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Research Paper

Batch reactor performance for the enzymatic synthesis of cephalexin: influence of catalyst enzyme loading and particle size

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A mathematical model is presented for the kinetically controlled synthesis of cephalexin that describes the heterogeneous reaction–diffusion process involved in a batch reactor with glyoxyl-agarose immobilized penicillin acylase. The model is based on equations considering reaction and diffusion components. Reaction kinetics was considered according to the mechanism proposed by Schroën, while diffusion of the reacting species was described according to Fick's law. Intrinsic kinetic and diffusion parameters were experimentally determined in independent experiments. It was found that from the four kinetic constants, the one corresponding to the acyl-enzyme complex hydrolysis step had the greatest value, as previously reported by other authors. The effective diffusion coefficients of all substances were about 5×10^{-10} m^2/s, being 10% lower than free diffusion coefficients and therefore agreed with the highly porous structure of glyoxyl-agarose particles. Simulations made from the reaction–diffusion model equations were used to evaluate and analyze the impact of internal diffusional restrictions in function of catalyst enzyme loading and particle size. Increasing internal diffusional restrictions decreases the Cex synthesis/hydrolysis ratio, the conversion yield and the specific productivity. A nonlinear relationship between catalyst enzyme loading and specific productivity of Cex was obtained with the implication that an increase in catalyst enzyme loading will not increase the volumetric productivity by the same magnitude as it occurs with the free enzyme. Optimization of catalyst and reactor design should be done considering catalyst enzyme loading and particle size as the most important variables. The approach presented can be extended to other processes catalyzed by immobilized enzymes.

Introduction

Penicillin G acylase (PGA) is a relevant industrial enzyme for the large-scale production of 6-aminopenicillanic acid (APA) and 7-amino-3-deacetoxicephalosporanic acid (ADCA), both being key precursors for the synthesis of β-lactam antibiotics. These intermediates are mostly produced from penicillin G and cephalosporin G using immobilized PGA [1]. PGA catalysts have also been evaluated for the synthesis of second generation β-lactam antibiotics from APA and ADCA [2,3], like amoxicillin and cephalexin, being an interesting application of this hydrolytic enzyme in reactions of synthesis.

The efficient utilization of PGA is crucial for its industrial application, so that immobilization onto solid carriers [4,5] or insolubilization by aggregation [6] is fundamental because stabilization occurs and reutilization is possible. Nevertheless, enzyme immobilization on solid carriers is accompanied by a decrease in catalytic efficiency as a consequence of the internal diffusional restrictions (IDR) of substrates and products within the catalyst [7,8]. Biocatalyst properties are essential in determining the impact of IDR and its consequence on enzymatic reaction. It is

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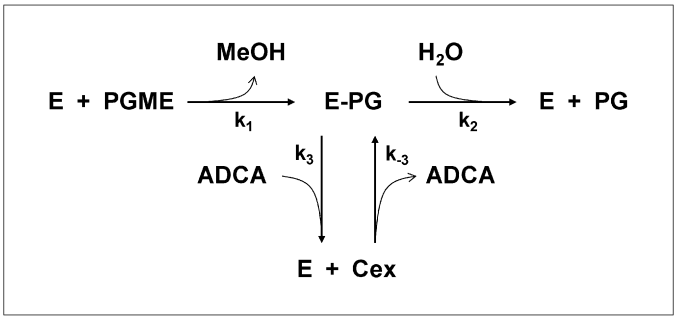

FIGURE 1

Scheme for the kinetically controlled mechanism of cephalexin synthesis. E: penicillin acylase; Cex: cephalexin; PGME: phenylglycine methyl ester; PG: phenylglycine; E-PG: acyl-enzyme complex; MeOH: methanol.

well known that the volumetric productivity increases linearly with an increment of enzyme loading and that the specific productivity is independent of enzyme loading when free enzyme is used. So, the gain–cost relationship is constant between obtained product and enzyme used; now it depends on scaling factors. When immobilized enzyme catalysts are used, the gain–cost relationship in terms of the obtained product versus the enzyme used is non-trivial because mass transfer limitations are present.

In this work a reaction–diffusion mathematical model is described and quantified. The system is formed by a biocatalyst of immobilized PGA on a glyoxyl-agarose matrix through multipoint covalent attachment. Reaction corresponds to the synthesis of cephalexin (Cex) under kinetic control from phenylglycine methyl ester (PGME) and ADCA in a batch reactor. The objective of this work is the validation of a reaction–diffusion model to evaluate and analyze, through computer simulations of a batch reactor with immobilized PGA catalysts, the effect of biocatalyst properties in the magnitude of IDR and its impact on reactor performance, described in terms of conversion yield and productivity.

Model

Kinetics

Synthesis of Cex under kinetic control has been developed from an adaptation of the model proposed by Schroën *et al.* [9] (Fig. 1), considering PGME as acyl donor. Defining A, B, P and C as molar concentrations of PGME, ADCA, Cex and PG, respectively, rate equations derived from this mechanism are:

$$\frac{dA}{dt} = E_0 \frac{-k_1 k_3 \cdot A \cdot B - k_1 k_2' \cdot A}{\Sigma} \quad (1)$$

$$\frac{dB}{dt} = E_0 \frac{k_2' k_{-3} \cdot P - k_1 k_3 \cdot A \cdot B}{\Sigma} \quad (2)$$

$$\frac{dP}{dt} = E_0 \frac{k_1 k_3 \cdot A \cdot B - k_2' k_{-3} \cdot P}{\Sigma} \quad (3)$$

$$\frac{dC}{dt} = E_0 \frac{k_2' k_{-3} \cdot P + k_1 k_2' \cdot A}{\Sigma} \quad (4)$$

$$\Sigma = k_1 \cdot A + k_2' + k_3 \cdot B + k_{-3} \cdot P \quad (5)$$

where E_0 corresponds to the total PGA concentration. Kinetic constant k_2 is multiplied by water concentration resulting in k_2'. The symbol Σ is the common denominator of the kinetic equations deduced from mechanism of Figure 1.

