Average Shear Rate Estimation in Conventional Stirred-Tank Bioreactor Using Non-Newtonian Fluid: An Experimental Approach

Rewari, Vinay
Biomedical Research Laboratory, Department of Biotechnology, K L E F University (Koneru Lakshmaiah Educational Foundation), Greenfields; Vaddeswaram, Guntur (Dist), Andhra Pradesh 522502, INDIA

Mathi, Pardhasaradhi
Upstream Process Development Lab, Gene therapy department, Reliance life sciences, Navi Mumbai, Maharashtra, 400701 INDIA

Botlagunta, Mahendran* +●
Biomedical Research Laboratory, Department of Biotechnology, K L E F University (Koneru Lakshmaiah Educational Foundation), Greenfields; Vaddeswaram, Guntur (Dist), Andhra Pradesh 522502, INDIA

ABSTRACT: *An experimental approach for the estimation of average shear rate (γ_{av}) in stirred tank bioreactor has been proposed for the turbulent flow regime. Based on the proposed methodology, the correlation for the estimation of γ_{av} was obtained as a function of agitation speed (N), superficial gas velocity (Vs) and the rheological properties of the non-Newtonian fluids. The γ_{av} estimated by the present method was found to be within the range of values calculated by correlations available in the literature. The γ_{av} increased with the increase of agitation and sparging in all the conditions tested. The correlation derived in the present work helps in estimation of estimation of γ_{av} as a function of bioreactor geometry along with physical conditions (N and Vs), and rheological properties (n and K) of non-Newtonian fluid in commercially available stirred tank bioreactor.*

KEYWORDS: *Average shear rate; Bioreactor; Non-Newtonian fluid; Rheology.*

INTRODUCTION

Biopharmaceutical products have revolutionized in the treatment of various diseases. These medications are manufactured using live microorganisms such as CHO cells, bacteria, yeast etc. in stirred tank bioreactor. As a result of increase in biomass, the culture broth exhibits a pseudo plastic non-Newtonian behavior [1,2] which can be described by power-law model. To enhance adequate mixing and mass transfer in stirred tank bioreactor, selection of the type of impeller is also an important criteria [3]. The impeller geometry influences

* *To whom correspondence should be addressed.*
+ *E-mail: drmahi_bt@kluniversity.in*
● *Other Address: School of Bioengineering, VIT Bhopal University, Madhya Pradesh, 466114, INDIA*
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the hydrodynamics of the culture broth and finally affecting the batch productivity [4]. The most commonly used impeller in the bioprocess is the Rushton turbine impeller [5,6] and it provides higher gas dispersion and higher volumetric mass transfer values [7].The increase in the biomass also increases the apparent viscosity (μ_{app}) of non-Newtonian fluid which can be mitigated by increasing the impeller agitation speed (N) and specific airflow rate (V_s). This in turn influences gas-liquid mass transfer and also causes increase in shear rate (γ_{av}). Increased shear rate changes the rheological properties of the culture and thus affecting the batch performance [8-10]. Hence, need has been created to consider shear as one of the parameters during process development [11-13].

A number of correlations were available in the literature for the estimation of shear rate as a function of impeller agitation speed [14-19]. The shear rate is also effected by rheological properties of the fluids like flow index (n) and consistency index (K) for non-Newtonian fluids [15,16,19,20]. Correlations have been proposed for the estimation of average shear rate in laminar flow regime. Few correlations described that the average shear rate (γ_{av}) in bioreactor is dependent on impeller agitation speed (N) and k [21]. Where k is a constant with a value of 11.4 for Rushton turbine impeller. The value of k depends on the geometry of the impeller. [22] defined that γ_{av} is a function of impeller speed (N) and flow index (n), a rheological property of non-Newtonian fluids. Correlations for the estimation of γ_{av} in the transition flow regime has been proposed by [17,18].

The present work was aimed to develop a methodology for the estimation of average shear rate, γ_{av}. In this method superficial gas velocity (V_s) was selected for the estimation of γ_{av}. Correlation proposed in the present work is more appropriate for the estimation of γ_{av} because it uses superficial gas velocity V_s (function of bioreactor geometry and specific air flow rate), impeller agitation N and rheological properties (K and n) of the non-Newtonian fluid.

