Simultaneous Co-Fermentation of Mixed Sugars: A Promising Strategy for Producing Cellulosic Biofuels and Chemicals

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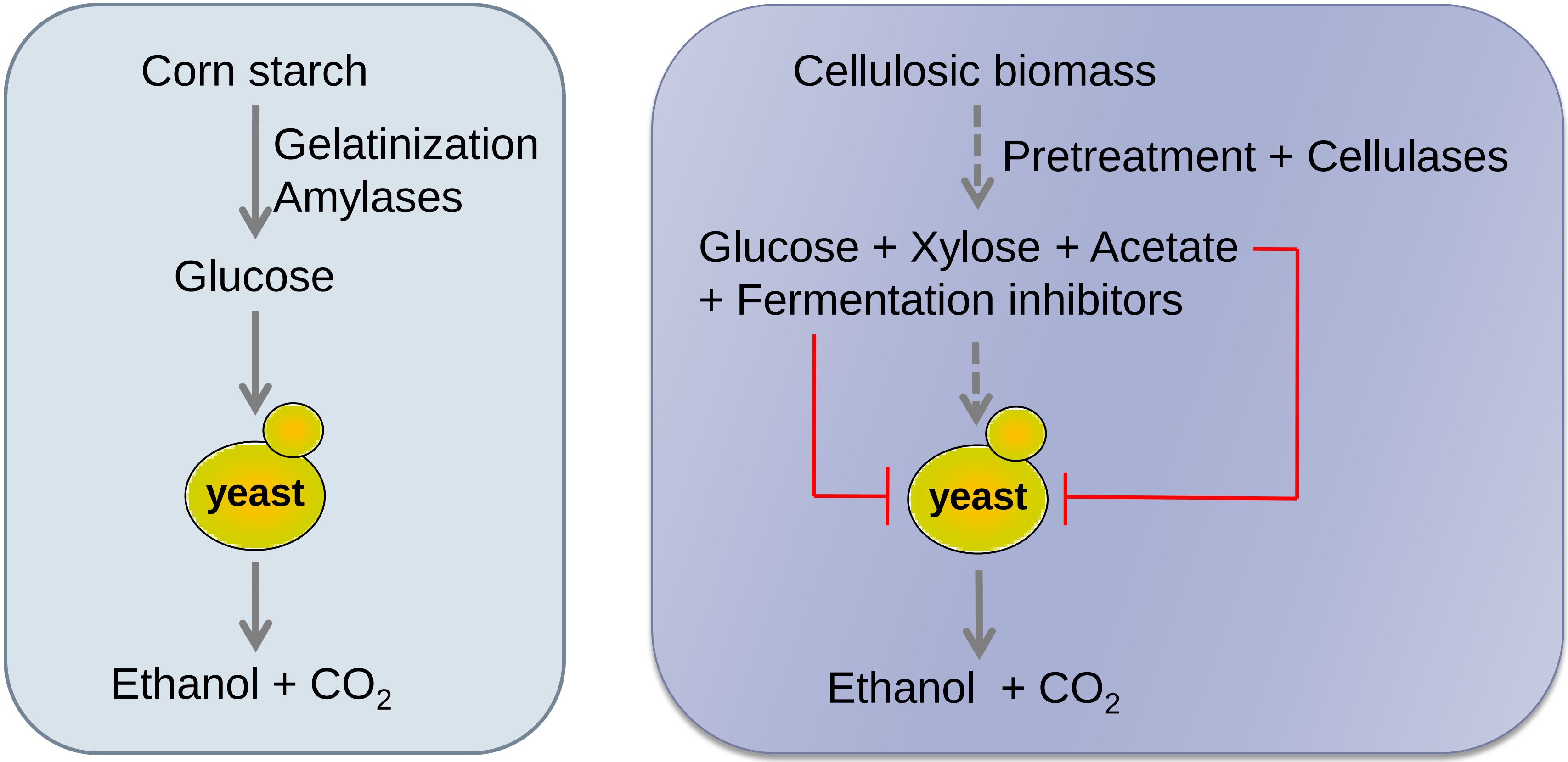
Corn ethanol vs. Cellulosic ethanol



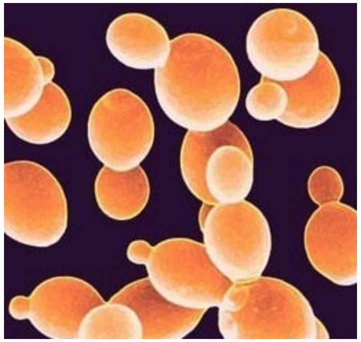
- Single sugar fermentation
- No fermentation inhibitors
- Easy high loading

- **Mixed** sugar fermentation
- Fermentation inhibitors
- Difficulties in high loading

Saccharomyces cerevisiae: a workhorse strain for industrial ethanol production

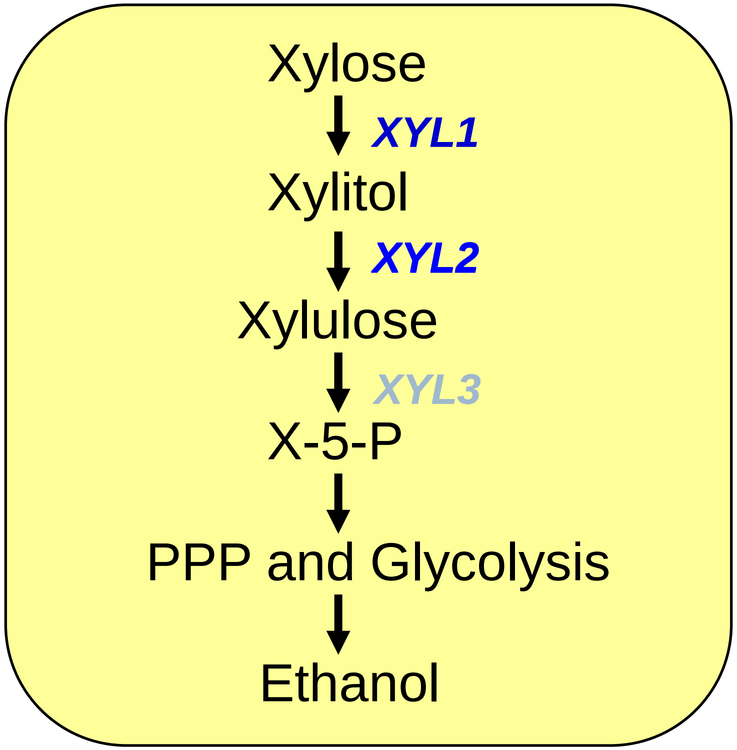
- The most widely used yeast since ancient times in baking and brewing
- Osmotolerant and ethanol-tolerant
- Numerous genetic/genomic tools are available
  - Overexpression / Knockout
  - Expression of heterologous enzymes

- *Cannot utilize xylose*
  - Not suitable for producing cellulosic biofuels



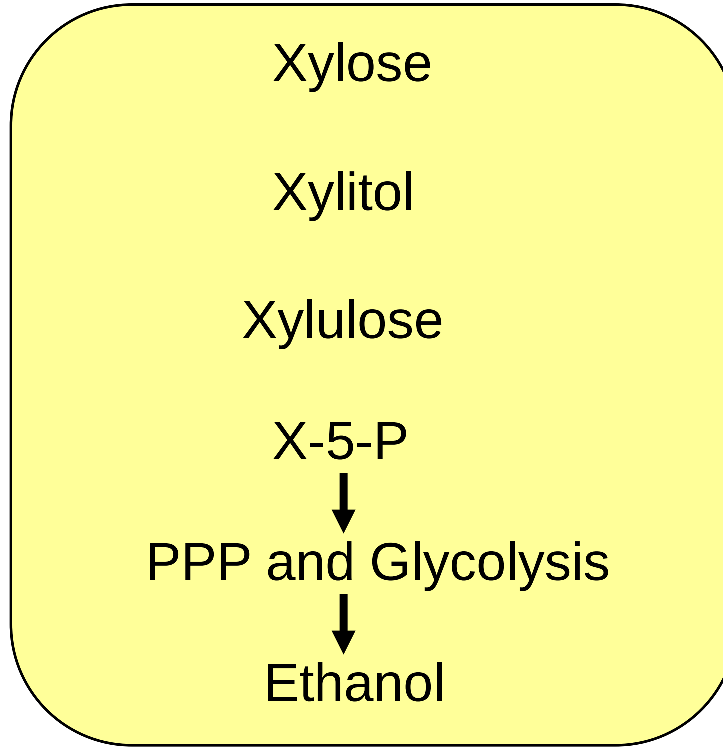
Basic strategy in metabolic engineering of xylose fermentation in *S. cerevisiae*

