



Research Article

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OPTIMIZATION OF REMOVAL EFFICIENCY AND ABSORPTION CAPACITY OF DYESTUFFS BY *ASPERGILLUS SP.* USING MANOVA

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ABSTRACT: Fungi is widely found on air, soil, water and organic substances in nature. Unlike single-celled bacteria, fungi form multicellular structures, sometimes even visible to the naked eye. In studies of *Aspergillus* sp. removal and dye removal in order to obtain information, examinations are made by painting. Basic dyes, when ionized, are charged with positive electricity. Basic dyes include malachite green (MG), methyl violet (MV), crystal violet (CV), and basic fuchsin (BF). The aim of this research is to determine the effects of different factors on the parameters of absorption capacity and the removal efficiency of malachite green (MG), methyl violet (MV), crystal violet (CV) and basic fuchsin (BF) from *Aspergillus* sp. The data analyzed using Minitab statistical software. MANOVA used as the statistical analysis. It is concluded that the temperature and initial concentration have significant effect on removal efficiency and absorption capacity.

Keywords: *Aspergillus* sp., Dyestuff, Multifactorial Experiments, MANOVA

1. INTRODUCTION

Fungi are among the most widely distributed organisms on Earth and are of great environmental and medical importance. Fungi is a plural form of fungus. Fungus is any of about 144,000 known species of organisms of the kingdom Fungi, which includes the yeasts, rusts, smuts, mildews, molds, and mushrooms. Many fungi are free-living in soil or water; others form parasitic or symbiotic relationships with plants or animals. Fungi are everywhere in very large numbers-in the soil and the air, in lakes, rivers, and seas, on and within plants and animals, in food and clothing, and in the human body. Together with bacteria, fungi are responsible for breaking down organic matter and releasing carbon, oxygen, nitrogen, and phosphorus into the soil and the atmosphere. Humans have been indirectly aware of fungi since the first loaf of leavened bread was baked and the first tub of grape must be turned into wine. Fungi are essential to many household and industrial processes, notably the making of bread, wine, beer, and cheese. Fungi are also used as food; for example, some mushrooms, morels, and truffles are epicurean delicacies and mycoproteins (fungal proteins), derived from the mycelia of certain species of fungi, and are used to make foods that are high in [1-4].

Dyestuffs are a group of organic pollutants and their industrial use is common. Dyestuffs is the general name of intensely colored and complex organic compounds that dissolve in certain environments and permanently give their color to the applied material. In order for a substance to be named as a dye, it must provide a continuous coloring on another substance. Unlike pigments, almost all of the dyestuffs are soluble compounds and are generally applied after being made into an aqueous solution in order to ensure a homogeneous color distribution [5]. Dyestuffs consist of two main components. The first is the functional groups that bind the dye to the yarn; the second is the chromophore groups that give color. Chromophore means coloring. A chromophore is an atom, group of atoms or electrons that provide a colored appearance in an organic molecule. It contains one or more links. These bonds are variable and provide the bright colored appearance of the paint by absorbing the light. Aromatic ring compounds containing chromophores in the dyestuff are called chromogen. Dyestuffs are generally complex, synthetic, high molecular weight and organic compounds. It is classified by considering various characteristics such as solubility, chemical structure and dyeing properties. Due to their chemical structure, they can resist heat, water and many chemicals, and color removal is very difficult due to their complex synthetic structure. Examples of dyestuffs include MG, MV, CV, and BF. These dyestuffs cause serious harm to people and the environment. For this reason, it is important to remove these dyestuffs [6-12].

In the present study, determining the factors and levels affecting the removal efficiency and absorption capacity of MG, MV, CV, and BF, which are dyestuffs, are examined. So, with *Aspergillus* sp., it is examined whether the factors and interactions, namely the independent variables, have a significant effect on the removal efficiency and absorption capacity of MG, MV, CV, and BF from fungi and dyestuffs. Thus, the effects of different factors on the parameters of MG, MV, CV, and BF removal efficiency and absorption capacity will be determined. It is considered to eliminate or minimize negative factors for removal efficiency and absorption capacity. Thus, by optimizing the removal efficiency and absorption capacity, with fungi of *Aspergillus* sp., it is aimed to see and analyze the results of the experiment of removing fungi and dyestuffs. So, the treatment combinations which give the maximum removal efficiency and maximum absorption capacity will be obtained. Multivariate analysis of variance (MANOVA) will be applied with the Minitab package program to determine the effects of different factors on the parameters of the removal efficiency and absorption capacity. In this way, it is aimed to make the necessary analyzes for the removal efficiency and absorption capacity of these dyestuffs as well as reducing the negative effects on people and the environment. For this purpose, it was planned to use 4 factors (initial concentration (mg/L), biosorbent amount (g/L), temperature (°C), time (minute)) in the experiment, and different levels for each factor. These levels; 50-100-150-200 mg/L for initial concentration, 0.1 - 0.2 - 0.4 - 0.6 - 0.8 - 1 g/L for biosorbent amount, 25-35-45 °C for temperature and 30-60-90-120-150-180 minutes for time. In our study, since there is more than one dependent variable, MANOVA (Multivariate ANOVA), that is, multivariate analysis of variance is used.

2. METHODS

2.1. Multivariate Analysis of Variance (MANOVA)

In Multivariate analysis of variance (MANOVA), the number of response variables is increased to two or more. In multivariate variance analysis, Wilks 'Lambda, Lawley - Hotelling Trace, Pillai' s Trace and Roy's Largest Root statistics are used [13].

In this study, MANOVA of the results of the experiments made by applying the necessary conditions will be done with Minitab. In addition to that, with Minitab, it is planned to draw the

necessary graphics according to the analysis of the observations obtained [14]. The best way to understand or improve a process is to measure that process accurately and analyze the measurement data correctly.

