Turkish Online Journal of Qualitative Inquiry (TOJQI)
Volume 12, Issue 7, July 2021: 9629 – 9646

Research Article

Evaluation of the Modified Moving Bed Biofilm Reactors in Basic Red 18 Removal from Wastewater

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Abstract

Basic Red 18 is vastly used in various industries including textile and cosmetic industries. Investigations on the removal of dyes have led to the use of biological methods as appropriate removal methods due to their low costs and economic nature. Moving bed biofilm reactor is one of the most economically efficient methods which reach optimal removal efficiency in less retention time. In this study, feed backward/feed forward moving bed biofilm reactor was designed for the first time using the moving bed biofilm reactors and used for wastewater treatment. In this system, two independent variables including retention time (in the range of 2h.10'-7h.50') and input concentration of pollutant (22.47-202.78 mg/l) were measured and the effect of each variable on removal percentage of dye were studied based on Response surface methodology. Following that, modeling and determining the optimum conditions for operating the system was carried out. The results showed that the optimum condition occurred at retention time of 6.21 h and input dye concentration of 74.77 mg/l. Based on this research, retention time has the greatest impact on the removal efficacy of this biological system and this system has a great potential for wastewater treatment containing Basic Red18.

Keywords: *Biodegradation, basic red 18, optimization, FB/FFMBBR, RSM.*

Introduction

Industrial wastewater contains chemical and toxic pollutants and their treatment to reduce the pollutants discharge in the hydrosphere is vital (Asadi et al. 2019, Sheikholeslami et al. 2018(a), Sheikholeslami et al 2018(b), Faghih Nasiri et al. 2018 (a), Yavari and Qaderi. 2018, Qaderi et al. 2018(a), Kumar et al. 2016, Babanezhad et al. 2017, Qaderi et al. 2015 (a), Qaderi et al. 2011). Textile industry has one of the most polluted wastewaters that have long been considered as a main challenging issue (Ghaly et al., 2014). Generally, production of one kilogram of fabric needs 100-200 liters of water (Tehrani &Amini, 2010). According to global statistics, about 40-50 million tons of materials are produced in the textile industry throughout the world. It can be realized through a simple analysis that 4-10 billion cubic meters of water in the world is polluted by this industry annually (Lackey et al., 2006). Textile wastewater discharge directly to the environment without treatment can

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have devastating effects on the natural water basins as well as the surrounding lands. The large amount of COD and BOD in the wastewater decrease the dissolved oxygen in the water and has a negative effect as well. Moreover, due to the dyes and chemical materials, it inhabits the photosynthesis process and changes the native biology of the wastewater discharge zone (Ranganathan & Karunagaran, 2007; Fathy et al., 2017). Reduction or removal of dye materials from textile wastewater is the main objective of several wastewater treatment studies. More than 10000 types of artificial dyes are produced in the world that is used in textile and paper industries (Maleki & Rezaee, 2009; Malakootian and Moridi, 2017). Since the presence of artificial and complex organic dyes in textile industry is effluent, the regulatory agencies adopt very common strict standards which have high costs for the wastewater treatment of textile industries (Rafatullah et al., 2010). One of the most commonly used dyes in these wastewaters is Basic Red18, which has the Molecular weight of 426.34 gr/mole, and its chemical formula is $C_{19}H_{25}Cl_2N_5O_2$.

This dye has detrimental environmental effects including the reduction of dissolved oxygen in the water and making its color cloudy or dark (Ojha et al., 2013; Mahmoodi, 2013). It is also threatening for the aquatic and endangers human health, specifically in terms of causing liver and kidney failure (Kabra et al., 2013). In different wastewater treatment like as physical-chemical and biological methods are used(Qaderi et al. 2015 (b), 2012, Qaderi and Ayati 2014, Faghih Nasiri et al. 2018 (b), Taghizadeh et al. 2019,PajoumShariatiet al. 2018). The physical methods for wastewater treatment include flotation of color through fine bubbles (Zongping et al., 2011), absorption of color by various absorbents (Zolgharnein *et al*., 2014) like clay (Nandi et al., 2009; Bouatay*et al*., 2014), activated carbon (Kilic et al., 2011), peat (Allen et al., 2004), rice hulls (Elmaghraby & Eldeeb, 2011), water hyacinth roots (Saltabaş et al., 2012), and other operations like filtration (Sheng &Ming, 1997) and reverse osmosis (Ramesh et al., 2007). The chemical methods include removal of dyes using coagulants (Sheng &Ming, 1997), and chemical oxidation using chemical materials like ozone (Ranganathan & Karunagaran, 2007; Tijani et al. 2014).

