|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **pH** | **Temperature** | **Concentration** | **Decolorization (RR)** | **Predicted (RR)** | **RY** | **Predicted** | **BB** | **Predicted** |
| 1 | 9 | 45 | 50 | 38.4 | 38.42611 | 61.1 | 60.26146 | 54.3 | 55.16332 |
| 2 | 7 | 35 | 100 | 78 | 78.06620 | 76.5 | 77.15975 | 80.1 | 81.07583 |
| 3 | 5 | 45 | 150 | 19.4 | 19.38233 | 18.2 | 17.27953 | 28.1 | 27.63487 |
| 4 | 7 | 18.18207 | 100 | 52.2 | 52.23198 | 54.9 | 55.0651 | 59.7 | 59.76515 |
| 5 | 7 | 35 | 15.91036 | 82.1 | 82.13071 | 85.4 | 85.43995 | 88 | 86.46958 |
| 6 | 7 | 51.81793 | 100 | 13.6 | 13.58439 | 20.7 | 20.77687 | 29.3 | 28.33165 |
| 7 | 7 | 35 | 100 | 78.1 | 78.06620 | 78.8 | 77.15975 | 80.8 | 81.07583 |
| 8 | 5 | 45 | 50 | 35.2 | 35.16338 | 38.4 | 39.17267 | 46.5 | 47.61705 |
| 9 | 5 | 25 | 50 | 58.3 | 58.24337 | 60.8 | 59.91058 | 62.4 | 62.40752 |
| 10 | 10.36359 | 35 | 100 | 31.6 | 31.53359 | 50.9 | 51.00458 | 50.1 | 49.14742 |
| 11 | 9 | 25 | 50 | 61.5 | 61.50610 | 70.1 | 70.84937 | 70.1 | 71.20379 |
| 12 | 9 | 45 | 150 | 26.6 | 26.64505 | 30.2 | 30.91832 | 36.4 | 37.03114 |
| 13 | 7 | 35 | 100 | 78.2 | 78.06620 | 76.8 | 77.15975 | 80 | 81.07583 |
| 14 | 9 | 25 | 150 | 49.5 | 49.52504 | 51.9 | 50.95623 | 60.1 | 59.62161 |
| 15 | 5 | 25 | 150 | 42.3 | 42.26232 | 46.8 | 47.46744 | 49.2 | 48.97534 |
| 16 | 7 | 35 | 184.0896 | 58.8 | 58.78566 | 50.1 | 50.30202 | 59.3 | 59.92722 |
| 17 | 7 | 35 | 100 | 77.9 | 78.06620 | 77.8 | 77.15975 | 80.5 | 81.07583 |
| 18 | 3.636414 | 35 | 100 | 22.6 | 22.68278 | 30.2 | 30.33738 | 33.8 | 33.84938 |
| 19 | 7 | 35 | 100 | 78.1 | 78.06620 | 75.8 | 77.15975 | 82.4 | 81.07583 |
| 20 | 7 | 35 | 100 | 78.1 | 78.06620 | 77.3 | 77.15975 | 82.5 | 81.07583 |

Y=b0+b1X1+b2X2+b3X3+b12X1X2+b13X1X3+b23X2X3+b11X12+b22X22+b33X3

Where: - b0: Intercept (constant term), - b1, b2, b3: Coefficients of individual variables, - b12,b13,b23: Coefficients of interaction terms, - b11,b22,b33: Coefficients of squared terms.

RR  
  
Estimate Std. Error t value Pr(>|t|)

(Intercept) 7.8066e+01 3.5711e-02 2186.0416 < 2.2e-16 \*\*\*

x1 2.6314e+00 2.3694e-02 111.0581 < 2.2e-16 \*\*\*

x2 -1.1490e+01 2.3694e-02 -484.9414 < 2.2e-16 \*\*\*

x3 -6.9405e+00 2.3694e-02 -292.9287 < 2.2e-16 \*\*\*

x1:x2 -6.1871e-15 3.0957e-02 0.0000 1.0000

x1:x3 1.0000e+00 3.0957e-02 32.3027 1.904e-11 \*\*\*

x2:x3 5.0000e-02 3.0957e-02 1.6151 0.1374

x1^2 -1.8016e+01 2.3065e-02 -781.1114 < 2.2e-16 \*\*\*

x2^2 -1.5966e+01 2.3065e-02 -692.2059 < 2.2e-16 \*\*\*

x3^2 -2.6898e+00 2.3065e-02 -116.6197 < 2.2e-16 \*\*\*

---

Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple R-squared: 1, Adjusted R-squared: 1

