APPLICATIONS OF PNEUMATIC BIOREACTORS IN WASTEWATERS TREATMENT
1. MIXING EFFICIENCY AND DISTRIBUTION IN SPLIT-CYLINDER GAS-LIFT BIOREACTOR FOR VISCOUS MEDIA

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Abstract

The distribution of mixing time for a split-cylinder gas-lift bioreactor has been investigated for simulated broths with apparent viscosities up to 26 cP. The results indicated important variation of mixing time on the height of riser or downcomer, as well as different behavior of dispersion flows in the two regions. Thus, the highest mixing efficiency for the ascending circulation has been recorded for the intermediary positions, placed above the sparger. Contrary, for the downcomer, the intermediary positions are associated with the lowest efficiency of mixing, due to the bubbles entrapping phenomenon. Moreover, at air superficial velocities over 1.05x10⁻³ m/s, the influence of air input rate on turbulence extent is continuously positive for the riser, while it became negative for the downcomer. By means of the experimental data and using MATLAB software, mathematical correlations for mixing time have been proposed for each circulation region, taking into consideration both the operational parameter and the distance from the bioreactor bottom. These equations offer a good concordance with the experiment, the average deviation being of 3.3% for the riser and 6.6% for the downcomer zone.

Key words: pneumatic bioreactor, gas-lift bioreactor, split-cylinder, mixing time, simulated broth

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1. Introduction

Pneumatic bioreactors are widely used in the biotechnological processes of chemicals productions or pollutants treatment, at laboratory or industrial scale (Chisti and Moo-Young, 1988; Gavrilescu, 1997; Gavrilescu and Chisti, 2005; Kilonzo and Margaritis, 2004). These bioreactors exploit the difference on hydrostatic pressure or density from different regions inside the broth to induce its circulation. The classification of pneumatic bioreactors considers three main types, namely bubble columns, gas-lift bioreactors and fluidized bioreactors. Among them, the gas-lift bioreactors are the most attractive for large-scale applications, due to their simplicity of construction (without any rotating devices), significant reduction of power consumption compared to stirred or hydraulic bioreactors, superior efficiency of heat and mass transfer rates related to specific power input, possibility to process high volumes of different media, easiness of maintaining the aseptic conditions during the fermentation cycle, as well as low operational and maintenance costs (Baque et al., 2012; Gavrilescu, 1997; Gavrilescu and Chisti, 2005; Li et al., 2009; Zimmerman et al., 2009).

Gas-lift bioreactors are designed as a variety of internal and external loop devices (split-cylinder...
or draft tube internal-loop, with or without impeller driven loop, packed bed, external loop, etc.) (Chisti and Moo-Young, 1988; Gavrilescu, 1997; Gavrilescu and Chisti, 2005; Kilonzo and Margaritis, 2004; Moraveji et al., 2013; Xu and Yu, 2008; Zimmerman et al., 2009). These devices induce the cyclic circulation of the broths, the cells being continuously recycled from the region of high turbulence (riser region) to that of lower turbulence (downcomer region). This flow pattern exposes the cells to fluctuating growth conditions, the uniform distribution of the nutrients and oxygen concentrations, temperature, and shear stress magnitude representing one of the main factors controlling the gas-lift bioreactors performance. The homogeneity of the aforementioned parameters in the whole bulk of broths could be improved by a more uniform distribution of the mixing intensity.

According to the simplest and most suggestive definition, given by Hiby, the mixing is the process through which the inhomogeneity of a system is decreased (Hiby, 1981). Thus, the complete mixed system corresponds to a perfect uniform distribution of its components. The mixing can be analyzed from the viewpoint of the homogenization level: macromixing, mesomixing, and micromixing (Bujalski et al., 2002).

Macromixing consists on the uniform distribution of the content in the whole bulk of broth due to the mechanical of pneumatic agitation of liquid. Mesomixing is directly related to the nutrients consumption rate. Therefore, when the feed rate of a certain nutrient is high and leads to its accumulation in the feed region, then a local inhomogeneity appears. However, this region could be considered perfectly mixed inside, due to the internal circulation (Baldyga and Bourne, 1992). Micromixing describes the mixing at molecular scale and is controlled by molecular diffusion. Regardless of the flow regime promoted in the whole bulk of broth, the flow remains laminar at micromixing scale, due to its coherent structure of the vortex sheets and vortex tubes types (Vincent and Meneguzzi, 1994). Mesoo- and micromixing are important mainly for the bioprocesses consisting on phase transformation or biochemical reactions.

