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KINETICS OF SILICON-LIMITED GROWTH IN THE FRESHWATER DIATOM *ASTERIONELLA FORMOSA*[1, 2]

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SUMMARY

Growth rates of two clones of the freshwater planktonic diatom Asterionella formosa *Hass. were measured under conditions in which external silicon concentrations controlled growth. Clone AfOH2 from Lake Ohrid, Yugoslavia, had a higher maximum growth rate ($\mu_{max} = 1.11$ doublings/day) and apparent half-saturation constant ($K_{si} + Si_o = 1.93$ μM Si) than clone L262 from Lake Windermere, England ($\mu_{max} = 0.61$ doublings/day; $K_{si} + Si_o = 1.09$ μM Si). K_{lim}, the silicon concentration at $\mu = 0.9\ \mu_{max}$, is 13.8 μM Si for clone AfOH2 and 6.5 μM Si for clone L262. These values agree well with published field observations showing* A. formosa *populations decreasing below 0.5 mg/l SiO_2 (= 8.4 μM Si). Calculations of yield gave a range of 0.5–1.5 μM Si/10^6 cells for clone AfOH2 and 0.6–1.9 μM Si/10^6 cells for clone L262.*

Key words: Asterionella*; diatom; growth kinetics; silicon*

INTRODUCTION

There have been several recent studies on the responses of planktonic marine diatoms to limiting concentrations of silicon (7, 16, 17), yet no comparable data exist for freshwater planktonic diatoms. Kilham (11) suggested that silicon may have an important role in the succession of planktonic diatoms, but there has been no physiological evidence to support this hypothesis for freshwater diatoms. *Asterionella formosa* Hass. is one species for which there is abundant field evidence for silicon having some controlling influence on population levels (11, 13–15). Work on marine diatoms has also suggested that there are intraspecific differences in physiological responses to silicon (7) and nitrate (3). Such data do not exist for freshwater species.

This study examines the kinetics of the growth response of 2 clones of *Asterionella formosa* to limiting concentrations of silicon and attempts to determine whether intraspecific differences in response do occur.

METHODS

Two clones of *Asterionella formosa* were used in the experiments. Clone L262 was isolated by J. W. G. Lund from Lake Windermere, England. Clone AfOH2 was isolated by me from Lake Ohrid, Yugoslavia, 7 June 1973. Both clones are unialgal but not axenic. A freshwater medium ("WC", 8) was used for all diatom cultures (without buffer or NH_4Cl). All cultures were maintained in a culture box at *ca.* 20 C and 55 μEin/m²/sec (4000 lx) illumination provided by cool white fluorescent bulbs on a 14:10 LD cycle. Experiments were also conducted under these conditions.

To condition the clones prior to each experiment, small inocula were grown in 250-ml batch cultures of complete WC for *ca.* 10 days. A portion (usually *ca.* 50 ml or 5×10^5 cells) of each clone was then transferred into polycarbonate fernbach flasks containing 1 l WC with only 5 μM Si and maintained 5 days in the culture box. At the end of this period the cells were presumed to be Si depleted. The ambient concentration of Si in each low-Si batch culture at the end of 5 days was less than 0.5 μM Si.

Polycarbonate 250 ml erlenmeyer flasks were used in all experiments. Each flask was filled with 150 ml of WC-Si and autoclaved. A stock solution of $Na_2SiO_3 \cdot 9H_2O$ containing 0.5 mM Si was sterile-filtered and various amounts added to each flask. Two separate experiments were conducted 8 days apart. The data were pooled for each clone. In each experiment there were 6 flasks of varying Si concentration for each clone. A known number of diatoms was added to each flask so there were initially *ca.* 500 cells/ml in each flask in the first experiment and initially *ca.* 250 cells/ml in each flask in the second experiment. Initial Si values were 0.15–11 μM Si. Each experiment

