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Determination of the Optimum Conditions for Removal of Congo Red Dye by Peroxidase Enzyme Plant

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Abstract

The pollution producing from textile industries effluents is growing since the years, due to at discharged lots of it in water without treatment. The resulting effluent is colourful, highly toxic, and poses a significant environmental hazard. This problem can be solved by using enzymic biological treatment, where the Congo red dye was used with concentrations (100,200,300,500) mg /L, pH values (3,4,5,6,7,8), and variable temperatures (25,35,45)°C, the best removal of Congo red (CR) dye under optimum conditions for degradation was at concentration of 100 mg/L, at (pH 6, 25 °C) with efficiency of 99.85 % using the peroxidase enzyme extracted from red radish plant, while the removal percentage decreased when increase dye concentration .

***Keywords*:** *Peroxidase enzyme, Congo red dye, Plant enzyme, Decolorization*.

1. Introduction

Water is one of the essential elements for life on earth, along with air and soil. It is regarded as one of the important elements for both human and industrial development. Industrial effluents like wastewater from the textile industry significantly damage water bodies by increasing demand for biochemical and chemical oxygen (BOD and COD), decreasing photosynthesis and plant growth, and interfering with the food chain of living things [1]. There are different ways to remove dyes from wastewater as (Physical, chemical, or biological) methods [2], as adsorption [3], Electro-oxidation [4], Fenton reaction [5], biodegradation [6], coagulation and precipitation [7], electrocoagulation [8]. Also, degradation by use bacteria, fungi, and plants to remove the color textile dyes [1]. where employ the enzymes extracted from it is, to dissolved this problem.

Using dye in the textile industry creates substances with high toxic levels and resilience to natural decomposition that are extremely harmful to humans and the environment. One technique used to regard textile dyes is biodegradation, an innovative approach to conventional chemical and physical treatments [9]. Changing, transforming, or mineralizing textile dye within certain limitations is possible using plant enzymes to be considered the most effective and stable technique [1]. The percentage of the lost dyes at discharge maybe reach 50 percent; when dyes are discharged without treatment [10]. It affects the environment for extended times, which causes unhealthy conditions for the photosynthetic of aquatic plants and affects all living bodies, which can be caused to carcinogenic [10]. Among these dyes are a group of azo dyes of different densities. It has high-water solubility, so they are easily transmitted to the organism through the food chain, causing kidney damage, muscle and nervous spasms, and high body temperature [11].

Moreover, the dyes are hurtful lead to genetic mutations or carcinogenic and teratogenic cells

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[12; 13], and they cause carcinogenic to the liver and spleen, dye distortions in human cells and DNA abnormalities in some animals [14], as well as block the penetration of sunlight into the aquatic environment and prevent the growth of life in it [15]. Biodegradation is the process of mineralizing or transforming the contaminant by the enzymes derived from plants, bacteria, and fungi [1]. The enzyme does as catalyze oxidation for electron from substrate is (BH_2), and produce oxidized organic material (B_2), [16]:

$$BH_2 + H_2O_2 \xrightarrow[peroxidase]{} B_2 + 2(H_2O)$$

Where this research aims to remove azo dyes by biodegradation using peroxidase enzyme extracted from plant origin, such as the removal of Congo red (CR) dye with a concentration of 100 mg/L , where it was decomposed with an efficiency of 99.85 % using the peroxidase enzyme extracted from red radish plant immobilized by sodium alginate after passing 24 hr., under optimum conditions for degradation for this dye where pH 6, temperature 25 °C , with enzyme ratio adding to dye was 2.5:3 (W:V) [17].

2. Methodology

The experimental work used in this study for determination optimum conditions for Congo red (CR) decolorization the peroxidase enzyme was extraction from red radish and immobilization it's, then batch experiments procedure for determination (Initial dye concentration, pH, temperature) for decolorization of Congo red, finally was use design of experiments program (DOE).

