EPSO Biofuels Workshop

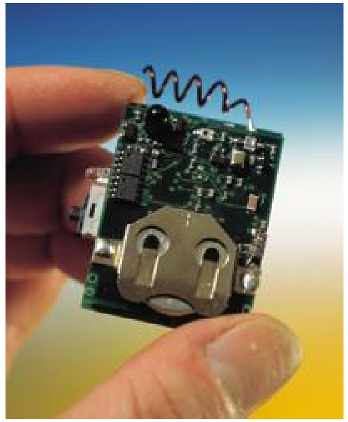
London, 28-29 May 2008

Metabolic engineering for production of fuels and chemicals from biomass

Merja Penttilä

VTT

Teknologiasta liiketoimintaa



VTT

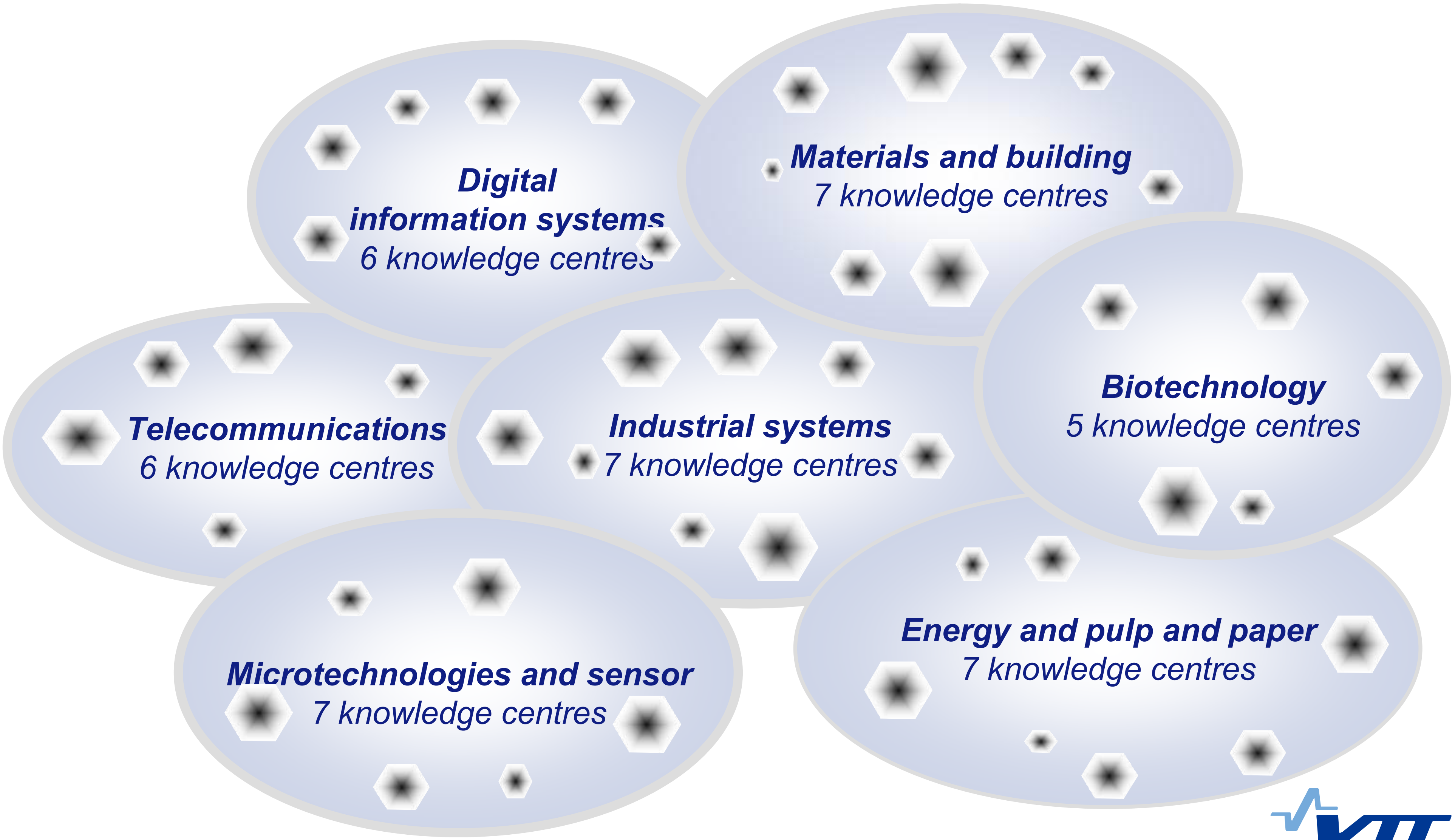
(Valtion Teknillinen Tutkimuskeskus)

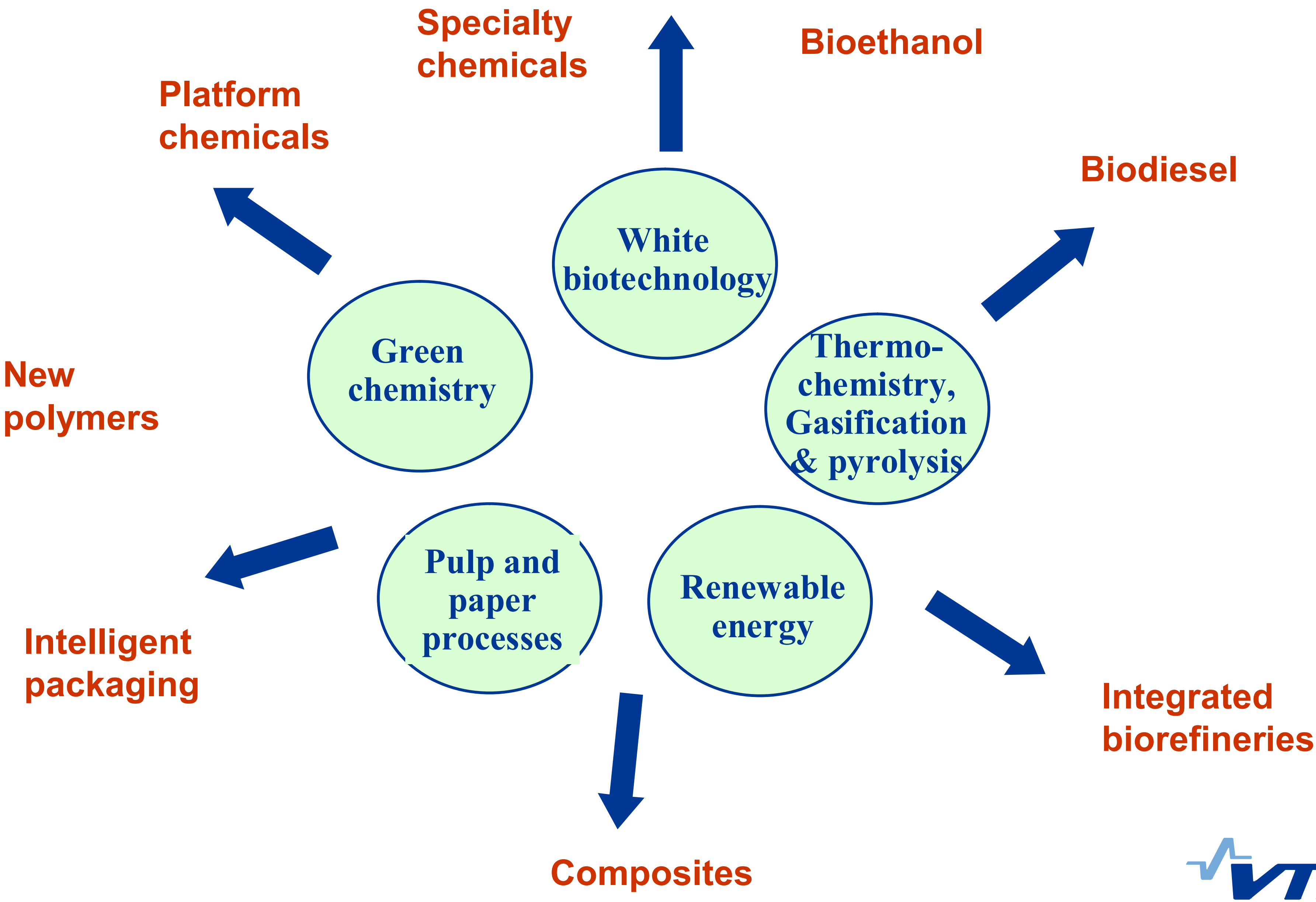
Through creating and applying technology, we actively enhance the competitiveness of industry and other business sectors, and thus increase the welfare of society.

- Governmental, not-for profit research organisation
- Biggest in Nordic countries
- Staff: ~ 2800
- R&D activities range from fundamental public research to confidential industrial projects

RESEARCH AND DEVELOPMENT

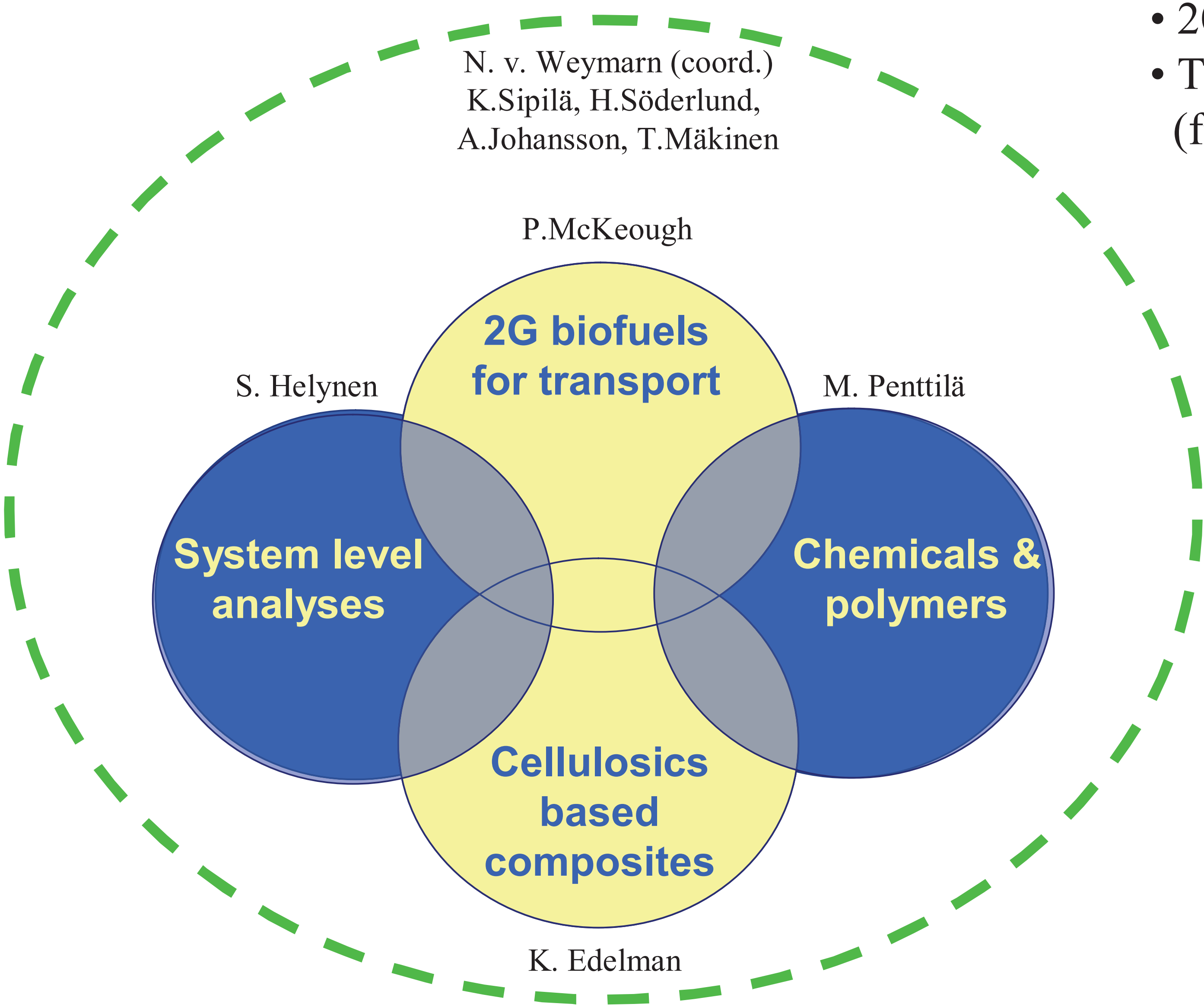
7 knowledge clusters and 45 knowledge centres



