Linking maximal shear rate and energy dissipation circulation function in airlift bioreactors

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Abstract

Pneumatic reactors are an important class of bioreactors widely used in biotechnological processes. The growing interest in these reactors is mainly related to their good mass transfer capacity, as well as lower operating costs, due to the simple mechanical structure. Knowledge of the transport phenomena and hydrodynamics of bioreactors is important to enable definition of the best bioreactor model and operating conditions for a specific bioprocess. Several performance parameters are used to evaluate bioreactors, with the imposed shear being one of the most difficult to quantify. For stirred tanks, the fragmentation of microorganisms has been well correlated with a hydrodynamic parameter called the "energy dissipation/circulation function" (EDCF). However, there have been no estimates of the EDCF for pneumatic bioreactors. The present work proposes a methodology to estimate the EDCF for different pneumatic bioreactors and operating conditions. The difficulty in estimating the EDCF for pneumatic bioreactors is in defining the volume of higher energy dissipation. Here, this was achieved employing the maximal shear rate obtained using computational fluid dynamics simulations. The estimated volume was validated by comparing pellet fragmentation in conventional and pneumatic bioreactors, under conditions that led to similar EDCF values.

1. INTRODUCTION

Airlift reactors are an important class of multiphase contactors that are widely used in the chemical industry, biotechnological processes, and biological wastewater treatment. The growing interest in these reactors is mainly related to their mixing capacity and heat and mass transfer characteristics, as well as lower design and operating costs, due to the simple mechanical structure and absence of moving parts (Behin, 2010).

Understanding of the transport phenomena involved in the operation of airlift bioreactors is very important in order to allow definition of the best operating conditions for a specific bioprocess. The most common performance parameters used for this task are the liquid circulation velocity, mixing time, gas holdup, bubble diameter, overall oxygen transfer coefficient (k_La), and shear rate ($\dot{\gamma}$) (de Jesus, Neto, & Maciel, 2017). Among all these variables, k_La and $\dot{\gamma}$ have been extensively applied to characterize cultivations in airlift bioreactors, while also enabling comparison of the performances of stirred tank and airlift bioreactors in the production of bioproducts (Thomasi, Cerri, & Badino, 2010; Michelin et al., 2013, Cerri & Badino, 2012).

Airlift bioreactors usually require less energy than stirred tank reactors, under similar oxygen transfer conditions. The turbulence of two-phase flow in airlift bioreactors not only provides favourable conditions for mass transfer, but can also be used for suspending solid particles (Trager, Qazi, Onken, & Chopra, 1989). Despite the importance of power input in the characterization of bioreactors, few studies have described its effect on the performance of cultivations in airlift bioreactors (de Jesus et al., 2017; Contreras, Garcia, Molina, & Merchuk, 1999; Grima, Chisti, & MooYoung, 1997; Merchuk & Berzin, 1995; Merchuk & Benzvi, 1992). The main application of this concept is focused on modelling the liquid circulation velocity (Mendes & Badino, 2016; Kilonzo, Margaritis, & Bergougnou., 2010; Hwang & Cheng, 1997; Bando, Fujimori, Terazawa, Yasuda, & Nakamura, 2000; Calvo & Leton, 1991; Calvo, Leton, & Arranz, 1991; Chisti, Halard & Mooyoung, 1988). In contrast, the power input has been extensively used to evaluate the performance of cultivations in stirred tank bioreactors.

Smith, Lilly, and Fox (1990) evaluated the effect of agitation on the fragmentation of *Penicillium chrysogenum* in bench (9-L) and pilot (90-L) scales of conventional bioreactors equipped with Rushton turbine impellers. Firstly, an unsuccessful attempt was made to correlate the penicillin production rate to the impeller tip speed, for both scales. Subsequently, it was observed that the breakup frequency ($1/\mathrm{t_C}$) could be used to characterize the penicillin production rate. As a result, a correlation was proposed where the turbulence dissipation rate ($\epsilon = \frac{P}{D^3}$) was combined with the frequency of exposure of the mycelium to the high shear zone (breakup frequency, $1/t_C$), defining what is currently called the energy dissipation/circulation function (EDCF) (**Equation 1**).

$EDCF = \frac{P}{D^3} \bullet \frac{1}{t_C}$ **(1)**

Once the energy dissipation/circulation function concept had been defined, it started to be widely used to correlate mycelium fragmentation to operating conditions in conventional bioreactors. Makagiansar, Shamlou, Thomas, and Lilly (1993) successfully correlated the EDCF to the specific penicillin production rate obtained in bench (5-L), pilot (100-L), and industrial scale (1,000-L) stirred tank bioreactors equipped with Rushton turbine impellers.

Justen, Paul, Nienow, and Thomas (1996) modified the definition of EDCF by including a geometrical factor (k) (**Equation 2**), whereby the relevant volume depends on the type of impeller and the volume swept by it while rotating, allowing the evaluation of different impeller geometries (paddle, Rushton turbine, and pitched blade). An excellent correlation was observed between this new EDCF concept and morphological parameters such as the mean total hyphal length and mean projected area, obtained from *P. chrysogenum* cultivations.

$EDCF = \frac{P}{k \bullet D^3} \bullet \frac{1}{t_C}$

(2)

Amanullah et al. (2000) determined EDCF values for several impeller models (Rushton turbine, Prochem Maxflow T, pitched blade, paddle, propeller, and Intermig set) during fragmentation tests of *P. chrysogenum* and *Aspergillus oryzae* , also observing a dependency among EDCF values and morphological parameters.

The selection of a bioreactor model for application in an aerobic bioprocess should consider not only its oxygen transfer capability, but also the shear environment provided by it, since biochemical processes are extremely sensitive to the shear intensity, when fragile animal cells, plant cells, and filamentous microorganisms are used (Contreras et al., 1999). Excessive shear can cause cell damage, leading to viability loss and even cell disruption or disintegration, so bioreactors should provide moderate or low shear, necessitating understanding of the shear distribution within the system.

Although there are some methodologies available for estimating the shear rates in conventional bioreactors (Campesi, Cerri, Hokka, & Badino, 2009; Buffo et al., 2016; Bustamante, Cerri, & Badino, 2013) and pneumatic bioreactors (Cerri, Futiwaki, Jesus, Cruz, & Badino, 2008; Thomasi et al., 2010; Nishikawa, Kato, & Hashimoto, 1977; Schumpe & Deckwer, 1987; Shi, Riba, & Angelino, 1990; Al-Masry & Chetty, 1996; Merchuk & Benzvi, 1992; Merchuk & Berzin, 1995; Grima et al., 1997), they involve indirect estimation of this parameter. The application of the EDCF concept can fill this gap, since it considers the specific power consumption, which is a variable associated with the shear level, and also takes into account the frequency of exposure to the high shear environment.

The nonexistence of the energy dissipation/circulation function for other types of bioreactors makes it difficult to compare the performance of the bioreactors in terms of the degree of cellular fragmentation. Therefore, the present work proposes a new way to estimate the EDCF for pneumatic bioreactors, considering two different approaches, namely fragmentation similarity and computational fluid dynamics simulations, for which very similar values were obtained.

2. MATERIALS AND METHODS

2.1. Bioreactors and experimental conditions

A 4-L conventional stirred tank bioreactor (STB) (New Brunswick Scientific, USA) was evaluated in this study. This device was equipped with two different dual-impeller configurations, either two Rushton turbines (RT-RT) or two Elephant Ear impellers (EEDP-EEUP). These impellers associations, with diameters of 0.076 and 0.080 m for RT and EE, respectively, were employed previously by Buffo, Esperança, Farinas, and Badino (2020b).

