2007 RITE International Symposium
-Technologies for mitigating global warming and the role of Japan –

18 January 2007

Biofuel technologies of RITE

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Current stage of Biofuel R&D

Cellulose resources (Soft-Biomass)

- Ethanol： Aiming for Industrialization (with Honda)
- Butanol： Fundamental
- H_2： Fundamental (with SHARP)
- Fatty acids: Investigative
  (Diesel)

Gasified organic wastes

- Ethanol etc.： Investigative

R&D schedule for industrialization

- R&D system： Cooperation with Honda
  - RITE： Biotechnology
  - Honda： Machinery and Engineering

- Future plans

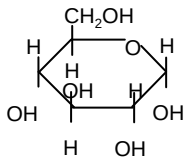
April 2007	Construct pilot plant
Summer 2007	FFV test run with Bioethanol
End of 2007	Complete data collection
2008 onwards	Design & Establish demonstration plant

Next-generation bioethanol production

Conventional Process

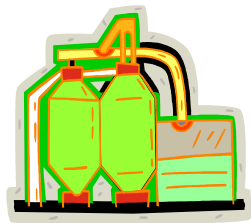
Corn / Sugar cane

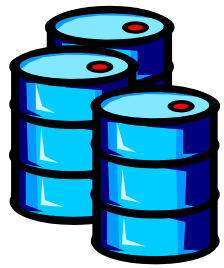
Sugar/Starch



glucose
oligosaccharide

Ethanol
fermentation

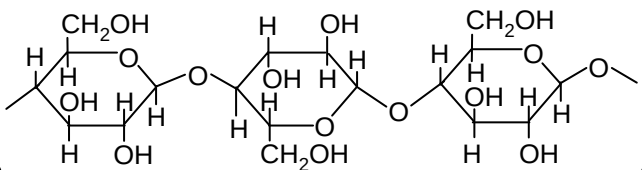




Bio-ethanol

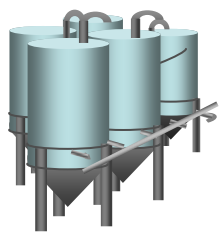
Future Process

Soft Biomass



Mixed Sugar

Saccharification

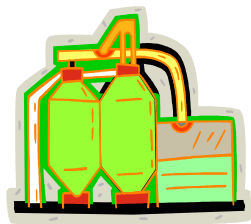


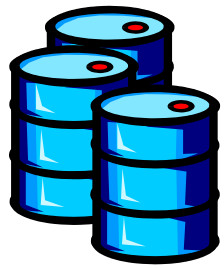
