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Acute toxicity test under optimal conditions of two commercial reactive dyes using the Fenton-like process:

Assessment of process factors by Box–Behnken design

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Abstract

Reactive dye has generally been used in woven cotton fabric dyeing industries. Some treatments of several reactive dyes may produce more toxicity than the original dyes. The objectives of this study were to find the optimal condition on dye degradation efficiency of commercial reactive red dye 36 (DR36) and reactive violet dye 30 (DV30) using Fenton-like reaction, and to determine acute toxicity by static bioassay method under the optimal condition. The experiment was designed by Box Behnken Design (BBD), in which an initial pH, catalyst dosage and initial concentration of H₂O₂ were considered as independent variables. The results showed that only an initial pH solution was the principal parameter which influenced decolorization of the reactive dyes. Other factors were much less significant. The optimal conditions were found to be given by pH 3, 1 g/L of catalyst dosage, 27.63 mM of concentration of H₂O₂ for DR36, and pH 3, 1.35 g/L of catalyst dosage, 45 mM of concentration of H₂O₂ for DV30. Ninety percent of both decolorization were achieved in 30 min. Acute toxicity tests of the treated solutions using freshwater fairy shrimps (*Streptocephalus sirindhornae*) revealed that the shrimps survived longer than 24 h, indicating that the treated solutions were not acutely toxic. The average leaked iron, ADMI value and total organic carbon were found to be less than 10 ppm, 5 ADMI and 9.17 ppm respectively, in the treated samples. This research demonstrated an efficient method for decolorization of the reactive dyes with low acute toxicity.

Keywords: freshwater fairy shrimps, immobilization, iron powder, commercial reactive dye, decolorization, response surface methodology

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1. Introduction

Global textile industries have grown unstoppably for several decades, more over 700,000 ton of about 10,000 types of dyes and pigments were annually produced (Lyu et al. 2016; Holkar et al. 2016). Consequently, dye, especially reactive dye, has become an important feedstock used in the industry. Since dye cannot be consumed totally in the dyeing process, the unreacted dye could remain in the wastewater discharge, causing the wastewater to have unpleasant appearance and toxicity. In Thailand it has been regulated that wastewater discharge should contain dye concentration less than 300 ppm (Nidheesh et al. 2018) and/or the color of the wastewater should be less than 300 ADMI unit. Some reactive dyes were also declared as high toxins promoting *carcinogenesis* and *mutagenesis* (Nasuha et al. 2016; Mahmood Reza Sohrabi et al. 2016).

To meet all the requirements of wastewater, several techniques have been used to reduce dye concentration before the wastewater can be discharged. Advanced oxidation processes (AOP) are some promising methods used for the purpose. These processes have been classified as photo-catalytic (Ayyob et al. 2020), ozonation (Powar et al. 2020), and Fenton reaction (Ertugay & Acar 2017). Among the AOPs, the Fenton reaction can extensively be used to decompose hard biodegradable organics, including different dyes (Nasuha et al. 2016; Youssef et al. 2016), and textile discharge (Ghanbari et al. 2014; Punzi et al. 2015). The reaction involves a reaction of ferrous ion with H_2O_2 to produce OH^\bullet free radicals having extremely strong oxidation capacity, especially in narrow pH range of 2.8-3.0 (Ghanbari et al. 2014; Glugoski et al. 2017). The narrow pH range makes the Fenton reaction difficult to implment (Wang et al. 2017). The heterogeneous Fenton-like catalytic technique has therefore been used more widely, as has been reported on laterite soil (Khataee et al. 2015), red mud (Dias et al. 2016), montmorillonite clay (Fida et al. 2017), zeolite (Rache et al. 2014) and zero valent iron nanoparticles (Vilardi et al. 2018).

Reactive dye such as DR36 and DV30 possesses prominent properties for their stabilities (Malade & Deshannavar 2018) and high level of washing fastness (Nallathambi & Venkateshwarapuram Rengaswami 2017). Nasuha et al. (2016) have studied decolorization of reactive black 5 using Fenton-like. Initial dye concentration, hydrogen peroxide concentration, initial pH of a solution and amount of initial catalyst were selected as the main factors for studying this reaction. Khataee et al. (2016) have also studied the effects of operating parameters of the reaction such as catalyst dosages, [pH], [H_2O_2] on their decolorization of Reactive Orange 29 dye. However, the

decolorization of these reactive dyes have been studied by considering one factor at a time (OFAT). Interaction effects of the parameters were not studied during the tests.

Statistical experimental design could be a better approach in multi-factor study. A systematic study using response surface methodology (RSM), like central composite design (CCD) and Box Behnken Design (BBD), could be used to set up and analyze the experimental data. Some of the advantages of the RMS include its ability to explain both individual and interaction effects, and optimizing decolorization condition. In addition, it's has successfully been applied to various oxidation processes to optimize the experimental design condition (Fu et al. 2009; Berkani et al. 2020).

In this study, the decolorization of DR36 and DV40 dye was implemented by BBD under three factors simultaneously, including pH, catalyst loading, and amount of H₂O₂. The study was carried out using iron powder in a Fenton-like reaction. The experiment was set up in a range of pH 3-7, Catalyst 0.01-1.5 g/L, and H₂O₂ 0.5-100 mM. The optimum condition and the most influential factor(s) in decolorization of the reactive dyes were the objective of this study. The water after the treatment was also tested for acute toxicity by using fairy shrimps (*Streptocephalus sirindhornae*).

2. Materials and methods

2.1 Materials

Reactive red dye 36 (DR36) and violet dye 30 (DV30) (without heavy metal, Dylon, England) used in this study were purchased in Thailand. The Fenton-like experiment were carried out using commercial iron-powder grade (99.64% Gammaco, Thailand). H₂O₂ 30% (QRëC, New Zealand). NaOH (98%wt Ajax Finechem Pty Ltd, Auckland, New Zealand) and H₂SO₄ (96%wt RCI Labscan Limited, Thailand) were used to adjust the solution pH to the desired levels. Ethanol (99.8% Analar NORMAPUR ® ACS, Reag.Ph.Eur. , France) was used to wash the catalyst. Nylon filter membrane (Syring filter 0.45 Micron CNW, China) was used to filter the sample solution before determining the color values using UV-Vis spectrophotometer (SPECORD, Analytik Jena, Germany). All solutions were prepared with deionized water (DI water).

