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Evaluation of the hydrodynamic regime of aerobic stirred bioreactors using the mixing distribution criteria 2. *Saccharomyces cerevisiae* broths

ANCA-IRINA GALACTION[1)], DAN CAŞCAVAL[2)], STEFĂNICA CĂMĂRUŢ[2)], MARIUS TURNEA[1)]

[1)] University of Medicine and Pharmacy, Faculty of Medical Bioengineering, Dept. of Biotechnology, 16 University Street, 700115 Iasi, Romania

[2)] Technical University "Gh. Asachi" of Iasi, Faculty of Chemical Engineering, Dept. of Biochemical Engineering, 71 D. Mangeron Avenue, 700050 Iasi, Romania,

** the corresponding author*

Abstract

When compared to 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 studies on mixing distribution for a stirred bioreactor and aerated S. cerevisiae broths indicated that the mixing time varies significantly on the broth height in direct relation with the biomass concentration, rotation speed and aeration rate.

The analysis of the influence of rotation speed indicated the existence of a critical rotation speed corresponding to the minimum of mixing time. The value of critical rotation speed increases with the biomass accumulation, from 300 rpm for $C_X \leq 75$ g/l d.w. to 400 rpm for $C_X \leq 150$ g/l d.w. Moreover, it is possible to reach a uniform mixing in whole bulk of the broth for a rotation speed between 300 and 500 rpm.

The influence of aeration is directly related to the change of relative contribution of pneumatic mixing to broth circulation. Except for the bottom region, for biomass concentration below 100 g/l d.w., the mixing time initially decreases to a minimum value with the increase of the flow rate, increasing then. The value of critical aeration rate increases from 150 to 300 l/h with biomass accumulation. For more concentrated suspensions (over 100 g/l d.w.) the influence of aeration rate is contrary, due to the appearing of flooding for the air flow rate of 150 l/h.

Keywords: mixing time, mixing distribution, stirred bioreactor, aerated broths, *Saccharomyces cerevisiae*.

Introduction

Due to the biomass accumulation, shear stress sensitivity of the biocatalysts (free or immobilized cells and/or enzymes), high viscosity or non-Newtonian rheological 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. 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. 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.

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 [1,2]. This parameter offers specific informations concerning the bulk mixing in the system (macromixing), but it cannot rigorously quantify the mixing at lower level (meso- and micromixing) [3]. 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.

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 [1]).

Although there is much information concerning the influence of feed position on mixing efficiency, respectively on mixing time [3-6], 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 must to determine the mixing time for different positions of electrode.

In the context of the above mentions, the aeration influence on mixing efficiency and distribution in bioreactors is complex and 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 [13,14].

Therefore, 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 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 mixing intensity distribution into aerated *S. cerevisiae* broths 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 [15].

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 64 mm (d) from the inferior one, this being the optimum distance for the Ruston turbine, as it was demonstrated in the previous works on *S. cerevisiae* broths [15]. The rotation speed was maintained below 600 rpm. The experiments have been carried out at Reynolds number lower than 8,200, 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, corresponding to an air superficial velocity of 0.84 - $5.02 \text{x} 10^{-3}$ m/s.

The *S. cerevisiae* suspensions having biomass concentration between 40 and 150 g/l d.w. have been used. 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 or morphological conformation 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 [1,16].

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 have been considered:

$$I = \frac{pH_{\infty} - 0.5\Delta pH}{pH_{\infty}} \text{x} 100 = 99\% \qquad \text{where } \Delta pH = 0.02.$$

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 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 placed at the four different positions mentioned in the previous papers [17]. The pH variations were recorded by the bioreactor computer-recorded system and were analyzed for mixing time calculation.

Results and discussion

The previous studies on mixing distribution for a stirred bioreactor and non-aerated *S. cerevisiae* broths indicated that the mixing time values are non-uniformly distributed into the broths. Thus, at lower rotation speed (below 300 rpm), the main stagnant zone is placed at the bioreactor bottom, owing to the deposition of yeasts cells. By increasing the impeller speed, the biomass is dispersed more uniformly into the broths bulk, that inducing the gradual diminution of mixing efficiency at the superior region [11].