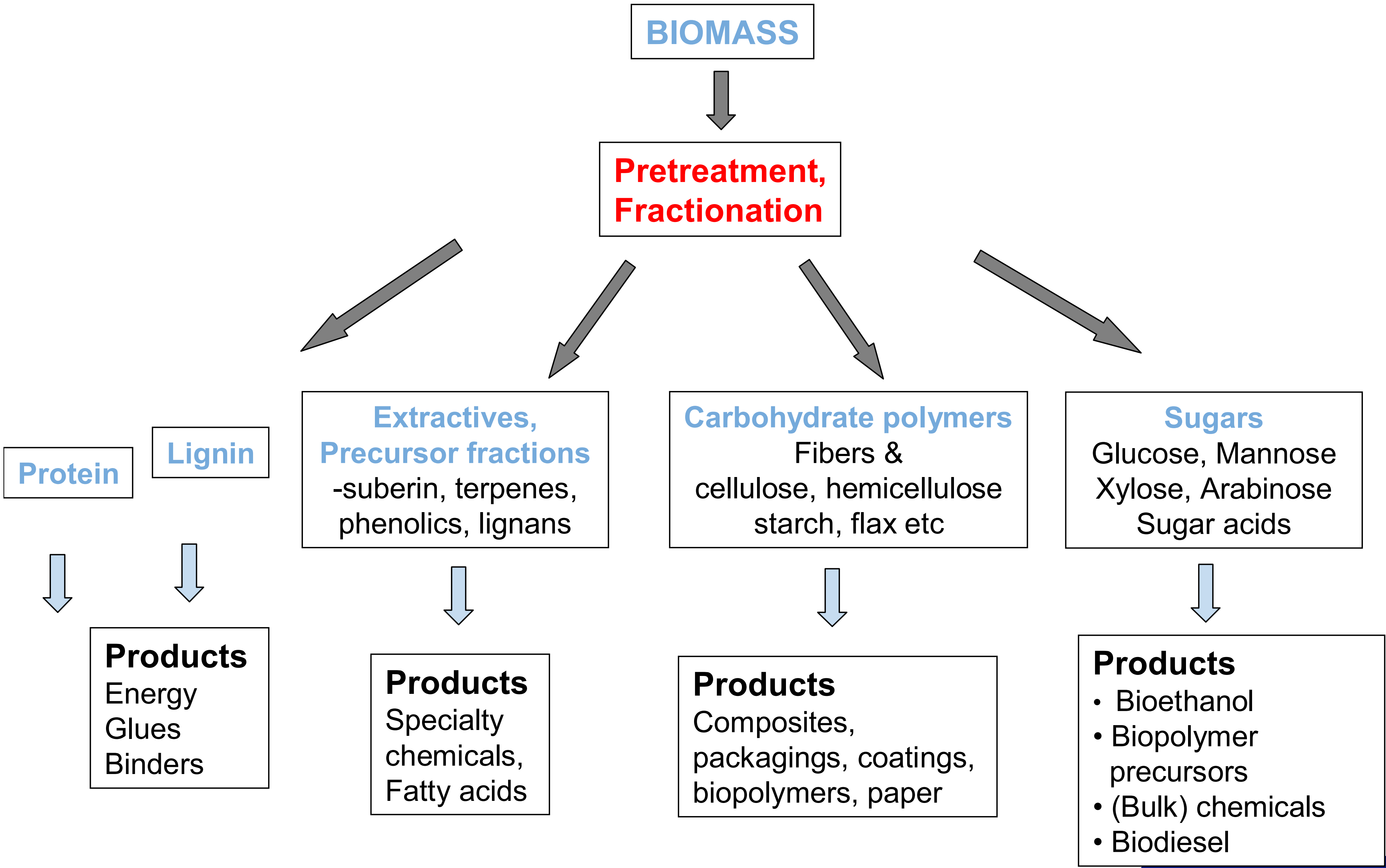

Specialty chemicals
Bioethanol
Platform chemicals
Biodiesel
White biotechnology
Green chemistry
Thermo-chemistry, Gasification & pyrolysis
New polymers
Pulp and paper processes
Renewable energy
Intelligent packaging
Integrated biorefineries
Composites

VTT Biorefinery Theme

- 2007-2011
- Total budget ~ 40 mill.€ (from VTT ~ 20 mill.€)



Good fibers, easy sugars, interesting extractives ?

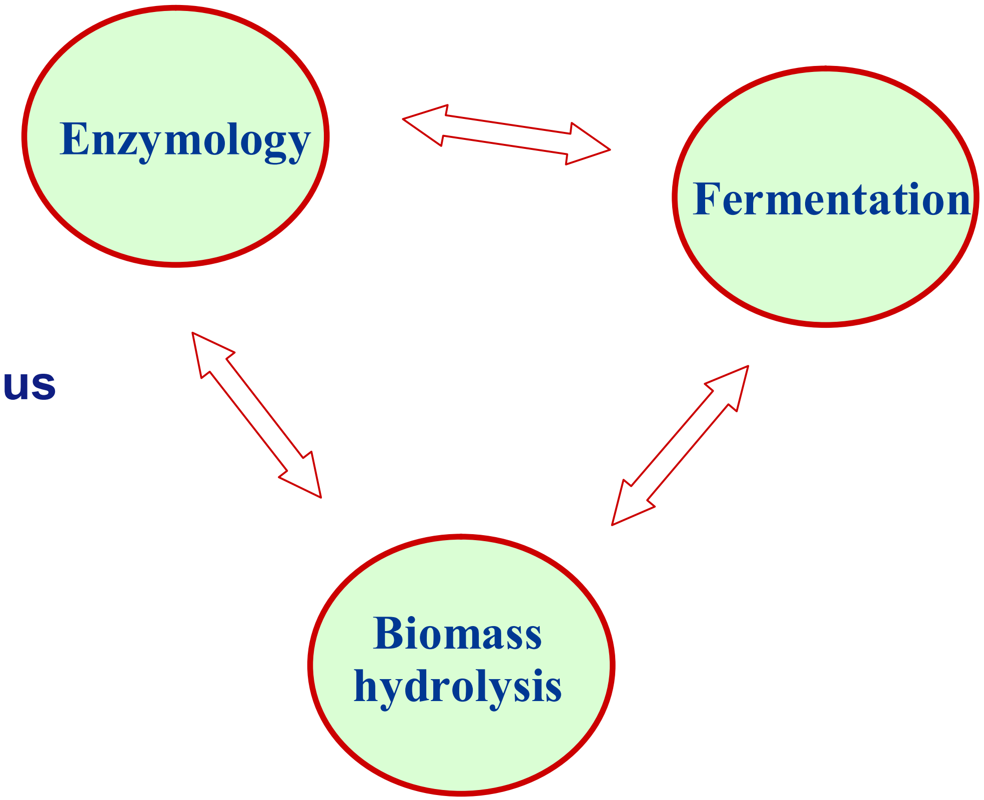


VTT is an expert in the essential disciplines of Industrial Biotechnology

Research started in the 70's

Glucanases
Cellulases

Filamentous fungus
Trichoderma reesei



Brewing

Baker's yeast
Saccharomyces cerevisiae

VTT is an expert in the essential disciplines of

Industrial Biotechnology / White Biotechnology

Last 25 years: > 1000 publications, > 100 patents

Metabolic engineering

e.g. production of bioethanol, xylitol and lactic acid with yeasts, pharmaceutical compounds with plants

Enzymology

e.g. structure - function of hydrolytic and oxidative enzymes, enzyme screening & protein engineering

Fermentation

e.g. pilot scale production of proteins and metabolites

Systems biology

mRNA, protein, metabolite, flux -level omics, TRAC, metabolomics software, biomarkers...

Protein production

e.g. Trichoderma molecular biology & production strain engineering

Fiber modification

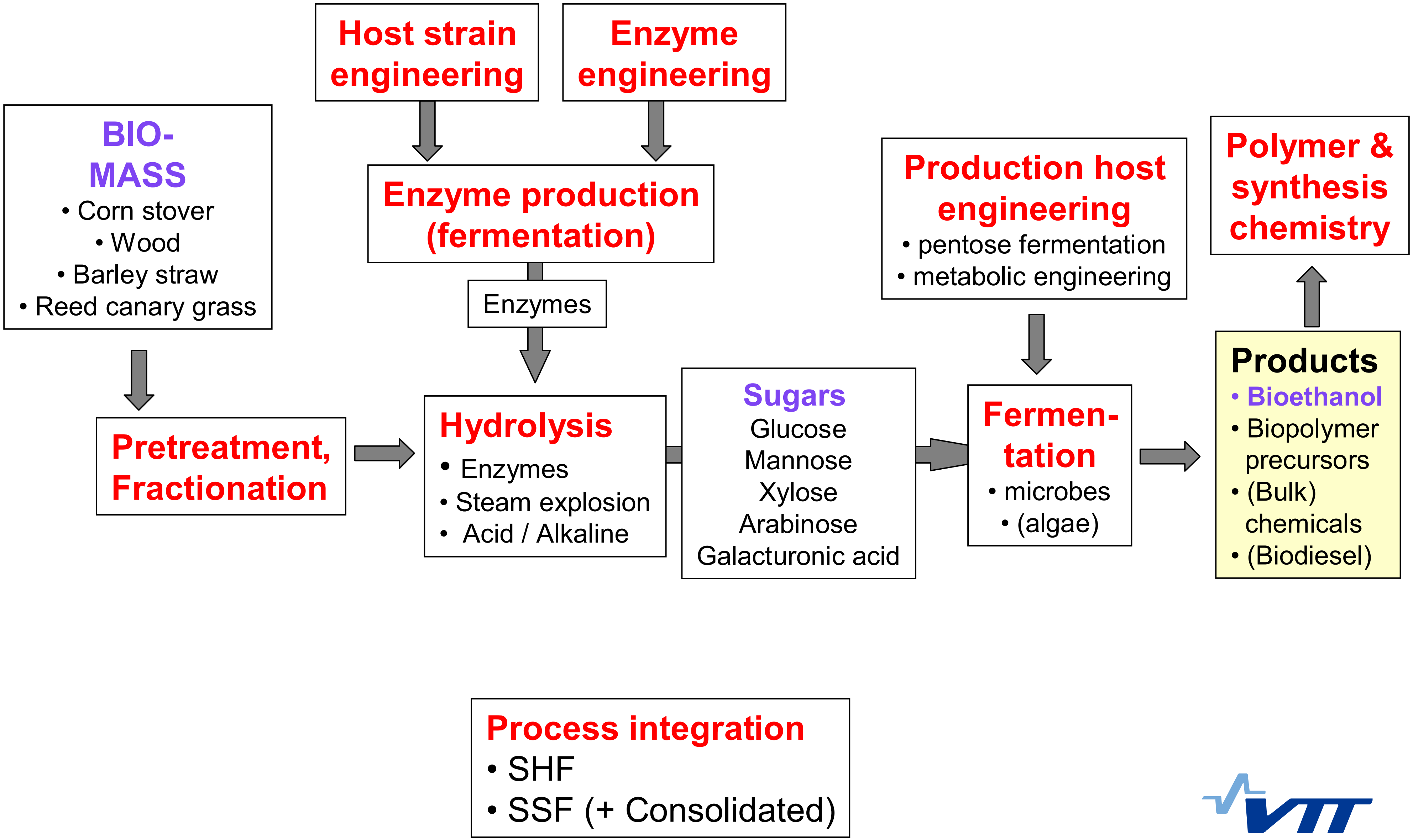
enzymatic fiber engineering

Biomass hydrolysis

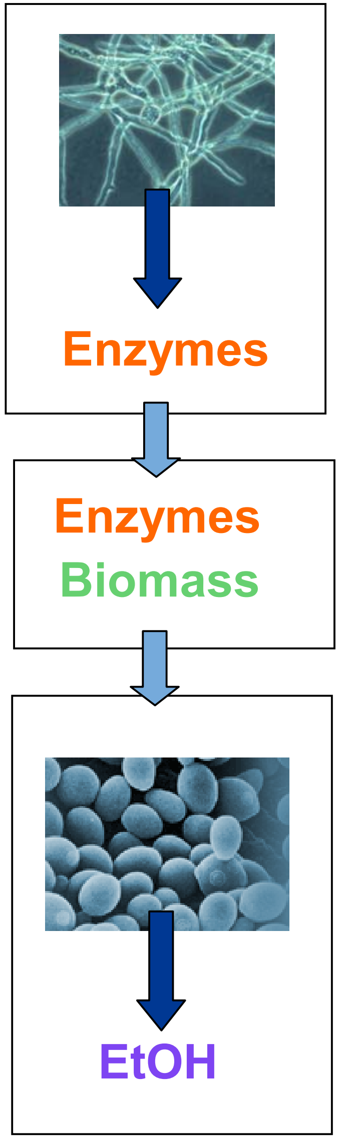
e.g. optimising enzymatic hydrolysis of biomass: soft wood, corn stover, canary grass, ..

