In addition to these two problems, engineering pathways require good quantitative information on the flow of metabolites in a cell. Structured models of cells can be used in conjunction with experiments to optimize the design of new pathways. Metabolic engineering has been a fertile area for engineering contributions.

A goal of the engineering approach has been to develop rational design techniques for metabolical engineering. Due to the highly nonlinear, dynamic nature of a cell and its metabolism, uninformed changes intended to improve production of a specific compound often fail to give the desired result. The problem of metabolic engineering is to express appropriate genes in the "right" amount. Too much of a key enzyme may result in little change, if it is not a rate-influencing step, as there is insufficient reactant to increase the flow of substrate (or *flux*) into the reaction product. If it is rate-influencing, the increased reaction of the substrate will alter fluxes of precursors in other pathways; sometimes these changes compromise the cell's ability to grow or to provide co-reactants when needed. Metabolic engineering (and gene therapy) require a delicate balance of activities and are a problem of quantative optimization rather than simple maximization of expression of a gene.

Metabolic control theory and the closely allied activity of *metabolic flux analysis* are mathematical tools that can be applied to such problems. A flux balance equation consists of the product of a stoichiometric matrix and an intracellular flux vector to yield an overall rate vector. Typically such equations are underdetermined and require assumptions (e.g., on energy stoichiometry) that may not be justified for a metabolically engineered cell. However, improved experimental techniques to measure the intracellular flux vector (e.g., mass spectrometry) coupled with genomic/proteomic information may provide the data to allow complete solution of the flux balance equations. One caution is that analysis of pathways in isolation can be misleading. An assumption of such analysis is that the products of the pathway do not influence inputs into that pathway. Given the complexity of a cell, it is difficult to assure that such an assumption is satisfied.

Metabolic control theory is based on a sensitivity analysis to calculate the response of a pathway to changes in the individual steps in a pathway. This approach allows calculations of flux control coefficients, which are defined as the fractional change of flux expected for a fractional change in the amount of each enzyme. This process involves a linearization, so that flux coefficients can vary significantly if growth conditions and the cell's physiological step change. An important result of the application of this theory to many cases is that it is rare for a single enzyme step to be rate-controlling. Typically several enzymatic steps influence rate. Maximization of flux through a particular pathway would require several enzyme activities to be altered simultaneously. The potential of such analysis to contribute to rational design of microbes is clear, but not yet routinely applicable.

Real progress has been made toward the industrial use of metabolically engineered cells. Processes to convert glucose from cornstarch into 1,3-propanediol, an important monomer in the polymer, polytrimethylene terepthalate, is poised for commercialization. A process to make 2-keto-L-gluconic acid as a precursor for vitamin C production is in the pilot stage (30,000 l), using metabolically engineered bacteria with glucose as a substrate. Other products from metabolically engineered cells may be new polyketides, modified polyhydroxyalkanoates (as biodegradable polymers), indgo, xylitol, and hybrid antibiotics.