Introduction to first generation biorefineries

Biorefinery: chemistry meets biotechnology

*The term **"biorefinery"** describes the process that entails refining of biomass in a commercial context for the production of fuels, chemicals, polymers, materials, food, feed and value-added ingredients.*

Biomass

Renewable products

Fuels

Chemicals

Drugs

Materials

…….more

Chemical compounds can be made biologically by

*-**engineering the cellular metabolism***

-transforming biomass, waste, side products, CO_2

Why biofuels and biobased chemistry?

In 2011, the global petroleum consumption reached 88 x 10^6 barrels per day that corresponds to ***12 x 10^6 t oil consumption per day.***

Only ***7-10%*** *of annual petroleum consumption is required for* ***chemical production***

The hystory:

The establishment of petroleum-derived commodity and speciality chemicals received significant investment by international corporations and subsidies by governments to reduce their initially high prices in order to compete with traditional materials.

Recent history:

Corn biorefineries for first generation bioethanol (biofuels)

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.

Fossil – based refineries

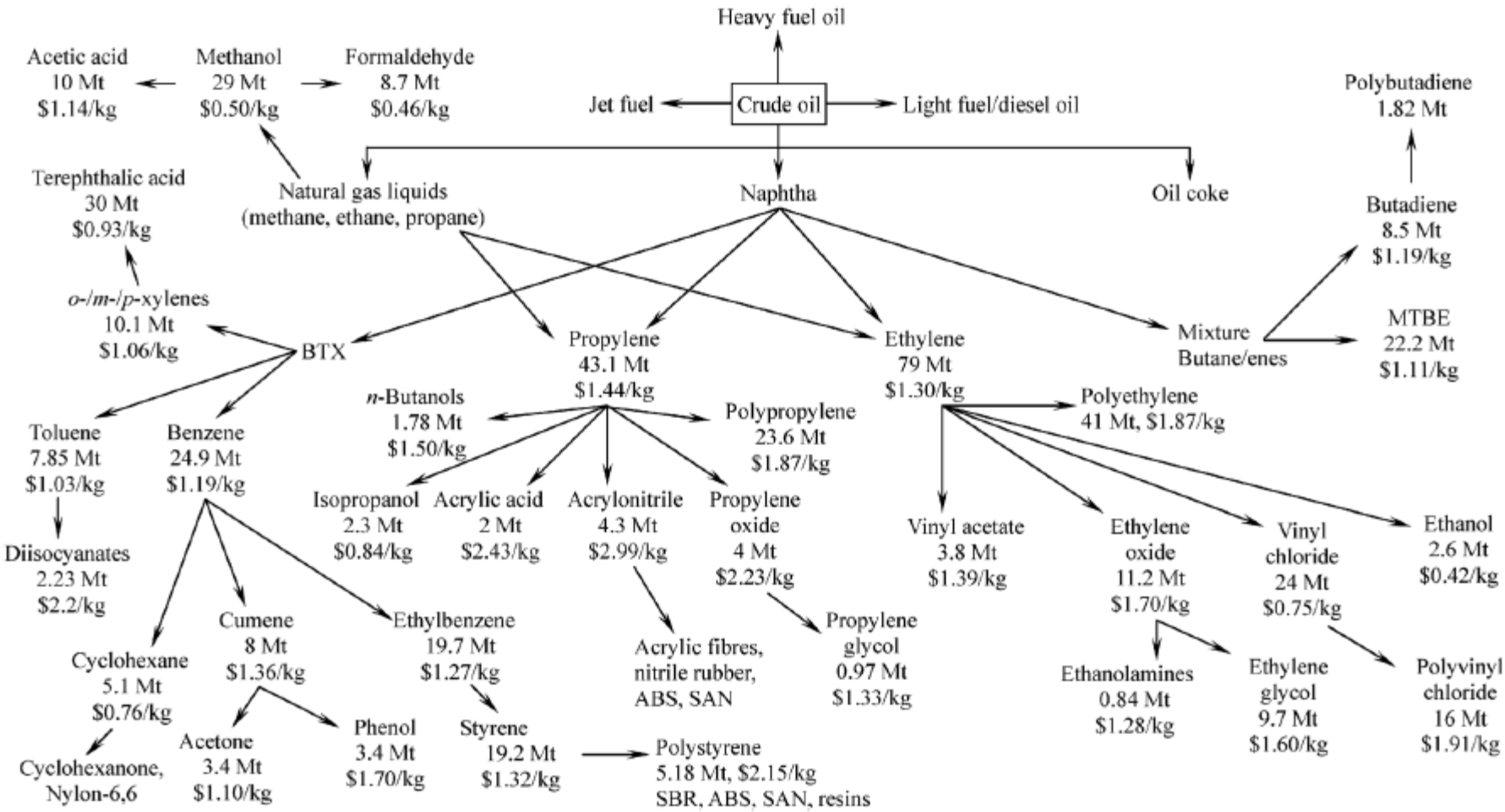
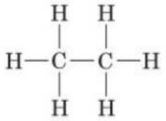


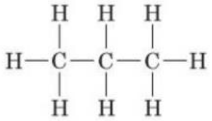
Fig. 1 Base chemicals and derivatives produced from petroleum (production capacities were taken from the journal *Chemical Engineering News*[4] and unit prices were taken from the *ICIS Indicative chemical prices*[5]).

What chemistry needs

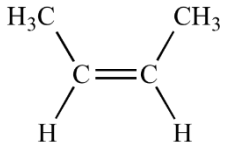
The chemical industry relies on six basic chemicals or chemical groups including

ethylene, propylene,

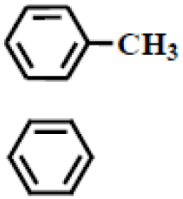




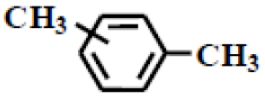
the C4 olefins (butadiene and butenes),



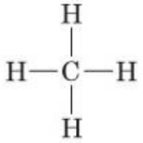
the aromatics (benzene and toluene),



the xylenes (ortho, meta and para)



and methane.

