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Comparative evaluation of radial impellers efficiency for bioreactors with stirred bed of immobilized cells
2. Pumper mixer and curved bladed turbine

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Abstract

The 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 by comparatively analyzing of them for three radial impellers: pumper mixer, curved bladed turbine and Rushton turbine. For the inferior region of the bioreactor, the efficiency of a certain impeller is directly related to the concentration and size of biocatalysts. For the superior region, apparently the pumper mixer and curved bladed induce the most intense circulation. The increase of biocatalysts size exhibits a favorable effect on mixing efficiency, owing to the friction forces between the alginate particles and to the particles collision, both controlling the suspension circulation for lower diameter of alginate particles.

Keywords: bioreactor, stirred bed, immobilized cells, yeasts, mixing, mixing time, radial impeller, Rushton turbine, pumper mixer, curved bladed turbine.

Introduction

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 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 of the bioreactor [1]. Due to the deposition tendency of the solid phase at the bioreactor bottom and 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 [1,2].

Because the 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. 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 effect for different double radial stirrers, there were selected the following optimum combinations of impellers 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 [3].

Therefore, the aim of our experiments is to comparatively study the efficiency of mixing for a bioreactor with stirred bed of immobilized yeast 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.

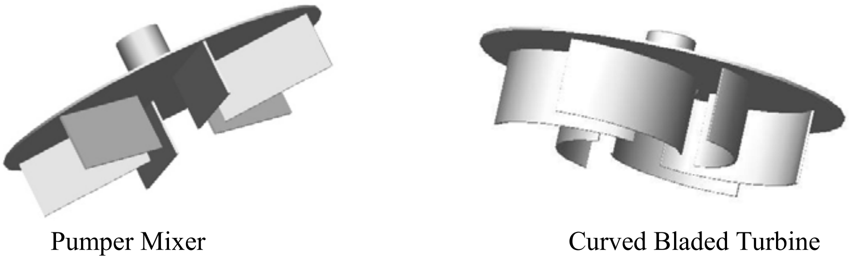
Due to the large amount of experimental data, this study consists of four parts. In the first one, the results obtained for the disperser sawtooth and Smith turbine have been discussed [4]. The conclusions those have been drawn were: the less efficient impeller was the disperser sawtooth, especially 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. It can also induce an uniform circulation of the suspension for certain values of rotation speed and biocatalyst volumetric fraction up to 15%, similar to the Rushton turbine. Furthermore, 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.

In this paper, the previous studies are continued for other two radial impellers, namely the pumper mixer and the curved 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 [5].

The mixing system consists in 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 [6].



Pumper Mixer

Curved 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 mm from the inferior one, this being the optimum distance form the Ruston turbine, as it was demonstrated in the previous works [6]. 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 biocatalyst 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 to the method given in the literature [7]. 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 suspension 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 tracer method [8]. 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 [9]:

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

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:

- 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-recording system and were analyzed to calculate the mixing time.

Results and Discussion

These experiments are carried out in the similar manner for the two types of radial impellers, the pumper mixer and the curved bladed turbine, in the purpose to select the optimum mixing system for bioreactors containing stirred/mobile suspensions of immobilized yeasts.

1. Pumper mixer

This impeller represents the component part of some equipments 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 [10].

Because 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 rotation speed compared with the previous two impellers [4].

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 the axial dispersion of the solid phase. But, not depending on the biocatalyst concentration, from Figures 2-4, two types of dependence between the mixing time and rotation speed can be distinguished, corresponding to the inferior and, respectively, superior regions inside the bioreactor.

For lower biocatalyst 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, increasing then to a maximum level. The further increase of rotation speed leads again to the decrease of mixing time. The existence of the minimum is due to the appearance and intensification of the hindrance effect on suspension circulation, induced either by the baffles or by the bioreactor wall, as in the case of simulated broths mixing [3]. Moreover, the collision of the alginate particles as well as the friction between these particles, amplify 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 biocatalyst size, the values of rotation speed corresponding to the two extreme points are 150 rpm and 250 rpm, respectively.

