Optimization of mixing in stirred bioreactors
3. Comparative analysis of shear promoted by the radial impellers for anaerobic simulated broths

Received for publication, June 15, 2007
Accepted, July 20, 2007

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Abstract

In order to select the optimum impeller combination for a given broth, the studies on mechanical mixing performances are continued by comparatively analyzing the shear forces promoted by seven types of radial impellers. The obtained results indicated the following order of the shear stress magnitude, valid for the domain of apparent viscosity of 1 - 60 cP: Rushton turbine << Paddle with 6 blades < Smith turbine << Curved bladed turbine < Pumper mixer ≈ Pitched bladed turbine << Disperser sawtooth.

Unlike to the shear rate variation, for each studied impeller the shear stress increases with the apparent viscosity increase up to 60 cP, over this level the modification of shear forces being made only by rotation speed changing, independently of the further increase of the apparent viscosity.

Keywords: stirred bioreactor, radial impeller, Rushton turbine, disperser Sawtooth, Smith turbine, pumper mixer, paddle with 6 blades, pitched bladed turbine, stirred bioreactor, shear rate, shear stress.

Introduction

The analysis of media circulation into a stirred vessel by means of the homogenization level at a given moment represents the base for selecting the most efficient mixing system, respectively for optimizing the mixing of fermentation broths [1]. Among the three homogenization levels indicated in the literature, each describing the mixing at a certain scale (macro-, meso- and micromixing), the macromixing is directly related to and depended on media circulation and offers information concerning the mixing intensity in bioreactors (meso- and micromixing become important especially for the systems in which phase transformation or chemical/biochemical reaction occurs) [2].

Compared to the chemical reactors, the difficulty of the analysis of mixing efficiency in bioreactors is amplified by the biomass accumulation, which has the characteristics of the solid phase (deposition tendency) and pronounced shear stress sensitivity, and by fermentation processes particularities, especially high viscosity or non-Newtonian rheology behavior of the broths, as well as the presence of gaseous phase, as the result of aeration or cells respiration. Due to the complexity of rheological behavior and high viscosity of broths, and, consequently, due to the flow patterns, in bioreactor is inevitably induced a non-uniform mixing distribution with the appearance of the stagnant regions.

Although the radial impellers, especially the Rushton turbine, are widely used in the large-scale stirred bioreactors, their use is limited by the high viscosity and non-Newtonian behavior of the broths. For example, in the case of filamentous fungus cultures with high apparent viscosity, it is recommended a double stirrer, provided on the same shaft with one Rushton turbine at the inferior region and one paddle with plane blades at the superior region [3]. The inferior stirrer promotes high turbulence and, therefore, avoids the biomass deposition, and the superior one creates high flow velocity of the broth.

For these reasons, the comparative analysis of the mixing efficiency induced by different impellers types for different broths is required. But, besides the intensification of the broths flow, for selecting a certain impeller or impellers combination it has to be taken into account its energetic efficiency and shear effects on biomass.

The efficiency and distribution of mixing induced by seven radial impellers have been previously comparatively analyzed [4,5]. The current studies have been carried out on anaerobic simulated broths of different apparent viscosities, without biomass. Therefore, the following impellers combinations have been selecting for reaching simultaneously high mixing intensity and uniform mixing distribution with lower power consumption:

- **water and similar media**: stirrer equipped at the inferior part with a disperser sawtooth, and at the superior part with a paddle with six blades
- **moderate apparent viscosity (below 25 - 30 cP)**: stirrer provided with a pitched bladed turbine at the inferior part and a Rushton turbine at the superior part
- **high apparent viscosity (up to 100 cP)**: stirrer equipped with an inferior pumper mixer and a superior disperser Sawtooth.

Thus, by using specific impellers combinations for a given broth, optimum mixing can be reached, from the viewpoint both of the broth circulation intensity, and of the power consumption. Besides these two criteria, for selecting a proper impeller the effects on biocatalysts (shear forces) have to be analyzed.

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 indicated in Table 1.

Table 1. Resistance of the biocatalyst to the shear forces [1].

Biocatalysts	Particle size	Resistance
Enzymes	nanometer	+ -
Micobial 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 fermentation processes, the mixing induces the transitory and/or low turbulent flow of the broths, the dynamic forces exceeding 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. The gradients of shear stress near the impellers are higher than the average shear stress [3]. Moreover, the shear rate controls the apparent viscosity of the non-Newtonian liquids, thus influencing the mass and heat transfer rates, as well as the power consumption for mixing.