2.2 Experimental

Iron-powder was sieved to the size range of 100-500 mesh. It was washed by Deionized water and ethanol, and then dried at 80°C for 1 hour. The obtained material was immediately used right after the preparation. Box–Behnken design (Software Minitab 16) was used to randomize the experimental values of the three factors at three levels as shown in Table 1. The studied factors included initial pH, catalyst dosage, and initial concentration of H₂O₂. The experiments were performed in a batch-wise system of 600 mL container, under room temperature. The decolorization of reactive dye (300 ppm) was carried out using different initial concentrations of H₂O₂ (0.5-100 mM), the catalyst dosage (0.01-1.5 g/L) and pH (3-7). One molar of H₂SO₄ or NaOH solution was used to adjust the solution pH.

During the test, the remaining concentration of the reactive dye in the solution was withdrawn 30 minutes after the beginning of the experiment, and analyzed for the percentage of decolorization efficiency (DE%) and the American Dye Manufacturers Institute (ADMI) value by UV-Vis spectrophotometer. The Non-Purgeable Organic Carbon (NPOC) method was used to determine the TOC value. These values were compared with those of the solution withdrawn prior to the commencement of the experiment.

The catalyst was further characterized for its surface area and composition by N₂ Adsorption-Desorption (ASAP 2010, Micromeritics, USA) and X-Ray Fluorescence Spectrometry (SEA1000A, Seiko Instruments GmbH, Germany), respectively. After 30 minutes of reaction, the solution was analyzed for iron leachate by Inductively Coupled Plasma-Optical Emission Spectrometer (Optima 8x00 Series, PerkinElmer, United States of America).

Table 1 Box-Behnken design for varying the factors

Factors	Symbol	Levels of factors		
		-1	0	1
Initial pH	(X ₁)	3	5	7
Catalyst dosage (g/L)	(X ₂)	0.01	0.755	1.5
Initial concentration of H ₂ O ₂ (mM)	(X ₃)	0.5	50.25	100

The Quadratic polynomial model equation (1) was obtained to analyze the data using the least square of error technique as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_3^2 + \beta_7 X_1 X_2 + \beta_8 X_1 X_3 + \beta_9 X_2 X_3 \pm \varepsilon \quad (1)$$

In which Y is dye removal efficiency (response function), β_i are regression coefficient, X_i are independent variables, and ε is value of the error.

2.3 Acute toxicity assays

Young freshwater fairy shrimps (*Streptocephalus sirindhornae Sanoamuang*) were obtained from a stock maintained at the Department of Fisheries, Khon Kaen University. The acute toxicity and immobilization tests were performed according to 202 of OECD Guidelines for Testing of Chemicals (OCDE, 1984) with modifications. For the tests, 20 of neonates were divided into five groups of four animals each. The first and second groups were exposed to both (DR36 and DV30) of the original dye diffusion (300 mg/L), the third and fourth groups in the treated water from the optimal condition in Fenton-like process (DR36 and DV30), and the final group in the water obtained from the source of fairy shrimps (control water).

The water obtained from the source of fairy shrimp culture was used as the control water. The acute toxicity assays can be assessed by counting the number of dead and surviving neonates at 0.5, 1, 6, 12, 24, 36, 48, 60 hours and compared with the control water. Prior to the test, the solution of every experimental batch was adjusted to the pH value of 7.66 before adding the neonates except the control water. This experiment did not have any light control. Therefore, the daytime was assumed to be approximately 12h, and 12h nighttime.

3. Results and discussion

3.1 Characteristics of catalysts

The specific surface area was determined to be 3.932 m²/g by the Brunauer–Emmett–Teller (BET) method using the N₂ Adsorption-Desorption Semisorb, (ASAP 2010, Micromeritics, USA). The purity of the iron-powder was found to be 99.64%wt using X-Ray Fluorescence Spectrometry.

3.2 Dye degradation

The values of dependent and independent factors, and the condition for every experimental data set which was obtained from Box–Behnken design (Table 1) are presented in Table 2. The decolorization efficiency (DE%) of DR36 and DV30 were calculated by Equation 2 and shown in Table 2.

$$DE\% = \frac{C_0 - C_t}{C_0} \times 100 \quad (2)$$

where DE% is the decolorization efficiency, C₀ is the initial concentration of the dye, and C_t is concentration of the dye at 30 min.

Table 2 Results of the Box–Behnken experimental design for dye decolorization efficiency (DE%) of DR36 and DV30.

Run	Initial pH	Catalyst dosage (g/L)	H ₂ O ₂ (mM)	%DE of DR36 dye 30 min	%/DE of DV30 dye 30 min
1	5	0.01	0.5	19.523	24.945
2	3	0.01	50.25	42.668	78.061
3	7	0.01	50.25	7.768	10.950
4	3	0.755	0.5	99.244	94.770
5	5	0.01	100	19.994	22.047
6	7	0.755	0.5	21.411	10.833
7	5	1.5	0.5	39.764	64.757
8	7	1.5	50.25	22.460	25.771
9	3	1.5	50.25	94.033	77.113
10	7	0.755	100	19.144	4.785
11	5	1.5	100	45.714	93.609
12	5	0.755	50.25	62.514	64.182
13	5	0.755	50.25	62.796	81.096
14	5	0.755	50.25	53.157	64.208
15	3	0.755	100	92.751	62.056

The coefficients of the response functions for various independent variables were obtained by correlating the experimental results with the response functions by using a Minitab 16 statistical software. The response functions for DR36 and DV30 were obtained by the Least Square of Error method as Equations 3 and 4, respectively for predicting the dye decolorization efficiency.

$$\%Y(DR36) = 55.43 - 30.25X_1 + 9.70X_2 - 3.9X_3 - 1.62X_1^2 - 20.06X_2^2 - 7.01X_3^2 - 8.21X_1X_2 + 3.34X_1X_3 + 1.76X_2X_3 \quad (3)$$

$$\%Y(DR36) = 69.83 - 32.46X_1 + 15.66X_2 - 1.6X_3 - 15.04X_1^2 - 6.81X_2^2 - 11.68X_3^2 + 3.94X_1X_2 + 6.67X_1X_3 + 7.94X_2X_3 \quad (4)$$

Figure 1 and 2 can be used to verify the accuracy of Equations 3 and 4, respectively. Figures 1a and 2a indicate that the data were distributed on straight lines independently. Figure 1B and 2B show the stability of variance to be dispersed around the zero. The histogram of the Figure 1C and 2C demonstrate that the standard deviation of the data was well distributed (bell-shape) and tend to the center. Lastly, Figure 1D and 2D show the standardized residual of the data with respect to the observation order. These two figures reveal that the order of the experiment was well distributed, indicating that the experimental design was well randomized.