Reaction–diffusion model

The model was built from mass balances for each substance within the catalyst particle. Reaction–diffusion equations were built up by combining mass transfer rates, as determined by Fick's law, and appropriate rate equations. Fick's law was derived considering spherical particles, corresponding to the regular shape of glyoxyl-agarose beads. Reaction–diffusion equations are then:

$$\frac{\partial A}{\partial t} = D_{eA} \cdot \left(\frac{\partial^2 A}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial A}{\partial r} \right) + E_0 \frac{-k_1 k_3 \cdot A \cdot B - k_1 k_2' \cdot A}{\Sigma} \quad (6)$$

$$\frac{\partial B}{\partial t} = D_{eB} \cdot \left(\frac{\partial^2 B}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial B}{\partial r} \right) + E_0 \frac{k_2' k_{-3} \cdot P - k_1 k_3 \cdot A \cdot B}{\Sigma} \quad (7)$$

$$\frac{\partial P}{\partial t} = D_{eP} \cdot \left(\frac{\partial^2 P}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial P}{\partial r} \right) + E_0 \frac{k_1 k_3 \cdot A \cdot B - k_2' k_{-3} \cdot P}{\Sigma} \quad (8)$$

$$\frac{\partial C}{\partial t} = D_{eC} \cdot \left(\frac{\partial^2 C}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial C}{\partial r} \right) + E_0 \frac{k_2' k_{-3} \cdot P + k_1 k_2' \cdot A}{\Sigma} \quad (9)$$

where D_{eX} corresponds to the effective diffusion coefficients of substance X and r is the radial dimension inside the catalyst particle. Boundary conditions are:

At $r = 0$:

$$\left.\frac{\partial A}{\partial r}(r,t)\right|_{r=0} = \left.\frac{\partial B}{\partial r}(r,t)\right|_{r=0} = \left.\frac{\partial P}{\partial r}(r,t)\right|_{r=0} = \left.\frac{\partial C}{\partial r}(r,t)\right|_{r=0} = 0 \quad (10)$$

At $r = R$:

$$\left.\left(\frac{\partial A}{\partial r}(r,t) + \frac{RV_b}{3V_c D_{eA}} \frac{\partial A}{\partial t}(r,t) \right)\right|_{r=R} = 0 \quad (11)$$

$$\left.\left(\frac{\partial B}{\partial r}(r,t) + \frac{RV_b}{3V_c D_{eB}} \frac{\partial B}{\partial t}(r,t) \right)\right|_{r=R} = 0 \quad (12)$$

$$\left.\left(\frac{\partial P}{\partial r}(r,t) + \frac{RV_b}{3V_c D_{eP}} \frac{\partial P}{\partial t}(r,t) \right)\right|_{r=R} = 0 \quad (13)$$

$$\left.\left(\frac{\partial C}{\partial r}(r,t) + \frac{RV_b}{3V_c D_{eC}} \frac{\partial C}{\partial t}(r,t) \right)\right|_{r=R} = 0 \quad (14)$$

where R is the radius of the catalyst particles, V_b is the liquid phase volume and V_c is the catalyst volume calculated from the mass and density of the catalyst. This boundary condition considers that the impact of external diffusional restrictions (EDR) is negligible, which is sound when the reactor is properly agitated.

Initial conditions in the batch reactor are defined as:

$$A(r,0) = B(r,0) = P(r,0) = C(r,0) = 0 \quad (15)$$

$$A(r = R, 0) = A_{b0} \quad (16)$$

$$B(r = R, 0) = B_{b0} \quad (17)$$

where b indicates in bulk liquid phase. Dimensionless equations obtained from Eqs. (6) and (7) allow defining the Thièle moduli for PGME and ADCA for a spherical catalyst.

$$\frac{\partial A}{\partial t} = D_{eA} \cdot \left(\frac{\partial^2 A}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial A}{\partial r}\right) - \frac{V_A \cdot A \cdot B}{K_A(1 + (B/K_B)) + A} \tag{18}$$

$$\frac{\partial a}{\partial \tau} = \left(\frac{\partial^2 a}{\partial \rho^2} + \frac{2}{\rho} \cdot \frac{\partial a}{\partial \rho}\right) - \frac{R^2 \cdot V_A \cdot B}{D_{eA} \cdot K_A}\left(\frac{a}{1 + a + b}\right) \tag{19}$$

$$\frac{\partial a}{\partial \tau} = \left(\frac{\partial^2 a}{\partial \rho^2} + \frac{2}{\rho} \cdot \frac{\partial a}{\partial \rho}\right) - 9\Phi_A^2 \sigma_A \tag{20}$$

$$\Phi_A = \frac{R}{3}\sqrt{\frac{V_A \cdot B}{D_{eA} \cdot K_A}} = \frac{R}{3}\sqrt{\frac{k_3 \cdot E_0 \cdot B}{D_{eA} \cdot K_A}} \tag{21}$$

$$\frac{\partial B}{\partial t} = D_{eB} \cdot \left(\frac{\partial^2 B}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial B}{\partial r}\right) - \frac{V_B \cdot A \cdot B}{K_B(1 + (A/K_A)) + B} \tag{22}$$

$$\frac{\partial b}{\partial \tau} = \left(\frac{\partial^2 b}{\partial \rho^2} + \frac{2}{\rho} \cdot \frac{\partial b}{\partial \rho}\right) - \frac{R^2 \cdot V_B \cdot A}{D_{eB} \cdot K_B}\left(\frac{b}{1 + a + b}\right) \tag{23}$$

$$\frac{\partial b}{\partial \tau} = \left(\frac{\partial^2 b}{\partial \rho^2} + \frac{2}{\rho} \cdot \frac{\partial b}{\partial \rho}\right) - 9\Phi_B^2 \sigma_B \tag{24}$$

$$\Phi_B = \frac{R}{3}\sqrt{\frac{V_B \cdot A}{D_{eB} \cdot K_B}} = \frac{R}{3}\sqrt{\frac{k_1 \cdot E_0 \cdot A}{D_{eB} \cdot K_B}} \tag{25}$$

where a and b are the dimensionless concentrations of A and B, respectively, and V_A and V_B correspond to the maximum rates of Cex synthesis when B and A are, respectively, the saturating substrate ($V_A = k_3 \cdot E_0$; $V_B = k_1 \cdot E_0$) and K_A and K_B are the Michaelis–Menten constants of PGME and ADCA, respectively ($K_A = k'_2/K_1$; $K_B = k'_2/k_3$). Notice that $V_A{\cdot}B$ and $V_B{\cdot}A$ are the maximum apparent rates of Cex synthesis. As seen, the PGME Thièle modulus (Φ_A) is a function of ADCA concentration and the ADCA Thièle modulus (Φ_B) is a function of PGME concentration.

Materials and methods

Materials

PGA from *Escherichia coli*, with 400 ± 20 IU_H/mL and 16.6 ± 1 mg/mL protein, was a gift from Antibióticos S.A. (León, Spain). Cross-linked 6% agarose spherical beads (Sepharose 6B-CL) was a product from GE Healthcare (Uppsala, Sweden). Penicillin G potassium salt was kindly provided by Natsus S.A. (Lima, Perú); (R)-(−)-2-phenylglycine methyl ester hydrochloride 97% pure (PGME) and Cex hydrate were from Sigma (St Louis, MO, USA); ADCA was kindly provided by Antibióticos S.A; (R)-(−)-2-phenylglycine (PG) was from Aldrich (Milwaukee, WI, USA). All other reagents were of analytical grade and purchased either from Sigma or Merck (Darmstadt, Germany).

