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Comparative evaluation of radial impellers efficiency for bioreactors with stirred bed of immobilized cells
3. Paddle with six blades and pitched bladed turbine

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Abstract

The comparative studies on 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 continued for the paddle with six blades and pitched bladed turbine. The obtained results indicated that the intensity of the mixing induced by these impellers is rather similar to that promoted by the Rushton turbine. For rotation speed over 200 rpm, value which is directly related to the biocatalysts volumetric fraction, both impellers can offer a uniform mixing of the suspension. The influence of the size of biocatalysts on suspension circulation depends on the flow streams promoted by the impellers. Therefore, in the case of the paddle with six blades, the optimum diameter of the alginate particles is the intermediary one, while for the pitched bladed turbine is the smallest one.

Keywords: bioreactor, stirred bed, immobilized cells, yeasts, mixing, mixing time, radial impeller, Rushton turbine, paddle with six blades, pitched bladed turbine.

Introduction

The mixing constitutes one of the main factors controlling the performances of the bioreactors with stirred/mobile beds of immobilized cells/enzymes, being in its turn influenced by many constructive and operational parameters. Therefore, the analysis and quantification of these influences on mixing efficiency and distribution are required for process optimization. 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.

Under these circumstances, the aim of our experiments is to study comparatively the mixing intensity for a bioreactor with stirred bed of immobilized yeasts cells equipped with seven 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 given suspension of biocatalysts.

Due to the large amount of experimental data, this study consists on four parts. In the first two, the results obtained for the disperser sawtooth and Smith turbine, on the one hand,

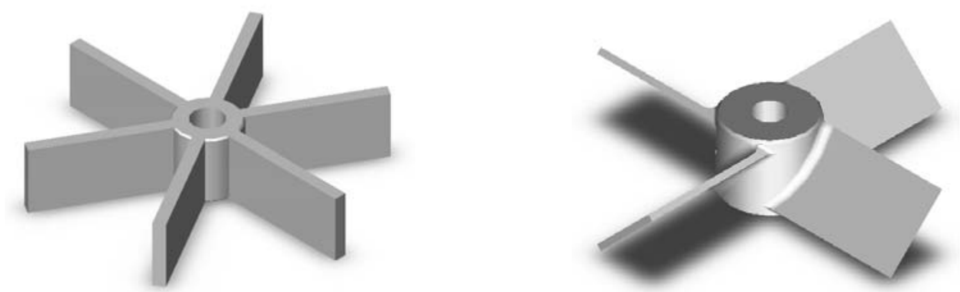
and for the pumper mixer and curved bladed turbine, on the other hand, have been discussed [1,2]. The conclusions those have been drawn were that the less efficient impeller was the disperser sawtooth, especially due to the low pumping capacity which cannot avoid the solid phase deposition at the bioreactor bottom. Contrary, the Smith turbine offers the most efficient mixing for a large domain of biocatalysts concentration and rotation speed [1]. Owing to their configuration, the last two impellers can induce an intense mixing only in the inferior region [2]. The uniform circulation can be reached for the Smith turbine, but only for a limited domain of rotation speed. The influence of biocatalysts size depends on the stream flows promoted by the impellers, respectively on the magnitude of the interaction between the alginate particles or of their deposition [1,2]

In this paper, the previous studies are continued for the last two considered radial impellers, namely the paddle with six blades and the pitched bladed turbine.

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 [3].

The mixing system consists on 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 [4].



Paddle with six blades — Pitched bladed 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 from the inferior one, this being the optimum distance form the Rushton turbine, as it was demonstrated in the previous works [4]. The rotation speed was maintained between 50 and 300 rpm, domain that avoids the “cave” formation at the broths surface and mechanical disruption of the biocatalysts particles.

In the experiments, suspensions of *S. cerevisiae* cells immobilized on alginate have been used. The immobilization has been carried out by cells inclusion into the alginate matrix, according with the method given in literature [5]. The following diameters of the biocatalyst spherical particles have been obtained: 4, 4.6 and 5.2 mm, respectively. The volumetric fraction of the immobilized cells into the suspension varied between 7 and 40%.

The experiments have been carried out at a temperature of 25^{o}C. Any mechanical lysis 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 [6,7]. 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.

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 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 for calculating the mixing time.

Results and Discussion

According with the experimental program previously presented [1,2], these studies have been carried out in the similar manner for the two types of radial impellers, the paddle with six blades and the pitched bladed turbine, in the purpose to select the optimum mixing system for bioreactors containing stirred/mobile suspensions of immobilized yeasts cells.

1. Paddle with six blades

Due to the modification of suspension concentration from the bioreactor bottom to the top and to the phenomena which appear during the suspension circulation, from Figures 2-4 it can be observed the differentiation of the correlations between the mixing time and the rotation speed on the bioreactor height, indifferent of the biocatalysts size.

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, decreasing then. The initial increase of mixing time could be the result of the streams interactions with bioreactor wall or/and baffles, phenomenon that is amplified by the presence of solid phase, due to the collisions between the particles or with the bioreactor internal elements. These effect lead to the hindrance of suspension circulation at lower rotation speed values. Over a certain level of rotation speed, these influences are diminished and, consequently, 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.

