



**“Sustainable Production and Products”
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**IMPROVEMENT OF XYLOSE UTILISATION FOR
BIOPROCESSES**

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Improvement of xylose utilisation for bioprocesses (XyloImpro)

AIMS OF THE RESEARCH

Biotechnological production of fuel bioethanol and other chemicals from lignocellulosic raw materials

Fermentation of the xylose fraction of lignocellulosics by the yeast *Saccharomyces cerevisiae*

Use of lignocellulosic raw materials technically and economically feasible

Background and significance

Global concern on **climate change** (CO₂ emissions), **cost and availability of oil**
Increase the use of renewable plant biomass as raw material in industrial processes to fuels, chemicals and materials

Biotechnologies provide **advantages over conventional chemical syntheses** (mild conditions, environmentally friendly, biodegradable products, regionally distributed and smaller scale production operations)

Worldwide strategies to promote the use of renewable resources and waste material, and the use of biotechnology

OECD (2001) biotechnology essential for sustainable development

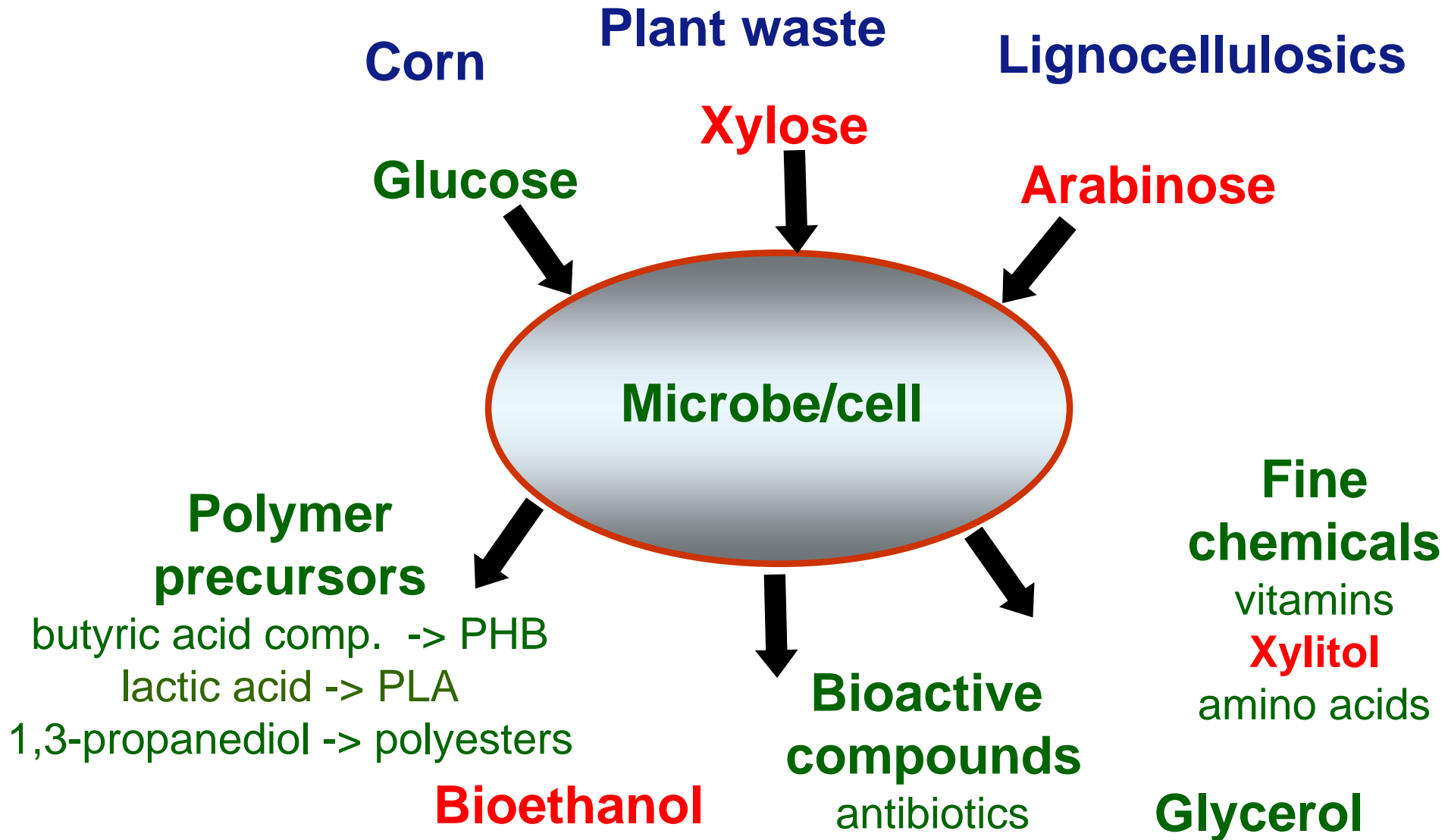
US National Research Council estimation; by 2010, 10% liquid fuels, 25% organic chemicals from renewables (presently 5%)