Scheffersomyces stipitis



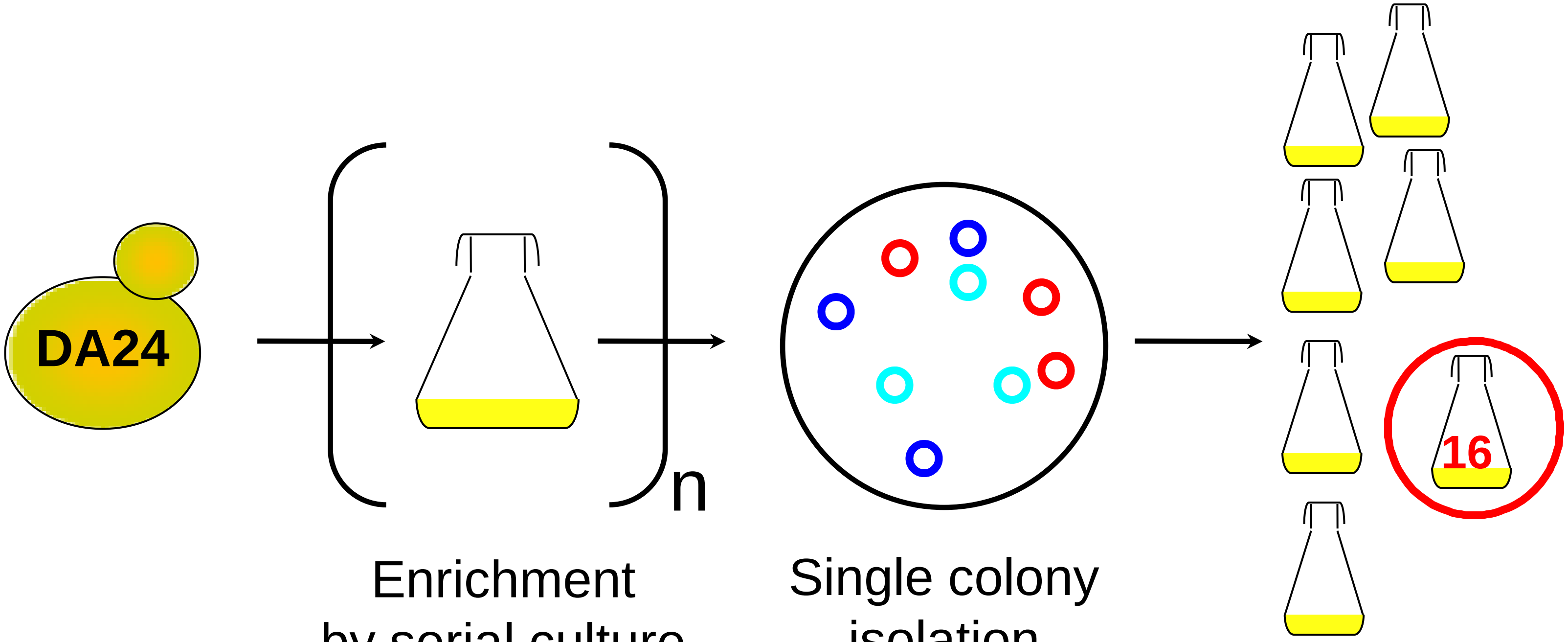
- Natural xylose fermenting
- **Low** ethanol tolerance

Saccharomyces cerevisiae



- **High** ethanol tolerance
- Amenable to metabolic engineering

Laboratory evolution of an engineered *S. cerevisiae* strain for further improvement



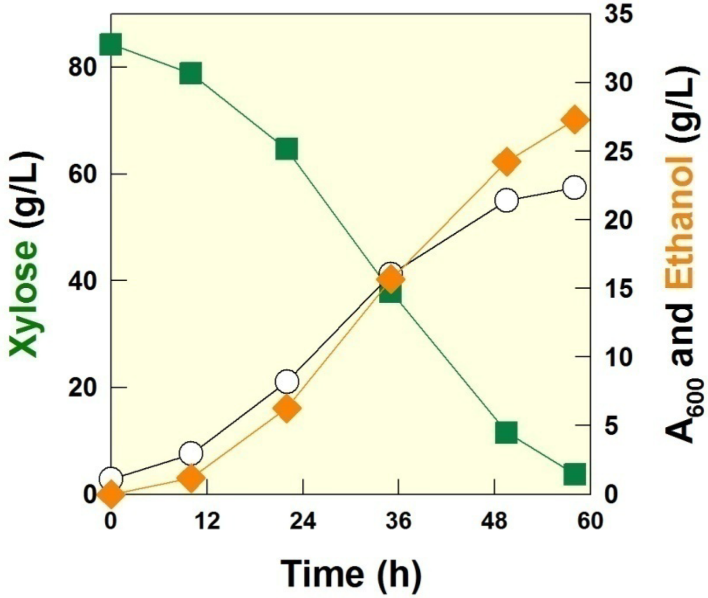
Enrichment
by serial culture
in 80 g/L of xylose

Single colony
isolation

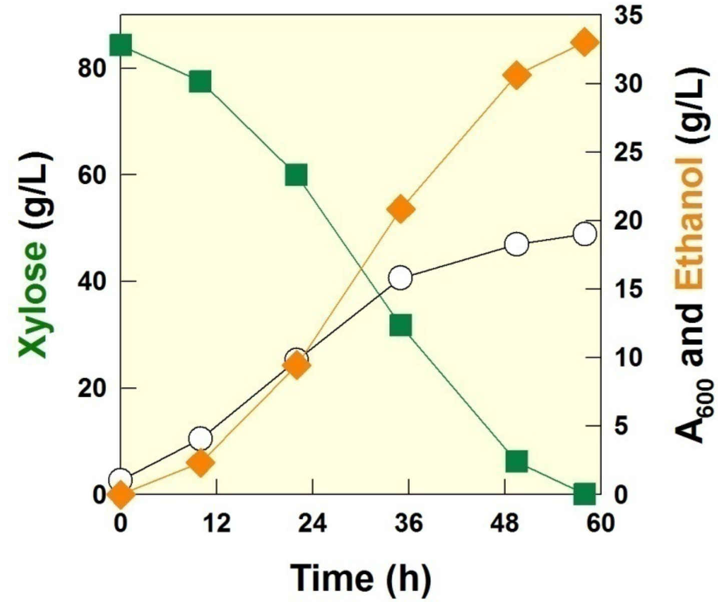
Evaluation

Comparison of xylose fermentation capability between engineered *S. cerevisiae* and *S. stipitis*

Engineered *S. cerevisiae*



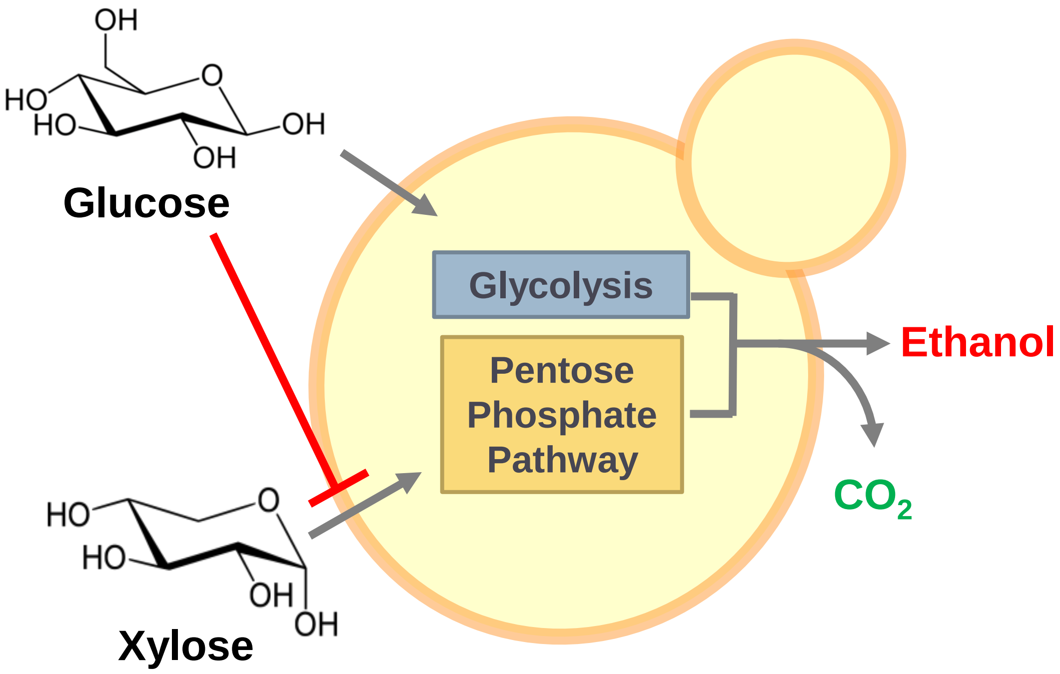
S. stipitis



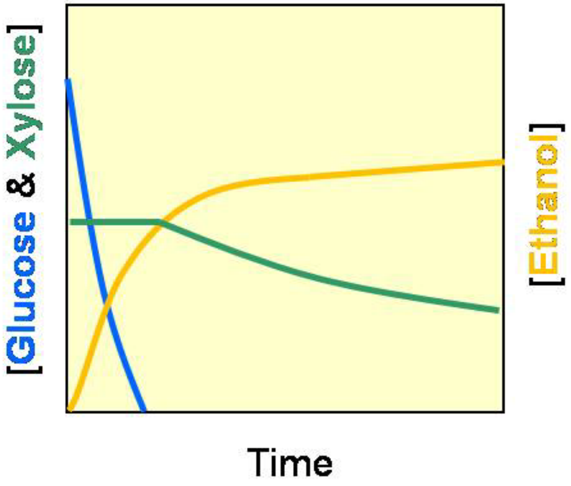
The engineered *S. cerevisiae* strain consumed xylose **almost as fast as *S. stipitis***, the fastest xylose-fermenting yeast

