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Institut de recherche en biotechnologie

Recherche sur les bioproduits
Vers une nouvelle chimie industrielle

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Outline

- NRC-BRI
- Bioproducts
- Biomass / Biorefineries
- Enzyme-catalyzed condensation
- Metabolic pathway engineering

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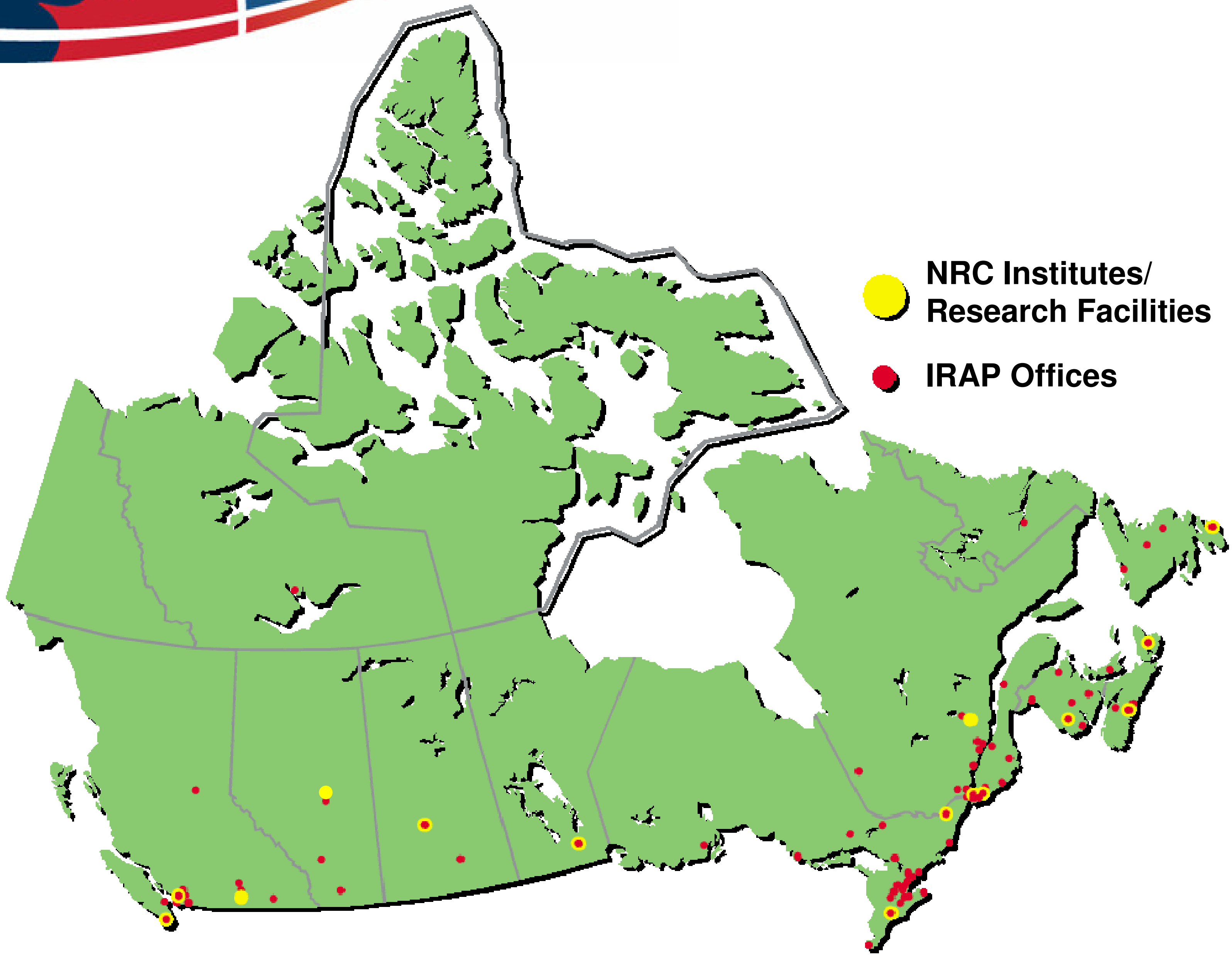
National Research Council



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NRC Institutes

Biotechnology Research Institute (Montréal)
Institute for Biodiagnostics (Winnipeg)
Institute for Biological Sciences (Ottawa)
Institute for Marine Biosciences (Halifax)
Plant Biotechnology Institute (Saskatoon)
Herzberg Institute of Astrophysics (Victoria and Penticton)
Institute for Aerospace Research (Ottawa)
Institute for Chemical Process and Environmental Technology (Ottawa)
Institute for Information Technology (Ottawa)
Institute for Marine Dynamics (St. John's)
Industrial Materials Institute (Boucherville)
Institute for Microstructural Sciences (Ottawa)
Institute for National Measurement Standards (Ottawa)
Institute for Nutrisciences and Health (Charlottetown)
Institute for Research in Construction (Ottawa)
National Institute for Nanotechnology (Edmonton)
Steacie Institute for Molecular Sciences (Ottawa and Chalk River)
NRC Innovation Centre (Vancouver)

NRC Technology Centres

Canadian Hydraulics Centre (Ottawa)
Thermal Technology Centre (Ottawa)
Centre for Surface Transportation Technology (Ottawa and Vancouver)

CISTI Canada Institute for Scientific and Technical Information (across Canada)
IRAP Industrial Research Assistance Program (across Canada)

National Program on Bioproducts (1st national program)
ABIP (Agricultural Bioproducts Innovation Program) with Agriculture Canada

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Director General

Human Ressources — Industrial Affairs

Health
Biomolecular NMR
Chemical Biology
Computational Chemistry and Biology
Enzymology
Genetics
Macromolecular Structures
Receptor, Signaling and Proteomics

Environment
Analytical Chemistry
Applied Ecotoxicology
Bioconversion/Sustainable Development
Bioengineering
Biosensors and Nanobiotechnology
Environmental Genetics
Environmental Microbiology

Bioprocess
Animal Cell Technology
Genomics and Gene Therapy Vectors
Microbial and EnzymaticTechnology

Bioproducts

Bioproducts are products (excluding biopharmaceuticals) that are made from biomass.
Biomass is any type of organic material that is available on a renewable or recurring basis. It includes such things as crops and trees, wood and wood wastes, aquatic plants and grasses, and municipal wastes.

-224 billion tonnes per year of new biomass worldwide
-15% of the energy used around the world
-35% of the energy needs of developing countries

Primer on Bioproducts. www.pollutionprobe.org (2004)

Bioproducts

Examples of readily available bioproducts and their origin

- Structural biomass
  - cellulose fibers from trees for paper
  - cellulose fibers from flax for textile (cotton)
- Energy storage
  - sugar from sugarcane (→ ethanol)
  - starch from corn or wheat (→ glucose → fructose or ethanol)
  - oil from canola, flax, etc. (→ biodiesel)
  - jojoba oil
- Bark and leaves
  - quinine from the bark of Cinchona trees
  - paclitaxel (Taxol™) from yew tree bark
  - artemisinin from the leaves of *Artemisia annua*

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Bioproducts

Man-made, historical examples

- Soap (Babylonia, 2800 BC)
- Cellulose derivatives
    - Nitrocellulose (cellulose nitrate): explosive (1832), photographic film (before 1948)
    - Cellulose acetate: film
    - Cellulose xanthate: Viscose Rayon, cellophane
- Nitroglycerin (glyceryl trinitrate, 1848)
- Linoleum (1860, polymerized linseed oil + wood dust)

Biofuels

	GHG reduction	Energy balance	Yield, m^3/ha	
Corn EtOH	20%	~1.4 (0.6 – 1.8)	3	needs fertilizers and energy
Biodisel	80%	~3.2 (1.4 – 4.1)	1 (canola) 5 (palm)	difficult to increase yield in North-America
Sugarcane EtOH	90%	~9 (8 – 10)	6 – 10	need the climate
Cellulosic EtOH	90%	3 – 5	9.5 – 14	technical-economic challenge
Coskata / GM		7.7	-	Syngas + fermentation
Butanol	-	-	-	not yet available

Main sources of biomass

- Municipal wastes
  - highly heterogeneous and variable
- Wastes from agriculture and forestry
  - mostly lignocellulose (except manure)
- Dedicated crops
  - glucose, starch, fibers, oil

→ **Biorefinery** (sugarcane ethanol)

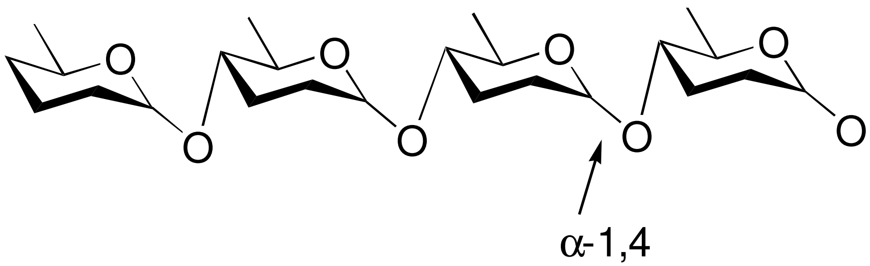
Transformation: mechanical, thermal, chemical, or biological

Municipal wastes

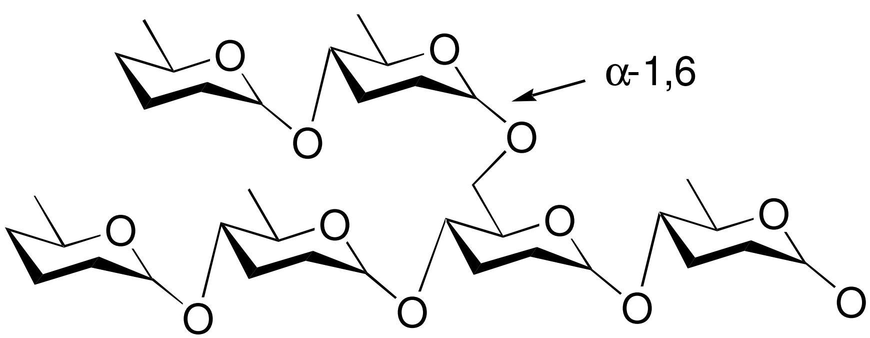
Because of their heterogeneity and variable composition, the best way to use municipal wastes is a degradative approach

- pyrolysis → $CH_4 + H_2$ + oil, tar and char
- gasification → $CO + H_2$ (syngas) → CH_3OH, C_2H_5OH or alkanes
- anaerobic digestion → $CH_4 + CO_2$ (biogas)