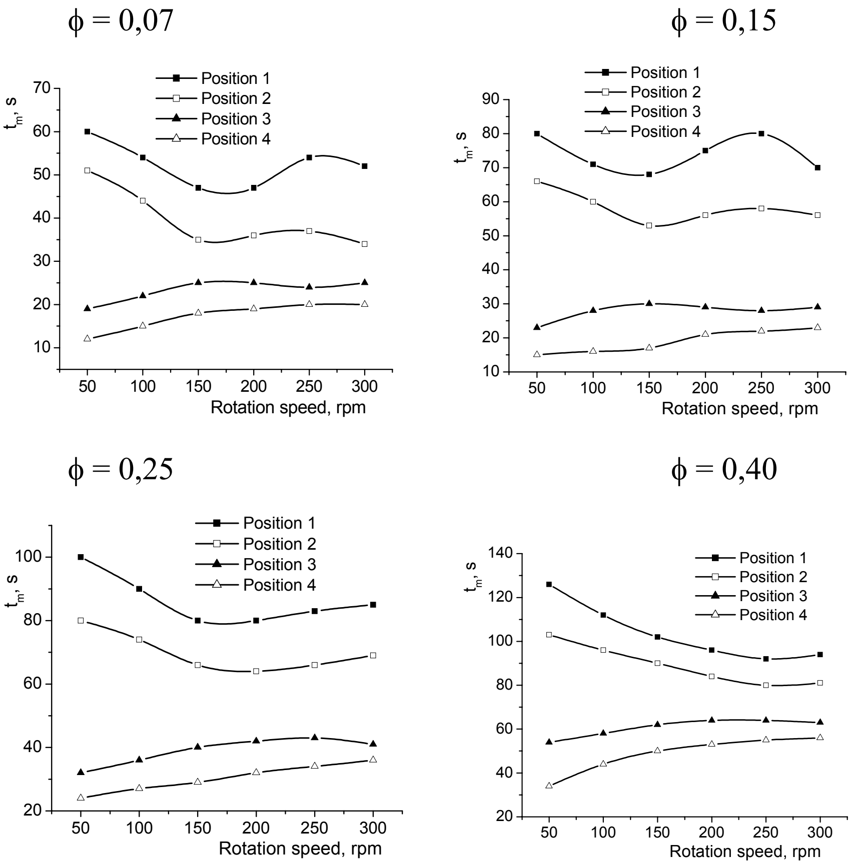

$\phi = 0{,}07$ $\phi = 0{,}15$ $\phi = 0{,}25$ $\phi = 0{,}40$

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

By increasing the volumetric fraction of the biocatalysts, the suspension circulation becomes more difficult, consequently the minimum level of 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 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 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. biocatalyst, the reduction of mixing time cannot be observed.

