

# **GENOMIC AND EVOLUTIONARY ENGINEERING STRATEGIES IN YEASTS FOR IMPROVED BIOETHANOL PRODUCTION FROM SUGARCANE**



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## Sustainability and Energy

PERSPECTIVE

# Ethanol for a Sustainable Energy Future

José Goldemberg\*

9 FEBRUARY 2007 VOL 315 SCIENCE [www.sciencemag.org](http://www.sciencemag.org)



## BIOFUELLING THE FUTURE



BUSINESS FEATURE

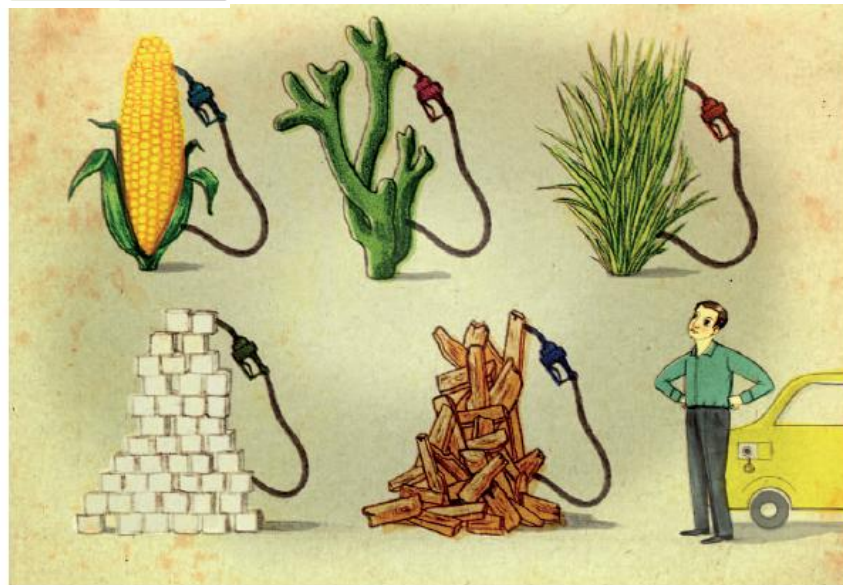
NATURE | Vol 444 | 7 December 2006

OUTLOOK BIOFUELS

23 JUNE 2011 | VOL 474 | NATURE | 59

## Drink the best and drive the rest

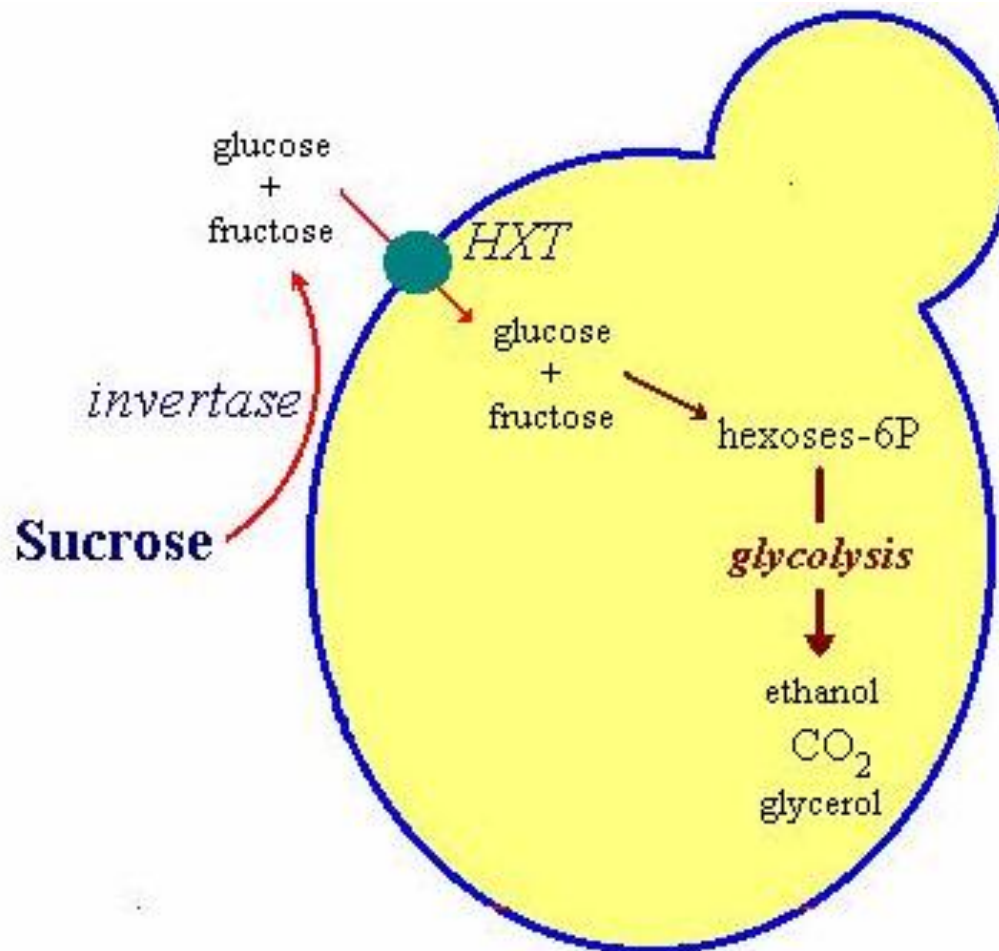
Brazil's sugar-cane ethanol industry is the world's best and able to get better, says **Emma Marris**.