Three different 5-L pneumatic bioreactor models were used in the present work: bubble column (BC), concentric-duct airlift (CDA), and split airlift (SA). These devices were equipped with different spargers: 84-hole cross-type spargers for the BC and CDA bioreactors, and a 76-hole wing-type sparger for the SA bioreactor. The sparger holes were 0.5 mm in diameter and were spaced 5 mm apart.

The bioreactor models are illustrated in **Figure 1** (stirred tank bioreactor and impellers) and **Figure 2** (pneumatic bioreactors). The detailed geometric dimensions are presented in **Table 1** .

The pneumatic bioreactors were evaluated for specific air flow rates (ϕ_{air}) ranging from 1 to 5 vvm, while the stirred tank bioreactor was operated at an impeller speed (N) of 400 rpm andϕ_{air} of 0.4 vvm.

Figure 1.

Figure 2.

Table 1.

2.2. Energy dissipation/circulation function (EDCF)

The energy dissipation/circulation function (EDCF) is an important hydrodynamic parameter and can be related to possible damage that a bioreactor may cause to cells (Buffo et al., 2020b; Smith et al., 1990; Hardy, Augier, Nienow, Beal, & Ben Chaabane, 2017). For stirred tank bioreactors, this parameter can be obtained considering the gassed power consumption (P_g) in a region of higher energy dissipation and shear stress, the volume V_C, which is close to the impellers, and the circulation time ($\mathrm{t_C}$) (**Equation 3**).

$$\mathrm{EDCF} = \frac{P_g}{V_C} \bullet \frac{1}{t_C}$$

(3)

For stirred tanks, V_C is normally related to the impeller diameter and geometry. However, for pneumatic bioreactors, no definition or estimation for this volume of higher energy dissipation was found in the literature. Despite this apparent lack, methods have been reported for estimation of the other two variables, P_g and $\mathrm{t_C}$, in pneumatic bioreactors. These are also important variables for the estimation of EDCF and have been determined experimentally for the pneumatic devices presented in **Section 2.1** .

The circulation time (t_C) was obtained using the method described by Vasconcelos et al. (2003), which consists of monitoring the time required for a spherical particle with the same density as the liquid to circulate through the bioreactor. A digital camera (Nikon D5200) and free VideoPad software were used for filming and analysis of the results, respectively.

The gassed power consumption was calculated using **Equation 4** , assuming isothermal gas expansion of the bubbles rising from the sparger holes to the fluid surface (Chisti, 1989).

$$P_g = Q_g \bullet P_a \bullet \ln\left(1 + \frac{\rho_d \bullet g \bullet h_d}{P_a}\right)$$

(4)

where, Q_g is the volumetric gas flow rate ($m^3.s^{-1}$), P_a is the atmospheric pressure (Pa),ρ_d is the gas-liquid mixture density ($kg.m^{-3}$), g is the gravitational acceleration ($m.s^{-2}$), and h_d is the gas-liquid dispersion height (m).

2.3. Evaluation of shear rate ($\dot{\gamma}$) in pneumatic bioreactors

Based on behaviour observed in stirred tank bioreactors, it is expected that the region of higher energy dissipation should also exhibit a high degree of shear. This region has been described using a characteristic volume (V_C), although values have not yet been established for pneumatic bioreactors.

In order to evaluate the axial shear rate profile in the pneumatic bioreactors, computational fluid dynamics (CFD) simulations were performed using the Euler-Euler approach and the mathematical modelling proposed by Rodriguez, Valverde-Ramirez, Mendes, Bettega, and Badino (2015), which had successfully predicted the liquid circulation velocity, global gas hold-up, and volumetric oxygen transfer coefficient for the same three pneumatic bioreactor geometries evaluated in the present work. Subsequently, Esperança et al. (2019) extended this approach and estimated the average shear rate ($\dot{\gamma}_{\mathrm{av}}$) for the CDA bioreactor, based on the mean interstitial liquid velocity and the interstitial liquid velocities in the riser and downcomer. More recently, Esperança et al. (2020) extended the investigation to the other pneumatic bioreactors (SA and BC) and showed that the highest shear rate values (maximum shear rate,$\dot{\gamma}_{\mathrm{max}}$) occurred close to the sparger holes. In the present work, this approach, which has provided satisfactory and consistent prediction of both global and local variables, was used to obtain the shear rate values along the axial profile, for all the pneumatic bioreactors.

The simulations were performed for the pneumatic bioreactors (BC, CDA, and SA) operating with three different fluids: distilled water (ρ_L= 997 kg[?]m^{-3}, μ_L= 8.49[?]10^{-4} Pa[?]s, σ = 0.072 N[?]m^{-1}) and glycerol solution (63% v[?]v^{-1}, ρ_L= 1157 kg[?]m^{-3},μ_L= 0.01 Pa[?]s, σ = 0.068 N[?]m^{-1}) as Newtonian fluids, and xanthan gum solution (0.2% w[?]v^{-1}, ρ_L= 1000 kg[?]m^{-3},K = 0.06 Pa[?]s^n, n = 0.36, σ = 0.0708 N[?]m^{-1}) as a non-Newtonian fluid. The specific air flow rates (ϕ_{air}) used were the same as those described in**Section 2.1** .

The computational geometry and numerical mesh were generated using GAMBIT v. 2.4.6 software and the simulations were performed using Fluent 14.5 software.

2.4. Pellet fragmentation assays

In recent work, Buffo, Esperanca, Bettega, Farinas, and Badino (2020a) used A*spergillus niger* pellets as a microbial model to compare the fragmentation caused by different bioreactors. Subsequently, Buffo et al. (2020b) performed assays of *A. niger* pellet fragmentation with different STB impeller configurations. In the present work, the same methodology described in the previous studies was applied for pellet fragmentation in the STB, with the two impeller configurations, and in the CDA bioreactor. Pellet fragmentation assays were performed during 4 h, with samples periodically removed for acquisition of stereoscopic images using a Biofocus XT-3H-BI microscope, at a magnification of 7.5x. The images were treated using free ImageJ software (rsbweb.nih.gov/ij), enabling determination of the temporal profiles for the pellet projected area (A), the variable used for fragmentation analysis. For each experimental point, more than 100 objects (pellets) were analysed in two different samples, with the standard deviation used as the statistical parameter.

3. RESULTS AND DISCUSSION

3.1 Definition of the characteristic volume (V_C) in pneumatic bioreactors

The characterization of mycelium fragmentation, using the energy dissipation/circulation function (EDCF) concept, requires knowledge of the characteristic volume (V_C), a region where a higher amount of energy is dissipated. In stirred tank bioreactors, the swept volume around the impellers corresponds to the region where this elevated energy dissipation is observed, in what is known as the impeller discharge zone (Li et al., 2002). This is also the region where the maximum shear rate occurs (Li et al., 2002) (**Figure 3**).

Figure 3.

According to Smith et al. (1990), "in the turbulent model of local isotropy it is the local energy dissipation rate that determines the shear stress level". Liu et al. (2016) used the Euler-Lagrange CFD approach to investigate the correlation between energy dissipation and shear rate/stress locations for stirred tank bioreactors, monitoring the trajectories of random particles released in a 3-L stirred tank. The authors defined the maximum shear stress and shear frequency (SSF) parameter, based on the EDCF concept. According to Liu et al. (2016), the SSF is the ratio between the maximum shear stress ($\tau_{\max}$) and the time required for a microorganism to be exposed to the high-shear condition ($\mathrm{t_C}$).

