EVALUATION OF THE HYDRODYNAMIC REGIME OF AEROBIC STIRRED BIOREACTORS USING THE MIXING DISTRIBUTION CRITERIA

3. SUSPENSIONS OF *PENICILLIUM CHRYSOGENUM* PELLETS

Stefănica Cămăruț[1], Anca-Irina Galaction[2], Dan Caşcaval[1*]

[1]Technical University "Gh. Asachi" of Iasi, Faculty of Chemical Engineering, Dept. of Biochemical Engineering, D. Mangeron 71, 700050 Iasi, Romania
[2]University of Medicine and Pharmacy "Gr.T. Popa" of Iasi, Faculty of Medical Bioengineering, Dept. of Biotechnology, University 16, 700115 Iasi, Romania, email: galact@from.ro

*Corresponding author: dancasca@ch.tuiasi.ro, Fax: 0040 (0)232 271 311

Received: 19/10/2007
Accepted after revision: 25/10/2007

Abstract: The study on mixing distribution for an aerobic stirred bioreactor and *P. chrysogenum* pellets broths indicated the significant variation of mixing time on the bioreactor height. The uniform mixing in whole bulk of fermentation broth can be reached only for 500 rpm and biomass concentration below 24 g/L d.w. The influence of aeration rate has to be correlated with the fungus concentration. At lower biomass concentration, below 16 g/L d.w., due to the appearance of flooding, the mixing time initially increased with the increase of aeration rate, reached a maximum value, decreasing then (the critical air flow rate varied with the position of pH electrode into the broth and fungus concentration, being of 150 - 300 L/h). For higher *P. chrysogenum* concentration, the increase of air flow rate lead to the continuous mixing intensification for all the considered positions into the fungus broth.

Keywords: *stirred bioreactor, mixing time, mixing distribution, fungus, Penicillium chrysogenum, pellets*

INTRODUCTION

Fungus is the most used class of microorganisms in biotechnology, possessing the ability to produce complex and unique molecules through metabolic pathways that are not entirely known. Fungus can biosynthesize from organic acids to enzymes, being the producers of about 20 most profitable compounds for the pharmaceutical industry. Among them, the profit due to penicillins, statins and cyclosporine A exceeds $1 billion annually each [1].

Table 1. *The use of fungus in biotechnology [1-7]*.

Product	**Strain sp.**	**Applications**
Organic acids (citric acid, gluconic acid, itaconic acid)	*Aspergillus, Penicillium*	Pharmaceuticals, food, chemicals
Alkaloids (anabasine, citisine, quinine, ergotamine, higrine, sparteine)	*Claviceps, Erythroxylon, Trichoderma*	Pharmaceuticals
Antibiotics (penicillins, cephalosporins, griseofulvin, fumagillin)	*Acremonium, Aspergillus, Cephalosporium, Cordyceps, Penicillium*	Pharmaceuticals
Enzymes (*asparaginase, amylase, catalase, cellulase, dextranase, β-glucanase, glucose oxidase, hemicellulase, lipase, pectinase, protease, tannase, xylanase*)	*Aspergillus, Endothia, Mucor, Penicillium, Phanerochaete, Pyricularia, Trametes, Trichoderma*	Pharmaceuticals, food, chemicals, detergents
Phytohormones (abscisic acid, gibberellic acid)	*Cercospora, Fusarium, Gibberella*	Agriculture, horticulture
Immunomodulators, immunosuppressants (cyclosporine A, mevinolin, polysaccharides)	*Cordyceps, Cylindrocarpon, Lentinula, Monascus, Tolypocladium, Trichoderma*	Pharmaceuticals
Pigments (antraquinones)	*Aspergillus, Curvularia, Drechslera, Trichoderma*	Textile dyes, cosmetics
Polysaccharides (β-glucan, lentinan, chitosan, chitin)	*Lentinula*	Cosmetics, pharmaceuticals, food
Statins (lovastatin, simvastatin, pravastatin)	*Aspergillus, Penicillium*	Pharmaceuticals
Steroids	*Achlya, Fomitopsis*	Pharmaceuticals
Toxins (mycoinsecticides, mycoherbicides: patulin, aflatoxins, ochratoxin, fumonisin)	*Agaricus, Amanita, Aspergillus, Coprinus, Cortinarius, Fusarium, Gyomitra, Metharizium, Penicillium, Psylocybe*	Agriculture, horticulture
Vitamins (vitamin B group, vitamin D group)	*Eremothecium, Nematospora, Pleurotus*	Pharmaceuticals, food, cosmetics

