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ARAŞTIRMA MAKALESİ

RESEARCH PAPER

Microbial Decolorization of Reactive Violet 1: Biosorption Optimization by Central Composite Design

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Abstract: Biological treatment of wastewater containing dyes utilizing aerobic, anaerobic, or combined aerobic-anaerobic biodegradation techniques is often utilized due to its relative affordability and lack of hazardous consequences. Different microorganisms including bacteria, fungi, and algae have been preferred to decolorize the different dyes Up to 10% of the dyes used in the textile industry remain as wastewater, and wastewater containing this dye must be treated appropriately before mixing with natural spring waters. The objective of variety search is to identify the factor combination with the highest desirability combination. Desirability functions are frequently employed in RSM optimization. In this study, four bacterial biomasses have been tested for their capacity to decolorize dyes. Screening studies included four distinct biomasses and four distinct reactive dyes. The bacterial strain #288 has a biosorption yield of about 70% on Reactive Violet 1. Response Surface Methodology classified under the central composite design was employed to maximize the biosorption percentage and biosorption capacity. At the end of the studies, Reactive Violet 1 was adsorbed by #288 with 93.7% biosorption yield and the biosorption capacity was estimated to be 325.7 mg/g. Fourier Transmission Infrared Spectroskopi and Scanning Electron Microscopy were used to characterize the untreated and dyeloaded biomass. In conclusion, strain #288 was shown to be an effective biomass in removing Reactive Violet 1 from textile wastewater.

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Keywords: Biosorption, environmental treatment, reactive azo dye, response surface methodology...

Reaktif Mor 1'in Mikrobiyal Dekolorizasyonu: Merkezi Kompozit Tasarim İle Biyosorpsiyon Optimizasyonu

Öz: Aerobik, anaerobik veya kombine aerobik-anaerobik biyolojik degradasyon teknikleri boya içeren atık suların iyileştirilmesinde oldukça sık kullanılmaktadır. Boya gideriminde fungus, bakteri ve alglerin yer aldığı mikroorganizmalar tercih edilmektedir. Tekstil endüstrisinde kullanılan boyaların %10'u atık su olarak kalmakta ve bu boya içeren atık suların doğal kaynak sularına karışmadan önce uygun şekilde iyileştirilmesi gerekmektedir. Bu alanda yapılan çalışmaların asıl amacı, iyileştirmede etkili faktörlerin kombinasyonlarını deneyerek en yüksek giderimi sağlayan istenirlik fonksiyounna ulaşmaktır. Bu istenirlik fonksiyonları da Yanıt Yüzey Metodolojisi optimizasyonunda sıklıkla kullanılmaktadır.

Bu çalışmada, dört bakteri biyokütlesinin renk giderimindeki kapasiteleri test edilmiştir. Tarama çalışmaları, dört farklı biyokütle ve dört farklı reaktif boya içermektedir. Bakteriyel suş #288'in, Reaktif Mor 1'de yaklaşık %70'lik bir biyosorpsiyon verimine sahip olduğu bulunmuştur. Biyosorpsiyon yüzdesini ve biyosorpsiyon kapasitesini maksimize etmek için merkezi kompozit tasarım altında sınıflandırılan Yanıt Yüzey Metodolojisi kullanılmıştır. Çalışmalar sonucunda Reaktif Mor 1 %93,7 oranında #288 kodlu bakteriyel suş tarafından adsorbe edilmiş ve biyosorpsiyon kapasitesi 325,7 mg/g olarak hesaplanmıştır. Muamele edilmemiş ve boya yüklü biyokütleyi karakterize etmek için Fourier Transmission Infrared Spektroskopi ve Taramalı Elektron Mikroskobi kullanılmıştır. Sonuç olarak, #288 suşunun tekstil atık sularından Reaktif Mor 1'i gidermede etkili bir biyokütle olduğu gösterilmiştir.

Anahtar kelimeler: Biyosorpsiyon, çevresel iyileştirme, reaktif azo boya, yanıt yüzey metodolojisi.

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INTRODUCTION

Biological treatment of wastewater containing dyes using aerobic, anaerobic, or combination aerobic-anaerobic biodegradation processes is frequently used due to its relative affordability and lack of harmful byproducts. A variety of microorganisms such as bacteria, fungi, and algae were utilized to decolorize and mineralize various colors (Roy et al., 2018). They have been identified as potential adsorbents for removing dyes, metals, aromatic compounds, insecticides, and other pollutants (Waheed et al., 2021). The biological adsorbents could be called biosorbents. They have a cheap operating cost, are non-hazardous, can regenerate, and have a high pollution intake capability. Many dye particles, metals, and other pollutants can be used as carbon or nitrogen sources by pure and mixed cultures of bacterial species (Khan et al., 2013; Pathak et al., 2015).

