



Lignin peroxidase immobilization on Ca-alginate beads and its dye degradation performance in a packed bed reactor system

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ABSTRACT

In this study, lignin peroxidase (LiP) was immobilized by covalent binding onto high-quality Ca-alginate beads using glutaraldehyde as a cross-linking agent. Response surface methodology (RSM) was employed to optimize the operating conditions for optimal immobilization efficiency (IE) and to scrutinize the interactive effects of the processing variables affecting the immobilization efficiency. Influences of various factors such as sodium alginate (0.8, 2.0, and 2.5%), CaCl₂ (0.9, 2.2, and 3.4%), and glutaraldehyde concentration (0.1, 0.2, and 0.3 M) on the IE were determined adopting a three-level-three-factor central composite design (CCD). Results indicated that Ca-alginate beads (average 2.0 mm diameter) developed using 2.0% (w/v) Na-alginate in 2.2% (w/v) CaCl₂ solution functionalized with 0.2 M glutaraldehyde registered the maximum immobilization efficiency (~96.31). Sodium alginate, CaCl₂, and its combination revealed the most significant effect ($p > F$ less than 0.05) on the IE of LiP. However, glutaraldehyde concentration was recorded to be non-significant with less influence on the LiP immobilization. Moreover, the optimally Ca-alginate immobilized LiP was also applied for the decolorization of a textile dye Remazol Brilliant Blue R (RBBR) in a packed bed reactor system (PBRS). The immobilized biocatalytic system was capable of effective dye decolorization in five consecutive batch operations retaining more than 80% dye removal efficiency after the 5th cycle. Taken together, the results manifest that Ca-alginate immobilized LiP might be an attractive choice as an industrial biocatalyst for bioremediation of textile dyes and effluents.

1. Introduction

Interest in bio-catalysis technology has profoundly increased in recent years because of the growing applications in the environmental sectors to tackle various types of emerging pollutants of high concern. In fact, numerous traditional processes have already been substituted with enzyme technology due to obvious benefits such as low toxicity, biodegradability, milder reaction conditions, energy savings, and no protection and deprotection steps (Adeel et al., 2018; Bilal et al., 2018a, 2019a; Bilal and Iqbal, 2019a; Feng et al., 2019; Zdarta et al., 2019). Soluble bio-catalysts render their uses relatively costly for larger-scale applications, and their recoveries are challenging. Therefore, significant efforts have been directed to develop insoluble immobilized enzymes for a range of industrial applications. The insolubilized enzyme presents the advantages of reduced production cost by enzyme reusability, high catalytic stability, and tolerance to unfavorable environmental conditions (Barbosa et al., 2014; Amin et al., 2017; Bilal et al., 2018b, 2018c;

Zdarta et al., 2018a; Bilal and Iqbal, 2019b, 2019c).

In the recent past, several immobilization supports such as agarose, sodium alginate, chitin/chitosan, polyacrylamide, polyvinyl alcohol, macroporous exchange resins, nanoporous silica gel, and hydrophobic sol-gels have been pursued to promote the traditional enzyme immobilization technology (Blandino et al., 2001; Krajewska, 2004; Asgher et al., 2014, 2017a; Bilal et al., 2017, 2019b; Wahba, 2017; Žuža et al., 2017; Zdarta et al., 2018b; Bracco et al., 2019; Martín et al., 2019). Immobilization in Ca-alginate microspheres is of particularly attractive owing to very simple and mild preparation conditions, low-priced, abundant availability, physiological inertness, non-toxicity, good biocompatibility, biodegradability, and paramount performance (Blandino et al., 2001; Won et al., 2005). Alginate is a naturally occurring polysaccharide consisting of repeating units of α -L-guluronic acid and β -D-mannuronic acid residues. Alginate-based immobilization matrix was usually prepared by cross-linking of guluronic acid with mannuronic acid residues using some divalent cations, i.e. Ba²⁺, Ca²⁺,

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Co²⁺ (Khani et al., 2006). Among various enzyme immobilization procedures, covalent attachment to a support material presents the advantages of tight enzyme fixation, minimum leaching and negligible product contamination with byproducts generated during the reaction (Bilal et al., 2018b; Bilal and Iqbal, 2019a). Moreover, multipoint covalent immobilization between the support and enzyme molecule may provide a high stabilization to biocatalyst (Bilal and Iqbal, 2019c). Covalent coupling of biocatalysts in the chitosan matrix is generally accomplished by the reaction of polymeric amino groups with the cross-linking reagent, i.e. glutaraldehyde (Migneault et al., 2004; Sofia et al., 2016; Žuža et al., 2017).

