

Ethanol production in a basket bioreactor with immobilized yeasts cells

2. Study on the mixing efficiency in the outer region of basket for a double Rushton turbine impeller

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Abstract

The studies on the mixing efficiency of medium with apparent viscosity between 1 and 75 cP in a double Rushton turbine stirred bioreactor with a fixed basket bed of immobilized cells of *S. cerevisiae* indicated that the presence of basket leads to the significant intensification of medium circulation compared to a conventional stirred bioreactor or to a single turbine basket bioreactor. The mixing time recorded for the medium circulation in the outer region of basket was for about 1.2 to 30 times lower than that previously reached in the absence of the cylindrical bed of immobilized cells. The optimum positions of the Rushton turbines on the stirrer shaft were found to be one inside the cylindrical bed, at its inferior extremity, and the other above the basket. This combination allows the possibility to reach the lowest mixing time values and the most important attenuation of the negative influence of the apparent viscosity increase on the medium hydrodynamics. The mathematical correlation describing the influence of the main factors on the mixing time was established for the optimum positions of turbines, and offers a good concordance with the experimental data (the average deviation was of $\pm 7.42\%$).

Keywords: basket bioreactor, mixing time, *Saccharomyces cerevisiae*, immobilized cells, ethanol, Rushton 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 conventional bioreactors and, therefore, their constructive and functional characteristics are rather similar with the former ones, they offer important advantages, namely as: the increase of the 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 the biocatalysts from the final broths, the diminution or avoidance of the inhibition processes [1-4].

Among these bioreactors, those with fixed beds of biocatalysts are intensively used at small and large scale due to their simple construction, lower cost for operation and maintenance, facile automatization and scaling-up, as well as due to the avoidance of the mechanical lysis of the immobilized cells or enzymes. The bioreactors with fixed beds of biocatalysts promote the intimate contact between the phases and their easier separation, the products with inhibitory effect being removed from the reaction zone (fixed bed), this leading to the reutilization of the biocatalyst without its preliminary regeneration. Moreover, these bioreactors allow large flow rates for the substrates with low solubility [5].

But, the bioreactors with fixed bed of biocatalysts have some disadvantages [1]. The flow inside the bed is laminar, thus leading to low rate of mass and heat transfer and inducing the back-mixing of reverse flow phenomenon. The turbulent flow could be reached only at high flow rate inside the bed, but this is less possible due to the resistance to flow induced by the biocatalysts. On the other hand, the solid particles from effluent can clog the biocatalyst bed, thus leading both to the reducing of the flow rate inside the bed, and to the biocatalyst inactivation. Another important undesirable phenomenon is the formation of the preferential flow channels inside the bed at the beginning of the feed with medium or during the bioreactor working. The formation of these channels induces the deviation from the plug flow and the inefficient conversion of the substrate.

The bioreactors of basket type are derived from the bioreactors with fixed beds, the biocatalysts particles being fixed in an annular cylindrical or conic bed, which is either static around the stirrer [6-9], or rotating [10-13]. Owing to its design, this bioreactor avoids either the disadvantages of the bioreactors with fixed beds, and the flooding/deposition or the mechanical disruption of the biocatalysts particles, phenomena that are encountered in the bioreactors with mobile beds.

The basket bioreactors have been used in food industry for ethanol and carboxylic acids production and for various enzymatic transformations [7,13-15], in pharmaceutical industry for antibiotics production [16] and for cells or tissue cultures [8,17,18].

In this bioreactor, the liquid phase flow combines the perfect mixed flow around the basket with plug flow inside the biocatalysts bed. Thus, the hydrodynamics of the medium around the basket exhibits an important influence on the transfer processes involved in the substrate conversion [19].

Nevertheless, only few experimental or simulated studies on the basket bioreactor hydrodynamics are given in literature, due both to the more complex flow compared with similar systems but without the basket, and to the difficult sampling and analysis of the data obtained for the flow inside the cylindrical or conic bed of biocatalyst [19]. For these reasons, the aim of our works is to describe and quantify the mixing efficiency and its distribution both in the outer region of the basket, between the basket and the bioreactor wall, and in the inner one, in which the stirrer is placed.

