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THE EFFECT OF MOLYBDENUM ON GROWTH RATES, PHOTOSYNTHETIC RATES AND NITROGEN ASSIMILATION IN FRESHWATER PLANKTON ALGAE

BY

LEE D. CARTIER

A Thesis
Submitted to the Faculty of Graduate Studies through the Department of Biology in Partial Fulfillment of the Requirements for the Degree of Master of Science at the University of Windsor

Windsor, Ontario, Canada

1973

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436558

FOR MY BELOVED WIFE PHYLLIS

Another damned, thick square book!
Always scribble, scribble, scribble!
Eh! Mr. Gibbon?

William Henry,
Duke of Gloucester

ABSTRACT

The effect of molybdenum on growth, photosynthesis and nitrate assimilation in two species of freshwater algae, *Navicula pelliculosa* and *Chlamydomonas reinhardtii* was examined. Molybdenum concentrations up to 1.0 ppm did not alter the growth rates in either species. Relative growth constants for *N. pelliculosa* and *C. reinhardtii* were 0.50 and 0.43 days respectively.

Photosynthetic rates in molybdenum starved cells of *N. pelliculosa* were significantly lower compared to non-starved cells, 0.0026 and 0.0045 mgC 10^6 cells^{-1} hr^{-1} respectively. For C. *reinhardtii*, there was no significant reduction in photosynthetic rates of molybdenum starved cells compared to non-starved cells, 0.0064 and 0.0056 mgC 10^6 cells^{-1} hr^{-1} respectively. Photosynthetic rates in both species of plankton algae, whether molybdenum starved or non-starved, were not altered by concentrations of molybdenum up to 1.0 ppm.

The K_m value for *N. pelliculosa* was lower than for *C. reinhardtii*, 14.9 and 148.0 µM respectively. Nitrate uptake in molybdenum starved cells of *N. pelliculosa* was significantly lower compared to non-starved cells, 7.83 and 3.67 µM 10^6 cells^{-1} hr^{-2} respectively. In *C. reinhardtii*, there was no reduction in nitrate uptake of molybdenum starved cells compared to non-starved cells, 3.30 and 3.82 µM 10^6 cells^{-1} hr^{-2} respectively. Nitrate uptake in both species of plankton algae, whether molybdenum starved or non-starved, were not altered by concentrations of molybdenum up to 0.8 ppm.

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Chapter 1

EFFECT OF MOLYBDENUM ON GROWTH CHARACTERISTICS OF TWO SPECIES OF FRESHWATER ALGAE

INTRODUCTION

It is recognized that many elements are important in the growth and physiology of plants. These elements may be put into two distinct groups. The macronutrients, such as phosphate and nitrate, are required in concentrations from 0.1-2.0 ppm. The micronutrients, such as molybdenum are required in concentrations from 0.001-0.01 ppm. The latter group, which are referred to as trace elements, are now accepted to be essential for the cultivation of algae (Wiessner 1962; Fogg 1965; Goldman 1966; Devlin 1966; Hutchinson 1967). For example, S. P. Chu (1942), in formulating seventeen defined media, incorporated seven trace elements into all of his media. Although it has been recognized that algae have a micronutirent requirement, little work has been done on the trace elements. The roles of vanadium, zinc, and boron are essentially unknown. Cobalt is thought to be involved in vitamin B_{12} synthesis and perhaps photosynthesis (Yagodin 1963; Godnev and Lipskaya 1965; Gorid'ko 1965; Feoktistova 1962). Steemann-Nielsen and Wium-Andersen (1970) have examined the effects of copper on photosynthesis.

For the past thirty years, biologists have realized the requirement of molybdenum by green plants (Arnon and Stout 1939). This element is a true micronutrient in that it is required only in trace quantities by plants. Arnon *et al* (1955) found, for instance, that 0.1 ppb of molybdenum significantly increase the growth rate of *Scenedesmus*. The requirement for molybdenum, however, appears to be linked to nitrate utilization. When ammonia is the nitrogen source, molybdenum does not appear to be required (Arnon *et al* 1955; Syrett 1962).

The concentration of molybdenum in natural waters is very low, ranging from 0.75-14.0 ppb in sea water (Head and Burton 1970) and 0.1-0.6 ppb in freshwater (Bachmann and Goldman 1964). Hutchinson (1957) suggested that the low natural concentrations of molybdenum could play a vital role as a limiting factor in aquatic ecosystems.

Goldman (1960) working on Castle Lake, an alpine lake in California, was the first to demonstrate this hypothesis. Since then other workers have examined the role of molybdenum in various physiological processes (Aparicio et al 1971).

For the most part, however, the work on molybdenum has been scarce. The reasons for this could be many but the lack of interest in molybdenum has left a large gap in our knowledge of how algae and especially the diatoms will respond to increased molybdenum concentrations in the ecosystem. A study of this nature is important now, as the use of molybdenum in industrial processes could result in an increase in the concentration of molybdenum in natural waters.

It is the purpose of this chapter to examine the effects of high concentrations of molybdenum on the growth rates of two species of freshwater algae: Chlamydomonas reinhardtii (Dangeard), Camb. 11/32a and Navicula pelliculosa (Breb) Hilse, Lewin 50.

MATERIALS AND METHODS

Axenic cultures of two species of freshwater algae, the green alga Chlamydomonas reinhardtii (+ strain, wild type) and the diatom Navicula pelliculosa were used in these experiments. Cultures were

obtained from the culture collection of algae at Indiana University (Starr 1964).

Media Used

Stock cultures were grown in modified Hughes medium as described by Allen (1968)(Appendix A). This medium was further modified by the addition of 1 ml/l of a vitamin mix solution containing 500 μg/ml thiamine-HCl, 2 μg/ml vitamin B_{12} and 1 μg/ml of biotin (Wallen 1970). In addition, the medium was buffered using a Tris buffer prepared by dissolving 10 g tris(hydroxymethyl)amino methane in 100 ml of glass distilled water. This was titrated with concentrated HCl to a pH of 7.7 at 21 C. An addition of 7 ml/l of Tris stock gave a final pH of 7.8-7.9. This medium will be referred to as modified Hughes medium #1.

The medium used for the determination of growth rates was the basic modified Hughes medium #1, except that the trace elements were prepared according to Chu #10 (Chu 1942). Unlike modified Hughes medium #1, Chu's trace elements do not contain molybdenum or copper. Copper is thought by Steeman-Nielson and Wium-Andersen (1970) to interfere with measurements of photsynthesis. This modification of Hughes basic medium will be referred to as modified Hughes medium #2.

Measurement of Growth Rate

Stock cultures of both _C. reinhardtii_ and _N. pelliculosa_ were grown in 1 liter foam stoppered erlenmeyer flasks containing 250 ml

of modified Hughes medium #1. The medium was sterilized by autoclaving for 15 minutes at 120 C and 15 psi.

Stock cultures were grown in a Percival incubator at 20±1 C with continuous shaking. The cultures were exposed to a light:dark regime of 15:9 hr. A light intensity of 2.61×10^4 ergs cm^{-2} sec^{-1} was obtained using a bank of 6 G.E. cool white flourescent bulbs.

A typical growth experiment consisted of six cultures grown in foam stoppered 125 ml erlenmeyer flasks containing 70 ml of sterile modified Hughes medium #2. The cultures were supplemented with 0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm and 1.0 ppm of molybdenum respectively as molybdic acid. No molybdenum was added to the controls as the medium contained sufficient molybdenum contaminant to give concentrations close to natural levels. The cultures were run in duplicate. Routine checks of both stock and experimental cultures were conducted to verify the absence of contamination (Appendix B).

The inocula for the experimental cultures were taken from the stock cultures in the exponential phase of growth. The volume was adjusted to provide an initial concentration of 2-5 x 10^4 cells ml^{-1}. Cultures were then incubated under the same conditions as the stock cultures.

Growth rates for _C. reinhardtii_ were determined by measuring the changes in optical density at 540 nm with a Spectronic 20 colorimeter. Optical density values were converted to cells ml^{-1} using a calibration curve (Appendix C).

Growth rates for _N. pelliculosa_ were determined using a A/O fine line haemocytometer. Before counting, the aliquot was sonicated for

60-80 seconds at 20% intensity using a sonic disrupter (Bronwill Biosonic). The diatoms were sonicated in order to separate clumps. This clumping effect appears to be common in freshwater diatom cultures and contributes many problems to the accurate determination of their numbers. Counts taken from the haemocytometer were extrapolated to give cell no. ml^{-1}.

The relative growth constant (k) and mean generation time (T) were calculated using the following formulae:

$$k = \frac{\log_e N - \log_e N_o}{t} = \frac{2.3\ (\log_{10} N - \log_{10} N_o)}{t}$$

$$T = \frac{\log_e 2}{k} = \frac{0.693}{k}$$

where: N = cell concentration at time t.

N_o = cell concentration at time t = 0.

t = time in days.

RESULTS

Chlamydomonas reinhardtii grew more slowly than *Navicula pelliculosa* (Table 1). The relative growth constant for *C. reinhardtii* was 0.43 days. The relative growth constant for *N. pelliculosa* was 0.50 days. Both species exhibit typical growth characteristics (Fig. 1).

The division rates and relative growth constants of *C. reinhardtii* grown in concentrations of molybdenum from 0.2-1.0 ppm are given in Table 2. No significant difference was found between the mean genera-

Figure 1 Growth of Chlamydomonas reinhardtii (●) and Navicula pelliculosa (○). Light intensity, 2.61×10^4 ergs cm^{-2} sec^{-1}. Temperature, 20±1 C.

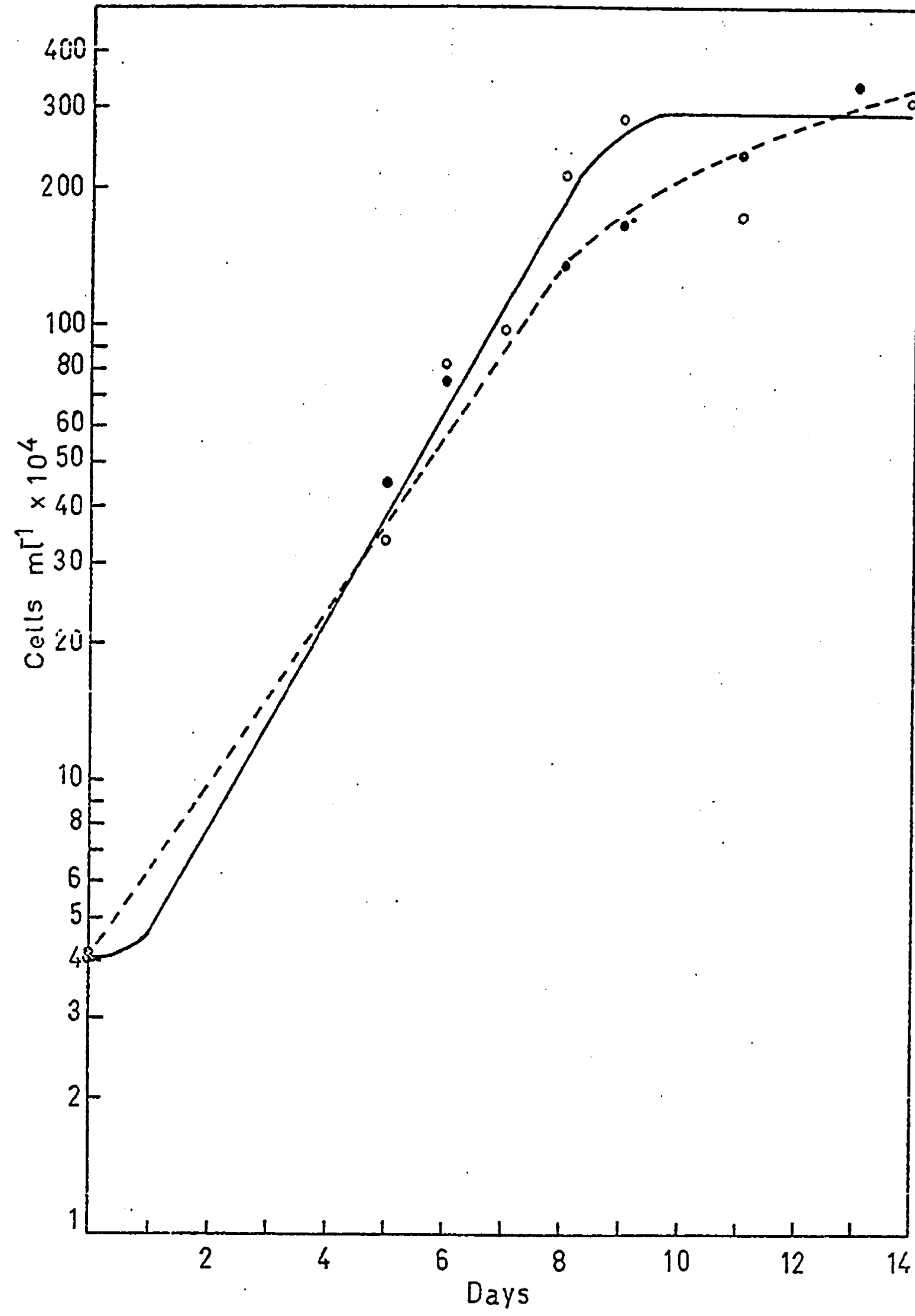

400
300
200
100
80
60
50
40
30
20
10
8
6
5
4
3
2
1
Cells ml^{-1} x 10^4
2
4
6
8
10
12
14
Days

