Scale-up of Aerobic Stirred Bioreactors Using the Mixing Time Criteria 1. Simulated Broths

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The paper is dedicated to the analysis of the scale-up of an aerobic stirred bioreactor from 4 L working volume to 40 L using the mixing time criterion, taking into account its required conditions and effects. Therefore, if the selected operating parameters allow obtaining similar values of mixing time in the larger bioreactor as in the reference one, it is possible to maintain also the specific power consumption by scaling-up. However, the shear forces in the larger bioreactor are amplified with 23 – 35 %, the most important increase being recorded for high viscous broths.

Key words:
Stirred bioreactor, mixing time, scale-up, power input, shear force

Introduction

The design of biotechnological processes requires also their scale-up from the laboratory level, at which the strain and its favorable growing conditions have been selected, to the pilot level, for establishing the optimum operating parameters, and, finally, to the industrial one, for reaching profits. The scale-up of fermentative processes was recognized as an essential problem from the first application of a fermentation at industrial scale (1940, Penicillin production). The difficulty of this translation to a larger scale arises from the multitude and complexity of the factors involved in the biochemical transformations occurring in the media. For example, the magnitude of the physical parameters depends significantly on the operating scale (mixing intensity, shear forces, mass and heat transfer rates, etc.), especially if the geometrical similitude is not respected by scaling-up.[1]

Ideally, the scale-up should lead to higher fermentation efficiency, product quality or, at least, to maintaining them at those corresponding to the inferior scale. However, the change of the geometrical characteristics and, implicitly, of the physical phenomena amplitude in the larger scale affect the process consistency and reproducibility simultaneously with the product quality, induce the diminution of the fermentation yields, lead to the accumulation of supplementary amounts of unwanted side compounds, all of them representing key issues in an industrial production.[2]

From the viewpoint of mixing, the scale-up of bioreactors enhances the difficulties in reaching the optimum hydrodynamics of the broths, as the result mainly of the increase of the distances and, consequently, of the extending of stagnant regions, owing to the larger volumes that have to be mixed. Therefore, the values of the mixing time could be increased by scaling-up.[3–5] In the case of aerobic stirred bioreactors, the reduction of the mixing efficiency by increasing the tank sizes leads to the non-uniform distribution of substrate and oxygen on the broth height. Thus, the microorganisms at the bioreactor bottom dispose of insufficient substrate amounts, whereas those of the top suffer from oxygen limitation, but are exposed to excessive substrate concentration (in this region the substrate inhibitory phenomenon could appear).[4,6,7] For these reasons, in the industrial bioreactors the appearance of the regions in which different stress factors act on the cells (reduction of limitative substrate concentration below the critical level, carbon dioxide accumulation, overheating, etc.) becomes possible.

One of the most useful criterions for characterization of the mixing intensity is the mixing time, defined as the time needed to reach a given mixing intensity at a given scale, when it starts from the completely segregated system.[8] This parameter could be used also as a scale-up criterion for bioreactors, either individually or in combination with other criteria.[2,9]

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Thus, the aim of these studies is to establish the operating conditions for maintaining the mixing efficiency and distribution inside the broth, by means of mixing time values, increasing the working volume of the aerobic stirred bioreactor by 10 times. For underlining the effect of fermentation broths behavior on scale-up, the experiments have been carried out for aerated broths without biomass (simulated broths) and with microorganisms (yeasts *Saccharomyces cerevisiae*, fungus *Penicillium chrysogenum* free mycelia and pellets). This paper presents the results obtained for simulated broths.

Materials and method

The experiments have been carried out in 50 L (40 L working volume) Fermac bioreactor, provided with computer-controlled and recorded parameters. The bioreactor characteristics and operating parameters are presented in Table 1. The geometric ratios are similar to those of the smaller bioreactor (5 L working volume), which is considered the reference.[10]

Table 1 – *Characteristics of bioreactor*

d mm	*d*/*D*	*H*/*D*	*b*/*d*	*l*/*d*	*h*/*d*	No. blades	No. baffles	*s*/*d*	*d'*/*d*	*l'*/*d*
124	0.40	2.2	0.12	0.28	1	6	3	0.20	0.11	1.44

The mixing system consists of a double stirrer and three baffles. The impeller diameter, *d*, was of 124 mm. The inferior stirrer was placed at 248 mm from the inferior one, this being the optimum distance as it was demonstrated in the previous works for simulated broths.[11] The rotation speed was maintained below 600 rpm. The experiments were carried out at Reynolds number lower than 10 250, domain which corresponds to the laminar and transitory flow regime, and avoids the cavity formation at the broths surface.

The sparging system consisted of a single ring sparger with 124 mm diameter, placed at 30 mm from the vessel bottom, having 28 holes with 1 mm diameter. The air volumetric flow rate varied from 225 to 1360 $\mathrm{L\ h^{-1}}$, corresponding to an superficial air velocity of $0.84 - 5.02 \cdot 10^{-3}\ \mathrm{m\ s^{-1}}$, similar to that used in previous studies on the reference bioreactor.[11]

In the experiments, simulated broths were used. These broths were carboxymethylcellulose sodium salt solutions with the apparent viscosity in the domain of $15 - 96 \cdot 10^{-3}$ Pa·s. 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 Ostwald type. Both the experiments and viscosity measurements were carried out at a temperature of 25 °C. Any viscosity change was recorded during the experiments.

The mixing efficiency was analyzed by means of the mixing time values. For mixing time determination, a solution of 2 $\mathrm{mol\ L^{-1}}$ KOH was used as tracer, recording the time needed to the medium pH to reach the value corresponding to the considered mixing intensity.[12] In this case, the following homogeneity criterion for mixing has been considered:

$$I = \frac{\mathrm{pH}_\infty - 0.5\Delta\mathrm{pH}}{\mathrm{pH}_\infty} \cdot 100 = 99\ \%,$$

where $\Delta\mathrm{pH} = 0.02$.

The tracer volume was 2 mL, the tracer being injected at the opposite diametral position to the pH-electrode (HA 405 Mettler Toledo), at 135 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 bioreactor bottom as follows:

- position 1: at 124 mm
- position 2: at 248 mm
- position 3: at 372 mm
- position 4: at 496 mm.

