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ORIGINAL PAPER

Comparative analysis of mixing distribution in aerobic stirred bioreactor for simulated yeasts and fungus broths

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Abstract The study on mixing distribution for an aerobic stirred bioreactor and simulated (solutions of carboxymethylcellulose sodium salt), yeasts (*S. cerevisiae*) and fungus (*P. chrysogenum* pellets and free mycelia) broths indicated the significant variation of mixing time on the bioreactor height. The experiments suggested the possibility to reach a uniform mixing in whole bulk of the real broths for a certain value of rotation speed or biomass concentration domain. For *S. cerevisiae* broths the optimum rotation speed increased to 500 rpm with the biomass accumulation from 40 to 150 g/l d.w. Irrespective of their morphology, for fungus cultures the existence of optimum rotation speed (500 rpm) has been recorded only for biomass concentration below 24 g/l d.w. The influence of aeration rate depends on the apparent viscosity/biomass concentration and on the impellers and sparger positions. By increasing the apparent viscosity for simulated broths, or biomass amount for real broths, the shape of the curves describing the mixing time variation is significantly changed for all the considered positions. The intensification of the aeration induced the increase of mixing time, which reached a maximum value, decreasing then, due to the flooding phenomena. This variation became more pronounced at higher viscosities for simulated broths, at higher yeasts concentration, and at lower pellets or filamentous fungus concentration, respectively. By means of the experimental data and using MATLAB software, some mathematical correlations for mixing time have been proposed for each broth and considered position inside the bioreactor. These equations offer a good agreement with the experiment, the maximum deviation being ±7.3% for *S. cerevisiae* broths.

Keywords Stirred bioreactor · Mixing time · Mixing distribution · Simulated broth · *Saccharomyces cerevisiae* · *Penicillium chrysogenum*

List of symbols

C_X	biomass concentration (g/l d.w.)
d	stirrer diameter (mm)
N	impeller rotation speed (s^{-1})
t_m	mixing time (s)
v_S	superficial air velocity (m/s)
pH_∞	pH value corresponding to perfect mixing
ΔpH	pH limits accepted for mixing time determination
$\alpha, \beta, \gamma, \delta$	parameters of empirical correlations (1) and (2)
η_a	apparent viscosity (Pa.s)

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Introduction

The simplest and most suggestive definition of mixing was given by Hiby [1] in (1981), who affirmed that

mixing is the process through which the inhomogeneity of a system is decreased. The complete mixed system corresponds to a perfect uniform distribution of its components.

The mixing could be characterized by means of mixing scale and mixing intensity [2]. The mixing scale represents the smallest dimension (volume, mass) of the analyzed system in which the inhomogeneity is allowed.

The mixing intensity is defined as the deviation from the perfect mixing. Since the perfect mixing is practically 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 [2].

The mixing can be analyzed also from the viewpoint of the homogenization level. Thus, three homogenization levels could be identified: macro-, meso- and micromixing [3]. Macromixing consists on the uniform distribution of the content in the whole bulk of the system by means of the liquid movement induced by stirrer agitation. Mesomixing occurs when the feed rate of a certain component is high and leads to its accumulation in the feed region, thus inducing a local inhomogeneity. Owing to the internal circulation, inside this region is perfectly mixed [4]. Micromixing consists on mixing at molecular scale and is controlled by molecular diffusion. Irrespective of the flow regime generated in the whole system, at micromixing scale the flow is laminar. Micromixing is achieved by coherent structure of flow, like the vortex sheets and vortex tubes [5]. Meso- and micromixing become important especially for the systems in which phase transformation or chemical/biochemical reaction occur.

One of the most useful criteria for the characterization of the mixing intensity is the mixing time, defined as the time needed to reach a given mixing intensity at a given scale, when it starts from the completely segregated system [2, 6]. This parameter offers specific information concerning the bulk mixing in the system (macromixing), respectively the flow inside the whole system, but it cannot allow rigorous quantification of the meso- and micromixing [3]. It can indicate the optimum hydrodynamic regime, the stirrer type that has to be used, or can predict the modification of mixing efficiency induced by scaling-up [7, 8].

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 solutions, suspensions, etc. The mixing time is the time needed for the considered parameter (pH value, temperature, absorption, etc.) to reach a constant value. As the perfect mixing can be reached after a long time period, for the mixing time determination a predefined level of homogeneity is admitted (ΔpH from Fig. 1).

The analysis of mixing in bioreactors is based on the same principles as previously mentioned. But, due to the biomass accumulation, sensitivity of the biocatalysts to shear stress (free or immobilized cells and/or enzymes), high viscosity or non-Newtonian rheological behavior of the broths, the analysis of the mixing in these systems becomes more difficult. Furthermore, most of the models applied for the mixing into the bioreactors can predict the mixing time values only for Reynolds number over 10,000, this flow regime being rarely reached in the large-scale bioreactors due to the microorganism's sensitivity to shear forces. For this reason, for Reynolds number below 10,000, these models need some corrections [2].

Although there is much information concerning the influence of feed position on mixing efficiency [3, 9–11], the relevance of these studies for bioreactors, especially for stirred ones, is questionable. For 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 broth characteristics or process conditions on the mixing efficiency could be more rigorously analyzed for each region inside the bioreactor.

As it was underlined in the literature, the electrode position doesn't affect the values of mixing time if the flow regime is turbulent [12–14]. But, at low rotation speed, significant variations of mixing time values were recorded with the change of electrode position, irrespective of the bioreactor scale. As the flow regime into the bioreactors is laminar or transitory, due to the microorganism's sensitivity to shear stress, the analysis

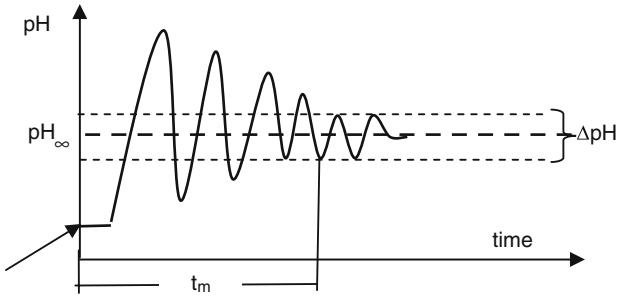

Fig. 1 Determination of mixing time

of mixing distribution requires the determination of mixing time for different positions of electrode.

