Investigation on the Transient Conditions of a Rotating Biological Contactor for Bioethanol Production

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Alcoholic fermentations of sucrose solutions were performed in a Rotating Biological Contactor with immobilized-yeast cells, and the results collected during the transient conditions of start-up are presented and discussed. The analysis and modeling of data constitute a preliminary semi-empirical approach to the study of dynamics of such a bioprocess. The investigation has been developed on the observations of the responses to variations in the operating conditions of substrate, product, suspended- and immobilized-cell concentrations either in the fermentation broth or within a synthetic spongy matrix.

Key words:
Start-up, dynamics, transient conditions, immobilized-cell reactor, alcoholic fermentation

Introduction and modeling

Microbial dynamics is recognized as being the way and the extent as how the material balance equations for biomass, substrate and product mass concentrations can describe the microbial behavior under the so-called "transient" non-stationary state.

Non-stationary state conditions take place during one of the following situations: a) batch cultivation; b) start-up of a continuous process; c) time periods following variations of one or more of the operating variables that are responsible for the switch off of steady-state (residence time, temperature, agitation, etc.); and d) change from preceding to new steady-state conditions. *Koga* and *Humphrey*[1] proposed that the mass concentration of biomass and substrate can exhibit, according to the starting conditions, one of six different transient responses.

From the material balance for biomass, we obtain a bioprocess under non-stationary conditions:[2,3]

$$\frac{\mathrm{d}\gamma_{\mathrm{X}}}{\mathrm{d}t} = \frac{\mu_{\max}\gamma_{\mathrm{S}}}{K_{\mathrm{s}} + \gamma_{\mathrm{S}}} - \frac{1}{\tau} \qquad (1)$$

where γ_X and γ_S are biomass and substrate concentrations, t the time, τ the hydraulic residence time referred to the whole reaction volume, K_s the saturation constant of the *Monod* equation, and μ_{max} the maximum value of the specific growth rate (μ). The second term of the right hand-side of this equation has to be taken into consideration only in continuous processes.

From the mass balance equation for the substrate is:[3,4]

$$\frac{1}{Y_{\mathrm{x/s}}} = \frac{m}{\mu} + \frac{1}{Y_{\mathrm{G}}} \qquad (2)$$

where $Y_{x/s}$ and Y_G are the macroscopic and the true yield of biomass production on consumed substrate and m is the specific rate of substrate consumption in maintenance processes.

Equation (2) has been confirmed experimentally by several authors using different carbon sources as limiting substrates.[3,4] By substitution of this equation into the mass balance for the substrate, we obtain:

$$\frac{\mathrm{d}\gamma_{\mathrm{S}}}{\mathrm{d}t} = \frac{\gamma_{\mathrm{S}_0} - \gamma_{\mathrm{S}}}{\tau} - \left(\frac{1}{Y_{\mathrm{G}}}\frac{\mu_{\max}\gamma_{\mathrm{S}}}{K_{\mathrm{s}} + \gamma_{\mathrm{S}}} + m\right)\gamma_{\mathrm{X}} \qquad (3)$$

where γ_{S_0} is the substrate mass concentration in the feeding solution. The term including the residence time should again be taken into account only in continuous processes.

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Equations (1) and (3) are at the basis of the description of microbial dynamics of suspended-cell systems and can be re-proposed, with required adaptations, for supported-biomass systems, as described in this paper. This allows avoiding the necessity of resorting to complicate models based on numerical solution of the equations describing the system.[5]

The Rotating Biological Contactor (RBC) is a reactor configuration widely used under aerobic and anoxic conditions in wastewater treatment,[6] mainly in the presence of industrial streams containing high concentration of low biodegradable substances.[7] The major advantage of this reactor configuration with respect to stirred chemostat is combination of fixed-film and suspended growth features[8] as well as efficient and cheap mixing,[9] which makes it particularly suited for systems in which gas-liquid transfer plays a significant role. Thanks to these characteristics, it was proposed as an interesting alternative for alcohol[9–15] and citric acid fermentations.[16,17]

This study is one of the few attempts to describe the transient dynamics of immobilized-cell RBC, particularly concerning the behavior of biomass entrapped within porous matrices. Such a type of immobilization procedure did in fact reveal to be the simplest one for modeling purposes.[9,18,19]

According to *Sassi* et al.,[20] the performance of a RBC can be evaluated through a certain number of hydrodynamic parameters.

