APPLICATION NOTE No. 456

A Beginner's Guide to CHO Culture: Bioprocess Modes – Batch, Fed-Batch, and Perfusion

Amanda Suttle and Ma Sha
Eppendorf Inc., Enfield, CT, USA
Contact: bioprocess-experts@eppendorf.com

Abstract

In this application note, we explain the differences between batch, fed-batch, and perfusion cell culture bioprocesses using Chinese Hamster Ovary (CHO) cells at bench scale. We explain the importance of tracking specific parameters to achieve inoculation of bioreactors at high viability and how we analyze our samples and adjust the bioreactor conditions throughout the runs. We discuss what our data obtained from sampling means in terms of growth rate, production yields and bioreactor feeding or perfusion rates. The principles in this beginner's guide to CHO culture may be applicable to other suspension cell culture processes at smaller or larger scales.

Introduction

Recombinant protein manufacturing in CHO cells represents more than 70 % of the entire biopharmaceutical industry [1]. In fact, human monoclonal antibodies (hmAbs) produced in CHO cells have played a major role in the therapeutic markets for decades. Certainly, one of the first human-mouse chimeric mAbs obtaining FDA approval was Rituximab, Roche's Non-Hodgkin's lymphoma, chronic lymphocytic leukemia, and rheumatoid arthritis treatment. Since that approval in 1997, there have been scores of chimeric, humanized, and human mAbs that have gained approval and entered the clinic. And there promises to be more, including recent FDA approval of several mAbs for the treatment of COVID-19.

In the development of any pharmaceutical production process, including those involving hmAbs produced in CHO cells, decisions regarding the best process parameters and methods are made based on various factors, such as media costs, process runtime, cell growth and viability as well as product yield and product quality. During process development, bioprocess scientists take these factors into consideration when choosing between a batch, fed-batch or perfusion bioprocess mode [2-8]. The process mode influences the availability of nutrients and the concentration of byproducts and therefore cell growth, viability, and product formation.

Cell growth curves

A culture's growth curve can typically be divided into several distinct phases (Figure 1). During the initial lag phase, growth is slow while cells are adapting to their new environment inside the bioreactor. During the exponential growth (or log) phase, the cell division continues at a constant rate. Once nutrients are depleted and by-products accumulate, cell growth starts to slow down and the culture enters the stationary growth phase. Typically, harvesting of the culture and product occurs at this time. The culture

then enters the death phase which is usually characterized by a steep decline in viable cell density. The duration of the exponential and stationary phases differ in batch, fed-batch, and perfusion bioprocesses (Figure 1), as explained in more detail below.

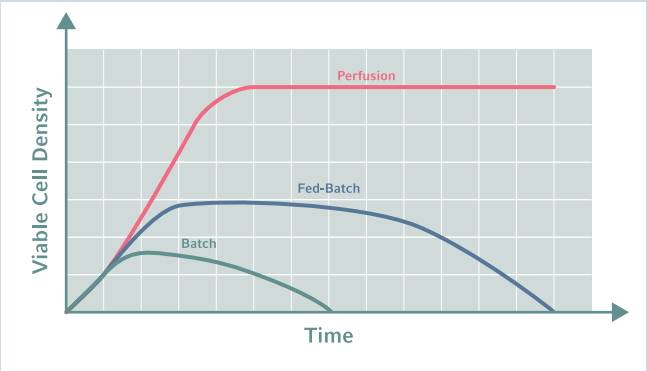

Fig. 1: Schematic representation of cell growth curves in batch, fed-batch, and perfusion bioprocesses.

Batch bioprocess

In a cell culture batch run, mammalian cells are inoculated into a fixed working volume of media in a bioreactor (Figure 2). Batch cultures are typically run at the maximum working volume of the vessel. Throughout the duration of the run, nutrients are gradually depleted and toxic byproducts accumulate inside the vessel. At some point the cells will stop growing and the proportion of viable cells will decline, because nutrients are consumed and toxic metabolites get concentrated. Major advantages of a batch culture are the ease of operation and process setup, and a lowered risk of contamination comparatively to other processes.

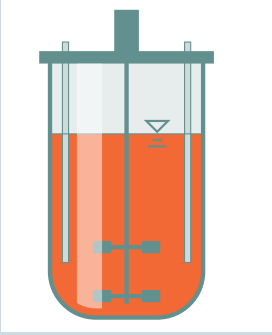

Fig. 2: Batch process. In a batch process the culture grows in the initially supplied batch of medium.

However, cell densities and production yields that can be achieved in batch cultures are much lower than those in fed–batch and perfusion processes. Batch cultures are a great starting point for beginners in the field looking to optimize culture conditions in the early stages of experimental development and design. A batch process can run for a duration of around 7 days.

Fed-batch bioprocess

A fed-batch culture is a modified version of a batch culture. Here the culture is fed to keep the concentration of nutrients constant (Figure 3). High substrate concentrations can be achieved, resulting in higher cell densities and product titers, and prolonged cell viability, but toxic metabolites still accumulate over time.

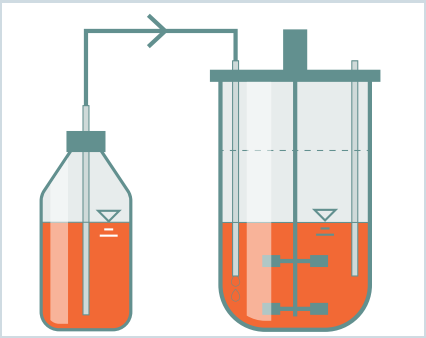

Fig. 3: In a fed-batch process the culture is fed to keep the concentration of nutrients constant.

