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# Packed-Bed Reactor Running on Babassu Oil and Glycerol to Produce Monoglycerides by Enzymatic Route using Immobilized *Burkholderia cepacia* Lipase

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**Abstract** The aim of this study was the glycerolysis of babassu oil catalyzed by immobilized lipase from *Burkholderia cepacia*, in a continuous packed-bed reactor. The best reaction conditions were previously established in batchwise via response surface methodology as a function of glycerol-to-oil molar ratio and reaction temperature. The reactor operated continuously for 22 days at 50 °C, and during the first 6 days, no significant decrease on the initial lipase activity was observed. Monoglycerides concentration was in the range from 25 to 33 wt.%. Subsequently, a progressive decrease in the activity was detected, and an inactivation profile described by Arrhenius model estimated values of 50 days and $1.37\times10^{-2}h^{-1}$, for the half-life and deactivation coefficient, respectively.

**Keywords** Continuous packed reactor · Glycerolysis · Lipase · Monoglycerides · Babassu oil · Operational stability · Response surface methodology

## Introduction

Monoglycerides (MG) are nonionic surfactants used in pharmaceutical, food, and cosmetic industries. Most of MG is manufactured by chemical glycerolysis of oils and fats at high temperatures using inorganic catalysts [1]. The limitations of this process are high energy consumption, formation of undesirable by-products, partial product degradation, and high investment capital [2]. Alternatively, the process can be performed using lipases a versatile catalyst that possess high stability in both aqueous and nonaqueous media and are readily commercially available [3].

Different methods for the enzymatic synthesis of monoglycerides have been described; (1) selective hydrolysis of triglycerides; (2) glycerolysis of triglycerides, and (3) direct

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esterification of glycerol with variable fatty acids [3]. However, due to its high yield and productivity, glycerolysis reactions seem to be more advantageous than the other reactions. Vegetable oils are considered to be readily available raw material and low cost source for several important fatty acids [4]. In addition, glycerolysis route has additional relevance in the present worldwide context, due to the sharp increase on the glycerol supplied in the market as a primary byproduct from biodiesel plants. Therefore, converting glycerol into value-added products provides an alternative for glycerol disposal and for its surplus problems [5].

Previous work carried out in our lab has identified that *Burkholderia cepacia* lipase is a potential lipase source to produce monoglycerides from the glycerolysis of babassu oil [6]. Pursing our interest in developing a feasible enzymatic process, an attempt was made to perform the process under continuous mode. For this, packed-bed reactor (PBRs) configuration was selected based on its suitability to perform typical lipase-catalyzed reactions [4]. PBRs are kinetically more favorable than continuous reactors with agitation as the disadvantage of the high mechanical stress due to the agitation can be avoided.

Prior to the continuous experiments, the optimization of glycerol to oil molar ratio and reaction temperature was carried out in batchwise. The experiments were analyzed through the methodology of statistical factorial design that makes possible to verify the influence of the variables and their interactions in the process yield with great economy of time, material, and resources [7].

The aim of the present study was to implement, in a continuous mode, the glycerolysis of babassu oil, in solvent-free media, catalyzed by an immobilized preparation of lipase from *B. cepacia*. The continuous glycerolysis as carried out at lab scale, in a continuous packed-bed reactor running on inert atmosphere seeking to avoid the oxidation of the feeding feedstock. This strategy also avoids the negative influence of the oxidized oil on both enzymatic activity and reaction rate [8].

## Experimental

### Materials

All experiments were carried out with a commercial lipase preparation from *B. cepacia* (Lipase PS—Batch number: 01022TD) purchased from Amano Pharmaceuticals (Nagoya, Japan) and used as received without further purification. Refined, bleached, and deodorized babassu oil was supplied by Cognis (Jacarei, SP, Brazil) and had the following composition in fatty acids (*w*/*v*): 3.5% octanoic, 4.5% decanoic, 44.7% lauric, 17.5% myristic, 9.7% palmitic, 3.1% steriac, 15.2% oleic, and 1.8% linoleic with average molecular weight 709.90 g/mol. Glycerol (propane-1,2,3-triol) was purchased from Merck (Darmstadt, Germany). Tetraethoxysilane was acquired from Aldrich Chemical Co. (Milwaukee, WI, USA). Epichlorohydrin, hydrochloric acid (minimum 36%), ethanol (minimum 99%), polyvinyl alcohol (molecular weight 72,000), and polyethylene glycol (molecular weight 1,500) were supplied by Reagen (Rio de Janeiro, RJ, Brazil). Solvents were of standard laboratory grade (Synth, São Paulo, SP, Brazil). All the other reagents were of analytical grade.

