Comparative evaluation of radial impellers efficiency for bioreactors with stirred bed of immobilized cells
1. Disperser sawtooth and smith turbine

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Abstract

The influences of the main factors on mixing efficiency and distribution for a bioreactor with stirred bed of S. cerevisiae immobilized cells in alginate (biocatalysts with 4, 4.6 and 5.2 mm diameters) have been comparatively analyzed for three radial impellers: disperser sawtooth, Smith turbine and Rushton turbine. The most efficient impeller, from the viewpoint of intensity and uniformity of suspension circulation, was found to be the Smith turbine. The optimum size of alginate particles was of 4.6 mm, because it compensates the friction specific to the smaller particles and the deposition of the bigger particles, the values of mixing time recorded for this size of biocatalysts using the Smith turbine being the lowest ones.

Keywords: bioreactor, stirred bed, immobilized cells, yeasts, mixing, mixing time, radial impeller, Rushton turbine, disperser sawtooth, Smith turbine.

Introduction

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 are rather similar with the second ones, they offer important advantages, namely as: the increase of the thermal, chemical and to the shear forces resistance of the biocatalysts, the increase of the number of the repeated biosynthesis cycles using the same particles of biocatalysts, the easier recovery of the 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 some 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 difference between the constructions of the two types of bioreactors consists on 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 that are 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], concentration of enzymes/cells into the particles [8,12,13], feed strategy [9,14,15]), among them the mixing efficiency and its distribution being the most important [16].

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

Because 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 mixing efficiency and distribution are required for process optimization.

One of the most useful criteria for characterization of the mixing intensity is the mixing time, t_m, defined as the time needed to reach a given mixing intensity at a given scale, when starting from the completely segregated situation [1,23]. This parameter offers specific informations 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 micromixing [23]. It can indicate the optimum hydrodynamic regime, the stirrer type that has to be used, or can predict the modification of mixing efficiency induced by scaling-up [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, power consumption or shear stress for different double radial stirrers, the following optimum combinations of impellers were selected for simulated broths: disperser sawtooth and paddle with six blades for water, pitched bladed turbine and Rushton turbine for broths with viscosity up to 30 cP, pumper mixer and disperser sawtooth for broths with higher viscosity [26].

By means of the previous results, the development of a comparative evaluation 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, power consumption and shear effect on biocatalysts particles have to be taken into account.

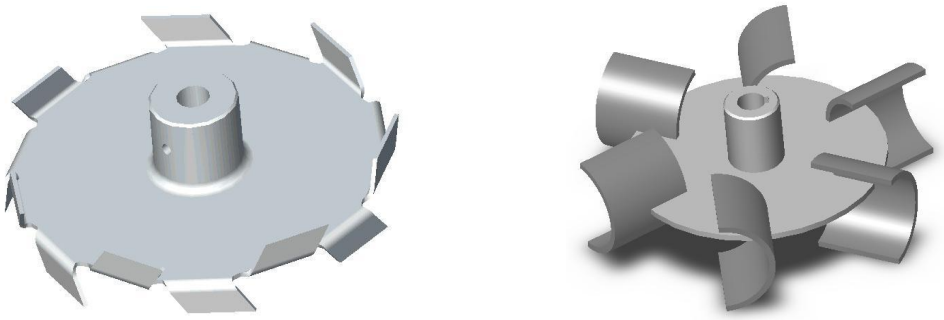
In this context, the aim of our experiments is to comparatively study the efficiency of mixing for a bioreactor with stirred bed of immobilized yeasts cells equipped with different radial impellers. This analysis will be made by means of the mixing time distribution obtained by vertically changing the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

Due to the large amount of experimental data, this study is constituted in four parts. In this one, the results obtained for the disperser sawtooth and Smith turbine are discussed.

Materials and method

The experiments have been carried out in 5 l (4 l working volume, ellipsoidal bottom) laboratory bioreactor (Biostat A, B. Braun Biotech International), with computer-controlled and recorded parameters. The bioreactor characteristics and operating parameters have been presented in the previous papers [27].

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



Disperser Sawtooth Smith Turbine

Figure 1. The radial impellers used in experiments.

The diameter of the two impellers on the shaft, d, was of 64 mm. The inferior impeller has been 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 form the Ruston turbine, as it was demonstrated in the previous works [28]. The rotation speed was maintained between 500 and 300 rpm, domain that avoids the "cave" formation at the broths surface and mechanical disruption of the biocatalysts particles.

In the experiments, *S. cerevisiae* cells immobilized on alginate have been used. The immobilization have been carried out by cells inclusion into the alginate matrix, according with the method given in literature [29]. The following diameters of the biocatalyst spherical particles have been obtained: 4, 4.6 and 5.2 mm. The volumetric fraction of the immobilized cells into the media varied between 7 and 40%.

The experiments have been carried out at a temperature of 25^{o}C. Any mechanical damage of the biocatalyst due to the shear forces was recorded during the experiments.

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

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

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, placed vertically from the bioreactor bottom as follows (Figure 2):

- 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.

Results and discussion

The previous studies on mixing inside of the bioreactors with stirred bed of *S. cerevisiae* immobilized 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 suspension concentration. For biocatalysts with intermediary size (particle diameter of 4.6 mm) the uniform circulation of the suspension have 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].

