Second generation biofules: beyond the competition for soil and food

First generation biofuels: ethanol from starch & grains

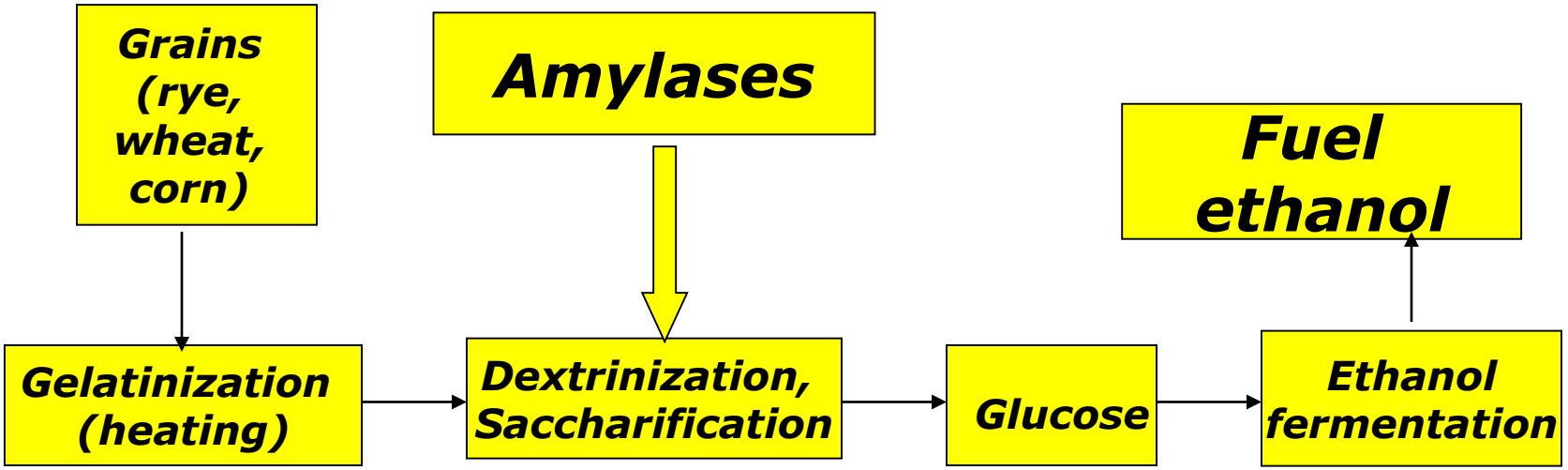


Table 17 Major ethanol producers and feedstocks utilised

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]

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

Lignocellulosic feedstocks composition

In general lignocellulosic feedstocks contain about 40% of the carbon bound as cellulose, 30% as lignin and 26% as hemicelluloses and other polysaccharides.

While cellulose is a uniform component of most types of cellulosic biomass, the proportions and composition of hemicelluloses and lignin differ between species.

...using a variety of sustainable (non-food) biomasses...

biochemtex

Non-food cellulosic crops

- ✓ Arundo donax (Giant reed)
- ✓ Miscanthus giganteus
- ✓ Panicum virgatum (Switchgrass)