VTT

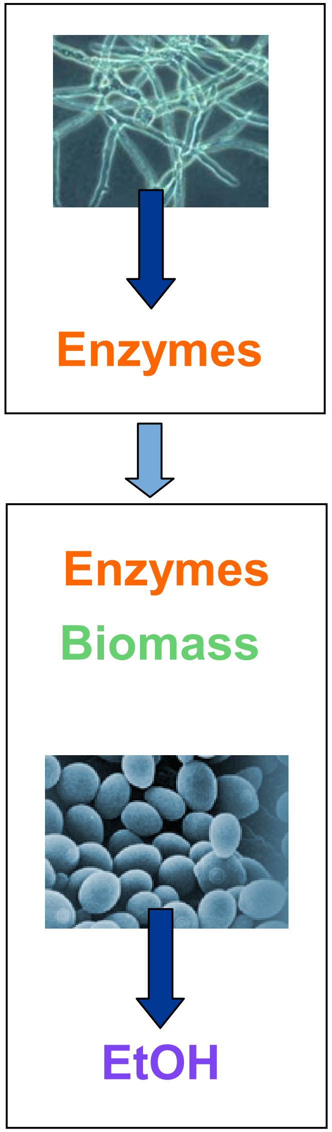
Biorefinery - Sugar platform – Essential technologies



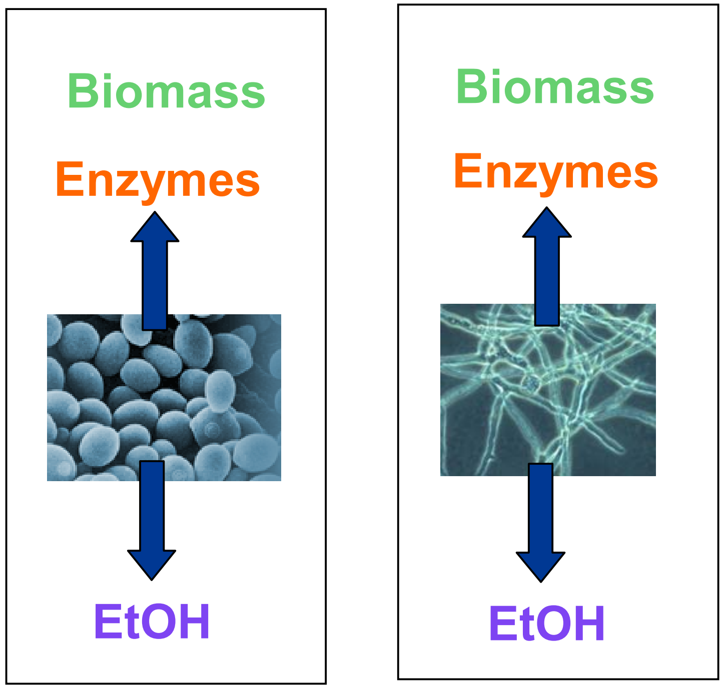
SHF
Separate hydrolysis and fermentation



SSF
Simultanous saccharification and fermentation



CBP
Consolidated bioprocessing



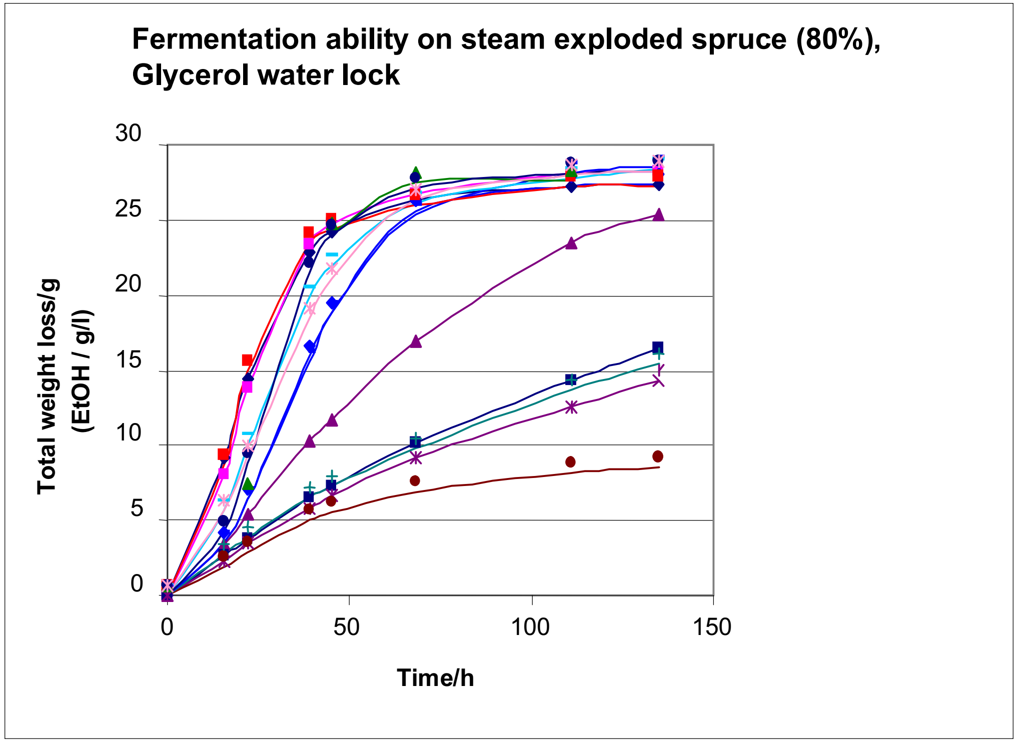
In SSF and CBP temperature for hydrolysis and fermentation needs to be compatible

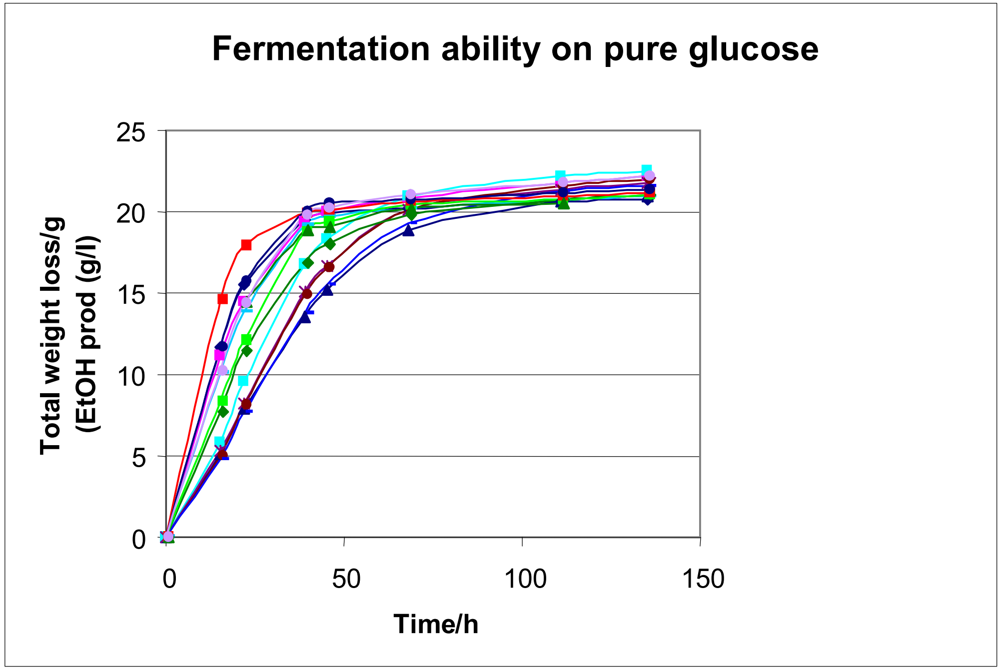
Requirements of the production organism for 2nd generation bioethanol

- Generally robust in large scale production (~ 1 000 m^3)
- Ethanol tolerant (> 8%)
- Good fermentation rate (>1g/L/h)
- Compatible with processes not prone to contaminations (low pH, high T)
- Require minimal nutrients
- Flexible with air (facultative anaerobes)

- Tolerant towards inhibitors in lignocellulosic hydrolysates (acetic acid, furfural, phenolic compounds)
- Able to ferment all major biomass C6 and C5 sugars (glucose, xylose, arabinose)
- Able to ferment certain sidestream sugars (arabinose, galacturonic acid)

Comparison of different *S.cerevisiae* yeast strains





Examples of microbes studied for bioethanol production

- *Zymomonas mobilis*; naturally ethanol producing

  engineered to ferment xylose and arabinose
- *E.coli*; utilises naturally xylose, engineered to produce ethanol
- *Klebsiella;* engineered to produce ethanol
- *Clostridia* (gas fermentation)

General drawbacks with bacteria: low inhibitor, low pH, ethanol tolerance and process robustness, more complex nutrient requirements

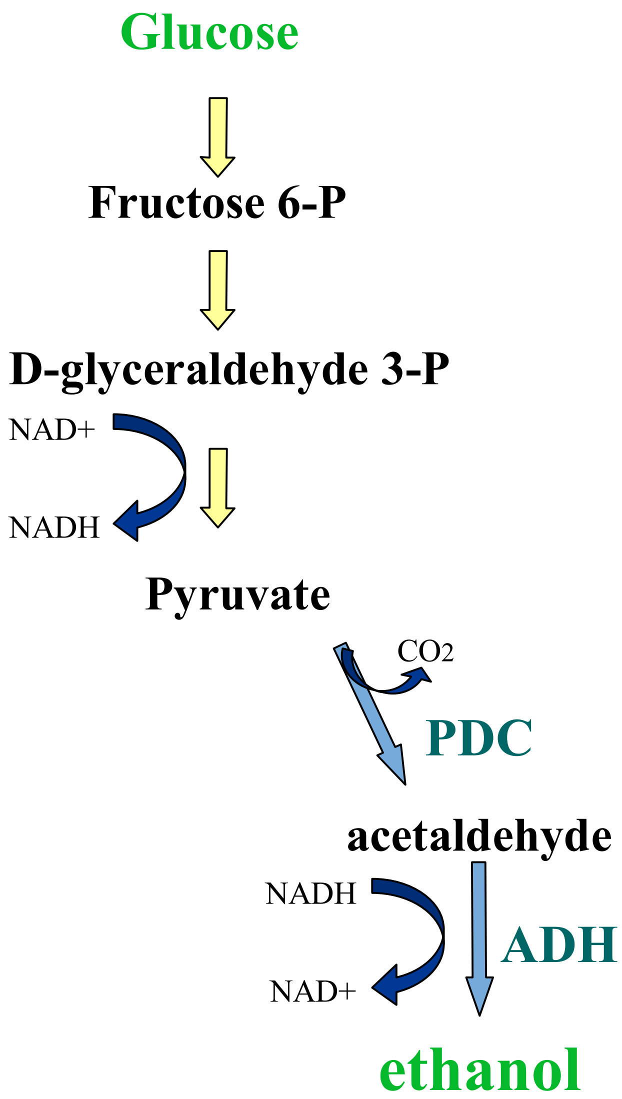
- *Pichia stipitis;* naturally xylose utilising
- *Saccharomyces cerevisiae;* current production host for ethanol, GRAS

