Selection of Algal Species For Use In Open Outdoor Mass Cultures*

MARELIE M.J. VAN VUREN AND JOHAN U. GROBBELAAR
Institute for Environmental Sciences, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300

Abstract

Microalgae can tolerate a wide spectrum of environmental conditions, have high growth rates and contain proteins in excess of 50% per unit of dry mass. Their use in food production, waste treatment and energy harvesting operations have become attractive possibilities in alleviating shortages and treating wastes. The suitability of an algal species for such uses depends on: nutritional value; high growth rates; harvestability; tolerance to environmental conditions; and resistance to infections. The use would largely determine the choice of alga and for this reason, algal species were subjected to various tests. Activation energy, Arrhenius equations, Q_{10}-values, yield coefficients, half-saturation constants, protein contents and growth rates were determined.

Introduction

Interest in the mass cultivation of microalgae has grown tremendously over the past 30 years. Before World War II, the prime interest of plant physiologists and biochemists was to unravel the mysteries of photosynthesis. In these studies, however, small quantities of algal material usually sufficed. The large scale production of algal biomass began in Germany in 1942 (Soeder, 1980), with the main emphasis on food production.

Chlorella and *Scenedesmus* (the well studied, hardy and easy to grow fresh-water algae) are the favourite types used in the mass cultivation of algae throughout the world, especially in Germany, Japan, United States, Taiwan, Czechoslovakia, Israel, France, USSR, Mexico, Netherlands, Belgium, and South Africa. Other algae that have been used include *Coelastrum* (Germany), *Micractinium* (California), *Dunaliella* (Israel), *Spirulina* (Mexico, Germany, Israel, France, USSR), *Anabaena* (USA, USSR). Investigations concerning the utilization of various algal species for use in mass algal cultures are few. The three major approaches to mass algal culture i.e. food production, waste treatment and bioenergy conversion (Grobbelaar, 1982) would mean different requirements of the algae used in each application. The criteria used to select an alga for each application can be grouped into two major sections, with various subdivisions. These are:

1. General characteristics, which include high specific growth rates, harvestability, and predator resistance (infection).

2. Specific characteristics which apply to the particular application, i.e.

   2.1 For food production (high protein content, good digestibility, and favourable amino acid content).

   2.2 For waste treatment (high nutrient uptake capability, possibility of heterotrophic growth, capability of tolerating low O_2 levels, and good resistance to bacterial contamination).

   2.3 For bioenergy conversion (high carbohydrate content for fermentation purposes, and low respiration and organic excretion rates).

Research undertaken at the Institute for Environmental Sciences is directed towards establishing which of the above characteristics occur in a large variety of algal species for eventual use in open mass algal cultures. It is aimed to use the results in order to select the most suitable alga for a specific application. Preliminary results of temperature, light intensity and nutrient concentrations will be reported on in the paper.

Materials and Methods

Although it is planned to test various algal species, only results obtained with *Scenedesmus bijugates* and a *Chlorella* species (isolated from the Amazon River) will be presented.

Stock cultures of the above were cultured in a modified BG-11 nutrient medium (Krüger & Eloff, 1978) at *ca.* 25°C and 80 μ Einst. m^{-2} s^{-1}. The influence of temperature on growth was determined in a temperature gradient incubator (Scientific Industries Inc., New York). The method of incubation was the same as Krüger & Eloff (1978) except for an air mixture containing 0,5% CO_2 which was used to enrich the air phase inside the culture tubes. Incubations were done at a temperature range from 0 to 50°C. Each tube contained 10 mℓ culture and turbidity, measured with a Klett-Summerson photoelectric colorimeter equipped with a 540 nm filter (green), was used as an indicator of biomass. Biomass estimations were made every 2 to 4 h. These were plotted on one cycle logarithmic paper and the exponential phase of growth was determined from these plots.