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

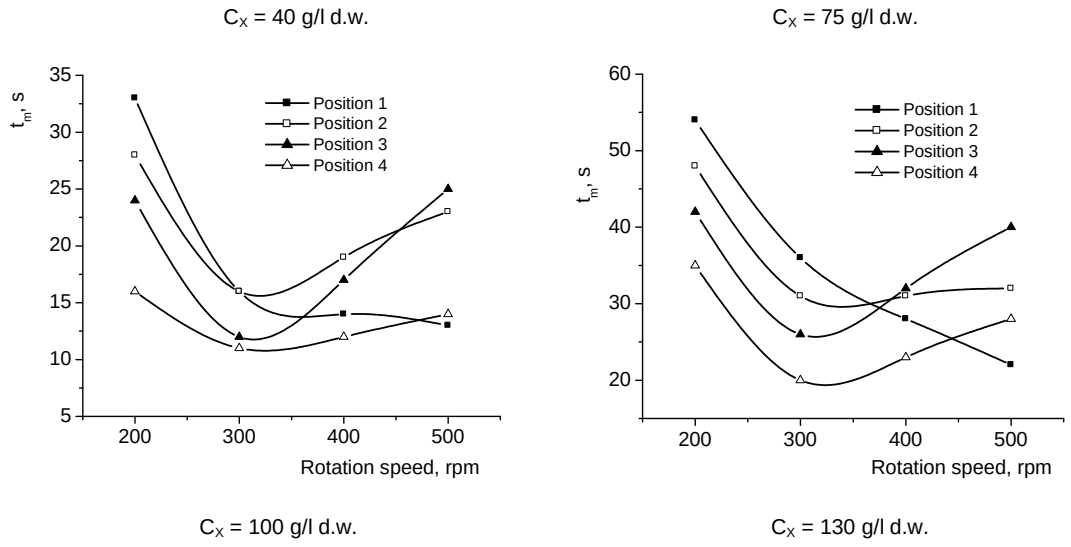
due to the cumulated pneumatic and mechanical agitation. The deviations from the obtained values for non-aerated broths depend on the constructive and operating characteristics of the bioreactor. Moreover, the influence of number and position of the stirrers on the shaft is unknown, and the influence of the gas flow rate is different for different rotation speed or Reynolds number [3,11,14].

In the case of yeasts suspensions, the influence of aeration on mixing intensity depends on the biomass concentration, in relation with the rotation speed. Thus, for position 1 of the pH electrode, the previous experiments carried out for biomass concentration below 75 g/l d.w. and rotation speed up to 400 rpm indicated that the mixing time continuously decreased with the increase of air volumetric flow rate for rotation. This variation of mixing efficiency with aeration rate was significantly modified for rotation speed values over 400 rpm. For this domain of agitation intensity, the mixing time was initially reduced with aeration increase, reached a minimum value, increasing then. The value *critical aeration rate* (150 - 200 l/h), which corresponds to the minimum mixing time, represents the limit of the aeration contribution to the mixing of broths [18].

This variation of mixing efficiency with aeration rate for rotation speed values over 400 rpm was considerably changed for more concentrated yeasts suspensions (over 75 g/l d.w.). The increase of aeration induced initially the increase of mixing time to a maximum value, followed by its decrease. At higher air flow rate values, the energy dissipated by the air exceeds that of the stirrer, appearing the flooding. At the flooding point (150 - 200 l/h), the rise velocity of the air strongly increased, inducing the simultaneous increase in the intensity of media circulation and the decrease of mixing time [18].

But, by placing the pH electrode in different regions inside the bioreactor it could be drawn more rigorous conclusions concerning the distribution of mixing intensity, as well on the effects of broths characteristics and/or fermentation conditions on mixing efficiency in a certain region inside the bioreactor. This approach leads to the recording of the mixing intensity variations in whole bulk of the broth, which could differ significantly from the above presented for aerated *S. cerevisiae* suspensions.

From Figure 1 it can be observed that the shape of the obtained curves differs from one position to another and it is modified by the biomass accumulation.