The hydraulic residence time for the liquid crossing each stage or section (τ_c) was defined as the quotient of the liquid working volume of each stage or section (V) and the feed flow rate (Q):

$$\tau_c = \frac{V}{Q} \tag{4}$$

The hydraulic residence time for mixing due to liquid film circulation induced by disc rotation (τ_c) was defined as:

$$\tau_c = \frac{V}{F_f} \tag{5}$$

where:

$$F_f = \frac{28.8 A Re_r^{3/2}}{\pi g^{1/2} d^{5/2} (\rho/\mu)^2} \tag{6}$$

is the liquid film flowrate, A the wetted area of discs per section, ρ is the liquid density, μ the liquid dynamic viscosity, g the gravity acceleration, d the disc diameter and

$$Re_r = \frac{\rho n d^2}{\mu} \tag{7}$$

the rotational Reynolds number, being n the rotational speed.

Finally, the hydraulic residence time for observed kinetics (τ_{obs}) was calculated according to the equation:

$$\tau_{obs} = \frac{(\gamma_S \gamma_X)^{1/2} V}{\Delta\gamma_S Q} \tag{8}$$

where $\Delta\gamma_S$ is the substrate mass concentration decrease.

Materials and methods

Microorganism

The culture of *Saccharomyces cerevisiae* (Baker's yeast, var. Vinal) used in our experiments was maintained on agar-malt slants. The cells were grown aerobically in shaken flasks on a rotary shaker at 30 °C and harvested at the stationary phase. The cells for the inoculum were centrifuged and suspended in the medium to produce a thick suspension.

Medium

The medium used for fermentations was a 100 kg m^{-3} sucrose solution containing 5 kg m^{-3} KH_2PO_4, 2 kg m^{-3} $(NH_4)_2SO_4$, 2 kg m^{-3} yeast extract, and 0.4 kg m^{-3} $MgSO_4 \cdot 7H_2O$; trisodium citrate dihydrate (0.03 mol dm^{-3}) and H_2SO_4 were used to buffer the medium at pH 5.0.

Immobilized-cell RBC

The fermentor employed for fermentations was a $2.7 \cdot 10^{-3}$ m^3-benchscale RBC, subdivided into 6 sections (Fig. 1), which was already described in detail.[9] The rotating disk shaft assembly was mounted onto a glass tank made of two superimposed semicylinders in order to allow for the arrangement of the spongy support on 6 disks. The upper side of the reactor contained four small holes utilized for the control and regulation of the equipment and for determination of the cell concentration in the support; the lower side, which contained the input and the output, was filled with culture broth.

The support material was a synthetic commercial sponge, which had not been treated in any special way before its use, except for sterilization. The main characteristics of the reactor as well as of the immobilizing support are listed in Table 1.

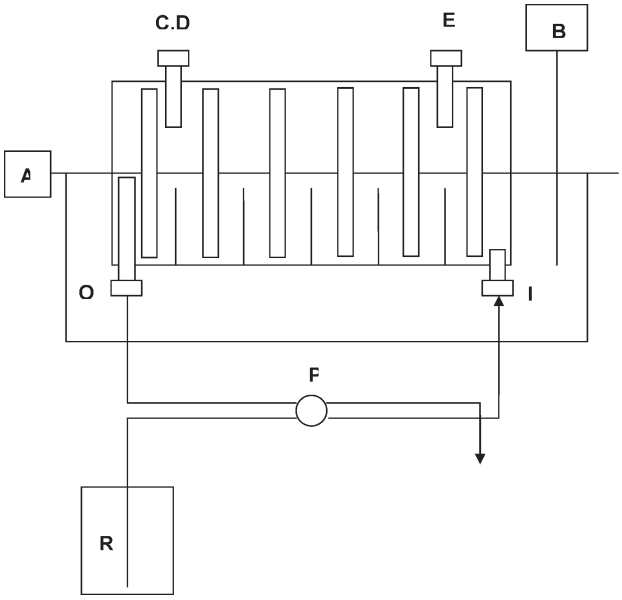


Fig. 1 – *Schematic diagram of the bench-scale experimental set-up. A = shaft stirrer; B = temperature regulation; C,D = pH control and regulation; E = thermometer; I = inlet; O = outlet; P = peristaltic pump; R = nutrient reservoir.*

