INFLUENCES OF EXTERNAL FACTORS ON THE ENERGY CONVERSION AND PRODUCTIVITY OF *SCENEDESMUS* SP. IN MASS CULTURE

J. C. WESSELIUS

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Dit proefschrift met stellingen van:

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De Rector Magnificus van de Landbouwhogeschool,
H. A. LENIGER

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(Met een samenvatting in het Nederlands)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS,
PROF. DR. IR. H. A. LENIGER,
HOOGLERAAR IN DE TECHNOLOGIE,
IN HET OPENBAAR TE VERDEDIGEN OP
VRIJDAG 15 JUNI DES NAMIDDAGS TE VIER UUR
IN DE AULA VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN

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Aan mijn ouders
Aan mijn gezin

STELLINGEN

1

Bij lichtverzadiging is de groei van *Scenedesmus* het hoogst bij een dagelijkse lichtperiode van 12 uur, omdat bij deze daglengte synchronisatie op kan treden.

dit proefschrift.

2

Het rendement van de lichtomzettingen bij *Scenedesmus* is bij vergelijkbare partiële CO_2-spanningen, temperatuur en lichtintensiteit lager dan bij landbouwgewassen met een gesloten gewasoppervlak, omdat de diffusiecoëfficiënt voor CO_2 in water veel hoger is dan die in lucht.

dit proefschrift.

3

Er zijn redenen om aan te nemen, dat bij algen de nitrietreductie en niet de nitraatreductie gebruik maakt van electronen die door de fotosynthese worden geleverd.

BEEVERS, L. and HAGEMAN, R. H.; Ann. Rev. Plant Physiol. **20**: 495–522 (1969).
PANEQUE, A., *et al*; Biochim. Biophys. Acta **162**: 149–151 (1968).
LOSADA, M., *et al*; in: METZNER, H., ed.; Progress in Photosynthesis Research Vol. III, H. Laupp Jr., Tübingen (1969): 1504–1509.
dit proefschrift.

4

De gevonden verschillen in fysiologische en biochemische eigenschappen tussen zon- en schaduwecotypen van zowel *Solanum* als *Solidago* wijzen op een afwijkende waterbalans.

BJÖRKMAN, O.; Physiol Plant. **21**:84–99 (1968).
BJÖRKMAN, O. and HOLMGREN, O.; Physiol. Plant. **16**:889–914 (1963).
GAUHL, E.; Carnegie Inst. Year Book 1967–1968: 482–487 (1969).
HOLMGREN, P.; Physiol. Plant. **21**: 676–698 (1968).

5

Er is onvoldoende grond om bij aanwezigheid van zuurstof in het voedingsmedium van al of niet heterocysten vormende blauwwieren N_2-fixatie in vegetatieve cellen te veronderstellen.

GORKOM, H. J. VAN, en DONZE, M.; Nature **234**: 231–232 (1971).
STEWART, W. D. P., *et al*; Nature **224**: 226–228 (1969).
STEWART, W. D. P., en LEX, M.; Arch. Mikrobiol. **73**: 250–260 (1970).
TAYLOR, B. F., *et al*; Arch. Mikrobiol. **62**: 336–348 (1969).

6

Analoog aan de GA- stimulering van secretie en synthese van hydrolytische enzymen zouden soortgelijke processen door GA, ABA en ethyleen bij de abscissie van bladeren plaats kunnen vinden.

BIGGS, R. H.; Hort. Sc. **6**: 36–40 (1971).
LEWIS, L. N., en VARNER, J. E.; Plant Physiol. **46**: 194–199 (1970).
ABELES, F. B., *et al*; Hort. Sc. **6**: 371–376 (1971).

7

De opvatting dat de invloed van ver-rode straling op de morfogenese slechts via het fytochroomsysteem zou werken is onvoldoende gefundeerd.

SCHNEIDER, M. J., en STIMSON, W. R.; Plant Physiol. **48**: 312–315 (1971).
MARONER, M., en UNSER, G., en MOHR, H.; Planta **105**: 267–272 (1972).
HARTMANN, K. M.; Photochem. Photobiol. **5**: 349–366 (1966).
BELLINI, E., en HILLMANN, W. S.; Plant Physiol. **47**: 668–671 (1971).

8

Het diffusiemodel van de fotosynthese is een simplificatie waarvan de bruikbaarheid twijfelachtig is, daar ademhaling, fotorespiratie en de fotosynthetische binding van CO_2 moeilijk in het model te plaatsen zijn.

CHARTIER, P.; Ann. Physiol. Végét. **8**: 167–196 (1966).
JONES, H. G., en SLATYER, R. O.; Plant Physiol. **50**: 283–288 (1972).
LAISK, A., in: Proceed. IBP/PP Techn. Meeting Třeboň, 1969; PUDOC, Wageningen (1970): 295–304.

9

Indien de ATP-behoefte voor de fotosynthetische reductie van CO_2 in C_4-planten groter is dan die in C_3-planten behoeft dit nog geen konsekwenties te hebben voor de efficiëncy van het fotosyntheseproces. Laatstgenoemde efficiëncy wordt namelijk uiteindelijk bepaald door de gesynthetiseerde eindprodukten.

BULL, T. A.; Crop Sci. **9**: 726–729 (1969).
HATCH, M. D.; Proceed. IBP/PP Techn. Meeting Třeboň, 1969; PUDOC, Wageningen (1970).

10

Denkbeelden als "natural selection", competitie tussen individuen van een soort en competitie tussen organen van een plant roepen de gedachte op dat de natuur volgens een strijd- en concurrentieprincipe is opgebouwd. Er is evenwel niet zoveel voor te zeggen de nadruk te leggen op integratie als verbindend element.

11

De enkeling heeft geen reden zijn verantwoordelijkheid inzake de milieuproblematiek uit gevoelens van machteloosheid of vermeende incompetentie ter zijde te schuiven. Een van de voorwaarden voor een oplossing moet namelijk in beginsel in eigen voelen en willen worden gezocht.

Proefschrift van J. C. WESSELIUS
Wageningen, 15 juni 1973

VOORWOORD

In de eerste plaats ben ik mijn wederzijdse ouders heel veel dank verschuldigd. Ik hoop, dat al Uw goede zorgen hun zin zullen krijgen door het feit dat ik werk kan doen dat ik met plezier verricht; het hier voor U liggende boekje moge daar een weerspiegeling van zijn.

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CONTENTS

1. INTRODUCTION

1.1. INTRODUCTORY STATEMENT

The sun is the major source of energy for life on earth. About one half of the total radiation energy received on the earth is in the visible part of the spectrum and can be used in photosynthesis. By way of modern agriculture it is possible to fix 1–2 % of the photosynthetically active radiation, calculated on a year basis. Only a small part of the year a closed crop surface exists. During this period, conversion of light energy is much better, reaching values of 5–10 %. The trend of the investigations found in literature is that longer periods of closed crop surfaces result in higher yields per unit area, cf. ALBERDA (1962), BROUWER (1962), GAASTRA (1962), DE WIT (1959, 1966).

Selection of special varieties may result in prolonging the period of optimal light utilization. Although the total yield/unit area will improve a little bit in this way, a light energy conversion much higher than 5–10 % is rather improbable.

Algae were believed to be better producers than higher plants. They offered advantages on higher plants: culture conditions were rather easy to maintain, they had a short generation time, and their nitrogen content could be very high (2–11 %) as compared with 1.5 % in maize (cf. KRAUSS, 1953).

It seemed attractive, therefore, to cultivate algae on a large scale as a source of food, especially to deal with the food shortage which exists in several parts of the world. A lot of investigations about mass culturing were carried out during the period 1945–1955, but after that interest waned (cf. BURLEW c.s. 1953). Yield values were disappointingly low which made algae less attractive as economic food producers. At some places they are used for the biological oxidation of metropolitan wastes. The production of algal material is a matter of secondary importance in these cases; they can be used e.g. as cattle fodder (cf. GOLUEKE and OSWALD 1964). In some East-European countries and in Japan, research on algal mass culture is still done, especially with the purpose to obtain a cheap protein source.

In order to provide the basis for a discussion of the possibilities of mass culturing of algae it seemed unavoidable to pay more attention to the culture requirements of the algae. Therefore, it seemed worth while to carry out a thorough investigation concerning the influence of external factors on energy conversion in mass cultures. In this way it should be possible to predict yield values throughout the growing season and to give maximum levels for light energy conversion under natural daylight which should be the ultimate aim.

1.2. Survey of the literature

An excellent survey about the efficiency of photosynthesis is given by Kok (1960). The original and later measurements of the quantum yield for photosynthesis as measured by Warburg and his school were discussed in the light of new material collected by Emerson and Lewis (1941), Kok (1948), Spruit and Kok (1955) and others.

Warburg and Negelein (1922, 1923) estimated the quantum yield for photosynthesis at 0.25 by way of manometric methods. However, there are strong arguments to support the assumption that in these measurements transistory effects, such as a large CO_2-gush in the first seconds of the light period may have interfered and have made the data less reliable (cf. Emerson and Lewis, 1939, Spruit and Kok, 1955).

Rabinowitch surveyed the maximum quantum yields Emerson and Lewis obtained for green and blue green algae (cf. Rabinowitch pp. 1095). The general trend was that maximum quantum yields of 0.10 and lower were obtained. Wassink (1946) measured quantum yields for O_2-production in horticultural plants reaching values ranging from $\gamma = 0.023$ up to 0.092. Gaastra (1959) measured photosynthetic rates with the infrared analyzer at various CO_2-levels. An average quantum yield of 0.10 was calculated for turnip, tomato and cucumber.

In practice, high quantum yields are only obtained at low light intensities. For the cucumber leaf Gaastra (1962) found that photosynthetic activity was rather constant at low light intensity, but varied strongly at higher light intensities.

Maximum photosynthetic efficiency can only be expected at low light intensities. At high light intensities, the diffusion of CO_2 from the air to the reaction centre in the cells may become a limiting factor. The large variation in the 'mesophyll resistance', (cf. Gaastra, 1959, 1962) is one of the factors considered in explaining the variation of the photosynthetic activity at high light intensities.

Thomas and Hill (1949) calculated an efficiency of 16% for alfalfa under light limiting conditions in the field. Gaastra (1962), using mercury light and leaves from different plant species, estimated the maximum photosynthetic efficiency to be 12.5%. Both authors used normal air, i.e. with 0.03% CO_2. Even with closed crop surfaces, lower values for the efficiency of light energy conversion during the growing season are reported, viz. 4–9% for sugar beet (Gaastra, 1958, 1962), and 6–7% for grassland (Alberda, 1962). Wassink (1948) calculated the efficiency in crop plants over the whole season to be 2% or less. Wassink's figures differ from the ones mentioned above since also periods with an incomplete leaf coverage of the soil are necessarily included.

It may be asked whether maximum efficiencies of light energy conversion in photosynthesis and in growth are equal. Kok (1952) measured efficiencies for growth in unicellular algae in a Warburg apparatus in sodium light, using diethanolamine as a CO_2-buffer. The algae were suspended in culture solutions with a high and a low nitrogen content. Photosynthesis, respiration, and energy

fixed were determined during the experiment. This enabled a comparison between the efficiencies for growth and photosynthesis. The efficiencies for growth varied largely, depending on environmental conditions. The best growth efficiencies were comparable with the maximum photosynthetic efficiencies of 20–25% in suitable culture solutions.

VAN OORSCHOT (1955) estimated the production rates of *Chlorella* in stirred solutions of 300 l volume, using natural daylight; there was no temperature control. He calculated the fixed energy by multiplying the daily production with the average caloric value of the material (measured in a caloric bomb). Dividing the fixed energy by the incident energy, he obtained the net photosynthetic efficiency which was 1–4%.

A decrease of light intensity by screening algal mass cultures resulted in better light energy conversion, ranging from 4.5% at 84% daylight to 7.7% at 25% daylight. The reason for this improvement is that light saturation of the photosynthetic apparatus in a single cell is already reached at 0.05 cal $\cdot$ cm$^{-2}\cdot$ min^{-1}, whereas the maximum photosynthetically active radiation (400–700 mμ) in the daylight amounts to 0.5–0.6 cal $\cdot$ cm$^{-2}\cdot$ min^{-1}. Exposing a single cell to natural daylight thus gives a saturation of photosynthesis for a large part of the day, and therefore a low efficiency of energy conversion. VAN OORSCHOT (1955) found energy conversions of about 8% in KOLLE dishes exposed to the south under an angle of 45 degrees. Probably, this is due to a better gas flow and a more suitable dimension of the culture vessel in relation to the volume of algal suspension cultivated in it.

ANSELL c.s. (1963a, 1963b) studied the efficiency of energy conversion of *Phaedatylum* cultures in fertilized sea water. Using flue gas to reduce pH and to deliver the necessary CO_2, they arrived at efficiency values ranging from 3.0–4.4% for *Phaeodactylum* and 1.0–1.3% for *Tetraselmis*. These figures are comparable with those obtained by VAN OORSCHOT in 300 l outdoor tanks.

For the cultivation of algae several culture methods have been devised. TAMIYA (1957) reviewed this subject until 1957. Algae have been cultivated in open ponds, trenches, boxes, or in closed circuits of some lucite material through which the suspension is driven by a pump. Settling of cells is mostly prevented by shaking or rotating the suspension or by bubbling a gas mixture of air and CO_2 through it. A research group in Czechoslovakia devised an open system in which algae are pumped on a glass platform which inclines a few degrees. On their way down, the fall is broken by vertical sheets. In this way the suspension becomes turbulent like water in a cascade (PROKEŠ and ZAHRADNÍK, 1969, 1970). Not all culture systems used proved to be successful. Experiments with open systems deal with losses of CO_2, especially when the suspension is violently shaken. The group of DAVIS, c.s. (1953) investigated several types of culture vessels; e.g. bottles (5 gallons): production rate 4.8 g$\cdot$m$^{-2}\cdot$ day^{-1}, shaking machine: production rate 8.2 g$\cdot$m$^{-2}\cdot$ day^{-1}, plastic and glass walled tubes: production rate 4.5–11.7 g$\cdot$m$^{-2}\cdot$day^{-1}.

Increase in turbulence resulted in nearly doubling the growth rate: from 25.2 to 43.2 g $\cdot$ m$^{-2}\cdot$ (12 hr)$^{-1}$. This experiment, performed in a closed system with

small losses of CO_2 and a good shaking device, indicated that also at high light intensities high energy conversions are possible.

By shaking a suspension, a flash effect is introduced which especially improves the light energy conversion at high light intensities. MYERS and GRAHAM (1959, 1961) attempted to overcome the difficulty of too high a light intensity in natural light fields by the use of diffusing cones. In this way, the light energy is distributed over a large surface. In the best examples, efficiency reached 10% of the incident energy, using small vessels with reflecting side walls and light entrance from above.

Chlorella has a rather low saturation level for photosynthesis ranging from 16,000 to 36,000 ergs $\cdot$ $cm^{-2} \cdot sec^{-1}$. Light adaption was associated with changes in chlorophyll content (cf. VAN OORSCHOT, 1955; STEEMANN NIELSEN and JÖRGENSEN, 1962) although no significant change in maximum rate of photosynthesis or growth was found (KOK and VAN OORSCHOT, 1954; STEEMANN NIELSEN and JÖRGENSEN, 1962). By selecting new strains with a higher saturation value, it would perhaps be possible to improve the yield per unit surface. SOROKIN and MYERS (1953) isolated a high temperature strain of *Chlorella* with an optimum growth temperature of 39 °C. It was expected that this would be connected with higher light saturation levels, which, however, was not the case. Light saturation of photosynthesis did not increase significantly whereas the growth rate at 39 °C was 15% higher as compared with a 'normal' Chlorella at optimum temperature.

Temperature adaption, in order to obtain strains with a higher saturation level, proved to be successful in *Chlorella* in the range of 20 to 40 °C (cf. VAN OORSCHOT, 1955). Yield values for cells adapted to 30 ° or 40 °C reached the same maximum level at the same light intensity, whereas the maximum yield measured at 45 ° was sub-optimal. KOK and VAN OORSCHOT (1954) investigated several *Chlorella* and *Scenedesmus* strains, including SOKORIN's high temperature strain in outdoor experiments. They did not find significant differences in yield between the different strains.

Summarizing, we conclude that one of the best means to diminish the disadvantage of too high a light intensity in natural light fields is an efficient stirring system. It is feasable that the efficiency of stirring at a certain speed decreases with increasing layer depth in which the algae are cultivated. We did not find experiments in literature, designed to explore the optimum relation between stirring velocity and layer depth, although more knowledge on this subject might be important for designers of culture vessels.

A lot of physiological data have been collected after the introduction of the synchronous cultures, by TAMIYA *et al.* (1953a). A programmed light and dark regime enabled these investigators to obtain a cell mass with uniform physiological properties, dividing in the dark and enlarging in the light. The growth of simple green algae, such as *Chlorella* and *Scenedesmus*, but also that of other species appeared to be cyclic and could be reproduced numerous times after a synchronous cell mass was obtained. A necessary requirement is a high light intensity which saturates the photosynthetic apparatus. Several investigators

used the new technique, which proved to be very successful. We refer here to SOROKIN (1957,1958; 1960a, b, c; 1961; 1964); LORENTZEN and RUPPEL (1959), METZNER and LORENTZEN (1960), PIRSON and LORENTZEN (1958), PIRSON and RUPPEL (1962), SENGER (1961, 1967). Although differences in technique caused many controverses, some of the experimental results which are well established may be mentioned here:

1) Temperature influences the duration of the growth cycle, a decrease in temperature causes an increase (cf. TAMIYA c.s., 1953a).
2) In principle, light energy determines the number of daughter cells. A minimum dry matter production or light exposure is required, before cell division starts (cf. MORIMURA, 1959). Provided the exposure is long enough, the number of daughter cells increased with increasing light energy input up to a certain maximum. Too low a light energy input or too low a temperature can disturb the synchronization, especially when the number of nuclei in the various cells is not uniform (cf. TAMIYA, c.s., 1961). The maximum rate of photosynthesis varies during the cycle. Maximum photosynthesis increases during the first hours of light till an optimum is reached, and declines after that. Somewhere in the middle of the dark period, cells brought back into the light give the lowest maximum level for photosynthesis (cf. SOROKIN, 1960a, 1960b).

SENGER and BISHOP (1967) reported that, under conditions of light limitation, differences in photosynthetic efficiency could be observed in synchronous cultures of *Scenedesmus obliquus*-D3. Photosynthesis and photoreduction of this strain, suspended in WARBURG buffer no. 9, were measured in a differential respirometer. Absorption of the suspension was measured in a RIEKE sphere using indian ink and methanol extracted cells as controls. The quantum requirement for photosynthesis (measured as O_2-evolution) was 10.7 for cells which had received 8 hours of light, and 16.6 on the average for cells which had received 16 hours of light. On the contrary, the ability to assimilate CO_2 of H_2-adapted *Scenedesmus*, poisoned during the experiment with DCMU, was not affected by the duration of the illumination; the quantum requirements in both cases mentioned above were 19.6 and 21.0 respectively. The authors concluded that the physiological 'age' of the cells affects the activity of photosystem II.

Models constructed to explain phenomena in synchronous systems as given by TAMIYA, SOROKIN and LORENTZEN, are helpful to understand the interplay of external factors in non-synchronous mass cultures of algae, but cannot be applied directly. Disturbing factors may be: 1) peculiarities of culture vessel and technique; 2) variation of light intensity in natural light fields, in comparison with a constant energy input in synchronous systems; 3) changes in spectral composition of the incident light in natural light fields; 4) the variation of daylength in nature.

The present investigation intends among other things to contribute in bridging the gap between laboratory experiments and semi-controlled experiments in the field.

1.3. Scope of the investigation

Some examples of discrepancies between the maximum photosynthetic efficiency and the actual growth efficiencies under the best conditions one could provide in the field, were mentioned. Kok's determinations of the maximum quantum yield and the growth yield (1952) indicated that differences in the pretreatment of the material could influence the growth yield.

The question can be raised whether the rather fragmentary knowledge collected already has a general value. It seemed worth while, therefore, to investigate the influence of external factors on algae cultivated in different types of culture vessels both in and outside the laboratory.

Effects of the composition of the culture solution on growth are described in literature for some special cases. In general, these solutions were used by several investigators using different culture vessels. Data concerning algal growth in the same solution but in different culture vessels are scarce. Experiments about the composition of the culture solution are described in Chapter 3. The optimum composition of the culture solution in relation to the type of culture vessel and the light intensities applied is discussed. Influences of inorganic precipitates owing to preferent consumption of certain ions and the effect of organic additions on growth were studied.

The influence of light intensity on photosynthesis is well known. In large cultures, the light field is difficult to describe which necessitates a lot of investigations before anything can be concluded. Chapter 4 starts with an attempt to define the light field under natural conditions. In growing cultures cell and pigment concentration vary constantly. It is necessary, therefore, to work at a constant optical density, or in a certain concentration range. Experiments, designed to explore the relation between daily incident radiation and growth rate or energy conversion had to fullfil these requirements. Starting the experiment each day at a fixed cell density, it was possible to estimate the amount of energy conversion per day. A large number of these data were used to estimate the net photosynthetic efficiency throughout the growing season. Finally, the effect of altering layer thickness, influencing the average light intensity in the vessel, was studied.

Day length, varying largely in the temperature zones, may interfere with the yield. In chapter 5, experiments dealing with the effect of day length on energy conversion are discussed.

Chapter 6 describes the influence of temperature on growth rate in algal mass cultures; the results will be compared with those of Davis *et al.* (1953) who found that different day and night temperatures influence the growth rate.

2. MATERIAL AND METHODS

2.1. ALGAL STRAINS AND THEIR PRECULTIVATION

The species used during this study were *Scenedesmus* and in a few experiments also *Chlorella*-A (isolated by KOK). *Scenedesmus* was reisolated in 1962 from an old *Scenedesmus*-D3 strain already present in the laboratory (denoted as *Scenedesmus* sp. K4).

In 1966, some fresh *Scenedesmus* strains were isolated from wild forms (strains K22 and K15). The K4, K15 and K22 strain did not differ significantly in morphological and physiological properties. They were cultivated on BEYERINCK agar slants. During the investigation, two ways of precultivation were used. Until the end of 1964, an inoculum from the agar slants was brought into a flask containing 100 ml of a solution of the composition: 12.6 mM KNO_3, 10 mM $MgSO_4 \cdot 7H_2O$, 9.3 mM KH_2PO_4, 8.3 mM glucose, 2.9 mM sodium citrate, 3 ppm $FeSO_4$. The algae were grown for 4 days in a light cabinet, at 30 °C in fluorescent light. The light intensity at the surface of the culture solution was about 0.7×10^4 ergs $\cdot$ cm$^{-2}\cdot$ sec^{-1}. Thereafter, the algae were decanted and resuspended in sterile water. From this suspension 5 ml was taken and inoculated into a flask containing 300 ml of a solution of the composition: 10 mM KNO_3, 2 mM $MgSO_4$, 1 mM KH_2PO_4, and 2 ml of a solution containing 3 g $FeSO_4$, 2 g sodium citrate and 1 g EDTA per litre, to which 1 ml A_4-solution and 0.1 ml B_7-solution (cf. ARNON, 1938) was added. The flasks were placed on the rocking table at 30 °C for 3 days. They were illuminated continuously from below by 4 'daylight' fluorescent tubes (40 W / 33 PHILIPS). The light intensity at the bottom of the flask was about 2×10^4 ergs $\cdot$ cm$^{-2}\cdot$ sec^{-1}. Air containing 5% CO_2, filtered through sterilized cotton wool, was continuously bubbled through the suspension at a rate of 1.5–2.0 l/flask/hour.

After the end of 1964, the precultivation was simplified by omitting the growth in the solution containing glucose. Tests indicated that in this way the growth rate did not decrease. The danger of infection was also smaller. Experiments, described in literature indicated that *Chlorella* metabolizes glucose quickly whereas *Scenedesmus* stored it in a metabolically inert product (cf. GRIFFITH, 1961). The procedure runs as follows. Algae were taken from the agar slants and suspended into a flask containing 300 ml of a solution of the composition 10 mM KNO_3, 2 mM $MgSO_4$, 1 mM KH_2PO_4, 1 ml A_4-solution and 0.1 ml B_7-solution. The low phosphate concentration proved advisable for a rapid start of growth. The flasks were placed on the rocking table, at a constant room temperature of 23 °C. Illumination from below warmed the suspension up to 30 °C. The light was provided continuously by 5 fluorescent tubes (PHILIPS TLM 125 W/33 RS). The light intensity at the bottom of a flask amounted to 7×10^4 ergs $\cdot$ cm$^{-2}\cdot$ sec^{-1} on the average. Air containing 4% CO_2, and filtered through cotton wool, was continuously bubbled through the suspension at a rate of

2.4–3.1 l/hour. After 4 days, the cell concentration was 1–2 µl packed cell volume per ml suspension. From this suspension, 5 ml was taken with a sterile pipette and introduced into a flask containing 300 ml suspension of the same general composition but with 15 mM KH_2PO_4 which proved to exert a buffering effect, favourable for continued growth.

The flasks were placed on the rocking table and treated as described above. After 3 days, the cells could be harvested; the cell concentration then amounted to 5–7 µl packed cell volume/ml suspension.

For reasons of simplicity, culture solutions are coded in the rest of this paper. A key is given here: KNO_3 = symbol N, $MgSO_4$ = S, KH_2PO_4 = P, NH_4Cl = A, NH_4NO_3 = AN, urea = U. Numbers behind the symbols, and separated by dots, denote the concentration in millimoles. So: NSP 10.2.1 is the culture solution already mentioned above, in which the algae are inoculated directly from the agar slants.

Thick inoculates start growing in NSP 10.2.15. The buffering action of the H_2PO_4-ions provides a good growth up to a cell concentration of 11 µl/ml (at this point pH = 6.8–6.9). A further discussion will be given in Chapter 3.

2.2. Culture vessels and technical equipment

For the experiments, the algae were cultivated in various types of vessels, e.g.

a) 11 Erlenmeyer bottles containing 300 ml culture solution. The experiments were made on a rocking table (amplitude 2 cm, 90 times/minute). Temperatures in the range of 20–40 °C could be maintained within ± 1.0 °C. The flasks were illuminated from below by 4 Philips fluorescent tubes (TL 40 W/33). The light intensity at the bottom of a flask, measured with a photocell calibrated against a Moll thermopile, was in the range of (2.5–3.6) × 10^4 ergs · cm^{-2} · sec^{-1}. Air from a compressor and CO_2 from a cylinder were mixed with the aid of flow meters in the composition: 95% air + 5% CO_2. The gas stream was introduced into a flask through a sterilized glass tube provided with a small cotton plug; this tube passing through the cotton plug in the neck of the bottle.

b) Fernbach bottles containing 500 ml solution. The experiments were made on the rocking table. Air containing 4% CO_2, filtered through cotton wool, was continuously bubbled through the suspension.

c) All-glass culture tubes with a volume of 650–750 ml, consisting of three concentric mantles. Through the outer mantle, water at constant temperature was pumped; temperatures in the range of 15–40 °C could be maintained within ± 0.5 °C. The algae were cultivated in the middle mantle. The thickness of the layer amounted to 0.5 cm on the average. Air containing 4% or 5% CO_2 was pressed through a glass filter into the algal compartment. Above the surface of the algal suspension was an overflow, used as a gas outlet only. The inner compartment contained a Philips fluorescent tube (30 W TL/34), the light intensity of which was measured with a photocell, calibrated against a Moll thermopile.

d) Tubes, closed with cotton plugs, in a thermostated water bath. Settling of the algae was prevented by continuous bubbling of air + 5% CO_2 through the bottom of the tube into the suspension. The temperature could be maintained in the range of 10–50 °C (± 0.5 °C). Through one of the glass front panels of the water bath illumination was provided by 4 PHILIPS fluorescent tubes (TLMF 120W/33RS). The light intensity at the surface of the culture tubes was measured with a photocell, calibrated against a MOLL thermophile, and amounted to 2.8×10^4 ergs · cm^{-2} · sec^{-1}.

e) 50 l demonstration models of 'MIELE' washing machines (cf. WASSINK, 1959).

Each machine consisted of a perspex vessel and top and a stirring device, alternately moving to and fro. Air containing 4% or 5% CO_2 was continuously bubbled through the suspension by way of a plastic tube with holes, at the bottom of the container. During the summer months, the washing machines were placed outdoors at a spot facing the south. In this position the possible 'sun' hours were from 7.30–18.00 hours M.E.T. The temperature could be adjusted by way of an electric coil with a maximum capacity of 2200W, while during hot days, the machines were cooled with tap water if necessary.

In winter, the machines were placed indoors in the laboratory. Each machine was illuminated from above by a PHILIPS HPLR mercury lamp. The light intensity at the surface of the suspension was measured with a photocell, calibrated against a MOLL thermophile. In some experiments, 2 PHILIPS HO 2000, 450W mercury lamps and reflection screens were added for illumination from aside.

2.3. MEASUREMENT OF THE ENERGY CONVERSION IN GROWING CULTURES

Growth of the algae was measured in different ways e.g., as increase in cell number, increase in packed cell volume or increase in dry matter.

Cell-numbers were estimated by counting in a haemocytometer (SCHRECK with improved double NEUBAUER ruling). In each count, the average cell number with its standard error was calculated.

Packed cell volume was estimated by centrifuging samples in 10 ml TROMMSDORFF tubes for 10 minutes in a HOMEF centrifuge at 1130 *g*.

Dry matter content of the suspension estimated by centrifuging four samples of 100 or 200 ml for 10 minutes at 2770 *g* in an A.H.T. centrifuge. Thereafter, cells were resuspended in distilled water and again centrifuged for 10 minutes at 2770 *g*. Thereafter, cells were dried for 24 hours at 70 °C and 15 cm Hg pressure. After that they were transferred to an exsiccator and allowed to dry further and cool over silica gel for 12 hours. Finally, they were weighed on a METTLER balance, with an accuracy of ± 0.1 mg.

Calculation of the photosynthetic efficiency. Photosynthetic efficiency was determined in these experiments as the total energy fixed during a certain period, divided by the incident energy. The fixed energy was determined by multiplying the dry matter increase with the caloric value of the material as measured in a bomb calorimeter. KOK (1952) estimated the caloric value of *Chlorella* as

5.6 kcal/g dry material. The average ash content amounted to 10%. Also for *Scenedesmus*, the caloric value was estimated as a reference.* According to SPOEHR and MILLNER (1949), the degree of reduction of organic material can be expressed as the amount of oxygen required for the oxydation of 1 g ash-free dry material. When the most highly reduced compound CH_4 receives a value of 100 on the reduction scale, the degree of reduction R of any compound can be expressed as the percentage of the degree of reduction of methane. When the material consists of c%C, h%H, o%O, n%N, the R-value amounts to: $R = 0.668c + 1.989h - 0.250n$. VAN OORSCHOT (1955) calculated the combustion energy of 1 g dry weight as $0.14 \times R$ kcal.

Micro-elementary analyses of 4 *Scenedesmus* samples were used to estimate the R-value and the combustion energy of 1 g ash free dry material, being 5.1 kcal/g on the average.

In the outdoor experiments the incident energy was measured with a KIPP solarimeter attached to a KIPP micrograph with an integrator. The amount of light between 400 and 700 nm, the region important for photosynthesis, may vary between 45 and 55% of the total global radiation (cf. RABINOWITCH, 1945; REESINCK and DE VRIES, 1942). We therefore assumed that the photosynthetically active part of the total global radiation was 50%.

The incident energy on a geometrically complex surface as that of a washing machine could not be obtained directly, because also the side walls had to be taken into consideration, and had to be 'translated' into an effective contribution to extension of the horizontally illuminated surface. For this purpose, we compared the growth rates in washing machines under light limitation. In one machine the vertical part of the wall was darkened up to the surface level of the algal suspension whereas the other machine received light over the whole surface. Under light limitation the growth rate per unit time is proportional to the light energy absorbed. Therefore, the ratio between the growth rates in the partially darkened machine and in the machine in which the total surface was illuminated was taken to represent the proportion between the illuminated surfaces considered as horizontal planes. This can be expressed as:

Prod. tot. ill.: Prod. ill. hor. plane = Surface total: Surface hor. plane.

