Bull. Environm. Contam. Toxicol. 20, 303-312 (1978)

Differential Elimination of Phenol by Diatoms and Other Unicellular Algae from Low Concentrations

D. Werner, and H. Pawlitz

Fachbereich Biologie, Universität Marburg, Lahnberge, D-3550 Marburg-Germany

Algae are essential constituents of all comprehensive saprobic systems (LIEBMANN 1969; FJERDINGSTADT 1971; SLADECEK 1973) due to their dominant role in aquatic biomass production. But our knowledge about the physiological basis of the occurrence of the indicator species in the various zones is still rather incomplete.

The number of new synthetic chemical substances released into the environment is about 3oo per year (HENSCHLER 1973). This is a significant fraction of the number of metabolic intermediates developed in organisms during the biological evolution over a period of several hundred million years. These environmental chemicals are also metabolized or degraded by the action of a complicated interplay of biotic and abiotic factors (KORTE et al. 1970). Also during these processes, biological as well as synthetic pollutants are diluted significantly, especially in a water environment. However, our information is very scarce as to which dilution and from which lowest concentration of the various pollutants is still taken up by microalgae or bacteria and enters the food chain. There are many biological phenolic compounds; we chose phenol itself, also an important chemical pollutant to determine the differential efficiency of several unicellular algae to eliminate this substance from low concentrations (between $1o^{-7}$ and $1o^{-9}$ M).

We used non-axenic cultures, since these conditions resemble more the environmental situation of algae in streams or lakes in elimination (not uptake !) of a pollutant such as phenol, taking cultures without algal inoculum as blanks.

0007-4861/78/0020-0303 $02.00

MATERIALS AND METHODS

1. Cultivation:

The algae were cultivated at 20°C in liquid culture in light-thermostats as described by WERNER (1966) in the following media:
Cyclotella cryptica, Nitzschia spec. strain 15, strain 16 and strain 23 according to WERNER (1969) but with 355 mg $SiO_2 \cdot l^{-1}$. Scenedesmus obliquus WDT and Chlorella pyrenoidosa 211-8b as described by BISHOP and SENGER (1971) with 1 ml trace element solution $\cdot l^{-1}$ from KRATZ and MYERS (1955).
Euglena gracilis (CRAMER and MYERS 1952); Anacystis nidulans (KRATZ and MYERS 1955); Chroococcus strain 6903 and 6910 in the following medium: (concentration as l^{-1}) 1500 mg Na_2NO_3; 40 mg KH_2PO_4; 75 mg $MgSO_4 \times 7\ H_2O$; 36 mg $CaCl_2 \times 2\ H_2O$; 6 mg citric acid; 6 mg NH_4Fe - citrate; 1 mg EDTA; 20 mg Na_2CO_3; Chlamydomonas reinhardii: per 1 medium): 1011 mg KNO_3; 247 mg $MgSO_4 \times 7\ H_2O$; 14.7 mg $CaCl_2 \times 2\ H_2O$; 521 mg $NaH_2PO_4 \times H_2O$; 89 mg $Na_2HPO_4 \times 2\ H_2O$; 61 µg H_3BO_3; 287 µg $ZnSO_4 \times 7\ H_2O$; 169 µg $MnSO_4 \times H_2O$; 2.49 µg $CuSO_4 \times 5\ H_2O$; 12.35 µg $(NH_4)_6Mo_7O_{24} \times 4\ H_2O$.

2. Uptake studies:

At the beginning of the experiments we added to 200 ml of growing cell suspension (optical density (1000 nm) between 0.3 and 0.5 in the PMQ II (Zeiss spectrophotometer) either 0.34 µCi = 5.1×10^{-8} M phenol or 2.0 µCi U-^{14}C-phenol (Amersham Buchler, Braunschweig)= $3. \times 10^{-7}$ M phenol. At the times indicated in the figures, 5 ml of the suspensions were centrifuged at 1000 x g for 10 min, 1 ml of the supernatant mixed with 5 ml dioxane scintillator and counted in the ^{14}C channel of a Liquid Scintillation Counter (Packard TriCarb model 3380) with quench correction by channel ratio of the external standard. As a blank, medium without algae was used.