The physical methods are not environmentally efficient since the pollutant is not destroyed and it only transfers from one environment to another environment (Fakhru et al., 2009). The chemical methods have high costs of treatment and in some instances, dissolution of raw materials to other substances is toxic (Robinson et al., 2001). Therefore, in this study, the biological method has been used for wastewater treatment. In the biological method, not only are the chemical materials of the wastewater destroyed, but also it is economically affordable (Zitomer& Tonuk, 2003; Ahmed et al., 2014). Biological treatment falls into aerobic and anaerobic methods. In the anaerobic process, the anaerobic bacteria are placed inside the anaerobic reactors to treat the wastewater. However, the implementation of this method is not affordable due to the high costs of reactor construction as well as its difficult and problematic application (Shaolan et al., 2010; Qaderi et al., 2011; Zitomer & Tonuk, 2003).

MBBR is one of the aerobic reactor systems, which has filter media floating inside it. The filter media are suitable for the growth and reproduction of microorganisms (Adriana et al., 2014). The use of this treatment system for municipal and industrial wastewater has recently been expanded successfully. Nowadays, this system is considered a great success for the treatment of paper industry (Jahren et al., 2002), industrial slaughterhouses (Johnson et al., 2000), printing equipment waste (Dong et al., 2014), dairy factories(Andreottola et al., 2002), etc.

However, this research used a combination of modified MBBR reactors in the form of FB/FFMBBR for wastewater treatment containing Basic Red 18 for the first time. Furthermore, modeling of the laboratory results and optimization of the reactor operation condition for achieving the highest efficiency was performed for the first time using RSM. The effectiveness of this system was evaluated through a statistical analysis and comparison of the results to the previous studies.

Materials and Methods

1. Purchase of Dyes and Other Chemicals

Basic Red 18 dye was purchased from Alvan Sabet Corporation in Iran and other compounds were purchased from Merk brand.

2. Reactors Structure

Each reactor was a cylinder with the volume of 2.75 liters and the internal diameter of 10 cm and height of 35 cm. Inside the reactor, a screen was installed at the height of 10 cm from the cylinder floor to prevent the filter media from coming down from the screen surface and provided a space for the establishment of aerators in a way that the filter media settling did not cause the cavitation of the pores. At the height of 30 cm from the floor, there were signs to characterize the highest level of effluent in the reactor. Thus, the effective height of the reactor was 30 cm and the effective volume of the reactor was 2.355 L. Filter media is one of the main parts of each MBBR reactor. Therefore, in this research, the Bee Cell 2000 filter media was used due to their greater specific surface area compared to other conventional filter media. The appearance of this filter media is shown in Figure 1 and its characterization is presented in Table 1.

Fig. 1. MBBR Filter Media

Table 1.
Characterization of Bee Cell 2000 Media (Qaderi et al, 2011)

Parameter	Amount
Substance	HDPE
Density (gr/cm^3)	0.95
Specific growth surface (m^2/m3)	650
Effective surface (mm^2/piece)	857
Total surface (mm^2/piece)	1800

One of the other parts of the MBBR system was the aeration system used for two purposes

1. Supplying the required oxygen used for biological reactions.
2. Mixing the filter media all over the reactor.

The aeration system included air pumps, interface hoses and aeration stones. The pumps used were from Sonic 9908 commercial brand, which is used in home aquariums. The compressed output air from the pumps was passed through the hose to the aeration rocks placed at the bottom of the reactor and injected into the reactors to completely mix the filter media all over the system. Aeration was conducted at a rate of 3.33 l/min per liter of liquid.

3. Biofilm Formation on Filter Media and Removal of Suspended Microorganisms

In moving bed bio film reactors, the microorganisms attached to the existing filter media have the main task of treatment. At the beginning of the operation, the opportunity was given to the microorganisms to adapt to Basic Red 18 pollutant. After the adaptation, about 50% of the system volume was filled with filter media and then the opportunity was given to the microorganisms to form for biofilm on the filter media. To remove the effect of the suspended microorganisms in the reactors, system wastewater was discharged slowly from the lowest output of each reactor. Then, through a very slow flow, the reactors were filled and following that, the experiments were conducted. The goal of this step was to remove the suspended microorganisms in the reactor and to review the removal efficiency of the bio film attached to the filter media.

4. Reactors Connection to Each Other and Wastewater Transfer in Each Run in Proportion to the Raw Wastewater Concentration

After the formation of bio film on the filter media, the reactors were connected to each other as shown in Figures 2, and the following steps were taken for each different amount of raw wastewater concentration.

Step 1: Both reactors were first filled up to the efficient volume with the input and then aeration was done to pass the retention time. Following that, the aerator was turned off for 30 minutes to remove the filter media and sludge from the wastewater. Next, the wastewater of reactor 2 was discharged and samples were taken from it for experiment. Then, the wastewater of reactor 1was transferred to reactor 2and reactor 1 was refilled with the wastewater with an initial dye concentration.

