

# Targeted discovery of novel cellulases and hemicellulases and their reaction mechanisms for hydrolysis of lignocellulosic biomass: DISCO

Theme 2: Food agriculture and biotechnology  
**KBBE 3-2-01 Lignocellulosic enzymes**

Kristiina Kruus  
VTT, Technical Research Centre of Finland



Business from technology

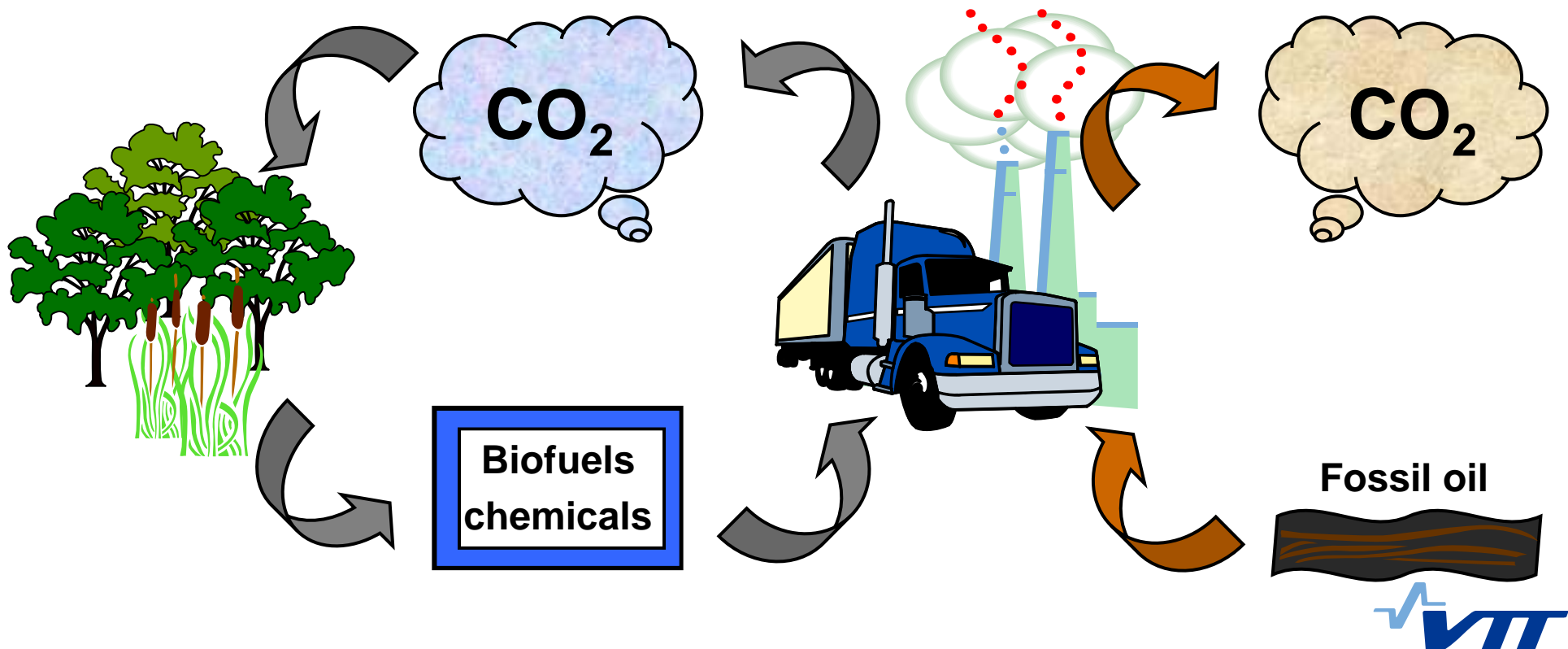
5th European Symposium on Enzymes in Grain Processing  
March 31 2008  
Norwich, UK

