ELECTRONIC SUPPLEMENTARY INFORMATION for

Complex Function by Design Using Spatially Pre-Structured Synthetic Microbial Communities: Degradation of Pentachlorophenol in the Presence of Hg(II)

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Supporting Information

Supporting Figures:

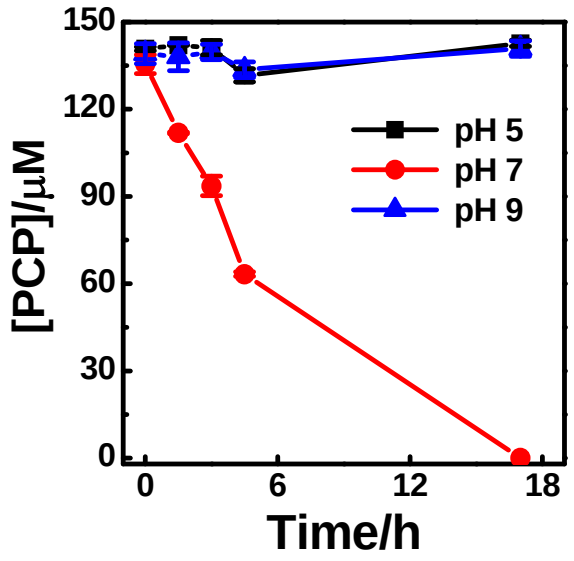

Figure S1: The optimal pH for degradation of PCP by *S. chlorophenolicum* was pH 7. *S. chlorophenolicum* cells were inoculated in PM media containing PCP (~140 μM, final concentration), under 3 different initial pH conditions. The pH of the media was adjusted to either 5 or 9 by using either HCl solution (1 M) or NaOH solution (1 M), respectively prior to inoculations. PCP was completely degraded at pH 7 (red solid circles), whereas PCP was not degraded in either pH 5 (black solid squares) or pH 9 (blue solid triangles). The initial cell density was adjusted to be ~10^7 CFU·mL^{-1}. Error bars indicate standard errors (n = 2). *S. chlorophenolicum* grew under all three pH conditions (data not shown).

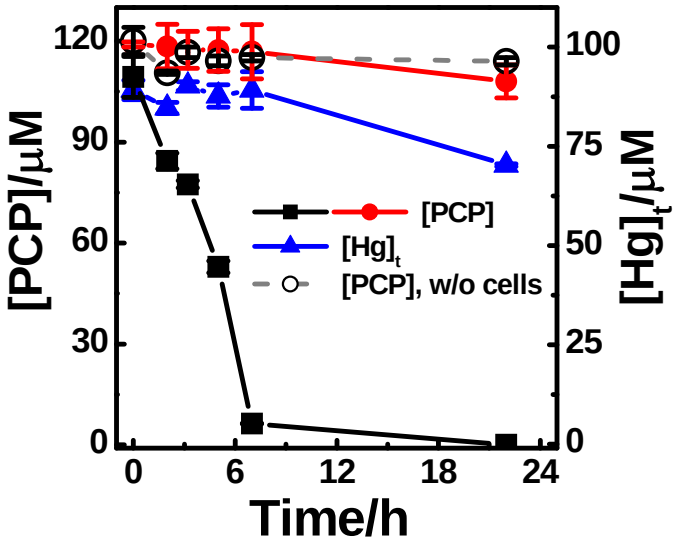

Figure S2: In the absence of Hg(II) ions, PCP was completely degraded by *S. chlorophenolicum*; whereas in the presence of the Hg(II) ions, *S. chlorophenolicum* cells did not degrade the PCP at all. The profiles of PCP degradation in a pure solution culture of *S. chlorophenolicum* cells show that PCP was completely degraded in the absence of mercury (black solid squares). In contrast, *S. chlorophenolicum* cells did not degrade the PCP at all in the presence of the Hg(II) ions (red solid circles). Since *S. chlorophenolicum* cells are extremely sensitive to the presence of Hg(II) ions, *S. chlorophenolicum* cells did not reduce the Hg(II) ions (blue closed triangles) and did not even grow in the presence of Hg(II) ions (see Figure S5). As a control, we confirmed that in the absence of *S. chlorophenolicum* cells (i.e. 'w/o cells'), PCP was not degraded (black open circle, grey dashed line). These results indicate that *S. chlorophenolicum* cells are not resistant to the Hg(II) ions and that they cannot reduce the Hg(II) ions. $[Hg]_t$ in the axis title indicates the total mercury concentration at all oxidation states. Approximately 10-15% of the initial amount of Hg(II) ions was spontaneously reduced in the media without any bacterial cells (Figure S3, black open triangles). In the seed culture of *S. chlorophenolicum* cells, PCP (50 μM, final concentration) was added in the culture broth to induce the PCP degradation pathway. The initial number of live *S. chlorophenolicum* cells in each experimental setup was adjusted to be ~10^8 $CFU \cdot mL^{-1}$. Error bars indicate standard errors (n = 2).