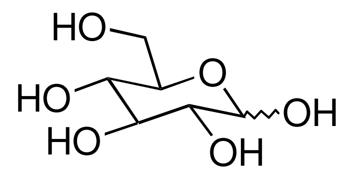
Starch



amylose 25-30% Dp ~600-1000



amylopectin 70-75%, Dp 10^3-10^4, α-1,6 every 20-25



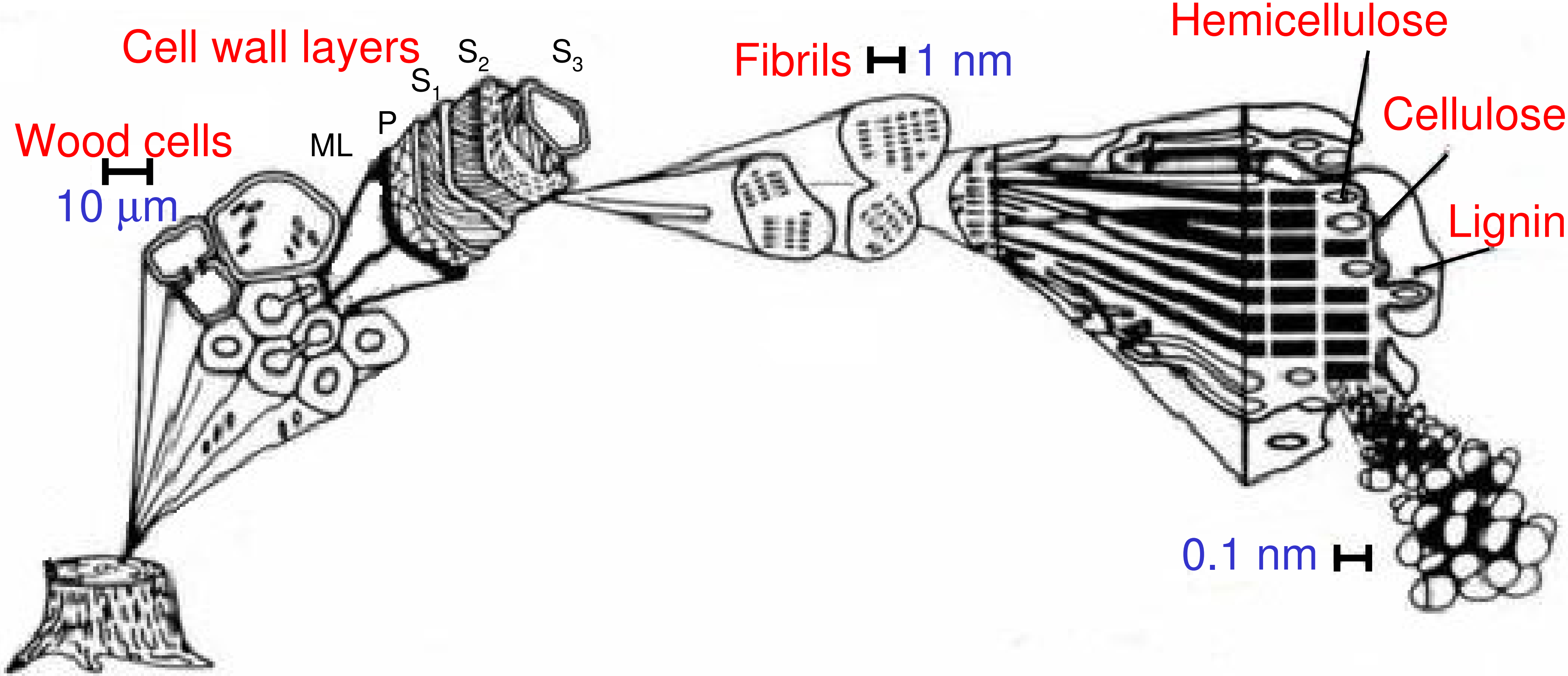
α-1,4 glucose polymer

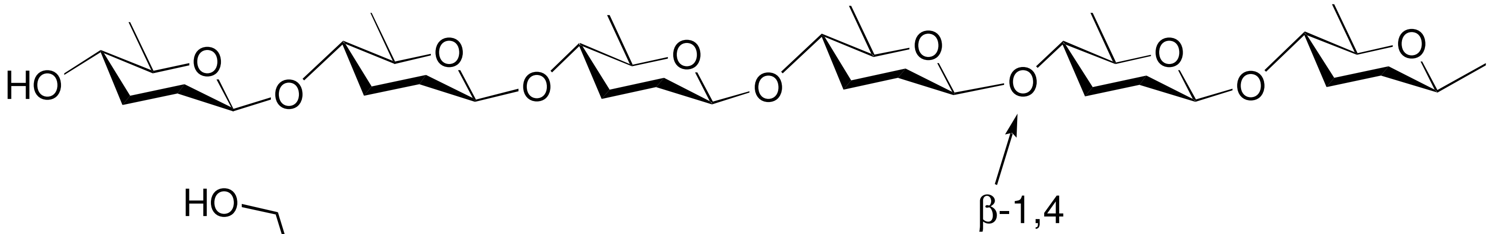
Easy enzymatic hydrolysis to obtain glucose

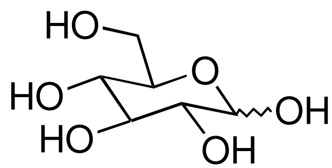
Long experience from fructose production (HFCS)

Glucose can be fermented to any desired product

Lignocellulose





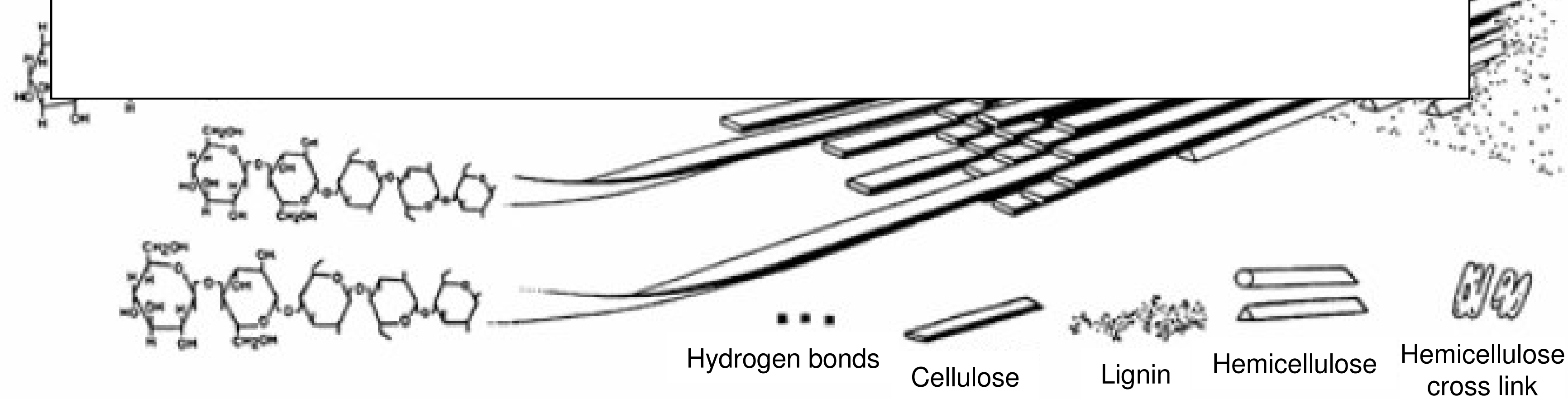


Cellulose Dp ~ 10^4

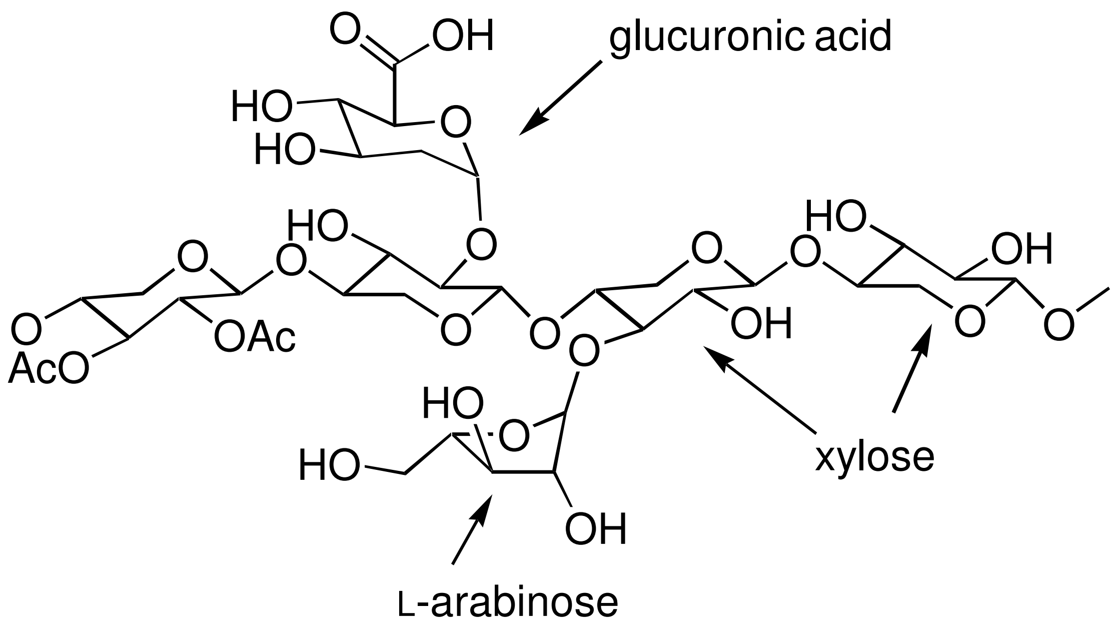
β-1,4 glucose polymer

Mammals cannot digest it

Fungi (red rot) and bacteria have cellulase complexes



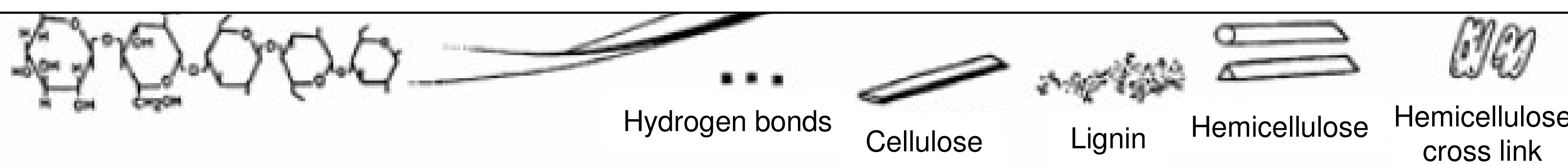
Hardwood hemicellulose
Dp 50-300

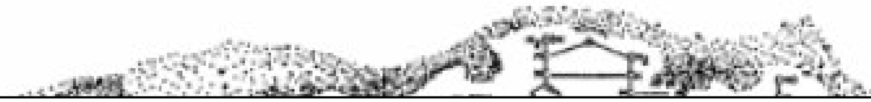


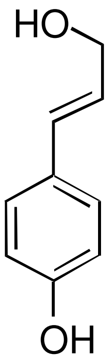
Mixed, branched polymer, mostly β-1,4 xylose

Hydrolyzed by xylanases and bases

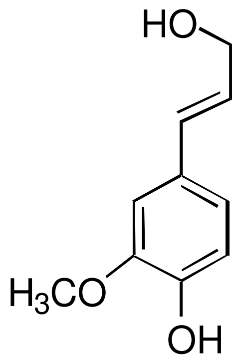
Hydrolysis products are difficult to ferment



