DAN CASCAVAL[1]
ANCA-IRINA GALACTION[2]
CORNELIU ONISCU[1]
FLORINA UNGUREANU[1]

[1]**Technical University "Gh. Asachi" of Iasi, Faculty of Industrial Chemistry, Dept. of Biochemical Engineering, Iasi, Romania**

[2]**University of Medicine and Pharmacy, Faculty of Medical Bioengineering, Dept. of Biotechnology, Iasi, Romania**

SCIENTIFIC PAPER

66.023:577+66.021.3:579.8

MODELING OF MIXING IN STIRRED BIOREACTORS 4. MIXING TIME FOR AERATED BACTERIA, YEASTS AND FUNGUS BROTHS

The mixing time for bioreactors depends mainly on the rheological properties of the broths, the biomass concentration and morphology, mixing system characteristics and fermentation conditions. For quantifying the influence of these factors on the mixing efficiency for stirred bioreactors, aerated broths of bacteria (P. shermanii), yeasts (S. cerevisiae) and fungi (P. chrysogenum, free mycelia and mycelial aggregates) of different concentrations have been investigated using a laboratory bioreactor with a double turbine impeller. The experimental data indicated that the influence of the rotation speed, aeration rate and stirrer positions on the mixing intensity strongly differ from one system to another and must be correlated with the microorganism characteristics, namely: the biomass concentration and morphology. Moreover, compared with non–aerated broths, variations of the mixing time with the considered parameters are very different, due to the complex flow mechanism of gas–liquid dispersions.

By means of the experimental data and using a multiregression analysis method some mathematical correlations for the mixing time of the general form:

$$t_m = a_1 \cdot C_X^2 + a_2 \cdot C_X + a_3 \cdot \lg V_a + \frac{a_4 \cdot N^2 + a_5 \cdot N + a_6}{a_7 \cdot L^2 + a_8 \cdot L + a_9}$$

were established. The proposed equations offer good agreement with the experiments, the average deviation being ±6.7% – ±9.4 and are adequate for the flow regime Re < 25,000.

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

The mixing time represents one of the most useful criteria for characterizing the mixing intensity and for biosynthesis scale–up. The mixing time, t_m, is defined as the time needed to reach a given mixing intensity at a given scale, starting from a completely segregated situation. This parameter depends on a multitude of geometrical (dimensions of the mixing system, dimensions of the bioreactor) and technological factors (fermentation conditions, physical characteristics of the medium, power consumption, dissipated energy). A general correlation that describes the mixing time is [1]:

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

The experimental measurement of mixing time uses tracers (acidic, alkaline or salt solutions, heated solutions, colored solutions), which are added to previously homogenized broths. The mixing time is the time needed for the considered parameter M (pH, temperature, absorption) not to exceed the considered range $M_\infty \pm 0.5 \times \Delta M$ [2]. For example, an alkaline pulse is added to the liquid, this method being used in the experiments in this study. The system can be regarded as completely segregated at t = 0. After a certain time, which is the mixing time, the pH remains in the considered range of deviation from that of an ideally mixed system. In this way, the mixing time can be related to the mixing intensity.

Numerous equations have been proposed in the literature for calculation of the the mixing time. These equations taking into account the type of fermentation (aerobe or non–aerobe), the rheological characteristics of the broths and the fermentation conditions [2–14]. Because of the complexity of the 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 times for Re > 10,000, this flow regime being rarely reached in large–scale bioreactors due to the microorganism sensitivity to shear stress. For Re < 10,000, these models need some corrections [7].

For these reasons, the aim of our experiments was to analyze the influence of some specific design and operational parameters of stirred bioreactors (geometrical characteristics, apparent viscosity or biomass concentration, rotation speed, aeration rate) on the mixing time. For emphasizing and quantifying the effects of biomass presence in the medium, studies were previously carried out for non–aerated and aerated simulated broths and models were developed for bacteria (*Propionibacterium shermanii*), yeast (*Saccharomyces cerevisiae*) and fungus (*Penicillium chrysogenum*, free mycelia and mycelial aggregates or pellets) suspensions [15–20]. By means of the obtained data for various values of the apparent viscosity or

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Author address: D. Cascaval, Technical University "Gh. Asachi" of Iasi, Faculty of Industrial Chemistry, Dept. of Biochemical Engineering, D. Mangeron Avenue 71, 6600 Iasi, Romania
E–mail: dancasca@from.ro, dancasca@ch.tuiasi.ro
Paper received: June 24, 2003
Paper accepted: March 22, 2004

biomass concentration and using the nlinfit function from the Statistics Toolbox of MATLAB correlations were established between mixing time and rotation speed, air volumetric flow and distance between the stirrers on the shaft for the considered systems. The proposed equations allow analysis of the mixing efficiency of stirred bioreactors under different fermentation conditions.

MATERIALS AND METHODS

The experiments were carried out in a 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.

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 200 and 600 rpm. The experiments were carried out for Reynolds number intervals below 25,000, which correspond to the laminar, transitory and low turbulent flow regime, and avoid "cave" formation at the broth surface (for rotation speeds above 700 rpm and L = 2d).

The sparging system consisted of a single ring sparger of 64 mm diameter, placed 15 mm from the vessel bottom, having 14 holes of 1 mm diameter. The air volumetric flow rate was varied from 75 to 450 l h^{-1}.

The following biomass suspensions were used:

– bacteria (*Propionibacterium shermanii*), the biomass concentration, C_X, varying between 30.5 and 120.5 g l^{-1} dry weight.

– yeast (*Saccharomyces cerevisiae*), C_X 43–150 g l^{-1} d.w.

– fungus (*Penicillium chrysogenum*), with two morphological conformations: free mycelia and mycelial aggregates (pellets, of the average diameter 1.6–1.8 mm); in both cases, the biomass concentration varied between 4 and 36.5 g l^{-1} d.w.

Due to the difficulty of the *in–situ* measurement of viscosity during the experiments, the viscosity was measured before and after each experiment using a modified Ostwald type viscometer [2, 21]. Both the experiments and viscosity measurements were carried out at 21°C. Any viscosity or morphological conformation change was recorded during the experiments.

