

THEORETICAL AND EXPERIMENTAL STUDY OF THE EFFECTS OF SCALE-UP ON MIXING TIME FOR A STIRRED-TANK BIOREACTOR

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Abstract - Mixing time is one of the criteria most widely used to characterize mixing intensity in bioprocesses. In bioreactors, mixing mainly depends on amount of energy consumed, reactor and stirrer shapes, airming speed and the rheology of the medium. In this work we experimentally determined the mixing times for a lab-scale bioreactor equipped with a stirrer propelled by two Rushton turbines. From these experiments we could obtain expressions to evaluate the effects of stirring speed, superficial gas velocity, specific power consumption and system geometry on mixing times under various flow regimes. The resulting correlations were employed to analyze the effect of scale-up on mixing times for the production of *Staphylococcus aureus* Smith.

Keywords: Stirred-tank bioreactor; Mixing time; Scale-up; *Staphylococcus aureus*.

INTRODUCTION

Mixing efficiency is one of the most significant factors, affecting both performance and scale-up in a bioreactor. Stirring is frequently decisive for the yield of fermentative processes and it is associated with many fermentator scaling problems. The effect of scale-up on a system's mixing efficiency affects its transfer processes. Therefore, concentration and temperature gradients as well as oscillations of pH, which cause substantial damage to the microorganisms, may occur. One of the parameters most widely used to characterize stirring in a bioreactor is mixing time, which is defined as the time a liquid needs to be stirred to obtain a specific degree of homogeneity after adding a pulse signal of a tracer.

There is information in the literature on how to predict mixing times for two-phase systems (liquid-gas) (Einsele and Finn, 1980; Van't Riet and Tramper, 1991; Vasconcelos et al., 1995). Most of the proposed correlations have been determined for turbulent-flow regimes ($N_{Re} > 10000$).

In this work the mixing time of a lab-scale stirred-tank bioreactor was experimentally determined, and the ways it was affected by operating conditions, such as stirring speed, superficial gas velocity and specific power consumption, were quantified. The resulting expressions, together with the results of previous work (Ducrós, 2003; Ducrós et al., 2001), were used to analyze the effect of scale-up on mixing times for the production of *Staphylococcus aureus* Smith. This strain is used in the formulation of vaccines

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developed to fight bovine mastitis, an illness that affects dairy bovine cattle (Calzolari et al., 1997).

MATERIALS AND METHODS

Installations and Equipment

The experiments were performed in a stirred-tank bioreactor (Applikon Instruments) with a 2.5 l

capacity, equipped with a six-blade stirrer propelled by two Rushton turbines with a diameter of 45 mm, and three baffles at 120° angles. The pH was measured with an autoclave-sterilizable Phoenix sensor with a Cole Palmer controller 29041-02. Air was introduced into the system through a distributor located under the stirrer. The details and dimensions of the fermenter are shown in Figure 1. The data were registered by means of a Linear 1200 recorder.

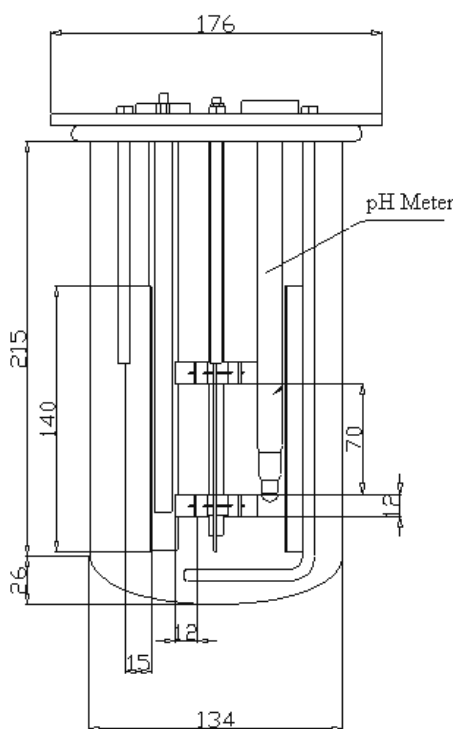


Figure 1: Diagrammatic representation of the bioreactor. (dimensions in mm.)

