Journal of Biotechnology 194 (2015) 100–109

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Determination of the maximum operating range of hydrodynamic stress in mammalian cell culture

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ARTICLE INFO

Article history:
Received 27 March 2014
Received in revised form 8 November 2014
Accepted 9 December 2014
Available online 18 December 2014

Keywords:
Hydrodynamic stress threshold
Mammalian cell culture
Scale-down
Oscillating stress
Maximum operating range

ABSTRACT

Application of quality by design (QbD) requires identification of the maximum operating range for parameters affecting the cell culture process. These include hydrodynamic stress, mass transfer or gradients in dissolved oxygen and pH. Since most of these are affected by the impeller design and speed, the main goal of this work was to identify a maximum operating range for hydrodynamic stress, where no variation of cell growth, productivity and product quality can be ensured. Two scale-down models were developed operating under laminar and turbulent condition, generating repetitive oscillating hydrodynamic stress with maximum stress values ranging from 0.4 to 420 Pa, to compare the effect of the different flow regimes on the cells behavior. Two manufacturing cell lines (CHO and Sp2/0) used for the synthesis of therapeutic proteins were employed in this study. For both cell lines multiple process outputs were used to determine the threshold values of hydrodynamic stress, such as cell growth, morphology, metabolism and productivity. They were found to be different in between the cell lines with values equal to 32.4 ± 4.4 Pa and 25.2 ± 2.4 Pa for CHO and Sp2/0, respectively. Below the measured thresholds both cell lines do not show any appreciable effect of the hydrodynamic stress on any critical quality attribute, while above, cells responded negatively to the elevated stress. To confirm the applicability of the proposed method, the obtained results were compared with data generated from classical small-scale reactors with a working volume of 3 L.

1. Introduction

Production of biopharmaceuticals is a delicate process considering the risks connected to the administration of the product into human beings and the possibility of life-threatening side effects (Attarwala, 2010; Schneider et al., 2006). To minimize these risks, the Quality by Design (QbD) concept (US Food and Drug Administration, 2009) aims at increasing the knowledge of the production process and characterizing the relationship between process conditions and critical quality attributes (CQA). This includes the understanding of the impact of raw materials variability and different production scales on the final product (Rathore, 2009; Seely and Seely, 2003). Key component of this concept is the design space that needs to be characterized for each process step resulting in a maximum operating range, which describes the overall process operational limits for which a defined product quality can be ensured. Mostly, this evaluation is performed at development scale using design of experiments (DoE), which raises the question how valid the results are at manufacturing scale (Eon-duval et al., 2014). The micro environment at manufacturing scale can vary substantially due to variations in mixing time, possibly inducing pH, oxygen and temperature gradients as well as differences for oxygen transfer and carbon dioxide removal (Amanullah and McFarlane, 2001; Li et al., 2010; Sieblist et al., 2011). A maximum operating range established at small scale (Eon-duval et al., 2012; Rouiller et al., 2012) needs to be proven valid across other scales. Therefore options are limited. Application of a similar strategy at large-scale is possible; however, it requires very high investment for the manufacturer. The alternative would be to define a maximum operating range of scale independent parameters, e.g. O_2 transfer, CO_2 removal, temperature or maximum hydrodynamic stress (Eon-duval et al., 2014; Sieck et al., 2014), instead of using scale dependent parameters like stirring speed or gas flow rate. In this way some process parameters will be different at different scales, but can be defined as unique scale independent parameters. This implies the use of scale independent

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http://dx.doi.org/10.1016/j.jbiotec.2014.12.003
0168-1656/

D	diameter of the impeller [m]
d_{nozzle}	diameter of the nozzle [mm]
N	impeller agitation rate [1/s]
N_P	impeller power number
N_{Pump}	centrifugal pump agitation rate [rpm]
q_i	specific production/consumption rate of i [pg/cell*day]
Q_{loop}	flow through the loop [L/min]
Q_{nozzle}	flow through the nozzle [ml/min]
Re_{nozzle}	Reynolds number of the nozzle
V	culture volume [L]
X_t	viable cell concentration at time t [10^6 cells/mL]

List of Greek symbols

ε	local energy dissipation rate of turbulent flow [W/kg]
$\langle\varepsilon\rangle$	mean energy dissipation rate [W/kg]
$\tau_{\exp}$	experimentally determined $\tau_{\max}$ [Pa]
τ_{IS}	inertial sub range of turbulent hydrodynamic stress [Pa]
$\tau_{\max}$	maximum hydrodynamic stress [Pa]
τ_{VS}	turbulent hydrodynamic stress [Pa]
v_{Tip}	impeller tip speed [m/s]

parameters in a DoE approach, to define a maximum operating range valid for multiple scales.