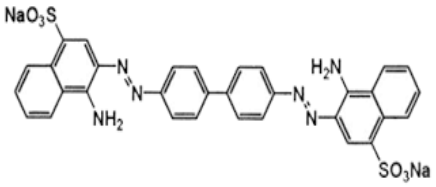
2.1. Material and Apparatuses

Materials and dye employed in this study are shown in Table (1) and Table (2).

Table 1,
Materials used

Material	Company, Origin
Sodium acetate ($C_2H_3NaO_2$) purity (99%)	BDH, England
Sodium Hydroxide (NaOH) purity (98%)	Fluka, Switzerland
Hydrochloric acid (HCl) purity (37%)	Fluka, Switzerland
Tris-Hydroxymethyl Amino Methane (Tris-HCl) purity (99.9%)	Fluka, Switzerland
Congo red (CR) (C32H22N6Na2O6S2) purity (99%)	Fluka, Switzerland
Na-alginate (NaC6H7O6) purity (99%)	Merck, Germany
Calcium Chloride (CaCl2) purity (≥97.0%)	Merck, Germany

Table 2,
Dye properties

Property / **Symbol**	**Congo Red dye** / **CR**
Structure	
Formula	**$C_{32}H_{22}N_6Na_2O_6S_2$**
Exterior	
Molecular weight	**696.7(g/mole)**
Solubility in water	**10 mg/mL 20°C**

Also, the following apparatuses was used in this study: (Shaker Incubator, LSI-3016A, Korea), (UV- Vis spectrophotometer, -APEL PD-303 UV Spectrophotometer - Japan) (Balance, Beakers, cylinders, flask, pipet, filtering paper).

2.2. Extraction of Peroxidase Enzyme and Immobilization it's

By using blender, fifty grams from red radish plant were homogenized in 30 mL of sodium acetate (pH 6, 0.1 M) for 5 minutes, The peroxidase enzyme extract by centrifuged filtered at a speed of 10000 rpm for ten minutes at 4°C, ratio of Enzyme to buffer 2:1 (W: V) as extracted ratio, Then the peroxidase activity and protein content were calculated at 470 nm [18]. By using heating, 5grams of 0.1M Na-alginate pH 6 was dissolved with 100 mL distilled water and cool it, then, added the extract peroxidase enzyme to Na-alginate gel was percentage 0.25 :1 (V: V) for immobilized the enzyme. $CaCl_2$ was Preparation by dissolved 0.2 M $CaCl_2$ solution by dissolved 2.2 g $CaCl_2$ powder in 100 mL from distilled water. In order to prepare the enzyme beads was use syringe needle, was instillation the immobilized peroxidase gel by Na-alginate in a $CaCl_2$ solution. then used in degradation of Congo red dye [19; 20].

2.3. Determination the optimum conditions for decolorization

Congo red dye (CR) was prepared in 100, 200, 300 and 500 mg/L by dissolved in distilled water and adjust the dye solution at pH 6. After determining the optimum concentration of the dye, the optimum pH was determined for decolorization, 100 mg/L Congo red dye (CR) was prepared with distilled water at pH values (3,4,5,6,7, and 8). After determining the concentration and pH of the dye, the optimum temperature was determined, 100 mg/L Congo red dye (CR) was dissolved in distilled water and adjusted the solution at pH 6. In each of the above three experiments to determine (dye concentration, pH, and temperature), where added the immobilized peroxidase beads to dye solution by ratio 2.5:3 (W : V), the solution were agitated at 25°C at 120 rpm using Shaker Incubator a thermostatic,LSI-3016A, Korea , then screened after 24 hr. at 540 nm with a UV-Vis spectrophotometer (APEL PD-303 UV Spectrophotometer - Japan) , the Decolorization efficiency for dye by immobilized peroxidase was assessed by observing the decrease in absorbance [18;21].

2.4. Design of experimental method (DOE)

In order to estimate optimum conditions for (pH, initial dye concentration(mg/L), amount of enzyme added to dye solution (g/mL) that they give best decolorization of Congo red dye, were using the batch system by applied the many values for these conditions, at 25 ºC, and 540 nm., then analyze it statistically. Design of experimental was used in 20 runs with factorial method, a different value of initial concentration has been encoded as(A), pH value as (B), ratio of enzyme adding dye as (C), and removal efficiency percent as input data to the program.