To the best of our knowledge, the EDCF concept has never been applied for the characterization of mycelial fragmentation in pneumatic bioreactors (airlift and bubble column), despite there being well-established methodologies for the determination of both gassed power consumption (P_g) and circulation time (t_C).

The determination of EDCF values for pneumatic bioreactors requires knowledge of the characteristic volume (V_C). Several studies have reported that the region around the sparger holes presents the highest shear rate (Esperanca et al., 2020; Pawar, 2017; Garcia, Paternina, Pupo, Bula, & Mare, 2014; Mavaddat, Mousavi, Amini, Azargoshasb, & Shojaosadati, 2014). This maximum shear rate ($\dot{\gamma}_{\max}$) is the result of the high gas injection velocity, which is determined by the sparger characteristics, considering the number and diameter of the holes (Esperanca et al., 2020).

When the maximum shear rate ($\dot{\gamma}_{\max}$) is used to evaluate the shear environments in pneumatic and stirred tank bioreactors, similar values are obtained, irrespective of bioreactor type, showing that the high shear condition is defined by the operating conditions, rather than only by the type of bioreactor (Esperanca et al., 2020).

Therefore, since there is a link between the locations of elevated energy dissipation and shear rate for stirred tanks, in the case of pneumatic bioreactors, the highest amount of energy would be expected to be dissipated close to the sparger holes.

In order to determine the characteristic volume (V_C) in pneumatic bioreactors, the axial shear rate profile was obtained from the CFD results (**Figure 4a**). This profile started at the bottom of the bioreactor and exhibited a peak of $\dot{\gamma}$, whose value was around 3 orders of magnitude higher than the average value ($\dot{\gamma}_{\mathrm{av}}$), for liquid heights (h) ranging from about 0.03 to 0.04 m (**Figure 4a**). A more accurate evaluation of the characteristic volume (V_C) was performed using the derivative of the shear rate in relation to the liquid height ($d\dot{\gamma}/dh$), (**Figure 4b**). The V_C started at the liquid height where $d\dot{\gamma}/dh$ began to increase faster, and ended at the liquid height where $d\dot{\gamma}/dh$ approached zero again (**Figure 4b**). The calculation of V_C assumed a cylindrical volume surrounding the sparger holes, with the diameter taken as the sparger diameter (**Figure 3b**). This approach was applied for the entire set of pneumatic bioreactor models (bubble column, concentric-duct airlift, and split airlift), types of fluid (distilled water, glycerol solution, and xanthan gum solution), and specific air flow rates (ϕ_{air}).

Figure 4.

Regardless of the pneumatic bioreactor model, fluid type, and specific air flow rate, the axial profile of the normalized shear rate ($\dot{\gamma}/\dot{\gamma}_{\mathrm{av}}$) showed that the highest shear rates occurred at liquid heights (h) ranging from 0.0220 to 0.0471 m (**Figure 5**). This range of heights corresponded to volumes ranging from 57.9 to 106.5 cm3, which accounted for between 1.2 and 2.8% of the total liquid volume.

Figure 5.

3.2 Estimation of EDCF for pneumatic bioreactors

Once the location of the characteristic volume (V_C) for pneumatic bioreactors had been identified and its value had been estimated, the EDCF could be estimated for the 5-L pneumatic bioreactors (**Equation 5**).

EDCF $= \frac{P_g}{V_C} \bullet \frac{1}{t_C}$

(5)

Knowing the experimental values of the dispersion density (ρ_d) and gas-liquid dispersion height (h_d), the gassed power consumption could be calculated using **Equation 4.** Subsequently, knowing P_g and the liquid circulation time (t_C), and assuming an average characteristic volume of 75 cm3, based on the range from 57.9 to 106.5 cm3 (corresponding to 1.5% of the total liquid volume), the EDCF could be estimated. **Figure 6** shows the EDCF according to the specific air flow rate (ϕ_{air}) for the CDA and SA bioreactors operated with Newtonian and non-Newtonian fluids. In this case, the frequency term was considered to be the inverse of the circulation time ($\frac{1}{t_C}$), as indicated by Esperanca et al. (2020).

Figure 6.

For the concentric-duct airlift (CDA) bioreactor, the EDCF ranged from 1.0 to 9.1 kW.m^{-3}.s^{-1}, while for the split airlift (SA) bioreactor it varied from 0.71 to 6.8 kW.m^{-3}.s^{-1}. Comparing the two airlift models, the EDCF values were slightly higher for the CDA device, compared to the SA system. Increase of the specific air flow rate acted to increase the EDCF, due to the combined effects of a shorter circulation time (t_C) and higher gassed power input (P_g).

Despite these results being estimates of EDCF, the values seemed to be reasonable, since they were of the same order of magnitude as EDCF values reported for stirred tanks (**Table 2**). Therefore, the estimated value of V_C also seemed to be appropriate.

It is important to highlight that in the scale-up of stirred tank bioreactors, lower specific power inputs are required, due to "cost considerations and practical restraints on motor, gearbox, and bearing design, among other" (Justen et al. 1996), resulting in longer circulation times. Hence, lower ECDF values are expected for larger stirred tanks, as the literature shows (**Table 2**).

Table 2.

3.3 Validation of V_C

estimation using fragmentation assays

In order to validate the pneumatic bioreactor characteristic volume (V_C) values estimated by CFD analysis, pellet fragmentation assays were performed using the 5-L concentric-duct airlift bioreactor at 5 vvm. For this operating condition, the expected EDCF values were in the range from 7.0 to 9.1 kW.m^{-3}.s^{-1}, depending on the fluid characteristics (**Figure 6**).

Pellet fragmentation assays were also performed using a 4-L stirred tank bioreactor at 400 rpm and 0.4 vvm, with different dual-impeller combinations: Rushton turbine/Rushton turbine (RT-RT), and Elephant Ear down-pumping/Elephant Ear up-pumping (EEDP-EEUP). The EDCF values for these impeller configurations and operating conditions were 5.3 and 5.0 kW.m^{-3}.s^{-1}, respectively (Buffo et al., 2020b). **Figure 7** shows the variation of the normalized pellet projected area (A/A_0) during the fragmentation assays performed using the stirred tank bioreactor (STB) with two different impeller combinations (RT-RT and EEDP-EEUP) and the concentric-duct airlift (CDA) bioreactor.

Figure 7.

The results showed that despite some differences in the pellet fragmentation profiles, the final A/A_0 values were very similar for all the bioreactors, with an average value of 0.77. This indicated that for these bioreactors, under the operating conditions employed, the fragmentations were equivalent.

Since the EDCF has been applied to describe the cellular fragmentation in stirred tanks, it was expected that the EDCF values would be the same for the CDA and STB systems, under the abovementioned operating conditions (**Equation 6a**).

$\mathrm{EDCF_{CDA}} = \mathrm{EDCF_{STB}}$ **(6a)**

Considering an average EDCF value of 5.15 kW.m^{-3}.s^{-1} for the stirred tank with RT-RT and EEDP-EEUP impeller configurations, and the general EDCF definition (**Equation 3**), then:

$\mathrm{EDCF_{CDA}} = \frac{P_g}{V_C}\frac{1}{t_C} = 5.15\ \frac{kW}{m^3 \bullet s}$

(6b)

Substituting in **Equation 6b** the gassed power input (P_g) value of 1.64 W (**Equation 4**) for the CDA bioreactor operating at 5 vvm and the circulation time (t_C) of 3.1 s (Mendes & Badino, 2016), the characteristic volume (V_C) in which there was the highest energy dissipation was calculated as being 103 cm^3. This volume represented about 2.1% of the liquid volume in the CDA bioreactor and was within the V_C range estimated using CFD simulation (from 57.9 to 106.5 cm3). This proximity between the V_C values obtained by the two methodologies evidenced the applicability of the analytical approach proposed in the present work.

