**Improved ethanol production in the presence of cadmium ions by a *Saccharomyces cerevisiae* transformed with a novel cadmium-resistance gene *DvCRP1***

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**Supplemental Material Summary**

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***Evaluation of Eq. (1)***: To evaluate the reliability of the model, the key elements of the regression equations were obtained by ANOVA using Design Expert. The R2 value of the model was 0.9983. The adjusted and predicted R2 values of the model were 0.9974 and 0.9943, respectively. For a good statistical model, the R2 value should be close to 1.0 and all three factors should be positive and close to each other, as was the case here. In addition, the model had an adequate precision value of 128.65, which suggested that it could be used to navigate the design space. The p-value of the model was <0.0001, which indicated that the factors had a significant effect on the response. The lack of fit p-value of the model was 0.14 (>0.05 indicates non significance). It should be noted that the lack of fit value was not significant relative to the pure error. ANOVA analysis confirmed that the model was good and fitted the experimental data well.

***Evaluation of Eq. (2)***: To evaluate the reliability of the model, the key elements of the regression equations were obtained by ANOVA using Design Expert. The R2 value of the model was 0.9959. The adjusted and predicted R2 values of the model were 0.9938 and 0.9874, respectively. For a good statistical model, the R2 value should be close to 1.0 and all three factors should be positive and close to each other, as was the case here. In addition, the model had an adequate precision value of 80.65, which suggested that it could be used to navigate the design space. The p-value of the model was <0.0001, which indicated that the factors had a significant effect on the response. The lack of fit p-value of the model was 0.49 (>0.05 indicates non significance). It should be noted that the lack of fit value was not significant relative to the pure error. ANOVA analysis confirmed that the model was good and fitted the experimental data well.

***Evaluation of Eq. (3)***: To evaluate the reliability of the model, the key elements of the regression equations were obtained by ANOVA using Design Expert. The R2 value of the model was 0.9969. The adjusted and predicted R2 values of the model were 0.9952 and 0.9904, respectively. For a good statistical model, the R2 value should be close to 1.0 and all three factors should be positive and close to each other, as was the case here. In addition, the model had an adequate precision value of 94.46, which suggested that it could be used to navigate the design space. The p-value of the model was <0.0001, which indicated that the factors had a significant effect on the response. The lack of fit p-value of the model was 0.27 (>0.05 indicates non significance). It should be noted that the lack of fit value was not significant relative to the pure error. ANOVA analysis confirmed that the model was good and fitted the experimental data well.

Table S1 Correlation of different variables for samples of 6-24 h

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | Transformant | Cadmium Concentration | OD | Glucose Concentration | Ethanol Concentration |
| Transformant | S | - | 0.000 | 0.348 | -0.262 | 0.221 |
| *P* | - | 0.500 | 0.001 | 0.008 | 0.022 |
| Cadmium Concentration | S | 0.000 | - | -0.589 | 0.351 | -0.562 |
| *P* | 0.500 | - | 0.000 | 0.001 | 0.000 |
| OD | S | 0.348 | -0.589 | - | -0.916 | 0.952 |
| *P* | 0.001 | 0.000 | - | 0.000 | 0.000 |
| Glucose Concentration | S | -0.262 | 0.351 | -0.916 | - | -0.924 |
| *P* | 0.008 | 0.001 | 0.000 | - | 0.000 |
| Ethanol Concentration | S | 0.221 | -0.562 | 0.952 | -0.924 | - |
| *P* | 0.022 | 0.000 | 0.000 | 0.000 | - |

\* There were 84 sets of data from Figure 1. S was Spearman correlation. Transformant was set as a dummy variable (empty vector pAJ401 was set as level 0 and pAJ401-*DvCRP1* was set as level 1). The results of 0.000 in the table means far less than 0.001.