For this reason, the aim of our studies is to establish the distribution of mixing efficiency inside the aerobic stirred bioreactor, by means of the mixing time values recorded at various positions of pH-electrode, as well as the influences of the broth characteristics 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 aerated broths without biomass (simulated broths) and with microorganisms (yeasts *Saccharomyces cerevisiae*, fungus *Penicillium chrysogenum* free mycelia and pellets). By means of the experimental data, some mathematical correlations between the mixing time and the considered parameters have been established for each position of the pH electrode. The proposed equations could be useful for mixing optimization or scaling-up.

Materials and methods

The experiments were 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 [15, 16].

The mixing system consisted of a double stirrer and three baffles. The impeller diameter, d, was 64 mm. The inferior stirrer was placed at 64 mm from the bioreactor bottom. The superior stirrer was placed on the shaft at the optimum distance from the inferior one according to the type of the studied broth, namely at 128 mm ($2d$) for the simulated broths, respectively at 64 mm (d) for the real broths, as it was demonstrated in the previous works [16]. The rotation speed was maintained below 600 rpm. The experiments were carried out at Reynolds number lower than a domain of 15,200, which corresponded to the laminar, transitory and low turbulent flow regime, and avoided the cavity formation at the broths surface.

The sparging system consisted 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, corresponding to an air superficial velocity of $0.84–5.02 \times 10^{-3}$ m/s.

In the experiments, simulated and real broths were used. The simulated broths were carboxymethylcellulose sodium salt solutions with the apparent viscosity in the domain of 15–96 cP. The following real broths were studied:

- yeasts (*S. cerevisiae*), C_X being of 40–150 g/l d.w.
- fungus (*P. chrysogenum*), with two morphological conformations: free mycelia and mycelial aggregates (pellets, with the average diameter of 1.6–1.8 mm); in both cases, the biomass concentration varied between 4 and 36 g/l d.w.

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°C. Any viscosity or morphology change was recorded during the experiments.

The mixing efficiency was analyzed by means of the mixing time values. For mixing time determination, a solution of 2 N KOH was used as tracer, being recorded the time needed to the medium pH to reach the value corresponding to the considered mixing intensity. In this case, the following homogeneity criterion for mixing was considered:

$$I = \frac{\mathrm{pH}_\infty - 0.5\Delta\mathrm{pH}}{\mathrm{pH}_\infty} \times 100 = 99\% \quad (1)$$

where $\Delta\mathrm{pH} = 0.02$.

The tracer volume was of 0.5 ml, the tracer being injected at the opposite diametral position to the pH electrode (HA 405 Mettler Toledo), at 65 mm from the stirrer shaft and 10 mm from the liquid surface. As the tracer solution density is close to the liquid phase density, the tracer solution flow follows the liquid flow streams and there were 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 calculating the mixing time.

The mathematical correlations, which describe the influences of considered factors on mixing time for various positions inside the broths, were developed on a PC 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 was carried out for three or four times; for identical conditions, the average value of mixing time was used. The maximum experimental errors were between ±4.08 and ±5.76%.

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 broths 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 contributions of pneumatic and mechanical agitation. The deviations from the obtained values for non-aerated broths depend on the constructive and operating characteristics of the bioreactor, as well as on the microorganisms' morphology. Moreover, the influence of number and position of the stirrers on the shaft is unknown, and the influence of the aeration rate is changed with the rotation speed or Reynolds number values modification [2, 16].

The accurate conclusions concerning the mixing distribution in bioreactors, aerobic or non-aerobic, can be drawn by analyzing comparatively the mixing into different broths types at similar operating conditions.

For simulated broths, irrespective 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 (Fig. 2). This evolution could be the result of the modification of mixing mechanism in the presence of bubbles. Thus, at low rotation speed, the contribution of pneumatic mixing to the circulation of dispersion is important, the increase of rotation speed intensifying additionally the broth agitation into the bioreactor. At higher rotation speed, the bubbles' retention time increases, the gas–liquid dispersion flow becomes more 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 that corresponds to the minimum of mixing time is called *critical rotation speed* [17].

Although the experimental curves are similar, the value of critical rotation speed is changed by changing the electrode position or by increasing the viscosity. Thus, the lowest value of mixing time has been recorded for positions 1 and 4, due to their location near to the stirrers. The variation 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 [16].

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

The lowest 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 effects of the flow streams generated by the two vicinal stirrers [15]. Therefore, owing to the distance between the stirrers, the stagnant regions can be formed in the intermediary positions, a phenomenon more pronounced at higher 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 amplifies additionally the agitation between the stirrers and extends the well-mixed regions. For the above reasons, the mixing intensity is lower in the positions 2 and 3 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 to 400 rpm with the increase of an apparent viscosity from 1 to 96 cP.