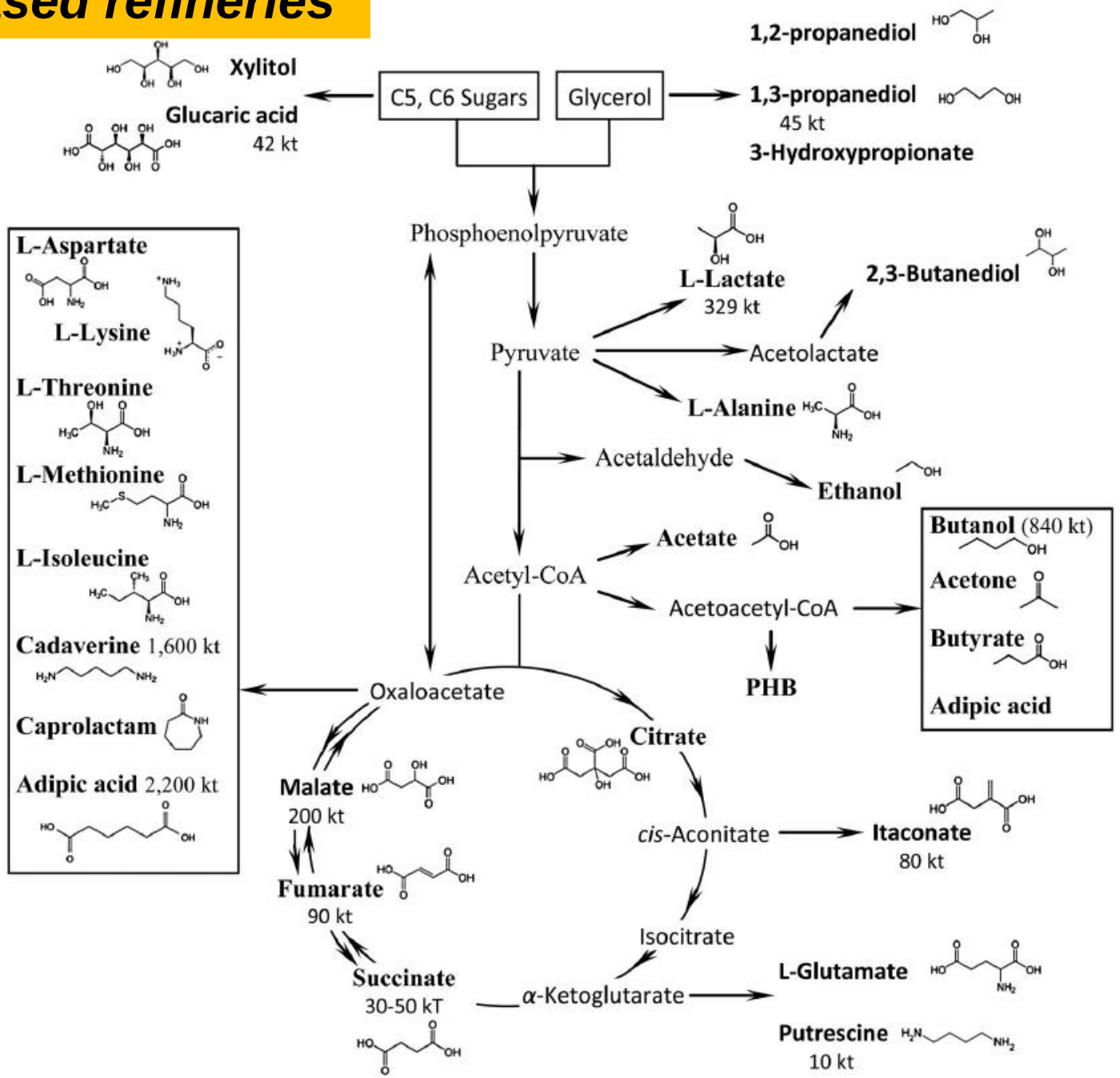


Biomass:

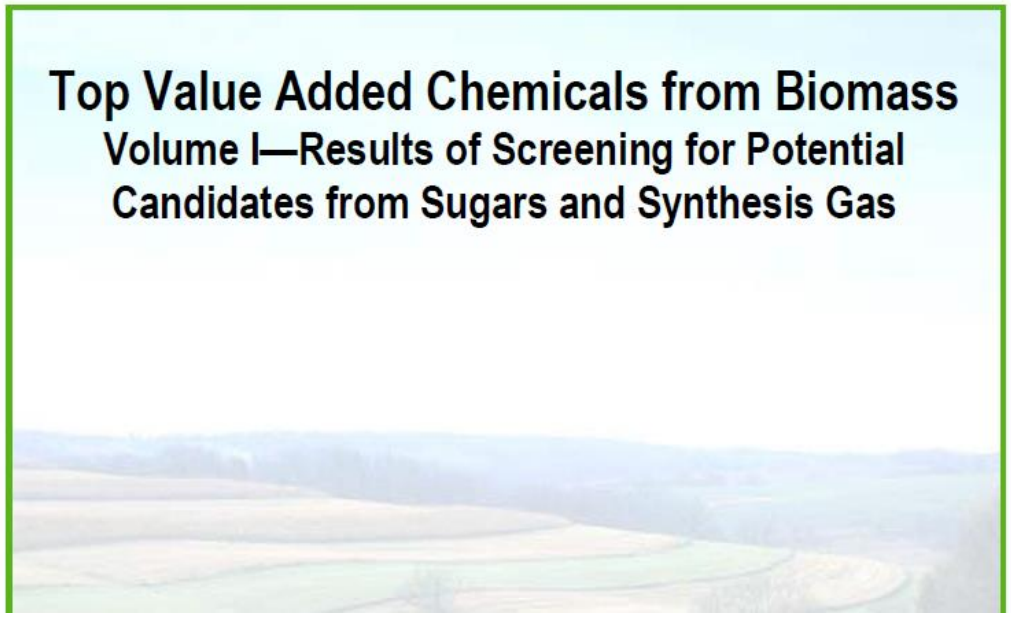
“all non-fossil-based living or dead organisms and organic materials that have an intrinsic ***chemical energy content”.***

In an industrial context, the term ***“refining”*** *describes fractionation of a given raw material into various fractions that could be converted into commodity and speciality products aiming to maximise the efficiency of resource utilisation.*

Bio – based refineries



Platforms for the production of renewable chemicals



Building Blocks
1,4 succinic, fumaric and malic acids
2,5 furan dicarboxylic acid
3 hydroxy propionic acid
aspartic acid
glucaric acid
glutamic acid
itaconic acid
levulinic acid
3-hydroxybutyrolactone
glycerol
sorbitol
xylitol/arabinitol

Such building blocks could either be produced from renewable carbon through green chemical conversion routes or via microbial conversions.

Efficient exploitation of biomass

- *it has been evaluated that production of chemicals and polymer resins from sugars and biomass result in two to four times more added value, create six to eight times more employment and require less percentage of feedstock compared to biofuel production.*

- ***Therefore, renewable carbon should be utilized for integrated production of fuels and chemicals.***

What Biomass for bio-based chemicals?

- *Industrial implementation of **first generation bioethanol and biodiesel** production was facilitated by **existing raw materials** (e.g., **corn, sugarcane, oilseed**) and facilities (e.g., **corn wet milling plants**) and the flexibility given by the utilisation of biofuels as blends with existing petroleum-derived fuels.*

- *Large-scale production of commodity chemicals and materials from **biomass** will require **feedstock availability**, flexibility and logistics, development of new technologies and unit operations, production of new building blocks, conversion of these building blocks into marketable products and significant investment to scale-up new processes.*

Some historical info:
the evolution of first generation biorefineries

1. ***Starch processing***
2. ***Fructose syrup***
3. ***Bioethanol***

Technological evolution leading to first generation bioethanol.

Corn biorefineries: from starch to sweeteners

*Refining started by simple **starch** extraction in 1948 by Thomas Kingsford, which was initially used as a **laundry aid, food ingredient and sweetener** production.*

*Advances in corn refining gradually led to more efficient starch and **glucose syrup** production coupled to the commercialisation of new products, such as crystalline **dextrose and corn oil**.*

*During the 20th century, corn refineries managed to resist competition through innovation in manufacturing a variety of **derivatised starches** and introduction of new technologies based on **enzymatic hydrolysis** for the production of a wide spectrum of **sweeteners**, **high fructose corn syrups** being the most important of these inventions.*

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Major biotransformations at industrial scale 1.