3. Results and Discussion

3.1. Effect of initial concentration for dye and operating time

Figure (1) Shows the best removal of Congo red was at a concentration of 100 mg/L, where it reached 99.85% after 24 hours, which is the ideal contact time for the degradation dye, using immobilize peroxidase enzyme. While the removal efficiency for each (200,300,500) mg /L were (99.16, 98.37, and 97.08) % respectively, after same time. It is clear from this that as dye concentration increases the percentage of dye removal decreases because a result of occupying the sorption sites in beads by the dye molecules during the operation, and the removal efficiency is low at high dye concentration [24]. Also, the contact period important in the decolorization process. where the efficacy of dye removal rises the longer the contact period [22].

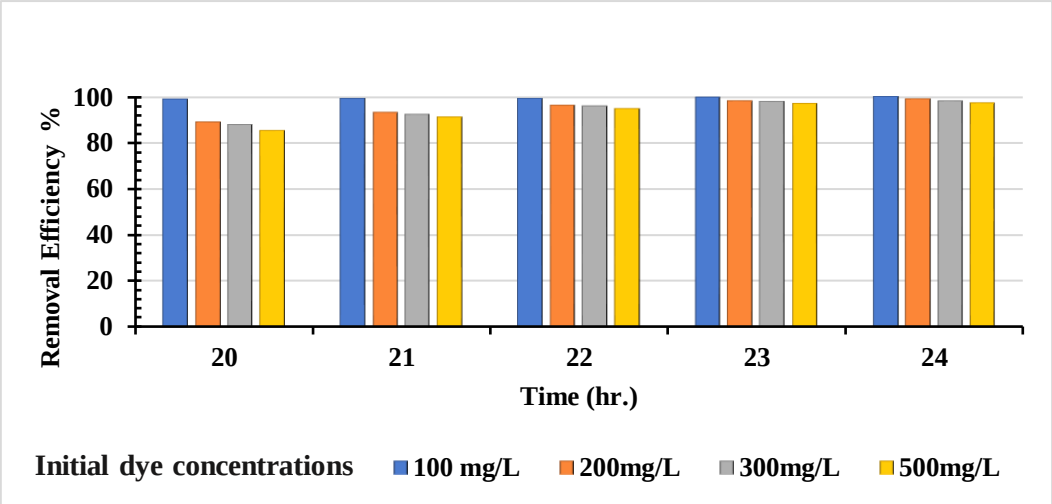


Fig. 1. Effect of initial concentration for dye and operating time

3.2. pH effect on decolorization

Figure (2) shows maximum value for decolorization for the Congo red (CR) was 99.8% at pH 6, after 24 hr., while the removal efficiency at use pH values (3, 4, 5, 7, and 8) were (87.6, 87.97, 89.05, 96.09, and 91.87) % respectively, after same time. Many of time, pH level is an important variable affecting sorption for dye, because it affects the absorbent's surface charge, surface binding sites, and degree of ionization [23].