At a given moment, the flow streams become strong and interact, reducing the positive effect created by rotation speed intensification, a phenomenon that is more pronounced at higher rotation speed values. By increasing the apparent viscosity, an additional effect was observed, namely the diminution of the turbulence

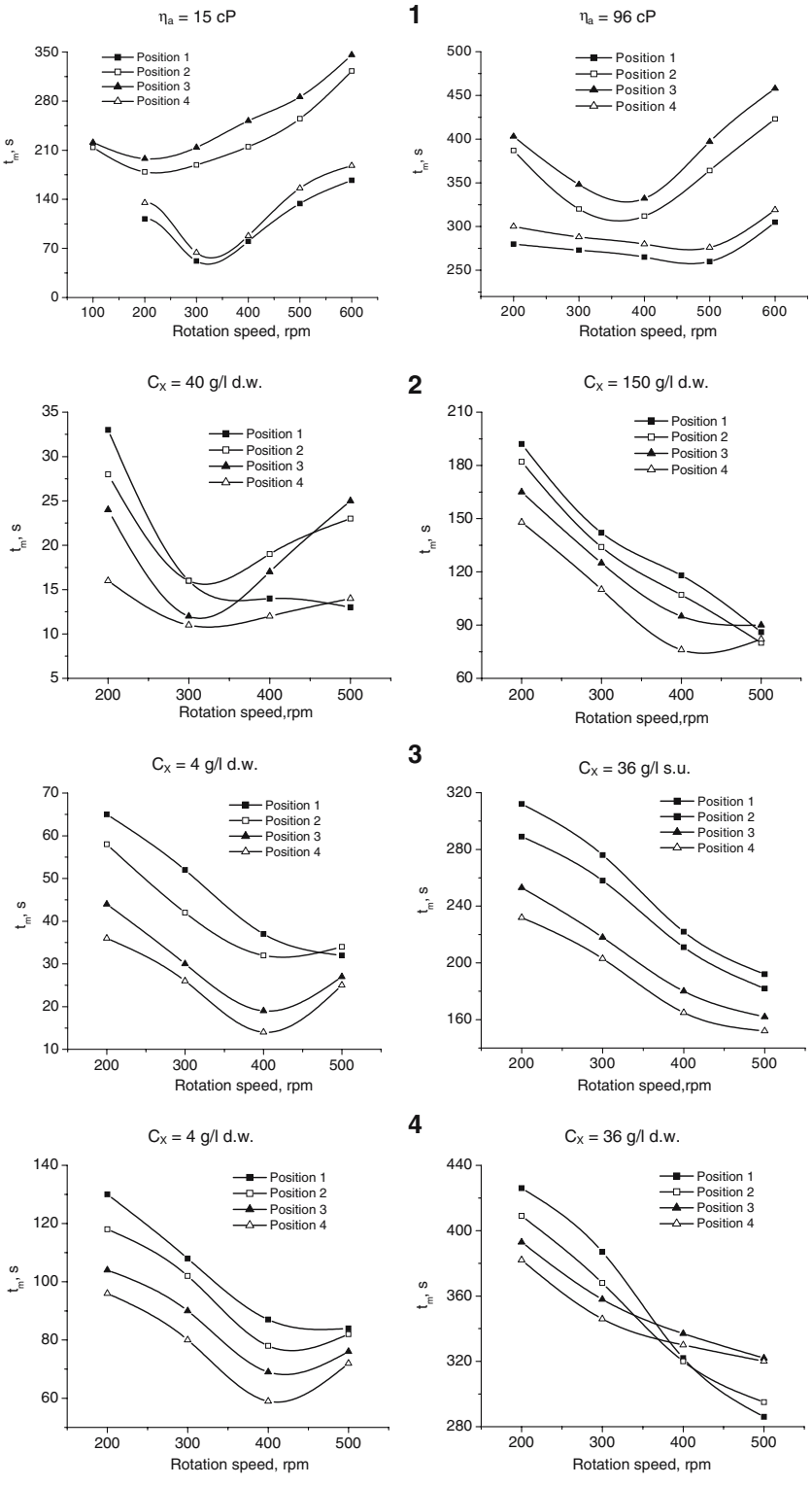

Fig. 2 Influence of rotation speed on mixing time distribution (aeration rate of 75 l/h; *1* Simulated broths, *2* *S. cerevisiae* broths, *3* *P. chrysogenum* pellets broths, *4 P. chrysogenum* free mycelia broths)

in the intermediary region, due to the accumulation of air around the stirrers. Therefore, the minimum value of mixing time becomes more evident, and its further increase is more significant than that recorded for positions 1 and 4.

The presence of biomass could change significantly the behavior of the analyzed systems from the viewpoint of broths mixing. Thus, for the *S. cerevisiae* suspensions it was observed that the shape of the plotted variations differs from one position to another

and it is modified with the biomass accumulation (Fig. 2(2)). Therefore, irrespective of the yeasts concentration, the increase of rotation speed leads to the intensification of mixing at the bioreactor bottom (position 1). For the other positions, at constant aeration rate, the variation of mixing time with the rotation speed is different. This parameter initially decreases with mixing intensification, reaches a minimum level, increasing then, this evolution being more pronounced for the regions placed nearly the turbine impellers (position 2 and 3). Similar to the simulated broths, this variation is the result of the change in relative importance of mechanical and pneumatic agitation, the contribution of pneumatic mixing being more important, especially in the regions with lower concentration of solid phase and lower rotation speed (the solid phase exhibits the tendency of deposition at the bioreactor bottom, in region 1). But, owing to the lower viscosity of yeast suspensions compared with the simulated broths, these effects are less significant. The critical rotation speed increases with the biomass accumulation from 300 rpm for $C_X \leq 75$ g/l d.w. to 400 rpm for $C_X \geq 75$ g/l d.w.

The yeast accumulation induces the reduction of the mixing intensity in whole bulk of the fermentation broth. Furthermore, the biomass growth leads to the gradual increase of its concentration also in the regions 2 and 3. Therefore, the variations of mixing time with the rotation speed obtained for these positions become similar to that recorded for position 1. It can be concluded that the relative contribution of mechanical mixing to broth circulation becomes more important by increasing the yeast concentration.