The pH variations were recorded by the bioreactor computer-recorded system and were analyzed for calculating the mixing time.

The mathematical correlations, which describe the influences of considered factors on mixing time obtained by scaling-up for various positions inside the broth were developed on a PC using MATLAB software. For the experimental data, a multiregression analysis was performed, the difference between the experimental and modeled value being reduced to a minimum by least-square fit method. By means of a MATLAB program, the regression coefficients and standard deviations were calculated.

Each experiment has been carried out for three times, for identical conditions, the average value of the considered parameters being used. The maximum experimental error was ± 5.06 %.

Results and discussion

The accumulation of biomass or biosynthesized product (extracellular polysaccharides, protein molecules etc.) in the fermentation processes leads to the continuous modification of the broths' rheological properties, promoting the appearance of heterogeneous regions inside the bioreactor. These effects become more pronounced at larger scale and promote the appearance of new stagnant regions or extension of existing ones.

For the simulated broths, the scale-up of bioreactor by respecting the geometrical similitude leads to the dependences between the mixing time and rotation speed similar to those corresponding to the inferior operating level.[11] Thus, according to Fig. 1, for apparent viscosities up to $96 \cdot 10^{-3}$ Pa·s and regardless of the pH electrode position on the liquid height, the increase of impeller rotation speed induces the initial decrease of mixing time to a minimum value followed by its increase. As it was previously concluded, [11] this evolution could be the result of the modification of mixing mechanism in presence of bubbles. Thus, at low rotation speed, the contribution of pneumatic mixing to the circulation of dispersion is important, the increase of rotation speed intensifying supplementary broth agitation into the bioreactor. At higher rotation speed, the bubbles retention time increases, the gas-liquid dispersion flow becomes more complex and its circulation velocity is lower than that of the flow streams created by mechanical mixing in non-aerated media. The value of the rotation speed that corresponds to the minimum of mixing time was called *critical rotation speed*.[11,13]

For apparent viscosity below $60 \cdot 10^{-3}$ Pa·s, it can be observed that the four curves can be considered by twos, one group for the extreme positions (positions 1 and 4), and the other for the intermediary positions (positions 2 and 3). As in the case of the reference bioreactor, the lowest values of mixing time were recorded for positions 1 and 4, owing to their location near to the stirrers. The variation plotted for position 1 indicates a more efficient mixing compared with position 4, being the result of the "bottom effect" which induces a better circulation of air-broth dispersion.[12]

Although for the mentioned domain of apparent viscosity the shape of the plotted variations are rather similar for all the considered positions, the value of critical rotation speed is lower for the intermediary regions and increases with the viscosity increase. This difference is the consequence of the more important relative contribution of mechanical mixing in positions 1 and 4 to the broth circulation, on the one hand, and the amplification of this contribution at more viscous media compared to the pneumatic mixing, on the other hand.

The lowest mixing intensities were recorded for positions 2 and 3. According to previous studies,[12] this variation is the result of the modification of mixing intensity in the intermediary region due to the cumulated or opposite effects of the flow streams generated by the two vicinal stirrers. Therefore, owing to the distance between the stirrers, the stagnant regions can be formed in the intermediary region, a phenomenon that becomes more pronounced at higher apparent viscosity and larger scale. The rotation speed acceleration promotes the intensification of broth circulation, thus reducing the volume of the stagnant region and, consequently, the values of mixing time for positions 2 and 3. The aeration strongly modifies and influences the circulation into the intermediary region, because it amplifies supplementary the agitation between the stirrers and extends the well-mixed regions. For the above reasons, the mixing intensity is lower in positions 2 and 3 and the critical rotation speed is inferior to those recorded for positions 1 and 4. At a given moment, the flow streams become strong and interact, diminishing the positive influence of the rotation speed intensification, an effect more pronounced at higher rotation speed values. By increasing the apparent viscosity, an additional effect was observed; namely the reduction of turbulence in the intermediary region, due to the air accumulation around the stirrers. Therefore, the minimum value of mixing time appears more evident, and its further increase more significant than that recorded for positions 1 and 4.

At higher apparent viscosity the bubbles coalescence becomes significant, the air is accumulated around the stirrers and the air hold-up increases (for apparent viscosity of $96 \cdot 10^{-3}$ Pa·s the air volumetric fraction was of 5.4 – 6.3 % at 300 rpm, becoming 15.7 – 16.4 % at 600 rpm, values for about 1.2–1.4 times greater than those obtained for the reference bioreactor[13]). The increase of air hold-up, as well as its non-uniform distribution inside the broth, diminish the broth circulation velocity and, respectively, attenuate the influence of the rotation speed increase on mixing intensification. For this reason, the variations recorded for apparent viscosity up to $60 \cdot 10^{-3}$ Pa·s are less evident compared to those for the smaller bioreactor, and the minimum value of the mixing time is not reached for broths more viscous.

The ratio between the mixing time corresponding to the reference bioreactor, t_{m0}, and that obtained for the larger bioreactor, t_{m1}, calculated for certain rotation speed and superficial air velocity, represents a suggestive quantification of the changes in mixing efficiency by scaling-up the bioreactor (Fig. 2). In all experimented cases, this ratio value is below 1, thus indicating the reduction of the mixing efficiency for the larger bioreactor. However, Fig. 2 reveals different behavior of water and low-viscous broths compared to the viscous ones. Therefore, for broths with apparent viscosity up to $15 \cdot 10^{-3}$ Pa·s, the increase

