Numerical simulation of scaling-up an inverted frusto-conical shaking bioreactor with low shear stress for mammalian cell suspension culture

Ning Ding · Chao Li · Meijin Guo · Ali Mohsin · Siliang Zhang

Received: 8 October 2018 / Accepted: 20 February 2019 / Published online: 8 March 2019
© Springer Nature B.V. 2019

Abstract Shear stress is one of the key factors affecting the large-scale culture of mammalian cells. In this study, numerical simulation based on computational fluid dynamics was used to conduct a flow-field analysis of 7, 50, 200, and 1200 L inverted frusto-conical shaking bioreactors. The results show that the shear rate, specific mass transfer area (a), and volumetric oxygen mass transfer coefficient (k_La) gradually decreased as the scale of the bioreactor increased. Through application of BHK21 and CHO cells in 7, 200, and 1200 L bioreactors, it was found that the cell density and antibody expression level increased as the volume of the bioreactor increased. Moreover, the antibody expression level in a 1200 L bioreactor was nearly 30% and 35% higher than that of 7 and 200 L bioreactors, respectively. The results demonstrate that the environment with a larger volume is more suitable for the growth and antibody expression of

CHO cells, indicating shear stress might be the most critical factor affecting the scale-up of mammalian cells.

Keywords Inverted frusto-conical shaking bioreactor · Scale up · Low shear stress · Computational fluid dynamics · Numerical simulation · Suspension cell culture

Abbreviations

A	Centrifugal acceleration (m/s^2)
α	Gas–liquid specific mass transfer area (m^{-1})
BHK	Baby hamster syrian kidney
CFD	Computational fluid dynamics
CHO	Chinese hamster ovary
DO	Dissolved oxygen (%)
Hr	Relative height of liquid level (dimensionless)
IFSB	Inverted frusto-conical shaking bioreactor
k_L	Oxygen mass transfer coefficient (m/h)
k_La	Volumetric oxygen mass transfer coefficient (h^{-1})
P/V_L	Specific power consumption (Kw/m^3)
SCBB	Sharp-point conical bottom bioreactor
ε	Energy dissipation rate (m^2/s^3)

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10616-019-00308-3>) contains supplementary material, which is available to authorized users.

N. Ding · C. Li · M. Guo (✉) · A. Mohsin · S. Zhang (✉)
State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China
e-mail: guo_mj@ecust.edu.cn

S. Zhang
e-mail: siliangz@ecust.edu.cn

Introduction

In recent years, many mammalian cell lines that can adapt to suspension cultures in serum-free and animal origin-free media have been identified, including CHOD, CHOK, HEK293, and NS0 (Porte et al. 2017; Leong et al. 2018; Leticia et al. 2015). Animal origin-free suspension culture media, including basal medium and fed-batch medium, have also been developed. In the small scale culturing, for example, a bench-top bioreactor with a working volume from 100 to 1000 mL or a 100 mL spinner, the dissolved oxygen (DO) tension and the pH of a suspension culture system can be maintained through sufficient surface aeration and by adding a non-carbon dioxide-dependent buffer such as 20-mM HEPES buffer instead of in situ pH and DO probes that can automatically adjusted pH and DO by a control system (Monteil et al. 2016). Therefore, several simple and easily optimized bioreactors for the suspension culture of mammalian cells have already been developed in past 20 years.

Currently, there are many types of bioreactors for the culturing of animal cells, whereas, for laboratory researchers, simple and easy-to-operate Erlenmeyer (Muller et al. 2005; Büchs 2001) and spinner flasks (Annathur et al. 2003; Hiedemann et al. 1994) are the first choices for small-scale suspension culturing of cells. However, whether it is a cone-shaped Erlenmeyer flask or a cylindrical spinner flask, the specific surface area of the culture remains relatively small, which is not only insufficient for effective oxygen supply in high-density suspension culture of mammalian cells, but also disadvantageous for the discharge of CO₂ produced by cells. Moreover, the scale up of bioprocesses developed in a cylindrical spinner is difficult as well. Therefore, our research group has developed an inverted frusto-conical shaking bioreactor (IFSB) for animal cell cultures (Hang et al. 2011). Comparatively, it outperforms the 5 L mechanically stirred bioreactor and the sharp-point conical bottom bioreactor (SCBB) in terms of cell-culture performance. This is due to less shear force below than 30%, resulting in more than 10% viable cell density. The reason might be that the culture media used in the IFSB has a large surface area, which is favorable for supplying oxygen and removing carbon dioxide, and the cells are subjected to a small shear force as they are suspended with the liquid flow. Recently, Zhu et al.