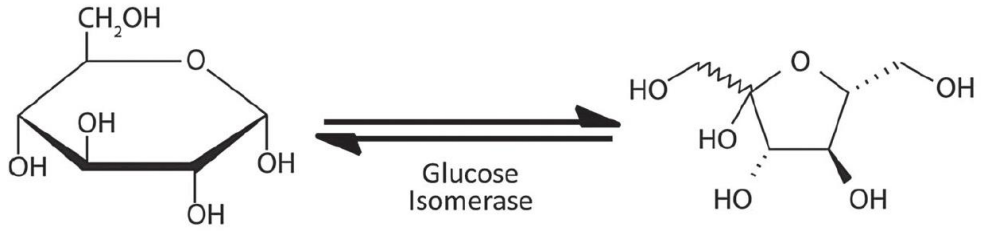
Fine-pharma chemistry

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

D-Glucose isomerase : biotransformation at largest scale in industry

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

reversible isomerization of D-glucose to D-fructose: 42% fructose



Glucose (aldose)

Mole wt=180 $C_6H_{12}O_6$
75% as sweet as Sucrose

Fructose (ketose)

Mole wt=180 $C_6H_{12}O_6$
160% as sweet as Sucrose

Scheme 1 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

D-Xylose isomerases (EC 5.3.1.5), commonly known as glucose isomerases, are widely distributed in nature and are produced by many microorganisms.

D-glucose isomerizing enzyme from *Pseudomonas hydrophila* were characterized in 1957. The enzyme had a preference for D-xylose, but also accepted D-glucose as an alternative substrate, albeit with a Km 160-fold higher.
Additional work led to the identification of a Streptomyces strain (YT-5) that expressed a xylose isomerase without the need for xylose induction.
There are ongoing efforts directed toward the discovery and engineering of glucose isomerases with improved thermostability, reduced dependence on metal ions for activity, lower pH optima and greater resistance to Ca2+ ions and other inhibitors.

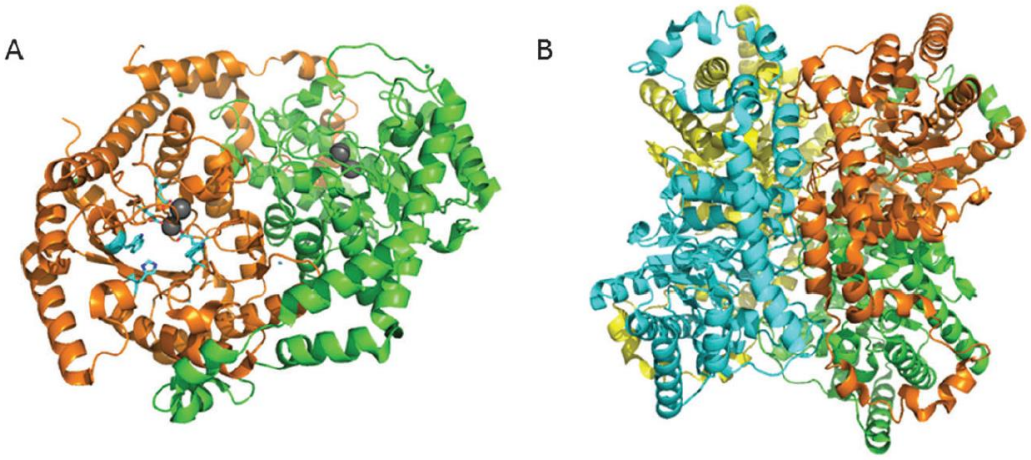
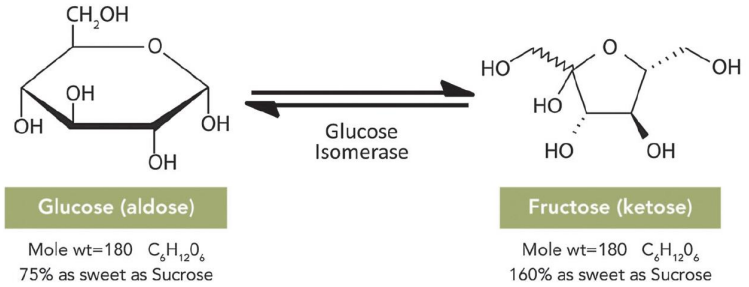


Fig. 3 Structure of Glucose Isomerase from *Streptomyces rubiginosus* in (A) dimeric form with metal ions depicted as grey spheres and active site residues in cyan, and (B) as a tetramer



Scheme 1 Isomerization of D-glucose to D-fructose.

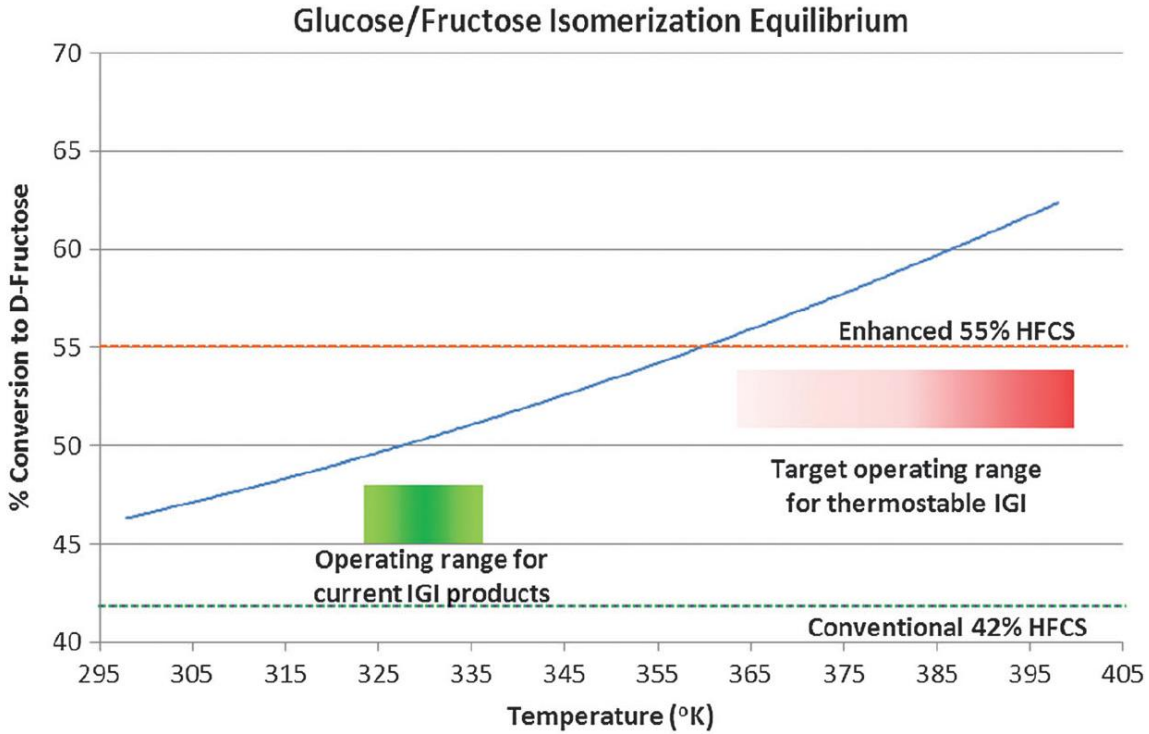


Fig. 2 The glucose/fructose equilibrium is shown as a function of temperature (blue line). The operating temperature of current IGI products, used to produce 42% HFCS, is shown by the green bar. Thermostable GI would need to operate at temperatures over 365 K (red bar) in order to produce 55% HFCS.