Step 2: Again the two reactors were used as batches during the retention time. The difference was that from then on, the route and flow of wastewater recycling was from reactor1 to 2 to transfer 50% of the reactors volume to each other during the retention time. After completing the retention time, the wastewater of reactor 2 was discharged and samples were taken from it for experiments after that, the wastewater was transferred from reactor 1 to reactor 2 and reactor 1 was refilled with the wastewater with an initial dye concentration.

Step 3: Step 2 was repeated over until the system reached to a steady state and has performance difference of less than 2% at least at two consecutive steps. The steady state efficiency for each initial concentration is presented in the results section.

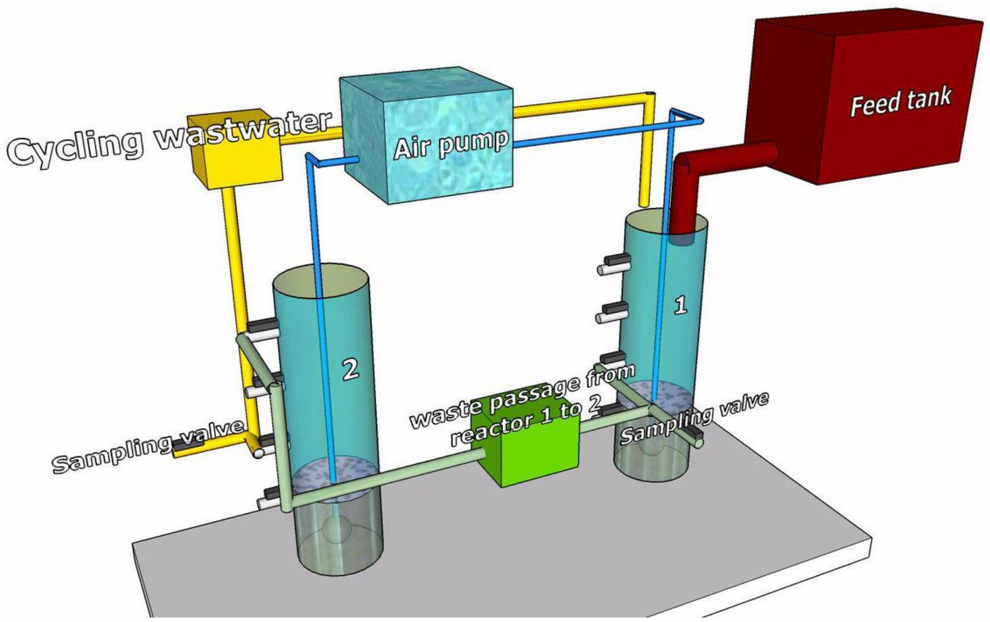


Fig. 2. The three-dimensional schematic view of FB/FFMBBR bioreactor

5. RSM and Dependent and Independent Variables

RSM consists of computational methods based on the experimental data and it used in previous researches (Khalegh and Qaderi 2019, Tavakoli Moghadam and Qaderi 2019, Tamadoni and Qaderi 2019, Qaderi and Babanezhad 2017, Qaderi et al. 2018, (a, b, c), Babanezhad et al. 2018, Ebrahimi Ghadi et al. 2018). In this research, to optimize the model and describe the system operation, various functions are used. In this research, the experimental design for the new biological system (FB/FFMBBR) was conducted for the treatment of wastewater containing Basic Red 18. Moreover, statistical analysis was used to determine the optimum conditions for treatment of wastewater containing Basic Red 18 in the system. To design the experiments and analyze the results, Design-Expert 7.0.0 software was used. Design requirements based on each of the variables are stated in Table 2 representing a summary of the design requirements based on the parameters of time, and initial dye concentration. Table 2 shows that A is the retention time and B is the initial dye concentration. Using this design, the optimum removal percentage was determined using the Quadratic model. Considering the conditions in Table 2, and considering the existence of two independent variables (retention time, input dye concentration in the raw wastewater) and the dependent variable of dye removal efficiency, the software offered 13 experiments during the main experimental period (Table 3).

Table 2.
Summary of parameter conditions in Response Surface Methodology experiment design

Factor	Name	Units	type	Low Actual	High Actual	Low Coded	High Coded	Mean	Std.dev.
A	Retention time	hr	Numeric	3	7	-1	1	5	1.569
B	initial dye concentration	mg/L	Numeric	70	180	-1	1	125	43.146

Table 3.