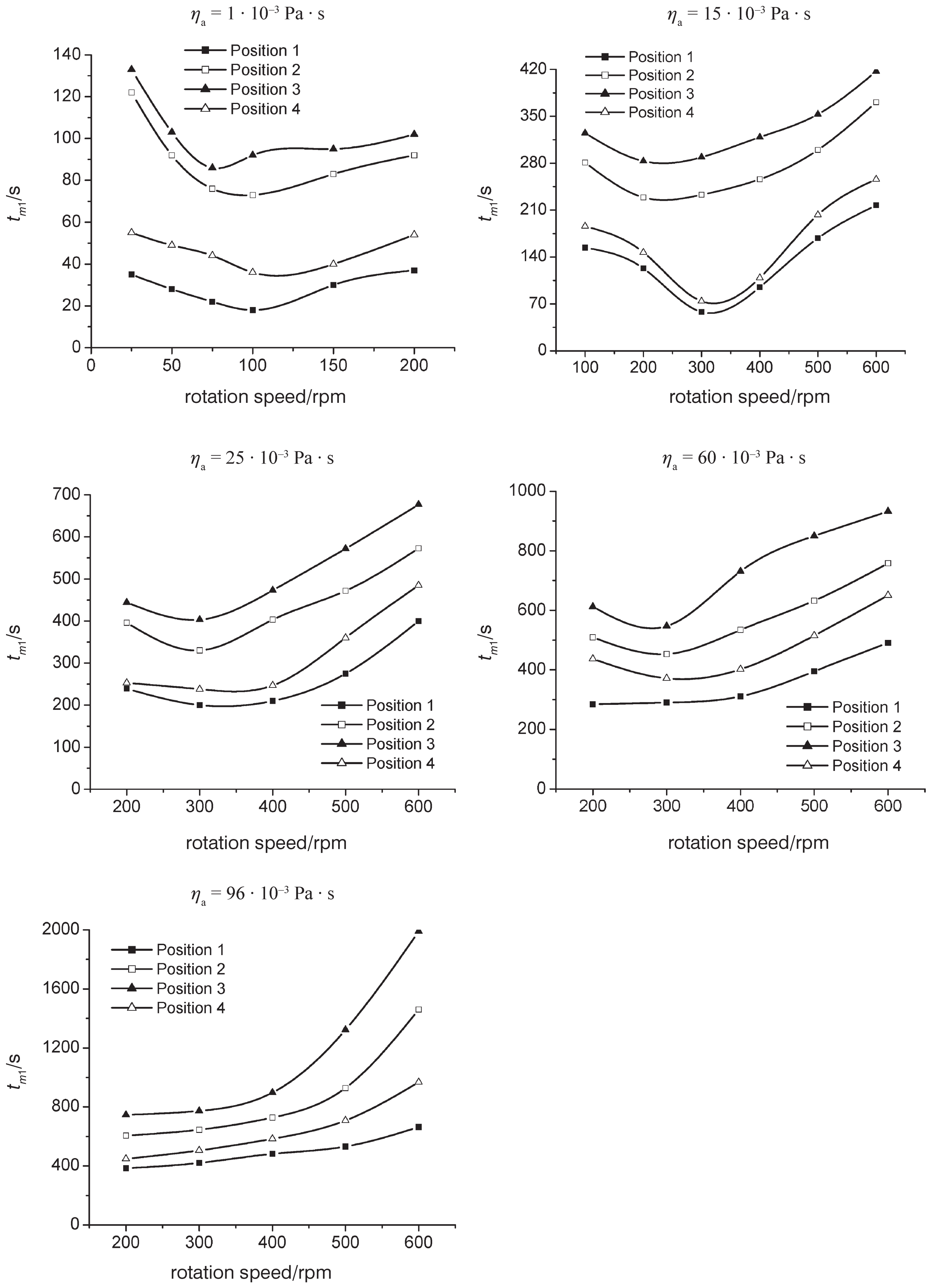
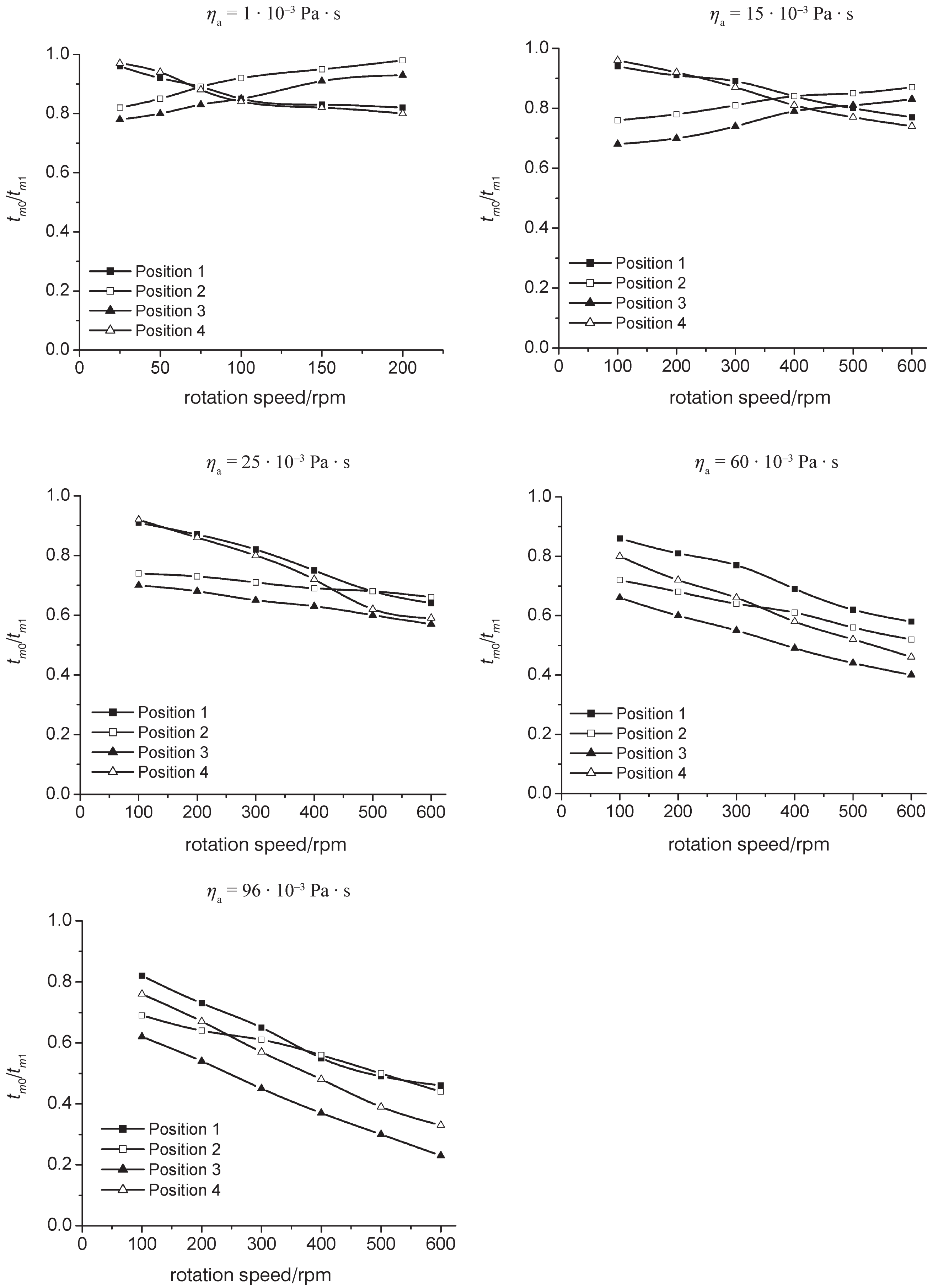


