

Scientific Paper:

Biotechnology Reports (2022) 33, e00694

High cell density cultivation of *E. coli* in shake flasks for the production of recombinant proteins

Snehal D. Ganjave¹, Hardik Dodia¹, Avinash Vellore Sunder¹, Swati Madhu¹, Pramod P. Wangikar^{1,2,3}

¹Department of Chemical Engineering, Indian Institute of Technology Bombay, Mumbai, India

²DBT-Pan IIT Center for Bioenergy, Indian Institute of Technology Bombay, Mumbai, India

³Wadhvani Research Center for Bioengineering, Indian Institute of Technology Bombay, Mumbai, India

Abstract:

Batch cultivation of recombinant bacteria in shake flasks typically results in low cell density due to nutrient depletion. Previous studies on high cell density cultivation in shake flasks have relied mainly on controlled release mechanisms. Here, we report a true fed-batch strategy to achieve high cell density of recombinant *E. coli* in shake flasks in 24 h by feeding a mixture of glycerol and yeast extract with a syringe pump. Feed composition and feed rate were obtained by cybernetic model-based, multi-objective optimization. Model parameters were estimated from time-course measurement of substrate, biomass, and dissolved oxygen levels. The optimized process yielded 20.7 g dry cell weight/L, in agreement with the model prediction. Volumetric protein productivity improved by 10–34-fold compared to batch cultivation with 2.8-fold further improvement when the fed-batch process was replicated in a 3 L bioreactor. The process has significance in the routine laboratory cultivations and in scaleup studies.

Keywords: High cell density cultivation, *E. coli*, cybernetic model, genetic algorithm, multi-objective optimization, recombinant protein production