Roum. Biotechnol. Lett., Vol. 7, No. 4, 2002, pp 817-828

ORIGINAL PAPER

Modelling of Mixing in Stirred Bioreactors
3. Mixing Time for Aerated Simulated Broths

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Received: 9th May, 2002; Accepted: 7th June, 2002

Abstract

The mixing time is one of the most useful criterion for mixing intensity of fermentation broths and for scale-up of biosynthesis processes. This parameter value depends mainly on the rheological characteristics of the broths, bioreactor construction and fermentation conditions.

This paper presents the experiments on mixing efficiency for aerated media for a laboratory stirred bioreactor with double turbine impeller. The effects of stirrer rotation speed, air volumetric flow rate and stirrers position on the shaft on mixing time for aerated water and simulated broths (CMCNa solutions) were analyzed. Compared with non-aerated broths, the results indicated that the variation of mixing time with the considered parameters are very different, due to the complex flow mechanism of gas-liquid dispersion, mechanism which is changed by changing the broths properties or fermentation conditions.

Keywords*:* stirred bioreactor, aerobic bioreactor, mixing time, turbine stirrer, viscous liquids, aerated media.

Introduction

The accumulation of biomass or biosynthesized product (extracellular polysaccharides, protein molecules) leads to the continuous modification of the medium rheological properties, producing the appearance of the heterogeneous regions in the bioreactor. In these conditions, one of the most important problems which must be solved is to establish the optimum hydrodynamic regime for the bioreactor.

The mixing time represents one of the most useful criterion for characterization of the mixing intensity and for biosynthesis processes scale-up. The mixing time, t_m, is defined as the time needed to reach a given mixing intensity at a given scale, when starting from the

completely segregated situation. This parameter depends on a multitude of geometrical (dimensions of mixing system, dimensions of bioreactor) and technological factors (fermentation conditions, physical characteristics of the medium, power consumption, dissipated energy), the general correlation that describes the mixing time being of the following type:

$$t_m = f\left(d/D, N, \eta, \rho, V_a/V, P/P_a, \varepsilon_T\right) \quad (1)$$

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 time needed for the considered parameter M (pH-value, temperature, absorption) not to exceed the considered range $M_\infty \pm 0.5x\Delta M$ [5]. For example, an alkaline pulse is added to the liquid, this method being used in this paper experiments. The system can be regarded as completely segregated at t = 0. After a certain time, which is the mixing time, the pH remains into the considered range of deviation from ideally mixed. In this way, the mixing time can be related to the mixing intensity.

For calculation of mixing time, numerous equations have been proposed in literature, these equations taking into account the type of fermentation (aerobe or non-aerobe), the rheological characteristics of the broths and the fermentation conditions [1-13]. Because of the complexity of rheological behavior of broths, of the flow phenomena and of the particularities of fermentation systems, the accuracy of the proposed models is very limited, especially for aerated broths. Furthermore, the most of these models can predict the mixing time values for Re > 10,000, this flow regime being rarely reached in the large-scale bioreactors due to the microorganisms sensitivity to shear stress. For Re < 10,000, these models need some corrections [6].

For these reasons, the aim of our experiments is to analyze the dependence between the mixing time, the rheological characteristics of broths, the fermentation conditions and the biomass concentration, for a stirred bioreactor. For emphasizing and quantifying the effects of the biomass presence in medium, the studies were carried out with simulated and real broths.

The previous results obtained for simulated broths indicated that, for water or liquids with low viscosity, the distance between the stirrers assembled on the shaft has to take into account the interference of the stirrers flow lines, and, for viscous liquids, the position of the stagnant region in the bioreactor [14]. The presence of biomass in fermentation broths considerably reduces the mixing efficiency, even at low viscosity level of the suspensions. The magnitude of this effect depends on the nature, concentration and morphology of biomass. The experimental studies concerning the mixing of bacteria (*Propionibacterium shermanii*), yeasts (*Saccharomyces cerevisiae*) and fungus (*Penicillium chrysogenum*, free mycelia and mycelial aggregates or pellets) non-aerated suspensions in a stirred bioreactor provided with double impeller emphasized that the value of mixing time increases as follows:

bacteria < yeasts << fungus pellets < fungus free mycelia.

Due to their higher viscosity and pronounced non-Newtonian behavior, the mixing time for fungus suspensions was significant higher than for bacteria and yeasts suspensions, at same biomass concentration and operational conditions. Furthermore, the mixing time for free mycelia suspensions was about 2 - 3 times greater than for mycelial aggregates, owing to the higher viscosity. But, the mycelial aggregates exhibit an accentuated tendency of solid-liquid separation, the influence of distance between the stirrers being more important in this case. Generally, contrarily to the mixing of simulated broths without biomass, for increasing the mixing efficiency of biomass suspensions, the stirrers have to be placed near the bottom of the vessel for avoiding the solid phase deposition [15, 16].

By means of the experimental data, for each of the non-aerated systems were established mathematical correlations which describe unitary the dependence between the mixing time and apparent viscosity or biomass concentration, stirrer rotation speed and distance between the stirrers [15].