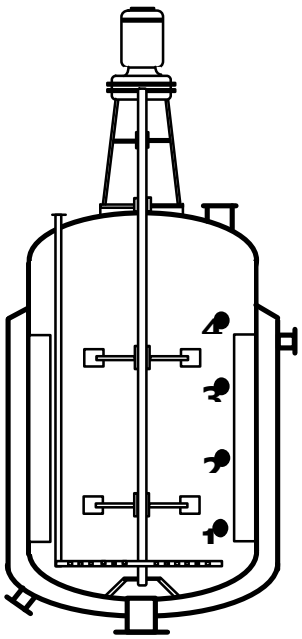


Figure 2. The considered positions inside the bioreactor.

These experiments are carried out in the similar manner for another two types of radial impellers, the disperser sawtooth and the Smith turbine, for selecting the optimum mixing system for systems containing suspensions of immobilized yeasts.

1. Disperser sawtooth

From Figures 3-5 important differences can be seen between the variations of mixing time recorded for the four considered positions inside the bioreactor. 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.

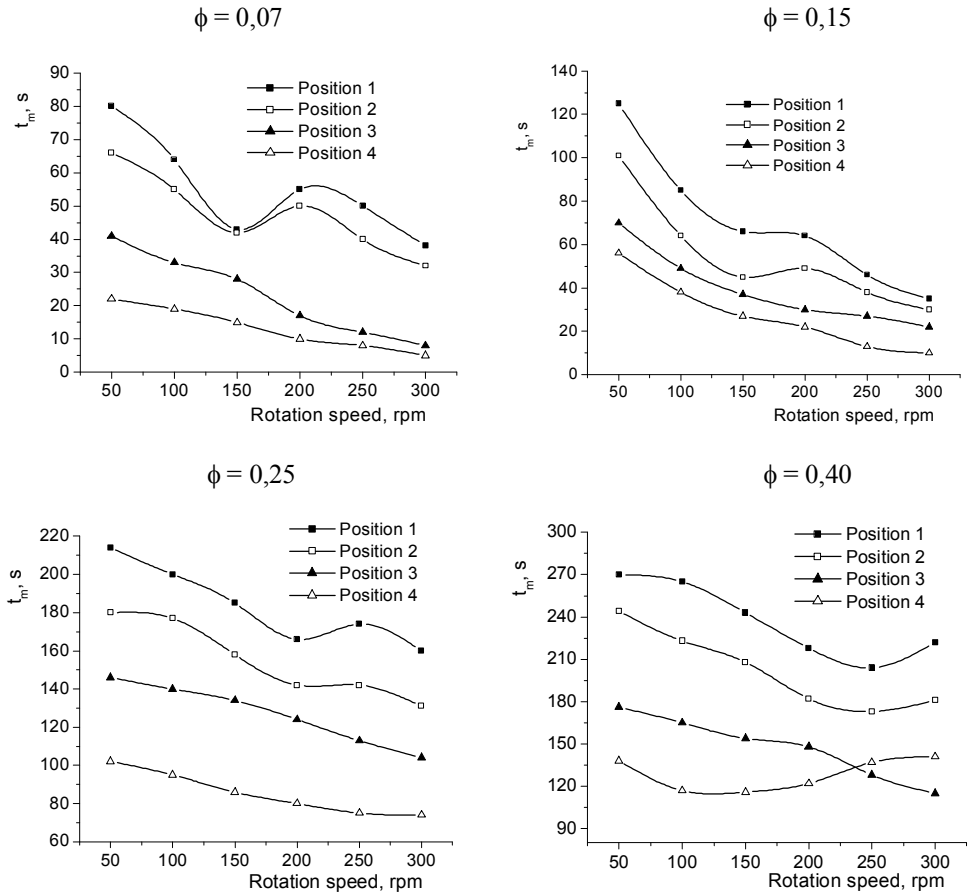


Figure 3. The influences of rotation speed on mixing time at different sensor positions and biocatalysts concentration for the disperser sawtooth (particle diameter of 4 mm).

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 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 of alginate particles, the rotation speed which correspond to the two extremes are 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.