Table S2 Experimental results for *S. cerevisiae* by the experimental design

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| No | Variables | | |  | Results (cultured for 24 h) | | |
| Initial OD | Cadmium Concentration (μmol/L) | Transformant |  | OD | Glucose Concentration (g/L) | Ethanol Concentration (g/L) |
| 1 | 0.11 | 0.00 | EV |  | 1.45 | 10.86 | 4.60 |
| 2 | 0.11 | 0.00 | EV |  | 1.46 | 10.71 | 4.73 |
| 3 | 0.02 | 50.00 | EV |  | 0.89 | 13.97 | 3.01 |
| 4 | 0.02 | 50.00 | EV |  | 0.92 | 13.89 | 3.11 |
| 5 | 0.20 | 50.00 | EV |  | 1.34 | 8.39 | 5.46 |
| 6 | 0.20 | 50.00 | EV |  | 1.34 | 9.55 | 5.02 |
| 7 | 0.01 | 175.00 | EV |  | 0.19 | 18.93 | 0.56 |
| 8 | 0.01 | 175.00 | EV |  | 0.14 | 19.09 | 0.44 |
| 9 | 0.11 | 175.00 | EV |  | 0.91 | 13.29 | 3.22 |
| 10 | 0.11 | 175.00 | EV |  | 0.92 | 13.22 | 3.29 |
| 11 | 0.11 | 175.00 | EV |  | 0.90 | 13.51 | 3.28 |
| 12 | 0.11 | 175.00 | EV |  | 0.91 | 13.45 | 3.26 |
| 13 | 0.11 | 175.00 | EV |  | 0.91 | 13.42 | 3.29 |
| 14 | 0.11 | 175.00 | EV |  | 0.92 | 13.49 | 3.30 |
| 15 | 0.11 | 175.00 | EV |  | 0.91 | 13.52 | 3.33 |
| 16 | 0.11 | 175.00 | EV |  | 0.88 | 13.49 | 3.20 |
| 17 | 0.24 | 175.00 | EV |  | 1.04 | 11.94 | 3.97 |
| 18 | 0.24 | 175.00 | EV |  | 1.01 | 12.40 | 3.82 |
| 19 | 0.02 | 300.00 | EV |  | 0.09 | 19.12 | 0.29 |
| 20 | 0.02 | 300.00 | EV |  | 0.10 | 19.11 | 0.31 |
| 21 | 0.20 | 300.00 | EV |  | 0.81 | 14.73 | 2.54 |
| 22 | 0.20 | 300.00 | EV |  | 0.79 | 14.44 | 2.35 |
| 23 | 0.11 | 350.00 | EV |  | 0.43 | 17.30 | 1.27 |
| 24 | 0.11 | 350.00 | EV |  | 0.43 | 17.13 | 1.22 |
| 25 | 0.11 | 0.00 | D |  | 1.41 | 10.04 | 5.02 |
| 26 | 0.11 | 0.00 | D |  | 1.45 | 10.38 | 4.89 |
| 27 | 0.02 | 50.00 | D |  | 0.96 | 12.99 | 3.48 |
| 28 | 0.02 | 50.00 | D |  | 0.98 | 12.67 | 3.65 |
| 29 | 0.20 | 50.00 | D |  | 1.27 | 9.10 | 5.58 |
| 30 | 0.20 | 50.00 | D |  | 1.20 | 8.94 | 5.71 |
| 31 | 0.01 | 175.00 | D |  | 0.57 | 15.84 | 2.12 |
| 32 | 0.01 | 175.00 | D |  | 0.61 | 16.05 | 2.04 |
| 33 | 0.11 | 175.00 | D |  | 1.01 | 12.28 | 3.82 |
| 34 | 0.11 | 175.00 | D |  | 0.99 | 12.14 | 3.96 |
| 35 | 0.11 | 175.00 | D |  | 1.00 | 12.36 | 4.01 |
| 36 | 0.11 | 175.00 | D |  | 1.01 | 12.45 | 3.99 |
| 37 | 0.11 | 175.00 | D |  | 1.00 | 12.27 | 3.99 |
| 38 | 0.11 | 175.00 | D |  | 1.00 | 12.15 | 4.03 |
| 39 | 0.11 | 175.00 | D |  | 1.01 | 11.92 | 4.05 |
| 40 | 0.11 | 175.00 | D |  | 1.00 | 12.38 | 3.85 |
| 41 | 0.24 | 175.00 | D |  | 1.08 | 11.20 | 4.31 |
| 42 | 0.24 | 175.00 | D |  | 1.11 | 11.13 | 4.13 |
| 43 | 0.02 | 300.00 | D |  | 0.39 | 17.09 | 1.39 |
| 44 | 0.02 | 300.00 | D |  | 0.37 | 17.39 | 1.30 |
| 45 | 0.20 | 300.00 | D |  | 0.92 | 12.46 | 3.78 |
| 46 | 0.20 | 300.00 | D |  | 0.94 | 12.61 | 3.80 |
| 47 | 0.11 | 350.00 | D |  | 0.76 | 14.42 | 2.75 |
| 48 | 0.11 | 350.00 | D |  | 0.78 | 14.22 | 2.81 |