* The author is much indebted to Prof. Dr. H. J. DEN HERTOG for kindly carrying out the micro-elementary analyses in his laboratory.

3. THE INFLUENCE OF THE COMPOSITION OF THE CULTURE SOLUTION ON GROWTH

3.1. INTRODUCTION

Detailed information about the mineral nutrition of *Scenedesmus* is given by ÖSTERLINDT (1949). KUZNETSOV and VLADIMIROVA (1965), with *Chlorella*, determined the uptake from an NO_3^--containing culture solution (as described by TAMIYA, 1953b). The highest uptake per gram dry weight was found for nitrogen; uptake of P, Mg, and K were of the same order of magnitude. The nitrogen uptake of *Chlorella* sp.-K remained stable under conditions of light limitation and high cell densities (1–10 g/l suspension). The uptake of nitrogen depended on the culture conditions, and was high in vessels with active growth and lower in vessels with lower growth rates (cf. KUZNETSOV and VLADIMIROVA, 1964). Nitrogen deficiency resulted in a lower level of energy conversion (cf. VAN OORSCHOT, 1955; BONGERS, 1956). Growth in media containing NH_4^+, yielded higher energy conversions than growth in media containing NO_3^- (cf. KOK, 1952).

It is a well known fact that pH increases when algae are cultivated in solutions containing NO_3^-, while pH decreases in cultures containing NH_4^+ (KRAUSS, 1953, TAMIYA, 1953). For this reason, TAMIYA proposed to apply nitrogen by way of urea; pH would then be more stable, and magnesium and phosphate would not precipitate. DAVIS *et al.* and TAMIYA *et al.* (1953) successfully used urea in mass cultures of *Chlorella*. Growth rates were about the same in media containing urea and nitrate (cf. DAVIS, 1953). The TAMIYA medium as improved by KUZNETSOV (1967) contained macro-elements in concentrations proportional to those in the algal biomass, while nitrogen was given as urea. The cultures appeared slightly unbalanced in K and S; however, on the whole the relative amounts of the macro-elements remained the same after excessive growth. Other sources of nitrogen, such as aspartic acid, glumatic acid and alanine were tried by ALGÉUS. Glumatic acid gave the best results, although growth was rather slow (cf. ALGÉUS, 1951).

The work of KUZNETSOV and VLADIMIROVA (1965) showed that light limited growth of *Chlorella* strains was highly independent of the salt concentration in the medium. The nitrogen uptake, however, was not the same in different culture vessels and probably depended on the average energy flux received by the cells. Therefore, the incident energy and the geometry of the culture vessel may be of interest in relation to the optimal culture solution. Data concerned with the relation between the optimal culture solution and the dimensions of the culture vessel are scarce in literature. The medium used by the Russian workers was well adapted for intensive cultivation, it contained high salt concentrations.

We have tried to find out the optimal composition of the culture solution for our special types of culture vessels, from the view-point of attaining the maxi-

mum energy conversion, and have investigated which other environmental factors are important in determining the energy yield.

3.2. Influence of KNO_3-concentrations on growth

The optimum KNO_3-concentration for the growth of some *Scenedesmus* strains was determined on the rocking table. In the course of the work it sometimes was necessary to reisolate the original *Scenedesmus* sp. because contamination had occurred. The growth rates of the related strains (called K, K4, K15, K22 and K23 in the following), showed no significant differences. KNO_3-concentrations ranged from 0 to 30 mM, while S and P remained at 2 and 1 millimolar respectively. In our notation, the solutions would be denoted as NSP 0–30.2.1. Growth was measured as the increase in dry weight in 3 days in continuous light with an intensity of 6×10^4 ergs $\cdot$ cm$^{-2}\cdot$sec^{-1}. The results of 3 experiments with *Scenedesmus* sp. K23 showed optimal production rates of 425 mg $\cdot$ l$^{-1}\cdot$ day^{-1} with NSP 5.2.1. With higher NO_3-concentrations growth rates decreased to 355 mg $\cdot$ l$^{-1}\cdot$ day^{-1}, a relative decrease of 16% (cf. fig. 3.2.1.)

Preliminary experiments with *Scenedesmus* sp., strain K, showed the same optimum concentration for KNO_3. The pH shift during the experiments was from 5.8 at the start to 8.8–9.2 at the end of the experiment. The pH tended to

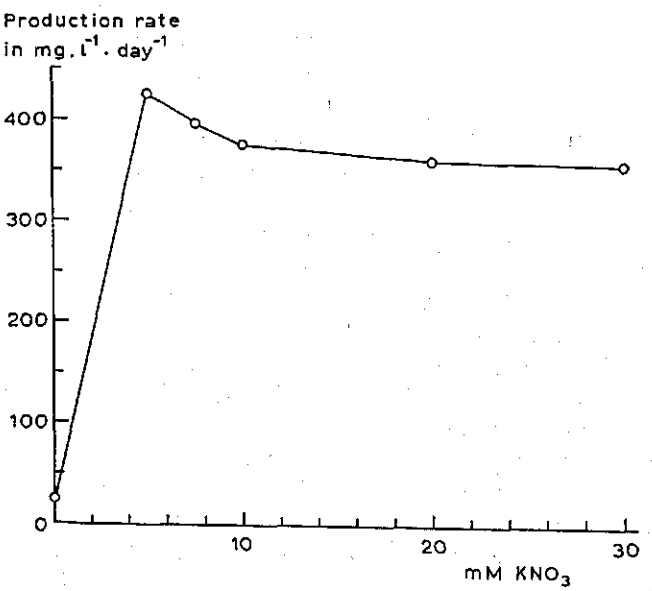

Fig. 3.2.1. Dry matter production of *Scenedesmus* sp., strain K23, as influenced by the KNO_3-concentration. Cultures on the rocking table in 1 litre erlenmeyer flasks, each containing 300 ml suspension. Temperature 30 °C. Growth was measured over 3 days in three experiments; each point is an average of 12 determinations; inoculation density 0.10 µl/ml.

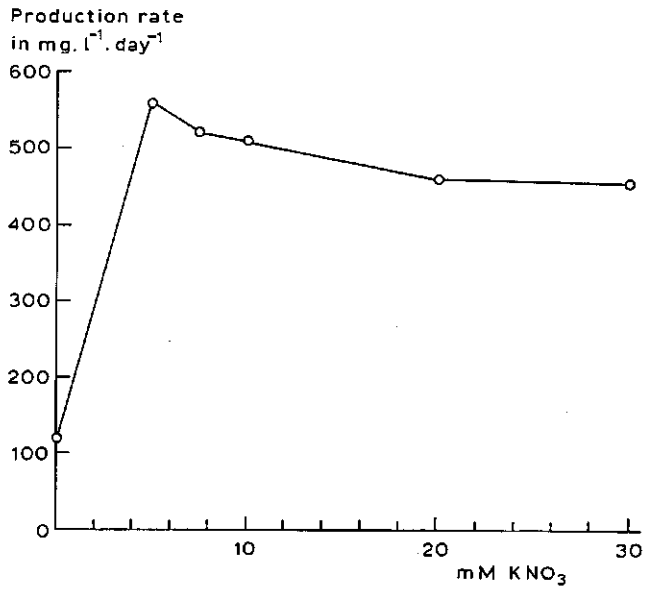

FIG. 3.2.2. Dry matter production of *Scenedesmus* sp., strain K23, as influenced by the KNO_3-concentration. Cultures on the rocking table; flasks contained 300 ml each. Inoculation density: 0.30 µl/ml, temperature 30 °C. Growth was measured over 3 days, algae were resuspended in fresh medium each day. Two parallel experiments; each point is an average of 8 determinations.

stabilize around these values which may be attributed to large quantities of CO_2 bound in CO_3^{--} and HCO_3^-. It is possible that the optimum KNO_3-concentration depends on the amount of biomass present. Therefore, we designed a similar experiment as described above with a three times higher inoculation density (0.30 µl/ml). To prevent exhaustion of NO_3^- from the solution, cells were centrifuged once a day, and resuspended in fresh medium. Also in this experiment, the growth rate was highest with 5 mM KNO_3 (cf. fig. 3.2.2).

Comparing figure 3.2.1 and fig. 3.2.2, we observe that the growth rate at 5 mM KNO_3 is higher when the inoculation density is increased. When NO_3^--concentrations above 5 mM were used, the relative decrease in growth rate was 21 % at most. The inhibition of the growth rate by high KNO_3-concentrations is of the same order of magnitude.

We investigated the possibility that a higher NO_3^--concentration might be toxic by osmotic action. The medium NSP 7½.2.1, in which N 7½ represents N given as KNO_3, was used as the basic culture solution. Besides KNO_3, the culture solution was enriched with KCl in the concentration range: 0–32½ mM. This experiment on the rocking table was repeated twice. The temperature was 30 °C, continuous light with an intensity of 6×10^4 ergs · cm^{-2} · sec^{-1} was used.

The average daily production rate decreased only slightly at KCl concentrations of 12½ mM and higher (cf. fig. 3.2.3). The average production rate in NSP

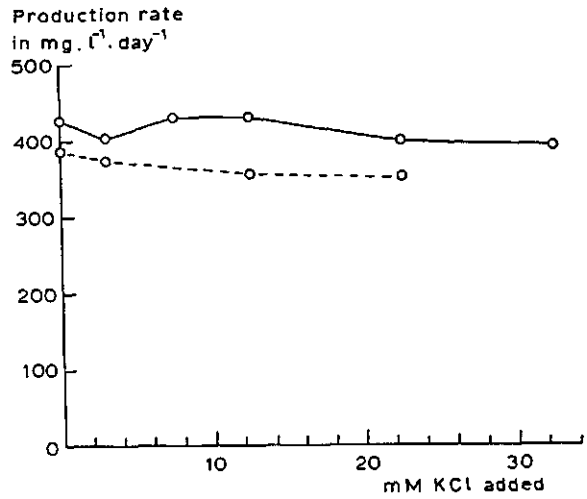

FIG. 3.2.3. Influence of the osmotic value of the culture solution on the production rates in *Scenedesmus* sp., strain K23. Cultures on the rocking table, 3 days in continuous light. Light intensity 60,000 ergs · cm^{-2} · sec^{-1}. Temperature 30°C. Basis culture solution NSP 7½.2.1. Osmotic value of the solution increased by adding: 0, 2½; 7½; 12½; 22½ and 32½ mM KCl (solid line); comparative amounts of KNO_3 only (cf. fig. 3.2.2): broken line.

30.2.1 was 90% of the one in NSP 7½.2.1, whereas the production rate in NSP 7½.2.1 + 22½ mM KCl was 94% of the production in NSP 7½.2.1. The trend of both curves was nearly the same. The experimental data suggest, that the decreased production rate above 7½ mM KNO_3 may well be interpreted as an osmotic phenomenon.

Growth rates in media with different nitrate concentrations were tested in other types of culture vessels: culture tubes with a diameter of 3.0 cm in a thermostated bath, and in 'continuous culture tubes' (described in METHODS). To prevent N-deficiencies, the cells were cultivated during short periods. Part of the cell suspension was used for dry matter determinations, the rest of the cells were brought into new medium. Average growth rates expressed as g · l^{-1} · day^{-1} did not differ significantly for the culture media used. The energy conversion is slightly better in NSP 5.2.1 and 10.2.1 than in the rest of the culture solutions, but the differences are insignificant (cf. Table 3.2.1).

Growth rates were also determined in 100 ml vessels with a diameter of 3 cm placed in a thermostated bath at 30°C. Light was received from a bank of 5 TL fluorescent tubes (120 W, PHILIPS TLMF/33 RS). The light intensity at the surface of the culture tubes was 6.4×10^4 ergs · cm^{-2} · sec^{-1}. Nitrogen concentration was varied between 3 and 30 mM. The increase in packed cell volume was taken as a measure for growth. The growth constants were about the same in all treatments. This means that the KNO_3-concentration had no influence on the growth rate under this set of experimental conditions (cf. fig. 3.2.4).

TABLE 3.2.1. Growth rates for *Scenedesmus* sp., strain K23, over periods of 7 hours; continuous light; 30°C. Media: NSP 5.2.1; 10.2.1; 20.2.1 and 30.2.1. Growth rates expressed as $g \cdot l^{-1} \cdot day^{-1}$. Incident energy in the range of (3–4) $\times 10^4$ ergs $\cdot cm^{-2} \cdot sec^{-1}$. Initial cell density 1.0 µl/ml. Cultures in 'continuous culture' tubes.

Medium NSP	Daily production rate ($g \cdot day^{-1} \cdot l^{-1}$)				Average production rate ($g \cdot day^{-1} \cdot l^{-1}$)	Average energy conversion (%)
	9/10	10/10	13/10	22/10		
5.2.1	1.14	1.08	0.73	0.88	0.96	8.0
10.2.1	1.22	1.08	0.71	0.69	0.92	8.1
20.2.1	1.06	0.99	0.70	0.74	0.87	7.3
30.2.1	0.97	1.19	0.75	0.98	0.97	7.0

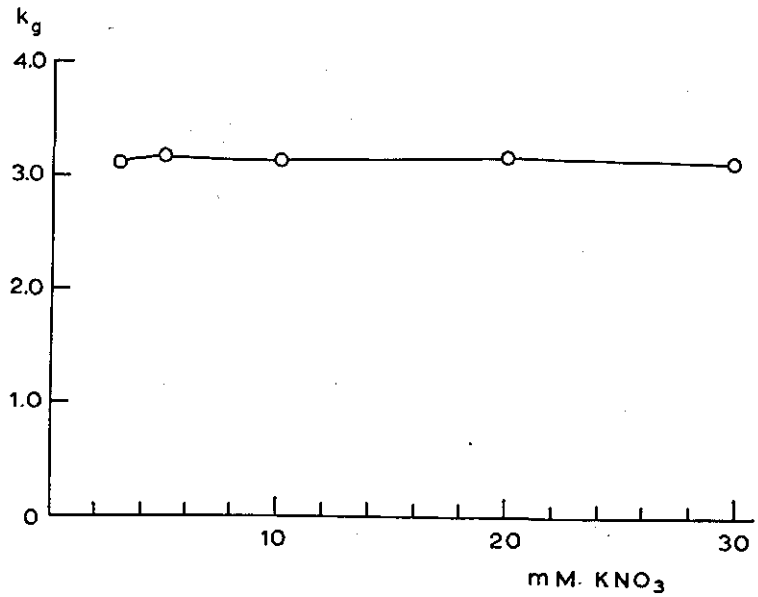

FIG. 3.2.4. Growth rates for *Scenedesmus* sp., strain K23, in relation to the KNO_3-concentration. Cultivation in 100 ml vessels in a thermostated bath at 30°C. Growth period 15½ hours. Media: NSP 3.2.1; 5.2.1; 10.2.1; 20.2.1; 30.2.1. Inoculation density 0.13 µl/ml, 0.17 µl/ml and 0.26 µl/ml packed cell volume per ml respectively. Light intensity 6.4×10^4 ergs $\cdot cm^{-2} \cdot sec^{-1}$, continuous light. Growth constants were calculated from TROMMSDORFF values with the formula:

$$K_g = \frac{24}{t} \times \ln \frac{\textit{Trommsdorff value end experiment}}{\textit{Tromsdorff value start experiment}}$$

3.3. THE EFFECT OF THE $MgSO_4$-CONCENTRATION ON GROWTH

The influence of the $MgSO_4$-concentration on the average daily production rate of *Scenedesmus*, strain K23, was investigated on the rocking table.

Starting with the optimum NO_3^--concentration and a variable $MgSO_4$-concentration, the average daily growth rates in: NSP 5.0.1, 5.1.1, 5.2.1, 5.5.1, 5.10.1 and 5.20.1 were estimated. Small amounts of cells were inoculated into 1 l erlenmeyer flasks, and grown for 3 days in continuous light at 30 °C. The results are presented in fig. 3.3.1. This is an average of 4 parallel experiments. A rather weak optimum was obtained at an $MgSO_4$-concentration of 2 mM. At higher $MgSO_4$-concentrations the average daily growth rate slightly decreased, or was hardly affected under the given set of experimental conditions.

Since pH increased in the course of the experiment, owing to the consumption of nitrate, the Mg^{++} was liable to precipitation with phosphate ions at pH 7.1. In such cases acid was added, since there was a chance that the $MgSO_4$-concentration would be sub-optimal at higher pH. However, this was not done in the present experiment, but the culture solution was buffered by increasing the KH_2PO_4-concentration to 15 mM (pH = 4.8). Average daily growth rates were investigated in three experiments on the rocking table. One experiment was continued for three days, two others for four days. The illumination was continuous, the temperature amounted to 30 °C. The media used in the experiment were: NSP 10.0.15, 10.2.15, 10.5.15, 10.10.15. The results from these experiments are demonstrated in fig. 3.3.2. At $MgSO_4$-concentrations in the range of

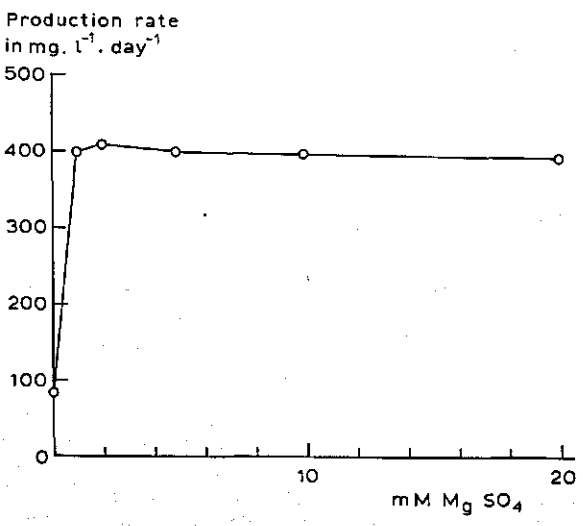

FIG. 3.3.1. Growth rates for *Scenedesmus* sp., strain K23, in cultures on the rocking table. $MgSO_4$-concentration were varied. Media: NSP 5.0.1; 5.1.1; 5.2.1; 5.5.1; 5.10.1 and 5.20.1. Duration experiment 3 days, temperature 30 °C, continuous light. Initial concentration 22 mg dry matter per litre.

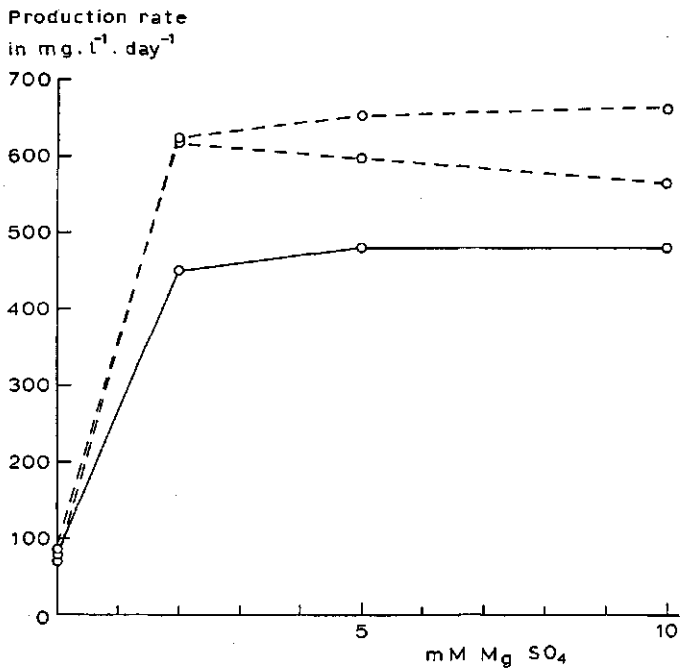

FIG. 3.3.2. Growth rates of *Scenedesmus* sp., strain K, as influenced by the $MgSO_4$-concentration in a buffered medium. Growth rates estimated over a 3 days period: O——O; growth rates estimated over a 4 days period in two experiments: O— —O. Experiments on the rocking table, continuous illumination, temperature 30°C.

2 to 10 mM the average daily growth rate remained nearly the same or decreased a little. Since the absorption of the incident light was imcomplete, the growth rate was a function of the biomass present. This explains the difference in average daily growth rate by cells cultivated for three and four days.

A comparison of fig. 3.3.1 and 3.3.2 shows that the optimum $MgSO_4$-concentration is 2 mM $MgSO_4$. Since the Mg^{++} remained mainly in soluble form in buffered media, and the optimum concentration is equal in buffered and non buffered media, we may conclude that there is no influence of precipitate formation under the conditions of these experiments.

3.4. GROWTH MEASUREMENTS AT VARIOUS KH_2PO_4-CONCENTRATIONS

The influence of the KH_2PO_4-concentration on the average daily growth rate was measured on the rocking table. *Scenedesmus* sp., strain K23, was cultivated during 72 hours at 30°C. The KH_2PO_4-concentration was varied in the range from 0 to 15 mM, yielding media between NSP $7\frac{1}{2}$.2.0 and $7\frac{1}{2}$.2.15. To keep the phosphate concentration fairly stable, the culture solution was renewed every

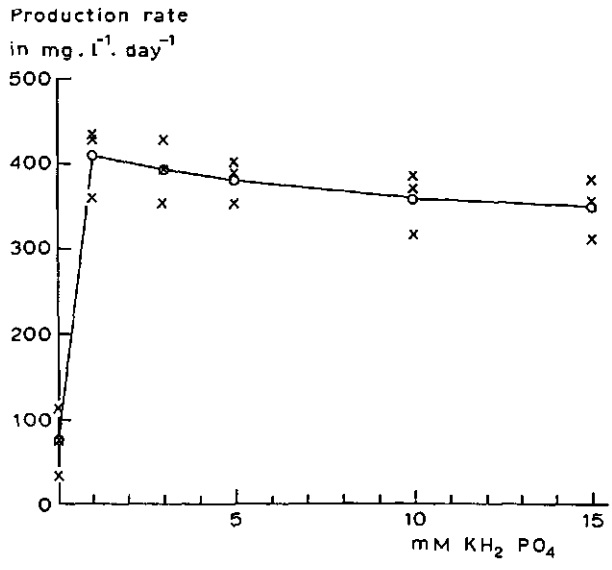

FIG. 3.4.1. Growth of *Scenedesmus* sp., strain K23, as influenced by the phosphate concentration. Cultures on the rocking table in continuous light, temperature 30 °C. Average production rates: O——O; individual measurements: ×.

day. In fig. 3.4.1 it appears, that the average daily growth rate is optimal at 1 mM under the given conditions.

For some experiments on the rocking table, in culture tubes, and in washing machines, the media NSP 25.2.1 or NSP 10.2.1 were used in order to avoid renewal of the medium, although these nitrate concentrations were supra-optimal for growth (cf. section 3.2). In the first experiment, the CALVIN medium NSP 25.2.1 was enriched with phosphate to the composition NSP 25.2.5. The growth of *Scenedesmus* sp., strain K4, was measured in these media. Cells were cultivated in washing machines at a temperature of 30 °C over a period of 11 days in the open. The growth curves are given in fig. 3.4.2.

The increase in dry weight was higher in NSP 25.2.1 than in NSP 25.2.5. Significant differences in growth were present after a period of 3 days. After the second day of the experiment, pH was always higher in NSP 25.2.1 than in NSP 25.2.5. This also demonstrates the higher growth rate in NSP 25.2.1 because the pH increase is due to an unbalanced uptake of K^+ and NO_3^- from the culture solution. After some days, the cultures became somewhat turbid, at first in the machine with NSP 25.2.1. It appeared that Mg^{++} interacted with the HPO_4^{--}-ions to form $MgHPO_4$ at pH 7.0–7.1. This drastically reduced the concentrations of Mg^{++} and HPO_4^{--} in solution. Since the precipitate formation occurred in both culture solutions, we composed a culture medium with a strong buffer action: NSP 10.2.15. When not all nitrate is taken up from the solution the buffering is sufficient to keep pH below the point where precipitation of

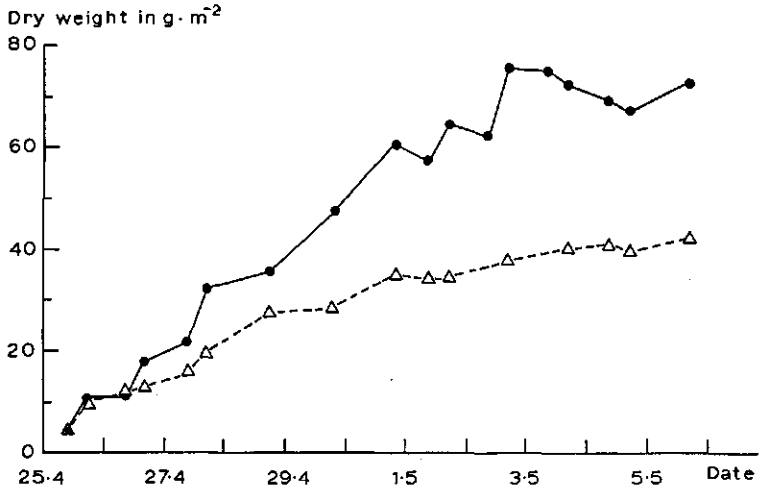

FIG. 3.4.2. Growth of *Scenedesmus* sp., strain K4, in washing machines as influenced by the phosphate concentration. Experiments in the open, temperature 30°C. Culture solution NSP 25.2.1 and NSP 25.2.5, depicted as: ●——● NSP 25.2.1; and △- - -△ NSP 25.2.5.

$MgHPO_4$ occurs. Thus, the growth rates in NSP 10.2.1 and NSP 10.2.15 were compared in cultures on the rocking table. The results are presented in Table 3.4.1. Three initial densities were applied.

In Table 3.4.1 growth data of *Scenedesmus* with two P-concentrations are compared in three otherwise unrelated experiments. In the $62\frac{3}{4}$ hour experiment no differences in growth rate between the two culture media were found. In the 24 hour experiments the growth rate in NSP 10.2.15 was higher than in NSP 10.2.1. It may be assumed that nearly all phosphate is precipitated in NSP 10.2.1 (24 hour series), but that in NSP 10.2.15 soluble phosphate is still present in excess in the culture solution. Available phosphate, which is equal to the equilibrium concentration of $MgHPO_4$ in solution, would then determine the growth rate in NSP 10.2.1.

TABLE 3.4.1. Growth of *Scenedesmus* sp., strain K, in cultures on the rocking table. Culture media: NSP 10.2.1 and NSP 10.2.15. Temperature 30°C; continuous illumination.

Duration (hours)	Number of determin.	Average growth rate NSP 10.2.1 ($mg \cdot l^{-1} \cdot day^{-1}$)	Inocul. dens. NSP 10.2.1 ($mg \cdot l^{-1}$)	Number of determin.	Average growth rate NSP 10.2.15 ($mg \cdot l^{-1} \cdot day^{-1}$)	Inocul. dens. NSP 10.2.15 ($mg \cdot l^{-1}$)
$62\frac{3}{4}$	8	450	21	7	445	24
24	8	730 ±19	170	7	995 ±43	180
24	8	437 ±11	850	7	597 ±26	900

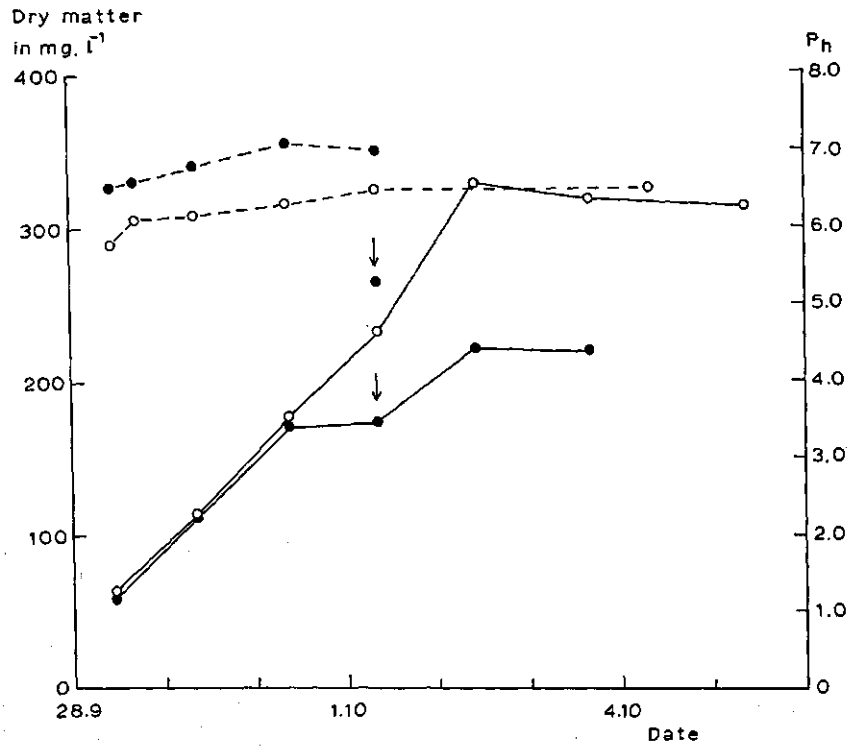

FIG. 3.4.3. Growth of *Scenedesmus* sp., strain K, in washing machines in the open. Culture media NSP 10.2.15 and NSP 10.2.1, temperature 30°C. Arrow: addition of diluted HCl in NSP 10.2.1 to a pH of 5.3. NSP 10.2.1: ●——●; NSP 10.2.15: ○——○; course of pH in NSP 10.2.1: ●- - -● and NSP 10.2.15: ○- - -○.

Similar effects were observed in washing machines. *Scenedesmus* sp., strain K, was cultivated in a machine with NSP 10.2.1 and NSP 10.2.15. Temperature during the experiment was 30°C. As can be seen in fig. 3.4.3, the growth rate was the same from 28/9 to 30/9. After that, the machine with NSP 10.2.1 had a lower growth rate. Simultaneous pH measurements showed a pH increase in both machines. The increase in pH in NSP 10.2.15 was smaller than in NSP 10.2.1, and pH did not exceed 6.5. In the machine with NSP 10.2.1, pH reached 7.0. Apparently, the slowing down of the growth rate in this medium was associated with the high pH. Acid addition (arrow in fig. 3.4.3) in the machine with NSP 10.2.1. resulted in a resumed growth.

3.5. THE INFLUENCE OF DIFFERENT N-SOURCES ON GROWTH

The use of NH_4^+ as a nitrogen source resulted in a pH decrease during the growth period. In preliminary experiments, it was observed that at pH levels below 4.0 the growth stopped, and the cells turned brown.

The growth rate in the media NSP 10.2.1 and ASP 10.2.1 at a temperature o 30 °C was compared in washing machines. Cells precultivated on the rockin table were divided in two parts. After washing of the cells in distilled water each sample was inoculated in the required culture medium. Productions wer measured once a day. Because the production rate per day varied, we totalle (if possible) the daily productions. In some of the experiments the culture ha to be diluted since cell density became too high. In such cases the NSP and AS pretreated cells were diluted to the same TROMMSDORFF level with fresh cultur solution. The results are collected in Table 3.5.1.

It is evident from the table that the net photosynthetic efficiencies in the AS series were always higher than in the NSP 10.2.1 series. On hot summer days th covers were removed from the washing machines to prevent overheating. At th same time, however, CO_2 escaped from the solution. This was concluded fro the fact that the ASP series showed an increase in pH which is the reversal of th normal course in this medium. Replacing the lid resulted in a decrease in pH The pCO_2 in the culture solution normally is in equilibrium with the 5% CO_2 i the gas stream. It is likely that the removal of the cover resulted in a lower equi librium concentration of CO_2 in the solution. This may have influenced the leve of net photosynthetic efficiency during the periods 26/5–29/5 and 21/8–27/8 The favourable effect of ammonium ions on growth, however, remained.