The STB swept volumes were calculated based on the geometric characteristics of the dual-impeller configurations, for comparison withV_C of the CDA system. The characteristic volumes for the STB equipped with different impeller combinations were higher than those observed for the CDA bioreactor (**Table 3**), corresponding to about 11.9% (average value) of the liquid volume. Hence, despite the higher power input observed for stirred tank bioreactors, this amount of energy is dissipated in a greater volume, resulting in similar EDCF values.

Table 3

The establishment, in this work, of the EDCF calculation for pneumatic bioreactors makes it possible to estimate this variable for different operating conditions, allowing comparison of filamentous microorganism cultivations in pneumatic and conventional bioreactors.

CONCLUSIONS

The energy dissipation/circulation function (EDCF) was successfully estimated, for the first time, for pneumatic bioreactors operating under different conditions. The values obtained were of the same order of magnitude as EDCF values for stirred tanks reported in the literature. A critical point in the methodology was definition of the characteristic volume (V_C) of higher energy dissipation, which was obtained by analysing the region of maximal shear rate. This approach proved to be satisfactory, since similar EDCF values were obtained for conventional and pneumatic bioreactors operating under conditions that resulted in similar *A. niger* fragmentation. The present work introduces a new methodology for calculation of an important parameter for pneumatic bioreactors, allowing comparisons to be made of the fragmentation of filamentous microorganisms in different bioreactors.

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References

Al-Masry, W. A. & M. Chetty (1996) On the estimation of effective shear rate in external loop airlift reactors: Non-Newtonian fluids.*Resources Conservation and Recycling,* 18**,** 11-24.

Amanullah, A., R. Blair, A. W. Nienow & C. R. Thomas (1999) Effects of agitation intensity on mycelial morphology and protein production in chemostat cultures of recombinant Aspergillus oryzae.*Biotechnology and Bioengineering* , 62 (4), 434-446.

Amanullah, A., P. Justen, A. Davies, G. C. Paul, A. W. Nienow & C. R. Thomas (2000) Agitation induced mycelial fragmentation of Aspergillus oryzae and Penicillium chrysogenum. *Biochemical Engineering Journal,* 5**,** 109-114.

Bando, Y., K. Fujimori, H. Terazawa, K. Yasuda & M. Nakamura (2000) Effects of equipment dimensions on circulation flow rates of liquid and gas in bubble column with draft tube. *Journal of Chemical Engineering of Japan,* 33**,** 379-385.

Behin, J. (2010) Modeling of modified airlift loop reactor with a concentric double-draft tube. *Chemical Engineering Research & Design,* 88**,** 919-927.

Buffo, M. M., L. J. Correa, M. N. Esperanca, A. J. G. Cruz, C. S. Farinas & A. C. Badino (2016) Influence of dual-impeller type and configuration on oxygen transfer, power consumption, and shear rate in a stirred tank bioreactor. *Biochemical Engineering Journal,*114**,** 133-142.

Buffo, M. M., M. N. Esperanca, R. Bettega, C. S. Farinas & A. C. Badino (2020a) Oxygen transfer and fragmentation of *Aspergillus niger*pellets in stirred tank and concentric-duct airlift bioreactors.*Industrial Biotechnology,* 16.

Buffo, M. M., M. N. Esperanca, C. S. Farinas & A. C. Badino (2020b) Relation between pellet fragmentation kinetics and cellulolytic enzymes production by *Aspergillus niger* in conventional bioreactor with different impellers. *Enzyme and Microbial Technology,* 139.

Bustamante, M. C. C., M. O. Cerri & A. C. Badino (2013) Comparison between average shear rates in conventional bioreactor with Rushton and Elephant ear impellers. *Chemical Engineering Science,*90**,** 92-100.

Calvo, E. G. & P. Leton (1991) A fluid dynamic model for bubble columns and airlift reactors. *Chemical Engineering Science,* 46**,**2947-2951.

Calvo, E. G., P. Leton & M. A. Arranz (1991) Prediction of gas hold up and liquid velocity in airlift loop reactors containing highly viscous newtonian liquids. *Chemical Engineering Science,* 46**,**2951-2954.

Campesi, A., M. O. Cerri, C. O. Hokka & A. C. Badino (2009) Determination of the average shear rate in a stirred and aerated tank bioreactor. *Bioprocess and Biosystems Engineering,* 32**,**241-248.

Cerri, M. O. & A. C. Badino (2012) Shear conditions in clavulanic acid production by *Streptomyces clavuligerus* in stirred tank and airlift bioreactors. *Bioprocess and Biosystems Engineering,*35**,** 977-984.

Cerri, M. O., L. Futiwaki, C. D. F. Jesus, A. J. G. Cruz & A. C. Badino (2008) Average shear rate for non-Newtonian fluids in a concentric-tube airlift bioreactor. *Biochemical Engineering Journal,* 39**,**51-57.

Chisti, M. Y., B. Halard & M. Mooyoung (1988) Liquid circulation in airlift reactors. *Chemical Engineering Science,* 43**,**451-457.

Chisti, Y. 1989. Airlift Bioreactors. Belfast, Northern Ireland: Elsevier Science Publishers Ltd.

Contreras, A., F. Garcia, E. Molina & J. C. Merchuk (1999) Influence of sparger on energy dissipation, shear rate, and mass transfer to sea water in a concentric-tube airlift bioreactor. *Enzyme and Microbial Technology,* 25**,** 820-830.

de Jesus, S. S., J. M. Neto & R. Maciel (2017) Hydrodynamics and mass transfer in bubble column, conventional airlift, stirred airlift and stirred tank bioreactors, using viscous fluid: A comparative study.*Biochemical Engineering Journal,* 118**,** 70-81.

Esperanca, M. N., C. E. Mendes, G. Y. Rodriguez, M. O. Cerri, R. Bettega & A. C. Badino (2019) Average shear rate in airlift bioreactors: searching for the true value. *Bioprocess and Biosystems Engineering,* 42**,** 995-1008.

Esperanca, M. N., C. E. Mendes, G. Y. Rodriguez, M. O. Cerri, R. Bettega & A. C. Badino (2020) Sparger design as key parameter to define shear conditions in pneumatic bioreactors. *Biochemical Engineering Journal,* 157.

Garcia, S., E. Paternina, O. R. Pupo, A. Bula & L. Di Mare (2014) CFD simulation of multiphase flow in an airlift column photobioreactor.*Global Nest Journal,* 16**,** 1121-1134.

Grima, E. M., Y. Chisti & M. MooYoung (1997) Characterization of shear rates in airlift bioreactors for animal cell culture. *Journal of Biotechnology,* 54**,** 195-210.

Hardy, N., F. Augier, A. W. Nienow, C. Beal & F. Ben Chaabane (2017) Scale-up agitation criteria for Trichoderma reesei fermentation.*Chemical Engineering Science,* 172**,** 158-168.

Hwang, S. J. & Y. L. Cheng (1997) Gas holdup and liquid velocity in three-phase internal-loop airlift reactors. *Chemical Engineering Science,* 52**,** 3949-3960.

