Naphthalene Assay

1. Take 1.6 mL of culture in exponential phase into a screw-cap tube, and add few crystals of naphthalene. (We use the culture directly for naphthalene assay. Never washed or centrifuged to concentrate the cells)

2. Incubate for 1 h at 30 °C with shaking (200 rpm).

3. Prepare fresh Fast Blue B (tetrazotized o-dianisidine) (4 °C fridge) solution (4.21 mM, 2 mg for 1 mL).

4. After 1 h (30 °C) incubation of cells with naphthalene, centrifuge the cells for 5 min at 5,800 x g.

5. Take out 1.3 mL of supernatant into a new tube and add 130 μL of Fast Blue B (4.21 mM) solution. Color change after adding Fast Blue B solution is very rapid and clear if there is sMMO activity.

6. Transfer 1 mL to the cuvette for measuring absorbance at 528 nm.