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One-Factor-at-a-Time (OFAT) Optimization of Victoria Blue R Dye Biodegradation by Pineapple Waste Garbage Enzymes

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ABSTRACT

Victoria blue R is a cationic triphenylmethane dye usually released from textile industries and reported to exhibit mutagenic and carcinogenic effects on aquatic organisms and humans. Hence, the objective of this study is to investigate the capability of pineapple waste garbage enzymes to biodegrade the Victoria blue R dye using One-Factor-At-a-Time (OFAT) optimization under the effect of Victoria blue R dye concentration (0.02 to 0.10 mg/mL), pH (1 to 7), and temperature (25°C to 49°C) via Design Expert 7.0 software. The results show that pineapple waste garbage enzymes gave the highest decolorization efficiency at a concentration of 0.07 mg/mL of Victoria blue R dye, pH 4.74, and 40.4°C. The ANOVA analysis suggests all models are quadratic, and the R-Squared values for the factors are 0.92, 0.95 and 0.92 for the concentration of Victoria blue R dye, pH and temperature, respectively. This work proposed that pineapple waste garbage enzymes can effectively remove Victoria blue R dye in wastewater applications.

1. Introduction

Synthetic dye is essential in a variety of industries involving textile, paper, and leather. An estimated 700,000 tons of various colorants are produced each year, and 100,000 tons are commercially available [1]. Usually, textile factories are released synthetic dyes either in treated or untreated effluents directly into the water bodies, thus posing serious ecotoxicological threats to living organisms [2]. Dye effluents are known to be present in the environment because of five major industries, i.e., textile, dyeing, paper and pulp, tannery and paint and dye manufacturers. Among them, the textile industry contributes more than half, 54% annually, releasing the highest amount of colouring into the environment. Generally, only 80% of dye can be absorbed into the fabrics. The remaining is usually released as dye effluents or wastewater and polluted with numerous hazardous chemical components. As Malaysia's textile and garment business has emerged rapidly in recent

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years, dye pollution has become an alarming challenge, being one of the most threatening factors toward marine life. Synthetic dyes can withstand natural deterioration. By decreasing light penetration into the water, dyes thrown into rivers may impair aquatic plants' photosynthetic activities and have disastrous consequences since there is less accessible dissolved oxygen in the water [3].

The use of enzymes for treating dye wastewater has gained significant attention due to their desirable properties, such as high efficiency, selectivity, and ability to operate under mild conditions when compared to other chemical catalysts. To illustrate, the use of enzymes in mild conditions can result in cost reduction as expensive equipment is not required to reach extreme operating conditions such as high pressure or temperature, which are often necessary for chemical catalysts. Additionally, enzymes are environmentally friendly as they are biodegradable and derived from natural sources, resulting in minimal environmental impact [4,5]. Previously, studies have shown the effectiveness of enzymes such as laccase, lignin peroxidase, NADH-preferring 2,6-dichloroindophenol (NADH–DCIP) reductase, tyrosinase, hexane oxidase, and aminopyrine N-demethylase toward the dye decolorization in wastewater [6]. For instance, laccase enzyme from *Megasporoporia* sp. achieved more than 26.1%, 19.7%, 13.6%, and 6.2% of decolorization for Malachite Green (MG), Safranin (S), Crystal Violet (CV), and Methylene Blue (MB) dyes, respectively [7].

The Malaysia Pineapple (*Ananas comosus*) is a typical tropic fruit with a total production of between 16 and 19 million tonnes around the world annually [8]. Generally, pineapple is consumed as fresh fruits or is processed into salads, fruit cocktail, jam, and can. During the pineapple processing, the pineapple wastes (peels, leaves and stems mainly), generally accounting for 50% (w/w) of total pineapple weight, are peeled off and discarded. In terms of circular economy perspective, a lot of nutrients contained in pineapple waste are lost when the residues are dumped off into the open environment, even though will cause negative environmental health issues [9]. Hence, the utilization of pineapple waste as a source for the fermentation of garbage enzymes is of great significance, especially in the prevention of biological resource waste and environmental pollution. Garbage enzyme (GE) is an organic solution produced by the simple fermentation of fresh vegetable waste, brown sugar, and water. GE can be utilized as a low-cost alternative to improve wastewater treatment processes by removing impurities and bacteria [10]. Pineapple wastes are a rich source of GE, which contains abundant proteases, amylases, and lipases. The pineapple and citrus peels-derived GE have high levels of protease, lipase, and amylase activity, which aid in the breakdown of TKN (Total Kjeldahl Nitrogen), COD (Chemical oxygen demand), and TP (Total phosphorus) in waste activated sludge and landfill leachate, increasing their solubilization [8,9]. Jiang *et al.,* [11] reported that adding GE effectively reduced the NH_3 emission and increased the TN (Total Nitrogen) content of the end compost. No information about the succession of garbage enzymes from pineapple waste for dye decolorization is available.