Justen, P., G. C. Paul, A. W. Nienow & C. R. Thomas (1996) Dependence of mycelial morphology on impeller type and agitation intensity.*Biotechnology and Bioengineering,* 52**,** 672-684.

Justen, P., G. C. Paul, A. W. Nienow & C. R. Thomas (1998) Dependence of Penicillium chrysogenum growth, morphology, vacuolation, and productivity in fed-batch fermentations on impeller type and agitation intensity. *Biotechnology and Bioengineering,* 59**,**762-775.

Kilonzo, P. M., A. Margaritis & M. A. Bergougnou (2010) Hydrodynamic characteristics in an inverse internal-loop airlift-driven fibrous-bed bioreactor. *Chemical Engineering Science,* 65**,** 692-707.

Li, Z. J., V. Shukla, K. S. Wenger, A. P. Fordyce, A. G. Pedersen & M. R. Marten (2002) Effects of increased impeller power in a production-scale Aspergillus oryzae fermentation. *Biotechnology Progress,* 18**,** 437-444.

Liu, Y., Z. J. Wang, J. Y. Xia, C. Haringa, Y. P. Liu, J. Chu, Y. P. Zhuang & S. L. Zhang (2016) Application of Euler-Lagrange CFD for quantitative evaluating the effect of shear force on Carthamus tinctorius L. cell in a stirred tank bioreactor. *Biochemical Engineering Journal,* 114**,** 212-220.

Makagiansar, H. Y., P. A. Shamlou, C. R. Thomas & M. D. Lilly (1993) The influence of mechanical forces on the morphology and penicillin production of Penicillium chrysogenum. *Bioprocess Engineering,*9**,** 83-90.

Mavaddat, P., S. M. Mousavi, E. Amini, H. Azargoshasb & S. A. Shojaosadati (2014) Modeling and CFD-PBE simulation of an airlift bioreactor for PHB production. *Asia-Pacific Journal of Chemical Engineering,* 9**,** 562-573.

Mendes, C. E. & A. C. Badino (2016) Hydrodynamics of Newtonian and non-Newtonian liquids in internal-loop airlift reactors.*Biochemical Engineering Journal,* 109**,** 137-152.

Merchuk, J. C. & S. Benzvi (1992) A novel approach to the correlation of mass transfer rates in bubble-columns with non-Newtonian liquids.*Chemical Engineering Science,* 47**,** 3517-3523.

Merchuk, J. C. & I. Berzin (1995) Distribution of energy dissipation in airlift reactors. *Chemical Engineering Science,* 50**,**2225-2233.

Michelin, M., A. M. O. Mota, M. L. T. M. Polizeli, D. P. da Silva, A. A. Vicente & J. A. Teixeira (2013) Influence of volumetric oxygen transfer coefficient (k(L)a) on xylanases batch production by Aspergillus niger van Tieghem in stirred tank and internal-loop airlift bioreactors.*Biochemical Engineering Journal,* 80**,** 19-26.

Nishikawa, M., H. Kato & K. Hashimoto (1977) Heat-transfer in aerated tower filled with non-Newtonian liquid. *Industrial & Engineering Chemistry Process Design and Development,* 16**,** 133-137.

Pawar, S. B. (2017) CFD analysis of flow regimes in airlift reactor using Eulerian-Lagrangian approach. *Canadian Journal of Chemical Engineering,* 95**,** 420-431.

Rocha-Valadez, J. A., E. Galindo, L. Serrano-Carreon (2007). The influence of circulation frequency on fungal morphology: A case study considering Kolmogorov microscale in constant specific energy dissipation rate cultures of *Trichoderma harzianum* . *Journal of Biotechnology* , 130, 394-401.

Rodriguez, G. Y., M. Valverde-Ramirez, C. E. Mendes, R. Bettega & A. C. Badino (2015) Global performance parameters for different pneumatic bioreactors operating with water and glycerol solution: experimental data and CFD simulation. *Bioprocess and Biosystems Engineering,*38**,** 2063-2075.

Schumpe, A. & W. D. Deckwer (1987) Viscous media in tower bioreactors - Hydrodynamic characteristics and mass-transfer properties.*Bioprocess Engineering,* 2**,** 79-94.

Shi, L. K., J. P. Riba & H. Angelino (1990) Estimation of effective shear rate for aerated non-Newtonian liquids in airlift bioreactor.*Chemical Engineering Communications,* 89**,** 25-35.

Smith, J. J., M. D. Lilly & R. I. Fox (1990) The effect of agitation on the morphology and penicillin production of Penicillium chrysogenum.*Biotechnology and Bioengineering,* 35**,** 1011-1023.

Tang, W. J., A. Pan, H. Z. Lu, J. Y. Xia, Y. Zhuang, S. Zhang, J. Chu, H. Noorman (2015). Improvement of glucoamylase production using axial impellers with low power consumption and homogeneous mass transfer.*Biochemical Engineering Journal* , 99, 167-176.

Thomasi, S. S., M. O. Cerri & A. C. Badino (2010). Average shear rate in three pneumatic bioreactors. *Bioprocess and Biosystems Engineering,* 33**,** 979-988.

Trager, M., G. N. Qazi, U. Onken & C. L. Chopra (1989). Comparison of airlift and stirred reactors for fermentation with *Aspergillus niger* . *Journal of Fermentation and Bioengineering,* 68**,**112-116.

Vasconcelos, J. M. T., J. M. L. Rodrigues, S. C. P. Orvalho, S. S. Alves, R. L. Mendes & A. Reis (2003). Effect of contaminants on mass transfer coefficients in bubble column and airlift contactors.*Chemical Engineering Science,* 58**,** 1431-1440.

Xia, J. Y., Y. H. Wang, S. L. Zhang, N. Chen, P. Yin, Y. P. Zhuang, J. Chu (2009). Fluid dynamics investigation of variant impeller combinations by simulation and fermentation experiment.*Biochemical Engineering Journal* , 43, 252-260.

Tables

Table 1 . Geometric characteristics of the bioreactors.

	Bioreactor	**Bioreactor**	**Bioreactor**	**Bioreactor**
	STB	BC	CDA	SA
V_L (L)	4	5	5	5
H (m)	0.307	0.600	0.600	0.600
H_L (m)	0.192	0.450	0.450	0.450
H_I (m)	0.078	-	-	-
H_1 (m)	-	-	0.350	0.350
H_2 (m)	-	-	0.045	0.045
D (m)	0.170	0.125	0.125	0.125
D_I (m) L (m)	-	-	0.080 -	- 0.124
	-	-		

Table 2. EDCF values reported for stirred tank bioreactors.