Ha et al. *PNAS*, **108**:504-509

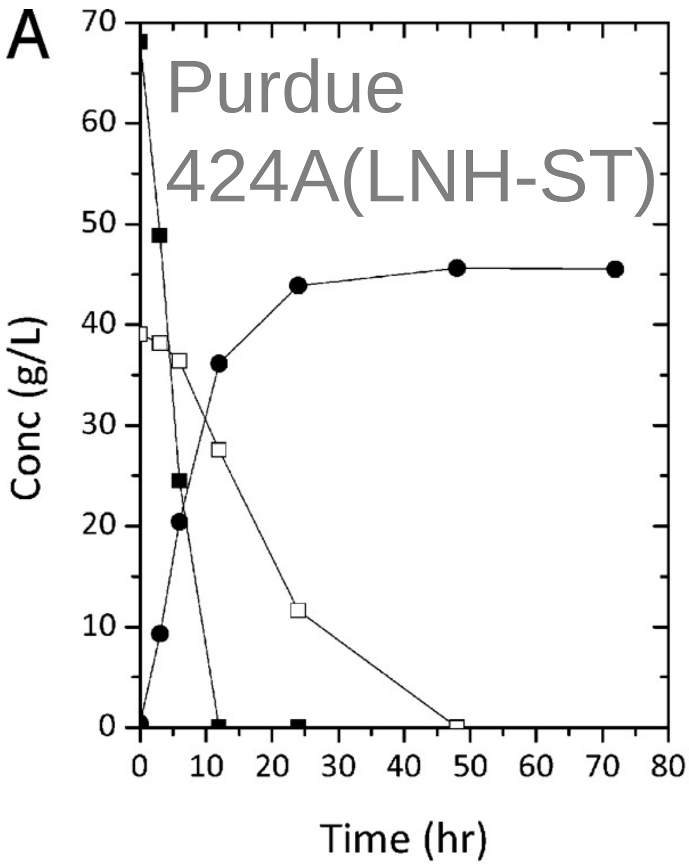
Why we want to co-ferment cellobiose and xylose?

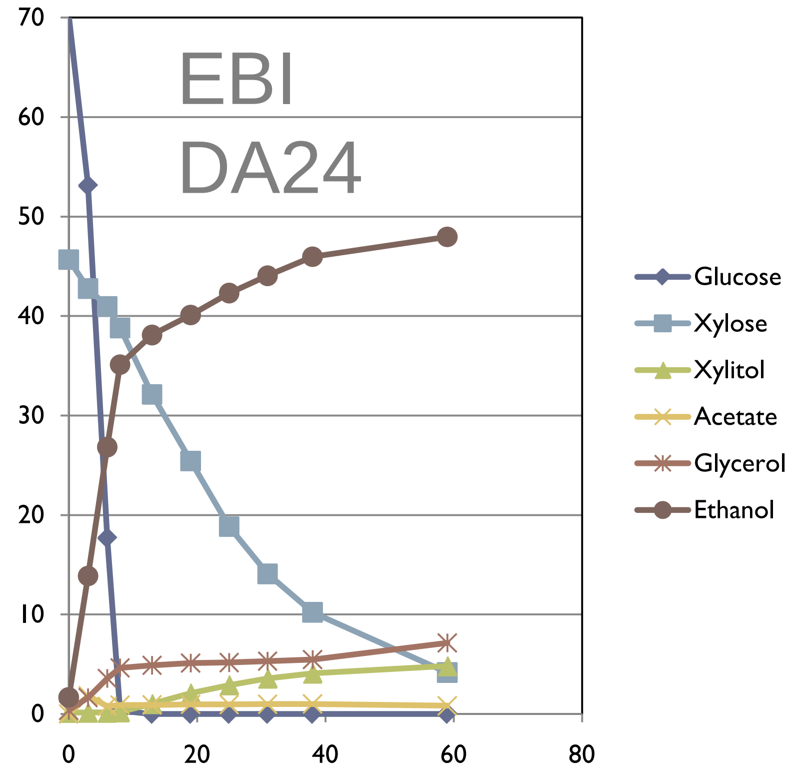


Typical fermentation profile of glucose and xylose mixture



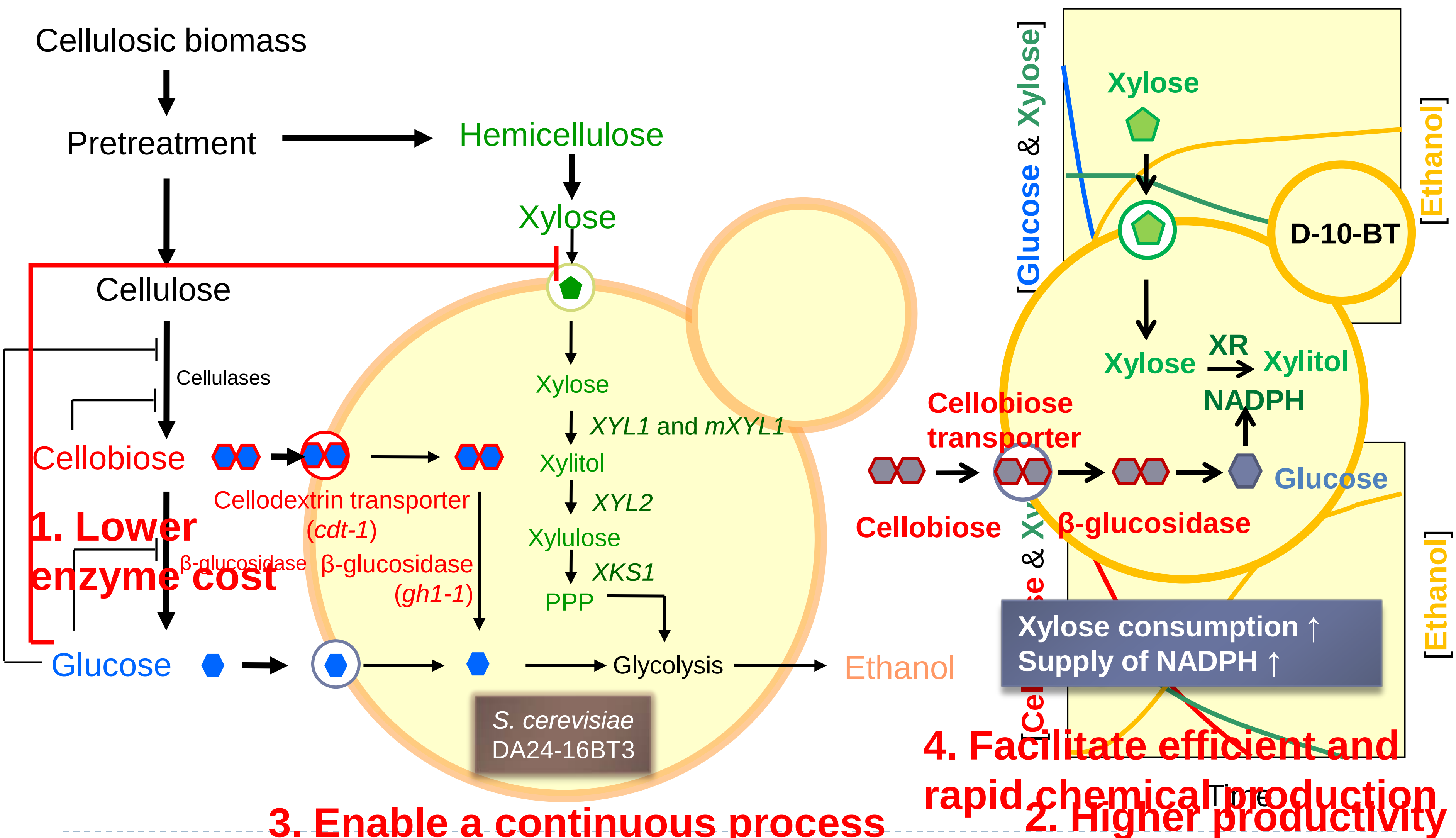
Engineered *S. cerevisiae* strains ferment xylose only after glucose depletion