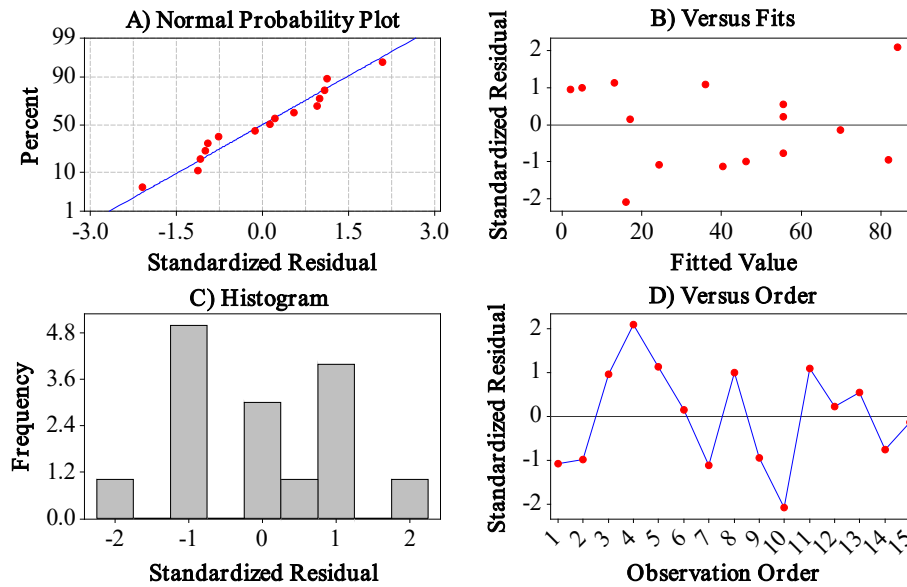


Fig. 1 Standard error compared to a) standardized residual b) versus fits c) Histogram d) Versus order.

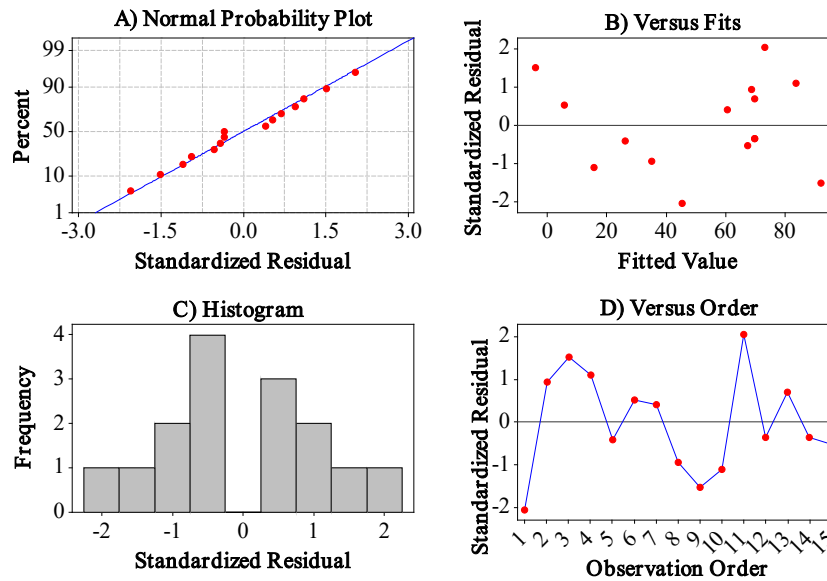


Fig. 2 Standard error compared to A) standardized residual B) versus fits C) Histogram D) Versus Order.
For DV30.

Based on the statistical data of Figures 1 and 2, it can be seen that the data obtained were well distributed and independent. In other words, no abnormal statistical data were observed. Therefore, Equations 3 and 4 can be used with some degree of confidence. Equations 3 and 4 were also analysed for variance by ANOVA test with 95% confidence interval. The F_{value} and P_{value} were considered for each variable presented in Table 3.

From Table 3, it should be noted that only terms of linear source, which have statistically significant effects on the DE% of DR36 and DV30. For DR36, the significant factors were found to be initial pH (X_1), the catalyst dosage (X_2) and the term of X_2^2 . Even though the initial concentration of hydrogen peroxide (X_3) was not significant, it is necessary for a Fenton-like reaction as the reducing agent. For DV30, it was found that only the initial pH was the significant factor, while other factors were insignificant, but they were needed for the reaction.

However, the applicability of Equations 3 and 4 must be judged by the term of Lack of fit which is used to indicate to the error of these equations. Table 3 shows that the Lack of fit values for DR36 and DV30 were not significant. Therefore, the equations are statistically reliable without a need to remove any factors from the equations (Ay et al. 2009), indicating the model can be used to forecast the DE%.

Table 3 ANOVA test for response function Y (DE%)

Source	DF	DR36			DV30		
		F-value	P-value	Result	F-value	P-value	Result
Regression	9	10.71	0.009	Significant	3.46	0.093	Insignificant
Linear	3	26.03	0.002	Significant	8.82	0.019	Significant
X ₁	1	69.76	0.000	Significant	21.42	0.006	Significant
X ₂	1	7.18	0.044	Significant	4.98	0.076	Insignificant
X ₃	1	1.16	0.331	Insignificant	0.05	0.828	Insignificant
Square	3	5.07	0.056	Insignificant	1.13	0.420	Insignificant
X ₁ ²	1	0.09	0.774	Insignificant	2.12	0.205	Insignificant
X ₂ ²	1	14.15	0.013	Significant	0.44	0.538	Insignificant
X ₃ ²	1	1.73	0.246	Insignificant	1.28	0.309	Insignificant
Interaction	3	1.03	0.453	Insignificant	0.42	0.749	Insignificant
X ₁ × X ₂	1	2.57	0.170	Insignificant	0.16	0.707	Insignificant
X ₁ × X ₃	1	0.42	0.543	Insignificant	0.45	0.531	Insignificant
X ₂ × X ₃	1	0.11	0.751	Insignificant	0.64	0.460	Insignificant
Residual	5						
Error							
Lace-of-fit	3	4.71	0.180	Insignificant	6.22	0.142	Insignificant
Pure-Error	2						
Total	14						

F-test 95% confidence ($\alpha=0.05$)			
Source	DF 1	DF 2	F critical
F _(0.05,DF1,DF2)	9	5	4.77
F _(0.05,DF1,DF2)	3	5	5.41
F _(0.05,DF1,DF2)	1	5	6.61
F _(0.05,DF1,DF2)	3	2	19.16

Comparison between the predicted and experimental values can be made by plotting the predicted values against the experimental values as shown in Fig.3A and 3B for DR36 and DV30, respectively. As shown in Fig. 3A, the equation obtained fits approximately well with experimental results (less than 10% error). Similarly, Fig 3B reveals similar results but with less accuracy (less than 15% error). It should be noted, however, for the range of 70- 80% of decolorization the model for DV30 can be used to predict decolorization with improved accuracy (less than 10% error). Consequently, it can be concluded that both of the models were in reasonably good agreement with the experiment.

