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Estimation of Bioreactor Efficiency Through Structured Hydrodynamic Modeling Case Study of a *Pichia pastoris* Fed-Batch Process

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Abstract

In this article, two theories are unified to investigate the effect of hydrodynamics on a specific bioprocess: the network-of-zones (NOZ) hydrodynamic structured modeling approach (developed by several researchers but applied to only a few bioprocesses) and the effectiveness factor η approach. Two process scales were investigated (20 and 500 L), and for each, hydrodynamics were quantified using an NOZ validated by homogeneity time measurements. Several impeller combinations inducing quite different hydrodynamics were tested at the 20-L scale. After this step, effectiveness factors were determined for each fermentation run. To achieve this, a perfectly mixed microbial kinetic model was evaluated by using simple Monod kinetics with a fed-batch mass balance. This methodology permitted determination of the effectiveness factor with more accuracy because of the relation with the perfect case deduced from the Monod kinetics. It appeared that for the small scale, η decreased until reaching a value of approx 0.7 (30% from the ideal case) for the three impeller systems investigated. However, stirring systems that include hydrofoils seemed to maintain higher effectiveness factors during the course of the fermentation. This effect can be attributed to oxygen transfer performance or to homogenization efficiency exhibited by the

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hydrofoils. To distinguish the oxygen transfer from the homogenization component of the effectiveness factor, these phenomena were analyzed separately. After determining the evolution of η_{O_2} linked to oxygen transfer for each of the fermentation runs, the NOZ model was employed to quantify substrate gradient appearance. After this step, another effectiveness factor, η_{mix}, related to mixing was defined. Consequently, it is possible to distinguish the relative importance of the mixing effect and oxygen transfer on a given bioprocess. The results have highlighted an important scale effect on the bioprocess that can be analyzed using the NOZ model.

Index Entries: Network-of-zones; effectiveness factors; oxygen transfer; mixing effect; gradient; impeller; homogeneity time.

Introduction

Microorganisms are extremely sensitive to environmental conditions such as pH level, substrate concentration, and dissolved oxygen (DO) level. These considerations led to the design of a stirred bioreactor for large-scale manipulation of microorganisms. Indeed, impeller systems tend to generate a high turbulence in the bulk of the stirred liquid and, thus, enhance transfer processes such as mass transfer, heat transfer, and kinetic energy transfer. Nevertheless, limitations to these actions appear when operating at large scale or with viscous broth.

Bioreactor efficiency is important for two reasons: first, to ensure a good homogeneity of the broth; and, second, to enhance the transfer efficiency such as oxygen transfer from the gas phase to the liquid phase and heat transfer. This article deals with the impact of chemical engineering parameters on the growth of a *Pichia pastoris* wild strain in a fed-batch stirred bioreactor. This microorganism is sensitive to both oxygen transfer and substrate homogenization (owing to the Pasteur effect at high substrate concentration). Traditional chemical engineering approaches involve the use of specific performance criteria in order to quantify the efficiency of these two phenomena: $k_l a$ for oxygen transfer and mixing time or homogeneity time for mixing efficiency. However, these performance criteria are not characteristic of the bioprocess because they are linked only with the physical performance of the stirring system and not with the dynamics of the process.

To quantify the efficiency of a bioreactor, a practical approach consists of employing an effectiveness factor, η (*1*). This factor represents the ratio of the reaction rate $r_{process}$ of the process to the reaction rate $r_{perfect}$ of a process without mixing and transport limitations:

$$\eta = r_{process}/r_{perfect}$$

When there is no problem associated with mixing or mass transfer, the biochemical reaction rate is limited only by the biologic system itself and $\eta = 1$.

In this article, we propose the use of a theoretical kinetics in order to fix the denominator of η at a reliable value. Indeed, the traditional approaches have not considered a real fermentation run to fix the reaction rate of the supposed perfect process. However, this run does not correspond to an ideal case because it is performed in real conditions and some uncertainties related to this assumption remain when calculating η.

When $\eta < 1$, mixing operation becomes limiting for the process reaction rate and problems owing to gradient appearance and oxygen transfer appear.

The process considered here is particularly sensitive to gradient formation and oxygen limitation. Indeed, *P. pastoris* is sensitive to glucose effects (shift from a respiratory to a fermentative metabolism, even in aerobic conditions, which lead to a lower cell yield), and fed-batch regulation must be tuned in this way. Nevertheless, the addition of substrate at the top of the bioreactor leads to gradient formation with an intensity depending on the mixing efficiency of the agitation system. We can thus describe the effectiveness factor as a function of two components:

$$\eta = f(\eta_{mix}, \eta_{O_2})$$

in which η_{mix} is the component of the η factor associated with the homogenization efficiency of the impeller system, and η_{O_2} is the oxygen transfer performance component of the impeller system. Distinction between the two components is important in order to orient the stirring system design and operating conditions.

At this stage of knowledge, we need reliable methods to predict mixing efficiency of a bioreactor. The simplest method consists of a mixing time measurement. This can be achieved by injecting a nonreactive tracer and following it in the vessel studied. However, experiments involving several probes are best for conducting homogeneity time measurements *(2).*

Improvements in structured hydrodynamic models have been accomplished. These models are based on tank-in-series concept in which the vessel is separated into several compartments that are individually considered perfectly mixed. Figure 1 gives an example of a fed-batch process. By using a traditional approach that consists of considering the vessel as perfectly mixed, the formation of gradients is neglected. It has been show that these gradients have a great impact on microbial kinetics *(3–6).* The structured approach takes into account the appearance of gradients along the vessel by breaking the black box (Fig. 1). With this approach, it is possible to see what happens inside the vessel and to follow several parameters at several locations. In the fed-batch process, it permits detection of the appearance of gradients along the vessel height. There has been great interest in this approach, and several variants can be found in the literature on the function of the type of impeller used (axial or radial), number of agitation stages, and number of perfectly mixed