Lau M. W., Dale B. E. *PNAS* 106:1368-1373

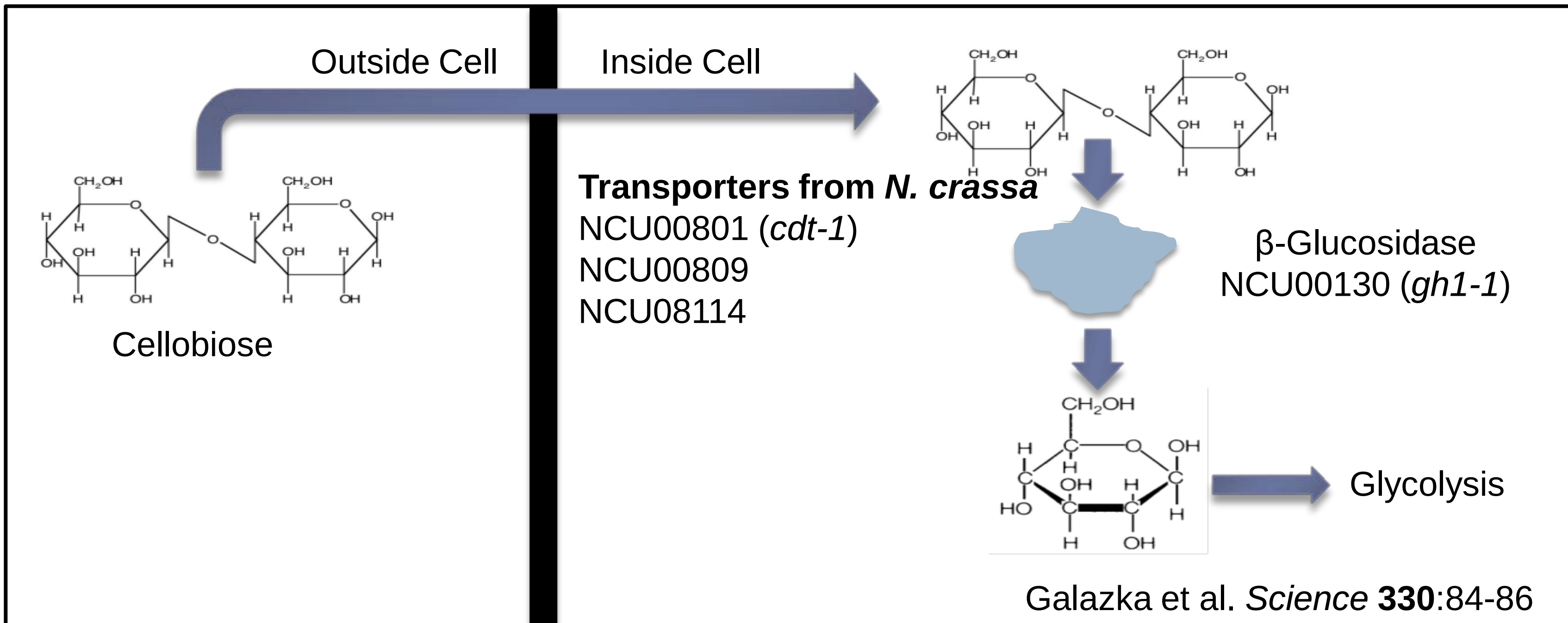
Grand scheme of co-fermentation of cellobiose and xylose in cellulosic hydrolysate



Ha et al. *PNAS*, **108**:504-509

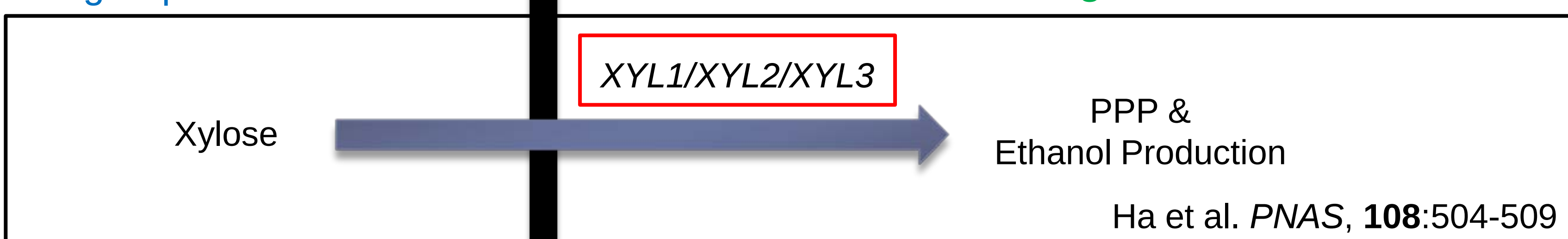
Synthesis of engineered yeast capable of co-fermenting cellobiose and xylose simultaneously

Cate group at UC-Berkeley

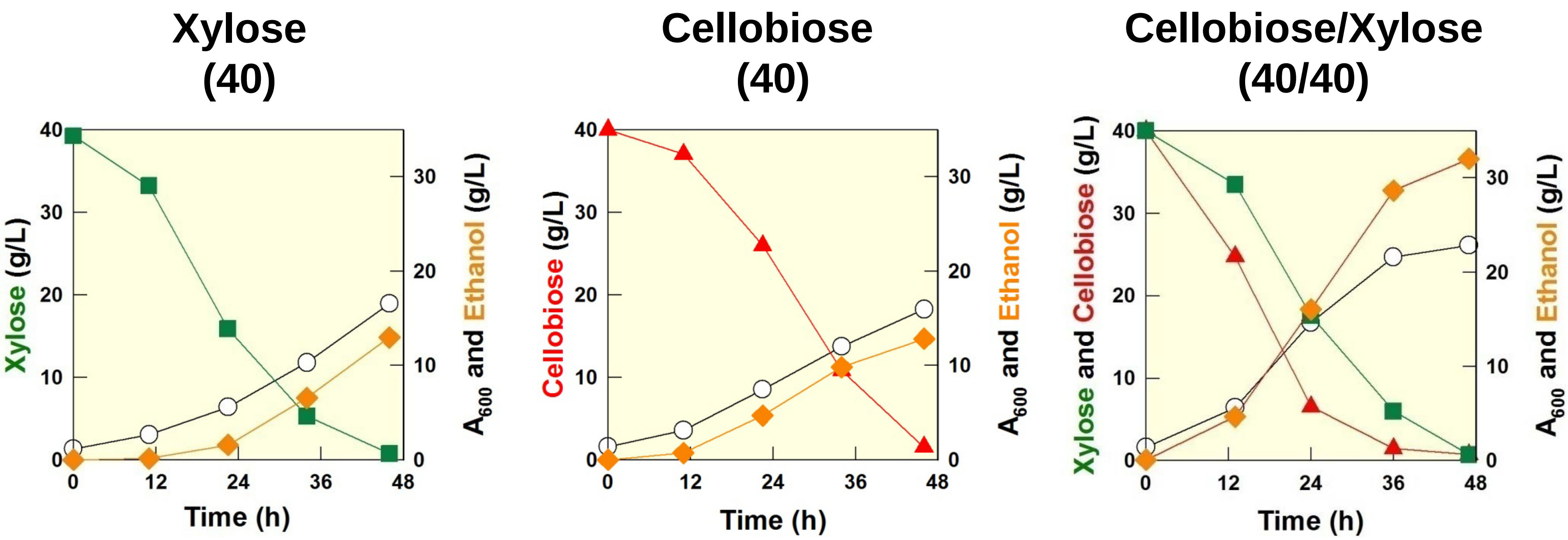


Jin group at UIUC

+ Xiaomin Yang at BP

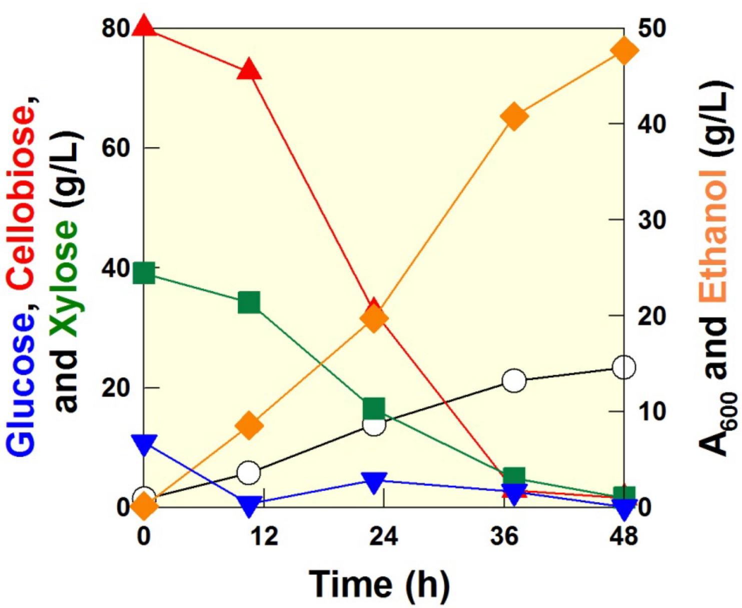


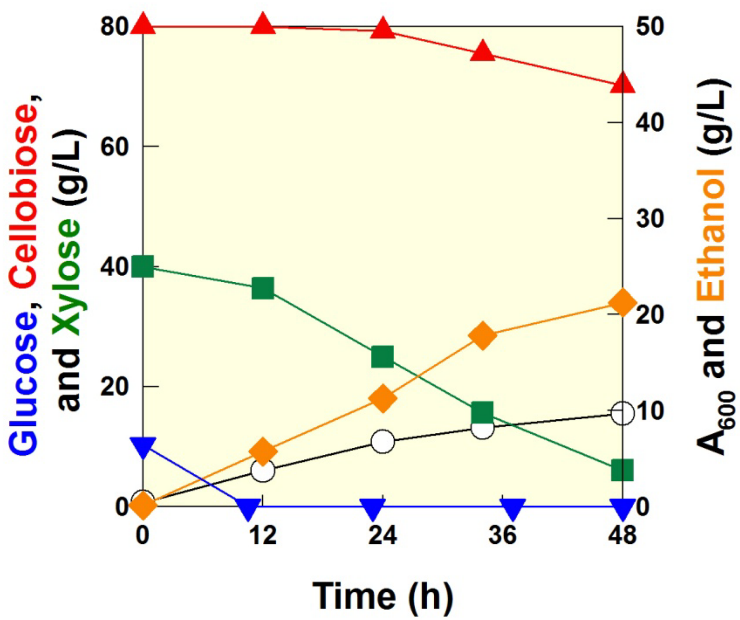
Co-fermentation of cellobiose and xylose by an engineered *S. cerevisiae* (DA24-16BT3)



