Modeling of Mixing Time for Bioreactors with Self-Inducing Impellers

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Abstract:

The mixing time is one of the most useful criterions for characterization of the broths mixing intensity and for scaling up of biosynthesis processes. This parameter value depends mainly on the presence of biomass in the broths, mixing system characteristics and operating conditions. For quantifying the influence of these factors on mixing efficiency, this study was carried out for simulated fermentation aerated broths consisted of carboxymethyl cellulose sodium salt (CMCNa) taking into account the broth's viscosity, rotation speed (rpm) and the stirrer geometry. The experimental results were used in obtaining a correlation to calculate the mixing time for Re <5000, domain of flow regime for which only few data are available from literature.

$$t_m = 1.6 x 10^3 x \frac{\mu_a^{0.43}}{N^{1.13} \ x \ L^{0.53}}$$

This model offer a good concordance with the experimental results, the average deviation being of 6.6% for CMCNa solutions and can be used for fermentation scaling up.

Introduction

The accumulation of biomass or biosynthesized product (extra cellular polysaccharides, protein molecules) lead to the continuous modification of the medium physical properties as well as the appearance of the heterogeneous region in the bioreactor .In these conditions ,one of the most important problems which must be solved is to establish the optimum hydrodynamic regime for the bioreactor.

The mixing time represents one of the most useful criterions for characterization of 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 the completely segregated situation*. This parameter depends on multitude of geometry (dimension of mixing system, dimension of bioreactor) and technological factors (fermentation conditions, medium composition physical characteristics of the medium). The general correlation which describes the mixing time being of the following type (1).

$$t_m = f (N, \mu, \rho, V_a /V, \ P, P_a)$$

The experimental measurement of mixing time uses the tracers (acidic, alkaline or salts solutions, heated solution, colored solutions) which are

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added to the homogenized broths. The mixing time is the time needed to the considered parameter, such as pH-value, to reach a constant value, as shown in Fig. (1). An alkaline is added to the liquid as a tracer pulse and the system can be regarded as completely segregated at t=0. As it 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.

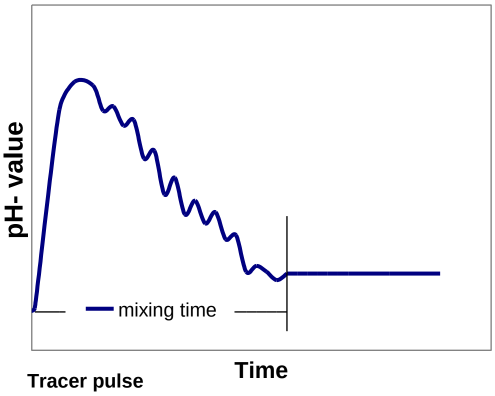

Fig. (1): Experimental method for mixing time.

For calculation of mixing time, numerous equations have been proposed in literature taking into account the type of formation (aerobe or non-aerobe), the rheological characteristics of the broths and the formation conditions (2-7) . For water or solutions with similar viscosity(2):

$$\frac{7.7}{t_m} - 1 = 1.4x10^{-4}\ \mathrm{N.lnN - 22.8.L\,.\,ln\ L - 19.4\,.L^{0.5}}$$

While for viscous solutions (Re <5000): $t_m = 2.7x10^3 . \frac{\mu_a^{0.53}}{\ln \mathrm{N.L^{o.6}} - 1.27}$

Because of the complexity of physical behavior of broths and of the particularities of formation systems, the accuracy of the proposed models of the particularities of fermentation system is very limited .Furthermore, the most of these models can predicted the mixing time of value for Re>10000 this flow regime being rarely reached in the large –scale bioreactor. For Re <1000, these models need some correction.(2).

For these reasons, the aim of our experiment is to analyze the dependence between the mixing time , the physical characteristics of broths and the fermentation condition for stirred bioreactor in the region Re <5000, domain of flow regime for which only few data are available from literature .

