Results & Discussions

Chapter-7

CHAPTER 7

7.1 Biodegradation of reactive red 120 in the microbial fuel cell by *Staphylococcus equoruma RAP1*: Statistical modelling and process optimization

A laboratory-scale Microbial Fuel Cell (MFC) is used for the degradation of reactive red 120 (RR120) using bacterial strain *Staphylococcus equorum* RAP1 isolated from wastewater treatment plants capable of degrading 100% dye (100 ppm) in 72 h at pH 7 efficiently. The effect of pH, concentration, and time on dye degradation and electricity generation were studied using Center Composite Design (CCD) combined with Response Surface Methodology (RSM)

7.1.1 Experiment design

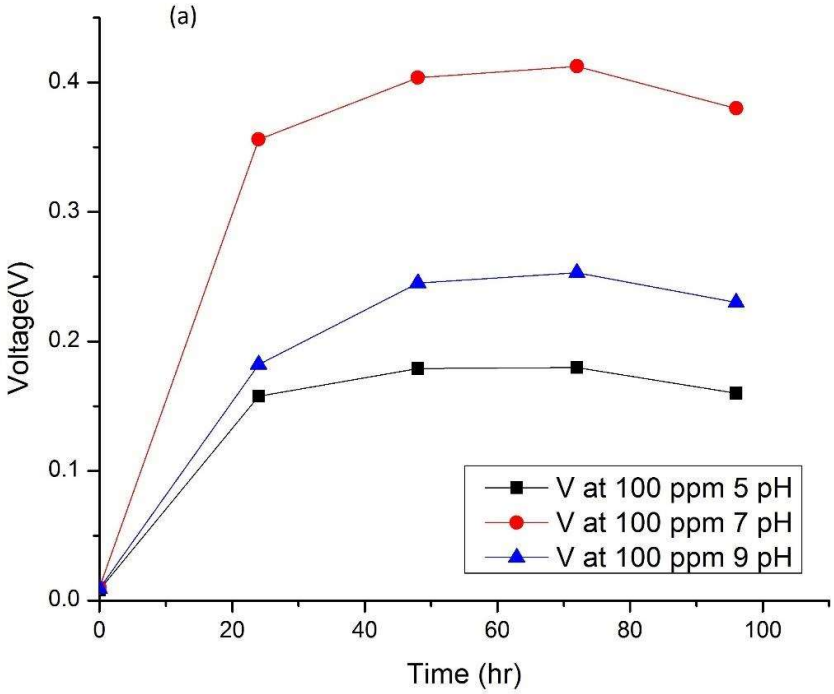
For dye biodegradation, samples were classified based on different combinations of concentrations of dye and pH. Once these are classified, each sample was run in MFC for four days. On every 4^{th} day of operation, the whole sample was removed and a new sample with 10 % inoculums was added in MFC. The sample was removed from the MFC chamber and subjected to centrifugation at 5000 rpm for 10 min. After centrifugation, samples were filtered with a 0.22 μ cellulose-based syringe filter and stored at 4° C for further analysis.

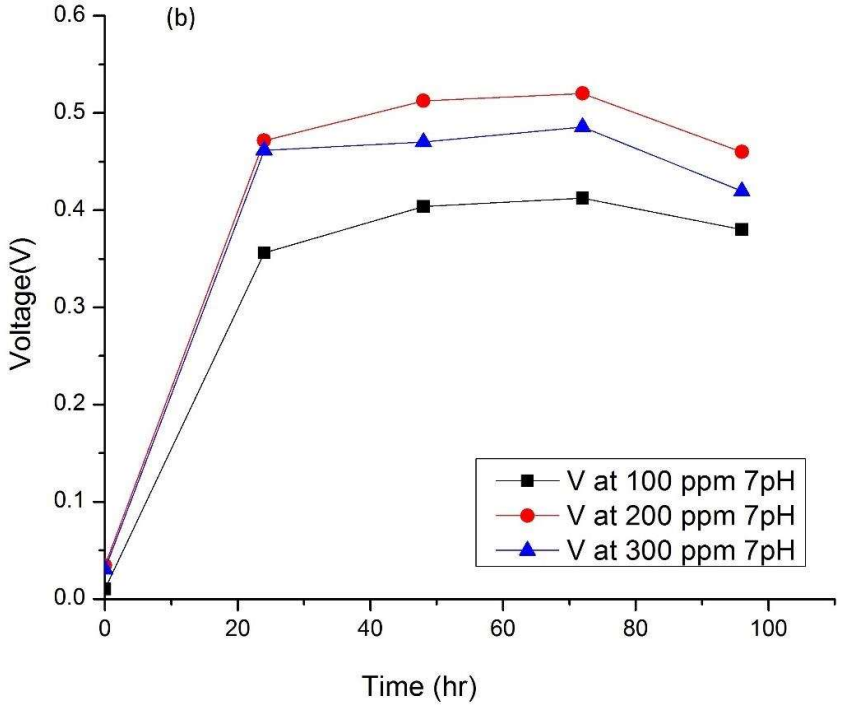
7.1.2 Result and Discussions

- **The operation of MFC**

MFC was operated at different combinations of pH and concentration of RR120 dye for 80 h. The initial concentration of dye (100, 200, 300 ppm) and pH (5.0,7.0,9.0) were chosen based on results of previous experiments with *Staphylococcus equorum* as

inoculums and literature review which show the better activity of this species at slightly higher than neutral pH(Nisar et al., 2017; Vijay et al., 2018). The experimental time was selected in such a way that the maximum degradation and voltage rise would be achieved in the MFC. On observation of a voltage drop, the whole anolyte was replaced with a new anolyte having different concentrations of dye and pH. Generally, it was done, on every 4^{th} day of MFC operation. All the data were recorded in triplicates and averages were taken for further calculation. **Figure 7.1(a)** showed the variation of voltage at a fixed initial concentration of dye with different pH. It is evident in **figure 7.1(a)** that at pH 7.0 a higher voltage was observed than at pH 5.0 and 9.0. Here voltage continuously increases for 72 h thereafter decreases due to a decrease in colony-forming unit/ml (CFU/ml). The operation of MFC was studied at varying concentrations of dye and a constant pH of 7.0. In **figure 7.1(b),** the increase of voltage was observed when the initial concentration of dye was increased to 200 ppm, and thereafter, the voltage drop was observed. The highest voltage of 0.52 V was obtained at 200 ppm at pH 7.0 after 72 h of operation of MFC. From **figure 7.1(c),** it was evident that the power density obtained in the case of pH 7.0 is higher than pH 5.0 and pH 9.0. In **figure 7.1(d),** the power density obtained within 20 h of operation for 200 and 300 ppm was approximately the same but after 20 h, the power density for 200 ppm was higher than both 100 and 300 ppm. It indicates that with the increased concentration from 100 to 200 ppm, the availability of dye promotes more voltage production but after 200 ppm voltage drop started, this phenomenon may be attributed to the toxicity of metabolites to microorganisms (Hiegemann et al., 2018; Ma et al., 2014).