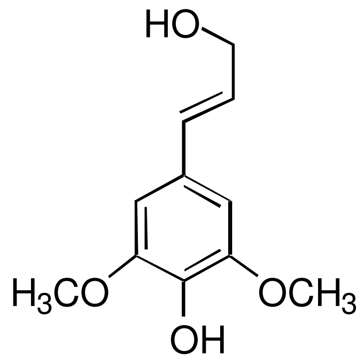




p-coumaryl alcohol
annual plants

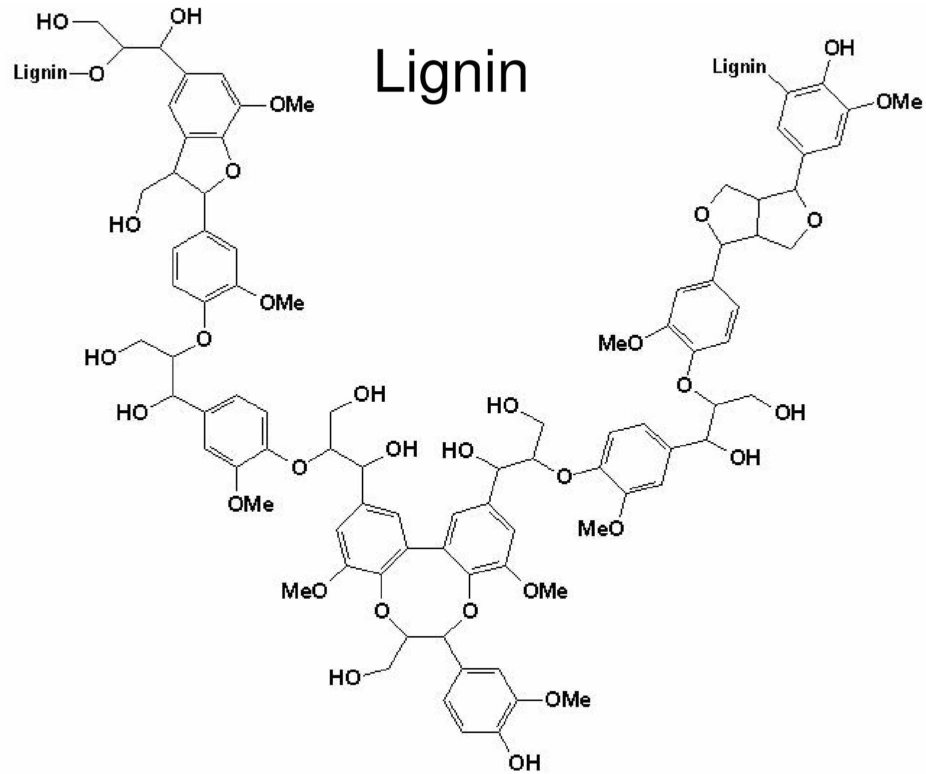


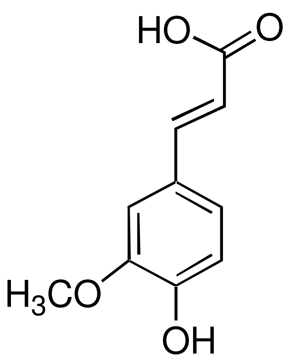
coniferyl alcohol
softwood



sinapyl alcohol
hardwood

Lignin





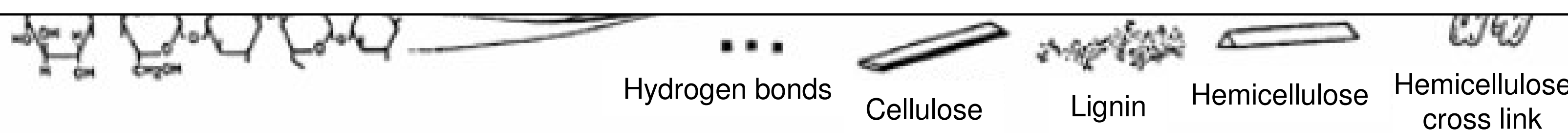
ferulic acid

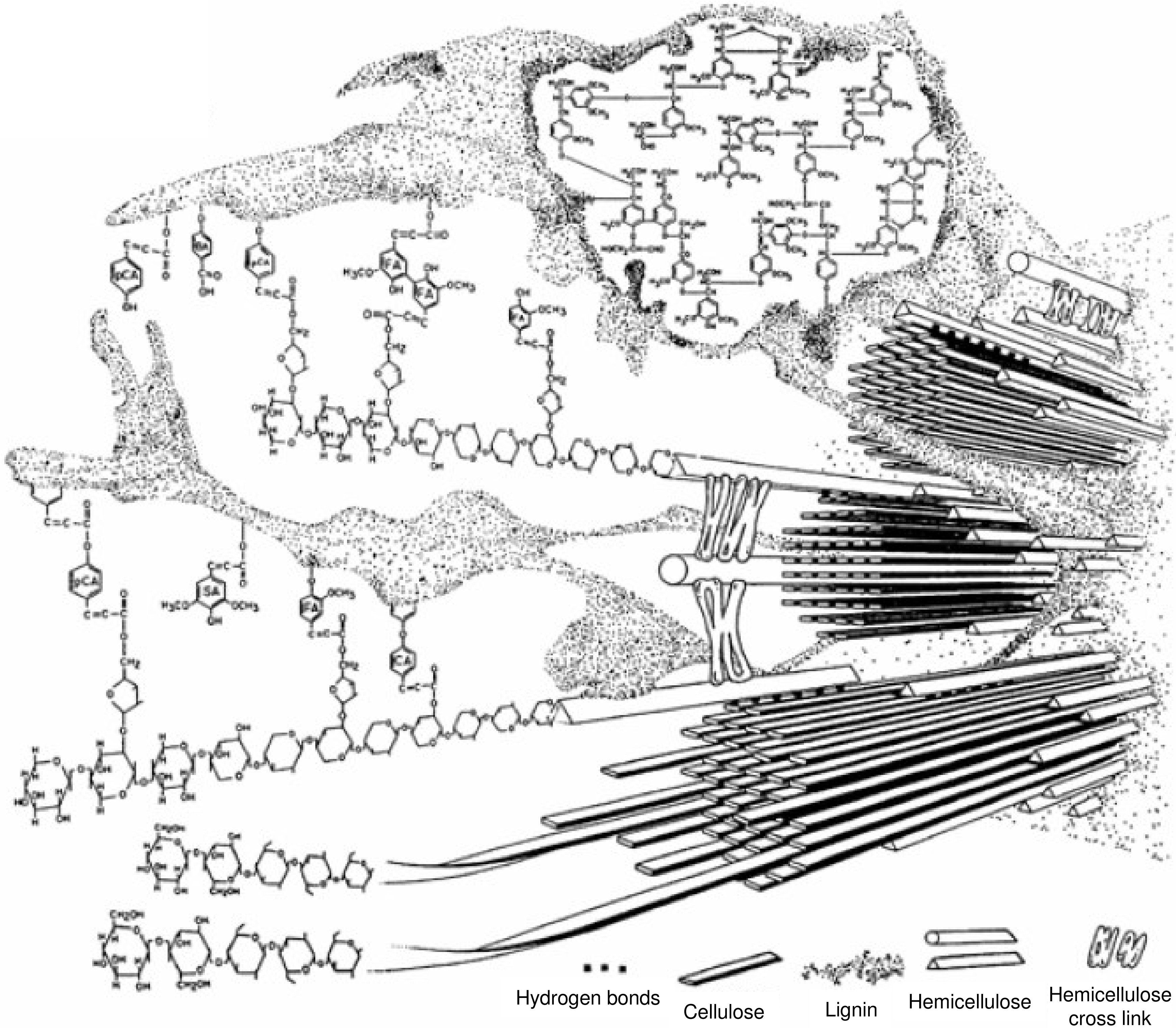
3-D polymer

Fungi (white rot) can hydrolyze it

Lignin + hemicellulose = matrix

Linked to hemicellulose through ferulic acid




Hydrogen bonds
Cellulose
Lignin
Hemicellulose
Hemicellulose cross link

Lignocellulose

Chemical component	Weight % (dry basis)		
	Softwoods	Hardwoods	Wheat straw
Cellulose	42 ± 2	45 ± 2	36 ± 5
Hemicellulose	27 ± 2	30 ± 5	27 ± 3
Lignin	28 ± 3	20 ± 4	11 ± 3
Extractives	3 ± 2	5 ± 3	26 ± 5

Thomas, R. J., Wood Structure and Chemical Composition, in “Wood Technology:Goldstein, Ed, ACS, 1-23, (1977)

Lignocellulose

Must separate components

- Liberate cellulose
  - fiber (paper, textile)
  - glucose
- Various processes
  - chemical
  - mechanical
  - steam explosion

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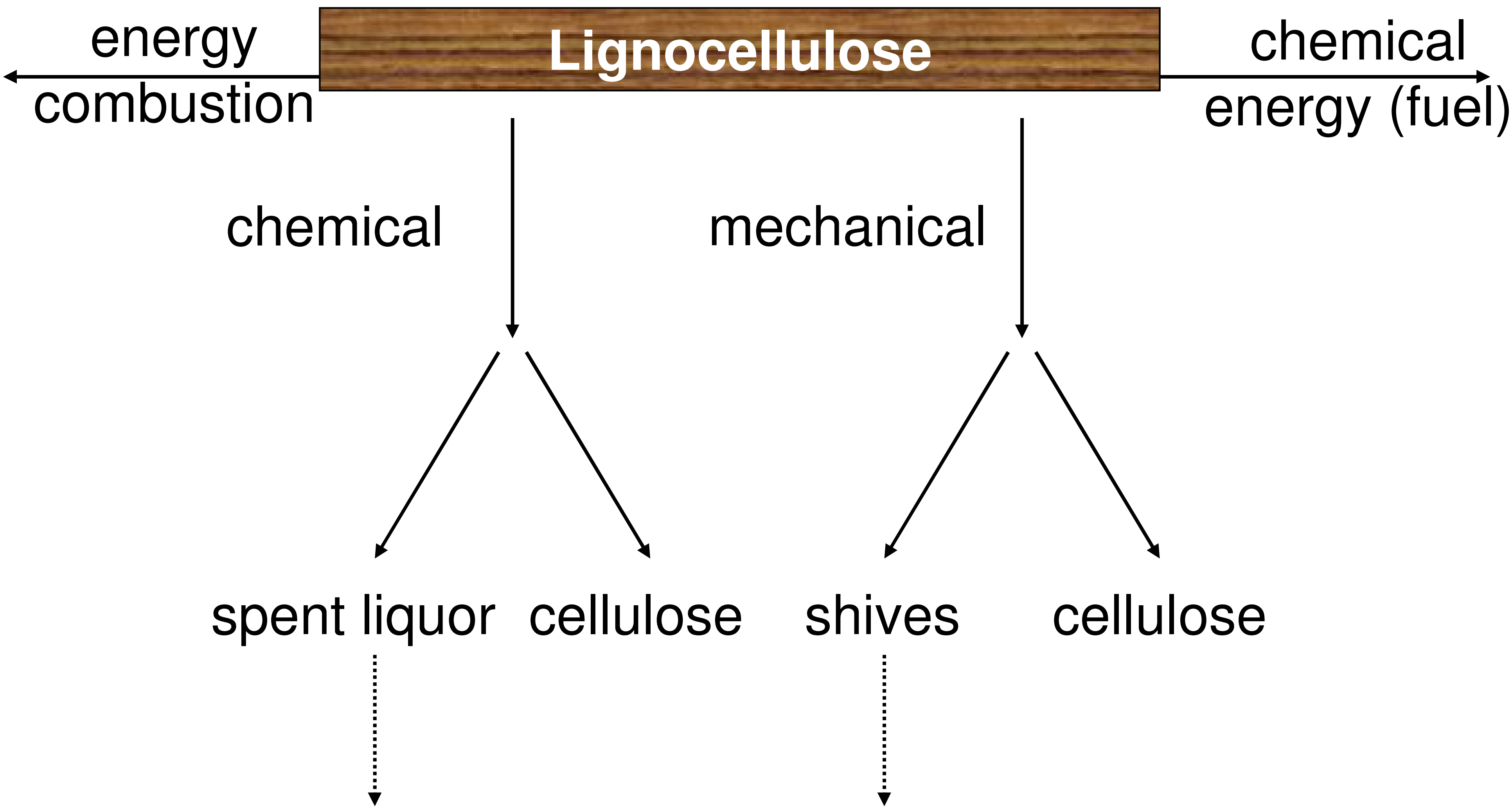
Lignocellulose



Lignocellulose

Chemical energy

- Ethanol (Iogen) →
- Gasification
- Anaerobic digestion

  DOE – Joint Genome Institute 250-300 microbial species in hindgut of higher termites. Produce H_2 and CH_4 from lignocellulose. Metagenomics is used to identify the enzymes involved.

  Nature, ***450***, *560 (2007)*

plant fiber (straw)

↓ steam explosion + separation ↘

cellulose + hemicellulose | lignin (energy)

↓ enzymatic hydrolysis

sugars

↓ fermentation + distillation

ethanol (340 L / t fiber)

Lignocellulose

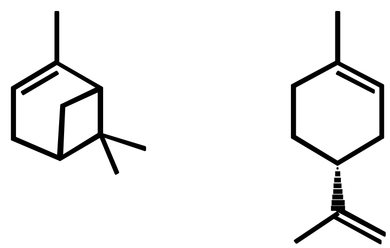
Spent liquors
- Used for energy: concentration followed by burning
- Anaerobic digestion to methane
- Lignosulfonates (reduce viscosity of sluries:
  concrete making, oil drilling mud)
- Efforts are put on valorization by fermentation

Shives
- Use in polymer composites
  Reinforcement or filling agent

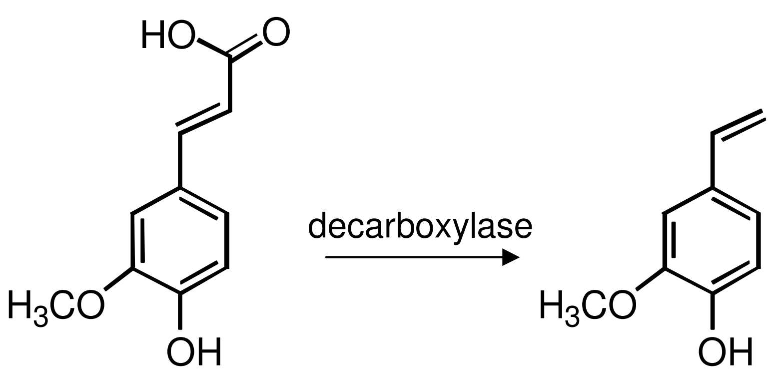
Lignocellulose

Lignocellulose

- Extractibles
  - Various classes of molecules
  - e.g. terpenes (turpentine)



α-pinene limonene



ferulic acid

vinylguaiacol (artificial clove)

