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Inhibitory Effect of Petroleum Oil on Photosynthetic Electron Transport System in the Cyanobacterium *Anabaena doliolum*

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Significant quantities of petroleum oils enter aquatic ecosystems from oil refineries, necessitating studies of their possible toxicity. Some attempts have been made to assess the impact of oils and hydrocarbons on algae which are primary producers in aquatic habitats (O'Brien and Dixon 1976; Vandermeulen and Ahern 1976). Although a majority of petroleum oils are toxic to algae, some are not (Coffey et al.1977; Fabregas et al. 1984). This difference is mainly due to great variability in chemical composition of test oils. This fact may be linked with the observation of Singh and Gaur (1990) that certain oils, or similar fractions of different oils, differ in their toxicity. Therefore for the proper management of aquatic ecosystems, it becomes necessary to explore the toxicity and mechanisms of commonly used petroleums such as diesel. Gaur and Singh (1989) reported that the hierarchy of toxicity of some oils to the cyanobacterium Anabaena doliolum was diesel>furnace oil>petrol>kerosene>crude. Virtually nothing is known about the site of action of oil and the mechanism of inhibition of photosynthetic electron transport, a process responsible for the generation of ATP and NADPH, which are essential for carbon fixation. The present study was an attempt to learn something about these aspects. The influence of diesel on photosynthetic O_2-evolution, $^{14}CO_2$ fixation, and electron transport system has been examined in Anabaena doliolum, a heterocystous cyanobacterium. A. doliolum and other heterocystous cyanobacteria are widely distributed in soil and aquatic ecosystems, and represent an important group of free-living nitrogen fixing microorganisms.

MATERIALS AND METHODS

Anabaena doliolum Bharadwaja was grown axenically in

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Allen and Arnon's medium (see Singh and Gaur, 1988) lacking combined source of nitrogen. The cultures received 2500 lux light in 14 h light and 10 h dark cycle at 24±1°C. All experiments were done in triplicates under axenic conditions.

The oil to be tested was extracted by the method of Boylan and Tripp (1971). One part of diesel, purchased locally, was slowly stirred by magnetic stirrer with 20 parts of sterilized basal medium. After 12 h, stirring was stopped and an aqueous phase containing water-soluble fraction was separated. Five concentrations, lethal concentration (LC_{50}), two below the LC_{50} and two higher than LC_{50} were prepared by dilution of aqueous oil extract with sterilized medium. Fluorescence spectroscopy was used to estimate the amount of oil in the aqueous extract of test oil (Singh and Gaur 1988).

Photosynthesis was measured by estimating light-induced O_2-evolution by the culture suspension with a Clark type O_2 electrode (Universal Biochem Model M 76 T, Madurai, India) at 25±1°C. The reaction vessel was illuminated with 12.5 $W\ m^{-2}$ at its outer surface.

Carbon fixation was estimated by measuring the uptake of ^{14}C from $NaH^{14}CO_3$ by the method of Gaur and Singh (1990), and expressed as dpm (disintegrations per minute).

Hill reaction assay was carried out within three hours of membrane preparation by the polarographic method (Lien 1978) and expressed in terms of either O_2 evolved or consumed:

Reaction 1. $H_2O \rightarrow$ ferricyanide (assayed as O_2-evolution). This reaction is dependent on a functional photosystem II and shows stronger dependence on the activity of photosystem I and the redox coupling between the two photosystems.

Reaction 2. $H_2O \rightarrow$ p-benzoquinone (assayed as O_2-evolution). The reduction of p-benzoquinone and the concomitant O_2-evolution are dependent on a functional photosystem II. This reaction does not require either PS I activity or a redox coupling between the two photosystems.

Reaction 3. Ascorbate - DCPIP (2, 6-dichlorophenol-indophenol, Na salt) $\rightarrow$ MV (Methylviologen): Assayed as O_2 consumption; the photoreduction of methylviologen by the artificial electron donor system ascorbate-DCPIP only requires the electron transport reactions associated with photosystem I. Addition of 0.01 ml of

5 mM 3-(3, 4 dichlorophenyl)-1, -1 dimethylurea) (DCMU) prevented PS II electron transport.

Chlorophyll a was extracted in 100% methanol at 4°C for 12 h, made upto known volume, and the absorbance recorded at 665 nm. The amount of chlorophyll a was estimated by using the extinction coefficient of 74.5.

