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Acclimation of Algal Species Following Exposure to Phenol

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Phenol is a constituent of coal tar formed during natural decay of organic matter. The production and use of phenol at the industrial level as well as the residential wood burning are the main anthropogenic sources of phenol in the environment. Phenol is degraded by abiotic reactions and microbial activity (WHO 1994). Therefore biological oxidation of industrial and municipal effluents can reduce the phenol considerably. However, levels as high as 75 mg/L is reported to be toxic to the aerobic treatment systems (Tolgyessy 1993). The aim of the present study was to investigate the growth response of algae *Oocystis pusilla, Chlorella pyrenoidosa* and *Oscillatoria quadripunctulata* on exposure to phenol, and to explore the possibility of developing phenol acclimated strains of algae for probable application to a bioreactor for effluent treatment.

MATERIALS AND METHODS

The test species *Oocystis pusilla, Chlorella pyrenoidosa* (unicellular green algae) and *Oscillatoria quadripunctulata* (filamentous blue-green alga) were isolated locally from a waste stream The axenic cultures of the green algal species were maintained in the laboratory in Ward and Parrish medium (Ward and Parrish 1982). *Oscillatoria quadripunctulata* was maintained in BG 11 medium (Stainer et al. 1971).

Algal assays were conducted in triplicate in 50 mL culture tubes containing 24 mL of the growth media with two concentration series of phenol. The test series were (a) 0.1, 1, 2, 4, and 5 mg/L phenol, and (b) 10, 25, 50, 75 and 100 mg/L. The test media were inoculated with exponential growth cultures at an initial cell density of $1x10^4$ cells/mL. The initial absorbance at 620 mu was measured for *O.quadripunctulata.* Control sets of cultures were maintained in the test medium free of phenol. All the test cultures were incubated for 8 days at a temperature 28 ± 3°C under cool white light from an assembly of 5x40 watts fluorescent lamps. The cell yield was determined as cell count of *O. pusilla* and *C.pyrenoidosa,* and absorbance of *O. quadripunctulata.* The data were analysed by one-way analysis of variance.

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The cells of *O. pusilla* and *C. pyrenoidosa* exposed to 4 mg/L phenol were centrifuged and inoculated to phenol free medium to check the viability. Following 8 day growth, the cells were harvested and exposed to successively higher concentrations of phenol in the order of 7, 10, 12, 15, 17, 20, 22 and 25 mg/L, each time checking the viability of the culture. At each level of exposure, growth was measured in terms of cell number or absorbance, chlorophyll *a* and productivity. Chlorophyll *a* was estimated spectrophotometrically following the procedure of Burnison (1980). Cell count was determined using a haemocytometer. Productivity was estimated by oxygen method (APHA 1992). At 25 mg/L phenol, the depletion of phenol in the cultures of the acclimated *O.pusilla* and *C. pyrenoidosa* and unacclimated *O. quadripunctulata* was followed at different time intervals of 10 min, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 24 hr, 48 hr, and 96 hr of exposure. The uninoculated test media was the control to verify the depletion of phenol by algae. The concentration of phenol was estimated by 4-amino antipyrene method (APHA 1992).

RESULTS AND DISCUSSION

The results of the direct exposure of the three test species to the lower concentration series of phenol is shown in Fig. 1. The eight day cell yield of *O.pusilla* decreased with the concentration of phenol ($p \leq 0.0$). Comparison with the control showed significant inhibition at >2 mg/L phenol. The cell yield of *C. pyrenoidosa* at different concentrations of phenol were significantly different from each other ($p \leq 0.01$) with growth inhibition at > 1 mg/L (Fig. la). Upon resuspension in phenol free medium, these cultures regained viability and the growth rates were similar to that of control cultures. The growth of *O.quadripunctulata* was significantly stimulated by phenol at 5 mg/L (Fig. lb). *The* exposure of *O. pusilla* and *C. pyrenoidosa* to higher concentration series of phenol showed growth inhibition in all cases. The higher concentration series of phenol stimulated the growth of *O. quadripunctulata* at 10 mg/L, and a significant growth inhibition occurred only at > 25 mg/L ($p \leq 0.01$).

