

Temperature Dependence of Population Growth in a Green Microalga: Thermodynamic Characteristics of Growth Intensity and the Influence of Cell Concentration

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Growth intensity of the green alga, *Scenedesmus obliquus*, was measured in autotrophic cultures, diluted once daily, between 20 and 30 °C in a light-dark cycle of 16 : 8 h at initial optical densities between 0.02 and 1.2. Arrhenius analyses of the results showed linear relationships between growth intensity and temperature below the temperature optimum. The temperature effects on growth, activation energy, deactivation energy and normalized Q_{10} values were significantly influenced by the amount of available light energy per unit biomass. The temperature dependence of nutrient-limited growth was not considered.

Introduction

Planktonic microalgae form the photosynthesizing vegetation of the open oceans, of the pelagic zones of deeper lakes, reservoirs and of some other types of freshwaters. In addition, they are of applied interest in aquaculture and aerobic wastewater treatment in ponds.

Prediction of the productivity of microalgal canopies has to be based on the knowledge of the complex relations between photosynthetic production ($\text{g m}^{-2} \text{d}^{-1}$), light energy input (m^{-2}), and temperature. The principles of these interrelationships have to be investigated under conditions where productivity is not limited by other external factors like nutrient supply and turbulence. Limitations of that kind appear to prevail under natural conditions to such an extent that the classic modelling of productivity of phytoplankton populations considered up to now only light intensity and light attenuation as the decisive factors [1–3], but not temperature.

Of course, temperature is one of the ecological key factors in microalgae, too. Ecological observations and laboratory experiments have led to the distinction between psychrophilic, mesophilic and

thermophilic microalgae, displaying optimum temperatures below 20 °C (or even below 10 °C), between 20 and 35 °C, and above 35 °C, respectively. After the pioneering work of Shelef [4] on growth kinetics of *Chlorella sorokiniana* TX-7105 in a chemostat, Tiwari *et al.* [5] were probably the first to publish a formula describing the combined action of temperature and light on autotrophic production of biomass and oxygen. A simplified version of their formula has been numerically adapted and applied to data from *Scenedesmus obliquus* growing under field conditions [6]. An analogous approach for the prediction of productivity in high-rate algal ponds [7] should also be mentioned here.

By cultivating periodically diluted suspensions of the green microalga, *Scenedesmus obliquus*, in a light-and-dark cycle of 16 : 8 hours at various constant temperatures, we obtained net daily yields which resulted from production during the light period minus the respiratory losses during the dark period as is the case under natural field conditions.

Besides describing the influence of temperature and biomass concentration on growth intensity and productivity (*i.e.* biomass increase per unit volume and day), it is the purpose of this paper to answer the question to what degree the temperature sensitivity of growth intensity, expressed as Arrhenius constant or Q_{10} value, and activation or deactivation

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energy depend on the relative light energy input per unit biomass and day. The mathematical analysis and modelling of growth-temperature curves of *Scenedesmus obliquus* will be dealt with in a subsequent publication [8].

Materials and Methods

Scenedesmus obliquus (Turp.) Kütz. (strain SAG 276-3a of the Göttingen Culture Collection) was grown axenically in mineral medium N8 containing nitrate as the sole nitrogen source in a light-dark-cycle of 16 : 8 h as described before [9]. The cultures were continuously gassed with 1 vol.% CO₂ in air and irradiated during the light periods from one side of the thermoconstant water bath with 6 fluorescent lamps (OSRAM 1/32, 40 W, 220 V, 100 cm long) which gave an irradiance of about 78 $\mu\text{E m}^{-2} \text{sec}^{-1}$ at the surface of the culture tubes (liquid volume: 300 ml/culture).

