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Effects of Crude Oil and Benzene on Growth, Photosynthesis and Glycollate Dehydrogenase of *Anacystis nidulans*

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ABSTRACT. Effects of Kuwait crude oil and benzene on growth, photosynthesis and glycollate dehydrogenase of *Anacystis nidulans* were studied. Crude oil completely inhibited growth, photosynthesis and enzyme activity at a concentration of 4 ml/l. However, benzene had its complete inhibitory effect at about 0.4 ml/l.

Key Words: *Anacystis nidulans,* Crude oil, Glycollate dehydrogenase, Photosynthesis.

Petroleum is considered one of the most important pollutant entering the sea and has a great potential for altering the natural environment. The effects of oils and extracts of crude oils on growth, photosynthesis and respiration of algae have been described (Baker 1970, 1971, Pulich *et al.* 1974, Soto *et al.* 1975, O' Brien and Dixon 1976, Kusk 1978, Prahl 1979).

Water-soluble fractions of used lubricating oil were reported for their inhibition of phytoplankton photosynthesis (Bate and Crafford 1985). The effects of Kuwait crude oil extracts on the growth of *Phaeodactylum tricornutum* (Lacase 1969) and Kuwait crude emulsion on *Monochrysis lusheri* growth (Strand *et al.* 1971) were discussed. Alexander and Schwartz (1980) have also studied the effect of Kuwait crude oil on glucose utilization by marine bacterial populations.

Glycollate is a product of photosynthesis in various algae, cyanobacteria, photosynthetic bacteria (Codd and Stewart 1973, Tolbert 1973, 1976, Codd and Sallal 1978, Al-Houty and Syrett, 1984). Due to the importance of photosynthetic microorganisms, particularly, algae and cyanobacteria as primary producers of fresh

water and marine environments and due to the lack of information on the effects of crude oil and hydrocarbons on the metabolism of glycollate, the present study was done on the effect of Kuwait crude oil and benzene on growth, photosynthetic electron transport system and on glycollate dehydrogenase of *Anacystis nidulans.*

Materials and Methods

Organism and growth conditions:

Anacystis nidulans 1405/1 was obtained from the Culture Collection of Algae and Protozoa, Cambridge, England. The organism was grown in 250 ml screw-capped conical flasks containing 100 ml of BG-11 medium (Stanier *et al.* 1971). Kuwait crude oil was added to the culture flasks in duplicate to give final concentrations of 1-6 ml/l. Benzene was added in duplicate to give 0.1-1.0 ml/l. Screw-capped culture falsks were used, in order to retain the volatile compounds. Culture flasks were incubated at 22 °C in an orbital shaker under constant illumination of 61 μ mol m^{-2} s^{-1} at the surface of the culture flasks.

Cell disruption and centrifugation:

Aliquots of about 10 ml culture were harvested by centrifugation at 5000 g for 20 min. The cell pellet was suspended in 5 ml of 75 mM Tricine - HCl buffer pH 7.5 containing 10 mM NaCl. Cells were disrupted by ultrasonication for 4x15 sec, punctuated by 15 sec rest periods in an ice-bath. Disrupted cells were centrifuged at 2500 g for 15 min and the supernatant was used to study the photosynthetic electron transport system. Spectrophotometric assays were carried out at 23-25°C. using a Pye Unicam SP 800 spectrophotometer.

Oxygen evolution :

Oxygen evolution was measured using a Rank Pt-Ag oxygen electrode at 25°C. 3.0 ml of cells was incubated in the dark for 10 min and then exposed to saturated light for another 10 min. Crude oil and benzene were added and the amount of oxygen evolved calculated. The oxygen electrode was calibrated according to Lessler (1970).