Table 1

Relative growth constants and mean generation times of *Chlamydomonas reinhardtii* and *Navicula pelliculosa*. Cultures were exposed to a light:dark regime of 15:9 hr. Light intensity was 2.61×10^4 ergs $cm^{-2}.sec^{-1}$. Cultures were grown at 20±1 C with continuous shaking.

	Growth Characteristics		
	k(days)	T (days)	*t (p=0.05)
C. reinhardtii	0.43	1.59	3.39
N. pelliculosa	0.50	1.37	

*t : "t" value of *C. reinhardtii* compared to *N. pelliculosa*

t (p=0.05) = 2.92

Table 2

Relative growth constants and mean generation time of _Chlamydomonas reinhardtii_ grown in molybdenum. Cultures were exposed to a light:dark regime of 15:9 hr. Light intensity was 2.61 x 10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

Molybdenum concentration (ppm)	Growth Characteristics k (days)	T (days)	*t (p=0.050
control	0.43	1.59	
0.2	0.48	1.45	2.514
0.4	0.46	1.50	1.327
0.6	0.48	1.44	2.738
0.8	0.48	1.45	2.455
1.0	0.48	1.44	2.738

*t : all "t" values are compared to control.

t (p=0.05) = 2.92

tion times at any concentration of molybdenum and the control. The mean generation times varied from 1.44 to 1.50 days.

The division rates and relative growth constants of N. pelliculosa grown in concentrations of molybdenum up to 1.0 ppm are given in Table 3. C. reinhardtii showed no significant difference between the mean generation times at all concentrations of molybdenum and the control. The mean generation times varied from 1.34 to 1.43 days.

DISCUSSION

Although molybdenum is required for the growth of green algae, the results presented here indicate that increasing the concentration of molybdenum present in the medium does not have an effect on the growth rate of C. reinhardtii (Table 2). Arnon et al (1955) however, reported that concentrations from 0.1 - 10 ppb stimulated favorable growth responses. He reported 0.1 ppb molybdenum to be the lowest acceptable level of molybdenum to give an adequate growth response, and stated that concentrations above 10 ppb could be inhibitory. Arnon demonstrated that molybdenum was an absolute requirement for cell division. Considering this fact, growth in the control cultures support Arnon's hypothesis that the molybdenum concentration required is very low (Table 2). Data presented here however, does not support Arnon's contention that concentrations above 10 ppb will inhibit growth, indeed concentrations as high as 1.0 ppm did not significantly inhibit growth (Table 2). This apparent discrepancy may be clarified

Table 3

Relative growth constants and mean generation times of <u>Navicula pelliculosa</u> grown in molybdenum. Cultures were exposed to a light:dark regime of 15:9 hr. Light intensity was 2.61 x 10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

Molybdenum concentration (ppm)	Growth Characteristics k (days)	T (days)	*t (p=0.05)
control	0.50	1.37	
0.2	0.52	1.34	0.334
0.4	0.51	1.36	0.286
0.6	0.53	1.30	1.429
0.8	0.49	1.42	1.320
1.0	0.48	1.43	0.768

*t : all "t" values are compared to control.

t (p=0.05) = 2.92

upon close examination of Arnon's data. Growth rates of Scenedesmus at 0.1, 1.0 and 10 ppb have almost identical mean generation times (ranging from 1.29 to 1.32 days respectively). Growth appeared to be slightly slower at 10 ppb, but one might question whether there is a significant difference in the growth response at the concentrations used. Arnon did not present any statistical analysis of his data. Goldman (1960) reported that the addition of 100 ppb of molybdenum to Castle Lake stimulated growth. These data support the results presented here that high concentrations of molybdenum do not inhibit the growth rates of green algae.

The fact that abnormally high concentrations of molybdenum do not affect the growth rate of C. reinhardtii is interesting (Table 2). Two different hypotheses could explain the observations.

1. As the concentration of molybdenum in the environment increases, the amount of molybdenum entering the cell increases until the division threshold is obtained. This threshold may be 0.1 ppm or 6.3×10^7 atoms per cell (Arnon et al 1955). As external concentrations increase beyond this threshold level, molybdenum entering the cell may be sequestered. The storage of molybdenum by the bacterium Nitrobacter has been reported by Finstein and Delwiche (1965). They reported that Nitrobacter could concentrate molybdenum 1 million fold from the medium. The sequestration of molybdenum would explain the lack of response to increased external concentrations.

2. Molybdenum uptake may involve a transport mechanism in the cell membrane. If this were the case, then saturation kinetics could be occurring close to 0.1 ppb molybdenum (Arnon et al 1955). This

hypothesis could also explain why no inhibition of growth appeared at high concentrations (Table 2).

The data presented in this study is not able to contribute any insight as to mechanisms. This problem will be examined in later chapters.

The effect of molybdenum on growth of diatoms has not been reported in the literature. The data presented in Table 3 indicate that there is no significant effect of molybdenum on the growth of N. pelliculosa in concentrations ranging from 0.2 to 1.0 ppm. The fact that molybdenum concentrations up to 1.0 ppm do not affect the growth rates in N. pelliculosa may be explained by the same argument used for C. reinhardtii. The question of molybdenum utilisation will be examined in Chapters 2 and 3.

The results obtained in this investigation are considerably different from the response of algae to copper reported by Steemann-Neilson and Wium-Andersen (1970). He reported that a copper concentration of 5 ppb inhibited growth in Chlorella, however, copper toxicity appears to be a function of cell density.

The mean generation times for N. pelliculosa and C. reinhardtii were 1.37 and 1.59 days respectively (Figure 1). These growth rates compare favorably with those reported for other algae (Fogg 1965). The difference in growth rates is not surprising since N. pelliculosa is smaller than C. reinhardtii (6-11 μ for N. pelliculosa and 18-25 μ for C. reinhardtii). Fogg (1965) states that there is a correlation between relative growth constants and the surface to volume ratio.

The greater the surface to volume ratio, the greater the rate of entry of materials important for growth and division into the cell.

On the basis of this study it appears that increasing molybdenum concentrations in the natural environment via industrial discharge may not affect the growth rates of indigenous algae. What the effects of long term exposure to high molybdenum concentrations will be is still questionable.

Chapter 2

EFFECT OF MOLYBDENUM ON PHOTOSYNTHETIC RATES IN TWO SPECIES OF FRESHWATER ALGAE

INTRODUCTION

It has been stated in Chapter 1 that molybdenum is related to nitrate metabolism. Several workers have examined nitrogen deficiency in relation to photosynthesis (Dugdale & Goering 1967; Thomas 1970; Schindler 1971; Sakamoto 1971) however, evidence has been reported that molybdenum may also be involved in photosynthesis (Arnon et al. 1955).

Arnon reported that molybdenum deficient Scenedesmus had a low chlorophyll content. He stated that the addition of 13 ppb molybdenum to these cells increased the chlorophyll content of the cells. This evidence indicates that molybdenum may have a significant effect on photosynthesis.

Goldman (1960) was the first to report that molybdenum additions could increase photosynthetic rates in an aquatic ecosystem. He noted that a supplement of 0.1 ppm of molybdenum significantly increased the rate of photosynthesis in Castle Lake. Since then it has been reported that molybdenum stimulated photosynthetic rates in other lakes (Goldman 1972; Allen 1972).

For the most part however, the amount of available information concerning the effects of molybdenum on photosynthesis is limited. The effect of molybdenum on photosynthetic rates of diatoms has not been examined. Also, the photosynthetic response of algae to high concentrations of molybdenum has not been examined.

The purpose of this chapter is to examine the effects of high concentrations of molybdenum on the photosynthetic rates of

two species of freshwater algae.

MATERIALS AND METHODS

Axenic cultures of two species of freshwater algae were used in this study; the green alga Chlamydomonas reinhardtii, and the diatom Navicula pelliculosa.

Media Used

Stock cultures of C. reinhardtii and N. pelliculosa were grown using using modified Hughes medium #1. The method of culture was the same as described in chapter 1.

Cultures for photosynthetic studies were grown on modified Hughes medium #2. The cultures were incubated at 20±1 C with continuous shaking. The cultures were incubated under a light:dark regime of 15:9 hr. A light intensity of 2.61 x 10^4 ergs cm^{-2} sec^{-1} was obtained using a bank of 6, G.E. cool white flourescent bulbs.

Measurements of Photosynthetic Rates

To study the effect of increasing concentrations of molybdenum on photosynthetic rates of C. reinhardtii, two 1.5 l cultures of the alga were grown in 2.8 l "low form" fernback flasks. Inocula for both cultures were taken from the stock cultures in their exponential phase of growth. The volume of the inocula were adjusted to give

a final cell concentration of 3-10 x 10^4 cells ml^{-1}. A typical experiment was as follows. To examine the effect of molybdenum starvation on photosynthetic rates, one culture was incubated for 48 hr without a molybdenum supplement. Molybdenum was removed from the inoculum by three successive aseptic centrifugations at 2300 rpm for 2-3 min on a model HN-S Centrifuge (International Equiptment Co.) and rinsed with sterile modified Hughes medium #2. The other culture was also incubated for 48 hr but with a molybdenum supplement of 0.4 ppm. At the end of the 48 hr growth period molybdenum was removed from this culture, as described above, and the pellet was transferred to 1.5 l of modified Hughes medium #2.

Six 180 ml aliquots were taken from each of the two cultures. Five were supplemented with 0.2, 0.4, 0.6, 0.8 and 1.0 ppm molybdenum respectively. One was used as a control and was not supplemented with molybdenum. Nine, 10 ml aliquots at each molybdenum concentration were placed in test tubes (6 light, 3 dark) and 0.5 μCi of $Na^{14}CO_3$ were added to each test tube. At 1, 3 and 6 hr intervals, two light and one dark sample of each molybdenum concentration were removed. Each sample was filtered through HA millipore filters (47 mm, 0.8 μ pore size) at 254 mm of Hg. The filters were placed into scintillation vials containing 15 ml TEG scintillation cocktail described by Wallen and Geen (1968). All experiments were performed in duplicate.

Radioactivity was determined using a Nuclear Chicago Mark II liquid scintillation counter. A counting time of 1-2 min was

sufficient to attain the minimum of 10,000 cpm. Efficiencies were determined using the channels ratio method of Peng (1966) and a standard quench curve.