Bio-fuel

出典　Corn写真：フリー百科事典

Next-generation bioethanol production

Ethanol fermentation



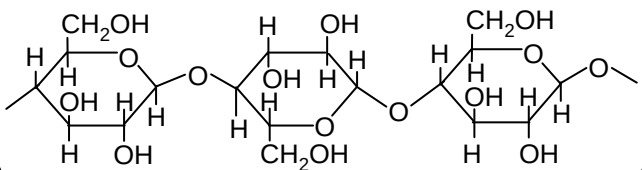


Bio-ethanol

Bio-fuel

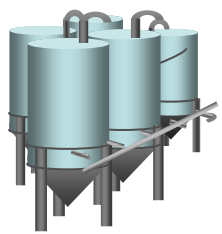
Future Process

Soft Biomass



Mixed Sugar

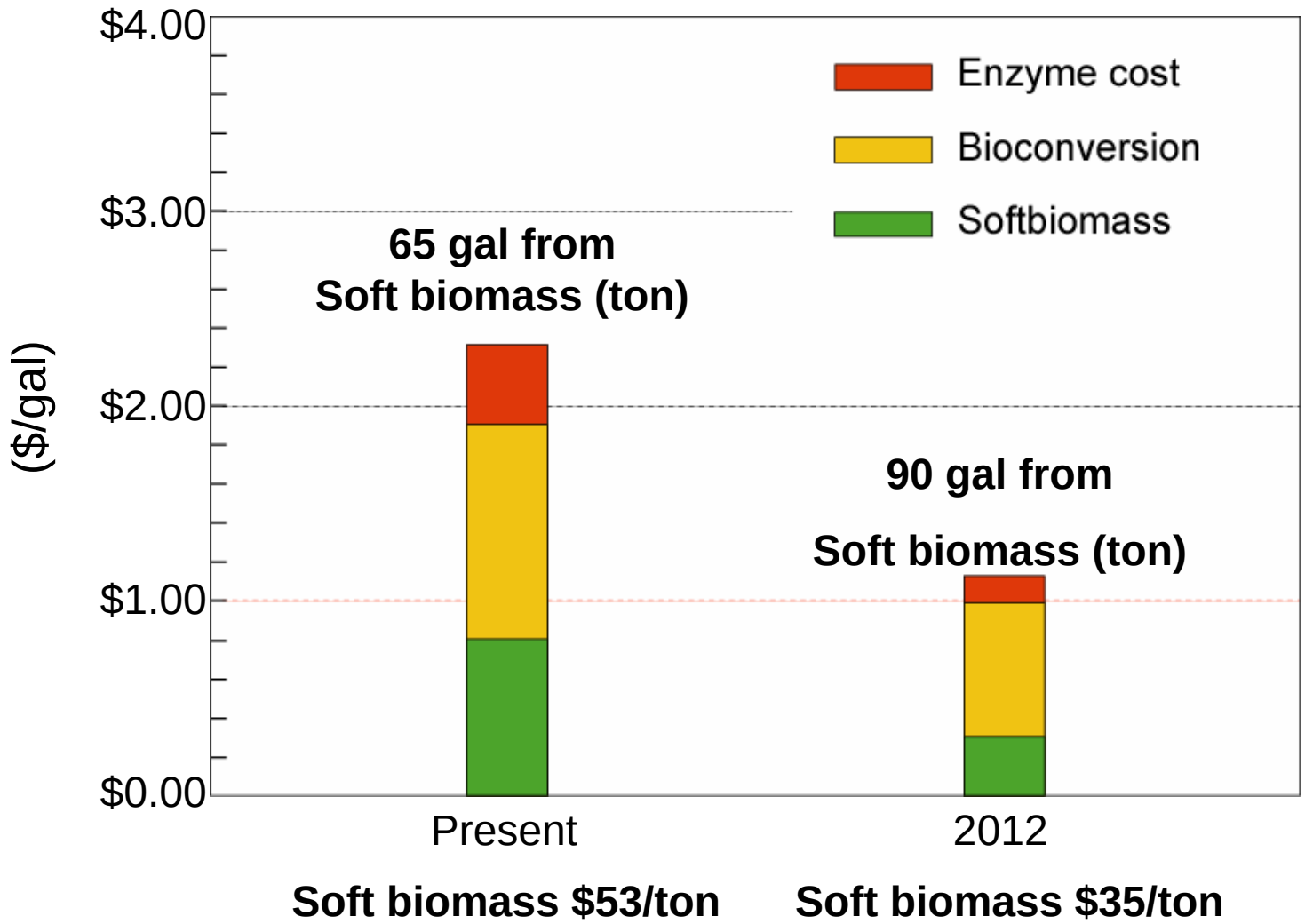
Saccharification



Bioethanol from cellulose

- Possibility of major cost reduction
  - **＜1 $/gallon**
- Reduce CO_2 emission
  - End to the LCA debate
  - 90% reduction compared to gasoline
- Others
  - Sharp rise in crude oil price
  - Less dependence on the mid-east
  - Backing agricultural sector

US DOE research target : Bioethanol cost



Ethanol production from soft biomass

Soft biomass

Pre-treatment

Enzymatic saccharification

Saccharifying enzyme

C6 & C5 sugars
(glucose, xylose, arabinose etc.)

Microorganism

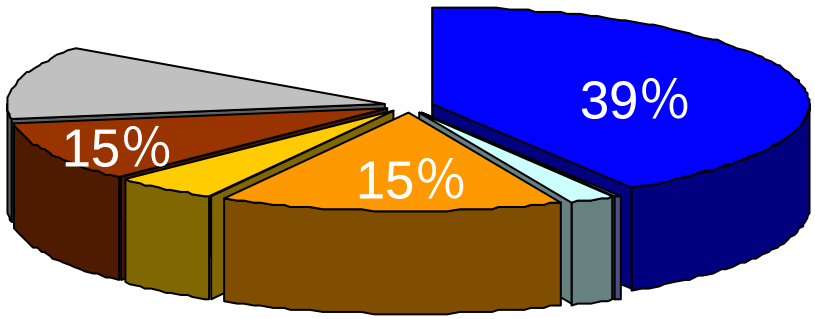
Distillation

Dehydration

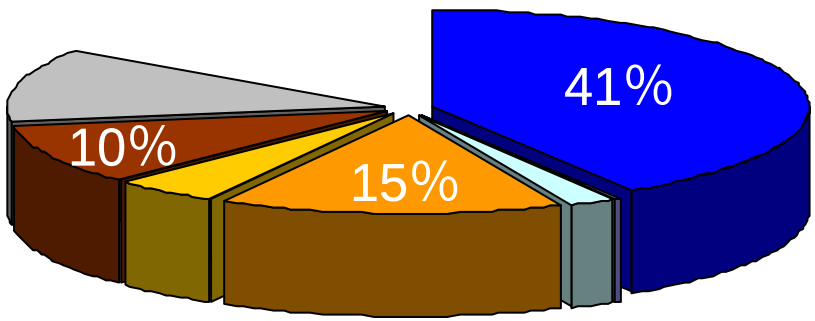
Bioethanol

Soft biomass composition

Corn stover



Rice straw



C6 sugars
- Glucose
- Mannose

C5 sugars
- Xylose
- Arabinose

- Lignin
- Ashes

Aristidou A., *Curr.Opin. Biotechnol*. (2000) 11: 187-198

Important traits for industrialization

- Utilization of C_6 & C_5 sugars
- Ethanol productivity >1 g/l/h
  >40 g/l
- Tolerance to “fermentation inhibitors”

