

## Supporting Material

### **Chitosan microbeads as supporter for *Pseudomonas putida* with surface displayed laccases for decolorization of synthetic dyes**

Zhiqiang Bai<sup>1,2</sup>, Xiaowen Sun<sup>1</sup>, Xun Yu<sup>1</sup> and Lin Li<sup>1,\*</sup>

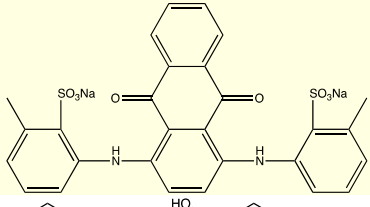
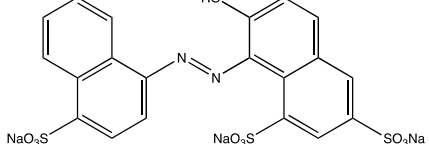
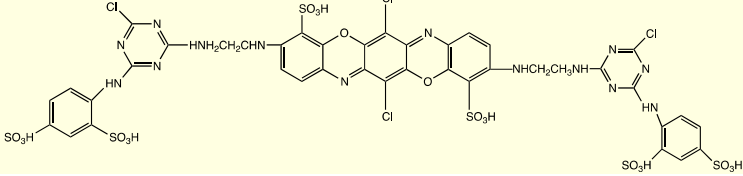
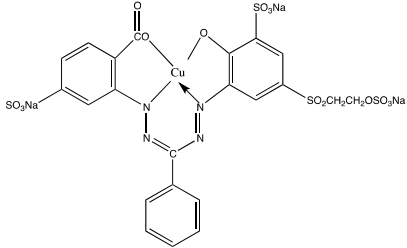
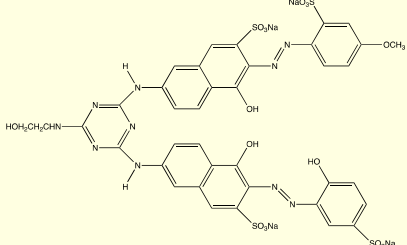
<sup>1</sup> State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology Huazhong Agriculture University, Wuhan 430070, China; baizq1987@126.com (Z.B.); sunxiaowen525@webmail.hzau.edu.cn (X.S.); yuxun@webmail.hzau.edu.cn (X.Y.); lilin@mail.hzau.edu.cn (L.L.)

<sup>2</sup> School of Life Sciences, Shanxi Datong University, Datong 037009, Shanxi Province, China

\* Correspondence: lilin@mail.hzau.edu.cn; Tel.: +86-27-8728 6952

## Supplementary Tables

**Table S1** Molecular formulas of five selected synthesized dyes

| Dyes                         | Dye molecular formula  | Dye category        | $\lambda_{\max}$ (nm) |
|------------------------------|--|---------------------|-----------------------|
| Acid Green 25<br>(AG25)      |    | Anthraquinone       | 636                   |
| Acid Red 18<br>(AR18)        |    | Azo                 | 506                   |
| Reactive Blue 198<br>(RB198) |    | Tribenzylidioxazine | 621                   |
| Reactive Blue 220<br>(RB220) |   | Phthalocyanine      | 612                   |
| Direct Red 243<br>(DR243)    |  | Azo                 | 527                   |

