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Application of Response Surface Methodology (RSM) for optimizing removal of Cr(VI) wastewater using Cr(VI)-reducing biofilm systems

Nurfadilah Mohammed and Wan Azlina Ahmad*

Chemistry Department, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia

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ABSTRACT

Response surface methodology (RSM) involving central composite design (CCD) was employed to obtain optimal conditions for Cr (VI) wastewater treatment by Cr (VI) reducing biofilm systems. On the basis of a CCD, RSM was used to determine the effect of initial metal concentrations (40-100 mgL^{-1}), nutrient supplementations (10-20%) and flowrate (3-6 $mLmin^{-1}$) on the levels of response, i.e. Cr (VI) reduction efficiency. A set of 20 experimental runs were needed for optimizing of the operating conditions. Quadratic regression models with estimated coefficients were developed to describe the Cr (VI) reduction. Analysis of variance (ANOVA) showed a high coefficient of determination (R^2) value of 0.9941, thus ensuring a satisfactory adjustment of the second-order regression model with the experimental data. Cr (VI) reduction had significant effect on all the three dependent variables. The experimental results show that Cr (VI)-reducing biofilm systems could effectively reduce Cr (VI), 100% at the optimum conditions of initial metal concentration of 100 mgL^{-1}, nutrient supplementation of 20% and flowrate of 3 $mLmin^{-1}$. The experimental observations were in reasonable agreement with the modelled values.

| Cr(VI)-reducing biofilm system| Cr(VI) wastewater| Central Composite Design(CCD) | Response surface methodology (RSM) |

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1. INTRODUCTION

Hexavalent chromium is often attributed to anthropogenic sources, since it is used in a number of industrial applications including electroplating, tanning, paper pulp production, pigment fabrication, wood preservation and industrial water cooling. Chromium can exist in several chemical forms with oxidation numbers ranging from -2 to +6. However, in the environment, Cr commonly exist in only two oxidation states Cr(VI) and Cr(III) which have different toxicity and transport characteristics [1]. Cr (VI) compounds have been declared as potent occupational carcinogens particularly lung carcinogen among workers in electroplating, stainless steel and pigment fabrication. Also, it is widely known to cause allergic dermatitis, adverse tissue damage and carcinogenic effects in humans and animals [2]. Cr (III), on the other hand, is more stable and is approximately 100 times less toxic and 1000 times less mutagenic than Cr (VI). Besides, Cr (III) is less soluble in water, and it is an essential dietary element [3]. Therefore, reducing Cr (VI) to Cr (III) is beneficial in eliminating the toxicity of Cr (VI) of wastewaters. Due to the severe toxicity of Cr (VI), the Malaysian Environmental Quality (sewage and industrial effluents regulation 1978) has set the maximum contaminant level for Cr (VI) in industrial effluent at 0.05 mg/L.

Conventional methods for removing Cr (VI) include chemical reduction to Cr (III) followed by a precipitation step under alkaline conditions, sorption onto various materials, including ion exchange and biosorption and membrane filtration [4, 5]. All these methods have specific disadvantages. For example, alkali precipitation produces large quantities of chemical sludge, whereas ion exchange and adsorption are generally costly and less specific for Cr (VI) removal in the presence of other ions [5].

Of late, the use of high performance biofilm reactors, packed with immobilized microorganisms, to reduce chromates to the less soluble trivalent chromium species has been gaining attention [6, 7, 8, 9]. Biofilms have been reported to be more resistant to Cr (VI) toxicity than suspended growth cells. Concentration gradients, complex formation and ion entrapment are possible reasons for the superior resistance [6].