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Materials and Methods :

The fermentor used in this work has a working capacity of 50 liters of liquid Fig.(1.a). The fermentor tank, the central draft tube, the aeration wheel disk and hub were made of stainless steel. The air ducts were also stainless steel tubes, which were welded to the disk. The assembly was tinned and the hub was turned to fit a vulcanite sleeve in the bottom guide bearing. The shaft was made of ¾ inch stainless steel pipe. The cap for the introduction of air was bored to a tight running fit.

The bioreactor mixing system consists of single or two turbines stirred with six evacuated bending blades Fig.(1.b). The stirrer's diameter (according to standard configuration) is of 84 mm and the length and height of blades of 18 mm and 8mm, respectively. In the experiments where a single stirrer was used, the turbine stirrer was placed at 40 mm from the bioreactor bottom. The second turbine stirrer, in the experiments with two stirrers, was placed at a distance varying between 42 and 168 mm (0.5d and 2d) from the first one. The rotation speed was maintained between 50 and 1000 rpm.

In the experiments, water and simulated fermentation broths have been used. The simulated broths consisted of carboxymethyl cellulose sodium salt (CMCNa) which having the apparent viscosity in the domain of 6.2-94 cp. The viscosity was measured before and after each experiment using a viscometer of Ostwald type. Both the experiments and viscosity measurement were carried out at a temperature of about 25 °C.

The values of mixing time have been determined by means of solution of 10% KOH as a tracer, by recording the time needed to the medium pH value to reach a constant level at whole in the fermentor. The tracer volume was of 15 ml, the tracer being injected at 10 mm from the liquid surface. The pH variation was measured at four different locations inside the bioreactor.

The parameters studied were the medium viscosity, rotation speed (rpm) and the stirrer geometry.