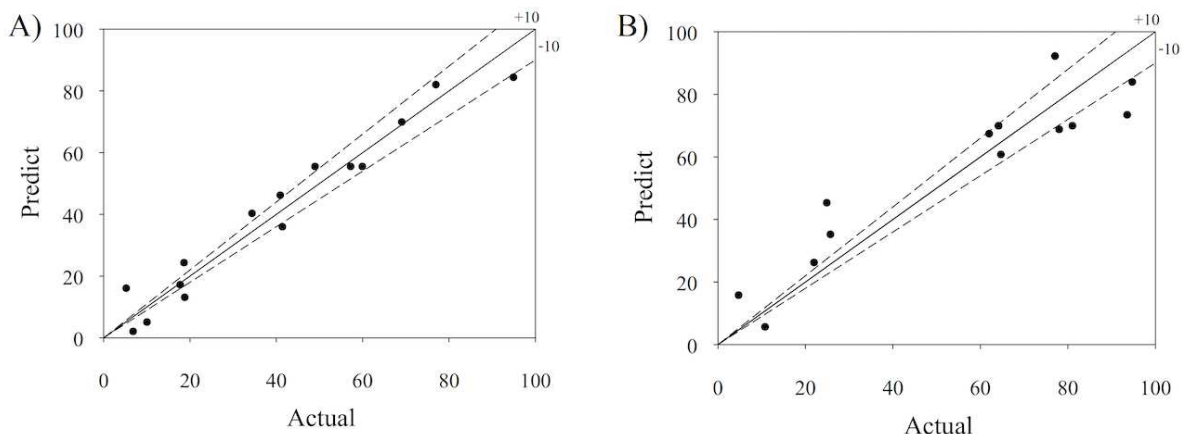


Fig. 3. Experimental and predicted equation results for decolorization A) DR36 B) DV30

3.3 Effects of parameters on decolorization efficiency

The initial pH (X_1) was found to be the most influential factor on the decolorization efficiency. The F-value can confirm this in Table 3 where the F_{value} values were found to be 69.76 and 21.42 for DR36 and DV30, respectively. On the contrary, the catalyst dosage (X_2) and the initial concentration of H_2O_2 (X_3) were much less influential on decolorization.

More interestingly, based on statistical results in Table 3, it should be noted that the interaction effects between the insignificant variables and significant variables were not significant. However, the interaction effects of the variables were shown to influence decolorization by response surface methodology (Fig.4).

The interaction effects of between (X_1) and (X_2) were depicted in Fig. 4A and Fig.4D, whereas Fig. 4B and Fig. 4E show the interaction effects between (X_1) and (X_3). It is seen that %DE increases with the catalyst dosage and H_2O_2 at a constant pH of 3. At other pH values, the %DE is slightly affected by the catalyst dosage and initial concentration of H_2O_2 . At the initial pH of 3, the decolorization efficiency is the highest. This finding was also observed by Khataee et al. (2015), who used the iron-rich laterite soil as the catalyst. This can be explained by the instability of H_2O_2 and depressed oxidation potential of hydroxyl radicals at higher pH (Fida et al., 2017). In addition, the alkaline solution causes the Ferrous ion (Fe^{2+}) to transform to be Iron (II) hydroxide ($\text{Fe}(\text{OH})_2$) according to Equation 5. Fe in this form is inactive (Ertugay & Acar 2017; Chu et al. 2012).



Fig. 4C and Fig. 4F show the DE% tends to the maximum value as X_2 and X_3 increase until some limits. The limits of X_2 were found to be 1.3g/l for DR36 and 1.5g/l for DV30. The limits were due to excessive active sites of the catalyst, resulting in excessive ferrous ions (Khataee et al., 2015; Fida et al., 2017). In theory, the ferrous ion can react with H_2O_2 to produce more hydroxyl radicals (Khataee et al. 2015; Ma et al. 2015), but excessive ferrous ions will result in some of the ions becoming the scavengers of the hydroxyl radicals (Li et al. 2017; Bouzayani et al. 2017) as illustrated in Equations 6-8.



where X is the solid catalyst support.

In a similar manner to the catalyst dosage, the DE% increases with H_2O_2 concentration until some limits. The H_2O_2 concentration over 60 mM tends to decrease the DE%. Excessive H_2O_2 will result in hydroxyl radical scavenging (Mahmood R. Sohrabi et al., 2017; Quadrado & Fajardo, 2017; Grisales et al., 2019) according to Equations 9 and 10.



In conclusion, the study of the effects of the parameters on decolorization efficiency, as presented in Figure 4, reveals that the most influential parameter is the initial pH, which should be controlled at pH 3 for the best dye decolorization efficiency. The other factors, namely catalyst dosage and concentration of H_2O_2 , should respectively be kept in the ranges of 0.9-1.2 g/L, and 20-35 mM of $[H_2O_2]$ for DR36, and 1.3-1.5 g/L, and 40-50 mM of $[H_2O_2]$ for DV30.