Unlike the yeasts, the fungus can grow on two morphological conformations: free mycelia and mycelial aggregates (pellets). Moreover, irrespective of the morphological structure, the accumulation of fungus biomass induces a significant increase of broths viscosity, but the magnitude of this influence depends on the fungus morphology. Thus, for 33.5 g/l d.w. *P. chrysogenum* strains used in these experiments, the apparent viscosity of suspension was of 172.5 cP for free mycelia and 88.4 cP for pellets. The values of viscosity cumulated with the stronger hyphal–hyphal interactions for filamentous conformation or with the deposition tendency for pellets induce differences between the values of mixing time recorded for the two morphological structures.

The influence of rotation speed on mixing efficiency for low concentrated *P. chrysogenum* pellet broths is similar to those previously recorded for *S. cerevisiae* broths (Fig. 2(3)). The contribution of pneumatic agitation to the broth circulation in the regions 3 and 4 is more pronounced than in the case of yeasts, owing to the superior tendency of pellets to deposition at the bioreactor bottom [16]. As the concentration of pellets in position 2 is higher than in the superior positions 3 and 4, the variation of mixing time recorded for position 2 is close to that for position 1.

The accumulation of fungus leads to the significant diminution of mixing intensity in the whole bulk of the broth, this effect being more important for the superior region of the bioreactor (for 400 rpm, by increasing the fungus concentration from 4 to 36 g/l d.w., the mixing time increased for six times for position 1, respectively for 10.8 times for position 4). The gradual increase of biomass amount also in positions 3 and 4 induces a similar behavior of these regions to that obtained for positions 1 and 2 from the viewpoint of the influences on mixing efficiency.

At a constant aeration level and for fungus concentration up to 24 g/l d.w., from Fig. 2(4) it can be observed that the shape of the obtained curves for *P. chrysogenum* free mycelia is rather similar to those of pellet cultures. But, in this case, due to the more uniform distribution of the mycelia, the influence of the rotation speed is the same for all considered positions of pH electrode. For both morphological conformations, the increase of rotation speed induces the dispersion of biomass also in the regions 2, 3 and 4. Therefore, the values of mixing time recorded for the four regions become closer. The critical rotation speed is of 400 rpm irrespective of the fungus morphology.

The accumulation of filamentous *P. chrysogenum* leads to the reduction of the mixing intensity, this effect being more pronounced for the superior regions, due to their position related to the impellers (for 400 rpm, by increasing the fungus concentration from 4 to 36 g/l d.w., the mixing time increased for 3.7 times for position 1, respectively for 5.6 times for position 4; the difference between the two positions is less important than that previously recorded for pellet suspensions in the same experimental conditions, due to the superior deposition tendency of pellets which amplifies the heterogeneity of the system).

Since the free mycelia biomass is more uniformly distributed into the broths, the position of the impellers controls more evidently the mixing distribution for a given level of fungus concentration. Therefore, it can be observed that by increasing the fungus concentration, respectively by increasing the apparent viscosity, the variations of mixing time are modified, the rotation speed influence becoming more pronounced for the inferior positions 1 and 2. For this reason, at rotation speed over 350–400 rpm and biomass concentration higher than 30 g/l d.w.,

the mixing intensity is superior in the positions 1 and 2, these results being opposite to those obtained for the pellet suspensions.

As mentioned above, the analysis of the mixing distribution for the four considered positions inside the bioreactor indicated that the highest values of mixing time have been recorded either for the region placed between the stirrers, in the case of simulated broths, or for the inferior region, in the case of yeasts and fungus pellets broths (Fig. 3).

For the *P. chrysogenum* free mycelia, due to the lower rate of biomass deposition and, therefore, to the more uniform distribution of biomass into the broth, the highest mixing time values have been recorded for the inferior region only for fungus concentration below 24 g/l d.w. and rotation speed below 400 rpm. If these limits are exceeded, the most efficiently agitated regions become those related to the positions vicinal to the impellers, respectively positions 1 and 2.