For these reasons, the previous studies for selecting the optimum impellers combination have to be developed with the comparative analysis of the shear rate and shear stress promoted by these impellers, in the purpose to identifying and avoiding the conditions which allow high shear forces to be induced. The experiments have been carried out for each type of radial impeller and fermentation broths formerly considered.

Materials and Method

The experiments have been carried out in 5 l (4 l working volume, V, ellipsoidal bottom) laboratory bioreactor (Biostat A, B. Braun Biotech International), with computer-controlled and recorded parameters. The bioreactor and mixing characteristics, and the operating parameters have been presented in the previous papers [4,5]. For mixing, we have used seven types of radial impellers.

Table 2. Mathematical correlations for shear rate [8].

Rheological behavior	Flow regime	
	Laminar	Transitory and turbulent
Newtonian	(1)	$\gamma = \sqrt{\frac{4 \cdot N_p \cdot \rho \cdot d^2}{\pi \cdot \left(\frac{D}{d}\right)^3 \cdot \eta}} \cdot \sqrt{N^3}$ (2)

Non-Newtonian (pseudoplastic)	(3)	(4)

where: C - constant in N_P equation; d - impeller diameter, m; D - bioreactor diameter, m; k - consistency index, $Pa.s^n$; n - flow index; N - rotation speed, s^{-1}; N_P - Power number; γ - shear rate, s^{-1}; η - viscosity, Pa.s; ρ - density, kg/m^3.

In the experiments, we have used water and simulated fermentation broths. The simulated broths consisted of carboxymethylcellulose sodium salt (Sigma Chemie GmbH) solutions having the apparent viscosity, in the domain of 15 - 96 cP. The apparent viscosity and rheological indexes of the broths have been determined by means of the Ostwald viscometer with variable pressure drop, using the Whorlow relationships [6,7]. Owing to the difficulty of *in-situ* measurement of viscosity during the experiments, the viscosity was measured before and after each experiment using this viscometer type. Both the experiments and viscosity measurements were carried out at a temperature of 25^{o}C. Any viscosity change was recorded during the experiments.

The shear rate has been calculated with mathematical correlations adequate for the flow regime and rheological behavior of the broths given in Table 2.

Results and discussion

The complex role of mixing in the bioreactor is to promote the broth circulation that can compensate the negative effects of the continuous modification of the medium rheological properties, due to the accumulation of biomass or biosynthesized product during the fermentation, on the transfer processes. In order to increase the efficiency of the mixing process, the bioreactors are provided with multiple agitator systems which consist on two or more identical or different impellers assembled on the same shaft, their number being in function of the broths height in the vessel. The mixing efficiency in the systems with multiple stirrers is directly related to the capacity of mixing to generate high turbulence and intense circulation into the whole fermentation broths. The distance between the impellers on the stirrer shaft controls the interactions of the generated flow streams, its optimum value depending on the nature and viscosity of the fermentation broths [9,10].

In the previous experiments the mixing efficiencies of seven double stirrers have been comparatively analyzed for non-aerated water and simulated fermentation broths with different apparent viscosities [7]. These studies have been finalized by proposing two combinations of different impellers for each media, one offering the most intense and uniform mixing, and the other requiring the lowest power consumption for a certain level of mixing intensity. Further on, for verifying these impellers combinations and selecting the optimum impellers combination among the two proposed for each broth, these studies have been developed by analyzing concomitantly the intensity of mixing and the energetically cost for mixing, in direct relation with the capacity of these stirrers to promote an uniform distribution of mixing into the bulk volume of the broths. Furthermore, for ensuring also an optimum mass transfer of the limiting substrate, the selection of the appropriate impellers combination have been made taking into account the possibility to obtain mixing time value below 30 s with lower power consumption (the value of 30 s represents the average superior limit of the duration of oxygen consumption from the broths in the microbial respiration process without addition of new amount of solved oxygen into the media, the oxygen being the limiting substrate with the lowest solubility into the fermentation broths [9]).

But, the mixing has to induce an intense circulation of the media without mechanically disrupting the biocatalysts, respectively without exceeding the maximum level of biocatalysts tolerance to the shear stress. Besides the broths viscosity and complex rheological behavior, this limitation represents one of the main causes of the inevitable appearance of stagnant regions into the bioreactor, indifferent of the constructive and operational characteristics of the used stirrers.

