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# **An effective toluene removal from waste-air by a simple process based on absorption in silicone oil (PDMS) and cross-linked Brassica rapa peroxidase (BRP-CLEAs) catalysis in organic medium: Optimization with RSM**

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## Environmental Progress & Sustainable Energy

### An effective toluene removal from waste-air by a simple process based on absorption in PDMS and BRP-CLEAs catalysis in organic medium: optimisation with RSM

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# An effective toluene removal from waste-air by a simple process based on absorption in PDMS and BRP-CLEAs catalysis in organic medium: optimisation with RSM

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### Highlights

- PDMS was efficient for toluene absorption with a good CLEAs stability.
- Toluene degradation with CLEAs of peroxidase was conducted in an organic phase with the minimum of reagents.
- Effect of different parameters was studied with Box-Behnken restricted duplicate design.
- Until 60% of the absorbed toluene was removed.

For Peer Review

## Abstract

In the present work, absorption in an organic solvent followed by enzymatic biodegradation by cross-linked peroxidase from *Brassica rapa* (BRP-CLEAs) was successfully applied as a solution for toluene removal from waste-air in batch experiments. Silicone oil (PDMS 47V20) showed a high capacity for toluene absorption, regarding its air-PDMS partition coefficient ( $2.08 \text{ Pa}\cdot\text{m}^3\cdot\text{mol}^{-1}$ ) when compared to air-water ( $666 \text{ Pa}\cdot\text{m}^3\cdot\text{mol}^{-1}$ ). Box-Behnken restricted duplicate design was employed for the optimization of the enzymatic degradation of toluene in PDMS. Effects of four independent variables, namely BRP-CLEAs activity, toluene and hydrogen peroxide concentrations and sonication, were studied by choosing two responses: conversion yield and initial rate of reaction. Results analysis showed that both hydrogen peroxide and toluene concentrations presented the largest effect on process while sonication had no effect. On other hand, coefficient of determination  $R^2$  and adjusted  $R^2$  were 0.828 and 0.770 for conversion and 0.919 and 0.911 for initial rate respectively. Optimum conversion determined was 60% of toluene removal.

**Keywords:** toluene, absorption, Box-Behnken design, BRP-CLEAs, enzymatic degradation.

### 1. Introduction

Coupling absorption in an organic phase prior to biodegradation by microorganisms in a TPPB is one of the techniques recently developed in order to treat hydrophobic VOCs with a limited solubility in water like toluene [1–4]. Advantages of TPPB are numerous but in return, several limits have to be overcome with this technology. For instance, the separation of the organic phase from the aqueous one can be complex; large interfacial areas, which are crucial for optimal mass transfer of substrate and for microbial adhesion, high cost of construction and apparently poor long-term stability have been underlined [5,6].

In order to introduce an enhancement to TPPB and overcome these problems, an alternative solution was proposed. This technology is based on VOCs degradation directly in the absorption phase without the need to introduce an aqueous phase. For this purpose, microorganisms are replaced by enzymatic biocatalysis. Enzymes, which are playing the role of biocatalysts could be stabilized in the organic phase by their immobilization as cross-linked enzyme aggregates (CLEAs) [7]. The proposed

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3 technology shows high conversion rates, relatively low operating costs, low energy  
4 requirements, and avoidance of cross media transfer of pollutants [8,9].  
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7 Due to the loss of activity in organic solvents and mass transfer limitations into enzyme  
8 aggregates [10,11], the conditions under which the highest performance of the proposed  
9 solution could be achieved must be optimized [12]. In biocatalyst engineering research,  
10 a number of statistically designed experimental models have been applied to optimize  
11 the reaction conditions in order to obtain the highest efficiency. The most popular one  
12 is the response surface methodology (RSM), which consists of a group of mathematical  
13 and statistical techniques that can be used to define the relationships between the  
14 response and the independent variables [12,13]. These statistical methods have shown  
15 their efficiency for the optimization of a wide range of enzymatic reactions  
16 comparatively to the common “one-factor-at-a-time (OFAT)” [14–16].  
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25 To our knowledge, up to now, no study in the literature described the degradation of  
26 toluene using class III peroxidase (plant peroxidase). As already demonstrated,  
27 peroxidase of turnips *Brassica rapa*, is capable to degrade different phenolic  
28 compounds with a considerable efficiency [17–19]. Hence, in the present work, a new  
29 exploration for this enzyme was investigated, namely the biooxydation of hydrophobic  
30 volatiles compounds. The aim of the present study was to find out the optimal  
31 conditions for the removal of toluene using *Brassica rapa* peroxidase immobilized as  
32 cross-linked enzyme aggregates (CLEAs) after absorption into silicone oil by using  
33 Box-Behnken designs (BBD). The optimal conditions under which the highest  
34 efficiency, in terms of yield and degradation rate, were determined.  
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## 43 **2. Materials and methods**

### 44 2.1. Chemicals and enzyme

45 Enzyme used in this study, described earlier [7] was extracted from turnips of *Brassica*  
46 *rapa*.  
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51 The silicone oil (polydimethylsiloxane or PDMS 47V20) was purchased from the  
52 Rhodia Company (Boulogne-Billancourt, France) with a viscosity of 20 mPa.s. This  
53 solvent is characterized by a molecular weight ranging between 2800 and 3200 g.mol<sup>-1</sup>,  
54 and a density of 950 kg.m<sup>-3</sup> at 25°C [20].  
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3 All chemicals and reactants used were of analytical grade, Glutaraldehyde (25%  
4 solution), hydrogen peroxide aqueous solution (50% w/w), toluene (98%) and guaiacol  
5 (98%) were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France).  
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## 8 9 2.2. BRP-CLEAs preparation