We investigated whether the enhanced efficiency values caused by NH_4^+ salts, also existed in other types of culture vessels. *Scenedesmus* sp., strain K15 was cultivated in 6 culture tubes during 3 days in continuous light. The tem perature was 30 °C. The media used were strongly buffered to prevent large pH shifts during the growth period. Three tubes contained the culture mediun NSP 10.2.20, three tubes a culture solution with the following composition NH_4NO_3–5 mM, $MgSO_4$–2 mM, KH_2PO_4–10 mM, K_2HPO_4–10 mM.

TABLE 3.5.1. Growth of *Scenedesmus* sp., strain K, in washing machines in NSP 10.2.1 an ASP 10.2.1. Culture temperature 30 °C. Productions are expressed as growth efficiencies Experiments in the open. Irradiated surface = 'apparent horizontal surface' (cf. Section 2. and 4.2).

Expt.	Dates	Photos. active radiation (kcal · m^{-2})	Irradiated surface (m^2)	Efficiency NSP 10.2.1 (%)	Efficiency ASP 10.2.1 (%)
64 B5	18/3–21/3	3340	0.37	1.3	5.1
64 B6	25/3–26/3	420	0.37	2.4	5.5
64 B11	20/5–22/5	3051	0.35	5.6	6.5
	26/5–29/5	4484	0.35	2.9	4.9
				2.7	3.5
64 B15	23/6–26/6	2624	0.33	0.6	4.9
				3.5	6.4
64 B19	21/8–24/8	4261	0.37	2.8	4.3
					3.7
	25/6–27/8	4380	0.37	3.0	5.2
					4.1

TABLE 3.5.2. The efficiency of *Scenedesmus* sp., strain K15, grown in buffered media containing KNO_3 or NH_4NO_3 as a nitrogen source. Experiment in 'continuous culture' tubes; three days continuous light. Temperature 30°C. Tubes 2, 3, 5: KNO_3; tubes 6, 7, 8: NH_4NO_3. Light intensity (I_0) in 10^4 ergs · cm^{-2} · sec^{-1}.

Tube no.	I_0 (10^4 ergs · cm^{-2} · sec)	Irradiated surface (cm^2)	Photosynthetically active radiation (kcal · $tube^{-1}$)	Production (g · $tube^{-1}$)	Energy fixed dry weight in g/tube. 5.05 (kcal)	Efficiency (%)
2	3.04	475	82.24	2.89	14.60	17.7
3	2.19	494	61.61	2.52	12.71	20.6
5	2.72	474	73.43	2.45	12.40	16.9
6	2.61	479	71.20	3.14	15.85	22.3
7	2.63	485	72.65	4.04	20.42	28.1
8	2.63	479	71.75	3.16	15.97	22.3

The results of this experiment are shown in Table 3.5.2. The average efficiency in media containing KNO_3 was 18.4%, and 24.2% in solutions containing NH_4NO_3. The relative increase due to ammonium ions then is 31.4%. This is in accordance with the theoretically calculated efficiency increase of 30% (cf. KOK, 1952).

Another possible N-source was urea which does not cause large shifts in pH. Growth of *Scenedesmus* sp., strain K, in urea concentrations ranging from 0–25 mM was measured in an experiment on the rocking table. After autoclaving of the culture solution a white precipitate was formed. It appeared that autoclaving caused a pH shift from about 5.6 to about 9.0. Growth was measured over a period of 3 days. Optimum growth was observed at 15 mM urea. After the growth period, pH in the urea containing media was highest in the series with 10 mM urea and decreased with increasing urea concentration.

The optimum nitrogen concentration seems to be higher in media containing urea than in media with KNO_3 (15 mM against 5 mM respectively). Since autoclaving could have interfered with these results, the experiments were repeated with urea, sterilized through a bacterial filter, added to the autoclaved other part of the solution. The urea concentration was varied within the range from 0 to 30 mM (USP 0–30.2.1). Determinations of the growth rate over a 3 days' growth period from 4 parallel experiments are demonstrated in fig. 3.5.1. A weak optimum was found at 15 mM urea. Experiments with KNO_3, described in section 3.2, showed growth rates of 400 to 500 mg · l^{-1} · day^{-1}, which is about the same order of magnitude as those obtained with urea.

It was of interest to compare the results obtained on the rocking table with growth data in the open. Growth rates of *Scenedesmus* sp., strain K were measured in 3 or 4 washing machines. The growth rates in NSP 10.2.1 and USP 5.2.1 were compared. The temperature was kept constant at 30°C. Repetition of an experiment by simply diluting the culture proved to be difficult. After some days,

TABLE 3.5.3. Growth of *Scenedesmus* sp., strain K, culture media containing KNO_3 or urea. Experiments in 1963 and 1964 in NSP 10.2.1 and USP 5.2.1; experiments in 1966 in NSP 10.2.15 and USP 5.2.1. Temperature 30°C. Experiments in washing machines in the open.

Period	Photosynthetically active radiation (kcal · m^{-2})	Efficiency NSP 10.2.1 (%)	Efficiency USP 5.2.1 (%)	Efficiency NSP 10.2.15 (%)
1963 – 13/9 –16/9	5775	1.2; 1.4	0.8	–
24/9 –26/9	2265	2.1	1.8; 1.9; 1.4	–
8/10–10/10	1915	3.4	4.1; 3.8	–
1964 – 20/2 –23/2	3180	4.0	2.7	–
4/3 – 6/3	1025	2.0	5.3	–
1966 – 22/8 –25/8	2341	–	5.1	4.2
25/8 –28/8	3133	–	6.3	4.4
7/9 –11/9	6108	–	5.4	4.8

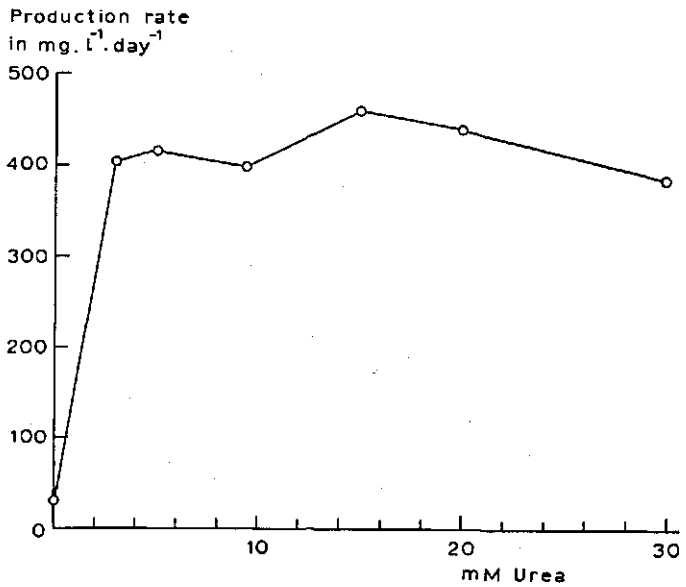

FIG. 3.5.1. Growth of *Scendesmus* sp., strain K23, as influenced by the urea concentration. Cultures in flasks with 300 ml medium on the rocking table. Duration of the experiments 3 days in continuous light, temperature 30°C. Four parallel experiments.

the cells turned yellow or brown. The cause of this phenomenon could not be detected. These experiments were repeated in 1966 with a small modification. Here *Scenedesmus* sp., strain K, was cultivated in NSP 10.2.15 and USP 5.2.1. The data from the experiments in 1963, 1964 and 1966 are presented in Table 3.5.3.

The average net photosynthetic efficiency per day in the '63–'64 series was 2.5% for NSP 10.2.1 and 2.6% for USP 5.2.1. The average net photosynthetic efficiency per day in the '66 series amounted to 4.5% for NSP 10.2.15 and 5.6% for USP 5.2.1, showing that the algae preferred urea over nitrate. The net photosynthetic efficiency in general was higher in the '66-experiments than in the one in 1963. This may have been partly due to an improvement of the experimental conditions in the washing machines after 1963, when the heating coils were isolated with plastic tape and plastic coating, preventing possible leakage of Cu^{++}-ions. Moreover, the fact that the 1963–1964 experiments were carried out in less favourable seasonal conditions (shorter days) may have lowered the efficiency values.

TABLE 3.5.4. Growth rates for *Chlorella*-A in washing machines containing NSP 10.2.15 and USP 5.2.1 respectively. Light from above from one mercury lamp per vessel (HPLR 400 W). Light intensity $(6–8) \times 10^4$ ergs $\cdot$ cm$^{-2}\cdot$ sec^{-1}; temperature 30°C.

Duration (days)	Expt.	Culture solution	Washing machine	Dry matter production (g $\cdot$ vessel$^{-1}\cdot$ day^{-1})	Relative growth rate (%)
3	66L18	NSP 10.2.15	W3	2.61	100
		USP 5.2.1	W4	4.49	139
2	66L19	USP 5.2.1	W3	3.60	138
		NSP 10.2.15	W4	3.22	100

The growth of *Chlorella*-A, a strain already used by KOK, was compared in NSP 10.2.15 and USP 5.2.1 in washing machines in an indoor experiment. Each machine received light from above by one HPLR 400W mercury lamp. Cell samples precultivated in NSP 10.2.15 were divided in two parts. One part was stored in containers at + 5°C, to prevent physiological changes. These cells were used for inoculation in experiment 66L19 (cf. Table 3.5.4). The other part was used directly in experiment 66L18. These experiments lasted 2 and 3 days respectively. Because the light intensity at the surface of the culture solutions was not exactly the same (in the order of $(6–8) \times 10^4$ ergs $\cdot$ cm$^{-2}\cdot$ sec^{-1}) each machine received one time the nitrate containing medium, and one time the urea containing culture solution. The collected growth rates (expressed in g $\cdot$ vessel$^{-1}\cdot$ day^{-1}), could be compared per machine. The results are collected in Table 3.5.4.

If we compare the growth rates in NSP and USP respectively, it is seen that urea stimulated the dry matter production; the growth stimulation amounted to ca. 38%. The effect is comparable with the data given in Table 3.5.3, although the relative increase in efficiency was smaller in the latter case. There may be a difference between the two species of algae, but also the culture conditions differ: outdoor experiments, and round-the-clock-illumination with a lower energy input in the laboratory; rendering a direct comparison of the data in Table 3.5.3 and Table 3.5.4 uncertain.

3.6. The influence of organic additions on *Scenedesmus* growth

Although *Scenedesmus* is an autotrophic organism, it might well be that the addition of yeast extract or soil extract would favour growth. To investigate the effect of yeast extract addition, an experiment on the rocking table (temperature 30 °C) was made.

In a preliminary experiment the growth of *Scenedesmus* over a period of 4 days was compared in the culture solutions: NSP 10.2.1 and NSP 10.2.1 + 0.1 or 0.5 g/l yeast extract. The average production rates over 4 days were: 0.1523 g/300 ml, 0.1672 g/300 ml and 0.1628 g/300 ml respectively. Although growth was higher in the yeast containing series, differences were not significant, due to the variation in production rates. Since light intensity varied from place to place on the rocking table, the same experiment with 3 series of 4 flasks each was repeated; the duration was 4 days. Each day, the place of each flask on the rocking table was different, to secure that the average light intensity received per series was as equal as possible. The results are collected in Table 3.6.1.

Table 3.6.1. Growth of *Scenedesmus* sp., strain K, on the rocking table as influenced by the addition of 0.1 g/l or 0.5 g/l yeast extract. Series A, B, and C were parallel experiments. Temperature 30 °C; duration of the experiment 4 days; light intensity 6×10^4 ergs · cm^{-2} · sec^{-1}.

Culture solution	Series A ($mg \cdot l^{-1} \cdot day^{-1}$)	Series B ($mg \cdot l^{-1} \cdot day^{-1}$)	Series C ($mg \cdot l^{-1} \cdot day^{-1}$)	Average series A+B+C ($mg \cdot l^{-1} \cdot day^{-1}$)
NSP 10.2.1	401.0 ± 73.4	424.0 ± 46.0	332.0 ± 64.8	385.7 ± 12.5
--- + 0.1 g/l	365.0 ± 43.9	389.0 ± 46.0	483.0 ± 56.7	412.3 ± 13.6
--- + 0.5 g/l	409.0 ± 59.0	375.0 ± 100.4	461.0 ± 107.0	414.7 ± 12.9

Each value in the series A, B and C is the average of 4 determinations. On the basis of 4 parallels, significant differences in growth rate by way of yeast extract can be noticed only in series C. Collecting all the results, it shows that the average production rates in NSP 10.2.1 with yeast extract are significantly higher than in the control, although the increase in production is not more than 10%. We conclude that yeast extract increases growth, but is of minor importance.

Besides yeast extract, also addition of soil extract prepared according to Pringsheim was preliminary tested. It did not show any effect if used in connection with our culture solutions.

3.7. Discussion

The experiments described in section 3.2 showed that there is an optimum for KNO_3 at a concentration of 5 mM in rocking table experiments; the growth

rates declined about 15% at concentrations higher than 5 mM. The situation of the optimum remained the same when using higher inoculation densities (cf. fig. 3.2.2). The results in 'continuous culture' tubes, and in 100 ml tubes in a thermostated bath, on the other hand, showed that the situation may be different if we use other types of culture vessels. In 'continuous culture' tubes a decrease in energy conversion was observed at higher KNO_3-concentrations which, however, was statistically insignificant. No effect of the composition of the culture solution on the growth rate was found in algal cultures under conditions of light saturation in a thermostated bath (cf. fig. 3.2.4). This shows that the optimum as observed in our experiments on the rocking table, is qua position only valid for this particular set of conditions. There is a difference in absolute growth rate: the cultures on the rocking table produced about 400–500 mg $\cdot$ $l^{-1}\cdot$ day^{-1}, the algae in the culture tubes 1.0 g $\cdot$ $l^{-1}\cdot$ day^{-1} on the average. For the following reasons it may be assumed that the cells in 'continuous culture' tubes received a higher average light energy flux than those on the rocking table. The light intensity on the surface of the vessels on the rocking table was (4–7) $\times$ 10^4 ergs $\cdot$ $cm^{-2}\cdot$ sec^{-1}, depending on the age of the fluorescent tubes. In 'continuous culture' tubes the intensities were (3–4) $\times$ 10^4 ergs $\cdot$ $cm^{-2}\cdot$ sec^{-1}. The algal compartment in the cultures tubes had a diameter of 0.5 cm, on the rocking table the algal layer was 2.8 cm (with 300 ml per 1 l flask).

The decline in growth rate at concentrations above 5 mM KNO_3 showed parallelism with the decrease in growth rate observed in experiments in which the KNO_3-concentration was kept at 7.5 mM, and the osmotic value of the culture increased with KCl. It has to be admitted that the decline in growth rate is slightly different with KCl as compared with that observed with KNO_3. This might indicate that the toxicities of KNO_3 and KCl differ. Apparently, the concentration of KNO_3 is only toxic in those cases where the growth rate is low. High KNO_3-concentrations then might osmotically inhibit the growth rate.

Apart from the 'osmotic explanation' of the decrease in growth rate, an alternative explanation may be considered in relation to the observations of KUZNETSOV and VLADIMIROVA (1964) and BONGERS (1956). KUZNETSOV and VLADIMIROVA (1964) with *Chlorella* sp., strain K, tried three different types of culture vessels in which they found different growth rates under conditions of light dependency. The NO_3^--uptake depended on the culture conditions. In vessels with high absolute growth rates the NO_3^--uptake was also at its maximum.

BONGERS (1956) found a competition between NO_3^-- and CO_2-uptake in weak light. In the absence of CO_2 the NH_4^+-excretion which then equals NO_3^--reduction was the same in weak and strong light, whereas NO_3^--uptake in the presence of CO_2 was higher in strong light than in weak light. Thus, in our experiments high KNO_3-concentrations might inhibit NO_3^--uptake especially in cases in which the growth rate is low, for instance at low light intensity in both thin and thick layers, or at high light intensity in deep layers. However, from our experiments it cannot be decided with certainty whether differences in cell behaviour towards nitrogen are due to the energy supply, which also depends on size and shape of the culture vessel, or to the osmotic conditions.

The nutrients $MgSO_4$ and KH_2PO_4 gave optimal yields above 2 mM and 1 mM respectively in cultures with optimal KNO_3-supply on the rocking table. In some cases the KH_2PO_4-concentration was increased to 15 mM to inhibit the formation of precipitates of $MgHPO_4$, that occur at pH 7.0. Under such conditions growth rates at $MgSO_4$-concentrations above 2 mM were independent of the $MgSO_4$-concentration. Precipitates were formed in washing machines under artificial light and in the open when no buffering agent was present or pH was not lowered by acid addition. A small increase in KH_2PO_4-concentration to 5 mM KH_2PO_4 was not sufficient to prevent precipitate formation. In washing machines the growth rate was found to be reduced in NSP 25.2.5 as compared with 25.2.1 (cf. fig. 3.4.2). The increase of the KH_2PO_4-concentration to 15 mM was favourable both in washing machines and in cultures on the rocking table at higher cell densities. The growth rates obtained in washing machines are lower than those on the rocking table (100–200 mg $\cdot l^{-1} \cdot day^{-1}$ and 400–500 mg $\cdot l^{-1} \cdot day^{-1}$ respectively). Nevertheless pH also increased in the washing machines. After the formation of precipitate, the growth rate declined in cultures with 1 and 5 mM KH_2PO_4 (cf. figs. 3.4.2 and 3.4.3). Apparently the growth rate depends on the equilibrium concentration of Mg^{++} or of HPO_4^{--}. Another possibility is that the uptake of other kations, as for instance Fe^{++}, is limiting growth at pH values of 7.0 and higher.

NH_4NO_3 as well as NH_4Cl stimulated growth and enhanced net photosynthetic efficiency as compared with KNO_3, provided pH was controlled. The elimination of the nitrate reduction step when ammonium salts are used probably is the cause of this phenomenon.

SYRETT and MORRIS (1963), with *Chlorella*, showed that NH_4NO_3 application results in a preferential uptake of NH_4^+-ions. Nitrate was taken up only when nearly all NH_4^+ was removed from the culture solution. In our experiments comparison of the growth rates in NH_4NO_3 and KNO_3 containing media resulted in a 30% higher energy conversion in the case of NH_4NO_3 (cf. Table 3.5.2). KOK (1952) also calculated an increase in photosynthetic efficiency amounting to 30% in media containing ammonium salts as compared with media containing KNO_3. The increase of 30% found in our experiments, therefore, is probably due to the uptake of the ammonium ion from NH_4NO_3. A second argument is the decrease in pH observed in NH_4NO_3 containing media, which also demonstrates the NH_4^+-uptake.

Nitrogen in the form of urea was favourable for growth in washing machines (cf. Table 3.5.3). The optimum urea concentration on the rocking table was 15 mM (cf. fig. 3.5.1). The absolute growth rates were comparable to those in media containing KNO_3. The stimulatory effect of urea in washing machines (cf. Table 3.5.3) was obvious only in experiments in 1966.

BAKER and THOMPSON (1962) described the urea metabolism in N-deficient *Chlorella* cells. They demonstrated that the amino acid labeling after C^{14}-urea addition was different from that observed with NH_4Cl. No urease activity could be detected, a fact also supported by earlier work of WALKER (1952) and HATTORI (1957). This would imply that hydrolysis of urea did not occur in *Chlorella*.

According to BAKER and THOMPSON (1962), urea is incorporated in glutamate directly, and less in alanine. This is a difference with NH_4Cl, which is metabolized mainly into alanine. In short: the literature gives evidence that a separate pathway for urea-N and ammonium-N may exist. However, urea shares the advantage that nitrate reduction is not required, which is particularly favourable in weak light. The higher nitrogen concentration which can be applied without harmful effects, and the absence of strong pH shifts are a recommendation to use urea as a nitrogen source. The culture then has to be grown under fairly aseptic conditions, however.

The addition of yeast extract gives a small, but significant growth increase. HUTNER and PROVASOLI (1964) described *Scenedesmus* as an autotrophic organism. It might well be that factors in the yeast extract facilitate some metabolic step in the cell. Nevertheless, yeast extract is only a factor of secondary importance for the growth of *Scenedesmus*.

Growth of *Scenedesmus*, in our hands, did not react to the addition of soil extract.

4. THE INFLUENCE OF LIGHT INTENSITY ON GROWTH

4.1. Introduction

Numerous data exist for production rates under natural conditions, which, however, are not always comparable because they have been collected in various types of culture vessels. Felföldy collected production rates from the literature ranging from 3.5 to 28.5 $g \cdot m^{-2} \cdot day^{-1}$. This is not the best way to compare these figures. To estimate a cultivation method on its merits it is necessary to consider growth efficiencies. Data about energy conversion in large cultures are scarce. Gummert, Meffert and Stratmann (1953) obtained efficiency values up to 2%, whereas van Oorschot (1955) obtained energy conversion values of 1–4% in 300 l vessels with a stirring motor. Tamiya (1953), with *Chlorella* cultivated in a closed circuit with a volume of 40 l, measured maxima of 24 g dry weight increase per unit per day. Light energy was given in klux-hour, and the exact surface was not given. We calculated the indicent energy with the erg/lux ratios estimated by Gaastra (1959), and the surface with the data given in the text. The efficiency calculated on the basis of the energy actually received was 2.6%. Russian investigators (cf. Semenenko and Vladimirova, 1961) claim to have obtained energy conversion values of 7% in open air trenches, which is rather high. Small outdoor cultures by Kok and van Oorschot (1954) gave average efficiency values of 8%.

Some general remarks about growth in large cultures have to be made. The growth curves generally have a sigmoid shape (cf. Myers, 1953).

In the light saturated region:

$$dN/dT = kN \tag{1}$$

where N is the cell number and k a constant. If there is light limitation, and respiration can be neglected:

$$dN/dT = k \tag{2}$$

During the exponential start and the sigmoid end of the growth curve photosynthetic efficiency does not reach high values. The initial part has incomplete light absorption which reduces photosynthetic efficiency on the basis of incident light. The final part is characterized by a relatively high respiration and a low energy flux per cell, which also reduces the overall efficiency. Our aim was to study those conditions, where photosynthetic efficiency was highest. This could be expected in the linear part of the growth curve. In this part of the growth curve, connecting the initial and the final ones, the growth rate and the efficiency reach the optimal compromise for the culture as a whole. Therefore, our studies have been concentrated especially on this part of the growth curve.

Eppley and Dyer (1965) reached the optimum growth rate in continuous cultures at a cell density where the relative growth rate was one half of the maximum relative growth rate (k_{max}).

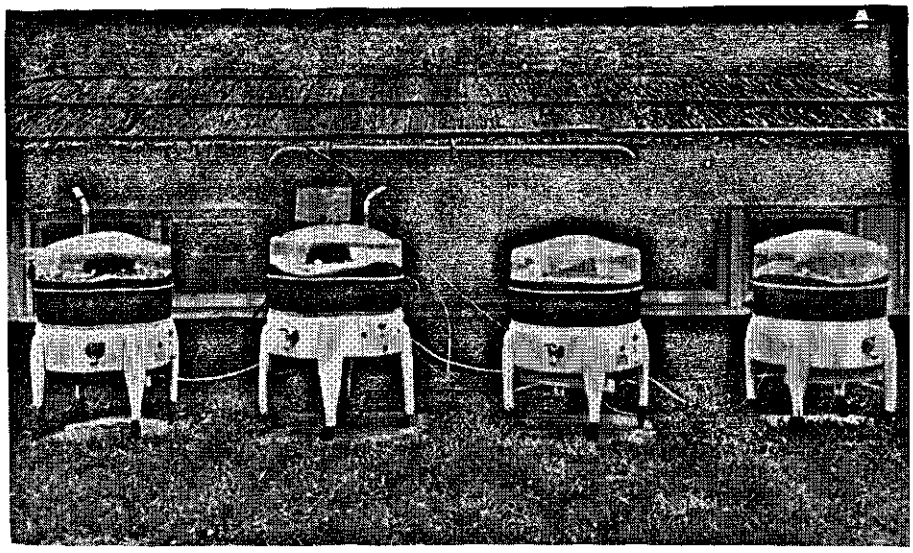

PLATE 1. Outdoor cultivation of algae in 50 l washing machines with temperature regulation.

This chapter starts with an attempt to describe the light fields for algal cultures in the open. The influence of the daily photosynthetically active radiation on yield is studied with one type of culture vessels, viz. washing machines. It is assumed that algal photosynthesis, as measured in cultures in the open, represents a simple integration of successive variable momentary photosynthetic rates. With the production data, an estimate is made for the average production and efficiency values all over the growing season. The average light intensity in the cultures may also be experimentally influenced by varying the thickness of layer of the algal suspension, the results for the growth rate are described in section 4.3.1.

4.2. LIGHT DISTRIBUTION PATTERNS ON HORIZONTAL AND VERTICAL SURFACES OCCURRING UNDER NATURAL CONDITIONS

The washing machines were set up on a site facing the South. The laboratory screened a large part of the northern and eastern sky. The duration of sunshine and the amount of free sky was calculated with a method normally used by architects to estimate the duration of sunshine on facades (cf. SWIERSTRA, 1954). Height and azimuth of the buildings were taken from a technical drawing and plotted on the circular projection of the sky, enabling to estimate the percentage of free sky. In this way the free sky, as seen from the place of the washing machines was estimated to be 68 %. The sun reached the culture vessels maximally 10.5 and minimally 4.5 hours per day. Because only the low sun elevations

were screened by buildings around the culture vessels, only a small percentage of the possible total sunlight was lost.

Light energy, received on a horizontal plane in the neighbourhood of the culture vessels was measured with a KIPP solarimeter, attached to an integrator. It was assumed that 50% of the total global radiation was within the range available for photosynthesis. The photosynthetically active radiation is composed of direct and diffuse light. The shape of the washing machines can be described as cylindrical. Different amounts of direct light are received on their upper and side surfaces.

The amount of diffuse light received over the surfaces of the washing machines was determined on days with totally overcast sky. The relative amount of light on a vertical plane facing the different directions was estimated with a cosinus corrected flat light meter and one cell of a spherical light meter*, both equipped with Se photocells. The intensity on the horizontal plane, measured with the one-sided spherical light meter was taken as 100. After calibrating the photocells in the horizontal position, the cosinus corrected flat light meter was held in the vertical plane while the other remained horizontal. Because the albedo of the overcast sky may vary with different elevations of the sun, we estimated the diffuse light distribution at different heights of the sun. Figures for diffuse light distribution are collected in Table 4.2.1. It can be concluded that the relative amount of diffuse light in % of $I_{horizontal}$ does not vary much.

TABLE 4.2.1. Relative light intensities on a vertical plane through the axis of a washing machine, in diffuse light. Figures are averages for the data obtained in different directions.

Sun's elevation in degrees	Relative light intensity on a vertical plane in % of *I horizontal*
61	43.4
48	46.4
48	43.5
48	40.5
40	42.0

The direct light energy on a vertical plane can be calculated if the incident radiation and the elevation of the sun are known. Only one half of the side walls is directly irradiated. Therefore, the energy received on the vertical side walls is:

$$A_{vert.} \times I_{vert.} = A_{vert.} \times \operatorname{cotg} h \times I_{hor.} \quad (1)$$

and on the horizontal plane:

$$A_{hor.} \times I_{hor.}$$

(where $A_{vert.}$ and $A_{hor.}$ represent the areas of a vertical plane through the axis of a washing machine andt he horizontal wall of a washing machine respectively,

* Unfortunately this was used since a second cosinus corrected flat light meter was not available.

and h is the height of the sun). Assuming that the total area is irradiated with $I_{hor.}$, the apparent surface which receives such an energy flux is called the 'apparent horizontal surface'.

$$App.\ hor.\ surf. = A_{hor.} + A_{vert.} \times \text{cotg}\, h \qquad (2)$$

The apparent horizontal surface at several elevations of the sun is calculated with formula 2 (cf. fig. 4.2.1). Especially at low elevations of the sun, such as may occur on bright winter days, the apparent horizontal surface is about two times higher as compared with mid-summer.

If we knew the total amount of light received on a horizontal plane and the ratio of diffuse light/direct light for various hours of the day, it would be possible with the aid of a computer to calculate the instantaneous light distribution over the horizontal and the vertical surfaces. However, the amount of direct and diffuse radiation at each moment is unknown. In order to obtain an average impression, we measured this ratio in front of the laboratory on a day with a half overcast sky. A flat photocell with a white dome was used to measure direct and diffuse light together, and a similar cell, placed in the shade of an opaque white screen, to measure diffuse light separately. The outcome of these measurements was 48.1 % direct sunlight and 51.9 % diffuse light.

For general use we did not worry about the proportion direct to diffuse light, but only considered the total energy received on a horizontal plane. Therefore,

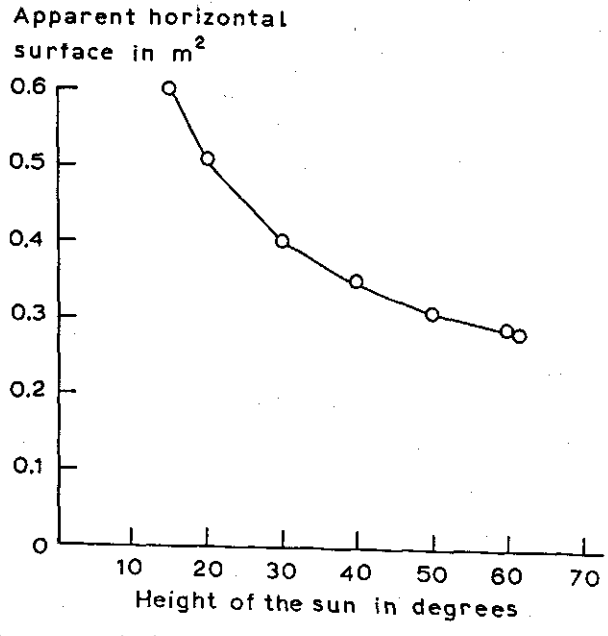

FIG. 4.2.1. The apparent horizontal surface as affected by the height of the sun, calculated with the formula: *Apparent horiz. surface* = $A_{horiz.} + A_{vert.} \cdot$ cotg h, where h is the height of the sun in degrees. $A_{horiz.} = 0.23$ m² and $A_{vert.} = 0.11$ m².

TABLE 4.2.2. Production values under conditions of light limitation in washing machines. The screened machine had the side walls darkened up to the surface of the culture solution. The horizontal surface s.s. was 0.23 m^2.

Date	Production unscreened g/vessel	Production screened g/vessel	Ratio	Apparent horizontal surface (m^2)
7/4–10/4	25.25	13.45	1.88	0.44
15/4–17/4 19/4–23/4	45.60	28.50	1.60	0.37
9/5–11/5 12/5–13/5	12.24	8.18	1.50	0.35
27/6–30/6	13.65	11.22	1.40	0.32

we looked for a method which gave us an average for the amount of light received on the side walls of the vessels.

To this purpose we measured the growth rates in cultures, where light was limiting the growth rate. We screened the side wall of one machine up to the surface of culture solution, whereas the other machine remained unscreened. Growth rates then should be proportional to the 'apparent horizontal surfaces'. By averaging several daily measurements, it was assumed that the results obtained would be representative for the average weather conditions during this special part of the year. The experiments were done in the end of April, the beginning of May and middle June (cf. Table 4.2.2).

It can be observed that the apparent horizontal surface varies in different parts of the year. The elevation of the sun being high in middle summer, should give a decrease in apparent horizontal surface as compared with spring or autumn (cf. fig. 4.2.1). This is more or less reflected in the data presented in Table 4.2.2.

In the case of only diffuse radiation it was possible to make independent estimates of the apparent horizontal surface. First, according to Table 4.2.1, the average diffuse radiation on a vertical plane is 43.1% of that on a horizontal plane; at high elevations of the sun it is rather constant. This yields an apparent horizontal surface of: $0.23 + 0.43 \times 0.32\ m^2 = 0.37\ m^2$ (direct measurements of the horizontal and vertical surface yielded 0.23 and 0.32 m^2 respectively). A second method for estimating the apparent horizontal surface makes use of the fact that the amount of free sky measured with the method of SWIERSTRA was 68%. The apparent horizontal surface would be: $0.68 \times (0.23 + 0.32)\ m^2 = 0.38\ m^2$. The values obtained in these two ways show good agreement and, moreover, are in the range of the experimental data of Table 4.2.2.