The design and development of a proficient immobilized biocatalyst onto a matrix is a multivariate process involving many variables that influence the immobilization efficiency. The classic approach establishes the optimal conditions by changing one parameter at a time while ensuring the other input constant. This method is considered single-dimensional, inefficient, cumbersome, and time-consuming. These drawbacks can be overcome by carrying out statistical optimization studies using response surface methodology (RSM), Plackett-Burman Design (PBD), and others. RSM is a versatile statistical tool with high suitability for building, improving, and optimizing bioprocess at the lowest cost and with the least number of experiments. The graphical representation of RSM function, i.e. response surface allows describing the individual as well as cumulative interactions of numerous factors as compared to traditional one-variable-at-a-time experimental design (Mohamad et al., 2015; Myers et al., 2016; Ejaz et al., 2018). In recent years, RSM has been a widely practiced approach for the biosynthesis and optimization of a wide-variety of value-able biotechnological products such as enzymes, chemicals. The central composite design is regarded as the most effective factorial design for estimating the response with a limited number of experiments.

Lignin peroxidase (LiP) is an important glycosylated enzyme with significant potential to biodegrade and bio-transform highly toxic phenolic compounds from bleach plant effluents. These enzymes have shown a range of industrial applications such as decolorization of dyes, bio-delignification for biofuel production, bioremediation of organic pollutants, biosensors development, and wastewater treatment (Asgher et al., 2014, 2017b; Bilal et al., 2019c). Most of the earlier studies reports the immobilization of various peroxidases including manganese peroxidase (MnP), versatile peroxidase (VP), horseradish peroxidase (HRP), and others, though using different methods for different purposes (Hibi et al., 2012; Bilal and Asgher, 2015; Bilal et al., 2016a, 2016b, 2019b; Bilal et al., 2019b). However, there is no much information available on the use and immobilization of lignin peroxidase (LiP). Therefore, to present the considerable catalytic potentialities of LiP, in the present work, LiP enzyme was immobilized onto Ca-alginate beads using glutaraldehyde as a bi-functional activating agent. Response surface methodology (RSM) based on a three-level-three-factor central composite design (CCD) was used to investigate the optimal values of immobilization parameters (i.e., sodium alginate, calcium chloride, and glutaraldehyde) to achieve the maximum enzyme immobilization. Finally, the optimally immobilized-biocatalyst was applied for the degradation and decolorization of a synthetic dye Remazol Brilliant Blue R (RBBR) from the aqueous solution and recycling ability of the immobilized biocatalytic system was evaluated a Packed Bed Reactor System (PBRS).

2. Materials and methods

2.1. Chemicals and reagents

Sodium alginate, calcium chloride anhydrous, and glutaraldehyde were obtained from Sinopharm Company, China. Remazol Brilliant Blue R (RBBR) dye was obtained from a local representative of Sigma-Aldrich, USA. During the study, the highest purity grade reagents and chemicals were used, and all necessary solutions were prepared in

List of Figures

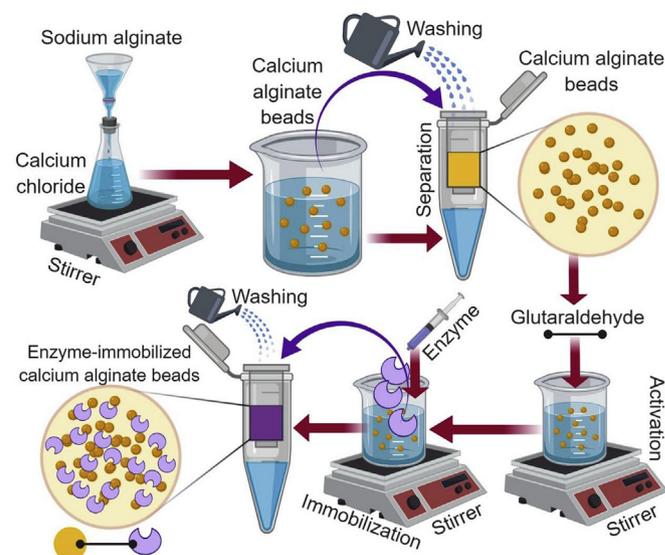


Fig. 1. Schematic representation of LiP covalent immobilization in Ca-alginate beads.

distilled water throughout the experiment.

