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MINI-REVIEW

Progress in fed-batch culture for recombinant protein production in CHO cells

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Abstract

Nearly 80% of the approved human therapeutic antibodies are produced by Chinese Hamster Ovary (CHO) cells. To achieve better cell growth and high-yield recombinant protein, fed-batch culture is typically used for recombinant protein production in CHO cells. According to the demand of nutrients consumption, feed medium containing multiple components in cell culture can affect the characteristics of cell growth and improve the yield and quality of recombinant protein. Fed-batch optimization should have a connection with comprehensive factors such as culture environmental parameters, feed composition, and feeding strategy. At present, process intensification (PI) is explored to maintain production flexible and meet forthcoming demands of biotherapeutics process. Here, CHO cell culture, feed composition in fed-batch culture, fed-batch culture environmental parameters, feeding strategies, metabolic byproducts in fed-batch culture, chemostat cultivation, and the intensified fed-batch are reviewed.

Key points

- *Fed-batch culture in CHO cells is reviewed.*
- *Fed-batch has become a common technology for recombinant protein production.*
- *Fed batch culture promotes recombinant protein production in CHO cells.*

Keywords CHO cell · Process development · Recombinant therapeutic proteins · Fed-batch culture

Introduction

In recent years, with the growing demand for recombinant therapeutic proteins (RTPs) in the pharmaceutical market, including recombinant antibody, the production of RTPs has developed rapidly. More than 120 recombinant antibodies have been approved by the European Medicines Agency (EMA) and the U.S Food and Drug Administration (FDA) with thousands of billion dollars in market value (Kaplon et al. 2020). A report has estimated that market value about monoclonal antibodies (mAbs) will has reached over US$ 300 billion by 2025 (Lu et al. 2020). Among the recombinant protein expression system, Chinese hamster ovary (CHO) cells are the preferred cells for RTP production (Ritacco et al. 2018; Gupta et al. 2021) due to several advantages: ① not susceptible to human virus infection with high safety (Lalonde and Durocher 2017); ② RTPs with post- translational modifications similar to those of human cells (Stach et al. 2019); ③ high-density CHO cells can accommodate suspension culture with the chemically defined serum-free medium (CD-SFM) and produce proteins that secrete into the culture medium (Ritacco et al. 2018; Dahodwala and Lee 2019).

Nowadays, mAbs production in mammalian cell culture is mainly performed with fed-batch processes in large-scale bioreactors (Schellenberg et al. 2022). CHO cells are usually cultivated in a fed-batch mode to achieve maximum cell density and product titer, with either continuous or bolus

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feed addition (Romanova et al. 2022). The initial cellular growth is supported by the basal medium. Concentrated feed medium is added in fed-batch culture to replenish nutrients and prolong the culture time (Ritacco et al. 2018; Chee Furng Wong et al. 2005). Feed medium can avoid the nutrients limitation, and it can promote higher production of recombinant proteins (Rish et al. 2022; Bibila and Robinson 1995; Lu et al. 2013). The culture time of cells in stationary phase after fed-batch treatment is longer than that of batch culture (Sellick et al. 2015). Fed-batch culture requires an understanding of the characteristics of cell growth and cell metabolism, and the key process parameters that affect the quality and yield of RTPs (Ghaffari et al. 2020). CHO cell grows at a high speed in exponential phase, with an accelerated consumption of glucose and amino acids, resulting in accumulation of lactate and ammonium (Horvat et al. 2020; Zagari et al. 2013). In fed-batch culture, temperature, pH, and other process parameters also have an impact on antibody expression and quality attributes (Kuwae et al. 2018; Pan et al. 2017; Graham et al. 2019). Fed-batch culture can improve the expression and production of RTPs in CHO cells, but traditional bolus feed delivery also causes accumulation of metabolic byproducts (Xiao et al. 2021). Therefore, a reasonable feeding strategy is quite important (Baik et al. 2015; Zhang et al. 2013; Mellahi et al. 2019). Fed-batch culture has focused on reducing the cost of RTP production, which has become the research focus in pharmaceutical industry (Ghaffari et al. 2020).

CHO cell culture

Adherent CHO cell grows under DMEM/F12 medium containing 10% fetal bovine serum (FBS) at 37℃, 5% CO_2 (Li et al. 2021). SFM is chosen to suspension culture, supporting a transition from adherent culture to suspension culture mode. The basal medium is used for the adherent culture including serum components from fetal bovine. Serum components are complex, and it is prone to contamination (Lalonde and Durocher 2017). SFM is divided into ordinary serum-free medium, xeno-free medium, animal-free medium, protein-free medium, and chemically defined serum-free medium (Gélinas et al. 2019; Yao and Asayama 2017). When it comes to cell suspension culture, SFM can effectively avoid disadvantages caused by serum culture, such as contamination and difficulty in purification.

