# Classi enzimatiche

- EC 1. Ossidoreduttasi. Catalizzano le reazioni redox in cui atomi, come l'idrogeno e l'ossigeno, o elettroni vengono trasferiti da una molecola all'altra.
- EC 2. Transferasi. Catalizzano le reazioni in cui avvengono trasferimenti di gruppi, come ad esempio quello metilico, acilico o glicosilico.
- EC 3. Idrolasi. Catalizzano reazioni di idrolisi.
- EC 4. Liasi. Classe di enzimi che addizionano o rimuovono un gruppo chimico senza l'intervento di reazioni di trasferimento, idrolisi, ossido-riduzione. Le l. possono agire sui legami del carbonio con il carbonio (decarbossilasi, aldolasi), del carbonio con l'ossigeno (idratasi, deidratasi), del carbonio con l'azoto, con lo zolfo (desulfidrasi) o con gli alogeni.
- EC 5. Isomerasi. Catalizzano reazioni in cui avvengono modificazioni strutturali o isomeriche (racemizzazioni e isomerizzazioni cis-trans di carboidrati).
- EC 6. Ligasi. Questi enzimi vengono chiamati anche sintetasi in quanto catalizzano il legame tra due molecole, utilizzando l'adenosina trifosfato (ATP) come donatore di energia.

*Classi enzimatiche*

L'NC-IUBMB (Nomenclature Committee of the International Union of Biochemistry and Molecular Biology), in associazione con JCBN (IUPAC-IUBMB Joint Commission on Biochemical Nomenclature), è responsabile della classificazione degli enzimi.

*BRENDA (Braunschweig Enzyme Database) è un data base ad accesso libero che viene preso come riferimento dalla maggior parte della comunità scientifica..*

Il numero EC è composto da quattro parti,

I primi tre numeri rappresentano la classe, la sottoclasse e la sotto-sottoclasse di appartenenza.

La quarta cifra è un numero seriale utilizzato per identificare un determinato enzima nella specifica sotto-sottoclasse.

EC 1.2.3.4 = ossalato ossidasi.

Ossidoreduttasi (classe 1), il quale agisce su aldeidi o donatori di gruppi osso (sottoclasse 2), appartenente alla sotto-sottoclasse in cui l'ossigeno è l'accettore (sotto-sottoclasse 3) Quarto: enzima classificato in questa determinata sotto-sottoclasse (numero seriale 4).

Classification of enzymes used in organic synthesis.

| Class | Enzyme | Common reaction |
|---|---|---|
| **1. Oxidoreductases** | Dehydrogenases | Oxidation of **alcohols** and aldehydes, reduction of **aldehydes and ketones**; oxidation of **C-C** single bonds, reduction of **C=C** double bonds |
| | Oxidases | Oxidation of alcohols and amines |
| | Mono- and dioxygenases | Hydroxylation, sulphoxidation, epoxidation, Baeyer-Villiger oxidation, |
| | Peroxidases | Oxidation, epoxidation, halohydration |
| | | |
| **2. Transferases** | Kinases | Phosphorylation (ATP-dependent) |
| | Sulphotransferases | Formation of sulphate esters |
| | Glycosyltransferases | Glycosidic bond formation |
| | Transketolases | Ketol (α-hydroxyketones) group transfer |
| | | |

| Class | Enzyme | Common reaction |
|---|---|---|
| **3. Hydrolases** | Esterase, lipases | Ester hydrolysis / synthesis |
| | Amidohydrolases (amidases or acylases) | Amide hydrolysis / synthesis |
| | Proteases | Peptide bond hydrolysis / synthesis |
| | Glycosidases | Glycosidic bond formation/hydrolysis |
| | Nitrilase (nitrile aminohydrolase) | Hydrolysis of nitrile to carboxylate |
| | Epoxide hydrolases | Hydrolysis of epoxides |
| | Phosphatases | Hydrolysis of phosphate esters |
| | Dehalogenases | C-halide hydrolysis |
| | | |

| Class | Enzyme | Common reaction |
|---|---|---|
| | | |
| **4. Lyases** | Aldolases | Aldol reaction (C–C bond) |
| | Oxynitrilase | Cyanohydrine formation |
| | | |
| **5. Isomerases** | Glucose isomerase | Isomerisation of carbohydrates, |
| | Mandelate racemase | Racemisation |
| | | |
| **6. Ligases** | | Not used at present for practical applications |

# Some enzymes require co-enzymes

Main coenzymes required by enzymes used in biocatalysis

| **Coenzyme** | **Reaction type** |
|---|---|
| Flavines | Oxygenation |
| Thiamine pyrophosphate | Decarboxylation, transchetolization |
| $NAD(P)^+$ /NAD(P)H | Hydrogenation/dehydrogenation |
| $NAD(P)^+$ /NAD(P)H | Oxygenation |
| ATP | Phosphorylation |
| Pyridoxal- phosphate | Modification of aminoacids |
| Metal-phorphyrin complexes | Peroxidation, oxygenation |

Several cofactors can be recycled effectively, including nucleoside triphosphates such as ATP in phosphoryl transfer reactions, nicotinamide adenine dinucleotide and its phosphate ($NAD^+$/NADH and $NADP^+$/NADPH) in oxidoreductions, acetylCoA in acyl transfer reactions, and sugar nucleotides in glycosyl transfer reactions. Many cofactor dependent reactions have been applied on preparative or industrial scales.

Nevertheless, hydrolases still remain the most widely employed enzymes due to their large availability and to the fact that they do not need organic coenzymes.

# Un esempio di deidrogenasi: alcol deidrogenasi nel metabolismo epatico dell'etanolo

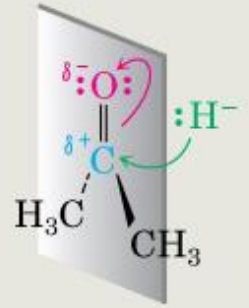
## Enzimi epatici che ossidano l'etanolo in aldeide acetica

- Alcool deidrogenasi (ADH)

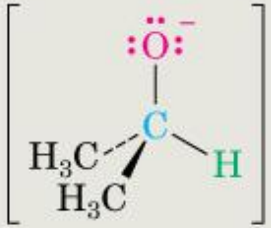
# Riduzione di carbonili ad alcoli

**FIGURA 19.1 MECCANISMO:** Una reazione di addizione nucleofila ad un'aldeide o ad un chetone. Il nucleofilo, per esempio qui lo ione idruro, si avvicina al gruppo carbonilico con un angolo di circa 45° rispetto al piano dell'orbitale $sp^2$, il carbonio carbonilico si reibridizza da $sp^2$ a $sp^3$ e si forma lo ione alcossido. Sulla destra, le mappe di potenziale elettrostatico mostrano come la densità elettronica si trasferisce dal nucleofilo all'ossigeno.

Una coppia di elettroni del nucleofilo attacca il carbonio elettrofilo del gruppo carbonilico, spingendo una coppia di elettroni del doppio legame C═O sull'ossigeno, portando ad uno ione alcossido. Il carbonio carbonilico si reibridizza da $sp^2$ a $sp^3$.

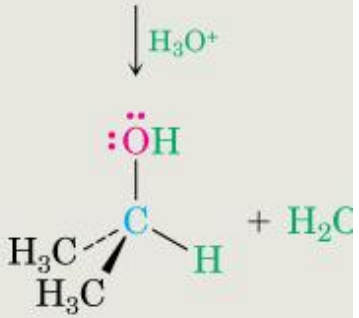



**Chetone**



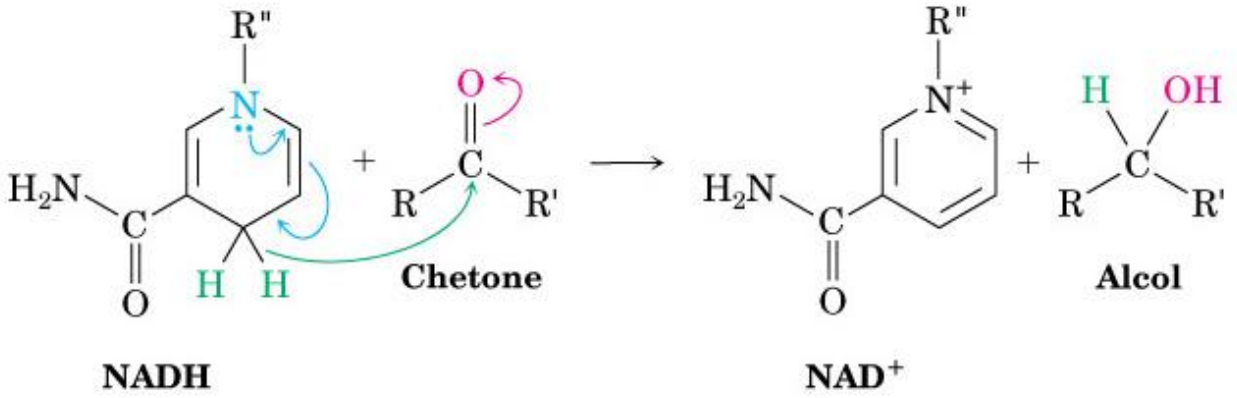

**Ione alcossido**

La protonazione dell'alcossido, derivante dall'addizione nucleofila porta al prodotto di addizione, un alcol neutro.