2.2. Optimization of LiP covalent immobilization in Ca-alginate beads

Immobilization of LiP was carried out using sodium alginate as support and glutaraldehyde as a cross-linker as portrayed in Fig. 1. A Central Composite Design (CCD) was used to examine the combined effect of three independent variables, namely sodium alginate (0.8, 2.0, and 2.5%), CaCl₂ (0.9, 2.2, and 3.4%), and glutaraldehyde concentration (0.1, 0.2, and 0.3 M) on LiP immobilization by selecting the immobilization yield (IY) as the response variable. A 2.0% sodium alginate solution was extruded dropwise into 0.2% CaCl₂ solution under continuous stirring, and the formed beads were hardened in the same solution for 2–4 h at room temperature. Good quality beads of uniform size (average 2.0 mm diameter, manually calculated using mm scale bar) and shape were collected, filtered, and washed with 100 mM tartrate buffer (pH 3.0). These Ca-alginate beads were incubated with different concentrations of glutaraldehyde solutions (v/v) following CCD for 2.0 h at room temperature to develop a cross-linked network. The cross-linked beads were then thoroughly washed with tartrate buffer (pH 3.0) to remove any unattached GLU from the surface of the beads. Finally, the beads were allowed to react with an enzyme solution for different times at 4 °C for immobilization. After the speculated time, the LiP-incorporated beads were washed, dried, and used for enzyme activity measurement. Percentage IY was designated as the ratio of the immobilized enzyme activity to the free enzyme activity. The protein concentration before and after immobilization was quantified by the Bradford method as reported earlier (Bradford, 1976).

2.3. Experimental design

A three-level-three-factor CCD was used in this study with 17 experimental runs to optimizing the operating parameters for optimal immobilization efficiency. The parameters and their levels are sodium alginate (0.8, 2.0, and 2.5), CaCl₂ (0.9, 2.2, and 3.4), and glutaraldehyde concentration (0.1, 0.2, and 0.3). The experimental runs and predicted data in terms of immobilization efficiency are presented Table 1. A second-order polynomial equation, which included all interaction terms, was employed to calculate the predicted response.

Table 1
Central composite design (coded values) of independent variables used for optimizing the immobilization of lignin peroxidase in calcium alginate beads.

Run	Sodium alginate (w/v)	Calcium chloride (w/v)	Glutaraldehyde (M)	Immobilization efficiency (%)
1	0	-1	0	91.22
2	1	0	-1	81.32
3	-1	0	0	74.36
4	1	-1	1	84.38
5	0	0	0	96.31
6	-1	-1	1	71.96
7	0	1	-1	91.07
8	-1	-1	0	81.61
9	-1	0	0	77.83
10	-1	-1	-1	83.29
11	0	0	1	91.26
12	1	1	1	81.92
13	0	1	-1	85.63
14	-1	1	1	78.03
15	1	-1	-1	74.57
16	1	1	0	76.93
17	0	-1	-1	88.37

2.4. Fourier transform infrared spectroscopy (FT-IR)

The FT-IR Spectroscopy (100 FTIR PerkinElmer Spectrometer) was performed to investigate the elemental/functional group attributes on the carrier (Ca-alginate beads) and carrier immobilized enzyme (LiP) at the optimum conditions. For a said purpose, both samples, i.e., Ca-alginate beads (before LiP immobilization was considered as a control sample), and Ca-alginate immobilized LiP beads (after LiP immobilization) were subjected to FT-IR analysis in the range of 500–4000 cm^{-1} with a resolution setting of 4 cm^{-1} .

2.5. Dye decolorization study by Ca-alginate immobilized LiP

Dye decolorization was carried out using a continuous packed bed reactor system (PBRS) containing immobilized LiP as reported in our earlier study (Iqbal and Asgher, 2013). Fig. 2 illustrates a schematic representation of as developed PBRS. Briefly, 5.0 g of Ca-alginate immobilized LiP biocatalyst was loaded into a glass column, and a solution of RBBR dye was passed through the column at a flow rate of 2.0 mL/min. The reactants from the LiP-immobilized column were collected, filtered, centrifuged (5000 \times g, 15 min) and clear supernatants were analyzed spectrophotometrically (UV-2600, SHIMADZO) at 595 nm to measure the percent decolorization using the relation given in Equation (1). The above-mentioned dye decolorization process was repeated for five successive times at regular intervals under the identical conditions.

$$\text{Percent decolorization} = \frac{A_i - A_t}{A_i} \times 100 \quad (1)$$

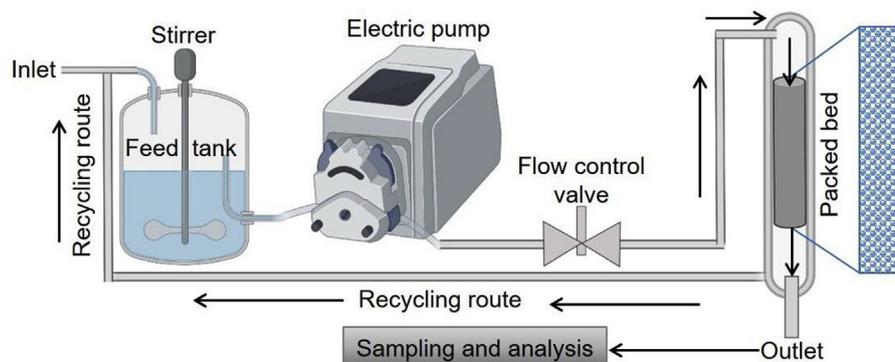


Fig. 2. A schematic representation of as developed PBRS.

where A_i = Initial absorbance of dye before enzymatic treatment and A_t = Final absorbance of dye after enzymatic treatment.