  - engineered to ferment xylose and arabinose

  - engineered for reduced byproduct formation, e.g. xylitol and glycerol

Drawbacks with engineerd *S.cerevisiae*: insufficient C5 fermentation rate, C5 uptake

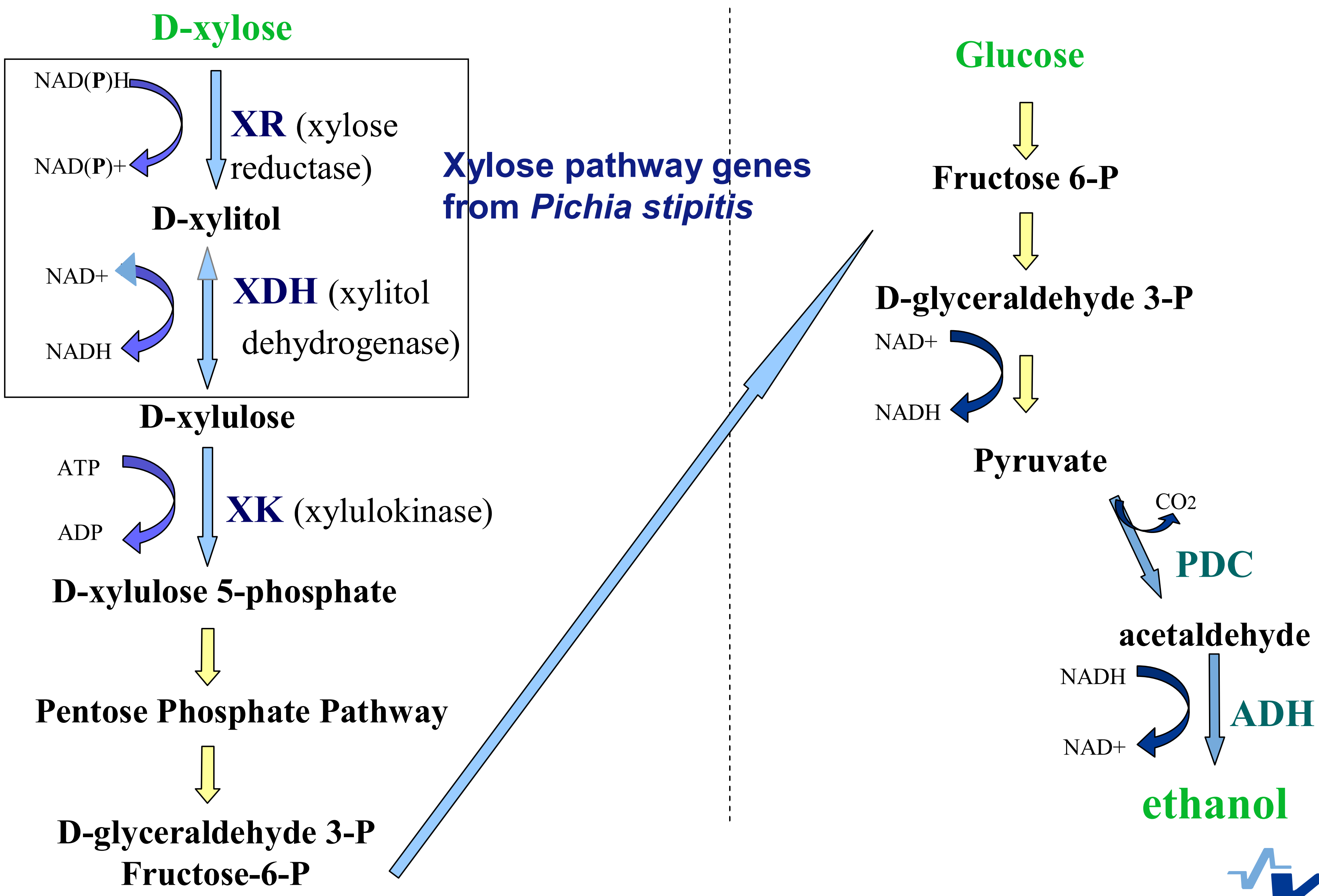
Glycolysis



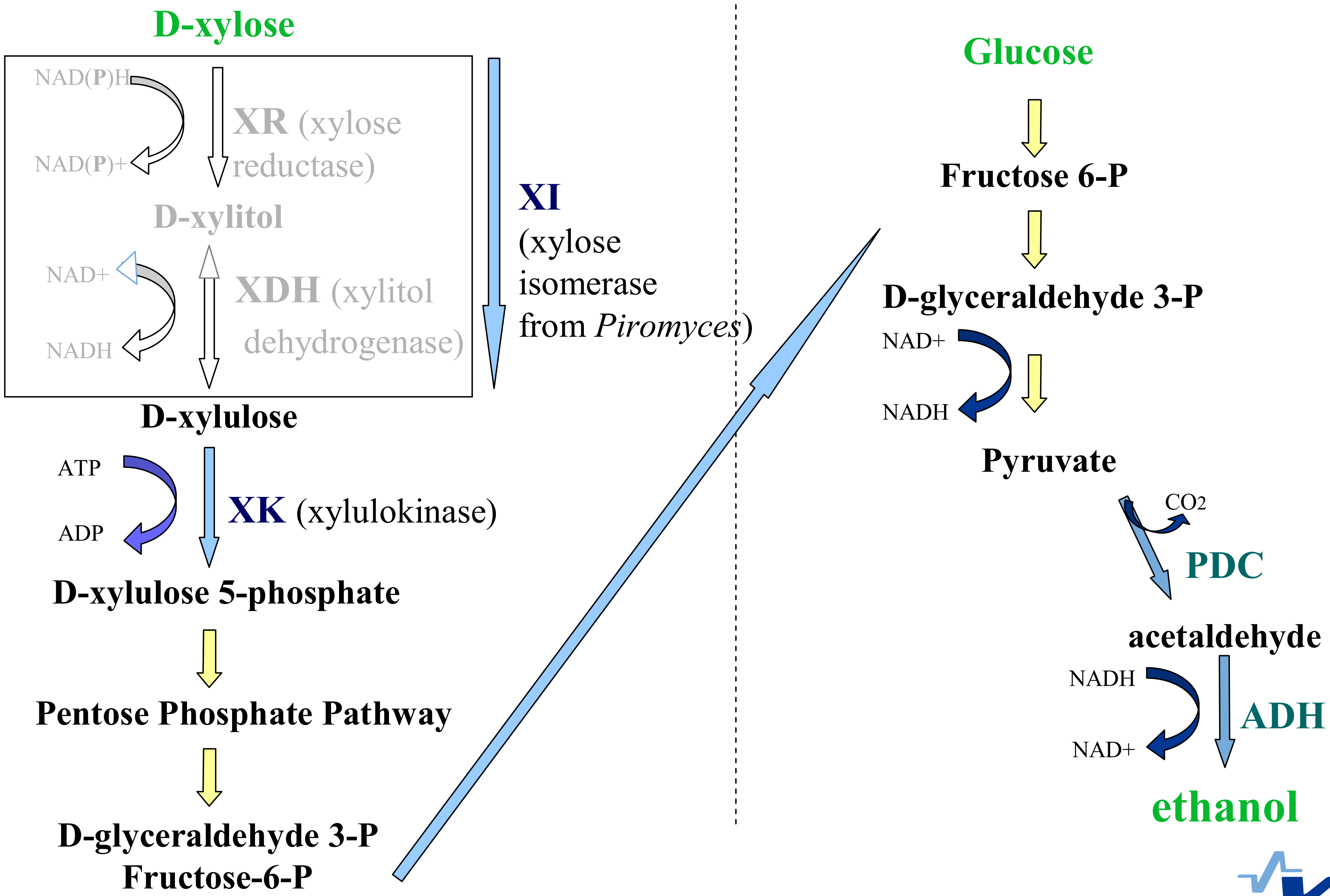
Glucose fermentation is efficient

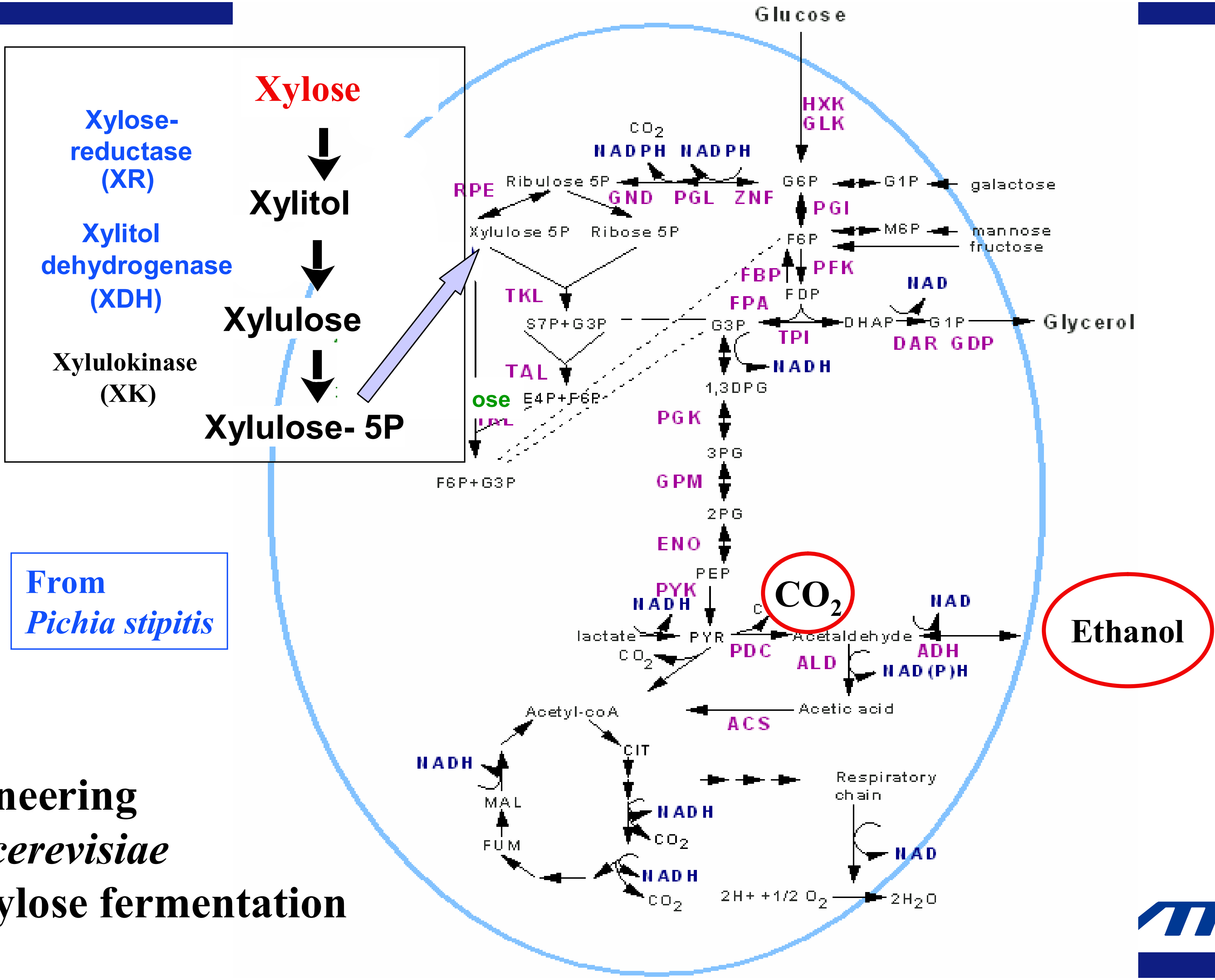
Saccharomyces cerevisiae yeast

Pentose utilisation | Glycolysis



