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Comparative evaluation of radial impellers efficiency for bioreactors with stirred bed of immobilized cells
4. Studies on mechanical effect on biocatalysts integrity

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Abstract

The comparative study on the mechanical disruption of S. cerevisiae immobilized cells in alginate (biocatalysts with 4, 4.6 and 5.2 mm diameters) for a bioreactor with stirred beds of biocatalysts and six radial impellers indicated the intensification of mechanical lysis with the increase of particles size and volumetric fraction. The mechanical lysis is produced mainly by the collision between the biocatalysts particles or between them and the impellers blades. The paddle with six blades and the pitched bladed turbine induced the less important effect on biocatalysts physical integrity, these impellers being recommended for the bioreactors with stirred beds of immobilized yeasts cells.

Keywords: bioreactor, stirred bed, immobilized cells, yeasts, mechanical lysis, radial impeller, disperser sawtooth, Smith turbine, pumper mixer, curved bladed turbine, paddle with six blades, pitched bladed turbine.

Introduction

The spectacular applications of the immobilized biocatalysts determined the design and construction of proper bioreactors, specific or derived from the "classical" ones. Although these bioreactors are constructively and functionally similar to the other types of bioreactors, they offer important advantages through the used immobilized cells or enzymes: thermal, chemical and to the shear forces resistance, increasing number of biosynthesis cycles using the same particles of biocatalysts, easier recovery of the biocatalysts, diminution or avoidance of the inhibition processes [1-6].

Among the bioreactors with immobilized biocatalysts, those with stirred/mobile beds are ones of the most attractive, owing to their constructive and operational similitude to the well-known stirred bioreactors. The applications of these bioreactors are directed to the production of pharmaceuticals [1,7,8], chemicals [9], solvents and biofuels [10,11], and treatment of wastewaters [12-16].

Mixing represents one of the main factors controlling the performances of bioreactors with stirred beds of biocatalysts, being influenced by many constructive and operational

parameters [4,5]. Because the immobilized cells or enzymes are sensitive to the forces generated by the impellers, the analysis of mechanical stability of biocatalysts are also required for process optimization.

Any microorganism, vegetal or animal cell, enzyme (free or immobilized) from a mixed media is the subject of the action of shear forces, their magnitude depending on the viscosity and velocity rate of the liquid, as well as on the size and structure of the solid particle. The sensibility of different biocatalysts to the shear forces are comparatively indicated in Table 1.

Table 1: Resistance of the biocatalysts to the shear forces [1].

Biocatalysts	Particle size	Resistance
Enzymes	nanometer	+ -
Microbial cells	1-10 μ	-
Animal cells	20-150 μ	+ + +
Vegetal cells	100 μ	+
Immobilized biocatalysts	15-50 mm	+ -
Vegetal cells associations	> 1 cm	+ +
Microbial associations	> 1 cm	+

For the most of the stirred bioreactors the dynamic forces exceed the viscous ones. In these circumstances, the mechanical lysis of the biocatalysts occurs in the regions with high shear stress, respectively either in the regions with high local shear rate gradients, or between two eddies. Moreover, even in these systems or in those with less intense circulation of the media, the mechanical disruption of the biocatalysts occurs in the regions with high concentration of solid phase, due to the collision between the particles or between the particles and the impellers.

For these reasons, the previous studies for selecting the optimum impellers combination for a bioreactor with stirred bed of immobilized yeasts cells have to be developed by analyzing comparatively the mechanical effects promoted by the impellers on the biocatalysts. The experiments presented in this paper were carried out for the six types of radial impellers previously selected and for *S. cerevisiae* cells immobilized on alginate with different particles size and concentration.

Materials and Method

The experiments have been carried out in 5l (4l working volume, ellipsoidal bottom) laboratory bioreactor (Biostat A, B. Braun Biotech International), with computer-controlled and recorded parameters [19].

The mixing system consists of a double stirrer and three baffles. Six types of radial impellers have been used (Figure 1).