The results obtained for the rheological indexes determination of the simulated fermentation broths, needed for the shear calculations, and for the analysis of the shear forces induced by these seven radial impellers are presented below.

1. Determination of the rheological indexes

For calculating the shear rates promoted by the used impellers, the rheological indexes of the simulated broths have been initially determined. In this purpose, the viscometer of Ostwald type with variable pressure drop and the calculation method proposed by Whorlow have been used [7]:

(5)

where: V - volume of the liquid, m^3

r - capillary radius, m

τ - shear stress, N/m^2

τ_p - shear stress at capillary wall, N/m^2, calculated with the expression:

$$\tau_p = \frac{r \cdot \Delta p}{2l} \qquad (6)$$

Δp - pressure drop which controls the liquid flow through the capillary, N/m^2

l - capillary length, m.

From the preliminary experiments it has been concluded that carboxymethylcellulose sodium salt solutions exhibit a pseudoplastic behavior for the considered apparent viscosity domain. Therefore, the flow of the simulated broths can be described by the following rheological equation (*power law*) [5,7]:

$$\tau = k \cdot \gamma^n \qquad (7)$$

the shear rate being calculated in function of the shear stress by means of the relationship:

$$\gamma = \left(\frac{\tau}{k}\right)^{\frac{1}{n}} \qquad (8)$$

By replacing the shear rate with the above correlation in equation (5), the Whorlow relationship becomes:

$$\frac{V}{t\pi r^3} = \frac{1}{\tau_p^3} \cdot \int_0^{\tau_p} \tau^2 \cdot \left(\frac{\tau}{k}\right)^{\frac{1}{n}} \cdot d\tau \qquad (9)$$

having the following solution:

$$\frac{V}{t\pi r^3} = \frac{1}{k^{\frac{1}{n}}} \cdot \frac{\tau_p^{\frac{1}{n}}}{3+\frac{1}{n}} \qquad (10)$$

The logarithm form of the expression (10) represents the equation of the straight line:

$$\ln \frac{V}{t\pi r^3} = \ln\left(\frac{1}{k^{\frac{1}{n}}} \cdot \frac{1}{3+\frac{1}{n}}\right) + \frac{1}{n} \cdot \ln \tau_p \qquad (11)$$

with the slope of 1/n and the intersection with the ordinate of $\ln\left(\frac{1}{k^{\frac{1}{n}}} \cdot \frac{1}{3+\frac{1}{n}}\right)$, thus allowing to calculate the flow and the consistency indexes.

The characteristic straight lines for the simulated broths are plotted in Figure 1.

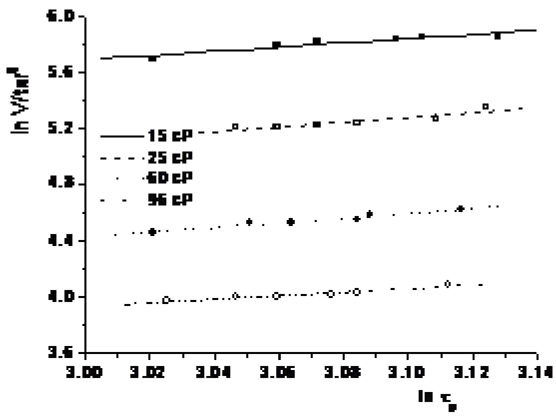


Figure 1. Linearization of the solution of Whorlow equation for the used simulated broths.

The rheological indexes of these broths have been calculated by means of the straight lines from Figure 1, the influence of the apparent viscosity, η_a, on these indexes being graphically described in Figure 2.

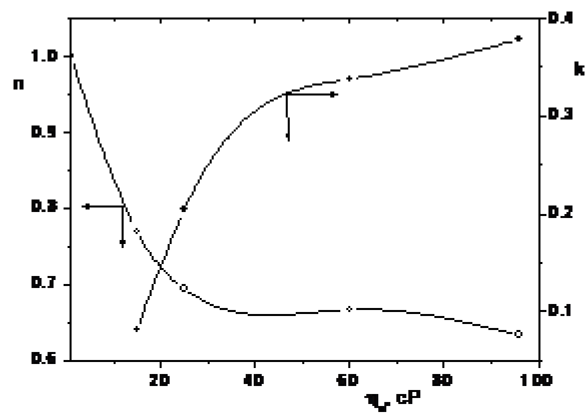


Figure 2. Influence of the apparent viscosity on the flow and consistency indexes.