Analysis

Activity assays for free and immobilized penicillin G were determined at pH 7.8 and 30°C in 100 mM phosphate buffer using pH-stat method (Mettler Toledo, DL50) to titrate the H^+ produced by the hydrolysis of 10 mM penicillin G as it is converted into phenylacetic acid; 50 mM NaOH was employed as titrant solution. One international unit of penicillin G activity (IU) was defined as the amount of enzyme that hydrolyzes 1 μmol of penicillin G per minute from 10 mM penicillin G solution under the above conditions. Substrates and products of synthesis of Cex were identified and analyzed by HPLC using a Jasco delivery system PU-2089plus with a Jasco UV 2075 UV-Vis detector and a LC-NetII/ADC Jasco HPLC/PC integrator. The column used was a Kromasil C_{18} (150 mm × 4.6 mm) from Análisis Vínicos (Madrid, España). Samples were eluted with a sonicated mixture of 10% acetonitrile and 90% 10-mM phosphate buffer pH 6.0 at a flow rate of 1 mL/min, and analyzed in the UV detector at 220 nm. Elution times were 1.6, 1.9, 3.3 and 7.4 min for ADCA, PG, Cex and PGME, respectively. Concentration of substrates and products were calculated from calibration curves using stock solutions. HPLC samples were always assayed in duplicate, differences among them never exceeding 3%.

Preparation of glyoxyl-agarose immobilized penicillin G acylase biocatalysts

Sepharose 6B-CL beads were sieved in two fractions with equivalent radius of 32.4 and 75.7 μm, respectively (The equivalent radius represents 50% of the cumulative volume of the particle size distribution.) An extra coarse fraction with equivalent radius of 168 μm was also obtained and used solely for the determination of the effective diffusion coefficients. Sieving, size distribution analysis and determination of equivalent radius were done as previously reported [10]. Glyoxyl-agarose immobilized PGA biocatalysts were prepared by multi-point covalent attachment of the enzyme, through ε-amino groups of lysine, to glyoxyl-agarose gel beads, as previously described [11]. Enzyme loads for both fractions were 150, 400 and 650 IU/g, which cover the range of actual industrial PGA biocatalysts [12,13]. In this way, six different

TABLE 1

PGA catalysts prepared from penicillin G acylase immobilization in glyoxyl-agarose particles: enzyme loading (E_0) and equivalent radius (R_e)

Catalyst	Specific activity (IU/g_{cat})	Expressed activity (% of offered)	E_0 (mM)	R_e (μm)
PGA150R30	131	87.3	0.0532	32.4
PGA400R30	321	80.2	0.1303	32.4
PGA650R30	499	76.8	0.2030	32.4
PGA150R72	116	77.3	0.0470	75.7
PGA400R72	262	65.5	0.1064	75.7
PGA650 R72	344	52.9	0.1399	75.7

biocatalysts were prepared as indicated in Table 1 (nomenclature indicates enzyme loading and mean particle size). Direct quantification of the immobilized enzyme activity after the process was determined by titration of the phenylacetic acid produced by hydrolysis of penicillin G.

Determination of intrinsic kinetic parameters of cephalexin synthesis

Intrinsic kinetic parameters were determined using the free enzyme because this represents a condition free from diffusional restrictions. Experimental conditions for determination of kinetic parameters of Cex synthesis were pH 7.4 and 14°C, corresponding to their previously determined optimum values [14]. The reaction rate constants (k_1, k'_2, k_3, k_{-3}) were determined from both PGME hydrolysis and Cex synthesis experiments, as proposed by Schroën *et al.* [9]. The constants k_1 and k'_2 were determined using data from PGME hydrolysis experiments based on the initial rate method at PGME concentrations between 10 and 300 mM with 0.130 ml of PGA in 10 ml of reaction medium. Samples of 200 μl were taken and immediately mixed with 200 μl of HCl 0.1 M to stop reaction. After 1 min, 200 μl of NaOH 0.1 M were added to neutralize. Samples were analyzed by HPLC. Rate constants k_3 and k_{-3} were determined using data from Cex synthesis experiments based on the progression curve method using 15, 30 and 45 mM of ADCA with a PGME/ADCA molar ratio of 3. Liquid medium volume, PGA volume and samples treatment were as in PGME hydrolysis. In both, PGME hydrolysis and Cex synthesis, models showed in Eqs. (1)–(5) were fitted to experimental data, minimizing the quadratic difference between measured and calculated concentrations using nonlinear fitting. Validation of PGME hydrolysis model was done using data from progression curves during 10, 20 and 30 mM PGME hydrolysis. Determination of enzyme concentration (E_0) was done using the active site titration method by determining enzyme activity after adding different amounts of phenylmethylsulfonylfluoride (PMSF) [15].

Effective diffusion coefficients

The effective diffusion coefficients of Cex, ADCA, PGME and PG were determined according to the Grunwald method [16]. A known mass of glyoxyl-agarose particles with equivalent radius of 168 μm were equilibrated with a known volume of a solution containing a known concentration of Cex, ADCA, PGME and PG in each case. Particles were filtered and contacted with a known volume of buffer solution. Samples were withdrawn and analyzed by HPLC. Experimental data with substance concentration through time were used for fitting effective diffusion coefficient from Eq. (26).

$$c_t = c_\infty - c_\infty e^{-(\pi^2 D_{ef}/R^2)t} \tag{26}$$

where c_t and c_∞ are the concentration at time t and the concentration at infinite time, respectively. Results were compared with free diffusion coefficients calculated from Vorlop's equation [16].

$$D_0 = \frac{1.7 \times 10^{-7} T}{M_r^{0.41} \mu} \ (\mathrm{cm^2/s}) \tag{27}$$

Diffusion–reaction model validation for PGME hydrolysis and Cex synthesis

PGA catalysts were used for validation of the reaction–diffusion model. Reactions were carried out with 0.15, 0.28 and 0.63 g of PGA650, PGA400 and PGA150, respectively, in 25 ml of liquid medium at 10 mM of PGME, for PGME hydrolysis, and 15 mM/45 mM of ADCA/PGME, for Cex synthesis. Samples were taken periodically and analyzed by HPLC. Data obtained from reaction–diffusion model predictions by simulation were compared with data from these experiments.