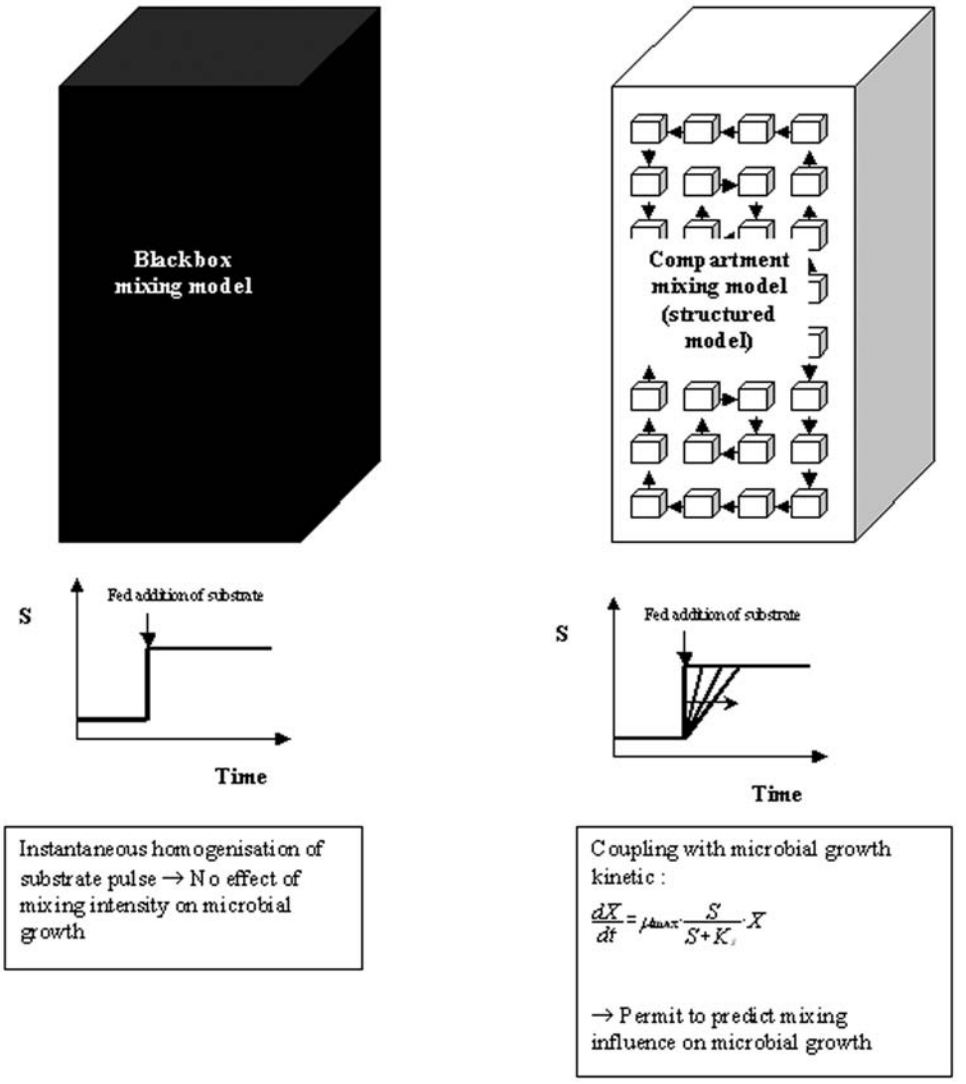

Fig. 1. Comparison of hydrodynamic black box (unstructured) model with structured NOZ model. The first model considers that a pulse of substrate fed to the top of the vessel is instantaneously mixed and, thus, substrate concentration is automatically homogenized after injection. The second model considers the internal dynamic of mixing by dividing the whole tank into a series of perfectly mixed elements. This second approach permit to consider substrate gradient appearance during a fed-batch process.

cells (this parameter being given in the case of a batch bioreactor). The complexity of the hydrodynamic model varies from considering single or a few perfectly mixed cells per agitation stage *(7–10)* to a network of interconnected cells per agitation stage *(11–13)*.

In the present, the efficiency of a fed-batch process involving cultivation a *P. pastoris* strain was studied. Several impeller combinations were tested

and fermentation at the pilot scale was also performed in order to investigate scaling effect. A network-of-zones (NOZ) model was constructed to estimate mixing efficiency for each of the agitation systems, and the oxygen transfer coefficient was followed during the fermentation runs. In a second step, effectiveness factor was calculated on the basis of the theoretical microbial curve obtained by the use of a Monod kinetics adapted to *P. pastoris* cultivation in fed-batch mode. Estimation of the effectiveness factor permitted determination of the appearance of mixing or transport limitations. A method to distinguish mixing efficiency from oxygen transfer efficiency based on the NOZ model, is discussed.

Materials and Methods

Experimental Strategy

During a first experimental step, chemical engineering experiments of homogeneity time were performed. The mixing curves obtained were used to validate the turbulent flow rate component (q_e) of the NOZ model.

During a second experimental step, several fermentation runs with a varying impeller system and scale were performed. The effectiveness factor was calculated as a function of time for each fermentation run.

At this level, NOZ simulations with fermentation stirring conditions permit quantification of the magnitude of heterogeneity. On this basis, it is possible to distinguish heterogeneity problems from oxygen transfer problems, and this distinction is useful for improving bioreactor efficiency.

Cultivation P. pastoris

P. pastoris CWBI F383 wild strain was grown on rich medium. Precultures were grown on 863 broth (20 g/L of glucose, 10 g/L of yeast extract, 10 g/L of casein pepton) in 150 and 500-mL shake flasks. The 500 mL flasks were used to inoculate fermentors.

Bioreactors

Stainless steel 20-L (D = 0.22 m) and 500-L (D = 0.62 m) strirred bioreactors (Biolaffite-France) were used. Temperature, DO, and pH were regulated by an automate (ABB). During culture, the pH was maintained at 5.5, temperature at 30°C, and DO as long as possible above 30% from saturation by varying stirrer speed (initial rotational speed of 150 rpm and maximum rotational speed of 450 rpm). Substrate (glucose) was fed at the top of the bioreactor with an exponential flow rate of $F = F_0 \cdot \exp^{ex \cdot (t\text{-}tstart)}$ (maximum flow rate, F_{max}). Off-gas analysis was performed using a CO_2 infrared analyzer (Servomex) and an oxygen paramagnetic analyzer (Servomex). Data were collected and used to compute the volumetric oxygen transfer coefficient ($k_l a$).