### Support Synthesis and Lipase Immobilization

A polysiloxane–polyvinyl alcohol hybrid composite ($SiO_2$–PVA) was prepared and activated with epichlorohydrin following previous methodology [9]. The properties of the activated support were as follows: 0.175 mm diameter, average pore diameter (22.91 Å);

surface area Brunauer-Emmett-Teller (BET) (461.00 $m^2g^{-1}$), and porous volume (0.275 $cm^3g^{-1}$). Epoxy $SiO_2$–PVA particles were used to immobilize lipase PS according to the procedure previously reported [9] achieving high retention of enzyme on the support (higher than 50%) and the following properties [10]: hydrolytic activity=1,600 U/g; esterification activity=30 U/g; water content=12%; optimum pH=8.5; optimum temperature 40–60 °C, and thermal stability (half-life at 60 °C=6.24 h).

## Glycerolysis Reactions

### Batch Glycerolysis Reactions

Preliminary studies were carried out in a batch reactor to optimize the glycerol-to-oil molar ratio and the reaction temperature on the formation of monoglycerides. A face-centered $2^2$ factorial design with three coded levels leading to 11 set of experiments was performed (Table 1). The reaction was carried out in a 50-mL conical flask with a working volume 30 mL containing the required amount of babassu oil and glycerol followed by addition of fixed amount of immobilized PS lipase (10 wt.%) having low water contents (<12%). Reaction mixtures and lipase immobilized were magnetically stirred (200 rpm) at different reaction temperatures (40–50 °C). The duration of the reaction was fixed at 24 h. Inert atmosphere was obtained by continuous sparging $N_2$ in the medium storage vessel, avoiding in this way exposure of the reaction medium to oxygen. The synthesis progress was monitored by overdraw samples during the reaction to quantify the products formed by gas chromatography (GC). Samples taken at regular intervals were treated for extraction of the water and glycerol followed previously methodology [11]. The "Statistica version 5.0" software (StatSoft Inc., USA) and Design Expert version 6.0 were used for regression and graphical analyses.

### Continuous Glycerolysis Runs

A PBR was tested for the glycerolysis at 50 °C using a mixture of glycerol and babassu oil at molar ratio of 15:1. PBR was a jacketed glass column (internal diameter=1.5 cm; height =5.5 cm, and total volume=10 mL). The temperature in the reactor was kept at 50 °C, by circulating water in the jacket. The substrate was continuously pumped (Sj-1211-Hatto) from a reservoir at 50 °C, through silicone tubing, to the bottom end of the bioreactor at flow rate of 0.028 mL $min^{-1}$. A schematic diagram of the PBR is shown in Fig. 1. An amount of 6.7 g biocatalyst was used, which corresponds to a bulk volume of 9.97 $cm^3$. Crystalline density of the immobilized derivative (dry weight) was 1.865 g $mL^{-1}$. The residence time was calculated according to Levenspiel [12] as described in Eq. 1:

$$\tau = \frac{V}{v_0} \tag{1}$$

**Table 1** Real values and coded of independent variables used to the face-centered $2^2$ factorial design.

| Independent variables | Coded levels | | |
|---|---|---|---|
| | −1 | 0 | +1 |
| $x_1$-Glycerol-to-oil molar ratio | 5 | 10 | 15 |
| $x_2$-Temperature, °C | 40 | 45 | 50 |

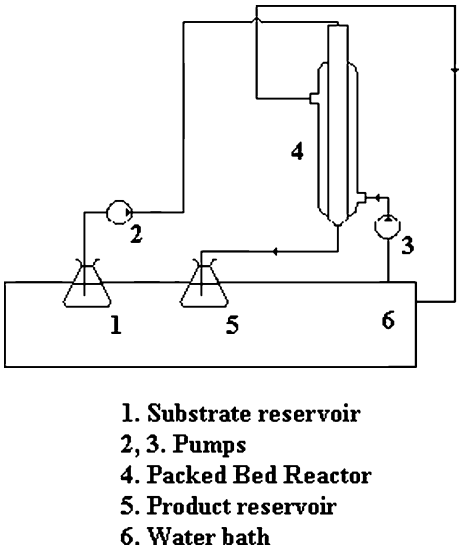


**Fig. 1** Schematic diagram of the PBR

where $\tau$=is the reaction time (minute), $V$=is the reactor working volume (milliliter), $v_o$= flow rate (milliliter per minute).