Process productivity is nowadays achieved by increasing cell densities beyond 20×10^6cells/mL, optimizing specific productivity and culture duration. In these conditions oxygen supply of state-of-the-art suspension cell cultures is challenging due to the combination of high cell density, increased specific oxygen uptake rate and reduced mass transfer caused by media components, i.e. surfactants. Compensation can be achieved by increased aeration combined with the proper level of agitation. Although the effect of hydrodynamic stress on mammalian cells is well-researched (Chisti, 2001, 2010) and mammalian cells show good resistance against stress, as shown by several authors (Al-Rubeai et al., 1995; Godoy-Silva et al., 2009b; Nienow, 2006; Nienow et al., 2013; Tanzeglock et al., 2009), different thresholds of hydrodynamic stress are tolerated by various cell lines. Due to this, any new process development within a QbD framework has to contain the response study of a culture to the above mentioned scale independent parameters. In particular, the lower limit will be determined by minimum O_2 transfer and CO_2 removal rates, and mixing performance of the bioreactor, while the upper limit of the maximum operating range will be defined by the threshold values for lethal or sub-lethal effects due to stress. In classical single vessel scale down models high stirring speed is used to reach the edge of failure for the hydrodynamic stress (Nienow, 2009; Nienow et al., 2013). While this is the simplest possible approach the application of high agitation drastically reduces the exposure period of cells to highest stress values present closed to the impeller. Furthermore, depending on the robustness of the investigated cells, to reach the stress threshold values it could require agitation rates outside of the technical limit of the used motor. Additionally vortex formation becomes very likely with the consequence of introducing air bubbles into the culture, a highly undesired effect because it can cause cell damage (Nienow, 1998).

Therefore, the goal of this study was to develop a scale-down system allowing us to cover a broad range of hydrodynamic stress avoiding the above mentioned limitations. After a detailed characterization of the proposed system it was used to investigate the effect of oscillating hydrodynamic stress on cell growth, productivity and product quality using two recombinant manufacturing cell lines. The first was derived from a Chinese hamster ovary (CHO) host cell line and the second from a mouse hybridoma (Sp2/0) host cell line. Since various approaches were applied previously in the literature, where cells were exposed to hydrodynamic stress, using laminar or turbulent conditions (Godoy-Silva et al., 2009b; Keane et al., 2003; Kunas and Papoutsakis, 1990; Oh et al., 1989; Sieck et al., 2013; Tanzeglock et al., 2009), both flow regimes were applied in the present study to identify an impact of the flow type on cells performance.

2. Materials and methods

2.1. Cell lines and bioreactor setup

Two model cell lines were used in this study. The first derived from a CHO host cell line producing a fusion protein, which is in clinical development. The second was derived from a Sp2/0 host cell line producing a commercialized monoclonal antibody.

Inoculum preparation for the CHO cells was started by thawing a cell bank vial and directly diluting it into proprietary animal-derived component free expansion media. The culture was kept at 37 °C and 90% humidity. Cell density after thawing was $0.5 \pm 0.1 \times 10^6$ cells/mL and at least three sub-cultivation steps were performed every week to keep the cell density below 2.5×10^6 cells/mL. Due to relatively high cell densities used for bioreactor seeding, cells were diluted into a second nutritionally rich expansion media, during the last 5 days of the inoculation preparation, to grow them to a cell density of more than 5×10^6 cells/mL. Independent of the bioreactor size, the duration of the expansion culture was always kept at 14 days. Cells were seeded with a density equal to $1.4 \pm 0.1 \times 10^6$ cells/mL into a bioreactor prefilled with animal-derived component free proprietary media. For this cell line a 3 L bioreactor (Sartorius Stedim, Germany) was used equipped with online temperature, pH and dissolved oxygen (DO) measurement probes (Mettler-Toledo, Switzerland) and one elephant ear impeller inclined by 45° from horizontal plane, pumping the liquid downwards (see Fig. 1). Agitation speed during the culture was set to 150 rpm and the volume average energy dissipation rate was calculated according to (Perry et al., 1997):

$$\langle\varepsilon\rangle = \frac{N_P D^5 N^3}{V} \quad (1)$$

where N_P is the power number of the impeller, D the impeller diameter, N the agitation rate and V the bioreactor volume. Considering N_P equal to 2.35 this results in $\langle\varepsilon\rangle$ being equal to 9.5×10^{-3} W/kg. Temperature was controlled at 37 °C for the whole cultivation process. Due to lactate production of the growing culture, the pH profile was characterized by a drift from the initial value of 7.2 down to 7.0 over the first 2 days. After this reduction, the pH was kept constant until the end of the culture by the addition of acid (lactic acid, 1 M) and base (NaOH, 0.5 M) when needed. To keep the dissolved oxygen (DO) at the set-point of 50% air saturation, a constant air flow of 5 mL/min combined with an oxygen on demand strategy was applied resulting in a total volumetric gas flow being below 40 mL/min (0.013 vvm). Glucose was fed daily from day 3 in order to keep the glucose concentration in the bioreactor between 2 g/L and 4 g/L. Additionally, a chemically defined bolus feed was added on day 6.

For the expansion of the Sp2/0 culture, cells were thawed into a complex proprietary media and were first cultured in orbitally shaken T-flasks and then, when an appropriate volume was reached, in spinner flasks (Integra, Switzerland). The culture was incubated at 37 °C and 90% humidity. Initial cell density was $0.5 \pm 0.1 \times 10^6$ cells/mL and sub cultivation was performed every 2 days to keep the cell density below $1.5 \pm 0.1 \times 10^6$ cells/mL. Cells