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Optimization of Victoria Blue R Dye Decolorization using Two-Level Factorial Analysis with Garbage Enzyme Pineapple Waste hybrid Nanoflowers (GPW-hNFs)

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ABSTRACT

In Malaysia, the textile industry poses a significant environmental challenge with its dye-containing effluents, and the disposal of ash from palm oil mills exacerbates the issue. This study addresses the critical need for efficient and sustainable methods to treat dye-containing industrial wastewater, focusing on Victoria Blue R (VBR) dye decolorization. The study explores the optimization of the decolorization process using Garbage Enzyme Pineapple Waste hybrid Nanoflowers (GPW-hNFs) through a two-level factorial analysis. The GPW-hNFs, synthesized from garbage enzyme derived from pineapple waste, serve as a promising enzymatic source for dye degradation. By systematically varying factors such as nanoflower amount, initial dye concentration, pH level, sonication time, and temperature, the study identifies key parameters influencing VBR dye decolorization. Employing statistical tools such as ANOVA and predictive modelling, the study reveals the significance of nanoflower amount, initial dye concentration, and their interaction (AC) in achieving optimal decolorization. The predicted optimum condition, validated experimentally, resulted in a remarkable 61.35% dye decolorization. The high accuracy (99.96%) underscores the efficacy of the two-level factorial analysis in optimizing GPW-hNFs for VBR dye decolorization, offering a promising avenue for sustainable wastewater treatment in the textile industry.

1. Introduction

Inadequate treatment of dye-containing effluents poses a serious threat to aquatic ecosystems, impacting water quality and aquatic life. The textile sector in Malaysia produces 22% of the country's industrial wastewater [1]. Manogaran *et al.,* [2] further highlight the detrimental influence of dye pollution on water resources in Malaysia, particularly in the Juru riverine area [3]. The discharge of synthetic dyes from industrial activities, especially in textile and dyeing processes, significantly

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contributes to environmental pollution [4-6]. The textile industry uses a substantial amount of water during dyeing, resulting in the production of concentrated wastewater containing contaminants in the form of organic compounds from chemicals like dyes [7]. Various methods are used for the treatment of textile wastewater, including physical [5-7], chemical [8], and biological [9] methods. Now, biological degradation of dyes using enzyme, like pineapple garbage enzyme [10], peroxidase [10] and laccase enzyme [11] has proven to be an effective, cost-efficient, and environmentally friendly method.

Enzymatic decolorization has emerged as a sustainable and effective approach for treating dye-contaminated wastewater [12]. Enzymes, with their specificity and catalytic efficiency, offer a targeted means of breaking down complex dye molecules into less harmful byproducts [13]. However, enzymatic wastewater treatment encounters challenges related to enzyme stability, recovery, and reusability. These limitations can be addressed by enzyme immobilization, which can stabilize enzymes, prevent enzyme inactivation, enhance enzymatic activity, and reduce susceptibility to microbial contamination [14].

In the new millennium, nanotechnology has shown promising applications such as the development of hybrid enzyme–inorganic nanoflowers with flower-like spherical structures, providing a simple, and adaptable technique for protein immobilization [15]. Nanoflowers show increased enzyme activity and stability when utilised as the organic element of hybrid nanoflowers, attributed to the greater specific surface area and enhanced immobilisation compared to the free enzyme [16]. In the current research of dye decolorization, many studies about nanoflower have been developed such as BSA-$Cu_3(PO_4)_2$ hybrid nanoflower (NF) [17], Myoglobin-Zn (II) assisted hybrid nanoflowers (MbNFs@Zn) [18], and laccase-lysine-CuSO4 [19]. These advancements have inspired researchers to design new hybrid nanostructures with flower-like shapes for dye decolorization applications.

Garbage enzyme (GE) is an organic solution produced by the simple fermentation of fruit or vegetable wastes, brown sugar, and water and is abundant in proteases, amylases, and lipases, making it inexpensive [20]. By taking into consideration the literature that reports the successful application of pineapple garbage enzyme in the treatment of Victoria Blue R dye [21] and eco-enzyme from orange and dragon fruit peels for the treatment of textile wastewater [22], further study should be conducted. Pineapple wastes consist of various enzymes such as bromelain, and xylanase [23,24]. After fermentation with molasses and water, these waste products yield garbage enzymes which typically contain protease, amylase, and lipase [9]. However, currently no information is available about the development of garbage enzyme from pineapple waste combined with metal ions to produce nanoflowers specifically for dye decolourization. While the treatment of Victoria Blue R dye by pineapple garbage enzyme shows significant potential, the optimization of crucial parameters is essential to enhance its efficiency and applicability [25].