**Alcol**

# Riduzione di gruppi carbonilici catalizzata da enzimi nei sistemi biologici




- Il coenzima dona $H^-$ e si ossida
- L'enzima catalizza la reazione (abbassa l'energia di attivazione)

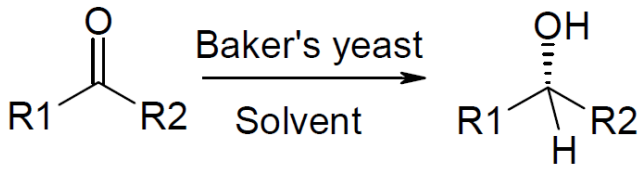



**Figure 1**. Baker's yeast catalyzed asymmetric reduction of prochiral ketones

## Whole Cells *vs* Isolated Enzymes

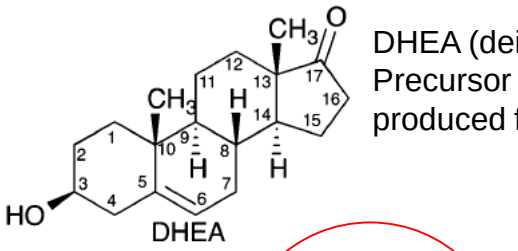
**Two ways in which coupled enzyme approach can be used:**

before it becomes toxic to cell

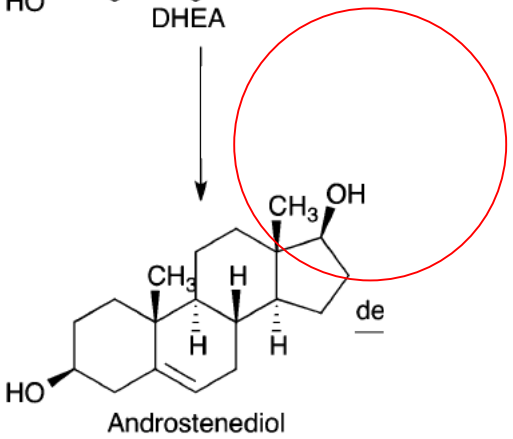
| | Pros | Cons |
|---|---|---|
| **Whole Cell:** | Cofactor recycling enzymes already present | Low substrate concentrations (typically 1-5 g $L^{-1}$) |
| | Convenient to operate Inexpensive | Tend to produce more by-products |
| **Isolated Enzymes:** | High substrate concentrations (typically 100-200 g $L^{-1}$) | Requires cofactor recycling hence additional cost and complexity |
| | Clean and efficient | |

lots of other enzymes present

# Steroids reduction using baker yeast



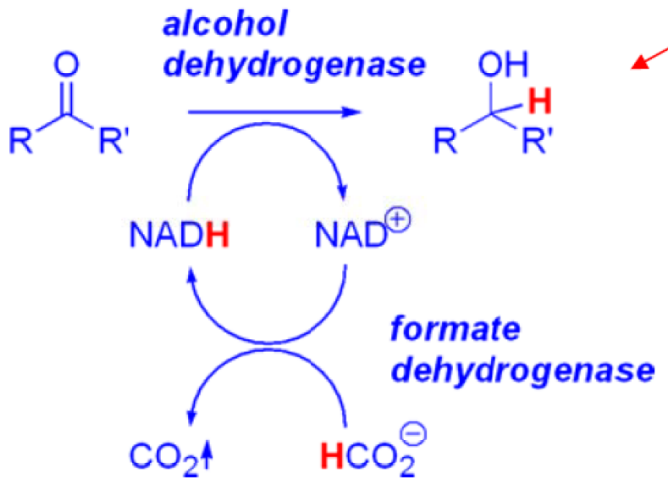

DHEA (deidroepiandrosterone), natural steroid (max in humans 15-20 anni)
Precursor of estrogens and androgens
produced from natural vegetal sources (tropical plant)




used as a radiation countermeasure: stimulates white cells and platelets production

# Ketons reduction using isolated enzymes

## Enzymatic use of NADH:




Coupled enzyme approach

Need to recycle NADH which is very expensive, use **formate** (very cheap!)

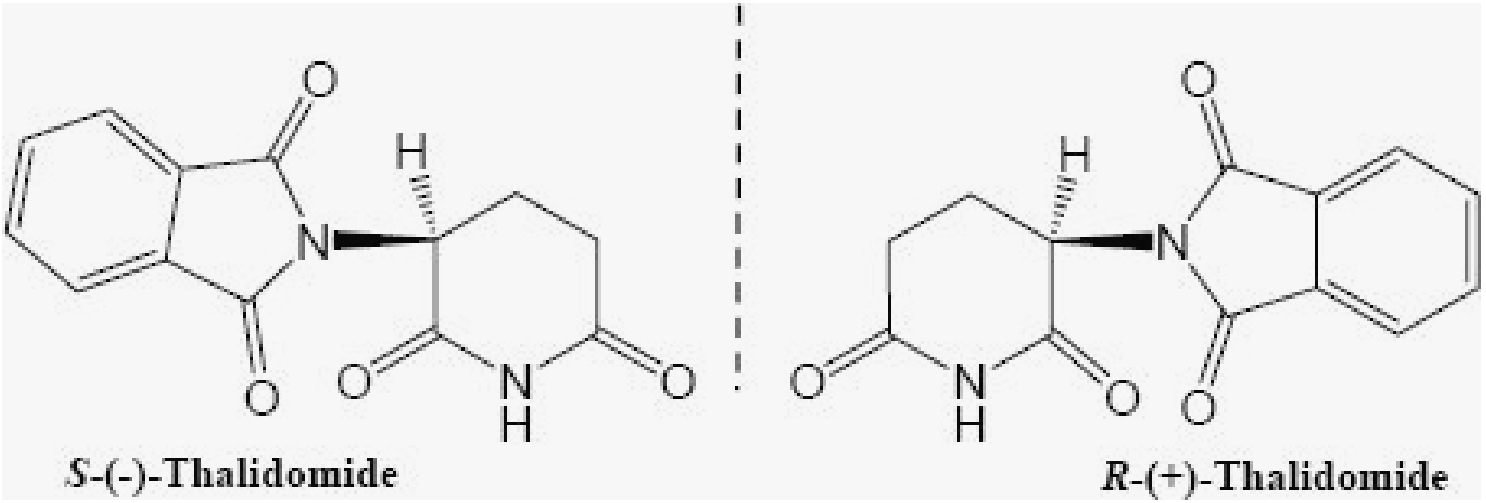
Now only need a catalytic amount of NADH, but need good turnover (ideally ~100,000).

# Chirality and pharmaceuticals

Chirality in biologically active molecules is of natural occurrence. Traditionally, it was common practice for a pharmaceutical company to market a chiral drug as the racemate, and as recently as 1985, more than 75% of chiral drugs were sold as the racemate.

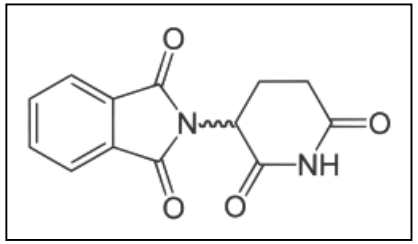
This policy implied that each dose of a drug is contaminated with an equal amount of an isomer, which usually has no therapeutic value but may have the potential to cause unsuspected deleterious side effects.

# The Thalidomide case




La Talidomide è un farmaco con proprietà ipnotico-sedative che è stato commercializzato per la prima volta in Germania nel 1956 per la terapia dell'influenza, e successivamente, in 46 Paesi, per la terapia dell'insonnia. È stato inoltre ampiamente utilizzato in donne in gravidanza nella terapia delle nausee mattutine grazie anche ad una pubblicità che sottolineava la "sicurezza" del prodotto.
I test preclinici su roditori ed i trial clinici non avevano infatti evidenziato effetti collaterali.
La vendita di Talidomide incrementò drasticamente in pochi anni e, entro il 1960, solo in Germania vennero prodotte circa 15 tonnellate di farmaco. In USA la Talidomide non ottenne l'autorizzazione all'immissione in commercio per una presunta associazione tra il farmaco e lo sviluppo di neuropatie periferiche.

# La Talidomide




Fin dall'inizio degli anni '60 si osservò un incremento di neonati con malformazioni congenite degli arti e fu ipotizzata una correlazione con l'assunzione materna di Talidomide in corso di gravidanza. Il farmaco venne pertanto ritirato dal commercio nel 1961; l'incidenza di malformazioni degli arti è ritornata nei limiti dopo il ritiro dal commercio, confermando l'effetto teratogeno della Talidomide.

È stato stimato che più di 6000 bambini (senza contare i casi di aborto spontaneo e morti fetali endouterine) siano nati con un'embriopatia da Talidomide, caratterizzata da difetti di riduzione degli arti di vario grado ed altri tipi di malformazioni congenite.

Studi ad hoc su animali di laboratorio (conigli e primati non umani) hanno inoltre confermato l'effetto teratogeno del farmaco, osservando il medesimo pattern malformativo osservato nell'uomo; il farmaco non è tuttavia risultato teratogeno in alcune specie animali (tra cui topi e ratti).

Tale episodio ha dimostrato che l'ambiente gioca un ruolo significativo nel determinare malformazioni congenite, ha demolito l'ipotesi che il feto sia protetto dalla "barriera placentare" rispetto ai farmaci assunti dalla madre ed ha confermato che l'effetto teratogeno è specie-specifico.