(a)
Voltage(V)
Time (hr)
V at 100 ppm 5 pH
V at 100 ppm 7 pH
V at 100 ppm 9 pH


(b)
Voltage(V)
Time (hr)
V at 100 ppm 7pH
V at 200 ppm 7pH
V at 300 ppm 7pH

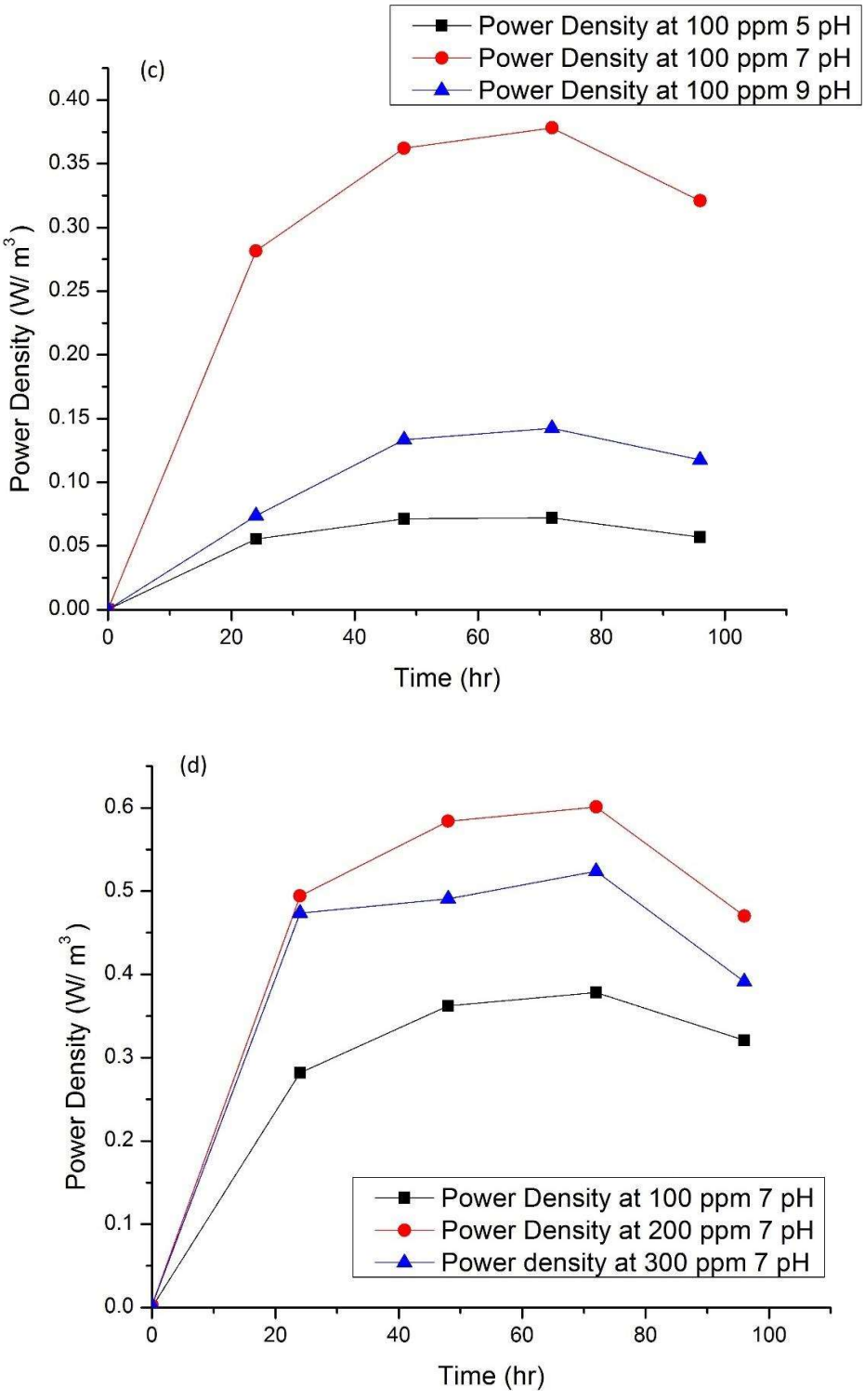


Figure 7.1 (a) voltage variation with time at different pH for 100 ppm dye; (b) voltage variation with time at pH 7.0 for different concentration of dye; (c) power density variation with time at different pH for 100 ppm dye; (d) power density variation with time at pH 7.0 for different concentration of dye.

Degradation was studied for RR120 at different concentrations (100 to 300 ppm) and pH 5.0 to 9.0, and it was clear that dye degradation increases with an increase in the concentration of dye. Maximum dye degradation obtained for 300 ppm at 7 pH was 197.70 ppm. The same pattern was reported by other researchers (Saha and Rao, 2019; Thung et al., 2018). But the % degradation follows the reverse order, with the increase in the concentration of dye, % degradation decreases (**Figure 7.2**). This phenomenon may be attributed to the toxicity of the degraded metabolites towards microorganisms at a higher concentration of dyes (Ghodake et al., 2011; Hiegemann et al., 2018). For the same concentration; better degradation was observed at pH 7.0 than 9.0 and 5.0 (Appendix 1). % Degradation of dyes follows the order for pH 7.0 >9.0 >5.0 and for concentration (ppm) 100>200>300. It was also clear from the graph that % degradation for 100 ppm of dye at pH 7.0 was maximum in all the cases and found to be approximately 100 % in 72 h.

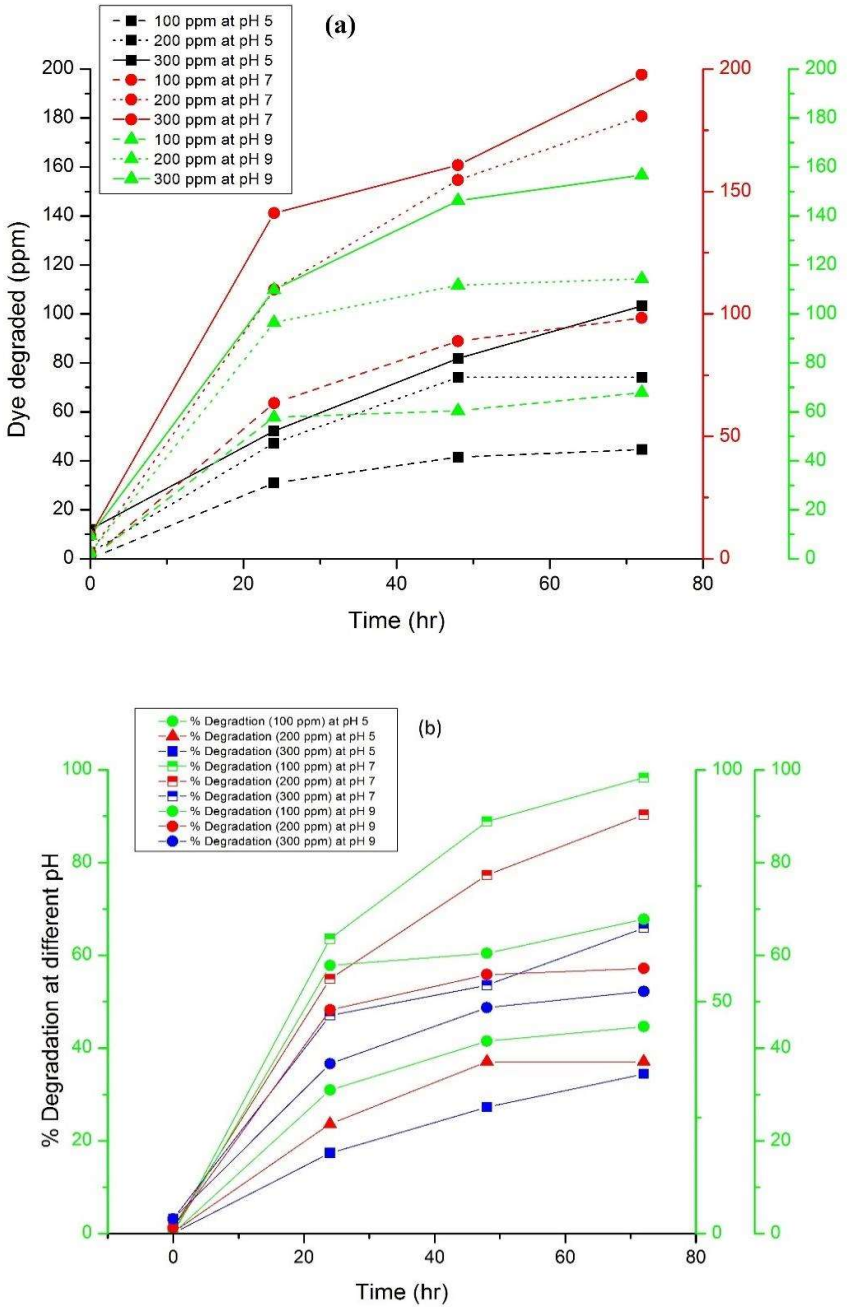


Figure 7.2 (a).Degradation of dye at different pH and concentration, and (b) % Degradation of dye at different pH and concentration.

The same pattern was followed for COD removal efficiency (**Figure 7.3**). With the increased concentration of dyes COD removal efficiency decreased and maximum COD removal efficiency, 79.75 % for 100 ppm of dye at pH 7.0 was obtained (Appendix 1).

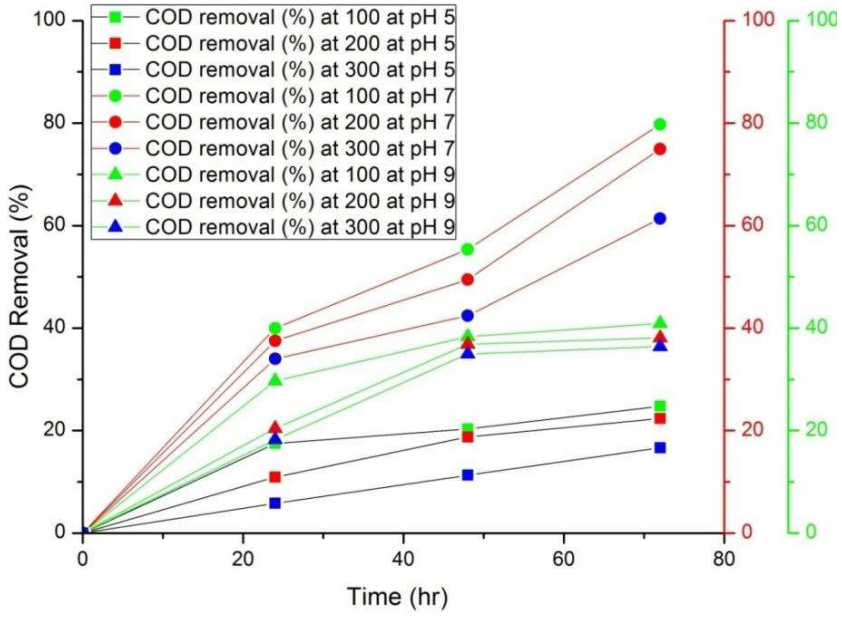


Figure 7.3 COD removal efficiency

- **Statistical modeling and Optimization of RR120 degradation & electricity generation**