Batch culture refers to a culture method that can harvest recombinant proteins at one time after adding SFM once. Due to short culture time and unsatisfactory cell density at the time of sample collection, RTP production is unideal. Fed-batch mode improves RTP production. It is reported that mAb titer in fed-batch culture has reached more than 10 g/L (Handlogten et al. 2018; Kelley 2009). In recent years, fed-batch process has become an important production process for the large-scale production of RTPs. However, maintaining high productivity of cells and quality attributes of protein is still a challenge. Therefore, fed-batch culture has shifted from the high-yield recombinant protein merely to the dual goals of high-yield and high-quality production (Ha et al. 2022).

Feed composition in fed-batch culture

Optimizing the critical feed composition and the concentrations of components are crucial to process development (Zou et al. 2020). In fed-batch industry, Raman spectroscopy with partial least squares regression (PLSR) or multiple linear regression (MLR) are applied to monitor and control the concentrations of glucose and amino acids (Kozma et al. 2018). Besides Raman spectroscopy, liquid chromatography-mass spectrometry (LC–MS) has been considered to analyze amino acids and inorganic salt (Hoang et al. 2021). For the first time, lipidomics analysis has been performed for lipid identification and lipid quantitation in fed-batch culture (Ali et al. 2018). In addition, feed composition has been linked with cell metabolism regulation to demonstrate fed-batch culture (Braasch et al. 2021) (Fig. 1).

The components of feed medium are similar to SFM, including amino acids, carbon sources, inorganic salts, trace elements, vitamins, lipids, and putrescine and yeast hydrolysate etc. (Table 1).

Amino acid

During upstream process optimization, amino acid is one of the major concerns (Fan et al. 2015). Amino acids deplete in batch culture, which is detrimental to RTP production (Ghaffari et al. 2020). Amino acids' feeding is related with tricarboxylic acid cycle (TCA cycle) (Rish et al. 2022). The TCA cycle depends on alternative metabolites to complement intermediates (Hong et al. 2018; Ghorbaniaghdam et al. 2014). Amino acids consumed from feed medium can be directed to the TCA cycle by conversion between the cytosol and the mitochondria. Furthermore, the catabolism of amino acids is supply for the TCA cycle. Amino acids in feed medium are limited. AMP that leads to boosting cell health is associated with the purine nucleotide cycle. Malate and glycine accumulation are from the TCA cycle. Cell proliferation has a negative impact with ammonia concentrations above 5 mM (Yang and Butler 2000), and ammonia is derived from the hydrolysis of amino acids (Rish et al. 2022). Thus, controlling feeding of amino acids may reduce byproducts accumulation (Rish et al. 2022). Although cells can synthesize non-essential amino acids, cell growth, and

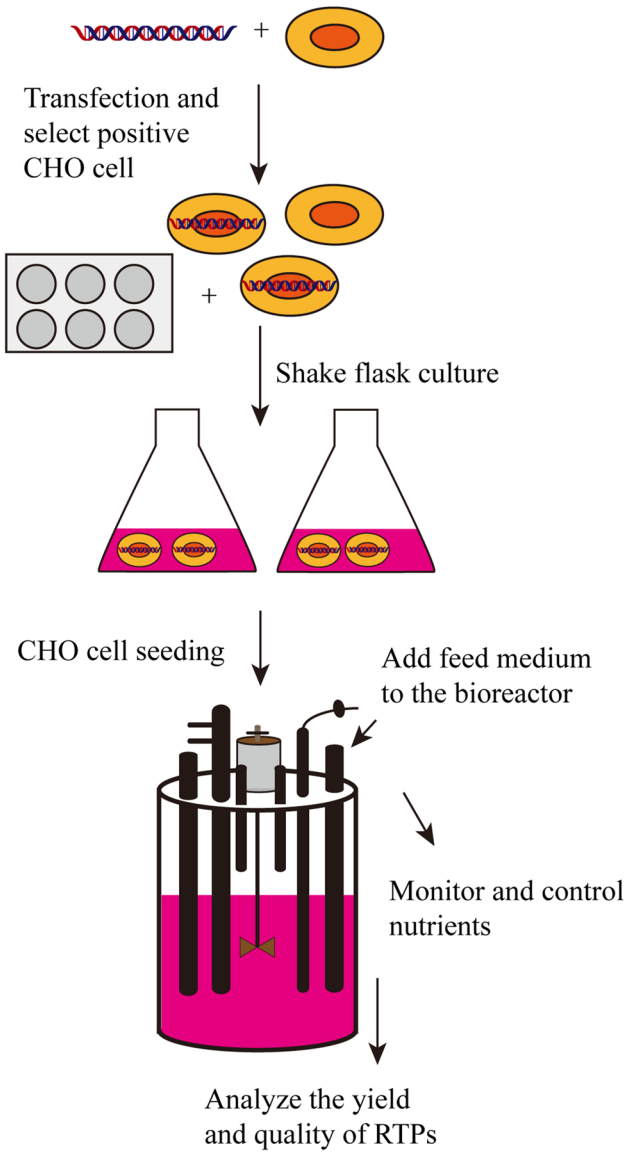

Fig. 1 Fed-batch culture in CHO cell. Gene of interest (GOI) is introduced in CHO cell via transfection. Positive CHO cells are used for shake flask culture. Then, CHO cells and feed medium are added in bioreactor. In fed-batch culture mode, nutrients are monitored and controlled to achieve the yield and quality of RTPs. The production of RTPs is indicated by fed-batch system.

the expression of recombinant proteins still rely on amino acids in feed medium (Horvat et al. 2020).