Organic dyes are widely utilized in the textile, paper, and paint industries, and it is estimated that 10,000 distinct textile dyes are accessible globally, with an annual production of approximately 7x109 tons (Benkhaya et al., 2020). Up to 10% of applied dyes in the textile sector do not bond to the fabrics and are eliminated as colored wastewaters, which must be adequately treated before discharge into different water bodies (Al-Zawahreh et al., 2021). Because of their high visible light absorption, colored pollutants have a deleterious impact on aquatic life's photosynthetic activity (Al-Zawahreh et al., 2021; Ribas et al., 2014). The one class of organic dyes is reactive dyes which are a highly successful family of synthetic dyes because of their simplicity of application, outstanding wetfastness qualities, reasonable price, and brilliance in hues. Reactive dyes have gained popularity due to their great wetfastness, brilliance, and color range. Many reactive dye molecules with mono or hetero-reactive groups are referred to as bifunctional dyes; these dyes have good fastness properties and a greater fixation value (Patel et al., 2023).

Response Surface Methodology (RSM) is a regression-based strategy that consists of a combination of mathematical and statistical methods for expressing the effects of specified factors on response variables. Central Composite Design (CCD) is one of the RSM techniques. Desirability functions are commonly used in RSM optimization, and the goal here is to find the factor combination with the highest desirability combination. Many researchers in biological processes have achieved successful findings using the RSM technique (Öge et al., 2023). However, it has been discovered that most statistical software is insufficient in approximating the global optimum outcome.

Different structures of synthetic dyes are frequently used in the textile industry during fiber processing, the

effluents produced are markedly variable in chemical composition, including organics, nutrients, compounds, salts, and various toxic substances (Chen et al., 2003; Ghodake et al., 2009). Various physicochemical operational parameters, such as agitation, oxygen, temperature, pH, dye structure, dye concentration, supplementation of different carbon and nitrogen sources, electron donor, and redox mediator, all have a direct impact on the bacterial decolorization performance of azo dyes in biological treatment processes. Thus, previous identification of the effect of each element on the bacterial decolorization of azo dyes is required to make the process more effective, quicker, and realistically applicable (Saratale et al., 2011).

In light of this knowledge, it was considered that the optimization of Reactive Violet 1 to maximize the biosorption and capacity in this research. In this context, central composite design from response surface methodology was used in optimization studies. The three levels of environmental parameters were studied. The characterization of biomass before and after biosorption by FTIR and SEM was carried out.

Scientific Publication Screening: There are some studies on the decolorization of Reactive Violet (RV) dyes in the literature. Moosvi and his colleagues utilize a bacterial mix culture to decolorize RV5. The decolorization rate was reported to be 94% after 37 hours under a wide range of pH (6.5-8.0) and temperature (25 °C - 40 °C) conditions (Moosvi et al., 2005). Jain and his colleagues investigated the decolorization of RV5 by a bacterial consortium. After 40 hours, the decolorization efficiency was determined to be 90%. The reactive dye was then characterized using several approaches (Jain et al., 2012). The haloalkaliphilic strain was employed by Prabhakar and colleagues to decolorize the RV1. To achieve the best decolorization rate, Box-Behnken from Response Surface Methodology was used. In 51 hours, the bacterium eliminated 96.33% of the RV1 (Prabhakar et al., 2019). Prabhakar and colleagues utilized the same haloalkaliphilic strain to demonstrate the effect of textile industrial solvent on RV1 elimination. However, the maximum decolorization rate obtained with alone dye was 96.8%. Other solvents had a negative impact on the removal reaction (Prabhakar et al., 2019).

MATERIAL AND METHOD

Biological Adsorbents: Four microorganisms were used as adsorbents isolated from industrial wastewater and deposited in glycerol stock in the laboratory. Bacterial strains were coded to be #288, #351, #201, and #HE1. Then every microorganism was incubated in Nutrient Broth with 100 ml. After inoculation of 10%, the microorganism cultures were incubated at 35 °C by shaking 150 rpm in 100

ml x 10 Erlen Mayer (Lab Companion SI 600R, USA). At the end of incubation, the cultures were centrifuged at 5000 rpm for 10 min (Beckman Coulter, Allegra X-30). The supernatants were removed, and pellets were left to dry at 50 °C. The dried pellets were crushed in mortar then their weighing was done.

Adsorbates: Four textile dyes including Reactive Blue 72 (RB72), Reactive Orange 12 (RO12), Reactive Violet 1 (RV1), and Reactive Yellow 85 (RY85) taken from a local textile company were used as an adsorbate. Before application, the dyes' maximum absorption wavelengths were determined, and all concentrations were prepared based on the standard curves of dyes. All spectrophotometric readings were practiced by the CPS Controller UV-VIS Spectrophotometer (Schimadzu UV-2550; Kyoto, Japan). The optimum wavelength enabled for RB72, RO12, RV1, and RY85 was 628 nm, 418 nm, 558.5 nm, and 422 nm, respectively.