Inert atmosphere was obtained by sparging $N_2$ in the medium storage vessel, avoiding in this way exposure of the reaction medium to oxygen. Samples were collected and storage at −2 °C up to carry out the chromatograph analyses. Results were expressed in molar fraction of monoglycerides, diglycerides, and triglycerides.

## Analytical Methods

Mono-, di-, and triacylglycerols were analyzed by GC using a Varian 3800 model (Varian, Inc. Corporate Headquarters, Palo Alto, CA, USA) equipped with flame ionization detector and with a 10 m×0.25 mm×0.12-μm CP Sil 5CB capillary column (Varian, Inc. Corporate Headquarters, Palo Alto, CA, USA). The chromatograms were processed using a Varian data integrator version 4.51 computational program. Hydrogen was used as the carrier gas with a flow rate of 2 mL $min^{-1}$. The detector and injector temperatures were 350 °C. The column temperature was set to 80 °C for 1 min and was then programmed at 20 °C $min^{-1}$ to 320 °C which was maintained constant for 2 min. Other conditions were split ratio of 1:20 and attenuation equal to 1. An organic phase was dissolved in hexane/ethyl acetate (proportion of 1:1) which contained tetradecane as internal standard, and the injection was carried out into the GC [11]. Each data point was repeated three times, and the standard error was lower than 5%.

## Results and Discussion

### Optimization of Batch Glycerolysis Reaction Conditions

The composition of the glyceride mixture upon lipase-catalyzed batch glycerolysis carried out under conditions established by the factorial design is shown in Fig. 2 and indicated that

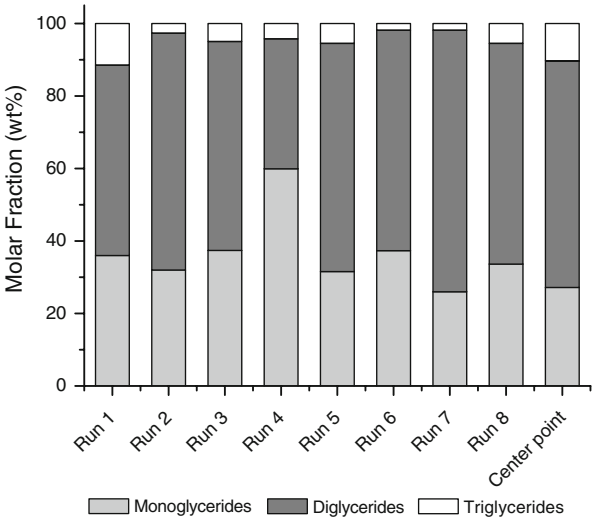


**Fig. 2** Formed product distribution in the glycerolysis of babassu oil under inert atmosphere catalyzed by PS lipase immobilized on $SiO_2$–PVA according to face-centered $2^2$ factorial design. The center point result is an average of three replicates

the reaction was addressed for preferential formation of monoglycerides and diglycerides with low residual of triglycerides level. The experimental matrix for the factorial design is shown in Table 2 together with results obtained for monoglycerides formation (percentage).

Results indicated that monoglycerides formation varied from 26 to 60 wt.%, and the highest concentration was achieved using both variables at highest levels (glycerol-to-oil molar ratio of 15:1 and 50 °C).

The experiment results displayed in Table 2 were used to estimate the main variable effects (molar ratio and temperature) and its interaction on the response variable monoglycerides formation (weight percentage). According to the Student's *t* test (Table 3), all variables and their interactions seems to have played a role in the MG formation within the experimental range studied. Its statistical meaning was significant and positive on the response variable at 95% probability level (Table 4).

From these results, the main effects were fitted by multiple regression analysis to a quadratic model, and the best response function can be described by Eq. 2.

$$y = 26.26 + 4.05x_1 + 9.50{x_1}^2 + 6.15x_2 + 4.88{x_2}^2 + 6.63x_1x_2 \quad (2)$$

where $\widehat{y}$ is the response variable (monoglycerides, weight percentage), and $x_1$ and $x_2$ represent the values coded for glycerol-to-oil molar ratio and temperature, respectively.

The response surface (Fig. 3) described by the second order model for babassu oil glycerolysis showed that the maximum monoglyceride formation can be reached at 50 °C and glycerol-to-oil molar ratio of 15:1.

