COMBINED ENGINEERING OF DISACCHARIDE TRANSPORT AND PHOSPHOROLYSIS FOR ENHANCED ATP YIELD FROM SUCROSE FERMENTATION IN *SACCHAROMYCES CEREVISIAE*

Wesley Leoricy Marques, Delft University of Technology, The Netherlands ; University of Campinas, Brazil ; University of São Paulo, Brazil.
w.l.marques@tudelft.nl
Robert Mans, Delft University of Technology, The Netherlands.
r.mans@tudelft.nl
Ryan K. Henderson, University of Groningen, The Netherlands
Eko Roy Marella, Delft University of Technology, The Netherlands.
Jolanda ter Horst, Delft University of Technology, The Netherlands.
Erik de Hulster, Delft University of Technology, The Netherlands.
Bert Poolman, University of Groningen, The Netherlands
Jean-Marc Daran, Delft University of Technology, The Netherlands.
Jack T. Pronk, Delft University of Technology, The Netherlands.
Andreas K. Gombert, University of Campinas, Brazil
Antonius J.A. van Maris, Delft University of TechnologyThe Netherlands ; KTH Royal Institute of Technology, Sweden

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Anaerobic industrial fermentation processes do not require aeration and intensive mixing and the accompanying cost savings are beneficial for production of chemicals and fuels. However, the free-energy conservation of fermentative pathways is often insufficient for the production and export of the desired compounds and/or for cellular growth and maintenance. To increase free-energy conservation during fermentation of the industrially relevant disaccharide sucrose by *Saccharomyces cerevisiae*, we first replaced the native yeast α-glucosidases by an intracellular sucrose phosphorylase from *Leuconostoc mesenteroides* (*Lm*SPase) (Figure 1). Subsequently, we replaced the native proton-coupled sucrose uptake system by a putative sucrose facilitator from *Phaseolus vulgaris* (PvSUF1). The resulting strains grew anaerobically on sucrose at specific growth rates of 0.09 ± 0.02 h^{-1} (*LmSPase*) and 0.06 ± 0.01 h^{-1} (*PvSUF1*, *LmSPase*). Overexpression of the yeast *PGM2* gene, which encodes phosphoglucomutase, increased anaerobic growth rates on sucrose of these strains to 0.23 ± 0.01 h^{-1} and 0.08 ± 0.00 h^{-1}, respectively. Determination of the biomass yield in anaerobic sucrose-limited chemostat cultures was used to assess the free-energy conservation of the engineered strains. Replacement of intracellular hydrolase with a phosphorylase increased the biomass yield on sucrose by 31%. Additional replacement of the native proton-coupled sucrose uptake system by PvSUF1 increased the anaerobic biomass yield by a further 8%, resulting in an overall increase of 41%. By experimentally demonstrating an energetic benefit of the combined engineering of disaccharide uptake and cleavage, this study represents a first step towards anaerobic production of compounds whose metabolic pathways currently do not conserve sufficient free-energy.

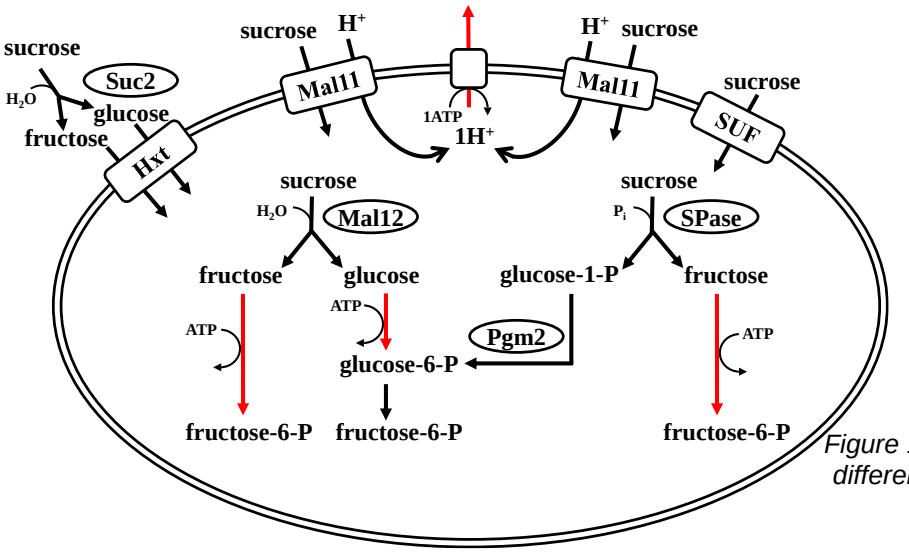

Figure 1 – Schematic representation of different strategies for the uptake and cleavage of sucrose.