Table 3 Examples of commercial immobilized glucose isomerase products[22,23,44]

Product	Producer	GI source	Description	Currently sold?
Optisweet® 22	Miles-Kali/Solvay	*S. rubiginosus*	Adsorpt with glu	
TakaSweet®	Miles Labs/Solvay	*Flavobacterium arborescens*	Polyami and sph	
Maxazyme® GI	Gist-Brocades	*A. missouriensis*	Crosslin	
Ketomax GI-100	UOP	*S. olivochromogenes*	Glutaral alumina	
Spezyme®	Genencor	*S. rubiginosis*	Crystalli DEAE-ce	
Sweetase®	Denki Kagku-Nagase	*S. phaeochromogenes*	Heat-tre	
Sweetzyme® T	Novozymes A/S	*B. coagulans* *S. murinus*	Gluteral containi	
GENSWEET®SGI	Genencor/DuPont	*S. rubiginosis*	Soluble anionic	
GENSWEET® IGI	Genencor/DuPont	*S. rubiginosis*	PEI/glut inorgani	

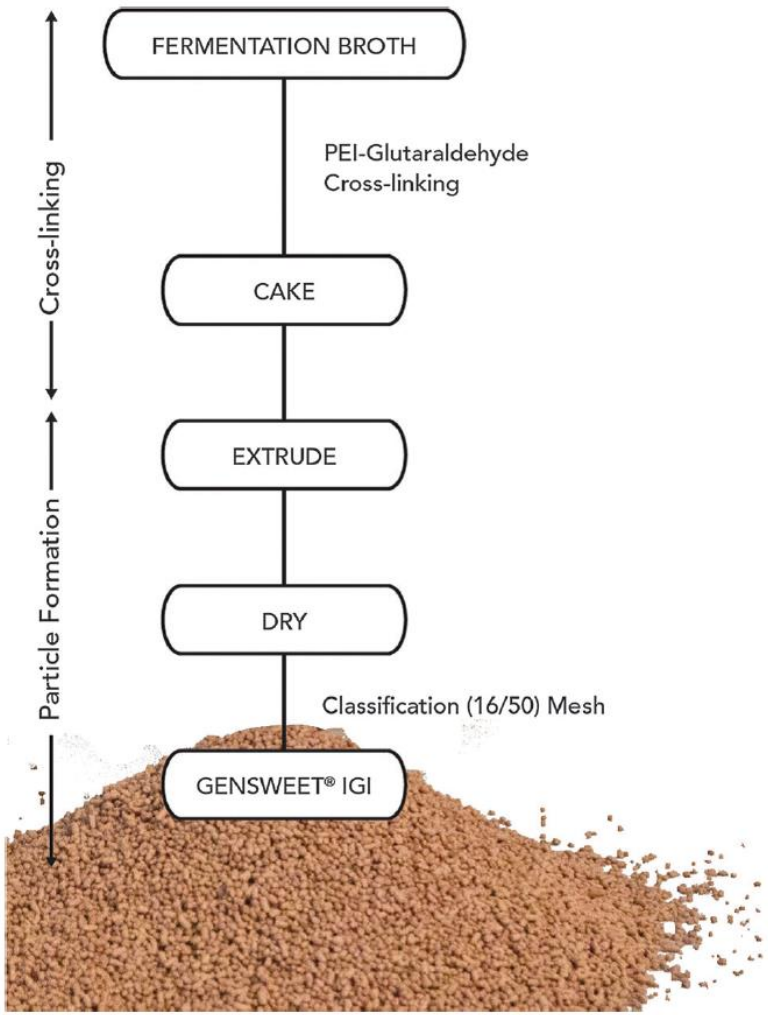


Fig. 4 Process for large scale immobilization of glucose isomerase.[53]

High-fructose corn syrups (HFCS)

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.*

Sucrose derived from sugar beet (40%) and sugarcane (60%) was the main sweetener in the world until 1976.

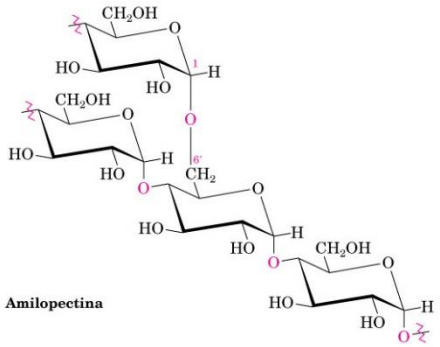
Enzymatic processing of starch

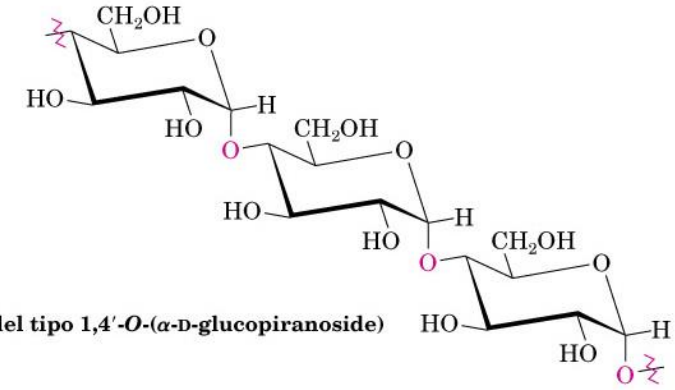
The native starch is semicrystalline in nature having varying size, shape, and granule size depending up on the botanical origin.
Industrial sources: maize, rice, wheat, cassava, and potato.

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

The number of α-D-glucose residues varies depending upon source of amylose and amylopectin.
Amylose: linear water-insoluble polymer of glucose subunits joined by α-1,4 bonds (~99%) and ~1% α-1,6 linkages, MW ~1 × 10^5 to 1 × 10^6.

Amylopectin: branched watersoluble polysaccharide (molecular weight ~1 × 10^7 to 1 × 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