The use of the fungus in biotechnology requires their strains isolation, the increase of their activity by genetically techniques, as well as their cultivation on specific media for biosynthesizing the desired products. The fermentation conditions exhibit a decisive influence on fungus growth and biosynthesis. The deviation from the optimum parameters could involve the significant alteration of fungus activity, morphology or biosynthetic compounds structures. For example, the modification of some media components concentration (polyelectrolytes, carbon dioxide) or of the aeration/mixing intensity could lead to the modification of mycelial aggregates size or of the formation of filamentous mycelia, with important influences on broths rheology or viscosity, on rate of heat and mass transfer [8].

The capacity of bioreactor to ensure the reduction of the temperature and concentration gradients inside the broths represents one of the most important conditions for an optimum fermentation process. From the viewpoint of the difficulty to reach the optimum hydrodynamic regime in the bioreactor, the exploitation of fungus cultures is the most complicate, on the one hand due to their higher apparent viscosity, and on the other hand due to their complex rheological behavior.

The establishing of the optimum hydrodynamic regime, the selection of the stirrer type that has to be used, or the prediction of the modification of mixing efficiency by scaling-up can be made by analyzing the mixing distribution into the bioreactor. This analysis becomes more important in the case of fungus broths, owing to their major influence on mixing efficiency compared with bacteria, yeasts or actinomicetes. The complexity of fungus broths rheology and their high apparent viscosity induce a non-uniform distribution of mixing intensity, with the inevitable appearance of the heterogeneous regions. Furthermore, because the most of the fungus fermentations are aerobic, the flow mechanism becomes more complicated due to the cumulated effects of the pneumatic and mechanical mixing. The aeration generates flow streams that are significant different from those induced by mechanical mixing into the non-aerated broths.

For establishing the mixing distribution and identifying the stagnant regions inside the broths, the values of mixing time must to be analyzed at different positions into the bioreactor. In this purpose, it is more appropriate to maintain the feed position of the tracer and to modify the position of the electrode used for mixing time determination [9].

Generally, the analysis of mixing efficiency for the aerated mechanical stirred systems is derived from that of non-aerated systems, due to the less complicated flow phenomena for the second ones. Because it has been assumed that the bubbles don't influence the broths flow, the values of mixing time calculated for aerated broths by means of the equations established for non-aerated systems differ significantly from the experimental ones (in most of these cases, the values of calculated mixing time were lower for about 1.2 - 2 times than the experimental data [10]). In this context, the aeration influence on mixing efficiency and distribution in bioreactors is complex and has to be distinctly analyzed. In most aerobic fermentations, the air is accumulated around the stirrers with the formation of cavities or compartmentalization of flow regions, that reducing the pumping capacity of the stirrer and modifying the distribution of mixing intensity compared with the non-aerated systems [11, 12].

Therefore, the aim of our studies is to establish the distribution of mixing efficiency inside the aerobic stirred bioreactor, by means of the mixing time values obtained at various positions of pH-electrode, as well as the influences of the broths characteristics and operating parameters on the change of active and stagnant regions positions. For underlining the effect of the biomass presence and morphology on mixing efficiency, the experiments have been carried out for aerated broths without and with microorganisms (bacteria, yeasts, fungus), or with microorganisms having different morphological conformations (fungus). In this paper, the obtained results for mixing intensity distribution into aerated *Penicllium chrysogenum* pellets (mycelial aggregates) broths are presented.

