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Sofia Nunes

Mario A. Torres-Acosta

Sarah Slack

Andrea Rayat

FROM FLASK TO LARGE SCALE HIGH CELL DENSITY PRODUCTION OF ω-TRANSAMINASE USING AUTO-INDUCTION MEDIA

Sofia Nunes, Department of Biochemical Engineering, University College London, United Kingdom
Sofia.nunes.18@ucl.ac.uk
Mario A. Torres-Acosta, Department of Biochemical Engineering, University College London, United Kingdom
Sarah Slack, Department of Biochemical Engineering, University College London, United Kingdom
Andrea Rayat, Department of Biochemical Engineering, University College London, United Kingdom

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For commercial manufacturing, the capacity to accelerate the development and scale-up of high cell density culture producing recombinant proteins while reducing the number of trials required is crucial. In addition, understanding how fermentation affects downstream processes (DSP) is critical to the development of a bioprocess pipeline. Bioprocess development can be accelerated by using ultra scale-down (USD) methodologies. USD intend to mimic large scale operations by using small quantities of material (~40 mL).
It is essential to develop production strategies, e.g., of an enzyme product, at different scales so that the relevant upstream conditions can be investigated for various DSP operations, particularly primary recovery steps by centrifugation or filtration.

The aim of this presentation is to show the development of a high cell density platform using auto-induction medium for the large-scale production of a recombinant enzyme, *transaminase* (*CV2025 ω-TAm*) in *E. coli* BL21, at high cell density while minimising the number of pilot scale trials. Additionally, this will demonstrate the applicability of USD investigations to predict primary recovery performance, which informed fermentation development.

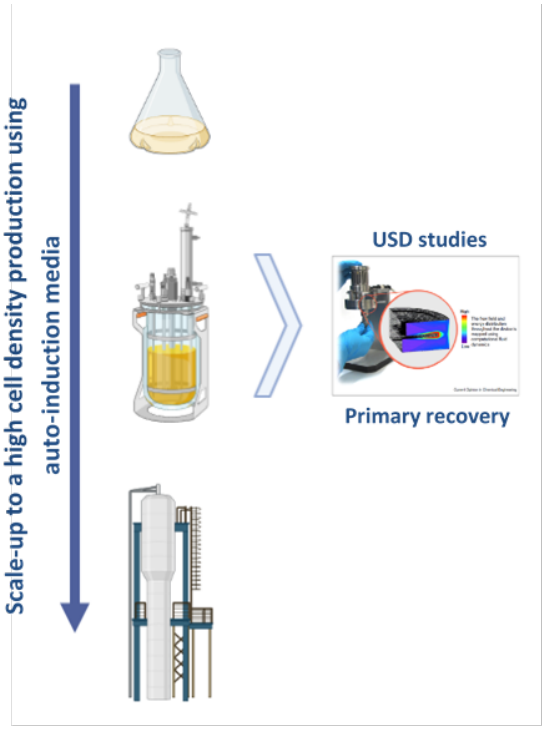

Figure 1 – Scheme of the experimental process: scale-up from flask to pilot scale with USD primary studies to inform fermentation development.

The study began with investigation of different media compositions including SM6GC and auto-induction medium at flask scale which showed higher cell density for the auto-induction medium. After scale-up to 1.2 L, further experiments were performed to assess repeatability, refine the final media composition, and determine the operation mode (batch vs fed-batch). An ultra scale-down DSP study, i.e., homogenization followed by flocculation and centrifugation, was used to assess the impact of using auto-inducible batch versus fed-batch on clarification. Interestingly, better cell growth was achieved in batch mode utilising auto-induction medium with 1:19 glucose to glycogen feed ratio. Furthermore, using the fed-batch mode resulted in weaker flocs and poorer centrifugation performance with supernatant OD increasing by 2-fold compared to feed from batch fermentation.

The third part of the study was to improve the induction strategy with the aim of increasing both cell density and enzyme product concentration. Thus, the inducer type, concentration and temperature were examined using flask cultures. The improved expression of *TAm* was found when the lactose concentration was adjusted. At the end, a scale-up to 20 L fermentation was completed achieving a final cell density was 20 g_{dcw} L^{-1} and *TAm* was expressed with an activity of 5.5 Units.mL^{-1} which are 4-fold higher than the flask production at the beginning of this study.