The bioreactor is inoculated at a lower working volume, which is often the minimum working volume of the vessel and is grown under batch conditions for a short time until certain criteria have been met to trigger the start of feeding. Typically, feeding is started when initial glucose has been depleted or there is a significant buildup of harmful byproducts, such as ammonia (> 3 mmol/L), inside the bioreactor. Once either or both of those criteria have been met, nutrients are added to the bioreactor via a prepared feed media bottle in specifically calculated increments every day until the feed bottle has been emptied and the maximum working volume has been met.

Media addition calculations are based on end or maximum working volume. Typically, in our lab, a fed-batch bioreactor is fed daily at 3 to 5 % of the total vessel working volume desired at the end of the run. It is important to calculate this parameter properly so that the volume of medium added to the culture is not too low, which can lead to a buildup of toxic byproducts in the vessel. Alternatively, adding too much medium to the bioreactor can cause the culture to be too diluted and affect overall cell growth and production. In addition to feeding fresh medium to your vessel, a glucose bolus feed might also be necessary in the exponential growth phase if your feed medium addition is not supplementing enough glucose to keep up with the demand.

After the bioreactor has reached the maximum working volume and the feed bottle has been emptied, the culture will

continue until viability and cell growth decline, signifying the end of the run. With the addition of fresh nutrients and dilution of toxic byproducts, fed-batch cultures can often double the cell growth and production yields compared to a batch run and can run for 2-3 weeks.

Perfusion bioprocess

In a perfusion process, a continuous medium exchange occurs. Like in fed-batch cultures, fresh medium is continuously added to the bioreactor to replace depleted nutrients throughout the run. However, in perfusion processes, used medium is also collected with the harvested product at the same rate that fresh medium is being added to the bioreactor (Figure 4). The addition of fresh medium and removal of spent medium at the same rate will allow the culture volume in the bioreactor to stay constant; therefore, the maximum working volume of the vessel does not limit the amount of fresh medium that can be added to the culture for the duration of the run. The rate of this media exchange process is referred to as vessel volume per day or VVD.

For suspension cultures like CHO, a cell retention device is needed to retain the cells inside the bioreactor when removing used media and product in perfusion processes. Hollow fiber filters using alternating tangential flow (ATF) are successfully used for the retention of suspension cells, and achieved great success in supporting high density cell culture when combined with existing stirred-tank bioreactors. The ATF perfusion device allows media and small molecules to pass through the hollow fiber filter column and to be collected in the harvest (waste) bottle while retaining cells inside the bioreactor. Simultaneously, fresh media is circulated through the growing culture, supplying critical nutrients to achieve high cell densities [9].

In a tangential flow filtration-based perfusion process, the liquid flows passes the pores of hollow fiber filters tangentially, rather than being forced through them orthogonally, thus reducing the likelihood of clogging. ATF devices use the same principle of tangential flow but reverses the direction of flow regularly to further minimize fouling. ATF perfusion is suitable for perfusion processes with suspension cells [9].

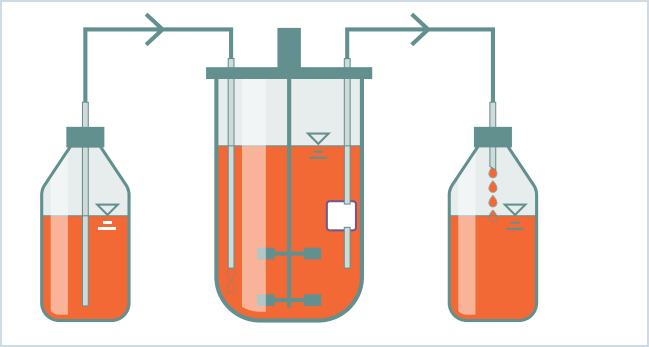

Fig. 4: In a perfusion process the culture medium is continuously exchanged while the cells are retained in the bioreactor.

The perfusion media exchange process is started once either glucose has been consumed, typically when concentration drops below 3 g/L in our lab, or there is a significant buildup of toxic byproducts, such as ammonia (> 3 mmol/L), inside the bioreactor.

A perfusion process can last three or more weeks, depending on the maximum VVD chosen to cap the medium consumption rate.

In this application note we describe how we set up a CHO cell culture bioprocess in batch, fed-batch, and perfusion mode, respectively. We compare the metabolic profiles and performance indicators like cell growth, product formation, and process costs.

Material and Methods

Cell line and medium composition

All experiments used a suspension CHO cell line from TPG Biologics, Inc., expressing a biosimilar hmAb. The cells were cultivated in Dynamis AGT Medium for the batch run. For the fed-batch and perfusion runs, cells were cultivated using CD-FortiCHO Medium (Thermo Fisher Scientific®). Both media were supplemented with 8 mM L-glutamine and 1 % Gibco® Anti-Clumping Agent and 1 % Antibiotic-Antimycotic (Thermo Fisher Scientific®). The feed media for both the fed-batch and perfusion runs were prepared with CD EfficientFeed C AGT. For the fed-batch, the media was supplemented the same as the initial culturing media.

For the perfusion run, the culture media was prepared as described above with one modification: the glutamine concentration in the perfusion media was changed to 2 mM. The glutamine feeding concentration was decreased to reduce ammonia production during the run.