(2018) has developed a three-dimensional CFD model to analyze the flow field in the IFSB-5L, the engineering parameters- P/V_L and k_La -were calculated at different filling volumes and shaking speeds under different the operating conditions. However, the numerical simulation of scaling up an inverted frusto-conical shaking bioreactor has not been reported until now.

In this study, numerical simulation based on computational fluid dynamics (CFD) was used to evaluate the fluid field performance of IFSB scaling up from 7 to 1200 L. In addition, the growth of BHK21 and recombinant antibody C12 expression by Chinese hamster ovary (CHO) cells in IFSBs with different sizes were tested.

Materials and methods

Cell culture

BHK21 cells (purchased from the Shanghai Institute of Cell Science, Chinese Academy of Sciences) were inoculated in IFSBs for batch culture (Yan et al. 2008), with an inoculation volume of 20%, an inoculation density of 2.0×10^5 cells/mL, and a rotation speed of 120 rpm, with the temperature controlled at 37 °C. Samples were taken for analysis after every 12 h.

A recombinant CHO-C12 cell line was generated as described in previous study (Wang et al. 2009) and stored in our laboratory, which were then subjected to fed-batch culture to express antibody C12. C12 is a mouse-human chimeric anti-epidermal growth factor receptor vIII antibody (Hu et al. 2011). The IFSBs had an initial inoculation volume size of 10%, an inoculation density of 1.0×10^6 cells/mL, and a rotation speed of 120 rpm, with the temperature controlled at 37 °C. pH value was set at 7.0 and DO was controlled in between 40 and 60% air saturation. The fed-batch medium was added starting from the fifth day at 300 mL/d. Samples were taken after every 24 h.

CFD simulation of bioreactor

Geometric model

The detailed geometric dimension of the 7 L inverted frusto-conical shaking bioreactor used in this study with a cone angle of 45° is shown in Fig. 1. The

Fig. 1 Geometric dimensions of 7 L bioreactor

diameter of the flask was 250 mm and the diameter of the flask neck was 90 mm. The parameters of the other bioreactors were scaled up proportionally, and are shown in Table 1.

Numerical simulation method

In this study, the method proposed by our group (Li et al. 2013) was used to simulate the flow-field characteristics in the oscillating bioreactors. The gas–liquid interface was simulated by the free-surface method, and the RNG k- ϵ model was used as the turbulence model. The meshing was performed using ANSYS ICEM CFD 15.0 software with a total of 1.5 million grids. ANSYS CFX PRE was used for modeling, and all boundaries were set as wall-boundary conditions. ANSYS CFX SOLVE was used to obtain the solution and ANSYS CFX POST was

applied for final data post-processing. The solving process was performed on a 96-core Dawning server and simulated using an unsteady-state method to obtain the motion state of the liquid. The initial liquid was in a static state and the liquid level was initialized by the step function, with the time step of the calculation being 0.05 s. The convergence criterion was the internal iteration residual being less than 0.0001, and the physical time calculated by the model was 15 s.

Analytical methods

Cell counting

Cell density: Cell density was determined using a hemocytometer, and the trypan blue staining method was used for distinguishing dead and viable cells.

Calculation of cell viability : cell viability (%)

$$= \frac{\text{number of unstained cells}}{\text{total number of cells}} \times 100\%.$$

Antibody C12 concentration determination

The chimeric anti-EGFRvIII C12 antibody concentrations in the supernatants were estimated by sandwich ELISA by following the method described in earlier study (Hu et al. 2011).

