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Kinetic modelling and simulation of laccase catalyzed degradation of reactive textile dyes

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Abstract

A kinetic model based on Michaelis–Menten equation was developed to simulate the dye decolourisation of Reactive Black 5 (RB5), Reactive Blue 114 (RB114), Reactive Yellow 15 (RY15), Reactive Red 239 (RR239) and Reactive Red 180 (RR180) dyes by commercial laccase. The unusual kinetic behavior of some of these reactions suggests that the kinetic model must consider the activation of the laccase-mediator system. Several reactions at different concentrations of each dye were performed in batch reactors and time courses were obtained. A LSODE code to solve the differential equation obtained from the batch reactor was combined with an optimization Fortran program to obtain the theoretical time courses. The time courses obtained from the developed program were compared with the experimentally obtained ones to estimate the kinetic constants that minimized the difference between them. The close correlation between the predicted and the experimental results seems to support the reliability of the established models.

Keywords: Reactive dyes; Commercial laccase; Kinetic constants; Modelling; Enzyme catalysis

1. Introduction

Considering both volume discharged and effluent composition, the wastewater generated by the textile industry is rated as the most polluting among all industrial sectors (Vandevivere et al., 1998). The presence of very low concentrations of dyes in effluent is highly visible and undesirable (Nigam et al., 2000). There are more than 100,000 commercially available dyes with over 7×10^5 ton of dyestuff produced annually (Robinson et al., 2001). The dyes do not bind completely to the fabric and depending on the class of the dye its loss in wastewaters could vary from 2% for basic dyes to as high as 50% for reactive dyes (Pandey et al., 2007), causing serious environmental problems. The presence of color in water will affect the transmission of light and photosynthesis and reduce aquatic diversity.

Many dyes are difficult to decolourise due to their complex structure and synthetic origin. A variety of physical, chemical and biological methods are presently available for the treatment of textile wastewater (Slokar and Marechal, 1998; Forgacs et al., 2004; Anjaneyulu et al., 2005). There is a growing recognition that enzymes can be used in many remediation processes to target specific pollutants for treatment (Durán and Esposito, 2000). Different studies show that extracellular ligninolytic enzymes of white-rot fungi can degrade a wide variety of recalcitrant compounds, such as dyes (Chivukula and Renganathan, 1995; Nyanhongo et al., 2002; Harazono et al., 2003; Lan et al., 2006). Laccase (*p*-diphenol oxidase, EC 1.10.3.2) catalyzes the oxidation of phenolic compounds and aromatic amines and accepts a broad range of substrates (Thurston, 1994; Claus, 2004). The number of substrates can be

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extended by addition of a redox mediator (Bourbonnais and Paice, 1990; Soares et al., 2001). The mediator is a compound with low molecular weight which acts as a kind of electron carrier. Once it is oxidised by the enzyme, it diffuses away from the catalytic system and in turn oxidises any substrate that, due to its size, could not be directly oxidised by the enzyme (Call and Mucke, 1997).

The kinetics of a reaction system can be studied by a mathematical modelling technique. The most important matter is to find an accurate model equation that has the capability to estimate the value of the reaction rate close to the experimentally observed one. The model of a certain reaction system should consider all the important parameters. Once formulated, it can be solved and the predicted behavior can be compared with experimental data. Any significant differences between the system performance and the behavior predicted by the model imply that there may be some other important effects not considered. The kinetic constants are estimated by fitting the model equation to the experimental data. The model can then be used for such purposes as predicting the system performance under different operating conditions, reactor design, scale-up, optimization and control of the system (Houng and Liau, 2006; Bas et al., 2007).

Various studies on dye degradation by laccase have been published (Wong and Yu, 1999; Abadulla et al., 2000; Campos et al., 2001; Kandelbauer et al., 2004). However, not so many papers have actually dealt with the kinetics of the reaction. Some works reported the values of kinetic constants of enzymatic dye degradation obtained from initial reaction rates (Almansa et al., 2004; Moldes and Sanromán, 2006), but to our knowledge there has been no study that determined the kinetic constants of enzymatic dye decolourisation from the entire time course data.

The aim of this work is to establish a mathematical model and to determine the kinetic constants that can adequately describe the kinetic behavior of five reactive textile dyes (Reactive Black 5 (RB5), Reactive Blue 114 (RB114), Reactive Yellow 15 (RY15), Reactive Red 239 (RR239) and Reactive Red 180 (RR180)) decolourisation by a commercial laccase. Success of the model was determined by comparing the time courses obtained experimentally with those obtained from the model.