Simulation

Eqs. (6)–(14) were discretized through Crank–Nicolson method of finite differences [17]. The simulation of Cex synthesis in a batch reactor was done by solving the resulting system of algebraic equations implemented on Python programming language (http://www.python.org/). A computational modulus was developed for the resolution algorithm whose documentation and code are available on-line (http://www.pypsdier.org/). Simulation of batch reactor operation for Cex synthesis generates conversion curves (bulk Cex concentration as a function of reaction time) and concentration profiles within the catalyst as a function of its radius and as a function of reaction time. Reaction of synthesis begins with a PGMA/ADCA molar ratio of 3 in the bulk liquid medium. However, within catalysts this molar ratio is different and varies in function of time and space. Molar ratio and synthesis/hydrolysis ratio were calculated from concentration profiles within the catalysts.

Maximum Cex concentration is obtained, as is usual in a kinetically controlled reaction, so conversion yield ($Y_{\max}$) and specific productivity (Q_{sp}) were calculated according to Eqs. (28) and (29) at the maximum Cex concentration obtained during synthesis, as defined by Illanes *et al.* [14].

$$\text{maximum conversion yield} \quad Y_{\max} = \frac{P_{b,\max}}{B_{b0}} \tag{28}$$

$$\text{specific productivity} \quad Q_{sp} = \frac{M_{\max}}{t_{\max} \cdot E_R} \tag{29}$$

where $M_{\max}$ is the maximum Cex mass obtained, $t_{\max}$ is the time when maximum Cex concentration is achieved and E_R is the total enzyme international units of activity (IU) in the reactor, determined as penicillin G hydrolysis activity [10]. Specific productivity is the amount of Cex obtained per unit of enzyme activity in the reactor and unit of time. This is a very useful parameter as a gain–cost estimate because it contains the amount of product per unit of enzyme used in reactor. The effect of IDR on batch reactor performance in the kinetically controlled Cex synthesis was analyzed in terms of these response variables which are affected by catalyst enzyme loading and particle size.

Results and discussion

Determination of parameters and model validation

Six PGA catalysts were prepared and characterized through enzyme loading and particle size determination (Table 1). Reaction rate constants k_1 and k'_2 were determined from PGME hydrolysis experiments showed in Figure 2a, where initial reaction rate method was used. Reaction rate constants k_3 and k_{-3} were determined from Cex synthesis experiments showed in Figure 2b, where progression curves of Cex synthesis at different initial concentrations of PGME and ADCA were used. Values of reaction rate constants are shown in Table 2. The higher value is obtained

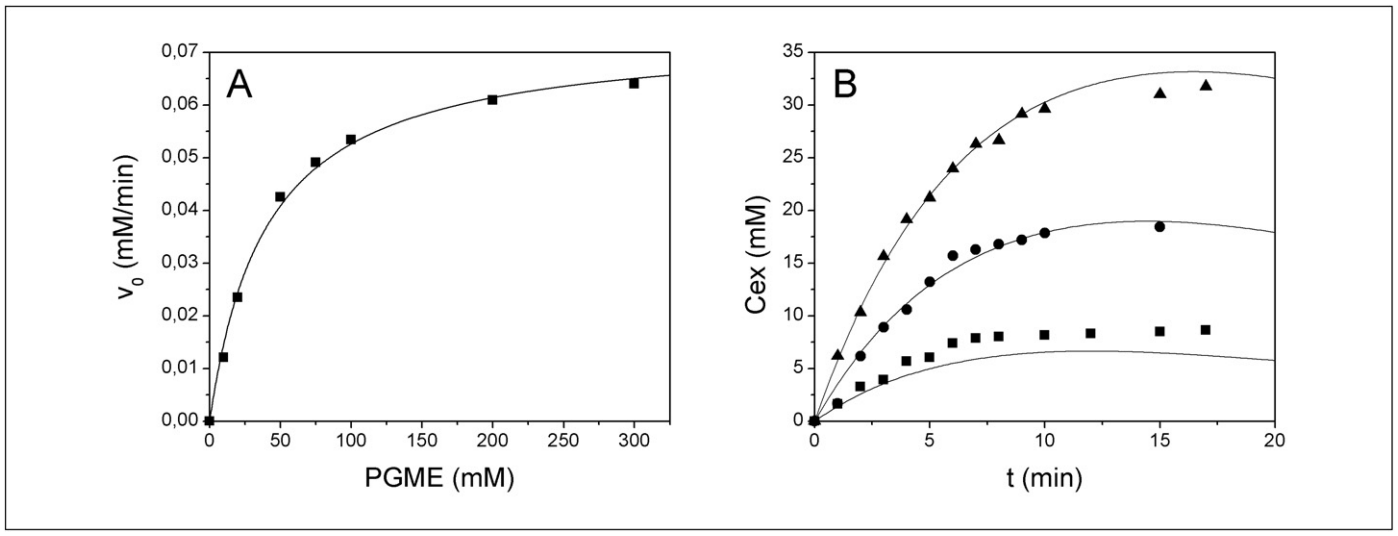

FIGURE 2

Initial rate of PGME hydrolysis **(a)** and Cex synthesis progression curves **(b)** experiments with PGA for the determination of reaction rate constants k_1, k'_2, k_3, k_{-3} at 14°C and pH 7.4. The Cex synthesis was done at different PGME/ADCA concentrations of 45/15 mM (squares), 90/30 mM (circles) and 135/45 mM (triangles).

for k'_2, corresponding to the nucleophilic attack to the acyl-enzyme complex by a water molecule (Fig. 1). These findings agreed with those obtained by Schroën *et al.* [3,9].

Effective diffusion coefficients of all substances were determined from effusion experiments. Results are presented in Table 3. As expected, because of the similar molecular weights, effective diffusion coefficients for all substances are in the same magnitude order and they are, in average, 10% lower than the free diffusion coefficients calculated from Eq. (27). These findings agreed with the highly porous structure of glyoxyl-agarose particles.

