# Scaling up and kinetic model validation of Direct Black 22 degradation by immobilized *Penicillium chrysogenum*

Ignacio Durruty, Jorge Froilán González and Erika Alejandra Wolski

## ABSTRACT

This research was undertaken to develop tools that facilitate the industrial application of an immobilized loofah–fungi system to degrade Direct Black 22 (DB22) azo dye. In laboratory-scale tests, the DB22, and loofah as support, were used. Assays without loofah were used as a free-cells control. The use of natural carriers to facilitate adhesion and growth of the fungi has shown favorable results. The degradation rate of immobilized cells increased twice as compared to free-cells control. At day 5 the decolorization was almost complete, while without loofah the total decolorization took more than 10 days. After 10 days, the extent of growth was nine times higher for the immobilized assays in comparison with the control flask. In subsequent experiments decolorization of DB22 was proven in a bench-scale reactor. A previously developed kinetic model was validated during the process. The model validation over free-cells assays gives an average normalized root mean squared error (ANRMSE) of 0.1659. Recalibration steps allowed prediction of the degradation with immobilized cells, resulting in an ANRMSE of 0.1891. A new calibration of the model during the scaling-up process yielded an ANRMSE of 0.1136 for DB22. The results presented encourage the use of this modeling tool in industrial scale facilities.

**Key words** | bench-scale bioreactor, Direct Black 22, fungi immobilization, model validation

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## INTRODUCTION

The textile industry is very relevant to the economic activity in Mar del Plata, Argentina. However, it produces a wastewater very difficult to treat, with an intense color, high pH, high salinity and high chemical oxygen demand (COD) due to the presence of azo dyes used for dyeing (Firmino *et al.* 2011; Sen *et al.* 2016). In Mar del Plata 32% of industrial wastes are dyes and solvents from the textile industry. Furthermore, seawater pollution has been detected as a consequence of illegal dumping of dyes over pluvial collectors from a dying facility (Anon 2007). There are regulations regarding the color limits in effluents, which vary in different countries. Even though the law regulating the wastewater discharges in Mar del Plata city (Law n°5965/1958, Buenos Aires Province) established the limits for several physicochemical parameters, it did not define any criteria for the color of the effluents or the presence of azo dyes, which results in an environmental risk.

Due to the textile wastewater complexity and chemical stability, several physicochemical treatment methods have been developed including coagulation, precipitation, activated carbon adsorption, filtration and oxidation. However, these traditional methods are expensive and usually not at all effective (Singh 2006; Sen *et al.* 2016). The fungal biological treatment has been found an attractive alternative since it is low-cost and effective (Rodríguez Couto 2009; Solís *et al.* 2012; Krastanov *et al.* 2013; Sen *et al.* 2016). Furthermore, the total mineralization can be achieved (Solís *et al.* 2012; Balapure *et al.* 2015). In addition, immobilized fungal cells have several advantages over free-growing cells. The immobilized preparations contain a higher concentration of biomass and have greater operational stability (Krastanov *et al.* 2013). Immobilized cell systems facilitate the separation of cells from the liquid medium. This leads to the possibility of biomass reuse and simplifies the operation of both the continuous culture and the subsequent downstream processes. Cell immobilization also decreases the apparent broth viscosity and makes the rheological features more favorable for oxygen supply and mass transfer. In addition, immobilized cultures tend to have a higher level of activity and are more resilient to

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environmental perturbations such as pH or exposure to toxic chemical concentrations than suspension cultures. Finally, immobilization protects the cells from shear (Rodríguez Couto & Toca-Herrera 2007; Rodríguez Couto 2009). Several immobilization beads and biosorbents have been used but, due to their closed embedding structures, the immobilization matrices based on polymeric gels usually have the inconvenience of restricted diffusion of reactants and degradation products (Vijayaraghavan & Yun 2007). A valid alternative is the use of a structural fibrous network like that of loofah sponge, which overcomes this problem, becoming therefore an attractive possibility in the treatment of industrial wastewaters (Iqbal & Saeed 2007; Saudagar *et al.* 2008). The immobilization matrix provided by the fibrous network of loofah sponge has extensive surface area, depressions and cavities making it ideally suited to allow microbial growth and prevent diffusive problems (Iqbal & Saeed 2007; Rodríguez Couto 2009).