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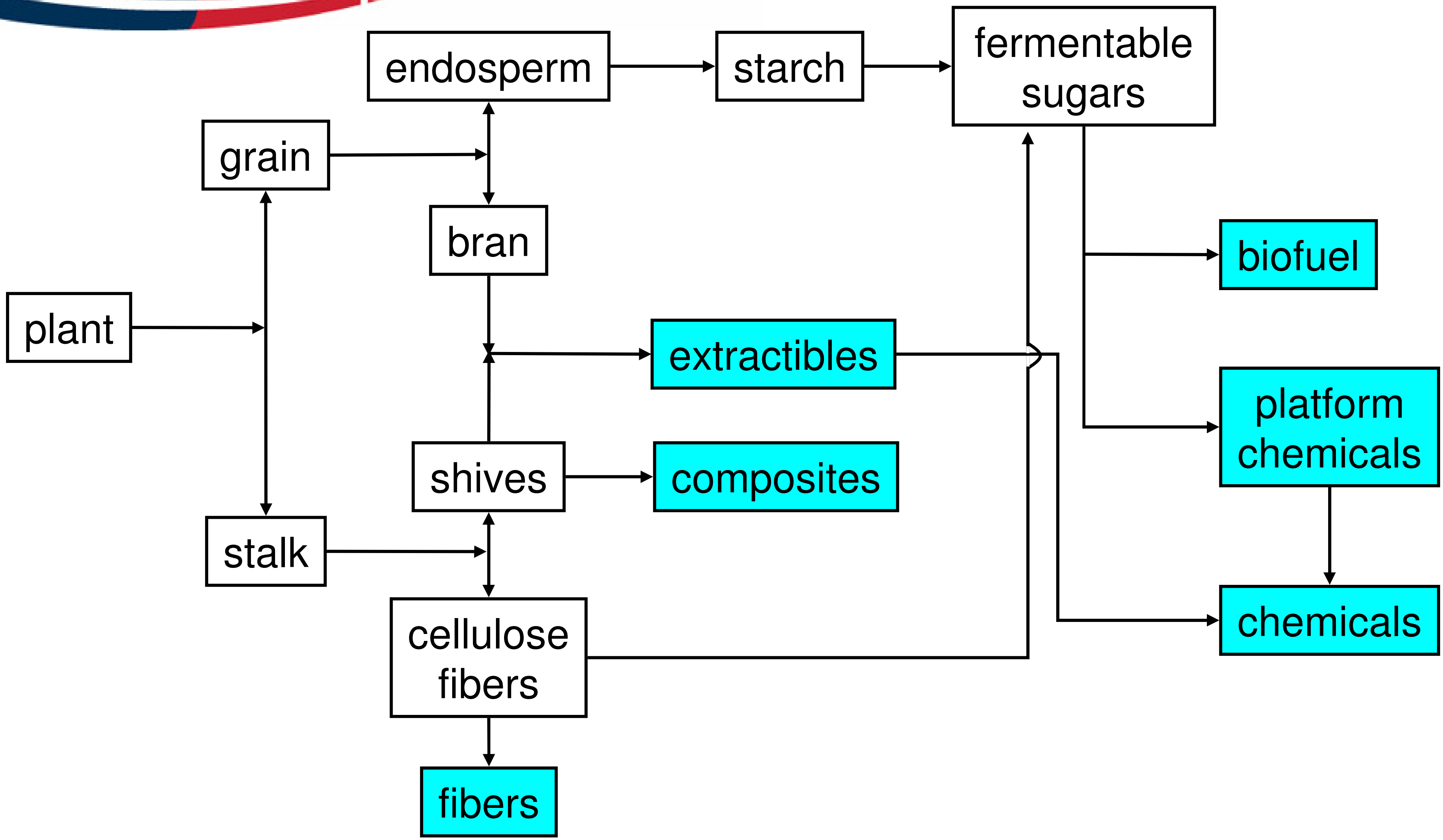
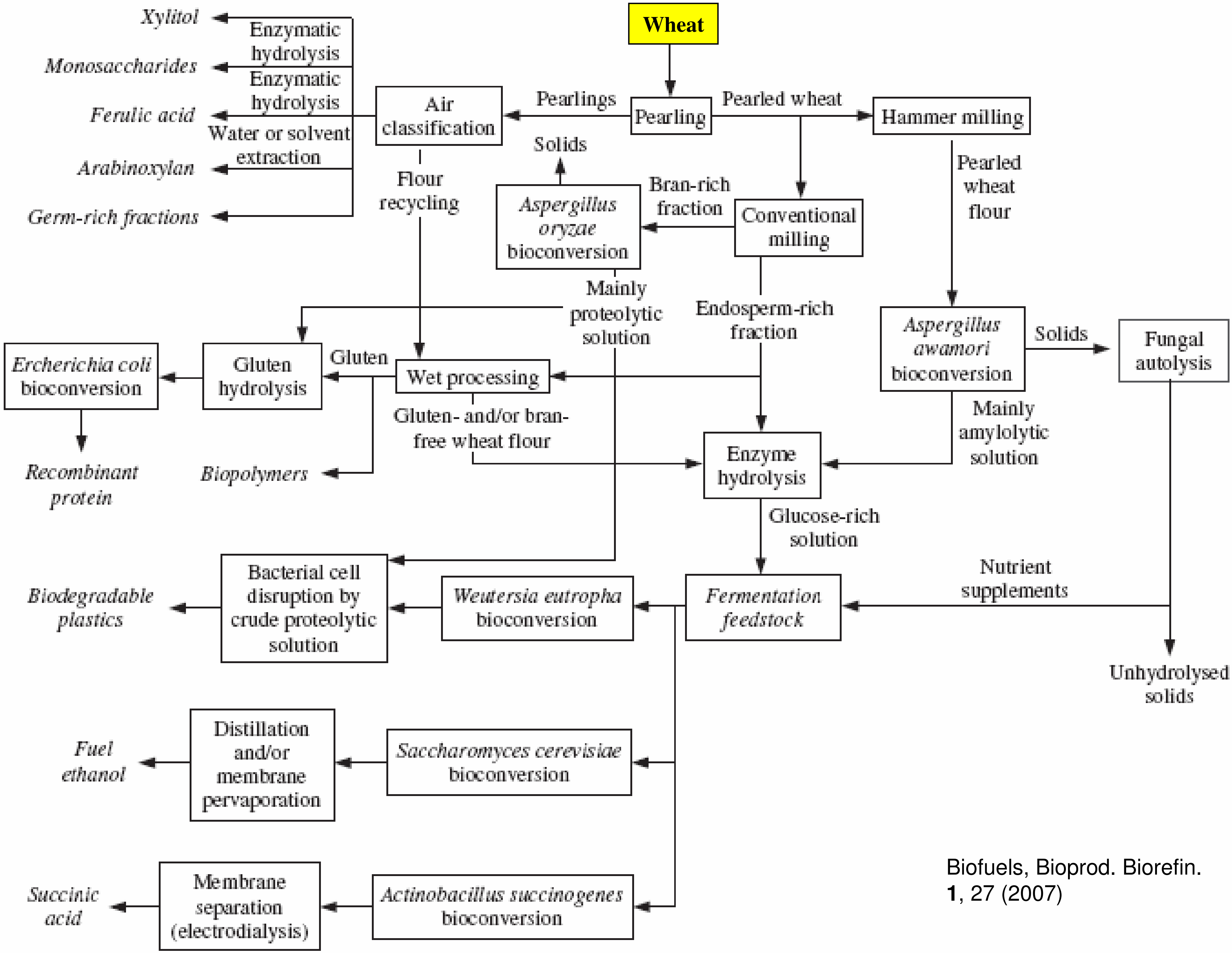
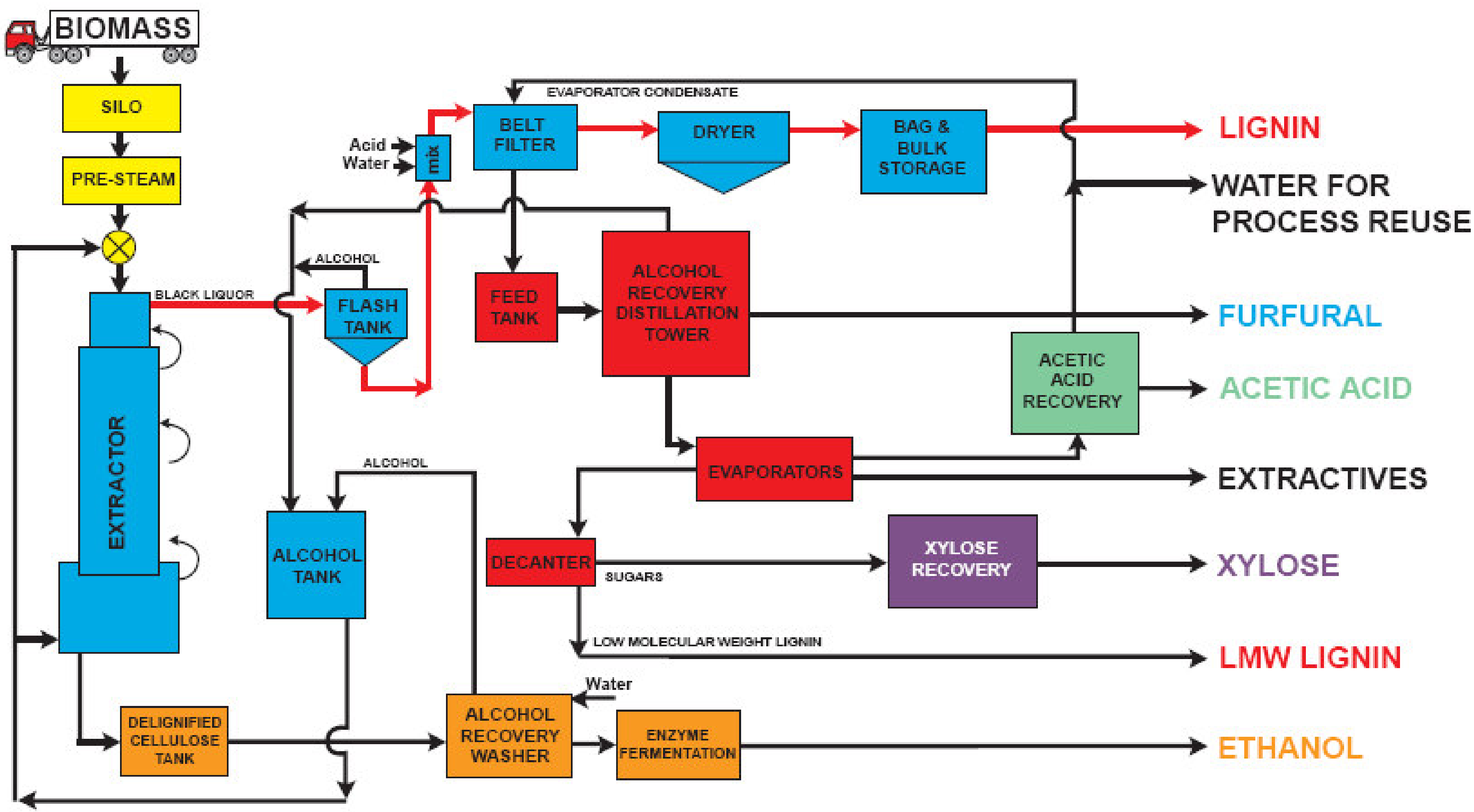
Biorefining





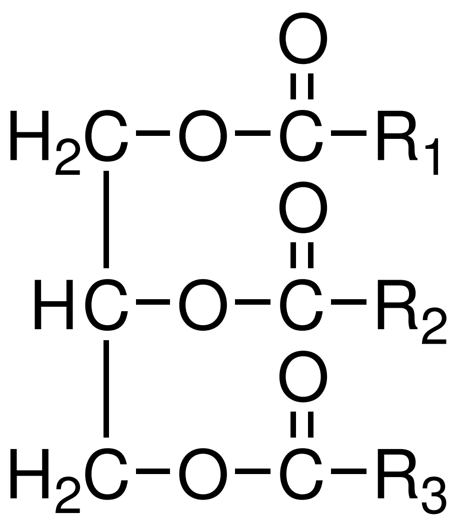
Biofuels, Bioprod. Biorefin. **1**, 27 (2007)

Commercial Lignol Biorefinery



Oil seeds

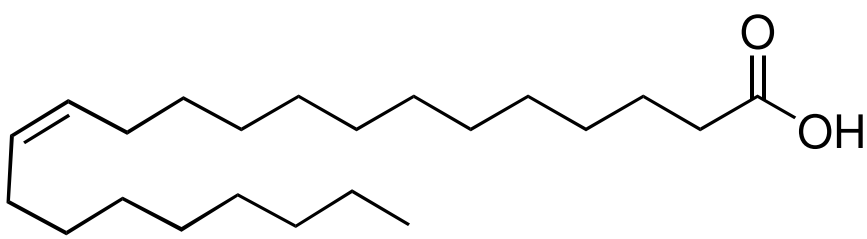
Store energy, mostly under the form of triglycerides

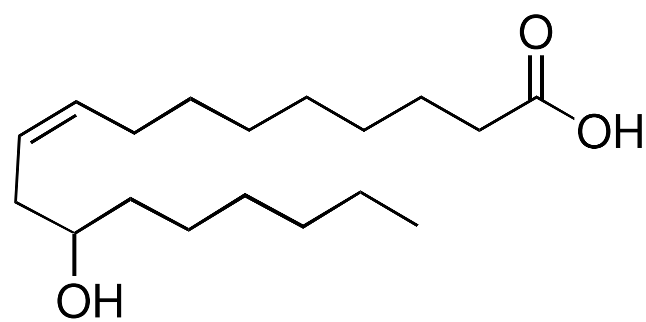


Oil seeds

Lubricants

Especially oils rich in long chain fatty acids (erucic (HEAR oil), ricinoleic (castor oil)) Castrol

