Biochemical Technologies for Biofuel Production: Challenges and Opportunities

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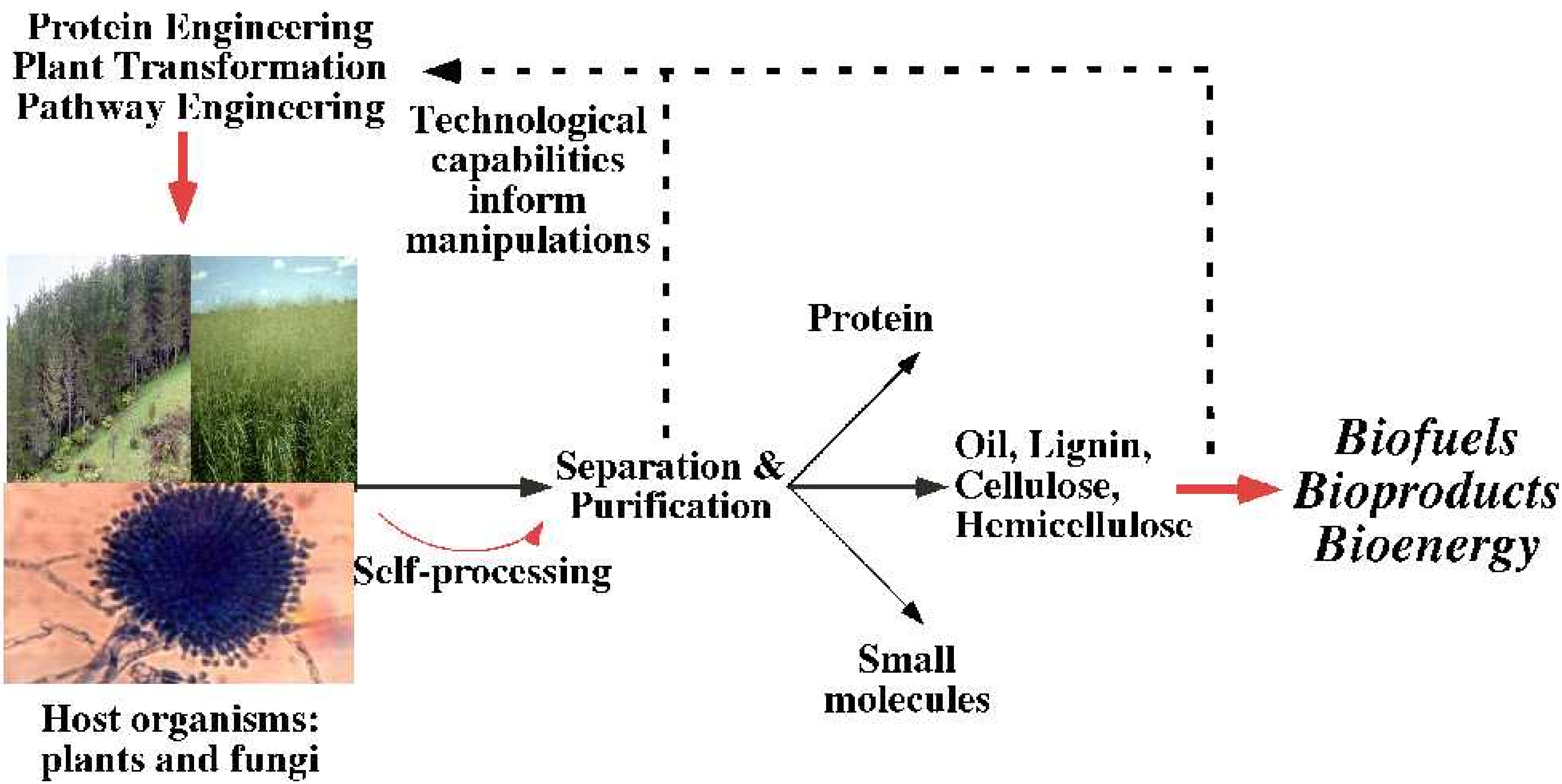
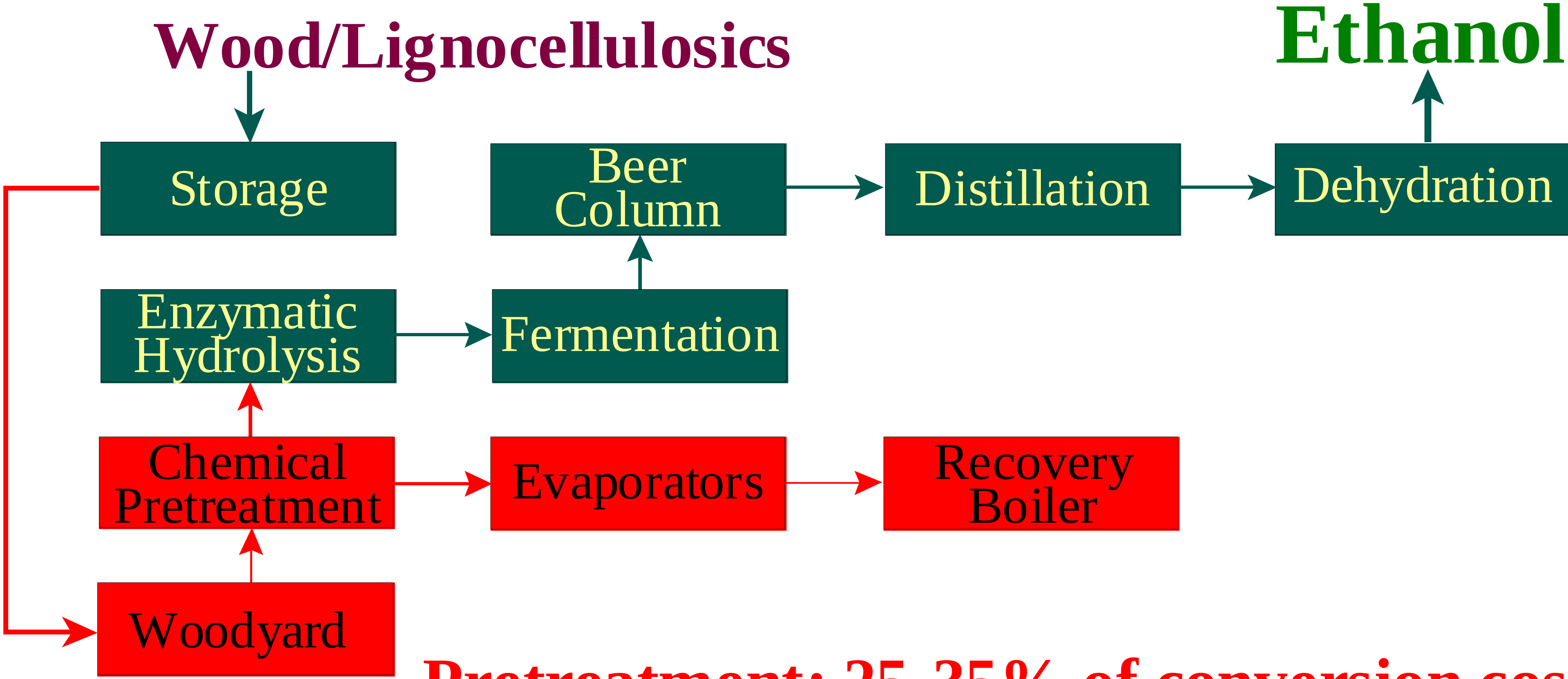
There are several opportunities upstream and downstream for biochemical processing



