, and that is when chirality is involved, although considerable progress in chiral synthesis has been achieved in recent years.

Enzymes display three major types of selectivities:

- **Chemoselectivity**: Since the purpose of an enzyme is to act on a single type of functional group, other sensitive functionalities, which would normally react to a certain extent under chemical catalysis, survive. As a result, biocatalytic reactions tend to be "cleaner" and laborious purification of product(s) from impurities emerging through side-reactions can largely be omitted.
- **Regioselectivity and Diastereoselectivity**: Due to their complex three-dimensional structure, enzymes may distinguish between the same functional groups which are chemically situated in different regions of the substrate molecule.
- **Enantioselectivity**: Since almost all enzymes are made from L-amino acids, enzymes are chiral catalysts. As a consequence, any type of chirality present in the substrate molecule is "recognized" upon the formation of the enzyme-substrate complex. Thus a prochiral substrate may be transformed into an optically active product and both enantiomers of a racemic substrate may react at different rates.

Advantages of Biocatalysts using Enzymes

- Enzymes are very **efficient** biocatalysis (high turnover number, high affinitiy for the substrate (microM range)
- Enzymes are **environmentally** acceptable.
- Enzymes act under **mild** conditions (pH 5-8, 20-40°C)
- Enzymes are **compatible** with each other (no side reaction)
- The conversions are carried out in **aqueous** solution

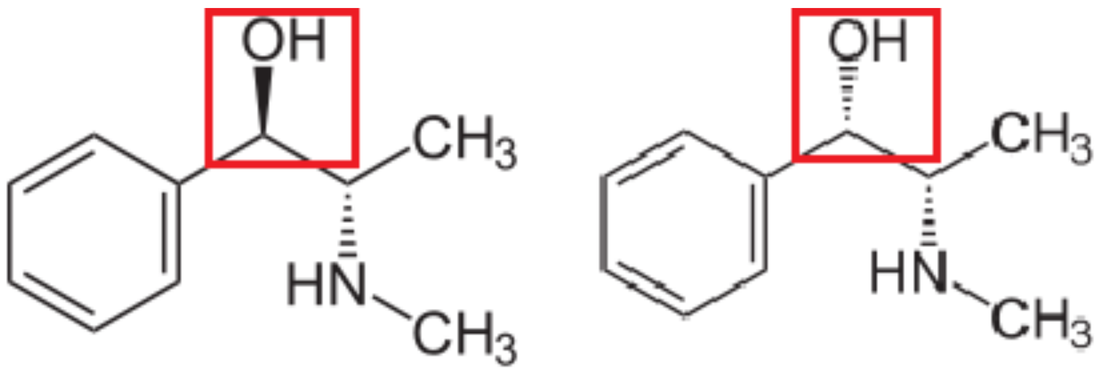
Disadvantages

- Enzymes are provided by nature in only **one enantiomeric form**
- Enzyme require **narrow** operation parameters
- Enzymes display their highest catalytic activity in water
- Enzymes are bound to their natural **cofactors**
- Enzymes are prone to **inhibition** phenomena
- Enzymes may cause **allergies**

WHY BIOCATALYSIS

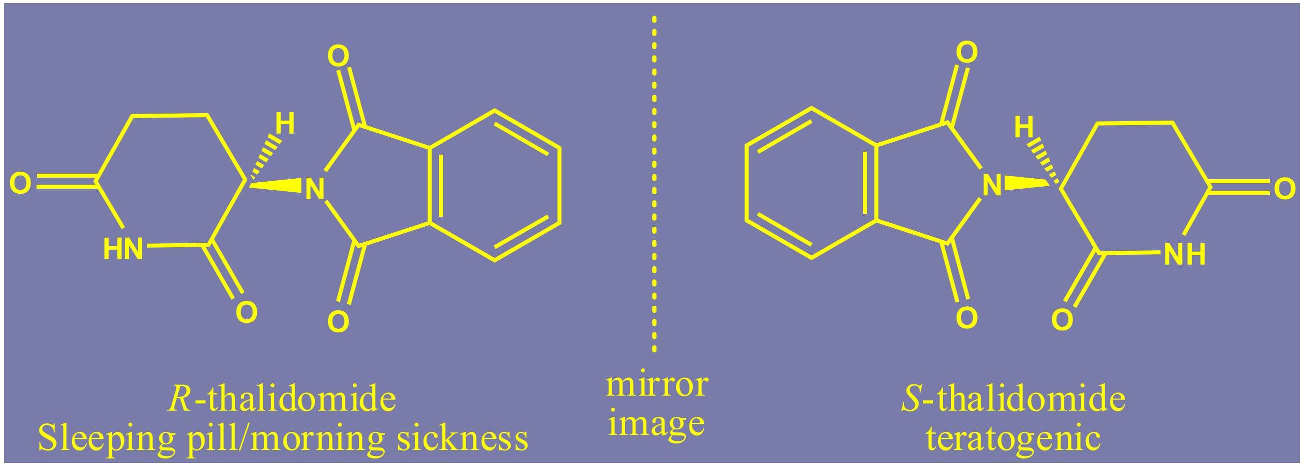
Enzymes and receptors in the body are chiral

- One stereoisomer is usually more potent
- Some stereoisomers may display different pharmacological effects



Ephedrine **Pseudoephedrine**

Thalidomide and the Role of Enantiomers and FDA approval



***S*-enantiomer causes Phocomelia**

http://engineering.cua.edu/biomedical/faculty/kirtley/synergy/ar_thalidomide909.jpg

BIOCATALYSIS: GENERAL FEATURES

USE OF PURIFIED ENZYMES

- USE OF WILD TYPE PROTEINS
- USE OF MUTANTS: PROTEIN ENGINEERING
- IMMOBILIZATION

USE OF WHOLE CELLS BIOCATALYSTS

- WILD-TYPE ORGANISMS
- RECOMBINANT ORGANISMS

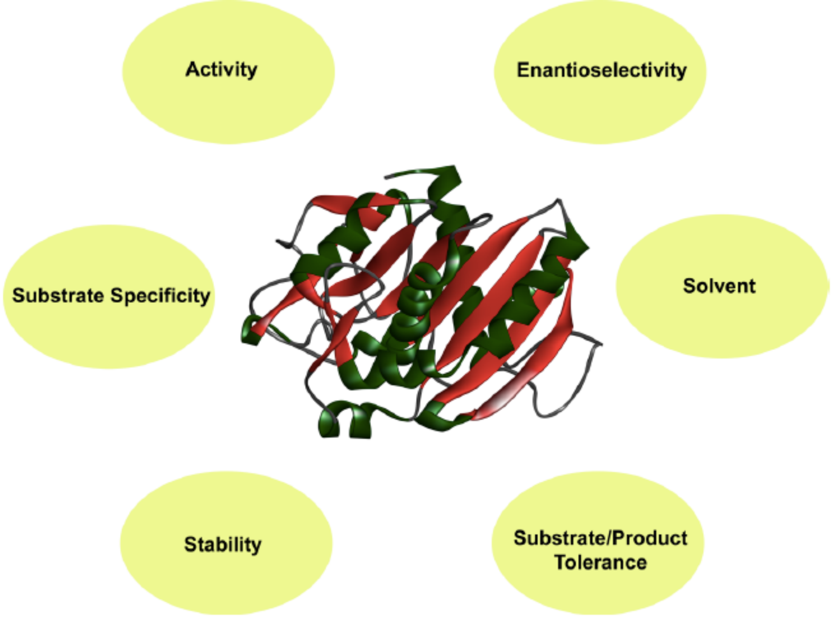
PROTEIN ENGINEERING

Native proteins are not well suited for industrial application

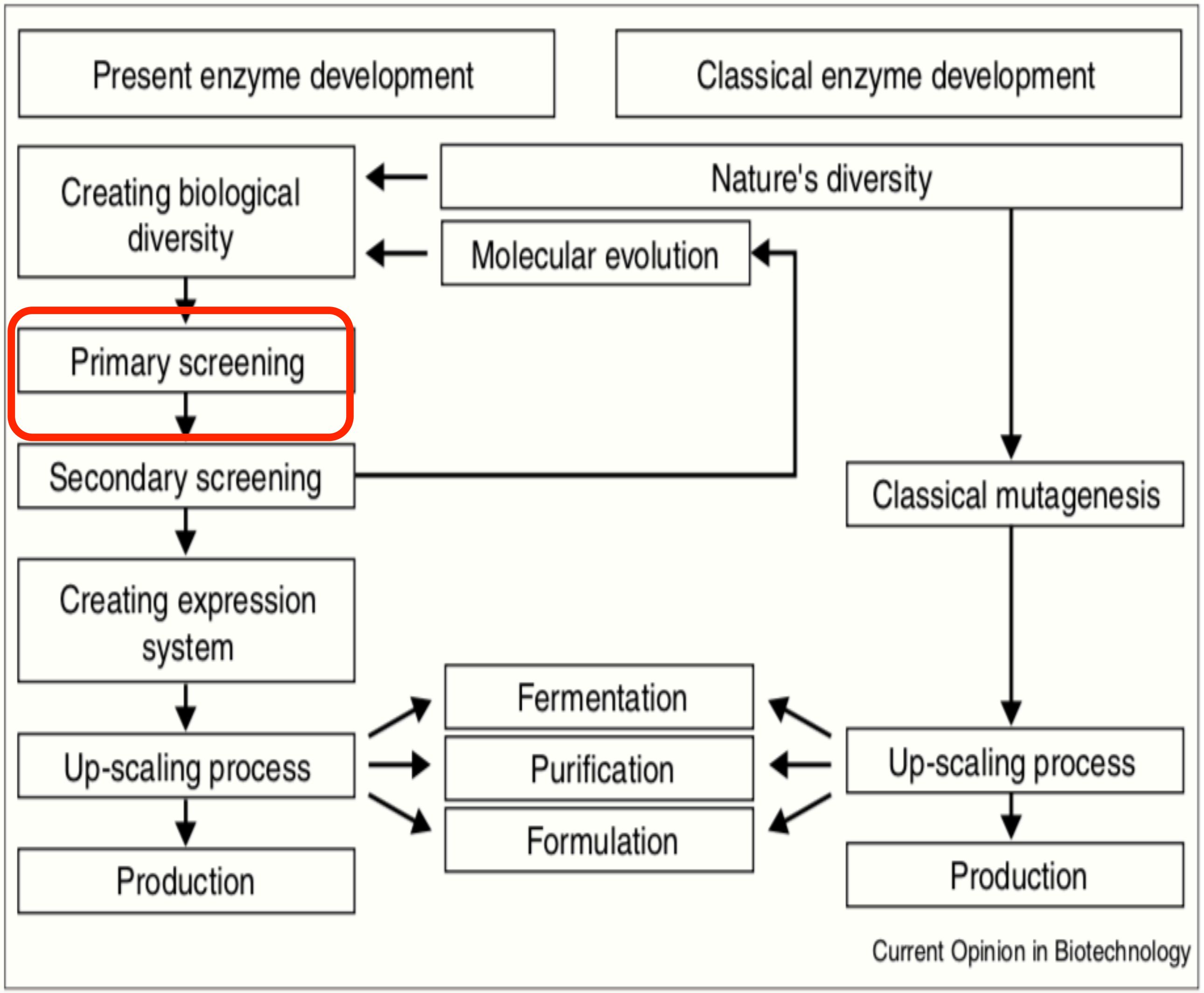
Native proteins are not optimized for medicinal purposes

- Increase the efficiency of enzyme-catalyzed reactions
- Eliminate the need for cofactor in enzymatic reaction
- Change substrate binding site to increase specificity
- Change the thermal tolerance
- Change the pH stability
- Increase proteins resistance to proteases (purification)
- Signal sequences - secretion

Figure 1. Evolvable enzyme properties for its successful utilization in industrial processes.