2.6. Enzyme activity assay

The activity of LiP was assayed by determining the H_2O_2 -mediated catalytic oxidation of veratryl alcohol to veratraldehyde in 100 mM tartrate buffer (pH 3.0) at room temperature (Tien and Kirk, 1988). A typical reaction combination (2.1 mL) includes 1.0 mL of tartrate buffer (100 mM, pH 3.0), 1 mL of veratryl alcohol (4 mM) as an assay substrate, 500 μL of H_2O_2 (0.2 M) and 100 μL of enzyme solution. The change in absorbance was recorded using a UV/Visible spectrophotometer at 310 nm. Blank test-tube comprising of all the assay mixture excluding enzyme solution was also run in parallel.

2.7. Statistical analysis

The data were analyzed using a Design-Expert® Software Version 11. Three major analytical steps, including analysis of variance (ANOVA), regression analysis, and plotting of response surface plots were executed to constitute the optimal conditions for immobilization efficiency.

3. Results and discussion

3.1. Optimization of immobilization parameters

An assortment of suitable immobilization conditions is important to maximize the biocatalyst attachment since the best-operating conditions facilitate the enzyme-support reaction. The main purpose of this work was to develop and evaluate a statistical tool to better apprehend the correlation between the immobilization parameters for optimal immobilization of LiP. Optimization of immobilization variables was carried out using a CCD procedure. The experimental runs and their experimental response as IE are listed in Table 1, whereas Table 2 portrays the coded values of all these parameters. Among the 17 experimental runs, experiment 5 (sodium alginate 2%, CaCl_2 2.2%, and glutaraldehyde concentration 0.2 M) exhibited the highest IE (96.31%), and the smallest IE (71.96%) was recorded in case of experimental run 6 (sodium alginate 0.8%, CaCl_2 0.9%, and glutaraldehyde concentration 0.3 M). The effects of individual factors and their mutual interactions were studied using a Design-Expert® Software Version 11. Fitting of the data and ANOVA indicate that LiP immobilization was appropriately represented with a quadratic polynomial model.

The quadratic polynomial model was found to be highly significant and adequate to illustrate the actual relationship between the operating parameters and response with a significant p -value (0.0391) from the ANOVA (Table 3). The computed model F-value of 4.06 indicates the model is significant at 95% confidence level. Moreover, the low value of the pure error and a suitable coefficient of determination revealed good reproducibility of the data and excellent correlations between the tested

Table 2

Range of coded and experimental (actual) values for central composite design used for optimization studies.

Coded value	Sodium alginate (w/v)	Calcium chloride (w/v)	Glutaraldehyde (M)
-1	0.8	0.9	0.1
0	2.0	2.2	0.2
1	2.5	3.4	0.3

independent variables.

The effects of different immobilization variables such as the concentration of sodium alginate, CaCl₂, and glutaraldehyde were assessed on the IE of LiP. The *p*-values denote the statistical significance of factors and are also imperative to understand the mutual interactions among the tested parameters. A *p*-value of less than 0.05 designates that the model terms are significant. A (sodium alginate), B (CaCl₂), AB, and AC parameters revealed the most significant effect (*p* > *F* less than 0.05) on the IE of LiP. However, C (glutaraldehyde concentration), and BC were recorded to be non-significant with less effect (*p* > *F* more than 0.05) on the IE (Table 3). In addition, the results in Fig. 3 demonstrates a high correlation between the experimental and predicted percent residual enzyme activity with different independent variables.

3.2. Effect of correlation between independent variables

Response surface plots scrutinized the correlation between tested operating parameters and IE. Fig. 4 portrays the effect of sodium alginate, CaCl₂, and their combined interaction on the IE of the enzyme. Increasing sodium alginate concentration was observed to increase the enzyme immobilization efficiency of LiP at any specified CaCl₂ concentration. This finding might be ascribed to the fact that alginate concentration is directly associated with the leaching of the immobilized enzyme, and leakage of the entrapped enzyme is decreased by increasing the alginate concentration. Sodium alginate concentration of 2.0% (w/v) and CaCl₂ of 2.2% (w/v) led to the highest IE. Among different concentrations tested, the highest residual activity of L-asparaginase was achieved as a sodium alginate (w/v) concentration of 1.98% (Bahraman and Alemzadeh, 2017). In contrary, manganese peroxidase enzyme exhibited the best IE (73.15%) at 4% (w/v) concentration of sodium-alginate (Bilal and Asgher, 2015). At very low concentration of Na-alginate, the entrapped enzyme escaped out due to the greater pore size of the beads as a result of less tightly cross-linked alginate network. Similarly, the IE again diminished at higher concentration of Na-alginate due to reduced gel permeability, the high viscosity of the gel network and substrate diffusional limitations (Blandino et al., 2000; Daassi et al., 2014; Bilal and Asgher, 2015). Since cross-linking between alginate anions and Ca²⁺ cations result in

Table 3

Analysis of variance for the fitted quadratic polynomial model for optimization of immobilization parameters.

