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Decolorization of a chromophore molecule with immobilized horseradish peroxidase

Descoloração de uma molécula de cromóforo com peroxidase de rábano imobilizada

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ABSTRACT

The enzymes can modify some effluent characteristics in order to increase the degradability, or the bioconversion of liquid effluents. The oxireductases, laccases and peroxidases have been used due their high potentiality in many environmental treatments of natural and synthetic organic compounds as dyes, phenols and polyphenolics molecules. The performance of immobilized horseradish peroxidase on aminopropyl glass beads was investigated in this work in a decolorization reaction of methylene blue colorant. The experiments were conducted in batch conditions during 3 hours, with different aqueous solutions of peroxide hydrogen (H_2O_2) concentration solutions (2-10 mg/L), methylene blue (ME) (5-20 mg/L) and the pH in the range from 4 to 8, according an experimental design proposed by the software STATISTICA®. After 3 hours of treatment the reduction of the color was 60% when comparing to the original color and 50% when the immobilized enzymes were reused in five sequential batch treatment cycles for a 10 mg/L of H_2O_2 solution, 20 mg/L of ME and pH 8.0. Working in continuous process with two microreactors in series the system showed a good performance with 97% of decolorization in the first 15 minutes. After 1 hour of continuous treatment the percentage of the color removing was around 70%.

Keywords immobilized horseradish peroxidase; enzymatic decolorization; sequential enzymatic microreactors**.**

RESUMO

As enzimas podem modificar algumas características do efluente para aumentar a degradabilidade, ou a bioconversão de efluentes líquidos. As oxiredutases, lacases e peroxidases têm sido utilizadas devido a sua alta potencialidade em diversos tratamentos ambientais de compostos orgânicos naturais e sintéticos, como corantes, fenóis e moléculas polifenólicas. O desempenho da peroxidase de rábano silvestre imobilizada em esferas de vidro de aminopropila foi investigado neste trabalho em uma reação de descoloração do corante azul de metileno. Os experimentos foram conduzidos em batelada por 3 horas, com diferentes soluções aquosas de soluções de concentração de peróxido de hidrogênio (H2O2) (2-10 mg / L), azul de metileno (ME) (5-20 mg / L) e pH no variam de 4 a 8, segundo um delineamento experimental proposto pelo software STATISTICA®. Após 3 horas de tratamento, a redução da cor foi de 60% quando comparada com a cor original e 50% quando as enzimas imobilizadas foram reutilizadas em cinco ciclos sequenciais de tratamento por lotes para uma solução de 10 mg / L de H2O2, 20 mg / L de ME e pH 8,0. Trabalhando em processo contínuo com dois microrreatores em série o sistema apresentou um bom desempenho com 97% de descoloração nos primeiros 15 minutos. Após 1 hora de tratamento contínuo, a percentagem de remoção de cor foi de cerca de 70%.

Palavras-chave peroxidase de raiz-forte imobilizada; descoloração enzimática; microreatores enzimáticos sequenciais

1.   INTRODUCTION

The organic and inorganic dyes are extensively used in the textile industries, due the variety of the color and tonalities, almost are easily applied and inexpensive in energy demand. Anthraquinones, phthalocyanines and azo dyes are reported as colorants that are used in many industries. Molecules with the function group -N=N-, called as azo dyes are known as the

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largest chemical class of colorants (66% of all colorants). The chemical and physico-chemical processes adopted for the treatment of the majority of liquid effluents have been investigating in order to achieve more efficient reducing grades of the hard recalcitrant molecules. One of these new used methods is the new advanced oxidations processes (AOPs) that deals with oxidant free radicals or apply ox reduction enzymes reactions. Among the methodologies applied for AOPs the enzymatic oxidation has been researching to degrade the liquid rejects provided from the textile industries. They are singled out as a promising strategy for the degradation of dyes, leading to effective results and in accordance with the concepts of technological sustainability (Stolz, 2001; Axelsson et al., 2006, Champagne & Ramsay, 2007). Methylene blue dye is an aromatic organic compound, heterocyclic and soluble in water, which has wide application in the production of paper and various materials such as nylons and polyesters. It is classified as a basic cationic dye and also because it releases colored cations in aqueous solution (Guaratini e Zanoni, 2000). The peroxidases enzymes (EC.1.11.1.7) were discovered by German scientist Schönbein in 1863. Oxidoreductases are able to catalyze oxidation reactions in a variety of phenols and aromatic amines through the presence of hydrogen peroxide. Many studies have shown the potential use of the enzyme horseradish peroxidase (HRP) in the treatment of colored effluents, and it was shown that the enzyme immobilization techniques improve the performance of the enzymatic reaction when compared to the free form. (Celebi et al., 2013, Veitch, 2004). The aim of this study was to evaluate the performance of the immobilized HRP on glass beads (aminopropyl) in decolorization of methylene blue (MB) in batch and continuous processes of aqueous waste treatment.