FUEL OPTIONS

## The ideal biofuel

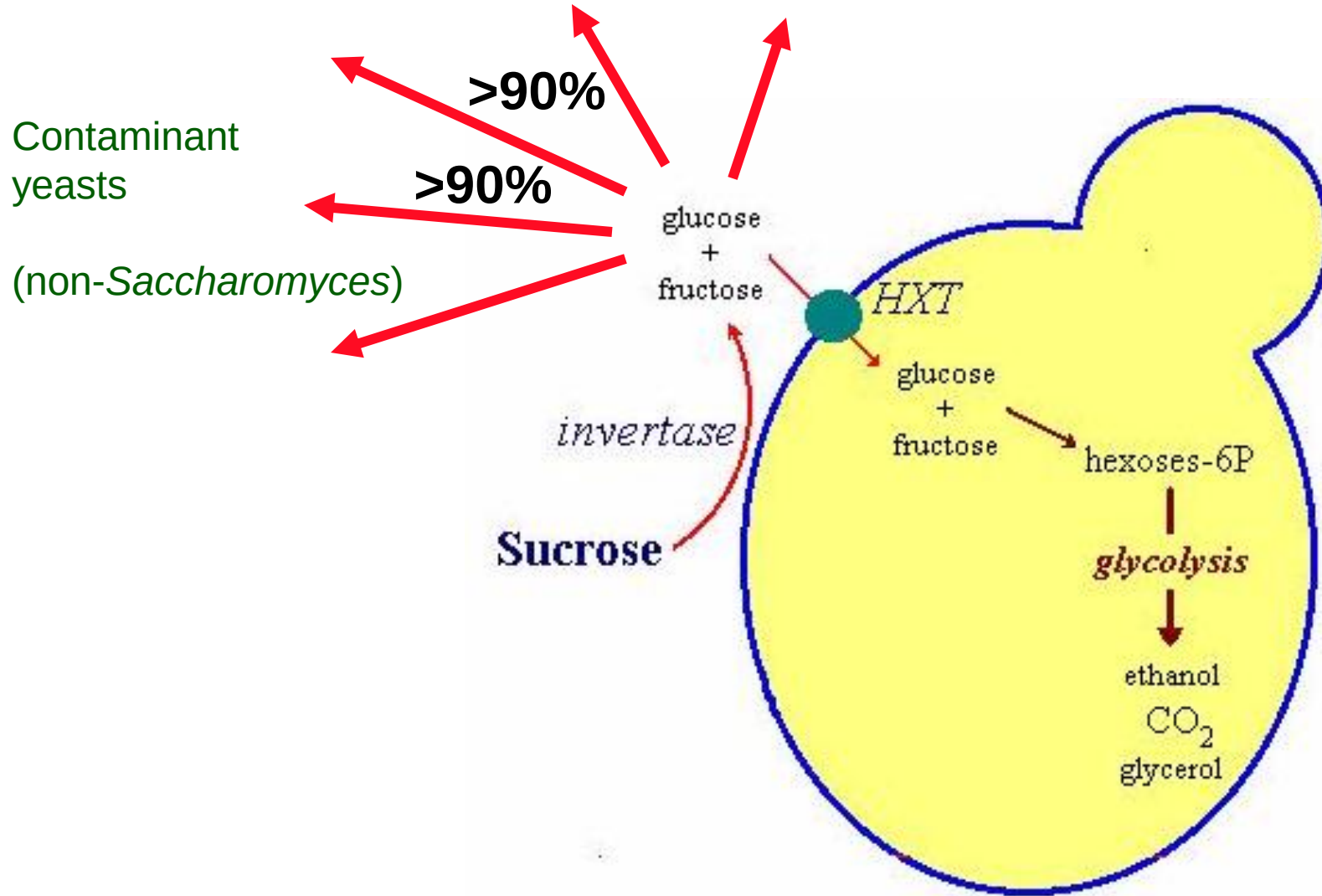




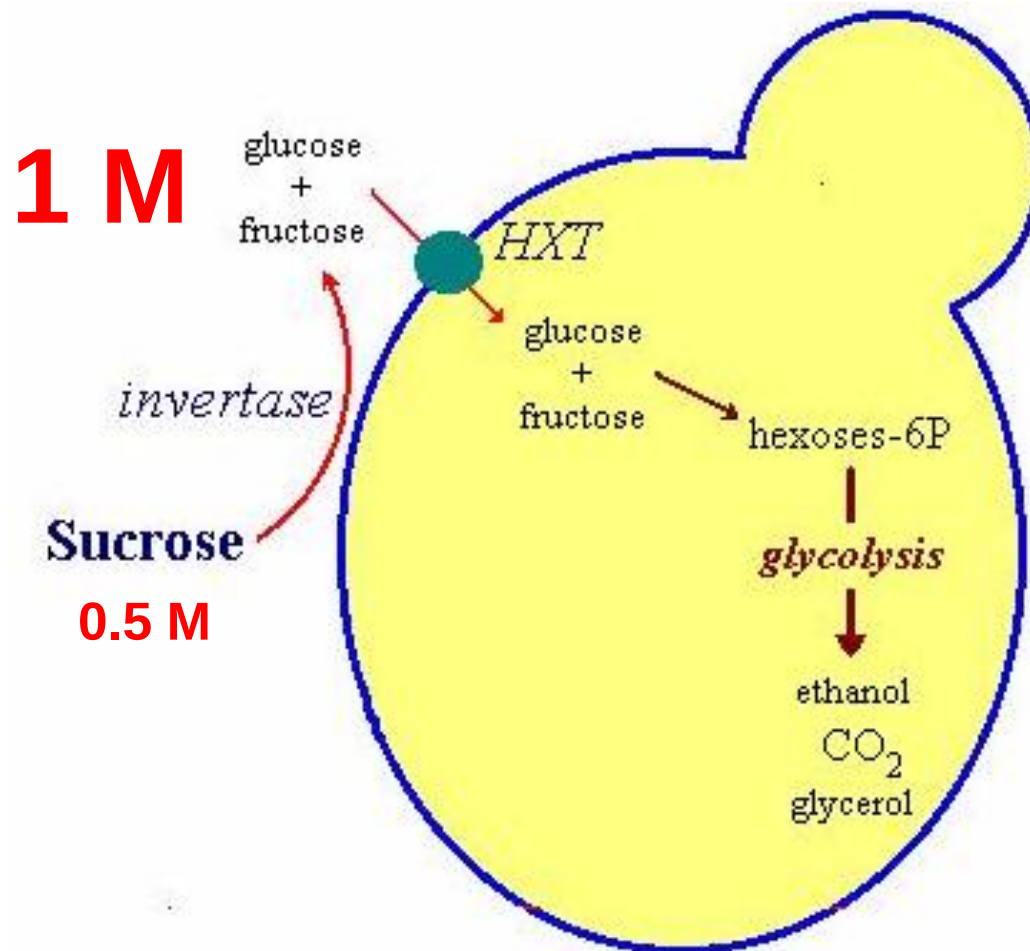
*Sucrose fermentation by S. cerevisiae*

Contaminant bacteria

Contaminant yeasts  
(non-*Saccharomyces*)