Experimental Conditions

The medium employed for all the experiments was distilled water. The effect of stirring speed in the range between 0.1667 and 13.33 r.p.s., which corresponds to a variation in Reynolds number of 0.337 to 27×10^3 , was studied. Stirring speed was controlled by means of an ADI 1012 controller (Applikon Instruments) that had an output signal in mA, which was proportional to the torque. This signal had been previously calibrated so as to evaluate the power of the stirring system.

The effect of airming speed was assessed using the following values for volumetric air flow per volume of medium: 0, 0.5, 1, 1.5, 2 v.v.m. Air flowrate was

registered by means of a mass flow meter AALBORG GFM17.

Experimental Determination of Mixing Times

Mixing time was experimentally determined using the pH-response technique (Van't Riet and Tramper, 1991). The tracer was a solution of hydrochloric acid (1 ml 3.9 M). It was introduced in pulses 10 mm from the liquid surface, and the variations in pH were registered as a function of time. Mixing time was determined using the homogeneity criterion based on the time required for the pH to reach $\pm 5\%$ of the final value. A typical experimental profile for the acid-pulse response obtained in this work is shown in Figure 2.

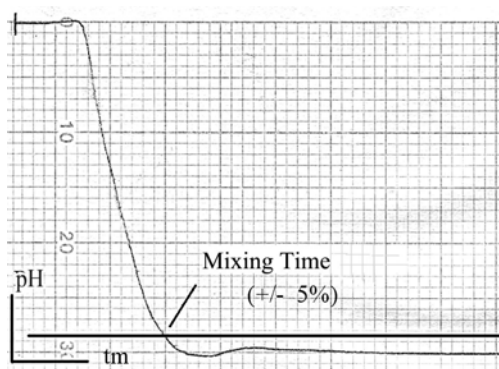


Figure 2: A typical curve for determination of mixing time, showing the response to an acid pulse

RESULTS AND DISCUSSION

Power Consumption

Experimental data obtained for power consumption as a function of Reynolds number, taking airting speed as a parameter, are shown in Figure 3. It can be noticed that, for laminar flow, the power number decreases as the Reynolds number increases, and the air flowrate does not affect the amount of power consumed. In contrast, in the case of turbulent regimes, the power number remains constant for systems without airting, and it decreases as airting increases.

Mixing Times

Experimental values for mixing times are plotted in Figure 4 as functions of stirring speed, taking volumetric air flow (expressed in v.v.m.) as a parameter.

It can be observed that the effect of airting on

mixing time depends on the reactor's operating regime. For low stirring speeds, which correspond to Reynolds numbers under 4000, the mixing time decreases as airting increases, while at high stirring speeds the effect is reversed with mixing time either increasing or remaining constant as the volumetric air flow increases. These results agree with those reported by Pandit and Joshi (1983) and Vasconcelos et al. (1995). These authors state that this phenomenon is produced by the opposing effects of the pneumatic and mechanical mixing mechanisms, respectively generated by the air distributor and the stirrer. For high stirring speeds (above 6.67 r.p.s.), the stirrer controls the flow regime so that any increase in superficial gas velocity negatively affects the mechanical stirring, without enough pneumatic stirring available to compensate for it. For low stirring speeds (below 2.08 r.p.s.), gas controls the regime, and the opposite effect prevails. In the range between 2.08 and 6.67 r.p.s., intermediate conditions can be observed with the negative effects of airting on stirring compensated for fairly well.