Because the previous results are adequate only for non-aerated broths, these studies have been developed for aerated simulated and real broths, at similar experimental conditions. In this paper are presented the experimental results for simulated broths consisted of carboxymethylcellulose sodium salt of different viscosities. The obtained data allow to correlate the mixing time with the geometrical and functional characteristics of the stirred bioreactor.

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 mixing system consists of two turbine stirrers and three baffles. The bioreactor and impeller characteristics are given in **Table 1**.

Table 1. Characteristics of bioreactor and impeller.

d, mm	d/D	H/D	w/d	l/d	h/d	No. blades	No. baffles	s/d	d'/d	l'/d
64	0.36	1.15	0.12	0.28	1	6	3	0.20	0.21	2.81

The upper stirrer was placed on the shaft at a distance varying between 32 and 128 mm (0.5d and 2d) from the lower one. The rotation speed was maintained between 0 and 700 rpm. The experiments were carried out for a Reynolds number intervals below 6,000, which correspond to the laminar and transitory flow, and avoid the "cave" formation at the broths surface (for rotation speed over 800 rpm and L = 2d).

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 0 to 400 $l.h^{-1}$.

In the experiments, water and simulated fermentation broths have been used. The simulated broths consisted of carboxymethylcellulose sodium salt (CMCNa) having the apparent viscosity in the domain of 8.25 – 268.7 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 21°C.

For determining the mixing time values the following homogeneity criteria for mixing was considered [6]:

$$I = \frac{pH_{\infty} - 0.5\Delta pH}{pH_{\infty}} x100 = 99\% \qquad (2)$$

where $\Delta pH = 0.02$.

The values of mixing time have been determined by means of a solution of 2N KOH as tracer, by recording the time needed to the medium pH-value to reach the value corresponding to the considered mixing intensity. The tracer volume was of 0.5 ml, the tracer being injected opposite to the pH electrode, at 10 mm from the liquid surface. The pH electrode was placed at 20 mm from the vessel bottom. Because the 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 variations were recorded by bioreactor computer-recorded system. Each experiment has been carried out three or four times, for identical conditions, the average value of mixing time being used. The maximum experimental error was of ± 3.2%.

Results and Discussions

For an aerobic bioreactor provided with one ore more stirrers the flow mechanism is complicated due to the combined action of mechanical and pneumatic mixing. Compared with the non-aerated systems, different flow patterns can exist when gas is sparged into the broth

Generally, the analyze of mixing efficiency for aerated mechanical stirred systems is derived from that of non-aerated systems, due to the less complicated flow phenomena for the second ones. In this case, it was assumed that the gas phase does not influence the flow of liquid phase in stirred vessels [7]. But, the values of mixing time for aerated broths calculated by means of the equations established for non-aerated systems are lower to the experimental ones for about 1.2 – 2 times [17].

The influence of aeration on mixing efficiency is much more complex and has to be distinctly analyzed. For numerous fermentations, owing to the decrease in pumping capacity of the stirrer, due to the cavity formation, and to the compartmentalization in regions around each of the stirrers, the aeration increases the mixing time compared with non-aerated systems. However, as it was affirmed in literature, the deviations from the values obtained for non-aerated broths depend on the constructive and functional characteristics of the bioreactor. Thus, 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 [7].

In this context, the aim of this paper is the study of the effects of stirrer rotation speed, air flow rate and stirrers position on the shaft on mixing time for aerated water and simulated broths having different viscosities.

1. Influence of stirrer rotation speed

Contrarily to the non-aerated media, for which the mixing time is reduced by increasing the rotation speed value, for aerated broths the influence of this parameter is different and must be related to the apparent viscosity, air flow rate and distance between the stirrers placed on the shaft.

For water, the mixing time initially decreases with the rotation speed, reaches a minimum value for 100 rpm increasing then, for all considered air flow rates or distances between the stirrers (**Figure 1.a**). 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 is considerable, the increase of rotation speed supplementary intensifying the broth circulation 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* [18].

The increase of the liquid phase apparent viscosity or the modification of fermentation conditions can lead to the change of the variation of mixing efficiency with rotation speed. As it can observed from **Figures 1.b, c and 2.a-c**, indifferent of the broth apparent viscosity, by increasing the stirrer rotation speed the mixing time reaches a minimum value for most of the studied systems.