The dependences plotted in Figure 2 suggest an exponential correlation between the rheological indexes and the apparent viscosity of the broths with the general expressions:

$$\mathbf{n} = \alpha \cdot \eta_a^{\beta}$$

$$\mathbf{k} = \alpha' \cdot \eta_a^{\beta'} \qquad (12)$$

The coefficients α, α’, β, β’ can be obtained by linearizing the equations (12), similar to the equation (10):

$$\ln n = \ln \alpha + \beta \cdot \ln \eta_a$$

$$\ln k = \ln \alpha' + \beta' \cdot \ln \eta_a \qquad (13)$$

and by plotting lnn, respectively lnk *vs.* lnη_a (Figure 3).

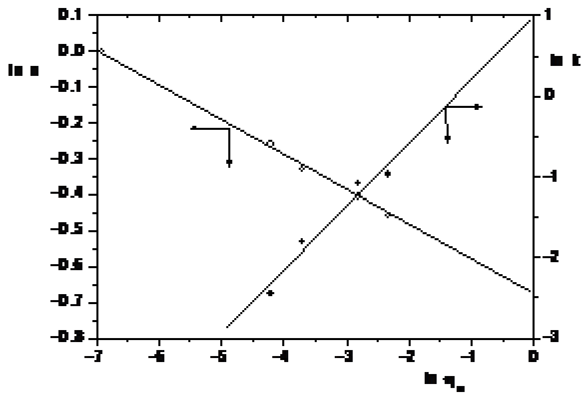


Figure 3. lnn and lnk *vs.* lnη_a.

Thus, the equations (13) can be explicitly written:

$$n = 0{,}486 \cdot \eta_a^{-0{,}1075}$$

$$k = 2{,}713 \cdot \eta_a^{0{,}7957} \qquad (14)$$

According with Table 3, the calculated values of flow and consistency indexes offer a good concordance with their experimental values, the average deviation being of ±1.52% for the flow index and of -9.04% for the consistency one.

Table 3. The experimental and the calculated values of flow and consistency indexes.

Viscosity, Pa.s		**$15x10^{-3}$**	**$25x10^{-3}$**	**$60x10^{-3}$**	**$96x10^{-3}$**
Flow index, n	Experimental	0.769	0.704	0.667	0.633
	Calculated	0.763	0.722	0.657	0.625
	Deviation, %	-0.78	+2.55	-1.49	-1.26
	Average deviation, %	±1.52			
Consistency index, k	Experimental	0.081	0.195	0.337	0.378
	Calculated	0.077	0.165	0.321	0.336
	Deviation, %	-4.93%	-15.38	-4.74	-11.11
	Average deviation, %	-9.04			

2. Calculation of the shear rate and shear stress generated by the radial impellers

By means of the equations (1)-(4) and using the values of the rheological indexes, the shear rate promoted by each studied radial impeller have been calculated for the simulated broths. The dependences between the shear rate and rotation speed are given in Figure 4. From this figure it can be seen that the highest values of shear rate are induced by Rushton turbine, and the lowest ones by disperser sawtooth, indifferent of the rotation speed or broths apparent viscosity.

From the information exiting in literature concerning the effects of the shear on the biocatalysts, it was concluded that the modification of the apparent viscosity or of rheological indexes leads to the modification of the mechanical forces which can disrupt the biocatalyst existing into the liquid [3]. For this reason, the analysis of the shear effect by means of the shear stress is more relevant.

For the studied radial impellers, the promoted shear stress has been calculated by using the *power law*, equation (7), for the pseudoplastic behavior, respectively with the relationship:

$$\tau = \eta \cdot \gamma \qquad (15)$$

for the Newtonian rheology (water).