Agricultural residues

- ✓ Wheat straw
- ✓ Rice straw
- ✓ Corn stover
- ✓ Sugarcane bagasse

Lignocellulosic crops

- ✓ Eucalyptus
- ✓ Poplar

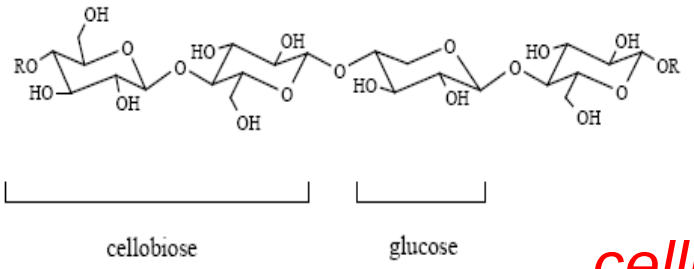




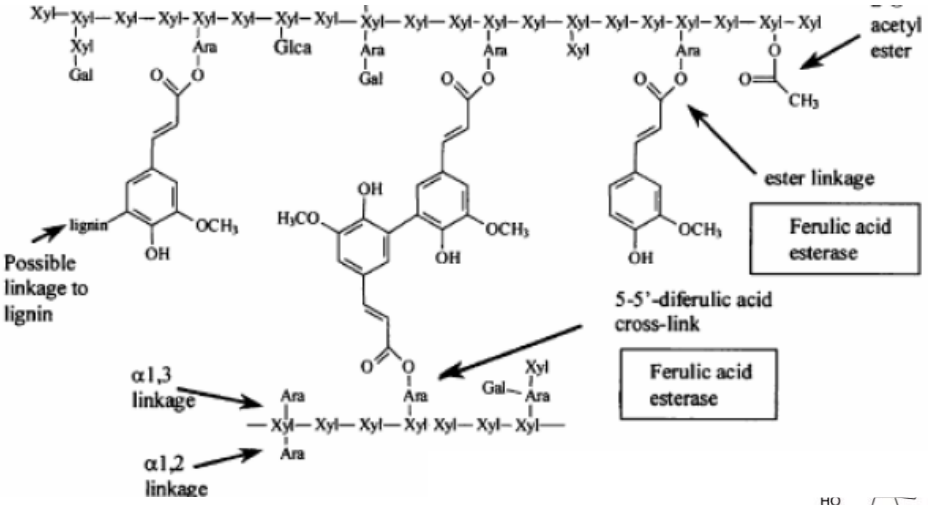


Might grow on marginal/abandoned land, creating additional income for farmers

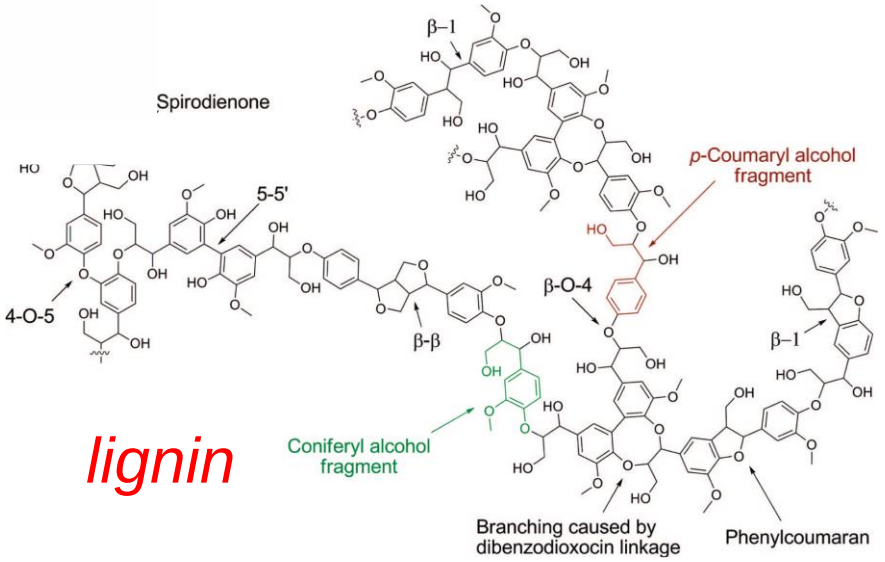
The chemistry of plant cell wall



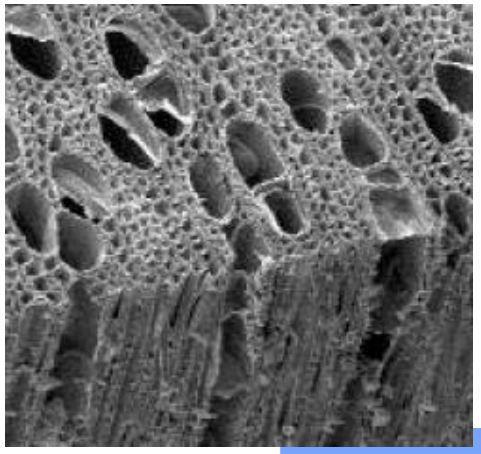
cellulose



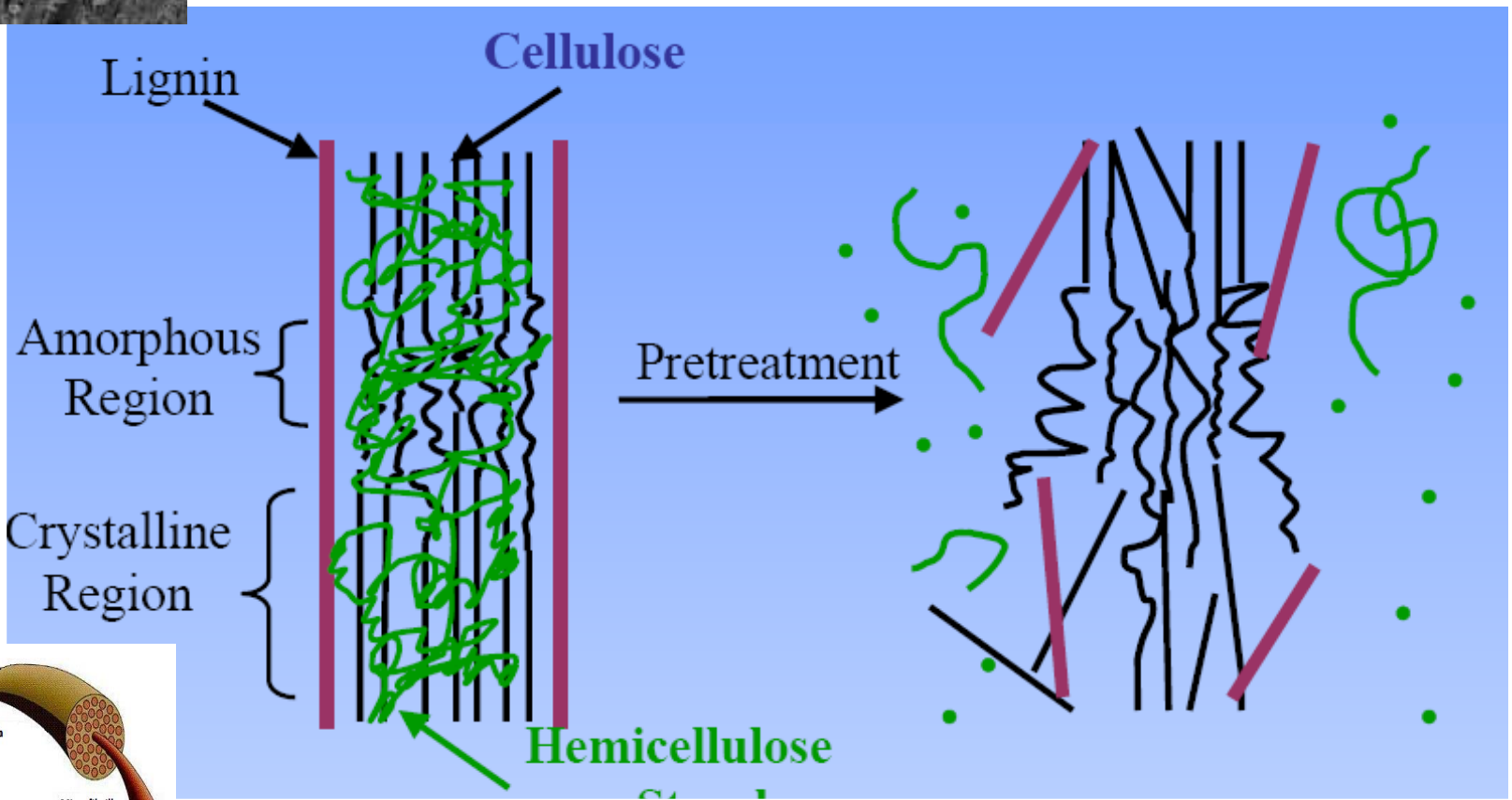
hemicellulose

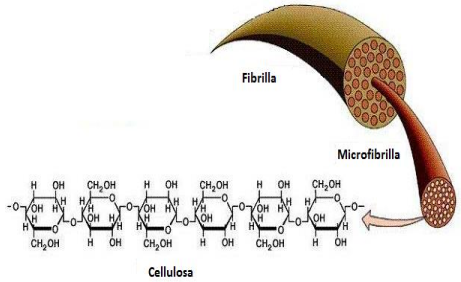


lignin



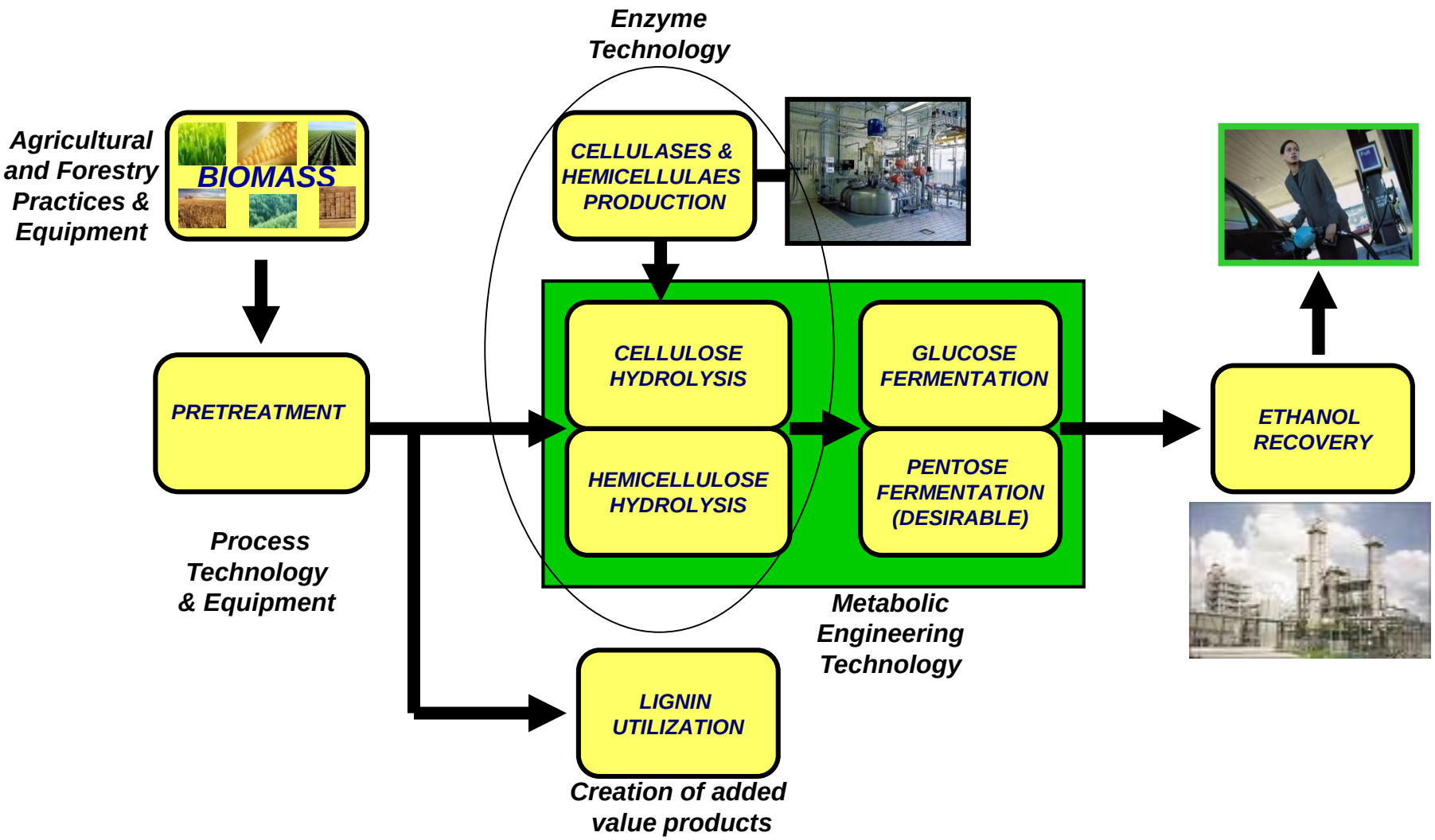
Pre-treatment of plant cell-wall: to allow complete enzymatic hydrolysis of polysaccharides





Fuel ethanol from lignocellulosic feedstocks: second generation bioethanol.

Beyond completion for food and land use

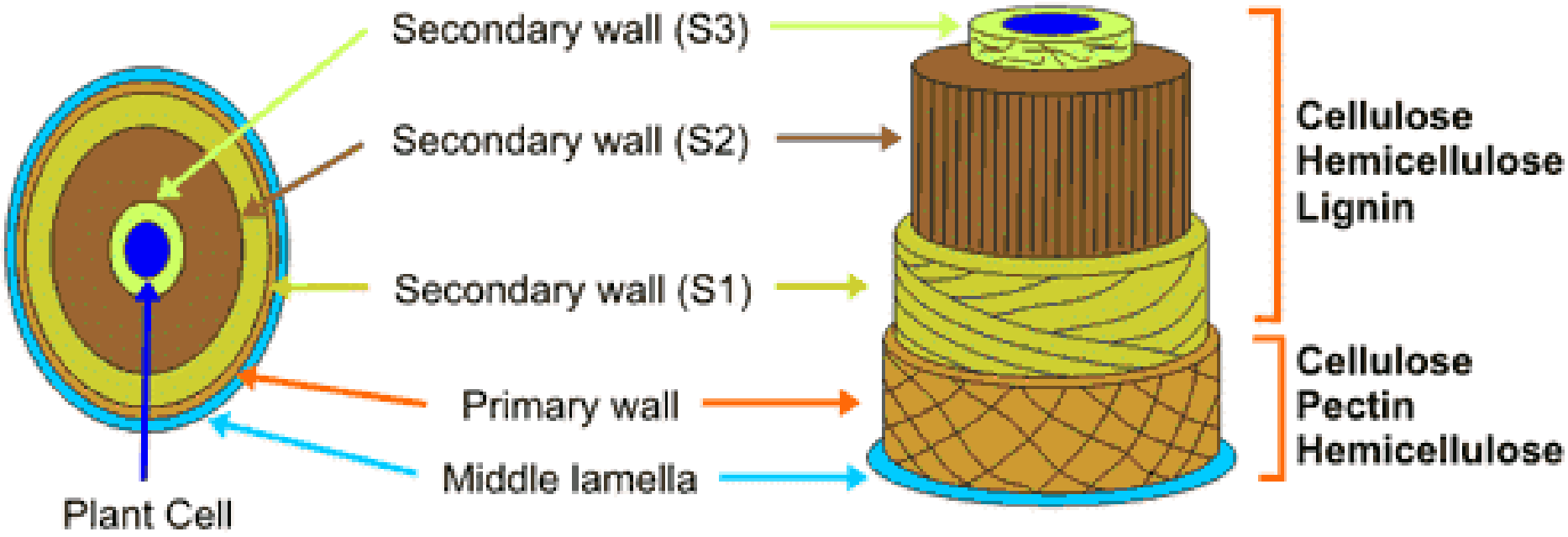


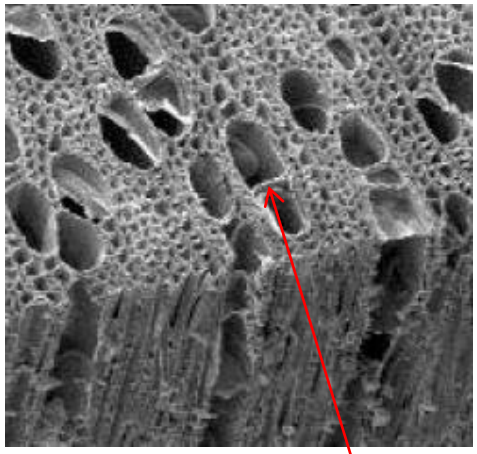
Plant cell wall

The cell walls contains layers of cellulose fibers interspersed within a hemicellulose packing. Adjacent cell walls are cemented together by ***pectins*** *in a layer called the* ***middle lamella****.*

The cell wall forms outside the plasma membrane initially as a thin ***primary cell wall.****.*

A more durable ***secondary cell wall*** *can form* ***between the primary cell wall and plasma membrane****.*





Tracheid
25 μm

Tracheids *are elongated cells of vascular plants that serve in the transport of water and mineral salts.*

Secondary cell wall

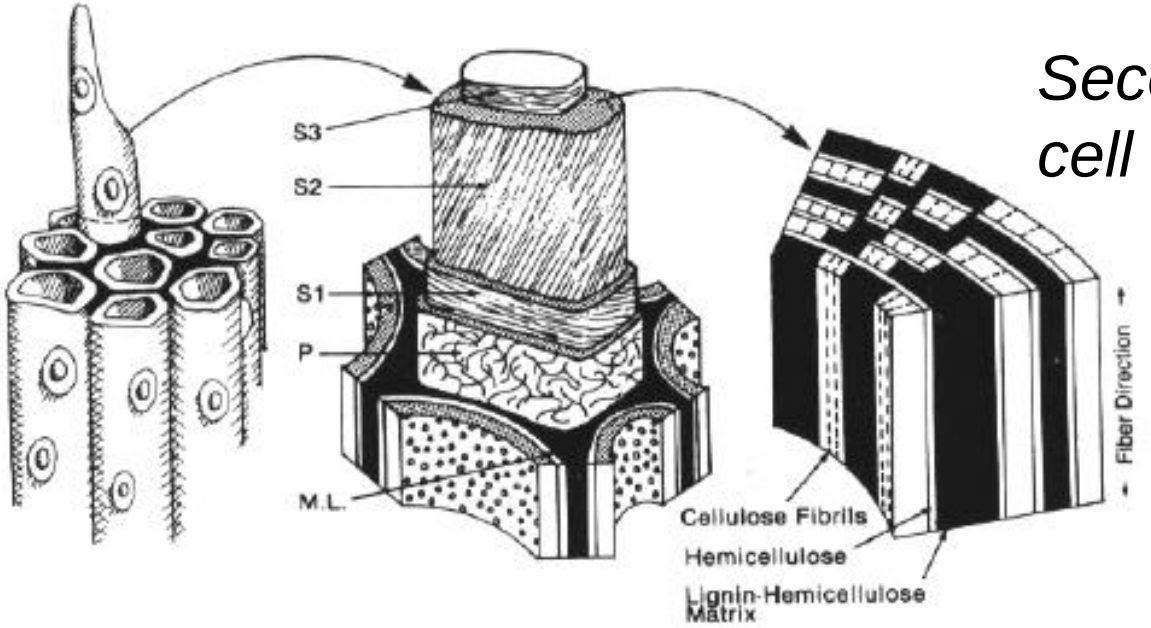
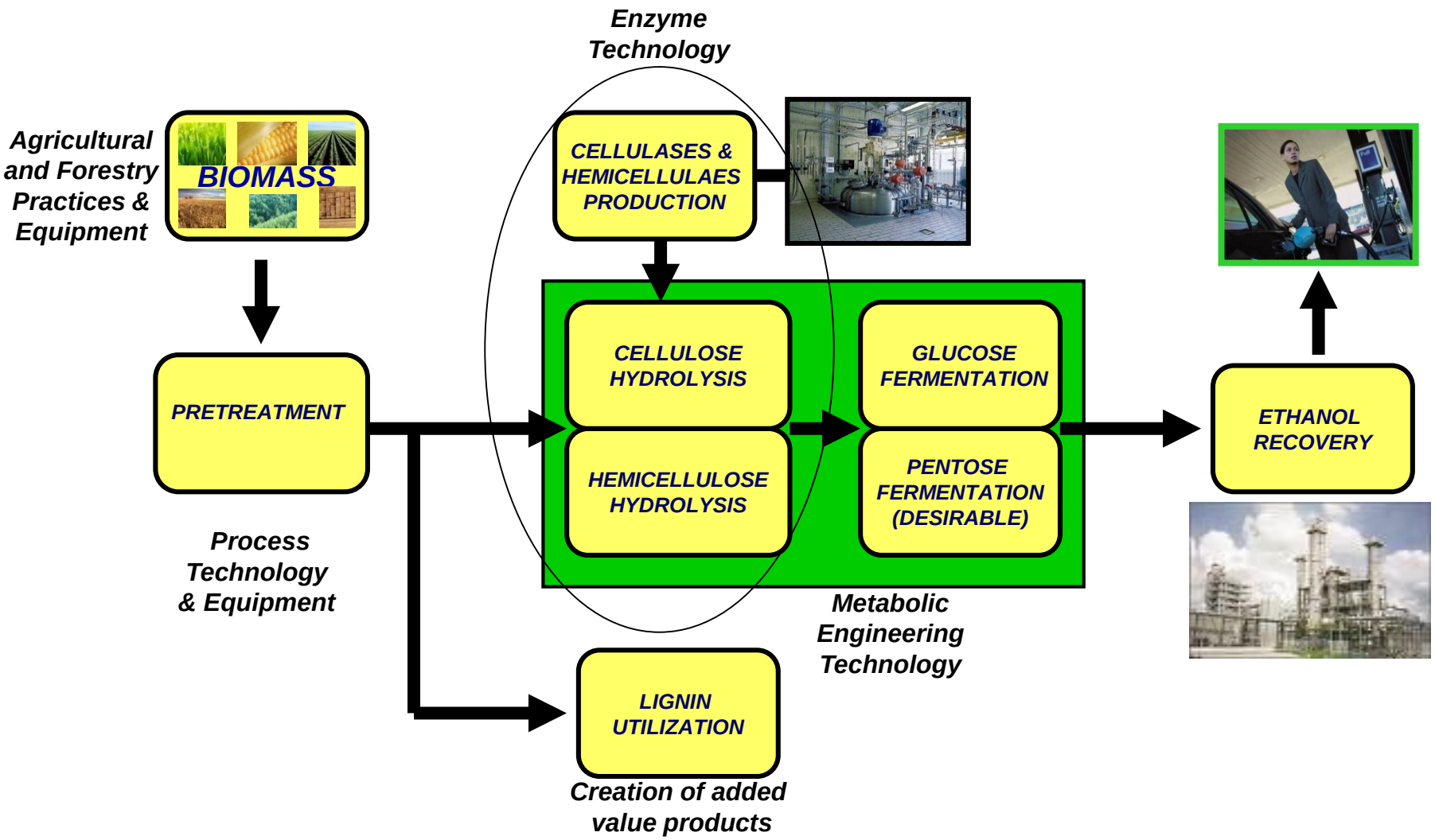


Fig. 2. Schematic illustration of the morphology of the tracheids, secondary wall layers and the relationship of the lignin, hemicelluloses, and cellulose in the secondary wall of a tracheid. Cell diameter is about 25µm. S1-S3, secondary cell wall layers; P, primary wall; M.L., middle lamella (Kirk & Cullen, 1998; partly from Goring, 1977).

Secondary cell wall *is a thicker additional layer of cellulose which increases wall rigidity. Additional layers may be formed by lignin in xylem cell walls, or suberin in cork cell walls. These compounds are rigid and waterproof, making the secondary wall stiff. Wood and bark cells of trees have secondary walls. Other parts of plants such as the leaf stalk may acquire similar reinforcement to resist the strain of physical forces.*

Fuel ethanol from lignocellulosic feedstocks: second generation bioethanol.

Beyond completion for food and land use



Pretreatment before enzymatic hydrolysis of cellulose

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Review

Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects

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Table 1
Overview of pretreatment methods for lignocellulosic feedstocks prior to enzymatic hydrolysis of cellulose.

