

# GROWTH OF BLUE-GREEN ALGAE IN THE MANUKAU (NEW ZEALAND) OXIDATION PONDS—I. GROWTH POTENTIAL OF OXIDATION POND WATER AND COMPARATIVE OPTIMA FOR BLUE-GREEN AND GREEN ALGAL GROWTH

W. F. VINCENT\* and W. B. SILVESTER†

Botany Department, University of Auckland, Auckland, New Zealand

(Received 14 December 1978)

**Abstract**—Water sampled from the Manukau oxidation ponds between 6 December 1973 and 12 July 1974 was tested for its ability to support blue-green algal growth. A local blue-green algal isolate of *Anabaena* grew well on membrane filtered pond water throughout the year, however the unfiltered water sustained *Anabaena* only when the resident green algal populations, in particular *Chlorella*, were low.

Temperature and pH optima for growth of *Anabaena* and the Manukau pond algal dominant, *Chlorella*, were found to be significantly different: 28–35°C and pH 9–10 and 23–28°C and pH 7–8 respectively. The ambient conditions of the ponds favoured growth of *Chlorella* over blue-green algae during the period of study.

## INTRODUCTION

Blue-green algal blooms are characteristic of highly productive nutrient rich waters and yet are seldom reported as dominant components of well established temperate sewage treatment ponds (Whitton, 1973).

During the first 2 years of operation of the Manukau oxidation ponds the blue-green alga *Microcystis aeruginosa* formed spectacular summer blooms and in subsequent years short-lived blooms of *Arthrospira* and *Oscillatoria* were recorded (Haughey, 1968, 1969). In later years the ponds have been dominated by green algae, predominately *Chlorella*, and bloom forming blue-green algae have not been detected. Absence of blue-green algae is considered an advantage in the efficient operation of an oxidation pond system as the surface scums produced by them reduce gaseous diffusion and light penetration, and release objectionable odours upon decomposition.

The absence of blue-green algae in a superficially highly favourable environment may be due to the peculiar biotic, nutritional or physical characteristic of oxidation ponds, and these are considered briefly here by way of introduction.

(a) Of the biotic factors that may control blue-green algae, pathogens such as cyanophage and bacteria have frequently been isolated from oxidation ponds (Safferman, 1973) and the previous occurrence of blue-green algae in the ponds increases the possi-

bility of residual pathogen populations. Other more subtle biotic controls may operate through competition and ecological succession which may be controlled by extracellular products from resident algae.

(b) Nutrition may control algal advantage although this is unlikely in the nutrient rich conditions. The highly organic substrate of the ponds may favour heterotrophic nutrition which is not common in blue-green algae. However some blue-green algae are capable of utilising organic compounds either in the dark or light (Kratz & Myers, 1955; Khoja & Whitton, 1971).

(c) Temperature and pH conditions of the ponds may well control the algal populations. Tropical oxidation ponds support high concentrations of blue-green algae (Singh, 1961) and Shapiro (1973) has shown that pH is important in controlling algal dominance.

The following paper investigates the ability of oxidation pond water to support various blue-green algae and the possible reasons for their current absence from the Manukau ponds.

## MATERIALS AND METHODS

### *Manukau oxidation ponds*

The sewage ponds total 525 ha and serve a population equivalent of 1.3 million persons. Sewage is received, treated by anaerobic digesters and sedimentation tanks before being released into the oxidation ponds.

### *Blue-green algal growth potential*

The ability of water sampled from the Manukau oxidation ponds between December 1973 and August 1974 to support blue-green algal growth was assessed using

\* Present address: Ecology Division, DSIR, P.O. Box 415, Taupo, New Zealand.

† Department of Biological Sciences, University of Waikato, Hamilton, New Zealand.

*Microcystis aeruginosa* (Cambridge collection) and a local species of *Anabaena* isolated from the Waikato River by Miss C. Lam. Freshly collected water from the main circulation channel of the plant was centrifuged at 16,000 *g* for 30 min at 10°C and then pressure filtered through a 0.45 µm membrane filter (Sartorius Membranfilter GmbH). Washed blue-green algal cells were inoculated into 100 ml quantities of undiluted or diluted filtered centrifuged pond water; unfiltered uncentrifuged pond water; and into sterile ASM inorganic nutrient media (Gorham *et al.*, 1964). The cultures were incubated under 2000 lux continuous fluorescent light in a constant temperature room at 28°C and growth was assayed by haemocytometer cell count. To facilitate accurate counting the filaments of *Anabaena* were broken up into unicells by a short period of low energy sonication (1 minute, power setting 3 on Kontes sonic disintegrator; Kontes Instruments, Vineland, New Jersey).