The diameter of the two impellers on the shaft 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 as it was demonstrated in the previous works [20]. In the purpose to avoid the "cave" formation at the broths surface, the rotation speed was of maximum 300 rpm.

In the experiments, *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 [21]. 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, ϕ, ranged between 0.07 and 0.40.

The experiments have been carried out at a temperature of 25°C.

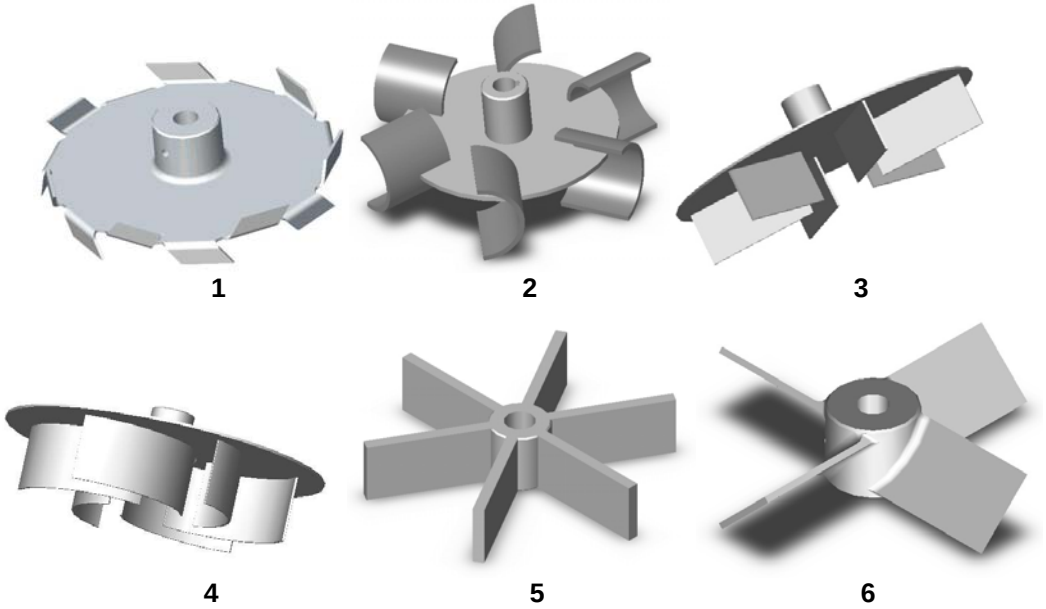


Figure 1. The radial impellers used in experiments (1 - Disperser sawtooth, 2 - Smith turbine, 3 - Pumper mixer, 4 - Curved bladed turbine, 5 - Paddle with six blades, 6 - Pitched bladed turbine).

The mechanical disruption of biocatalysts has been analyzed using the turbidimetric method. Thus, the variation of turbidity during the mixing process, which is directly related to the extent of mechanical damage of solid particles, has been measured with the turbidimeter of Hach 2100N type for each considered impeller and biocatalyst particles. The duration of experiments and interval between the sampling are indicated in the Table 2.

Table 2: Temporal parameters of the experiments.

Rotation speed, rpm	**Overall duration**, min	**Sampling interval**, min
50	180	30
100	150	25
150	120	20
200	90	15
250	60	10
300	Max. 120	Min. 5

Each experiment has been carried out three times, for identical conditions, the maximum experimental error being of between ±6.06%.

Results and Discussion

The study on the physical integrity of the biocatalysts underlined that the extent of the particles mechanical disruption is strongly influenced by the size and concentration of biocatalysts, mixing duration and intensity, in direct correlation with the type of the impeller.

The analysis of the magnitudes of the mechanical lysis effects induced by the considered radial impellers was made by means of the variations plotted in Figures 2 and 3, the results being comparatively given in Tables 3 and 4.

Table 3: Relative magnitudes of the mechanical lysis effects induced by the six radial impellers after 40 min.