Pretreatment methods	Main effect	Used chemicals	By-product formation
Acid-based methods	Hydrolysis of hemicelluloses to monosaccharides	Involve catalysts such as H_2SO_4, SO_2, HCl, H_3PO_4	Aliphatic carboxylic acids, phenylic compounds, furans, etc. (see Fig. 1)
Hydrothermal processing	Solubilization of hemicelluloses without complete hydrolysis	No additives	Acetic acid, minor amounts of furan aldehydes
Mild alkaline methods	Removal of lignin and a minor part of hemicelluloses	Involve alkali such as NaOH, $Ca(OH)_2$, NH_3	Acetic acid, hydroxy acids, dicarboxylic acids, phenolic compounds
Oxidative methods	Removal of lignin and part of hemicelluloses	Involve oxidants such as H_2O_2 and O_2 (alkaline conditions), and O_3	Aldonic and aldaric acids, furoic acid, phenolic acids, acetic acid
Chemical pulping processes	Methods that target lignin and to some extent hemicelluloses	Kraft pulping, sulfite pulping, soda pulping, organosolv pulping	Aliphatic acids
Alternative solvents	Dissolution of specific lignocellulosic components or the whole biomass	Ionic liquids	Dependent on solvent and conditions

Acid hydrolysis is one of the most promising pretreatment methods with respect to industrial implementation. It is usually performed with mineral acids, but organic acids and sulfur dioxide
are other options.

It results in high recovery of the hemicellulosic sugars in the pretreatment liquid, and in a solid cellulose fraction with enhanced enzymatic convertibility. Acid pretreatment has also some drawbacks, such as high cost of the materials used for construction of the reactors and
formation of inhibitory by-products.

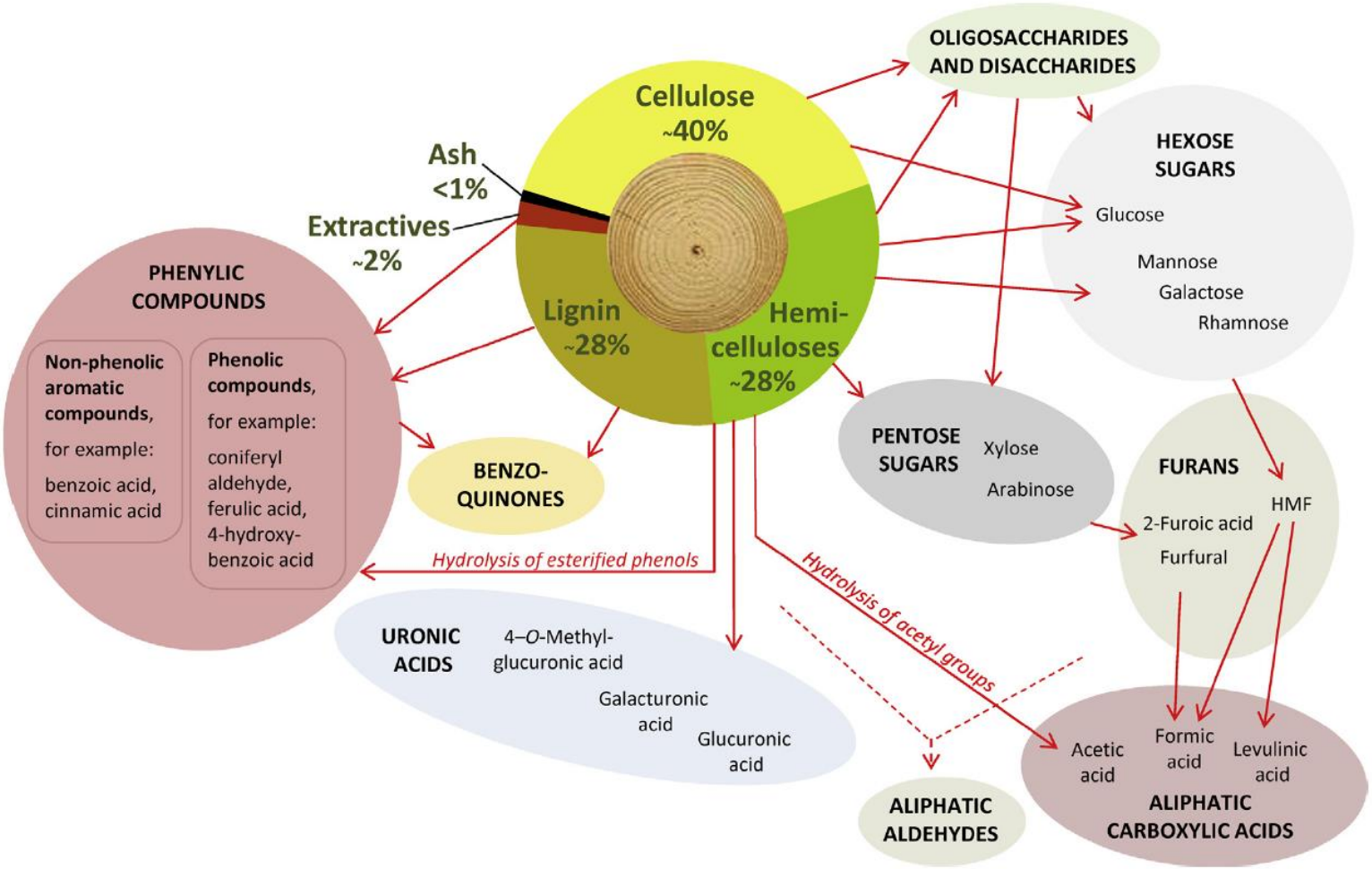


Fig. 1. Degradation products from lignocellulose as a result of pretreatment under acidic conditions. Numbers indicate fractions of constituents of wood of Norway spruce. Red arrows indicate tentative formation pathways. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Steam explosion is a successful pretreatment option that involves heating lignocellulose with superheated steam followed by a sudden decompression.

The high-pressure steam modifies the cell wall structure, yielding a slurry, which upon filtration renders a filtrate with hemicellulosic sugars and a cellulose-rich filter cake containing also lignin and residual hemicellulose.

Steam explosion can be assisted by impregnation with an acid catalyst, for instance sulfuric acid or sulfur dioxide. If no impregnating agent is used, the process is catalyzed through autohydrolysis.

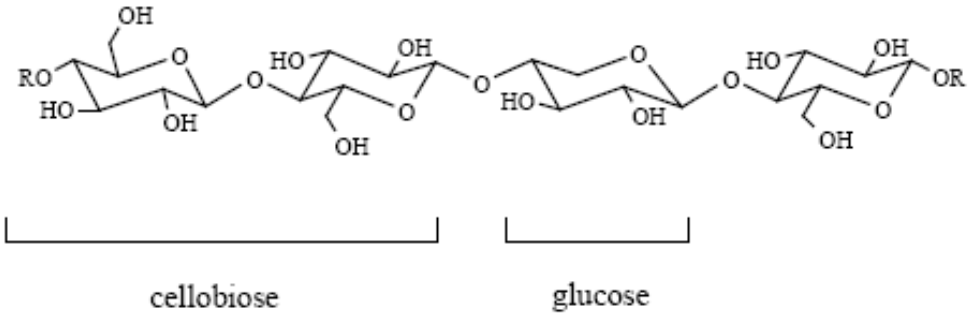
Acetic acid and uronic acids released from hemicellulose, and formic and levulinic acids resulting from sugar degradation contribute to acidification, and can inhibit downstream biochemical processes.

Cellulases: Cellulose enzymatic degradation

The polysaccharide consists of D-glucose residues linked by ß-1,4-glycosidic bonds to form linear polymeric chains of over 10 000 glucose residues.

The individual chains adhere to each other along their lengths by hydrogen bonding and van der Waals forces, and crystallise shortly after biosynthesis.

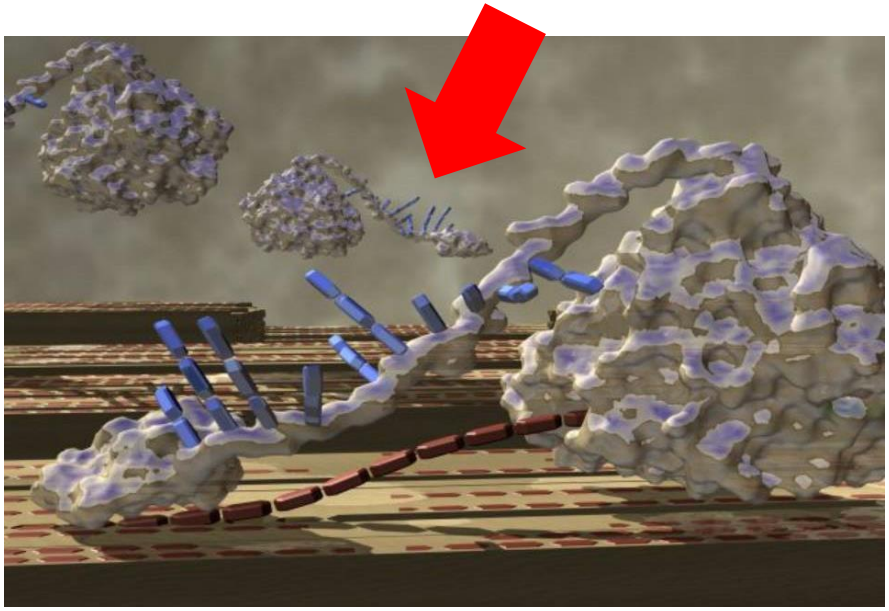
Multiple enzyme systems are required to efficiently degrade cellulose.

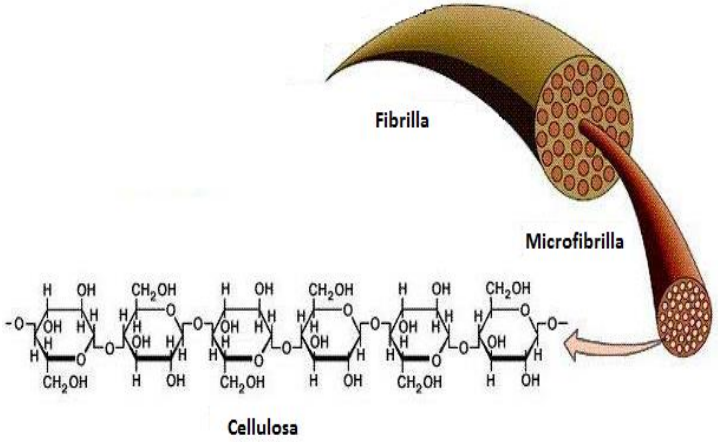


cellulose

Mechanism of cellulose hydrolysis

All cellulolytic enzymes share the same chemical specificity for ß-1,4-glycosidic bonds, which they cleave by a general acid-catalysed hydrolysis. A common feature of most cellulases in different fungal genera is a domain structure with a catalytic domain linked with an extended linker region to a cellulose-binding domain

