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SCIENTIFIC PAPER

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PREDICTION OF MIXING TIME FOR ANAEROBIC STIRRED BIOREACTORS

The mixing time is one of the most useful criteria for the mixing intensity of fermentation broths and for the scale–up of biosynthesis processes. This parameter value mainly depends on the rheological properties of the broths, biomass concentration and morphology, mixing system characteristics and fermentation conditions.
For quantifying the influence of these factors on the mixing efficiency for stirred bioreactors, these studies were carried out for non–aerated simulated broths and suspensions of bacteria (Propionibacterium shermanii), yeasts (Saccharomyces cerevisiae) and fungus (Penicillium chrysogenum, free mycelia and mycelial aggregates) of different apparent viscosities or biomass concentrations, using a laboratory bioreactor with a double impeller. By means of the experimental data and using a multiregression analysis method, some mathematical correlations for mixing time were established. The proposed equations are adequate for the transitory or turbulent flow regime of Re < 5,000 for simulated broths and of Re < 25,000 for biomass suspensions.

The accumulation of biomass or biosynthesized product (extracellular polysaccharides, protein molecules) leads to the continuous modification of the medium rheological properties, producing the appearance of heterogeneous regions in the bioreactor. In these conditions, one of the most important problems which must be solved is the establishment of the optimum hydrodynamic regime for the bioreactor.

The mixing time represents one of the most useful criteria for characterizing the mixing intensity and for biosynthesis processes scale–up. The mixing time, t_m, is defined as the time needed to reach a given mixing intensity at a given scale, when starting from a completely segregated situation. This parameter depends on a multitude of geometrical (dimensions of the mixing system, bioreactor dimensions) and technological factors (fermentation conditions, medium composition, physical characteristics of the medium), the general correlation that describes the mixing time being of the following type [1]:

$$t_m = f(N, \eta, \rho, V_a/V, P/P_a, C_x) \quad (1)$$

The experimental measurement of mixing time uses tracers (acidic, alkaline or salt solutions, heated solutions, colored solutions) which are added to previously homogenized broths. The mixing time is the time needed for the considered parameter (pH–value, temperature, absorption) to reach a constant value (Figure 1).

In the example from Figure 1, an alkaline pulse is added to the liquid, this method being used in the experiments in this paper. In this case of a single pulse, the system can be regarded as completely segregated at t = 0. As can be observed, after a certain time, which is the mixing time, the pH reaches a constant value. In this way, the mixing time can be related to the mixing intensity.

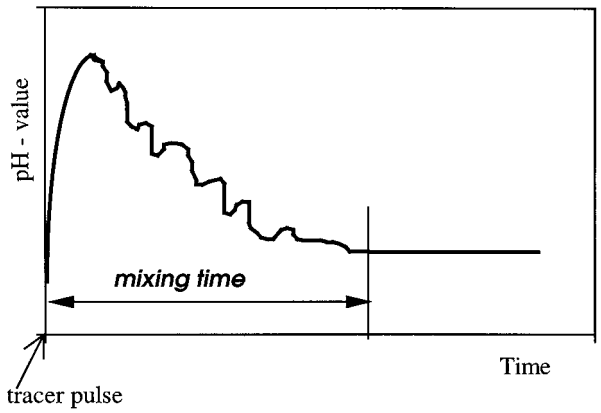

Figure 1. Experimental method for mixing time

Numerous equations have been proposed in the literature for calculation of the mixing time. These equations taking into account the type of fermentation (aerobic or non–aerobic), the rheological characteristics of the broths and the fermentation conditions [2–7]. Because of the complexity of the rheological behavior of broths and of the particularities of fermentation systems, the accuracy of the proposed models is very limited. Furthermore, most of these models can predict the mixing time values for Re > 10,000, this flow regime being rarely reached in large–scale bioreactors. For Re < 10,000, these models need some corrections [7].

The increase of broth viscosity, as a result of biomass or biosynthesized product accumulation, leads to an increase of the mixing time. But, the effect of viscosity must be related to the geometrical and functional characteristics of the bioreactor, as well as to the presence of solid particles (free or immobilized cells, immobilized enzymes, substrate) which manifest the tendency of deposition at the vessel bottom.

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For these reasons, the aim of our experiments is to analyze the dependence between the mixing time, the rheological characteristics of the broths, the fermentation conditions and the biomass concentration for a stirred bioreactor. For emphasizing and quantifying the effects of the biomass presence in the medium, the studies were carried out with simulated and real broths. The simulated broths were prepared by means of carboxymethylcelullose sodium salt solutions of different viscosities. Because the results obtained for simulated broths are adequate for liquids without solid particles, respectively biomass, these studies have been developed for non–aerated suspensions of bacteria (*Propionibacterium shermanii*), yeasts (*Saccharomyces cerevisiae*) and fungus (*Penicillium chrysogenum*, free mycelia and mycelial aggregates or pellets).

The experimental data have been correlated in some mathematical models that describe the influence of considered factors (apparent viscosity, biomass concentration, rotation speed, distance between the stirrers) on mixing time, for each type of considered system. The established equations offer a good concordance with the experiments and can be used for fermentation system scale–up.

EXPERIMENTAL

The experiments were carried out in a 5 dm^3 (4 dm^3 working volume) laboratory bioreactor (Biostat A, B. Braun Biotech International), with computer–controlled and recorded parameters. The bioreactor mixing system consists of two turbine stirrers with six blades. The stirrer diameter, d, is 64 mm and the length and height of the blades are 18 mm and 8 mm, respectively. The inferior stirrer was placed on the shaft at 40 mm from the bioreactor bottom and the second one at a distance varying between 32 and 128 mm (0.5d and 2d) from the first one. The rotation speed was maintained between 50 and 1000 rpm.

