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Effect of Pretreatment of Biosorbent in Biosorption: A Comparative Study

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Abstract

The textile industry generates large amounts of wastewater with strong BOD/COD and salt load, which are often seen in dark colour. An alternative treatment for this type of wastewater is the biosorption, where it involves a passive uptake of both the organic and inorganic compounds, including dye or its derivatives, using non-growing/living microbial mass. Biosorbent pretreatment, autoclaving techniques and combined with the chemical processes, such as acidification, were discussed. The response surface methodology (RSM) is used for researching and developing the effect of pH, contact time, dosage, and biosorbent size in the biosorption process in synthetic textile wastewater using *Bjerkandera adusta*. When the pH was 4, and the contact time, biosorbent dosage and biosorbent size were 90 minutes, 3000 mg/L and 0.4 mm, respectively, the optimal removal circumstance was able to be verified, at 53.55%, and 81.3% of colour removals were demonstrated through the experimental procedure. This leads to the high acceptance of the experimental findings and model forecast. In the optimisation of experimental parameters, the quadratic model estimated both R^2 and R^2_{adj} correlation coefficients quite satisfactorily as 0.988, 0.977, 0.926, and 0.783, respectively. It is more effective to combine the autoclaving technique with chemical processes than adopting just the autoclaving method. The two-sided t-test was used to identify any significant variations in the preparation techniques of biosorbents using $p < 0.05$. The biosorbent study using scanning electron microscopy (SEM) and characterisation of surface functional group using Fourier-transform infrared (FTIR) spectroscopy confirms the results obtained.

Keywords: *Bjerkandera adusta*, Synthetic textile wastewater, Biosorbent, Response surface methodology, Colour removal efficiency

1 Introduction

The textile dyeing process uses a large amount of water, making it one of the biggest liquid pollutant generators (1, 2). Towards the turn of the decade, the world population is estimated to be 11 billion due to its exponential growth from only 1 billion in the 1800s to more than 7 billion today. With a per capita intake of textiles and clothing of 7 kg per person per year, the global annual demand for textile products has exceeded 49 billion kg (3), where the discharge is between 115–175 kg COD/ton of completed textile (4). Manufacturing license in Malaysia (Malaysia Investment Performance, MIDA, 2011) has documented a total of 662 licensed textile and 1,000 clothing mills on a small scale. The treatment of wastewater produced by textile companies creates an important environmental challenge, particularly in terms of the expense of quality of the resulting textile effluent (5). During the dyeing process, textile dyeing factories use adequate quantities of different colours or additional chemicals and ultimately emit robust textile wastewater (6,7). Azo dyes currently reflect the better standard of synthetic dyes by global standards (around 70 percent), and their large-scale production may be related to their use by various manufacturing sectors (8). Various techniques for treating textile waste are being adopted, including physical-chemical methods, such as adsorption, filtering, biological processes, chemical flocculation, coagulation, and sedimentation (9). Although most of these approaches are highly effective, some are rather expensive and produce sludge that requires further treatment (e.g., sludge treatment). The ability of fungi to remove a large selection of contaminants that are intransigent, such as synthetic dyes, has been approved (10,11). The highest rate of degradation was recorded for *Bjerkandera adusta*, whose strain could completely decolourise a large number of colourants and detoxify synthetic wastewaters (7). *Bjerkandera adusta*, *Phanerochaete chrysosporium*, and *Pleurotus ostreatus* exhibited the best decolourisation properties (12), while Heinling et al. (1997) stated that *Phanerochaete chrysosporium*, *Trametes versicolor*, and *Bjerkandera adusta* had demonstrated their capability to decolour all of the colours tried (13). Through its production of ligninolytic enzymes, *Bjerkandera adusta* indicated that this strain could be beneficial for biotechnological advancement (14). Some methods of pretreatment may enhance the adsorption capacity of biomass materials. Other techniques of pretreatment include autoclaving,

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which might break the structure of fungi and reveal the potential colour of the binding sites (15).

Response surface methodology (RSM) was chosen as the analytical instrument for optimising multiple variables and predicting better performance. The method allows the determination of optimal conditions under predetermined factor preferences, such as high removal at the optimal condition and/or with the least number of experiments (16).