PROTEIN ENGINEERING



PROTEIN ENGINEERING

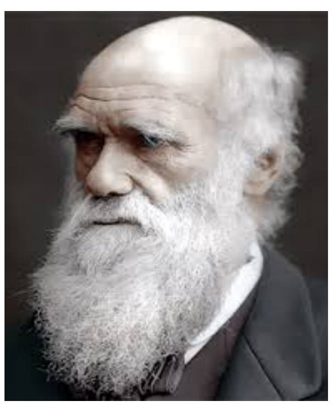
Site-directed mutagenesis

- STRUCTURE-BASED DESIGN
  Analysis of 3D structures/models
- SITE-DIRECTED MUTAGENESIS
- DNA SEQUENCING
- EXPRESSION
- FUNCTIONAL CHARACTERISATION
- STRUCTURAL DETERMINATION

Protein engineering→ Mutagenesis

↗

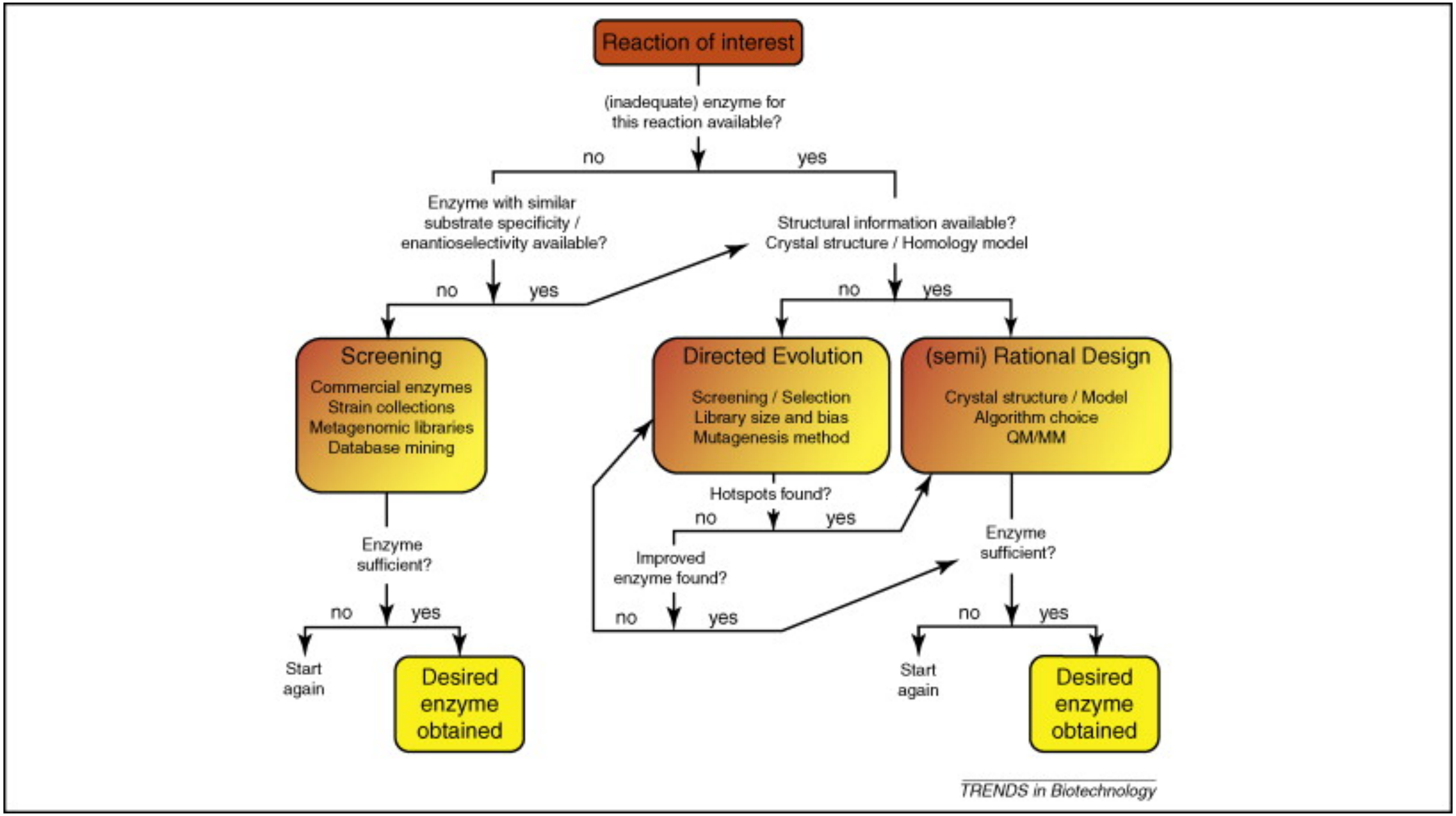
↘



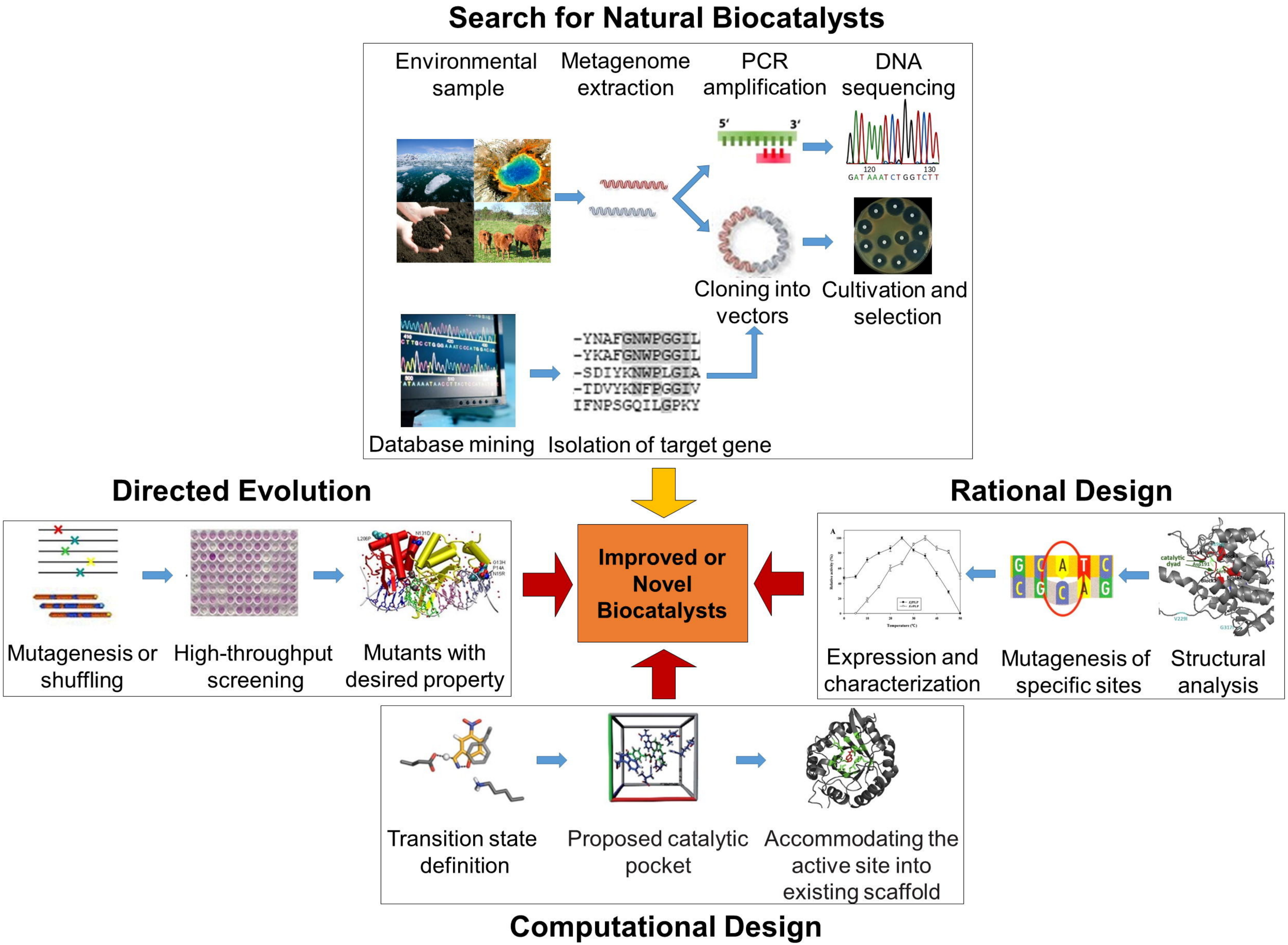
Random Forced evolution

- RANDOM MUTAGENESIS
  Error prone PCR / Homologous gene family
- RECOMBINATION
  DNA shuffling
- FUNCTIONAL SCREENING OF LIBRARY
  Selection (*in vivo*) / Screening (*in vitro*)
- DNA SEQUENCING
- FUNCTIONAL CHARACTERISATION
- STRUCTURAL DETERMINATION

