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Microalgae in Petrochemical Effluent: Growth and Biosorption of Total Dissolved Solids

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The petrochemical wastewater characteristics include oils, solvents, high biochemical oxygen demand, suspended solids, aromatics, phenols, sulphides, halogenated and polycyclic aromatic compounds and detergents. The phenols, aldehydes, chlorinated and aromatic hydrocarbons in the effluent are biocides, and very toxic to fish (Chivers 1984). The pollution of drinking water sources is a major health concern. In the petrochemical effluent treatment, lagoons are usually used where algae play a major role. At times, even after secondary treatment there is high concentration of total dissolved solids in the effluent, which make it unsuitable for recycling in the process plant. Algae can be used in the assessment of pollution (Wong 1995), or abatement of pollution (de la Noue et al. 1992; Venketaraman et al. 1994; Subramanian and Uma 1997). Low molecular weight hydrocarbons were found to stimulate or inhibit phytoplankton growth, depending upon the species (Dunstan 1975). Less attention has been paid to the effects of phenolic effluents to algae, in particular to fresh water algae (Tripathi and Pandey 1990). The present study was to investigate the growth response of both phenol acclimated and non-acclimated laboratory cultures of microalgae, *Oocystis pusilla*, *Chlorella pyrenoidosa* and *Oscillatoria quadripunctulata* to a petrochemical effluent, and to assess the subsequent effect on the biosorption of total dissolved solids (TDS).

MATERIALS AND METHODS

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

The effluent was collected from the discharge point of a petrochemical industry for four months from March to June 1995, and analysed for pH, biochemical oxygen demand, nitrate, phosphate, ammonia, phenol and total dissolved solids following standard methods (APHA, 1992). The effluent collected in the summer month of May was filtered through GF/C filter paper (0.45μm). A portion of the

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effluent was diluted with boiled, cooled and aerated fresh water to make the dilution series of 20, 40, 60 and 80 percentage of the effluent. These test dilutions along with undiluted effluent were taken in 250 mL borosilicate conical flasks, properly sterilized and plugged with non-absorbent cotton. Each test dilution was taken in triplicate for statistical evaluation of the data. Exponential growing cultures of *O. pusilla* and *C. pyrenoidosa* were inoculated into these test dilutions so as to give an initial cell count of $1x10^5$ cells mL^{-1}. The test species were similarly inoculated to the maintenance medium to serve as control. The test cultures were incubated for 96 hours under photosynthetically active radiation of intensity 160 $\mu Em^{-2}s^{-1}$ using a fluorescent lamp assembly set on 12:12 L/D cycle at 28 ± 2^0 C. The cell count was determined in a haemocytometer after 96 hr. Similar assay was conducted using *O. quadripunctulata* simultaneously where the optical density at 620 nm was measured instead of cell count as the alga has filamentous habit. The specific growth rate was calculated for *O. quadripunctulata* from the optical density at 620 nm using the equation $\mu = \ln (n_2/n_1)/(t_2-t_1)$ where μ = specific growth rate, and n_1 and n_2 are absobances of the culture suspension at time t_1 and t_2 respectively (Beg et al. 1982).

Subcultures of the three test species were raised in phenol containing culture medium so that they were acclimated to grow in phenol of 25 mgL^{-1} (Joseph and Joseph 1999). The phenol acclimated cultures were assayed against 1000 mL of above GF/C filtered and undiluted effluent simultaneously in the same way as the previous assay. The 96 hr cell count/ absorbance of both assays were compared to that of control (respective growth medium without phenol).

The total dissolved solids content of the effluent was determined initially and after 96 hr retention in test cultures and the effluent controlshowing algal growth.

RESULTS AND DISCUSSION

The effluent characteristics are given in Table 1. The concentrations of nitrate, ammonia and phosphate varied from 0.073 - 2.460 mgL^{-1}, 0.008 - 5.690 mgL^{-1} and 0.038 - 8.4 mgL^{-1} respectively.

Table1. Chemical characteristics (in mgL^{-1}) of the petrochemical effluent.

Parameter	March 1995	April 1995	May 1995	June 1995
pH	8.350	8.730	8.430	8.750
BOD	72.430	39.800	43.600	35.400
Nitrate	0.870	1.380	0.073	2.460
Phosphate	0.600	1.170	0.008	5.690
Ammonia	8.400	0.038	4.320	0.011
Phenol	0.520	0.430	0.780	1.380
TDS	423.000	398.000	411.000	427.000