The main aim of this research is to assess the effects of pH, biosorbent dosage, contact time, and biosorbent size in the biosorption process in synthetic textile wastewater using *Bjerkandera adusta* via two pretreatment methods of biosorbent; for instance, the process of autoclaving and combining autoclaving with chemical processes. RSM was applied, as it was among the most sophisticated and appropriate variable techniques used in analytical advancement. RSM was used for planning experiments, building models, and defining the optimal conditions.

2 Methods

2.1 Synthetic Textile Effluent

The following contents of the synthetic wastewaterwere used to test the performance: K_2HPO_4 0.58 g/L, NH_4Cl 0.16 g/L, KH_2PO_4 0.23 g/L, $MgSO_4 \cdot 7H_2O$ 0.09 g/L, $CaCl_2 \cdot 2H_20$ 0.07 g/L, trace solution 1 ml/L, and EDTA 0.02 g/L. The carbon sources considered in this experiment were: sodium acetate (0.5 g/L), glucose (0.5 g/L) and ethanol (0.125 g/L). The trace element was composed of $FeCl_3 \cdot 4H_2O$ (1.5 g/L), H_3BO_3 (0.15 g/L), $MnCl_2 \cdot 4H_2O$ (0.12 g/L), $ZnCl_2$ (0.12 g/L), $CoCl_2 \cdot 6H_2O$ (0.15 g/L), $CuCl_2 \cdot 2H_2O$ (0.03 g/L), $NaMoO_4 \cdot 2H_2O$ (0.06 g/L), and KI 0.03 g/L (17). For this study, the azo dyes were mixed at a concentration of 60 mg/L consist of Reactive Black 5, Reactive Blue 4 and Disperse Orange 2.

2.2 Pretreatment of Biosorbents

The *Bjerkandera adusta* fungi were cultivated in a static condition on malt extract agar (prepared in the laboratory following the DMZS media number 90 description) at a pH of 5.6 and a temperature of 25°C. At the end of 7 days (18), when sporulation had taken place, the fungal biomass was autoclaved to eliminate the fungal biomass at 121°C and 103.42 kPa for 45 min (19). Pretreatment method A (autoclaved) the autoclaved non-viable biomass was properly washed with distilled water and dried for 36 hours at 70 ± 1°C in the oven. Then, the dried fungal biomass was crushed into a powder form and sieved to a granular size between 0.2–0.6 mm. Next, it was transferred to 50 mL of 0.1 M HCl of autoclaved non-viable biomass (about 10.0 g) for pretreatment method B (a combination of autoclaved-chemical methods), and the mixture was stirred at 250 rpm and room temperature for 1 h (20). Subsequently, the non-viable acid-treated biomass was appropriately washed with distilled water, and then powdered in pretreatment method A.

2.3 Batch Testing

A batch testing was conducted by stirring 50 ml of synthetic textile wastewater together with the biosorbents in a flask at a room temperature of 25°C ± 2°C and shaking speed of 160 rpm (21). After stirring the flask for predetermined time intervals, the flasks were extracted and resuspended to obtain the liquid-solid separation prior to its analysis. In addition to the biosorbent used in this work, the SEM (TM3000 Hitachi High Technologies America, USA) was used to study the morphological structure, whereas FTIR spectroscopy (Nicolet iS10 FT-IR Spectrometer, USA) was used to investigate the chemical characterisation.

2.4 Experimental Design

RSM was used to determine the relationship between colour removal and the critical variables (e.g., pH, biosorbent dosage, contact time, and biosorbent size). In this experiment, a four-factor with a five-level CCD was used, which required almost 30 studies. The factors were: A: pH (4–8); B: biosorbent dosage; C: contact time (30–150 minutes); and D: biosorbent size (0.2–0.6 ± 0.05 mm). The experiments were selected randomly for statistical evaluations, and every sample was duplicated using a software package called the Design-Expert Version 11 (Stat-Ease, Statistical Made Easy, MN, USA).

3 Results and Discussion

The RSM can be in the form of a variety of mathematical and applied mathematical approaches. RSM promotes the practice of experimental data-based polynomial equations, which must characterise the knowledge conduct set with the goal of creating relevant mathematical forecasts (22). The expected value of information was achieved in this research by using the Design-Expert software to fit the model technique. After combining this information with distinct models such as cubic, ANOVA demonstrates that all responses were better represented by the mathematical quadratic polynomial model (Table 1).