Performance of biological Cr(VI) reduction process in biofilm reactors can be affected by factors such as initial hexavalent chromium [6], hydraulic residence time [10] and appropriate nutrient supplementation [9,11]. Other

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Corresponding author at: Chemistry Department, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia
E-mail addresses: azlina@kimia.fs.utm.my (Wan Azlina Ahmad)

appropriate nutrient supplementation [9,11]. Other researchers have reported that the Cr (VI) reducing activity of the microbial cells may vary in the presence of different carbon sources [12]. Orozco et al. 2009 reported that when nutrients are sufficiently available, high metal removal can be obtained, as the microorganism is able to respond well to the toxicity stress [11]. Hence, it is evident that the biological Cr(VI) removal process requires further optimization.

The process efficiency may be increased by the optimization of these factors. Response surface methodology (RSM) has been proposed to determine the influence of individual factors and their interactive influences. The RSM is a statistical technique for designing experiments, building models, evaluating the effects of several factors, and searching optimum conditions for desirable responses. With RSM, the interactions of possible influencing parameters on treatment efficiency can be evaluated with a limited number of planned experiments [13]. In conventional approach, optimization is usually carried out by varying one variable a time while keeping all other variables fixed at a specific set of conditions. It is not only time-consuming, but also usually incapable of reaching the true optimum due to ignoring the eventual interactions among variables [15]. This paper reports the combined effect of three process parameters; initial metal concentration, flowrate and nutrient supplementation on the optimization of chromium removal from simulated Cr(VI) solutions by the Cr (VI)-reducing biofilm systems using Central Composite Design in Response Surface Methodology (RSM) by Design Expert Version 8.0.2.

2. EXPERIMENTAL

2.1 Simulated Cr (VI) solution preparation

A 1000 mgL^{-1} stock solution of Cr(VI) was prepared by dissolving 2.833g of potassium dichromate $K_2Cr_2O_7$ (GPR ™) in 1.0 L of de-ionized water. The stock solution was diluted with de-ionized water to obtain the desired concentration range of Cr (VI) solutions.

2.2 Liquid pineapple waste

Liquid pineapple waste (LPW) was obtained from Lee Pineapple Industry, Tampoi, Johor, Malaysia. It was supplied externally to the Cr(VI) solution depending upon the requirement before being pumped through the bioreactor. Upon using, LPW was filtered using a sieve to separate the remaining solid particle. LPW functions as carbon source and electron donor for microorganisms in Cr(VI) reduction.

2.3 Laboratory scale bioreactor

In this study, a packed - bed PVC column with the following dimensions was used as the bioreactor; 5 cm i.d. and 52 cm in length (Figure 1). Inert stone was placed at the top and bottom of the column to provide sufficient flow distribution and also to retain the column content. The total volume entering the column was 1.021 L. The reactor was operated in a down-flow mode. The column consisted of three sections; bottom, middle and top biofilm sample collection ports. The PVC column was packed with rubber wood shavings.

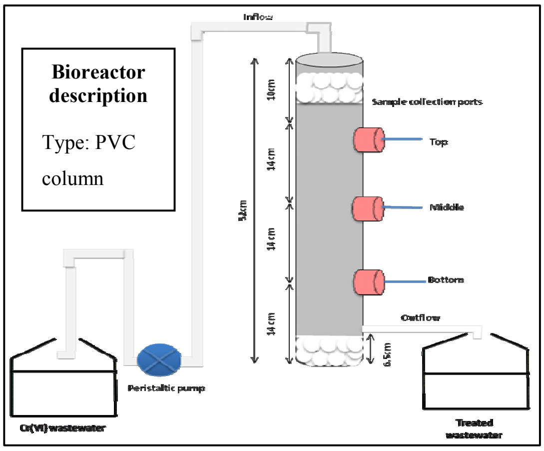


Fig 1: Bioreactor setup for Cr(VI) reduction process

2.4 Bioreactor startup and operation

The column was first rinsed with 1 bed volume i.e. 1000 mL of deionized water in a down-flow mode at 3 mL min^{-1} using peristaltic pump (Eyela MP - 1000D). *Acinetobacter haemolyticus*, 1 L grown for 12 h in Nutrient broth (NB), was continuously pumped for 24 h into the bioreactor using the same flowrate as the rinsing step. Then, fresh NB medium was supplied continuously for 3 days to promote bacteria growth and development of biofilm. Once the system stabilized, experimental design for optimization of parameters by Cr(VI)-reducing biofilms was conducted.