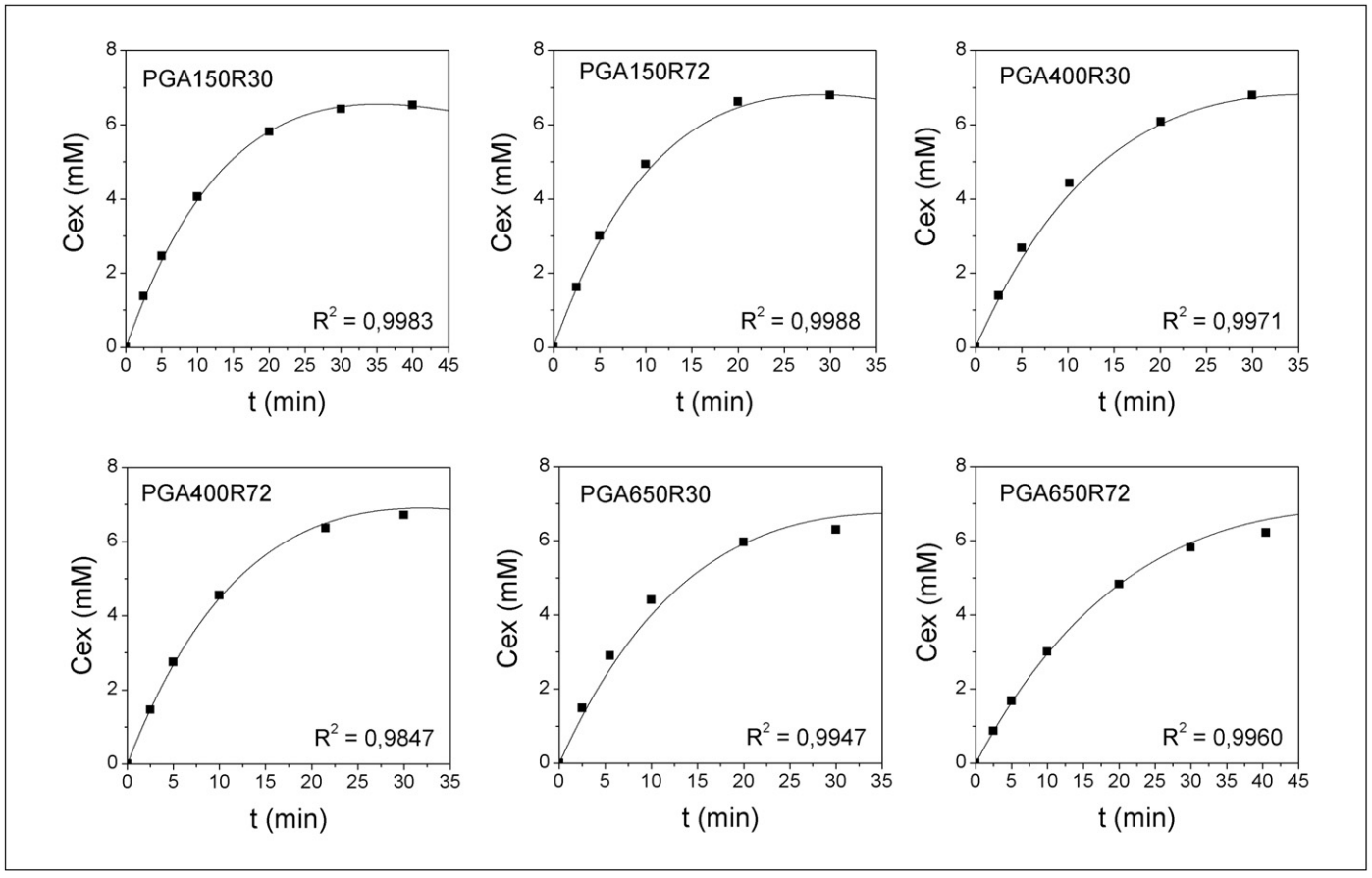

FIGURE 3

Progression curves of Cex synthesis with PGA catalysts at 14°C and pH 7.4 from PGME/ADCA concentration of 45/15 mM for the validation of the reaction–diffusion model.

TABLE 2

Kinetic parameters for the kinetically controlled synthesis of cephalexin with immobilized penicillin acylase at 14°C and pH 7.4

Parameter	Value	Units
k_1	56.5	$(\text{mM min})^{-1}$
k_2'	3407	$(\text{min})^{-1}$
k_3	101.1	$(\text{mM min})^{-1}$
k_{-3}	14.3	$(\text{mM min})^{-1}$

TABLE 3

Effective diffusion coefficients of substrates and products of cephalexin synthesis at 14°C and pH 7.4

Substance	Symbol	Value $(\text{m}^2/\text{s}) \times 10^{10}$
PGME	D_{eA}	5.65
ADCA	D_{eB}	5.71
Cex	D_{eP}	5.09
PG	D_{eC}	5.68

Reaction–diffusion model was validated separately and independently for PGME hydrolysis and Cex synthesis. Model predictions obtained from batch reactor simulations were compared with experimental data with all PGA catalysts. Progression curves for Cex synthesis are shown in Figure 3. As indicated by R^2 values, at least 98% of Cex concentration variation in time is explained by the reaction–diffusion model. With this data the model was validated with PGA catalysts with different enzyme loadings and particle sizes.

Thièle moduli

From the kinetic and diffusion parameters, PGME and ADCA Thièle moduli were calculated from Eqs. (21) and (25) to evaluate the impact of IDR on Cex synthesis. Results are shown in Figure 4. As stated before, both substrates moduli depend on the substrate concentration of the other substrate, so in Figure 4 moduli were calculated for two substrate concentrations to observe the effect of substrate concentration on the Thièle modulus. Maximum concentrations analyzed correspond to the optimal condition previously reported for the synthesis of Cex [14], this is, 600 mM PGME and 200 mM ADCA. Thièle moduli increase with the concentrations of both substrates indicating a greater impact of IDR. This effect is due to the dependence of maximum apparent rates ($V_A \cdot B$ and $V_B \cdot A$) on ADCA and PGME concentrations, as described in Eqs. (21) and (25). As a consequence of using a PGME/ADCA molar ratio of 3, ADCA Thièle moduli are approximately $\sqrt{3}$ times the PGME moduli because differences between the corresponding diffusion coefficients are small (Eqs. (21) and