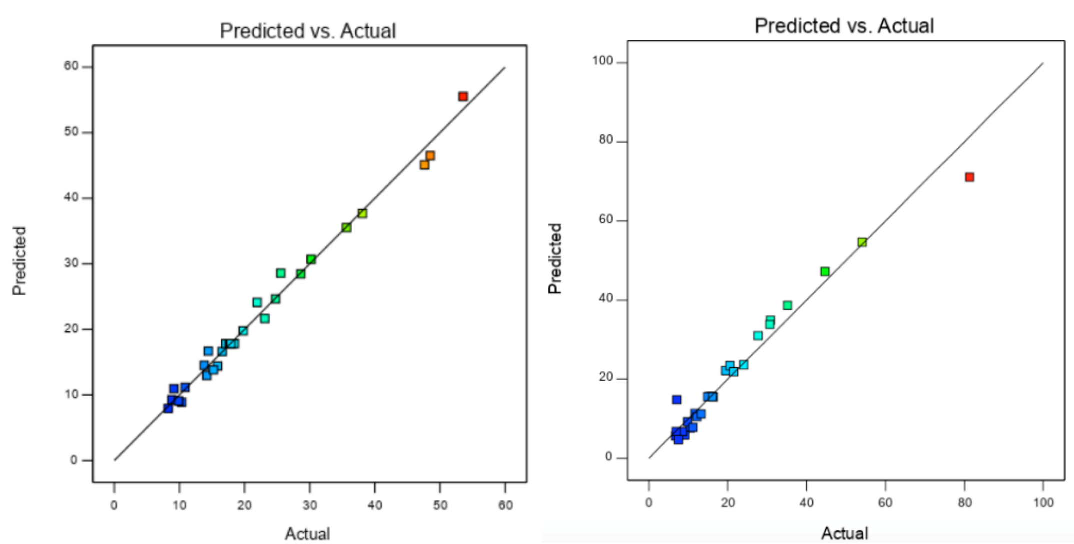
The predicted R^2 value for pretreatment method A of 0.9345 is based on the rational consensus with the adjusted R^2 value equivalent to 0.9776; this implies that the difference is below 0.2 (23). In addition, sufficient accuracy also tests the signal-to-noise ratio. A ratio of above 4 is ideal in this circumstance, where it sets a satisfactory signal by a ratio value equivalent to 37.058. This model can, therefore, be used to manage research methods (Figure 1). The predicted R^2 value of 0.7820 is also reasonably under the arrangement, with the adjusted R^2 of 0.9266 for pretreatment method B, at which the difference is less than 0.2. The 21,461 ratio shows an appropriate signal; hence, this model is suitable to be used for navigating the design room.

The highest colour removal was 53.55% and 81.3% for biosorbent preparation methods A and B, respectively, based on the experimental results. This condition is achieved when the pH, contact time, biosorbent dosage, and biosorbent size were 4, 90 minutes, 3000 mg/L, and 0.4 mm, respectively. The primary means of comparison is the percentage difference between biosorbent preparation methods A and B. A research by Farah., J et al. (2007) using baker's yeast as iosorbent (dried-autoclaved pretreatment) has achieved approximately 80% removal of Astrazon Blue in a contact time of 4 h, shaking speed of 150 rpm, biosorbent dosage of 6600 mg/L, initial pH of 7, and biosorbent size of 0.210 mm (24).

Table 1: Variance analysis (ANOVA) and model statistics discovered using *Bjerkandera Adusta* from Dye Biosorption Process

