Bull. Environ. Contam. Toxicol. (1988) 41:776–780

Bulletin of Environmental Contamination and Toxicology

Effect of Assam Crude on Photosynthesis and Associated Electron Transport System in *Anabaena doliolum*

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Expansion of the petroleum industry due to increased demand for oil products has caused alarming problems of oil pollution in aquatic enironments. Due to this, attempts have been made to assess the impact of oils and hydrocarbons on algae representing an extremely important group of primary producers in aquatic systems (see O'Brien and Dixon 1976; Vandermeulen and Ahern 1976). Petroleum oils have been found to inhibit phtosynthesis (light-induced O_2 evolution or ^{14}C incorporation) of laboratory cultures as well as natural phytoplankton populations (Vandermeulen and Ahern 1976), though in exceptional cases stimulatory effect has been observed at low concentration (Gordon and Prouse 1973; Parsons et al. 1976). Pulich et al. (1974) suggested that the toxic effects of oils possibly occur either on photosystem I (PS I) or II (PS II) of the 'Z' scheme, or perhaps but less likely, on the CO_2 fixation side of photosynthesis. Armstrong and Calder (1978) suggest that the primary effect of petroleum may be through direct action on the energy yielding electron transport system; however, they did not estimate the activity of photochemical electron transport.

Since no information is available establishing the action of oils on PS I or II, or on the redox coupling between the two photosystems, the present study was undertaken. The influence of Assam crude on photosynthetic O_2 evolution as well as upon its electron transport system in Anabaena doliolum, a heterocystous blue-green alga (cyanobacterium), has been examined. 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 (Desikachary 1959).

MATERIALS AND METHODS

Anabaena doliolum, obtained from the Centre of Advanced Study in Botany, Banaras Hindu University, was used as the test organism. The test alga was cultivated in Allen and Arnon's

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(1955) medium in 14h light and 10h dark cycle, and at a temperature of 24±1°C. The cultures received 5Wm^{-2} light at the surface of the vessels.

Photosynthesis was measured by estimating light-induced O_2 evolution of the culture suspension with a Clark type O_2 electrode (Rank Brothers, Cambridge) at 24±1°C. The reaction vessel received 40Wm^{-2} illumination at its outer surface. The amount of chlorophyll a was estimated following the method given by Mackinney (1941).

One part of sterilized Assam crude was slowly stirred by a magnetic stirrer with 20 parts of sterilized culture medium. After 12h stirring was stopped and the aqueous phase containing water-soluble fractions was separated. This solution was found to have 12.7 mg l^{-1} concentration of oil as determined by fluorescence emission method (excitation at 340nm, and emission at 463nm).

Filaments of A. doliolum were harvested from the culture suspension by centrifugation. The harvested filaments were washed by resuspending in Tricine-NaOH buffer (50mM; pH 7.5) followed by centrifugation. The washed cells were resuspended in cold Hepes-NaOH buffer (50mM; pH 7.5) and stored in a ice-water bath. A portion of diluted suspension was kept over a ice-water bath and sonicated for about 1 min. The cell-free preparation was stored in a ice-water bath and kept in the dark. Assay for Hill reaction was carried out within three hours of membrane preparation.

Hill reaction was assayed by Polarographic method (Lien 1978) in terms of either O_2 evolution or consumption:

Reaction 1. H_2O → p-benzoquinone (PBQ): Assayed as O_2 evolution, this reaction is dependent on PS II and does not have an absolute requirement for PS I or the redox coupling between the two photosystems.

Reaction 2. (H_2O → ferricyanide): This was assayed as O_2 evolution. The reaction is absolutely dependent on PS II and shows a stronger dependence on the activity of PS I and redox coupling between PS I and PS II.

Reaction 3. Ascorbate-DCPIP (2,6-dichlorophenol-indophenol, Na salt) → MV(Methylviologen): Assayed as O_2 consumption, this reaction is dependent on electron transport reactions associated with PS I. DCMU (0.01ml; 5mM) addition prevented PS II electron transport.

RESULTS AND DISCUSSION

Table 1 shows photosynthetic O_2 evolution by intact cells of A. doliolum treated with various concentrations of test oil.

Though lower concentrations were stimulatory, higher concentrations were always inhibitory, and the extent of inhibition depended on the amount of oil in the medium. Such high oil concentrations exist in refinery effluents (Gaur and Kumar 1985) and the areas of oil spills (see O'Brien and Dixon 1976). Crude oils from other parts of the world also exert stimulatory and inhibitory effects on algae (O'Brien and Dixon 1976).