An empirical relationship between the responses in terms of concentration of dye degraded (ppm)and design parameters using RSM is given in **equation 7.1**. The detailed design of experiments with two responses (Response 1 and Response 2) with their predicted and actual results for the degradation of RR120 is shown in **table 7.1.**

Table 7.1: Design table for Face Centered Central Composite design for MFC

Std	A: pH	B: The concentration of RR 120 (ppm)	C: Time (h)	Response 1 The concentration of dye degraded (ppm)		Response 2 Current density (A/m^3)	
				Actual Value	*Predicted Value*	*Actual Value*	*Predicted Value*
1	5.0	100	24	30.90	30.90	0.35	0.3094
2	9.0	100	24	57.54	56.23	0.4	0.4734

3	5.0	300	24	52.48	51.28	0.22	0.1734
4	9.0	300	24	70.79	70.79	0.36	0.3374
5	5.0	100	72	44.66	43.65	0.4	0.3754
6	9.0	100	72	67.60	69.18	0.56	0.5394
7	5.0	300	72	102.32	104.71	0.27	0.2394
8	9.0	300	72	125.89	125.89	0.38	0.4034
9	5.0	200	48	74.13	74.13	0.33	0.4725
10	9.0	200	48	112.20	109.64	0.69	0.6365
11	7.0	100	48	89.12	91.20	0.89	0.9025
12	7.0	300	48	162.18	158.48	0.69	0.7665
13	7.0	200	24	109.64	112.20	1.03	0.9996
14	7.0	200	72	181.97	177.82	1.08	1.07
15	7.0	200	48	154.88	154.80	1.04	1.03
16	7.0	200	48	154.8	154.80	1.04	1.03
17	7.0	200	48	154.88	154.80	1.04	1.03
18	7.0	200	48	154.81	154.80	1.04	1.03
19	7.0	200	48	154.88	154.80	1.04	1.03
20	7.0	200	48	154.76	154.80	1.04	1.03

- **Model response in terms of Concentration of dye degraded**

Response 1 is for the concentration of dye degraded and its empirical relation is shown in **equation 7.1**.

$$\textbf{Concentrationofdyedegraded} = 152.42 + 12.71\mathrm{A} + 22.20\mathrm{B} + 20.08\mathrm{C} - 1.18\mathrm{AB} + .0412\mathrm{AC} + 10.21\mathrm{BC} - 56.16\mathrm{A}^2 - 24.30B^2 - 3.83C^2 \quad \textbf{(7.1)}$$

Based on the Box-Cox plot analysis, the model was transformed into a log10 scale and is given in **equation 7.2**.

$$\log_{10}\text{Concentrationofdyedegraded} = 2.19 + 0.0840A + 0.1203B + 0.0995C - 0.0294AB - 0.0167AC + 0.0393BC - 0.2324A^2 - 0.1141B^2 - 0.0426C^2 \quad \textbf{(7.2)}$$

The adequacy of the model was tested by Analysis of variance (ANOVA) and the results are shown in **Table 7.2**. According to Zhang et al. and Pereira et al. (Pereira et al., 2018; Q. Zhang et al., 2017), larger F values, and low p-value signify that the predicted model is significant. Overall P-values are less than 0.0001 for A, B, C, AB, BC, AC, A^2, B^2, and C^2 and F value (1699.69) indicate model terms are significant and the Quadratic model is the best fit for response 1 (degradation of dye) and independent variable (Padmanaban et al., 2018). With the results of ANOVA, it was observed that linear term pH (A), concentration (B) and time (C), and interactive term concentration and time have positive attributes whereas, the interactive term pH and concentration, pH and time and quadratic terms of pH, concentration and time have negative attributes for the response 1. The **Predicted model has an R^2** of 0.99 with the **Adjusted R^2** of 0.99 along with a coefficient of variation of 0.39 and an **Adequate Precision** value of 137.37 a ratio greater than 4 which is desirable. The relationship between the actual and predicted value of $\log_{10}$ (concentration of dye degraded) is shown in **table7.1 and figure 7.4**.

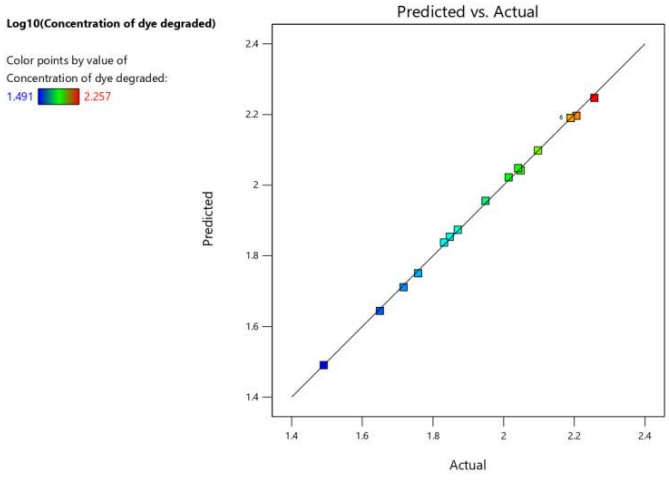


Figure7.4 Actual vs Predicted value of RR120 dye

Table 7.2: Analysis of variance (ANOVA) for the degradation of RR120 by *Staphylococcus equorum* RAP1 terms of concentration of dye degraded

Source	Sum of	df	Mean	F-value	p-value
Model	0.9277	9	0.1031	1699.69	< 0.0001
A-pH	0.0706	1	0.0706	1164.51	< 0.0001
B-Concentration of dye	0.1447	1	0.1447	2385.66	< 0.0001
C-Time	0.0990	1	0.0990	1631.76	< 0.0001
AB	0.0069	1	0.0069	113.64	< 0.0001
AC	0.0022	1	0.0022	36.57	0.0001
BC	0.0124	1	0.0124	204.00	< 0.0001
A^2	0.1485	1	0.1485	2449.35	< 0.0001
B^2	0.0358	1	0.0358	590.40	< 0.0001
C^2	0.0050	1	0.0050	82.33	< 0.0001
Residual	0.0006	10	0.0001		
Lack of Fit	0.0006	5	0.0001		
Pure Error	0.0000	5	0.0000		
Cor Total	0.9283	19			

- **Analysis of operational parameters of Response surface methodology**

With the help of the regression equation, the two dimensional (contour) and three dimensional (3D surface) plots had been developed for a better understanding of the interactive effects of two variables on the responses (Jegan Durai et al., 2020; Muniyasamy et al., 2020).

Interactive effect of pH (A) & concentration of dye (B) towards the Concentration of dye degraded:

From **figure 7.5 (a, b)**, the interactive effect of pH and concentration of dye can be observed as significant with $P < 0.0001$ along with their negative attributes on the dye degradation. As dye concentration increased from 100 ppm to 300 ppm, the degradation of dye decreased. But with an increase in pH from 5 to 7, degradation of dye increased

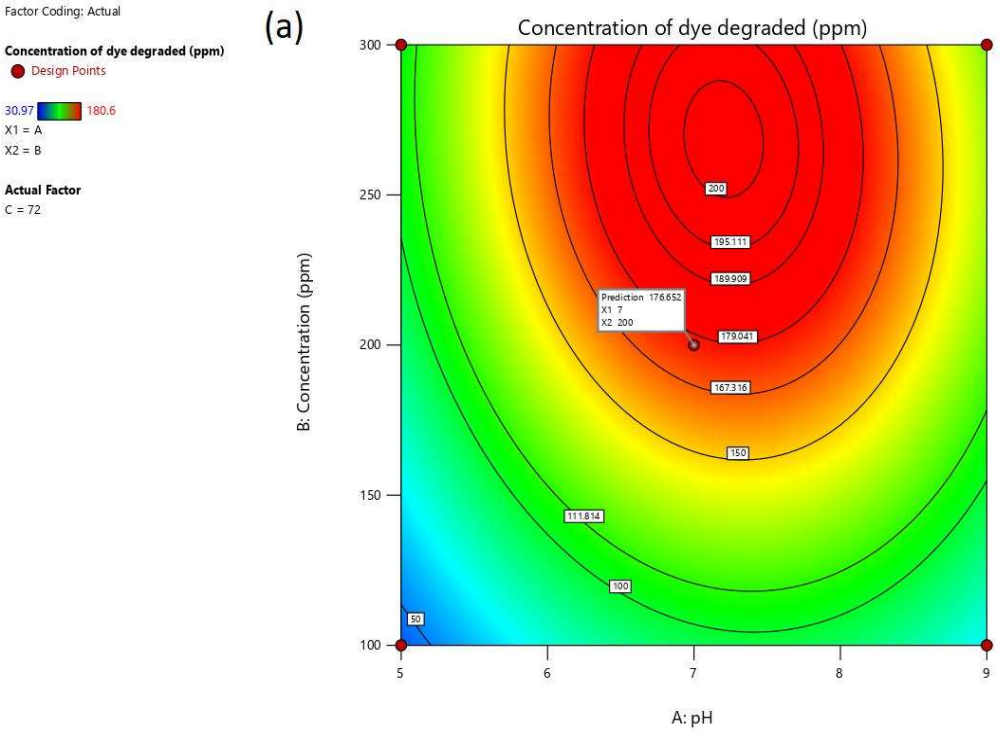
but further increase in pH resulted in a reduction of the degradation of dye. At pH 7.0 and 200 ppm of dye, 176.52 ppm of dye gets degraded. As the pH reached acidic (5.76), only 130.95 ppm of dye gets degraded. In slightly basic (pH 8.53), dye degradation increased to a value of 145.88 ppm than acidic but lower than pH 7. This behavior is supported by literature reported earlier (Padmanaban et al., 2016; U. Roy et al., 2018).