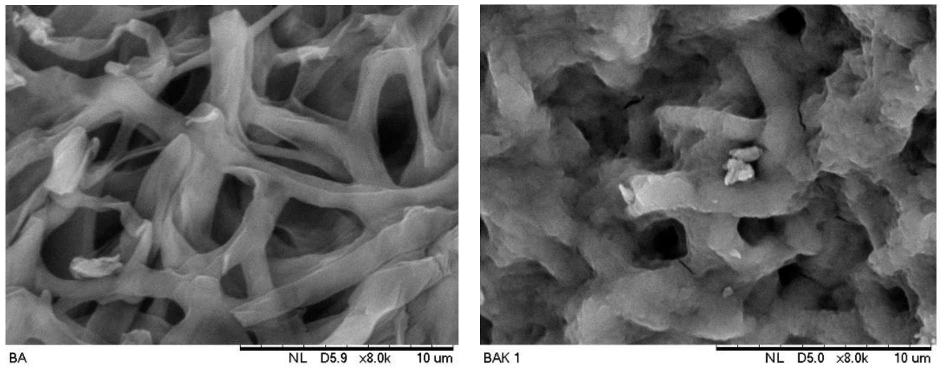
Source	Pre-treatment Methods A*)					Pre-treatment Methods B				
	Sum of square	DF	Mean square	F-value	P-value	Sum of square	DF	Mean square	F-value	P-value
Model	4223.68	14	301.69	91.5	< 0.0001	7282.5	14	520.18	27.14	< 0.0001
A-pH	2953.93	1	2953.9	895.88	< 0.0001	4746.9	1	4746.9	247.70	< 0.0001
B-Weight Biosorbent	570.57	1	570.57	173.04	< 0.0001	444.71	1	444.71	23.20	0.0002
C-Contact Time	14.41	1	14.41	4.37	0.054	37.68	1	37.68	1.97	0.1812
D-Size of Biosorbent	84.15	1	84.15	25.52	0.0001	231.57	1	231.57	12.08	0.0034
AB	103.43	1	103.43	31.37	< 0.0001	202.71	1	202.71	10.58	0.0054
AC	1.69	1	1.69	0.5125	0.485	14.04	1	14.04	0.7328	0.4054
AD	66.02	1	66.02	20.02	0.0004	154.44	1	154.44	8.06	0.0124
BC	0.6889	1	0.6889	0.2089	0.6542	12.66	1	12.66	0.6604	0.4291
BD	7.59	1	7.59	2.3	0.15	19.87	1	19.87	1.04	0.3247
CD	0.555	1	0.555	0.1683	0.6874	7.22	1	7.22	0.3769	0.5485
A^2	412.39	1	412.39	125.07	< 0.0001	1283.1	1	1283.1	66.95	< 0.0001
B^2	1.71	1	1.71	0.5199	0.482	9.36	1	9.36	0.4882	0.4954
C^2	0.2812	1	0.2812	0.0853	0.7743	11.64	1	11.64	0.6076	0.4478
D^2	11.02	1	11.02	3.34	0.0875	5.52	1	5.52	0.2878	0.5995
Residual	49.46	15	3.3			287.47	15	19.16		
Lack of Fit	48.35	10	4.83	21.76	0.0017	286.24	10	28.62	116.63	< 0.0001
Pure Error	1.11	5	0.2221			1.23	5	0.2454		
Cor Total	4273.14	29				7570.0	29			

*) Astuti and Muda, (2018)



(a) **(b)**

Figure 1: Graph of The Predicted Vs. Actual Color Removal Process Using *Bjerkandera Adusta* (a.) Methods A and (b.) Methods B



(a) **(b)**

Figure 2: (a) Methods A and (b) Methods B biosorbent process magnification powers (8000X)