4.2.1. *The light distribution in washing machines in the open*

We assume that the light distribution in the washing machines follows BEER's law:

$$ln\ I/I_o = k \cdot c \cdot d$$

in which k is a constant characteristic for the algal suspension, c = cell concentration, d = thickness of layer.

The constant k was measured for 7 different *Scenedesmus* samples. Each sample was diluted to cell concentrations in the range of 0.2 to 1.0 µl packed cell volume/ml. Absorption-maxima were measured in a BECKMAN spectrophotometer (type DU), using a piece of filter paper directly between the slit and the glass box. Preliminary experiments indicated that this modified SHIBATA method (cf. SHIBATA, 1955) largely reduced the error produced by scattering.

The absorption spectrum for *Scenedesmus* sp. showed a minimum at 550 nm, whereas the extinction observed at 800 nm can be attributed to scattering. The absorption of chlorophyll in vitro is very low at 550 nm as compared with the maximum at 670–680 nm. The absorption minimum at 550 nm and the measured extinction at 800 nm both increased when the concentration was enhanced. We have considered these minima as reference points for scattering. The line drawn through these points enabled to estimate the scattering value at each wavelength by interpolation. Preliminary experiments indicated that chlorophyll determinations with acetone extracts, and *in vivo*-estimates did not show significant differences between the maximum absorption values in the red part of the spectrum (cf. fig. 4.2.1.1) provided the *in vivo*-determinations were corrected for scattering.

Because *in vivo* and *in vitro* measurements were comparable, the value of k was estimated *in vivo* for reasons of simplicity. If the maximum extinction (normally between 680 and 670 nm) was plotted against cell concentration (in µl/ml) the slope of the regression line represented the factor k. The factor k was determined in 7 concentration series as described above. The average value for k was considered as the best estimate for k.

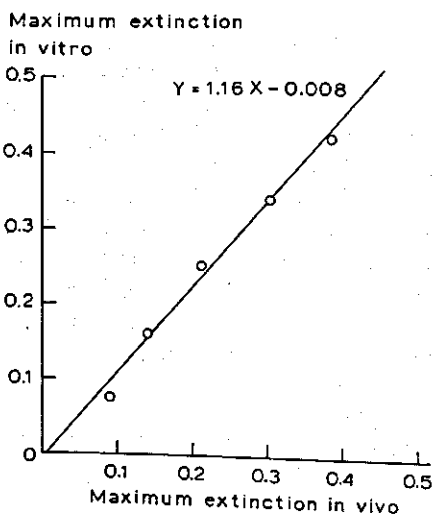

FIG. 4.2.1.1. Comparison between chlorophyll determinations *in vivo*, with a modified SHIBATA method, and *in vitro* in acetone extracts from the same *Scenedesmus* samples. *In vivo* measurements were corrected for scattering (reference points 800 and 550 nm).

Regression lines:

$y = 0.344\ x + 0.007$ $\qquad$ $y = 0.371\ x - 0.007$

$y = 0.291\ x - 0.007$ $\qquad$ $y = 0.309\ x + 0.008$

$y = 0.285\ x + 0.002$ $\qquad$ $y = 0.266\ x - 0.006$

$y = 0.241\ x + 0.039$ $\qquad$ $\bar{k} = 0.301 \pm 0.031$

With this $\bar{k}$-value it is possible to calculate the light intensities which exist in the culture vessel. In fig. 4.2.1.2 we can see that light saturation for photosynthesis (0.05 cal · cm^{-2} · min^{-1}), cf. VAN OORSCHOT (1955) is at smaller depths when the cell density is increased. Without stirring, the average light intensity can be estimated from the graph. The stirring movement of the cells can improve the efficiency of light utilization, provided the stirring is very quick. It was not possible to measure the movement of the cells directly. Since the particles were very small and immobile it was assumed that their movement was mainly determined by the streaming in the water, caused by the stirring motor.

Streaming was measured with a plastic diver, just floating in the water. The average time needed by the diver to travel from surface to surface was 9.5 sec. on the average. Taking into consideration that the depth of layer was 19.5 cm, the velocity of the streaming was: 2 × 19.5: 9.5 cm/sec = 4.1 cm/sec.
We assumed that the average velocity of cells was comparable to the velocity of the water stream, thus: 4.1 cm/sec.

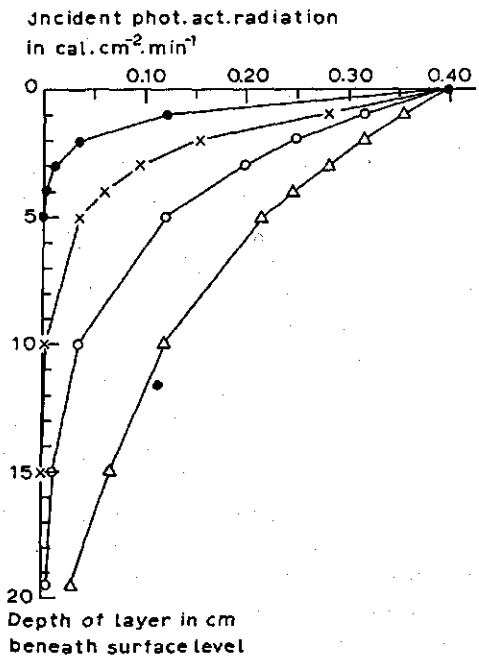

FIG. 4.2.1.2. The light distribution in a washing machine as a function of TROMMSDORFF value calculated with LAMBERT-BEER's law. Density 0.4 µl/ml: △——△; 0.8 µl/ml: ○——○; 1.6 µl/ml: ×——×; 4.0 µl/ml: •——•. On the abscis the photosynthetically active radiation in cal · cm^{-2} · sec^{-1}, on the ordinate the number of cm beneath the water level are given.

TABLE 4.2.1.1 Periods that the average cell receives incident radiation above the saturation point of photosynthesis (T_L) and below this point (T_D), calculated for washing machines when using the indicated cell concentrations.

Concentration (μl/ml)	T_L (sec)	T_D (sec)	$T_L/(T_L+T_D)$
0.4	8.525	0.975	0.90
0.8	4.47	5.03	0.47
1.6	2.14	7.36	0.23
4.0	0.88	8.62	0.09

If we calculate the ratio:

$$\frac{\textit{Time during which the cells receive light intensity above saturation}}{\textit{Time during which the cells receive light intensity below saturation}}$$

we obtain the data presented in Table 4.2.1.1.

Assuming that the light intensity at the surface of the vessel amounted to 0.4 cal · cm^{-2} · min^{-1}, the light distribution was calculated. Because the suspension was shaken, the cells moved 4.1 cm/sec in the vertical direction. We can now calculate the average time, a cell receives a light intensity above and below saturation of photosynthesis (0.05 cal · cm^{-2} · min^{-1}), see Table 4.2.1.1.

As can be observed in Table 4.2.1.1, above cell concentrations of 0.8 μl/ml cells remained more than 50% of the time under light limited conditions.

Light intermittency can be obtained by screening some part of the light by a rapidly rotating disc with incisions. If yield in intermittent light is compared with yield in continuous light with an intensity of:

$T_l/(T_l + T_d) \cdot I$ during the same total time

where T_l = flash duration in intermittent light; T_d = duration of dark period in intermittent light, the ratio between these two values can be calculated. RABONOWITCH (1956) discussed whether an enhancement by light intermittency could be expected. This was not found. The gain in efficiency reached at T_l-values of 0.1 sec and less was annihilated by the shortness of each light period.

In a culture with saturating light intensities at the top and low light intensities at the bottom, stirring will help anyhow since it increases the energy flux received by an average cell. Furthermore, it decreases the light intensity received by an average cell at the top of the culture with a factor $(T_l/T_l + T_d) \cdot I$. To obtain enhancement in energy conversion in the light saturated part of the culture, it is favourable if T_l is short as compared with T_d. In washing machines this is so above 0.8–0.9 μl packed cell volume/ml suspension (cf. Table 4.2.1.1).

KOK (1953) determined the effect of flash duration on the relative efficiency of the flash at several $T_l/T_l + T_d$ ratios. Flash durations in the order of milliseconds would be necessary to obtain maximum photosynthetic efficiency. The highest cell concentration of Table 4.2.1.1, viz. 4.0 μl/ml, is about the maximum which can be reached in washing machines (5.0 μl/ml in the middle of

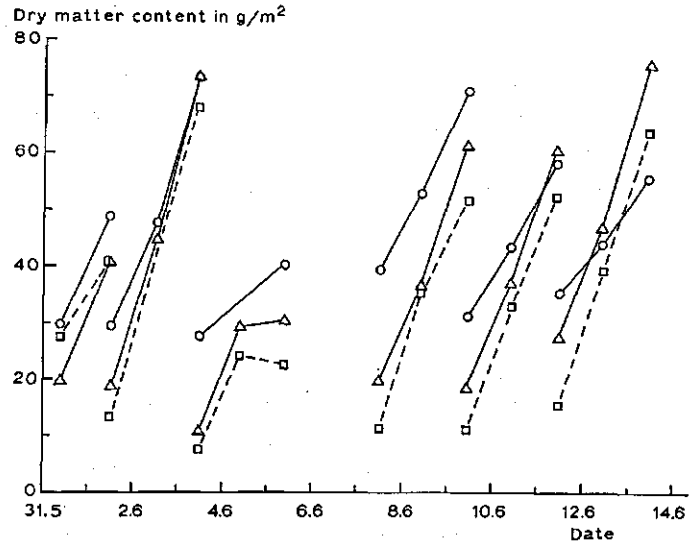

FIG. 4.3.1. Dry matter in washing machines, calculated as $g.m^{-2}$, for *Scenedesmus* sp., strain K, cultures. Medium NSP 10.2.1, temperature 30 °C. Average initial cell densities: ○——○ 1.5 µl/ml; △——△ 0.95 µl/ml; □ - - - □ 0.6 µl/ml. Experiment June 1963.

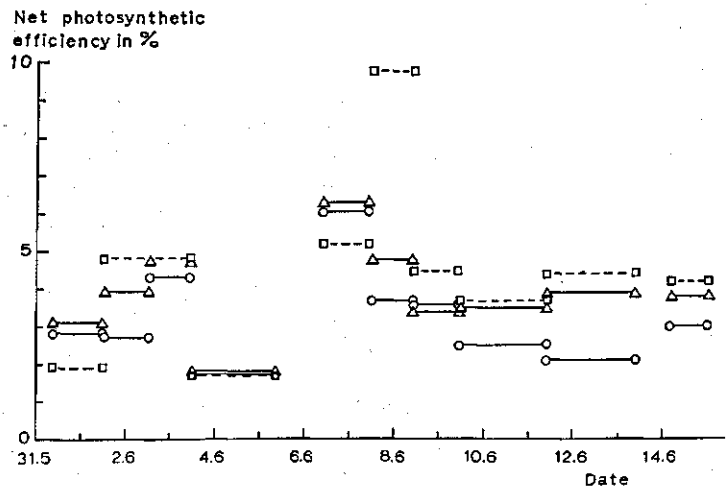

FIG. 4.3.2. Net photosynthetic efficiency values based on incident light for *Scenedesmus* sp., strain K, cultivated in washing machines. Temperature 30 °C, medium NSP 10.2.1. Experiments June 1963. Average initial densities: ○——○ 1.5 µl/ml; △——△ 0.95 µl/ml; □- - -□ 0.6 µl/mi.

summer). In this case the flash duration (T_l) is about 1 sec and the $T_l/T_l + T_d$ ratio 0.10.

It was derived from graphs presented by KOK (cf. BURLEW, 1953) that the relative efficiency of the flash in this case would have been 20%, which is comparable with an energy conversion of $0.2 \times 25\% = 5\%$ at a cell concentration of 4.0 µl/ml (in washing machines). It has to be borne in mind that a relatively long dark period unfavourably affects this enhancement. A decrease in depth of layer, and shortening of the dark period, would therefore be favourable with the same stirring rate.

4.3. INFLUENCE OF DAILY PHOTOSYNTHETICALLY ACTIVE RADIATION ON YIELD UNDER SEMI-CONTROLLED FIELD CONDITIONS

Daily yield in a growing culture can be estimated by sampling a thinly inoculated culture every day until no further growth is observed. When natural daylight is used, it is not easy to obtain a clear idea of the relationship between daily energy input and production rate.

Growth is a function of net photosynthesis provided no other processes interfere and limit dry matter production. Net photosynthesis depends on the amount of absorbed light in the culture under conditions of light limitation. It is a function of the optical density of the suspension and of the input of light energy. The energy flux varies frequently. Therefore, a well defined absorbing system is necessary to explain light influences on growth.

Some washing machine experiments were designed in June and July 1963 to solve the question whether there was an influence of the initial cell density on the growth rate of *Scenedesmus* sp., strain K. The algae were cultivated in NSP 10.2.1 at a temperature of 30 °C. Initial densities of the cell suspension were in the range of 0.5–1.5 µl/ml in each of three washing machines. The results, presented in fig. 4.3.1, show that the increase in dry matter in most cases is fairly linear with time. The daily growth rate appears to be somewhat lower at the highest initial concentration. In general, the slopes of the growth curves are similar for various initial concentrations. Differences in initial concentration are reflected especially in the early readings. The fact that the growth rate further shows increasing independence of this initial concentration suggests that the light intensity during the main part of the growth period acted as the limiting factor, the more so since CO_2 and concentration of nutrients were supplied at the optimal level.

Net photosynthetic efficiency values based on incident light were calculated for this experiment and are shown in fig. 4.3.2. Net photosynthetic efficiency values decreased, when the initial cell density was increased. The highest value was 4.2% for an initial concentration of 0.6 µl/ml, and 3.0% on the average for the initial concentration of 1.5 µl/ml. Since light intensity acted as a limiting factor during the main part of each growth period, initial cell density can only influence the average cell density which is reached during such a growth period.

TABLE 4.3.1. Growth of *Scenedesmus* sp., strain K, in washing machines. Natural daylight, culture medium NSP 10.2.1, temperature 30 °C continuously. Dilution every morning or afternoon to the TROMMSDORFF values of 0.5 and 1.0 µl/ml. Figures in brackets denote the duration of the experiment in days.

Month	Average photos. act. rad. (kcal·m^{-2})	Eff. (%) 0.5 µl/ml	Average photos. act. rad. (kcal·m^{-2})	Eff. (%) 1.0 µl/ml
June '63 (12)*	2492	4.2	2492 (12)*	3.8
June '63 (8)*	1288	5.7	–	–
Sept. '64 (8)	1437	4.4[1]	1225 (6)[4]	4.5
Apr./May '65 (8)	1277	6.6[2]	–	–
May '65 (8)	1971	4.1[3]	1685 (13)[5]	3.1

* Earlier experiment, added for comparison only. The cultures were diluted to TROMMSDORFF 0.5–0.6 µl/ml over periods varying from 1 to 3 days.

Standard deviations:
1) Sept. '64: $s = 1.11$ ($n = 16$).
2) April–May '65: $s = 1.33$ ($n = 16$)
3) May '65: $s = 0.52$ ($n = 24$)
4) Sept. '64: $s = 1.30$ ($n = 11$)
5) June '65: $s = 1.10$ ($n = 13$)

The average energy flux per cell decreases at higher cell densities, so that the explanation would be that photosynthesis was more compensated by respiration at higher cell densities than at lower ones.

In a new series of washing machine experiments, the cultures were diluted daily to fixed TROMMSDORFF values of 0.50, or 1.0 µl/ml at the same time of the day. The culture temperature was 30 °C, the culture solution was NSP 10.2.1, unless stated otherwise. Net photosynthetic efficiencies were averaged for a growth period. The results are collected in Table 4.3.1.

The figures in the table indicate that the average efficiency values over longer periods vary. Daily dilutions to the level of 1.0 µl packed cell volume per ml tend to produce lower net photosynthetic efficiency values as compared with the 0.5 µl/ml series. The net photosynthetic efficiencies per day (Table 4.3.1) for the '64 and '65 experiments were averaged. The corresponding daily growth rates as a function of light energy input per day are given in fig. 4.3.3.

The general trend of the points was submitted to the rank correlation test of KENDALL. The correlation between light intensity and production rates was significant in fig. 4.3.3. left and middle, and probably significant in the right curve. This means that the culture as a whole behaved as under conditions of light limitation. The experiments which were started at a TROMMSDORFF value of 1.0 µl/ml (cf. Table 4.3.1) also showed growth rates proportional to the light energy input. We conclude that initial cell densities of 0.50 µl/ml and higher are sufficient to create light limited growth in washing machines.

Literature shows that the physiological properties of synchronous algae do not remain the same, but depend on 'physiological age'. A correlation between

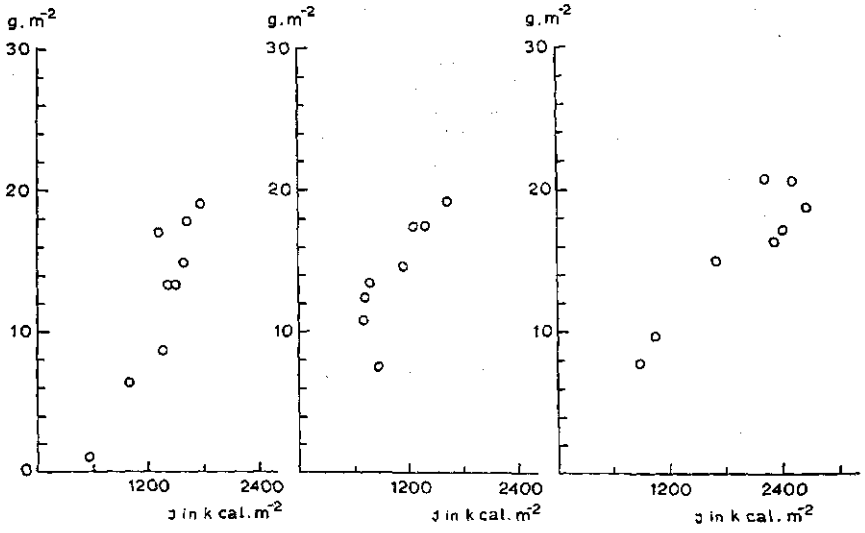

FIG. 4.3.3. Daily production rates for *Scenedesmus* sp., strain K, cultivated in washing machines. Daily dilutions to 0.50 µl packed cell volume per ml. Medium NSP 10.2.1, temperature 30°C continuously; left part: experiment Sept. '64; middle part: experiment April–May 1965; right part: experiment May 1965. Each point average of 3 to 4 parallel determinations.

cell size and photosynthetic activity was shown in synchronous algal cultures (cf. SOROKIN, TAMIYA, LORENTZEN). The question arises to what extent variations in cell size occur in daily diluted cultures. Cell length and width determinations are given for the September experiment in 1964, with an initial cell density of 1.0 µl/ml (cf. Table 4.3.1 and fig. 4.3.4). Small cells just after cell division have a length of 7–8 µ, large cells just before division: 12–13 µ. The fluctuations which are shown in fig. 4.3.4. are therefore rather strong. The maximum discrepancy in cell length, i.e. from 9–12.6 µ, is an increase of about 70% of the maximum possible difference. In analogy with synchronous cultures, variation in maximum and light limited photosynthetic rate, and in respiration might also be possible in daily diluted cultures.

If we assume that there is a variability in physiological properties, arresting of growth by cooling may keep the cells in the same physiological status as they had in the preculture period. We therefore stored a large amount of cells at 5°C in the dark. Samples from this cell mass were used to inoculate 1 litre erlenmeyer flasks. Growth of these inoculations on the rocking table was determined after 3 days. There was no significant difference in growth rate of these inoculations within a period of 10 days. We concluded that the physiological properties remained the same during a cooling period of at least 10 days.

In the following experiment, we tried to establish whether the variability was due to the dilution technique. Cells were precultivated in washing machines in natural daylight, and stored afterwards at 5°C in plastic containers. In an ex-

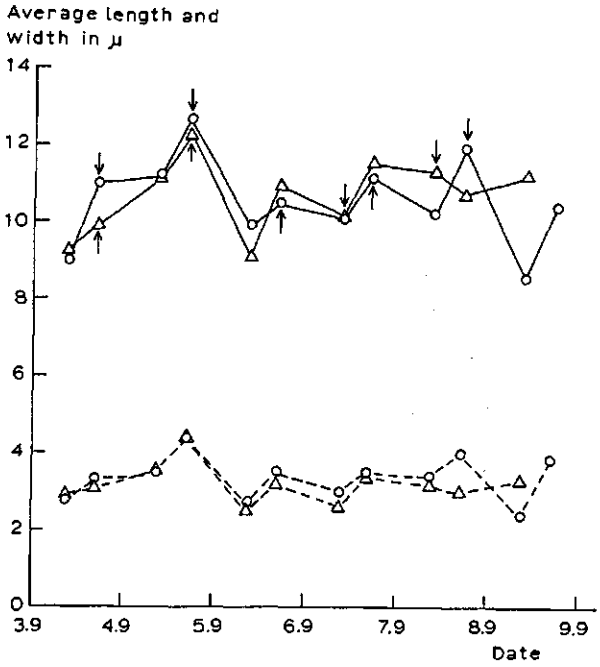

FIG. 4.3.4. Average length and width for *Scenedesmus* sp., strain K, cultivated in two washing machines. Measurements on 25 cells. The arrows denote the dilutions to TROMMSDORFF 1.0 μl/ml. Medium NSP 10.2.1, temperature 30 °C. Experiment Sept. '64. O——O, Δ——Δ, length; O- - -O, Δ- - -Δ, width.

periment in natural daylight samples from this cell mass were used as an inoculum for one washing machine each day. Another machine was diluted each day to the same cell density as present in the machine with the cooled cells. The culture solution was ANSP 5.2.1 (to prevent pH interactions), the culture temperature 30 °C. The concentration at the start of the experiment was 0.25 μl packed cell volume/ml. The yield as a function of the photosynthetically active radiation per day followed a normal BLACKMAN saturation curve (cf. fig. 4.3.5). The highest net photosynthetic efficiency was 7.0%, and it decreased with increasing light energy input. At the higher incident light energies the yield in the daily diluted suspension was somewhat lower as compared with the cooled cells. In general, the differences between the two curves are in the range of 10–20%. An influence of the dilution technique seems less evident. Cultures in washing machines (depth of layer 19.5 cm) are in general so thick that light intensity will not be sufficient to saturate photosynthesis in large parts of the machine. Under such conditions, variable maximum photosynthetic rates would be important in the top layers only.

Respiration seems to be the crucial factor under light limited conditions. Knowledge of respiratory activity might, therefore, be important.

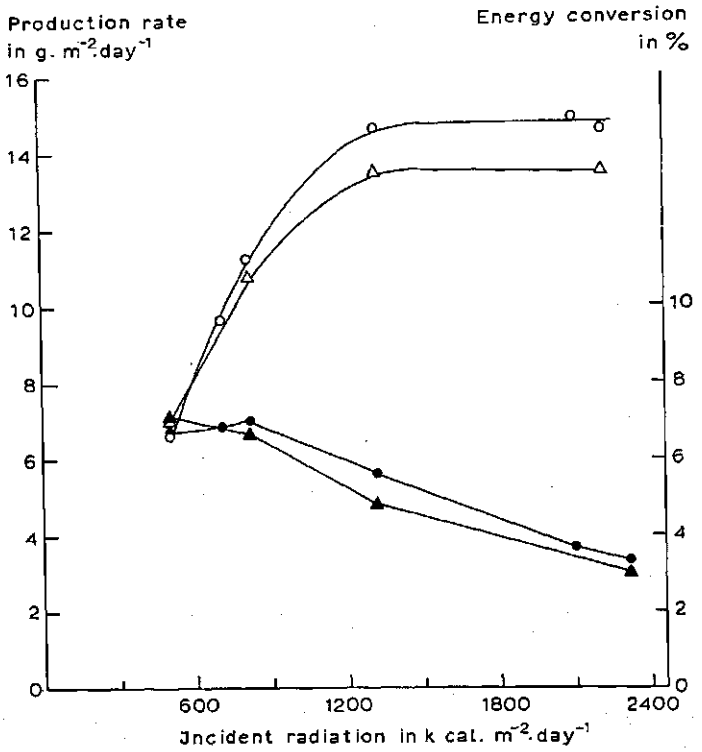

FIG. 4.3.5. Production rates and net photosynthetic efficiency values for *Scenedesmus* sp., strain K, in washing machines as a function of the daily photosynthetic active radiation. Daily dilutions to a TROMMSDORFF value of 0.25 µl/ml; culture medium ANSP 5.2.1; temperature 30 °C. Production rates cooled cells: O——O; daily diluted cells: △——△; efficiency values cooled cells: ●——●; daily diluted cells: ▲——▲.

Respiratory activity, measured as O_2-uptake per unit of cell volume or dry weight was estimated in three types of experiments.

A) A washing machine experiment under continuous mercury light (HPLR 400 W) in the laboratory. The culture medium was NSP 10.2.15, the temperature was 30 °C.

B) An experiment in the open in washing machines. Each day the culture was diluted to the level of 0.50 µl packed cell volume per ml. The culture medium was NSP 10.2.15 and the temperature 30 °C; experiment made in April.

C) As B, but diluted to a TROMMSDORFF value of 1.0 µl/ml; experiment carried out in May and June.

Respiration was measured in cell samples resuspended in WARBURG buffer no. 9, 0.1 M, in a WARBURG apparatus.

In experiment A), the respiration per unit cell volume was estimated in six samples taken at different times from a growing culture of *Scenedesmus* sp., strain K22 (cf. Table 4.3.2).

TABLE 4.3.2. Respiration in mm^3/hour per unit TROMMSDORFF value measured in cultures of *Scenedesmus* sp., strain K22. Cells cultivated in washing machines under HPLR 400 W mercury light, temperature 30 °C, culture medium NSP 10.2.15. Expt. of Dec. 1966.

Sampling date	Respiratory activity ($mm^3 \cdot h^{-1}$)
8/12	1.41 ± 0.63
9/12	2.68 ± 0.15
12/12	2.00 ± 0.30
13/12	2.15 ± 0.22
14/12	2.42 ± 0.16
16/12	1.99 ± 0.37

$\bar{x} = 2.11 \pm 0.45$ mm^3/mm^3 cell volume/hour
$s = 0.43$ mm^3/mm^3 cell volume/hour

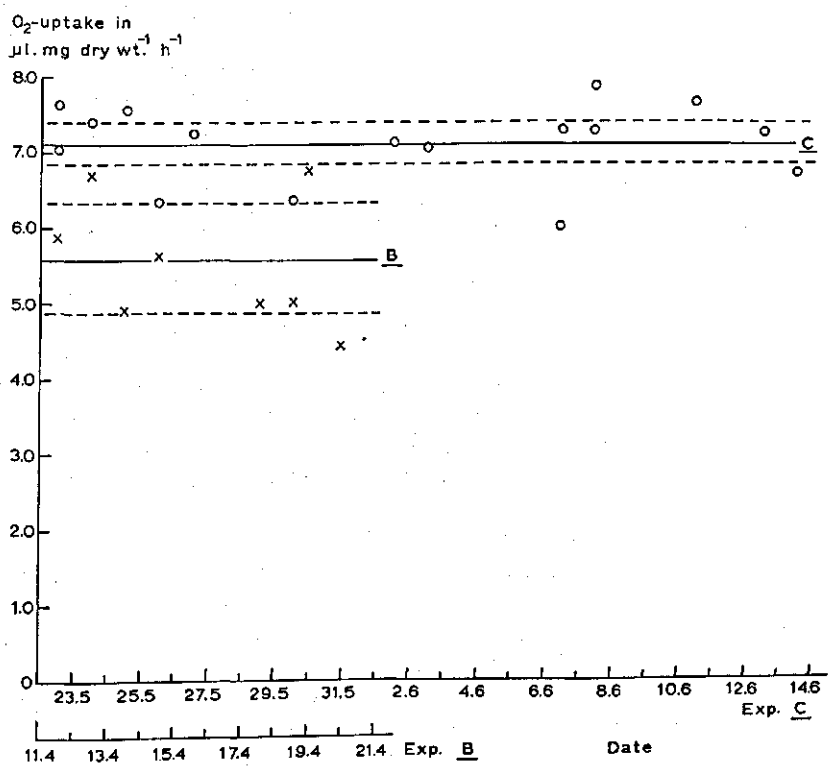

FIG. 4.3.6. Respiration in µl O_2-uptake per mg dry weight per hour. Manometric determinations in WARBURG buffer no. 9 at 30 °C, measured in morning samples taken from cultures of *Scenedesmus* sp., strain K22. Natural daylight, temperature in washing machines 30 °C, medium NSP 10.2.15. Experiment B: April 1967, diluted each day to TROMMSDORFF value 0.50 µl/ml, (×); experiment C: May and June '67, diluted each day to TROMMSDORFF value 1.0 µl/ml, (O).

The culture was diluted to its initial concentration on December 9th and 14th after the measurements. The respiratory activity was variable. The differences proved to be significant with the test of WILCOXON. Errors caused by the WARBURG and TROMMSDORFF determinations are considered to be 5% each as a minimum. The average respiratory activity per unit of packed cell volume amounted to 2.1 $\pm$ 0.4 mm^3/mm^3 cell volume.

Since TROMMSDORFF value is a parameter expressing the packed cell volume present in 1 ml suspension, it depends on cell size. In fig. 4.3.4, it was shown that cell length and width were variable parameters in a growing culture. Therefore, TROMMSDORFF value seems to be less preferable as a basis for respiratory activity as compared with dry weight.

In the experiments, described above under B) and C), the respiration was determined in samples taken from *Scenedesmus* sp., strain K22 cultures in the open, just after a dilution in the morning. Respiration in mm^3 O_2-uptake per hour per mg dry weight present was estimated for 8 samples in experiment B) and for 16 samples in experiment C). The averages were compared. The results are given in fig. 4.3.6.

Differences between the two averages were significant when the t-test was applied. The respiratory activity was highest in the culture with an initial concentration of 1.0 µl/ml. The average incident photosynthetically active radiation in June is about 1.4 times higher than in April (cf. DE VRIES, 1955). The cell density at the start of the experiment in June, however, was about two times higher than in April. When light absorption follows more or less BEER'S law, and when the incident energy in April would have been the same as in June, the energy flux for an average cell in June would have been $1/e^2$ lower than in April. The overall effect thus generally is a decrease in energy received by an average cell in June.

We may assume that the difference in respiratory activity is due to a higher carbohydrate level in experiment C). This assumption, however, can only be supported by the shorter duration of the dark period, since the average light energy received per cell is probably lower in experiment C) than in experiment B).

The phenomenon can also be explained by assuming that the difference in respiratory activity is due to a difference in cellular composition. The respiration per unit cell volume decreased for *Chlorella pyrenoidosa* in synchronous cultures during the course of cell development (cf. SOROKIN, 1964). Respiration per cell, however, increased during the course of cell development. This means that larger cells have higher respiratory rates. Since dry matter and TROMMSDORFF value were determined in experiments B) and C), the dry matter content per mm^3 packed cell volume could be calculated from determinations in experiments B) and C).

The dry matter content in experiment C) was: 130 ± 14.8 mg/ml packed cell volume.
The dry matter content in experiment B) was: 173 ± 12.0 mg/ml packed cell volume.
($s_b = 22.6$; $s_c = 27.8$; $F = 1.23$; $\varphi_1/\varphi_2 = 13/7$; $t = 4.6$, significant).

The overestimation of real cell volume by TROMMSDORFF determinations is highest in large cells. A high dry matter content per mm³ TROMMSDORFF value indicates a small cell size, a low dry matter content per mm³ Tromsdorff value a larger cell size. This would mean that the average cell size is larger in experiment C) than in experiment B).