One of the most useful criterion for the characterization of mixing efficiency is the mixing time, defined as the time needed to reach a given mixing intensity at a given scale, considering the system to be completely segregated at the initial moment (Bujalski et al., 2002; Caçaçval et al., 2007; Galaction et al., 2007; Geisler et al., 1991; Nienow, 1997; van’t Riet and Tramper, 1991). This parameter offers specific information concerning the bulk mixing in the system, macromixing, suggesting the optimum hydrodynamic regime, the proper stirrer type, or predicting the modification of mixing efficiency induced by scaling-up (Nienow, 1997; Oniscu et al., 2002). However, it does not allow to quantifying rigorously the meso- and micromixing (Bujalski et al., 2002).

The mixing time is experimentally measured by tracers method, consisting on the addition of acidic, alkaline, salts, heated or colored solutions to the beforehand homogenized media or broths (Oniscu et al., 2002; van’t Riet and Tramper, 1991). Therefore, the mixing time is defined as the time needed for the related parameter (pH-value, temperature, absorption, etc.) to reach a constant value. Because the perfect mixing can only be reached after a long time period, for the mixing time determination a predefined level of homogeneity is admitted.

The analysis of mixing is similar and based on the above mentioned principles for all types of bioreactors. However, the change of the broths rheological characteristics and behavior during the biomass accumulation, the high viscosity or non-Newtonian rheology behavior of the broths, as well as the sensitivity of the cells to shear stress amplify the complexity of the mixing analysis in these fermentation systems.

Regardless of the gas-lift bioreactor design, the studies on mixing efficiency reported in literature are related especially to the average value of mixing time for the whole bulk of broths, or to the influence of tracer feed position on this parameter (Bujalski et al., 2002; Chisti and Moo-Young, 1988; Moraveji et al., 2012; Moraveji et al., 2013; Sukan and Vardar-Sukan, 1987). Due to the cyclic flow patterns and, implicitly, to the existence of regions with different turbulence extent, the relevance of these studies for pneumatic bioreactors, especially for gas-lift ones, is questionable. Therefore, the analysis of distribution of mixing intensity inside the gas-lift bioreactor could offer more relevant information concerning the correlation between the cells growth rate and environmental conditions, namely the variation of pH, temperature, substrate and oxygen concentrations, and inhibitory effects inside the broth. In this manner, the stagnant regions could be easily identified and the influence of broth characteristics or process conditions on the mixing efficiency could be more rigorously analyzed for each region inside the gas-lift bioreactor.

According to the literature, for the turbulent flow regime, the electrode position does not affect the values of mixing time (Rielly and Pandit, 1988; Ruszowski, 1994; Thyn et al., 1976). However, at low broths flow velocity, significant variations of mixing time values have been recorded by modifying the electrode position (Caçaçval et al., 2007). Furthermore, most of the models describing the mixing into the bioreactors predict the mixing time values only for Reynolds number over 10,000 (van’t Riet and Tramper, 1991). As the result of the microorganisms’ sensitivity to shear stress, the flow regime into the bioreactors is laminar or transitory. Therefore, the flow regime corresponding to Reynolds number over 10,000 cannot be applied especially in the large-scale bioreactors, the models needing some corrections (van’t Riet and Tramper, 1991). In this purpose, the analysis of mixing...
distribution in the gas-lift bioreactors requires the determination of mixing time for different positions of electrode on the broths height, both in the riser region and downcomer one.

For these reasons, the aim of our studies is to establish the distribution of mixing efficiency inside the split-cylinder gas-lift bioreactor, by means of the mixing time values recorded at various positions of pH-electrode, as well as the influences of the broths characteristics, bioreactor geometry, and operating parameters on the interchange of active and stagnant regions positions.

For underlining the effect of the biomass presence and morphology on mixing efficiency, the experiments have been carried out for viscous simulated broths (without biomass) and real broths (containing bacteria, yeasts and fungus biomass). By means of the experimental data, mathematical correlations between the mixing time and the considered parameters have been established both for the riser and downcomer zones.

This study has been focused on the analysis of mixing intensity and its distribution in viscous simulated broths.

2. Materials and methods

The experiments have been carried out in 6 l (5 l working volume) laboratory split-cylinder gas-lift bioreactor FerMac 310/60 (Electrolab), with computer-controlled and recorded parameters (Fig. 1). The bioreactor reactor consists of a glass cylinder with 0.49 m height and 0.125 m diameter. For dividing the flow into the riser and downcomer regions with similar cross areas, a rectangular stainless steel baffle having 0.285 m height, 0.125 m width, and 2x10^{-3} m thickness was diametrically inserted into the glass column. The baffle was located at a distance of 0.04 m from the bottom of the reactor.

