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DYNAMIC RESPONSE OF FERMENTATIVE METABOLISM OF *Escherichia coli* TO AEROBIC AND ANAEROBIC GLUCOSE PULSES

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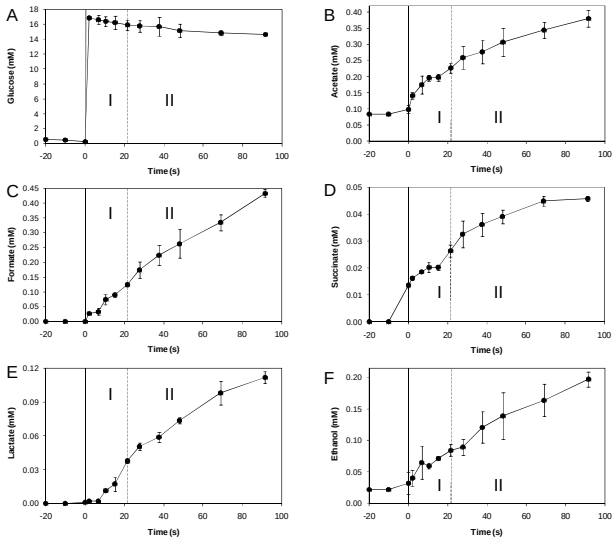
Introduction. Imperfect mixing in large-scale bioreactors leads to presence of spatial gradients. In the case of fed-batch cultures, substrate gradients are commonly encountered [1, 2]. Zones of high glucose concentrations at the substrate feeding point can be combined with anaerobiosis due to increased respiratory activity during exposure to glucose-concentrated regions. The accumulation of by-products due to glucose/oxygen gradients is a strongly undesired effect as it represents a waste of carbon skeletons, triggers transcriptional regulation processes and causes loss of productivity in industrial cultures [1]. In this study, the response of the fermentative metabolism of *E. coli* to glucose gradients was analyzed for the first time under fully aerobic and fully anaerobic conditions, in a time frame of 92 s.

Methodology. *E. coli* strain W3110 was cultivated in glucose-limited chemostat. A fraction of the exit flow was connected to a mini-plug flow reactor (PFR) [2] in which a continuous glucose pulse was applied. The PFR was divided into two hemispherical channels to allow mass transfer by diffusion through a permeable membrane. Fully aerobic or fully anaerobic conditions were controlled in the PFR by manipulating the composition of the gas injected. Eleven samples were taken along the PFR by means of a computerized sampling system. The samples were immediately cooled down to 0 ℃ and the filtered supernatants analyzed. Organic acids and glucose were quantified by enzymatic assays and ethanol by GC.

Results and Discussion. Formate and acetate were accumulated as fast as 2 s after exposure of *E. coli* to glucose gradients even during fully aerobic conditions. This can be attributed mainly to overflow metabolism. The respiration rate increased from 3.78 in the chemostat to 15.6 mmol g^{-1} h^{-1} in the PFR. As shown in fig. 1, glucose overflow combined with anaerobiosis caused the accumulation of formate, acetate, lactate, ethanol and succinate, which were also detected after 2 s of exposition of *E. coli* cells to the gradients. The rates of mixed-acid fermentation metabolites production and glucose uptake during exposures to glucose gradients were faster during the first 22 s of exposition, and then the intensity decreased for the last 70 s. A detailed characterization of the metabolic response will be shown.

Figure 1. Extracellular metabolite concentrations during glucose pulses combined with anaerobiosis to an E. coli culture



Conclusions. The experimental approach was useful for analyzing the metabolic response in times as short as those that cells can spend in concentrated regions of a large-scale bioreactor. The results obtained are useful for understanding the phenomena occurring in large-scale bioreactors and for the design of strains with an improved behaviour under large-scale conditions.

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