C5 Yeast Company

Ethanol production from second generation feedstocks by yeasts

Jan de Bont

IUMS, Sapporo, Japan

September 8, 2011

a DSM company
BRIGHT SCIENCE. BRIGHTER LIVING.

C5 Yeast Company towards Fuel Ethanol

- C5 Yeast Company originates from the former Royal Nedalco, which belonged to Royal Cosun
- Potential seen in second generation (=cellulosic) ethanol
- It has invested in R&D for second generation ethanol
  - In house (Bergen op Zoom, The Netherlands)
  - Universities (Delft, Groningen, Wageningen, Stuttgart)
  - Technology providers
  - R&D departments of Companies
- The company very recently has been acquired by DSM

Press Release by DSM

DSM strengthens yeast technology leadership for 2G biofuels
Heerlen, NL, 28 June 2011 08:15 CET

Royal DSM, the global Life Sciences and Materials Sciences company, today announces that it has reached an agreement to acquire C5 Yeast Company B.V. (Bergen op Zoom, Netherlands) from Royal Cosun. The acquisition will allow DSM to combine C5 Yeast Company's business with its own advanced yeast and enzyme technologies for second generation biofuels, further increasing its leadership position in this field.

http://www.dsm.com/en_US/cworld/public/media/pages/press-releases/45_11_dsm_strengthens_yeast_technology_leadership_for_2g_biofuels.jsp

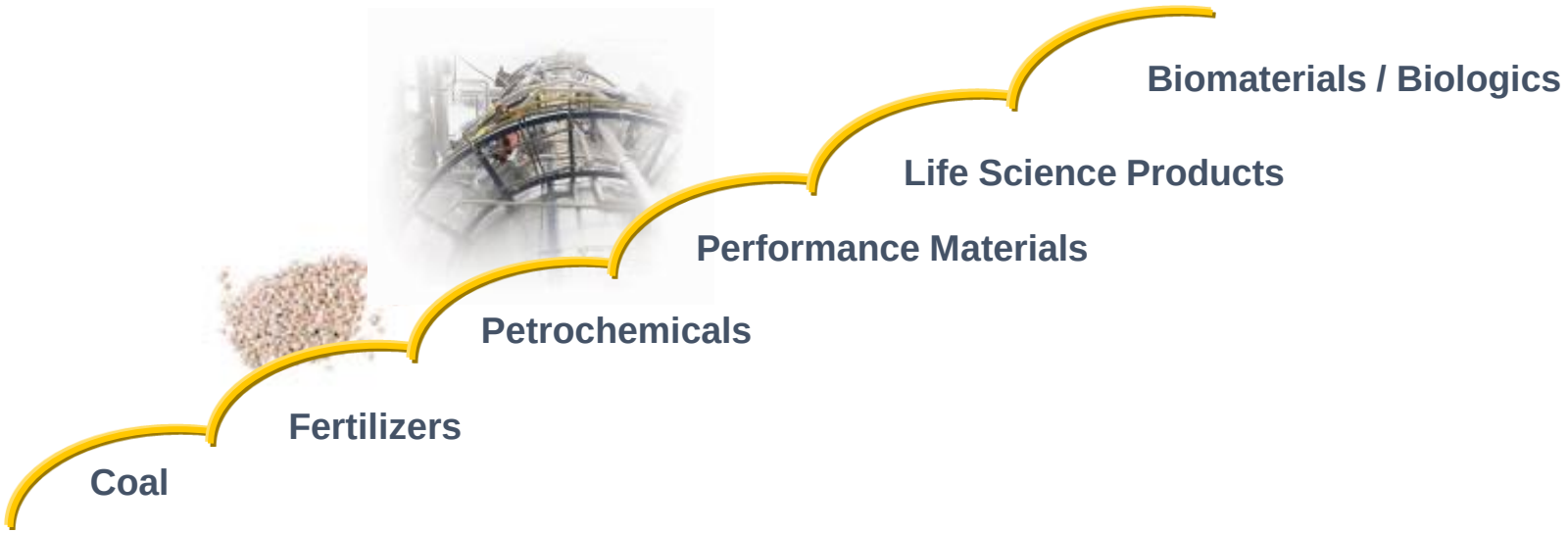
The C5YC R&D-team

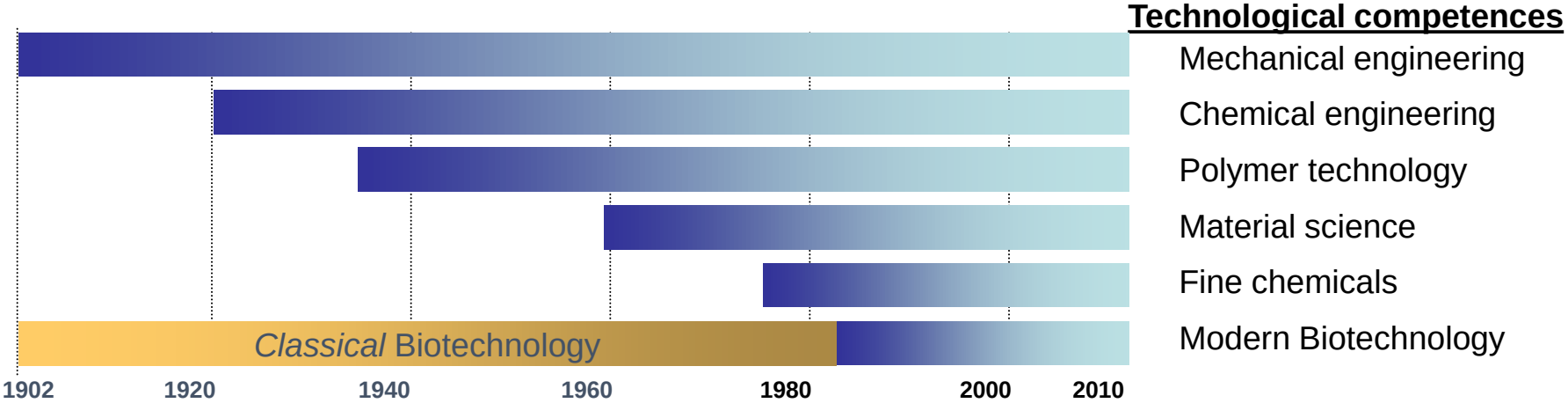
Official start under DSM ownership
August 25, 2011

DSM:
Presence in the world

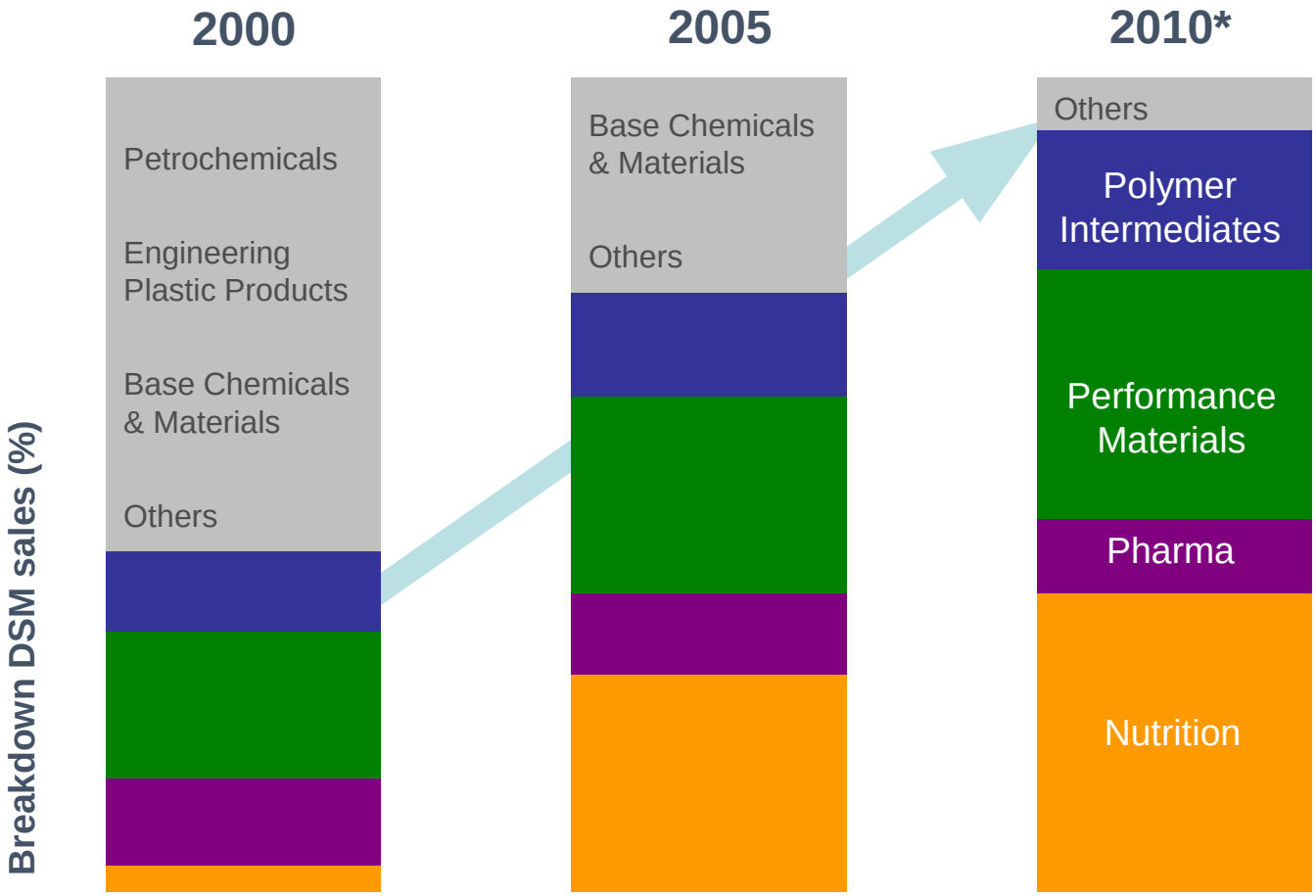
Yeast Company

DSM:
100 years of successful transformations





DSM: Transformation completed



* Excluding discontinued

DSM:
Focused platforms and pipelines

Yeast Company

Global shifts

Climate & Energy

Health & Wellness

Innovation Platforms (Examples - Business Groups)

- Bio processing ingredients
- Food/feed ingredients
- (Bio) Manufacturing
- Bio-based clean / green materials
- Sports & Life Protection Materials

Emerging Business Areas (DSM Innovation Center)

- Bio-based Products & Services
- Advanced Surfaces
- Biomedical

DSM:
Focused platforms and pipelines

Yeast Company

Global shifts

imate ergy

Health & Wellness

Innovation (Examples - Business Groups)

- **Bio processing ingredients**
- **Foo inc**
- **(Bio) Manufacturing**
- **Bio-based clean / green materials**
- **Sports & Life Protection Materials**

En usiness Areas (DSM Innovation Center)

- **Bio-based Products & Services**
- **Advanced Surfaces**
- **Biomedical**

EBA Bio-based Products and Services

Yeast Company

Strategy
Bio-based Products & Services

- License and service models to cellulosic ethanol industry
- Production of bio-based chemicals from traditional crops
- Focus on green materials with better performance

Aspirations

- Sales in 2020 > € 1bn
- High EBITDA margin

Work at C5 Yeast Company

1. General aspects ethanol production

2. Hemicellulolytic enzymes

3. Industrial yeast capable of xylose/arabinose (C5-sugars) fermentations

Background to 1st generation ethanol

- 1st generation ethanol is currently produced from digestible C6 sugars from:

