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CASE STUDY No. 002

Recombinant Protein Production in *E. coli*: From Shaker to Bioreactor

Researchers' affiliations

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Equipment

> New Brunswick Innova® 44 shaker
> BioFlo® bench scale bioreactor

Challenge

The enzyme L-asparaginase (L-ASNase) is relevant in the biopharmaceutical field as well as the food industry. To be economically viable, production processes need to deliver high protein yields. The study aimed at defining a detailed process for the production of L-ASNase in *E. coli* BL 21 (DE3) in shake flasks and bioreactors.

Strategy

To develop a highly productive bioprocess, the medium composition, inducer concentration, induction time, and culture feeding were optimized. Process optimization started in shake flasks before the process was transferred to a bioreactor.

Results

Process transfer from shake flask to bioreactor led to a 27-fold increased cell concentration. The L-ASNase activity per gram cells in the bioreactor indicates, that induction can be optimized further.

Conclusion

The study showcases an experimental workflow for the optimization of protein production. Shake flasks wer useful for optimizing a range of process parameters. Bioreactors have enabled a significant increase in productivity.

Shake flask culture

L-ASNase activity up to 99 U/g cells

1 Medium selection

> Evaluation: L-ASNase activity (U/g cells)
> Tested: Semi-defined, defined, complex media

Result

> Highest L-ASNase activity in defined medium
> This has most reproducible medium composition
> Defined medium was chosen for further experiments

2 Test of induction conditions

> Evaluation: L-ASNase activity (U/g cells)
> Tested: > Lactose and IPTG as inducer
> > Three inducer concentrations each
> > Four induction times

Result

> Best induction time was in the middle of the exponential phase (5 h)
> Lactose was chosen as inducer for further experiments
> Best inducer concentrations were 0.45 mmol (IPTG) and 10 g/L (lactose)

Cell dry weight: 1,8 g/L

Bioreactor culture

L-ASNase activity up to 42 U/g cells

3 Batch: Evaluation of kinetic parameters

> Evaluation: Process characteristics in batch process

Result

> *E. coli* growth curve, glucose consumption and acetate production were characterized

Cell dry weight: 11 g/L

4 Fed-batch: Process optimization

Fed-batch process with exponential feed rates
> Evaluation: L-ASNase activity (U/g cells), biomass
> Tested: Three lactose concentrations (10, 20, 30 g/L)

Result

> Induction with 30 g/L lactose led to highest L-ASNase activity
> Exponential feed rates supportet high biomass formation

Cell dry weight: 59 g/L

Read the full story

Barros et al. Development of Processes for Recombinant L-Asparaginase II Production by *Escherichia coli* BI21 (De3): From Shaker to Bioreactors. Pharmaceutics, 2020

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