Results and discussion

Liquid surface morphology of IFSBs

Changes in liquid surface morphology can characterize the gas–liquid contact area and the liquid motion pattern in bioreactors as well as the mixing

Table 1 Parameters for scaling up the inverted frusto-conical shaking bioreactor

Parameters	7 L	50 L	200 L	1200 L
Volumetric scaling-up-fold	1.0	7.143	28.571	171.43
One-dimensional scaling-up-fold	1.0	1.926	3.057	5.555
Filling volume (L)	3.0	21.4	85.7	514.3
Eccentricity (radius) (cm)	2.5	5.0	10.0	20.0
Rotation speed (rpm)	60–120	40–100	30–75	20–50
Initial liquid height (m)	0.128	0.247	0.391	0.711

performance of the bioreactors (Tan et al. 2011; Zhang et al. 2008). As shown in Figure S1, regardless of the size of the bioreactor, the centrifugal force acted on the liquid increased gradually with increasing rotation speed, and both the height of the liquid level and the degree of liquid-surface depression increased significantly, indicating that an increase in the rotation speed can remarkably enhance mixing and mass transfer in bioreactors. By comparing the changes in the liquid surface morphology of bioreactors of different sizes, it can be found that, despite of the different sizes, there is no significant difference among different bioreactors regarding the liquid surface morphology under the rotation speed set in this study. Moreover, since the liquid level reached near the electrode mouth at the highest rotation speed, the speed could not be further increased. To further determine the relationship between the change in the liquid level and the operating conditions, we plotted the relative change in the liquid level (i.e., the height of the liquid level after the rotation divided by the initial height of the liquid level) to the centrifugal acceleration of the liquid (i.e., a parameter that includes both the influences of the rotation radius and the rotation speed) (Figure S2). It can be seen from Figure S2 that the relative height of the liquid level was correlated with the centrifugal acceleration to some extent, but it was not related to the size of the bioreactor. As the centrifugal acceleration increased, the relative height of the liquid level gradually increased. By fitting the relative change in the liquid level with the centrifugal acceleration, the following relationship was found:

$$H_r = 1.1637A^{0.306},$$

where H_r is the relative height of the liquid level (dimensionless) and A is the centrifugal acceleration (m/s^2), which can be calculated based on the eccentricity and rotation speed of the shaker. The correlation coefficient of this fitting equation was 0.973, implying a good fitting effect. Based on this equation, the height of the liquid level of the bioreactor at any rotation speed and eccentricity can be calculated, which can be used to guide the selection of the shaker as well as the setting of the liquid volume and rotation speed.

Flow-field analysis

Figure S3 shows the velocity-field distribution of bioreactors of different sizes with different rotation speeds. As the rotation speed increased, the movement speed of the liquid in the bioreactor gradually increased. Although the angular speed of the liquid movement in the bioreactor was the same at any part of the bioreactor, the liquid near the bioreactor wall had a larger moving radius, and the centrifugal force was greater, so the liquid near the bioreactor wall (where the liquid level is higher) had a movement speed higher than the liquid in the middle of the bioreactor. The liquid in the middle of the bioreactor was subjected to the lowest centrifugal force, so it exhibited the lowest movement speed. Comparing the bioreactors of different sizes showed that, although the bioreactors were different in size, due to their different rotation radii and shaking speeds there was no significant difference among different bioreactors regarding the movement speed of the liquid inside them.

Turbulent dissipation rate

The energy dissipation rate can characterize the liquid turbulence intensity and liquid power consumption within the bioreactor. It can be seen from Figure S4 that as the rotation speed increased, the power applied to the bioreactor by the shaker gradually increased, so the energy dissipation rate in the bioreactor also increased. The distribution of energy dissipation rate was similar to the distribution of velocity contour presented in Figure S3. This is mainly because the liquid near the wall of the flask was moving fast, so the turbulence intensity was strong and the energy was dissipated fast as well. As shown in Fig. 2, there was a correlation between the energy-dissipation rate and the centrifugal acceleration; that is, as the centrifugal acceleration increased, the energy-dissipation rate also increased. By conducting a fitting analysis, it was found that the two adhere to the following relationship:

$$\varepsilon = 1.1637A^{0.306},$$

where ε is the energy dissipation rate (m^2/s^3). The correlation coefficient of this fitting equation was 0.859, showing a good fitting effect.