The significant effect of the glycerol-to-oil molar ratio on the MG synthesis can be explained by considering that alcohol in excess is needed to favor the MG accumulation in the reaction medium, instead of diglycerides or triglycerides [13]. In the present work, the highest level of glycerol corresponded to the best condition to lipase-catalyzed MG production.

The interaction effect of the variables was also statistically significant at the same confidence level (95%). Thus, the increase in the MG formation at highest glycerol-to-oil molar ratio was more pronounced when the temperature undertook the highest value (50 °C). This may explained by considering that, in this process, low temperatures impair the

**Table 2** Experimental design and results according to the face-centered $2^2$ factorial design carried out to evaluate the influence of the variables glycerol-to-oil molar ratio and temperature on the MG concentrations obtained in the glycerolysis of babassu oil.

| Runs | Coded values | | Real values | | Monoglycerides[a] (%) |
|---|---|---|---|---|---|
| | $x_1$ | $x_2$ | Glycerol-to-oil molar ratio | Temperature (°C) | |
| 1 | –1 | –1 | 5 | 40 | 36.00 |
| 2 | +1 | –1 | 15 | 40 | 31.99 |
| 3 | –1 | +1 | 5 | 50 | 37.37 |
| 4 | +1 | +1 | 15 | 50 | 59.90 |
| 5 | –1 | 0 | 5 | 45 | 31.54 |
| 6 | +1 | 0 | 15 | 45 | 37.30 |
| 7 | 0 | –1 | 10 | 45 | 26.00 |
| 8 | 0 | +1 | 10 | 50 | 33.59 |
| 9 | 0 | 0 | 10 | 45 | 26.49 |
| 10 | 0 | 0 | 10 | 45 | 28.02 |
| 11 | 0 | 0 | 10 | 45 | 26.96 |

[a] Maximum concentration

homogeneity, restraining the contact between the lipase and the hydrophobic substrates that represents an obstacle to improve the MG yield. Thus, 50 °C was the optimum temperature value that could result in high MG production while preventing the lipase from thermal deactivation effects. Critical temperature at which the enzyme starts to deactivate was found to be 60 °C revealing a biocatalyst half-life of 6.24 h as previously reported [10].

In agreement with these results, the maximum MG formation (60%) can be obtained, working at the highest level for glycerol-to-oil molar ratio 15:1 and central temperature level ($T$=50 °C), being these reaction conditions used for the subsequent tests.

These results are favorable compared with those reported in the literature dealing with production of MGs by enzymatic route using different feedstocks and biocatalysts [14, 15], evidencing also that the use of inert atmosphere is a valid strategy to obtain high formation of monoglycerides. However, this trend is dependent on the lipase source and its thermal stability data. For the lipase used in the present work (*B. cepacia* lipase immobilized on epoxy $SiO_2$–PVA) that has satisfactory thermal stability [10], high temperature values

**Table 3** Estimated effects, standard errors, and Student's $t$ test for monoglycerides production according to the face-centered $2^2$ factorial design.

| Variables | Effects | Standard errors | $t$ | $p$ |
|---|---|---|---|---|
| Mean | 26.26* | ±0.40 | 65.32 | 0.000* |
| Glycerol-to-oil molar ratio ($x_1$) | 8.09* | ±0.64 | 12.65 | 0.006* |
| Glycerol-to-oil molar ratio ($x_1^2$) | 19.01* | ±0.98 | 19.30 | 0.003* |
| Temperature ($x_2$) | 12.29* | ±0.64 | 19.21 | 0.003* |
| Temperature ($x_2^2$) | 9.76* | ±0.98 | 9.91 | 0.010* |
| $x_1.x_2$ | 13.27* | ±0.78 | 16.93 | 0.003* |

*$p<0.05$

$R^2$ =0.9635

**Table 4** Analysis of variance for the model representing the monoglycerides formation (weight percentage) according to the face-centered $2^2$ factorial design.

| Factor | SS | DF | MS | *F* | |
|---|---|---|---|---|---|
| | | | | Value probability>*F* | |
| Model | 879.59 | 5 | 175.92 | | |
| $x_1$ | 98.25 | 1 | 98.25 | 159.96 | 0.0013 |
| $x_1^2$ | 228.81 | 1 | 228.81 | 372.51 | 0.0121 |
| $x_2$ | 226.57 | 1 | 226.57 | 368.86 | 0.0021 |
| $x_2^2$ | 60.30 | 1 | 60.30 | 98.17 | 0.0298 |
| $x_1\ x_2$ | 176.09 | 1 | 176.09 | 286.69 | 0.0036 |
| Residual | 33.32 | 5 | 6.66 | | |
| Lack of fit | 32.09 | 3 | 10.70 | 17.42 | 0.0548 |
| Pure error | 1.23 | 2 | 0.61 | | |
| Cor total | 912.92 | 10 | | | |

