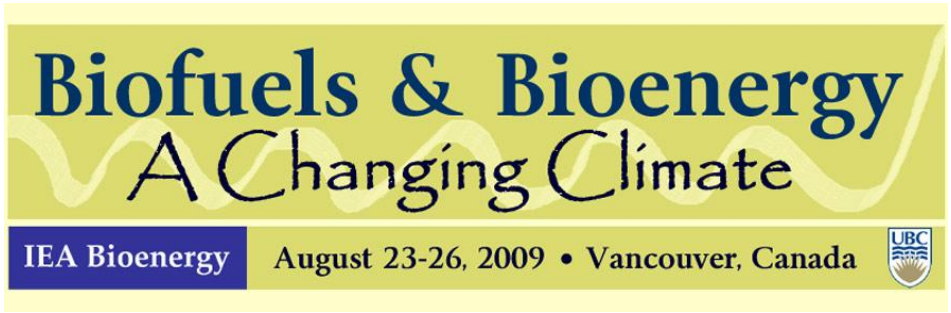


Metabolic engineering of the ethanol fermentation by *Saccharomyces cerevisiae* away from glycerol formation towards alternative products

Vishist Kumar Jain, Benoit Divol, Bernard Prior*, Florian Franz Bauer

Institute for Wine Biotechnology,

Stellenbosch University, Stellenbosch, South Africa

***Presenting author (bap@sun.ac.za)**

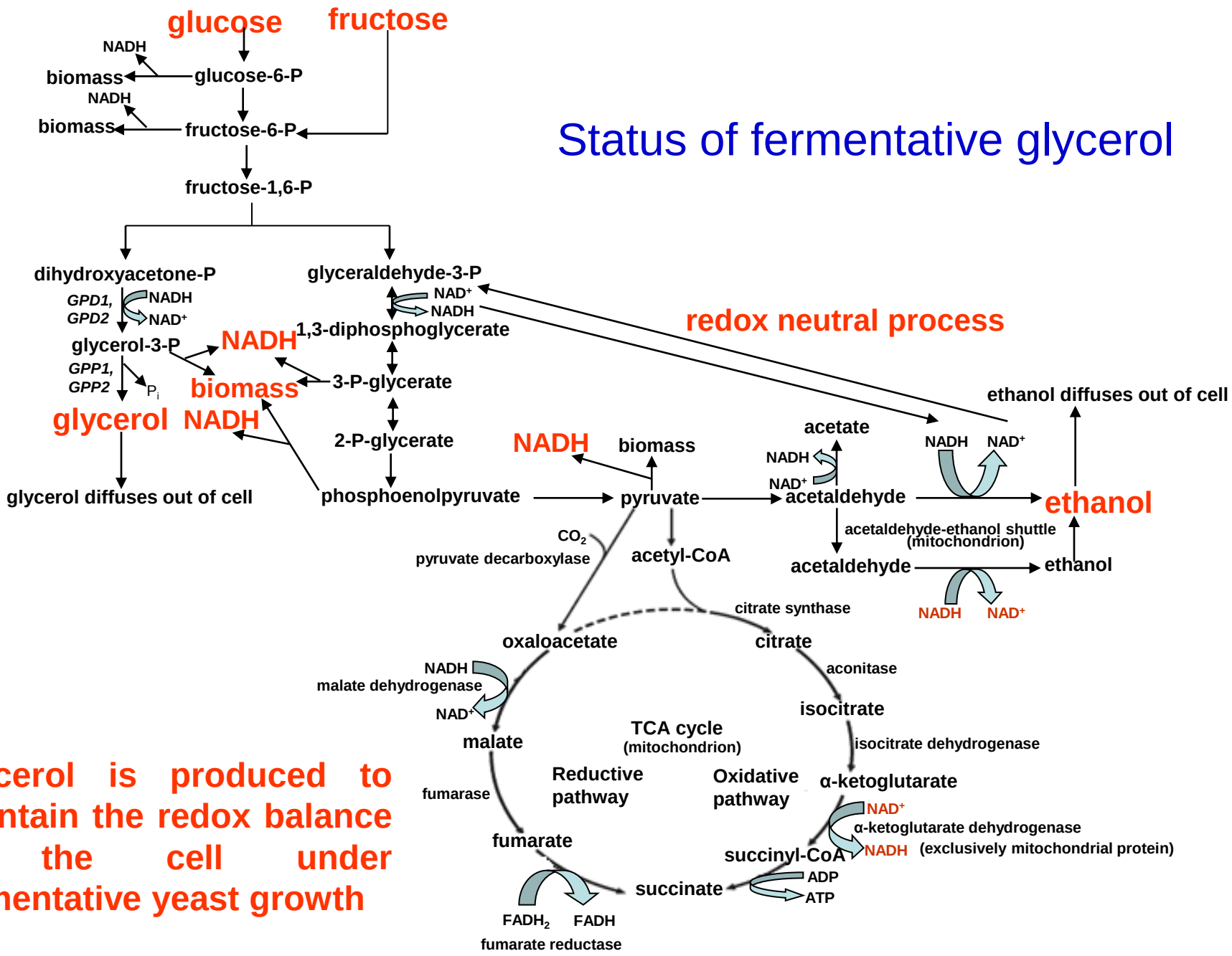
Introduction

1. Glycerol is a major byproduct of the ethanol fermentation by *Saccharomyces cerevisiae* and typically 2-3 % of the sugar fermented ends up as glycerol.