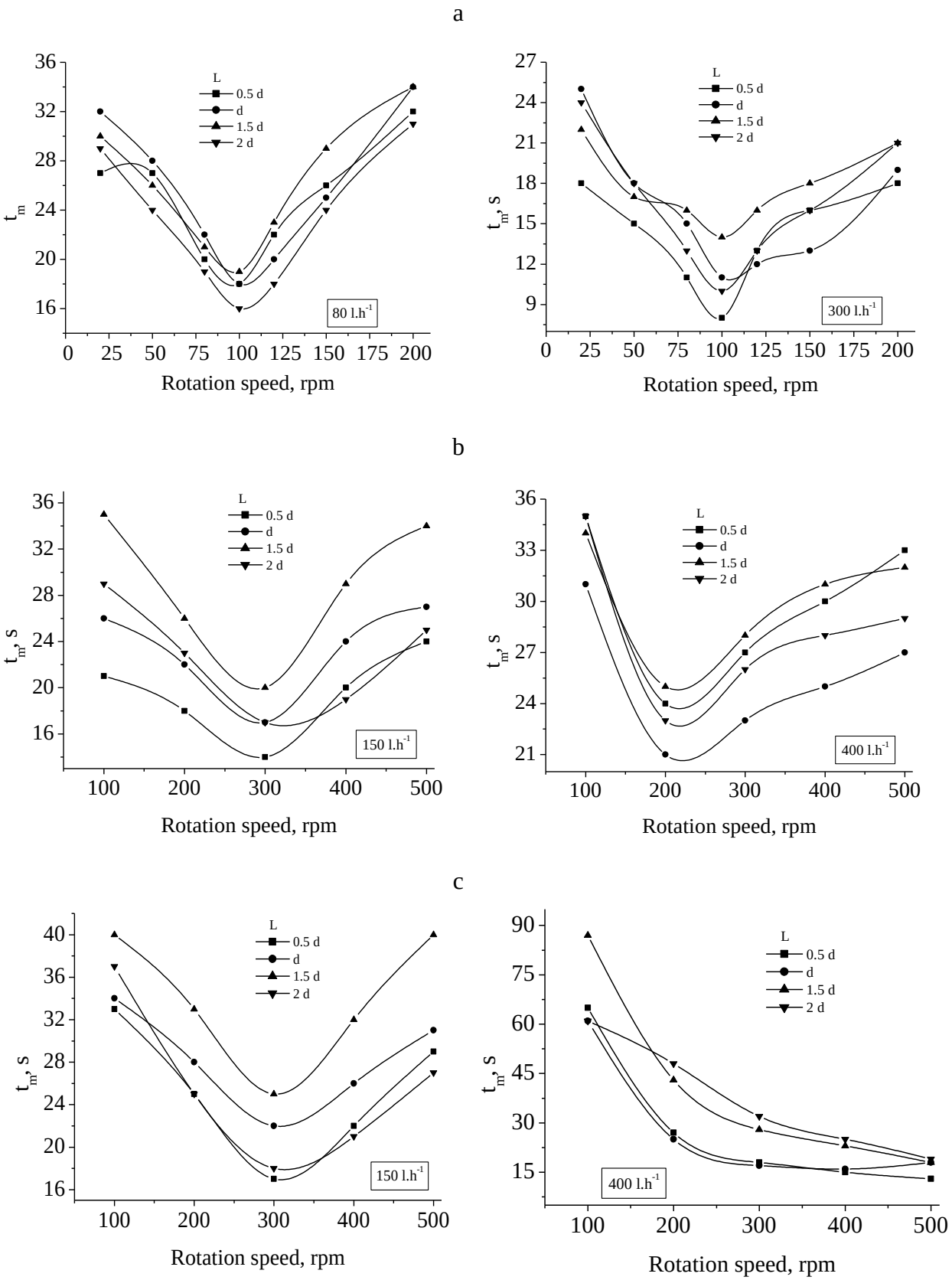


Figure 1. Influence of rotation speed on mixing time value (a - η = 1 cP, b - η_a = 8.25 cP, c - η_a = 31.9 cP).