In this paper, the results recorded for the outer region are discussed by means of the mixing time recorded for an impeller with two Rushton turbines on its shaft. The basket bed contains *S. cerevisiae* cells immobilized on alginate, biocatalyst used in the ethanol production.

Materials and method

The experiments were carried out in 10 l (8 l working volume) laboratory bioreactor Fermac 310/60 (Electrolab), with computer-controlled and recorded parameters. The bioreactor characteristics are given in Table 1.

Table 1. Characteristics of bioreactor

| d, mm | d/D | H/D | w/d | l/d | h/d | No. blades | No. baffles | s/d |
|----------|------|------|------|------|------|---------------|----------------|------|
| 82 | 0.41 | 1.95 | 0.20 | 0.33 | 0.50 | 6 | 4 | 0.18 |

The cylindrical bed of basket type has the inner diameter of 102 mm, height of 100 mm and the bed thickness of 8 mm. The basket was placed centered around the stirrer, at 120 mm from the bioreactor bottom (Figure 1).

The basket was filled with *S. cerevisiae* cells immobilized on alginate. The immobilization was carried out by cells inclusion into the alginate matrix, according to the method given in literature [20]. The biocatalyst spherical particles having 4 mm diameter were obtained.

For underlining the influence of the medium viscosity on mixing efficiency, water and carboxymethylcellulose sodium salt (CMC) solutions have been used. The CMC solutions have the apparent viscosity in the domain of 25-75 cP. Owing to the difficulty of *in-situ* measurement of viscosity during the experiments, the viscosity was measured before and after each experiment using a viscometer of Viscotester 6 Plus type (Haake). Both the experiments and viscosity measurements were carried out at a temperature of 28°C. Any viscosity change was recorded during the experiments.

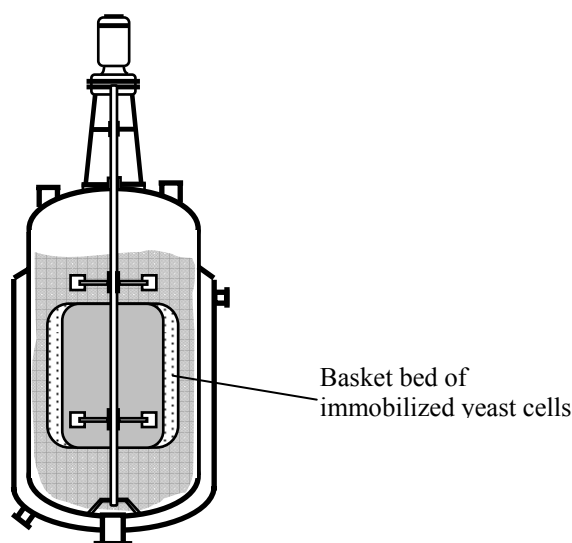


Figure 1. The experimental basket bioreactor

Two mixing systems have been used (A and B), both consisting of two Rushton turbines on the same shaft. One of these turbines (inferior or superior, respectively) is fixed and the other is placed successively in four positions on the stirrer shaft (Table 2, Figure 2). The rotation speed varied from 50 to 300 rpm.

