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Evaluation of the hydrodynamic regime of aerobic stirred bioreactors using the mixing distribution criteria.
1. Simulated broths

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Abstract

Broths circulation inside the aerobic stirred bioreactors provided with single or multiple impellers is complex, due to the cumulated pneumatic and mechanical agitation. The studies on mixing distribution for aerated simulated broths underlined that the mixing intensity varies inside the broth from one region to other. Moreover, contrary to the experiments carried out for non-aerated broths, in the case of aerated broths it cannot be observed a uniform distribution of the mixing intensity, indifferent of the rotation speed value.

The modification of the rotation speed, at a constant level of air flow rate, indicated the existence of a critical rotation speed which corresponds to the minimum of mixing time. The value of critical rotation speed varies between 100 and 500 rpm, depending on the position inside the bioreactor and on the apparent viscosity.

The critical flow rate, which corresponds to the minimum of mixing time, decreases from 300 to 100 l h^{-1} with the increase of apparent viscosity from 15 to 96 cP. The supplementary intensification of aeration leads to the increase of mixing time to a maximum value, follows by its decrease, due to the flooding phenomena.

Keywords: mixing time, mixing distribution, stirred bioreactor, aerated broths, simulated broths.

Introduction

According to the mixing definition given by Hiby (1981), the completely mixed system corresponds to a uniform distribution of its components, this being the ideal case [1]. The mixing could be characterized by means of mixing scale and/or mixing intensity [2]. The mixing scale represents the smallest dimension (volume, mass) of the mixed system in which the non - homogeneity is allowed. The mixing intensity is defined as the deviation from complete mixing. Because the perfect mixing is reached after an infinite period of time, the mixing intensity could be defined as the deviation from complete mixing after a prescribed finite amount of time.

One of the most useful criteria for characterization of the mixing intensity is the mixing time, t_m, defined as the time needed to reach a given mixing intensity at a given scale, when starting from the completely segregated situation [2,3]. This parameter offers specific

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information concerning the bulk mixing in the system (macromixing), but it cannot rigorously quantify the mixing at lower level (meso- and micromixing) [4]. By means of the mixing time it can be established the optimum hydrodynamic regime, the stirrer type that has to be used, or it can be predicted the modification of mixing efficiency induced by scaling-up [5,6].

Due to the biomass accumulation, shear stress sensitivity of the biocatalysts (free or immobilized cells and/or enzymes), high viscosity or non-Newtonian rheology behavior of the broths and to the presence of gaseous phase, as the result of aeration or cells respiration, the analysis of mixing distribution in fermentation systems is considerably complicated. Generally, the studies on mixing time for bioreactors and the corresponding mathematical models have been proposed for Reynolds numbers over 10,000, this flow regime being rarely reached in the large-scale bioreactors, due to the microorganisms sensitivity to shear stress. For this reason, these models need some corrections for lower Reynolds number (laminar and transitory flow regime) [2].

The complexity of broths rheology and their high apparent viscosity, the flow behavior inside the bioreactors and the particularities of the fermentation processes induce a non-uniform distribution of mixing intensity, with the inevitable appearance of the heterogeneous regions in the bioreactor. Furthermore, in the case of aerobic stirred bioreactors provided with single or multiple impellers, the flow mechanism becomes more complicated due to the cumulated effects of the pneumatic and mechanical mixing. The aeration generates flow streams that are significant different from those induced by mechanical mixing into the non-aerated broths.

Generally, the analysis of mixing efficiency for the aerated mechanical stirred systems is derived from that of non-aerated systems, due to the less complicated flow phenomena for the second ones. Because it has been assumed that the bubbles don't influence the broths flow, the values of mixing time calculated for aerated broths by means of the equations established for non-aerated systems differ significantly from the experimental ones (in most of these cases, the values of calculated mixing time were lower for about 1.2 - 2 times compared with the experimental data [2]).

Although there is much information concerning the influence of feed position on mixing efficiency, respectively on mixing time [4,7-9], the relevance of these studies for bioreactors, especially for the stirred ones, is questionable. From technical reasons, the nutritive or buffer solutions are fed at the liquid surface proximity. Therefore, for establishing the mixing distribution into the bioreactor is more appropriate to maintain the feed position of the tracer and to modify the corresponding electrode position. In this manner, the stagnant regions could be easily identified and the influence of broths characteristics or process conditions on the mixing efficiency could be more rigorously analyzed for each region inside the bioreactor.