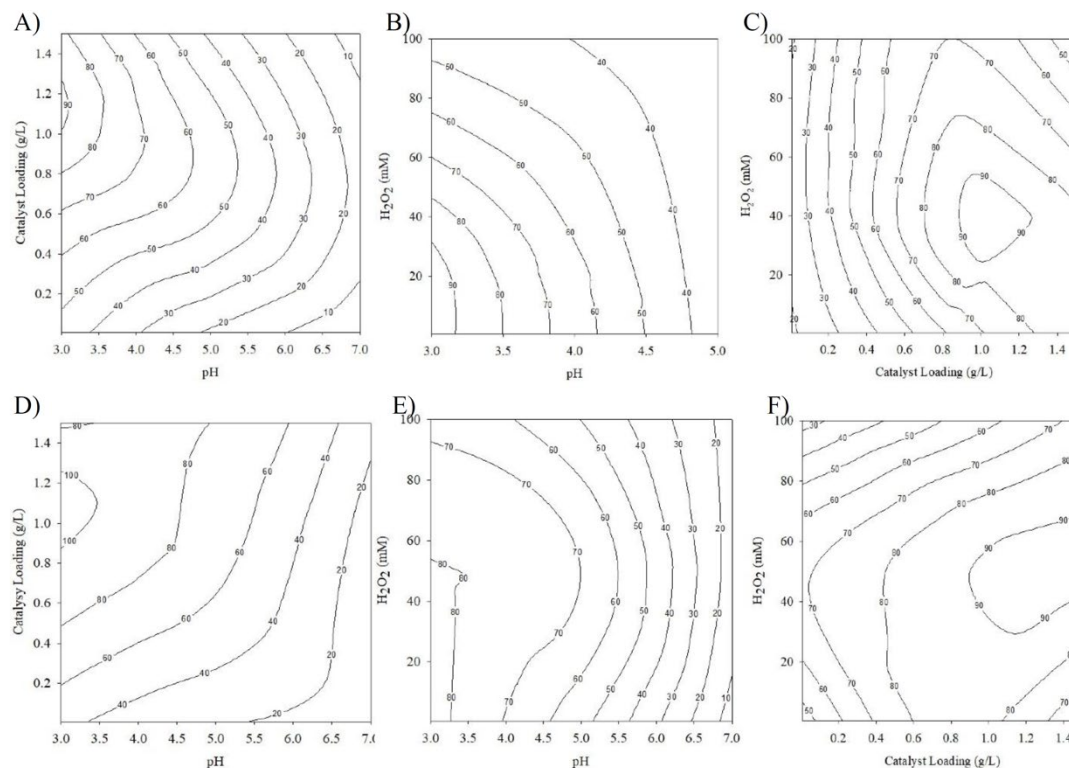


Fig. 4 Contour plot illustrations, the interaction effect of variables on dye decolorization efficiency in the Fenton-like process in white (A-C) For DR36 and (D-F) for DV30.

3.4 Optimal condition and asseveration of the findings

The optimal conditions of dyes were predicted by using Equations 3 and 4 for DR36 and DV30, respectively. These equations were obtained by the Least Square method based on the numerical values generated from the experimental data by Minitab 16 software. The optimal conditions were found to be pH 3, 1 g/L of catalyst dosage, 27.63 mM of H₂O₂ For DR36, and pH 3, 1.35 g/L of catalyst dosage, 45 mM of H₂O₂ for DV30. Under these predicted conditions, the best of DE% were found to be 89.38% and 92.33% for DR36 and DV30, respectively.

In order to verify the accuracy of the optimal conditions, three decolorization tests were repeated for DR36 and three tests for DV30. The average experimental results of DE% were 91.86% with a standard deviation of 7.24% and 92.97% with a standard deviation of 8.88% for DR36 and DV30, respectively. The experimental results were, therefore, comparable with the predicted values with the standard deviations as described.

Furthermore, under these optimal conditions, the average values of leached iron, TOC, and ADMI unit were also determined. These results were less than 10 ppm, 9.17 ppm, 5 ADMI, respectively. The treated water was therefore in compliance with American Dye Manufacturing Institute, and Thailand's Industrial Discharge Water standards.

3.5 Acute toxicity

Fig. 5 shows the results of acute toxicity test of the treated water with the optimal condition. The test was performed by counting fatalities of the fairy shrimps exposed to the treated water and the original untreated water. The result showed, after 24 hours, that no deaths were observed in all of the batches. This leads to the conclusion that there was no difference in acute toxicity of the treated and untreated water.

However, when the toxicity test reached 48 h, only 25% of the neonates in the original, untreated water with DR36 died, whereas the fatalities in the case of DV30 were 27%. In the case of treated waters and cultured water were found not any death. At 60 h, the treated water showed a slightly better survival rate than the original, untreated water. The survival rate was found to be 76% for treated waters (both DR36 and DV30), while the original, untreated water showed 74% and 72% for DR36 and DV30, respectively. No fatality was observed in the control water.

In conclusion, only a slight difference in toxicity between the treated and untreated water. Fernandes et al. (2018). also observed a similar result in their acute toxicity test (24 h). Therefore, the Fenton-like reaction under the optimal condition could be well applied to treat wastewater from the dyeing industry.

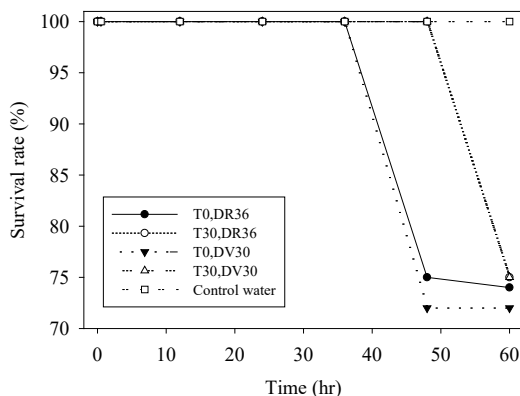


Fig. 5 Effects of different treated water and untreated water with survival rate (%) of fairy shrimp (T0 is original dye at 300 ppm, T30 is treated water, and control water is water from a freshwater fairy shrimp

culture.

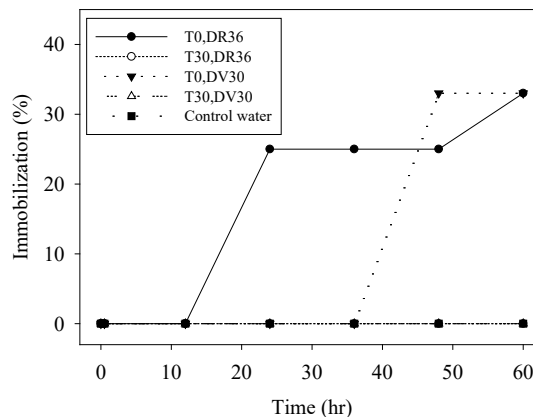


Fig. 6. Effects of different treated water and untreated water with immobilization (%) of fairy shrimp, T0 is original dye at 300 ppm, T30 is treated water, and control water is water from a freshwater fairy shrimp culture

The immobilization test of the fairy shrimps was also carried out and presented in Fig.6. The test mainly involved observing the movement of the laboratory animals. The experiment result revealed no abnormal movement of the fairy shrimps in the control water and all the treated water. However, only in the untreated water (DR36), 25% of immobilization was observed at 24 h, while in the untreated water (DV30), 33% of immobilization was observed at 48 h. This result indicated the treated water was less harmful to the fairy shrimps than the untreated water.