EXPERIMENTAL

The experiments have been carried out in 5 L (4 L working volume, 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 the previous papers [13].
The mixing system consists on a double stirrer and three baffles. The impeller diameter, d, was of 64 mm. The inferior stirrer has been placed at 64 mm from the bioreactor bottom. The superior stirrer was placed on the shaft at a distance of 64 mm (d) from the inferior one, this being the optimum distance for the Rushton turbine, as it was demonstrated in the previous works [13]. The rotation speed was maintained below 600 rpm. The experiments have been carried out at Reynolds number lower than 470, domain that avoids the cavity formation at the broths surface.
The sparging system consists of a single ring sparger with 64 mm diameter, placed at 15 mm from the vessel bottom, having 14 holes with 1 mm diameter. The air volumetric flow rate was varied from 75 to 450 L/h, corresponding to an air superficial velocity of $0.84 - 5.02 \times 10^{-3}$ m/s.
The *P. chrysogenum* pellets suspensions having biomass concentration between 4 and 36 g/L d.w. have been used. The experiments were carried out at a temperature of 24 ^{o}C. Any morphological conformation change was recorded during the experiments.
The mixing efficiency has been analyzed by means of the mixing time values. The experimental measurement of mixing time uses the tracers (acidic, alkaline or salts solutions, heated solutions, colored solutions) which are added to the beforehand homogenized broths. The mixing time is the time needed for the considered parameter (pH-value, temperature, absorption etc.) to reach a constant value. Because the perfect mixing can be reached after a long period, for the mixing time determination a predefined level of homogeneity is admitted [10, 14].
For mixing time determination, a solution of 2N KOH has been used as tracer, being recorded the time needed to the medium pH-value to reach the value corresponding to the considered mixing intensity. In this case, the following homogeneity criterion for mixing has been considered:

$$I = \frac{pH_{\infty} - 0.5\Delta pH}{pH_{\infty}} x100 = 99\% \qquad (1)$$

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 placed at the four different positions mentioned in the previous papers [9]. The pH variations were recorded by the bioreactor computer-recorded system and were analyzed for mixing time calculation.

RESULTS AND DISCUSSION

The previous studies on the influence of operating conditions on mixing efficiency of non-aerated *P. chrysogenum* pellets in stirred bioreactors indicated that the mixing intensity continuously increased with the impeller rotation speed, being considerably lower than that obtained for the cultures of bacteria or yeasts [13].
Compared with the non-aerated fermentation systems, in the case of aerobic stirred bioreactors provided with single or multiple impellers the broths flow becomes more complex due to the cumulated pneumatic and mechanical agitation. The deviations from the obtained values for non-aerated broths depend on the constructive and operating characteristics of the bioreactor, as well as on the microorganisms morphology. For aerated cultures of *P. chrysogenum* pellets, the mixing was initially intensified by increasing the rotation speed, decreasing then [15]. The critical rotation speed, which corresponds to the minimum level of mixing time, was between 400 and 500 rpm, depending on biomass concentration. The existence of minimum mixing time for fungus broths was more evidently compared with the bacteria or yeasts suspensions, due to the higher viscosity of fungus broths [15].
The aeration rate influence is correlated with the fungus concentration. Therefore, at constant rotation speed, the previous experiments underlined that at lower biomass concentration (4 g/L d.w.) the intensification of aeration initially induced the increase of mixing time to a maximum value, followed by its decrease. This variation is due to the appearance of the flooding for the air flow rate of 150 - 200 L/h [15]. In the case of more concentrated *P. chrysogenum* broths (30 g/L d.w.), the aeration effect was contrary to the above presented one, the increase of aeration leading to the continuous intensification of broths circulation. This phenomenon that was more accentuated for free mycelia suspension than for pellets one, due to the higher viscosity of the former one.
The previous results have been obtained for position 1 of the pH electrode. But, by placing the pH electrode in different regions inside the bioreactor it could be drawn more rigorous conclusions concerning the distribution of mixing intensity, as well as on the effects of broths characteristics (concentration, morphology) and/or fermentation conditions on mixing efficiency in a given region inside the bioreactor. This approach leads to the recording of the mixing intensity variations in whole bulk of the broth, which could differ significantly from the above presented for aerated *P. chrysogenum* suspensions.
At a constant aeration level, from Figure 1, it can be observed that the shape of the obtained curves differs from one position to another, the four experimental curves can

be paired up in three groups, one corresponding to the inferior region (position 1), one corresponding to the intermediary region (position 2) and the other to the superior region (positions 3 and 4). Thus, the increase of aeration rate leads to the mixing intensification, consequently the mixing time decreases in the inferior region. For the other regions inside the bioreactor, the mixing time initially decreases and reaches a minimum value, increasing then is increasing, this evolution being more pronounced for the superior region.