**Table S2** L<sub>25</sub> (4<sup>5</sup>)-orthogonal test of *P. putida* MB285 immobilization on the CTS-MBs

| Run | Factors <sup>a</sup> |   |   |   | Levels |    |    |    | Enzyme activity (U kg <sup>-1</sup> ) <sup>b</sup> |
|-----|----------------------|---|---|---|--------|----|----|----|--|
|     | A                    | B | C | D | A      | B  | C  | D  |  |
| 1   | 1                    | 1 | 1 | 1 | 0.5    | 2  | 4  | B5 | 3.25   |
| 2   | 1                    | 2 | 2 | 2 | 0.5    | 4  | 12 | B4 | 3.09   |
| 3   | 1                    | 3 | 3 | 3 | 0.5    | 6  | 20 | B3 | 7.51   |
| 4   | 1                    | 4 | 4 | 4 | 0.5    | 8  | 28 | B2 | 4.15   |
| 5   | 1                    | 5 | 5 | 5 | 0.5    | 12 | 36 | B1 | 6.02   |
| 6   | 2                    | 1 | 2 | 3 | 1.0    | 2  | 12 | B3 | 3.41   |
| 7   | 2                    | 2 | 3 | 4 | 1.0    | 4  | 20 | B2 | 3.78   |
| 8   | 2                    | 3 | 4 | 5 | 1.0    | 6  | 28 | B1 | 5.22   |
| 9   | 2                    | 4 | 5 | 1 | 1.0    | 8  | 36 | B5 | 6.39   |
| 10  | 2                    | 5 | 1 | 2 | 1.0    | 12 | 4  | B4 | 4.53   |
| 11  | 3                    | 1 | 3 | 5 | 2.0    | 2  | 20 | B1 | 4.90   |
| 12  | 3                    | 2 | 4 | 1 | 2.0    | 4  | 28 | B5 | 2.18   |
| 13  | 3                    | 3 | 5 | 2 | 2.0    | 6  | 36 | B4 | 6.76   |
| 14  | 3                    | 4 | 1 | 3 | 2.0    | 8  | 4  | B3 | 3.99   |
| 15  | 3                    | 5 | 2 | 4 | 2.0    | 12 | 12 | B2 | 4.69   |
| 16  | 4                    | 1 | 4 | 2 | 5.0    | 2  | 28 | B4 | 3.25   |
| 17  | 4                    | 2 | 5 | 3 | 5.0    | 4  | 36 | B3 | 3.67   |
| 18  | 4                    | 3 | 1 | 4 | 5.0    | 6  | 4  | B2 | 5.38   |
| 19  | 4                    | 4 | 2 | 5 | 5.0    | 8  | 12 | B1 | 5.91   |
| 20  | 4                    | 5 | 3 | 1 | 5.0    | 12 | 20 | B5 | 7.29   |
| 21  | 5                    | 1 | 5 | 4 | 8.0    | 2  | 36 | B2 | 4.47   |
| 22  | 5                    | 2 | 1 | 5 | 8.0    | 4  | 4  | B1 | 5.06   |
| 23  | 5                    | 3 | 2 | 1 | 8.0    | 6  | 12 | B5 | 6.23   |
| 24  | 5                    | 4 | 3 | 2 | 8.0    | 8  | 20 | B4 | 7.51   |
| 25  | 5                    | 5 | 4 | 3 | 8.0    | 12 | 28 | B3 | 6.81   |

<sup>a</sup> A: glutaraldehyde concentration; B: immobilization reaction time; C: immobilization reaction temperature (°C); D: prepared CTS-MB materials (CTS-MB1 to CTS-MB5).

<sup>b</sup> Whole-complex laccase enzyme activity, which was measured and calculated based on the wet weight of the prepared bacCTS-MB complexes.