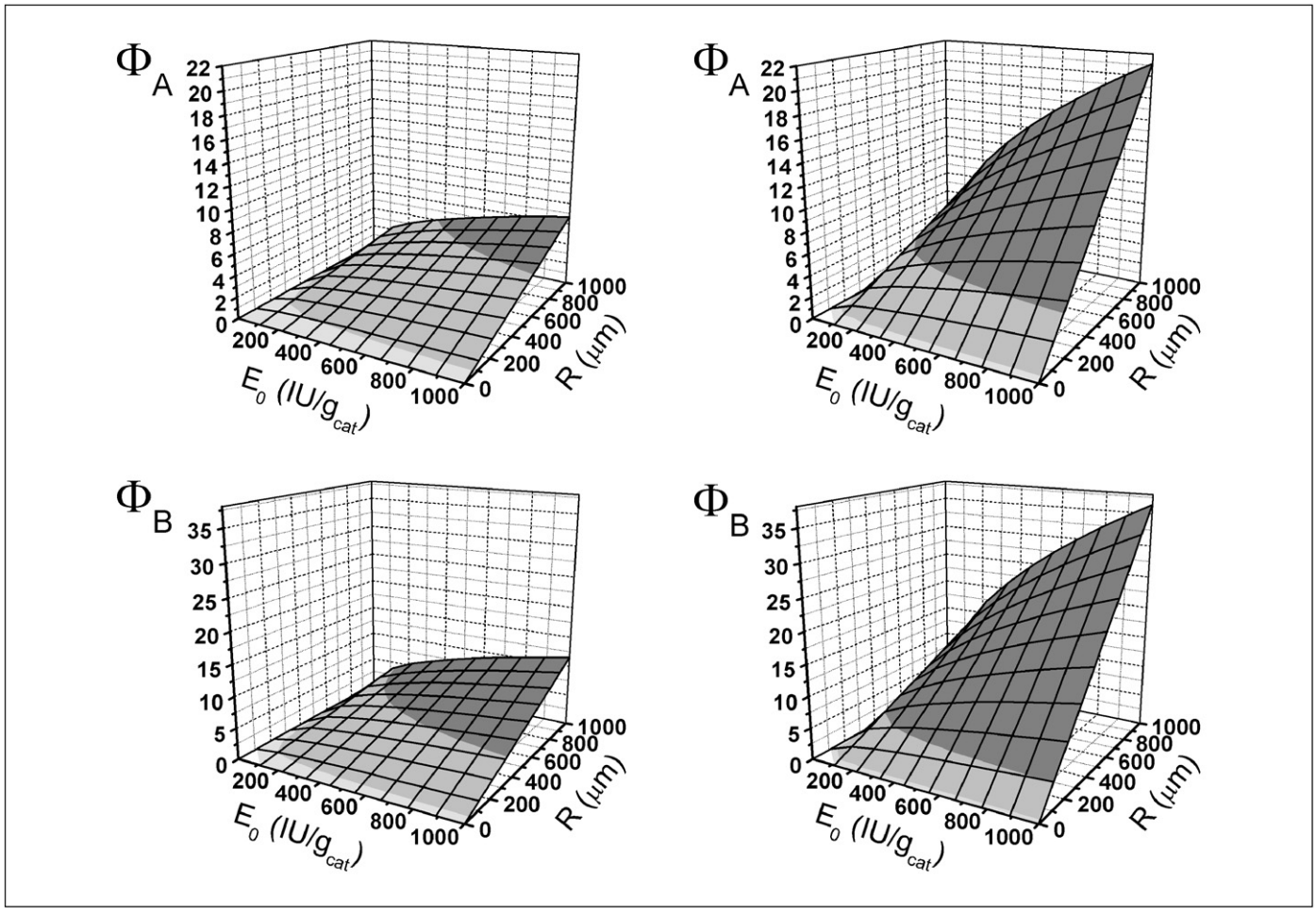

FIGURE 4

Thièle moduli (Φ_A and Φ_B) as a function of catalyst enzyme loading (E_0) and particle radius (R) at 20 mM (left) and 200 mM (right) ADCA concentration, and at 60 mM (left) and 600 mM (right) PGME concentration. Clear gray: $\Phi_A < 0.4$; medium gray: $0.4 < \Phi_A < 4$; dark gray: $\Phi_A > 4$.

(25) and Table 3) so that ADCA is more diffusionally restricted than PGME. Therefore, ADCA Thiële modulus was chosen to characterize the effect of IDR on Cex synthesis. In general terms, a PGA catalyst of an average loading of 400 IU/g_{cat} [11–13] will be essentially free of IDR for catalyst particles smaller than 100 μm; a moderate impact of IDR will occur for particles between 100 and 200 μm, and strong diffusion restrictions will ensue with catalyst particles bigger than 200 μm, as defined by Levenspiel for Thiële moduli smaller than 0.4, between 0.4 and 4 and greater than 4, respectively [18]. In this analysis Thiële moduli are affected by substrates concentrations; however, the effect of substrates mass transfer on Cex synthesis must be analyzed during reaction and quantified by process variables as yield and productivity.

Batch reactor operation

All kinetic and diffusion parameters were used in the reaction–diffusion model for simulating the batch reactor operation. Time course of substrate conversion yield at different initial concentrations of PGME and ADCA and ADCA Thiële moduli were obtained from liquid bulk concentration of Cex during batch reactor operation with the same amount of enzyme activity. These results are presented in Figure 5 showing than an increase in Thiële modulus produces a significant decrease in maximum yield and increase in time to achieve it, so reducing Cex productivity, while conversion yield increases with substrates concentration making the effect of the value of Thiële modulus less pronounced as mass transfer rate towards the catalyst porous matrix is increased. Nevertheless, these effects are the consequence of the combined effect of reaction and mass transfer inside the catalyst particle. In this way, an analysis of the time course of Cex synthesis is needed to understand the effect of IDR on conversion yield and productivity.

Profiles within the catalyst

The effect of IDR on the progression curves of Cex synthesis during batch reactor operation is explained by the behavior of reaction and mass transfer inside the catalyst particle. The simulation allows obtaining the behavior of reaction within the catalyst as a function of particle radius and time. This is shown in Figure 6 for the case represented in Figure 5d with $\Phi_B = 10$.

The reaction begins with a PGME/ADCA molar ratio of 3; however this molar ratio varies with respect to catalyst particle radius and time. Before the maximum Cex bulk concentration is reached, approximately at 8 min, a maximum in PGME/ADCA molar ratio with respect to the catalyst radius is obtained within the catalyst, which explains the maximum Cex concentration observed in Figure 5. After that maximum is reached in the bulk liquid phase, PGME/ADCA molar ratio is less than three for all radii inside catalyst, which significantly affect Cex production by favoring its hydrolysis. Cex hydrolysis/synthesis ratio within the catalyst particle is analyzed in Figure 6b. It can be appreciated that Cex synthesis is predominant at the beginning of the reaction and at the external half of the catalyst spherical particle. This is favored