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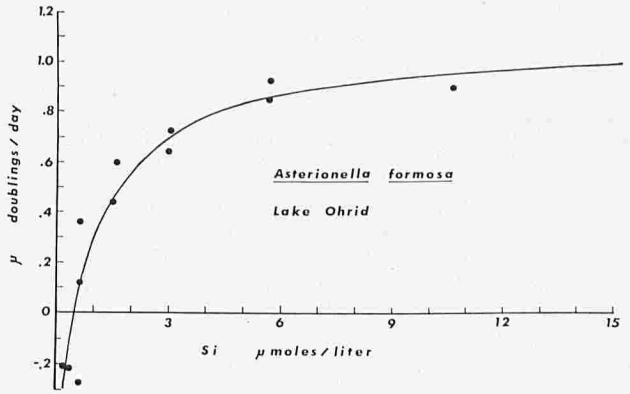

FIG. 1. Growth rates of *Asterionella formosa* clone AfOH2 as a function of Si concentration.

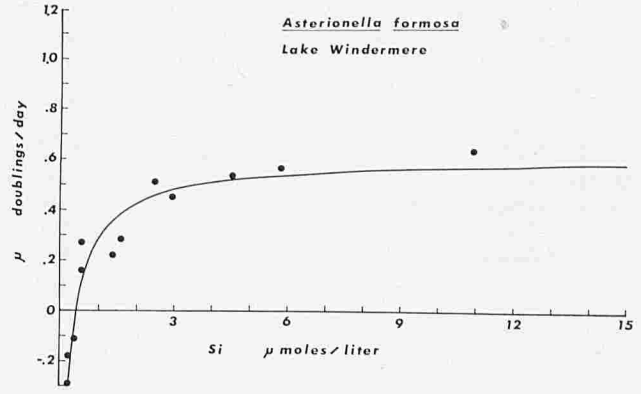

FIG. 2. Growth rates of *Asterionella formosa* clone L262 as a function of Si concentration.

was continued for 5 days. Samples for counting and Si determinations were taken at the same time each day (5 h into the light cycle). The flasks were shaken twice each day.

Counting was done with a Sedgwick-Rafter chamber on samples preserved with Lugol's acetate solution. When cell numbers were low (<1000/ml) the entire chamber was counted. At higher concentrations of cells, strips of the chamber were counted using the calibrated Whipple-Disk method described by Guillard (6). Growth rates were calculated in doublings/day by a linear least squares regression through log transformed data. There was no lag phase in growth observed. All of the growth curves had correlation coefficients of 0.8 or better.

Silicon was measured by a modified Strickland-Parsons (18) silico-molybdate method. The sample size was reduced to 10 ml, the molybdate reagent to 4 ml and the reducing reagent to 6 ml. Readings were made using 10 cm path-length cells in a Spectronic 100, allowing accurate determinations to *ca.* 0.05 μM Si. Calibration standards were Na_2SiF_6 solutions of 0.1, 1.0, 4.0 and 10.0 μM Si.

The half-saturation constants (K_{si}) were determined using the iterative procedure of Bliss and James (2). A Fortran IV program (9) obtained from Yale University Computer Center was used to calculate the half-saturation constants. Because of the observed threshold phenomenon, Si_o, defined in (1), was calculated by an iterative procedure which assumed that the best Si_o would yield a K_{si} with the least variance.

$$\mu = \mu_{max} \frac{(Si - Si_o)}{(Si - Si_o + K_{si})} \quad (1)$$

RESULTS

Figures 1 and 2 give the growth rates in doublings/day vs. initial silicon concentrations for each clone of *Asterionella formosa*. The hyperbolic relationships are adequately described by eq. 1. Table 1 gives the kinetic information for each clone. The maximum growth rate of clone AfOH2 from Lake Ohrid (μ_{max} = 1.11 doublings/day) and the apparent half-saturation constant ($K_{si} + Si_o$ = 1.93 μM Si) were higher than those of clone L262 from Lake Windermere (μ_{max} = 0.61 doublings/day; $K_{si} + Si_o$ = 1.09 μM Si). The K_{lim} (silicon concentration where $\mu = 0.90\ \mu_{max}$; above this concentration the nutrient is presumed to be non-limiting to growth; 5) for clone AfOH2 is 13.8 μM Si, whereas the K_{lim} for clone L262 is reached at 6.5 μM Si.