Among dye degradation by fungi, the most widely studied are the ligninolytic fungi or white rot fungi (WRF) (Solís *et al.* 2012). However white rot fungi have two major limiting factors that hinder their applicability in the industry, namely: they require a lignocellulosic substrate and many species have slow growth kinetics (Saroj *et al.* 2014), which encourages the study of other kinds of fungi (Saroj *et al.* 2014; Durruty *et al.* 2015). Furthermore, the use of carriers has demonstrated good results on azo-dye decolorization using WRF (Rodríguez Couto 2009; Krastanov *et al.* 2013) but has not been investigated so far on non-WRF (Rodríguez Couto 2009; Solís *et al.* 2012) and this work represents the very first report on such use. Filamentous fungi have a natural tendency to adhere to surfaces and, in this way, become immobilized (Rodríguez Couto & Toca-Herrera 2007; Rodríguez Couto 2009).

Most of the research studies on dye degradation are conducted at Erlenmeyer flask scale (Rodríguez Couto 2009; Singh *et al.* 2010). Nevertheless, before an industrial application can be implemented, fungal bioreactors which can be operated under industrial conditions must be developed (Donati & Paludetto 1997; Blánquez *et al.* 2008; Anastasi *et al.* 2010). Many works studied the treatment of textile wastewaters in different reactor systems (Lourenco *et al.* 2015; Santos & Boaventura 2015; Jegatheesan *et al.* 2016). These studies are critical to allow the scaling up from the laboratory to the pilot plant (Anastasi *et al.* 2010; Zhang & He 2015). During the scaling-up process, experiments should be followed by a sound statistical and mathematical modeling analysis in order to demonstrate and improve the applicability (Donati & Paludetto 1997). The validation of the kinetics models on different conditions or during scaling up is of paramount importance since the model could fit adequately the data that have been used for fitting identification, but could perform poorly with new incoming data (Donoso-Bravo *et al.* 2011).

In this work, the main objective was to study loofah-immobilized fungi systems for the azo-compound degradation by *Penicillium chrysogenum* at laboratory scale and at bench scale, as a first step of scaling up. Finally the cross-validation of a previous developed kinetic model was performed.

## MATERIALS AND METHODS

### Azo dye

In engineering science it is customary to design for the worst case scenario. Since DB22 showed longer decolorization times among the previously tested dyes (Durruty *et al.* 2015), it was selected in this work to develop industrial application tools (scaling-up process, model validation and immobilization). Figure 1 shows the chemical structure of Direct Black 22 (DB22). The dye has a complex chemical structure, is one of the most common dyes used in the textile industry and its degradation by *P. chrysogenum* has already been proven (Durruty *et al.* 2015). In addition kinetic parameters of the proposed model are available for this dye (Durruty *et al.* 2015). The azo dye was kindly provided by Gabriela Fioramonti, manager of Gama S. A. (Mar del Plata, Argentina).

### Microorganism

A *P. chrysogenum* ERK 1 isolate (GenBank, accession numbers HQ336382 and HQ336383) was maintained in potato

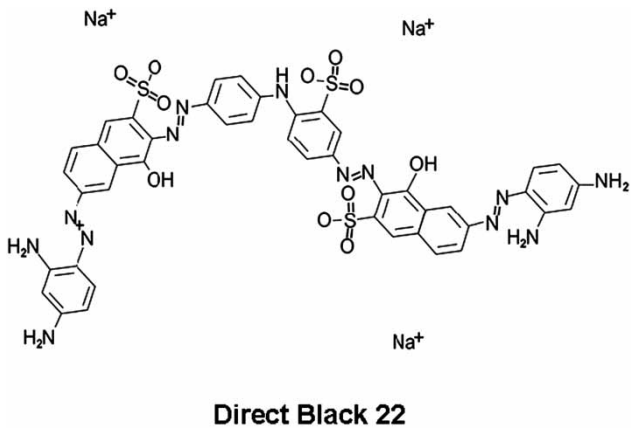


**Figure 1** | Chemical structure of Direct Black 22 (DB22).

dextrose agar (PDA, Gibco) at room temperature for 14 days. This fungus was isolated from commercial crop soils from Balcarce, Buenos Aires province, Argentina, as described by Wolski *et al.* (2012a).