The respiratory losses per day can be computed with the data from fig. 4.3.6:

Experiment B) respiratory activity 5.57 ml $\cdot g^{-1} \cdot hour^{-1}$ = 5.57 ml × 24 ml CO_2 = 133.6 ml CO_2 per g dry weight per day (30°C) = 5.37 mM CO_2. We may assume that each mole O_2 taken up by respiration evolves 120 kcal (cf. KOK, 1959). The average energy loss per day then is 645 cal $\cdot g^{-1} \cdot day^{-1}$. We determined the caloric value of the material to be 5.08 kcal/g ash free dry weight (cf. Chapter 2). The respiratory losses then amounted to 12.7%.

Experiment C): respiratory activity 7.11 ml $\cdot g^{-1} \cdot hour^{-1}$ = 170.6 ml $\cdot g^{-1} \cdot day^{-1}$ = 6.85 mM $CO_2 \cdot g^{-1} \cdot day^{-1}$. This amount of CO_2 represents a respiratory loss of 16.2% per day.

In the above computation average values and by no means extremes for respiratory activity were applied. The upper and lower limit for respiratory activity – as predicted by these experiments – would be for expt. B: $\bar{x}_b \pm s_b \times 1.96$; and for experiment C: $\bar{x}_c \pm s_c \times 1.96$ (for 95% of all cases). Roughly estimated, respiratory level in washing machines may vary between 16.2 + 2.4% = 18.6%, and 12.7–4.0% = 8.7%.

In order to show the effect of respiration on the production rate, the data from fig. 4.3.3 left, are corrected for respiratory losses. Since the initial concentration was 0.5 µl/ml, respiratory activity was assumed to be 12.7%. The production corrected for respiration was calculated from the production rate. Because the cultures were diluted in the morning, the light period was always split by a dark period. To simplify the calculations, it was assumed that all the light was received in one continuous period followed by a dark period.

If a = dry matter in g present at the start of the experiment
Δa = dry matter increase in g/day
X = number of hours light per day
R = respiratory loss per g dry weight present,
the average dry matter present during the light period is:

$$\frac{a + (a + \Delta a)}{2},$$

and the respiratory loss during the light period:

$$(a + \tfrac{1}{2}\Delta a) \cdot \frac{X}{24} \cdot R \qquad (1)$$

In our assumption the dry matter present at the start of the dark period would be: $(a + \Delta a)$ and the respiratory loss during the night:

$$(a + \Delta a) \cdot \frac{24 - X}{24} \cdot R \qquad (2)$$

By combining formulae 1 and 2 the production corrected for respiration (P) is:

$$P = (a + \tfrac{1}{2}\Delta a) \cdot \frac{X}{24} \cdot R + (a + \Delta a) \cdot \frac{24 - X}{24} \cdot R + \Delta a \qquad (3)$$

TABLE 4.3.3. Measured production rates for *Scenedesmus* sp., strain K, taken from fig. 4.3.3 left and corrected for respiration with formula (3). Culture medium NSP 10.2.1, temperature 30 °C, initial cell density 0.50 µl/ml.

Incident radiation (kcal · m^{-2})	Measured production (g · m^{-2})	Production corrected for respiration (g · m^{-2})	Respiratory loss (%)
540	0.04	1.62	97.5
980	6.82	9.49	28.0
1310	16.95	20.16	16.0
1350	8.26	10.67	22.5
1400	11.35	13.42	15.5
1480	10.18	12.74	20.0
1575	15.40	19.75	22.0
1640	15.05	17.55	14.3
1765	17.10	20.04	14.6

Table 4.3.3 shows that an incident radiation of 540 kcal · m^{-2} is nearly the compensation point of photosynthesis under this set of experimental conditions. The respiratory losses in the range of 1000 to 1800 kcal · m^{-2} incident radiation are between 28 and 15 % of the gross production.

The data collected in Table 4.3.1 and fig. 4.3.3 indicate that cultures diluted to a level of 1.0 µl/ml have a lower energy conversion than cultures diluted to 0.5 µl/ml. This could be ascribed to a difference in respiratory activity and/or a difference in average energy input per cell.

A lot of data were available for *Scenedesmus* cultures in washing machines growing to a steady state level. In fig. 4.3.7 the energy conversion in cultures of *Scenedesmus* sp., strain K, in nitrate containing media were collected over the period from 1962 to 1965. The bulk of the experimental material was obtained between March and October. The highest energy conversions were reached in July or August. The scarce data collected in late fall and early spring are insufficient to obtain a clear idea of the energy conversion in winter. At best they might indicate that energy conversion in cultures kept at 30 °C is lower in winter than in summer. Energy conversions collected during 117 days under comparable conditions i.e. 30 °C and 5 % CO_2, resulted in a general average of 4.02 %.

The incident radiation, measured by the Physics Department of the Agricultural University, is valid for an open field. Since no light measurements of ourselves exist for the period 1962–1965, we had to use their figures. In a later year the incident energy measured by the Physics Department was compared with light measurements at the place of the experiment. The results are presented in fig. 4.3.8. On the average the total global radiation at the place of the experiment was 75 % from the energy measured by the Physics Department. The screening by the surrounding buildings explains why the incident radiation is lower than that in the open field. The average energy conversion figures over the period 1962–1965, viz. 4.02 %, were calculated on the basis of the determinations

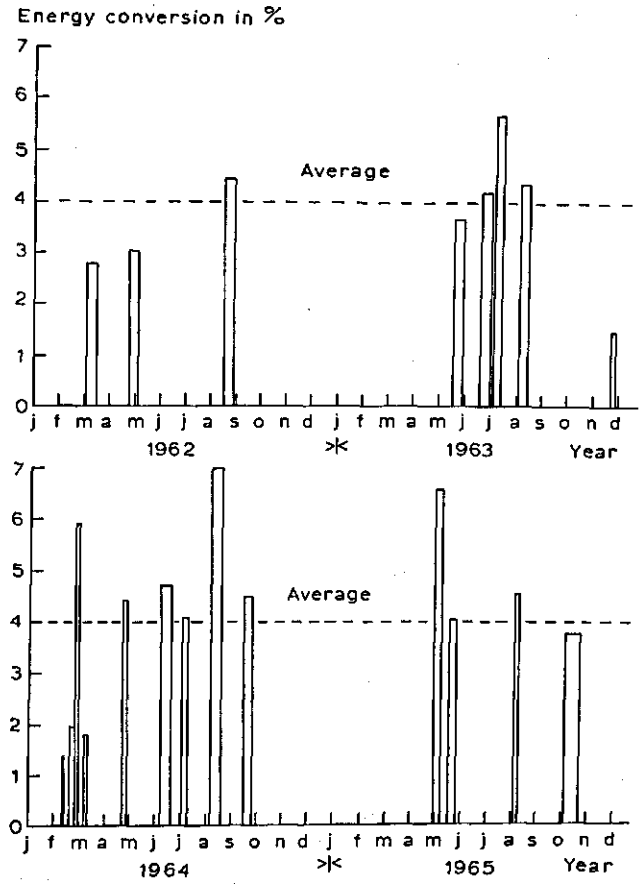

FIG. 4.3.7. Net energy conversion for *Scenedesmus* sp., strain K, over the period 1962–1965. Culture medium NSP 10.2.1, temperature 30°C.

from the Physics Department; the real energy conversion would then be: $100/75 \times 4.02\% = 5.4\%$. No correction was made for reflection and absorption by the perspex mantle of the culture vessel. Measurements indicated that around 10% of the energy was lost on the surface of the vessel. This brings the average energy conversion to 6.0%, and the maximum efficiency obtained in these experiments to 10.2%. Comparison of the non-corrected average energy conversion of 4.02% with the energy conversion in daily diluted cultures (cf. Table 4.3.1) shows that about the same values are found. Furthermore, when the high energy conversions in July and August each year are considered, it appears that cultures growing to the steady state level have no lower energy conversion than the daily diluted ones. As was shown in fig. 4.3.6, the rate of respiration per unit dry weight was higher in cultures which were diluted each day to the TROMMSDORFF value of 1.0 µl/ml than to the ones diluted to 0.5 µl/ml.

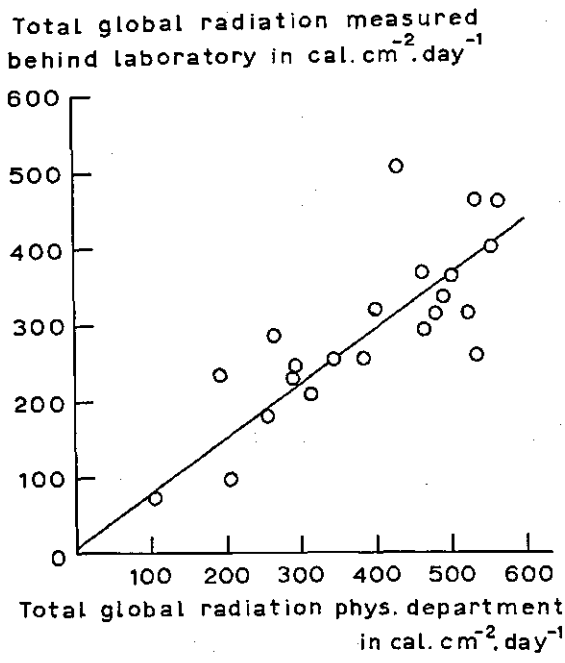

FIG. 4.3.8. Simultaneous measurements of total global radiation on a horizontal plane in an open field (Physics Department), and behind the laboratory with thermopile and integrator. Measurements 1966.

Extrapolation of these results to cultures growing towards a steady state level, where an average cell receives a steadily decreasing energy input, would suggest that the energy conversion of the culture decreases owing to an increase in biomass and an increased respiratory activity. On the basis of this reasoning, the energy conversion on the basis of incident light would be higher in daily diluted cultures than in cultures growing to a steady state level. As was shown above, this was not the case. Therefore, the behaviour of respiratory activity in daily diluted cultures cannot be extrapolated to non-diluted cultures. Further research on this subject may start with the hypothesis that a decrease in photosynthetic activity which is caused by a decreased energy input or by altered physiological properties may be associated with comparable alterations in respiratory activity.

Influence of light intensity on yield in laboratory experiments

Experiments described in the foregoing part demonstrated that the energy conversion in washing machines in the open is not better than in a conventional crop with a closed leaf surface and maximum activity.

The growth rate under light limited conditions depends on the average energy received per cell, and thus on the geometry of the culture vessel. When the depth of layer in a culture vessel is altered or the surface of the vessel is screened, the energy input per cell is affected. The efficiency of the stirring may be better if the depth of the layer is changed. Artificial illumination, which in many cases is

more one-directional than natural light, was easier than the complex situation which existed in the field.

In washing machines the depth of layer was decreased. Two machines were placed under HPLR 400W mercury lamps. They received light via the upper surface only. The energy supply on the upper surface was the same in both machines. One machine always contained the normal volume of 50 l, and served as a control. The other machine contained either 50, 40, 30 or 25 l nutrient solution. Growth rates, expressed as mg $\cdot$ l$^{-1}\cdot$ day^{-1} were compared for each run, which lasted 2–4 days. All were initially inoculated with the same total amount of cell material. Relative growth rates per l were expressed as percentages of the 50 l vessel. The results are presented in fig. 4.3.9.

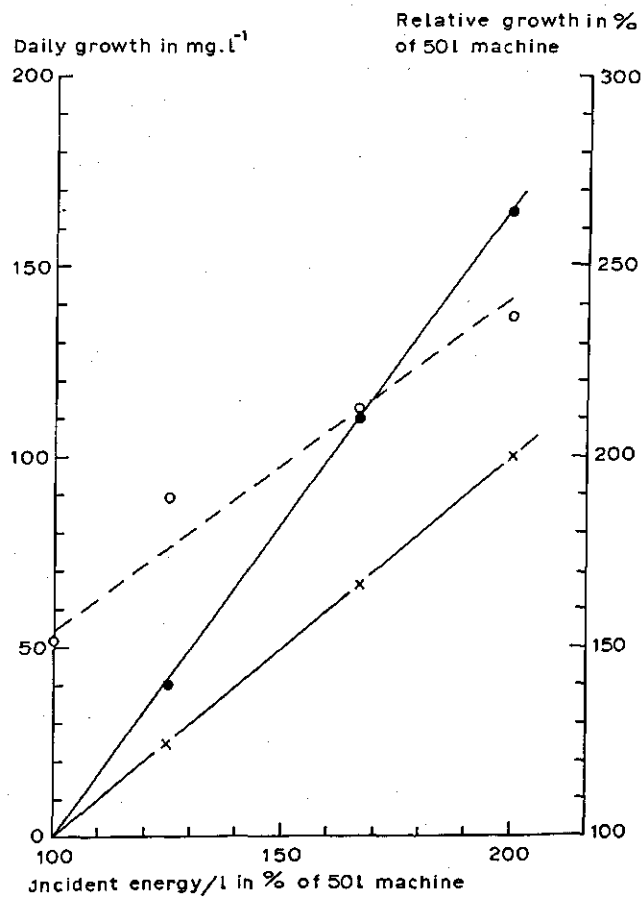

FIG. 4.3.9. Daily growth rates of *Scenedesmus* sp., strain K, in washing machines with different volumes of culture solution. Illumination with two HPLR 400 W Hg-lamps from above; O– – –O daily growth rate in mg dry weight $\cdot$ l$^{-1}\cdot$ day^{-1}; ×——× daily growth rate per litre in % of the 50 l vessel (without enhancement); ●– – –● daily growth rates in % of the 50 l vessel calculated from O– – –O.

The energy flux per unit surface is the same in the vessels with 50, 40, 30 or 25 l volume. It can be seen that the growth rates per litre increased roughly linearly with the energy received per litre volume. This means that the culture as a whole behaved as under light limited conditions. The production per vessel in % of the 50 l vessel increased more than expected when the depth of layer was decreased. This means an increased energy conversion on the basis of incident light which probably should be ascribed to a stirring effect.

In another experiment a washing machine received an energy input of 12.95×10^4 ergs $\cdot$ cm$^{-2}\cdot$ sec^{-1} continuously which, in part, was decreased by wire screens. The efficiency values were: 100% light intensity – 8.4%; 45% light intensity – 10.1% efficiency; 22.4% light intensity – 14.1% efficiency. Thus, the energy conversion improved when the light intensity decreased. Probably this means that light saturation was obtained for part of the cells.

The influence of depth of layer on yield was also investigated on the rocking table. Volumes of 100, 200, 300, 400 and 500 ml were used. The corresponding depths of layer were: 0.8; 1.6; 2.3; 3.0; and 4.0 cm. Erlenmeyer flasks were inoculated with *Scenedesmus* sp., strain K, at an initial concentration of 0.1 µl/ml (see also legend fig. 4.3.10). The experiment lasted 4 days, the illumina-

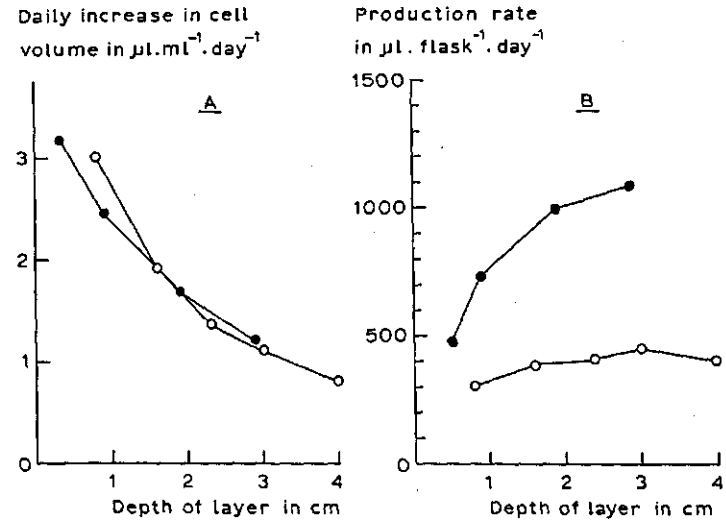

FIG. 4.3.10a. Daily increase in cell volume as a function of the depth of layer in *Scenedesmus* sp., strain K, cultivated on the rocking table. Intensity of incident light at the bottom of the vessels was 8×10^4 ergs $\cdot$ cm$^{-2}\cdot$ sec^{-1}. Light–dark ratio 16/8, temperature 30°C, culture medium NSP 10.2.15 and cultivated in 1 l Erlenmeyer flasks (growth period of 4 days) ○——○; cultivated in Fernbach bottles (growth period of 3 days) ●——●. Irradiated surface of erlenmeyer flask 133 cm², Fernbach bottle 290 cm².

FIG. 4.3.10b. Total production per flask (µl $\cdot$ ml^{-1} $\cdot$ day^{-1}) as a function of the depth of layer. *Scenedesmus* sp., strain K, was cultivated on the rocking table. Light–dark ratio 16/8, temperature 30°C, culture medium NSP 10.2.15. Erlenmeyer flasks (growth period 4 days) ○——○; Fernbach bottles (growth period of 3 days) ●——●.

tion was discontinuous. Light/dark ratios (L/D) of 16/8 were applied. Growth was measured as increase in TROMMSDORFF value (cf. fig. 4.3.10a) and as increase in packed cell volume per flasks (cf. fig. 4.3.10b). A similar experiment was made in FERNBACH bottles. In contrast with the 1 litre Erlenmeyer flasks these vessels have a partially vertical side wall. In this case the medium was NSP 10.2.15. The flasks received 3 days discontinuous light with an L/D ratio of 16/8. Also these results are presented in fig. 4.3.10a, b.

In both experiments the production per 100 ml suspension was highest in the 100 ml vessels and decreased more or less exponentially with increased volumes. The production per flask increased up to a depth of layer of about 3 cm in both cases. Since the algal density at the start of the experiment was low, the results may be partly due to differences in absorption.

In order to eliminate the possibility of differences in light absorption, in subsequent experiments flasks were inoculated with the same total cell volume in different quantities of NSP 10.2.15. Three parallel experiments with a duration of 1 day each were carried out under the same conditions as described in fig. 4.3.10. Increase in packed cell volume followed the same trend as in fig. 4.3.10a. The total production per flask was somewhat higher in the flasks with 200 ml than with the other volumes, although the differences between the treatments were statistically insignificant. These results imply that the differences in total production given in fig. 4.3.10b were due to the differences in total cell volume per flask at the start of the experiments. No indications for a favourable effect of a certain depth of layer in combination with the shaking rate could be found.

We have attempted at computing the energy conversion in a flask on the rocking table. The data presented in Table 3.4.1 are reliable production rates under conditions of light limitation. The best production was obtained with NSP 10.2.15, viz. 995 mg $\cdot$ $l^{-1}\cdot$ day^{-1} or 330 mg $\cdot$ $flask^{-1}\cdot$ day^{-1}. The light intensity was 6×10^4 ergs $\cdot$ $cm^{-2}\cdot$ sec^{-1}, the irradiated surface was 132.7 cm^2. If we consider that the depth of layer was 2.5 cm, the absorption coefficient $k =$ 0.30 ml $\cdot$ $\mu l^{-1}\cdot$ cm^{-1} (cf. section 4.2), and that the absorption in the flask was 95% of the incident light, the calculated energy conversion was 9.2%. Respiration will be high at a temperature of 30 °C and at these high densities. Moreover the light intensity at the surface of the flasks was above light saturation (the saturating light intensity for *Chlorella* at 25 °C was 3.5×10^4 ergs $\cdot$ $cm^{-2}\cdot$ sec^{-1}, cf. VAN OORSCHOT, 1955).

Energy conversion was also estimated in water cooled culture tubes which were inoculated with 8–10 μl packed cell volume/ml. The intensity of the fluorescent light was between 1.96×10^4 and 5.13×10^4 ergs $\cdot$ $cm^{-2}\cdot$ sec^{-1}. The energy conversion as a function of the light intensity is given in fig. 4.3.11.

Net photosynthetic efficiency reached the theoretical maximum at light intensities lower than 20 kcal/day. The net efficiencies decreased also in culture tubes when the light intensity at the surface of the suspension was increased.

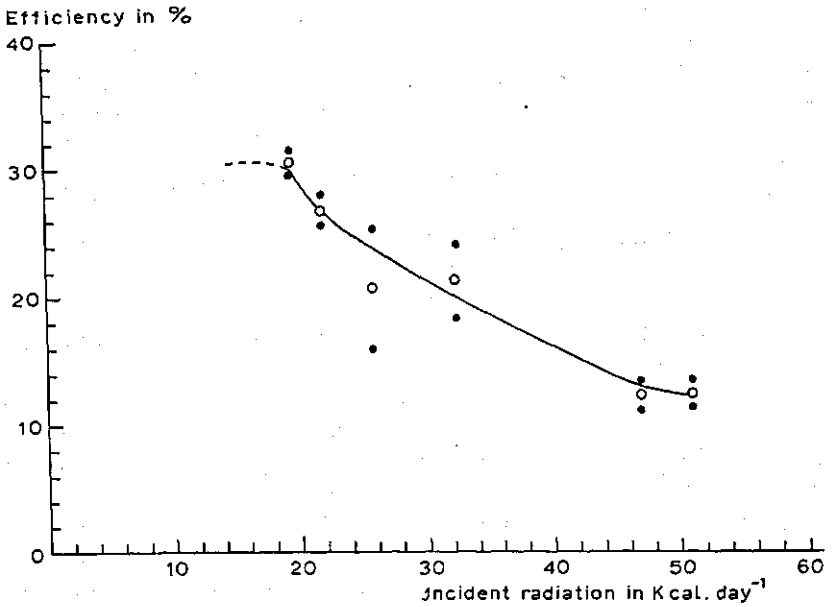

FIG. 4.3.11. Net photosynthetic efficiency for *Scenedesmus* sp., strain K, grown at low light intensity in 'continuous culture' tubes. Culture medium NSP 10.2.1, temperature 30 °C, L/D = 24/0. Dry weight increase over a period of 1 day used for the calculation of net efficiency; • individual measurements; ○ average efficiency values.

4.4. DISCUSSION

The apparent maximum efficiency in the open was rather low as compared with the theoretical value. The high efficiencies obtained by KOK (1952) in a WARBURG apparatus were measured in monochromatic Na-light of rather low intensity. They were based on the amount of absorbed energy. Since there is a difference in spectral composition between sunlight and Na-light, KOK (1952) postulated that the efficiency in sunlight had to be lower than the observed 25% under laboratory conditions. SEMENENKO *et al* (1966), with *Chlorella* sp., strain K, investigated the influence of the spectral composition on energy conversion. They composed equi-energetic fields by mixing red and blue light in different proportions. Cell division and productivity under conditions of light limitation increased up to 120% and 140% respectively when the light quality was changed in favour of the red component. Their results suggest, that the number of quanta in a certain energy flux influence the energy conversion.

Since daylight is variable in composition owing to cloudiness and moisture content of the air, the measured energy flux is difficult to evaluate as to its quantum content. Light measurements with a thermopile integrate the energy in all wave-lengths. When the Russian investigations are correct this may imply

that thermopile measurements alone do not give sufficient information to calculate the energy conversion. The quantum density in the different parts of the spectrum may be difficult to assess.

Our own experiments are evaluated in terms of incident energy.

The energy conversion in nitrate containing media (NSP 10.2.1) was estimated over the period 1962–1965 (cf. fig. 4.3.7). The average net energy conversion (mainly determined in the summer months) was 4.02% under conditions of light limitation. Maxima of 6–7% were obtained in July and August. These measurements were carried out in the open. Screening by the buildings determined the ratio between direct and diffuse radiation. On the average, 75% of the energy measured in the open field was received at the site of the experiment. Considering a reflection of 10% on the surface of the vessel, the real net energy conversion would be 6.0% on the average, with a maximum of 10.2% in July and August.

High energy conversions were also found in agricultural crops during short periods. KAMEL (1959), with barley and sugar beet, found net energy conversions of 13.6 and 10.0% respectively for periods of about a fortnight, confirming earlier computations by GAASTRA (1958). These values were higher than those in our algal cultures. It cannot be excluded that a more efficient energy conversion is possible in other types of culture vessels.

The data of fig. 4.3.1, 4.3.2, 4.3.3, and 4.3.5 suggest that the culture as a whole was light limited at an initial concentration of 0.5 µl/ml. In cultures with an initial cell density of 0.25 µl/ml light saturation was observed at an energy input of 1200–1500 kcal $\cdot$ m$^{-2}\cdot$ day^{-1} (cf. fig. 4.3.5). When the culture could grow during a couple of days before it was diluted, as in the experiments described in figures 4.3.1 and 4.3.2, a decrease in average energy conversion during the growth period was observed when the initial concentration was increased. Under conditions of light limitation the initial cell density can only affect the average cell density reached during the growth period, which may have consequences for the total respiration.

The average efficiency for cultures diluted to 0.50 µl/ml (cf. Table 4.3.1) increased when the incident radiation decreased. The same tendency was observed with the cultures diluted each day to a cell density of 1.0 µl/ml.

NICHIPOROVITCH and MALOFEEV (1965) arrived at the conclusion that an increase in leaf area ratio tended to produce a higher energy conversion at high light intensities provided that the crop had a closed canopy. This would mean in algal cultures that an increase in initial cell density would produce higher efficiency values. A consultation of Table 4.3.1 shows that a doubling of the initial cell density was associated with lower energy conversions as compared with the 0.50 µl/ml series. Our conclusion would be that other factors than light might interfere with the results, as for instance daylength, respiration.

The factor respiration appeared to be important in our cultures. Daily dilutions to 0.5 µl/ml or 1.0 µl/ml showed significantly different respiratory rates/g dry weight in morning samples in April and June respectively (cf. fig. 4.3.6). Respiratory losses per day (in percent of ash-free dry weight present) amounted

to 12.7% in the April experiment and 16.7% in the June one. An open question still is the course of respiration in non-diluted cultures. The gradual increase in cell density caused a decreased energy supply per cell in the culture. The data in fig. 4.3.2. and fig. 4.3.6 might suggest that diluted cultures also have lower energy conversion with higher initial concentrations, since respiration becomes higher. Measurements over longer periods, as in fig. 4.3.7, do not show lower energy conversions if the cultures are kept growing without dilution. Does this mean that respiration adapts to a lower energy input in non-diluted cultures? There are indications in the literature that this might occur. LOOMIS (1967) mentioned work of LUDWIG and SAEKI with corn. They found that corn transferred from a wide to a narrow spacing adapted its respiration to lower values in a couple of days.

Algae are appropriate to investigate the influence of density on respiratory activity. The experiments in culture tubes described in fig. 4.3.11 show that the theoretical maximum efficiency value (20–25%) can be reached in cultures provided the light intensity is sufficiently low. Higher light intensities resulted in efficiency values of 9–10% in experiments on the rocking table.

As described in part 4.2, an enhancement of the stirring velocity might increase the energy conversion. The results of DAVIS (in BURLEW, 1953) show that the energy conversion in strong light increases with increased stirring velocity. The only case in which we could detect an influence of quicker stirring was in washing machines with varied depths of layer (cf. fig. 4.3.9). The experimental data obtained on the rocking table did not show significant differences in growth rate when the volume in the flasks was varied. Since stirring in washing machines was more rapid, and the depth of layer 7 times higher as compared with the flasks on the rocking table, a decrease in depth of layer had a relatively stronger effect on the $T_l/T_l + T_d$ ratio in washing machines than on the rocking table. The rather high values of the standard deviation on the rocking table are possibly due to TROMMSDORFF measurements used as a growth indicator. A possible small increase in growth rate might be obscured by the high standard deviation of the measurements.

Discussing once more the low efficiency values in the open, we stated in part 4.2.1 that the $T_l/T_l + {}^{l}T_d$-ratio was rather high in washing machines. A decrease in depth of layer with the same stirring velocity would have been favourable, since it decreased the ratio $T_l/T_l + T_d$. The stirring is not without any effect since efficiency values of 6.0% on the average could be obtained. The high level of respiration and the stirring rate seem to be limiting factors for the energy conversion in washing machines.

5. EFFECT OF DAYLENGTH ON YIELD

5.1. INTRODUCTION

Literature does not present experiments specifically dealing with effect of light duration. Using 300 l vessels, VAN OORSCHOT (1955), with *Chlorella*, found a net photosynthetic efficiency of 4 % in July, and a lower value in autumn and spring. On the other hand, GUMMERT, MEFFERT and STRATMANN (1953) reported a maximum photosynthetic efficiency for *Chlorella* in October, when the algae were cultivated in open air trenches. Because the authors mentioned above worked under natural conditions, temperature, light intensity, and light duration effects cannot well be separated in their experiments. It might well be that light duration interferes with net photosynthetic efficiency via changes in respiratory level when the light-dark ratio is changed.

On the other hand, the existence of a life cycle in algae (cf. TAMIYA, 1953b; SOROKIN, 1964; LORENTZEN and RUPPEL, 1959), an inconstant photosynthetic rate (SOROKIN, 1964) and a decreasing maximum quantum efficiency (SENGER and BISHOP, 1967) do not exclude the possibility that changes in the light-dark ratios interfere with processes other than respiration only.

Since in the temperature zones daylength is a strongly variable factor it seemed worth while to investigate to what degree effects of daylength on yield could be observed.

5.2. EFFECT OF DAYLENGTH ON GROWTH IN THIN AND THICK LAYERS

Cell concentration, light intensity, and depth of layer affect the level in the vessel at which the photosynthetic apparatus is saturated, and that at which photosynthesis is compensated by respiration. Therefore, we considered 4 combinations of depth of layer and cell concentration:

a) thick layers and high cell concentrations, in washing machines.
b) thick layers and low cell concentrations in the same type of culture vessels.
c) thin layers and high cell concentrations, in 'continuous culture' tubes as described in Chapter 2.
d) thin layers and low cell concentrations, also in culture tubes.

We will subsequently consider the various cases.

a) Thick layers, high cell concentrations. In experiments made indoors in the laboratory high cell concentrations and thick layers were used. Washing machines were illuminated from above by 1 HPLR mercury lamp 400 W (PHILIPS), and by four HO-2000, 450 W mercury lamps (PHILIPS) from aside. Cells were precultivated under continuous light during a 4 days' growing period on the rocking table. After that, cells were aerated overnight in the dark

TABLE 5.2.1. Growth of *Scenedesmus* sp., strain K, as a function of daylength. Washing machines were inoculated with 0.05 µl/ml algae in NSP 10.2.1. Culture temperature 30 °C. Daylength treatments started after 1 day in continuous light.

Treatment L/D	(a) Production ($mg \cdot l^{-1}$/24 hours)	(b) Production ($mg \cdot l^{-1}$/24 hours)	s_a	s_b	φ
24/0	187.1 ± 15.8	7.80 ± 0.66	24.2	1.01	5+4
16/8	152.2 ± 11.0	8.14 ± 0.69	17.7	1.11	3+2+5
12/12	105.3 ± 5.3	8.78 ± 0.44	8.1	0.68	4+5
8/16	38.8 ± 8.9	4.85 ± 1.11	12.4	1.55	3+5

at room temperature. In this way all possible cell divisions were effectuated before inoculation of the washing machines. The influence of daylength on growth was estimated in several experimental series run after each other. Light limited growth rates were measured. The results are collected in Table 5.2.1.

Column φ denotes the number of degrees of freedom. Parallel experiments could be combined since the standard errors did not differ significantly as measured with the F-test. Production was highest in the L/D = 24/0 series, and lowest in the L/D = 8/16 series. Only in the 8/16 series, the production per light hour was significantly lower than under the other conditions. Since we were measuring production rates, it lies at hand to ascribe the lower production rates under conditions of L/D = 8/16 to a relatively increased respiration, resulting in lower productions per light hour with shorter daylengths.

An alternative would be that the cells adapt themselves to a certain light regime and behave differently if they are transferred to other light conditions. A change in cellular composition, for instance, was observed when cells precultivated at L/D = 24/0 were transferred to L/D = 16/8 (this was one of the L/D = 16/8 experiments from Table 5.2.1). Fig. 5.2.1 shows the change in frequency distribution of the cell mass which occurs in the course of time. The normal distribution found at the start of the experiment changes to a preponderance of large cells at the end of the second light period (40 hours) and smaller cells at the end of the dark period (48 hours).