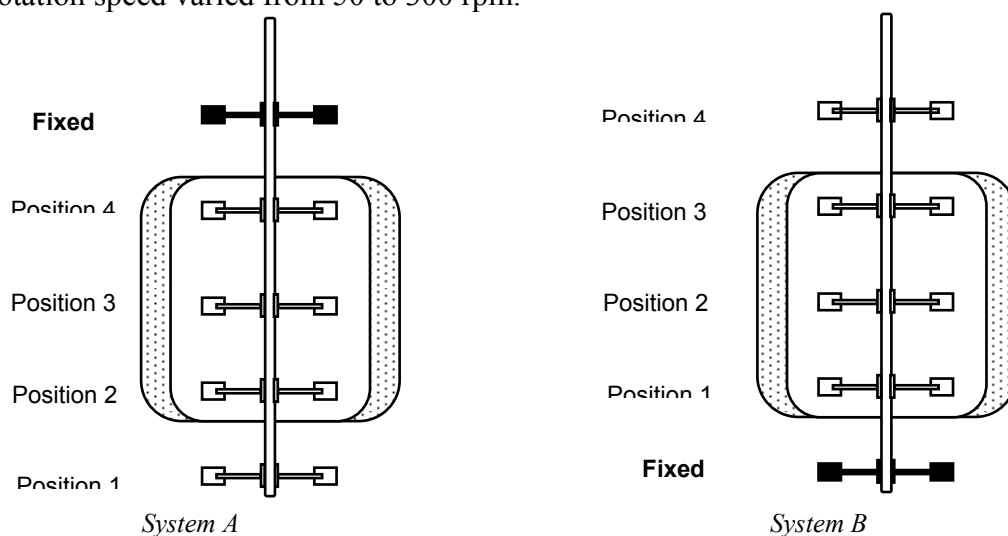


Figure 2. The studied mixing systems

The mixing efficiency was analyzed by means of the mixing time values obtained for various operating conditions, using the tracer method [21]. Thus, a solution of 2N KOH was 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, was considered [21]:

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

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

The tracer volume was of 0.5 ml, the tracer being injected at the opposite diametral position to the pH-electrode, 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 variations were recorded by the bioreactor computer-recorded system and were analyzed to calculate the mixing time. The pH probe was of HA 405 DPAS SC-K8S/325 type (Mettler Toledo).

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

Each experiment was carried out at least three times for identical conditions and the value of mixing time was taken as an average. The maximum experimental error was of $\pm 6.06\%$.

Results and discussion

The mixing systems provided with a cylinder around the stirrer are known to offer superior mixing efficiency, due to the extending of the turbulence, respectively to the increase of the velocity of medium flow streams [1]. Thus, the previous experiments on the hydrodynamics of medium with apparent viscosity between 1 and 75 cP in a stirred bioreactor with a fixed basket bed of immobilized cells of *S. cerevisiae* and one Rushton turbine indicated the significant intensification of medium circulation compared with a similar stirred bioreactor without the basket. Moreover, the optimum position was found to be inside the cylindrical bed, at the superior extremity of the basket. This position leads to the lowest mixing time values and to the most important attenuation of the negative influence of the apparent viscosity increase on the medium circulation.

The obtained results recorded for the two mixing systems, plotted in Figures 3 and 4, indicate the intensification of medium circulation in the outer region of the basket compared with the previously studied case. But, the effect induced is directly related to the positions of the stirrers on the shaft.

For system A, with the inferior Rushton turbine mobile, the lowest values of mixing time are reached for position 2, respectively inside the basket at its inferior extremity, opposite to the fixed stirrer (Figure 3). Contrary, the less efficient mixing was recorded for the closest turbines positions (position 4). Intermediary values of mixing time have been obtained for positions 1 and 3, but they differ from the viewpoint of the dependence on the rotation speed.