Corn

Wheat

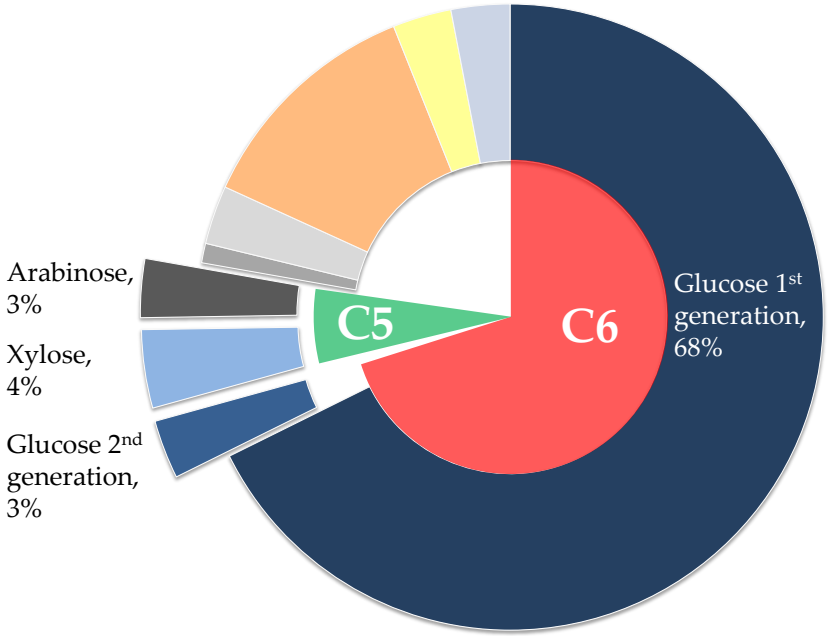
Sugar cane

Sugar beet

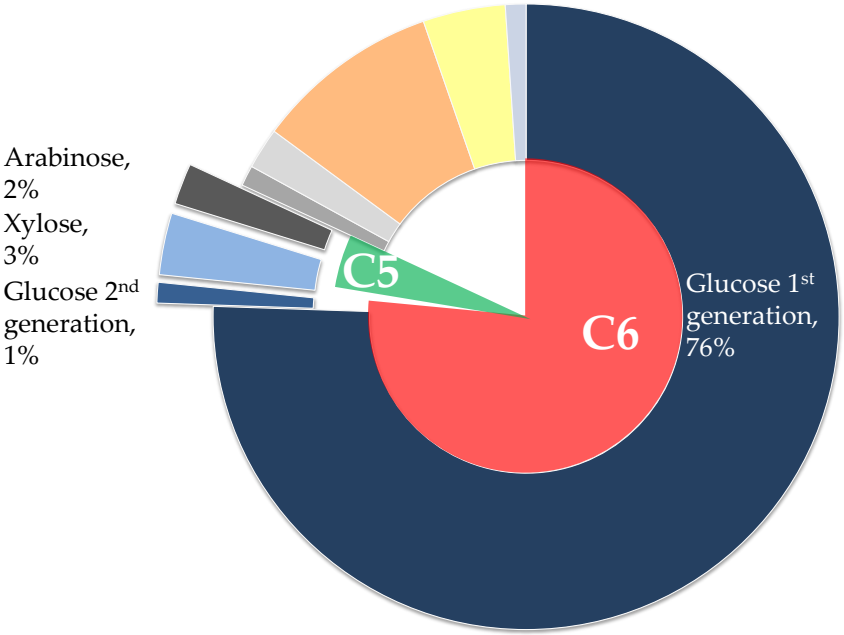
- This ethanol production is based on mature yeast fermentation technology

Sugar composition of wheat and of corn kernels

Wheat (kernel)

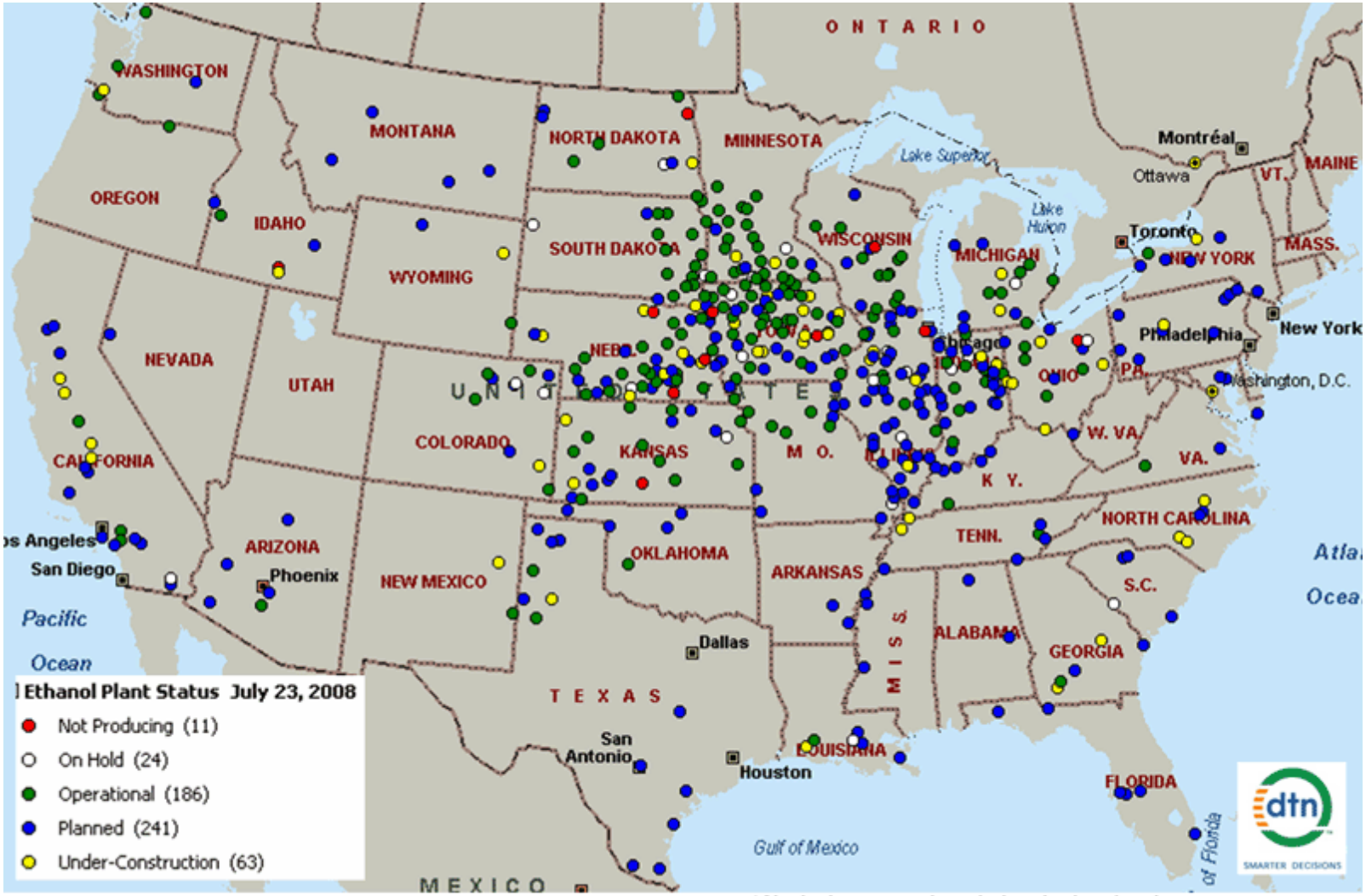


Corn (kernel)



Ethanol plant locations in the USA

Yeast Company



*Alaska has one ethanol plant in the planning stage
*Hawaii has one plant in the planning stage and one is on hold

Background to 2nd generation ethanol

- Prices of 1st generation biomass are increasing while the market for ethanol is growing
- Producers and governments consider alternative sources of biomass to produce ethanol
- 2nd generation biomass sources are widely available and they provide a huge opportunity in producing ethanol

Background to 2nd generation ethanol

- 2nd generation feedstocks include:
  - Stover, cobs and fiber from corn
  - Straw and bran from wheat
  - Bagasse from sugar cane
  - Pulp from sugar beet
  - Grasses
  - Empty fruit bunches from oil palm and glycerol
  - Woody materials

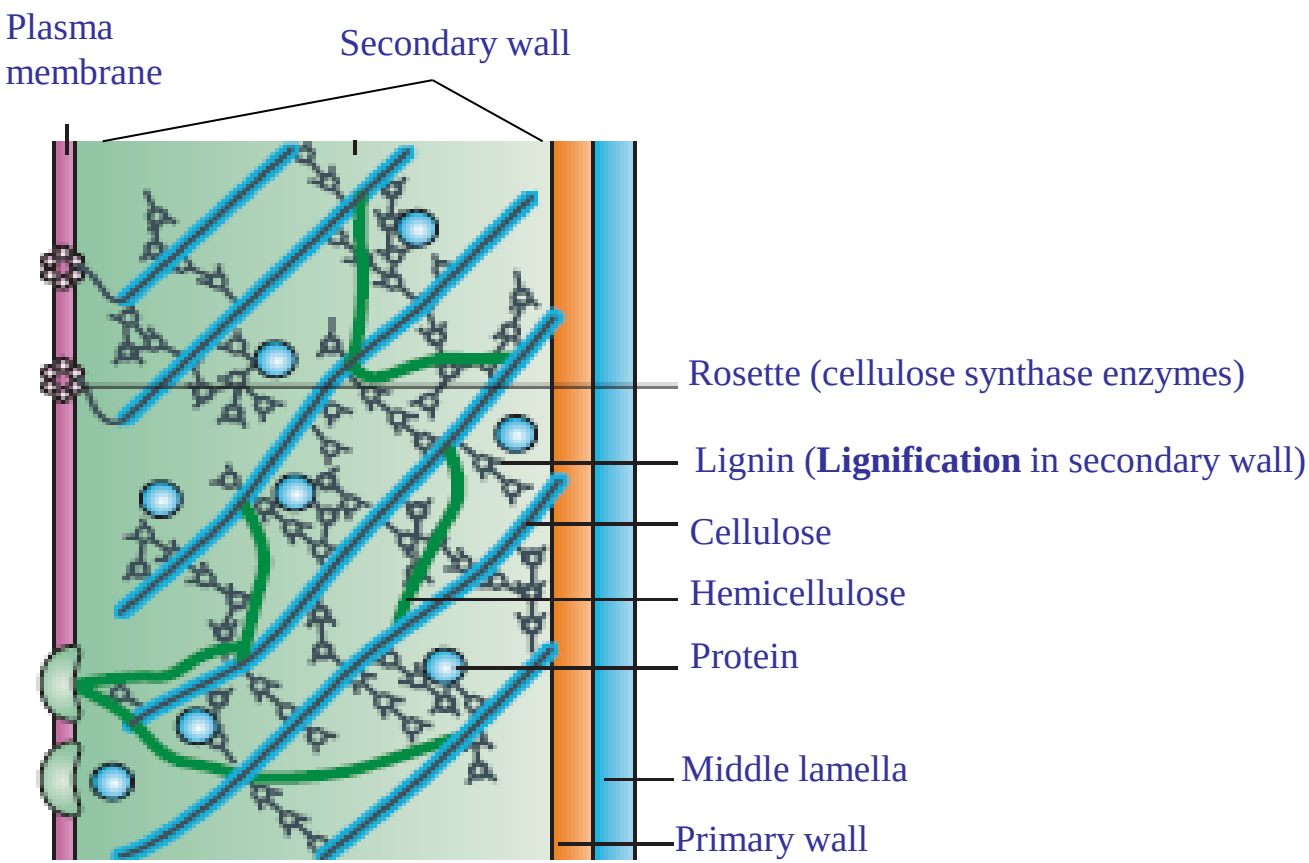
Wheat bran

Grasses

Sugar beet pulp

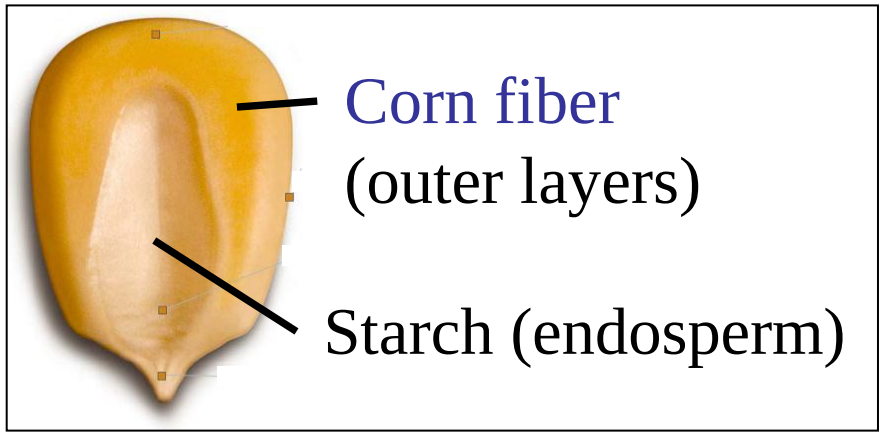
Bagasse

Plant cell wall architecture



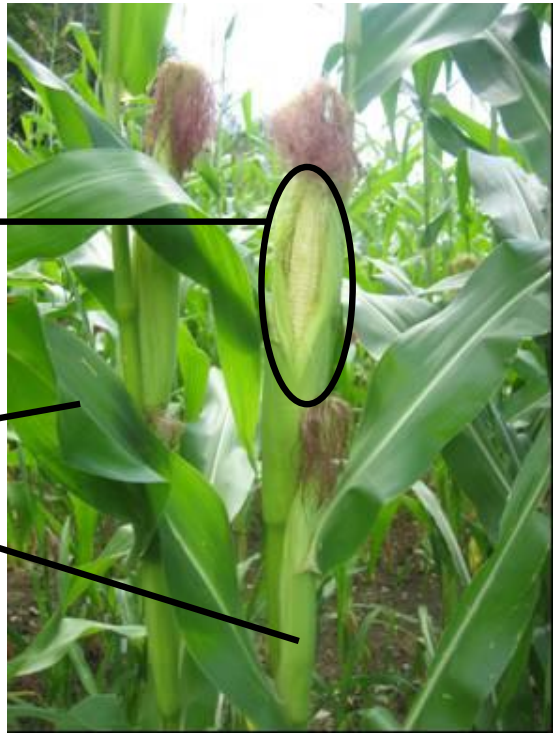
Sticklen, M.B. (2008). "Plant genetic engineering for biofuel production: towards affordable cellulosic ethanol." Nature Reviews **9**(Genetics): 433-443