Le conseguenze pratiche furono un incremento della ricerca di base ed epidemiologica nell'ambito della teratogenesi ambientale e dei controlli per contenere l'introduzione di nuovi agenti chimici, con possibile effetto teratogeno.

Review TRENDS in Biotechnology Vol.25 No.2


# Biocatalysis for pharmaceutical intermediates: the future is now

David J. Pollard[1] and John M. Woodley[2]

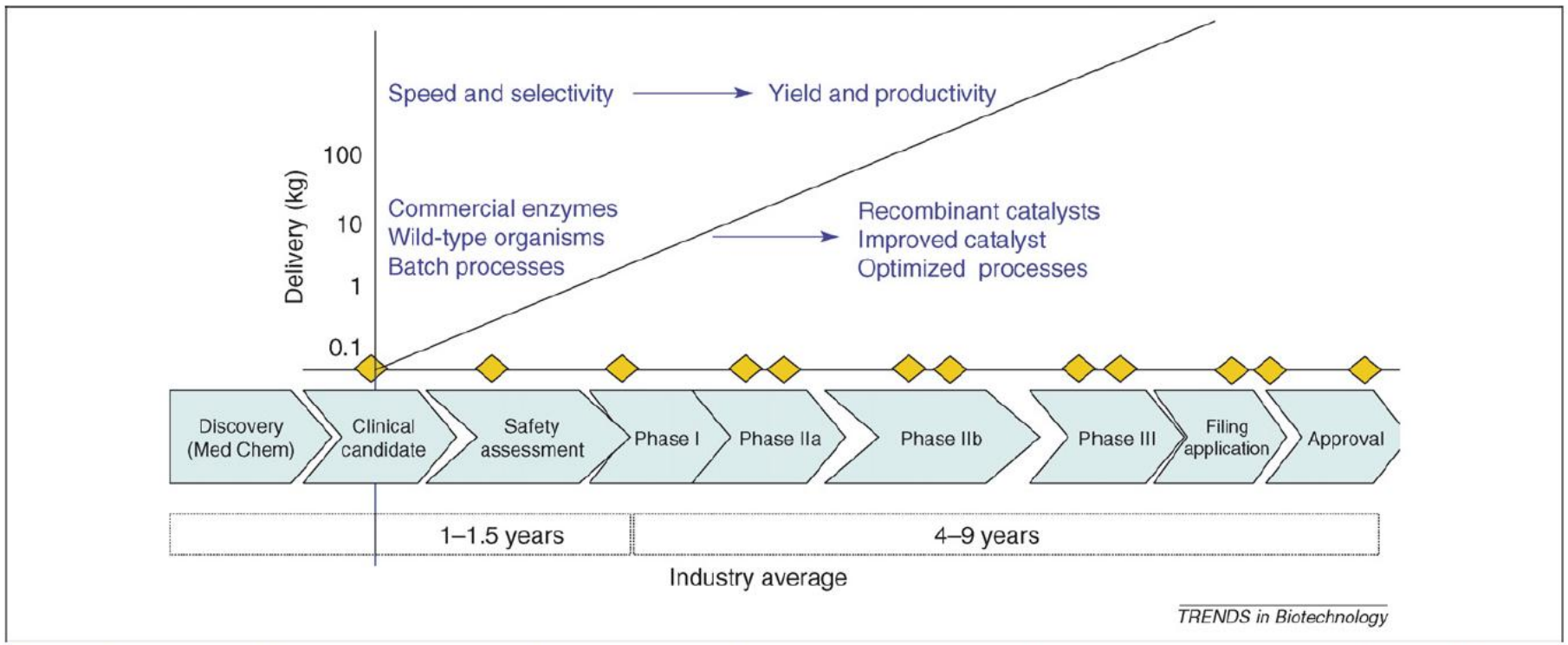
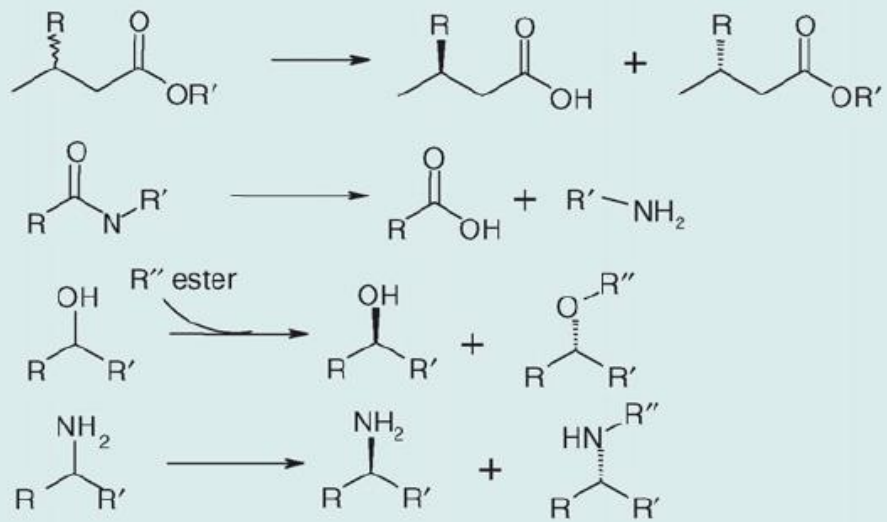



**Figure 2.** Development timeline for pharmaceutical products and processes. Initial development requires rapid synthesis of compounds to provide material for safety assessment. Later stage enables development time for optimized processes using recombinant catalysts. Abbreviation: Med Chem, medicinal chemistry.

Some examples of aplications of biocatalysts in the synthesis of drugs at industrial scale

## Established chemistries

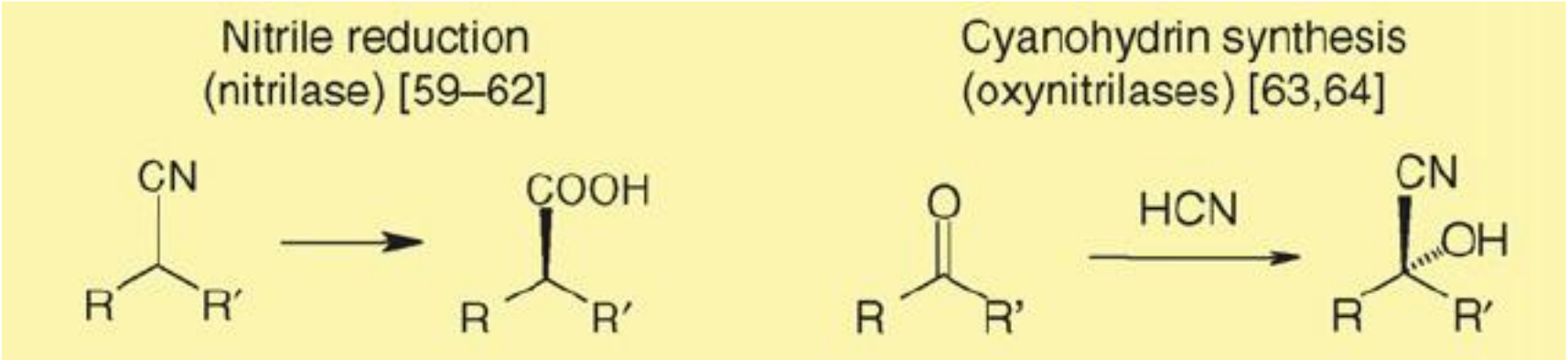
Resolution (lipase/protease) [20,21,34,56]



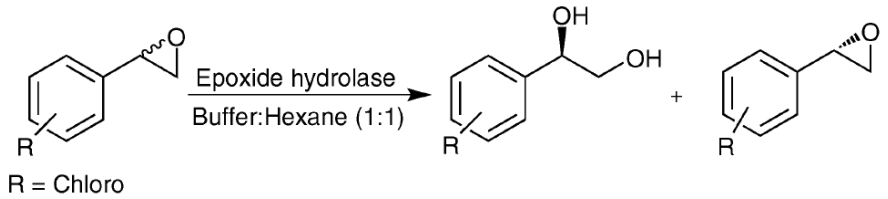

Hydrolysis (lipase/protease) [56,57]


Ketone reduction (ketoreductase) [49–52,58]

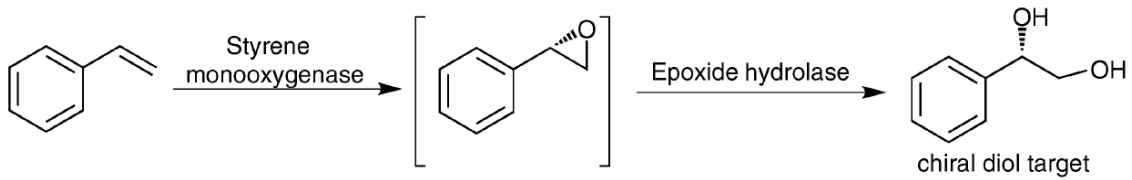
# Playing with nitrile group



# Playing with epoxides



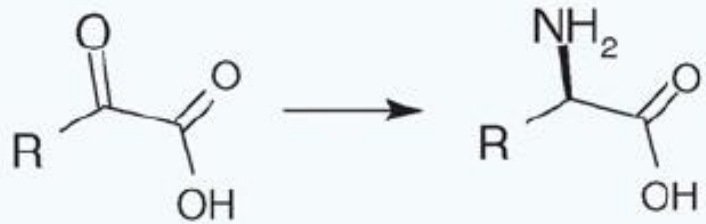

**Scheme 6** Biocatalytic kinetic resolution of racemic 2-, 3-, and 4-chlorostyrene oxides using epoxide hydrolase.




**Scheme 7** Asymmetric dihydroxylation of an aryl olefin *via* a tandem monooxygenase and epoxide hydrolase biocatalysis approach.