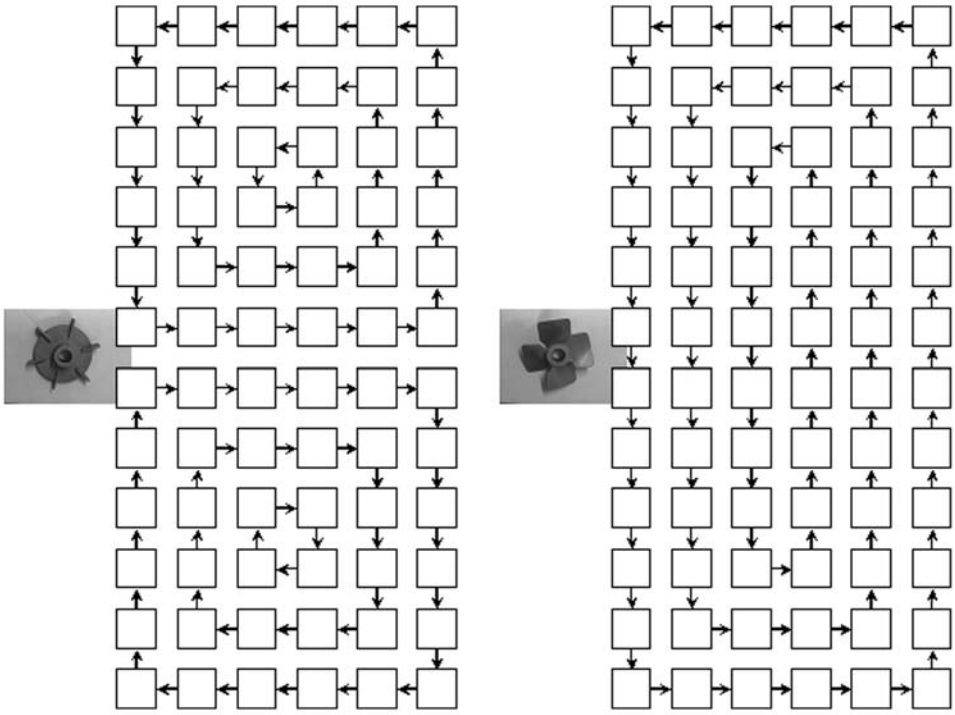

Fig. 2. Distinction between radial flow pattern (left) induced by rushton turbine and axial flow pattern (right) induced by A315 hydrofoil. Only the q_c circulation component is represented.

In the case of the 500-L vessel, only the TD6-TD6 stirring system was used. For experiments conducted in the 20-L vessel, several impeller combinations were tested (see Fig. 2 for details). The mixing efficiency of these impellers was previously tested in an experimental Perspex stirred vessel.

Microbial growth was followed by optical density (OD) measurements at a wavelength of 540 nm. Plate count and final dry matter measurements were also performed in order to convert OD measurements into grams of biomass per liter.

Experimental Vessel

Homogeneity time experiments were performed in a Perspex vessel (D = 0.24 m). Air is injected through a pipe sparger (2-mm holes). Two experimental fluids of varying rheologic properties were used: water and aqueous solutions of carboxymethylcellulose (CMC) (Fluka). CMC solutions were made at weak concentration in order to show the impact of a slight increase in the viscosity on the mixing process, as can be observed when operating with a high-cell-density fermentation such as a *P. pastoris* fed-batch process. A pulse of heated fluid (100 mL) was added at the top of the vessel near a baffle in order to produce a temperature fluctuation of approx 0.3°C. The temperature curves were recorded by several thermosensors disposed

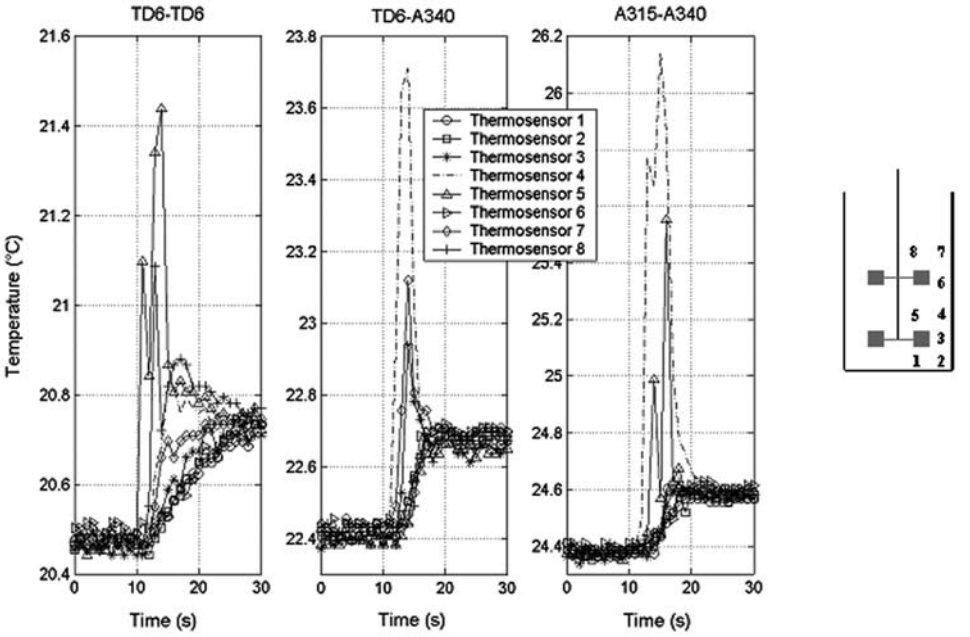

Fig. 3. Comparison of mixing curve obtained for different impeller combinations tested (working fluid: water; N = 270 rpm). On the right, the location of the thermosensors in the vessel is shown.

on the baffle. Data were recorded each second by a labview 3.1. station. Thermosensors are made of copper-constantan (response time of 0.45 s). Typical mixing curves can be seen in Fig. 3. Homogeneity time was calculated as described in the literature *(2)*. Mixing curves were used to adjust the parameter q_e of the NOZ model. Mixing time experiments were also performed in the 500-L vessel using the conductivity technique (Conducell electrode with an acquisition interval of 2 s).

Design of NOZ Mathematical Model

The model contains a constant number of perfectly mixed zones (72) per stirring stage. The number of zones is justified by a geometric network of 2-cm-sided cells. The adjustable parameters are the circulation flux between cells, q_c, and turbulence flow, q_e (this parameter also includes the pneumatic component of mixing in the case of aeration). The circulating flow is oriented depending on the global pattern generated by the type of impeller used (radial or axial), as can be seen in Fig. 2. The NOZ model consists of a mass balance on the perfectly mixed cells interconnected by circulating and/or turbulence flows. For a given zone n, the mass balance equation has the following form:

$$V \cdot \frac{dC_n}{dt} = Q_c \cdot (C_{n+i} - C_n) + Q_e \cdot (C_{n+i} + C_{n-j} - 2C_n)$$

It results in a set of ordinary differential equations (one for each cell or zone) that can be resolved numerically by a Runge-Kutta routine.

