IMPACT OF SEVERAL STRATEGIES TO IMPROVE ETHANOL YIELD ON THE ATP MAINTENANCE RATE (μ_{ATP}) OF *Saccharomyces cerevisiae*.

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Introduction. . Ethanol is a commodity with a large and fastly growing market. To readily assess attempts at improving fermentation performance, a simple stoichiometric model of the yeast metabolism is set up.

We reviewl batch culture experimental results of various strategies for improving the yield of ethanol on glucose. The SSMCIM is used to calculate the ATP maintenance rate as also to analyze the impact of the different experimental strategies on the metabolism of the yeast and its ethanol yield on glucose.

Methods.

Stoichiometric modeling of the lumped metabolism is a standard tool for pathway flux analysis (Schügerl, 2000).

Results

The lumped reactions network scheme is shown as Figure 1. Three key metabolites are highlighted: NAD, ATP and glycerol 3P.

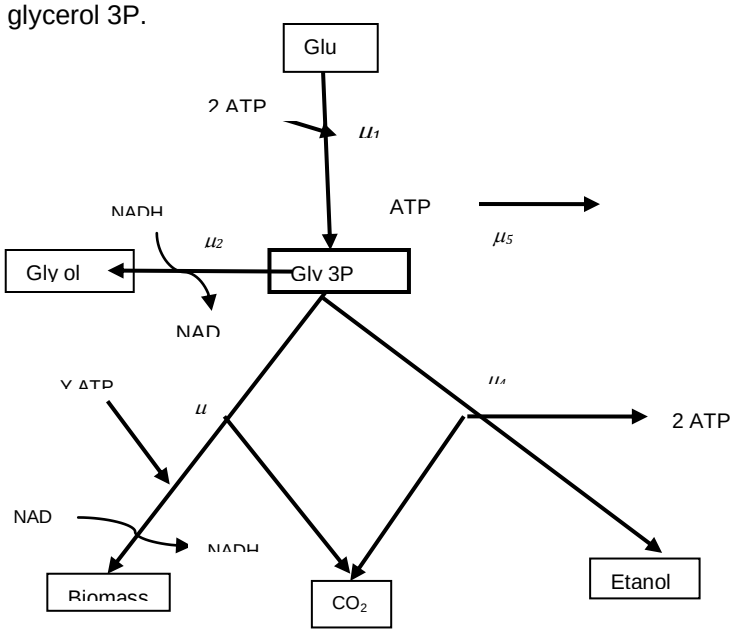

Fig.1 Schematic of the anaerobic metabolism of *S. cerevisiae*, Reaction μ_i is a metabolic pathway flux: i=1 for glycolisis,2 for glycerol production, 3 for biomass synthesis, 4 for ethanol production and 5 for ATP used for maintenance (μ_{ATP}).

The schematic points to the physiological necessity of glycerol reduction to provide oxidized NAD for biomass synthesis. The three key metabolites are conserved by relating their intracellular concentrations to the 5 metabolic flux rates. A solution for the resulting equations system is:

$$\mu_{ATP} = 3.14\mu_4 - 1.759\mu_1 \quad (1)$$

which relates μ_{ATP} to ethanol production and glucose uptake rates.

Converti et al.(1985) tested two yeast species at low, medium and high substrate concentrations. The batch kinetics is reported as also the biomass and ethanol yields on glucose. Nissen et al. (2000) mutated a *Saccharomyces* strain to impair Glycerol 3 P dehydrogenase (GPDH) synthesis in order to delete pathway coded μ_2 in Figure 1. They assayed the result in aerobic batch cultivation. The ethanol yield and the Monod kinetics parameters are reported though the biomass kinetics is not.. In this work the dynamic biomass concentrations were simulated. Pagliardini et al. (2010) tried to reduce glycerol formation by fine-tuning the expression of GPDH and carried out fed batch cultures with a wild and the mutant strains. They reported the kinetics and the ethanol and biomass yields obtained. Inei (2010) batch-cultured *S. cerevisiae* in the presence of 50 and 200 mg/L zeolite Velfor 100 and reported the kinetics for glucose, ethanol and biomass.

The concentration profiles were differentiated and the dynamic specific rates were calculated, and their values used to calculate $_{\mu ATP}$ using equation 1.

In some of the cases analyzed here, negative $_{\mu ATP}$ rates were found, pointing to a limitation of the structure of this model. Though ATP is formed and used during normal metabolic operation, in extreme, stressful, situations *de novo* ATP synthesis must occur to cope with ATP depletion. If this rate is considered in the model, negative $_{mATP}$ rates are in fact *de novo* ATP synthesis rates.

Table 1 shows the ethanol yields on glucose (g/g) and the maximum *de novo* ATP synthesis calculated.

Table 1. Main author, ethanol yield (g/g) and maximum *de novo*.ATP synthesis (mol ATP/mol C biomass –h) for the processes reviewed.

Author	Converti	Nissen	Pagliardini	Inei
$Y_{e/g}$	0.4	0.36	0.45	0.51
Max de nvo	0	0	2.0	3.0

Conclusions. ATP maintenance and/or ATP *de novo* synthesis correlate with ethanol yields approaching the maximum.

Further experimental work is needed to validate the model predictions.

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