When light intensity is constant, it is possible to describe the maximum growth rate solely as a function of temperature by applying the Arrhenius equation (Goldman & Carpenter, 1974):

$$\mu = Ae^{-E/RT} \quad \ldots\ldots 1$$

in which A = constant; the Arrhenius frequency factor (day^{-1}); E = activation energy or temperature characteristic (cal mol^{-1}); R = universal gas constant (cal $°K^{-1}$ mol^{-1}); and T =

*Revised paper, originally presented at *Symposium on aquaculture in wastewater*, 24–26 November 1980, CSIR Conference Centre, Pretoria

temperature (°K). The energy of activation was determined by employing the logarithmic version of the Arrhenius equation:

$$\log_e \mu = \log_e A - E/2{,}303\ RT \quad \ldots\ldots 2$$

and plotting log μ against 1/T. The gradient of the line equalled $-E/2{,}303R$. The gradients of the lines were computed by linear regression analysis. Once the activation energy was calculated for a particular organism, the Arrhenius frequency factor, A, could be calculated. The temperature coefficients (Q_{10}) of the test organisms at temperature ranges, were calculated by the following equation being deducted from the Arrhenius equation (Pirt, 1975):

$$\log Q_e = \frac{E}{2{,}303R} \times \frac{10}{(T+10)T} \text{ (Calculated from } 10-20°C) \quad \ldots\ldots 3$$

The influence of light intensity was determined in a high light intensity incubator with constant temperatures as described by Van Vuren (1979). Biomass was determined with a Coulter Counter Model B (Serial No B 3502).

The response to N, P and K were determined in batch cultures of 100 mℓ in volume. Before a nutrient was assayed, the cells were first starved for a week prior to inoculation. Air was bubbled through the cultures and biomass was determined as turbidity with the Klett-Summerson photo-electric colorimeter or as total organic carbon using a Beckman Model 915A Organic Carbon Analyzer. Exponential growth was determined as for the temperature experiments after plotting biomass against time. The half-saturation constant (K_s) was determined from the plots of growth rate versus nutrient concentration. Crude protein was calculated from total organic nitrogen x 6,25.

Results

Temperature

An Arrhenius plot of the influence of temperature on growth rate of *Scenedesmus bijugates* is shown in Figure 1. Two slopes, A-B and B-C, can be seen with the intersection at 33,5°C. Between 5 and 33,5°C a Q_{10} of 1,38 and activation energy of 5,25 k cal mol^{-1} were calculated. The growth rate decreased rapidly above 33,5°C. *S. bijugates* therefore has an optimum temperature at 33,5°C and a maximum of about 43°C at a light intensity of 150 μ Einst. m^{-2} s^{-1}.

The influence of different light intensities at various temperatures on the growth rate of *S. bijugates* are shown in Figure 2 where Arrhenius plots are presented. The growth rates are lower than those presented in Figure 1 for the same organism. This is attributed to C-limitation as air was supplied to the cultures and not air enriched with CO_2, and the measurements were made 12 hourly and not 2 to 4 hourly as in the first case. These results may, therefore, not reflect optimum conditions. However, the influence of different light intensities on the growth rate at various temperatures can be seen. An increase in the light intensity, raised the optimum temperature by about 5°C.