Ferricyanide - Hill reaction:

The ferricyanide-Hill reaction was used to measure transfer of electrons from water to ferricyanide, via photosystem II, according to Nishimura *et al.* (1964), with minor modifications. The reaction mixture contained 2.3 ml buffer (50 mM Tricine, 0.4 M sucrose and 10 mM NaCl, pH 7.5), 1 μ mol $MgCl_2$ 1umol potassium ferricyanide and 0.5 ml cell- free extract. Crude oil and benzene were added to the reaction mixture. The reaction mixture was illuminated in cuvettes by saturating

light (120 μ mol m^{-2} s^{-1}), provided by a Leitz pradovit tungsten filament lamp projector. The net photoreduction of ferricyanide was measured at 420 nm spectrophotometrically.

Mehler reaction:

The Mehler reaction with the 2,6-dichlorophenol-indophenol (DCPIP)/ ascorbate couple as electron donor was used to measure the transfer of electrons from DCPIP/ ascorbate to methylviologen via photosystem I (Schmid *et al.* 1975). The reaction mixture contained 2.3 ml buffer (75 mM Tricine, 0.2 M KCl pH 7.5), 2.5 μ mol DCPIP, 60 umol ascorbate, 2.5 μ mol KCN, 0.1μ mol 1 DCMU, 0.1μ mol methylviologen and 0.5 ml cell extract. Crude oil and benzene were added. The reaction mixture was equilibrated in the oxygen electrode in the dark for 5 min and light dependent oxygen consumption measured in saturated illumination.

NADP - Photoreduction:

NADP-photoreduction with water as electron donor was used to measure the transfer of electrons from water to NADP, via photosystem II and I according to the method of Rowell (1974). The reaction mixture contained: 2.2 ml 75 mM Tricine buffer containing 10 mM NaCl. pH 7.5, 1 μ mol NADP, 3 μ mol KCN, 5 μ mol $MgCl_2$ and 0.5 ml cell-free extract. Crude oil and benzene were added. The net photoreduction of NADP measured at 340 nm in the spectrophotometer.

Glycollate dehydrogenase:

Glycollate formation was measured according to the method of Codd and Schmid (1972).

Chlorophyll$_a$ and protein determinations:

Chlorophyll$_a$ was measured according to Kirk (1967). Protein was measured according to Lowry *et al.* (1951), using crystalline bovine serum albumin as a standard. Each value in the tables of the growth, photosynthesis and enzyme assay represents the mean of duplicate experiments.

Results

Effects of crude oil

Preliminary experiments were conducted to find out the range of concentrations on which crude oil and benzene have a visible effect on the growth of *A. nidulans.* Various concentrations of Kuwait crude oil were studied for their effects on growth, photosynthesis and glycollate dehydrogenase.