2. Glycerol consist of 7-10 % of the ethanol produced

3. Elimination of glycerol from the ethanol fermentation process and possible replacement with other valuable products could have significant commercial advantages.

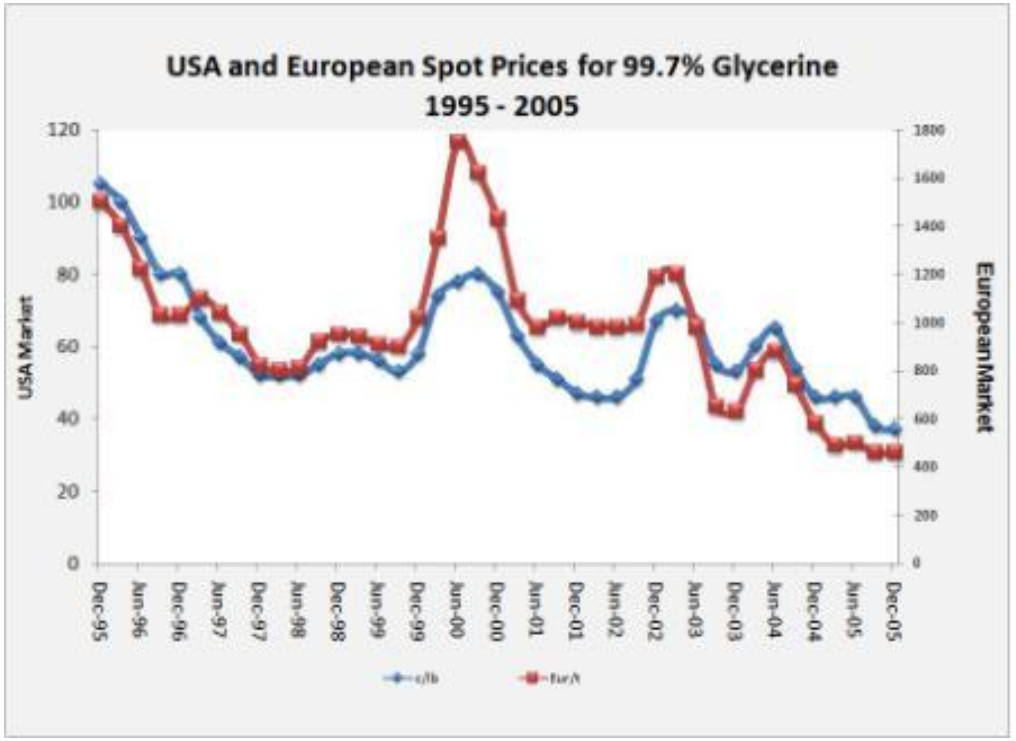
Status of fermentative glycerol



Glycerol is produced to maintain the redox balance in the cell under fermentative yeast growth

Commercial glycerol

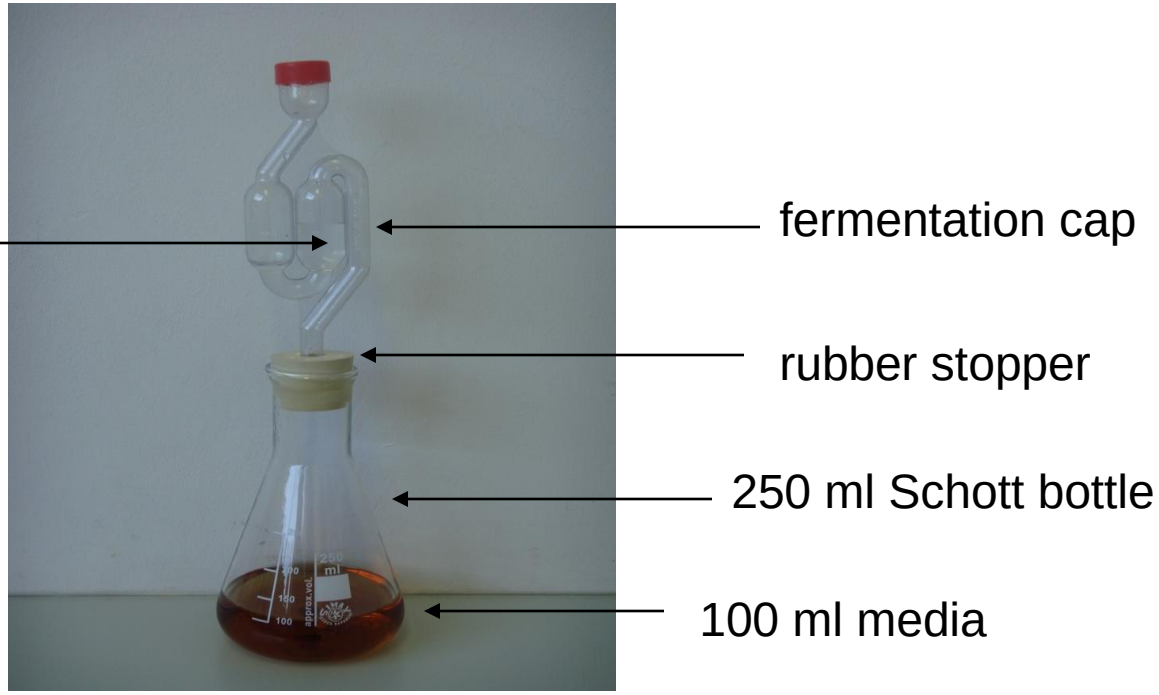
- Commercial glycerol is mainly produced as a by-product in soap and oleochemicals production, and more recently, biodiesel
- There is an oversupply at present resulting in price collapse
- As recently as 2003 glycerol prices were around $1200 per tonne. Today prices are around $600 per tonne and falling.