### Inocula

As was described by Durruty *et al.* (2015), the fungus was inoculated directly from the PDA plate into 150 mL of liquid mineral salt (LMS) medium with 200 mg/L of azo dye, which had been previously sterilized. The composition of LMS medium was glucose 9 g/L, $MgSO_4 \cdot 7H_2O$ 0.1 g/L, $K_2HPO_4$ 1 g/L, $NH_4NO_3$ 1 g/L, KCl 0.1 g/L and 25 μL of trace elements solution (in mg/L: $MnSO_4$ 15.4, $FeCl_3$ 40, $ZnSO_4 \cdot 7H_2O$ 6.3, $CuSO_4 \cdot 5H_2O$ 2.5, $(NH_4)_6 \cdot MO_7 \cdot O_{24} \cdot 4H_2O$ 0.5). The fungus was grown for 8 days and filtered. This is the time during which the fungus grows actively. It was incubated in darkness at room temperature with orbital shaking at 120 rpm. The filtered conidia solution was used as inoculum in the decolorization assays. Inocula substrate ratio (ISR) was defined as the volumetric relation between the conidia solution and the final volume.

### Immobilization

The fungus was immobilized by attachment using loofah as carrier as described by Iqbal & Edyvean (2005). The use of a natural support instead of an artificial inert one to attach fungi is advantageous because immobilization material mimics the natural habitat of the fungi (Rodríguez Couto & Toca-Herrera 2007; Rodríguez Couto 2009).

The loofah was cut into cubes of $2.54 \times 2.54$ cm and autoclaved twice to kill spores. The carrier was inoculated with conidia solution to allow the nucleation and growth of attached *P. chrysogenum*. The fungal immobilization proceeded as the degradation assays were carried out. The attachment was evaluated by weighing the carrier before and after the degradation assay.

### Laboratory-scale assays

Decolorization/degradation of DB22 tests were performed in Erlenmeyer flasks with a working volume of 150 mL of LMS medium, with a final concentration of 200 mg/L of azo compounds, which is the concentration of the azo dyes present in real wastewater (Durruty *et al.* 2015). One gram of loofah per Erlenmeyer (6.7 g/L) was used as carrier in immobilization assays. All Erlenmeyer flasks were inoculated with 50 mL of conidia solution (ISR = 1/3). The inoculated flasks were incubated at room temperature (25 °C) with orbital shaking at 120 rpm. The initial pH was 7 and the run was carried out without pH control other than that provided by the media composition. All the assays started saturated with oxygen (8 mg/L). Tests without carrier were used as free-cells control and a non-inoculated assay with carrier was performed as sorption test.

### Bench-scale assays

The bench-scale bioreactor consisted of a heterogeneous stirred tank of 1.7 L capacity with 1 L of work volume with temperature, aeration and stirring control. Loofah was used as natural carrier for immobilizing the microorganism as in the laboratory-scale tests; however in this case, the carrier was used as a nest. The stirring speed was set at 150 rpm and air was supplied at 200 mL/min (0.2 vessel volume per minute (VVM)) and temperature fixed to 25 °C (room temperature). The initial pH was 7 and the run was carried out without pH control other than that provided by the media composition. The reactor started saturated with oxygen (8 mg/L).The same carrier/final-volume ratio and ISR were used in both flasks and reactor experiments.

### Biomass and growth substrate

Fungal growth was determined by measuring the mycelium dry weight. The fungal mycelium was filtered onto a Whatman GF/A filter, rinsed twice with distilled water, and dried at 100 °C until constant weight. For the immobilized assays, the sample was weighed together with the carrier. The mass of carrier had been previously weighed and subtracted from the fungus-plus-carrier mass. Biomass was expressed as milligrams of dry weight per volume of reactor (L).