N-lactoyl-leucine (Lac-Leu) and N-lactoyl-isoleucine (Lac-Ile) can replace unmodified leucine and isoleucine, respectively. Lac-Leu and Lac-Ile improved the solubility in culture medium. Adding 2×concentrated feed medium with a 70% mixture of two above-mentioned N-Lactoyl amino acids (Lac-AA) increased the yield of recombinant protein by 58%, compared with the addition of 1×concentrated feed medium. Schmidt et al. found that 2×concentrated medium reduced the volume of feed medium (Schmidt et al. 2021). According to metabolic flux analysis (MFA), Xing et al. optimized feed medium to produce antibody fusion protein (B1) in CHO cells. The concentration of alanine, arginine, glutamine and glycine were reduced, while the concentration of methionine, tryptophan, asparagine and serine were increased. Finally, B1 protein titer was ameliorated by 27% in fed-batch culture (Xing et al. 2011). Fouladiha et al. used the computational modeling method to design a feeding strategy in CHO cells. To study the effect of the production of mAb in CHO cells, 15 metabolites were analyzed by Plackett – Burman (PB) design. The results showed that the production of mAbs could be significantly improved by 2 times after threonine treatment (Fouladiha et al. 2020). Hecklau et al. reported that S-sulfo-L cysteine (SSC) replaced cysteine and was highly stable in fed-batch culture; feed medium supplemented with SSC prolonged cell viability and enhanced product titers by 78%. Meanwhile, this strategy reduced ammonium (NH_4^+) accumulation (Hecklau et al. 2016). Zhang et al. took advantage of high concentration tyrosine and low concentration tyrosine experimental groups, and they cultured CHO cells expressing anti-CD20 IgG1 in bioreactor. Apart from feed medium containing tyrosine, 88 mM tyrosine solution was added in the high concentration tyrosine group. High concentration tyrosine promoted the cell growth and reduced the osmolality of bioreactor culture environment and increased the production of mAbs (Zhang et al. 2020a).

Amino acids are key components of feed medium, and their concentration and properties can affect yield and quality of RTPs in fed-batch culture. Amino acids involved in fed-batch culture require a strict design. However, some high concentration amino acids are easy to precipitate due to the limitation of the solubility. Modified amino acids are based on the original structure. We need to comprehensively consider the characteristics of CHO cells and establish the best addition strategy.

Carbon source

Glucose is the primary carbon source of the TCA cycle in exponential phase, and glucose consumes fast at the later stage of the exponential phase (Kirsch et al. 2022). However, glucose consumption causes accumulation of pyruvate and lactate, which leads to inhibition of cell growth. Wilkens et al. found that 3.6 g/L glucose concentration shortened CHO cell culture time and lactate concentration attained 2.7 g/L after 120 h. Although both 1.08 g/L glucose and 2.52 g/L galactose are applied to diminish accumulation of lactate, and tissue plasminogen activator (t-PA) production was increased to 100% when 1.08 g/L glucose and 2.52 g/L galactose was added to CHO cell culture, final concentration of glucose approached zero

Table 1 The components in fed-batch culture

Category	Additives	Effect	Reference
Amino acid	Lac-Ile	Improved yield of protein	Schmidt et al. (2021)
	Lac-leu	Improved yield of protein	Schmidt et al. (2021)
	Alanine	Inhibited the TCA pathway	Xing et al. (2011)
	Arginine	Increased cytotoxic level	Xing et al. (2011)
	Glutamine	Increased ammonium production	Xing et al. (2011)
	Glycine	Increased ammonium production	Xing et al. (2011)
	Methionine	Prevented amino acid limitation	Xing et al. (2011)
	Tryptophan	Prevented amino acid limitation	Xing et al. (2011)
	Asparagine	Decreased ammonium production	Xing et al. (2011)
	Serine	Provided a carbon donor	Xing et al. (2011)
	Threonine	Increased antibody production	Fouladiha et al. (2020)
	SSC	Promoted protein productivity	Hecklau et al. (2016)
	Tyrosine	Enhanced antibody production	Zhang et al. (2020a)
Carbon resource	Glucose	Increased byproduct accumulation	Wilkens et al. (2011)
	Glutamine	Accumulated lactic acid	Sun et al. (2021)
	Galactose	Improved protein expression and sialic acid content	Xiao et al. (2019)
	Fructose	Promoted cell growth	Wlaschin and Hu (2007)
Inorganic salt	Na^+	Regulated osmolality	Zhu et al. (2005)
Trace element	Selenite	Increased antibody production	Zhang et al. (2006)
	Fe (Fe^{2+})	Enhanced acidic charge variants	Chung et al. (2019)
	Zn (Zn^{2+})	Promoted protein production	Graham et al. (2020)
	Cu (Cu^{2+})	Improved antibody titer	Xu et al. (2016)
Vitamin	Vitamin C	Decreased phosphorylation level	Hou et al. (2019)
	Nicotinamide	Decreased phosphorylation level	Hou et al. (2019)
	B vitamins	Improved antibody titer	Vijayasankaran et al. (2013)
	Vitamin K	Decreased Gla level	Lee et al. (2017a)
Lipid	Ethanolamine	Promoted antibody titer	Zhang et al. (2013)
	Lipid mixture	Promoted antibody titer	Zhang et al. (2013)
Other additives	Putrescine	Increased antibody production	Zhang et al. (2013)
	3-MA	Increased protein production	Jardon et al. (2012)
	AIP	Improved antibody titer	Braasch et al. (2021)
	YE	Increased protein production	Hu et al. (2018)
	deoxyuridine	Increased antibody production	Takagi et al. (2017)
	thymidine	Increased antibody production	Takagi et al. (2017)
	deoxycytidine	Increased antibody production	Takagi et al. (2017)
	MPPB	Increased antibody production	Aki et al. (2021)