Inoculum preparation

Optimizing culture parameters at the shake flask stage is key to preparing ideal inoculum with reproducible results. Flask culture with high viability (90 % or higher) is a sign of healthy cells. Cells exhibiting high viability before inoculation are less likely to result in a prolonged lag in culture when growing them in the bioreactor. Keeping track of this information can help quickly detect changes in viability or cell growth from flask to flask (Figure 5).

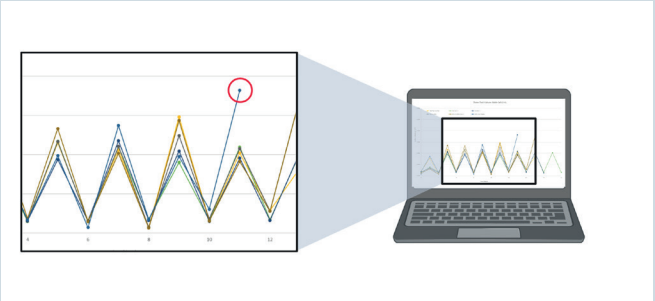

Fig. 5: Tracking cell growth and viability helps quickly detect and eliminate outliers amongst flask cultures during inoculum preparation.

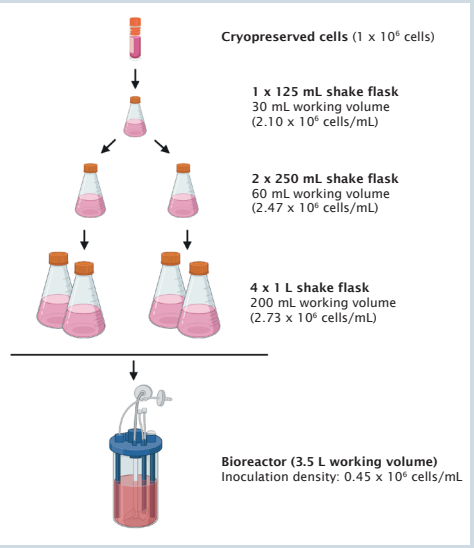

Fig. 6: Cell expansion for the inoculation of ATF perfusion run

Created with biorender.com

The bioreactor inoculum for each experiment was prepared by cultivating the cells in single-use baffled polycarbonate shake flasks (Corning®) in a New Brunswick S41i CO_2 Incubator Shaker (Eppendorf) set at 125 rpm and 8 % CO_2 with passive humidification. Cells from a cryopreserved stock vial were inoculated at a density of 0.3 x 10^6 cells/mL in a 125 mL flask with a 20 % fill volume. After one week of passaging every other day, the culture volume was scaled-up by increasing the flask size from 125 mL to 250 mL, and finally 1 L, while keeping the cell density, percentage fill, and all other parameters constant. Using this method, each bioreactor was inoculated with cells that were at approximately the same passage and duration of culture post-thaw. Each experiment had an inoculation density ranging from 0.3 x 10^6 cells/mL to 0.5 x 10^6 cells/mL. An example of the flask culture expansion process is shown for the ATF perfusion run in Figure 6.

Bioreactor control system

For this study, a BioFlo® 320 bioreactor control system was used (Figure 7). The BioFlo 320 was set up based on the operating manual [10].

Eppendorf BioBLU® Single-Use Bioreactors were used for all processes in this study. The cell culture BioBLU bioreactors are equipped with a pitched-blade impeller

Fig. 7: BioFlo 320 bioreactor control system

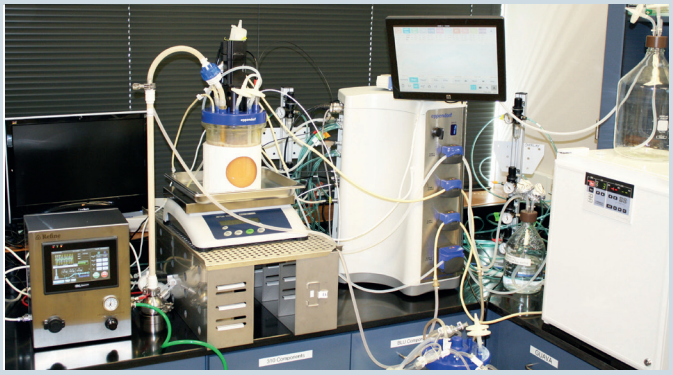

Fig. 8: Connection of the BioFlo 320 bioreactor control station with ATF cell retention device

Attention: In case of a rupture of the ATF membrane the pressure inside the vessel can increase.
> To avoid rupture of the vessel, maintain an unrestricted air vent
> This can be done by installing a pressure relief valve on the head plate of the bioreactor
> It needs to be ensured that there is no filter between safety valve and vessel to enable an unrestricted air vent

and non-invasive sensors for the measurement of DO, temperature, and optical measurement of pH that drastically reduce contamination risks.