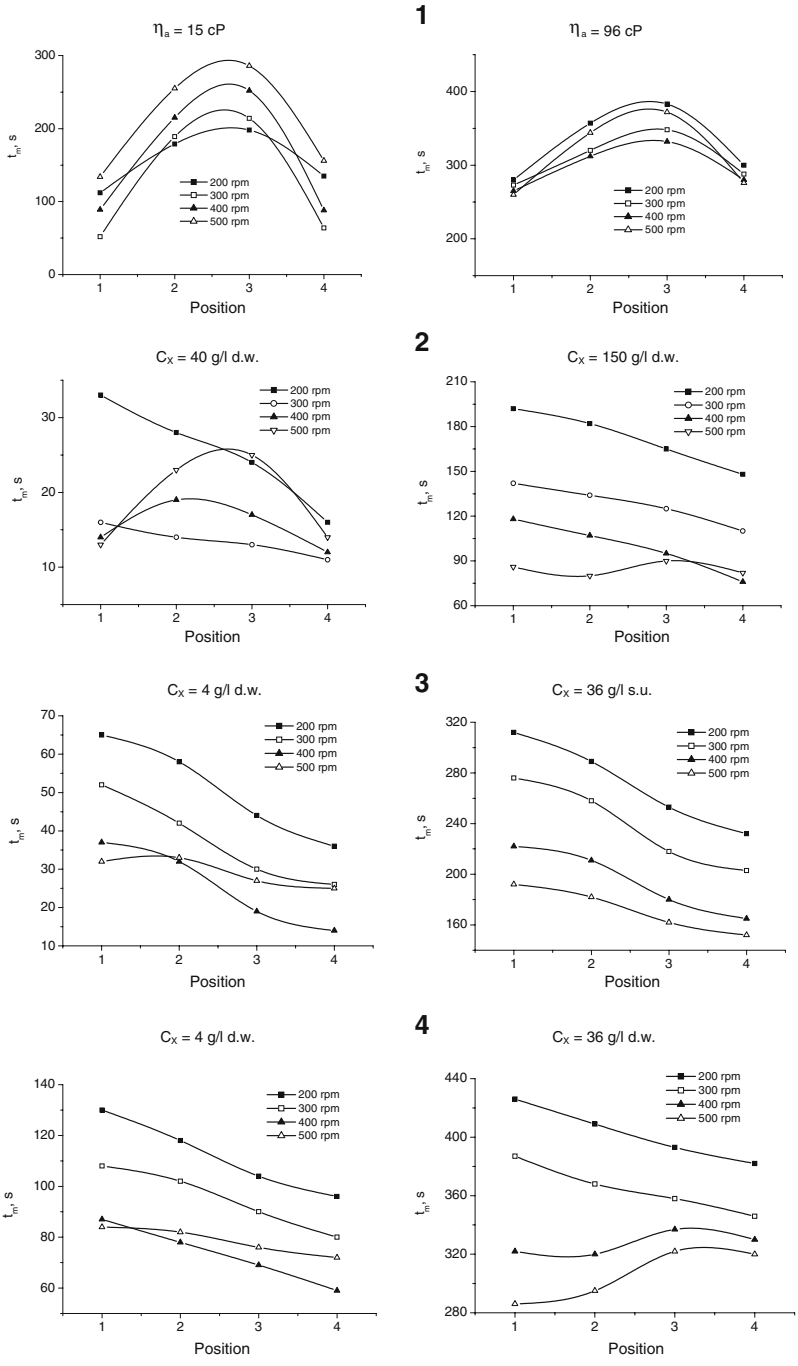

Fig. 3 Variation of mixing time with the position of pH electrode (aeration rate of 75 l/h; *1* Simulated broths, *2* *S. cerevisiae* broths, *3* *P. chrysogenum* pellets broths, *4 P. chrysogenum* free mycelia broths)

These results confirm and underline the decisive influence of solid phase presence and morphology on broth circulation.

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 which is directly related to broth apparent viscosity (250–300 rpm for apparent viscosity up to 60 cP, 400 rpm for higher viscosities [18]). But, irrespective of the rotation speed level, 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 recorded (Fig. 3(1)). A slight flattening of the obtained curves could be seen with the increase of the apparent viscosity, suggesting a more uniform distribution of mixing time in the viscous liquids, but concomitantly with the reduction of mixing efficiency.

Figure 3(2) suggests the existence of an optimum rotation speed, which corresponds to the uniform mixing of the *S. cerevisiae* broths. The value of the optimum rotation speed increases from 300 rpm for *S. cerevisiae* concentration up to 75 g/l d.w. to 500 rpm for suspensions more concentrated than 130 g/l d.w.

For both *P. chrysogenum* pellets and free mycelial cultures, the existence of an optimum rotation speed of 500 rpm has been recorded only for biomass concentration below 24 g/l d.w. (Fig. 3(3, 4)). The accumulation of biomass over this level induces the heterogeneous distribution of mixing, effect that becomes more accentuated at higher fungus concentration (36 g/l d.w.).

The observed differences between the yeast and fungus broths are the consequence of the significant higher viscosity of fungus broths, even at low biomass concentration, and of the superior tendency of deposition of pellets, both reducing the mixing efficiency and amplifying the system heterogeneity.

The nature of the influence of aeration rate for simulated broths depends strongly on the apparent viscosity and less on the position of pH electrode. Thus, the dependence between the mixing efficiency and aeration rate is considerably changed with the increase of viscosity, important differences between the values of mixing time being recorded for the four positions (Fig. 4) (Fig. 4(1)). For the above-presented reasons, the lowest values of mixing time have been obtained for positions 1 and 4.

At higher apparent viscosity, the increase of aeration rate initially leads to the mixing intensification; therefore, the mixing time decreases and reaches a minimum value, followed by its increase. At lower air flow rate, the bubbles' coalescence rate is high because the low turbulence cannot hinder this process. In these conditions, the heterogeneous distribution of air in the liquid phase, the reduction of air hold-up and the rise of bubbles through preferential central routes were observed, 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 a minimum value.

The value of air volumetric flow that corresponds to the minimum of mixing time is called *critical flow rate* and depends mainly on liquid apparent viscosity [17]. For all the considered positions, the value of critical flow rate is reduced from 300 to 150 l/h with the increase of the apparent viscosity from 15 to 96 cP.

For the apparent viscosity over 60 cP, the supplementary increase of air flow rate leads to the significant increase of mixing time to a maximum value, a phenomenon that is more pronounced at higher viscosity. This variation is the result of the formation of smaller bubbles having lower rise velocity, thus leading to the increase of gas hold-up value and to the decrease of dispersion circulation velocity. 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) the rise velocity of the air increases, determining the simultaneous intensification of media circulation. The flooding phenomenon is less pronounced in the intermediary positions because the air accumulation is not possible as in the positions 1 and 4.

In the case of real broths, the influence of aeration rate depends on the biomass concentration and on its dispersion in different regions inside the bioreactor, as well as on the microorganisms' type and morphology.

As the sparging system is placed at the bioreactor bottom, for *S. cerevisiae* concentration below 100 g/l d.w., the intensification of aeration leads to a decrease of mixing time at the inferior region, a phenomenon that is the result of the increase of the contribution of pneumatic mixing to the cells' dispersion (Fig. 4(2)).