As it was underlined in literature, the electrode position doesn't affect the values of mixing time if the flow regime is turbulent [10-12]. But, at low rotation speed, there were recorded significant variations of mixing time values with the change of electrode position, indifferent of the bioreactor scale [13]. Because the flow regime into the bioreactors is laminar or transitory, due to the microorganisms sensitivity to shear stress, the analysis of mixing intensity distribution into the broths needs to determine the mixing time for different positions of electrode.

For these reasons, the aeration influence on mixing efficiency and its distribution in bioreactors has to be distinctly analyzed. In most aerobe fermentations, the air is accumulated around the stirrers with the formation of cavities or compartmentalization of flow regions, that reducing the pumping capacity of the stirrer and modifying the distribution of mixing intensity compared with the non-aerated systems [14,15].

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The aim of our studies is to establish the distribution of mixing efficiency inside of the aerobic stirred bioreactor, by means of the mixing time values obtained at various positions of pH-electrode, as well as the influences of the broths characteristics and operating parameters on the change of active and stagnant regions positions. For underlining the effect of the biomass presence on mixing efficiency, the experiments have been carried out for aerated broths without and with microorganisms (bacteria, yeasts, fungus). In this paper, the results obtained for aerated simulated broths without biomass are discussed.

Materials and Method

The experiments have been carried out in 5 l (4 l working volume, ellipsoidal bottom) laboratory bioreactor (Biostat A, B. Braun Biotech International), with computer-controlled and recorded parameters. The bioreactor characteristics and operating parameters have been presented in the previous papers [16,17].

The mixing system consists on a double stirrer and three baffles. The impeller diameter, d, was of 64 mm. The inferior stirrer has been placed at 64 mm from the bioreactor bottom. The superior stirrer was placed on the shaft at a distance of 128 (2d) mm from the inferior one, this being the optimum distance for the Ruston turbine, as it was demonstrated in the previous works [16]. The rotation speed was maintained below 600 rpm. The experiments have been carried out at Reynolds number lower than 2,700, domain that avoids the cavity formation at the broths surface (for rotation speed over 700 rpm).

The sparging system consists of a single ring sparger with 64 mm diameter, placed at 15 mm from the vessel bottom, having 14 holes with 1 mm diameter. The air volumetric flow rate was varied from 75 to 450 l h^{-1}, corresponding to an air superficial velocity of 0.84 - 5.02×10^{-3} m s^{-1}.

In the experiments, water and simulated fermentation broths have been used. The simulated broths consisted of carboxymethylcellulose sodium salt solutions having the apparent viscosity in the domain of 15 - 96 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 Ostwald type. Both the experiments and viscosity measurements were carried out at a temperature of 24^{o}C. Any viscosity change was recorded during the experiments.

The mixing efficiency has been analyzed by means of the mixing time values. The experimental measurement of mixing time uses the tracers (acidic, alkaline or salts solutions, heated solutions, colored solutions) which are added to the beforehand homogenized broths. The mixing time is the time needed for the considered parameter (pH-value, temperature, absorption etc.) to reach a constant value. Because the perfect mixing can be reached after a long time period, for the mixing time determination a predefined level of homogeneity is admitted [2,18].

For mixing time determination, a solution of 2N KOH has been used as tracer, being recorded the time needed to the medium pH-value to reach the value corresponding to the considered mixing intensity. In this case, the following homogeneity criteria for mixing has been considered:

$$I = \frac{pH_{\infty} - 0.5\Delta pH}{pH_{\infty}} x100 = 99\% \qquad \text{where } \Delta pH = 0.02.$$

The tracer volume was of 0.5 ml, the tracer being injected opposite to the pH electrode, at 65 mm from the stirrer shaft and 10 mm from the liquid surface. Because the

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tracer solution density is close to the liquid phase density, the tracer solution flow follows the liquid flow streams and there are no errors due to tracer buoyancy.

The pH electrode was introduced at four different positions, placed vertically from bioreactor bottom as follows:

- position 1: at 20 mm
- position 2: at 70 mm
- position 3: at 120 mm
- position 4: at 170 mm.

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

Results and Discussion

The accumulation of biomass or biosynthesized product (extracellular polysaccharides, protein molecules etc.) in the fermentation processes leads to the continuous modification of the medium rheological properties, promoting the appearance of the heterogeneous regions in the bioreactor. Compared with the non-aerated fermentation systems, in the case of aerobic stirred bioreactors provided with single or multiple impellers the broths flow becomes more complex due to the cumulated pneumatic and mechanical agitation. The deviations from the obtained values for non-aerated broths depend on the constructive and operating characteristics of the bioreactor. Moreover, the influence of number and position of the stirrers on the shaft is unknown, and the influence of the gas flow rate is different for different rotation speed or Reynolds number values [2,15].