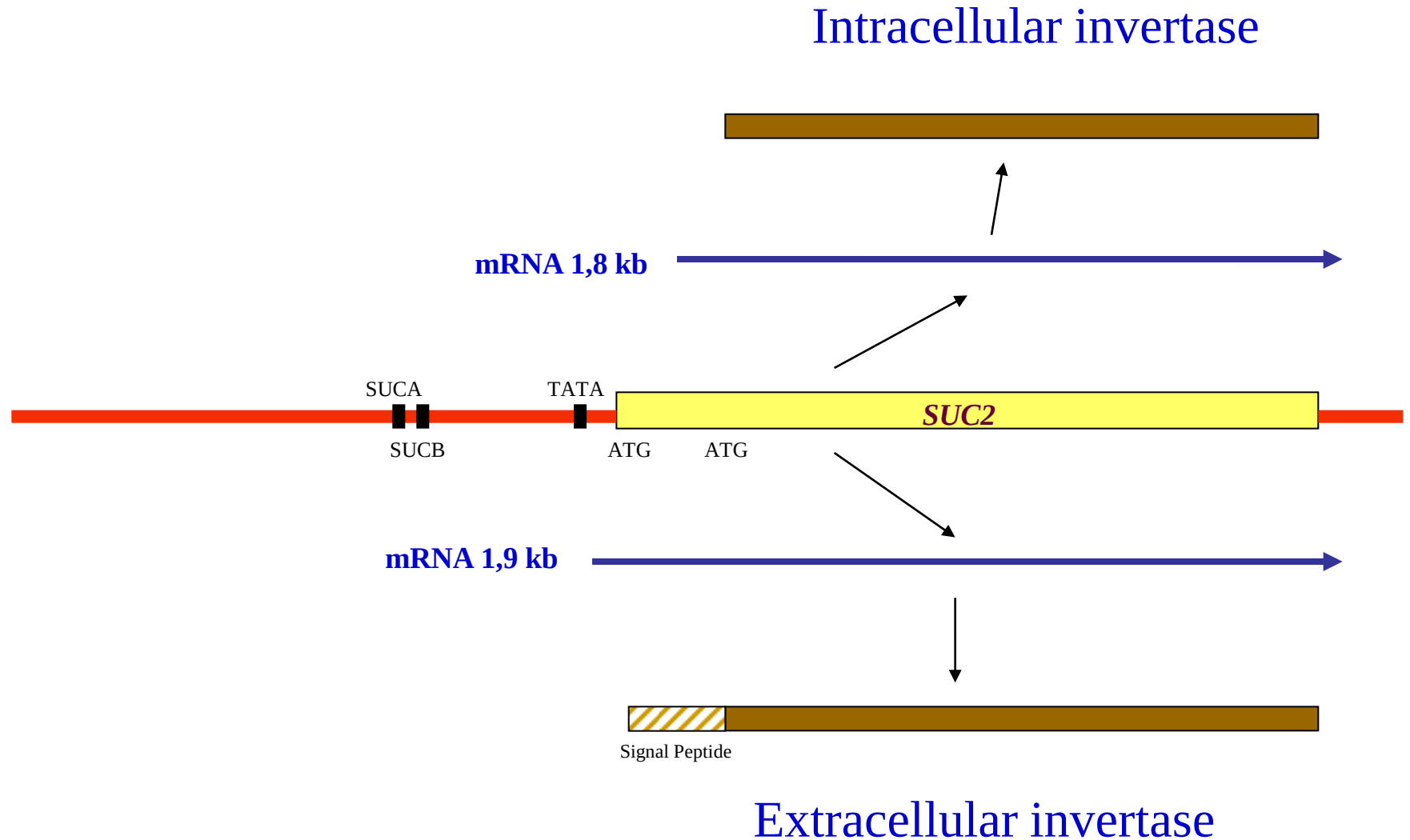


***Inefficient sucrose fermentation by *S. cerevisiae****



*High sucrose + High invertase  
= osmotic shock*

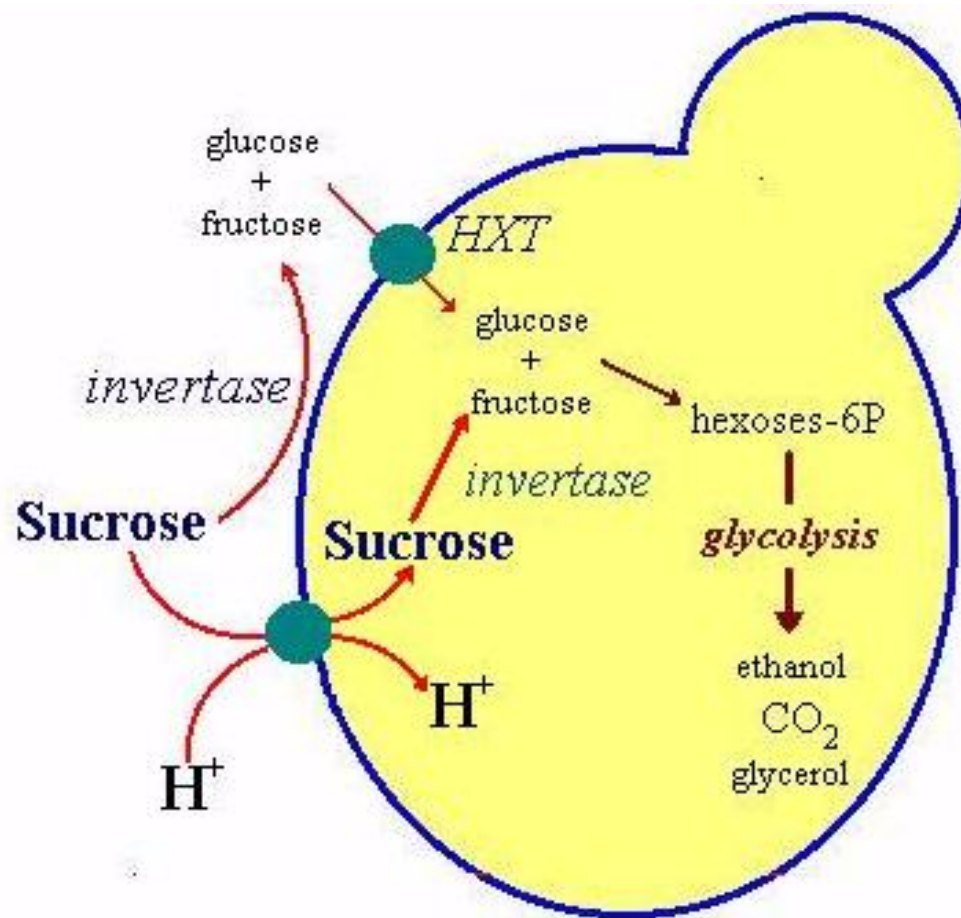
# *SUC* genes (*SUC1-SUC5* *SUC7-SUC10*)





**AGT1**  $K_m$  7 mM

**MALx1**  $K_m$  100 mM



## Active sucrose uptake

JOURNAL OF BIOSCIENCE AND BIOENGINEERING  
Vol. 89, No. 2, 212-214, 2000

### Kinetics of Active Sucrose Transport in *Saccharomyces cerevisiae*

BORIS U. STAMBUK,<sup>1\*</sup> ANDERSON S. BATISTA,<sup>1</sup> AND PEDRO S. DE ARAUJO<sup>2</sup>

*Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina,*

# Sucrose Fermentation by *Saccharomyces cerevisiae* Lacking Hexose Transport

Anderson S. Batista Luiz C. Miletto Boris U. Stambuk

## Microbial Cell Factories



Open Access

Research

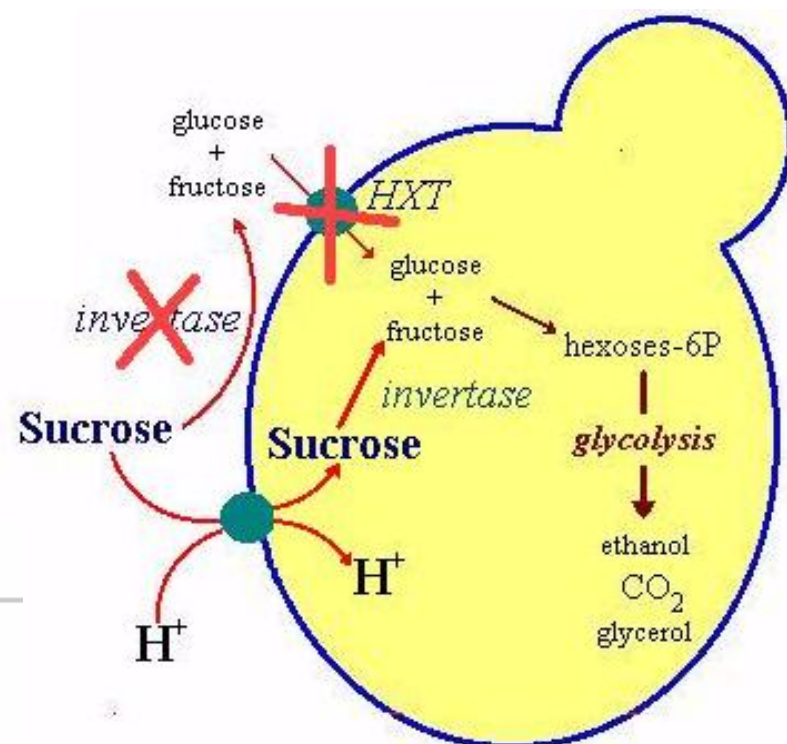
Switching the mode of sucrose utilization by *Saccharomyces cerevisiae*

Fernanda Badotti<sup>1</sup>, Marcelo G Dário<sup>1</sup>, Sergio L Alves Jr<sup>1</sup>, Maria Luiza A Cordioli<sup>1</sup>, Luiz C Miletto<sup>1,3</sup>, Pedro S de Araujo<sup>2</sup> and Boris U Stambuk\*<sup>1</sup>

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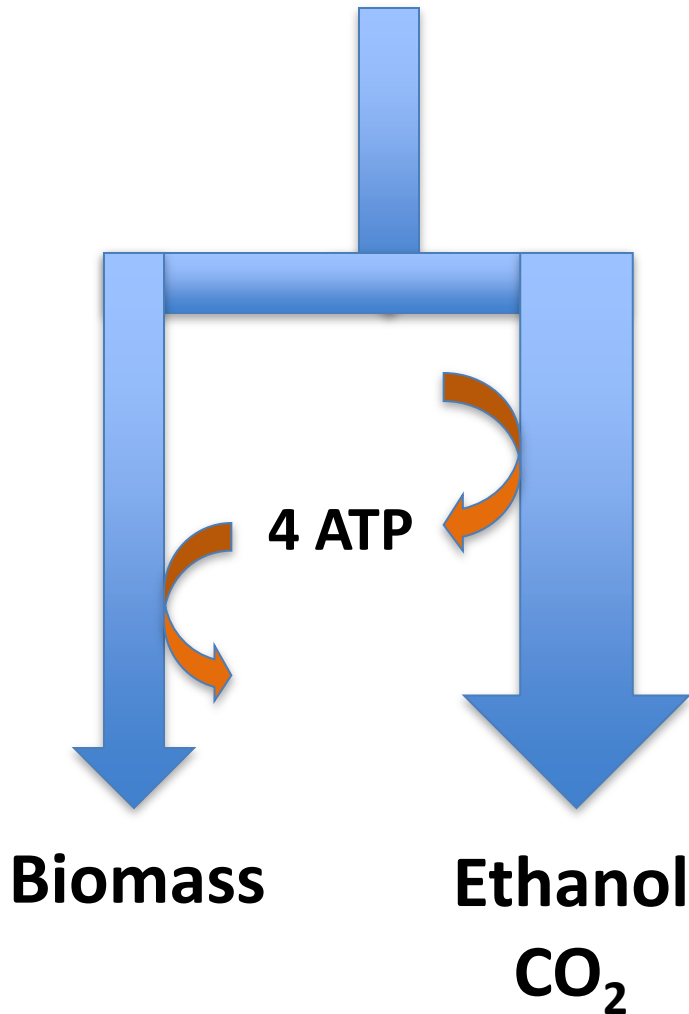
\* Corresponding author