Source	Sum of squares	Degree of freedom	Mean square	F-value	p-value	
Model	652.51	9	72.5	4.06	0.0391	Significant
A-Sodium alginate	4.78	1	4.78	0.2677	0.0208	
B-Calcium chloride	0.6573	1	0.6573	0.0368	0.0533	
C-Glutaraldehyde	0.2513	1	0.2513	0.0141	0.0989	
AB	0.9328	1	0.9328	0.0522	0.8258	
AC	65.15	1	65.15	3.65	0.0978	
BC	1.32	1	1.32	0.0741	0.7933	
A ²	414.6	1	414.6	23.21	0.0019	
B ²	4.52	1	4.52	0.2531	0.6304	
C ²	0.0008	1	0.0008	0	0.9949	
Residual	125.05	7	17.86			
Lack of fit	104.23	5	20.85	2	0.3657	Not significant
Pure error	20.82	2	10.41			
Cor total	777.56	16				

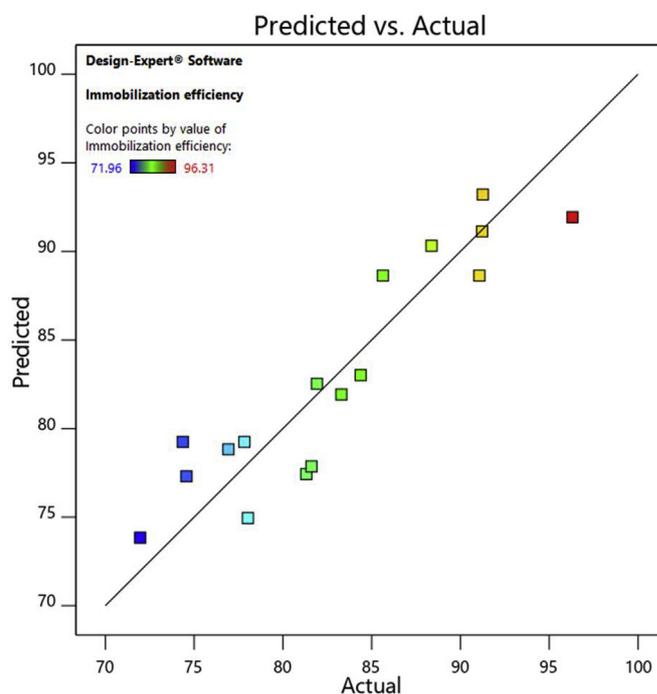


Fig. 3. Predicted versus experimental values of percent residual enzyme activity with different independent variables.

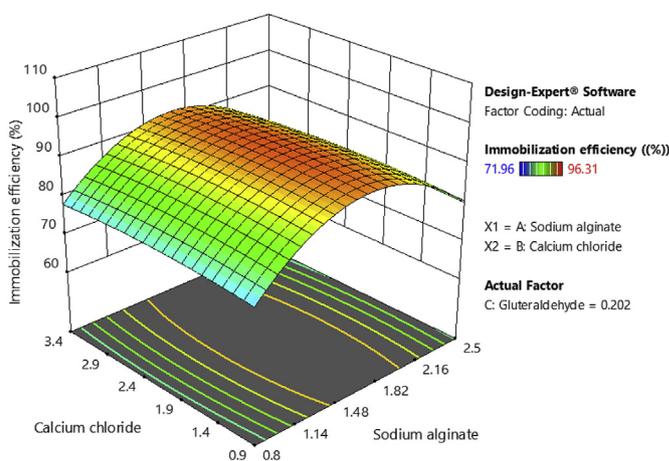


Fig. 4. Influence of sodium alginate concentration, calcium chloride concentration and their combined interaction on the immobilization efficiency.

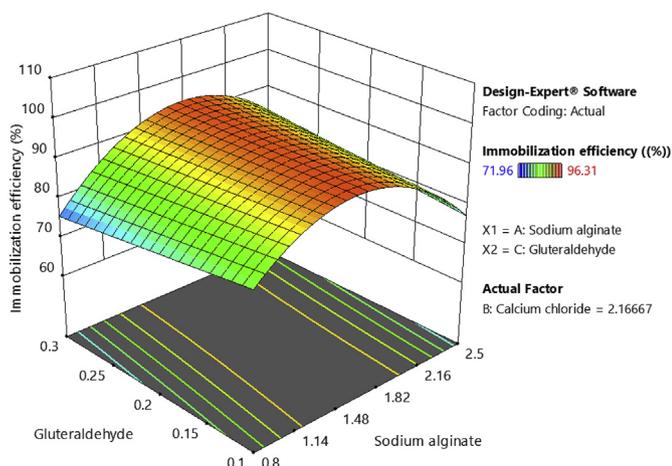


Fig. 5. Influence of sodium alginate concentration, glutaraldehyde concentration and their combined interaction on the immobilization efficiency.

