Effect of pH variation on the acetate “switch” and biomass growth in an *Escherichia coli* aerobic fermentation: global gene expression profiles analysis

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The objective of the present work is to gain a better understanding of pH response in *E. coli* cultures and how this response might impact acetate accumulation and biomass growth, in order to improve our understanding on the physiology and molecular mechanisms of the bacterium. In this work, batch fermentations in a 5 dm³ bioreactor system were conducted with *E. coli* K12 strain to study the effect of different transmembrane pH (pH 7.0, 7.5 and 8.0) on global gene expression before and after the changeover to acetate utilization. Transcriptional responses were analyzed by microarray technology. The results show that the metabolite profiles were clearly different. The highest cell concentration of 2.43 g/kg was obtained at pH 7.5, even though a higher concentration of acetate than for pH7.0 was found, possibly because of associated changes in the expression of several key genes or to a the lower ΔpH. The accumulation of the metabolic by-product acetic acid was highest at pH 8 operation (3.16 g/kg). In addition, small alterations in the pH of the medium had significant influence in the acetic acid concentration profiles and in cell growth. The present work showed that improvements in cell growth are not totally dependent on reduction of acetate accumulation and that a change of only 0.5 pH units induced considerable metabolic change. The integration of the information obtained provides a better understanding of how *E. coli* cells are able to adapt their metabolism to pH alterations.

Constraint-based approach for *in silico* *Escherichia coli* combined regulatory/metabolic modelling

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The annotation of the *Escherichia coli* genome sequence allowed the development of *in silico* models based on the reconstruction of metabolic networks, able to predict phenotypic behaviour under different environmental and genetic conditions. The constrained-based approach has been used to analyze the capabilities of a reconstructed metabolic network, described by a solution space that contains all possible steady-state metabolic flux distributions in the network. Narrowing down the range of the solution space by including regulatory constraints can simplify the search for the best flux distribution. Studies have shown that metabolic enzymes are differentially expressed under different nutrient conditions and certain metabolites influence the activities of transcription factors. This explicitly establishes the links between specific metabolites and transcription factors. The regulatory network consists of a large number of regulatory elements of interacting genes and proteins organized in a hierarchical organization. The present study leads to the reconstruction of an existing genome-scale model of *E. coli*, incorporating transcriptional regulation described by Boolean logic equations, and subsequently analysed by flux balance analysis. The addition of regulatory constraints improved the predictive capacity of flux balance models, which contributes to accurately identify the phenotypic behaviours under different environmental conditions.