3. EXPERIMENTAL STUDY

Malachite green (MG), methyl violet (MV), basic fuchsin (BF) and crystal violet (CV) can be given as examples of dyestuffs, which are common and organic pollutants for industrial use. MG is a green-colored form that does not contain malachite minerals and is a toxic chemical substance used in dyeing. MV is the name given to the group consisting of similar pH indicators and dye chemicals. MV types are mostly used in textile, for dyeing products with purple color. BF magenta dye, it is a mixture of rosaniline, pararosaniline, novel fuchsin and magenta II [15-19]. In the experiments in this study, there are 4 factors and the levels of these factors. Factors and levels for the removal efficiency (%) and absorption capacity (mg/g) are as follows: 50-100-150-200 mg/L for initial concentration, 0.1 - 0.2 - 0.4 - 0.6 - 0.8 - 1 g/L for biosorbent amount, 25-35-45 °C for temperature and 30-60-90-120-150-180 minutes for time.

With *Aspergillus* sp., the factors affecting the removal efficiency and absorption capacity of dyestuffs such as MG, MV, CV, and BF are examined. With *Aspergillus* sp., necessary experiments for the removal efficiency and absorption capacity of MG, MV, CV, and BF from the dyestuffs by using fungi microorganism will be carried out with the help of a team.

4. RESULTS

There are 4 factors in the experiment. These factors are initial concentration (mg/L), amount of biosorbent (g/L), temperature (°C), time (minutes). Different levels will be used for each factor. These levels; 50-100-150-200 mg/L for initial concentration, 0.1, 0.2, 0.4, 0.6, 0.8, 1 g/L for biosorbent amount, 25-35-45 °C for temperature and for time 30, 60, 90, 120, 150, 180 minutes. In addition, there are two dependent variables in this experiment. These dependent variables; removal efficiency (%) and absorption capacity (mg/g).

Table 1. Variables and their levels

Factor	Units	Levels
Biosorbent Amount (A)	g	0.1 - 0.2 - 0.4 - 0.6 - 0.8 - 1
Time (B)	min	30 - 60 - 90 - 120 - 150 - 180
Temperature (C)	°C	25 - 35 - 45
Initial Concentration (D)	mg / L	50 - 100 - 150 - 200

4.1. Results for the Malachite Green (MG)

Minitab uses s , m and n to calculate the F-statistics for Wilks, Lawley-Hotelling, and Pillai's tests. The F-statistic is exact if $s=1$ or 2 . If $s \neq 1$ or 2 , the F-statistic is approximate. The formulas for calculating s , m and n in Minitab are as follows:

$$\begin{aligned}
 s &= \min(p, q) \\
 m &= .5(|p - q| - 1) \\
 n &= .5(v - p - 1)
 \end{aligned}$$

p number of responses
 q df of the hypothesis
 v df for E
 E error matrix

Table 2. MANOVA Tests for Temperature (°C)

Criterion	Test Statistic	F	Num	Denom	P
Wilks'	0.73394	1.42	4	34	0.25
Lawley-Hotelling	0.36036	1.44	4	32	0.24
Pillai's	0.26765	1.39	4	36	0.26
Roy's	0.35426				

$$s = 2 \quad m = -0.5 \quad n = 7.5$$

Since the results of four different multivariate statistics for the temperature variable are $p > 0.05$, it is not significant at the 0.05 level. There is no significant difference in terms of dependent variables. In other words, changes in temperature levels do not make a significant difference on the removal efficiency and absorption capacity of MG.

Table 3. MANOVA Tests for Initial Concentration (mg/L)

Criterion	Test Statistic	F	Num	Denom	P
Wilks'	0.0835	13.9	6	34	0
Lawley-Hotelling	7.77342	20.7	6	32	0
Pillai's	1.18387	8.7	6	36	0
Roy's	7.33701				

$$s = 2 \quad m = 0 \quad n = 7.5$$

Since the results of four different multivariate statistics for the initial concentration variable are $p < 0.05$, it is significant at the 0.05 level. There is significant difference in terms of dependent variables. In other words, the change in the initial concentration levels creates a significant difference in the removal efficiency and absorption capacity of MG.