Table 1 – *Characteristics of the immobilized-cell RBC and the support employed for alcoholic fermentation of sucrose-based aqueous solutions*

total reactor volume (V_t)	$2.7 \cdot 10^{-3}$ m^{-3}
volume of each stage (V)	$0.45 \cdot 10^{-3}$ m^{-3}
rotational speed (n)	0.0583 rad s^{-1}
rotational Reynolds number (Re_r)	1,266
support specific surface area (s)	3.14 m^2 kg^{-1}
support mass concentration (γ)	24.1 kg m^{-3}
support porosity (ε)	12.6 kg_{H2O} kg_{sup}^{-1}
total area of the support (A_t)	0.204 m^2
mixing hydraulic residence time (τ_m)	1,126 s
disc diameter (d)	0.147 m
diameter of unwetted part of disc (d_w)	0.075 m

The lower part of the fermentor was filled with the thick-cell suspension in the presence of the rotating disks. Periodic aseptic renewals of the medium allowed the aerobic growth of the yeast within the cavities of the support. Aeration was ensured by introducing sterile air into the upper section of the fermentor.

Operating conditions

The pH of the fermentation broth was controlled at 5.0 ± 0.1 by a Leeds and Northrup apparatus and regulated by a Sigma pump, which injected a fine stream of 1.0 mol dm^{-3} sodium carbonate solution. All the experiments were carried out at 30 ± 0.5 °C.

In order to exclude atmospheric oxygen, the surface of the medium was blanketed with carbon dioxide by gently blowing the gas into the fermentor. The gas was first passed through sterile cotton and sterile water before entering the fermentor. A variable-speed peristaltic pump allowed a controlled volumetric flow of the medium into the reactor during the continuous experiments.

The fermentor and the fermentation medium were sterilized separately by autoclaving at 120 °C for 20 min.

Analytical procedures

The free-cell concentration was determined by filtering a known volume of culture broth through autoclavable filters with 0.45 μm pore diameter. The filters were dried at 105 °C for 1.5 h before and after the filtration and weighted. The immobilized cell mass concentration was determined applying the same methodology to cells eluted from sponge samples, periodically withdrawn from the reactor.

Ethanol concentration was determined with a gas chromatograph Fractovap model C Type ATC/t (Carlo Erba, Milan), with a column packed with Chromosorb W coated with Carbowax 1500. The column was kept at 130 °C and the detector at 190 °C. Helium at 1.5 bar was used as a carrier gas.

Sucrose was determined by the colorimetric method for determination of sugars according to *Dubois* et al .[21]

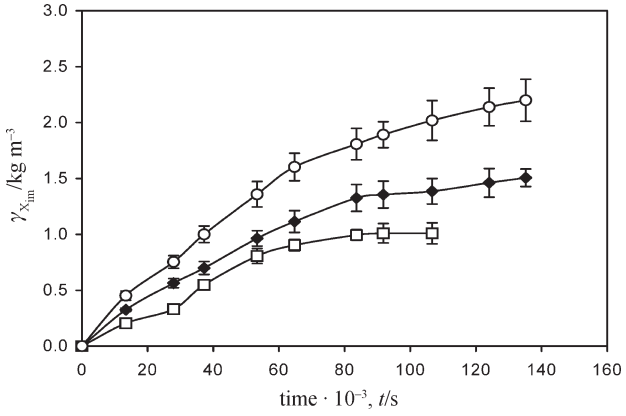
All tests were performed in triplicate and the results expressed as means values. Statistical analysis was done using standard deviations of the experimental data from the means values.

Results and discussion

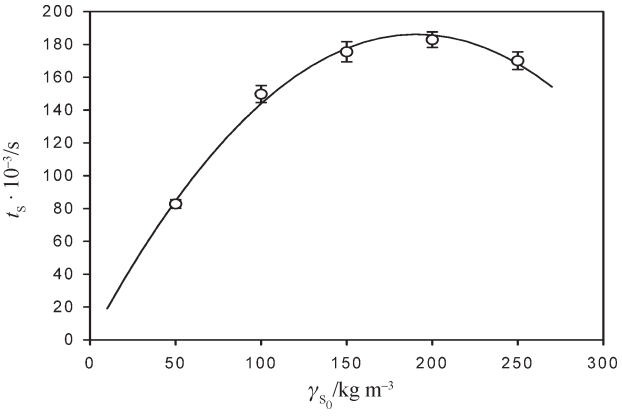
Cell entrapment in batch processes

Batch process is undoubtedly the simplest case of transient response of a biosystem, i.e. of bioprocesses under non-stationary conditions. The study of cell entrapment kinetics during batch processes can also provide useful information on the preliminary start-up phases of continuous processes, before the achievement of steady-state conditions.