Dien BS *et al*. *Appl Microbiol Biotechnol* (2003) 63: 258-266

Comparison of ethanol producing microorganisms

	C5 sugar fermentation	Productivity (g/l/h)	Product concentration (g/l)	Tolerance against inhibitors
Saccharomyces	×	○ (1-2 g/l/h)	◎ (>100 g/l)	×
Zymomonas	○ (Recombinant)	○ (1-5 g/l/h)	◎ (>100 g/l)	×
Escherichia coli	○	○ (1-2 g/l/h)	△ (50-60 g/l)	×
RITE strain ***(Corynebacterium)***	○ (Recombinant)	◎ (>10 g/l/h)	○ (>70 g/l)	◎

RITE strain

Coryneform Bacteria

Under oxygen deprivation:

- Growth-arrested
- Maintains main metabolic capabilities

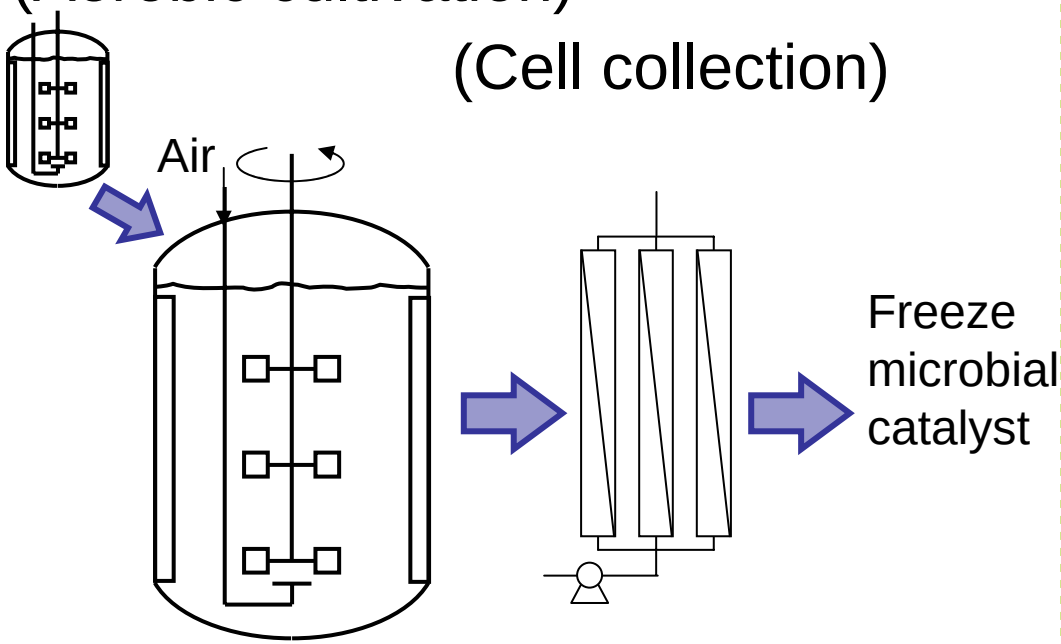
RITE Bioprocess

JP-Patent 3869788

Microbial catalyst preparation

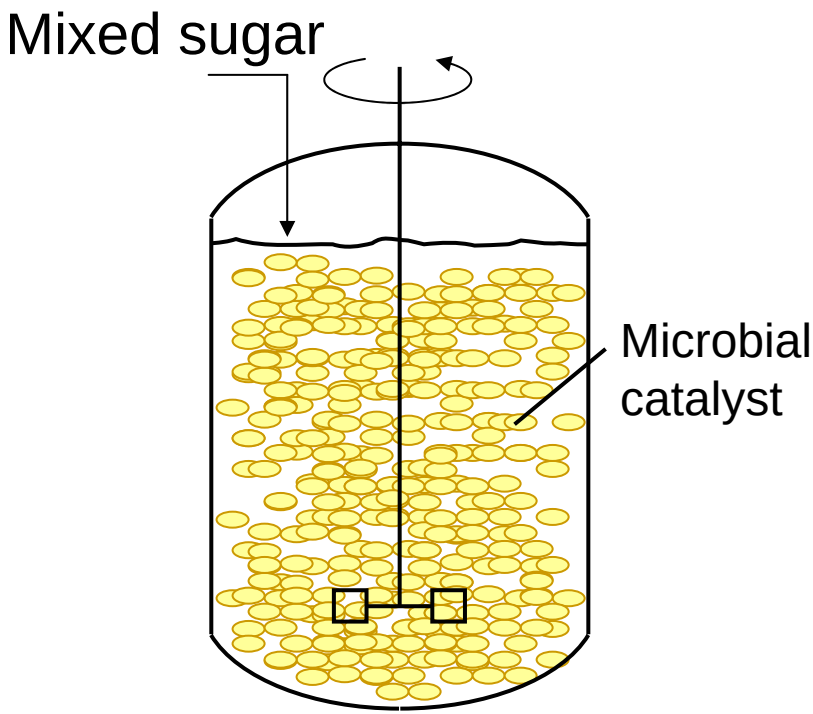
(Aerobic cultivation)