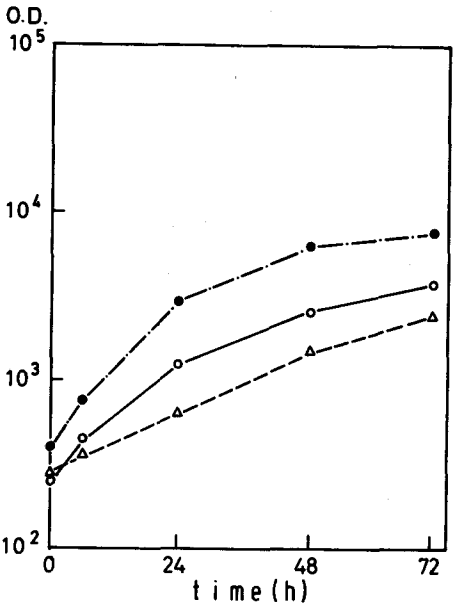

Figure 1.

Growth of diatoms in the presence of 5.1 x 10^{-8} M phenol (20 000 lux, 20°C)

● -.- ● Cyclotella cryptica

o —— o Nitzschia spec. str. 15

△ --- △ Nitzschia spec. str. 16

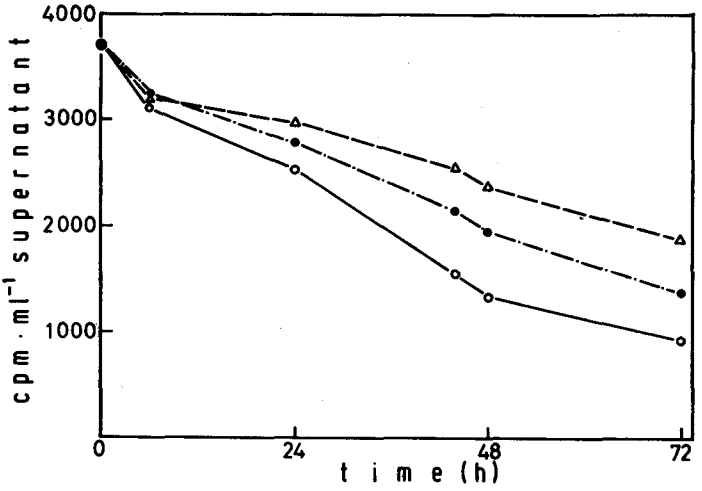

Figure 2.

Elimination of phenol (concentration 5.1 x 10^{-8} M = 1.7 nCi.ml^{-1}) from the medium by diatoms. Symbols as Fig. 1.

RESULTS AND DISCUSSION

The growth curves (Fig. 1) of the diatom species used in the elimination experiments indicate by their continued logarithmic growth (up to about 3 x 10^3 units of optical density)that the concentration of phenol used (5.1 x 10^{-8} M) is not inhibitory. Further experiments with unlabelled phenol showed no inhibition and cytological damage in Cyclotella cryptica with concentrations up to 4 x 10^{-4} M (36 mg · l^{-1}). The elimination from the low concentration (Fig. 2)

proceeds, on the other hand, with a linear rate in all three species used during the 72 h experiment. Phenol is eliminated after that time by the most effective strain, Nitzschia spec. D-15 down to a concentration of about 1.4 x 10^{-8} M. The elimination kinetics by the diatom species, starting from 3 x 10^{-7} M concentration, is quite different (Fig. 3).