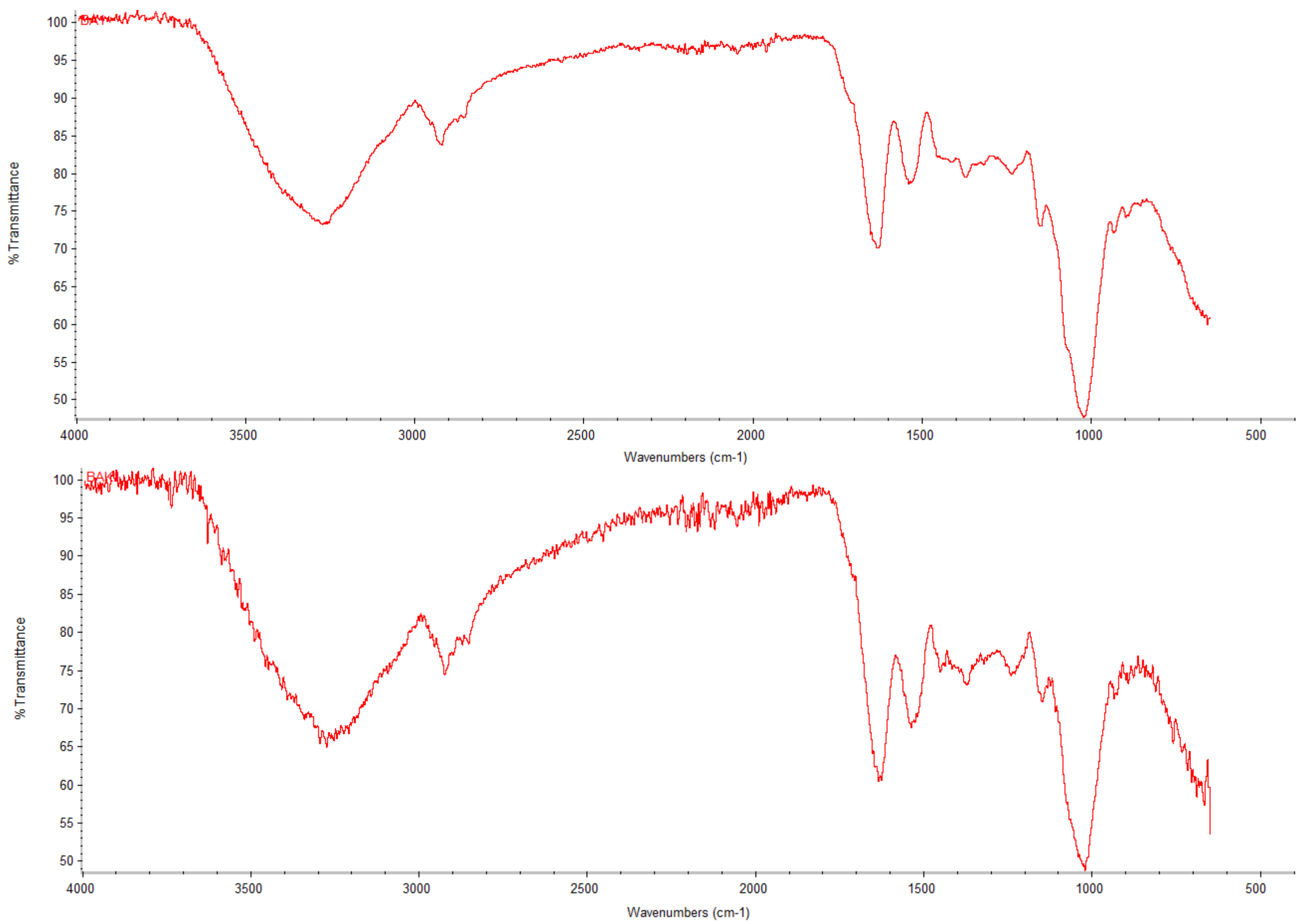


Figure 3: FTIR spectrum of biosorbent *Bjerkandera adusta* Methods A and Methods B

The initial concentration of Astrazon Blue (single pure dye) used was 100 mg/L. Meanwhile, Khalaf 's research findings showed that the dried-autoclaved pretreatment of biomass *A. niger* and *Spirogyra sp*. has a total dye removal of 88% and 85%, respectively, at a pH of 3, the temperature of 30°C and biosorbent dosage of 8000 mg/L after an 18-h contact time (25). Compared with the findings of this study, other researchers required higher doses of biosorbents and longer contact time.

Worku and Sahu (2014) had also carried out the colour removal work with various adsorbent pretreatment methods using rice husk. It was found that the maximum dye removal was 94% at pH 2; adsorbent dose 50,000 mg/L; dye initial concentration 100 mg/L; and a contact time 160 minutes for the physicochemical treatment of rice husk and 73% at an adsorbent dosage of 50,000 mg/L, pH 2, dye initial concentration 50 mg/L, and 80 minute of contact time for the physically treated rice husk

(26). The colour removal is quite high compared to this result, but it required a higher dose of adsorbent (i.e., 50,000 mg/L). However, a research conducted by Worku and Sahu is in line with the findings of this study, where the adsorbent pretreatment method had resulted in higher colour removal. This result is consistent with the results of previous studies, whereby it was learnt that autoclaving can break down the structure of fungi and expose the potential binding sites for dyes, while the pretreatment of acid can change the surface of negatively charged fungal biomass to become positively charged and thus, increasing the attractiveness between the fungal biomass and the colourants/dyes (15).

A t-test is a type of descriptive statistics used to assess whether the two group means differ, which can be attributed to specific characteristics. Two-sided t-tests were used to identify any crucial differences between the preparation methods, autoclaving, and autoclaving techniques in combination with the chemical process, and colour removal efficiencies at $p < 0.05$. The findings showed a significant difference between the means of the methods, as the value of t calculated had exceeded the t table.