Conversion of traditional lignocellulosics to ethanol

(Chemical pretreatment + enzymatic saccharification + fermentation)



Pretreatment: 25-35% of conversion cost

Pretreatment is necessary because lignin in plant cell walls limits enzyme's accessibility to polysaccharides.

Upstream Processing: Making "better" biomass

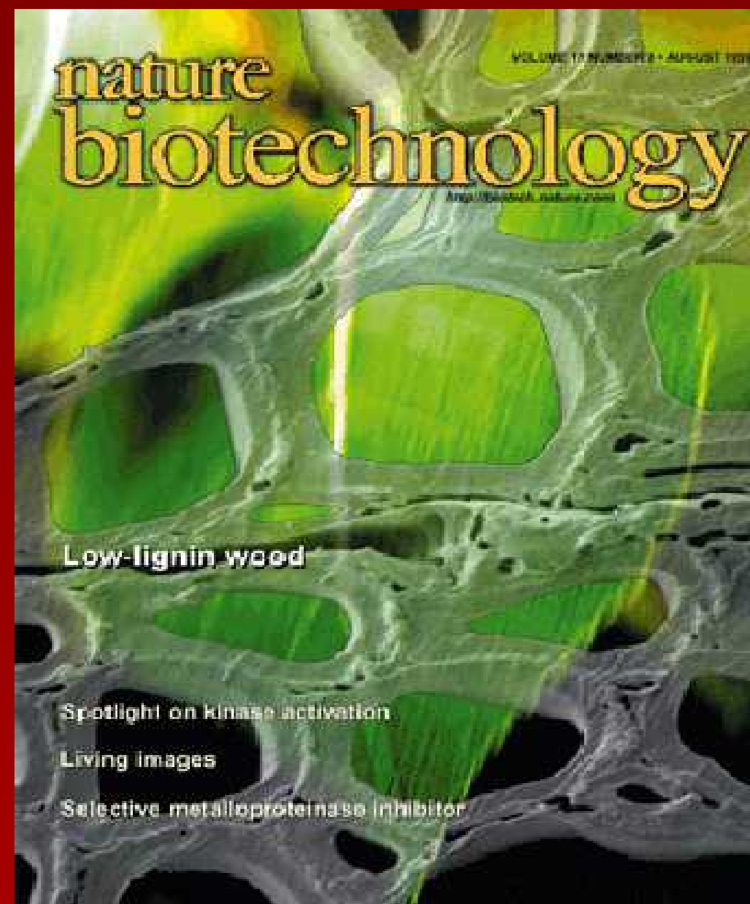
Antisense *4CL*-mediated lignin reduction in Trees