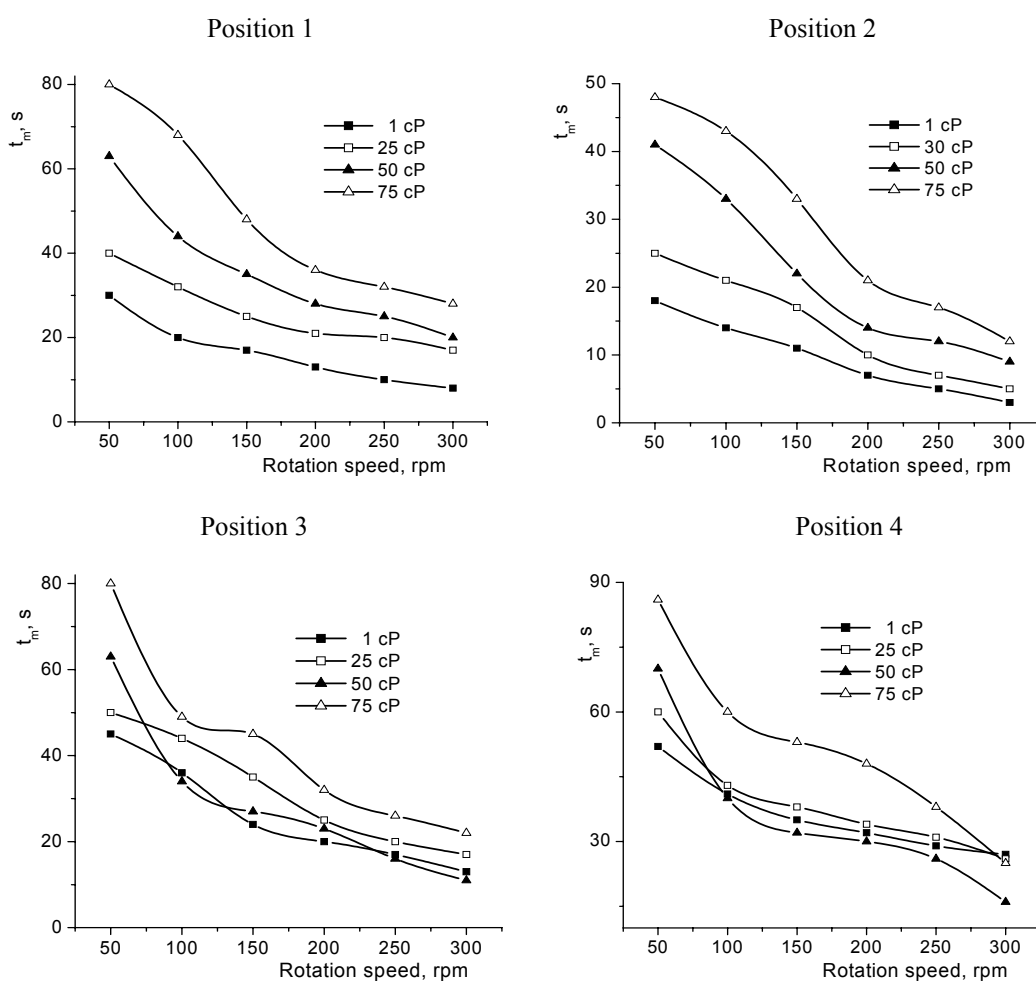


Figure 3. Influence of rotation speed and apparent viscosity on mixing time for system A

Similar to the basket bioreactor with a single Rushton turbine, these results could be the consequence of the acceleration or deceleration of the flow streams induced by the impeller, due either to the basket wall, or to the streams interference. Owing to the presence of two stirrers on the shaft, these phenomena are amplified, thus modifying either the magnitude of the above mentioned effects or the optimum position of the mobile stirrer, compared to the previously studied basket bioreactor with immobilized yeasts cells.

Therefore, because the presence of the cylindrical bed intensifies the medium circulation [1,22], the reduction of mixing time appears as evidently for the positions inside the basket, respectively for the positions 2, 3 and 4. But, according to the experimental results, the values of mixing time lower than those corresponding to position 1 can be reached only if the Mobil stirrer is situated in the inferior region inside the basket (position 2). As it was previously demonstrated [22], by placing the turbine at the basket inferior extremity, the inferior flow regions induced by the impeller is generated outside the cylindrical bed, this avoiding the flow streams interference and leading to the increase of flow velocity of the medium. For position 3 of the mobile turbine on the stirrer shaft, both flow regions generated by the Rushton turbine are situated entirely inside the inner region of the basket, therefore the flow streams are accelerated and interfere, thus leading to the hindrance of the medium circulation compared to the position 2. Furthermore, due to the presence of the second stirrer, the magnitude of the streams interference is increased.

The lowest efficiency of mixing has been reached for position 4, as the result of the interference of the flow streams generated by the two vicinal Rushton turbines, phenomenon that is amplified by reducing the apparent viscosity. Thus, by increasing the rotation speed, the values of mixing time recorded for water and medium with viscosity of 25 cP exceed those obtained for more viscous CMC solutions.

The above discussed phenomena constitute the cause also for the variation of mixing time with rotation speed observed for the system B (Figure 4). For the above mentioned reasons, the most efficient mixing is reached if the mobile stirred is placed inside the basket at its superior extremity (position 3). But, owing to the more important “bottom effect” which hinders the flow of medium promoted by the inferior fixed turbine, the turbulence generated by this stirrers combination is less intense comparatively to that corresponding to the optimum position for the system A (position 2).