The "start-up" of a continuous process is the time period preceding the achievement of steady--state conditions, whose duration depends on the time required to achieve maximum cell concentration within the support. After this time, the substrate available either for growth or product formation is depleted, no further growth is possible, and biomass is subject to decay, unless fresh substrate is fed.



F i g . 2 – *Time behavior of entrapped-biomass concentration during batch fermentation processes carried out in a RBC.* γ_{S_0} *(kg m*$^{-3}$*): (□) 44; (◆) 100; (○) 200. Sucrose-based synthetic media. Support: spongy material. T = 30 °C.*



F i g . 3 – *Dependence of the start-up duration of alcoholic fermentation in a RBC on the starting concentration of substrate. Sucrose-based synthetic medium. Support: spongy material. T = 30 °C.*

Fig. 2 illustrates typical sigmoid curves of cell entrapment during batch immobilized-cell processes, which are characterized by the well-known lag, exponential and stationary phases and are qualitatively similar to those usually observed for suspended-biomass systems. As observed by *Aiba* et al.,[2] this situation approaches the balanced growth, which is defined as the growth taking place at a specific rate coincident with that of one of the cell components (μ_{ci}).

The likeness to the dynamics of suspended-cell systems should not be surprising having in mind that, during the start-up, the support does not play any effective role of biomass entrapment. Unless transfer from the bulk to the pore is limited by excessively small pore size, it does in fact simply host within its pores the same poor cell concentration as the suspended-cell compartment does. As a consequence, during this phase, the curves of suspended- and entrapped-biomass concentrations are practically coincide, making reference to the whole reactor volume.

It is evident from the curves in Fig. 2 that duration of start-up remarkably increased with the starting concentration of substrate (γ_{So}). This is in agreement with the assumption that the higher γ_{So} the longer the time required to reach conditions of substrate depletion and biomass decay.

However, this description is rather simplistic because it does not take into account possible cell growth inhibition due to increases in the osmotic pressure, viscosity and density of culture broth, already reported for increasing γ_{So}.[12] Such inhibiting phenomena, which appear even at relatively low γ_{So} values are even more evident in Fig. 3, where the start-up time (t_s) is plotted versus γ_{So} for the alcoholic fermentation of a synthetic sucrose solution in a RBC with biomass entrapped within a porous matrix. It can be described by the quadratic term of the empirical relationship:

$$t_s = a\,\gamma_{S_0} - b\,\gamma_{S_0}^2 \qquad (9)$$

where a and b are empiric parameters that depend on all the independent variables of the bioprocess under consideration.

The presence of this term described a deviation from the linearity observed at low starting substrate level ($\gamma_{S_0} < 100$ kg m^{-3}). For the case under consideration, using biomass entrapped within porous support, the start-up period preceding the continuous fermentation of sucrose furnished $a = 0.54$ m^3 s kg^{-1} and $b = 5.04 \cdot 10^6$ m^6 s kg^{-2}.

Cell entrapment in continuous processes

Colonies of large size can develop within the pores by continuous feeding the nutrient solution to the reactor, during which substrate is uninterruptedly available to cell metabolism.

Because of the continuous substrate availability, biomass grows within the support pores up to the achievement of a constant maximum value referred to as “cell hold-up”. This phase can still be considered as belonging to the start-up operation; in fact, we can believe steady-state conditions are ensured in a biosystem only when also biomass concentration within the support achieves a constant value depending on the selected residence time. From this aspect, the immobilized-cell systems largely differ from the suspended-biomass ones, because cell growth within the immobilizing support is a process lasting much longer than cell metabolism leading to the formation of the desired product. When the concentrations of all chemical species present in the broth (mainly biomass, substrate and product) reach constant values under the given operative conditions, steady-state conditions are en-

sured and maintained indefinitely, unless perturbations of the regime take place.