Aspen

Hybrid poplar

Eucalyptus

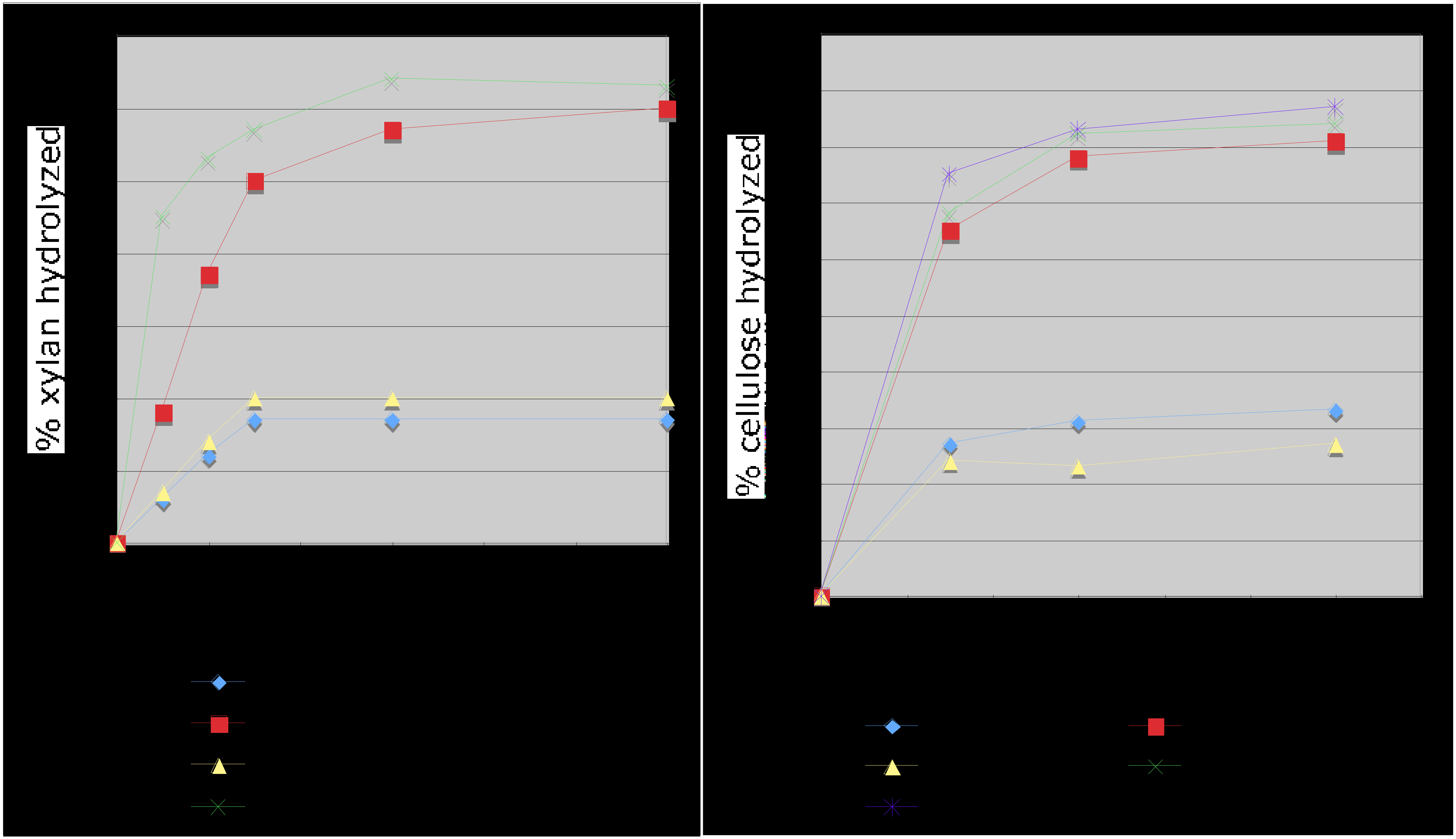
Loblolly pine



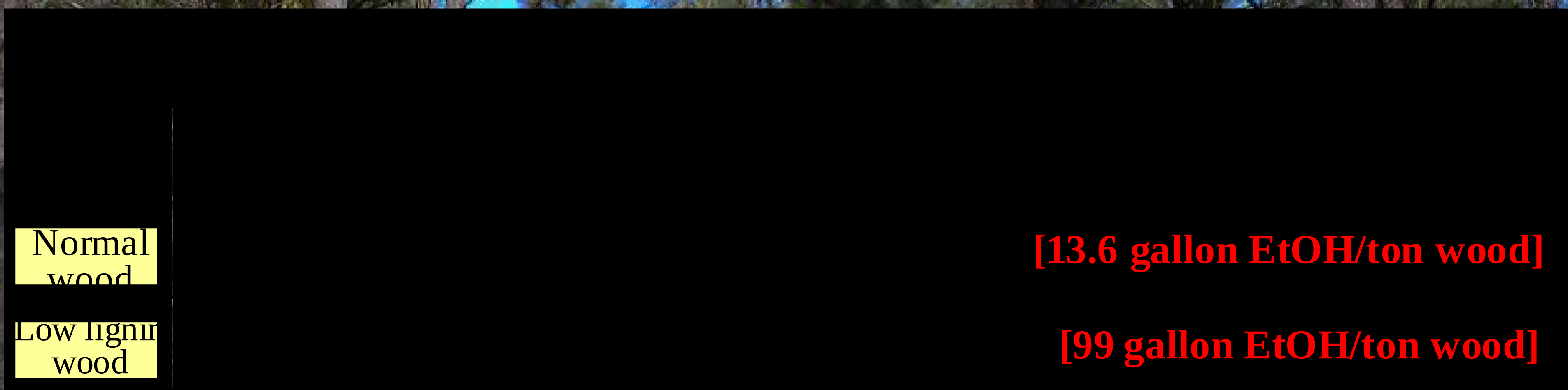
Genetic Engineering of Lignin Synthesis in Trees

	Normal wood	Genetically modified wood
Cellulose	43%	54%
Lignin	22%	10%
Lignin S/G	2.1	6.5

Transgenic *Populus* wood with reduced lignin can be directly hydrolyzed by enzymes without chemical pretreatment