One test only was carried out on *Arthrospira platensis* which formed a minor bloom in experimental ponds in March–April 1974. *Arthrospira* was isolated on ASM agar plates from experimental pond D during late April. Growth was exceedingly poor on ASM and other inorganic defined media and for the test *Arthrospira* cultures were washed and inoculated into 100 ml quantities of filtered and unfiltered pond water collected on 6 May, 1974. Growth was assayed by haemocytometer counts of cell units. As cross walls were difficult to detect a cell unit was defined as one spiral of a filament.

As a basic test for the presence of organisms pathogenic towards *Anabaena* 75 ml quantities of autoclaved ASM with 25 ml additions of either sterile deionised water, filtered pond water or unfiltered pond water (collected from the Manukau ponds 13 February, 1974) were inoculated with *Anabaena* and growth was monitored by cell count over 8 days.

#### Environmental optima

The optimum temperature and pH regimes for the growth of the local *Anabaena* isolate and the *Chlorella* dominant in the Manukau ponds were compared with the ranges normally encountered within the ponds. Growth was monitored by cell count for each species in static batch culture in ASM under continuous fluorescent light at four temperatures maintained to within ±1°C by water baths. Optimum pH ranges were established by inoculating each alga into 100 ml quantities of autoclaved ASM initially adjusted to pH 5.5, 6.5, 7.5, 8.5, 9.5 or 10.5 in 100 ml Buchner flasks each containing a pH electrode. Every 12 h the flasks were shaken and the pH readjusted to the correct value by addition of sterile, dilute NaOH or HCl through an injection port. Variation in pH of the media was maintained within 0.5 pH units.

Growth rate constants were calculated from the cell count data for the first 4–5 days of exponential growth by the formula:

$$k = \frac{2.3 \log_{10} (N_2/N_1)}{T_2 - T_1}$$

where *k* is the growth constant (natural log increments per day) and *N*<sub>1</sub> and *N*<sub>2</sub> are the cell concentrations at days *T*<sub>1</sub> and *T*<sub>2</sub> respectively.

## RESULTS

### Blue-green algal growth potential

At the first sampling time (6 December, 1973) a range of dilutions of filtered pond water were tested for their ability to support both *Anabaena* and *Microcystis*. Full strength pond water supported growth that was not significantly less than that found with

Table 1. Initial and final cell count data (mean and standard error of three replicates) for growth of *Anabaena* and *Microcystis* for 4 days in filtered pond water, collected from Mangere, 6 December, 1973

Treatment	Algal numbers 10 <sup>5</sup> ml <sup>-1</sup>	
	<i>Anabaena</i>	<i>Microcystis</i>
Initial	1.6	3.5
100%	5.7 (0.68)	5.5 (0.59)
50%	6.9 (1.35)	2.9 (1.86)
20%	2.5 (0.76)	2.3 (1.98)
10%	1.3 (1.10)	2.1 (1.72)
ASM	6.1 (0.48)	6.7 (0.43)
Unfiltered	0.3 (0.08)	0.22 (0.02)

Diluted with sterile deionised water to various concentrations and incubated as static cultures (shaken twice daily) under 2000 lux fluorescent light at 28°C. Final cell count data for ASM and unfiltered pond water treatments presented for comparison.

the standard ASM nutrient solution (Table 1). Dilution resulted in an equivalent reduction in algal growth with *Anabaena* showing a significantly higher growth rate than *Microcystis*. In this experiment (Table 1) both *Anabaena* and *Microcystis* showed a significant decline in cell numbers when inoculated into unfiltered pond water retaining its resident populations.

A more extensive experiment on unfiltered water followed both the resident populations and the inoculated blue-green algae at various dilutions (Table 2). In all but one isolated case the blue-green algal numbers fell significantly below the inoculum level. Green algal numbers showed distinct changes in culture with *Micractinum* and *Scenedesmus* increasing and *Chlorella* declining in most cultures. Large increases in *Ankistrodesmus* were generally only observed in those cultures inoculated with *Anabaena* or *Microcystis*.