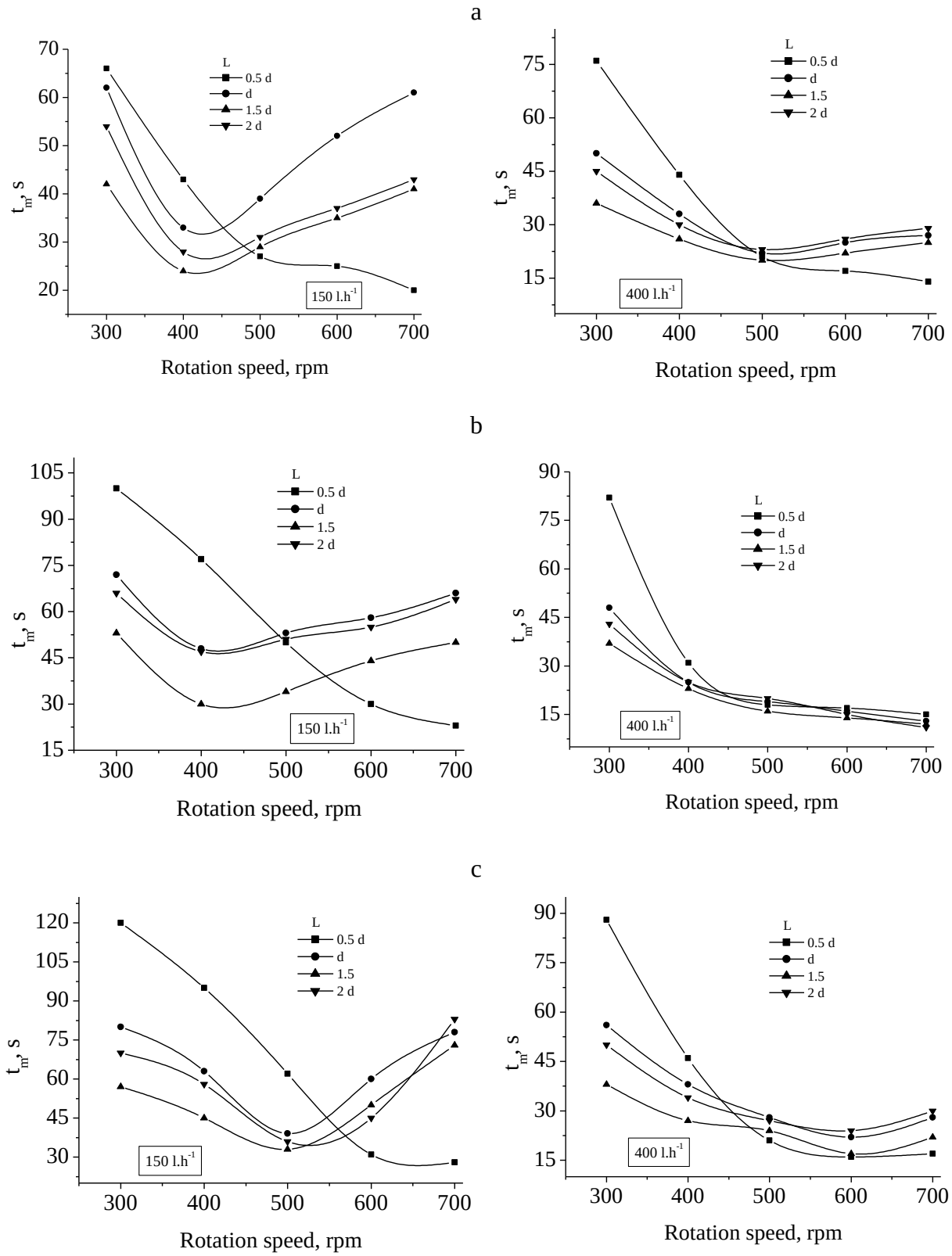


Figure 2. Influence of rotation speed on mixing time value (a - η_a = 98.5 cP, b - η_a = 187.3 cP, c - η_a = 268.7 cP).

But, the value of critical rotation speed depends on the apparent viscosity and air flow rate. Therefore, with the increase of apparent viscosity from 8.25 to 268.7 cP, the value of critical rotation speed increases from 300 to 500 rpm, for air flow rate between 80 and 400 $l.h^{-1}$, or between 200 and 600 rpm, for air flow rate over 400 $l.h^{-1}$. As it was earlier mentioned, this variation is the result of the change in broth circulation into bioreactor, as a consequence of the increase in gas hold-up, and in a less intensive mixing, respectively. Thus, for an apparent viscosity of 98.5 cP and 300 rpm, the gas volumetric ratio was of 2.6-3%, becoming 11-12% for 700 rpm.

Although this variation of mixing time is characteristic for most of the considered systems, it can be modified function of the stirrers position on the shaft and aeration rate.

2. Influence of air volumetric flow rate

The aeration influence on mixing intensity strongly depends on the apparent viscosity of liquid phase. For water and liquids with close viscosity, the mixing time continuously decreases with air flow rate, the magnitude of this effect being function of rotation speed value (**Figure 3.a**).

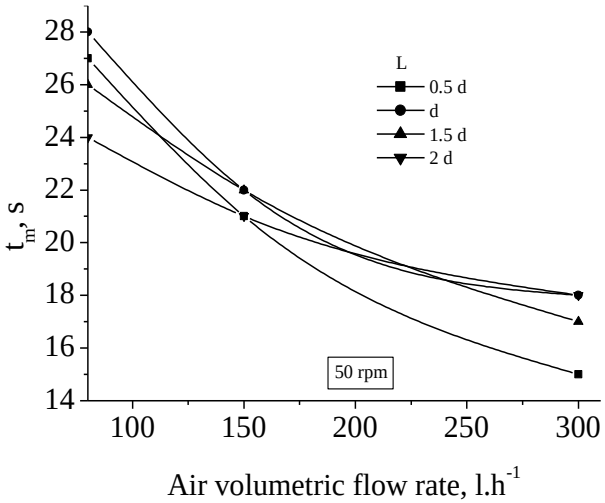
This variation of mixing efficiency with aeration rate is considerably changed for viscous media. Up to about 10 cP the coalescence characteristics of the liquid phase are modified, this determining the change in bubbles circulation velocity, bubbles dispersion and hold-up compared with non-aerated media [19]. Thus, at lower aeration rate, with the increase of viscosity bubbles coalescence increases, very large bubbles are formed, which can reach 80 mm diameter for a distance of 0.5d between the stirrers. In this circumstances, 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 (**Figures 3.b, c and 4.a-c**). The value of air volumetric flow that corresponds to the minimum of mixing time, called *critical flow rate*, depends on liquid apparent viscosity, rotation speed and stirrers position on the shaft. Thus, for most of the studied systems, the mixing time minimum value was reached for a critical flow rate of 150 – 200 $l.h^{-1}$. But, for apparent viscosities lower than 100 cP, the increase of rotation speed can lead to the modification of the critical flow rate to higher values (250 – 300 $l.h^{-1}$ for rotation speed values over 300 rpm).

