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Chemical Industry & Chemical Engineering Quarterly 16 (1) 47–64 (2010) CI&CEQ

ANCA-IRINA GALACTION[1]
ANCA-MARCELA LUPĂŞTEANU[2]
MARIUS TURNEA[1]
DAN CAŞCAVAL[2]

[1]University of Medicine and Pharmacy "Gr.T. Popa" of Iasi, Faculty of Medical Bioengineering, Dept. of Biotechnologies, Iasi, Romania
[2]Technical University "Gh. Asachi" of Iasi, Faculty of Chemical Engineering and Environmental Protection, Dept. of Biochemical Engineering, Iasi, Romania

SCIENTIFIC PAPER

UDC 66.023:66.098:582.282.3

DOI: 10.2298/CICEQ090407002G

COMPARATIVE ANALYSIS OF MIXING EFFICIENCY AND DISTRIBUTION INDUCED BY RADIAL IMPELLERS IN BIOREACTORS WITH STIRRED BED OF IMMOBILIZED CELLS

The influences of the main factors on the mixing efficiency and distribution for a bioreactor with stirred/mobile bed of immobilized S. cerevisiae cells in alginate (biocatalyst particles with 4, 4.6 and 5.2 mm diameters) have been comparatively analyzed for six radial impellers: a disperser sawtooth, Smith turbine, a pumper mixer, a curved bladed turbine, a paddle with six blades, a pitched bladed turbine vs. Rushton turbine. The most efficient impellers, from the viewpoint of intensity and uniformity of the suspension circulation were found to be the Smith turbine, the paddle with six blades and the pitched bladed turbine. The mathematical correlations describing the influence of the main factors on mixing time were established for each studied impeller offering a good concordance with the experimental data (the average deviations vary from ±7.9% for pitched bladed turbine to ±12.1% for disperser sawtooth).

Key words: bioreactor; stirred bed; immobilized cells; mixing time; radial impeller.

The spectacular applications of the immobilized biocatalysts determined the design and construction of some proper bioreactors, specific or derived from the "classical" ones. Although these bioreactors are derived from the "classical" bioreactors and, therefore, their constructive and functional characteristics being rather similar with the second ones, they offer important advantages, namely as: the increase of thermal, chemical and to the shear forces resistance of the enzymes or cells, the increase of the number of the repeated biosynthesis cycles using the same particles of biocatalysts, the easier recovery of biocatalysts from the final broths, the diminution or avoidance of the inhibition processes [1-4].

The bioreactors using immobilized biocatalyst can be designed as column, stirred, gas-lift or membrane bioreactors. They are operated in batch, continuous or semicontinuous systems, with fixed, mobile/stirred, expanded or fluidized bed [5]. Among them, the bioreactors with stirred/mobile bed of immobilized biocatalysts are ones of the most studied and applied bioreactors owing to their very similar constructive and operational characteristics to those of the well-known stirred bioreactors. The main constructive difference between the two types of bioreactors is the presence at the bottom of the former ones of a sieve which avoids the biocatalysts particles washout. The models describing the flow or the heat and mass transfer in stirred bioreactors, as well as their design and optimization, can be easily adapted for the stirred-bed bioreactors. But, these models are valid only for the continuous phase from the bioreactor [5]. Due to the deposition tendency of the solid phase at the bioreactor bottom to the internal diffusion of the substrate or product into the biocatalyst particle, the mixing and, consequently, the flow of these suspensions, as well as the mechanism and kinetics of the processes occurring into the solid phase become more complex than in the homogeneous systems, thus new models having to be established for the biocatalyst phase [5,6].

The performances of the fermentation processes carried out in the bioreactors with stirred bed of biocatalysts are influenced by specific or general factors (the size of the particles [7], geometrical and operational characteristics of the vessel [7-11], the concentration of enzymes/cells into the particles [8,12,13], feed strategy [9,14,15]), with the mixing efficiency and its distribution being the most important of them all [16].

Corresponding author: D. Caşcaval, Technical University "Gh. Asachi" of Iasi, Faculty of Chemical Engineering and Environmental Protection, Dept. of Biochemical Engineering, 71 D. Mangeron, 700050 Iasi, Romania.
E-mail: dancasca@ch.tuiasi.ro
Paper received: 7 April, 2009.
Paper revised: 3 August, 2009.
Paper accepted: 27 October, 2009.

These bioreactors have been used for the production of pharmaceuticals [1,17,18], chemicals [19], solvents and biofuels [20,21], whereas the current studies are mainly focused on the treatment of the Industrial or municipal wastewater [8,9,12-14].

Since mixing constitutes one of the main factors controlling these bioreactors performances, being in its turn influenced by many constructive and operational parameters, the analysis and quantification of these influences on the mixing efficiency and distribution are required for the process optimization.

One of the most useful criteria for characterization of the mixing intensity is the mixing time, t_m, which characterizes the intensity of segregation, being defined as the time needed to reach a given mixing intensity at a given scale, when starting from the completely segregated situation [1,23]. This parameter offers specific information concerning the bulk mixing in the system (macromixing), respectively the flow inside the whole studied system, but it cannot allow rigorous quantification of the meso- and micro-mixing [23]. It can indicate the optimum hydrodynamic regime, the stirrer type that is recommended to be used or can predict the modification of the mixing efficiency induced by scaling-up [24,25].

Although the radial impellers, especially the Rushton turbine, are widely used in the large-scale stirred bioreactors, their applications are limited by the high viscosity and non-Newtonian behavior of the broths. Thus, by comparing the information concerning the distribution of circulation intensity, the power consumption or shear stress for different double radial stirrers, the following optimum combinations of impellers were selected for simulated broths: a disperser sawtooth and paddle with six blades for water, a pitched bladed turbine and Rushton turbine for broths with viscosity up to 30 cP, a pumper mixer and disperser sawtooth for broths with higher viscosity [26]. These results suggest that a comparative analysis of the mixing induced by different radial impellers for systems containing immobilized cells is required. For selecting the optimum impellers combination, the data on the suspension circulation inside the bioreactors, the power consumption and shear effect on biocatalysts particles have to be taken into account.

In this context, the aim of our experiments was to comparatively study the efficiency of mixing for a bioreactor with stirred bed of immobilized yeasts cells equipped with different radial impellers. This analysis was made by means of the mixing time distribution obtained by vertically changing the position of the pH--sensor into the broth in correlation with the energy consumption. Using the experimental data, the most efficient impeller or impellers combination was selected for a certain fermentation broth. Moreover, some mathematical models describing the mixing intensity through mixing time were proposed for the considered radial impellers.

EXPERIMENTAL

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 [27]. The bioreactor characteristics are given in Table 1.

The mixing system consists of a double stirrer and three baffles. Six types of radial impellers were used (Figure 1), the experimental data being compared with the previous ones obtained for the Rushton turbine [28].

Table 1. Characteristics of bioreactor and impellers

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	2.15	0.12	0.28	1	6	3	0.20	0.21	2.81

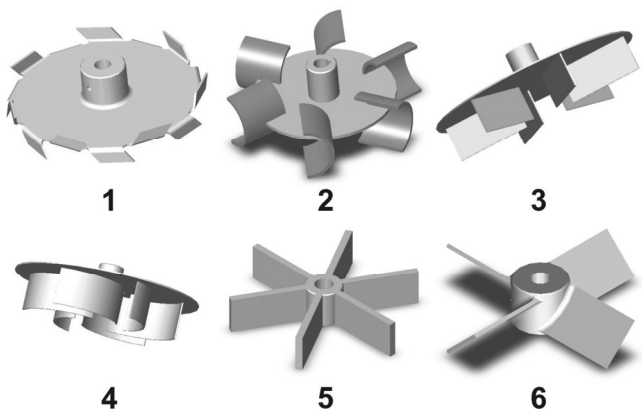

Figure 1. The radial impellers used in experiments (1 - disperser sawtooth, 2 - Smith turbine, 3 - pumper mixer, 4 - curved bladed turbine, 5 - paddle with six blades, 6 - pitched bladed turbine).

The diameter of the two impellers on the shaft, *d*, was of 64 mm. The inferior impeller was placed at 64 mm from the bioreactor bottom. The superior impeller was placed on the shaft at a distance of 32 mm from the inferior one, this being the optimum distance from the Ruston turbine, as it was demonstrated in previous works [28]. The rotation speed was maintained between 50 and 300 rpm (Reynolds number between 180 and 1260), domain that avoids the vortex formation at the broths surface and mechanical disruption of the biocatalysts particles.

In the experiments, *S. cerevisiae* cells immobilized on alginate were used. The immobilization was carried out by cell inclusion into the alginate matrix, according to the method given in literature [29]. The following diameters of the biocatalyst spherical particles were obtained: 4, 4.6 and 5.2 mm. The volumetric fraction of the immobilized cells into the media was varied between 7 and 40%. As it was established in the previous studies, these suspensions exhibit the dilatants behavior, their apparent viscosity being measured using the rotative viscometer of Haake Viscotester 6 Plus type [28]. The apparent viscosity varied from 2.7 cP, for suspensions containing 7% volumetric fraction of particles of 5.2 mm diameter, to 18.9 cP, for the most concentrated suspensions of the smallest size particles [28].

The experiments were carried out at the temperature of 25 °C. Any mechanical damage of the biocatalyst due to the shear forces was recorded during the experiments.