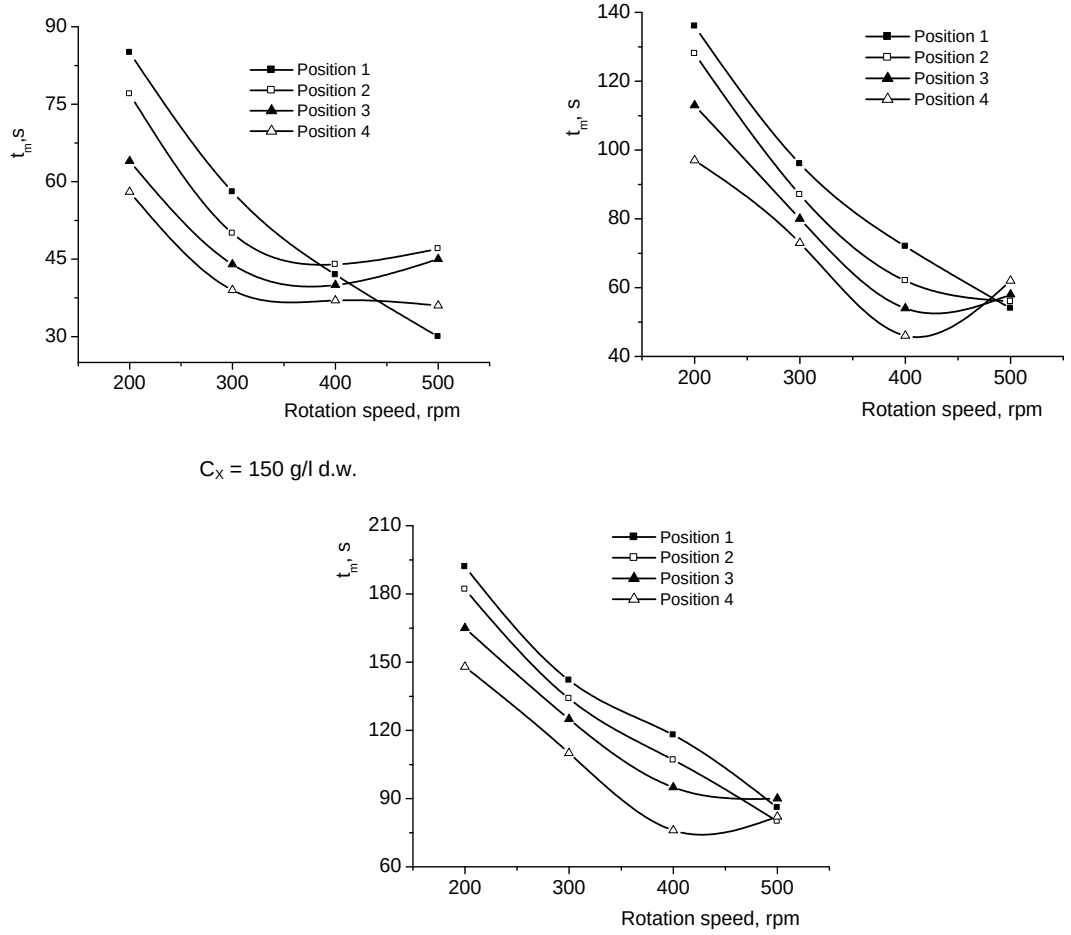


Figure 1. Influence of rotation speed on mixing time (aeration rate of 75 l/h).