10 BRP-CLEAs used in this study were prepared from crude solution of peroxidase  
11 extracted from turnips of *Brassica rapa* [7]. The enzyme solution was filtrated through  
12 cotton cheesecloth and the filtrate obtained was diluted at a concentration of 74.88  
13 IU.mg<sup>-1</sup>; then it was precipitated using three volumes of ice-cold acetone. The collected  
14 enzyme in the mixture was crosslinked by glutaraldehyde solution (25% in water) at a  
15 concentration of 2%; it was kept under agitation for 3 hours; then centrifuged in order  
16 to separate CLEAs from the residual solution. BRP-CLEAs obtained were washed by  
17 ultrapure water and stored in phosphate buffer (50mM, pH = 7) under 4°C until use.  
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## 26 2.3. Peroxidase assay

27 The peroxidase activity in the crude solution and BRP-CLEAs was evaluated by the  
28 guaiacol assay [21].  
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31 Reaction mixture consisted of 3.9 mL of 50 mM sodium phosphate buffer pH =7.0 and  
32 4.05 µL of guaiacol (98%), mixed with 2 mg of CLEAs or 90 µL of peroxidase solution.  
33 To initiate the reaction, 5 µL of hydrogen peroxide (0.8 M) was added quickly to the  
34 solution and colour development was monitored at 470 nm, which is the maximum  
35 wavelength of tetraguaiacol absorption. The measurement was conducted using a  
36 Thermospectronic UV–VIS spectrophotometer (with a molar extinction coefficient  
37 value of 4279 M<sup>-1</sup>.cm<sup>-1</sup>) (Marne-la-Vallée, France). One unit (1.0 IU) of peroxidase  
38 activity was defined as the amount of enzyme protein that catalyses the oxidation of 1.0  
39 µmol of hydrogen peroxide per minute at 25°C and pH = 7.  
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## 48 2.4. Toluene absorption in PDMS 47V20

49 Before starting toluene biodegradation experiments with BRP-CLEAs, toluene was  
50 absorbed from air into silicone oil PDMS 47V20. The absorption efficiency was  
51 assessed by determining the partition coefficient of toluene  $H_{\text{toluene}}$  (gas/water) and  
52 (gas/PDMS 47V20) at 25°C.  
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The partition coefficient  $H$  is defined as the slope of the linear range of curve representing the equilibrium between the concentration of toluene in liquid ( $C_{\text{liquid}}$ ) and gas ( $C_{\text{gas}}$ ) phase according to the following equation:

$$H_{\text{toluene}} = RT(C_{\text{gas}}/C_{\text{liquid}}) \quad (\text{Eq. 1}).$$

Where  $T$  (in K) is the operating temperature and  $R$  (in  $\text{Pa}\cdot\text{m}^3\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ ) is the universal ideal gas constant.

The experimental technique for the Henry's constant estimation is called the static method. A known volume of toluene is injected by the mean of a microsyringe through a septum rubber into 22 mL glass vials hermetically closed containing 10 mL of pure water or PDMS 47V20. The vials are placed in a swivel support for 3 days at  $25^\circ\text{C}$ . Once the equilibrium between the two phases is reached, the concentration of toluene in the gas phase ( $C_{\text{gas}}$ ) is determined by analysing a sample of 500  $\mu\text{L}$  of gas using a Perkin Elmer-Clarus 480 gas chromatography with flame ionisation detector. Temperatures of the injector, the oven and the detector are 250, 80 and  $250^\circ\text{C}$  respectively, while the flow rate of the carrier gas is 21  $\text{mL}\cdot\text{min}^{-1}$ . The liquid concentration ( $C_{\text{liquid}}$ ) is deduced from the mass balance.

## 2.5. Experimental design and statistical analysis

Any study of experimental design could be organized in three essential steps: (1) performing statistically designed experiments, (2) estimating the coefficients in a mathematical model, and (3) predicting the response and checking the adequacy of the model [15,22].

The model used in RSM is generally a full quadratic equation or a reduced form of this equation. The second order approximation can be written as follows (Eq. 2):

$$Y = a_0 + \sum_{i=1}^k a_i x_i + \sum_{i=1}^k a_{ii} x_i^2 + \sum_{i=1}^k \sum_{j=i+1}^k a_{ij} x_i x_j + \varepsilon \quad (\text{Eq.2})$$

At initial stage and from preliminary studies (data not shown), it was found that for toluene degradation by peroxidase, four independent variables could affect the conversion yield ( $Y_1$ ) and the rate of the reaction ( $Y_2$ ): the BRP-CLEAs activity ( $x_1$ ), the toluene and hydrogen peroxide concentrations ( $x_2, x_3$ ) and sonication ( $b$ ). For the



first three variables, variation is coded (resp.  $x'_1, x'_2, x'_3$ ) with three levels, -1, 0, +1 while the sonication has a binary effect  $b'$  which is coded by two levels -1, +1 without centre point. The range and levels of experimental variables investigated are presented in Table 1.