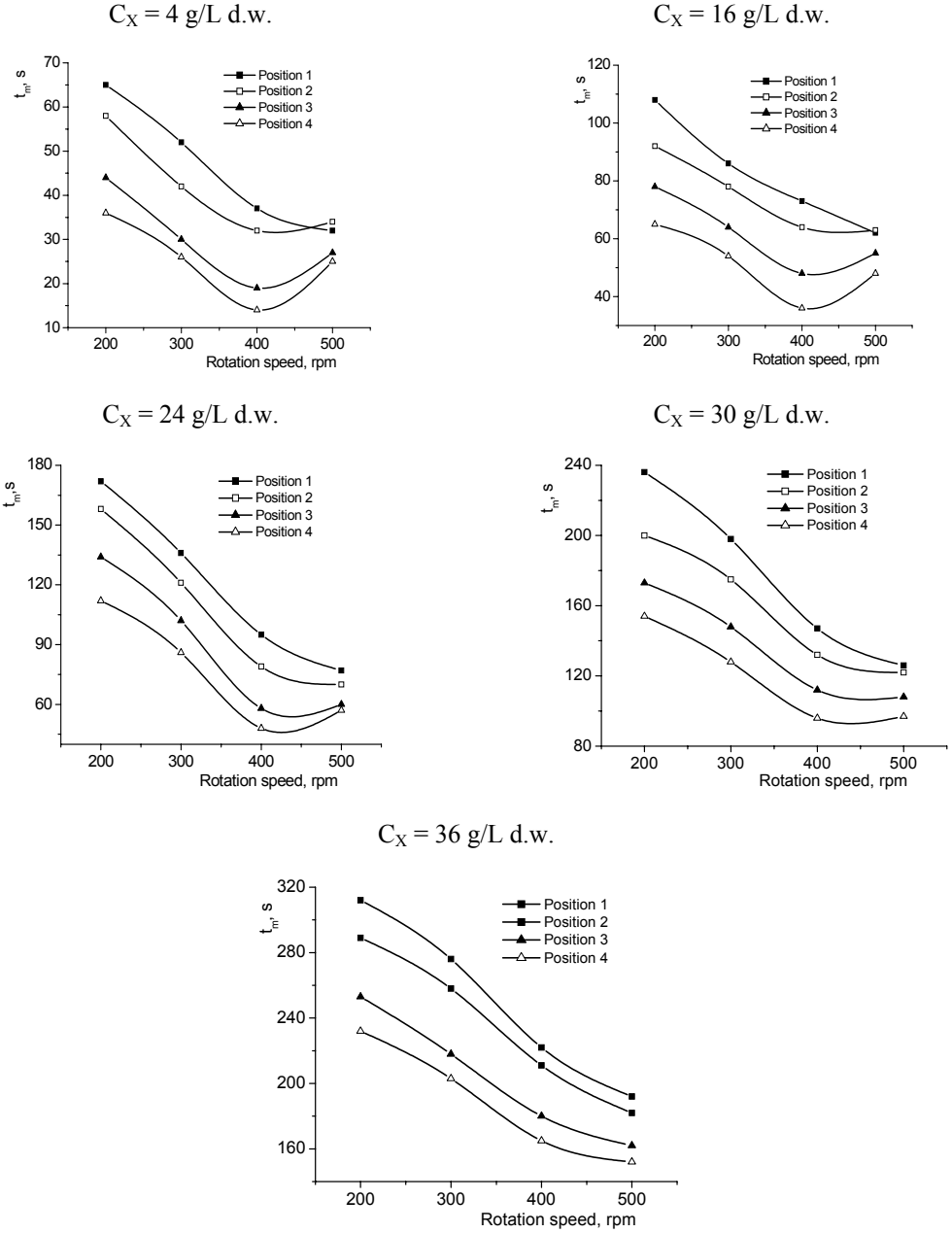

Figure 1. *Influence of rotation speed on mixing time (aeration rate of 75 L/h)*

This variation is the result of the change in relative importance of mechanical and pneumatic mixing. At lower rotation speed, the contribution of pneumatic mixing is more important, especially in the regions placed there away from the impellers [13]. In this case, the increase of rotation speed intensifies the mixing. At higher value of rotation speed, the gas hold-up increases, the flow of dispersion becomes more complex and its circulation velocity is inferior to that induced by the mechanical agitation in non-aerated systems. Moreover, the increase of rotation speed induces the dispersion of biomass into the whole bulk of the broth, respectively in the regions 2, 3 and 4, therefore the values of mixing time recorded for these regions become closer. The *critical rotation speed*, corresponding to the minimum of mixing time [15], is 400 rpm.