Inorganic CO_2 concentrations were measured according to the method of Saunders et al (1962). A mixed indicator prepared by mixing methyl orange and brom cresol green in the ratio of 2:1 was used in place of the methyl orange indicator outlined in the method.

Cell concentration was measured using an A/O fine line haemocytometer. In each case the average of 12 counts was taken and the value obtained extrapolated to give cell concentration as cells $liter^{-1}$.

Photosynthetic rates were measured as mg C 10^6 $cells^{-1}$ hr^{-1} and were calculated using the following formula:

$$\text{mg C } 10^6 \text{ cells}^{-1} \text{ hr}^{-1} = \frac{{}^{14}C_L - {}^{14}C_D}{{}^{14}C_T} \times \frac{1.06 \times CO_2}{t \times \frac{N}{10^6}}$$

where:

${}^{14}C_L$ = Average dpm of both light tubes

${}^{14}C_D$ = dpm of dark tube

${}^{14}C_T$ = Amount of added activity as dpm

1.06 = conversion factor of ${}^{14}C$ to ${}^{12}C$

CO_2 = concentration of CO_2 as mg $liter^{-1}$

t = time in hr.

N = cell number expressed as cells $liter^{-1}$

The method used to study photosynthetic rates in N. pelliculosa was the same as for C. reinhardtii.

RESULTS

Effect of Molybdenum on Photosynthetic Rates in *Chlamydomonas reinhardtii*

Carbon uptake expressed as mg C 10^6 cells^{-1} in the controls of both non-molybdenum starved and molybdenum starved cells are presented in Figure 2. No difference occurred in carbon uptake between starved and non-starved cells. Carbon uptake was linear with time. The rate of photosynthesis expressed as mg C 10^6 cells^{-1} hr^{-1} corresponds to the slope of the linear regression line. Photosynthetic rates for non-molybdenum starved and moybdenum starved cells at increasing molybdenum concentrations are given in Table 4. There was no significant difference between the controls of starved and non-starved cells or between molybdenum concentrations from 0.2 to 0.8 ppm. The photosynthetic rate at 1.0 ppm molybdenum in starved cells showed an apparent significant difference. However, it is felt that this particular observation is doubtful based on the time curve and is probably an artifact of the experimental procedure.

The effect of increasing molybdenum concentrations on photosynthetic rates of non-molybdenum starved cells are presented in Table 5. No significant difference in photosynthetic rates compared to the controls, occurred at molybdenum concentrations tested except 0.8 ppm. Since neither 0.6 ppm nor 1.0 ppm molybdenum altered the photosynthetic rate, this difference is doubtful and probably an artifact of experimental procedure.

The effect of increasing molybdenum concentrations on photo-

Figure 2 Photosynthetic rates in molybdenum starved (o) and non-molybdenum starved (•) cells of *Chlamydomonas reinhardtii*. Molybdenum starved cells were incubated 48 hr. without a molybdenum supplement. Non-molybdenum starved cells were incubated 48 hr. with a molybdenum supplement of 0.4 ppm. Light intensity, 2.61×10^4 ergs cm^{-2} sec^{-1}. Temperature 20±1 C.

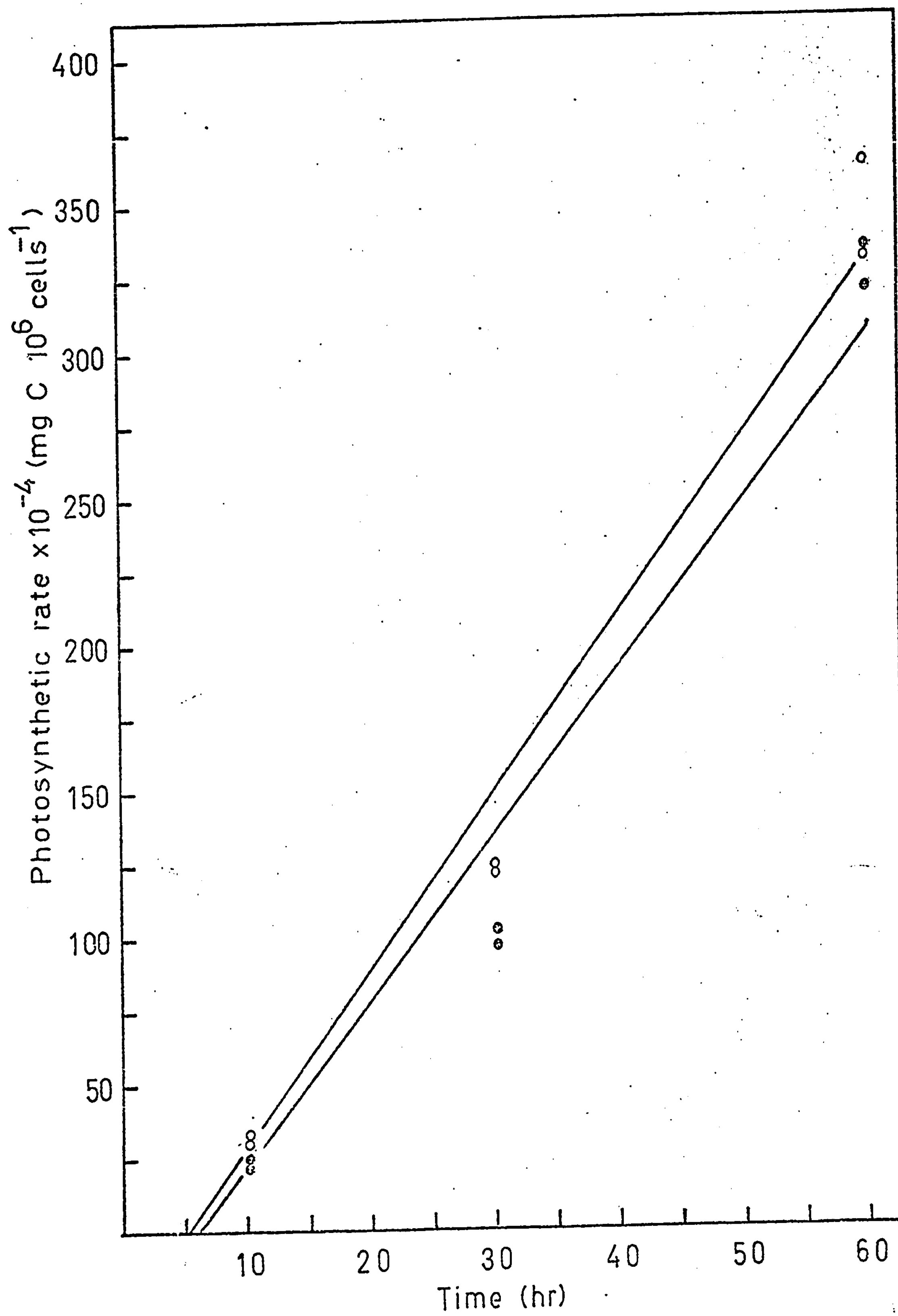

400
350
300
250
200
150
100
50
Photosynthetic rate x10^-4 (mg C 10^6 cells^-1)
10
20
30
40
50
60
Time (hr)

Table 4

Photosynthetic rate in Chlamydomonas reinhardtii. Molybdenum starved cells were incubated 48 hr.without a molybdenum supplement. Non-molybdenum starved cells were incubated 48 hr.with a molybdenum supplement of 0.4 ppm. Cultures were exposed to a light:dark regime of 15:9 hr. Light intensity was 2.61 x 10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

Molybdenum concentration (ppm)	Photosynthetic rate (mg C 10^6 $cells^{-1}$ hr^{-1})		
	non starved cells	starved cells	F (p = 0.05)*
control	0.0056	0.0064	1.1691
0.2	0.0053	0.0070	4.0690
0.4	0.0047	0.0050	0.3195
0.6	0.0057	0.0060	0.1901
0.8	0.0036	0.0035	0.1246[a]
1.0	0.0052	0.0040	5.1900

*F : All F values are starved cells compared to non-starved cells.

F (p=0.05) = 4.96

[a]F (p=0.05) = 5.12

Table 5

Photosynthetic rate in non-molybdenum starved cells of *Chlamydomonas reinhardtii*. Non-molybdenum starved cells were incubated for 48 hr. with a molybdenum supplement of 0.4 ppm. Cultures were exposed to a light:dark regime of 15:9 hr. Light intensity was 2.61 x 10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

Molybdenum concentration (ppm)	Photosynthetic rate (mg C 10^6 $cells^{-1}$ hr^{-1})	
	Rate	F (p=0.05)*
control	0.0056	
0.2	0.0053	0.0996
0.4	0.0047	1.1800
0.6	0.0057	0.0139
0.8	0.0036	8.0497
1.0	0.0052	0.0423

*F: All F values are compared to control.

F (p=0.05) = 4.96

sythetic rates of molybdenum starved cells are given in Table 6. Photosynthetic rates at 0.4 ppm and 1.0 ppm were significantly different from the controls, while all other concentrations showed no significant difference. The differences at 0.4 and 1.0 ppm molybdenum are felt to be artifacts for the same reasons given above.

Effect of Molybdenum on Photosynthetic Rates of Navicula pelliculosa

Carbon uptake in the controls over six hours in N. pelliculosa for both non-molybdenum starved and molybdenum starved cells are presented in Figure 3. The rate of photosynthesis as mg C 10^6 $cells^{-1}$ hr^{-1} was calculated as the slope of the carbon uptake curve. The photosynthetic rate in starved cells is significantly lower than for the non-molybdenum starved cells, 0.0026 and 0.0045 mg C 10^6 $cells^{-1}$ hr^{-1} respectively. Photosynthetic rates for both starved and non-starved cells at increasing molybdenum concentrations are given in Table 7. Photosynthetic rates in starved cells at all concentrations of molybdenum are significantly lower than for non-starved cells.

Photosynthetic rates at increasing molybdenum concentrations compared to the control in non-molybdenum starved cells are given in Table 8. It is demonstrated from these data that molybdenum concentrations as high as 1.0 ppm neither increase nor decrease photosynthesis in non-molybdenum starved cells.

Photosynthetic rates in molybdenum starved cells are presented in Table 9. As for the non-molybdenum starved cells, no significant difference was observed in the photosynthetic rates at any molybdenum

Table 6

Photosynthetic rate in molybdenum starved cells of _Chlamydomonas reinhardtii_. Molybdenum starved cells were incubated for 48 hr. without a molybdenum supplement. Cultures were exposed to a light:dark regime of 15:9 hr. Light intensity was 2.61 x 10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