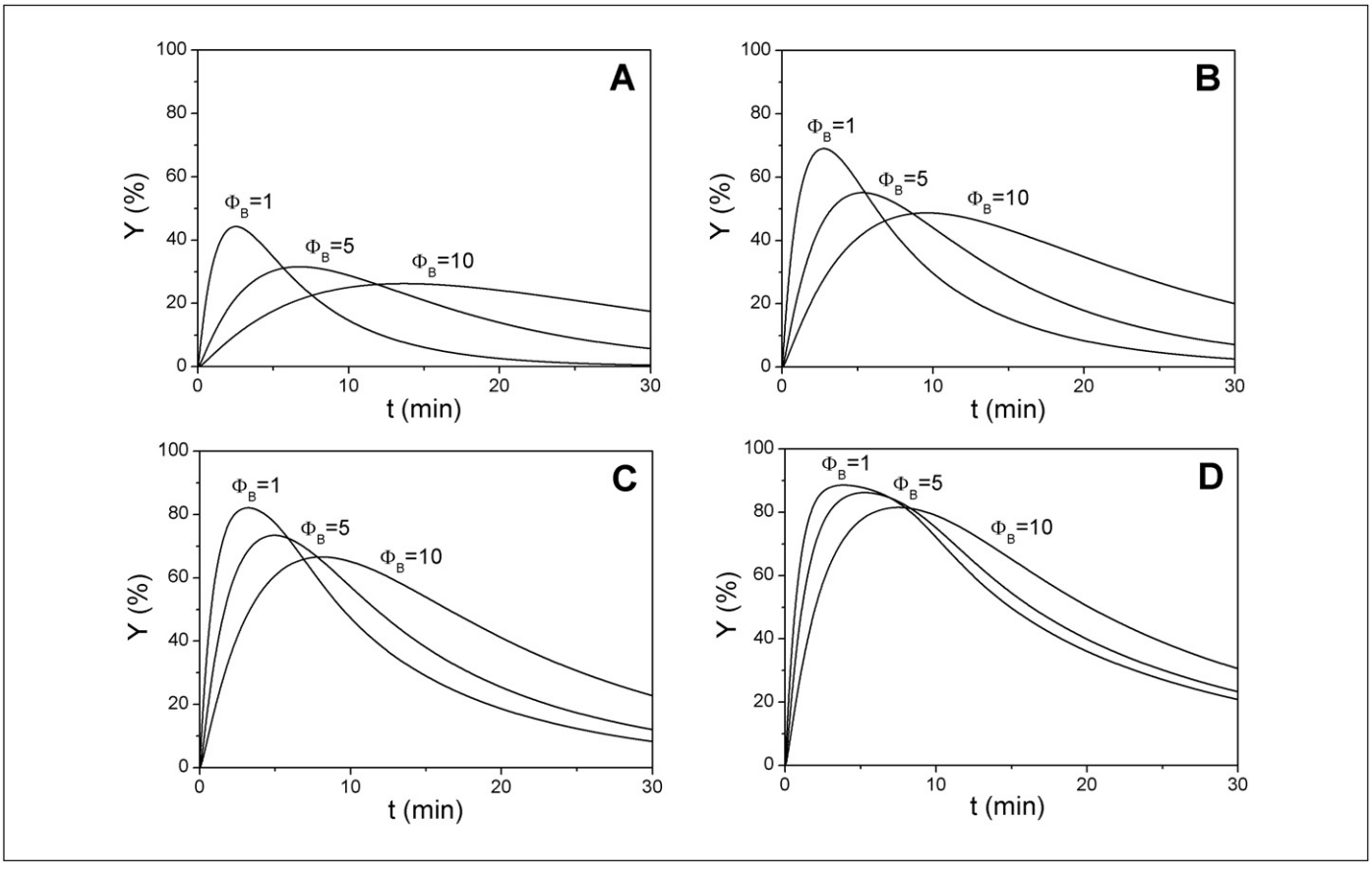

FIGURE 5

Time course of substrate conversion yield (*Y*) for Cex synthesis in batch reactor from **(a)** 20 mM, **(b)** 50 mM, **(c)** 100 mM and **(d)** 200 mM ADCA concentrations at a PGME/ADCA molar ratio of 3 for different values of ADCA Thiële modulus (Φ_B). Φ_A is 0.6, 2.90 and 5.8 when Φ_B is 1, 5 and 10, respectively.

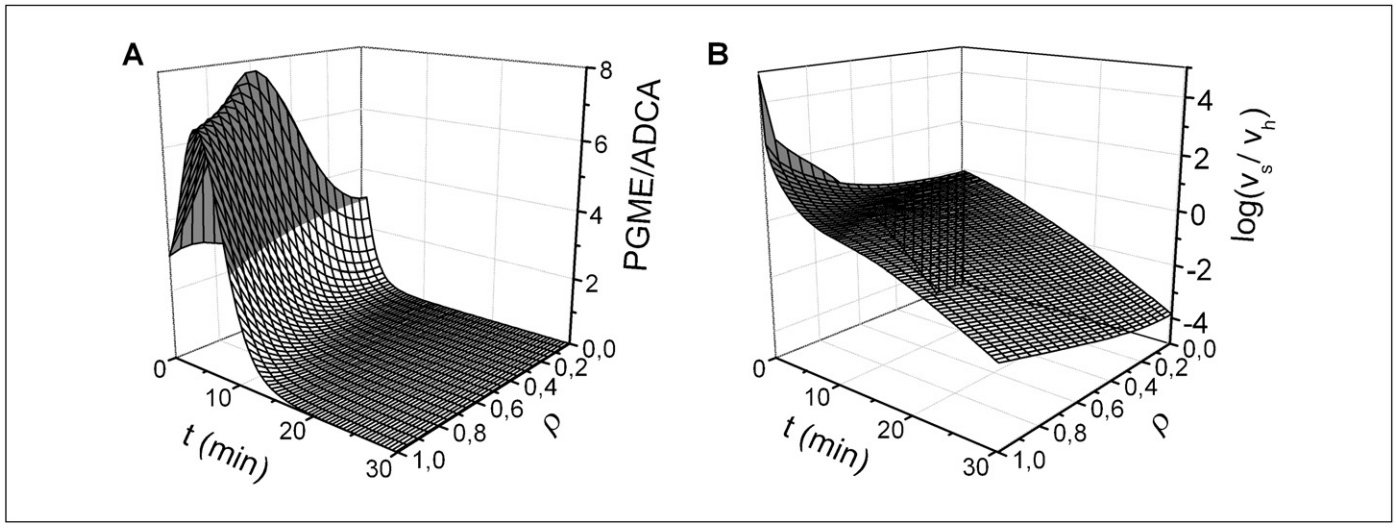

FIGURE 6

PGME/ADCA ratio **(a)** and Cex synthesis/hydrolysis ratio **(b)** as a function of dimensionless catalyst radius (ρ) and reaction time during Cex synthesis from 200 mM ADCA and PGME 600 mM with ADCA Thiele modulus (Φ_B) 10. Gray surface indicates PGME/ADCA ratio over three.

by a high PGME/ADCA molar ratio, as seen in Figure 6a. The most important observation from these results is that an increase in the magnitude of IDR favors hydrolysis over synthesis so impairing Cex conversion yield and productivity as observed in Figure 5. The way in which this is reflected on reactor performance is now analyzed in terms of response variables during reactor operation.