The mixing efficiency was analyzed by means of the mixing time values, using the tracer method [30]. Thus, for mixing time determination, a solution of 2N KOH was used as tracer, and the time needed for the media pH to reach the value corresponding to the considered mixing intensity was recorded. In this case, the following homogeneity criterion for mixing, I, was considered [31]:

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

where: pH_{∞} - pH-value corresponding to perfect mixing; ΔpH - allowed deviation from perfect mixing ($\Delta pH = 0.02$).

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. 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 introduced at four different positions and placed vertically from the 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 to calculate the mixing time.

The mathematical correlations, which describe the influences of the considered factors on mixing time for each impeller combination were developed on a PC using MATLAB software. For the experimental data, a multiregression analysis was performed. A nonlinear equation form that may be liniarized by applying logarithmic function, the difference between the experimental and modeled value being reduced to a minimum was chosen. By means of a MATLAB program, the regression coefficients and standard deviation were calculated.

Each experiment was carried out at least triplicates for identical conditions and the value of mixing time was taken as an average. The maximum experimental error was between ±3.31 and ±4.33%.

RESULTS AND DISCUSSION

The analysis of the mixing efficiency and distribution

Previous studies on mixing inside the bioreactors with stirred beds of immobilized *S. cerevisiae* cells in alginate indicated that the mixing efficiency and its distribution are controlled by the size and volumetric fraction of the biocatalyst particles [28]. In function of the characteristics of biocatalyst particles and operational parameters of the bioreactor, the uniform mixing in the whole bulk of the suspension could be reached. Thus, for particles with 4 mm diameter and volumetric fraction up to 15%, the optimum rotation speed is of 100 rpm increasing to 200 rpm for particles with 5.2 mm diameter and the same domain of the suspension concentration. For biocatalysts with an intermediary size (particle diameter of 4.6 mm), the uniform circulation of the suspension has been obtained even for more concentrated suspension (of maximum 20 vol%), the optimum rotation speed varying from 150 to 200 rpm with the volumetric fraction increase from 7 to 20% [28].

These experiments are carried out in the similar manner for another six types of radial impellers, namely a disperser sawtooth, Smith turbine, a pumper mixer, a curved bladed turbine, a paddle with six blades and a pitched bladed turbine for selecting the optimum mixing system for suspensions of immobilized yeasts.

Disperser sawtooth

The important differences between the variations of mixing time recorded for the four considered positions inside the bioreactor can be seen in Figure 2. Indifferent of the particles size, the dependences can be grouped in two categories for volumetric fraction of biocatalysts up to 25%, over this level three types of variations being observed.

Thus, for lower biocatalysts concentration, the shape of the obtained variations is similar for the inferior positions 1 and 2, respectively for the superior ones 3 and 4. For the inferior regions, the increase of the rotation speed leads to the initial reduction of mixing time, to a level corresponding to 150 rpm, followed by its increasing in the rotation speed domain of 150-200 rpm. Over 200 rpm the mixing time is again reduced. This trend is maintained also for more concentrated suspensions of biocatalysts, but the minimum and maximum of mixing time become less evident. Moreover, by increasing the volumetric fraction

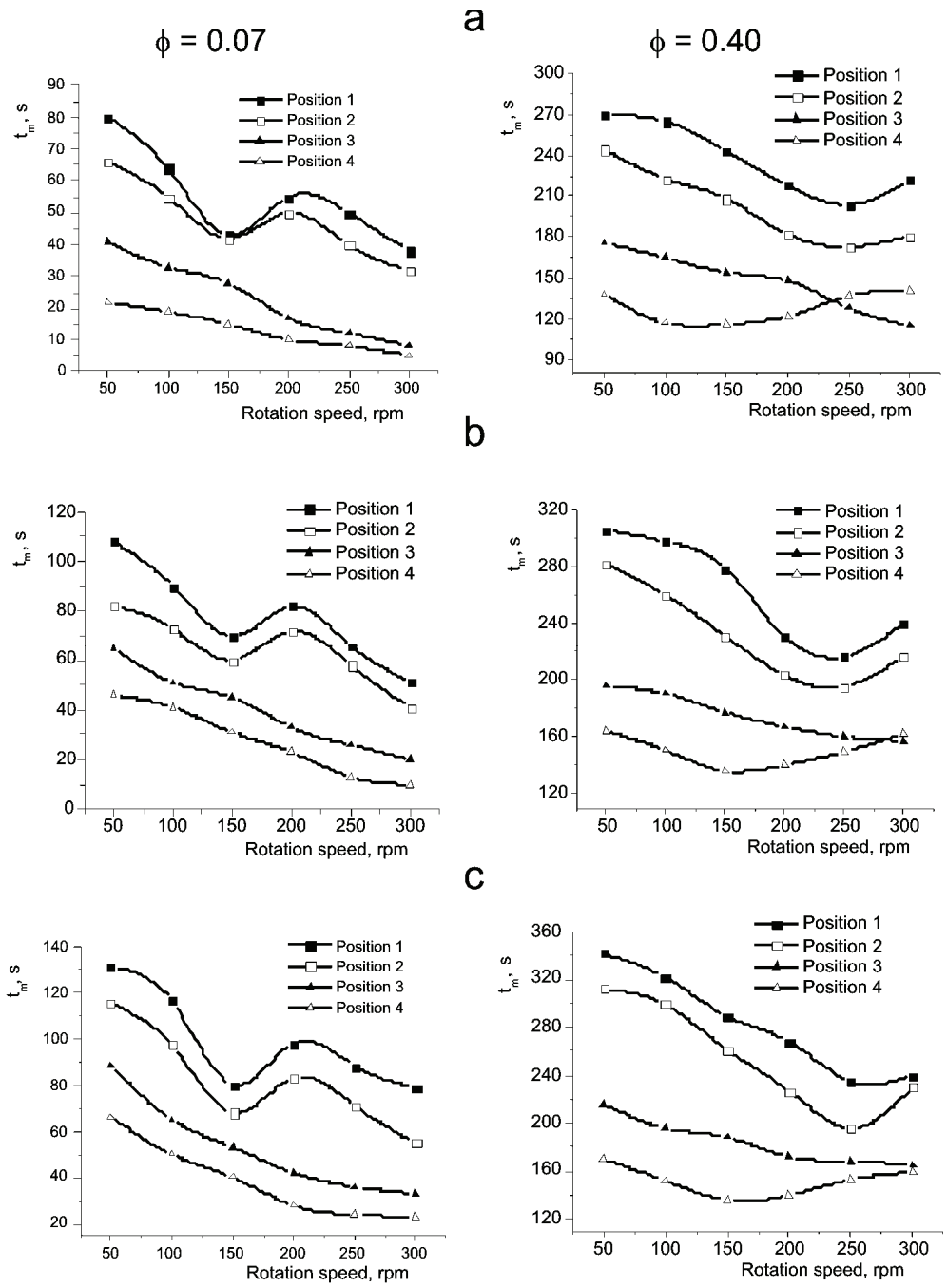

Figure 2. The influences of the rotation speed on mixing time at different sensor positions and biocatalysts concentration for the disperser sawtooth (particle diameter: a - 4, b - 4.6 and c - 5.2 mm).

of alginate particles, the rotation speed which corresponds to the two extremes is moved to higher values. Consequently, for 40 vol% immobilized cells, the maximum level of mixing time cannot be reached for the experimental rotation speed domain.

For biocatalysts concentration below 15 vol%, the intensification of rotation speed continuously improves the suspension circulation at the bioreactor top. The increase of the solid phase amount leads to the stronger differentiation of the mixing time variations for the positions 3 and 4. Therefore, the shape of the recorded curves for position 3 remains similar for the entire domain of alginate particles concentration. But, for the superior position 4, the obtained results indicate a minimum level for the mixing time, then this parameter increasing. The rotation speed which corresponds to the maximum efficiency of mixing is displaced to lower values by concentrating the suspension. Consequently, for biocatalysts volumetric fraction over 40% and rotation speed over 200 rpm the mixing time for position 4 is rather equal with that for position 3, becoming higher for the alginate particles with diameter of 4.6 mm.

The above presented data differ significantly from the previous ones obtained for simulated fermentation broths without solid phase [26]. The presence of the solid phase modifies the system behavior, owing to the appearance of a supplementary phenomenon, namely its deposition. In this case, the mixing has to avoid the deposition tendency of the biocatalysts and to uniformly disperse the broths components.

The highest concentration of the solid phase is in the inferior region of the bioreactor. For this reason, the biggest of mixing time values have been recorded for positions 1 and 2, respectively. The particular variation of the mixing time corresponding to the inferior region can be the result of the interference of the streams induced by the impellers placed at 0.5*d* on the shaft, the phenomenon that is amplified by the "bottom effect" and the solid phase collision. Therefore, the hindrance of the suspension circulation is more important than for the simulated broths without the solid phase. The increase of the rotation speed over the level needed to reach the maximum mixing time diminishes these negative effects, thus leading to the intensification of the suspension circulation in this region. The accumulation of the solid phase diminishes the influence of the rotation speed and, consequently, the magnitude of the above discussed phenomena is reduced at higher biocatalysts concentration.