	OD (A_{600})	Ethanol (g/L)	Y_{EtOH} (g/g)	P_{EtOH} (g/L·hr)
Xylose 40	16	13	0.33	0.28
Cellobiose 40	17	13	0.33	0.28
Cellobiose/xylose 40/40	**23**	**32**	**0.40**	**0.70**

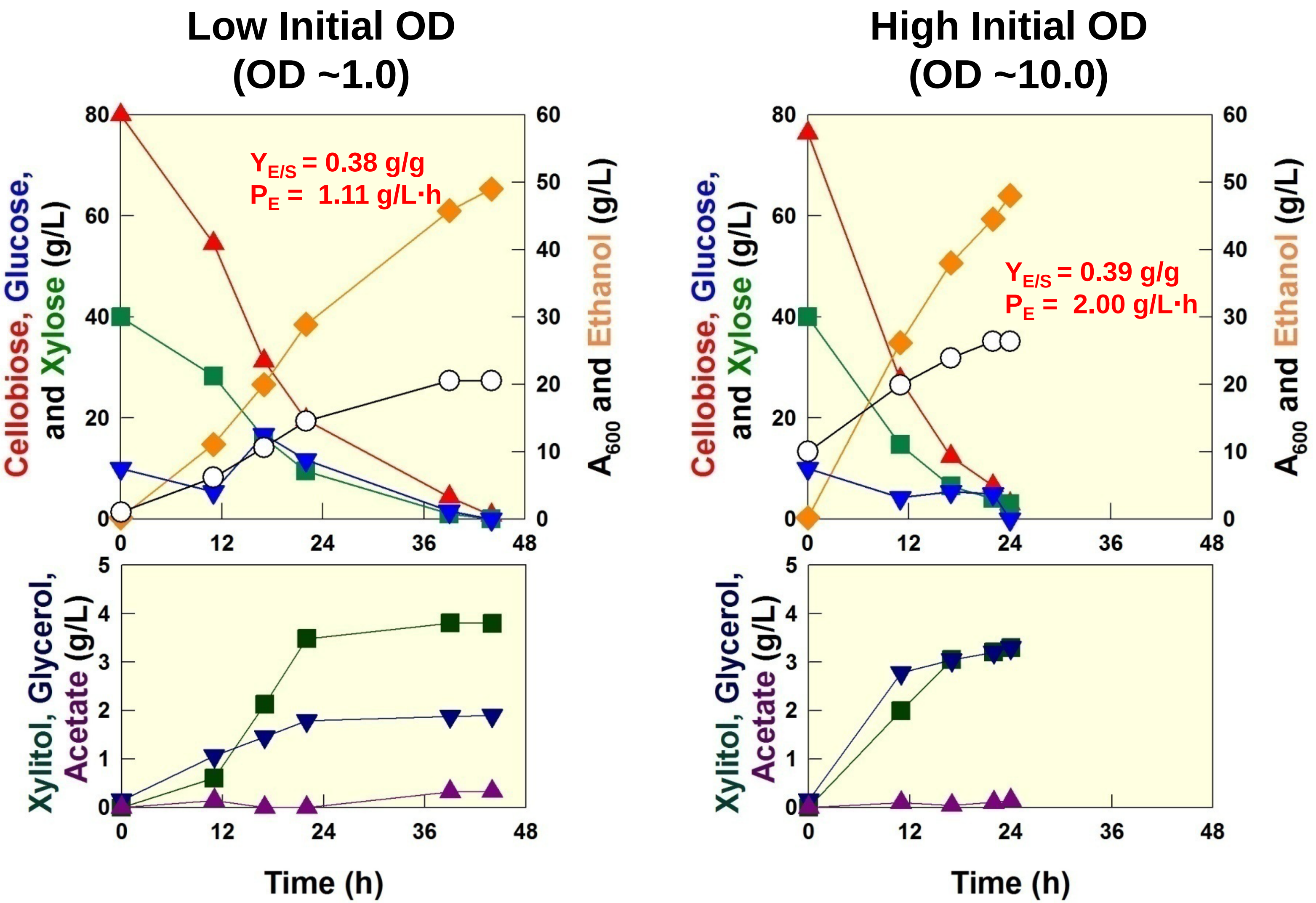
Co-fermentation of glucose, cellobiose, and xylose by strain DA24-16BT3 and *S. stipitis*





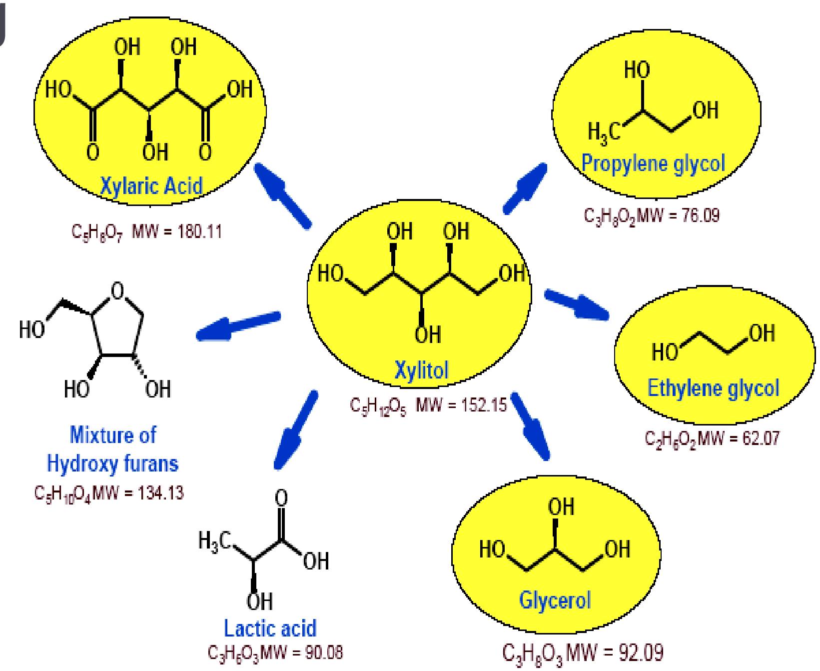
	OD (A_{600})	Ethanol (g/L)	Y_{EtOH} (g/g)	P_{EtOH} (g/L·hr)
DA2416-BT3	**25**	**48**	**0.38**	**0.99**
S. stipitis	19	25	0.38	0.55

Co-fermentation by an engineered industrial strain (HP111BT)