Even though garbage enzymes seem to be a reasonable solution for dye treatment, they still have some constraints, primarily when the enzymes are used in a harsh environment. Thus, creating long-lasting and sustainable dye wastewater treatment methods is essential for studying various variables affecting dye decolorization by employing waste enzymes. The optimization process may increase the enzyme's effectiveness and lower the enzyme necessary to decolorize dye effectively. For instance, optimization using the one-factor-at-a-time (OFAT) statistical method would be an excellent solution to test the optimal conditions for dye removal by garbage enzymes. Next, studying the factors could reduce the cost of implementation to ensure that garbage enzymes can be a more

affordable and attractive option than commercial enzymes while identifying the most cost-effective conditions for enzyme activities [8].

This study aims to prepare the pineapple waste garbage enzymes from the pineapple peels and stems and followed by the investigation of different factors, including the effect of concentration of Victoria blue R dye (0.02 – 0.10 mg/mL), pH (1 - 7), temperature (25°C - 49°C) against the efficiency of dye removal using one-factor-at-a-time (OFAT) optimization in Design Expert 7.0 software.

2. Methodology

2.1 Material and Chemicals

The materials used in the experiment were pineapple peels and stems, unsulfured blackstrap molasses, and deionised water. Pineapple peels, stems, and unsulfured blackstrap molasses were obtained from the local market, Tunas Manja Sdn Bhd around Kuantan. Next, Victoria Blue R (25G) was procured from Sigma Aldrich while the remaining chemicals were purchased from Merck and retrieved from the Universiti Malaysia Pahang (UMP) laboratory. All the aqueous solutions were prepared using deionised water obtained from UMP laboratory.

2.2 Sample Collection and Synthesis of Pineapple Waste Garbage Enzymes

The pineapple wastes, such as peels and stems were sliced into smaller pieces within 1cm in diameter to increase the surface area of the reaction in a fermentation jar. Then, 100g of unsulfured molasses, 300g of pineapple wastes, and 1L of dechlorinated water were mixed at the ratio of 1:3:10 in the 1.5 L airtight fermentation jar with a little space on the top. The sample was duplicated and acted as a control of the experiment. The jar stayed away from any UV source and always stayed in a dark and cold area since sunlight may disrupt the microbes' activity. The mixture was applied for 15 minutes of sonication time and fermented at pH 7, 37°C, and 2 days of incubation time. After fermentation, the mixture was filtered through filter paper to obtain the pineapple waste garbage enzymes, a concentrated dark brown liquid with a sour odour [9].

2.3 Dye Decolorization Efficiency of the Pineapple Garbage Enzymes in Victoria Blue R

Victoria blue R dye was chosen to investigate the dye decolorization efficiency of the pineapple waste garbage enzymes. The dye stock was initially prepared and kept in the dark at room temperature. To begin the experiment, a fixed ratio of 1:3 (10 ml Victoria blue R dye: 30 ml pineapple waste garbage enzymes) was mixed into a 50 ml centrifuge tube and then incubated for 24 hours at the experimental conditions without shaking. The test sample, after decolorization, was collected for absorbance readings at 599 nm wavelength using a UV-vis spectrophotometer (Model: Thermo Scientific™ GENESYS™ 50 UV-Visible). Samples before and after decolorization were inserted into vials, and the calibration was done using pure pineapple waste garbage enzymes as the blank solution. Hence, the respective absorbance was determined and applied to find the decolorization efficiency using the equation presented in Eq. (1) [10].

$$\text{Dye decolorization } \% = \frac{\text{Initial absorbance} - \text{Final absorbance (A)}}{\text{Initial absorbance (A)}} \times 100\% \quad (1)$$

2.4 One-Factor-at-a-Time (OFAT) Optimization

One-factor-at-a-time (OFAT) optimization was used to evaluate the effects of the independent variables: concentration of Victoria blue R dye (0.02 – 0.10 mg/mL), pH (1 - 7), temperature (25℃ - 49℃) on dye decolorization (%). Each parameter was randomized in 16 experimental runs by Design Expert 7.0 (Stat-Ease Inc., Minneapolis, MN, USA). One-way analysis of variance (ANOVA) was employed to determine the statistical significance of the model. Probability values of $p \leq 0.05$ were considered statistically significant.