Amilopectina


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

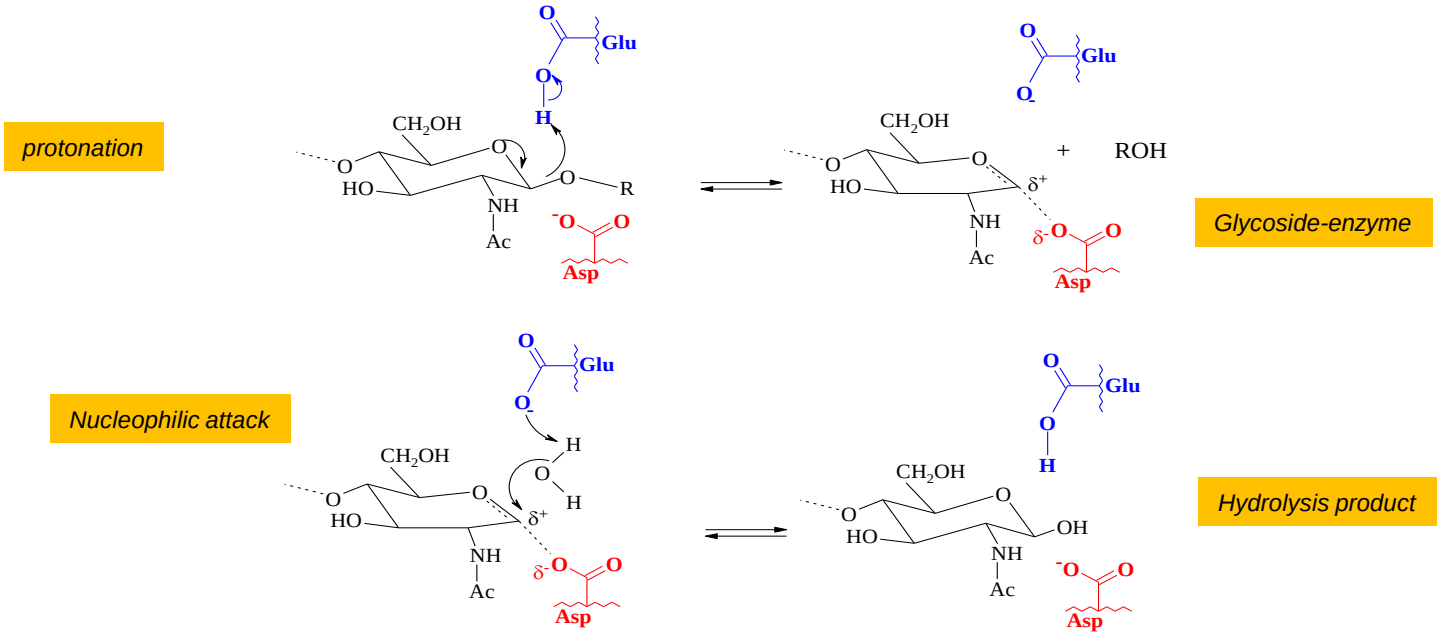
Enzymatic processing of starch

Glycosidases (hydrolases)

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

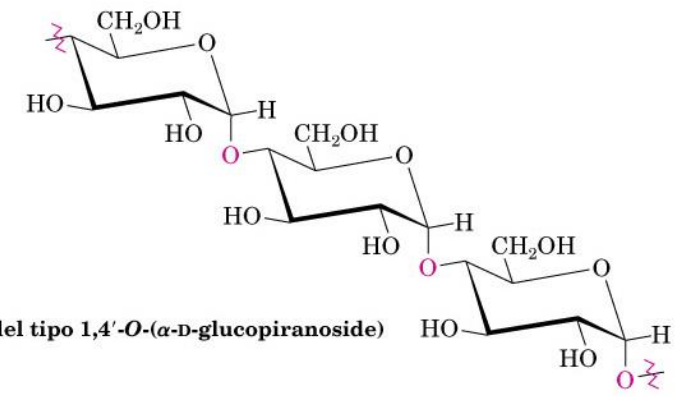
The hydrolysis of glycosides occurs by retaining or inverting the stereocenter involved in the hydrolysis, but always with high stereoselectivity.

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.



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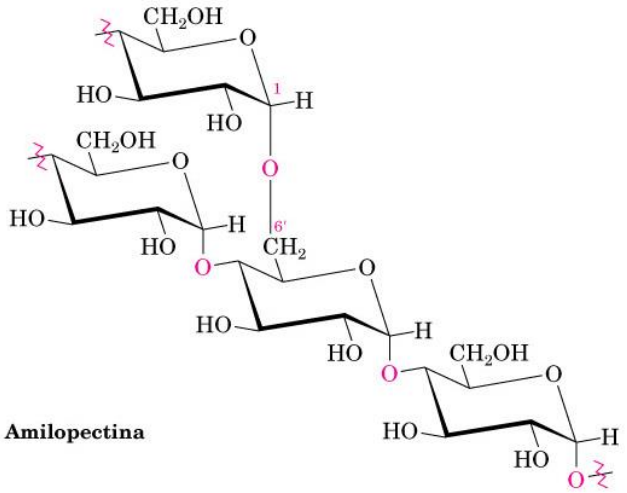
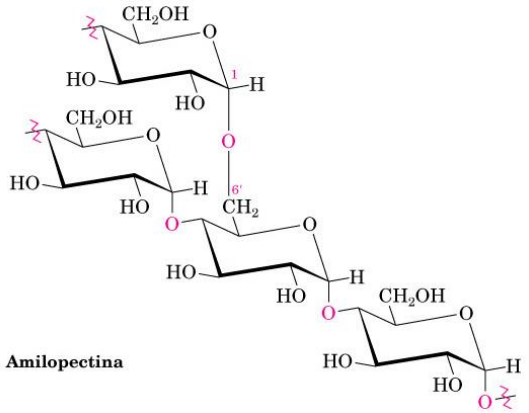


Amilopectina

Enzymatic processing of starch: endoamylases

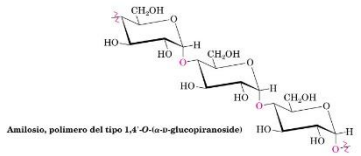
***Endoamylases**:*
*act randomly at the interior of starch molecule to release **linear and branched oligosaccharides** of varying chain lengths (e.g., α-amylase),*

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



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

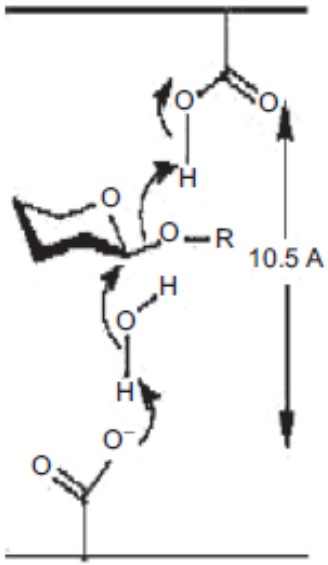
α-glucosidase hydrolyzes oligosaccharides (especially maltose) more rapidly than polysaccharides, which are hydrolyzed relatively slowly, or not at all.

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



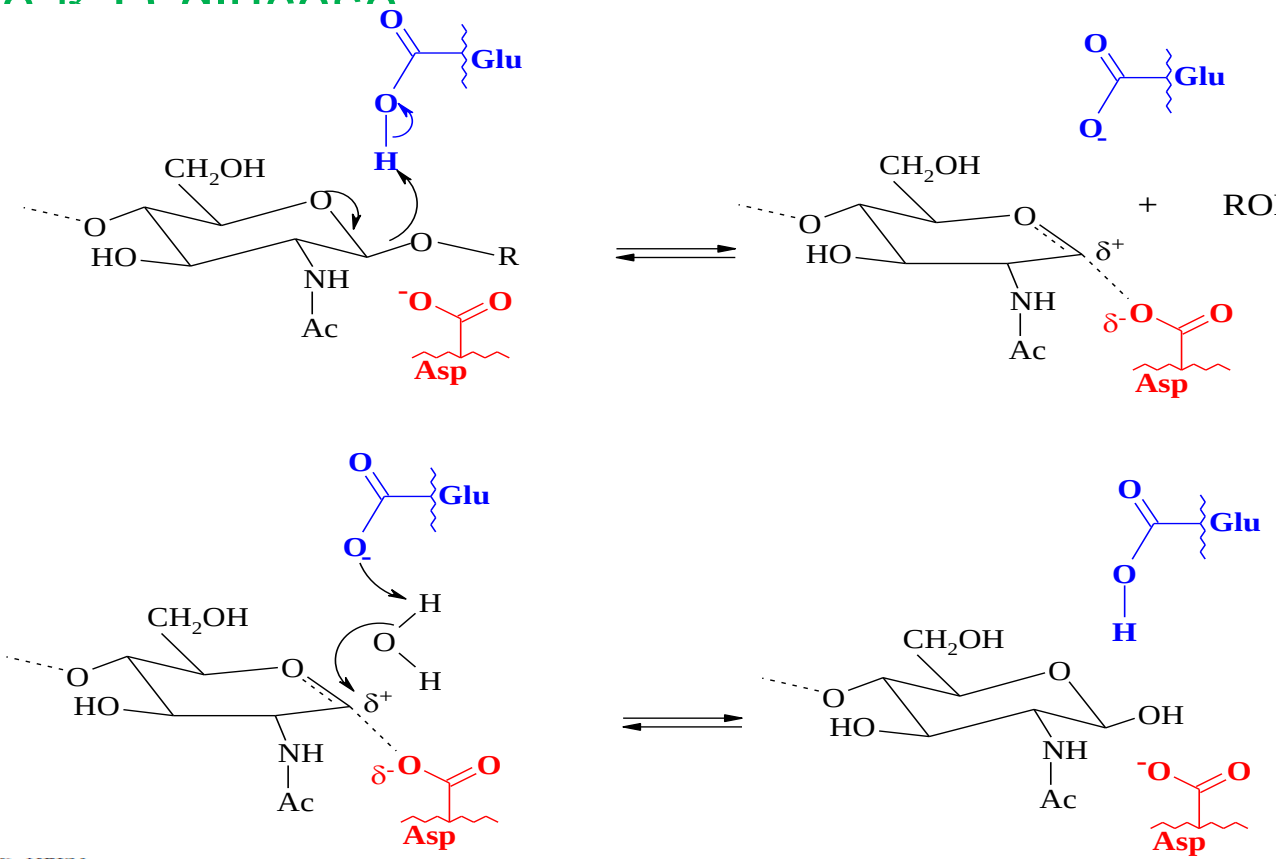
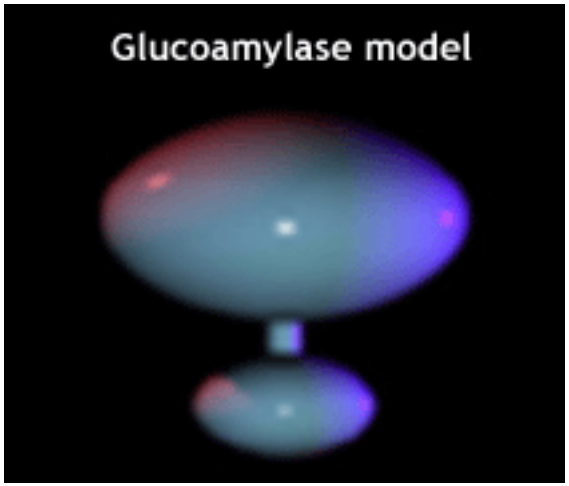