Corn and co-products



Corn Cobs
(after removal of kernels)

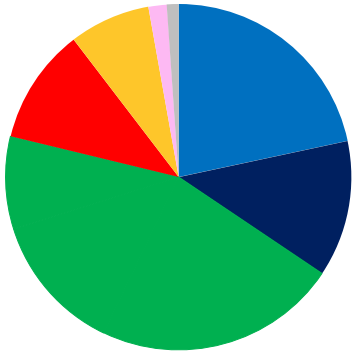
Corn Stover
(remaining plant material on land)



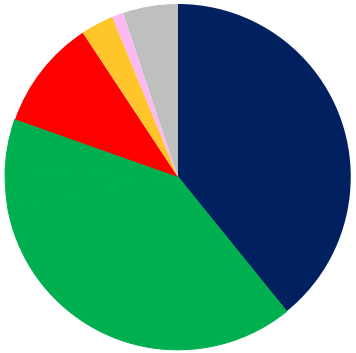
Feedstock composition

Examples of feedstocks under consideration for cellulosic ethanol production

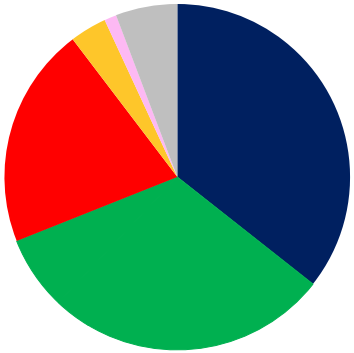
corn fiber



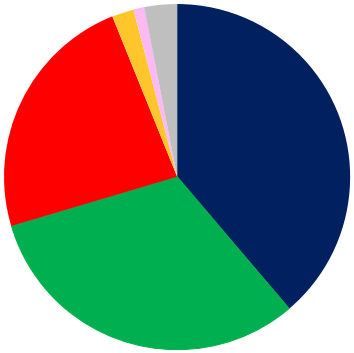
corn cobs



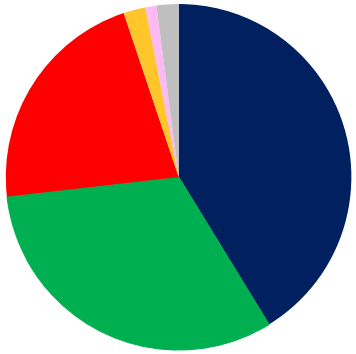
corn stover

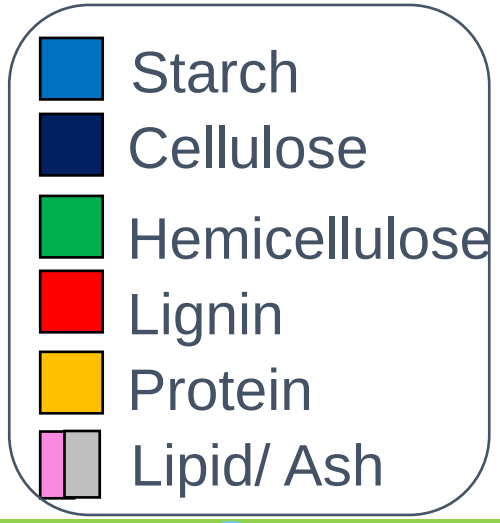


switch grass



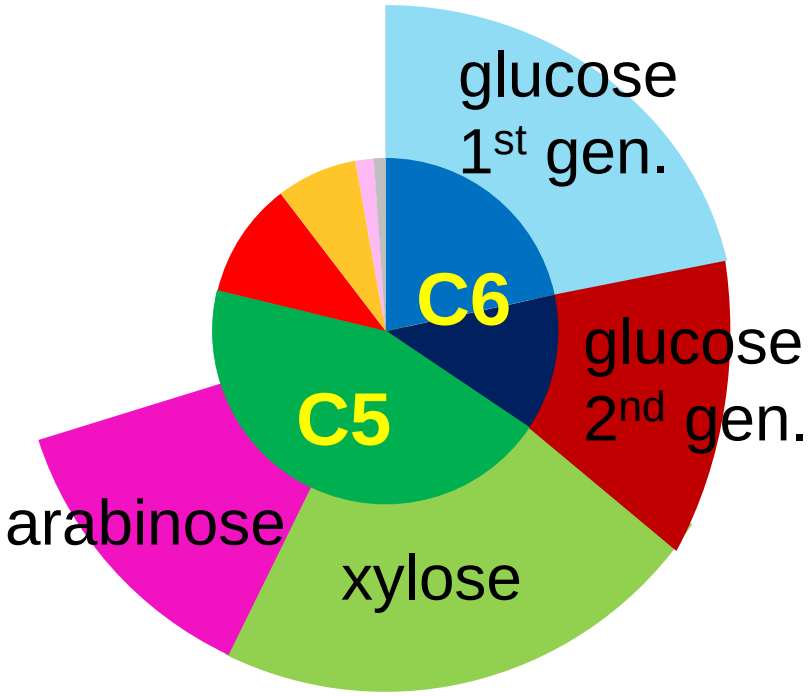
bagasse (sugar cane)