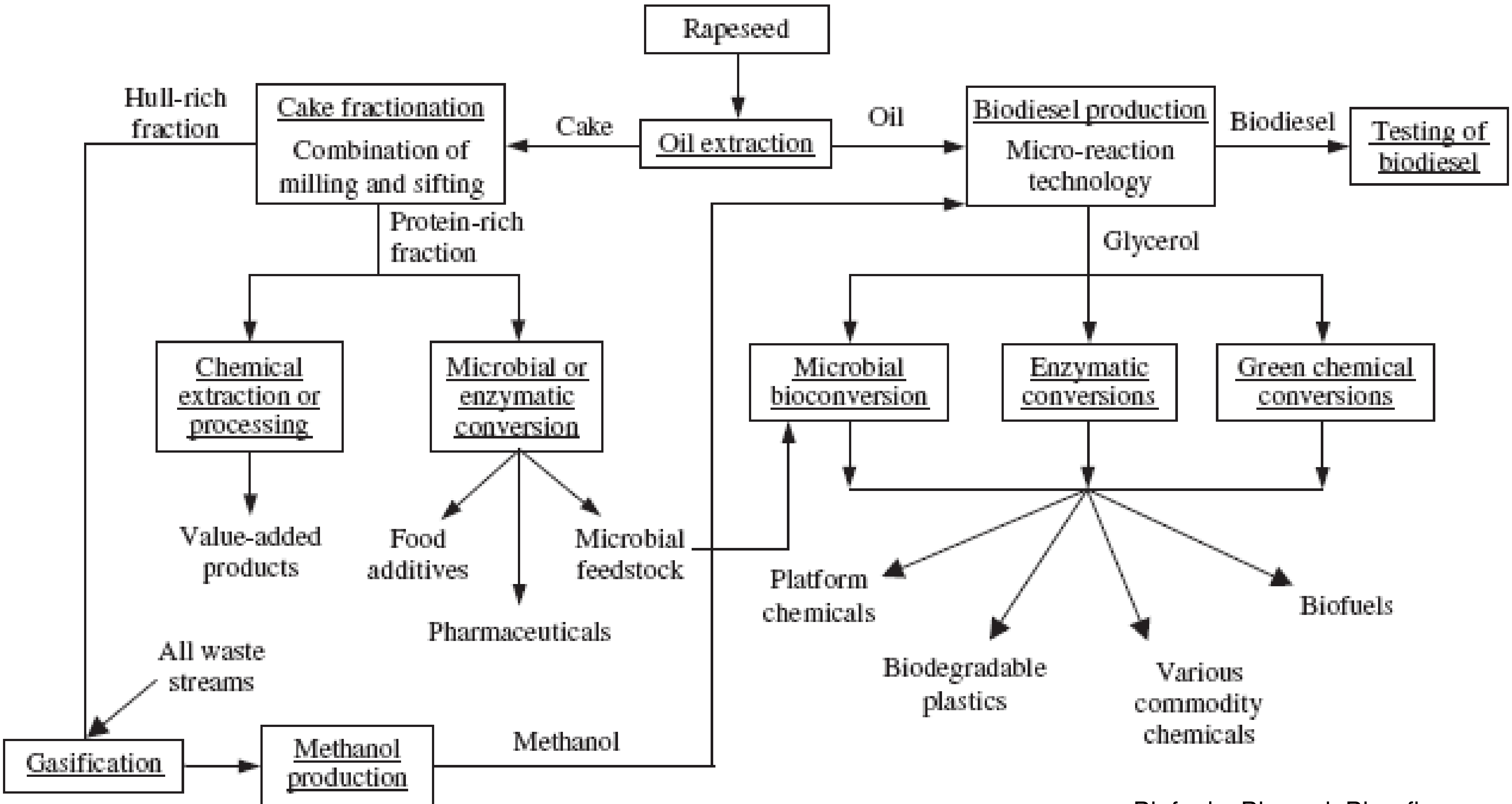




Biodiesel

triglycerides + methanol ⟶ fatty acid methyl esters + glycerol

Biorefining



Biofuels, Bioprod. Biorefin. **1**, 30 (2007)

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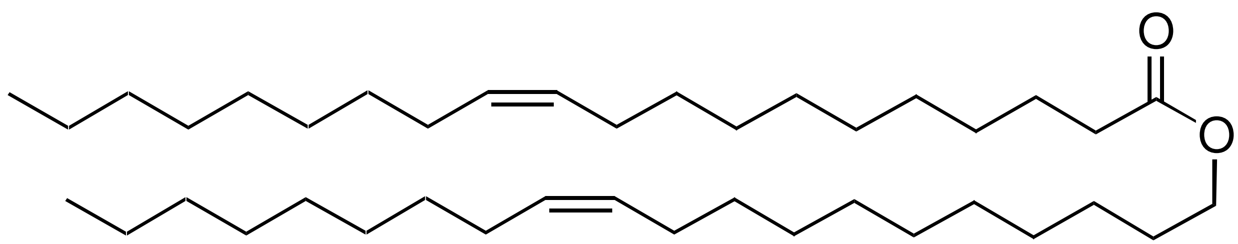
Wax esters

Jojoba (*Simmondsia chinensis*) oil contains esters of fatty acids ($C_{18:1}$, $C_{20:1}$ and $C_{22:1}$) and fatty alcohols ($C_{20:1}$, $C_{22:1}$ and $C_{24:1}$)



- Personal care (cosmetics, skin care)
- High pressure, high temperature lubricants
- Polymer plasticizers

- It is possible to synthesize new wax esters with different properties from fatty acids and fatty alcohols

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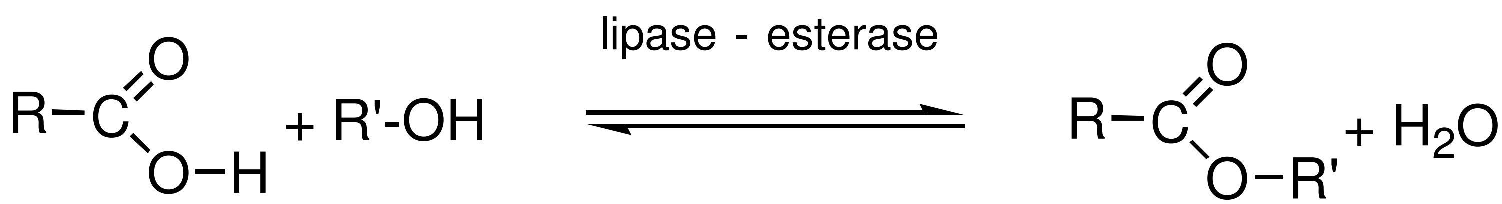
Enzyme-catalyzed condensation

Hydrolases can catalyze other reactions

CONCERNING LIPASE, THE FAT-SPLITTING ENZYME, AND THE REVERSIBILITY OF ITS ACTION.

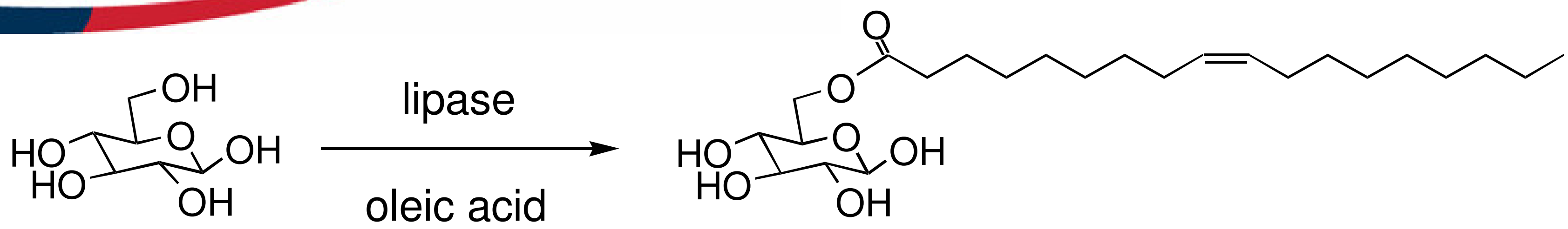
BY J. H. KASTLE AND A. S. LOEVENHART.

Am. Chem. J. **24**, 491 (1900)



- Low water environment will favor synthesis
  - Organic solvents
  - Pure substrates
- Water is important
  - Product / substrate (competitive inhibitor)
  - 3D structure and intramolecular interactions

Biosurfactants



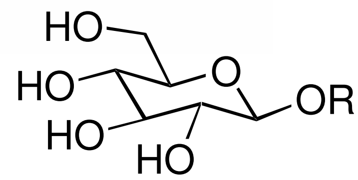
glucose

- need to mix polar and hydrophobic molecules
- 2-methyl-2-butanol
- azeotropic distillation under reduced pressure
- dehydration of the condensate before recycling

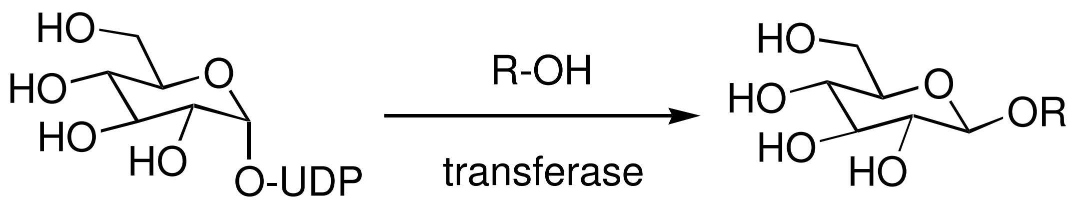
- green process:
  - renewable feedstock
  - very good atom economy (no derivatives, little by-products)
  - easy solvent reuse

Ducret, A., Lortie, R., Trani, M., Canada Patent 2,149,935 (02/01/2001); US Patent 5,635,614 (06/03/1997)

Biosurfactants



alkylglucosides



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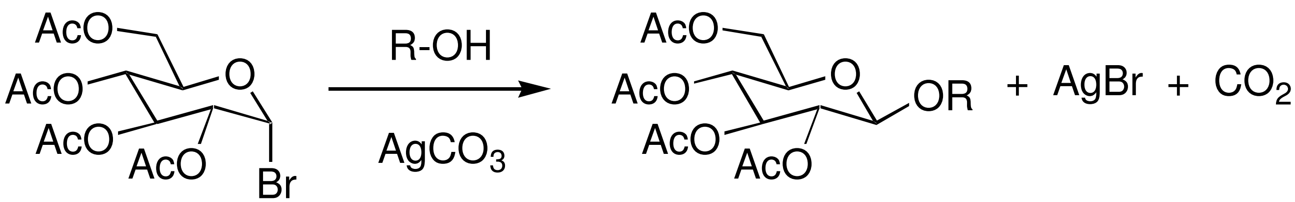
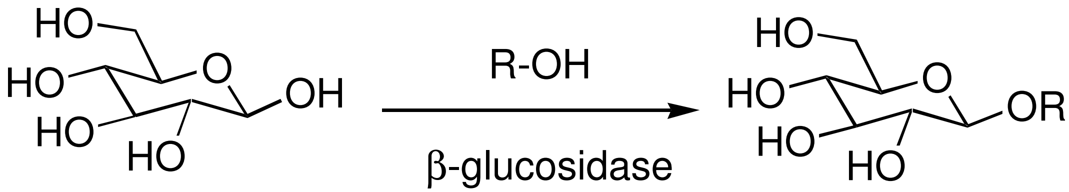


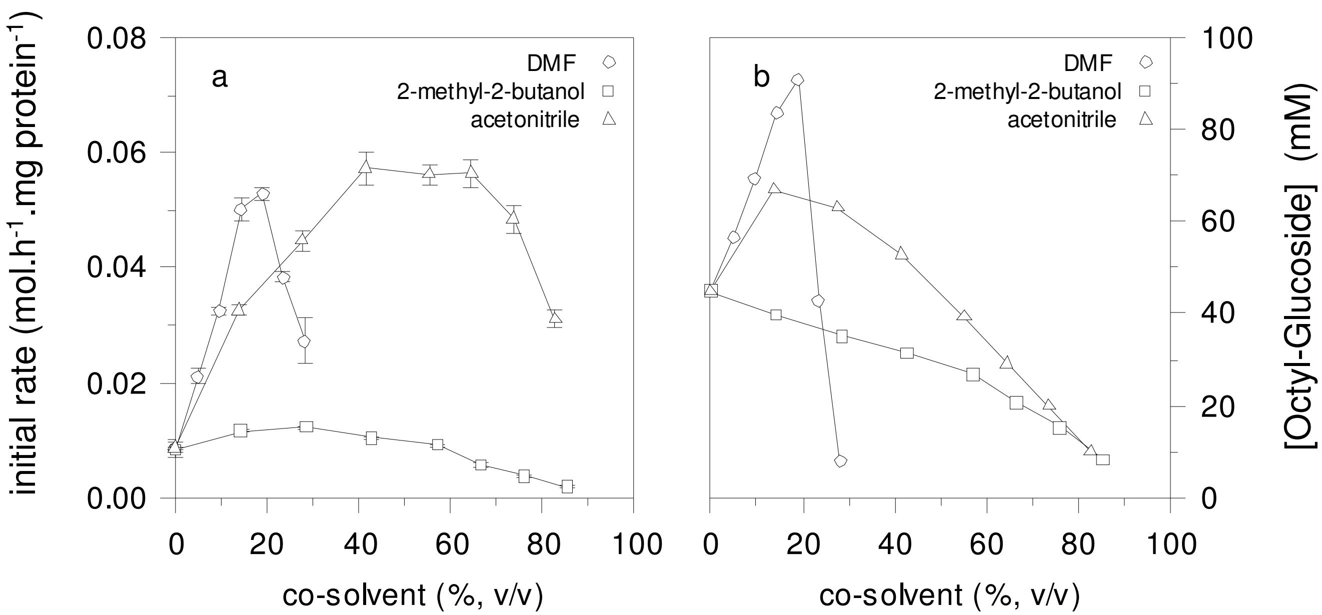
20 kg waste / kg



< 2 kg waste / kg
low yield, slow

Biosurfactants

Use of co-solvents to improve rate and yield



Solvent	ε
water	78
octanol	10.3
2-methyl-2-butanol	5.8
DMF	36.7
CH_3CN	37.5

300 mg D-glucose, 2 mL 1-octanol:co-solvent, 200 mg immobilized enzyme, a_{W0} = 0.75

Ducret, A., Carrière, J.-F., Trani, M., Lortie, R. Can. J. Chem.. **80**, 653 (2002)