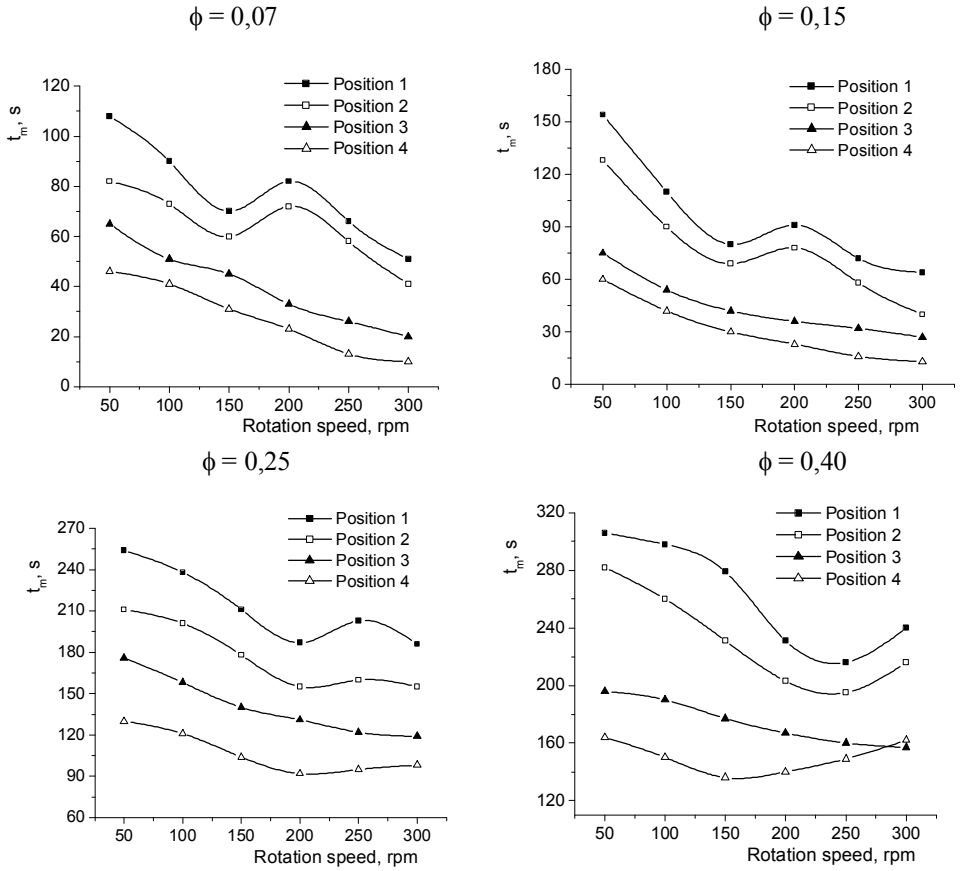
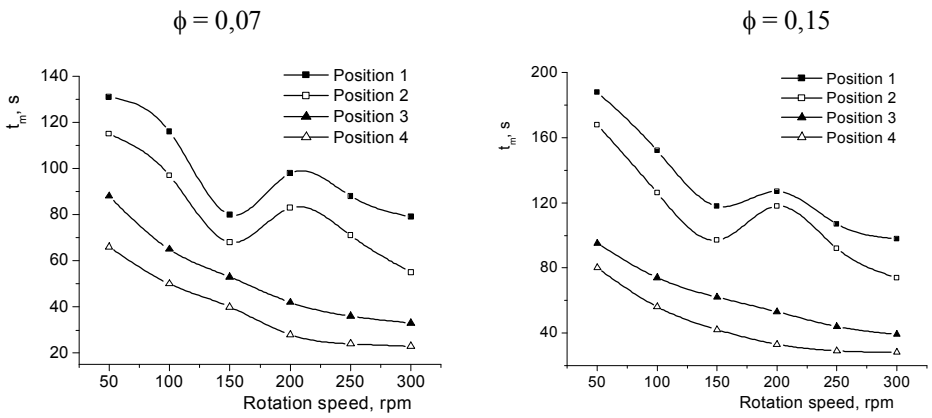


Figure 4. The influences of rotation speed on mixing time at different sensor positions and biocatalysts concentration for the disperser sawtooth (particle diameter of 4.6 mm).

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 variation 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 mixing time, this parameter increasing then. 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.



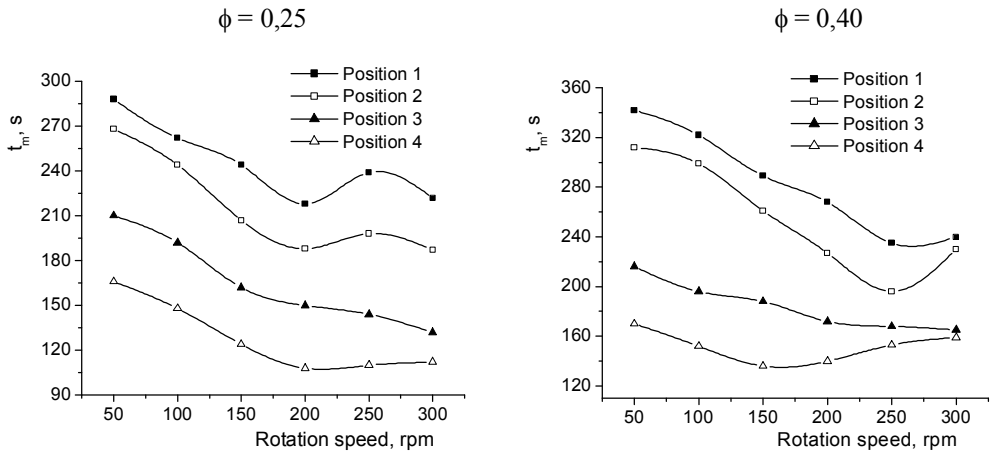


Figure 5. The influences of rotation speed on mixing time at different sensor positions and biocatalysts concentration for the disperser sawtooth (particle diameter of 5.2 mm).