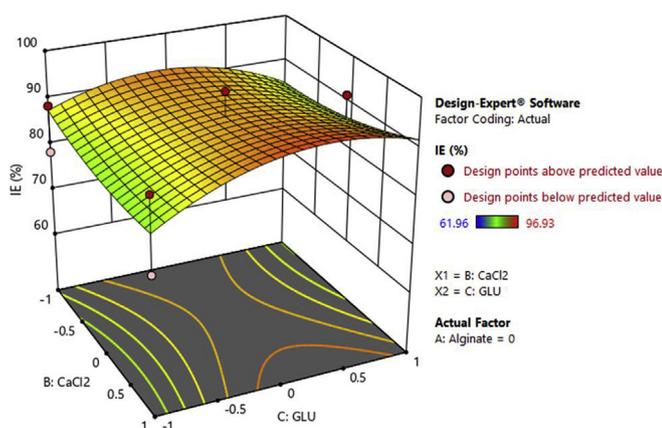


Fig. 6. Influence of glutaraldehyde concentration, calcium chloride concentration and their combined interaction on the immobilization efficiency.

gelation, therefore the concentration of CaCl_2 is also a major parameter for enzyme immobilization. Varying concentration of CaCl_2 concentration showed a notable effect on the enzyme immobilization yield but was less pronounced as compared to sodium alginate. The influence of sodium alginate, glutaraldehyde and their mutual interaction on the IE of the enzyme is displayed in Fig. 5. Apparently, 2.0% (w/v) sodium alginate at a glutaraldehyde concentration of 0.2 M had the maximum IE. As represented in Fig. 6 that varying concentrations of CaCl_2 and glutaraldehyde had a lesser influence on IE. During glutaraldehyde reaction, generation of aldehyde groups on the support surface may react with an amino group of the enzyme as well as other functional groups on the surface (phenols, thiols, and imidazoles) (Srivastava and Anand, 2014). At a lower concentration of glutaraldehyde, lesser aldehyde groups were generated resulting in lower immobilization efficiency (Srivastava and Anand, 2014). On the other hand, initially increasing concentration of glutaraldehyde improved the enzyme immobilization, but at an elevated concentration, the immobilization efficiency was again reduced due to steric hindrance (Asgher et al., 2017a; Kumari and Kayastha, 2011). The maximum IE of LiP enzyme (96.31%) from the model was elucidated as 2.0% (w/v) sodium alginate concentration, 2.2% (w/v) CaCl_2 and 0.2 M glutaraldehyde within the experimental ranges.

3.3. Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectroscopy was used to study the surface chemistry and

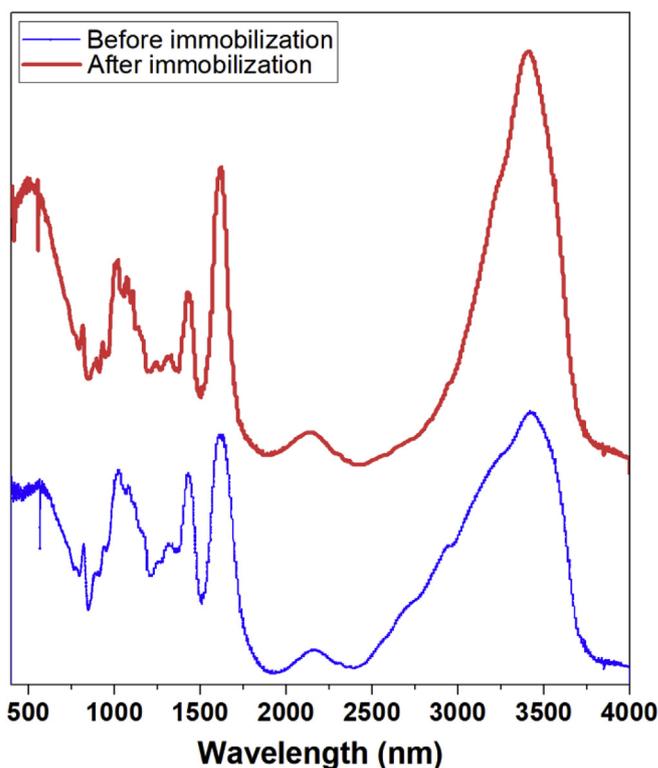


Fig. 7. FT-IR spectrum of as developed both samples, i.e., Ca-alginate beads (before LiP immobilization), and Ca-alginate immobilized LiP beads (after LiP immobilization).