Bioreactors and process parameters

For the batch culture, a BioBLU 10c was used at a working volume of 10 L. The batch culture was agitated at 126 rpm. A BioBLU 3c was used for the fed-batch culture with a starting working volume of 1.5 L and an ending working volume of 3 L with an agitation of 100 rpm (Table 1).
For the perfusion run, a BioBLU 5c Single-Use Bioreactor equipped with a pitched-blade impeller was used with a working volume of 3.5 L and an agitation of 100 rpm. For medium harvest, the bioreactor was equipped with a dip tube already installed inside the vessel with an AseptiQuik® connector (Colder Products Company). The dip tube was then connected to a XCell® ATF 2 (Repligen®) via silicone tubing and a CPC AseptiQuik fitting (3/8" hose barb fitting). The filter had a pore size of 0.2 µm. The filter setup was connected to an ATF controller (C24 Controller, Repligen). The ATF circulation rate was set to 1 L/min [11].

Because a temperature shift to 32 °C is common practice

Table 1: Process parameters for each experiment in this study

Parameter	Batch process	Fed-batch process	Perfusion process
Bioreactor	BioBLU 10c Single-Use Bioreactor	BioBLU 3c Single-Use Bioreactor	BioBLU 5c Single-Use Bioreactor with ATF dip tube
Bioreactor control system		BioFlo 320	
Process duration	7 days	12 days	14 days
Starting working volume	10 L	1.5 L	3.5 L
Ending working volume	10 L	3 L	3.5 L
Inoculation density	0.5 x 10^6 cells/mL	0.3 x 10^6 cells/mL	0.45 x 10^6 cells/mL
Agitation	126 rpm	100 rpm	100 rpm
Temperature	37 °C	37 °C; shift to 32 °C at day 3	37 °C; shift to 32 °C at day 7
DO sensor		polarographic sensor	
DO setpoint		50 %	
pH sensor	potentiometric sensor	optical sensor	optical sensor
pH setpoint	pH 7.0 (deadband = pH 0.2) via a cascade to CO_2 (acid) and 0.45 M sodium bicarbonate (base)		
Gassing strategy	3-gas auto mixing, 0.05 SLPM - 1 SLPM	3-gas auto mixing; 0.05 SLPM - 1 SLPM	3-gas auto mixing; 0.05 SLPM - 5 SLPM
Feeding strategy	n/a	3 % of total volume per day	0.2 VVD - 1.7 VVD
Glucose bolus feed target	n/a	> 3 g/L	> 3 g/L
ATF circulation rate	n/a	n/a	1 L/min; 1.2 L/min from day 12 to prevent clogging

for increasing CHO cell protein production, the temperature shift was utilized for increased hmAb production in some of the culture processes: In the fed-batch run, the temperature was decreased to 32 °C on initiation of feeding; in the ATF perfusion process, the temperature was shifted to 32 °C on day 7 [11].

Feeding and perfusion control

For the fed-batch run, the bioreactor was fed 3 % of the total volume per day. The feed media was prepared as described previously in the media composition section.

The glucose concentration target for the fed-batch and perfusion runs was to keep glucose levels 3 g/L or above. When the glucose concentration dropped below 3 g/L, the culture was fed by pumping in the appropriate amount of 200 g/L sterile glucose (bolus feed) into the culture twice daily. This was in addition to replenishing the glucose throughout the perfusion and fed-batch processes via media feeding.

The perfusion rate was between 0.2 VVD and 1.7 VVD. The rationale behind perfusion rate adjustment was to keep the ammonia concentration in the bioreactor < 4 mM. The perfusion rate was adjusted based on ammonia level at the time of sampling.

ATF perfusion device setup

The ATF device was turned on a few hours prior to inoculation to allow the media to circulate through the filter at the setpoint used for the run (1 L/min), as recommended by the manufacturer. This is an important step to properly wet the filter by media circulation and allow for any air bubbles to gradually work themselves out of the filter prior to inoculation. The ATF circulation of media and cells were started five hours before it was needed to initiate perfusion so that the cells could adjust to the alternating tangential flow-related stress, as recommended by the manufacturer. The connection of the ATF to the BioFlo 320 controller is shown in Figure 8.

More details on this specific perfusion process can be found in our publication titled, "Comparing Culture Methods in Monoclonal Antibody Production" [11]. Additionally, a description of a small scale ATF perfusion run using the DASGIP® Parallel Bioreactor System can be found in our Application Note 410 [9].

Sensor calibrations

The BioFlo 320 controller supports a range of Mettler Toledo® dissolved oxygen (DO) sensor technologies: polarographic (either analog or digital ISM®) or optical. Based on sensor type, calibrations will vary. For an in-depth look into DO sensor calibrations, please see "A Guide to Calibration on the BioFlo 120 and BioFlo 320: Dissolved Oxygen Sensors" [12].

In addition to using the calibration guide for reference, the BioFlo 320 also includes an Auto Calibration feature for DO sensors that will automatically calibrate the sensor once it has stabilized in pre-culture conditions. Auto Calibration also allows for process reproducibility between users which is extremely important for scale-up processes.

In this study, DO was measured using a polarographic sensor (Mettler Toledo) for all experiments and was controlled at 50 % by sparging air and/or oxygen.

For the batch culture, a potentiometric (gel-filled) pH sensor was used. Fed-batch and perfusion cultures used an optical pH sensor that is compatible with the BioBLU bioreactors. For all experiments, the pH was automatically controlled at 7.0 (deadband = 0.1 or 0.2) via a cascade to CO_2 (acid) and 0.45 M sodium bicarbonate (base).

The potentiometric pH sensor is calibrated outside of the vessel prior to sterilization. It is calibrated based on a 2-point calibration method on the bioprocess controller. For cell culture, a pH 7.00 buffer is used to set ZERO, and a pH 10.00 buffer to set SPAN. The sensor is then sterilized in a steam sterilizer (autoclave) pouch and inserted aseptically into the vessel in a laminar airflow cabinet (hood). For the optical pH sensor compatible with the BioBLU bioreactors, follow the instructions from the user manual.