Similar tests conducted in February 1974 showed that filtered undiluted pond water was capable of supporting better *Anabaena* growth than standard ASM medium (Fig. 1). However, in unfiltered pond water *Anabaena* concentration dropped sharply to undetectable levels after five days while *Chlorella* numbers rose slightly and *Euglena* remained constant (Fig. 2).

Over the period 6 December to 12 July five separate samplings of the ponds confirmed that filtered pond water was always able to support vigorous *Anabaena* growth (Table 3). However, while *Anabaena* growth was inhibited in unfiltered water for the December and February collections subsequent samplings allowed significant blue-green algal growth. *Anabaena* growth was low in March, but in May, when resident green algae were at their lowest, growth of *Anabaena* on crude pond water was equivalent to ASM control but lower than on filtered pond water (Table 3). By July when the green algal population had risen again *Anabaena* was again inhibited. *Arthrospira platensis* formed a minor bloom in late summer 1974 in two experimental oxidation ponds but

Table 2. Changes in green algal and blue-green algal concentrations (mean and standard error three replicates) after 4 days' growth in unfiltered Mangere pond water (collected 6 December) of various dilutions with sterile deionised water. Initial and final cell counts. Incubated under 2000 lux, 28°C, static cultures (shaken twice daily)

Pond water concentration	Cell concentration $10^4 \text{ ml}^{-1}$						
	<i>Anabaena</i>	<i>Microcystis</i>	<i>Chlorella</i>	<i>Euglena</i>	<i>Ankistrodesmus</i>	<i>Scenedesmus</i>	<i>Microactinium</i>
100%	A* 2.7 (0.8)		44.0 (7.0)	—†	2.8 (1.6)	2.5 (0.2)	15.0 (8.8)
	M 15.0 (7.0)	2.2 (0.2)	15.0 (7.0)	0.2	2.6 (0.3)	0.7 (0.7)	6.6 (4.7)
	C 15.8 (11.0)		15.8 (11.0)	—	0.8 (0.3)	1.0 (0.3)	15.3 (8.8)
50%	A 4.5 (1.8)	4.5 (0.3)	12.0 (1.3)	—	6.2 (1.2)	5.5 (2.0)	3.7 (2.7)
	M 4.3 (1.8)		4.3 (1.8)	—	2.6 (0.8)	2.2 (1.3)	4.7 (1.1)
	C 23.0 (5.0)	1.0 (0.5)	29.6 (12.0)	—	0.5 (0.2)	1.3 (0.7)	1.5 (1.2)
20%	A 4.2 (2.2)		11.0 (4.0)	—	1.0 (0.5)	2.2 (0.2)	—
	M 7.0 (5.0)	1.0 (0.5)	4.2 (2.2)	—	3.0 (1.4)	8.6 (0.6)	—
	C 2.5 (1.9)	1.0 (0.5)	7.0 (5.0)	—	1.5 (0.3)	0.8 (0.8)	0.3 (0.3)
10%	A 4.7 (2.0)	1.0 (0.5)	6.0 (5.0)	—	0.6 (0.2)	0.8 (0.8)	1.0 (0.6)
	M 1.3 (0.2)		1.3 (0.2)	—	1.0 (0.5)	2.8 (1.2)	—
	C 16.0	35.0	60.0	2.0	1.5 (0.5)	0.5 (0.8)	1.0 (1.0)
Initial					0.5	—	1.0

\* A: Inoculated with *Anabaena*.

M: Inoculated with *Microcystis*.

C: Uninoculated control.

† Less than  $10^3$  cells per ml.

Table 3. Initial and final cell concentrations of *Anabaena* (mean and standard error for three replicates) grown for four days on ASM, and on filtered and unfiltered pond water sampled at various times throughout the year. All cultures under 2000 lux fluorescent light, 28°C

Date	Inoculum	<i>Anabaena</i> concentration		Initial algal concentration within pond water		
		ASM	Filtered	Unfiltered	<i>Chlamy.</i>	<i>Chlorella</i> <i>Microactinium</i>
6 December*	1.6	6.1 (0.48)	5.7 (0.7)	0.3 (0.1)	—	6.0
13 February†	1.0	24.0	28.0	0.1	—	1.9
13 March	1.0	18.1 (2.1)	9.8 (1.4)	4.3 (0.01)	0.1	0.6
17 May	1.0	20.0 (1.8)	24.3 (1.8)	7.1 (2.1)	—	0.1
12 July	1.0	14.2 (0.71)	10.2 (2.7)	2.4 (0.78)	0.4	3.2

\* 6 December—static cultures all others on shaker 125 rev/min.

