Fed-batch Mode in Shake Flasks by Controlled Release Technique

AACHENER VERFAHRENSTECHNIK

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I. Introduction and Motivation

Most industrial production processes are performed in fed-batch operational mode. In contrast, the screenings for microbial production strains are run in batch mode which results in completely different physiological conditions than relevant for production conditions. This may lead to wrong selections of strains. Silicone elastomer discs containing glucose crystals were developed to realize fed-batch fermentation in shake flasks (Fig. 1). No other device for feeding was required. Glucose was fed in this way to H. polymorpha cultures controlled by diffusion [1].

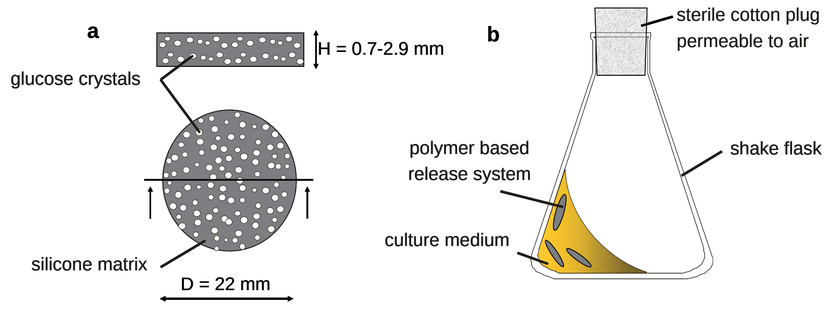

Fig. 1: Slow-release fed-batch technique.
a: Cut and top-view of a silicone elastomer disc with enclosed glucose crystals.
b: regular shake flask filled with a culture suspension and three silicone elastomer discs as polymer based release systems feeding glucose.

II. Materials and Methods

Two strains of H. polymorpha were investigated in shake flasks: the wild type strain (DSM 70277) and a recombinant strain RB11 pC10-FMD (PFMD-GFP) [2]. The oxygen transfer rate (OTR) and respiratory quotient (RQ) of the cultures were monitored online in shake flasks with a Respiration Activity Monitoring System (RAMOS) [3]. Offline measurements were performed like biomass by gravimetry, green fluorescent protein (GFP) by fluorimetric analysis. pH-drift was detected with a regular probe and the metabolite dynamics of glucose, ethanol and acetic acid were analyzed by HPLC. The slow-release discs can be obtained at AC Biotec GmbH [4].

III. Reduction of Overflow-Metabolism

Cultivation of H. polymorpha was performed under strictly aerobic conditions in YNB and Syn6-MES mineral medium. With the slow-release technique overflow metabolism could be reduced. Therefore, anaerobic byproduct formation could be decreased. This led to an increase of 85% in biomass yield (Fig. 2). To date, 23.4 g/l cell dry weight could be achieved in shake flask. Biomass yields of 0.38-0.47 were obtained which are in the same magnitude of laboratory scale fermentors equipped with a substrate feed pump.

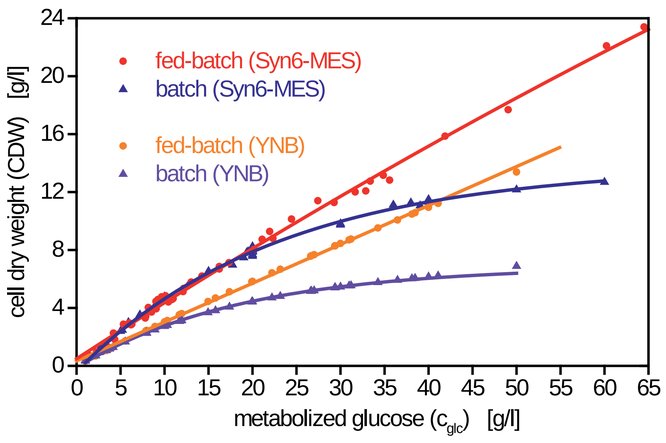

Fig. 2: Cell dry weights of H. polymorpha in batch mode and slow-release fed-batch mode using silicone elastomer discs.
Glucose as sole carbon source. Syn6-MES medium: pH_α = 6.4; YNB medium: pH_α = 4.3. Culture conditions: T = 37°C; d_0 = 50 mm; n = 350 rpm; V_L = 12.5 ml.