The simulated broths used consisted of carboxymethylcellulose sodium salt (CMCNa) having the apparent viscosity in the domain of 6.2 – 94 x 10^{-3} Pa·s.

The biomass suspensions were prepared using the following microorganism types:

– bacteria (*Propionibacterium shermanii*), the biomass concentration, C_X, varying between 30.5 and 120.5 g dm^{-3} dry weight;

– yeasts (*Saccharomyces cerevisiae*), C_X being of 43 – 157.5 g dm^{-3} d.w.;

– fungus (*Penicillium chrysogenum*), with two morphological conformations: free mycelia and mycelial aggregates (pellets, with the average diameter of 1.6 – 1.8 mm); in both cases, the biomass concentration varied between 4 and 36.5 g dm^{-3} d.w.

Although the viscosity of non–Newtonian liquids, like CMC solutions of biomass suspensions, varied with the shear rate, respectively with the impeller speed, owing to the difficulty of *in–situ* measurement of viscosity during the experiments, the viscosity was measured before and after each experiment using an Ostwald type viscometer. The obtained results were related to the ex–situ values of viscosity. Any viscosity or morphological conformation change was recorded during the experiments. Both the experiments and viscosity measurements were carried out at 21°C.

The values of mixing time were determined by means of a solution of 10% KOH as tracer, by recording the time needed for the medium pH–value to reach a constant level. The tracer volume was 0.5 x 10^{-3} dm^3, the tracer being injected at 10 mm from the liquid surface. The pH variations were recorded by a bioreactor computer–recorded system.

The mathematical model, which describes the mixing intensity through the mixing time, was developed on a PC compatible computer using MATLAB facilities. Multiregression analysis of the experimental data was performed. A non–linear equation form was chosen that may be linearized by applying a logarithmic function, the difference between the experimental and modeled value being reduced to a minimum. The regression coefficients and standard deviation were calculated by means of a MATLAB program.

RESULTS AND DISCUSSION

Mixing time for non–aerated simulated broths

For increasing the efficiency of the mixing process, bioreactors are provided with multiple agitator systems which consist of two or more identical or different stirrer types assembled on the same shaft, the number of stirrers being a function of the broth height in the vessel. The correct position of the stirrers on the shaft represents a determinant factor for the mixing efficiency in these systems. The distance between the stirrers controls the interactions with other stirrers, its optimum value depending on the nature and viscosity of the fermentation broths. The literature indicates that the optimum distance between two stirrers is (1÷2) d. An incorrect position can generate inefficient mixing, due to the following reasons [8]:

– the interference of flow streams created by adjacent stirrers, especially for small distances between the stirrers

– the formation of non–agitated regions between adjacent stirrers, as a result of to large distances between them.

Compared to a single stirrer system, analyzed in our previous papers [9], the use of a two stirrer system leads to a significant reduction of the mixing time, the effect being more pronounced for viscous liquids and which must be related to the stirrer position.

As can be seen from Figure 2, regardlens of the liquid viscosity, the mixing time strongly decreases with rotation speed, tending to a constant value depending

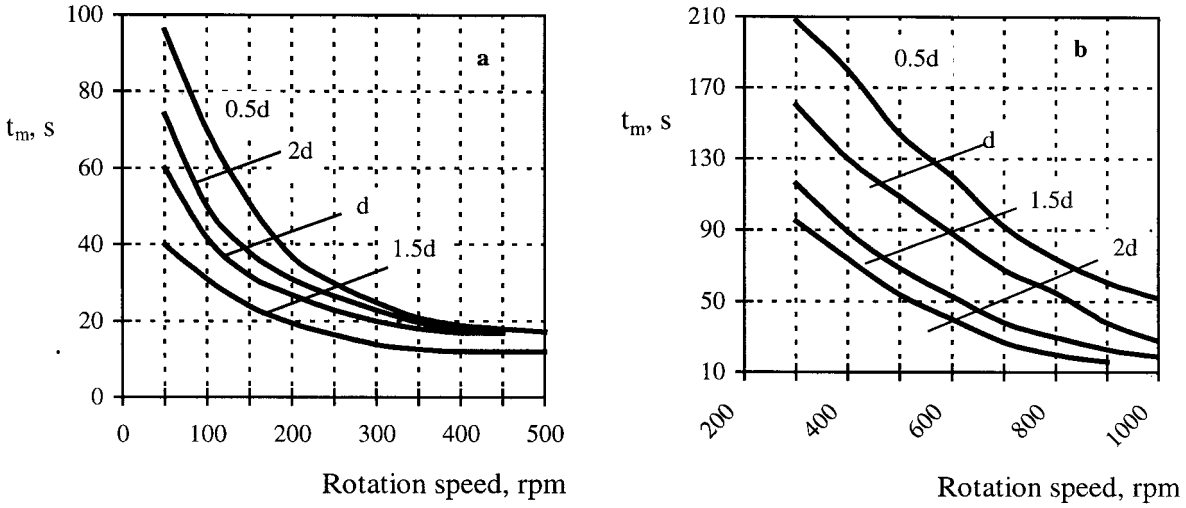

Figure 2. The influence of rotation speed on mixing time for a two stirrer system (a – water, b – C MC Na solution with η_a = 94x10^{-3} Pa·s).

both on the viscosity and on the distance between the stirrers. Owing to its low viscosity, a constant value of the mixing time for water is reached at a rotation speed range of 300 – 400 rpm. The lowest value of the mixing time is of 12 s and it was obtained for 400 rpm and a distance between the stirrers of 1.5 d.