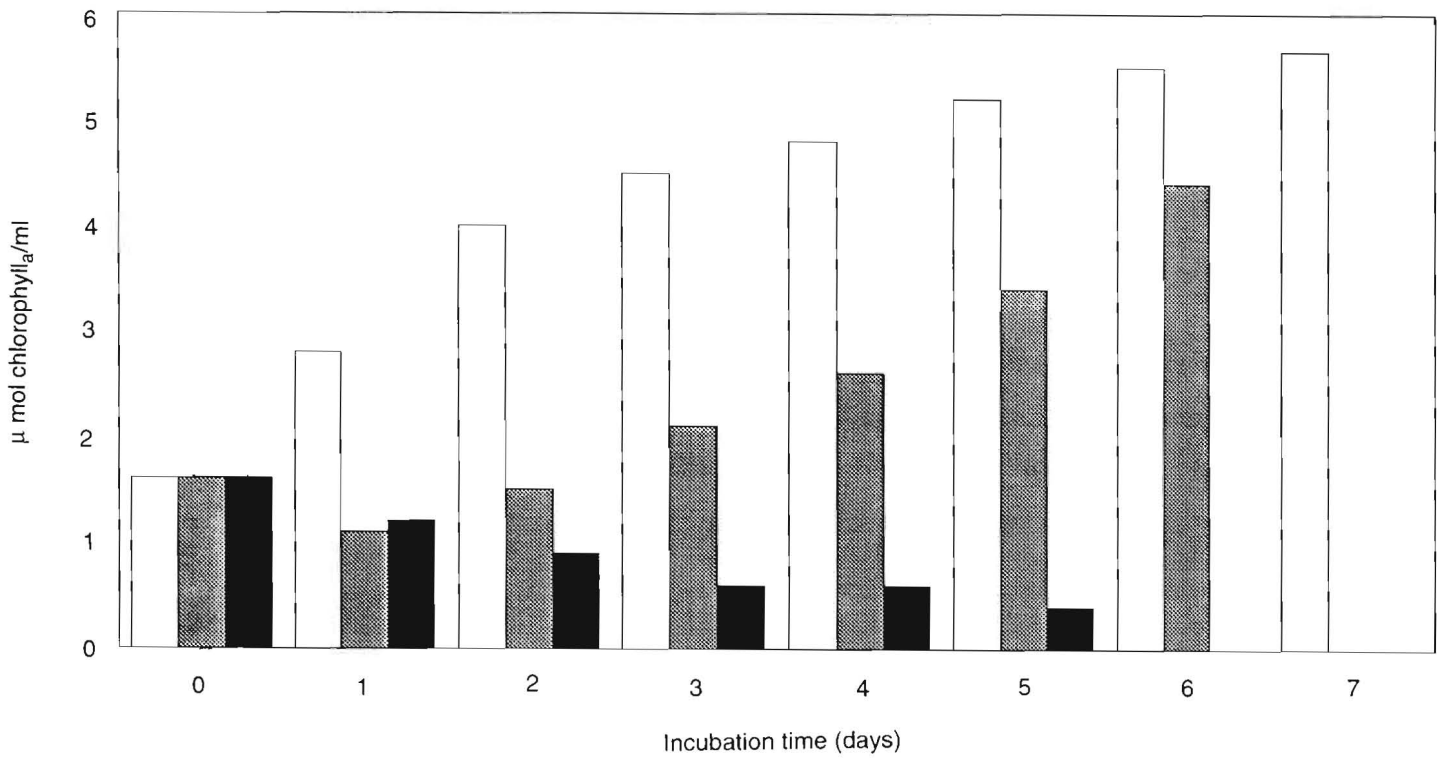

Fig. 1. Effect of crude oil and benzene on the growth of *A. nidulans*.

□control, ■ 4 ml/l, oil, ■ 0.4 ml/l benzene.

Complete inhibition of growth was obtained with 4ml/l crude oil and the subsequent higher concentrations (Fig.1) At 1.0-3.0 ml/l crude oil exerted 4-49% decrease in the growth of *A. nidulans* compared with the control culture.

Oxygen evolution was severely inhibited by 3 ml/l crude oil after 7 days of growth (Table 1). However, complete inhibition of oxygen evolution was obtained within 24h due to the addition of 4 ml/l. At 1 ml/l, crude oil caused a 29% inhibition of oxygen evolution after 7 days of growth (Table 1).

Concentrations of crude oil (1 µl/ml and 2 µl/ml of reaction mixture) similar to those that inhibited growth were tested for their effects upon direct addition to *in vitro* preparations catalyzing the following photosynthetic reactions: oxygen evolution, photosystem II, photosystem I and NADP-photoreduction (Table 2). Crude oil was found not to have any immediate effect on any of the photosynthetic reactions (Table 2). However, glycollate dehydrogenase activity was inhibited by 33-54% using 1-2 ml/l of crude oil (Table 3). Complete inhibition was obtained with 4ml/l. This respiratory enzyme was less affected than was whole cell photosynthesis, taking the percentage inhibition in considaration (Tables 1 and 3).

Effects of benzene

Benzene 0.6-1.0 ml/l inhibited the growth of *A. nidulans* completely (Fig.1). However, 0.1 ml/l caused 51% inhibition after 7 days of growth in the tightly capped culture flasks (Fig 1).