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 solid phase is in the inferior region of the bioreactor. For this reason, the biggest values of mixing time have been recorded for positions 1 and 2, respectively. The particular variation of mixing time corresponding to the inferior region can be the result of the interference of the streams induced by the impellers placed at 0.5d on the shaft, phenomenon that is amplified by the "bottom effect" and solid phase collision. Therefore, the hindrance of suspension circulation is more important than for the simulated broths without 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 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 discussed results, the particular variation recorded for position 4 is due especially to the deposition of solid phase and less to the friction forces between the alginate particles, 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 mixing efficiency and its distribution in suspensions of immobilized *S. cerevisiae* in alginate for a double stirrer

of Rushton turbine type [28] and one of disperser sawtooth type, 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. Thus, for biocatalysts particles with 4 mm diameter and 7% vol. concentration, the disperser sawtooth is more efficient for the entire domain of rotation speed, and only for rotation speed over 100 rpm for more concentrated suspensions. For particles with diameter of 4.6 mm, this stirrer is more efficient only for rotation speed higher than 150 rpm for biocatalysts concentration up to 7% vol., and only for rotation speed over 200 rpm for higher concentration of biocatalysts. In the case of the biggest particles, the disperser sawtooth generates a more intense circulation only for rotation speed over 200 rpm, if the suspension concentration is below 15% vol. For solid phase concentration over 15% vol., the Rushton turbine becomes more efficient not depending on particles size.
- **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 solid phase dispersed in the superior region.

The Figure 6 doesn't indicate any possibility to reach an uniform mixing in the whole bulk volume of the suspension if the disperser sawtooth is used. 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 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.

2. 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 disperser sawtooth, the correlations between the mixing time and 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 (Figures 7-9).

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, decreasing strongly then. 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 streams interference and solid phase collision with the baffles or bioreactor wall. The relative importance of these phenomena is maximum at 150 rpm, for the rest of the rotation speed domain the mixing intensity for position 2 being closer or superior to that obtained for position 1.

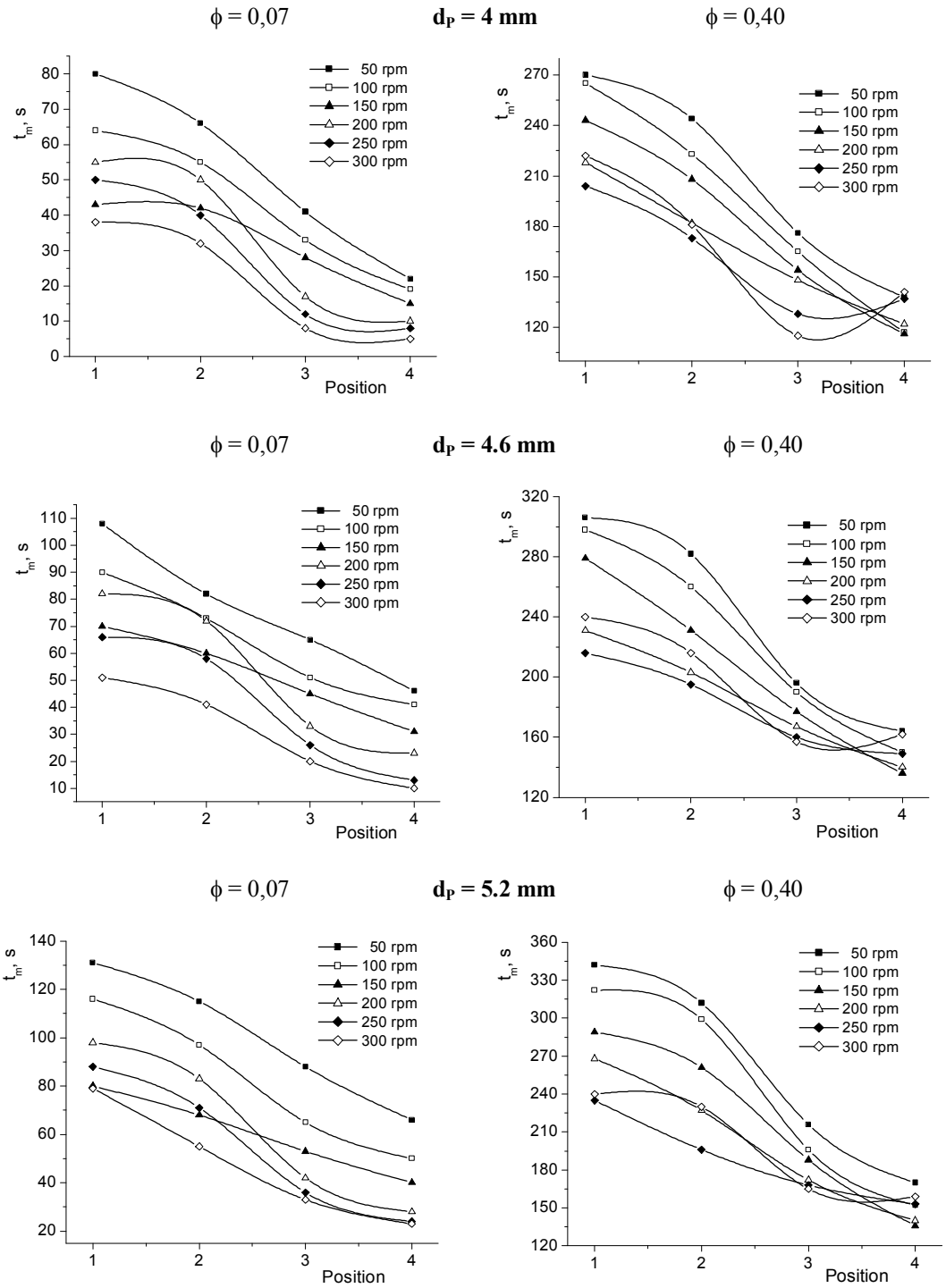


Figure 6. Variation of mixing time with the position inside the biocatalysts suspension for the disperser sawtooth.