Growth substrate concentration (mainly glucose) was determined by COD. COD is an indirect measure of organic matter commonly used in water and wastewater analysis (APHA 1998; Balapure *et al.* 2015; Jegatheesan *et al.* 2016). The COD was determined using method 5520 (closed reflux method) (APHA 1998).

### Kinetic model

A previously developed kinetic model of azo-compound degradation by *P. chrysogenum* (Durruty *et al.* 2015) was used in this work. The model uses Monod kinetics (Monod 1949) to predict the microbial growth (X) and

growth substrate (G) depletion.

$$\frac{dX}{dt} = \mu \cdot X = \frac{\mu_{max} \cdot G}{K_M + G} \cdot X \quad (1)$$

$$\frac{dG}{dt} = -\frac{1}{Y_{X/G}} \frac{\mu_{max} \cdot G}{K_M + G} \cdot X \quad (2)$$

where $\mu_{max}$ is the maximum specific growth rate, $K_M$ is the half constant saturation and $Y_{X/G}$ is the growth yield coefficient. Durruty *et al.* (2015) found that growth parameters are the same for all the azo-compounds studied.

The decolorization/degradation of the azo-compound ($S_i$) follows a Michaelis–Menten type equation associated with biomass growth.

$$\frac{dS_i}{dt} = b.\mu.X = \frac{b_{max,i} S_i}{K_{Si} + S_i} \frac{\mu_{max} \cdot G}{K_M + G} \cdot X \quad (3)$$

where $b_{max}$ is the maximum decolorization ratio, $K_S$ is the half saturation constant and the subscript $i$ represents the azo-compound.

Several mathematical models of textile wastewater degradation have been proposed on laboratory scale (Sandhya & Swaminathan 2006; Durruty *et al.* 2015). Among these, the non-structured models based on global parameters which are easy to estimate have demonstrated both their ability to predict the behavior of real systems at a minimum computational cost and their capability to be used in the design and optimization of reactors in wastewater treatment systems (Farhadi *et al.* 2012; Durruty *et al.* 2013; Durruty & Ayude 2014). The previously developed kinetic model (Durruty *et al.* 2015) used in this work falls into this non-structured kind of model and the parameters $b$ and $\mu_{max}$ considered both kinetic and substrate diffusive phenomena.

### Statistical and informatics tools

Non-linear regressions were performed with Origin 8.0® (OriginPro, v. 8.0724; Origin Lab Corporation, Northampton, MA 01060, USA). Once the kinetic parameters were set, the profiles were modeled using a fourth-order Runge–Kutta algorithm coupled to the regression in order to integrate the differential equations simultaneously (MathCad 14.0.0.163, Parametric Technology Corporation, Needham, USA).

To use the model as an evaluation tool two criteria were used: regression coefficient ($R^2$) of those parameters re-fitted on this work and root mean squared error (RMSE) for every output variable in all the cases. The RMSE is a common criterion to quantify the mean difference between simulated and measured values in the case of non-linear models (Kobayashi & Salam 2000; Quilot *et al.* 2005).

$$\text{RMSE} = \sqrt{\frac{1}{n}\sum_{i=0}^{n}(x_i - y_i)^2} \quad (4)$$

The normalization of the RMSE facilitates the comparison between datasets or models with different scales. With this aim, in the present work the previously used dimensionless normalized RMSE (NRMSE) was applied (Collard & McNally 2009; Han *et al.* 2010; Appeltant *et al.* 2011). NRMSE values greater than 0.5 were considered not to be relevant, values between 0.25 and 0.5 were considered not suitable, values lower than 0.25 were considered suitable, values lower than 0.2 were considered good and values lower than 0.1 were considered very good.

$$\text{NRMSE} = \frac{\sqrt{\frac{1}{n}\sum_{i=0}^{n}(x_i - y_i)^2}}{\bar{x}} \quad (5)$$

The global goodness-of-fit of the model was computed by averaging the NRMSE (ANRMSE) values of all variables.