\* EV was Empty vector pAJ401; D was pAJ401-*DvCRP1*

Table S3 The p-values of the terms in Eq. (1), Eq. (2) and Eq. (3)

|  |  |  |  |
| --- | --- | --- | --- |
| Terms | OD  Eq. (1) | Glucose Concentration  Eq. (2) | Ethanol Concentration  Eq. (3) |
| A | < 0.0001 | < 0.0001 | < 0.0001 |
| B | < 0.0001 | < 0.0001 | < 0.0001 |
| C | < 0.0001 | < 0.0001 | < 0.0001 |
| AB | < 0.0001 | 0.2810 | 0.0899 |
| AC | < 0.0001 | < 0.0001 | 0.0004 |
| BC | < 0.0001 | < 0.0001 | < 0.0001 |
| A2 | < 0.0001 | < 0.0001 | < 0.0001 |
| B2 | < 0.0001 | 0.0030 | < 0.0001 |
| ABC | 0.5677 | 0.0038 | 0.0466 |
| A2B | 0.0024 | < 0.0001 | < 0.0001 |
| A2C | 0.0111 | 0.1310 | 0.7265 |
| AB2 | < 0.0001 | < 0.0001 | < 0.0001 |
| B2C | 0.0004 | 0.0051 | 0.0121 |
| A3 | 0.0139 | < 0.0001 | < 0.0001 |
| AB2C | < 0.0001 | < 0.0001 | < 0.0001 |
| A3C | < 0.0001 | < 0.0001 | 0.0132 |

\* A p-value >0.1 indicated the model term are not significant and a lower p-value suggested more significant.