The air sparger consists of a 0.05 m long perforated tube 5x10^{-3} m in diameter placed at 0.08 m from the bottom of the bioreactor. The sparger has 5 orifices with 1 mm diameter. The air flow rate varied between 20 and 100 l/h (corresponding to a superficial velocity of 0.45 - 2.25x10^{-3} m/s). This domain of aeration rate has been selected for avoiding the slugging effect in the lower viscous broths, being respected for all viscosities considered in the experiments.

In the experiments, water and viscous simulated broths have been used. The simulated broths were carboxymethylcellulose sodium salt solutions with the apparent viscosity in the domain of 5 - 26 cP. Owing to the difficulty of in-situ measurement of viscosity during the experiments, the viscosity was measured before and after each experiment using a viscometer of Viscotester 6 Plus type (Haake).

Both the experiments and viscosity measurements were carried out at a temperature of 30°C. Any viscosity change was recorded during the experiments.

![Fig. 1. Schematic diagram of the experimental split-cylinder gas-lift bioreactor (1 - riser region, 2 - downcomer region, 3 - baffle, 4 - pH probe positions)](image)

The mixing efficiency has been analyzed by means of the mixing time values. For mixing time determination, a solution of 2N KOH has been used as tracer, being measured the time needed to the pH to reach the value corresponding to the considered mixing intensity. In this case, the following homogeneity criterion for mixing has been considered (Eq. 1):

\[
I = \frac{pH_{\infty} - 0.5\Delta pH}{pH_{\infty}} \times 100 = 99\% 
\]  

where \(\Delta pH = 0.02\).

The tracer volume was of 0.5 ml, the tracer being injected in the riser region, at 0.05 m from the bioreactor central axis and 0.38 m from the liquid surface. Because the tracer solution density is close to that of liquid phase, the tracer solution flow follows the liquid flow streams without errors due to the tracer buoyancy.

The pH electrode (HA 405 Mettler Toledo) was introduced initially in the riser region, then in the downcomer one. In both cases, it was placed at four different positions on the liquid height, varying from bioreactor bottom as follows (Fig. 1):

- position 1: at 0.06 m
- position 2: at 0.16 m
- position 3: at 0.26 m
- position 4: at 0.36 m.

The pH variations were recorded by the bioreactor computer-recorded system and were analyzed for calculating the mixing time.

The mathematical correlations, which describe separately the influences of considered factors on mixing time for the simulated broth at various positions inside the riser and downcomer regions have been developed using MATLAB software. For the experimental data, a multiregression analysis was performed, the difference between the experimental and modeled value being reduced to a minimum by least-square fit method. By means of a MATLAB program, the regression coefficients and standard deviations were calculated.
Each experiment has been carried out for three or four times, for identical conditions, the average value of mixing time being used. The maximum experimental errors were between 5.34 and 6.42%.

3. Results and discussion

The rheological characteristics or behavior of the broths are continuously changed during the fermentation process, as the result of biomass or high weight molecular products accumulation. Thus, the appearance and increase of the stagnant regions volume in the bioreactor cannot be avoided.

The heterogeneity and, implicitly, complexity of the broth hydrodynamics are more pronounced in the case of internal-loop gas-lift bioreactors, due to the cyclic pattern of fluid flow. In this case, besides the rheological properties of the broth or solid phase (biomass, biocatalysts) concentration and deposition tendency, the efficiency and distribution of mixing are controlled by a lot of geometrical and operational characteristics of the bioreactor: height, riser and downcomer cross areas ratio, height of gas separation and clearance regions, gas input rate (Gavrilescu, 1997; Merchuk and Gluz, 1999). Therefore, the accurate conclusions concerning the mixing distribution in this type of bioreactors can be drawn by analyzing comparatively the mixing in different regions for various broths at similar operating conditions.

The riser region is associated with the largest velocity of liquid phase, most intense mixing, and allows to reaching the largest rate of mass and heat transfer, as well as the highest shear stress level. As it can be observed from Fig. 2, the increase of aeration rate leads to the decrease of mixing time for all considered apparent viscosities and positions inside the riser.

Generally, this positive effect on mixing intensity is more pronounced for the domain of air superficial velocity varying between $1.05 - 1.95 \times 10^{-3}$ m/s. The less important influence of aeration rate observed for its values placed in the outer of the mentioned range becomes more obvious by increasing the apparent viscosity of the fluid.