Koga and *Humphrey*[1] demonstrated, by "phase-plane" analysis, that, whenever Y_G, m, K_s and μ_{max} can reasonably be considered constant, the solutions for γ_S and γ_X converge to only one point each, therefore no significant oscillations should occur. In other words, the equation of *Monod*, appearing in equations (1) and (3), turns out to be very stable. The transient responses of γ_X, γ_S and γ_P often show only one maximum or one minimum before reaching the steady state. The values of the above kinetic parameters and yield can be evaluated in chemostat for a continuous culture under steady-state conditions[22] by determination of $Y_{x/s}$ and γ_S values at different residence times, being $\mu = 1/\tau$. It can be surprising that the parameters estimated at the stationary state would be extended to transient situations.

Once again, the behaviors of suspended- and immobilized-cell systems appear to be quite similar. Fig. 4 shows the time trends of product and substrate concentrations during continuous alcohol fermentation of sucrose by *S. cerevisiae* at hydraulic residence times for the liquid crossing the whole RBC (τ) and each stage (τ_c) of 32,800 and 5,460 s, respectively. As expected, the transient responses of sucrose and ethanol concentrations were characterized by only one maximum or one minimum, which suggests the occurrence of a situation analogous to that of suspended-biomass system.

On the contrary, Fig. 5 shows a peculiar response of the entrapped-biomass concentration to the continuous feeding of sucrose, which can be explained by the occurrence of: a) a starting phase of biomass adaptation to the support microenvironment; b) a subsequent phase of exponential growth within the support, without any physical limitation; c) a third phase during which the growth is limited by the pore size; and d) a final phase of stationary growth. This behavior is a consequence of the high γ_X value in the immobilized-cell system and makes the analytical solutions of this variable less sensitive to variations of other parameters.

Suspended-biomass followed the typical *Monod*-type behavior in CSTR and was washed out for $\tau \leq 1/\mu_{max}$. For this reason, the concentration of suspended biomass decreased with increasing the time up to reaching a very low constant value, resulting from the continuous growth and the renewal of entrapped biomass. In this way *Doran* and *Bailey*[23] justified the presence of free cells in immobilized-cell columns. Total biomass level within the RBC was much higher than in a suspended-biomass system, which means that the immobilization had

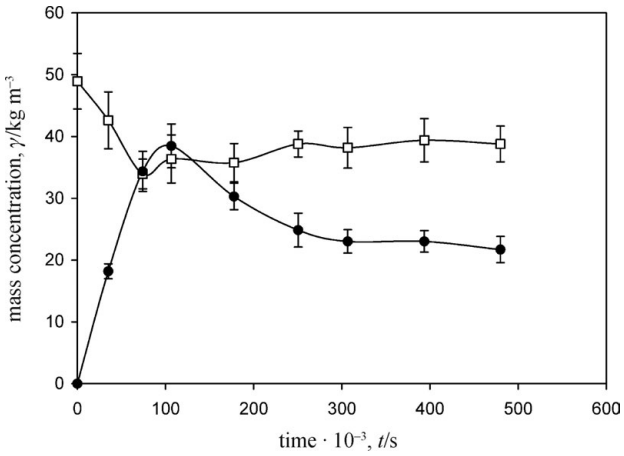


Fig. 4 – *Time behaviors of (●) sucrose and (□) ethanol concentrations in a RBC with spongy support during the start-up of fermentation of sucrose-based medium. T = 30 °C; γ_{S_0} = 100 kg m^{-3}; τ_c = 5,460 s.*

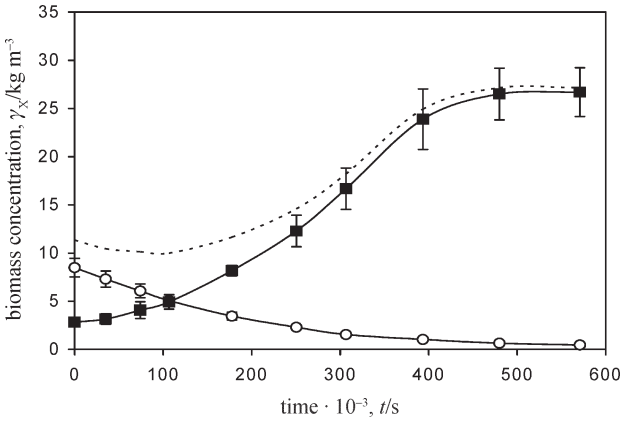


Fig. 5 – *Time behaviors of (○) suspended-, (■) immobilized- and (—) total cell concentrations in RBC with spongy support during the start-up of fermentation of sucrose-based medium. T = 30 °C; γ_{S_0} = 100 kg m^{-3}; τ_c = 5,460 s.*

the macroscopic effect of decreasing the residual γ_S value.