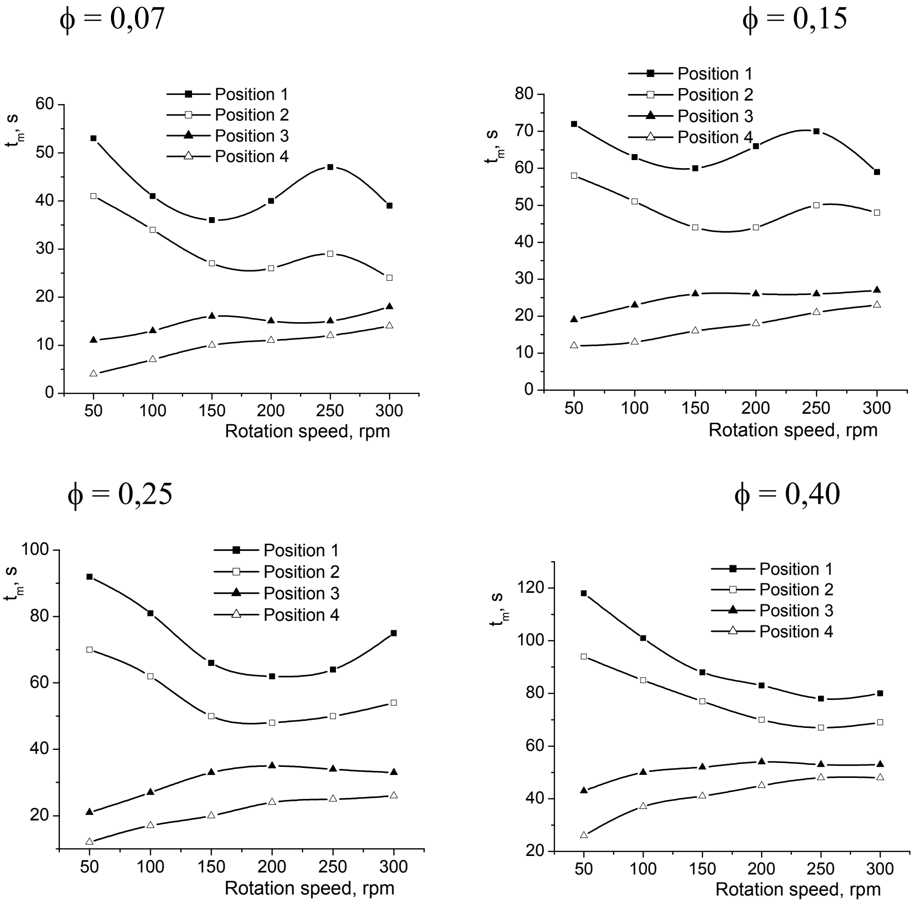


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Figure 3. The influences of rotation speed on mixing time at different sensor positions and biocatalysts concentration for the pumper mixer (particle diameter of 4.6 mm).

The mixing efficiency for position 4 is continuously reduced by the rotation speed increase, phenomenon 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 biocatalyst 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, process which becomes more important at higher concentration of biocatalyst, 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 result 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 biocatalyst particles, from Figures 2-4 it can be observed 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, effect which is more pronounced for biocatalysts with lower size.

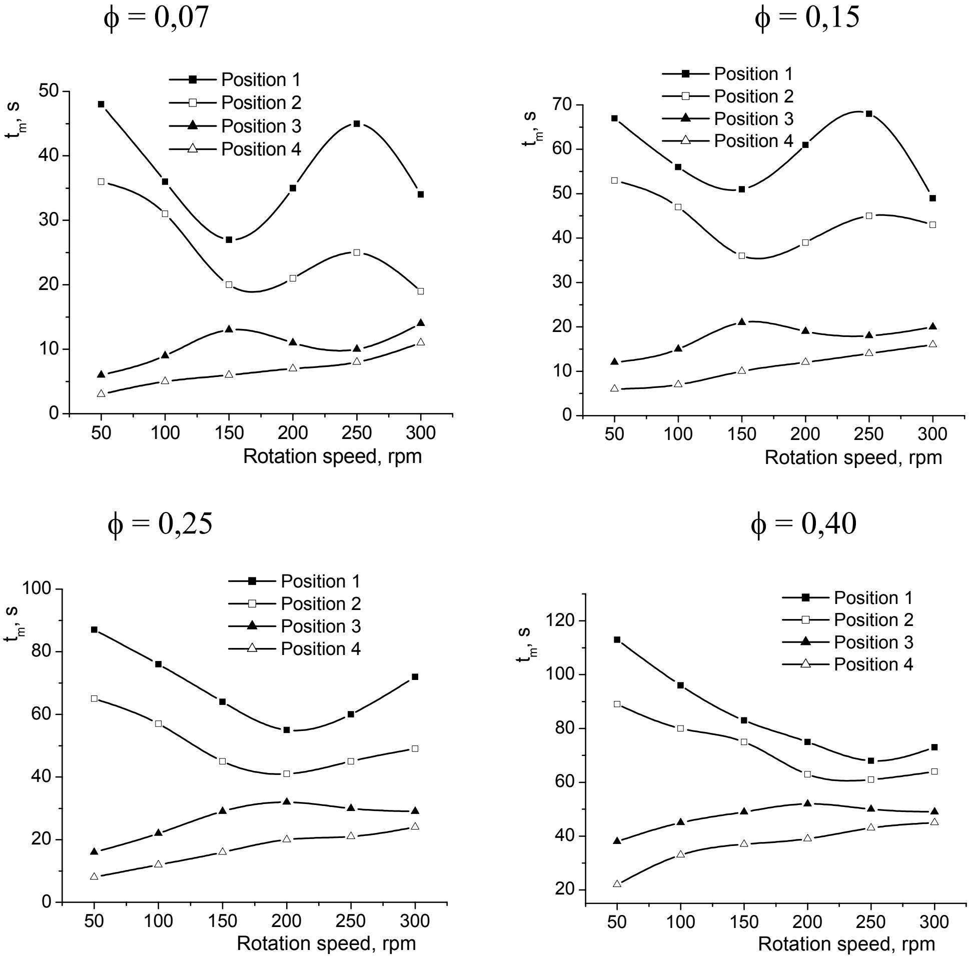
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Figure 4. The influences of rotation speed on mixing time at different sensor positions and biocatalysts concentration for the pumper mixer (particle diameter of 5.2 mm).