Reference	Volume (L)	Impeller	N (rpm)	ϕ_{air} (vvm)	EDCF (kW.m^{-3}.s^{-1})
Smith et al. (1990)	9 90	RT-RT-RT RT-RT-RT	800-1,200 350-565	0.54 0.70	11.0-58.4 3.3-14.4
Makagiansar et al. (1993)	5 100 1,000	RT-RT-RT RT-RT-RT RT-RT-RT	700-1,300 265-530 300-400	0.5 0.5 0.5	4-180 3.5-37 6-17
Justen et al. (1996)	1.4 1.4/20/180	Various (Pd, RT, PMT, Pp, PB, Int) RT	145-2,054^{+} 250-1,443^{++}	Ungassed Ungassed	9.5-1,300 1.8-400
Justen, Paul, Nienow, and Thomas (1998)	6	Pd RT PB	280 600-1,400 1,150-1,800	1 1 1	13.7-24.2§ 7.3-471.0§ 88.5-1,875§
Amanullah et al. (1999)	1.4	RT, PMT, PB	145-2,054^{+}	Ungassed	2-1,300
Amanullah et al. (2000)	1.4 1.4	RT, PMT, PB Various (Pd, RT, PMT, Pp, PB, Int)	145-2,054^{+} 120-1,500^{+}	Ungassed Ungassed	2-1,300 10-525
Li et al. (2002)	80,000	RT-RT-RT			19.4-26.4 (control) §,¶ 6.6-54.8 (high power) §,¶
Rocha-Valdez, Galindo, and Serrano-Carreón (2007)	10	RT-RT	275-660	Ungassed	3-96
Xia et al. (2009)	35	Three different impeller associations	300-600	-	8-18
Tang et al. (2015)	50	RT-RT-RT Whu-Whu-Whu	350 470	1	˜15 ˜11
Liu et al. (2016)	3	RT-RT	100-400	Ungassed	1-100 (SSF)
Hardy et al. (2017)	2.5 80,000 -130,000	Several (Pp, Pt, Ct, Pd)	800-1,700	0.5	100-1500 ˜1
Buffo et al. (2020b)	4	Two impellers RT-RT EEDP-EEUP	400-1,000	0.4-1.2	5-100

Legend: RT: Rushton turbine; Pd: Paddle; PMT: Prochem Maxflow T; Pp: Propeller; PB: Pitched blade; Int: Intermig set; Pt: Profiled triblade; Ct: Centripetal turbine; Whu: Wide-blade hydrofoil up-pumping.

$^{+}$Impeller speed ranges were different for each impeller type.

$^{++}$Impeller speed ranges were different for each bioreactor scale.

§EDCF values obtained during the cultivations.

¶Control cultivations: $D_i/T = 0.38$; higher impeller speed. Higher power cultivations: $D_i/T = 0.43$; lower impeller speed.

Table 3. Comparison of CDA and STB parameters.

Reference	**Bioreactor**	**Operating conditions**	**EDCF (kW.m^{-3}.s^{-1})**	**P_g (W)**	**t_C (s)**	**V_C (L)**	**V_C/V (%)**
Buffo et al. (2020b)	STB (RT-RT)	400 rpm 0.4 vvm	5.3^{+}	4.22^{+}	1.81^{+}	0.439	11.0
(Buffo et al. (2020b)	STB (EEDP-EEUP)	400 rpm 0.4 vvm	5.0^{+}	3.51^{+}	1.37^{+}	0.512	12.8
Present study	CDA	5 vvm	5.15^{++}	1.64	3.10	0.106	2.1

$^{+}$Values obtained from Buffo et al. (2020b).

$^{++}$Calculated as the average of both STB values.

Figure legends

Figure 1. Schematic geometries of the bioreactors: (a) stirred tank bioreactor (STB) and (b) Rushton turbine (RT) and Elephant Ear (EE) impellers.

Figure 2. Schematic geometries of the pneumatic bioreactors: (a) bubble column (BC), (b) concentric-duct airlift (CDA), and (c) split airlift (SA).

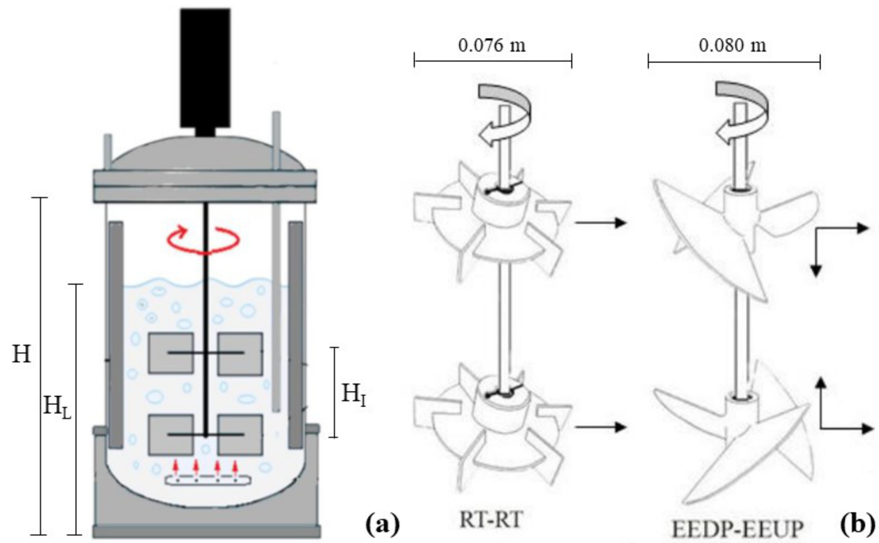
Figure 3. Regions of elevated energy dissipation and shear rate in (a) a stirred tank bioreactor and (b) a pneumatic bioreactor.

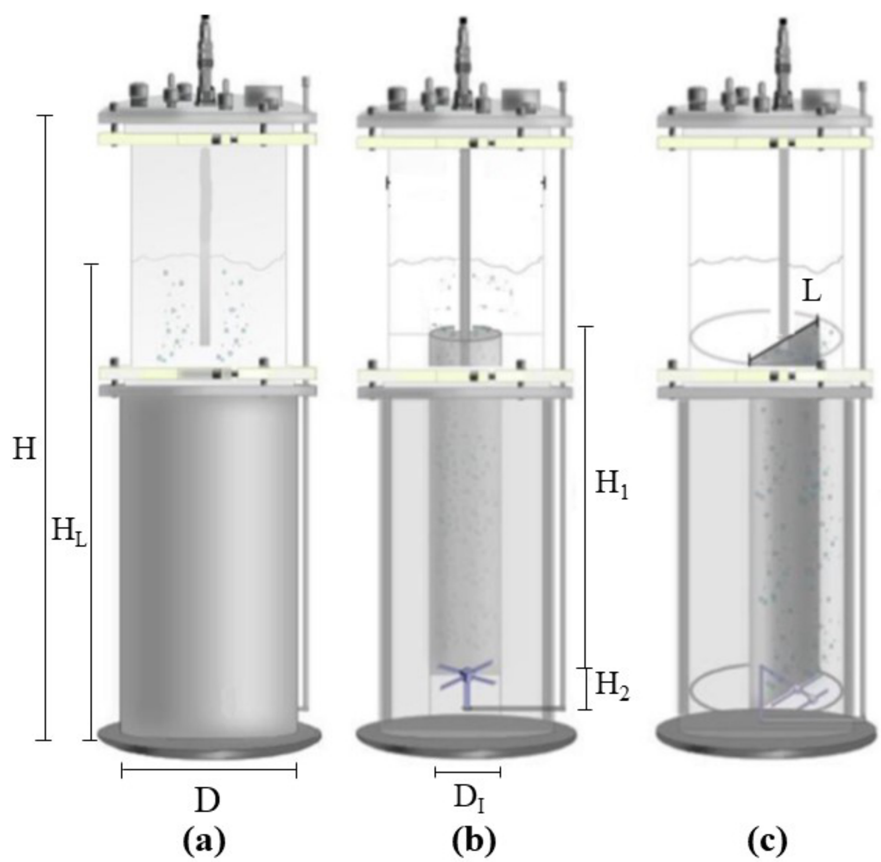
Figure 4. Determination of the characteristic volume in a 5-L concentric-duct airlift bioreactor operated with glycerol solution at 3 vvm: (a) shear rate as a function of liquid height; (b) derivative of shear rate as a function of liquid height.