RSM experiments for the main experimental period

Run	A: Retention time (hr.)	B: initial dye concentration (mg/L)
1	5	125
2	5	125
3	7	70
4	5	47.22
5	5	125
6	3	70
7	5	125
8	2.17	125
9	5	125
10	5	202.78
11	7	180
12	3	180
13	7.83	125

6. Preparation of Sludge, Edition of Microorganisms, Concentration and Composition of Raw Wastewater

To set up the pilots, the returned sludge from the activated sludge pond of the municipal wastewater treatment plant was used. About 40 percent of the volume of the bioreactors was filled with this sludge and the rest was filled with water. After setting up the bioreactors, experiments were conducted and their results are presented in Table 4.

Table 4.
Characterization of sludge used in the reactors

Parameter	Amount
pH	7/5
Temperature (°C)	21
Total suspended solid (mg/L)	4500

The pH was measured periodically and kept constant in the range of 6.8- 2.8 at all stages of the study. Moreover, during the operation process, the bioreactors temperature was kept constant from21-25°C. At the beginning of each period, the bioreactors were fed with carbon/nitrogen/phosphor with the ratio of C:N:P= 100:5:1. The carbon in the adaptation period was supplied by glucose and Basic Red 18 and in the main tests experiments from Basic Red 18. In the adaptation phase of this study, the order of the composition of the nutrient inputs from glucose to Basic Red 18 for feeding the system was used (see Table 6). In this period, the retention time was 7.8 hours and any combination of input was repeated until the system reached to the steady state. After the adaptation of the microorganisms, in the main experiments of RSM, Basic Red 18 was used as the main carbon source in feeding all reactors. In this research, urea as the source of nitrogen and the compounds of K_2HPO_4 and KH_2PO_4 (phosphate buffer salts) as the sources of phosphorus to supply the ratio of C:P:N= 100:5:1 were injected to the system. The other compounds used in the artificial wastewater formulation were also used as insignificant elements to increase the efficiency and activity of microorganisms, the characteristics of which are presented in Table (5).

Table 5.
Chemical Composition of synthetic wastewater

Ingredients	Compound name	Chemical formula	Adaptation concentrate (mg/L)
Carbon source	Glucose	$C_6H_{12}O_6.6H_2O$	0-200
	basic red 18	C19H25Cl2N5O2	0-200
Nutrients	Urea	H_2NCONH_2	10
	Potassium hydrogen phosphate	K_2HPO_4	1
	Potassium dihydrogen phosphate	KH_2PO_4	1
Micronutrients	Magnesium sulphate	$MgSO_4.7H_2O$	5
	Calcium chloride	$CaCl_2$	3.75
	Iron(III)chloride	$FeCl_3 \cdot 6H_2O$	1
	Sodium molybdate	$Na_2MoO_4.2H_2O$	1.26

7. Reactors Feeding During the Adaptation Period

The order of the composition of the nutrient inputs from glucose to Basic Red 18 for feeding the system in the adaptation period are presented in Table 6.

Table 6.
The used nutrients (glucose to Basic Red 18) for feeding the system in the adaptation period

RUN	COD of basic red 18 (mg/L)	COD of glucose(mg/L)
1	0	200
2	3	180
3	6	160
4	9	140
5	12	120
6	15	100
7	18	80
8	21	60
9	24	40
10	27	20
11	30	0

8. Experiments Reference

All the experiments performed in this research were done based on the guidelines presented in the book entitled "Standard Methods for Examination of Water and Wastewater" (Greenberg et al., 2000). The dye of Basic Red 18 was determined by a spectrophotometer at the wavelength of 530 nm.

9. Microscopic Examination

In order to study the microorganisms in bioreactors, microbial specimens went under microscopic examination, and the microorganisms in reactors went under microbial cultivation to identify the resistant species.

Results and Discussion

In the present research, the effects of two independent variables of retention time (hrt) (at the range of 2 hours and 10 minutes to 7 hours and 50 minutes) and input concentration of pollutants (in the range of 47.22 to 202.78 mg/L) on each factor were evaluated. Table 7 shows the test results. The first column represents the retention time, and the second column represents the concentration of pollutants in different experiments, and the third column represents the removal percentage related to each experiment.

Table 7.
The experiments designed by Response surface methodology

Run	A: Retention time (hr)	B: initial dye concentration (mg/L)	Response (Removal Percent (%))
1	5	125	62
2	5	125	62
3	7	70	80
4	5	47.22	80
5	5	125	62
6	3	70	57
7	5	125	62
8	2.17	125	49
9	5	125	62
10	5	202.78	54
11	7	180	59
12	3	180	45
13	7.83	125	71

Table 7 shows that the removal efficiency is equal to 80% at the retention time of 5 hours and at the initial concentration of 47.22 mg/l. For modeling the results, using the design expert software and the response surface method, the optimal model was extracted.