EXPERIMENTAL SECTION

Bioreactor configuration

The study was performed in a commercially available stir-tank bioreactor (5 L) (Sartorius, Germany). The un-baffled bioreactor had a tank diameter, (T) of 0.160 m and a tank height (H) of 0.32 m and was equipped with two Rushton turbine impellers (Di = 0.064 m) fixed at a distance of one impeller diameter. Gasses were introduced into the bioreactor by using a stainless-steel ring sparger (diameter of drilled holes 1.0 mm). The ratio of diameter of impeller to diameter of tank (Di:Dt) was 0.4. Fig. 1a and b shows the pictorial representation of the bioreactor and the impeller used in this work.

Fluids

Glycerol solutions (Sigma Aldrich, St. Louis, Missouri, USA) were used as Newtonian fluid and Carboxy methyl cellulose (Sigma, C5678) was used as non-Newtonian fluid. The dynamic viscosity of the Newtonian fluid and rheological parameters of the non-Newtonian fluid were determined by using a viscometer (Bohlin Visco88 and TA-instruments AR2000) fitted with a coquette configuration. Table 1 shows the dynamic viscosity and rheological properties of the fluid used in the current work.

Experimental determination of K_La

The determination of the overall volumetric mass-transfer coefficient value of a bioreactor is essential to establish its aeration efficiency and to quantify the effect of operating variables on the provision of oxygen. The, K_La estimation was done by following the static gassing-out method. The non-Newtonian fluid was deoxygenated by sparging nitrogen gas until the dissolved oxygen level reached below 5% of the saturation. The variation in the dissolved oxygen concentration, (C_L) in the liquid phase was detected using an oxygen probe. The dissolved oxygen concentration in the bioreactor liquid phase was measured by means of an oxygen probe inserted vertically and placed at 2 cm under the liquid level, the DO probe fitted with a Teflon membrane and with an electrolytic solution of Na_3PO_4 in the cell. The oxygen probe signals were measured using an A/D converter and a recorder on a PC. After that the nitrogen gas flow was turned off and the flow switched to the air flow with a specific volumetric flow rate using the rotameter. Then the dissolved oxygen concentration was recorded with respect to time as air was distributed into the bioreactor and until the water became saturated with oxygen. The dissolved oxygen was monitored until saturation, C* was reached. Gas composition was constant. The system was isothermal, and the effect of the dynamics of the dissolved oxygen electrode was negligible.

Table 1: Dynamic viscosity (μ) and rheological properties (K and n) of the Newtonian and non-Newtonian fluid.

Newtonian Fluids		Non-Newtonian Fluids		
Glycerol Solution (GS)	μ x 10^3 (Pa s)	Carboxy Methyl Cellulose (CMC) solution	K (Pa s^n)	n (dimensionless)
GS-1	1.35	CMC-1	0.147	0.929
GS-2	2.72	CMC-2	0.174	0.807
GS-3	7.19	CMC-3	0.262	0.799
GS-4	9.85	CMC-4	0.346	0.715
GS-5	14.1	CMC-5	0.421	0.681

