Process Biochemistry 46 (2011) 35–45

Contents lists available at ScienceDirect

Process Biochemistry

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

Performance of a vortex flow bioreactor for cultivation of CHO-K1 cells on microcarriers

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ARTICLE INFO

Article history:
Received 15 June 2009
Received in revised form 19 April 2010
Accepted 3 May 2010

Keywords:
Taylor vortex flow
Oxygen transfer
Animal cell culture
Shear stress
Bioreactor
Microcarriers

ABSTRACT

In biochemical processes involving cultivation of animal cells, the design of a bioreactor is important because it should enable the provision of appropriate oxygen and nutrients to the culture medium at low shear stress. The aim of this study is to evaluate a new bioreactor designed and built based on the concept of Taylor vortex flow (Taylor vortex flow bioreactor – TVFB) employed in the cultivation of animal cells. The overall mass transfer volumetric coefficient (K_La) was determined in order to evaluate the oxygen transfer. Mass transfer correlation under Taylor vortex flow regimes were estimated considering the overall volumetric oxygen transfer coefficient operating conditions and the geometry of the bioreactor. Experimental cultures were performed with CHO-K1 (*Chinese Hamster Ovary*) cells attached to microcarriers in TVFB. Cell culture carried out in the bioreactor exhibited a maximum viable cell concentration (X_{max}) of 1.0×10^6 cells/mL with $\mu_{max} = 2 \times 10^{-2}\ h^{-1}$. Nevertheless, the results of this study suggest that Taylor vortex flow regimes provide an effective oxygen transfer required for animal cell culture with low hydrodynamics shear.

1. Introduction

In the last three decades, the number of products and product applications derived from culture of animal cells has increased. This growing interest is due to its capacity to synthesize highly valuable complex molecules within the medical and clinical fields, as viral vaccines, monoclonal antibodies and recombinant proteins [1]. The high demand for such products has led to large-scale processes involving suspensions of cells in large bioreactors [2–4]. Among the most relevant problems that were resolved successfully in the scaling up of such processes are those related with mixing, mass transfer (particularly oxygen gas and carbon dioxide) and shear stress [3].

The supply of oxygen in large bioreactors is a major concern, due to its low solubility in water (app 7.8×10^{-3} g/L at 25 °C) [5]. Because of the large volumes, oxygen and nutrients transfer within the liquid medium become a critical design issue, since these parameters are dependent on a good mixing to ensure the survival of the cells.

In conventional microbial bioreactors, this problem can be solved using higher agitation rates and/or higher air flow rates. However, for mammalian cell cultures these alternatives may not be appropriate because of the physiological characteristics of animal cells. The absence of cell walls in mammalian cells and their relatively large size make them particularly sensitive to shear forces. The cell damage caused by stirring [6–12], bubble formation and disruption [13–18] and foaming [18,19] has been extensively reported. Thus, the hydrodynamic shear stress is an important factor and should be considered in the cultivation of animal cells, especially those that are anchorage-dependent and grow on microcarriers. This occurs because cells adhered to the surfaces of microcarriers are less robust with respect to the fluid-mechanical forces than cells freely suspended in culture medium [8,18].

Since its introduction in 1967 by van Wezel [20], microcarrier culture has been applied in growing primary cell lines and established cell lines such as CHO and VERO for the production of recombinant proteins. The aim of using microcarriers is to enhance the production of anchorage-dependent cells over an increased area available for cell growth. The use and importance of microcarrier cultures for anchorage-dependent animal cells is well documented [21–24]. However, cell cultivation on microcarriers has a major challenge, which is the efficient mixing and oxygenation of large-scale cultures [18]. When using non-porous microcarriers, the cells grown on the bead surface are vulnerable to damages. Cherry and Papoustakis [25] indicated three mechanisms that may be strong enough to damage the cell or interfere with its normal function. Those mechanisms can be summarized as collisions of the cell-covered microcarrier with the impeller or other reactor parts, collisions with other microcarriers, and interaction

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doi:10.1016/j.procbio.2010.05.002

Nomenclature

a	interfacial area for the transfer of oxygen, (m^2/m^3) $a = \frac{\text{area for oxygen transfer(membrane outer surface facing the fluid)}}{\text{reactor wet volume}}$
C_{O_2}	dissolved oxygen concentration in the medium, (mol/m^3)
C^*	dissolved oxygen concentration in equilibrium with the gas-phase oxygen concentration, (mol/m^3).
D	diffusivity of oxygen in the liquid, (m^2/s)
D_m	diffusion coefficient of oxygen through the silicone tubular membrane wall, (m^2/s)
DO	dissolved oxygen concentration, (% of air saturation)
d	annular gap width, $(d = r_o - r_i)$ (m)
d_{in}	inner diameter of the silicone tubular membrane, (m)
d_{out}	outer diameter of the silicone tubular membrane (m)
k_L	liquid-phase mass-transfer individual coefficient, (m/s)
k_g	gas phase mass-transfer individual coefficient, (m/s)
k_m	mass transfer individual coefficient through the membrane wall, (m/s)
K_La	overall volumetric liquid-phase mass-transfer coefficient for dilute systems (1/h)
K	overall mass transfer coefficient, (m/h)
K_L	overall liquid-phase liquid-film mass-transfer coefficient, (m/h)
L	axial length of the reactor, (m)
r_o	outer (stationary) cylinder radius, (m)
r_i	inner (rotating) cylinder radius, (m)
Q	air flow rate in the silicone tubular membrane, (mL/min)
Sh	Sherwood number, defined by Eq. (11)
Sc	Schmidt number; ratio of viscosity and mass diffusivity, $Sc = \frac{\mu_L}{\rho_L D}$
Re_{air}	air-side Reynolds number, defined by Eq. (10)
Re_θ	rotational Reynolds number, defined by Eq. (1)
$Re_{\theta,c}$	critical Reynolds number for the onset of Taylor vortices, defined by Eq. (2)
Ta	Taylor number, defined by Eq. (4)