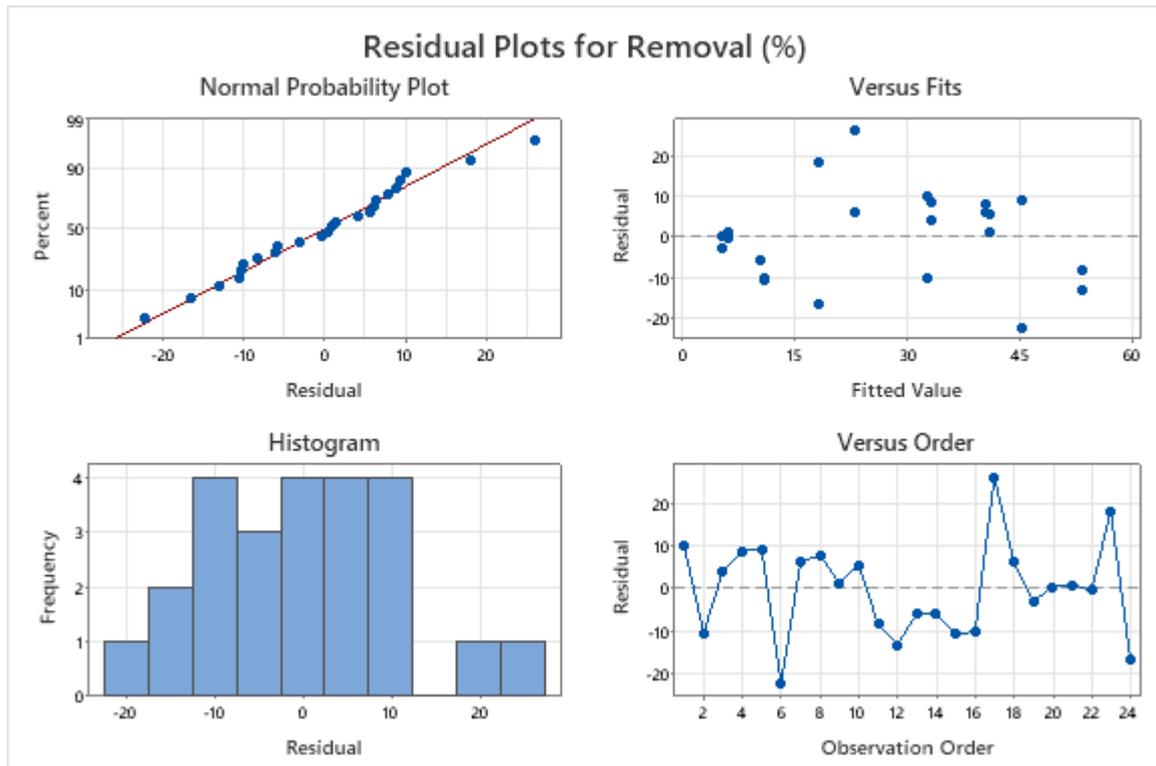


Fig. 1 Residual Plots for Removal Efficiency (%) for Malachite Green (MG)

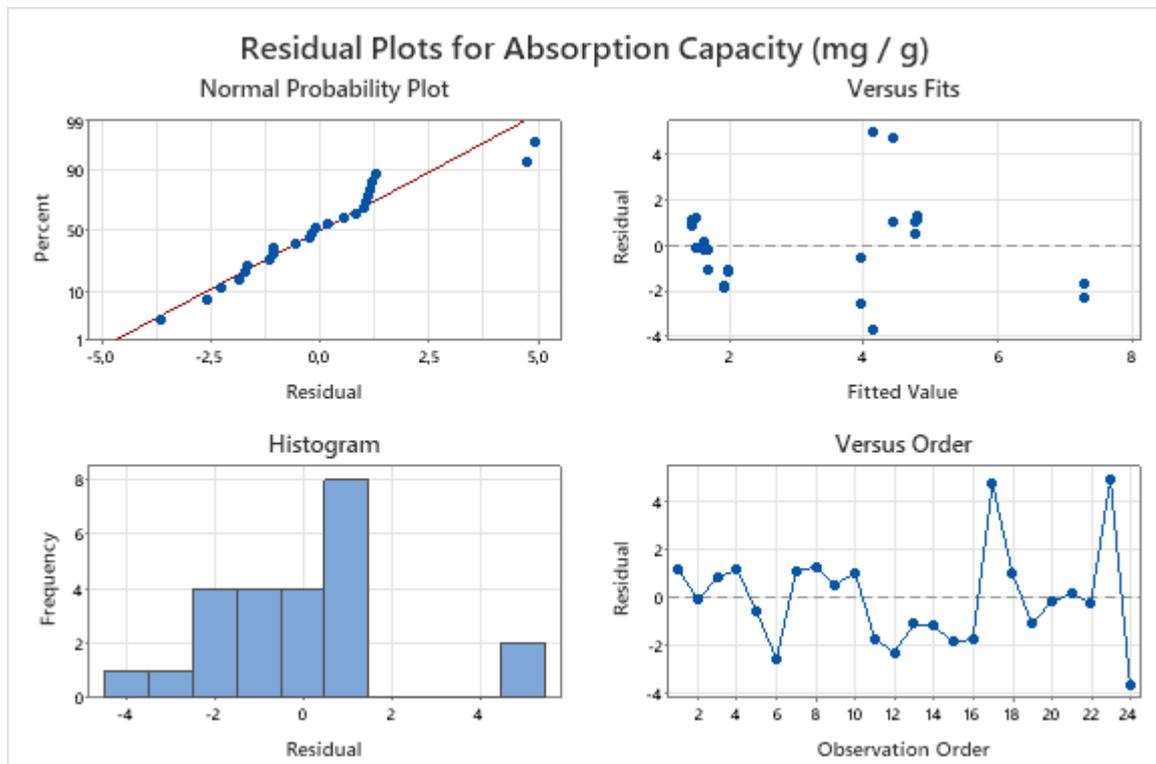


Fig. 2 Residual Plots for Absorption Capacity (mg/g) for Malachite Green (MG)

Table 4. Means for Initial Concentration (mg/L)

Initial Concentration (mg / L)	N	Mean	StDev	95 % CI
50	6	37.03	12.5	(25.43, 48.64)

100	6	45	3.07	(33.39, 56.61)
150	6	14.8	20.08	(3.19, 26.41)
200	6	9.9	13.19	(-1.71, 21.51)

Pooled St. Dev. = 13.6282

When we analyze the factors given in Table 1, the factor that is significant is the initial concentration and there are 4 levels related to it. These levels are 50, 100, 150 and 200 mg/L. When the initial concentration levels are examined, it is seen that the removal efficiency and absorption capacity is higher at the level of 100 mg/L. That is, the factor that is significant is the initial concentration and the corresponding removal at the 100 mg/L level is high and should be adopted.

It was observed that the removal efficiency and absorption capacity of 0.8 g amount of biosorbent was high in the single factor experiment performed in two repetitions.

It was observed that the removal efficiency and absorption capacity of 120 minutes was high in the single factor experiment performed in two repetitions.

4.2. Results for the Methyl Violet (MV)

MANOVA conducted for temperature and the results are given in Table 5.

Table 5. MANOVA Tests for Temperature (°C)

Criterion	Test Statistic	F	Num	Denom	P
Wilks'	0.26251	14	2	10	0
Lawley-Hotelling	2.80935	14	2	10	0
Pillai's	0.73749	14	2	10	0
Roy's	2.80935				

$$s = 1 \quad m = 0 \quad n = 4$$

Since the results of four different multivariate statistics for the temperature variable are $p < 0.05$, it is significant at the 0.05 level. There is significant difference in terms of dependent variables. In other words, the change in the temperature levels creates a significant difference on the removal efficiency and absorption capacity of MV.