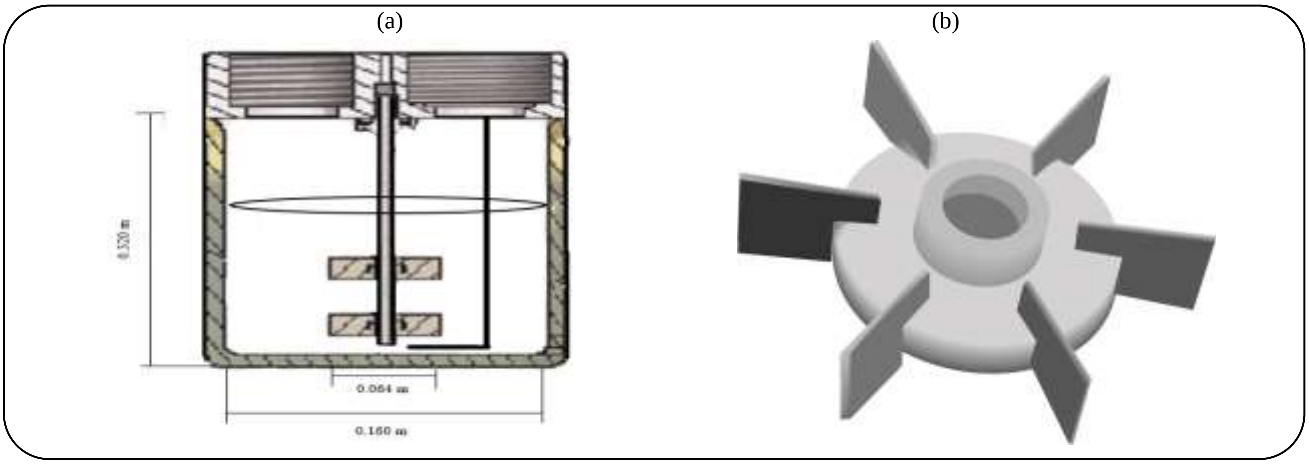

Fig. 1: Schematic view of the (a) bioreactor and (b) impeller (Rushton turbine impeller) used in this study:

The rate of oxygen transfer from gas to liquid phase was given by the empirical relationship [23].

$$\frac{dCL}{dt} = KLa\,(C*-CL) \quad (1)$$

The K_La values were calculated from the slope of the plot of ln(C*-C_L) versus time, t. A turbulent flow regime is generated at higher agitation speed where the Reynolds number (Re) > 5 x10^3. In this study, the Reynolds number (Re) was considered over a wide range of 25,000 to 75,000. K_La was estimated in triplicates with the above mentioned Re range and gas flow rate ranging from 2.0 x 10^{-5} m/s to 2.0 x10^{-4} m/s.

Method for estimation of average shear rate (γ_{av})

The average shear rate γ_{av} in the stirred tank bioreactor was estimated by following the method proposed by [24] in which mass transfer coefficient (K_La) was used as a characteristic parameter to estimate γ_{av} in concentric tube airlift bioreactor. Later this methodology was modified for stirred tank reactors by [19, 25]. The specific energy dissipation rate is dependent on shear rate γ and shear stress τ [26,27]

$$\frac{P}{V} = \tau\,\gamma \quad (2)$$

Where P is the power input and V is the fluid volume. For Newtonian fluids, dynamic viscosity (μ) is the ratio of shear stress and shear rate.

$$\mu = \frac{\tau}{\gamma} \quad (3)$$

Equation (2) can be written as

$$\frac{P}{V} = \mu\gamma^2 \quad (4)$$

For non-Newtonian fluids obeying the power law [28],

$$\tau = K\gamma^n \ \ (n < 1) \quad (5)$$

Where, K is the consistency index and n is the flow behavior index. Analogous to Newtonian fluids, apparent viscosity (μ_{app}) of non-Newtonian fluids can be described as

$$\mu_{app} = \frac{\tau}{\gamma} = K\,\gamma^{n-1} \qquad (6)$$

For non-Newtonian fluids, the equation corresponding to Equation (4) becomes

$$\frac{P}{V} = \mu_{app}\gamma^2 \qquad (7)$$

$$\frac{P}{V} = K\gamma^{n+1} \qquad (8)$$

The K_La values were correlated with correlation developed by [29].

$$KLa = \alpha\left(\frac{P}{V}\right)\beta\, Vs^{\delta} \qquad (9)$$

Where P is the mechanical agitation power (W), V = liquid volume (m^3), V_s. = gas superficial velocity (m/s), α = is a constant, β and δ are exponents. Substituting the value of equation 8 in equation 9,

$K_La = \alpha\,(K\,\gamma_{av}^{n+1})^{\beta}Vs^{\delta}$

$$\gamma_{av} = \left(\frac{KLa}{\alpha\, Vs^{\delta}K^{\beta}}\right)^{\frac{1}{\beta(n+1)}} \qquad (10)$$

The K_La for the non- Newtonian fluid was estimated by following the methodology proposed by [30]

$$K_La = b\, N^cV_s^dK^e \qquad (11)$$

Where N is the impeller, Vs = gas superficial velocity, b = is a constant, c, d and e are exponents.