## Drivers for lignocellulosic biomass utilization for energy and chemicals

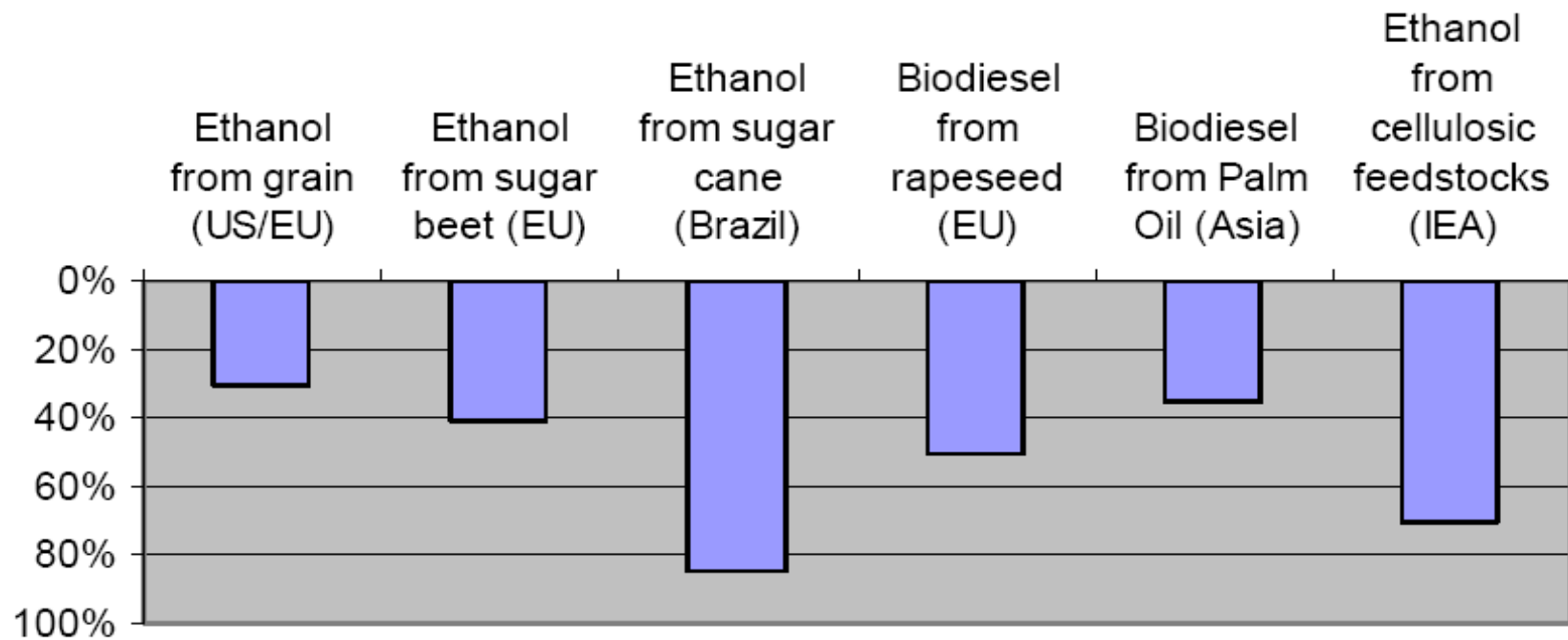
- ❑ Exhaustion of fossil energy resources
- ❑ Climate change and a need to reduce green house gas emissions
- ❑ Kyoto protocol
- ❑ EC strategy and directives to promote alternative fuels for transport
  - ❑ EU Biofuels Directive, 5.75% by 2010
  - ❑ EU Biofuels for Transport - Technology Platform: vision 25 % in 2030
  - ❑ EU Green Paper 2020: 10% natural gas, 8 % biofuels 2 % hydrogen
  - ❑ EU Biomass Action Plan (2005/628)
- ❑ Security of fuel supply and national raw materials, indigenous energy sources
- ❑ Need for raw materials not competing with food production

# THE ERA OF RENEWABLES

- Carbon dioxide is one of the major greenhouse gases
- Utilization of renewables should lead to a closed carbon dioxide cycle
- Renewables include agricultural and forest based raw materials and wastes
- According to the World Energy Council conversion of 5% of the world's biomass (a total of 13.5 billion tones) to cellulosic EtOH would meet 26% of the current energy needs (2007).