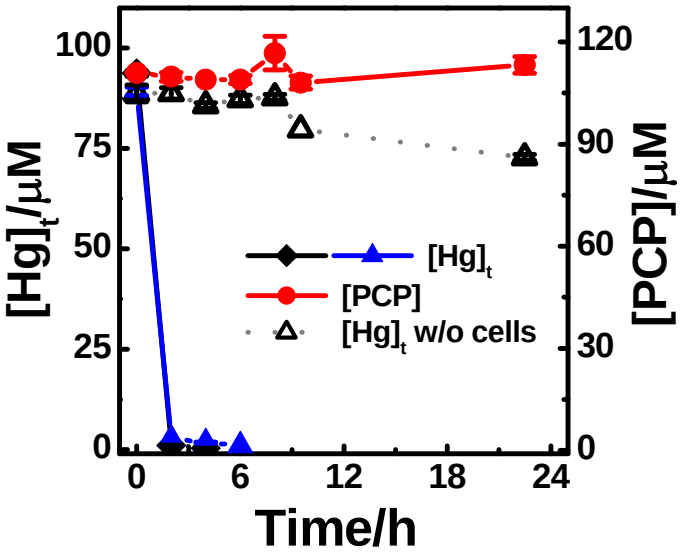

Figure S3: Hg(II) ions were completely reduced by *R. metallidurans* cells regardless of the presence or the absence of PCP. The profiles of Hg(II) ion reduction in a pure culture of *R. metallidurans* cells show that Hg(II) ions were completely reduced in 6 h (black solid diamonds) in the absence of PCP. *R. metallidurans* cells reduced Hg(II) ions regardless of the presence of the PCP (blue solid triangles), but they did not degrade PCP (red solid circles). As a control, we confirmed that in the absence of *R. metallidurans* cells (i.e. 'w/o cells'), Hg(II) ions were not reduced within 6 h (black open triangles, grey dotted line). However, approximately 10-15% of the initial amount of Hg(II) ions was spontaneously reduced in the media over 24 h without any bacterial cells. These results indicate that *R. metallidurans* cells are resistant to PCP, but that they cannot degrade it. $[Hg]_t$ in the axis title indicates the total mercury concentration at all states. In the seed culture of *R. metallidurans* cells, $HgCl_2$ solution (50 μM, final concentration) was added in the culture broth to induce the production of mercury reductases. The initial number of live *R. metallidurans* cells in each experimental setup was adjusted to be ~10^8 CFU·mL^{-1}. After 6 h, $[Hg]_t$ decreased below the limit of detection (0.37 μM). Error bars indicate standard errors (n = 2).