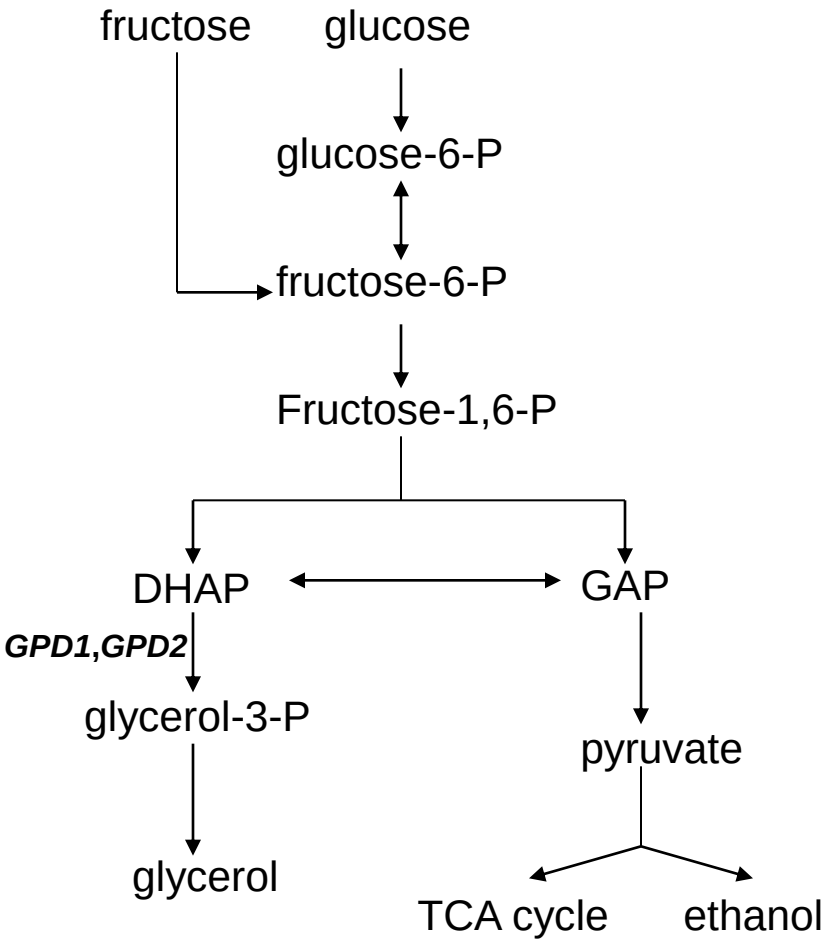
Heming, M.P.D, "*Glycerine Market Report*", no 71, December 2005

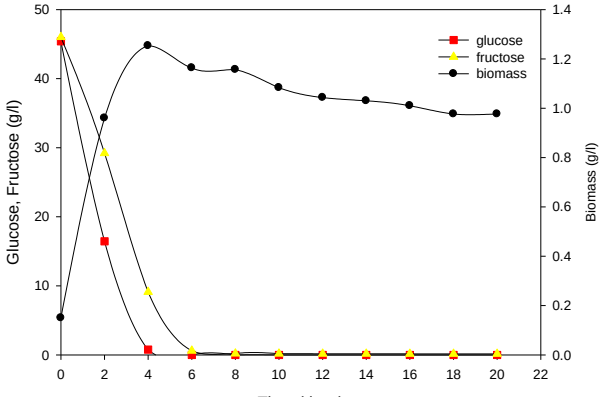
Cultivation conditions

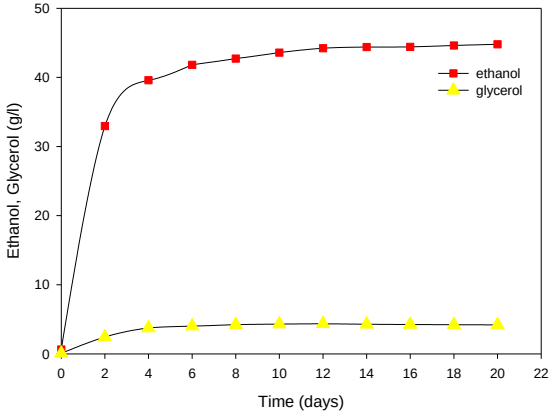
- *S. cerevisiae* BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*)
- Grown without agitation in 10% total sugar (5% glucose and 5% fructose) and 6.7 g/l yeast nitrogen base medium for 20 days.
- pH and temperature maintained at 3.5 and 30ºC



Typical *S. cerevisiae* fermentation on 10 % sugar (5 % glucose & 5 % fructose)