The interference of the flow streams for positions 1 and 2 leads to higher values of mixing time for lower viscous medium. Likewise the system A, the most inefficient mixing is recorded for the closest positions of the Rushton turbines on the shaft. On the other hand, owing to the “bottom effect”, the medium circulation for position 1 becomes less intense than that observed for the similar position 4 for the mixing system A.

The increase of the apparent viscosity leads to the significant reduction of the mixing intensity. Thus, as it can be seen from Figure 5, for the rotation speed of 200 rpm, the mixing time reached for the most viscous CMC solution was for about 1.5-2.4 times greater than that obtained for water in the case of system A, respectively for about 1.8-2.3 times greater than that for water for system B. For both systems, the influence of the apparent viscosity was lower than in the previous studied case of a single Rushton turbine on the shaft [22].

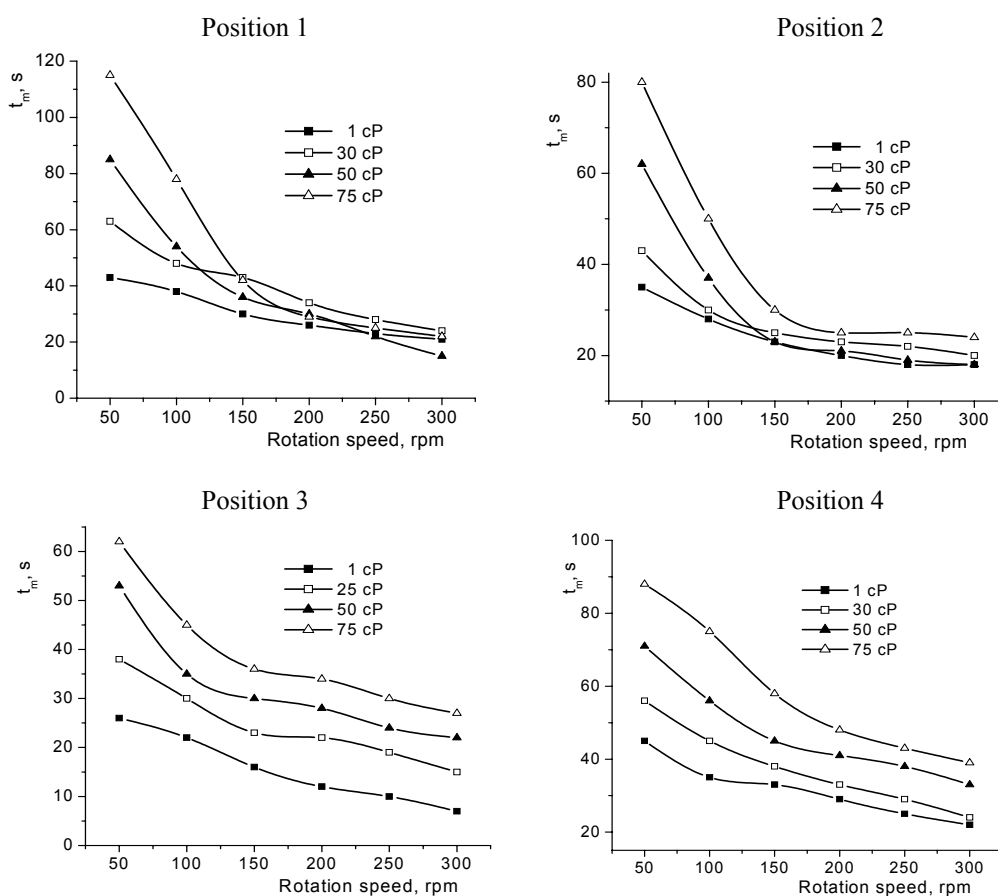


Figure 4. Influence of rotation speed and apparent viscosity on mixing time for system B

Besides the most intense circulation of the medium, position 2 for system A and position 3 for system B, respectively, offer the most important attenuation of the influence of apparent viscosity on the mixing time. By comparing this effect magnitude for the two positions, and taking into consideration also the induced mixing intensity, the position 2 for the system A can be recommended as the optimum stirrers combination.