With regard to the m value to use in equation (3), it has been adopted the value report by *Roels* and *Kossen*[24] for *S. cerevisiae* anaerobic maintenance on glucose ($4.16 \cdot 10^{-5}$ C-mol C-mol$_{DM}^{-1}$ s^{-1}), supposing that, under conditions of excessive substrate, m can reasonably be considered to be the same as in a suspended-biomass system. For modeling purposes, we assumed for yeast the average composition proposed by *Dekkers* et al.,[25] corresponding to a biomass molar mass of 0.0251 kg C-mol^{-1}, and the values of $\mu_{max} = 3.33 \cdot 10^{-5}$ s^{-1} and $K_s = 9.42$ kg m^{-3} previously determined in chemostat under the same conditions.[11]

Assuming for an immobilized-cell system a dependence of the actual maximum specific growth rate ($\mu_{max,im}$) on the total area available for biomass attachment per unit volume (A_t), we obtained:

$$\mu_{max,im} = \mu_{max} + c\,A_t \quad (10)$$

being c a correlation parameter.

From this equation we estimated, for our immobilized-cell system under the selected conditions (Table 1), $\mu_{max,im} = 8.08 \cdot 10^{-5}$ s^{-1}, i.e. a value more than twice that obtained with the suspended-biomass system ($\mu_{max} = 3.33 \cdot 10^{-5}$ s^{-1}).[11]

RBC Hydrodynamics

The final part of this study was devoted to the influence of typical hydrodynamic parameters on the RBC performance under different flowrate conditions. In order to inquire the mixing conditions inside the system, the hydraulic residence times for liquid crossing each stage (τ_c) and for mixing due to liquid film circulation induced by discs rotation (τ_m) were compared with that for observed kinetics (τ_{obs}) (Table 2). First of all, it should be noticed that the rotational Reynolds number did not vary (Re_r = 1,266) owing to the constant rotational speed adopted in this study (n = 0.0583 rad s^{-1}) and that its value was indicative of a regime close to turbulence inside the reactor. Even at the highest flowrates, both τ_m/τ_{obs} and τ_m/τ_c ratios were always lower than 1, which confirms that circulation was faster than both substrate disappearance and feed crossing, and that mixing conditions were satisfactory. Finally, it should be noticed that the τ_c/τ_{obs} ratio was always lower than 1, as was expected by the fact that crossing is a serial mechanism of the observed kinetics of substrate uptake.[20]

Table 2 – *Main hydrodynamic parameters calculated for the immobilized-cell RBC under the experimental conditions tested in this work (T = 30°C; γ_{S_0} = 100 kg m^{-3})*

$Q \cdot 10^9$/m^3 s^{-1}	41.2	82.4	165	247	330
τ_c (s)	10,920	5,460	2,730	1,820	1,365
τ_m/τ_c (–)	0.103	0.206	0.412	0.619	0.825
τ_{obs} (s)	11,027	7,615	6,757	6,522	6,750
τ_c/τ_{obs} (–)	0.990	0.717	0.404	0.279	0.202
τ_m/τ_{obs} (–)	0.102	0.148	0.167	0.173	0.167

Conclusions

In conclusion, comparison of the results of alcoholic fermentation previously obtained in chemostat with those collected in this work using an immobilized-cell RBC demonstrated that the dynamic models describing cell behaviors during the start-up of suspended-biomass processes can be extended to immobilized-cell systems, provided that the increase in cell growth effectiveness is taken into consideration.

Such a theoretical semi-empirical approach, which requires multistage scaling up for successful real-scale application, greatly simplifies the modeling with respect to complex dynamic models.

The hydrodynamic study of the system confirmed that circulation was faster than both substrate disappearance and feed crossing, and therefore mixing conditions were satisfactory.

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