Table S4 Remaining data for *S. cerevisiae* by the experimental design

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No |  | OD | |  | Glucose Concentration (g/L) | |  | Ethanol Concentration (g/L) | |  | Intracellular GSH Concentration (mg/L) |  | GSH/OD |
|  | 6 h | 12 h |  | 6 h | 12 h |  | 6 h | 12 h |  | 24 h |  | 24 h |
| 1 |  | 0.89 | 1.16 |  | 17.18 | 15.23 |  | 1.58 | 2.85 |  | 25.88 |  | 17.84 |
| 2 |  | 0.89 | 1.17 |  | 17.25 | 15.33 |  | 1.61 | 2.62 |  | 24.94 |  | 17.07 |
| 3 |  | 0.09 | 0.32 |  | 19.72 | 18.61 |  | 0.32 | 0.73 |  | 16.13 |  | 18.14 |
| 4 |  | 0.08 | 0.36 |  | 19.55 | 18.28 |  | 0.32 | 0.88 |  | 17.46 |  | 19.08 |
| 5 |  | 0.77 | 1.09 |  | 17.23 | 14.00 |  | 1.51 | 2.87 |  | 25.47 |  | 19.08 |
| 6 |  | 0.83 | 1.13 |  | 16.87 | 14.53 |  | 1.67 | 2.97 |  | 26.01 |  | 19.41 |
| 7 |  | 0.02 | 0.06 |  | 20.00 | 19.14 |  | 0.21 | 0.23 |  | 2.53 |  | 13.32 |
| 8 |  | 0.02 | 0.05 |  | 19.76 | 19.42 |  | 0.21 | 0.20 |  | 1.81 |  | 12.48 |
| 9 |  | 0.29 | 0.50 |  | 19.02 | 18.02 |  | 0.74 | 1.18 |  | 11.20 |  | 12.28 |
| 10 |  | 0.33 | 0.55 |  | 18.85 | 17.76 |  | 0.78 | 1.23 |  | 12.16 |  | 13.20 |
| 11 |  | 0.28 | 0.48 |  | 19.11 | 17.95 |  | 0.74 | 1.17 |  | 11.68 |  | 12.98 |
| 12 |  | 0.29 | 0.50 |  | 19.33 | 17.87 |  | 0.72 | 1.26 |  | 11.80 |  | 12.92 |
| 13 |  | 0.30 | 0.49 |  | 19.03 | 17.85 |  | 0.76 | 1.17 |  | 11.56 |  | 12.65 |
| 14 |  | 0.29 | 0.51 |  | 18.69 | 17.77 |  | 0.73 | 1.24 |  | 11.80 |  | 12.81 |
| 15 |  | 0.29 | 0.50 |  | 18.59 | 17.83 |  | 0.77 | 1.25 |  | 11.47 |  | 12.63 |
| 16 |  | 0.29 | 0.48 |  | 18.85 | 18.01 |  | 0.76 | 1.13 |  | 12.33 |  | 13.95 |
| 17 |  | 0.53 | 0.87 |  | 17.54 | 16.00 |  | 1.11 | 2.33 |  | 16.50 |  | 15.87 |
| 18 |  | 0.56 | 0.82 |  | 17.88 | 15.98 |  | 1.03 | 2.09 |  | 15.17 |  | 14.96 |
| 19 |  | 0.04 | 0.07 |  | 19.20 | 19.21 |  | 0.22 | 0.22 |  | 0.96 |  | 10.79 |
| 20 |  | 0.03 | 0.06 |  | 19.46 | 19.43 |  | 0.23 | 0.22 |  | 0.36 |  | 3.53 |
| 21 |  | 0.48 | 0.66 |  | 18.12 | 16.88 |  | 0.83 | 1.30 |  | 9.56 |  | 11.80 |
| 22 |  | 0.49 | 0.66 |  | 18.34 | 17.17 |  | 0.89 | 1.28 |  | 8.79 |  | 11.20 |
| 23 |  | 0.23 | 0.34 |  | 18.79 | 18.35 |  | 0.58 | 0.77 |  | 7.70 |  | 18.12 |
| 24 |  | 0.21 | 0.34 |  | 18.45 | 18.43 |  | 0.64 | 0.83 |  | 6.44 |  | 14.87 |
| 25 |  | 0.91 | 1.18 |  | 17.26 | 14.71 |  | 1.60 | 2.72 |  | 26.49 |  | 18.81 |
| 26 |  | 0.88 | 1.19 |  | 17.05 | 14.50 |  | 1.59 | 2.86 |  | 25.72 |  | 17.79 |
| 27 |  | 0.17 | 0.68 |  | 19.38 | 17.10 |  | 0.34 | 1.46 |  | 16.73 |  | 17.46 |
| 28 |  | 0.17 | 0.69 |  | 19.34 | 16.90 |  | 0.38 | 1.50 |  | 16.49 |  | 16.88 |
| 29 |  | 0.86 | 1.11 |  | 16.47 | 14.15 |  | 1.70 | 2.97 |  | 25.47 |  | 20.06 |
| 30 |  | 0.89 | 1.12 |  | 16.56 | 14.19 |  | 1.80 | 3.05 |  | 27.93 |  | 23.35 |
| 31 |  | 0.02 | 0.11 |  | 19.51 | 19.24 |  | 0.00 | 0.24 |  | 9.27 |  | 16.29 |
| 32 |  | 0.03 | 0.12 |  | 19.27 | 19.20 |  | 0.00 | 0.28 |  | 11.20 |  | 18.33 |
| 33 |  | 0.43 | 0.85 |  | 18.36 | 15.79 |  | 0.85 | 2.13 |  | 15.05 |  | 14.84 |
| 34 |  | 0.43 | 0.86 |  | 18.00 | 15.59 |  | 0.82 | 2.13 |  | 15.05 |  | 15.14 |
| 35 |  | 0.46 | 0.86 |  | 17.82 | 15.68 |  | 0.81 | 1.99 |  | 15.05 |  | 15.10 |
| 36 |  | 0.44 | 0.85 |  | 17.96 | 15.64 |  | 0.85 | 2.06 |  | 15.69 |  | 15.53 |
| 37 |  | 0.45 | 0.85 |  | 17.99 | 15.70 |  | 0.80 | 2.16 |  | 16.01 |  | 15.99 |
| 38 |  | 0.46 | 0.86 |  | 18.39 | 15.53 |  | 0.88 | 2.17 |  | 16.25 |  | 16.25 |
| 39 |  | 0.48 | 0.86 |  | 18.34 | 15.55 |  | 0.90 | 2.20 |  | 16.49 |  | 16.26 |
| 40 |  | 0.47 | 0.85 |  | 18.34 | 15.75 |  | 0.84 | 2.12 |  | 15.52 |  | 15.58 |
| 41 |  | 0.66 | 0.98 |  | 17.12 | 14.75 |  | 1.31 | 2.53 |  | 17.10 |  | 15.82 |
| 42 |  | 0.69 | 1.03 |  | 17.11 | 15.03 |  | 1.29 | 2.40 |  | 16.89 |  | 15.26 |
| 43 |  | 0.07 | 0.15 |  | 19.19 | 18.77 |  | 0.16 | 0.36 |  | 5.06 |  | 13.11 |
| 44 |  | 0.07 | 0.15 |  | 19.39 | 18.77 |  | 0.16 | 0.34 |  | 4.82 |  | 13.10 |
| 45 |  | 0.49 | 0.75 |  | 17.96 | 15.95 |  | 0.81 | 1.69 |  | 11.32 |  | 12.28 |
| 46 |  | 0.49 | 0.76 |  | 17.63 | 16.42 |  | 0.76 | 1.68 |  | 11.68 |  | 12.47 |
| 47 |  | 0.31 | 0.44 |  | 18.24 | 17.80 |  | 0.49 | 0.90 |  | 10.84 |  | 14.30 |
| 48 |  | 0.31 | 0.46 |  | 18.63 | 17.73 |  | 0.54 | 1.05 |  | 9.75 |  | 12.50 |