4. Conclusion

A Box–Behnken design was used to find the optimal condition for dye decolorizing efficiency by considering the three variables, including initial pH, catalyst dosage and initial concentration of hydrogen peroxide. The initial pH was found to be the most influential parameter on dye decolorization efficiency. The Least Square of Error method was used to create the models for predicting the dye decolorizing efficiency for DR36 and DV30, respectively. The model can be used to predict the optimal decolorization conditions for DR36 and DV30. The optimal conditions were experimentally verified, and it was found that the predicted DE% was in good agreement with the experimental values. More importantly, the treated water was in compliance with the American Dye Manufacturing Institute and Thailand's

Industrial Wastewater Discharge standards. The toxicity test revealed no acute toxicity. Therefore, the Fenton-like reaction using iron-powder as the catalyst is suitable for removing DR36 and DV30.

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-Ethical Approval: Compliance with Ethical Standards

-Consent to Participate: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

-Consent to Publish: Not applicable

-Authors' Contributions: Natwat Srikhao planed, designed the experiments, collected data, interpreted, analyzed, and wrote a manuscript. Arthit Neramittagapong, Pongsert Sriprom, and Sutasinee Neramittagapong designed the experiments, provided chemicals and instruments, interpreted, analyzed, discussed, and wrote a manuscript. Somnuk Theerakulpisut and Nurak Grisdanurak interpreted, analyzed, discussed, and wrote a manuscript

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-Competing Interests: The authors declare that they have no competing interests.

-Availability of data and materials: Not applicable

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Figures

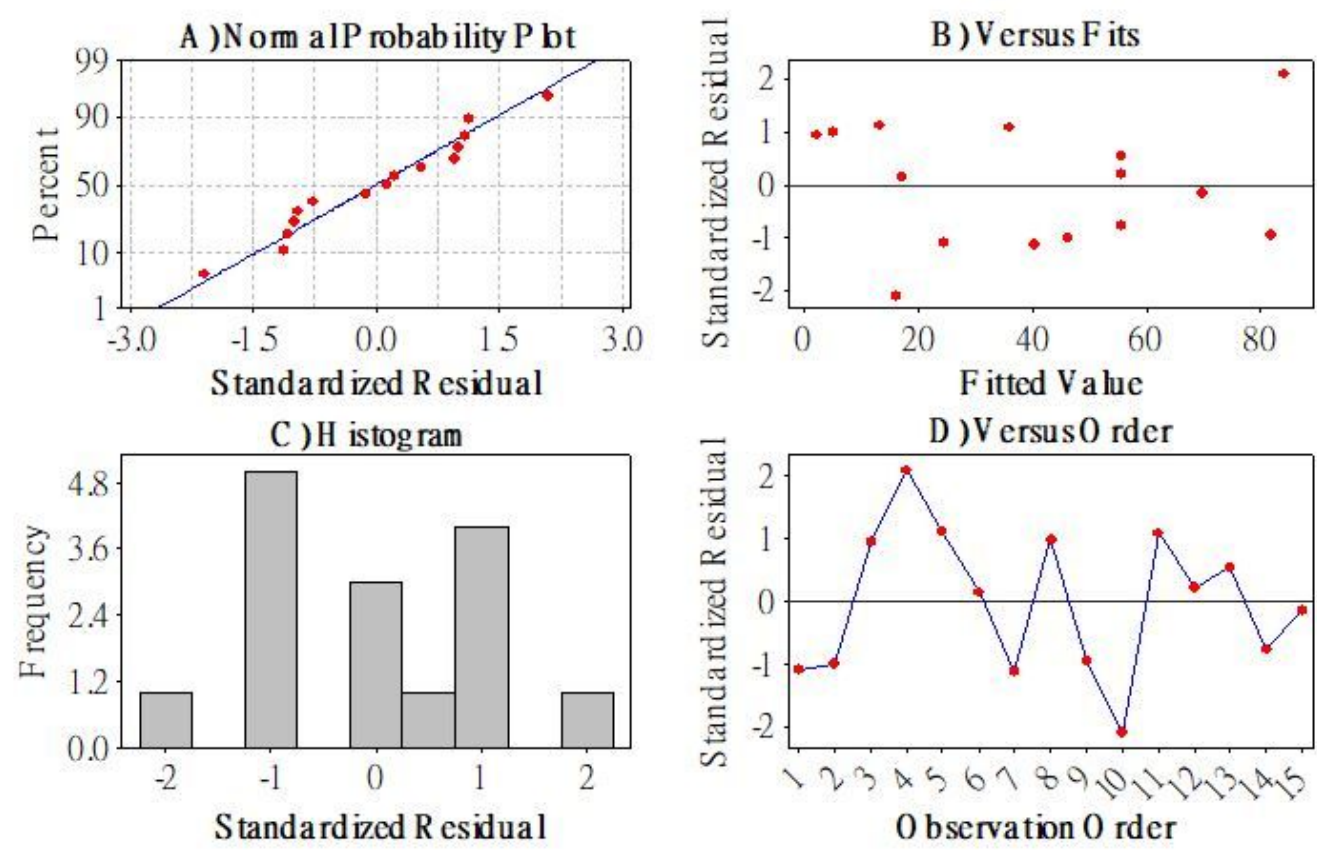


Figure 1

Standard error compared to a) standardized residual b) versus fits c) Histogram d)Versus order.