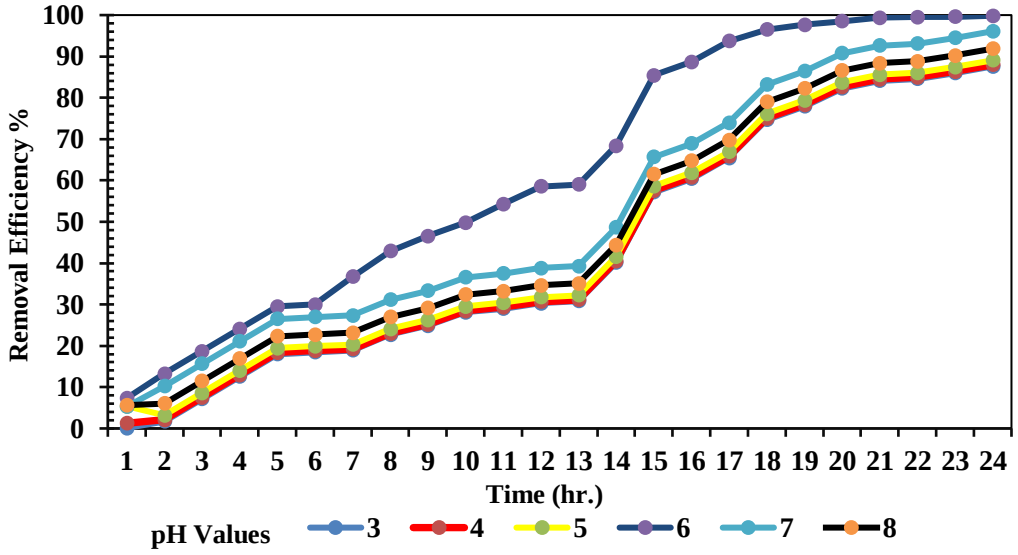


Fig. 2. pH effect on decolorization.

3.3. Effect of temperature on decolorization

Figure (3) shows after the experiment was conducted at temperatures (25, 35, and 45) °C, respectively, was the optimum temperature for decolorization of 100 mg/L Congo red (CR) dye is 25 °C, where the removal efficiency was 99.8 % after 24 hr., and (82.6, 80.7) % for (35,45) °C. Figure (4.A; B) shows the results before and after removed Congo red dye. Where the higher of temperature, the enzyme will lose its enzymatic activity causing a fall in removal efficiency due to the kinetic of the molecules raise leading to increase interaction in enzyme active sites, until enzyme maximum activity at optimum temperature, with increment in temperature, the enzyme activity will decreasing because denaturation enzyme molecule and disturbance in the 3 -dimensional conformation for enzyme , leading to happening changes in enzyme active sites [24].

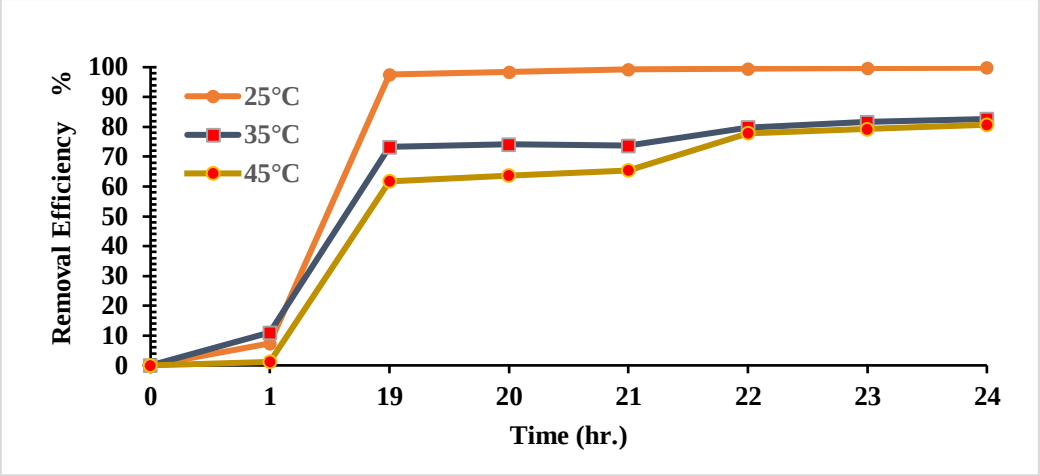


Fig. 3. Effect of temperature on decolorization at (25, 35,45) °C

Fig. 4. (A; B). The results before and after removed Congo red dye.

3.4. Design of experimental method (DOE)

Design of experimental is a piece of software designed to help with the design and interpretation of multi-factor experiments. In polymer processing, using the software to help for design an experiment to see how a property, such as tensile strength varies with changes in the processing conditions [25]. The results show below for design experiment program to get the best removal efficiency for Congo red dye by batch experiment system at different conditions for three factors initial concentration dye(mg/L), pH, and (enzyme to dye) ratio (g/mL) as shows in Table (3).