Although the positions 3 and 4 are placed at a higher distance from the impellers and, therefore, from the region in which the turbulence is generated, the mixing time in these regions is lower than that for the inferior positions, due to the lower concentration of biocatalysts. The influence of the rotation speed in the superior region is reduced by the increase of the solid phase amount, due to its dispersion in the whole bulk volume of the media with the mixing intensification. But, for particles concentration over 15 vol%, the dependence between the mixing time and rotation speed differs from position 3 to 4. According to the above results, the particular variation recorded for position 4 is due especially to the deposition of the solid phase and less to the friction forces between the alginate particles, the effect promoted by the low pumping capacity of the disperser sawtooth. The increase of particles size from 4 to 5.2 mm amplifies the magnitude of the variations obtained for positions 1 and 2 and attenuates those recorded for positions 3 and 4, this demonstrating the above conclusion.

By analyzing comparatively the influence of the rotation speed on the mixing efficiency and its distribution in suspensions of immobilized *S. cerevisiae* in alginate for a double stirrer of the Rushton turbine type [28] and one of the disperser sawtooth type, the important differences have been observed as follows:

- Positions 1 and 2: indifferent of the size and concentration of the biocatalysts particles, the Rushton turbine is more efficient for the entire rotation speed domain used, owing to the lower dispersing capacity and to the more pronounced streams interference of the disperser sawtooth.
- Position 3: the disperser sawtooth induces a more intense mixing for biocatalysts volumetric fraction below 15%, but only for rotation speed values directly related to the alginate particles size and concentration.
- Position 4: in all cases, the disperser sawtooth promotes the most intense circulation.

But, these conclusions should be prudently analyzed, because the mixing promoted by the disperser sawtooth for the superior positions is apparently more intense due to the low amount of the solid phase dispersed in the superior region.

Moreover, the use of this impeller does not offer any possibility to reach a uniform mixing in the whole bulk volume of the suspension. Contrary, the uniform dispersion of the solid phase can be obtained for the Rushton turbine at optimum rotation speed values of 100-200 rpm, but only for the biocatalysts concentration below 15 vol% [28].

The increase of the biocatalysts particles size exhibits a negative influence on the mixing time. Therefore, for the disperser sawtooth it can be concluded that the solid phase deposition controls the efficiency of mixing.

Smith turbine

This type of impeller disperses the gases better than the Rushton turbine, being recommended for aerobic fermentation processes.

As in the case of the disperser sawtooth, the correlations between the mixing time and the rotation speed are of two types due to the deposition tendency of biocatalysts. The differences between the variations corresponding to the four positions are diminished with the increase of the solid phase concentration (Figure 3).

For lower volumetric fraction of biocatalysts, the mixing time initially decreases by accelerating the rotation speed to 100 rpm, increases for rotation speed varying from 100 to 150 rpm then decreasing strongly. This variation has been recorded for positions 1, 2 and 3, but its amplitude differs from one position to another. Therefore, the highest value of mixing time, corresponding to 150 rpm, is reached for position 2, as the result of the stream interference and the solid phase collision with the baffles or the bioreactor wall. The relative importance of these phenomena is at 150 rpm maximum, for the rest of the rotation speed do-

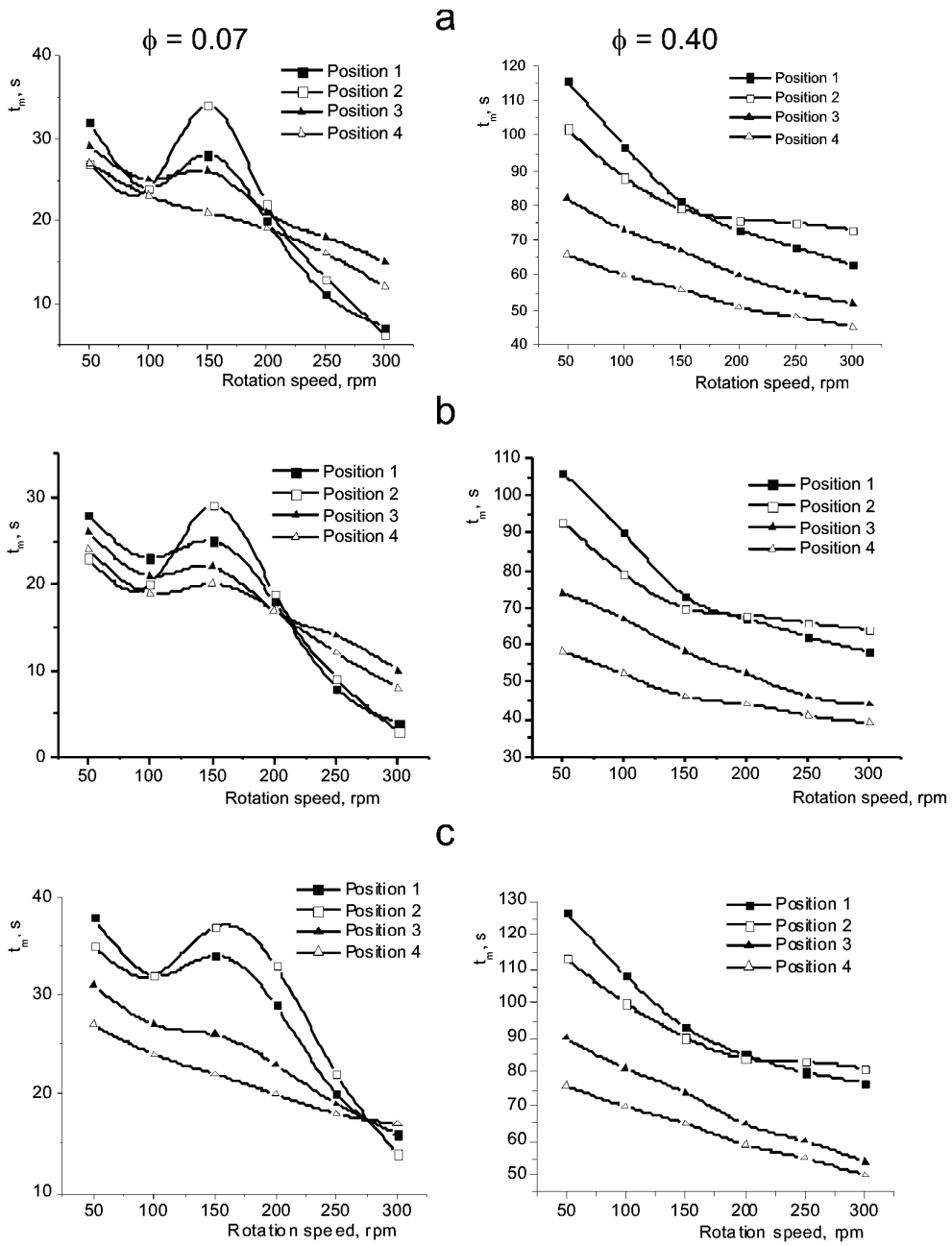

Figure 3. The influences of the rotation speed on mixing time at different sensor positions and biocatalysts concentration or the Smith turbine (particle diameter: a - 4, b - 4.6 and c - 5.2 mm).

main the mixing intensity for position 2 being closer or superior to that obtained for position 1.

The variation of the mixing time for position 3 is similar, but the influence of the mentioned phenolmena is significantly attenuated, owing to the distance from the bottom region with the concentrated deposit of the solid phase. By intensifying the agitation, the biocatalysts are also dispersed in the superior region, and, consequently, their volumetric fraction increases in region 3. For the rotation speed higher than 200 rpm, this process, cumulated with the lower turbulence transmitted in position 3, leads to the increase of mixing time over the values recorded for positions 1 and 2.

The mixing for the position 4 is continuously improved by the rotation speed increasing, but it becomes less efficient compared with the positions 1 and 2 for the rotation speed over 200 rpm, due to the dispersion of the solid phase also in the superior region. Although the position 4 is placed at the highest distance from the superior impeller, the mixing time for the position 4 was inferior to that related to the position 3 for the entire domain of the rotation speed. This result can be attributed either to the lower concentration of biocatalysts in this region, or to the propagation in the position 3 of the negative effect of streams interference and particles collision.

These variations are maintained for biocatalysts concentration up to 15 vol%. But, the rotation speed corresponding to the maximum mixing time for the positions 1, 2 and 3 is moved to higher values with the particles concentration increase, becoming 200 rpm. Moreover, the suspension circulation in the position 3 is more intense than that in the position 1 or 2 for a larger domain of the rotation speed, this underlining that for concentrated suspensions of biocatalysts, the alginate particles deposition and collision, as well as the streams interference, significantly reduce the relative efficiency of the mixing in the inferior region of the bioreactor. For the same reason, the mixing time obtained for position 4 continuously decreases with the rotation speed acceleration and reaches the lower values comparatively with the other three positions.

For the biocatalyst volumetric fraction over 15%, the shape of the variations describing the rotation speed influence on the mixing time in the considered regions inside the bioreactors become closer, the intensity of the suspension circulation increasing with the acceleration of the rotation speed. The lowest values of the mixing time have been recorded for position 4. On the other hand, for the rotation speed below 200 rpm the highest mixing time values have been obtained for position 1, or for position 2, over this level of the rotation speed. These results, as well as the comparison with the mixing induced by Smith turbine in simulated fermentation broths without solid phase [26], suggest that the circulation velocity of the suspension is reduced by the alginate particles collision with the baffles, a phenomenon amplified in more concentrated suspensions.