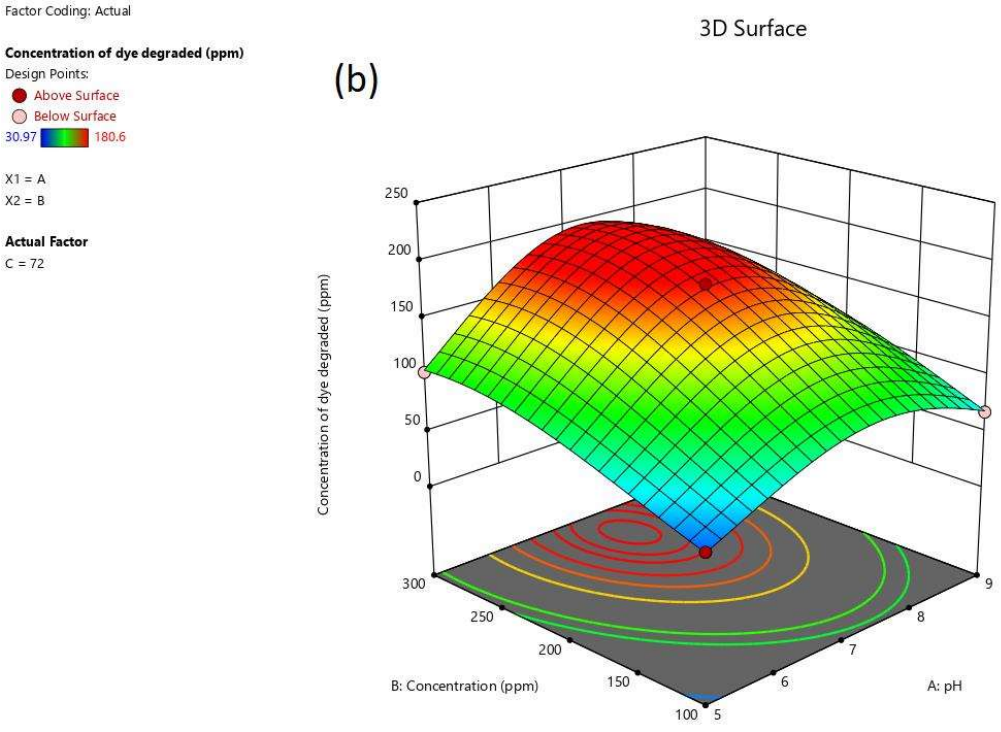
Interactive effect of pH (A) and time (C) towards the Concentration of dye degraded:

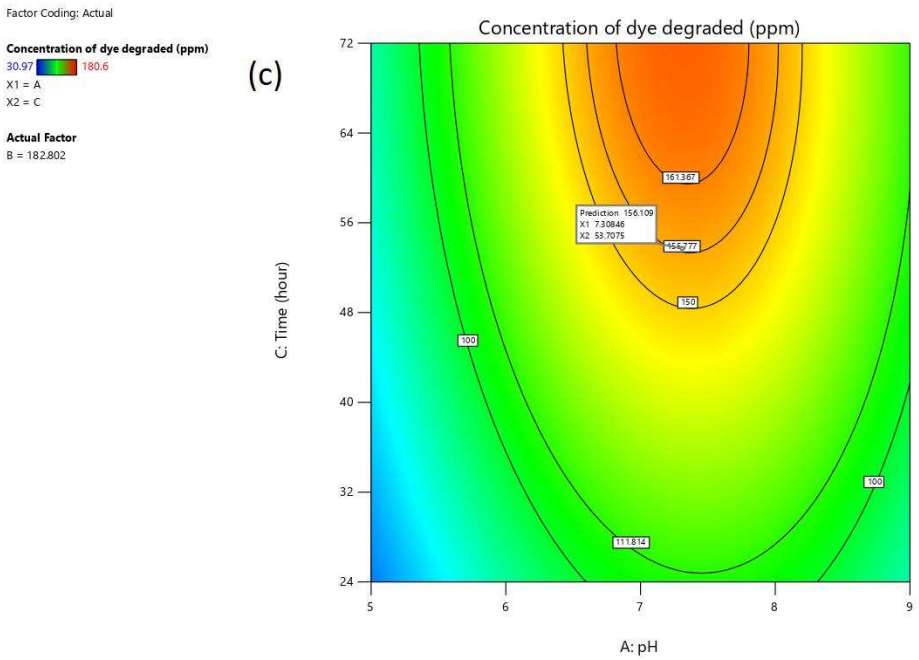
Figure 7.5 (c, d), shows the interactive effect of pH and time effect on dye degradation which is significant P<0.0001. At a fixed concentration of 182.87 ppm, at pH 6.97 degradation increased from 129.61 ppm to 157.16 ppm as time increased from 36.73 h to 59.7 hr. But as the pH increased from acidic (5.8) to neutral (6.8), dye degradation increased from 105.96 ppm to 141.69 ppm but a further increase in pH (8.3) leads to a decrease in dye degradation to only 130.08 ppm. Overall, it has a negative interaction with the dye degradation process. The same pattern is observed by many researchers(Kaur et al., 2015; Muniyasamy et al., 2020).

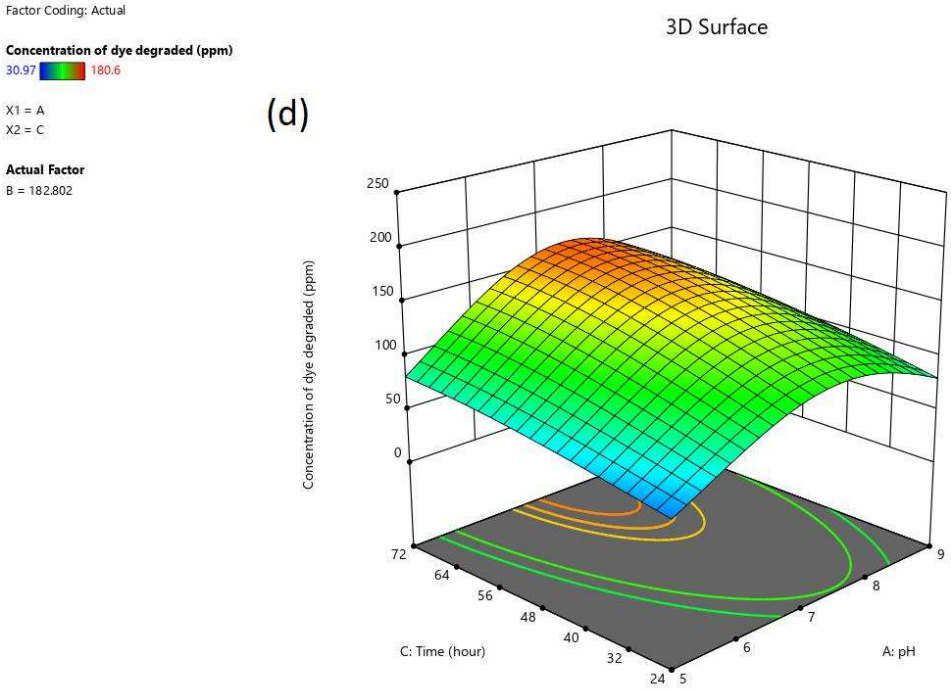
Interactive effect of time (C) and concentration (B) towards the Concentration of dye degraded:

Figure 7.5 (e, f), shows the interactive effect of time and concentration with a P-value <0.0001 which is significant. This interaction has a positive effect on dye degradation. As time increased from 38.06 h to 53.39 h dye degradation also increased from 139.84 ppm to 163.55ppm for 197 ppm of dye at pH 7.17. This work is supported by previously reported literature(Kaur et al., 2015; U. Roy et al., 2018).