after 160 h and cell viability was decreased (Wilkens et al. 2011). During the cell culture, the minimum glucose concentration needs to be controlled at 2 ~ 3 g/L (Sun et al. 2021).

Glutamine is the secondary carbon source in the early exponential phase of cells, but it increases lactate levels (Kirsch et al. 2022). It was found that the expression of Fc-fusion protein in CHO cells was increased by 43% and the total sialic acid content of cells was increased by 37% when galactose was supplemented with 40% replacement ratio and glucose carbon source was replaced (Xiao et al. 2019). CHO cells transfected with GLUT5 fructose transporter were cultivated in fed-batch culture system, and feed medium containing 14.5 g/L fructose was added. Fructose improved cell growth, and it decreased accumulation of lactate. Wlaschin et al. indicated that CHO cells had an ability to replace carbon source through the expression of GLUT5 fructose transporter (Wlaschin and Hu 2007).

According to the glucose content in the culture medium, the high concentration glucose solution can be added to the CHO cell culture medium. Because the high concentration of carbon source also leads to metabolic byproducts accumulation, so the concentration of carbon source should be strictly controlled. Proper alternative carbon sources can avoid inhibition of cell growth by byproducts after glucose consumption.

Inorganic salts and trace elements

Fed-batch culture also requires inorganic salts to regulate pH and osmolality in cell culture environment. Zhu et al. used 2L bioreactor for fed-batch culture, and they added Na^+ to control osmolality and partial pressure of CO_2 (pCO_2). Both 140 ~ 160 mmHg pCO_2 and 400 mOsm/kg osmolality decreased the viable cell density (VCD) of CHO cells by

18%, but these factors clearly augmented the production of protein B1 (Zhu et al. 2005). In cell culture, selenium (Se) in the form of selenite can protect cells from oxidative damage. Zhang et al. found that the VCD exceeded 1×10^7 cells/mL and product titer of approximately 3 g/L was achieved in 14-day fed-batch cultures with the addition of selenite (Zhang et al. 2006).

Gangwar et al. found that Zn, Cu, Fe, and Mn impact charge heterogeneity, and reduce accumulation of metabolic byproducts in cell culture. Fe, Mn, and Ni enhance the acidic variants while Cu inhibits it. Zn reduces the basic variants (Gangwar et al. 2021). Chung et al. found that Ferrous sulfate (Fe^{2+}) in feed medium stored for 7 weeks can reduce the charge heterogeneity, but it improved the yield of mAb expressed by CHO cells (Chung et al. 2019). Graham et al. found that both 50 and 100 μM zinc ions (Zn^{2+}) added in bioreactor promoted the production of β-Glucuronidase (GUS) in CHO cells and harvested 2 times increase in specific activity of GUS (Graham et al. 2020). 5 μM copper sulfate (Cu^{2+}) in feed medium obtained the final mAb titer of 11.9 ± 0.6 g/L to express IgG1 in CHO cells, which was 4.8 times higher than the final mAb titer of conventional fed-batch culture (Xu et al. 2016).

Vitamin

Vitamin enhances productivity in CHO cells, and impacts charge heterogeneity. Gangwar et al. also indicated that vitamins can inhibit the acidic variants while it enhances the basic variants (Gangwar et al. 2021). Hou et al. found that increasing rate of vitamin C and nicotinamide feeding can reduce the phosphorylation level in CHO cells to 3% and attenuated the hydroxylation level to 9.4% (Hou et al. 2019). Adding B vitamins including 0.005 g/L vitamin B2, 0.0035 g/L pyridoxine (vitamin B6), 0.03 g/L pyridoxal (vitamin B6), 0.0985 g/L folic acid and 0.024 g/L vitamin B12 can improve packed cell volume (PCV) and antibody titer (Vijayasankaran et al. 2013). Lee et al. added 10 g/L vitamin K solution prepared with 13% polysorbate 20 (PS20) in 4-day fed-batch culture for cultured CHO cells expressing recombinant human factor II (rhFII). When the final concentration of vitamin K was 0.1 mg/L, gamma-carboxyglutamate (Gla) level was decreased (Lee et al. 2017a).