Theoretical ethanol yields from wildtype and transgenic *Populus* wood



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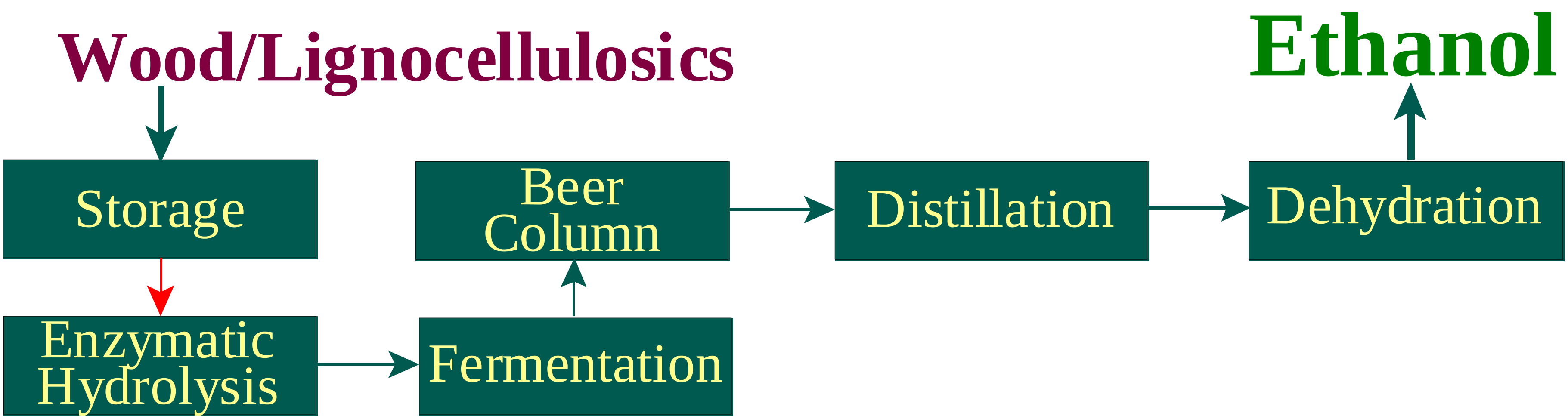
Conversion of low lignin wood to ethanol

(Direct enzymatic saccharification + fermentation)

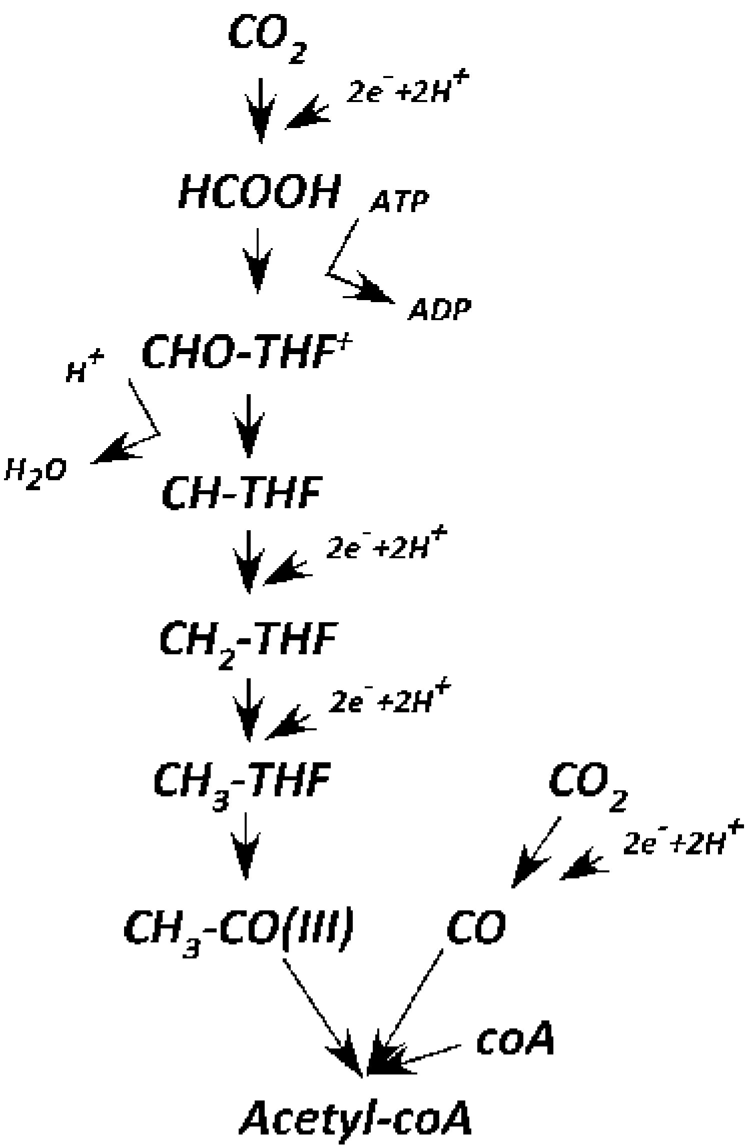
A process would be similar to current ethanol production from corn grain