The previous studies on mixing distribution into a stirred bioreactor for non-aerated simulated broths indicated that the values of mixing time varied inside the broths [19]. Thus, the stagnant regions are mainly placed between the stirrers on the shaft, their formation being the result either of the too long distances between the stirrers, or of the interference of flow streams created by the adjacent stirrers. For certain rotation speeds between 250 and 400 rpm, function of the apparent viscosity of the broths, the values of mixing time recorded for different regions become rather equal, that suggesting a uniform distribution of mixing intensity in whole bulk of the fermentation broth.

For most of the aerated simulated broths, the maximum value of mixing time has been recorded at a distance of 1.5d between the stirrers for low viscous liquids, respectively d for higher viscous ones, as the results of the flow compartmentalization and of the increase of retention time of bubbles into the broths, both phenomena reducing the dispersion velocity [14,15].

The previous results have been obtained for a fixed position of pH electrode, placed at position 1, and offer a generally view on mixing efficiency into the broths. The modification of electrode position offers the possibility for establishing the accurate conclusions concerning the mixing intensity distribution into the bioreactor, as well as the effects of broths characteristics and/or fermentation conditions on mixing efficiency in a certain region inside the bioreactor.

As it can be observed from Figure 1, indifferent of the apparent viscosity and pH electrode position, the intensification of mixing leads to the initial decrease of mixing time to a minimum value, follows by its increase. This evolution could be the result of the modification of mixing mechanism with the increase of rotation speed in presence of bubbles. Thus, at low rotation speed, the contribution of pneumatic mixing to dispersion circulation is considerable, the increase of rotation speed supplementary intensifying the broth agitation

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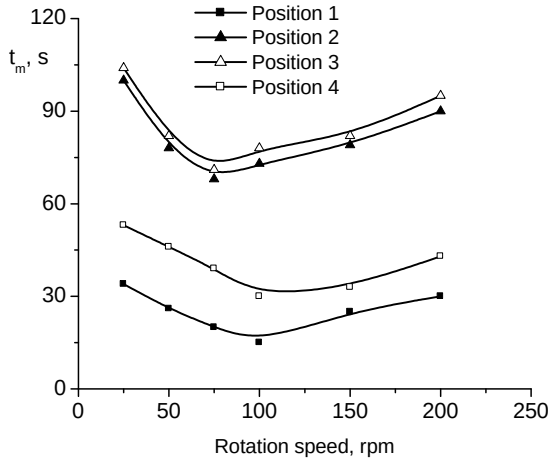
into the bioreactor. At higher rotation speed, the bubbles retention time increases, the gas-liquid dispersion flow becomes complex and its circulation velocity is lower than that of the flow streams created by mechanical mixing in non-aerated media. The value of the rotation speed which corresponds to the minimum of mixing time is called *critical rotation speed* [14,15].

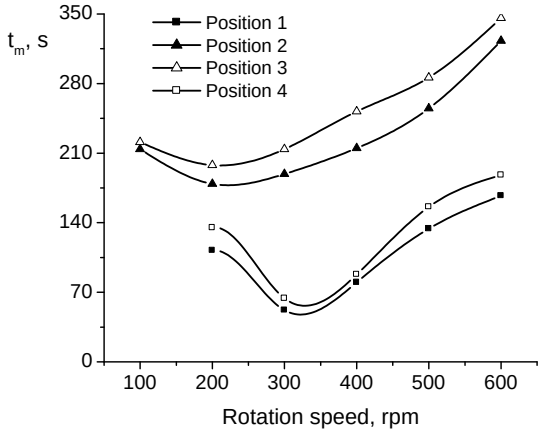
Although the experimental curves are similar, the value of critical rotation speed is changed by changing the electrode position or by increasing the viscosity. Indifferent of the apparent viscosity level, the four experimental curves can be paired up in two groups, one corresponding to the extreme regions (position 1 and 4) and the other to the intermediary regions (position 2 and 3). Thus, the lowest value of mixing time has been recorded for positions 1 and 4, due to their location near to the stirrers. Among these two variations, that plotted for position 1 indicates a more efficient mixing compared with position 4, as the result of the "bottom effect" which induces a better circulation of air-broth dispersion [17].