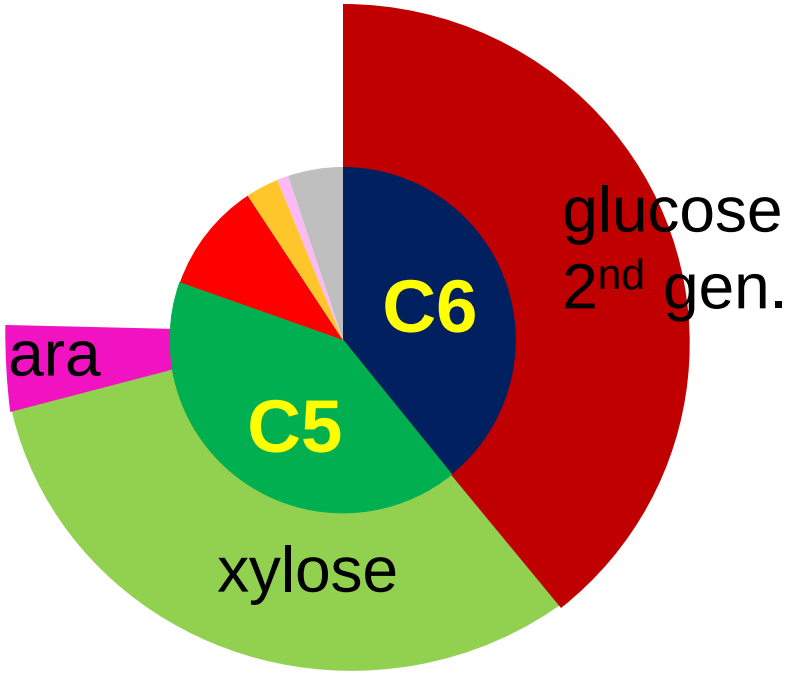


Feedstock composition of Corn Fiber and Corn Cobs

corn fiber



corn cobs



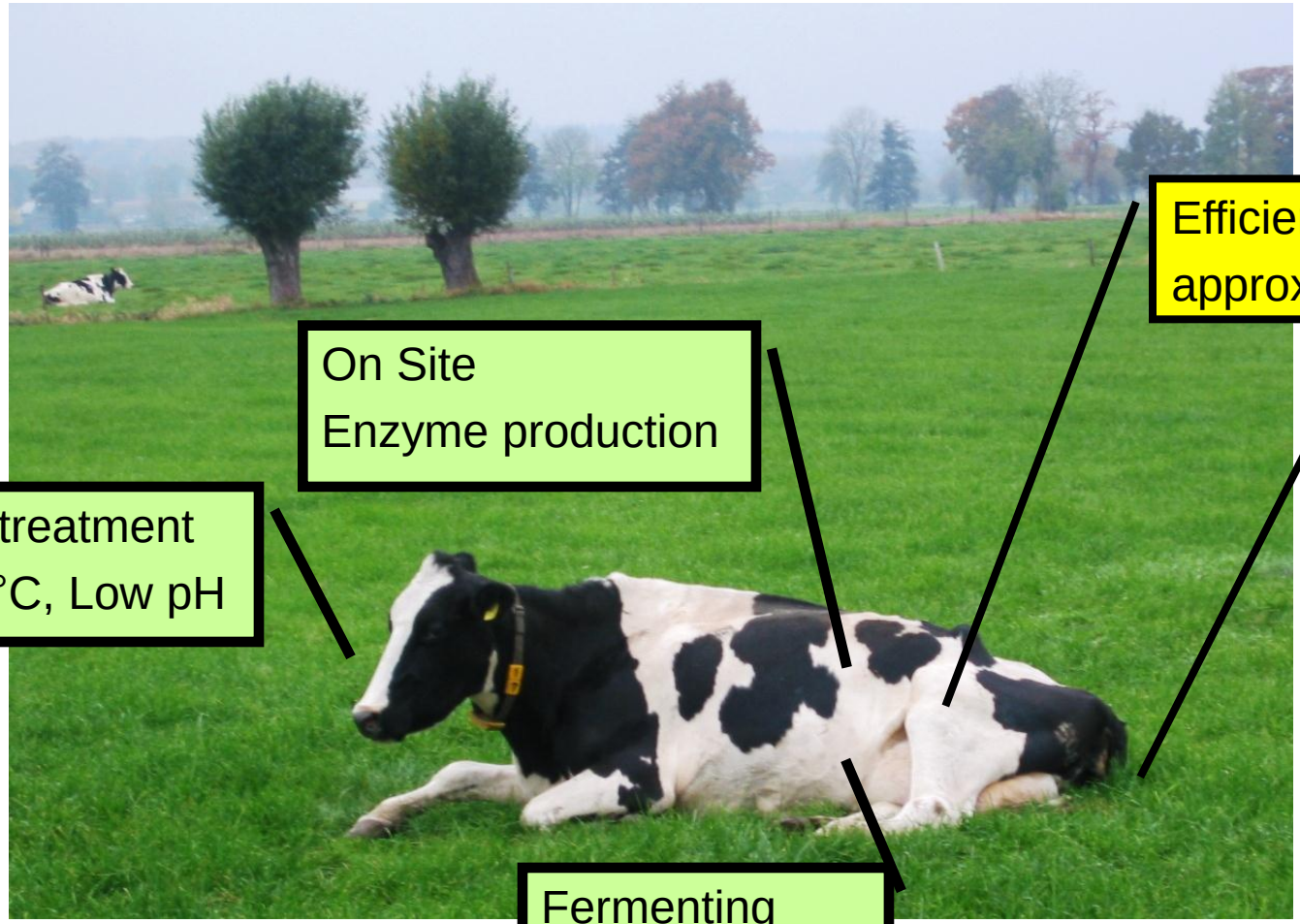
Major Technological Issues

1. Pretreatment

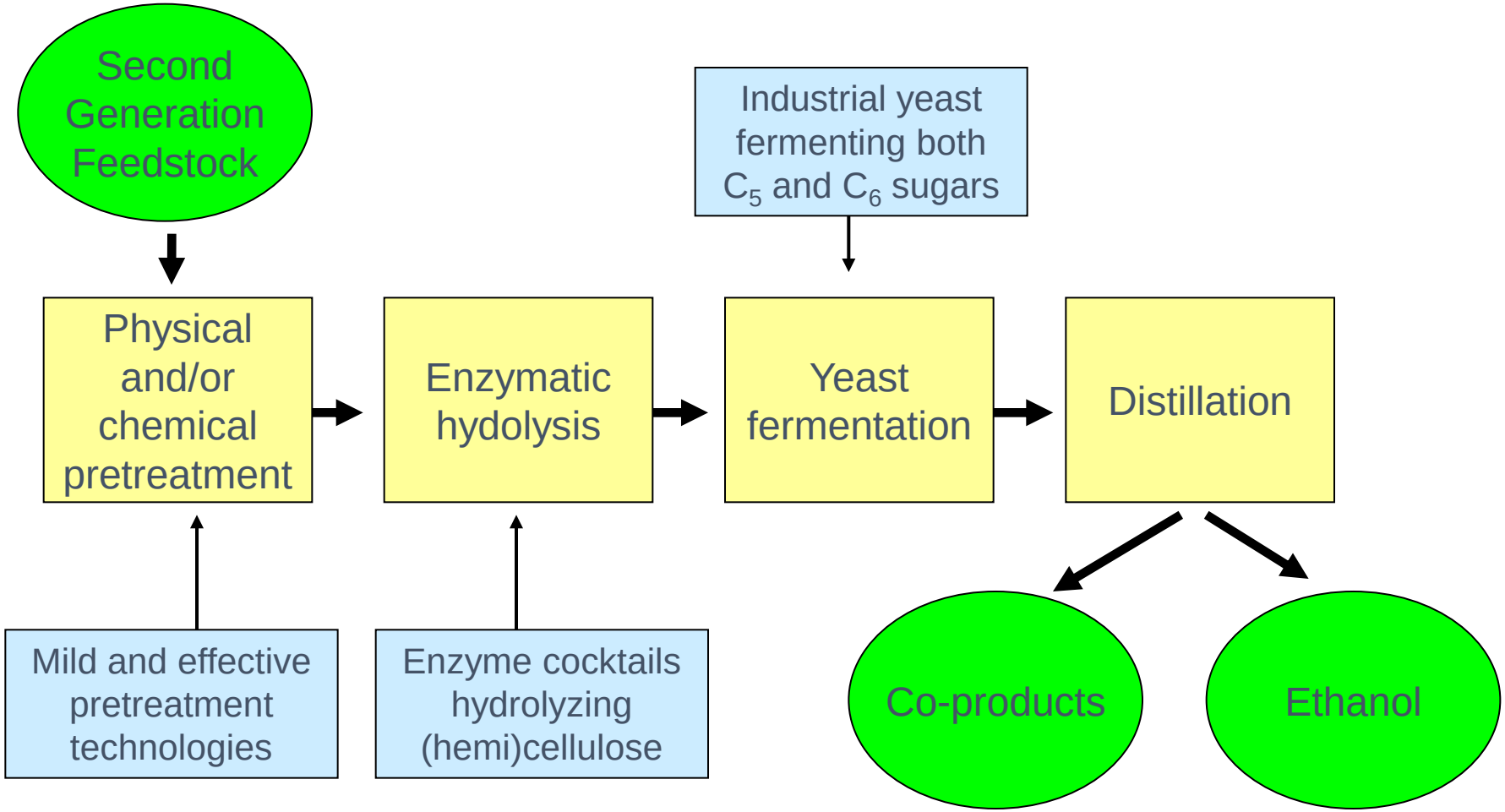
2. Hydrolytic polymer-degrading enzymes

3. Industrial yeast capable of C5 fermentations

Pretreatment & enzymatic hydrolysis

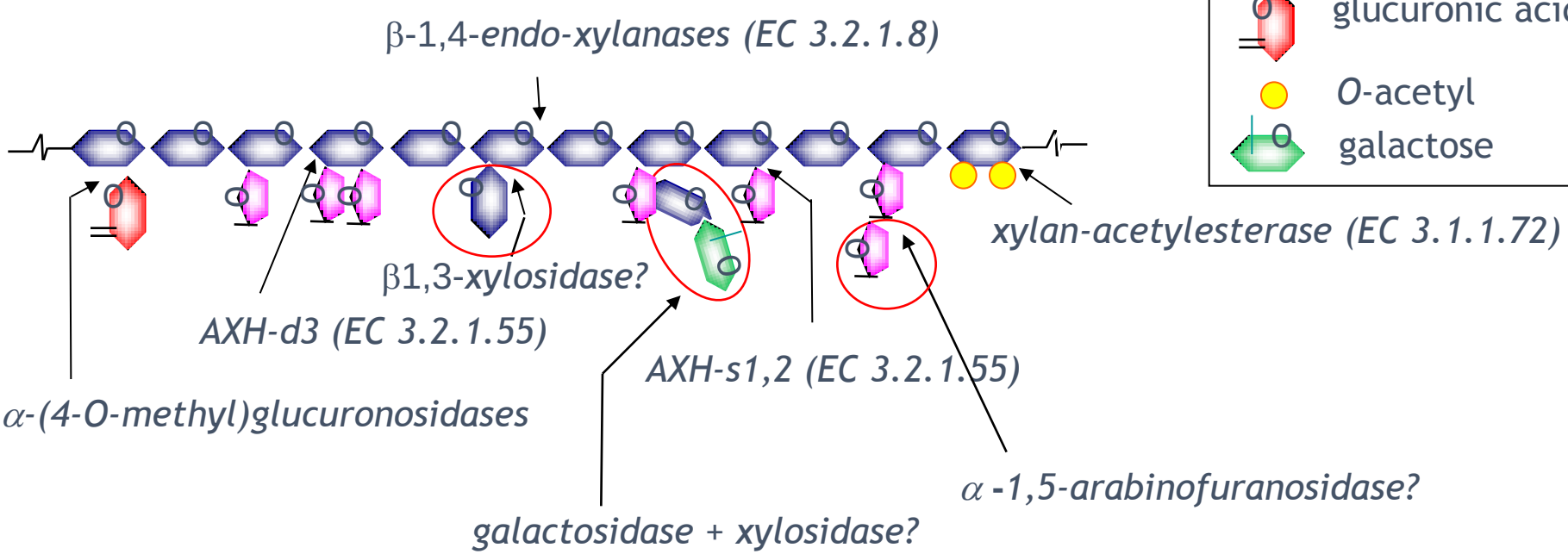


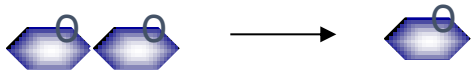
Simplified scheme production ethanol from second generation feedstocks



Enzymes and hemicellulose

Pretreatment can liberate substantial amounts of sugars from hemicellulose, but application of enzymes holds promise in establishing production processes with less severe conditions





β-xylosidase (EC 3.2.1.37)

Extra: *Feruloyl esterase (EC 3.1.1.73)*

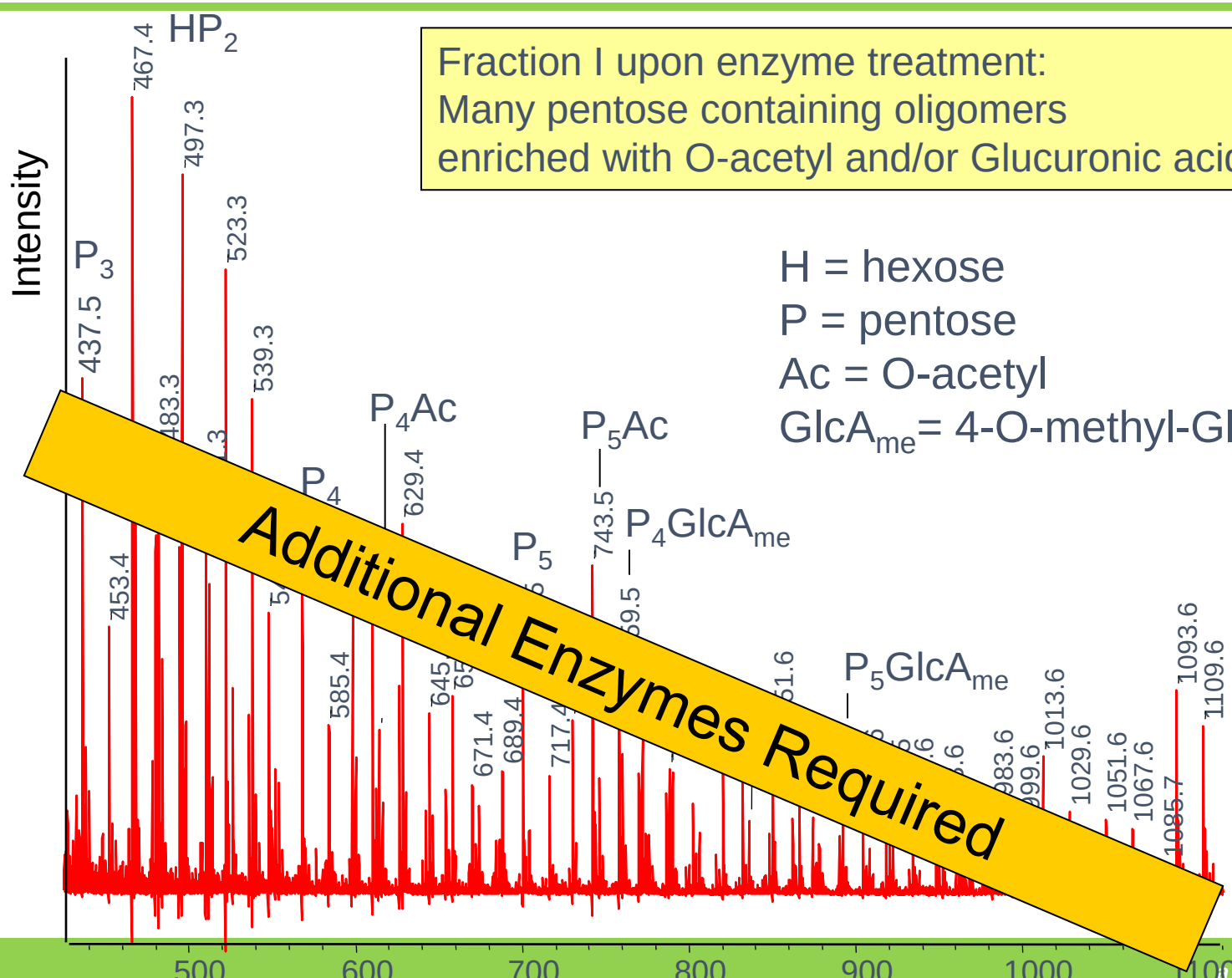
Approach enzyme work

- Determine remaining oligomeric structures after pretreatment and enzymatic hydrolysis
- Interactions with enzyme companies for testing preparations

Oligomers in Liquid Maldi-TOF MS

Fraction I upon enzyme treatment:
Many pentose containing oligomers
enriched with O-acetyl and/or Glucuronic acid side groups