Substituting the value of equation 11 in equation 10, γ_{av} is obtained as a function of agitation, gas velocity and rheological parameters of the non- Newtonian fluid

RESULTS AND DISCUSSION

Experimental K_La data for Newtonian fluid was correlated according to equation 9. The experimental values of K_La were in the range of 0.06–0.084 min^{-1}. The experimental values were fitted to the correlation in equation 9. The constant α and exponentials (β and δ) were estimated by least square non-linear regression by following Marquardt's procedure [31]. The proposed correlation along with correlation coefficient (R^2) is shown below

$$K_La = 0.055\,(P/V)^{0.320}(V_s)^{0.532} \qquad (12)$$

$(R^2 = 0.999)$

High value of the correlation coefficient R^2 shows that good fits were obtained indicating that the correlation can be used for accurate estimation of K_La.

For non-Newtonian fluids, experimental K_La data were correlated according to equation 11. The experimental value of K_La was in the range of 0.02–0.063 min^{-1}. The experimental values were fitted to the correlation in equation 11. The constant b and exponentials (c, d and e) were estimated by least square non-linear regression by following Marquardt's procedure [31]. The proposed correlation along with correlation coefficient (R^2) is shown below

$$K_La = 0.9\,(P/V)^{0.364}(V_s)^{0.631}K^{0.530} \qquad (13)$$

$(R^2 = 0.999)$

Fig. 2 shows the comparison between the experimental and calculated data of K_La and the difference were below 7.5%. Figure1 and high value of the correlation coefficient R^2 shows that good fits were obtained indicating that the correlation can be used to accurately estimate K_La in agitated and aerated tanks with non- Newtonian fluids.

Effect of apparent viscosity (μ_{app}) on K_La is shown in Figure 3. It can be observed that the K_La increased with increase in flow index (n) and decrease of consistency index (K).According to Equation(6), apparent viscosity (μ_{app}) increases with the increase in K and decrease of n. Increase in the μ_{app} negatively influenced the K_La as it generates resistance between the gas- liquid mass transfer.

After estimation of the values of parameters of equation 9 and equation 11, average shear rate correlation was equated as a function of impeller agitation (N), superficial gas velocity (V_s) and rheological parameters (K and n) of the fluid.

$$\gamma_{av} = (16.2\, N^{0.364}\, V_s^{0.099}K^{0.210})^{1/0.320(n+1)} \qquad (14)$$

As expected, the exponent of N is 3.7 times higher than V_s. Hence, γ_{av} is strongly influenced by agitation speed compared to specific air flow rate. The Equation (14) is better suited for estimation of γ_{av} as it uses experimental values of K_La and also involves superficial gas velocity which is a function of bioreactor geometry and air flow rate. To validate the correlation proposed in the present work for estimation of γ_{av}, it was compared with earlier reported correlations using an intermediate CMC solution (CMC-3) as non-Newtonian fluid at different agitation speed N and V_s= 2.0 x 10^{-5} m/s. Table 2 describes various

Table 2: Correlations for estimation of average shear rate, γ_{av}.

References	Proposed Correlation
[14]	$\gamma_{av}= k N$
[17]	$\gamma_{av}= 33.3N$
[18]	$\gamma_{av}= 33.1N^{1.4}$
[19]	$\gamma_{av}= 1.571 (\frac{2.876}{1-n})K(\frac{0.609}{1-n}) N \frac{1.343}{1-n}$
[22]	$\gamma_{av}= k (\frac{4n}{3n+1})\frac{n}{n-1} N$
[25]	$\gamma_{av}= (3.14 N^{0.653} Ø_{air}^{-0.2} K^{0.751} n^{1.193})\frac{1}{0.620(1-n)}$