F-statistic: 1.474e+05 on 9 and 10 DF, p-value: < 2.2e-16

Analysis of Variance Table

Response: Decolorization

Df Sum Sq Mean Sq F value Pr(>F)

FO(x1, x2, x3) 3 2555.4 851.80 1.1110e+05 < 2.2e-16

TWI(x1, x2, x3) 3 8.0 2.67 3.4869e+02 2.053e-10

PQ(x1, x2, x3) 3 7605.1 2535.05 3.3065e+05 < 2.2e-16

Residuals 10 0.1 0.01

Lack of fit 5 0.0 0.00 4.3750e-01 0.8073

Pure error 5 0.1 0.01

Stationary point of response surface:

x1 x2 x3

0.03732157 -0.36184679 -1.28656369

Stationary point in original units:

pH Temperature Concentration

7.074643 31.381532 35.671816

Eigenanalysis:

eigen() decomposition

$values

[1] -2.673498 -15.965815 -18.032673

$vectors

[,1] [,2] [,3]

x1 0.032571056 -0.0004549876 0.999469319

x2 0.001879791 0.9999981556 0.000393969

x3 0.999467655 -0.0018659614 -0.032571851

pH Temperature Concentration Predicted\_ Decolorization

1371 7.210526 31.84211 72.36842 84.51802

> print(snr)

[1] 834.7405

### Final Equation in Terms of Actual Factors

Decolorization =

-296.73818868268

63.373005920267 pH

10.01703853974 Temperature

0.002876567796865 Concentration

-1.3143262192325 e-15 AB

0.01 AC

0.00010000000000001 BC

-4.5040946158149 A²

-0.15965768797819 B²

-0.0010759355924165 C²

RY

Estimate Std. Error t value Pr(>|t|)

(Intercept) 77.15975 0.42758 180.4551 < 2.2e-16 \*\*\*

x1 6.14440 0.28369 21.6587 9.835e-10 \*\*\*

x2 -10.19395 0.28369 -35.9331 6.619e-12 \*\*\*

x3 -10.44657 0.28369 -36.8236 5.191e-12 \*\*\*

x1:x2 2.53750 0.37066 6.8459 4.481e-05 \*\*\*

x1:x3 -1.86250 0.37066 -5.0248 0.0005181 \*\*\*

x2:x3 -2.36250 0.37066 -6.3737 8.101e-05 \*\*\*

x1^2 -12.90073 0.27617 -46.7135 4.871e-13 \*\*\*

x2^2 -13.87300 0.27617 -50.2341 2.362e-13 \*\*\*

x3^2 -3.28407 0.27617 -11.8916 3.181e-07 \*\*\*

---

Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple R-squared: 0.9987, Adjusted R-squared: 0.9975

F-statistic: 835.6 on 9 and 10 DF, p-value: 3.515e-13

Analysis of Variance Table

Response: Decolorization

Df Sum Sq Mean Sq F value Pr(>F)

FO(x1, x2, x3) 3 3425.2 1141.72 1038.7539 9.053e-13

TWI(x1, x2, x3) 3 123.9 41.30 37.5796 9.382e-06

PQ(x1, x2, x3) 3 4717.2 1572.42 1430.6098 1.836e-13

Residuals 10 11.0 1.10

Lack of fit 5 5.5 1.09 0.9864 0.5058

Pure error 5 5.5 1.11

Stationary point of response surface:

x1 x2 x3

0.3350283 -0.1993530 -1.6137868

Stationary point in original units:

pH Temperature Concentration

7.670057 33.006470 19.310659

Eigenanalysis:

eigen() decomposition

$values

[1] -3.03754 -12.25619 -14.76407

$vectors

[,1] [,2] [,3]

x1 -0.1086425 0.8293400 0.54808024

x2 -0.1202966 0.5363235 -0.83539563

x3 0.9867753 0.1566916 -0.04149926

pH Temperature Concentration Predicted\_Decolorization

992 7.631579 33.94737 56.57895 87.49172

print(snr)