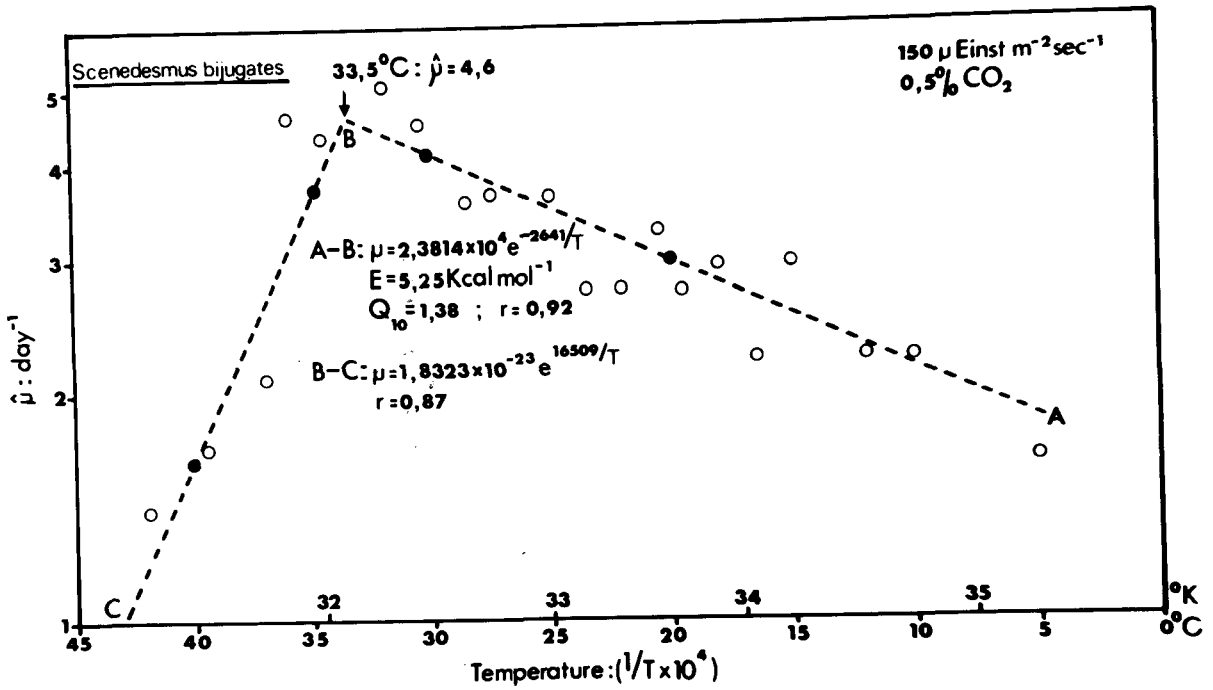

Figure 1
Arrhenius plot of the influence of temperature on growth rate (μ) of Scenedesmus bijugates

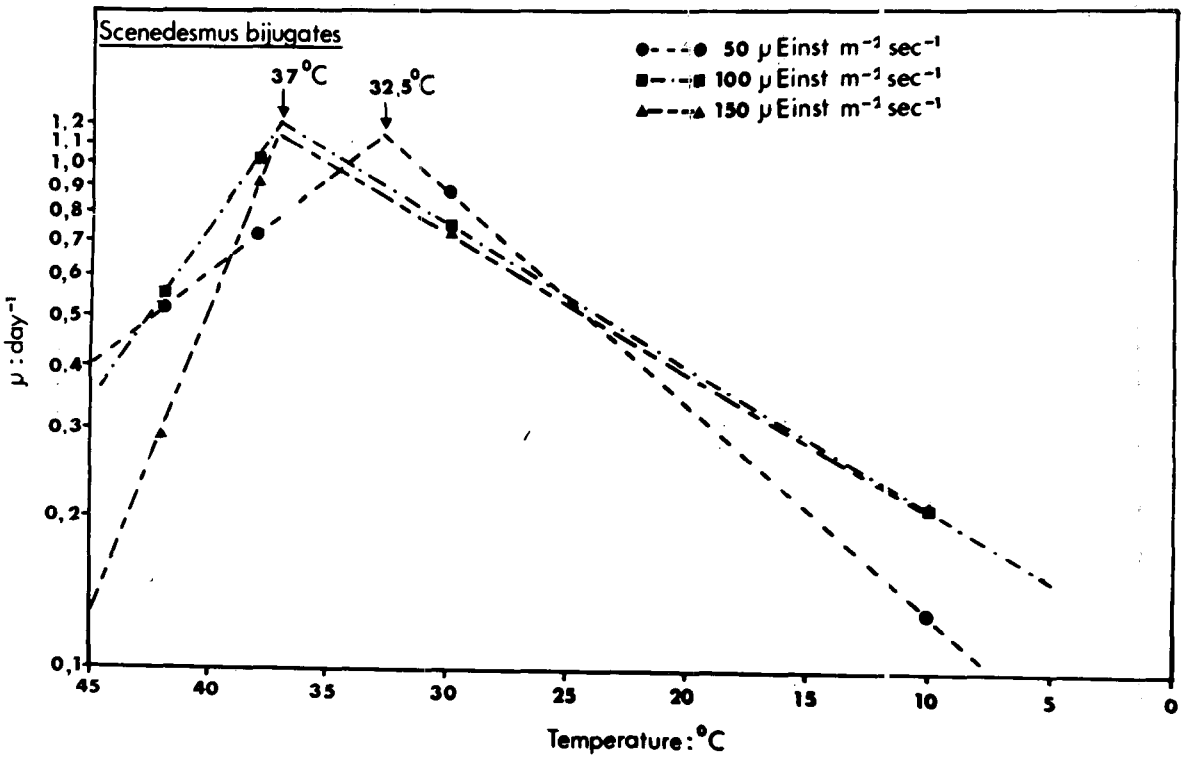

Figure 2
Arrhenius plots of the influence of temperature on the growth rate of S. bijugates *at different light intensities*