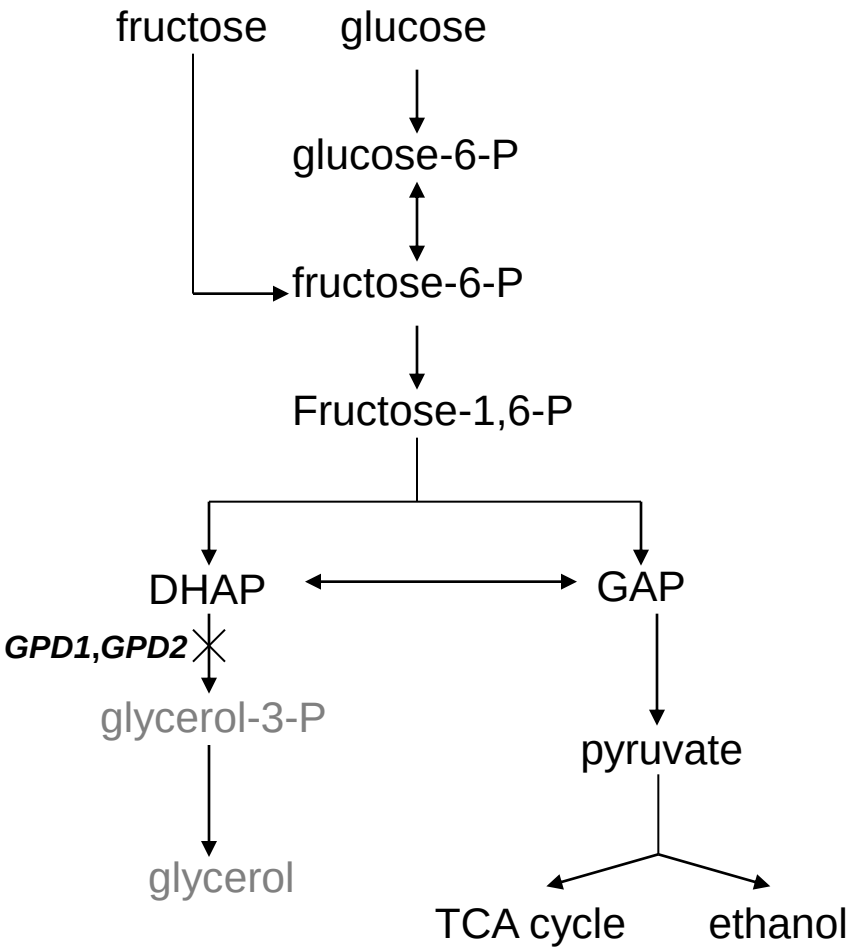


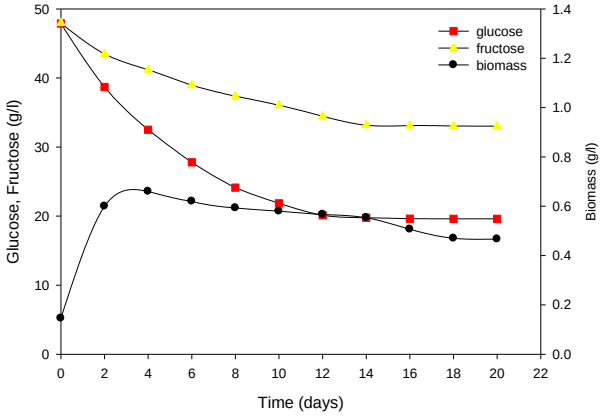


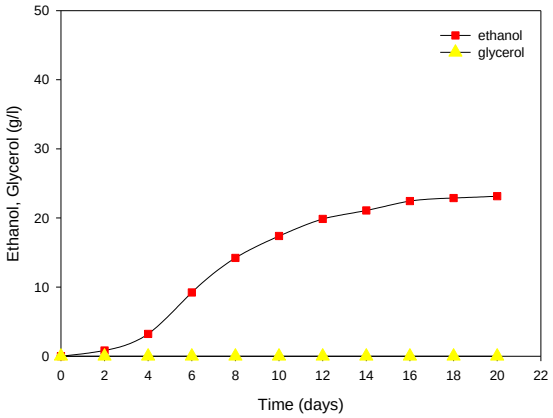


Ethanol: 45.2 g/l (1.76 moles/mole sugar)
Glycerol: 4.1 g/l (0.08 mole/mole sugar)

Deletion of glycerol synthesizing genes limits fermentation due to NADH accummulation