1. Effect of Retention Time and Initial Dye Concentration

In Figure 3, the effects of the retention time and the initial dye concentration on the removal efficiency are presented. As shown in Figure 3, at the retention time of 7 hours and at the dye concentration of 70 mg/l, the removal efficiency was obtained to be 80%. However, the removal efficiency of the system at the retention time of 2 hours and 17 minutes and the initial concentration of 125 mg/l was obtained to be 62%. As shown in this Figure, the removal efficiency has a synergistic relationship with the retention time and an antagonistic relationship with the initial dye concentration.

The previous research on the pollutant removal through biological methods using pumice stone as biofilm bed have shown that the removal efficiency of MBBR decreases significantly with the increase of the ratio of the pollutant in put concentration (Sharbatmalaki & Borghei, 2005, Borghei et al., 2010).

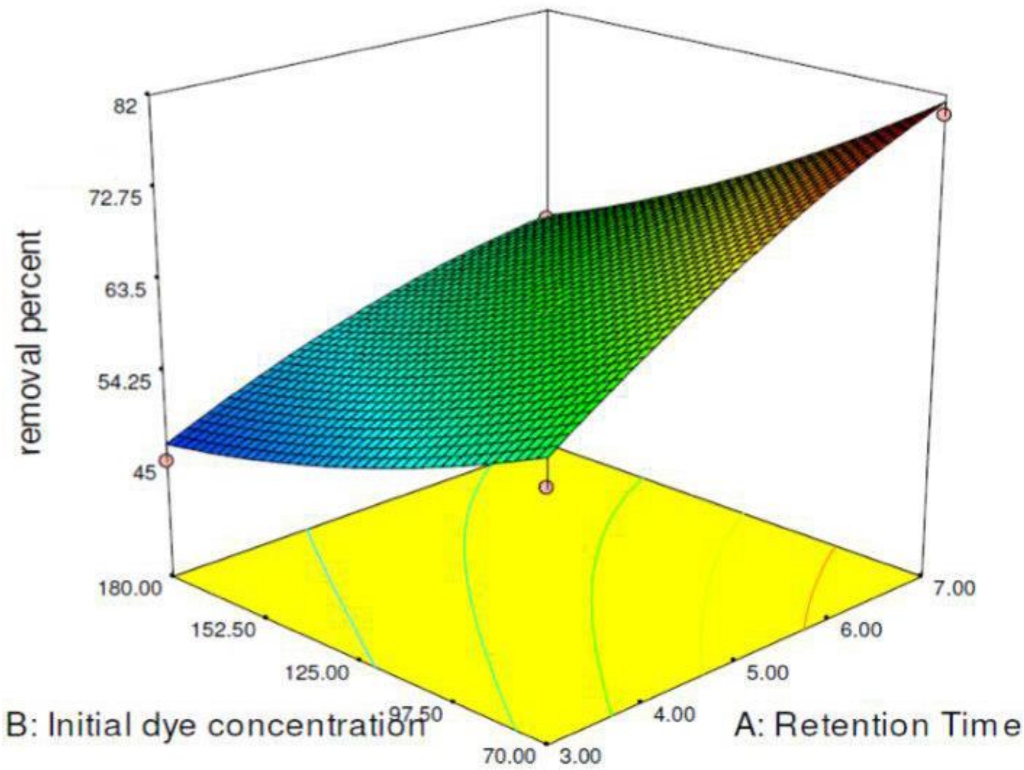


Fig. 3. The effect of retention time and initial dye concentration on the efficiency of dye removal

2. One-dimensional Figure

2.1. Effect of Retention Time

One-dimensional charts show the effects of each of the two independent variables on the removal efficiency. Figure 4 presents the effect of retention time on the removal efficiency percentage at dye concentration of 25 mg/l. Thus, when the retention time increases, the removal efficiency increases significantly. According to Figure 4, the percentage of removal efficiency at the retention time of 7 hours is equal to 71%. In general, in biological treatment method, a long time is needed for converting the organic materials to living cells. As this time increases, the contact time between the bacteria and the pollutant increases and so does the removal efficiency. Accordingly, the biological absorption capacity is directly related to the retention time. Based on the previous studies on the moving bed biofilm reactor efficiency in the treatment of municipal and industrial wastewaters, the removal efficiency increases by increasing the retention time (Mohammadyari & Balador, 2008).

In another study on the removal of the organic matter and dye from the textile industry wastewater using active granular carbon in the carbon-dioxide process, it has been concluded that with the increase of the hydraulic retention time, the dye removal efficiency increases significantly (Alizadeh & Borghei, 2005).