[1] 74.46268

Decolorization =

-227.37869260219

45.646612606905 pH

8.2760780782229 Temperature

0.34954447336252 Concentration

0.126875 AB

-0.018625 AC

-0.004725 BC

-3.2251814241121 A²

-0.1387299752058 B²

-0.0013136293889245 C²

BB

Estimate Std. Error t value Pr(>|t|)

(Intercept) 81.07583 0.49938 162.3544 < 2.2e-16 \*\*\*

x1 4.54814 0.33132 13.7271 8.172e-08 \*\*\*

x2 -9.34523 0.33132 -28.2057 7.292e-11 \*\*\*

x3 -7.89109 0.33132 -23.8168 3.868e-10 \*\*\*

x1:x2 -0.31250 0.43290 -0.7219 0.486904

x1:x3 0.46250 0.43290 1.0684 0.310458

x2:x3 -1.63750 0.43290 -3.7827 0.003586 \*\*

x1^2 -13.99273 0.32254 -43.3836 1.017e-12 \*\*\*

x2^2 -13.09117 0.32254 -40.5883 1.973e-12 \*\*\*

x3^2 -2.78509 0.32254 -8.6350 5.995e-06 \*\*\*

---

Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple R-squared: 0.9979, Adjusted R-squared: 0.996

F-statistic: 531.1 on 9 and 10 DF, p-value: 3.369e-12

Analysis of Variance Table

Response: Decolorization

Df Sum Sq Mean Sq F value Pr(>F)

FO(x1, x2, x3) 3 2325.6 775.20 517.0784 2.911e-11

TWI(x1, x2, x3) 3 23.9 7.98 5.3237 0.01887

PQ(x1, x2, x3) 3 4816.4 1605.46 1070.8814 7.778e-13

Residuals 10 15.0 1.50

Lack of fit 5 8.7 1.74 1.3816 0.36574

Pure error 5 6.3 1.26

Stationary point of response surface:

x1 x2 x3

0.1437232 -0.2758612 -1.3236360

Stationary point in original units:

pH Temperature Concentration

7.287446 32.241388 33.818199

Eigenanalysis:

eigen() decomposition

$values

[1] -2.715223 -13.133921 -14.019854

$vectors

[,1] [,2] [,3]

x1 0.02153071 -0.15715293 0.987339549

x2 -0.07896790 0.98421571 0.158377751

x3 0.99664462 0.08137812 -0.008780825

pH Temperature Concentration Predicted\_Decolorization

1371 7.210526 31.84211 72.36842 87.86436

print(snr)

[1] 68.31146

### Final Equation in Terms of Actual Factors

Decolorization =

-241.32349286168

51.333011032679 pH

8.6661724331737 Temperature

0.14723552270033 Concentration

-0.015625000000001 AB

0.0046250000000002 AC

-0.003275 BC

-3.4981834500525 A²

-0.13091172654197 B²

-0.0011140365273613 C²

Forward primer

minimum free energy of **-289.90** kcal/mol is given below.

Reverse

minimum free energy of **-334.50** kcal/mol

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Structural element** | | **δG** | **Information** | |
| ***Forward* δG =** **-308.90 kcal/mol** | | External loop | -1.30 | | 10 ss bases & 2 closing helices. |
| Stack | -0.90 | | External closing pair is U 893-A 937 |
| Multi-loop | 1.50 | | External closing pair is A897-U933 9 ss bases & 3 closing helices. |
| Helix | -6.50 | | 5 base pairs. |
| Hairpin loop | 6.40 | | Closing pair is A912-U925 |
| Interior loop | 0.40 | | External closing pair is G27-C878 |
| Bulge loop | 0.50 | | External closing pair is G61-C824 |
|  | **Structural element** | | **δG** | **Information** | |
| ***Reverse* δG = -**337.80  **kcal/mol** | | External loop | -0.50 | | 31 ss bases & 1 closing helices. |
| Stack | -2.10 | | External closing pair is G13-U1016 |
| Multi-loop | 2.20 | | External closing pair is G18-U1011 11 ss bases & 4 closing helices. |
| Helix | -11.20 | | 6 base pairs. |
| Hairpin loop | 4.00 | | Closing pair is G993-C1003 |
| Interior loop | -1.0 | | External closing pair is G53-C955 |
| Bulge loop | 0.40 | | External closing pair is G586-C947 |