2. Methods

2.1. Chemicals and enzyme

Textile dyes: Reactive Black 5 (Remazol Black B), Reactive Blue 114 (Levafix Brilliant Blue E-BRA), Reactive Yellow 15 (Remazol Yellow GR), Reactive Red 239 (Remazol Brilliant Red 3BS) and Reactive Red 180 (Remazol Brilliant Red F3B) were kindly provided by DyStar (Portugal) and were used for degradation experiments without any further purification.

Enzyme: Commercial laccase formulation (DeniLite IIS; 120 U/g) from genetically modified *Aspergillus* was kindly provided by Novozymes. This formulation is used for indigo dye decolourisation in denim finishing operations and includes a buffer and an enzyme mediator.

2.2. Dye decolourisation kinetics

Preliminary studies of optimization of laccase-catalyzed decolourisation of reactive dyes by response surface methodology enabled us to establish the optimal conditions of pH, temperature and enzyme mass used in reactor for each degradation. However, the commercial laccase formulation is a heterogeneous mixture. Reactions with the optimal enzyme mass showed that it was not possible to get reproducible results with this amount of enzyme. The amount of enzyme used was not representative of the composition of the mixture. Assays were carried out where the enzyme mass was increased five times, obtaining, by this way, reproducible results. However, unexpectedly, the reactions with more enzyme loads exhibited an apparent lesser degradation. In order to understand this phenomenon, a batch without dye, i.e., with only the enzyme and a buffer was carried out under stirring. It was observed that, after a short period of time, some turbidity appeared that increased with time. This fact was not observed when a small amount of enzyme was used. Thus, this turbidity (apparent colour) masks the effective degradation and contributes for the observed lesser degradation. To cope with this problem, the enzyme and buffer solution absorbances were measured along time at the maximum absorbance wavelength of each dye and their values were subtracted from the corresponding absorbance value of the dye degradation reaction.

So, to study the kinetic behavior of the five reactive textile dyes, five different concentrations of each dye (from 25 mg/L to 125 mg/L) and 432 U/L of commercial laccase were incubated in 50 mL Erlenmeyer flasks at 35 °C with phosphate buffer (50 mM/ pH 7.0) under stirring. For each assay duplicate runs were made.

After taking a zero sample, decolourisation was started by the addition of laccase. Samples were withdrawn at certain time intervals that increased as the reaction proceeded. The samples were subsequently analyzed by UV–vis spectrophotometry.

2.3. Determination of dye concentration

Dye concentration was determined through a calibration curve by reading the absorbance of the samples at the maximum absorbance wavelength for each dye: Reactive Black 5 (579 nm), Reactive Blue 114 (593 nm), Reactive Yellow 15 (416 nm), Reactive Red 239 (542 nm) and Reactive Red 180 (540 nm). UV–vis spectrophotometer (Thermo, model UV1) was used in all experiments. By measuring the amount of the substrate remaining overtime it is possible to obtain the concentration versus time plot which is known as progress curve of the enzymatic reaction or time course.

2.4. Kinetic modelling: optimization and simulation

2.4.1. Kinetics of enzymatic reactions

In 1913, Michaelis and Menten proposed a reaction mechanism for enzymatic reactions. As a starting point, it is assumed that the enzyme and substrate combine to form a complex, which then dissociates into product and free enzyme as follows:

$$E + S \overset{K_{MS}}{\leftrightarrow} ES \overset{k_2}{\rightarrow} E + P \quad (1)$$

where E, S, ES and P are enzyme, substrate, enzyme–substrate complex and product, respectively. K_{MS} and k_2 represent Michaelis–Menten constant and catalytic rate constant, respectively. The dependence of the reaction rate (v) on the substrate concentration is represented by the Michaelis–Menten equation and can be derived as

$$v = k_2[ES] = \frac{V_{max}[S]}{K_{MS} + [S]} \quad (2)$$

where V_{max} is the maximum reaction rate and [ES] and [S] are concentrations of the enzyme–substrate complex and of the substrate, respectively.