Fig. 2 Relationship between energy dissipation rate and centrifugal acceleration

According to the equation, the energy-dissipation rate of this bioreactor at any rotation speed and eccentricity can be calculated, and then the power consumption of the bioreactor and various parameters related to power consumption, e.g. mass transfer, mixing, and shear, can be estimated as well (Tan et al. 2011; Maier et al. 2004).

Shear rates of IFSBs

Shear rate is an important factor because the animal cells are very sensitive to shear, and that's why shear rate is often used as a standard factor for scale-up culturing (Collignon et al., 2016; van Oers et al. 2015; Wang et al. 2018). Figure S5 shows the distribution of shear rates of bioreactors of different sizes with different rotation speeds. The areas with a relatively large shear rate were mainly concentrated near the flask wall and the liquid surface, which is consistent with the results reported by Li et al. (2013). As the rotation speed increased, the shear rate in the bioreactor increased substantially. Comparing bioreactors of different sizes showed that as the bioreactor size increased, the shear rate gradually decreased. However, overall the shear rate of this type of bioreactor is significantly lower than that of mechanical agitation bioreactors (Wu et al. 2006). As shown in Fig. 3, at the same centrifugal acceleration, the larger the size of the bioreactor, the smaller the average shear rate. Since animal cells are sensitive to shear, if the entire scale-up process is mainly affected by the shear rate, it can be predicted that by increasing size of the bioreactor, the growth state of the cells could be gradually improved.

Fig. 3 Relationship between shear rate and centrifugal acceleration

Mass transfer coefficients of IFSBs

The gas–liquid mass transfer in an oscillating bioreactor mainly depends on the gas exchange on the liquid surface, and mass transfer in such bioreactors is generally weaker than that of mechanical agitation bioreactors (Li et al. 2013; Maier et al. 2004). Figure 4 shows the changes in the oxygen mass transfer coefficient (k_L), gas–liquid specific mass transfer area (a), and volumetric oxygen mass transfer coefficient ($k_L a$) of different bioreactors at different rotation speeds. It can be seen from Fig. 4a that k_L was correlated with the centrifugal acceleration, and it was found through fitting analysis that the two fulfill the following equation:

$$k_L = 0.1368 \ln A + 0.0997,$$

where k_L is the mass transfer coefficient (m/h). The correlation coefficient of the fitting equation was 0.989, indicating goodness of fit of proposed model.

As the centrifugal acceleration increased, the mass transfer coefficient was increased significantly. However, there were no significant differences among bioreactors of different sizes in terms of the maximum mass transfer coefficient. It can be seen from Fig. 4b that as the centrifugal acceleration increased, the gas–liquid specific mass transfer area gradually increased. Nonetheless, as the size of the bioreactor increased, the gas–liquid specific mass-transfer area gradually decreased, resulting in a decrease in the volumetric oxygen mass transfer coefficient with the increase of the bioreactor volume (Fig. 4c). Thus, as the size of the bioreactor increases, the bioreactor might face an

Fig. 4 Relationship between relevant mass-transfer parameters and centrifugal acceleration

increasingly prominent oxygen-supply problem. However, because the oxygen consumption of animal cells is usually small (Garcia-Ochoa et al. 2010; Schmid 1996), whether oxygen supply becomes a limiting factor at large scale L should be determined by an analysis of the oxygen consumption of cells in the culture system, which is usually expressed by the oxygen-uptake rate (OUR).

Animal cell culture in different size bioreactors from 7 to 1200 L.

Growth of BHK21 cells cultivated in IFSBs

BHK21 cells were cultured for 120 h in the 7, 200, and 1200 L bioreactors. The cell density reached its maximum at 96 h in all three cases. The maximum cell density was the highest in the 1200-L bioreactor,

and the cell density was still high at the end of the culture (Table 2), indicating that the 1200 L bioreactor not only can increase the cell-culture density, but also allows the high cell density to be maintained for a relatively longer period of time.

Growth and recombinant antibody C12 expression of CHO cells

The CHO-12 cells were cultured in the 7, 200, and 1200 L bioreactors. The results are shown in Table 3. As the volume of the bioreactor increased, the cell density and antibody expression level both increased. The antibody expression level in the 1200 L bioreactor was increased by 30% and 35%, respectively, compared with that of the 7 and 200 L bioreactors. This suggests that a large-volume environment is more suitable for the growth and antibody expression of CHO cells in high cell density suspension culture.