The mixing time was determined by the following homogeneity criteria for mixing [1,2]:

$$I = \frac{pH_\infty - 0.5\Delta pH}{pH_\infty} \times 100 = 99\% \qquad (2)$$

where $\Delta pH = 0.02$.

The values of the mixing time were determined by means of a 2N KOH solution as the tracer, by recording the time needed for the medium pH–value to reach the value corresponding to the considered mixing intensity. The tracer volume was 0.5 ml. The tracer was injected opposite to the pH electrode, 10 mm from the liquid surface. The pH electrode was placed 20 mm from the vessel bottom. Because the tracer solution density was similar to the liquid phase density, the tracer solution flow followed the liquid flow streams and there were no errors due to tracer buoyancy. The pH variations were recorded by a bioreactor computer – recorded system. Each experiment was carried out three or four times, under identical conditions, always for the average mixing time. The maximum experimental error was ±3.9%.

RESULTS AND DISCUSSION

The flow mechanism of an aerobic bioreactor equipped with one or more stirrers is complicated due to the combined action of mechanical and pneumatic mixing. Compared with non–aerated systems, different flow patterns can exist when gas is sparged into the broth. The flow mechanism becomes more complicated in the presence of solid particles (free or immobilized cells, immobilized enzymes, substrate particles), which tend to settle at the vessel bottom and/or adsorb to the bubble surface. Furthermore, biomass accumulation causes an increase of the broth viscosity thus affecting the mixing intensity, but the magnitude of this effect depends on the type, concentration and morphology of the used microorganism.

Generally, the mixing efficiency of aerated mechanically stirred systems is analyzed and compared to that of non–aerated systems, due to the less complicated flow phenomena of the latter ones. However, prediction of the mixing time for aerated broths by means of the equations established for non–aerated systems yields values about 1.2–2 times lower compared with the experimental data [5]. Thus, the aeration influence on mixing efficiency must be distinctly analyzed.

For numerous fermentations, due to the decrease in the pumping capacity of the stirrer, cavity formation, and compartmentalization in regions around each of the stirrers, aeration increases the mixing time compared with non–aerated systems. However, as confirmed in the

Table 1. Characteristics of the 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

literature, deviations from the obtained values for non–aerated broths depend on the design and functional characteristics of the bioreactor. Thus, the influence of the number and position of the stirrers on the shaft is unknown, and the influence of the gas flow rate is different for different rotation speeds or Reynolds number values [6].

The differences between the real flow of mechanically mixed aerated broths and the theoretical flow models for aerobic conditions are amplified by the presence and concentration of the biomass. For this reason, the aim of these studies was to analyze and quantify the influences of biomass type, concentration and morphology on the mixing time, in correlation with the design and operational characteristics of the aerobic stirred bioreactor. The experiments were carried out for bacteria, yeast and fungus cultures, under different fermentation conditions.

Due to the small size of bacterial cells, even for large biomass amounts, the viscosity of bacteria suspensions remained low. Thus, for the *P. shermanii* biomass concentration domain of 30.5–120.5 g l^{-1} d.w., the viscosity increased only 3.1 times compared to water [15]. For this reason, excluding the fermentation conditions, the mixing intensity is controlled by the amount of biomass in the broths, the viscosity exhibiting a negligible influence on these systems.

This conclusion is also valid for yeast (*S. cerevisiae*) suspensions, the viscosity reaching a level of 0.046 $g \cdot cm^{-1} \cdot s^{-1}$ for a biomass concentration of 150 g l^{-1} d.w.

On the other hand, the performance of a bioreactor containing a fungus fermentation broth is greatly affected by the rheological properties of the broth. These properties are controlled mainly by the biomass concentration, its growth rate and morphology. Some of the morphological characteristics, such as the geometry (length, diameter, branching frequency) and the flexibility of hyphae and the hyphal–hyphal interactions can influence the mixing efficiency. Generally, the fungus suspensions exhibit pseudoplastic or Bingham plastic behavior [16,17].

Unlike bacteria and yeasts, fungi can grow on two morphological conformations: free mycelia and mycelial aggregates (pellets). Regardles of the morphological structure, the accumulation of fungus biomass causes a significant increase of the broth viscosity and controls the rheological behavior, but the magnitude of this influence depends on the fungus morphology. Thus, for the *P. chrysogenum* strains used in these experiments, the apparent viscosity of the suspension was of 1.725 $g \cdot cm^{-1} \cdot s^{-1}$ for free mycelia and 0.884 $g \cdot cm^{-1} \cdot s^{-1}$ for pellets, at a biomass concentration of 33.5 g l^{-1} d.w. Consequently, the values of the mixing time for non–aerated fungus suspensions were significantly higher compared to those obtained for bacteria or yeast suspensions (for a biomass concentration of 30 g l^{-1} d.w., 400 rpm and a distance between the stirrers of d, the mixing time was 460 s for *P. chrysogenum* free mycelia and 175 s for mycelial aggregates, compared with 14 s for *P. shermanii* and 16 s for S. cerevisiae [17]).

Influence of the impeller rotation speed

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

As stated in previous papers for aerated water and simulated broths without any solid phase, the mixing time initially decreases with rotation speed increase, reaches a minimum value, subsequently increasing [20]. The value of the rotation speed corresponding to the minimum of the mixing time (*critical rotation speed*) depends on the apparent viscosity and aeration rate. This evolution is the result of a modification of the mixing mechanism with the increase of rotation speed in the presence of bubbles.

