

Increase protein yield with the ShakeReactor

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Introduction

Fed-batch is the standard operation mode for high performance protein production using microbial cultures. Implementing fed-batch usually requires bioreactors which are resource intensive to use and have low throughput.

Batch operated shake flasks are widely used instead of bioreactors for production of proteins in early stage R&D, due to their ease of use and the ability to parallelize 100's of shake flask cultures.

Protein titers in batch shake flasks is low, due to nutrient depletion and the lack of pH control, which makes it impossible to achieve high cell densities. Therefore multiple batch shake flasks are often used in parallel to produce enough of the needed protein.

The ShakeReactor

The patented atSpiro ShakeReactor can turn flasks into bioreactors in 15 minutes. The ShakeReactor adds:

- Online monitoring of dissolved oxygen, pH, temperature and humidity
- Fed-batch and pH control.
- Automation of induction and additions.

to shake flask cultivations in 500 mL and 1 L flasks.

Everything in contact with the cultivation is single use and supplied sterile and ready to use. The 3 pumps utilize single-use containers, each with a volume of 50 mL, and both the dissolved oxygen and pH sensors are single-use. The ShakeReactor is fully wireless and battery powered, batteries for cultivations up to 10 days and 18 days are available.

The ShakeReactor enables anyone to perform fed batch cultivation with pH control, without losing parallelization or having to use expensive and cumbersome bioreactors.

On the following page two cases are shown where the ShakeReactor was used to increase recombinant protein production in E. coli.



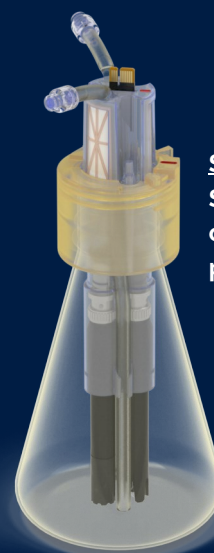
Liquid containers
Three with 50 mL
volume each



Battery
10 day battery life



ShakeReactor
Online monitoring
3 piston pumps



Sensor module
Single use dissolved
oxygen and pH
probe

Case 1: Peanut allergen produced in E. coli

Using the ShakeReactor a research group producing a his-tagged peanut allergen protein using E. Coli achieved

- Higher titers
- With less manual labor

Their protocol consisted of an 8 hour batch fermentation in shake flasks with manual IPTG induction after 2 hours.

Their protein yield was increased many fold by implementing a simple feeding procedure using the ShakeReactor, as can be seen from the SDS-page gel in figure 1.

What's more is with automatic induction of the ShakeReactor no manual interference was needed during the fermentation.

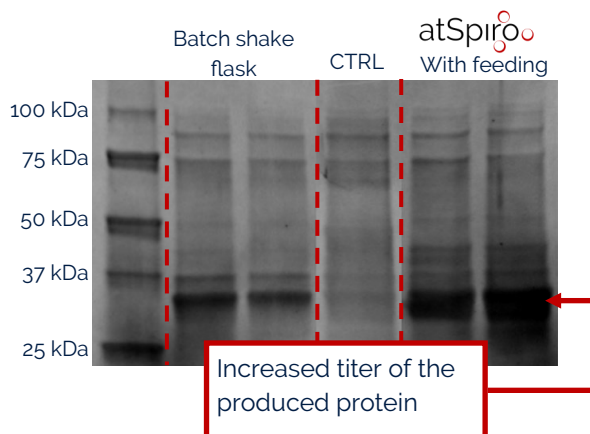


Figure 1 - SDS page showing the protein of interest (31.23 kDa) after purification from the batch shake flask protocol and fed-batch protocol using the ShakeReactor

Case 2: Antibody fragment produced in E. coli

An antibody fragment (mAb) was expressed using an E. Coli platform with IPTG induction. The mAb was produced in a 19 hour fermentation with induction at 12 and 15 hours.

Two setups were compared:

- Batch shake flask
- ShakeReactor with exponential feeding and two sided pH control.

Both fermentations were performed in 500 mL baffled flasks with 100 mL of LB broth with trace elements added.

Cell harvest was performed by centrifugation and cell lysis was performed by sonication.

Antibody titer was measured using a Gyrolab® xPanda. Total mAb produced in both setups can be seen in figure 2.

A 857 % increase in mAb production was observed, demonstrating that lack of nutrients and pH control is a major factor causing low protein yields in batch shake flasks.

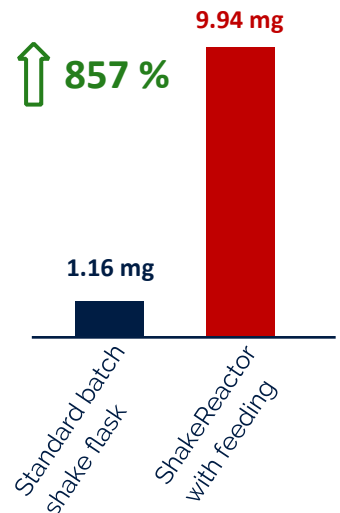


Figure 2 - Protein titer after cell harvest and lysis of cells in both standard batch shake flask and ShakeReactor with feeding and pH control.

Same incubator foot print, 8 times the production

- Increase protein production per flask with easy to implement fed-batch
- ShakeReactor has the same footprint as a 500 mL shake flask
- Automize workflows with pre-programmed induction, additions, and pH control.