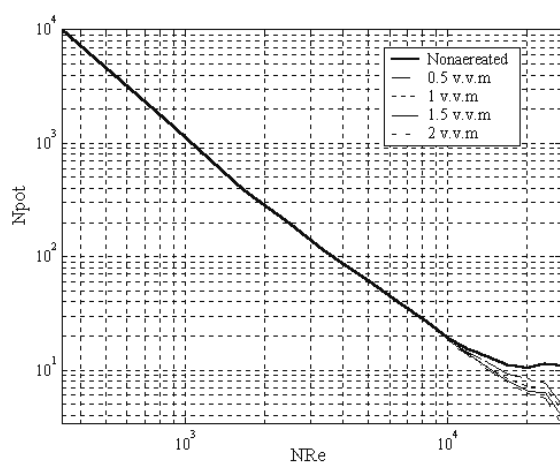


Figure 3: Power number as a function of Reynolds number

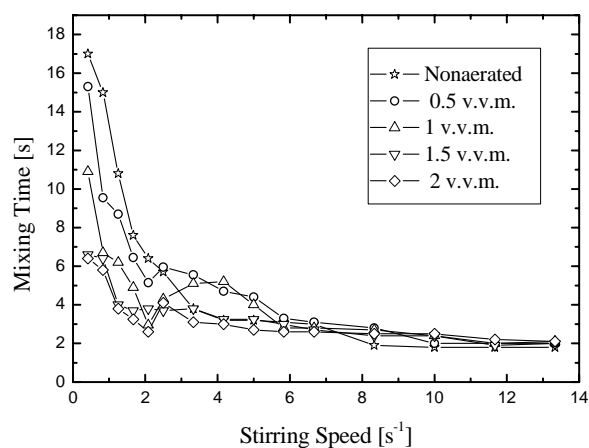


Figure 4: Mixing time as a function of stirring speed

Mixing-Time Correlations

Laminar Flow

The experimental data were adjusted by means of a regression method in order to describe the mixing time as a function of stirring and airming speeds for laminar flow. The proposed fitting model is shown in Equation (1).

$$t_m = \frac{c_1}{c_2 N \ln(N/60) + c_3} \quad (1)$$

the following values were found for the parameters:

$$c_1 = 21.451 \quad \text{Chi}^2 = 6.8 \times 10^{-3} \quad (2)$$

$$c_2 = -2.454 Q^2 + 1.62 Q + 0.216 \quad \text{Chi}_2 = 1.95 \times 10^{-6} \quad (3)$$

$$c_3 = 7.665 Q^2 + 5.084 Q + 0.805 \quad \text{Chi}_2 = 2.043 \times 10^{-1} \quad (4)$$

Finally, the resulting correlation for laminar flow may be expressed as given in Equation (5).

$$t_m = \frac{21.451}{\left(-2.454 Q^2 + 1.62 Q + 0.216\right) N \ln(N/60) + \left(7.665 Q^2 + 5.084 Q + 0.805\right)} \quad (5)$$

This equation holds for Reynolds numbers lower than 4000. In Figure 5 the model given by Equation (5) is represented in three dimensions. It can be observed that mixing time strongly depends on stirring speed. Besides, it is also clear that gas controls the regime because an increase in gas velocity causes a decrease in mixing time.

The correspondence between the theoretical mixing-time values estimated with Equation (5) and the experimental data is shown in Figure 6, where it can be observed that the percentage of deviation never exceeds 20%.

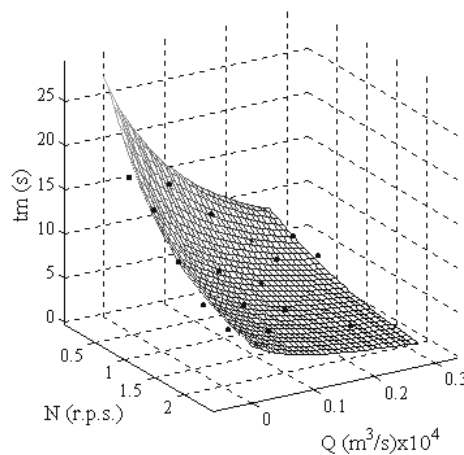


Figure 5: Correlation and experimental results for mixing time as a function of air flow and stirring speed Turbulent Flow