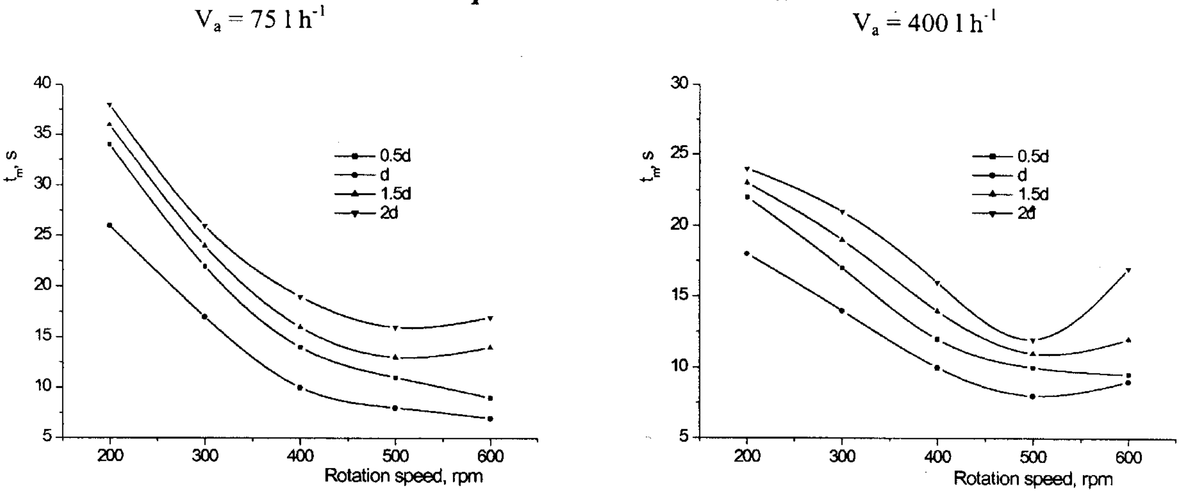
For real broths, the influence of fermentation conditions on the mixing efficiency must be distinctly analyzed either for each type of cultivated strain, and for different morphological structures of the same microorganism.

The mixing time of *P. shermanii* broths initially decreases with increasing rotation speed, reaches a minimum level, then slowly increases. This variation is less pronounced compared to that obtained for aerated simulated broths without any solid phase and is the result of the change in the relative importance of mechanical and pneumatic mixing. Thus, at lower rotation speed, the contribution of pneumatic mixing is more important, all the more so as the sparger position diminishes the deposition tendency of the biomass. In this case, the increase of rotation speed intensifies mixing.

At higher values of the rotation speed, gas hold–up increases, the flow of the dispersion becomes more complex and its circulation velocity is inferior to that induced by mechanical agitation in non–aerated systems. But, due to the lower viscosity of bacteria suspensions compared with the simulated broths, these effects were less significant. The value of the critical rotation speed was 500 rpm, the existence of a minimum value of the mixing time becoming more evident at higher bacterial cell concentrations and aeration rates (Figure 1).

For the same rotation speed value, the mixing time increases with increasing biomass concentration, this effect being more important at lower aeration rates. For example, for the considered domain of *P. shermanii* cell concentrations, a distance between stirrers d and 500 rpm, the mixing time increases about 6 times at 75 l h^{-1} air volumetric flow rate and about 3.8 times at 400 l h^{-1}.

Propionibacterium shermanii



Saccharomyces cerevisiae

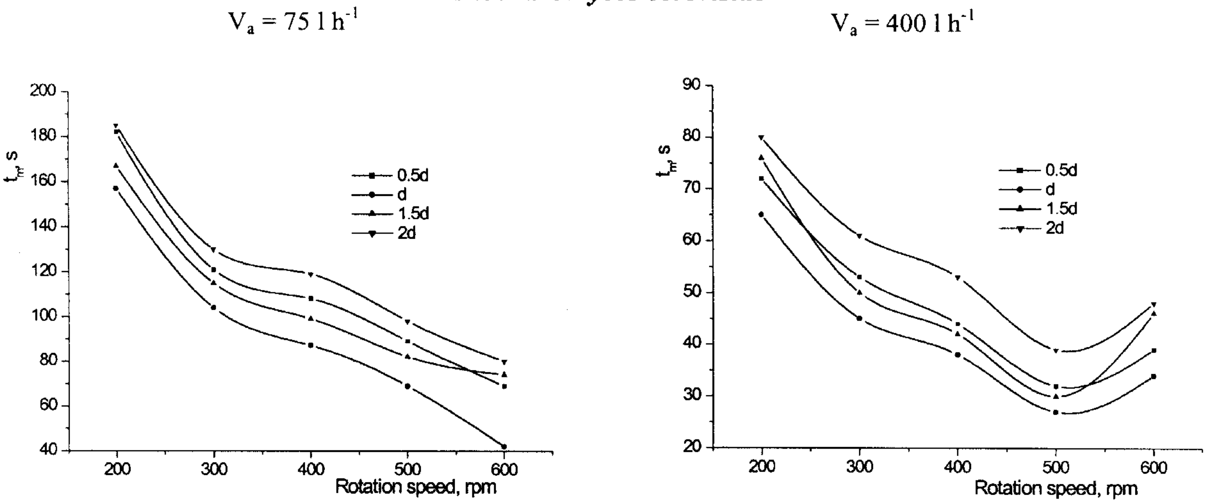


Figure 1. Influence of the impeller rotation speed on the mixing time for P. shermanii (C$_X$ = 45 g l^{-1} d.w.) and S. cerevisiae broths (C$_X$ = 150 g l^{-1} d.w.).

Although the rotation speed influence on the mixing efficiency for *S. cerevisiae* broths is similar to that observed for bacteria broths, the values of the mixing time were larger due both to the higher viscosity of yeast suspensions and the larger size of yeasts cells, leading an increase of the biomass deposition rate (Figure 1).

Therefore, the difference between the mixing times corresponding to the lowest and highest level of *S. cerevisiae* cell concentrations is more pronounced (at 500 rpm and a distance d between the stirrers on the shaft, the mixing time increases about 9.8 times for an aeration rate of 75 l h^{-1}, and about 4.5 times for 400 l h^{-1}).

Due to the considerably higher viscosity of *P. chrysogenum* broths, the mixing times were significantly higher compared to those obtained for the *P. shermanii* and *S. cerevisiae* broths. Furthermore, besides the common parameters influencing the mixing (rotation speed, air flow rate, impeller geometry, biomass concentration), fungus morphology exhibits a significant effect.

As may be seen from Figure 2, although the overall variations of the mixing intensity with rotation speed are similar to those obtained for bacteria and yeasts, the influence of the rotation speed is stronger and the existence of a minimum value is more evident for fungus suspensions, especially for the pellet morphology. This phenomenon is due both to the lower apparent viscosity of *P. chrysogenum* pellet suspensions compared to free mycelia ones, and to a more pronounced tendency of pellet deposition.