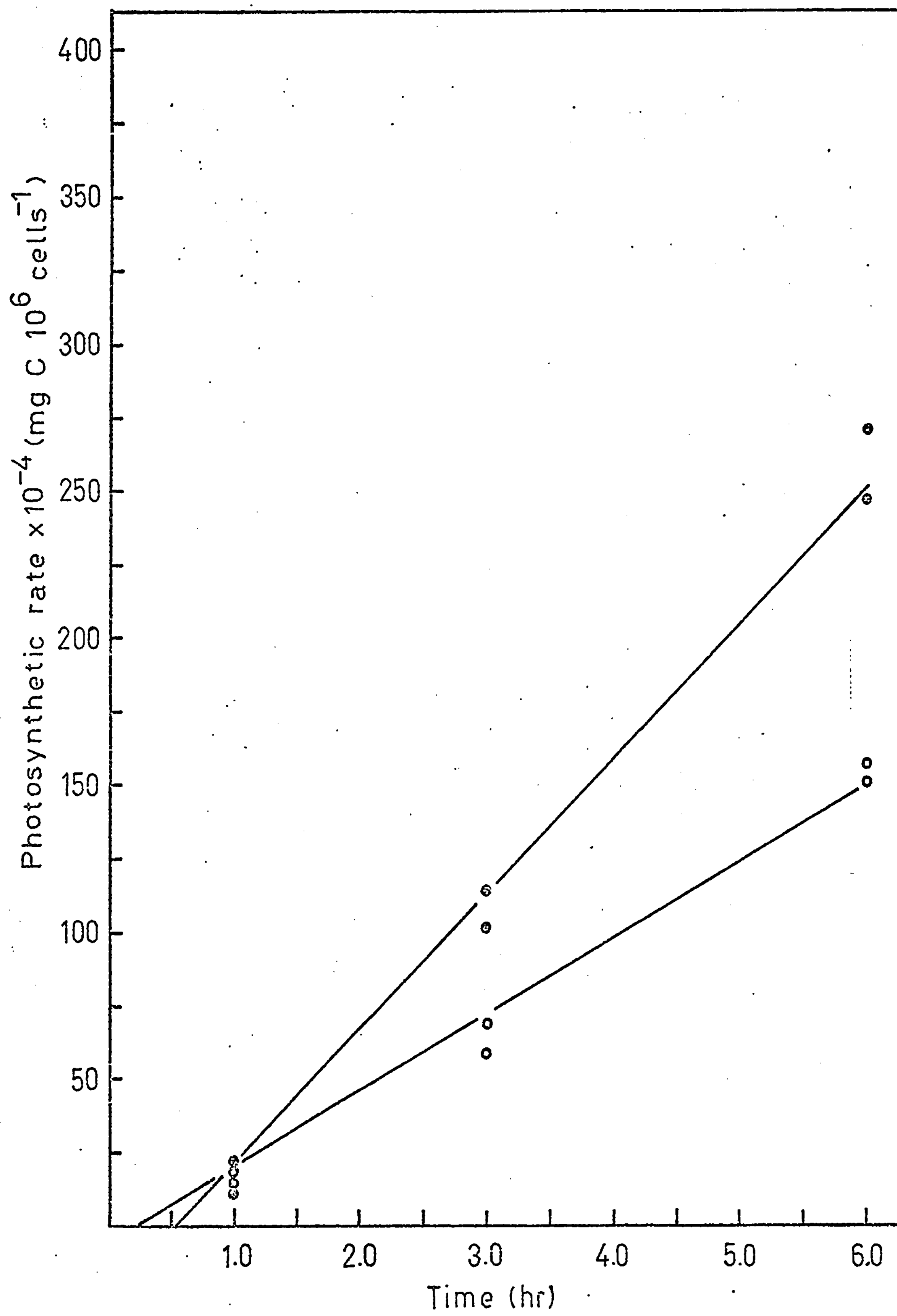
Molybdenum concentration (ppm)	Photosynthetic rate (mg C 10^6 $cells^{-1}$ hr^{-1})	
	Rate	F (p=0.05)*
control	0.0064	
0.2	0.0070	1.0172
0.4	0.0050	9.1362
0.6	0.0060	0.5291
0.8	0.0035	3.0072[a]
1.0	0.0040	39.6463

*F: All F values are compared to control.

F (p=0.05) = 4.96

[a]F (p=0.05) = 5.12

Figure 3 Photosynthetic rates in molybdenum starved (o) and non-molybdenum starved (●) cells of *Navicula pelliculosa*. Molybdenum starved cells were incubated 48 hr. without a molybdenum supplement. Non-molybdenum starved cells were incubated 48 hr. with a molybdenum supplement of 0.4 ppm. Light intensity, 2.61 x 10^4 ergs cm^{-2} sec^{-1}. Temperature 20±1 C.



Photosynthetic rate x 10^{-4} (mg C 10^6 cells^{-1})
400
350
300
250
200
150
100
50
1.0
2.0
3.0
4.0
5.0
6.0
Time (hr)

Table 7

Photosynthetic rate in Navicula pelliculosa. Molybdenum starved cells were incubated 48 hr. without a molybdenum supplement. Non-molybdenum starved cells were incubated 48 hr. with a molybdenum supplement of 0.4 ppm. Cultures were exposed to a light:dark regime of 15:9 hr. Light intensity was 2.61×10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

Molybdenum concentration (ppm)	Photosynthetic rate (mg C 10^6 $cells^{-1}$ hr^{-1})		
	non-starved cells	starved cells	F (p=0.05)*
control	0.0045	0.0026	43.4189
0.2	0.0045	0.0028	64.2362
0.4	0.0046	0.0025	109.045
0.6	0.0040	0.0024	36.970
0.8	0.0043	0.0024	23.194
1.0	0.0040	0.0023	105.667[a]

*F: All F values are starved cells compared to non-starved cells.

F (p=0.05) = 4.96

[a]F (p=0.05) = 5.32

Table 8

Photosynthetic rate in non-molybdenum starved cells of *Navicula pelliculosa*. Non-molybdenum starved cells were incubated for 48 hr. with a molybdenum supplement of 0.4 ppm. Cultures were exposed to a light:dark regime of 15:9 hr. Light intensity was 2.61×10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

Molybdenum concentration (ppm)	Photosynthetic rate (mg C 10^6 $cells^{-1}$ hr^{-1})	
	Rate	F (p=0.05)*
control	0.0045	
0.2	0.0045	0
0.4	0.0046	0.1060
0.6	0.0040	2.3760
0.8	0.0043	2.0077
1.0	0.0040	2.5012[a]

*F: All F values are compared to control.

F (p=0.05) = 4.96

[a]F (p=0.05) = 5.32

Table 9

Photosynthetic rate in molybdenum starved cells of Navicula pelliculosa. Molybdenum starved cells were incubated for 48 hr. without a molybdenum supplement. Cultures were exposed to a light:dark regime of 15:9 hr. Light intensity was 2.61×10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

Molybdenum concentration (ppm)	Photosynthetic rate (mg C $10^6 cells^{-1}$ hr^{-1})	
	Rate	F (p=0.05)*
control	0.0026	
0.2	0.0028	0.7730
0.4	0.0025	0.3422
0.6	0.0024	0.9379
0.8	0.0024	1.0112
1.0	0.0023	2.9276

*F: All F values are compared to control.

F (p=0.05) = 4.96

concentration tested.

Comparison of Photosynthetic Rates in C. reinhardtii and N. pelliculosa

Comparison of photosynthesis in C. reinhardtii and N. pelliculosa is given in Table 10. Although the rate of photosynthesis in N. pelliculosa (0.0045 mg C 10^6 $cells^{-1}$ hr^{-1}) is lower than C. reinhardtii (0.0056 mg C 10^6 $cells^{-1}$ hr^{-1}) in non-molybdenum starved cells, it is not a significant difference. Photosynthetic rates in molybdenum starved N. pelliculosa (0.0026 mg C 10^6 $cells^{-1}$ hr^{-1}) are significantly lower than C. reinhardtii (0.0064 mg C 10^6 $cells^{-1}$ hr^{-1}). These data demonstrate that the green alga fixes more carbon per unit time than the diatom in a molybdenum deficient environment.

DISCUSSION

From the data presented in Table 4, it is clear that molybdenum does not affect the rate of photosynthesis in Chlamydomonas reinhardtii. No decrease in the rates of photosynthesis were observed in cells starved of molybdenum for 48 hr before photosynthesis was measured compared to cells grown in a medium containing molybdenum. In addition, the data indicate that molybdenum in varying concentrations up to 1.0 ppm does not affect the rate of photosynthesis in cells starved for 48 hr before the initiation of a photosynthesis experiment nor in cells which were not deprived of molybdenum (Tables 5 and 6).

Two possibilities could explain these observations. Either molybdenum is not involved in the photosynthetic pathways or the

Table 10

Photosynthetic rates in control cultures of both *Chlamydomonas reinhardtii* and *Navicula pelliculosa*. Molybdenum starved cells were incubated 48 hr. without a molybdenum supplement. Non-molybdenum starved cells were incubated 48 hr. with a molybdenum supplement of 0.4 ppm. Cultures were exposed to a light:dark regime of 15:9 hr. Light intensity was 2.61×10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

	Photosynthetic rate (mg C 10^6 $cells^{-1}$ hr^{-1})			
	non-starved cells	F^* (p=0.05)	starved cells	F^* (p=0.05)
N. pelliculosa	0.0045	2.3405	0.0026	127.1309
C. reinhartii	0.0056		0.0064	

*F: F values compared to *C. reinhardtii*

F (p=0.05) = 4.96

concentration of molybdenum required for photosynthesis is very low and the cells are able to store sufficient molybdenum to satisfy the requirement over a period of 48 hr. As was suggested in Chapter 1, on growth, traces of molybdenum impurities may be present in the medium to which no molybdenum was added. However, a 48 hr starvation period, the time required for two cell divisions would essentially remove any molybdenum impurities.

Photosynthesis in algae has been examined by Gibbs et al (1970). Gibbs does not report any molybdenum involvement in the enzyme systems of the carbon reduction cycle. Arnon et al (1955) have suggested that molybdenum may be involved in chlorophyll synthesis in the green alga Scenedesmus. If molybdenum affects chlorophyll synthesis, cells of C. reinhardtii starved of molybdenum for periods longer than 48 hr may show a reduction in the photosynthetic rate, due to reduced chlorophyll content. The cell may be able to utilize sequestered molybdenum to support chlorophyll synthesis over the 48 hr starvation period.

This study is unable to determine the role of molybdenum in photosynthesis of C. reinhardtii, if indeed, molybdenum is involved at all. Most important, however, for purposes of this study, abnormally high concentrations of molybdenum do not inhibit photosynthesis in C. reinhardtii.

All cultures of Navicula pelliculosa starved of molybdenum for 48 hr have a lower photosynthetic rate than non-molybdenum starved cells (Table 7). These data indicate that molybdenum may play a role in photosynthesis in diatoms. It is possible that

molybdenum is not involved directly in the photosynthetic reactions but rather in some other metabolic pathway associated with photosynthesis. As has been discussed above, molybdenum is thought to be involved in chlorophyll synthesis. Bové and Bové (unpublished data quoted by Arnon 1958) observed a 50% reduction in photosynthesis on a per unit chlorophyll basis in Nostoc cultures deprived of molybdenum. Furthermore, they observed that insufficient molybdenum resulted in an increase in sugar accompanied by a decrease of glutamine and amino acids, particularly aspartate and glutamic acid. Dugdale and Goering (1967) have demonstrated a correlation between carbon uptake and nitrogen assimilation in shipboard cultures of marine phytoplankton. Eppley et al (1971 a, b) and McCarthy (1972) have demonstrated diel periodicity in nitrogen assimilation in marine diatoms. The accumulation of sugar and decrease in total CO_2 fixation in Nostoc and the reduction in photosynthetic rates in N. pelliculosa may be a result of impaired nitrate assimilation when molybdenum in the culture medium was low. The early precursors in chlorophyll synthesis are firmly established (Lascalles 1965). Glycine is a precursor of δ amino levulinic acid, a precursor of protoporphyrin. Inhibition of nitrate assimilation may result in a decrease in the glycine pool available for chlorophyll synthesis and hence result in a decrease in chlorophyll.

Varying concentrations of molybdenum up to 1.0 ppm in either non-molybdenum starved or molybdenum starved cells do not alter the rate of photosynthesis (Tables 8 and 9). The photosynthetic rate in non-molybdenum starved cells suggest that once the threshold

concentration is satisfied the cell carries out photosynthesis at an optimum rate. This rate is unaffected by molybdenum concentrations as high as 1.0 ppm. Incubation periods longer than 6 hr would be required before the starved cells may achieve optimum photosynthetic rates.

The mode of action of molybdenum on photosynthesis in *N. pelliculosa* is very complex, and further study is required to pinpoint its precise function in carbon uptake. The possibility exists that molybdenum may be involved in an associated metabolic process such as chlorophyll synthesis or nitrogen assimilation.