Turbulence flow, q_e is modeled by backmixing flow between each adjacent zone in the vertical direction. The value of this parameter is estimated by adjustment on the experimental homogeneity curve.

The circulation flow, q_c, is determined by using the following correlation, which comes from dimensional analysis:

$$Q_c = N_{qc} \cdot N \cdot d^3$$

For turbulent flow ($Re > 10{,}000$), circulation number, $N_{qc,}$ remains constant. In the case of an aerated vessel, the formation of gaseous cavities at the back of impeller blades induces a drop in pumping capacity of the agitation system. This drop is similar to a power drop and can be estimated by computing the aerated power using correlations found in the literature *(12,13).*

For transitional flow, the influence of viscosity on circulation capacity of the impeller must be taken into account. For the TD6 impeller, the Norwood and Meztner *(14)* correlation can be employed (this correlation was previously validated for several working fluids such as CMC and xanthan solutions [*12*]*).*

Results and Discussion

Chemical Engineering Experiments

The three impeller systems were first tested for mixing efficiencies. To achieve this, homogeneity time measurements were made as described earlier. As shown in Fig. 4, a radial impeller combination took longer to achieve a given degree of homogeneity (here, 85%); previous studies have shown similar results *(15).* This was owing to the compartmentalization effect induced by radial projection of liquid by the turbine. Replacement of the upper impeller with a propeller (axial) eliminated this effect and reduced the homogeneity time. The three impeller combinations tested showed different hydrodynamic behaviors, and mixing experiments highlight only the impact of these differences on the efficiency of homogenization. Indeed, analysis of typical mixing curves shows pronounced divergence between fluid mechanics induced by the impeller combinations (Fig. 3). Several investingators have interpreted these differences as an exchange flow between agitation stages, with this exchange flow more pronounced in the case of the axial impeller *(8).*

It can thus be concluded that the radial or axial behavior exerted by the impeller greatly influences the hydrodynamic differences induced by the balance between the recirculating and turbulence components of the impeller. This *a priori* knowledge will be exploited for elaboration of the NOZ model first by representing the circulation flows following

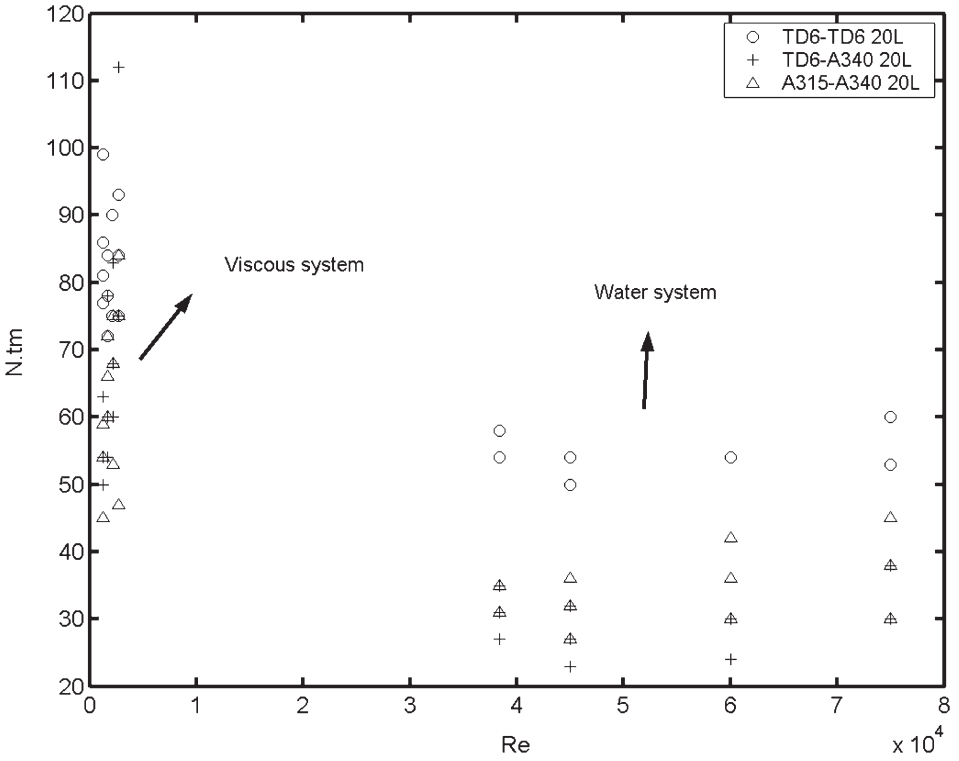

Fig. 4. Dimensionless plot synthesizing homogeneity time results obtained for water and CMC solution (viscous system) under varying operating conditions for the three impeller combinations tested.

a radial or an axial pathway, and second by considering a circulating component (q_c) and a turbulence component (q_e).

Values of homogeneity time were used to adjust the q_e parameter of the NOZ model for different operating conditions and for different impeller combinations. This model was used to operate simulations in order to estimate substrate gradient magnitude for each agitation system tested. To achieve this, the NOZ model was coupled with a simple Monod kinetics in order to take into account the impact of the substrate consumption rate.

Biochemical Engineering Experiments

The use of an effectiveness factor is an original way to investigate the impact of mixing operation on microbial processes. Referring to the definition of η, one can define the microbial kinetic corresponding to a perfectly mixed case with a stronger theoretical basis by employing a simple Monod kinetics coupled with mass balance equations corresponding to a fed-batch operation *(16).* Parameters related to calculation of the theoretical microbial kinetics are given in Table 1 and were obtained during a previous batch process. One thus obtains a theoretical microbial growth curve that

Table 1
Kinetic Parameters Used to Determine the Theoretical Microbial Growth Curve in Perfectly Mixed Condition for Calculation of η

μ_{max}	0.32 h^{-1}
K_s	0.025 g/l
m_s	0.013 h^{-1}
$Y_{x/s}$	0.42
S_a	370 g/l
Q_o	0.005 l/h
Q_{max}	0.25 l/h
exp	0.32
t_{start}	9 h

corresponds to the following ideal assumptions: the bioreactor is perfectly mixed, oxygen is in excess, and only the carbon source is limiting. Therefore, the effectiveness factor calculated on the basis of this theoretical growth curve is representative of the mixing and mass transfer efficiency of the apparatus chosen to achieve a given bioprocess. Figure 5 compares microbial kinetics obtained on a theoretical basis and those obtained during fermentation experiments. One can see that after the initial batch phase (10 h), there was a divergence from the ideal case for all mixing systems considered.