At the beginning of each light period all cultures were diluted back to their respective optical density (O.D.) values ranging from 0.02 to 1.20. Algal dry matter concentration (DW) and O.D. at 560 nm were determined as described earlier [9]. Optical densities were measured, however, in cylindrical cuvettes using a Medico photometer (Lange, Berlin). For the given strain and the given conditions the O.D. value 0.1 corresponded approximately to a biomass (DW) concentration of 100 mg/l. The linear correlation between DW and O.D. extended up to O.D. = 0.8. Denser suspensions were diluted with fresh medium accordingly.

Growth intensity and productivity were determined from the initial values after dilution (DW₀, O.D.₀) and the corresponding values after 24 h (DW₂₄, O.D.₂₄). Because of the irregular time course of the increase of biomass concentration and of O.D. in *Scenedesmus* 276-3a [10] the growth kinetics were not analysed in further detail. At O.D.₀ values < 0.1 the cultures were completely synchronous [10], and at higher initial biomass concentrations a transition to group synchrony [11] occurred.

Growth intensity (GI) was determined from:

$$\text{GI} = \frac{\text{O.D.}_{24}}{\text{O.D.}_0} - 1 \quad (1)$$

where O.D. could also be replaced by DW. The term GI replaces the former "daily increment

factor" [9] which has the disadvantage of being 1 instead of 0 at zero growth. A growth rate μ should not be calculated from data on autotrophic organisms growing in a light-and-dark cycle, because they reach neither constant exponential growth nor true steady states.

Productivity (P) was determined as $\text{DW}_{24} - \text{DW}_0$ ($\text{mg l}^{-1} \text{d}^{-1}$). The cultures were grown at 20 °C, and after obtaining 2 to 4 representative growth values in a quasi-steady state, the temperature was increased stepwise so that corresponding values could be obtained at each temperature level.

Goldman and Carpenter [12] pointed out that it is possible to describe the growth rate (μ) solely as a function of temperature, if light intensity is constant, by using the Arrhenius equation:

$$\mu = A e^{-E/RT} \quad (2)$$

where A = constant which is the Arrhenius frequency factor per day, E = activation or deactivation energy or temperature characteristic ($\text{kJ} \cdot \text{mol}^{-1}$), R = universal gas constant, and T = absolute temperature in °K.

In the present study GI is used instead of μ . By computer fitting of log GI against $1/T$, the gradient of the straight lines equal $-E/2.303 R$ which allows by equation (2) the determination of E . From the latter the temperature coefficients Q_{10} are calculated after [13] using the equation:

$$\log Q_{10} = \frac{E}{2.303 R} \frac{10}{(T + 10) T} \quad (3)$$

For normalization Q_{10} was always calculated for the temperature range 10 to 20 °C. This was done even though no measurements were made below 20 °C, for standardization and comparison purposes.

For the numerical fitting of measured values to the individual Arrhenius equations and for statistical evaluation of the fits, κ_0 and E were calculated according to:

$$\ln \text{GI} = (\kappa_0 - E/R \cdot T^{-1}) \quad (4)$$

where κ_0 is again the Arrhenius frequency factor.

Results

Average growth intensities as calculated from O.D. measurements are summarized in Table I, the

Table I. Average growth intensities of *Scenedesmus* 276-3a at different initial optical densities (O.D.₀) and temperatures. Measured experimental values (EXP) are compared with values (MOD) calculated according to equation (4) in order to give an indication of the reliability of the individual figures.