2.5 Experimental design for optimization of parameters

The optimization of Cr (VI) removal by Cr(VI) reducing biofilms system was carried out by three chosen independent process variables using 2^3 factorial experimental design with six star points (a=1) and six replicates at centre points, according to central composite design (CCD). The ranges and the levels of the variables investigated in the research are given in Table 1. The Cr(VI) percentage removal (%) was taken as the response of the design experiments.

Three factors were studied and their low and high levels are given in Table 1. Percentage removal was studied with a standard RSM design called central face composite design (CCD). Nineteen experiments were conducted in duplicate according to the scheme mentioned in Table 2. Design Expert Version 8.0.2 (Stat Ease, USA) was used for regression and graphical analysis of the data obtained. The optimum values of the selected variables were obtained by solving the regression equation and by analyzing the response surface contour plots and 3D. The variability in dependent variables was explained by the multiple coefficient of determination, R^2 and the model equation was used to predict the optimum value and subsequently to elucidate the interaction between the factors within the specified range.

2.6 Experimental procedures

In order to obtain optimum Cr(VI) reduction by Cr(VI)-reducing biofilm system, Cr (VI) aqueous solution with Cr (VI) ranging from 40-100 $\mathrm{mgL^{-1}}$ was mixed with (10-20 %) of LPW in 1L flask. Then the mixing solution was adjusted to pH 6.5-7.2 using 15%v/v NaOH. The solution was then pumped into the column at a down-flowrate mode at (3-6 $\mathrm{mLmin^{-1}}$). The effluents from bioreactor were collected and analysed spectrophotometrically at 540 nm using diphenylcarbazide method (DPC) [15]. The Cr(VI) percentage removal was determined as the difference between the initial metal ion concentration and effluent collected from the bottom of bioreactor in a single pass of the column.

Table 1: Independent variables: coded and real value in center composite rotatable design

Variables	Unit	Symbol	level				
			-α	-1	0	+1	+α
Nutrient supplementation (LPW)	%	A	5	10	15	20	25
Flowrate	$\mathrm{mLmin^{-1}}$	B	1.5	3	4.5	6	7.5
Initial Cr(VI) concentration	$\mathrm{mgL^{-1}}$	C	10	40	70	100	130

Table 2: Central Composite Design (CCD) for three variables together with the observed response

A: Nutrient supplementation (%)		B: Flowrate ($\mathrm{mLmin^{-1}}$)		C: Initial Cr(VI) concentration ($\mathrm{mgL^{-1}}$)		Percentage removal of Cr (VI) (%)	
Coded	Actual	Coded	Actual	Coded	Actual	Actual Value	Predicted Value
-1	10	-1	3	-1	40	90 ± 1.41	90.47
1	20	-1	3	-1	40	100 ± 1.41	101.10
-1	10	1	6	-1	40	80 ± 5.65	81.10
1	20	1	6	-1	40	90 ± 2.26	89.22
-1	10	-1	3	1	100	75 ± 1.27	76.10
1	20	-1	3	1	100	100 ± 1.55	99.22
-1	10	1	6	1	100	60 ± 1.62	59.22
1	20	1	6	1	100	80 ± 1.55	79.85
-2	5	0	4.5	0	70	70 ± 1.55	69.21
2	25	0	4.5	0	70	100 ± 1.76	100.46
0	15	-2	1.5	0	70	100 ± 2.82	99.21
0	15	2	7.5	0	70	70 ± 2.82	70.46
0	15	0	4.5	-2	10	100 ± 7.07	99.21
0	15	0	4.5	2	130	75 ± 10.6	75.46
0	15	0	4.5	0	70	80 ± 5.65	81.14
0	15	0	4.5	0	70	83 ± 2.12	81.14
0	15	0	4.5	0	70	82 ± 2.82	81.14
0	15	0	4.5	0	70	81 ± 1.41	81.14
0	15	0	4.5	0	70	80 ± 2.82	81.14