Although the variations of mixing time for the four positions are similar, the relative importance of the friction between the alginate particles *vs.* their deposition can induce the modification of these variations shapes. Thus, due to the equilibrium existing between the friction forces, specific to smaller particles, and the deposition to the bioreactor bottom, specific to the bigger ones, the minimum and maximum of mixing time are attenuated for the particles with the intermediary diameter of 4.6 mm. In the case of alginate particles with 5.2 mm diameter, the pronounced tendency of particles deposition to the bioreactor bottom leads to the amplification of these extremes points and to the more evident differentiation between the positions 1 and 2 compared with positions 3 and 4. For the smallest biocatalysts (diameter of 4 mm), the deposition tendency is considerably diminished, therefore the variation recorded for position 3 is rather similar to those corresponding to positions 1 and 2.

The comparative analysis of the mixing promoted by Smith and Rushton turbines indicates that the former one offers higher efficiency in the most of the studied cases:

- Position 1: indifferent of the biocatalysts size, the Smith turbine is more efficient for particles concentration below 25 vol%.
- Position 2: in the case of biocatalysts with diameter of 4 mm, the Smith turbine offers a more efficient mixing for the entire considered domain of rotation speed of solid phase concentration.
- Positions 3 and 4: in all cases, the Smith turbine promotes the most efficient mixing.

Contrary to the disperser sawtooth, the use of the Smith turbine offers the possibility to reach the uniform circulation into the whole bulk volume of the suspension for a certain domain of particles concentration and rotation speed values in relation with the biocatalysts size, as in the case of Rushton turbine. Therefore, for biocatalysts with diameters of 4 and 4.6 mm, the uniform mixing is obtained only for volumetric fraction up to 15%, at 100 and 200 rpm for the concentration of 7 vol%, respectively at 250 rpm for more concentrated suspensions. The uniform dispersion of the solid phase is reached for particles with 5.2 mm diameter only for the concentration below 7% at 250 rpm.

Since the biocatalyst with intermediary diameter of 4.6 mm diminishes the friction forces between the particles compared with the smaller biocatalysts, and also the deposition tendency compared with the bigger ones, the lowest values of mixing time have been recorded for the size of alginate particles.

Pumper mixer

This impeller represents the component part of some equipment used for liquid-liquid extraction, owing to its superior capacity to disperse the two liquid phases by promoting an intense circulation. The induced flow is similar to the radial flow created by the Rushton turbine, but the amplitude of the streams depends on the ratio between the blades diameter, d, and the disc diameter, d_D. Thus, for $d/d_D < 2$, the stirrer promotes an intense circulation in the region under the disc plane, similar to the flow stream from the inferior region of the radial circulation induced by the Rushton turbine. In this case, the impeller acts as a centrifugal pump. For $d/d_D \geq 2$, the flow is identical to that generated by the Rushton turbine [32].

Since the studied impeller possesses the ratio d/d_D of 1, the induced flow is more intense in the region below the impeller disc. This phenomenon cumulated with the deposition tendency of the biocatalysts lead to the different dependences between the mixing time and the rotation speed compared with the previous impellers.

In all studied cases, the highest values of mixing time have been recorded for position 1, due to the presence of important amount of biocatalysts and to the specific flow streams that do not allow to axially dispersing the solid phase. But, indifferent of the biocatalysts concentration, two types of dependence between the mixing time and rotation speed, corresponding to the inferior and, respectively, superior regions inside the bioreactor can be distinguished in Figure 4.

For lower biocatalysts concentration, up to 15 vol%, the experimental data indicated the sinusoidal variation of the mixing time for the inferior positions 1 and 2. Thus, the mixing time initially decreases with the rotation speed acceleration, reaches a minimum value then increasing to a maximum level. The further increase of the rotation speed leads again to the decrease of the mixing time. The existence of the minimum is due to the appearance and intensification of the hindrance effect on the suspension circulation, induced either by the baffles or by the bioreactor wall, as in the case of simulated broths mixing [26].

Moreover, the collision of the alginate particles, as well as the friction between these particles, amplifies the hindrance effect. The increase of the rotation speed counteracts this negative effect, this being the reason for the decrease of the mixing time and for its maximum level. Indifferent of the biocatalysts size, the values of the rotation speed corresponding to the two extreme points are 150 and 250 rpm, respectively.

By increasing the volumetric fraction of the biocatalysts, the suspension circulation becomes more difficult and consequently the minimum level of the mixing time is reached at higher rotation speed (200 rpm for biocatalysts concentration of 25 vol%, and 250 rpm for 40 vol%) this leading to the disappearing of the maximum value of mixing time.

For the superior positions, the influence of the rotation speed is considerably changed from the above discussed. Thus, at the position closer to the impellers region, namely position 3, and lower concentration of the solid phase, the mixing efficiency has a sinusoidal variation opposite to those recorded for positions 1 and 2 and less evident. This variation amplitude is diminished by increasing the alginate particles amount in the suspension, and, consequently, for 40 vol% biocatalysts the reduction of mixing time cannot be observed.

The mixing efficiency for position 4 is continuously reduced by the rotation speed increase, phenolmenon that becomes more pronounced at higher concentration of biocatalysts. The rotation speed influence is the result of the weak propagation of the flow streams in the superior region, this effect magnitude being enhanced by the biocatalysts dispersion from the bioreactor bottom. Therefore, the intensification of the circulation below the impeller disc leads to the hindrance of the circulation in the region superior to the impeller, the process which becomes more important at higher concentration of biocatalysts, due to the diminution of the turbulence. On the other hand, the significant difference between the mixing times recorded from the inferior and superior regions is not the consequence of the more intense mixing in the superior region, but of the heterogeneous distribution of the biocatalysts on the bioreactor height, the solid phase being concentrated at the bioreactor bottom.

Although the above presented variations are similar for all three diameters of the biocatalysts particles, Figure 4 shows that the magnitude of the recorded phenomena is amplified with the increase of the particles size. This influence can be attributed to the negative effect on the suspension circulation induced by the friction between the particles and their collision, the effect which is more pronounced for biocatalysts with the lower size.

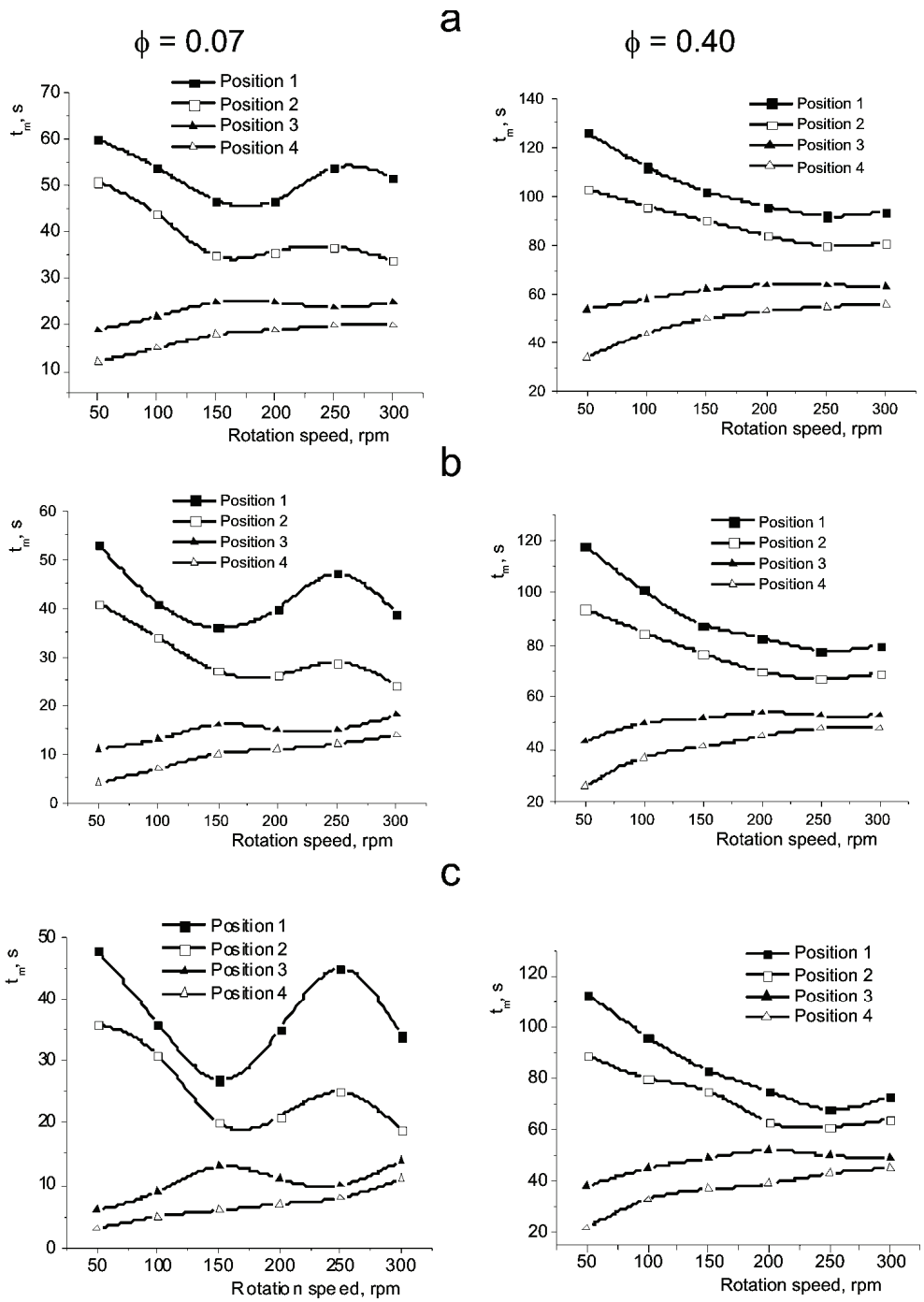

Figure 4. The influences of the rotation speed on mixing time at different sensor positions and biocatalysts concentration for the pumper mixer (particle diameter: a - 4, b - 4.6 and c - 5.2 mm).