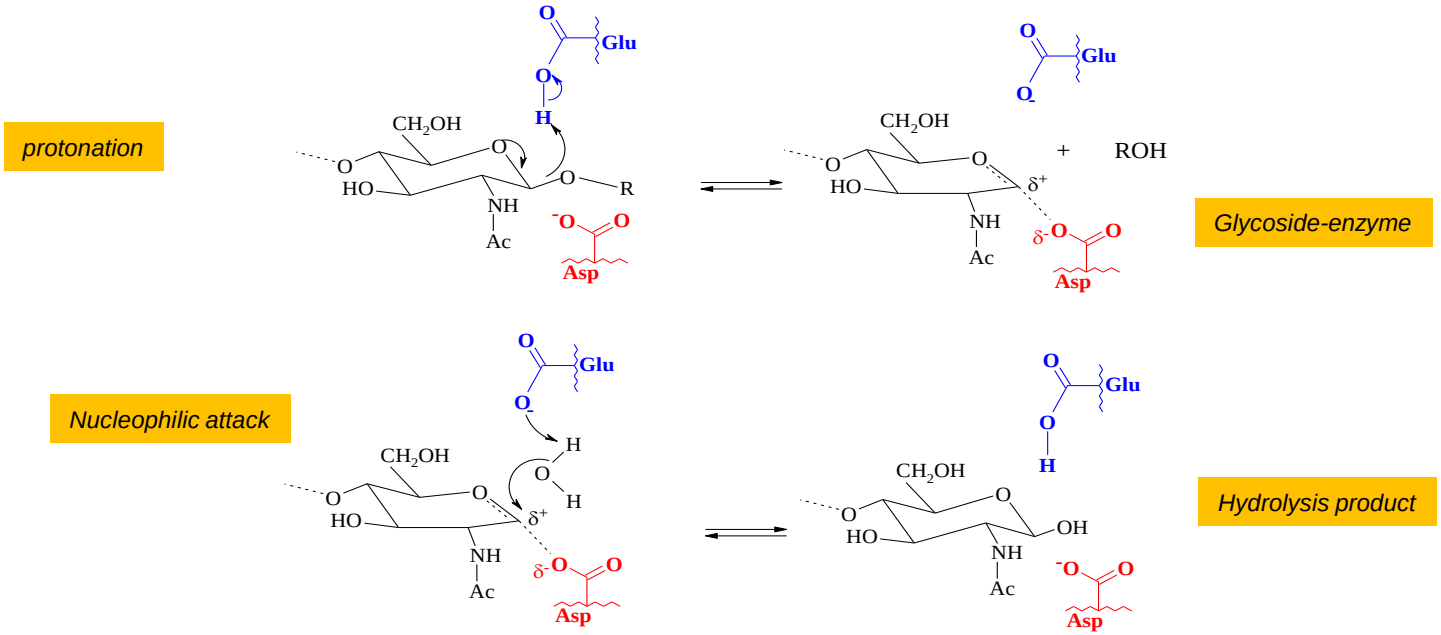


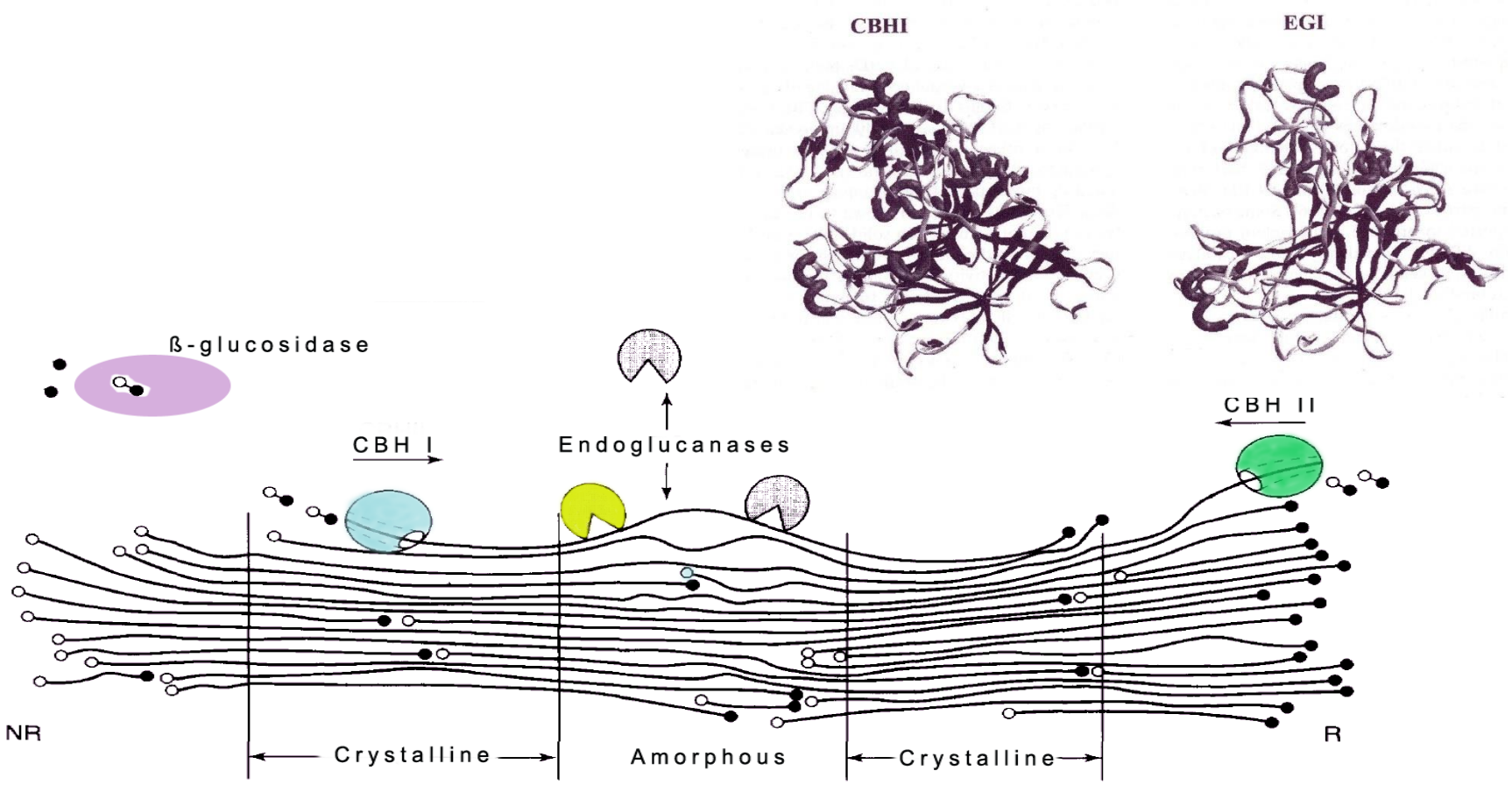


Ref: Himmel, M. et al, NREL (2000)

Mechanism of Glycosidases (hydrolases)

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.





The complementary activities of endo- and exotype enzymes lead to synergy. Endoglucanases (EGs, E.C. 3.2.1.4) attack cellulose microfibrils preferentially in the amorphic parts of the fibril . The catalytic region of the enzyme is groove-shaped that enables the attachment of the enzyme and the hydrolysis in the middle part of the cellulose fibre.

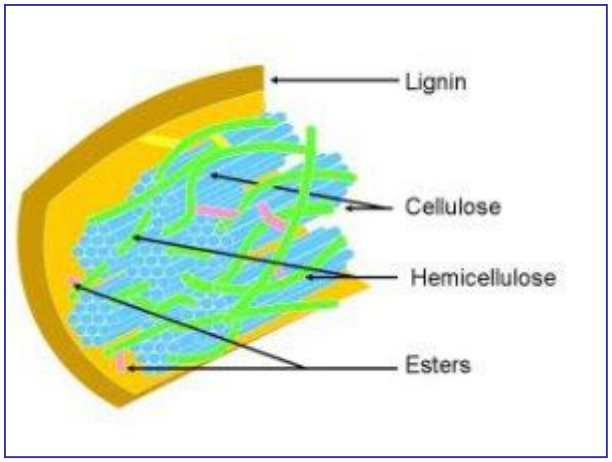
Cellobiohydrolases (CBHs, E.C. 3.2.1.91) are exo-type enzymes that attack cellulose fibres from both reducing and non-reducing ends. The product of CBH action, cellobiose is hydrolysed by ß-glucosidases (E.C. 3.2.1.21) to two glucose units.

Hemicellulose

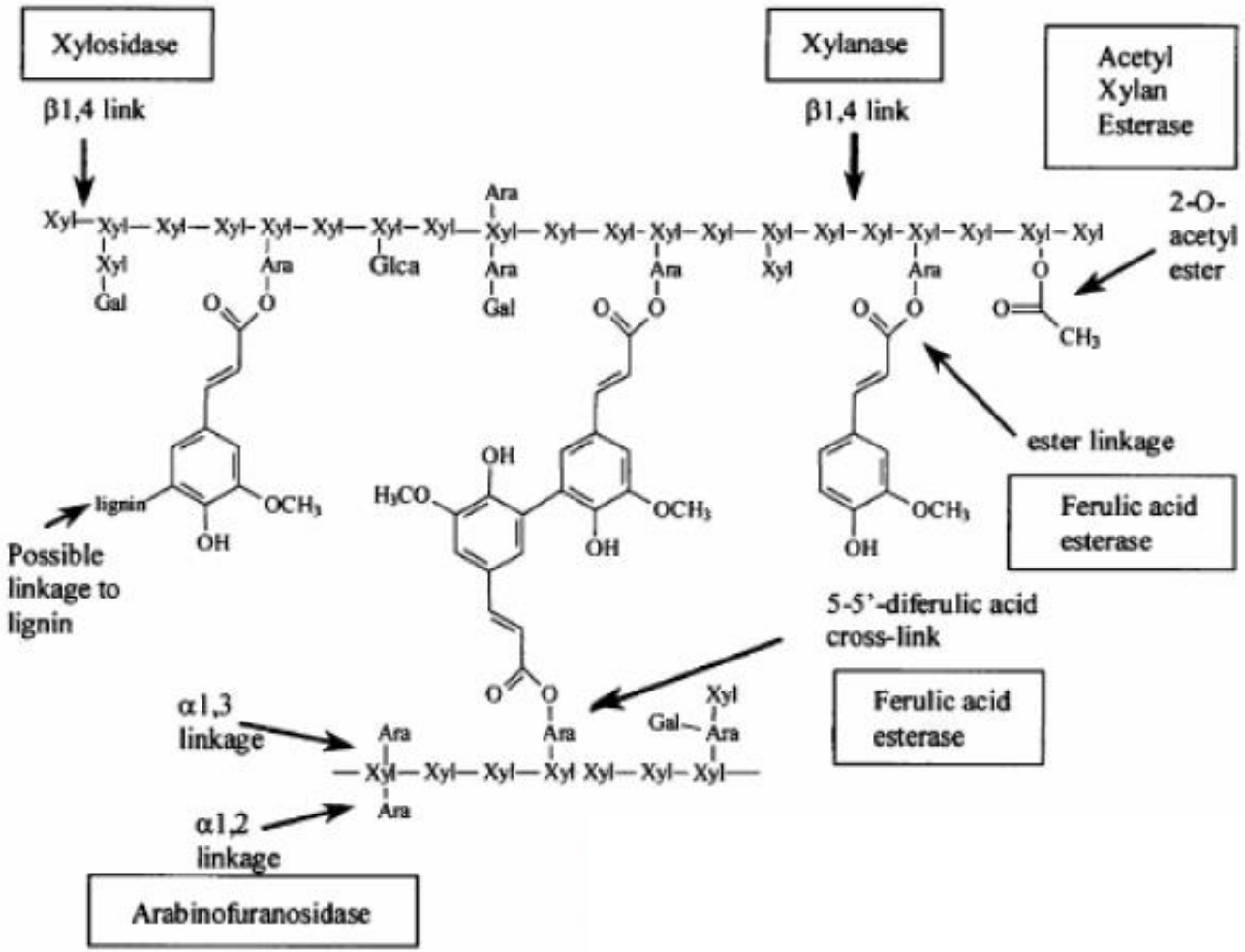
Hemicellulose consists of several different sugar units and substituted side chains in the form of a low molecular weight linear or branched polymer.

This polymer is more soluble than cellulose with a DP (degree of polimerzation) of less than 200.

Hemicellulose can be hydrolyzed by weak acid: it is not crystallin but rather a gel.



***Branched* polymers contain *neutral and/or acidic side groups*. These groups render hemicelluloses noncrystalline or poorly crystalline, so that they exist more like a gel than as oriented fibres.**

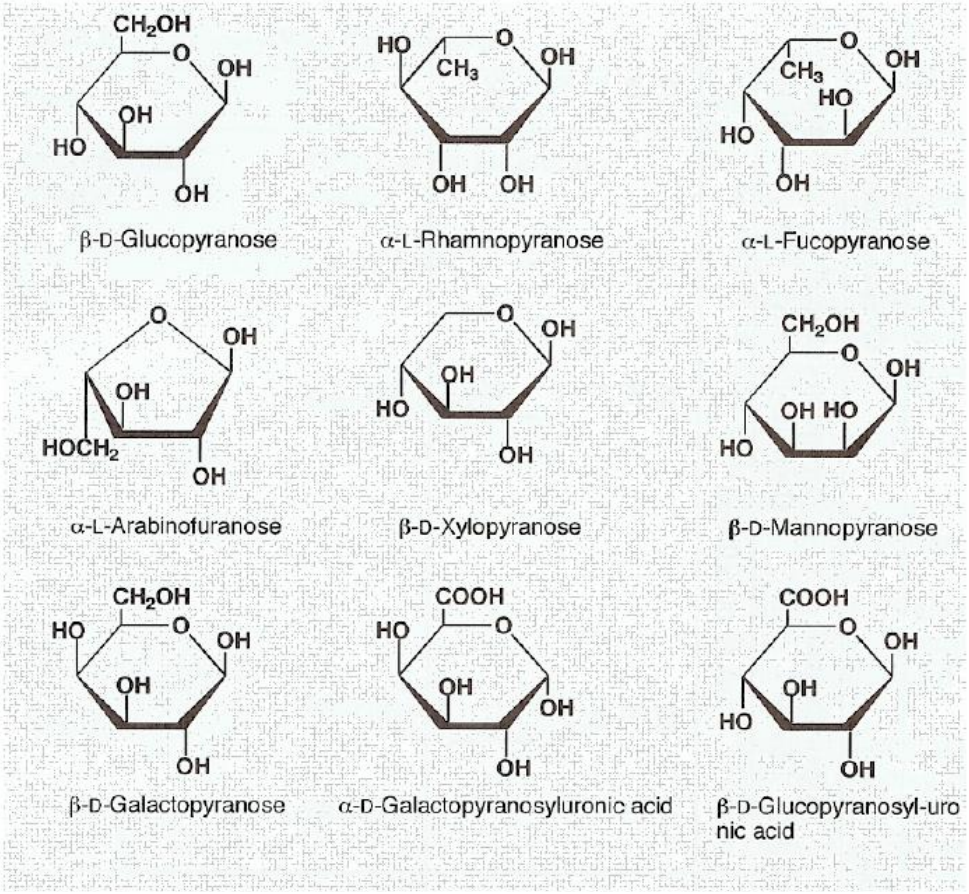


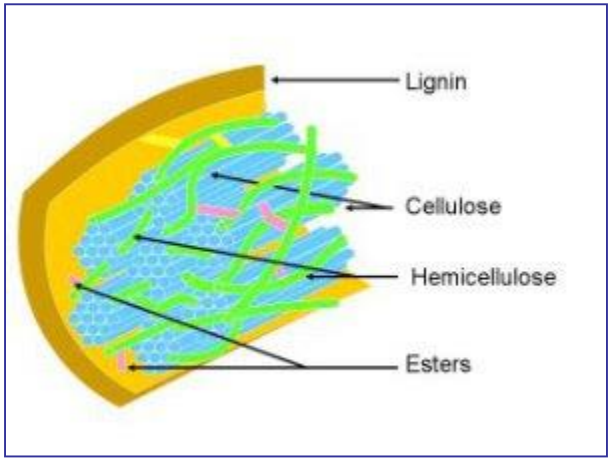
Hemicelluloses form a matrix together with pectins and proteins in primary plant cell walls and with lignin in secondary cell walls.

