

Rewiring metabolism for maximum lipid production in oleaginous yeast *Yarrowia lipolytica*

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Project Goals: We focused on achieving a fundamental understanding of the metabolism of the oleaginous yeast *Yarrowia lipolytica* and developing tools to characterize and engineer it towards cost-effective lipids production. More specifically, we aimed to improve its fermentation characteristics towards the development of a cost-effective process which converts renewable resources to lipids for biodiesel production. The conversion yield and volumetric productivity on various carbon sources are the key metrics for optimization.

Production of lipids by microbial fermentation of carbohydrate feedstocks outcompetes oil crops in terms of productivity, however, presently achievable carbohydrates-to-lipids process yields are not yet at a point that can support cost-effective production of lipids and biodiesel. To maximize process yields, one needs to maximize lipid content as well as capture as many of the electrons generated from the catabolism of the available substrate as possible. We show that overall lipid process yield, Y , is significantly improved via introduction of synthetic pathways that effectively recycle glycolytic NADHs into cytosolic NADPH and acetyl-CoA to be used for lipid synthesis. Strain construction is guided by a quantitative model that predicts Y from the non-lipids biomass yield, lipid content, and yield of lipid synthesis pathway, Y_L , with Y_L becoming the key driver of process yield maximization at high lipid content. In total, thirteen rationally designed strain constructs were evaluated in shake flask and bioreactor experiments to identify the best strain that achieved a lipid titer of close to 100 g/L with a productivity of 1.2 g/L/h and an overall process yield 0.27 g FAME/g glucose. These figures of merit advance the commercialization opportunities of carbohydrate-based biodiesel production.