functional group involvement of as developed samples before and after immobilization. The recorded FTIR spectrum is given in Fig. 7, which indicates the presence of several functional groups involved in the process. Briefly, the appearance of extensive absorption peaks at the wavelength region $3300\text{--}3500\text{ cm}^{-1}$ attributes to the OH-stretching of hydroxyl groups (-OH) or NH stretching vibration and at $2800\text{--}2900\text{ cm}^{-1}$ attributes to the -CH stretch. The increase in the intensity of characteristics sharp peak at the region $1600\text{--}1725\text{ cm}^{-1}$ designate the aldehyde group that indicates the presence of polyglutaraldehyde, which was used a crosslinking purpose. More specifically, the major peak clearly observed at 1640 cm^{-1} confirms the presence of Amide I ($\nu\text{ C=O}$, $\nu\text{ CN}$), related to peptide bonds (CONH) (Andrade et al., 2018). The absorption bands at 1433 and 1027 cm^{-1} are due to the vibrational stretch of C-N and C-O in amino acid (Yedurkar et al., 2016), indicating the presence of an enzyme in the polymeric beads.

3.4. Application of Ca-alginate immobilized LiP for dye decolorization

The use of immobilized biocatalysts in industrial applications appears an economically viable technology because immobilization may enable enzyme recycling in repeated batches. Herein, the dye-decolorizing ability of Ca-alginate immobilized LiP was evaluated in a packed bed reactor to degrade Remazol Brilliant Blue R (RBBR) dye, and results are shown as UV-vis absorption spectra before and after enzyme treatment (Fig. 8). Notably, the immobilized enzyme treatment led to more than 90% removal of the tested dye. The principal objective of immobilization is to maximize the advantages of enzyme catalysis by repeated uses in the continuous batch processes. Consequently, the Ca-alginate immobilized LiP was used for six successive dye removal cycles. Results revealed that the immobilized biocatalytic system retained higher than 70% of operational efficiency even after six consecutive uses. A gradual decline in catalytic performance of the immobilized LiP by increasing the number of dye removal runs might be ascribed to the