In an experiment on the rocking table we investigated whether the previous light period had an influence on the growth rate under different daylengths. Cells were inoculated from agar slants in NSP 10.2.1 and received 5 days of continuous light. After that, 5 ml of equal cell density were inoculated in NSP 10.2.15. These flasks received the pretreatments: L/D = 24/0, 16/8, 12/12, or 8/16 during three days. The cultivated cells were stored in the dark at 5 °C and then used for inoculation at 1.0 µl packed cell volume per ml. Each treatment used for the determination of production rates consisted of 7 parallels. The duration was one day for each treatment. Table 5.2.2 shows the results.

The differences between the individual production rates were tested for significance. Let us subsequently consider the experimental treatments in relation to the pretreatment. Treatment L/D = 24/0 showed the highest production rates

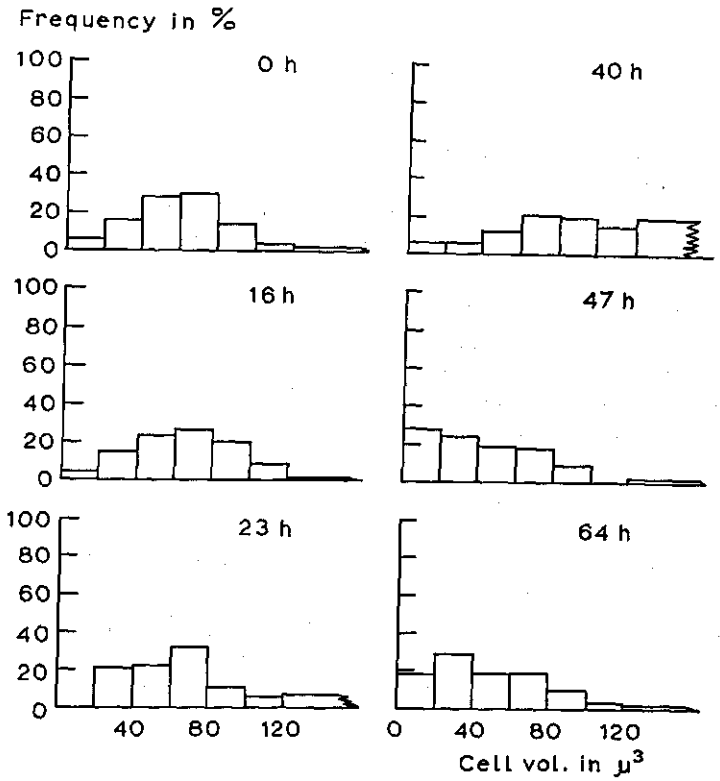

FIG. 5.2.1. Frequency distributions of cell volume calculated from length and width measurements with the formula: $V = 1/6\,\pi\, lW^{-2}$ Cells precultivated at L/D = 24/0 and aerated during 12 hours in the dark at room temperature were transferred to L/D = 16/8. Light periods started at 0, 24 and 48 hours after the start of the experiment *Scenedesmus* sp., strain K, temperature 30 °C, NSP 10.2.1.

TABLE 5.2.2. The influence of the light regime during precultivation on the growth of *Scenedesmus* sp., strain K, under several daylengths.

Precultivation 1) 5 days NSP 10.2.1.; L/D = 24/0; 3 days NSP 10.2.15; L/D = 24/0.
2) 5 days NSP 10.2.1; L/D = 24/0; 3 days NSP 10.2.15; L/D = 16/8.
3) 5 days NSP 10.2.1; L/D = 24/0; 3 days NSP 10.2.15; L/D = 12/12.
4) 5 days NSP 10.2.1; L/D = 24/0; 3 days NSP 10.2.15; L/D = 8/16 .

Temperature 30 °C ± 0.5 °C; NSP 10.2.15 precultivated cells kept at 5 °C in the dark and used for inoculation at 1.0 μl packed cell volume per ml. Each treatment one day growth.

Pretreatment (L/D)	Treatment			
	L/D = 24/0 (mg · l⁻¹ · day⁻¹)	L/D = 16/8 (mg · l⁻¹ · day⁻¹)	L/D = 12/12 (mg · l⁻¹ · day⁻¹)	L/D = 8/16 (mg · l⁻¹ · day⁻¹
24/0	528.3 ± 71.5	391.3 ± 54.8	242.3 ± 31.4	154.3 ± 21.9
16/8	585.3 ± 68.3	363.3 ± 58.3	194.0 ± 59.7	149.0 ± 17.6
12/12	433.0 ± 113.4	258.0 ± 47.8	210.0 ± 32.2	132.5 ± 20.4
8/16	481.6 ± 26.4	295.0 ± 62.3	152.3 ± 23.1	-

under the pretratment L/D = 16/8, although the difference with the pretreatment 24/0 was statistically insignificant. Pretreatment L/D = 16/8 had a significantly higher production rate than 12/12 and 8/16. Treatment L/D = 16/8 shows the highest production rate with a pretreatment of L/D = 24/0, which does not significantly differ from pretreatment L/D = 16/8. Significant differences in production rates exist between pretreatments L/D = 24/0 and 12/12 and 8/16 respectively. Treatment 12/12 does not show significant differences between the pretreatments L/D = 24/0, 16/8 and 12/12. Significant differences in production rate existed between L/D = 24/0 and L/D = 8/16, and also between L/D = 12/12 and L/D = 8/16. Treatment L/D = 8/16 showed no significant differences in production rates for the several pretreatments. The last series is omitted owing to a defect of the rocking table machinery.

We conclude that pretreatment influences the growth of algae under different light regimes. An extreme shortening of the daylength, e.g. from continuous light to L/D = 8/16, was unfavourable for growth.

Experiments in washing machines, as described in Table 5.2.1 showed a decreased production under conditions of L/D = 8/16 and a pre-treatment at L/D = 24/0. If we do not attribute the lower production at L/D = 8/16 to a relatively increased respiration alone, but also to the pretreatment, we may expect that photosynthesis is favoured by certain pretreatments. The cultures on the rocking table received a higher energy input per cell than the ones in washing machines. Differences in photosynthetic activity due to the pretreatment would be first expected under the best illumination, thus on the rocking table.

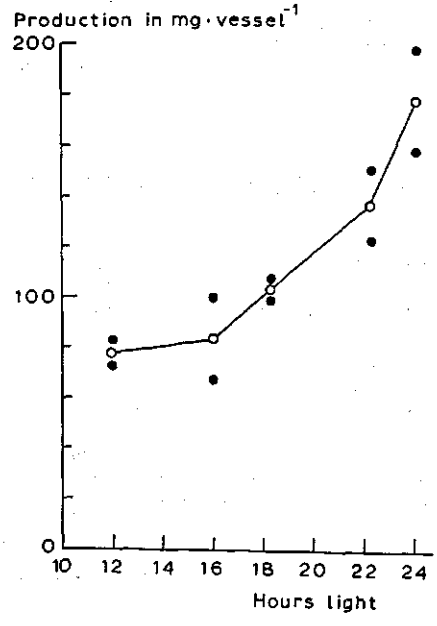

FIG. 5.2.2. Effect of daylength on yield of *Scenedesmus* sp., strain K, in washing machines. Temperature 30°C, culture medium NSP 10.2.15. Illumination by 6 TL fluorescent tubes 125 W (PHILIPS), 46 cm above the water level. Initial cell density 0.10 µl/ml.

b) Thick layers, low cell concentrations. An experiment was carried out in two washing machines, illuminated with six (PHILIPS) 125 Watt fluorescent tubes, 46 cm above the water level. Cells precultivated at L/D = 24/0 in washing machines to the density of 0.95 µl packed cell volume per ml, were stored at 5 °C. Each day, the machines were inoculated with this suspension (initial concentration: 1 g dry weight per 50 litre). The cell suspension was subjected each day to another light treatment. Light-dark ratios of L/D = 24/0; 22.5/1.5; 18.5/5.7*; 16/8 and 12/12 were applied. Dry matter production over a period of 24 hours was estimated. Since the cell mass used for inoculation remained physiologically stable during cooling there was no objection to collect the production rates in one graph (cf. fig. 5.2.1). Growth is more or less exponential in relation to duration of the light period, between 12 and 24 hours of light.

In thick layers with a low cell concentration (light saturation for the majority of the cells), increase in daylength will result in increased light absorption, owing to algal growth. Growth rates then can be calculated with the formulae:

$$W = W_o \cdot e^{kt} \qquad (1)$$

in which W_o and W are dry weights at time 0 and t respectively, and k is a growth constant.

Maximum increase in cell number in one day, as given in literature under conditions of light saturation, is 8-fold for *Scenedesmus*. The same is true for dry weight increase (cf. TAMIYA, c.s., 1953). Substituting $W/W_o = 8$ in formula 1, the calculated maximum value for k would be 0.087/hour (which in practice means hours of light). If we neglect respiration, and consider the growth rates at several daylengths (cf. fig. 5.2.2) as part of an exponential curve in continuous light, k as resulting from our observations in this experiment can be calculated by substituting the growth data in formula 1. The results of these calculations are collected in Table 5.2.3.

TABLE 5.2.3. Values of k for growth of *Scenedesmus* sp., strain K, in two washing machines as influenced by daylength. Illumination by six TL fluorescent tubes (PHILIPS) 125 W; 46 cm above the water level. Culture temperature 30°C; NSP 10.2.15. Inoculation with material precultivated in washing machines up to a concentration of 0.95 µl/ml. Initial concentration 0.10 µl/ml.

L/D	k/hour	average k/hour
24/0	0.044; 0.041	0.043
22.5/1.5	0.043; 0.037	0.040
18.5/5.7	0.042; 0.042	0.042
16/8	0.046; 0.030	0.038
12/12*	0.064; 0.053	0.058

* In this case the initial concentration was 0.75 g/50 l instead of 1.0 g/50 l, owing to a dilution error.

* This particular experiment lasted 24 hours and 12 minutes.

The k-values from L/D = 24/0 to 16/8 are about the same, but lower than the maximum value of 0.087/hour. The observed variation can be due to the fact that the precision of the dry matter estimation is questionable at the low cell densities of 1 g/50 litre suspension. The k-values, in general, are too low to assume complete light saturation. Considering that the depth of layer in washing machines was 20 cm, it is understandable that already at very low concentrations part of the cells have light limited growth. Nevertheless, the production increased more than proportional with increasing daylength, owing to an apparent increase in efficiency based on incident light energy by increased light absorption.

c) Thin layers, high cell concentrations. The influence of daylength on algae grown at increasing cell concentrations was investigated at light-dark ratios (L/D) of 8/16, 12/12, 16/8, and 24/0 in culture tubes. The temperature during the experiment was 30 °C. The pretreatment was under continuous light. The results are presented in Table 5.2.4.

At the initial concentrations of 3.16 µl packed cell volume per ml and higher the energy conversion attained high values. This may be due to the probability that light energy was limiting in this range of concentrations.

If we average the energy conversions obtained with initial concentrations of 3.16 µl/ml and higher, the energy conversion shows a tendency to decrease from L/D = 24/0 to L/D = 8/16. Differences between L/D = 24/0, 16/8 and 12/12 were insignificant. Nevertheless, energy conversion at L/D = 24/0 was significantly higher than at L/D = 8/16. It can be observed in Table 5.2.1 that cells pretreated with an L/D = 24/0 show significant differences in growth rate be-

TABLE 5.2.4. Net photosynthetic efficiencies for *Scenedesmus* sp., strain K, in culture tubes as influenced by daylength and inoculation density. Temperature 30 °C; NSP 10.2.15. The measurements started with different initial cell densities (in µl packed cell volume/ml) and were all run independently for 24 hours.

TROMMSDORFF value at start (µl/ml)	Efficiency L/D = 24/0 (%)	Efficiency L/D = 16/8 (%)	Efficiency L/D = 12/12 (%)	Efficiency L/D = 8/16 (%)
3.16	18.8	–	13.3	–
	14.6	–	12.8	–
4.97	19.2	9.9	–	7.2
	12.4	13.4	–	4.8
5.30	23.1	19.3	–	13.3
	17.2	–	–	12.4
8.12	19.4	–	11.0	13.6
	16.4	–	17.7	13.3
13.40	17.0	16.0	–	16.8
	14.9	13.9	–	13.7
Average	17.3 ± 2.2	14.5 ± 4.3	13.7 ± 4.5	11.9 ± 3.3

tween the treatments L/D = 24/0 and L/D = 8/16. The results of the experiments described in Table 5.2.4 give analogous results. This would mean that respiration, especially at short daylength, diminishes the efficiency of energy conversion in this different experimental set up.

d) Thin layers, low cell concentrations. Energy conversions in a series of L/D treatments, obtained with *Scenedesmus* sp. cultivated at low cell densities in culture tubes, are presented in Table 5.2.5.

Cell density was low at the start of the experiment; we assumed light saturation and exponential growth. Under these conditions the energy conversion on the basis of incident light will depend on the amount of absorbed energy, which is a function of the cell density, unless an influence of light-dark treatment is present. We took as a zero hypothesis that the average cell density over the growth period is the only relevant factor in determining the energy conversion

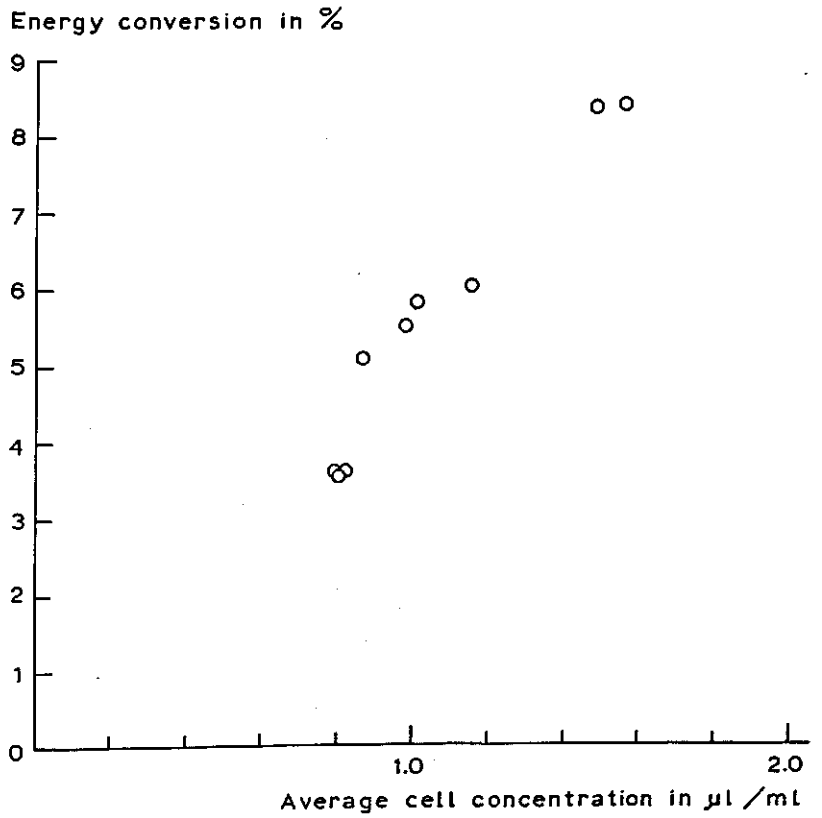

FIG. 5.2.3. Energy conversion as a function of average cell concentration for *Scenedesmus* sp., strain K. Tubes were inoculated with low initial cell concentrations. Temperature 30°C; NSP 10.2.15. Data from Table 5.2.5 used for the calculation.

TABLE 5.2.5. Growth of *Scenedesmus* sp., strain K, expressed as increase in TROMMSDORFF value (μl/ml) and net photosynthetic efficiencies as influenced by daylength and inoculation density. Temperature 30 °C; NSP 10.2.15. Experiments A, B and C were run independently for 24 hours.

Expt.	L/D = 24/0				L/D = 12/12				L/D = 8/16			
	Start	End ($\mu l \cdot ml^{-1}$)	Average	Effic. (%)	Start	End ($\mu l \cdot ml^{-1}$)	Average	Effic. (%)	Start	End ($\mu l \cdot ml^{-1}$)	Average	Effic. (%)
A	0.59	4.36	1.60	8.9	0.59	1.68	0.99	5.6	0.59	1.30	0.88	5.6
	0.59	3.77	1.50	7.9	0.59	1.52	0.95	5.4	0.59	1.24	0.85	4.6
	Average		*1.55*	*8.4*			*0.97*	*5.5*			*0.86*	*5.1*

Expt.	L/D = 24/0				L/D = 16/8				L/D = 12/12			
	Start	End ($\mu l \cdot ml^{-1}$)	Average	Effic. (%)	Start	End ($\mu l \cdot ml^{-1}$)	Average	Effic. (%)	Start	End ($\mu l \cdot ml^{-1}$)	Average	Effic. (%)
B	0.48	2.79	1.16	6.4	0.48	1.55	0.86	4.1	0.48	1.22	0.77	3.3
	0.48	2.73	1.14	5.6	0.48	1.30	0.79	3.1	0.48	1.40	0.82	3.8
	Average		*1.15*	*6.0*			*0.82*	*3.6*			*0.80*	*3.55*

Expt.	L/D = 24/0				L/D = 16/8				L/D = 8/16			
	Start	End ($\mu l \cdot ml^{-1}$)	Average	Effic. %	Start	End ($\mu l \cdot ml^{-1}$)	Average	Effic. %	Start	End ($\mu l \cdot ml^{-1}$)	Average	Effic. %
C	0.48	4.55	1.48	8.9	0.48	2.27	1.04	6.5	0.48	1.42	0.82	5.7
	0.48	4.37	1.45	7.9	0.48	2.01	0.98	5.2	0.48	1.25	0.77	4.45
	Average		*1.47*	*8.4*			*1.01*	*5.85*			*0.80*	*5.1*

under conditions of light saturation. We may then expect a linear relationship between average cell density and energy conversion in terms of incident light, irrespective of the applied L/D ratios. The average cell concentrations, calculated as the geometric mean of the TROMMSDORFF values at the start and the end of the growth period, and energy conversions for the different L/D ratios are presented in fig. 5.2.3.

The distribution of the data was tested with KENDALL's correlation test. The zero hypothesis, viz. that the distribution of the data was produced by chance could be rejected ($p < 0.01$). These data do not allow to conclude, therefore, that there is an influence of daylength during the first 24 hours after a change from continuous light to a certain light/dark treatment.

Influences of daylength were investigated in this chapter on cultures with increasing optical density. Identical results had to be expected when the cell density was kept at a constant level. This could be made in similar tubes which

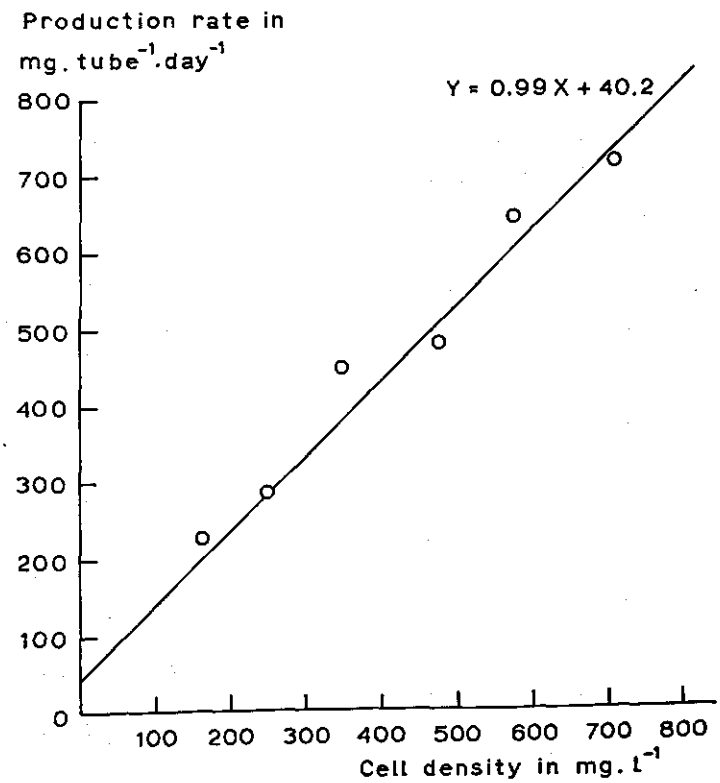

FIG. 5.2.4. Production of *Scenedesmus* sp., strain K22, in a continuous culture tube as a function of cell concentration. Temperature 30°C; L/D = 24/0; NSP 10.2.15; light intensity 3.4×10^4 ergs · cm^{-2} · sec^{-1}.

were fitted out with the specific design for continuous culture so that growth was kept at constant optical density.

Fig. 5.2.4 shows that, operating this design at different cell densities, a linear relationship exists between production rate and cell density in the range of 160 to 700 mg dry matter per litre (TROMMSDORFF 1.1–4.6 μl/ml). These results imply that photosynthesis was light saturated in this case, and also at the cell concentrations in Table 5.2.5 and fig. 5.2.3. In the following experiment cells were kept at a constant optical density of about 2.0 μl/ml. A series of measurements with this technique was carried out with different light regimes, viz. L/D = 24/0, 16/8, 12/12, and 8/16 at a temperature of 25 °C. Production rates were measured at intervals over a total period of 5 to 8 days. The production rate per hour light is given in fig. 5.2.5.

The production rate at L/D = 12/12 was significantly higher and that at L/D = 24/0 significantly lower than in the other treatments tested. The total production per vessel per light period was highest at L/D = 12/12 and decreased at

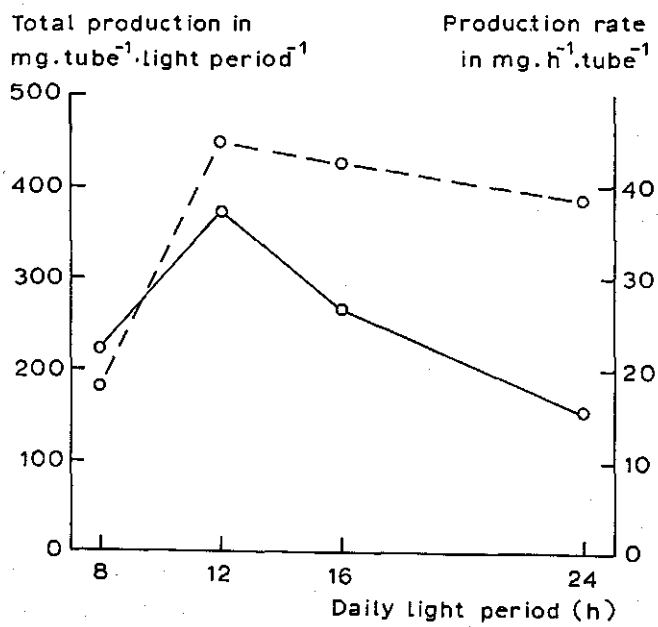

FIG. 5.2.5. Production per hour light O——O, and total production per light period O- - -O for *Scenedesmus* sp., strain K23, kept at a constant density of 2.0 μl/ml in a continuous culture tube. Light intensity was 3.4×10^4 ergs · cm^{-2} · sec^{-1}, temperature 25 °C, culture medium NSP 10.2.15.

shorter and longer daylengths. Apparently, continuous illumination is disadvantageous when the culture is kept at a constant low cell density. This phenomenon did not occur in the experiments lasting one day as for instance in Tables 5.2.3 and 5.2.4. In cultures kept at a constant cell density, the energy flux received by an average cell remains nearly the same. A production rate which depends on the light/dark treatment suggests, therefore, that the photosynthetic activity or the efficiency of dry matter production is not the same when different light/dark ratios are applied. Similarly, we concluded from the experiments described in Table 5.2.2 that certain pretreatments with different L/D regimes influenced photosynthetic activity. Apparently, a longer lasting pretreatment or a treatment over a period of a week as in this experiment, induces the production rate to become dependent on the light/dark regime applied. Since comparable phenomena are well known for synchronous algal cultures, we conclude that a long range of certain light/dark ratios induces synchronisation to a certain extent. Experiments lasting one day were independent of the daylength which can be explained as an absence of synchrony.

5.3. Discussion

First of all we will draw attention to the fact that the energy conversions in our experiments are based on incident light. This is a reason for the decrease in energy conversion in thin suspensions with shorter daylengths (cf. fig. 5.2.2 and 5.2.3).

In thin suspensions at light saturation in experiments lasting 24 hours, the light absorption decreases in as much as the daylength is shorter.

In thick suspensions, with partial or total light limitation it must be born in mind that the influence of respiration on yield can be expected to be considerable. In this respect the cause of a decreased energy conversion at L/D = 8/16, as compared with L/D = 24/0 (cf. Table 5.2.1, 5.2.4) was partially attributed to respiration. It has to be stated that these explanations are based on measurements of dry weight increase. Alterations in chemical composition, owing to prolonged light-dark treatments and a possible influence on yield were not taken into consideration.

According to several investigators, photosynthetic activity is not constant in synchronous cultures of algae; cf. Nihei et al (1964); Sorokin (1964); Metzner and Lorentzen (1960); Senger and Bishop (1967). Sorokin (1960c, 1961) found that desynchronisation unfavourably influenced the photosynthetic rate. Photosynthetic activity thus appeared coupled with cell development. Since light duration may influence development, an influence of light duration on photosynthetic activity can be expected (cf. Sorokin, 1960a; 1960b).

The results presented in Tables 5.2.3 and 5.2.4 were obtained with cells, pretreated at L/D = 24/0 which results in a normal distribution of all cell sizes, i.e. a desynchronisation. Growth rates under other light/dark ratios were measured during one day immediately after the growth period, or with these cells cooled

at 5 °C. Preliminary experiments indicated that the physiological properties of cooled cells remained stable during a period of at least 10 days and we may safely assume that cells pretreated in this way were completely desynchronised.

This situation profoundly altered by shifts from one light treatment to another (cf. Table 5.2.2). These data suggest that the pretreatment can influence the growth rate at different light-dark ratios. Cells in these experiments were pretreated during 3 days, and experiments started with dark cells in the L/D = 16/8, 12/12 and 8/16 series. Adaptation to a certain light regime during 3 days normally is sufficient to synchronise the algae. Therefore, we have good reason to assume that these cultures were partially synchronised. A shift in light regime might then cause desynchronisation. In this way photosynthetic rates may be lower, resulting in lower production rates. We concluded that a strong discrepancy between pretreatment and treatment unfavourably affects the production rate.

We found that *Scenedesmus* could be synchronised at L/D = 12/12 and 14/10 at 30 °C. LORENTZEN and RUPPEL (1959); SENGER (1961) used L/D ratios of 16/12; and BONGERS (1958), with *Scenedesmus*, L/D ratios of 14/10 to synchronise them. Since these light/dark ratios were favourable to establish synchronisation, we expect that they are also close to the optimum for production.

In all the experiments described so far, the optical density and thus the light absorption of the total cell mass changed. A variation in physiological properties of the cells, for instance a higher or lower saturation rate of photosynthesis (cf. SOROKIN, 1964) which is, of course, mainly restricted to the exponential start of the growth curve, may have been obscured for this reason. In the experiment described in fig. 5.2.4, optical density combined with the existing light intensity ensured light saturation during the whole experiment. It was shown that production rates and total production were not the same under the light/dark treatments used, but showed an optimum for L/D = 12/12.

It strengthens the idea that a light/dark ratio which is in harmony with the cycle of cell development, enables the cell to optimal production under the given set of conditions.

6. THE INFLUENCE OF TEMPERATURE ON YIELD

6.1. INTRODUCTION

KOK and VAN OORSCHOT (1954) found that strains of *Chlorella* and *Scenedesmus* could be adapted to temperature. In this way 'high' and 'low' temperature adapted forms could be cultivated. 'Low temperature forms' brought to a high temperature gave significantly lower absolute growth rates than 'high temperature cells'. 'High temperature cells' brought to a lower temperature gave lower absolute growth rates than low temperature adapted cells.

In most of the experiments described in the foregoing chapters, the temperature during growth was 30 °C. In order to prevent adaptation phenomena cells were precultivated at the same temperature (28–30 °C).

Temperature is a diffuse environmental factor influencing many processes more or less connected with growth, e.g., the saturation rate of photosynthesis, the rates of respiration and cell division. Synchronous cultures enable to separate in time increase in organic matter and cell division.

MORIMURA (1959) investigated the effect of temperature on synchronous cultures of *Chlorella pyrenoidosa*. The life cycle was shortened when the temperature was increased. This was partly due to an increased rate of photosynthesis, partly to a more rapid rate of cell division.

SOROKIN (1960d), with low and high temperature forms of *Chlorella pyrenoidosa*, investigated the effect of temperature and light on cell division. With a high energy input the minimum temperature required for cell division was higher than with a low energy input. It was concluded that cell division was inhibited by high light intensities.

SOROKIN and KRAUSS (1962), with the high temperature strain of *Chlorella pyrenoidosa*, confirmed that an increase in temperature shortened the 'generation time'. High temperatures during the dark period as compared with the light period accelerated the division to an earlier completion. A lower temperature during the dark period retarded cell division.

Extreme temperatures (40 °C and higher) tend to stop cell division. SEMENENKO *et al.* (1967), with *Chlorella* sp. K cultivated at 43 ° and 38 °C and extreme high light intensities, found an increase in dry weight per unit number of cells and a reduction of cell division in the cells cultivated at 43 °C as compared with those at 38 °C. Respiration at 43 °C was doubled, and photosynthesis at 43 °C reduced to one third as compared with 38 °C.

Optimum temperatures for growth and photosynthesis are different. TRUKHIN and MIKRYAOVA (1969), with *Chlorella*, measured changes in respiratory activity in cells precultivated at different temperatures. Photosynthesis was light limited. Rates of respiration at 39 °C for cells precultivated at low temperatures were 2–3 times higher than those from cells precultivated during a couple of days at 39 °C.

DAVIS and DEDRICK (in BURLEW, 1953), with *Chlorella*, measured growth rates in full sunlight. Cultures grown at constant temperature were compared with cultures receiving the same day temperature but different night temperatures. A combination of 25 °C day temperature and 20 °C or 15 °C night temperature showed an increase in growth rate as compared with a series receiving 25 °C continuously. Night temperatures higher than 25 °C or lower than 15 °C in combination with 25 ° C day temperature reduced the growth rate as compared with a series receiving 25 °C continuously. The phenomenon was typical for high light intensities since it was not found in cultures grown in the shade or with diffuse light.

The work of SOROKIN and MORIMURA mentioned above suggests that cell division is strongly temperature dependent. A lower night temperature retards cell division in synchronous cultures. SOROKIN et al, (1961), with *Chlorella pyrenoidosa*, compared rates of growth and photosynthesis in synchronous and non-synchronous cultures. The saturation level for photosynthesis was higher in synchronous than in non-synchronous cultures under comparable experimental conditions. The number of daughter cells was about two times higher in synchronous cultures than in non-synchronous ones.

METZNER and LORENTZEN (1960), SENGER and BISHOP (1967), SENGER (1970), SOROKIN (1960), found that the photosynthetic capacity in different species of *Chlorella* was high in cells early in their life cycle, and low in cells at the end of the light period.

Considering the evidence from literature there was reason to believe that:

a) Cell division was a temperature dependent process.
b) Photosynthesis was high in synchronous cultures briefly exposed to light, and therefore containing small cells.

We might deduce from b that photosynthetic capacity in non-synchronous cultures depends on the ratio: cells just divided / total number of cells being high when this ratio is high and low when it is low.

According to a), a decrease in night temperature unfavourably affects cell division. This might influence the rate of photosynthesis during the following light period.

Since DAVIS' results were in conflict with the experimental evidence from SOROKIN (1957, 1965), and MORIMURA (1959), we decided to reinvestigate the effect of temperature on growth. Photosynthesis, dry matter production and respiration were measured in cultures receiving various constant temperatures. Growth rates for cultures receiving constant day temperatures and non-regulated night temperatures were compared with growth rates in cultures receiving a constant day- and night temperature.