Screening of dye biosorption: In dye biosorption studies, four textile dyes (RB72, RO12, RV1, RY85) were treated by four bacterial isolates coded with #288, #351, #201 and #HE1. The experimental setup was designed in a 25 mL working volume with a pH 2 dye solution (Akar et al., 2009). 2.5 mg (0.1 g/L) biomass was used, and incubation was completed at 25 °C by 150 rpm for 30 min. The initial dye concentrations were prepared as 50 ppm. The experiments were practiced in triplicate. At the end of the experimental steps, biosorption % and biosorption capacity were calculated based on Equation 1 (Razmovski et al., 2008) and Equation 2 (Wang et al., 2016).

C₀=initial dye concentration

C_t= dye concentration at "t" time

Biosorption % =
$$\frac{C_0 - C_t}{C_0} x 100$$
 (1)

C₀= initial dye concentration

C_t= dye concentration at "t" time
q=biosorption capacity

 $q = \frac{C_0 - C_t}{m} X V$ (2)

Response surface methodology in optimization study for dye biosorption: The central composite design was used to optimize the parameters of incubation time, biomass amount, and initial dye compound concentration for biosorption % and biosorption capacity. Table 1 lists the three factors and their levels.

Table 1. Parameters and their levels.

Parameter	Unit	-1	0	+1
Incubation time (A)	min	30	60	90
Biomass (B)	g/L	0.2	0.6	1
Dye concentration (C)	g/L	50	125	200

The best-fitting models were identified using quadratic regression, in which inconsequential model parameters were removed from the models and only variables with significant values were chosen for model creation using Central Composite Design. Design-Expert version 11.0 (Stat-Ease In., Minneapolis, USA) was used for the computational work, which included the assignment of experimental points, randomization, analysis of variance fitting of the quadratic models and graphical representations, as well as optimization.

Characterization of adsorbent

Fourier Transmission Infrared Spectroscopy: IR spectra in the 4000-400 cm⁻¹ region were obtained using a KBr pellet by Bruker Tensor FT-IR spectrometer (Aytar Celik et al., 2021; Cengiz et al., 2014; Dolphen et al., 2007).

Scanning Electron Microscope: To determine the morphology and to detect the surface element of biomass before and after biosorption was analyzed by FE-SEM (Hitachi Regulus 8230 FE-SEM) (Aytar Celik et al., 2021; Aytar et al., 2016).

Statistical Analysis: The averages and standard deviations of the data are shown (n = 3). Using the package program of JMP (version 6.0.0), analysis of variance was carried out. The Student's t-test was used to establish the significance ratings between the averages (p<0.001 was regarded as significant).

Design-Expert version 11.0 (Stat-Ease In., Minneapolis, USA) was used to analyze experimental data according to the ANOVA test.

RESULTS AND DISCUSSION

Microbial Adsorbent Selection: The bacterial particles were used in the decolorization of dyes. All results have been commented according to statistical analysis. The selection was made based on biosorption efficiency and biosorption capacity. Both the biosorption efficiency and biosorption capacity were calculated and then the values were given in Table 2. Based on the biosorption efficiency and biosorption capacity except for #HE1 and #351 isolates, other adsorbents have the potential to decolorize all dyes. #202 and #288 particles were more significant for decolorization. However, the most biosorption yield belongs to isolate #288 with 69.20% biosorption on Reactive Violet 1. Under this biosorption yield, the biosorption capacity was found as highest value (Table 2).

Optimization study for dye biosorption: The results of the statistical examination of the parameters' effects on biosorption % and biosorption capacity are presented in Table 3. Biosorption yield (%) and biocapacity were computed for each experimental set and evaluated as a response within the scope of experiments in the design matrix.

Table 2. Biosorption % and biosorption capacity of adsorbents.

		RB72	RO12	RY85	RV1
#202	Biosorption (%)	14.74± 0,01 ^b	20.36± 0,04 ^b	21.47± 0,03 ^b	30.52± 0,00 ^b
	Biosorption capacity (mg/g)	$78.83 {\pm}\ 10{,}11^{\mathrm{B}}$	$102.68\pm20,\!86^{\mathrm{B}}$	$106.50 {\pm}\ 18{,}37^{\mathrm{B}}$	$139.86 \pm 3,79^{\mathrm{B}}$
#288	Biosorption (%)	23.75 ± 0.01^a	29.14 ± 0.05^{a}	50.02 ± 0.03^a	$69.20\pm0,17^{a}$
	Biosorption capacity (mg/g)	$127.00\pm6,04^{A}$	146.90± 26,36 ^A	$248.17 \pm 15,48^{A}$	317.18± 79,23 ^A
#351	Biosorption (%)	18.02 ± 0.03^{b}	13.33 ± 0.00^{bc}	$7.81 \pm 0.00^{\circ}$	17.91 ± 0.03^{b}
	Biosorption capacity (mg/g)	$89.33 \pm 18,27^{\mathrm{B}}$	$70.20 \pm 4{,}10^{\mathrm{BC}}$	$38.21 \pm 4,23^{\circ}$	85.22± 17,52 [°]
# HE 1	Biosorption (%)	13.54 ± 0.02^{b}	$8.71 \pm 0,00^{\circ}$	0.75 ± 0.01^{d}	17.40 ± 0.05^{b}
	Biosorption capacity (mg/g)	$67.16\pm12,11^{\mathrm{B}}$	$45.91\pm 2,06^{\circ}$	$3.65 \pm 6,51^{D}$	$82.81\pm28,02^{D}$

^{**}a-d: Within each column, different superscript lowercase letters show differences between the different biosorptions (p < 0.001)

Table 3. Design matrix and analysis results of biosorption % and biosorption capacity under different conditions.

