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SCIENTIFIC PAPER

66.023:577+66.011:621.224.2

OPTIMIZATION OF MIXING IN STIRRED BIOREACTORS

2. Selection of optimum impeller combinations for non-aerated simulated broths

Although radial impellers, especially the Rushton turbine, are widely used in stirred bioreactors, their applicability is limited by the high apparent viscosities of the broths. For optimizing mechanical mixing by selecting the appropriate impeller for a specific fermentation broth or process, the comparative analysis of the mixing efficiency, energy costs and shear effects on the biocatalysts is required.
By means of this analysis, three different combinations of radial impellers for water and viscous simulated broths were selected for athaining optimum mixing in a bioreactor. The proposed impellers combinations offer the most intense and uniformly distributed mixing and the lowest specific power consumption required for reaching a maximum level of mixing time of less than one minute.

Key words: Stirred bioreactor, Radial stirrer, Rushton turbine, Disperser Sawtooth, Smith turbine, Pumper mixer, Paddle with 6 blades, Pitched bladed turbine, Stirred bioreactor, Mixing time, Power consumption, Specific power input.

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

One of the most useful criteria for characterizing the mixing intensity is the mixing time, tm, defined as the time needed to reach a given mixing intensity at a given scale, when starting from a completely segregated situation [1,3]. This parameter offers specific information concerning bulk mixing in the system (macromixing) and the flow inside the whole studied system, but it cannot allow the rigorous quantification of meso– and micromixing [2]. It can indicate the optimum hydrodynamic regime, the impeller type that must be used, or can predict the modification of mixing efficiency induced by scaling–up [4,5].

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

Although radial impellers, especially the Rushton turbine, are widely used in 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, a double stirrer is recommended, provided on the same shaft with one Rushton turbine in the lower region and one paddle with plane blades in the upper region [6]. The lower stirrer promotes high turbulence and, therefore, avoids biomass deposition, and the upper one creates a high flow velocity of the broth.

For these reasons, a comparative analysis of the mixing efficiency induced by different impeller types for different broths is required. But, beside intensification of the broth flow, for selecting a certain impeller or impeller combination, its energy efficiency and the shear effects on the biomass must be taken into account.

In this context, the mixing efficiency and distribution for a laboratory stirred bioreactor provided with seven different double impellers of the radial type were discussed in the previous study [7]. The experiments were carried out for non–aerated simulated fermentation broths with apparent viscosities up to 96 cP and followed variation of the mixing time at different regions inside the bioreactor, the mixing uniformity, and

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the power consumption needed to reach a certain level of broth circulation intensity. Therefore, four pairs of radial impeller combinations were selected to reach either the minimum mixing time values, or a uniform distribution of mixing with the lowest energy costs for a certain apparent viscosity domain. The purpose of the studies presented in this paper is to select the optimum impeller combination among the previously proposed ones for a certain fermentation broth by analyzing concomitantly the experimental data regarding the mixing intensity, mixing distribution and energy efficiency. As in earlier studies, these experiments were carried out from non–aerated water to highly viscous simulated fermentation broths.

MATERIALS AND METHOD

The experiments were carried out in a 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 characteristics and operating parameters have been presented in previous papers [8].

The mixing system consists of a double stirrer and three baffles. The double stirrer has two identical or different radial impellers selected among the seven impellers used in the previous experiments [7].

Water and simulated fermentation broths were used in the experiments. The simulated broths consisted of carboxymethylcellulose sodium salt (Sigma Chemie GmbH) solutions having an apparent viscosity in the range of 15–96 cP.

The mixing efficiency was analyzed by means of the mixing time values, t_m [1–3,8]. The values of the mixing time were determined for different positions in the broths. For this purpose, a solution of 2M KOH was used as a tracer, and the time needed for the medium pH–value to reach the value corresponding to the considered mixing intensity was reached. In this case, the following homogeneity criterion for mixing was considered:

$$I = \frac{pH_\infty - 0.5\ \Delta pH}{pH_\infty} \times 100 = 99\% \quad \text{where } \Delta pH = 0.02.$$

The tracer volume was of 0.5 ml, the tracer being injected opposite 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 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–recorded system and were analyzed for mixing time calculations.