Figure 6 shows the evolution of the effectiveness factor along each fermentation run performed for different hydrodynamic conditions. After the initial batch step (the feed pump was only activated after 10 h), η tended to decrease. This can be attributed to mixing limitation, which is transduced by the appearance of substrate gradient and by oxygen depletion. We therefore need a new parameter to make the distinction between mixing and mass transfer phenomena.

The first step is to manage the oxygen transfer coefficient obtained by gas balance analysis in order to define an effectiveness factor related to mass transfer alone. We can thus define:

$$\eta_{O_2} = k_l a / k_l a_{perfect}$$

with $k_l a_{perfect}$ being the oxygen transfer coefficient ensuring no oxygen limitation during the culture. It can be easily calculated knowing that in a perfect situation, 1 g of oxygen must be used to assimilate 1 g of glucose. Taking into account the time and the volume of culture, we obtain $k_l a_{perfect}$ = 900 h^{-1}. Figure 7 shows the evolution of η_{O_2} for all the fermentation runs. Regulation of DO by controlling the stirring speed improved η_{O_2} (shift from 0.2 to 0.4 for the 20-L scale); this impact was less pronounced at the 500-L scale. Indeed, in this case oxygen because limiting faster than for the

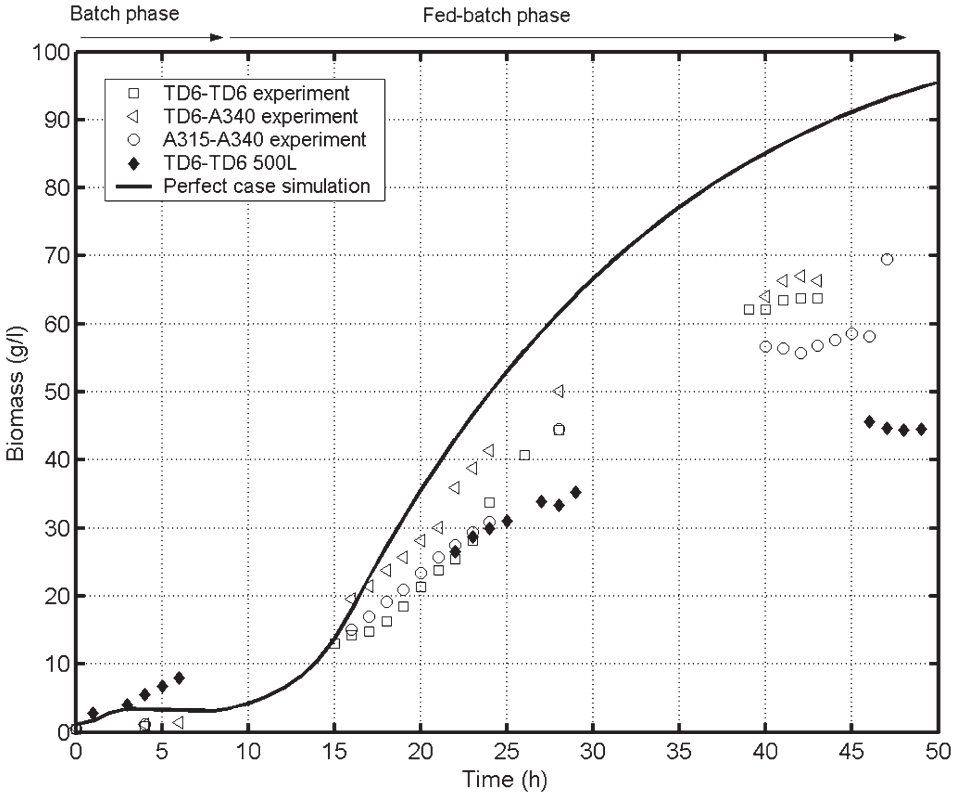

Fig. 5. Microbial growth curves obtained during fermentation runs at 20 L and 500 L scales and comparised with theoretical growth curve.

20-L vessel owing to the lower aeration capacity of the larger system, as can be seen in Fig. 7. It can also be seen that the TD6-TD6 system at the 20-L scale showed a drop in performance at 16 h. At this time, growth was exponential and the DO requirement was maximal. The drop recorded for the TD6-TD6 system was owing to an inability of the DO regulation system to react based on agitation speed (this phenomenon can be visualized at the top Fig. 7, which shows the stirrer speed evolution during the culture). In general, η_{O_2} remained at a low value and DO was irremediably a limiting factor of *P. pastoris* growth for high-cell-density cultures.

Computation of Effectiveness Factor Related to Homogenization Using NOZ Model

The effectiveness factor does not permit directly mixing effects to be distinguished from mass transfer effects. In our case, the mixing effect was very important because substrate was added continuously during the culture. We thus need a parameter to estimate mixing efficiency in the particular case of a fed-batch process. By using NOZ coupled with a simple Monod microbial kinetics, it is possible to evaluate glucose gradient in a

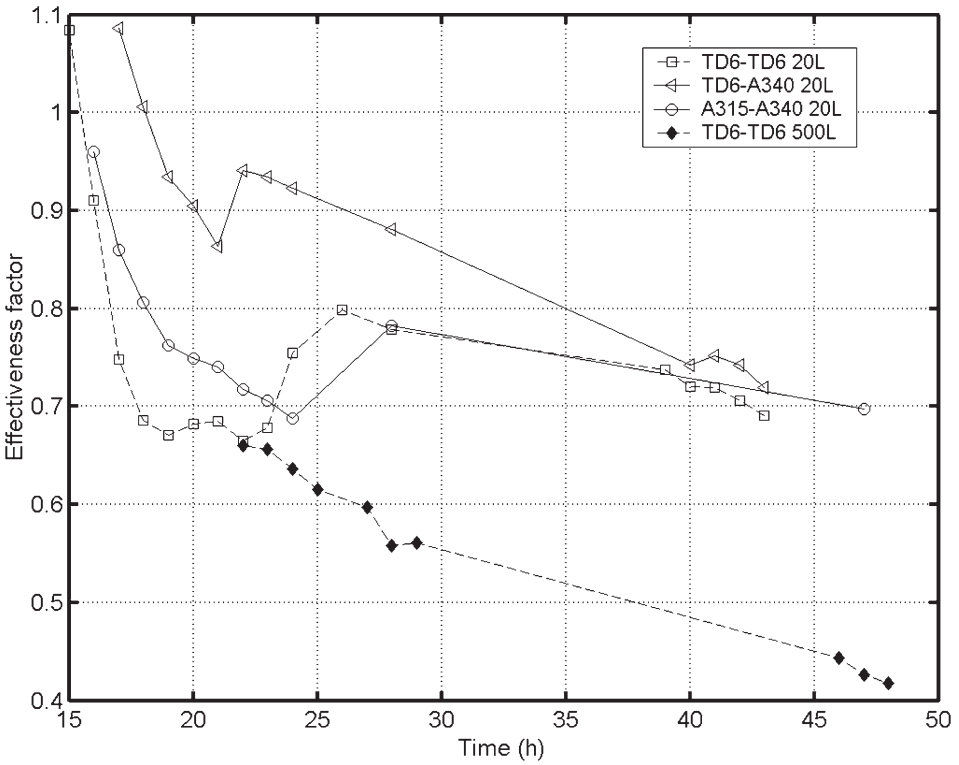

Fig. 6. Variation in effectiveness factor, η with time for each stirring system considered.