Table 1. Effect of Assam crude on photosynthetic O_2 evolution ($\mu M\ O_2 . mg^{-1}\ Chl\ a . h^{-1}$) by *A. doliolum*.

Oil Concentration mg l^{-1}	O_2 evolution Mean (SEM)
Control	542.28 (14.21)
1.0	637.56 (16.65)^b
2.5	592.68 (17.48)^a
5.0	532.60 (18.10)
7.5	486.38 (11.12)^a
10.0	412.10 (12.56)^b

Values significantly different from control have been marked: $^aP<0.05$, $^bP<0.01$ (Student's 't' test).

In order to find out the site of action of Assam crude, assay for various types of Hill reactions dependent on the activity of PS I, II, and both the photosystems, were made (Table 2). The reaction 1, $H_2O \rightarrow PBQ$, dependent on PS II alone, was not affected by low concentration of test oil; however, higher concentrations significantly inhibited the reaction. The activity of PS I (Ascorbate - DCPIP $\rightarrow$ MV) was not influenced by oil at any concentration.

The reaction with ferricyanide as the electron acceptor, requiring PS II and showing stronger dependence on PS I and the redox coupling between the two photosystems, was slightly stimulated at 1.0 mg l^{-1} of oil, but, inhibition in the medium did not cause any apparent effect on either PS I or II, stimulation occurred in Hill reaction requiring ferricyanide and O_2 evolution by the intact cells. It seems difficult to correlate these two instances of stimulation and some more information is needed to explain this phenomenon.

The study shows that the test oil primarily acted upon PS II of the photosynthetic electron transport. Majority of chemically diverse herbicides also display specific action on PS II (see Dodge 1983). This similarity had in the past led to large-scale use of petroleum oils as herbicidal agents (Van Overbeek and Blondeau 1954). PS II herbicides act by clocking electron tranport at the secondary electron acceptor "Q_B" thought to be a non-covalently bound plastoquinone (Gardner 1987). It has been further suggested that the polar portion of herbicides bind to a protein component of

Table 2. Effect of test oil on Hill activity by membrane preparation from *A. doliolum* (μ M O_2 evolved or consumed. mg^{-1} Chl *a*.h^{-1}).

Oil concentration mg l^{-1}	Reaction 1 $H_2O \rightarrow$ PBQ	Reaction 2 $H_2O \rightarrow$ Ferricyanide	Reaction 3 Ascorbate-DCPIP $\rightarrow$ MV
Control	63.94 (2.44)*	176.17 (16.57)	217.26 (9.48)
1.0	68.72 (2.16)	204.54 (10.95)^a	234.80 (10.46)
2.5	57.85 (2.38)$_b$	180.76 (11.28)	231.65 (8.64)
5.0	52.83 (1.83)^b	162.84 (5.35)^a	229.13 (11.42)
7.5	46.30 (2.72)^c	131.24 (7.75)^a	226.50 (8.15)
10.0	40.23 (2.53)^c	124.96 (5.58)^b	221.94 (9.52)

*Mean (SEM)
Values significantly different from control have been marked: $^aP<0.05$, $^bP<0.01$, $^cP<0.005$ (Student's 't' test).

around 32 kD, and makes the electron transport thermodynamically unfavourable (see Dodge 1983). Similar details about the action of oil are needed, and it would be worthwhile to see if the physiological system of algae resistant to oils is similar to their herbicide-resistant counterparts (Golden and Sherman 1983).

Since crude oils from different geographical locations have many compounds in common (Burks 1982), and also that the cyanobacterial cells are akin to chloroplasts of higher plants (Lang 1964), inhibition of PS II by petroleum oils may occur widely in photosynthetic organisms. The inhibition of PS II will in turn hamper CO_2 fixation due to reduced supply of NADPH and ATP. Assam crude inhibited ^{14}C incorporation by *A. doliolum* (Gaur, 1987), and this could *inter alia* be ascribed to disruption of PS II activity.

Acknowledgments. We thank the Head, Department of Botany, North-Eastern Hill University, for facilities, and the Council of Scientific & Industrial Research, New Delhi, for financial support.

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Received February 25, 1988; accepted April 13, 1988.