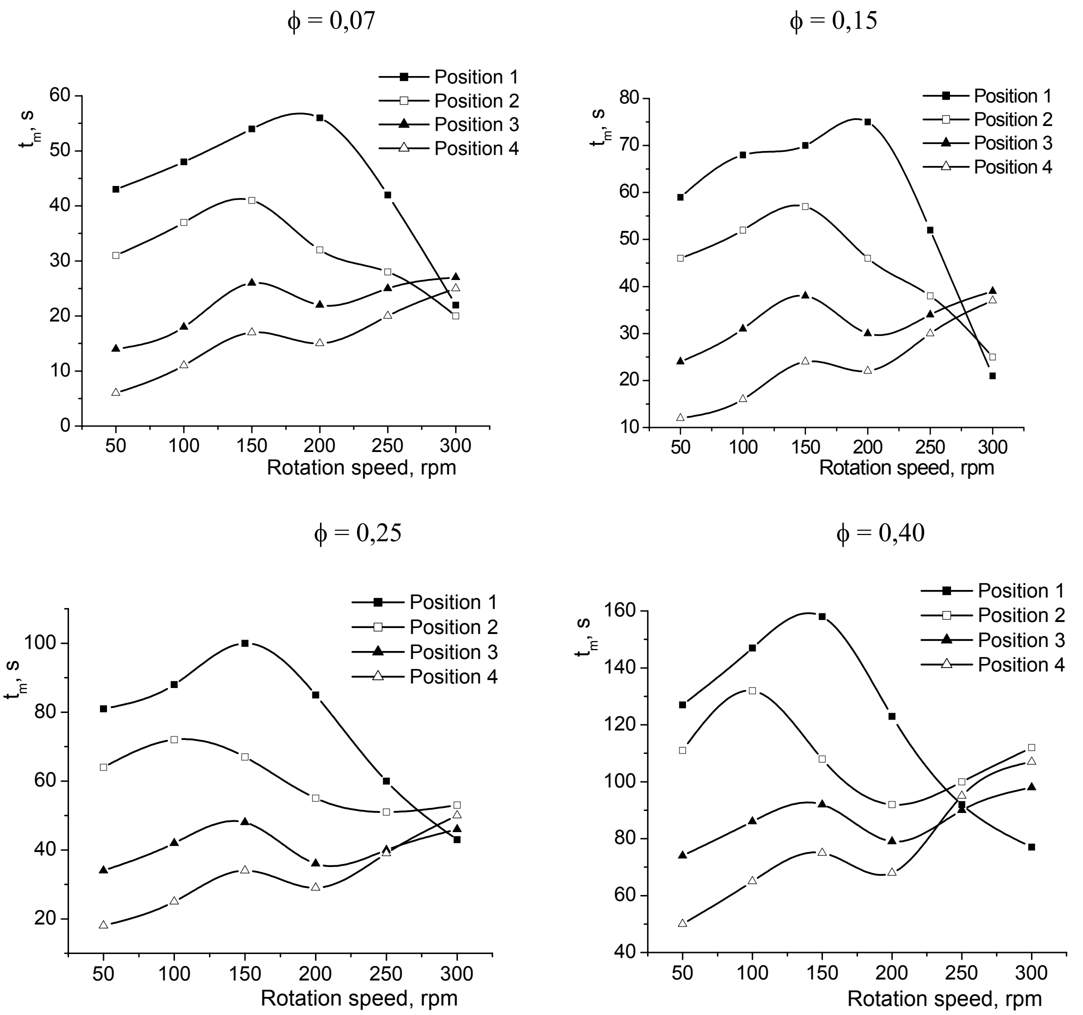


Figure 2. The influences of rotation speed on mixing time at different sensor positions and biocatalysts concentration for the paddle with six blades (particle diameter of 4 mm).

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 mixing time recorded for position 1, which maintains its general shape for the entire domain of biocatalysts concentration, the variation of mixing time for position 2 is gradually changed with the increase of biocatalysts concentration. Therefore, in the domain of alginate particles volumetric fraction of 25-40% and rotation speed over 200 rpm, the mixing time increases again, 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 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.