2. MATERIALS AND METHODS

2.1. HORSERADISH PEROXIDASE IMMOBILIZATION

The enzyme horseradish peroxidase (HRP) (EC.1.11.17) was given from Toyobo do Brazil. The lyophilized enzyme was solved in phosphate buffer pH 7.0, and filtered with INLAB paper, and dialyzed with SPECTRUM* (12000 – 14000 kD) membrane at 8ºC during 24 h. After the treatment the enzyme in buffer solution was maintained in freezer conditions.

The enzyme activity was measured with the 4-aminoantipirine method as described by Nicell e Wright, 1997, and the concentration in solution was determined with the Bradford method (Bradford, 1976).

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The enzyme horseradish peroxidase was immobilized on aminopropyl glass beads (porosity of 700 Å and mesh in the range of 80-120, Sigma) as described by Alhadeff et al. (2005). Glutaraldehyde (2.5%, v/v) was used as a crosslink agent that gives the carbonyl group which react with the enzyme nucleophilic groups (ZAIA et al., 1998) to form stable covalent bonds. Enzymatic solutions with 150 U/L were prepared and added with 0.4 g of amynopropyl glass beads that were previous treated with the bifunctional linker glutharaldehyde.

The percentage of immobilized enzyme on the beads was obtained with equation (1):

$$\text{Immobiliza tion Efficiency } (\%) = \left(\frac{[\text{Protein}]_{before} - [\text{Protein}]_{after}}{[\text{Protein}]_{before}} \right) 100 \qquad (1)$$

Where [Protein]$_{before}$ means the free protein concentration before the immobilization and [Protein]$_{after}$ the protein concentration of the enzymatic solution after the immobilization procedure.

2.2. DECOLORIZATION EFFICIENCY

All the reactions with the colorant methylene blue were in tem milliliters of total volume, with a concentration in a range from 5 to 20 mg of MB/L (linear absorbance response at 660 nm, $R^2 = 0.9921$), buffer solution of pH from 4.0 to 8.0 and H_2O_2 solution from 2 to 10 mg/L, sequentially added into a Becker of 50 mL. The batch decolorization reaction has from 1 to 3 hours, with constant mixing at 60 rpm at 30ºC (Pereira, 2014) in a shaker CIENTEC (Model CT-712). The absorbances of the methylene blue solutions were measured before and after de decolorization reactions were obtained using a spectrophotometer UV (SHIMADZU UV SPECTROPHOTOMETER – Model UV-1800) at 660 nm. Color removal efficiency was determined with equation (2):

$$\textit{Efficiency of color removal (\%)} = \left(\frac{[\textit{Absorbance}]_{before} - [\textit{Absorbance}]_{after}}{[\textit{Absorbance}]_{before}} \right) 100 \qquad (2)$$

Where [Absorbance]$_{before}$ means the absorbance for the free protein solution before the immobilization and [Absorbance]$_{after}$ the absorbance for the protein solution after the immobilization procedure.

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2.3. EXPERIMENTAL DESIGN

Intending evaluate the pH influence, the methylene blue concentration (MB) and the peroxide hydrogen concentration (H_2O_2), in the color removal, an experimental design with the software STATISTICA® was used to define the better conditions for the enzymatic reaction. The fractional factorial model 2^{k-p} with 3 central points, and p=1 (considered premise) as shown in table 1.

Tabel 1. Factorial fractionary design 2^{k-p}.