Upon inoculation to successively higher concentrations of phenol, the cell count, chlorophyll *a,* and productivity of *O. pusilla* were nearly the same as or higher than the control. The growth of *C. pyrenoidosa* was inhibited upon re-exposure to phenolic media at 7 and 10 mg/L. However, following a second exposure to the test medium at 10 mg/L, the growth was revived and attained a cell yield higher than the control. The chlorophyll *a* and productivity increased and the trend was observed up to 25 mg/L phenol (Fig. 2).

The concentration of phenol in the control decreased to 21.1 mg/L after 24 hr (Fig. 3). The phenol concentration of the test media inoculated with *O. pusilla* and *C. pyrenoidosa* was 17 mg/L and that of *O. quadripunctulata* was 3.40 mg/L after 24 hr.

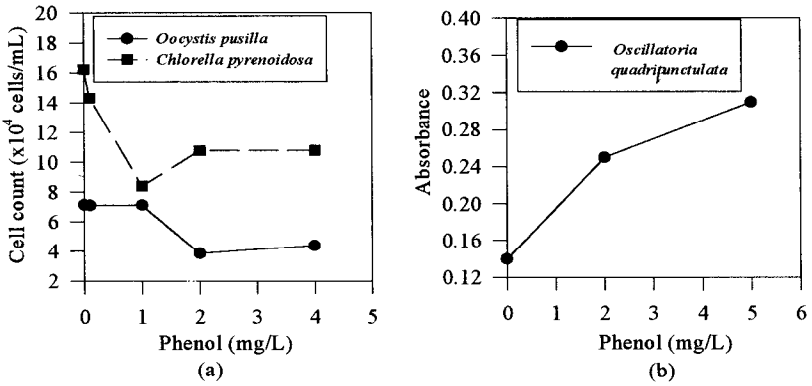

Figure 1. Growth of *Oocystis pusilla, Chlorella pyrenoidosa* and *Oscillatoria quadripunctulata* on direct exposure to phenol for 8 days.

The amount of phenol in the control as well as that inoculated with algae reduced by > 90% after 48 hr. The depletion of phenol in the control reveals that phenol undergoes natural degradation within 48 hr. Phenol degradation in the environment is attributred to abiotic process or microbial activity (WHO 1994). The study clearly shows that the algae can speed up the phenol depletion, the rate of which is species specific. Thus *O. quadripunctulata* could deplete phenol by 84% in 24 hr against 15.6% depletion in control.

The results reveal that the phenol toxicity depends on various sensitivities of the species and their pre-exposure to the contaminant. The growth of blue-green alga *O. quadripunctulata* is stimulated at the lower test doses of phenol up to 25 mg/L. Many blue-green algae are reported to metabolise phenols. *Oscillatoria* sp., strain JCM can oxidise biphenyl (Cernigla et al. 1980). According to Megharaj et al. (1991) *Nostoc linckia* can degrade phenol. A marine cyanobacterium *Phormidium valderianum* BDU 3050 was able to degrade phenol completely at 100 mg/mL by its intracellular oxidase and lactase enzymes (Subramanian and Uma 1997). This organism was identified for use in phenolic effluent treatment.

The green algal species were very sensitive to phenol, as the growth inhibition was evident at very low concentrations as 2 mg/L and 1 mg/L for *O. pusilla* and *C. pyrenoidosa*, respectively. In a similar study on the differential response of the green algal species to solvents, *C. ellipsoidoea* was reported to be the most sensitive species, the growth being inhibited at 0.05%, 0.1% and 0.2% phenol concentrations (Tadros et al. 1994).