By increasing the apparent viscosity the mechanical agitation becomes more important relative to the pneumatic one. But, at higher viscosity the bubbles coalescence becomes important, the air is accumulated around the stirrers and the air hold-up increases (for apparent viscosity of 96 cP the air volumetric fraction was of 2.6 - 3% at 300 rpm, becoming 11 - 12% at 700 rpm [14]), thus reducing the dispersion velocity and, consequently, the influence of rotation speed increase on mixing intensification. These two opposite phenomena generate the increase of critical rotation speed from 100 rpm for water to 500 rpm for simulated broths with viscosity of 96 cP. Furthermore, the minimum value of mixing time becomes less evident at higher viscosities.

η_a = 1 cP



η_a = 15 cP



η_a = 25 cP

η_a = 60 cP

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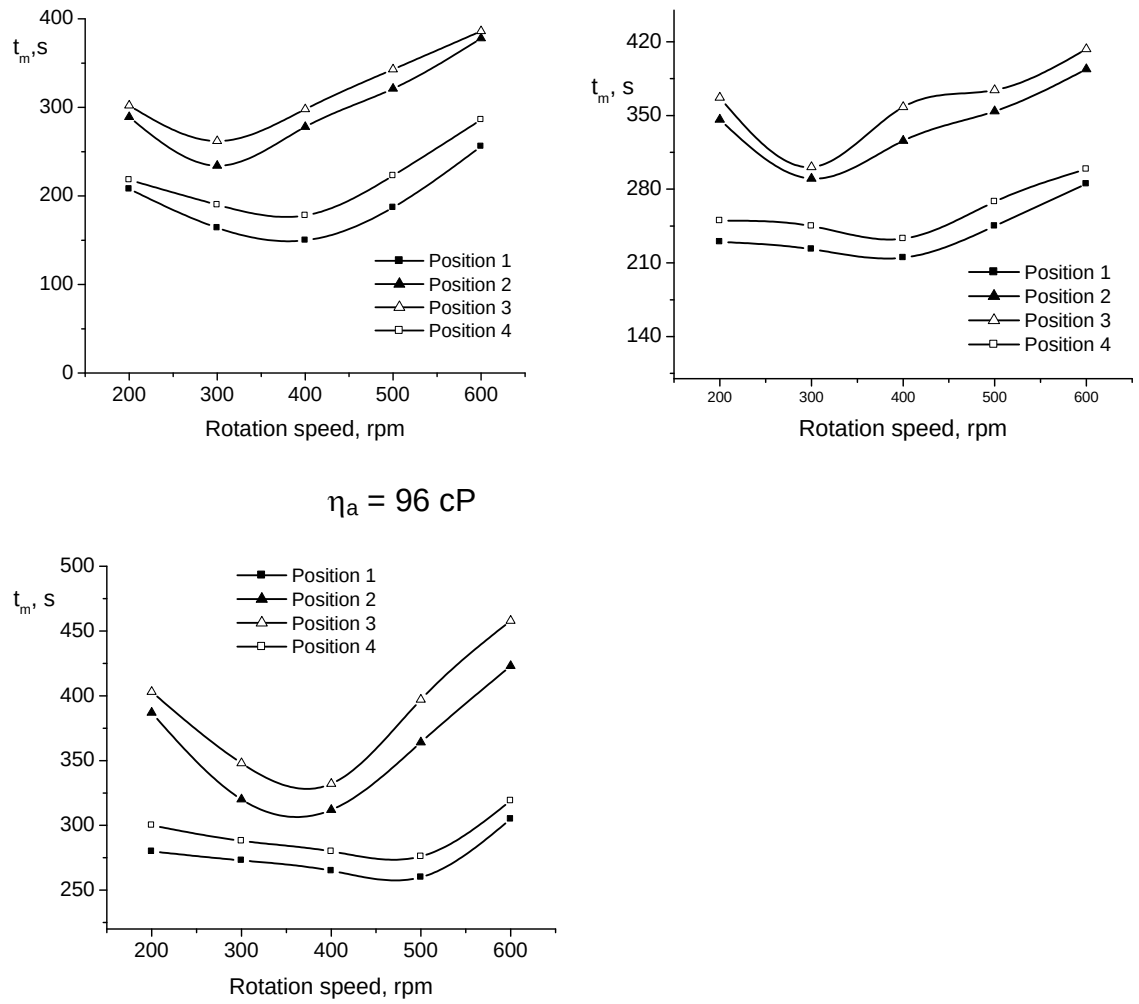

Figure 1. Influence of rotation speed on mixing time (aeration rate of 75 l h^{-1}).