A Box-Behnken restricted duplicate experimental design (BBD) was used to determine the optimal conditions for factors significantly affecting toluene removal in the range of conditions investigated. In this case, the Box-Behnken design is defined for the three variables ( $x_1, x_2, x_3$ ) and will be executed for the two levels of  $b$ . The equation (2) is then written as follows:

$$Y = a_0 + a_1 \cdot x_1 + a_2 \cdot x_2 + a_3 \cdot x_3 + a_4 \cdot b + a_{12} \cdot x_1 \cdot x_2 + a_{13} \cdot x_1 \cdot x_3 + a_{23} \cdot x_2 \cdot x_3 + a_{14} \cdot x_1 \cdot b + a_{24} \cdot x_2 \cdot b + a_{34} \cdot x_3 \cdot b + a_{11} \cdot x_1^2 + a_{22} \cdot x_2^2 + a_{33} \cdot x_3^2 + \varepsilon \quad (\text{Eq. 3})$$

Where  $Y$  is the predicted response,  $a_1, a_2, a_3, a_4$  are the linear regression coefficients,  $a_{12}, a_{13}, a_{23}, a_{14}, a_{24}, a_{34}$  are the interaction terms and  $a_{11}, a_{22}, a_{33}$  are the quadratic terms respectively.  $x_1, x_2, x_3, b$  are the independent variables and  $\varepsilon$  is the random error or uncertainties between predicted and measured values.

A total number of 26 experiments  $(3 \cdot 2^2 + 1) \cdot 2$  according to the Box-Behnken design were necessary to resolve the equation (3) and to estimate the regression coefficients (Table 2).

## 2.6. Toluene degradation experiments

Twenty-six experiments of toluene degradation by BRP-CLEAs in PDMS 47V20 were carried out in closed agitated glass vials. Mixtures were prepared by introducing 10 mL of PDMS 47V20 with the desired quantity of BRP-CLEAs into hermetically closed vials equipped with rubber septa and aluminium capsules. A precise volume of pure toluene (98%) was then injected into vials through septa using 10  $\mu\text{L}$  syringes and mixtures were incubated under agitation at 25°C in a thermostatic laboratory oven.

When equilibrium between the gas phase and PDMS 47V20 was reached, the reaction was started by injecting 25  $\mu\text{L}$  of fresh hydrogen peroxide solution. The same volume of  $\text{H}_2\text{O}_2$  was reinjected into vials every 60 minutes for 4 hours of reaction time.

Sonication is considered as a factor that enhances BRP-CLEAs dispersion in silicone oil as shown in Fig. 1. Experiments with sonication were performed by incubating vials before H<sub>2</sub>O<sub>2</sub> addition in a sonication bath (Elmasonic S150) for 15 minutes.

The initial and residual toluene concentration in the gas phase was quantified by injecting a withdrawn sample of 500 µL of gas phase from vials in a GC (gas chromatography) under specified conditions (section 1.4).

At any time, mass balance for toluene could be written as follows (Eq. 4):

$$m_{\text{tot}} = m_{\text{gas}} + m_{\text{PDMS}} \quad (\text{Eq. 4})$$

Where:

$m_{\text{tot}}$  : the total mass of toluene in vials;  $m_{\text{gas}}$  : mass of toluene in the gas phase;

$m_{\text{PDMS}}$  : mass of toluene in the PDMS.

Concentration of toluene in PDMS 47V20 at a given time  $t$  was estimated using the dimensionless partition coefficient of toluene ( $K$ ) which is a constant defined by equation (5):

$$K = \frac{C_{\text{gas}}}{C_{\text{PDMS}}} = \frac{H}{RT} \quad (\text{Eq. 5})$$

$C_{\text{gas}}$ ,  $C_{\text{PDMS}}$  are the toluene concentrations (mg.L<sup>-1</sup>) in the gas phase and the PDMS respectively.

The two responses  $Y_1$  and  $Y_2$ , which represent the conversion (%) and the rate of reaction (mg.L<sup>-1</sup>.h<sup>-1</sup>) respectively, were calculated as follows:

$$Y_1 = \frac{C_{\text{PDMS},0} - C_{\text{PDMS},f}}{C_{\text{PDMS},0}} \times 100 \quad (\text{Eq. 6})$$

$$Y_2 = \frac{C_{\text{PDMS},0} - C_{\text{PDMS},f}}{\Delta t} \quad (\text{Eq. 7})$$

Experiments were similarly joined with control experiments with and without BRP-CLEAs in order to evaluate the abiotic degradation of toluene resulting from adsorption, sonication, chemical or photochemical oxidation. All experiments were performed in duplicate.

### 3. Results and discussion

### 3.1. Absorption of toluene

Prior to the biodegradation tests, toluene was absorbed in PDMS 47V20. During the absorption step, toluene was transferred from the gas phase to the liquid phase until reaching a thermodynamic equilibrium. The absorption efficiency was estimated by determination of the partition coefficient of toluene between the two phases. Mean values for toluene partition between gas/water and gas/PDMS 47V20 phases were determined as  $666 \text{ Pa}\cdot\text{m}^3\cdot\text{mol}^{-1}$  and  $2.08 \text{ Pa}\cdot\text{m}^3\cdot\text{mol}^{-1}$  respectively. This result shows that PDMS 47V20 presents a stronger absorption capacity compared to water which could be due to its non-polar character.