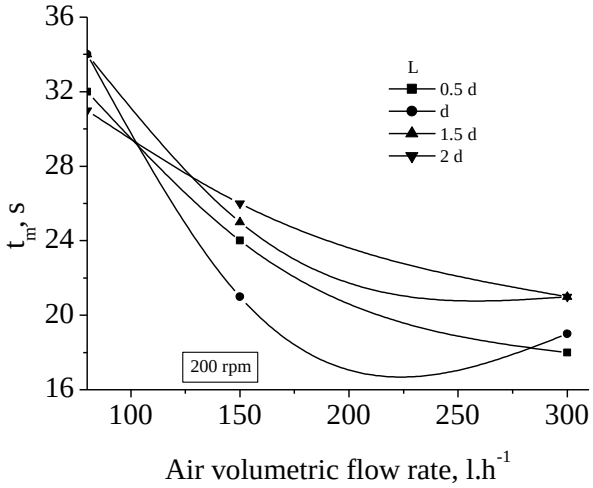
For constant rotation speed value, the supplementary increase of aeration rate induces the increase of mixing time. This variation is the result of the formation of smaller bubbles having lower rise velocity, thus leading to the increase in gas hold-up value and to the decrease of dispersion circulation velocity. The phenomena was more evidently observed for the experimental conditions for which the mechanical agitation exhibits a significant role in dispersion and retention of bubbles in media.

At higher air flow rate values, the mixing time reaches a maximum, reducing then. The maximum value corresponds to the flooding point, when the energy dissipated by the air exceeds that of the stirrer. At this moment, 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 some different rotation speed domains controlled by the apparent viscosity. Thus, the maximum was reached at rotation speed values below 200 rpm for an apparent viscosity lower than 31.9 cP, at rotation speed below 400 rpm for apparent viscosity between 31.9 and 98.5 cP, and at rotation

speed below 600 rpm for higher apparent viscosities. The flooding aeration rate was of 300 $l.h^{-1}$.

a





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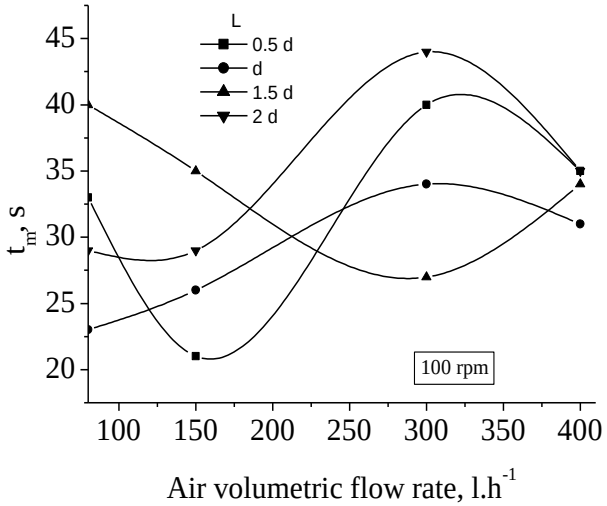
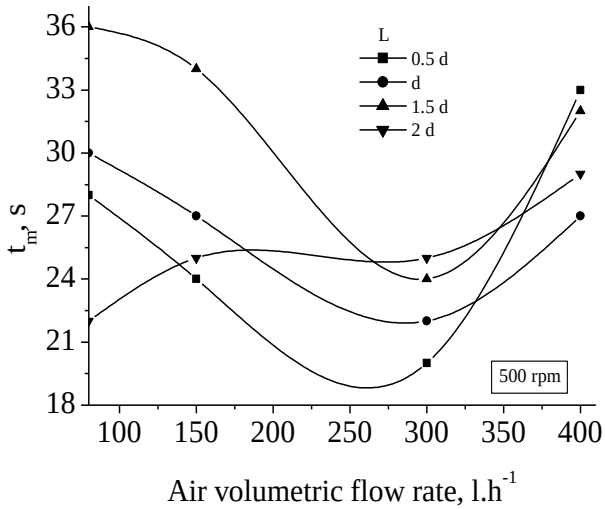
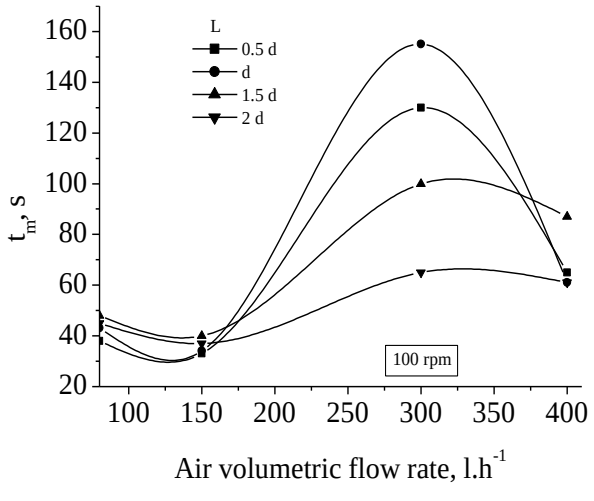