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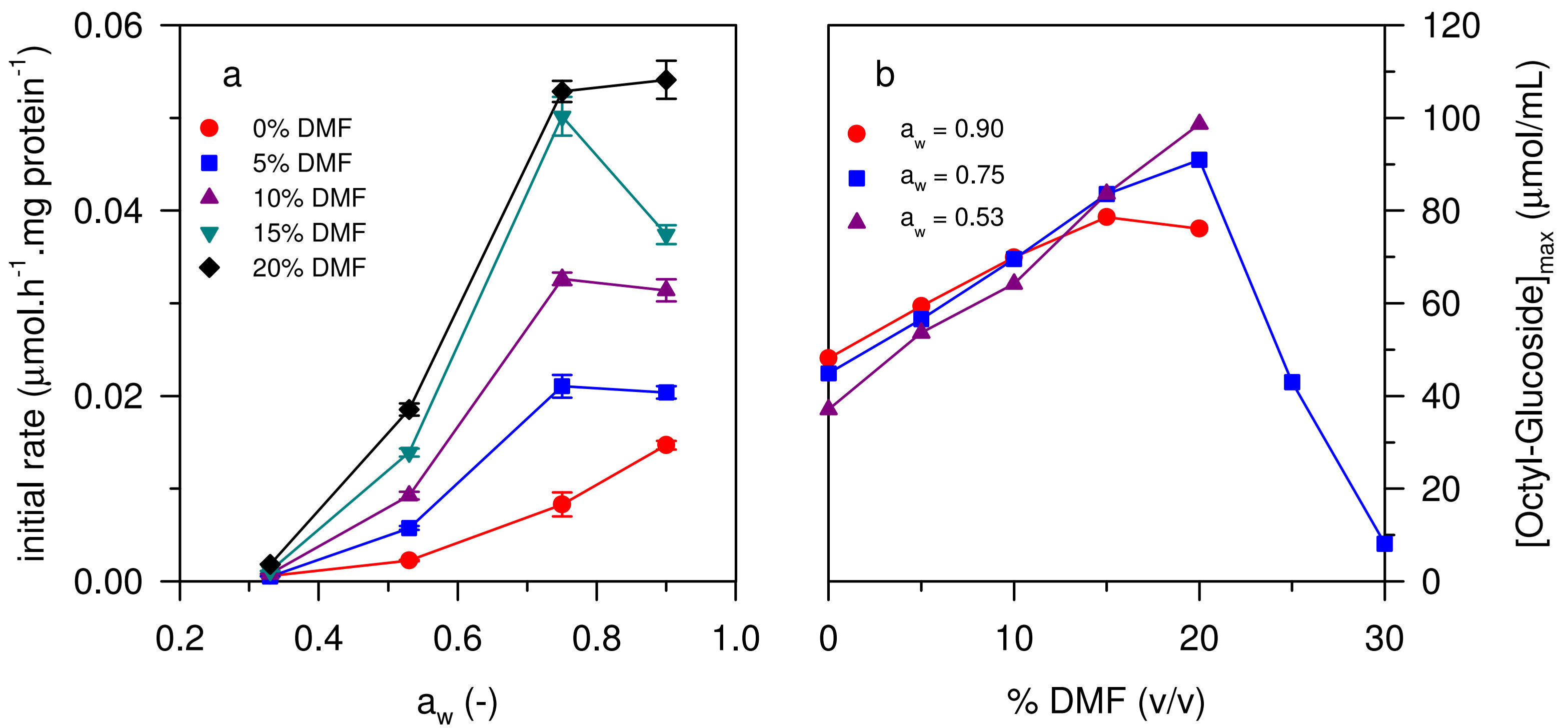
Biosurfactants

Effect of a_W and DMF to improve rate and yield



300 mg D-glucose, 2 mL 1-octanol:DMF,
200 mg immobilized almond
β-glucosidase preparation

Ducret, A., Carrière, J.-F., Trani, M., Lortie, R. Can. J. Chem. **80**, 653 (2002)

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Biosurfactants

- Can be done with β-glucosidase from almond
- β-glucosidase from almond does not have constant properties
- It has not been cloned or sequenced
- Other plant β-glucosidases have been cloned
- We decided to work with an enzyme from maize
- It is as active as the best almond enzyme preparations

- It is not inhibited by glucose BUT
- It is not stable in reaction conditions

- We are in the process of modifying it by protein engineering

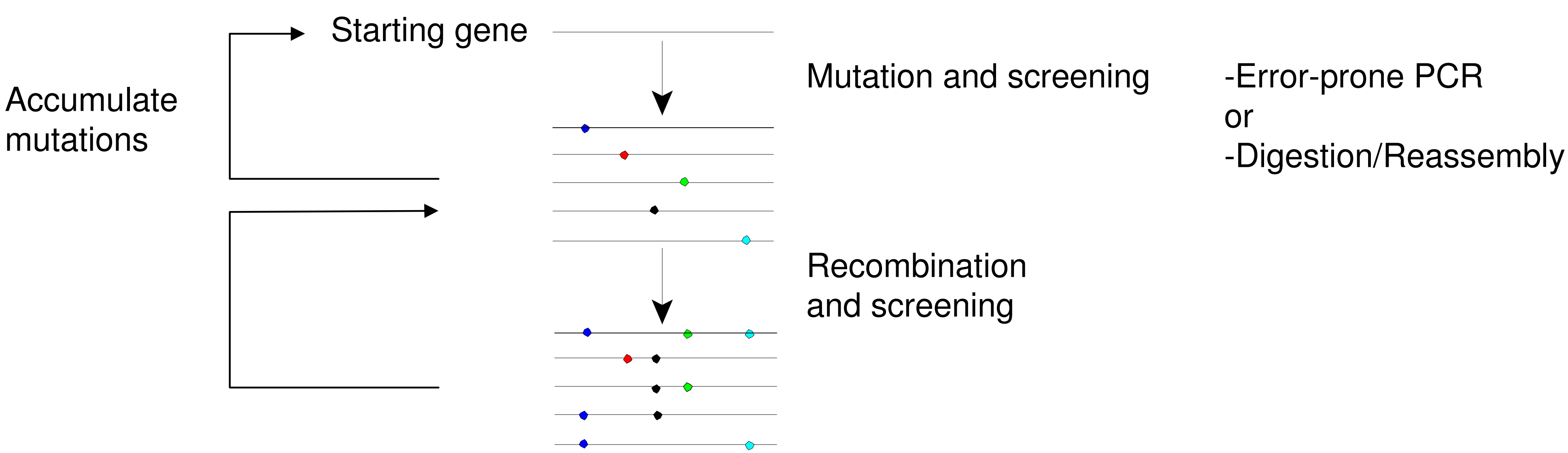
Enzyme engineering

Site-directed mutagenesis

- Enough should be known to design mutations

Directed evolution

- Iterative process, based on the creation of diversity and the selection of desired properties
- Mimics natural evolution, at a faster pace
- Can select properties not relevant to natural environments



Industrial fermentation

- Biomass (bakers yeast, lactobacillus, etc.)
- Enzymes (lipases, cellulases, amylases, etc.)
- Metabolites (ethanol, amino acids, vitamins, lactic acid, CoQ10, etc.)
- Biopharmaceuticals (insulin, HBV, etc.)
- Biotransformation (steroids, etc.)

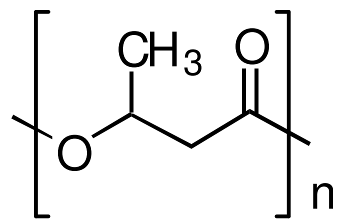
Metabolic engineering

Change metabolic pathways of microorganisms to produce commodity chemicals or pharmaceuticals (or their precursors) from biomass (glucose)

- Bioplastics
- 1,3-Propanediol
- Chemical intermediates
- Artemisinin

Bioplastics

Polyhydroxyalkanoates

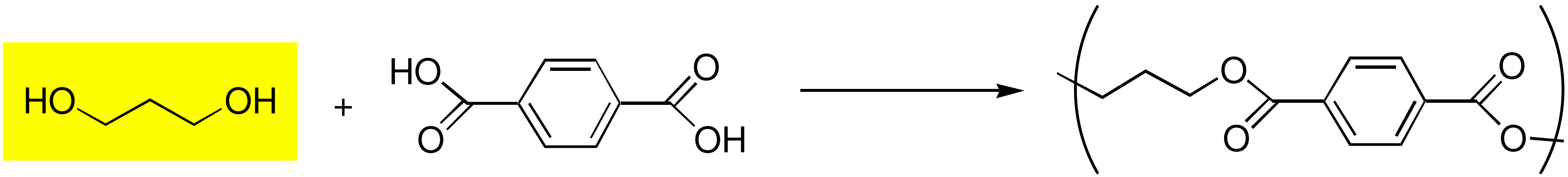


poly-3-hydroxybutyrate

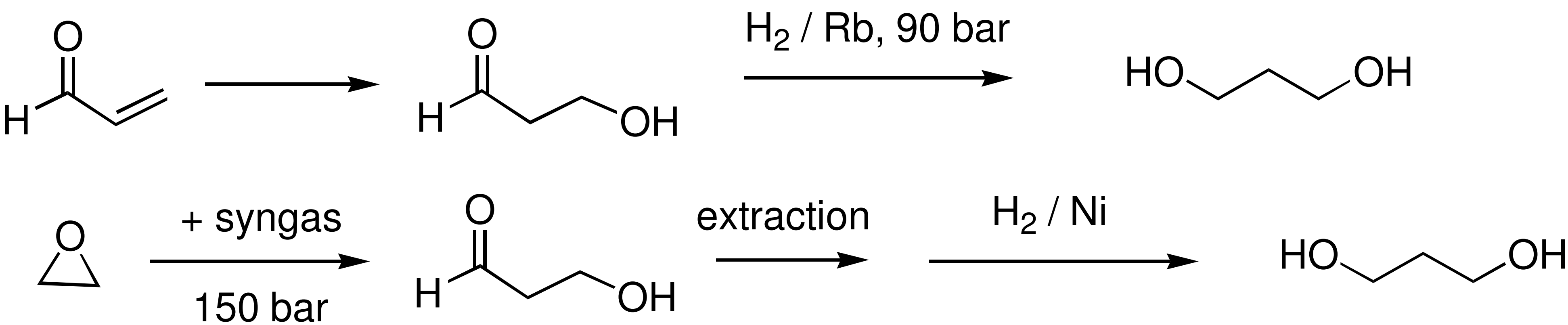
Produced by some microorganismes for energy storage *Alcaligenes eutrophus* from glucose or starch, *Methylobactrerium extorquens* from methanol (D. Groleau, NRC-BRI)

The genes from *A. eutrophus* were expressed in *E. coli* and it can produce 80% (dry weight) PHB. (A. Sinsky, MIT, Metabolix)