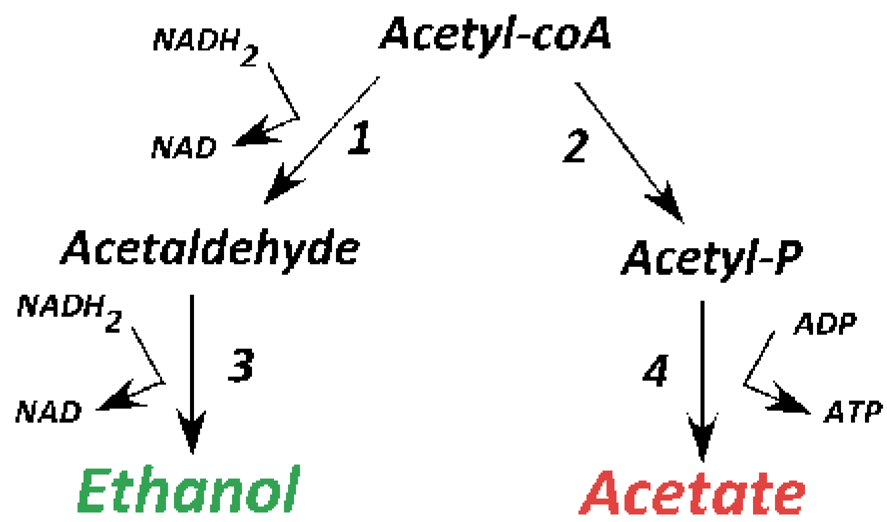


Downstream processing: gas-phase CO_x adsorber capable of biofuel synthesis

- Advantages of microbial synthesis of ethanol from gas-phase CO_x (CO_2 or CO)
  - Recover gas-phase CO_x as useful fuel
  - Make C-C bonds from CO_x
  - CO_x can be from fermentation exhaust + H_2 or biomass gasification (syn-gas)
  - Less water use, better mass transfer than submerged syn-gas fermentation process
  - Higher total gas-phase carbon conversion efficiency to liquid fuel

Autotrophic acetogen/ethanologen (Clostridia ljungdahlii)



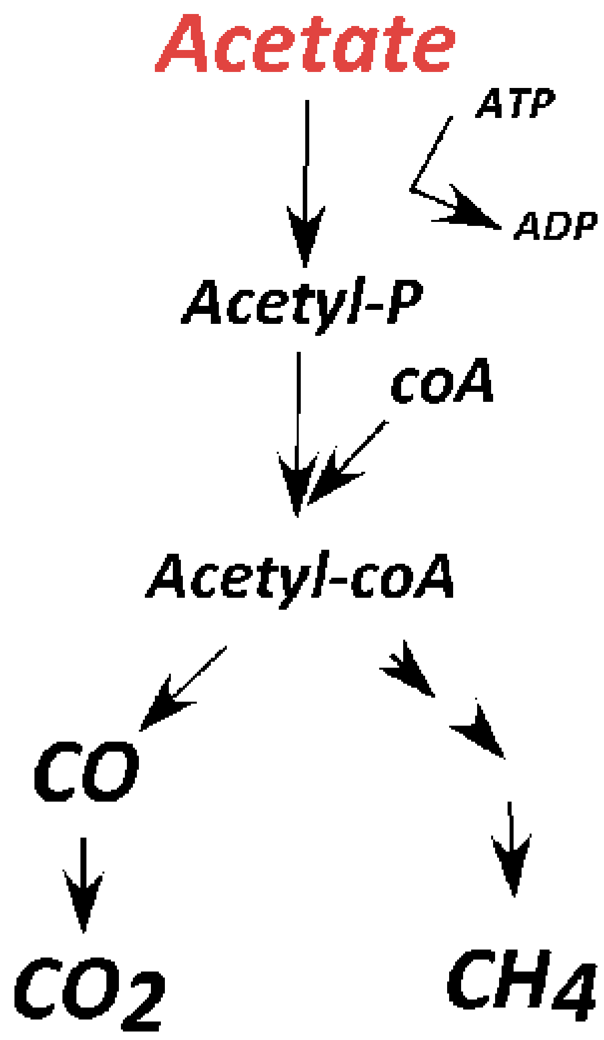


Anabolic pathway

1. Acetaldehyde dehydrogenase
2. Alcohol dehydrogenase
3. Phosphotransacetylase
4. Acetate kinase

Aceticlastic methanogen (Methanosaeta)