PROTEIN ENGINEERING

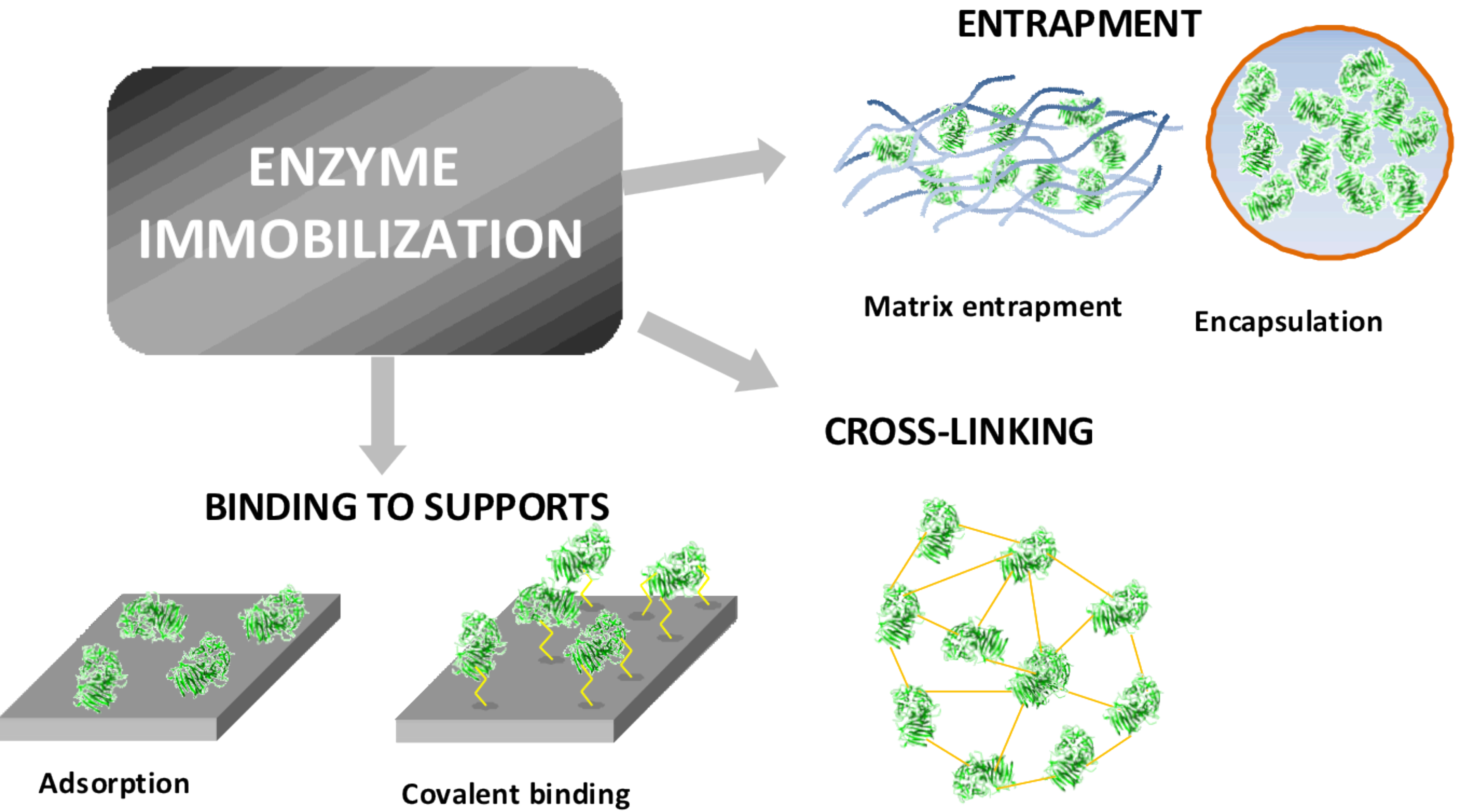


Trends in Biotechnology 2010 28, 46-54DOI: (10.1016/j.tibtech.2009.10.001)



From: Yang G, Ding Y. Recent advances in biocatalyst discovery, development and applications. Bioorg Med Chem. 2014, 22(20):5604-12. doi: 10.1016/j.bmc.2014.06.033.

IMMOBILIZATION



From http://www.franciscoploulab.eu/p/research-lines.html

Enzyme use in industry

Industry	Enzyme class	Application
Detergent (laundry and dish wash)	Protease	Protein stain removal
	Amylase	Starch stain removal
	Lipase	Lipid stain removal
	Cellulase	Cleaning, color clarification, anti-redeposition (cotton)
	Mannanase	Mannanan stain removal (reappearing stains)
Starch and fuel	Amylase	Starch liquefaction and saccharification
	Amyloglucosidase	Saccharification
	Pullulanase	Saccharification
	Glucose isomerase	Glucose to fructose conversion
	Cyclodextrin-glycosyltransferase	Cyclodextrin production
	Xylanase	Viscosity reduction (fuel and starch)
	Protease	Protease (yeast nutrition – fuel)
Food (including dairy)	Protease	Milk clotting, infant formulas (low allergenic), flavor
	Lipase	Cheese flavor
	Lactase	Lactose removal (milk)
	Pectin methyl esterase	Firming fruit-based products
	Pectinase	Fruit-based products
	Transglutaminase	Modify visco-elastic properties
Baking	Amylase	Bread softness and volume, flour adjustment
	Xylanase	Dough conditioning
	Lipase	Dough stability and conditioning (*in situ* emulsifier)
	Phospholipase	Dough stability and conditioning (*in situ* emulsifier)
	Glucose oxidase	Dough strengthening
	Lipoxygenase	Dough strengthening, bread whitening
	Protease	Biscuits, cookies
	Transglutaminase	Laminated dough strengths

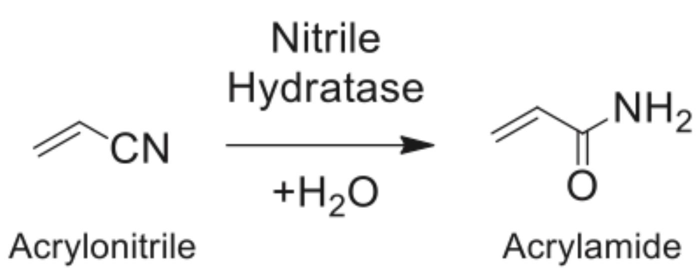
Industry	Enzyme class	Application
Animal feed	Phytase	Phytate digestibility – phosphorus release
	Xylanase	Digestibility
	β-Glucanase	Digestibility
Beverage	Pectinase	De-pectinization, mashing
	Amylase	Juice treatment, low calorie beer
	β-Glucanase	Mashing
	Acetolactate decarboxylase	Maturation (beer)
	Laccase	Clarification (juice), flavor (beer), cork stopper treatment
Textile	Cellulase	Denim finishing, cotton softening
	Amylase	De-sizing
	Pectate lyase	Scouring
	Catalase	Bleach termination
	Laccase	Bleaching
	Peroxidase	Excess dye removal
Pulp and paper	Lipase	Pitch control, contaminant control
	Protease	Biofilm removal
	Amylase	Starch-coating, de-inking, drainage improvement
	Xylanase	Bleach boosting
	Cellulase	De-inking, drainage improvement, fiber modification
Fats and oils	Lipase	Transesterification
	Phospholipase	De-gumming, lyso-lecithin production
Organic synthesis	Lipase	Resolution of chiral alcohols and amides
	Acylase	Synthesis of semisynthetic penicillin
	Nitrilase	Synthesis of enantiopure carboxylic acids
Leather	Protease	Unhearing, bating
	Lipase	De-pickling
Personal care	Amyloglucosidase	Antimicrobial (combined with glucose oxidase)
	Glucose oxidase	Bleaching, antimicrobial
	Peroxidase	Antimicrobial

Fine and bulk chemical industries

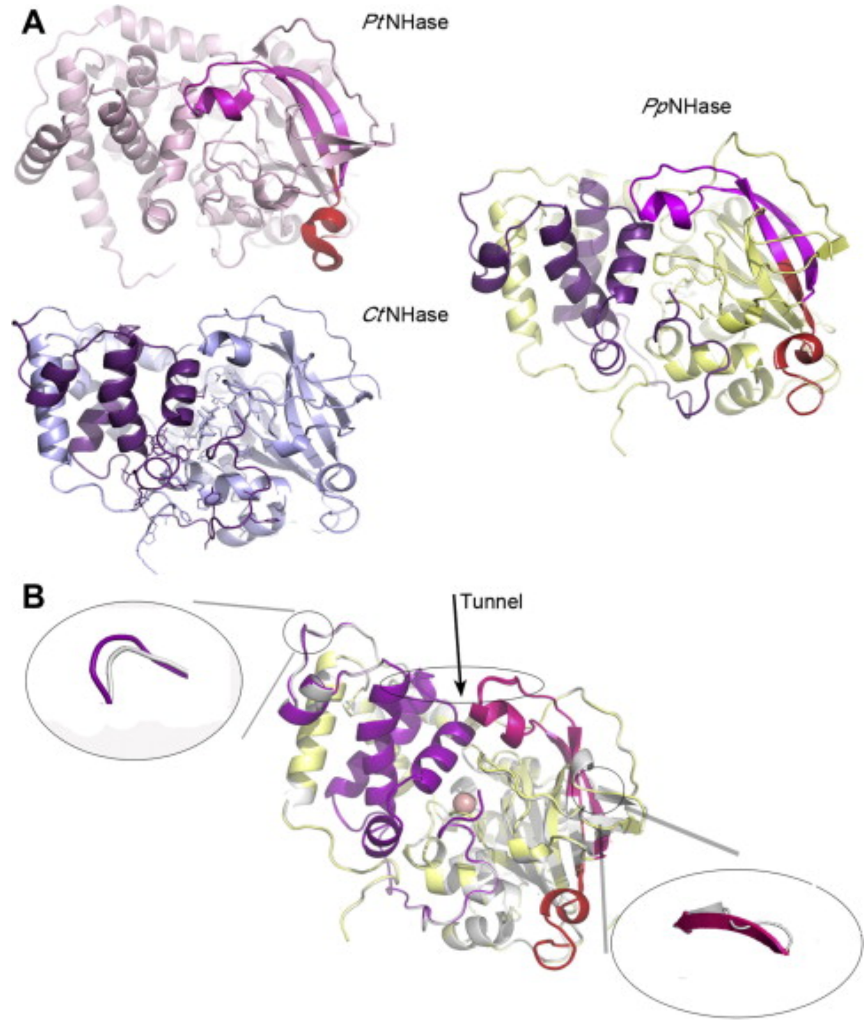
- Acrylamide is an important commodity chemical for synthesizing polyacrylamide used for petroleum recovery, wastewater treatment, papermaking, pesticide formulation, soil erosion prevention, and gel electrophoresis
- Traditionally, acrylamide can be produced chemically by oxidizing acrylonitrile using copper and sulfuric acid as a catalyst at high temperature
- However, both methods are known to cause several types of environmental pollution.
- The discovery of **nitrile hydratase** (EC 4.2.1.84) and its application in nitrile hydration has offered a novel process for the production of acrylamide