1,3-Propanediol

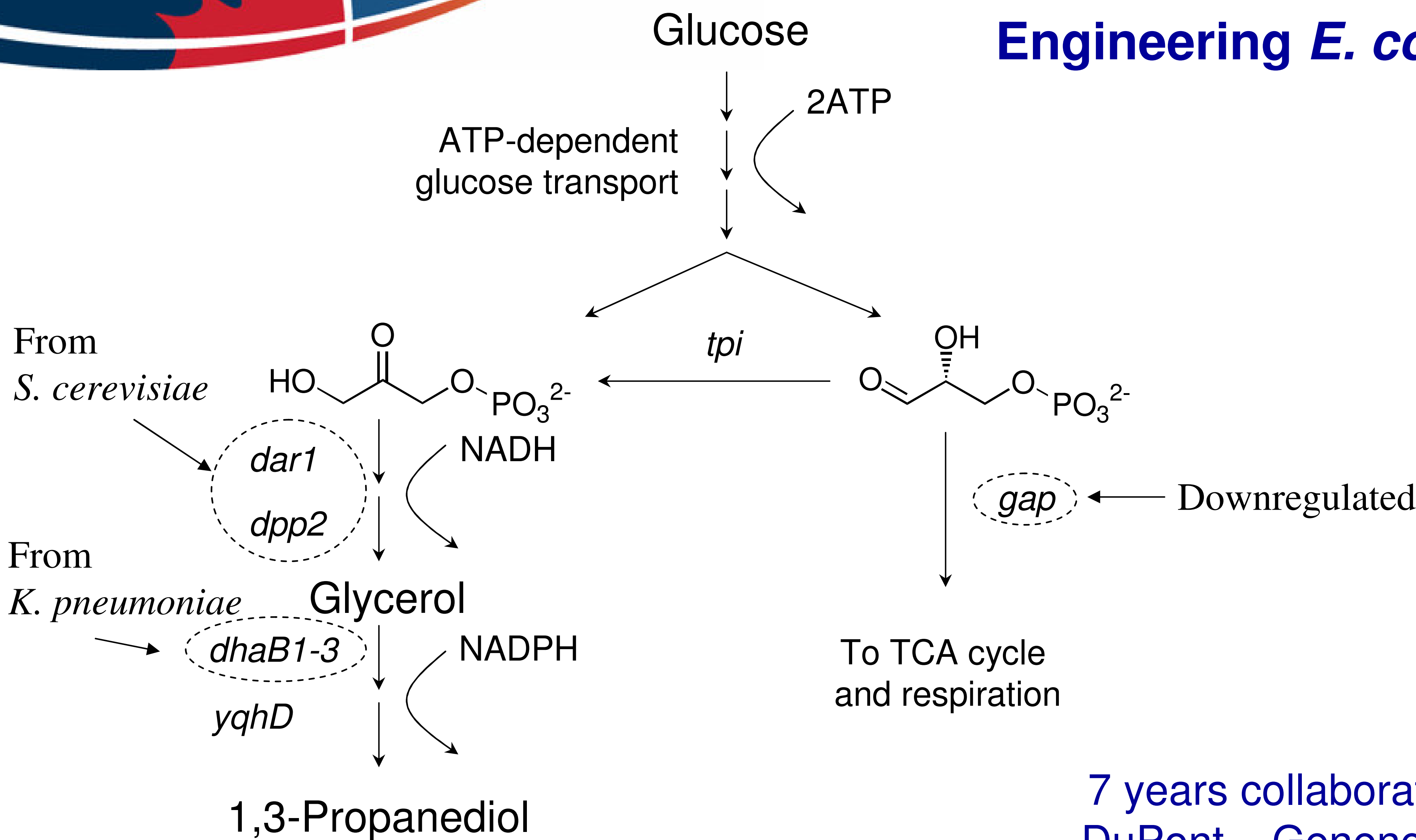


Polypropylene terephtalate
Projected: 1 Mt / y in 2014
Sorona™



1,3-Propanediol

Engineering *E. coli*



7 years collaboration
DuPont – Genencore
3.5 g/L·h 135 g/L

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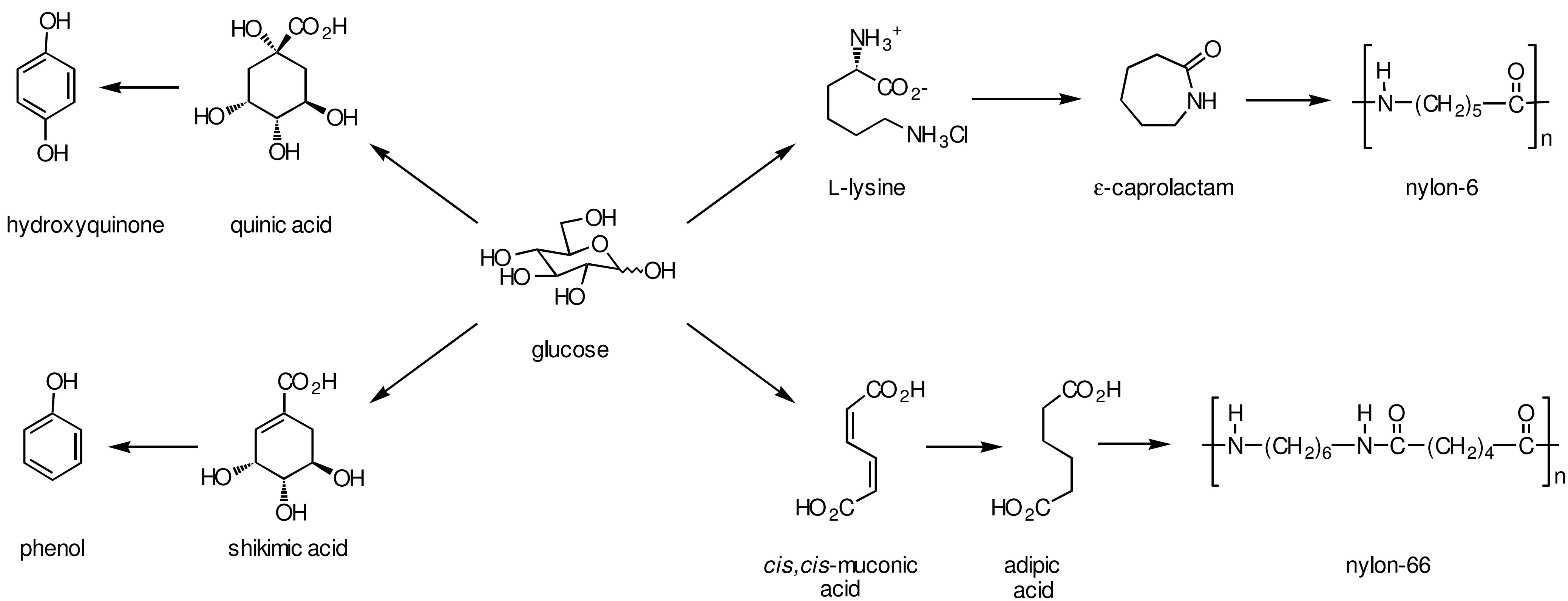
Intermediates from glucose



John Frost
Michigan State University

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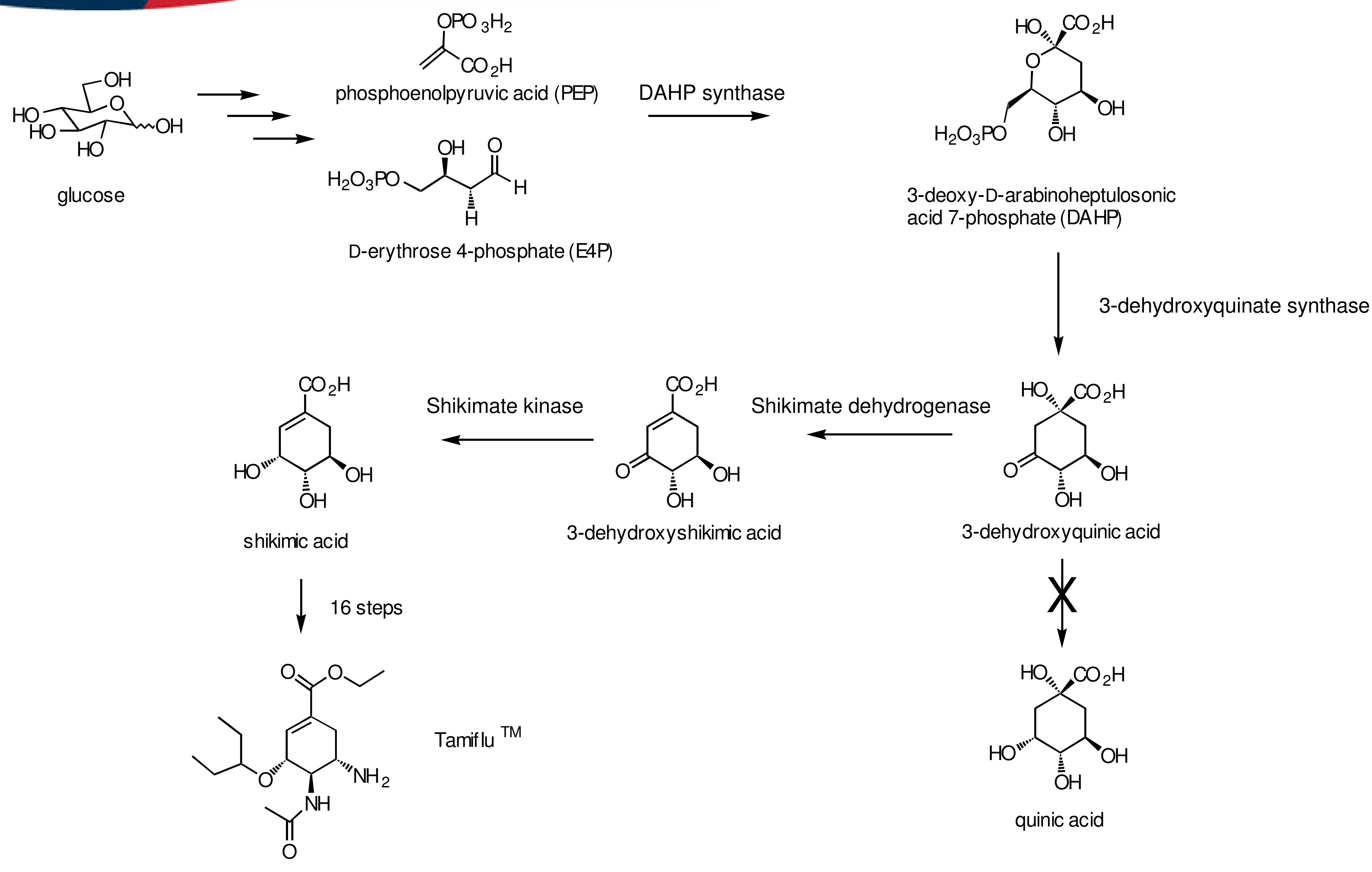
Intermediates from glucose



Intermediates from glucose

- This replaces the extraction of shikimic acid from *Illicium* trees

- Strains are tested in fed-batch fermenters ASAP
- The concentration and yield were improved

1999	27 g/L	14 %
2001	52 g/L	18 %
2003	87 g/L	36 %

The maximum theoretical yield is 43 %

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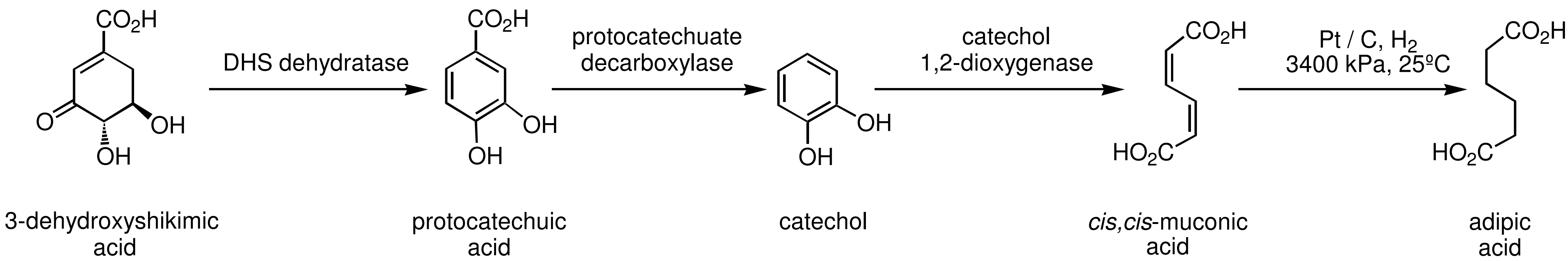
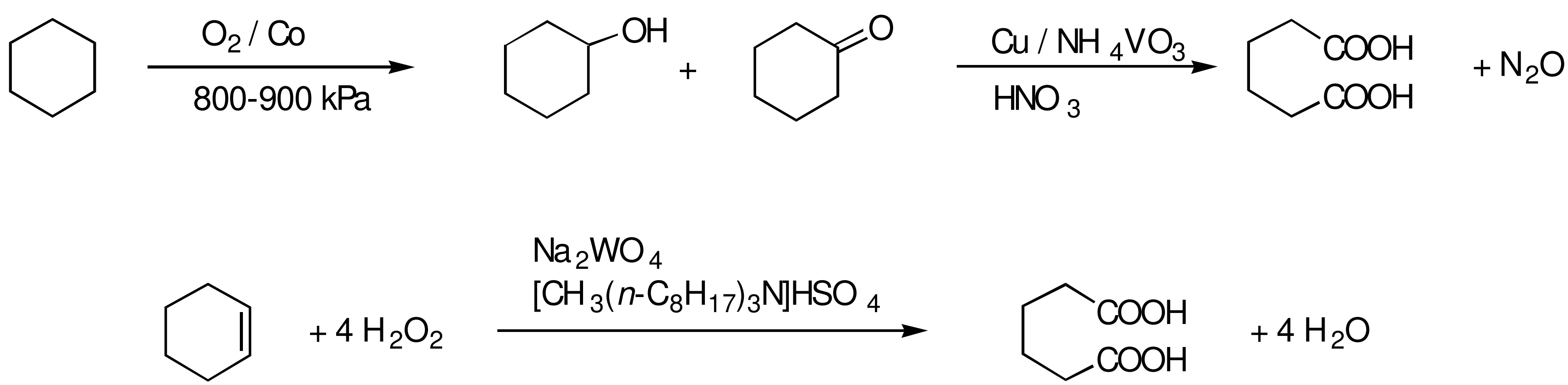
Intermediates from glucose