Table 1. Effect of crude oil on oxygen evolution of *A. nidulans*

Incubation time (d)	Crude oil (ml/l)						
	0	1	2	3	4	5	6
0	29.5*	29.5	29.5	29.5	29.5	29.5	29.5
1	57.0	36.0	32.0	24.0	0.0	0.0	0.0
2	73.0	47.5	35.0	21.0	0.0	0.0	0.0
3	84.0	52.0	39.0	19.0	0.0	0.0	0.0
4	92.0	61.3	37.2	18.0	0.0	0.0	0.0
5	99.0	65.0	39.0	12.0	0.0	0.0	0.0
6	100.0	67.3	42.0	9.0	0.0	0.0	0.0
7	101.0	72.0	41.5	7.0	0.0	0.0	0.0

*Oxygen evolution measured as µ mol oxygen evolved/mg chlorophyll/h.

Table 2. Direct effects of Kuwait crude oil and benzene on the photsynthetic electron transport system of *A. nidulans*

	Oxygen evolution (μ mol oxygen evolved/mg chlorophyll/h)	Photosystem II (μ mol ferricyanide reduced/mg chlorophyll/h)	Photosystem I (μ mol oxygen uptake/mg chlorophyll/h)	NADP-photoreduction (μ mol NADP reduced/mg chlorophyll/h)
Control	11.2	82.0	8.3	116.0
1 μl/ml Crude Oil	11.2	81.0	8.1	115.0
2 μl/ml Crude Oil	11.1	82.0	8.15	115.0
0.1 μl/ml Benzene	8.2	70.0	6.8	99.0
0.2 μl/ml Benzene	6.1	64.0	5.9	79.5

Table 3. Effect of crude oil on glycollate dehydrogenase activity of *A. nidulans*

Incubation time (d)	Crude oil (ml/l)						
	0	1	2	3	4	5	6
0	2.3*	2.3	2.3	2.3	2.3	2.3	2..3
1	3.6	3.3	2.75	2.5	2.1	0.0	0.0
2	4.75	4.0	3.3	2.3	2.0	0.0	0.0
3	5.7	4.65	3.3	2.2	1.2	0.0	0.0
4	6.6	5.1	3.5	2.5	0.75	0.0	0.0
5	7.25	5.7	3.8	2.1	0.25	0.0	0.0
6	7.9	5.5	4.0	1.9	0.0	0.0	0.0
7	8.4	5.6	3.9	1.5	0.0	0.0	0.0

*Glycollate dehydrogenase activity measured as μ mol glyoxylate reduced/mg protein.

Oxygen evolution was inhibited completely by benzene at 0.4 ml/l. At 0.1-0.2 ml/l benzene caused 55-87% inhibition of photosynthesis after 7 days of growth (Table 4). Upon direct addition to *in vitro* preparations, 0.1-0.2 ml/l benzene caused the following percentage inhibitions: 26-45% of oxygen evolution, 14-22% of PSII, 18-29% of PSI and 14-31% of NADP-photoreduction (Table 2). Glycollate dehydrogenase activity was inhibited completely after 5 days of growth using 0.4 ml/l (Table 5).

This enzyme acitivity was less affected by benzene than was with cell photosynthesis (Tables 4 and 5).

Table 4. Effect of benzene on oxygen evolution of *A. nidulans*

Incubation time (d)	Benzene (ml/l)							
	0	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**1.0**
0	29.5*	29.5	29.5	29.5	29.5	29.5	29.5	29.5
1	57.0	35.0	28.0	18.0	0.0	0.0	0.0	0.0
2	73.0	41.4	26.5	12.2	0.0	0.0	0.0	0.0
3	84.0	46.8	25.0	9.0	0.0	0.0	0.0	0.0
4	93.0	45.0	26.0	7.2	0.0	0.0	0.0	0.0
5	99.0	49.0	23.0	7.0	0.0	0.0	0.0	0.0
6	100.0	48.0	19.0	6.0	0.0	0.0	0.0	0.0
7	101.0	45.0	13.5	0.0	0.0	0.0	0.0	0.0

*Oxygen evolution measured as μ mol oxygen evolved/mg chlorophyll/h.