Covalent hemicellulose-lignin bonds involving ester or ether linkages form lignin-carbohydrate-complexes (LCCs)

Hemicellulose

Hemicelluloses include xylan, glucuronoxylan, arabinoxylan, glucomannan, xyloglucan





Cellulose *vs.* Hemicellulose

	Cellulose	Hemicellulose
Monomer	Pure glucose	Mixed sugars
Polymer chain length	Long (5μm)	Short
M.W.	High (10000 units)	Low (hundred units)
Polymer topology	Linear	Branched
Side groups substitution	No substitution	On C_2, C_3, and C_6
Polymer morphology	Crystalline + amorphous	Amorphous
Solubility	Low	High
Reactivity	Less reactive	More reactive
Hydrolysis	Partial	Readily (susceptible)

TABLE 3-5. The Major Hemicellulose Components

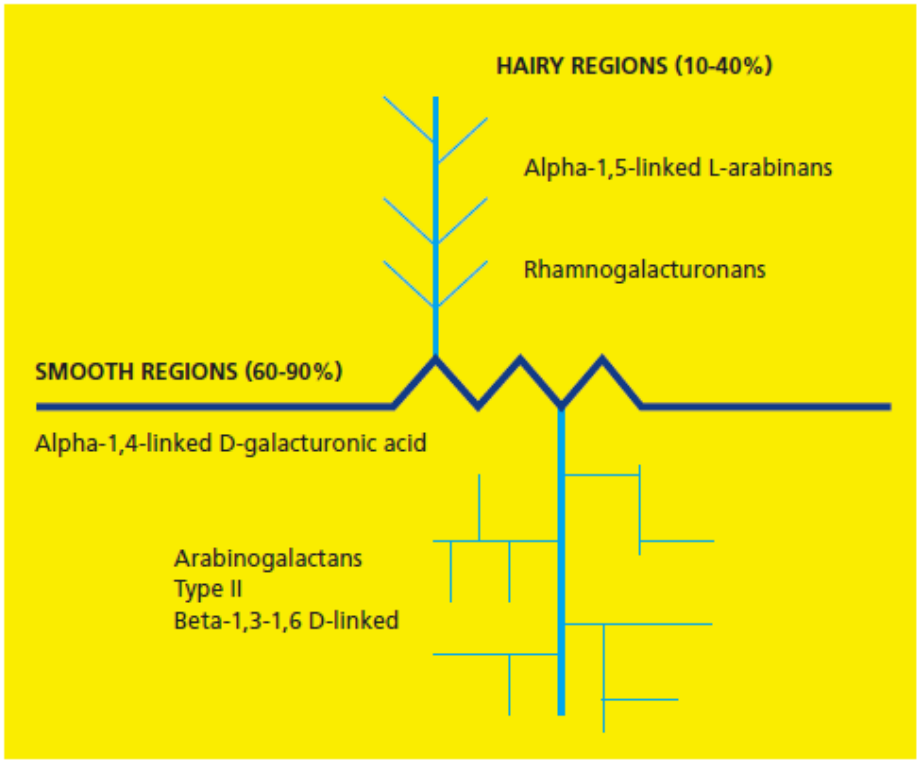
Hemicellulose type	Occurrence	Amount (% of wood)	Composition			Solubility[a]	$\overline{DP}_n$
			Units	Molar ratios	Linkage		
Galactoglucomannan	Softwood	5-8	β-D-Manp	3	1 → 4	Alkali, water*	100
			β-D-Glcp	1	1 → 4		
			α-D-Galp	1	1 → 6		
			Acetyl	1			
(Galacto)glucomannan	Softwood	10-15	β-D-Manp	4	1 → 4	Alkaline borate	100
			β-D-Glcp	1	1 → 4		
			α-D-Galp	0.1	1 → 6		
			Acetyl	1			
Arabinoglucuronoxylan	Softwood	7-10	β-D-Xylp	10	1 → 4	Alkali,	100
			4-O-Me-α-D-GlcpA	2	1 → 2	dimethylsulfoxide*,	
			α-L-Araf	1.3	1 → 3	water*	
Arabinogalactan	Larch wood	5-35	β-D-Galp	6	1 → 3, 1 → 6	Water	200
			α-L-Araf	2/3	1 → 6		
			β-L-Arap	1/3	1 → 3		
			β-D-GlcpA	Little	1 → 6		
Glucuronoxylan	Hardwood	15-30	β-D-Xylp	10	1 → 4	Alkali,	200
			4-O-Me-α-D-GlcpA	1	1 → 2	dimethylsulfoxide*	
			Acetyl	7			
Glucomannan	Hardwood	2-5	β-D-Manp	1-2	1 → 4	Alkaline borate	200
			β-D-Glcp	1	1 → 4		

[a] The asterisk represents a partial solubility.

Hardwood: from dicot angiosperm
Softwood: from gymnosperms (e.g. conifers)

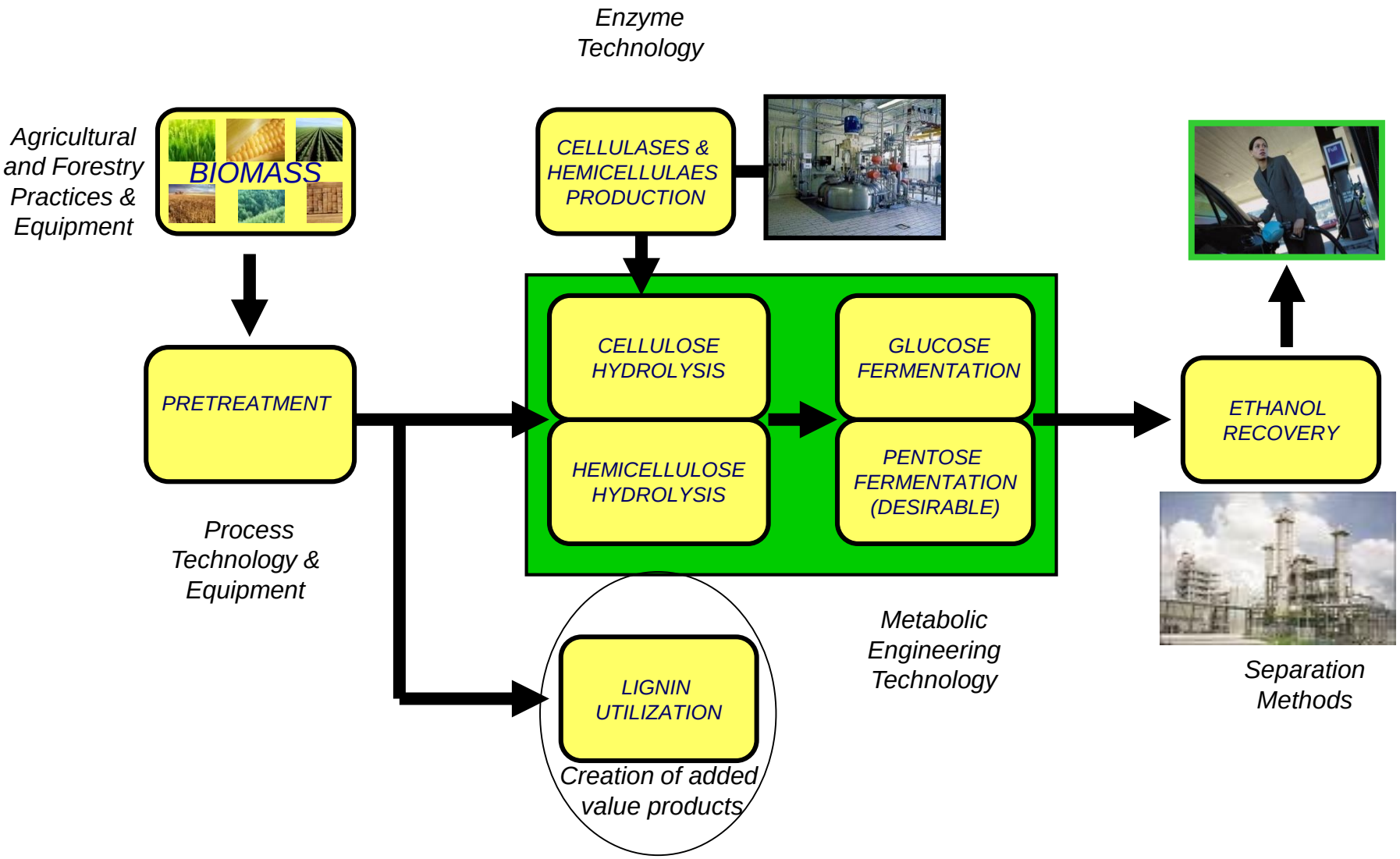
Pectin

- Group of amorphous polymers.
- rich in **galacturonic acid**
- They exist in nature both in a **methylesterified** and in a free **acidic** form.
- Polymers also contain neutral sugars, notably D-galactose, L-arabinose, L-rhamnose, D-xylose,



The hairy regions (shown as branches), are more difficult to break down

Lignin from biorefineries



Use of lignin

The processing of 140 million tons cellulose and pulp in paper production lead to 50 million tons lignin

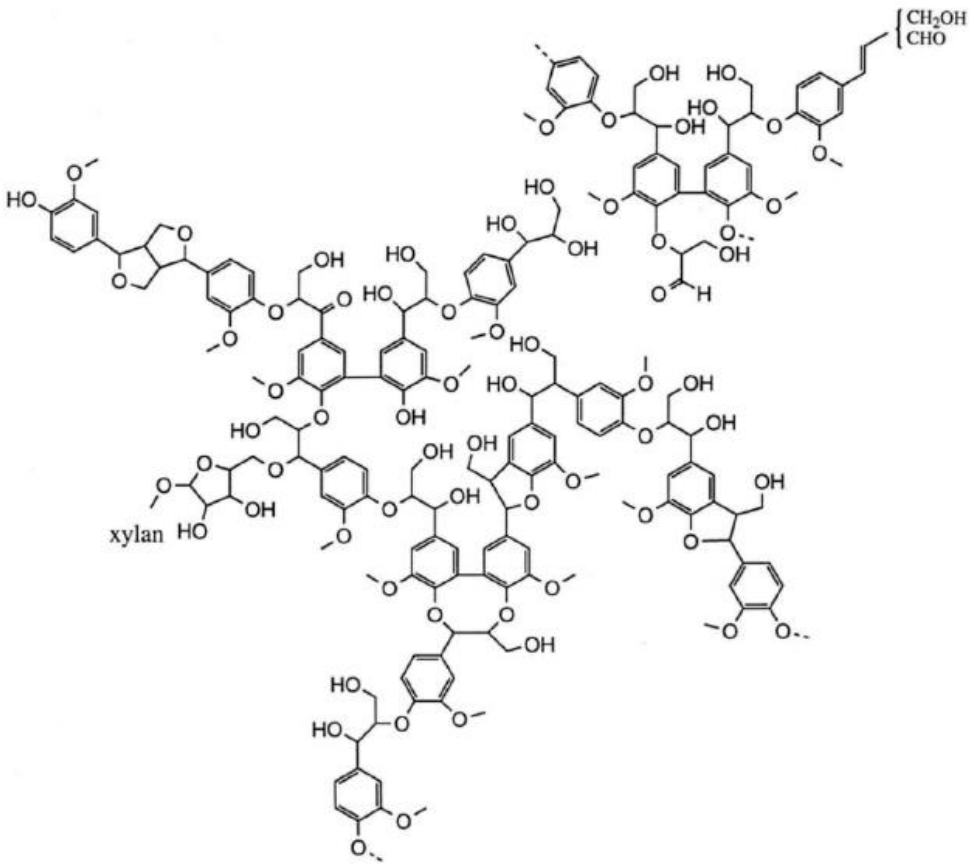
About 95% is burned
Only 5% reutilized

Opportunities that arise from utilizing lignin fit into three categories:

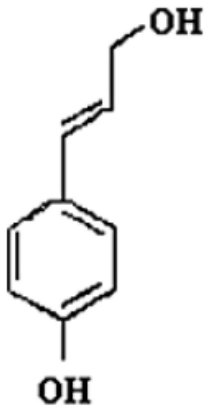
- *power, fuel (near-term)*
- *macromolecules (medium-term; <10y)*
- *aromatics and miscellaneous monomers (long-term; >10y)*

Lignin

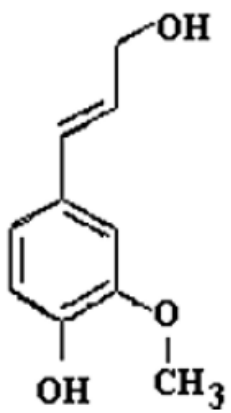
*Lignin is a **branched polymer** of **substituted phenylpropane units** joined by carbon-carbon and ether linkages. Biosynthesis of lignin formation proceeds via **polymerisation of the free radical forms of precursors**.*



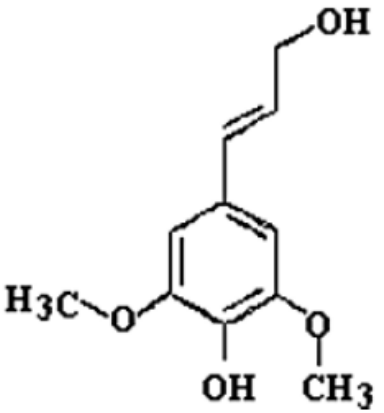
Lignin precursors



p-coumaryl alcohol

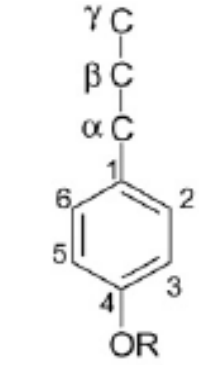


Coniferyl alcohol

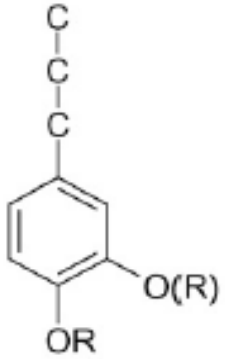


Synapil alcohol

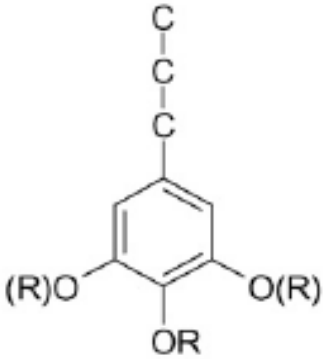
In the final polymer they form:



p-Hydroxyphenyl



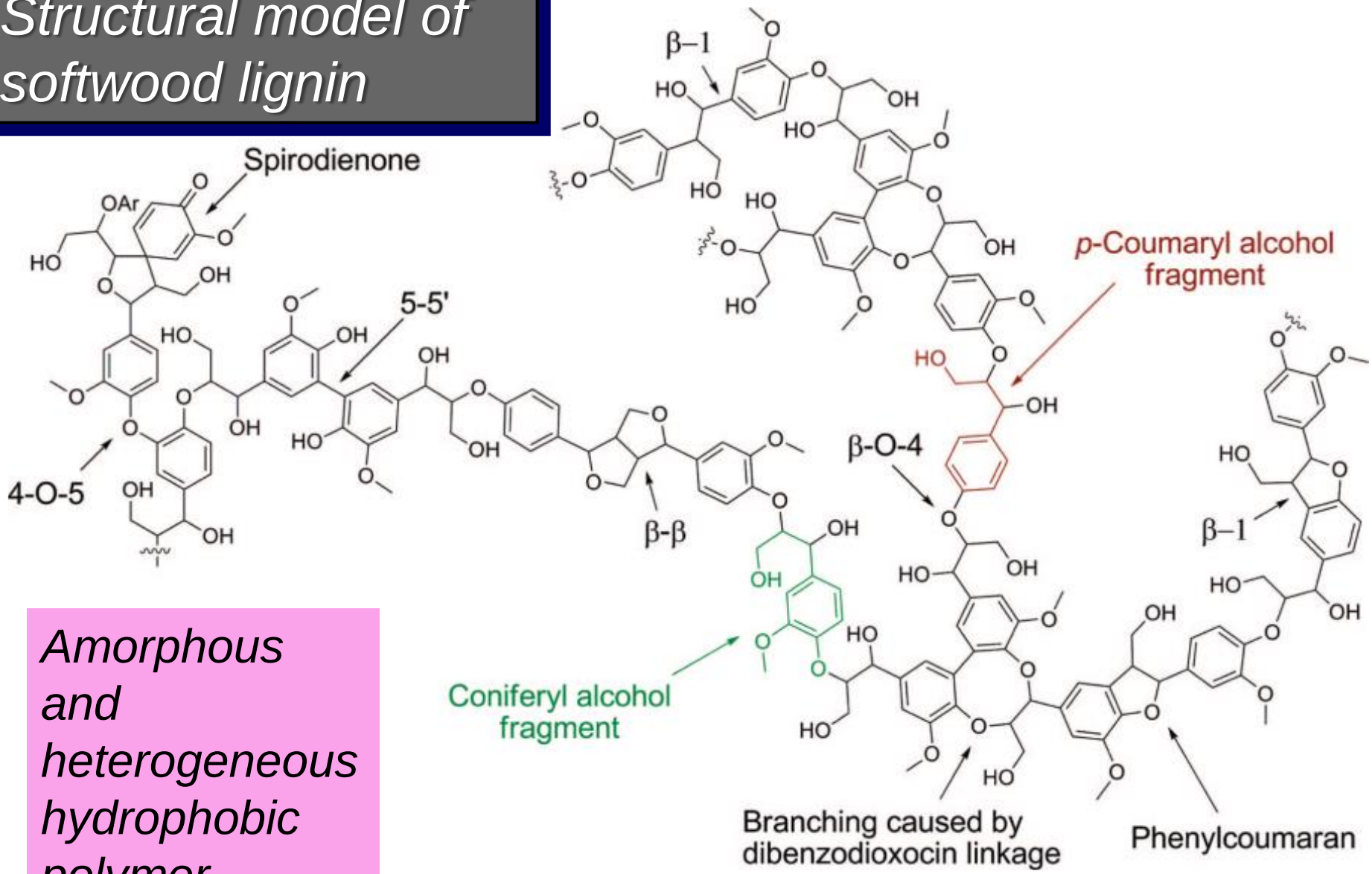
Guaiacyl



Syringyl

R=H,alkyl,aryl

Structural model of softwood lignin



Amorphous and heterogeneous hydrophobic polymer

G. Brunow, "Oxidative coupling of phenols and the biosynthesis of lignin", In: Lewis N.G. and Sarkanen S. Ed, "Lignin and lignan biosynthesis", 1998 American Chemical Society, Washington, DC, p.131.

Enzymes for lignin degradation: laccases (oxidative enzymes)

*Since **white-rot fungi** are the only organisms capable of efficient lignin degradation, their ligninolytic enzyme system has been studied extensively.*

Lignin polymer structure is irregular, which means that the degradative enzymes must show lower substrate specificity compared to the hydrolytic enzymes in cellulose or hemicellulose degradation.

Enzymes for lignin degradation

Plant laccases and peroxidases catalyse the generation of radical formations.

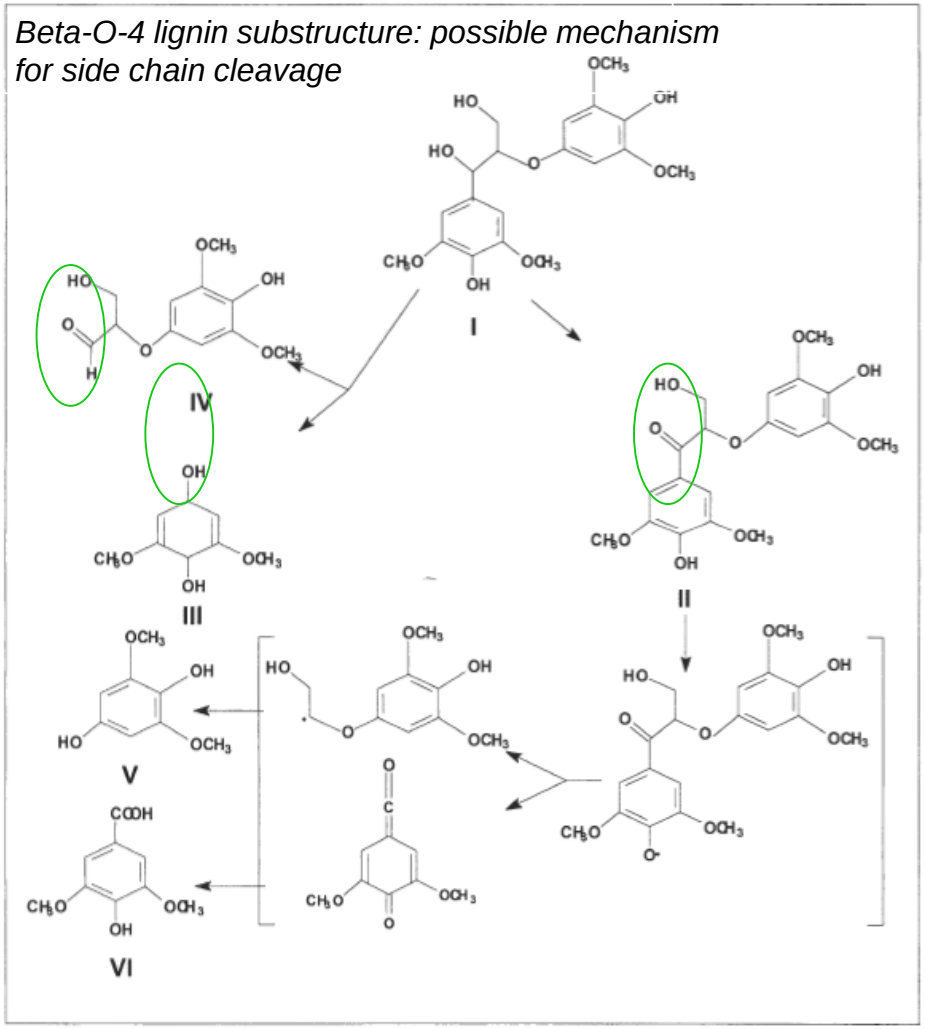
*Since **white-rot fungi** are the only degradation, their ligninolytic enzyme*

Lignin polymer structure is irregular, enzymes must show lower substrat enzymes in cellulose or hemicellulo

Because lignin consists of interunit enzymes must be oxidative rather

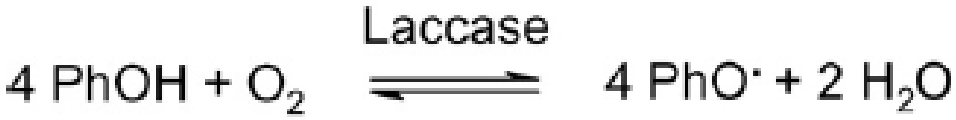
The major consequence of enzymati lignin-related phenols is oxidative c

Beta-O-4 lignin substructure: possible mechanism for side chain cleavage



LACCASES

Benzenediol oxygen oxidoreductases, EC 1.10.3,2: glycoproteins members of the "blue" multi-Cu oxidase family.

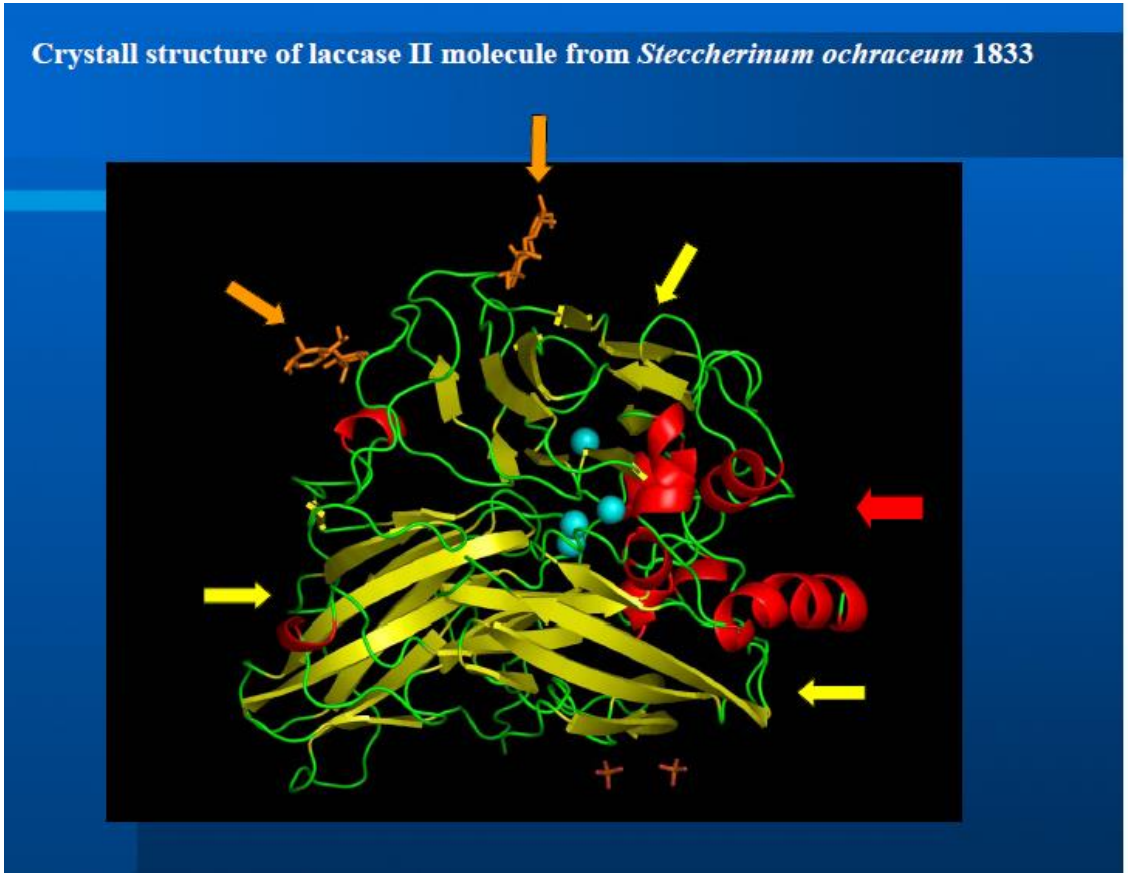


Laccases oxidize aromatic compounds, in particular PHENOLS and AMINES, giving reactive radicals.

"Laccases: blue enzymes for green chemistry" S. Riva Trends Biotechnol., 24, 219-226 (2006)

Laccases: limitations

1. Laccase is a large and glycosilated molecule (MW 70,000) which cannot penetrate deep into wood

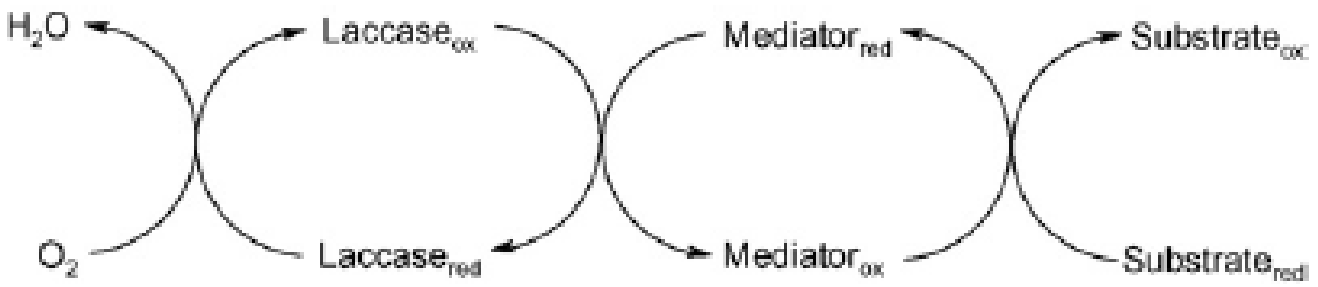


2. Has a rather low-redox potential (~0.5–0.8 V): it is unable to oxidize nonphenolic (C4-etherified) lignin units, which have a high-redox potential (>1.5 V)

3. Because of these limitations, laccase alone can only oxidize phenolic lignin units (<20% of all lignin units in native wood) at the substrate surface.