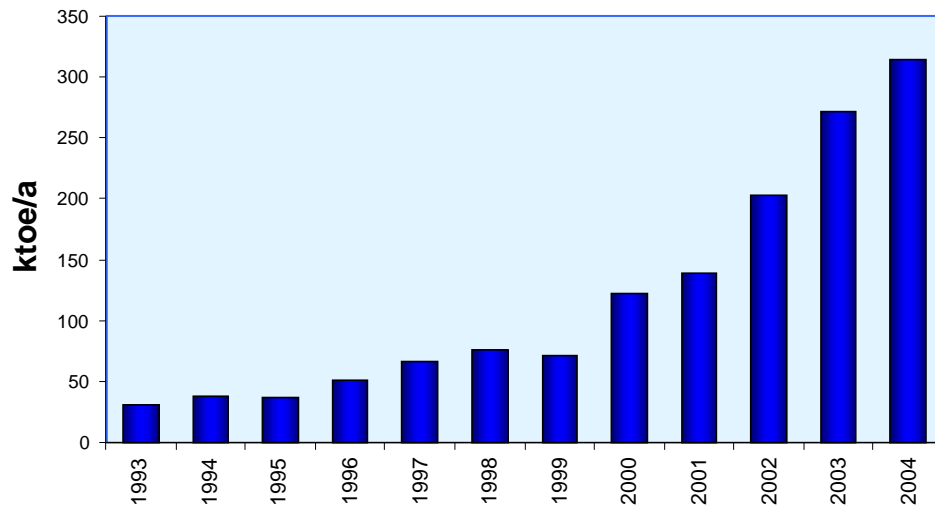
# GREENHOUSE GAS REDUCTIONS



## Production trends of biofuels in the EU

### Fuel ethanol production in EU, 1993-2004

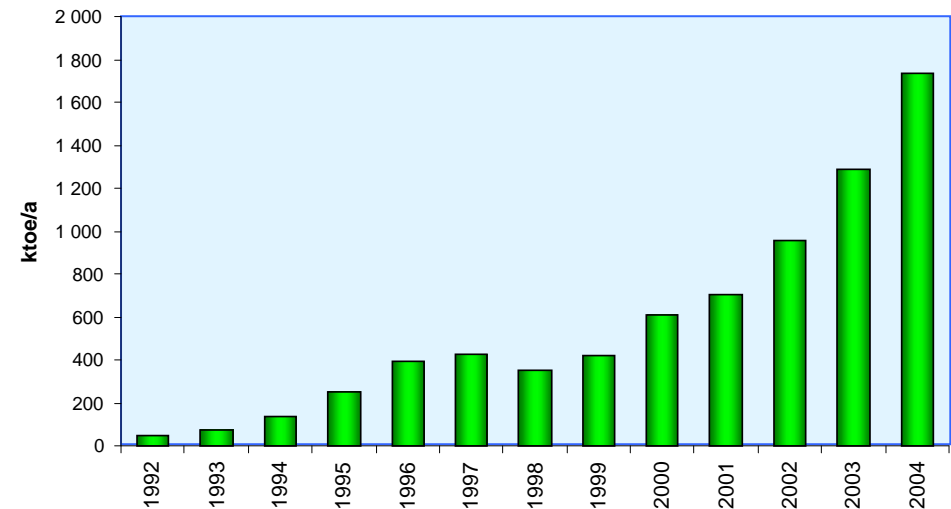
Ethanol tonne ~ 0.64 toe



source: System Solaires EurObserv'ER 2005 & <http://europa.eu.int>

