

## **Metabolic Flux and Sensitivity Analysis in *Aspergillus niger***

A comprehensive metabolic network comprising three compartments (cytoplasm, mitochondrion and the external compartment) was developed for *Aspergillus niger*. The metabolic flux network includes carbohydrate and amino acid metabolism in both anabolic and catabolic reactions. According to experimental observations, the time course of fermentation was divided into five phases. Each phase had unique physiological properties. The network was used to form a set of linear algebraic equations based on the stoichiometry of the reactions by assuming pseudo-steady state for intracellular metabolites. The metabolic flux model consists of 136 metabolites and 265 reactions, of which 190 represent biochemical conversions and 75 represent transport processes between different intracellular compartments and between the cell and the extracellular medium. Linear programming was used for the optimization of the specific growth rates in combination with the measured input and output fluxes of the key metabolites to evaluate corresponding intracellular flux distributions throughout the batch fermentations. Sensitivity analysis revealed that phosphate uptake was the growth limiting flux during the early stages of the fermentation. Later, the uptake rate of ammonia had a significant effect on the specific growth rate. Among the amino acids, proline and tyrosine had the highest logarithmic sensitivity. The solution of the flux network was very sensitive to sulphate and to a lesser degree to ammonia, proline, and ammonia uptake rates during the early stages of exponential phase when glucose was the primary carbon source. The proposed model is able to predict correctly the specific growth rate and oxygen uptake rate with good precision. This verifies the validation of the model to predict a realistic flux distribution. The results of the metabolic flux analysis may be employed for medium design in continuous or fed-batch operations involving high density culture.