RESULTS AND DISCUSSION

The effect of various concentrations of aqueous extract of test oil on photosynthetic O_2-evolution and CO_2 fixation by intact cells of A. doliolum are given in Figs. 1 and 2. Results are expressed as a percentage with respect to controls. Different doses of test oil (0.5, 1.0, 1.3, 2.0 and 3.0 mg l^{-1}) significantly decreased the photosynthetic O_2-evolution and $^{14}CO_2$ fixation. The test oil inhibited both the processes in a concentration dependent fashion. Prolonged incubation caused severe inhibition of O_2 evolution as inhibition occurred just after application of test oil (Fig. 1). Similar results were observed in case of $^{14}CO_2$ fixation (Fig. 2). The present findings agree with those of Gaur and Singh (1990) where prolonged incubation caused severe inhibition of $^{14}CO_2$ fixation exposed to Assam crude. Our observations suggest that for testing the effect of oil, O_2-evolution is a more sensitive parameter than is $^{14}CO_2$ fixation.

Photosynthetic O_2-evolution requires an effective electron transport chain. The site of inhibition of electron flow can often be detected in isolated chloroplasts by using artificial electron donors or acceptors. Hill-activities were measured in the presence and absence of test oil (Table 1). Hill-activities with ferricyanide as electron acceptor (Reaction-1) requiring PS II and showing stronger dependence on PS I, and redox coupling between the two photosystems were immediately inhibited by application of test oil in a concentration dependent manner. In contrast, the reaction with benzoquinone (Reaction-2), dependent on PS II, was greatly inhibited by test oil. The activity of PS I (Reaction-3), measured with methylviologen as electron-acceptor, was not influenced by test oil at any concentration. These results demonstrate that photosynthetic activity of isolated thylakoid membranes is rapidly and severely inhibited by test oil. By contrast, in whole cells, the inhibitions of both O_2 evolution and $^{14}CO_2$ fixation were delayed (Figs. 1 and 2). The difference in test oil response times between the two systems may be due to limitation imposed upon test oil uptake by diffusion and adsorption properties of whole cells. As far as the site of action of test oil on photosynthetic electron transport chain is concerned we found that oil

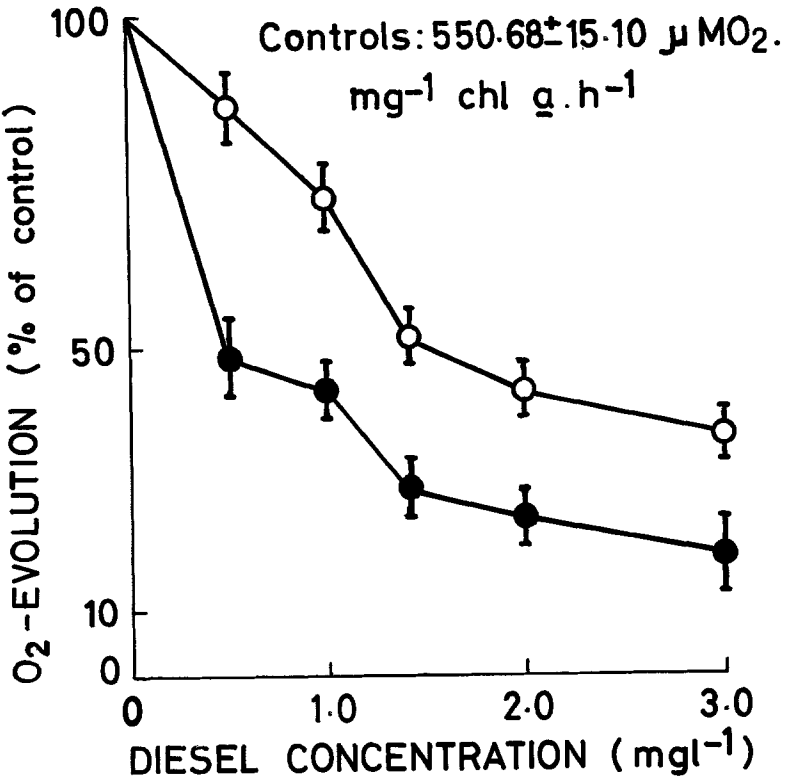

Figure 1. Photosynthetic O_2 Evolution Following Application of Test Oil (% of control). Vertical Lines Represent the Standard Deviation for the Mean (n=3). o = immediately after application; ● = 4 hrs after application.