† 13 February—extracted from Figs. 1 and 2—less than  $10^4$  cells per ml; *Ankis*: *Ankistrodesmus*; *Chlamy*: *Chlamydomonas*.

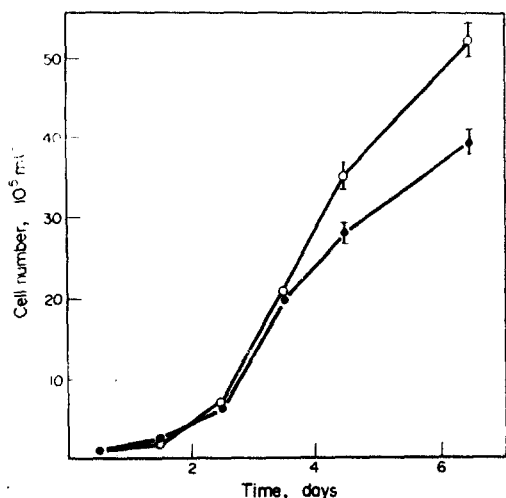


Fig. 1. Growth of *Anabaena* in filtered Mangere pond water (collected 13 February) and in ASM. Each point represents the mean of three replicates  $\pm$  one standard error.  $\bullet$ — $\bullet$  ASM;  $\circ$ — $\circ$  filtered pond water. Incubated under 2000 lux fluorescent light at 28°C shaken at 125 rev/min.

was not detected in the adjacent main ponds. This organism was isolated from the experimental pond into unialgal culture and tested for growth potential in main pond water sampled 6 May, 1974, both filtered and unfiltered. At this stage the main ponds contained low concentrations of *Chlorella* and *Euglena* ( $6.1 \times 10^4$  and  $6.5 \times 10^4 \text{ ml}^{-1}$  respectively). *Arthrospira* like *Anabaena*, was capable of vigorous growth in filtered water ( $k = 0.22$ ). However, in crude pond water *Arthrospira* was completely inhibited while *Chlorella* continued to grow ( $k = 0.11$ ).

Tests for pathogens

Tests during March, May and July showed that *Anabaena* was able to grow in unfiltered pond water (Table 3) and thus pathogens were not significant.

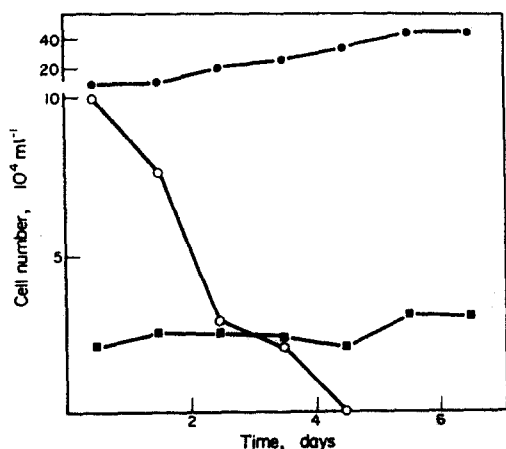


Fig. 2. Growth of *Anabaena* and green algal dominants in unfiltered pond water collected 13 February. Incubated under 2000 lux fluorescent light, 28°C, shaken at 125 rev/min.  $\bullet$ — $\bullet$  *Chlorella*;  $\blacksquare$ — $\blacksquare$  *Euglena*;  $\circ$ — $\circ$  *Anabaena*.