Table 2 shows the yield data calculated by 3 methods. Yield A gives the cells/μM Si taken up. Yield B is the μM Si taken up per cell produced. Yield C gives μM Si per 10^6 cells. Generally the data illustrate that the number of cells produced for a given amount of Si decreases with increasing Si concentration because the amount of Si/cell increases at higher concentrations. There was no detectable Si taken up at the lower concentrations and hence no yields could be calculated.

DISCUSSION

Figures 1 and 2 indicate that the rate of growth of *Asterionella formosa* is dependent on the external silicon concentration in the range 0.4–15 μM. There is a threshold concentration (Si_o) below which growth ceases or becomes negative (death rate greater than birth rate). Paasche (17) observed a similar threshold concentration for Si uptake in experiments on the marine diatom *Thalassiosira pseudonana* Hasle & Heimdal. He suggested that this was possibly a form of Si measurable by the molybdate method, but unusable by the diatoms; however, there is no evidence for this hypothesis. This threshold Si level for growth of *T. pseudonana* was not observed by Guillard, Kilham and Jackson (7). The growth of some other

TABLE 1. *Experimentally determined kinetic quantities for* Asterionella formosa.

Clone (Source)	μ_{max} doublings/day	Confidence interval 95%	$K_{si} + Si_o$ μM Si	Confidence interval 95%	Si_o μM Si	K_{lim} μM Si
Clone AfOH2 (Lake Ohrid)	1.11	0.78–1.44	1.93	1.19–3.05	0.45	13.8
Clone L262 (Lake Windermere)	0.61	0.48–0.74	1.09	0.82–1.43	0.41	6.5

TABLE 2. *Yield data for* Asterionella formosa.

Clone; Experiment	Si μM			Yield		
				A	B	C
	Initial	Taken up by day 5	Cells/l produced by day 5	Cells/μM Si taken up	μM Si taken up/cell produced	μM Si/10^6 cells
AfOH2	1.56	0.26	1.7×10^5	0.7×10^6	15.3×10^{-7}	1.53
Experiment 1	2.95	0.95	8.5×10^5	0.9×10^6	11.2×10^{-7}	1.12
	5.70	1.50	1.37×10^6	0.9×10^6	10.9×10^{-7}	1.09
Experiment 2	3.00	0.75	1.53×10^6	2.0×10^6	4.9×10^{-7}	0.49
	5.65	1.90	2.79×10^6	1.5×10^6	6.8×10^{-7}	0.68
	10.60	2.30	2.94×10^6	1.3×10^6	7.8×10^{-7}	0.78
L262	1.60	0.38	4.87×10^5	1.3×10^6	7.8×10^{-7}	0.78
Experiment 1	2.95	1.05	9.37×10^5	0.9×10^6	11.2×10^{-7}	1.12
	5.85	1.85	9.90×10^6	0.5×10^6	18.7×10^{-7}	1.87
Experiment 2	2.50	0.18	3.18×10^5	1.8×10^6	5.7×10^{-7}	0.57
	4.55	0.95	5.27×10^5	0.6×10^6	18.0×10^{-7}	1.80
	11.00	0.55	4.62×10^5	0.8×10^6	11.9×10^{-7}	1.19

species of freshwater diatoms such as *Cyclotella meneghiniana* Kütz (unpublished observations) also do not exhibit a threshold value of Si for growth. The threshold phenomenon is not well understood.