In a molybdenum deficient environment, dominated by diatom populations, the rate of photosynthesis may be reduced, resulting in lower productivity. This affect would probably reflect the impairment of the diatoms' metabolism by molybdenum deficiency. The addition of molybdenum to this environment may stimulate photosynthesis but not significantly increase the division rate of the diatoms (chapter 1). This may have potential applications in the operation of fish hatcheries and fish stocking programs of various government agencies. By supplementing molybdenum in concentrations up to 0.1 ppm, the primary productivity of the hatchery or lake might be increased without increasing the phytoplankton standing crop. The higher productivity could increase fish yields in hatcheries and allow government agencies to increase the productivity of unproductive lakes without causing algal blooms, thus providing more lakes adequate for fish stock.

CHAPTER 3

THE HALF SATURATION CONSTANTS FOR NO_3 IN TWO SPECIES OF FRESHWATER ALGAE AND THE EFFECTS OF HIGH CONCENTRATIONS OF MOLYBDENUM ON ASSIMILATORY RATES

INTRODUCTION

The importance of nitrate nitrogen in the ecology of algae has been discussed by several ecologists (Hutchinson 1957, Ruttner 1960, Keeney 1972). It is through the process of nitrate assimilation that nitrogen is incorporated into algal protoplasm. The utilization of nitrate via nitrate assimilation has been reviewed by Syrett (1962). The first step in the assimilatory mechanism is the reduction of nitrate to nitrite. This is accomplished by the enzyme nitrate reductase.

The ability of an alga to assimilate ambient nitrate nitrogen will determine its success in competing with other algae for nitrogen. The rate at which nitrate is assimilated is dependent on the enzyme kinetics of nitrate reductase. The half saturation constant is the measurement of the affinity of the enzyme for the substrate. By comparing the K_m of different groups of algae, one should be able to asses how they will relate to different concentrations of nitrate (Eppley and Thomas 1969, Eppley _et al_ 1969).

Although Eppley _et al_ (1969) have examined the half saturation constants for various marine phytoplankters, no data have been published which compares half saturation constants for fresh water algae.

Nitrate reductase is now known to contain molybdenum. Furthermore, molybdenum is the reducing agent responsible for the reduction process (Aparicio _et al_ 1971, Vega _et al_ 1971). Considering this

fact, the ambient molybdenum concentration could have an effect on the rate of nitrate uptake by algae. Arnon et al (1955) reported that the addition of 1 ppb of molybdenum significantly increased the rate of nitrate assimilation in molybdenum deficient cells. No data are available, however, concerning the effects of high concentrations of molybdenum on nitrate assimilation.

The purpose of this chapter is twofold. First, to determine how two different groups of freshwater algae respond to different nitrate concentrations by comparing their half saturation constants. Second, to examine the effects of high concentrations of molybdenum on the uptake rates of nitrate in these two species of algae.

MATERIALS AND METHODS

Axenic cultures of the green alga *Chlamydomonas reinhardtii* and the diatom *Navicula pelliculosa* were used in this investigation.

Media Used

Stock cultures of *C. reinhardtii* and *N. pelliculosa* were grown using modified Hughes medium #1. The method of culture was the same as described in chapter 1.

Experimental cultures were grown on modified Hughs medium #2. The cultures were incubated at 20±1 C with continuous shaking. The cultures were incubated under a light:dark regime of 15:9 hr. A light intensity of 2.61×10^4 ergs cm^{-2} sec^{-1} was obtained using a bank of 6, G.E. cool white fluorescent bulbs.

Determination of Half Saturation Constants

To determine the half saturation constants for *C. reinhardtii* and *N. pelliculosa* nitrogen free batch cultures of these species were prepared in the following way. The inocula for the cultures were taken from the stock cultures in their exponential phase of growth. Their volumes were adjusted to give a final cell concentration of 6-12 x 10^4 cells ml^{-1}. Molybdenum was removed from the inocula by successive centrifugations and rinsings in nitrogen free medium. Cells were incubated in the nitrogen free medium for 48 hr. At the end of the 48 hr incubation period, 1 μM of NO_3 as $NaNO_3$ was added to the cultures and they were incubated for another 5 hr. This preincubation was done to compensate for the problems outlined by Eppley and Thomas (1969). Immediatly following the 5 hr preincubation period, six, 100 ml aliquots were removed from the batch cultures and placed into 125 ml erlenmeyer flasks. The six flasks of *C. reinhardtii* were supplemented with 8, 20, 50, 75, 100 and 150 μM of nitrate respectively. The six flasks of *N. pelliculosa* were supplemented with 1.5, 8, 10, 12, 14 and 18 μM of nitrate respectively. A 100 ml aliquot was also taken from each batch culture to determine the concentration of nitrate remaining after the 5 hr preincubation period. Concentrations of nitrate in the experimental flasks were corrected to include this value. All flasks were incubated for 1 hr with continuous shaking. Following the 1 hr incubation period the samples were filtered through HA millipore filters (47 mm, 0.8 μ pore) at 254 mm Hg. The filtrate was

refridgerated at 4 C and assayed for nitrate within 3-12 hr. Nitrate in the samples was reduced to nitrite by passing the samples through a column containing copper coated cadmium filings and analysed using the method of Wood et al (1967). All values reported have been corrected for any discrepancy occurring between the columns.

Cell concentration was measured using an A/O fine line haemocytometer. In each case the average of 12 counts was taken and the value obtained extrapolated to give cell concentration as cells . liter^{-1}.

Nitrate uptake rates were taken as the amount of nitrate removed from the medium 10^6 cells^{-1} hr^{-1}. The rate of uptake (v) was determined using the following formula:

$$v = \frac{V_m S}{K_m + S}$$

where: v = rate of uptake (µM NO_3 10^6 cells^{-1} hr^{-1})

V_m = maximum rate

S = nitrate concentration (µM)

K_m = Michaelis-Menten half saturation constant.

The half saturation constant for N. pelliculosa was determined using the Lineweaver-Burke plot (1/v vs 1/S). The K_m value for C. reinhardtii was calculated using a Wolfe plot (S/v vs S).

Molybdenum Effects on Nitrate Kinetics

To study the effects of molybdenum on the nitrate assimilation mechanism of *C. reinhardtii*, two batch cultures were prepared. One culture did not contain a molybdenum supplement. The preparation of the cultures was the same as that used in the photosynthesis experiments in chapter 2. In addition, nitrogen was removed from the batch cultures as described above. Following the 48 hr incubation period, 1 µM of NO_3 was added to the cultures and they were pre-incubated for 5 hr. Molybdenum was removed from non-molybdenum starved cells by centrifugation and the pellet reinoculated into nitrogen free Hughes medium #2. Three, 400 ml aliquots were taken from each batch culture and placed into 500 ml erlenmeyer flasks. These flasks were supplemented with 0.4 and 0.8 ppm molybdenum respectively. One was used as a control and was not supplemented with molybdenum. Each flask was supplemented with 70 µM of nitrate. A correction for nitrate remaining after preincubation was made. At 0.5, 1.0, 1.5 and 2.0 hr periods, 100 ml aliquots were removed, filtered and frozen at -20 C to preserve samples for analysis at a latter date. *N. pelliculosa* was treated the same as *C. reinhardtii* with the exception that 15 µM NO_3 was added rather than 70 µM. Analysis of nitrate after thawing the samples at room temperature was identical to that outlined for the K_m measurements.

The velocity of uptake was calculated as µM NO_3 removed from the medium 10^6 cells^{-1} hr^{-1}.

RESULTS

Nitrate Kinetics

Nitrate uptake in Chlamydomonas reinhardtii and Navicula pelliculosa exhibit typical Michaelis- Menten kinetics (Figure 4 and 5). Uptake rates are given as µM 10^6 $cells^{-1}$ hr^{-1}, and nitrate concentrations are in µM. Lineweaver-Burke plot and Wolfe plot are shown in Figures 6 and 7 respectively. The K_m for nitrate in C. reinhardtii was considerably higher than for N. pelliculosa; 148.0 and 14.9 µM respectively.

Molybdenum Effects on Nitrate Kinetics

The rate of nitrate uptake in C. reinhardtii and N. pelliculosa decreased with time. The decrease was a non-linear function. Nitrate uptake was maximum after 30 min of incubation, then decreased hyperbolically to a constant rate after 120 min. These hyperbolic functions were transformed into linear functions by plotting the inverse of the velocity of uptake (1/v) versus time (hr). The slope of the curve is a function of the rate of deceleration of uptake kinetics. The graphs of nitrate uptake over a 2 hr period for C. reinhardtii and N. pelliculosa controls are presented in Figures 8A and 8B respectively.

Nitrate uptake rates for C. reinhardtii over a two hour incubation period, in both molybdenum starved and non-molybdenum starved cells are given in Table 11. There was no significant

Figure 4. Michaelis-Menten curve for nitrate uptake in *Chlamydomonas reinhardtii*. Light intensity was 2.61 x 10^4 ergs cm^{-2} sec^{-1}. Temperature was 20±1 C.

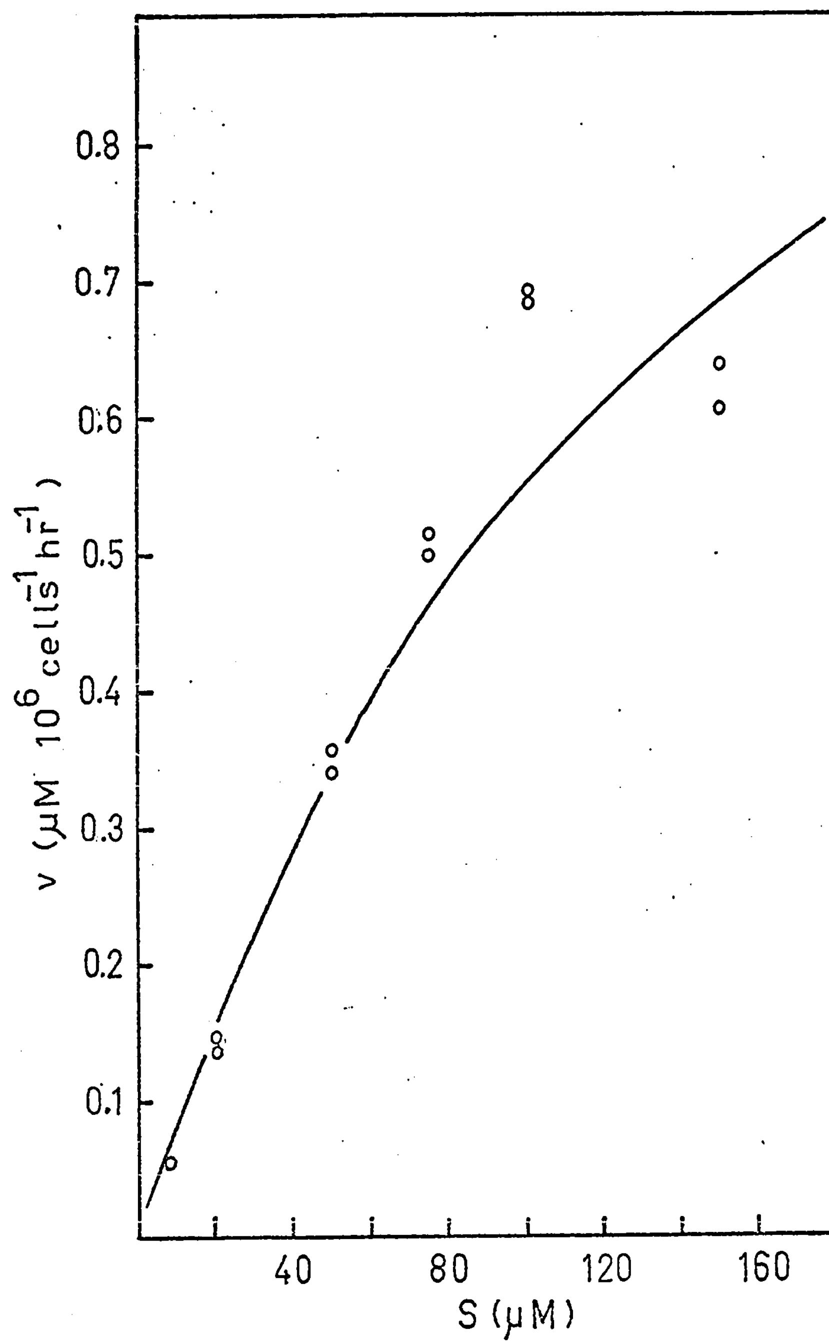

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
v (μM 10⁶ cells⁻¹ hr⁻¹)
40
80
120
160
S (μM)

Figure 5. Michaelis-Menten curve for nitrate uptake in *Navicula pelliculosa*. Light intensity was 2.61×10^4 ergs cm^{-2} sec^{-1}. Temperature was 20±1 C.