## RESULTS AND DISCUSSION

In the present work, degradation of DB22 by *P. chrysogenum* at laboratory scale, immobilized on loofah, was tested. Figure 2 shows the mycelium of the fungus grown over loofah carrier in both Erlenmeyer and reactor scale after the decolorization process. This figure shows how the fungus grows macroscopically. In the no-carrier controls the fungal mycelia growths take the form of spheres (Figure 2(a)); however, in the presence of the carrier, all the mycelia growths attached to the loofah and no suspended mycelia were observed (Figure 2(b)).

Figure 3 shows the results of DB22 decolorization/degradation by *P. chrysogenum* at laboratory scale. The concentration of DB22 ($S_{DB22}$), the total organic load expressed as COD (G) and biomass (X) are depicted versus time for both the immobilized fungus on loofah and free-cells fungus. At day 5 the decolorization was almost complete, while without loofah the total decolorization took more than 10 days, indicating that the decolorization rate improved by more than twice in the presence of carrier. Furthermore, the fungal growth was improved in the presence of loofah as natural carrier. The growth was nine


**Figure 2** | Fungal growth: (a) no-carrier control after 15 days at laboratory scale, (b) with loofah as carrier after 5 days at laboratory scale, (c) reactor scale using a unique piece of loofah as carrier, after 5 days, and (d) detail of (c).

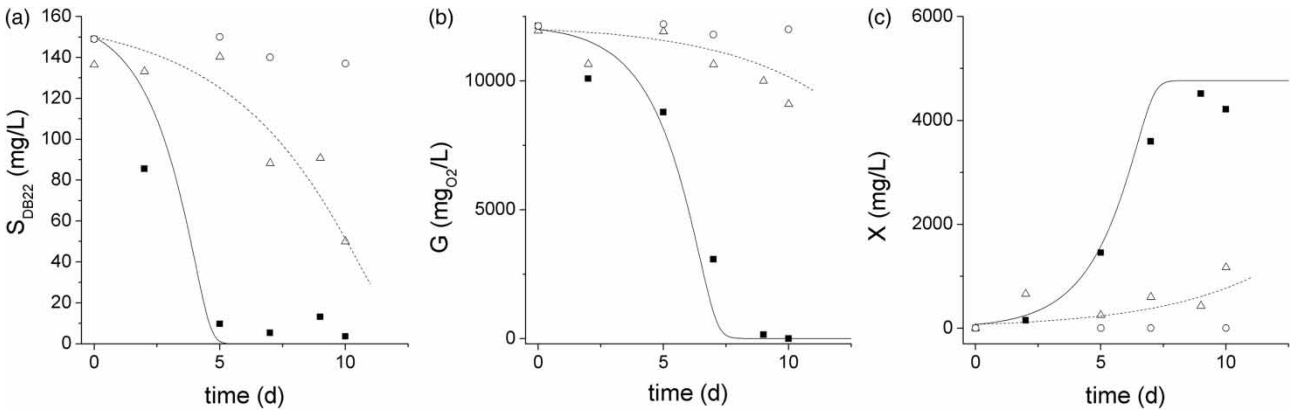


**Figure 3** | Laboratory-scale DB22 degradation assays by *P. chrysogenum*: (a) dye concentration ($S_{DB22}$), (b) growth substrate as COD (G) and (c) biomass concentration (X). The solid squares show the results of the loofah-immobilized assays, empty triangles represent the free-cells control and empty circles represent sorption control. Solid lines represent the prediction of immobilized assay and dashed lines represent the predictions for the free-cells control assay.

times higher for DB22, for immobilized assays, in comparison with the control at day 10. In both conditions, with and without loofah, the initial pH was 7 and fell to a minimum to nearly 4, to later freely rise again to nearly 7. As was reported by Durruty *el at.* (2015), the fall in the pH was coincident with the presence of organic acids detected by Fourier transform infrared analysis. The same results were observed during azo-dye degradation by Elisangela

et al. (2009) with the bacterium *S. arlettae*. Afterwards, the return to pH 7 could be explained by the consumption of these compounds.