One promising strategy is the application of two-level factorial analysis. A two-level factorial design is a systematic and effective method in experimental design and statistical analysis for examining the influence of numerous factors on a specific response variable. This strategy involves manipulating factors at two levels (high and low) to analyse their impact systematically [25]. By assessing all feasible combinations of factor settings, it determines the primary effects (the individual influence of factors) and interactions (the degree to which these factors influence one another's effects). This approach minimizes the number of tests needed to optimize a process, product, or system, making it a valuable tool for decision-making and quality improvement. This study aims to prepare Garbage Enzyme Pineapple Waste hybrid Nanoflowers (GPW-hNFs) followed by the investigation of different factors, including the quantity of GPW-hNFs (0.05 to 0.15 g), pH levels (5.4 to 9.4), temperature (27 to 47 °C), sonication time (20 to 40 minutes), and initial dye concentration

(0.02 to 0.10 mg/mL), against the decolorization percentage using two-level factorial analysis in Design Expert 7.0 software.

2. Methodology

The methodology for this study encompassed three phases, which are the preparation of Garbage Enzyme Pineapple Waste hybrid Nanoflowers (GPW-hNFs), the adsorption experiment and the application of a two-level factorial design to optimize Victoria Blue R dye decolorization.

2.1 Preparation of Garbage Enzyme Pineapple Waste hybrid Nanoflowers (GPW-hNFs)

The initial step involved the preparation of garbage enzyme (GE) using a mixture of pineapple, water, and molasses, with a specific ratio and subsequent incubation for a duration of 2 days. Following a modified method from existing literature [26], nanoflowers were formed. A stock solution of 120 mM $CaCl_2$ was prepared, and in a controlled environment, a solution consisting of a phosphate-buffered saline (PBS) at pH 7.4, GE, and $CaCl_2$ underwent a sequence of mixing, vertexing, sonication, and incubation, leading to the formation of GPW-hNFs. The successful synthesis of the nanoflowers was indicated by the precipitate, which was subsequently collected, washed, and dried.

2.2 Adsorption Experiment

The adsorption test was initially conducted at fixed parameters (0.1g of GPW-hNFs, pH 7.4, sonication 30 min, and a temperature of 37 °C). The absorbance of different concentrations of Victoria Blue R dye, ranging from 0.02 to 0.1 mg/mL, was measured. The mixture was incubated for 3 hours, with 2 mL samples taken every 30 minutes. Each sample was then centrifuged for 10 minutes at 10,000 rpm. The supernatant obtained was measured spectrophotometrically using a UV-vis spectrophotometer (Model: Thermo Scientific™ GENESYS™ 50 UV-Visible) at λmax of 610 nm. To construct the calibration curve, the measured absorbance values were plotted against the respective concentration of Victoria Blue R dye in different concentrations.

2.3 Design of Experiment by a Two-Level Full Factorial Design

We formulated a two-level factorial design to identify the most effective parameter combinations for decolorizing Victoria Blue R dye. The selected parameters included the quantity of GPW-hNFs (ranging from 0.02 to 0.18 g), initial dye concentration (ranging from 0.02 to 0.10 mg/mL), pH levels (ranging from pH 5.4 to 9.4), sonication time (ranging from 20 to 40 minutes), and temperature (ranging from 27 to 47 °C). Each parameter was set at two doses, upper and lower, chosen based on previous experiments and literature review. For example, Lee *et al.,* [21] found that pineapple waste garbage enzymes achieved the highest decolorization efficiency at a Victoria Blue R dye concentration of 0.07 mg/mL, pH 4.74, and 40.4 °C.

Additionally, Jalani *et al.,* [9] reported the highest activities for lipase, amylase and protease of pineapple garbage enzyme were achieved at sonication time from 20 to 35 minutes. Meanwhile, Shelar *et al.,* [27] observed that the optimal dose for ZnS nanocatalysts was 1 g/L when working with a 20 mg/L concentration of Victoria Blue R dye. The temperature for the Victoria Blue R dye decolorization was set to not exceed 47 °C because an excessive temperature may cause degradation of nanoflower and hinder the decolorization process [28]. For this study, a fractional factorial design (FFD) (2^{5-1}) was utilized to assess the impact of five independent variables, which are the amount of

nanoflower (A), concentration (B), pH level (C), sonication time (D) and temperature (E) on the dye decolorization percentage (%). The lower value for each parameter was coded as -1, while the higher value was coded as +1, and the experiment was conducted according to Table 1.