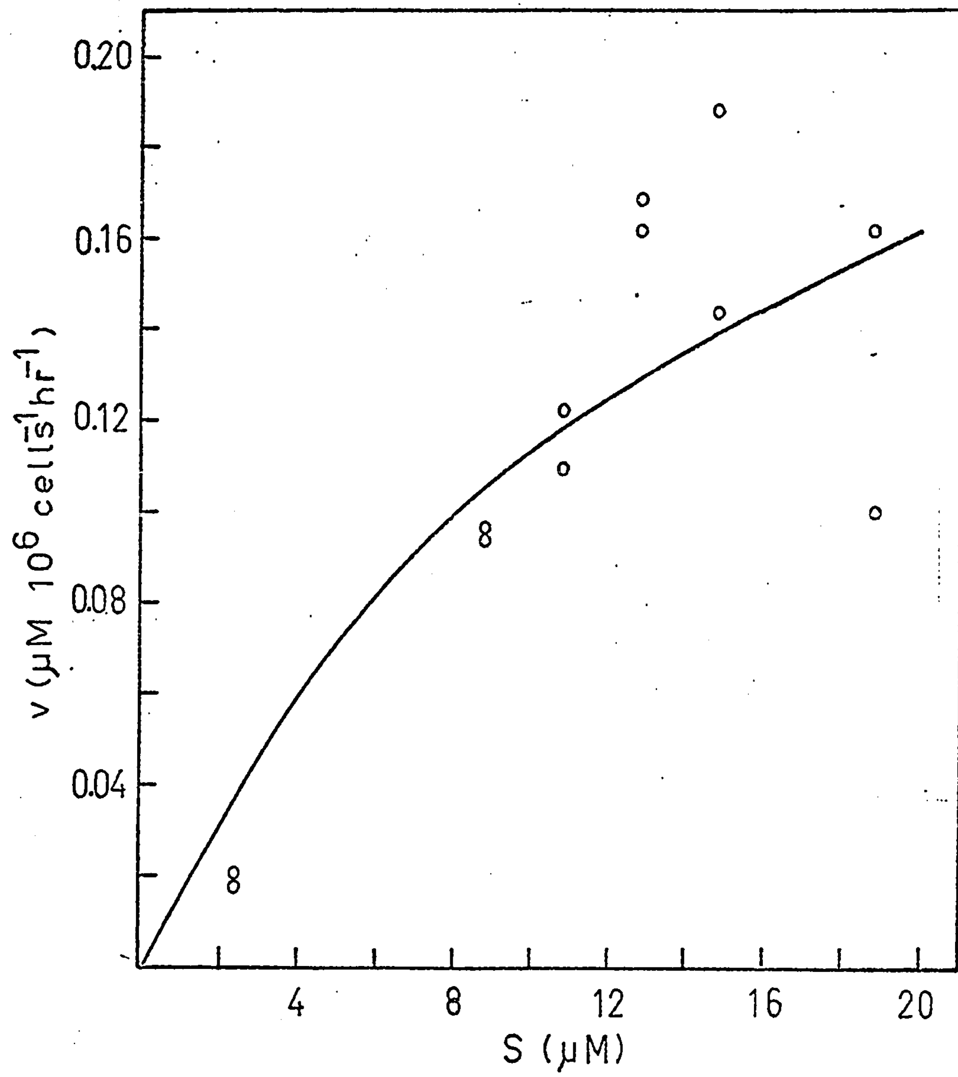

0.20
0.16
0.12
0.08
0.04
v (μM 10^6 cells^-1 hr^-1)
4
8
12
16
20
S (μM)

Figure 6. Wolfe plot for the K_m determination in *Chlamydomonas reinhardtii*. Light intensity was 2.61×10^4 ergs cm^{-2} sec^{-1}. Temperature was 20±1 C.

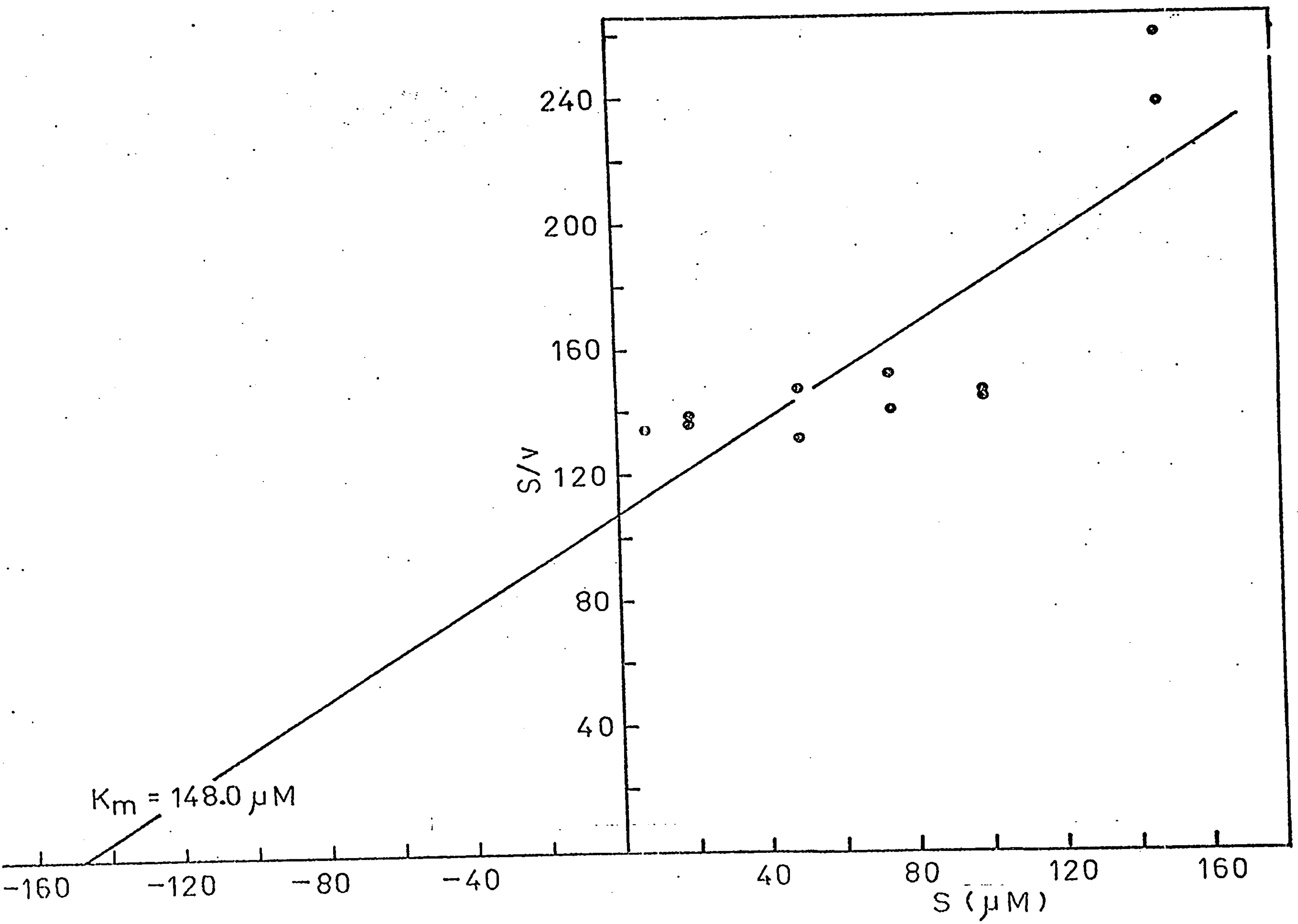
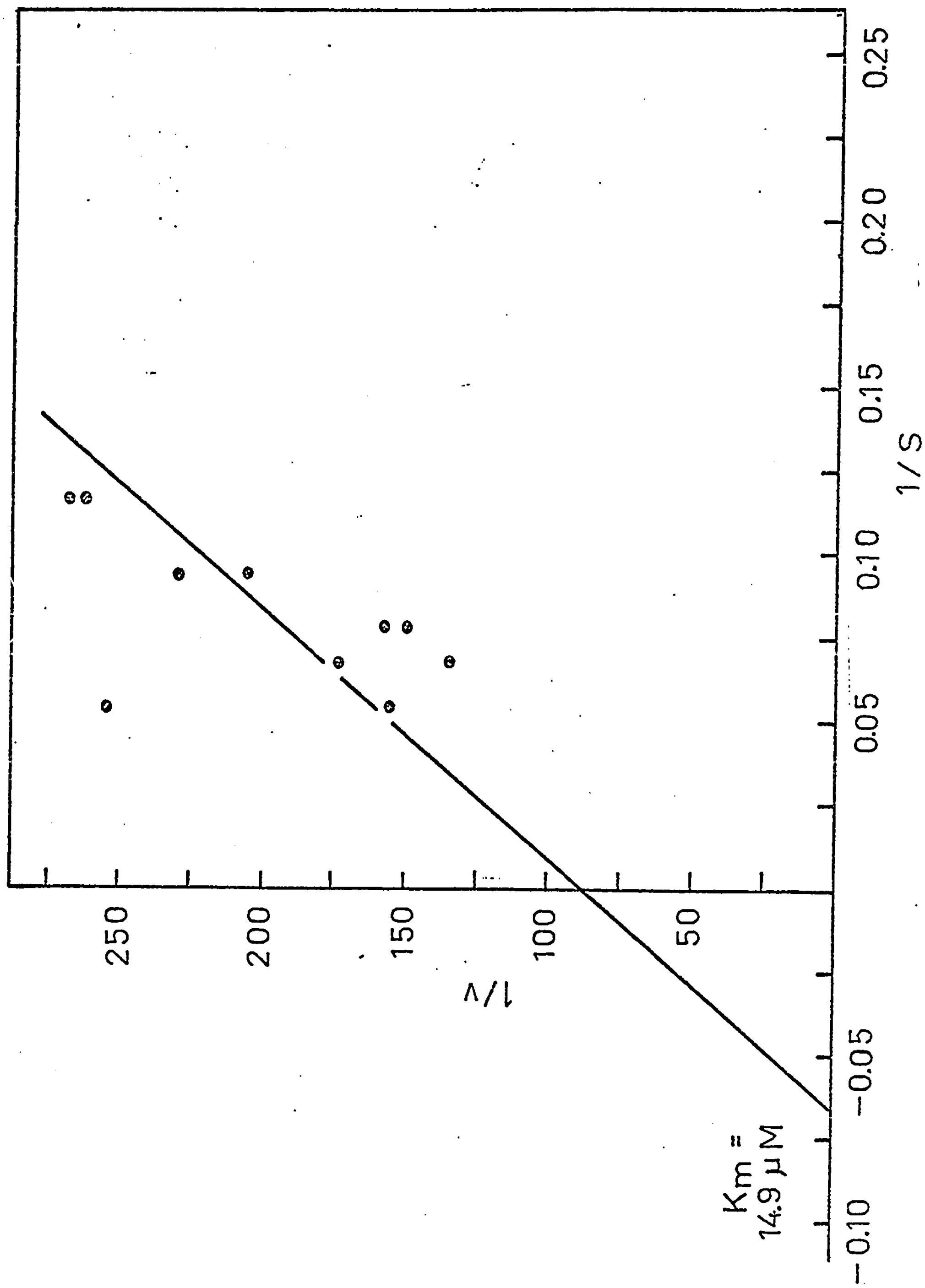

240
200
160
S/v
120
80
40
K_m = 148.0 μM
−160
−120
−80
−40
40
80
120
160
S (μM)

Figure 7. Lineweaver-Burke for the K_m determination in Navicula pelliculosa. Light intensity was 2.61×10^4 ergs cm^{-2} sec^{-1}. Temperature was 20±1 C.