H = hexose
P = pentose
Ac = O-acetyl
$GlcA_{me}$= 4-O-methyl-Glucuronic acid



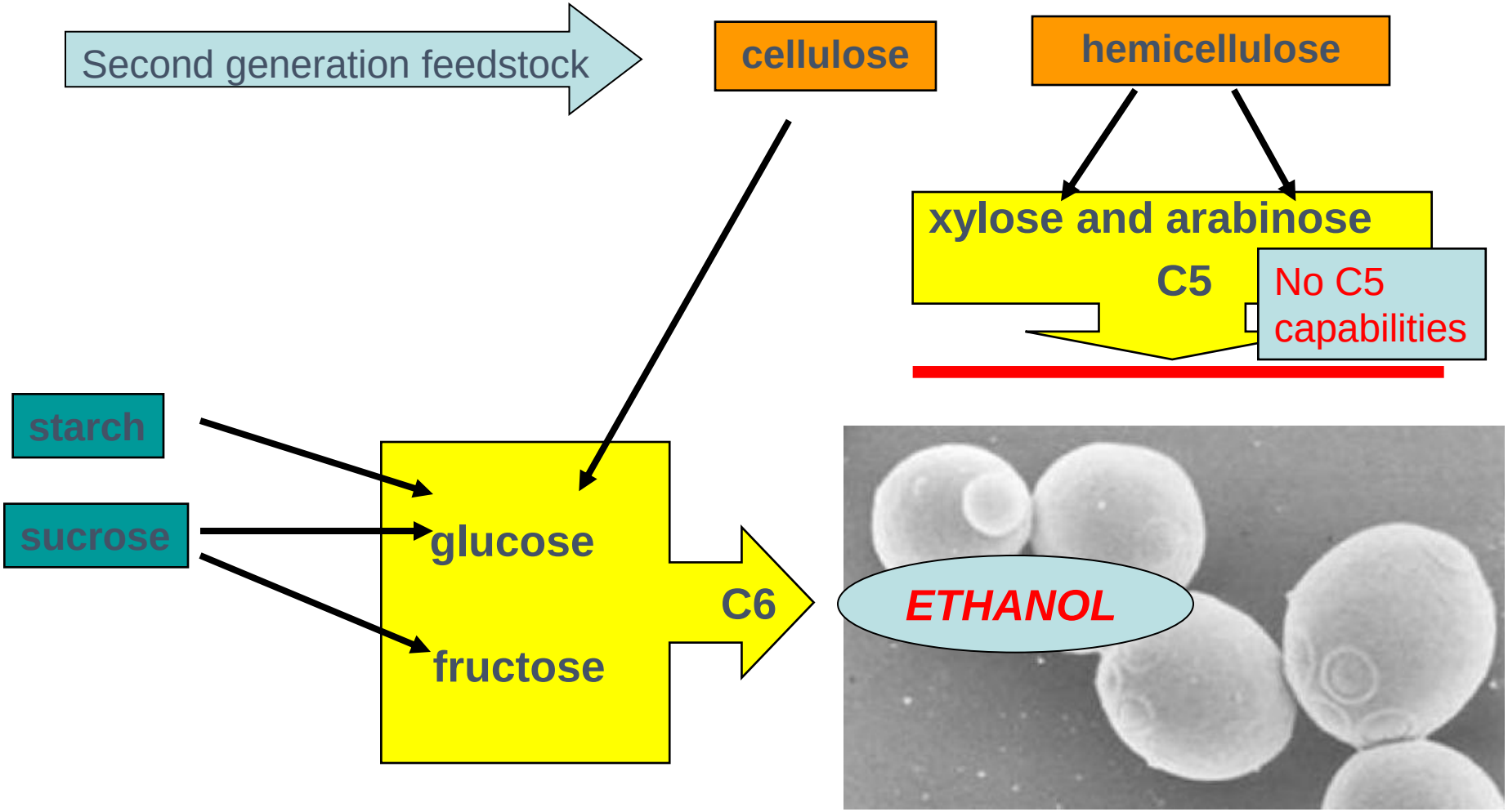
Additional Enzymes Required

Producing Ethanol with Yeast

Yeast Company

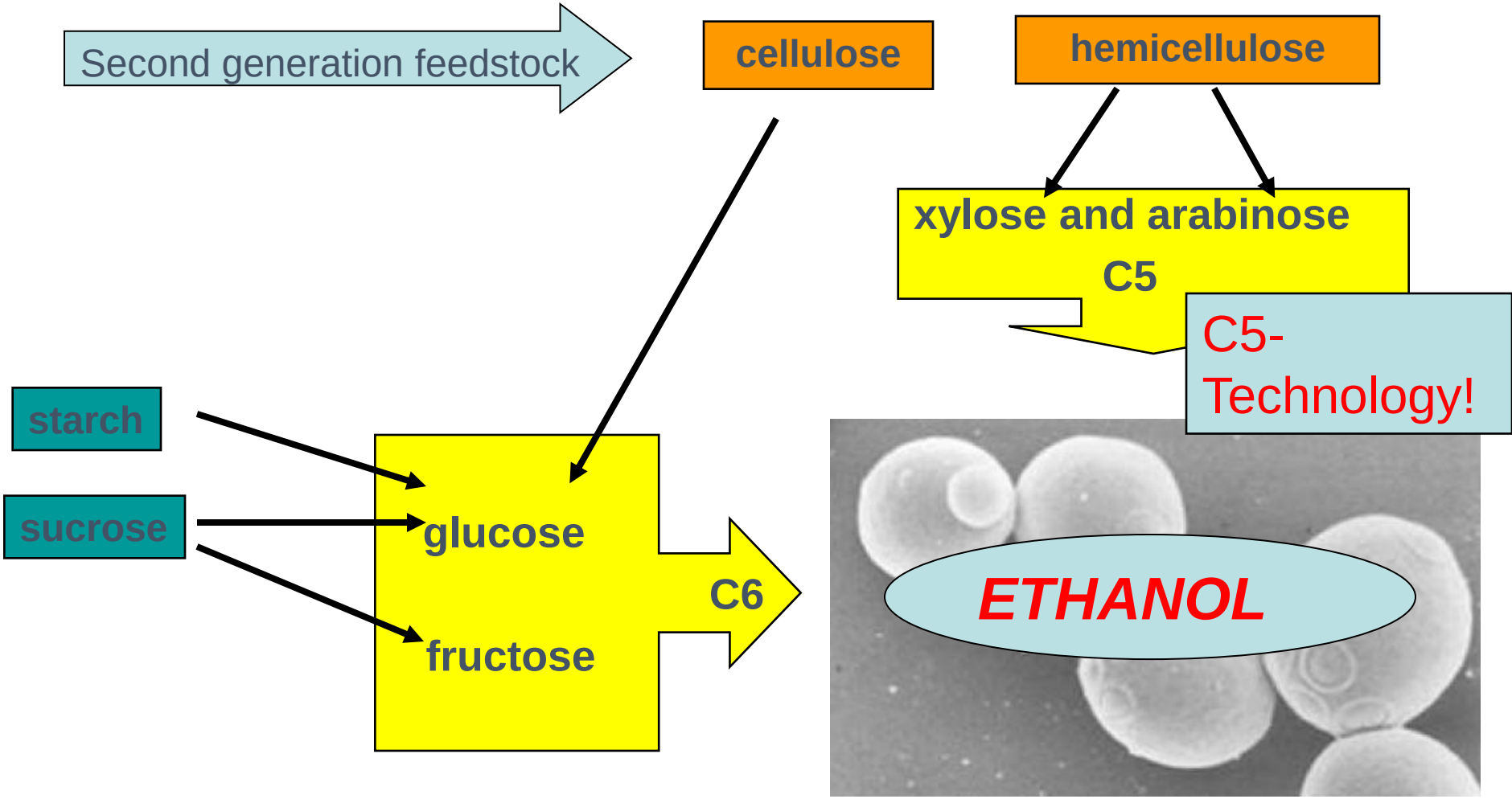
- The production of ethanol with yeast is a proven process for 1st generation feedstocks.
- The bulk of the fuel ethanol producers produce feed, and occasionally food products as well. Application of yeast (*S. cerevisiae*) is commonly accepted in this industry.
- Agricultural biomass such as corn fibers, corn cobs and corn stover can be made available at ethanol plants that now run on yeast.
- Relatively low capital investment is required when these second generation feedstocks can be fermented also by yeast in conjunction with yeast 1st generation processes.

Fermentation by natural *S. cerevisiae*



Fermentation by modified *S. cerevisiae*

Yeast Company

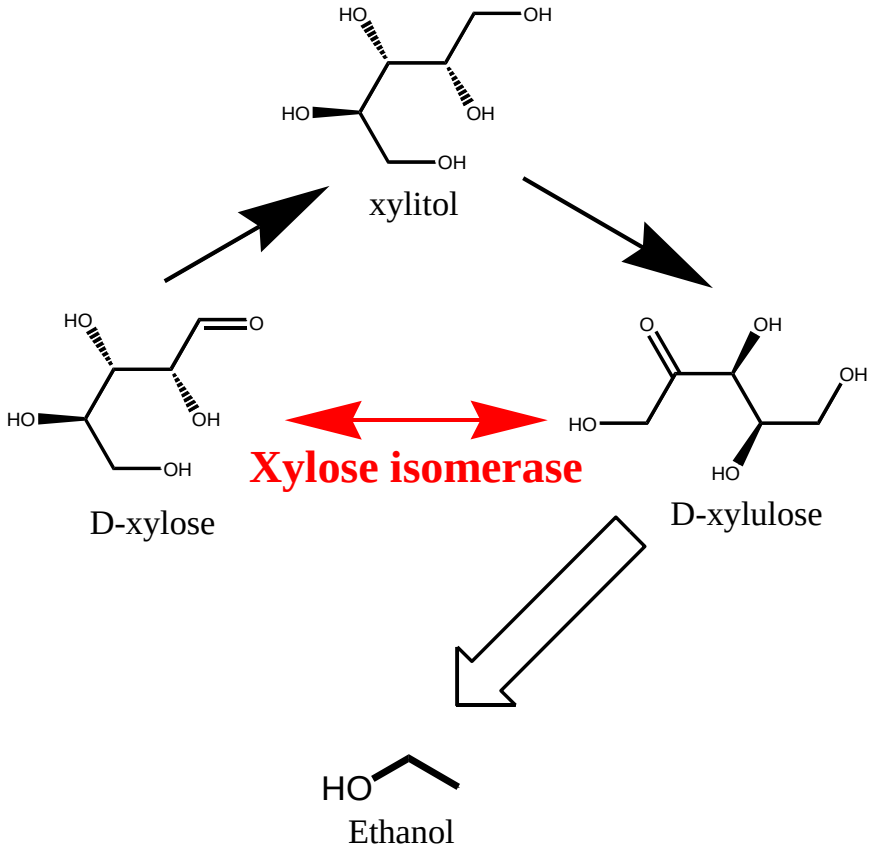


C5 Technology: Where did it start?

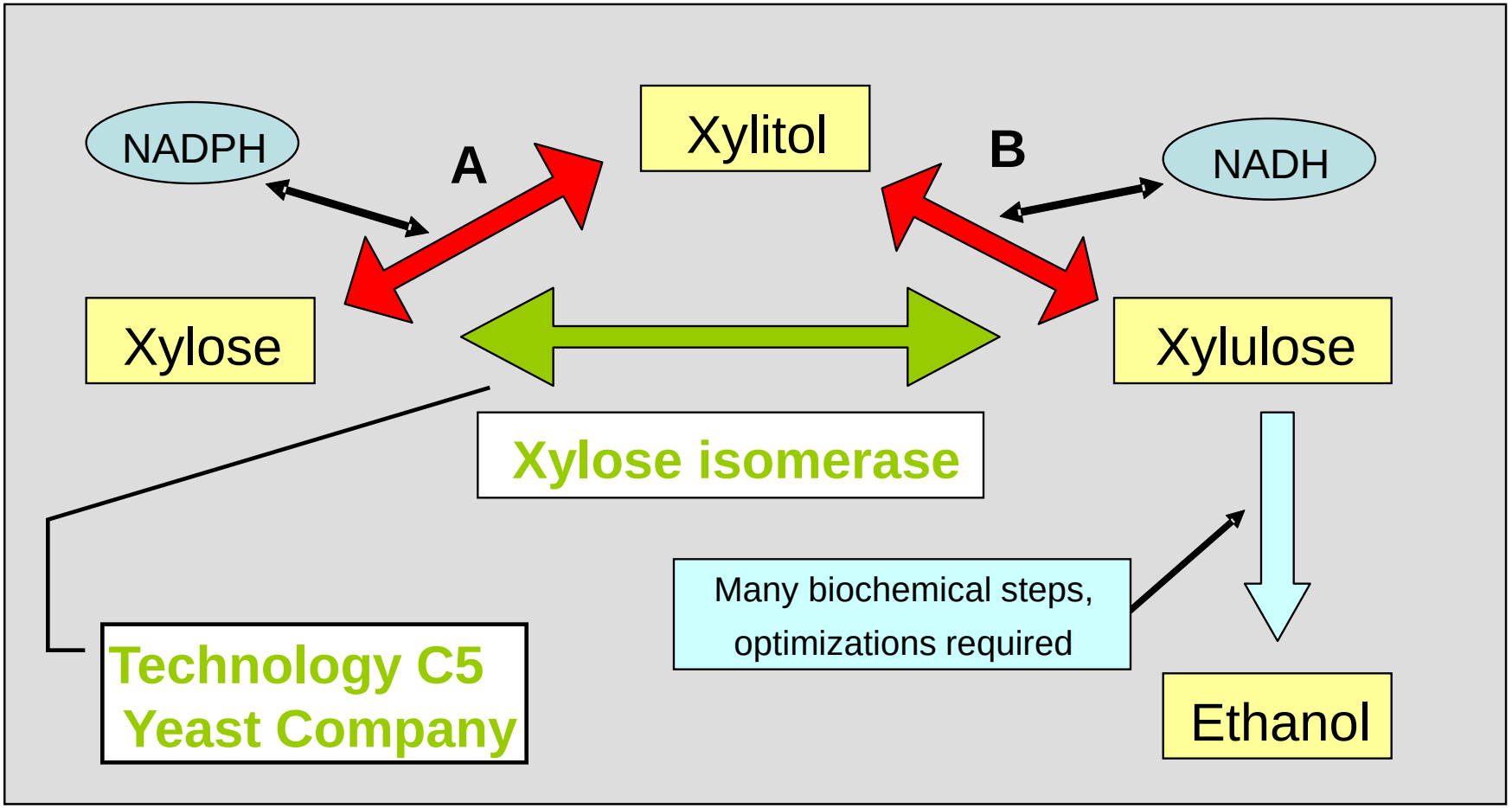
Kuyper, M, Toirkens, M.J., Diderich, J.A., Winkler, A.A., Van Dijken, J.P. and Pronk, J.T.

Evolutionary engineering of mixed-sugar utilization by a xylose-fermenting *Saccharomyces cerevisiae* strain.

FEMS Yeast Research 5, 399-409 (2005).