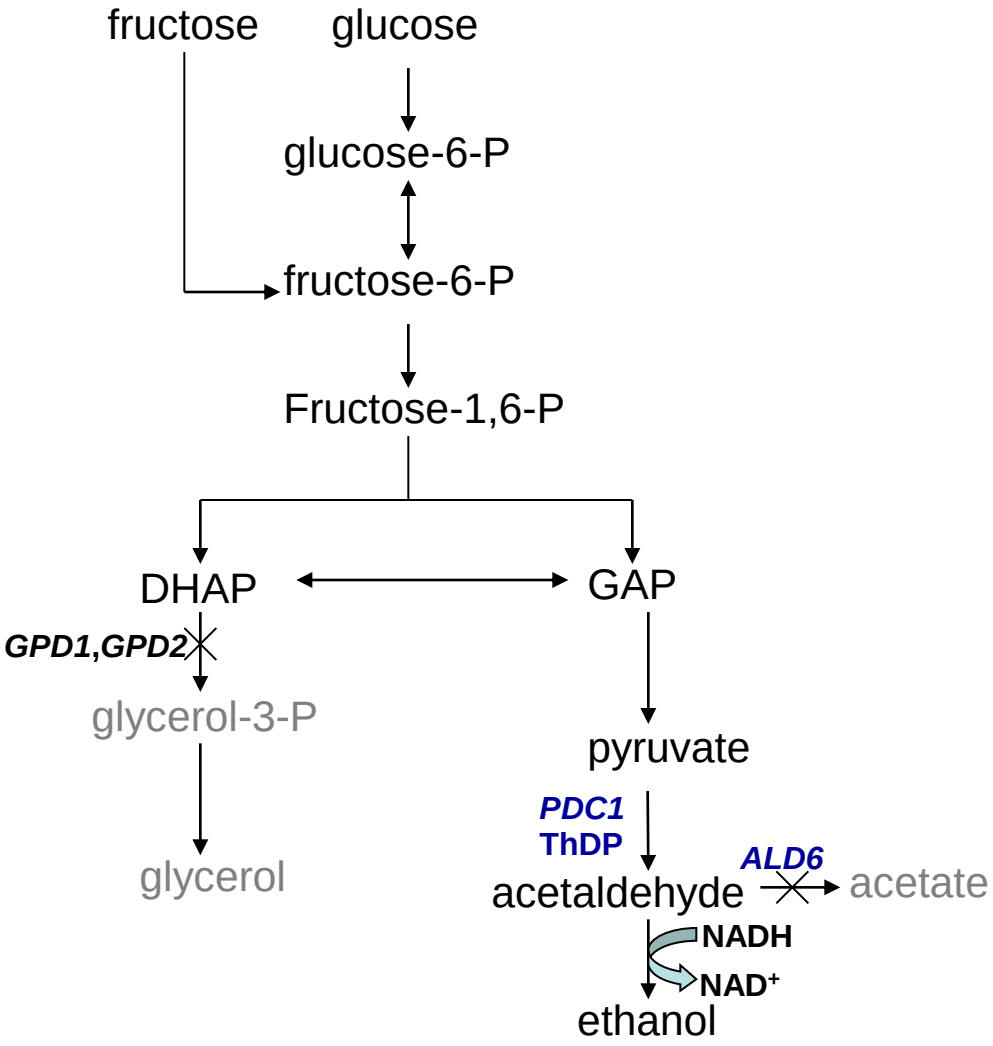
Ethanol: 22.9 g/l (1.89 moles/mole sugar)
Glycerol: 0 g/l (0 mole/mole sugar)

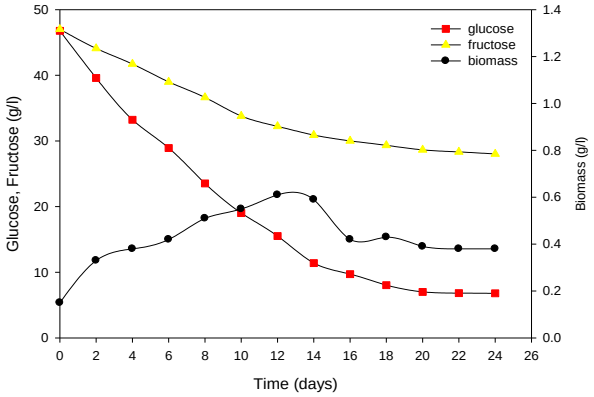
Objective

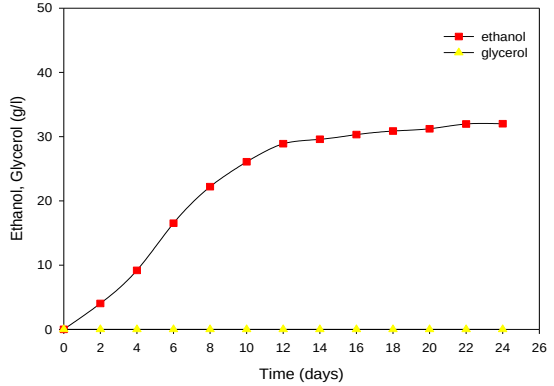
- Investigated the possibility of converting excess NADH to NAD^+ by transforming a glycerol synthesizing double mutant (*gpd1Δgpd2Δ)* with genes that could restore the redox balance in the yeast.

- The double mutant was transformed with genes that could lead to the formation of other valuable products

Regeneration of NAD$^+$ by *S. cerevisiae gpd1Δgpd2Δ* by overexpression of native pyruvate decarboxylase (*PDC1*) and deletion of *ALD6*





