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Deoxygenation of Water Following Use of the Herbicide Terbutryn Simulated in a Batch Culture System

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Control of aquatic weeds with herbicides may lead to deoxygenation of the treated water (BROOKER 1974). Deoxygenation is a complex process in which cessation of photosynthetic oxygen evolution by the plants is an important factor (ROBSON et al. 1978). In addition, release of nutrients from dying plants and the biomass of plant residues available for decomposition stimulate growth of microorganisms and so increase the biological oxygen demand of the system. The importance of each factor and the sequence of events resulting in deoxygenation are influenced by variables such as water type, plant and microbial biomass and climatic conditions. In order to study the process in detail a system has been developed using water supplemented with dilute inorganic nutrients and a dense suspension of the alga Stichococcus sp. to simulate a bloom. Experiments are done in fermenter vessels, one treated with herbicide and the other left as control. Use of this apparatus enables conditions within the vessels to be monitored and controlled if required, thus allowing effects of individual factors to be studied. This paper describes the system and its use to investigate effects of the herbicide terbutryn on microbial populations and oxygen levels in water from a small reservoir. Terbutryn was cleared for use against aquatic weeds in Britain in 1974. However, under certain conditions, its use has led to severe reduction of dissolved oxygen level in treated water (ROBSON et al. 1978).

MATERIALS AND METHODS

As replication was not possible in individual experiments, the investigation was repeated six times during the period January to June 1980 using freshly collected water samples.

Herbicide

Terbutryn (2-(tert-butylamino)-4-(ethylamino)-6-(methylthio)-S-triazine) was recrystallized from the formulated product (Prebane 500L, Ciba-Geigy Agrochemicals).

A 100 ppm solution of the purified herbicide in methanol was diluted to 10 ppm with reservoir water and used in the experiment at a final concentration of 0.1 ppm, a level recommended for aquatic weed control. Terbutryn residues in the culture were measured by gas chromatography or by high pressure liquid chromato-

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graphy (BYAST et al. 1977).

Stichococcus sp.

The alga was isolated from a small, shallow lake at Widford, Oxfordshire. It was grown at room temperature in 5 l aerated Beneckes medium (NH_4NO_3 0.2 g; K_2HPO_4, 0.1 g; $MgSO_4$, $7H_2O$, 0.1 g; $CaCl_2$, $6H_2O$, 0.1 g; $FeCl_3$, $6H_2O$, trace; Distilled water, 1000 ml; pH 7.0; FLENT 1958). The culture was continuously illuminated by warm white fluorescent light from a 150 cm, 65/80 W tube and was not shaded from normal daylight. After 7 days the culture was harvested by centrifugation (1000*g* for 10 min), washed once with Beneckes medium and resuspended in the same medium (50 ml) to give a final concentration of $3.2 \pm 0.1 \times 10^8$ cells/ml.

Water samples

Water (c. 10 l) was collected from the top 30 cm of a small (52 x 33 x 3 m deep) butyl-rubber lined reservoir fed from a natural spring and partially colonised with Chara sp. and Cladophora sp. The latter became free-floating during the summer, occasionally covering up to 30% of the surface. Successions of algal blooms also occurred. The reservoir contained trout and sticklebacks.

Batch culture system

The culture vessels (3.5 l capacity) were sterilized empty but all subsequent manipulations were made without sterile precautions. Freshly collected water (2,670 ml) was placed in each vessel, maintained at $15 \pm 1^{\circ}C$ and stirred at 100 rpm. Ports on the top of the vessels were left open to allow contact between water and atmosphere. Each vessel was illuminated for 15 h/day by four 300 mm, 8W, cool white fluorescent tubes. Two tubes were placed approximately 120 mm from each side of the vessel giving a light intensity of c.8000 Lux at the glass surface. Oxygen level (Mackereth electrode), pH, temperature and redox potential (Platinum spade electrode, calomel remote reference electrode) in the water were continuously recorded. Curves in Fig. 1 are transcribed from these recordings using readings taken at discrete intervals. Redox potentials are presented with reference to the calomel electrode and are corrected to pH 8.0 at $15^{\circ}C$. The approximation that at $15^{\circ}C$ a fall in pH of one unit causes the potential to become more positive by 57 mv is used in the correction (JACOB 1970).

