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Modeling perfusion at small scale using ambr15TM

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Abstract

Reaching cell densities higher than 80 million with the minimum possible perfusion rates is a goal for an increasing proportion of processes developed by biopharmaceutical companies. With the goal of fulfilling the industry needs for better commercial and customized perfusion media, SAFC evaluated different small-scale perfusion models to achieve an efficient work flow that can accommodate perfusion systems. SAFC successfully uses an optimized work flow for the development of media and feeds for fed-batch cell culture that integrates high-throughput screening, statistical tools and bench-top bioreactor scale studies. In this model, 96-deep well plates are used for the initial high throughput screening, followed by further development in spin tubes or shake flasks. At this time, there is no commercially available cell separation device that can be used for scales of 30mL or lower. The application of the 96-deep well plate or spin tubes model for perfusion showed to have severe limitations, specifically when trying to optimize processes to extremely low cell specific perfusion rates (CSPR). In order to develop media that can sustain the desired high densities and productivity at the desired low CSPRs, we needed a representative model that provided enough throughput to apply our statistical analysis. With this goal, we evaluated an alternative small scale model using the automation and process control offered by the ambr15TM. In this work, we show how ambr15TM fits in the work flow for perfusion media development and its comparability to a chemostat bioreactor.

Material and Methods

In this study, batch model and chemostat at small scale were performed and compared to a chemostat bioreactor. Batch mode was performed in spin tubes (TPP, Switzerland) and small scale chemostat was performed in ambr15TM (Sartorius, Germany). The working volumes were 30 and 14mL, respectively. The small scale cultures were compared to a 5L bioreactor scale chemostat (BioStat B, Sartorius, Germany). Dilution rates of 0.22, 0.26, 0.3 were evaluated in small scale, while 0.28 followed by 0.2 were evaluated at the bioreactor scale. Media exchanges were started on day 1 for small scale models and on day 5 for bioreactor scale.

An in-house generated CHOZN® GS cell line producing IgG1 was used in this study. Cells were scaled up in EXCELL® CD Fusion. The carry over in all experiments was between 5-10%. Four different chemically defined SAFC's media known to have different growth and production profiles were used during the course of this study.

Cell viability and density were measured using Vi-cell XR (Beckman Coulter, California), and titers were measured using ForteBio Octet (Pall Co., California).

Equations used for this study were as follow:

$$CSPR = \frac{V}{\int_0^t V(t) \times X(t)} \qquad \mu = \frac{\ln(X/X_0)}{(t_1 - t_0)} \quad \text{where} \quad X = X_1 + (X_1 + X_2) \times 0.5 \times D \times (t_1 - t_0)$$

$$D = \frac{F \times N}{V} \qquad q_p = P \times CSPR \quad \text{for continuous processes or,}$$

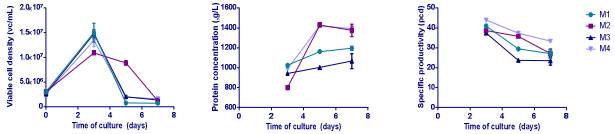
$$q_p = \frac{P}{IVCD} \quad \text{for batch processes.}$$

CSPR: cell specific perfusion rate (nL/cell*d)
D: dilution rate (vvd)
F: volume of medium exchanged (mL)
IVCD: integral viable cell density (cell*d/mL)
μ: growth rate (d^{-1})
N: number of media exchanges per day
P: IgG concentration (mg/L)
q_p: specific productivity (pcd)
X: cell density (vc/mL)
V: working volume (mL)

Small scale models

Figure 1. High seed density batch culture

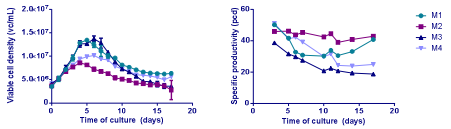
- Peak cell densities between 10-15x10⁶vc/mL were observed. Culture longevity was inversely correlated to the peak cell density (Fig. 1A)
- Cells stopped producing at the peak density with the exception of M4 (Fig. 1B).
- Specific growth rates ranked as M3 > M4> M1 > M2 (data not shown)
- Specific productivity ranked as M4 > M2 ≥ M1 > M3 (Fig. 1C)



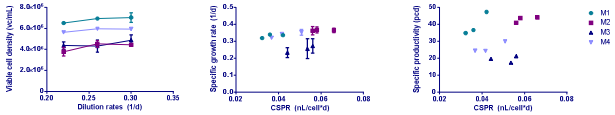
Cell growth (1A), protein production (1B) and specific productivity (1C)