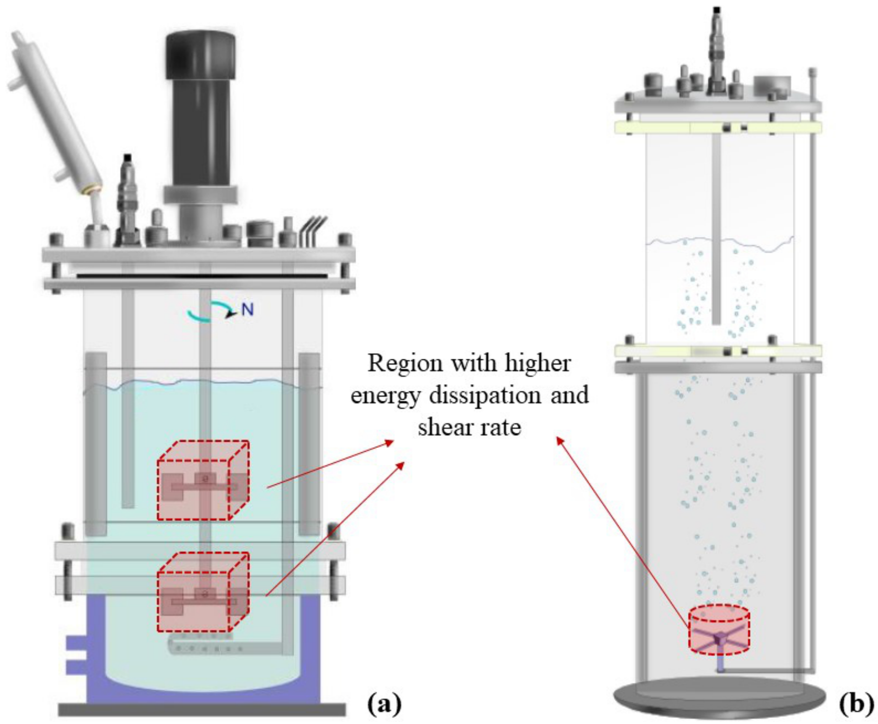
Figure 5. Normalized shear rate, as a function of liquid height, for pneumatic bioreactors operated with different fluids and specific air flow rates.

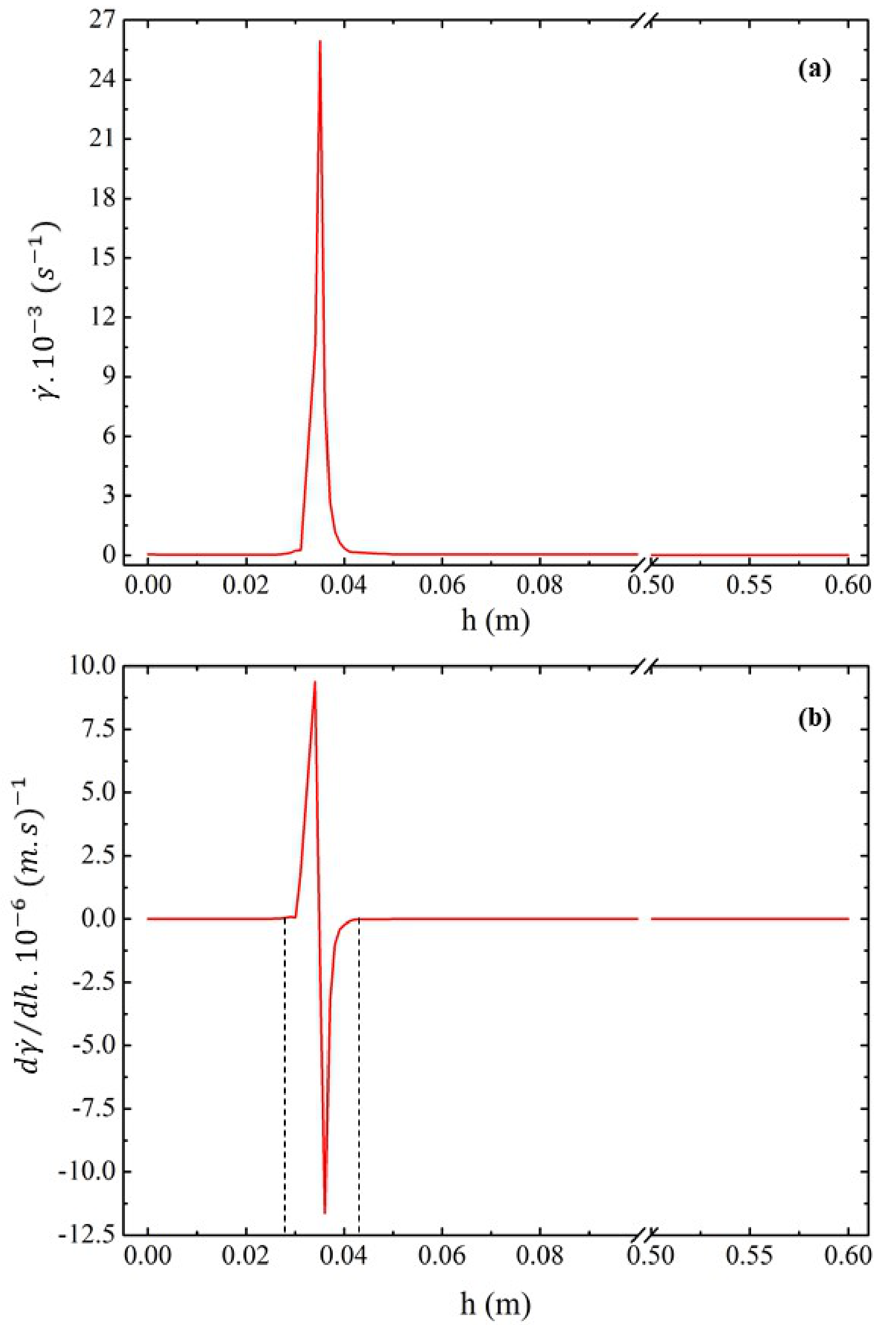
Figure 6. Energy dissipation/circulation function (EDCF), according to specific air flow rate (ϕ_{air}), for the 5-L airlift bioreactors.

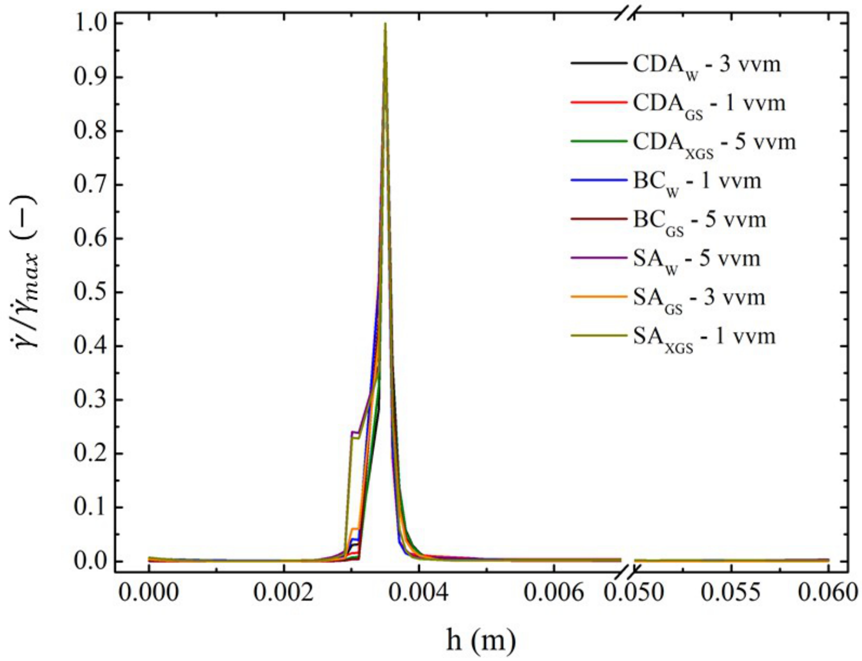
Figure 7. Temporal variation of the normalized pellet projected area for the different bioreactors.