Lipid

Ethanolamine is one of the components of the phospholipid structure in mammalian cell membranes (Spens and Häggström 2007). As for TNFR-Fc production in CHO cells, the addition of the feed medium supplemented with ethanolamine and lipids resulted in a maximum VCD of 4.3×10^6 cells/mL and the antibody titer of 378 mg/L, which was threefold higher than batch culture (Zhang et al. 2013). Therefore, lipid can appropriately supplement in feed medium.

Other components

Putrescine is essential for cell growth (Jänne et al. 2004). Zhang et al. confirmed that putrescine was designed by PB design and it sharply increased mAb production. A significant increase in TNFR-Fc production was observed after adding feed medium with the concentration of 41.67 mg/L of putrescine (Zhang et al. 2013). Jardon et al. found that autophagy negatively affected recombinant proteins production. The autophagy inhibitor 3-methyl adenine (3-MA) in fed-batch culture resulted in a 2.8-fold increase in t-PA production compared with control group without 3-MA (Jardon et al. 2012). Autophagy-inducing peptide (AIP), a derivative of the autophagy protein Beclin 1, facilitated the production of mAbs in CHO cells by regulating cellular autophagy. The addition of 3 μM of AIP in fed-batch culture ameliorated the expression level of human IgG1, and IgG1 concentration exceeded 1200 μg/mL (Braasch et al. 2021). The yield of Fc-fusion protein (Fc) in fed-batch culture raised twofold by Yeast hydrolysate (YE), and YE also increased CHO cells size and intracellular nucleotide content (Hu et al. 2018). CHO cells expressing human mAb and Fab fragments were cultivated in 14-day fed-batch culture, and the concentration of 25 mg/L deoxyuridine was added at day 2. VCD and protein production were increased. Takagi et al. harvested 9.2 g/L human mAb and over 4 g/L Fab fragment antibody with deoxyuridine, thymidine, and deoxycytidine (Takagi et al. 2017). Since deoxyuridine, thymine, and deoxycytidine are all pyrimidine nucleotides, exogenous nucleoside may give a platform for efficient antibody production by providing nucleosides precursors through the remedial synthesis pathway of pyrimidine nucleotides. Aki et al. screened 4-(2,5-dimethyl-1H-pyrrol-1-yl) -N- (2,5-dioxopyrrolidin-1-yl) benzamide (MPPB). The mAb concentration of MPPB treatment was increased to 1098 mg/L, which was 1.5-fold higher than the mAb concentration of control group (Aki et al. 2021). Further studies in fed-batch culture facilitate the development of more additives, besides these above components.

Fed-batch culture environmental parameters

Culture parameters can affect CHO cell growth, recombinant protein expression, protein glycosylation, cell metabolites, and other factors (Butler 2005). Bioreactors are designed for the cultivation of suspension culture for RTP production

in CHO cells (Schmid et al. 2022). Cells are expanded by the cryovial or shake flask, and then grows in bioreactor (Hernández Rodríguez and Frahm 2020; Schmid et al. 2022). For fed-batch culture, culture environmental parameters in bioreactor are usually optimized through design-of-experiments (DoE) or one-factor-at-a-time (OFAT) studies, like temperature, dissolved oxygen (DO), and pH (Weng et al. 2020; Schneider et al. 2019).

Culture temperature

Culture temperature downshifting is generally performed in exponential growth phase. The culture temperature is switched from 37 ℃ to a lower temperature ranged from 29 ℃ to 35 ℃ for the sake of decreasing cell apoptosis and promoting antibody synthesis (Moore et al. 1997; Kou et al. 2011). Low temperature has a positive impact on integral cell density, cell viability, and cell-specific productivity (McHugh et al. 2020).

Torres et al. cultured CHO cell expressing human erythropoietin (hEPO) using a fed-batch culture process. They observed that low temperature not only had the advantage of improving the specific productivity of CHO cell expressing recombinant proteins, but also significantly increased the gene encoding the specific transcription activator of unfolded protein response, reducing the possibility of protein degradation; Hypothermia also enhanced the expression of hEPO by regulating the cell cycle (Torres et al. 2021). Cell culture temperature can significantly affect the VCD and protein expression in CHO cells. When CHO cells expressing anti-CD20 mAb were cultured at 35 °C, 33 °C, and 31 °C with the addition of feed medium at days 3, 6, and 9, 35 °C can increase cell density and expression of anti-CD20 mAb (Kong et al. 2020). Stiefel et al. that the effect of shifting temperature from 37 °C to 30 °C on miRNA expression in CHO cells was investigated. Low temperature condition increased IgG production through upregulating the expression of miRNAs related with inhibition of apoptosis, promotion of cell growth and protein production in CHO cells (Stiefel et al. 2016). Thus, low temperature is one of the most normal factors of cell culture.