3. Results and discussion

3.1 Effect of Initial Concentration of Victoria Blue Dye

Figure 1 shows the decolorization percentage at different concentrations of dye (0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL). Other parameters are constant at pH 7 and room temperature (25°C). The decolorization efficacy increases gradually as the amount of dye substrates increases. It reaches the highest decolorization at 0.06 mg/ml, which is 59%. Then, any additional dye substrates after reaching the equilibrium state will not affect the rate of reaction of enzymes, which concluded that the graph should be stagnant and then decrease as the concentration increases [12].

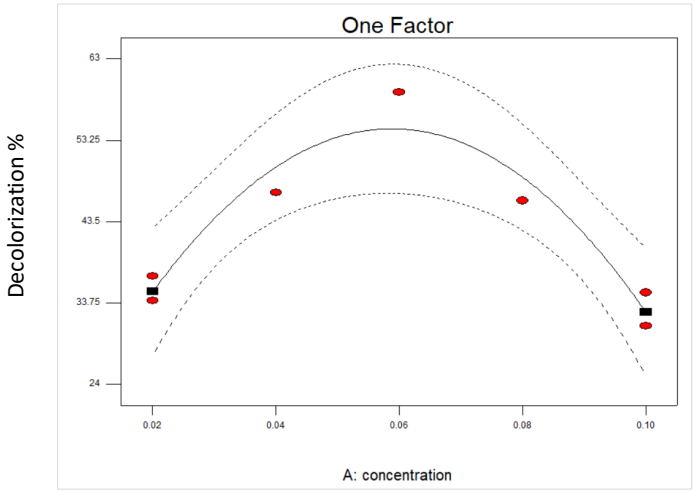

Fig. 1. Graph of decolourisation against the concentration of Victoria blue dye

Table 1 shows the ANOVA analysis for Response Surface Quadratic Model using Design Expert 7.0. It showed that the lack of fit is not significant (Acceptable).

Table 1
ANOVA for response surface quadratic model for concentration of Victoria blue dye analysis

Parameters	*Sum of squares*	*Degree of freedom*	*Mean square*	*F value*	*p-value*	
Model	535.94	2	267.97	21.66	0.0071	significant
Concentration	6.72	1	6.72	0.54	0.5019	
A^2	529.22	1	529.22	42.78	0.0028	
Residual	49.49	4	12.37			
Lack of Fit	36.99	2	18.49	2.96	0.2526	not significant
Pure Error	12.50	2	6.25			
Cor Total	585.43	6				

At the same time, the R-Squared value in Table 2 verified that the value is more than the acceptable range for the biological experiment. Later, optimization was performed using the numerical method given in the software.

Table 2
R-Squared values for the concentration of Victoria blue dye analysis

Parameters	*Value*
Std. Dev.	3.52
Mean	41.29
C.V. %	8.52
PRESS	154.54
R-Squared	0.92
Adj R-Squared	0.87
Pred R-Squared	0.74
Adeq Precision	9.53

The predicted value suggested by the software was 0.07 mg/ml with a decolorization efficiency of 51.75%, as shown in Table 3. An experimental result at the optimum condition was repeated. Comparing the observed and predicted values shows that the error was within the acceptable range of 5.31%.

The degree to which dye concentration can significantly influence enzymes' decolorized dye. Higher dye concentration may cause lower decolorization since the dye molecules are more difficult to break down. Thus, when the concentration of dye increases, the decolorization rate decreases [12]. This is because enzymes can only break down a limited amount of dye molecules until they are overwhelmed and unable to keep up with the rate of decolorization when the concentration is high. Conversely, if the dye concentration is too low, the enzyme could not have enough substrate to function correctly, resulting in a slower decolorization rate. An ideal dye concentration is required to achieve the highest rates of enzyme decolorization. The type of enzyme being employed might also impact the ideal dye concentration. Certain enzymes might function more effectively at higher dye concentrations, whereas others might only be able to partially degrade dye molecules, resulting in slower decolorization rates at higher dye concentrations.