### 3.2. Statistical analysis of the model

The BBD was selected to optimise toluene degradation by BRP-CLEAs in PDMS 47V20. The different combinations between the four variables are given in Table 2 and analysis was performed by using the statistical analysis of variance (ANOVA). Basically, analysis of adequacy of the proposed model to represent the rate of reaction ( $Y_1$ ) and conversion ( $Y_2$ ) was performed by different statistical tests based on sequential model sum of squares, Fisher test and P values for each contribution and for the full model. Results are represented in Tables 3 and 4 for the two responses respectively. The quality of the fit is given by the correlation coefficient ( $R^2$ ) and the adjusted  $R^2$  [23].

ANOVA results of the quadratic model represented on Tables 3 and 4 indicate that the quadratic model fits well the experimental data. The statistical evaluation based on F values compares the variations in the data that might be explained by the model, respectively the factors or their interactions, with the unexplained, i.e. residual, variations in the observed data [24]. The higher the F values obtained the more significant is the corresponding term [25]. The associated P-value is used to estimate whether  $F_{\text{exp}}$  is large enough to indicate statistical significance [26]. It is usual practice, to consider that a P value greater than 0.05 indicates that the term is statistically insignificant. Insignificant terms were removed from the model using the backward elimination regression and the resulting ANOVA are shown on Tables 5 and 6.

After the backward elimination of insignificant terms, an increase in the model adequacy can be noticed as the following F-values were recorded: 17.65 and 26.52 for conversion ( $Y_1$ ) and reaction rate ( $Y_2$ ) respectively (with a very low P values). Further,

the model F values were much greater than the theoretical  $F_{0.05}$  values (2.84 and 2.57 for  $Y_1$  and  $Y_2$  respectively) indicating that the model was significant for this type of reaction.

As seen in Tables, 5 and 6, the terms including sonication (parameter b) were dropped from the model. Hence, it could be concluded that sonication of the mixture has no significant effect on toluene degradation by BRP-CLEAs in PDMS 47V20.

In this case, the empirical equation for conversion as function of variables in coded terms is given by Eq. 8 and with the uncoded factors by Eq. 9:

$$Y_1 = 35.36 + 12.21 x'_1 + 12.09 x'_3 - 11.61 x'^2_1 + 9.96 x'_1 x'_3 \quad (\text{Eq. 8})$$

$$Y_1 = 5.571 + 3.336 x_1 + 10.71 x_3 - 0.2031 x^2_1 + 17.58 x_1 x_3 \quad (\text{Eq. 9})$$

For the reaction rate, the empirical equation as function of variables in coded terms is given by Eq. 10 and with the uncoded factors by Eq. 11:

$$Y_2 = 0.55 + 0.21 x'_1 + 0.39 x'_2 + 0.18 x'_3 - 0.22 x'^2_1 + 0.24 x'_1 x'_2 + 0.15 x'_1 x'_3 + 0.27 x'_2 x'_3 \quad (\text{Eq. 10})$$

$$Y_2 = 0.294 + 0.031 x_1 - 0.07 x_2 - 3.95 x_3 - 0.0039 x^2_1 + 0.00915 x_1 x_2 + 0.268 x_1 x_3 + 1.04 x_2 x_3 \quad (\text{Eq. 11})$$

It was found that the linear terms  $x_1$ ,  $x_3$  that reflect BRP-CLEAs activity and hydrogen peroxide concentration affect very significantly the conversion of toluene as well as the reaction rate (P values  $\leq 10^{-4}$ ). Furthermore, for reaction rate ( $Y_2$ ), toluene concentration ( $x_2$ ) had the most significant effect. This result seems to be rational since the rate of any enzymatic reaction is defined as a function of the initial concentration of substrate [27].

From all the quadratic terms of the three variables, only BRP-CLEAs activity ( $x^2_1$ ) showed a significant effect on the process. Among all binary interactions, only the one between BRP-CLEAs and  $[H_2O_2]$ , can be considered as having a significant effect on degradation yield. However, all binary interactions had a significant influence on the reaction rate (Eq. 10 and 11) with  $P < 0.05$ .

Consequently, a general analysis of the two equations led to understand that hydrogen peroxide concentration ( $x_3$ ) has the largest effect on toluene degradation by BRP-CLEAs.

### 3.3. Quality of the regression.

The fit of BBD is checked by the correlation coefficient  $R^2$  and the adjusted  $R^2$  after the elimination of insignificant terms. For the conversion ( $Y_1$ ), the model shows  $R^2$  value of 0.828 and an adjusted  $R^2$  value of 0.770. While for the rate of reaction ( $Y_2$ ), values were 0.919 and 0.911 respectively. Both values were sufficiently high to explain that the model results in a good correlation between the experimental and predicted data.

The plots of the predicted values calculated from the model of  $Y_1$  (conversion) and  $Y_2$  (rate of reaction) against experimental values are represented in Fig. 2 and Fig. 3 respectively. The fact that some predicted  $Y_2$  values are negative clearly points to one of the limitation of such empirical models. It can however be noticed that the majority of the points for both responses are close to the diagonal; this distribution reflect a satisfied agreement between predicted and experimental data.

### 3.4. Effect of variables on conversion and reaction rate

In order to understand the effect of different variables on both responses, 3-D plots of surface response were constructed, considering the two most influential factors.

Figure 4 depicts the effect of hydrogen peroxide concentration and BRP-CLEAs activity on the conversion of toluene in silicone oil. It was found that hydrogen peroxide concentration presented the largest effect on toluene degradation. The variation of its concentration from the lowest value (0.025M) to the highest one (0.175 M) induced the augmentation of conversion of toluene from 13 to approximately 60%. At the same time, keeping BRP-CLEAs activity as high as 8.56 IU.mL<sup>-1</sup> led to the highest conversion yield.