Pump calibrations

Pump calibrations are an important step to any process. Calibrating with the correct tubing that is used in a process will help with pump rate accuracy throughout the run. Additionally, making sure that the tubing length and inner diameter (ID) for a particular run is mimicked in the calibration process to make sure the pumps are calibrated at user parameters.

This is especially important in processes like fed-batch and perfusions which rely heavily on pumps to deliver nutrients or remove wastes at a specific rate. For example, if an addition pump is not properly calibrated, this can lead to over pumping nutrients, and potentially, overflowing a vessel causing culture dilution. Alternatively, it can also lead to under feeding or under removal of wastes that can lead to culture lag or premature death.

The pump's operating minimum and maximum range should also be taken into consideration when choosing the right pump for each task in a specific process. Pump calibration processes vary between controllers. The

controller's operator manual should be consulted to ensure proper pump calibrations.

Aseptic connection of addition and harvest bottles

At the bioreactor stage, it is extremely important to connect addition and harvest bottles aseptically. Maintaining aseptic conditions can be done in several different ways. For example, a laminar airflow cabinet can be used to make aseptic connections, however, it is not always practical to make connections inside a hood.

One way to keep aseptic connections outside the hood is by using a tube welder. We use the SCD®-II Sterile Tubing Welder from Terumo (Figure 9). The Terumo welder reaches temperatures up to 300 °C to maintain sterility while welding. The tube welder requires the use of a special type of tubing made of weldable material like C-Flex. We like to prepare our bottles with silicone tubing (for our pump heads) and add a reducer to a piece of C-Flex tubing, usually 4 to 8 inches in length, for welding. An example of the silicone to C-Flex tubing set up can be seen in Figure 9.

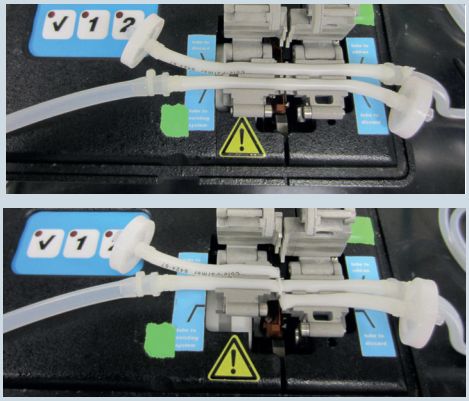

Fig. 9: Tube welding. Silicone tubing is extended with weldable connectors made of C-Flex® via straight connectors. A: Before welding B: After welding

CPC AseptiQuik Connectors from Colder Products Company (CPC) are an easy alternative to investing in a tube welder (Figure 10). We used these connectors in our ATF run featured in this beginner's guide for the ATF filter connection to our bioreactor.

These connectors allow the user to make sterile connections to the bioreactor without the added risk of contamination, via a sterile boundary membrane on each side half of the connector. AseptiQuik Connectors come in a variety of hose barb sizes and other types of connection ends.

When using an AseptiQuik connector, we integrate one side of the connector onto our tubing assemblies and autoclave them. We also integrate a connector onto our bioreactors. Depending on using either glass or single-use bioreactors, this is either assembled in a way to connect it to our single-use bioreactors after autoclave inside the hood or directly onto our glass bioreactor prior to autoclave. Once the AseptiQuiks are sterilized, we are able to connect them together. For first time users, I highly recommend visiting CPC's website. They have great resources, including how-to videos, that will guide you through the process step by step for every type of connector they offer.

Sampling and analytics

For each experiment, the bioreactor was sampled twice daily, one in the morning and one in the evening, to check offline values such as cell density, viability, glucose, ammonia (NH_3), lactate, and hmAb concentration. To collect the highest quality sample from the growing culture in the bioreactors, a sterile 5 mL syringe was connected to the sample port Luer Lock and a dead volume of 4 mL was removed. After the dead volume was removed, a second 4 mL sample as taken using a new syringe to provide a fresh, viable sample for analytics [13]. It is important to collect enough dead volume from the sampling line to ensure the best sample is taken from the bioreactor that accurately depicts the growing culture.

The cell density and viability were measured via the trypan blue exclusion method using a Vi-Cell® XR Viability Analyzer from Beckman-Coulter®. The in-vessel pH values read on the controllers were checked offline using an Orion Star® 8211 pH-meter (Thermo Fisher Scientific) to verify that the pH sensor values were accurate. By checking the offline pH value, the pH was able to be restandardized, if necessary, to prevent any discrepancies between online and offline measurements. An offline pH check is important to minimize any sensor drift that can happen throughout an experiment. Glucose, ammonia, lactate, and hmAb were measured using a Cedex® Bio Analyzer (Roche Diagnostics®) [13]. Measuring these analytics twice daily helps keep track of ammonia build up and glucose levels, which are especially important to monitor in processes such as fed-batches and perfusions.

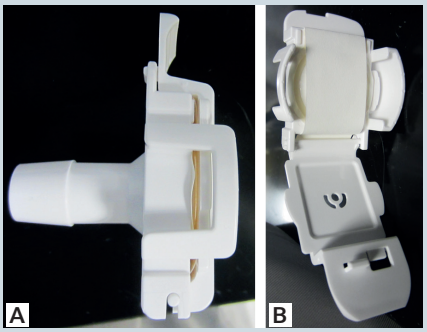

Fig. 10: AseptiQuik genderless connector. **A.** The connector closed and ready for autoclave. **B.** The sterile boundary membrane of the AseptiQuik connector after opening the tab.