3. RESULTS & DISCUSSION

3.1 Response surface methodological approach

The results for each trial performed as per the experimental plan is given in Table 2. The application of the response surface surface methodology based on the estimates of the parameters indicated an empirical relationship between the response and input variables expressed by the following quadratic model (Eq. 1).

$$\%Cr(VI)\ removal = +81.14 + 7.81 * A - 7.19 * B - 5.94 * C + 0.93 * A^2 + 0.93 * B^2 + 1.55 * C^2 - 0.62 * A * B + 3.13 * A * C - 1.8 * B * C$$

Where A, B, C are three independent variables. A, B, and C are the coded values of the operation variables nutrient supplementation , flowrate and initial Cr(VI) concentrations.

Table 3 shows the results of the quadratic model for percentage Cr(VI) removal in the form of analysis of variance (ANOVA). The value of R^2; 0.9941 and adjusted R^2; 0.9883. The value of R^2 and adjusted R^2 is close to 1 which is very high and has advocated a high correlation between the observed values and predicted values. The associated Prob.> F value for the model is lower than 0.05 (i.e. α= 0.05, or 95 % confidence), which indicates that the model is considered to be statistically significant. The examination of the fit summary output revealed that the quadratic model is statisically significant for the response and therefore it will be used for futher analysis. Regression model provides an excellent explanation of the relationship between the independent variables (factors) and the response (Cr(VI) percentage removal %). From Anova analysis, lower value of the coefficient of variation (C.V= 1.54 %) indicates a better precision and reliability of the experiments carried out. The CV as the ratio of the standard error of estimate to the mean value of the observed response (as a percentage) is a measure of reproducibility of the model and as a general rule a model can be considered reasonably reproducible if its CV is not greater than 10% [16].

Table 3: ANOVA table for the RSM model

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	2545	9	282.78	169.65	< 0.0001	significant
A	976.56	1	976.56	585.87	< 0.0001	
B	826.56	1	826.56	495.88	< 0.0001	
C	564.06	1	564.06	338.4	< 0.0001	
A^2	20.29	1	20.29	12.17	0.0068	
B^2	20.29	1	20.29	12.17	0.0068	
C^2	56.94	1	56.94	34.16	0.0002	
AB	3.12	1	3.12	1.87	0.2041	
AC	78.13	1	78.13	46.87	< 0.0001	
BC	28.12	1	28.12	16.87	0.0026	
Residual	15	9	1.67			
Lack of Fit	8.2	5	1.64	0.96	0.5285	not significant
Pure Error	6.8	4	1.7			
Cor Total	2560	18				

DF:degrees of freedom of variance source

3.2 Effects of parameters

For reduction of Cr(VI) efficiency, nutrient supplementation was found to have greatest effect on the response, with the highest F value of 976.56. While both the flowrate and initial Cr(VI) concentration exhibited less effect regarding percentage of Cr(VI) removal. Figs 2-4 show the three-dimensional response surfaces which were generated to show the effects on the percentage of Cr (VI) removal. These graphs represent the effect of 2 variables at their studied range with the third one maintained at its fixed level.