Table 5. Effect of benzene on glycollate dehydrogenase activity of *A. nidulans*

Incubation time (d)	Benzene (ml/l)							
	0	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**1.0**
0	2.0*	2.0	2.0	2.0	2.0	2.0	2.0	2.0
1	3.6	3.0	2.6	2.3	2.1	1.8	1.3	0.0
2	5.4	3.3	2.7	2.3	1.8	1.4	0.9	0.0
3	5.8	4.0	3.0	2.1	1.5	1.1	0.7	0.0
4	6.6	4.1	3.2	2.2	1.3	0.9	0.5	0.0
5	7.3	4.3	3.4	4.2	0.75	0.5	0.2	0.0
6	8.1	4.45	3.6	1.6	0.5	0.2	0.0	0.0
7	8.6	4.4	3.6	1.3	0.0	0.0	0.0	0.0

*Glycollate dehydrogenase activity measured as μ mol glyoxylate reduced/mg protein.

Discussion

Growth of *A. nidulans* which was measured as chlorophyll$_a$ concentrations was inhibited by Kuwait crude oil or benzene. On a volume basis, crude oil was almost 10 fold less inhibitory than benzene, on growth, photosynthesis and the respiratory enzyme glycollate dehydrogenase. This is possibly due to the dilution of the oil fractions which have inhibitory effects (Fig.1 and Tables 1-5). Benzene was found to have more inhibition on photosystem I than II upon their direct addition to the reaction mixtures, although crude oil had no immediate effect on these photosynthetic reactions (Table 2).

Kuwait crude oil at a concentration of 1% (v/v) was found to cause 19% inhibition to the growth of *Phaeodectylum tricounutum* (Lacaze 1969). However , the effects of particular oil and oil fractions on the growth response and photosynthesis of phytoplanktons species are variable (Mironov 1968, Kuass *et al.* 1973, Pulich *et al.* 1974). Possible mechanisms by which hydrocarbons impair the ability for photosynthesis have been described by Van Overbeek and Blodeau (1954) and Baker (1970).

Kuwait crude oil and benzene were found to inhibit glycollate dehydrogenase activity over the same range of concentrations used to inhibit growth and photosynthesis of *A. nidulans.* However, like growth and photosynthesis, glycollate dehydrogenase was more sensitive to benzene than to crude oil. This may explain the possible effects of crude oil and aromatic hydrocarbons on the glycollate metabolism in cyanobacteria, since these organisms are considered as one of the primary producers in fresh water environments.

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تأثير البترول الخام والبنزين على النمو والتركيب الضوئي وانزيم الجلايكوليت ديهيدروجينيز للسيانوبكتيريا *Anacystis nidulans*

عبد الكريم جبر السلال

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تمت دراسة تأثير البترول الخام والبنزين على نمو السيانوبكتيريا المسماة *Anacystis nidulans* وكذلك على التركيب الضوئي وانزيم الجلايكوليت ديهيدروجينيز ، حيث تبين بأن البترول الخام يؤدي إلى قتل هذا النوع من السيانوبكتيريا عند تركيز ٤ سم٣/ لتر ، بينما البنزين يؤدي إلى نفس النتيجة ولكن عند تركيز ٤ ,٠ سم٣/ لتر .

ليس للبترول الخام أي تأثير على تفاعلات التركيب الضوئي عند إضافته مباشرة إلى هذه التفاعلات دون أي فترة حضانة بينما هنالك تأثير واضح للبنزين على هذه التفاعلات عند إضافته مباشرة حيث يصل هذا التأثير إلى ٤٥٪ من الاحباط لإنتاج الأكسجين في احدى تفاعلات التركيب الضوئي . كذلك قد تبين أن انزيم جلايكوليت ديهيدروجينيز أقل تأثراً بالبنزين من تفاعلات التركيب الضوئي .