Besides the differences between the profile shape of mixing time with rotation speed, by comparing the results obtained for pumper mixer with those for Rushton turbine [6], the following conclusion can be drawn:

Positions 1 and 2: indifferent of the biocatalyst particles size, for biocatalyst concentration up to 15% vol., the Rushton turbine promotes the more 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; but, this result should be prudently analyzed, because the mixing promoted by the pumper mixer for the superior positions is only apparently more intense, due to the low amount of solid phase dispersed in these regions.

The complete non-uniform distribution of the mixing intensity inside the bioreactor, as a result of both the sinusoidal and opposite variation of mixing time for the studied positions, and of the significant difference between the concentrations of solid phase in these positions, is underlined in the Figure 5. The uniform mixing could be considered to be reached only for concentrated suspensions, for the particles with the higher size and rotation speed over 200 rpm.

Contrary to the two impellers previously studied [4], the increase of the biocatalyst size influences favorably the mixing. As it was above mentioned, 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 suspension circulation compared to the phenomenon of particle deposition.

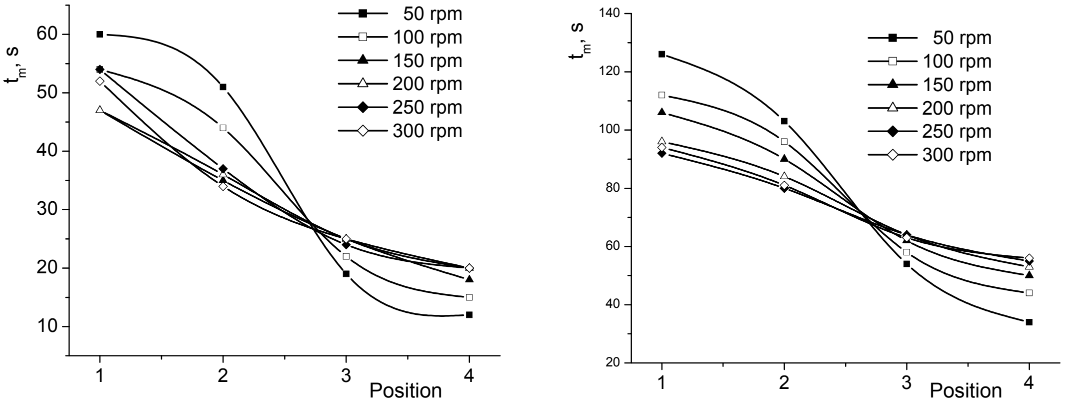
2. Curved bladed turbine

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 [10].

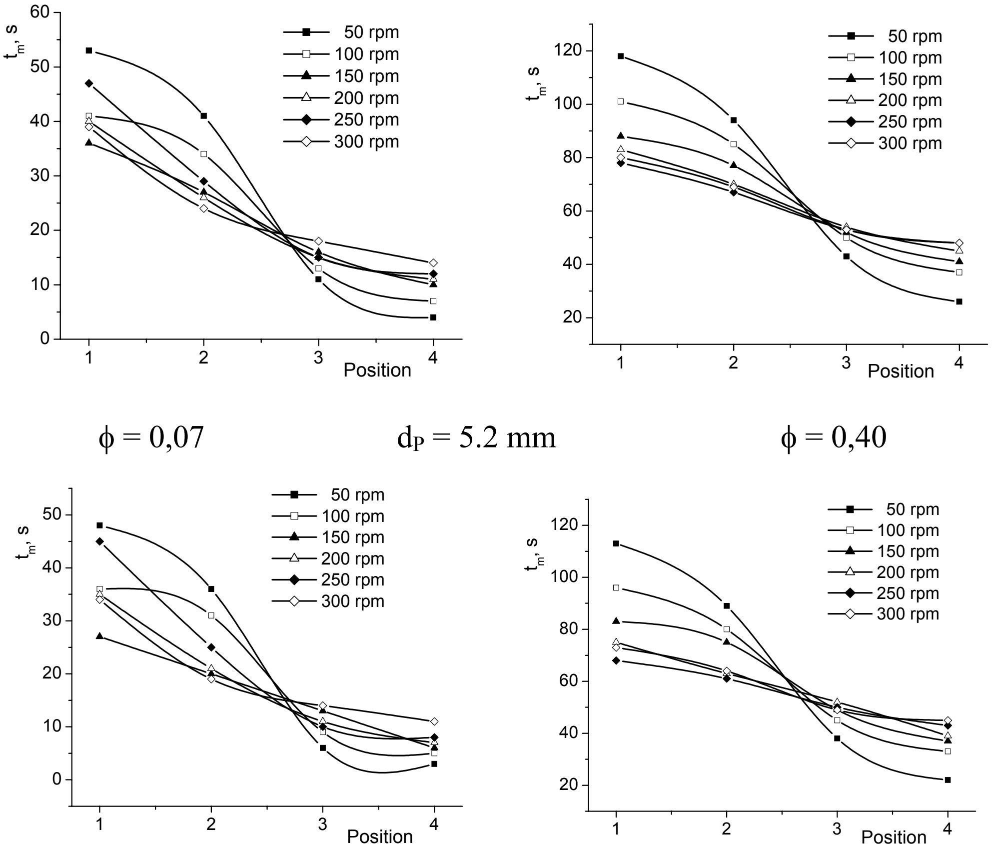
Although its construction is quite similar to the pumper mixer one, the experiments indicated significant differences between the two impellers concerning the variation of mixing time with the rotation speed, as in the case of the impellers of Rushton turbine and Smith turbine types [4]. Thus, according to the Figures 6-8, 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 biocatalyst size, the mixing time initially decreases with the increase of the rotation speed, reaches a minimum value, increasing then. The minimum level could be the result of the hindrance of 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 bioreactor wall.