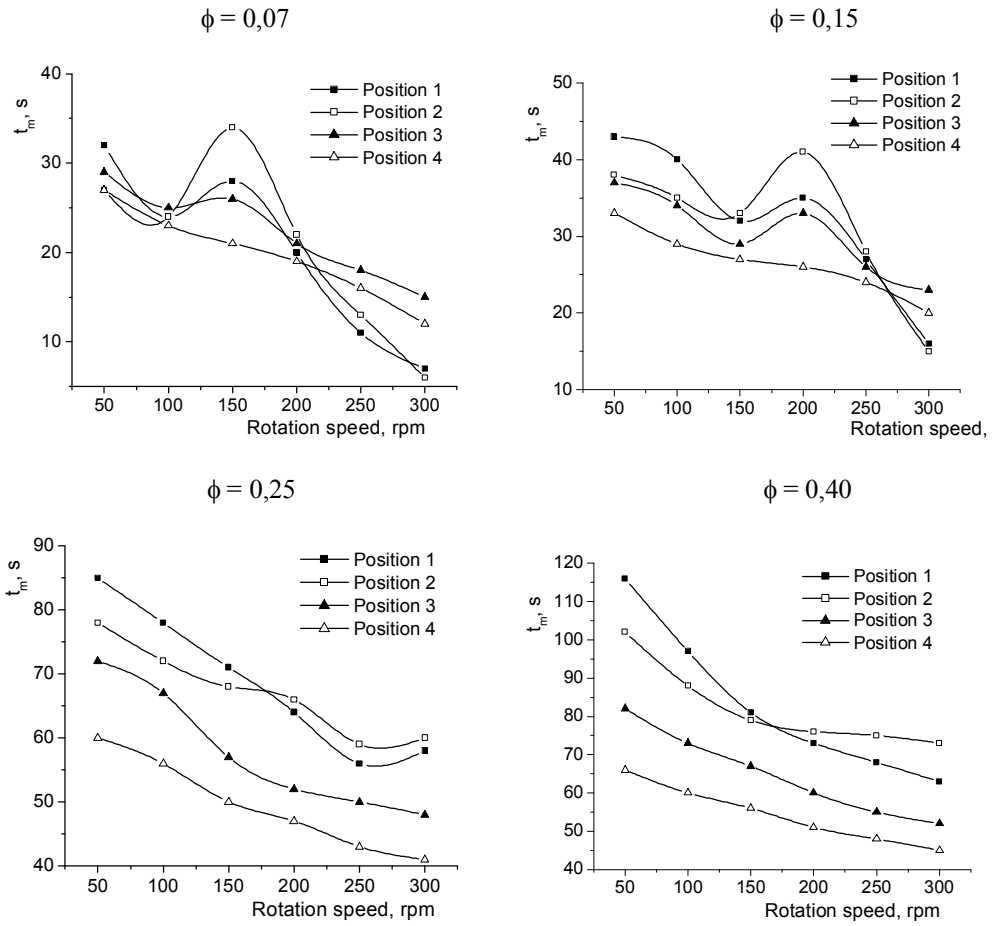
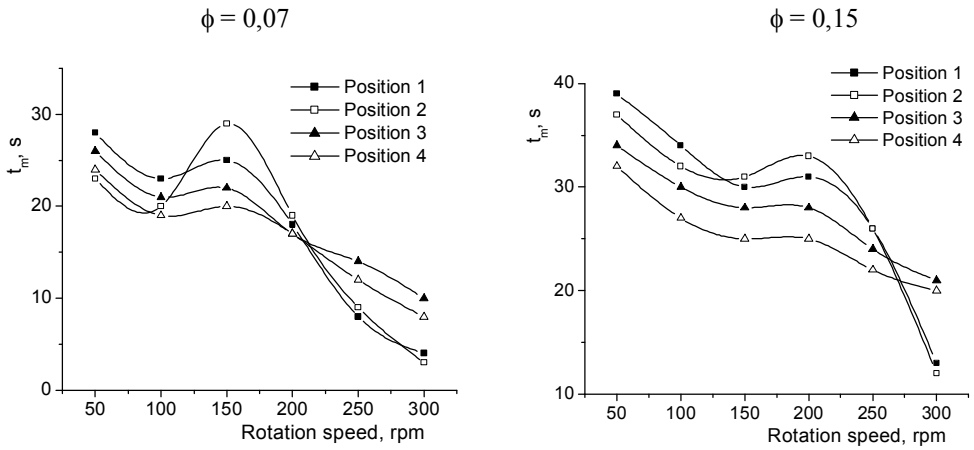


Figure 7. The influences of rotation speed on mixing time at different sensor positions and biocatalysts concentration for the Smith turbine (particle diameter of 4 mm).