Preliminary studies showed that the degradation of some reactive dyes by commercial laccase is not complete. So, to describe the kinetics of these enzymatic decolourisations the irreversible (Eq. (2)) and the reversible (Eq. (3)) forms of Michaelis–Menten equation were employed (Murzin and Salmi, 2005)

$$v = \frac{V_{max}\left([S] - \frac{[P]}{K_{eq}}\right)}{K_{MS}\left(1 + \frac{[P]}{K_{MP}}\right) + [S]} \quad (3)$$

where K_{eq} is the equilibrium constant and K_{MP} is the Michaelis–Menten constant for product. [P] is the product concentration.

2.4.2. Batch reactor balance

The differential equation obtained from the mass balance to a batch reactor is given by

$$-\frac{d[S]}{dt} = v \cdot W_E \cdot \frac{1}{V_L} \quad (4)$$

where W_E is the enzyme mass, V_L is the volume of the reactor and t is the time.

2.4.3. Activation of the laccase-mediator system

In general enzymatic reactions, the reaction rate increases with the increase of substrate concentration. When the substrate concentration increases up to a certain high value, the reaction rate reaches a plateau and keeps constant even if more substrate is used. On preliminary studies of dye degradation reactions it was possible to observe for some reactive dyes an unusual kinetic behavior: there is a short period of time in the beginning of the reaction that does not follow the Michaelis–Menten equation. At this point an increase in the concentration of substrate causes only a very small increase in the rate – the slope is less than predicted. Preliminary results have also shown that pure laccase did not decolourise the five reactive textile dyes under study, indicating that the presence of a mediator to oxidise the dyes was required. The oxidation was not carried out by the enzyme directly, but rather by the oxidised form of the mediator. So, the time period described seems to correspond to an induction time where an activation effect of the laccase-mediator system occurs. This situation is similar to a perfectly agitated solution where the enzyme and the mediator are present, but initially they cannot oxidise the substrate. They need a period of time to be activated, that is represented by an exponential term. So, in order to predict this period for some of the dyes a new term, taking into account this induction time, was added to the equations previously presented

$$v = \frac{V_{max}[S]}{K_{MS} + [S]}(1 - e^{-kt}) \quad (5)$$

$$v = \frac{V_{max}\left([S] - \frac{[P]}{K_{eq}}\right)}{K_{MS}\left(1 + \frac{[P]}{K_{MP}}\right) + [S]}(1 - e^{-kt}) \quad (6)$$

where k is the rate constant of the activation period of the laccase-mediator system.

2.4.4. Estimation of the reaction kinetics and kinetic constants

To predict dye concentrations versus time profile in the batch reactor, the system of equations obtained from the batch reactor balance (Eq. (4)) and from the proposed kinetics for the enzymatic reactions (Eqs. (2), (3), (5) or (6)) was solved using a Fortran program with integration by LSODE solver (Livermore solver for ordinary differential equations) based on Adams backward differentiation formula methods. The initial concentrations of dye and variables were fed to the program. This program was combined with an optimization algorithm to estimate the kinetic constants of the proposed model by minimizing the difference between the predicted time courses and the ones obtained experimentally. The function to minimize between both time courses was the sum of squared relative residuals and was calculated as

$$F = \sum_{1}^{n} \left| \frac{C_{calc} - C_{exp}}{C_{exp}} \right|^2 \quad (7)$$

where C_{calc} is the concentration calculated using the model equation, C_{exp} is the experimental concentration and n is the total number of experimental or calculated points. The function was minimized through an optimization loop based on an adaptative random search algorithm that varies the values of the kinetic constants until a global minimum within the optimization criteria is achieved (Salcedo

et al., 1990; Salcedo, 1992). This procedure was repeated for each dye studied.

3. Results and discussion

3.1. Kinetic modelling and estimation of kinetic constants

A kinetic model to simulate the decolourisation of each of the five reactive textile dyes in a batch reactor by a commercial laccase formulation containing a specific mediator was proposed. Some studies related to dye degradation present kinetic constants values based on initial reaction rates (Soares et al., 2002; Kandelbauer et al., 2004). In this work, the kinetic constants of the proposed models were estimated by comparing the experimental time courses with the predicted ones for each dye.

Experimental time courses were obtained in duplicate at five different initial dye concentrations for each dye. For RB5, RB114 and RY15, it was observed that initially the dye concentration almost do not decrease, probably corresponding to the activation time of the laccase-mediator system. So, the kinetic model for these dyes must present an exponential term to predict this induction time. For RB5 and RB114 the dye concentration decreases with the increase of time until reaching a plateau where no more dye is degraded, while the experiments with RY15, RR239 and RR180 presented complete degradation.