Figure 5 shows the effect of the interaction between CLEAs activity and toluene concentration on the average rate of reaction during the 4 hours of the experiments when maintaining hydrogen peroxide concentration at fixed levels ((a) for  $[H_2O_2]=0.025$  M and (b) for  $[H_2O_2]=0.175$  M)). It is clear that the rate of reaction increases with toluene concentration from 43.18 to 691.1 mg.L<sup>-1</sup>, which is apparently in agreement with a first order enzymatic reaction. Hence, it could be noticed that at a concentration of 0.175 M of  $H_2O_2$ , reaction rate increases more rapidly than at 0.025M, which indicate that brassica rapa peroxidase in this type of reaction needs more hydrogen peroxide comparing to other polymerization reactions [28,29]. However, for

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3 low concentrations of toluene and 0.175M of H<sub>2</sub>O<sub>2</sub>, the initial rate of reaction was only  
4 slightly affected by increasing BRP-CLEAs activity.  
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#### 7 **4. Conclusion**

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9 The present study investigated a new innovative process for the elimination of volatile  
10 organic compounds by an integrated process gathering absorption and enzymatic  
11 degradation. The step of absorption was efficiently realised in PDMS 47V20 which  
12 presented a high affinity for toluene. The biodegradation step was evaluated by RSM  
13 based on Box-Behnken design. Initial rate of reaction and conversion yield were studied  
14 as functions of four variables, namely: BRP-CLEAs activity, toluene and hydrogen  
15 peroxide concentrations and sonication.  
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18 It was shown from the ANOVA analysis of the second-order regression model that  
19 experimental data were in relative agreement with predicted values. Analysis of the  
20 effect of each parameter leads to conclude that sonication had no effect on the  
21 elimination efficiency while hydrogen peroxide and toluene concentrations represent  
22 the key factors affecting the process efficiency. Moreover, the impact of BRP-CLEAs  
23 activity appears clearly with increasing toluene concentration for a fixed value of  
24 hydrogen peroxide.  
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### Figure captions

**Fig. 1.** Effect of sonication on BRP-CLEAs dispersion in PDMS 47V20: (A) before sonication, (B) after sonication.

**Fig. 2.** Plot of predicted values versus the experimental values for the conversion of toluene by BRP-CLEAs



1  
2  
3 **Fig. 3.** Plot of predicted values versus the experimental values for the reaction rate.  
4

5 **Fig. 4.** Response surface plot of the effect of BRP-CLEAs activity and hydrogen  
6 peroxide concentration on toluene conversion.  
7

8 **Fig. 5.** Response surface plot of the effect of BRP-CLEAs activity and toluene  
9 concentration on toluene reaction rate. (a) for  $[\text{H}_2\text{O}_2]= 0.025 \text{ M}$  and (b)  $[\text{H}_2\text{O}_2]= 0.175$   
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**Table legends**

**Table 1.** Variables and their levels.

**Table 2.** Complete design layout in coded values and obtained responses.

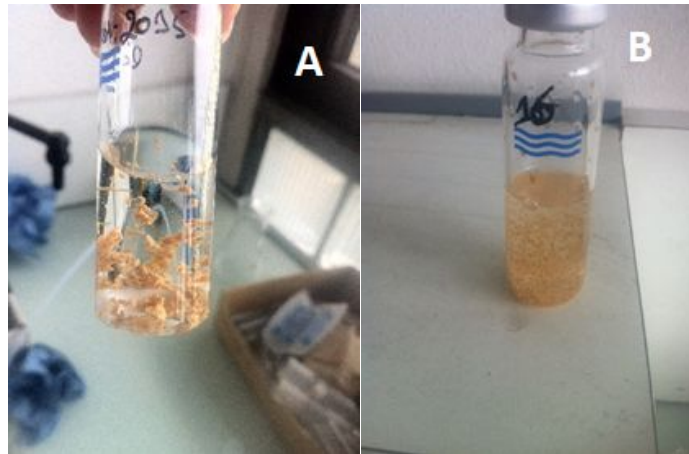
**Table 3.** ANOVA analysis of BBD for the conversion of toluene by BRP-CLEAs in PDMS 47V20. (\*: significant at 5% confidence level)

**Table 4.** ANOVA analysis of BBD for the rate of toluene degradation by BRP-CLEAs in PDMS 47V20. (\*: significant at 5% confidence level)