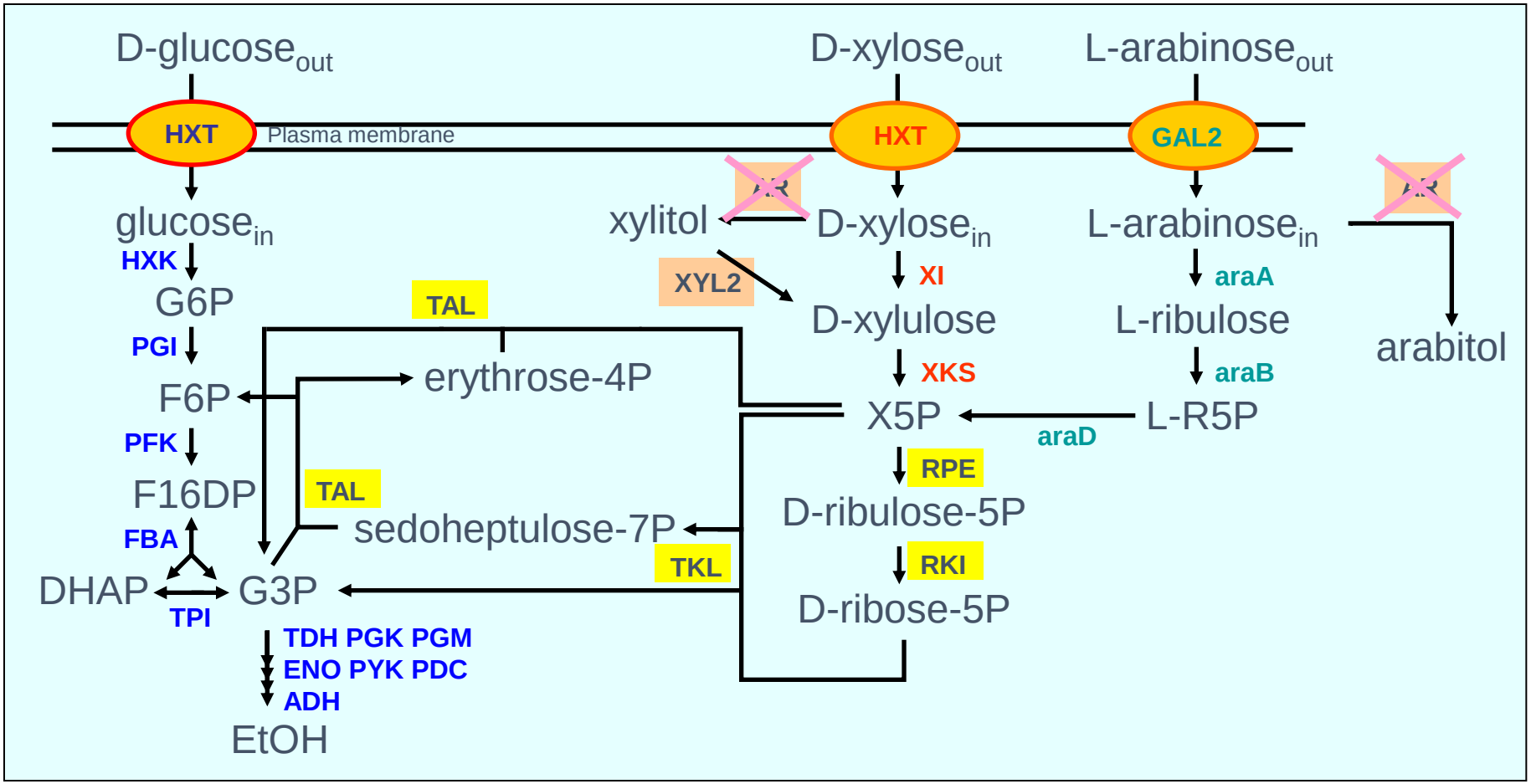


Key in xylose-fermenting *S.cerevisiae*



Enabling C5 Metabolism in *S. cerevisiae*

Yeast Company



Xylose isomerase

- Xylose isomerase (EC 5.3.1.5)
  - alternative name glucose isomerase
  - widely spread in Nature
  - not found in yeast
  - tetramer: 4 identical subunits
  - one subunit: around 440 amino acids

- Xylose isomerase is difficult to express in *S. cerevisiae*
  - Functional Expression of a Bacterial Xylose Isomerase in *Saccharomyces cerevisiae*

    D. Brat, E. Boles and B. Wiedemann

    Applied and Environmental Microbiology, Apr. 2009, p. 2304

Xylose isomerase

Yeast Company

- Xylose isomerase (EC 5.3.1.5)
  - alternative name glucose isomerase
  - widely spread in Nature
  - not found in yeast
  - tetramer: 4 identical subunits
  - one subunit: around 440 amino acids

- Xylose isomerase is difficult to express in *S. cerevisiae*
  - Functional Expression of a Bacterial Xylose Isomerase in *Saccharomyces cerevisiae*

    D. Brat, E. Boles and B. Wiedemann

    Applied and Environmental Microbiology, Apr. 2009, p. 2304

- **Rationale for expression behavior?**

Amino acid sequence Xylose Isomerase

Primary structure with conserved regions (Prosite)

M A K E Y F P Q I Q K I K F E G K D S K N P L A F H Y Y D A E K E V M G K K M K D W L R F A M A W W H T L C A E G A D Q

F G G G T K S F P W N E G T D A I E I A K Q K V D A G F E I M Q K L G I P Y Y C F H D V D L V S E G N S I E E Y E S N L

MOTIF1

K A V V A Y L K E K Q K E T G I K L L W S T A N V F G H K R Y M N G A S T N P D F D V V A R A I V Q I K N A I D A G I E

MOTIF2 MOTIF3

L G A E N Y V F W G G R E G Y M S L L N T D Q K R E K E H M A T M L T M A R D Y A R S K G F K G T F L I E P K P M E P T

MOTIF4 MOTIF5 MOTIF6

K H Q Y D V D T E T A I G F L K A H N L D K D F K V N I E V N H A T L A G H T F E H E L A C A V D A G M L G S I D A N R

MOTIF7

G D Y Q N G W D T D Q F P I D Q Y E L V Q A W M E I I R G G G F V T G G T N F D A K T R R N S T D L E D I I I A H V S G

MOTIF8 MOTIF9

M D A M A R A L E N A A K L L Q E S P Y T K M K K E R Y A S F D S G I G K D F E D G K L T L E Q V Y E Y G K K N G E P K

Q T S G K Q E L Y E A I V A M Y Q

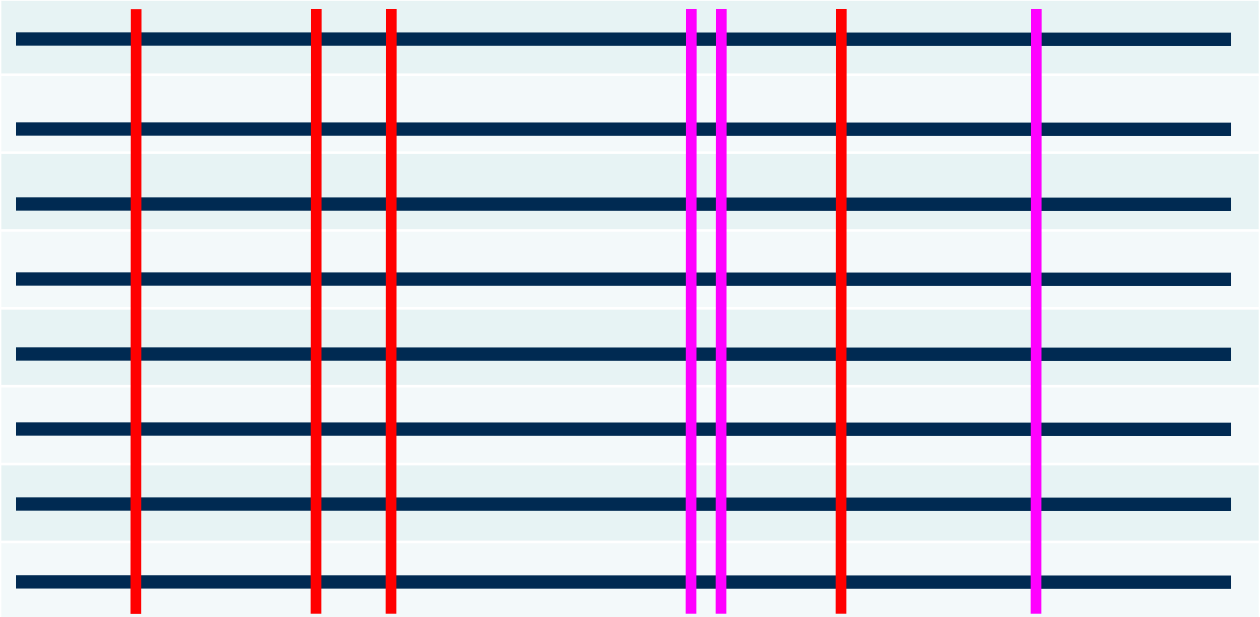
Active site: conserved in all xylose isomerases