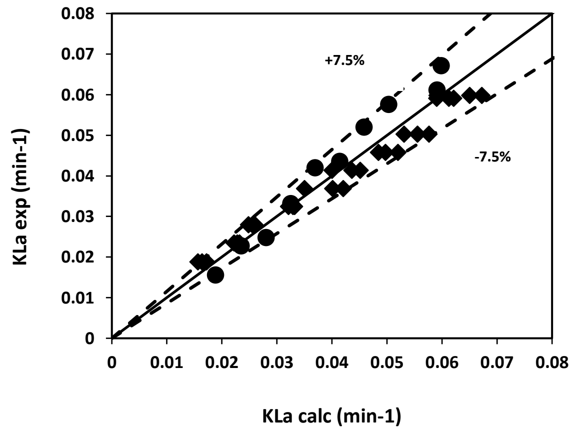

Fig. 2: Comparison between calculated and experimental data of KLa estimation at V_s=1.0×10^{-4} m/s (♦) experimental values, (●) correlation values.

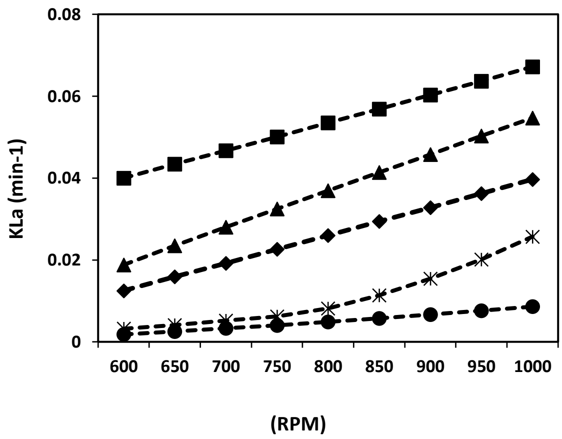

Fig. 3: Estimated K_La values at different agitation speed for non-Newtonian fluids at V_s= 1.0 x10^{-4} m/s. (■) CMC-1, (▲) CMC-2, (♦) CMC-3, (×) CMC-4, (●) CMC-5.

correlations available in the literature for evaluation of γ_{av} and Fig. 3 shows the profile of γ_{av} obtained from the correlations.

It can be observed from figure 4 that the values of γ_{av} calculated by the correlation proposed in the present work are within the values predicted by [17] and [18], indicating that the proposed correlation estimated γ_{av} is closer to the literature value. The γ_{av} estimated by using [14] and [22] was different as compared to other authors because these correlations were proposed for laminar flow regime while equation predicted for the estimation of γ_{av} by [17] and [18] were proposed for transitional flow regime. Hence the values of γ_{av} predicted by using these correlations were extrapolated for turbulent flow regime. A similar system set-up to that of present work was used by [19] and [25] for the estimation of γ_{av}, however their studies were performed in baffled bioreactor and the size of the impeller and the flow rate of gas used were different as compared to the present work.

To illustrate the effect of agitation speed N and V_s on γ_{av}, Fig. 5 shows the γ_{av} as a function of N at different V_s. It can be observed that the increase in agitation speed N, positively affected shear rate γ_{av}. Considering the influence of V_s on γ_{av}, increase in gas flow rate resulted increase in the γ_{av}. Increase in V_s creates higher number of bubbles which further burst of the surface of the fluid and causing an increase in γ_{av} [32]. A similar behavior has been reported by [19] in which high values of V_s resulted in increase of γ_{av}

In all the bioprocess the culture broth obeys the power law model (Eq. 6) in which the flow index (n) decreases with the culture while and consistency index (K) rises and increasing the apparent viscosity (μ_{app}) of the culture [33]. At the end of the stationary phase of the culture,