### Biodiesel production in EU, 1992-2004

Biodiesel tonne ~ 0.9 toe



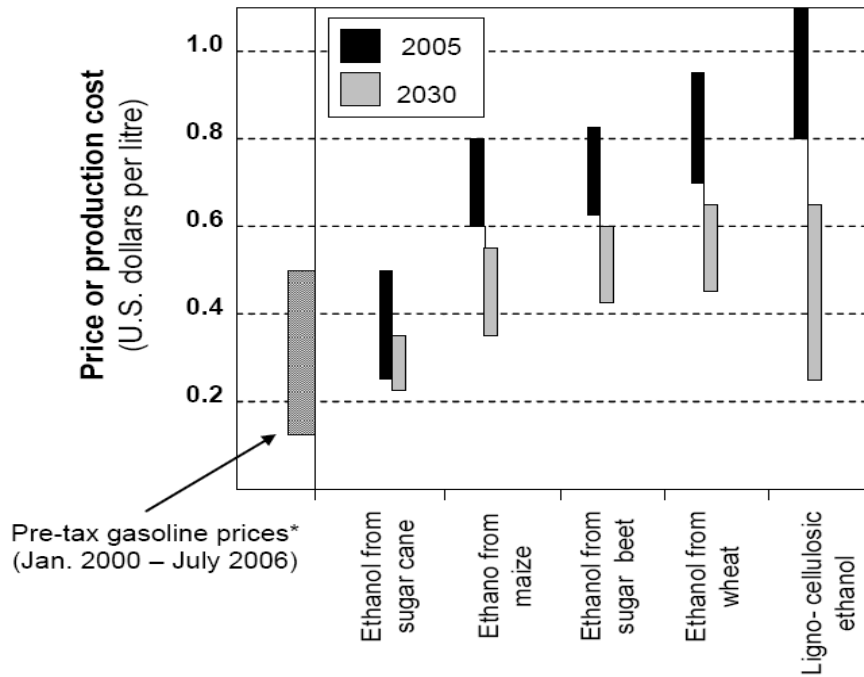
Source: European Biodiesel Board

Fast growth, but total amount still quite low!

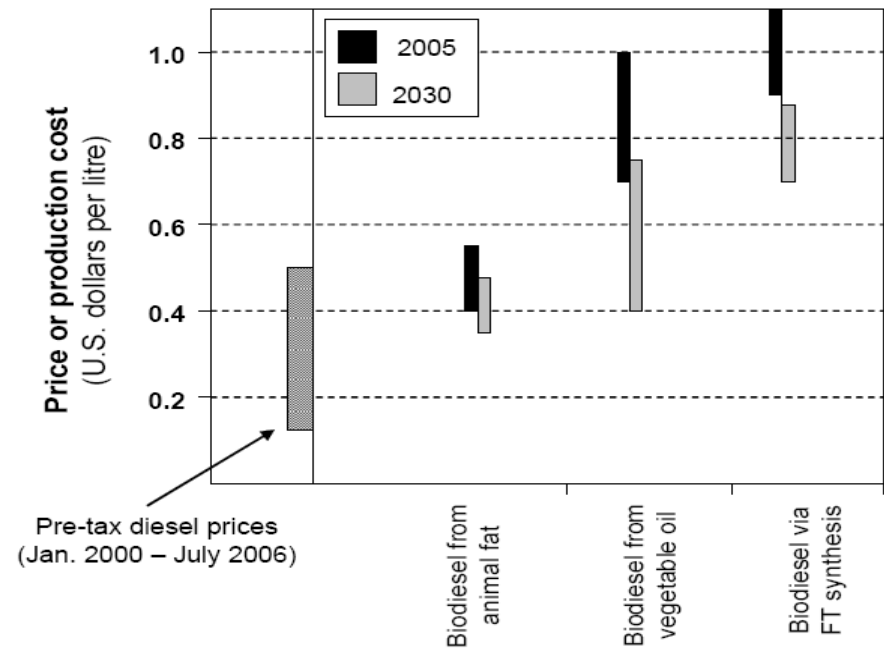
Source: EurObservER, System Solaires 2005, European Biodiesel Board