c



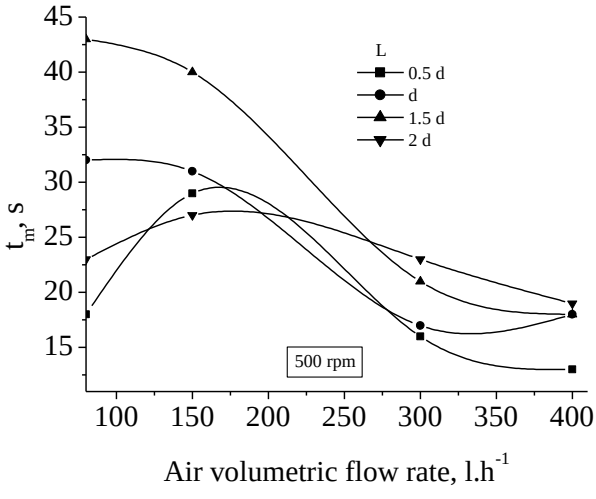
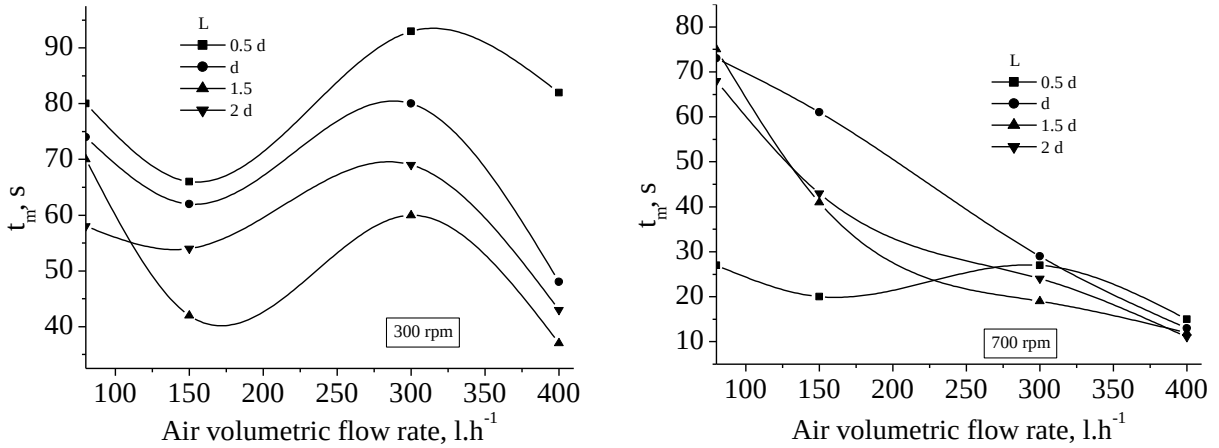


Figure 3. Influence of air volumetric flow rate on mixing time value (a - η = 1 cP, b - η_a = 8.25 cP, c - η_a = 31.9 cP).

a



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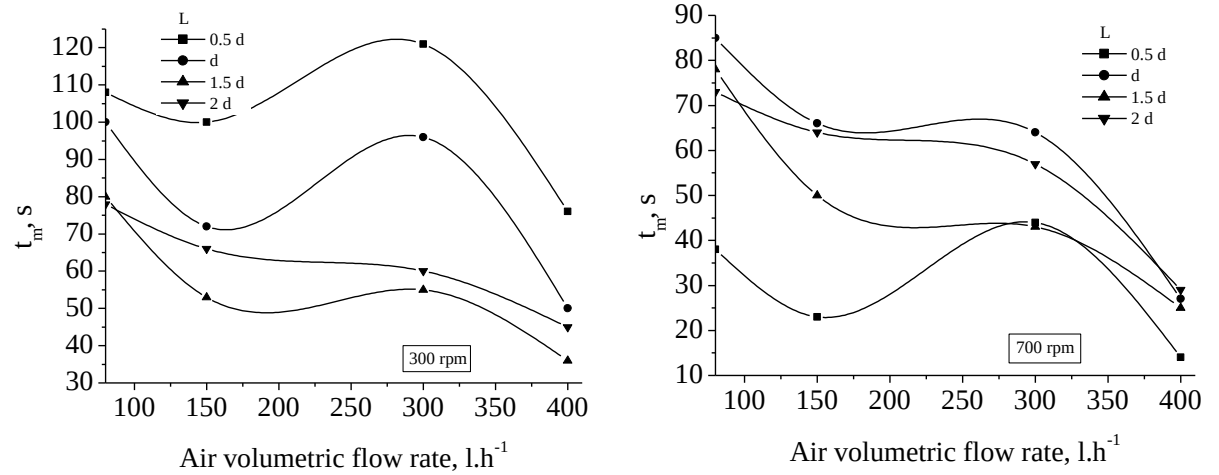