Experiment	H_2O_2 (mg/L)	AM (mg/L)	pH
1	6.0	12.5	6.0
2	6.0	12.5	6.0
3	10.0	20.0	8.0
4	6.0	12.5	6.0
5	2.0	20.0	4.0
6	2.0	5.0	8.0
7	10.0	5.0	4.0

2.4. CONTINUOUS DECOLORIZATION IN MICROREACTORS

The microreactors used in this work, that had a cylindrical geometry, were made in acrylic, with 0.91 mL of total void volume (20 mm of high and 7.6 mm of inside diameter), and a ratio of high/inside diameter of 2.5 (Alhadeff et al., 2004). The system that worked with two microreactors in series, which were connected by a polycarbonate three vials valve used to take samples of the discolored effluent from the first microreactor. The methylene blue aqueous solution (20 mg/L) was mixed with a magnetic flat plate (MARCONI®) and the continuous flux controlled by a peristaltic PUMP drive PD5001 HEIDOLF®. The conditions of the experiment was investigated working with pH 8.0, and concentration of H_2O_2 and MB of 10 mg/L and 20 mg/L, respectively. The performance of the continuous decolorization process was studied with both microreactors packed with the horseradish peroxidase immobilized on the aminopropil glass beads. The residence time of each microreactor was measured considering the period of the time that the continuous methylene blue solution had to go through the immobilized enzymes packed microreactor. The period the time was defined as zero when the colored solution was at the entrance of the packed microreactor and the end when the flux was at the exit of the packed microreactor. Working with a volumetric flow of 0.78 mL/min the experimental residence time measurements were 7.0 s and 8.6 s for the first one (R_1) and the second (R_2) microreactor respectively. The continuous discoloring

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experiments was conducted during 3 hours and aliquots were taken at the exit of the microreactors (R_1) and (R_2) at each 15 minutes. Figure 1 shows details of the two microreactors system, details of the microreactor was reported previously (Alhadeff et al., 2004).
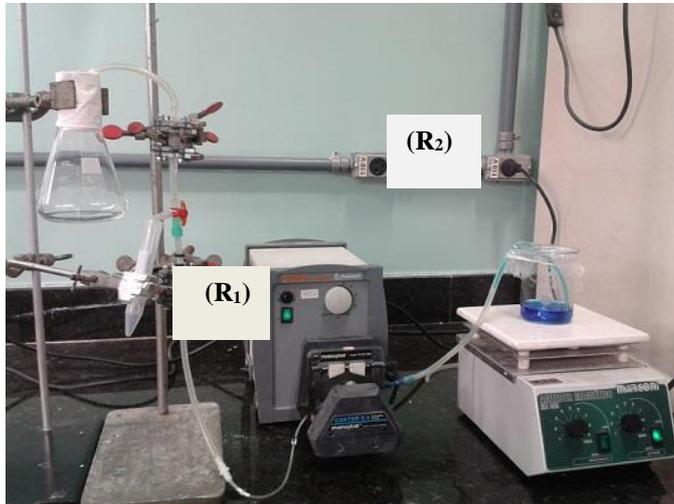

Figure 1 . The two immobilized enzymes microreactors system.

3. RESULTS AND DISCUSSION

3.1. ENZYME IMMOBILIZATION

Enzyme activity was measured in pH 4.0, 6.0, 7.0 and 8.0 and after the different pH enzyme solutions were adapted to the immobilization method used in this study. The immobilization efficiency was evaluated to define the best pH condition for the covalent bond horseradish peroxidase immobilization on amino propel glass beads. Table 2 shows the values of the enzymatic solutions activity, protein concentration and the specific activity for each pH investigated. pH 7.0 provides the major ratio activity/mass of enzyme (U/mg) and the higher enzymatic activity solution (U/ml). The enzymatic solution was diluted to achieve an activity around 150 U/ml as the immobilization method recommended.

