Supplementary Materials

**Decolorization and detoxification of Direct Blue 2B by indigenous bacterial consortium**

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## S1 Optimization designs

Response surface methodology (RSM) is a proven tool to screen studies for optimizing the region of the impact factors, and could obtain the most efficient parameters with a minimum number of experiments (Lim et al., 2013). Box-Behnken design (BBD), as a class of rotatable or nearly rotatable second-order designs, are more efficient than conventional method based on three-level incomplete factorial designs (Das and Mishra, 2017). Therefore, the optimization for DB2 decolorization was evaluated by RSM using BBD in this study. The decolorization efficiency at 48 h was considered as dependent variable (response) for optimal experiments. Parameters of pH, salinity and temperature were chosen as independent variables based on our preliminary survey. The plan of BBD in coded levels of the three independent variables was shown in Table S1. Design Expert Software 8.0.6 was used for the regression and graphical analysis of data obtained. Analysis of variance (ANOVA) was performed to obtain the interaction between variables and responses.

## S2 Optimization study using RSM

### *Box-Behnken analysis*

RSM was used to analyze the effects of the three independent factors (temperature, pH and NaCl) and their interactions on DB2 decolorization and predict and optimize their responses. The experimental data and predicted values of responses were obtained from quadratic model fitting techniques (Table S2). The predicted model equation fitted with experimental results was obtained as Eq. (3):

Y = +88.59 + 18.92A + 1.55B + 1.67C - 3.64AB - 2.38AC -3.64BC

-21.15A2 - 12.71B2 - 6.47C2 (3)

Where, Y is the predicted response and A, B, C are the coded values of the independent variables incubation temperature (ºC), pH and NaCl (g l-1), respectively.

Analysis of variance (ANOVA) was used to evaluate the significance of the fit of the second-order polynomial for DB2 decolorization. The ANOVA statistics for the response are shown in Table S3. P-value lower than 0.05 indicates that the model is significant and p-value higher than 0.1000 indicates that the model is not significant ([Paz et al., 2017](#_ENREF_35)). The model F-value of 937.82 corresponding to P-value < 0.0001, indicated that the model was adequate. In this case, A, B, C, AB, AC, BC, A2, B2, C2 were significant model terms. The lack of fit F-value of 3.95 implied the Lack of Fit was not significant relative to the pure error. There was a 10.88% chance that a lack of fit F-value this large could occur due to noise. Thus, this model could be used to navigate the design space.

Moreover, the determination coefficient R2 indicates the correlation between the experimental and predicted values ([Qu et al., 2010](#_ENREF_37)). An R2 value close to 1 indicates a high degree of correlation between the observed and predicted responses ([Zhao et al., 2010](#_ENREF_51)). A close value of R2 (0.9992) and adjusted R2 (0.9981) was observed, suggesting the adequacy of the model. The model is reasonably reliable and reproducible when the coefficient of variation (C.V) is less than 10% ([Das & Mishra, 2017](#_ENREF_10)). Thus, C.V.% = 1.23 together with low standard deviation of 0.84 indicated a high level of accuracy and an excellent consistency of the model for the experimental results.

## S3 Simulated microcosm studies

The effectiveness of YHK microbial consortium to degrade DB2 in soil environments was evaluated using soil microcosms. Microcosms containing 40 g of soil collected from industrial areas, were set up in a 200 mL glass flask at 35 °C. There were five different treatments to the soils as shown in Table S4. Sterilized soils were prepared by baking at 121 °C for one hour under high pressure. All samples were sprayed with 20 % mass fraction of deionized water and were inoculated with 10 % YHK microbial consortium. All soil treatments were in triplicate.

**Table S1.** Experimental design levels of three variables for Box-Behnken design.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Independent variables | Symbols | Units | Coded and Actual levels | | |
| Coded level | Low (-1) Average (0) High (+1) | | |
| Temperature | A | ºC | 25 | 35 | 45 |
| pH | B | - | 4.5 | 7.5 | 10.5 |
| NaCl | C | g l-1 | 0 | 20 | 40 |

**Table S2.** Box-Behnken design with experimental as well as predicted responses of dependent variable.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Run No. | Temperature  (ºC) | pH | NaCl  (g l-1) | Decolorization rate (%) | |
| Experimental | Predicted |
| 1 | 25 | 10.5 | 20 | 39.86 | 40.15 |
| 2 | 35 | 7.5 | 20 | 89.24 | 88.59 |
| 3 | 25 | 7.5 | 0 | 37.06 | 36.38 |
| 4 | 35 | 7.5 | 20 | 87.81 | 88.59 |
| 5 | 35 | 10.5 | 40 | 69.5 | 69.73 |
| 6 | 35 | 7.5 | 20 | 88.83 | 88.59 |
| 7 | 45 | 4.5 | 20 | 72.87 | 72.58 |
| 8 | 45 | 7.5 | 0 | 75.98 | 76.50 |
| 9 | 35 | 4.5 | 0 | 65.04 | 64.81 |
| 10 | 45 | 7.5 | 40 | 73.05 | 73.73 |
| 11 | 25 | 7.5 | 40 | 43.71 | 43.19 |
| 12 | 25 | 4.5 | 20 | 31.08 | 31.99 |
| 13 | 45 | 10.5 | 20 | 71.14 | 70.23 |
| 14 | 35 | 10.5 | 0 | 73.19 | 73.58 |
| 15 | 35 | 7.5 | 20 | 88.83 | 88.59 |
| 16 | 35 | 7.5 | 20 | 88.26 | 88.59 |
| 17 | 35 | 4.5 | 40 | 73.09 | 72.70 |