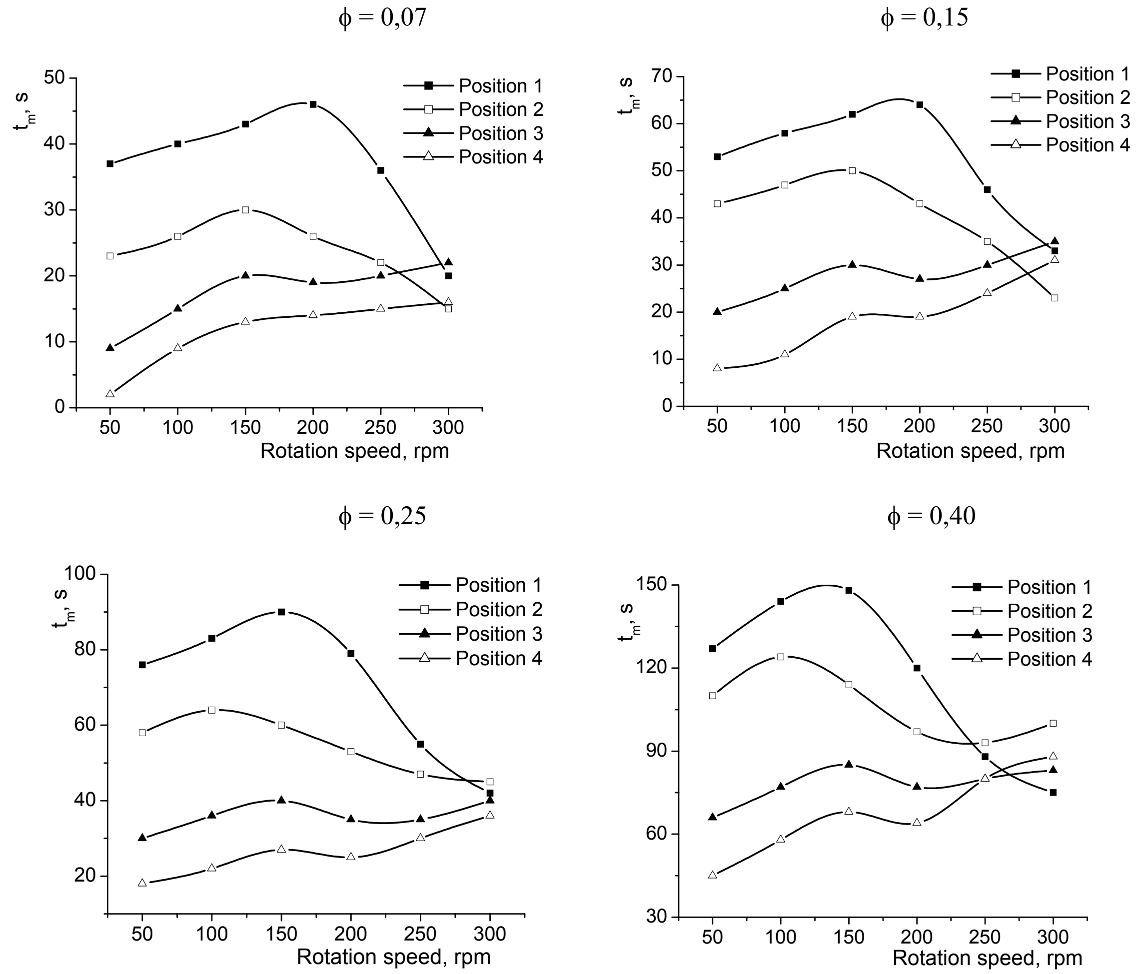


Figure 3. The influences of rotation speed on mixing time at different sensor positions and biocatalysts concentration for the paddle with six blades (particle diameter of 4.6 mm).

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 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 in the inferior region too. This evolution has been recorded only for a restricted domain of 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 rotation speed over 200 rpm, the mixing efficiency for position 4 becomes very close or inferior to that recorded for position 3.