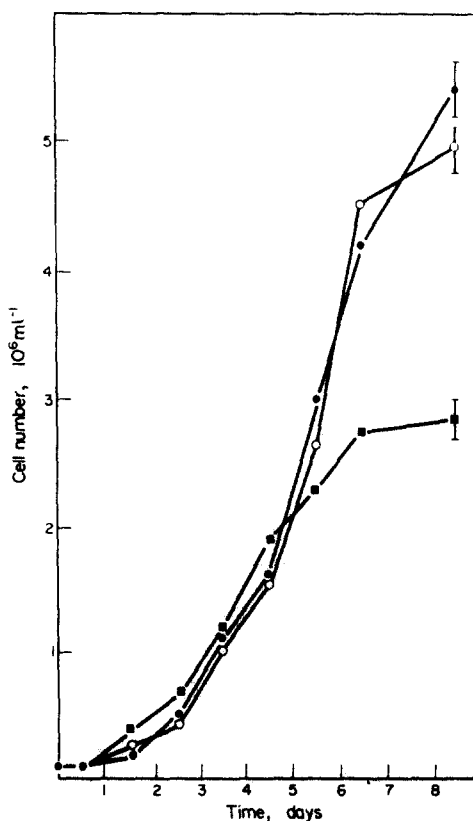


Fig. 3. Test for pathogenicity of Mangere pond water, collected 13 February, towards blue green algae: growth of *Anabaena* in 75 ml of ASM plus the following 25 ml additions; sterile deionised water  $\blacksquare$ — $\blacksquare$ ; filtered pond water  $\bullet$ — $\bullet$ ; unfiltered pond water  $\circ$ — $\circ$ . Incubated on shaker at 125 rev/min, under 2000 lux continuous fluorescent light, at 28°C. (Means of three replicates  $\pm$  standard error.)

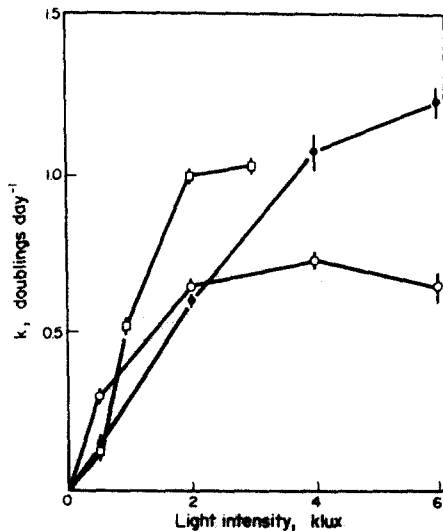


Fig. 4. Mean growth rates ( $k$ ) of *Anabaena*, *Microcystis* and *Chlorella* in relation to light intensity at 28°C. Algae were grown in static cultures, shaken each 12 h in 500 ml flasks containing 100 ml ASM medium under continuous cool white fluorescent light. Means and standard errors for three replicates for *Microcystis*  $\square$ — $\square$ ; *Anabaena*  $\circ$ — $\circ$  and *Chlorella*  $\bullet$ — $\bullet$ .

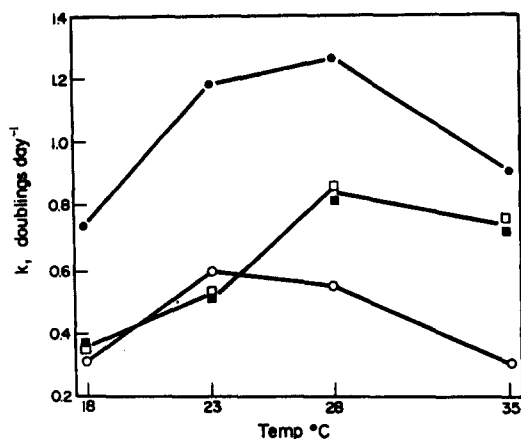


Fig. 5. Mean growth rate ( $k$ ) of *Anabaena* (squares) and *Chlorella* (circles) at 2000 lux (open) and 6000 lux (closed) continuous cool white fluorescent light. Algae were grown in water baths in 500 ml flasks in 100 ml ASM medium shaken each 12 h.

Tests were conducted during February when crude pond water strongly inhibited *Anabaena* growth. The results (Fig. 3) show that addition of a 25 ml inoculum of unfiltered pond water to an *Anabaena* culture resulted in increased growth and thus there is no evidence for pathogenic organisms being responsible for the poor blue-green algal growth at that time.

#### Comparative environmental optima

Environmental optima for growth of blue-green algae and *Chlorella* are distinctly different. While both blue-green species were light saturated at 2000 lux, *Chlorella* saturated at around 4000 lux or more (Fig. 4). Similarly temperature optima were significantly different with *Anabaena* showing optimum growth between 28°C and 35°C and *Chlorella* between 23°C and 28°C (Fig. 5). Further, as shown by Fig. 5, light and temperature appear to act independently on both *Anabaena* and *Chlorella*.