A program using the LSODE code to solve the differential equation obtained from the batch reactor balance (Eq. (4)) considering the proposed kinetics for the enzymatic reaction (Eq. (2) for RR239 and RR180, Eq. (5) for RY15 and Eq. (6) for RB5 and RB114) was combined with an optimization program (Salcedo et al., 1990; Salcedo, 1992) to obtain the theoretical time courses. The algorithm developed adjusts automatically the kinetic constants so that the output response (predicted by the model) and the input values (experimental data) were as close as possible according to the objective function (Eq. (7)). Estimation was made and the results were compared with the corresponding experimental value. This process was repeated while the relative errors between the estimated and the experimental values decreased.

The obtained kinetic constants for each dye considering the selected kinetic model are shown in Table 1, which were used to draw the continuous lines in Figs. 1–5.

In a system without a mediator, the Michaelis–Menten constant K_{MS} is the equilibrium constant of the enzyme–substrate complex decomposition, so, by analogy, in a system with a mediator the K_{MS} constant is probably the equilibrium constant of the enzyme-mediator/substrate complex decomposition. So, it is a measure of the enzyme-mediator system affinity to the substrate. The lesser the value of K_{MS}, the larger is the affinity of the enzyme-mediator system to the substrate. Comparing the K_{MS} values of each dye, the laccase-mediator system affinities for the dyes decrease according to the following order: RB5 > RB114 > RY15 > RR239 > RR180. This result is easy to confirm in Figs. 1–5 by the time that each dye takes

Table 1
Proposed model, kinetic data and average sum of squared relative residuals (SSRR) for RB5, RB114, RY15, RR239 and RR180 degradation by commercial laccase

	RB5	RB114	RY15	RR239	RR180
Kinetic equation	(6)	(6)	(4)	(2)	(2)
V_{max} (mg/g min)	20.000	18.677	37.500	1.190	3.460
K_{MS} (mg/L)	3.1384	21.283	99.000	165.000	650.000
K_{MP} (mg/L)	300.0	450.0	–	–	–
K_{eq}	15.000	2.278	–	–	–
k (min^{-1})	0.192	0.067	0.081	–	–
SSRR/n	8.836×10^{-2}	4.948×10^{-3}	918.548	6.233	6.823

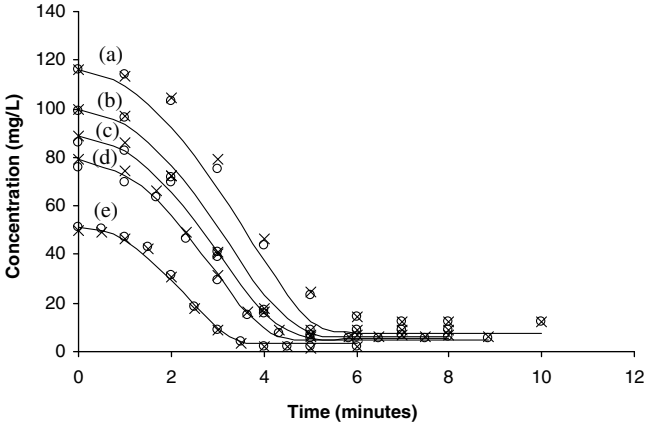

Fig. 1. Comparison of experimental (× run 1; ○ run 2) and simulated (continuous line) time courses of RB5 degradation by commercial laccase under different dye initial concentrations: (a) 116.0 mg/L and 115.8 mg/L; (b) 99.5 mg/L and 99.3 mg/L; (c) 88.5 mg/L and 85.8 mg/L; (d) 78.9 mg/L and 76.0 mg/L and (e) 49.9 mg/L and 51.5 mg/L.