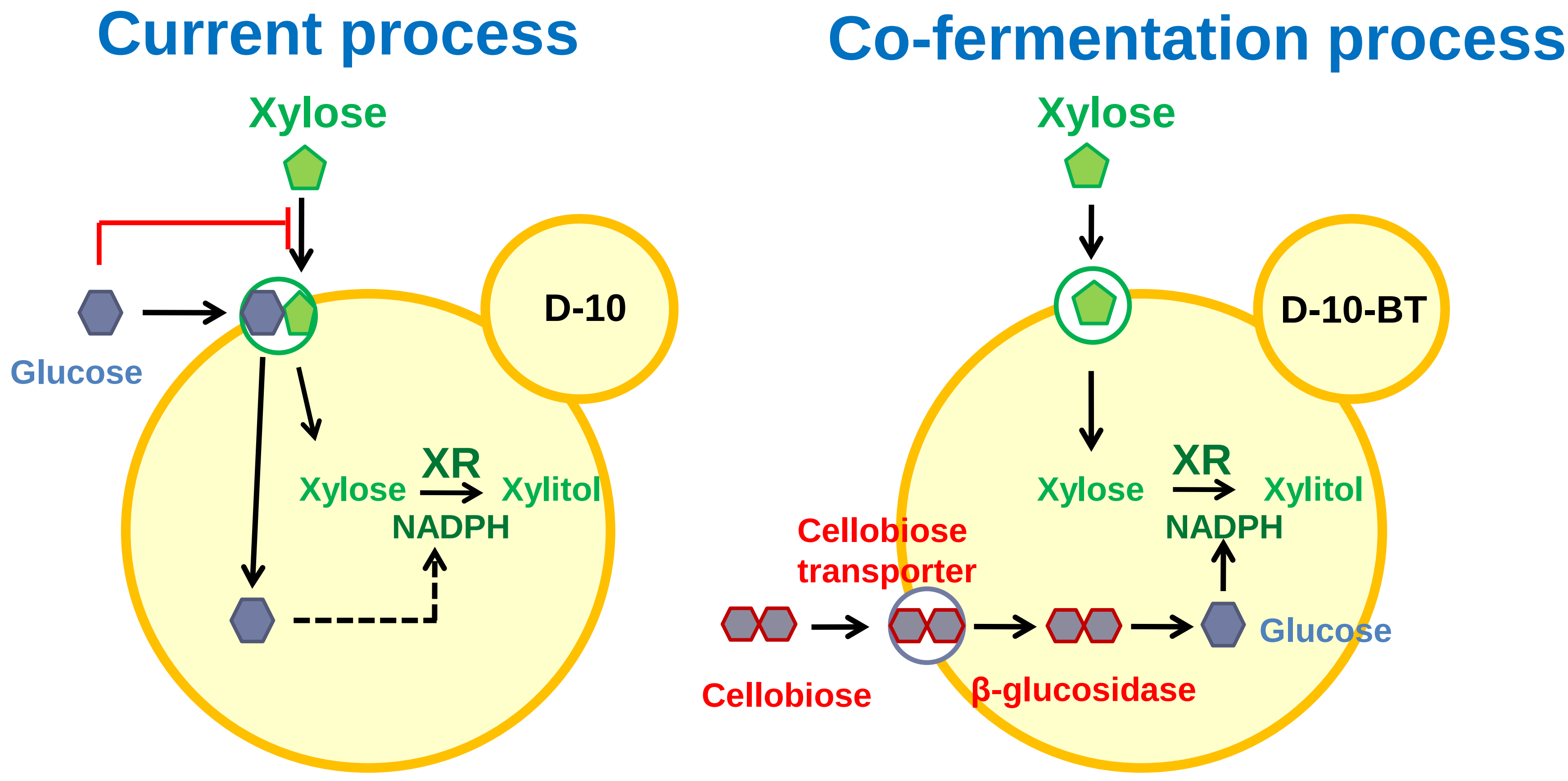


Xylitol: a functional sweetener and chemical

- A very popular food additive in Asian market
  - Sugar substitute with lower calorie (2.4 cal/g)
  - Better sensory with a cooling effect
  - Good for diabetic patients and prevents dental caries
- Selected as one of the top value-added chemicals from biomass by US-DOE

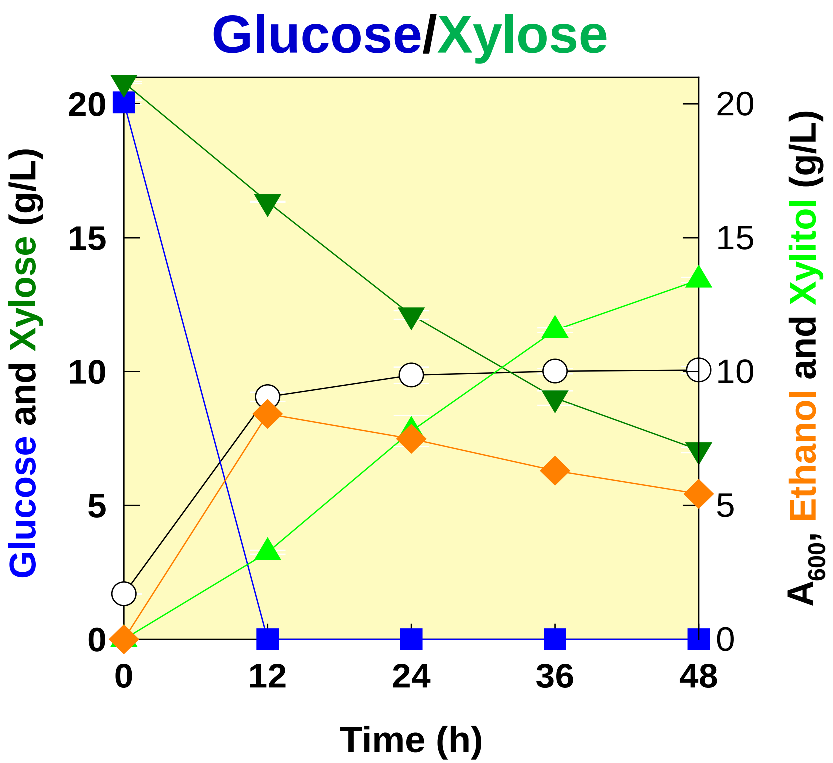


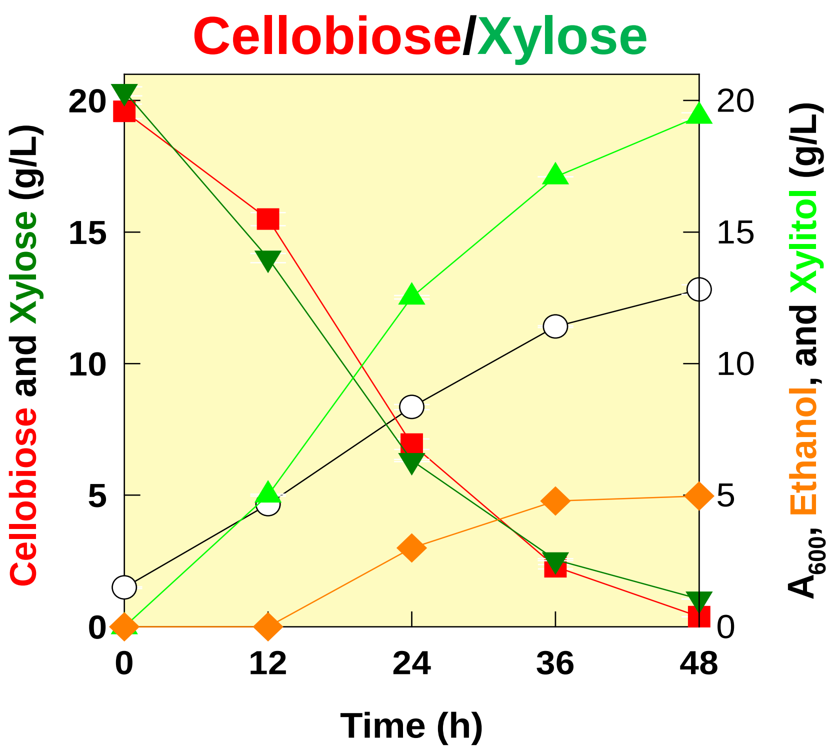
Xylitol production through co-utilization of xylose and cellobiose



Xylose consumption ↑
Supply of NADPH ↑

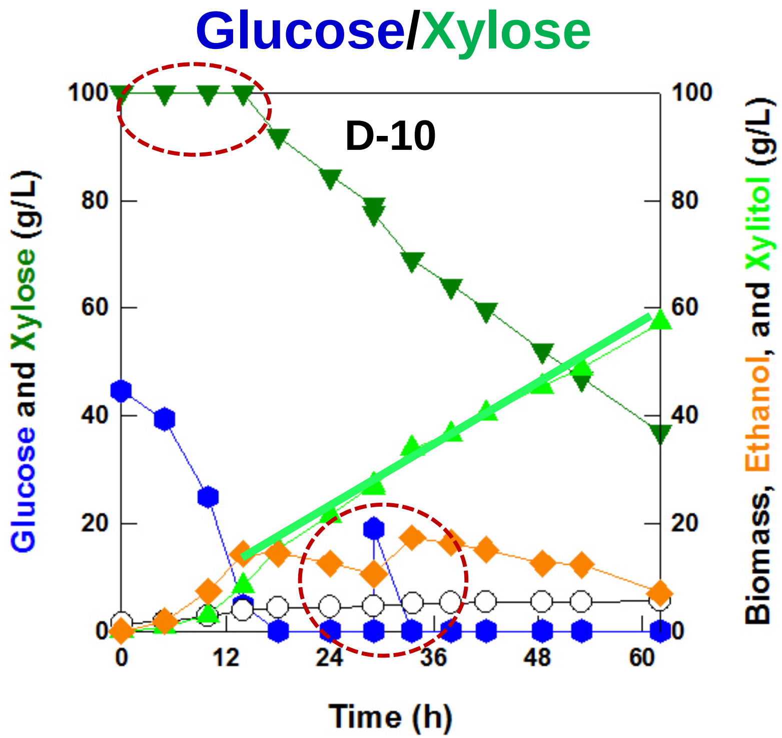
Enhanced production of xylitol without glucose repression