Besides the differences between the shapes of the variations of mixing time with the rotation speed, by comparing the results obtained for the pumper mixer with those for the Rushton turbine [28], the following conclusion can be drawn:

- Positions 1 and 2: indifferent of the biocatalyst particles size, for the biocatalyst concentration up to 15 vol%, the Rushton turbine promotes the most intense mixing for the entire rotation speed domain used; by increasing the solid phase concentration, the pumper mixer becomes more efficient.

- Positions 3 and 4: for all considered cases, the pumper mixer offers the possibility to reach the lowest mixing times.

The uniform mixing could be considered to be reached only for concentrated suspensions, for the

particles with the higher size and the rotation speed over 200 rpm.

Contrary to the impellers previously studied, the increase of the biocatalyst size favorably influences the mixing. As it was mentioned above, due to the pumper mixer configuration which induces specific flow streams, the friction and collision between the alginate particles exhibit a more pronounced influence on the suspension circulation compared to the phenolmenon of particle deposition.

Curved bladed turbine

Similar to the pumper mixer, this impeller is used for dispersing the liquid phases in the extraction process. The generated circulation streams depend in the same manner on the ratio between the blades diameter and the disc diameter [32].

Although its construction is quite similar to the pumper mixer one, the experiments indicated significant differences between the two impellers concerning the variation of mixing time with the rotation speed, as in the case of the impellers of Rushton turbine and Smith turbine types. Thus, according to the Figure 5, the variation of mixing time for the inferior region is contrary to that for the superior one.

For the positions 1 and 2 and indifferent of the biocatalysts size, the mixing time initially decreases with the increase of the rotation speed, reaches a minimum value and then increases. The minimum level could be the result of the hindrance of the suspension circulation which appears if the rotation speed exceeds a certain value, this phenomena being induced by the friction between the particles or by the baffles and/or the bioreactor wall.

The rotation speed corresponding to the minimum mixing time, called *critical rotation speed*, is moved to higher values with the increase of biocatalysts volumetric fraction. Therefore, for position 1, the critical value is of 200 rpm for 25 vol% alginate particles, becoming 250 rpm for more concentrated suspensions. For position 2, the critical rotation speed is lower than that for position 1, due to the more intense circulation of the suspension promoted both by the propagation of the flow streams generated by the inferior impeller, and to the diminished concentration of the solid phase (150 rpm for biocatalysts concentration up to 25 vol%, 200 rpm for more concentrated suspensions).

But, the mixing time recorded for the superior positions increases to a maximum level with the rotation speed intensification, decreasing for higher rotation speed. As in the case of pumper mixer, this dependence is due to the increase of the solid phase amount in the superior region by its dispersing by the impellers. When the mixing efficiency for the positions 1 and 2 is reduced, the biocatalysts are less efficiently dispersed, and, consequently, the mixing time for the positions 3 and 4 decreases. The presented variation is less evident for the position 3, the closest to the impellers, and is more important at higher volumetric fraction of the alginate particles.

The above discussed influence of the rotation speed on mixing time becomes more pronounced with the increase of biocatalysts particles size, this underlining that for this impeller the suspension circulation is controlled mainly by the friction between the particles, similar to the pumper mixer, and not by the solid phase deposition.

Indifferent of the diameter of alginate particles and the rotation speed, the less efficient mixing has been reached for the position 1, owing to the highest concentration of the solid phase in this region. The significant difference between the inferior and superior regions is due only to the heterogeneous distribution of the solid phase into the bioreactor. By increasing the biocatalyst concentration and the rotation speed, this difference is attenuated.

Comparative to the pumper mixer, the use of the curved bladed turbine leads to the lower mixing time for the same experimental conditions, this suggesting the more efficient dispersion of the solid phase.

Analyzing the mixing promoted by the curved bladed turbine and the Rushton turbine for the suspensions of immobilized yeasts cells the following results have been obtained:

- Positions 1 and 2: only for the biocatalyst concentration below 15 vol% the Rushton turbine is more efficient, but its efficiency becomes close to that of the curved bladed turbine by increasing the particles size.
- Positions 3 and 4: indifferent of the concentration and size of biocatalysts and of the rotation speed, the curved bladed turbine is more efficient than the Rushton turbine.

The experiments indicated that for this impeller the uniform circulation inside the whole bulk of suspension cannot be reached.

Similar to the pumper mixer, due to the induced flow streams, the increase of the alginate particles size exhibits a negative effect on the mixing, the effect being more pronounced than in the previous case. The friction between the particles, which controls the suspension circulation at the bioreactor bottom, is more important at the higher turbulence promoted by the curved bladed turbine.

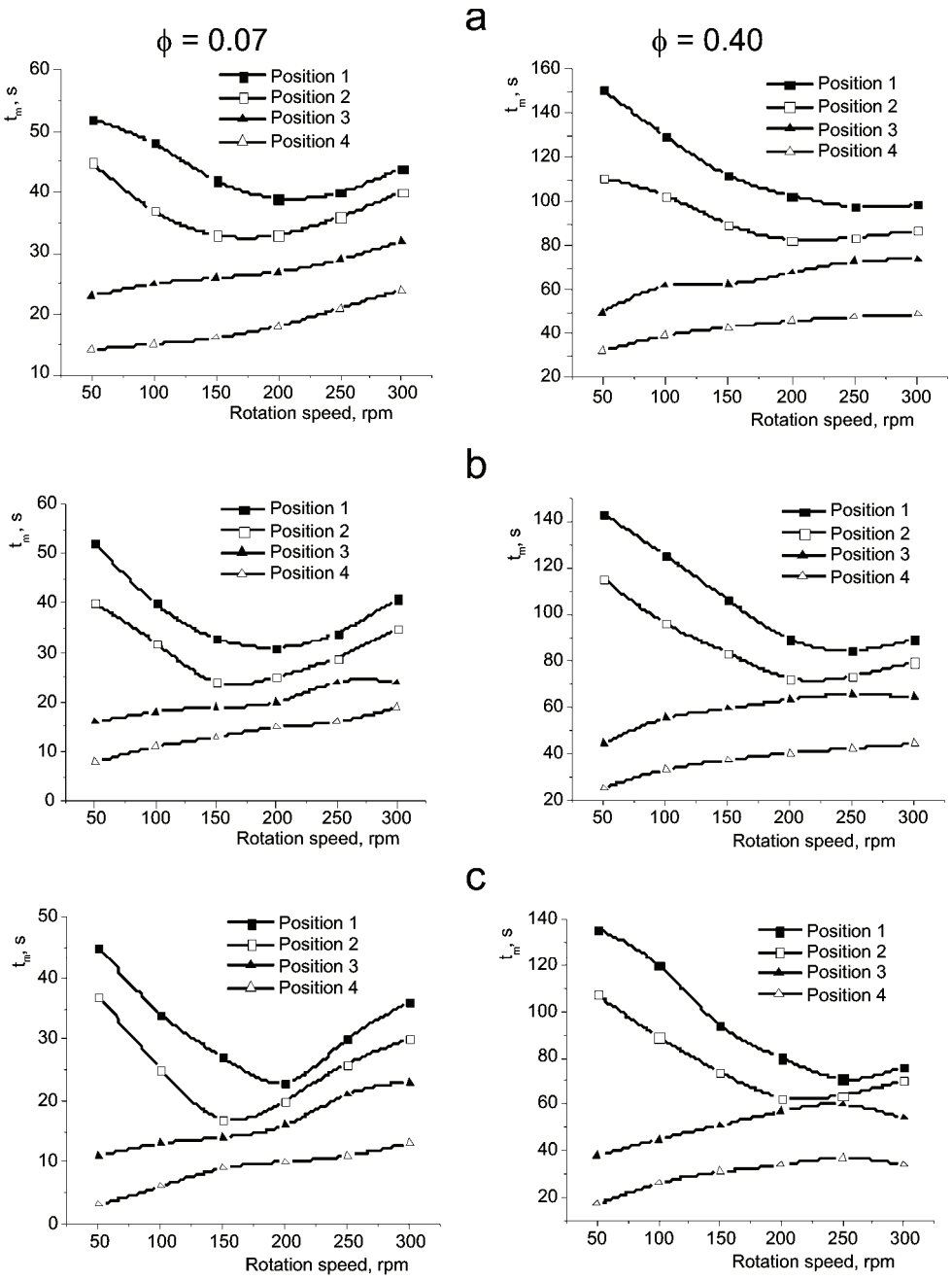

Figure 5. The influences of the rotation speed on mixing time at different sensor positions and biocatalysts concentration for the curved bladed turbine (particle diameter: a - 4, b - 4.6 and c - 5.2 mm).

Paddle with six blades

Due to the modification of the suspension concentration from the bioreactor bottom to the top and to the phenomena which appear during the suspension circulation, the differentiation of the correlations between the mixing time and the rotation speed on the bioreactor height, indifferent of the biocatalysts size can be observed in Figure 6.