By increasing the liquid viscosity, an increase of both the rotation speed corresponding to a constant level of the mixing time and of the final value of the mixing time was observed. For apparent viscosities greater than 20x10^{-3} Pa·s, a constant level of the mixing time cannot be reached, even at a rotation speed of 1000 rpm, this effect being more pronounced at distances smaller than d between the two stirrers.

The distance between the two stirrers influences the mixing of water and a liquid with higher viscosity in two different ways. Thus, for all rotation speed values, the lowest mixing time values for water were reached for a position of the stirrers at 1.5 d. In this case, for a certain rotation speed, the mixing time increases in the following order (Figure 3a):

$$1.5 \cdot d < 2 \cdot d < 0.5 \cdot d$$

This variation can be the result of two phenomena: at low distances between the stirrers (0.5d), a stagnant region is formed at the top of the liquid, while at longer distances (2d), the interference of flow lines leads to the formation of poorly agitated regions between the stirrers.

On the other, the data for CMCNa viscous solutions indicated that the mixing time continuously decreased with distance between the stirrers, the obtained order being (Figure 3b):

$$2 \cdot d < 1.5 \cdot d < d < 0.5 \cdot d$$

For the considered system, this evolution is specific for liquids with a higher viscosity than that of water and it is due to the reduction of the volume of the superior stagnant region by outdistancing the stirrers. In this case, the presence of a stagnant region controls the

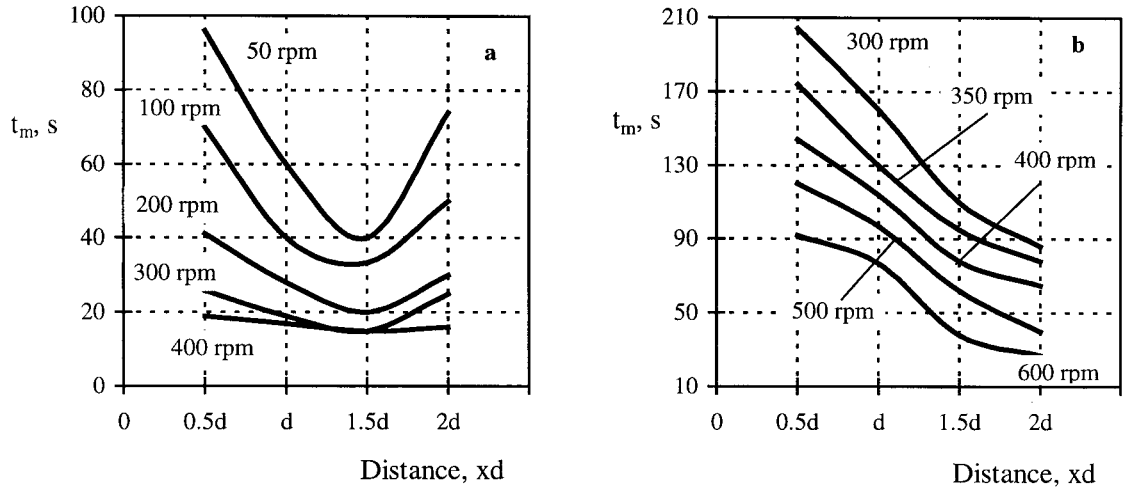

Figure 3. The influence of distance between the stirrers on mixing time for a two stirrer system (a – water, b – C MC Na saltion with η_a = 94x10^{-3} Pa·s).

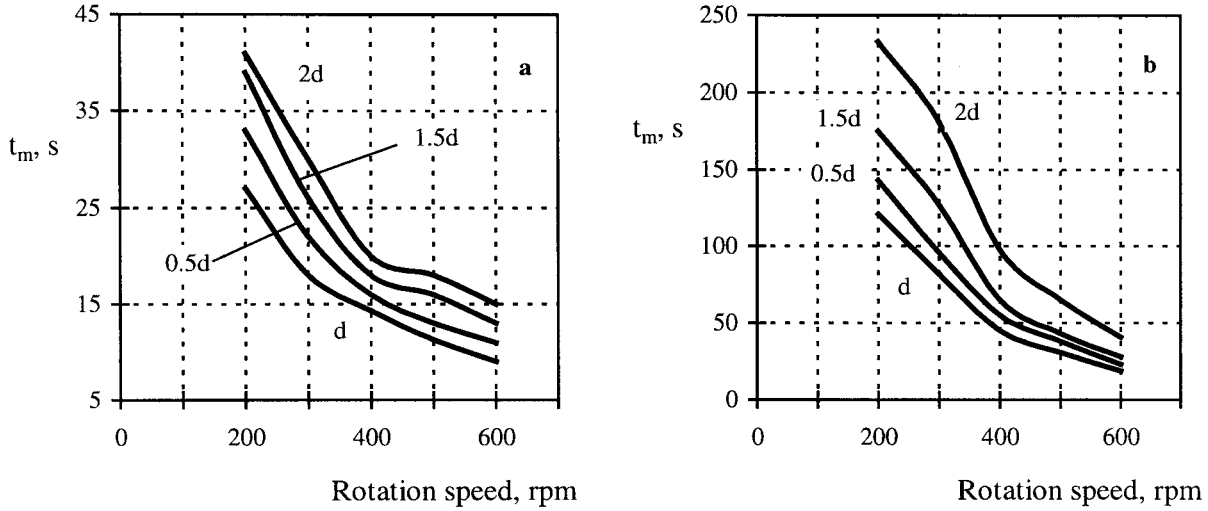

Figure 4. The influence of rotation speed on mixing time for Propionibacterium shermanii suspensions (a – C_X = 30.5 g dm^{-3} d.w., b – C_X = 120.5 g dm^{-3} d.w.).

mixing efficiency, the favorable effect created by placing the stirrer in this region being more important than the negative effect due to the inefficient mixing induced between the stirrers. Thus, for viscous liquids, the low mixing intensity is mainly the result of stagnant region formation and less the effect of interactions between the flow streams.