Table 6. MANOVA Tests for Initial Concentration (mg/L)

Criterion	Test Statistic	F	Num	Denom	P
Wilks'	0.00182	74.8	6	20	0
Lawley-Hotelling	47.4563	71.2	6	18	0
Pillai's	1.91	77.8	6	22	0
Roy's	31.59829				

$$s = 2 \quad m = 0 \quad n = 4$$

Since the results of four different multivariate statistics for the initial concentration variable are $p < 0.05$, it is significant at the 0.05 level. There is significant difference in terms of dependent variables. In other words, the change in the initial concentration levels creates a significant difference in the removal efficiency and absorption capacity of MV.

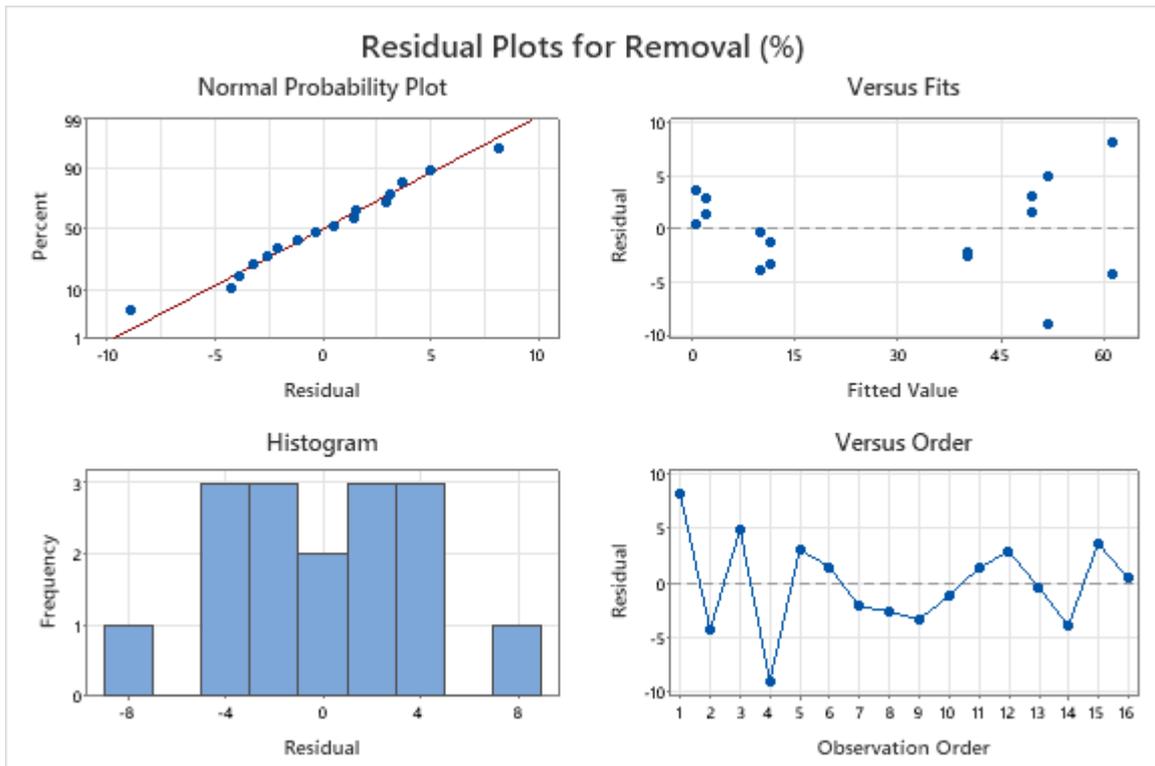


Fig. 3 Residual Plots for Removal Efficiency (%) for Methyl Violet (MV)

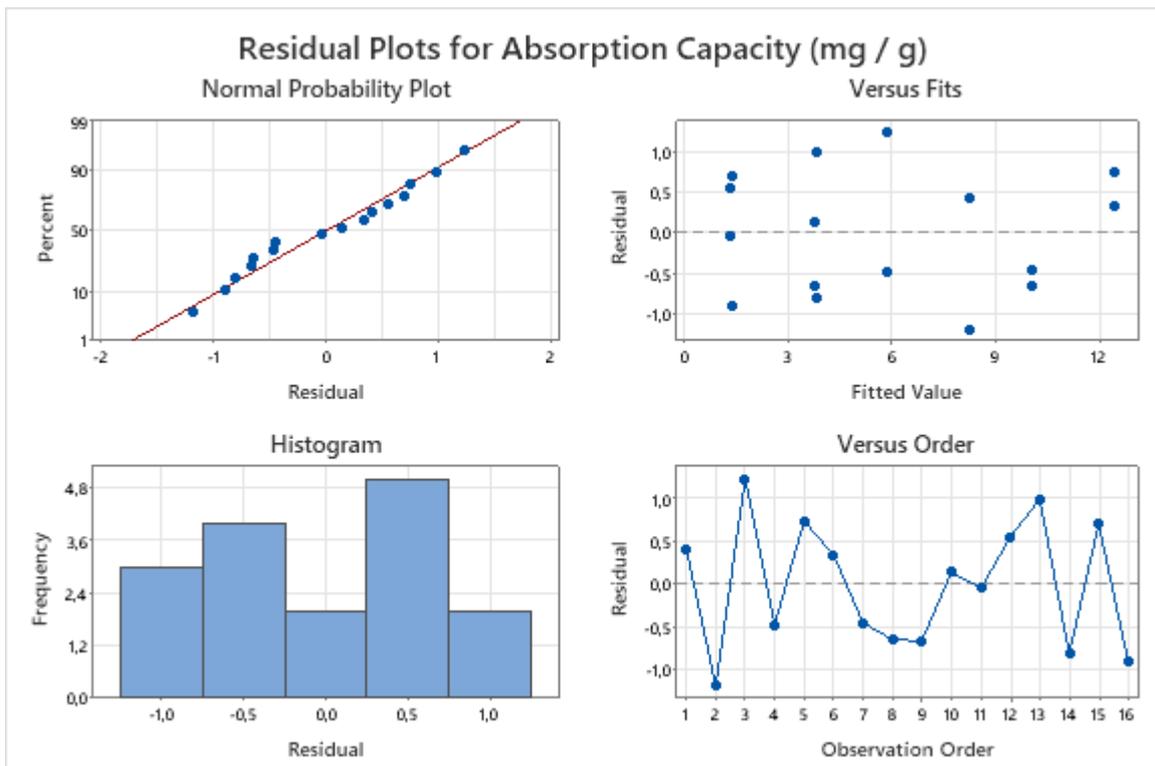


Fig. 4 Residual Plots for Absorption Capacity (mg/g) for Methyl Violet (MV)