Osmolality

Nutrient supplementation and metabolic byproducts accumulation are able to increase extracellular osmolality at the late stages of culture in fed-batch culture (Alhuthali et al. 2021). Both nutrient and alkaline supplemented can increase the osmolality of the culture environment (Ha et al. 2022). In fed-batch process, NaCl maintained high osmolality. It increased the titer of mAb, reduced galactosylation, and increased the proportion of high-mannose glycosylation (man5) (Qin et al. 2019). Xiao et al. found that continuous feeding resulting in osmolality below 450 mOsm/kg reduced the high-mannose glycosylation to less than 5% and increase the yield of mAb to 10 g/L (Xiao et al. 2021). Lee et al. investigated the effect of high osmolality on protein glycosylation in CHO cells. Osmolality reached over 400 mOsm /kg after adding betaine, and Fc-fusion protein sialylation in CHO cells decreased (Lee et al. 2017b). Inorganic salt and high feed volume can lead to high osmolality in fed-batch culture. Although high osmolality increased the cell-specific productivity (q_p) of CHO cells, but it increased the proportion of high mannose and inhibited the growth of CHO cells. Romanova et al. launched proteome study of hyperosmolality-exposed CHO cells together with control cells, and harvested cell samples at days 2, 6, and 8 in fed-batch culture, respectively. Whole protein lysates of hyperosmolality-exposed cell group and control cell group sampled on days 2, 6, and 8 were analyzed by western blot. Septins protein was obviously up-regulated by about 2-threefold at day 6 after treated with hyperosmolality, and up-regulated about 1-threefold at day 8 (Romanova et al. 2022).

Exposure of CHO cell to highly concentrated feed medium causes an increasing osmolality over 300 mOsm/kg in respect of cell physiology, morphology, and proteome (Romanova et al. 2022). In fed-batch culture, appropriate additives can obtain feasible osmolality. Furthermore, an available feeding strategy is also used for avoiding hyperosmolality.

pH

The pH of cell culture requires optimization to produce high-quality recombinant protein. The pH of CHO cell growth ranges from 7.2 to 7.6 (Michl et al. 2019). CHO cells expressing mAb against vascular endothelial growth factor (VEGF-MA) were cultured in 3 L bioreactor and the culture conditions were optimized. The pH was adjusted by adding sodium bicarbonate. When the pH value was set to 7.10, the yield of VEGF-MA increased to 4.1 g/L, and the charge heterogeneity, glycosylation level and protein purity were 26.1%, 59.1%, and 95.1% respectively (Feng and Shi 2020). It is necessary to follow the standard pH range of CHO cells when it comes to designing the pH in fed-batch culture. If the pH of cell culture maintains below 6.8 or above 7.6, CHO cell growth is negatively affected. The pH of cell culture is usually interactively regulated by CO_2 and inorganic salts to improve CHO cell growth, which can achieve large-scale recombinant proteins production.

Dissolved oxygen

DO is one of the key environmental parameters in large-scale process, which affects cell growth and recombinant protein production (Chotigeat et al. 1994; Restelli et al. 2006). Most of DO values in CHO cells are usually in the range of 20 to 50% (Fleischaker and Sinskey 1981). Ventini et al. reported that the final recombinant thyroid-stimulating hormone (rTSH) titer was 1.6 mg/L when DO value was 20% in fed-batch culture mode (Ventini et al. 2011). To improve the yield and quality of human IgG1 mAb, Mahé et al. adjusted the DO value to 40%, and CHO cells expressing human IgG1 mAb were cultured in fed-batch culture supplied with Copper Acetate and Ferric Citrate. The yield of mAb reached more than 10 g/L in 14 days fed-batch culture, Copper Acetate and Ferric Citrate diminished man5 content and acidic charge variants of mAb and increased the proportion of G1F (Mahé et al. 2022).

Feeding strategy

In addition to adjusting the composition of feed medium and setting reasonable process parameters, it is necessary to design and develop available feeding strategy to produce the high-yield and high-quality of recombinant proteins (Horvat et al. 2020). The control criterion and the mode of feeding should be taken into consideration in the aspect of feeding strategy. Keeping the concentration of one or two main nutrients at an ideal level is completed by control criterion. According to the stoichiometric feed composition, the rest of nutrients are added to culture system (Costa et al. 2014). Feeding strategy is usually divided into two addition methods: continuous feeding and bolus feeding. Bolus feeding has the advantages of simple operation, low facility requirements and low production costs. Bolus feed delivery strategy increases the expression of mAbs in CHO cells and is the most widely used fed-batch culture method for large-scale mammalian cell culture. However, the addition of a large amount of feed medium leads to accumulation of metabolic byproducts and the increase of osmolality. Continuous feeding can deal with two problems above, and it can adjust the feeding rate to maintain the concentration of nutrients, which is based on the nutrients consumption. Therefore, continuous feeding has more advantages in the aspect of cell culture (Xiao et al. 2021; Kamachi and Omasa 2018).