inhibits electron flow in PS II but not in PS I. This is consistent with the findings of Singh and Gaur (1988) who used Assam Crude. However, the toxicity of diesel (LC_{50} 1.3 mg l^{-1}) used in our experiment was approximately seven fold greater than that of Assam Crude (LC_{50} 9.01 mg l^{-1}), with significant differences in amount and toxicity of aromatic, paraffinic and asphaltic constitutents (Singh and Gaur 1990). Therefore, it may be concluded that irrespective of toxicity and constituents, petroleum oil primarily appears to act on PS II in general. This may be due to the binding of petroleum oil to certain proteinaceous molecules in the photosynthetic membrane. The inhibition of $^{14}CO_2$ fixation may be mainly due to a reduced supply of NADPH and ATP because of severe inhibition of photosynthetic electron transport system.

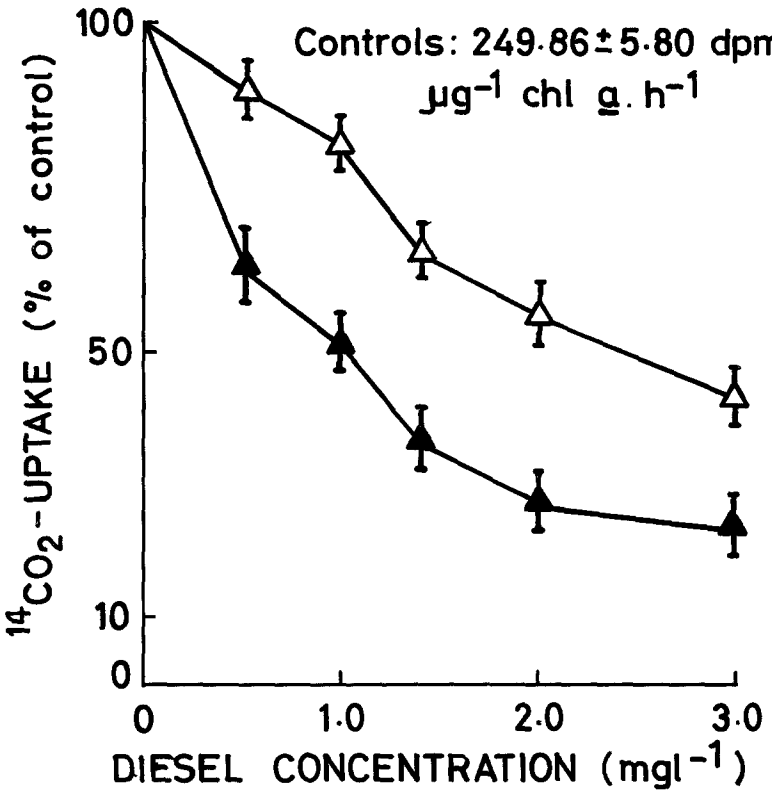

Figure 2. $^{14}CO_2$ Fixation Following Application of Test Oil (% of control). Vertical Lines Represent the Standard Deviation for the Mean (n=3). △ = 30 min after application; ▲ = 4 hrs after application.

Table 1. Hill-reactions assayed after application of various concentrations of test oil $_{-1}$ in *A. doliolum* (µM O_2 evolved or consumed mg $^{-1}$ chl *a* h^{-1}).

Oil concentration mg l^{-1}	Reaction-1 H_2O-Ferricyanide	Reaction-2 H_2O-PBQ	Reaction-2 Ascorbate-DCPIP-MV
Control	180.7±8.6*	68.9±3.2	210.2±8.1
0.5	75.9±2.4	27.0±1.1	213.6±10.5^{ns}
1.0	59.8±3.7	21.4±1.0	210.5±13.2^{ns}
1.3	46.8±1.8	15.2±0.4	213.7±8.6^{ns}
2.0	36.9±1.2	11.2±0.5	215.1±11.3^{ns}
3.0	22.5±0.8	6.1±0.2	208.3±9.0^{ns}

*Mean ± Standard Deviation
All the values are significantly different from control at $p<0.05$ (Student's 't' test), except those marked with ns.

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