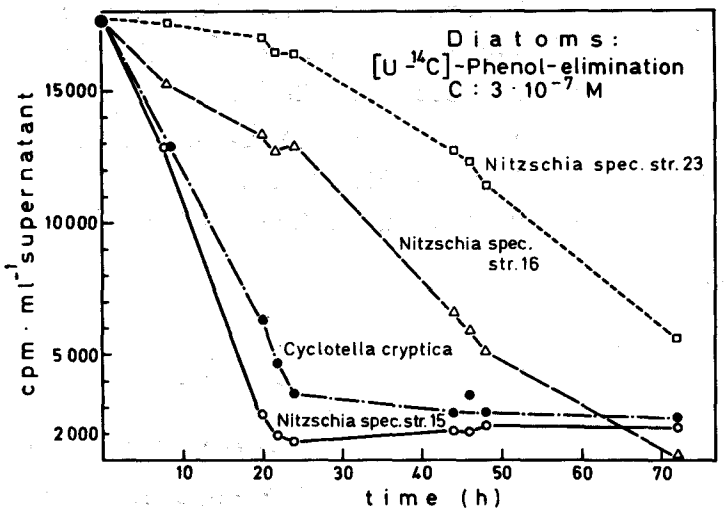

Figure 3. Elimination of phenol (concentration 3 x 10^{-7} M = 10 nCi·ml^{-1}) by diatoms.

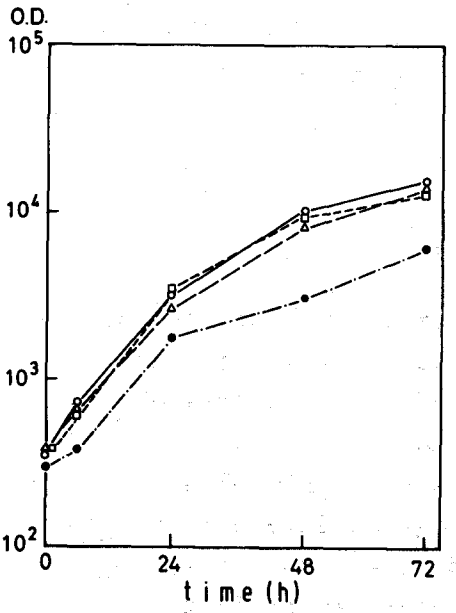

Figure 4.

Growth of green algae and flagellates in the presence of 5.1 x 10^{-8} M phenol (20 000 lux, 20° C)

● —— o Scenedesmus obliquus

□ --- □ Chlamydomonas reinhardii

▲ --- △ Chlorella vulgaris

● -.- ● Euglena gracilis

Cyclotella cryptica and Nitzschia strain D-15 show a linear and rapid elimination during the first 24 h down to a concentration of 2-3 x 10^{-8} M phenol, but then no further significant effect. The two other Nitzschia strains show a slightly increased activity during the first 6 h, then a constant elimination rate during a further 72 h period. Less than 2 x 10^{-8} M phenol is left by Nitzschia strain D-16 after that time in the medium.

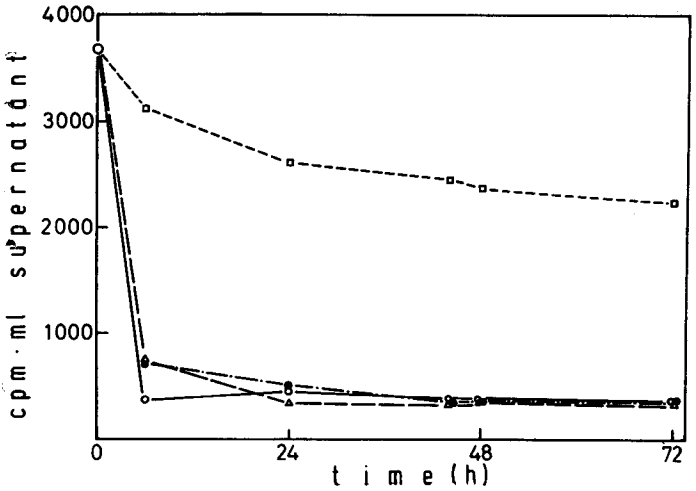

Figure 5.
Elimination of phenol (concentration 5.1 x 10^{-8} M = 1.7 nCi·ml^{-1}) from the medium by green algae and flagellates.
Symbols as Fig. 4.