Mixing time for non–aerated biomass suspensions

Although biomass accumulation leads to an increase of broth viscosity and to a reduction of the mixing efficiency, this effect magnitude differs as a function of microorganism type and/or morphology. For emphasizing and quantifying the influence of biomass presence in the medium, correlated with the geometrical and operational characteristics of the bioreactor, on the mixing time, comparative studies were carried out for bacteria, yeasts and fungus non–aerated suspensions.

Bacteria suspensions (*Propionibacterium shermanii*)

Owing to the small size of bacterial cells, even for high biomass concentration, the viscosity of bacteria suspensions remains at lower values. Thus, for a *P. shermanii* biomass concentration range of 30.5 – 120.5 g dm^{-3} d.w., the viscosity increased only 3.1 times compared to the viscosity of water. For this reason, except the fermentation conditions, the mixing intensity is controlled by the amount of biomass in the broths, the viscosity exhibiting a negligible influence.

As can be seen from Figure 4, increase of the bacterial biomass concentration leads to an increase of the mixing time, the effect being more pronounced at lower rotation speed values (for example, for 200 rpm and a distance between the stirrers equal to their diameter d, the mixing time was found to increase about 5 times for the considered range of biomass concentrations).

The reduction of the mixing efficiency is a result of the deposition tendency of the microbial cells at the

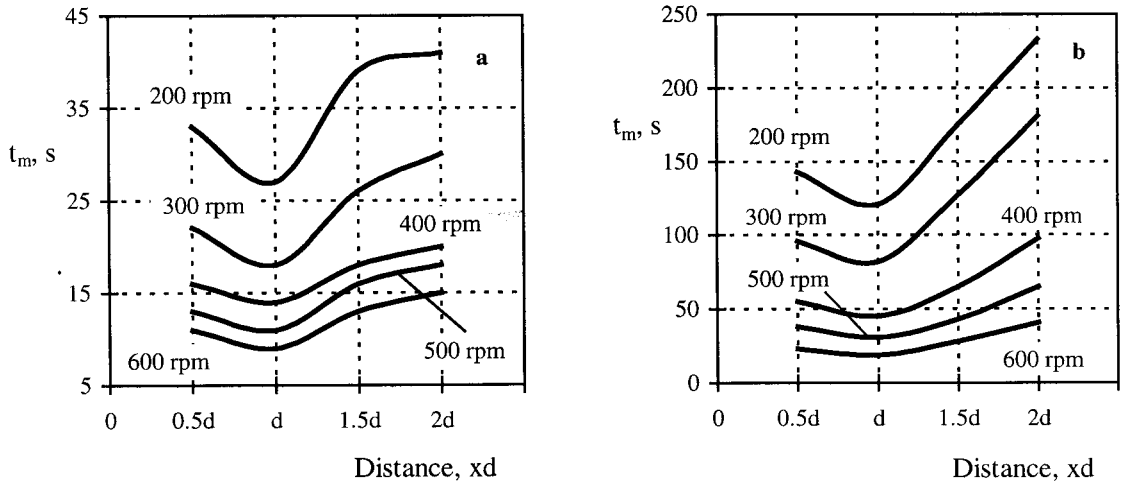

Figure 5. The influence of distance between the stirrers on mixing time for Propionibacterium shermanii suspensions (a – C_X = 30.5 g dm^{-3} d.w., b – C_X = 120.5 g dm^{-3} d.w.).

bioreactor bottom, phenomena that are amplified with solid phase accumulation. This conclusion is suggested by the variation of the mixing time with the distance between the stirrers (Figure 5).

Thus, it may be observed from Figure 3 that the lowest values for the mixing time are reached if the two stirrers are placed in the inferior region of the bioreactor, the order of mixing time increase being as follows:

$$d < 0.5 \cdot d < 1.5 \cdot d < 2 \cdot d$$

Yeast suspensions (*Saccharomyces cerevisiae*)

Similar to bacteria broths, the yeast concentration exhibits a reducion effect on the suspension viscosity (the viscosity reaches a level of 4.5×10^{-3} Pa·s for a cell concentration of 157.5 g dm^{-3} d.w.), having a significant influence on the mixing time (Figure 6).

Compared to the bacteria suspension case, for the same values of biomass concentration, the mixing efficiency was lower, because of the greater size of yeast cells and, consequently, of the superior deposition rate of the solid phase. Therefore, the differences between the mixing time values obtained for different stirrers positions on the shaft are more pronounced, as indicated in Figure 7.

The order of mixing intensity decrease with the distance between the stirrers is similar to that obtained for bacteria suspensions.

