Application of Kuhner Feeding Technology in CHO cell cultures

AppNote by Kuhner shaker

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In this application note the influence of glutamine releasing FeedBeads® on the growth and productivity of CHO cells is reviewed. A detailed protocol of application is presented and the performance of the FeedBeads® for different filling volumes are discussed. The results demonstrate that the glutamine releasing FeedBeads® can be used to optimize a CHO cultivation process and that the filling volume will impact the release kinetics and yield.

FeedBeads® for continuous nutrient release

FeedBeads® are polymer-based discs which enable continuous release of nutrient at a defined rate. Nutrients, like amino acids (e.g. glutamine), which are normally susceptible to degradation in solution, are protected by the polymer matrix. After adding to a cultivation media, the substrate is released in a controlled way to the media. The release rate itself is dependent on the substrate loading on the FeedBead®, the number of FeedBeads® applied in the cultivation experiment and the chemical environment of the cultivation. In this way, the FeedBeads® technology represents a powerful tool to improve bioprocess development and to decrease development time and costs.

High substrate concentrations in cell culture cultivation?

Mammalian cultivations that begin with a high nutrient concentration (e.g. 100 mM glucose) and receive subsequent bolus feeding can alter the metabolic profiles of batch and fed-batch processes, yielding an inefficient cell metabolism (i.e. the “Warburg” effect) (Zhou & Kantardjieff, 2014). These conditions have been linked to an increase in the concentration of unwanted metabolic byproducts (e.g. lactate or ammonia), which have been shown to limit cell growth and productivity in mammalian cell processes (Eibl et al., 2008; Ozturk and Hu, 2005). By maintaining a low concentration of substrate over time, these metabolic inefficiencies can be avoided and the output from a process improved (Jeude et al., 2006). Keil et al. (2019) recently demonstrated the positive impact of FeedBeads® on the growth and productivity of microalgae (Chlorella vulgaris) in a shake flask process.

In this application note, we review the impact of FeedBeads® on the growth and productivity of chinese hamster ovary (CHO) cells in shake flasks with different fill volumes and compare these results with a reference control.

Application of FeedBeads® in a CHO cell cultivation

Preculture

The preculture of CHO DP-12 cells was performed in Erlenmeyer shake flask cultures with 40 mL culture volume (total volume: 125 mL, baffled, 0.2 µm VentCap, Corning, USA). The medium of the preculture (TC-42, 0.1 mg L^{-1} LONG R3 IGF-1, Xell AG, Germany) was supplemented with 42 mmol L^{-1} glucose, 6 mmol L^{-1} glutamine and 0.2 µmol L-1 methotrexate (MTX, all Sigma-Aldrich, Germany). The incubator (LT-XC, Kuhner AG, Switzerland) was set to: 37°C, 85% humidity, and 5% CO2. Shaking was set to 200 rpm with an orbital diameter of 25 mm. To ensure cell growth after inoculation, the preculture medium was prewarmed for at least one hour in the incubator at 37°C.

Main cultivation

Erlenmeyer shake flasks (baffled, 0.2 µm VentCap, Corning, USA) with 40, 80, and 150 mL culture volume (32%, 32%, 30% of total volume, respectively) were used. The medium (TC-42, 0.1 mg L^{-1} LONG R3 IGF-1, 42 mmol L^{-1} glucose,0.2 µmol L^{-1} MTX) was supplemented with an initial glutamine concentration of 0.5 mmol L^{-1} and prewarmed in the incubator for at least one hour.

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One glutamine FeedBead® (equals to 0.2 mmol) was added to each flask. The culture medium was inoculated with the preculture to achieve an initial viable cell density of 0.3*10 ^{6} cells mL^{-1}. As reference, two 40 mL shake flask cultures (TC-42, 0.1 mg L^{-1} LONG R3 IGF-1, 42 mmol L^{-1} glucose, 6 mmol L^{-1}, 0.2 µmol L^{-1} MTX) were performed without FeedBead® addition.

Note

The FeedBeads® are delivered sterile and can be simply added to the medium with a tweezer under sterile conditions. The glutamine FeedBead® was added 30 min before inoculation to ensure initial cell growth. To reduce the duration of the lag-phase, the medium should be inoculated with exponentially growing cells.

Analytics

Sampling was performed every 24 h and cell density (Z2 cell counter, Beckman Coulter, USA) as well as cell viability (DAPI method, CytoFLEX, Beckman Coulter, USA) were measured.