Table 7. Means for Temperature (°C)

Temperature (°C)	N	Mean	StDev	95 % CI
35	8	33.16	26.82	(14.41, 51.92)
45	8	23.66	22.45	(4.91, 42.42)

Pooled St. Dev. = 24.7331

Temperature is found significant factor for removal efficiency and absorption capacity. Temperature has 2 levels; 35 °C and 45 °C. When the temperature levels are examined, it is seen that the removal efficiency and absorption capacity is higher at the level of 35 °C. That is, the factor that is significant is the temperature and the corresponding removal efficiency and absorption capacity at the 35 °C level is high and should be adopted.

Table 8. Means for Initial Concentration (mg/L)

Initial Concentration (mg/L)	N	Mean	StDev	95 % CI
50	4	56.67	10.87	(48.81, 64.54)
100	4	44.92	8.2	(37.06, 52.79)
150	4	6.8	3.13	(-1.06, 14.66)
200	4	5.25	3.64	(-2.61, 13.11)

Pooled St. Dev. = 7.21714

Initial concentration is also found significant factor for removal efficiency and absorption capacity. It has 4 levels; 50, 100, 150 and 200 mg/L. When the initial concentration levels are examined, it is seen that the removal is higher at the level of 50 mg/L. That is, the factor that is significant is the initial concentration and the corresponding removal at the 50 mg/L level is high and should be adopted.

It was observed that the removal efficiency and absorption capacity of 0.4 g amount of biosorbent was high in the single factor experiment performed in two repetitions.

It was observed that the removal efficiency and absorption capacity of 180 minutes was high in the single factor experiment performed in two repetitions.

It was observed that the removal efficiency and absorption capacity of 150 mg/L initial concentration was high in the single factor experiment performed in two repetitions.

4.3. Results for the Crystal Violet (CV)

Table 9. MANOVA Tests for Temperature (°C)

Criterion	Test Statistic	F	Num	Denom	P
Wilks'	0.42645	6.73	2	10	0.01
Lawley-Hotelling	1.34493	6.73	2	10	0.01
Pillai's	0.57355	6.73	2	10	0.01
Roy's	1.34493				

$s = 1 \quad m = 0 \quad n = 4$

Since the results of four different multivariate statistics for the temperature variable are $p < 0.05$, it is significant at the 0.05 level. In other words, the change in the temperature levels creates a significant difference in the removal efficiency and absorption capacity of CV.

Table 10. MANOVA Tests for Initial Concentration (mg/L)

Criterion	Test Statistic	F	Num	Denom	P
Wilks'	0.00132	88.3	6	20	0
Lawley-Hotelling	261.70315	393	6	18	0
Pillai's	1.65095	17.3	6	22	0
Roy's	259.80641				

$s = 2 \quad m = 0 \quad n = 4$

Since the results of four different multivariate statistics for the initial concentration variable are $p < 0.05$, it is significant at the 0.05 level. In other words, the change in the initial concentration levels creates a significant difference in the removal efficiency and absorption capacity of CV.