For the considered biomass concentration, 500 rpm and d, the distance between stirrers, the values of the mixing time increased as follows:

- *P. chrysogenum* pellets:
  18.7 times at 75 l h^{-1} aeration rate
  13.5 times at 400 l h^{-1}
- *P. chrysogenum* free mycelia:

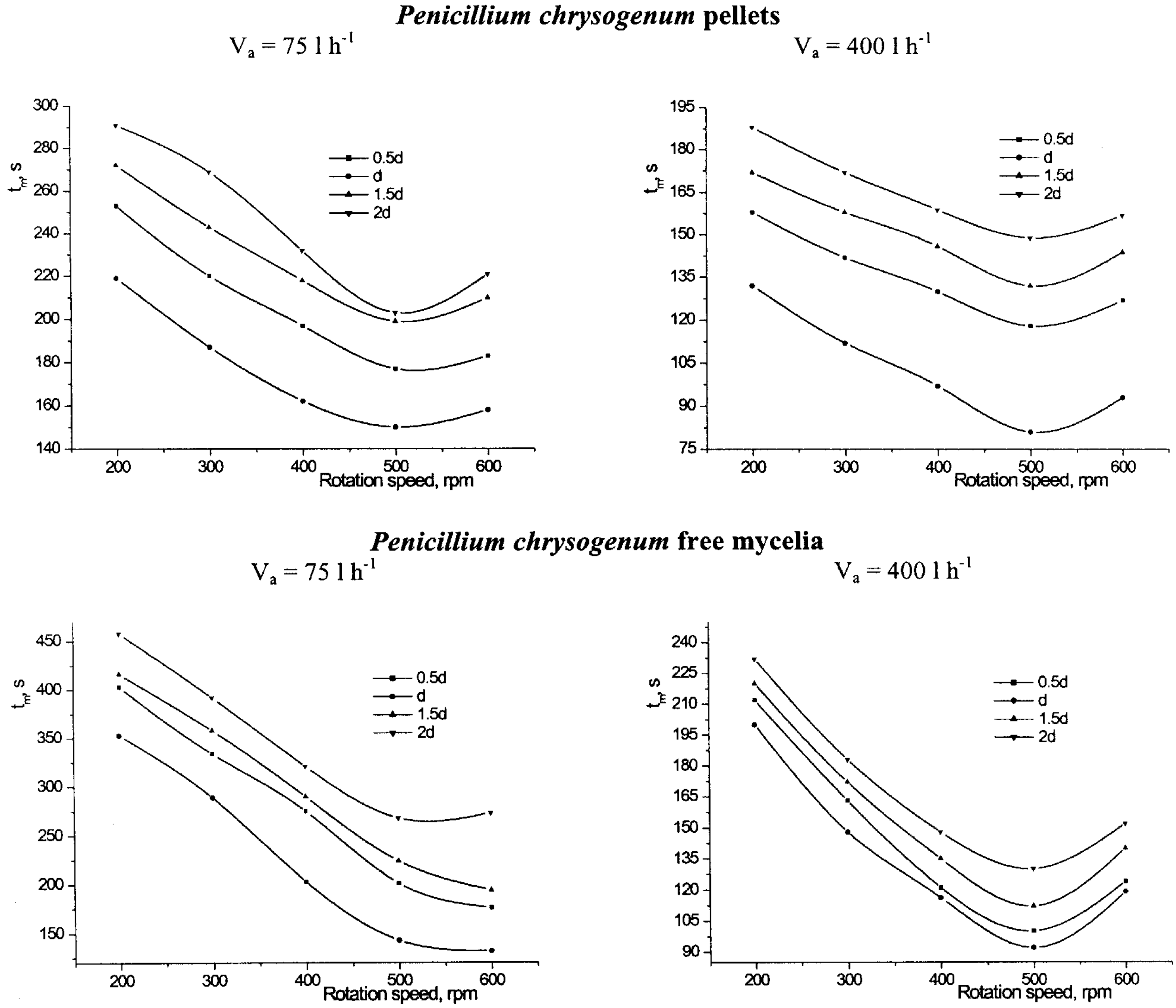


Figure 2. Influence of the impeller rotation speed on the mixing time for P. chrysogenum pellets (C_X = 36. 5 g l^{-1} d.w.) and free mycelia broths (C_X = 36. 5 g l^{-1} d.w.).

15.9 times at 75 l h^{-1}

7.7 times at 400 l h^{-1}

These results indicate the stronger unfavorable effect of biomass deposition on the circulation velocity of the air – broth dispersion compared with the effect of the apparent viscosity. However, due to the hyphal – hyphal interactions and, consequently, to the higher apparent viscosities, the mixing efficiency for free mycelia broths is about 2–3 times greater than for pellets broths.

Influence of the aeration rate

Generally, as the experimental results indicated for a stirred bioreactor, aeration reduces the mixing time compared with the non-aerated system, this phenomenon being the effect of the pneumatic mixing contribution to the broth circulation.

The aeration influence on the mixing intensity strongly depends both on the apparent viscosity of the liquid phase, and on the concentration and characteristics of the solid phase in the broths. From Figure 3, depicted for *P. shermanii* and *S. cerevisiae* suspensions, it may be observed that the mixing time continuously decreases with increasing air volumetric flow rate for rotation speeds below 400 rpm, regardless of the cell concentration and stirrer positions on the shaft. In this range of rotation speeds, the contribution of mechanical agitation is less important, the pneumatic mixing considerably intensifying the broth circulation.