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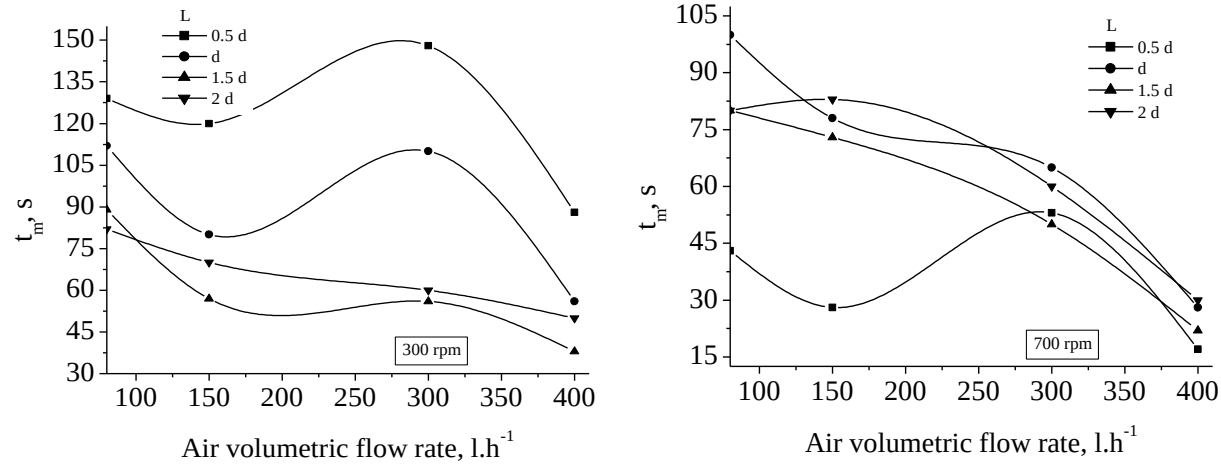

Figure 4. Influence of air volumetric flow rate on mixing time value (a - η_a = 98.5 cP, b - η_a = 187.3 cP, c - η_a = 268.7 cP).

On the other hand, as it can be observed from Figures 3 and 4, the mixing time either continuously increases with air flow rate after reaching its minimum value, for rotation speed greater than 200 rpm and apparent viscosity below 31.9 cP, or decreases for entire considered aeration domain, for rotation speed greater than 400 rpm and apparent viscosity between 31.9 and 98.5 cP. The observed variation of mixing intensity indicated that in the first case the flooding is avoided at higher rotation speed, and the aeration contributes positively at mixing, in the second one.

3. Influence of distance between the stirrers

Excepting water, for which the variation of mixing time with rotation speed or aeration rate is similar for all positions of the stirrers on the shaft, the distance between the stirrers can modify the evolution of mixing time with the considered parameters.

In the previous works was stated that for non-aerated water the minimum values of mixing time are reached for a distance between the stirrers of 1.5d [14,15]. As it can be seen from Figures 3.a, the lowest values of mixing time for aerated water are obtained for a distance of 0.5d - d, as the result of a more homogeneous distribution of bubbles in liquid phase, especially at higher rotation speed level. Contrarily to the non-aerated CMCNa solutions, for which a distance of 2d leads to the minimum mixing time, for the most of studied aerated viscous solutions the minimum was reached for 0.5d, too. But, from Figures 2.a-c it can observed that for the apparent viscosities over 31.9 cP the variation of mixing time with rotation speed for 0.5d completely differs compared with the others positions of the stirrers on the shaft. Thus, for the entire domain of aeration rate level and a distance of 0.5d the mixing efficiency continuously increases with stirrer rotation speed. For viscous liquids, this evolution is explained by the tendency of bubbles accumulation and coalescence in the region around the stirrer, phenomena that is more pronounced for lower distance between the stirrers. As it was earlier mentioned, the accumulation of air around the stirrer reduces the dispersion circulation velocity. By increasing the rotation speed, the very large bubbles formed in the stirrer region are dispersed and uniformly distributed in liquid phase. The rise velocity of the bubbles in the upper compartment of the bioreactor is higher if the stirrers are closely placed on the shaft.

The highest values of mixing time were obtained for a distance of 1.5d for water, and d for viscous simulated broths, as the effect of the compartmentalization of flow regions and increase of retention time of bubbles in the liquid phase, both reducing the dispersion circulation velocity.

Conclusions

The flow mechanisms of aerated and non-aerated are very different, this being the result of a different influence of stirrer rotation speed and construction and air volumetric flow rate on mixing time.