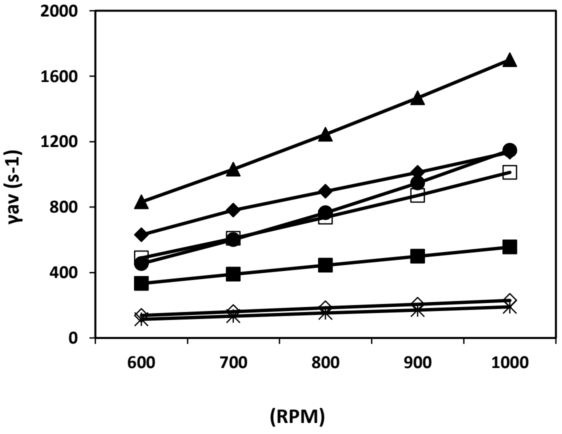

***Fig. 4:** Comparison of average shear rate (γ_{av}) in agitated bioreactors with Rushton turbine impellers as function of agitation speed N for Carboxy methyl cellulose solution (CMC-3) aerated at 2.07x10^{-5} m/s. (■) [17], (▲) [18], (♦) present work, (×) [14], (◊) [22], (●) [19], (□) [25].*

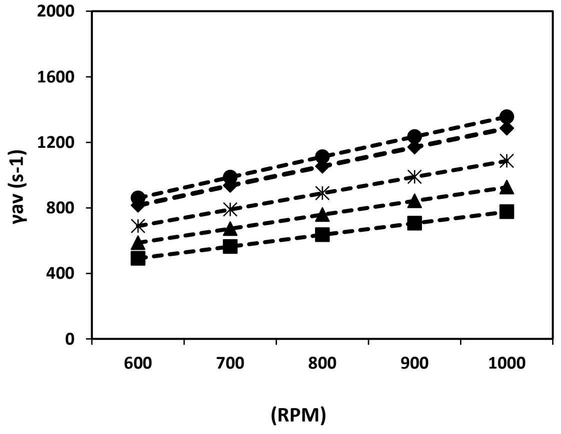

***Fig. 5:** Average shear rate (γ_{av}) as function of agitation speed N and V_s for Carboxy methyl cellulose solution (CMC-2) aerated at (●) 1.0 x10 $^{-4}$ m/s, (♦) 8.03 x10 $^{-5}$ m/s, (×) 4.01 x10 $^{-5}$ m/s, (▲) 2.00 x10 $^{-5}$ m/s, (■) 1.01 x10 $^{-5}$ m/s.*

apparent viscosity (μ_{app}) decreases due to higher cell death and increasing the average shear rate (γ_{av}). In this context, a similar behavior of apparent viscosity (μ_{app}) with average shear rate (γ_{av}) has been reported by [19] while working with *S.clavuligerus* in stirred tank bioreactor.

CONCLUSION

Good fits were obtained for the K_La correlations derived for newtonian and non-newtonian fluids. In the present work, a correlation was derived for the estimation of average shear rate (γ_{av}) as a function of agitation speed (N), superficial gas velocity (V_s) and rheological properties of the culture broth in the turbulent flow regime. Most of the correlations available in the literature for estimation of γ_{av} were developed for laminar and transient flow regime. γ_{av} predicted by the current method was within the range of values estimated from correlations available in the literature for turbulent flow regime, hence indicating that the present method predicts reliable values of γ_{av} The choice of V_s as a characteristic parameter was appropriate for the estimation of average shear rate (γ_{av}) because it considers bioreactor geometry and dimensions along with gas flow rate (Q). Commercially available stirred tank reactors vary among themselves on the basis of different geometry and thus generating diverse hydrodynamic patterns. Considering bioreactor geometry in the evaluation will provide better understanding of the shear generated by the impellers inside the bioreactors. γ_{av} increased proportionately with increase of N and Vs while increase in μ_{app} resulted in reduction of $\gamma_{av.}$ Hence, N and V_s needs to be optimized to protect the cells from shear generated by the impeller in stirred tank reactor.