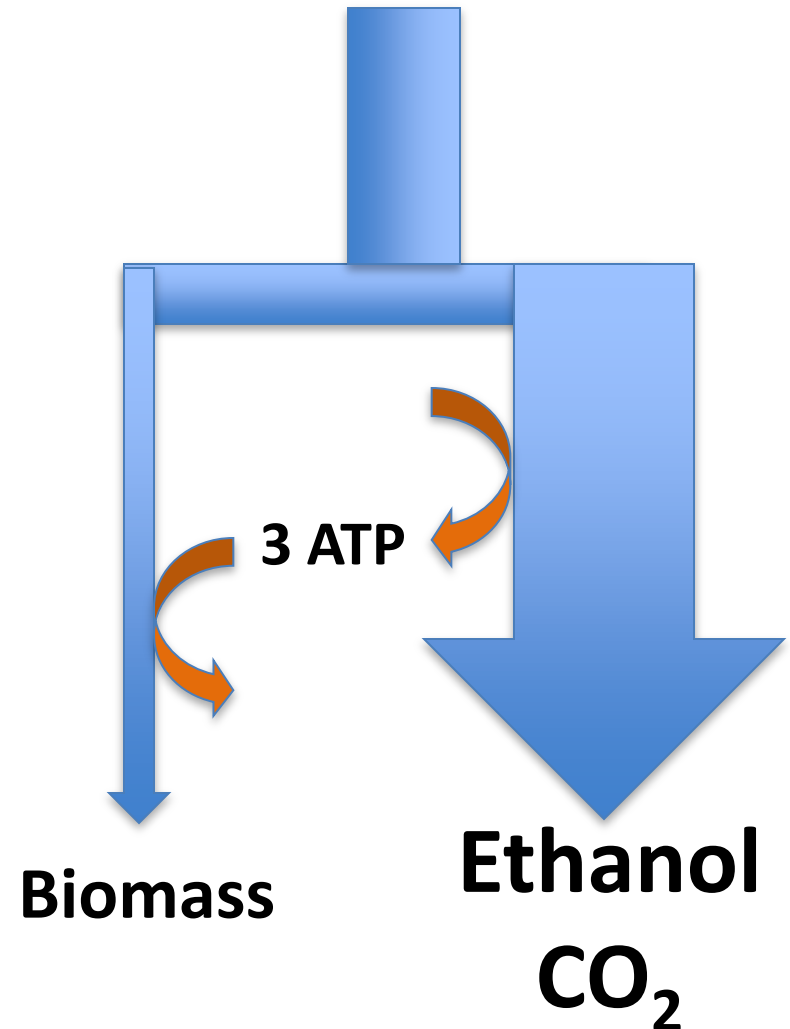
## *Extracellular invertase*

**Sucrose**

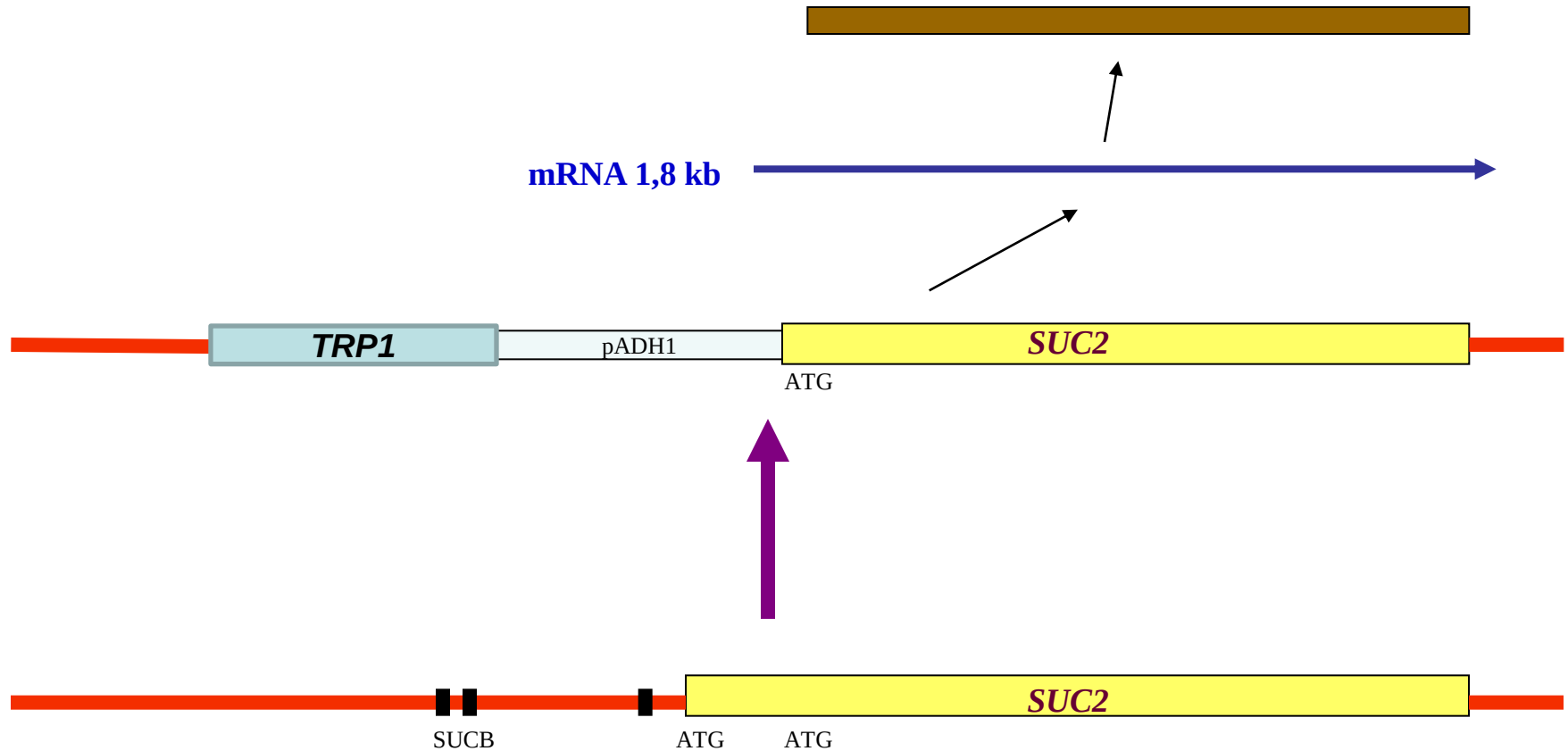


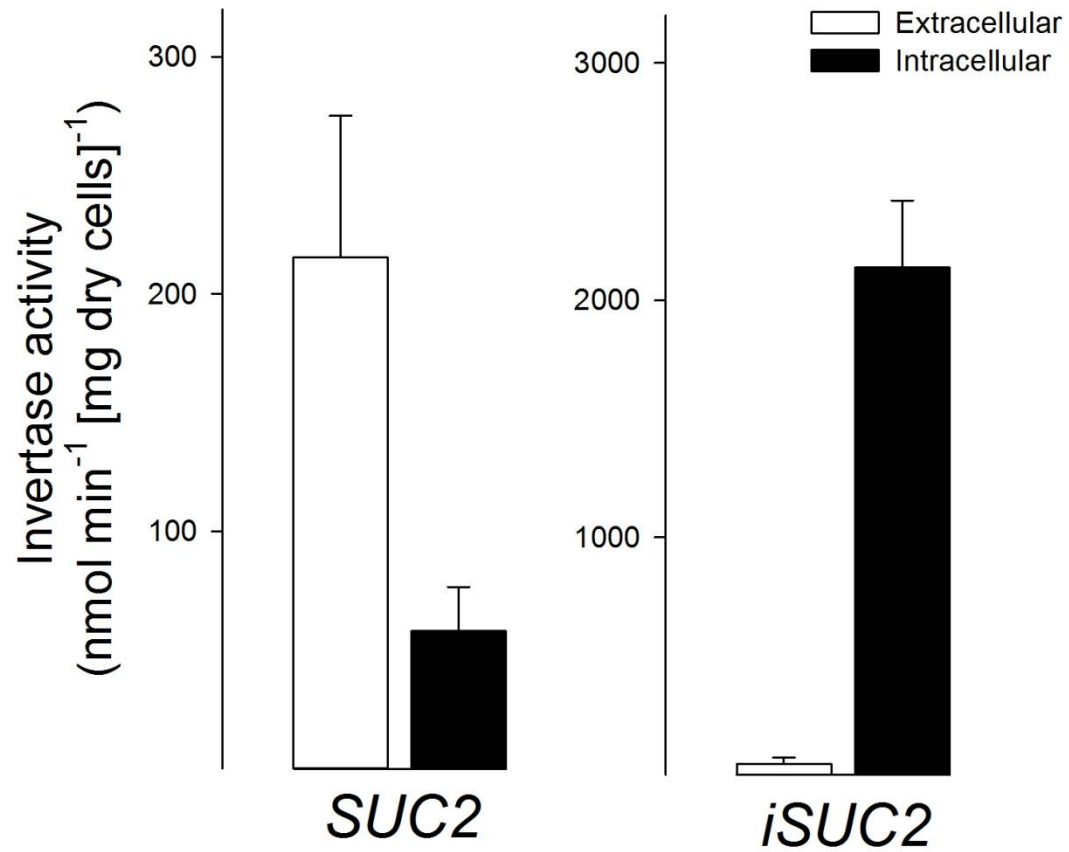