(Cell collection)



Growth by cell division

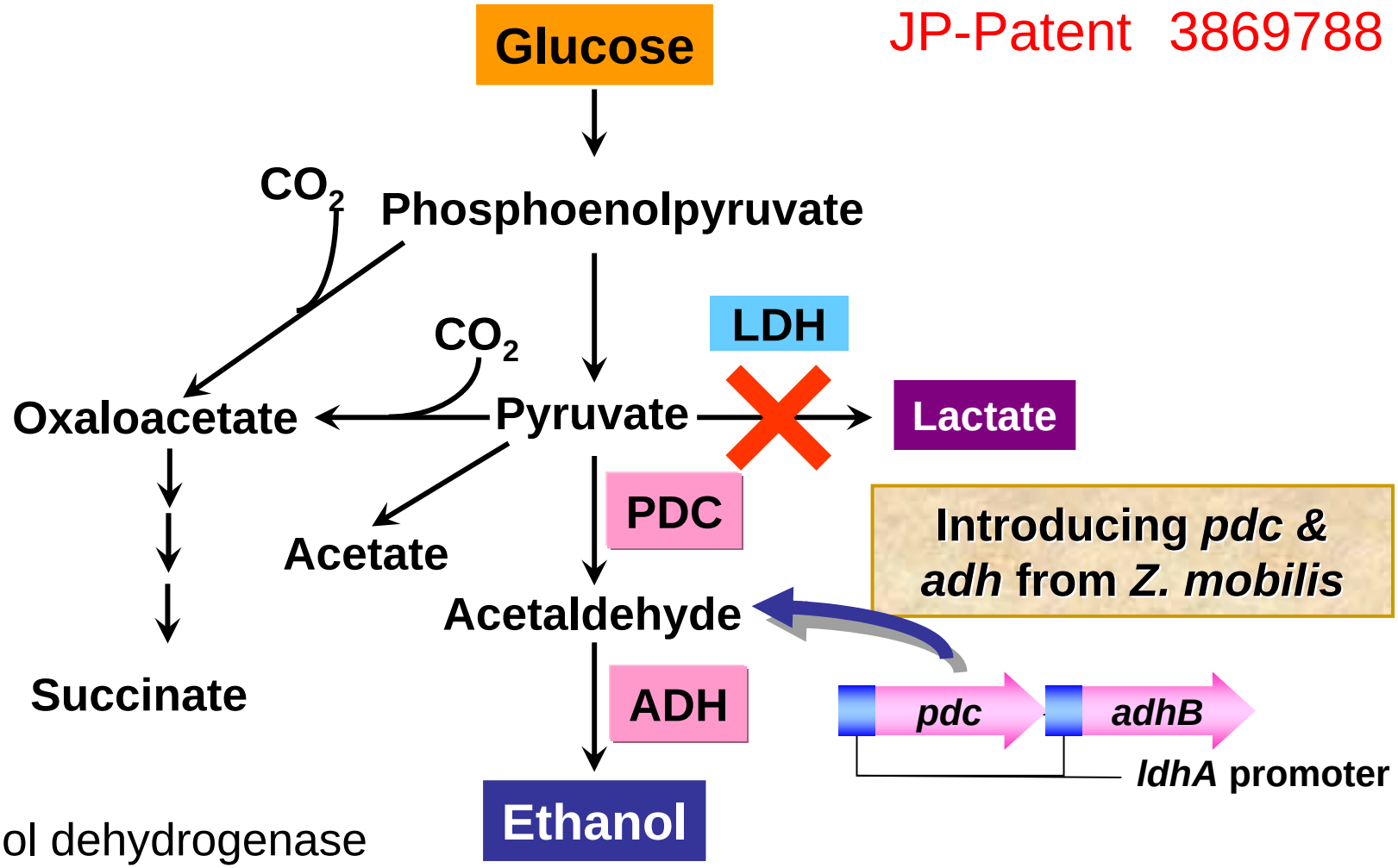
Bioconversion



Growth-arrested cells

Developing ethanol producing strain

JP-Patent 3869788



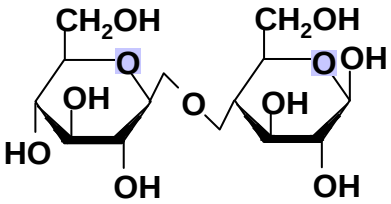
ADH：alcohol dehydrogenase
PDC：pyruvate decarboxylase
LDH：lactate dehydrogenase

J Mol Microbiol Biotechnol (2004) 8: 243-254

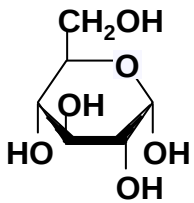
Introducing ability to utilize sugars derived from biomass

Cellulose

Cellobiose (C_6-C_6)



Glucose (C_6)

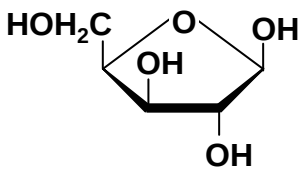


Adaptive mutant for cellobiose uptake ability 1)

