Toward Synthetic Biology Strategies for Adipic Acid Production: An in Silico Tool for Combined Thermodynamics and Stoichiometric Analysis of Metabolic Networks

Adipic acid, a nylon-6,6 precursor, has recently gained popularity in synthetic biology. Here, 16 different production routes to adipic acid were evaluated using a novel tool for network-embedded thermodynamic analysis of elementary flux modes. The tool distinguishes between thermodynamically feasible and infeasible modes under determined metabolite concentrations, allowing the thermodynamic feasibility of theoretical yields to be assessed. Further, patterns that always caused infeasible flux distributions were identified, which will aid the development of tailored strain design. A review of cellular efflux mechanisms revealed that significant accumulation of extracellular product is only possible if coupled with ATP hydrolysis. A stoichiometric analysis demonstrated that the maximum theoretical product carbon yield heavily depends on the metabolic route, ranging from 32 to 99% on glucose and/or palmitate in *Escherichia coli* and *Saccharomyces cerevisiae* metabolic models. Equally important, metabolite concentrations appeared to be thermodynamically restricted in several pathways. Consequently, the number of thermodynamically feasible flux distributions was reduced, in some cases even rendering whole pathways infeasible, highlighting the importance of pathway choice. Only routes based on the shikimate pathway were thermodynamically favorable over a large concentration and pH range. The low pH capability of *S. cerevisiae* shifted the thermodynamic equilibrium of some pathways toward product formation. One identified infeasible-pattern revealed that the reversibility of the mitochondrial malate dehydrogenase contradicted the current state of knowledge, which imposes a major restriction on the metabolism of *S. cerevisiae*. Finally, the evaluation of industrially relevant constraints revealed that two shikimate pathway-based routes in *E. coli* were the most robust.