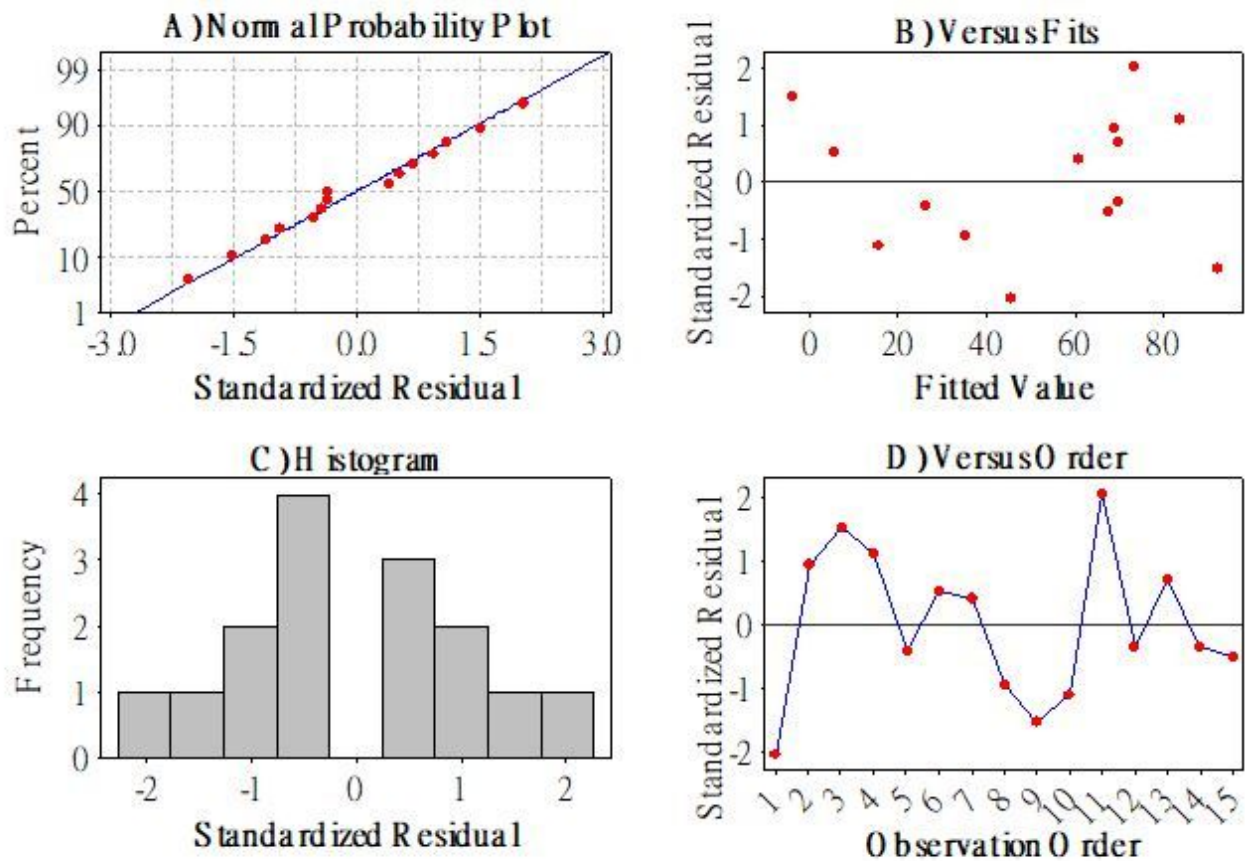


Figure 2

Standard error compared to A) standardized residual B) versus fits C) Histogram D) Versus Order. For DV30.