# COST ESTIMATIONS FOR 2030

## Ethanol



## Biodiesel



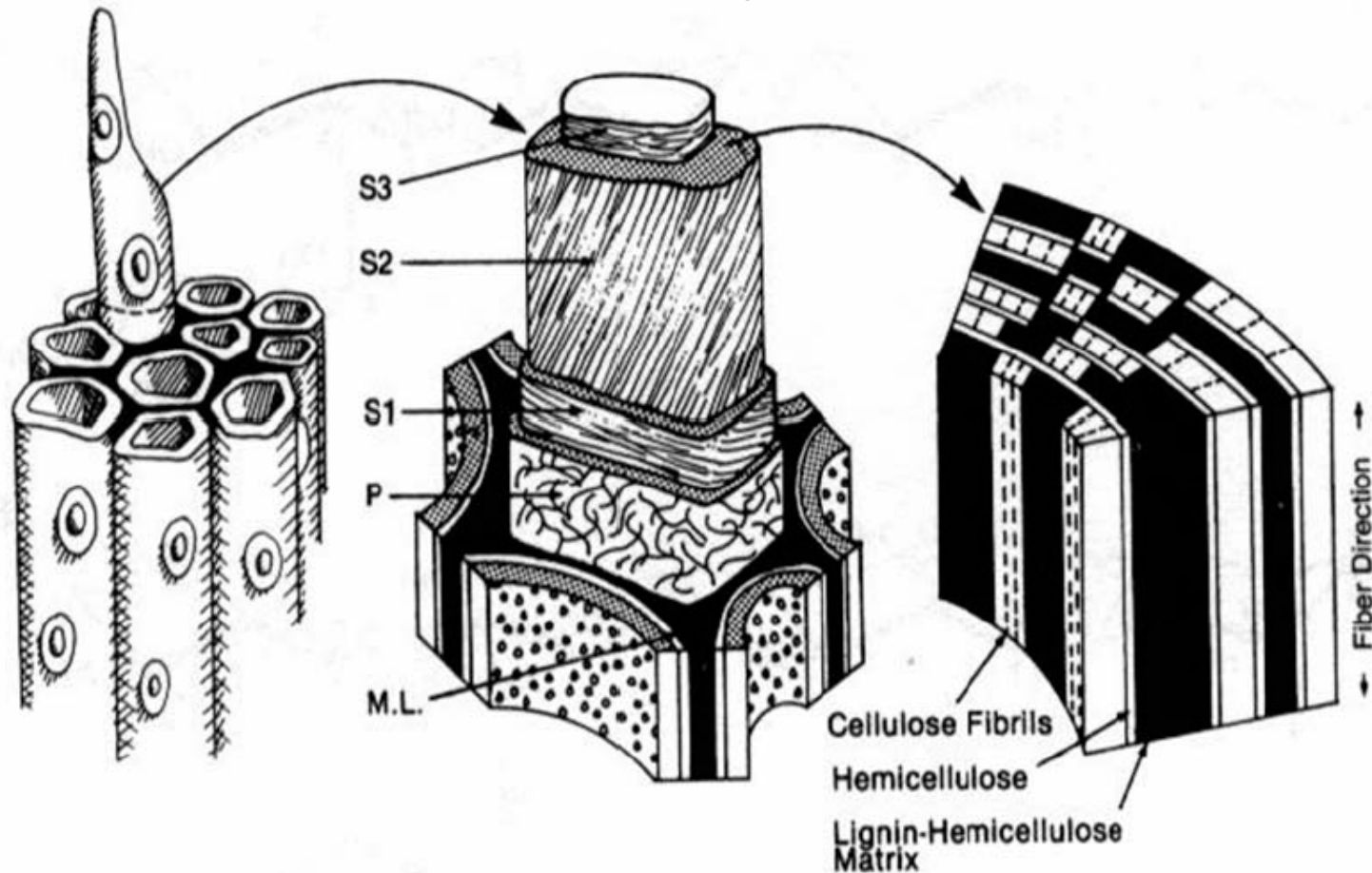
## Composition of various lignocellulosic biomass (%)



Raw material	Sugars		
	Hexoses	Pentoses	Lignin
Bagasse	33	30	29
Birch wood	40	33	21
Corn wastes	42	39	14
Nut shells	38	36	16
Barley straw	41	16	11
Pine wood	41	10	27
Rice straw	32	24	13
Wheat straw	30	24	18