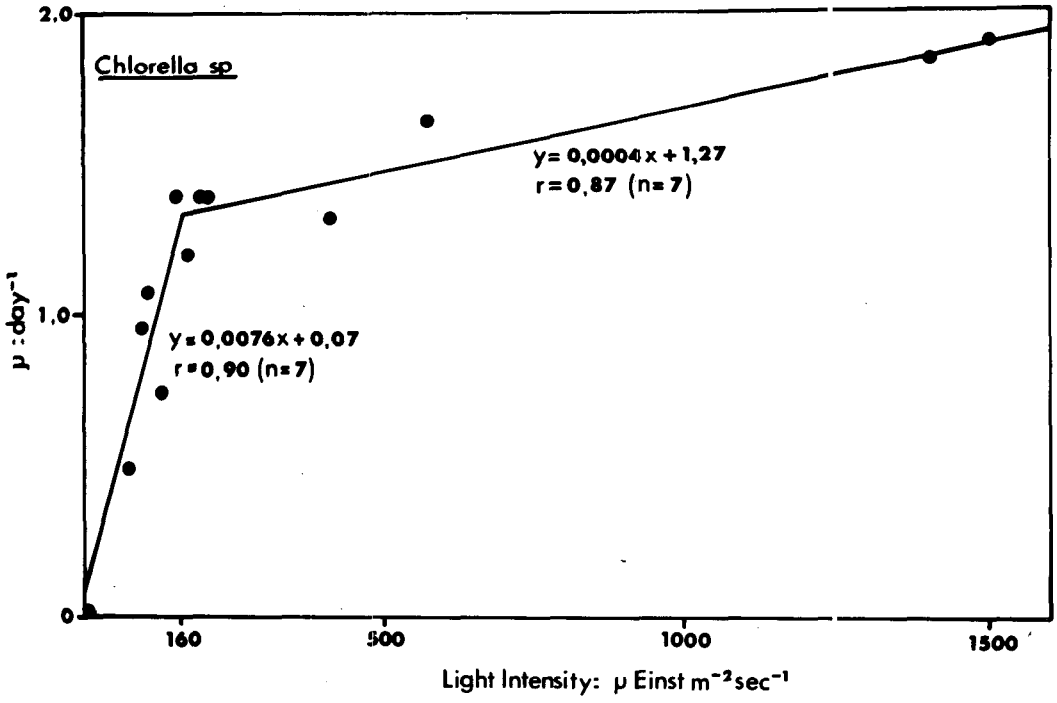

Figure 3
The influence of different light intensities on the growth rate of a Chlorella *sp. at 20°C*

Light intensity also appears to have an influence on the maximum and minimum temperatures (Figure 2) which can be tolerated. The angle between the two slopes decreases with increased light intensity. The angle at 50 μ Einst. m^{-2} s^{-1} is 112°, at 100 μ Einst. m^{-2} s^{-1} is 95° and at 150 μ Einst. m^{-2} s^{-1} it is 82°.

Light intensity

The influence of different light intensities on the growth rate of a *Chlorella* sp. at 20°C is shown in Figure 3. The light dependent and light saturated sections of the curve can be seen with the inflection point at 160 μ Einst. m^{-2} s^{-1}. This intersection light intensity is defined as the parameter I_k and can vary from about 20 to 300 μ Einst. m^{-2} s^{-1} (Harris, 1978).

Nutrients

A typical Monod type of relationship between N (Figure 4 & 5) and P (Figure 6) and growth rate was found. Maximum growth of *S. bijugates* occurred at concentrations greater than 20 mg NO_3—N ℓ^{-1} with a half-saturation constant (K_s) of 1,8 mg NO_3—N ℓ^{-1} (Figure 4). The maximum growth of a *Chlorella* sp. was at NO_3—N concentrations greater than 20 mg ℓ^{-1} (Figure 5) and PO_4—P concentrations greater than 5 mg ℓ^{-1} (Figure 6). The K_s-values for this alga were 3 mg NO_3—N ℓ^{-1} and 0,38 mg PO_4—P ℓ^{-1} respectively.