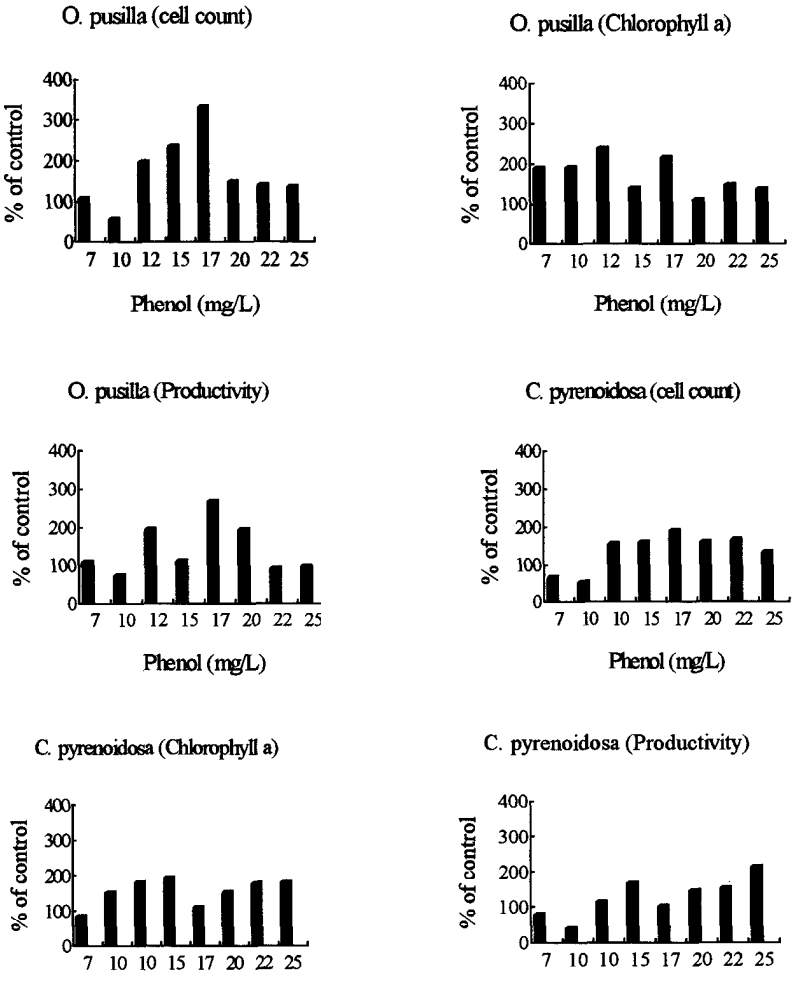

Figure 2. Growth of *Oocystis pusilla* and *Chlorella pyrenoidosa* on successive exposure to phenol for 8days.

Acute toxicity level of phenol to *C. pyrenoidosa* was reported to be 10-30 mg/L in terms of 96 hr LC 50 (Moore and Ramamoorthy 1984). Tisler and Zagorc (1995) observed that *Scenedesmus quadricauda* had only a low sensitivity to phenol as the 24 hr EC 50 value was 403 mg/L. Shigoeka et al. (1988) also indicated high EC SO values for *Selenastrum capricornutum* and *Chlorella vulgaris* following 4 day growth in phenol.