$\phi = 0,07$ $d_P = 4$ mm $\phi = 0,40$



$\phi = 0,07$ $d_P = 4.6$ mm $\phi = 0,40$

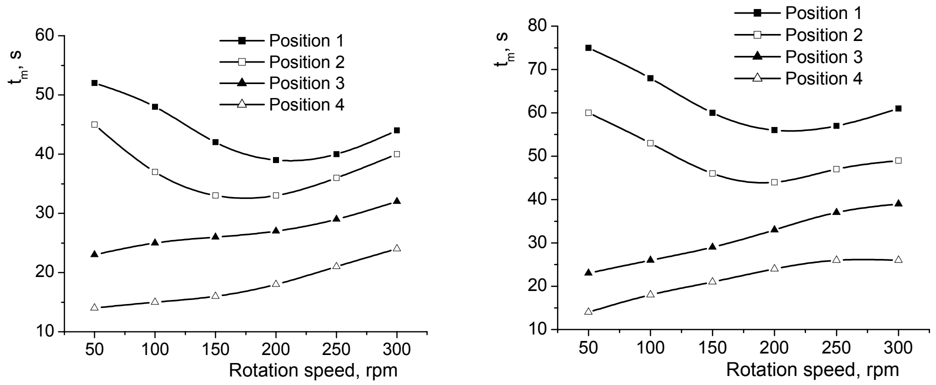


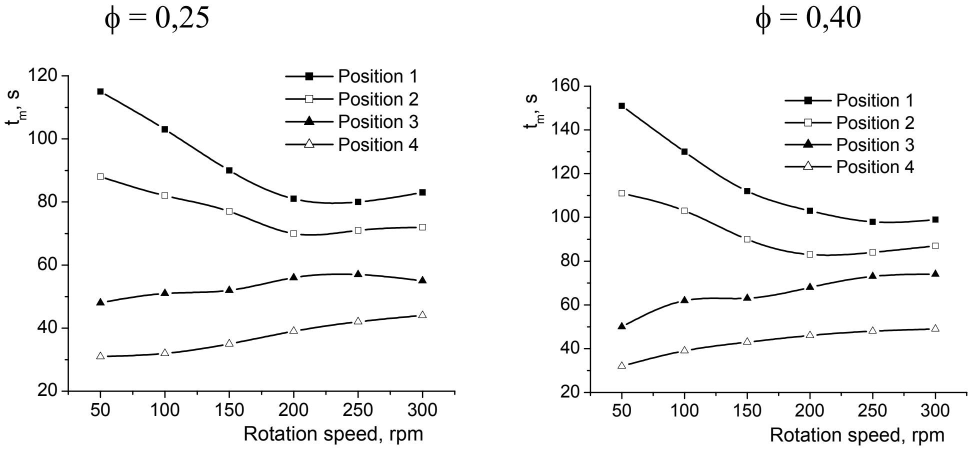
ϕ = 0,07 dP = 5.2 mm ϕ = 0,40

Figure 5. Variation of mixing time with the position inside the biocatalyst suspension for the pumper mixer.