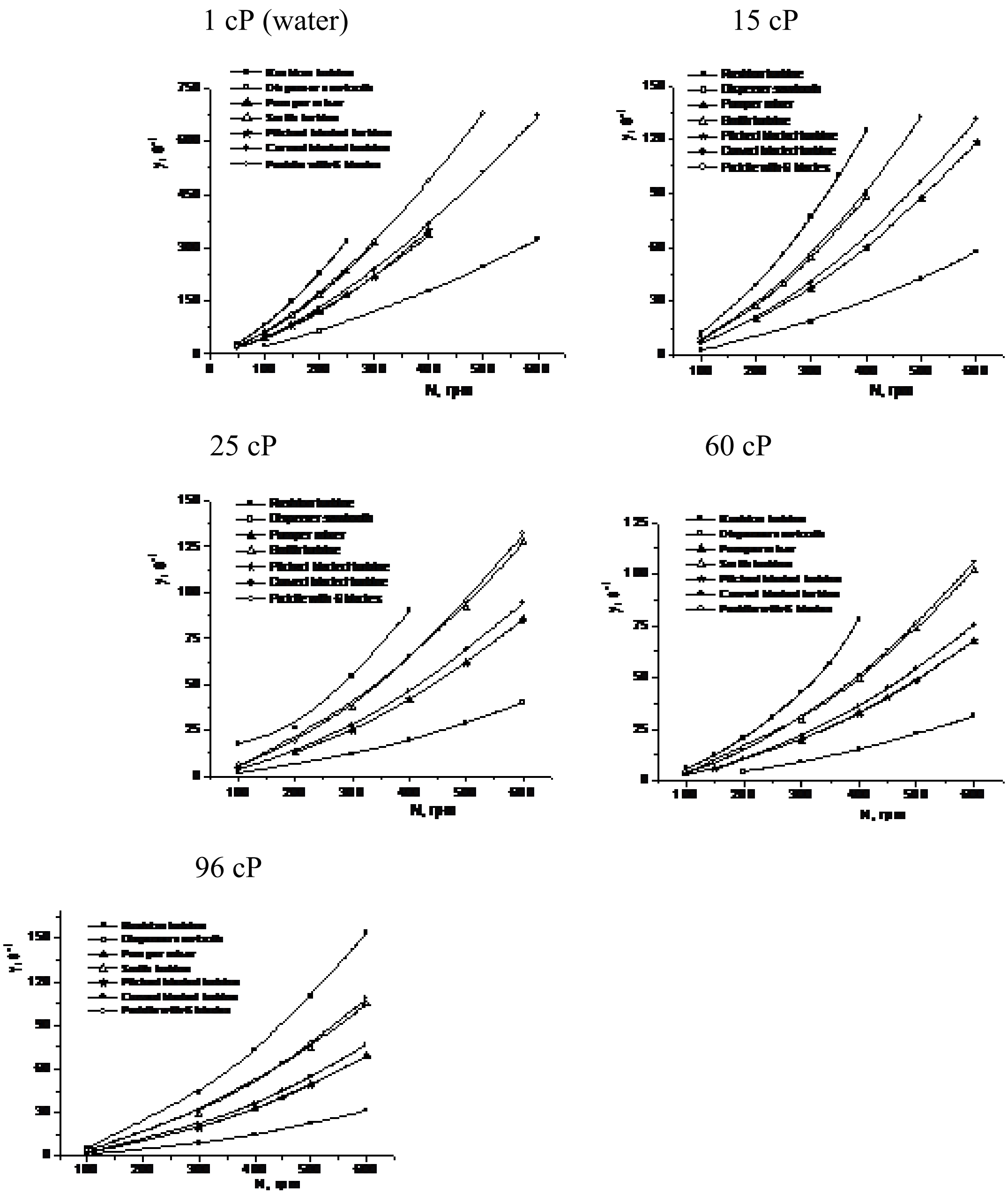
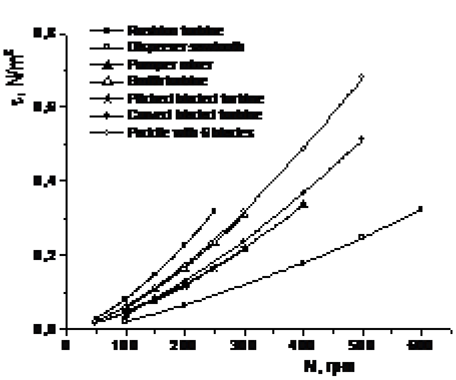


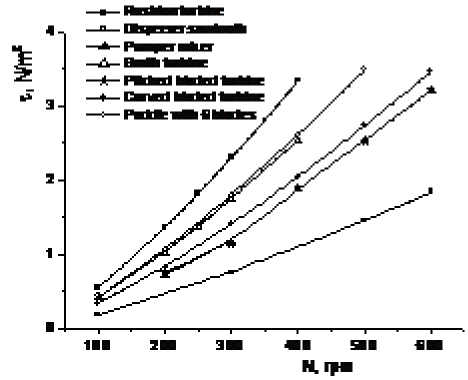
Figure 4. Influence of rotation speed on shear rate, for different viscosities of the simulated broths.