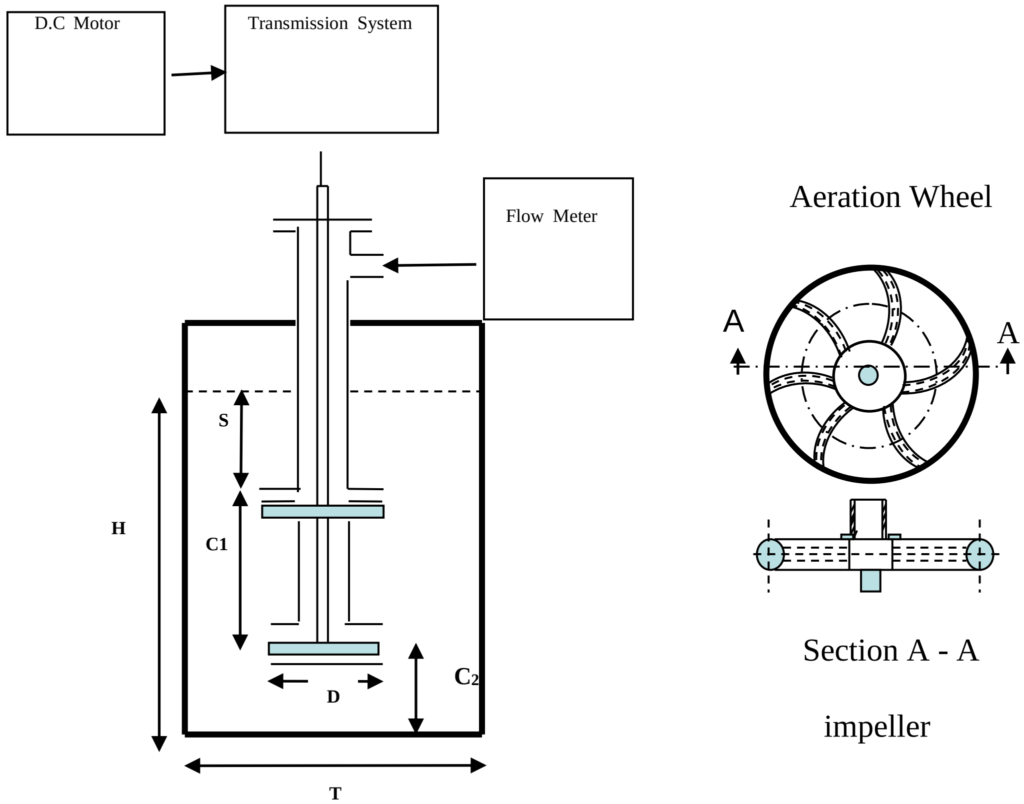

Fig.(1.a) Schematic diagram of the experimental system.
(T=50 cm, D=18cm, C1=(5 – 15) cm , C2=4 cm , S=10 cm, H=60 cm.)

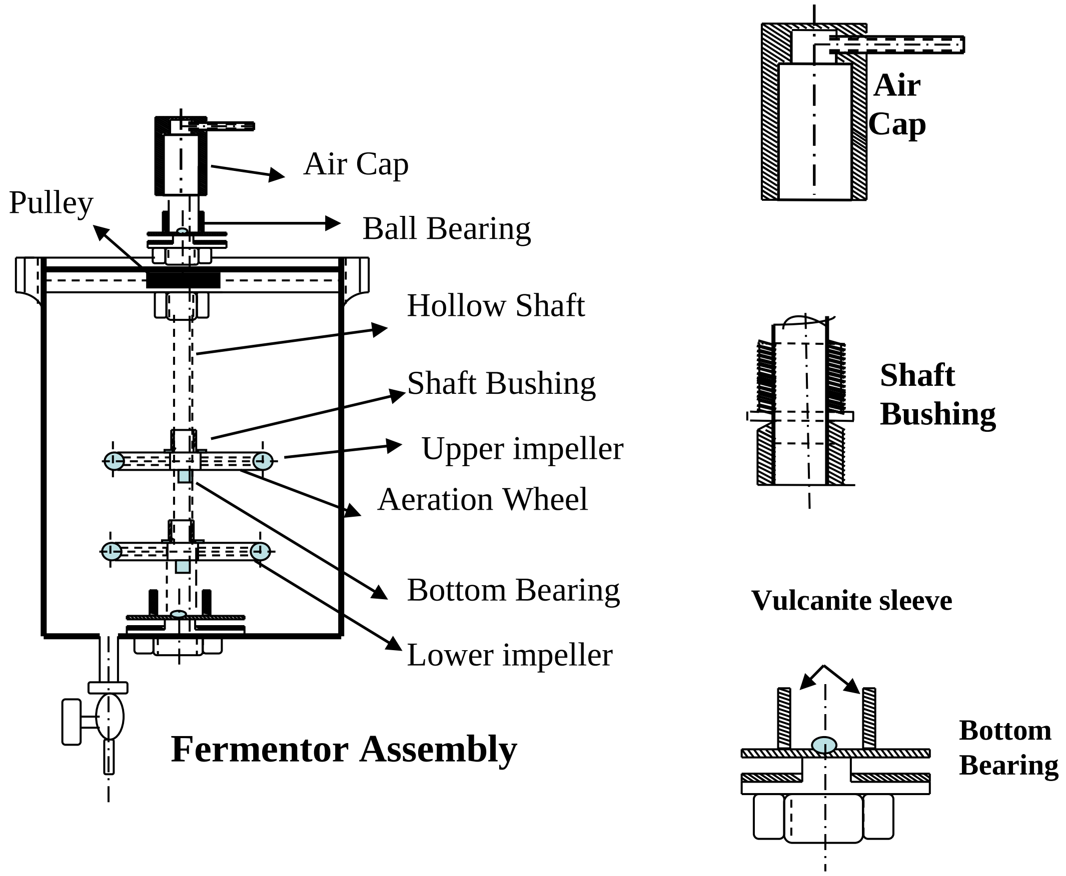

Fig.(1.b) Laboratory Experimental Fermentor with Mechanical Aerator .