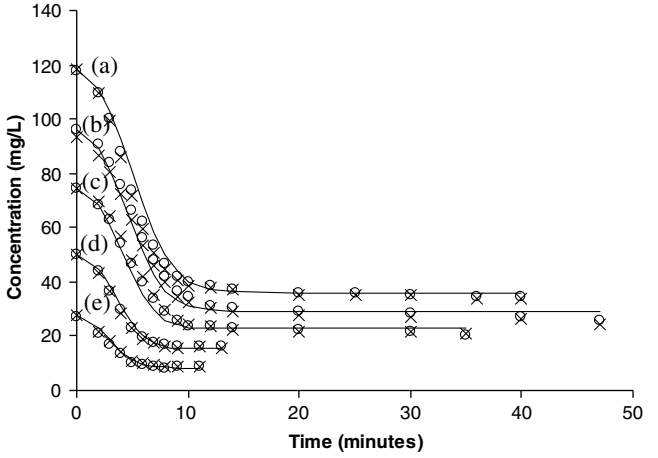

Fig. 2. Comparison of experimental (× run 1; ○ run 2) and simulated (continuous line) time courses of RB114 degradation by commercial laccase under different dye initial concentrations: (a) 118.4 mg/L and 117.8 mg/L; (b) 93.2 mg/L and 96.2 mg/L; (c) 74.6 mg/L and 74.1 mg/L; (d) 50.3 mg/L and 50.4 mg/L and (e) 27.6 mg/L and 27.3 mg/L.

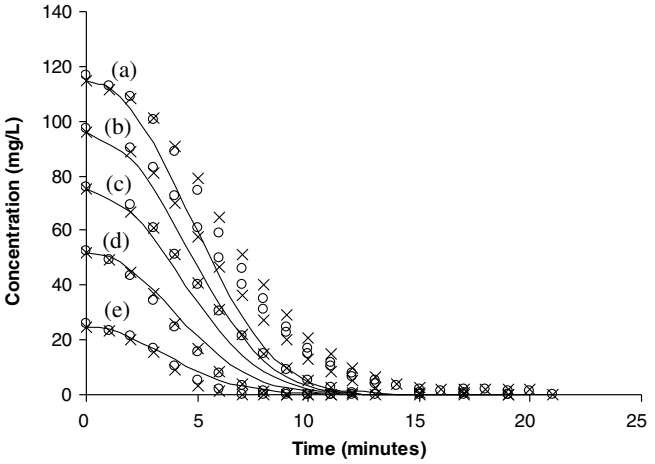

Fig. 3. Comparison of experimental (× run 1; ○ run 2) and simulated (continuous line) time courses of RY15 degradation by commercial laccase under different dye initial concentrations: (a) 116.8 mg/L and 116.9 mg/L; (b) 95.9 mg/L and 97.4 mg/L; (c) 75.3 mg/L and 76.1 mg/L; (d) 47.7 mg/L and 49.1 mg/L and (e) 25.3 mg/L and 25.6 mg/L.

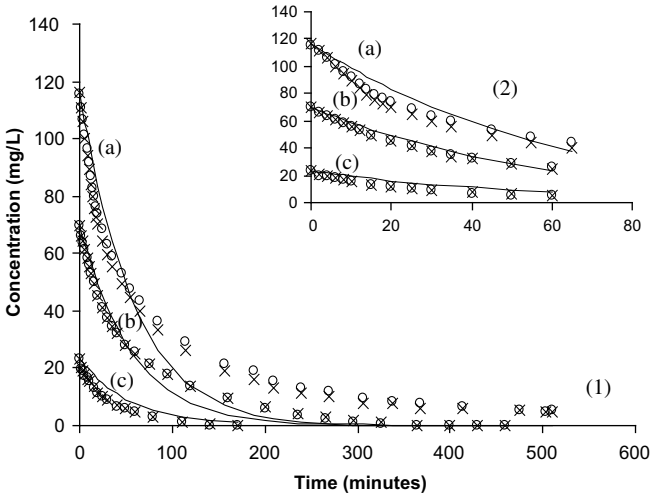

Fig. 5. (1) Comparison of experimental (× run 1; ○ run 2) and simulated (continuous line) time courses of RR180 degradation by commercial laccase under different dye initial concentrations: (a) 116.3 mg/L and 115.8 mg/L; (b) 69.9 mg/L and 69.8 mg/L and (c) 23.4 mg/L and 23.0 mg/L. (2) Scale amplification of (1).