## Emerging chemistries

Transamination (transaminase) [53,65,66]

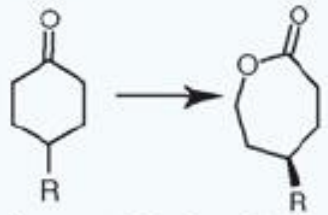



Enoate reduction (enoate reductase) [67–69]


Hydroxylation (cytochrome P450) [70,71]


Dihdroxylation (cytochrome P450) [72,73]

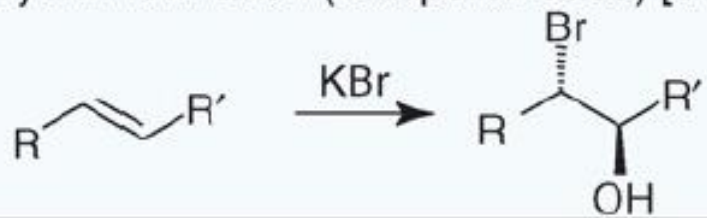
Baeyer-Villiger (monooxygenase) [74]



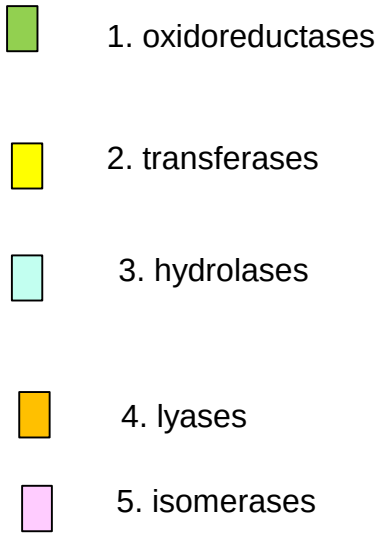

Hydrolysis (epoxide hydrolase) [75–77]


Epoxidation (haloperoxidase, cytochrome P450) [78–80]


Halohydrin formation (haloperoxidase) [81–82]



# Industrial technical applications of enzymes




| Industry | Enzyme class | Application |
|---|---|---|
| **Detergent (laundry and dish wash)** | Protease | Protein stain removal |
| | Amylase | Starch stain removal |
| | Lipases | Lipid stain removal |
| | Cellulase | Cleaning, color clarification, anti-redeposition (cotton) |
| | Mannanase | Mannanan stain removal (reappearing stains) |
| **Cellulose/Starch** | Amylase | Starch liquefaction and saccharification |
| | Cellulase | Cellulose saccharification |
| | Xylanase | Viscosity reduction |
| | Pullulanase | Saccharification |
| | Glucosidase | Saccharification |
| | Glucose isomerase | Glucose to fructose conversion |
| | Cyclodextrin-glycosyltransferase | Cyclodextrin production |

| | | |
|---|---|---|
| **6. Ligases** | | Not used at present for practical applications |

hydrolases

isomerases

transferases

oxidoreductases

lyases

| | | |
|---|---|---|
| **Food (including dairy)** | Protease | Milk clotting, infant formulas (low allergenic), flavor |
| | Lipase | Cheese flavor |
| | Lactase (β-galactosidase) | 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 and phospholipase | Dough stability and conditioning (*in situ* emulsifier) |
| | Glucose oxidase | Dough strengthening |
| | Lipoxygenase | Dough strengthening, bread whitening |
| | Protease | Biscuits, cookies |
| | Transglutaminase | Laminated dough strengths |

hydrolases

isomerases

transferases

oxidoreductases

lyases

| | | |
|---|---|---|
| **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 |

hydrolases

isomerases

transferases

oxidoreductases

lyases

| | | |
|---|---|---|
| **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 |
| **Leather** | Protease | Bating (macerazione) |
| **Personal care** | Lipase | De-pickling |
| | Amyloglucosidase | Antimicrobial (combined with glucose oxidase) |
| | Glucose oxidase | Bleaching, antimicrobial |
| | Peroxidase | Antimicrobial |

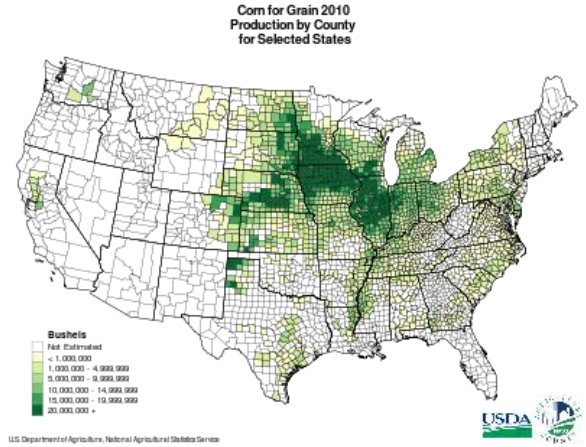
Adapted from: Ole Kirk et al., Current Opinion in Biotechnology 2002, 13, 345.

# Major biotransformations at industrial scale 1.

Fine-pharma chemistry

| Production scale [tpy] | Product | Enzyme | Reactor | Company |
|---|---|---|---|---|
| > 1 000 000 | high-fructose corn syrup (HFCS) | glucose isomerase | fixed-bed, IME | various |
| > 100 000 | lactose-free milk | lactase | fixed-bed, IME | various |
| > 10 000 | acrylamide | nitrilase | batch reactor | Nitto Co. |
| | cocoa butter* | lipase (CRL) | fixed-bed, IME | Fuji Oil |
| > 1,000 | | | | |
| | nicotinamide | nitrilase | 3-stage batch | Lonza Guangzhou |
| | D-pantothenic acid | aldonolactonase | | Fuji Pharma-ceuticals |
| | (S)-chloropropionic acid | lipase | | Dow Chemical |
| | 6-aminopenillanic acid | penicillin amidase | fixed-bed, IME | various |
| | 7-aminocephalo-sporanic acid | glutaryl amidase | Kundl/Hoechst | |
| | aspartame® | thermolysin | soluble enzyme | Tosoh/DSM |
| | L-aspartate | aspartase | fixed-bed, IME | various |
| | D-phenylglycine | hydantoinase/ (carbamoylase) | resting cells | Kanegafuchi |
| | D-*p*-OH-phenyl-glycine | hydantoinase/ carbamoylase | resting cells | Recordati |

# High-fructose corn syrups (HFCS)
# The largest enzymatic process at industrial level
# HFCS set the technological basis for
# “first generation bioethanol biorefineries”




## 1.Corn biorefineries

Refining started by simple **starch extraction** in 1948 by Thomas Kingsford, which was initially used as a **laundry aid, food ingredient and sweetener** production. With the development of glucoamylase in the 1940s and 1950s it became a straightforward matter to produce glucose syrups. However, D-glucose has only about 70% of the sweetness of sucrose, on a weight basis, and is comparatively insoluble. Batches of glucose syrup at the final commercial concentration (71%) must be kept warm to prevent crystallisation or diluted to concentrations that are microbiologically insecure.

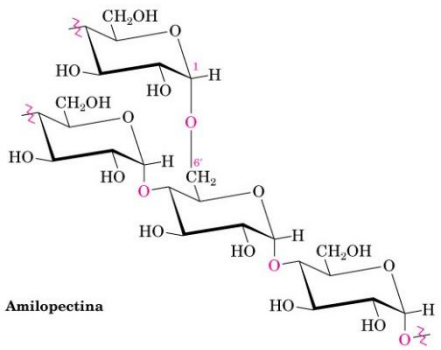
# Enzymatic processing of starch

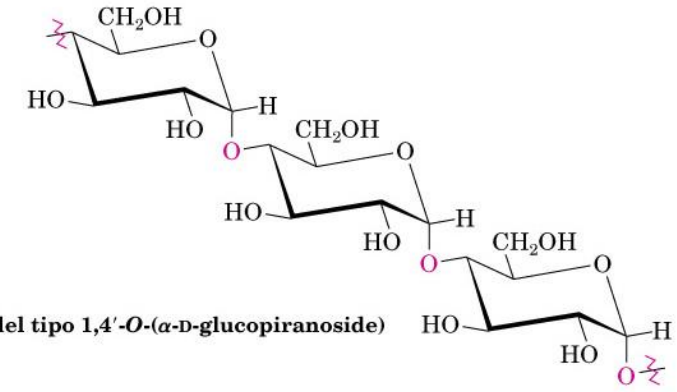
The native starch is semicrystalline in nature having varying size, shape, and granule size depending up on the botanical origin.
Industrial sources: maize, rice, wheat, cassava, and potato.

Starch: heterogeneous polysaccharide made by **amylose (15–25%)** and **amylopectin (75–85%)**, containing **α-D- glucose** as the sole monomer.

**Amylose:** linear water-insoluble polymer of glucose subunits joined by **α-1,4 bonds** (~99%) and ~1% α-1,6 linkages, MW ~$1 \times 10^5$ to $1 \times 10^6$.