Figure 1. The proposed mechanism of action glycosidic bond creates a product with the alp general acid-catalyzed leaving group departur sugar ring (Sinnott, 1990; McCarter and Withers, 1994).

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

High-fructose corn syrups (HFCS)

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

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Sucrose derived from sugar beet (40%) and sugarcane (60%) was the main sweetener in the world until 1976.

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%

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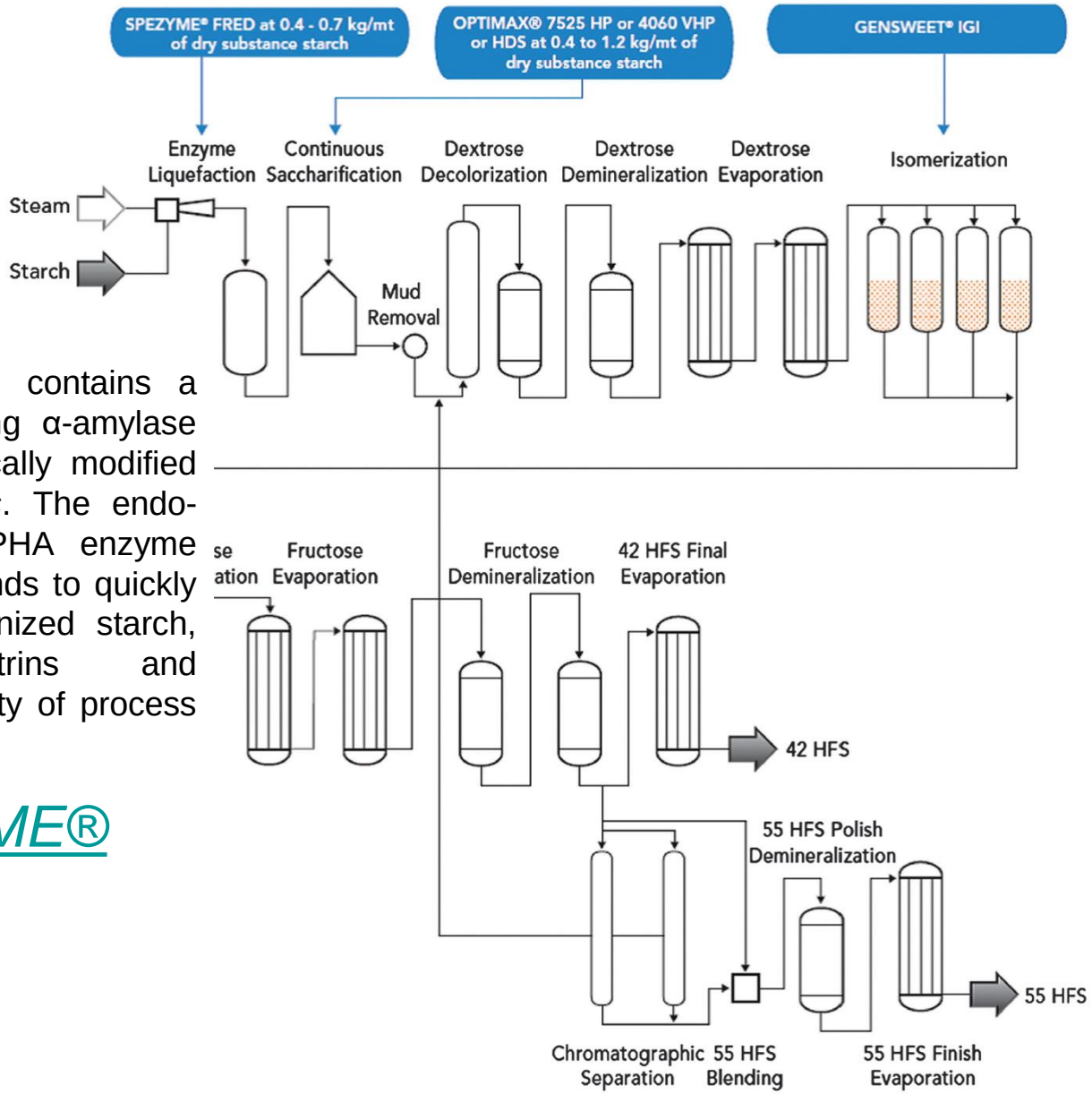
Raffreddamento

↓

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

glucoamylase and pullulanase



SPEZYME® ALPHA enzyme contains a thermostable starch hydrolyzing α-amylase that is produced by a genetically modified strain of *Bacillus licheniformis*. The endo-amylase in SPEZYME® ALPHA enzyme hydrolyzes α-1,4-glucosidic bonds to quickly reduce the viscosity of gelatinized starch, producing soluble dextrins and oligosaccharides under a variety of process conditions.