RITE strain

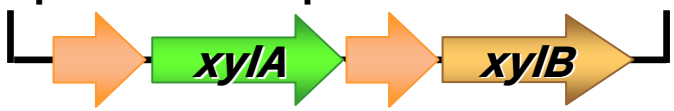
Hemicellulose

Xylose (C_5)



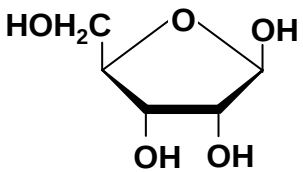
Chromosomal integration for xylose metabolic ability 2)

promoter promoter



xylose isomerase xylulokinase

Arabinose (C_5)



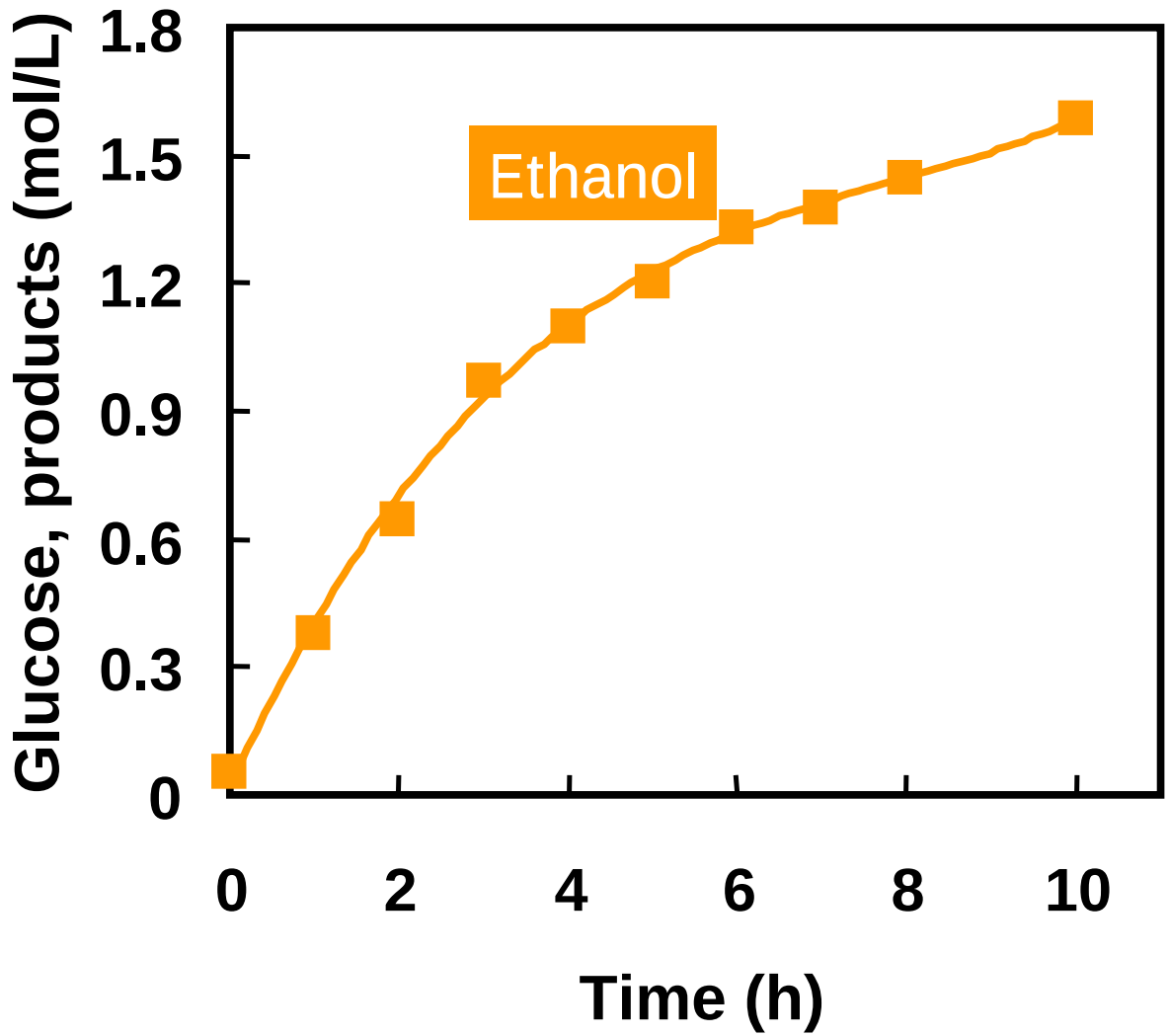
Chromosomal integration for arabinose metabolic ability

L-arabinose isomerase

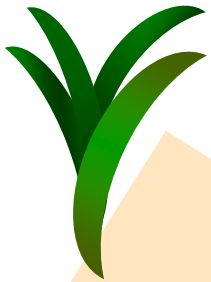
L-ribulokinase L-ribulose-5-P-4-epimerase