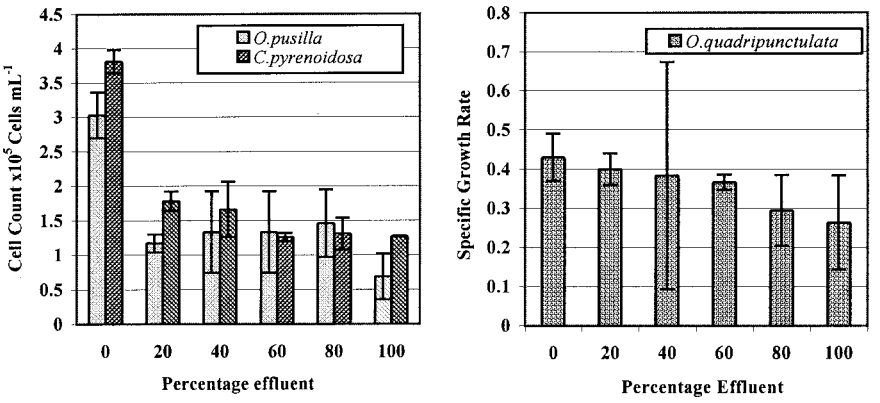

Figure 1. Growth of *Oocystis pusilla*, *Chlorella pyrenoidisa* and *Oscillatoria quadripunctulata* in a dilution series of petrochemical effluent for 96 hours.

The effluent inhibited the growth of the three species of algae with the increasing concentration (Fig.1). The percentage inhibition of growth of *O. pusilla* in 20% of the effluent was 61%, while in the undiluted effluent it was 77%. The percentage inhibition of growth of *C. pyrenoidosa* in the dilution series varied from 53-67%. The degree of growth depression of O. *quadripunctulata* in the undiluted effluent was only 39%.

The phenol acclimated cultures of the algae thrived in the effluent whereas the growth of the unacclimated cultures was depressed (Table 2). The cell count of the phenol acclimated and unacclimated *O. pusilla* in the undiluted effluent was 22% and 148%, and that of *C. pyrenoidosa* was 33% and 144% respectively, when compared to the control. The percentage growth of accclimated *O. quadripunctulata* in the effluent was 139% of the control.

Table. 2. Mean cell count / absorbance (% of control) of phenol acclimated and non-acclimated *Oocystis pusilla*, *Chlorella pyrenoidosa* and *Oscillatoria quadripunctulata* after growth in the petrochemical effluent for 96 hours (n=3).

Test species	Cell count/ absorbance (% of control)	
	Phenol Acclimated	Non-acclimated
O. pusilla	22.64	148.51
C. pyrenoidosa	33.33	44.36
O. quadripunctulata	61.12	139.39

The initial mean total dissolved solids content of the effluent was 411 mgL^{-1} (Table 3). Upon retention for 96 hours apparently there was no change in the dissolved solids content of the control. The effluent inoculated with *O. pusilla* and *C. pyrenoidosa* did not show any significant change in the total dissolved solids as

evidenced by the mean TDS values. *O. quadripunctulata* reduced the TDS to 277 mgL^{-1} in 96 hours (Table 3).

Table. 3. The effect of phenol acclimated *Oocystis pusilla*, *Chlorella pyrenoidosa* and *Oscillatoria quadripunctulata* on the biosorption of Total Dissolved Solids (TDS) from the petrochemical effluent (n=3).

TDS (mgL^{-1})	Effluent control	*O. pusilla*	*C. pyrenoidosa*	*O. quadripunctulata*
Initial	411	411	411	411
96 hr	427	446	477	277

The petrochemical effluent inhibited the growth of the test species of algae. The growth of *O. pusilla* was suppressed by 77%, *C. pyrenoidosa* by 67% and *O. quadripunctulata* by 39%. According to Walsh and Merrill (1984) suppression of growth after 48 or 72 hours is a best indicator of toxicity. The observed toxicity may be traced to and explained by the bioavailable contaminants absorbed by algal cells. Wong et al. (1995) reported that the stress effects of organic toxicants on the fine structure of algae is the abnormal build up of starch grains, and mass destruction of organelles. The interference with oxidative phosphorylation, photosynthesis, respiration, and protein and nucleic acid synthesis occurred in *Chlorella* cells exposed to toxicants such as DDT (Hoagland and Duke 1982).

The growth of the pure cultures were inhibited by the effluent whereas the phenol acclimated cultures were found to be growing in the effluent. In a study on the degradation of azodyes by algae it was found that the unacclimatd algae display a lower azoreductase activity compared with the acclimated cultures of algae (Jingi and Houtain 1992). Species of *Oscillatoria* were also reported to degrade dyes from wastewater.

The reduction of the total dissolved solids content in the effluent samples inoculated with *O.quadripunctulata* may be firstly attributed to the biodegradative capacity reported in many blue green algae (Subramanian and Uma 1997). According to Shashirekha et al. (1997) a marine cyanobacterium *Phormidium valderianum* effectively degraded phenol. Secondly, cyanobacteria are also efficient in the removal of nutrients from the effluents (de la Noue et al. 1992). The nutrient uptake by many algal species are either promoted or inhibited by environmental contaminants (Boyle 1984). Even then, to explain the processes involved in the absorption of total dissolved solids by algae evidenced by this study needs further research, as the fate after absorption is important from the point of effluent treatment. In our earlier studies, *O. quadripunctulata* was found to be tolerant to phenol and efficient in the absorption of phenol from the growth medium (Joseph and Joseph 1999). The results of the present study suggest *O. quadripuctulata* as a potent tool to be investigated, in the development of new techniques for the biological treatment of petrochemical effluents.

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