The *P. chrysogenum* biomass accumulation induces the reduction of the mixing intensity in whole bulk of the fermentation broth, this effect being more pronounced for the superior regions, due to their position related to the impellers (for 400 rpm, by increasing the fungus concentration from 4 to 36 g/L d.w., the mixing time increased for 6 times for position 1, respectively for 10.8 times for position 4.

The biomass accumulation leads to the gradual increase of its concentration also in the regions 3 and 4, consequently the variations of mixing time with the rotation speed obtained for these positions becomes similar to those recorded for the positions 1 and 2. It can be concluded that by increasing the fungus concentrations the contribution of mechanical mixing to the broth circulation becomes more important.

According to the above mentions, the analysis of the mixing distribution for the four considered positions into the bioreactor indicated that the highest mixing time values have been recorded for the inferior region only for fungus concentration below 24 g/L d.w. and rotation speed below 400 rpm (Figure 2). The experimental dates were indicated that the obtained mixing time for the first position increased was for 1.2 – 1.8 times bigger compared with the values of mixing time for position 4.

By comparing these results with those obtained for simulated broths without biomass, for which the highest values of mixing time have been obtained for the intermediary positions 2 and 3, due to the interference of the flow streams generated by the impellers [9], it can be underlined the decisive influence of solid phase presence through the biomass deposition which induces the heterogeneous distribution of mixing.

Also, for biomass concentration up to 24 g/L d.w., Figure 2 suggests the existence of an optimum rotation speed of 500 rpm which corresponds to the uniform mixing into the whole bulk of fungus pellets broth. The accumulation of biomass over the above mentioned level promotes the heterogeneous distribution of mixing, effect that becomes more pronounced at higher fungus concentration.

These results differ from those obtained for other aerated microorganisms cultures. For example, for *S. cerevisiae* broths it can be reached an uniform dispersion of the cells also at high biomass concentration (150 g/L d.w.). In this case, the value of the optimum rotation speed increased from 300 rpm for yeasts concentration below 75 g/L d.w. to 500 rpm for suspensions more concentrated than 130 g/L d.w. [16]. The observed differences are on the one hand the consequence of the significant higher viscosity of fungus broths, even at low biomass concentration, (the apparent viscosity of the *P. chrysogenum* pellets suspension of 33.5 g/L d.w. is 88 cP [13], compared with 7 cP for the *S. cerevisiae* suspension of 150 g/L d.w.[9]), and on the other hand of the biomass deposition.