Metal-binding site: conserved in all xylose isomerases

Comparison of xylose isomerases from various sources

Yeast Company

Schematic representation

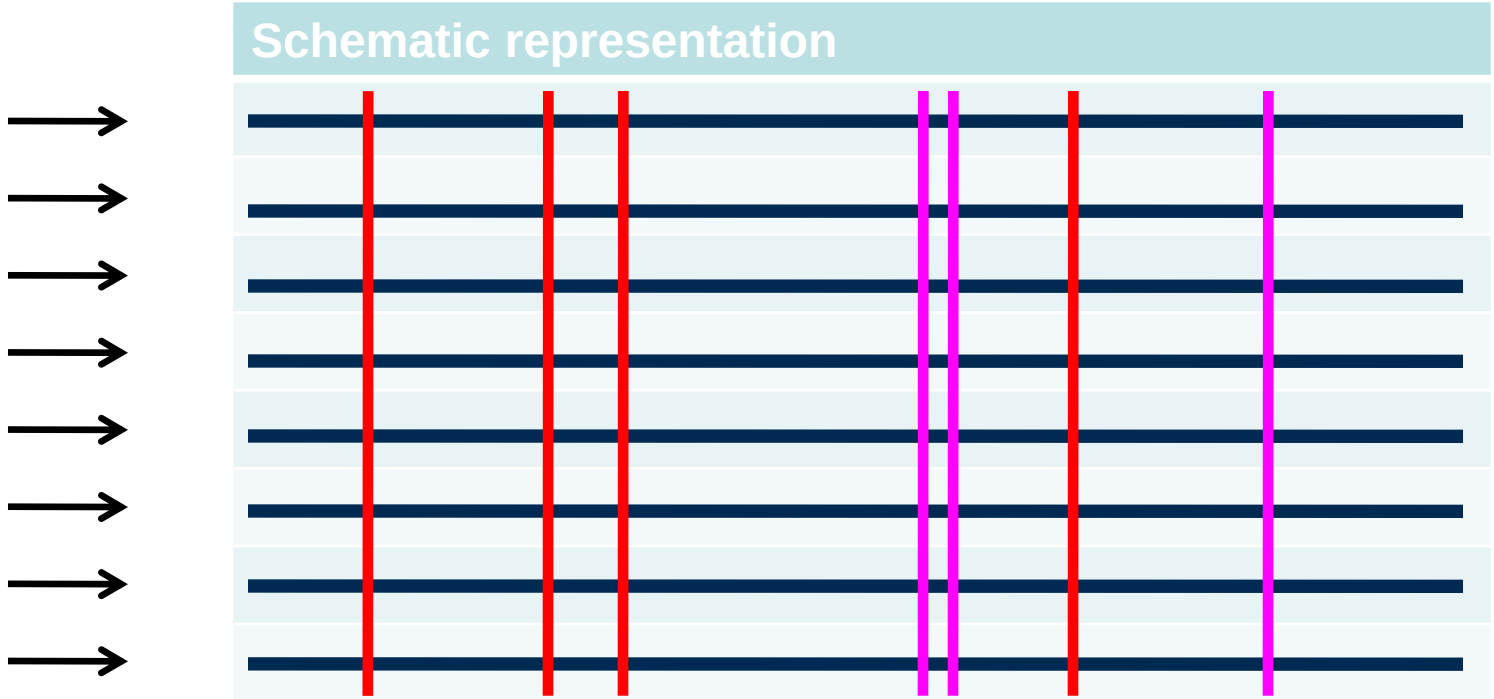


: conserved amino acids active site

: conserved amino acids metal-binding site

Comparison of xylose isomerases from various sources

Eight sequences of xylose isomerase

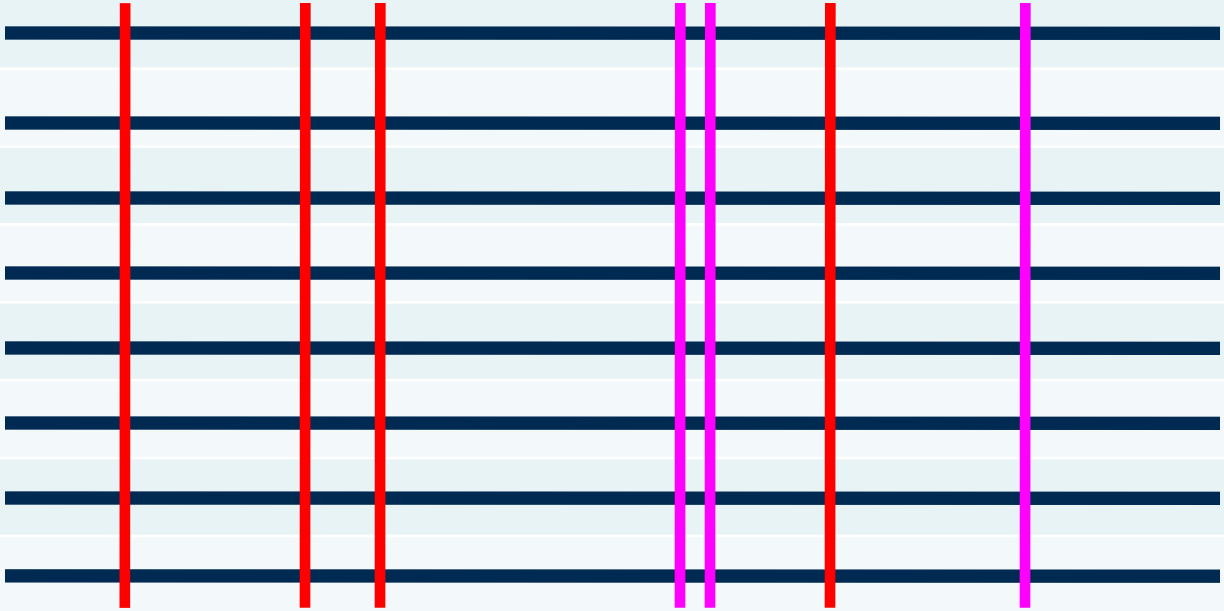


: conserved amino acids active site

: conserved amino acids metal-binding site

Xylose isomerase expression in *S. cerevisiae*

Origin	Active	Schematic representation
Piromyces	yes	
Bacteroides	yes	
Ciona	yes	
Clostridium	yes	
Physcomitrella	no	
Arabidopsis	no	
Haemophilus	no	
Arthrobacter	no	

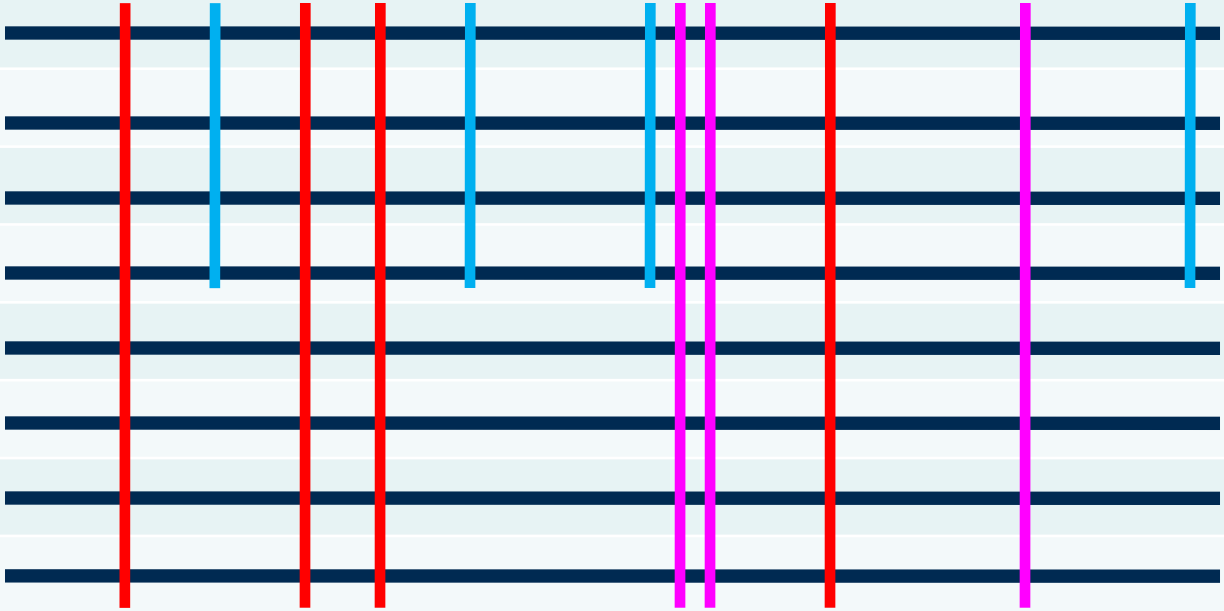


: conserved amino acids active site

: conserved amino acids metal-binding site

Xylose isomerase expression in *S. cerevisiae*

Origin	Active	Schematic representation
Piromyces	yes	
Bacteroides	yes	
Ciona	yes	
Clostridium	yes	
Physcomitrella	no	
Arabidopsis	no	
Haemophilus	no	
Arthrobacter	no	



: conserved amino acids active site

: conserved amino acids metal-binding site

: conserved amino acids in xylose isomerase active in yeast

Amino acid sequence

Yeast Company

Primary structure with conserved regions

M A K E Y F P Q I Q K I K F E G K D S K N P L A F H Y Y D A E K E V M G K K M K D W L R F A M A W W H T L C A E G A D Q

F G G G T K S F P W N E G T D A I E I A K Q K V D A G F E I M Q K L G I P Y Y C F H D V D L V S E G N S I E E Y E S N L

MOTIF1

K A V V A Y L K E K Q K E T G I K L L W S T A N V F G H K R Y M N G A S T N P D F D V V A R A I V Q I K N A I D A G I E

MOTIF2 MOTIF3

L G A E N Y V F W G G R E G Y M S L L N T D Q K R E K E H M A T M L T M A R D Y A R S K G F K G T F L I E P K P M E P T

MOTIF4 MOTIF5 MOTIF6

K H Q Y D V D T E T A I G F L K A H N L D K D F K V N I E V N H A T L A G H T F E H E L A C A V D A G M L G S I D A N R

MOTIF7

G D Y Q N G W D T D Q F P I D Q Y E L V Q A W M E I I R G G G F V T G G T N F D A K T R R N S T D L E D I I I A H V S G

MOTIF8 MOTIF9

M D A M A R A L E N A A K L L Q E S P Y T K M K K E R Y A S F D S G I G K D F E D G K L T L E Q V Y E Y G K K N G E P K

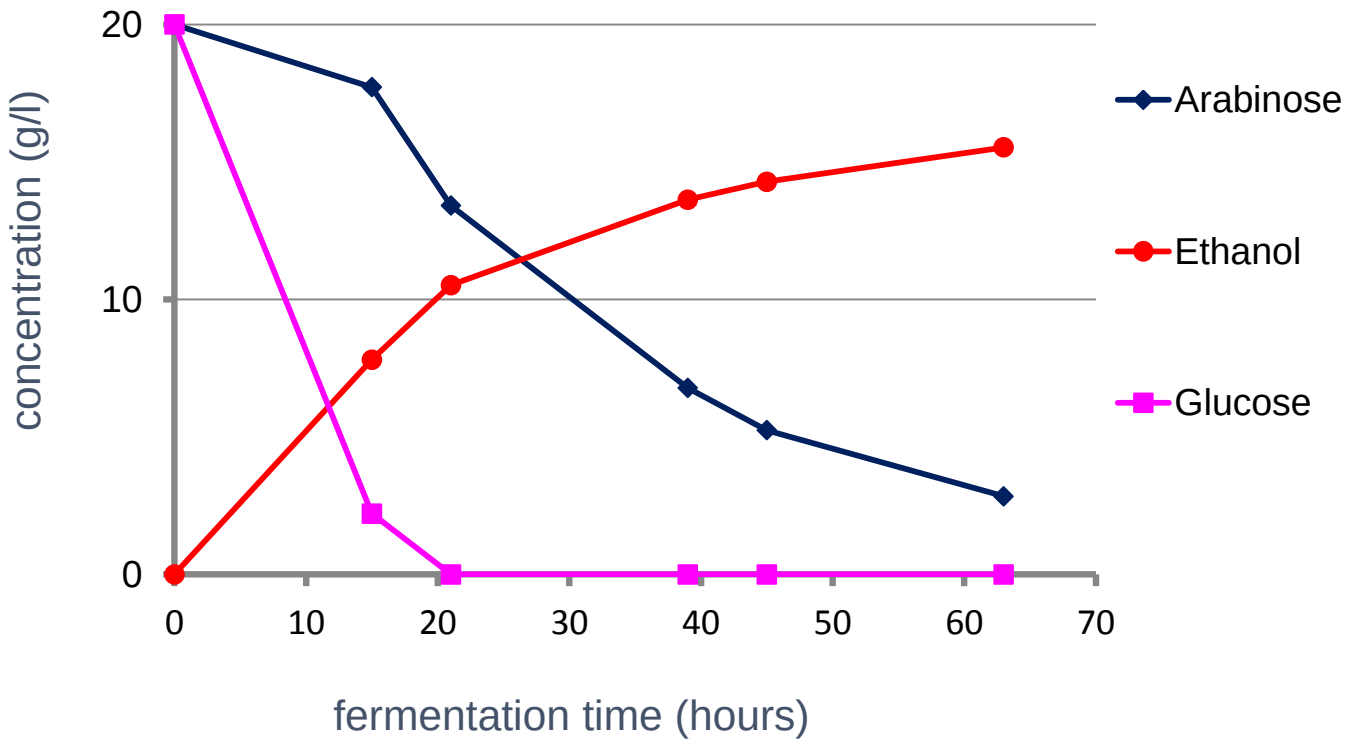
Q T S G K Q E L Y E A I V A M Y Q

Active site: conserved in all xylose isomerases

Metal-binding site: conserved in all xylose isomerases

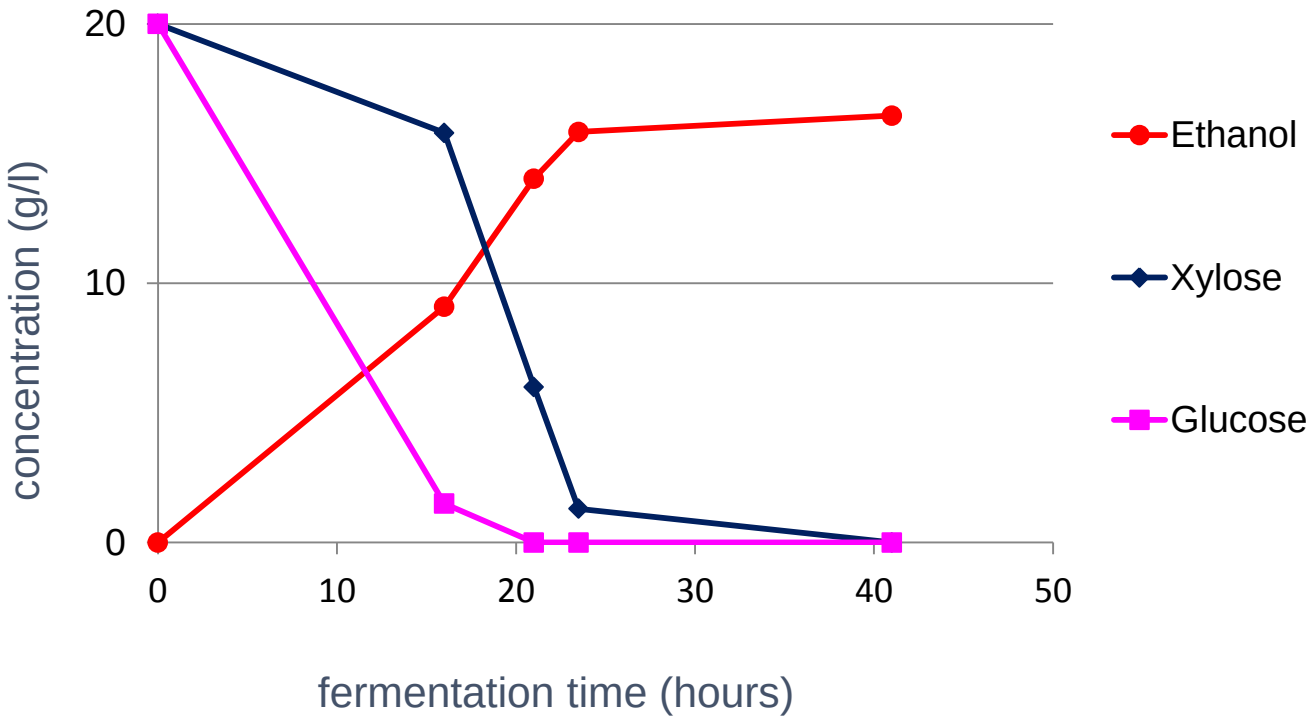
Conserved in Xylose Isomerases active in yeast (patented)