To promote the high expression of anti-HER2 mAb in CHO cells, Feed 4 was added at 5% of the initial culture volume at days 3, 5, 7, and 9. Peak VCD was 17.1×10^6 cells/mL in fed-batch culture and mAb yield reached 437 mg/L (Yuan et al. 2020). In order to improve the expression of human mouse chimeric anti epidermal growth factor receptor variant III (EGFR vIII) antibody C12 in CHO cells, continuous feeding strategy was adopted and feed medium was added to the bioreactor from day 3. Compared with bolus feeding methods, the continuous feeding method reduced the ratio of cell metabolic byproducts, culture osmolality and high mannose type, improved the VCD and viability of CHO cells, and the antibody production exceeds 10 g/L (Xiao et al. 2021). Horvat et al. used continuous feeding to optimize fed-batch culture process to culture CHO cells. From day 4, glucose solution with a concentration higher than 400 g/L was supplemented to keep final glucose concentration less than 5.55 mmol/L. From day 5, feed medium containing serine was continuously added (Horvat et al. 2020). Continuous feeding can reduce the osmolality and mannosylation ratio of the cell culture, but it increased the mAb yield. If the VCD and cell viability of CHO cells show a downtrend in the late exponential phase or in the early stationary phase, feed medium should be added in cell culture. Great feed strategy has a positive impact on RTP production.

Monitoring and control system is introduced in fed-batch culture, which maintains key cellular nutrients at an ideal level. Bolus feed delivery strategy is widely used for fed-batch culture, but it cannot adjust the medium concentration to the dynamic nutrient consumption rate of cells at different metabolic stages (Xie and Wang 1994a, b).

Raman spectroscopy is used for monitoring nutrient and byproducts (glucose, lactate, and amino acid) (Domján et al. 2022). Dynamic feeding strategies are used to control the delivery of feed medium via Raman spectroscopy. In an early phase of the development, Domján et al. established a partial least squares (PLS) calibration model that took the cell metabolic behavior and nutritional consumption into account. Subsequently, the nutrients are maintained at the desired level (Domján et al. 2022). For mAb production in CHO cells, Eyster et al. developed Raman spectroscopy to control lactate and glucose in fed-batch culture, and established PLS model for predicting glucose and lactate concentrations. Three feeding strategies were evaluated in terms of cell metabolism, productivity, and product quality. Ammonium levels were reduced by 68% through controlling lactate at 2 g/L, while mAb galactosylation levels were increased by approximately 50% (Eyster et al. 2021) .

Metabolic byproducts in fed-batch culture

In the late stages of cell culture, fed-batch culture usually stores byproducts such as lactate and ammonium (Brunner et al. 2017; Fan et al. 2015). High concentrations of

metabolic byproducts are detrimental to cell growth and antibody expression (Torres et al. 2018; Zhang et al. 2019; Konakovsky et al. 2016; Karengera et al. 2018). Glucose is transferred to the cytoplasm by transport protein GLUT1 in CHO cells, and a large amount of pyruvate is generated through glycolysis. Part of pyruvate using lactate dehydrogenase is further converted to lactate (Li et al. 2012). To culture CHO cells expressing Fc-fusion protein and reduce lactate accumulation in fed-batch culture, galactose is used as a carbon source to reduce accumulation of lactate (Xiao et al. 2019). CHO-K1 cells expressing anti-HIV antibody VRC01 were cultured in fed-batch culture. Feed medium was added daily starting from day 3. After 12 h of cell inoculation, 10 mM and 30 mM NH_4Cl were added to the CHO cell culture medium. Compared with the control group, VCD and culture time of group treated with 30 mM NH_4Cl were significantly reduced, and byproducts including lactate, glucose, ammonium, and glutamine were accumulated. Particularly, ammonium had a negative impact on antibody titer and cell-specific productivity (Chitwood et al. 2021).

The addition of TCA cycle intermediates including alpha-ketoglutarate (α-KG), malate, and succinate to CHO cells can reduce accumulation of lactate and ammonium in fed-batch culture and improve cell-specific productivity and antibody titer (Zhang et al. 2020b). Xiao et al. designed continuous feeding strategy in fed-batch culture. They found that continuous feeding reduced accumulation of lactate and ammonium because of massive nutritional feed medium in a 7-L bioreactor. Finally, the production of C12 antibody was promoted (Xiao et al. 2021). In fed-batch culture, byproducts should be avoided. It is advisable for fed-batch culture to choose available feeding strategies to reduce the probability and concentration of metabolic byproducts accumulation.

Chemostat cultivation

For improving cell-specific productivity of RTPs, successful strategies require to manipulate culture temperature and glucose concentration in media. Unfortunately, changes in cell culture affect the specific growth rate, especially in both operational variables. To produce recombinant human tissue plasminogen activator (rh-tPA) in CHO cell, Vergara et al. utilized chemostat cultivation and set three groups about feeding media with 20 mM, 30 mM, and 40 mM glucose concentrations. A total of 40 mM glucose limited cell growth, but it increased cell-specific productivity of rh-tPA and cells in the G2/M phase. Furthermore, glucose consumption and lactate production rates were reduced when temperature condition was 33 °C. Vergara et al. indicated that a reduced specific growth rate together with high feed glucose rapidly improves protein productivity in CHO cell (Vergara et al. 2018).