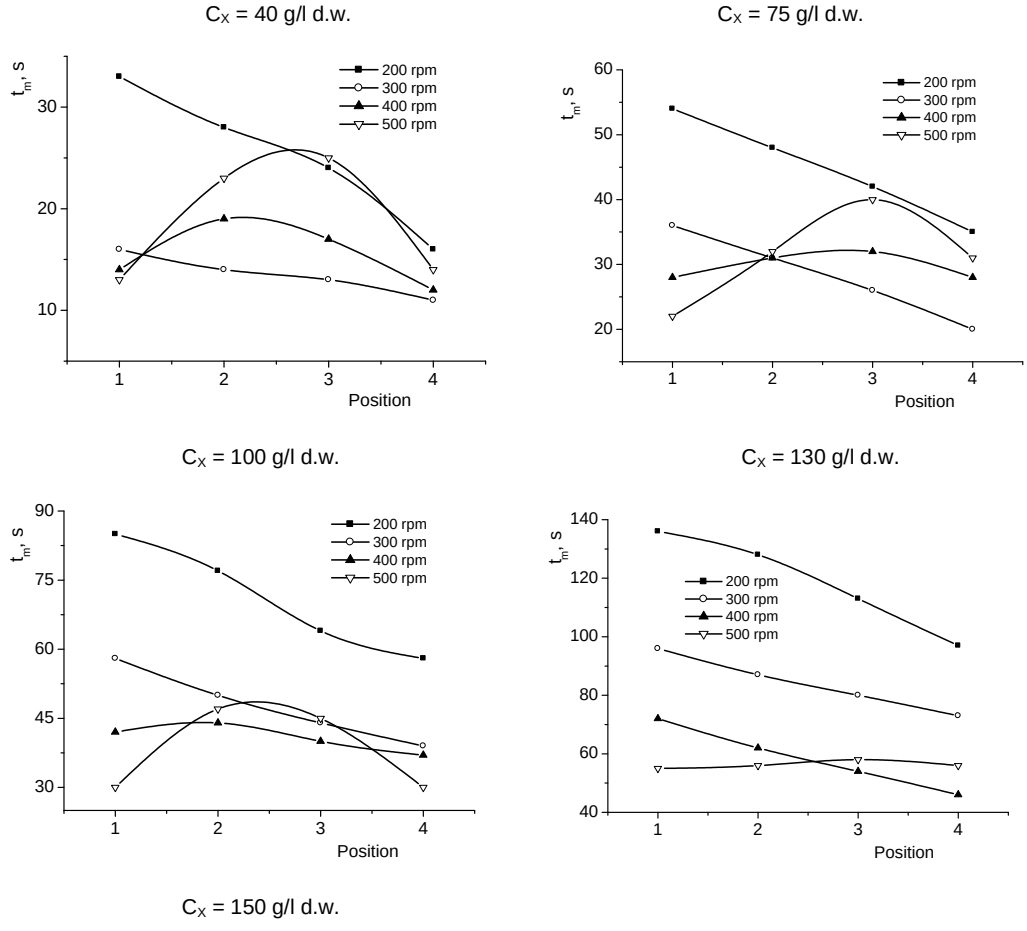
Therefore, indifferent of the yeasts concentration, the increase of rotation speed leads to the intensification of mixing at the bioreactor bottom (position 1). For the other positions, the variation of mixing time with the rotation speed, at constant aeration rate, is different. Thus, this parameter 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). This variation is the result of the change in relative importance of mechanical and pneumatic mixing. At lower rotation speed, the contribution of pneumatic mixing is more important, especially in the regions with lower solid phase concentration (the solid phase exhibits the deposition tendency at the bioreactor bottom, in region 1). In this case, the increase of rotation speed intensifies the mixing. At higher value of rotation speed, the gas hold-up increases, the flow of dispersion becomes more complex and its circulation velocity is inferior to that induced by the mechanical agitation in non-aerated systems. But, owing to the lower viscosity of yeasts suspensions compared with the simulated broths, these effects are less significant. The *critical rotation speed*, corresponding to the minimum of mixing time [13,14], is moved to higher values with the biomass accumulation, being of 300 rpm for $C_X \leq 75$ g/l d.w., and becoming of 400 rpm for $C_X \geq 75$ g/l d.w.

The biomass accumulation induces the reduction of the mixing intensity in whole bulk of the fermentation broth. Furthermore, the biomass accumulation leads to the gradual increase of its concentration also in the regions 2 and 3, consequently 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 by increasing the yeasts concentration the contribution of mechanical mixing to broth circulation becomes more important.

In the simulated broths, by increasing the apparent viscosity the bubbles are accumulated and coalescence around the impellers, thus leading to the increase of the air hold-up [17]. These phenomena has not been observed into the *S. cerevisiae* cultures, on the one hand due to the low 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 be adsorbed to the bubbles surface, avoiding their coalescence.