Results

Batch Run

For the batch run, the bioreactor was inoculated at 0.5 x 10^6 cells/mL. The culture in the BioBLU 10c reached a peak density of 14.1 x 10^6 cells/mL on day 5 (Figure 11A).

The metabolic profile and antibody production for the batch run is shown in Figure 11B. The culture reached a peak antibody production of 195 mg/L on day 6. Ammonia gradually increased throughout the run until it reached toxic levels of 12 mmol/L on day 6. The lactate concentration remained under 2 g/L for the duration of the run.

Fed-batch Run

For the fed-batch run, the bioreactor was inoculated at 0.3 x10^6 cells/mL. The maximum ammonia level was targeted for 3 mmol/L and monitored daily. Feeding was started on day 3, when those levels reached close to 3 mmol/L. The bioreactor was fed 3 % of the total volume per day until the feed bottle was empty. The fed-batch culture reached a peak density over 20 x 10^6 cells/mL on day 9 (Figure 12A).

The metabolic profile and antibody production for the fed-batch is shown in Figure 12B. Peak antibody concentration was 1550 mg/L on day 15. Ammonia was over 6 mmol/L for the first few days of feeding. With culture feeding it got diluted and remained low until feeding was complete.

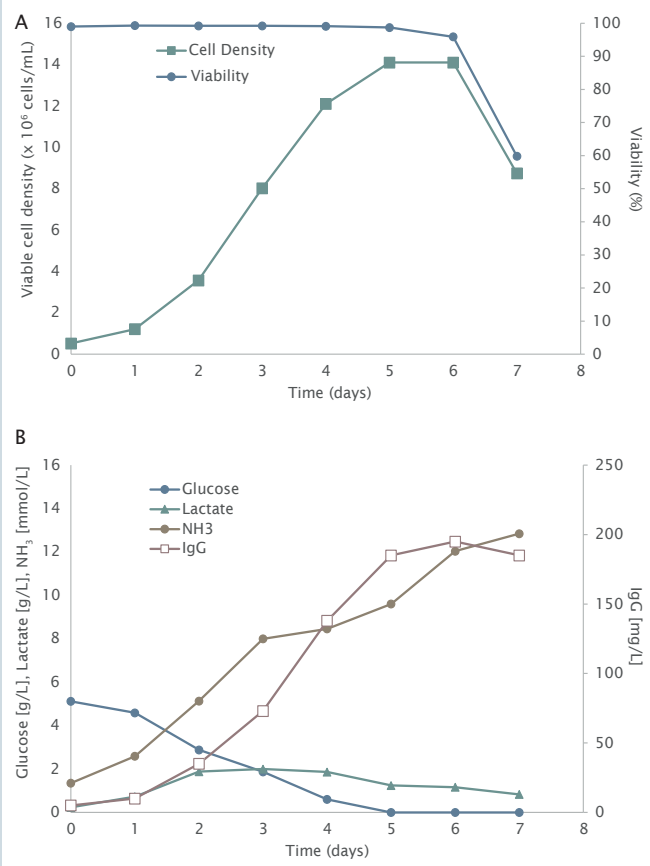

Fig. 11: CHO cell bioprocess in batch mode

A. Viable cell density and viability.

B. Metabolic profile (concentrations of glucose, lactate, and NH_3) and IgG concentration.

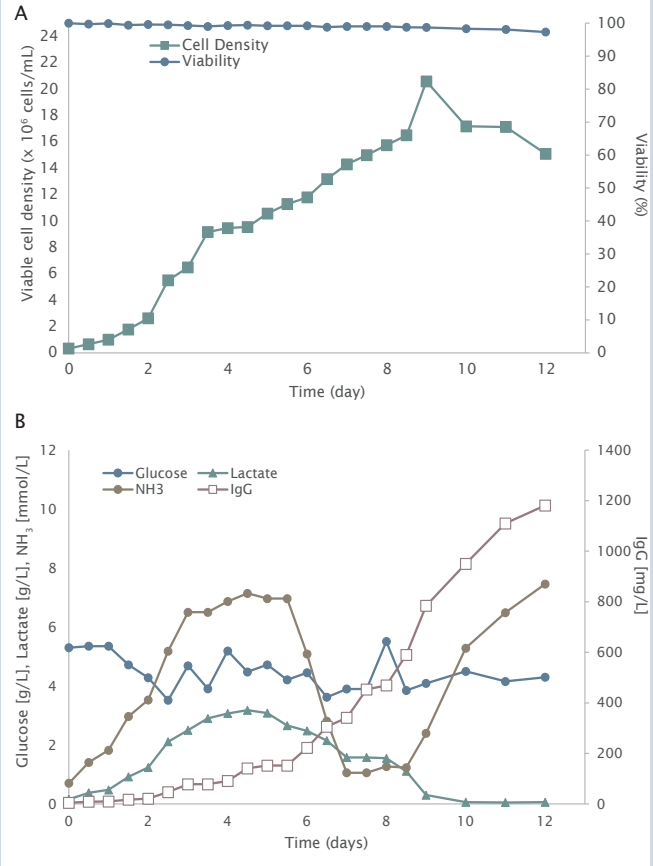

Fig. 12: CHO cell bioprocess in fed-batch mode

A. Viable cell density and viability.

B. Metabolic profile (concentrations of glucose, lactate, and NH_3) and IgG concentration.

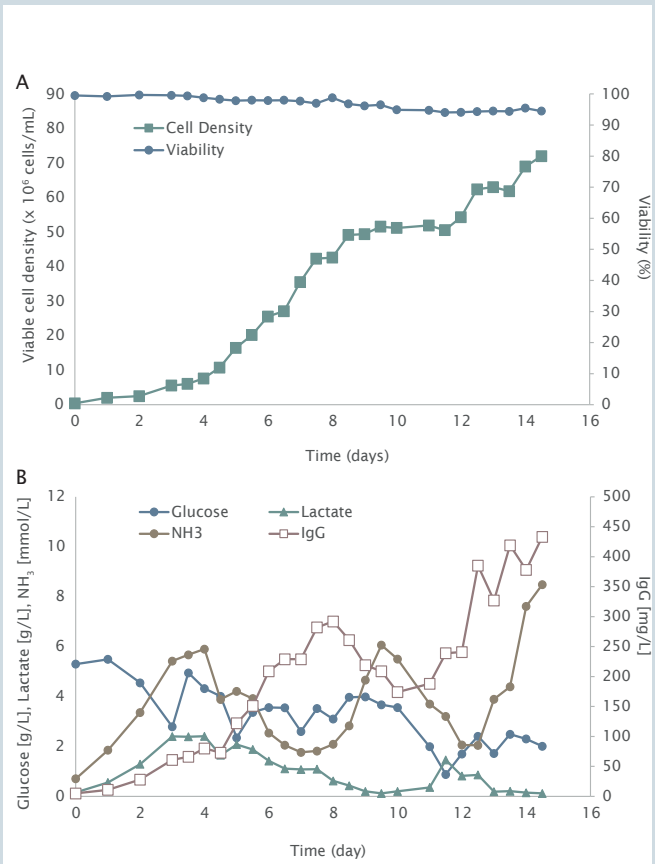

Fig. 13: CHO cell bioprocess in perfusion mode
A. Viable cell density and viability.
B. Metabolic profile (concentrations of glucose, lactate, and NH_3) and IgG concentration.

Ammonia increased to toxic levels by day 15 when the culture entered the decline phase. Lactate concentration remained under 3 g/L for the duration of the run.

ATF Perfusion

The culture was inoculated at a density of 0.45 x 10^6 cells/mL. On day 12, the culture reached a peak cell density of 72 x 10^6 cells/mL. At that time point, 90 % of the cells were viable (Figure 13A).