RESULTS AND DISCUSSION

The complex role of mixing in the bioreactor is to promote 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. For increasing the efficiency of the mixing process, bioreactors are provided with multiple agitator systems which consist of two or more identical or different impellers assembled on the same shaft, their number being a function of the broth height in the vessel. The mixing efficiency in systems with multiple stirrers is directly related to the capacity of mixing to generate high turbulence and intense circulation in the whole fermentation broth. 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 [3,8].

The mixing must induce an intense circulation of the media without mechanically disrupting the cells, without exceeding the maximum level of cell tolerance to the shear stress. Besides the broth viscosity and complex rheological behavior, this limitation represents one of the main causes of the inevitable appearance of stagnant regions in the bioreactor, regardles of the constructive and operational characteristics of the used stirrers.

In previous experiments the mixing efficiencies of seven double stirrers were comparatively analyzed for non–aerated water and simulated fermentation broths with apparent viscosities between 1 and 96 cP [7]. The stirrers consisted of two identical radial impellers placed on the same shaft at a given position. These studies were finalized by proposing two combinations of different impellers for each medium: one that could lead to the most intense and uniform mixing, and an other that would require the lowest power consumption for a certain level of mixing intensity. For selecting the optimum impeller combination of the two proposed for each fermentation broth, these studies were developed by analyzing concomitantly the intensity of mixing and the energy cost for mixing, directly related to the capacity of these stirrers to promote a uniform distribution of mixing in the bulk volume of the broths. Furthermore, for also ensuring optimum mass transfer of the limiting substrate, selection of the appropriate impeller combination was made taking into account the possibility to obtain mixing times lower than 30 s with lower power consumption (the value of 30 s represents the average upper limit of the duration of oxygen consumption from broths in the microbial respiration process without the addition of a new amount of solved oxygen into the medium, the oxygen being the limiting substrate with the lowest solubility in fermentation broths [3]).

The previous results obtained for non–aerated water suggested that the lowest values of mixing time that are uniformly distributed could be obtained using a double stirrer consisting of a pitched bladed turbine at the lower part and a paddle with six blades at the upper one (combination A). From the viewpoint of power consumption, the most efficient mixing of water can be obtained with a double stirrer equipped at the lower part with a disperser sawtooth, and at the upper part with a paddle with six blades (combination B) [7].

From analysis of the influence of the rotation speed on mixing time for the two impeller combinations, it can be observed that for combination A the contribution of the two impellers on the stirrer shaft on medium circulation is similar, the plotted variations being close to those previously recorded for each individual impeller (Figure 1).

In the case of combination B, the shape of the curves is rather similar to those obtained for an individual paddle with six blades, thus indicating a more important contribution of this impeller to water mixing compared with a disperser sawtooth.

Unlike combination B for which the mixing time strongly varies on bioreactor height, the use of combination A allows a uniform mixing intensity in the broths at 200 rpm. But, the specific power input needed for reaching the same level of mixing intensity is lower for combination B (for mixing times below 30 s, the power consumption of combination B is about 2 times lower than that for combination A).

Analyzing these results and taking into account the recorded values of the mixing time, which are lower for combination B (except position 1 of the pH electrode), it can be concluded that a double stirrer of the combination B type is required for optimum agitation of non–aerated water and simulated broths with similar rheological characteristics. The lower energy cost and the superior mixing intensity that are obtained using combination B can compensate for the non–uniform distribution of mixing in the bioreactor, all the more so as combination A offers uniform mixing only for a single value of the rotation speed.

Increase of the apparent viscosity leads to a modification of the impeller combinations that can offer maximum efficiency of mixing. Thus, for simulated

