PHENOTYPIC DESIGN CHOICES FOR ENHANCED TWO-STAGE MICROBIAL PRODUCTION PROCESSES

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Microbial metabolism can be harnessed to produce a broad range of industrially important chemicals. Many microorganisms naturally produce some important compounds but do so with low efficiency. To target a more diverse range of chemicals, pathways for non-natural products can be designed and implemented. However, in order to improve these microbes toward the target of industrial production, their metabolism must be engineered by controlling metabolic flux through key pathways. The merits of microbial production processes are often measured using three key variables: titer, rate and yield (TRY). Each of these variables has an impact on the economic viability of any microbial production process. Previous research into improving these TRY metrics have examined the efficacy of decoupling microbial growth from chemical production to achieve enhanced production rates. However, there has been limited research into the choice of microbial phenotype for the growth and production stages of two-stage production processes. Moreover, the substrate uptake rates of microbes drop significantly upon reducing the growth rate, adding to the need for intelligent phenotype selection while designing strains for two-stage processes. In this work, we present a two-stage optimization framework that scans the phenotypic space of microbial metabolism to identify the correct choice of phenotypes during growth and production stages, along with the optimal time to switch between these stages to achieve required TRY values. Through this framework and using *Escherichia coli* as a model organism, we compare the performance of two-stage fermentation processes where dynamic pathway regulation is involved with one-stage fermentation processes that have static intervention strategies implemented for a range of naturally produced chemicals. Our results indicate that while one stage processes are better at achieving optimal yields, two-stage processes outperform them in achieving optimal production rates even after incorporating the effects of reduced substrate uptake rates during the production stage. We anticipate that this optimization framework would be invaluable in designing microbial strains and fermentation processes for industrial chemical production.