$x_1$ molar ratio, $x_2$ temperature, *SS* sum of squares, *DF* degrees of freedom, *MS* mean square

usually increases the product formation, in which MG concentration increased to about 60% when the temperature level rose from 40 to 50 °C. Kaewthong et al. [14] reported the formation of about 21% of MG in 24 h using this same lipase immobilized on a different support (Accurel EP 100) to mediate the glycerolysis of palm oil in the absence of inert atmosphere. In another example, Pawongrat et al. [15] described the formation of 24.5% MG enriched with polyunsaturated fatty acids in 24-h reaction, from the glycerolysis of tuna oil using lipase of *Pseudomonas fluorescences* immobilized on Accurel-EP100.

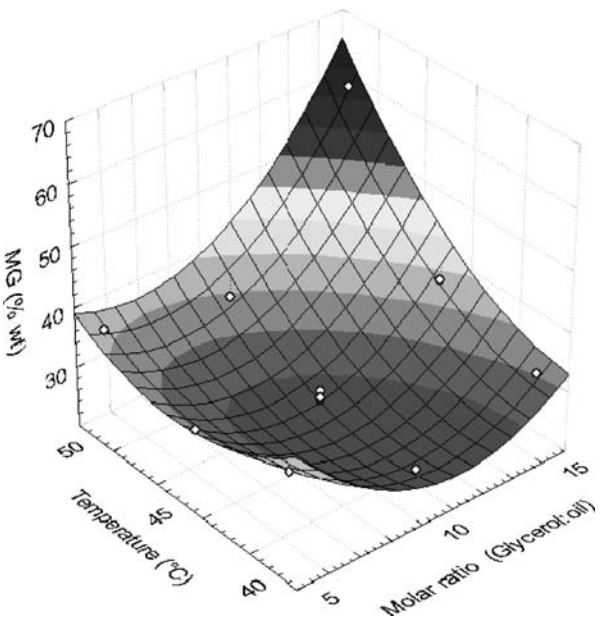


**Fig. 3** Response surface described by the model *y* that represents the glycerolysis of babassu oil in the formation of monoglycerides (weight percentage) catalyzed by PS lipase as a function of molar ratio ($x_1$) and temperature ($x_2$) according to Eq. 2

## Continuous Glycerolysis in PBR

PBR are the most frequently used for processes catalyzed by immobilized lipase due to its high efficiency, low cost, and ease construction and operation [16]. The glycerolysis of babassu oil was carried out in a PBR under the conditions previously established in tests performed batchwise (glycerol-to-oil molar ratio of 15:1 at 50 °C and inert atmosphere). The reactor was packed with 6.70 g of lipase PS immobilized on $SiO_2$–PVA at descending flow rate of 0.028 mL/min. The inert atmosphere was guaranteed by sparging $N_2$ in the feed medium storage. Results are shown in Fig. 4.

The formation of the monoglycerides was 30.5%, and the steady state was reached in four residence times (24 h). During the first 6 days, no significant decrease in the initial activity was observed, and monoglycerides concentration varied from 25 to 33 wt.%. Subsequently, a progressive decrease in the activity was detected, and a reduction of 20% in the mixture was obtained after 22 days of operation.

These results were also favorable comparable with those reported in the literature using packed-bed reactors [14, 17, 18]. In the work carried by Yang et al. [17], 12% of MGs was obtained in continuous glycerolysis of sunflower oil using Novozym 435 as catalyst. The system was considered to be stable for 30 days. In another work [13] dealing with continuous glycerolysis palm oil using lipase PS immobilized on Accurel EP100 as biocatalyst, the formation of MGs was in the order of 14%. Therefore, in the present work, highly satisfactory behavior of the experimental system for the continuous synthesis of MGs was verified.

## Operational Stability of the Immobilized Derivative

A parameter of fundamental importance, when working with processes that involve immobilized enzymes, is its operational stability. This stability depends on a series of factors, such as linkage of the enzyme from the support, obstruction of the pores for sludge or by products, support loss for attrition, and obstruction of the fixed bed, causing bypass [19]. A high stability of the lipase would turn continuous glycerolysis more attractive to be used at industrial plants, mainly due to the high cost of the lipases.