1) *Microbiology* **149**:1569-1580. 2003. 2) *Appl. Environ. Microbiol.* **72**:3418-3428. 2006

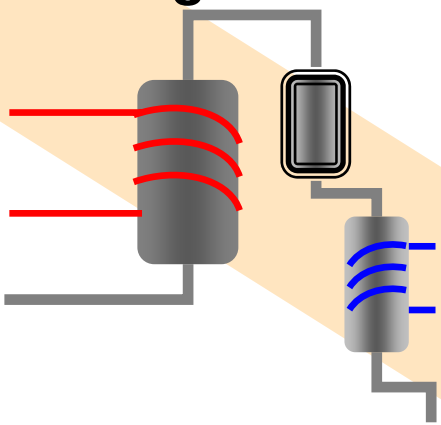
Ethanol production by RITE bioprocess



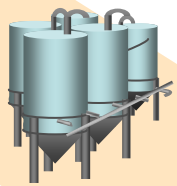
What is “fermentation inhibitors”?



Cellulose
Hemicellulose
Lignin

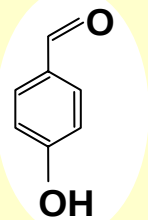


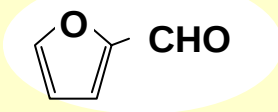
Pre-treatment



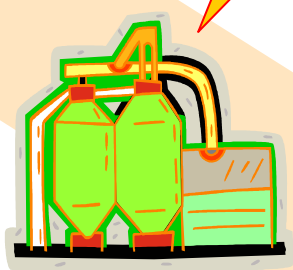
Enzymatic saccharification

Fermentation Inhibitors

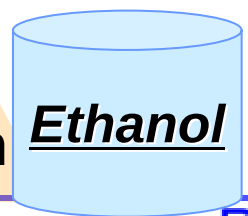




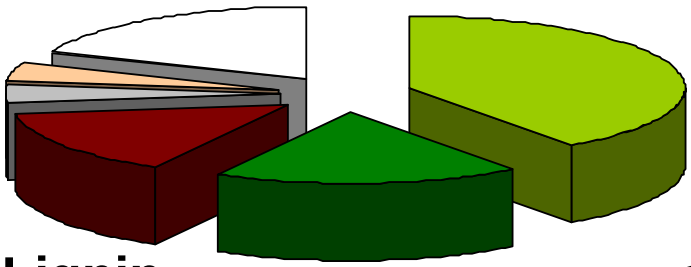
Inhibit ethanol fermentation



Ethanol fermentation



Major “fermentation inhibitors”



Biomass

Cellulose

Hexose

Lignin

Hemicellulose

Pentose

Furans

Phenols

Acetic acid

Furfural

4-HB

4-hydroxybenzaldehyde

Vanillin

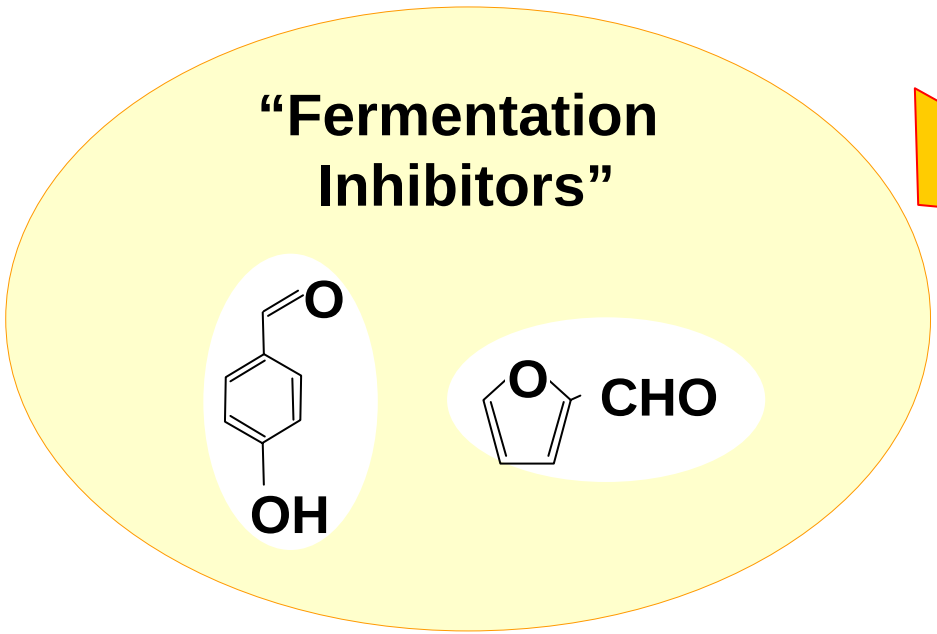
5-HMF

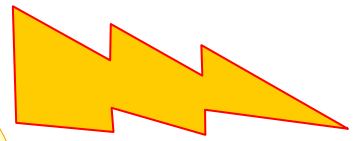
5-hydroxymethyl-
2-furaldehyde

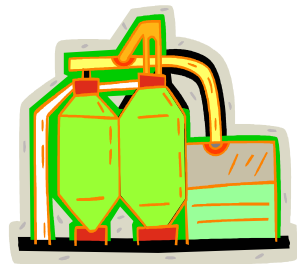
Syringaldehyde

Adapted from E. Palmqvist, B. Hahn-Hägerdal Bioresource Technology 74 (2000) 25-33