Furthermore, glucose, glutamine, lactate, ammonia and antibody (Ab) concentrations were quantified. See (Möller et al., 2019) for details about the cultivation and analytics.

Results & conclusion

The release kinetics of the FeedBeads® and their impact on the growth and productivity of the CHO cell cultivation over time is presented in Figure 1. The concentrations of the viable cell density (Fig. 1 A), antibody (Fig. 1 B), glutamine (Fig. 1 C), and ammonia (Fig. 1 D) are shown for batch cultivation with continuous glutamine release and working volumes of: 40, 80, and 150 mL (32%, 32%, 30% of total volume). Data from a reference batch cultivations are also shown in parallel and error bars represent the standard deviation from three independent experiments . For more detail of the reference batch cultivation, see Möller et al., 2019. The growth profile shown in Fig. 1 A indicates that the amount of released glutamine from the FeedBead® is sufficient to run shake flask cultivations of CHO cells and that the swirling of the FeedBead® tablets in solution did not negatively impact cell growth.

Preculture

Working volume:	40 mL 0.1 mg L^{-1} 42 mmol L^{-1} 6 mmol L^{-1} 0.2 µmol L^{-1}	TC-42 LONG R3 IGF-1 glucose glutamine MTX
Shaking conditions:	200 rpm, 25 mm shaking diameter, 37°C, 85% relative humidity	
Duration:	Between 2 and 3 days	
Final viable cell density:	Approx. 2*10^{6} cells mL^{-1}	

Mainculture

Working volume:	40, 80, 150 mL 0.1 mg L^{-1} 42 mmol L^{-1} 6 mmol L^{-1} 0.2 µmol L^{-1}	TC-42 LONG R3 IGF-1 glucose glutamine MTX
Shaking conditions:	200 rpm, 25 mm shaking diameter, 37°C, 85% relative humidity	
Duration:	8 days	
Final viable cell density:	Approx. 12*10^{6} cells mL^{-1}	

The maximum viable cell densities obtained on the sixth day of the cultivation (t=144h) for the three filling volumes tested were similar to the control; however, cell growth was prolonged in the FeedBead® cultivations for an additional full day (t=168 h).

The productivity and final antibody concentration (Fig. 1 B) were in the same range for all cultivations . While the concentration of glutamine (7.6 mmol L^{-1}) in the reference cultivation (Fig. 1 C) begins to decrease immediately after inoculation, the concentration of glutamine in the FeedBeads® cultures increases consistently for the first 24-48 h.