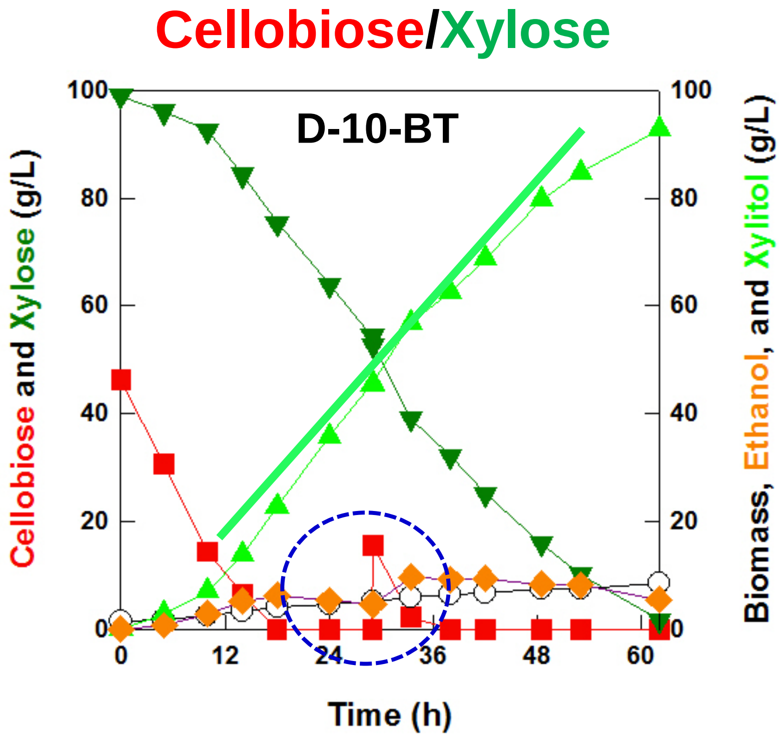




	OD (A_{600})	Xylitol (g/L)	$P_{Xylitol}$ (g/L·hr)	Xylitol production per sugar consumed (g/g)	Fermentation conditions
Glucose/Xylose 20/20	10	13	0.28	0.67	80 rpm, 50mL
Cellobiose/Xylose 20/20	13	19 (46%↑)	0.40 (43%↑)	1.0	

pH controlled bioreactor fermentation





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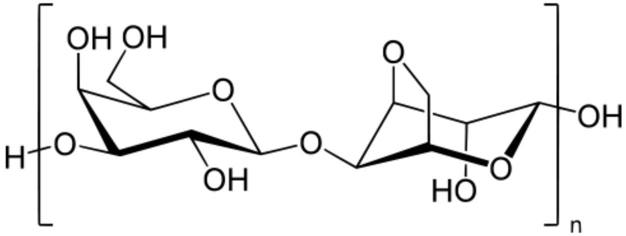
	Cell mass (g/L)	Xylitol (g/L)	$P_{Xylitol}$ (g/L-hr)	Xylitol production per sugar consumed (g/g)	Fermentation conditions
glucose/xylose 40/100	5.5	49	0.92	0.77	500 rpm, 2vvm pH 5.5
cellobiose/xylose 40/100	7.4	85 (73%↑)	1.60 (74%↑)	1.4	

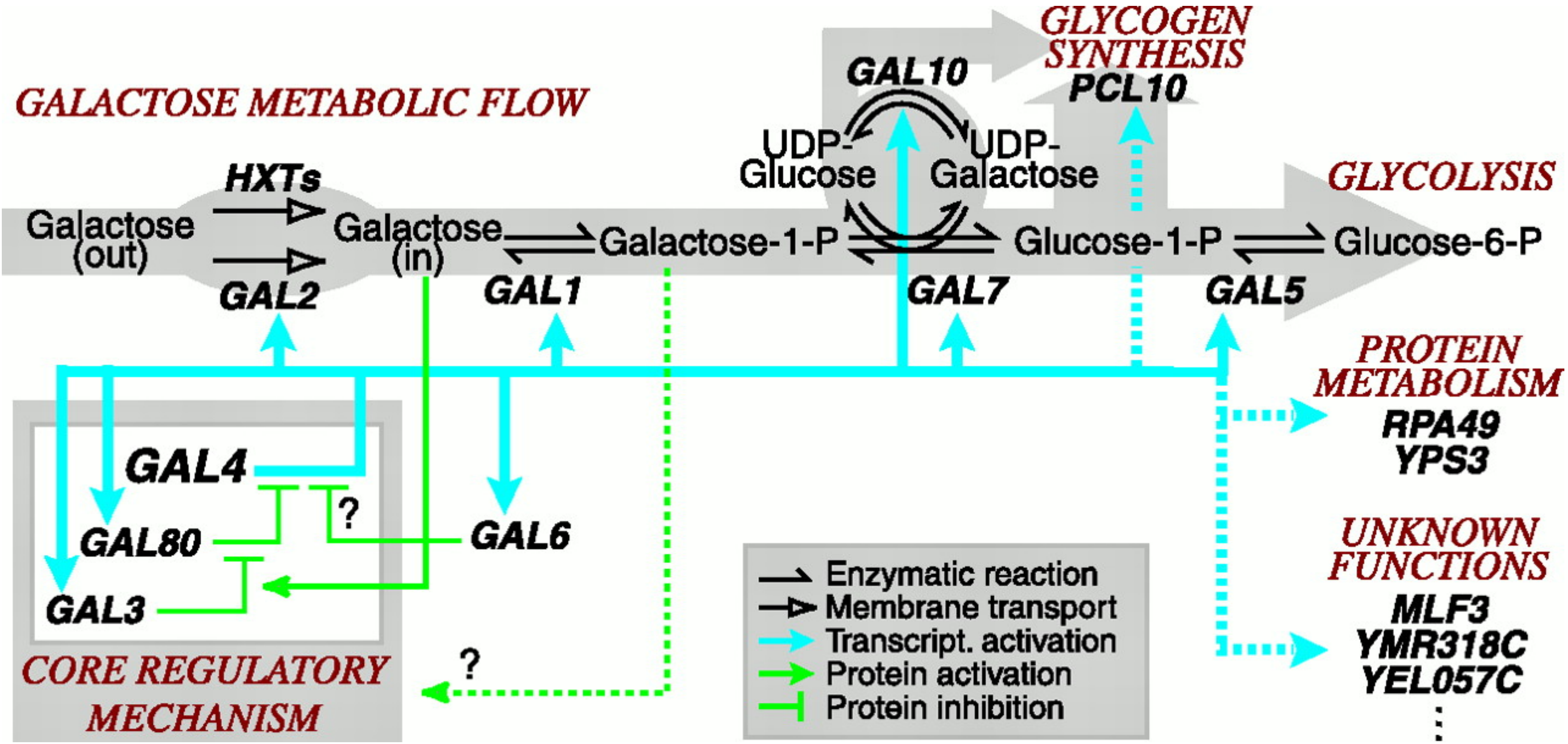
Why do we study galactose metabolism?

- Galactose is a major sugar in marine biomass



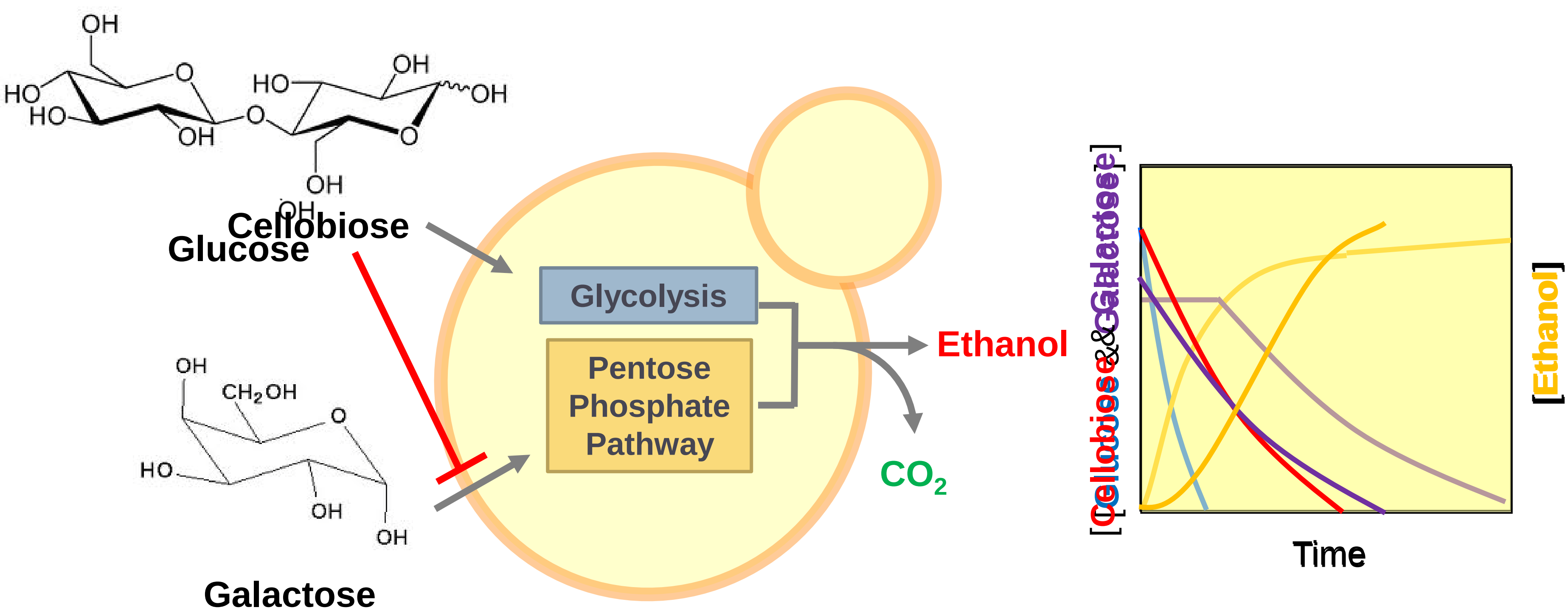
- Marine plant biomass has several attributes that would make it an attractive renewable source for the production of biofuels
  - Higher production yields per unit area
  - Can be depolymerized relatively easily compared to lignocellulosic biomass
  - Higher carbon dioxide fixation rates than terrestrial biomass