Growth rates in relation to pH are presented in Fig. 6. *Chlorella* grew well in the range pH 5–9 with optimum in the range pH 7–8. In contrast *Anabaena* grew between pH 6 and 11 with a much higher optimum in the range pH 9–10. Growth at pH 6 was extremely poor for *Anabaena* with a doubling time exceeding 300 h while *Chlorella* showed no growth whatsoever at the pH optimum for *Anabaena* growth.

#### DISCUSSION

Manukau pond water when filtered was capable of supporting vigorous blue-green algal growth throughout the period of tests (December–July). This period included late summer, the only period in which temperatures are high enough to support vigorous blue-green algal blooms. The growth of blue-greens was entirely suppressed in mixed cultures in early and mid-summer when green algal populations were at a maximum while in autumn when green algae had declined the unfiltered pond water supported intense

growth of *Anabaena* in culture. However by this time of year pond temperatures drop to 20°C or below which would severely inhibit blue-green algal growth.

Several explanations may be offered for the ability of the blue-green species to grow well in filtered, but not unfiltered pond water:

(i) The pond water contained parasitic microorganisms which selectively attack these blue-green algae. This explanation is rejected for cyanophage since virus particles are small enough to pass through the 450 nm pores of the membrane filter (e.g. cyanophage LPP-1 has a head diameter of 60 nm—Safferman, 1973) and high growth rates were recorded on the filtered water. The experiments in which unfiltered pond water was added to cultures of *Anabaena* on ASM indicated that filtration did not remove any fungus or bacterium pathogenic towards blue-green algae. However this test was made under conditions optimal for growth of *Anabaena* and it is possible that pathogenic organisms exist in the pond water which attack only cells which are not in a phase of healthy, active growth. It is known, for example, that the phycomyxete *Rhizosiphon anabaenae* only attacks unhealthy or senescent cells of *Anabaena planktonica* (Paterson, 1960).

(ii) The unfiltered pond water contained zooplankton which removed the blue-green algae by predation. Although rotifers, protozoans and crustaceans were recorded in the pond water, their numbers were low, and therefore unlikely to cause the rapid decline in blue-green algal concentration in the early and mid-summer samples. Furthermore, zooplankton

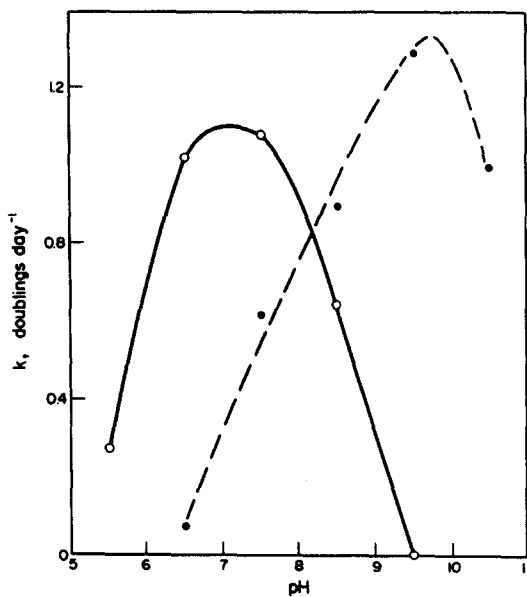


Fig. 6. Mean growth rates ( $k$ ) of *Anabaena* and *Chlorella* in relation to pH at 28°C and 2000 lux continuous fluorescent light. The algae were grown in single 500 ml flasks with ASM medium each containing a pH electrode. Every 12 h the culture pH was adjusted by addition of NaOH or HCl through sidearm. *Chlorella* ○—○; *Anabaena* ●—●.

generally prefer green algae rather than blue-green algae as food sources (Hutchinson, 1967).

(iii) One or more of the green algal components of the unfiltered pond water produces an extracellular product which inhibits growth of blue-greens. This is unlikely because of the vigorous growth of blue-green algae on the filtered water, although it is possible that such a product might be highly labile and readily lost during the filtration and centrifugation processes. To further test this possibility a wide range of interaction tests were performed and are reported in part II of this work (Vincent & Silvester, 1979). In the March filtered pond water sample, growth of *Anabaena* was unusually much lower than the ASM control. This sample was characterised by low total green algal concentration and an unusually high *Chlamydomonas* count and it is possible that this alga may produce compounds inhibitory towards *Anabaena*.