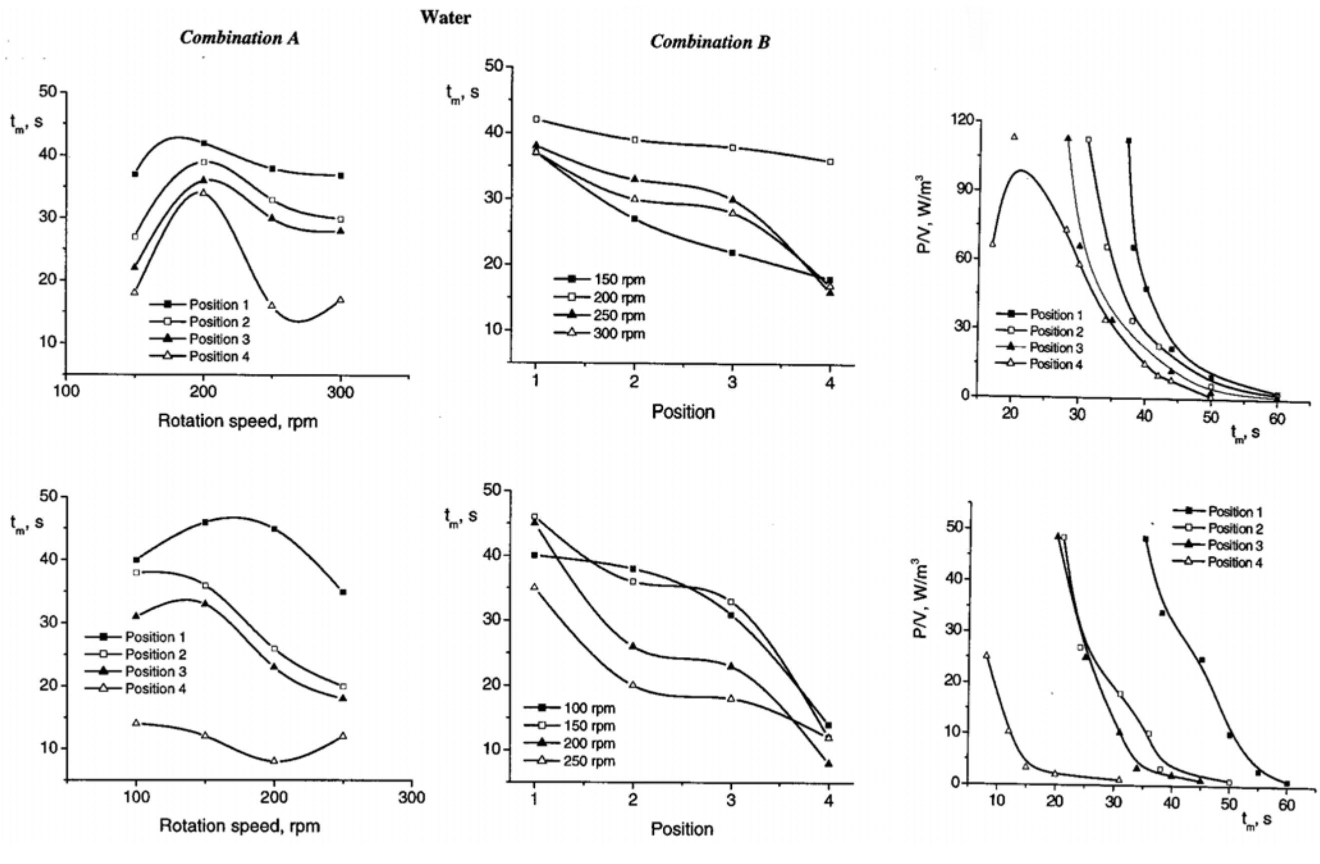

Figure 1. Comparative variations of the intensity, uniformity and power consumption of non–aerated water mixing with the impeller combinations A and B