DuPont™ SPEZYME®

Fig. 5 Layout of a corn wet milling plant.[53]

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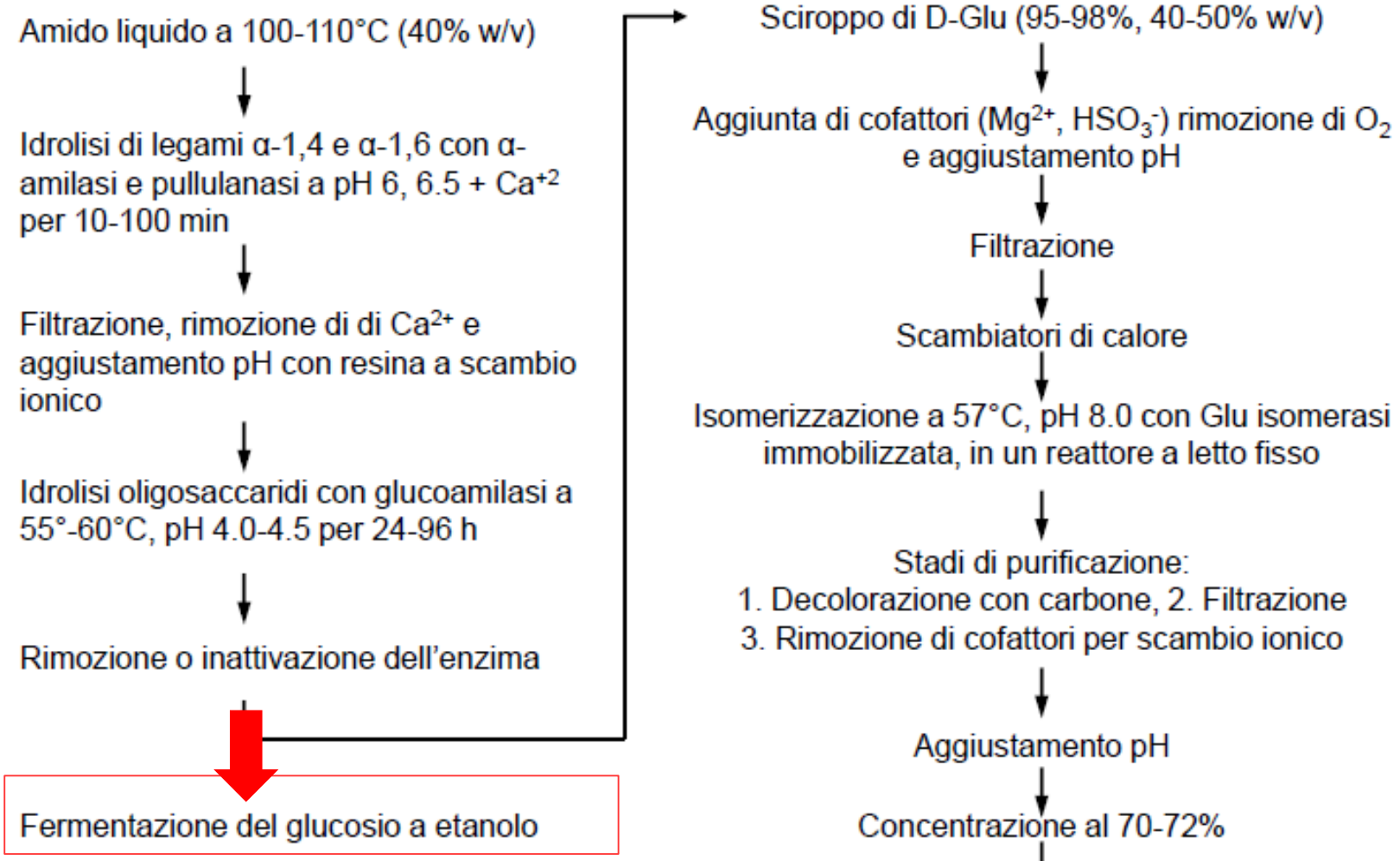
However, they do not provide sustainable renewable resources for widespread fuel and chemical production due to direct competition with food applications.

Technological evolution of Corn biorefineries: from starch to sweeterners to bioethanol



First generation biofuels: ethanol from starch & grains

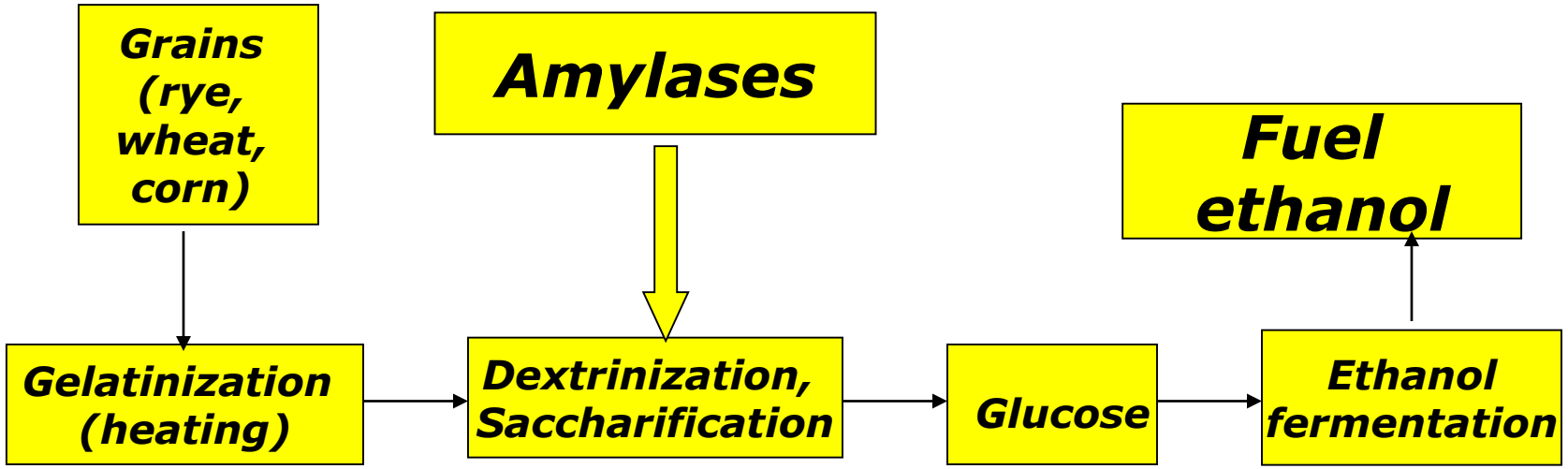
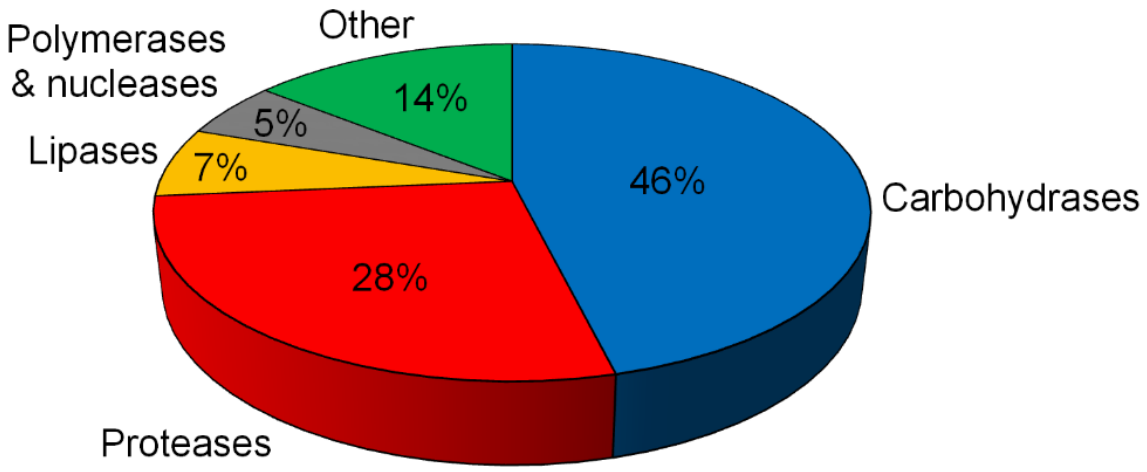