Impeller type	**Relative magnitude of the effect** (1 - minimum, 6 - maximum)						**Relative sum**
	1	***2***	***3***	***4***	***5***	***6***	
Disperser sawtooth		***		*	*	*	21
Smith turbine	*		*	*	**	*	24
Pumper mixer		*	*	*	**	*	25
Curved bladed turbine		*	**	*	*	*	23
Paddle with six blades	**	*	***				13
Pitched bladed turbine	***	*	**				10

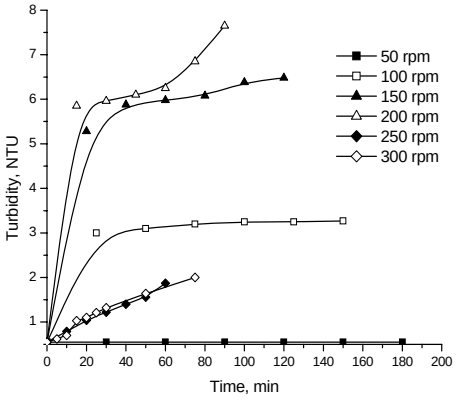
The results given in Tables 3 and 4 lead to the following order of the degree of biocatalysts disruption for the mixing duration of 40 min:

Pitched bladed turbine < Paddle with six blades << Disperser sawtooth < Curved bladed turbine < Smith turbine < Pumper mixer

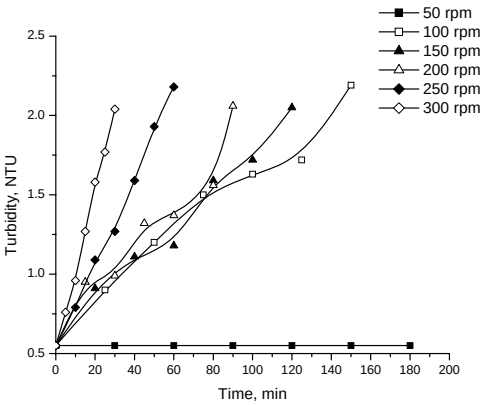
respectively, for the duration of 80 min:

Pitched bladed turbine < Paddle with six blades << Curved bladed turbine < Disperser sawtooth < Smith turbine ≅ Pumper mixer