## *Intracellular invertase*

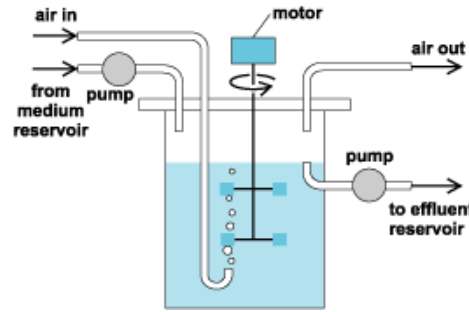
**Sucrose**



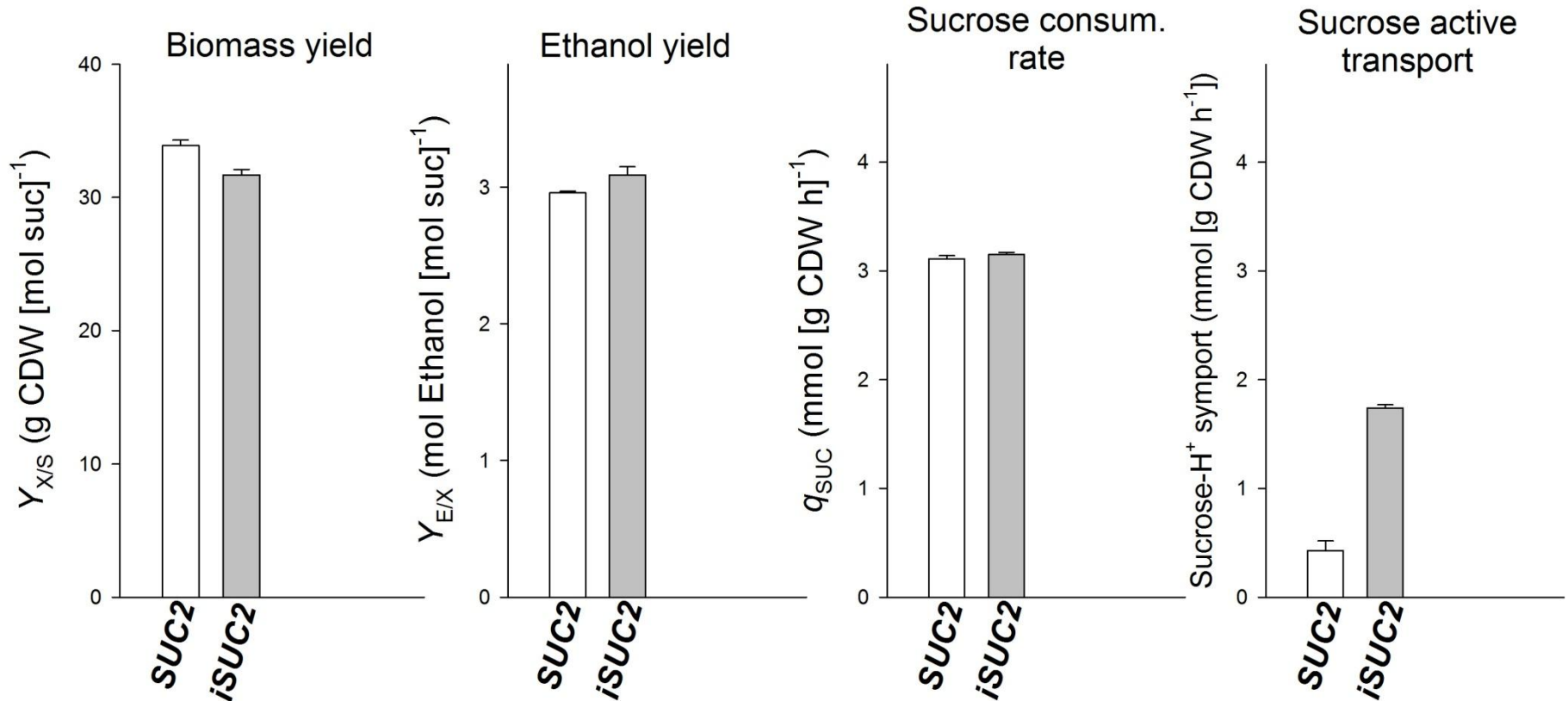
## Intracellular invertase