The variation of mixing time for position 3 is similar, but the influence of the mentioned phenomena is significantly attenuated, owing to the distance from the bottom region with concentrated deposit of solid phase. By intensifying the agitation, the biocatalysts are dispersed also in the superior region, and, consequently, their volumetric fraction increases in region 3. For 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.



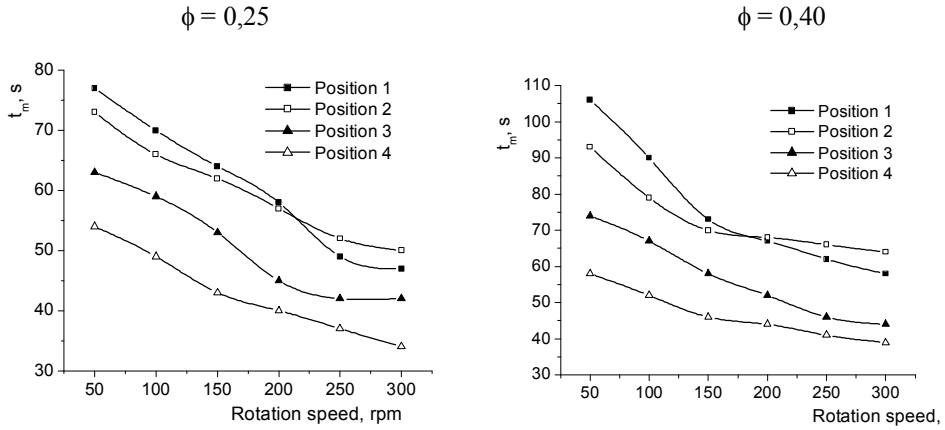
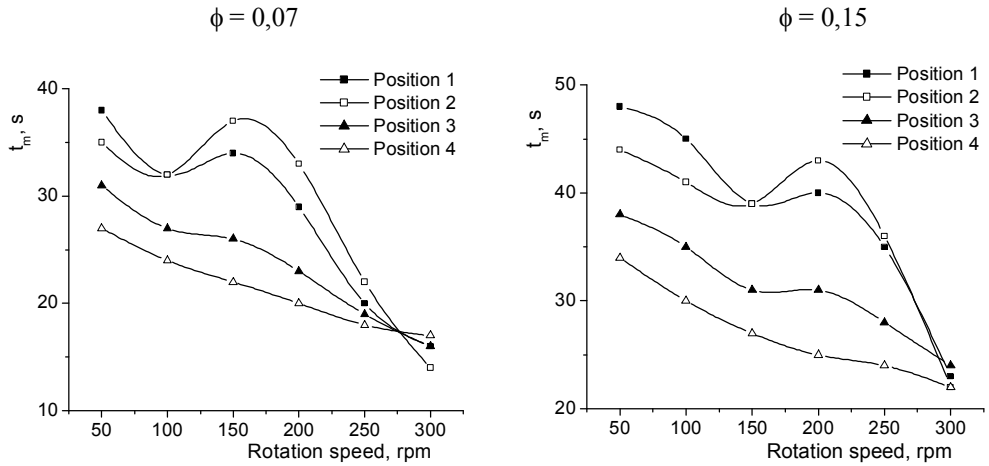


Figure 8. The influences of rotation speed on mixing time at different sensor positions and biocatalysts concentration for the Smith turbine (particle diameter of 4.6 mm).

The mixing for the position 4 is continuously improved by rotation speed increasing, but it becomes less efficient compared with the positions 1 and 2 for rotations 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 rotation speed. This result can be attributed either to the lower concentration of biocatalysts in this region, and to the propagation in the position 3 of the negative effect of streams interference and particles collision.



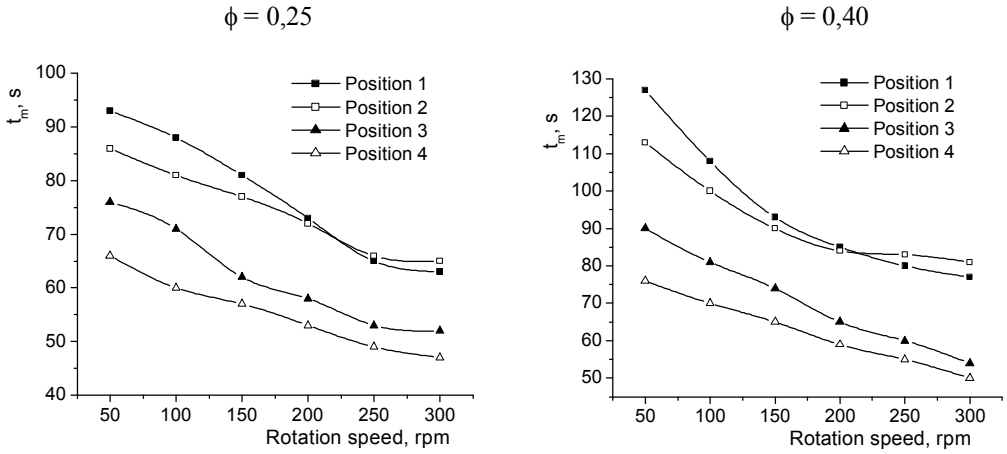


Figure 9. The influences of rotation speed on mixing time at different sensor positions and biocatalysts concentration for the Smith turbine (particle diameter of 5.2 mm).