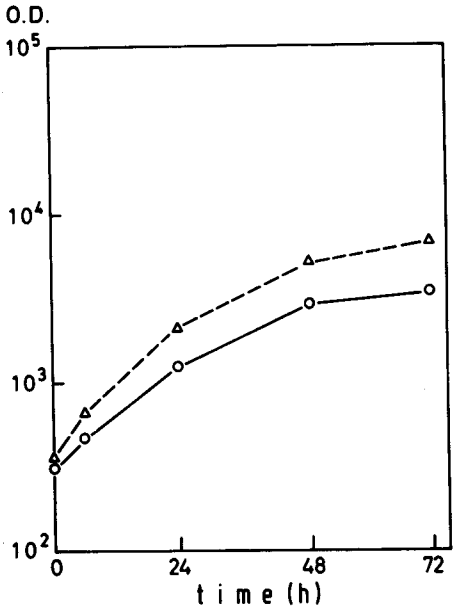

Figure 6.

Growth of blue-green algae in the presence of 5.1 x 10^{-8} M phenol.
(2o ooo lux, 2o° C)
o —— o Anacystis nidulans
Δ --- Δ Chroococcus strain 691o

The comparison of the efficiency of diatoms with four different species of green algae indicated remarkable differences. The growth rate (Fig. 4) also remains unchanged up to an optical density of about 3 x 10^{3} units after addition of the phenol. The elimination of this compound by Scenedesmus obliquus, Chlorella pyrenoidosa and Euglena gracilis proceeds very rapidly in the first 6 h to concentrations of 5-8 x 10^{-9} M. This residual concentration remains fairly constant during the following 66 h. Chlamydomonas reinhardii is ineffective in eliminating phenol. The suspension with the same optical density as the other green algae eliminates only about one sixth of the phenol of those other algae during the first 6 h of the experiments (Fig. 5).

The capacity of the blue-green alga Anacystis nidulans to eliminate phenol (Fig. 7) is comparable to that of the most effective alga, while Chroococcus strain 6910 is significantly less active. The growth of both strains is similar (Fig. 6).

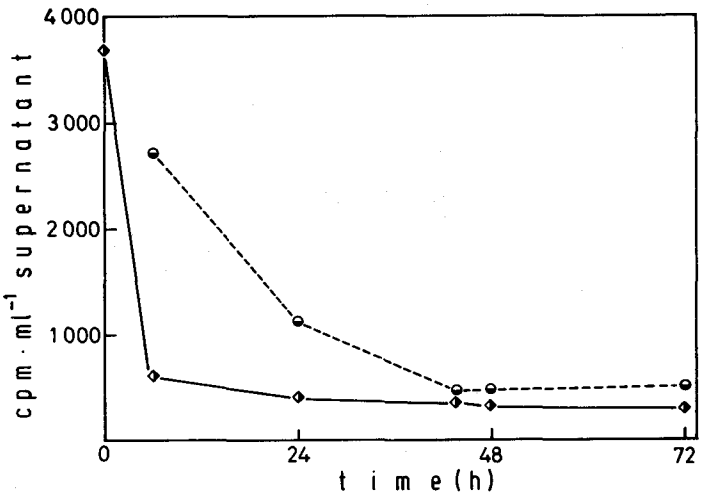

Figure 7. Elimination of phenol (concentration 5.1 x 10^{-8} M = 1.7 nCi · ml^{-1}) from the medium by blue-green algae. Symbols as Fig. 6.

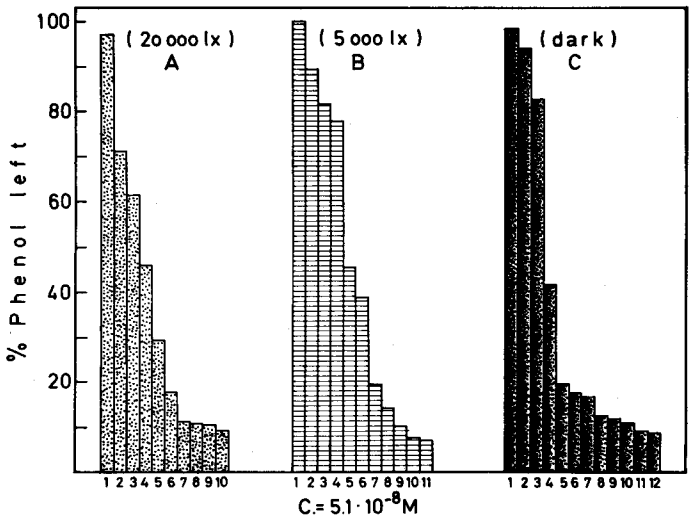

Figure 8.