For alginate particle concentration below 25 vol%, the variations of mixing time can be related to two regions, inferior and superior ones. Thus, for positions 1 and 2, by intensifying the rotation speed, the mixing time initially increases, reaches a maximum level and then decreases. The initial increase of the mixing time could be the result of the stream interactions with the bioreactor wall or/and baffles, the phenomenon that is amplified by the presence of the solid phase due to the collisions between the particles or with the bioreactor internal elements. These effect leads to the hindrance of the suspension circulation at lower rotation speed values. Over a certain level of the rotation speed, these influences are diminished and, conse-

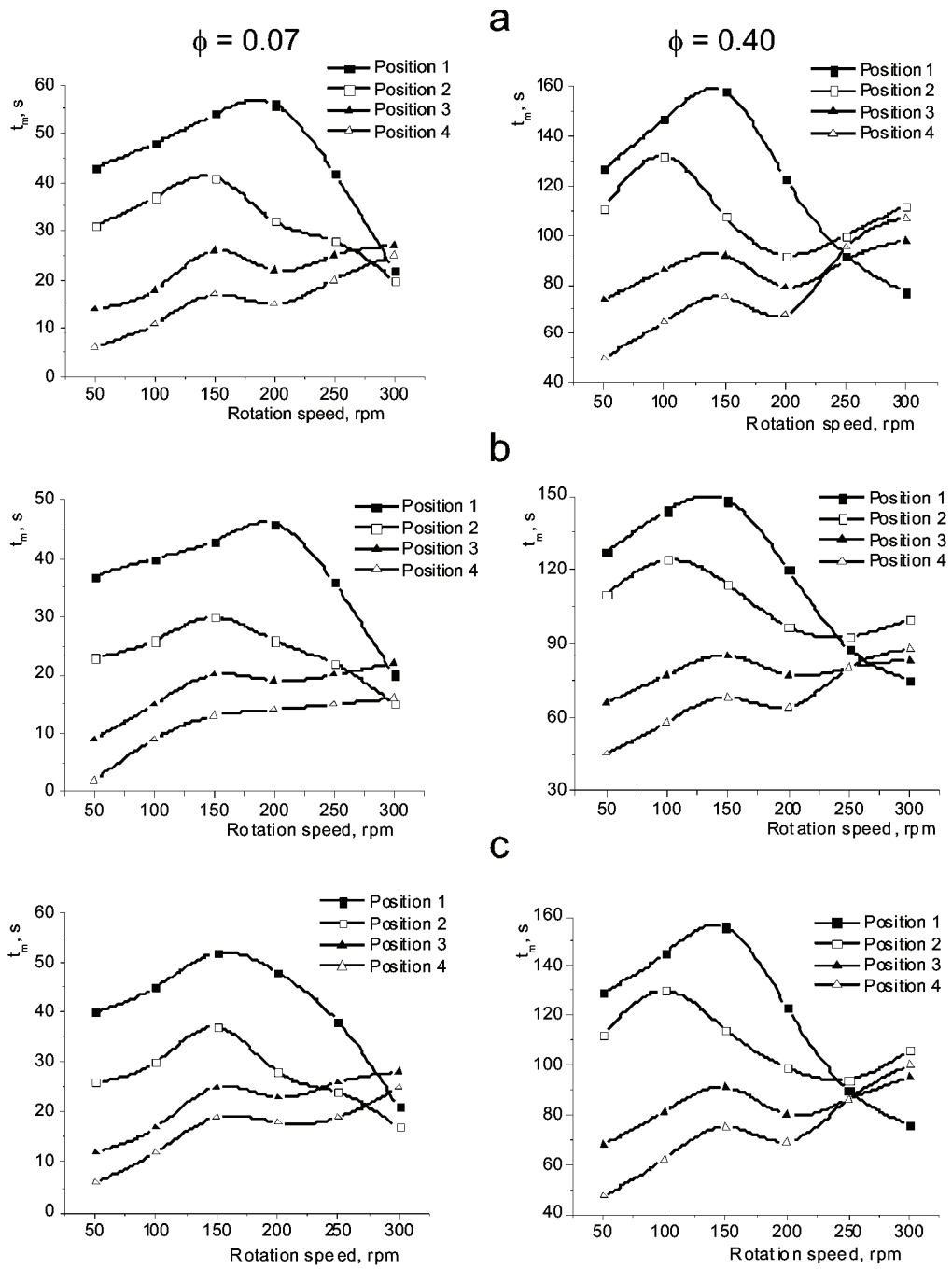

Figure 6. The influences of the rotation speed on mixing time at different sensor positions and biocatalysts concentration for the paddle with six blades (particle diameter: a - 4, b - 4.6 and c - 5.2 mm).

quently, the mixing time is reduced. In this domain of biocatalysts volumetric fraction, the maximum value of mixing time is reached at 200 rpm for position 1 and at lower rotation speed for position 2, owing to the more intense mixing in the region above the first impeller.

By increasing the volumetric fraction of the biocatalysts, the rotation speed corresponding to the minimum mixing efficiency from the inferior region is moved to lower values (for suspension concentration over 25 vol%, this rotation speed becomes 150 rpm for position 1 and 100 rpm for position 2, respectively). Contrary to the variation of the mixing time recorded for position 1, which maintains its general shape for the entire domain of biocatalysts concentration, the variation of the mixing time for position 2 is gradually changed with the increase of the biocatalyst concentration. Therefore, in the domain of alginate

particles volumetric fraction of 25-40% and the rotation speed over 200 rpm, the mixing time increases again, the evolution that is more pronounced for higher concentration of biocatalysts. This variation could be considered to be the consequence of the interferences of flow streams induced by the two impellers, cumulated with the hindrance of the suspension circulation by the baffles. This conclusion is supported by the absence of these effects in position 1, this region of the bioreactor being not provided with baffles and being not placed between the two impellers.

The shapes of the curves recorded for the superior positions 3 and 4 are similar, the increase of the biocatalysts concentration only amplifying some effects of the rotation speed, without any modification of the general variations. For both positions, the increase of the rotation speed induces the initial increase of mixing time, due to the dispersion of the solid phase from the bioreactor bottom. At 150 rpm, the mixing time reaches its maximum value, then decreasing due to the intensification of circulation also in the inferior region. This evolution has been recorded only for a restricted domain of the rotation speed (150-200 rpm), for higher values the mixing time increasing again. The further increase occurs concomitantly with the intensification of mixing in the inferior positions, this suggesting that the variation is the result of the increase of biocatalysts concentration in the positions 3 and 4 due to their dispersion from the inferior region. This effect is more important for position 4, which is placed at the longest distance from the second impeller and, consequently, the transmitted turbulence is considerably diminished. For this reason, for immobilized cells concentration over 25 vol%. and the rotation speed over 200 rpm, the mixing efficiency for position 4 becomes very close or inferior to that recorded for position 3.

In all studied cases, up to 250 rpm, the less efficient mixing has been obtained for position 1, due both of the higher concentration of biocatalysts, and to the implication of the “bottom effect” on the streams interference, cumulated with the particles collision and friction, as well as with their deposition [28]. The appearance of negative effects generated by the rotation speed intensification on the other positions, especially by increasing the solid phase concentration in these positions placed outside from the impellers region, leads to the increase of the relative mixing efficiency in position 1 for the rotation speed over 250 rpm. Moreover, the difference between the mixing time recorded for position 1 and those for the other three positions is accentuated with the increase of the suspension concentration.

Excepting the maximum values of the mixing time obtained for positions 1 and 2, the mixing intensity induced by the paddle with six blades is similar or rather superior to those promoted by the previously analyzed impellers. But, for the other two positions, the mixing efficiency is lower (the comparison with the pumper mixer and curved bladed turbine is not accurate, because they disperse poorly the biocatalysts in the superior region).

The shapes of the above discussed dependences are similar for all considered sizes of the alginate particles, but the recorded variation are more pronounced for the smallest and biggest particles (the negative phenomena of the particle interactions and their deposition are reciprocally compensated in the case of the biocatalysts with intermediary size).

The analysis of the influence of the rotation speed on mixing of immobilized yeasts cells suspension for the Rushton turbine [28] and paddle with six blades leads to the following comparative results:

- Position 1: the Rushton turbine is more efficient, indifferent of the size and volumetric fraction of the biocatalysts.
- Position 2: for solid phase concentration up to 15 vol%, the intensities of the mixing promoted by the two impellers are similar; for more concentrated suspensions, the paddle with six blades is recommended.
- Positions 3 and 4: although the mixing time increases with the increase of the rotation speed for the paddle with six blades, this impeller offers a more intense circulation of the suspension for the entire domain of the biocatalysts concentration and of the rotation speed.

The paddle with six blades can promote a uniform mixing in the whole volume of suspension at 250-300 rpm, the optimum rotation speed becoming lower with the increase of alginate particles concentration. These values of the rotation speed are higher than those required by the Rushton turbine for uniform mixing [28].

The lowest values of the mixing time have been reached for the biocatalysts particles with the intermediary diameter of 4.6 mm, due to the equilibrium established between the friction forces, specific to smaller particles with higher interfacial area, and deposition to the bioreactor bottom, specific to the bigger ones.