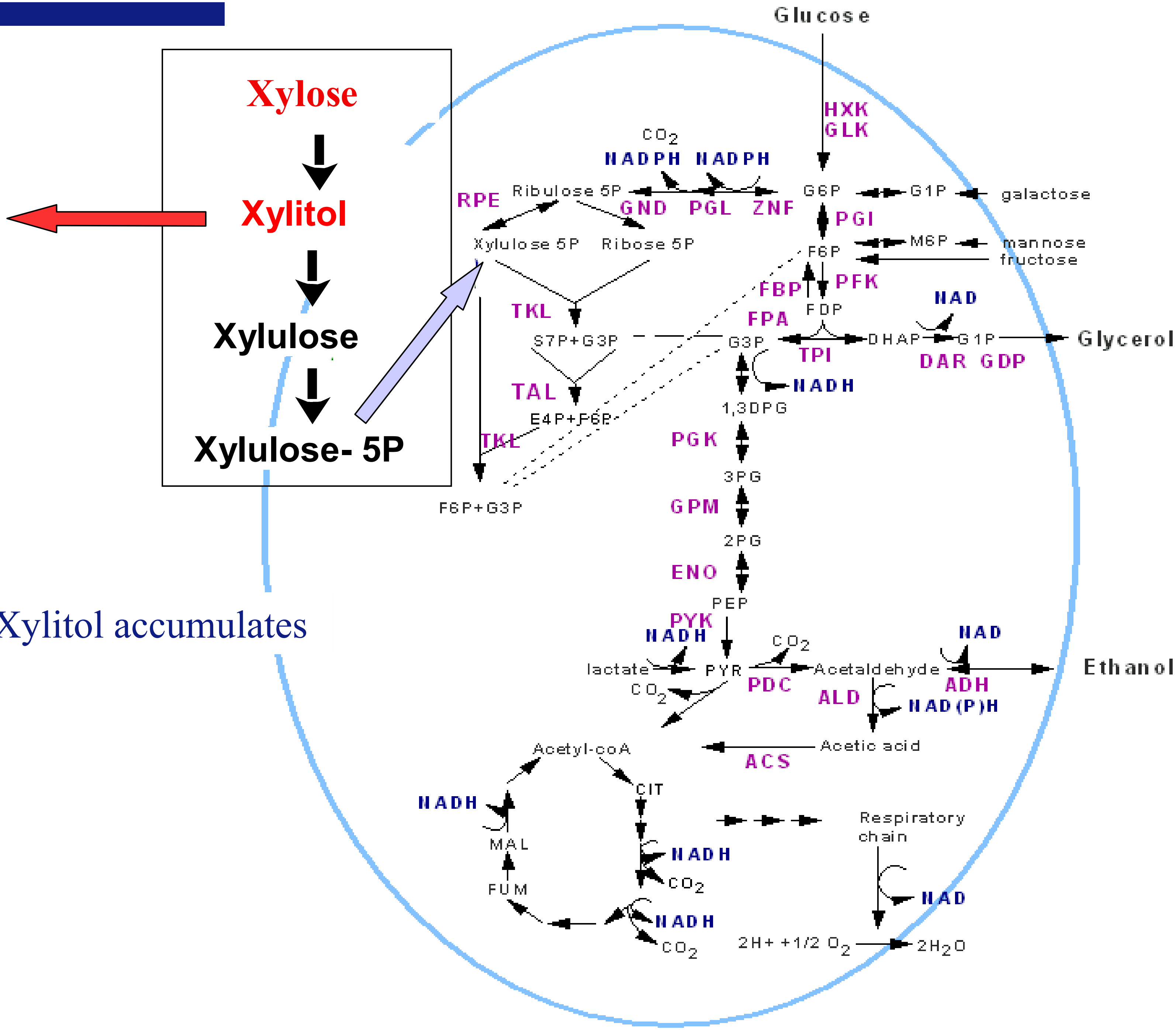
Pentose utilisation — Glycolysis





From
Pichia stipitis

Engineering of *S.cerevisiae* for xylose fermentation



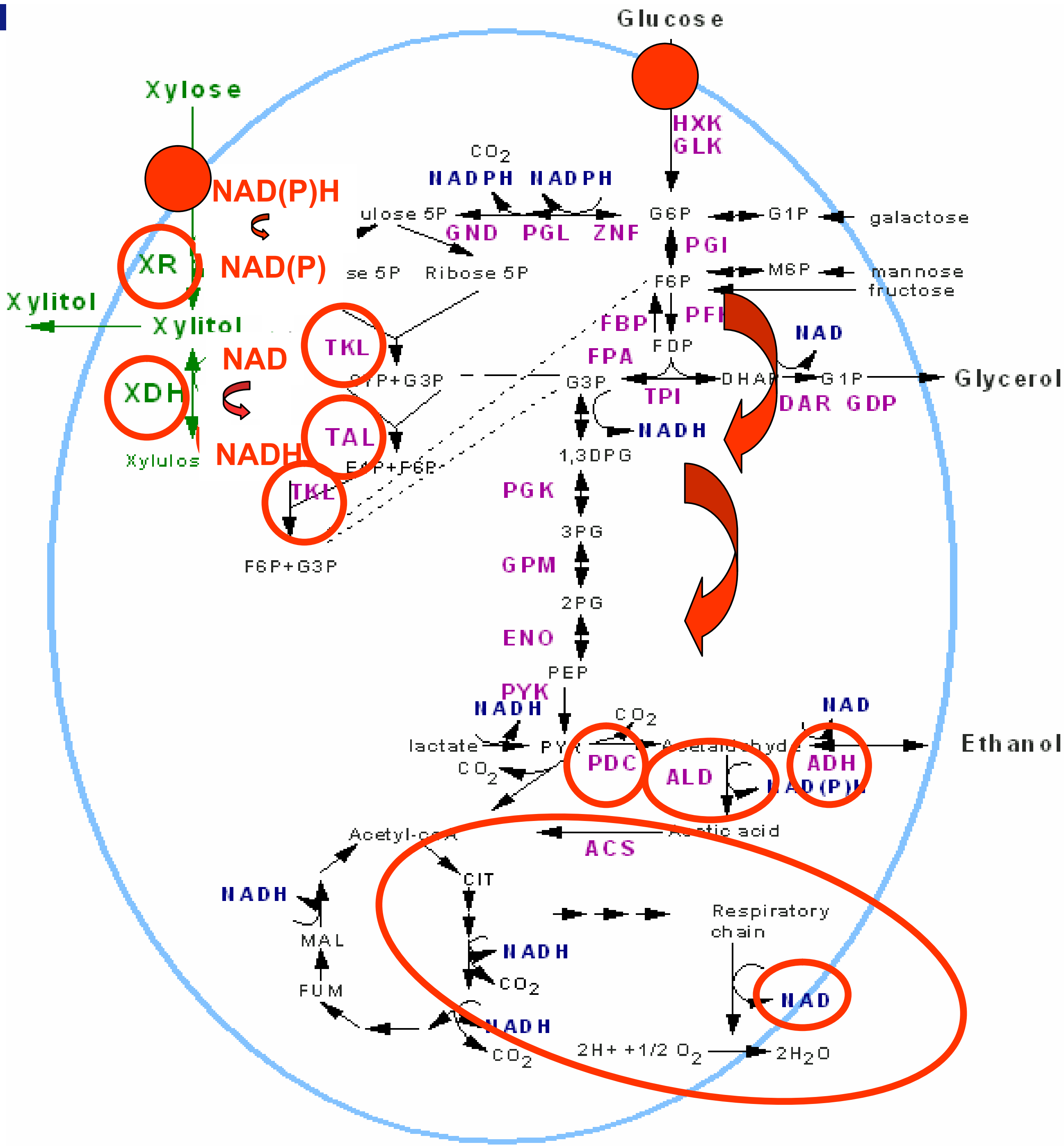
- Xylitol accumulates

Bottle neck suspects in xylose fermentation in recombinant *S.cerevisiae*

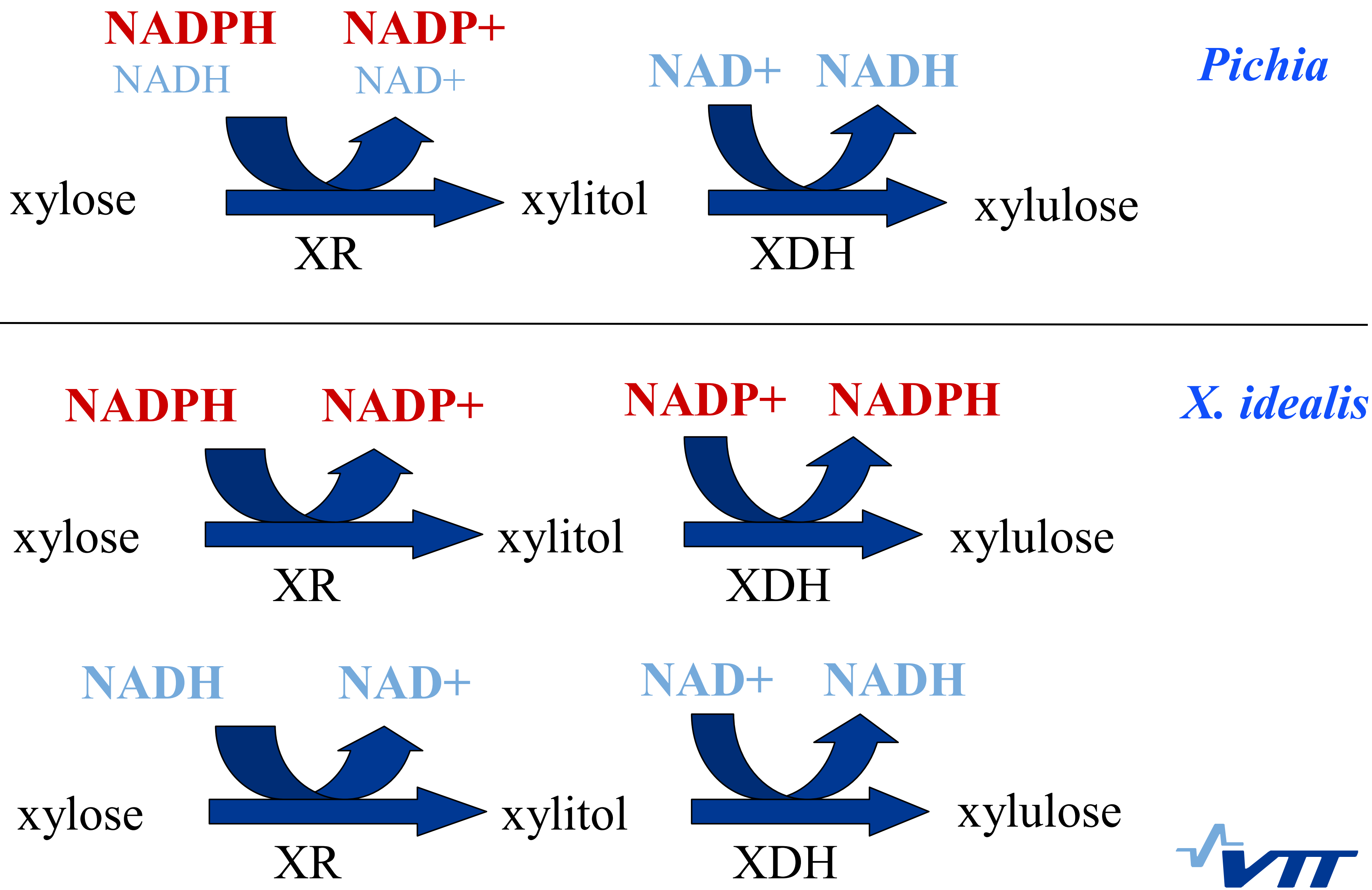
- ***sugar uptake***
- ***inefficient PPP***
- ***general regulatory issues?***

