Partner in der Forschung

ZENTRUM ANGEWANDTE CHEMIE
Institut für Technische Chemie

Institut für Technische Chemie, Callinstr. 3, 30167 Hannover

Gene expression of *Saccharomyes cerevisiae* in different metabolic states

C. Klockow, F. Stahl, B. Hitzmann
University of Hannover, Institute for Technical Chemistry

Introduction

The metabolism of cells depends significantly on enviromental parameters such as nutrien and oxygen concentration or cell density. In consequence, the gene expression should also differ under varying conditions.

The yeast *Saccharomyces cerevisiae* is an especially well qualified organism for this investigation. In dependence on the surounding conditions, it changes into different metabolic states. The concentration of substrate in combination with the amount of dissolved oxygen determines wether the organism shows an oxidative or a reductive metabolism.

Methods

Experiments

To examine the dependence of the gene expression on the substrate concentration, fed-batch cultivations of yeast cells are run with different glucose concentrations. A two liter stirred tank reactor (Biostat B, Sartorius) is used. To keep the concentration constant at the intended set points for several hours, a glucose measurement and control system was established, which is based on a mathematical model of the bioprocess.

The results of two cultivations, which were carried out with glucose set points of 70 mg/L and 500 mg/L, are shown. From the samples taken during the experiments, RNA was isolated, labeled, transcriped to cDNA and hybridised on whole genome yeast chips.

Data processing and analysis

The data processing consists of the following steps

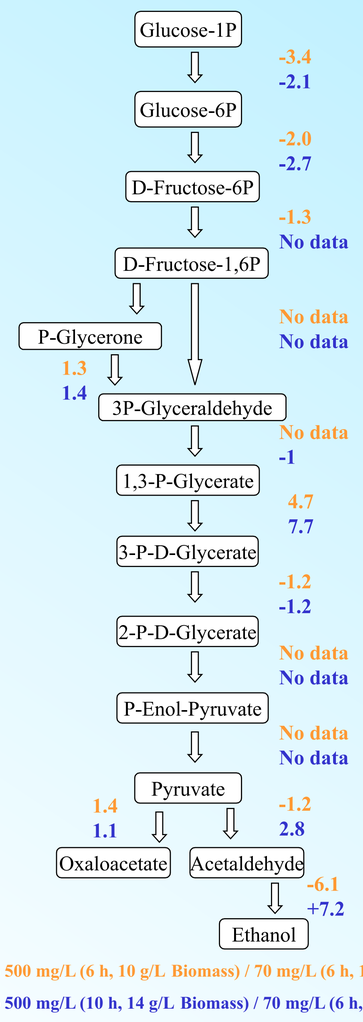
- image processing (commercial software ImaGene)
- substraction of local background from signal (median)
- removal of badly shaped spots (Matlab)
- all signals below a chosen threshold are given a value of 1 (Matlab)
- array-to-array standarisation (Matlab)
- addition of Cy3 and Cy5 signals (Matlab)

Results

The gene expression of two cultivations – with glucose set points of 70 mg/L and 500 mg/L - will be discussed in deal. With a concentration of 70 mg/L, the cells are in an oxidative metabolic state. The growth is limitated by the substrate concentration. At a concentration of 500 mg/L, the yeast is in an oxidative-reductive state, which means that ethanol is produced.

The focus is set on the glycolysis which represents a well known pathway. The figure shows the process of the metabolism of glucose to oxaloactetate and ethanol, respectivly. Oxaloacetate can be considered as the entrance to the citrate cycle. The ethanol concentration in the media was measured by GC. In the cultivation with a glucose set point of 70 mg/L, no ethanol was produced. In the second considered cultivation, with a set point of 500 mg/L, an ethanol concentration of 8 g/L was reached after 6 hours. After 10 hours, 11 g/L were obtained.

The resulting ratios of the genes involed in the glycolysis are shown in the figure on the right hand side.



The genes involved in the first part of the pathway are not regulated or slightly downregulated for the higher glucose concentration. Obviously, the amount of enzymes expressed at the lower substrate concentration is sufficient to process a higher magnitude of substrate as well.

The expression of the phosphoglycerate kinase is increased. Possibly this enzyme represents a bottel-neck in the metabolism of glucose and its production is increased in order to allow a higher glucose metabolism.

The regulation of the gene for the pyruvate decarboxylase is inconsistent. It is downregulated for the first data set and upregulated for the second. Further investigations are necessary to shed light on this aspect.

The same applies for the alcohol decarboxylase. Considering also the data, which are not shown, the pathway was downregulated mostly. As this enzyme is mainly responsible for the degradation of ethanol, a downregulation is to be expected. However, it also catalyses the production of ethanol. The mechanism of the regulation is complex and further investigations are required.

Outlook

- The analysis of the gene expression will be extended to all other metabolic pathways. Possibly, interesting co-regulations can be found.
- The data sets of further experiments will be compared to each other to see, if minor changes in the substrate concentration also result in a differend gene expression level.
- The influence of the biomass concentration will be considered.