Table 2. The pH influence on the enzymatic activity value.

pH	A (U/mL)	Conc (mg/ml)	$A_{specific}$ (U/mg)
4.0	319.6	3.570	89.5
6.0	712.3	2.426	293.6
7.0	952.0	2.500	380.8

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8.0	699.0	2.398	291.5

The results showed surface limitation of the functionalized glass bead, after glutaraldehyde treatment, in receiving the amino groups from the enzyme horseradish peroxidase (~40.0000 kDa) to form the covalent linkage, as was previous observed by Alhadeff (2005). Figure 2 shows the behavior of the immobilization efficiency with the pH and the respective enzymatic load expressed as mg HRP/mg of support.
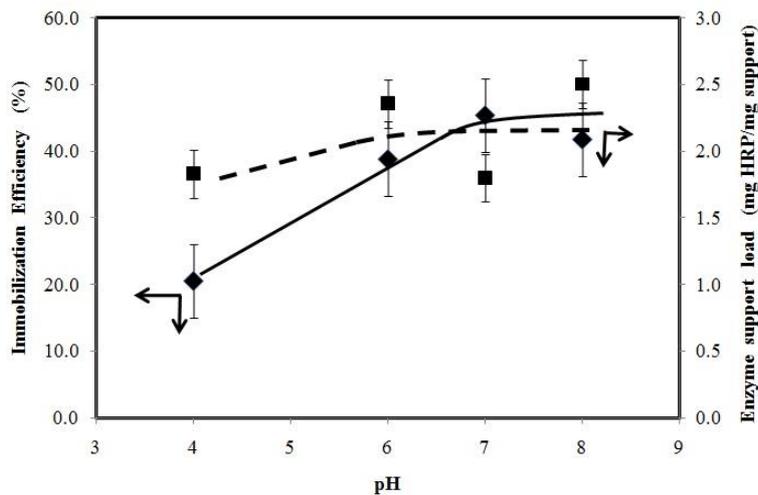

Figure 2 . Influence of pH on the immobilization efficiency (◆) and the enzyme load on the support (■).

The higher specific activity of 380.8 U/mg was verified for the pH 7.0 and a load of immobilized enzymes of 1.8 mg HRP/mg of support. The media immobilization efficiency in acid environment (pH 4.0) was around 20% of HRP retention, and as pH increase for neutrality the efficiency reached the maximum value of 45.4% (pH 7.0) for horseradish peroxidase covalent linking to the glutaraldehyde aminopropyl glass beads.

3.2. EXPERIMENTAL DESIGN RESULTS ANALYSES

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The results obtained for the experimental design are shown in table 3.

Table 3. The percentage decoloration after 1, 2 and 3 hours of continuous process.

Experiment	Decoloration[1] (%)	Decoloration[2] (%)	Decoloration[3] (%)
1	37.6	39.6	40.6
2	36.3	38.2	39.5
3	58.5	59.3	60.4
4	35.4	36.7	38.2
5	12.1	12.7	13.3
6	56.1	57.0	58.9
7	23.6	24.8	23.2

[1]- after 1 hour of reaction; [2] – after 2 hours of reaction; [3] – after 3 hours of reaction.

The higher color decolorization percentage was observed at pH 8.0 (experiments 3 and 6) achieving values around 59%. Working at pH 6.0 the decolorization was above 35% (experiments 1, 2, and 4). For the experiments made in acid environment (pH 4.0) the lowers results were obtained, approximately 23% and 13% for experiments 7 and 5, respectively. The strong influence of the pH can be confirmed with the ANOVA statistical analysis, for the p-values data shown in table 4, with 95% of confidence grade.

Table 4. Statistical p-values (STATISTICA®).

Factor	p - value
Curvature	0.311164
H_2O_2	0.024562
Methylene Blue	0.054444
pH	0.000781

The mathematic model that correlate methylene blue decolorization with immobilized HRP as a function of the pH and peroxide hydrogen concentrations evaluated by the equation (3).