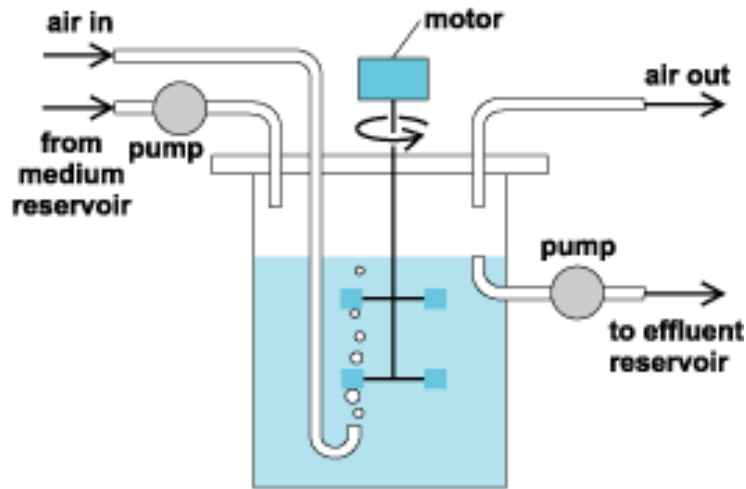




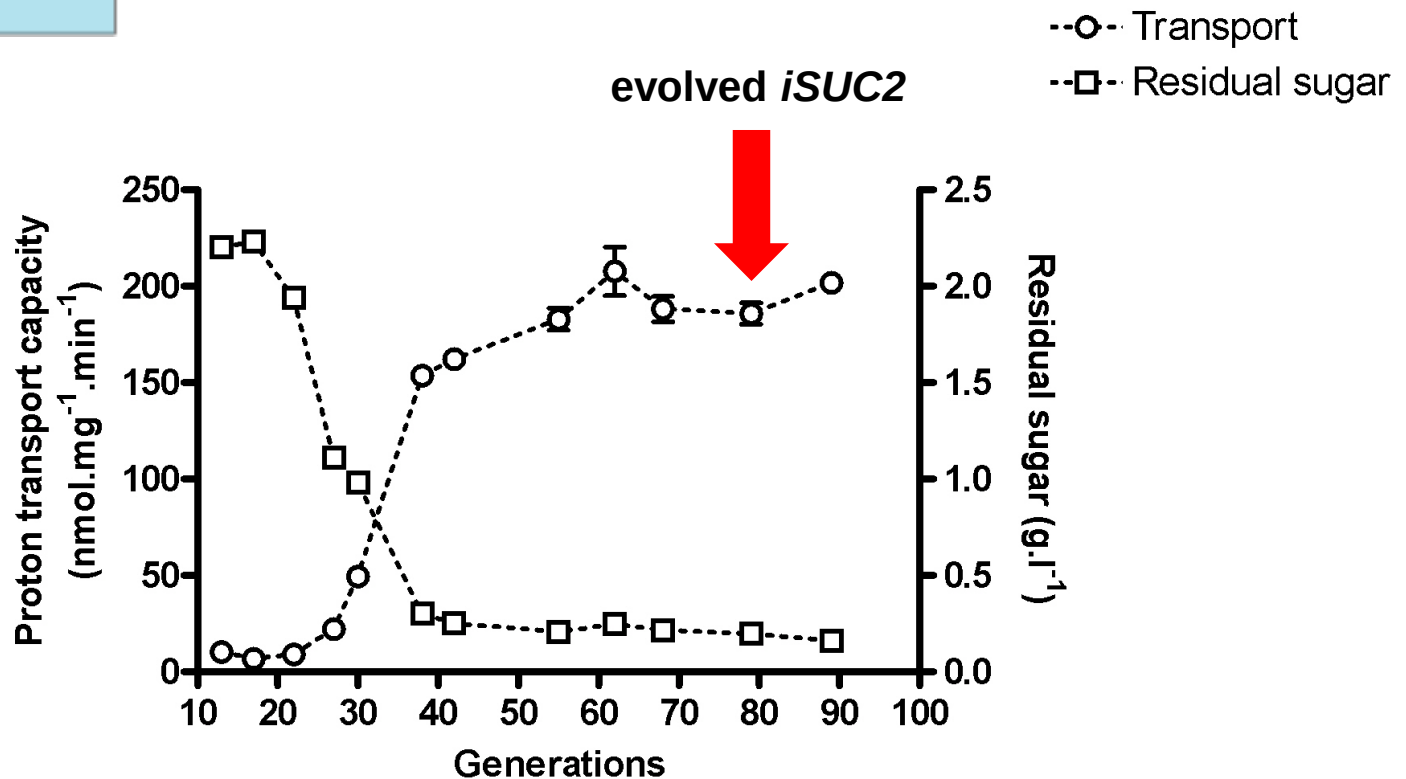
## Sucrose-limited anaerobic chemostats ( $D = 0.1 \text{ h}^{-1}$ )



