**Immobilization and Characterization of L-Asparaginase over Carbon Xerogels**

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**Model Equations:**

$Y(CX-4)=-363.27+0.17X\_{1}+119.92X\_{2}+447.25X\_{3}+0.11X\_{1}X\_{2}-0.83X\_{1}X\_{3}+57.71X\_{2}X\_{3}-0.01X\_{1}^{2}-10.47X\_{2}^{2}-1501.96X\_{3}^{2}$ (S1)

$Y(CX-13)=-440.42+2.56X\_{1}+111.42X\_{2}+922.57X\_{3}-0.36X\_{1}X\_{2}-3.94X\_{1}X\_{3}+41.98X\_{2}X\_{3}-7.46X\_{2}^{2}-1790.42X\_{3}^{2}$ (S2)

$Y(CX-30)=110.83-4.80X\_{1}+20.78X\_{2}+573.48X\_{3}-0.24X\_{1}X\_{2}-0.17X\_{1}X\_{3}+43.50X\_{2}X\_{3}-3.60X\_{2}^{2}-1564.96X\_{3}^{2}$ (S3)

**Tables**

**Table S1.** Factor levels for a central composite design to evaluate the ASNase immobilization over CX.

|  |  |  |
| --- | --- | --- |
| **Factors** | **Parameters** | **Coded level** |
| **-1.68** | **-1** | **0** | **+1** | **+1.68** |
| *X1* | Time (min) | 9.6 | 30.0 | 60.0 | 90.0 | 110.4 |
| *X2* | pH | 4.32 | 5.00 | 6.00 | 7.00 | 7.68 |
| *X3* | ASNase Concentration (mg·mL-1) | 0.02 | 0.09 | 0.20 | 0.31 | 0.38 |

**Table S2.** Central composite experimental design plan.

|  |  |  |  |
| --- | --- | --- | --- |
| **Run** | ***X1*** | ***X2*** | ***X3*** |
| 1 | -1 | -1 | -1 |
| 2 | 1 | -1 | -1 |
| 3 | -1 | 1 | -1 |
| 4 | 1 | 1 | -1 |
| 5 | -1 | -1 | 1 |
| 6 | 1 | -1 | 1 |
| 7 | -1 | 1 | 1 |
| 8 | 1 | 1 | 1 |
| 9 | -1.68 | 0 | 0 |
| 10 | 1.68 | 0 | 0 |
| 11 | 0 | -1.68 | 0 |
| 12 | 0 | 1.68 | 0 |
| 13 | 0 | 0 | -1.68 |
| 14 | 0 | 0 | 1.68 |
| 15 | 0 | 0 | 0 |
| 16 | 0 | 0 | 0 |
| 17 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 |
| 19 | 0 | 0 | 0 |