Nomenclature

C*	Dissolved oxygen saturation concentration, mg/L
C_L	Initial dissolved oxygen concentration, mg/L
CHO	Chinese hamster ovary
Di	Diameter of impeller, m
H	Tank height, m
K_La	Volumetric mass transfer coefficient, min^{-1}
K	Consistency index, Pa S^n
N	Agitation speed, rps
n	Flow behavior index
Np	Power number
P	Power, Watts
Q	Gas flow rate, m^3/s
Re	Reynolds number
T	Tank diameter, m
Vs	Superficial gas velocity, m/s
vvm	Vessel volume per minute
V	Working volume, m^3
α,β,δ	Parameters of equation 9, dimensionless
b,c,d,e	Parameters of equation 11, dimensionless
γ	Shear rate, s^{-1}
γ_{av}	Average shear rate, s^{-1}

μ	Dynamic viscosity, Pa s
μ_{app}	Apparent viscosity, Pa s
τ	Shear stress, Pa

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REFERENCE

[1] Sassi G., Ruggeri B., Specchia V., Gianetto A., Citric Acid Production by *Aspergillus niger* with Banana Extract, *Bioresour. Technol* **37**, 259–269 (1991)

[2] Justen P., Paul GC., Nienow AW., Thomas CR., Dependence of Mycelial Morphology on Impeller Type and Agitation Intensity, *Biotechnol Bioeng*., **52**, 672–684 (1996).

[3] Garcia-Ochoa F., Gomez E., Mass Transfer Coefficient in Stirred Tank Reactors For Xanthan Gum Solutions. *Biochemical Engineering Journal*. **1**: 1-10 (1998).

[4] Tang W., Pan A., Lu H., Xia J., Zhuang Y., Zhang S., Chu J., Noorman H., Improvement of Glucoamylase Production Using Axial Impeller with Low Power Consumption and Homogenous Mass Transfer, *Biochemical Engineering Journal*, **99** 167-176 (2015).

[5] Okabe M., Kuwajima T., Satoh M., Kimura K., Okamura K., Okamoto R., Preferential and High Yield Production of Cephamycin C by Dissolved Oxygen Controlled Fermentation, *J. Ferment. Bioeng*. **73(4)**: 292-296 (1992).

[6] Rosa JC., Baptista-Neto A., Hokka CO., Badino AC., Influence of Dissolved Oxygen and Shear Conditions on Clavulanic Acid Production by *Streptomyces clavuligerus*, *Bioprocess Biosyst. Eng*., **27**: 99-104 (2005).

[7] Kresta S.M., Brodkey R.S., Turbulence in Mixing Applications. In: Paul E.L., Atiemo-Obeng V.A., Kresta SM. (Editors), "Handbook of Industrial Mixing: Science and Practice", Wiley-Interscience, New York, pp. 19-87 (2003).

[8] Oh S.K.W., Nienow A.W., Al-Rubeai M., Emery A.N., Further Studies of the Culture of Mouse Hybridomas in an Agitated Bioreactor with and Without Continuous Sparging. *Journal of Biotechnology*. **22**: 245–270 (1992).

[9] Meijer J.J., ten Hoopen, H.J.G., Luyben K., Libbenga K.R., Effects of Hydrodynamic Stress on Cultured Plant Cells: A Literature Survey, *Enzyme and Microbial Technology*, **15**: 234–238 (1993).

[10] Chisti Y., Hydrodynamic Damage to Animal Cells, *Crit. Rev. Biotechnol*., **21(2)**: 67–110 (2001).

[11] Zhang Z., Chisti Y., Moo-Young M., Effects of the Hydrodynamic Environment and Shear Protectant on Survival of Erythrocytes In Suspension, *Journal of Biotechnology*, **43**: 33-40 (1995).

[12] Martens D.E., de Gooijer C.D., C.A.M. van der velden-de Groot., Beuvery EC., Tramper J., Effect of Dilution Rate on Growth, Productivity, Cell Cycle and Size, and Shear Sensitivity of a Hybridoma Cell in a Continuous Culture, *Biotechnol Bioeng*., **41**: 429-439 (1993)

[13] Bowen R.L., Unraveling the Mysteries of Shear-Sensitive Mixing Systems, *Chem. Eng*., **93**: 55–63 (1986).