Greek letters

α	empirical parameter used in Eq. (9)
β	empirical parameter used in Eq. (9)
γ	empirical parameter used in Eq. (9)
γ_w	shear rate at the inner cylinder wall (1/s), defined by Eq. (3)
Γ	aspect ratio, $\Gamma = \frac{L}{b}$
δ	wall thickness of the silicone tubular membrane, (m)
η	radius ratio, $\eta = \frac{r_i}{r_o}$
μ_L	liquid-phase viscosity, (kg/m s)
μ	specific growth rate (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})
ν	liquid kinematic viscosity, (m^2/s)
ν_{air}	air kinematic viscosity, (m^2/s)
ρ_L	liquid-phase density, (kg/m^3)
τ	shear stress, (N/m^2)
τ_e	response time of oxygen probe (s)
ω	inner cylinder rotational velocity, (1/s)

with turbulent fluid eddies that are smaller than or the same size as the microcarrier. Cherry and Papoutsakis and Croughan et al. estimated, using the Kolmogorov eddy-length scale, the size of these smallest eddies. This correlation is based on the Kolmogorov theory of isotropic turbulence. From Kolmogorov's theory, an eddy-length scale can be determined in which it is assumed that a majority of the energy associated with the turbulence is dissipated. If this length scale is of the order of a microcarrier with cells attached (200 μm), or a cell diameter (10 μm) for a suspension cell culture, cell damage is expected [26].

To overcome the difficulty of obtaining an optimal compromise solution between low shear stress and effective mass transfer, this paper presents a new bioreactor design for animal cell cultivation, based on Taylor vortices flow: the Taylor vortex flow bioreactor (TVFB).

The secondary flow pattern that appears above a critical rotation rate in the gap between an inner (rotating) and an outer (generally stationary) cylinder is named Taylor vortex flow [27]. This flow is characterized by the formation of a series of counter-rotating toroidal vortices, superposed to the tangential flow in the annulus [28,29]. The vortices are capable of fulfilling the gap between the inner and outer cylinders in a stable and reproductive way and may provide a good mixing together with good heat and mass transfer characteristics [28,30]. The critical rotation of the inner cylinder, above which the laminar Couette flow becomes unstable, depends only on the ratio between the radii of cylinders, the kinematic viscosity of the medium and the radius of the inner cylinder, for a Newtonian fluid and negligible end effects (infinite length of the apparatus).

Taylor–Couette flow has been used to improve the performance of chemical and biochemical reactors [28–39]. One important reason for using vortex flow in biochemical reactors is its ability to promote a gentle, but still efficient, stirring, which is ideal when using shear-sensitive cells such as mammalian cells. As previously mentioned, for adherent cells the efficient mixing and oxygenation of the medium is a major challenge [18]. Taylor vortices may provide a way to conciliate effective mass transfer and mixing with low shear stress. Moreover, the rotation of the inner cylinder supplies an additional degree of freedom for the operation of the bioreactor, thus facilitating the fluidization of the microcarrier particles – a difficult task for conventional fluidized bed bioreactors [27].

Resende et al. [38], studying residence time distributions in a two-phase continuous Taylor Vortex Flow Reactor (VFR), showed that a VFR with radius ratio $\eta = 0.664$, and aspect ratio $\Gamma = 14.9$ could sustain the homogeneous fluidization of agarose gel particles with an average diameter of 214 μm, up to a 10%-load of the reactor (in volumetric basis). In this study, the authors also found that increasing the viscosity of the medium facilitated the fluidization of the particles. Furthermore, the vortices, and not the axial flow, were responsible for maintaining the particles in suspension, even for very low axial flow rates. This may be a significant operational advantage of the VFR over conventional reactors.

The particular TVFB studied here uses a tubular silicone membrane around its inner cylinder, allowing the diffusion of oxygen gas through the wall and into the culture medium, without the formation of air bubbles. These tubular membranes are made of materials, as silicone and Teflon that have high gas permeability allowing high gas transfer rates between the membrane and the culture medium [40].

Due to the complexities in defining optimal operating conditions of TVFB, because it is a new design of equipment, the approach adopted in this work was: (1) building a prototype bioreactor; (2) validation of fundamental design features such as the formation of Taylor vortex and the achievement of appropriate K_La values, and (3) evaluation of the performance of the bioreactor using the most