(iv) Growth inhibition in crude pond water was due to the inability of the blue-green algae to compete with dense green algal populations. The correlation of high green algal numbers with blue-green algal inhibition and lack of evidence for pathogens, grazing or extracellular products effects renders algal competition as the most likely explanation.

Both temperature and pH are known to be important factors controlling bloom formation by blue-green algae and the environmental optima for the local *Chlorella* and *Anabaena* isolates fall within the range normally reported for these groups. In Canadian lakes it has been noted that blue-green algae are seldom found until water temperatures reach about 15°C and blooms rarely develop until temperatures rise to 23–26°C (Hammer, 1964). The effect of temperature on *Anabaena* growth is very marked with an optimum between 28 and 35°C. The annual temperature range of the ponds is 15–30°C with temperatures, rarely exceeding 25°C (Haughey, 1965), thus favouring growth of *Chlorella*. Similarly the pH of the pond water remains in the range of 7.5–9.0 (Haughey, 1965) favouring *Chlorella*. Shapiro (1973) has shown that pH is crucial in determining the relative abundance of green and blue-green algae and believes this is associated with CO<sub>2</sub> availability.

From this preliminary study it may be concluded that the ponds are outside the range of optimal blue-green algal growth for a great deal of the year. Although a large number of factors interact in allow-

ing algal dominance, the current situation, whereby a dominant green algal flora is maintained will persist as long as stable pond loading conditions prevail and the physical and chemical parameters stabilise. However an oxidation pond is inherently an unstable system and short term perturbations may have far reaching effects on the relative dominance of biota.

*Acknowledgements*—This work was supported by a research scholarship from the Auckland Regional Authority, Research Advisory Committee, and by research grants from the University of Auckland Research Committee. We are grateful to Miss Catherine W. Y. Lam for assistance with algal isolation and culture.

#### REFERENCES

- Gorham P. R., McLachlan J. S., Hammer V. T. & Kim W. K. (1964) Isolation and culture of toxic strains of *Anabaena flos-aquae* (Lyngb.) de Breb. *Verh. Int. Verein. Limnol.* **15**, 796–804.
- Hammer V. T. (1964) The succession of bloom species of blue-green algae and some causal factors. *Verh. Int. Verein. Limnol.* **15**, 829–836.
- Haughey A. W. V. (1965) Phytoplankton in sewage treatment ponds. MSc thesis, University of Auckland, New Zealand.
- Haughey A. W. V. (1968) The planktonic algae of Auckland sewage treatment ponds. *N.Z. J. Mar. Freshwat. Res.* **2**, 721–766.
- Haughey A. W. V. (1969) Further planktonic algae of Auckland sewage treatment ponds. *N.Z. J. Mar. Freshwat. Res.* **3**, 245–261.
- Hutchinson G. E. (1967) *A Treatise on Limnology*. Wiley, New York.
- Khoja T. & Whitton B. A. (1971) Heterotrophic growth of blue-green algae. *Arch. Mikrobiol.* **79**, 280–282.
- Kratz W. A. & Myers J. (1965) Nutrition and growth of several blue-green algae. *Am. J. Bot.* **42**, 282–287.
- Paterson R. A. (1960) Infestation of chytridiaceous fungi on phytoplankton in relation to certain environmental factors. *Ecology* **41**, 416–424.
- Safferman R. S. (1973) Phycoviruses (Edited by Carr N. G. & Whitton B. A.). In *Biology of Blue-green Algae*. Blackwell, London.
- Shapiro J. (1970) A statement on phosphorus. *J. Wat. Pollut. Control Fed.* **42**, 772–775.
- Shapiro J. (1973) Blue-green algae: why they become dominant. *Science* **179**, 382–384.
- Singh R. N. (1961) *The Role of Blue-Green Algae in the Nitrogen Economy of Indian Agriculture*. Indian Council of Agricultural Research, New Delhi.
- Vincent W. F. & Silvester W. B. (1979) Growth of blue green algae in the Manukau (New Zealand) oxidation ponds. II: Experimental studies on algal interaction. *Water Res.* **13**, 717–723.
- Whitton B. A. (1973) Freshwater plankton. In *Biology of the Blue-Green Algae* (Edited by Carr N. G. & Whitton B. A.). Blackwells, London.