Previous reports showed an improvement in azo-dye degradation when WRF was immobilized (Rodríguez Couto 2009; Krastanov *et al.* 2013). However, this is the first study that demonstrates such improvement with a non-basidiomycete fungus. The increase of azo-dye degradation rate generally has been attributed to an increase in laccase activity due to the immobilization (Rodríguez *et al.* 1999) or to the carrier matrix sorption of pollutant (Krastanov *et al.* 2013). However, laccase activity was not detected in plate activity assays for this *P. chrysogenum* strain (Wolski *et al.* 2012a). In addition, a sorption test was performed in this work and no significant differences were observed with respect to the case of no-carrier control ($p < 0.05$). Since the degradation rate depends on the amount of biomass (Blanch & Clark 1996), the improvement in decolorization rate is considered a side consequence of the fungal growth.

Moreover, it is important to mention that catechol dioxygenase activity was detected in plate assays (Wolski *et al.* 2012b) for this fungus during the degradation of phenol as a sole carbon source, and this enzyme could be also involved in some step during the azo-dye degradation pathway. In addition, phytotoxicity of the degradation products was already been tested in previous work by Durruty *et al.* (2015) showing that biological degradation did not increase the toxicity of the untreated azo dyes.

The kinetic model used in this work was originally developed to be applied in the free-cells azo-compound degradation by *P. chrysogenum* (Durruty *et al.* 2015), and was cross-validated in this work on free-cells and immobilized assays. A cross-validation is mandatory under new conditions. In the same context, short calibration steps may also be performed regularly during the validation of the model (Béline *et al.* 2007; Batstone *et al.* 2009).

The parameter values used to predict the output variables' behavior in every assay are summarized in Table 1 and model predictions are also shown in Figure 3 as solid lines. In free-cells controls the values previously fitted (Durruty *et al.* 2015) were used as cross-validation; however, the kinetic parameters must be refitted to predict the immobilized behavior (Donoso-Bravo *et al.* 2011). In this work, the initial condition used to initialize the differential equations was 70.73 mg/L of biomass, obtained by averaging the initial values.

The model prediction capability was evaluated using RMSE. Table 2 shows the values of RMSE for X, G and S. The predictions for growth substrate present a very good performance with an error lower than 10% (NRMSE $< 0.1$) and predictions for S present a good performance with an error near to 10% of the mean. The values for RMSE of X predictions are comparable with those of other assays. However, the low value of the mean ($\bar{x}$) makes the NRMSE not suitable for DB22. Nevertheless, since the ANRMSE values are lower than 0.2 (good fitness) this cross-validation confirms the capability of the model to predict these data. The predictions are also shown in Figure 3 as dashed lines.

To predict the growth during immobilized assays, the model must be recalibrated and parameters $\mu_{max}$, $K_M$ and $Y_{X/S}$ must be refitted ($R^2 = 0.876$). On the other hand, the model was able to predict the azo-compound degradation with original $b_i$ and $K_S$ values once growth parameters

**Table 1** | Kinetic parameters

| Parameter[a] | | Direct Black 22 | Reference | Validation |
|---|---|---|---|---|
| $\mu_{max}$ | Free cell | 0.273 ± 0.023 | Durruty *et al.* (2015) | Cross-validated |
| $[d^{-1}]$ | Immobilized | 0.715 ± 0.071 | This work | Recalibrated |
| $K_M$ | Free cell | 1631 ± 112 | Durruty *et al.* (2015) | Cross-validated |
| $[mg_{O2} \cdot L^{-1}]$ | Immobilized | 1542 ± 231 | This work | Recalibrated |
| $Y_{X/S}$ | Free cell | 0.380 ± 0.023 | Durruty *et al.* (2015) | Cross-validated |
| $[mg_X/mg_{O2}]$ | Immobilized | 0.391 ± 0.043 | This work | Recalibrated |
| $b_{max}$ | Free cell | 0.179 ± 0.011 | Durruty *et al.* (2015) | Cross-validated |
| $[mg_S/mg_X]$ | Immobilized | 0.179 ± 0.011 | Durruty *et al.* (2015) | Cross-validated |
| $K_S$ | Free cell | 25.162 ± 6.118 | Durruty *et al.* (2015) | Cross-validated |
| $[mg_S \cdot L^{-1}]$ | Immobilized | 25.162 ± 6.118 | Durruty *et al.* (2015) | Cross-validated |

[a]All the parameters were fitted at room temperature (25 °C).