EU 7th FW strong support for research on utilisation of biomass

European Chemical Industry “A vision for 2025: Industrial or White Biotechnology: A driver of Sustainable growth in Europe”

EU directives; renewable proportion in transportation fuels to 5.75% by 2010, biodiesel, bioethanol (or as ETBE) up to 10% feasible in present day cars

Municipal waste



The bioethanol concept

World wide interest in production of an alternative biofuel

93% obtained by fermentation

2/3 into fuel ethanol

over 90% from crops (cane sugar, beet sugar, corn)

Ethanol to fuel; replace fossil fuels

gasohol E5/E10, E85

Lignocellulose as **cheaper** substrate for ethanol production

Fermentation of **hexose and pentose** sugars to ethanol

Energy efficiency of the process higher (positive)

with 2nd generation raw materials

(corn stover, straw, canary grass, woody materials)

Major challenges in utilisation of lignocellulosics in bioprocesses

Pre-treatment of lignocellulosics

Enzymatic hydrolysis to release the sugars from biomass polymers

Microbial conversion of all sugars present in plant biomass

Lignocellulosics rich in pentoses, e.g. xylose (20-30%)

Use of pentose sugars essential to increase the product yield and economic feasibility

Yeast *Saccharomyces cerevisiae* unable to utilise pentoses

Metabolic engineering

Oxidoreductive pathway (xylose reductase, xylitol dehydrogenase)

Isomerisation pathway (xylose isomerase)

Aims and approaches

Further develop and improve *S. cerevisiae* for production of bioethanol (and other chemicals) from the xylose fraction of plant biomass

S. cerevisiae; (most) desired process organism for industrial scale – excellent hexose-fermenting microbe, robust, high ethanol tolerance, tolerance to acids and inhibitors, extensively used in large scale processes, GRAS

Improvement of **xylose transport to the cell**

Engineering of **cellular redox metabolism** for improved xylose fermentation

Understanding of **cell responses and regulatory mechanisms** involved in xylose utilisation

Improvement of xylose transport to the cell

No specific xylose transporters found in eukaryotic microbes that improve xylose uptake capacity of *S. cerevisiae* (bacterial ABC transporters, eukaryotic facilitated diffusion carriers, proton symporters; *C. intermedia*)

S. cerevisiae; Hexose transporters transport xylose; low affinity, glucose preferred substrate

Anaerobic metabolism (fermentation) calls for efficient uptake of the substrate

Engineering of cellular redox metabolism for improved xylose fermentation

Different cofactor specificities of xylose reductase [NAD(P)H] and xylitol dehydrogenase (NAD⁺)

Cofactor imbalance will eventually halt cellular metabolism

Transhydrogenase cycles, heterologous activities (*K. lactis* GAPDH)

Redox balancing is important in almost all metabolic engineering efforts

Understanding of cell responses and regulatory mechanisms involved in xylose utilisation

Previous studies (transcriptomics, proteomics, flux analyses) on overall metabolic responses of yeast utilising xylose

Xylose does not provide clear signals to the cells

Neither glucose repressed (fermentative, glucose metabolism)
nor glucose de-repressed (respiratory e.g. ethanol metabolism) state

Comparison of oxidoreductive (XR-XDH) and isomerisation (XI) pathways

Comparison of xylose-not naturally-metabolising (*S. cerevisiae*) and xylose-naturally-metabolising (*Pichia stipitis*) yeasts

Bioreactor cultivations (micro/an/aerobic), systems biology methodology