For the other positions, the mixing time initially decreases to a minimum value with the increase of the flow rate, followed by its increasing. At constant rotation speed, the supplementary increase of air volumetric flow rate induces the formation of smaller bubbles having lower rise velocity, thus leading to the increase in gas hold-up and to the decrease of dispersion circulation velocity. This phenomenon was more evidently observed for the experimental conditions for

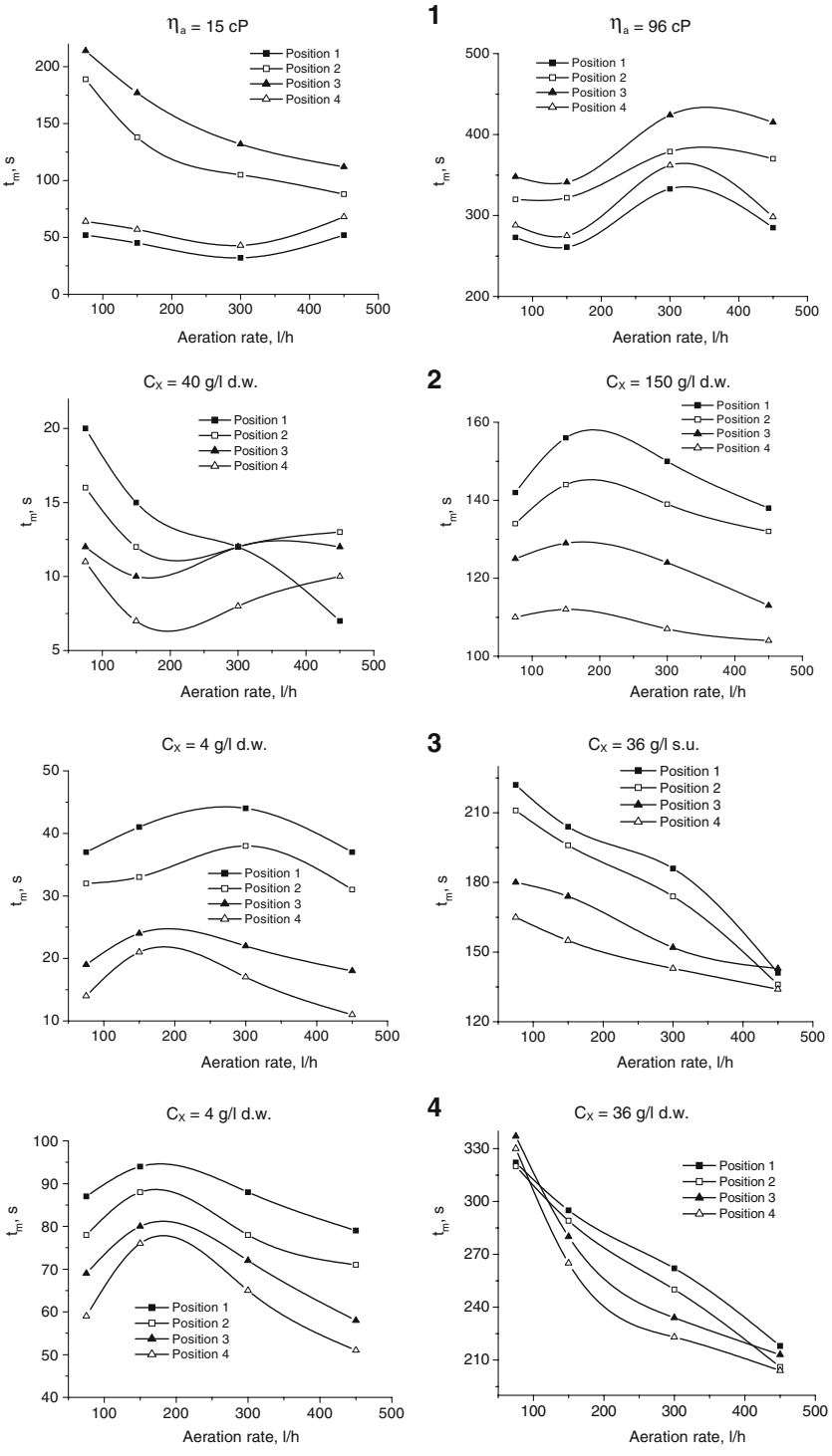

Fig. 4 Influence of aeration rate on mixing time distribution (rotation speed of 400 rpm; *1* Simulated broths, *2 S. cerevisiae* broths, *3 P. chrysogenum* pellets broths, *4 P. chrysogenum* free mycelia broths)

which the mechanical agitation exhibits a significant role in dispersion and retention of bubbles in media, respectively for positions 2 and 3. The accumulation of biomass attenuates this phenomenon, the recorded variations becoming similar for C_X = 100 g/l d.w. The critical aeration rate increases from 150 l/h for 40 g/l d.w. *S. cerevisiae* to 300 l/h for 100 g/l d.w.

At constant rotation speed, this variation of mixing efficiency with aeration rate is considerably changed for more concentrated yeast suspensions (over 100 g/l d.w.). Thus, the increase of aeration determines initially the increase of mixing time to a maximum value. This variation is more important for the regions with higher amount of biomass (positions 1 and 2) and is

due to the adsorption of the cells to bubbles surface, therefore avoiding the coalescence of the bubbles. As mentioned earlier, the small bubbles formed by air dispersion and mechanical agitation exhibit a negative effect on broth circulation, reducing the broth circulation velocity, and therefore the mixing intensity. At higher air flow rate values, the flooding appears, the corresponding value of air volumetric flow is 150 l/h.

In the simulated broths, by increasing the apparent viscosity the bubbles are accumulated and form a coalescence around the impellers, thus leading to the increase of the air hold-up. This phenomenon has not been observed in the *S. cerevisiae* cultures, on the one hand due to the lower apparent viscosity of these broths even at high biomass concentration (for C_X = 150 g/l d.w. the apparent viscosity was of 7 cP), and on the other hand due to the tendency of the cell to hinder the bubbles' coalescence.

The flooding phenomenon was recorded also for *P. chrysogenum* broths with biomass concentration below 16 g/l d.w., irrespective of the morphological conformation. For *P. chrysogenum* pellets, the value of flooding point is correlated with the biomass amount (Fig. 4(3)). Therefore, for C_X = 4 g/l d.w., the critical air flow rate is 150 l/h for the inferior region, and 300 l/h for the superior one, becoming 300 l/h for both regions at 16 g/l d.w.