**Table S3.** ANOVA statistics of quadratic model for decolorization of DB2 dye.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Source | Sum of  Squares | Degree of  freedom | Mean Square | F-Value | P-value  (Prob > F) |
| Model | 5996.44 | 9 | 666.27 | 937.82 | < 0.0001 |
| A-Temperature | 2496.77 | 1 | 2496.77 | 3514.36 | < 0.0001 |
| B-pH | 16.85 | 1 | 16.85 | 23.72 | 0.0018 |
| C-NaCl | 8.16 | 1 | 8.16 | 11.49 | 0.0116 |
| AB | 27.62 | 1 | 27.62 | 38.87 | 0.0004 |
| AC | 22.94 | 1 | 22.94 | 32.30 | 0.0007 |
| BC | 34.46 | 1 | 34.46 | 48.50 | 0.0002 |
| A2 | 2386.16 | 1 | 2386.16 | 3358.67 | < 0.0001 |
| B2 | 514.19 | 1 | 514.19 | 723.75 | < 0.0001 |
| C2 | 226.74 | 1 | 226.74 | 319.15 | < 0.0001 |
| Residual | 4.97 | 7 | 0.71 |  |  |
| Lack of Fit | 3.72 | 3 | 1.24 | 3.95 | 0.1088 |
| Pure Error | 1.25 | 4 | 0.31 |  |  |
| Correlation Total | 6001.42 | 16 |  |  |  |

**Table S4.** The first-order kinetics constants of DB2 decolorization at (a) varying culture temperatures; (b) different initial dye concentrations.

(a) Varying temperature (ºC)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Constants | 25 | 30 | 35 | 40 | 45 | 50 |
| k1 | 0.0252 | 0.0396 | 0.0479 | 0.0465 | 0.0363 | 0.0214 |
| R2 | 0.9745 | 0.9845 | 0.9786 | 0.9823 | 0.9566 | 0.9761 |

(b) Different dye concentration (mg l-1)

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Constants | | 50 | 100 | 200 | 400 | 600 | 800 | 1000 | 1200 | 1500 |
| K1 | 0.0642 | 0.0528 | 0.0455 | 0.0342 | 0.0318 | 0.0283 | 0.0278 | 0.0181 | 0.0161 |
| R2 | 0.9688 | 0.9813 | 0.9811 | 0.9818 | 0.9859 | 0.9846 | 0.9971 | 0.9869 | 0.9838 |

**Table S5**. Experimental set up for microcosms study to access the competence of YHK for its ability to degrade DB2 in soil environment.

|  |  |  |
| --- | --- | --- |
| Experimental sets | Experimental parameter (DB2 100 mg/g) | Comment |
| Set A | Sterilized soil amended with DB2 | Determination of abiotic loss of DB2 |
| Set B | Sterilized soil amended with DB2 and inoculated with YHK | Determination DB2 degradation in absence of indigenous microbial communities |
| Set C | Non sterile soil amended with DB2 | Determination of the intrinsic ability of the soil to degrade DB2 |
| Set D | Non sterile soil amended with DB2 and inoculated with YHK | Determination synergy between indigenous microbiome and YHK in degradation of DB2. |
| Set E | Sterilized soil (without dye) inoculated with YHK | Determination of ability of YHK to grow in the soil. |

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**Figure S1.** Effect of glucose (a) and YE (b) on DB2 degradation by consortium YHK under static condition; condition: pH = 7.5, T = 35 ºC, CDB2 = 100 mg l-1.

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**Figure S2**. Three-dimensional response surface graph and its corresponding contour plot for the effect of pH and Temperature(a), NaCl concentration and Temperature(b) and NaCl concentration and pH (c) on decolorization of DB2 using microbial consortium YHK.

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**Figure S3** UV–vis spectra of DB2 and degraded products of DB2 at varied time intervals under static condition; condition: pH = 7.5, T = 35 ºC, CDB2 = 50 mg l-1.

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**Figure S4.** Therelative abundances of bacteria in all samples at the phylum level under various conditions: (a) temperature, (b) pH, (c) NaCl concentrations, and (d) dye concentrations.



**Figure S5**. Soil microcosm study evaluating removal of DB2 in soil ecosystems. (A) Sterilized soil amended with DB2, (B) sterilized soil inoculated with YHK and amended with DB2, (C) non sterilized soil amended with DB2, (D) non sterilized soil inoculated with YHK and amended with DB2, (E) sterilized soil inoculated with YHK.

**References**

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