Fig. 1 – *Influence of rotation speed on mixing time* ($v_S = 0.84 \cdot 10^{-3}$ *m* s^{-1})



F i g . 2 – *Influence of rotation speed on mixing times ratio ($v_S = 0.84 \cdot 10^{-3}$ m s^{-1})*

of rotation speed for the intermediary positions induces the approach of the mixing time values recorded for the two bioreactors. But using the same media, the differences between the mixing times obtained for positions 1 and 4 are amplified by increasing the rotation speed. For more viscous simulated broths, regardless of the considered position inside the bioreactor, the acceleration of rotation speed reduces the mixing efficiency in the larger bioreactor, an effect that becomes more pronounced at higher viscosity.

The discussed variation of ratio t_{m0}/t_{m1} is the result of the aeration contribution in the acceleration or deceleration of the stirred broth circulation. The bubbles hinder the broth circulation especially in the region around the impellers, the magnitude of this phenomenon being amplified by scaling-up. Because for water and low-viscous broths the air hold-up in the region between the two impellers is inferior to that for positions 1 and 4, the increase of the rotation speed leads to the values of ratio t_{m0}/t_{m1} closer to 1. This influence is changed for more viscous broths, due to the increase of air hold-up and, implicitly, to the reduction of broths circulation velocity in larger bioreactor.

In this context, for the variation of viscosity from 1 to $96 \cdot 10^{-3}$ Pa·s and 600 rpm, the mixing time value increases about 2.4–4.3 times by scaling-up the bioreactor from 4 to 40 L working volume. The most important reduction of mixing efficiency was recorded for the intermediary positions.

The distribution of the mixing times ratio on the broth height confirms the above conclusions. Thus, except water and broths with apparent viscosity below $15 \cdot 10^{-3}$ Pa·s, the closest ratio values to 1 have been obtained for the impellers regions, regardless of the rotation speed (Fig. 3).

Unlike the reference bioreactor, the influence of the superficial air velocity is similar for all considered viscosities (Fig. 4). Thus, the mixing time is reduced continuously with the aeration rate increase, regardless of the region inside the broth. Contrary to the smaller bioreactor, the intensification of aeration induces the intensification of broth circulation without reaching the minimum level of mixing time. Due to the drawbacks of scale-up from the viewpoint of mixing, in the case of larger bioreactor the increase of aeration rate exhibits a favorable effect, by its supplementary contribution to the amplification of turbulence, even if t_{m1} values remain higher than those of t_{m0}.

The positive influence of aeration in the larger bioreactor is also suggested by the variation of ratio t_{m0}/t_{m1} plotted in Fig. 5, and becomes more pronounced with the increase of apparent viscosity. In these circumstances, it is possible to reach ratio values close to 1 at higher aeration rate, especially for the regions around the impellers, this result indicating similar mixing conditions in the two bioreactors.

By means of the experimental data, some mathematical correlations which describe unitary the influence of apparent viscosity, rotation speed and superficial air velocity on mixing time at different positions inside the broth have been established for the aerobic stirred bioreactor. According to the above discussions, these correlations have been proposed for the extreme and, respectively, intermediary positions:

– Positions 1 and 4

$$t_{m} = 1.89 \cdot 10^{4} \cdot \frac{\eta_a^{0.533} \cdot v_S^{0.093}}{N^{1.585 \cdot \ln 0.733}}, \text{ s} \qquad (1)$$

– Positions 2 and 3

$$t_{m} = 20.325 \cdot \frac{\eta_a^{0.403}}{v_S^{0.137} \cdot N^{2.285 \cdot \ln N - 1.232}}, \text{ s} \qquad (2)$$

The proposed models, similar to those established for the reference bioreactor,[11] offer a good concordance with the experimental data, the maximum deviation being of ± 9.45 %.

In order to identify the operating parameters of the larger bioreactor for reaching in every considered position the mixing time values equal to those recorded for the smaller bioreactor, the values of ratio t_{m0}/t_{m1} for the two position pairs were calculated using the following relationships:

– Positions 1 and 4

$$\frac{t_{m0}}{t_{m1}} = 15.8 \cdot \frac{v_S^{0.312}}{N^{\frac{4.006 - 1.2 \ln N}{1.585 \cdot \ln N - 0.733}}} \qquad (3)$$

– Positions 2 and 3

$$\frac{t_{m0}}{t_{m1}} = 1.30 \cdot 10^{4} \cdot \frac{v_S^{1.594}}{N^{\frac{2.119 - 0.694 \ln N}{2.285 \cdot \ln N - 1.232}}} \qquad (4)$$

The primary analysis of eqs. (3) and (4) indicates that the viscosity is not involved in bioreactor scaling-up using the mixing time criterion. The variations of the ratio t_{m0}/t_{m1} in function of rotation speed and superficial air velocity for the two position pairs are plotted in Fig. 6. The shaded regions are related to the levels of bioreactor operating parameters corresponding to the rather equal values of mixing time in the two bioreactors (ratio t_{m0}/t_{m1} is about 1). As it can be observed from Fig. 6, the indicated domains of combination between rotation speed and superficial air velocity do not coincide, their superposition being possible only for a limited