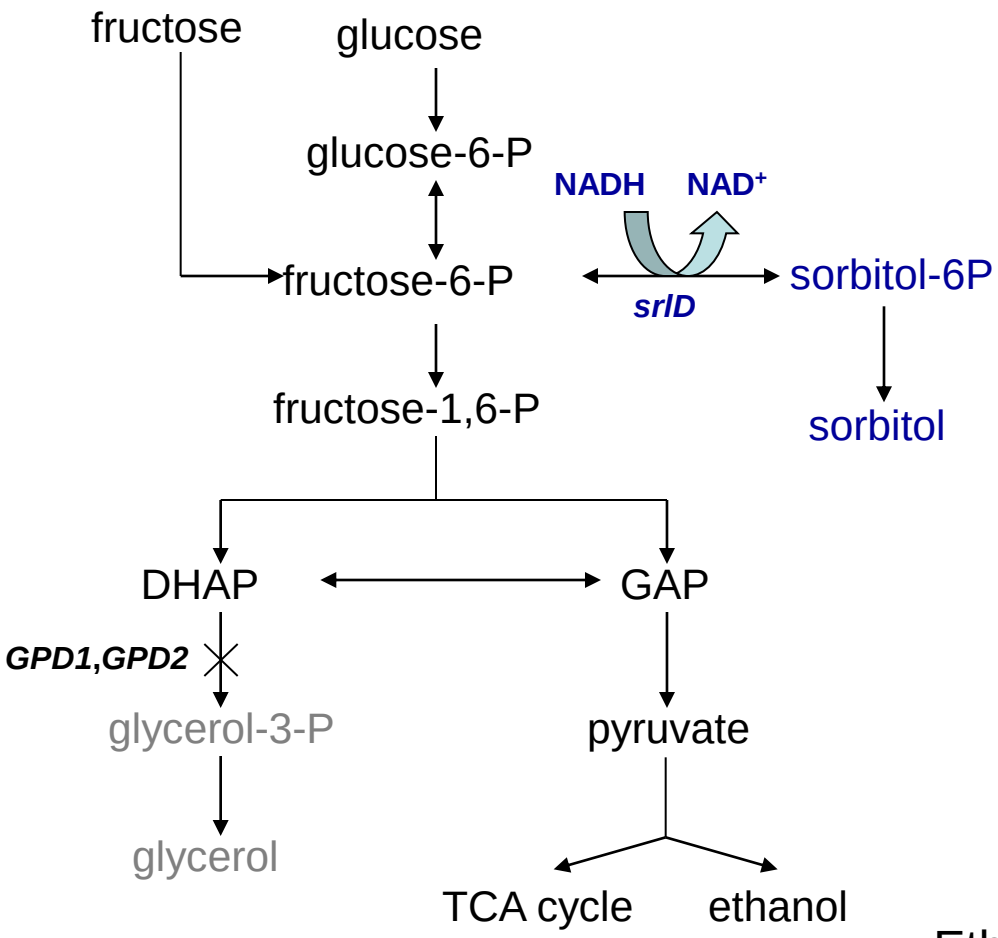


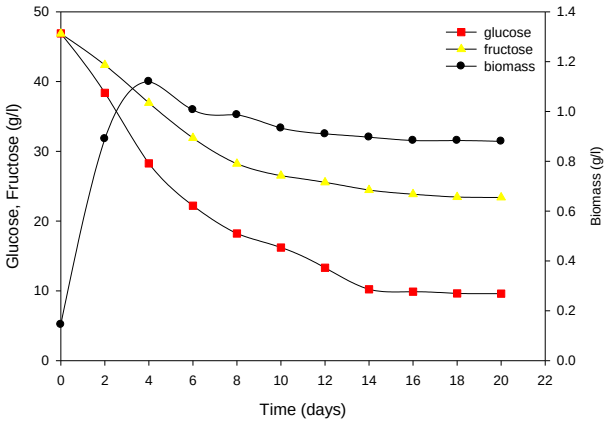
Ethanol: 32.0 g/l (1.92 moles/mole sugar)
Glycerol: 0 g/l (0 mole/mole sugar)

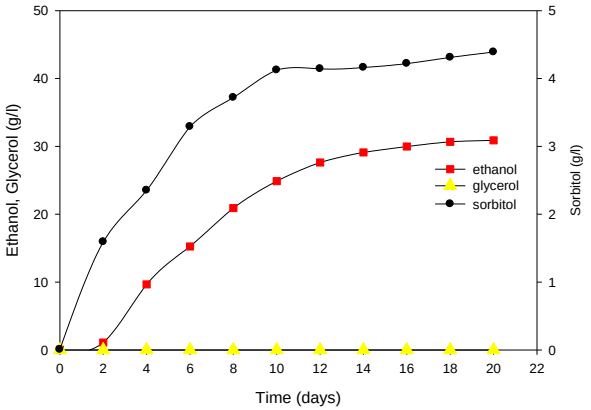
Sorbitol as an alternative product

- Most important polyol with applications in foods, pharmaceuticals and cosmetics, as well as feedstock for ascorbic acid
- More than 750,000 tons produced worldwide (2001)
- Sorbitol is facing competition from glycerol as an alternative as the price of glycerol declines

Redirection of metabolic flux to sorbitol (1) by overexpression of *E. coli* sorbitol-6-phosphate dehydrogenase (*srlD*) gene