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 of 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 rotation speed, this underlining that 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 concentrated suspensions of biocatalysts. For the same reason, the mixing time obtained for position 4 continuously decreases with rotation speed acceleration and reaches the lower values comparatively with the other three positions.

For biocatalysts 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 rotation speed. The lowest values of mixing time have been recorded for position 4. On the other hand, for rotation speed below 200 rpm the highest mixing time values have been obtained for position 1, over this level of rotation speed for position 2. 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, 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 and of their deposition can induce 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 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. For more concentrated suspensions, the Smith turbine induces a more intense circulation only for biocatalysts with 4 and 5.2 mm diameters and rotation speed up to 150-200 rpm, then the mixing efficiency becoming similar to that of Rushton turbine. But, for the biocatalysts with intermediary size (4.6 mm), the Rushton turbine is recommended for rotation speed over 100 rpm and volumetric fraction higher than 25%.
- **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. For the biggest alginate particles, this impeller induces an intense mixing only for solid phase concentration over 25% vol. and rotation speed below 150 rpm, the mixing efficiency being rather similar to that promoted by the Rushton turbine in the rest. But, the efficiency of the Smith turbine for particles with diameter of 4.6 mm is the same as in the position 1.
- **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, both correlated with the biocatalysts size, as in the case of Rushton turbine (Figure 10). 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 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 concentration below 7% at 250 rpm.

Because the biocatalyst with intermediary diameter of 4.6 mm diminishes the friction forces between the particles compared with the smaller biocatalysts, and the deposition tendency compared with the bigger ones, the lowest values of mixing time have been recorded for these size of alginate particles (Figure 11).

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 three radial impellers (disperser sawtooth, Smith 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 reduce of the mixing efficiency.

2. The Smith turbine offers the most efficient mixing for a large domain of biocatalysts concentration and rotation speed. It can also induce an uniform circulation of the suspension for certain values of 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.

These studies will be developed for other radial impellers that could be used for bioreactors with stirred bed of immobilized cells.