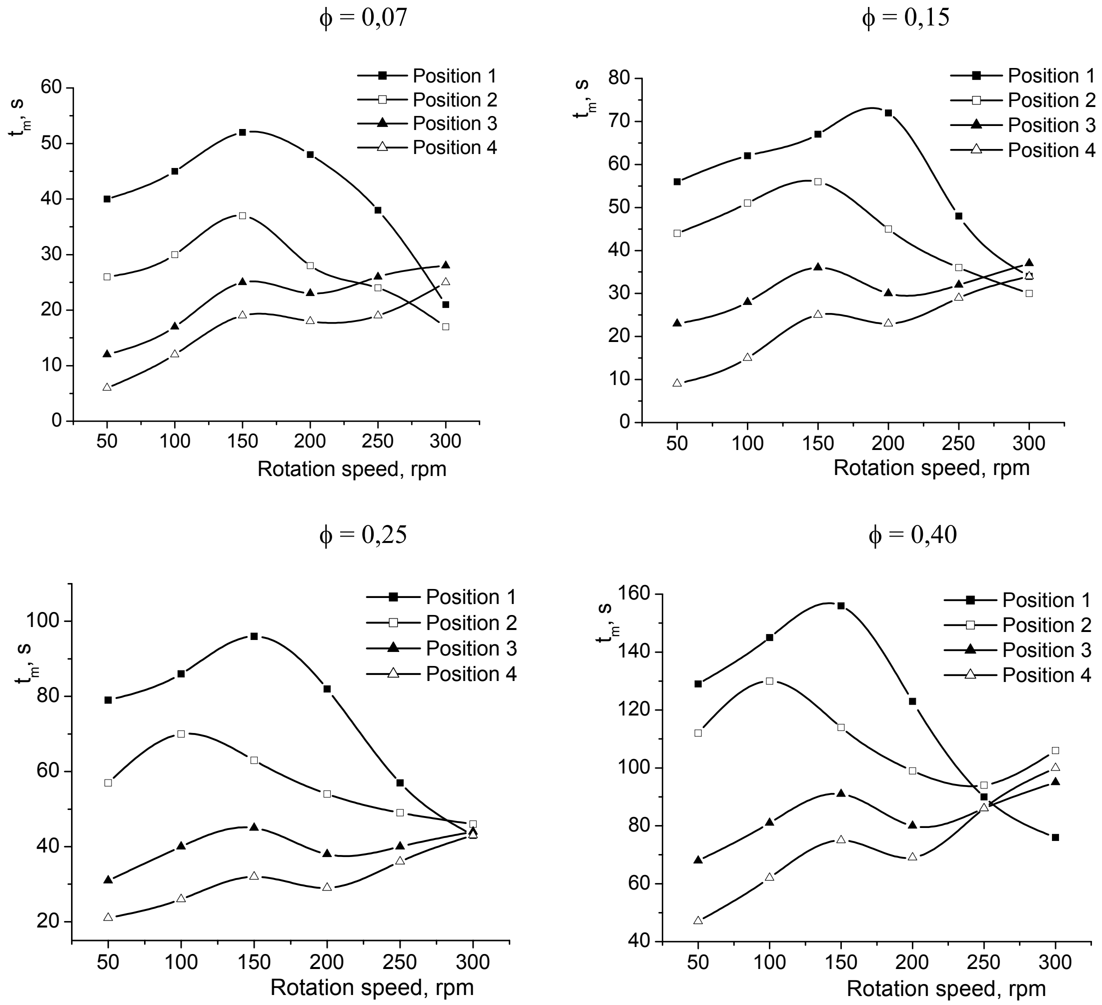


Figure 4. The influences of rotation speed on mixing time at different sensor positions and biocatalysts concentration for the paddle with six blades (particle diameter of 5.2 mm).

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 [4]. The appearance of the 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 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 suspension concentration.

Excepting the maximum values of 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 studied impellers [1,2]. 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 [2]).

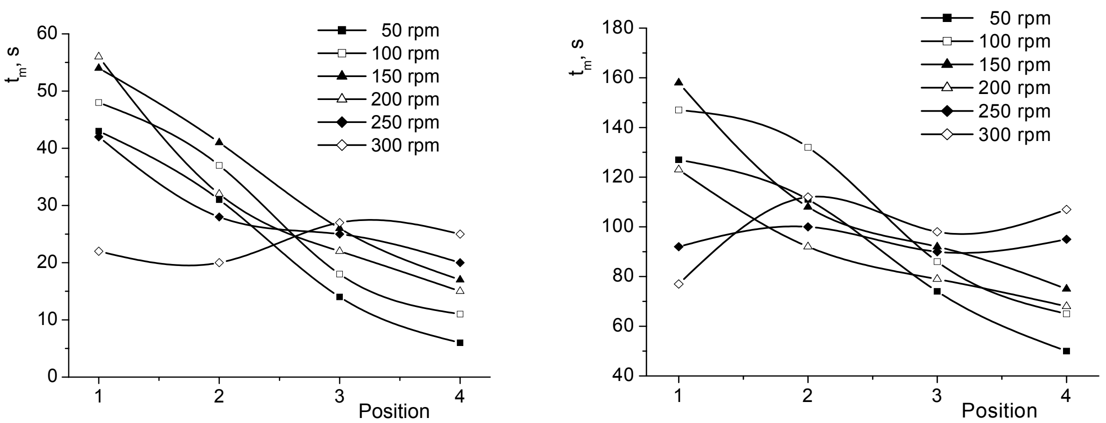
The shapes of the above discussed dependences are similar for all considered sizes of the alginate particles, but the recorded variation are more pronounced for the smallest and biggest particles (the negative phenomena of the particles interactions and their deposition are reciprocally compensated in the case of the biocatalysts with intermediary size [1]).

The analysis of the influence of rotation speed on mixing of immobilized yeasts cells suspension for the Rushton turbine [4] and paddle with six blades leads to the following comparative results:

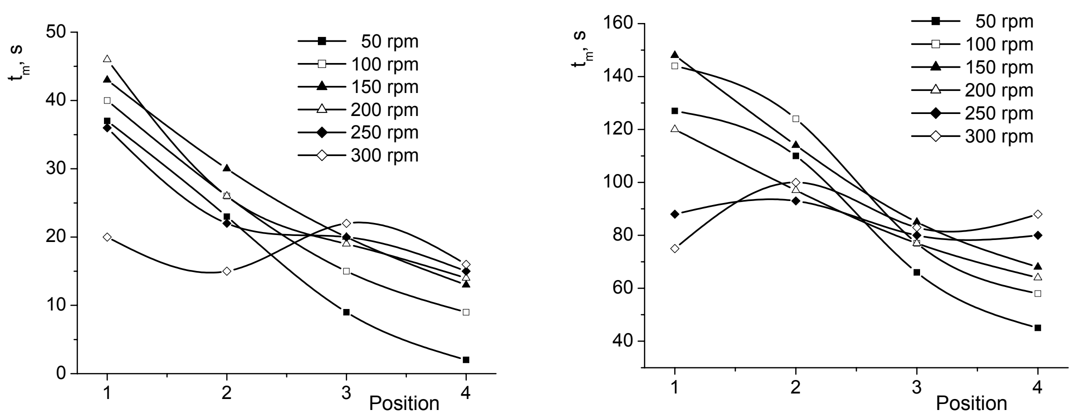
- **Position 1:** the Rushton turbine is more efficient, indifferent of the size and volumetric fraction of the biocatalysts.
- **Position 2:** for solid phase concentration up to 15% vol., the intensities of the mixing promoted by the two impellers are similar; for more concentrated suspensions, the 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, contrary to the Rushton turbine, this impeller offers a more intense circulation of the suspension for the entire domain of the biocatalysts concentration and of rotation speed (only for rotation speed over 250-300 rpm and biocatalysts concentration over 25% vol., the efficiencies of the two impellers become rather similar).