**Amylopectin:** branched watersoluble polysaccharide (molecular weight ~$1 \times 10^7$ to $1 \times 10^9$) comprising short α-1,4-linked (~95%) linear chains of 10–60 glucose units and **α-1,6-linked** (~5%) side chains containing 15–45 glucose units, which forms the bulk of starch molecule



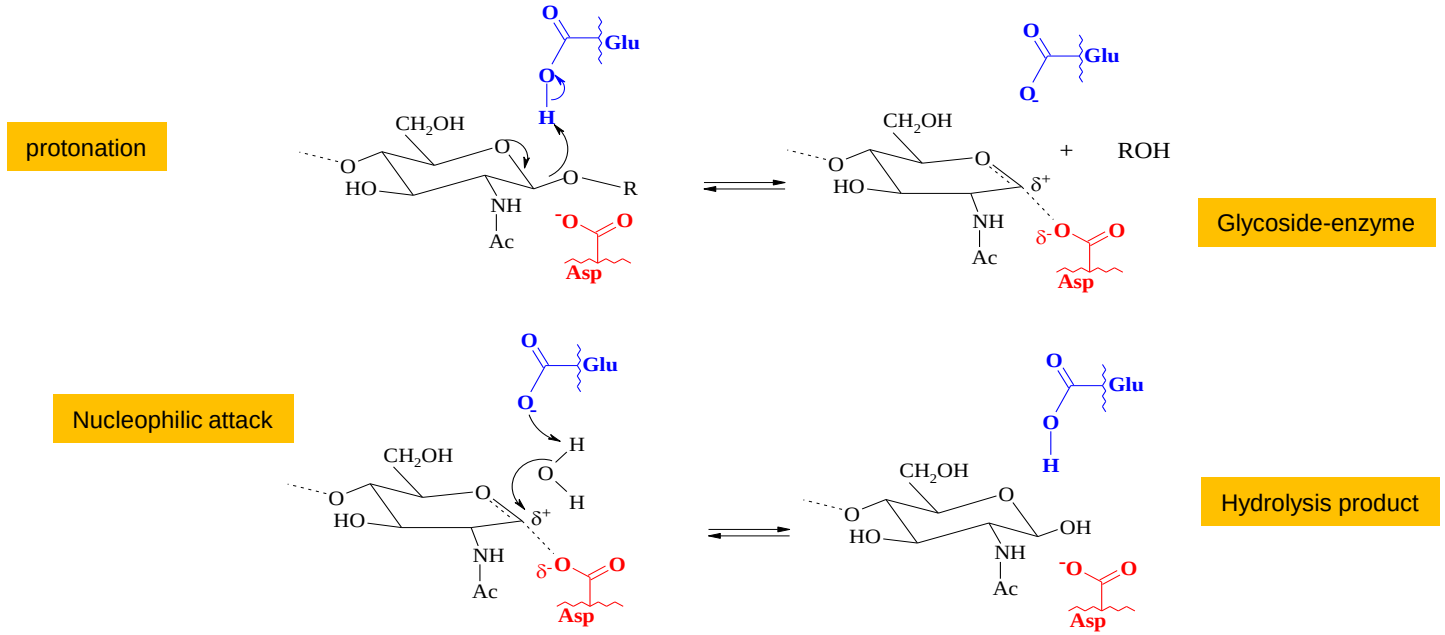
# Enzymatic processing of starch

## Glycosidases (hydrolases)

The enzymatic hydrolysis of poli- and oligosaccharides is catalysed by different glycosidases enzymes (hydrolases). They are regioselective, and enantioselective.

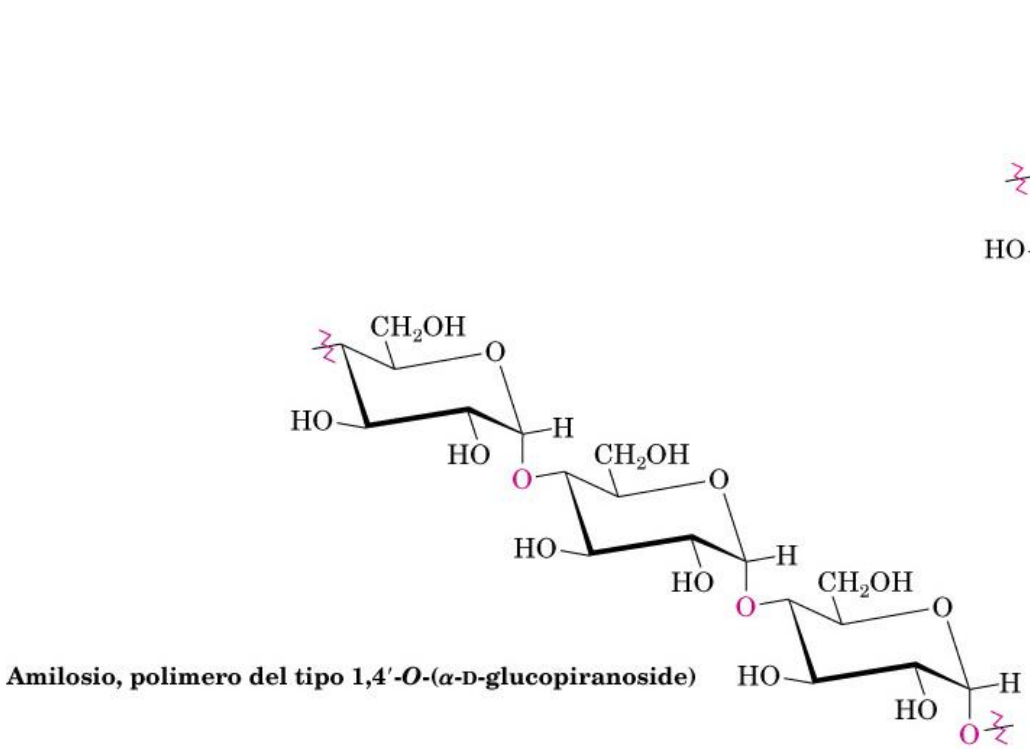
The hydrolysis of glycosides occurs by retaining or inverting the stereocenter involved in the hydrolysis, but always with high stereoselectivity.

Mechanism: the glycosidic oxygen is protonated by the acid catalyst (i.e. the carboxylic function of a glutamic residue occurring on the glycosidase) and nucleophilic assistance to the departing aglycone is provided by a base (i.e. the charged carboxylate function of an aspartic residue); the resulting glycoside-enzyme is finally hydrolysed by water generating a stereocenter with the same configuration.

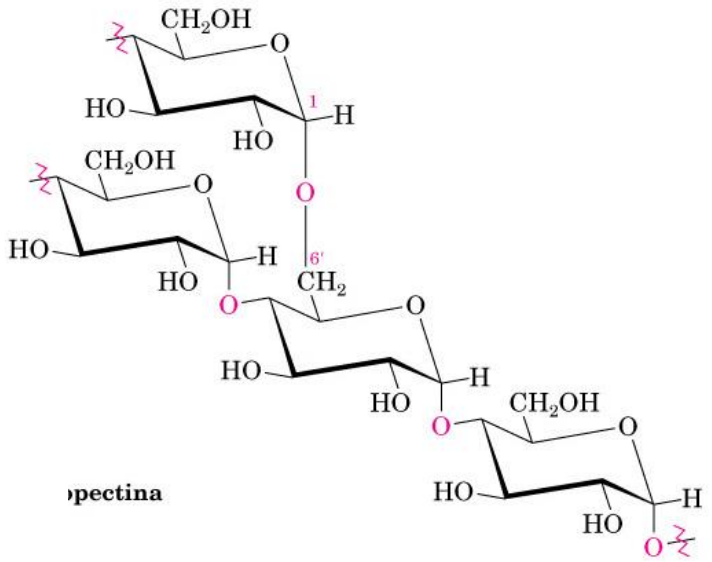


# Enzymatic processing of starch: amylases

**Enzymatic starch hydrolysis requires the coordinated action of different endo- and exo-amylases: each of these enzymes having a specific role.**



Amilosio, polimero del tipo 1,4′-*O*-(α-D-glucopiranoside)

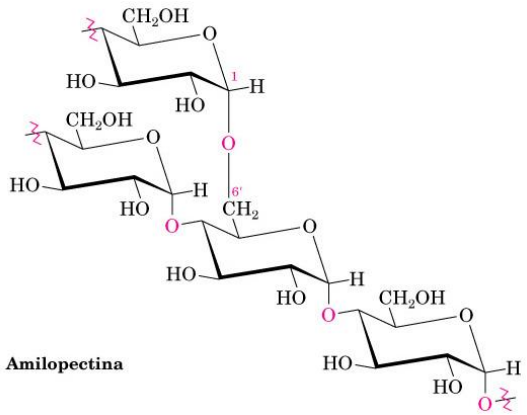


pectina

# Enzymatic processing of starch: endoamylases

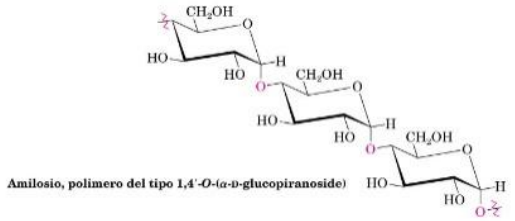
**Endoamylases**:
act randomly at the interior of starch molecule to release **linear and branched oligosaccharides** of varying chain lengths (e.g., α-amylase),

**Pullulanases:**
endo-acting enzymes capable of hydrolyzing α-1, 6-glycosidic linkages in starch and amylopectin,



Amilopectina

# Enzymatic processing of starch: exoamylases




Exoamylases act in a sequential manner starting from the **nonreducing** end of the starch molecule

a) cleave every glycosidic bond from the non-reducing end successively to produce glucose.
**glucoamylase** [EC 3.2.1.3] **inverting** mechanism to release **β-D-glucose**
**α-glucosidase** [EC 3.2.1.20] retaining mechanism to yield **α-D-glucose**

b) **β-amylase** [EC 3.2.1.2] breaks every alternate glycosidic linkage, thereby producing **maltose** as the end product

α-glucosidase hydrolyzes oligosaccharides (especially maltose) more rapidly than polysaccharides, which are hydrolyzed relatively slowly, or not at all.

