Chemical Engineering Science 126 (2015) 160–168

Contents lists available at ScienceDirect

Chemical Engineering Science

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

Micro-bubble aeration in turbulent stirred bioreactors: Coalescence behavior in Pluronic F68 containing cell culture media

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HIGHLIGHTS

- Sauter diameter of micro-bubbles increases with increasing specific power inputs.
- Bubble sizes were shown not to be affected by variations in the sparging rate.
- Bubble coalescence was modeled for various stirrer configurations and two scales.
- Pitched blade impeller provides higher k_La values than a Rushton impeller.
- FBRM technology provides reliable measurement of micro-bubble size variations.

ARTICLE INFO

Article history:
Received 30 August 2014
Received in revised form
31 October 2014
Accepted 6 December 2014
Available online 16 December 2014

Keywords:
Turbulence
SF-900 II culture medium
Coalescence
FBRM
Pluronic F68
Oxygen transfer

ABSTRACT

Micro-bubble aeration in stirred bioreactors represents a suitable method that allows sufficient oxygen transfer without the necessity of intense stirring. However, understanding of micro-bubble behavior in real culture media remains a crucial factor in the interpretation of possible cell/bubble interactions and the scale up of bioprocesses. The present study focuses the impact of various stirrer configurations, sparging rates, as well as 2 different bioreactor scales on micro-bubbles in SF-900 II insect culture medium, which represents a suitable example for Pluronic F68 supplemented culture media commonly applied in cell culture processes. Obtained results for the first time demonstrate a coalescence determined increase in bubble Sauter diameter d_{32} from 187 to 211 μm with increasing stirring intensities. The development of an empirical model reveals $d_{32} \propto V^{0.4}(P/V)^{0.4}$, were P represents the stirrer induced power input and V the medium volume. The results were interpreted a consequence of micro-bubble entrapment in turbulent flow structures. Additional studies on the volumetric oxygen transfer coefficient k_La demonstrate a strong impact of axial flow impellers and indicate that increases in k_La can be attributed to an improvement of the gas–liquid oxygen transfer rather than an increased specific gas surface area due to bubble breakup.

1. Introduction

The cultivation of cell cultures in sparged and stirred tank reactors (STRs) is still the approach of choice for the production of biologically active recombinant products in research and industry. However, due to the sensitiveness of eukaryotic cells to environmental conditions, hydrodynamic stress can cause detrimental effects on culture performance. On the one hand, power input via stirring should be high enough to provide sufficient mass transfer. On the other hand, intensive stirring should be avoided in order to prevent damaging effects on cells. In terms of sufficient oxygen supply, which is predominantly determined by the volumetric mass transfer coefficient k_La, the latter requirement can be an issue. This is especially the case when cells with comparatively high oxygen uptake rates such as baculovirus infected Sf9 or High-5 insect cells are employed as expression systems (Kioukia et al., 1995; Pamboukian et al., 2008). One opportunity to overcome this issue is the application of sintered or ceramic micro-spargers suitable to generate gas bubbles in the micro-scale. Micro-bubbles provide a high gas-phase surface area per medium volume, and therefore

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http://dx.doi.org/10.1016/j.ces.2014.12.020
0009-2509/

increased k_La values compared to common sparger systems (Czermak et al., 2009; Nehring et al., 2004). Nowadays, micro-spargers are commonly available as a basic configuration for cell culture bioreactors even in disposable systems (Eibl et al., 2010). However, several detrimental effects are mentioned in the context of micro-bubble aeration. Cell damage based on cell/bubble interactions is mainly attributed to bubble rupture at the liquid surface, where cells entrapped in a killing volume close to the gas bubble are stressed by high energetic fluid jets formed during bubble rupture (Boulton-Stone and Blake, 1993; Garcia-Briones et al., 1994; Tramper et al., 1988; Trinh et al., 1994). Moreover, cell damage strongly depends on the bubble size and the size related specific killing volume, were small bubbles generate more intense hydrodynamic forces than larger ones (Wu and Goosen, 1995). Foam formation as well as the entrapment of cells in a foam layer at the liquid surface represents another problem. However, all the factors mentioned above are strongly dependent on the culture medium of choice. For instance, mass transfer as well as the behavior of bubbles in STRs is determined by the presence of surface-active substances such as electrolytes or the cell protective agent Pluronic F68, which are usually part of the most culture media applied in animal cell culture. Both factors play an important role in the coalescence behavior of gas bubbles (Sieblist et al., 2013, 2009; Toye et al., 2010). Hence, information about gas bubble sizes in micro-bubble aerated and stirred bioreactors filled with the culture medium of choice, as well as their behavior at varying process conditions, are crucial in order to gain insights regarding cell stress based on cell/bubble interactions.