Design Expert 7.0 was employed (Stat-Ease Inc., Minneapolis, MN, USA) to randomize each parameter in 16 experimental trials to identify which factors have more impact than others. The statistical significance of the model was assessed using a two-way analysis of variance (ANOVA). Statistically significant probability values were defined as $p < 0.05$. Pareto charts were generated to visually analyze the statistical importance of each response coefficient. The percentage change in Victoria Blue R dye decolorization is calculated using the formula in Eq. (1):

$$\text{Decolorization } (\%) = \frac{D_i - D_f}{D_i} \times 100\% \quad (1)$$

The equation provided represents the initial and final readings of dye (D_i and D_f, respectively) obtained using a UV-vis spectrophotometer (Model: Thermo Scientific™ GENESYS™ 50 UV-Visible).

Table 1
Range values for two-level factorial design

Factor	Name	Units	Minimum	Maximum	Low coded	High coded	Mean	Standard deviation
A	Amount of NF	g	0.02	0.18	-1	+1	0.1	0.080
B	Concentration of dye	mg/mL	0.02	0.10	-1	+1	0.060	0.040
C	pH level		5.4	9.4	-1	+1	7.4	2.00
D	Sonication time	Min	20	40	-1	+1	30	10
E	Temperature	°C	27	47	-1	+1	37	10

3. Results

3.1 Calibration Curve of Victoria Blue R (VBR) Dye

According to Beer-Lambert's law, the dye concentration is directly proportional to the absorbency. Therefore, to determine the dye decolorization efficacy, a calibration curve was prepared consisting of absorbance against different concentrations of Victoria Blue R dye. Figure 1 shows a straight line of the absorbance versus the concentration of Victoria Blue R dye suggesting the validity of Beer-Lambert law. The molar absorption coefficient (ε) of Victoria Blue R dye was found to be 10.015 $mL \cdot mg^{-1} \cdot cm^{-1}$ at 37 °C.

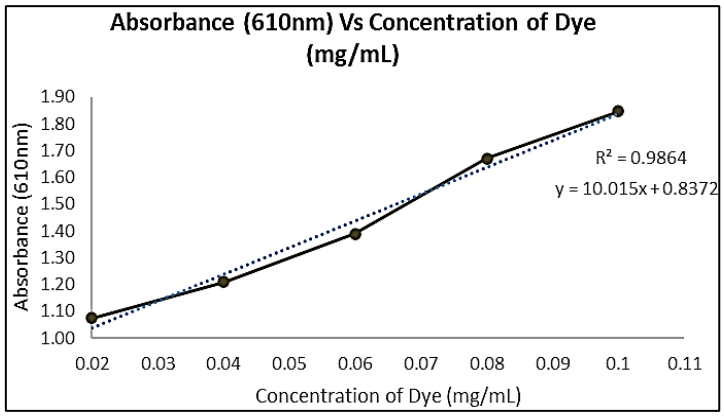


Fig. 1. Standard curve of methylene blue at concentration ranges from 0.02 to 0.1 mg/mL at fixed parameters (0.1g of GPW-hNFs, pH 7.4, sonication 30 min, at temperature 37 °C)