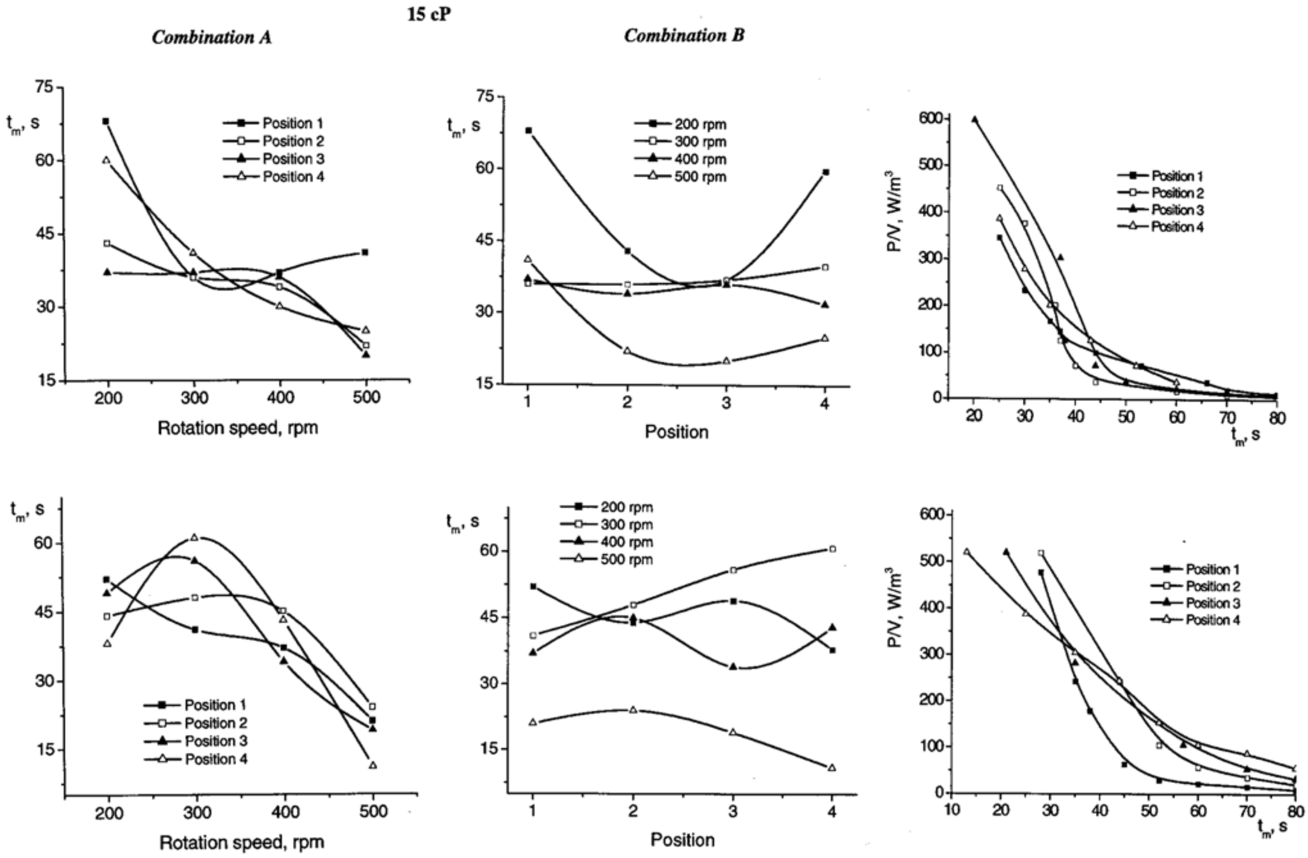

Figure 2. Comparative variations of the intensity, uniformity and power consumption of non–aerated simulated broths mixing with the impeller combinations A and B for 15 cP

broths with apparent viscosities of 15 and 25 cP, the previous studies suggested as potential optimum stirrers the following two combinations: a pitched bladed turbine at the lower part and a Rushton turbine at the upper part (combination A), for the most intense and uniform mixing, and lower pumper mixer and an upper pitched bladed turbine (combination B), for the lowest power consumption needed for a given level of liquid circulation velocity [7].

As observed in Figure 2, plotted for 15 cP, the mixing time variation with rotation speed recorded for combination A indicates that each impeller exhibits a more important influence on mixing in its immediate vicinity. Thus, the recorded curves for positions 1 and 2 are similar to those previously obtained for a double stirrer of the pitched bladed turbine type [7]. By increasing the distance from these regions, the curve shapes are changed, for positions 3 and 4 becoming similar to those previously recorded for a double Rushton turbine stirrer [8,9].

For combination B, the medium flow streams induced by the lower impeller interferes with and is strongly influenced by the broth circulation promoted by the upper impeller (probably because the pumper mixer promotes an intense circulation in the region under the disc plane for d/dD < 2 [7,10]). Therefore, the variation of mixing time is rather similar to those recorded in the earlier studies for a stirrer with two pitched bladed turbines [7], this similarity becoming more pronounced with increasing distance from the lower impeller.

The use of combination A allows the reaching of uniform mixing for the rotation speed range 300–400 rpm. Some uniform mixing could also be obtained in the case of combination B, but the amplitude of the mixing time variation between its extreme values is 7–8 s at this rotation speed, significantly higher than for the former combination.

From the comparative analysis of the power consumption, it can be observed that the energy demands of the two combinations are similar for mixing time values lower than 20 s. With the increasing mixing time, the difference becomes more pronounced and favorable for combination A. Thus, for reaching a mixing time of 60 s, the energy required by combination B is about 3 times greater than that for combination A. The mixing in position 1 is the exception from the above conclusion, because in this region combination B requires lower energy consumption for reaching mixing times longer than 50 s.