**Table 2** | Root mean squared error (RMSE), normalized root mean squared error (NRMSE) and average normalized root mean squared error (ANRMSE) for different assays

| Assay | | | Variable | RMSE | NRMSE | ANRMSE |
|---|---|---|---|---|---|---|
| Laboratory scale | DB22 | Free cell | X | 130.37 mg/L | 0.2999 (ns) | 0.1659 (g) |
| | | | G | 749.5297 $mg_{O2}$/L | 0.0699 (vg) | |
| | | | S | 13.63725 mg/L | 0.1281 (g) | |
| | | Immobilized | X | 403.4194 mg/L | 0.1738 (g) | 0.1891 (g) |
| | | | G | 1058.162 $mg_{O2}$/L | 0.1854 (g) | |
| | | | S | 9.9159 mg/L | 0.2079 (s) | |
| Bench scale | DB22 | Table 1 | G | 4107.204 $mg_{O2}$/L | 0.4577 (ns) | 0.5056 (ns) |
| | | | S | 21.2229 mg/L | 0.4491 (ns) | |
| | | Optimized | G | 1130.718 $mg_{O2}$/L | 0.1260 (s) | 0.1136 (g) |
| | | | S | 4.7768 mg/L | 0.1011 (s) | |

The goodness-of-fit was labeled based on NRMSE as not-relevant (NRSME > 0.5) not-suitable (ns, NRSME = 0.25–0.5); suitable (s, NRSME < 0.25); good (g, NRSME < 0.20) and very good (vg, NRSME < 0.10).

were refitted. Thus, the decolorization sub-model was validated for the immobilized assay without recalibration with NRMSE near to 20% for DB22. This gives mathematical support to the statement that the presence of a natural carrier facilitates the fungal growth and the improvement of decolorization rate is a side consequence. The model predictions obtained after solving the model using the Runge–Kutta method with the parameters shown in Table 1 are also shown in Figure 3 as solid lines.

The value of $\mu_{max}$ obtained in this work was 2.61 times higher than the previous one obtained for a non-immobilized system (Table 1). However, the difference in values obtained for $K_M$ and $Y_{X/S}$ fall into the fitting error and therefore they cannot be considered different. This strongly suggests that the presence of loofah as carrier increases the growth rate but does not affect either the microorganism affinity for the substrate or the yield.

Figure 4 shows the DB22 decolorization, the COD depletion and biomass growth together with pH evolution in the reactor assay. The fungal growth in the reactor can also be seen in Figure 2(c) and 2(d). The results show how, in the reactor assay, the fungus was able to grow attached to the carrier and decolorize the azo-compounds, and the pH shows the same behavior as in laboratory-scale assays.

The model was also tested to predict the reactor performance. When parameters fitted to laboratory-scale immobilized assays (Table 1) were used, the model overestimated the experimental rates (dashed lines in Figure 4) and the ANRMSE results were not relevant. For this reason, an optimization step had to be done and the growth parameters $\mu_{max}$ and $K_M$ had to be recalibrated. This procedure is usual during the model validation for industrial applications, when operational variables affect the degradation rates (Béline *et al.* 2007; Donoso-Bravo *et al.* 2011; Durruty *et al.* 2015). After calibration, $\mu_{max}$ and $K_M$ values were 0.572 $d^{-1}$ and 5,500 [$mg_{O2}$/L], respectively. The model prediction after the recalibration step is shown