Experiments Number	FACTORS			RESPONSES		
	Incubation time (min) A	Biomass (g/L) B	Dye conc. (g/L) C	Biosorption (%)	Biosorption capacity (mg/g)	
1	30	0.2	50	70	201	
2	90	0.2	50	82	236	
3	30	1	50	100	58	
4	90	1	50	92	55	
5	30	0.2	200	39	400	
6	90	0.2	200	42	424	
7	30	1	200	99	199	
8	90	1	200	99	199	
9	30	0.6	125	93	194	
10	90	0.6	125	95	207	
11	60	0.2	125	58	369	
12	60	1	125	98	123	
13	60	0.6	50	84	80	
14	60	0.6	200	69	231	
15	60	0.6	125	98	205	
16	60	0.6	125	98	205	
17	60	0.6	125	98	205	
18	60	0.6	125	98	205	
19	60	0.6	125	98	205	
20	60	0.6	125	98	205	

The quadratic regression model equations were fitted with the selected parameters. These equations related to parameters were represented in Eq.3 and Eq.4 for biosorption percent and biosorption capacity, respectively, based on the coded levels of these parameters. The basic factors were kept constant for the model. Except this, a dual interaction was found between biomass and dye concentration.

$$Y_{Biosorption~(\%)} = 94.96 + 0.90A + 19.70B - \\ 8.00C + 9.62BC - 8.81B^2 - 10.31C^2$$

$$Y_{Biosorp~cap} = 201.49 + 6.90A - 99.60B +$$
(3)

 $82.30C - 12.75BC + 54.06B^2 - 36.44C^2$

The ANOVA results of the statistical evaluation are shown in Table 4. It shows that the R^2 values for biosorption % and biosorption capacity were found to be very close to the value of 1. However, the best model belongs to biosorption capacity according to R^2 . The

relevance of the model has been checked by determination coefficient (R²). The R² value of 0.9362 and 0.98 depict the acceptable validity to the predicted values for the response, respectively, biosorption % and biosorption capacity. The adjusted R² value of 0.9067 and 0.97 is a reasonable agreement with predicted values. The predicted R² was used to evaluate how well a regression model predicts. Although it is preferred to use biosorption efficiency, the predictability of biosorption capacity turned out to be more reliable. On the other hand, the prediction of biosorption efficiency was acceptable for following experiment.

The parameter that affects the model's response more than the others in regression equations is the one with the highest coefficient. If the coefficient is positive, the parameter has a proportional effect on the response. If the coefficient is negative, the parameter has an inverse effect on the answer. When Eq (3) was examined, it was seen that the parameter that has the most effect on biosorption was

^{*}A-D: Within each column, different superscript uppercase letters show differences between the different biocapacities (p < 0.001)

^{**}Data are presented as mean \pm standard deviation (n = 3)

biomass amount. However, it was found that biomass amount is proportional to the biosorption and inversely proportional to the biosorption capacity, so, as the biomass amount increases, biosorption increases, but biosorption capacity decreases (Hu et al., 2020) (Figure 1). The same

results have been obtained by Hu et al., on Pb²⁺ biosorption from aqueous solutions. As expected, high biomass amount has huge potential to decolorize the dye; but when the biomass decolorizes the dye at the high biomass concentration, it could not absorb all dye to its surface.

Table 4. ANOVA results of statistical evaluation.

	Sources of variations	Degree of freedom	Sum of Squares	Mean square	f -value	p-value		
	Regression model	6	6735.44	1122.57	31.77	0.0001		
	Lack of fit	1	459.36	57.42				
Biosorption	Error	1	0.00	0.00				
	Corrected total	19	7149.80					
	$R^2 = 0.9362$; Adj- $R^2 = 0.9067$; Pred $R^2 = 0.7988$							
Biosorption capacity	Regression model	6	1.781E+05	29690.65	155.37	< 0.0001		
	Lack of fit	8	2484.29	476.10				
	Error	5	0.00	0.00				
	Corrected total	19	1.806E+05					
	$R^2 = 0.98$: Adi- $R^2 = 0.97$: Pred I	$R^2 = 0.9534$						

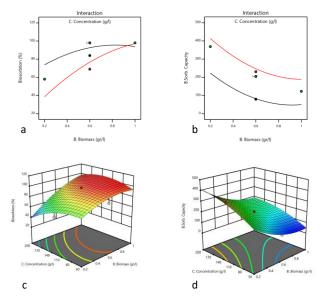


Figure 1: Dual interaction on biosorption % and biosorption capacity. ac: dye concentration and biomass interaction on biosorption %. Two and three-dimensional graphs, respectively. b-d: dye concentration and biomass interaction on biosorption capacity. Two and three-dimensional graphs, respectively.