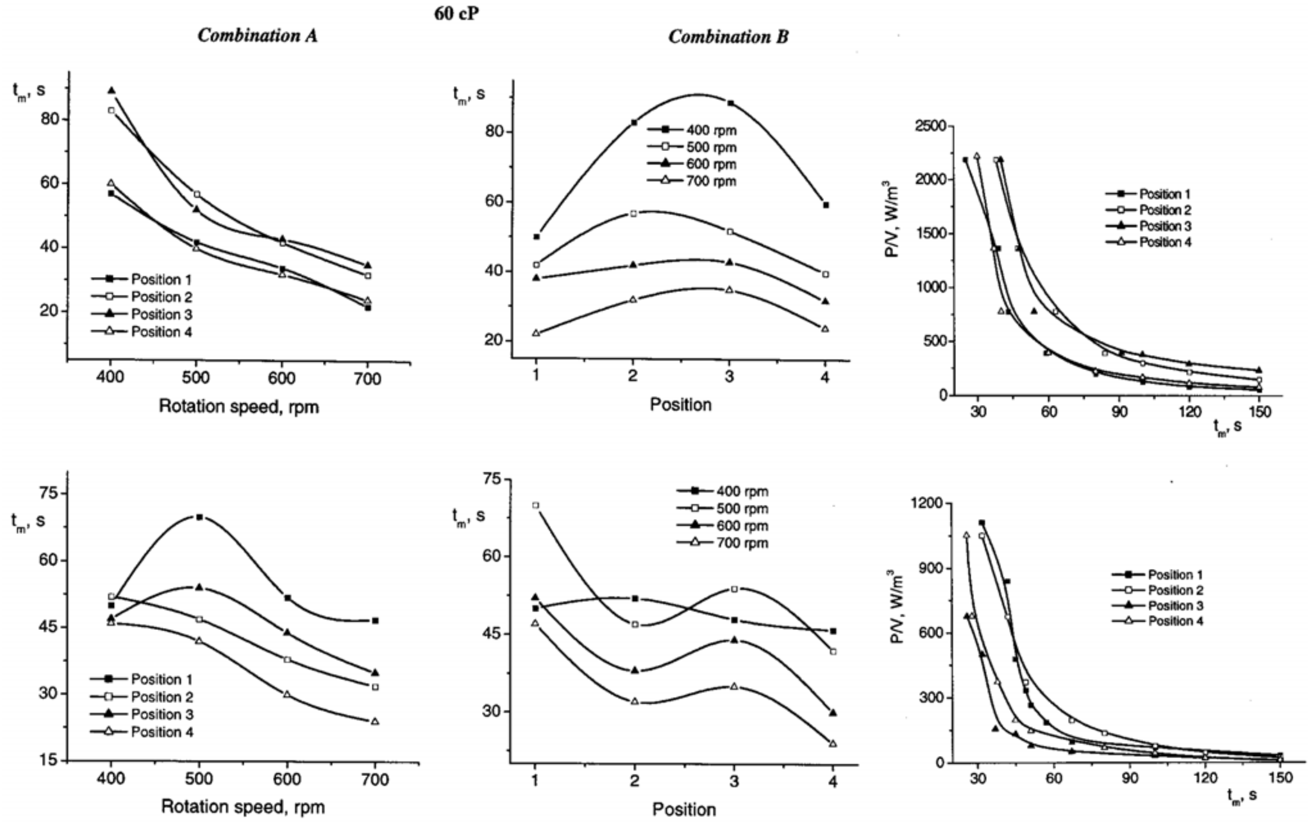

Figure 3. Comparative variations of the intensity, uniformity and power consumption of non–aerated simulated broths mixing with the impeller combinations A and B for 60 cP

By means of these results and of those obtained for an apparent viscosity of 25 cP, a stirrer equipped with a pitched bladed turbine at the lower part and a Rushton turbine at the upper part (combination A) can be selected for optimum mixing of simulated fermentation broths having an apparent viscosity lower than 25–30 cP.