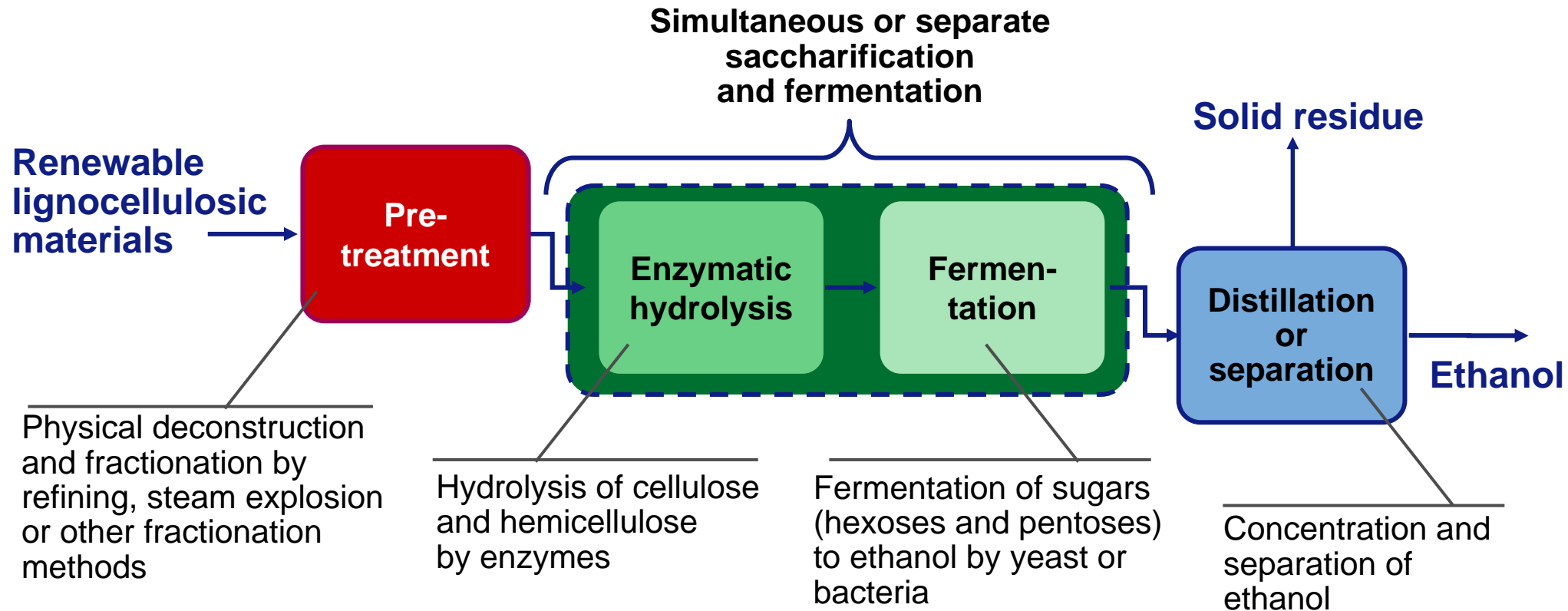
## THE RAW MATERIAL - THE CHALLENGE

Diameter of each tracheid is approximately 30  $\mu\text{m}$  (left), wood cell wall layers S1-S3: secondary cell wall layers, P: primary wall, M.L. middle lamella (middle) and lignin-carbohydrate complex of the secondary cell wall (right)



Adapted from Kirk and Cullen (1998).

# MAIN PROCESS STEPS IN LIGNOCELLULOSE-TO-BIOETHANOL PROCESS



# Enzymes participating lignocellulose hydrolysis

- ❑ **Cellulases**
  - ❑ Endo- $\beta$ -1.4-glucanases, cellobiohydrolases, and  $\beta$ -glucosidases
  - ❑ Fungal cellulases e.g. *Trichoderma*, *Humicola*, *Acremonium*
  - ❑ Bacterial cellulases e.g. *Clostridium thrmocellum*
  
- ❑ **Hemicellulases**
  - ❑ Backbone degrading enzymes
  - ❑ Enzymes affecting on the side chains
  - ❑  $\beta$ -xylosidases degradation of xylooligos
  
- ❑ **Lignin modifying enzymes**
  - ❑ Laccases
  - ❑ Peroxidases
  - ❑  $H_2O_2$  producing enzymes like aryl alcohol oxidase
  
- ❑ **Other helper enzymes/proteins**
  - ❑ Swollenin

# Development of improved enzymes and enzyme mixtures and hydrolysis technologies

## Enzyme properties

- ❑ Specific activity
- ❑ Thermal activity and stability
- ❑ End- product inhibition
- ❑ Non-specific binding

## Enzymes for efficient hydrolysis

- ❑ Composition of cellulase mixture
- ❑ Role of additional enzymes (hemicellulases, ligninases)
- ❑ Efficient production hosts systems

## Hydrolysis technologies

- ❑ Separate/simultaneous/stepwise
- ❑ Operational temperature
- ❑ Optimal use of enzymes - recycling