For water and simulated viscous media the increase of rotation speed value leads to the decrease of mixing time, which reaches a minimum value, corresponding to the critical rotation speed, increasing then. The value of critical rotation speed depends on aeration rate and varies between 300 and 500 rpm, for air flow rate between 80 and 400 $l.h^{-1}$, or between 200 and 600 rpm, for air flow rate over 400 $l.h^{-1}$.

In most of the studied systems, the variation of mixing time with gas flow rate indicates a sinusoidal dependence. Thus, with increasing the aeration rate the mixing initially decreases,

reaches a minimum value, and increases to a maximum value. For lower rotation speed or higher apparent viscosity values, the mixing time decreases then by supplementary increase of aeration rate.

The lowest values of mixing time for aerated water are obtained for a distance of 0.5d – d between the stirrers placed on the shaft, and 0.5d for CMCNa solutions, as the result of a more homogeneous distribution of bubbles in liquid phase. Furthermore, the variation of mixing efficiency with aeration rate or rotation speed for a distance of 0.5d is completely different those obtained from the other positions of the stirrers, the mixing efficiency continuously increasing with stirrer rotation speed.

The observed phenomena are the results of change of pumping capacity of the stirrer, due to the cavity formation, compartmentalization in regions around each of the stirrers, coalescence and dispersion of bubbles and flooding. These lead to a very complex flow mechanism of the gas-liquid dispersion, mechanism which is changed by changing the broths properties or fermentation conditions.

Notations

d - stirrer diameter
d' - pH electrod diameter
D - bioreactor diameter
h - distance from the inferior stirrer to the bioreactor bottom
H - bioreactor height
l - impeller blade length
l' - pH electrode immersed length
L - distance between the stirrers
N - impeller rotation speed
P - power consumption for mixing of non-aerated broths
P_a - power consumption for mixing of aerated broths
pH_∞ - value of pH at $t = \infty$
ΔpH - allowed deviation from ideally mixed
s - baffle width
t_m - mixing time
V_a - volumetric air flow rate
V - volume of medium
ε_T - energy dissipated
η_a - apparent viscosity
ρ - density

References

1. C.J. HOOGENDOORN, A.P. DEN HARTOG, *Chem. Eng. Sci.*, **22**, 1689 (1967).
2. Y.T. SHAH, G.J. STIEGEL, M.M. SHARMA, *A. I. Ch. E. J.*, **24**, 369 (1978).
3. A. EINSELE, *Proc. Biotechnol.*, **7**, 13 (1978).
4. R. MANFREDINI, V. CAVALLERA, L. MARINI, G. DONATI, *Biotechnol. Bioeng.*, **25**, 3115 (1983).
5. J.J. HEIJNEN, K. VAN'T RIET, *Chem. Eng. J.*, **28**, B21 (1984).

6. P. VERLAAN, J. TRAMPER, K. VAN'T RIET, CH.K.A.M. LUYBEN, *Chem. Eng. J.*, **33**, B43 (1986).
7. K. VAN'T RIET, J. TRAMPER, *Basic Bioreactor Design,* M. Dekker Inc., New York, pp. 183 (1991).
8. H.-J. REHM, G. REED (Eds.), *Biotechnology*, vol.4, VCH, Weinheim, pp. 299 (1991).
9. B. MAYR, P. HORVATH, E. NAGY, A. MOSER, *Biotechnol. Bioeng.*, **43**, 195 (1994).
10. A.G. PEDERSEN, M. BUNDEGARD-NIELSEN, J. NIELSEN, J. VILLADSEN, *Biotechnol. Bioeng.*, **44**, 1013 (1994).
11. A.W. NIENOW, *Trans. I. Chem. E*., **A74**, 417 (1996).
12. R. POHORECKI (Ed.), *Fluid Mechanics Problems in Biotechnology*, **51**, 3 (1998).
13. A. LUBBERT, S.B. JORGENSEN, *J. Biotechnol.*, **85**, 187 (2001).
14. C. ONISCU, A.I. GALACTION, D. CASCAVAL, F. UNGUREANU, *Roum. Biotechnol. Lett.*, **6**, 43 (2001).
15. D. CASCAVAL, C. ONISCU, A.I. GALACTION, F. UNGUREANU, *Chem. Industry J.*, **55**, 367 (2001).
16. C. ONISCU, A.I. GALACTION, D. CASCAVAL, F. UNGUREANU, *Biochem. Eng. J.* (in press).
17. A. EINSELE, R.K. FINN, *Ind. Eng. Chem. Proc. Des. Dev*., **19**, 600 (1980).
18. D. CASCAVAL, C. ONISCU, A.I. GALACTION, *Inginerie biochimica si biotehnologie.2. Bioreactoare,* InterGlobal, Iasi (2002).
19. J.J.HEIJNEN, K. VAN'T RIET, D.J. WOLTHUIS, *Biotechnol. Bioeng*., **22**, 1945 (1980).