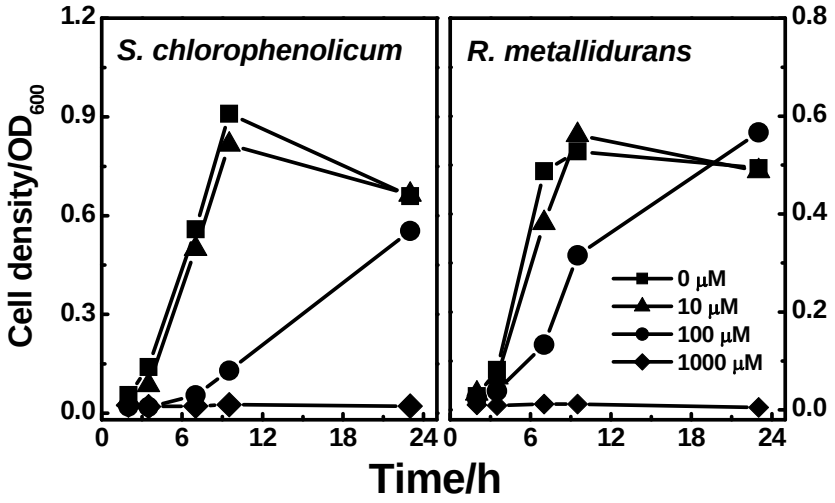

Figure S4: Both *S. chlorophenolicum* and *R. metallidurans* cells can grow in the presence of PCP at concentrations below 1000 μM. Growth profiles are shown of *S. chlorophenolicum* (left) and *R. metallidurans* (right) species in the presence of PCP at concentrations of 0 μM (square), 10 μM (triangle), 100 μM (circle), and 1000 μM (diamond). The PCP degradation pathway was not induced in either species because the PCP solution was not added to the culture broth in the seed culture period for either species. Cultivations were performed in a 96-well plate. The initial cell density of both species was adjusted to be ~0.03 optical density units at 600 nm.

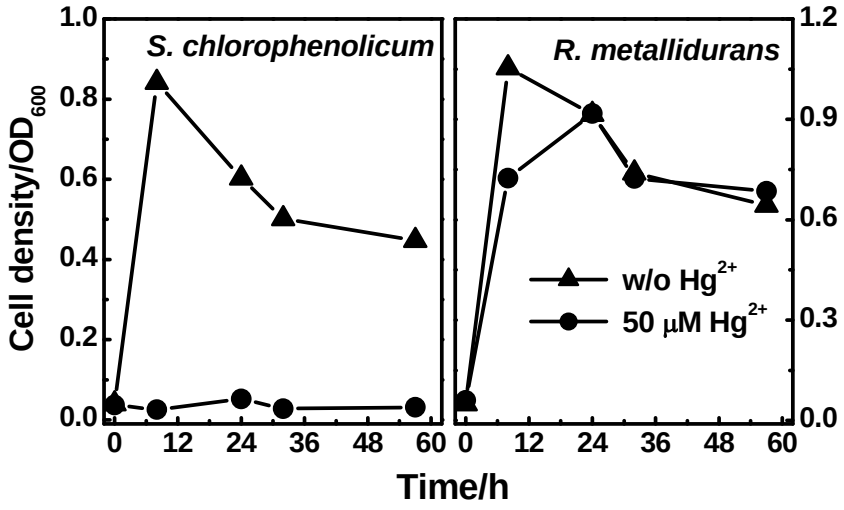

Figure S5: In the absence of Hg(II) ions in the PM media, both species grew, but in the presence of Hg(II) ions, *S. chlorophenolicum* did not grow even at a very low concentration of Hg(II) ions. Growth profiles are shown of *S. chlorophenolicum* (left) and *R. metallidurans* (right) species in the presence and absence of Hg(II) ions. In the absence of Hg(II) ions in the PM media, both species grew quickly and gradually decreased their densities (triangles). In the presence of Hg(II) ions at 50 μM, *S. chlorophenolicum* did not grow at all (left, circles). However *R. metallidurans* grew robustly in the presence of Hg(II) ions even at 50 μM (right, circles). Cultivations were performed in a 96-well plate. Mercury reductases were not induced in either species because the $HgCl_2$ solution was not added to the culture broth in the seed culture period for either species. The initial cell density of both species was adjusted to be ~0.03 optical density units at 600 nm.