Pitched bladed turbine

Although this impeller generally induces an axial flow, in certain conditions it could generate a radial flow, similar to the above impellers. Thus, for the ratio between the impeller diameter, d, and the bioreactor diameter, D, greater than 0.2 and higher rotation

speed, the broth circulation is changed from an axial flow to a radial one [33]. Since for the used experimental equipment the ratio *d*/*D* is 0.36 [27,30], it could be assumed that the conditions for a radial circulation of the agitated liquid are respected.

Figure 7 indicates two types of correlations between the mixing time and the rotation speed, one corresponding to position 1, and the other for positions 2-4.

In the inferior region, position 1, indifferent of the biocatalysts size and concentration, the mixing time initially decreases with the increase of the rotation speed. Over a certain value of the rotation speed, which increases from 150 rpm, for alginate particles concentration below 15 vol%, to 200 rpm, for more concentrated suspensions, the mixing time increases due to the hindrance of the circulation in this region induced by the "bottom effect", particles collision and friction?

The variations of the mixing intensity for the other three positions are similar. Thus, by intensifying the rotation speed, the mixing time increases to a ma-

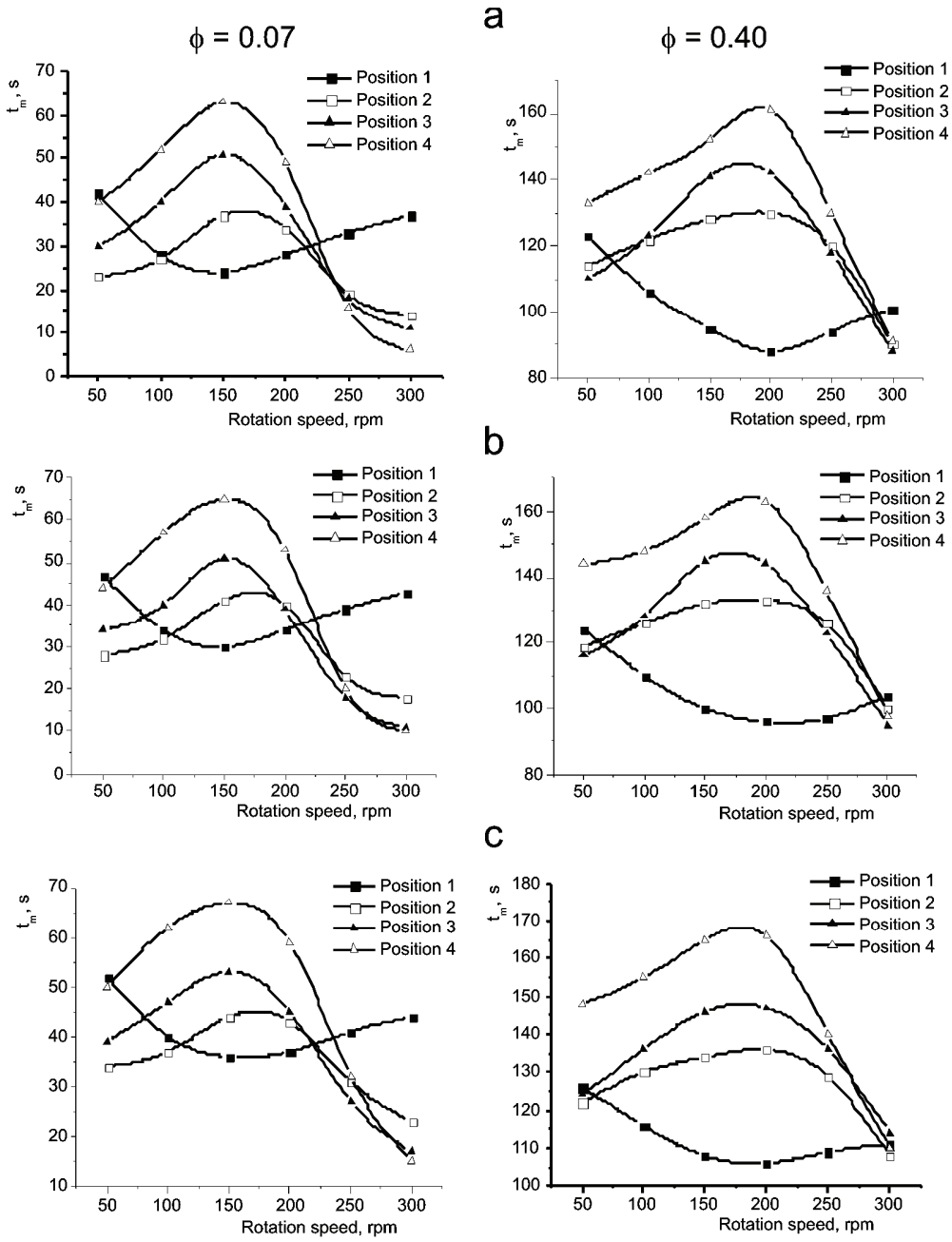

Figure 7. The influences of the rotation speed on mixing time at different sensor positions and biocatalysts concentration for the pitched bladed turbine (particle diameter: a - 4, b - 4.6 and c - 5.2 mm).

ximum value, being reduced then. The value of the rotation speed corresponding to the maximum level of mixing time can be related to the change of axial flow streams, less extended, to the radial ones, more extended and intense. This rotation speed is of 150 rpm for suspensions with volumetric fraction up to 25%, increasing to 200 rpm for higher concentrations of biocatalysts.

Contrary to the simulated fermentation broths, without a solid phase [26], at low rotation speed, the less efficient mixing of the immobilized yeasts cells suspensions is reached for position 4, due both to the rapid dispersion of the biocatalysts on the vertical direction through the axial flow streams, and to the longer distance from the impellers. For the same reasons, the mixing intensity in the position 3 is rather similar, but higher, to that recorded for the position 4, this position being closer to the impellers.

According as the flow streams become of radial type, at the rotation speed over 150-200 rpm, the circulation of the suspension in the superior region is more and more intensified, this region becoming the most efficient mixed for the rotation speed higher than 200-250 rpm.

The shape of the variation of mixing time with the rotation speed recorded for position 2 is similar to those for positions 3 and 4, above discussed. But, in this case the increase of mixing time is the result both of the vertical flow streams interference due to the axial circulation induced by the two impellers at lower rotation speed and of the interactions between the alginate particles. The modification of the flow streams from axial to radial, at higher rotation speed, leads to the intensification of the mixing also in this region.

Because the streams promoted by the pitched bladed turbine possess an important component of an axial type, especially at low rotation speed, the mixing efficiency is mainly controlled by the solid phase deposition. For this reason, the highest values of mixing time have been reached for the biocatalysts with the highest diameter. Moreover, the variation of mixing time *vs.* rotation speed for the particles with 5.2 mm diameter is more flattened.

By analyzing comparatively the mixing promoted by the pitched bladed turbine and the Rushton turbine for these suspensions of biocatalysts, the following conclusions have been drawn:

- Position 1: for the smallest particles of biocatalysts, indifferent of their concentration, the intensity of the mixing promoted by the pitched bladed turbine is comparable to that induced by the Rushton turbine; for bigger alginate particles, the Rushton turbine is more efficient, but any significant difference between the two impellers has been recorded.

- Position 2: the pitched bladed turbine offers a more intense circulation of the suspension only for the rotation speed domain over the value corresponding to the maximum of mixing time (150-200 rpm).

- Positions 3 and 4: for the entire domain of size and concentration of biocatalysts, the pitched bladed turbine is more efficient.

The experimental data suggested that the pitched bladed turbine can promote the uniform circulation in whole bulk of suspension in certain experimental conditions, the optimum rotation speed being of 200 rpm for biocatalysts concentration below 25 vol%, becoming 250-300 rpm for more concentrated suspensions.

MATHEMATICAL CORRELATIONS FOR MIXING TIME

By means of the experimental data, mathematical correlations which describe the influence of size and volumetric concentration of biocatalysts particles, as well as of the rotation speed on the mixing time have been established for the six investigated radial impellers. The explicit values of the coefficients were calculated for each considered positions by the multi-regression method using MATLAB software. Thus, the following correlations have been obtained:

Disperser sawtooth

Position 1:

$$t_m = -0.21\frac{\Phi^{1.251}N^3}{d_p^{0.635}} + 1.974\times10^2\frac{e^{1.735}N^2}{d_p^{1.123}} + 2.179\times10^2\frac{N}{d_p^{0.324}} \quad (2)$$

Position 2:

$$t_m = -3.51\frac{\Phi^{0.811}N^3}{d_p^{0.694}} + 45.06\frac{e^{1.7114}N^2}{d_p^{1.026}} + 5.835\times10^2\frac{N}{d_p^{0.815}} \quad (3)$$

Position 3:

$$t_m = 15.974\frac{d_p^{1.022}\Phi^{1.765}}{N^{0.415}} \quad (4)$$

Position 4:

$$t_m = 18.058\frac{d_p^{1.183}\Phi^{2.154}}{N^{0.445}} \quad (5)$$

Smith turbine

Position 1:

$$t_m=-1.2\times10^{-2}\frac{\Phi^{1.851}N^3}{d_p^{0.335}}-15.853\frac{e^{0.135\Phi}N^2}{d_p^{0.723}}+ +1.461\times10^2\frac{N}{d_p^{0.524}} \quad (6)$$

Position 2:

$$t_m=-0.293\frac{\Phi^{0.851}N^3}{d_p^{0.635}}-74.136\frac{e^{1.435\Phi}N^2}{d_p^{1.223}}+ +1.739\times10^2\frac{N}{d_p^{0.724}} \quad (7)$$