Fine and bulk chemical industries

- *Rhodococcus rhodochrous* J1 overexpressing nitrile hydratase efficiently converts acrylonitrile into acrylamide at up to 45% (W/W) under mild conditions
- This biotranformation process produces over 650,000 t annually in Japan
- Cui et al. recently engineered nitrile hydratase from *Pseudomonas putida* NRRL-18668, and showed improvements in thermal stability and catalytic activity of 3.5- and 1.5-fold, respectively

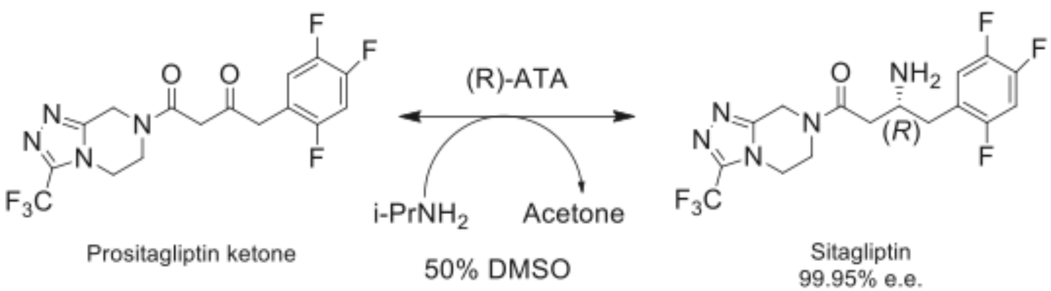


Scheme 1. Conversion of acrylonitrile into acrylamide using nitrile hydratase.

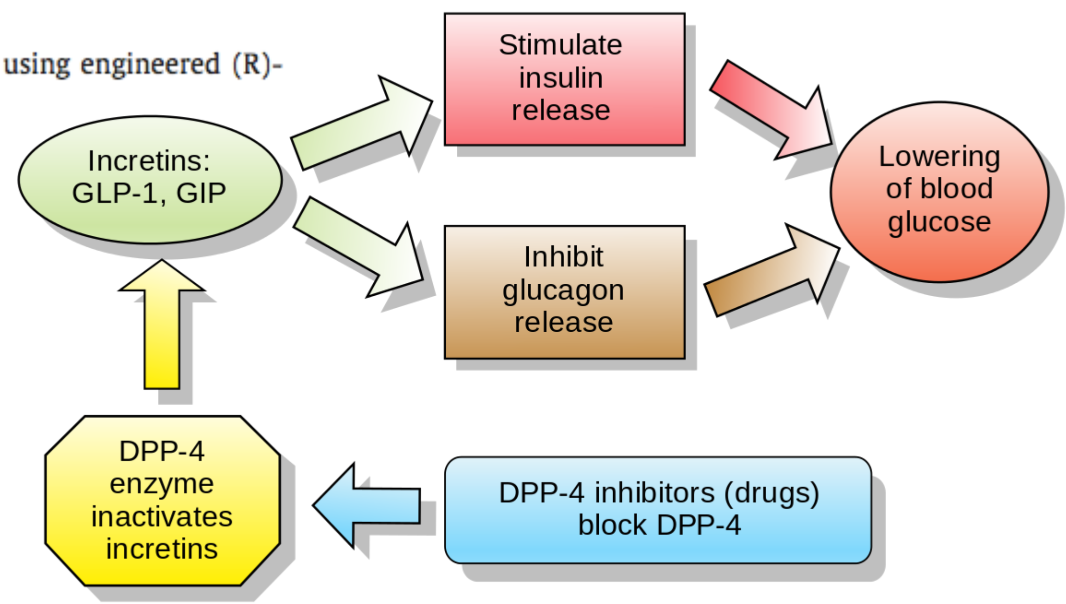


Pharmaceutical industry

- One of the most successful examples in the practical application of enzymes in the pharmaceutical industry is the anti-diabetic compound, sitagliptin
- Sitagliptin is a drug for type II diabetes that has been marketed under the trade name Januvia by Merck
- Researchers at Codexis and Merck engineered **R-selective transaminase** (R-ATA, ATA-117) from *Arthrobacter* sp. for the asymmetric amination of prositagliptin ketone.
- By applying a substrate walking, modeling, and mutation approach, they were able to overcome the limitation of the substrate's size for the enzyme.

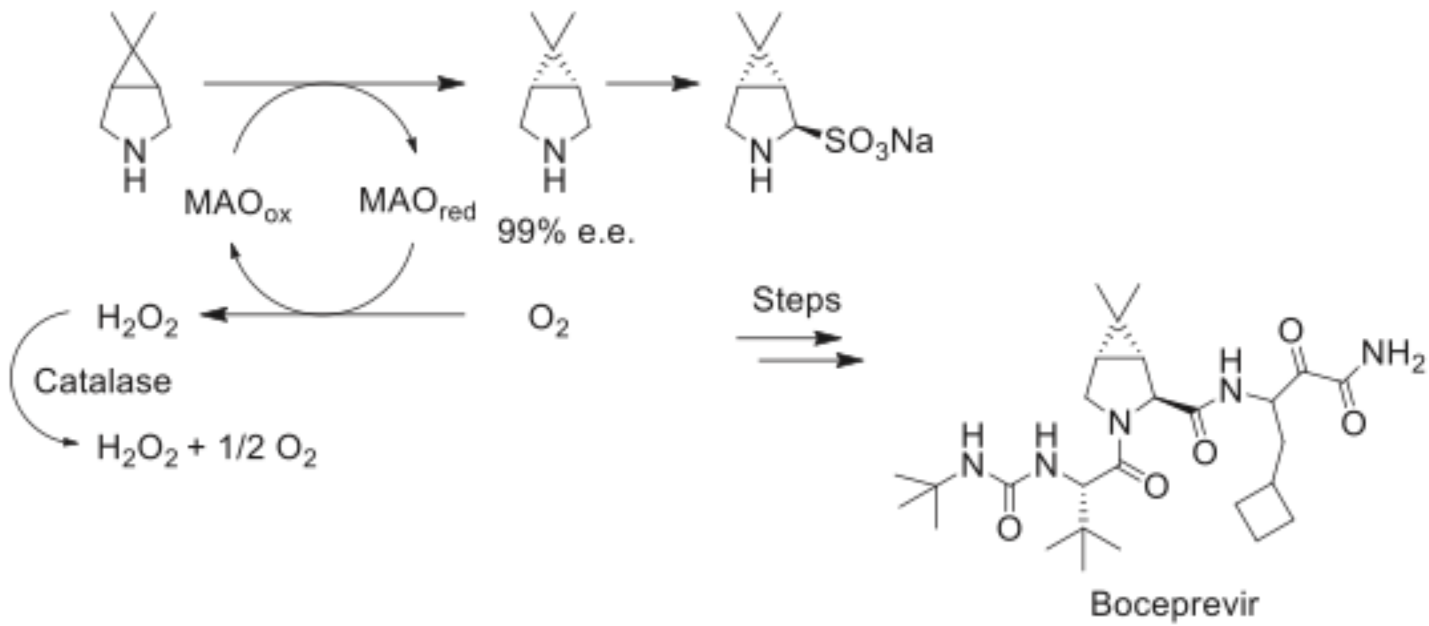


Scheme 6. Synthesis of sitaglipin from prositagliptin ketone using engineered (R)-selective ATA.



Pharmaceutical industry

- Another example of a chiral amine synthesis is boceprevir which is a clinically used drug for chronic hepatitis C infections under the trade name Victrelis by Merck.
- In the synthesis of boceprevir, an efficient and enantio-pure desymmetrisation of a bicyclic proline intermediate is highly required.
- Codexis and Merck have employed monoamine oxidase (MAO) from *Aspergillus niger* for the asymmetric amine oxidation of the intermediate



Scheme 7. Enantiopure desymmetrisation of bicyclic proline intermediate using engineered MAO in the synthesis of boceprevir.