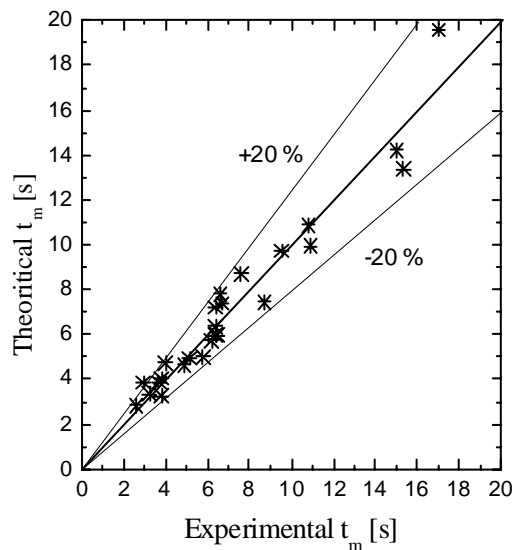


Figure 6: Comparison between experimental results and those of Equation (5)

For Reynolds numbers above 10000 and for aired systems, several equations to describe the dependence of mixing time on operating conditions, fluid properties and bioreactor properties have been proposed in the literature. One of the most frequently used is the equation proposed by Van't Riet (1991), whose general expression is

$$t_m = \left[a \frac{\rho_L D^5}{P_G} \left(\frac{D}{d_a} \right)^4 \right]^b \quad (6)$$

where $a = 216$ and $b = 0.33$.

The experimental results were analyzed by means of a MATLAB implementation of a nonlinear fitting method in order to set the values for coefficients a and b . The resulting expression is shown in Equation (7).

$$t_m = \left[6.08 \frac{\rho_L D^5}{P_G} \left(\frac{D}{d_a} \right)^4 \right]^{0.332} \quad (7)$$

In Figure 7, the theoretical mixing-time values obtained with Equation (7) are compared with the experimental values. Besides, the upper and lower bounds for a 20% error are shown.

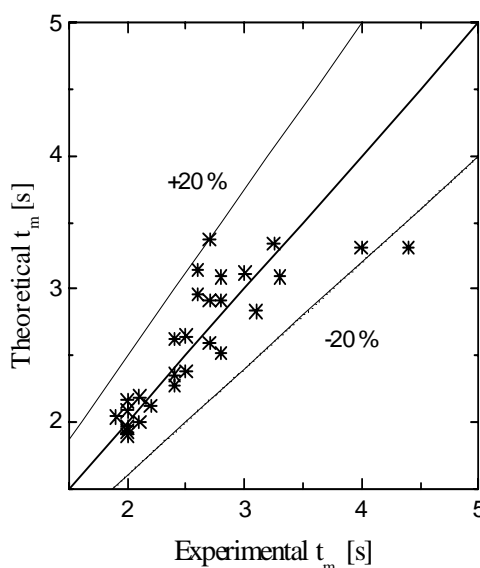


Figure 7: Comparison between experimental results and those of Equation (7)

Effects of Scale-Up on Mixing Times

Scaling up is a standard procedure for the design and construction of a large-scale system from experimental results gathered with small-scale equipment.

Analysis of scale-up for the production of strains of *Staphylococcus aureus* Smith and its capsular polysaccharide reported in a previous article (Ducrós, 2003; Ducrós et al., 2001) was taken as a basis to assess the effect of scale-up on mixing time. The same bioreactor and an identical geometric configuration were employed on that occasion. The analysis of production scale-up was developed following one of the criteria proposed by Hubbard (1987). According to this researcher, besides considering the geometric resemblance, the scale-up may be carried out by regarding the simultaneous constancy of both the k_{La} value and the volumetric air flow expressed in v.v.m. as additional criteria. The scale-up was performed for the following operating conditions: a stirring speed of 8.33 r.p.s. and a volumetric air flow of 1 v.v.m. The latter as well as the volumetric oxygen-transfer coefficient

$k_{La}=0.0012 \text{ sec}^{-1}$ was kept constant.