The rotation speed corresponding to the minimum mixing time, called *critical rotation speed*, is moved to higher values with the increase of biocatalyst 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 solid phase (150 rpm for biocatalysts concentration up to 25% vol., 200 rpm for more concentrated suspensions).

ϕ = 0,07 ϕ = 0,15



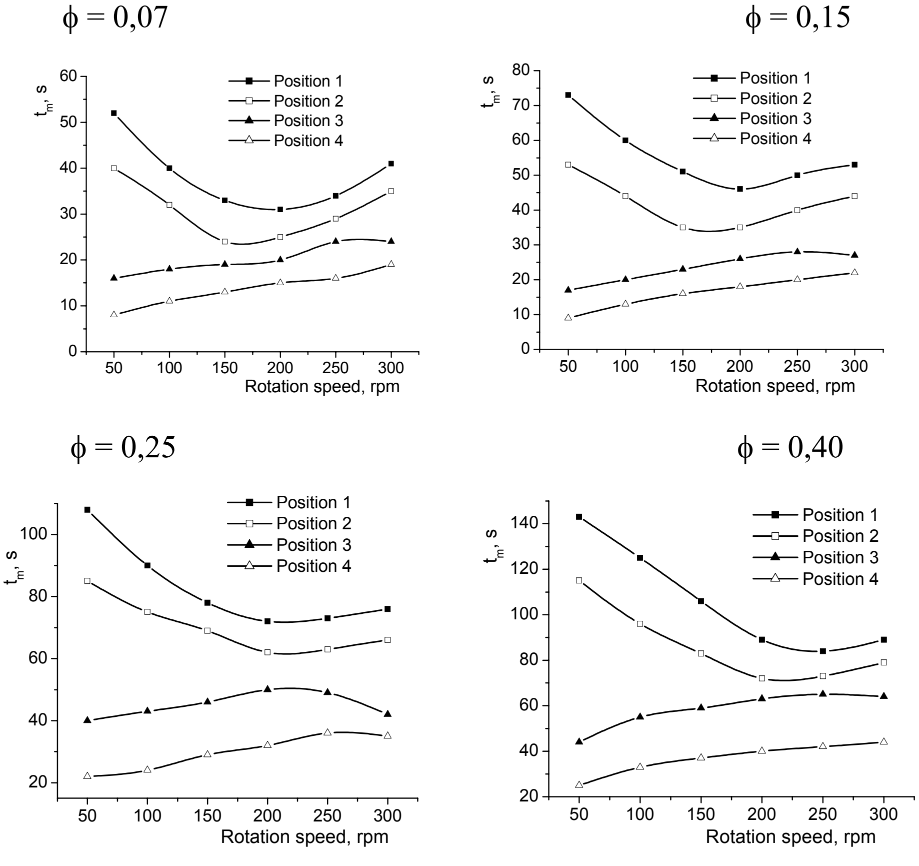


ϕ = 0,25

ϕ = 0,40

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

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 efficient 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.