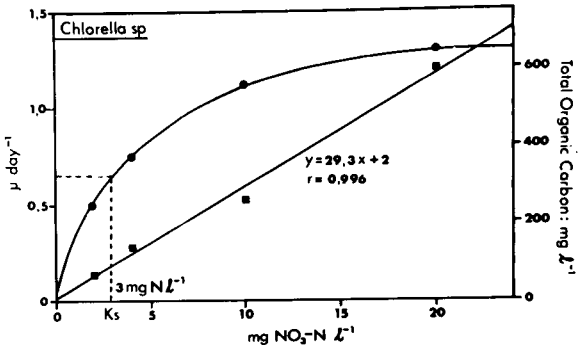

Figure 5
The influence of various NO_3—N concentrations on the growth rate and Total Organic Carbon content of a Chlorella *sp. (after Grobbelaar, 1982 in press)*

The influence of various K-concentrations on the crude protein content of *S. bijugates* is shown in Figure 7. The protein values above 580 mg K ℓ^{-1} were calculated from an equation derived of measurements below 580 mg K ℓ^{-1}. The crude protein content varied between 30 and 47% per dry mass, with the maximum protein content at K-concentrations above 2 254 mg ℓ^{-1}.

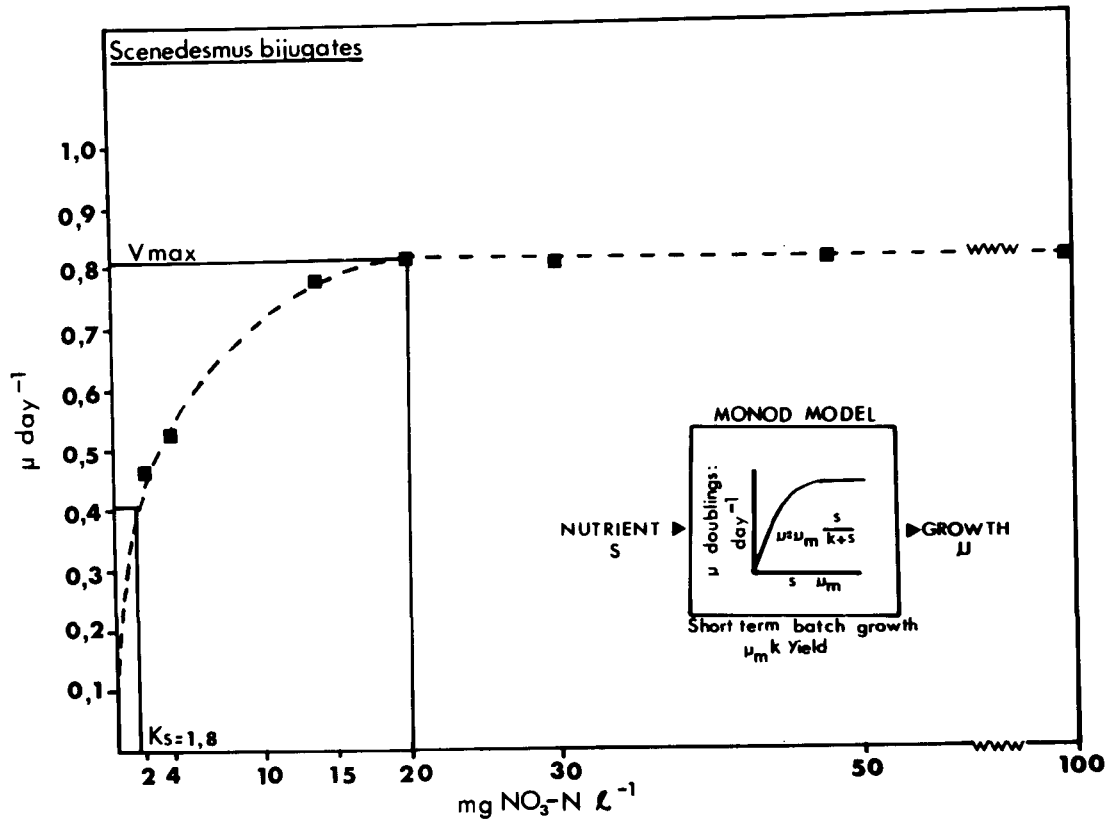

Figure 4
The influence of various NO_3—N concentrations on the growth rate of Scenedesmus bijugates *(Temperature 25°C; I = 80 μ Einst. m^{-2} s^{-1}*