**Table S3.** Central Composite design matrix with the experimental data (Exp.) and predicted (Pred.) values of *RRA* obtained after ASNase immobilization onto CX-4, CX-13 and CX-30, as a function of the coded factors *X1*, *X2*, *X3*, respectively time (min), pH and enzyme concentration (mg·mL–1).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Run** | ***X1*(min)** | ***X2*** | ***X3*(mg·mL**-1**)** | **Relative Recovered Activity (%)** |
| **CX-4** | **CX-13** | **CX-30** |
| **Exp.**  | **Pred.** | **Exp.**  | **Pred.**  | **Exp.**  | **Pred.**  |
| 1 | 30.0 | 5.00 | 0.09 | 43.0 | 42.1 | 33.3 | 29.6 | 70.0 | 96.9 |
| 2 | 90.0 | 5.00 | 0.09 | 42.4 | 29.3 | 47.9 | 55.3 | 74.1 | 45.9 |
| 3 | 30.0 | 7.00 | 0.09 | 51.2 | 48.0 | 49.5 | 59.3 | 52.6 | 74.6 |
| 4 | 90.0 | 7.00 | 0.09 | 52.3 | 48.9 | 41.3 | 41.5 | 62.4 | 53.0 |
| 5 | 30.0 | 5.00 | 0.31 | 56.8 | 66.3 | 83.7 | 95.2 | 110.9 | 134.3 |
| 6 | 90.0 | 5.00 | 0.31 | 33.3 | 42.6 | 66.9 | 68.9 | 93.6 | 85.5 |
| 7 | 30.0 | 7.00 | 0.31 | 78.4 | 97.6 | 139.0 | 143.3 | 89.0 | 131.2 |
| 8 | 90.0 | 7.00 | 0.31 | 80.6 | 87.6 | 58.1 | 73.5 | 124.6 | 111.7 |
| 9 | 9.6 | 6.00 | 0.20 | 95.7 | 84.0 | 126.4 | 119.0 | 242.2 | 180.8 |
| 10 | 110.4 | 6.00 | 0.20 | 61.9 | 64.9 | 91.1 | 81.9 | 80.1 | 121.7 |
| 11 | 60.0 | 4.32 | 0.20 | 42.0 | 42.1 | 68.9 | 64.3 | 83.6 | 82.0 |
| 12 | 60.0 | 7.68 | 0.20 | 93.5 | 84.8 | 105.0 | 93.0 | 103.5 | 85.3 |
| 13 | 60.0 | 6.00 | 0.02 | 0.0 | 15.3 | 0.0 | -2.5 | 0.0 | -0.01 |
| 14 | 60.0 | 6.00 | 0.38 | 91.9 | 68.1 | 93.7 | 79.6 | 100.5 | 80.8 |
| 15 | 60.0 | 6.00 | 0.20 | 82.4 | 93.0 | 94.6 | 99.7 | 110.5 | 93.8 |
| 16 | 60.0 | 6.00 | 0.20 | 91.8 | 93.0 | 93.4 | 99.7 | 95.5 | 93.8 |
| 17 | 60.0 | 6.00 | 0.20 | 65.8 | 93.0 | 110.8 | 99.7 | 91.3 | 93.8 |
| 18 | 60.0 | 6.00 | 0.20 | 140.3 | 93.0 | 105.8 | 99.7 | 94.8 | 93.8 |
| 19 | 60.0 | 6.00 | 0.20 | 83.2 | 93.0 | 90.9 | 99.7 | 73.5 | 93.8 |