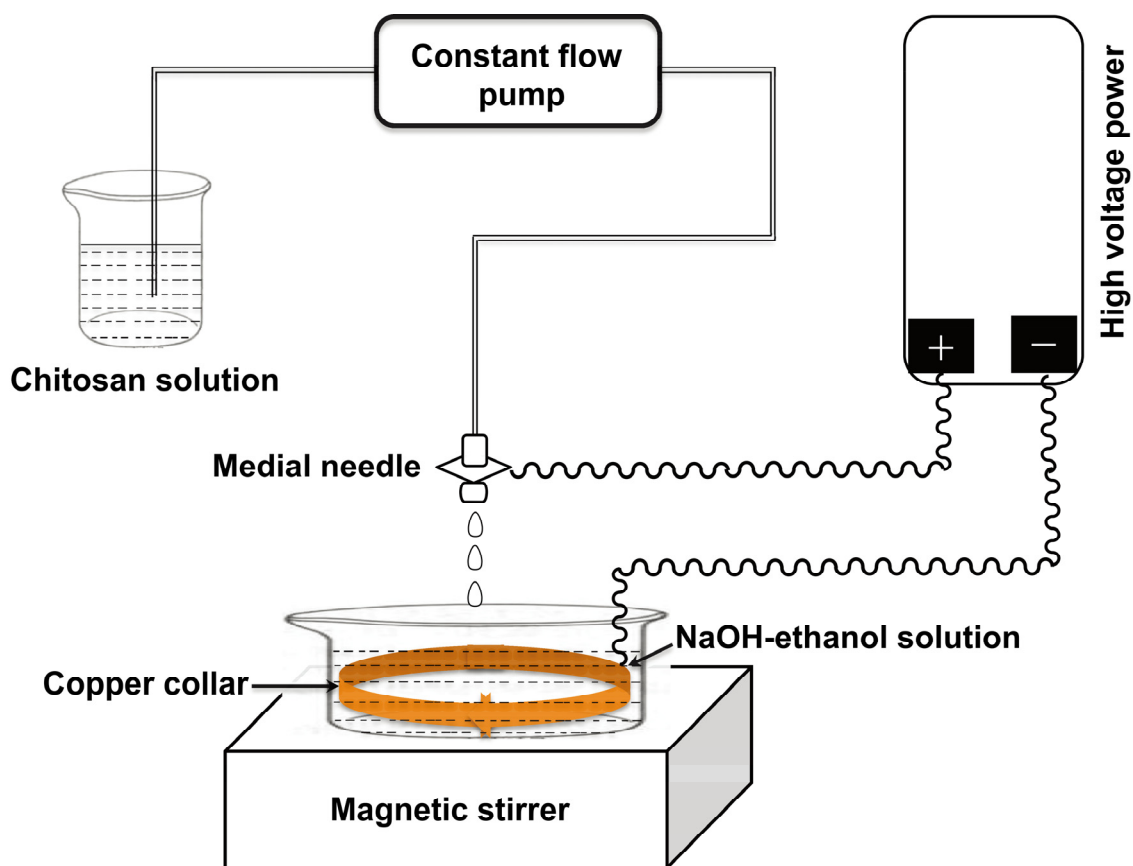
**Table S3** Significance analysis of the factors in the L<sub>25</sub>-orthogonal test of *P. putida* MB285-immobilization

| Levels  | <i>A</i> <sup>a</sup> | <i>B</i> | <i>C</i> | <i>D</i> |
|---|-----------------------|----------|----------|----------|
|   | Mean                  | Mean     | Mean     | Mean     |
| 1   | 4.804                 | 3.856    | 4.442    | 5.068    |
| 2   | 4.666                 | 3.556    | 4.620    | 5.028    |
| 3   | 4.458                 | 6.220    | 6.198    | 5.078    |
| 4   | 5.100                 | 5.590    | 4.322    | 4.448    |
| 5   | 6.016                 | 5.822    | 5.462    | 5.422    |
| Laccase enzyme activity (U g <sup>-1</sup> , wet weight) <sup>b</sup> | 1.558                 | 2.664    | 1.876    | 0.974    |
| Rank  | 3                     | 1        | 2        | 4        |