Compared with the suspensions of *P. chrysogenum* pellets, the existence of the flooding point is more evident for the free mycelial cultures, owing to the more uniform distribution of biomass into these broths and to the higher viscosity of them (Fig. 4(4)). In this case, the value of critical volumetric flow is depended only on the mycelia amount, being the same for all the considered regions inside the bioreactor (for 4 g/l d.w. filamentous fungus the critical air flow rate was 150 l/h, and 300 l/h for 16 g/l d.w.).

For both morphological conformations, the existence of flooding point becomes less pronounced and the shapes of the plotted curves are gradually changed with the fungus growth, being observed differences between the inferior positions 1 and 2 and the superior ones 3 and 4.

For fungus concentration over 16 g/l d.w., the increase of aeration leads to the continuous intensification of mixing in the inferior region. The variation of mixing time becomes contrary for the superior region, the increase of air flow rate inducing the slow increase of mixing and the flattening of its variation compared with the lower concentrated suspension of fungus. For mycelia concentration over 30 g/l d.w., the influence of aeration becomes similar in whole bulk of the broth, but it is more important for the inferior region.

In these systems, the highly apparent viscosity of fungus suspensions controls the mixing efficiency, the mechanical agitation is poor, especially in the region away from the impellers, and the relative contribution of pneumatic mixing to the broths circulation is more important.

Contrary to the simulated broths, where the bubbles coalesce and their accumulation around the stirrers is enhanced at higher viscosity, this phenomenon has not been recorded in the *P. chrysogenum* free mycelia or pellets cultures, especially because the biomass hinder the bubbles' coalescence. But, at higher biomass amount and aeration rate, the increase of air hold-up has been observed also for free mycelial suspensions, as the result of the hindrance of bubbles rising (for 300 l/h and 400 rpm, by increasing the biomass concentration from 4 to 36 g/l d.w., the air volumetric fraction increased from 2.3 to 8.4% for *P. chrysogenum* pellets, respectively from 4.1 to 12% for free mycelia).

The cumulated influence of rotation speed and aeration rate on mixing time has been plotted in Fig. 5 for the studied broths and for the positions with the most different behavior from the viewpoint of the correlation between the mixing time and the considered parameters.

The experimental data have been included in some mathematical correlations, which describe unitarily the influence of apparent viscosity or biomass concentration, rotation speed and superficial air velocity on mixing time at different positions of the pH electrode for the aerobic stirred bioreactor. The general expression of the proposed equations is

$$t_m = \alpha \cdot \eta_a^{\beta} \cdot N^{\gamma} \cdot v_S^{\delta} \tag{2}$$

for simulated broths, or

$$t_m = \alpha \cdot C_X^{\beta} \cdot N^{\gamma} \cdot v_S^{\delta} \tag{3}$$

for yeast and fungus broths.

The influence and the relative importance of the considered variables are suggested by the coefficients α, β, γ and δ values. The values of these coefficients are specific for each microorganism type or morphology and were calculated by the multiregression method using MATLAB software. Thus, the following correlations have been established:

(a) simulated broths:

- Positions 1 and 4

$$t_m = 3.00 \times 10^5 \cdot \frac{\eta_a^{0.533} \cdot v_S^{0.029}}{N^{4.006-1.2\cdot\ln N}}, \text{ s} \tag{4}$$