Table 17 Major ethanol producers and feedstocks utilised

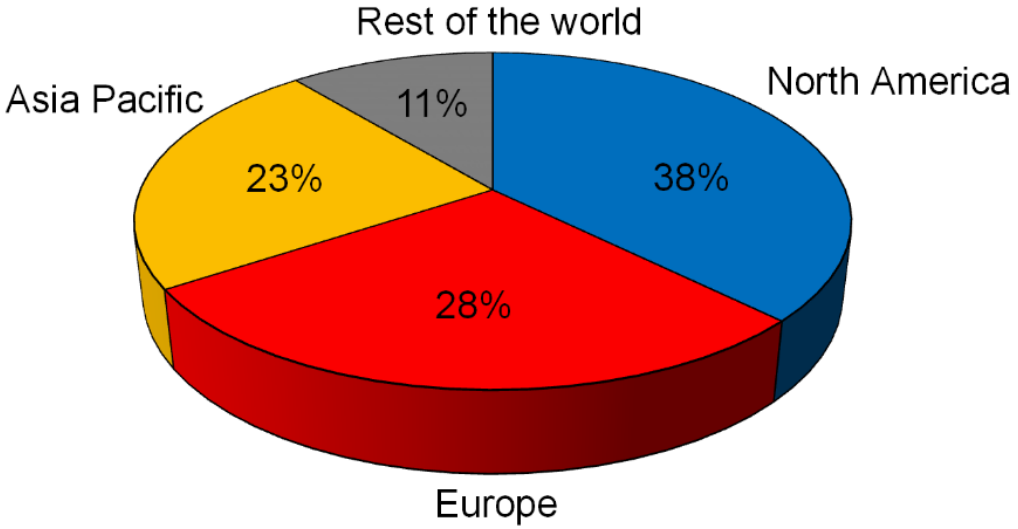
Region	Raw materials[a]	Million gallons[b]
USA	Corn (98%), sorghum (2%)	14 887
Brazil	Sugarcane (100%)	5557
Europe	EU-27: wheat (48%), sugar beet (29%)	1179
Asia	China: corn (70%), wheat (30%)	952 (China: 555)
Canada	corn (70%), wheat (30%)	449

[a] Balat and Balat.[265] [b] 2013 ethanol industry outlook.[266]

2013 Global enzyme market revenue by product



2013 Global enzyme market revenue by region



Total 2013: 4412 USD million

Data extracted from the Grand View Research Enzyme Market Report

Enzyme market

*The **biocatalyst market for specialty enzymes**, where pharmaceuticals are the prominent application, is accounted only for **$230 M**.*

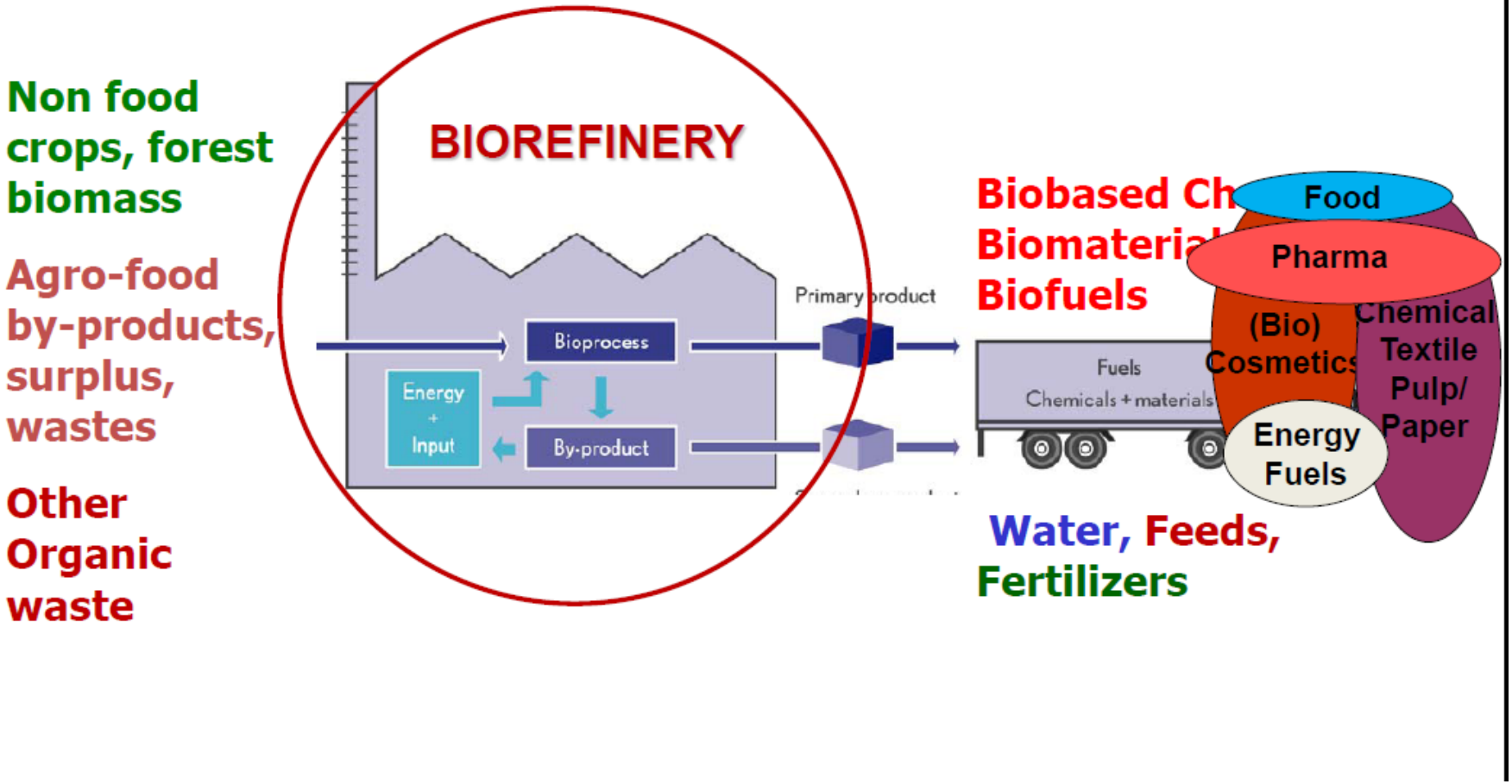
*Although these figures do not take into account own production and usage of enzymes by companies, it is evident that **specialty enzymes represent a limited portion of the global industrial enzyme market**, which in 2013 was valued at around **$4.4 B** and by 2020 it is expected to reach **111.7 kt by volume**.*

Efficient exploitation of biomass

- *it has been evaluated that production of chemicals and polymer resins from sugars and biomass result in two to four times more added value, create six to eight times more employment and require less percentage of feedstock compared to biofuel production.*

- ***Therefore, renewable carbon should be utilized for integrated production of fuels and chemicals.***

Integrated biorefineries with «cascade» transformations



Biomass platforms for lignocellulosic feedstocks for second generation biofuels

Options

- Agricultural crop residues
- Dedicated biomass crops
- Residues from forestry and the forest products industry
- Municipal solid wastes
- Food processing wastes