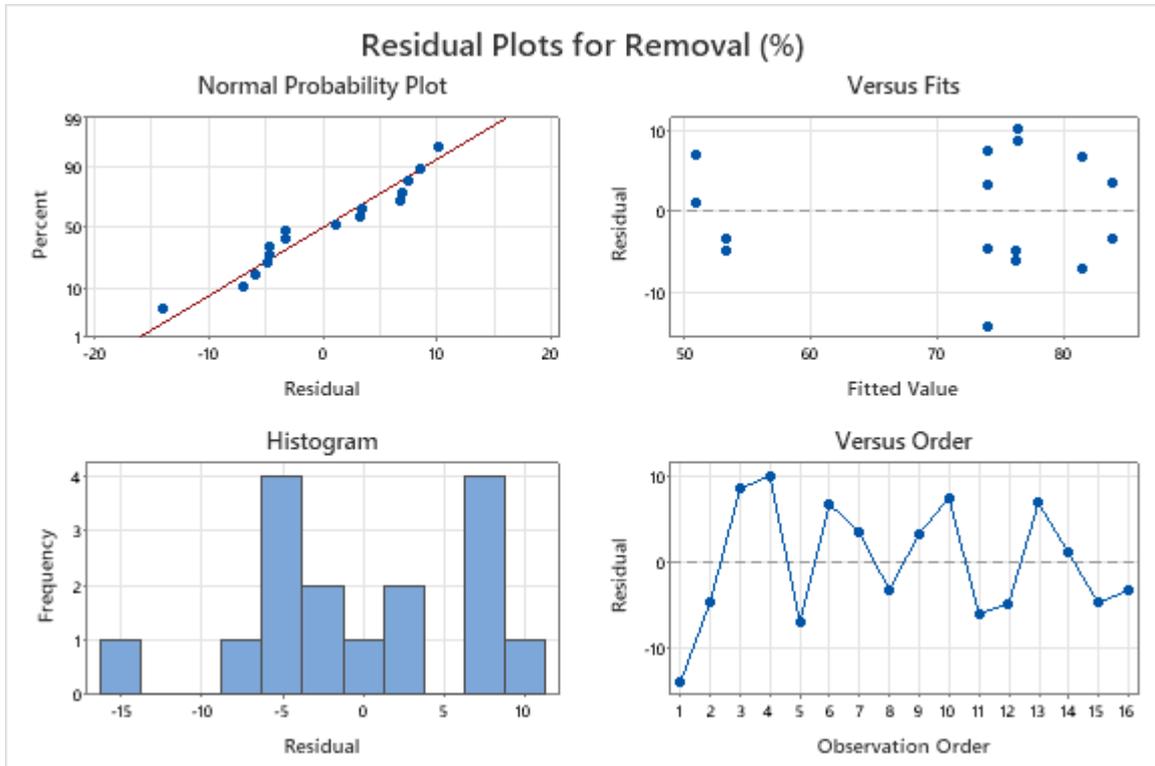


Fig. 5 Residual Plots for Removal Efficiency (%) for Crystal Violet (CV)

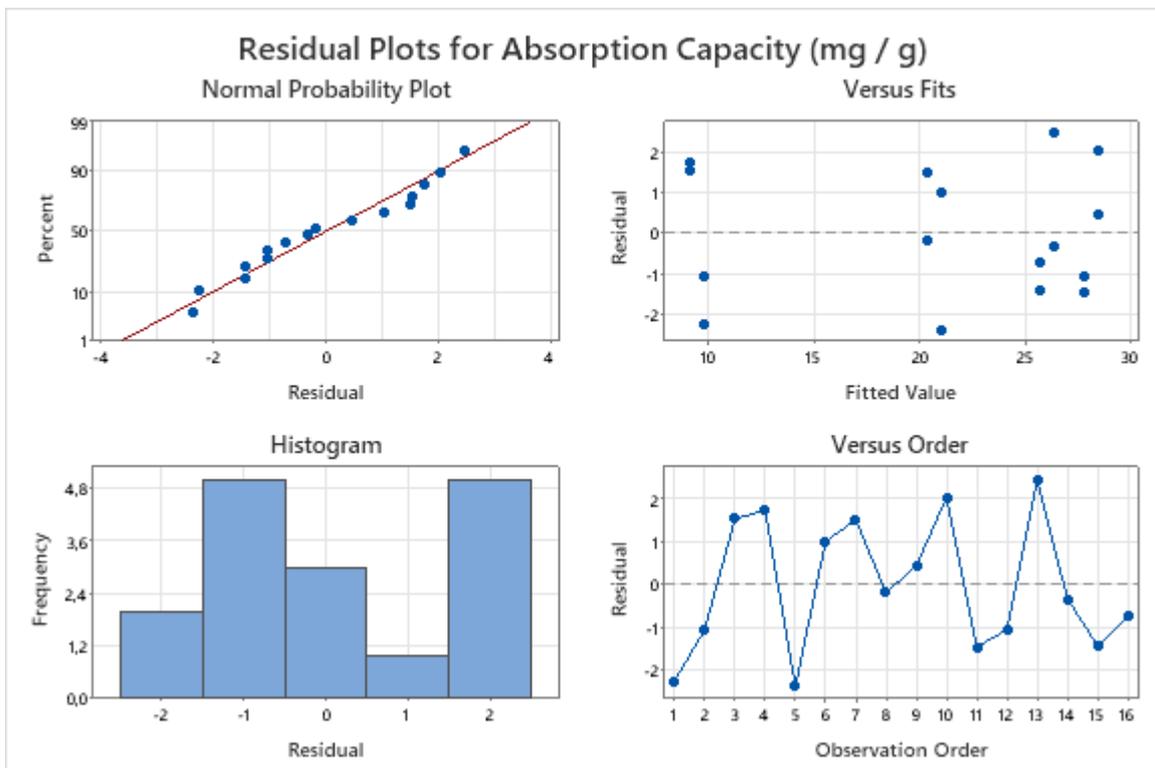


Fig. 6 Residual Plots for Absorption Capacity (mg/g) for Crystal Violet (CV)

Table 11. Means for Temperature (°C)

Temperature (°C)	N	Mean	StDev	95 % CI
35	8	70.14	12.58	(59.35, 80.92)
45	8	72.51	15.7	(61.73, 83.30)

Pooled St. Dev. = 14.2252

Temperature is found significant factor for removal efficiency and absorption capacity. Temperature has 2 levels; 35 °C and 45 °C. When the temperature levels are examined, it is seen that the removal efficiency and absorption capacity is higher at the level of 45 ° C. That is, the factor that is significant is the temperature and the corresponding removal efficiency and absorption capacity at the 45 °C level is high and should be adopted.

Table 12. Means for Initial Concentration (mg/L)

Initial Concentration (mg/L)	N	Mean	StDev	95 % CI
50	4	75.22	12.76	(66.66, 83.79)
100	4	82.75	6.4	(74.18, 91.32)
150	4	75.18	5.19	(66.61, 83.74)
200	4	52.15	4.09	(43.58, 60.72)

Pooled St. Dev. = 7.86646

Initial concentration is also found significant factor for removal efficiency and absorption capacity. It has 4 levels; 50, 100, 150 and 200 mg/L. When the initial concentration levels are examined, it is seen that the removal efficiency and absorption capacity is higher at the level of 100 mg/L. That is, the factor that is significant is the initial concentration and the corresponding removal efficiency and absorption capacity at the 100 mg/L level is high and should be adopted.

It was observed that the removal efficiency and absorption capacity of 0.6 g amount of biosorbent was high in the single factor experiment performed in two repetitions.

It was observed that the removal efficiency and absorption capacity of 90 minutes was high in the single factor experiment performed in two repetitions.

It was observed that the removal efficiency and absorption capacity of 100 mg/L initial concentration was high in the single factor experiment performed in two repetitions.

4.4.Results for the Basic Fuchsin (BF)

MANOVA is conducted for temperature and the results are given in Table 13.