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 (Figure 5). These values of rotation speed are higher than those required by the Rushton turbine for uniform mixing [4].

ϕ = 0,07 **d_P = 4 mm** ϕ = 0,40



ϕ = 0,07 **d_P = 4.6 mm** ϕ = 0,40



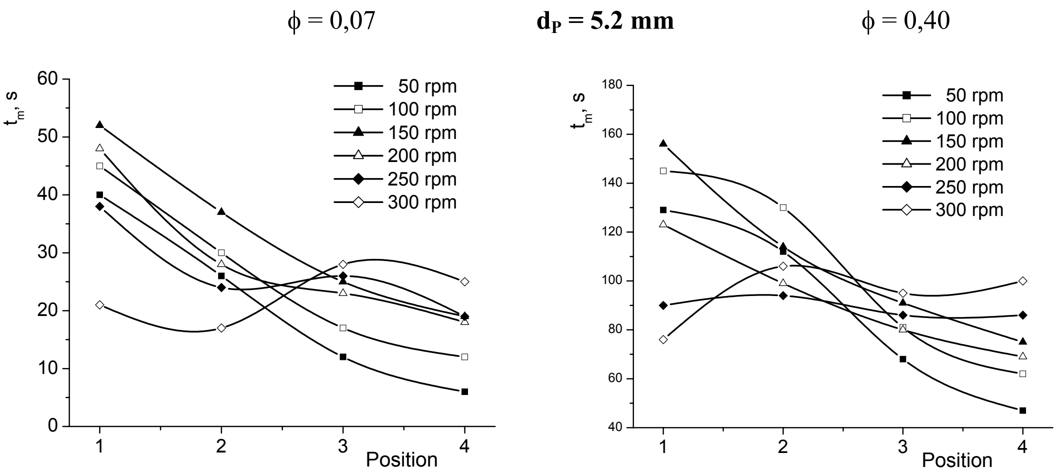


Figure 5. Variation of mixing time with the position inside the biocatalysts suspension for the paddle with six blades.

The lowest values of 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.

2. 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 axial flow to radial one [8]. Because for the used experimental equipment the ratio d/D is 0.36 [3,6], it could be assumed that the conditions for a radial circulation of the agitated liquid are respected.

The Figures 6-8 indicate two types of correlations between the mixing time and rotation speed, one corresponding to position 1, and the other for positions 2, 3 and 4.

In the inferior region, position 1, indifferent of the biocatalysts size and concentration, the mixing time initially decreases with the increase of rotation speed. Over a certain value of rotation speed, which increase 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 mixing intensity for the other three positions are similar. Thus, by intensifying the rotation speed, the mixing time increases to a maximum value, being reduced then. The value of 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 solid phase [9], 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 rather similar, but higher, to that recorded for the position 4, this position being closer to the impellers. According as the suspension circulation becomes of radial type, at 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 rotation speed higher than 200-250 rpm.