Lower mixing intensities have been recorded for positions 2 and 3. As it was previously concluded, this variation is the result of the modification of mixing intensity in the intermediary region due to the cumulated or opposite effect of the flow streams created by the two stirrers [16]. Therefore, owing to the distance between the stirrers, the stagnant regions can be formed in the intermediary positions, phenomena that become more pronounced by increasing the apparent viscosity. The rotation speed acceleration promotes the intensification of broth circulation, that reducing the volume of the stagnant region and, consequently, the values of mixing time for positions 2 and 3. The aeration strongly modifies and influences the circulation into the intermediary region, because it supplementary amplifies the agitation between the stirrers and extends the well-mixed regions. For these reasons, in the positions 2 and 3 the mixing intensity is lower and the critical rotation speed is inferior to those recorded for positions 1 and 4. The value of critical rotation speed for the intermediary region also increases from 75 rpm at 400 rpm with the increase of apparent viscosity from 1 cP to 96 cP.

At a given moment, the flow streams become strong and interact, reducing the positive effect created by rotation speed intensification, phenomena that is more pronounced at higher rotation speed values. By increasing the apparent viscosity, it was observed a supplementary effect, namely the diminution of the turbulence in the intermediary region, due to the accumulation of air around the stirrers. Therefore, the minimum value of mixing time

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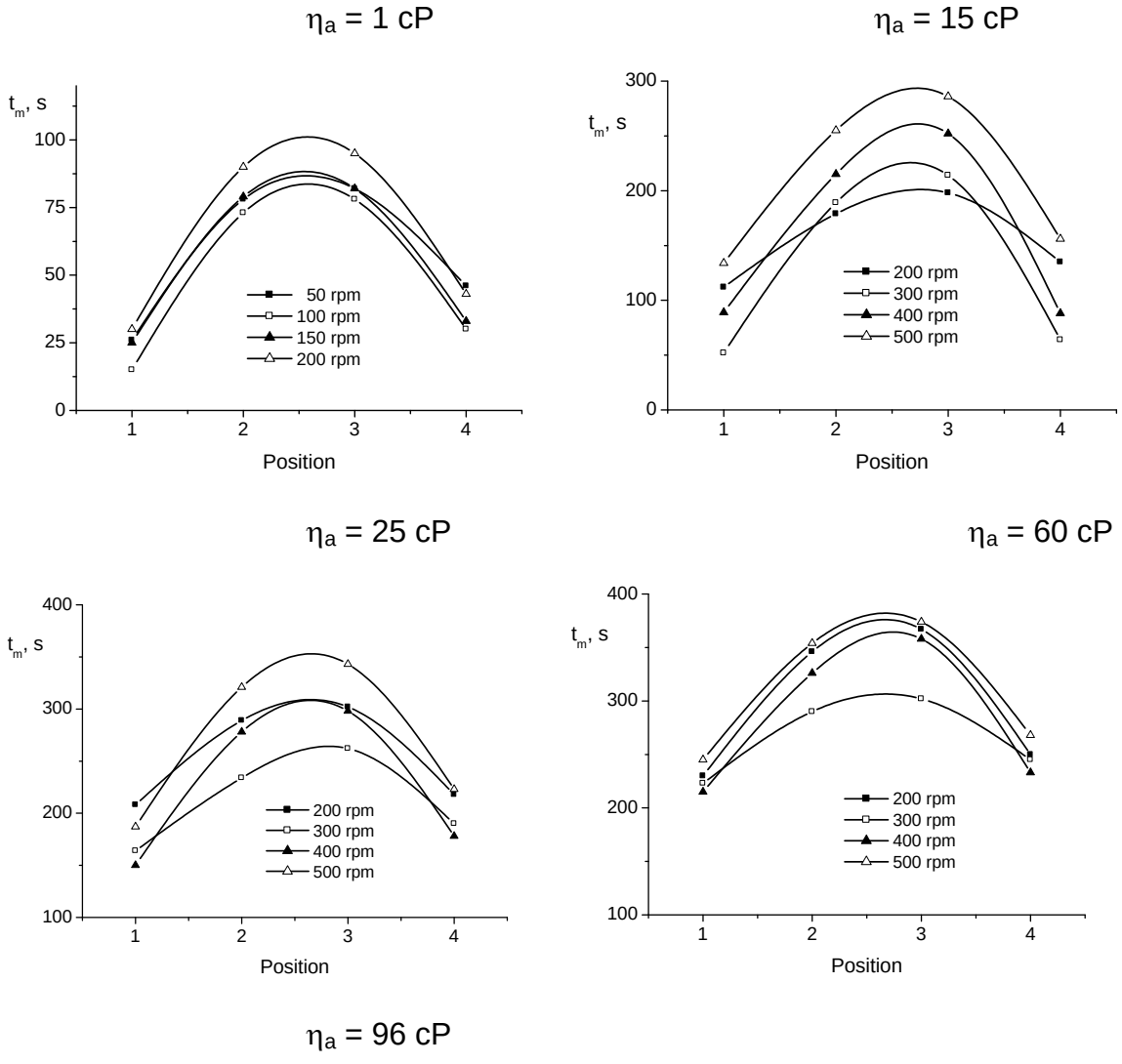
becomes more evident, and its further increase more significant than that recorded for positions 1 and 4.

