Metabolic engineering and *in silico* modelling for evaluating ethylene glycol assimilation as a carbon source in *Saccharomyces cerevisiae*.

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Abstract: Polyethylene terephthalate (PET) is one of the most used polymers in the packaging industry. Consequently, its waste has a negative impact creating major environmental problems. This work aims to evaluate the consumption of ethylene glycol (EG), a PET monomer that can be obtained after PET enzymatic hydrolysis, as an alternative carbon source in *Saccharomyces cerevisiae* through metabolic engineering and *in silico* modelling.

Previous studies showed that *S. cerevisiae* primarily converts EG into glycolic acid (GA) while also forming the toxic intermediate glycolaldehyde (GAH). In fact, when GAH is added to the medium as the initial substrate, cells mainly detoxify GAH in to EG and, in a minor scale also, oxidate it to GA, creating a dynamic equilibrium among the three molecules (Senatore et al. 2025). Time-course data were used to model reaction dynamics with *ODEs,* and a custom *Particle Swarm Optimization* approach was used to estimate the unknown kinetic constants. This allowed the model to reproduce the experimental trends and simulate reactions directionality.

To prompt EG utilization as a carbon source, a growth-coupling selection strain was implemented by engineering a strain that express the bacterial pathway known as TaCo, which converts GAH to 2-phosphoglycerate (2PG), into *S. cerevisiae* (CER.TaCo). A knockout of the *GPM1* gene was introduced to block glycolytic 2PG formation, forcing CER.TaCo *Δgpm1* to rely on EG assimilation via the TaCo pathway. The control strain CEN.PK113-7D *Δgpm1*, as previously described (Papini et al. 2010), cannot grow in minimal medium with glucose but only with ethanol and glycerol. In this study, EG was tested as an alternative carbon source, and growth assays showed that it enhances growth in several nutritional condition. EG could partially replace ethanol in the mutants but could not substitute glycerol. Moreover, when TaCo pathway was expressed, there was a significant decrease in the percentage of EG oxidised to GA, suggesting that EG may instead be partially diverted toward biomass precursor formation. Flux Balance Analysis was performed on two strains (CEN.PK113-7D *wt* and *Δgpm1*) grown in minimal media with various carbon-source combinations. Using the Yeast8 model in COBRApy, simulated biomass values were compared between strains and different experimental conditions. Overall, this study lay foundation for further optimization towards the use of PET monomers as feedstock, in a circular bioeconomy framework.