(%) Decolorization $= 3.45312[H_2O_2] + 19.72312$**pH** $+ 36.42102$ (3)

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The linear profile had a coefficient correlation (R^2) of 0.99851, a good value for the mathematical model adjustment. The figure 3 showed the surface response levels considering the decolorization percentage as a function of the pH and H_2O_2 concentration (mg/L).
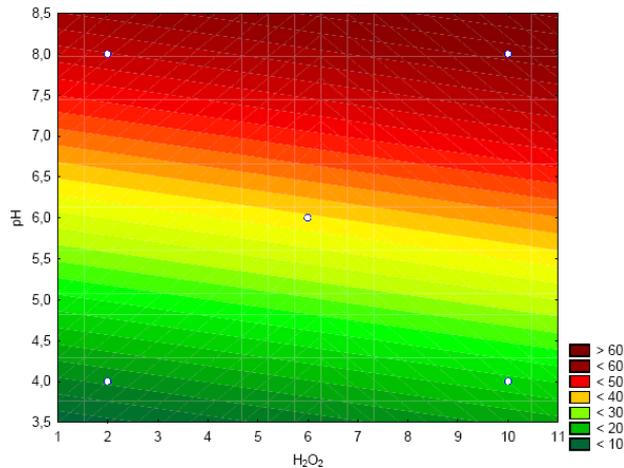

Figure 3. Response surface plot for the color reduction percentage of the dye methylene blue as a function of pH and H_2O_2 concentration.

The figure 4 shows that for pH 8.0 the decolorization percentage fitted around 60% in the range from 1 to 10 mg/L of H_2O_2. The statistical analyses showed that levels upper 60% of decolorization could be achieved when the peroxide hydrogen solution had 10 mg/L . The immobilized HRP decolorization reaction performance was investigated working in pH 7.0 the value considered good for HRP oxireduction reaction, and 45.4% of decolorization was observed for immobilized HRP (1.8 mg HRP/g aminopropyl beads). After this first batch, a sequential batch reaction (3 cycles) with the reuse of the same immobilized enzymes using the conditions operations as described by Pereira et al. (2014) (10 mg MB/L and 6.0 mg H_2O_2/L) for free HRP methylene blue decolorization reaction. The same immobilized enzymes were tested in different concentrations conditions (10 mg H_2O_2/L and 20 mg MB/L). The results of the batch experiments are shown in figure 4.
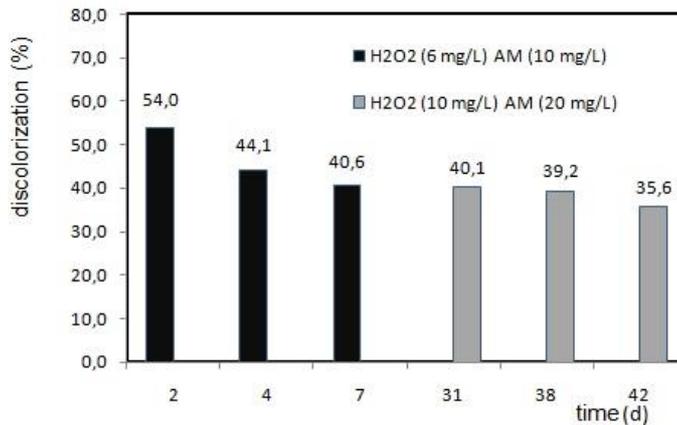
Figure 4. Sequential batch decolorization of the methylene blue solution with reuse of the immobilized enzymes.

The color percentage remove as can be observed in figure 3 fitted in a range of 50% to 35%, with a decrease of action in 10% after the third cycle. Reduction of 40% was obtained after 5 cycles of reutilization of the immobilized HRP, and a little percentage of the color removing of 35.6% after the six sequential batch cycles. The results for the batch sequential reuses were in accordance to those obtained with the experimental design and the statistical analyses for the factorial planning made before. For pH 8.0, after 6 days, for a second reuse of the immobilized enzymes, 53.7 % of decolorization was observed in the batch process. The figure 5 showed the global performance of the two microreactors system, and a softly declining of the color removing percentage during the three hours of experiment monitoring. The initial 98% decolorization percentage stays upper 70% during the first hour of the continuous process, working with tow microreactor in series. A decrease of approximately 50% of the capability of the enzymatic methylene blue decolorization was observed after 100 minutes of continuous process.
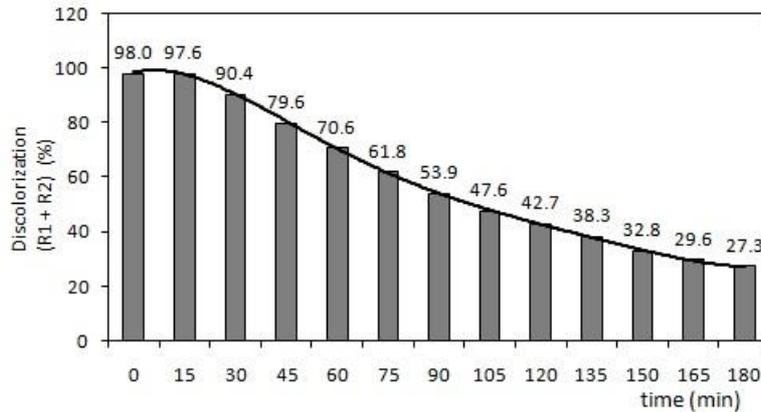
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Figure 5. Percentage of the total discolored reaction.