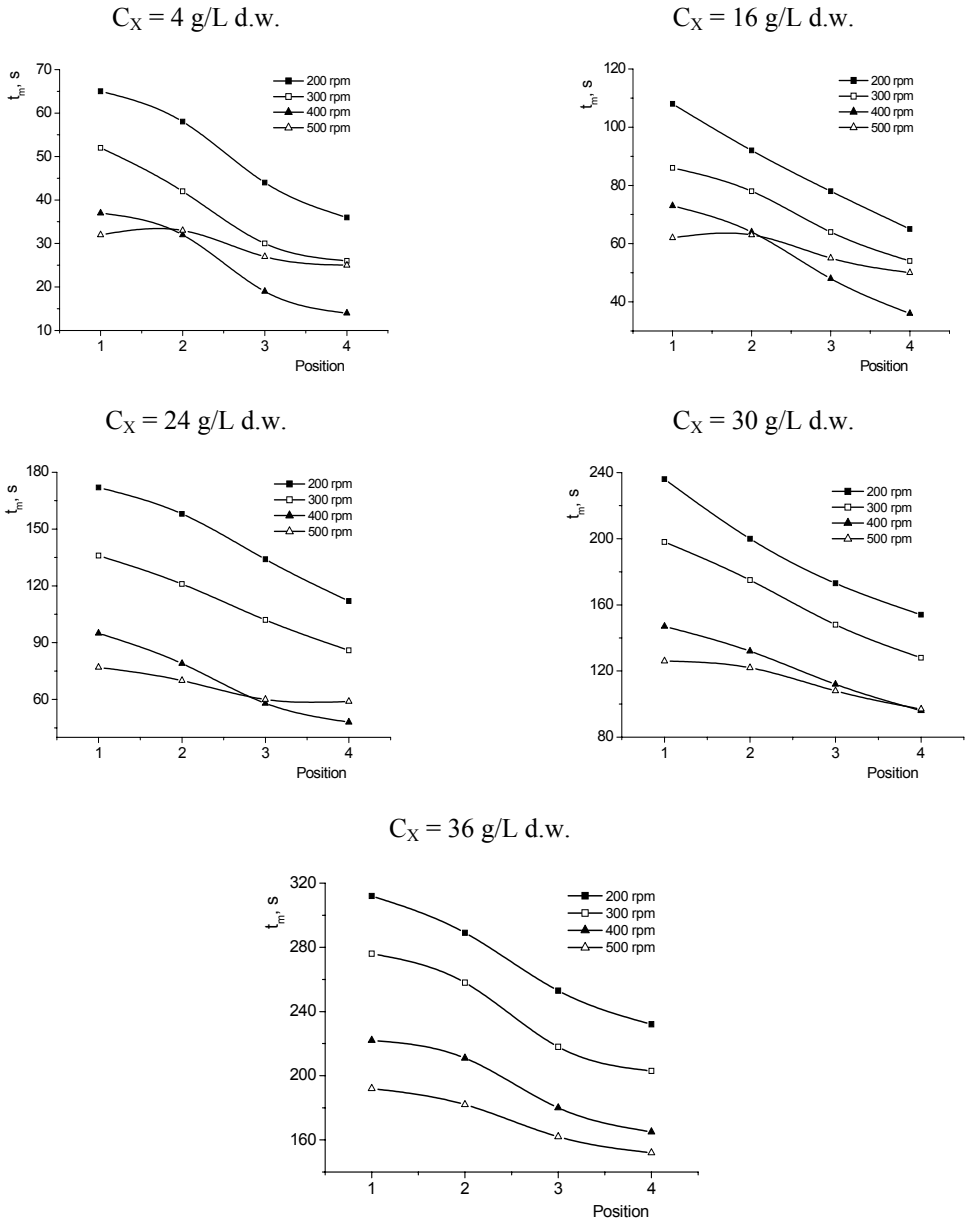

Figure 2. *Variation of mixing time with pH electrode position (aeration rate of 75 L/h)*

At constant rotation speed, the influence of aeration rate mainly depends on the biomass concentration. From Figure 3 it can be observed that the shape of the curves describing the correlation between the mixing time and the air flow rate is significantly modified by increasing the fungus concentration.