IV. Avoidance of Catabolite Repression

Well-functioning repression system of a microorganism usually leads to hardly detectable product formation in batch mode. This means that the optimum strain may not be found in commonly performed batch screening. GFP yield of *H. polymorpha* RB11 pC10-FMD (P_{FMD}-GFP) could be increased by a factor of 35 in Syn6-MES mineral medium (Fig. 3). In fed-batch mode 88 mg/l GFP was synthesized with 35.9 g/l fed glucose. In contrast, only 2.5 mg/l with 40 g/l metabolized glucose was revealed in batch mode.

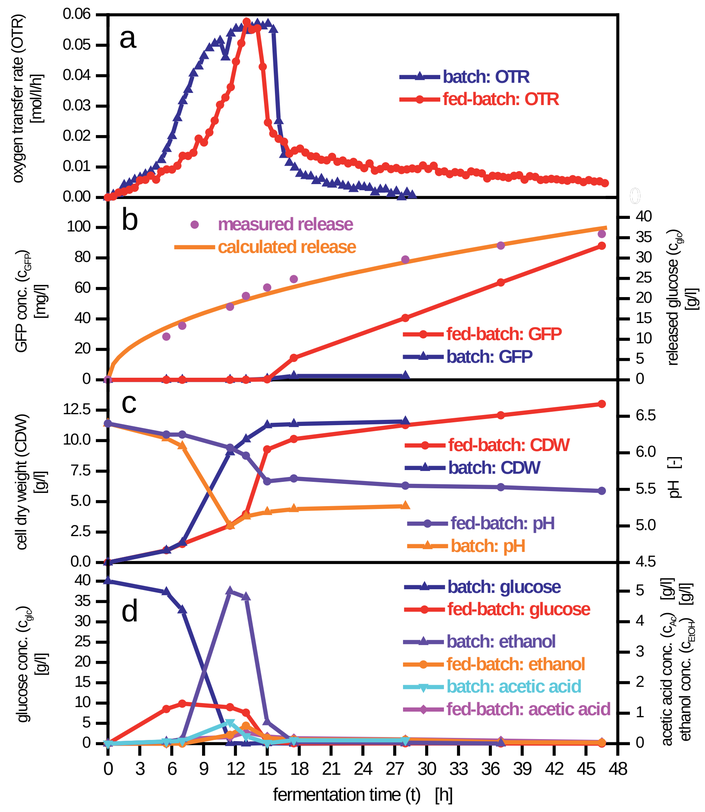

Fig. 3: Comparison of batch and slow-release fed-batch mode in shake flask cultures of *H. polymorpha* RB11 pC10-FMD (P_{FMD}-GFP) in Syn6-MES medium.
a: oxygen transfer rate (OTR). **b:** green fluorescent protein formation and released glucose. **c:** cell dry weight (CDW) and pH. **d:** dynamics of glucose, ethanol, acetic acid. Culture conditions: pH_α= 6.4; T = 37°C; d_0 = 50 mm; n = 350 rpm; V_L = 12.5 ml. Glucose as sole carbon source in batch with 40 g/l and fed-batch with released glucose from 5 silicone elastomer discs (D = 22 mm; H = 1.8 mm; 20% (w/w) glucose).

V. Conclusion

The application of easy to use diffusion based slow-release technique is a very promising option to improve bioprocesses. On the one hand, the application of release systems in primary screenings would most likely enable to find optimal functional strains for the expression of desired products in fed-batch processes. On the other hand, the technique could accelerate process development by enabling a very high number of simultaneous, repeatable small scale cultivations in fed-batch mode providing preliminary information for suitable feeding rates in scaled-up lab-scale fermentations.

References

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