Summary

The methods developed in this research enable the detection of the labelling distribution directly in the anabolic intra cellular metabolites. The selective LC-ESI-MS/MS measurement method fulfils the criteria for complex biological samples and is validated according to DIN 32 645. It allows the delicate acquisition of 25 intra cellular anabolic metabolites. The analytes are accurately separated without prior derivatisation within 37 min (basic amino acids 65 min) and are detected by Mass Spectrometry. Subsequently, the adaptation of the developed method for quantification of the ^{13}C-labelling distribution in cytoplasmic metabolites is demonstrated. The appropriate quantifying strategy is evaluated by a systematic approach investigating external calibration, standard addition, internal standard and isotope dilution.

Preparation of the sample is of utmost importance for “quantitative” metabolome analysis. If the metabolic state at the sampling point is not conserved during sample preparation, it is impossible to capture the momentary metabolic conditions. Studies comparing sampling procedures, show that a rapid inactivation of enzyme activity is essential to maintain the metabolic state of the sample. Various methods have been considered for the extraction of intra cellular metabolites in this research. The results for chloroform disruption are less error-prone, show less matrix effects and the extraction of the majority of metabolites is more effective than for other methods tested. Meanwhile chloroform treatment is established as a standard preparation procedure for the extraction of metabolites from bacteria as well as mammals.

Various L-threonine producers are compared with *E. coli* wild type MG 1655. As described in the literature, metabolite pools in *E. coli* wild type are subject to regulation, resulting in adjusted pool sizes. In contrast, threonine biosynthesis of production strains is genetically modified, causing accumulation of certain intermediates. Especially, homoserine was found to accumulate in threonine producing *E. coli* BKIIM. Based on these results homoserine kinase is over expressed in strain *E. coli* PthrB. The resulting optimised mutant reflects the change at genome level in the intra cellular pool sizes. In addition to an increased L-threonine concentration the genetic modification also has an unexpected effect on peripheral product metabolism.

In addition to regular fed-batch experiments, ^{13}C-labelling experiments were carried out on metabolically stationary and isotopically instationary conditions using labelled Glucose ($U^{13}C_6$-Glucose) as substrate. It has been possible, for the first time, to gain insight into cytoplasmic production processes, under industrial fed-batch conditions. Applying the developed techniques, allows a time resolved illustration of the labelling incorporation, thus labelling dynamics directly in the intra cellular pools can be detected under *in vivo* conditions. Despite the fact that both threonine producers investigated carry the same threonine operon, many

differences can be derived from the raw data already. With the exception of the amino acids alanine, aspartate and asparagine, one of the strains shows a much faster reaction on the ^{13}C-pulse. While one *E. coli* threonine producer shows a rapid accummulation of intra cellular aspartate, the other presents the fastest kinetics in the alanine pool. Besides threonine biosynthesis, the preference seems to lie on glutamate biosynthesis and especially the transaminase reactions in one of the strains. In summary, it can be derived that central metabolism (substrate uptake) and threonine biosynthesis are fundamentally different in both L-threonine producers. A comparision of the intra cellular states of both *E. coli* L-threonine producers investigated, show significant differences in product metabolism (e.g. L-threonine breakdown to glycine and conversion of the enzyme homoserine kinase). Therefore, it is confirmed that, the methods developed in this research reveal limiting reaction steps in the pathway. The applied techniques are not limited to biosynthesis of amino acids only, but can equally be applied to other metabolic pathways.

The results obtained from this research are consistent with the state of knowledge on bacterial metabolic networks. The developed and presented methods can be applied to map metabolic pathways relevant to industrial production processes. The labelling experiments show that the $^{13}C_6$-glucose pulse, fast sampling, sample preparation and metabolome analysis can be successfully applied to illustrate genetic modifications in the metabolome of *E. coli*. Thus, mathematically very complex instationary ^{13}C-metabolic flux analysis is not necessary to locate promising targets for Metabolic Engineering. The approaches and opportunities described are not limited to the analysis of bacterial metabolism, but can also be used for analysis of intermediates in mammalian and plant cells. The product under investigation is not only limited to amino acids. The techniques can equally be applied to investigate other production processes, e.g. secondary metabolites of precursors such as amino acids, organic acids, and carbohydrates or aromatic metabolites e.g. shikimate - phytochemicals e.g. terpenes (aroma or synthetic chemicals), alkaloids, steroids, glycosides, ployketides, prostaglandins, pyrimidines (pharmaceuticals) and vitamins (food). The natural variations of metabolic activities in comparable fermentations should be averaged by multiple cultivations. The application of these methods to products of higher molecular weight such as peptides or proteins is also conceivable and deserves future research.