Temperature [°C]	O.D. ₀ 1.2		0.8		0.4		0.2		0.1		0.02	
	EXP	MOD	EXP	MOD	EXP	MOD	EXP	MOD	EXP	MOD	EXP	MOD
20			0.64	0.58	1.40	1.47	2.40	2.46	3.38	3.35	5.80	5.86
24	0.40	0.42	0.67	0.81	1.77	1.78	3.47	3.32	4.97	4.99	9.17	9.29
28	0.72	0.64	1.18	1.10	2.52	2.15	4.38	4.46	7.12	7.34	15.95	14.54
30	0.79	0.79	1.27	1.29	2.29	2.35	5.18	5.15	8.78	8.88	17.60	18.11
30	0.73	0.79	1.34	1.29	2.40	2.35	5.09	5.15	9.21	8.88	17.38	18.11
32	0.59	0.61	1.04	1.15	2.42	2.58	4.57	5.93	8.60	10.70	19.86	26.73
33	0.35	0.38	0.91	0.73	2.61	2.70	4.76	6.47	9.39	11.79	17.57	9.85
34	0.29	0.24	0.45	0.47	1.69	1.59	3.40	2.36	5.64	3.43	3.18	3.66
35	0.14	0.15	0.25	0.30	1.05	0.88	1.06	0.87	0.74	1.01	1.05	1.37
36	0.09	0.09	0.21	0.19	0.42	0.48	0.20	0.32	0.31	0.30	0.58	0.51

Table II. Average productivities at different optical densities (O.D.₀) and temperatures. For definition of productivity (expressed in mg dm⁻³ d⁻¹) it is referred to the methods section.

Temperature [°C]	O.D. ₀					
	1.2	0.8	0.4	0.2	0.1	0.02
20	n. d.	511	585	440	340	115
24	495	537	697	686	500	183
28	871	933	1000	876	713	319
30	916	1040	988	1026	899	339
32	725	844	954	917	855	404
33	476	730	991	932	964	352
34	366	443	677	678	564	64
35	168	200	418	212	74	21
36	108	173	165	50	31	12

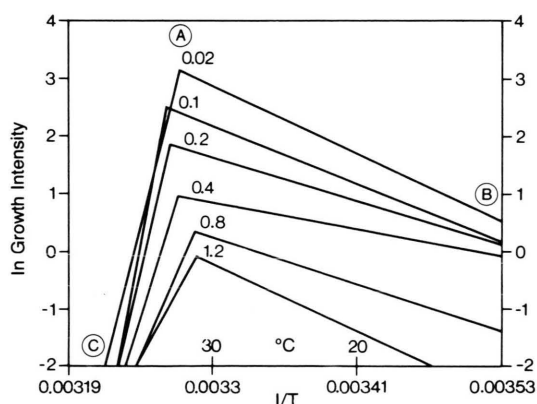


Fig. 1. Arrhenius plots of the growth intensity measurements done at various temperatures and initial optical densities of *Scenedesmus obliquus* (strain 276-3a).

results of productivity determinations at the various temperatures in Table II. Arrhenius plots of GI are shown in Fig. 1, whilst activation energies, deactivation energies, and Q_{10} values are given in Table III. The productivity measurements could also have been used for these calculations; the only difference would have been a change in the frequency factor (A).

The Arrhenius plots (Fig. 1) clearly show a distinct optimum (A) for each of the initial optical densities (O.D.₀) plotted in Fig. 3. The temperature optima (T_{opt}) in dependence from O.D.₀ vary between about 31 and 33 °C, with a maximum shift of the optimum between O.D.₀ 0.1 and 0.2. Above T_{opt} the influence of temperature becomes expectedly negative and is governed by deactivation. Although cultures appeared bleached above 33 °C, an occurrence of dead cells was not noticed.

The dependence of productivity upon both temperature and O.D.₀ (Fig. 2) shows a distinct optimum area. There is little difference between the response of productivity to temperature between the cultures with O.D.₀ values greater than 0.2, except for the slightly lower productivity at the highest O.D.₀ at 20 and 24 °C. Productivity reached a maximum of variable position at O.D.₀ values between 0.4 and 0.8. A definite optimum of productivity relative to O.D.₀ became apparent above 32 °C, especially at 35 °C (peak at O.D.₀ = 0.4 in Fig. 2). The productivities were low at 36 °C with a maximum at an O.D.₀ of 0.8.