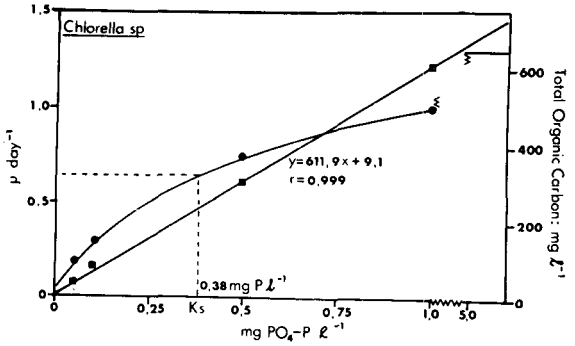

Figure 6
The influence of various PO_4– P concentrations on the growth rate and Total Organic Carbon content of a Chlorella *sp. (after Grobbelaar, 1982 in press)*

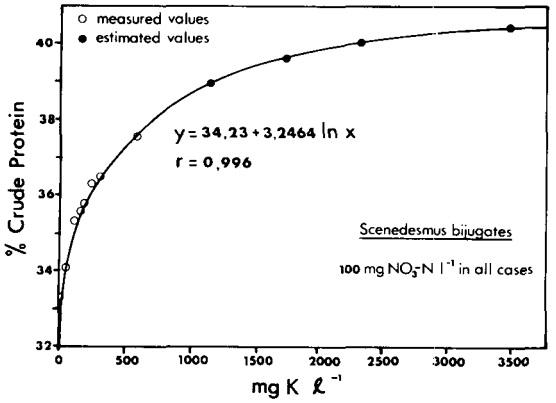

Figure 7
The influence of various K concentrations on the crude protein content of S. bijugates

Discussion

An important aspect of mass algal cultures is the development of mathematical models, especially in terms of establishing design criteria, and identifying research needs (Grobbelaar, 1981). The use of reliable parameter values (e.g. Q_{10}, I_k, K_s etc.) are of cardinal importance in model building, calibration and eventual verification. Results obtained from investigations concerning influences of temperature, light intensity and nutrients on the growth rate, are not only important for selecting suitable algal types, but also for modelling.

Temperature influences the rates of biochemical processes and the distribution of algal species in nature. There exists an interplay between temperature, light intensity and nutrients. Because of this, it is often difficult to isolate the specific influence and relate response of e.g. growth rate to one such factor. An Arrhenius equation describes the relationship between temperature and growth rate best if no other factor is limiting (Goldman and Carpenter, 1974) and a plot of $\log_e \mu$ against $1/T$ (μ = maximum specific growth rate and T = °Kelvin) gives a straight line.

Sorokin (1960) and Krüger and Eloff (1978) recorded changes in the slopes at different temperatures. Sorokin (1960) attributed these changes to the existence of different master reactions. These have, however, not been identified. Krüger and Eloff (1978) offer no explanation for the four changes in slopes, except for possibly limiting conditions which could be responsible for one of these changes. The results presented in Figures 2 and 3 show a steady increase in growth rate with increased temperature up to an optimum temperature, whereafter the growth rate decreases with increased temperature.

The increase between points A and B is gradual, compared to the decrease between points B and C. No slope changes were recorded at temperatures below 33°C. It is therefore possible that some or other factor became limiting during the experiments of Krüger and Eloff (1978). The activation energy of 5,25 k cal mol^{-1} is well within the reported range for algae and corresponds to those obtained by Krüger and Eloff (1978) at temperatures between about 13 and 25°C. The Q_{10} value of 1,38 (Figure 1) appears to be somewhat low because Eppley (1972) recorded 1,88 and Goldman and Carpenter (1974) 2,08 and 2,19 for a variety of organisms. It should however be noted that much higher growth rates were recorded than those recorded by the previous authors. Harris (1978) showed that the Q_{10} value of phytoplankton is greatly influenced by light intensity. Referring to work of Piccinin (1976), a Q_{10} of only 1,0 was recorded at 6,4 μ Einst. m^{-2} s^{-1}; 1,675 at 99 μ Einst. m^{-2} s^{-1} and 2,0 at 1 280 μ Einst. m^{-2} s^{-1}. It is therefore reasonable to expect higher Q_{10} values for *S. bijugates* at saturating light intensities. Our experiments were done at 150 μ Einst. m^{-2} s^{-1}.