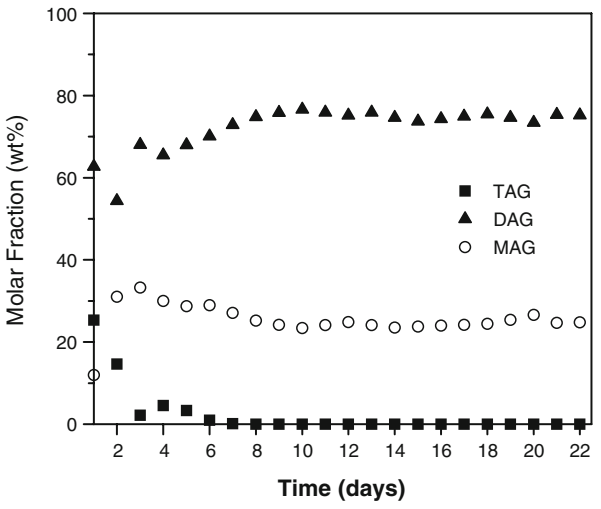


**Fig. 4** Glyceride profile in the continuous glycerolysis of babassu oil using PS lipase immobilized on $SiO_2$–PVA under inert atmosphere. Monoglycerides (*circles*), diglycerides (*triangles*), triglycerides (*squares*)

The experimental results showed in Fig. 4 were normalized following Arrhenius equation (Eq. 3) and fitted to a deactivation model of first order (Fig. 5).

$$\frac{A}{A_0} = e^{-Kd*t} \tag{3}$$

where $K_d$ is the deactivation coefficient, $t$ is the reaction time, $A_0$ is the maximum MG concentration, and $A$ is the MG concentration at a given time ($t$).

Half-life of the biocatalyst ($t_{1/2}$) was estimated by Eq. 4, in which $t_{1/2}$ is the half-life, and $K_d$ is the deactivation coefficient.

$$t_{1/2} = \frac{\ln(2)}{Kd} \tag{4}$$

High operational stability of the biocatalyst is required for industrial application of lipase-catalyzed glycerolysis under continuous operation. In this work, a half-life of 50 days and a deactivation coefficient of $1.37\times0^{-2}\mathrm{h}^{-1}$ were estimated. These values were favorable comparable with those reported by Osorio et al. [20] that found a half-life time of 17 days for Novozym 435, a lipase preparation commercially available in the immobilized form, using fluidized bed reactor running in palm stearin with soybean oil to produce fat blends.

## Conclusions

The objective of the present work was to develop an efficient system for the enzymatic production of monoglycerides from babassu oil running on inert atmosphere to avoid the oxidation of the oily raw materials during the reaction. Glycerolysis of babassu oil was operated under discontinuous and continuous (PBRs) mode in solvent-free system, using immobilized lipase as biocatalyst. The lipase used was *B. cepacia* (PS) immobilized by covalent binding on a hybrid support $SiO_2$–PVA that combines physical–chemical attributes of organic and inorganic materials. Under continuous mode, monoglycerides concentration varied from 25 to 33 wt.%, and biocatalyst half-life was 50 days, which revealed higher performance when comparison is made with data reported in the literature [20]. Results also

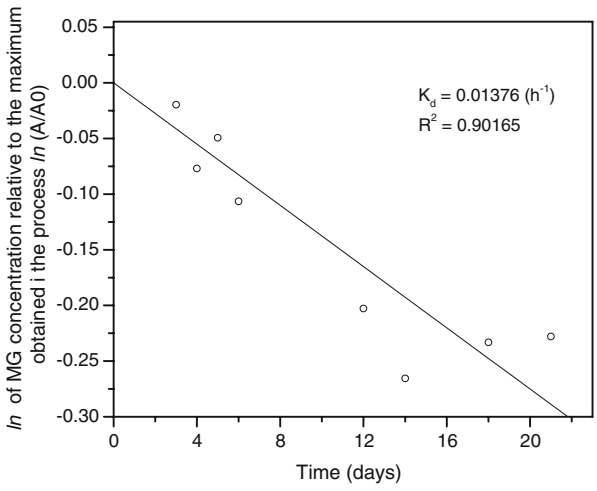


**Fig. 5** Deactivation model adjustment for MG formation in the continuous glycerolysis of babassu oil catalyzed by lipase PS immobilized on $SiO_2$–PVA carried out in packed-bed reactor at 50 °C

demonstrated good potential for using the PRB in solvent-free system due to the high operational stability of the immobilized derivative measured in the presence of substrate and products.

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