Position 3:

$$t_m=4.859\times10^2\frac{d_p^{0.688}\Phi^{0.205}}{N^{0.283}} \quad (8)$$

Position 4:

$$t_m=8.533\times10^2\frac{d_p^{0.666}\Phi^{0.344}}{N^{0.269}} \quad (9)$$

Pumper mixer

Position 1:

$$t_m=-1.8\times10^{-2}\frac{\Phi^{1.352}N^3}{d_p^{0.325}}-1.801\times10^2\frac{e^{1.234\cdot\Phi}N^2}{d_p^{0.896}}+ +2.022\times10^2\frac{N}{d_p^{0.432}} \quad (10)$$

Position 2:

$$t_m=0.669\frac{\Phi^{0.765}N^3}{d_p^{0.639}}-2.945\times10^2\frac{e^{1.428\cdot\Phi}N^2}{d_p^{1.264}}+ +2.113\times10^2\frac{N}{d_p^{0.784}} \quad (11)$$

Position 3:

$$t_m=2.8\times10^{-3}\frac{d_p^{0.693}N^{0.212}}{\Phi^{1.892}} \quad (12)$$

Position 4:

$$t_m=9.3\times10^{-4}\frac{d_p^{0.788}N^{0.43}}{\Phi^{2.445}} \quad (13)$$

Curved bladed turbine

Position 1:

$$t_m=0.142\frac{\Phi^{0.781}N^3}{d_p^{0.866}}-2.406\times10^2\frac{e^{1.314\cdot\Phi}N^2}{d_p^{1.306}}+ +2.674\times10^2\frac{N}{d_p^{0.715}} \quad (14)$$

Position 2:

$$t_m=0.302\frac{\Phi^{0.81}N^3}{d_p^{0.736}}-1.906\times10^2\frac{e^{1.224\cdot\Phi}N^2}{d_p^{1.196}}+ +1.778\times10^2\frac{N}{d_p^{0.685}} \quad (15)$$

Position 3:

$$t_m=1.17\times10^{-2}\frac{d_p^{0.633}N^{0.226}}{\Phi^{1.638}} \quad (16)$$

Position 4:

$$t_m=8.6\times10^{-4}\frac{d_p^{0.654}N^{0.432}}{\Phi^{2.434}} \quad (17)$$

Paddle with six blades

Position 1:

$$t_m=-1.737\frac{\Phi^{0.863}N^3}{d_p^{0.725}}+14.249\frac{e^{1.184\cdot\Phi}N^2}{d_p^{1.241}}+ +3.488\times10^2\frac{N}{d_p^{0.644}} \quad (18)$$

Position 2:

$$t_m=-1.125\frac{\Phi^{0.829}N^3}{d_p^{0.646}}+60.216\frac{e^{1.152\cdot\Phi}N^2}{d_p^{1.183}}+ +1.394\times10^2\frac{N}{d_p^{0.67}} \quad (19)$$

Position 3:

$$t_m=46.693\frac{d_p^{0.771}N^{0.23}}{\Phi^{0.163}} \quad (20)$$

Position 4:

$$t_m=44.098\frac{d_p^{0.929}N^{0.583}}{\Phi^{0.104}} \quad (21)$$

Pitched bladed turbine

Position 1:

$$t_m=0.591\frac{\Phi^{0.913}N^3}{d_p^{0.873}}-282.974\frac{e^{1.141\Phi}N^2}{d_p^{1.286}}+ 153.180\frac{N}{d_p^{0.665}} \quad (22)$$

Position 2:

$$t_m=-0.941\frac{\Phi^{0.856}N^3}{d_p^{0.699}}-40.757\frac{e^{1.202\cdot\Phi}N^2}{d_p^{1.358}}+ +1.569\times10^2\frac{N}{d_p^{0.625}} \quad (23)$$

Position 3:

$$t_m = 9.477\times10^3 \frac{d_p^{0.932}\Phi^{0.616}}{N^{0.222}} \quad (24)$$

Position 4:

$$t_m = 42.544\times10^4 \frac{d_p^{0.832}\Phi^{0.762}}{N^{0.352}} \quad (25)$$

The proposed equations offer a good concordance with the experimental data, the average deviations being as follows: ±12.1% for disperser sawtooth, ±8.5% for Smith turbine, ±10.3% for pumper mixer, ±9.1% for curved bladed turbine, ±8.4% for paddle with six blades and ±7.9% for pitched bladed turbine.

CONCLUSIONS

By analyzing comparatively the mixing intensity and its distribution into a bioreactor with stirred bed of yeast cells immobilized in alginate (particles with 4, 4.6 and 5.2 mm diameters) using six radial impellers (disperser sawtooth, Smith turbine, pumper mixer, curved bladed turbine, paddle with six blades, pitched bladed turbine) *vs.* Rushton turbine, the following conclusions can be drawn:

1. The less efficient impeller was the disperser sawtooth, especially due to the low pumping capacity which cannot avoid the solid phase deposition at the bioreactor bottom. Therefore, the increase of the biocatalysts size led to the significant reduction of the mixing efficiency.

2. The Smith turbine offers the most efficient mixing for a large domain of the biocatalyst concentration and rotation speed. It can also induce a uniform circulation of the suspension for certain values of the rotation speed and biocatalysts volumetric fraction up to 15%, similar to the Rushton turbine.

The most efficient mixing has been obtained for biocatalyst particles with 4.6 mm diameter, due to the equilibrium existing between the friction forces, specific to smaller particles, and deposition to the bioreactor bottom, specific to the bigger ones.

4. Over the biocatalysts concentration of 15 vol%, the curved bladed turbine induces more efficient mixing than the Rushton turbine.

5. Contrary to the disperser sawtooth and Smith turbine, the increase of biocatalysts size exhibits a favorable effect on the mixing efficiency promoted by the pumper mixer or curved bladed turbine, owing to the friction forces between the alginate particles and to the particles collision which control the suspension circulation at low diameter of alginate particles.

6. Comparatively to the above mentioned four impellers, the analysis of the efficiency of the paddle with six blades and pitched bladed turbine *vs.* Rushton turbine underlined that the intensity of the mixing induced by the first two impellers is closer to that promoted by the Rushton turbine. Furthermore, both impellers can offer a uniform mixing of the suspension for the rotation speed over 200-250 rpm.

Without taking into account the power consumption and the shear effect on biocatalysts, which constitute the aims of the future studies, the impellers of Smith turbine, paddle with six blades and pitched bladed turbine types could represent an advantageous alternative for mixing the beds with immobilized cells.

7. Using the experimental data, some mathematical correlations for the mixing time have been established for each studied impeller. The proposed equations offer a good prediction of real behavior, the average deviations varying between ±7.9% for pitched bladed turbine and: ±12.1% for disperser sawtooth.

Notations

d - Impeller diameter, m
d_p - Biocatalyst particle diameter, m
d' - Oxygen electrode diameter, m
D - Bioreactor diameter, m
h - Distance from the inferior stirrer to the bioreactor bottom, m
H - Bioreactor height, m
l - Impeller blade length, m
l' - Oxygen electrode immersed length, m
s - Baffle width, m
w - Impeller blade height, m
t_m - Mixing time, s
ϕ - Biocatalysts volumetric fraction

Acknowledgement

This work was included in the Grant PNCDI II 21-048/2007 supported by the National Centre for Programs Management (CNMP).

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ANCA-IRINA GALACTION[1]
ANCA-MARCELA LUPĂŞTEANU[2]
MARIUS TURNEA[1]
DAN CAŞCAVAL[2]

[1]University of Medicine and Pharmacy "Gr.T. Popa" of Iasi, Faculty of Medical Bioengineering, Dept. of Biotechnologies, Iasi, Romania
[2]Technical University "Gh. Asachi" of Iasi, Faculty of Chemical Engineering and Environmental Protection, Dept. of Biochemical Engineering, Iasi, Romania

NAUČNI RAD

KOMPARATIVNA ANALIZA EFIKASNOSTI MEŠANJA U BIOREAKTORIMA SA RADIJALNIM MEŠALICAMA I SUSPENDOVANIM IMOBILISANIM ĆELIJAMA

U radu je analiziran uticaj glavnih faktora na efiksanost mešanja i uniformnost suspenzije u bioreaktoru sa suspendovanim imobilisanim ćelijama kvasca S. cerevisiae na alginatu (prečnik čestica biokatalizatora: 4,0, 4,6 and 5,2 mm), koji je opremljen radijalnom mešalicom tipa zupčaste turbinske, Smitove turbinske, turbinske sa ravnim ili zakrivljenim lopaticama ispod diska, lopataste sa 6 lopatica ili sa 4 iskošene lopatice ili Ruštonove turbinske mešalice. Pokazano je da su u pogledu intenziteta mešanja i uniformnosti cirkulacije suspenzije najefiksnije mešalice Smitova turbinska, lopatasta sa 6 lopatica i lopatasta sa 4 iskošene lopatice. Za svaku ispitivanu mešalicu određene su matematičke jednačine koje opisuju uticaj glavnih faktora na vreme mešanja, koje se dobro slažu sa eksperimentalnim podacima (srednje devijacije variraju od ±7,9% za lopatastu mešalicu sa 4 iskošene lopatice do ±12,1% za zupčaste turbinsku mešalicu).

Ključne reči: bioreaktor; suspenzija; imobilisane ćelije; vreme mešanja; radijalne mešalice.