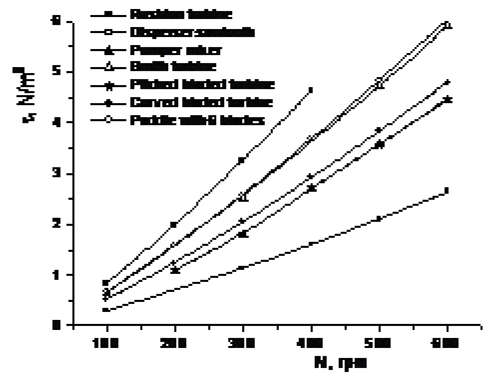
The influence of the rotation speed on shear stress is presented in Figure 5, being similar to that recorded for the shear rate. Moreover, indifferent of the broths viscosity, the magnitude of shear stress induced by the radial impellers varies identically with that of shear rate, the order being as follows:

Rushton turbine << Paddle with 6 blades < Smith turbine << Curved bladed turbine < Pumper mixer ≈ Pitched bladed turbine << Disperser sawtooth.

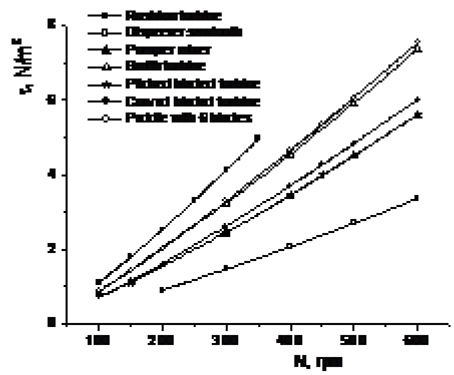
1 cP (water) 15 cP







25 cP 60 cP



96 cP

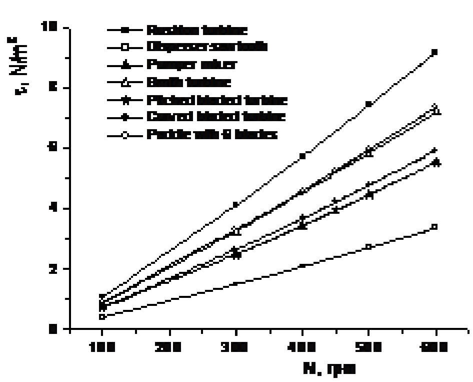


Figure 5. Influence of rotation speed on shear stress, for different viscosities of the simulated broths.

But, unlike the influence on shear rate, the increase of the apparent viscosity leads to the increase of the shear stress, for each impeller, thus suggesting that in viscous and/or non-Newtonian media the mechanical forces which act on the biocatalyst are significant higher than those acting in low-viscous and/or Newtonian media (Figure 6).

From Figure 6 it can be observed that in all cases the shear rate is initially strongly amplified by the increase of the apparent viscosity up to 60 cP, over this level the shear stress increase becoming slower. Analyzing this dependence it can be concluded that for the studied broths and apparent viscosities over 60 cP the shear stress induced by a given radial impeller can be modified only by changing the rotation speed, without any influence of the further increase of viscosity or of rheological indexes modification.

Conclusions

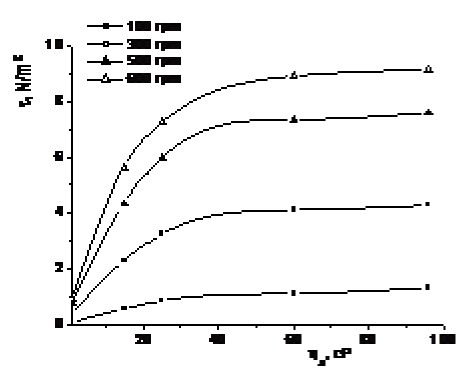
The role of mixing is to induce an intense circulation of the media without mechanically disrupting the biocatalysts, respectively without exceeding the maximum level of biocatalysts tolerance to the shear stress. The shear forces are controlled by the mixing intensity, apparent viscosity and rheological characteristics of the broths, in relation with the used impeller type.