- ***redox imbalance;***

problematic in particular in anaerobic conditions, where respiration is not functioning in NAD+ regeneration



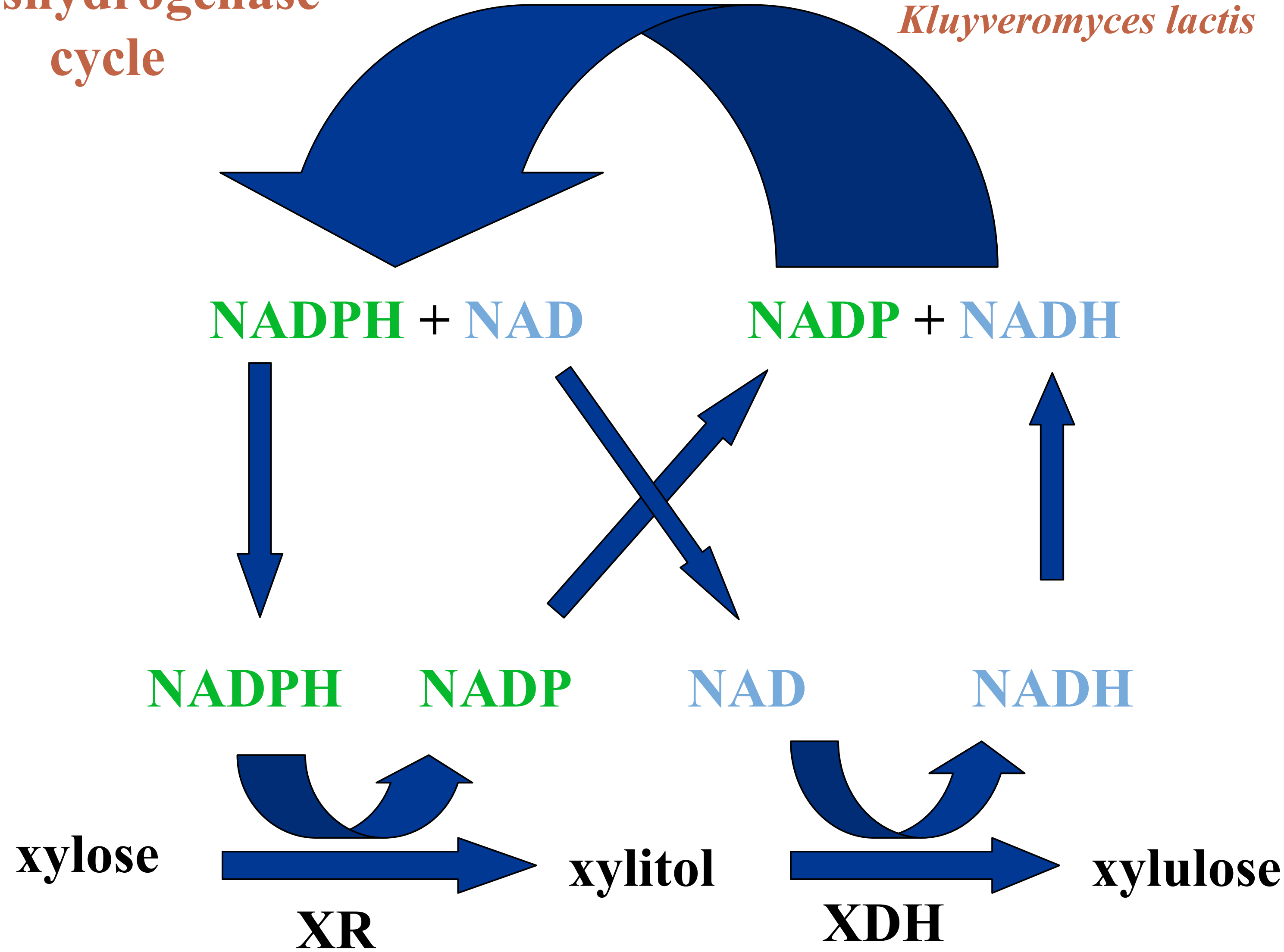
Cofactor imbalance in the xylose pathway



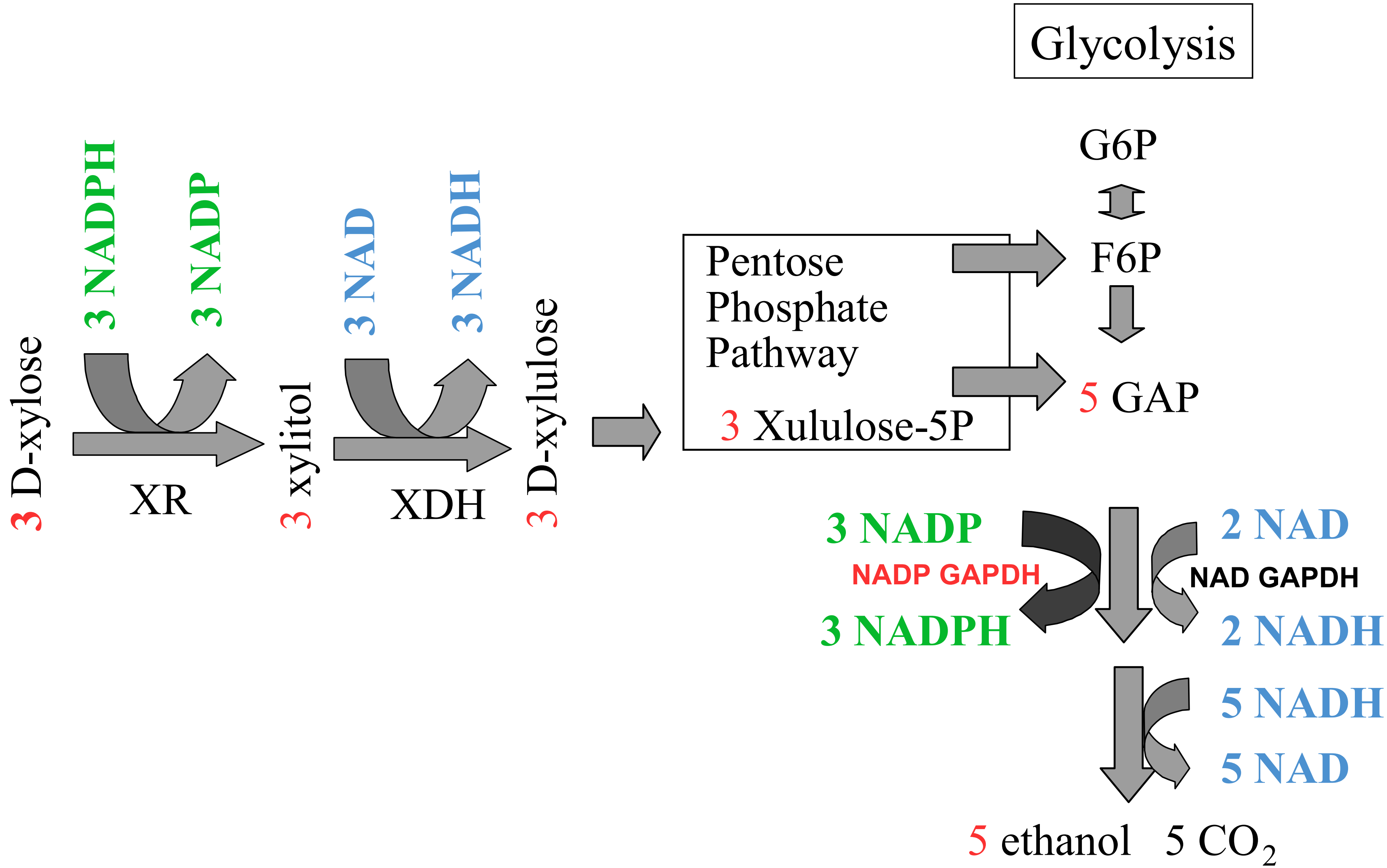
Engineering of cellular redox balances

- coexpression NAD & NADP-dep. GDH
- constitutive expression of malic enzyme
- **expression of NADP-dep GAPDH of *Kluyveromyces lactis***

Transhydrogenase cycle



Cofactor balancing with the NADP dependent glyceraldehyde-P dehydrogenase

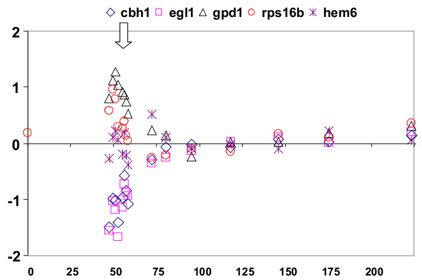


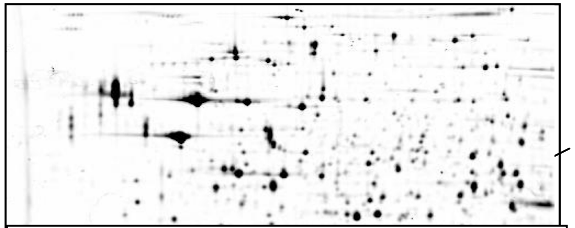
Significant reduction in xylitol/EtOH ratio

VTT Systems Biology Experimental platform

Transciptomics

- commercial oligo-nucleotide arrays
- rapid at line analysis of selected transcripts by TRAC





Proteomics

- 2D electrophoresis
- P-proteomics 2D + MS
- Mitochondrial and nuclear proteomics

Fermentation facilities

for batch, steady-state and transient experiments

- engineered strains
- specific culture conditions

Metabolic flux analysis

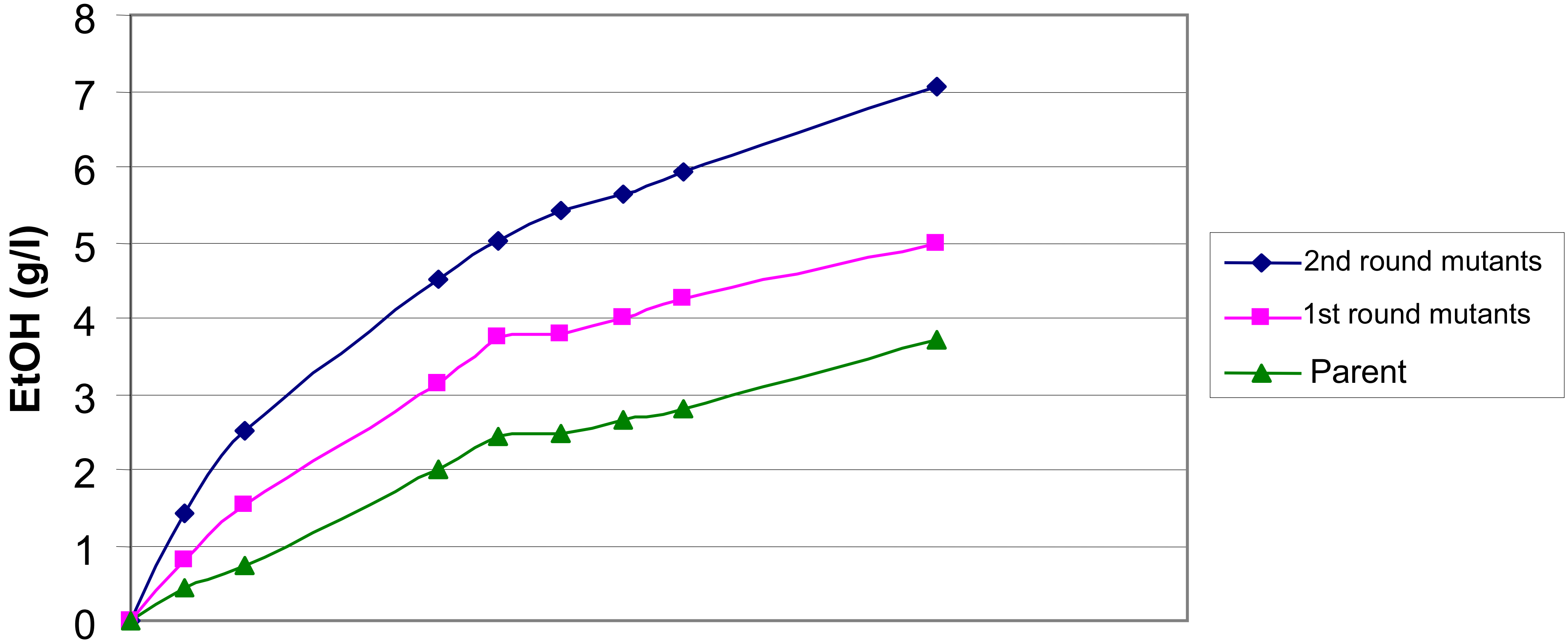
- metabolic flux analysis (MFA)
- ^{13}C tracer analysis (NMR, MS)