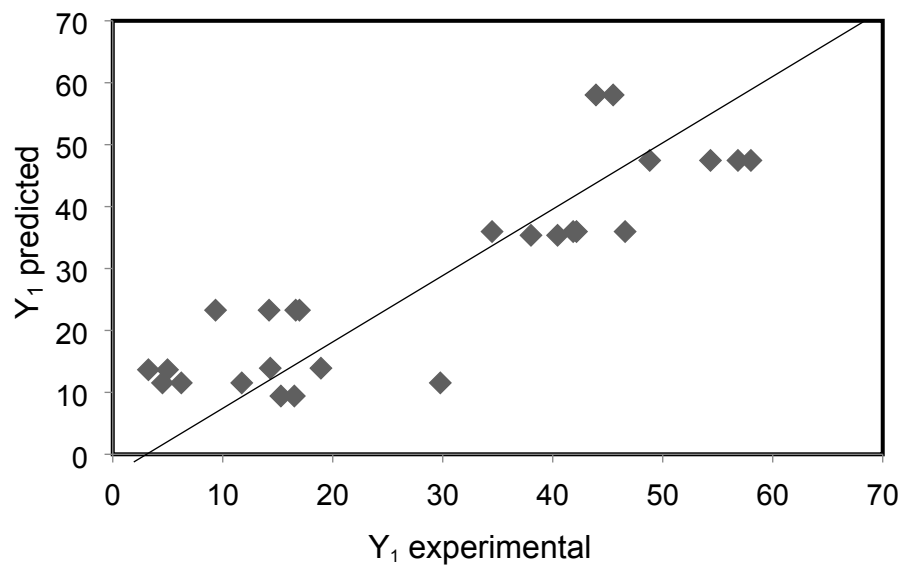
**Table 5.** Resulting ANOVA for the reduced BBD model (response: Conversion %) (\*: significant at 5% confidence level)

**Table 6.** Resulting ANOVA for the reduced BBD model (response: rate of reaction  $\text{mg.L}^{-1}.\text{min}^{-1}$ ). (\*: significant at 5% confidence level)

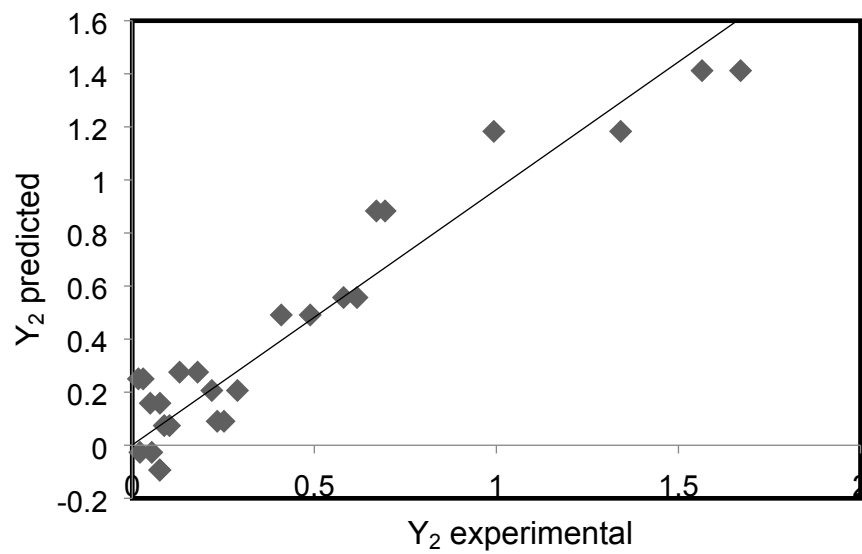
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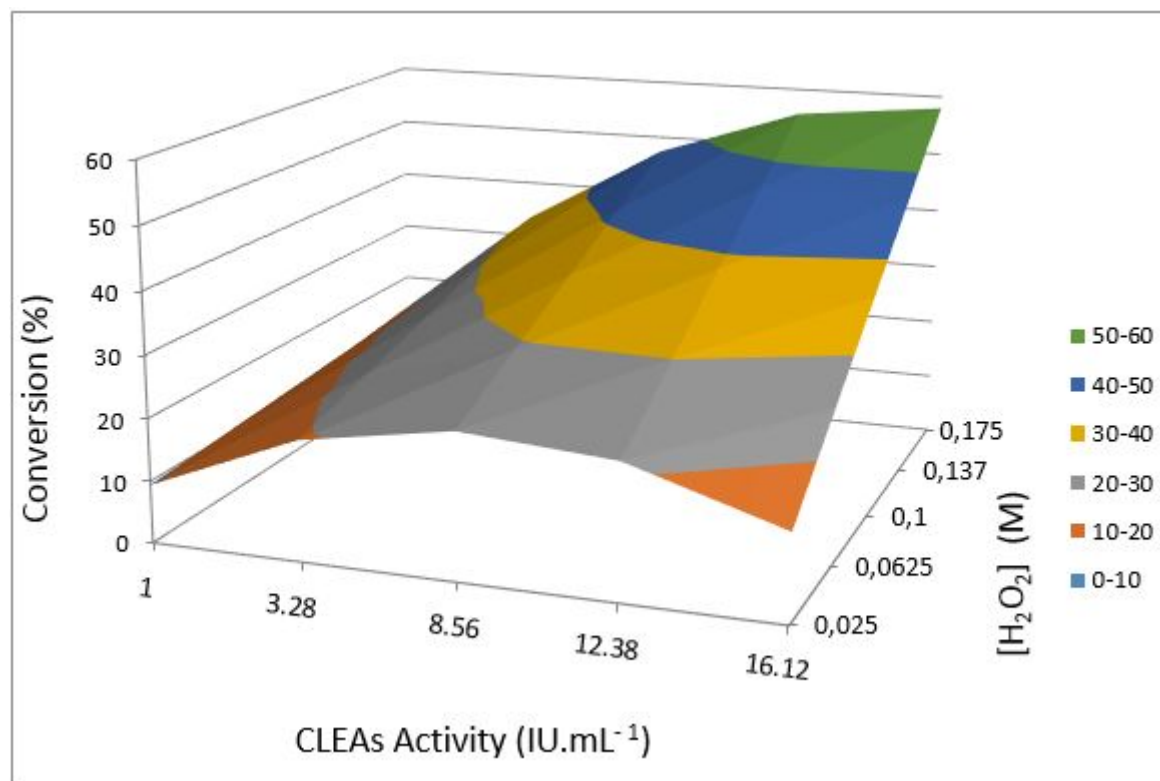
**Fig. 1.** Effect of sonication on BRP-CLEAs dispersion in PDMS 47V20: (A) before sonication, (B) after sonication.