A pattern is also evident in the slopes A–B of the Arrhenius curves for GI (Fig. 1), and hence of the

Table III. Arrhenius reaction parameters, their standard errors, activation energies, deactivation energies, and Q_{10} values at various O.D.₀ values of *Scenedesmus* 276-3a. The reaction parameters for activation (slopes A–B in Fig. 1) and deactivation (slope A–C) were calculated after equation (4). The standard error estimate is on the 90% significance level.

O.D. ₀	Activation parameter		Standard error		Deactivation parameter		Standard error		Energies (kJ/mol) for activation		Q_{10} values for	
	α_0	E/R	α_0	E/R	α_0	E/R	α_0	E/R			activation	deactivation
1.20	31.55	9.64	27.50	8.28	−145.05	−44.11	46.74	14.36	80.12	−366.74	3.20	$4.9 \cdot 10^{-3}$
0.80	23.62	7.08	15.84	4.74	−138.73	−42.38	63.89	19.62	58.89	−352.32	2.35	$6.0 \cdot 10^{-3}$
0.40	14.67	4.19	6.24	1.88	−182.99	−56.35	109.48	33.68	34.82	−468.49	1.66	$1.1 \cdot 10^{-3}$
0.20	23.33	6.58	3.86	1.16	−307.24	−94.64	241.11	74.19	54.68	−786.81	2.21	$1.1 \cdot 10^{-5}$
0.10	30.72	8.65	3.59	1.08	−376.60	−116.06	264.23	81.28	71.93	−964.89	2.84	$8.4 \cdot 10^{-7}$
0.02	35.96	10.02	7.63	2.29	−302.26	−93.24	147.27	45.23	83.35	−775.20	3.35	$1.3 \cdot 10^{-5}$

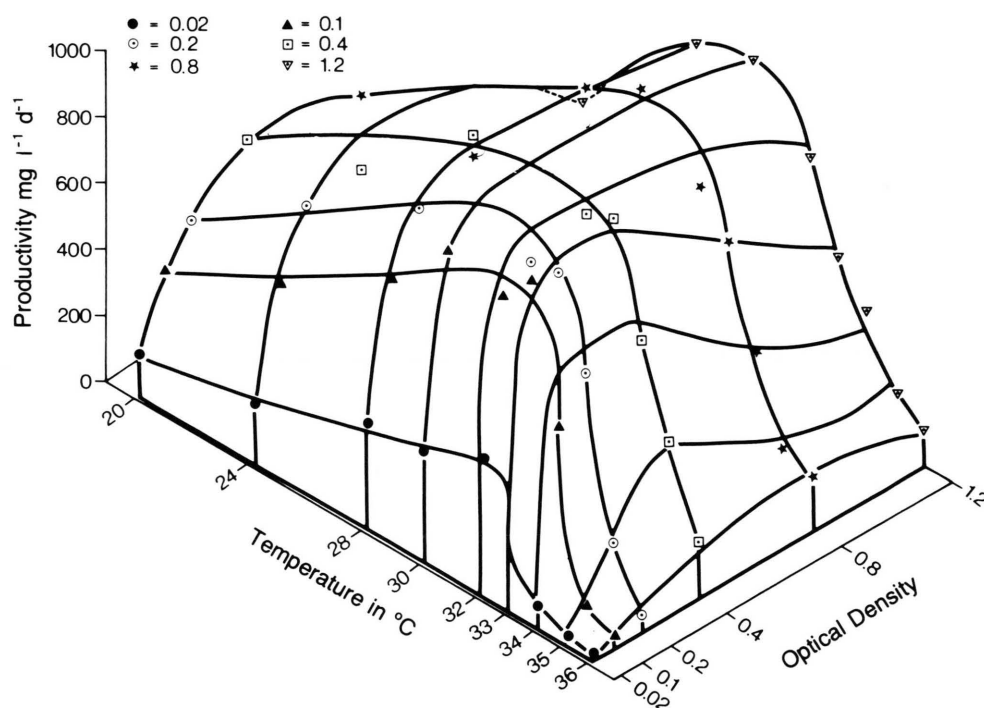


Fig. 2. Three-dimensional plot of the productivities as influenced by temperature and initial optical densities. All the cultures were subjected to the same light intensity.