For simulated fermentation broths with an apparent viscosity of 60 cP, the previous experiments indicated as a possible optimum combination either a double Rushton turbine stirrer (combination A), for high mixing intensity regardles of the energy cost for mixing, or a stirrer equipped with lower a pumper mixer and an upper disperser Sawtooth (combination B) [7]. As above mentioned, in the case of two different impeller combinations an increase of the apparent viscosity leads to a more accentuated separation between the broth circulation regions generated by each individual impeller. Therefore, Figure 3 shows that for combination B the variation of mixing time with rotation speed in positions 1 and 2, respectively 3 and 4, is almost identical with that previously recorded for a double stirrer of the pumper mixer type, or a disperser Sawtooth type, respectively [7].

By comparing the results plotted in Figure 3 for the two impeller combinations it can be seen that the most efficient stirrer for these liquids is that having a pumper mixer at the lower part and a disperser Sawtooth at the upper one (combination B). Thus, this combination allows a lower mixing time, uniform distribution of mixing in the bulk volume of the broth for 400 rpm and requires the lowest power consumption for these purposes (the specific power input is about 2–2.5 times lower compared with a double Rushton turbine stirrer). The use of combination A can lead only to relative homogenization of the broth at the bioreactor height, the difference between the extreme values of the mixing time being 8 s for a rotation speed of 600 rpm.

The above combination B is also recommended for optimum mixing of simulated fermentation broths with higher viscosity. Thus, the previous experiments indicated as possible optimum combinations for liquids with an apparent viscosity of 96 cP that at the lower part have a Smith turbine and at the upper part a Rushton turbine (combination A), for generating the most intense liquid circulation, as well as that equipped with lower pumper mixer and an upper disperser Sawtooth (combination B), for the lowest power consumption [7]. As in the previously analyzed case, the high viscosity reduces the flow stream interferences, the result being the delimitation of regions in which broth circulation is strictly controlled by the corresponding individual impeller (Figure 4). For this reason, variations of the