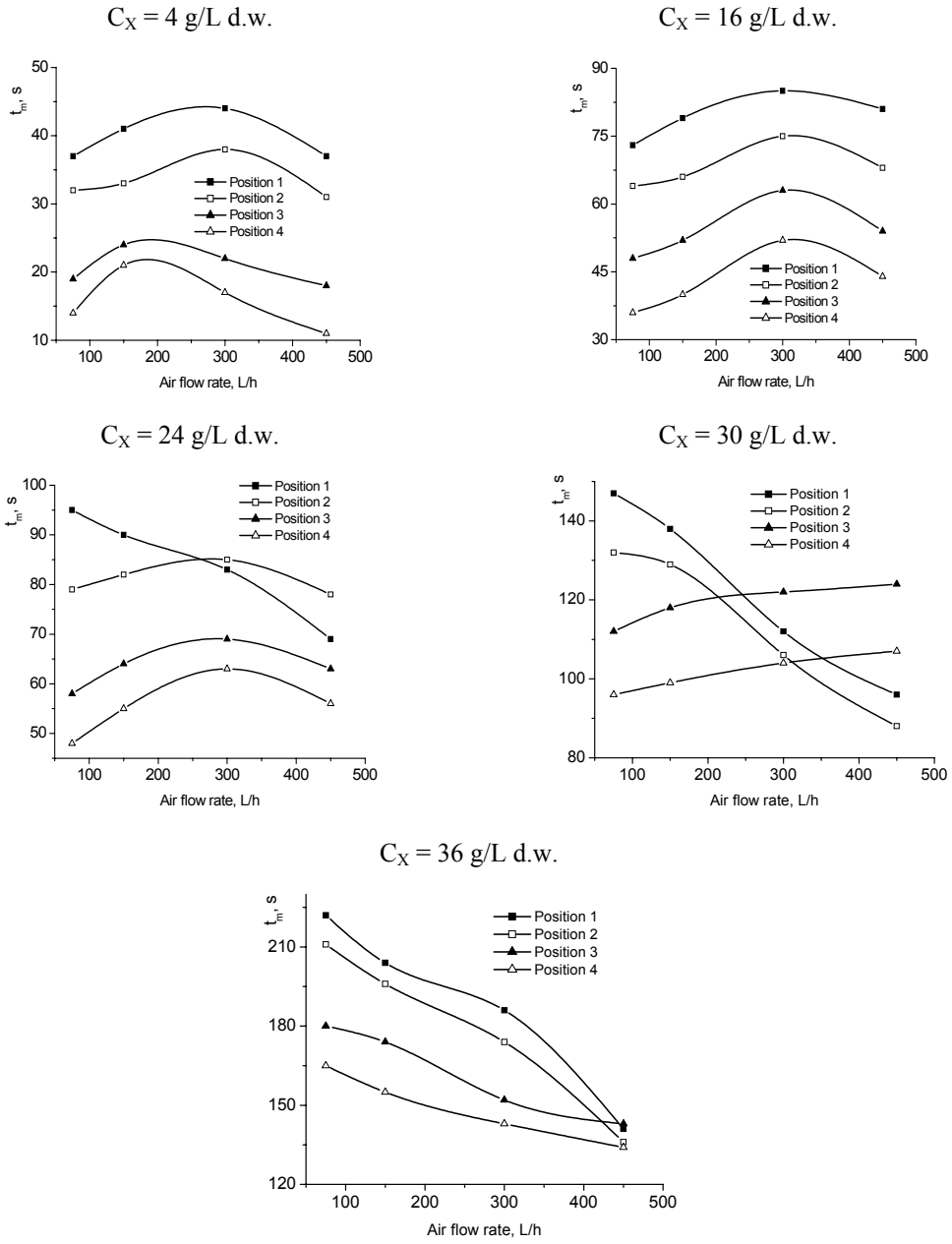

Figure 3. *Influence of aeration rate on mixing time (rotation speed of 400 rpm)*

For the biomass concentration below 16 g/L d.w., indifferent of the pH electrode position, the increase of aeration initially induces the increase of mixing time to a maximum value, followed by its decrease. This variation is the result of the formation of small bubbles, due to the air dispersion and mechanical agitation, as well as to the biomass presence which avoids the bubbles coalescence, their rise velocity being reduced by the high apparent viscosity. These small bubbles exhibit a negative effect on broths circulation, reducing its velocity, and, therefore, the mixing intensity. At higher air flow rate values, the energy dissipated by the air exceeds that of the stirrer,

appearing the flooding [15]. At the flooding point, the rise velocity of the air strongly increases, generating the simultaneous increase of the velocity of media circulation and the decrease of mixing time. The value of air volumetric flow corresponding to the flooding point is depended only on the pellets amount, being the same for the all considered regions inside the bioreactor. For 4 g/L d.w. pellets fungus the critical air flow rate was 150 L/h, becoming 300 L/h for 16 g/L d.w.

With the biomass accumulation, the existence of flooding point becomes less pronounced and the shapes of the plotted curves are gradually changed, being observed differences between the inferior positions 1 and 2 and the superior ones 3 and 4. For fungus concentration over 16 g/L d.w., the intensification of aeration leads to the continuous intensification of mixing in the inferior region. The variation of mixing time is contrary for the superior region, the increase of air flow rate inducing the slow increase of mixing and the flattening of its variation compared with lower concentrated suspension of fungus. For fungus concentration over 30 g/L d.w., the influence of aeration becomes similar in whole bulk of the broth, but it is more important for the inferior region.

In these systems, the high apparent viscosity of fungus suspensions controls the mixing efficiency, the mechanical agitation is poorly, especially in the region placed far from the impellers, and the relative contribution of pneumatic mixing to the broths circulation is more important.

Contrary to the simulated broths, where the bubbles coalescence and their accumulation around the stirrers are enhanced at higher viscosity [9], the mentioned phenomena has been not recorded in the *P. chrysogenum* pellets cultures, especially due to the avoiding of the bubbles coalescence by the biomass. But, at higher biomass amount and aeration rate, the increase of air hold-up has been observed also for pellets suspensions, as the result of the hindrance of bubbles rising (for 300 L/h and 400 rpm, the air volumetric fraction increased from 2.3% for 4 g/L d.w. *P. chrysogenum* pellets to 8.4% for 36 g/L d.w.).

CONCLUSIONS

The studies on mixing distribution for a stirred bioreactor and aerated *P. chrysogenum* pellets broths underlined the different behavior of these systems compared with simulated broths or with other microorganisms suspensions, from the viewpoint of the correlation between the mixing time and the considered parameters (biomass concentration, rotation speed, aeration rate, position into the broths).