Pharmaceutical industry

Enzyme	Use
Asparaginase	For leukaemia
Collagenase	For Skin ulcers
Glutaminase	For Leukaemia
Hyaluronidase	For Heart attack
Lysozyme	For Infection
Ribonuclease	As Antiviral
β-Lactamase	For Penicillin allergy
Streptokinase	For dissolving Blood clots
Trypsin	For Inflammation
Uricase	For Gout
Urokinase	For dissolving Blood clots

BIOCATALYSIS: GENERAL FEATURES

USE OF PURIFIED ENZYMES

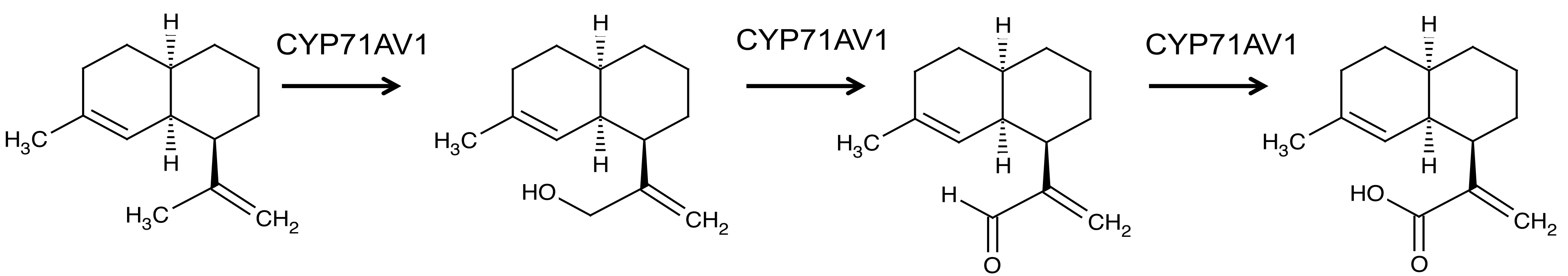
- USE OF WILD TYPE PROTEINS
- USE OF MUTANTS: PROTEIN ENGINEERING
- IMMOBILIZATION

USE OF WHOLE CELLS BIOCATALYSTS

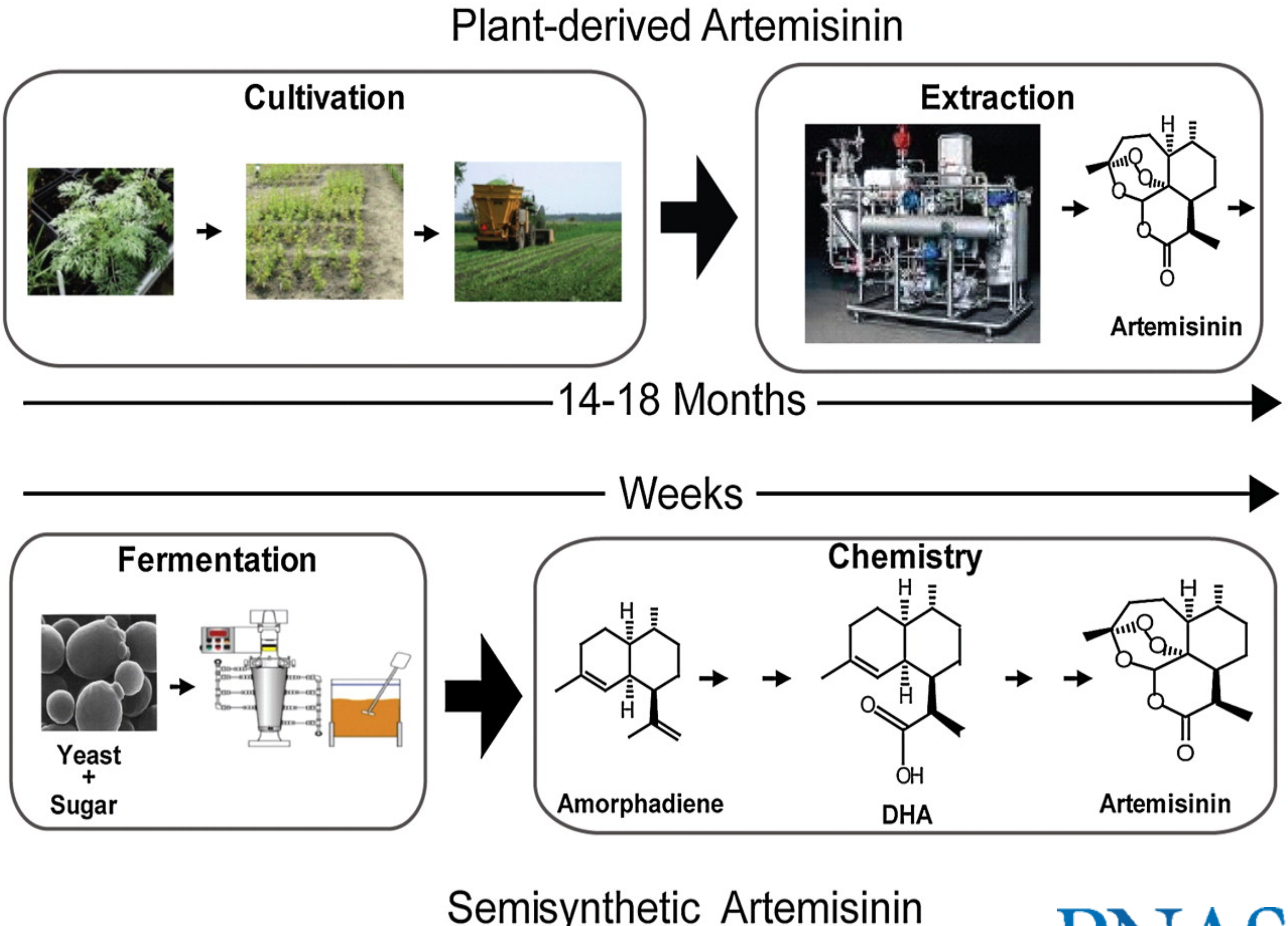
- WILD-TYPE ORGANISMS
- RECOMBINANT ORGANISMS

Pharmaceutical industry

- Conversion of amorphadiene in artmenisic acid (antimalaria) by enzymes from *Artemisia annua*



Amorphadiene | Artemisinic alcohol | Artemisinic aldehyde | Artemisinic acid



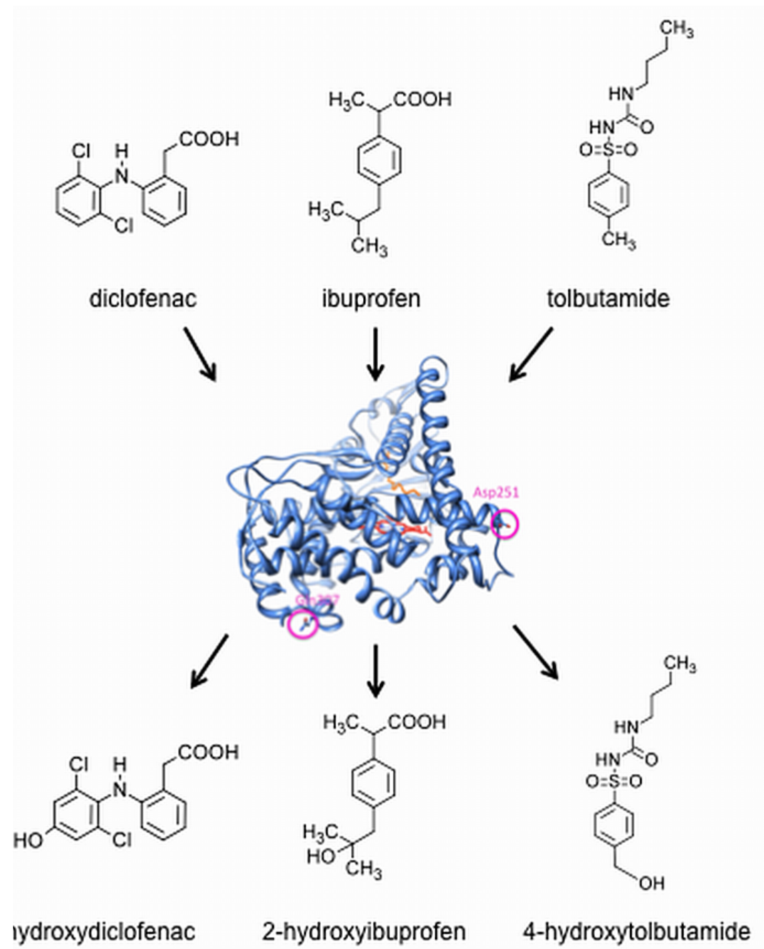
Patrick J. Westfall et al. PNAS 2012;109:3:655-656

Pharmaceutical industry

- The possibility that the metabolites themselves can be reactive or toxic makes it necessary to synthesize preparative amounts of these reaction products, a process that is sometimes difficult and expensive to achieve by classical synthetic methods.
- The use of human cytochromes P450 to produce drug metabolites is mainly limited by the fact that they are difficult to handle and unstable

- A new biocatalyst, able to mimic the substrate specificity and the metabolite profile of human P450 2C, has been generated through random mutagenesis applied to P450 BM3.

- The mutant shows new catalytic abilities toward diclofenac, ibuprofen and tolbutamide respect to the WT protein.

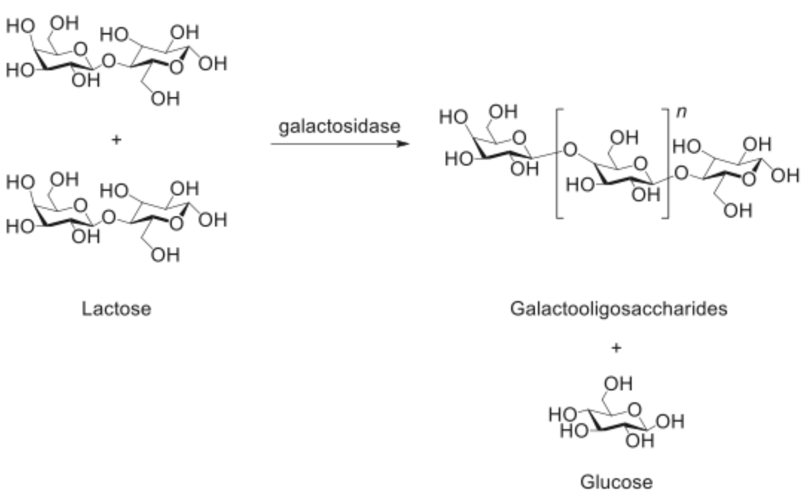


Food industry

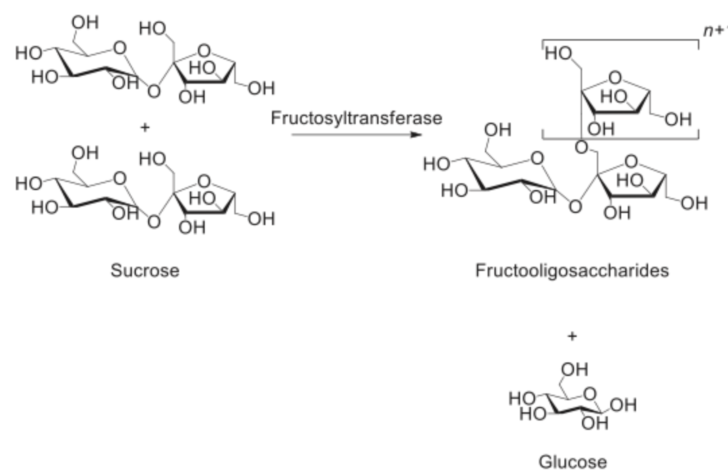
- Most uses of biocatalysis have focused on hydrolytic reactions for debranching, improving the solubility, and clarification.
- With the increasing request for nutritional aspects, a significant amount of attention has been paid to the functionality of foods **beyond the primary function of nutrient supply**.
- A recent trend in the food industry is to develop functional foods such as **prebiotics, low-calorie sweeteners, and rare sugars.**
- Prebiotics are dietary substances composed of non-starch polysaccharides and oligosaccharides, including inulin, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), lactulose, and breast milk oligosaccharides.
- According to a Global industry Analysis (GIA) report, by 2015, the prebiotic market has reached nearly $225 million and $1.12 billion in the USA and Europe, respectively

