Naphtalene Assay <- Warwick/New Englang

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Brusseau, G.A., Tsien, H.-C., Hanson, R.S., and Wackett, L.P. (1990). Optimization of trichloroethylene oxidation by methanotrophs and the use of a colorimetric assay to detect soluble methane monooxygenase activity. Biodegradation *1*, 19–29.
To the samples containing naphthalene
Add 100 ul of freshly hydrated tetrazotized o-dianisidine (4.21 mM)
Samples were sacrificed at time intervals by adding 100/zl of freshly hydrated tetrazotized o-dianisidine (4.21 mM). Heat-killed and sterile media controis were also tested. If formed, the colored product was clearly visible to the naked eye or readily monitored by recording the absorption spectrum over the range of 430 to 650 mm with a Beckman DU-70 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA). This adduct, as well as azo dyes formed from synthetic 1-naphthol and 2-naphthol, proved to be unstable in the mineral salts media, and phosphate and organic buffers
used in these studies. However, the intensity of color formation immediately following the addition of tetrazotized o-dianisidine was proportional to the naphthol concentration

Graham, D.W., Korich, D.G., LeBlanc, R.P., Sinclair, N.A., and Arnold, R.G. (1992). Applications of a colorimetric plate assay for soluble methane monooxygenase activity. Applied and Environmental Microbiology *58*, 2231–2236.

Plates were incubated at 30°C under a 25% methane-air atmosphere for 7 to 21 days to allow development of colonies of different age and size. Every 2 to 3 days the incubator (BBL anaerobic jar) was opened and replenished with air and methane. Plates were removed intermittently, and colonies were screened for sMMO activity by the following procedure. A few naphthalene crystals were sprinkled in the lid of the plate, and the plate was stored inverted at 30°C for
15 min in air. The plates were then opened and lightly sprayed with freshly prepared, 5-mg/ml o-dianisidine (tetrazotized; zinc chloride complex; Sigma Chemicals Ltd.), for 2
to 3 s. The lid was replaced, and the plate was stored for 15 min in the presence of the dye. If naphthol was produced by the colonies, a purple-red color appeared upon contact with the dye. The color, once formed, remained stable for at least 24 h at room temperature and up to a month at 4°C.

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Fast blue B salt, Sigma product no. D9805

Reagents:
http://www.sigmaaldrich.com/catalog/product/sial/d9805?lang=en&region=CA
http://www.sigmaaldrich.com/catalog/product/aldrich/147141?lang=en&region=CA
Reference:
https://books.google.ca/books?id=3T72JboguyMC&pg=PA145&lpg=PA145&dq=methane+monooxygenase+naphthalene+sigma&source=bl&ots=6uqKlrtcIU&sig=FgD14jUiRQcmEySuTB_PbWG9zok&hl=en&sa=X&ved=0ahUKEwir3NPUm4XNAhVL62MKHe00Du4Q6AEIQTAF#v=onepage&q=methane%20monooxygenase%20naphthalene%20sigma&f=false

> **The standard naphthalene oxidation test is performed as follows: A few crystals of naphthalene (□ 50 mg) are scattered across the lid of the Petri dish and the plate is inverted over it, bacteria side down. The plate is sealed in a plastic sandwich**
> **box and placed in an incubator at 30 □C for 1–4 h or at 45 C for 40 min. The optimum temperature for wild-type Ms. trichosporium sMMO is 30 □C; the use of a higher incubator temperature is effective because the local high**
> **temperature on the base of the box helps to vaporize some of the naphthalene, while the plate remains sufficiently cool for the enzyme not to be inactivated. The plates are removed from the incubator and a freshly prepared solution of tetrazotized o-dianisidine (fast blue B salt, Sigma product no. D9805; 5 mg mL□ 1) is added dropwise onto the colonies. A purple or pink color that develops instantly or within a few minutes indicates sMMO activity.**