Engineering Conferences International
ECI Digital Archives

Microbial Engineering II Proceedings

4-3-2022

mDOE for development of optimized fed-batch cultivation for the production of PET degrading enzymes in E. coli

Lisa Fohler

Follow this and additional works at: https://dc.engconfintl.org/microbial_ii

mDOE FOR DEVELOPMENT OF OPTIMIZED FED-BATCH CULTIVATION FOR THE PRODUCTION OF PET DEGRADING ENZYMES IN E. COLI

Lisa Fohler, Austrian Center of Industrial Biotechnology (ACIB), University of Natural Resources and Life Sciences (BOKU), Austria
lisa.fohler@students.boku.ac.at

Key Words: enzymes, PET degradation, sustainability, mDOE, recycling

Plastic production increases exponentially, with an estimated 448 million tonnes manufactured in 2015, about 200 times more than in 1950. About 80 % of all produced plastic accumulates in landfills eventually, and natural degradation takes hundreds of years [1]. PET, the largest plastic waste fraction, is typically recycled by thermo-mechanical means, resulting in a loss of mechanical stability. A more attractive alternative for recycling of PET waste is the enzymatically catalysed depolymerization und subsequent production of virgin PET [2]. The key to this recycling approach is the availability of sufficient amounts of thermostable, PET-degrading enzymes, which implies extremely high yield and cost efficiency requirements on the enzyme production processes used. So far, the production of PETases proofed difficult, with literature describing expression conditions of 16 °C to prevent rapid cell lysis due to assumed cell membrane degradation by the enzyme [3]. Expression temperatures this low are not feasible for big-scale production, therefore a new and more viable process has to be established. To tackle this challenge we identified three key tasks: cloning and selection of an expression system with which high titres can be achieved, set-up and optimization of fed-batch production using mDOE and lastly, implementing the gathered knowledge of the previous task to produce the enzyme in continuous culture. For the first task, the selected enzyme, PHL7, was cloned in the enGenes pENGX plasmid, equipped with N-terminal signal sequence for periplasmic expression and expression enhance tag and C-terminal 6His-tag for purification and analytics. The performance of 5 different signal sequences (spa, ompA, lamB, pelB and dsbA) was evaluated and the best performing leader selected. In addition, the influence of the C-terminal 6His-tag on expression and enzyme activity was examined. The expression host, E. coli enGenes X-press V2, was selected for its growth decoupling properties, redirecting all resources of the cell to recombinant protein production upon induction [4]. For the second task, set-up and optimization of fed-batch production, the critical input parameters and their corner points for the mDOE were identified in µ-scale bioreactor cultivation. These parameters were then used for a machine-learning aided process optimization based on a 33 fractional factorial design to maximize product titre of active enzyme. The insight about the expression behaviour will be used to implement a stable continuous production process, since we see it as a promising way to produce sufficient amounts of PET degrading enzymes.

References:

[1] R. Geyer, J. R. Jambeck, and K. L. Law, “Production, use, and fate of all plastics ever made,” Sci. Adv., vol. 3, no. 7, pp. 19–24, 2017.

[2] V. Tournier et al., “An engineered PET depolymerase to break down and recycle plastic bottles,” Nature, vol. 580, no. 7802, pp. 216–219, 2020, doi: 10.1038/s41586-020-2149-4.

[3] C. Sonnendecker et al., “Low Carbon Footprint Recycling of Post-Consumer PET Plastic with a Metagenomic Polyester Hydrolase,” ChemSusChem, 2022, doi: 10.1002/cssc.202101062.

[4] P. Stargardt, L. Feuchtenhofer, M. Cserjan-Puschmann, G. Striedner, and J. Mairhofer, “Bacteriophage Inspired Growth-Decoupled Recombinant Protein Production in Escherichia coli,” ACS Synth. Biol., vol. 9, no. 6, pp. 1336–1348, 2020, doi: 10.1021/acssynbio.0c00028.