Conclusions

In this study, we investigated the fluidic field characteristics of different size IFSBs. It can be seen from the CFD simulation results that there was no significant difference among bioreactors of different sizes in terms of liquid surface morphology, flow field distribution, energy dissipation rate, and mass transfer coefficient (k_L). The shear rate, specific mass transfer area (a), and volumetric oxygen mass transfer coefficient ($k_L a$) gradually decreased as the size of the bioreactor increased. By culturing cells in the 7, 200, and 1200 L bioreactors, it was found that the 1200 L bioreactor outperformed the small-volume bioreactors in terms of the culture density and antibody expression level of cells, which is similar to the CFD simulation results. Although the mass-transfer ability of the 1200 L bioreactor is relatively poor based on the CFD simulation results, nevertheless, the cell density is higher, indicating that the shear of the bioreactor may be the most critical factor affecting the scale-up of culturing animal cells.

Author's contribution This study was designed by ND and CL. The experiments were performed by ND, CL and MG. The paper was written by ND and CL. AM participated in manuscript writing and polished the English thoroughly. MG and SZ

Table 2 Comparison of highest cell densities during BHK21 cell cultivation in Inverted Fruco-conical shaking bioreactors

Bioreactors	Seed density ($\times 10^5$ cells ml $^{-1}$)	Highest cell density ($\times 10^6$ cells ml $^{-1}$)	Cell density at end of culture ($\times 10^6$ cells ml $^{-1}$)
1200 L	2.0 $\pm$ 0.2	6.5 $\pm$ 0.7	6.4 $\pm$ 0.2
200 L	2.0 $\pm$ 0.2	6.2 $\pm$ 0.3	5.8 $\pm$ 0.1
7 L	2.0 $\pm$ 0.2	6.1 $\pm$ 0.6	5.5 $\pm$ 0.2

Table 3 Comparison of maximum cell density and produced antibody C12 by rCHO-C12 cells grown in 7, 200, and 1200 L bioreactors

Bioreactors	Culture duration (h)	Ab C12 (mg/L)	X _{max} (10 ⁶ cells/mL)
7 L	300	980.56	6.51
200 L	300	1020.94	6.50
1200 L	300	1328.40	6.75

participated in the data discussion. All authors read and approved the final manuscript.

Funding This research was supported by grants from the National High Technology Research and Development Program of China (2012AA021201) and the Fundamental Research Funds for the China Central Universities (Nos. 22221818014 and 22221817014) and the 111 Project (B18022) to Meijin Guo.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Availability of data and materials All supporting data are included within the article and its Additional file.

Consent for publication All authors consented to publication of the present manuscript.

Ethics approval and consent to participate The authors declare that this is not a study involving human participants and reporting health related outcomes.

References

- Annathur GV, Pierce JL, Combs RG, Rathore AS, Banerjee A, Steinmeyer DE (2003) Improvements in spinner flask designs for insect-cell suspension culture. *Biotechnol Appl Biochem* 38:15–18
- Büchs J (2001) Introduction to advantages and problems of shaken cultures. *Biochem Eng J* 7:91–98