The increase of the rotation speed, at a constant level of air flow rate, induces the initial reducing of mixing time to a minimum level, followed by its increasing, this evolution being more pronounced for the suspensions with higher biomass amount. The fungus accumulation leads to the significant decrease of mixing efficiency in the whole bulk of broth, the rotation speed influence becoming more pronounced for the inferior positions 1 and 2.

For biomass concentration up to 24 g/L d.w., it was observed the existence of an optimum rotation speed of 500 rpm which corresponds to the uniform mixing into the whole bulk of pellets broth, similar to the free mycelia suspensions. The supplementary

accumulation of biomass induces the heterogeneous distribution of mixing, effect that is more accentuated at higher fungus concentration.
The influence of aeration rate depends especially on the biomass concentration and on the sparger position. Therefore, for biomass concentration below 16 g/L d.w., the increase of aeration initially induces the increase of mixing time to a maximum value, followed by its decrease, for all considered positions in the bioreactor. This variation is due to the appearance of flooding, the flooding point value increasing from 150 L/h, for 4 g/L d.w. mycelia, to 300 L/h, for 16 g/L d.w.
The existence of flooding point becomes less pronounced and the shapes of the plotted variations are gradually changed with the biomass accumulation, being observed differences between the inferior and the superior positions. Thus, for fungus concentration over 16 g/L d.w., the intensification of aeration leads to the continuous intensification of mixing in the inferior region, contrary to the superior one. For pellets fungus concentration over 30 g/L d.w., the influence of aeration becomes similar in whole bulk of the broth, being more important for the inferior region.

NOTATIONS

C_X - biomass concentration, g/L d.w.
d - stirrers diameter, mm
pH_∞ - pH-value corresponding to perfect mixing
ΔpH - pH-limits accepted for mixing time determination
t_m - mixing time, s

REFERENCES

1. Wainwright, M.: *An Introduction to Fungal Biotechnology*, Wiley, Chichester, **1995**, pp. 43.
2. Arora, D.K., Elander, R.P., Mukerji, K.G.: *Handbook of Applied Mycology*, *Fungal Biotechnology*, Marcel Dekker, New York, **1992**, **4**, pp. 144.
3. Leatham, G.: *Frontiers in Industrial Mycology*, Chapman & Hall, London, **1993**, pp. 101.
4. Carlile, M.J., Watkinson, S.C.: *The Fungi*, Academic Press, London, **1994**, pp. 203.
5. Hobson, D.K., Wales, D.S. : *J. Soc. Dyers Colour.*, **1998**, **114,** 42.
6. Moss, M.O.: *Fungal metabolites*, in *Encyclopedia of Life Science*, Macmillan Publishers Ltd., London, **2002**, pp. 325.
7. Galaction, A.I., Caşcaval, D.: *Metaboliţi secundari cu aplicaţii farmaceutice, cosmetice şi alimentare*, Venus, Iaşi, **2006**, pp. 25.
8. Oniscu, C., Caşcaval, D.: *Inginerie biochimică şi biotehnologie. 1. Ingineria proceselor biotehnologice*, InterGlobal, Iaşi, **2002**, pp. 231.
9. Caşcaval, D., Galaction, A.I., Cămăruţ, Ş., Turnea, M.: *Roum. Biotechnol. Lett.*, **2006**, **11,** 2537.

10. Van't Riet, K., Tramper, J.: *Basic Bioreactor Design*, M. Dekker Inc., New York, **1991**, pp. 183.
11. Oniscu, C., Galaction, A.I., Caşcaval, D.: *Roum. Biotechnol. Lett.*, **2002**, **7**, 817.
12. Caşcaval, D., Oniscu, C., Galaction, A.I., Ungureanu, F.: *Chem. Ind.*, **2002**, **56**, 506.
13. Oniscu, C., Galaction, A.I., Caşcaval, D., Ungureanu, F.: *Biochem. Eng. J.*, **2002**, **12**, 61.
14. Bujalski, W., Jaworski, Z., Nienow, A.W.: *Trans. Int. Chem. Eng.*, **2002**, **80**, 97.
15. Caşcaval, D., Oniscu, C., Galaction, A.I., Ungureanu, F.: *Chem. Ind.*, **2004**, **58**, 128.