According to the above mentions, the analysis of the mixing distribution for the four considered positions into the bioreactor indicated that the lowest mixing time values have been recorded for the superior region, owing to its low content in biomass (Figure 2). This result confirms and underlines the decisive influence of solid phase presence on broth circulation. Moreover, for the positions 2 and 3 and rotation speed over 400 rpm, the mixing intensity reaches a minimum level, as the result of the interference of the flow streams generated by the two impellers and, consequently, of the reducing of broth circulation velocity. This effect is less pronounced at higher yeasts concentration.



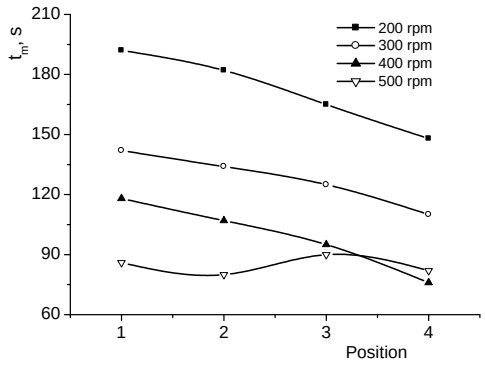
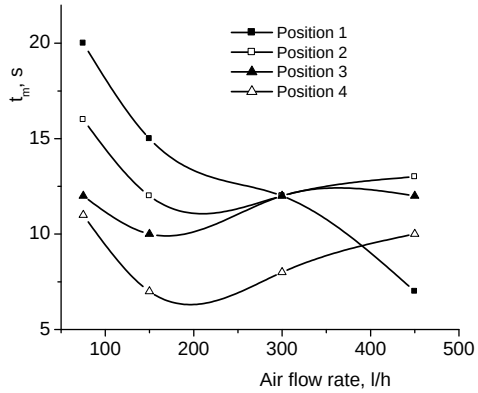


Figure 2. Variation of mixing time with pH electrode position (aeration rate of 75 l/h).

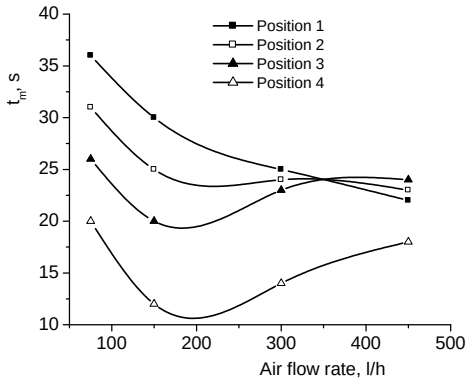
Figure 2 suggests the existence of an optimum rotation speed which corresponds to the uniform mixing into the whole bulk of the yeasts broth. The value of the optimum rotation speed increases from 300 rpm for *S. cerevisiae* concentration up to 75 g/l d.w. to 500 rpm for suspensions more concentrated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

At constant rotation speed, the influence of aeration rate mainly depends on the biomass concentration and on its dispersion in different regions inside the bioreactor. From Figure 3 it can be observed that the shape of the curves describing the correlation between the mixing time and the air flow rate is significantly changed by increasing the biomass concentration.

C_X = 40 g/l d.w.



C_X = 75 g/l d.w.



C_X = 100 g/l d.w.

C_X = 130 g/l d.w.