Food industry

- Galacto-oligosaccharides are health promoting ingredients that show prebiotic properties, but are poorly digestible.
- Additionally, many other health benefits have been reported, including an improvement in defecation, the stimulation of mineral absorption, colon cancer prevention, and protection against certain pathogenic bacterial infections.
- The production of galacto-oligosaccharides was achieved through an enzymatic reaction of lactose with β-galactosidases from various microbial, yeast, and fungal sources, leading to the structural diversity of galacto- oligosaccharides
- fructo-oligosaccharides (FOS) are used as an artificial sweetener and dietary fiber with low caloric levels, promoting the growth of *Bifidobacterium* in the human colon. In addition, it has an important role in the stimulation of calcium and magnesium absorption, and a lowering of the cholesterol, phospholipid, and triglyceride levels in human serum

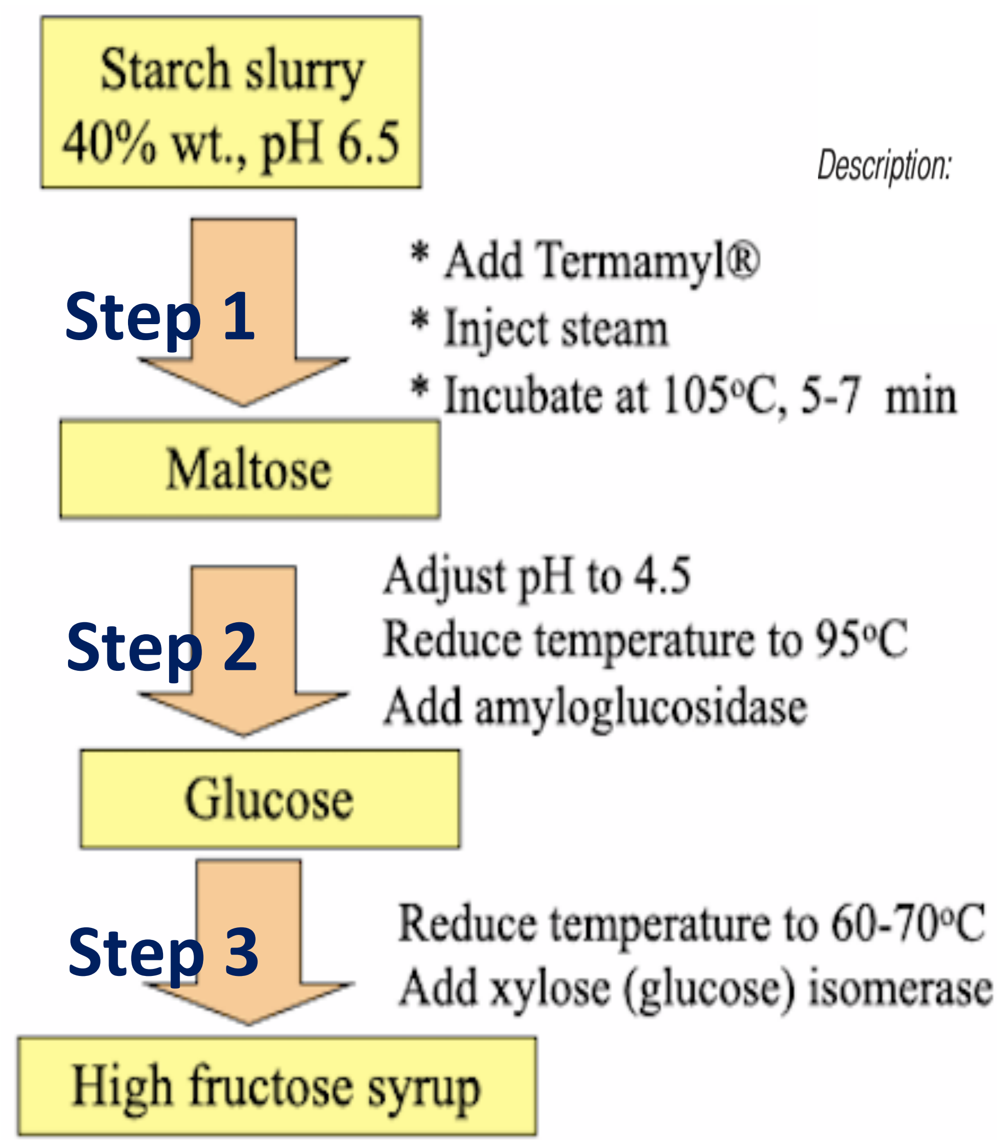


Scheme 13. Synthesis of galactooligosaccharide from lactose using β-galactosideas.



Scheme 14. Synthesis of fructooligosaccharides from inulin and sucrose by fructosyltranferase.

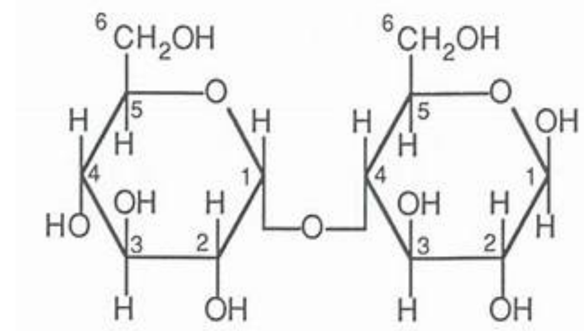
Food industry



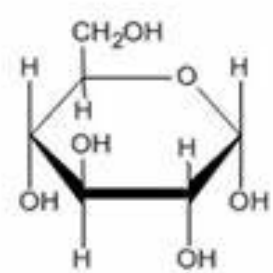
Termamyl®

Description:

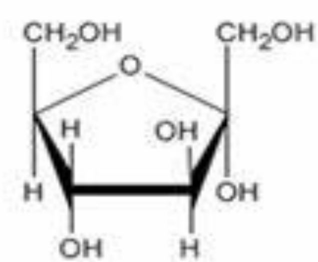
Termamyl® is a liquid enzyme containing outstanding heat-stable alpha amylase, expressed and produced by a genetically modified laboratory strain of *Bacillus licheniformis*. It is an enzyme that hydrolyses 1,4 alpha glucosidic linkages in amylose and amylopectin. Starch is rapidly broken down to soluble dextrins and oligosaccharide,



Maltose



Glucosio

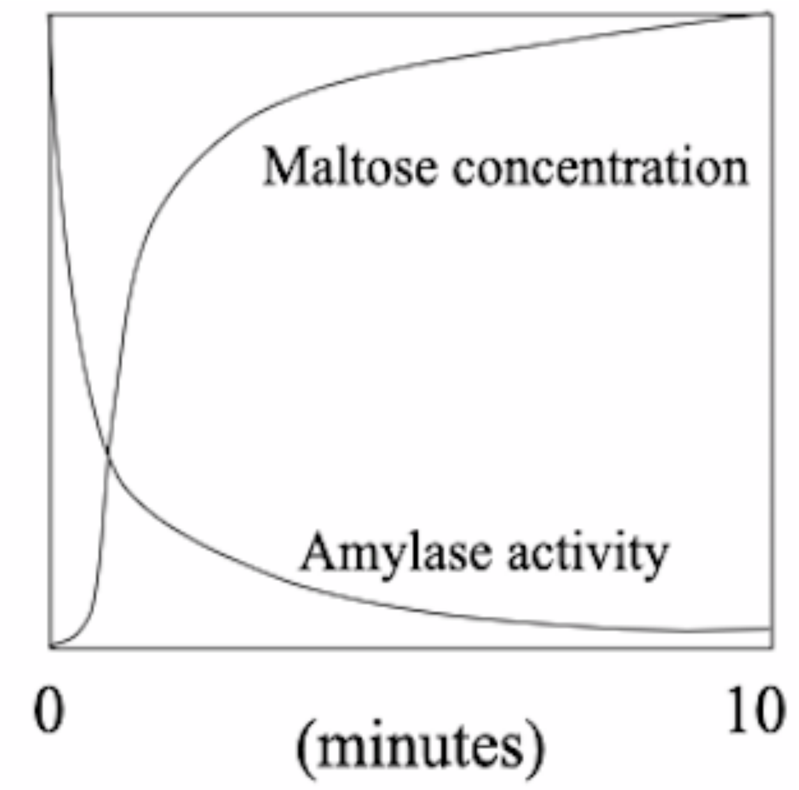


Fruttosio

Food industry

Step 1 : Termamyl®

- Termamyl® is an alpha-amylase which hydrolyzes the 1-4 alpha glycosidic bonds
- The high temperature swells the starch granules, making the amylose and amylopectins chains more accessible to the enzyme
- Termamyl® is stable at high temperatures for short reaction times (5-7 min)
- The process is called "batch" because the enzyme is not re-used



Cosmetic industry

- A variety of the ingredients used in the cosmetic industry are produced from petrochemical-based raw materials. Recently, however, the cosmetic industry has faced a challenge because of increasing consumer demands for natural and eco-friendly cosmetics.
- Accordingly, the cosmetic industry promotes basic research and eco-friendly processes using enzymes for developing more effective cosmetic products.
- Arbutin is the most common skin-lightener, and is known to inhibit melanogenesis without causing melano-cytotoxicity. As an enzymatic approach to producing arbutin, various enzymes have been used, including α-amylase, α-glucosidase, transglucosidase, sucrose phosphorylase, and dextransucrase
- Recently, a high production yield was achieved using amylosucrase from *Deinococcus geothermalis* (DGAS) (EC 2.4.1.4), which belongs to glycoside hydrolase family 13 that catalyzes the synthesis of amylose-like glucans from sucrose