Efficiency of unicellular algae in eliminating phenol (5.1×10^{-8} M) from the medium. Per cent of phenol left in the medium after 72 h (2o ooo lux and 5 ooo lux) and after 48 h (dark experiments)

A: 1: Blank (without algae)
2: Chlamydomonas reinhardii
3: Nitzschia spec. strain 16
4: Cyclotella cryptica
5: Chroococcus strain 691o
6: Nitzschia spec. strain 15
7: Euglena gracilis
8: Scenedesmus obliquus
9: Chlorella vulgaris
1o: Anacystis nidulans

B: 1: Blank
2: Chlamydomonas reinhardii
3: Nitzschia spec. st. 23
4: Chroococcus st. 691o
5: Nitzschia spec. st. 15
6: Chroococcus st. 69o3
7: Cyclotella cryptica
8: Scenedesmus obliquus
9: Chlorella vulgaris
1o: Euglena gracilis
11: Anacystis nidulans

C: 1: Blank
2: Chlamydomonas reinhardii
3: Nitzschia spec. strain 23
4: Nitzschia spec. strain 16
5: Cyclotella cryptica
6: Scenedesmus obliquus
7: Euglena gracilis
8: Chlorella vulgaris
9: Chroococcus strain 691o
1o: Chroococcus strain 69o3
11: Nitzschia spec. st. 15
12: Anacystis nidulans

Fig. 8 summarizes the experiments already described and several others using the same methods, comparing the elimination capacity of 11 different algal strains in the light and in the dark. Anacystis nidulans, Euglena gracilis, Chlorella pyrenoidosa and Scenedesmus obliquus are the most efficient strains leaving under the various conditions not more than about 5 x $1o^{-9}$ M phenol in the medium.

Chlamydomonas reinhardii and the Nitzschia strains 16 and 23 are by far the least effective species. With all strains used, the phenol elimination proved to be light independent.

Other studies on the uptake and metabolism of phenol by bacteria and unicellular algae were mainly centred on those concentrations, which significantly stimulated or inhibited growth and metabolic activity. Pseudomonas aeruginosa gave no increase in respiration at concentrations less than 1 x $1o^{-4}$ M compared to 6 times higher respiratory activity at about 8 x $1o^{-3}$ M phenol (RIBBONS 197o). Growth of Chlorella vulgaris was affected by some phenolic substances at concentrations as low as 5 x $1o^{-5}$ M, but phenol itself inhibited growth only at the rather high concentration of $1o^{-3}$ M (DEDONDER and VAN SUMERE 1971). Even sludge samples were affected by concentrations of about $1o^{-4}$ M phenolics (BAIRD et al. 1974), whereas conditioned sludges can metabolize concentrations up to 5oo mg · l^{-1} (about 5 x $1o^{-3}$ M (Mc KINNEY et al. 1956). The present work shows that ^{14}C labelled phenol is eliminated by some algae in concentrations as low as 5 x $1o^{-9}$ M which is one hundred times lower than the concentration that can be determined chemically using 4-nitroaniline (MERCK 1976).

This finding might be specially important when using aquatic organisms as indicators (SOEDER 1972, VILEGAS and DEGINER 1974), and for the elimination of pesticides (KEIL and PRIESTER 1969; SEIDEL 1974, REDDY and KHAN 1975) from low concentrations well beyond the range ($1o^{-3}$ - $1o^{-7}$ M) used in most other recent work.

The present work does not exclude the possibility, that semi-symbiotic associations of algae with bacteria, located at the surface of the algal cells, are responsible for the high efficiency of elimination from low concentrations.

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