This variation of the mixing efficiency with aeration rate is significantly modified for rotation speeds above 400 rpm. Thus, at low concentrations of biomass for both strains, the mixing time is initially reduced with aeration increase, reaching a minimum value, and then increasing. The value of the air flow rate that corresponds to the minimum mixing time is called the *critical aeration rate* and represents the limit of the

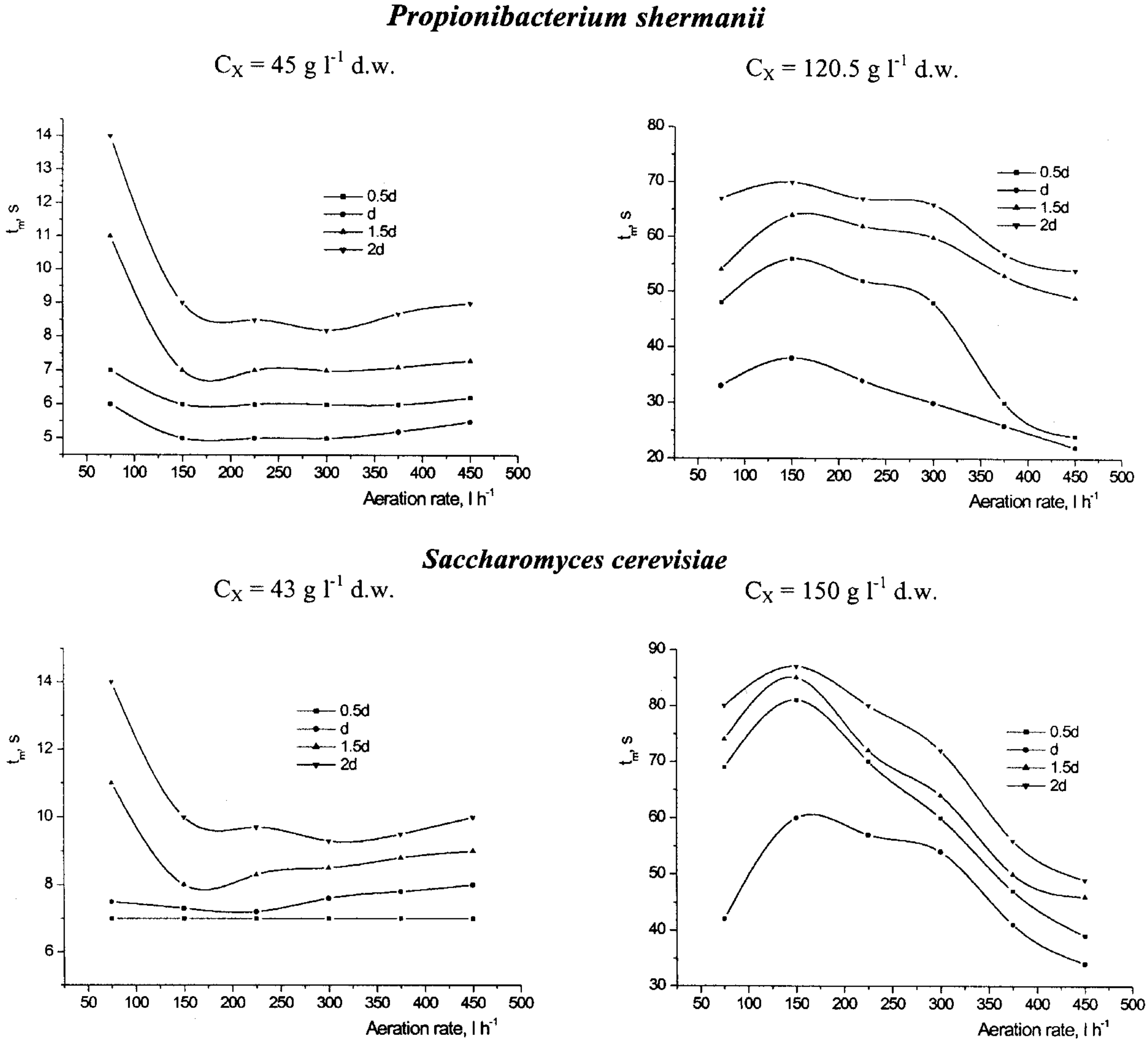


Figure 3. Influence of the aeration rate on the mixing time for P. shermanii and S. cerevisiae broths at 600 rpm.

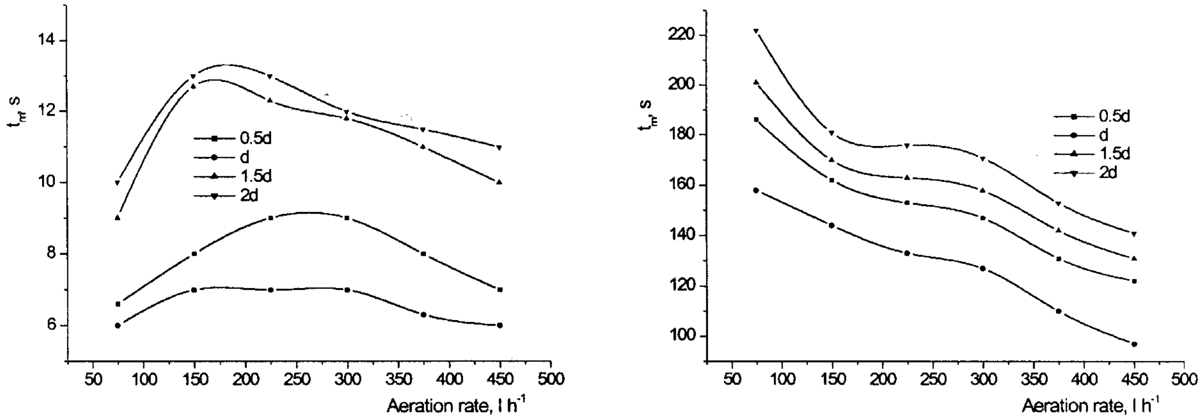
aeration contribution to the mixing of broths. For bacteria and yeast suspensions the value of the critical aeration rate was of 150–200 l h^{-1}.

For constant rotation speeds, the supplementary increase of the air volumetric flow rate causes the formation of smaller bubbles with a lower rise velocity, thus leading to an increase in the gas hold-up and to a decrease of the dispersion circulation velocity. The phenomenon was more evidently observed for the experimental conditions under which mechanical agitation exhibits a significant role in the dispersion and retention of bubbles in the medium.