After 22 h algal suspension (25 ml) and Beneckes medium (275 ml) were added and 2 h later 30 ml terbutryn (treated vessel) or 30 ml reservoir water (control vessel). Algae, bacteria and protozoa were counted in samples taken at the start of the experiment, before addition of the algal suspension, after addition of terbutryn and, unless stated otherwise, every 24 h for 7 days thereafter. The volume removed at each sample time (usually 25 ml) was replaced with reservoir water collected at the start of the experiment and stored at $5^{\circ}C$. Approximately 300 ml of sample were replaced during the experiment.

Counts of microbial populations

Stichococcus sp. Viable cells were counted by a dilution-plate technique (GREAVES et al. 1978) using three replicate plates of each dilution. Algae native in the reservoir water were rarely observed on the plates due to the large dilutions required to obtain countable numbers of Stichococcus sp.

Bacteria. Total cell counts were made using fluorescein isothiocyanate (GREAVES et al. 1978). A sample (10 µl) was spread over a 1 cm^2 area on a microscope slide and 10 graticule areas counted in the smear. Viable cells were counted by a modified dilution-plate technique using casein, peptone, starch agar FRY & HUMPHREY 1978; JONES 1979). Petri dishes containing the medium were dried overnight at 40°C and ten 10 µl drops of appropriately diluted sample then spotted on the agar surface. The number of colonies formed from each drop were counted after 8 days incubation at 19±2°C.

Protozoa. Numbers of protozoa were estimated using a modification of SINGH's Ring method (DARBYSHIRE 1973). Soil-extract fluid (100 µl) was dispensed into all the wells on a microtitration plate with the exception of those in the first row. The 8 wells of this row each received 100 µl of sample. Micro-diluters (25 µl) were then used to prepare dilution series from the sample (GREAVES et al. 1978). The plates were incubated at 19±2°C and samples from each well examined microscopically for protozoa after 7 and 14 days.

RESULTS

Similar patterns of change in oxygen level, redox, pH and microbial populations were observed in control and herbicide treated water in all 6 experiments. Details of one experiment are presented with noteworthy data from the other experiments.

On addition of terbutryn, oxygen level in the water started to fall within 10 min and declined increasingly for 128 h (Fig. 1A). Complete deoxygenation was maintained for 10 h followed by recovery to the initial oxygen level. Similar results were obtained in the other experiments although, in one case, complete deoxygenation occurred only 24 h after addition of the herbicide. The duration of complete deoxygenation also varied between 5 min and 20 h. Recovery always started suddenly and oxygen concentration rose rapidly.

Oxygen levels in the control vessel showed the normal diurnal photosynthesis cycle but this was absent in the treated vessel.

Redox response following herbicide treatment was very similar to that of oxygen (Fig. 1B) and was lowest just prior to the onset of recovery. In the control little variation in redox occurred apart from a slight drop at 70 h.

In the control vessel pH rose for 120 h and was then constant (Fig. 1C) whereas in the treated vessel it fell from 8.0 to 6.8 at