Figure 2. Bolus-chemostat using ambr15™

- Peak cell densities between 8-14x10⁶vc/mL were observed (Fig. 2A). Comparable peak cell density was observed for a given medium in all dilution rates. However, the peak was slightly higher and it was reached one day sooner at lower dilution rates (data not shown). The steady state cell density was comparable for a given medium at the three different dilutions performed (data not shown).
- Peak cell density ranked as M1 ≥ M3 > M4 > M2 (Fig. 2C)
- At steady state growth rates were balanced with the dilution rates, however differences in CSPR were observed. At comparable CSPRs specific growth rate ranked as M1> M4> M3 ≥ M2 (Fig. 2D)
- Specific productivity decreased after the initial growth phase in all media but M2 (Fig. 2B). Once steady state was reached, specific productivity was constant for media M2 and M4, increased for media M1 and decreased for medium M3. Specific productivity at steady state and comparable CSPRs ranked as M1 > M4 ≥ M2 > M3 (Fig. 2E)



Cell growth (2A) and specific productivity (2B) at D 0.22vvd



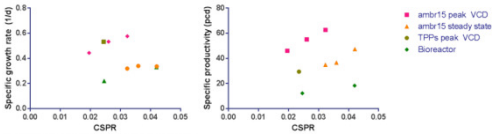
VCD at steady state (2C), specific growth rate (2D) and specific productivity (2E) at D 0.22, 0.26 and 0.3vvd

3. Comparison of TPPs batch, ambr and bioreactor chemostat

- The growth profile in the bioreactor chemostat was comparable to ambr15TM chemostat (Fig. 2A). Initially cells grew to a higher peak cell density (9x10⁶vc/mL) and as growth rate decreased it reached a steady state of 6.7x10⁶vc/mL at 0.3vvd (Table 1).
- The steady state cell density was comparable between the bioreactor (6.7x10⁶vc/mL) and the ambr15™ (7.0x10⁶vc/mL) at a dilution rate of 0.28 or 0.30vvd, respectively . However, the cell density was higher in the bioreactors (8.1x10⁶vc/mL) when compared to ambr15™ (6.5x10⁶vc/mL) at a dilution rate of 0.2 or 0.22vvd, respectively.
- At the higher dilution rates of 0.28 or 0.3vvd, specific growth rates and CSPRs were comparable between the bioreactor and the ambr15™. However, at the lower dilution rates of 0.2 or 0.22vvd while growth rate were comparable, significant differences in CSPRs were observed (Fig. 3A).
- When comparing chemostats with batch TPPs at equivalent CSPR (Konstantinov et al., 2006), significant differences in growth rates were observed (Fig. 3A). However, in the chemostat growth rate is initially higher (0.7d^{-1}) and as CSPR decreases, growth rate gradually decreases until it stabilizes to the dilution rate. During the growth phase of the chemostat, specific growth rates were comparable between ambr15™ and TPPs at equivalent CSPR (Fig. 3A).
- Specific productivity was comparable between ambr15™ at steady state and TPPs at peak cell density, but not during ambr15™ growth phase (Fig. 3B). Specific productivity was significantly lower in the bioreactor than in ambr15™.

Table 1. Bioreactor chemostat

Dilution rate	0.28vvd	0.2vvd
Avg CSPR (nL/cell*d)	0.042	0.024
Avg VCD (10⁶vc/mL)	6.72	8.13
Avg μ (1/d)	0.33	0.22
Avrg q_p (pcd)	18.31	12.22



Specific growth (3A) productivity (3B)

Conclusions and future work

- The use of batch TPPs or well plates allows for high throughput screening that can be used for the selection of media to be used for perfusion. However, this work shows that observations made on TPPs do not always transfer to a chemostat system. It is not known at this time if this observation is due to differences between a dynamic and a continuous process or due to process parameters such as pH and DO and this is currently being investigated.
- Differences when ranking different media based on their specific growth and productivity between TPPs and ambr15™ were found. The ranking in specific productivity was more similar between TPPs and ambr15™ if the comparison is made on the growth phase of the chemostat. While the absolute values are not comparable (Fig. 3B) the ranking and behavior on the stability of productivity was comparable (Fig. 1C and 2B). This fact underlines the need for a model that can represent a steady state as opposed to a dynamic model.
- The chemostat run in ambr15™ is a semi-chemostat. Because of the discontinuity of the model when compared to a fully continuous process, limitations on growth and potentially productivity can become visible at higher dilution rates than in bioreactors (Figure 3A and B).
- Work published by Heltmann (2015) and Henry et. al (2008) showed comparability in specific rates between chemostat and perfusion at similar CSPR. In this work we showed that ambr15™ can be used as a tool to model a chemostat and evaluate different media that ultimately is going to be used in perfusion mode. We expect that this model is going to allow us to evaluate media with very different characteristics in order to perform media component optimization at a relatively high throughput.

Bibliography

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