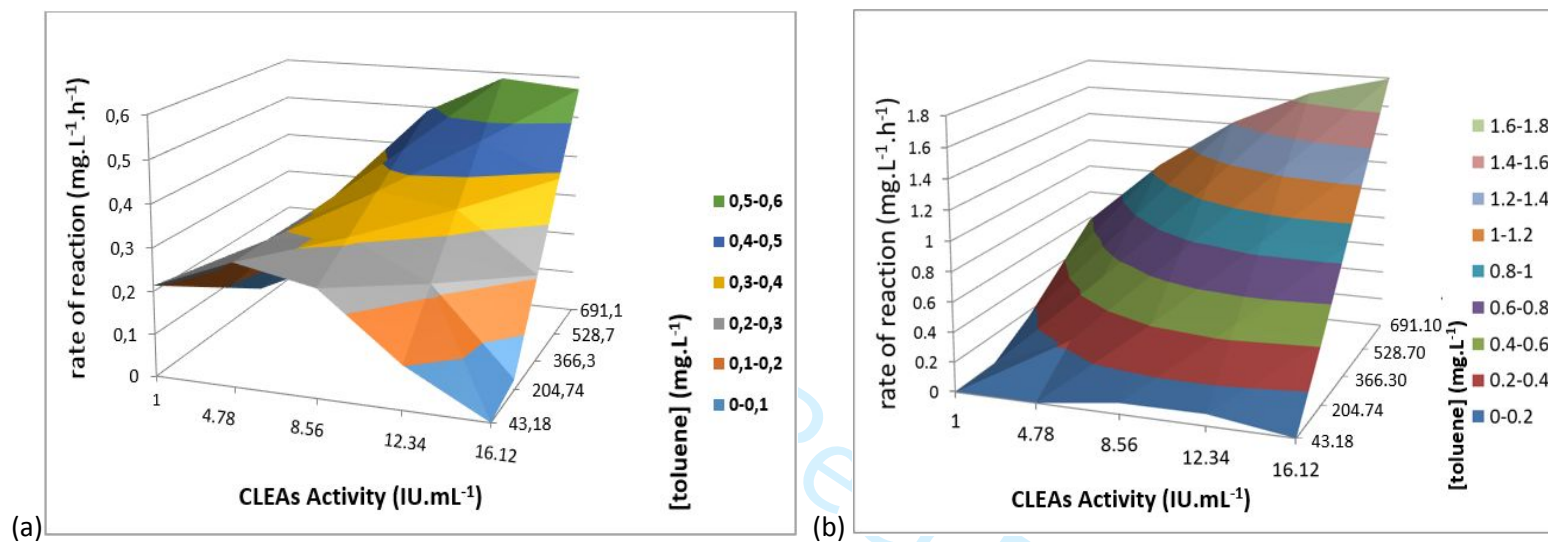
**Fig. 2.** Plot of predicted values versus the experimental values for the conversion of toluene by BRP-CLEAs



**Fig. 3.** Plot of predicted values versus the experimental values for the reaction rate.



**Fig. 4.** Response surface plot of the effect of BRP-CLEAs activity and hydrogen peroxide concentration on toluene conversion.



**Fig. 5.** Response surface plot of the effect of BRP-CLEAs activity and toluene concentration on toluene reaction rate. (a) for [H<sub>2</sub>O<sub>2</sub>] = 0.025 M and (b) [H<sub>2</sub>O<sub>2</sub>] = 0.175 M

**Table 1.** Variables and their levels.

Factor	Variable	unit	Level		
			min	center	max
			-1	0	+1
$x_1$	CLEAs activity	IU.mL <sup>-1</sup>	1	8.56	16.12
$x_2$	[toluène]	mg.L <sup>-1</sup>	43.18	366.30	691.10
$x_3$	[H <sub>2</sub> O <sub>2</sub> ]	M	0.025	0.1	0.175
<b>b</b>	-		with	-	without

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**Table 2.** Complete design layout in coded values and obtained responses.

Run	Factors level with coded terms				Responses	
	$x'_1$	$x'_2$	$x'_3$	$b'$	$Y_1$ (%)	$Y_2$ ( $mg \cdot L^{-1} \cdot h^{-1}$ )
1	0	0	0	1	40.4	0.617
2	1	0	1	1	45.5	0.694
3	1	0	-1	-1	18.9	0.289
4	1	1	0	1	46.6	1.34
5	1	-1	0	-1	42.2	0.0759
6	-1	0	1	1	4.98	0.0760
7	-1	0	-1	-1	15.3	0.233
8	-1	1	0	1	4.52	0.130
9	-1	-1	0	-1	29.8	0.0536
10	0	-1	1	1	48.8	0.0879
11	0	-1	-1	-1	9.34	0.0168
12	0	1	1	1	58.0	1.67
13	0	1	-1	-1	14.2	0.409
14	0	0	0	-1	38.0	0.580
15	1	0	1	-1	43.9	0.670
16	1	0	-1	1	14.3	0.219
17	1	1	0	-1	34.5	0.993
18	1	-1	0	1	41.9	0.0753
19	-1	0	1	-1	3.24	0.0495
20	-1	0	-1	1	16.5	0.252
21	-1	1	0	-1	6.23	0.179
22	-1	-1	0	1	11.7	0.0211
23	0	-1	1	-1	56.8	0.102
24	0	-1	-1	1	16.6	0.0299
25	0	1	1	-1	54.3	1.56
26	0	1	-1	1	17.0	0.489