- Acetate is sole energy source
- Anaerobic
- Genome sequencing underway
- CO_2 and CO potential for recycle

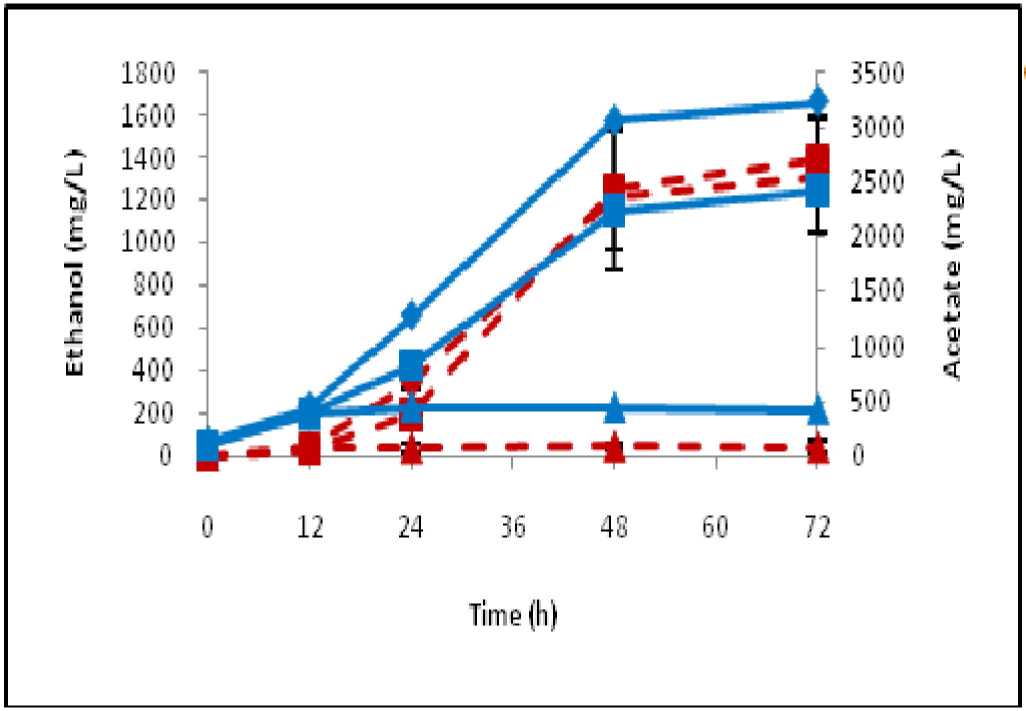


C. ljungdahlii –OTA1 vs. WT ethanol production on mRCMfs

- Reinforced Clostridial Medium with 5 g/L fructose in batch (50 ml media in 160 ml serum bottles) and reducing agents under 1 atm syngas (20 % CO_2, 20 % CO, 10 % H_2, 50 % N_2) (mRCMfs)
- Ethanol to acetate ratio