According to the above mentions, the analysis of the mixing intensity distribution for the four considered positions inside the bioreactor indicated that the highest values of mixing time have been obtained for the region placed between the stirrers (Figure 2).

From the previous studies on non-aerated simulated broths, it was observed that the uniform mixing in whole bulk of fermentation broth can be reached for a certain rotation speed value depending on broth apparent viscosity (250 - 300 rpm for apparent viscosity up to 60 cP, 400 rpm for higher viscosities [19]). But, in the case of aerated broths, owing to the non-uniform dispersion of the air bubbles into the broths and to the air accumulation around the stirrers, any uniform distribution of mixing intensity on the bioreactor height is indicated in Figure 2, indifferent of the rotation speed level. It could be seen a slight flattening of the obtained curves with the increase of the apparent viscosity, that suggesting a more uniform distribution of mixing time in the viscous liquids, but concomitantly with the reduce of mixing efficiency . The value of rotation speed corresponding to the most flattened curve increases from 200 rpm for an apparent viscosity of 15 cP to 400 rpm for 96 cP. However, it could not be admitted that the mixing intensity is uniform distributed into the broths, as in the case of non-aerated media.



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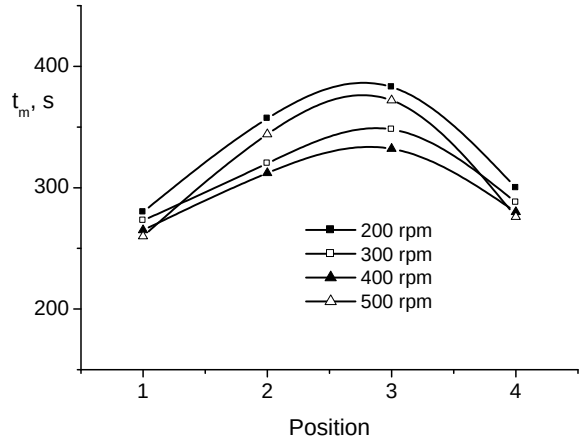
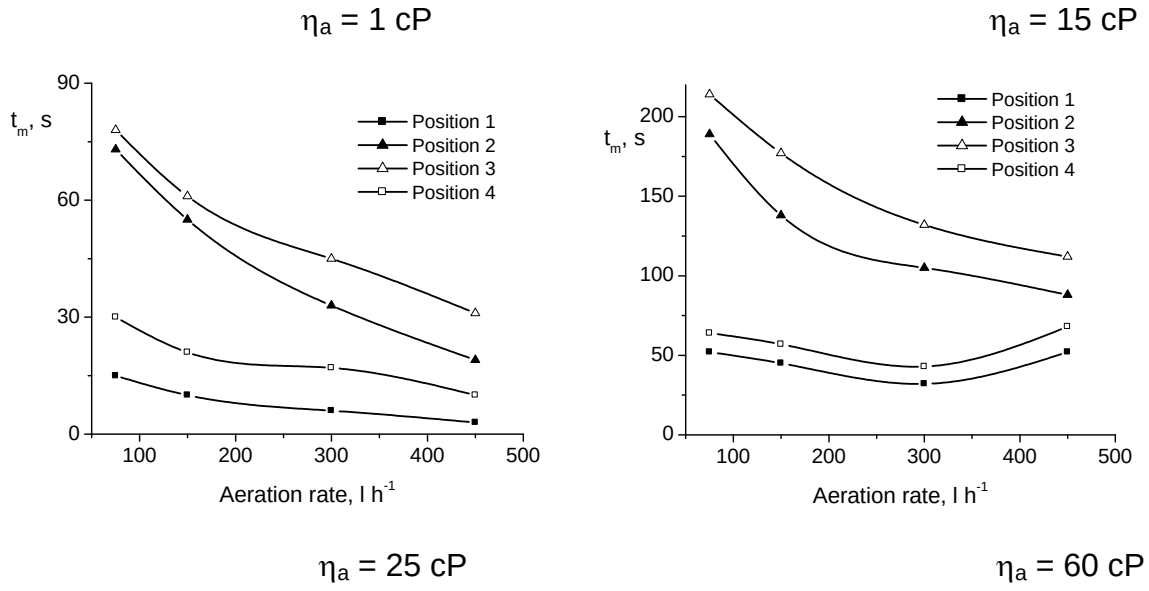