Fungus suspensions (*Penicillium chrysogenum*)

The performance of a bioreactor containing a fungus fermentation broth is greatly affected by the rheological properties of the broth. These properties are controlled mainly by the biomass concentration, its growth rate and morphology. Among the morphological characteristics, such as the geometry of the hyphae (length, diameter, branching frequency) and flexibility,

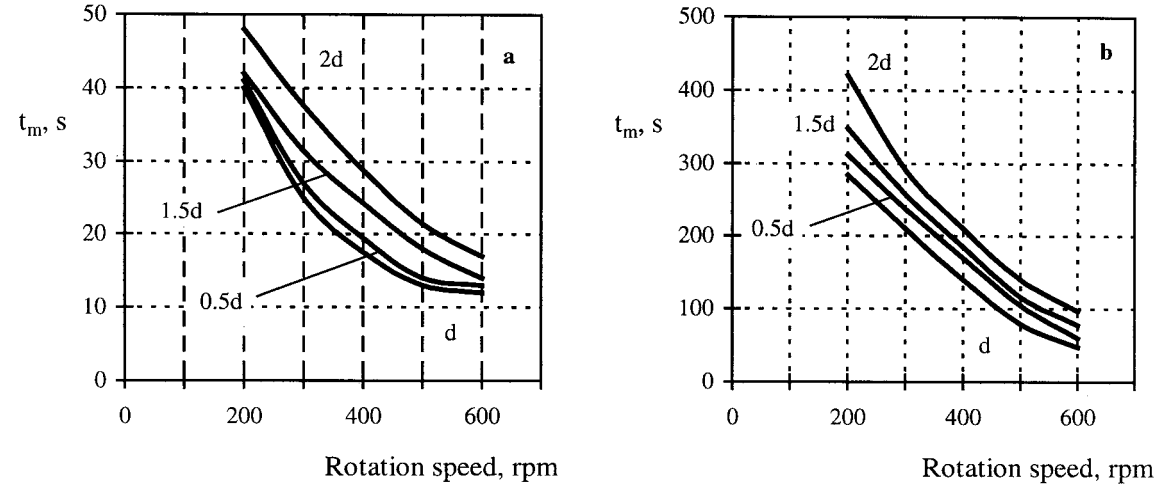

Figure 6. The influence of rotation speed on mixing time for Saccharomyces cerevisiae suspensions (a – Cx = 43 g dm^{-3} d.w., b – Cx = 157.5 g dm^{-3} d.w.).

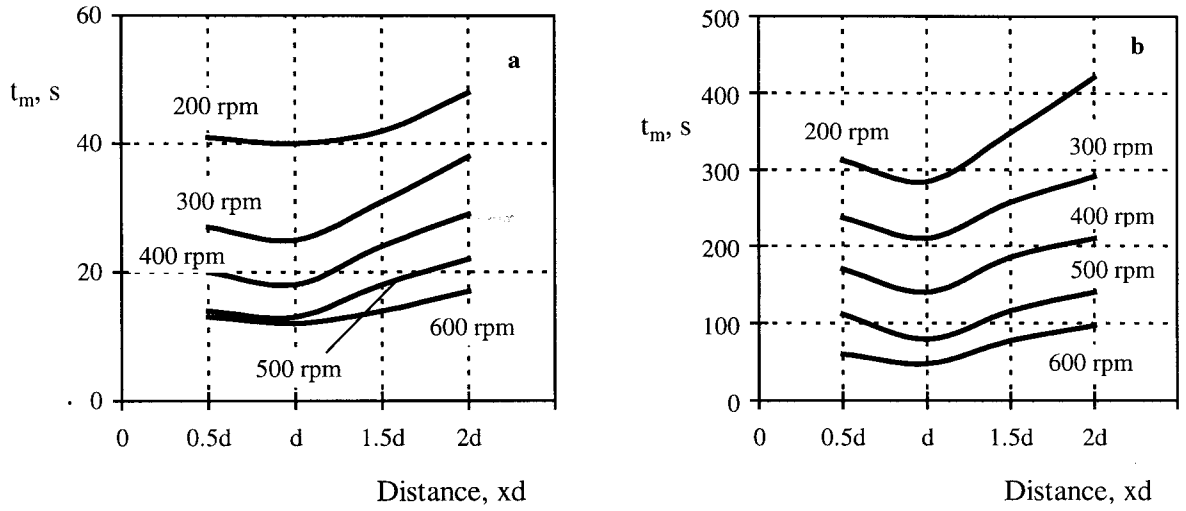

Figure 7. The influence of distance between the stirrers on mixing time for Saccharomyces cerevisiae suspensions (a – Cx = 43 g dm^{-3} d.w., b – Cx = 157.5 g dm^{-3} d.w.).

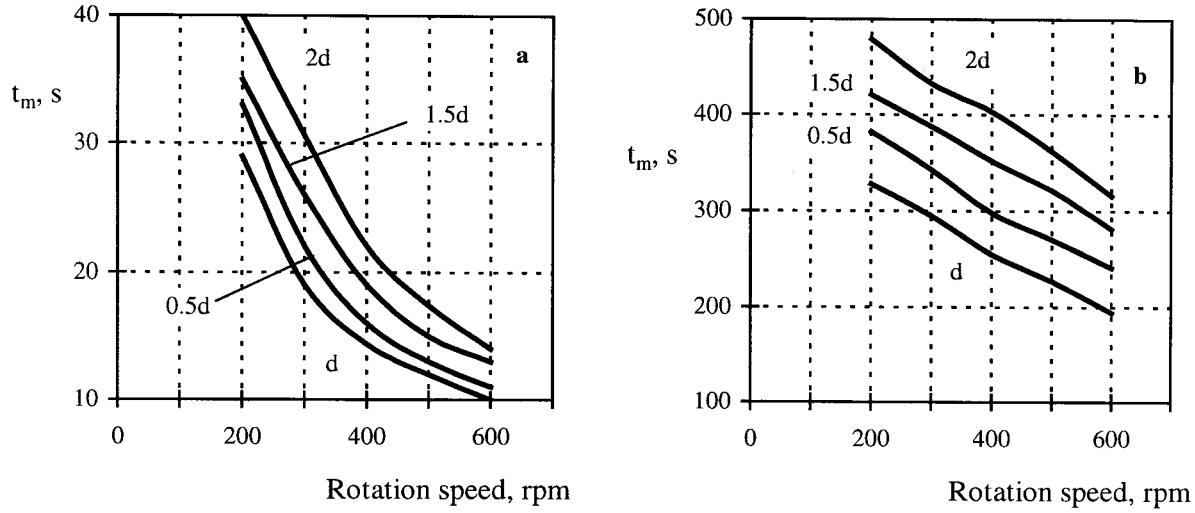

Figure 8. The influence of rotation speed on mixing time for Penicillium chrysogenum pellet suspensions (a – C_X = 5 g dm^{-3} d.w., b – C_X = 36.5 g dm^{-3} d.w.).

hyphal–hyphal interactions can influence the mixing efficiency.