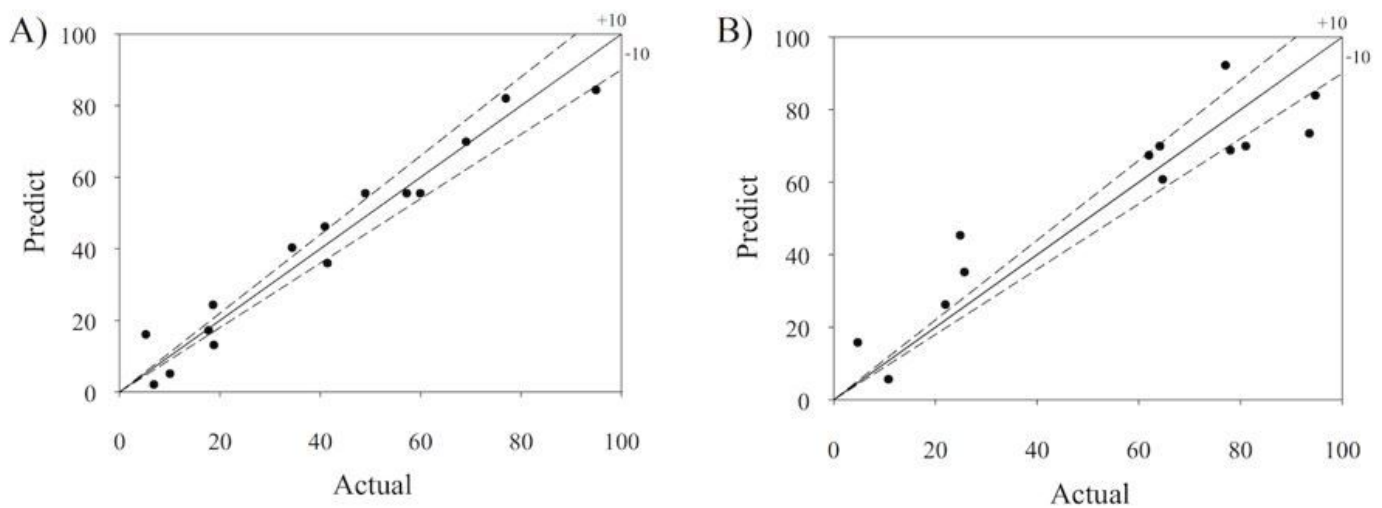


Figure 3

Experimental and predicted equation results for decolorization A) DR36 B) DV30

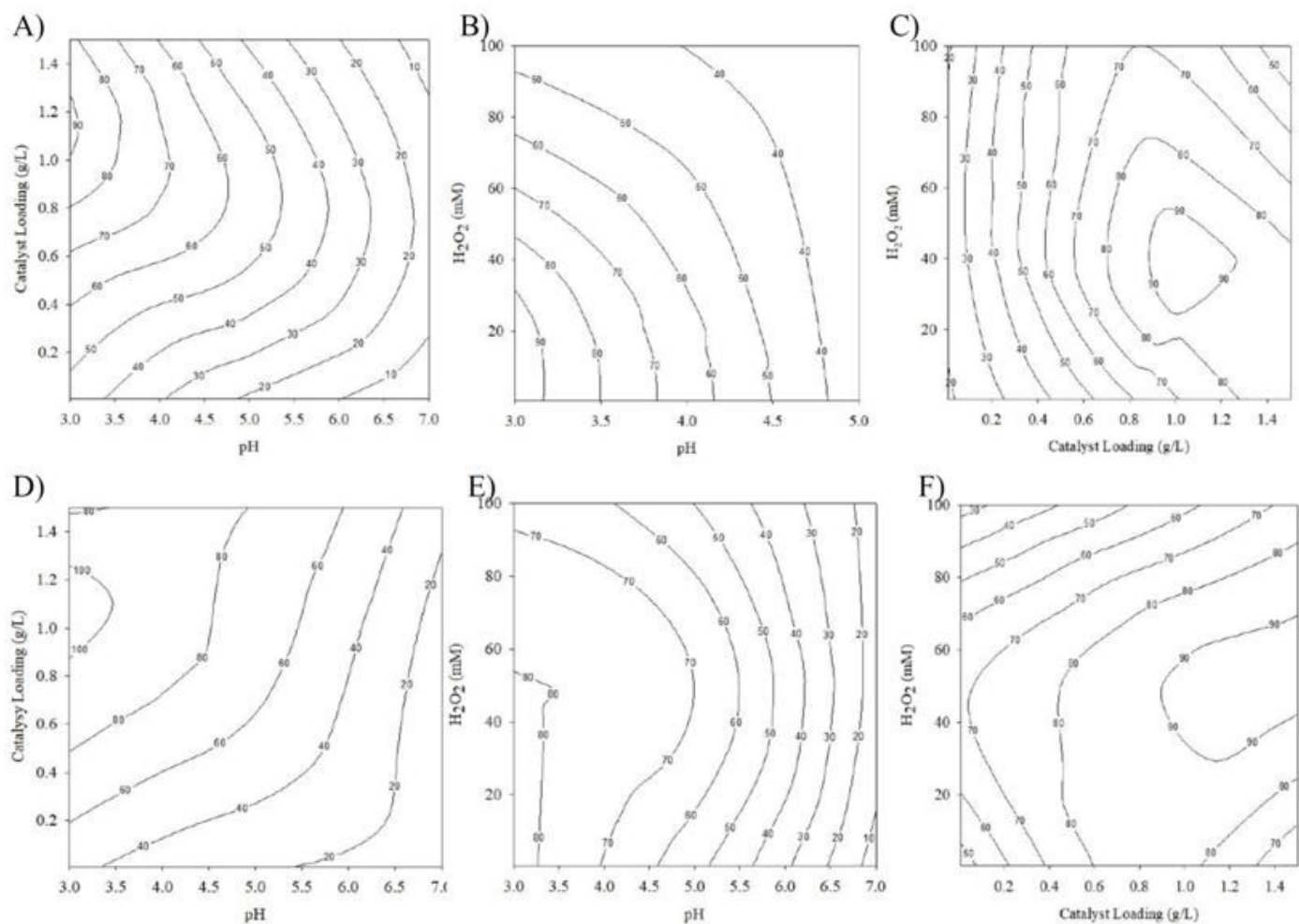


Figure 4

Contour plot illustrations, the interaction effect of variables on dye decolorization efficiency in the Fenton-like process in white (A-C) For DR36 and (D-F) for DV30.

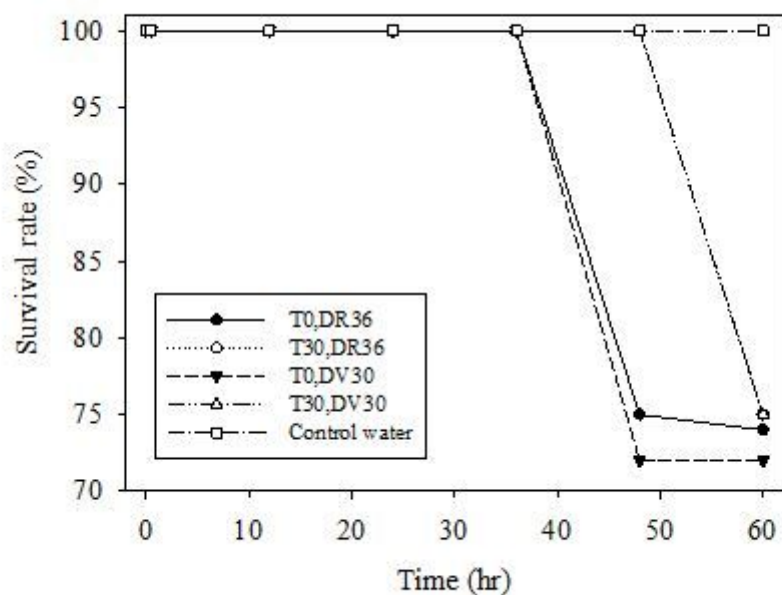


Figure 5

Effects of different treated water and untreated water with survival rate (%) of fairy shrimp (T0 is original dye at 300 ppm, T30 is treated water, and control water is water from a freshwater fairy shrimp

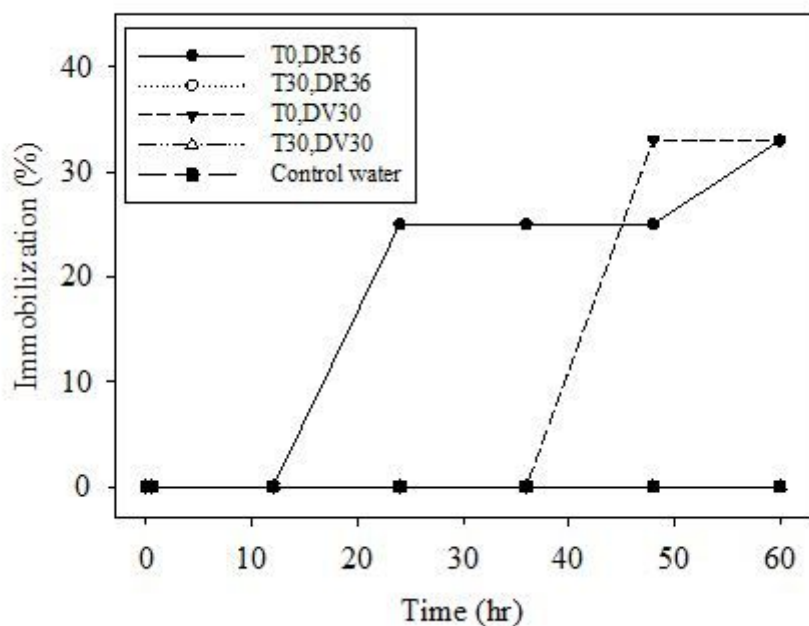


Figure 6

Effects of different treated water and untreated water with immobilization (%) of fairy shrimp, T0 is original dye at 300 ppm, T30 is treated water, and control water is water from a freshwater fairy shrimp culture