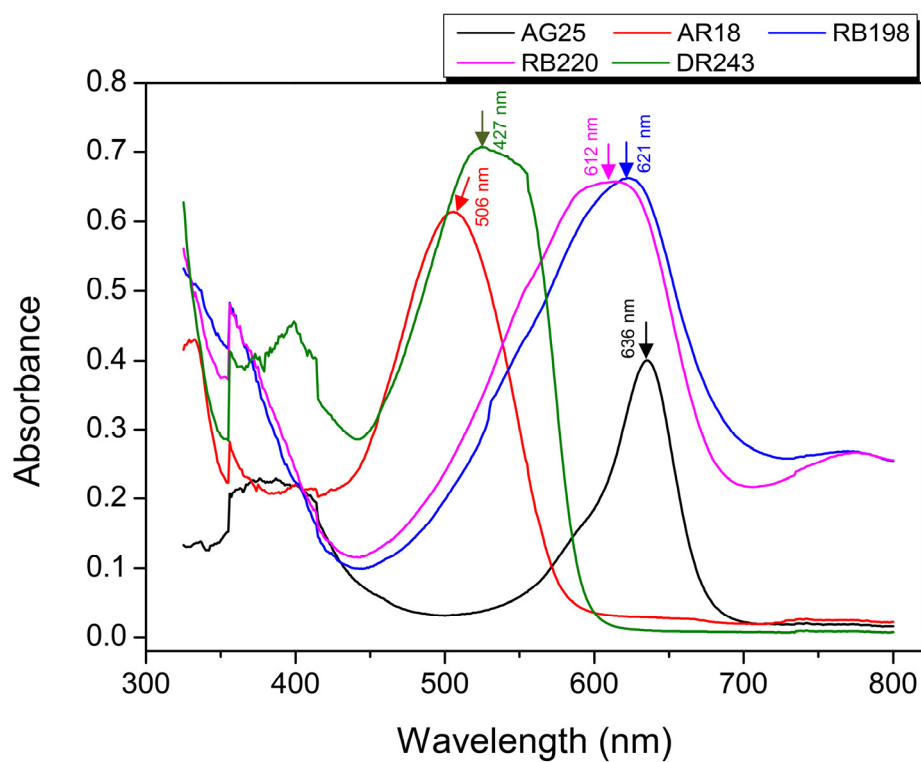
<sup>a</sup> *A*: glutaraldehyde concentration; *B*: immobilization reaction time; *C*: immobilization reaction temperature (°C); *D*: prepared CTS-MB materials (CTS-MB1 to CTS-MB5).

<sup>b</sup> Range of the corresponding whole-complex laccase enzyme activities of each factor.