The ammonium concentration target was < 4 mM and the glucose concentration > 3 g/L in the course of the run. On day 3 the ammonia concentration exceeded 3 mM and perfusion was started at 0.5 vessel volumes per day (VVD). Based on the ammonia concentrations determined offline, the perfusion rate was gradually increased up to 1.7 VVD. The ammonia concentration stayed below 6 mmol/L until day 3 when perfusion was started. Ammonia reached over 8 mmol/L at day 14. The lactate concentration remained below 2.5 g/L for the duration of the run. When the glucose concentration dropped below 3 g/L, the culture was fed by pumping in the appropriate amount of 200 g/L sterile glucose into the culture twice daily, in addition to replenishing the glucose throughout the perfusion process (Figure 13B).

The IgG concentrations in the bioreactor increased up to approximately 433 mg/L. As expected, IgG production steadily increased following the cell growth profile (Figure 13B).

Discussion and Conclusion

Shown in Figure 14, we compared the growth curves from our batch, fed-batch and perfusion runs. Our batch culture ran for a total of 7 days and reached a peak density of 14.1 x 10^6 cells/mL on day 5. The fed-batch culture ran for 12 days and reached a peak density of 21 x 10^6 cells/mL on day 9. Our perfusion culture ran the longest and reached the highest density of all bioprocess modes by day 15. A batch culture takes significantly less run time than a fed-batch or perfusion and is the easiest process to execute. A batch culture is a great way for a beginner to start their optimization process without added complexity or equipment costs. Using this process, it is an easy way to test different parameters like inoculation densities, varying media compositions or gassing strategies, especially at small scale. However, culture growth and yield production are limited in batch processes. A fed-batch has increased yield and process time with a higher complexity but can be overall more cost efficient than running batch cultures. As you see in Table 2, we achieved a significantly higher IgG yield in the fed-batch run than in the batch process. Fed-batch processes are great for those looking to achieve higher cell growth and yield productions in comparison to batch runs. Fed-batch cultures also do not require additional equipment costs to run.

Perfusion can achieve higher cell density and yields than a batch or a fed-batch culture. In our example, we obtained almost trice the amount of IgG per bioreactor

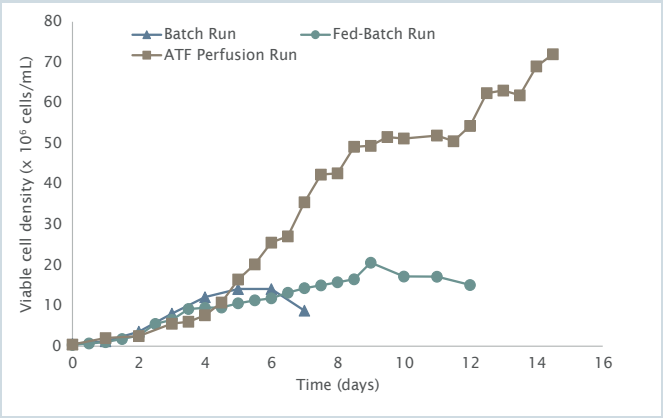

Fig. 14: Growth curve comparison for batch, fed-batch and perfusion runs

volume compared to the fed-batch run. However, perfusion processes are also associated with higher equipment costs and media consumption.