2. Materials and methods

2.1. Vessel configuration and medium

Experiments were conducted in a 3 L glass bioreactor (Applikon, Netherlands) with a diameter $D=130$ mm and overall vessel height $H_V=250$ mm. The vessel was filled with 1615 mL of SF-900 II serum-free insect cell culture medium (Life Technologies, USA), which corresponds to a filling height to vessel diameter ratio of $H/D=1$. The reactor was equipped with 3 baffles (blade width to vessel diameter ratio $B/D=0.1$). Immersion depth of the baffles at $H/D=1$ was 110 mm, corresponding to an immersion depth to filling height ratio of $I/H=0.85$. In order to mimic culture close conditions, the vessel was additionally equipped with two sensor dummies, each 12 mm in diameter, as well as a temperature probe. The immersion depth of the probe dummies at $H/D=1$ was 100 mm (immersion depth to filling height ratio $I_D/H=0.77$). Micro-bubble aeration was facilitated by a commonly available sintered micro-sparger with 15 μm pore size (Applikon, Netherlands). Formation of foam was prevented by the addition of Antifoam C (Sigma-Aldrich, USA). Gentamycin (PAA, Austria) was added to a final concentration of 10 mg/mL to prevent bacterial contamination. Prior to measurement start the medium was preheated to 28 °C, unless not stated otherwise. 28 °C represents the regular cultivation temperature for insect cells. Three different stirrer configurations were tested with varying stirrer diameter to vessel diameter ratios d/D. For each stirrer configuration, the stirrer was mounted at a stirrer height to filling height ratio of $h/H=0.54$. For experiments in a larger bioreactor scale, a 7 L vessel (Applikon, Netherlands) was utilized ($D=160$ mm, $H_V=350$ mm) with a filling volume of 3011 mL, corresponding to an H/D ratio of 1. All the remaining ratios mentioned for the 3 L scale were kept constant for the 7 L scale in order to generate geometric similarity. The experiments were performed at different sparging rates φ between 0.01 and 0.04 vvm. Out of these sparging rates the superficial gas velocities can be calculated using the applied medium volume V and cross sectional area A of the bioreactor:

$$v=\frac{\varphi V}{A} \tag{1}$$

The specific power input of each stirrer was chosen in a way that bubble entrainment from the air/liquid surface was prevented. All measurements were performed in the turbulent flow regime ($Re>10^4$). Specific power inputs were calculated according to the following equation:

$$\frac{P}{V}=\frac{Ne\, n^3\, d^5 \rho}{V} \tag{2}$$

where Ne is the power number, n is the stirring rate, and ρ the liquid density. The density of SF-900 II insect culture medium at 28 °C was determined as 1013 kg/m^3. Three different stirrer configurations were tested in this study. A 6-blade Rushton impeller (Applikon, Netherlands), $d=60$ mm, determined power number $Ne=4$. Two so called "elephant-ear impellers", hereinafter referred to as $3\times45°$ pitched blade impellers (Applikon, Netherlands), downward pumping (vortex), $d=60$ mm and 74 mm with determined power numbers of $Ne=2.3$ and 2.4, respectively (for further information see Supplementary data).

2.2. Focused beam reflectance measurement (FBRM) technology and particle vision and measurement (PVM) technology

Gas bubble measurements were facilitated using a G400 FBRM-Probe (Mettler-Toledo, Switzerland), which was inserted via the head plate of the vessel. The function principle of the probe is already described elsewhere (Druzinec et al., 2013; Sparks and Dobbs, 1993; Tadayyon and Rohani, 1998). Briefly, a circulating laser beam is backscattered once a particle gets crossed. The 180° backscattered light is transferred to a photo diode were the light is detected. From the rotation velocity of the laser beam multiplied by the detection time a characteristic chord length C of the particle can be calculated (Sparks and Dobbs, 1993; Tadayyon and Rohani, 1998). Due to the high rotation velocity of the laser of 2 m/s, several thousand of particles can be measured within seconds, which emphasizes the statistical significance of the measurements (Hoepfner et al., 2010; Wollny and Sperling, 2007).

Measurements were recorded every 10 s in the macro display distribution mode. All measured chord lengths are sorted into different size-classes. Each size-class covers a certain range of chord lengths within the overall measurement range between 0 and 1024 μm, resulting in a characteristic chord length distribution. Due to the nature of the FBRM-technology, the mean chord length of a spherical particle differs from the actual diameter. For instance, two particles with equal sizes but different scattering and absorption coefficients may cause different chord lengths. Moreover, a single circular particle can be passed by the laser at different positions other than the position of maximum diameter, resulting in a particle specific chord length distribution rather than a single diameter. Therefore, the mean chord length of a spherical particle is always smaller than the actual particle diameter (Tadayyon and Rohani, 1998). For an analysis of the chord length distribution, the following equation is applied (Tadayyon and Rohani, 1998, 2000):

$$\overline{C}_\delta=\frac{\sum m_i\times C_i^{\delta+1}}{\sum m_i\times C_i^{\delta}} \tag{3}$$

where m_i represents the number of particles counted in each size-class and C_i the corresponding chord length midpoint of each class. Various weight-factors δ can be applied by the iC FBRM 4.3 software (Mettler-Toledo, Swizerland). While a weight-factor of $\delta=0$ leads to an expression that equals the arithmetic mean