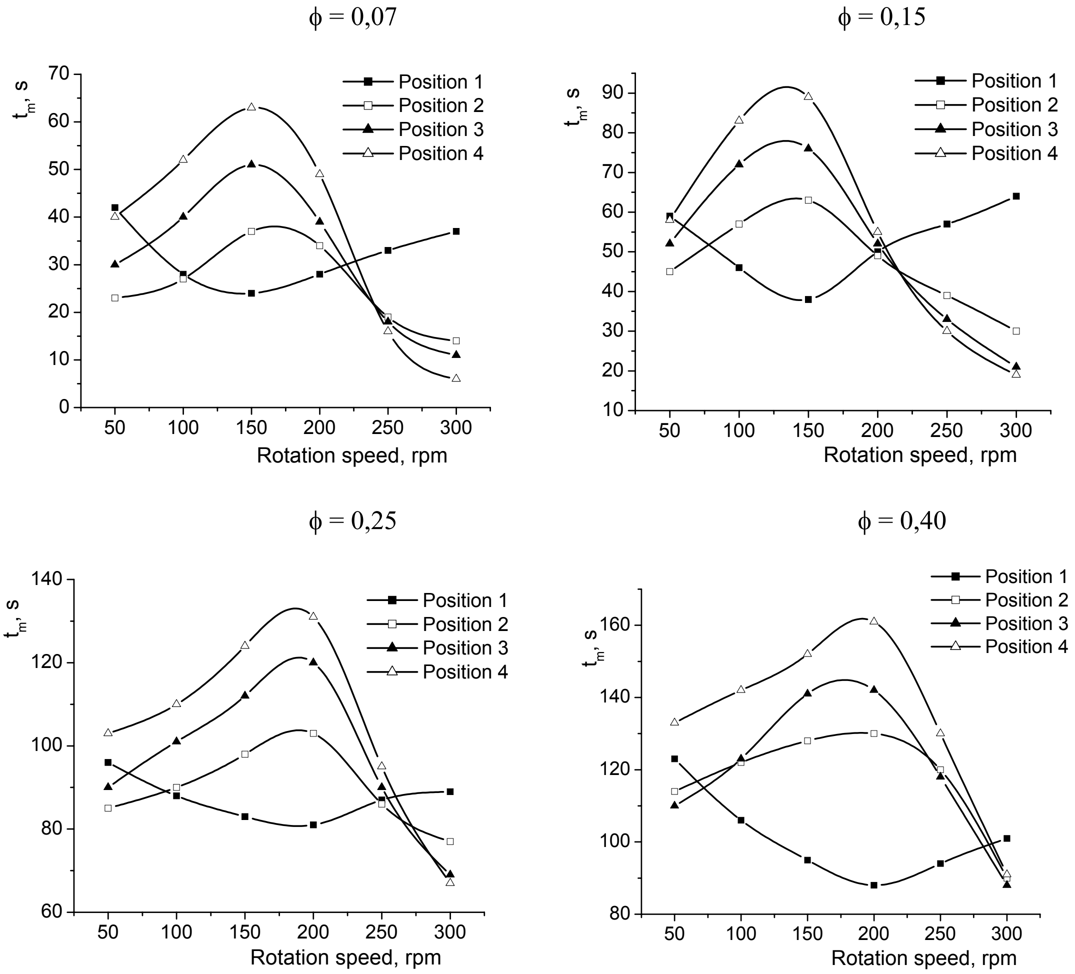


Figure 6. The influences of rotation speed on mixing time at different sensor positions and biocatalysts concentration for the pitched bladed turbine (particle diameter of 4 mm).

The shape of the variation of mixing time with rotation speed recorded for position 2 is rather 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 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.

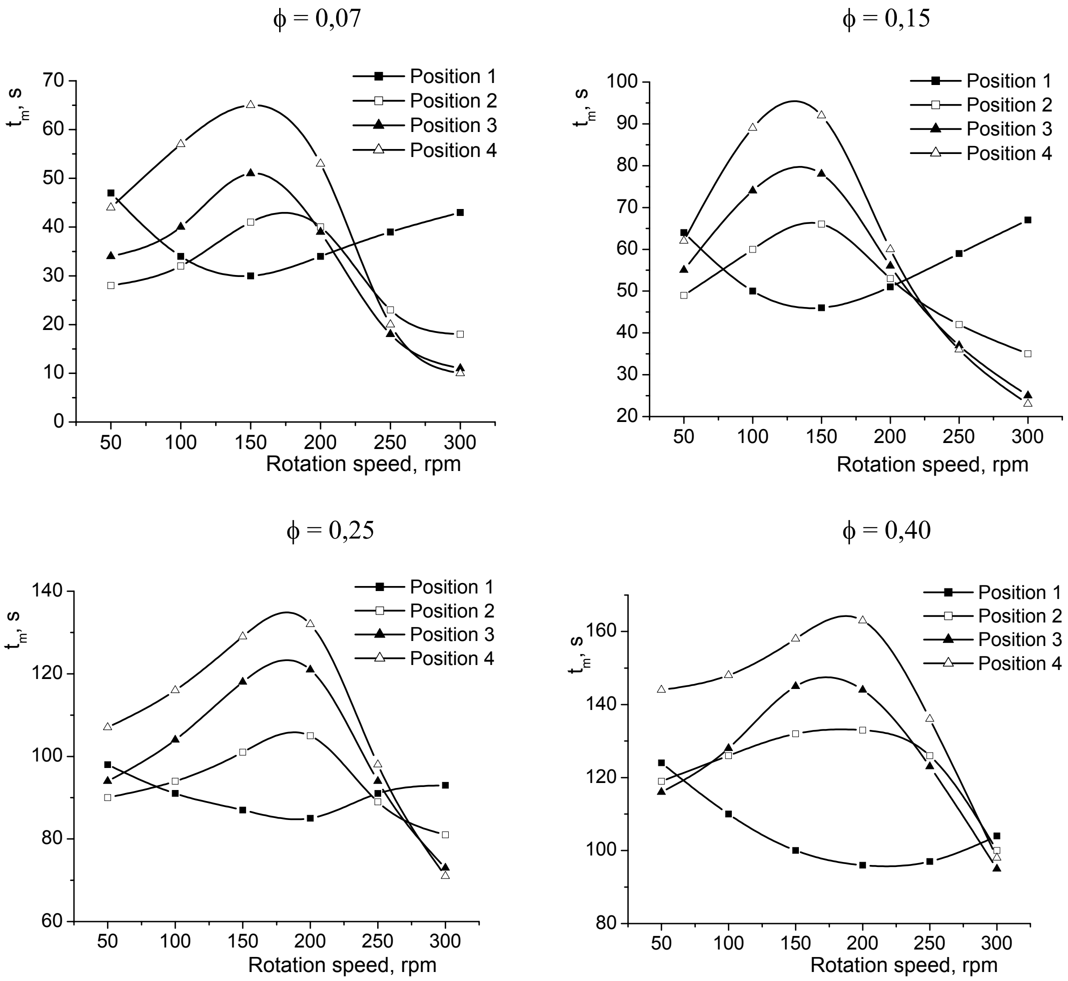


Figure 7. The influences of rotation speed on mixing time at different sensor positions and biocatalysts concentration for the pitched bladed turbine (particle diameter of 4.6 mm).