Table 3
Comparison of predicted and experimental results for concentration

Parameters	*Value*
Concentration of Victoria Blue	0.07 mg/ml
Predicted decolorization	51.75 %
Experimental decolorization	49 %
Error	5.31%

3.2 Effect of pH Values

The effect of pH of Victoria blue R dye decolorization efficiency was investigated from pH 1 to pH 7 at an optimum concentration (0.07 mg/ml) and room temperature (25°C). From Figure 2, the decolorization response slowly emerged and peaked at pH 2.50 before gradually reducing as it approached pH 7. Hence, it is evident that pineapple waste garbage enzymes had the highest decolorization which was 77% in an acidic environment.

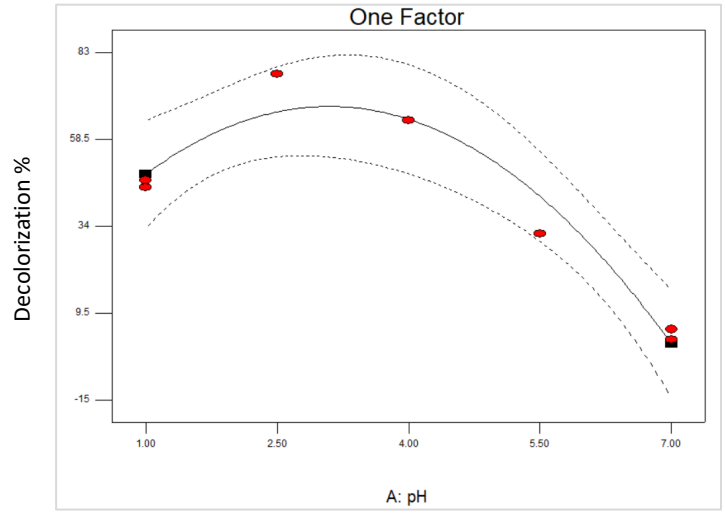

Fig. 2. Graph of decolorization against the pH

Table 4 shows the ANOVA analysis for Response Surface Quadratic Model.

Table 4
ANOVA for response surface quadratic model for pH

Parameters	Sum of Squares	Degree of freedom	Mean Square	F value	*p*-value	
Model	4485.57	2	2242.79	34.87	0.0029	significant
pH	2568.06	1	2568.06	39.93	0.0032	
A^2	1917.52	1	1917.52	29.81	0.0055	
Residual	257.29	4	64.32			
Lack of Fit	250.79	2	125.39	38.58	0.0253	significant
Pure Error	6.50	2	3.25			
Cor Total	4742.86	6				

The lack of fit was significant (rejected), while the R-Squared value was accepted (Table 5). However, it was an exceptional case for the effect of pH as the lowest pH value for a solution was pH 1, and it was impossible to add any points below pH 1.

Table 5
R-Squared values for pH analysis

Parameters	*Value*
Std. Dev.	8.02
Mean	38.86
C.V. %	20.64
PRESS	609.10
R-Squared	0.95
Adj R-Squared	0.92
Pred R-Squared	0.87
Adeq Precision	12.46

An optimization was carried out, and the suggested optimization value was predicted to be at the maximum pH of 4.74, with a decolorization efficiency of 55.53%, as shown in Table 6. The comparison shows a significant difference of 4.77 %. This result is in agreement with Theng *et al.,* [13] which obtain pH 2 as the favourable condition to remove Congo red dye from water using cassava leaf powder.

The pH of the dye solution significantly influenced the dye decolorization by the action of enzymes. Enzymes have a preferred pH range for their activity. In the case of fermented garbage enzymes, the pH of the solution is usually acidic [14]. Hence, outside of this pH range, the catalytic ability of enzyme could be diminished or even completely inhibited, thus suggesting that the acidic condition favours Victoria blue R dye decolorization. This meant that the enzyme was more capable of breaking down the dye molecules only if the dye could completely dissolve in a low pH solution, which was around pH 2-5.

Table 6
Comparison of predicted and experimental results for pH

Parameters	*Value*
pH	4.74
Predicted decolorization	55.53 %
Experimental decolorization	53 %
Error	4.77

3.3 Effect of Temperature

The effect of temperature (25℃, 31℃, 37℃, 43℃, and 49℃) on the dye decolorization efficiency was investigated at optimum concentration (0.07 mg/ml) and optimum pH values (4.74) obtained from the previous experiment. The decolorization efficiency increased as temperature increased despite decreasing after surpassing the optimum value of 37°C, as shown in Figure 3.