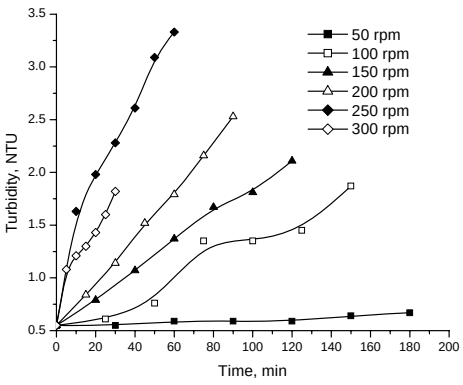
Disperser sawtooth



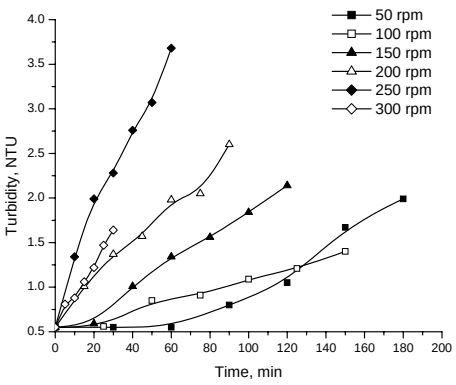
Smith turbine



Pumper mixer



Curved bladed turbine



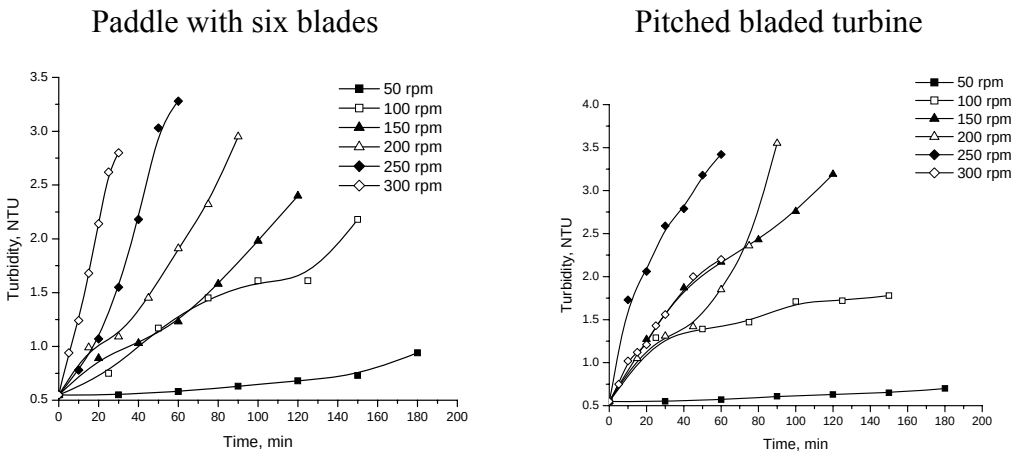
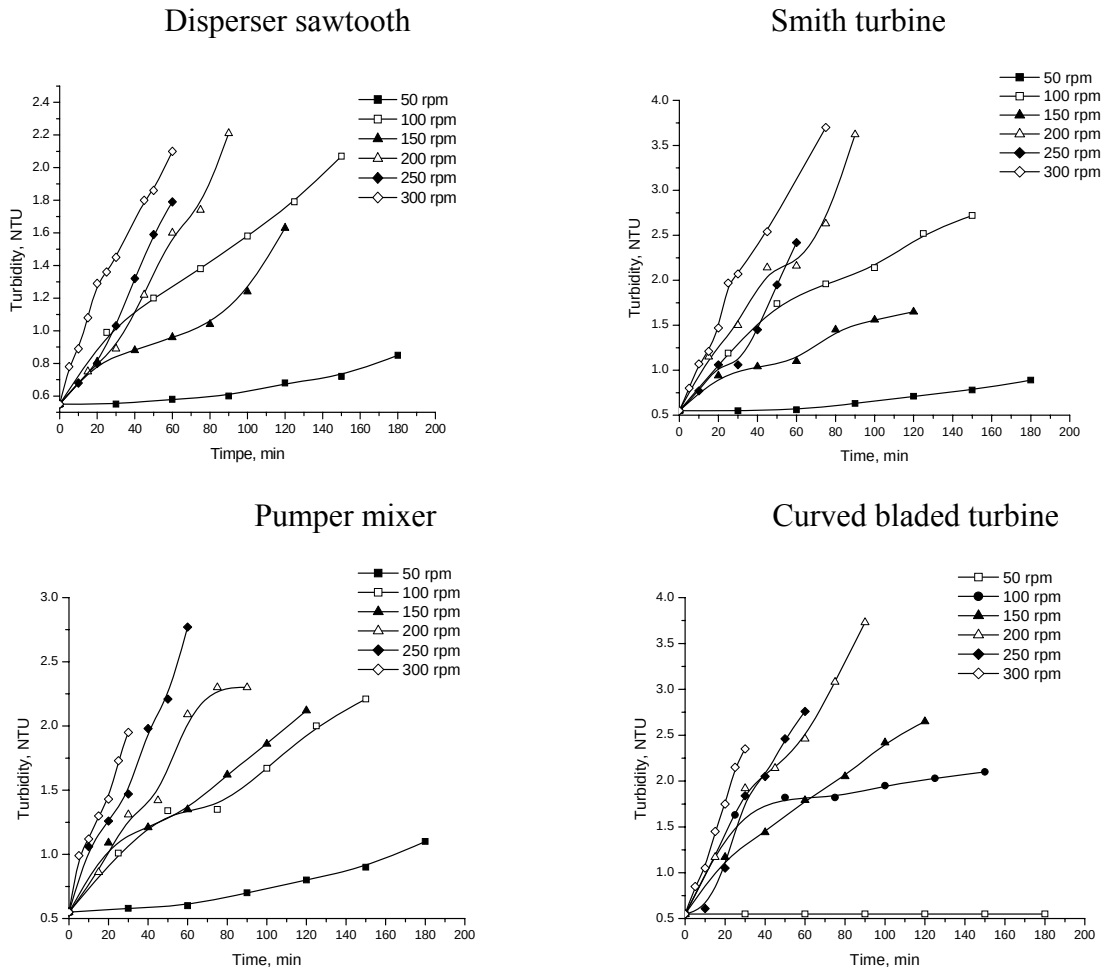