Inhibition mechanism







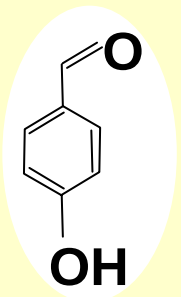
Ethanol fermentation

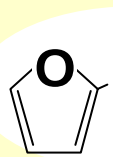
Inhibit

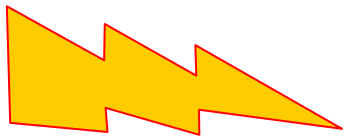
Growth or Ethanol formation

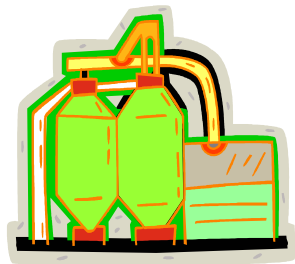
Inhibition mechanism

“Fermentation Inhibitors”









Ethanol fermentation

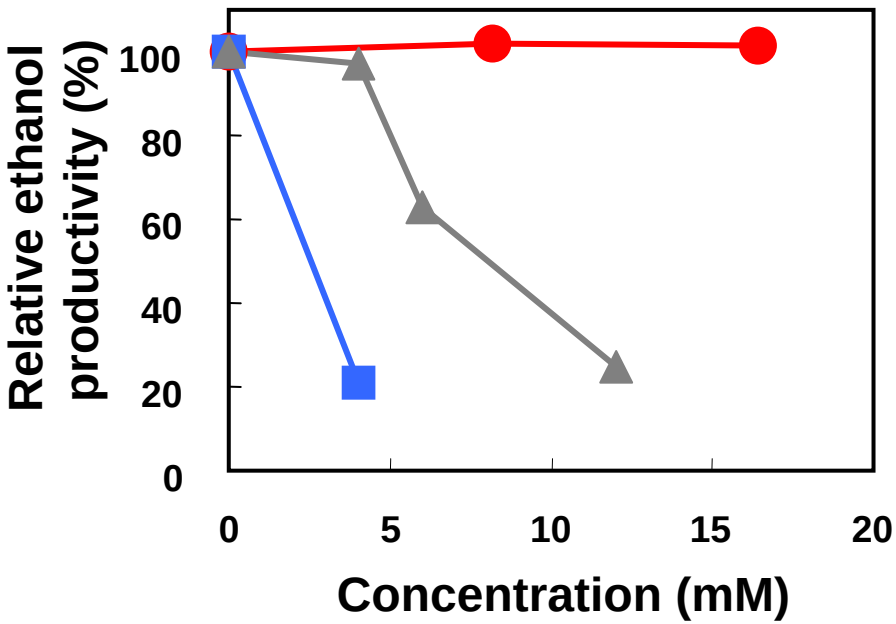
Growth Inhibition

No inhibition of ethanol production!

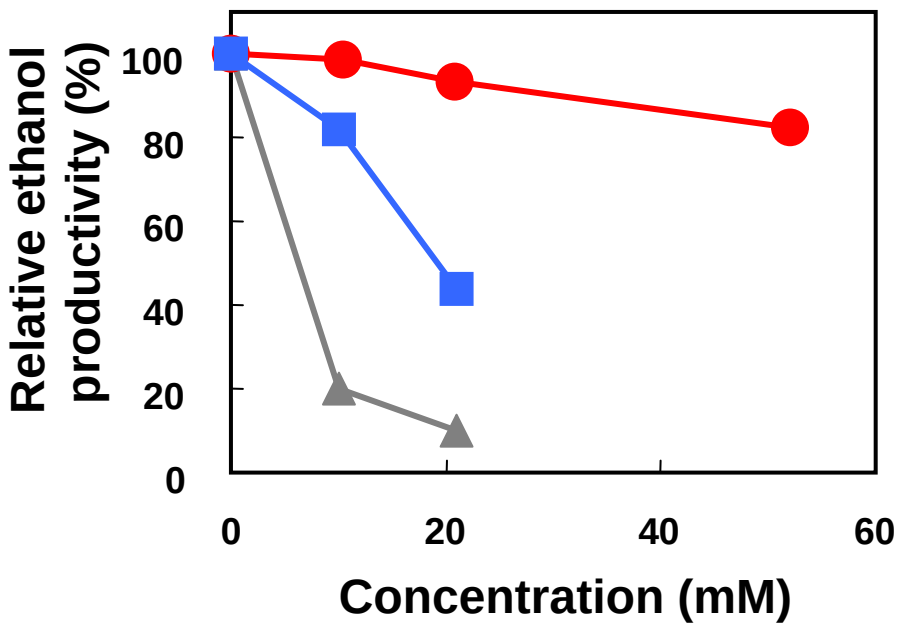
Inhibition effects

●RITE Bio-Process ■*Z. mobilis*※ ▲*S. cerevisiae*※

4-HB



Furfural



※Klinke et al. Appl Microbiol Biotechnol vol.66 (2004) 10-26

Current stage of Biofuel R&D

Cellulose resources (Soft-Biomass)