Table 3,
Design experiment program to get the best Removal Efficiency for Congo red dye by Batch Experiment System

Run	Factor 1 A:C0 (mg/L)	Factor 2 B: pH	Factor3 C:(Enzyme: Dye) ratio (g/mL)	Response1 Removal efficiency (R)(%)
1	282.4	6.9	2.0	99.4
2	550.0	5.5	1.4	96.9
3	817.6	4.0	2.0	98.5
4	550.0	8.0	1.4	98.9
5	100.0	5.5	1.4	94.1
6	550.0	5.5	1.4	99.3
7	817.6	6.9	0.7	99.5
8	282.4	4.0	0.7	98.4
9	282.4	4.0	2.0	98.9
10	550.0	5.5	1.4	99.5
11	282.4	6.9	0.7	99.1
12	550.0	5.5	0.3	99.2
13	550.0	5.5	1.4	99.4
14	817.6	7.0	2.0	99.8
15	550.0	5.5	1.4	99.6
16	550.0	5.5	2.5	99.7
17	1000.0	5.5	1.4	99.8
18	817.6	4.0	0.7	99.5
19	550.0	3.0	1.4	99.1
20	550.0	5.5	1.4	99.4

3.5. Analysis of variance (ANOVA)

Statistical significance of the factors was established by analysis of variance (ANOVA) results showed the quadratic model in Table (4), this Table it is showed the ability of applied this model with a highly significant model P-value, which was less than 0.05 and high F-value of 50.77. The experimental values obtained from the lab experiments were compared with the predicted results calculated by statistical design software. The value of coefficient R^2 is equal to 0.9068, the predicted R^2 of 0.9068 is in reasonable agreement with the adjusted R^2 of 0.9545; i.e., the difference is less than 0.2, that indicated that the experimental data fits with the quadratic model. While, [26] found in (ANOVA) analysis that the factors significant on degradation of Congo red to get on (R^2=0.9900) at the use (27.21 mg/L Congo red concentration, 2.07 Unit for peroxidase enzyme activity, 0.15 mmol/L H_2O_2) give the maximum degradation of 58.13%. In general, the peroxidase enzyme of vegetable origin is effective in removing azo dyes and gives satisfactory results, where [27] found that effects of factors (pH, enzyme dose, and dye concentration) on degradation of violet azo dye by peroxidase enzyme from plant origin after done 20 experiments with different combinations, where observed that the decolorization reach 87.95 %, with the fit of quadratic model that was obvious by high R^2 value of 0.9903.

The F-values in Table (4) shows that the initial concentration is the most effective factor in color removal, followed by the pH factor, and then the enzyme added to the dye solution is less than the other two factors.

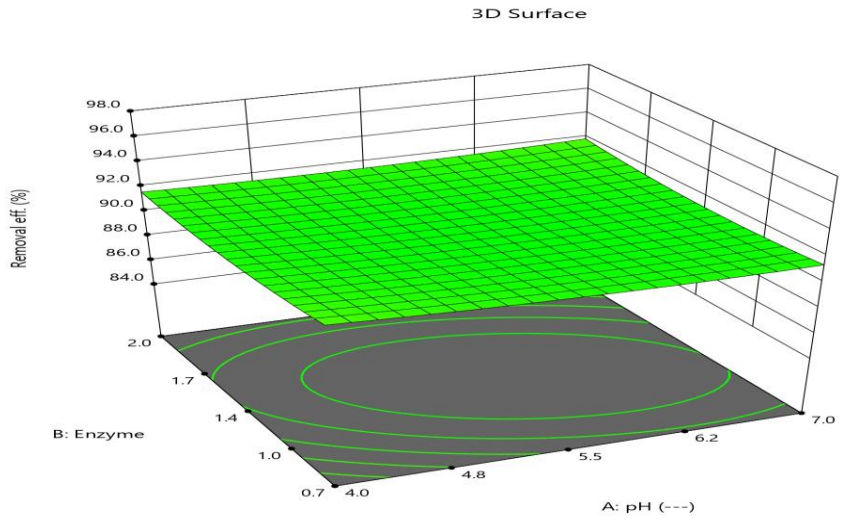
Table 4,
Removal efficiency of Congo red dye is the target in this table.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	151.15	8	18.89	50.77	< 0.0001	significant
A-pH	0.0417	1	0.0417	0.1120	0.7442	
B-Enzyme	0.0157	1	0.0157	0.0423	0.8408	
C-C0	146.36	1	146.36	393.32	< 0.0001	
AB	0.3200	1	0.3200	0.8599	0.3737	
AC	0.0800	1	0.0800	0.2150	0.6519	
BC	0.9800	1	0.9800	2.63	0.1329	
A^2	0.7607	1	0.7607	2.04	0.1806	
B^2	2.83	1	2.83	7.60	0.0187	
Residual	4.09	11	0.3721			
Lack of Fit	1.74	6	0.2897	0.6151	0.7157	not significant
Pure Error	2.35	5	0.4710	-	-	-
Cor Total	155.25	19	-	-	-	-