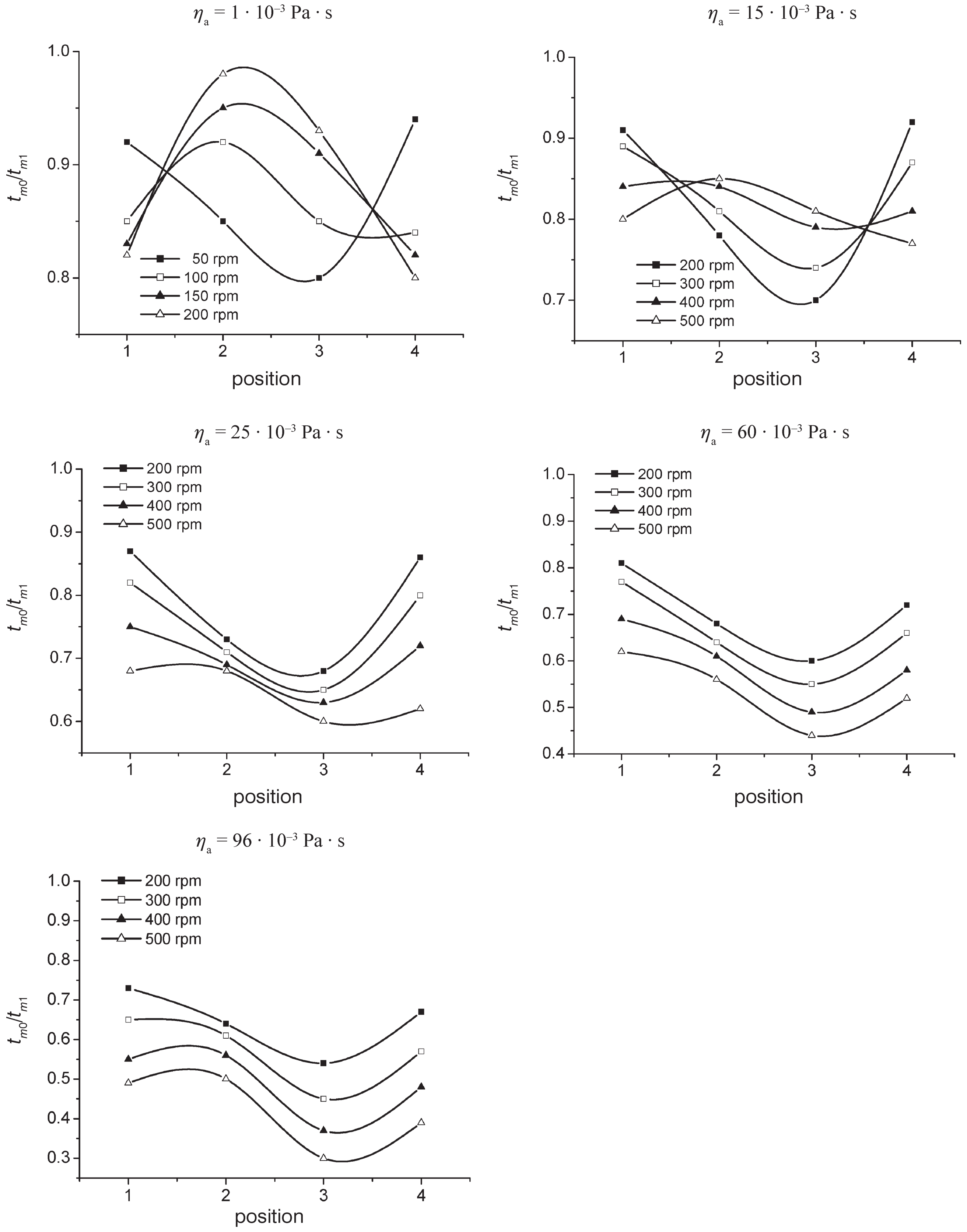


Fig. 3 – *Variation of mixing times ratio on the broth height*

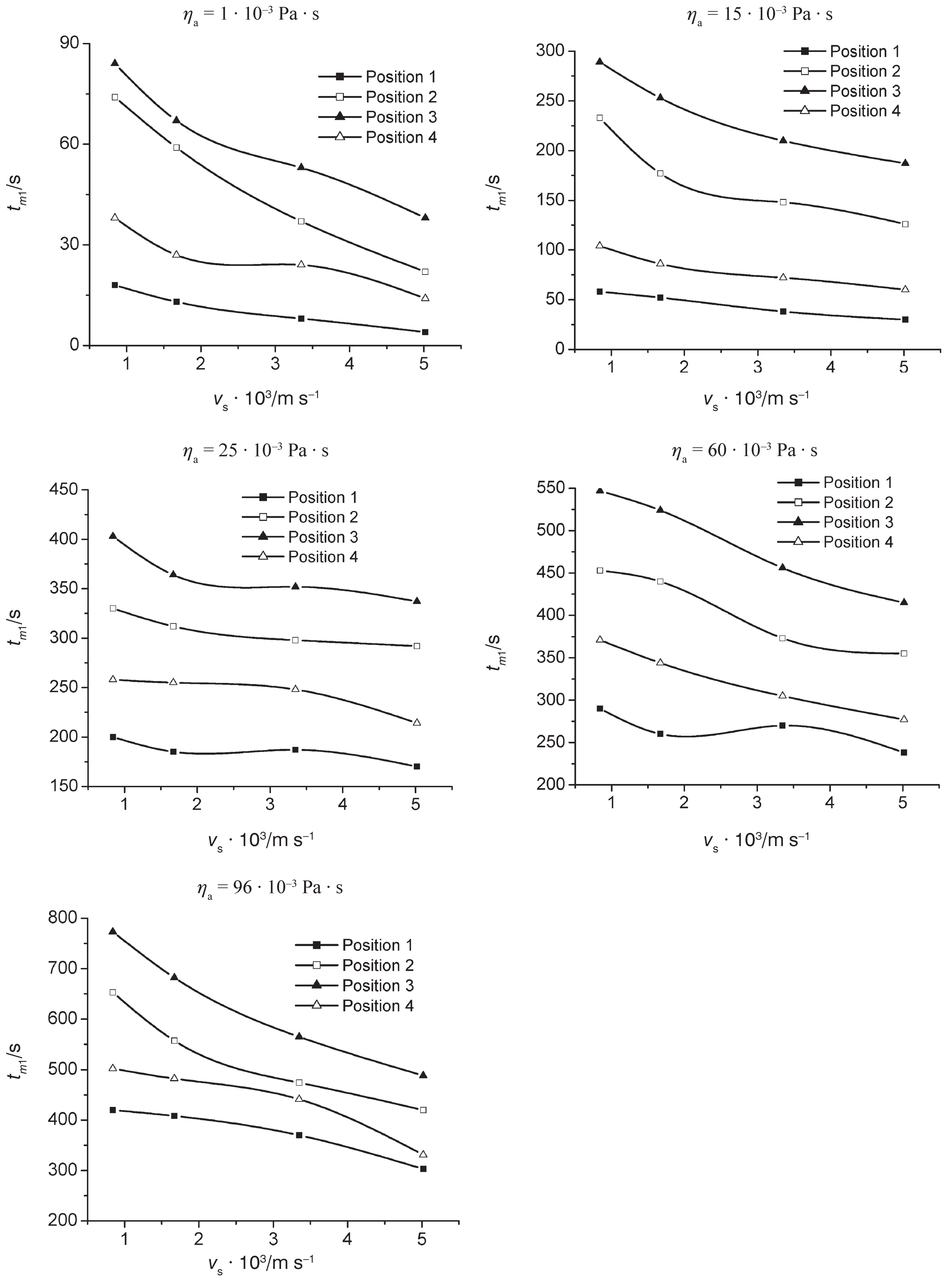
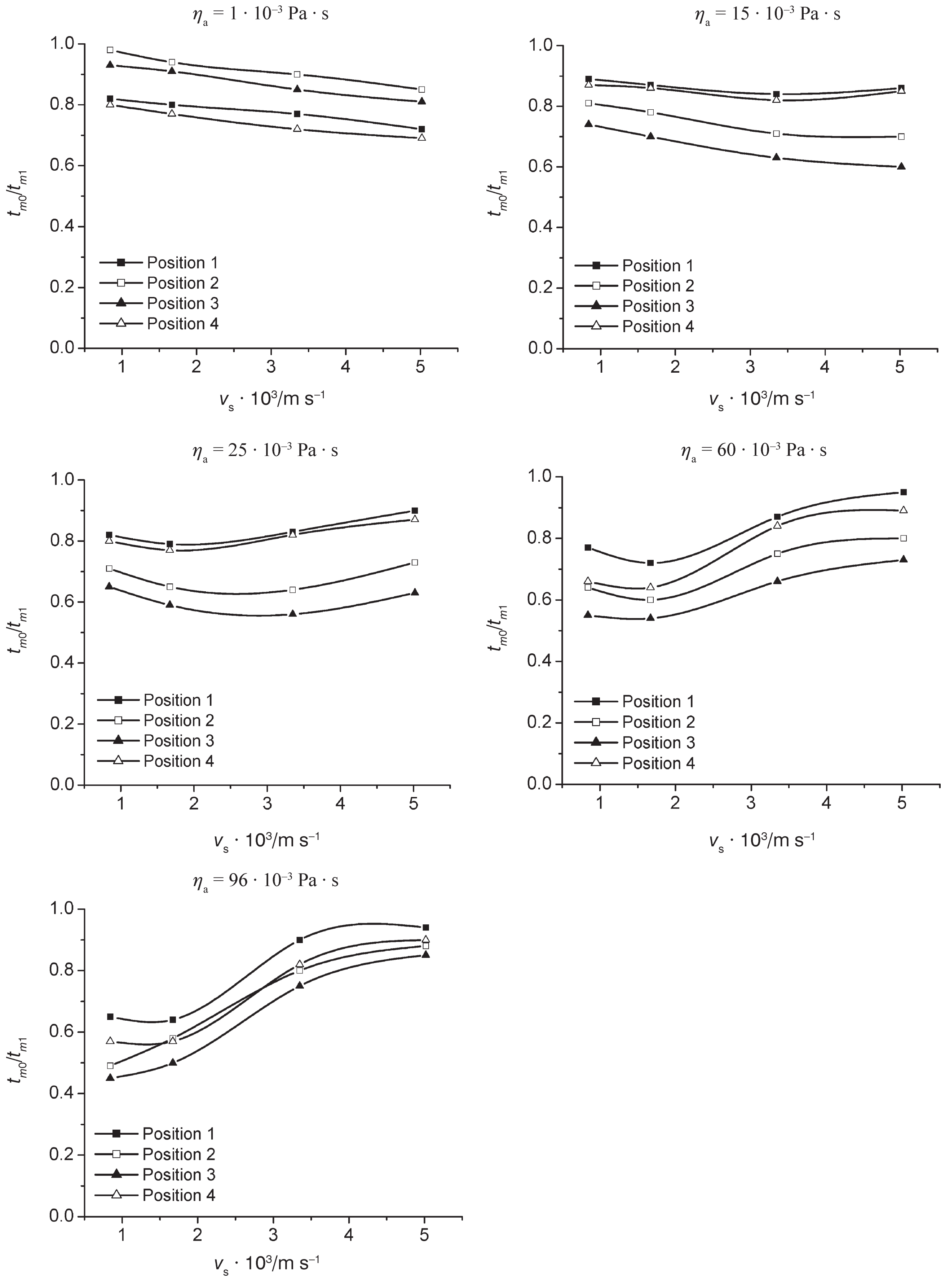


Fig. 4 – *Influence of superficial air velocity on mixing time (N = 300 rpm)*



F i g . 5 – *Influence of superficial air velocity on mixing times ratio ($N = 300$ rpm)*