Ludmila Golovleva, G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russia

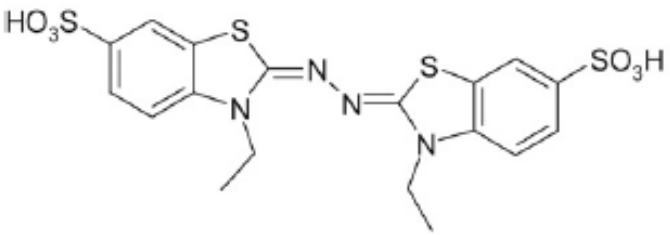
LACCASES and CHEMICAL MEDIATORS



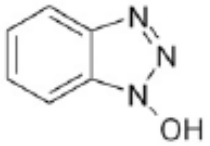
- *Therefore, laccase is often applied with an **oxidation mediator**, a small molecule able to extend the effect of laccase to nonphenolic lignin units and to overcome the accessibility problem.*

- *In these so-called LMS, the mediator is **first oxidized by laccase** and then **diffuses** into the cell wall, oxidizing lignin inaccessible to laccase*

Mediators: examples



A



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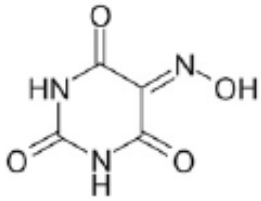

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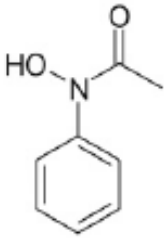
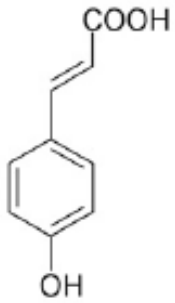
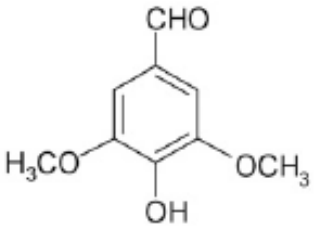


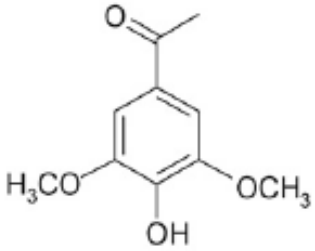
D



E



F



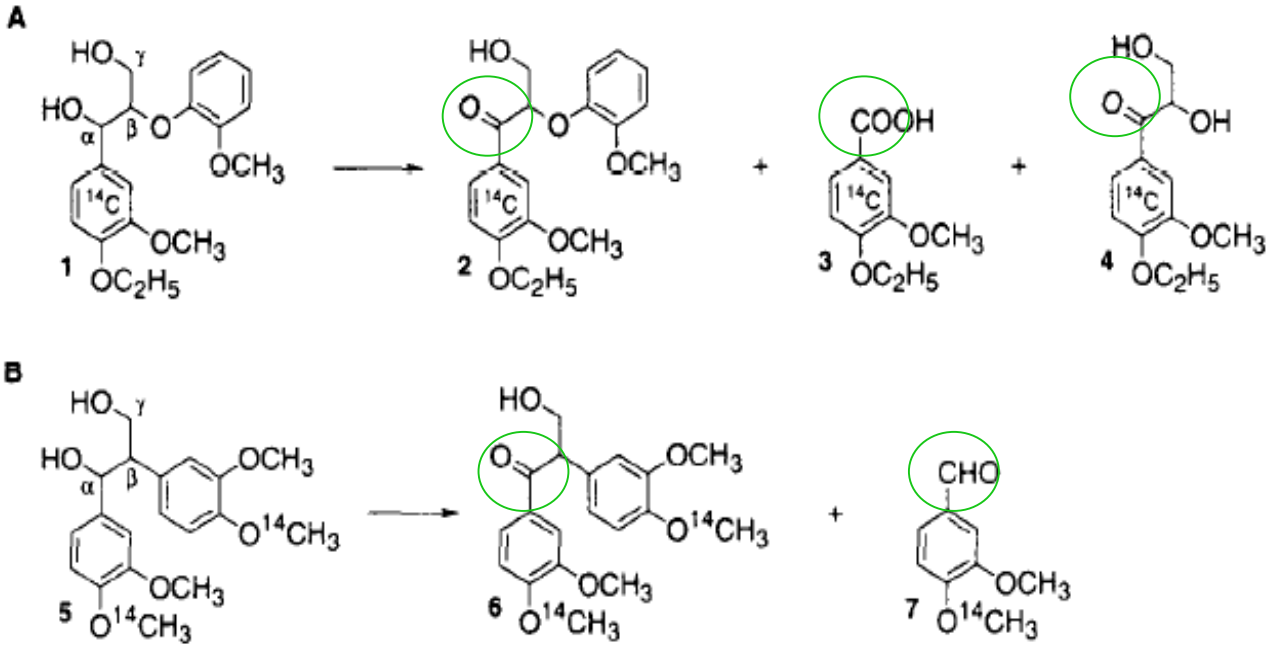
G

p-coumaric acid → *Natural mediators*

syringaldehyde → *Natural mediators*

acetosyringone → *Natural mediators*

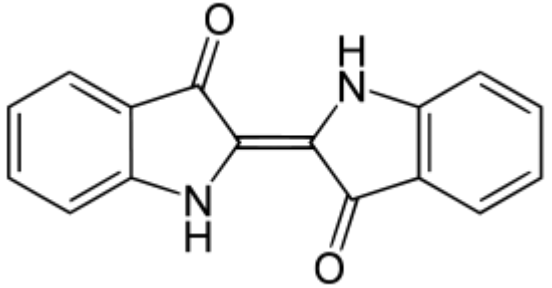
Oxidation of non-phenolic lignin models in the presence of mediators

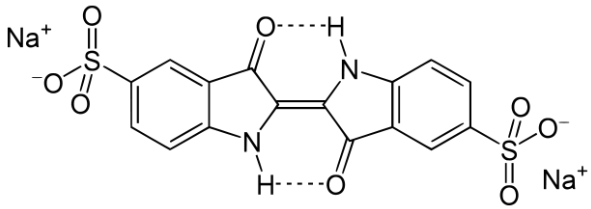


Journal of Biotechnology 81 (2000) 179-188

Bio-bleaching:
Hypochlorite damages fabrics and pollutes

***Indigo dye**: hystorically extracted from the leaves of certain plants, and this process was important economically because blue dyes were once rare. A large percentage of indigo dye produced today – several thousand tons each year – is synthetic. It is the blue often associated with denim cloth and blue jeans.*





***Indigo carmine**, or **5,5'-indigodisulfonic acid sodium salt**, derived from indigo by sulfonation, which renders the compound soluble in water. It is approved for use as a food colorant in the U.S and E.U., It has the E number **E132**. It is also a pH indicator.*

blue at pH 11.4 and yellow at 13.0

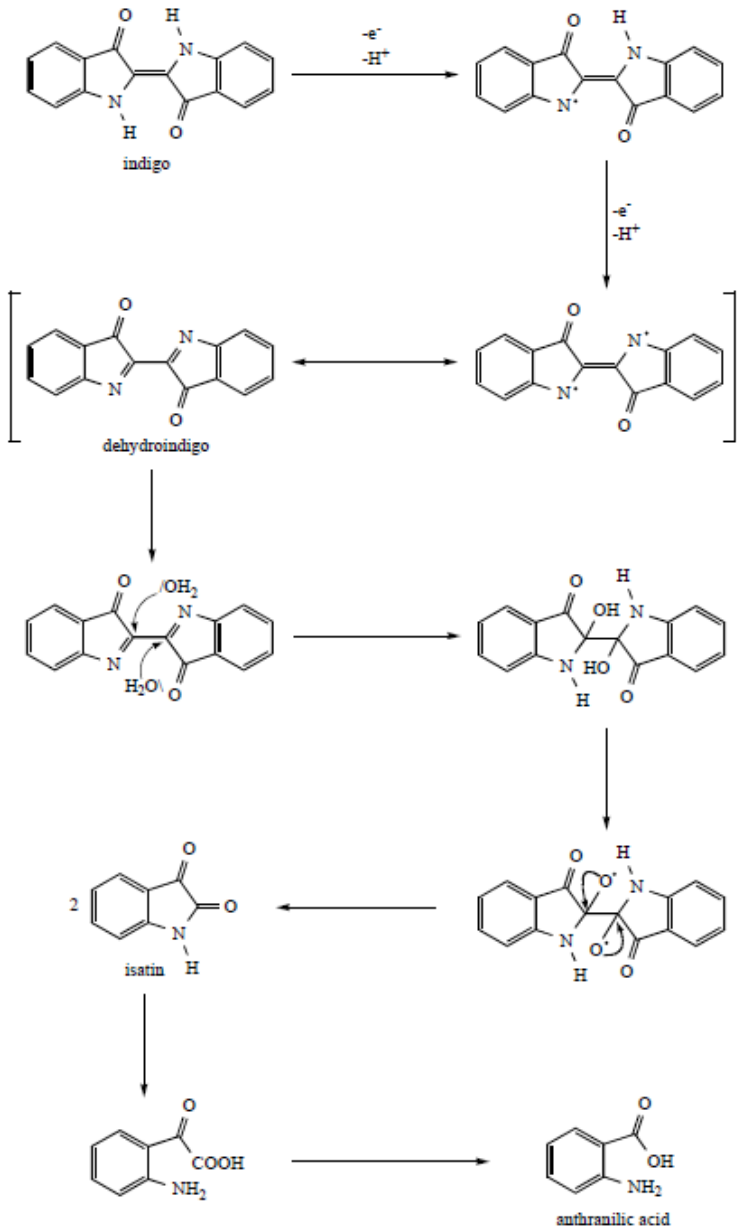


Fig. (4). A possible mechanism for laccase-catalysed degradation of indigo dye (Figure re-printed from Campos *et al.*, (2001) Journal of Biotechnology 89, 131-139 [19], *with kind permission of Elsevier Ltd.*).

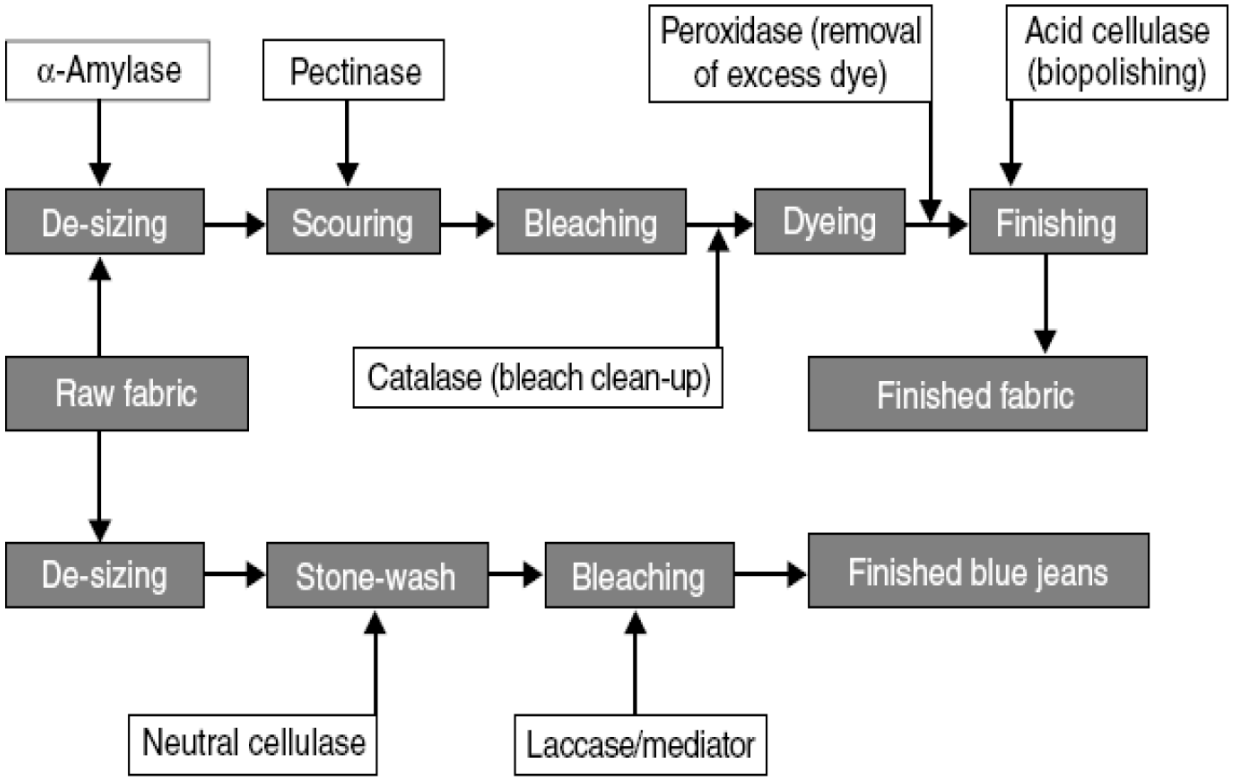


Fig. (5). Enzymes used in various unit operations in textile wet processing and the manufacturing of denim (Figure re-printed from Kirk *et al.,* (2002) Current Opinion in Biotechnology **13**, 345-351 [24], *with kind permission of Elsevier Ltd.*).

Enzyme Microb Technol. 2011 Jun 10;49(1):100-4. doi: 10.1016/j.enzmictec.2011.03.005. Epub 2011 Mar 26.

Decolorization of indigo carmine by laccase displayed on Bacillus subtilis spores.

Cho EA[1], Seo J, Lee DW, Pan JG.

Author information

Abstract

Blue multicopper oxidases, laccases displayed on the surface of Bacillus spores were used to decolorize a widely used textile dyestuff, indigo carmine. The laccase-encoding gene of Bacillus subtilis, cotA, was cloned and expressed in B. subtilis DB104, and the expressed enzyme was spontaneously localized on Bacillus spores. B. subtilis spores expressing laccase exhibited maximal activity for the oxidation of 2,2'-azino-bis (3-ethylthiazoline-6-sulfonate) (ABTS) at pH 4.0 and 80°C, and for the decolorization of ***indigo carmine at pH 8.0 and 60°C****. These results suggest that laccase displayed on B. subtilis spores can serve as a powerful environmental tool for the treatment of textile dye effluent.*

Italian second generation bioethanol biorefineries

MOSSI GHISOLFI GROUP

Beta Renewables has invested over $200 million in the development of the PROESA™ process. The company has built the world's first commercial-scale cellulosic ethanol facility in Crescentino

PROESA is a 'second-generation' technology for using non-food energy crops or agricultural waste and turning them into different types of sugary liquids, and is designed to produce them at a lower cost than competing approaches.