*X1* – contact time (min); *X2* – pH; *X3* – ASNase concentration

**Table S4.** Analysis of variance (ANOVA) for the fitted quadratic polynomial models of *RRA* values obtained after ASNase immobilization onto CX-4, CX-13 and CX-30.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Source** | **Sum of Squares (SS)** | **df** | **Mean Square (MS)** |  | ***F*-value** |  | ***p*-value** |
| **CX-4** | **CX-13** | **CX-30** |  | **CX-4** | **CX-13** | **CX-30** |  | **CX-4** | **CX-13** | **CX-30** |  | **CX-4** | **CX-13** | **CX-30** |
| (1) *X1* (L) | 440.36 | 1660.35 | 4224.52 | 1 | 440.36 | 1660.35 | 4224.52 | 0.7930 | 10.8903 | 3.2876 | 0.3964 | **0.0092** | 0.1032 |
| *X1* (Q) | 585.14 | 0.95 | 5634.08 | 1 | 585.14 | 0.95 | 5634.08 |  | 1.0538 | 0.0063 | 4.3845 |  | 0.3314 | 0.9387 | 0.0658 |
| (2) *X2* (L) | 2208.20 | 1000.83 | 12.99 | 1 | 2208.20 | 1000.83 | 12.99 |  | 3.9768 | 6.5645 | 0.0101 |  | 0.0773 | **0.0306** | 0.9221 |
| *X2* (Q) | 1490.42 | 757.37 | 175.92 | 1 | 1490.42 | 757.37 | 175.92 |  | 2.6841 | 4.9676 | 0.1369 |  | 0.1358 | 0.0528 | 0.7199 |
| (3) *X3* (L) | 3378.00 | 8133.37 | 7883.20 | 1 | 3378.00 | 8133.37 | 7883.20 |  | 6.0835 | 53.3469 | 6.1348 |  | **0.0358** | **0.0000** | **0.0352** |
| *X3* (Q) | 4494.57 | 6386.71 | 4879.48 | 1 | 4494.57 | 6386.71 | 4879.48 |  | 8.0943 | 41.8905 | 3.7973 |  | **0.0192** | **0.0001** | 0.0831 |
| 1L by 2L | 93.67 | 944.35 | 428.50 | 1 | 93.67 | 944.35 | 428.50 |  | 0.1687 | 6.1940 | 0.3335 |  | 0.6909 | **0.0345** | 0.5778 |
| 1L by 3L | 59.78 | 1351.57 | 2.42 | 1 | 59.78 | 1351.57 | 2.42 |  | 0.1077 | 8.8649 | 0.0019 |  | 0.7503 | **0.0155** | 0.9663 |
| 2L by 3L | 322.41 | 170.59 | 183.18 | 1 | 322.41 | 170.59 | 183.18 |  | 0.5806 | 1.1189 | 0.1426 |  | 0.4656 | 0.3177 | 0.7145 |
| Error | 4997.48 | 1372.16 | 11564.92 | 9 | 555.28 | 152.46 | 1284.99 |  |  |  |  |  |  |  |  |
| Total SS | 16963.41 | 21500.03 | 36806.00 | 18 |  |  |  |  |  |  |  |  |  |  |  |
| CX-4: *R2* = 0.7054; CX-13: *R2* = 0.93618; CX-30: *R2* = 0.68579*X1:* Time (min); *X2*: pH; *X3*: ASNase Concentration (mg·mL-1)Df, degrees of freedom; L, linear; Q, quadratic |

**Table S5.** Experimental and predicted relative recovered activity (*RRA*) and immobilization yield (*IY*) maximum values at critical process conditions for the ASNase immobilization onto carbon xerogels with different pore sizes, namely 4, 13 and 30 nm (CX-4, CX-13 and CX-30, respectively).

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample** | **Critical Value Conditions** | **Predicted Values** | **Experimental Values** |
| **Time (min)** | **pH** | **Enzyme Concentration (mg·mL**-1**)** | ***RRA* (%)** | ***IY* (%)** | ***RRA* (%)** | ***IY* (%)** |
| CX-4 | 49.0 | 6.73 | 0.26 | 100 | 100 | 97 ± 4 | 99.2 ± 0.4 |
| CX-13 | 116 | 5.18 | 0.19 | 85 | 100 | 89 ± 2 | 99.9 ± 0.1 |
| CX-30 | 67.8 | 6.90 | 0.28 | 100 | 1.5 | 100 ± 5 | 0 ± 8 |

**Figures**

(b) – CX-13

(c) – CX-30

(a) – CX-4

**Figure S1.** Pareto chart of standardized effects for the Central Composite design for ASNase immobilization onto (a) CX-4, (b) CX-13 and (c) CX-30. (1) time; (2) pH; (3) enzyme concentration.

(a)

(b)

(c)

**Figure S2.** Response surface plots for *RRA* of immobilized ASNase over CX as a function of pH and time with an enzyme concentration of 0.2 mg·mL-1. (a) CX-4; (b) CX-13; (c) CX-30.

(a)

(b)

(c)

**Figure S3.** Response surface plots for *RRA* of immobilized ASNase over CX as a function of enzyme concentration and time, at pH 6. (a) CX-4; (b) CX-13; (c) CX-30.

**Figure S4.** Initial reaction rates (*v0*) for free (♦) and immobilized ASNase (■) (0.26 mg·mL-1) onto CX-4 by physical adsorption. The solid lines represent the experimental data fit to the Hill equation.