Factor Coding: Actual
Concentration of dye degraded (ppm)
Design Points
30.97
180.6
X1 = A
X2 = B
Actual Factor
C = 72
(a)
Concentration of dye degraded (ppm)
B: Concentration (ppm)
A: pH
Prediction 176.652
X1 7
X2 200
200
195.111
189.909
179.041
167.316
150
111.814
100
50


Factor Coding: Actual
Concentration of dye degraded (ppm)
Design Points:
Above Surface
Below Surface
30.97
180.6
X1 = A
X2 = B
Actual Factor
C = 72
3D Surface
(b)
Concentration of dye degraded (ppm)
B: Concentration (ppm)
A: pH


Factor Coding: Actual
Concentration of dye degraded (ppm)
30.97
180.6
X1 = A
X2 = C
Actual Factor
B = 182.802
(c)
Concentration of dye degraded (ppm)
Prediction 156.109
X1 7.30846
X2 53.7075
161.367
156.777
150
100
111.814
C: Time (hour)
A: pH


Factor Coding: Actual
Concentration of dye degraded (ppm)
30.97
180.6
X1 = A
X2 = C
Actual Factor
B = 182.802
(d)
3D Surface
Concentration of dye degraded (ppm)
C: Time (hour)
A: pH

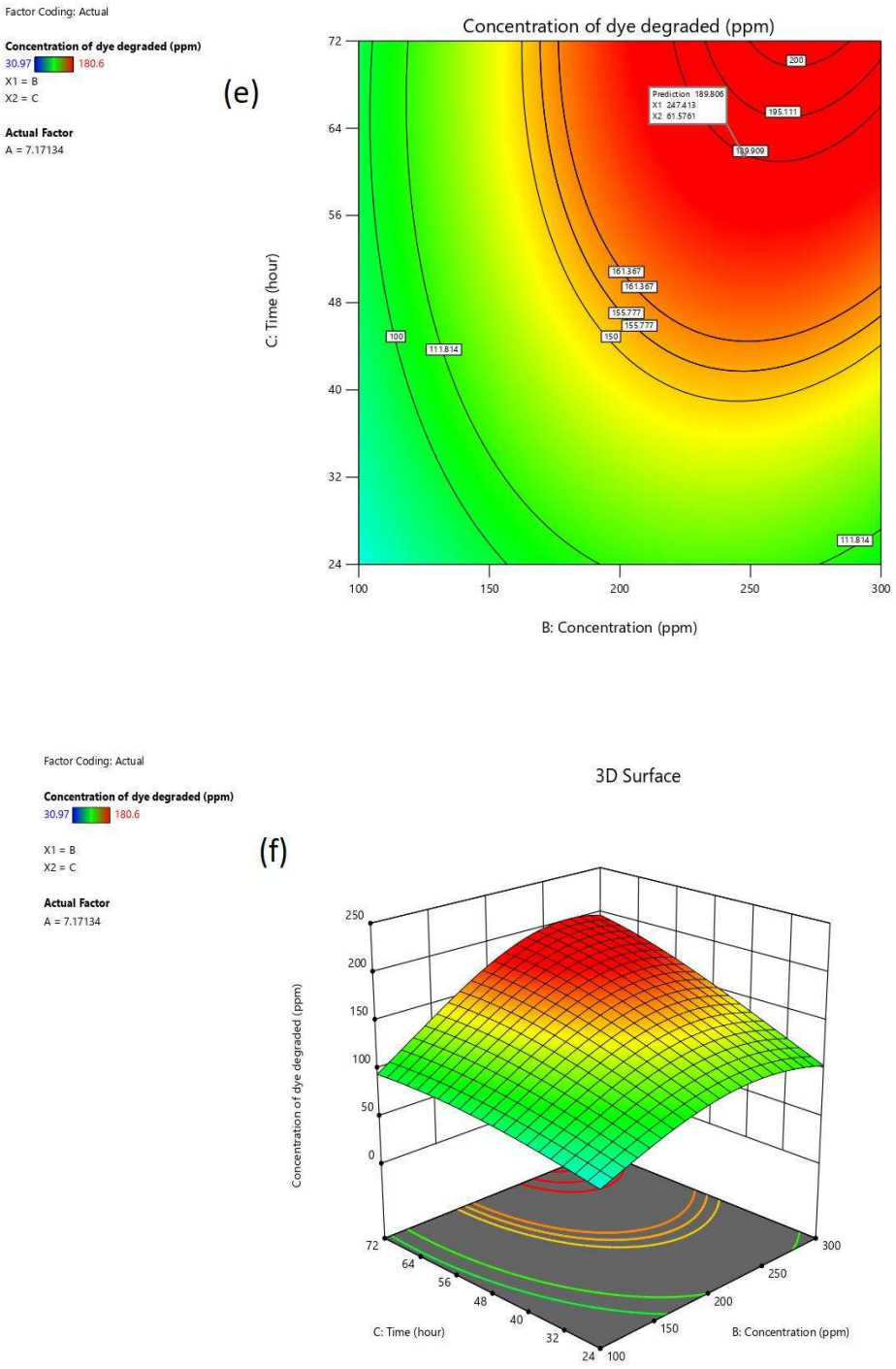


Figure 7.5 (a) 2D Contour plot and 3(b) 3D Response surface plot for the degradation of RR120 dye as a function of the concentration of dye and pH, (c) 2D Contour plot and (d) 3D Response surface plot for the degradation of RR120 dye as a function of time and pH (e) 2D Contour plot and 5(f)3D Response surface plot for the degradation of RR120 dye as a function of time and concentration of dye

- **Model response in terms of current density:**

Response 2 is for current density and its empirical relation is reduced for its significant parameters and shown in **equation 7.3.**

$$\boldsymbol{Currentdensity} = 1.03 + 0.0820A - 0.0680B + 0.0330C - 0.4781A^2 - 0.1981B^2 \quad \textbf{(7.3)}$$

The adequacy of the model was tested by Analysis of variance (ANOVA) and the results are shown in **Table 7.3.** The **Model F-value** of 132.72 implies that the model is significant. **P-values** less than 0.05 indicate model terms are significant and the Quadratic model is the best fit for response 2. In this case, A, B, A^2, B^2 are significant model terms. In this, pH and time have positive attributes whereas quadratic terms pH and concentration have negative attributes on response 2 (current density). The interactive effect of pH, concentration, and time does not affect response 2. The **Predicted model has an R^2** of 0.94 with an **adjusted R^2** of 0.97 along with the coefficient of variation of 7.97 % and an **Adequate Precision** value of 29.43 which is greater than 4 which is desirable for the model to be the best fit. The relationship between actual and predicted value current density is shown in **table 7.2 and figure 7.6.**

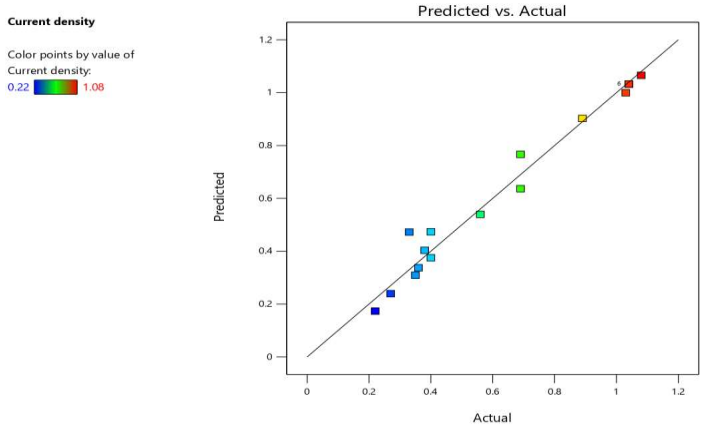


Figure 7.6 Predicted Vs actual value of current density

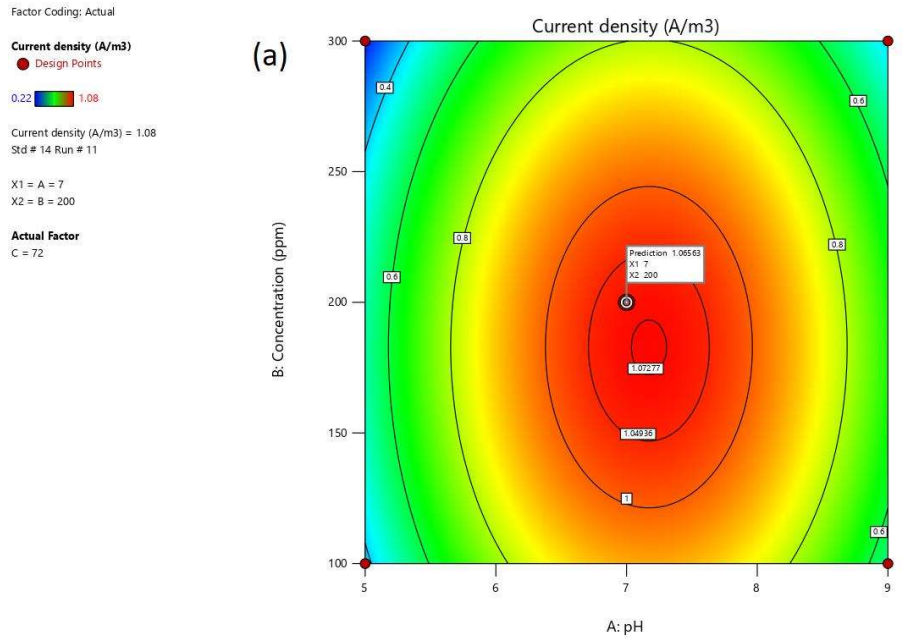
Table 7.3: Analysis of variance (ANOVA) for the degradation of RR120 by *Staphylococcus equorum* RAP1 terms of Current density