Table 13. MANOVA Tests for Temperature (°C)

Criterion	Test Statistic	F	Num	Denom	P
Wilks'	0.78544	1.09	4	34	0.38
Lawley-Hotelling	0.26427	1.06	4	32	0.39
Pillai's	0.22155	1.12	4	36	0.36
Roy's	0.22468				

s = 2 m = -0.5 n = 7.5

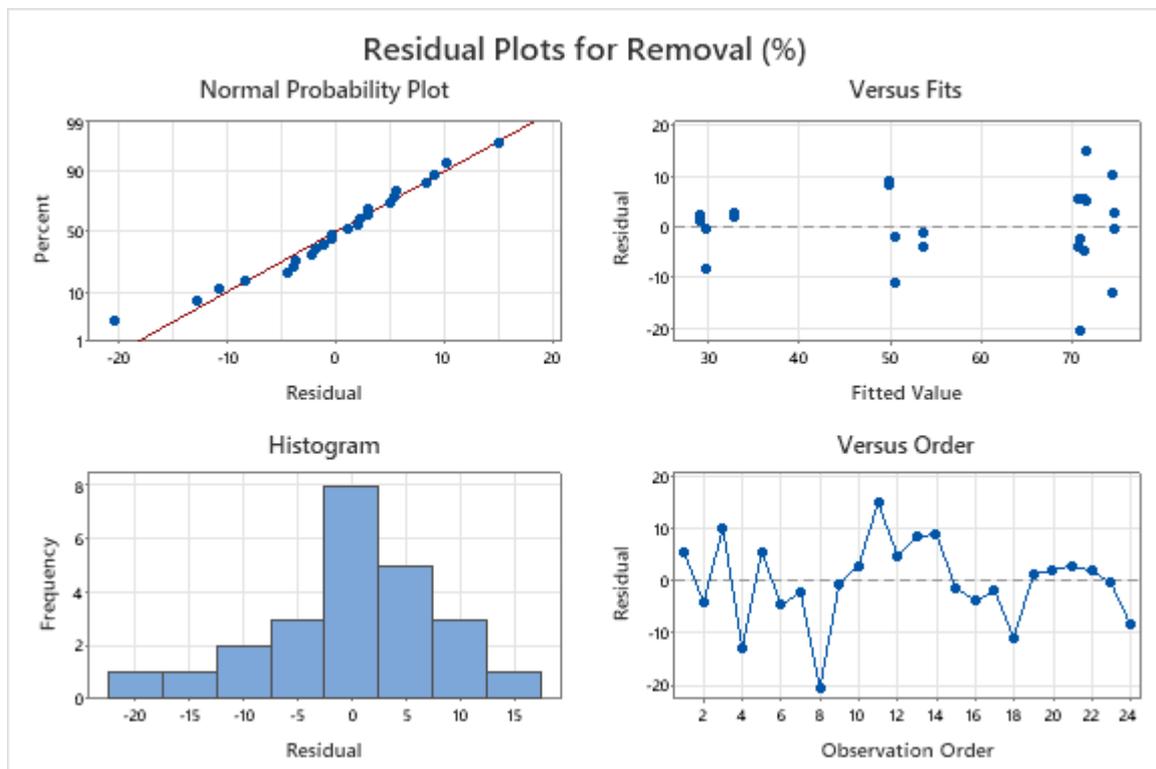
Since the results of four different multivariate statistics for the temperature variable are $p > 0.05$, it is not significant at the 0.05 level. There is no significant difference in terms of dependent variables. In other words, changes in temperature levels do not make a significant difference in the removal efficiency and absorption capacity of BF.

Table 14. MANOVA Tests for Initial Concentration (mg/L)

Criterion	Test Statistic	F	Num	Denom	P
Wilks'	0.00479	76.2	6	34	0
Lawley-Hotelling	82.35769	220	6	32	0
Pillai's	1.59598	23.7	6	36	0
Roy's	80.80533				

$$s = 2 \quad m = 0 \quad n = 7.5$$

Since the results of four different multivariate statistics for the initial concentration variable are $p < 0.05$, it is concluded that concentration is significant ($p < 0.05$). In other words, the change in the initial concentration levels creates a significant difference in the removal efficiency and absorption capacity of BF.