[14] Metzner A.B., Otto R.E., Agitation of Non-Newtonian Fluids, *AIChE*, **3**: 3–10 (1957)

[15] Robertson B., Ulbrecht J.J., "Measurement of Shear Rate on an Agitator in a Fermentation Broth", In: Ho CS, Oldshue JY (ed), Biotechnology Processes, AIChE, New York. (1987)

[16] Wichterle K., Kadlec M., Zack L., Mitschka P., Shear Rates on Turbine Impeller Blades, *Chemical Engineering. Communications*, **26**: 25–32 (1984).

[17] Kelly W., Gigas B., Using CFD to Predict the Behavior of Power Law Fluids Near Axial-Flow Impellers Operating in the Transitional Flow Regime, *Chemical Engineering Science*. **58**: 2141–2152 (2003).

[18] Perez, J.A.S., Porcel E.M.R., Lo´pez J.L.C., Sevilla J.M.F., Chisti Y., Shear Rate In Stirred Tank and Bubble Column Bioreactors, *Chemical Engineering Journal*. **124**, 1–5 (2006).

[19] Campesi A., Cerri M.O., Hokka C.O., Badino A.C., Determination of the Average Shear Rate in a Stirred and Aerated Tank Bioreactor, *Bioprocess Biosystems Engineering*, **32**: 241–248 (2009).

[20] Chisti M.Y., Moo-Young M., Airlift Reactors: Characteristics, Applications and Design Considerations, *Chemical Engineering Communications*, **60**: 195–242 (1987)

[21] Metzner A.B., Feehs R.H., Ramos L.H., Tuthill J.D., Otto RE., Agitation of Viscous Newtonian and Non-Newtonian fluids. *AIChE*. **7(1)**: 3-9 (1961).

[22] Calderbank P.H., Moo Young M.B., The Prediction of Power Consumption in the Agitation of Non-Newtonian Fluids, *Chemical Engineering Research and Design*. **37**: 26-33 (1959).

[23] Stanbury P.F., Whitaker A., Hall S.J., "Principles of Fermentation Technology", Elsevier (2013)

[24] Cerri M.O., Futiwaki L., Jesus C.D.F., Cruz AJG., Badino AC., Average Shear Rate for Non-Newtonian Fluids in a Concentric Tube Airlift Bioreactor, *Biochemical Engineering Journal*, **39**: 51-57 (2008).

[25] Bustamante M.C.C., Cerri M.O., Badino A.C., Comparison between Average Shear Rates in Conventional Bioreactor with Rushton and Elephant Ear Impellers. *Chemical Engineering Science,* **90**: 92-100 (2013)

[26] Metz B., Kossen N.W.F., van Suijdam J.C., The Rheology of Mould Suspension, *Advances in Biochemical Engineering*, **11**: 103-156 (1979).

[27] Nagata S., "Mixing Principles and Application", John Wiley & Sons Inc., New York, USA (1975)

[28] Coulson J.M., Richardson J.F., "Chemical Engineering", Vol. 1, 4th ed., Pergamon Press, Oxford, pp. 227–263 (1990).

[29] Cooper C., Fernstrom G.A., Miller S.A., Performance of Agitated Gas-Liquid Contactors, *Industrial & Engineering Chemistry*. **36**: 504-509 (1944).

[30] Badino A.C., Facciotti M.C.R., Schmidell W., Volumetric Oxygen Transfer Coefficients (kLa) in Batch Cultivations Involving Non-Newtonian Broths, *Biochemical Engineering Journal.* **8(2)**: 111-119 (2001).

[31] Marquardt D.W., An Algorithm for Least-Squares Estimation of Nonlinear Parameters, *Journal of the Society for Industrial and Applied Mathematics.* **11(2)**: 431-441 (1963)

[32] Li Z.J., Shukla V.,Wenger K.S., Fordyce A.P., Pedersen A.G., Marten M.R., Estimation of Hyphal Tensile Strength in Production-Scale *Aspergillus oryzae* Fungal Fermentation, *Biotechnology and Bioengineering*. **77**: 601-613 (2002).

[33] Doran P.M., "Bioprocess Engineering Principles", Elsevier Science and Technology Books (2013).