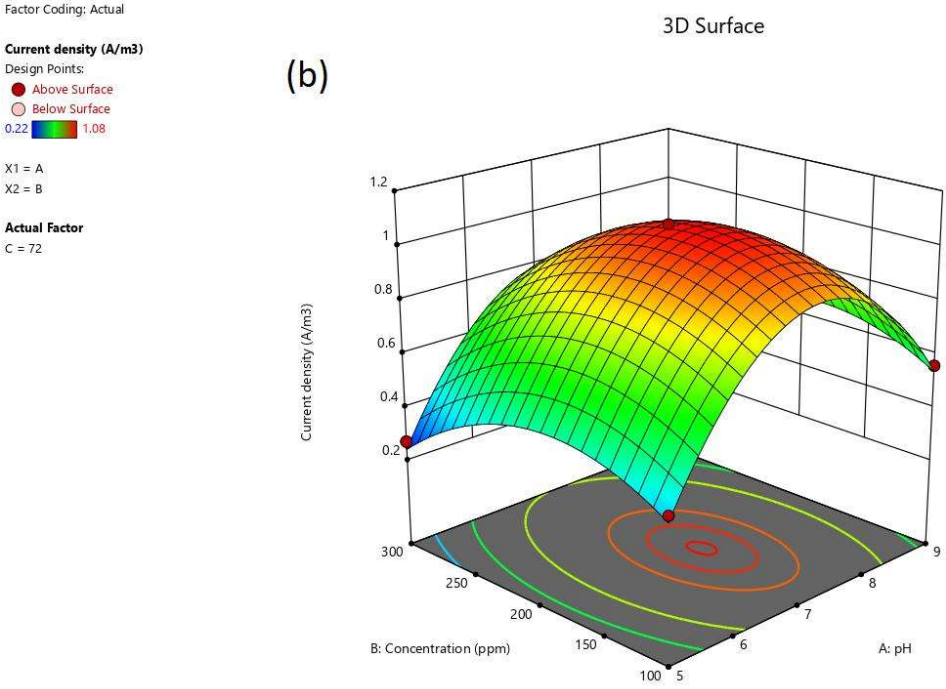
Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	2.03	5	0.4064	132.72	< 0.0001
A-pH	0.0672	1	0.0672	21.96	0.0004
B- Concentration of dye	0.0462	1	0.0462	15.10	0.0016
C-Time	0.0109	1	0.0109	3.56	0.0802
A^2	0.7315	1	0.7315	238.90	< 0.0001
B^2	0.1256	1	0.1256	41.02	< 0.0001
Residual	0.0429	14	0.0031		
Lack of Fit	0.0429	9	0.0048		
Pure Error	0.0000	5	0.0000		
Cor Total	2.07	19			

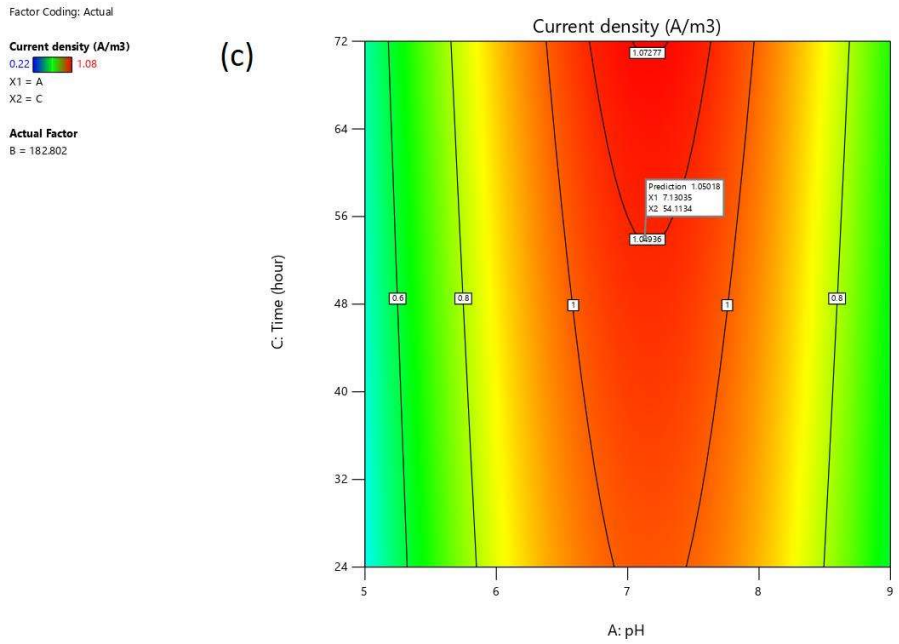
- **Analysis of operational parameters of Response surface methodology**

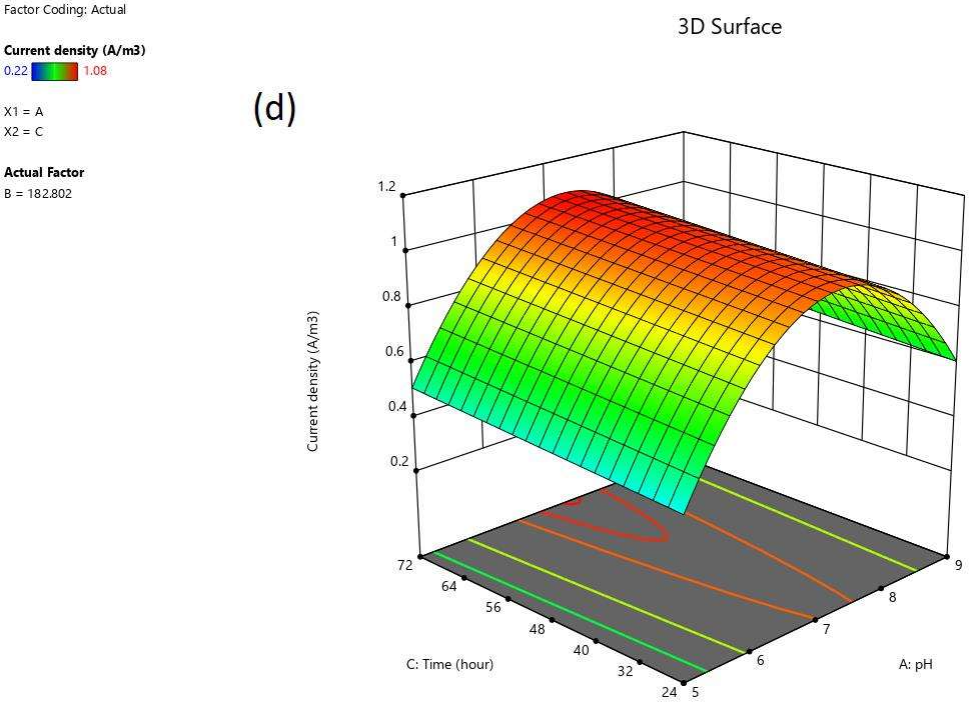
Interactive Effect on Current Density

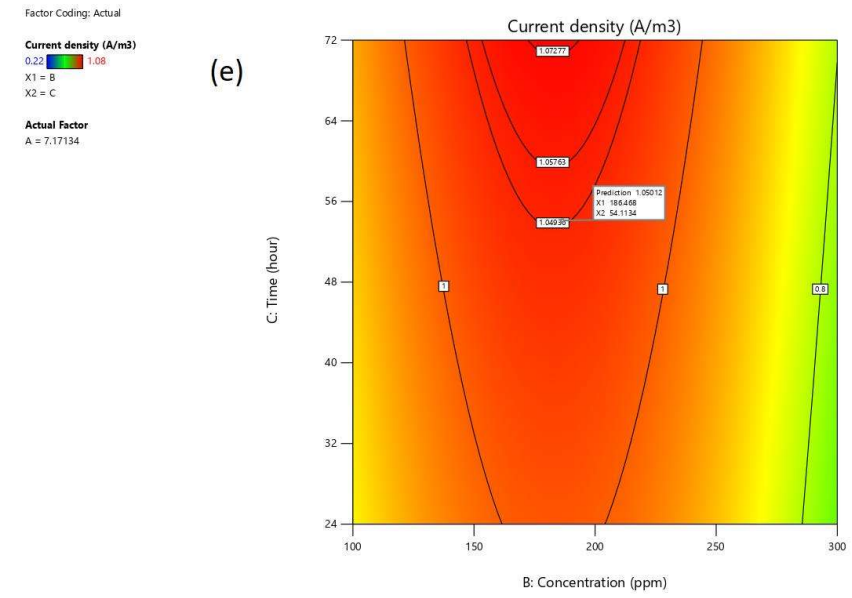
Figure 7.7 showed the 2D contour and 3D surface plots for current density. Parameters A(pH), B(concentration), C(time) has a single and quadratic effect on current density and has a significant value with $P<0.05$ but the AB, AC, and BC do not have any significant effect on current density.