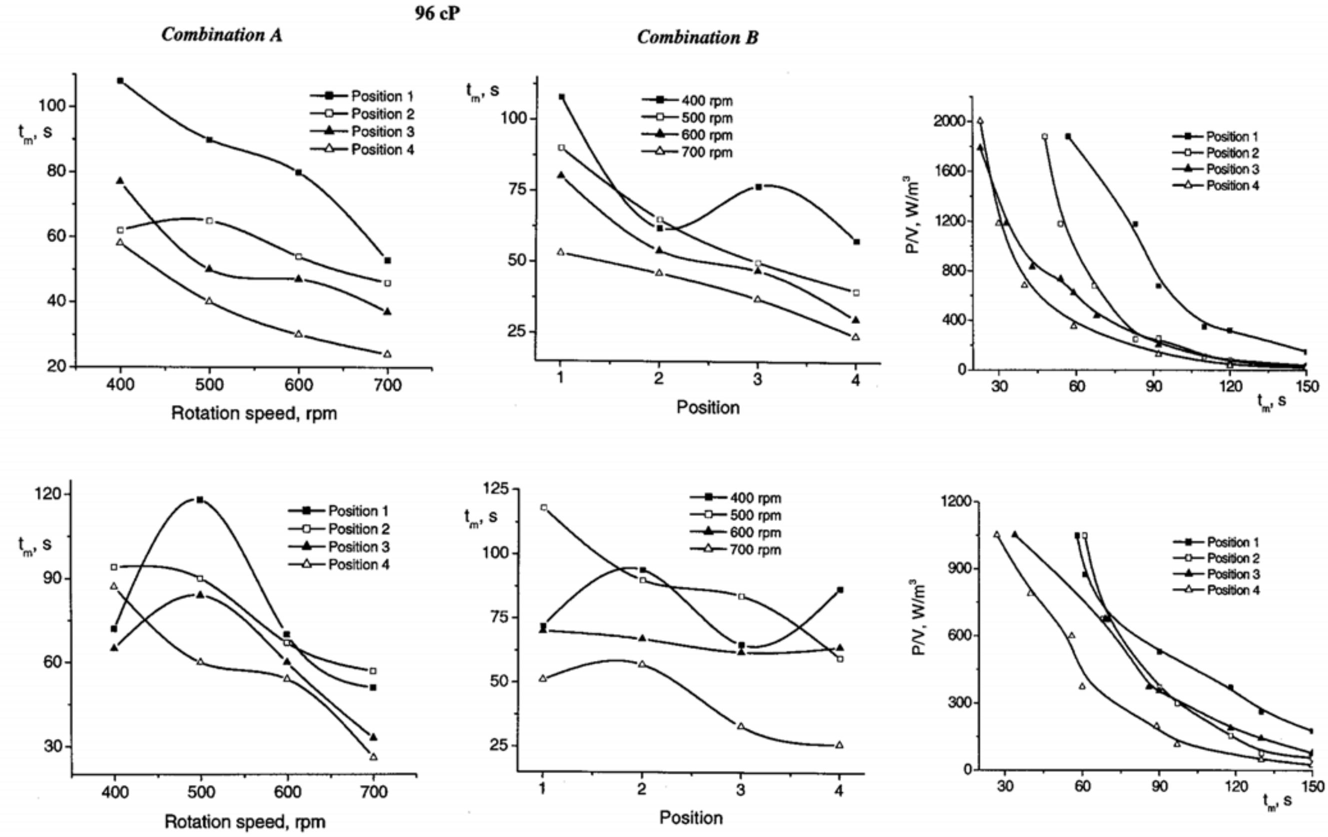

Figure 4. Comparative variations of the intensity, uniformity and power consumption of non–aerated simulated broths mixing with the impeller combinations A and B for 96 cP

mixing time for the lower and upper regions are similar to the previous ones for double stirrers with two identical impellers of the above indicated types [7].

Although combination A induces more intense mixing, combination B offers better homogenization of the broth (unlike combination A, for which the mixing intensity strongly varies on the bioreactor height, at 600 rpm combination B generates uniform circulation of the broth in the bioreactor). Moreover, the specific power input needed by combination B is about 1.8–2 times lower than that for combination A. The difference between the power consumptions of the two impellers is reduced with an increase of the mixing time to 90 s, over this limit the combination becomes energetically more efficient.

CONCLUSIONS

Among the functions of mixing, namely the uniform dispersion of the biomass and the reduction of the concentration and temperature gradients, the critical functions consist on the uniform distribution of the major nutritive elements and the promotion of the optimum circulation of the broths, on passing of the biomass through the active regions with a frequency required by the metabolic process rates. For achieving these functions, mixing systems must be adapted to the particularities of the biosynthesis processes which occur in bioreactors. In the case of stirred bioreactors, this assertion indicates the use of different types of impellers according to the apparent viscosity of the broths and with the type, morphology, concentration and shear sensibility of the cultivated microorganisms.

The comparative analysis of the mixing induced by seven radial impellers enabled the selection of different combinations with the purpose of finding the optimum stirrer from the viewpoint of mixing intensity, mixing distribution and power consumption. Therefore, by studying the mixing efficiency for non–aerated water and simulated fermentation broths with apparent viscosities up to 100 cP, the following optimum combinations of different radial impellers were selected:

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

For broths containing biomass, the analysis and selection of the optimum impeller combination must also to take into account the shear effect on the cells, this being the aim of our future experiments.

NOTATIONS

d – impeller diameter, m

d_D – disc diameter (for a pumper mixer), m

P/V – specific power input, W/m^3

t_m – mixing time, s

REFERENCES

[1] Van't Riet, K., Tramper, J., Basic Bioreactor Design, M. Dekker Inc., New York, 1991, pp. 183

[2] Bujalski, J.M, Ph.D. Thesis, University of Birmingham, 2003, pp. 22

[3] Cascaval, D., Oniscu, C., Galaction, A.I., Biochemical engineering and biotechnology. 2. Bioreactors, InterGlobal, Iasi, 2002, pp. 53

[4] Kramers, H., Baars, M., Knoll, W.H., Chem. Eng. Sci. **2** (1953) 35

[5] Nienow, A.W., Chem. Eng. Sci. **52** (1997) 2557

[6] Oniscu, C., Cascaval, D., Biochemical engineering and biotechnology. 1. Biotechnological processes engineering, InterGlobal, Iasi, 2002, pp. 261

[7] Cascaval, D., Galaction, A.I., Folescu, E., Chem. Ind. Chem. Eng. Q. (2006) in press.

[8] Oniscu, C., Galaction, A.I., Cascaval, D., Ungureanu, F., Biochem. Eng. J. **12** (2002) 61

[9] Cascaval, D., Oniscu, Galaction, A.I., Ungureanu, F., Chem. Ind. **55** (2001) 367

[10] Post, T.A., Giralico, M.A., Greaves, M.C., Fraser, G.M., Proceedings of The 2nd Annual Copper Hydromet Roundtable, Vancouver, 1996, pp. 295