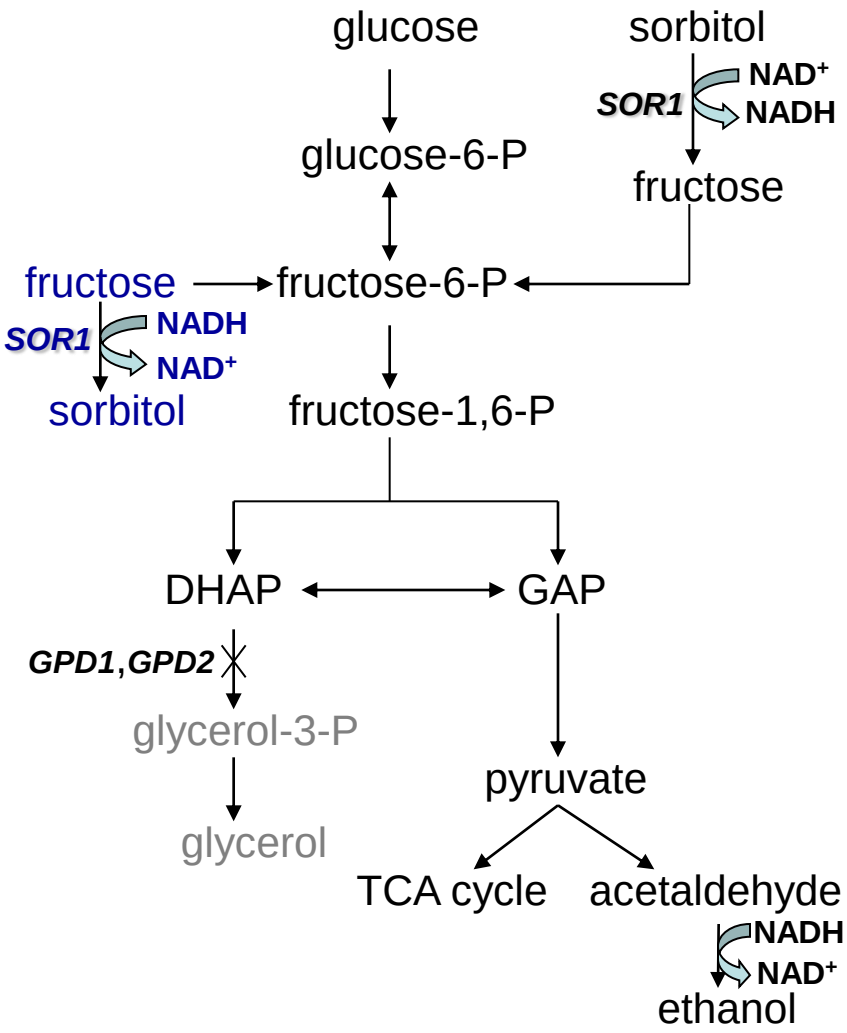


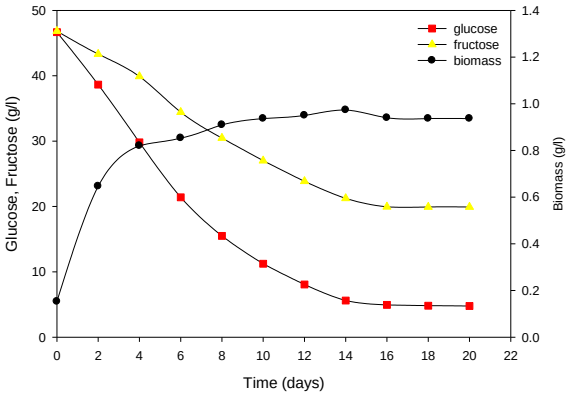


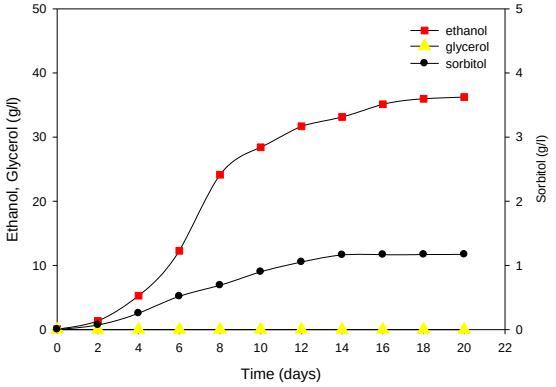


Ethanol: 30.9 g/l (1.79 moles/mole sugar)
Glycerol: 0 g/l (0 mole/mole sugar)
Sorbitol: 4.4 g/l (0.065 mole/mole sugar)

Redirection of metabolic flux to sorbitol (2) by overexpression of native sorbitol dehydrogenase (*SOR1*) gene