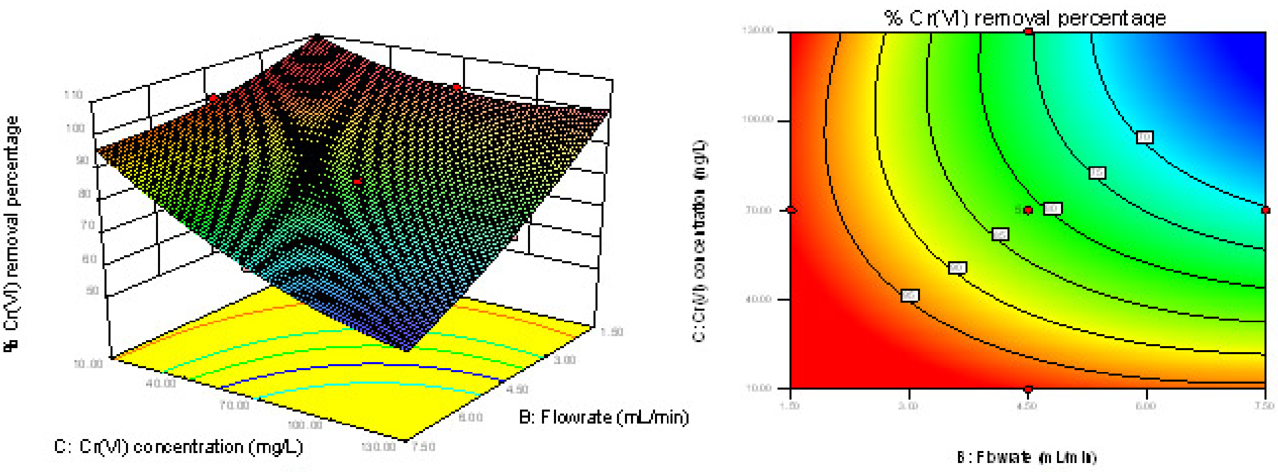
3.2.1 Effect of initial Cr(VI) concentration and flowrate

The effect of initial Cr(VI) concentration and flowrate on Cr(VI) removal percentage is shown in the form of 3D plots and surface contour. Figs 2 (a) and (b) show that with increasing initial Cr(VI) concentration and flowrate, the percentage of Cr(VI) removal decreased.

When Cr(VI) concentrations increased from 40 mg/L^{-1} to 100 mg/L^{-1}, overall Cr(VI) percentage removal decreased from 95% to 70% at fixed nutrient supplementation (15 %) (Fig 2b). The decrease in Cr(VI) removal percentage can be attributed to the fact that the synthesis of the enzyme involved in Cr(VI) reduction by *A. haemolyticus* was induced at high Cr(VI) concentration. The enzymes responsible for Cr(VI) reduction were probably inactivated and could not be synthesized at a high Cr(VI) toxic effect. From the previous studies, *A. haemolyticus* was able to grow in LB broth supplemented with Cr(VI) concentration up to 150 mg/L^{-1}. However, the bacterial growth percentage dropped to 48% at 110 Cr(VI) mg/L^{-1} indicating the apparent Cr(VI) toxicity [17].

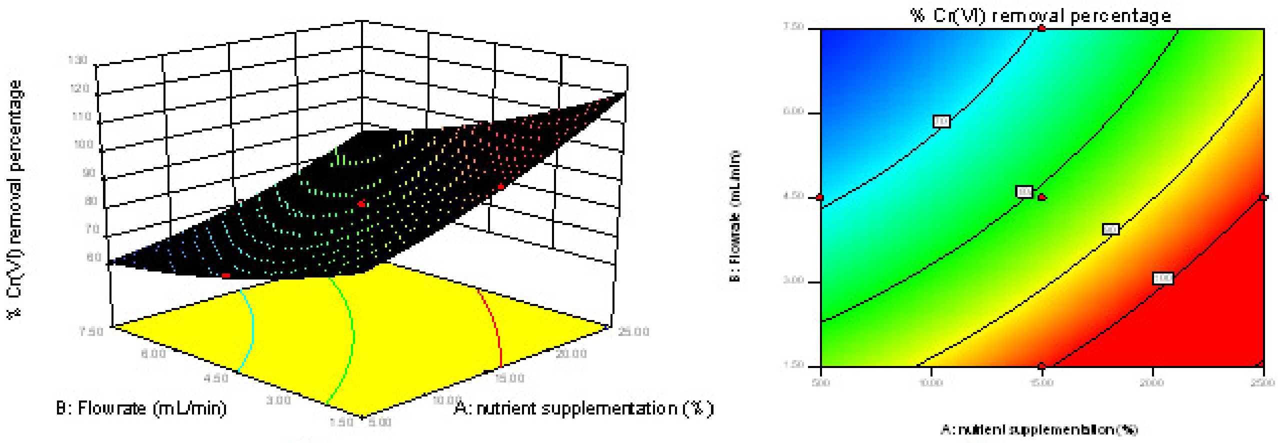
3.2.2 Effect of flowrate and nutrient suplementation