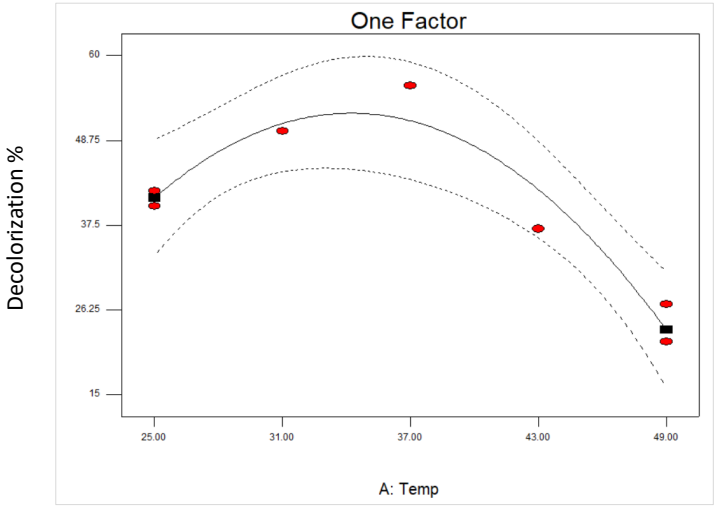

Fig. 3. Graph of decolorization against temperature

It showed a good fit for the quadratic model and acceptable R-Squared values, as seen in Tables 7 and 8.

Table 7
ANOVA of response surface quadratic model for temperature

Parameters	*Sum of squares*	*Degree of freedom*	*Mean square*	*F value*	*p-value*	
Model	790.85	2	395.42	23.96	0.0059	significant
A-Temp	346.72	1	346.72	21.01	0.0102	
A^2	444.13	1	444.13	26.91	0.0066	
Residual	66.01	4	16.50			
Lack of Fit	51.51	2	25.75	3.55	0.2197	not significant
Pure Error	14.5	2	7.25			
Cor Total	856.86	6				

Table 8
R-Squared values for temperature

Parameters	*Value*
Std. Dev.	4.06
Mean	39.14
C.V. %	10.38
PRESS	198.47
R-Squared	0.92
Adj R-Squared	0.88
Pred R-Squared	0.77
Adeq Precision	10.44

The decolorization efficiency between predicted and experimental values after optimization did show 4.34 % of error difference, as shown in Table 9. Temperature is a crucial factor in the enzyme-mediated decolorization of dyes [15]. Enzymes have a preferred temperature range in which they operate most effectively, and temperatures outside of this range could either cause denaturation of the enzyme or a reduction in enzyme activity [16,17]. Both of which diminished the enzyme's ability to decolorize. In theory, higher temperatures could speed up enzymatic activities by boosting the kinetic energy of the dye and enzyme molecules. However, the ideal temperature for enzyme activity

could vary depending on the specific enzyme and dye system [18]. Onder *et al.,* [19] reported that the highest decolorization rate could be achieved by peroxidase at 30℃ for the Naphthol Blue Black (NBB) dye from a range of temperatures studied from 25-70℃. Additionally, results presented by Pandey *et al.,* [20] further proved that the optimum decolorization rate of dye in the aqueous phase could be achieved at 30℃ by Laccase.

Table 9
Comparison of predicted and experimental result for temperature

Parameters	*Value*
Temperature	40.4 ℃
Predicted decoloriz*ation*	47.28 %
Experimental decoloriz*ation*	45.23 %
Error	4.34 %

4. Conclusions

In this study, the application of pineapple waste garbage enzymes to the Victoria blue R dye removal was investigated using One-factor-at-a-time (OFAT) optimization in Design Expert 7.0 software by varying control variables such as concentration of Victoria blue R dye, pH, and temperature. The function of these variables in terms of Victoria blue R dye removal was well explained by the results obtained from OFAT optimization. The study found that pineapple garbage enzymes work the best at 0.07 mg/ml concentration of Victoria blue, pH 4.74 and 40.4℃. The ANOVA analysis using Design Expert suggests the three models are quadratic, and the R-Squared values for the factors are 0.92, 0.95 and 0.92 for the concentration of Victoria blue, pH and temperature, respectively. The experimental and the predicted values did not show significant differences, reflecting the accuracy and applicability of OFAT optimization. It can be concluded that the pineapple waste garbage enzymes can effectively be used as a locally abundant enzyme extracted from waste to remove dyes from textile dying industry effluent.

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