Figure 2. Variation of magnitude of mechanical lysis of biocatalysts in time (particles diameter = 4 mm, volumetric fraction $\phi = 0.25$).



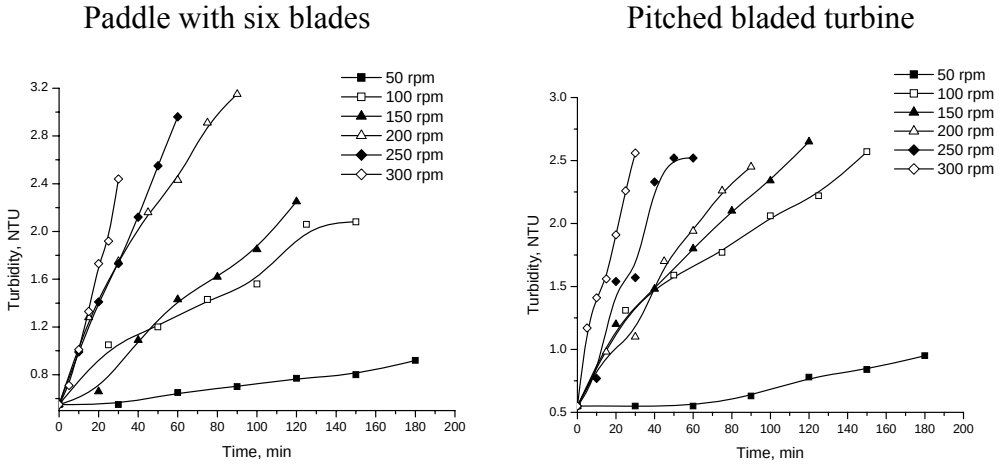


Figure 3. Variation of magnitude of mechanical lysis of biocatalysts in time (particles diameter = 5.2 mm, volumetric fraction $\phi = 0.25$).

As it can be seen from these sequences, the duration of mixing does not change significantly the order of the importance of mechanical lysis of the biocatalysts particles.

Table 4: Relative magnitude of the mechanical lysis effects induced by the six radial impellers after 80 min.

Impeller type	**Relative magnitude of the effect** (1 - minimum, 6 - maximum)						**Relative sum**
	1	***2***	***3***	***4***	***5***	***6***	
Disperser sawtooth			**	***		*	24
Smith turbine	*	*		*		***	25
Pumper mixer		*	*	*	**	*	25
Curved bladed turbine		*	*		***	*	23
Paddle with six blades	*	****	*				12
Pitched bladed turbine	****	*	*				9

The obtained order cannot be attributed only to the variation of mixing intensity, respectively to the variation of induced shear stress magnitude. According to the previous studies [20], the most intense mixing has been recorded for Smith turbine, this justifying the position of this impeller in the two sequences. For pumper mixer and curved bladed turbine, the degree of biocatalysts disruption is important, owing to the deposition of particles at bioreactor bottom [20] and, implicitly, to the collision between them or with the impellers.