6.2. The effect of constant day temperatures and non-regulated night temperatures on growth

The rate of photosynthesis of *Scenedesmus* sp., strain K22, was measured in a Warburg apparatus. Cells precultivated at 30 °C in continuous light were brought at pH 4.8 in the culture medium in order to dissolve precipitates formed during growth. After washing with distilled water, cells were resuspended in Warburg buffer no. 9, 0.1 M. Measurements were made over periods of ¾ hour at various temperatures. Duplicate runs were made successively. In fig. 6.2.1 the relation between the maximum rate of photosynthesis and the temperature under the conditions of our experiment is given. A maximum is reached at 35 °C. Rates of photosynthesis at 40 °C were difficult to measure, however. They showed a decline in time, which was not the case at the other temperatures. This introduces un uncertainty in the average values at 40 °C.

Respiration of *Scenedesmus* sp., strain K22, precultivated in continuous light at 30 °C, was measured at 15, 20, 25, 30, 35, and 40 °C (cf. fig. 6.2.2). The measuring period was 1 hour. Each point in fig. 6.2.2 is an average of 5 parallel measurements in rather thick suspensions. The highest rates of respiration were measured at 35 °C. At 40 °C, the respiratory activity declined during the measurements. This was not the case at the other temperatures.

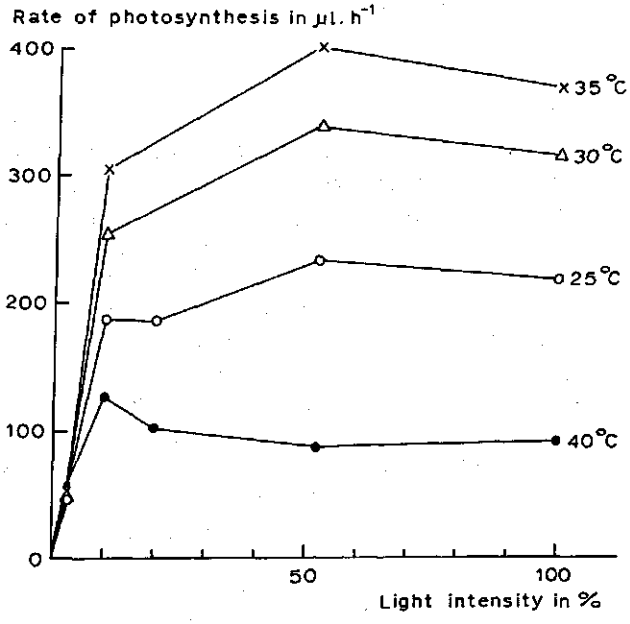

Fig. 6.2.1. Rates of photosynthesis of *Scenedesmus* sp., strain K22, in a Warburg vessel. Light intensity was reduced with wire screens. Figures denote the temperature applied. Warburg buffer no. 9, 0.1 M; volume (V_f) = 15 ml, Trommsdorff value: 0.67 µl/ml.

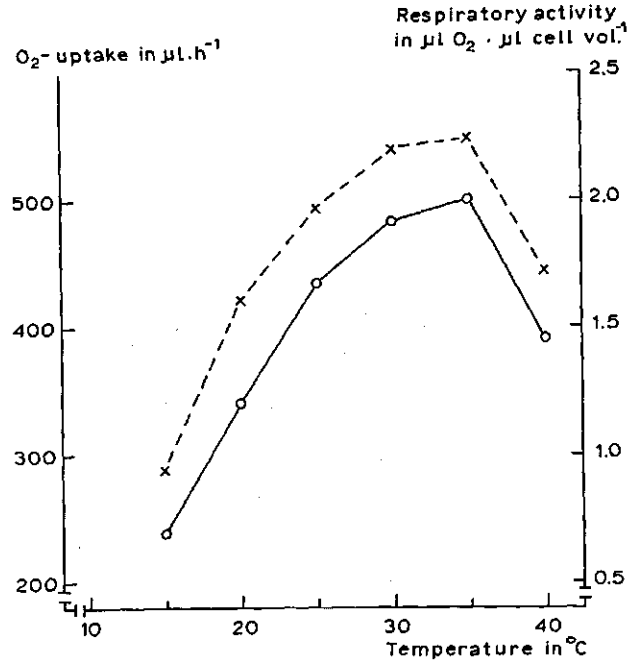

FIG. 6.2.2. Rates of respiration and respiratory activity of *Scenedesmus* sp., strain K22 cells, as a function of temperature. $V_f = 15$ ml, TROMMSDORFF value 14.75 µl/ml. Rate of respiration ×---×; respiratory activity O——O.

The decrease of both photosynthesis and respiration at 40 °C as a function of time is therefore probably due to a destruction or inactivation of the enzyme systems of photosynthesis and respiration (KUYPER, 1910).

Growth implies several processes fundamentally different from photosynthesis. Therefore, it cannot be accepted *a priori* that the optimum temperature for growth is the same for several light/dark ratios. To this purpose we measured the growth rate of *Scenedesmus* with the light/dark ratios 24/0 and 8/16 at several temperatures. Cells precultivated in continuous light were stored at 5 °C and used for inoculating culture tubes to a cell density of 0.2 µl packed cell volume per ml suspension, in NSP 10.2.15. The results are collected in fig. 6.2.3. Highest growth rates were measured at 35 °C. In Chapter 5, we discussed the influence of a shift from one light treatment to another on growth (cf. Table 5.2.2). In essence the same occurred in this experiment. Before the measuring period the cells received a pretreatment in continuous light. The L/D = 8/16 cells were transferred from a round the clock light regime to a shorter light period. This may explain why the absolute growth rate in the L/D = 8/16 series is lower than the expected value, calculated from the continuous light series. We calculated the relative values for the growth constant k_g (k_g at 35 °C in 24/0 and 8/16 kept at 100). There is no large difference in behaviour be-

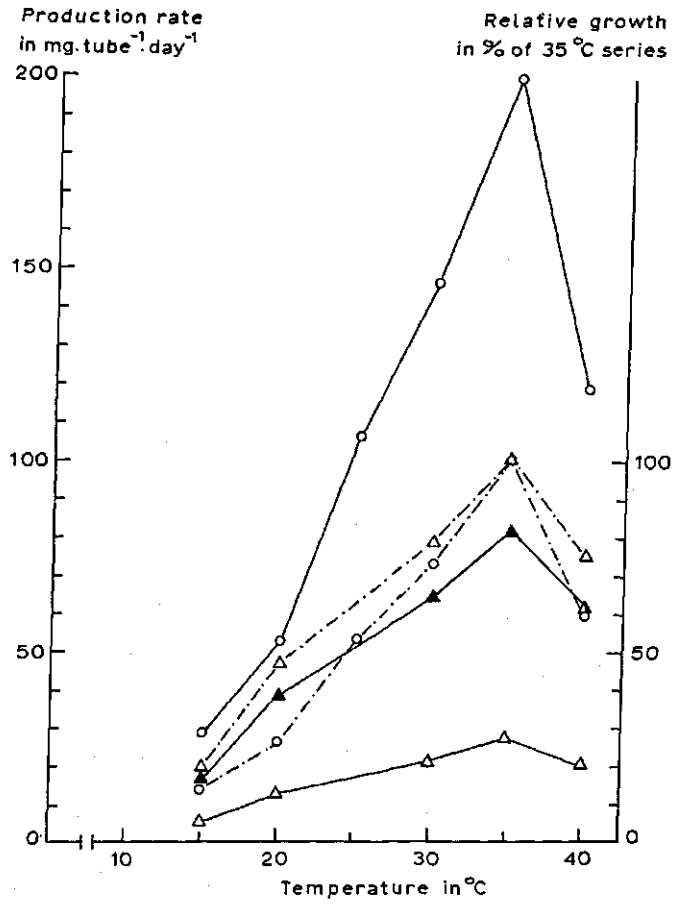

FIG. 6.2.3. Growth of *Scenedesmus* sp., strain K22, as a function of temperature. ○——○ daylength 24/0; △——△ daylength 8/16; ▲——▲ production 8/16 series calculated for 24 hours light; ○-·-○ relative growth rate L/D = 24/0; △-·-△ relative growth rate L/D = 8/16. Cells were precultivated in continuous light and stored at 5 °C; initial cell density 0.20 µl/ml; NSP 10.2.15; cultivation in 'continuous' culture tubes.

tween the short day treated cells and the continuously illuminated ones. The optimum is not altered and remains at 35 °C. Essentially, as for photosynthesis, the growth rate decreased at 40 °C. Since the rates of photosynthesis and respiration decline in time, and growth will be highly dependent on these processes we assume that the decrease in growth rate at 40 °C is caused by a gradual destruction or inactivation of the enzymatic apparatus. A constant temperature of 35 °C thus seems optimal also for dry matter production irrespective of daylength.

In an attempt to test MILLNER's results (Davis, 1953), *Scenedesmus* was cultivat-

TABLE 6.2.1. Growth of *Scenedesmus* sp., strain K, in washing machines. Growth at a constant temperatures as compared with constant day temperature and variable night temperatures. Day temperature 30 °C. Culture solutions as indicated.

Medium	Incident radiation (kcal · m^{-2})	Duration	Efficiency 30 °C continuously (%)	Efficiency variable night temperature (%)
NSP 10.2.1	1478	10/7 –13/7	8.6; –	9.9; 8.6
NSP 10.2.1	6112	13/7 –16/7	3.4; 3.2	5.2; 5.5
NSP 10.2.1	7482	16/7 –18/7	1.9; 1.7	2.4; 3.0
NSP 10.2.1	6635	18/7 –22/7	2.3; 1.9	3.0; 3.1
NSP 10.2.1	1632	22/7 –24/7	2.3; 3.9	4.6; 5.2
NSP 10.2.1	3423	12/8 –15/8	4.65; –	5.6; 5.6
NSP 10.2.1	8515	15/8 –19/8	2.5; –	3.8; 4.2
NSP 10.2.1	1870	17/9 –20/9	3.8; –	4.7; –
ANSP 5.2.1	4215	7/8 –10/8	4.3; 5.2	4.7; 5.1
NSP 10.2.15	2401	31/5 – 2/6	4.7; –	4.6; –
NSP 10.2.15	6216	4/7 – 6/7	3.9; –	2.4; –
NSP 10.2.15	3302	8/7 –11/7	5.3; –	4.9; –
NSP 10.2.15	5500	11/10–17/10	2.7; –	2.5; –
NSP 10.2.15	3860	20/10–24/10	4.7; –	4.7; –
NSP 10.2.15	2500	29/10– 3/11	2.0; –	1.9; –

ed in washing machines in natural daylight. Two machines were kept at a constant temperature of 30 °C; the other two were kept at 30 °C during the day while at night the heating device was disconnected. Experiments were carried out in July and August. The culture solution was NSP 10.2.1. One of two times per day pH was adjusted to 5.8. On hot days, the cover was taken from the vessels to prevent overheating. The experimental results are collected in Table 6.2.1. Efficiency values were lower in the constant temperature series as compared with those obtained with variable night temperatures when NSP 10.2.1 was used. The efficiency values, obtained with the two temperature treatments mentioned above, were about the same in the culture medium ANSP 5.2.1. With NSP 10.2.15 a constant temperature was slightly better than variation in night temperature.

No influence of temperature on growth would be expected under conditions of light limitation for the culture as a whole. Nevertheless, three series of measurements at a constant temperature of 20 ° and 30 °C respectively, showed an increase in energy conversion in the 30 ° series (cf. Table 6.2.2). Its origin has to be sought in the high light intensity at the surface of the algal suspension in daylight. Although the light intensity is assumed to decrease exponentially within the solution, owing to mixing, part of the cells is always light saturated and has for this reason a rate of photosynthesis which is temperature dependent. Illustrated with a BLACKMAN curve for photosynthesis this would mean that a deviation from the 'normal' shape occurs, viz.: a long linear range for light

TABLE 6.2.2. Growth of *Scenedesmus* sp., strain K, in washing machines at a constant temperature of 20°C. and 30°C. Medium NSP 10.2.15.

Duration	Incident radiation (kcal · m^{-2})	Efficiency 20°C (%)	Efficiency 30°C (%)
28/4–30/4	6756	3.5	4.1
2/5– 4/5	4541	2.8	5.3
5/5– 7/5	2712	4.1	5.0

limitation and a sharp bending with light saturation would be altered in a longer transition zone from light limitation towards light saturation. In the transition zone influence of temperature and CO_2 concentration can still be expected. The variable night temperature is favourable in the case of NSP 10.2.1, suggesting a higher rate of photosynthesis as compared with the constant temperature series. Since this phenomenon did not occur in NSP 10.2.15 (cf. Table 6.2.1) and in ANSP 5.2.1, the reason has to be sought in the differences in composition of the culture solutions. NH_4^+- or NO_3^- containing culture solutions differ in that they give rise to a decrease and increase of pH respectively. In using NSP 10.2.15, the culture solution does not show strong pH shifts and remains more acidic than NSP 10.2.1. In the case of NSP 10.2.1, pH increased during the day. Then the equilibrium: $H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{--}$ will shift to the right. During the night, CO_2 in solution converts HPO_4^{--} in $H_2PO_4^-$ again, whereas HCO_3^- is formed, so that CO_2 is bound. At lower night temperatures the culture solution has a higher capacity to dissolve CO_2 as compared with a culture at higher constant temperature. This CO_2 can interact with HPO_4^{--} thus increasing the $H_2PO_4^-$-concentration and the amount of potentially available CO_2. Solutions containing NH_4^+ or buffered media as NSP 10.2.15 become acidic or less alkaline. In these cases the beneficial pH effect of the extra available CO_2 at low temperature is lower since less HCO_3^- is formed at lower pH; or the phosphate concentration is so high that hardly any limitation by phosphate can be expected.

For comparison, pH values were determined in morning samples from cultures receiving constant temperature or variable night temperature (cf. Table 6.2.3). Although the efficiency values were higher in the series with a variable night temperature (cf. Table 6.2.1), which would have given rise to a higher pH than in the constant temperature series, pH was slightly lower at the variable night temperature. This may be due to a higher amount of available CO_2 as compared with the constant temperature series.

Measurements of photosynthesis of *Scenedesmus* in a diaferometer, which were made together with Mr. G. A. PIETERS in this laboratory, indicated that CO_2 dependency started below partial pressure of 2% CO_2 in an air stream, equilibrated with the water phase at 30°C (cf. fig. 6.2.4 and PIETERS, 1972).

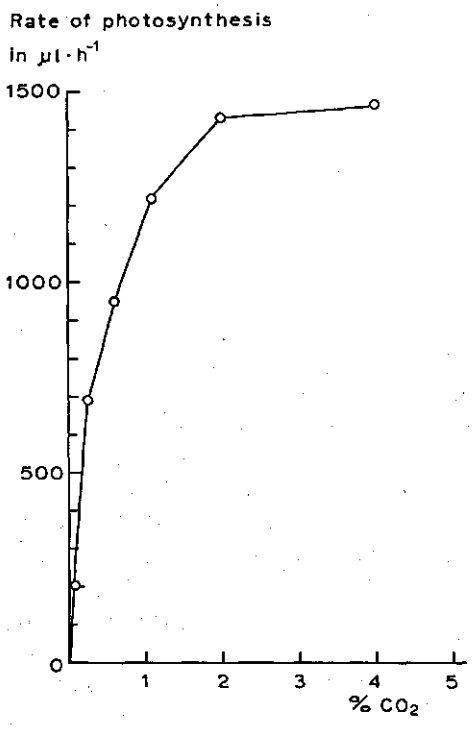

FIG. 6.2.4. Maximum rates of photosynthesis of *Scenedesmus* as a function of pCO_2 as measured in a diaferometer. Algal cells, suspended in distilled water, were sprayed over 100 cm^2 filter paper so that the average cell density was 4.14 $\mu l \cdot cm^{-2}$. Light intensity: 4.74×10^4 ergs $\cdot cm^{-2} \cdot sec^{-1}$, temperature 25.2°C.

TABLE 6.2.3. Measurements of pH in cultures receiving a constant temperature of 30°C or a variable night temperature. *Scenedesmus* sp. K, was cultivated in washing machines in NSP 10.2.1. Measurements during experiments described in Table 6.2.1.

Sampling time	pH constant temperature	pH variable night temperature
13/8 – 9.00 hours	6.9	6.55; 6.45
14/8 – 9.00 hours	7.0	6.65; 6.85
15/8 – 8.50 hours	6.75	6.65; 6.85
16/8 – 8.45 hours	6.75	6.55; 6.55
17/8 – 8.30 hours	6.85	6.65; 6.60
18/8 – 9.00 hours	6.15	6.1; 6.15
19/8 – 8.45 hours	6.15	6.0; 6.0
21/8 – 11.15 hours	6.4	5.9

6.3. Discussion

The influence of temperature on dry matter production, photosynthesis and respiration was investigated. The optimum temperature for growth, photosynthesis and respiration was 35 °C. No influence of daylength on the position of the optimum temperature could be found in our experiments. At 40 °C respiration and photosynthesis declined in time, which was not the case at the lower temperatures. The lower rates of photosynthesis and respiration were ascribed to a deterioration or partial destruction of the enzymatic apparatus.

Millner's experiments, suggesting a favourable effect of low night temperatures in combination with higher day temperatures and strong light could be partially confirmed in our experiments (cf. Table 6.2.1 – NSP 10.2.1 series). In NSP 10.2.15, however, a constant temperature was more favourable for growth than a variable night temperature, whereas in ANSP 5.2.1 the growth rates under the two conditions were about the same. We concluded from our experiments in Table 6.2.2, describing an increase of the energy conversion by an increase of the culture temperature, that the light limited conditions existing for the culture as a whole did not exclude a temperature effect for that part of the cells which was under light saturated conditions. In a Blackman curve this would mean an extended transition zone between light limited and light saturated conditions. In the transition zone temperature and CO_2-effects are possible.

Measurements of pH as given in Table 6.2.3 showed that higher pH values, which would be expected in the faster growing NSP 10.2.1 cultures with variable night temperature, were not found. Since the solubility of CO_2 is temperature dependent we concluded that the total available amount of CO_2 was higher in the cultures with a variable night temperature than in the constant temperature cultures. Although the culture was continuously aerated with an air mixture containing 4 % CO_2, CO_2 may be a rate limiting factor since the cover was taken from the vessels on hot days to prevent excessive heating when the culture was illuminated, or HPO_4^{--} may be limiting owing to precipitation at high pH. Indirect evidence, obtained from an experiment about the effect of ammonium-or nitrate salts on growth rate indicated that removal of the cover resulted in an increase of pH in all the experimental series. Since uptake of ammonium salts will decrease pH (cf. Chapter 3) this is an abnormal effect, probably caused by CO_2 escaping from the culture solution. Because we did not pay attention to this phenomenon during the experiment with NSP 10.2.1, we could not exactly retrace when the covers were taken from the vessels. In the series with ANSP 5.2.1 and NSP 10.2.15 the cooling was established with tap water flowing through copper tubing (isolated from the culture suspension with plastic tape and 'plastoderm' coating). In these series a CO_2 depletion or phosphate deficiency could hardly be expected, therefore.

In this context an interesting field for further research would be to investigate the effect of partial pressures of CO_2 on the rate of photosynthesis and especially on the rate of growth. Østerlind (1951a, 1951b) found that *Scenedes-*

mus could use HCO_3^- and CO_3^{--} as a carbon source. Differences in diffusion of CO_2 and HCO_3^- from water to cell and possible interference of membrane permeability still have to be explored.

Reasons why a constant temperature is more favourable for growth of algal mass cultures than a variable night temperature, as was the case in the NSP 10.2.15 series, may be summarized as follows:

1) Rates of photosynthesis and respiration increased up to 35 °C.
2) The 'generation time' decreased with increasing temperature.
3) Cell division is strongly temperature dependent.

The question of temperature adaptability, which was investigated by KOK and VAN OORSCHOT (1954) and VAN OORSCHOT (1955) still remains. They found that it was possible to adapt certain species cultivated at low temperatures to high temperature conditions. Cells transferred from a lower pretreatment temperature to a higher culture temperature showed a relatively lower growth rate than algae precultivated at the same temperature as in the experiment. Under all circumstances, however, absolute growth rates at 30 °C were higher than at 20 °C. Adaptation of respiration to temperature was found in *Chlorella* by TRUKHIN and MIKRYAOVA (1969). This was a rather fast process lasting one day, whereas KOK and VAN OORSCHOT (1954) measured adaptation times of one to three weeks to convert low temperature forms into high temperature forms. We would suggest two alternatives for further research:

a) A population of 'low' temperature forms may contain a few 'high' temperature cells; this would imply certain (slight) genetical differences. The predominance then merely is a question of the existing temperature conditions. Then the generation time simply is the number of divisions to reach dominance of the adapted types to the existing temperature. This may be best investigated in synchronous cultures. We would expect that the time needed to adapt high temperature cells into the low temperature modification is longer than the reversed operation. The reason for this would be that the generation time increases with a decreasing temperature (cf. MORIMURA, 1959, SOROKIN and KRAUSS, 1962).

b) The temperature adaptations are variations on an enzymatic pattern only. The adaptation time could then be interpreted as the time required to induce the synthesis of an enzyme system, necessary to protect the cells against the effect of high or low temperatures.

7. GENERAL DISCUSSION

Algal photosynthesis as measured in the laboratory is influenced by light intensity, CO_2 content and mineral supply.

The influence of the salt concentration on the growth rate of *Scenedesmus* was described in Chapter 3. Growth rates were nearly independent of the salt concentration in a broad range of concentrations of SO_4^{--} and $H_2PO_4^-$ (cf. figs. 3.3.1 and 3.4.1). *Scenedesmus* was more strictly in its demands concerning KNO_3. Initial concentrations of 5 mM were optimal for growth on the rocking table.

The application of NH_4^+ salts is favourable for growth, provided the solution is well buffered. This is in accordance with experiments of KOK (1952) and VAN OORSCHOT (1955) with *Chlorella*. Using NH_4^+, photosynthetic efficiency could be increased by about 30%, as compared with media containing nitrogen as KNO_3 (cf. Table 3.5.2). *Scenedesmus* sp., strain K, could be cultivated in washing machines in media containing urea. A comparison of growth in washing machines in 1963/64 and '66 using NSP 10.2.1 and USP 5.2.1 (cf. Table 3.5.3) showed no big differences in energy conversion between these media. Less favourable experimental conditions and shorter daylengths probably caused low efficiency values in these experiments. In the '63–'64 experiments the efficiency values as such were low, indicating other growth limitations. In general, a comparison of the growth rates in NSP 10.2.15 and USP 5.2.1 showed preference for USP 5.2.1. The average efficiencies were 4.5% in NSP 10.2.15, and 5.6% in USP 5.2.1, which is a relative increase in energy conversion of 25% in media containing urea. Growth of *Chlorella*-A cultivated in washing machines in artificial light, was 38% higher in USP 5.2.1 than in NSP 10.2.15. Since the growth stimulation by urea was more pronounced in longer lasting experiments with high cell densities, it is concluded that urea acts as a growth stimulator under light limited conditions by bypassing the rate limiting NO_3^- reduction. The growth of *Scenedesmus* sp., strain K23 on the rocking table was highest in the concentration range of 5 to 15 mM urea (cf. fig. 3.5.1)

With respect to KCl it is of interest that rather high concentrations are tolerable (cf. fig. 3.2.3) which may partly be a reason that *Scenedesmus* occurs in brackish waters.

It was already stated that *Scenedesmus* is rather non-selective in its salt demands. Several authors give formulae for the best composition of the culture solution which mutually differ rather profoundly (cf. KRAUSS and THOMAS, 1954, KRAUSS 1958; PRATT, 1941; DAVIS *et al.*, 1953; ØSTERLIND, 1949). This may be connected with the fact that the light conditions in the vessel, i.e. irradiated surface and volume of the suspension, influence the potential growth rate and, therefore, also the requirement of nutrients. The actual growth rates are functions of the average light energy received per cell as applied by the various authors mentioned above. The dissimilarities in technical equipment do

not guarantee comparable light fields, and thus similar light energies per cell. It is therefore difficult to suggest an optimum composition of the growth medium without specification of the type of culture vessel. Laboratory experiments perhaps offer the opportunity to calculate the average light energy received per cell. This appeared extremely difficult in natural light fields. In order to obtain an idea about the average light energy received per cell, the actual growth rates per day were estimated. Together with the knowledge of the average cell composition, this appears sufficient to calculate the nesessary amounts of salts to sustain uninhibited growth. In essence, this method was already applied by KUZNETSOV (1967), who used media containing urea. The use of sterile conditions is highly advisable with such media. An application in closed systems (cf. TAMIYA, 1957), therefore, seems to be best.

The occurrence of an NO_3^--optimum for growth in cultures on the rocking table and the absence in cultures in a thermostated bath or in 'continuous culture' demonstrate that the optical properties of the culture unit may alter the behaviour towards a major nutrient.

BONGERS (1956) found that NO_3^--uptake was light dependent within a range of light intensities. Comparison of the growth rates in cultures in flasks on the rocking table, in 'continuous culture' tubes, and in flasks in a thermostated bath show that the growth rate on the rocking table is about two times lower than the one in 'continuous culture' tubes although light intensity is about two times higher than in these vessels (cf. fig. 3.2.2 and Table 3.2.1). The depth of layer in these culture tubes is about four times smaller than that in flasks on the rocking table, so that the average energy received per cell is higher in 'continuous culture' tubes than in flasks on the rocking table. In the thermostated bath the depth of layer in the flasks was comparable with that on the rocking table but the optically thin suspensions which existed during the shortly lasting experiment (cf. fig. 3.2.4) also resulted in higher average light intensities in the flasks in the thermostated bath than in flasks on the rocking table.

Therefore, the different behaviour of cells towards NO_3^- in the various culture vessels are probably due to differences in the average energy received by an individual cell. This can induce differences in the rate of NO_3^--uptake.

The effects of light intensity and light duration were mainly studied in Chapters 4 and 5.

VAN OORSCHOT (1955) calculated the total photosynthetic yield by integrating the rates of photosynthesis over all depths. Formulae as he derived can only be applied with vessels of an easy geometrical shape, e.g. rectangular boxes, receiving light from above as in VAN OORSCHOT's case. When more than one surface is illuminated, it becomes very complicated to introduce the effect of stirring in the formulae for yield determination.

We attempted to describe the light field by assuming that BEER's law was valid. The complicated surface of the washing machines was converted into the model of a rectangular box with the same depth of layer, receiving light via the horizontal surface only. Therefore, to fit the model, the horizontal surface of a

washing machine had to be extended as a correction for the amount of light received on the vertical plane of the machine. The effective surface in the model (= 'apparent horizontal surface') was determined by comparing the light limited growth rates in washing machines illuminated on the horizontal surfaces only, and on the entire surface respectively. Since the growth rates depend on the amount of energy actually received under these conditions, the ratio between the growth rates also represents the ratio between the effective surfaces. This method had the drawback that the apparent horizontal surface had to be estimated separately for different parts of the year, because the maximum elevation of the sun affects the results (cf. Table 4.2.2). Moreover, we had to consider the stirring velocity in its effects on the motion of the cells. The movement of the cells was more or less circular in a vertical plane as could be seen with a plastic diver.

The aim of the stirring mechanism was to improve the yield per unit surface. Going from surface to bottom of the culture vessel, part of the cells will receive an energy input above the saturation point of photosynthesis. The rates of photosynthesis of the rest of the cells depend on the light intensity. Stirring introduces a sort of 'flash' effect, which, however, is different from the flash effect obtained with rotating discs, in that the transition from light to darkness and vice versa is of a completely different nature. During transition from light saturation to light dependent conditions part of the enzymatic apparatus regulating the CO_2 fixation is 'free' to convert a high light received just before within a short flash. With some restrictions knowledge derived from flash experiments can be applied. In section 4.2.1, the intermittency factor for equal energy and equal total time was already discussed (cf. RABINOWITCH, 1956):

$$I_{IE} = \frac{\textit{Yield in intermittent light}}{\textit{Yield in continuous light with equal total energy and equal total duration}}$$

To obtain enhancement in energy conversion, the movement of the cells had to be in the order of seconds or less, to obtain I_{IE} values higher than 1.

KOK (1953) found that flash durations in the order of msec were necessary to obtain a maximum utilisation of the absorbed energy.

Incident energies of direct sunlight amount to 0.6 cal.cm^{-2} · min^{-1} at most (cf. DE VRIES, 1955), which is about ten times the saturation value for photosynthesis. This means in our model that the duration of the flash has to be 1/10 of the total time used for one rotation in the vessels provided its absolute value is sufficiently low. This was approached for cell concentrations of 4.0 µl packed cell volume per ml suspension (cf. Table 4.2.1.1).

Considering that the duration of the flash in this case could be estimated at about 1 sec, maximum utilisation of the energy received during the flash could not be expected. This would mean that: a) The stirring velocity had to be increased, which is impossible in the existing apparatus; b) The depth of layer had to be decreased to about 4 cm, and the cell concentration had to be increased at the same time if the stirring velocity was not altered.

When designing a new apparatus for mass cultivation, theoretically it would

be the easiest way to use thin layers of the order of a few cm, and high cell densities. In this case a simple stirring device would be sufficient. With accelerated stirring, the danger of mechanical injuries increases.

The daily production rates, described in section 4.3 demonstrated that the production in washing machines was light limited at initial densities of 0.5 µl/ml and higher. The situation then is more or less comparable with a standing crop with closed leaf canopy.

Comparing the average productivity of an algal culture and a conventional crop, it appears that they are of the same order of magnitude (200–240 kg·ha^{-1}·day^{-1} representing about 10–12 g C·m^{-2}·day^{-1}), cf. ALBERDA (1962), GAASTRA (1962), KOK (1952, 1953), VAN OORSCHOT (1955) SIBMA (1968).

In this respect, the discussion of STEEMANN-NIELSEN (1960, 1961) about the productivity in communities of terrestrial and water plants is of interest. He concluded that the productivity in the water always had to be inferior to that of terrestrial plants. He based this on the higher photosynthetic capacity of leaves in comparison with algae. If we consider a collection of primary productivities in natural waters, STEEMANN-NIELSEN's statement is certainly correct (cf. Table 7.1). The production rates are expressed in g C·m^{-2}·day^{-1}. According to KRAUSS (1953) an average green alga contains 49.5–70.2% C. The average C-content of *Scenedesmus*, as measured in 4 samples was 47.7% of total water and ash-free dry matter content (elementary analysis of our own material).

By multiplying the production rates, expressed as g C·m^{-2}·day^{-1}, with 2 they represent the production rate of dry matter in g·m^{-2}·day^{-1}. It appears that the production rates are very low, except in the Tsymlyanskii Reservoir and in the Southampton measurements of ANSELL *et al* (1963a). The low values, however, are averages calculated over periods of half a year or a year. The possibility cannot be excluded that the production rate is higher if we consider for instance June or July in particular (cf. for instance the measurements in Southampton). The two exceptions with higher production rates, mentioned above, were in eutrophic waters.

The conclusion would be that the mineral supply in natural waters may be a rate limiting factor. Moreover, especially in deep waters, temperature stratification may occur which prevent the mixing of the fertile bottom water with the layers on the surface (cf. TALLING, 1957, 1963).

Some other work about energy conversion in higher plant communities may be mentioned here.

WASSINK (1948) calculated the efficiency values in agricultural crops over the entire growing season. A maximum of 2% of the incident energy was reached.

WASSINK, KOK and VAN OORSCHOT (1953) with *Chlorella*-A, found that the energy conversions of this strain under natural conditions were in the same order of magnitude as the energy conversion measured in a 1 m^2 plot of grass on three successive harvests. Cultures under semi-controlled conditions give growth rates comparable with the highest production rates in agricultural crops.

