Dynamic flux balance analysis of high cell density fed-batch culture of E. coli BL21 (DE3) with mass spectrometry-based spent media analysis

Pramod Wangikar[1], Hardik Dodia[1], Vivek Mishra[2], Prajval Nakrani[2], Charandatta Muddana[2], Anant Kedia[2], Sneha Rana[1], and Deepti Sahasrabuddhe[1]

[1]Indian Institute of Technology Bombay Department of Chemical Engineering
[2]Clarity Bio Systems India Pvt Ltd

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Abstract

Dynamic flux balance analysis (FBA) allows estimation of intracellular reaction rates using organism-specific genome scale metabolic models (GSMM) and by assuming instantaneous pseudo steady states for processes that are inherently dynamic. This technique is well-suited for industrial bioprocesses employing complex media characterized by a hierarchy of substrate uptake and product secretion. However, knowledge of exchange rates of many components of the media would be required to obtain meaningful results. Here, we performed spent media analysis using mass spectrometry (MS) coupled with liquid (LCMS) and gas chromatography (GCMS) for a fed-batch, high cell density cultivation of *E. coli* BL21(DE3) expressing a recombinant protein. Time course measurements thus obtained for 246 metabolites were converted to instantaneous exchange rates. These were then used as constraints for dynamic FBA using a previously reported GSMM, thus providing insights into how the flux map evolves through the process. Changes in TCA cycle fluxes correlated with the increased demand for energy during recombinant protein production. The results show how amino acids act as hubs for the synthesis of other cellular metabolites. Our results provide a deeper understanding of an industrial bioprocess and will have implications in further optimizing the process.

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