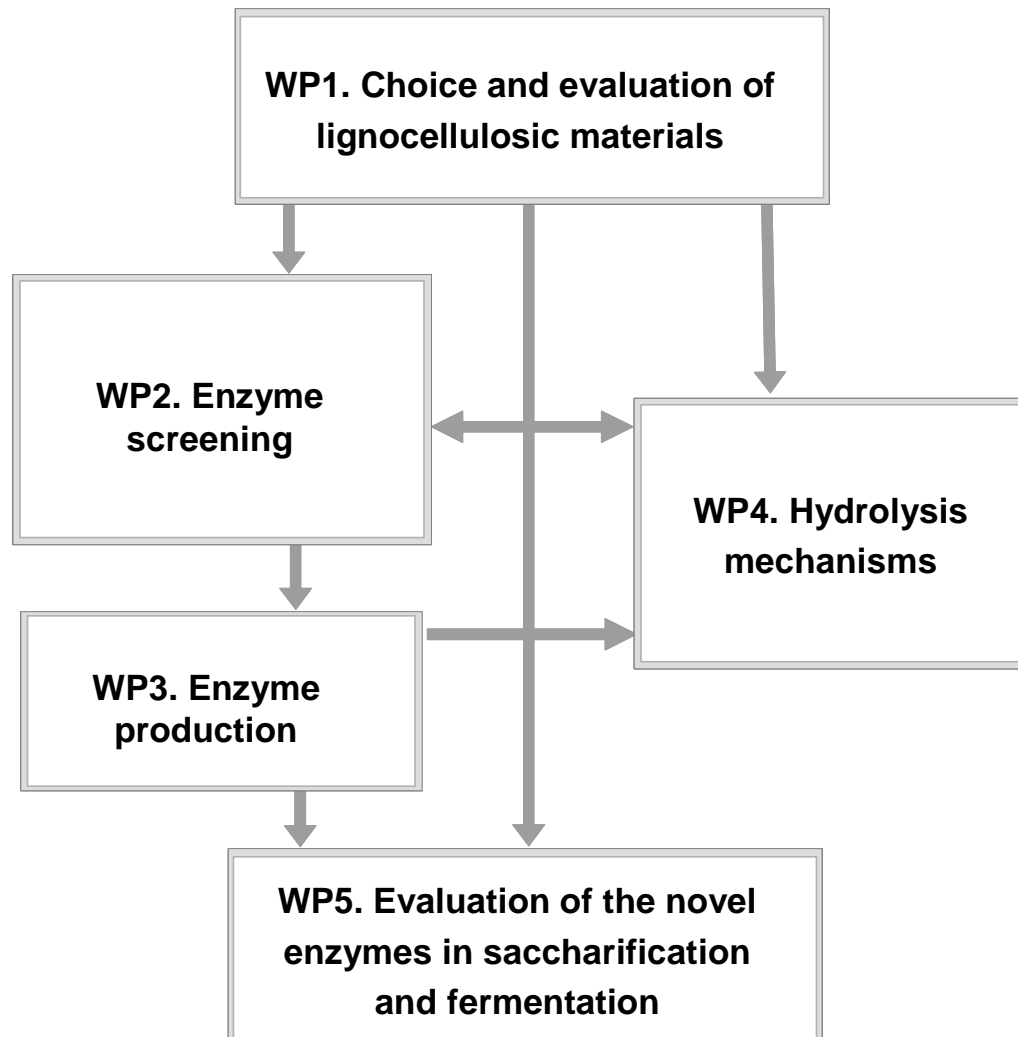


# DISCO Targeted **discovery** of novel cellulases and hemicellulases and their reaction mechanisms for hydrolysis of lignocellulosic biomass

## Aim

- ❑ to develop more efficient and therefore more cost-effective cellulosic and hemicellulosic enzymes to enhance the hydrolysis of pre-treated lignocellulosic biomass
- ❑ through the identification of novel cellulases and hemicellulases with high catalytic and synergistic activity and low affinity on lignin
- ❑ elucidation of enzymatic hydrolysis mechanisms focusing on the limiting structural factors and synergy between different cellulase and hemicellulase components
- ❑ to demonstrate the proof of concept with the cellulolytic enzymes in pilot scale using the most relevant European feedstock

## Schematic diagram describing the main inter-relationships between the 5 technical workpackages in the DISCO project.



## DISCO partnership

- ❑ Excellent combination of academic partners and industry from 7 different countries
- ❑ Totally 11 partners, from which 5 are industrial
- ❑ Expertise on lignocellulosic biomass and analyses, enzymology of lignocellulosic enzymes, enzyme discovery, enzyme production, pretreatment and hydrolysis technologies
- ❑ Whole value chain represented
- ❑ Dissemination of results: commercial and scientific exploitation