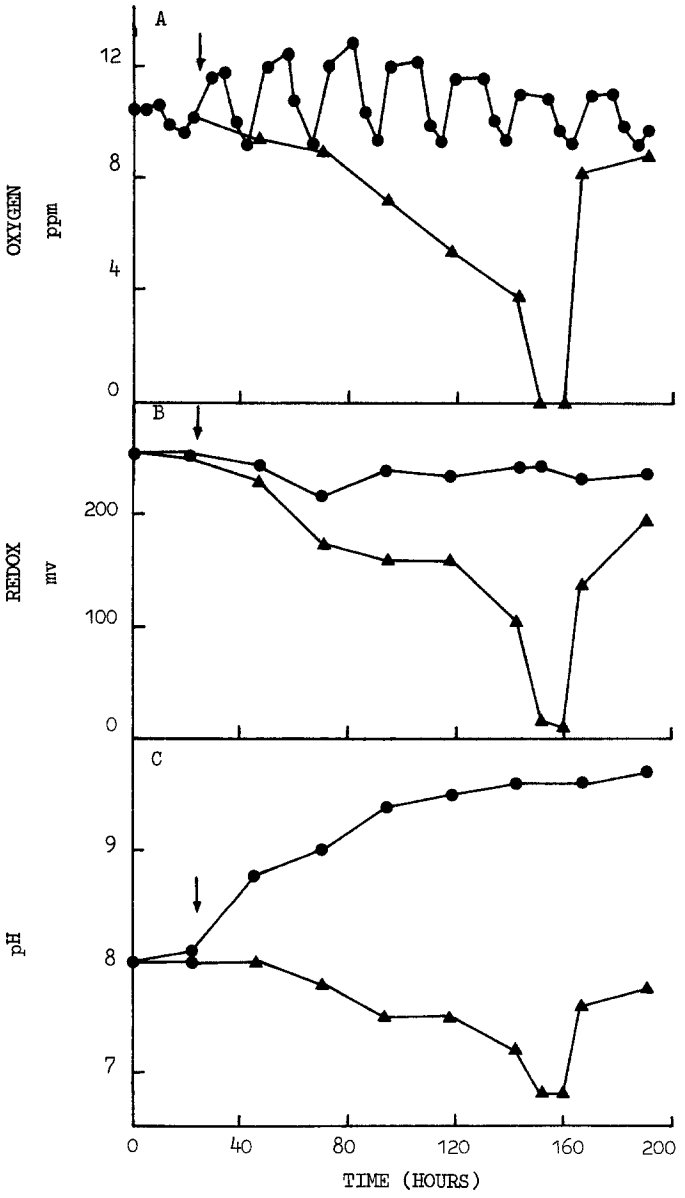

Fig. 1. The effect of terbutryn on (A) oxygen level, (B) redox potential and (C) pH. ↓ time of addition of Stichococcus sp. and Beneckes medium (22 h) and terbutryn (24 h). ● , control; ▲ , terbutryn.