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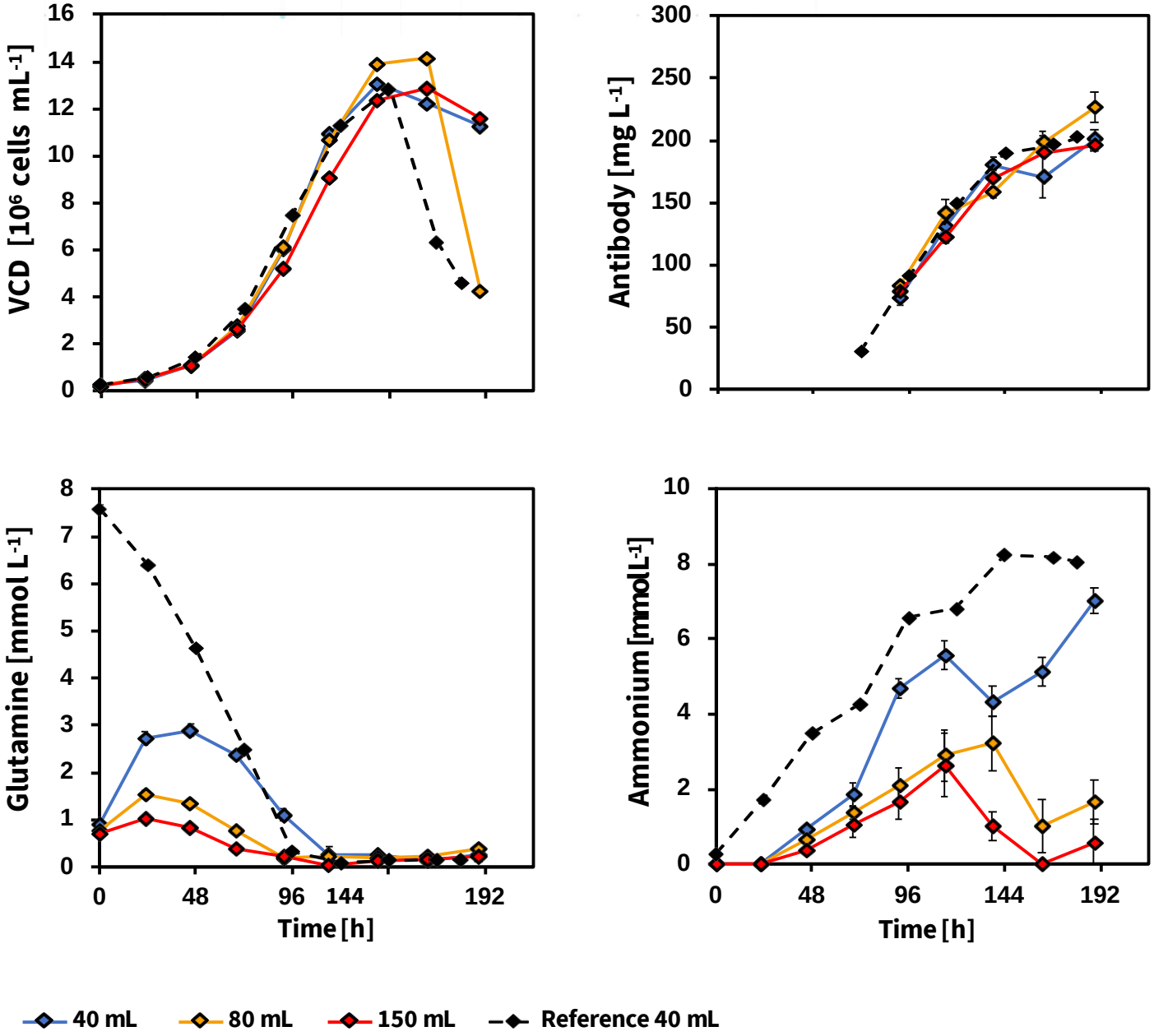

Figure 1.

Viable cell density (A) and concentrations of: antibody (B), glutamine (C), and ammonia (D) are shown for 40 mL (blue), 80 mL (orange), and 150 mL (red) FeedBead® CHO cultivations in parallel with the control cultivation (black). Error bars represent the standard deviation (N=3).

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Once the glutamine consumption of the CHO cells exceeds the glutamine release from the FeedBeads® at ~24 h for the 80 and 150 mL conditions and ~48 h for the 40 mL condition, the concentration of glutamine in solution decreases until glutamine-limited conditions are reached at ~120 h for all conditions. For the FeedBead® cultivations, the 40 mL fill-volume yielded the highest concentration of glutamine at 3 mmol L^{-1}, with while the 80- and 150-mL fill-volume conditions yielded 1.6 mmol L^{-1} and 1 mmol L^{-1} concentrations of glutamine, respectively.

The concentrations of ammonia in solution (Fig. 1 D) also varied with respect to fill volume. While the control cultivation yielded 8.2 mmol L-1 ammonia , the FeedBead® cultivations yielded: 7.0 mmol L^{-1}, 1.7 mmol L^{-1}, and 0.6 mmol L^{-1} for fill volumes of 40, 80, and 150 mL, respectively. These results indicate that the concentration of ammonia produced by the FeedBead® cultivations was lower than the control. Similar reports of low ammonium concentrations following a sustained feed of glutamine at low concentrations have been shown by others (e.g. Ozturk and Hu, 2005), which highlights one of the key advantages of the FeedBead® technology. The courses of the glucose and lactate concentration were not influenced.

Conclusion regarding glutamine FeedBeads®:

1.Glutamine-releasing FeedBeads® can be used to improve CHO-based processes.

2.The slow addition of glutamine via FeedBead® technology can yield lower concentrations of ammonium over an 8-day process.

3.Glutamine released from FeedBeads® is sufficient to maintain cell growth and antibody formation in 40, 80, and 150 mL working volumes for processes lasting up to 8 days.

4.FeedBeads® prolong cell growth for an additional 24-hrs compared to the control. Sustained feeding of glutamine at low concentrations does not inhibit antibody production in CHO.

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