The variation of the mixing efficiency with aeration rate for rotation speeds above 400 rpm is considerably different for concentrated bacterial and yeast cell suspensions. As may be seen from Figure 3, the increase of aeration initially induces an increase of the mixing time to a maximum value, followed by a decrease of the mixing time. This variation is due to the adsorption of cells to the bubble surface, thus avoiding bubble coalescence. As mentioned earlier, the small bubbles formed by air dispersion and mechanical agitation exhibit a negative effect on broth circulation, and reduce the mixing efficiency.

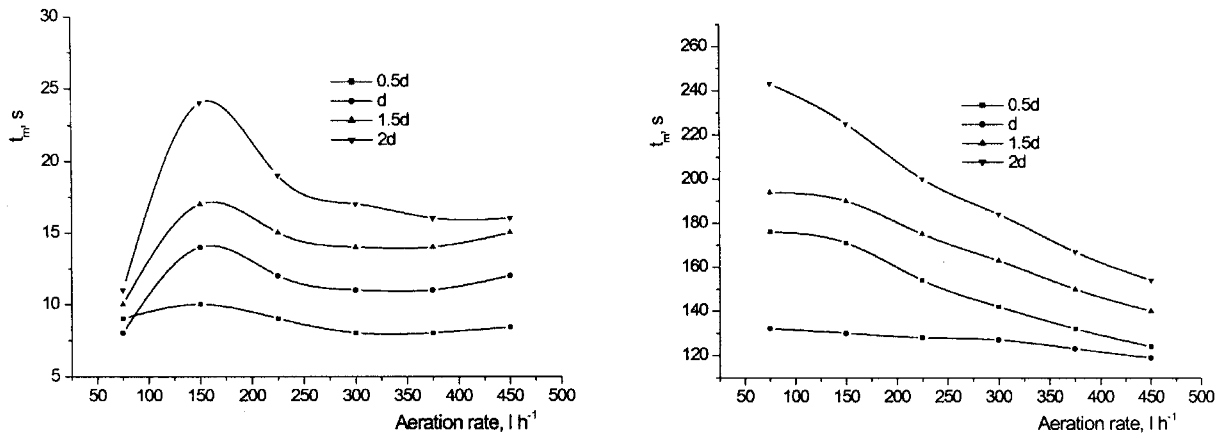
At higher air flow rates, the energy dissipated by the air exceeds that of the stirrer, and flooding appears. At the flooding point, the rise velocity of the air strongly increases, inducing a simultaneous increase in the intensity of medium circulation and a decrease of the mixing time. The value of the air volumetric flow corresponding to the flooding point for *P. shermanii* and *S. cerevisiae* broths was 150–200 l h^{-1}.

Regardles of the concentration and morphology of *P. chrysogenum*, for stirrer rotation speeds below 400 rpm, the mixing time decreased with aeration increase, this variation being similar to those for bacteria and yeasts (Figure 4).



***Penicillium chrysogenum* pellets**

$C_X = 4$ g l^{-1} d.w. $C_X = 30$ g l^{-1} d.w.



***Penicillium chrysogenum* free mycelia**

$C_X = 4$ g l^{-1} d.w. $C_X = 30$ g l^{-1} d.w.

Figure 4. Influence of the aeration rate on the mixing time for P. chrysogenum pellets and free mycelia broths at 600 rpm.

However, for rotation speeds above 400 rpm, the dependence of the mixing time on the aeration rate is completely different to that observed for bacteria and yeasts. Thus, for lower *P. chrysogenum* concentrations and both morphological conformations, the mixing time initially increases with aeration rate, reaches a maximum value and decreases. This variation can be explained by the formation of small bubbles. Due to the presence of the solid phase that avoids the bubbles to coalescece, the rise of these bubbles is strongly hindered by the high apparent viscosity of fungus broths. After the flooding point (at an aeration volumetric rate of 200–300 l h^{-1} for *P. chrysogenum* pellets, and 150 l h^{-1} for free mycelia), the mixing time was reduced due to the intensification of dispersion circulation.

Accordingly as the biomass concentration increases, the variation of the mixing time with aeration rate gradually changes, for 33.5 g l^{-1} d.w. *P. chrysogenum* a continuous reduction of the mixing time with aeration increase was observed. In these systems, the high apparent viscosity of the fungus suspensions controls the mixing efficiency, mechanical agitation is poor and the relative contribution of pneumatic mixing to the broth circulation is important. Due to the higher apparent viscosity which considerably reduces the relative contribution of mechanical agitation to broth mixing, these phenomena are more pronounced for *P. chrysogenum* free mycelia.

Influence of the distance between the stirrers

For all the studied systems and operating conditions, the lowest values of the mixing time were obtained for a distance d between the stirrers placed on the shaft. The order of the increase of the mixing time function on the stirrer position is:

$$d << 0.5\,d < 1.5\,d < 2\,d$$

The difference between the mixing intensity induced by placing the stirrers at a distance d and that