Galactose metabolism is tightly regulated in *S. cerevisiae* (strong glucose repression)

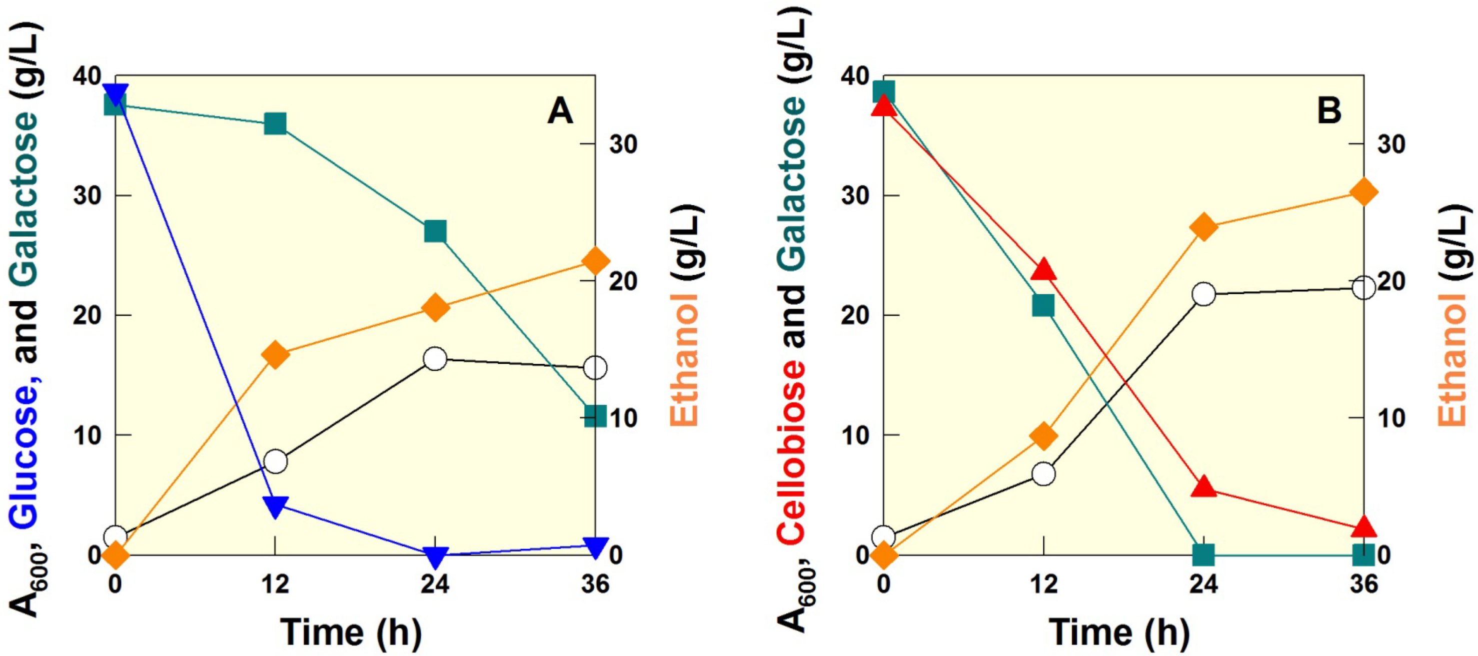


From Ideker et al. *Science* (2001) 292, 929-934

Improvement of galactose fermentation through co-fermentation with cellobiose



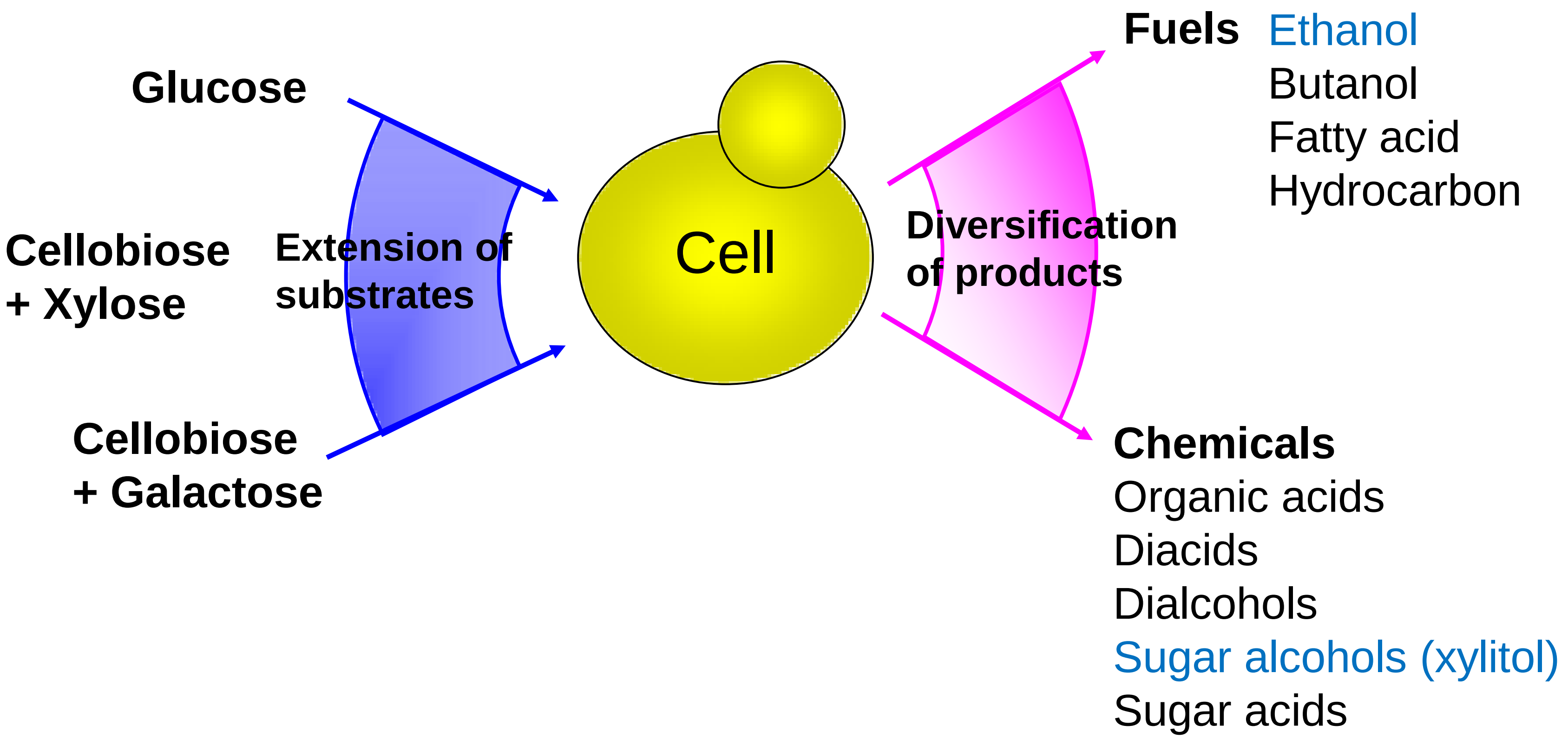
Comparison of sequential fermentation (A) and co-fermentation (B)



	OD (A_{600})	Ethanol (g/L)	Y_{EtOH} (g/g)	P_{EtOH} (g/L·hr)
glucose/galactose (40 g/L and 40 g/L)	16	21	0.34	0.60
cellobiose/galactose (40 g/L and 40 g/L)	22 (38% ↑)	27 (29% ↑)	0.36 (6% ↑)	0.74 (23% ↑)

Ha et al. *Appl. Environ. Microbiol.* 77,5822-5826

Numerous applications of co-fermentation for producing fuels and chemicals



Summary

- Optimization of the xylose metabolic pathway and laboratory evolution drastically improved ethanol yield and productivity of engineered *S. cerevisiae*

- Co-fermentation of non-fermentable carbon sources (cellobiose and xylose) is possible by metabolic engineering
  - Cellodextrin transporter and intracellular β-glucosidase

- Engineered industrial *S. cerevisiae* showed impressive ethanol production capability

- Cellobiose and galactose co-fermentation is also feasible

- Various chemicals can be produced using the co-fermentation technology
  - Enhanced production of xylitol from cellulosic hydrolysate

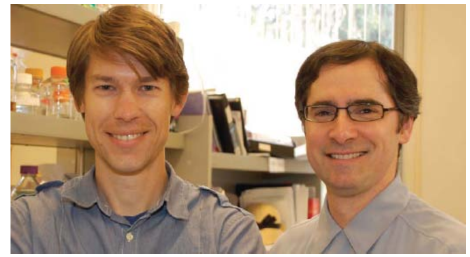
Acknowledgements

Jin lab members

Suk-Jin Ha
Won-Heong Lee
Hyo-Jin Kim
Soo Rin Kim
Josh Quarterman
Qiaosi Wei
Eun-Joong Oh
Heejin Kim

EBI-Berkeley

Jamie Cate
- **Jon Galazka**



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