[sucrose] = 25 g L $^{-1}$



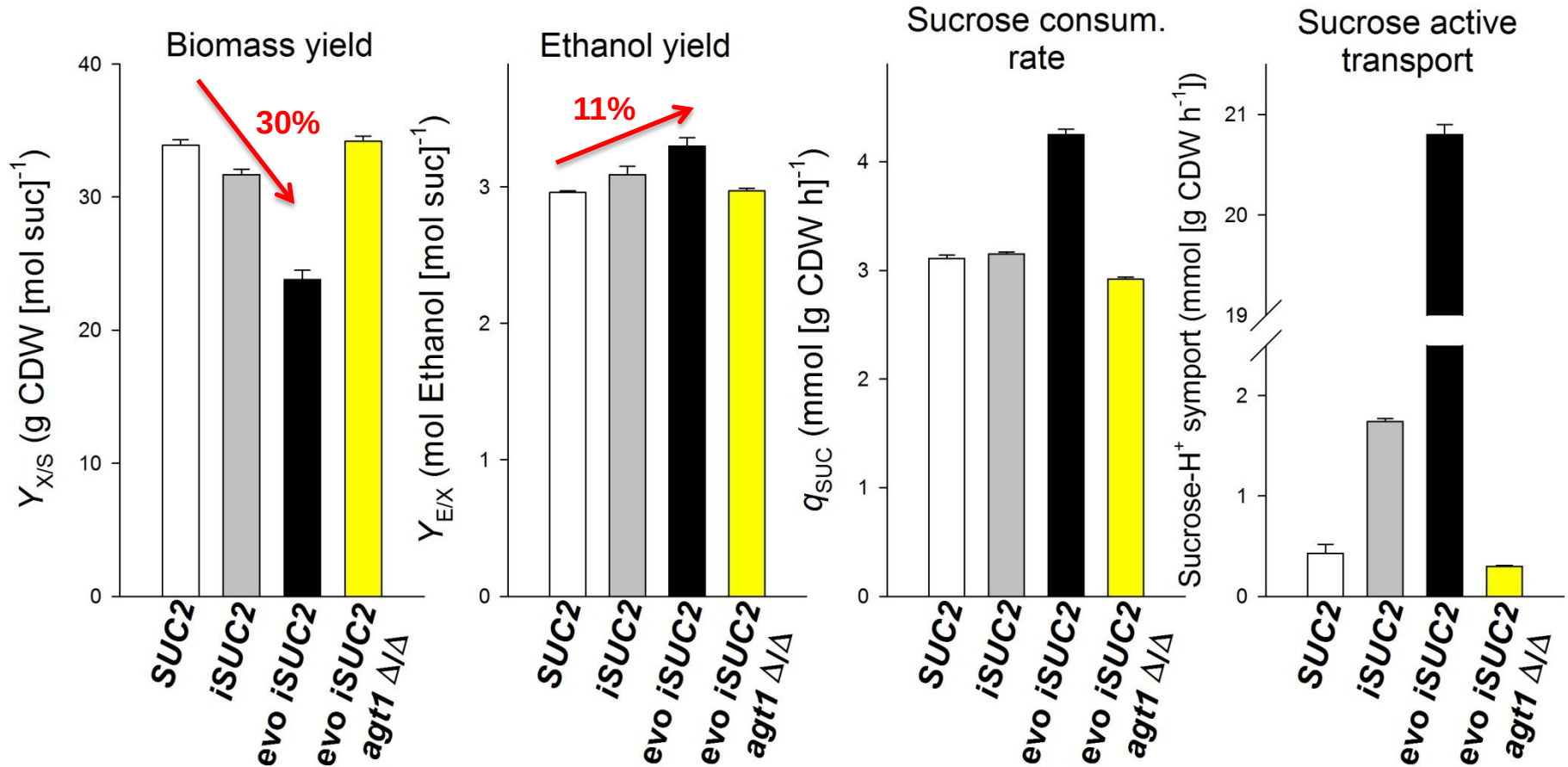
## Evolutionary engineering



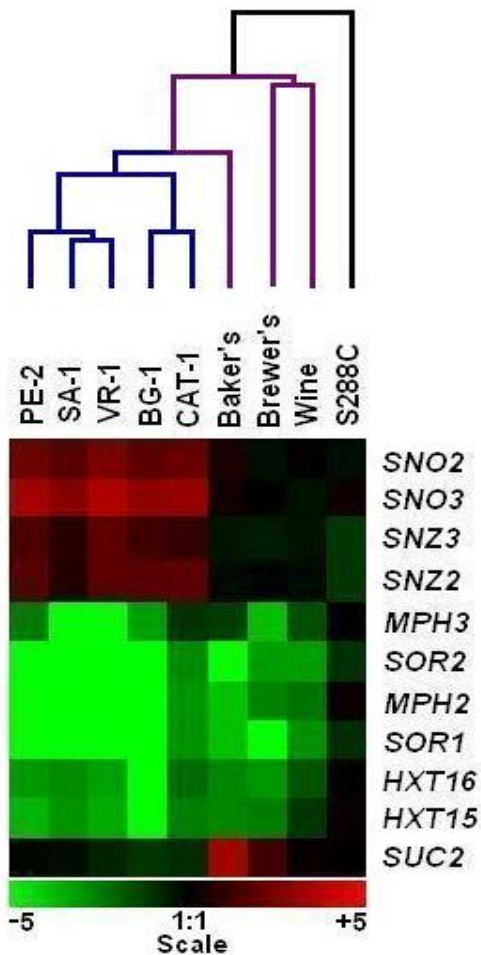




# evolved *iSUC2* strain ferments sucrose efficiently

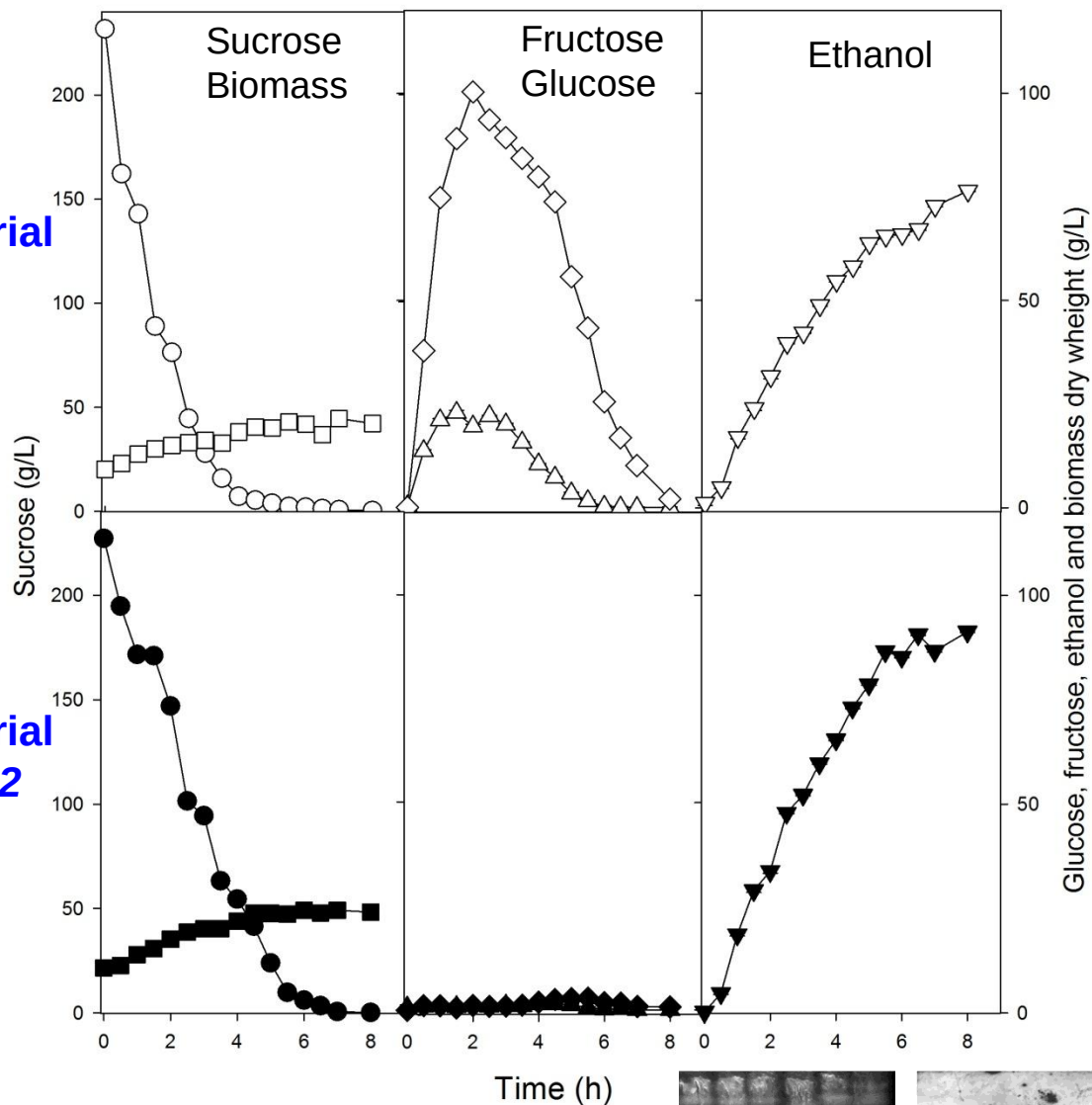


evolved *iSUC2* strain duplicated *AGT1*



Industrial

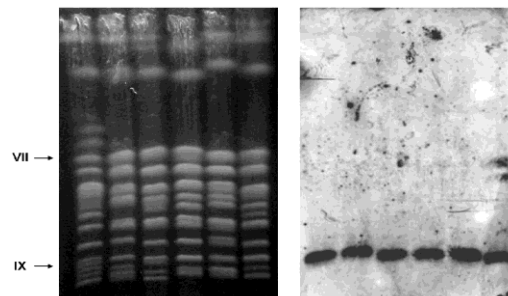
Industrial  
*iSUC2*



## Industrial fuel ethanol yeasts contain adaptive copy number changes in genes involved in vitamin B1 and B6 biosynthesis

Boris U. Stambuk, Barbara Dunn, Sergio L. Alves, Jr, et al.

Genome Res. 2009 19: 2271-2278 originally published online November 6, 2009



## **Conclusions:**

- Possible to modify the topology & energetics of sucrose utilization by yeasts**
- Evolutionary engineering allowed improved kinetics of sucrose transport & fermentation**
- Modifications introduced into a diploid industrial strain, allowing efficient sucrose fermentation**
- Industrial world?**



**Marcelo G. Dário**  
**Júlio César A. do Espírito-Santo**  
**Gabriela Müller**  
**Paulo S. Schlölg**  
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**Thiago O. Basso**  
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**Delft University of Technology**