Since higher cell densities can be achieved with perfusion processes, using smaller bioreactors and optimizing process parameters could potentially reduce space requirements and investment costs while still achieving the highest production yields over batch or fed-batch processes. Perfusion cultures can add many layers of challenges into its processes and often requires additional in-process optimization to fine-tune process parameters. However, once established, high cell culture growth and production yields are achievable [14].

Overall, the choice between processes depends on many factors such as experimental needs and budget. We hope that this application note will help our beginner cell culture scientists confidently choose their bioprocess mode that is well suited to their needs.

Table 2: Run comparison of each bioprocess mode

Run type	Typical duration	Media consumption/ ending working volume [L]	Equipment costs	Process complexity	Peak cell density	Total hmAB produced [g]	Total hmAB/ ending working volume [g/L]
Batch	~ 1 week	1	costs for bioreactor control system and bioreactor	Low	14 x 10^6 cells/mL	1.95 g	0.195 g/L
Fed-batch	~ 2 weeks	1	costs for bioreactor control system and bioreactor	Medium	21 x 10^6 cells/mL	4.5 g	1.5 g/L
ATF perfusion	~ 2 to 4 weeks	14.4	costs for bioreactor control system and bioreactor; additional costs for hollow fiber filters and ATF perfusin control device	High	72 x 10^6 cells/mL	11.5 g	3.3 g/L

Literature

[1] K.P. Jayapal, K.F. Wlaschin, M.G.S. Yap, W.-S. Hu, Recombinant protein therapeutics from CHO cells—20 years and counting, Chem. Eng. Prog. (2007), 103(7): 40–47.
[2] Sherman, M., Lam, V., Carpio, M., Hutchinson, N., Fenge, C., Continuous cell culture operation at 2,000-L scale. Bioprocess Int (2016), 14(S7): 22-28.
[3] Luo J, Vijayasankaran N, Autsen J, Santuray R, Hudson T, Amanullah A, Li F., Comparative metabolite analysis to understand lactate metabolism shift in Chinese hamster ovary cell culture process. Biotechnol Bioeng (2012) 109(1): 146-56.
[4] Dean J1, Reddy P., Metabolic analysis of antibody producing CHO cells in fed-batch production. Biotechnol Bioeng (2013), 110(6): 1735-47.
[5] Li, F., Vijayasankaran, N., Shen, A., Kiss, R., Amanullah, A., Cell culture processes for monoclonal antibody production. mAbs (2010), 2(5): 466-477.
[6] Zhang, H., Wang, H., Liu, M., Zhang, T., Wang, X., et al., Rational development of a serum-free medium and fed-batch process for a GS-CHO cell line expressing recombinant antibody. Cytotechnology (2013), 65: 363-378.
[7] De Jesus M1, Wurm FM., Manufacturing recombinant proteins in kg-ton quantities using animal cells in bioreactors. Eur J Pharm Biopharm (2011), 78(2): 184-8.
[8] Sauer, P.W., Burky, J.E., Wesson, M.C., Sternard, H.D., Qu, L., A high-yielding, generic fed-batch cell culture process for production of recombinant antibodies. Biotechnol Bioeng (2000), 67(5): 585-597.
[9] Suttle, A, Willard, S and Sha, M. Small Scale Perfusion Using an ATF Cell Retention Device with DASGIP® Parallel Bioreactor System. Eppendorf Application Note 410. 2019.
[10] Eppendorf BioFlo 320 Operating Manual, Eppendorf. Inc.
[11] Willard, Stacey S, et al. Comparing Culture Methods in Monoclonal Antibody Production. BioProcessing International, Mar. 2017, 15 (3): 38–46.
[12] Willard, S. A Guide to Calibration on the BioFlo® 120 and BioFlo® 320: Dissolved Oxygen Sensors. Eppendorf Short Protocol 40. 2017.
[13] Suttle, A and Sha, M. CHO Cell Culture in Eppendorf BioBLU® 10c Single-Use Vessels. Eppendorf Application Note 361. 2018.
[14] Pollock, James et al. Fed-batch and perfusion culture processes: economic, environmental, and operational feasibility under uncertainty. Biotechnology and bioengineering vol. 110,1 (2013): 206-19.

Ordering information

Description	Order no.
BioFlo® 320, base control station, with water connection	1379963011
BioBLU® 3c Single-Use Bioreactor, cell culture, macrosparger, 1 pitched-blade impeller, optical pH, sterile	1386000300
BioBLU® 10c Single-Use Bioreactor, cell culture, macrosparger, 1 pitched-blade impeller, optical pH, sterile	1386141000
BioBLU 5c Single-Use Bioreactor, cell culture, macrosparger, 1 pitched-blade impeller, optical pH, sterile with ATF dip tube	inquire

Your local distributor: www.eppendorf.com/contact
Eppendorf SE · Barkhausenweg 1 · 22339 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com