**Glucoamylase are therefore preferred for starch processing**

Majority of the enzymes employed in starch saccharification are of microbial origin, except beta-amylase, which is generally derived from plants

# Enzymatic processing of starch: glucoamylase glycosidases – amylases- exoamylases

glucoamylase [EC 3.2.1.3] inverting mechanism to release β-D-glucose

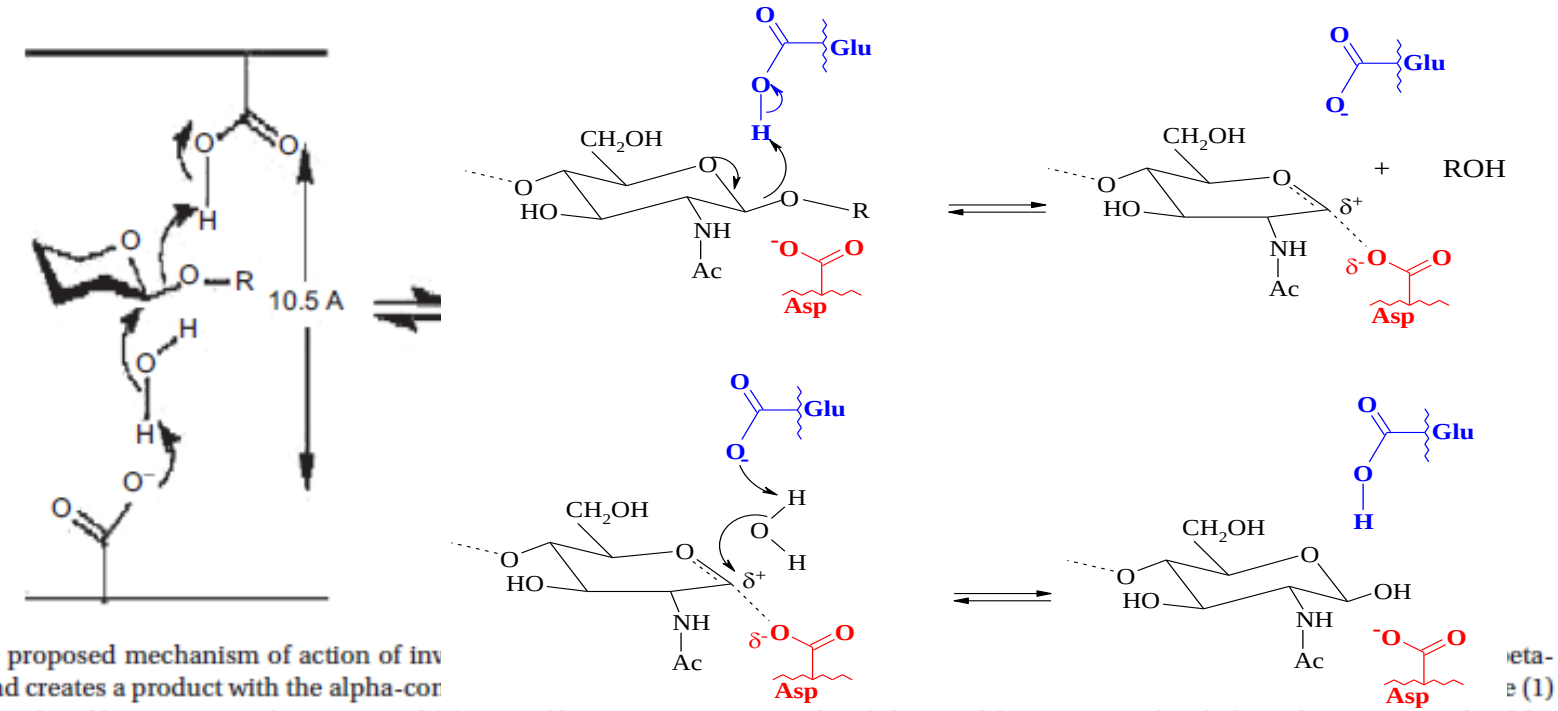
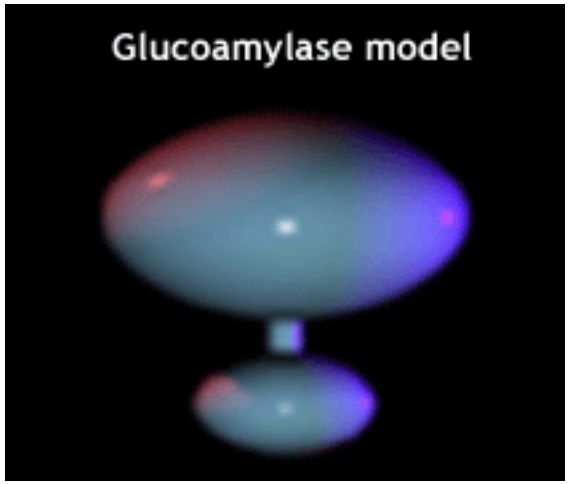



**Figure 1.** The proposed mechanism of action of inv
glycosidic bond creates a product with the alpha-cor
general acid-catalyzed leaving group departure and (2) general base-assistance to nucleophilic attack by a water molecule from the opposite side of the sugar ring (Sinnott, 1990; McCarter and Withers, 1994).

# Enzymatic processing of starch: glucoamylase glycosidases – amylases- exoamylases




Glucoamylase is a multi-domain enzyme. The smaller **starch-binding domain** (SBD) is attached to the larger catalytic domain by a heavily glycosylated linker. The binding domain provides the **hydrophilic surface** for interacting with starch

RSC Advances

PAPER



Cite this: *RSC Adv.*, 2022, **12**, 35676

# 2,5-Furandicarboxaldehyde as a bio-based crosslinking agent replacing glutaraldehyde for covalent enzyme immobilization†

Chiara Danielli,[ab] Luuk van Langen,[b] Deborah Boes,[bc] Fioretta Asaro,[a] Serena Anselmi,[d] Francesca Provenza,[de] Monia Renzi[e] and Lucia Gardossi*[a]

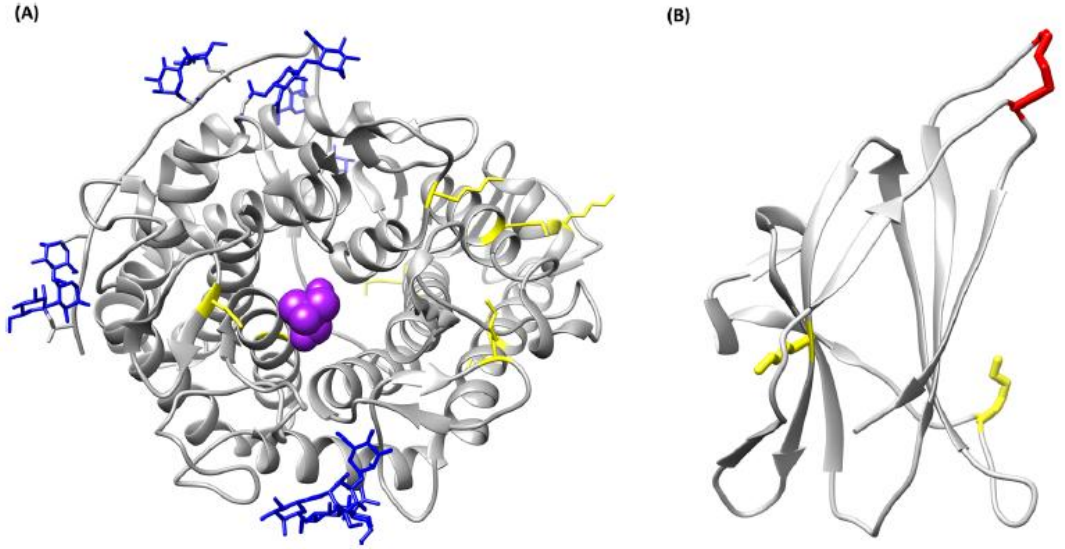
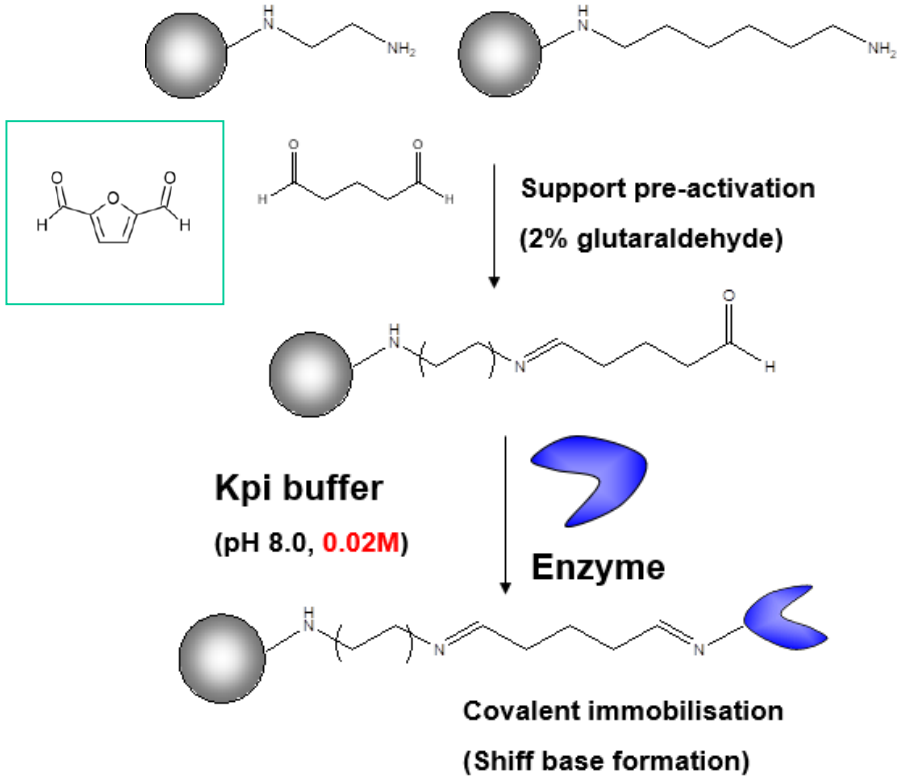