1/v
250
200
150
100
50
1/S
−0.10
−0.05
0.05
0.10
0.15
0.20
0.25
Km =
14.9 µM

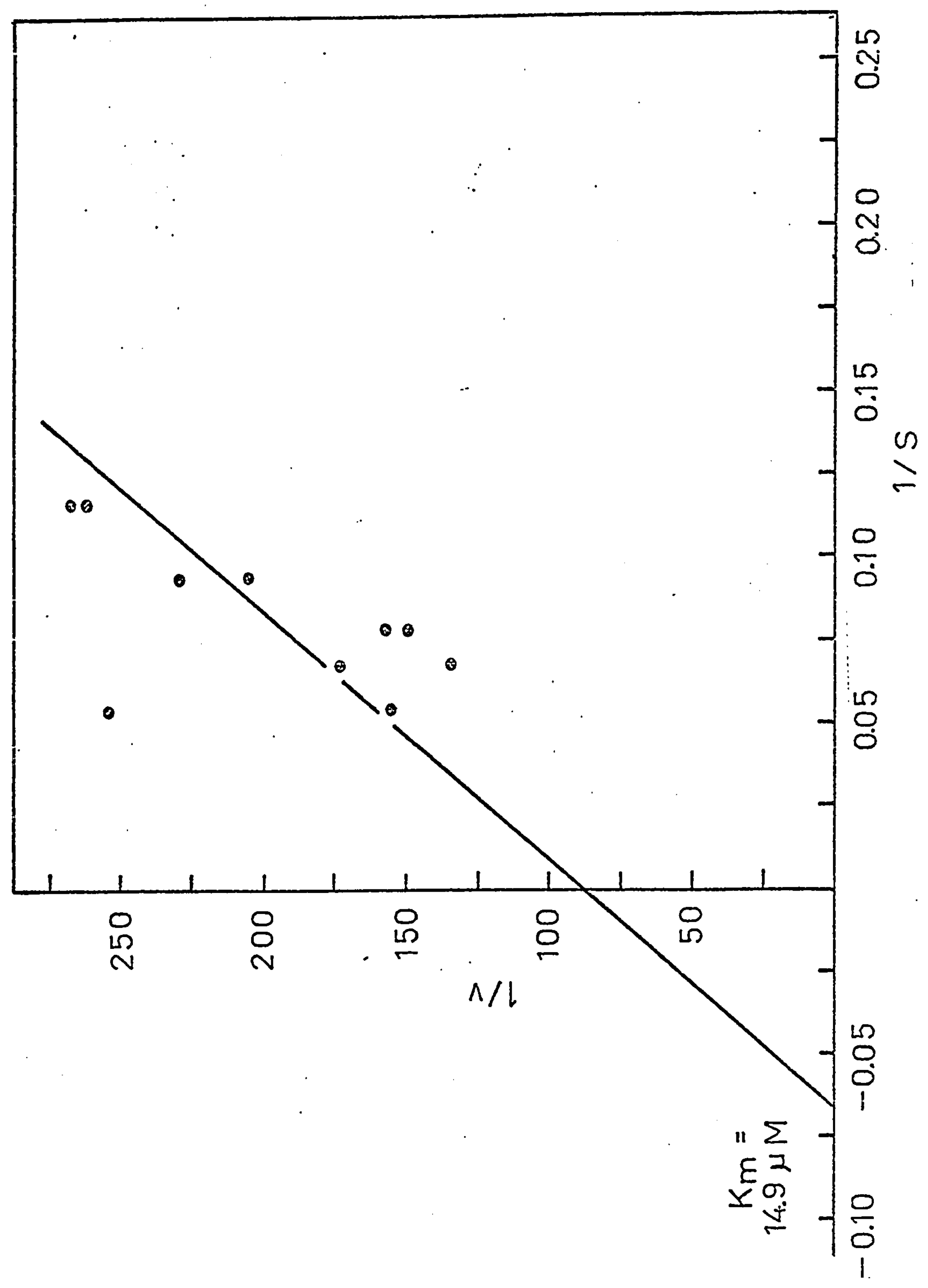

1/V
1/S
250
200
150
100
50
−0.10
−0.05
0.05
0.10
0.15
0.20
0.25
Km =
14.9 μM

Figure 8. A. Deceleration curve for nitrate uptake in molybdenum starved (o) and non-molybdenum starved (●) cells of _Chlamydomonas reinhardtii_. Molybdenum starved cells were incubated 48 hr without a molybdenum supplement. Non-molybdenum starved cells were incubated 48 hr with a molybdenum supplement of 0.4 ppm. Light intensity was 2.61 x 10^4 ergs cm^{-2} sec^{-1}. Temperature was 20±1 C.

B. Deceleration curve for nitrate uptake in molybdenum starved (o) and non-molybdenum starved (●) cells of _Navicula pelliculosa_. Culture conditions were the same as for _C. reinhardtii_.

Table 11

Deceleration of nitrate uptake in *Chlamydomonas reinhardtii*. Molybdenum starved cells were incubated 48 hr without a molybdenum supplement. Non-molybdenum starved cells were incubated 48 hr with a molybdenum supplement of 0.4 ppm. Cultures were exposed to a light:dark regime of 15:9 hr. Light intensity was 2.61 x 10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

Molybdenum concentration (ppm)	Nitrate uptake deceleration (μM 10^6 $cells^{-1}$ hr^{-2})		
	non-starved cells	starved cells	F (p=0.05)*
Control	3.82	3.30	1.699
0.4	4.52	4.13	0.470
0.8	4.86	3.63	5.403

*F: All F values compared to non-starved cells

F (p=0.05) = 4.75

difference in uptake rates of starved and non-starved cells. The molybdenum concentration of the medium did not alter the uptake rates in non-starved cells (Table 12). Results of nitrate uptake in molybdenum starved cells are presented in Table 13. A concentration of 0.4 ppm molybdenum in molybdenum starved cells appears to be slightly inhibitory, however 0.8 ppm of molybdenum was not inhibitory and the result at 0.4 ppm is believed to be a statistical artifact.

Nitrate uptake rates for N. pelliculosa over a two hr incubation period, in molybdenum starved and non-molybdenum starved cells are presented in Table 14. The rate of uptake in molybdenum starved cells of the control was significantly lower, compared to non-starved cells. At molybdenum concentrations of 0.4 and 0.8 ppm, no apparent significant difference in the slope was observed. However, uptake rates after 30 min of incubation demonstrated a considerable reduction in the rate of uptake in molybdenum starved cells (Table 18).

The rate of uptake in non-molybdenum starved and molybdenum starved cells are presented in Tables 15 and 16 respectively. There is no significant difference in the slopes at any concentration of molybdenum examined.

A comparison of nitrate uptake over a two hr incubation period in C. reinhardtii and N. pelliculosa are given in Table 17. No significant difference was observed in the non-starved cells. There was a significant difference in the slopes of the deceleration curves for the starved cells.

Table 12

Deceleration of nitrate uptake in non-molybdenum starved cells of Chlamydomonas reinhardtii. Non-molybdenum starved cells were incubated for 48 hr with a molybdenum supplement of 0.4 ppm. Cultures were exposed to a light: dark regime of 15:9 hr. Light intensity was 2.61×10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

Molybdenum concentration (ppm)	Nitrate uptake deceleration (μM 10^6 $cells^{-1}$ hr^{-2})	
	Rate	F (p=0.05)*
Control	3.82	
0.4	4.52	1.180
0.8	4.86	2.897

*F: All F values are compared to control

F (p=0.05) = 4.75

Table 13

Deceleration of nitrate uptake in molybdenum starved cells of *Chlamydomonas reinhardtii*. Starved cells were incubated for 48 hr without a molybdenum supplement. Cultures were exposed to a light:dark regime of 15:9 hr. Light intensity was 2.61 x 10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

Molybdenum concentration (ppm)	Nitrate uptake deceleration (μM 10^6 $cells^{-1}$ hr^{-2})	
	Rate	F (p=0.05)*
Control	3.30	
0.4	4.13	13.423
0.8	3.63	1.020

*F: All F values are compared to control

F (p=0.05) = 4.75

Table 14

Deceleration of nitrate uptake in Navicula pelliculosa. Molybdenum starved cells were incubated 48 hr without a molybdenum supplement. Non-molybdenum starved cells were incubated 48 hr with a molybdenum supplement of 0.4 ppm. Cultures were exposed to a light: dark regime of 15:9 hr. Light intensity was 2.61 x 10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

Molybdenum concentration (ppm)	Nitrate uptake deceleration (μM 10^6 $cells^{-1}$ hr^{-2}) non-starved cells	starved cells	F (p=0.05)*
Control	3.67	7.83	9.233
0.4	5.41	11.22	2.261
0.8	3.86	21.52	1.758

*F: All F values are compared to non-starved cells.

F (p=0.05) = 4.84

Table 15

Deceleration of nitrate uptake in non-molybdenum starved cells of *Navicula pelliculosa*. Non-molybdenum starved cells were incubated for 48 hr with a molybdenum supplement of 0.4 ppm. Cultures were exposed to a light: dark regime of 15:9 hr. Light intensity was 2.61×10^4 ergs.cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

Molybdenum concentration (ppm)	Nitrate uptake deceleration (µM 10^6 cells^{-1} hr^{-2})	
	Rate	F (p=0.05)*
Control	3.67	
0.4	5.41	2.079
0.8	3.86	0.018

*F: All F values are compared to control

F(p=0.05) = 4.75

Table 16

Deceleration of nitrate uptake in molybdenum starved cells of Navicula pelliculosa. Starved cells were incubated for 48 hr without a molybdenum supplement. Cultures were exposed to a light:dark regime of 15:9 hr. Light intensity was 2.61×10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

Molybdenum concentration (ppm)	Nitrate uptake deceleration (μM 10^6 $cells^{-1}$ hr^{-2})	
	Rate	F (p=0.05)*
Control	7.83	
0.4	11.22	0.610
0.8	21.52	0.840

*F; All Fvalues are compared to control

F (p=0.05) = 4.96

Table 17

Deceleration of nitrate uptake in control cultures of *Chlamydomonas reinhardtii* and *Navicula pelliculosa*. Molybdenum starved cells were incubated 48 hr without a molybdenum supplement. Non-molybdenum starved cells were incubated 48 hr with a molybdenum supplement of 0.4 ppm. Cultures were exposed to a light: dark regime of 15:9 hr. Light intensity was 2.61 x 10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

	Nitrate uptake deceleration (μM 10^6 $cells^{-1}$ hr^{-2})			
	non-starved cells	F^* (p=0.05)	starved cells	F^* (p=0.05)
C. reinhardtii	3.82		3.30	
N. pelliculosa	3.67	0.024[a]	7.83	23.538[b]

*F: All F values compared to *C. reinhardtii*

[a]F (p=0.05) = 4.74

[b]F (p=0.05) = 4.84

Table 18

Nitrate uptake rates in Chlamydomonas reinhardtii and Navicula pelliculosa after 0.5 hr incubation. Molybdenum starved cells were incubated 48 hr without a molybdenum supplement. Non-starved cells were incubated with a molybdenum supplement of 0.4 ppm. Cultures were exposed to a light:dark regime of 15:9 hr. Light intensity was 2.61×10^4 ergs cm^{-2} sec^{-1}. Cultures were grown at 20±1 C with continuous shaking.