The table 5 has the resume of the operational conditions and the results obtained in this work for both strategies used for the enzymatic decolorization of the colorant methylene blue with the immobilized HRP.

Table 5. Comparison of the different conduction process: batch and continuous.

Process	Cycles or Microreactors Number	mg HRP / g support	Flow (mL/min)	Decolorization (%)
Batch	3	2.7	-	60 - 50
Continuous	2	1.3 (R1) ; 1.1 (R2)	0.78	≈ 71

In order to compare the results obtained operating in sequential batch cycles end in continuous process de decolorization percentage values were determined after 60 minutes of reaction time. For the sequential batch experiments, after one hour of the first batch, 58.5% of decolorization was observed, and a decrease of 17% of decolorization action after the third cycle. For the continuous process the percentage of the color removing stayed above 70% for the effluent of the second microreactor working in series after 60 minutes of analyses monitoring. After 15 minutes from the beginning of the enzymatic continuous treatment, a

color removing of 80% was obtained in the effluent of the first microreactor and 97% in the effluent of the second one. The figure 7 shows a photograph of the samples used in this work to measure the absorbance and monitor the continuous experiment. The color of the original methylene blue aqueous solution (20 mg/L) (Figure 6(1)) and the discolored samples after the treatment (Figure 6(2)), collected directly from the effluent of the first microreactor (R_1), and from the effluent from the second microreactor (R_2) at 0, 15 and 30 minutes of continuous enzymatic treatment process.
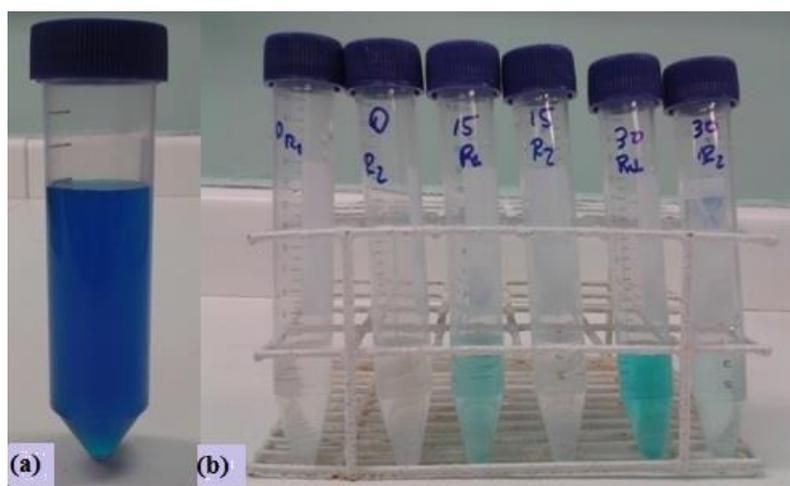

Figure 6. Methylene blue solution (20 mg/L): (a) original sample before enzymatic treatment;   (b) Samples after 0, 15 and 30 minutes of continuous process from the effluents of each microreactors   (R1 and R2) operating in series.

Table 6 shows the results for different synthetic dyes decolorization using enzymatic procedure with immobilized peroxidases in batch and continuous process reported into the literature in the last six years.