- Ethanol： Aiming for Industrialization (with Honda)
- Butanol： Fundamental
- H_2： Fundamental (with SHARP)
- Fatty acids: Investigative

  (Diesel)

Gasified organic wastes

- Ethanol etc.： Investigative

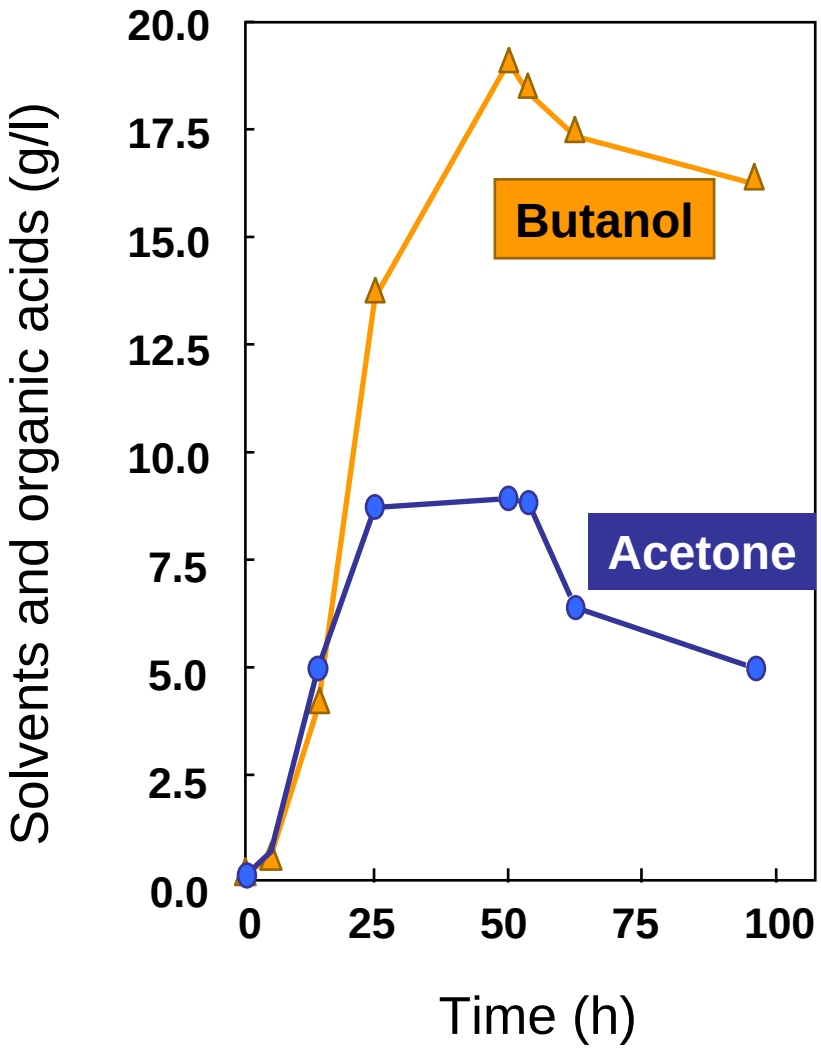
Butanol production history

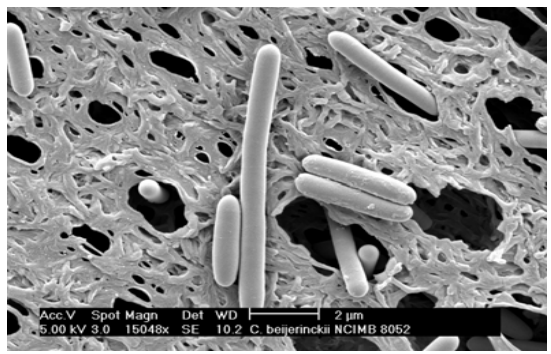
BP, Dupont & British Sugar

- Aiming for market introduction in 2007 by AB fermentation

1912	Acetone-Butanol production (AB fermentation) with *Clostridium*
1930s	Large-scale production world wide
1950s	Shift to petrochemical processes

Acetone-Butanol fermentaion

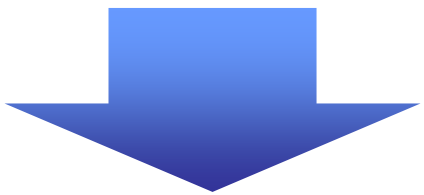




Clostridium beijerinckii BA101
(Hans P. Blaschek, Uni. of Illinois)

Problems with butanol fermentation

- **Low concentration (*ca.* 20 g/L)**
- **Low productivity**
- **Low growth rate**



Require an innovative production strategy

Summary

- **Ethanol**

  Be the top-runner

  in ethanol production from soft biomass

- **Butanol**

  Develop an innovative process