Q_{10} values which are shown against O.D.₀ in Fig. 3. The hyperbolic relation reaches a minimum at an O.D.₀ of 0.4. The range of Q_{10} values is also of interest, because a 51% difference was calculated between the lowest and highest values (Table III).

Unlike the stimulating influence of temperature below T_{opt} , temperature stress above T_{opt} leads to a pronounced drop of GI. This is expressed by the negative slopes A–C of the Arrhenius curves (Fig. 1). The relation between the respective deactivation energies and O.D.₀ (Table III) is char-

acterized in principle by an inverse, logarithmic correlation ($E_{deact.} = f(\log O.D._0^{-1})$) between O.D.₀ values of 0.1 and 1.2 and a maximum at O.D.₀ = 0.1.

The experimentally imposed differences in biomass concentration (expressed as O.D.₀) implied that the effective light energy per cell decreased with increasing O.D.₀. It is therefore considered important to have some indication of the light availability per cell. Since this was not measured directly, the inverse of O.D.₀ was considered to be one possibility, and another is to calculate the rela-

tive light availability as a percentage, assuming 100% illuminance at $O.D._0 = 0$, and using the formula:

$$\ln I_{Z_i} = \ln I_{Z_0} - \varepsilon Z_i \tag{5}$$

where I_{Z_i} = illuminance at a depth Z_i , I_{Z_0} = illuminance at the surface and ε = attenuation coefficient (in this case it was substituted with the O.D. measurements).

At all relative light availabilities, GI below T_{opt} increased with temperature (Fig. 4), the response being the greatest at the highest relative light availability and *vice versa*. The response of GI to temperature was maximal at temperatures approaching T_{opt} . Above T_{opt} the GI increased slightly with an increase in available light, but then remained fairly constant at a low rate.

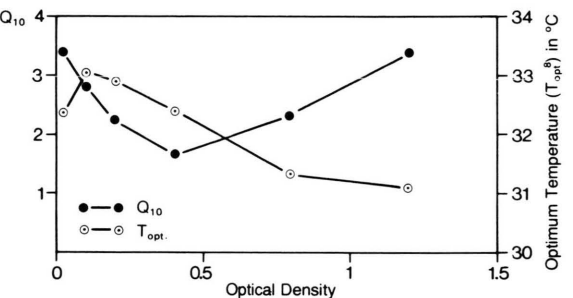


Fig. 3. Variations in the temperature coefficient (Q_{10}) and optimum temperature for maximum growth intensity in cultures with various initial optical densities.

Productivity also increased below T_{opt} with increasing temperature (Fig. 4), but it decreased at levels of higher light availability. At 20 °C the peak is at about 40% relative light availability, at 24 °C it is at 60% and at 32 °C about 65%. These results can be explained by the fact that significant quantities of light will not be utilized at the low optical densities (*i.e.* high light availability), simply because there is not enough biomass present to utilize the available light. At higher $O.D._0$, a portion of the cells will be light starved and consequently photosynthetically less active with the result that biomass will actually be lost due to respiration. The peak then represents the density where the interplay between these two factors is at a minimum. The entire effect is strongly temperature dependent, as shown by the increase in the productivities with increased temperature. Above T_{opt} the peak appears to be more pronounced (Fig. 4), decreasing rapidly in both directions. This clearly indicates the inhibiting influence of temperature, both in the light limited and light saturated regions.

Dependence of growth intensity on $1/O.D._0$ (Fig. 5) shows the typical hyperbolic rate dependence on light intensity with light-limited and saturated sections. The marked influence which temperature below T_{opt} has on the growth intensities can clearly be seen. Light saturation had probably not been reached at 30 °C. Above T_{opt} , GI was strongly reduced due to the combined action of temperature and light stress [8].