bioreactor. However, it is a purely subjective method, because the microbial growth is directly influenced by substrate repartition into the bioreactor. Indeed, if we take a given value of growth rate to compare the mixing efficiency of different apparatuses, the results do not depend on the microbial activity but are influenced only by the homogenization efficiency of the apparatus. This is an artifact created by the coupling of the NOZ and microbial growth models. To obtain a realistic model coupling microbial growth and fluid mechanics, we need an equation relating the influence of the fluctuation of substrate concentration on specific growth rate. This is a very ambitious task and is beyond the context of this article. We therefore focuse here on the fluid mechanics part of the problem.

The microbial kinetic can be coupled to the NOZ model, with the growth rate being the same for all the experiments. The results obtained depend only on the chemical engineering performance of the impeller systems and are not influenced by the microbial growth.

Simulations performed for each stirring system are shown in Fig. 8. It can be seen that systems including hydrofoils (A340 or A315) were more efficient in minimizing the substrate gradient inside the vessel. For all the simulations, the zone directly receiving substrate pulse was

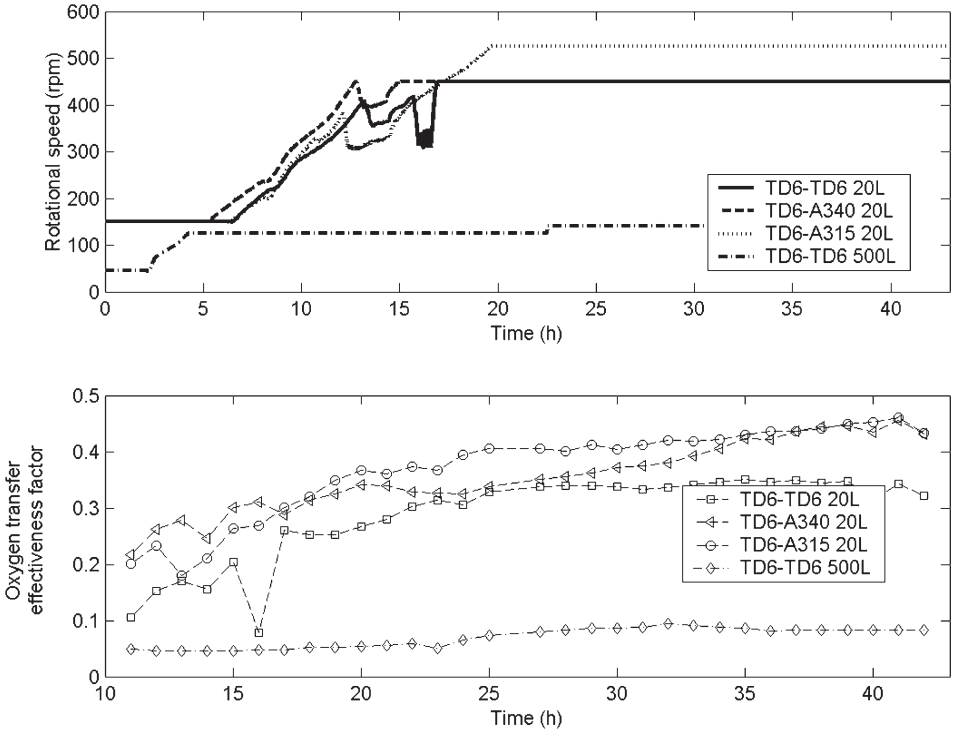

Fig. 7. Variation of the oxygen transfer effectiveness factor η_{O_2} with time for each stirring system considered. The top panel shows, the increase in stirring speed induced by the regulator in order to maintain the DO at 30% from saturation.

constantly subjected to a higher substrate concentration than in the bulk of the vessel.

An objective way to define the effectiveness factor related to the mixing component is to consider the inhomogeneity curve (2). On this basis, a parameter specific to the homogenization of the system at a given microbial reaction rate can be defined as

$$\eta_{\text{mix}} = 1 - i$$

in which i is the degree of inhomogeneity as described in the literature *(2)*. This parameter was modified in order to be specific about the microbial process:

$$i = s/\text{acceptable gradient}$$

in which s is the mean absolute deviation of the different zones of the model from a homogeneous substrate concentration. Here, the originality comes from the fact that the degree of inhomogeneity is calculated on the basis of an acceptable gradient for the bioprocess. We obtain a parameter more reliable than the traditional performance criteria used to quantify