Figure 2. Influence of pH electrode position on mixing time (aeration rate of 75 l h^{-1}).

The influence of aeration rate depends strongly on the apparent viscosity and less on the position of pH electrode. For water, the mixing time is continuously reduced with the increase of the air flow rate for all considered regions inside the bioreactor. But, the dependence between the mixing efficiency and aeration rate is considerably changed at higher viscosities, appearing important differences between the values of mixing time recorded for the four positions (Figure 3).



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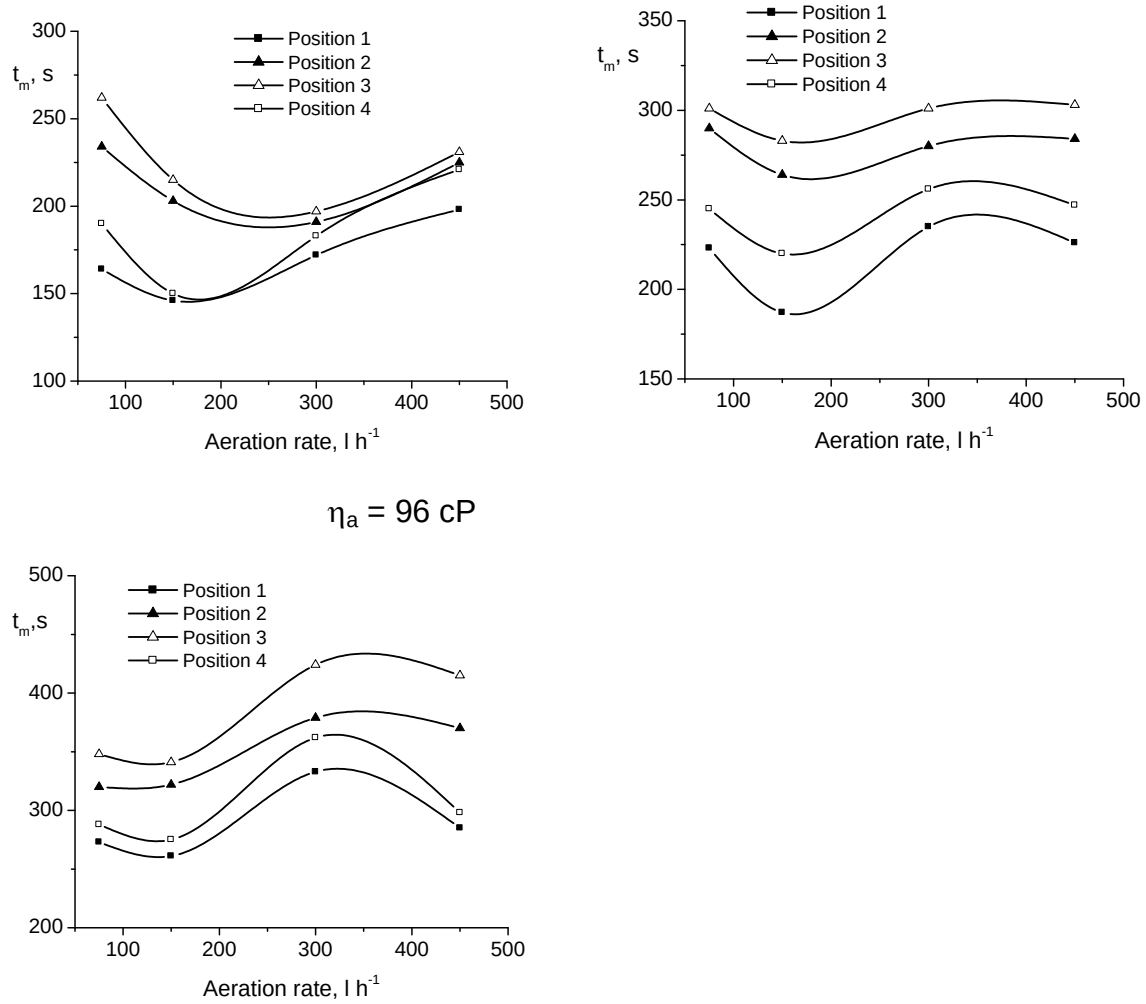

Figure 3. Influence of aeration rate on mixing time (rotation speed 300 rpm).