Verification Experimental: The optimum conditions were determined to maximize both biosorption % and biosorption capacity. Incubation time, 90 min, biomass measurement, 04022 g/L, and initial dye concentration 134.04 g/L, and under these conditions, the model estimated that a maximum of 82.2754% and 281.025 were obtained at the end of biosorption. In the optimum conditions given by the model, biosorption % and biosorption capacity were found as 93.7% and 325.7 mg/g respectively at the end of verification experiments. The two values are very close to the predicted values by the model. This suggest the model is reliable according to these values.

Characterization of Biomasses

The Determination of Functional Group: RV1 may have been biosorbed onto the adsorbent via functional

groups consisting of -SO₃, hydroxyl, amide, and carboxyl groups, according to IR spectra of untreated and dyeloaded biomasses. These findings suggest that the functional groups on the biosorbent surface may be involved in the biosorption process. The functional groups found in the untreated biomass are most likely involved in the biosorption of the dyestuffs under consideration.

Dolphen and coworkers have reported the wavelength of functional group peaks. These wavelengths were identified for functional groups which are identified following. $1558~\rm cm^{-1}$ for -NH₂, $1621~\rm cm^{-1}$ for amide II, $1654~\rm cm^{-1}$ for amide I, $1205~\rm cm^{-1}$ and $1258~\rm cm^{-1}$ for -CNH, $1025~\rm cm^{-1}$ for C-N, $3363~\rm cm^{-1}$ and $3438~\rm cm^{-1}$ for N-H (Dolphen et al., 2007).

As shown in Figure 2, the FT-IR spectra of untreated biomass and dye-loaded biomass were utilized to identify biosorption sites. Three peaks appeared in the range of 3600-4000 cm⁻¹ for untreated and dye-loaded biomasses. The change in the number of peaks at the range of 2000-3600 cm⁻¹ was determined, three peaks increased seven peaks for dye-loaded biomass. At the range of 800-2000 cm⁻¹, it increased the number of peaks. The dye-loaded biomass has more peaks. Amide I functional group was detected for untreated and dye-loaded biomasses at 1653 and 1657 cm⁻¹. -CNH functional group was detected for untreated and dye-loaded biomasses at 1237 and 1231 cm⁻¹. At 2359 and 2367 cm⁻¹, the -OH group was detected for untreated and dye-loaded biomasses, respectively.

The bands found at 898-651 cm⁻¹ following biosorption were linked to biosorption properties for aromatic skeletal groups (Aytar Celik et al., 2021). Furthermore, the 673.6 cm⁻¹ band was found solely in dyeloaded biomass in this study.

Surface Morphology: The surface of the untreated biomass is heterogeneous, smooth, and porous, as seen in the SEM image in Figure 3a-3b. SEM image of the dyeloaded biomass looks as loading rounder and fuller with dye in Figure 3c-3d.

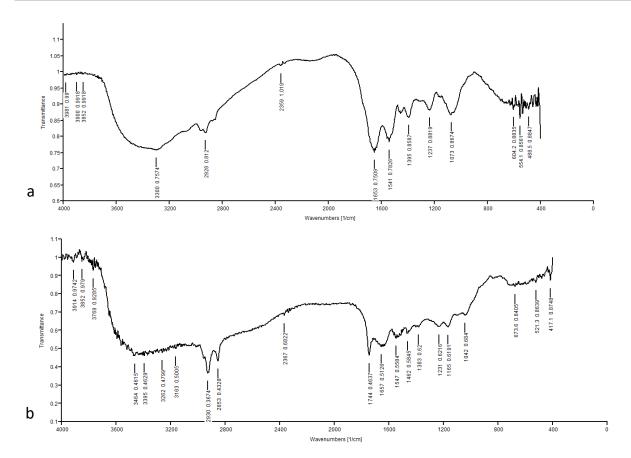


Figure 2: FTIR spectrum, a: FTIR spectra of untreated biomass, b: FTIR spectra of dye-loaded biomass.

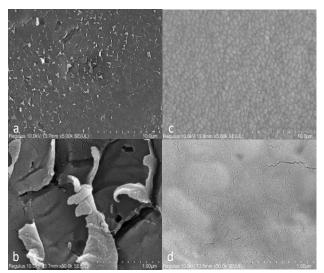


Figure 3: Scanning electron micrograph of untreated and dye-loaded biomass. a-b: untreated biomass at $10~\mu m$ and $1\mu m$ scale. c-d: dye-loaded biomass at $10~\mu m$ and $1\mu m$ scale.

SEM-EDX images present the elemental convent of untreated and dye-loaded biomasses (Figure 4). When the presence percentage of carbon was decreased, the percentage of oxygen and nitrogen was increased. The result has showed that, azo dye (RV1) absorbed by biomass. So mean that, SEM micrographs indicated that #288 can also play a role in the decolorization process.