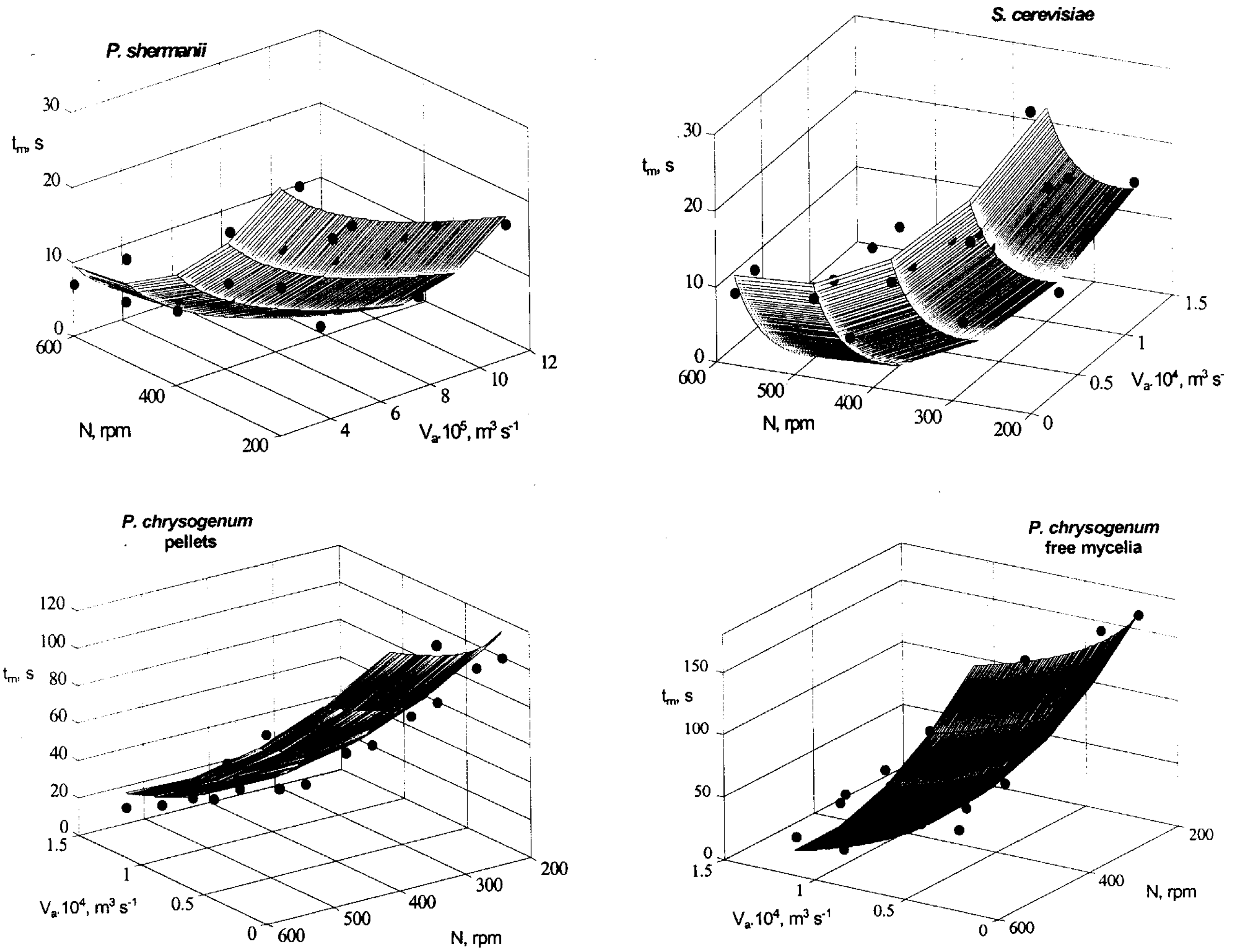


Figure 5. Surfaces plotted for equations (4) – (7) for L = d and C_X = 45 g l^{-1} d.w. P. shermanii, C_X = 43 g l^{-1} d.w. S. cerevisiae, C_X = 16 g l^{-1} d.w. P. chrysogenum pellets, C_X = 16 g l^{-1} d.w. P. chrysogenum free mycelia (• – experimental data).

obtained for the other positions of the stirrers on the shaft becomes more significant for fungus broths (Figures 1 and 2). Due to solid phase deposition, the distance of 0.5 d or d between the stirrers can facilitate the mixing. But, if the stirrers are too close (0.5 d), bubbles are accumulated and coalescece around the stirrer. The phenomenon of air accumulation in the region around the stirrer is amplified at higher biomass concentrations and reduces the dispersion circulation velocity.

Modeling of mixing for aerated simulated broths

The experimental data were included in mathematical correlations which describe the influence of aeration, rotation speed and the distance between the stirrers on the mixing time for aerobic stirred bioreactors containing bacteria, yeast and fungus broths. The general expression of the proposed equations is:

$$t_m = a_1 \cdot C_X^2 + a_2 \cdot C_X + a_3 \cdot \lg V_a + \frac{a_4 \cdot N^2 + a_5 \cdot N + a_6}{a_7 \cdot L^2 + a_8 \cdot L + a_9} \quad (3)$$

The influence and the relative importance of the considered variables are suggested by the coefficients $a_1, \ldots, a_9$. The values of these coefficients are specific for each microorganism type or morphology and were calculated by means of the *nlinfit* function from the Statistics Toolbox of MATLAB. This function uses non–linear least–squares data fitting by the Gauss–Newton method. Thus, the following correlations were established:

a. **bacteria** (*P. shermanii*) (Re < 25.000):

$$t_m = 5.54 \cdot 10^{-3} \cdot C_X^2 - 0.30 \cdot C_X - 8.40 \cdot \lg V_a + \frac{9.85 \cdot 10^{-4} \cdot N^2 - 1.15 \cdot N - 1.43 \cdot 10^2}{3.29 \cdot 10^2 \cdot L^2 - 38.89 \cdot L + 7.20} \quad (4)$$

b. **yeasts** (*S. cereviasiae*) (Re < 23.000):

$$t_m = 9.40 \cdot 10^{-3} \cdot C_X^2 - 1.26 \cdot C_X - 12.12 \cdot \lg V_a + \frac{8.04 \cdot 10^{-4} \cdot N^2 - 0.90 \cdot N - 34.82}{1.47 \cdot 10^2 \cdot L^2 - 19.75 L + 4.25} \quad (5)$$

c. **fungus** (*P. chrysogenum*) pellets (Re < 20.000):

$$t_m = 0.11 \cdot C_X^2 + 0.48 \cdot C_X - 23.20 \cdot \lg V_a + \frac{1.10 \cdot 10^{-2} \cdot N^2 - 11.99 \cdot N - 4.43 \cdot 10^3}{1.13 \cdot 10^3 \cdot L^2 - 1.33 \cdot L + 35.19} \quad (6)$$

free mycelia (Re < 15.000):

$$t_m = 0.15 C_X^2 + 0.24 \cdot C_X - 41.20 \cdot \lg V_a + \frac{1.31 \cdot 10^{-2} \cdot N^2 - 14.73 \cdot N - 5.47 \cdot 10^3}{3.61 \cdot 10^2 \cdot L^2 - 42.01 \cdot L + 23.90} \quad (7)$$

The proposed models offer good agreement with the experimental data, the average deviation being ±6.7% for *P. shermanii*, ±9.1% for *S. cerevisiae*, ±7.4% for *P. chrysogenum* pellets and ±9.4% for *P. chrysogenum* free mycelia (Figure 5).