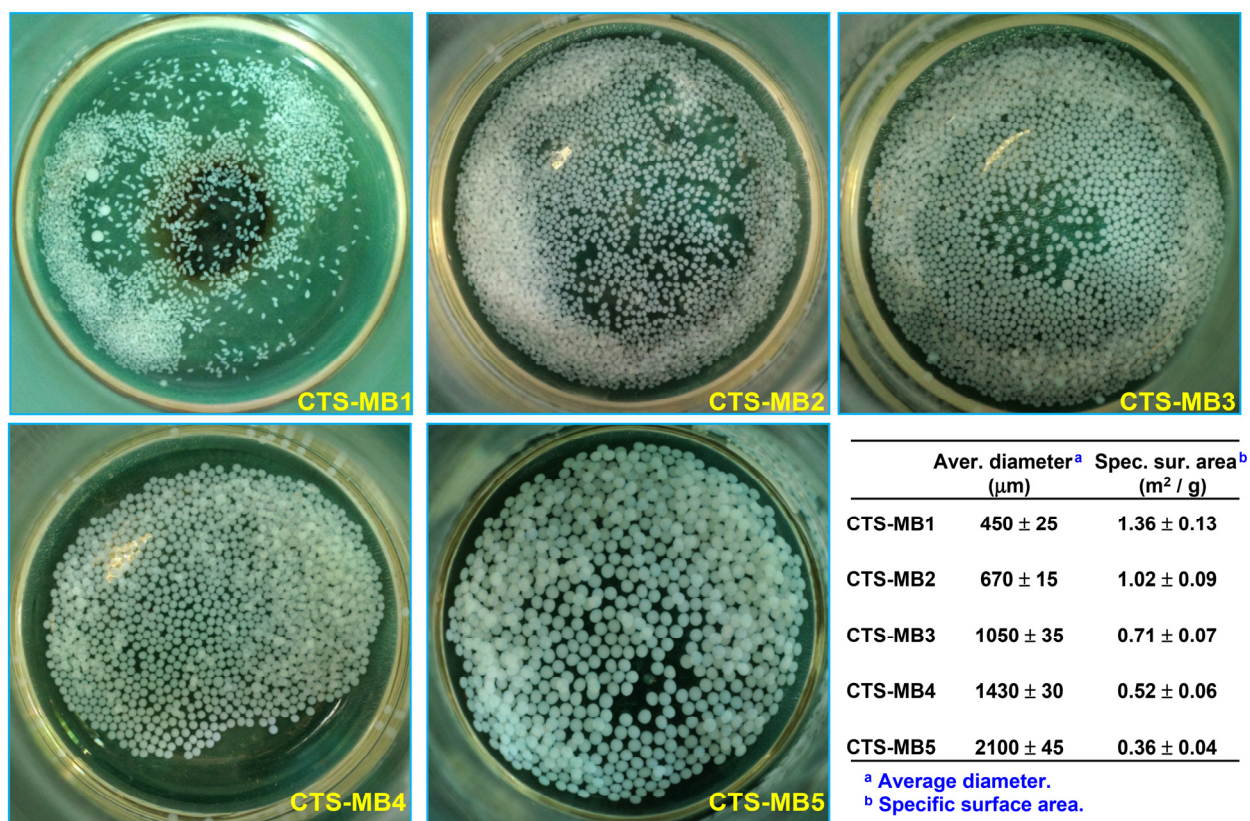
## Supplementary Figures



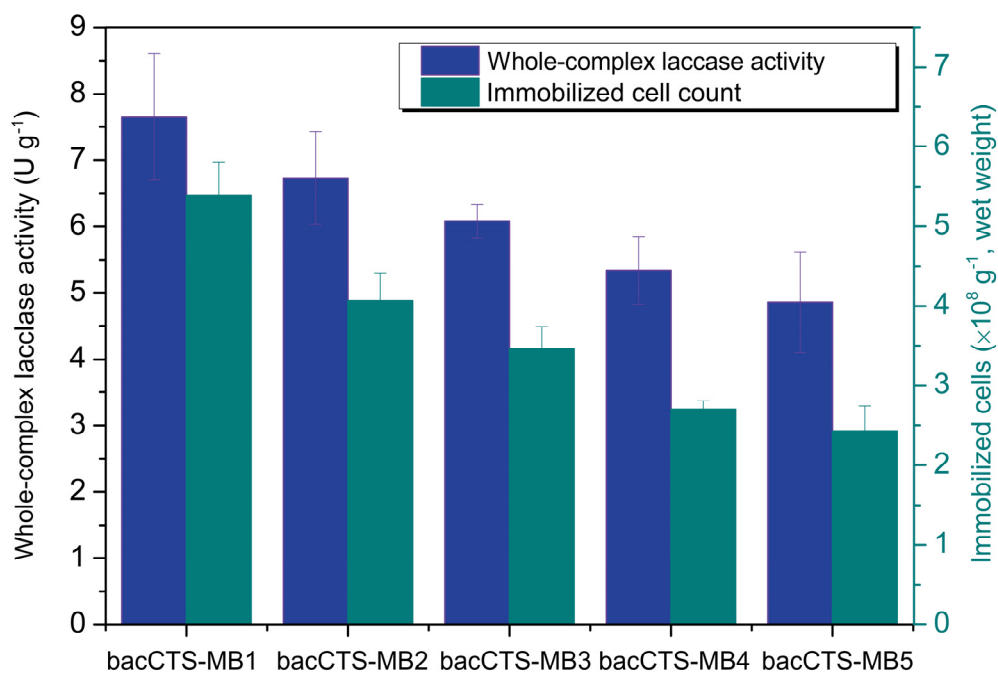
**Figure S1.** Schematic illustration of the self-assembled facile device for the preparation of various chitosan microbeads with different particle sizes.



**Figure S2.** Full wavelength (325 nm to 800 nm) scanning curves of five synthesized dyes indicating their maximal adsorbent peaks (arrows), which were used as the OD values for the dye absorbance measurements.



**Figure S3.** Morphological images of the five as-prepared CTS-MB materials and their average diameters and specific surface area values.



**Figure S4.** Whole-composite laccase enzyme activity and immobilized *P. putida* cell counts of the five prepared bacCTS-MB materials.