Arabinose-fermenting RN1015



Mineral medium
0.2 g/l yeast pitch

Xylose-fermenting RN1001



Mineral medium
0.2 g/l yeast pitch

Marker-free strain

Fermentation rates in 2008

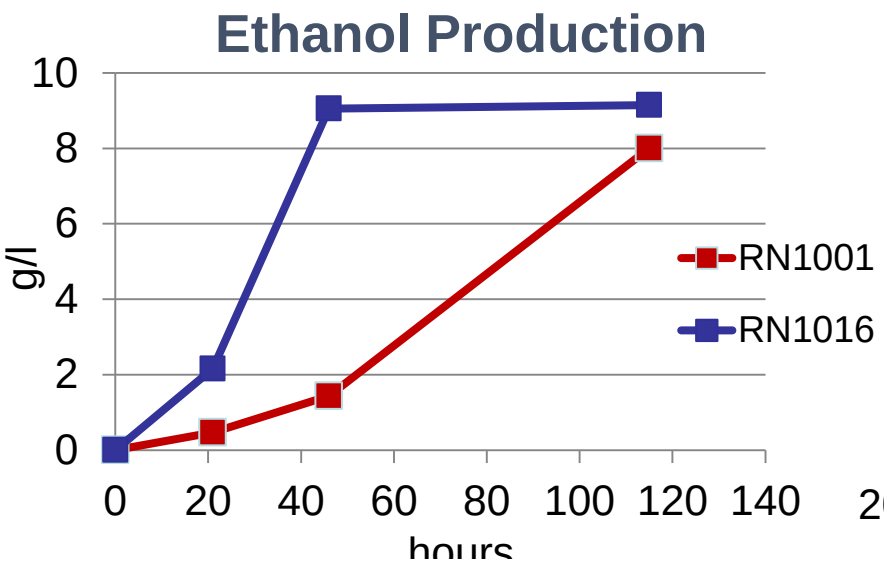
Strain	Compound	Rate (g sugar.(g yeast. h)$^{-1}$)
RN1001/1015	Glucose	3.0
RN1001	Xylose	1.4
RN1015	Arabinose	0.7

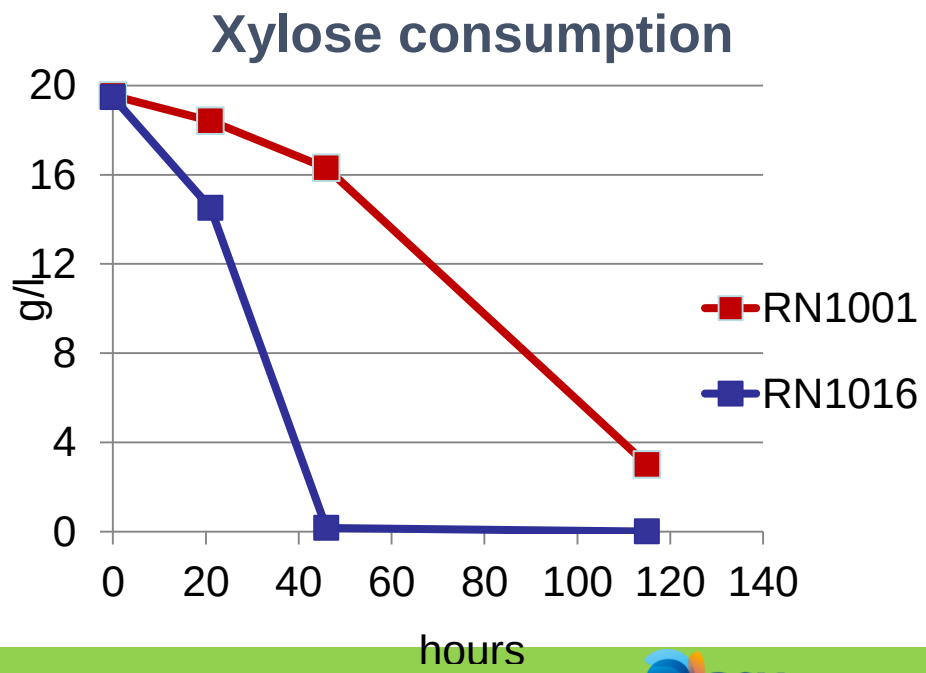
Optimization Xylose-fermenting strain since 2008

- Several routes have been taken to optimize the performance of the original xylose-fermenting strain

- Key aspects:
  - Rate of xylose consumption
  - Tolerance to acetic acid

Xylose consumption and ethanol production at 3g/l Acetic acid





Mineral medium
0.5 g/l yeast pitch

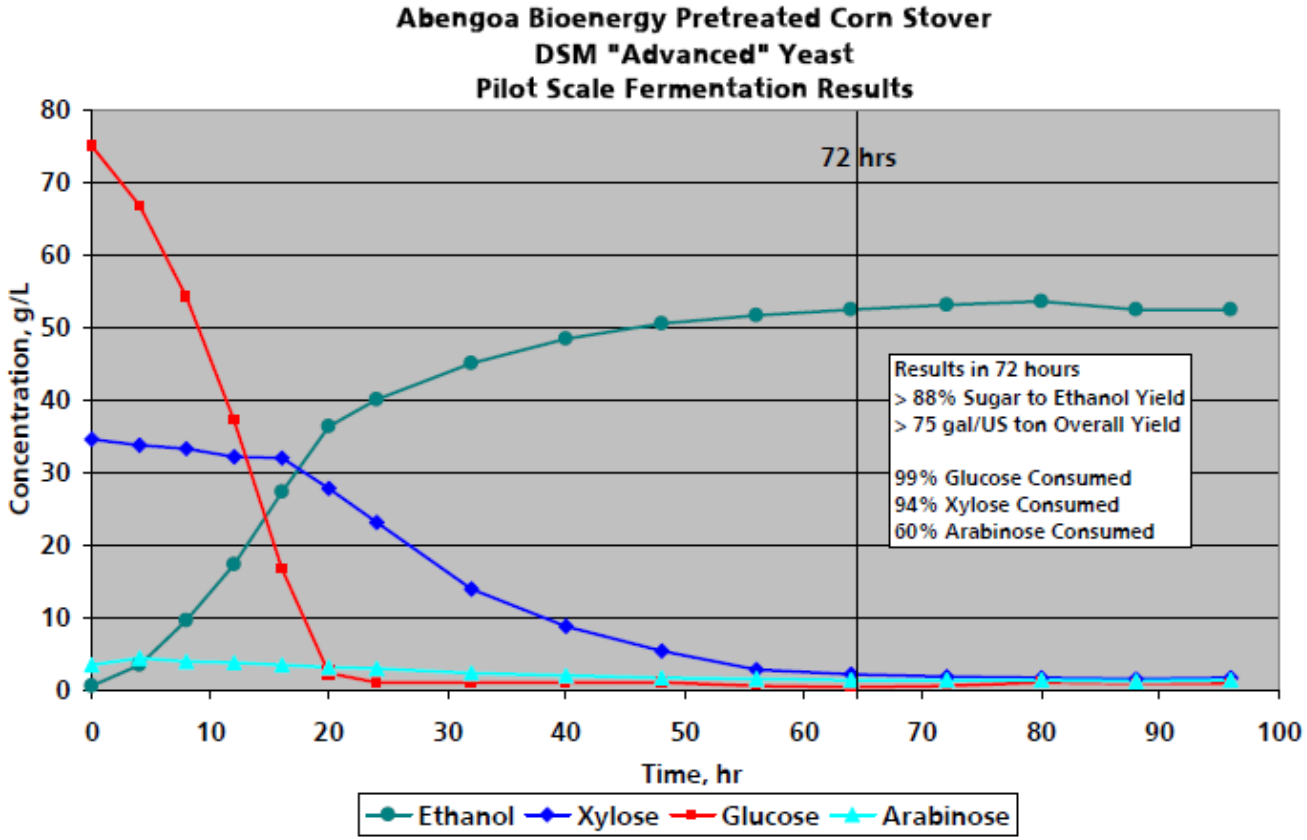
Application test at ABNT (York, NE, USA)

Yeast Company

ABENGOA BIOENERGY

Enabling Technology Breakthroughs

Significant Breakthroughs in Fermentation Recently



Science. Solutions. Service.

a DSM company

Issues Industrial Yeast Strains

- The current C5 Yeast Company strain is performing very well
- Several tons of yeast that exhibit C5 Yeast Company's Technology have been produced successfully by a commercial partner
- This yeast has been tested successfully at pilot scale

Nevertheless:

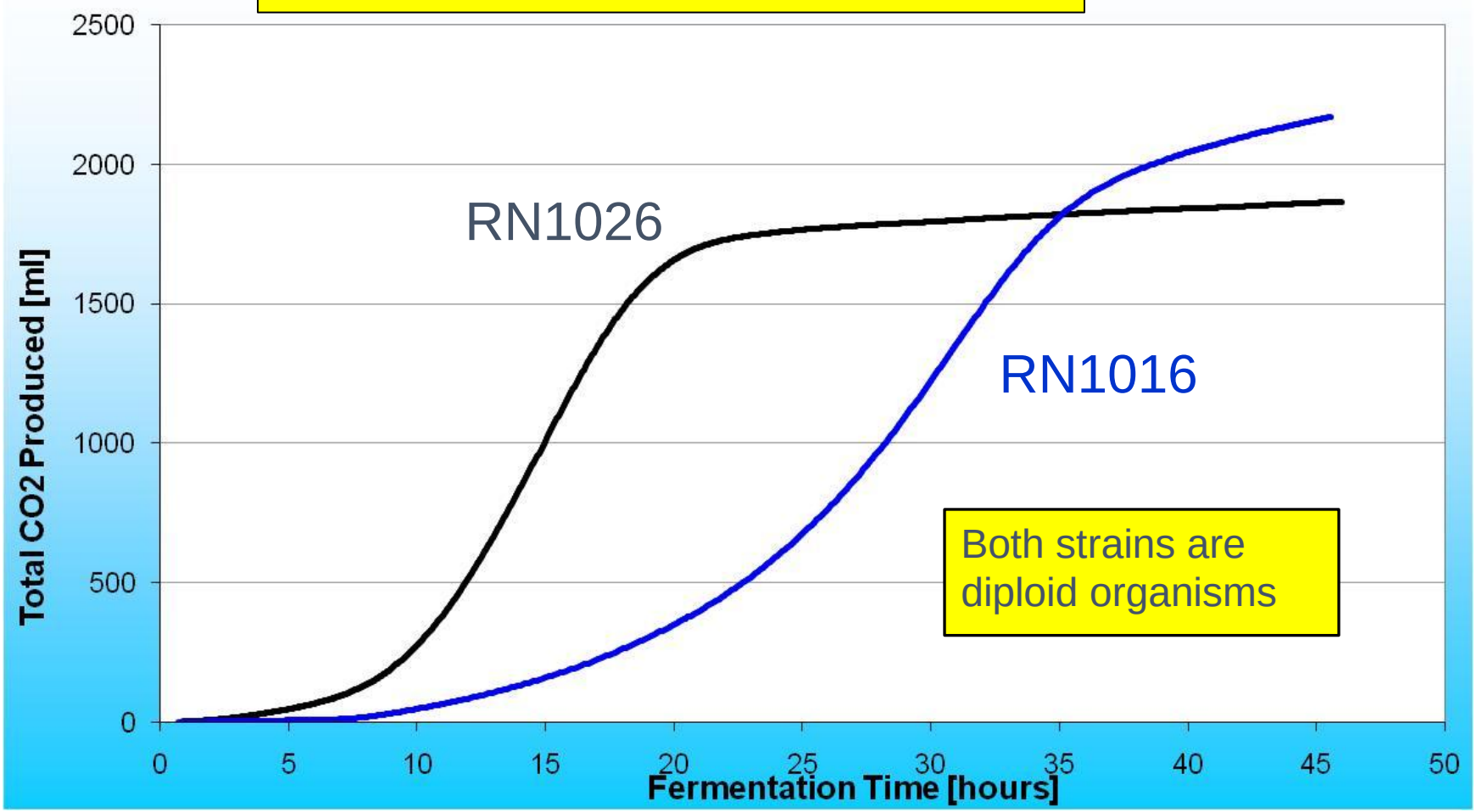
- Better hosts may be available that are more suitable in toxic hydrolysates

Industrial hosts

- Several strains have been tested for their potential as better hosts
- Promising strains have been obtained
- These strains are used as hosts for the C5-technology

Other strain on corn stover hydrolysate

Glucose only for RN1026
Glucose plus xylose for RN1016



Both strains are diploid organisms

Conclusions

- C5 Yeast Company yeast is available and has been produced at ton scale.
- C5 Yeast Company collaborates with first-movers in the ethanol industry around the world.
- C5 Yeast Company yeast is being successfully trialed at pilot scale at leading ethanol producers and technology suppliers.
- C5 Yeast Company continues to further develop and improve its yeast strains to resist higher inhibitor concentrations, etc.