The lower portions of the fitted curves for both clones of *A. formosa* are essentially identical up to a Si concentration of 1.0 μM. Clone AfOH2 has a higher growth rate than clone L262 at Si levels above this concentration. Previous experiments by Hughes and Lund (10) on the growth rate of *A. formosa* from the English Lake District (growth conditions 17 C, 30 μM Si, 12:12 LD) showed a growth rate (calculated from their graphs) of 0.68 doublings/day, which is remarkably good agreement with the present experiments (0.61 doublings/day). The lower growth rates of clone L262 may have been a result of the larger size of the clone (cells *ca.* 80 μm long as compared to 60 μm for AfOH2; see 17). There may also be different optima for each clone for light and temperature (although light was at saturating levels; 1, 19, 20). Eppley *et al.* (4) have discussed the interaction of nutrient concentrations, light and temperature with respect to kinetic properties of algae. Guillard *et al.* (7) suggested that the differences they observed in the Si growth kinetics of 2 clones of *Thalassiosira pseudonana* were a result of adaptation to the environments from which they were isolated. The Si levels reported for Lake Ohrid (12) and Lake Windermere (13, 14) are similar, in the range 0–2 mg/l, which makes the distinction between the 2 clones difficult to explain on this basis alone. In Lake Ohrid the Si concentration is below 0.5 mg/l (8.4 μM Si) for all but October (12), whereas in Lake Windermere Si concentration is below 0.5 mg/l for at most a few summer months (13, 14). This may be the reason for the lack of blooms of *Asterionella formosa* in Lake Ohrid. Lake Windermere is mesotrophic, and Lake Ohrid is oligotrophic, so adaptation to differences in light, temperature and other nutrients may be important in determining the growth characteristics observed for these 2 clones.

Yield data were calculated by 3 methods (Table 2). Cells/μM Si were determined as a function of Si removed from the medium. Generally the number of cells produced for a given amount of Si decreased with increasing concentration. AfOH2 expt. 1 did not show this trend, and if both experiments for each clone were combined the trend is also not as clear. Silicon/cell was expressed as a function of the number of cells produced and as the amount necessary to make 10^6 cells. Generally the amount of Si/cell increases with increasing Si concentration (again AfOH2 expt. 1 did not show this trend). These experiments are certainly not adequate to establish that there is an increased cellular nutrient content associated with increased growth rates at higher nutrient concentrations. The amounts of Si necessary to produce 10^6 cells of *A. formosa* clone L262 (0.78–1.87 μM Si) agree well with the value of 1.66 μM Si per 10^6 cells (100 μg/10^6 cells) reported by Hughes and Lund (10) in their experiments on *A. formosa* from the English Lake District.

Finenko and Krupatkina-Akinina (5) have introduced the concept of K_{lim} which is the concentration of the limiting nutrient at $\mu = 0.90\ \mu_{max}$. Above this concentration the nutrient is presumed to be nonlimiting. Below it it may be limiting. For clone AfOH2, K_{lim} is 13.8 μM Si and for clone L262, K_{lim} is 6.5 μM Si (0.4 mg/l Si). This latter figure compares well with 0.5 mg/l that is the Si level at which the natural populations of *A. formosa* cease to grow (i.e., birth and death processes are equal) in the English Lake District (10, 13, 14). Such data are unfortunately not available for Lake Ohrid. If these kinds of relationships between physiological data and field observations can be demonstrated for other species (and clones) of diatoms, we will have a useful tool to develop more realistic models for seasonal successions of phytoplankton.

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MORPHOLOGY OF *PLACENTOPHORA* (SOLIERIACEAE, GIGARTINALES: RHODOPHYTA), A NEW GENUS BASED ON *SARCODIOTHECA COLENSOI* FROM NEW ZEALAND[1]

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SUMMARY

The only member of the red algal family Solieriaceae known from New Zealand is the endemic Sarcodiotheca colensoi *(Hook. & Harv.) Kylin. This study shows that it differs in several respects from the type* S. furcata *(Setch. & Gard.) Kylin; thus a new genus* Placentophora *is created for the New Zealand alga. Although* P. colensoi *nov. comb. is retained in the Solieriaceae on the basis of vegetative, spermatangial, tetrasporangial, carpogonial-branch and early gonimoblast features, it differs from typical members of that family in its pattern of later carposporophyte development. After a single gonimoblast initial is cut off from the auxiliary cell towards the center of the thallus, further gonimoblasts develop from the initial as ramifying, radiating filaments. These filaments enter an extensive "nutritive-cell" region surrounding the auxiliary cell, form numerous connections to the "nutritive" cells, and incorporate most of them into a central placenta of interconnected and variously-fused vegetative and gonimoblast cells. Carposporangia then form in short chains around the periphery of the placenta. The cystocarp lacks both a central fusion cell and a sterile-celled investment,*

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