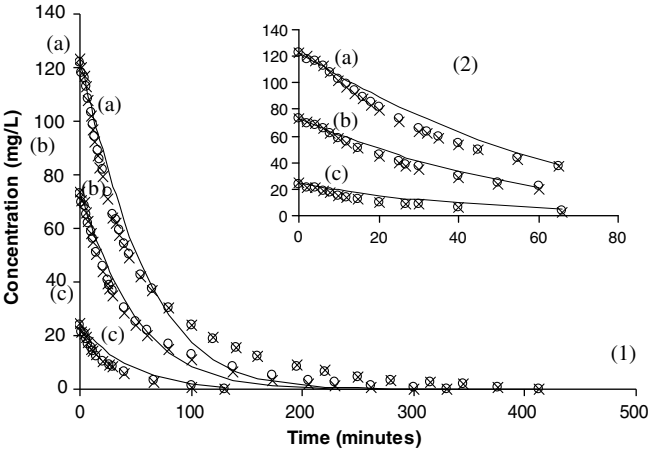

Fig. 4. (1) Comparison of experimental (× run 1; ○ run 2) and simulated (continuous line) time courses of RR239 degradation by commercial laccase under different dye initial concentrations: (a) 123.0 mg/L and 122.2 mg/L; (b) 73.7 mg/L and 72.8 mg/L and (c) 24.3 mg/L and 24.0 mg/L. (2) Scale amplification of (1).

to reach the equilibrium. A larger dyes degradation time corresponds to a more difficult decolourisation (less affinity of laccase-mediator system for the dye), probably due to the structure of the dye or to the difference of redox potential between the dye and the laccase-mediator system (Almansa et al., 2004; Kandelbauer et al., 2004; Zille et al., 2004). The velocity of dyes degradation can also be evaluated by the value of V_{max} – the theoretical maximal velocity, which is proportional to the kinetic constant k_2. According to the values of V_{max} presented in Table 1 for each dye studied, the degradation of dyes RB5, RB114 and RY15 by commercial laccase is faster than the degradation of RR239 and RR180, that present lower values of V_{max}.

The activation of the laccase-mediator system is another important aspect to be considered. The present study enables one to evaluate the activation rate of the laccase-mediator system on RB5, RB114 and RY15 dyes degradation by inspection of the k values obtained in the kinetic model (Table 1). According to the results, the activation of the laccase-mediator system is faster in the degradation of RB5 and decreases in the following order: RB5 > RY15 > RB114. To our knowledge, this is the first report on the activation of the laccase-mediator system on dyes degradation by enzyme laccase.

In order to evaluate the adequacy of the proposed model for the kinetics of dye decolourisation by commercial laccase, the time courses calculated by the kinetic models were compared with the experimental ones. These comparisons for RB5, RB114, RY15, RR239 and RR180 degradation are presented in Figs. 1–5, respectively. For RR239 and RR180 only three different concentrations are presented to avoid overloading the graphs.

The comparisons show that the model proposed, including an induction period and reversible reaction (Eq. (6)), describes with remarkable accuracy the RB114 degradation (Fig. 2) and less well the RB5 degradation (Fig. 1). The degradation of the remaining dyes seems to be irreversible since the experimentally measured concentrations, after subtracting the turbidity communicated by the enzyme support, tends to zero. In these cases, whether with (RY 15, Fig. 3) or without (RR239, Fig. 4 and RR180, Fig. 5) an induction period, the proposed models, although exhibiting a correct qualitative behaviour, present some systematic quantitative deviations, more evident for the yellow dye RY15. The results in Table 1 show that Eq. (5) seems not to be applicable to RY15; this is probably due to the fact that both this dye and the enzyme plus buffer solution show their maximum absorbances at close wavelength

values. Note that the absorbance of the enzyme plus buffer solution was subtracted during the treatment of the results, probably masking the dye absorbance results for this case. It should be noted that if the turbidity (apparent colour) due to the enzyme support were not subtracted, the last models would do a better job. Since this apparent colour is not very reproducible, some work has to be done yet in order to try to understand what is really happening. Nonetheless, the proposed mathematical models with the parameters values displayed in Table 1, can be considered to represent within reasonable accuracy the observed dyes degradations and the overall behaviour of these systems. In principle, the models can be used whenever the commercial laccase is used in the degradation of reactive dyes. But the fact that the behavior of the commercial laccase changes with the colour of the dye indicates that they should be used with care.

4. Conclusions

Mathematical models based on Michaelis–Menten equation were proposed for reactive dyes degradation by commercial laccase in a batch reactor.

In this study, kinetic constants were determined by minimizing the difference between the time courses predicted by the model and experimental ones. The similarity between the experimental data and the predicted values for all dyes studied indicates that the proposed models could simulate successfully the kinetic behavior of reactive dyes degradation by commercial laccase.

The models allow to examine the effects of the considered process parameters on decolourisation of reactive dyes by commercial laccase and can be used to predict the time courses of the substrate consumption and the product formation under different substrate concentrations. The knowledge of the kinetic models of these reactions also provides an emergent tool that can be applied to the simulation and design of enzymatic bioreactors.

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