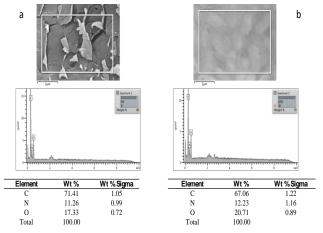


Figure 4 SEM-EDX value and graph. a: untreated biomass b: dye-loaded biomass.

DISCUSSION

To sum up, #288 coded as a bacterial strain was used to decolorize the Reactive Violet 1 in this study. The initial dye concentration, biomass measurement, and incubation time were optimized by Central Composite Design. 134 g/L dye was removed 93% biosorption yield in 90 min using 0.1 g/L biomass. In this condition, biosorption capacity was found as 325.7 mg/g. Untreated and dye-loaded biomasses were characterized by FTIR and SEM. According to IR spectra, characteristic functional

groups were determined for untreated and dye-loaded biomasses. SEM analyzed surface morphologies of untreated and dye-loaded biomasses. On the other hand, levels of carbon, nitrogen, and oxygen were measured by EDX for untreated and dye-loaded biomasses. Based on these results, the comparison with other studies in the literature is outlined below.

Ganoderma cupreum AG-1 was used to decolorize azo dye Reactive Violet 1 in a study by Gahlout and co-workers. The environmental and medium conditions were optimized and took advantage of the presence of ligninolytic enzymes including laccase etc. At lower concentrations, it was found that the percentage of decolorization was higher and might reach up to 98% at the lowest concentration of 0.1 g/L. They analyzed degradation products; therefore, they used control dye and products. Degraded product has major changes in the fingerprint region based on FTIR spectrum (Gahlout et al., 2013).

Bello and his friends studied on adsorption of Remazol Brilliant Violet-5R on carbon derived from cocoa pod husks (CPHAC). They examined the effects of concentration of dye, time, temperature, and pH. Isotherm models were studied to the fitted model. They found Freundlich model for this adsorption. CPHAC was good at acidic pH and at the end of time evaluation, CPHAC is good at adsorbing dye with 97,8% removal at 100 mg/l. On the other hand, they characterized the adsorbent by FTIR, SEM. Characteristic peaks of CPHAC were determined and intermolecular bonds due to high temperature (Bello et al., 2012).

A maximum 93% decolorization of Reactive Violet 5R by the bacterial consortium JW-2 was observed at 37 °C, according to Moosvi and coworkers. They added that the pace at which Reactive Violet 5R dye decolorizes diminishes with a greater increase or decrease in temperature from the ideal (Moosvi et al., 2007)

Zuorro and his friends investigated the adsorption of azo dyes on non-living microalga which was identified as Nannochloropsis oceanica. They chose the Reactive Violet 5 (RV5) as a dye that is used widely in textile dyeing. At 10, 25, and 40 °C, the kinetics of RV5 biosorption on the biomass were researched. The Temkin equation was found to accurately reflect equilibrium data. Then the kinetic model was fitted to experimental setups. Nannochloropsis oceanica biomass removed above 95% of dye in a few hours. In summary, the results show that Nannochloropsis oceanica's non-living cells can be a successful biosorbent for the treatment of textile wastewaters including azo dyes. The biomass has been evaluated by FTIR before and after dye adsorption. The standard peaks were determined according to the literature. They have decided the peaks belonging

methyl/methylene groups, Amide I, Amide II, carbohydrates, and hydroxyl/ether (Zuorro et al., 2017).

Mohammad Hanapi et al., have been reported to remove colors from the textile dye effluents by *Bacillus cereus*. In order to establish the ideal treatment process conditions for the wastewater degradation of textile dyes, Central Composite Design (CCD) from Response Surface Methodology (RSM) was utilized for this content. With low pH, agitation, a medium initial concentration of bacterial inoculum, and ten days of biodegradation, the maximum decolorization rate was 88.67%. The researchers chose different factors to analyze in RSM. Therefore, the interaction between pH and agitation was found as significant (Mohammad Hanaoi et al., 2021).

Maximum RV1 biosorption (%) onto PSP obtained by means of the Box-Behnken method was approximately 83.22%. The initial dye concentration was chosen as 100 mg/L. The best operating conditions were as follows: pH: 2.0, biosorbent amount: 0.6 g (in 25 mL dye solution), reaction time: 56.9 min, and temperature 27.3 °C. The dual interaction has been reported between pH-temperature; and pH-biosorbent amount (Akar et al., 2016).

Consequently, To the best of our knowledge, the wastewater of textile dye is one of the general environmental problems. Every finding or suggested solution is very important to treat these waters. In this study, four different bacterial biomasses have been evaluated due to their ability for dye decolorization. Four different biomasses and four different reactive dyes were used in screening studies. According to our results, #288 coded bacterial strain has the highest biosorption yield at approximately 70% on Reactive Violet 1. The central composite design from response surface methodology was used to maximize the biosorption % and biosorption capacity. The chosen factors have been determined differently from the literature. Dual interaction has been reported between dye concentration and biomass amount. At the end of experiments, the biosorption of Reactive Violet 1 by #288 was increased to 93.7%, and biosorption capacity was calculated as 325.7. This result shows that #288 is good on adsorption of Reactive Violet 1 from textile wastewaters.