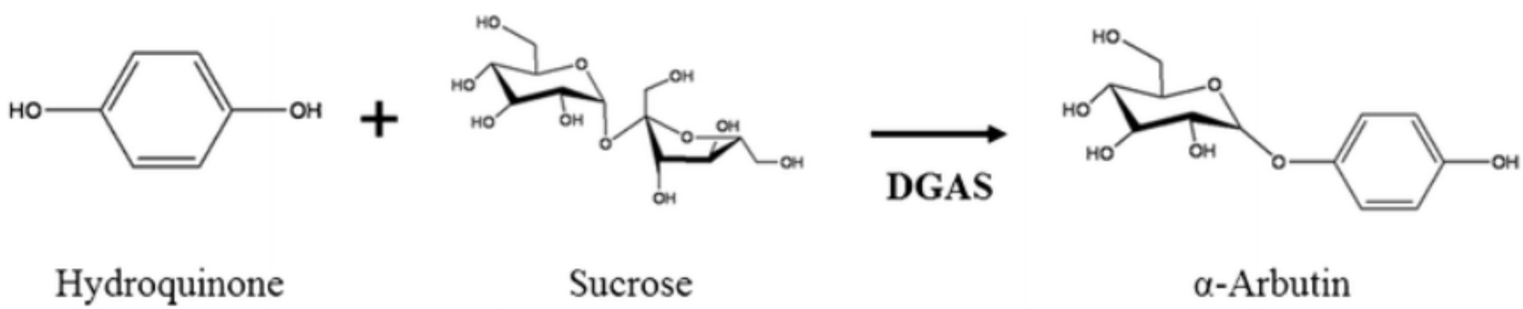
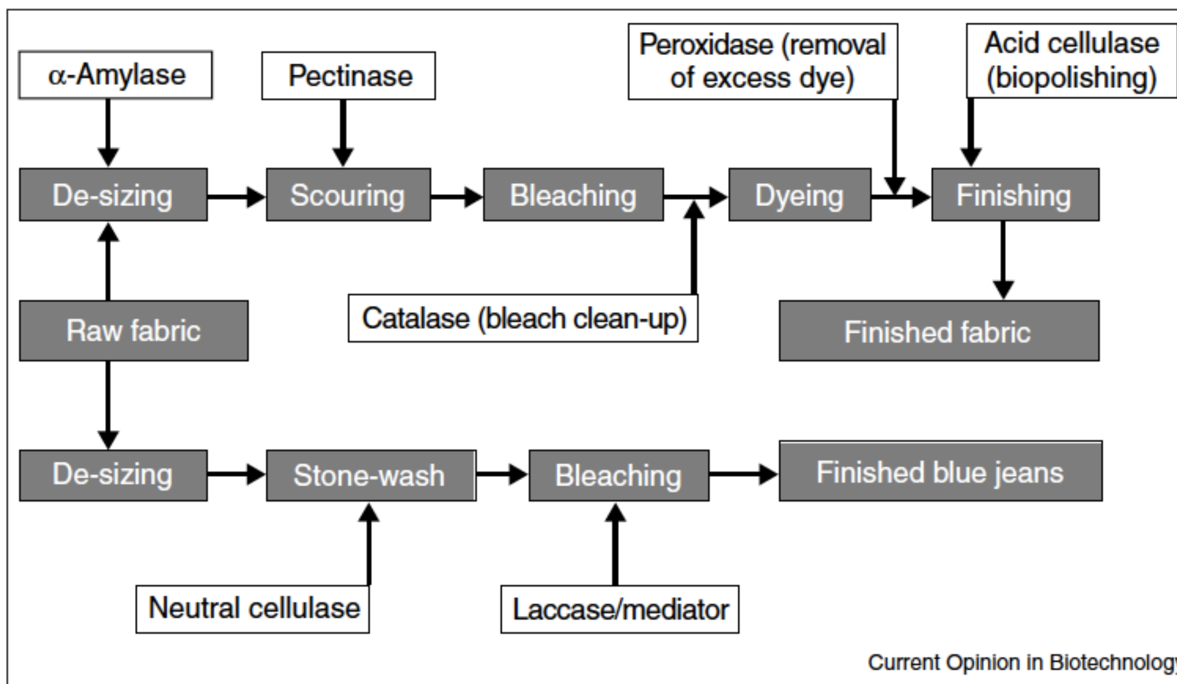


Fig. 5

Transglycoslyation reaction of HQ acceptor by DGAS. The glucosyl unit of sucrose is transferred to HQ via α-linkages

Textile industry

- In the textile industry, prior to conversion into fabric and yarn, cotton undergoes various processes including refining, bleaching, dyeing, and polishing.
- These processes consume large amounts of energy, water, and resources, discharging huge amounts of waste.
- For the development of cleaner processes, the use of enzymes is rapidly growing.
- Typical examples include the staining of jeans using cellulase from *Trichoderma viride*, and a bio-carbonization process in the case of wool.
- Cellulase and protease are used in the polishing step for clear dyeing, the improvement of color and surface vividness, and resistance to wrinkles



Pulp and paper industries

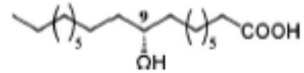
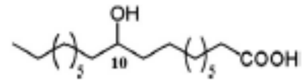
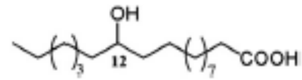
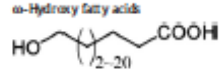
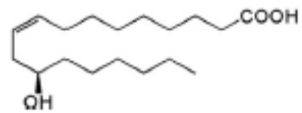
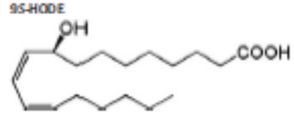
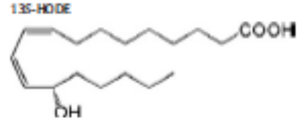
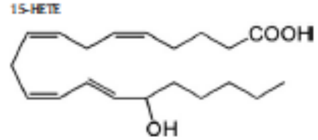
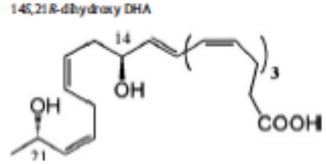
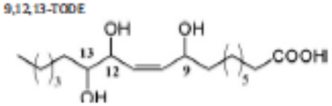
- In the pulp and paper industries, xylanase and ligninase are used to enhance the quality of the pulp by removing lignine and hemicelluloses, which are typical.
- In pulp production, lipase is also employed for degrading the pitch in wood, the presence of which causes a serious problem in the manufacturing process.
- The recycling of printed papers such as newspaper using cellulase was also developed.
- In the paper making process, lignin causes a dark color, and the removal of lignin is required for making bright paper.
- The chemical pulping process requires the addition of a large amount of alkali chemicals and chlorine. The use of **laccase** was shown to avoid elemental chlorine, and significantly reduces the amount of waste that causes ozone depletion and acidification, as well as high energy consumption.

HYDROXY FATTY ACIDS

Hydroxy fatty acids are widely used in chemical, food, and cosmetic industries as starting materials for the:

- synthesis of polymers and as additives for the manufacture of lubricants, emulsifiers, and stabilizers.
- They have antibiotic, anti-inflammatory, and anticancer activities and therefore can be applied for medicinal uses.
- Microbial fatty acid-hydroxylation enzymes, including P450, lipoxygenase, hydratase, 12-hydroxylase, and
- diol synthase, synthesize regio-specific hydroxy fatty acids.

Table 1
Uses and properties of hydroxy fatty acids.

HFA	Usage or status	Feature	Reference
9R-Hydroxystearic acid 	– Stereo-selective inhibitor against human histone deacetylase 1 (colon cancer) – Apoptosis inducer and regulation of cell division	– Endogenous by-product of lipid peroxidation	Parolin et al. (2012)
10-Hydroxystearic acid 	– Lubricant – Precursor of flavor (lactone)	– Similar physico-chemical properties with 12-HSA	Joo and Oh (2012)
12-Hydroxystearic acid 	– Acrylic polymer, rubber, wax, and grease – Coating (food container, drug, car, and variant) – Hydrophobic drug delivery – Cosmetics and personal care (Emollient) – Shrink resistant for wool, leather softener	– High melting point, thermostable, and impact strength – Gel state at room temperature – Safety (no toxic or hazardous)	Gow (2010)
ω-Hydroxy fatty acids 	– Polymeric monomer of plastic manufacture – Antibacterial, antifungal, and anticancer	– Polyester-like property, degradable	Metzger and Bornscheuer (2006)
Recinoleic acid 	– Polyester polyols, polyurethane, and plasticizer – Emulsifier, lubricant, and dispersant – Precursor of flavor and fragrance – Laxatives, drug delivery, food additives, and personal care	– Advanced flexibility, heat, chemical resistance than petroleum derived polymers. – Biocompatibility and degradability – Safety (no toxic or hazardous)	Biermann et al. (2011), Tunaru et al. (2012)
9S-HODE 	– Increased in atherosclerosis as major component of oxidized low density lipoprotein and also increased in rheumatoid arthritis – Antifungal activity	– PPAR-γ ligand (agonist) – G2A receptor ligand under stress	Itoh et al. (2008), Jira et al. (1997)
13S-HODE 	– Inhibition of cancer cell adhesion – Reduce artherogenesis – Precursor of fragrance – Antifungal activity	– Reduced 13S-HODE level in colon and lung cancer cell/down regulate PPAR-δ mediated apoptosis	Shureiqi et al. (2003)
15-HETE 	– Inhibition of cancer cell proliferation, adhesion, and metastasis – Anti-inflammation – Human actin ligand	– PPAR-γ ligand – Reduced in lung cancer tissue – Precursor of lipoxin	Moreno (2009)
14S,21R-dihydroxy DHA 	– Wound healing and angiogenesis activity in diabetes – Remedies mesenchymal stem cell function	– Biosynthesized from DHA by lipoxygenase and P450	Tian et al. (2011)
9,12,13-TODE 	– Antifungal activity to phytopathogenic fungi – Adjuvant for influenza vaccine	– Self-defensive product in plant	Nagai et al. (2010)

(continued on next page)

http://ac.els-cdn.com/S0734975013001183/1-s2.0-S0734975013001183-main.pdf?_tid=67941576-4487-11e7-a1dd-00000aacb35d&acdnat=1496073580_3731fcd1f117dfb28e7b946a1c258401

Fermentation

French chemist Louis Pasteur was the first zymologist, when in 1857 he connected yeast to fermentation.