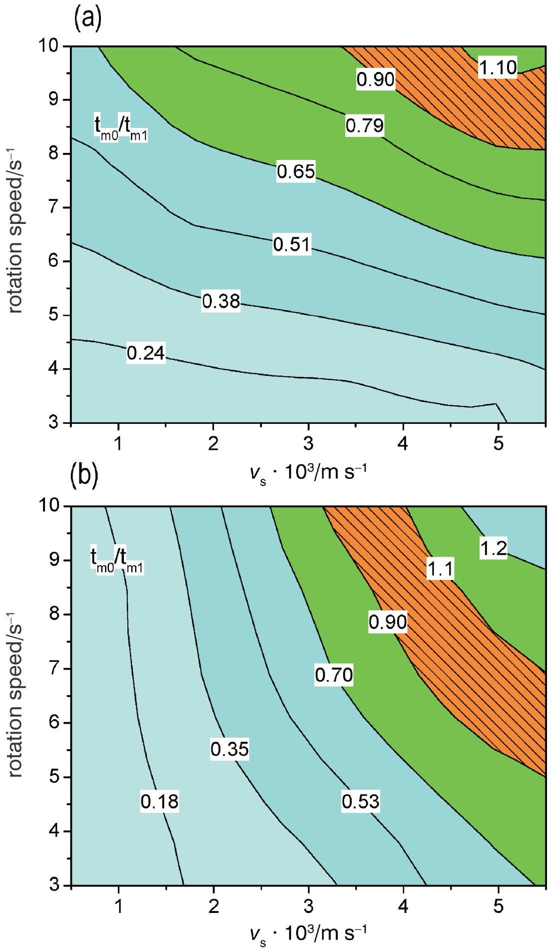


Fig. 6 – *Cumulated influence of rotation speed and superficial air velocity on mixing times ratio ((a) – positions 1 and 4, (b) – positions 2 and 3)*

number of these parameters pairs (Fig. 7). Therefore, it can be stated that for scaling-up this aerobic stirred bioreactor using the mixing time criterion, the values of rotation speed and aeration rate have to be maintained in the domain plotted in Fig. 7.

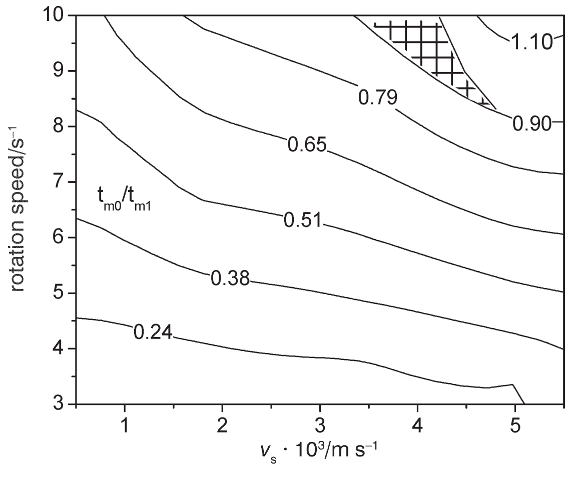


Fig. 7 – *Combinations between rotation speed- superficial air velocity values corresponding to the same mixing time in the two bioreactors*

The use of mixing time criterion also requires the analysis of the simultaneous effects induced by scaling-up, namely the modification of specific power input and of shear forces magnitude at larger scale. The specific power consumption is the parameter, which indicates the turbulence degree and media circulation in bioreactor and includes a wider range of influences than by taking into account only the rotation speed or power input. For non-aerated systems, the calculation of power consumption for stirring by Rushton turbine is made using the power number, N_P:[14]

$$N_P = \frac{P}{\rho \cdot N^3 \cdot d^5} = \frac{6}{\mathrm{Re}^{0.15}} \qquad (5)$$

The power consumption for mechanical stirring of aerated media can be determined by means of the value obtained for non-aerated media, using the equation proposed by Hughmark:[15]

$$\frac{P_a}{P} = \left(\frac{g \cdot w \cdot V^{2/3}}{N \cdot d^4}\right)^{0.2} \cdot \left(\frac{N \cdot V}{D_a}\right)^{0.25} \qquad (6)$$

Taking into consideration the geometrical similitude between the two bioreactors, the ratio between the specific power inputs for the reference bioreactor, $(P_a/V)_0$, and for the larger one, $(P_a/V)_1$, can be calculated by means of the following expression:

$$\frac{\left(\frac{P_a}{V}\right)_0}{\left(\frac{P_a}{V}\right)_1} = 7.22 \cdot \frac{\left(\frac{N_0}{N_1}\right)^{2.9}}{\left(\frac{v_{S0}}{v_{S1}}\right)^{0.25}} \qquad (7)$$

For establishing the values of this ratio, the specific power input $(P_a/V)_1$, using eq. (7), and the mixing time, t_{m1}, using eqs. (1) and (2), have been initially calculated for a certain apparent viscosity and for the pairs of rotation speed – superficial air velocity indicated in the shaded region from Fig. 7. Then, for the obtained values of mixing time, the corresponding specific power consumption $(P_a/V)_0$ was calculated for the same apparent viscosity using the previous relationships.[11]

The influence of impeller rotation speed and aeration rate for the larger bioreactor on the specific power inputs ratio is presented graphically in Fig. 8, for an apparent viscosity of $60 \cdot 10^{-3}$ Pa·s. The plotted results suggest the possibility to respect also the similitude of specific power consumption by scaling-up the bioreactor taking into consideration the mixing time criterion (for positions 1 and 4 the value of $(P_a/V)_0/(P_a/V)_1$ varied from 0.88 to 1.11, and for positions 2 and 3 from 0.86 to 1.13,

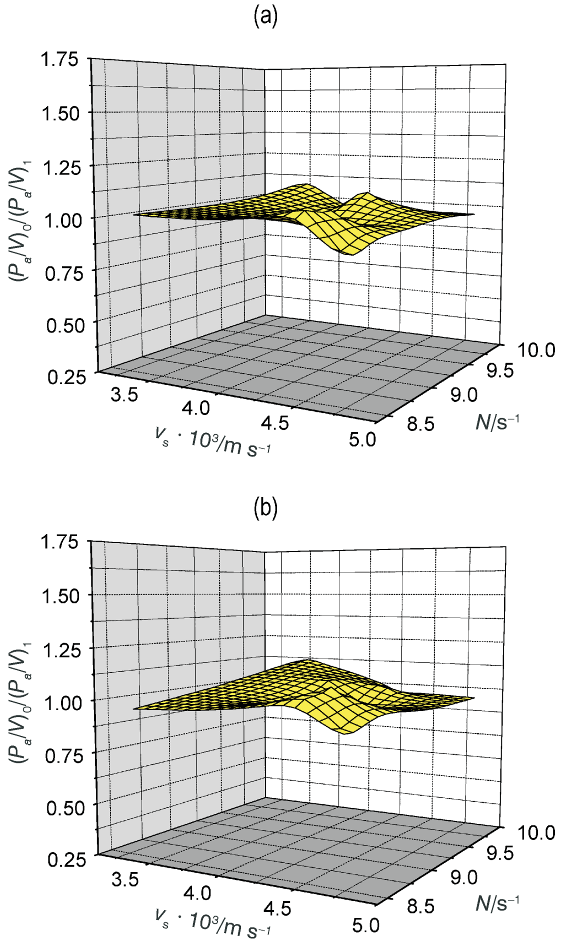


Fig. 8 – *Cumulated influence of rotation speed and superficial air velocity on specific power inputs ratio ((a) – positions 1 and 4, (b) – position 2 and 3)*