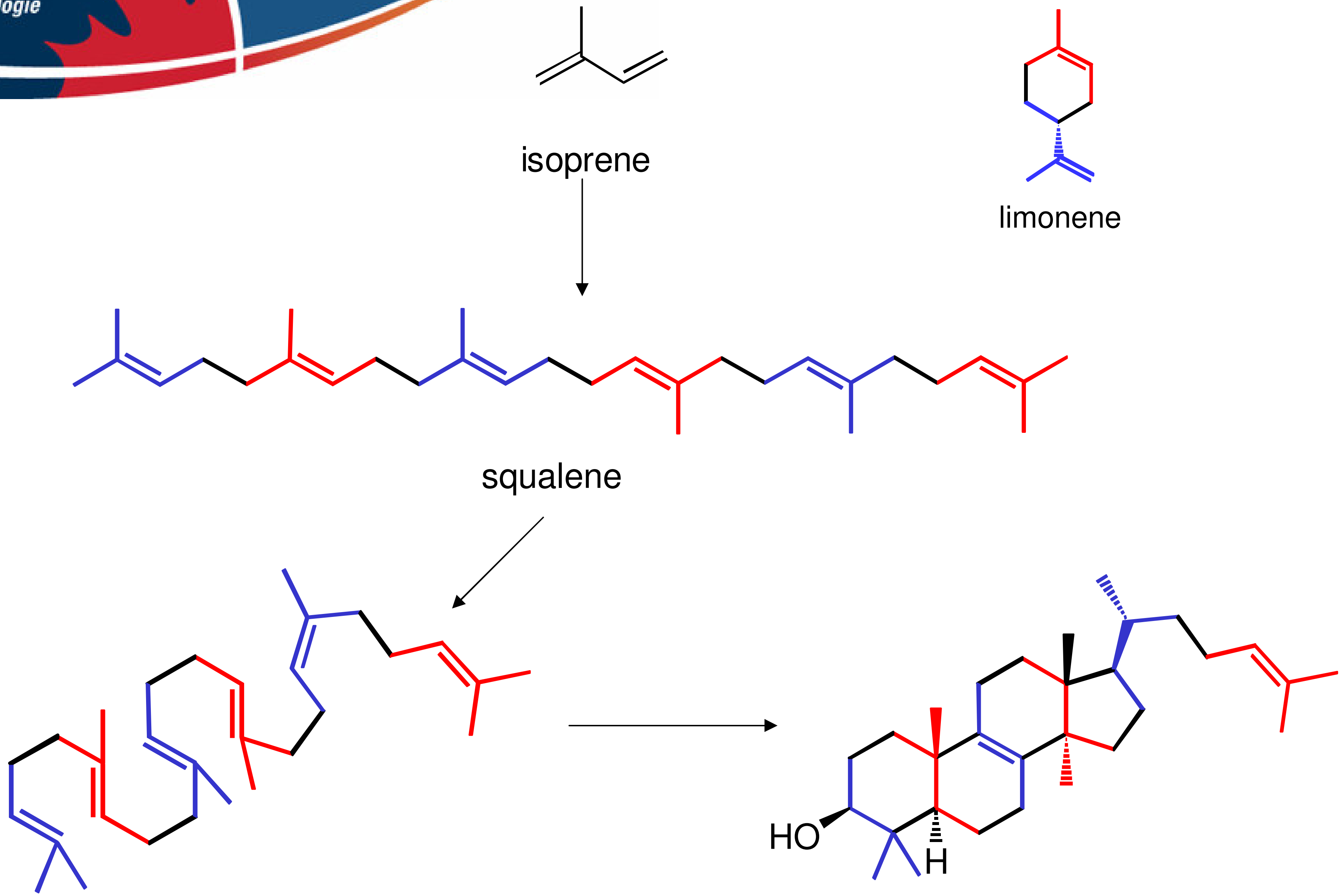
Production of adipic acid from glucose



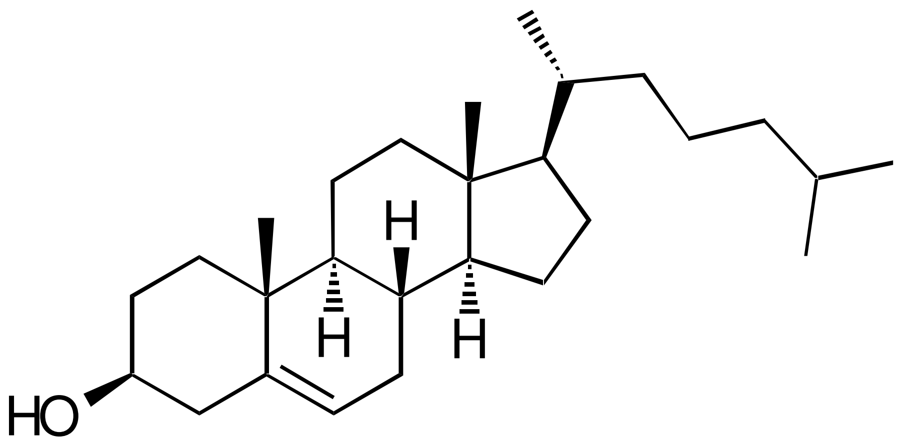
Petrochemical production of adipic acid



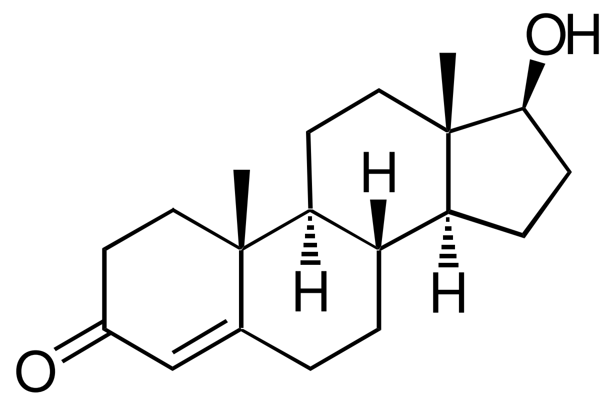
Terpenoids/isoprenoids



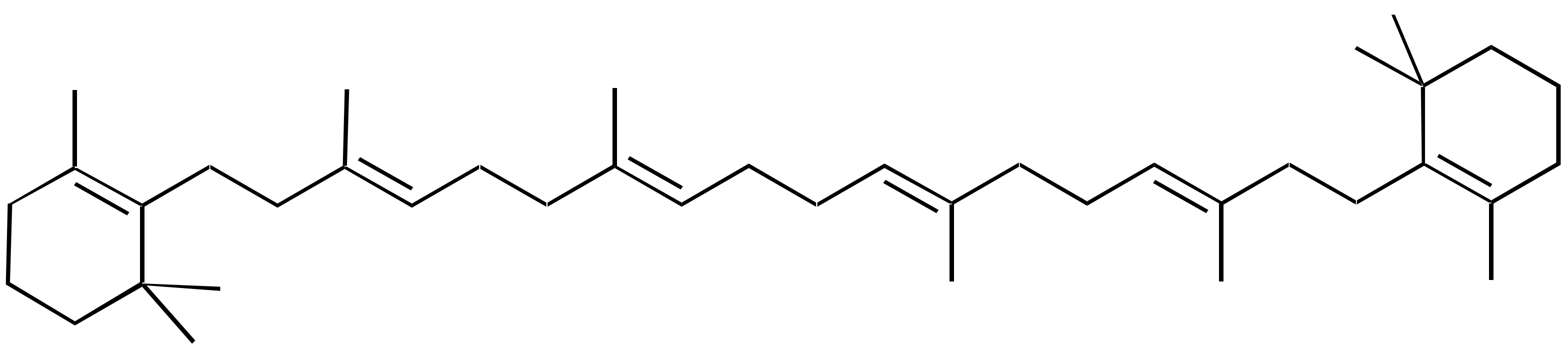
Terpenoids/isoprenoids



cholesterol



testosterone



β-carotene

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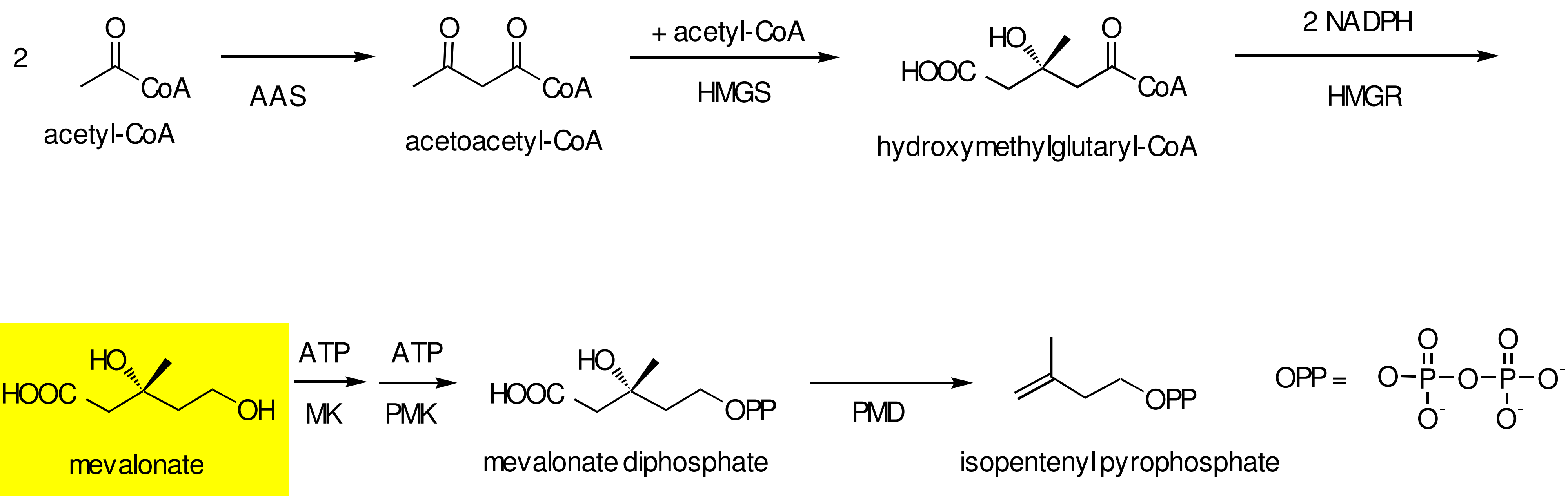
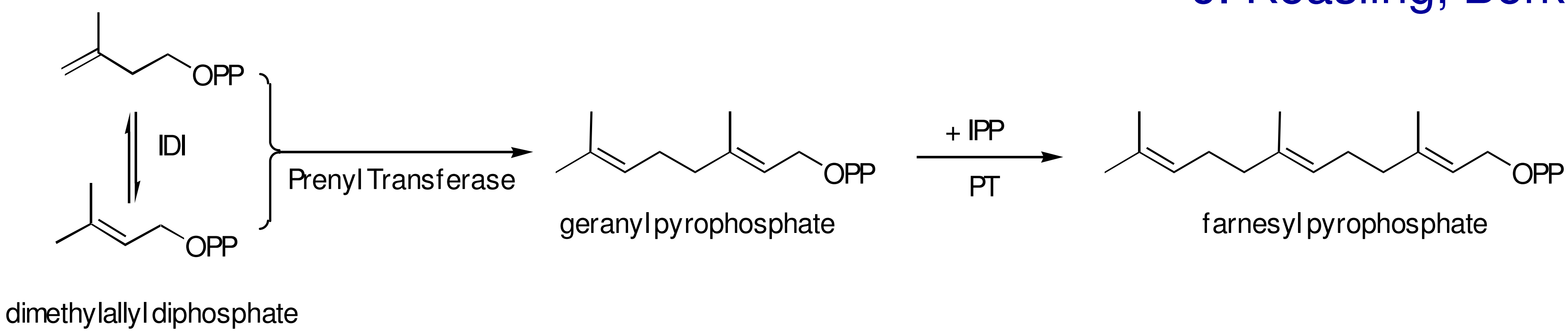
Terpenoids/isoprenoids

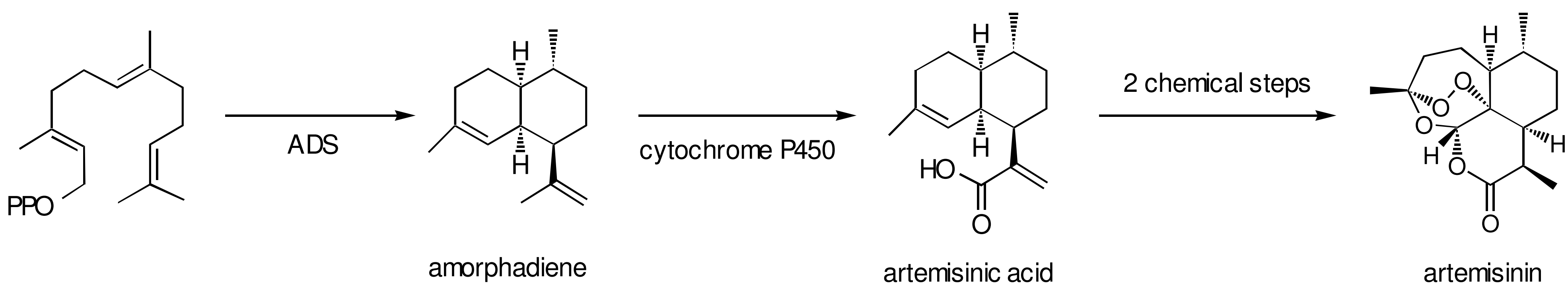
Synthesis of isopentenyl building blocks *via* the mevalonate pathway
V. Martin & J. Keasling, Berkeley



Terpenoids/isoprenoids

Synthesis of artemisinic acid and artemisinin
J. Keasling, Berkeley





Terpenoids/isoprenoids

- Artemisinin is a potent anti-malaria drug
- Extracted form *Artemisia annua*
    - Limited supplies
    - High cost
- The goal is to bring the cost to 0.10 $/g

Metabolic pathway engineering

Multidisciplinary endeavor

- Analyze the metabolic fluxes
- Add/delete genes
- Identify bottlenecks
- Correct the situation
    - Verify level of expression of enzyme
        - Promoter
        - Optimize DNA / RNA
    - Modify the enzyme for better activity
- Work in fermenters **ASAP**

- Next step: **Synthetic Biology**
    - Assemble compatible pieces of pathways

Conclusion

- "Bioproducts" covers a broad range of products
  - Nature, volume and price
- The development of new bioproducts is multidisciplinary
  - Biology (plant, cellular, and molecular)
  - Agronomy
  - Engineering
  - Chemistry
- Delocalize production (rural economy)
- Minimize the use of fossil carbon (GHGs)
- A new chemistry will emerge and replace petrochemistry