0.076 m
0.080 m
H
H_L
H_I
(a)
RT-RT
EEDP-EEUP
(b)

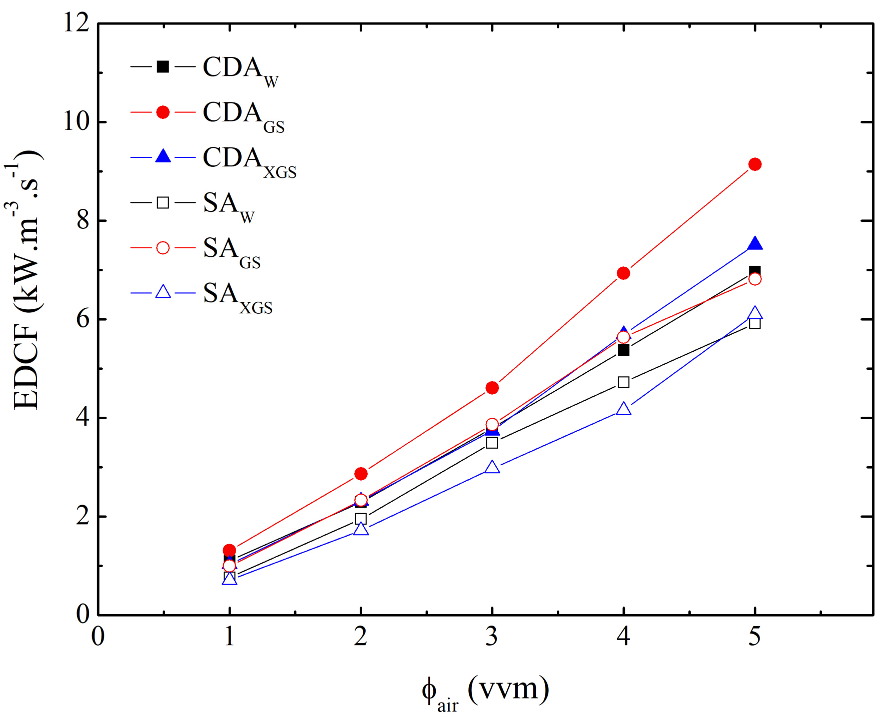

H
H_L
H_1
H_2
L
D
D_I
(a)
(b)
(c)


N
Region with higher energy dissipation and shear rate
(a)
(b)

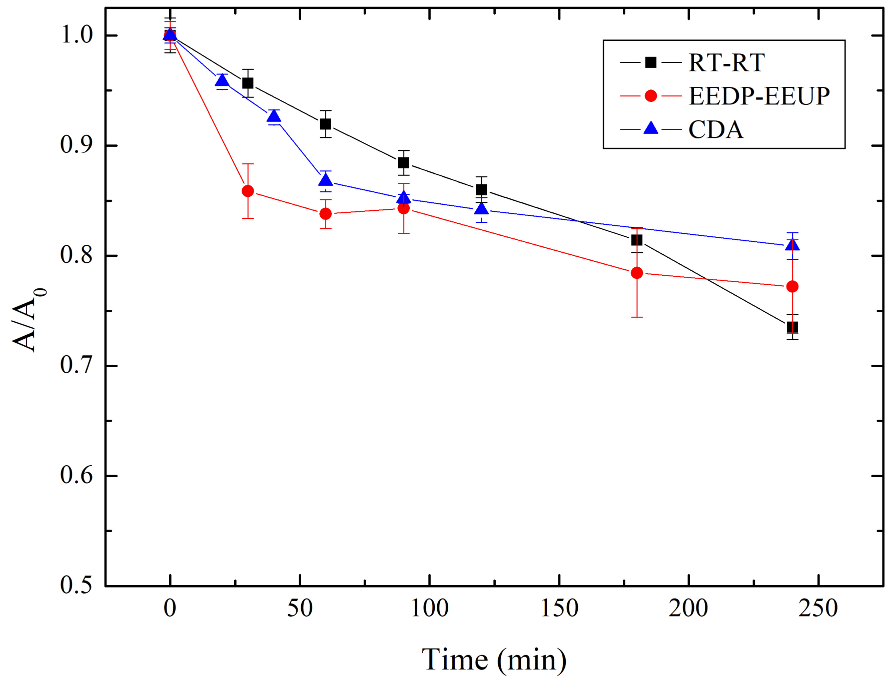

(a)
$\dot{\gamma}.10^{-3}\ (s^{-1})$
h (m)
(b)
$d\dot{\gamma}/dh\,.10^{-6}\ (m.s)^{-1}$
h (m)

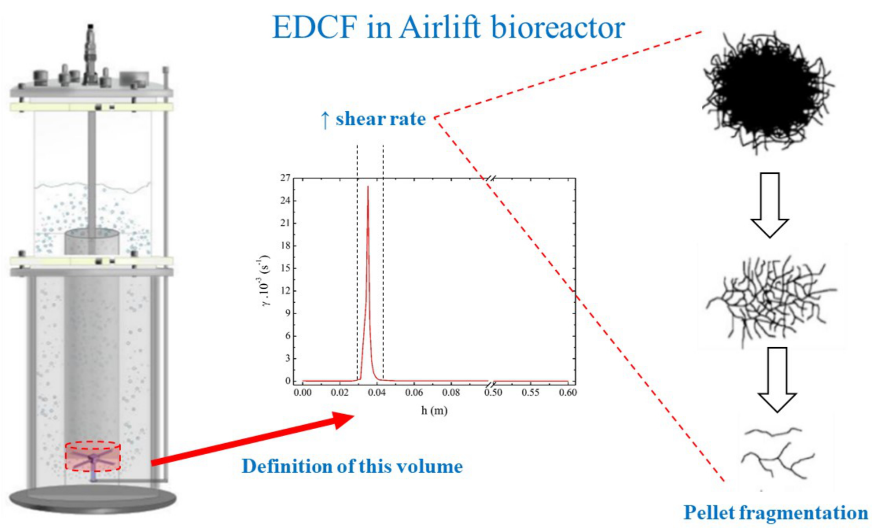


$\dot{\gamma}/\dot{\gamma}_{max}$ (−)
h (m)
CDA$_W$ - 3 vvm
CDA$_{GS}$ - 1 vvm
CDA$_{XGS}$ - 5 vvm
BC$_W$ - 1 vvm
BC$_{GS}$ - 5 vvm
SA$_W$ - 5 vvm
SA$_{GS}$ - 3 vvm
SA$_{XGS}$ - 1 vvm



EDCF (kW.m^{-3}.s^{-1})
ϕ_{air} (vvm)
CDA$_W$
CDA$_{GS}$
CDA$_{XGS}$
SA$_W$
SA$_{GS}$
SA$_{XGS}$


RT-RT
EEDP-EEUP
CDA
A/A0
Time (min)
0.5
0.6
0.7
0.8
0.9
1.0
0
50
100
150
200
250


EDCF in Airlift bioreactor
↑ shear rate
h (m)
Definition of this volume
Pellet fragmentation