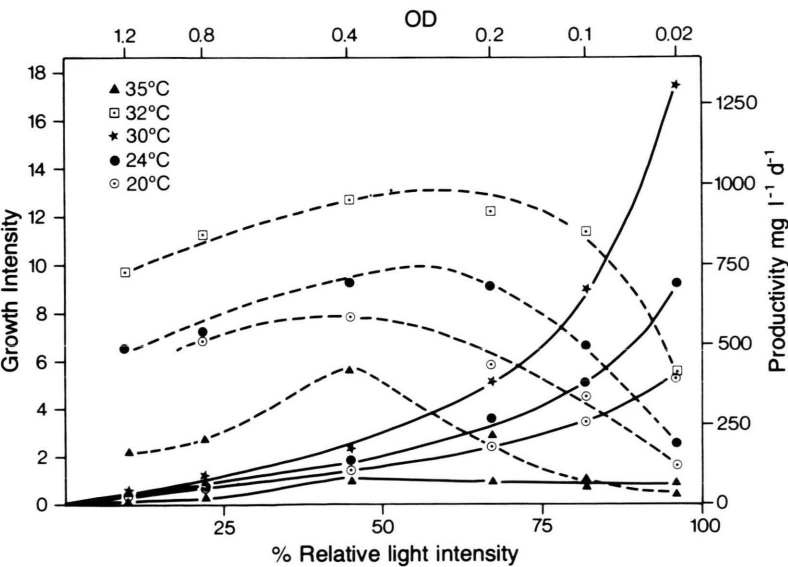


Fig. 4. Growth intensity (solid lines) and production rates (broken lines) at various temperatures against the percentage relative light intensity.

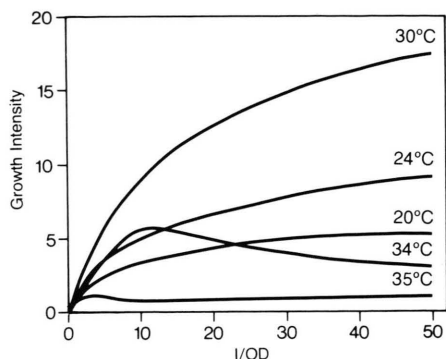


Fig. 5. Growth intensity against the inverse of optical density at various temperatures.

Discussion

The importance of irradiance and temperature interactions in determining growth intensity and productivity of microalgae is obvious from all data presented in this paper. For instance, growth intensities at temperatures just above T_{opt} depend on light availability, becoming more reduced as temperature increases. This is clearly shown by the results of 34 and 35 °C (Fig. 5) and reminds of the findings by Sorokin and Krauss [14], who found under constant illumination that growth decreased sooner at higher light intensities above the optimal temperatures.

The activation energies of 34.82 to 83.35 kJ mol⁻¹ agree well with reported values for other algae, *e.g.* a mesophilic strain of *Chlorella pyrenoidosa* [3] and various *Microcystis* species [15] (curves B and C). Christophersen [16] reported a general range of 12.6 to 84 kJ mol⁻¹ for heterotrophic microorganisms which grow in a normal temperature range. These activation energies correspond to enzyme-catalyzed reactions. Above and below the normal temperature range, much higher activation energies were found.

The deactivation energies varied between -367 and -965 kJ per mol which is again typical for enzyme-catalyzed reactions [17]. The same holds for the ratio between activation energy and deactivation energy (0.07 for O.D.₀ from 0.1 to 0.4 and 0.22 for O.D.₀ = 1.2; Table III).