Results and Discussion

The increase of broths viscosity, as the result of the biomass or biosynthesized product accumulation, leads to the increase of the mixing time. But, the effect of viscosity must be related to the geometrical and the functional characteristics of the bioreactor.

Initial, for establish the cumulated influence of these factors on the mixing intensity, the values of mixing time obtained for water and CMCNa solutions with different viscosities were comparatively analyzed, in the absence of solid phase, for a stirred bioreactor provided with a single and two stirrers, respectively.

1. Single stirrer system

The use of a single stirrer in this bioreactor determines a low level of mixing intensity, phenomena which is suggested either by the high value of mixing time or by the formation of a stagnant region at the top of the bioreactor (for an apparent viscosity of 66 cP and Re=5, the volume of the stagnant region was of about 65 -68% of the total volume of liquid).

As it can be observed from Fig**.** 2, indifferent of the liquid viscosity value, the mixing time is strongly reduced with the increase of rotation speed, then remaining at a constant level. This constant value indicates the maximum mixing intensity that can be reached in this system for a certain fermentation broths.

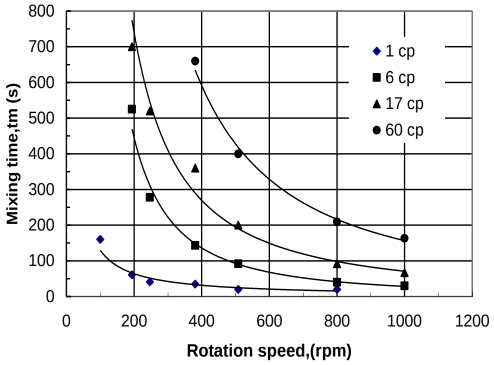

Fig.(2) Influence of viscosity and rotation speed on mixing time for single stirrer system .

The minimum values of mixing time as well as the corresponding rotation speed value are controlled by viscosity. Thus the minimum mixing time varied between 15 s for water and 160 s for a solution of CMCNa with an apparent viscosity of 60 cp, values obtained for 450 rpm for water and at 1000 rpm for CMCNa solution respectively. The differences due to the increase of viscosity become more significant at low rotation speed values. Practically below a rotation speed level of 150-200 rpm, the mixing time for CMCNa solution is extremely high. This phenomenon is the result of the appearance of stagnant region in which the tracer diffusion is possible mainly by molecular mechanism. For this reason, the results from this domain of mixing intensity have an unsatisfactory precision.

In fact for lower value of mixing time for fermentation broths with apparent viscosity over 50 cp the rotation speed over 500rpm is required, this level of viscosity being reached as early as the first steps of microorganisms growth at industrial scale .But, this rotation speed level can create shear forces or variety of other mechanical forces due to collision with the agitator or other objects in the bioreactor which can damage the cells or other free or immobilized biocatalysts.

2- <u>Tow stirrers system</u>

For increasing the efficiency of mixing process, the bioreactors are provided with multiple agitator system which consists of two or more identical or different stirrers assembled on the same shaft, the number of stirrers being in function of the broths height in the vessel. The correct position of the stirrers on the shaft represents a determinant factor of the mixing efficiency in these systems. The distance between stirrers controlled the interactions with other stirrers, its optimum value being depended on the nature and viscosity of fermentation broths. Literature indicates that the optimum distance between two stirrers is of (1/2) d (i.e. half stirrer diameter), the correct position can generating an efficient mixing, due to the following reasons (8):

a. The interference of flow streams by the adjacent stirrers, especially for small distances between the stirrers.
b. The formation of non-agitated regions between the adjacent stirrers, as a result of two long distances between them.

As it can be seen from Fig. (3), indifferent of the liquid viscosity, the mixing time strongly decrease with rotation speed, tending to a constant value depending both on viscosity and on distance between the stirrers. Owing to its low viscosity, the constant value of mixing time for CMCNa solution with μ=15.2 cp is reached at a rotation speed domain of 300-400 rpm. The lowest value of mixing time is of 20 s which was obtained for 300 rpm and a distance between the stirrers of 2 d.