- Collignon ML, Delafosse A, Calvo S, Martin C, Marc A, Töye D, Olmos E (2016) Large-Eddy simulations of microcarrier exposure to potentially damaging eddies inside mini-bioreactors. *Biochem Eng J* 108:30–43
- Garcia-Ochoa F, Gomez E, Santos VE, Merchuk JC (2010) Oxygen uptake rate in microbial processes: an overview. *Biochem Eng J* 49:289–307
- Hang HF, Guo YX, Liu J, Bai L, Xia JY, Guo MJ, Hui M (2011) Computational fluid dynamics modeling of an inverted frustoconical shaking bioreactor for mammalian cell suspension culture. *Biotechnol Bioprocess Eng* 16:567–575
- Hiedemann R, Rise U, Lutkemeyer H et al (1994) The super-spinner: a low cost animal cell culture bioreactor for the CO₂ incubator. *Cytotechnology* 14:1–9
- Hu SW, Deng L, Wang HM, Zhuang YP, Chu J, Zhang SL, Li ZH, Guo MJ (2011) Bioprocess development for the production of mouse-human chimeric anti-epidermal growth factor receptor vIII antibody C12 by suspension culture of recombinant Chinese hamster ovary cells. *Cytotechnology* 63:247–258
- Leong DSZ, Teo BKH, Tan JGL, Kamari H, Yang YS, Zhang PK (2018) Application of maltose as energy source in protein-free CHO-K1 culture to improve the production of recombinant monoclonal antibody. *Sci Rep* 8:4037. <https://doi.org/10.1038/s41598-018-22490-8>
- Leticia LC, Lecina M, Schucht R, Wirth D, Hauser H, Cairó JJ (2015) Hek293 as a recombinant protein factory: three different approaches for protein production. *BMC Proc* 9:74–79. <https://doi.org/10.1186/1753-6561-9-s9-p74>
- Li C, Xia JY, Chu J, Wang YH, Zhuang YP, Zhang SL (2013) CFD analysis of the turbulent flow in baffled shake flasks. *Biochem Eng J* 70:140–150
- Maier U, Losen M, Büchs J (2004) Advances in understanding and modeling the gas-liquid mass transfer in shake flasks. *Biochem Eng J* 17:155–167

- Monteil DT, Juvet V, Paz J, Moniatte M, Baldi L, Hacker DL, Wurm FM (2016) A comparison of orbitally-shaken and stirred-tank bioreactor: pH modulation and bioreactor type affect CHO cell growth and protein glycosylation. *Biotechnol Prog* 32:1174–1180
- Muller N, Girard P, Hacker DL, Jordan M, Wurm FM (2005) Orbital shaker technology for the cultivation of mammalian cells in suspension. *Biotechnol Bioeng* 89:400–406
- Porte M, Havard J, Moreau V, Vjetrovic J, Stock F, Erbacher P (2017) Improving transient CHO and HEK-293 expression systems with a powerful transfection solution for high protein production yields. *PEGS Eur.* <https://doi.org/10.13140/rg.2.2.28765.10728>
- Schmid G (1996) Insect cell cultivation: growth and kinetics. *Cytotechnology* 20:43–56
- Tan R-K, Eberhard W, Buchs J (2011) Measurement and characterization of mixing time in shake flasks. *Chem Eng Sci* 66:440–447. <https://doi.org/10.1016/j.ces.2010.11.001>
- van Oers MM, Pijlman GP, Vlak JM (2015) Thirty years of baculovirus–insect cell protein expression: from dark horse to mainstream technology. *J Gen Virol* 96:6–23
- Wang HM, Jiang H, Zhou M, Xu ZB, Liu SG, Li ZH (2009) Epidermal growth factor receptor vIII enhances tumorigenicity and resistance to 5-fluorouracil in human hepatocellular carcinoma. *Cancer Lett* 279:30–38
- Wang H, Xia J, Zheng Z, Zhuang Y-P, Yi X, Zhang D, Wang P (2018) Hydrodynamic investigation of a novel shear-generating device for the measurement of anchorage-dependent cell adhesion intensity. *Bioprocess Biosystems Eng.* <https://doi.org/10.1007/s00449-018-1964-6>
- Wu J, Graham LJ, Noui Mehidi N (2006) Estimation of agitator flow shear rate. *AIChE J* 52:2323–2332
- Yan H, Guo MJ, Huang M, Zhang M, Zhang SL (2008) Adaptation of baby hamster kidney (BHK21) cells to culture with low serum medium and comparison of cell primary metabolism. *Chem Bioeng* 25:21–26
- Zhang XW, Stettler M, Reif O, Kocourek A, De Jesus M, Hacker DL, Wurm FM (2008) Shaken helical track bioreactors: providing oxygen to high-density cultures of mammalian cells at volumes up to 1000 L by surface aeration with air. *New Biotechnol* 25:68–75
- Zhu LK, Zhang XT, Cheng K, Lv ZH, Zhang L, Meng QY, Yuan SJ, Song BY, Wang ZL (2018) Characterizing the fluid dynamics of the inverted frustoconical shaking bioreactor. *Biotechnol Prog* 34:478–485

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.