The analysis of the variation of mixing time on liquid height indicated that its lowest values are reached for the intermediary positions, namely positions 2 and 3 (Fig. 3). These positions are placed above the sparger and correspond to the regions with the most intense turbulence. At lower apparent viscosity, up to 19 cP, the mixing is more efficient for position 2, the closest one to the sparger. For more viscous simulated broths, the lowest values of mixing time are recorded for position 3.

This variation of the relative mixing intensity is the result of the effect of bubbles coalescence on bubble rising velocity and, implicitly, on turbulence extent. The process of bubbles coalescence occurs in viscous media and becomes more important by increasing the air input rate. At low aeration rate and less viscous media, the small bubbles formed by air dispersion exhibit a negative effect on broths circulation, due to their low rising velocity (Caşcaval et al., 2007; van’t Riet and Tramper, 1991). This effect becomes more important by increasing the apparent viscosity of liquid phase.

For viscous simulated broths, at higher aeration rate, it was observed the significant bubbles coalescence, this phenomenon leading to the heterogeneous distribution of air in the liquid phase and acceleration of bubbles rising velocity through central route (Caşcaval et al., 2007; van’t Riet and Tramper, 1991). Consequently, the mixing time is reduced due to the amplification of turbulence, the magnitude of this effect increasing with the distance from the sparger.

The fluid flow velocities in the regions of bottom clearance and top gas separation, namely positions 1 and 4, are lower than those corresponding to the intermediary positions, thus leading to lower mixing intensity (Gavrilescu and Chisti, 2005; Li et al., 2009; van’t Riet and Tramper, 1991). Owing to the larger fluid section area, the media circulation velocity is lower in position 4 compared to position 1, and, consequently, the mixing time values are superior in the gas separation region (Fig. 3).

The above discussed phenomena are supported by the dependence between mixing time and apparent viscosity of simulated broths, plotted in Fig. 4. For all regions considered in the riser, it can be observed that the mixing time increases by increasing the apparent viscosity up to 12 - 19 cP, reaches a maximum value, decreasing then for more viscous media. For the above mentioned reasons, at higher apparent viscosities, the mixing related to position 3 becomes more intense than that corresponding to position 2, this differentiation being amplified by increasing the aeration rate.

The broth hydrodynamics in the downcomer region is more complex and is controlled by the number and behavior of bubbles entrapped in the fluid circulating downward. For all considered positions, Fig. 5 indicates that the fluid velocity in the downcomer region is lower than that corresponding to the riser. Moreover, contrary to the variations plotted in Fig. 1, by increasing the aeration rate, the mixing time recorded for downcomer decreases, reaches a minum value, followed by its increase. This variation becomes more obvious at higher apparent viscosity and characterizes mainly the flow for the intermediary regions 2 and 3.

According to the previous results reported in literature (Chisti and Moo-Young, 1988; Gavrilescu, 1997; Merchuk and Gluz, 1999), this behavior is the result of the change of the relative importance of bubbles disengagement in the gas separation region and their entrapping into the fluid which circulates downward. Thus, at low air superficial velocity, the dispersion flow velocity in the riser is low and allows for virtually complete separation of the bubbles at the top of gas-lift bioreactor.

As it was above discussed, the dispersion flow velocity in the riser is accelerated by intensifying the
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Due to the buoyancy tendency of the bubbles, as well as to their accumulation, stratification, and coalescence, the descending velocity of the dispersion is reduced and, implicitly, the mixing intensity is affected.

These phenomena are more important for the regions farther from bioreactor bottom or top and are amplified in the case of more viscous simulated broths. For the same reason, by increasing the apparent viscosity, the value of air superficial velocity related to the minimum mixing time is reduced.

**Fig. 2.** Influence of air superficial velocity on mixing time for water and viscous simulated broths at different pH sensor positions in the riser
Fig. 3. Variation mixing time on liquid height in the riser for water and viscous simulated broths at different air superficial velocities.

The descending bubbles could reach the bottom zone of the gas-lift bioreactor and are recirculated by the riser stream, inducing the increase of the turbulence in this region. This effect was observed for position 1 in the downcomer for simulated broths with apparent viscosity up to 12 cP (Fig. 5).

The variations of mixing time in the considered points on the height of downcomer region are plotted in Fig. 6 for different air rate input and apparent viscosity. Fig. 6 suggests that the most intense mixing is reached at the bottom and top regions, namely positions 1 and 4, due to the recirculating effect induced in these regions by the split device.