Unlike bacteria and yeasts, fungi can grow in two morphological conformations: free mycelia and mycelial aggregates (pellets). Moreover, regardless of the morphological structure, the accumulation of fungus biomass induces a significant increase of the broth viscosity, but the magnitude of this influence depends on the fungus morphology. Thus, for the *P. chrysogenum* strains used in these experiments, the apparent viscosity of the suspension was of 172.5x10^{-3} Pa·s for free mycelia and 88.4x10^{-3} Pa·s for pellets, at a biomass concentration of 33.5 g dm^{-3} d.w. These differences are reflected in the values of the mixing times corresponding to the two morphological structures and are the result of the stronger hyphal–hyphal interactions for the filamentous conformation (Figures 8 and 10). The strength of these interactions correlated with the biomass concentration determines the rheological behavior and properties of fungus suspensions and, consequently, the mixing intensity.

Owing to the considerably higher viscosity of fungus fermentation broths, the values of the mixing time were significantly higher than those obtained for the other studied microorganisms. For example, for a biomass concentration of 33.5 g dm^{-3} d.w., 400 rpm and a distance between the stirrers of d, the mixing time was 460 s for free mycelia and 175 s for mycelial aggregates, compared to 14 s for bacteria and 16 s for yeasts.

For both fungus morphological conformations, it may be seen from Figures 9 and 11 that the order of the mixing time increase with the distance between the stirrers was the same:

$$d < 0.5 \cdot d < 1.5 \cdot d < 2 \cdot d$$

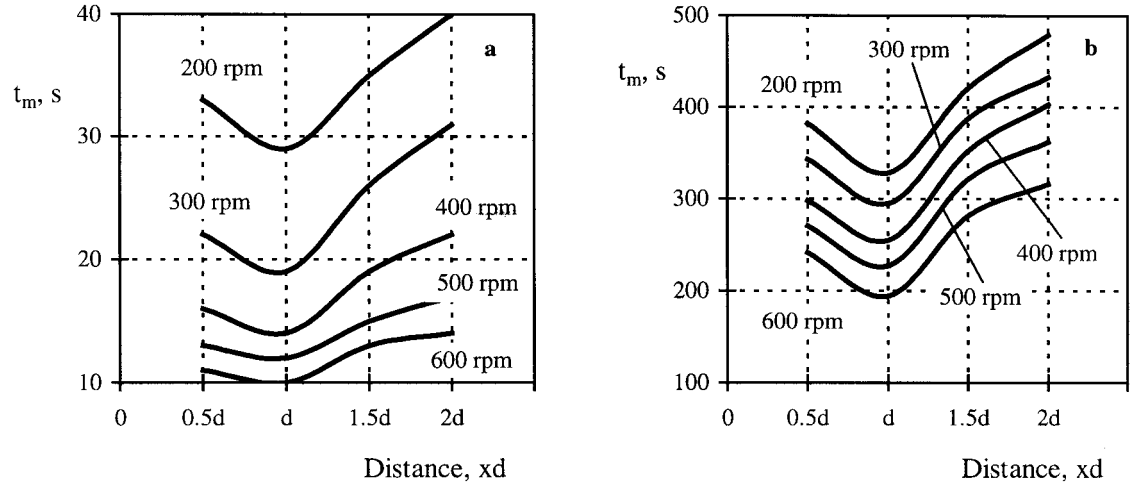

Figure 9. The influence of rotation speed on mixing time for Penicillium chrysogenum free mycelia suspensions (a – C_X = 4 g dm^{-3} d.w., b – C_X = 33.5 g dm^{-3} d.w.).