The influence of the flowrate and nutrient supplementation at fixed Cr(VI) concentration (70 mg/L^{-1}) on the removal of Cr(VI) is shown in Fig 3. The Cr(VI) percentage removal decreased from 100 % to 70% when there is an increase in flowrate from 3 to 6 mLmin^{-1} (Fig 3b). Maximum Cr(VI) removal percentage was obtained when there is an increase in nutrient supplementation. Juang et al., 2009 reported that changes in convective mass and residence time in the reactor caused by flow rate [18]. Residence time was reported as the more predominant effect. In this experiment, with further increase in flowrate from 3 to 6 mLmin^{-1} the substrate will only pass through immobilized cells without interacting well with the bacterial cells causing the drop in Cr(VI) percentage removal. Besides that, high flow rate will increase shear stress on immobilized bacteria to slide and roll over a solid support, which may lead to detachment [19]. Hence by selecting an appropriate flowrate, it is possible to achieve a very high reaction rate or even complete conversion of the substrate in a single pass of the column.



(a) (b)

Fig 2: 3D plot (a) and Contour plot (b) showing effect of initial Cr(VI) concentration and flowrate at fixed nutrient supplementation (15 %) on the Cr(VI) percentage removal.



(a) (b)

Fig 3: 3D plot (a) and Contour plot (b) showing effect of flowrate and nutrient suplementation at fixed Cr(VI) concentration (70 mg/L^{-1}) on the Cr(VI) percentage removal.

3.2.3 Effect of nutrient supplementation and Cr(VI) concentration

Biological Cr(VI) removal using immobilized cells in packed bed reactor requires additional nutrient supplementation to maintain the number of growing cells in bioreactor during Cr(VI) reduction. In this study, LPW was chosen due to a good level of total carbohydrates, a good substrate for the development of microorganisms and other components that could act as an electron donor for Cr(VI) reduction. The presence of high levels of several important micronutrients for microbial development makes it an important and promising substrate, since it does not require supplementation [20]. Besides that LPW contains organic acids such as citric acid, malic acid and oxalic acid [21] which enhance overall Cr(VI) reduction capacity by supplying electron for reduction of Cr(VI). It was also reported by Cerventas *et al*., 2008 that Cr(VI) may also be reduced by unspecific reactions associated with organic compounds such as amino acids, nucleotides, sugars, vitamins, organic acids, or glutathione. However, selecting an inexpensive and effective nutrient (carbon source) is the key to reducing cost in remediation [22].

Fig 4 shows the effect of nutrient supplementation and Cr(VI) concentration at fixed flowrate value (4.5 mLmin^{-1}) on the Cr(VI) removal percentage. Cr(VI) removal percentage increases as the nutrient supplementation increases from 70 to 95%. Microorganisms require sufficient nutrient (carbon source) to produce energy in enzymatic reduction of Cr(VI) to Cr(III). Other than that, microorganisms consume nutrients (carbon source) to reproduce new cells that might be killed due to high toxicity of Cr(VI). To optimize the Cr(VI) reduction, it is critical to determine the concentration of nutrient supplementation required to achieve the desired treatment level. The minimization of nutrient supply decreases the residual nutrient concentration in the treated effluent that usually contains high level of organic matter especially when agricultural/food processing waste was used. The minimization is important in order to reduce treatment cost and post-treatment requirements.