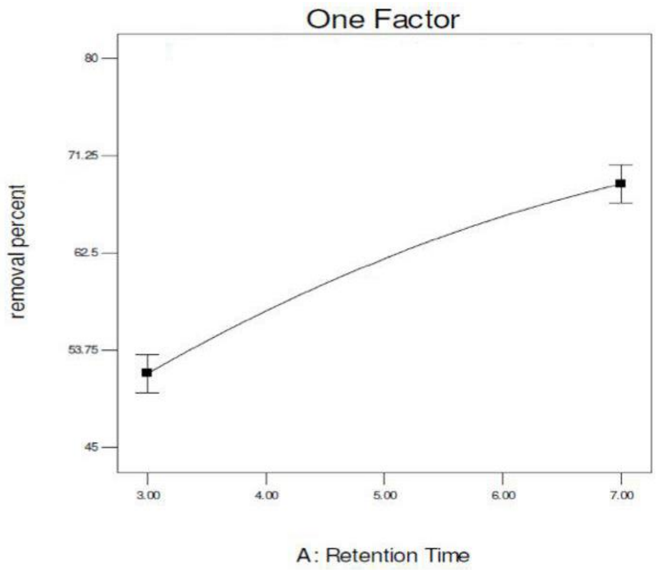


Fig. 4. The effect of retention time on the efficiency of dye removal (input dye concentration = 25 mg/l)

2.2. Effect of Initial Dye Concentration

In Figure 5, the effect of the input concentration of the pollutant on the removal efficiency has been investigated at the retention time of 5 hours. The figure clearly shows that by increasing the initial dye concentration to 2.5 times, the efficiency decreases to 1.3 times. Given the fact that the dye removal process through biological treatment depends on the bacteria, their effectiveness in removing the organic material depends on the toxicity of the nutrients in the system. According to the previous studies, the removal percentage has an inverse relationship with the input pollutant concentration. At the retention time of5 hours and the input concentration of 180 mg/l, the removal efficiency reaches to less than 60%. And at the concentration of 70 mg/l, this amount reaches to more than 71%. It can be concluded that the removal efficiency is highly dependent on the initial concentration of dye (Tchobanoglous et al., 2003).

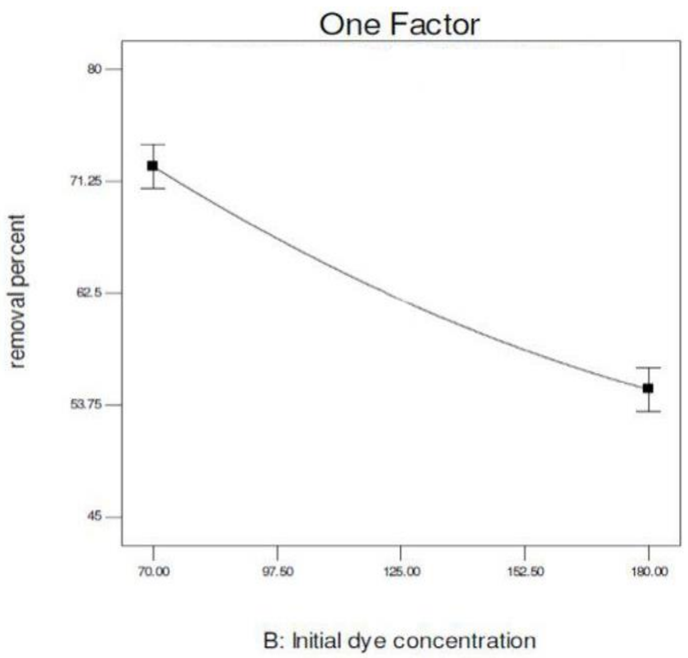


Fig. 5. The effect of initial dye concentration on the dye removal efficiency (retention time = 5 hours)

3. Interaction of Initial Dye Concentration and Retention Time

The interaction of the independent variables can be synergistic or antagonistic. Figure 6 shows the interaction of independent variables and the percentage of removal changes. According to Figure 6,at the retention time of 3 hours, the removal efficiency decreases significantly with the increase of the initial input concentration, and at the retention time of 7 hours, this amount decreases with the increase of the initial input concentrations, but the slope of the retention time of 7 hours is more than 3 hours. Therefore, these two variables have a synergistic effect.