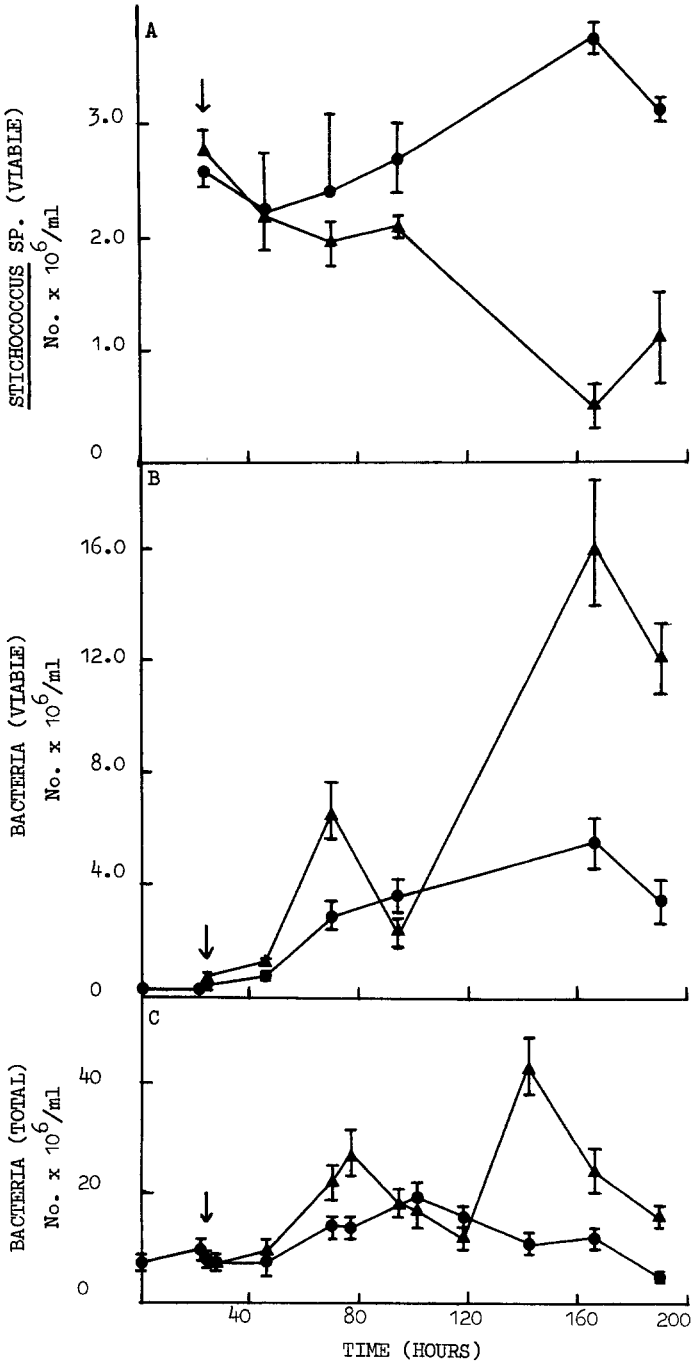

Fig. 2. The effect of terbutryn on viable counts of (A) Stichococcus sp., and (B) bacteria and total counts of (C) bacteria. ↓ time of addition of Stichococcus sp. and Beneckes medium (22 h) and terbutryn (24 h). ●, control; ▲, terbutryn. Bars = standard errors.

the start of recovery and then rose to 7.7.

The concentration of terbutryn in the culture did not fall during the experiment.

Counts of microbial populations

Stichococcus sp. Viable cell numbers increased in the control but decreased in the treated vessel (Fig. 2A). However, at the end of the experiment, terbutryn had not killed all the algal cells.

Bacteria. Total populations in the control rose to a maximum at 94 h and then declined (Fig. 2C). In the treated vessel peaks occurred at 70 and 142 h. The latter was statistically significant and corresponded to the start of the rapid fall in oxygen concentration. Numbers fell markedly during reoxygenation of the water.

Results were similar for viable cell counts (Fig. 2B) except that the second peak in the treated vessel occurred at 166 h. However, viable bacteria were not counted in the sample taken at 142 h.

Approximately 95% of colonies growing on dilution-plates of herbicide-treated samples taken at 166 and 180 h were very small ($<$1.0 mm) and translucent. They were absent or rare on plates from control and treated samples taken at other sampling times. This type of colony also occurred in the other experiments and was first noted when the oxygen concentration had fallen to approximately 2 ppm. The incidence of this colony type was always high throughout the period of complete deoxygenation. Numbers then quickly declined although in one investigation these colonies were still apparent 4 days after reoxygenation.

Protozoa. Numbers of protozoa, principally flagellates, were very low in both vessels ($<$10/ml) except for a transient increase to 120/ml in the treated vessel at 168 h. In two experiments numbers of protozoa were high (1×10^5/ml) following deoxygenation. There were, however, wide fluctuations in the other three experiments.

DISCUSSION

Addition of terbutryn to reservoir water supplemented with dilute inorganic nutrients and a dense algal suspension caused similar changes in oxygen level, redox, pH and microbial populations in six experiments despite the water samples being taken during the period January to June and therefore differing in microbial composition and activity. The time between herbicide addition and complete deoxygenation and the duration of this state before recovery varied between experiments and may have been due to these differences.

The onset of recovery was always sudden and the oxygen level in the water rapidly returned to the initial level. This sudden

transition between deoxygenation and reoxygenation may have been due to inhibition of bacteria by their own metabolic products formed during deoxygenation or, possibly, by nutrient limitation. These factors could lead to the reduction in bacterial numbers observed during reoxygenation. The rate at which recovery occurred is a balance between oxygen input to the system by diffusion from the air and the photosynthetic activity of surviving algal cells and the removal of oxygen by microbial respiration and oxidation reactions associated with algal autolysis.

The role in deoxygenation of the bacterium producing small colonies on count plates is unclear at present. However, as it proliferated when oxygen was very low or absent, its metabolism was almost certainly fermentative at that time.

The laboratory microcosm used in the experiments has features in common with the natural ecosystem. Water with a natural microbial population is used at a temperature and lighting regime which approximate to those occurring in the U.K. during the spring. It is important, however, that in future experiments sediment and aquatic macrophytes are included in the system as the microorganisms specific to those habitats may affect the deoxygenation process.

The ability of the microcosm to successfully mimic the natural ecosystem is shown by the similarity of results obtained with those found in a study of the use of terbutryn to eradicate a heavy infestation of the filamentous alga Spirogyra sp. at Widford Lake, Oxfordshire (ANON 1980). The deoxygenation of the water that occurred after terbutryn treatment was primarily caused by the herbicide stopping photosynthesis in the alga while not affecting its respiration. The microbial decomposition of plant residues was not appreciable until after deoxygenation was established. Further work using the microcosm may indicate the relative importance of these and of other environmental factors during deoxygenation.

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