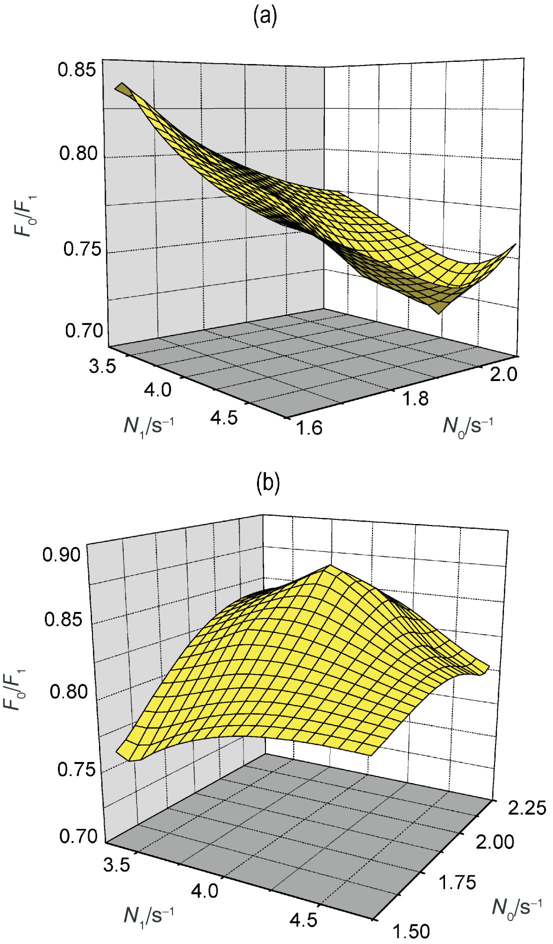


Fig. 9 – *Cumulated influence of rotation speeds in the two bioreactors on shear forces ratio ((a) – positions 1 and 4, (b) – position 2 and 3)*

respectively). Similar variations have been recorded for the other experimented viscosities.

Another parameter directly related to the broths circulation intensity is the shear force promoted by mechanical mixing, considering its contribution to the broths circulation to be decisive. The modification of shear force magnitude by scaling-up can be described by the ratio between $N \cdot d$ for the smaller and, respectively, larger bioreactors.[16] For the studied cases, the ratio of shear forces for the two bioreactors was estimated by means of rotation speeds, which lead to the mixing time values included in the shaded part of Fig. 7:

$$\frac{F_0}{F_1} \propto 0.516 \cdot \frac{N_0}{N_1} \tag{8}$$

The influences of the rotation speeds corresponding to the similar mixing times for the two bioreactors on the shear forces ratio, F_0/F_1, are simultaneously plotted in Fig. 9 for the four analyzed positions inside the broth. For both position groups, the ratio F_0/F_1 is below 1, this result suggesting the amplification of the shear forces by scaling-up using the mixing time criterion. Therefore, for broths with apparent viscosity of $60 \cdot 10^{-3}$ Pa·s, for positions 1 and 4 the average value of this ratio is 0.78, respectively 0.81 for the intermediary positions 2 and 3. These values indicate an average increase of the shear forces magnitude for about 1.23–1.28 times in the larger bioreactor. Although this conclusion is valid for the entire considered domain of apparent viscosity, the shear forces ratio decreases from 0.88 for water to 0.74 for the most viscous broth, this variation underlining the major role of the viscosity increase in the promotion of higher shear forces, especially at larger operating scale.

At the same time, from Fig. 9 it is obvious that the variation of ratio F_0/F_1 with the rotation speeds differs from one position group to another. For the extreme positions 1 and 4, the maintaining of the mixing time values in the larger bioreactor as in the reference one leads to the reduction of this ratio with the acceleration of rotation speeds and, implicitly, to the intensification of shear forces. However, in the case of positions 2 and 3, the increase of rotation speeds induces initially the slow amplification of the shear forces in the smaller bioreactor, followed by a significant similar effect in the larger bioreactor. These differences are the consequence of the different contribution of mechanical mixing to the broth circulation in the regions around the impellers and in the intermediary region. The aeration, the air accumulation in the impellers regions, as well as the flow streams interference in the intermediary region concur with these variations.

Conclusions

The study on the scale-up of an aerobic stirred bioreactor for simulated broths with apparent viscosities below $96 \cdot 10^{-3}$ Pa·s underlined that it is possible to apply the criteria of value and distribution of mixing time. If the geometrical similitude between the bioreactors is respected, the use of mixing time leads to similar specific power input for the smaller and larger operating scales.

However, by scaling-up, the magnitude of the shear forces is increased about 1.23 – 1.35 times, this effect being more pronounced at higher apparent viscosities.

List of symbols

b – impeller blade height, mm
d – stirrer diameter, mm
d' – pH electrode diameter, mm
D – bioreactor diameter, mm
Q_a – volumetric air flow rate, $m^3\ s^{-1}$
F – shear force, Pa
h – distance from the inferior stirrer to the bioreactor bottom, mm
H – bioreactor height, mm
l – impeller blade length, mm
l' – pH electrode immersed length, mm
L – distance between the stirrers, m
N – impeller rotation speed, rpm
N_P – Power number
P – power consumption for mixing of non-aerated broths, W
P_a – power consumption for mixing of aerated broths, W
Re – Reynolds number
(P_a/V) – specific power input, W m^{-3}
s – baffle width, mm
t_m – mixing time, s
v_S – superficial air velocity, m s^{-1}
V – volume of medium, m^3
pH_∞ – pH-value corresponding to perfect mixing
ΔpH – pH-limits accepted for mixing time determination
η_a – apparent viscosity, Pa·s
ρ – density, kg m^{-3}

Subscripts

0 – for the reference bioreactor
1 – for the studied (larger) bioreactor

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