Metabolite analysis

- LC-MS: central C-metabolites
- GC-MS: organic & amino acids
- NMR with cryogenic probes

Data integration and modelling, identification of biomarkers

Classical mutagenesis is a useful tool in strain improvement



Ethanol production of mutants in anaerobic (glycerol lock flasks), cultures on xylose, mean values of several mutants

Xylose fermentation to bioethanol by recombinant *Saccharomyces cerevisiae*

XR-XDH pathway (e.g. VTT)

- **~ 70% yield of EtOH from xylose of the theoretical in anaerobic conditions (strain with various genetic modifications)**
- **Hydrolysate tolerant yeasts ferment biomass hydrolysates**

XI pathway (e.g. Delft Univ.)

- **XI from an anaerobic fungus *Piromyces***
- **Close to theoretical yields of ethanol (strain with various genetic modifications)**

Rates of xylose fermentation still need improvement

"2nd generation" raw materials under study

Whole crop approach

- Straw, hulls
- Corn stover, cobs
- Wood chips, bark

Industrial process sidestreams

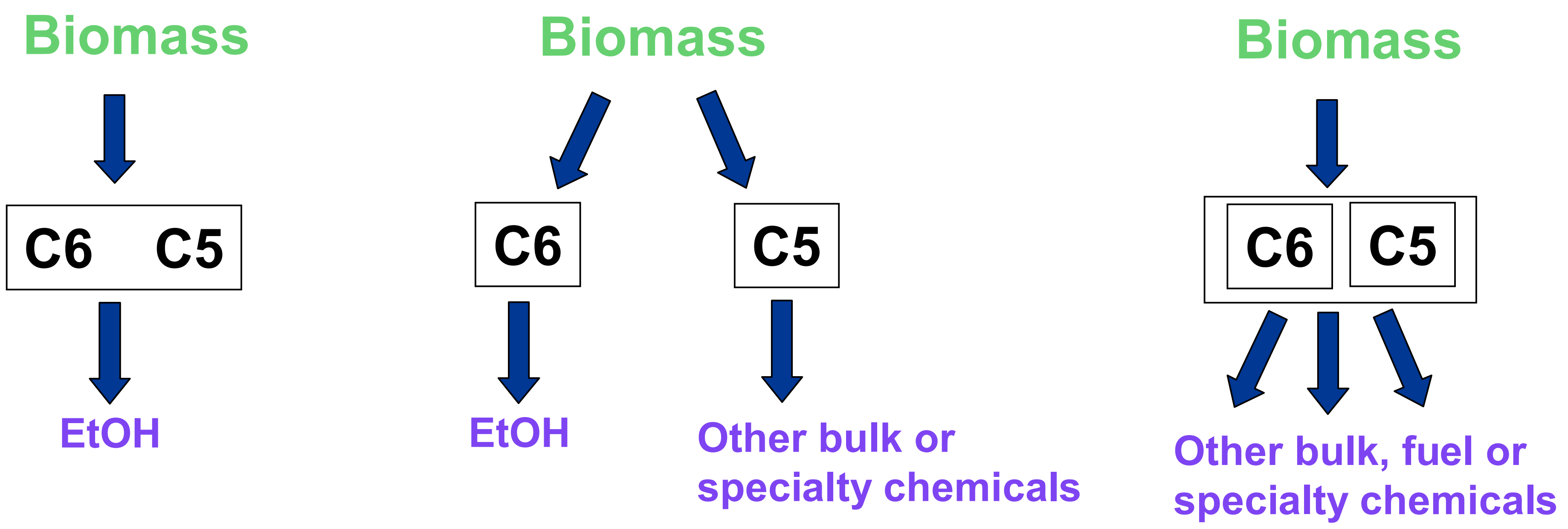
- Wheat bran, spent grain
- Bagasse
- Beet pulp
- Food industry waste (peels etc, oil industry waste)
- P&P waste streams
- Recycled paper

Dedicated energy plants

- *Miscanthus*
- *Sorghum*
- *Reed canary grass*

Etc

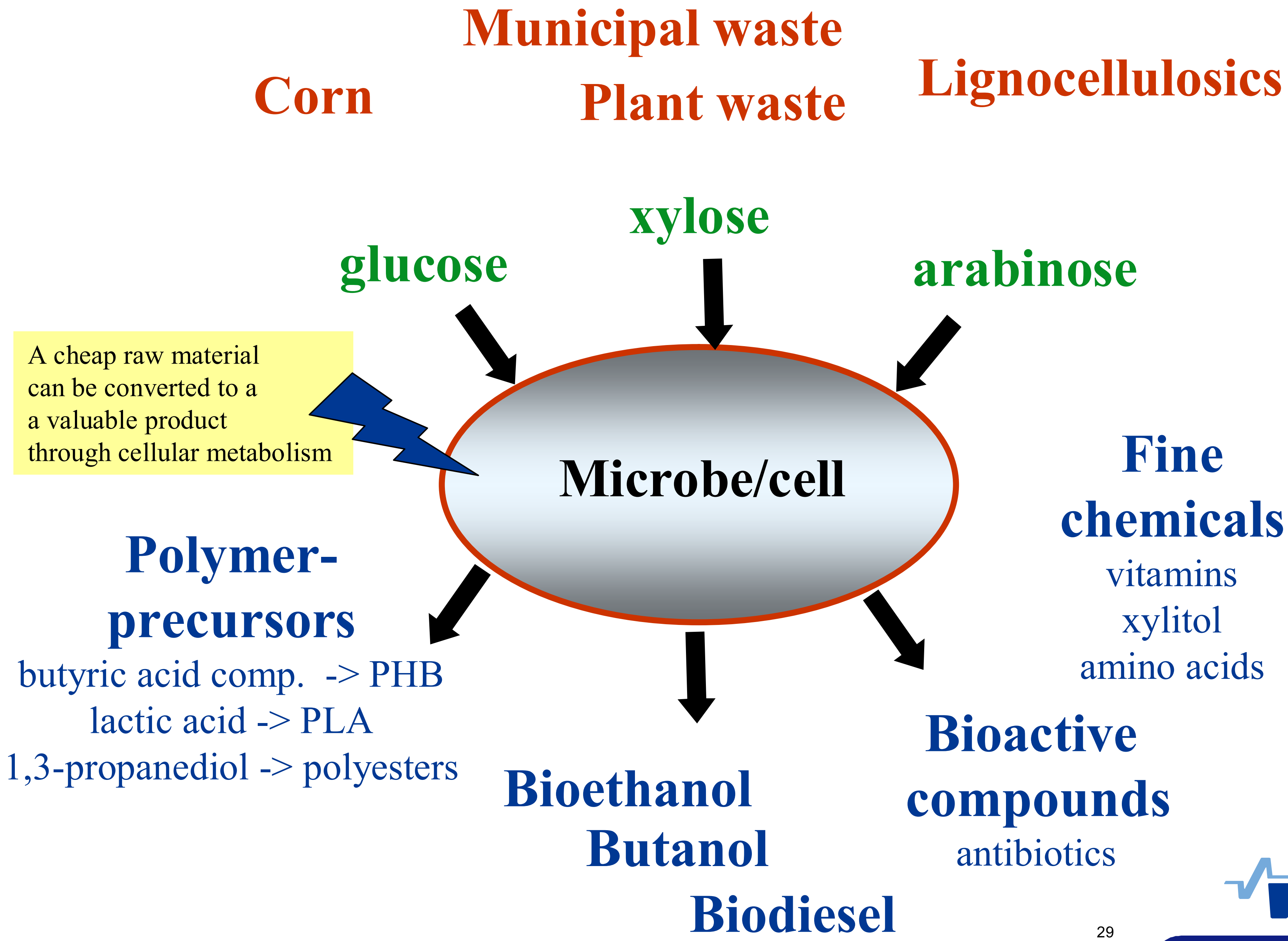
Process options



- efficient co-fermentation needed
- high yield of bioethanol per biomass
- in principle a simple process

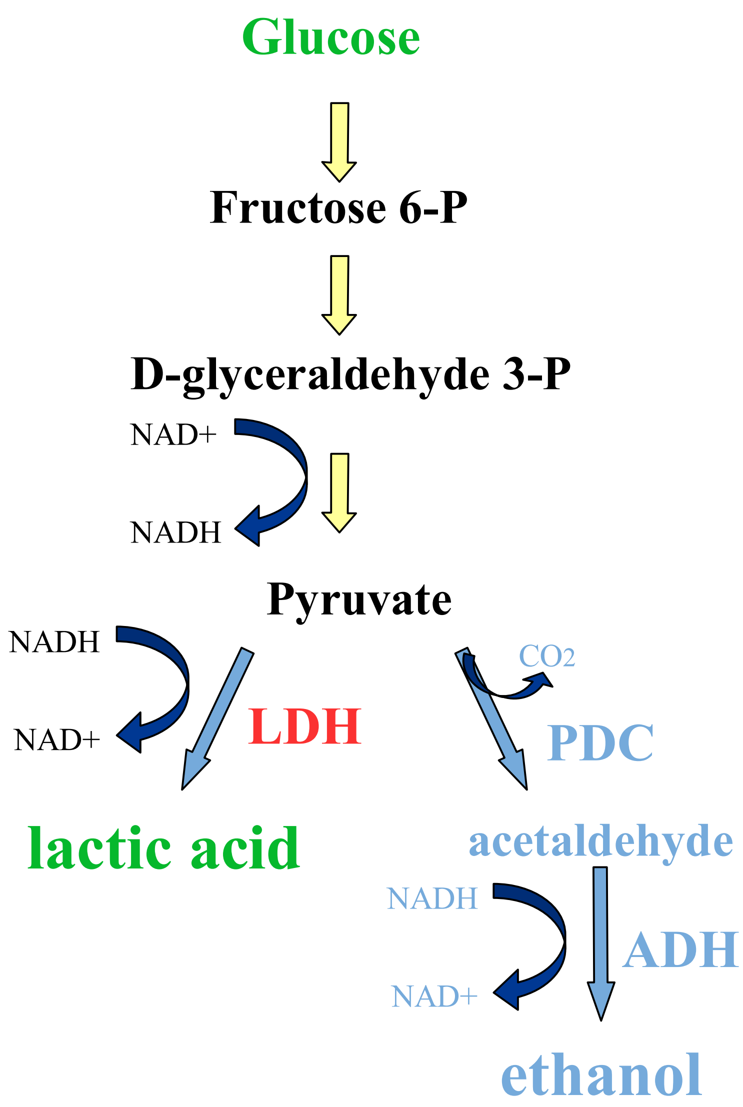
- separation of sugar fractions needed
- economics of bioethanol biorefinery can be improved
- novel product options

- infrastructure and biomass treatment methods available
- bioethanol process easily convertible to other products
- flexible product options