The intensification of the medium circulation promoted by the presence of the basket inside the bioreactor can be quantify by means of the ratio between the mixing time obtained in the absence of the basket [22], t_{m0} , and that corresponding to the studied basket bioreactor, t_{mB} . The variation of this ratio with the apparent viscosity, plotted in Figure 6 for 200 rpm and both analyzed mixing systems, indicates that the cylindrical bed of immobilized yeast cells amplifies considerably the turbulence inside the medium, t_{m0} being for several times greater than t_{mB} .

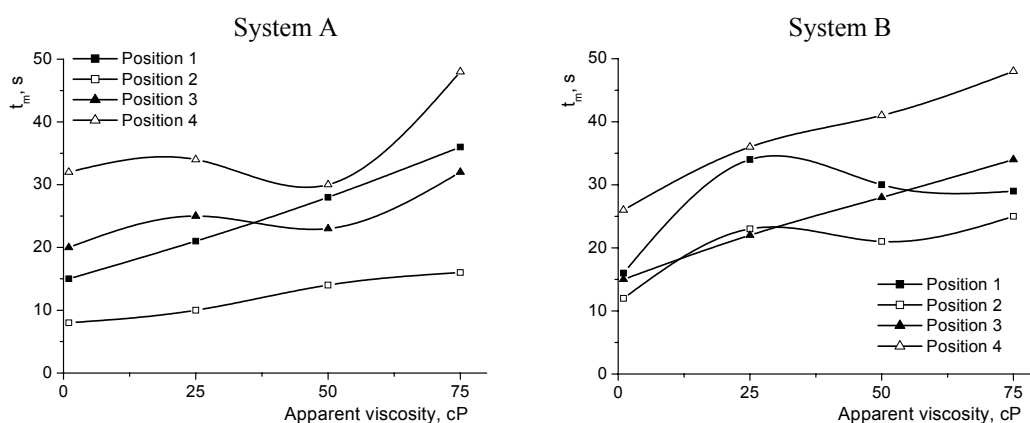


Figure 5. Influence of apparent viscosity on mixing time for the two considered mixing systems at 200 rpm

For the system A, the ratio t_{m0}/t_{mB} varied between 1.2 and 30, and for the system B between 1.2 and 19. The lowest effect of turbulence intensification has been reached for water and CMC solutions with apparent viscosity up to 25 cP (the ratio t_{m0}/t_{mB} was of 1.2-7), while the most important effect for apparent viscosity over 50 cP. Among the two possible optimum stirrers combinations (position 2 for system A and position 3 for system B, respectively), the favorable effect induced for the position 2 system A was the most important.

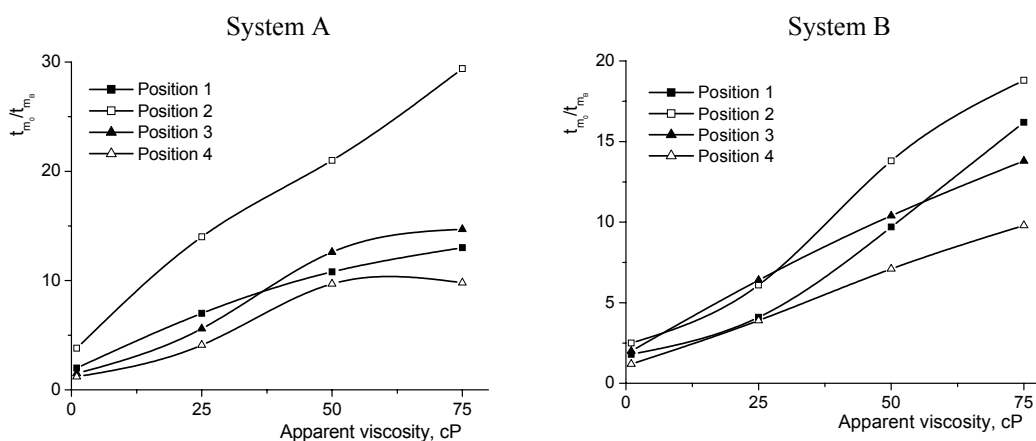


Figure 6. Influence of apparent viscosity on ratio t_{m0}/t_{mB} for the two considered mixing systems at 200 rpm

The cumulated influences of the rotation speed and apparent viscosity on the mixing efficiency for the optimum position of the Rushton turbine on the stirrer shaft, namely position 2 for system A, are plotted in Figure 7.