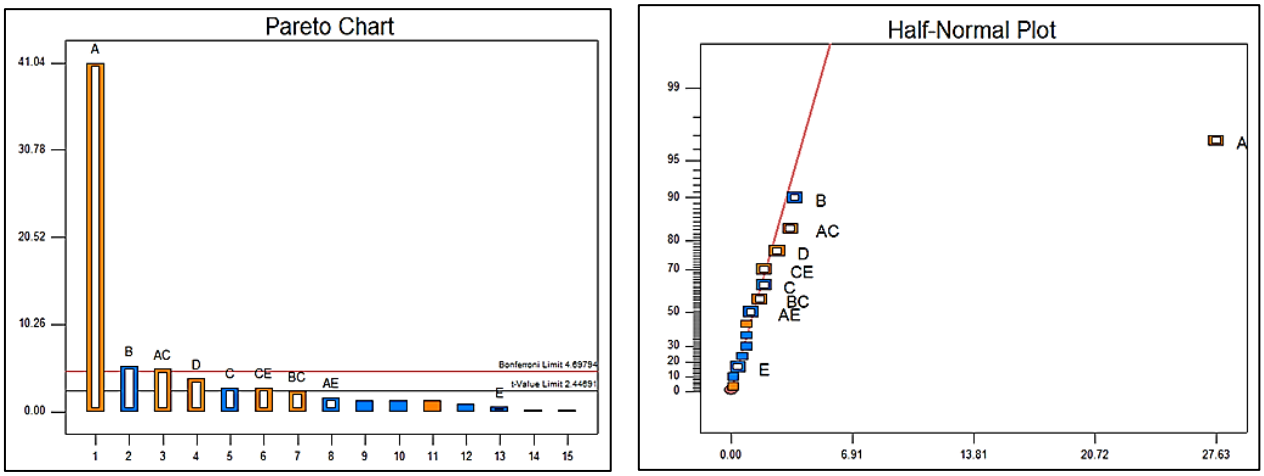
3.2 Screening of Significant Dye Decolorization Variables using FFD

Table 2 shows the results of Victoria Blue R (VBR) dye decolorization, which ranged from 22% to 59%. Based on Table 2, the highest dye decolorization percentage was at 59 % with variables 0.18 g of nanoflower, 0.02 mg/mL initial dye concentration, pH level 9.40, 40 minutes sonication time, and a temperature of 27 °C. This study aligns with Dadi *et al.,* [29] who similarly examined the use of organic-inorganic nanoflowers for dye decolorization applications. Furthermore, Qin *et al.,* [30] also presented the effectiveness of laccase enzymes in the decolorization of Ramazol Brilliant Blue R, which supports the relevance of the enzymatic decolorization process in this study. The significant factors were determined by the Pareto chart (Figure 2(a)) and half-normal probability plots (Figure 2(b)).

Table 2
Design matrix and the responses of two-level factorial design

Run	Amount of nanoflower (g)	Initial dye concentration (mg/mL)	pH level	Sonication time (minutes)	Temperature (°C)	Dye decolorization (%)
1	0.02	0.02	5.40	20.00	47.00	32
2	0.18	0.02	5.40	20.00	27.00	57
3	0.02	0.10	5.40	20.00	27.00	28
4	0.18	0.10	5.40	20.00	47.00	50
5	0.02	0.02	9.40	20.00	27.00	22
6	0.18	0.02	9.40	20.00	47.00	56
7	0.02	0.10	9.40	20.00	47.00	24
8	0.18	0.10	9.40	20.00	27.00	54
9	0.02	0.02	5.40	40.00	27.00	35
10	0.18	0.02	5.40	40.00	47.00	57
11	0.02	0.10	5.40	40.00	47.00	27
12	0.18	0.10	5.40	40.00	27.00	55
13	0.02	0.02	9.40	40.00	47.00	30
14	0.18	0.02	9.40	40.00	27.00	59
15	0.02	0.10	9.40	40.00	27.00	25
16	0.18	0.10	9.40	40.00	47.00	56

The height of the bars on the Pareto chart shows the standardized effects of the investigated variables on dye decolorization. Based on the chart, the amount of nanoflower (A), the initial concentration of Victoria Blue R dye (B), and the interaction between the nanoflower amount and pH level (AC) were the most influential factors in decolorizing the Victoria Blue R dye as they exceeded the Bonferroni limit line of 4.69794. On the contrary, the temperature (E), the interaction between nanoflower amount and temperature (AE) were insignificant as they fell below the t-value limit line of 2.44691. Besides that, other standardized effects, which were D, C, CE, and BC, also had significant effects on dye decolorization, as they were in between Bonferroni and t-values limit line. The half-normal probability plot in Figure 2(b) shows the magnitude effects of these factors, which consist of individual variables and their interactions, from smallest to largest, as standardized effects along the x-axis. The amount of nanoflower (A), dye concentration (B), and interaction AC deviated from the main group of standardized effects that lie near or along the linear line. Therefore, this deviation further confirms that A, B, and AC were the significant factors influencing dye decolorization in this study.