Municipal waste
Corn
Plant waste
Lignocellulosics
xylose
glucose
arabinose
A cheap raw material can be converted to a a valuable product through cellular metabolism
Microbe/cell
Fine chemicals
vitamins
xylitol
amino acids
Polymer-precursors
butyric acid comp. -> PHB
lactic acid -> PLA
1,3-propanediol -> polyesters
Bioethanol
Butanol
Biodiesel
Bioactive compounds
antibiotics

Production of lactic acid, PLA monomer

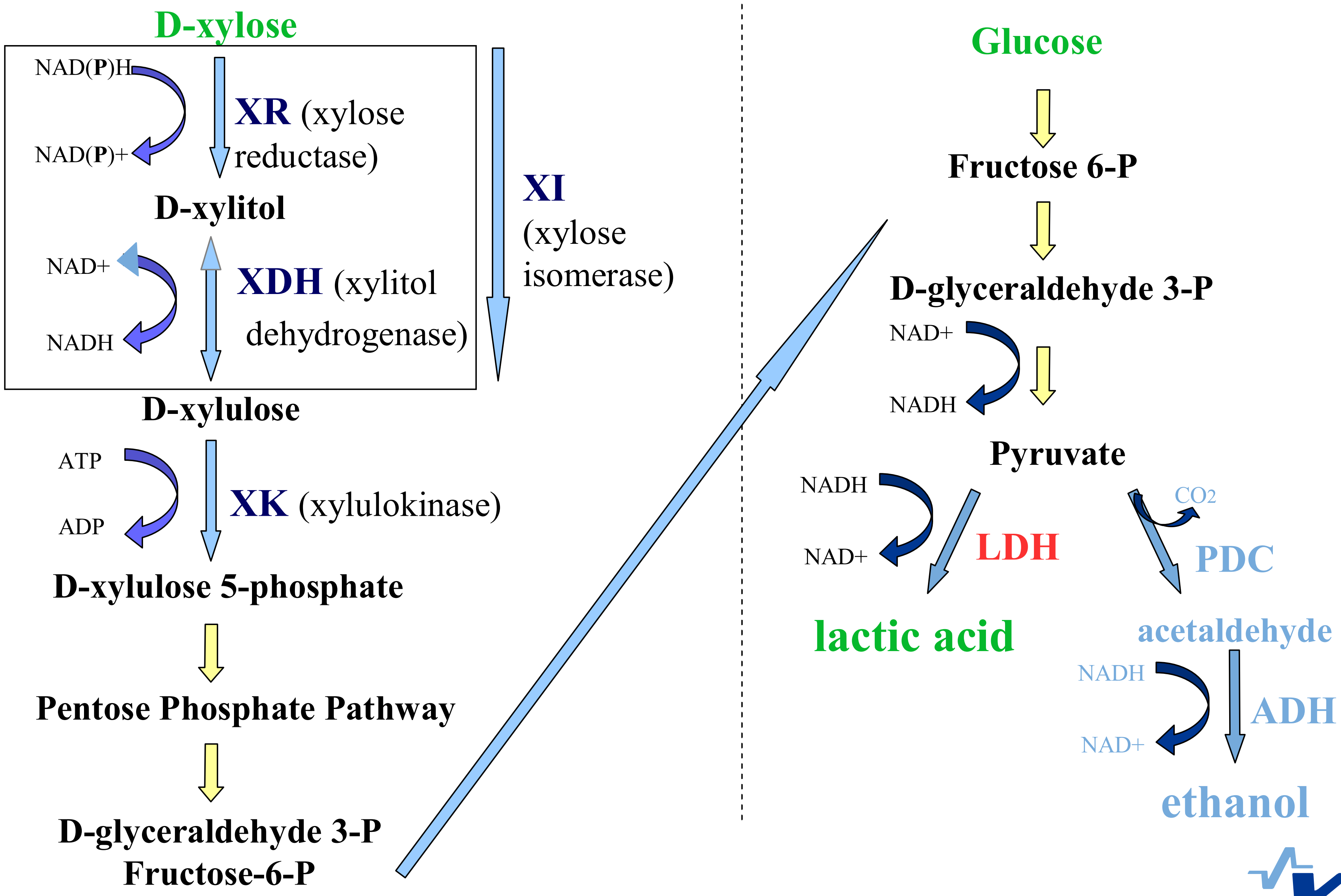
Glycolysis



Lactic acid production from glucose

- introduction of bacterial lactate dehydrogenase (LDH)

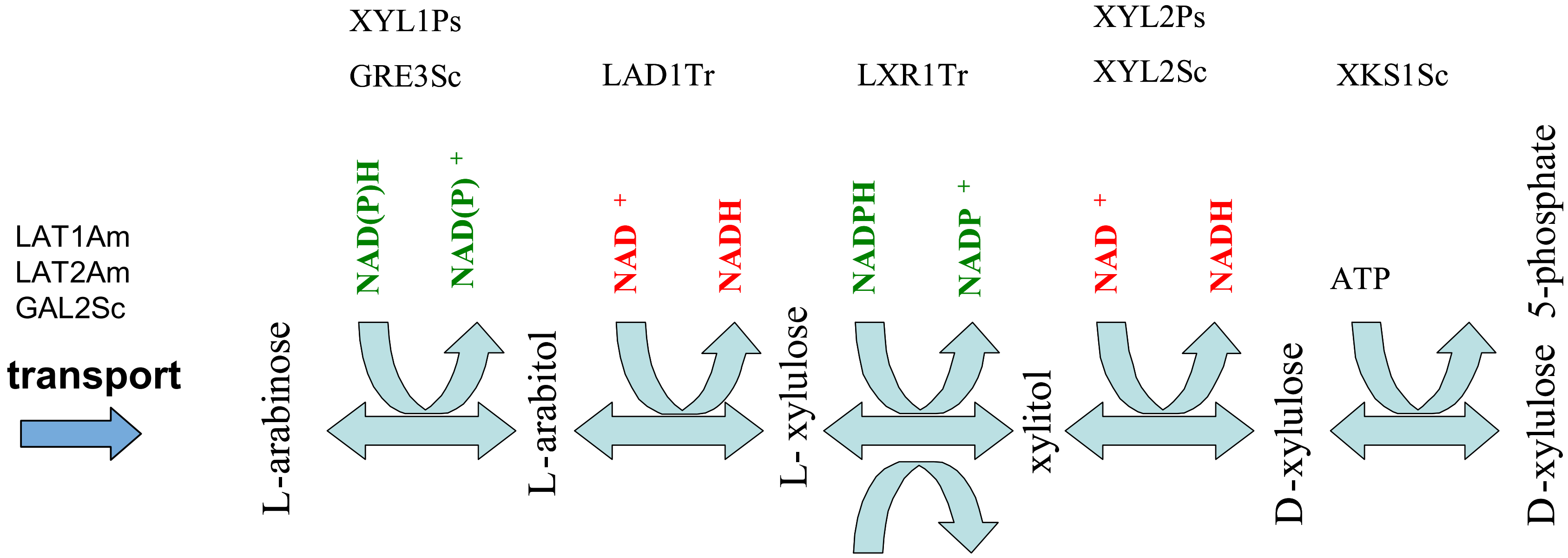
Pentose utilisation — Glycolysis



VTT has also characterised the fungal pathways for

- L-arabinose utilisation
- D-galacturonic acid utilisation

and expressed these pathways in *S.cerevisiae*



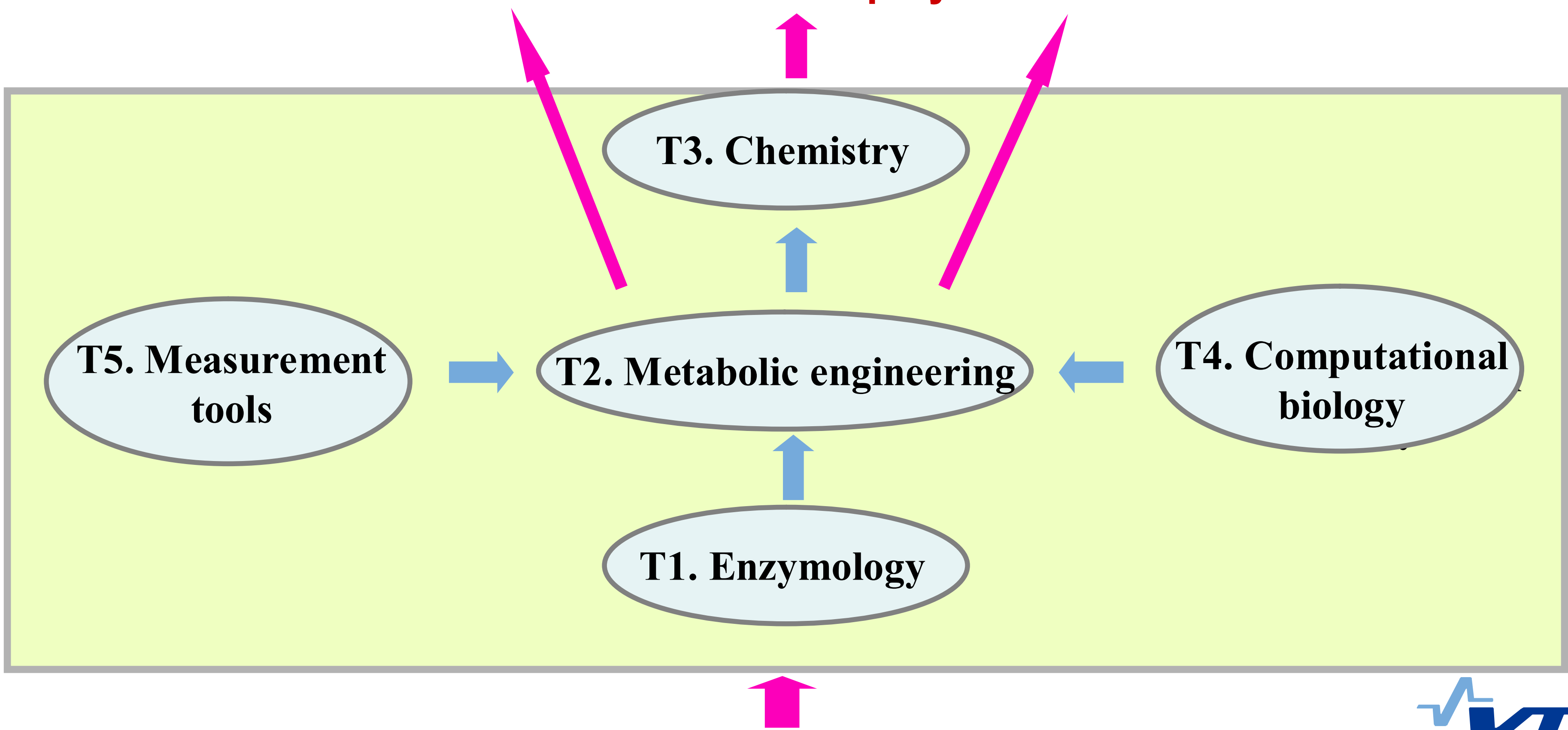
The fungal pathway for L-arabinose utilisation with the cofactors involved. The genes studied are shown above and below the pathway with their origin indicated (Ps= P.stipitis, Sc=S.cerevisiae, Tr=T.reesei, Am=Ambrosiozyma monospora).

Centre of Excellence status

by the basic science funding body Academy of Finland

2008-2013 in White Biotechnology – Green Chemistry

Chemicals and polymers



C5 & C6 sugar fractions from plant biomass

Issues

- Pretreatment methods under rapid development
  - Reduced enzyme requirements, consolidated processes
  - Reduced toxicity
  - Separation of sugar streams
- Feedstock development
  - Reduced lignin, “self-hydrolysis”
  - Altered sugar composition
- New product options
  - Essential for biorefinery concepts and their economy
  - Market need & volumes need to be considered
  - New challenges for metabolic engineering

- Organisms robustness & performance remain always key issues
  - Process and end product tolerance
  - Rate and yield of production