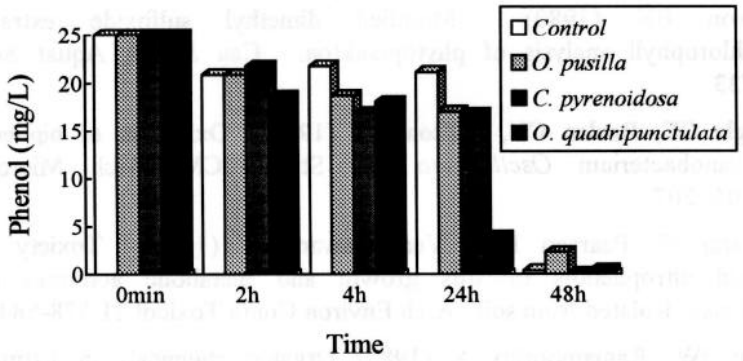

Figure 3. Phenol removed from the growth medium by algae

The present investigation also revealed that phenol at the doses does not induce an algicidal effect as the exposed cultures retained their vitality upon m-exposure to phenol free medium. Furthermore, they could be gradually acclimated to grow in the phenolic medium. In a study on the acclimation of algal communities to zinc toxicity, it was concluded that the acclimation of the test organism to a toxicant has an important effect on its response to toxicity (Wang 1986). The tolerance of algae to phenol on repeated exposure is species specific, the mechanism involved may be either storage or metabolic utilisation. However, it is evident that algae are capable of reducing the phenol load of the medium, the extent of removal being species specific. *O. pusilla* could remove 32% of the phenol in 24 hr, against 15.6% of the control. The phenol removal effected by *C. pyrenoiodsa* was 32% and that by *O. quadripunctulata* was 84% in 24 hr. The results obtained with *O. quadripunctulata* are more promising. It may be concluded that tolerance to a particular effluent or chemical can be induced by sufficient pre-exposure. The investigation has also identified *O. quadripunctulata* as a suitable organism for the biotreatment of phenolic effluent.

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REFERENCES

American Public Health Association (APHA) (1992) Standard methods for The examination of water and wastewater. American Public Health Association, Washington D.C.

Burnison BK (1980) Modified dimethyl sulfoxide extraction for chlorophyll analysis of phytoplankton. Can J Fish Aquat Sci 37:728-733

Cernigla CE, Baalen CV, Gibson DT (1980) Oxidation of biphenyl by the cyanobacterium *Oscillatoria sp.,* Strain JCM. Arch Microbiol 125: 205- 207

Megharaj M, Pearson HW, Venkateswarlu K (1991) Toxicty of phenol and nitrophenols towards growth and metabolic activities of *Nostoc linckia* isolated from soil. Arch Environ Conta Toxicol 21:578-584

Moore JW, Ramamoorhty S (1984) Organic chemicals in natural waters: applied monitoring and impact assessment. Springer-Verlag, New York

Shigoeka T, Sato Y, Takeda Y, Yoshida K, Yamauchi F (1988) Acute toxicity of chlorophenols to green algae *Selenastrum capricornutum* and *Chlorella vulgaris* and quantitative structure-activity relationships. Environ Toxicol Chem 7:847-854

Stainer RY, Kunisawa R, Mandel M, Cohin-Bazire G (1971) Purification and properties of unicellular blue-green algae (order Chrococcales). Bacteriol Rev 35:171-205

Subramanian G, Uma L (1997) Role of cyanobacteria in pollution abatement. In: Sinha MP (ed) Recent Advances in Ecobiological Research, Vol 1. A.P.H. Publishing, New Delhi, p 435-443

Tadros MG, Philips J, Patel H, Pandiripally V (1994) Differential response of green algal species to solvents. Bull Environ Contam Toxicol 52:333-337

Tisler T, Zagorc-Koncan J (1995) Relative sensitivity of some selected aquatic organisms to phenol. Bull Environ Contam Toxicol 54:717-724

Tolgyessy P (1993) The ecotoxicology of water pollutants. In: Tolgyessy J (ed) Chemistry and Biology of Water, Air and Soil, Environmental Aspects. Studies in Environmental Science 53. Elsevier Science, Amsterdam p 742-756

Wang W (1986) Acclimation and response of algal communities from different sources to zinc toxicity. Water Air Soil Pollut 28: 335-349

Ward GS, Parrish PR (1982) Mammal of methods in aquatic environment research Part 6 Toxicity tests. FAO FishTech Pap (185) p 23

World Health Organization (WHO) (1994) International Programme on Chemical Safety Environmental Health Criteria 161: Phenol