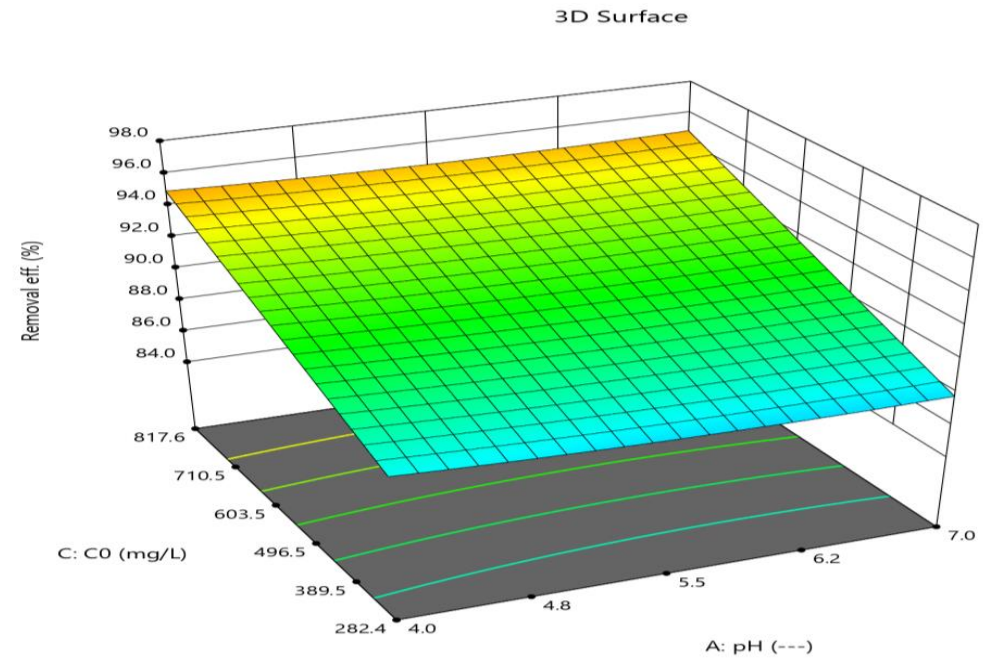
The following equation was used in this work to evaluate the terms of Actual Factors for removal efficiency by the design experiment program.

$$\text{Removal Efficiency (R)(\%)} = 90.42791 - 1.59003B - 2.68865\ C + 0.013541\ A + 0.201133\ B * C + 0.000251\ B * A - 0.001955\ C * B + 0.103457\ B^2 + 0.984972\ C^2$$