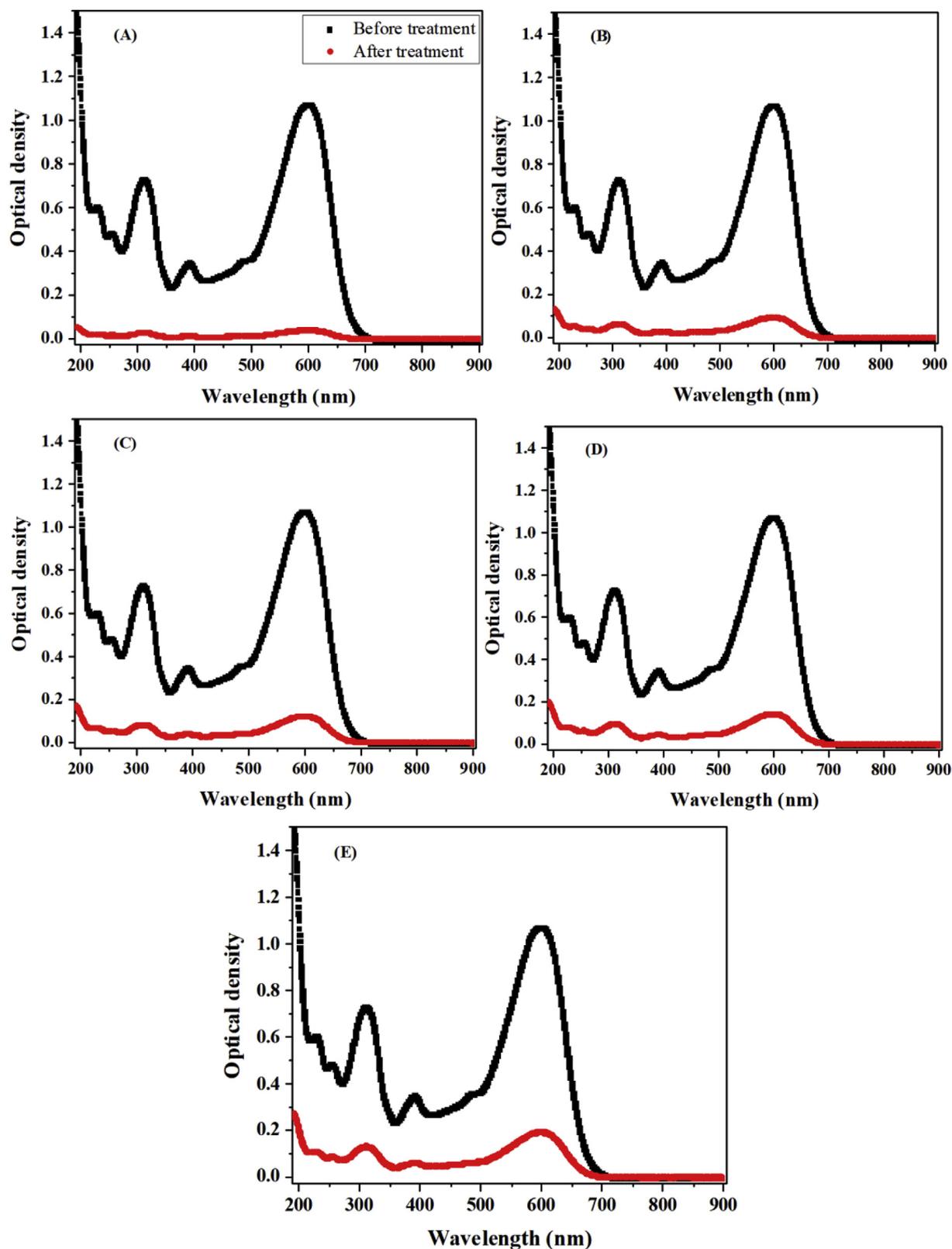


Fig. 8. UV-vis absorption spectra of RBBR dye before and after Ca-alginate immobilized LiP treatment. Where, (A) dye treatment/removal cycle 1, (B) dye treatment/removal cycle 2, (C) dye treatment/removal cycle 3, (D) dye treatment/removal cycle 4, and (E) dye treatment/removal cycle 5.

enzyme leaching from the Ca-alginate network or due to the accumulation of highly active free radicals, which entangled the catalytic site of enzyme leading to enzyme inactivation or product inhibition. In an earlier study, Sofia et al., (2016) immobilized a purified LiP from *Schizophyllum commune* IBL-06 onto glutaraldehyde cross-linked

chitosan microspheres and employed for the decolorization of various reactive dyes. The chitosan-immobilized enzyme decolorized 69.86%, 95.43%, 89.71%, 63.76%, 83.87% and 87.54% of Reactive T blue GWF, Sandal-fix Turq blue GWWF, Sandal-fix Foron blue E2BLN, Sandal-fix Black CKF, Sandal-fix Golden yellow CRL, and Sandal-fix Red

C4BLN dyes, respectively, within 6 h at 30 °C. It also showed higher than 70% and 35% of its original activity after three and seven repeated batches, respectively. The nanoporous gold immobilized LiP resulted in 87.2%, 75.5%, and 84.6% conversion of pyrogallol red, rhodamine B, and fuchsine dye, respectively (Qiu et al., 2009). Recently, Shaheen et al. (2017) entrapped LiP from *Ganoderma lucidum* IBL-05 in Ca-alginate beads under optimized conditions of Na-alginate (4.0%), CaCl₂ (0.2 M) and glutaraldehyde (0.02%). The resulted developed LiP-based biocatalytic system exhibited potential dye decolorization (80–93%) towards a range of Sandal reactive dyes. In addition, water quality parameters (i.e., BOD, COD, and TOC), and toxicity of dye solutions (heamolytic and brine shrimp lethality) was also substantially reduced by treatment with Ca-alginate immobilized LiP (Shaheen et al., 2017). It can be inferred that Ca-alginate beads-immobilized LiP may be an effective biocatalyst for bioremediation of dye pollutants or dye-harbing industry wastewater effluents.

4. Conclusions

In this investigation, lignin peroxidase was successfully immobilized onto glutaraldehyde cross-linked high-quality Ca-alginate beads by covalent linkage. Response surface methodology based on central composite design appeared to be a noteworthy tool to optimize processing parameters for the optimal immobilization. A second-order polynomial model was used to scrutinize the correlation between the immobilization parameters such as sodium alginate, CaCl₂, and glutaraldehyde concentration. Results revealed that sodium alginate, CaCl₂, and its combination revealed the most significant effect ($p > F$ less than 0.05) on the IE of LiP. However, glutaraldehyde concentration was recorded to be non-significant with less effect on immobilization efficiency. Maximum immobilization efficiency of LiP was achieved by Ca-alginate beads (average 2.0 mm diameter) developed under optimal conditions of 2.0% (w/v) Na-alginate in 2.2% (w/v) CaCl₂ solution and cross-linked with 0.2 M glutaraldehyde. Further, a noteworthy potential of the immobilized LiP for decolorization/degradation of a textile dye RBBR in repeated batch cycles suggests its usefulness for bioremediation purposes.

Conflicts of interest

Authors declare that they have no conflict of interest.

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