Factor Coding: Actual
Current density (A/m3)
Design Points
0.22
1.08
Current density (A/m3) = 1.08
Std # 14 Run # 11
X1 = A = 7
X2 = B = 200
Actual Factor
C = 72
(a)
Current density (A/m3)
Prediction 1.06563
X1 7
X2 200
1.07277
1.04936
1
B: Concentration (ppm)
A: pH
300
250
200
150
100
5
6
7
8
9


Factor Coding: Actual
Current density (A/m3)
Design Points:
Above Surface
Below Surface
0.22
1.08
X1 = A
X2 = B
Actual Factor
C = 72
(b)
3D Surface
Current density (A/m3)
1.2
1
0.8
0.6
0.4
0.2
B: Concentration (ppm)
300
250
200
150
100
A: pH
5
6
7
8
9


Factor Coding: Actual
Current density (A/m3)
0.22
1.08
X1 = A
X2 = C
Actual Factor
B = 182.802
(c)
Current density (A/m3)
1.07277
Prediction 1.05018
X1 7.13035
X2 54.1134
1.04936
0.6
0.8
1
1
0.8
C: Time (hour)
72
64
56
48
40
32
24
5
6
7
8
9
A: pH


Factor Coding: Actual
Current density (A/m3)
0.22
1.08
X1 = A
X2 = C
Actual Factor
B = 182.802
(d)
3D Surface
Current density (A/m3)
1.2
1
0.8
0.6
0.4
0.2
72
64
56
48
40
32
24
C: Time (hour)
5
6
7
8
9
A: pH



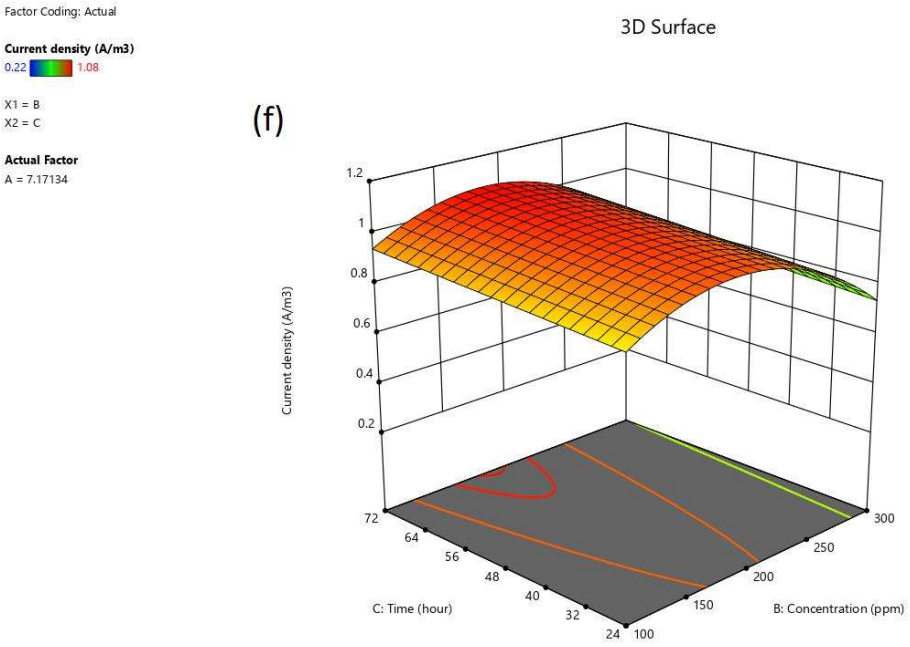


Figure 7.7 (a) 2DContour plot and (b)3D Response surface plot for current density as a function of concentration and pH,(c) 2DContour plot and (d)3D Response surface plot for current density as a function of time and pH(e) 2D Contour plot and (f)3D Response surface plot for current density as a function of time and concentration

Optimized numerical solutions for degradation of dye and current density:

Various responses along with their variables were optimized and solutions are given in **Table7.4**. Variables like pH, concentration, and time were kept in the range while response degradation of dye and current density were maximized using an optimization technique. From the various solution, when the pH was kept 7.19 with a concentration of dye 203.46 in 72 h resulted in maximum dye degradation 180.6 with the 1.06 A/m^3 current density. A quadratic model was found to be the best fit for these parameters. The predicted and actual response (**Table7.2**) has an error of ±0.5%, which suggests that the model is suitable for predicting the behavior of dye in the microbial fuel cell**.**

Table 7.4 Optimized numerical solutions for degradation of dye and current density

Number	pH	The concentration of dye (ppm)	Time (h)	The concentration of dye degraded (ppm)	Current density (A/m^3)	Desirability
1	7.197	203.460	72.000	180.600	1.066	0.992
2	7.189	203.514	72.000	180.599	1.066	0.992
3	7.171	203.667	72.000	180.601	1.066	0.992
4	7.224	203.301	72.000	180.598	1.066	0.992
5	7.147	203.903	71.999	180.602	1.066	0.992
6	7.218	202.210	71.999	179.900	1.067	0.991

- **Analysis of degraded metabolites of RR 120**

Functional group identification using FTIR analysis

FTIR spectra are given in **figure 7.8(a)** showed considerable variation between the treated and the control dye sample. Spectra **(Figure 7.8(a))** obtained at 3854.54 cm^{-1} is due to -OH stretching of benzene substituted alcohol and at 3448.39 cm^{-1} is due to –NH stretching of amine group present in RRR120 dye. -N=N- stretching of the azo bond was obtained at 1637.18 cm^{-1} and 1560.28 cm^{-1} was due to -N=N- stretching of N present inside the benzene ring. The peak obtained at 1028.49 cm^{-1} and 499.80 cm^{-1} was due to C-O

stretching of meta substituted Azobenzene and Fingerprint region of -C=C- out of plane ring bending respectively. It can be observed in **figure 7.8(b),** there are significant increases in transmittance value at 1635.56 cm^{-1} which is a characteristics peak of azo bond present in the dye which can be concluded that some of the dye presents in MFC get biodegraded and it confirms the breakdown of azo bond preset in the dye. Some new peaks were also obtained at 1419.14cm^{-1} and a shift in the peak was obtained at 500.10 cm^{-1} and 469.02 cm^{-1} which is due to Aromatic ring stretching, fingerprint region and corresponds to the -OH out of plane bending vibration and Fingerprint region of -C=C- out of plane ring bending respectively. These significant peak shifts and the generation of new peaks confirmed the biodegradation of RR120 dye in MFC(Srikantan et al., 2018; Subbaiah et al., 2019)**.**

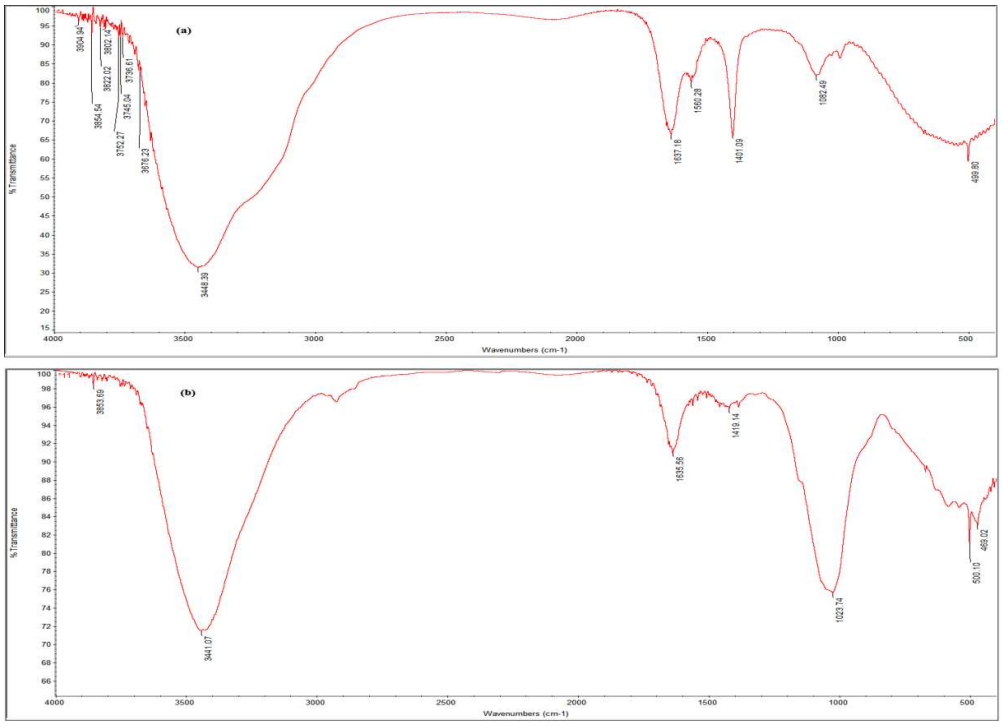
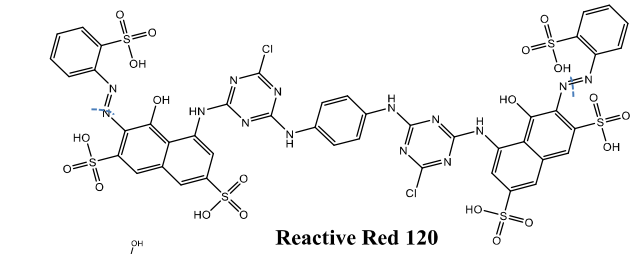