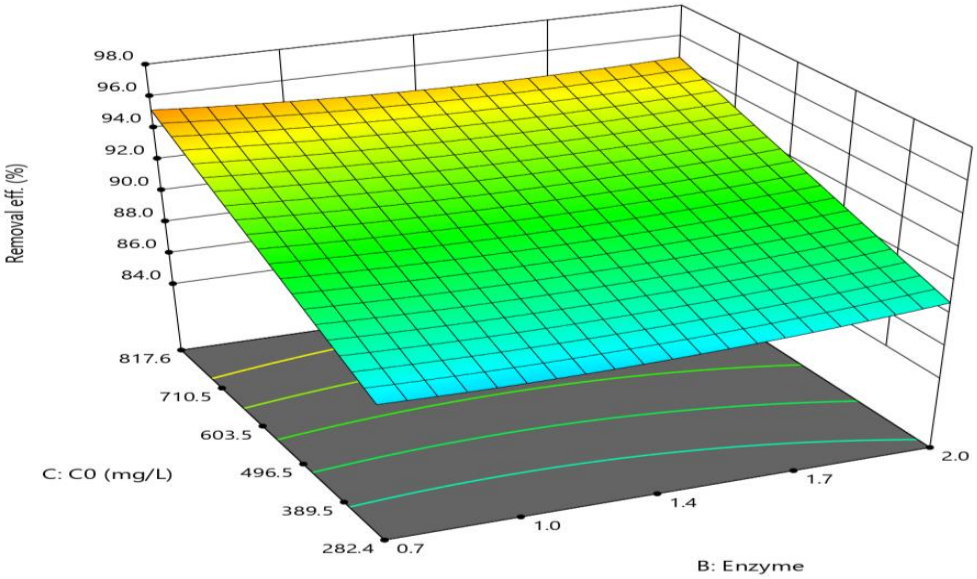
Where:
A: Initial concentration for dye (mg/L).
B: pH
C: Enzyme: dye (g/mL), where the ratio of dye is stable at 3 while the enzyme ratio is change such as in Table (3). Where Figures (5. A; B; C) shows the results for these experiments.



(A)



(B)



(C)

Fig. 5. (A; B; C) Effect of factors on removal efficiency of Congo red dye.
Where shows the results for these experiments and the influence of each (initial concentration, pH, and enzyme ratio adding to dye) on removal efficiency, the findings indicate that initial dye concentration of 817.6 mg/L, pH from (4 to 7), and enzyme adding ratio to dye was (0.7 to 2) to achieve a removal efficiency of reach 95 percent of the Congo red dye.

4. Conclusion

This study concluded that peroxidase enzyme extracted from red radish plant is a good source for azo dye decolorization such as Congo red dye, at the optimal conditions for decolorization dye pH 6.0, temperature 25 °C, after 24 hr., at 540 nm, when enzyme adding to dye by ratio (2.5:3) (W: V). Peroxidase immobilized enzyme by sodium alginate can give a good result when using to remove the Congo red azo dye from wastewater resulting from many industries as textile industry which cause healthy and environmental serious problems as cancer and genetic distortions.

Notation

CR	Congo red dye
BH_2	Substrate
B_2	Oxidized organic material
DNA	Deoxyribonucleic acid
H_2O	Water
hr.	Time
pH	Acidic degree
°C	Temperature
W	Weight
V	Volume
UV-Vis	Ultraviolet–visible
$CaCl_2$	Calcium Chloride
DOE	Design of experimental
ANOVA	Analysis of variance

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تحديد الشروط المثلى لإزالة صبغة الكونغو الحمراء بواسطة انزيم البيروكسيديز النباتي

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الخلاصة

يتزايد التلوث الناتج عن المخلفات السائلة للصناعات النسيجية منذ سنوات، بسبب تصريف الكثير منها في المياه المفتوحة دون معالجة. المخلفات الناتجة تكون ملونة وشديدة السمية وتشكل خطراً بيئياً كبيراً. يمكن حل هذه المشكلة باستخدام المعالجة البيولوجية الأنزيمية، حيث تم استخدام صبغة الكونغو الحمراء بتراكيز (100،200،300،500) مجم / لتر وقيم الأس الهيدروجيني (3،4،5،6،7،8) ودرجات حرارة متغيرة (25،35 45) درجة مئوية، كانت أفضل إزالة لصبغة الكونغو الحمراء (CR) في الظروف المثلى للتحلل بتركيز 100 مجم / لتر، عند الاس الهيدروجيني (pH 6) و 25 درجة مئوية بكفاءة 99.85٪ بواسطة استخدام إنزيم البيروكسيداز المستخلص من الفجل الأحمر ، بينما تنخفض نسبة الإزالة عند زيادة تركيز الصبغة.