According to Sorokin [3] activation energy increases with an increase in the light intensity. Krüger and Eloff [18] found the opposite for *Microcystis* sp. and explained it in terms of the possible higher light sensitivity of their test organisms. Our results displayed in Table III show both of these

phenomena: the activation energy reached a minimum at an O.D.₀ of 0.4, increasing in both directions, *i.e.* with more and less available light energy (see also Fig. 3, Q_{10} values). With an increase in activation energy, photosynthetic production must be assumed to work against a greater rate of counterreactions the exact nature of which is as yet open to speculation. It is well known that photorespiration is light dependent and increases with light intensity [19]. At a light availability higher than required for optimal productivity (Fig. 4) increasing rates of photorespiration could be responsible for the "metabolic friction" which net photosynthetic production has to cope with. If, on the other hand, light availability becomes so restricted that an increasing statistical part of the algal population approaches the compensation point or even falls below it, dark respiration has to be made up for by increased gross production. This can work only at temperatures at which the Q_{10} of photosynthesis is larger than that of respiration as is generally the case within the physiologically beneficial range of temperatures. Another possible reason for the increased activation energy required at pronounced light limitation could consist in the perhaps stressful cyclic travelling between light saturation and extreme light limitations in our highly turbulent cultures, where complete adaptation to a constant irradiance [20] cannot occur. The time scale of the cyclic travellings of cells in our cultures is in the order of seconds, *i.e.* much above the msec range required for the "flashing light effect" [21].

The curves A–C in Fig. 1 present the stressed growth of *Scenedesmus obliquus* above T_{opt} . The slopes of the lines should represent the deactivation or death rate as applied in mathematical formulations of temperature vs. growth. The decrease of the slopes in Fig. 1 and of the deactivation energies (Table III) with increasing initial O.D. indicate that the temperature stress is less at higher biomass concentrations, *i.e.* at low light availability. This is contrary to the findings of Krüger and Eloff [18] who found that dense suspensions of *Microcystis* were more sensitive to light than less dense suspensions. In their experimental set-up, CO₂ could have been limiting at higher cell densities and that they in fact measured the result of a nutrient depletion.

For each initial biomass concentration (expressed as O.D.₀) another Arrhenius equation could be produced to describe the temperature dependence

of growth intensity (Fig. 1; Table III), since the constants vary considerably with external conditions for growth. Therefore, the interpretation of a single measurement of growth intensity vs. temperature becomes almost meaningless. It could *e.g.* be concluded that the positive temperature response of *Scenedesmus obliquus* was low (see Table III, O.D.₀ of 0.4) with a Q_{10} of 1.66. On the other hand it could have been high (see Table III, O.D.₀ of 0.02 or 1.2) with a Q_{10} of 3.2. Since the Q_{10} value is the exponent of an exponential equation, the minute Q_{10} values of temperature deactivation (Table III) indicate an enormous temperature sensitivity of the heat stress above T_{opt} . The minimum of deactivation Q_{10} at O.D.₀ = 0.10 is not statistically significant (*cf.* the size of standard errors for deactivation energy in Table III).

The observed interrelations between growth intensity, temperature and available light as well as their satisfactory explicability by Arrhenius functions may well reflect some important characteristics of temperature response in algae, if not in plants, in general. However, the same strain for which we described in this paper temperature optima of 31–33 °C, may be exposed to transient temperature maxima of 45 °C under field conditions without being impaired [22]. Obviously constant

laboratory conditions are not a reliable basis to predict correctly temperature hardness in nature.

We have studied the productivity of *Scenedesmus obliquus* (SAG 276-3a) also in large-scale mass cultures under unmanipulated field conditions. A reliable prediction of biomass yields ($\text{g m}^{-2} \text{d}^{-1}$) for a wide spectrum of climatic situations was only possible by incorporating the appropriate temperature coefficients into the deterministic calculation model [6, 23]. If this is not done as, for instance, in the more simplified models of algal productivity [1], the actual yields are grossly misestimated [23].

Admittedly, massive populations of *Scenedesmus* are found in nature only in fishponds and other eutrophic, shallow waters, and temperature coefficients for most other species and types of planktonic algae are unknown. However, the principles laid down in this paper for the temperature response of autotrophic microalgal growth should be applicable to all cases in which productivity is not primarily limited by nutrient supply.

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