For the above presented reasons, the lowest values of mixing time have been obtained again for positions 1 and 4. In these regions, the increase of aeration rate initially leads to the mixing intensification, consequently the mixing time decreases and reaches a minimum values, then increasing. At lower air flow rate, the bubbles coalescence rate is high, because the reduced turbulence cannot hinder this process. In these conditions, it were observed the heterogeneous distribution of air in the liquid phase, the reduce of air hold-up and the rise of bubbles through preferential central routes, this resulting in higher values of mixing time. The increase of air flow rate leads to the intensification of gas-liquid dispersion circulation, therefore to the decrease of mixing time, which reaches o minimum value.

The value of air volumetric flow that corresponds to the minimum of mixing time, called *critical flow rate*, mainly depends on liquid apparent viscosity [14,15]. For positions 1 and 4, the value of critical flow rate is reduced from 300 l h^{-1} to 150 l h^{-1} with the increase of the apparent viscosity from 15 to 96 cP (Figure 3).

For constant rotation speed value, the supplementary increase of aeration rate induces the increase of mixing time. This variation is the result of the formation of smaller bubbles having lower rise velocity, thus leading to the increase in gas hold-up value and to the decrease of dispersion circulation velocity.

The minimum value of mixing time becomes more evident with the increase of apparent viscosity. This variation cumulated with the decrease of critical flow rate at higher

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viscosities underlines the major role of the viscosity in the formation of small bubbles with low rise velocity, which diminish the mixing efficiency.

In these regions, for apparent viscosity over 60 cP, the supplementary increase of air flow rate leads to the significant increase of mixing time to a maximum value, phenomena that is more pronounced at higher viscosity. The maximum value corresponds to the flooding point, when the energy dissipated by the air exceeds that of the stirrer [2]. At this moment (300 l h^{-1}), the rise velocity of the air increases, determining the simultaneous increase in the intensity of media circulation. The existence of the maximum value of mixing time, respectively of the flooding phenomena, is specific to the viscous broths, as the result of the inefficient mechanical agitation which allows the air accumulation around the stirrers [15].

For the intermediary positions 2 and 3 and broths with apparent viscosity up to 15 cP, the dependence between the mixing efficiency and the aeration rate is similar to that observed for water. At higher viscosities, the increase of air flow rate exhibits an effect similar to that observed for positions 1 and 4, namely the reduction of the mixing time to a minimum, follows by its increase. The critical flow rate varies from 300 l h^{-1} for broths with apparent viscosity lower than 25 cP to 150 l h^{-1} for more viscous broths.

Evidently, the flooding phenomena appears also in these regions, being less pronounced than that produced for positions 1 and 4, because the air accumulation is not possible in the intermediary positions.

Conclusions

The studies on mixing distribution for an aerobic stirred bioreactor and simulated broths underlined that the dependence between the mixing time and the considered factors (apparent viscosity, rotation speed, aeration rate) differs from one region inside the broth to another.

The modification of the rotation speed, at a constant level of air flow rate, indicated the existence of a critical rotation speed corresponding to the minimum of mixing time. The value of critical rotation speed depends on pH electrode position and increases along with the apparent viscosity, varying between 100 and 500 rpm (the lower values have been recorded for the intermediary positions).

Contrary to the experiments carried out for non-aerated broths using identical conditions, the uniform distribution of the mixing intensity cannot be observed in the case of aerated broths, indifferent of the rotation speed value.

At constant rotation speed, the increase of aeration rate leads to the initially decrease of the mixing time to a minimum value, follows by its increase. The critical flow rate, which corresponds to the minimum of mixing time, decreases from 300 to 100 l h^{-1} with the increase of apparent viscosity. The supplementary intensification of the aeration could induce the increase of mixing time, which reaches a maximum value, decreasing then, due to the flooding phenomena. This variation becomes more pronounced at higher viscosities and for the extreme positions 1 and 4.

Notations

d - stirrers diameter, mm
pH_∞ - pH-value corresponding to perfect mixing
ΔpH - pH-limits accepted for mixing time determination
t_m - mixing time, s
η_a - apparent viscosity, cP

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