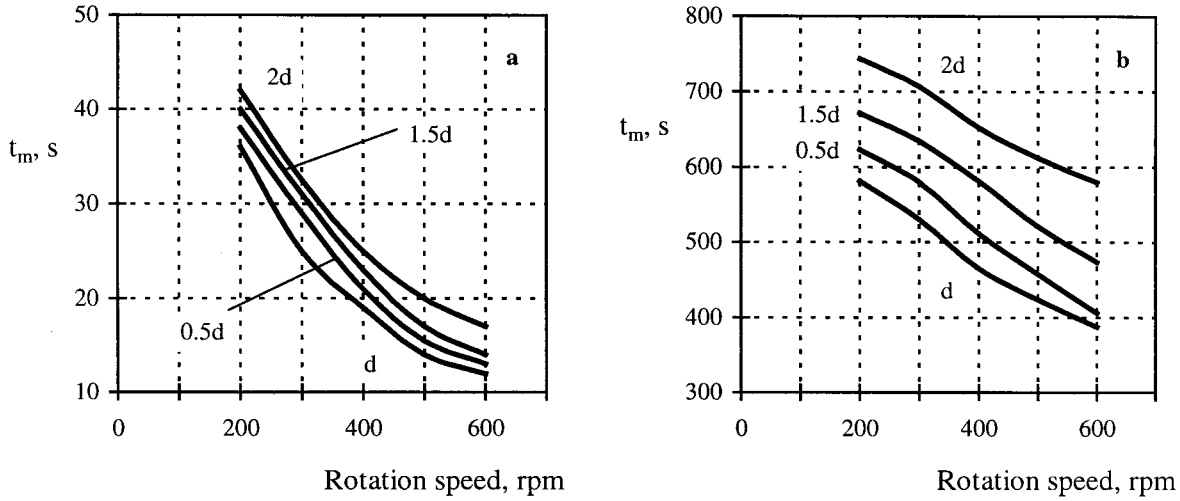

Figure 10. The influence of distance between the stirrers on mixing time for Penicillium chrysogenum pellet suspensions (a – C_X = 5 g dm^{-3} d.w., b – C_X = 36.5 g dm^{-3} d.w.).

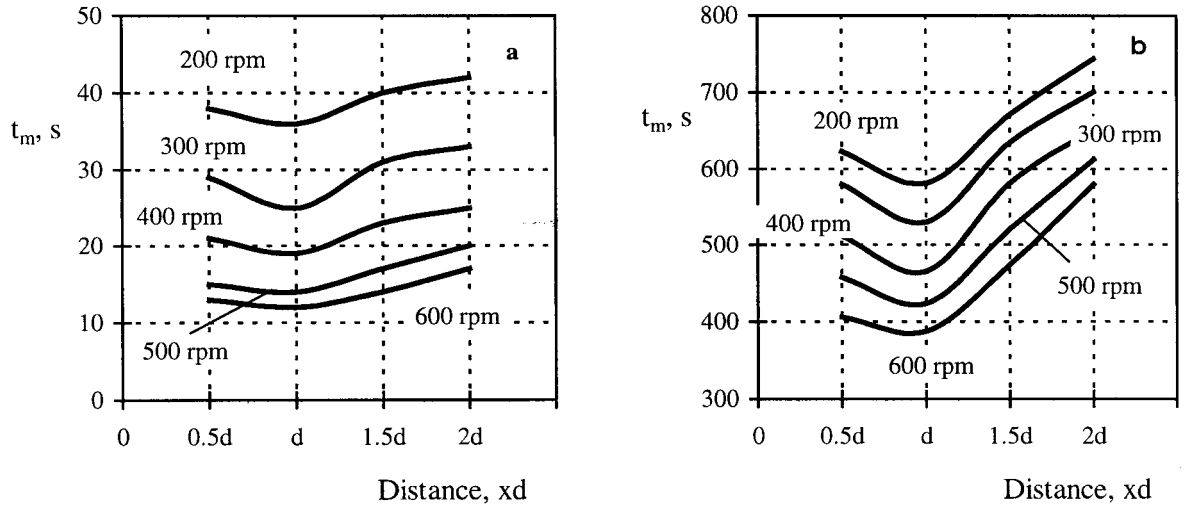

Figure 11. The influence of distance between the stirrers on mixing time for Penicillium chrysogenum free mycelia suspensions (a – C_X = 4 g dm^{-3} d.w., b – C_X = 33.5 g dm^{-3} d.w.).

Although for the same concentration level of biomass and operational conditions mixing is more diminished in systems containing free mycelia (the mixing time being about 2 – 3 times greater than for the pellet suspensions), pellet formation accelerates the deposition of biomass at the bioreactor bottom. For this reason, in the case of pellet suspensions, more pronounced differences between the values of the mixing time for various distances between the stirrers on the shaft were obtained.

Practically, for fungus suspensions, the presence of biomass affects the mixing intensity on the one hand by the high apparent viscosity of the broth, the effect being more important in the case of free mycelia, and on the other hand, by the accentuated tendency of solid phase deposition, the effect being more important in the case of pellets.

Correlations for the mixing time

For describing the influence of the apparent viscosity for simulated broths, microorganism concentration for biomass suspensions, rotation speed and distance between the stirrers on the mixing time, mathematical correlations for each considered system were established by analyzing the experimental data using the multiregression method.

For water and viscous liquids without biomass, owing to the different variation of mixing time, two types of equations are proposed:

a. for water or solutions with similar viscosity:

$$\frac{7.7}{t_m} - 1 =$$

$$= 1.4 \cdot 10^{-4} \cdot N \cdot \ln N - 22.8 \cdot L \cdot \ln L - 19.4 \cdot L^{0.5} \quad (2)$$

b. for viscous solutions (Re < 5,000):

$$t_m = 2.7 \cdot 10^3 \cdot \frac{\eta_a^{0.53}}{N^{1.25} \cdot L^{0.64}} \quad (3)$$

These models offer good concordance with the experimental results, the average deviations being 5.7% for water and 6.6% for CMCNa solutions.

A general expression of the proposed equations for biomass suspensions is:

$$t_m = \alpha \cdot \frac{C_X^{\beta} \cdot L^{\delta}}{N^{\gamma}} \quad (4)$$

where the coefficients α, β, γ and δ depend on the microorganism type and morphology. By means of the experimental data, the following correlations were established:

a. bacteria (Re < 25,000):

$$t_m = 9.92 \cdot 10^2 \cdot \frac{C_X^{0.98} \cdot L^{0.22}}{N^{1.17}} \quad (5)$$

b. yeasts (Re < 23,000):

$$t_m = 1.35 \cdot 10^2 \cdot \frac{C_X^{1.57} \cdot L^{0.28}}{N^{1.21}} \quad (6)$$

c. fungus: *free mycelia* (Re < 15,000):

$$t_m = 7.25 \cdot 10^2 \cdot \frac{C_X^{1.42} \cdot L^{0.18}}{N^{0.84}} \quad (7)$$

d. fungus: *mycelial aggregates* (Re < 20,000):

$$t_m = 1.59 \cdot 10^2 \cdot \frac{C_X^{1.39} \cdot L^{0.23}}{N^{0.64}} \quad (8)$$

The proposed models offer an average deviation of ±10.5% for *P. shermanii* suspensions, (6.6% for *S. cerevisiae* suspensions, ±8.5% for *P. chrysogenum* free mycelia suspensions and ±4.5% for *P. chrysogenum* pellet suspensions.