ϕ = 0,07

ϕ = 0,15

ϕ = 0,25

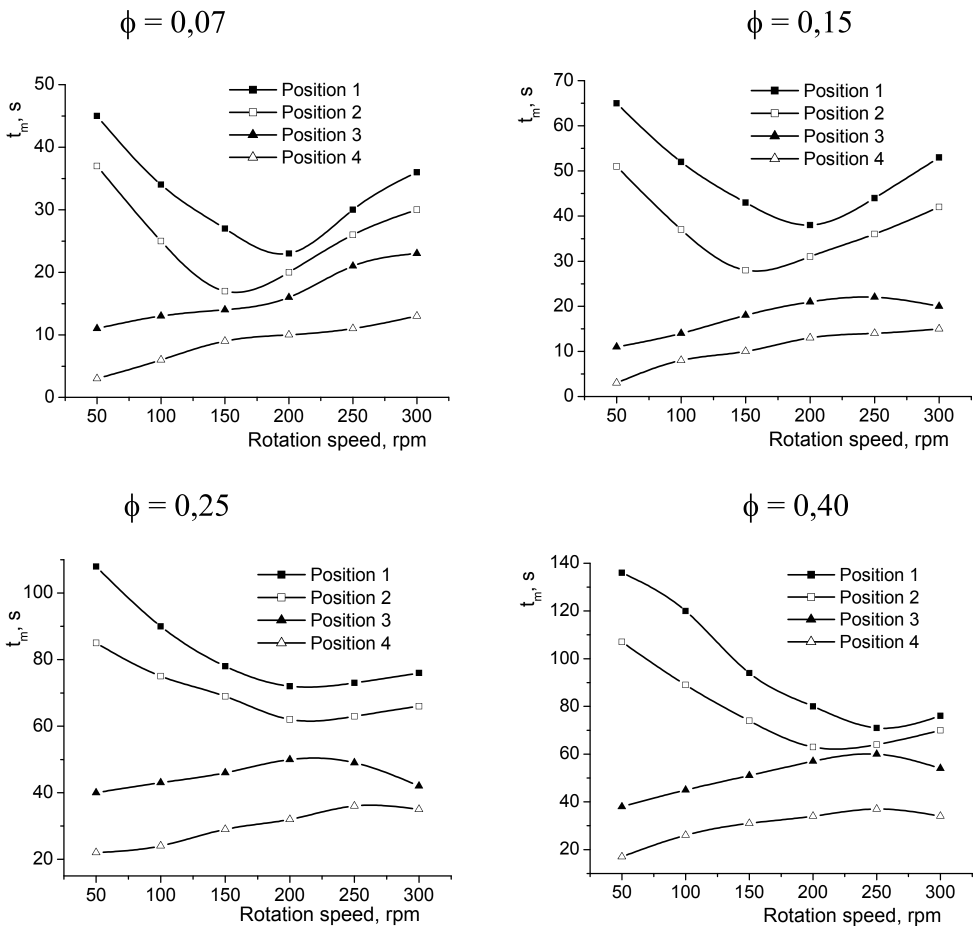
ϕ = 0,40

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

The above discussed influence of rotation speed on mixing time becomes more pronounced with the increase of biocatalyst 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 rotation speed, the less efficient mixing has been reached for the position 1, owing to the highest concentration of solid phase in this region. The significant difference between the inferior and superior regions is the result only of the heterogeneous distribution of the solid phase into the bioreactor. By increasing the biocatalyst concentration and the rotation speed, this difference is attenuated.

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


$\phi = 0,07$

$\phi = 0,15$

$\phi = 0,25$

$\phi = 0,40$

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

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

Positions 1 and 2: for 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 particle size; for more concentrated suspensions, the curved bladed turbine offers the most intense mixing.

Positions 3 and 4: irrespective of the concentration and size of biocatalysts and of the rotation speed, the curved bladed turbine is more efficient than the Rushton turbine; but, as in the previous case, this conclusion is false, because the mixing promoted by the curved bladed turbine for these positions is more intense only because the amount of dispersed solid phase is low.

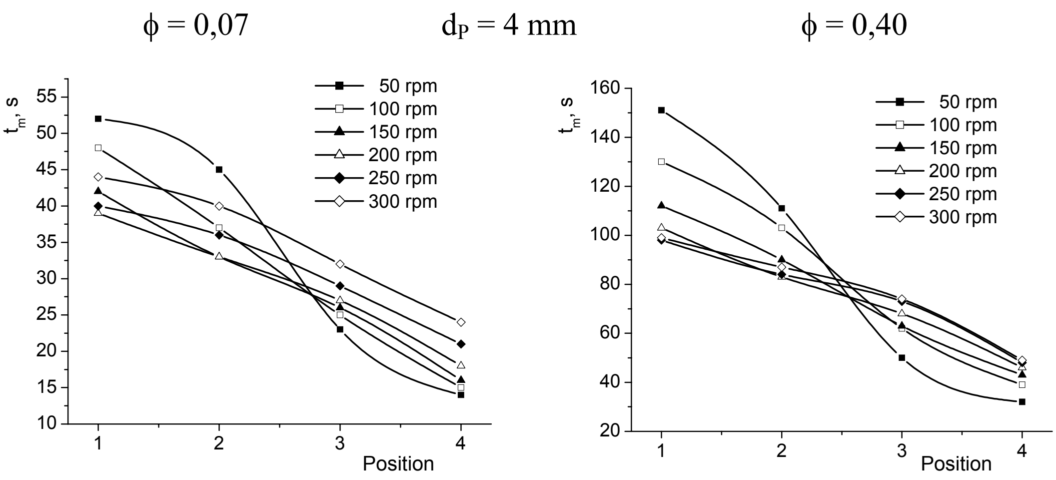
From Figure 9 it can be seen that neither for this impeller the uniform circulation inside the whole bulk of suspension cannot be reached.