The measurements in *Scenedesmus*-cultures over the period 1962–1965

TABLE 7.1. *Primary productions in some natural waters collected from literature.*

Place invest. Method.	Production $gC \cdot m^{-2} \cdot day^{-1}$	Author.	Reference
ENGLISH CHANNEL Decrease nitrate Decrease phosphate Decrease CO_2	0.21–0.55	STEEMANN-NIELSEN	Ann. Rev. Plant Physiol. **11** 341–363 (1960)
KATTEGAT (^{14}C)	0.17	STEEMANN-NIELSEN	Ibid.
LIMFJORD	0.29		
GREAT BELT	0.16		
DYBSÖFORD	0.02		
SOUTH ATLANTIC OCEAN (^{14}C)	Min.: 0.08 Max.: 0.27	STEEMANN-NIELSEN	Ibid.
LAKE BAIKAL (^{14}C)	Min.: 0.02–0.04 Max.: 0.20–0.26	KUZNETSOV ROMANENKO GLAZUNOV.	Doklady Akad. Nauk SSSR **156** (6): 399–402 (1964)
RYBINSKOE RESERV.	0.33	POTOTSKAYA TSYBA	Doklady Akad. Nauk SSSR **155** (3): 234–236 (1963)
GORKII RESERV.	0.48		
KUIBYSHEV RESERV.	0.93		
TSYMLYANSKII RES.	2.50		
(Mod. O_2-method)	(0.81–1.17%)		
LAKE BALATON (Diatom Bloom) Glucose determin.	0.62–1.01	FELFÖLDY	Ann. Biol. Tihany **28** 99–104 (1961)
SOUTHAMPTON Fertilized seawater *Phaeodactylum* O_2-method Decrease phosphate Increase packed cell volume	March: 2.2 May–Aug.: 4.9 Oct –Nov.: 2.0 (3.0–4.4%)	ANSELL, RAYMONT, LANDER, CROWLEY, SHACKLEY	Limnol. Ocean. **8** (2): 184–206 (1963)

suggest that the energy conversion can be as high as in crops with a closed leaf canopy, and that even over longer periods. If we restrict ourselves to algae forming autospores, as *Chlorella* and *Scenedesmus*, we may even say that these species are superior to higher plants in that they are not bound to an annual production cycle and that with suitable techniques high productivity may be obtained over prolonged periods. We, therefore, do not agree with STEEMANN-NIELSEN that the productivity in the water necessarily is inferior to that of terrestrial plants.

The experiments about the effect of energy input on the energy conversion at various initial concentrations suggest that the efficiency in non-diluted cultures declines when the initial concentration is increased.

As a comparison, the experiments of KAMEL (1959), about the influence of plant density on net photosynthetic efficiency in barley and sugar beet are of interest. With close spacing high efficiency values were reached early in the

season. Later on, the efficiency values declined and the highest values were found in the normal spacing.

NICHIPOROVITCH and MALOFEEV (1965), with kale, investigated the effect of light intensity on the efficiency of photosynthesis at various plant densities, for every leaf area ratio which was chosen, the energy conversion decreased with higher light intensities. An increase of plant density in the range 0.25–10 m^2 ground surface resulted in higher energy conversion values using high light intensity.

Plant densities and algal densities were compared on the basis of the amount of chlorophyll per cm^2 irradiated surface (cf. Table 7.2). The transition from light saturated to light dependent growth in washing machines occurred above 0.5 µl packed cell volume per ml. A chlorophyll content of 4 mg/dm^2 for single leaves (cf. GABRIELSEN, 1948) and from 1–5% on dry weight basis in *Chlorella* (cf. VAN OORSCHOT, 1955) were used for the calculations.

It appears that algae in dense packing contain an amount of chlorophyll which is comparable with that in a plant density with a closed leaf surface, normally given in literature as having a leaf area ratio of 5. Light limitation in washing machines started above TROMMSDORFF values of 0.5 µl/ml, i.e. 38 µg chlorophyll per cm^2, a figure well comparable with the 40 µg chlorophyll/cm^2 of GABRIELSEN (1948) at which energy conversion was independent of chlorophyll concentration.

We found in our experiments that net energy conversion decreased with an increase in initial chlorophyll concentration (in the range of 0.6–1.5 µl/ml). It may be expected that in experiments within higher plants an increase in plant density will be favourable up to a certain plant density. After that a decrease in efficiency will follow caused by respiratory processes.

In some cases observed differences in efficiency may be connected with differences in respiratory activity in the culture as a whole (cf. Table 4.3.1, fig. 4.3.2). Average respiratory activity in samples taken from cultures in washing machines under continuous mercury light was 2.11 µl $CO_2 \cdot \mu l^{-1} \cdot hour^{-1}$ (cf. Table 4.3.2).

TABLE 7.2. Chlorophyll content of *Scenedesmus* in a 20 cm layer of water, surface 1 cm^2, irradiated with natural daylight. Average chlorophyll content taken as 2.5% on dry weight basis; 1 µl algae is equivalent with 151 µg dry weight on the average. Maximum energy conversion in leaves is independent of the chlorophyll concentration above 40–100 µg chlorophyll/cm^2 leaf (cf. GABRIELSEN, 1948).

TROMMSDORFF value ($\mu l \cdot ml^{-1}$)	Cell number ($\times 10^6 \cdot ml^{-1}$)	Chlorophyll content ($\mu g \cdot cm^{-2}$)
0.4	1.0	25.0
0.5	1.8	37.8
0.7	5.7	52.9
0.8	6.9	60.4
1.1	10.9	83.1
2.6	20.5	196.3

The dry weight equivalent for 1 µl algae was 151 µg as determined in experiments. Using this conversion factor, the O_2 -uptake in Table 4.3.2 was 2.11 µl/151 µg/hour = 14.0 µl/mg/hour. A difference in respiratory activity was found in series of morning samples collected in cultures in washing machines in April and June being 5 µl/mg/hour and 7 µl/mg/hour respectively (cf. fig. 4.3.6). It could be made plausible that respiratory activity was highest in the morning samples in June, containing larger cells than in the April series. It is evident, however, that the respiratory activity in the morning samples is lower than in the culture in continuous light. Does literature present evidence that respiratory activity changes when algal cells are illuminated?

NIHEI *et al.* (1964), with synchronous cultures of *Chlorella ellipsoidea* measured respiratory activities in the course of cell development. They found that respiratory activity per unit of cell number increased during the light period.

Cell division, which depends on the duration of the light period and the intensity of the incident radiation, decreases the average cell size (cf. TAMIYA, *et al.*, 1953a, 1961; PIRSON and RUPPEL, 1962; LORENTZEN and RUPPEL, 1959; SOROKIN, 1964; SENGER, 1961).

It was shown in fig. 5.2.1 that cell size changed when cells were transferred from continuous light to an L/D treatment of 16/8. Smaller cells than in the suspension at the start of the experiment were predominant in morning samples, larger cells at the end of the light period. Fig. 4.3.4 is an example for the observation in many experiments that cell length and width increase during the day. Changes in cellular composition thus occurred in our experiments. Table 4.3.2 and fig. 4.3.6 show that respiratory activity is not the same under several experimental conditions. A cause for this phenomenon cannot be given on the basis of the present material. Evidence for the existence of a relationship between the rates of photosynthesis and respiration in growing algal cultures was subject of an investigation which will be published later on.

It can be stated that, in general, production is influenced by daylength when the respiration of the cell mass cannot be neglected. In practice, these effects could be observed in deep layers with rather high cell concentrations. An appreciably decreased production rate was observed at an L/D ratio of 8/16 (cf. Table 5.2.1).

The growth rate of algae in thin layers (comparable with light saturated conditions) was more rapid during long days than during short ones, owing to the fact that the higher growth rates during long days caused improved light absorption.

Transitions from one daylength regime to another influenced the production rate (cf. Table 5.2.2), we concluded from our experiments that a strong discrepancy in light regime between pretreatment and treatment unfavourably affected the production rate. Cells cultivated in 'continuous culture' tubes at a constant optical density and light/dark ratios ranging from L/D = 8/16 to continuous light showed an optimum growth at L/D = 12/12 (cf. fig. 5.2.5). It was concluded that long term action of a certain daylength treatment induced a partial synchronisation of the cell mass. This explanation is illustrated with

experiments with synchronous algal cultures taken from literature.

Synchronised algae, exposed to shorter or longer daylengths show disturbances in their cell division pattern. SOEDER (1968), with synchronous *Chlorella* cultures, found that prolonging of the light period caused a delay in the start of cell division. The same was found with the high temperature strain of *Chlorella pyrenoidosa* by SOROKIN and KRAUSS (1965).

Shortening of the light period in synchronous algal cultures divided the cell mass in groups with a normal and a prolonged cycle (cf. PIRSON, 1962; SENGER, 1961).

It is evident from literature that rates of photosynthesis and respiration are coupled with cell development (cf. SOROKIN and KRAUSS, 1965; NIHEI *et al*, 1964; SPEKTOROVA *et al.*, 1968).

The conclusion would be that a delay or disturbance of cell division in synchronous algae, owing to shortening or prolonging of the light period, may influence the rate of photosynthesis and respiration at the start and during the following light period. Therefore, our conclusion that a partial synchronisation of the algal mass occurs when daylength regimes in the vicinity of the 'generation time' are applied, seems plausible in the light of literature.

In Chapter 6, we discussed the influence of temperature on yield. Photosynthesis, dry matter production, and multiplication are influenced in a similar way be temperature, resulting in an optimum around 35 °C.

The favourable effect of a decreased night temperature as described by WENT (1948) in higher plants, was also found by DAVIS and collaborators (1953). They found such effects in cultures in the open when there was direct sunlight. Laboratory experiments of the same group (cf. BURLEW, 1953) indicated that a growth promotion by low night temperatures could not be obtained in low intensity cultures. DAVIS *et al* supposed that only high light energy input was more efficiently utilized when the night temperature was lower than the day temperature.

In our experiments evidence for a stimulatory effect of low night temperature was only apparent in non-buffered cultures in daylight. The effect was not observed when buffered or NH_4NO_3 containing media were used. In non-buffered cultures CO_2- or phosphate deficiency may have existed at constant temperature. In general, in our experiments no evidence for a stimulatory effect of low night temperatures on the growth rate could be found. Arguments against growth stimulation by low night temperatures are found in articles dealing with the effect of temperature on cell division in synchronous algal cultures (cf. MORIMURA, 1959; SOROKIN and KRAUSS, 1962).

Cell division is a temperature dependent process. A decrease in temperature during the night probably causes a phase shift in cell development when a comparison is made with a culture receiving a high temperature continuously. Thus, coupling of photosynthesis with cell development (cf. NIHEI *et al*, 1964; SPEKTOROVA *et al*, 1968) will be influenced by low night temperature. In this respect it is less likely that a stimulatory effect of low night temperature, as described by DAVIS *et al.*, is due to a more efficient cell division. Other possibil-

ities are: 1) that the culture solution in the series with low night temperatures stores large amounts of CO_2 in the free and bicarbonate form, which induces higher amounts of available CO_2 as compared with a series receiving a high temperature continuously; 2) that rebalancing of cellular composition, or restoration of the photosynthetic apparatus during the night period is especially favoured by low night temperature.

KOK (1952) found that cells at the end of the light period contained relatively high amounts of carbohydrates and lipids.

A decrease in photosynthetic efficiency, coupled with a decrease in N- content was found by BONGERS (1956) in N-deficient cultures. There are no direct experiments about the influence of cellular N-content on photosynthetic efficiency in complete media.

The maximum rates of photosynthesis for *Chlorella pyrenoidosa* reached an optimum in the middle of the light period (cf. SOROKIN, 1961). Since the N-content is relatively highest at the end of the dark period (cf. KOK, 1952), it is less likely that N-content and maximum rate of photosynthesis are directly coupled.

At the end of this discussion we would like to give a personal view concerning the possible use of algae.

In parts of the world with a large increase in the size of human population and food shortage, the production of organic material rich in proteins is a necessity. The area which can be cultivated already is a limiting factor for food production at some places or may become so in the future. Additional food production in shallow lakes and ponds might be helpful, therefore.

As was shown in this thesis, rather high energy conversion values were obtained over relatively long periods during the summer months. In temperate regions, cultivation of *Scenedesmus* for nutritional purposes should be restricted to this part of the year. The energy input in winter is too low and too variable in the Netherlands to maintain high production rates. It is perhaps possible to extend the culture period in winter by using stable communities of algae which are adapted to low temperatures. The thermic pollution of natural waters in industrialised countries might prevent too low a temperature during winter. The existence of algal communities in arctic and antarctic waters might be a proof that growth in winter is possible, provided that a combination of adequate species is selected.

The algal product is rich in proteins (cf. KOK, 1952; VAN OORSCHOT, 1955) although part of the proteins are not directly digestible. Rather intricate and thus expensive cultivation apparatus are needed to obtain yields which are comparable with the maximum growth rate in modern agricultural crops, although these high production rates can be maintained over longer periods than in normal agriculture. It would be unrealistic, however, to recommend algae as a possible source for nutrition in those countries of the world which already have an agricultural overproduction, i.e. in the Western European countries and the U.S.A. In subtropical and tropical countries with a lack of area useful for agriculture and a high population pressure, stable communities

of algae can be a possible source for nutrition since light and temperature are adequate all over the year and food shortage urges to use all possible reserves.

The eutrophication of inland waters in industrialised countries offers a substratum for 'algal blooms' which can be a menace for a healthy water flora and fauna. The existence of such 'algal blooms' illustrates that algae can be used in principle for the removal of inorganic and organic substances in heavily contaminated water. Waste water treatment by algae is already used in the U.S.A. (cf. GOLUEKE and OSWALD, 1964). Removal of the algae from the water and composting of the filtered material or use of the algal residue as cattle fodder might offer possibilities to purify waste water and to delay the flow of soluble fertilizing agents which else would have been lost. These methods can be employed in temperate regions as well as in tropical areas.

8. SUMMARY

Experiments about the influence of external factors on the energy conversion in mass cultures of *Scenedesmus* are described in this thesis. Several types of culture vessels were used in the laboratory as well as in the open. Demonstration models of MIELE washing machines with a volume of 50 *l* were used for experiments in the open. In the laboratory the algae were cultivated in flasks on the rocking table, in washing machines under mercury light, in 'continuous culture' tubes and in small culture tubes with a volume of 100 ml in a thermostated bath. A mixture of 5% CO_2 and air was continuously bubbled through the suspension, and the temperature was kept constant in all culture methods.

The growth rate of *Scenedesmus* was independent of the concentrations of SO_4^{--} and $H_2PO_4^-$ above a certain minimum. This was not the case with KNO_3 (cf. Chapter 3). An optimum for growth was found in culture media containing KNO_3 at 5 mM in flasks on the rocking table. The average light intensity received by an individual cell was low in this case. The inhibition of growth by KNO_3-concentrations higher than 5 mM could only partially be explained osmotically. No optimal KNO_3-concentration was found in other types of culture vessels with a comparatively high average light intensity for an individual cell. It was concluded that tolerance for KNO_3 depended on the energy supply for the individual cell. Therefore, the incident radiation, the density of the algal suspension and shape and dimensions of the culture vessel are of importance.

An increase of the energy conversion of 30% was found when the rate of photosynthesis was light limited and when ammonium salts in well buffered media or urea was used instead of KNO_3. It was concluded, that the yield at low light intensities can be improved with about 30% by bypassing nitrate reduction.

The growth rate was slightly enhanced by the addition of yeast extract, whereas the growth rate was not influenced by soil extract in our experiments.

Section 4.2 describes the light distribution over the surfaces of the washing machines. The theoretical models for calculation of the total production per unit of surface, which are described in literature suppose that light only enters via the upper surface. The presence of horizontal and vertical surfaces in washing machines necessitated to adapt such models to the situation existing in these machines. The total global radiation measured in the field only was valid for a horizontal plane. To estimate light distribution for any complex surface is theoretically possible when the ratio between direct and diffuse light is known for every moment of the day. Calculation of the distribution of the incident radiation was simplified in our case in that the energy supply over the vertical surface was expressed as if it was an enlargment of the horizontal surface, This surface was called 'apparent horizontal surface'.

Growth rates depend on the incident energy under light-limited conditions.

Algal production in washing machines with incident radiation via the horizontal surface only, was compared with that in washing machines with incident radiation over the total surface. When light limitation exists, the ratio between these productions measured over a period of several days enabled to estimate the 'apparent horizontal surface' for several parts of the growing season. Since the contribution of the vertical surface depended on the height of the sun, it is feasible that a minimum 'apparent horizontal surface' was obtained around the longest day of the year. Decrease of light energy in the vessels was calculated with LAMBERT-BEER's law. The extinction coefficient k was determined experimentally. The stirring was insufficient to expect an appreciable increase in energy conversion. An increase in algal density at the start of the experiments decreased the energy conversion, which was atributed to respiratory losses (cf. Ch. 4.3).

A comparison between three series of experiments showed that respiration varied significantly under the different experimental conditions. Daily respiratory losses were determined by means of WARBURG measurements and dry matter determinations. These losses ranged from 8.7% to 18.6% of the total amount of dry matter present.

The energy conversion corrected for shading by the buildings, reflection and absorption by the culture vessels was 6.0% on the average for the period from March to October in the years 1962–1965. Energy conversion was 10.2% on the basis of incident light in July and August.

The efficiency of stirring was computed on the basis of the stirring velocity. A relative utilisation of 20% of the maximum efficiency of the flash, i.e. 5% on the basis of photosynthetically active radiation, was expected.

The energy conversion could be enchanced in washing machines under mercury light by decreasing the volumes of the suspension in the vessels (cf. section 4.3.1). A more effective stirring was obtained in using smaller depths of layer at the same rate of stirring.

Influence of daylength on the energy conversion in mass cultures were examined in the four combinations: thick/thin layers; high/low cell densities (cf. Ch. 5). The energy conversion on the basis of incident light decreased with shorter daylengths under light saturated conditions in experiments lasting 24 hours, i.e. in thin/thick layers with low cell densities.

The culture as a whole was light limited in thick layers with high cell densities. A lower energy conversion as compared with continuous light was obtained only with a light/dark ratio of 8/16. This was atributed to respiratory losses.

A completely different situation occurred when cultures were exposed to a fixed light/dark treatment during several days. A strong discrepancy in light/dark ratio between pretreatment and experiment caused a decrease in energy conversion in cultures in flasks on the rocking table. Optimum absolute growth rates and growth rates per hour light were found at light/dark ratios of 12/12 in 'continuous culture' tubes in which the algal suspension was kept at a constant optical density.

It was concluded from the experiments described in Chapter 5 that cells

pretreated in continuous light exhibited a simple reaction pattern during the first 24 hours after the pretreatment. An increase in energy conversion on the basis of incident light was found when the cell concentration at the start of the experiment was increased, at relatively high average light intensities for the individual cell. A decrease in energy conversion can be expected in thick layers and high cell concentrations at very short daylengths (i.e. L/D = 8/16), which is caused by respiratory losses. Longer lasting pretreatments with intercalation of dark periods were most favourable when the daylength approximated a light/dark ratio which is necessary for synchronisation; i.e. L/D = 12/12, causing partial synchronisation. A temperature optimum of 35 °C was found for photosynthesis, respiration and growth when cells were precultivated at 30 °C (cf. Ch. 6). No shift in temperature optimum was obtained with shorter daylengths.

Higher energy conversion was found in cultures receiving variable night temperatures, when non-buffered culture solutions containing KNO_3 were utilised. Culture solutions containing NH_4NO_3 did not show differences in energy conversion between the two temperature treatments, whereas a buffered culture solution containing KNO_3 showed highest efficiencies in the constant temperature series. The increase in energy conversion at variable night temperatures in non-buffered culture solutions was attributed to a higher amount of total available CO_2, or soluble phosphate, as compared with cultures kept at a constant temperature. When phosphate or CO_2 do not limit growth, as is the case in buffered culture solutions, a low night temperature probably inhibits cell division which reduces the efficiency.

The results of the experiments described so far are compared with literature in Chapter 7. Considering the energy conversion values obtained in our experiments and the efficiencies measured in agricultural crops with a closed crop surface, it is concluded that algae in dense packing contain a comparable amount of chlorophyll per unit of surface. The energy conversion is about the same in algal cultures and in crops with a closed canopy. An increase in efficiency would theoretically be possible in higher plants if the CO_2 concentration would have been increased.

At last a possible application of algae as food plants or as purifiers of waste waters is discussed.

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10. SAMENVATTING

In dit proefschrift worden experimenten beschreven over de invloed van milieufaktoren op de energieomzettingen bij de fotosynthese in massakulturen van *Scenedesmus* sp. Er werd met verschillende soorten kweekvaten zowel buiten als binnen het laboratorium gewerkt. Voor de buitenproeven werden demonstratiemodellen van wasmachines gebruikt met een inhoud van 50 liter. In het laboratorium werden de algen gekweekt in kolven op de schudmachine, in wasmachines onder kwiklampen, in 'continue kweekbuizen', en voorts in kleine kweekbuizen van 100 ml, die in een bad met een constante temperatuur werden geplaatst. Bij alle kweekmethodes werd een mengsel van lucht met 5% CO_2 door de algensuspensie geleid, terwijl de temperatuur constant gehouden kon worden.

De groeisnelheid van *Scenedesmus* was boven een zekere minimum concentratie onafhankelijk van de gebruikte concentraties SO_4^{--} en $H_2PO_4^-$; dit gold echter niet voor KNO_3 (zie Hfdst. 3).

Het gebruik van ammonium zouten in goed gebufferde voedingsoplossingen of van ureum gaf in vergelijking met KNO_3 een relatieve verbetering van de energieomzettingen van 30%; dit alles echter indien de fotosynthesesnelheid door de lichtenenergie werd beperkt. Hieruit kan worden geconcludeerd, dat bij lage lichtintensiteiten de opbrengst met 30% kan worden verhoogd door de nitraatreductie te vermijden.

In kolven op de schudmachine, waar de gemiddelde lichtintensiteit die door een individuele cel ontvangen werd laag was, werd een optimum voor de groei gevonden bij 5 mM KNO_3. Remming van de groei bij KNO_3-concentraties hoger dan 5 mM was slechts ten dele osmotisch te verklaren. In andere soorten kweekvaten, waar de gemiddelde lichtintensiteit die door elke cel ontvangen werd hoog was, werd geen optimale KNO_3-concentratie gevonden. Er werd geconcludeerd dat de tolerantie voor KNO_3 afhing van de energietoevoer voor de individuele cel. De ingestraalde lichtintensiteit, de dichtheid van de algensuspensie, en de vorm en afmetingen van het kweekvat zijn in dit verband dus van belang.

Toevoeging van gistextract aan het voedingsmedium gaf een geringe maar significante stijging van de groeisnelheid. Grondextract had geen invloed op de groeisnelheid in onze proeven.

In hoofdstuk 4, paragraaf 2, wordt de lichtverdeling over het oppervlak van wasmachines aan een nader onderzoek onderworpen.

De modellen, die in de literatuur beschreven zijn om de produktie per eenheid van oppervlakte te berekenen, berusten op de veronderstelling dat het licht alleen van boven op een oppervlak valt. Het voorkomen van zowel een horizontaal als een gebogen verticaal oppervlak bij wasmachines maakte dit model niet zonder meer bruikbaar. De totale globale straling, zoals die met de normale metingen wordt verkregen geldt slechts voor een horizontaal vlak. Alleen hier-

mee is de lichtverdeling over een complex oppervlak zonder meer niet te berekenen, omdat de verhoudingen tussen de direkte en de diffuse hemelstraling op ieder moment van de dag feitelijk mede gegeven zouden moeten zijn.

In ons geval werd de berekening van de instraling over beide oppervlakken vereenvoudigd door de onbekende energietoevoer via het verticale vlak zo uit te drukken alsof het een vermeerdering van het horizontale oppervlak betrof; voor dit laatste waren de instralingsgegevens immers bekend. Het zo verkregen horizontale oppervlak werd 'schijnbaar horizontaal oppervlak' genoemd.

Bij lichtlimitering is de groei afhankelijk van de ingestraalde hoeveelheid energie. Onder deze omstandigheden werden produktie vergeleken tussen machines met alleen een verlicht bovenvlak en machines met een totaal verlicht oppervlak. De verhouding van deze produkties over periodes van langere duur gaf de mogelijkheid het 'schijnbaar horizontale oppervlak' te berekenen voor verschillende periodes van het jaar. Aangezien de bijdrage van het verticale vlak sterk afhankelijk is van de zonshoogte is het begrijpelijk dat een minimum 'schijnbaar horizontaal oppervlak' om en nabij de langste dag werd gevonden. De lichtverzwakking werd met behulp van de wet van LAMBERT-BEER berekend, waarbij de extinctiecoëfficiënt k werd bepaald. De roersnelheid bleek onvoldoende snel te zijn om aanzienlijke verhogingen van de energieomzettingen te verwachten. Indien bij beënting de dichtheid van de algensuspensie verhoogd werd bleek hierdoor de energieomzetting van de netto-fotosynthese te dalen. Dit verschijnsel werd aan ademhalingsverliezen toegeschreven (zie 4.3). De laagdikte van 20 cm in wasmachines zorgde er voor dat reeds bij lage dichtheden van de algensuspensie lichtlimiteringen bestonden. Hierdoor werden de relatieve ademhalingsverliezen bij hogere beëntingsdichtheden steeds groter. Bij vergelijking van 3 series proeven waarin de ademhalingssnelheid werd bepaald bleek dat deze significant verschilde onder de verschillende omstandigheden. De dagelijkse ademhalingsverliezen werden met behulp van WARBURG metingen en droge stof bepalingen berekend. De verliezen lagen tussen 8,7% en 18,6% van het aanwezige droge gewicht.

De gemiddelde energieomzetting van de lichtenergie werd berekend over de periode van maart tot oktober in de jaren 1962 tot 1965. Deze bedroeg, gecorrigeerd voor reflektie en absorptie van de kweekvaten gemiddeld 6,0%, terwijl in juli en augustus rendementswaarden van 10,2% werden gemeten.

Op grond van de roersnelheid en de laagdikte in de kweekvaten is te verwachten, dat de gemiddelde lichtintensiteit die door de zon wordt ingestraald met een efficiency van 20% van het maximale rendement (5% van de fotosynthetisch actieve energie) wordt omgezet. Het nuttig effekt van het roeren kon worden verhoogd in wasmachines onder kwiklicht waarin de volumina werden verkleind (zie hoofdstuk 4 paragraaf 3.1). De geringere laagdikte bij kleinere volumina gaf met dezelfde roersnelheid een effektievere roering.

Invloeden van daglengte op de energieomzettingen in massakulturen werden nagegaan in de vier combinaties: dikke laag/dunne laag, hoge/lage celdichtheid (zie hoofdstuk 5). Indien gedurende 24 uur werd belicht zonder dat de lichtenergie de groei beperkte veranderde de energieomzetting op basis van de *inge-*

straalde lichtenergie naarmate de daglengte korter werd, d.i. in dunne en dikke lagen met lage celconcentraties. In dikke lagen met hoge celdichtheden heerste gehele of gedeeltelijke lichtlimitering. Alleen bij zeer korte dagelijkse lichtperioden van 8 uur licht werd een lager rendement gevonden dan in continu licht, hetgeen werd toegeschreven aan ademhalingsverliezen.

De situatie wijzigde zich indien langere tijd een bepaald licht-donker ritme aan de kulturen werd gegeven. In kulturen in kolven op de schudmachine gaf een grote afwijking in licht-donker verhouding tussen voorbehandeling en experiment een lager rendement. In 'continue kweekbuizen' waarin de optische dichtheid van de celsuspensie constant kon worden gehouden bleek een dag-nacht ritme van 12 uur licht-12 uur donker absoluut, en eveneens per uur licht optimaal voor de groei te zijn. We concludeerden uit de proeven die in hoofdstuk 5 besproken zijn, dat cellen, voorgekweekt in continu licht, gedurende 24 uur na de voorbehandeling een eenvoudig reaktiepatroon vertoonden. Bij relatief hoge lichtintensiteiten voor de gemiddelde individuele cel was een verhoging van de celconcentratie gunstig voor het bereiken van een hoger rendement. In dikke lagen met een relatief hoog aandeel van de ademhaling verlaagde een korte dag (L/D = 8/16) het energierendement. Langer durende voorbehandelingen met tussenschakeling van donkerperiodes waren het gunstigst indien de daglengte de tijd benaderde waarbij celsynchronisatie mogelijk is, d.i. L/D = 12/12.

In hoofdstuk 6 werd gevonden dat het temperatuuroptimum voor de fotosynthese, ademhaling en groei bij 35 °C lag indien de cellen waren voorgekweekt bij 30 °C. Een kortere daglengte veroorzaakte geen verschuiving van het temperatuuroptimum voor de groei. Variabele nachttemperatuur gaf met een ongebufferde, KNO_3 bevattende voedingsoplossing hogere rendementen dan een constante temperatuur van 30 °C. In NH_4NO_3 bevattende voedingsoplossingen was er geen verschil tussen de twee temperatuurbehandelingen terwijl een gebufferde KNO_3 bevattende voedingsoplossing hogere rendementen opleverde bij constante temperatuur. De rendementsverhoging bij variabele nachttemperatuur in ongebufferde oplossingen werd toegeschreven aan een grotere hoeveelheid beschikbaar CO_2, of fosfaat in opgeloste vorm dan in kulturen bij constante temperatuur. Wanneer fosfaat of CO_2 de groei niet limiteren, zoals in gebufferde oplossingen, remt een lage nachttemperatuur waarschijnlijk de celdeling, waardoor het rendement lager is dan bij een constante temperatuur.

In hoofdstuk 7 worden de resultaten van de proeven vergeleken met gegevens uit de literatuur. Een vergelijking van de door ons verkregen gegevens met energieomzettingen van landbouwgewassen met een gesloten gewasoppervlak laat zien, dat algen in dichte pakking een vergelijkbare hoeveelheid chlorophyll per eenheid van oppervlakte en een vergelijkbaar rendement opleveren. Hierbij valt op te merken dat bij hogere planten wellicht nog enige opbrengstverhoging zou kunnen optreden indien de CO_2-concentratie verhoogd zou worden.

Tenslotte wordt de mogelijke toepassing van algen als voedselplant en waterzuiveraar besproken.

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CURRICULUM VITAE

In navolging van een bestaande gewoonte volgt hier een overzicht van mijn studie.

De middelbare opleiding aan het Heymanslyceum te Groningen werd in 1954 afgesloten na het behalen van het einddiploma H.B.S.-B. Hierna werd de studie voortgezet aan de Rijksuniversiteit te Groningen. Na een onderbreking ter vervulling van de militaire dienst werden in 1959 het candidaatsexamen, en in 1962 het doctoraalexamen in de biologie behaald. Als specialisaties werden plantenfysiologie, dierfysiologie en biochemie gekozen.

Gedurende de doctoraalstudie werd bij Prof. Dr. L. DE RUITER gewerkt over het voedingsgedrag van normale muizen, en muizen met chemisch beschadigde hersencentra. Bij Prof. Dr. M. H. VAN RAALTE werd gewerkt over de invloed van remstoffen op de worteladembaling van granen, terwijl bij Prof. Dr. M. GRUBER en Prof. Dr. M. H. VAN RAALTE de opname van ^{32}P en de ademhaling in wortels van granen werden bestudeerd. Tenslotte onderzocht ik bij Prof. Dr. M. GRUBER de remming van aceetaldehyde op gistcarboxylase. Dit onderzoek kon met behulp van een Z.W.O.-subsidie in 1962 gedurende korte tijd worden voortgezet aan het Physiologisches Chemisches Institut van de universiteit te Freiburg (Dld.) onder leiding van Prof. Dr. H. HOLZER.

Vanaf december 1961 ben ik werkzaam aan het Laboratorium voor Plantenfysiologisch Onderzoek van de Landbouwhogeschool van Prof. Dr. E. C. WASSINK. Onder diens leiding wordt door mij onderzoek verricht over energieomzettingen bij algen en hogere planten.