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 without any significant difference between the two impellers.
- **Position 2:** the pitched bladed turbine offers a more intense circulation of the suspension only for rotation speed domain over the value corresponding to the maximum of mixing time (150-200 rpm); in the rest of rotation speed domain the Rushton turbine is recommended.
- **Positions 3 and 4:** for the entire domain of size and concentration of biocatalysts, the pitched bladed turbine is more efficient; but, as in the previous studied systems [1,2], this conclusion is erroneous, because the concentration of solid phase in these position is lower than that reached by its dispersing using the Rushton turbine (the axial component of the flow generated by the pitched bladed turbine does not allow to dispersing efficiently the biocatalysts in the superior region of the bioreactor).

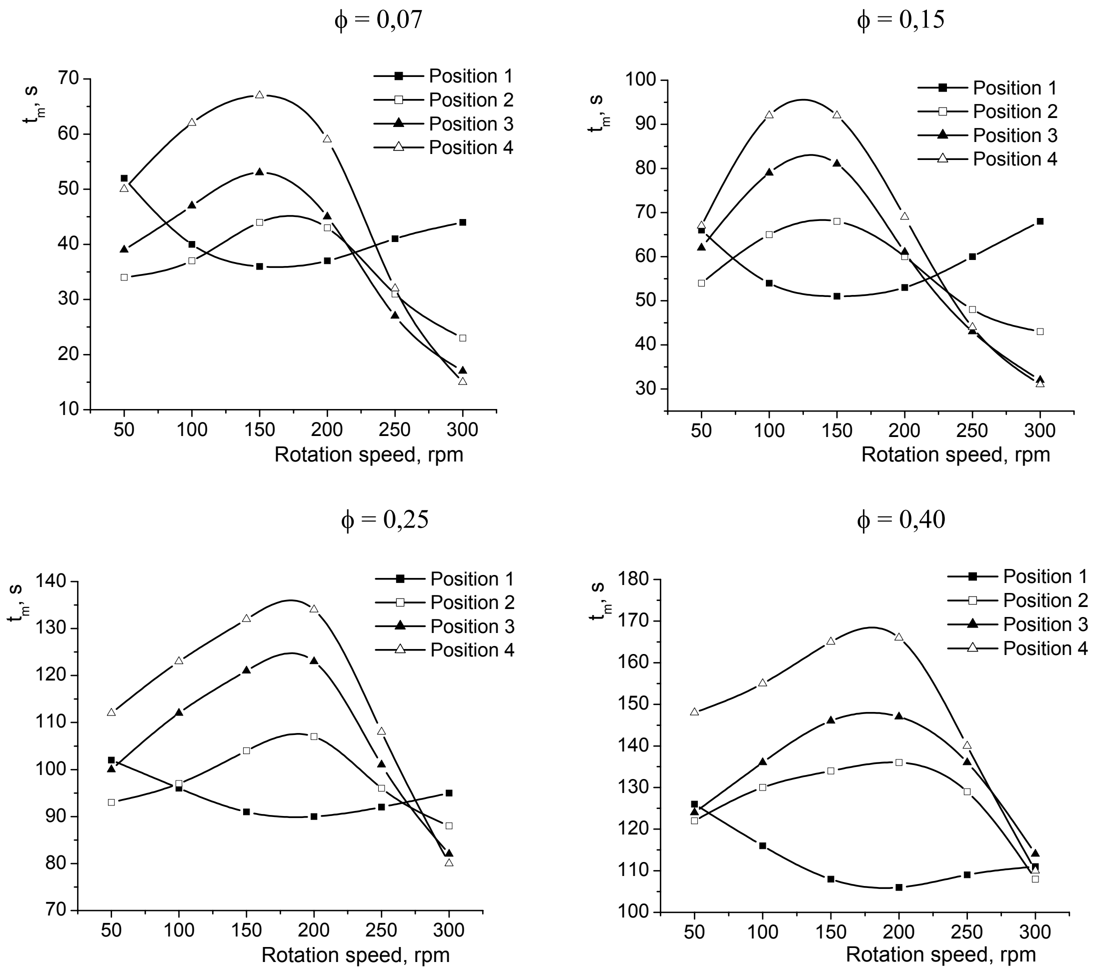
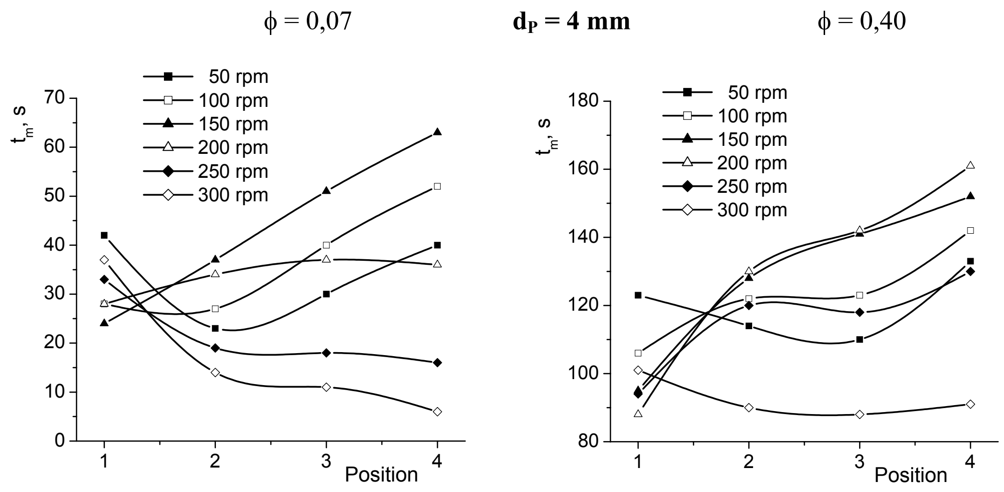


