Strategies to improve plasmid stability in *Escherichia coli* cell factories

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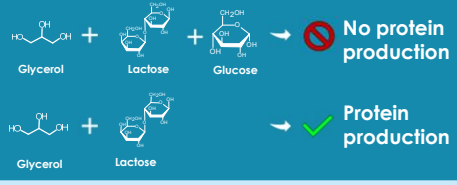
Introduction

Escherichia coli cell factories for recombinant protein production typically harbor a plasmid containing the gene of interest. Maintaining the plasmid requires selective pressure, typically achieved through the use of antibiotics. The choice of a specific antibiotic resistance plays a critical role in the overall stability and scalability of the production process, making it a key factor in determining the feasibility of the process.

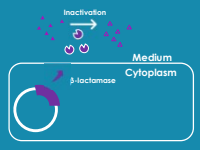
Process principles

The system is based on autoinduction for protein production and a β-lactam antibiotic as selective pressure for plasmid loss

Autoinduction [1]



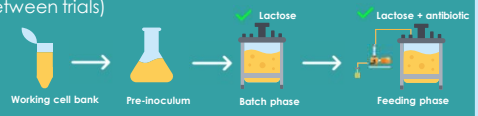
β - lactam selection [2]

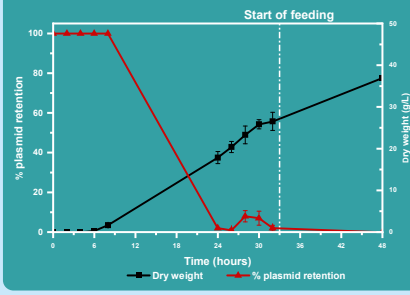


Existing pilot scale process

Our work begun on an existing industrial process, that had major issues of reproducibility between batches in laboratory scale (2L, 3.5X difference in productivity between worse and best trial), and even worse results in the pilot plant (150L, 9X difference between trials)

Process overview:



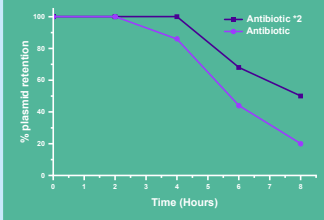


- Almost complete plasmid loss at the end of the batch phase
- Feeding antibiotic does not solve the issue, as the accumulated β-lactamase quickly degrades it

Shake flask

Process optimization

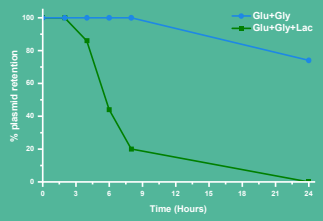
Increase antibiotic concentration



Minor improvement

Does not solve the issue by itself

Remove lactose from batch phase

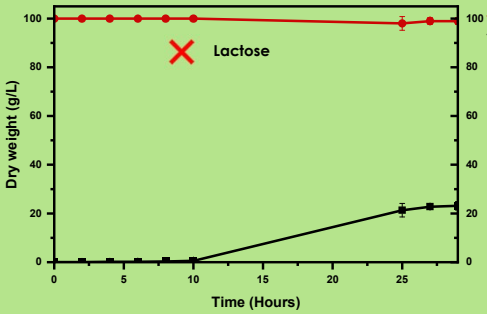


Induction contributes to plasmid loss

Removing lactose from the batch phase, thus limiting protein production only in the feeding phase, proved to be the best solution, and is the most desirable in the industrial setting as well, as it reduces usage of both antibiotic and inducer

2L bioreactor

Process transfer: Batch with no lactose



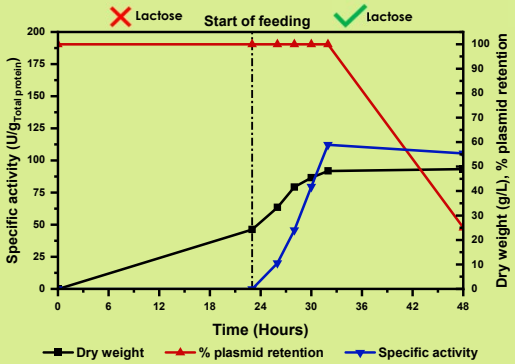
We achieve **100% plasmid retention** at the end of the batch phase

Cell growth is only 10% less than batch with lactose

During process transfer in a 2L bioreactor, we were able to achieve an even better plasmid retention rate than the flask experiments, this could be due to the lower growth temperature (25°C instead of 37°C) of the reduced stress of growing in a bioreactor

Process transfer: Fed-batch

2L bioreactor



Plasmid is maintained in the first hours of feeding

Minor loss of activity at 48h

A short feeding phase may prove beneficial to maximize protein production, as cells reach their maximum concentration after about 8h, but more studies are needed to identify the peak of protein production.

This process has been **scaled up to 150L** in a pilot plant and has high reproducibility

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Our website:

[1] Studier, F. W. (2005). Protein production by auto-induction in high-density shaking cultures. *Protein expression and purification*, *41*(1), 207-234.

[2] King, D. T., Sobhanifar, S., & Strynadka, N. C. (2017). The mechanisms of resistance to β-lactam antibiotics. In *Handbook of antimicrobial resistance* (pp. 177-201). Springer, New York, NY.