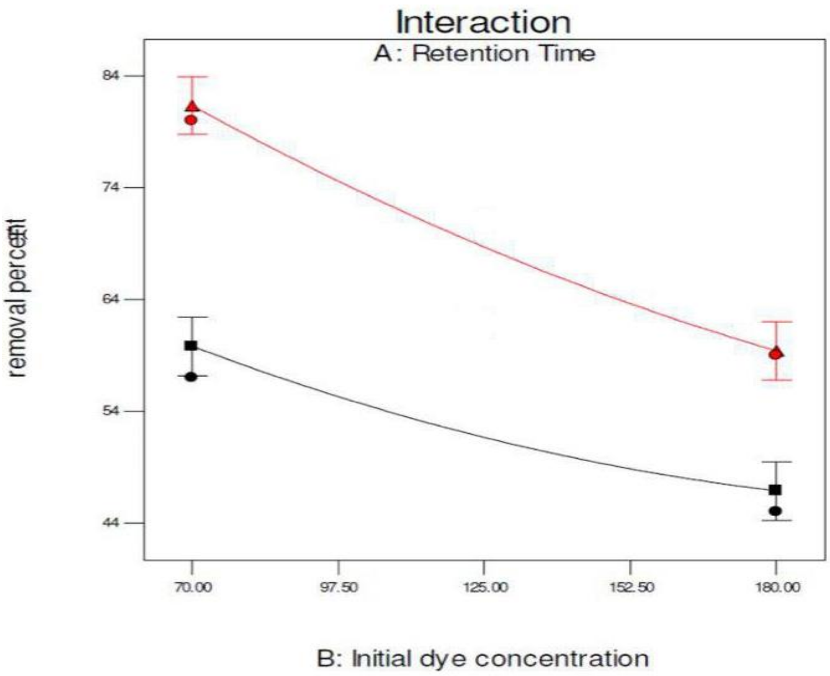


Fig. 6. The interaction of retention time and initial dye concentration on the dye removal efficiency (retention time = 3 and 7 hours)

4. Mathematical Model

According to the studies conducted, the mathematical model this the reactor is presented as follows in Equation 1.

Removal percent = +45.13989 + 11.34511 × Retention Time – 0.19576 × Initial dye concentration – 0.020455 × Retention Time × Initial dye concentration – 0.45312 × Retention Time^2 + 5.57851E-004 × Initial dye concentration^2

As shown in this equation, the retention time has the most effect on the pollutant removal efficiency.

5. Analysis of Variance

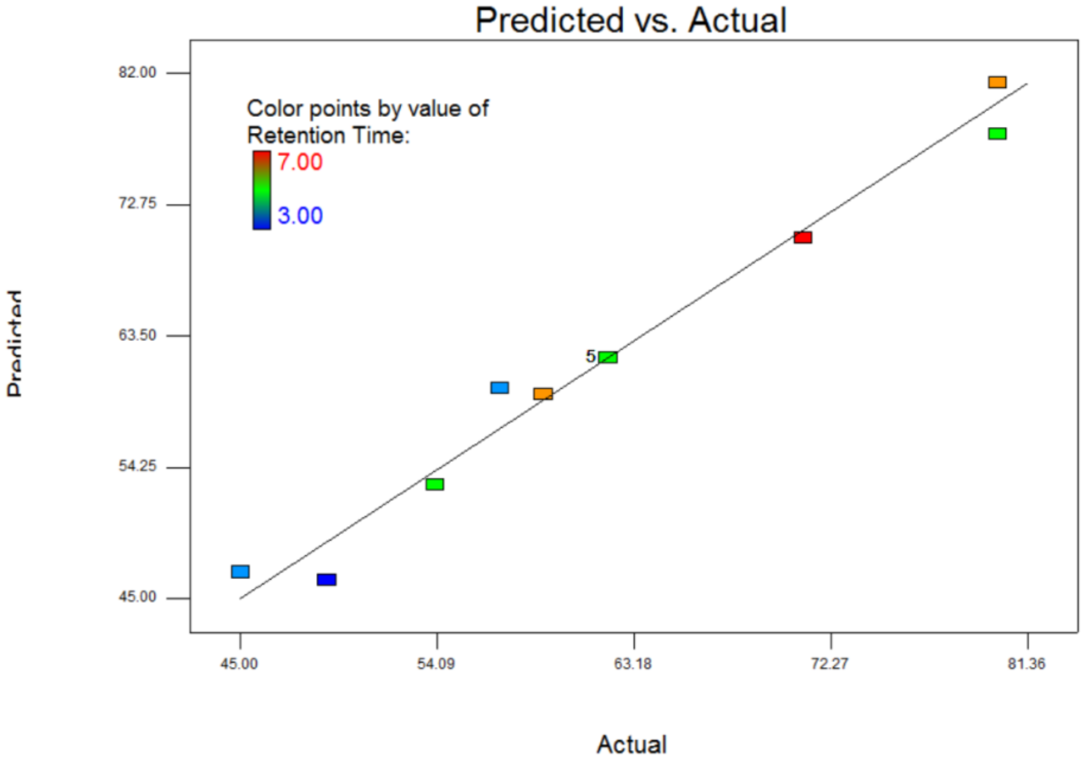
Table 8 shows the results of ANOVA. The sum of the squares and degrees of freedom of each parameter are provided. The output of ANOVA is F statistic expressed in the "F Value" column of Fisher statistics. The most important results in this Table are p-values in the last column which is less than 0.0001. According to the data, this mathematical model is statistically significant. Moreover, the two variables of retention time and initial dye concentration have statistically significant effects on the removal efficiency.