\* 6 h, 12 h and 24 h were culture time. The value of variables could be seen in Table S2.

Table S5 Correlation of different variables for samples cultured for 6 h

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Transformant | Initial OD | Cadmium Concentration | OD | Glucose Concentration | Ethanol Concentration |
| Transformant | S | - | 0.000 | 0.000 | 0.183 | -0.262 | 0.096 |
| *P* | - | 0.500 | 0.500 | 0.106 | 0.036 | 0.258 |
| Initial OD | S | 0.000 | - | 0.000 | 0.820 | -0.825 | 0.797 |
| *P* | 0.500 | - | 0.500 | 0.000 | 0.000 | 0.000 |
| Cadmium Concentration | S | 0.000 | 0.000 | - | -0.383 | 0.265 | -0.458 |
| *P* | 0.500 | 0.500 | - | 0.004 | 0.035 | 0.001 |
| OD | S | 0.183 | 0.820 | -0.383 | - | -0.941 | 0.960 |
| *P* | 0.106 | 0.000 | 0.004 | - | 0.000 | 0.000 |
| Glucose Concentration | S | -0.262 | -0.825 | 0.265 | -0.941 | - | -0.906 |
| *P* | 0.036 | 0.000 | 0.035 | 0.000 | - | 0.000 |
| Ethanol Concentration | S | 0.096 | 0.797 | -0.458 | 0.960 | -0.906 | - |
| *P* | 0.258 | 0.000 | 0.001 | 0.000 | 0.000 | - |

\* There were 48 sets of data from Table S4. S was Spearman correlation. Transformant was set as a dummy variable (empty vector pAJ401 was set as level 0 and pAJ401-*DvCRP1* was set as level 1). The results of 0.000 in the table means far less than 0.001.