- The German Eduard Buchner, winner of the 1907 Nobel Prize in chemistry, later determined that fermentation was actually caused by a yeast secretion that he termed *zymase*.

The Brewer, designed and engraved, in the Sixteenth Century, by J. Amman.

The research efforts undertaken by the Danish Carlsberg scientists greatly accelerated the gain of knowledge about yeast and brewing.

Carlsberg

Commercially Important Fermentation

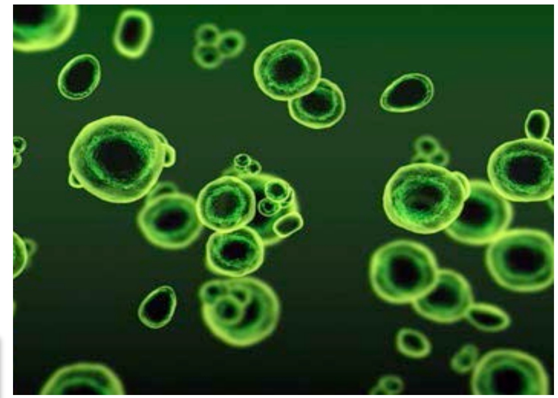
- Microbial cells or Biomass as the product: Eg. Bakers Yeast, Lactic acid bacillus, Bacillus sp.
- Microbial Enzymes: Catalase, Amylase, Protease, Pectinase, Glucose isomerase, Cellulase, Hemicellulase, Lipase, Lactase, Streptokinase etc.
- Microbial metabolites :
  - Primary metabolites – Ethanol, Citric acid, Glutamic acid, Lysine, Vitamins, Polysaccharides etc.
  - Secondary metabolites: All antibiotic fermentation
- Recombinant products : Insulin, HBV, Interferon, Streptokinase
- Biotransformations: Eg. Phenyl acetyl carbinol,Steroid Biotransformation

BIOFUELS

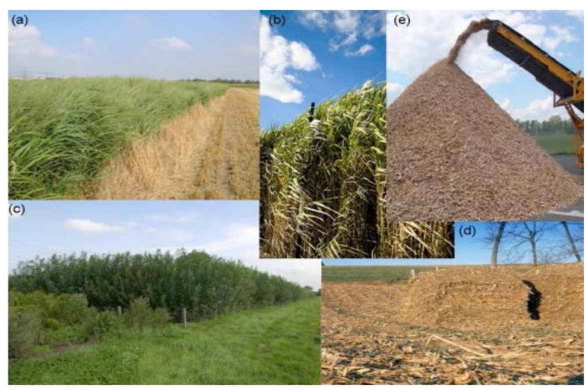
Development of biofuels

1° generation

food-for-food vs.
food-for-fuel



3° generation



2° generation

BIOFUELS

1st Generation Biofuels	2nd Generation Biofuels
Feedstocks: sugars, grains, seeds	**Feedstocks: lignocellulose (crop residues, grasses, woody crops)**
➢ **Biodiesel** **(fatty acid methyl & ethyl ester)** – rapeseed (RME), soybeans (SME), sunflowers, jatropha, coconut, palm, recycled cooking oil	➢ **Ethanol via enzymatic hydrolysis** **(cellulosic ethanol)**
➢ **Pure plant oils** **(straight vegetable oil)**	➢ **Thermochemical fuels** – Fischer-Tropsch fuel (FT) – methanol, MTBE, gasoline – dimethyl ether (DME) – mixed alcohols – hydrogen – hydrothermal upgrading oils (HTU) – pyrolysis oils **: via gasification**
➢ **Bioethanol** – from grains or seeds: corn, wheat, potato – from sugar crops: sugar beets, sugarcane	

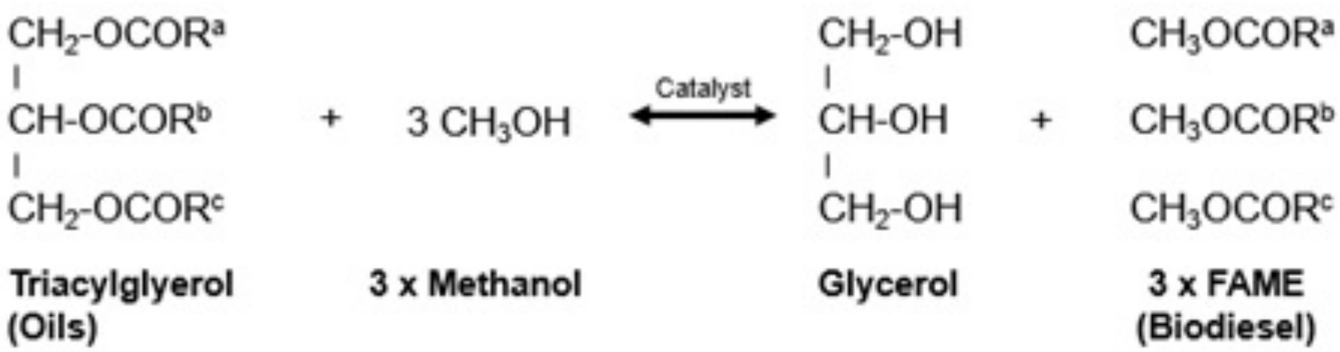
E. D. Larson, Biofuel Technologies Overview (2007)

Bioalcohols

- Alcohol fuels are produced by fermentation of sugars derived from wheat, corn, sugar beets, sugar cane, molasses and any sugar or starch that alcoholic beverages can be made from (like potato and fruit waste, etc.).

Biodiesel

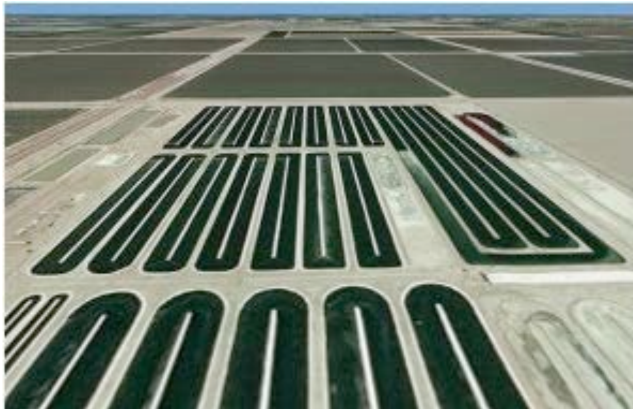
- It is produced from oils or fats using transesterification and is a liquid similar in composition to mineral diesel. Its chemical name is fatty acid methyl (or ethyl) ester (FAME). Oils are mixed with sodium hydroxide and methanol (or ethanol) and the chemical reaction produces biodiesel (FAME) and glycerol.



Third generation biofuel

- **Algae fuel**, also called **oilgae** or **third generation biofuel**, is a biofuel from algae. Algae are low-input/high-yield (30 times more energy per acre than land) feedstocks to produce biofuels and algae fuel are biodegradable.

Photobioreactor

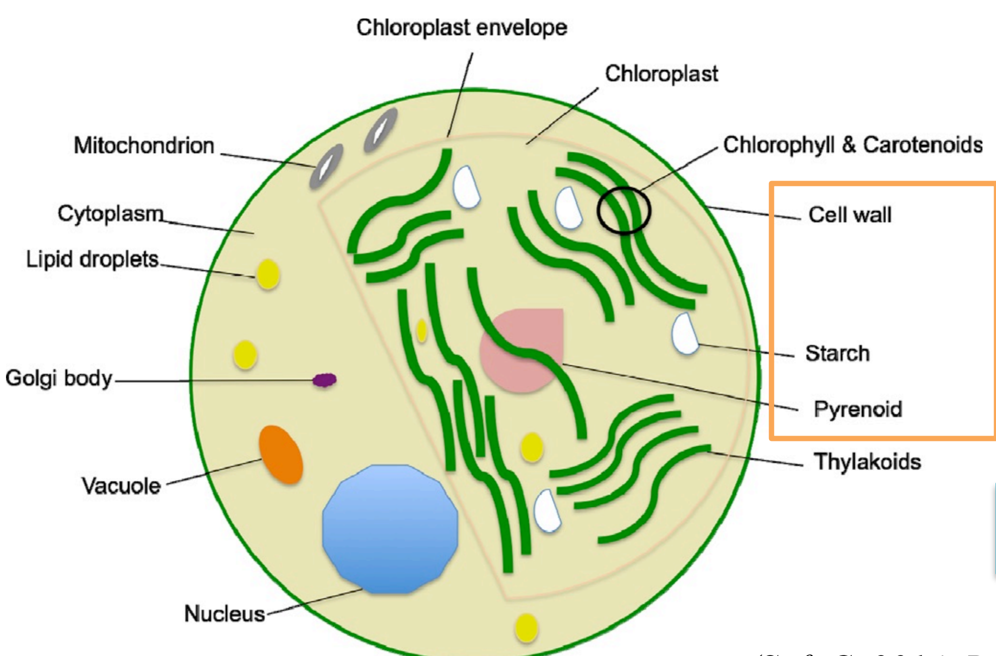


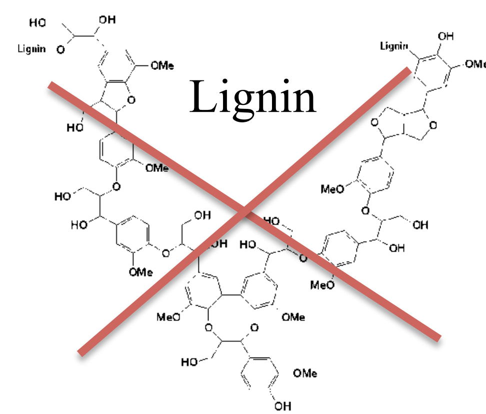
Open pound system

Third generation biofuel

General composition:

Protein : ~ 50 % → Non-energy uses
Lipids: 2-40 % → Biodiesel *(extraction)*
Carbohydrates: 10-40 % → Bioethanol *(fermentation)*





Sources of polysaccharides

(Safi C.,2014, Renewable and Sustainable Energy Reviews)

FINAL EXAM

- 3 questions with limited space (1 page maximum)

Examples:

1. Biosynthesis of neurotransmitters from amino acids;
2. Heme degradation
3. Regulation of metabolism by insulin
4. Describe the 2 main mechanism of hormone action

Oral exam only in case of necessity to increase the grade (but it can also decrease)