Analyzing the corresponding coefficients, which represent the square of the correlation coefficients for the proposed equations, it may be concluded that the considered factors influence the mixing time to an extent of 96.1% for *P. shermanii*, 95.4% for *S. cerevisiae*, 96.8% for *P. chrysogenum* pellets and 94.9% for *P. chrysogenum* free mycelia The remaining 3.9%, 4.6%, 3.2% and 5.1% respectively can be attributed to the effect of other factors, namely: the number, position and geometry of the baffles, temperature, etc.

CONCLUSIONS

Due to the complex flow behavior of the broths into aerobic stirred bioreactors, the influence of the geometrical and operating characteristics of the bioreactor on the mixing time are very different compared with non–aerated systems.

By studying the mixing efficiency for bacteria, yeast and fungus aerated broths, the following conclusions may be drawn:

1. At the same biomass concentrations and under identical fermentation conditions, the highest mixing times were obtained for fungus broths, as a result of the higher apparent viscosity, and the more pronounced tendency of biomass deposition.

2. The variation of mixing time with impeller rotation speed indicated the existence of a critical rotation speed (500 rpm) that corresponds to the maximum of the mixing intensity.

3. The influence of aeration on mixing efficiency depends on the broth viscosity, concentration and morphology of the cultivated strain, as well as on the rotation speed. Thus, an increase of the air flow rate can lead to opposite effects for different microorganism types or fermentation conditions.

4. The most efficient mixing was obtained for a distance between the stirrers on the shaft equal to their diameters. This position of the stirrers avoids solid phase deposition and air accumulation around the impeller.

5. The influence of the studied parameters was included in mathematical correlations which allow the prediction of the mixing time for aerobic stirred bioreactors, the average deviation from the experimental results being ±6.7 – ±9.4%.

NOTATIONS

C_X	– biomass concentration, g l^{-1} dry weight
d	– stirrer diameter, mm
d'	– pH electrode diameter, mm
D	– bioreactor diameter, mm
h	– distance from the inferior stirrer to the bioreactor bottom, mm
H	– bioreactor height, mm
l	– impeller blade length, mm
l'	– pH electrode immersed length, mm
L	– distance between the stirrers, m
N	– impeller rotation speed, rpm
P	– power consumption for the mixing of non–aerated broths, W
P_a	– power consumption for the mixing of aerated broths, W
pH_∞	– value of pH at t = ∞
ΔpH	– allowed deviation from ideally mixed
Re	– Reynolds number
s	– baffle width, mm
t_m	– mixing time, s
V	– volume of the medium, m^3
V_a	– volumetric air flow rate, $m^3\ s^{-1}$
w	– impeller width, mm
ε_T	– dissipated energy, W m^{-3}
η_a	– apparent viscosity, Pa·s
ρ	– density, kg m^{-3}

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IZVOD

ISPITIVANJE MEŠANJA U BIOREAKTORU SA MEŠANJEM
4. Vreme mešanja aerisanih suspenzija bakterija, kvasaca i gljiva

(Naučni rad)

Dan Cascaval[1], Anca–Irina Galaction[2], Corneliu Oniscu[1], Florina Ungureanu[1]
[1]Tehnički univerzitet "Gh. Asachi", Fakultet za industrijsku hemiju, Jaši, Rumunija
[2]Univerzitet medicine i farmacije, Fakultet za medicinsko bioinženjerstvo, Jaši, Rumunija

Vreme mešanja kod bioreaktora zavisi uglavnom od reoloških osobina fermenatcione komine, koncentracije i morfologije biomase, karakteristika sistema u kome se ostvaruje mešanje i uslova pod kojima se realizuje fermentacija. U cilju kvantifikacije navednih uticaja na efikasnost mešanja u bioreaktoru sa mešanjem izvedena su prethodna ispitivanja sa suspenzijama bakterija (*P. shermanii*), kvasca (*S. cerevisiae*) i gljiva (*P. chrysogenum*, slobodne micele i agregati micela) različitih koncentracija u laboratorijskom bioreaktoru sa dve turbinske mešalice na jednoj osovini. Eksperimentalni rezultati su pokazali da je uticaj brzine obrtanja mešalice, intenziteta aeracije i pozicije mešalica na osovini značajno različit od jednog do drugog ispitivanog sistema i da se on može korelisati u funkciji karakteristika mikroorganizama, pre svega, koncentracije biomase i njene morfologije. Nadalje, kada se napravi poređenje sa neaerisanim fermentacionim smešama istog sastava, promena vrednosti u parametru koji se definiše kao vreme mešanja je različita, pre svega, zbog kompleksnog mehanizma pri mešanju disperzije gas–tečnost.

Primenom multiregresione analize, na osnovu izmerenih vrednosti vremena mešanja (t_m), izvedena je korelaciona zavisnost opšteg oblika:

$$t_m = a_1 \cdot C_X^2 + a_2 \cdot C_X + a_3 \cdot \lg V_a + \frac{a_4 \cdot N^2 + a_5 \cdot N + a_6}{a_7 \cdot L^2 + a_8 \cdot L + a_9}$$

gde je: C_x – koncentracija biomase; V_a – zapreminski protok vazduha za aeraciju fermentacione komine, N – broj obrtaja mešalice, L – rastojanje između dve turbinske mešalice, i a_1–a_9 odgovarajuće vrednosti konstanti. Korelacija ovog oblika daje dobro slaganje sa eksperimentalnim podacima, sa srednjom disperzijom od ±6.7% do ±9.4 za ispitivane fermentacione medijume, pri režimu mešanja u bioreaktoru koji je okarakterisan odgovarajućim Rejnoldsovim kriterijumom mešanja, Re < 25000.

Ključne reči: Reaktor sa mešanjem • Bioreaktor • Vreme mešanja • *Propionibacterium shermanii* • *Saccharomyces cerevisiae* • *Penicillium chrysogenum* • Slobodne ćelije •
Key words: Stirred bioreactor • Mixing time • *Propionibacterium shermanii* • *Saccharomyces cerevisiae* • *Penicillium chrysogenum* • Pellets • Free mycelia •