Different methods and supports were investigated looking for the ideal environment condition of pH and temperature in order to identify high levels of dyes decolorization reaction with the immobilized enzyme. Investigations were also through the conditions of the process if in batch, continuous or sequential continuous process. Continuous flow working with fixed bed column bioreactors was also studied and this process strategy seems to be interesting and promote high percentages of decolorization when adapted this way to a sequential continuous flow process as was reported by Bilal et al. (2016, 2017a, 2017b).

Table 6. Dye decolorization with immobilize horseradish peroxidase.

Dye	Decolorization (%)[e]	support	Decolorization Process	Reference
Azo	61.2	Polyurethane cubes	static	Malani et al. (2013)
DR19[a]	78.4	Alginate-pedtin beads	batch	Jamal et al. (2013)
	91.2		continuous	
DR19	88.2	Diethilaminoethyl cellulose	batch	Jamal e Goel. (2014)
	69.4		continuous	
LB16, RRV9, RRN4[b]	84.0, 51.0, 46.0	Alginate beads	Sequential bacth in bioreator	Yanto et al. (2014)
RR120, RB4, RO16[c]	72.4, 87.2, 79.6	Alginate beads	Sequential batch in bioreactor	Bilal et al. (2016)
RBBR, RB5, CR, CV[d]	82.2, 97.8, 94.3, 87.4[d]	chitosan beads	Sequential batch in bioreactor	Bilal et al. (2017a)
methyl orange	~90	polyvinyl alcohol-alginate beads	batch	Bilal et al. (2017b)
methylene blue	~ 60 ~ 70	aminopropyl glass beads	batch continuous	This work

[a] Dispersed red 19 (DR19); [b] Levafix blue (LB16), Reactive remazol violet (RRV9) and Reactive remazol navy4 (RRN4); [c] Reactive Red 120 (RR 120), Reactive Blue 4 (RB 4) and Reactive Orange 16 (RO 16); [d] Remazol Brilliant Blue R (RBBR), Reactive Black 5 (RB5), Congo Red (CR) and Crystal Violet (CV), percentages for total organic carbon reduction; [e] best value described.

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Malani et al. (2013) reported a sonoenzymatic decolorization of an azo dye with immobilized horseradish peroxidase at high pressure in presence of polyethylene glycol (PEG) attained the highest of 61.2%. The enzyme was immobilized in cubes of polyurethane foam and 0.1% (w/v) glutaraldehyde solution was added as a cross-linking agent. In a static process the immobilized horseradish peroxidase was reused for 3 cycles, and the best result obtained was 51.5% at ambient static pressure. Jamal et al. (2013) studying the Concanavalin A-peroxidase conjugate immobilized by entrapment with alginate-pectin gel beads obtained 78.4% of color removing percentage for the colorant Dispersed Red 19 (DR19) after 1 hour in batch process. The same authors working with continuous process with two columns in series, the first with the immobilized enzyme, and the second with activated silica achieved 91.2% of decolorization after 20 days of continuous process.

Jamal and Goel (2014) studied the decolorization reaction of synthetic dies with peroxidase adsorbed on diethylaminoethyl cellulose in batch sequential cycles and in continuous process. The authors after five reuses, in stirred batch process at 40ºC, decolorize up to 88.2% of DR19 dye. For the immobilized enzyme in a vertical packed column, the continuous removal of dye color could decolorize DR19 to 69.4% at 37ºC after 50 days of operation. The samples were taken at an interval of 10 days from the column outlet and after submitted to centrifugation to in the sequence be analyzed in a spectrophotometer for the dye color removal. Yanto et al. (2014) worked with a vertical reactor to investigate the ability of immobilized enzymes from Trametes versicolor U97 or Pestalotiopsis sp. NG007 to decolorize three textile dyes (Lefavix Blue 16 (LB16), Reactive Remazol Violet 9 (RRV9), and Reactive Remazol Navy 4 (RRN4)). The authors working in pH 4.5 in sequential batch performed in vertical reactor system obtained decolorization values around 40 % after 5 cycles. A higher percentage was attained when glutaraldehyde was used with the calcium alginate immobilization method (75 %).