**Fig. 7** Residual Plots for Removal Efficiency (%) for Basic Fuchsin (BF)

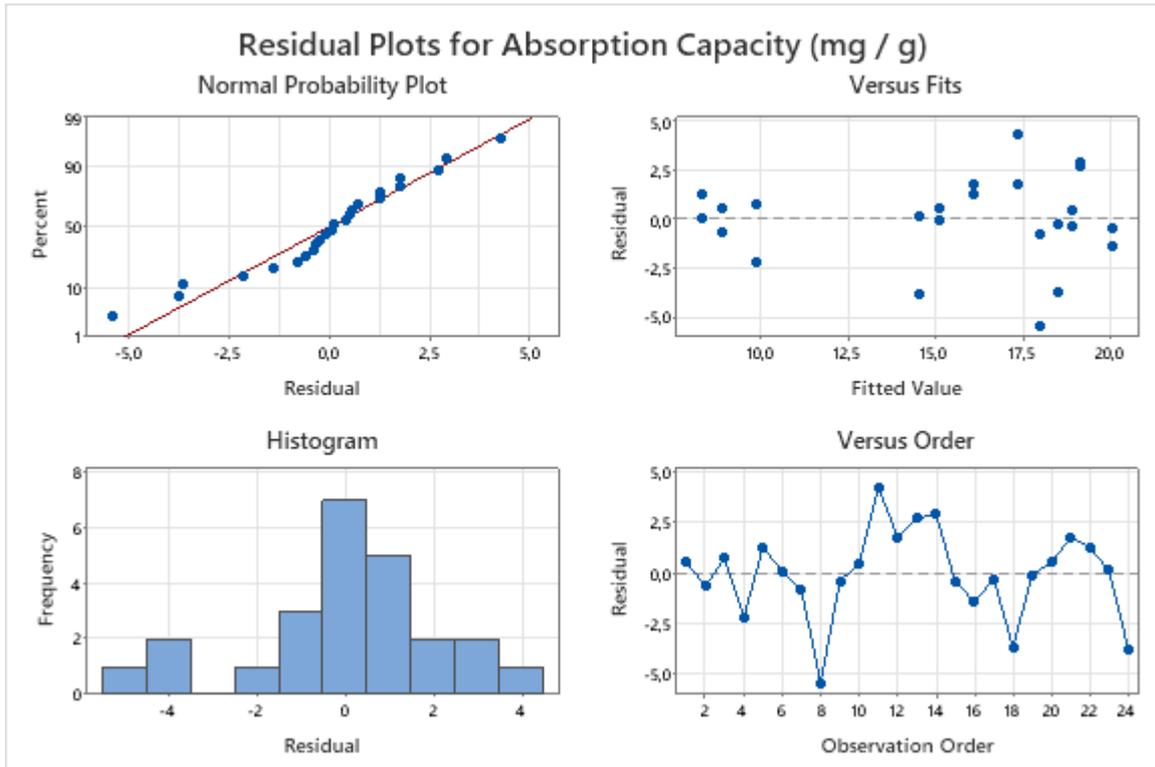


Fig. 8 Residual Plots for Absorption Capacity (mg/g) for Basic Fuchsin (BF)

Table 15. Means for Initial Concentration (mg/L)

Initial Concentration (mg/L)	N	Mean	StDev	95 % CI
50	6	72.12	8.52	(64.76, 79.47)
100	6	72.43	12.22	(65.08, 79.79)
150	6	51.3	7.09	(43.95, 58.65)
200	6	30.57	5.1	(23.21, 37.92)

Pooled St. Dev. = 8.63422

When we analyze the factors above, the factor that is significant is the initial concentration and there are 4 levels related to it. These levels are 50, 100, 150 and 200 mg/L. When the initial concentration levels are examined, it is seen that the removal efficiency and absorption capacity is higher at the level of 100 mg/L. That is, the factor that is significant is the initial concentration and the corresponding removal efficiency and absorption capacity at the 100 mg/L level is high and should be adopted.

It was observed that the removal efficiency and absorption capacity of 0.6 g amount of biosorbent was high in the single factor experiment performed in two repetitions.

It was observed that the removal efficiency and absorption capacity of 120 minutes was high in the single factor experiment performed in two repetitions.

5. RESULTS AND DISCUSSION

In this study, the effects of the factors on the removal efficiency and absorption capacity of MG, MV, CV, and BF from *Aspergillus* sp. were examined. We defined the dependent variables, factors and the levels of these factors in the experiments. Multivariate Analysis of Variance (MANOVA) with Minitab was used for optimum removal efficiency and optimum absorption capacity combination with these factors.

The factor that is significant for MG is the initial concentration and there are 4 levels related to it. These levels are 50, 100, 150 and 200 mg/L. When the initial concentration levels are examined, it appears that the removal efficiency and absorption capacity is 45 % at the 100 mg/L level. It is seen that the removal efficiency and absorption capacity are higher at the level of 100 mg/L. That is, the factor that is significant is the initial concentration and the corresponding removal efficiency and absorption capacity at the 100 mg/L level are high and should be adopted.

The factors that are significant for MV are the temperature and the initial concentration. Temperature levels are 35 °C and 45 °C. When the temperature levels are examined, it appears that the removal efficiency and absorption capacity is 33.16 % at the 35°C level. It is seen that the removal efficiency and absorption capacity are higher at the level of 35 °C. The temperature is significant factor and the corresponding removal efficiency and absorption capacity at the 35 °C are high and should be adopted. Initial concentration has 4 levels and these levels are 50, 100, 150 and 200 mg/L. When the initial concentration levels are examined, it appears that the removal efficiency is 56.67% at the 50 mg/L level. It is seen that the removal efficiency and absorption capacity are higher at the level of 50 mg/L. The initial concentration and temperature are found as significant factors. Hence, the corresponding removal efficiency and absorption capacity at the 50 mg/L level are high and should be adopted. So, 50 mg/L initial concentration and 35 °C temperature should be adopted for MV.

The factors that are significant for CV are the temperature and initial concentration. Temperature has 2 levels related to it. These levels are 35°C and 45 °C. When the temperature levels are examined, it appears that the removal efficiency is 72.51 % at the 45 °C level. It is seen that the removal efficiency and absorption capacity is higher at the level of 45 °C. The factor that is significant is the temperature and the corresponding removal efficiency and absorption capacity at the 45 °C are high and should be adopted. Initial concentration has 4 levels which are 50, 100, 150 and 200 mg/L. When the initial concentration levels are examined, it appears that the removal efficiency 82.75% at the 100 mg/L level. It is seen that the removal efficiency and absorption capacity are higher at the level of 100 mg/L. The factor that is significant is the initial concentration and the corresponding removal efficiency and absorption capacity at the 100 mg/L level is high and should be adopted. So, 100 mg/L initial concentration and 45 °C temperature should be adopted for CV.

The factor that is significant for BF is the initial concentration with 4 levels. These levels are 50, 100, 150 and 200 mg/L. When the initial concentration levels are examined, it appears that the removal efficiency is 72.43 % at the 100 mg/L level. That is, the factor that is significant is the initial concentration and the corresponding removal efficiency and absorption capacity at the 100 mg/L level are high and should be adopted.

As a result, with this study, MG, MV, CV, and BF which are dyestuffs, it is put forward reveal the relationship between the factors and levels as a result of the analyzes for the removal efficiency and absorption capacity. Temperature and initial concentration will be useful for removal efficiency and absorption capacity. It is thought that the temperature and initial concentration parameters determined during the experiment will be useful for the subsequent studies for removal efficiency and absorption capacity.

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