The intensified fed-batch

In the biomanufacturing industry, intensified processes require more time to develop compared with conventional fed-batch.

Schulze et al. performed standard fed-batch (sFB) and intensified fed-batch (iFB) processes in 15 mL and 250-mL bioreactors, starting with 4% (vol / vol) feed medium A and 4% (vol / vol) feed medium B. The final glucose concentration was 5 g/L. Cell concentrations inoculated by N-1 perfusion reached 1×10^8 cells/mL, which are used for the iFB. In 15 mL bioreactors, two iFBs inoculated at 2.5 and 5×10^6 cells/mL were from N-1 perfusion. Compared with sFB process, similar titers were reached in shorter process times, but respectively, the space–time yield (STY) was dramatically increased by 16% and 36% for the 2.5 and 5×10^6 cells/mL methods. In 250-mL bioreactors, iFB inoculated at 5×10^6 cells/mL added 2.5 mM butyric acid (BA) after 48 h. Compared with iFB inoculated at 5×10^6 cells/mL without BA, KPI was found by raising the STY by 50% after BA treatment. The average cell-specific productivity was increased from 25 to 37 pg/cell/day through BA supplementation (Schulze et al. 2022a, b).

Discussion

RTPs are fast developing. About 40 novel antibodies are introduced each year. How to implement high-level expression and production of RTPs is a challenge which limits RTP drugs development. These are state-of-the-art strategies for boosting RTP production and quality, such as optimizing gene sequence, expression vector, and glycosylation (Zhang et al. 2022).

A report has shown that the biosimilar development of pembrolizumab can be directed by Quality by design (QbD) (Jaffar-Aghaei et al. 2022). QbD is an efficient but challenging approach for the development of biosimilar due to the complex relationship among process, quality, and efficacy (Zhang et al. 2020c). To ensure critical quality attributes (CQAs) about the production of mAbs, an understanding of the production process is highlighted by QbD (Gibbons et al. 2022). Jaffar-Aghaei et al. found a pharmaceutical measurement guided by QbD to undertake the Pembrolizumab biosimilar candidate PSG-024 (Keytruda®) in fed-batch culture. Currently, Keytruda® has become the ace of therapeutic mAbs (Jaffar-Aghaei et al. 2022).

Despite the fact that glucose concentration above 1 g/L had brought 10 g/L mAb titer, Handlogten et al. still pointed out that bolus feeding was defective (Handlogten

et al. 2018). Recently, the final mAb titer of 4 g/L was reached by the glucose concentration of 12 g/L in fed-batch platform process (Schellenberg et al. 2022); meanwhile, the final glucose concentration was 5 g/L. This glucose feeding strategy also promoted cell growth efficiently. Cysteine greater than 2.5 mM with low cell densities resulted in counteracted oxidative stress, but Komuczki et al. presented that high cysteine concentration with a higher cell concentration can counteract oxidative stress (Komuczki et al. 2022). Wang et al. reported that an upturn in terms of the product titer was influenced by temperature shifted to 33℃ in 14-day fed-batch culture, and 100% normalized titer was set as the highest titer on day 14. Fed-batch optimization is available and nevertheless, optimizing all process parameters is impossible on account of limited resources and timeline (Wang et al. 2022).

Biosimilars will enter the markets in the context of COVID-19 (Schulze et al. 2022a, b; Tichy et al. 2022), it depends on a robust and scalable manufacturing with excellent productivity. PI is a key trend involving process strategies. Along with the advance of perfusion and other processes, fed-batch culture has been studied increasingly (MacDonald et al. 2022; Schulze et al. 2022a, b). Presently, there is still some room for improvement in fed-batch culture (Wang et al. 2022).

Summary and prospect

Overall, fed-batch culture has harvested high-yield and high-quality protein production, prolonging cell culture time. Feed medium is replenished continuously or intermittently in fed-batch culture. In recent years, fed-batch culture has made considerable progress. Feed components optimization is still essential for process development. Simultaneously, fed-batch culture should consider culture parameters, nutrient consumption and accumulation of metabolic byproducts, etc. With in-depth exploration of RTP production in CHO cells, we still take these indicators into account in the future, such as cell growth, the yield and quality of RTPs. However, fed-batch culture has defects in the aspect of process development, choosing optimal parameters is challenging, including temperature, pH, dissolved oxygen, basal and feeding medium, and additives. Exploring a series of great process parameters are constrained by limited factors. With the wide application of multi-omics, new monitoring and analysis technology, artificial intelligence together with other methods, intensified fed-batch culture will further improve the yield and quality of recombinant protein production.

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Declarations

Human and animal rights and informed consent This paper does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare no competing interests.

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