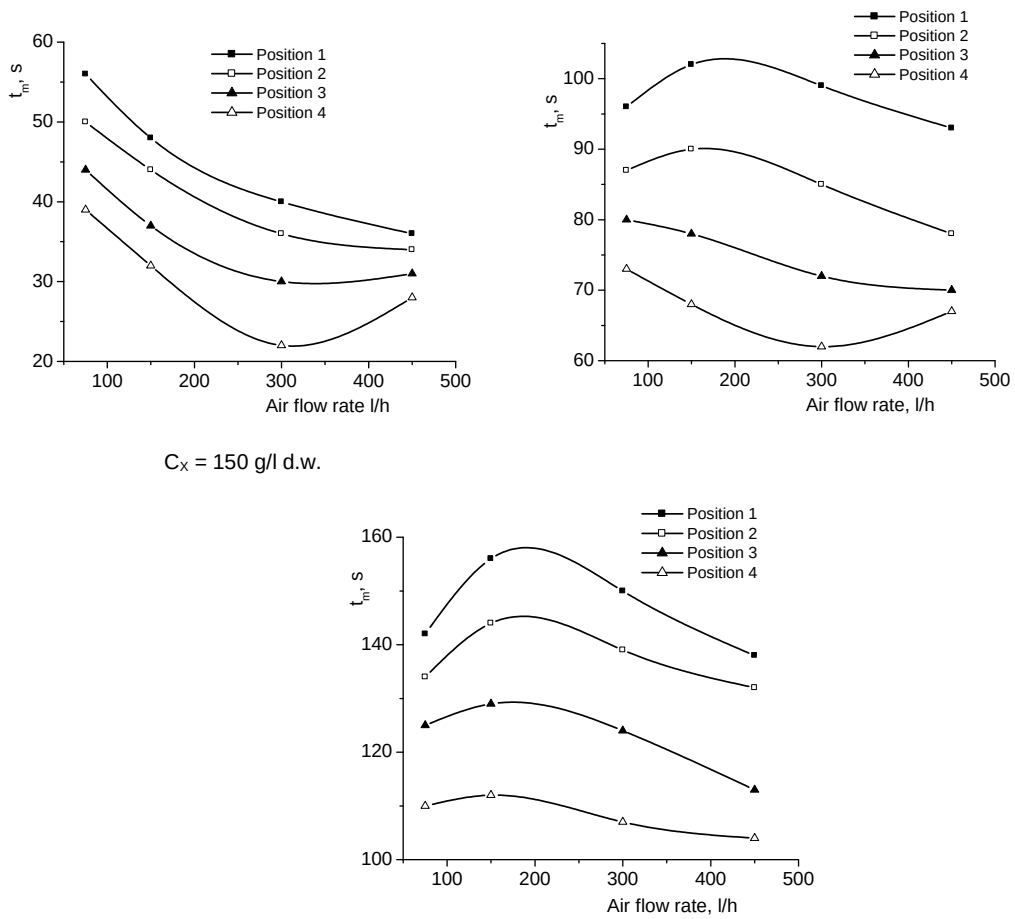


Figure 3. Influence of aeration rate on mixing time (rotation speed 300 rpm).

Therefore, for biomass concentration below 100 g/l d.w., the intensification of aeration at the inferior region leads to decrease of mixing time, phenomena that is the result of the increase of the contribution of pneumatic mixing to the cells dispersion, the sparging system being placed at the bioreactor bottom.

For the other positions, the mixing time initially decreases to a minimum value with the increase of the flow rate, follows 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 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 phenomena, the recorded variations becoming similar for C_X = 100 g/l d.w. The value of air flow rate that corresponds to the minimum mixing time, called *critical aeration rate* [17], increases from 150 l/h for 40 g/l d.w. *S. cerevisiae* to 300 l/h for 100 g/l d.w.

This variation of mixing efficiency with aeration rate for constant rotation speed is considerably changed for more concentrated yeasts suspensions (over 100 g/l d.w.). As it can be seen from Figure 3, the increase of aeration determines initially the increase of mixing time to a maximum value, followed by its decrease. 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, that avoiding the bubbles coalescence. As it was earlier mentioned,

the small bubbles formed by air dispersion and mechanical agitation exhibit a negative effect on broths circulation, reducing the broth circulation velocity, and, therefore, the mixing intensity.

At higher air flow rate values, the energy dissipated by the air exceeds that of the stirrer, appearing the flooding [18]. At the flooding point, the rise velocity of the air strongly increases, inducing the simultaneous increase of the velocity of media circulation and the decrease of mixing time. The value of air volumetric flow corresponding to the flooding point was of 150 l/h.

Conclusions

The studies on mixing distribution for a stirred bioreactor and aerated *S. cerevisiae* broths indicated that the dependence between the mixing time and the considered factors (biomass concentration, rotation speed, aeration rate) differs on the broth height.

The modification of the rotation speed, at a constant level of air flow rate, underlined the existence of a critical rotation speed corresponding to the minimum of mixing time. The existence of the minimum level of mixing time is more evident for regions 2, 3 and 4 and lower biomass concentration. The value of critical rotation speed increases with the biomass accumulation, from 300 rpm for $C_X \leq 75$ g/l d.w. to 400 rpm for more concentrated yeasts suspensions.