Table S6 Correlation of different variables for samples cultured for 12 h

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Transformant | Initial OD | Cadmium Concentration | OD | Glucose Concentration | Ethanol Concentration |
| Transformant | S | - | 0.000 | 0.000 | 0.295 | -0.367 | 0.292 |
| *P* | - | 0.500 | 0.500 | 0.021 | 0.005 | 0.022 |
| Initial OD | S | 0.000 | - | 0.000 | 0.671 | -0.694 | 0.705 |
| *P* | 0.500 | - | 0.500 | 0.000 | 0.000 | 0.000 |
| Cadmium Concentration | S | 0.000 | 0.000 | - | -0.576 | 0.511 | -0.567 |
| *P* | 0.500 | 0.500 | - | 0.000 | 0.000 | 0.000 |
| OD | S | 0.295 | 0.671 | -0.576 | - | -0.972 | 0.986 |
| *P* | 0.021 | 0.000 | 0.000 | - | 0.000 | 0.000 |
| Glucose Concentration | S | -0.367 | -0.694 | 0.511 | -0.972 | - | -0.977 |
| *P* | 0.005 | 0.000 | 0.000 | 0.000 | - | 0.000 |
| Ethanol Concentration | S | 0.292 | 0.705 | -0.567 | 0.986 | -0.977 | - |
| *P* | 0.022 | 0.000 | 0.000 | 0.000 | 0.000 | - |

\* There were 48 sets of data from Table S4. S was Spearman correlation. Transformant was set as a dummy variable (empty vector pAJ401 was set as level 0 and pAJ401-*DvCRP1* was set as level 1). The results of 0.000 in the table means far less than 0.001.

Table S7 Correlation of different variables for samples cultured for 24 h

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Transformant | Initial OD | Cadmium Concentration | OD | Glucose Concentration | Ethanol Concentration | Intracellular GSH | GSH/OD |
| Transformant | S | - | 0.000 | 0.000 | 0.256 | -0.313 | 0.340 | 0.221 | 0.253 |
| *P* | - | 0.500 | 0.500 | 0.040 | 0.015 | 0.009 | 0.065 | 0.042 |
| Initial OD | S | 0.000 | - | 0.000 | 0.59 | -0.618 | 0.602 | 0.425 | 0.038 |
| *P* | 0.500 | - | 0.500 | 0.000 | 0.000 | 0.000 | 0.001 | 0.399 |
| Cadmium Concentration | S | 0.000 | 0.000 | - | -0.699 | 0.659 | -0.660 | -0.824 | -0.708 |
| *P* | 0.500 | 0.500 | - | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| OD | S | 0.256 | 0.590 | -0.699 | - | -0.976 | 0.973 | 0.926 | 0.591 |
| *P* | 0.040 | 0.000 | 0.000 | - | 0.000 | 0.000 | 0.000 | 0.000 |
| Glucose Concentration | S | -0.313 | -0.618 | 0.659 | -0.976 | - | -0.987 | -0.905 | -0.576 |
| *P* | 0.015 | 0.000 | 0.000 | 0.000 | - | 0.000 | 0.000 | 0.000 |
| Ethanol Concentration | S | 0.340 | 0.602 | -0.660 | 0.973 | -0.987 | - | 0.904 | 0.576 |
| *P* | 0.009 | 0.000 | 0.000 | 0.000 | 0.000 | - | 0.000 | 0.000 |
| Intracellular GSH | S | 0.221 | 0.425 | -0.824 | 0.926 | -0.905 | 0.904 | - | 0.759 |
| *P* | 0.065 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | - | 0.000 |
| GSH/OD | S | 0.253 | 0.038 | -0.708 | 0.591 | -0.576 | 0.576 | 0.759 | - |
| *P* | 0.042 | 0.399 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | - |

\* There were 48 sets of data from Table S2 and S4. S was Spearman correlation. Transformant was set as a dummy variable (empty vector pAJ401 was set as level 0 and pAJ401-*DvCRP1* was set as level 1). The results of 0.000 in the table means far less than 0.001.