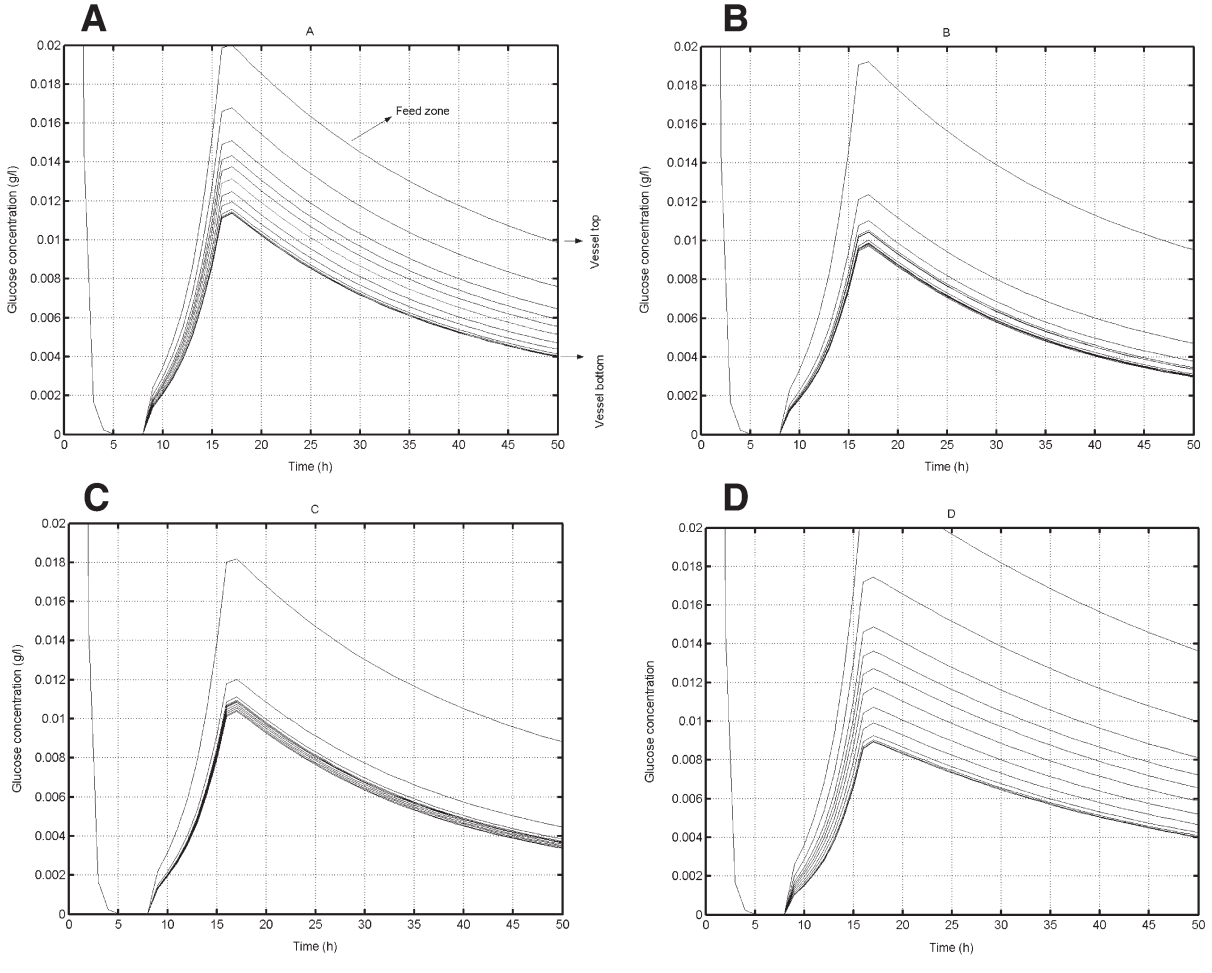

Fig. 8. Substrate gradients observed for each stirring system when coupling NOZ model to a simple Monod microbial growth model: **(A)** TD6-TD6 system at 20 L scale; **(B)** TD6-A340 system at 20 L scale; **(C)** A315-A340 system at 20 L scale; **(D)** TD6-TD6 system at 500 L scale).

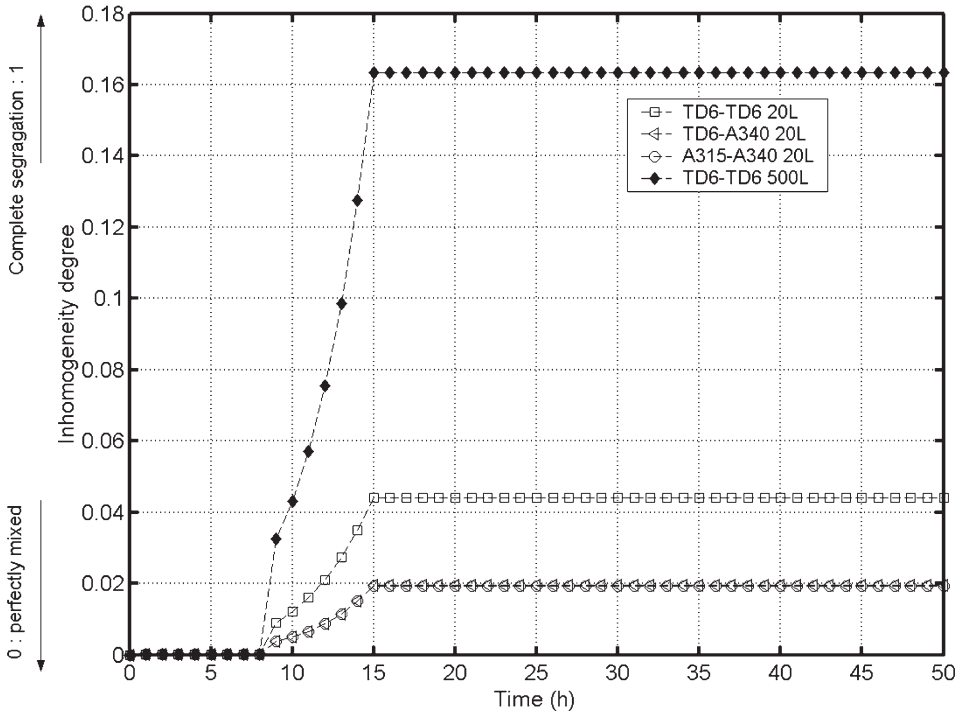

Fig. 9. Evolution degree of inhomogeneity computed using NOZ model for each stirring system considered. Calculations were performed on the basis of an acceptable gradient of 10 mg/L.

mixing efficiency (mixing time) because related to the dynamic of a fed-batch bioprocess.

The evolution of the degree of inhomogeneity is shown in Fig. 9. During the exponential phase of the feed, the tendency for the formation of gradient was greater for all the systems tested. Working at the 500-L scale showed a degree of inhomogeneity of 16%, which is four times greater than at the 20-L scale with the same impeller system. At this scale, the system comprising hydrofoil presented a slightly lower degree of inhomogeneity than the double turbine (TD6-TD6) system. This effect can be attributed to the well-known compartmentalization effect induced by radial impellers such as the TD6.

Figure 10 presents the evolution of each of the systems tested. It can be seen that this factor is acceptable from a biochemical engineering point of view for each scale considered (>80%). However, the sensitivity of *P. pastoris* to gradient exposure is not known, and it is not possible to obtain a maximal tolerable gradient to compute the η_{mix} value limiting for the bioprocess. Further research is necessary to investigate this effect.