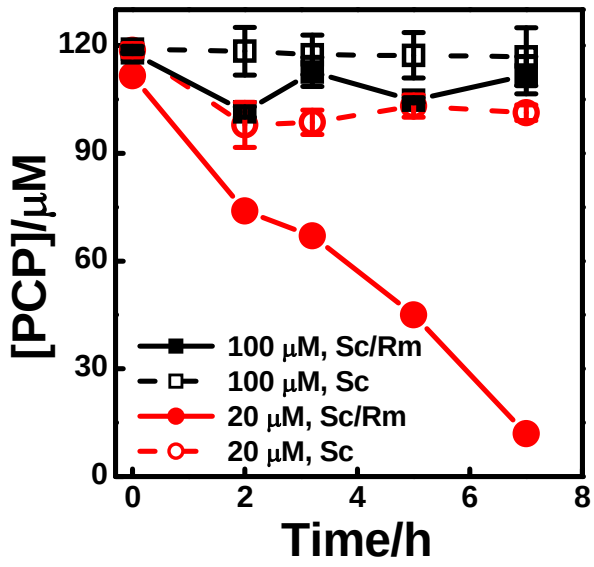

Figure S6: At a low concentration of Hg(II) ions (20 μM), the well-mixed community of *R. metallidurans* and *S. chlorophenolicum* degraded PCP, but the monoculture of *S. chlorophenolicum* did not. At a higher concentration of Hg(II) ions (100 μM), neither the well-mixed community nor the mono-culture degraded PCP. The graph shows degradation of PCP in a well-mixed co-culture of *R. metallidurans* and *S. chlorophenolicum* or a mono-culture of *S. chlorophenolicum*, grown in the presence of both PCP and Hg(II) ions. At 100 μM concentration of Hg(II) ions, *S. chlorophenolicum* can not degrade PCP regardless of the presence (black solid squares, solid line) or the absence (black open squares, dashed line) of *R. metallidurans*. However, at 20 μM concentration of Hg(II), *S. chlorophenolicum* can degrade PCP in the presence of *R. metallidurans* cells (red solid circles, solid line) but not in the absence of *R. metallidurans* (red open circles, dashed line). These results demonstrate that there is a critical concentration of Hg(II) ions for the degradation of PCP in a well-mixed co-culture of *S. chlorophenolicum* and *R. metallidurans* cells. 'Sc' or 'Rm' indicates the mono-culture of either *S. chlorophenolicum* cells or *R. metallidurans* cells, respectively, whereas 'Sc/Rm' means the well-mixed co-culture of both species. The initial cell density of either *S. chlorophenolicum* or *R. metallidurans* cells in each experimental setup was adjusted to be ~10^8 CFU·mL^{-1}. Error bars indicate standard errors (n = 2).

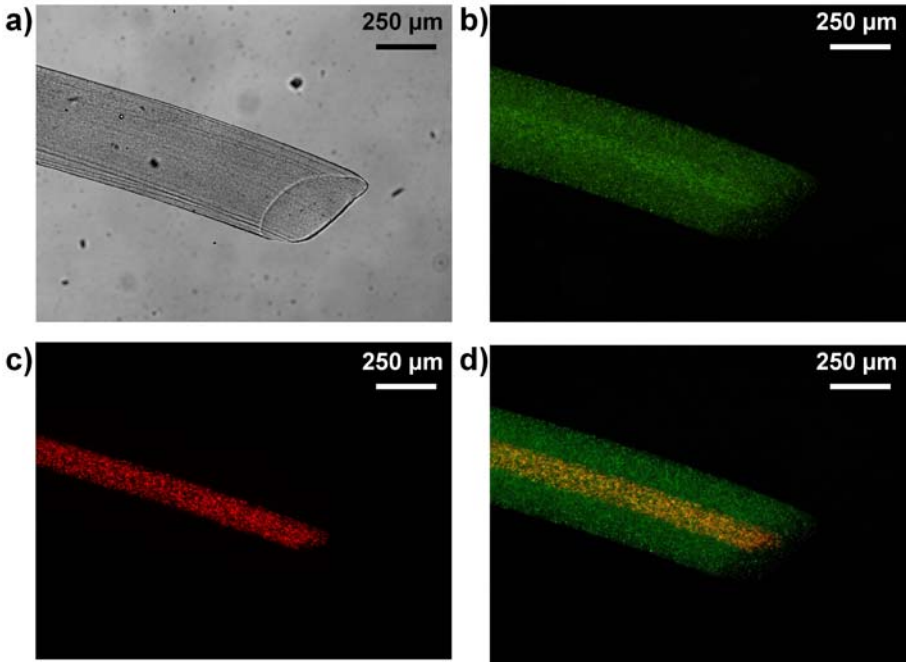

Figure S7. Images of core-shell structured fibers a) A bright field image of the core-shell structured fiber with one end cut by a blade. b) A fluorescent image taken with an L5 filter indicates GFP-labeled *E. coli* cells are in the shell layer. c) A fluorescent image taken with a TX2 filter indicates RFP-labeled *E. coli* cells are in the core part. d) An overlay of b) and c) images highlights the core-shell structure in the fiber. None of the cells in the core material are found in the shell or vice versa.