Mossi Ghisolfi background

SANDRO COBROR
Head of Public Affairs
Biochemtex spa

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1953 - 1979	1979 - 2000	2000 - TODAY	
Packaging Manufacturing Phase	Chemical Specialty Manufacturing Phase	PET expansion phase	*Renewables*
HDPE and PVC packaging production	Development and production of PET resins for food packaging	Acquisition of PET Shell activities and Rhodia from Rhone Poulenc Acquisition of Chemtex from Mitsubishi Corporation Construction of the world's largest plants for PET production in Altamira (Mexico) and Suape (Brasil) Plans announced for a new plant in Corpus Christi (Texas, USA)	2006 -2008 - Lab scale technology development for 2nd gen ethanol 2009 - Pilot plant for cellulosic ethanol 2011 - Beta Renewables is founded, dedicated to sustainable chemistry. 2012 - Beta Renewables and Novozymes partnership. Oct 2013 - World's 1st commercial-scale biofuel plant from non-food biomass (40.000 ton/year)

Guido Ghisolfi

...using a variety of sustainable (non-food) biomasses...

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Non-food cellulosic crops

- ✓ Arundo donax (Giant reed)
- ✓ Miscanthus giganteus
- ✓ Panicum virgatum (Switchgrass)

Agricultural residues

- ✓ Wheat straw
- ✓ Rice straw
- ✓ Corn stover
- ✓ Sugarcane bagasse

Lignocellulosic crops

- ✓ Eucalyptus
- ✓ Poplar







Might grow on marginal/abandoned land, creating additional income for farmers

The cellulosic biorefinery today ….

Non food Biomass → PROESA® Technology → Fermentable Sugars → Fermentation → Separation → Ethanol (up to -85% GHG saving)

Separation → Lignin → Energy Production → Steam & Power generation

2nd Gen Ethanol Plant (Crescentino – Italy) – Awarded FP7 support

Efficient exploitation of biomass

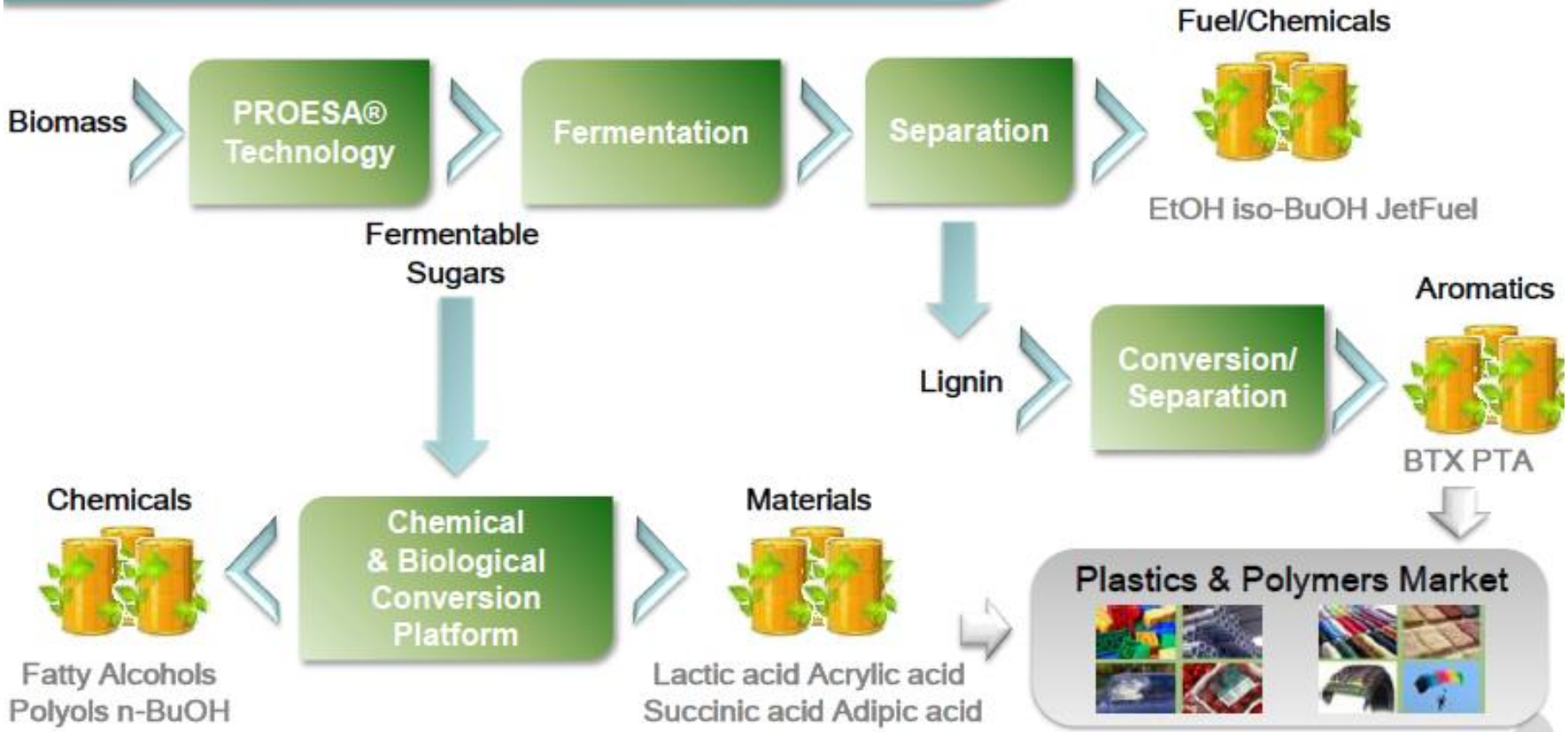
- *it has been evaluated that production of chemicals and polymer resins from sugars and biomass result in two to four times more added value, create six to eight times more employment and require less percentage of feedstock compared to biofuel production.*

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

The Biorefinery opportunity -

SANDRO COBROR
Head of Public Affairs
Biochemtex spa

The cellulosic biorefinery tomorrow



We want to develop the Cellulosic Biorefinery Concept

MOSSI GHISOLFI GROUP

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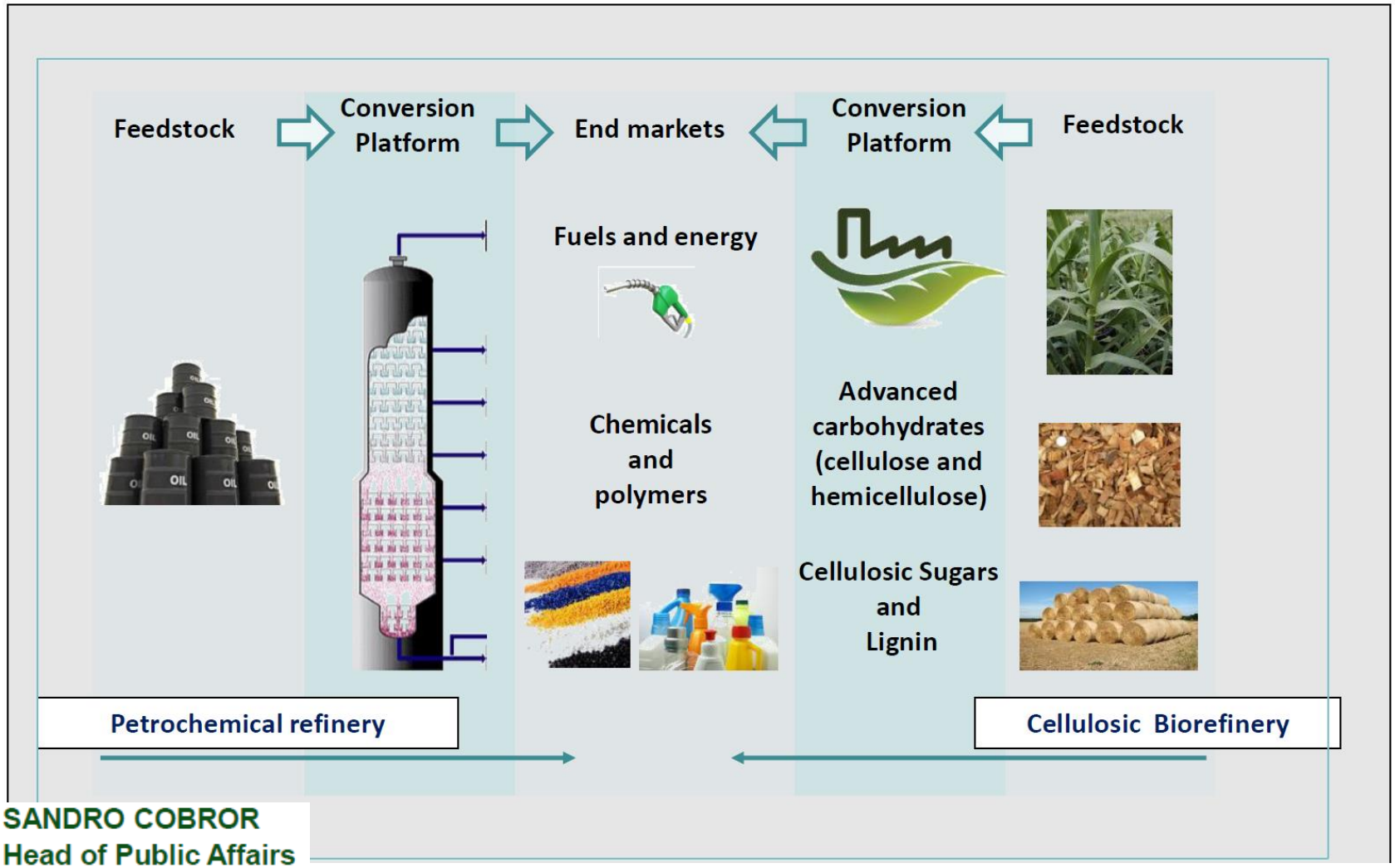
The biorefinery concept is similar to today's oil refinery, which produces multiple fuels and chemicals from crude oil.



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Annexes

Any biofuel capable to address all the policy obligations and long term expectations?

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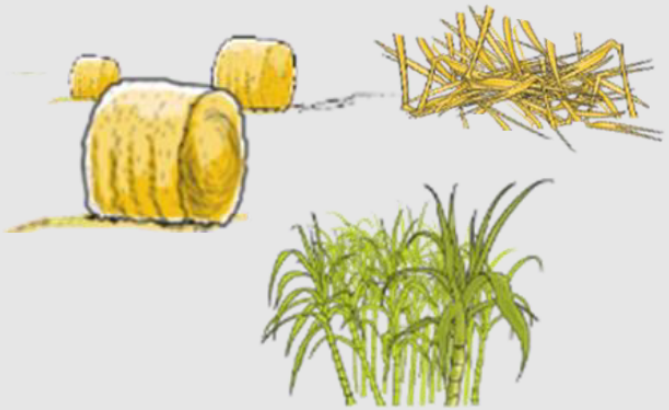
According to the current policy framework and expectations, post-2020 allowed biofuels shall feature at least:

- ✓ No competition vs food
- ✓ High GHG saving vs fossil
- ✓ Minimal use of land
- ✓ Price competition
- ✓ Technology innovation
- ✓ Benefits for rural areas



THIS IS WHAT WE CALL **ADVANCED BIOFUELS** !

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Head of Public Affairs
Biochemtex spa

EU vision is a sustainable, low carbon and climate friendly economy

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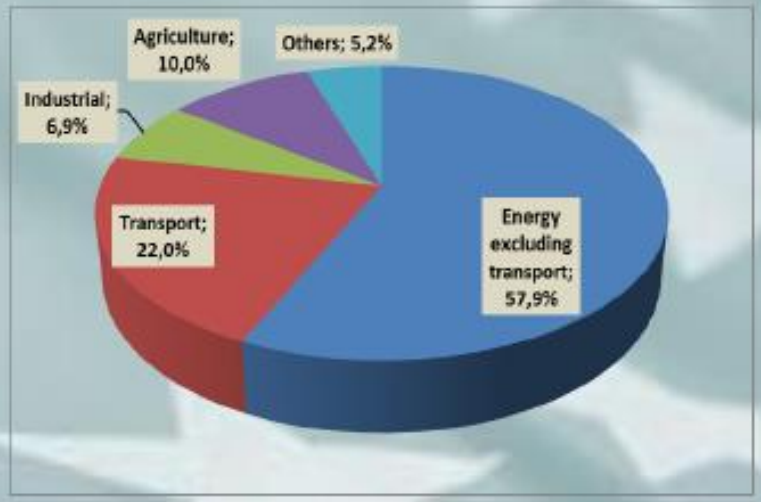
- In feb 2015 EC launched the Energy Union Package: low carbon technology, efficiency and job creation are the pillars and EU target -40% GHG emissions by 2030.
- BUT….
  - "Latest data shows that the EU imported 53% of its energy at a cost of around EUR 400 billion"
  - "94% of transport relies on oil products, of which 90% is imported"
  - 22% of GHG emissions relate to transport

In last 20 yrs transport emissions increased by 20% while all other sectors'emissions decreased by around 15-20%



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What is EU doing to address the trasport issue

- 2009: Renewable Energy Directive sets 10% renewable energy in the transport sector by 2020. Only sustainable biofuels can be used to meet the target.
- 2012: ILUC Proposal. The Commission published a proposal to limit global land conversion for biofuel production, and raise the climate benefits of biofuels used in the EU. The use of food-based biofuels to meet the 10% renewable energy target of the RED should be limited.

Italy's approach to advanced biofuels

January 2013: IT Governement signed an agreement (Protocollo d'Intesa) with Gruppo Mossi Ghisolfi to foster the deployment of second and third generation biorefineries in Italy

May 2014: IT Government signed an agreement with MG to build up 3 cellulosic ethanol plants in the South of Italy

October 2014: DM set minimum quota of adv biofuels from 2018 on (from 0,6% in 2018 to 1% in 2022). This translates into around 180ktoe/y in 2018 and 300ktoe/y in 2022.