Figure 8. The influences of rotation speed on mixing time at different sensor positions and biocatalysts concentration for the pitched bladed turbine (particle diameter of 5.2 mm).



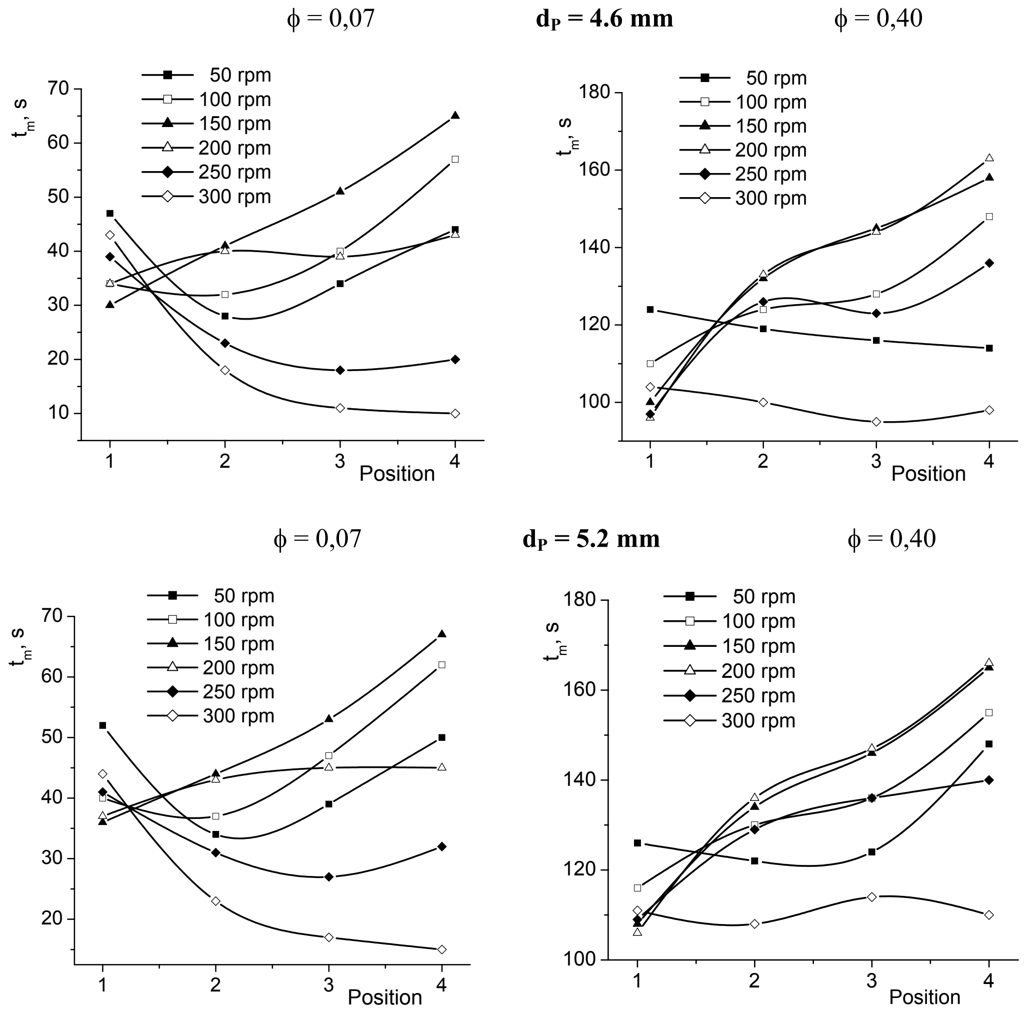


Figure 9. Variation of mixing time with the position inside the biocatalysts suspension for the pitched bladed turbine.

The Figure 9 suggests that the pitched bladed turbine can leads to 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., and becoming 250-300 rpm for more concentrated suspensions.

Conclusions

Comparatively to the previously studied 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 [1,2].

Furthermore, both impellers can offer a uniform mixing of the suspension for rotation speed over 200-250 rpm.

The influence of the size of biocatalysts on suspension circulation depends on the flow streams promoted by the impellers. Therefore, in the case of the paddle with six blades, the optimum diameter of the alginate particles is the intermediary one, while for the pitched bladed turbine is the smallest one.

Abbreviations

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

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