In 2016, Bilal et al., reported the use of immobilized horseradish peroxidase in continuous decolorization process, working with a packed bed column and the enzyme entrapment made with calcium alginate and glutaraldehyde as a cross-linking agent. For three different synthetic dies the authors found percentages of decolorization between 72.4% to 87.2%, working with a packed bed bioreactor, in sequential continuous cycles, with a flow of 2 ml/min in each cycle. After seven reused cycles the immobilized enzyme activity remained at 40%. Recently, Bilal et al. (2017a) studied the decolorization of four different dies in a column packed with immobilized horseradish peroxidase in chitosan beads and obtained from

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76 up to 84 % loss of total organic content as a measurement of carbon mineralization, or degradation efficiency. Decolorization was also determined using the continuous flow process (2 mL/min) and percentages ranges from 97.8% - 82.2% to 63.2 % - 37.5% in six reuses of the same beads column. Bilal et al. (2017b) also studied the immobilized horseradish peroxidase on dye decolorization using polyvinyl alcohol-alginate support material in order to degrade methyl orange in batch mode. The authors reported complete degradation of the methyl orange and 10 reuses cycles with retention of 64 % of the immobilized enzyme activity. Yanto et al.(2014) and Bilal et al. (2016, 2017a) reported that immobilized horseradish peroxidase in vertical packed bed bioreactors systems was used as a sequential batch. In each cycle the bioreactor systems worked with continuous flow rate for a period of time. Yanto et al. (2014) worked with a peristaltic pump connected to one bioreactor (49 ml) system to maintain a continuous constant flow rate of 1.5 ml/min (residence time of 32.7 min) during 360 minutes, in each cycle. The authors compared the performance of the HRP immobilized in alginate beads, with and without cross linking agent, for the dye decolorization process. Bilal et al. (2016, 2017a) with a valve to control the flow rate used a Packed Bed Reactor System (PBRS) in successive cycles.

Bilal et al. (2018) reported the relevance of the peroxidases enzymes and the potential uses on the new bioeconomy. The authors described different horseradish uses (biosensors, in organic and polymer synthesis, and as an agent for removal, degrading or mineralizing aromatic, phenol, poliphenols compounds, etc.) and the processes that can be developed taken the enzyme bio-catalysis as a sustainable solution for the industrial residues treatment. One of the challenges to the industrial enzyme bio-catalysis reality goes through the improvement of the operational/functional stability, explicitly H_2O_2, and the methods for enzyme immobilization associated to the continuous process operation were reported (Bilal et al, 2017c) with success.

The results obtained in this study for two packed microreactors working in series (each one with 0.91 mL of void volume), both of them with the immobilized horseradish peroxidase (approximately 2 mg of HRP/g of support), in continuous treatment process, could remove upper 70% of the color after 60 minutes in only one cycle. The experiments run with two microreactors showed the potential to apply immobilized horseradish peroxidase in continuous synthetic dye decolorization as a option for the treatment of textile industrial aqueous residues. In this study, working with a flow rate of 0.78 ml/min, each packed microreactor had a residence time of 7.0 s (R1) and 8.5 s (R2), and after 90 min of continuous operation the

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percentage of dye decolorization decreased from 98.0% to around 54%, for the second microreactor effluent measurements. Both microreactors working in series with continuous process operation, adapted with a three vial valve in order to collect intermediary samples, showed that the total immobilized enzyme have to be optimize, and also de microreactor dimensions project design to achieve higher decolorization grades. In this study the second packed bed microreactor actuated as a second stage complement to the decolorization performance for the first one.

The results pointed to the continuity of this research aiming to define the best parameters conditions to develop a robust and sustainable biocatalyst able to be used in the aqueous residue generated by the textile industry.

4. CONCLUSIONS

Experiments in batch conditions, as was proposed by experimental design plan, could confirm levels of decolorization around 60% for pH 8.0, after 3 hours of contact with the same immobilized enzymes.   A decolorization degree in the range of 50% - 35% for five sequential reuse of the same immobilized enzymes, for baffled pH 7.0 methylene blue solution, was observed into a period of 40 days. For the continuous system that works with two microreactors in series a performance of 98 % of decolorization was observed. Comparing both strategies of decolorization process, batch and continuous running, after 60 minutes, 58.5% was observed for batch conditions, and 70.6% for the continuous experiment.

ACKNOWLEDGEMENTS

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