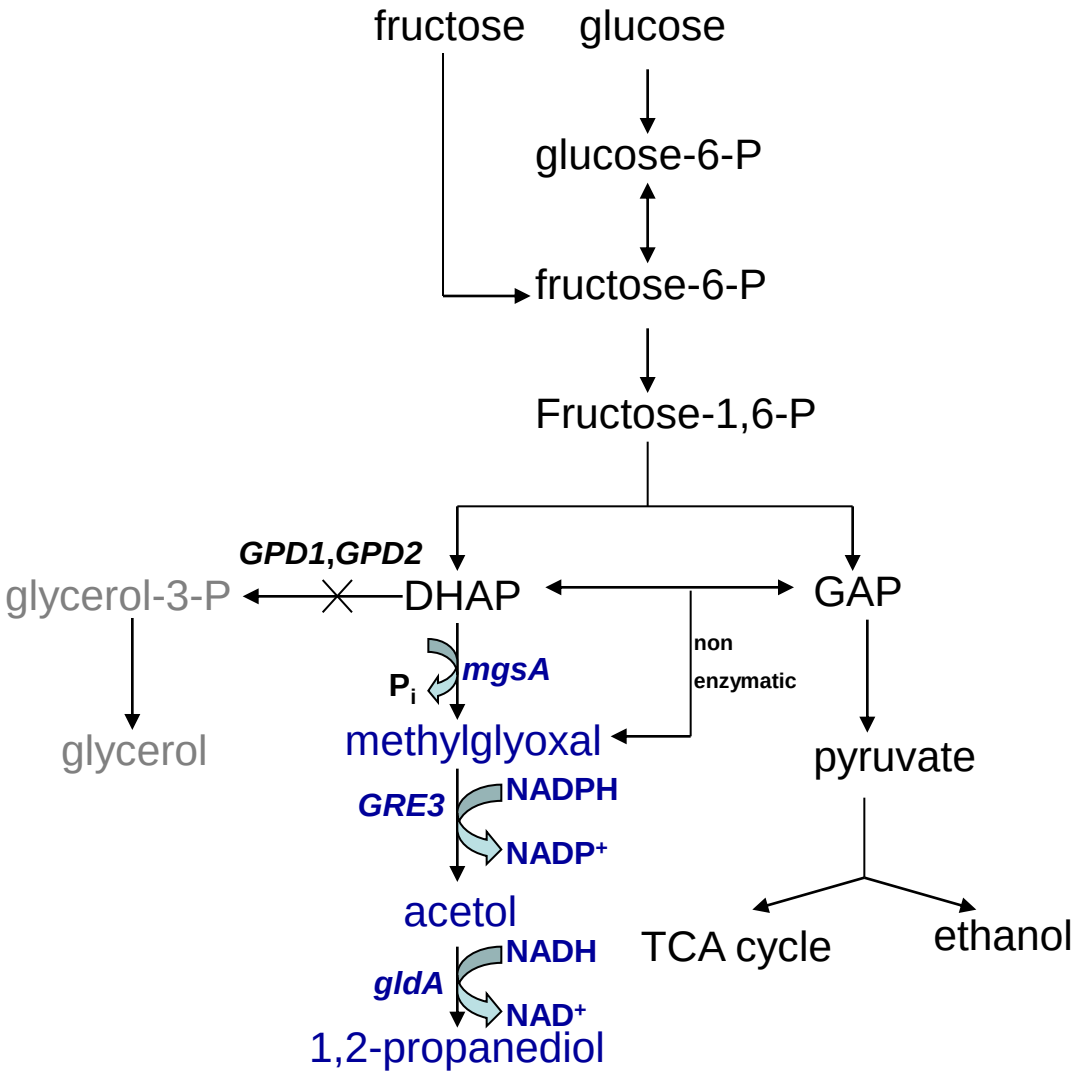


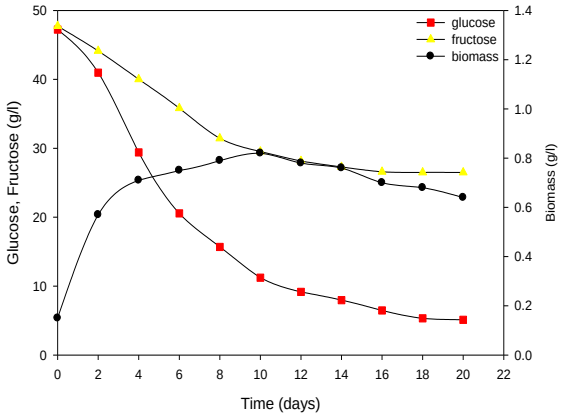
Ethanol: 36.3 g/l (1.84 moles/mole sugar)
Glycerol: 0 g/l (0 mole/mole sugar)
Sorbitol: 1.2 g/l (0.015 mole/mole sugar)

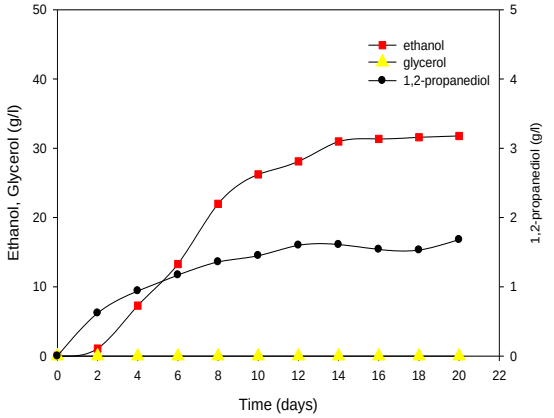
Propane-1,2-diol

- Industrially propylene glycol is produced by propylene oxide hydration
- More than one million tons produced annually (2004). The market is growing at >10% per annum
- Worldwide propane-1,2-diol (also known as propylene glycol or 1,2-propanediol) is mainly used for the production of unsaturated polyester resins.
- Used as an industrial solvent, an antifreeze & in cleaning agents, paint and surfactants
- Propane-1,2-diol is an approved additive in foods, in cosmetics and as a solvent in pharmaceutical preparations

Redirection of metabolic flux to 1,2 propanediol by overexpression *E. coli* methylglyoxal synthase (*mgsA*) and glycerol dehydrogenase (*gldA*) and native aldose reductase (*GRE3*) genes







Ethanol: 31.8 g/l (1.81 moles/mole sugar)
1,2 propanediol: 1.7 g/l (0.047 mole/mole sugar)

Summary of metabolites in g/l (mole/mole subtstrate used)

Metabolite	Wild type	Double deletion	*PDC1 overexp. ALD6 delete*	*srlD* gene overexp.	*SOR1* gene overexp.	*mgsA, gldA, GRE3 overexp.*
Ethanol	45.2 (1.76)	22.9 (1.89)	32.01 (1.92)	30.9 (1.79)	36.25 (1.84)	31.8 (1.81)
Glycerol	4.1 (0.08)	0	0	0	0	0
Sorbitol	0	0	0	4.39 (0.065)	1.17 (0.015)	0
Propane-1,2 diol	0	0	0	0	0	1.68 (0.047)

Conclusion

- Glycerol formation can be eliminated during fermentation but no alternative redox balancing pathway was as efficient at the glycerol pathway in maintaining fermentation.

- Alternative products such as sorbitol and 1,2 propanediol can be produced instead of glycerol but these genetic manipulations had negative effects on fermentative ability

- Ethanol yields but not concentrations were improved in mutants