Figure 7.8 (a) FTIR spectra of RR120 dye (Control) and (b) FTIR spectra of degraded metabolites

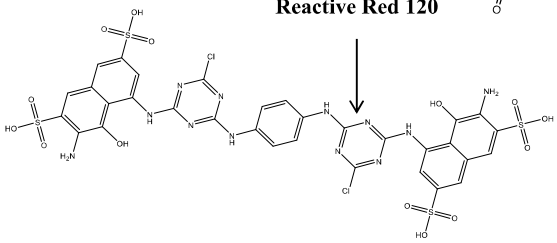
Degradation pathway using LCMS analysis

During the biodegradation of RR120, many stable and unstable metabolites are formed. But only those that are stable can be detected in LCMS analysis. A possible mechanism of RR120 degradation could be the cleavage of N=N azo bonds which generates many unknown and known intermediate. LCMS Spectra for pure RR120 dye and its metabolites is given in supplementary data LCMS spectra for metabolites evident that smaller molecular weight compounds are formed which is due to biodegradation of RR120(Appendix 1). These spectra revealed 2-amino-3-phenylpropanoic acid (m/z 165.2) and 2-amino-3-(2-hydroxyphenyl) propanoic acid (m/z = 181.3),3-amino-5-((4-amino-6-chloro-1,3,5-triazin-2-yl)amino)-4-hydroxynaphthalene-2-sulfonic acid (m/z = 383.1) etc. as known intermediates. Swarnkuma et al. (Swarnkumar and Osborne, 2020) had reported the same compounds as degraded products when RR120 get decolorized using *Pseudomonas guariconensis*. The complete catabolic pathway of RR120 degradation using *Staphylococcus equorum* has to be studied. Many pathways are proposed by researchers in which many amino derivatives are produced as intermediates such as 2-amino-3-phenylpropanoic acid, 2-(2-amino-2-formylethyl)-3-hydroxybenzoic acid, 2-(2-amino-2-formylethyl)-3-hydroxybenzoic acid, 2-amino-3-(2-hydroxyphenyl) propanoic acid, 2-amino-3-(2,6-dihydroxyphenyl) propanoic acid, 3-hydroxyphthalic acid, benzene-1,4-diamine, 2-hydroxybenzoic acid, 8-aminonaphthalene-1,2,3,6-tetraol, 3-Amino-5 [(4-amino-6-chloro-1,3,5-triazin-2-yl)amino]-4- hydroxynaphathalene -2-sulfonic acid, 3-Amino-4-hydroxynaphthalene-2-7-disulfonic acid, -chloro-1,3,5-triazine-2,4-diamine (Oturkar et al., 2011; Pirkarami and Olya, 2014; Swarnkumar and Osborne, 2020). The proposed pathway for biodegradation of RR120 by *Staphylococcus equorum* RAP1 is given in **figure 7.9** and these metabolites were are reported to be used by bacteria for their growth and maintained which could be the reason for its complete

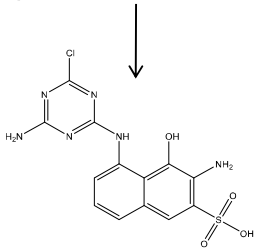
mineralization. The complete catabolic pathway for metabolites of RR120 has to be studied.



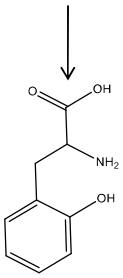
Reactive Red 120



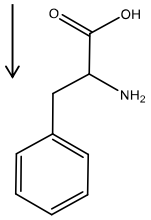
5,5'-(((1,4-phenylenebis(azanediyl))bis(6-chloro-1,3,5-triazine-4,2-diyl))bis(azanediyl))bis(3-amino-4-hydroxynaphthalene-2,7-disulfonic acid)
Molecular Weight: 999.75
m/z: 996



3-amino-5-((4-amino-6-chloro-1,3,5-triazin-2-yl)amino)-4-hydroxynaphthalene-2-sulfonic acid
Molecular Weight: 382.78
m/z: 383.1



2-amino-3-(2-hydroxyphenyl)propanoic acid
Molecular Weight: 181.19
m/z: 181.3



2-amino-3-phenylpropanoic acid
Molecular Weight: 165.19
m/z: 165.2

Figure 7.9 Proposed mechanism of biodegradation of RR120 dye by *Staphylococcus equorum RAP1*

- **Coulombic efficiency (%CE)**

At the end of the experiment %, CE was calculated and found to be 2.25 % for *Staphylococcus equorum* for 300 ppm dye concentration at 1000Ω resistance. A very low value indicated that most of the electron released during dye degradation was not only utilized for electricity production, rather utilized for biomass growth and other metabolites (Koroglu et al., 2016; Sulonen et al., 2014; M. Zhang et al., 2019).

- **Phytotoxicity assay**

Germination % in case of control (distilled water), treated RR 120 dye and RR 120 dye **(Figure 7.10)**wastewater was found to be in the range of 91-96%, 74-88 %, and 31-58% respectively. Most of the seeds were germinated in the case of control whereas treated dye had more number of germinated seeds than untreated. There was also a difference in length of the plume and radical in treated dye wastewater and untreated dye which confirmed that the treatment of dye in MFC toxicity was decreased. The significant difference in length of the plume and radical in control and treated dye wastewater could be attributed to the presence of intermediates that arises due to dye degradation.

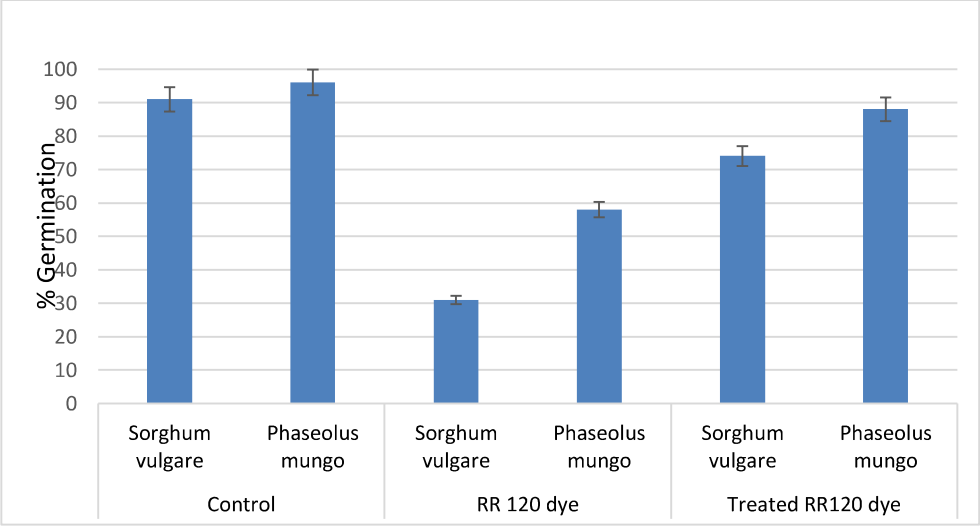


Figure 7.10 Phytotoxicity effect of treated dye samples using *Staphylococcus equorum* in MFC

7.1.3 Concluding Remarks

The present study successfully demonstrated the degradation of reactive red 120 dyes by *Staphylococcus equorum* RAP1 in MFC. Almost 90 % dye (100 ppm) was removed in 72 h of operation at pH 7.0. With the increases in concentration from 100 to 200 ppm voltage and power, density gets increased but thereafter both decreases due to toxicity of dye towards microorganism. A maximum voltage of 0.52 V at 200 ppm was observed at 7 pH after 72 h of operation. During this, maximum dye degradation was observed for 300 ppm initial concentration of dye and found to be 197.91 ppm. COD also get reduced to a sufficient value and maximum COD removal (%) 79.75 % was observed at 100 ppm of dye. Predicted R^2 values for dye degradation were found to be 0.99 and for current density, R^2 was found to be 0.94 with P-value <0.05. A quadratic model was found to be the best fit for the experiment. 3D Response surface plot showed the significant interactive effect of all three parameters on the dye degradation and current density. Through the Box-Cox design, parameters were optimized. The optimized solution was further confirmed with the experiments and the predicted and actual value had a very low difference with the error of ±0.5%. Center Composite Design combined with RSM methodology was effective for determining the optimum degradation condition for Reactive Red 120. Experimental results demonstrated approximately the same condition for the removal of dye as predicted by CCD design methodology. Significant peak shifts and absence of aromatic ring in FTIR data and metabolites detected in LCMS after degradation confirms the removal of dye by biodegradation in MFC. Phytotoxicity studies also revealed that the degraded dye was less toxic and this MFC technology gives the novel solution for the treatment of dye along with electricity generation. Furthermore, a study is needed to establish these phenomena.