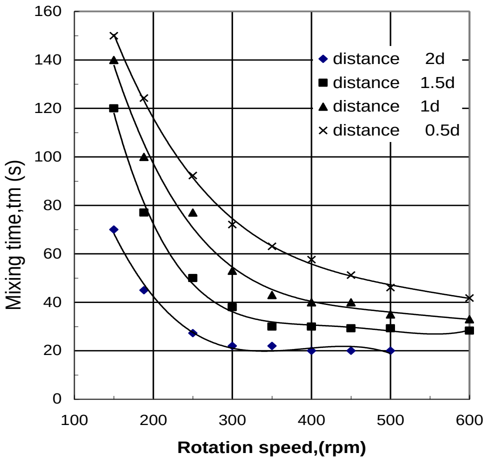

Fig.(3) Influence of rotation speed on mixing time for two stirrers system (CMCNa solution with μ=15.2 cp)

Figures (4-6) show that by increasing the liquid viscosity, it was observed the increase both of the rotation speed corresponding to the constant level of mixing time and of the final value of mixing time. For apparent viscosities greater than 20 cp, the constant level of mixing time can not be reached even at a rotation of 1000 rpm, this effect being more pronounced at distances below 1d between the two stirrers.

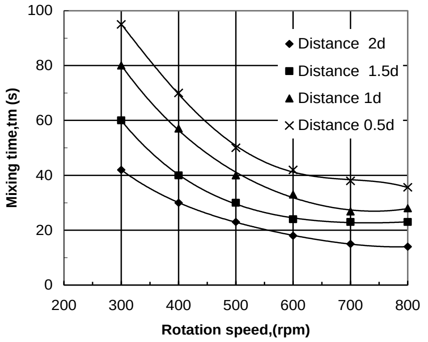

Fig. (4) Influence of rotation speed on mixing time for two stirrers system CMCNa solution with μ=25 cp).

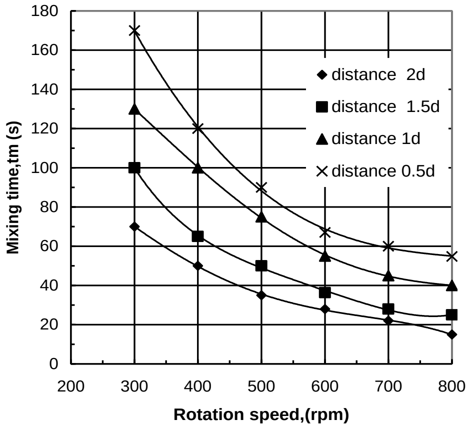

Fig.(5) Influence of rotation speed on mixing time for two stirrers system (CMCNa solution with μ=58.7 cp).

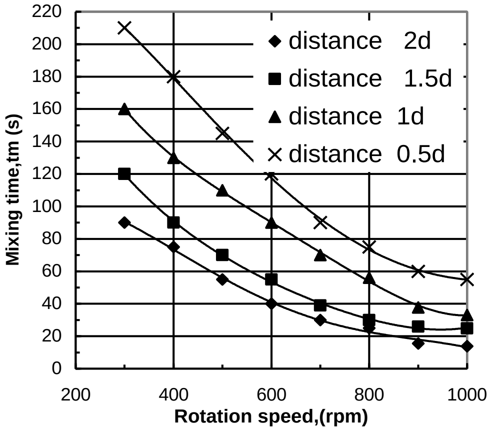

Fig.(6) Influence of distance between the stirrers on mixing time for two stirrers system.(CMCNa solution with μ=15.2 cp).

As it was expected, in all these situations, the results indicated that the mixing intensity for the two stirrers system is superior to that obtained for the single stirrer system, the difference becoming more significant with increase of apparent viscosity.