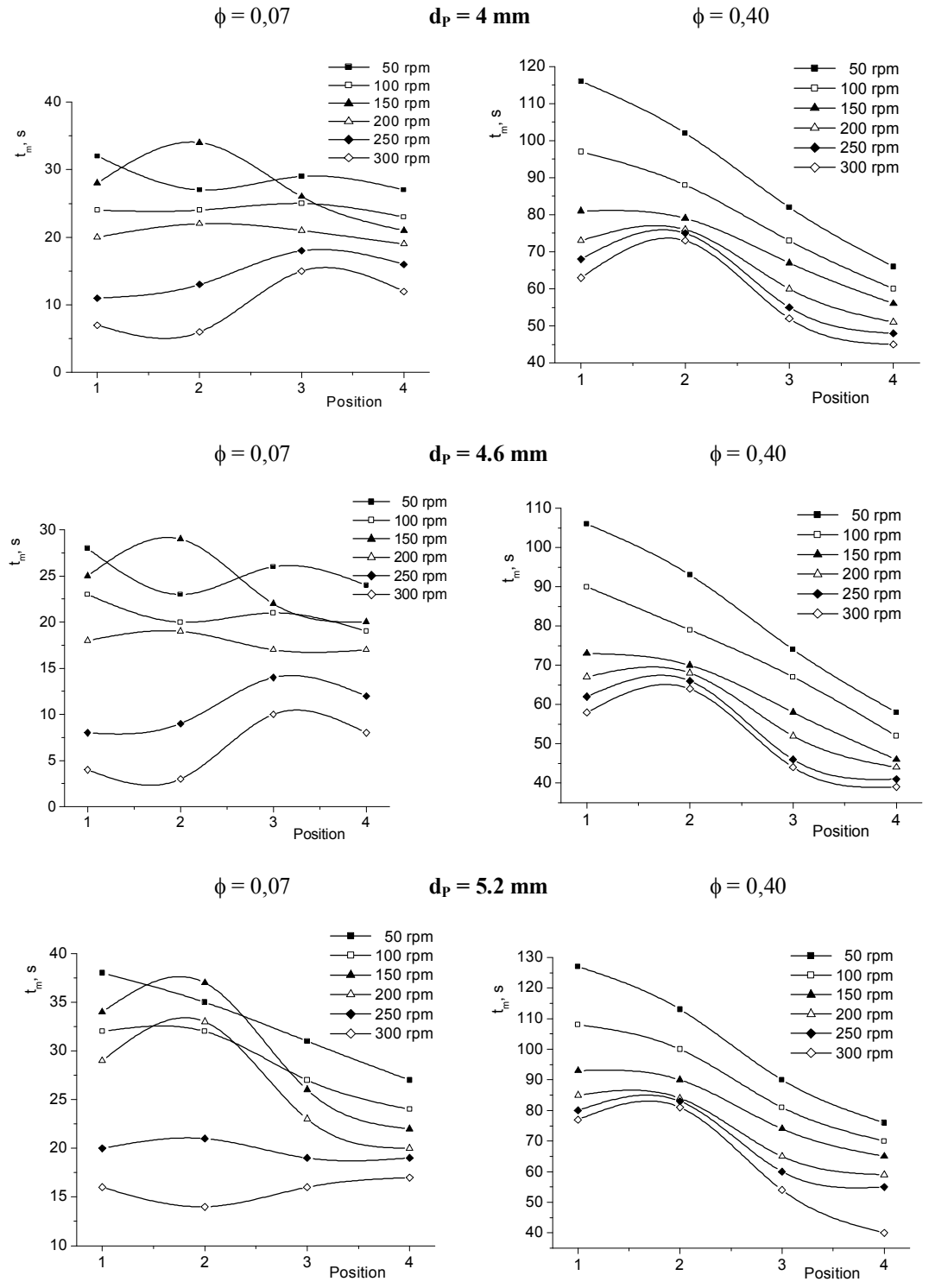


Figure 10. Variation of mixing time with the position inside the biocatalysts suspension for the Smith turbine.

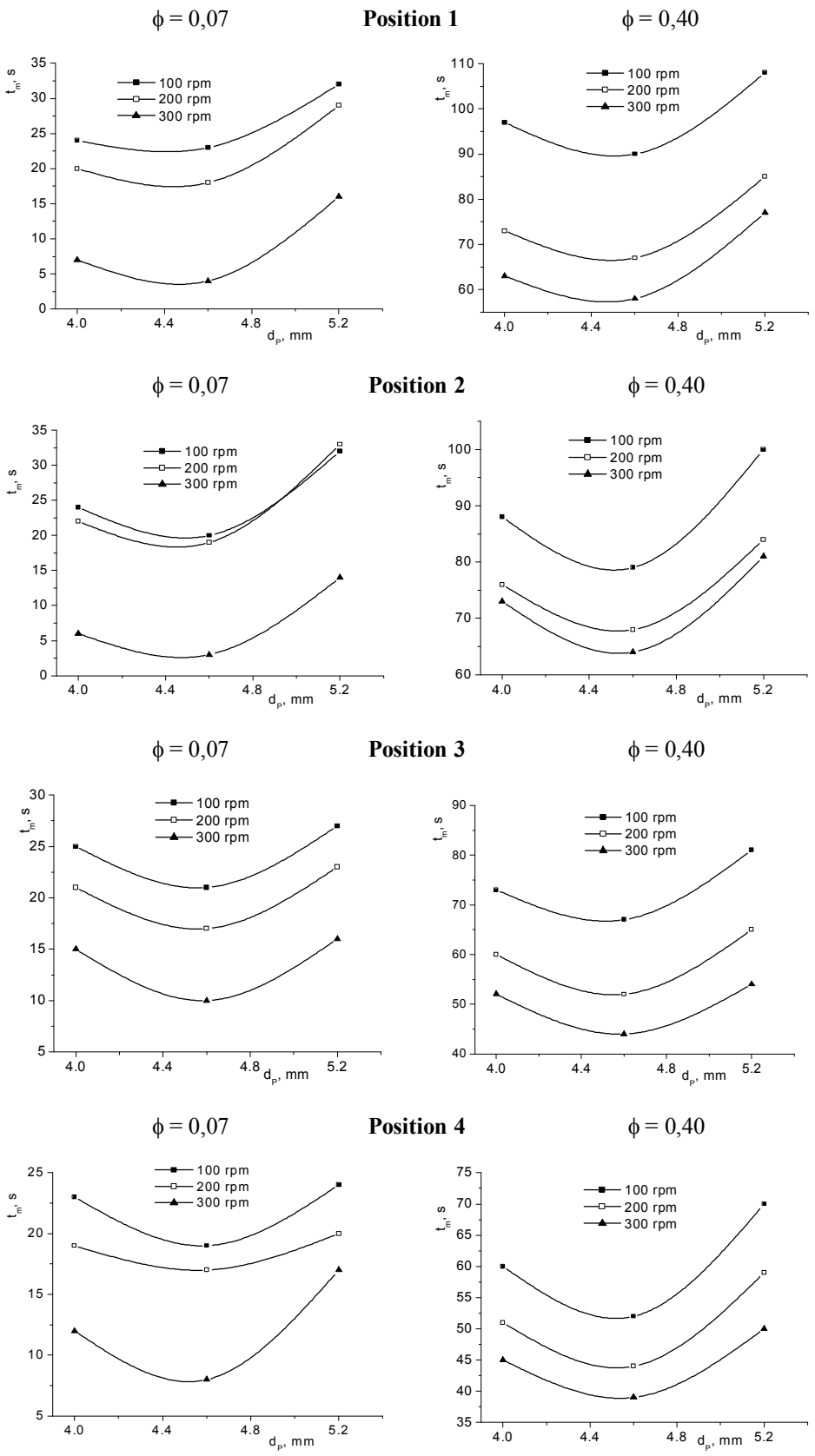


Figure 11. Variation of mixing time with the alginate particles diameter for the Smith turbine.

Notations

d - impeller diameter, mm
d_P - biocatalyst particle diameter, mm
t_m - mixing time, s
ϕ - biocatalysts volumetric fraction, %

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