Time (h)	OTA1	WT
24	0.19	0.12
48	**0.40**	0.08
72	**0.36**	0.09

Results: *C. ljungdahlii*-OTA1 EtOH + Acetate Production in O_2 Exposed Cultures

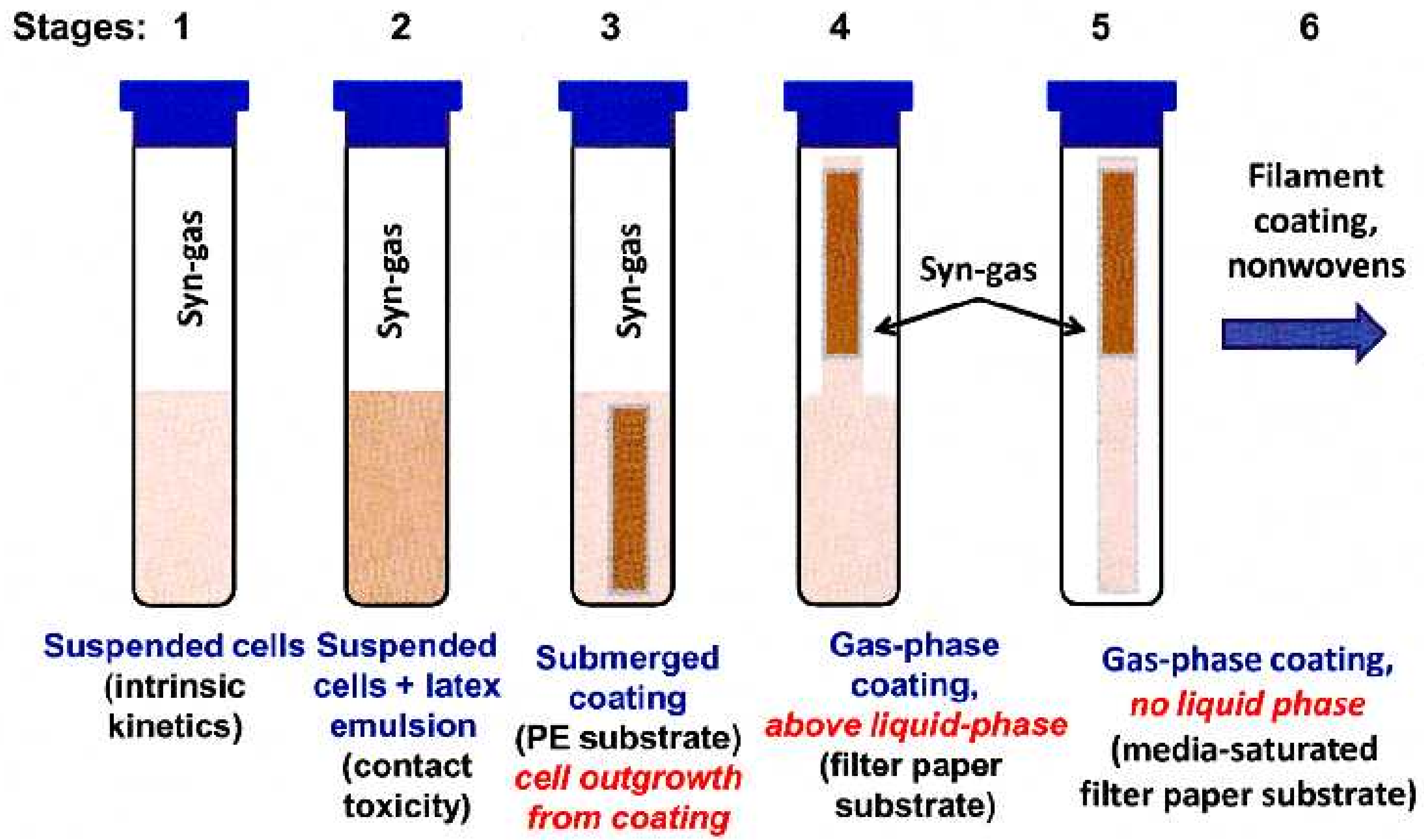


Dashed red lines, ethanol (diamond, 0 % O_2; square, 6 % O_2; triangle, 12 % O_2) Solid blue lines, acetate (diamond, 0 % O_2; square, 6 % O_2; triangle, 12 % O_2).

Ethanol to acetate ratios for *C. ljungdahlii*-OTA1 in control and oxygen exposed cells.

Time (h)	0 % O_2	6 % O_2	12 % O_2
12	0.08	0.08	0.20
24	0.27	0.23	0.17
48	0.40	0.56	0.19
72	0.41	0.57	0.18

Stages in Development of *C. ljungdahlii* –OTA1 Latex Coatings for Gas-Phase Syn-Gas Conversion



Results: *C. ljungdahlii*-OTA1 Latex Coatings

- **Non-toxic latex coating emulsion, anaerobic coating methods developed**
  - Polyester coatings (non-porous substrate)
  - Media-saturated filter paper coatings
- **Media-submerged coatings consume syn-gas, produce EtOH, but demonstrate cell outgrowth** (sealant topcoat development needed)
- **Reproducible syn-gas consumption by coatings above liquid-phase and on saturated filter paper without liquid-phase demonstrated** (no outgrowth)
- **Gas-phase coating reactivity, EtOH + acetate yield** (experiments in progress)

Summary

- Biochemical pathway manipulation
  - Has potential to improve ease of processing of material
  - Offers unique end products and product selectivity
  - Can reduce water utilization
  - Can improve biofuel yield
  - Is a productive tool in process engineering