According to the above discussion, the entrapping of the bubbles in the descending stream hinders the broths circulation and leads to the...
increase of mixing time for aeration rates over $1.05 \times 10^{-3}$ m/s. The magnitude of this effect is enhanced for viscous media and leads to the differentiation of dependences plotted in Fig. 6 in two distinct groups which correspond to apparent viscosity values lower or higher than 5 cP. Because it is very difficult to reach the uniform circulation in the downcomer region for viscous broths, the increase of apparent viscosity in the experimental range cumulated with the bubble accumulation in the downcomer reduce the turbulence in the intermediary positions 2 and 3 (Fig. 7).

**Fig. 4.** Influence of apparent viscosity on mixing time for different air superficial velocities and pH sensor positions in the riser
Fig. 5. Influence of air superficial velocity on mixing time for water and viscous simulated broths at different pH sensor positions in the downcomer

The relative negative influence of bubble entrapping on velocity of broth downward circulation is more important for position 3 for apparent viscosity below 19 cP, becoming significant for position 2 at higher viscosities and aeration rate.

The experimental data have been included in two mathematical correlations which describe the influence of apparent viscosity, superficial air velocity, and position on the bioreactor height on mixing time separately for the riser and downcomer regions. The general expression of the proposed equations is (Eq. 2):

$$t_m = \alpha \cdot \eta_a^\beta \cdot v_s^\gamma \cdot h^\delta$$  \hspace{1cm} (2)
The influence and the relative importance of the considered variables are suggested by the coefficient $\alpha$, $\beta$, $\gamma$ and $\delta$ values. The values of these coefficients are specific for each circulation region created by the split device inside the air-lift bioreactor, being calculated by the multiregression method using MATLAB software.

Thus, the following correlations have been established:

- **riser region**

$$t_m = 4.186 \cdot \frac{\eta_0^{0.242}}{v_s^{0.516}} \cdot h^{0.124}, \text{ s} \quad (3)$$

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**Fig. 6.** Variation mixing time on liquid height in the downcomer for water and viscous simulated broths at different air superficial velocities
The proposed models are in concordance with the experimental data, the maximum deviation being of 4.47% for the riser region and 8.92% for the downcomer one. Analyzing the corresponding determination coefficients, which represent the square of correlation coefficients for the proposed equations, it can be concluded that the considered factors influence the mixing efficiency and distribution in an average extent of 95.8%. The rest of 4.2% can be attributed to the effects of other factors, namely: sparger position, ratio of cross areas
of riser and downcomer flow regions, height of gas separation or bottom clearance regions, etc.

4. Conclusions

The studies on mixing intensity and distribution for viscous simulated broths in a split-cylinder gas-lift bioreactor revealed the different behavior of broth flow in the riser and downcomer regions, not only from the point of view of upward and downward circulation velocities.

Therefore, in the riser, the most intense mixing has been reached in the intermediary positions, placed above the sparger. The increase in the viscosity of the liquid phase exhibited a negative effect on mixing efficiency, but the mixing time decrease at apparent viscosities over 12 cP, due to the higher rising velocity of larger bubbles formed by coalescence.

The distribution of mixing intensity in the downcomer zone is different, the highest turbulence being induced in the positions 1 and 4, namely at the bottom and top of the dispersion descending stream. The positive influence of aeration rate on turbulence extent was recorded only for air superficial velocity up to 1.05×10⁻³ m/s. For higher values of air input rate the extent was recorded only for air superficial velocity.

The distribution of mixing intensity in the downcomer zone is different, the highest turbulence being induced in the positions 1 and 4, namely at the bottom and top of the dispersion descending stream. The positive influence of aeration rate on turbulence extent was recorded only for air superficial velocity up to 1.05×10⁻³ m/s. For higher values of air input rate and apparent viscosity, the phenomenon of bubbles entrapment in the descending stream became significant, this leading to the hindrance of broth circulation and, implicitly, to the increase of mixing time.

The influences of the considered factors on mixing intensity have been included in two mathematical correlations established for the riser region and for the downcomer one, respectively. The proposed equations allow to predicting the mixing time on the height of the internal-loop gas-lift for the two circulation regions of bioreactor, and offer a good agreement with the experimental results.

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Nomenclature

- distance from the bioreactor bottom, m
- pH-value corresponding to the perfect mixing determination
- pH-limits accepted for mixing time
- mixing time, s
- air superficial velocity, m/s
- parameters of empirical correlation fit (2)
- apparent viscosity, Pa.s

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