For the considered system, this evolution is specific to the liquids with higher viscosity than water and it is due to the reduce of the volume of the superior stagnant region by outdistancing the stirrers. In this case, the presence of the stagnant region controls the 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 the viscous liquids, the low mixing intensity is mainly the result of the stagnant region formation and less the effect of the interactions between the flow streams.

Mathematical correlation for mixing time

For establishing mathematical correlations between mixing time, apparent viscosity, rotation speed and distance between the stirrers, the experimental data for the two stirrers system were analysis using the multiregression method. Owing to the different variation of mixing time for CMCNa solutions, the equation has been proposed is:

$$t_m = 1.6 x 10^3 x \frac{\mu_a^{0.43}}{N^{1.13}\ x\ L^{0.53}}$$

For (Re < 5000)

Which gives an average deviation of 6.6% for CMCNa solutions.

This models offer a good concordance with the experimental results than that published by (Ashwin et al.)(2):

$$t_m = 2.7 x 10^3 . \frac{\mu_a^{0.53}}{\ln N . L^{o.6} - 1.27}$$

Compared with the models from literature (2-5), models settled, generally, for Re > 10000, the equation proposed for viscous solution is adequate for Re < 5000, the flow regime which is preferred at industrial scale fermentation, because it avoid the mechanical damage of biocatalysts.

A mathematical model which describes the experimental data of mixing intensity through mixing time was developed on a PC computer using MATLAB facilities in which the difference between the experimental and modeled value being reduced to a minimum. By means of a MATLAB program the regression coefficients and standard deviation were calculated.

Conclusions:

In the case of stirred bioreactors with multiple agitations, the experimental results indicate that for water or liquids with low viscosity, the distance between the stirrers assembled on the shaft has to take into account the interference of the stirrers 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).

The obtained equation permits the calculation of mixing time for aerated liquids, for a viscosity domain below 100 cp. Because this equation is adequate for Re < 5000, it represent a development of the models from literature for the transitory flow regime, regime which is specific to the large-scale bioreactor.

Notations:

d- Stirrer diameter, (m)
L- Distance between the stirrer, (m)
N- Rotation speed, (rpm)
P- Power consumption for mixing of non-aerated broths, (w)
P_a- Power consumption for mixing of aerated broths, (w)
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, (cp)
ρ- Density, (Kg $m^{-3)}$
cp- centi poise

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النمذجة الرياضية لزمن الخلط للمفاعلات الحيوية ذات الخلاطات الساحبة للهواء

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الخلاصة

يعد زمن الخلط احد اهم العوامل المهمة في تحديد كفاءة الخلط للاوساط الغذائية في المفاعلات الحيوية وكذلك في عمليات توسيع الانتاج. يعتمد هذا الزمن على طبيعة الوسط الغذائي والظروف التشغيليةللعملية التحضيرية وكذلك على الشكل الهندسي للخلاط المستخدم في عملية المزج. استخدم في هذا البحث ملح صوديوم كاربوكسي مثيل سليلوز كمادة مشابهة للوسط الغذائي ذات التخمر الهوائي وتمت دراسة تاثير كل من لزوجة الوسط الغذائي ، سرعة تدوير الخلاط والشكل الهندسي للخلاط على زمن الخلط . استخدم لهذا الغرض مخمر ذات الخلاط الساحب للهواء. تم تحليل النتائج العملية للبحث رياضيا باستخدام برنامج MATLAB بهدف الحصول على موديل رياضي يربط زمن الخلط مع العوامل المذكورة اعلاه في مدى من رقم رينولد اصغر من 5000(Re<5000) وهو المدى الشائع لتشغيل المفاعلات الحيوية للاغراض الصناعية.

$$t_m = 1.6 x 10^3 x \frac{\mu_a^{0.43}}{N^{1.13} \text{ x } L^{0.53}}$$

اعطت هذه المعادلة تطابق جيد مع النتائج العملية المستحصلة من البحث وبمعدل حيود 6.6%.