**Table 3.** ANOVA analysis of BBD for the conversion of toluene by BRP-CLEAs in PDMS 47V20 . (\* : significant at 5% confidence level)

	<i>Sum of squares</i>	<i>df</i>	<i>F<sub>exp</sub></i>	<i>P Value</i>
<i>Model</i>	6828.08	13	4.46	0.00705 *
<i>Mean</i>	3077.97	1	26.1	0.000256 *
$x'_1$	2388.23	1	20.2	0.000723 *
$x'_2$	29.70	1	0.252	0.624
$x'_3$	2339.07	1	19.8	0.000784 *
$x'_1*x'_1$	780.04	1	6.62	0.0243 *
$x'_2*x'_2$	4.56	1	0.0387	0.847
$x'_3*x'_3$	155.52	1	1.32	0.272
$x'_1*x'_2$	96.6	1	0.820	0.382
$x'_1*x'_3$	795.06	1	6.74	0.0233 *
$x'_2*x'_3$	0.279	1	0.00237	0.961
$b'$	0.000176	1	1.49 10 <sup>-6</sup>	0.999
$b'*x'_1$	40.8	1	0.346	0.567
$b'*x'_2$	80.5	1	0.684	0.424
$b'*x'_3$	3.71	1	0.0315	0.861
<i>Residual</i>	1413.62	12		0.00025 *

**Table 4.** ANOVA analysis of BBD for the rate of toluene degradation by BRP-CLEAs in PDMS 47V20. (\* : significant at 5% confidence level)

	<i>Sum of squares</i>	<i>df</i>	<i>F<sub>exp</sub></i>	<i>P value</i>
<i>Model</i>	5.36	13	10.5	0.000120 *
<i>Mean</i>	0.717	1	18.3	0.00105 *
$x'_1$	0.706	1	18.1	0.00111 *
$x'_2$	2.49	1	63.8	3.79 10 <sup>-6</sup> *
$x'_3$	0.554	1	14.2	0.00267 *
$x'_1*x'_1$	0.258	1	6.63	0.0242 *
$x'_2*x'_2$	1.92 10 <sup>-5</sup>	1	0.000493	0.982
$x'_3*x'_3$	0.0116	1	0.297	0.595
$x'_1*x'_2$	0.474	1	12.1	0.00448 *
$x'_1*x'_3$	0.185	1	4.74	0.0500
$x'_2*x'_3$	0.601	1	15.4	0.00200 *
$b'$	0.00906	1	0.232	0.638
$b'*x'_1$	0.00713	1	0.182	0.676
$b'*x'_2$	0.0168	1	0.430	0.523
$b'*x'_3$	0.000636	1	0.0163	0.900
<i>Residual</i>	0.468	12		

**Table 5.** Resulting ANOVA for the reduced BBD model (response: Conversion %) (\* : significant at 5% confidence level)

	<i>Sum of squares</i>	<i>df</i>	<i>F<sub>exp</sub></i>	<i>P value</i>	<i>Coef.</i>	<i>St.dev.</i>
Model	6352.25	4	17.65	1.74 10 <sup>-6</sup> *		
Mean	12505.57	1	138.99	1.00 10 <sup>-10</sup> *	35.4	3.0
$x'_1$	2388.23	1	26.54	4.18 10 <sup>-5</sup> *	12.2	2.4
$x'_3$	2339.07	1	25.99	4.74 10 <sup>-5</sup> *	12.1	2.4
$x'^2_1$	829.87	1	9.22	0.00626 *	-11.6	3.8
$x'_1*x'_3$	795.06	1	8.83	0.00726 *	10.0	3.4
Residual	1889.45	21				

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**Table 6.** Resulting ANOVA for the reduced BBD model (response: rate of reaction  $\text{mg.L}^{-1}.\text{min}^{-1}$ ) (\* : significant at 5% confidence level)

	<i>Sum of squares</i>	<i>df</i>	<i>F<sub>exp</sub></i>	<i>P value</i>	<i>Coef.</i>	<i>St.dev.</i>
Model	5.31	7	26.52	$3.03 \cdot 10^{-8}$ *		
Mean	3.10	1	108.22	$4.84 \cdot 10^{-9}$ *	0.557	0.054
$x'_1$	0.706	1	24.67	$9.96 \cdot 10^{-5}$ *	0.210	0.042
$x'_2$	2.49	1	87.00	$2.57 \cdot 10^{-8}$ *	0.395	0.042
$x'_3$	0.554	1	19.35	0.00034 *	0.186	0.042
$x'^2_1$	0.303	1	10.61	0.00437 *	-0.222	0.068
$x'_1*x'_2$	0.474	1	16.56	0.00071 *	0.244	0.060
$x'_1*x'_3$	0.185	1	6.46	0.02040 *	0.152	0.060
$x'_2*x'_3$	0.601	1	21.00	0.00023 *	0.274	0.060
Residual	0.515	18				

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