The SEM analysis was carried out to clarify the morphological characteristics, along with the surface characteristics of the adsorption samples. The analysis also modelled the surface porosity and structure/texture of the processed biosorbent. In addition, the adsorption capacity relies on the particle size, distribution of pores, and surface area or porosity of micro/mesoporous materials (27).

Biosorbent *Bjerkandera adusta* had undergone the SEM analysis at a power magnification of 8000× and 2000× before and after the biosorption process, respectively. After some interaction with the pollutant dye, the controlled and treated biosorbents were monitored and confirmed to have experienced some changes, such as surface structure and size of pores. The size of the biosorbent pores *Bjerkandera adusta* method A was between 2.74–8.30 μm and changed to 1.31–2.14 μm after biosorption. Meanwhile, the size of the biosorbent pores *Bjerkandera adusta* method B had varied from 2.55–4.81 μm and became 1.14–4.25 μm after biosorption. The dye deposits between biosorbents *Bjerkandera adusta* are indicated as crystalline. Therefore, the SEM results showed the most excellent bond with the colour being explored. These findings support the previous research by Mahmoud et al. (2017) using baker's yeast (28). The biosorbent pores *B. adusta* method B has a pore size smaller than those in method A. Small pore size expands the surface of the adsorption to produce better colour removal (Figure 2).

Biosorbent functional group *Bjerkandera adusta* was evaluated using FTIR spectroscopy to detect functional groups that could bind to the synthetic textile wastewater with dyes. The sample of adsorbent IR spectrum was captured between 4000–400 cm^{-1}. Figure 3 displays the biosorbent pretreatment methods A and B in the FTIR spectra, where it can be seen as identical. As for the spectra obtained for biosorbent pretreatment methods A and B, the bands have shown between 3800–3700 cm^{-1} could be due to the (O-H) vibrations in alcohol and phenol groups in hydroxyl groups, while the latter might suggest that the hydrogen-bonded OH is chemisorbed to water (29). The O-H stretching adsorption band in the structure of crystal of the biosorbent is also detected between 3200–3000 cm^{-1}, indicating the presence of an OH group. From Figure 3, the most prominent bands can be found at 3800, 3200, 2900, 1600, 1500, 1100, and 1000 cm^{-1}. A weak absorption peak can be seen at 2920 cm^{-1}; however, it typically indicates aliphatic groups (symmetric and asymmetric stretch/CH_3 vibration). A cluster of water molecules was assigned to free the hydroxyl on biosorbents *Bjerkandera adusta* represented by the peak at 1640 cm^{-1}. The peak at 1160 cm^{-1} may imply the presence of a C-O stretching band of alcohol, hydroxyl, or ether. The intensity of the peaks gradually increases in the pretreatment of combining autoclave and chemical processes.

Some new peaks were detected at 1800 cm^{-1} and 1450 cm^{-1}; this datum shows that in pretreatment method B, the carboxyl groups have appeared, implying that the combination process has succeeded. The findings indicate that the biosorption process was affected by the presence of functional groups on the biosorbent *Bjerkandera adusta* surface. The results of this research are similar to those outlined by Mahmoud (28) and K. Jain (30). The FTIR and SEM analyses also confirmed the dye-biosorbent interactions and found that functional groups such as carboxyl, amine, amide, and hydroxyl on the surface of biosorbent were liable for the biosorption of a reactive dye (RB49) (31).

4 Conclusion

A study was carried out to investigate the effects of pH, biosorbent dosage, contact time, and biosorbent size in the biosorption process with *Bjerkandera adusta* in synthetic textile wastewater using two methods of biosorbent pretreatment, i.e., autoclaving and combination of autoclaving with chemical processes. Research findings showed that variables, such as pH, biosorbent dosage, and biosorbent size, were the key factors that influenced colour removal in the biosorption with *Bjerkandera Adusta*. Experimental values are as expected under optimal conditions, demonstrating the suitability of the model and the achievement of RSM in optimising the state of the biosorption process using *Bjerkandera adusta*. The two-sided t-test was used with $p < 0.05$ to identify the important differences in the biosorbent pretreatment technique, in which it was found that the best biosorbent preparation method was the combination of autoclaving with chemical processes. The assessment of SEM and FTIR analyses has led to the conclusion that the colour removal technique was conducted via the biosorption process.

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