	Nitrate uptake rate (µM 10^6 $cells^{-1}$ hr^{-1})			
	N. pelliculosa		C. reinhardtii	
Molybdenum concentration (ppm)	non-starved cells	starved cells	non-starved cells	starved cells
Control	0.464	0.376	0.561	0.525
0.4	0.449	0.154	0.579	0.423
0.8	0.346	0.086	0.377	0.435

DISCUSSION

It was reported in Chapter 1 that the division rate in *Navicula pelliculosa* was greater than in *Chlamydomonas reinhardtii*. Considering the surface to volume ratios of the two species, the diatom may have a greater affinity for nutrients than the larger green alga. This is supported by the nitrate kinetics of the two species. *N. pelliculosa*, with a lower K_m (14.92 μM), has a greater affinity for low concentrations of nitrate than *C. reinhardtii*, which had a K_m of 148.0 μM.

The K_m value reported here for *N. pelliculosa* is slightly higher than the K_m values reported for marine diatoms by Eppley *et al* (1969). Nitrate is often a limiting factor in seawater, whereas nitrate concentrations in freshwater, especially eutrophic lakes, is much greater (Winner *et al* 1970; Dugdale and Goering 1967). It is possible that the higher K_m values reflect the differences in freshwater and marine environments. Manny (1969) has also reported a higher nitrate uptake rate in the green alga *Chlamydomonas* compared to the nitrate uptake rate of *Nitzschia*.

The importance of molybdenum to the enzyme nitrate reductase in algae is now well established (Aparicio *et al* 1970; Vega *et al* 1971). Arnon *et al* (1955) reported a threshold requirement of 1.0 ppb molybdenum for *Scenedesmus*. Vega *et al* (1971) reported concentrations as low as 2.59 ppb supported adequate nitrate utilization in *Chlorella*. The data presented in this study also demonstrates that the threshold level of molybdenum in *C. reinhardtii*

is very low, as adequate nitrate uptake was evident in the controls (Table 11). A 48 hr molybdenum starvation period does not reduce nitrate uptake rates in *C. reinhardtii*. Vega *et al* (1971) reported that the nitrate reductase complex was formed in the absence of molybdenum in *Chlorella*, and suggested that it was not required for *de novo* synthesis of the enzyme. The fact that molybdenum starvation does not reduce the rate of nitrate uptake suggests that sufficient molybdenum is stored by the cell to compensate for the 48 hr molybdenum starvation period.

The data presented in Table 12 and 13 demonstrate that high molybdenum concentrations do not affect nitrate uptake in *C. reinhardtii*. The fact that high molybdenum concentrations do not inhibit uptake rates supports the data of Vega *et al* (1971). They reported concentrations up to 16.2 ppm did not inhibit enzyme activity. Vega reported a 20 fold increase in nitrate reductase activity in cell free extracts of *Chlorella* for concentrations as high as 16.2 ppm. The data presented in this study does not support this result. The activity of the cell free extracts gives the potential for enzyme activity under constant physiological conditions. The same enzyme system, when operating in the cell may not attain this full potential. This may be the situation in *C. reinhardtii* as the data clearly indicate that molybdenum concentrations up to 0.8 ppm do not increase *in vivo* nitrate uptake rates.

The mode of action of molybdenum in *C. reinhardtii* appears to be a functional one. That is, molybdenum is only required for the reduction of nitrate to nitrite and not for the synthesis of the enzyme. Once the threshold requirement for molybdenum is attained

(*ca* 1.0 ppb, Arnon *et al* 1955), the enzyme operates optimally, and is not affected by higher concentrations.

A 48 hr molybdenum starvation period reduced the rate of nitrate uptake in *N. pelliculosa*. After the 2 hr incubation in molybdenum, the rate of nitrate uptake approaches that of the non-molybdenum starved cells (Figure 8B). This lag period suggests that molybdenum may be involved, directly or indirectly, in the synthesis of nitrate reductase. It is possible that during the synthesis of nitrate reductase, molybdenum is transported actively into the cell. As synthesis of the enzyme is completed, the transport mechanism is again inactivated, through a feedback mechanism.

In *N. pelliculosa,* high concentrations of molybdenum do not affect the nitrate uptake rates. An active transport mechanism would explain why high molybdenum concentrations do not affect nitrate uptake in *N. pelliculosa*.

The mode of action of molybdenum in *N. pelliculosa* seems more complex than *C. reinhardtii*. Molybdenum is not only incorporated into the apoenzyme, but may also be involved in the synthesis of the enzyme complex.

Eppley and Coatsworth (1968), Eppley and Thomas (1969) and Eppley *et al* (1969) have formed the hypothesis that in marine phytoplankton, where nitrate is often limiting, the algal species having the lower K_m would be more successful in competition for the substrate. The same hypothesis may be applied to a nitrate limited freshwater environment. Considering the K_m values determined in this study one could predict the diatoms to be more successful in competition for nitrate

than the green alga.

The results of this chapter support the observed effects of molybdenum on growth and photosynthesis in the two algal species.

This study has attempted to provide more information concerning how increasing molybdenum concentrations affect the physiology and ecology of algae. This has essentially been a background study, and as such provides more questions than answers. The author feels that further investigations with molybdenum are necessary if ecologists are to be able to fully understand the ecology of algae.

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APPENDIX A

Algal Media

Modified Hughes Medium #1

Taken from Allen (1968).

$NaNO_3$	1.5 g/l
K_2HPO_4	0.039 g/l
$MgSO_4.7H_2O$	0.075 g/l
$NaCO_3$	0.02 g/l
$CaCl_2$	0.027 g/l
$Na_2SiO_3.9H_2O$	0.058 g/l
EDTA	0.001 g/l
Citric Acid	0.006 g/l
Fe Citrate	0.006 g/l
0.8 M Tris-HCl Buffer, pH 7.7	7 ml/l

1 ml/l of trace metal solution containing the following dissolved in 1.0 l distilled water.

H_3BO_3	2.86 g	$Na_2MoO_4.2H_2O$	0.391 g
$MnCl_2.4H_2O$	0.181 g	$CuSO_4.5H_2O$	0.079 g
$ZnSO_4.7H_2O$	0.222 g	$Co(NO_3)_2.6H_2O$	0.049 g

1 ml/l of vitamin solution containing

thiamine.HCl	500 µg/ml
vitamin B_{12}	2.0 µg/ml
biotin	1.0 µg/ml

Modified Hughes Medium #2

The same as Hughes medium #1 except for the trace metal solution. 1 ml/1 of trace metal solution containing the following dissolved in 1.0 1 distilled water

H_3BO_3	2.0 g	LiCl	1.0 g
$MnSO_4 \cdot 7H_2O$	2.0 g	$CoCl_2 \cdot 6H_2O$	1.0 g
$ZnSO_4 \cdot 7H_2O$	2.0 g		

APPENDIX B

Contamination Checks

A technique of selective enrichment of possible microbial contamination (bacteria and molds) by heterotrophic growth was used. Fifteen ml of T Soy broth (BBL Div. of BioQuest) was used for such sterility checks. From 2-4 drops of algal culture were added to a screw top vial and incubated about 3 weeks at 20±1 C. The appearance of cloudiness and turbidity typical of bacterial growth or discolouration and tuft formation, typical of mold growth was taken as evidence of possible contamination, subject to confirmation by phase microscopy and transfer onto nutrient agar (Difco Laboratories). In the absence of any visible changes in the sterility check vial, the algal culture tested was considered to be free from contamination. Periodic sterility checks were routinely maintained on all algal stock cultures. Every stock culture used to provide inoculum for an experimental culture was subjected to such a check in triplicate vials. Each experimental culture was submitted to such a check prior to use in an experiment. Any cultures which became contaminated were discarded. All data presented in this study is assumed to be from axenic cultures.

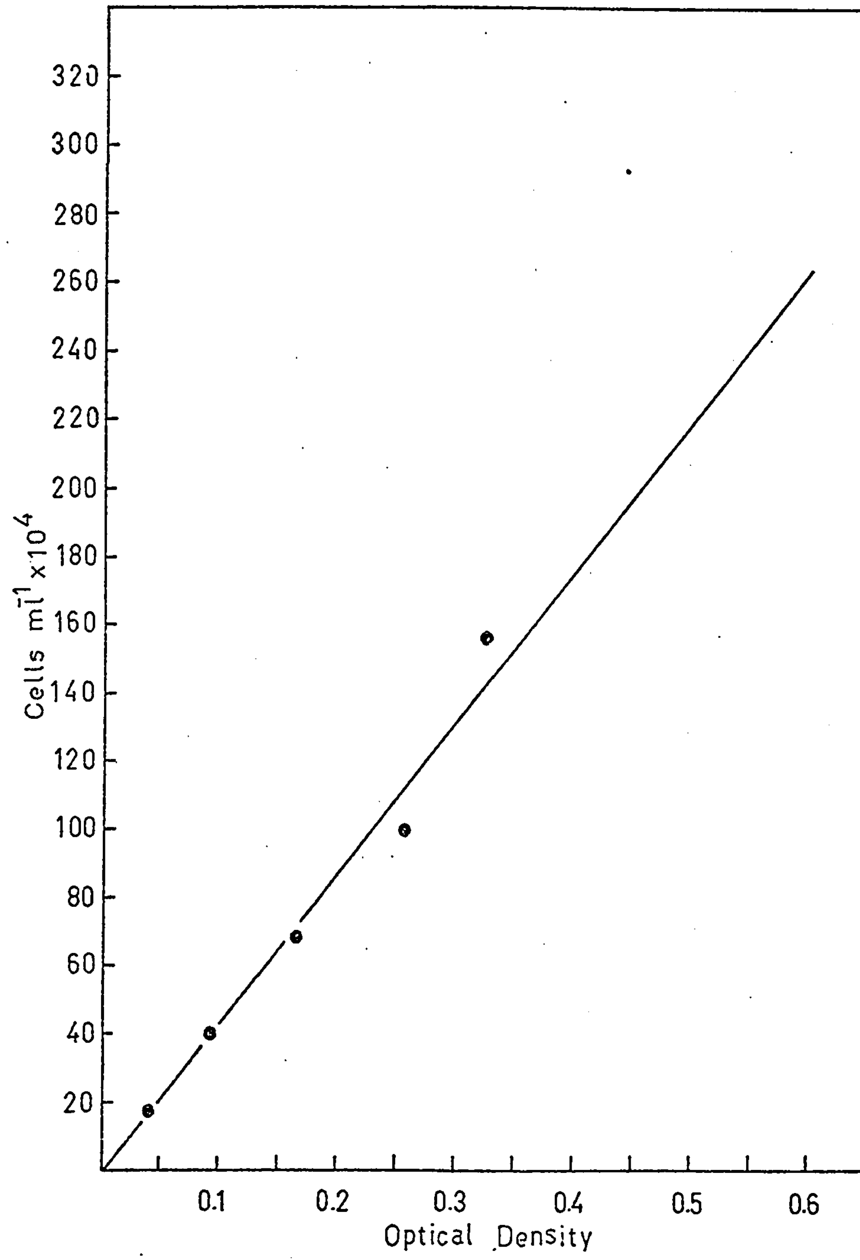
APPENDIX C

Calibration Curve for the Conversion of Optical Density to Cell Number in *Chlamydomonas reinhardtii*

Growth rates for *C. reinhardtii* were determined by measuring the changes in optical density at 540 nm with a Spectronic 20 colorimeter. Optical density values were converted to cells ml^{-1} using the calibration curve presented in Figure 9.

The calibration curve was constructed by measuring the optical densities of six dilutions of a stock culture: 100, 75, 50, 25, 10 and 0 %. Cell concentration at each dilution was determined using an A/O fine line haemocytometer. The calibration dilutions were performed in triplicate.

Figure 9. Calibration curve to convert optical density values to cells ml^{-1} in *Chlamydomonas reinhardtii*.



320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
Cells ml⁻¹ x10⁴
0.1
0.2
0.3
0.4
0.5
0.6
Optical Density

CURRICULUM VITAE

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END OF REEL

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