Table 8.
The results of ANOVA

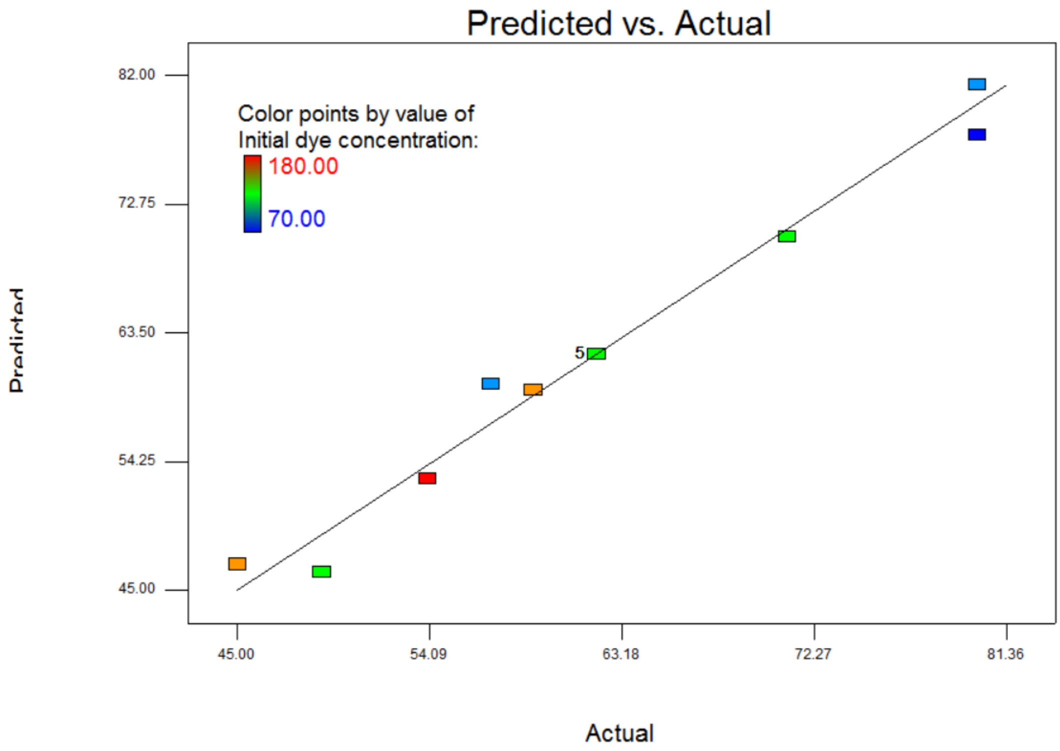
Source	Sum of squares	Df	Mean squares	F value	P-value prob>F
Model	1257.69	5	251.54	64.65	<0.0001
A-Retention time	579.92	1	579.92	149.06	<0.0001
B- Initial dye concentration	608.47	1	608.47	156.40	<0.0001
AB	20.25	1	20.25	5.20	0.0565
A^2	22.85	1	22.85	5.87	0.0458
B^2	19.81	1	19.81	5.09	0.0586
Residual	27.23	7	3.89		
Lack of fit	27.23	3	9.08		
Pure error	0.000	4	0.000		
Cor total	1284.92	12			

6. Optimization and Validation

Figures 7 represent the validity of this research. According to these two Figures, all the test results are consistent with the modeling results. The optimal amount was obtained at the retention time of 6.21 hours and at the initial concentration of 74.77 mg/l. According to the mathematical modeling, the optimal removal efficiency was obtained to be 73.75 percent. Comparing this modeling with the experimental results indicate that the error was less than 5%, indicating the high validity of the modeling.



(A)



(B)

Fig. 7. Predicted values based on the actual amounts of removal efficiency by changing A:the retention time, B:the initial dye concentration

7. Investigation of the Growth of Colonies

In cultivated plates, colonies of cauliflower were grown. After Gram staining and photographing the stained slabs, there were bacillus in the colonies. In the illustrations presented in Fig. 8, a sample of observed colonies and microorganisms after Gram staining are presented.

A B

Fig. 8. Images of microbial examination. A) A sample of observed colonies; B) A sample of observed bacillus

Conclusion

In this research, treatment of wastewater containing Basic Red 18 which has been significantly observed in the textile industries was studied. In the new FB/FFMBBR system, the best removal efficiency was obtained to be 80% of the pollutant, at the retention time of 5 hours and a concentration of 22.24 mg/l. The biological modeling results of this combination show that the removal of this dye is highly dependent on the retention time, and the removal efficiency increases with the increase of the retention time and reduction of the initial dye concentration. It was found that the proposed system (FB/FFMBBR) has a high potential to remove Basic Red 18 and can be studied for treatment of other pollutants from the wastewater.

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