Although the lowest mixing efficiency is reached when the disperser sawtooth is used [20], this impeller is also included in the category of the impellers promoting important mechanical lysis (Tables 3 and 4). This result is due to the inefficient mixing, which allow the solid phase deposition and accumulation at the bioreactor bottom, the impeller conformation enhancing the biocatalysts disruption by collision with its blades.

Contrary to the disperser sawtooth, the pitched bladed turbine and paddle with six blades induce an intense circulation of the suspension [20]. But, their blades configurations lead to the slipping of the flow streams [22], avoiding the impact with the impellers, thus diminishing the mechanical lysis of the biocatalysts particles.

Therefore, the mechanical damage of the immobilized yeasts cells in the studied systems is produced mainly by the collision between the particles or with the impellers blades, and less by the shear forces generated by suspension circulation.

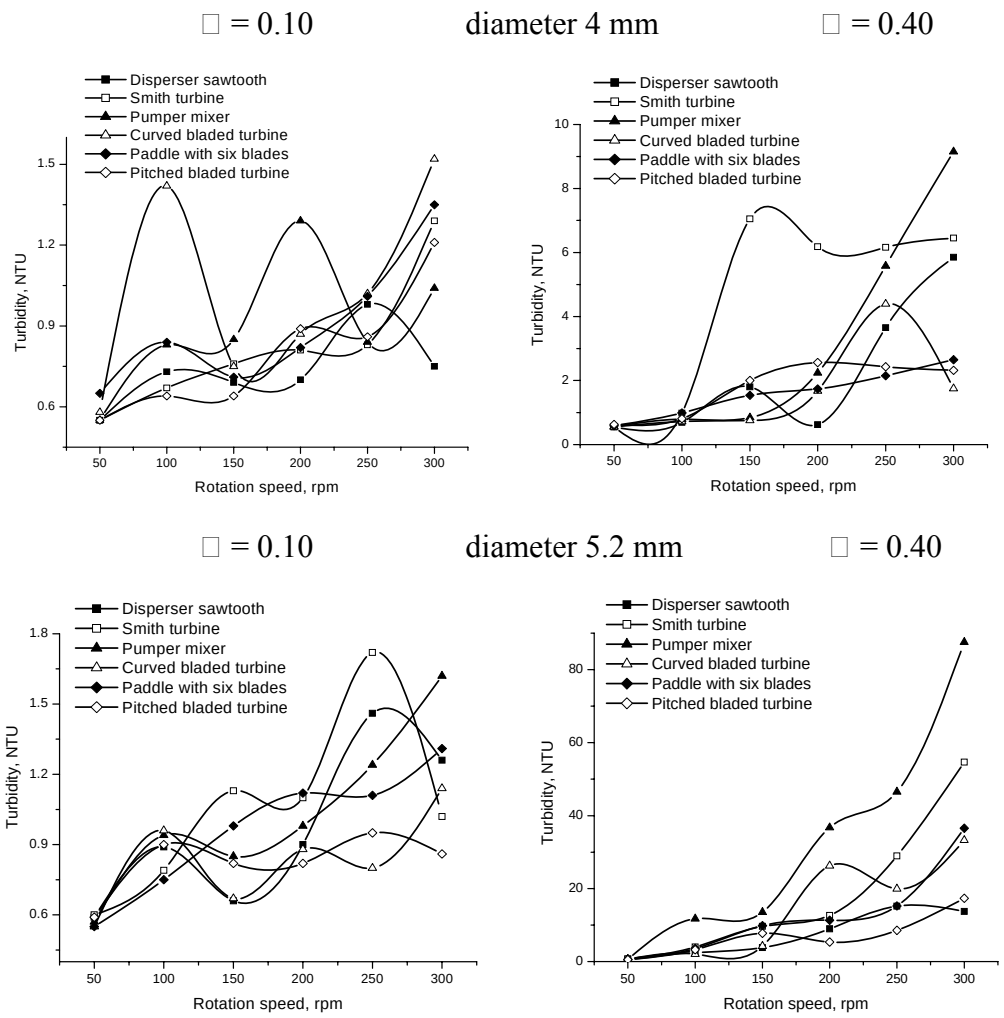


Figure 4. Influence of rotation speed on magnitude of mechanical lysis of biocatalysts.