Similar to the pumper mixer, due to the induced flow streams, the increase of the alginate particle size exhibits a negative effect on mixing, this 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.

Conclusions

By continuing the comparative study on 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 (pumper mixer, curved bladed turbine *vs.* Rushton turbine), the following conclusions can be drawn:

1. The most efficient impellers from the two studied was the curved bladed turbine.
2. For the inferior region, the Rushton turbine promotes the most intense circulation, but only for biocatalysts concentration below 15% vol. Over this value of concentration, the bladed curved turbine induces more efficient mixing.
3. The lower amount of solid phase dispersed in the superior region leads to the false result that the pumper mixer or curved bladed turbine can be recommended for mixing of suspensions of immobilized yeast cells in alginate, because the mixing promoted by these two impellers into the whole bulk of suspensions is non-uniform.
4. Contrary to the disperser sawtooth and Smith turbine previously studied [4], the increase of biocatalyst size exhibits a favorable effect on mixing efficiency, due to the friction forces between the alginate particles and to the particle collision which control the suspension circulation at low diameter of alginate particles.



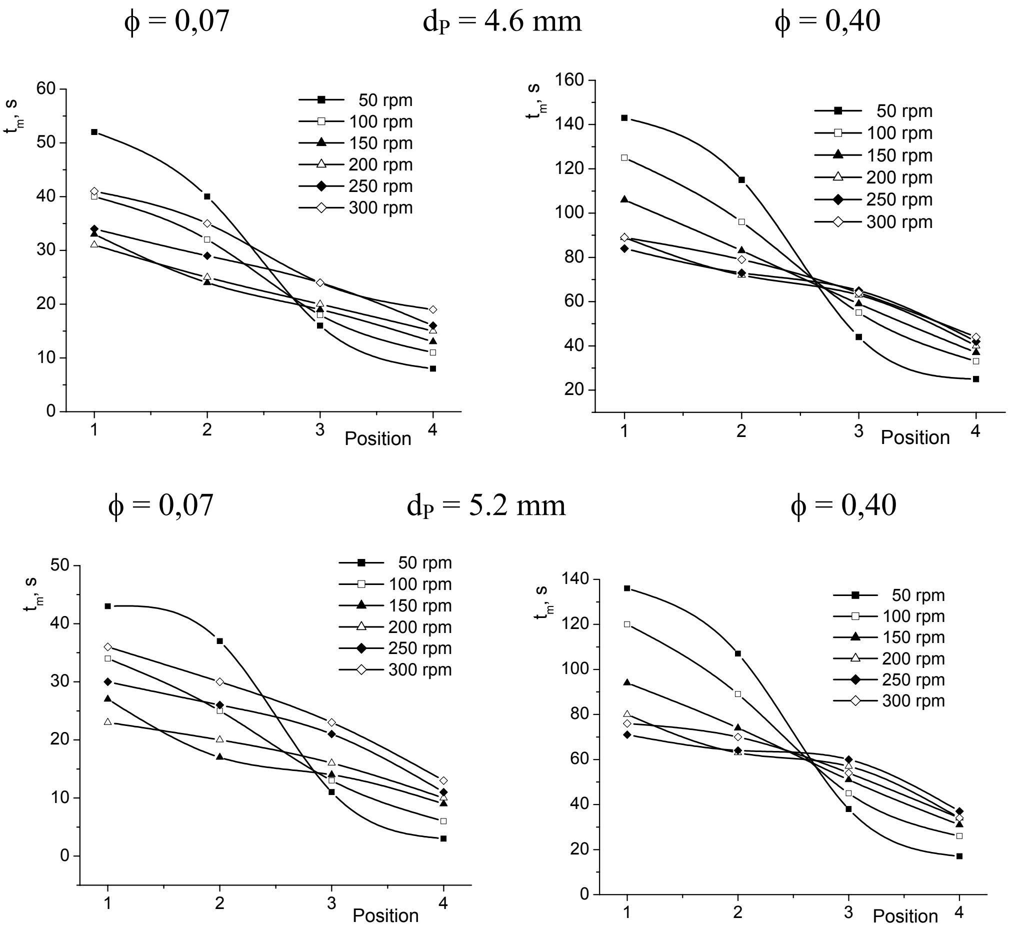


Figure 9. Variation of mixing time with the position inside the biocatalyst suspension for the curved bladed turbine.

Notations

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

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