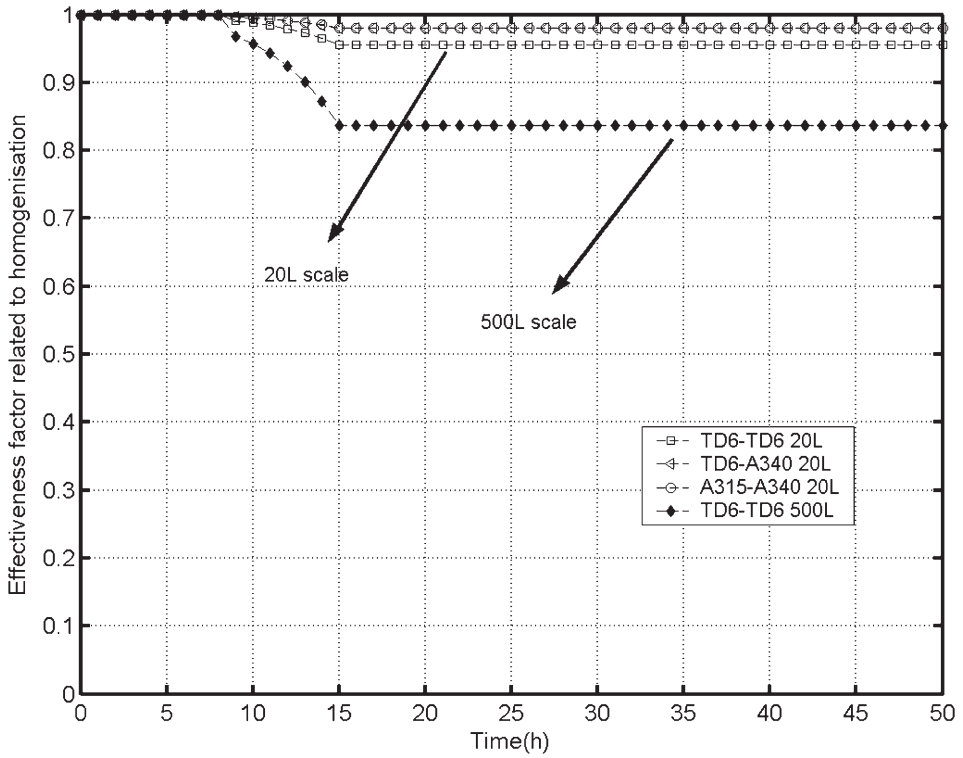

Fig. 10. Variation in homogenization effectiveness factor η_{mix} with time for each stirring system considered.

Conclusion

The experiments conducted in this study highlight the influence of homogenization efficiency and mass transfer on a bioprocess conducted in a stirred vessel operating with a low-viscosity broth. To achieve this, an NOZ model was elaborated to integrate fluid dynamics in the microbial process and thus to facilitate the study of mixing and mass transfer for a given set of operating conditions. Indeed, these two factors are implied in the efficiency of bioreactors and a method to distinguish them is necessary. We developed a method involving the concept of the effectiveness factor developed by Diaz et al. (*1*). The effectiveness factor was divided into two components to deal separately with oxygen transfer and homogenization. These factors were expressed in comparison with a perfectly mixed simulated growth curve. This approach permits more reliable results.

The effectiveness factor related to homogenization efficiency was more difficult to express. To facilitate its implementation, an NOZ model was used to express substrate gradient concentration in a stirred vessel. The NOZ model has been extensively studied in the literature, but only a few articles deal with application to bioprocesses. The methodology presented herein permits to this application by using an integrated effectiveness factor.

The experiments and simulations with NOZ demonstrated very different hydrodynamic behaviors among the impeller combinations tested. The chemical engineering data obtained are in accordance with related literature *(15,18).* Scale-up showed a significant decrease in microbial growth, which can be attributed to hydrodynamic and oxygen transfer. At this stage of the study, we tried hybrid radial-axial (TD6-A340) or complete axial (A315-A340) impeller combinations at a larger scale, because these configurations promote better mixing efficiency.

However, during the scaling-up, heterogeneity will become more and more important, leading to substrate gradient accompanied by an oxygen gradient. Comparison of experiments conducted in 500-L and in 20-L bioreactors have highlighted this effect. The structured component of the effectiveness factor related to homogeneity will thus have a critical importance at large scale because both carbon substrate and oxygen must be homogenized *(17).* NOZ has proven to be an efficient tool for estimating the appearance of gradients and can be used for estimating the mixing part of the effectiveness factor.

Analysis of effectiveness factor components led to the conclusion that stirring systems composed of hydrofoil(s) present better oxygen transfer effectiveness factor and homogenization effectiveness factor. Our results showed a slightly positive impact on microbial growth. This implies that hydrofoils could be used to design a stirring system at a greater scale in which mixing efficiency is necessary to ensure a good homogenization of both DO concentration and substrate concentration.

To obtain a more reliable η_{mix} value, the acceptable gradient intensity must be known. To achieve this, it is necessary to know the substrate gradient sensitivity of *P. pastoris*. Apparatus for such experiments is described in the literature *(4,6),* and future studies are needed.

In conclusion, a method for comparing the impact of homogenization and oxygen transfer on bioprocesses was proposed. The efficiency of such a methodology was tested on a *P. pastoris* fed-batch process and led to a reliable basis for comparison of process performance (several impeller combinations and two scales were investigated). The great advantage of this approach that it can directly link chemical engineering variables and microbial kinetics.

Nomenclature

d = impeller diameter (m)
ex = exponential factor of substrate feed pump
F = fed-batch pump feed rate (m^3/s)
F_o = initial fed-batch feed rate (m^3/s)
F_{max} = maximum feed rate (m^3/s)
i = degree of inhomogeneity
K_s = affinity constant (g/L)

n = number of circulation loops of NOZ model
N = impeller rotational speed (s^{-1})
N_{qc} = circulation without (dimensionless)
η = circular, number dimensions
q_c = circulating flow rate of NOZ model ($q_c = Q_c/n$) (m^3/s)
q_e = turbulence backmixing flow rate of NOZ model (m^3/s)
Q_c = circulating flow rate (m^3/s)
Re = Reynolds number (dimensionless)
s = mean absolute deviation
S = substrate concentration (g/L)
S_a = substrate concentration in the feed (g/L)
X = biomass concentration (g/L)
Y_{xs} = substrate-to-biomass conversion yield
η = effectiveness factor (%)
η_{mix} = homogenization component of effectiveness factor (%)
η_{O_2} = oxygen transfer component of effectiveness factor (%)
μ = viscosity ($Pa \cdot s$)
μ_x = growth rate (h^{-1})
μ_{xmax} = maximum growth rate (h^{-1})

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