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REFERENCES

- Akar, S.T., Akar, T. & Çabuk, A. (2009). Decolorization of a textile dye, Reactive Red 198 (RR198), by Aspergillus parasiticus fungal biosorbent. *Brazilian Journal of Chemical Engineering*, 26(2), 399-405. https://doi.org/10.1590/S0104-66322009000200018.
- Akar, T., Turkyilmaz, S., Celik, S. & Akar, S.T. (2016). Treatment design and characteristics of a biosorptive decolourization process by a green type sorbent. *Journal of Cleaner Production*, 112, 4844-4853.
- Al-Zawahreh, K., Barral, M.T., Al-Degs, Y. & Paradelo, R. (2021). Comparison of the sorption capacity of basic, acid, direct and reactive dyes by compost in batch conditions. *Journal of Environmental Management*, 294. DOI: 10.1016/j.jenyman.2021.113005
- Aytar Celik, P., Abutaha, A.M.K., Nural Yaman, B., Cakmak, H., Hosgün, S. & Cabuk, A. (2021). Efficient removal of Reactive Orange 13 with magnetic Mucor circinelloides from mill scale. *Desalination And Water Treatment*, 226, 347-361. DOI: 10.5004/dwt.2021.27236
- Aytar, P., Bozkurt, D., Erol, S., Özdemir, M. & Çabuk, A. (2016). Increased removal of Reactive Blue 72 and 13 acidic textile dyes by *Penicillium ochrochloron* fungus isolated from acidic mine drainage. *Desalination and Water Treatment*, 57(41), 19333-19343. DOI: 10.1080/19443994.2015.1098567
- Bello, O.S., Siang, T.T. & Ahmad, M.A. (2012). Adsorption of Remazol Brilliant Violet-5R reactive dye from aqueous solution by cocoa pod husk-based activated carbon: kinetic, equilibrium and thermodynamic studies. *Asia-Pacific Journal of Chemical Engineering*, 7(3), 378-388. DOI: 10.1002/apj.557
- Benkhaya, S., M' rabet, S. & El Harfi, A. (2020). A review on classifications, recent synthesis and applications of textile dyes. *Inorganic Chemistry Communications*, 115, 107891. DOI: 10.1016/j.inoche.2020.107891
- Cengiz, G., Aytar, P., Şam, M. & Çabuk, A. (2014). Removal of Reactive Dyes using Magnetically Separable *Trametes versicolor* Cells as a New Composite Biosorbent. *Separation Science and Technology*, 49(12), 1860-1871. DOI: 10.1080/01496395.2014.903496
- Chen, K.C., Wu, J.Y., Liou, D.J. & Hwang, S.C.J. (2003). Decolorization of the textile dyes by newly isolated bacterial strains. *Journal of Biotechnology*, 101(1). DOI: 10.1016/S0168-1656(02)00303-6
- Dolphen, R., Sakkayawong, N., Thiravetyan, P., & Nakbanpote, W. (2007). Adsorption of Reactive Red 141 from wastewater onto modified chitin. *Journal of Hazardous Materials*, 145(1–2), 250-255. DOI: 10.1016/j.jhazmat.2006.11.026

- **Gahlout, M., Gupte, S. & Gupte, A.** (2013). Optimization of culture condition for enhanced decolorization and degradation of azo dye reactive violet 1 with concomitant production of ligninolytic enzymes by Ganoderma cupreum AG-1. *3 Biotech*, *3*(2), 143-152. DOI: 10.1007/s13205-012-0079-z
- Ghodake, G., Jadhav, S., Dawkar, V. & Govindwar, S. (2009). Biodegradation of diazo dye Direct brown MR by Acinetobacter calcoaceticus NCIM 2890. International Biodeterioration and Biodegradation, 63(4). DOI: 10.1016/j.ibiod.2008.12.002
- Hu X, Cao J, Yang H, Li D, Qiao Y, Zhao J, et al. (2020).

 Pb2+ biosorption from aqueous solutions by live and dead biosorbents of the hydrocarbon-degrading strain Rhodococcus sp. HX-2. *PLoS ONE* 15(1): e0226557. DOI: 10.1371/journal.pone.0226557
- Jain, K., Shah, V., Chapla, D. & Madamwar, D. (2012).

 Decolorization and degradation of azo dye –
 Reactive Violet 5R by an acclimatized indigenous bacterial mixed cultures-SB4 isolated from anthropogenic dye contaminated soil. *Journal of Hazardous Materials*, 213-214, 378-386. DOI: 10.1016/j.jhazmat.2012.02.010
- Khan, R., Bhawana, P. & Fulekar, M.H. (2013).