The experiments suggested the possibility to reach an uniform mixing in whole bulk of the broth for a certain value of rotation speed. The optimum rotation speed depends on yeasts concentration and varies between 300 to 500 rpm for *S. cerevisiae* accumulation from 40 to 150 g/l d.w.

The influence of aeration has to be correlated with the biomass concentration and region inside the bioreactor. Thus, by increasing the *S. cerevisiae* concentration, the shape of the curves which describe 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. For biomass concentration below 100 g/l d.w., the intensification of aeration at the inferior region leads to decrease of mixing time, phenomena that is the result of the increase of the contribution of pneumatic mixing to the cells dispersion, the sparging system being placed at the bioreactor bottom. For the other positions, the mixing time initially decreases to a minimum value with the increase of the flow rate, follows by its increasing. The value of critical aeration rate increases from 150 l/h for the yeasts concentration of 40 g/l d.w. to 300 l/h for 100 g/l d.w.

For more concentrated biomass suspensions (over 100 g/l d.w.) the influence of aeration rate is contrary to the above presented, due to the appearing of flooding, for the air flow rate of 150 l/h.

Notations

C_X - biomass concentration, g/l d.w.
d - stirrers diameter, mm
pH_∞ - pH-value corresponding to perfect mixing
ΔpH - pH-limits accepted for mixing time determination
t_m - mixing time, s

References

1. K. VAN'T RIET, J. TRAMPER, *Basic Bioreactor Design*, M. Dekker Inc., New York, 1991, pp. 183.
2. D. CAŞCAVAL, C. ONISCU, A.I. GALACTION, *Biochemical Engineering and Biotechnology. 2.Bioreactors*, InterGlobal, Iasi, 2002, pp. 53.
3. J.M. BUJALSKI, *Ph.D. Thesis*, University of Birmingham, 2003, pp. 22.
4. R. GEISLER, A. MERSMANN, H. VOIT, *Int. Chem. Eng.*, **31**, 642-648 (1991).
5. J.M. BUJALSKI, Z. JAWORSKI, W. BUJALSKI, A.W. NIENOW, *Proceed. 7th UK Conference on Mixing*, Bradford, 2002, pp.124.
6. M. ASSIRELLI, W. BUJALSKI, A.W. NIENOW, A. EAGLESHAM, *Proceed. 7th UK Conference on Mixing*, Bradford, 2002, pp. 218.
7. J. THYN, V. NOVAK, P. POCK, *Chem. Eng. J.*, **12**, 211-218 (1976).
8. C.D. RIELLY, A.B. PANDIT, Proceed. 6th European Conference on Mixing, Pavia, 1988, pp. 69.
9. S. RUSZOWSKI, *Proceed. 8th European Conference on Mixing*, Cambridge, 1994, pp. 283.
10. O. STENBERG, *Ph.D. Thesis*, University of Göteborg, 1984, pp. 116.
11. A.I. GALACTION, E. FOLESCU, D. CAŞCAVAL, *Roum. Biotechnol. Lett.*, **10**, 2291-2299 (2005).
12. A.I. GALACTION, E. FOLESCU, D. CAŞCAVAL, *Rev. Chimie*, **56**, 985-993 (2005).
13. C. ONISCU, A.I. GALACTION, D. CAŞCAVAL, *Roum. Biotechnol. Lett.*, **7**, 817-828 (2002).
14. D. CAŞCAVAL, C. ONISCU, A.I. GALACTION, F. UNGUREANU, *Chem. Ind.*, **56**, 506-513 (2002).
15. D. CAŞCAVAL, C. ONISCU, A.I. GALACTION, F. UNGUREANU, *Chem. Ind.*, **55**, 367-375 (2001).
16. W. BUJALSKI, Z. JAWORSKI, A.W. NIENOW, *Trans. Int. Chem. Eng.*, **80**, 97-105 (2002).
17. D. CAŞCAVAL, A.I. GALACTION, S. CAMARUT, M. TURNEA, *Roum. Biotechnol. Lett.*, **11**, 2537-2548 (2006).
18. D. CAŞCAVAL, C. ONISCU, A.I. GALACTION, F. UNGUREANU, *Chem. Ind.*, **58**, 128-137 (2004).