The results presented in Figure 2 clearly show the close relationship between temperature and light intensity and its influence on the growth rate. Grobbelaar (1982) has used a deterministic production model to construct three-dimensional graphs showing this interaction. The data presented in Figure 2, however, show a decrease in the angle between the two slopes with increased light intensity. Similar influences can be seen in the plots of Beljanin and Trenkensu (1977), and Tetik and Necas (1977). The latter authors remarked that light intensities which would inhibit *Calamydomonas geitleri* at high temperatures, would not do so at low temperatures. The results presented in Figure 2, indicate that *S. bijugates* would have a greater temperature tolerance at low light intensities than at higher light intensities. The ecological implication of this observation is not clear.

The effect of light intensity on the growth rate is similar to many examples shown in the literature — see e.g. Harris (1978). The value of 160 μ Einst. m^{-2} s^{-1} for I_k at 20°C agrees well with that of Harris (1978), plotted from Talling (1957).

K_s values for N and P are considered important characteristics of organisms living in N- or P-limiting environments. There appear to be considerable differences in K_s-values reported in the literature, ranging for N from 1,35 — 2 250 μg N ℓ^{-1} (see e.g. Zevenboom and Mur, 1978), and for P from 0,216 — 9,27 μg P ℓ^{-1} (see e.g. Steemann Nielsen 1978). The K_s values given in Figures 4, 5 and 6 are considerably higher, being 3 000 and 1 570 μg N ℓ^{-1} and 380 μg P ℓ^{-1}. A possible explanation may well be that great care was taken in the cultures to ensure that nothing but the factor tested for was limiting and that the growth rate was determined from the logarithmic phase. We have found great discrepancies in results, when for example two pre-determined times were chosen for biomass determinations for growth rate calculations. It was necessary to estimate biomass changes at intervals as short as two hours in some cases, before certainty of logarithmic growth could be obtained. This was especially so at very low nutrient concentrations.

Mostert and Grobbelaar (1981) have shown that the protein content of algae could be manipulated by varying the N-content of the growth medium. This was especially prominent at nitrogen concentrations below 25 mg ℓ^{-1}. The importance of potassium in the growth medium has generally been neglected. Pribil and Marvan (1970) demonstrated the importance of good K supply to algae for good growth and cell division. The results shown in Figure 7 clearly demonstrates the importance of K-concentration on the crude protein content of the cells. In these experiments nitrogen was supplied as NO_3-N at a constant concentration of 100 mg ℓ^{-1}. The rest of the nutrients were as in BG-11 nutrient medium.

The protein content could be manipulated from 30 to 47% over a K-concentration range of 22 mg to 2 254 mg ℓ^{-1}. The potassium content of most nutrient solutions is below 10 mg ℓ^{-1} (see e.g. Rodhe, 1978) and may reach 100 mg ℓ^{-1} in some cases. The results shown in Figure 7 demonstrate the importance of a good potassium supply to algae. Potassium is an important activator of enzymes (O'Kelley, 1974) and research on its role in algal inorganic nutrition is needed.

Conclusions

Results on the influence of temperature, light intensity, and nutrient supply on the growth rate of algae are important. Especially for:

1. their characterization in terms of growth constants such as μ, I_k, K_s for N and P and optimum conditions;
2. understanding of the response of an organism to environmental changes and conditions; and for
3. establishing parameter values for use in mathematical model building.

It is envisaged to devise a series of tests, to which prospective algal strains could be subjected, in order to classify them according to their possible utilization, as described in the introduction of this paper. The problem of using these results from "standard" tests are, however, recognized. Ruzicka and Simmer (1970) listed several imperfections of laboratory algal tests, and these will be avoided as far as possible.

The work reported on in this paper is in its initial phase and is continuing.

Acknowledgements

The authors are indebted to Professor D.F. Toerien, director of the Institute for Environmental Sciences for his keen interest and support and the University of the O.F.S. and Council for Scientific and Industrial Research for financial aid.