* A: Amount of NF, B: Concentration of dye, C: pH level, D: Sonication time, E: Temperature

(a) (b)

Fig. 2. (a) The Pareto chart representing the significance of the independent variables (b) The half normal plot for determination of extraction variables

3.3 ANOVA Analysis

An analysis of variance (ANOVA) is a valuable tool for assessing the impact of specific input parameters (independent variables) on a set of experimental outcomes. It is particularly useful for designing experiments, screening data, and interpreting the findings [30]. ANOVA can be used to determine the impact of each control factor on the experimental error, providing numerical insights into the importance of each variable. This process ensures that no significant factors are overlooked, thereby enhancing the accuracy of the forecast. *P*-values were used to assess the importance of each coefficient, with lower *p*-values indicating a greater relevance of the associated coefficient [31]. Similarly, from Table 3, it is clearly seen that the amount of nanoflower (A) was the most significant factor as it has the smallest *p*-value, followed by the initial dye concentration (B), and the interaction AC. In contrast, the pH value (C), the sonication time (D), and the temperature (E) were insignificant, as their *p*-values were greater than 0.05, indicating that these factors do not significantly influence the dye decolorization model.

Table 3
ANOVA table on the dye decolorization of VBR

Source	Sum of squares	Mean squares	F-Value	*p*-value prob>F
Model	3222.56	358.06	197.55	0.0001 (significant)
A-nanoflower amount	3052.56	3052.56	1684.17	0.0001
B-Concentration	52.56	52.56	29.00	0.0017
C-pH	14.06	14.06	7.76	0.0318
D-Sonication time	27.56	27.56	15.21	0.0080
E-Temperature	0.56	0.56	0.31	0.5976
AC	45.56	45.56	25.14	0.0024
AE	5.06	5.06	2.79	0.1457
BC	10.56	10.56	5.83	0.0523
CE	14.06	14.06	7.76	0.0318

3.4 Influence of Decolorization Parameters on Dye Decolorization of VBR

The interaction effects between factors are shown in Figure 3. In this figure, the red line represents the highest pH level used in this study, which is at 9.4, while the black line represents the lowest pH value in this study which is at 5.4. To achieve the highest dye decolorization between the two variables shown in Figure 3, other factors were set as constant, where the dye concentration is at 0.07 mg/mL, the sonication time is at 30 minutes, and the temperature is at 37 °C. From Figure 3, it was clearly seen that the dye decolorization percentage (59%) was achieved by the red lines at the maximum amount of nanoflower (NF) at 0.18 g with a pH level of 9.4. This result highlights the critical role of pH level and nanoflower amount in the dye decolorization process. The pH level has a strong influence on the ionic species present in solution and electrostatic forces, which are essential to many adsorption processes. The ionization state of both the enzyme and the support material may change as a function of pH, resulting in their respective net charges [32].

Lee *et al.,* [21] achieved up to 55.53% of Victoria Blue R (VBR) decolorization by using pineapple garbage enzyme at pH 4.74. Besides, Jalani *et al.,* [9] also used pineapple garbage enzyme at pH 4.3, but extended the incubation time up to 48 hours to achieve 90% decolorization of Victoria Blue R (VBR) dye. The addition of calcium chloride has shifted the optimum pH of Garbage Enzyme Pineapple Waste hybrid Nanoflowers (GPW-hNF) to 9.4, increasing the decolorization efficiency to 59%. Similarly, the lipase ZC12/$Ca_3(PO_4)_2$ hybrid nanoflowers have an optimum pH at 8 after incorporation with calcium ions [33]. The decolorization efficiency of Victoria Blue R dye is also highly dependent on the amount of nanoflowers. The more amount of nanoflowers will provide a larger surface area for the interaction between the nanoflower and the dye, consequently the quicker the dye will decolorize until it reaches an equilibrium point. After this point, further increases in nanoflower amount can lead to a decline in decolorization efficiency due to saturation effects or aggregation of the nanoflowers, which prevents access to active sites [34]. Furthermore, the amount of free pineapple garbage enzyme used in the previous study [9] is much higher than the amount of GPW-hNF to decolorize the same concentration of Victoria Blue R (VBR) dye, indicating the higher catalytic ability of GPW-hNF to oxidize dye in small quantity.