Yield and specific productivity

Specific productivity, defined by Eq. (29), was used to evaluate reactor performance under mass transfer limitations in PGA catalysts. Maximum conversion yield was defined as the maximum concentration of Cex obtained with respect to initial ADCA concentration, the stoichiometric limiting substrate in the kinetically controlled Cex synthesis. As seen in Figure 7a, maximum conversion yield is strongly affected by initial substrates concentration, as previously reported [11,19,20]. The effect of IDR on maximum conversion yield is milder but, anyhow, a significant variation from 91 to 74% at 200 mM ADCA and 600 mM PGME is obtained when Φ_B increases from 1 to 10, which is a significant variation from a process perspective. As observed in Figure 7b, both initial ADCA concentration and ADCA Thiele modulus (Φ_B) strongly affect specific productivity. The effect is stronger at high ADCA concentration. These results show the impact of IDR characterized by the Thiele modulus, which is a function of catalyst enzyme loading and particle size (Eq. (25)). Specific productivity was then analyzed as a function of catalyst enzyme loading and particle size. Initial conditions used for batch reactor simulation were 200 mM ADCA and 600 mM PGME with a catalyst concentration of 38 g/l corresponding to 25,000 IU/l. With this analysis, an approximation to the process gains versus enzyme expense was done. Specific

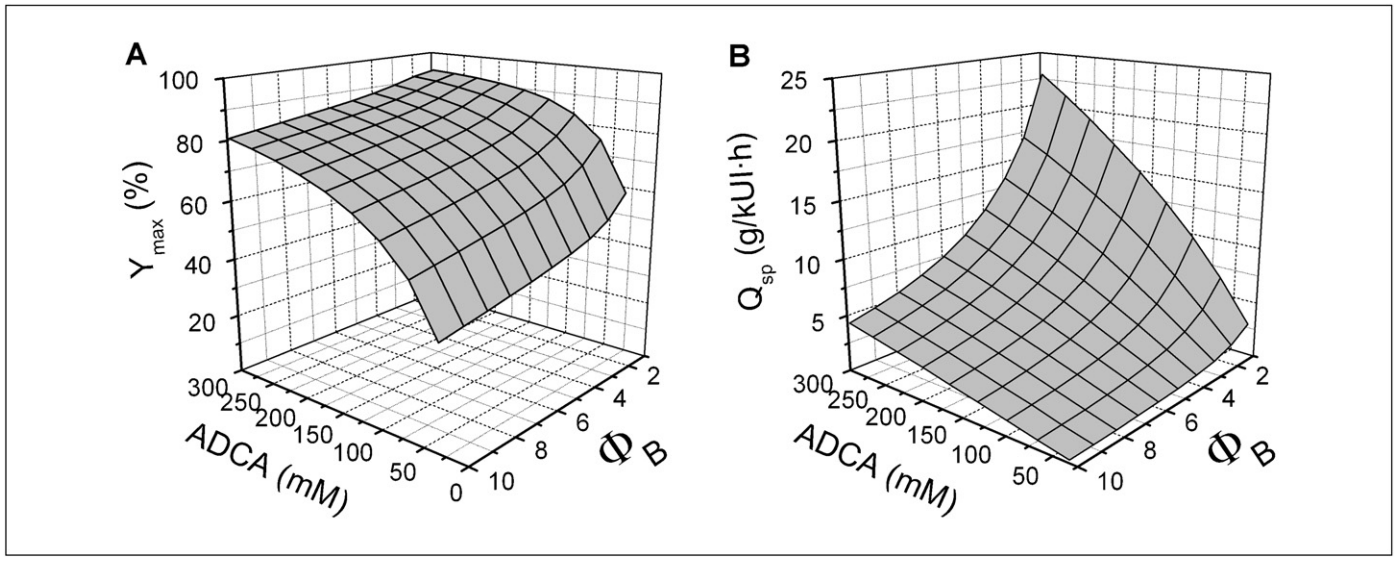

FIGURE 7

Maximum conversion yield (Y_{max}) **(a)** and specific productivity (Q_{sp}) **(b)** of Cex synthesis as a function of ADCA initial concentration and ADCA Thiele modulus (Φ_B), at PGME/ADCA molar ratio of 3.

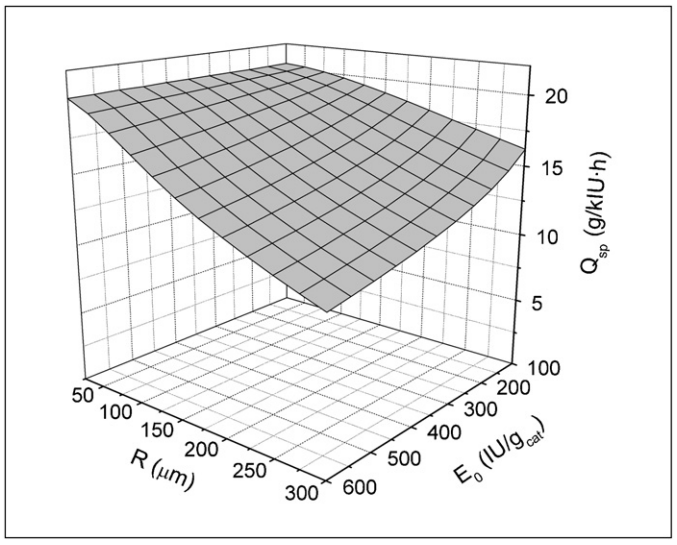

FIGURE 8

Specific productivity of Cex synthesis as a function of catalyst enzyme loading and particle size at 200 mM ADCA initial concentration at PGME/ADCA molar ratio of 3.

productivity as a function of catalyst enzyme loading and particle size is presented in Figure 8, showing that increasing catalyst enzyme loading increases the amount of Cex obtained per unit of enzyme in a non-linear fashion, on the contrary from when the free enzyme is used. In this way, a gain–cost estimate for product obtained and enzyme expense emerges. From these results we can compare two PGA catalysts of 400 UI/g_{cat} with different particle sizes of 25 and 300 μm in a reaction volume of 1 m^3. The production of Cex would vary from 323 to 190 kg/h, which is a very significant difference that will affect reactor and catalyst design during process optimization.

Conclusions

The presence of IDR in PGA-glyoxyl agarose catalysts affects the kinetically controlled synthesis of Cex, decreasing yield and productivity. These effects are a consequence of mass transfer limitations within the catalyst particle that favor hydrolysis reactions of the acyl donor PGME and the product Cex over the reaction of synthesis. Catalyst enzyme loading and particle size are relevant characteristics of catalyst design that strongly affect the specific productivity of Cex, by modulating the magnitude of IDR. A nonlinear relationship between catalyst enzyme loading and specific productivity of Cex was obtained with the implication that an increase in catalyst enzyme loading will not increase the volumetric productivity by the same magnitude as it occurs with the free enzyme. In consequence, the gap between gain and cost narrows when higher enzyme loading and particle size are used. This is a relevant finding that needs to be adopted during the optimization of catalyst and reactor design considering catalyst enzyme loading and particle size as the most important variables. The approach presented can be extended to other processes catalyzed by immobilized enzymes.

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