References

BELJANIN, V.N. and TRENKENSU, A.P. (1977) Growth and spectrophotometric characteristics of the blue-green alga *Synechococcus elongatus* under different temperature and light conditions. *Arch. Hydrobiol. Suppl.* **51** Algological studies 18 46–66.

EPPLEY, R.W. (1972) Temperature and phytoplankton growth in the sea. *Fish. Bull.* **70** 1063–1085.

GOLDMAN, J.C. and CARPENTER, E.J. (1974) A kinetic approach to the effect of temperature on algal growth. *Limnol. Oceanogr.* **19** 756–766.

GROBBELAAR, J.U. (1981) Deterministic production model for describing algal growth in large outdoor mass algal cultures. *In: Wastewater for Aquaculture,* Grobbelaar J.U., C.J. Soeder and D.F. Toerien (Eds.). UOFS Publ., Series C, No 3, 173–181.

GROBBELAAR, J.U. (1982) Potential of algal production. *Water S.A.* **8**(2) 79–85.

HARRIS, G.P. (1978) Photosynthesis, productivity and growth: The physiological ecology of phytoplankton. *Arch. Hydrobiol., Beih. Ergebn. Limnol.* **10**(I–IV) 1–171.

KRÜGER, G.H.J. and ELOFF, J.N. (1978) The effect of temperature on specific growth rate and activation energy of *Microcystis* and *Synechococcus* isolates relevant to the onset of natural blooms. *J. Limnol. Soc. Sth Afr.* **4** 9–20.

O'KELLEY, J.C. (1974) Inorganic nutrients. In: W.D.P. Stewart (Ed.) Algal physiology and biochemistry. *Botanical Monographs* **10** 610–635.

MOSTERT, E.S., and GROBBELAAR, J.U. (1981) Protein manipulation. *In: Wastewater for Aquaculture.* Grobbelaar, J.U., C.J. Soeder, and D.F. Toerien (Eds.). UOFS, Publ., Series C, No. 3, 86–90.

PICCININ, B.B. (1976) Photosynthesis by natural phytoplankton populations and its interrelationship with light and temperature. M.Sc.-thesis, McMaster University Hamilton, Ontario, 119 pp.

PIRT, S.J. (1975) Principles of microbe and cell cultivation. Blackwell Scientific Publications, Oxford, 274 pp.

PRIBIL, S., and MARVAN, P. (1970) Der Verlauf des Mineralnährstoffbedarfes in der Kultur van *Scenedesmus quadricauda* (Turp.) Breb. 1 Kalium-Aufnahme. *Algol. Studies* (Trebon) **1** 41–56.

RODHE, W. (1978) Algae in culture and nature. *Mitt. Internat. Verein. Limnol.* **21** 7–20.

RUZICKA, J. and SIMMER, J. (1970) Measurement of productivity of algal strains by characteristic constants. *Algol. Studies* (Trebon) **1** 33–40.

SOEDER, C.J. (1980) Massive cultivation of microalgae: Results and Prospects. *Hydrobiologia* **72** 197–209.

SOROKIN, C. (1960) Kinetic studies of temperature effects on the cellular level. *Biochim. Biophys. Acta* **38** 197–204.

STEEMANN NIELSEN, E. (1978) Productivity and Modelling. Growth of the unicellular alga *Selenastrum capricornutum* as a function of P. With some information also on N. *Verh. Internat. Verein. Limnol.* **20** 38–42.

TALLING, J.F. (1957) Photosynthetic characteristics of some freshwater plankton diatoms in relation to underwater radiation. *New Phytol.* **56** 29–50.

TETIK, K. and NECAS, J. (1977) Growth characteristics of *Chlamydomonas geitleri. Arch. Hydrobiol/Suppl.* **51**, *Algological studies* **19** 164–183.

VAN VUREN, M.M.J. (1979) Die invloed van ligintensiteit en ligkwaliteit op die groei van *Microcystis.* M.Sc.-thesis, University of the Orange Free State, 102 pp.

ZEVENBOOM, W. and MUR, L.C. (1978) N-uptake and pigmentation of N-limited chemostat cultures and natural population of *Oscillatoria agardhii. Mitt. Internat. Verein. Limnol* **21** 261–274.