3.3 Optimization and verification of models

Fig 5 summarizes the optimal levels of the variables for Cr(VI) removal and predicted data of response (Cr (VI) removal percentage). Verification experiments were conducted under optimized conditions (initial Cr (VI) concentration 100 mgL^{-1}, flowrate 3 mLmin^{-1} and nutrient supplementation 20%). The maximum Cr (VI) removal percentage is shown in table 4 and it was found that the values given in the experiments are in accordance with the suggested model given by RSM. The Cr (VI)-reducing biofilms system was able to completely reduce 100 mgL^{-1} Cr (VI) at flowrate of 3 mLmin^{-1} using 20 % nutrient supplementation. Complete Cr (VI) reduction was achieved when Cr (VI) solutions was pumped through in a single pass of the biofilm reactor.

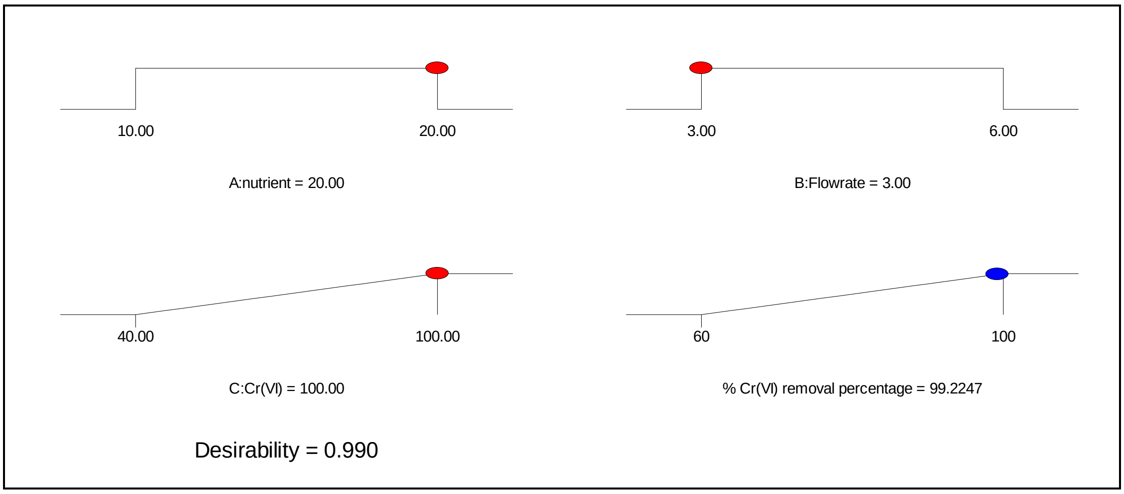


Fig 5: Suggested values of optimized conditions for Cr (VI) removal percentage given by RSM

Table 4: Predicted and experimental value for the responses at optimum condition

Independent variables	Optimized values	Cr(VI) percentage removal	
		Predicted	Experimental
Initial Cr(VI) concentration Flowrate Amount of nutrients	100 mgL^{-1} 3 mLmin^{-1} 20%	99.22%	100%

4. CONCLUSION

A Central Composite Design (CCD) was employed to study the effects of three influencing factors, which were initial Cr(VI) concentration, flowrate and nutrient supplementation, on the Cr(VI) removal efficiency by Cr(VI)-reducing biofilm system. From ANOVA analysis, the amount of nutrients with the highest F value of 976.56 was found to have the greatest effect on the response. While both the flowrate and initial Cr(VI) concentration exhibited less effect regarding percentage of Cr(VI) removal. The experimental results show that Cr (VI)-reducing biofilm systems could effectively reduce Cr (VI), 100% at the optimum conditions of initial metal concentration of 100 mgL^{-1}, nutrient supplementation of 20% and flowrate of 3mLmin^{-1}.

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