For each microorganism studied, the relative importance of the considered factor influence on the mixing efficiency is suggested by the values of the coefficients from the corresponding equation. Thus, from the proposed equations it may be observed that the influence of the biomass concentration is greater in the case of yeasts and fungus broths. However, for fungus cultures and both morphological structures, owing to the higher viscosity of the suspensions, the rotation speed effect is less important compared to bacteria and yeast broths.

The distance between the stirrers becomes an important factor in the case of suspensions with a pronounced tendency of deposition, such as bacteria, yeasts and fungus pellet cultures.

Contrary to the simulated broths, an increase of the distance between the inferior and the superior stirrers reduces the mixing intensity of broths containing biomass, the exponent of the term L from equations (5)–(8) being positive. The position of the stirrers on the shaft must avoid the formation of a stagnant region, in the case of simulated broths, or the deposition of solid phase, in the case of biomass suspensions.

Compared to models from the literature, models set, generally for Re > 10,000, the proposed equations are adequate for transitory and low turbulent flow regimes which are preferred in industrial scale fermentation, because they avoid mechanical damage of the biocatalysts. The proposed equations can be used for fermentation scale–up, by means of the mixing time criterion, for anaerobic mechanical stirred bioreactors provided with a double impeller.

CONCLUSIONS

In the case of stirred bioreactors with a multiple impeller, the experimental results indicated that, for water or liquids with low viscosity, the distance between the stirrers assembled on the shaft must take into account the interference of the stirrer flow lines, and, for viscous liquids, the position of the stagnant region in the bioreactor. These conclusions are valid for fermentation broths which contain no solid particles (cells or other free or immobilized biocatalysts, substrate).

Compared to the simulated broths, the presence of biomass in the fermentation broths considerably reduces the mixing efficiency, even at low viscosity levels of the suspensions. The magnitude of this effect depends on the nature, concentration and morphology of the biomass. The experimental studies concerning the mixing of bacteria, yeasts and fungus non–aerated suspensions emphasized that the value of the mixing time increases as follows:

bacteria < yeasts << fungus pellets <
< fungus free mycelia.

Furthermore, contrary to the mixing of simulated broths without biomass, for increasing the mixing efficiency of biomass suspensions, the stirrers must be placed at the bottom of the shaft to avoid solid phase deposition.

The mathematical correlations established by means of the experimental data describe the dependence between the mixing time and the considered factors (apparent viscosity, biomass concentration, stirrer rotation speed, distance between the stirrers) and offer good concordance with the experimental data, being useful for fermentation systems scale–up.

NOTATIONS

C_X – biomass concentration, g dm^{-3} d.w.
d – stirrer diameter, m
L – distance between the stirrer, m
N – rotation speed, rpm
P – power consumption for mixing non–aerated broths, W
Pa – power consumption for mixing aerated broths, W

Re – Reynolds number $Re = \frac{N \cdot d^2 \cdot \rho}{\eta_a}$

t_m – mixing time, s

V_a – volumetric air flow rate, $m^3 s^{-1}$

V – volume of medium, m^3

η_a – apparent viscosity, Pa·s

ρ – liquid or suspension density, $kg\ m^{-3}$

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IZVOD

PROCENA VREMENA MEŠANJA U BIOREAKTORIMA KOJI RADE POD ANAEROBNIM USLOVIMA

(Naučni rad)

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Vreme mešanja je jedan od najvažnijih kriterijuma kojim se definiše intenzitet mešanja fermentacionih medijuma pri povećanju razmere biorektora. Vrednost ovog parametra u velikoj meri zavisi od reoloških osobina fermetacione komine, koncentracije biomase i njenih morfoloških karakteristika, karakteristike mešanja i uslova pod kojima se izvodi fermentacija.

Ispitivanja koja su sprovedena u ovom radu imala su za cilj kvantifikaciju uticaja navedenih veličina na efikasnost mešanja u biorektorima sa meheničkim mešanjem sa dve mešalice. Rad bioreaktora je realizovan pod anaerobnim uslovima pri čemu su korišćene simulirane fermentacione komine sa suspenzijom bakterija (*Propionibacterium shermanii*), kvasca (*Saccharomyces cerevisia*) i gljiva (*Penicillium chrysogenum*) različitih prividnih viskoziteta ili koncentracija biomase. Na osnovu eksperimentalnih podataka i primenom multiregresione analize izvedena je korelaciona zavisnost koja definiše vreme mešanja u funkciji koncentracije biomase, rastojanja između mešalica i brzine rotacije osovine sa mešalicama. Predložene korelacione zavisnosti su primenljive za prelazni ili turbulentni režim (Re < 5,000) u slučaju medijuma korišćenih kao simulirane fermenatcione komine ili pri Re < 25,000 za medijume sa suspendovanom biomasom.

Ključne reči: Vreme mešanja • Biorekatori sa mehaničkim mešanjem • Simulirane fermentacione komine • Anaerobne kulture • Matematički modeli •

Key words: Mixing time • Stirred bioreactor • Simulated broths • Anaerobic cultures • Mathematical models •