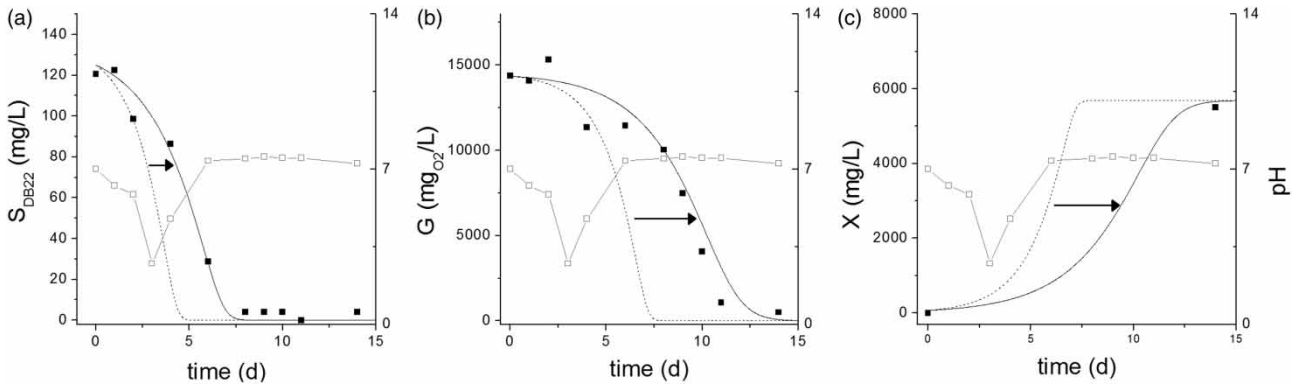


**Figure 4** | Bench-scale DB22 degradation by *P. chrysogenum* assays. DB22 concentration ($S_{DB22}$), COD (G) and biomass concentration (X). Dots represent the experimental data. Dashed lines represent the model prediction using the parameters obtained from laboratory assay (Table 1). Solid lines represent the model prediction after the growth parameters' recalibration. Empty gray squares represent the pH measured during degradation.

in Figure 4 as solid lines. Furthermore, ANRMSE fell from 0.4577 to 0.1136 (Table 2). Figure 4 also shows the model prediction of $X$. Experimental values could not be measured over time in the case of the reactor since it would require ending the assay. However, the final value shows the same biomass yield. In that sense the model is presented as an effective tool to predict the fungal growth during the scale-up process.

The recalibration required seems to indicate that under reactor conditions the specific growth rate is slower and inoculum–substrate affinity is much lower. These phenomena can be attributed to issues derived from scaling up: increases in substrate mass transfer resistance and reduced oxygen transport (Donati & Paludetto 1997). The differences in geometry and shaking system (Erlenmeyer's flask orbitally shaken versus the reactor with torispherical bottom and magnetically shaken), the different aeration devices (Erlenmeyer superficially aerated versus reactor bubbled by a deep tube) and VVM in reactor lower than reported in the literature (Moldes *et al.* 2003; Enayatzamir *et al.* 2009; Pazdzior *et al.* 2009) seem to indicate an insufficient oxygenation, detrimental to aerobic fungal growth. A parametric study which evaluates dissolved oxygen and decolorization rates to different aeration rates and rpm agitation is mandatory to discriminate between these effects and will be performed in future research at bench scale to approach the industrial application.

Despite the complications inherent to the scaling up, the degradation performance showed by the reactor was similar to those observed in laboratory assays and encourages the industrial scale application. Furthermore, the immobilized treatment at bench scale was faster than the non-immobilized treatment at laboratory scale. This clearly indicates that the immobilized treatment at bigger scale presents an improvement over the free-cells assay.

## CONCLUSIONS

The immobilization of *P. chrysogenum* on loofah increases significantly the degradation rate of BD22 at laboratory scale as well as at bench scale, with respect to the non-immobilized treatment. The use of a reactor is the first step of scaling up with a view to an industrial application. The kinetic model was successfully cross-validated with fresh data using RMSE as evaluation tool. The degradation performance showed by the reactor was similar to those observed in laboratory assays and encourages the industrial-scale application.

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