The analysis of the influence of rotation speed on mechanical disruption degree, plotted in Figure 4, suggests a variation contrary to that recorded for mixing time in the experimented rotation speed domain [20]. The sinusoidal variation of turbidity with rotation speed increase is recorded especially for lower sizes and volumetric fractions of the biocatalysts. By increasing the diameter and concentration of immobilized cells particles, the dependence between the turbidity and mixing intensity becomes direct proportional. Furthermore, for the same rotation speed values, the magnitude of mechanical lysis is significantly enhanced with the increase of the size and concentration of biocatalysts. Thus, the accumulation of solid phase by deposition at bioreactor bottom exhibits a negative effect on particles integrity.

From the above reasons, the increase of biocatalysts diameter leads to the intensification of disruption effect, phenomenon that is amplified at higher volumetric fraction (Figure 5). Although this influence was recorded for all considered radial impellers, at biocatalysts concentration lower than 25% vol. the influence of particle size is considerably diminished for Smith turbine, pitched bladed turbine and paddle with six blades. This

differentiation is due to the more intense circulation of the suspension promoted by this three impellers and, implicitly, to the avoidance of the particles deposition.

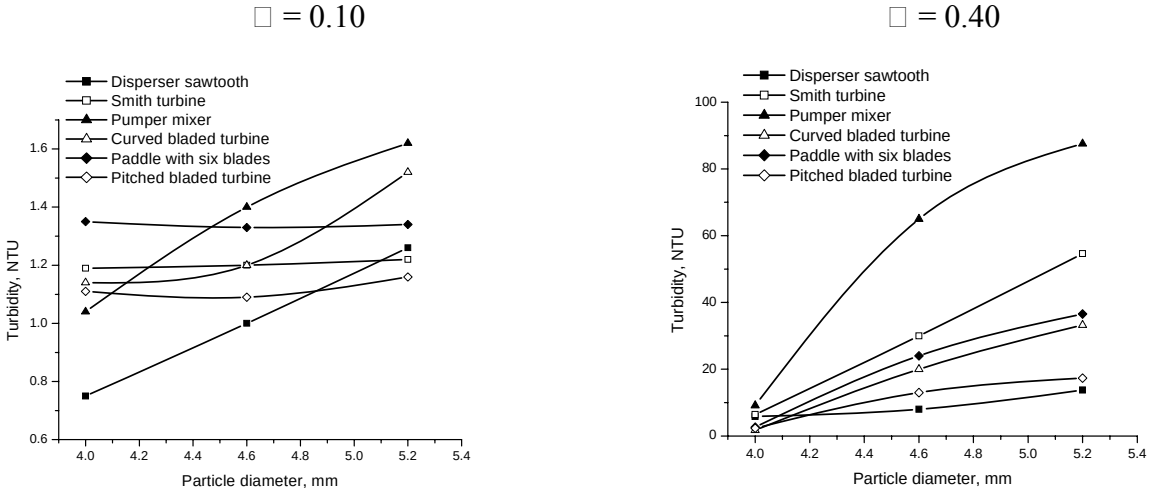


Figure 5. Influence of particles diameter on magnitude of mechanical lysis of biocatalysts.

Conclusions

The experiments on mechanical effects on physical integrity of immobilized *S. cerevisiae* cells induced by six radial impellers underlined the significant contribution of the collision between the biocatalysts particles or between them and the impellers. This mechanism of biocatalysts disruption is supported by the influence of particles size and volumetric fraction.

The most diminished effects have been generated by the pitched bladed turbine and paddle with six blades impellers. These results, cumulated with those previously obtained by studying the efficiency and distribution of mixing, recommend this two impellers to be used in the bioreactor with stirred bed of immobilized yeasts cells.

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