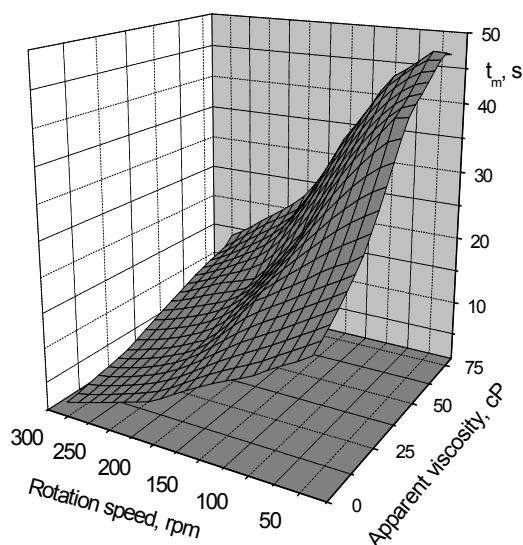


Figure 7. Cumulated influences of rotation speed and apparent viscosity on mixing time for the optimum position 2 system A

By means of the experimental data, the mathematical correlation which describes the influence of rotation speed and apparent viscosity of the medium on the mixing time has been established for the most efficient position, position 2 system A. The explicit values of the coefficients were calculated by the multiregression method using MATLAB software. Thus, the following correlation has been obtained:

$$t_m = e^{(-0.0238 \cdot N^3 + 3.4359 \cdot e^{\eta_a} \cdot N + 0.2684)}, s \quad (2)$$

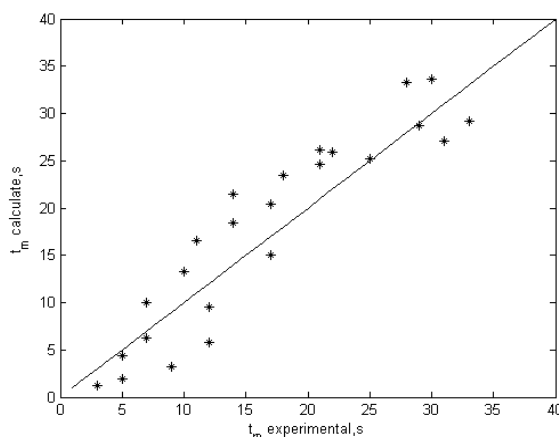


Figure 8. Comparison between the experimental and calculated values of mixing time for the optimum position 2 system A

The proposed equation offers a good concordance with the experimental data, the average deviation being of $\pm 7.42\%$ (Figure 8).

Conclusions

The experiments on the hydrodynamics of medium with apparent viscosity between 1 and 75 cP in a double Rushton turbine stirred bioreactor with a fixed basket bed of immobilized cells of *S. cerevisiae* indicated the significant intensification of medium circulation compared to a similar basket bioreactor with a single impeller. Moreover, the

mixing time recorded in the investigated basket bioreactor was for about 1.2 to 30 times lower than that reached in the similar conventional stirred bioreactor.

By means of the analysis of the influence of the two turbines position on the stirrer shaft on the mixing efficiency in the outer region of the basket, the optimum combination was found to be that consisting in one superior stirrer outside the basket and the other inside the basket at its inferior extremity. This combination leads to the lowest mixing time values and to the most important attenuation of the negative influence of the apparent viscosity increase on the medium circulation.

The selected system for mixing will be used in the further experiments for ethanol or biosolvents production in this basket bioreactor.

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Notations

- d - impeller diameter, mm
- D - bioreactor diameter, mm
- h - distance from the bioreactor bottom to the inferior impeller, m
- H - bioreactor height, mm
- l - impeller blade length, mm
- N - rotation speed, s⁻¹
- s - baffle width, mm
- w - impeller blade height, mm
- t_m - mixing time, s
- η_a - apparent viscosity, Pa.s

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