For developing and completing the previous studies focused on the selection of the optimum impellers combination for a given broth [4,5], the shear forces promoted by seven types of radial impellers have been comparatively analyzed. The obtained results indicated the following order of the shear stress magnitude, valid for the entire domain of apparent viscosity considered in the experiments:

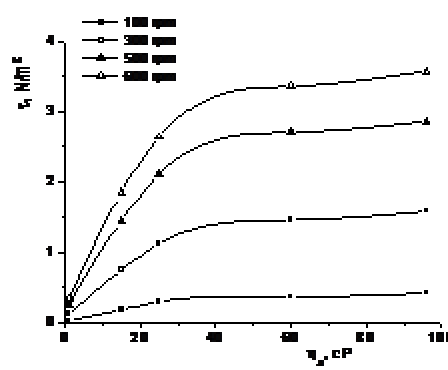
Rushton turbine << Paddle with 6 blades < Smith turbine << Curved bladed turbine < Pumper mixer ≈ Pitched bladed turbine << Disperser sawtooth.

For all studied impellers and contrary to the shear rate variation, the shear stress increases with the apparent viscosity increase up to 60 cP, over this level the modification of shear forces being made only by rotation speed changing, independently by the further increase of the apparent viscosity.

Rushton turbine

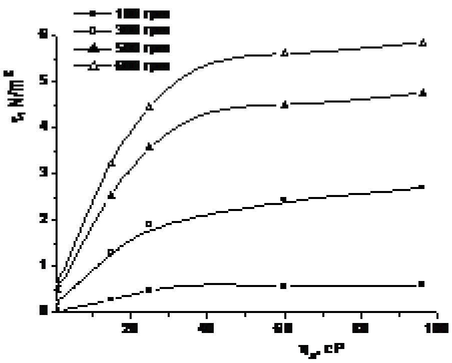


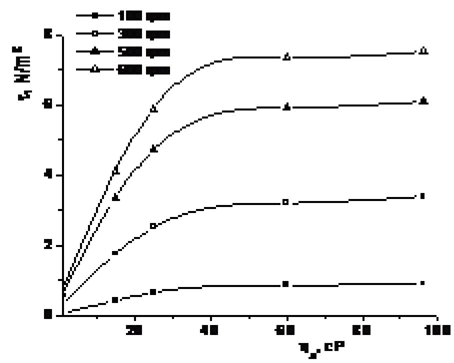
Disperser sawtooth



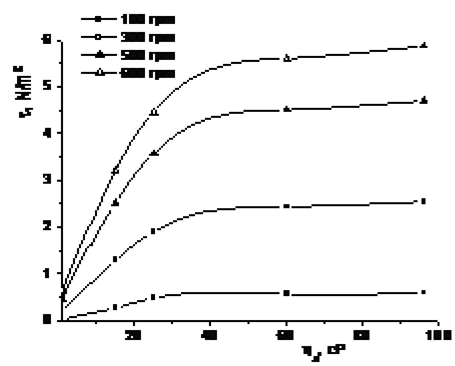
Pumper mixer

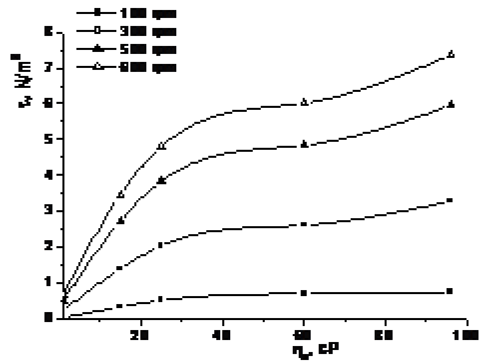
Smith turbine





Pitched bladed turbine Curved bladed turbine





Paddle with 6 blades

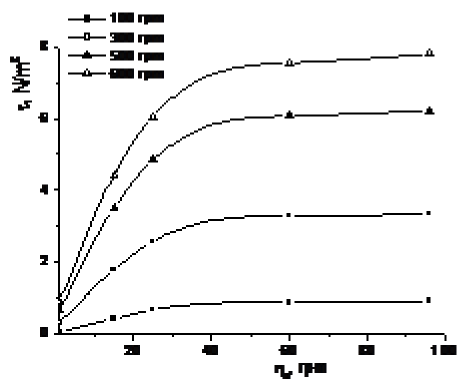


Figure 6. Influence of apparent viscosity on shear stress.

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