 Microbial decolorization and degradation of synthetic dyes: A review. In *Reviews in Environmental Science and Biotechnology* (Vol. 12, Issue 1). DOI: 10.1007/s11157-012-9287-6.
- Mohammad Hanapi NH., Sayid Abdullah SHY., Ismail A. & Juahir H. (2021). Central Composite Design: a Response Surface Methodology Approach in Biodegradation of Textile Dye Wastewater. Journal of Korean Society of Environmental Engineers, 43(6), 461-475.
- Moosvi, S., Keharia, H. & Madamwar, D. (2005).

 Decolourization of textile dye Reactive Violet 5
 by a newly isolated bacterial consortium RVM
 11.1. World Journal of Microbiology and
 Biotechnology, 21(5), 667-672. DOI:
 10.1007/s11274-004-3612-3
- Moosvi, S., Kher, X. & Madamwar, D. (2007). Isolation, characterization and decolorization of textile dyes by a mixed bacterial consortium JW-2. *Dyes and Pigments*, 74(3), 723-729. DOI: 10.1016/j.dyepig.2006.05.005
- Öge, E., Nural Yaman, B. & Buruk Şahin, Y. (2023). Optimization of biodegradation yield of reactive blue 49: An integrated approach using response surface methodology based marine predators algorithm. *Journal of Microbiological Methods*, 206, 106691. DOI: 10.1016/j.mimet.2023.106691
- Patel, M. J., Tandel, R. C., Sonera, S. A., & Bairwa, S. K. (2023). Trends in the synthesis and application of some reactive dyes: A review. *Brazilian Journal of Science*, 2(7), 14-29. DOI: 10.14295/bjs.v2i7.350
- Pathak, V.V., Kothari, R., Chopra, A.K. & Singh, D.P. (2015). Experimental and kinetic studies for phycoremediation and dye removal by Chlorella

- pyrenoidosa from textile wastewater. *Journal of Environmental Management*, **163**. DOI: 10.1016/j.jenvman.2015.08.041
- Prabhakar, Y., Gupta, A. & Kaushik, A. (2019). Effect of some Organic co-pollutants on Decolorization of Reactive Violet 1 dye by an Indigenous Microbial Strain from Textile Wastewater. Society for Environment and Development, 159-168.
- Prabhakar, Y., Gupta, A. & Kaushik, A. (2019). Enhanced decolorization of reactive violet dye 1 by halo-alkaliphilic Nesterenkonia strain: Process optimization, short acclimatization and reusability analysis in batch cycles. *Process Safety and Environmental Protection*, 131, 116-126. DOI: 10.1016/j.psep.2019.09.004
- **Razmovski, R. & Šćiban, M. (2008).** Biosorption of Cr (VI) and Cu (II) by waste tea fungal biomass. *Ecological Engineering*, **34**(2), 179-186.
- Ribas, M.C., Adebayo, M.A., Prola, L.D.T., Lima, E.C., Cataluña, R., Feris, L.A., Puchana-Rosero, M. J., Machado, F.M., Pavan, F.A. & Calvete, T. (2014). Comparison of a homemade cocoa shell activated carbon with commercial activated carbon for the removal of reactive violet 5 dye from aqueous solutions. *Chemical Engineering Journal*, 248, 315-326. DOI: 10.1016/j.cej.2014.03.054
- Roy, U., Manna, S., Sengupta, S., Das, P., Datta, S., Mukhopadhyay, A. & Bhowal, A. (2018). Dye Removal Using Microbial Biosorbents. In Green Adsorbents for Pollutant Removal. Environmental Chemistry for a Sustainable World; Crini, G., Lichtfouse, E., Eds.; Springer: Cham, Switzerland, 2018; Volume 19. DOI: 10.1007/978-3-319-92162-4 8
- Saratale, R. G., Saratale, G. D., Chang, J. S., & Govindwar, S.P. (2011). Bacterial decolorization and degradation of azo dyes: A review. *Journal of the Taiwan Institute of Chemical Engineers*, 42(1), 138-157. DOI: 10.1016/j.jtice.2010.06.006
- Waheed, A., Baig, N., Ullah, N. & Falath, W. (2021). Removal of hazardous dyes, toxic metal ions and organic pollutants from wastewater by using porous hyper-cross-linked polymeric materials: A review of recent advances. *Journal of Environmental Management*, 287, 112360.
- Wang, B., Zhou, K., Liu, H. et al. (2016). Biosorption Behavior and Reuse Potential of Waste Biomass of Aspergillus fumigatus, previously Used in Humic Acid Biosorption, in Removal of Reactive Blue 49. *Environmental Process.* 3, 843-856. DOI: 10.1007/s40710-016-0188-5
- **Zuorro, A., Maffei, G. & Lavecchia, R. (2017).** Kinetic modeling of azo dye adsorption on non-living cells of Nannochloropsis oceanica. *Journal of Environmental Chemical Engineering*, **5**(4), 4121-4127. DOI: 10.1016/j.jece.2017.07.078