Whenever a change of scale of one or two magnitudes is carried out, as in the case under study, it is necessary to analyze how other important features of the bioreactor are modified (Humphrey, 1998). One of these characteristics is mixing time.

The variations in specific power and stirring speed with bioreactor size obtained by Ducrós (2003) are shown in Figure 8. Taking those results as the basis for the calculations and applying Equation (7), as obtained in this work, Figure 9, which shows the evolution of mixing time with bioreactor diameter, was plotted. From this picture it is possible to evaluate the effect of scale-up on mixing times.

For each diameter value shown on the horizontal axes in Figures 8 and 9, the real dimensions of the bioreactor were obtained by employing the geometric resemblance criterion and the dimensions of the experimental bioreactor already shown in Figure 1.

It can be observed that mixing time is a variable that would not cause problems in production scale-up because this parameter never exceeds 30 seconds, even for large diameters.

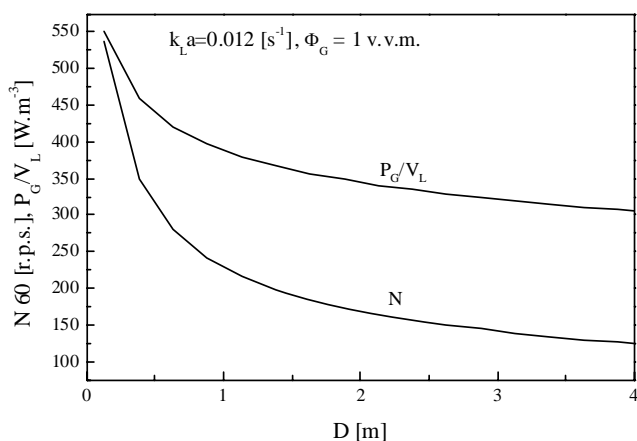


Figure 8: Specific power and stirring speed as functions of reactor diameter

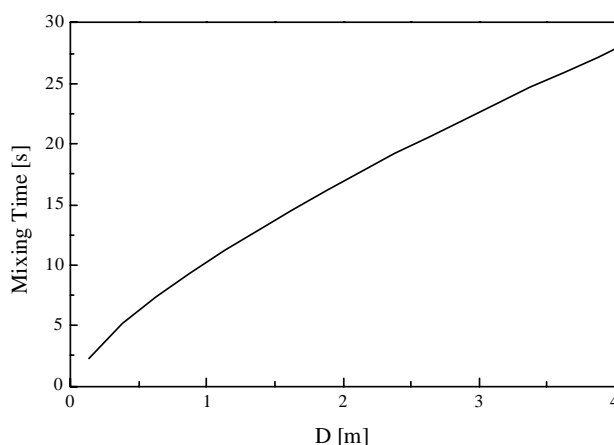


Figure 9: Mixing time as a function of reactor diameter

CONCLUSIONS

In this work we have presented an experimental study of mixing times in a stirred-tank bioreactor equipped with two Rushton turbines. The experiments were carried out for a wide range of stirring and airming speeds. This study enabled the determination of expressions to estimate mixing times for various flow regimes. Through analysis of the experimental data it was possible to observe that, for low stirring speeds (laminar flow), increases in airming speed resulted in decreases in mixing times, while at high stirring speeds (turbulent flow), the opposite effect prevailed. The expressions obtained in this work, together with the results from a previous article (Ducrós, 2003), allowed us to evaluate the effects of scale-up on mixing times for the production of *Staphylococcus aureus* Smith and its capsular polysaccharide. It can be concluded that this parameter would not cause problems in the scale-up process because it never exceeds 30 seconds for a bioreactor with a four-meter diameter.

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NOMENCLATURE

d_a	Stirrer diameter, m
D	Reactor diameter, m
N_{pot}	Power number
N_{Re}	Reynolds number
P_G	Power under airming conditions, W
Q	Volumetric air flowrate, m^3/s
N	Stirring speed, r.p.s.
t_m	Mixing time, s

Greek Letters

ρ	Medium density, kg/m^3
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