Fig. 2 Tridimensional models of the structure of glucoamylase from *Aspergillus niger*. In yellow, lysine residues; in blue, glycan residues; in purple, TRIS inhibitor in the catalytic pocket; in red, disulfide bonds. (A) Catalytic domain (PDB ID: 3EQA), (B) starch binding domain (PDB ID: 5GHL).



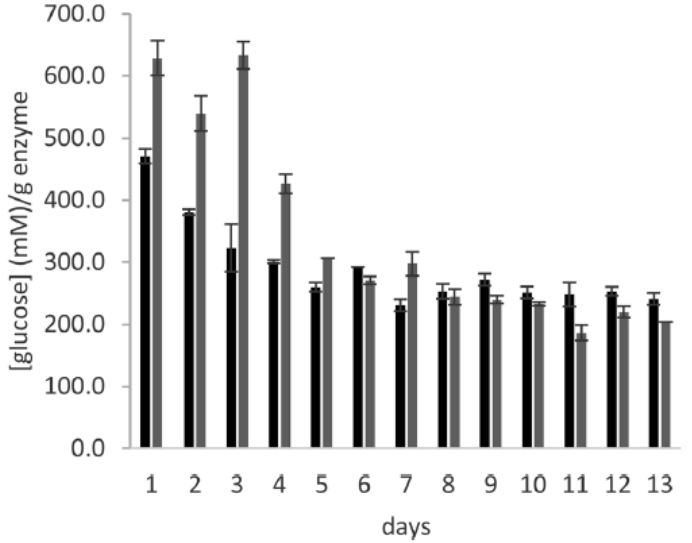




Fig. 3 Glucose concentration per g of dry enzyme preparation in the effluent of the continuous flow column experiment for glucoamylase immobilized with 0.2 µmol $g_{carrier}^{-1}$ of glutaraldehyde (in grey) and DFF (in black). Reaction conditions: 25% maltose solution in 10 mM citrate buffer, pH 4.5, 25 °C.

**Immobilization of glucoamylase on the carrier.**

10 g of carrier ReliZyme HA403/M were suspended in 16 mL of potassium phosphate buffer (25 mM, pH 7, 1.6 mL $g_{carrier}$ −1). Then, 200 mmol dialdehyde g $_{carrier}$ −1 were added to the mixture. The mixture was shaken at 25 °C for 2 hours. The supernatant was then removed, and the activated carrier was rinsed 3 times with 20 mL of demineralized water.

The activated carrier was resuspended in 16 mL of potassium phosphate buffer (25 mM, pH 7, 1.6 mL $g_{carrier}$ −1). 5 g of Dextrozyme GA (commercial glucoamylase solution; 120 U $g_{carrier}$ −1 referred to the amount of wet carrier as provided by the manufacturer) were added to the reaction mixture. The mixture was kept shaking at 25 °C for 24 hours. The supernatant was then removed and tested for residual enzyme activity. The immobilized enzyme was rinsed 3 times with 20 mL of demineralized water. The immobilized enzyme was stored at 4 °C in potassium phosphate buffer (25 mM, pH 7).

# 2. High-fructose corn syrups (HFCS)

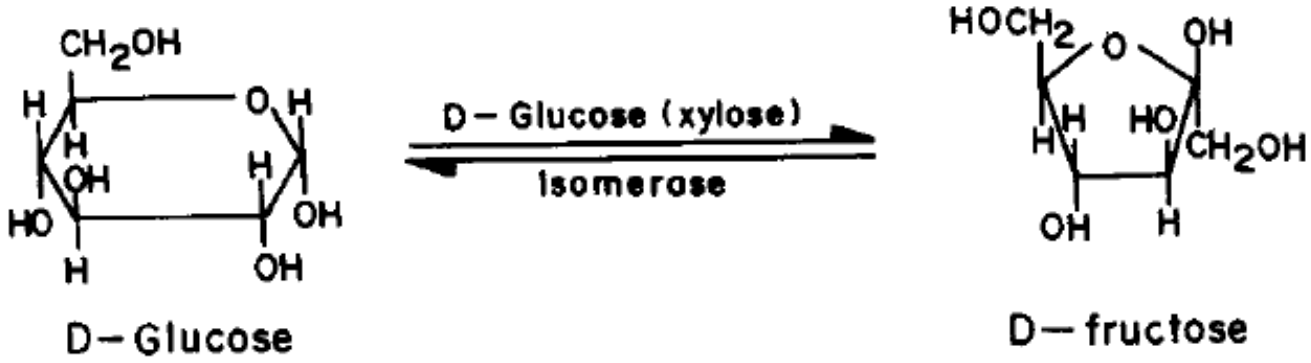
Fructose is 30% sweeter than sucrose, on a weight basis, and twice as soluble as glucose at low temperatures.

A 50% conversion of glucose to fructose overcomes both problems giving a stable syrup that is as sweet as a sucrose solution of the same concentration.

The production of HFCS was developed first in Japan and later in the United States.

It gained commercial importance in the United States because of the lack of supply of sucrose after the **Cuban revolution** in 1958, and it continues to be one of the most important industrial enzymes to this day.

D-Glucose isomerase catalyzes *in vitro* the reversible isomerization of D-glucose to D-fructose




The isomerisation is possible by chemical means but not economical, giving tiny yields and many by-products
e.g. 0.1 M glucose 'isomerised' with 1.22 M KOH at 5°C under nitrogen for 3.5 months gives a 5% yield of fructose but only 7% of the glucose remains unchanged, the majority being converted to various hydroxy acids.
No alternative to enzymes!

# Isomerizzazione glucosio

Amido liquido a 100-110°C (40% w/v)

↓

Idrolisi di legami α-1,4 e α-1,6 con α-amilasi e pullulanasi a pH 6, 6.5 + $Ca^{+2}$ per 10-100 min

↓

Filtrazione, rimozione di di $Ca^{2+}$ e aggiustamento pH con resina a scambio ionico

↓

Idrolisi oligosaccaridi con glucoamilasi a 55°-60°C, pH 4.0-4.5 per 24-96 h

↓

Rimozione o inattivazione dell'enzima

↓

Sciroppo di D-Glu (95-98%, 40-50% w/v)

↓

Aggiunta di cofattori ($Mg^{2+}$, $HSO_3^-$) rimozione di $O_2$ e aggiustamento pH

↓

Filtrazione

↓

Scambiatori di calore

↓

Isomerizzazione a 57°C, pH 8.0 con Glu isomerasi immobilizzata, in un reattore a letto fisso

↓

Stadi di purificazione:
1. Decolorazione con carbone, 2. Filtrazione
3. Rimozione di cofattori per scambio ionico

↓

Aggiustamento pH

↓

Concentrazione al 70-72%

↓

Raffreddamento

↓

Isoglucosio (>50% Glu, ca 42% Fru)

# 3. Idrolisi dell'amido per ottenere bioetanolo

## Isomerizzazione glucosio

Amido liquido a 100-110°C (40% w/v)

↓

Idrolisi di legami α-1,4 e α-1,6 con α-amilasi e pullulanasi a pH 6, 6.5 + $Ca^{+2}$ per 10-100 min

↓

Filtrazione, rimozione di di $Ca^{2+}$ e aggiustamento pH con resina a scambio ionico

↓

Idrolisi oligosaccaridi con glucoamilasi a 55°-60°C, pH 4.0-4.5 per 24-96 h

↓

Rimozione o inattivazione dell'enzima

↓

Fermentazione del glucosio a etanolo

Sciroppo di D-Glu (95-98%, 40-50% w/v)