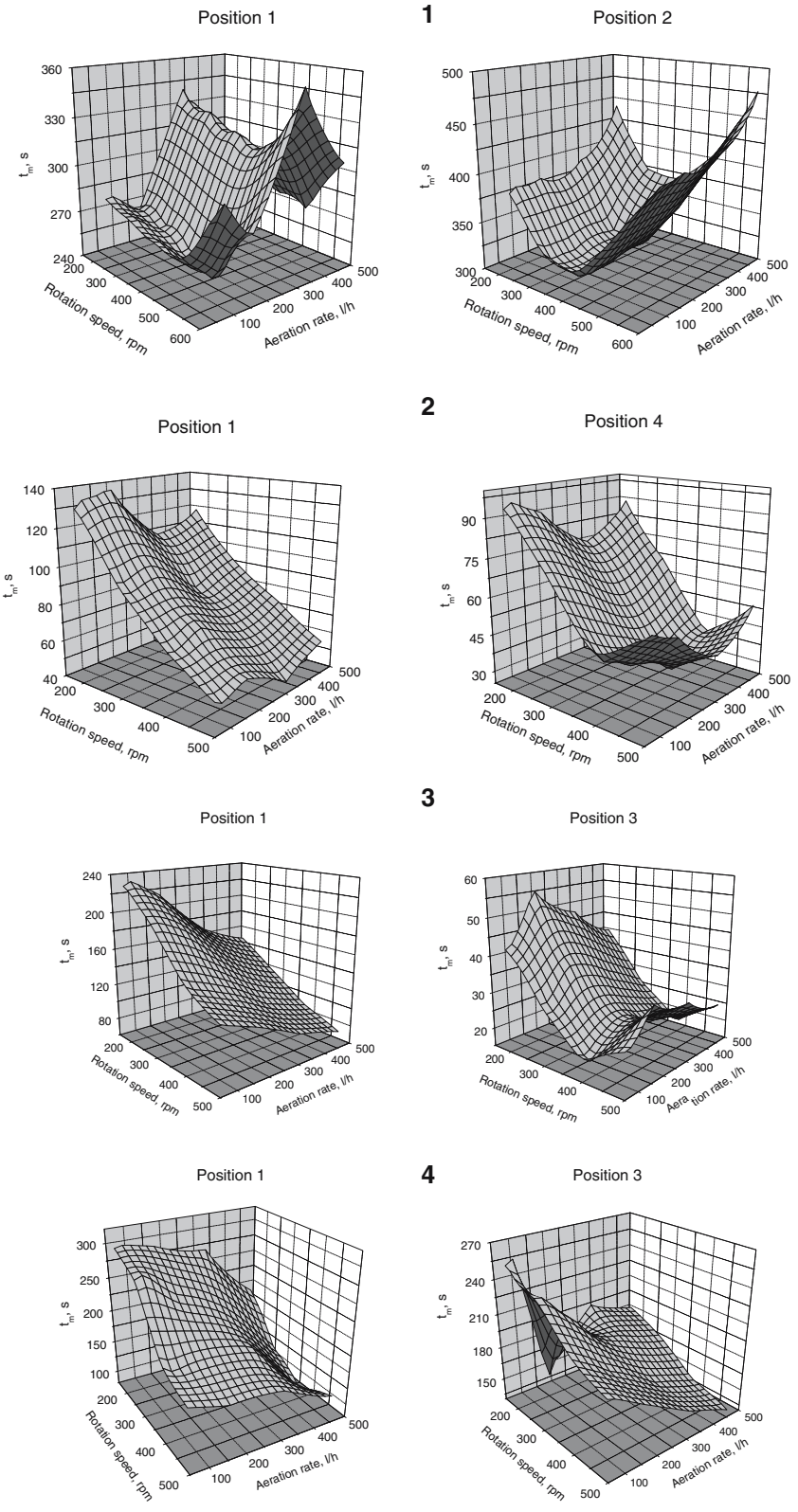

Fig. 5 Cumulated influence of rotation speed and aeration rate on mixing time (*1* Simulated broths, *2* *S. cerevisiae* broths, *3* *P. chrysogenum* pellets broths, *4 P. chrysogenum* free mycelia broths)

– • Positions 2 and 3

$$t_m = 2.64 \times 10^5 \cdot \frac{\eta_a^{0.403}}{N^{2.119-0.694\cdot\ln N} \cdot v_S^{0.086}}, \text{ s} \quad (5)$$

(b) yeasts (*S. cerevisiae*):

– • Position 1

$$t_m = 9.45 \cdot \frac{C_X^{0.716}}{N^{1.164} \cdot v_S^{0.237}}, \text{ s} \quad (6)$$

– • Positions 2, 3 and 4

$$t_m = 16.98 \cdot \frac{C_X^{1.422}}{N^{4.721-1.28\cdot\ln N} \cdot v_S^{0.083}}, \text{ s} \quad (7)$$

(c) fungus (*P. chrysogenum*) pellets:

– • Position 1

$$t_m = 34.65 \cdot \frac{C_X^{0.571}}{N^{0.703} \cdot v_S^{0.108}}, \text{ s} \quad (8)$$

– • Positions 2, 3 and 4

$$t_m = 75.85 \cdot \frac{C_X^{0.722}}{N^{1.646-0.309\cdot\ln N} \cdot v_S^{0.019}}, \text{ s} \quad (9)$$

(d) fungus (*P. chrysogenum*) free mycelia:

– • Position 1

$$t_m = 61.23 \cdot \frac{C_X^{0.415}}{N^{0.547} \cdot v_S^{0.127}}, \text{ s} \quad (10)$$

– • Positions 2, 3 and 4

$$t_m = 51.68 \cdot \frac{C_X^{0.458}}{N^{0.56-0.056\cdot\ln N} \cdot v_S^{0.096}}, \text{ s} \quad (11)$$

The proposed models offer a good agreement with the experimental data, the maximum deviation being of ±6.8% for simulated broths, ±7.3% for *S. cerevisiae*, ±5.4% for *P. chrysogenum* pellets and ±4.6% for *P. chrysogenum* free mycelia.

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 extent of 97.8% for simulated broths, 96.6% for *S. cerevisiae*, 97.2% for *P. chrysogenum* pellets and 97.9% for *P. chrysogenum* free mycelia. The rest of 2.2, 3.4, 2.8, 2.1%, respectively, can be attributed to the effect of other factors, namely: number, position and geometry of baffles, sparger diameter, etc.

Conclusions

The studies on mixing distribution for an aerobic stirred bioreactor underlined the different behavior of the simulated and real broths from the viewpoint of the correlation between the mixing time and the considered parameters (biomass concentration, rotation speed, aeration rate, position inside the bioreactor).

The modification of the rotation speed, at a constant level of air flow rate, demonstrates 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, apparent viscosity or biomass concentration and morphology.

Excepting the simulated broths, the experiments suggested the possibility to reach a uniform mixing in whole bulk of the broth for a given value of rotation speed or biomass concentration domain. For yeasts, the optimum rotation speed varied between 300 and 500 rpm, for *S. cerevisiae* growth from 40 to 150 g/l d.w. For fungus, irrespective of the morphology, the existence of the optimum rotation speed of 500 rpm has been observed only for biomass concentration below 24 g/l d.w.

The influence of aeration rate depends especially on the apparent viscosity or biomass concentration and on the impellers and sparger positions. By increasing the apparent viscosity or biomass amount, the shape of the curves describing the correlation between the mixing time and the air flow rate is significantly changed for all the considered positions of pH electrode, as the result of the change of relative contribution of pneumatic mixing to broth circulation. The intensification of the aeration induces the increase of mixing time to a maximum value, decreasing then, due to the flooding phenomena. This variation became more pronounced

at higher viscosities for simulated broths, at higher yeast concentration or at lower pellets or filamentous fungus concentration.

The influences of the considered factors on mixing intensity have been included in some mathematical correlations established by means of the experimental data. The proposed equations allow predicting the mixing time in different regions of the stirred bioreactors for aerated simulated, yeast and fungus broths, and offer a good concordance with the experimental results.

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