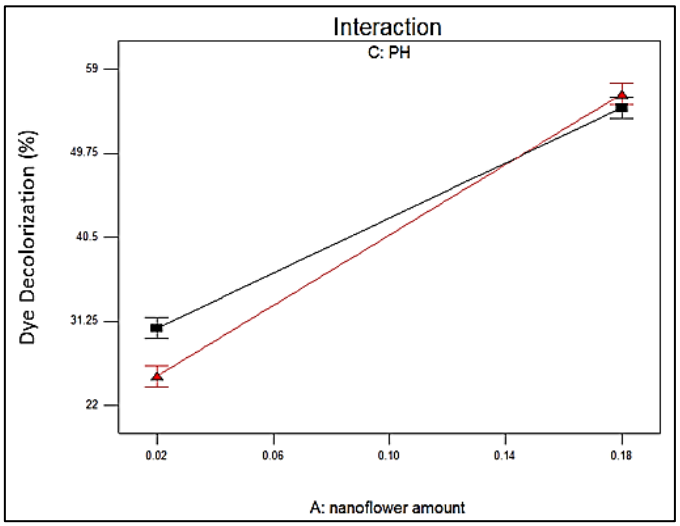


Fig. 3. Effect of amount of nanoflower (A) and pH Level (C) on the dye decolorization (%) of VBR dye

3.5 Modelling Statistics

The statistical analysis of antioxidant activity is shown in Table 4. The coefficient of determination (R^2) for dye decolorization was 0.9966, indicating a reasonable fit between the model and the experimental data. Besides that, the predicted R^2 of 0.9761 was in reasonable agreement with the adjusted R^2 of 0.9916, which suggested a high degree of correlation between the observed and predicted values. The adjusted R^2 value confirmed that the model was highly significant. The predicted R^2 value indicates that this model will remain accurate for future data, even if new observations are introduced to the decolorization model. Moreover, the adequate precision ratio of 32.649, which exceeds the desirable threshold of 4, indicates that this model can effectively navigate the design space. Therefore, it can be concluded that the selected variables factors were significant for the dye decolorization of Victoria Blue R (VBR) dye.

Table 4
Statistical analysis of dye decolorization (%) of VBR

R-squared	0.9966
Adj R- squared	0.9916
Pred R-squared	0.9761
Adeq R-squared	32.649

3.6 Optimum Results from Design Expert

The predicted optimum dye decolorization percentage, as generated by Design Expert (Table 5) was 59.1212 mg/L, where the amount of nanoflower is 0.18g, the initial concentration of Victoria Blue R (VBR) dye is at 0.02 mg/mL, the pH level is at 6.00, the sonication time at 39.66 minutes, and the temperature at 32.34 °C. To validate the model developed by the Design Expert, an additional confirmation experiment was conducted under these optimal conditions. As shown in Table 5, the experimental result obtained was 61.35%, while the predicted value was 59.1212%. The difference between the experimental and predicted values was minimal, with only a 0.04% error more than the predicted value. This reflects the accuracy of 99.96% between the experimental and predicted results. This high level of accuracy shows that two-level factorial analysis was highly effective in predicting the dye decolorization of Victoria Blue R (VBR) dye by GPW-hNFS.

Table 5
Comparison of dye decolorization of VBR between the predicted and the experimental values at optimized conditions

Response	Experimental	Predicted
Dye decolorization	61.35 %	59.1212 %

*Amount of nanoflower = 0.18 g; Concentration of VBR = 0.02 mg/mL; pH Level 6.00; Sonication time= 39.66 minutes; Temperature = 32.34 °C

4. Conclusions

In the present study, a two-level factorial design screening was successfully utilized to determine the significant factors influencing dye decolorization, which are the amount of nanoflower (A), the initial dye concentration (B), and the interaction between the amount of NF with pH value (AC). From the ANOVA result obtained, the amount of NF (A), initial dye concentration (B) and interaction AC are very significant ($p < 0.05$). The highest dye decolorization was achieved at 59.1212% at the optimal extraction condition generated by the Design Expert with 0.18 g of NF, 0.02 mg/mL of dye

concentration, pH level 6.00, 39.66 minutes sonication time, and 32.34 °C temperature. In the adsorption experiment, the dye concentration is directly proportional to the absorbency and follows Beer-Lambert's law. The optimized condition was proved to have an accuracy of 99.96% as the experimental results show highest dye decolorization at 61.35 %. In conclusion, the dye decolorization of Victoria Blue R (VBR) dye is suitable to test the efficacy of the GPW-hNFs in dye decolorization application. In this study, the results obtained align with the trends observed in previous studies, confirming the effectiveness of hybrid nanoflower to decolorize dye. Nevertheless, future studies should focus on optimizing the parameters using Response Surface Methodology (RSM) to find their optimal level in order to maximize the decolorization response.

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