↓

Aggiunta di cofattori ($Mg^{2+}$, $HSO_3^-$) rimozione di $O_2$ e aggiustamento pH

↓

Filtrazione

↓

Scambiatori di calore

↓

Isomerizzazione a 57°C, pH 8.0 con Glu isomerasi immobilizzata, in un reattore a letto fisso

↓

Stadi di purificazione:
1. Decolorazione con carbone, 2. Filtrazione
3. Rimozione di cofattori per scambio ionico

↓

Aggiustamento pH

↓

Concentrazione al 70-72%

↓

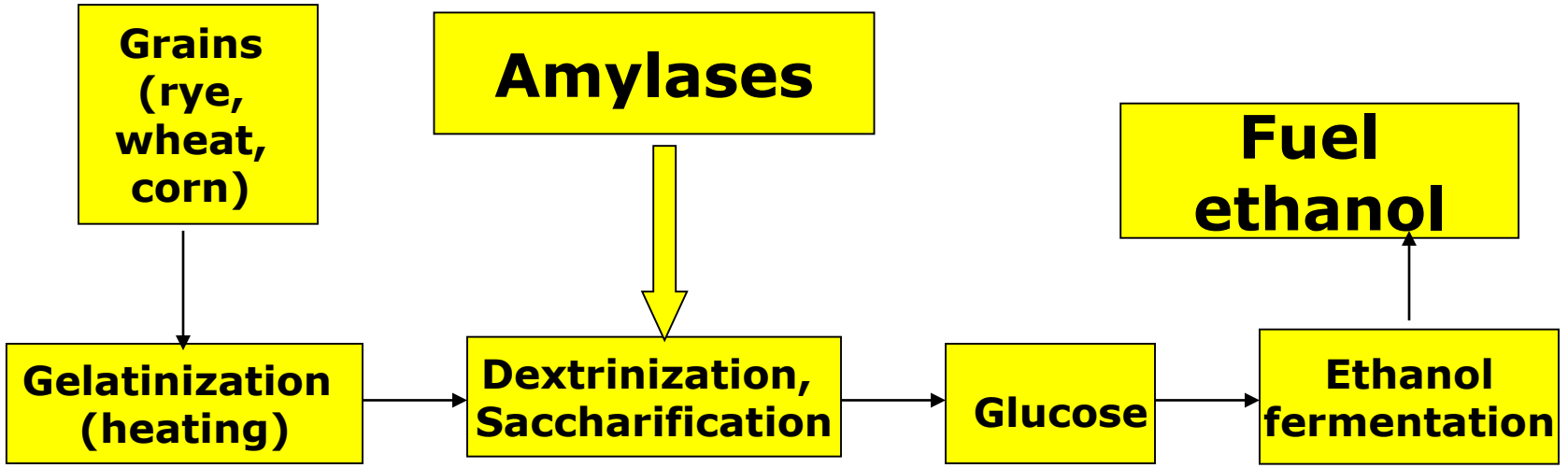
Raffreddamento

↓

Isoglucosio (>50% Glu, ca 42% Fru)

# First generation biofuels:

## ethanol from starch & grains




Nowadays, corn wet milling is one of the main fuel ethanol production processes in the USA. The advantages of using cereals and other agricultural products for fuel and chemical production lies on the available infrastructure and expertise in collection, distribution and processing.

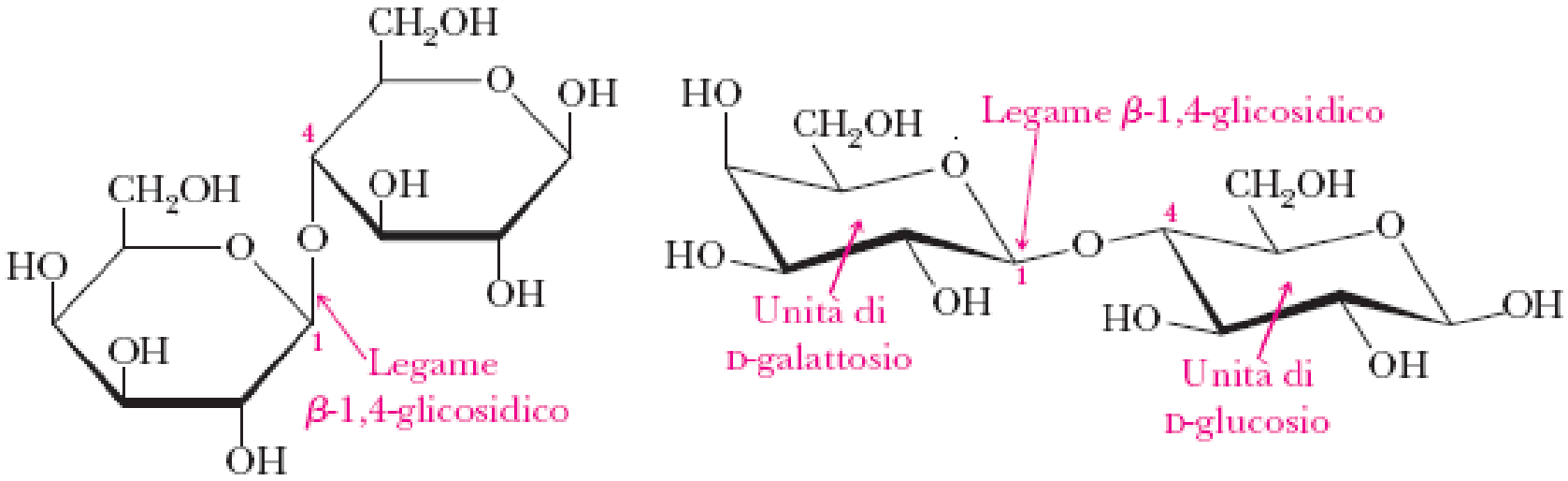
**However, they do not provide sustainable renewable resources for widespread fuel and chemical production due to direct competition with food applications.**

# Major biotransformations at industrial scale 1.

Fine-pharma chemistry

| Production scale [tpy] | Product | Enzyme | Reactor | Company |
|---|---|---|---|---|
| > 1 000 000 | high-fructose corn syrup (HFCS) | glucose isomerase | fixed-bed, IME | various |
| > 100 000 | lactose-free milk | lactase | fixed-bed, IME | various |
| > 10 000 | acrylamide | nitrilase | batch reactor | Nitto Co. |
| | cocoa butter* | lipase (CRL) | fixed-bed, IME | Fuji Oil |
| > 1,000 | | | | |
| | nicotinamide | nitrilase | 3-stage batch | Lonza Guangzhou |
| | D-pantothenic acid | aldonolactonase | | Fuji Pharma-ceuticals |
| | (S)-chloropropionic acid | lipase | | Dow Chemical |
| | 6-aminopenillanic acid | penicillin amidase | fixed-bed, IME | various |
| | 7-aminocephalo-sporanic acid | glutaryl amidase | Kundl/Hoechst | |
| | aspartame® | thermolysin | soluble enzyme | Tosoh/DSM |
| | L-aspartate | aspartase | fixed-bed, IME | various |
| | D-phenylglycine | hydantoinase/ (carbamoylase) | resting cells | Kanegafuchi |
| | D-*p*-OH-phenyl-glycine | hydantoinase/ carbamoylase | resting cells | Recordati |

**β-Galactosidase hydrolyses lactose into glucose and galactose, is a widely used in food technology, mainly in the dairy industry.**




Lattosio

Lactose intollerance: lack of beta-galactosidase

**Lactose Intolerance: Inability to hydrolyze lactose in the upper GI track thus allowing a readily fermentable sugar into the lower GI track where it is rapidly acted upon by the flora. This produces moderate to sever GI disturbances.**

**Lactase: Enzyme catalyzing hydrolysis of lactose into glucose and galactose thus relieving the symptoms of consuming lactose by lactose intolerant people.**

Enzymatic hydrolysis of lactose is one of the most important biotechnological processes in the food industry.

It is realised by enzyme β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23), trivial called lactase.

The enzymatic hydrolysis of lactose proposes several benefits and advantages of industrial application, including:
– development of lactose hydrolysed products to present one of the possible approaches to diminish the lactose intolerance problem, prevalent in more than half of the world's population,
– formation of galacto-oligosaccharides during lactose hydrolysis to favour the growth of intestinal bacterial microflora,
– improvement in the technological and sensorial characteristics of foods containing hydrolysed lactose,
– better biodegradability of whey after lactose hydrolysis

**Tab. 1.** Properties of microbial β-galactosidases.

| Microorganism | | Production of enzyme | pH-optimum | T-optimum [°C] | References |
|---|---|---|---|---|---|
| Fungi | *Aspergillus niger* | E | 3,0–4,0 | 55–60 | [7] |
| | *Aspergillus oryzae* | | 5,0 | 50–55 | [8, 9, 10] |
| Yeasts | *Kluyveromyces lactis* | I | 6,5–7,0 | 30–35 | [8, 11, 12] |
| | *Kluyveromyces fragilis* | | 6,6 | 30–35 | [8,12] |
| Bacteria | *Escherichia coli* | I | 7,2 | 40 | [7, 13] |
| | *Lactobacillus thermophilus* | | 6,2 | 55 | [7] |
| | *Leuconostoc citrovorum* | | 6,5 | 66 | [14] |
| | *Bacillus circulans* | | 6,0 | 65 | [8] |

I - intracellular, E - extracellular.