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FORMATION OF BIOMASS, TOTAL PROTEIN, CHLOROPHYLLS, LIPIDS AND FATTY ACIDS IN GREEN AND BLUE–GREEN ALGAE DURING ONE GROWTH PHASE.*

MARGRET PIORRECK and PETER POHL

Institut für Pharmazeutische Biologie, Universität Kiel, Grasweg 9, D-2300 Kiel, Germany

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Key Word Index—Blue–green algae; green algae; biomass; mass culture; lipids; fatty acids; protein; ageing cultures.

Abstract—Batch cultures (8–32 l.) of *Chlorella vulgaris* and *Scenedesmus obliquus* and of *Anacystis nidulans* and *Microcystis aeruginosa* were grown in media containing 0.001 % KNO_3 and at several stages in growth sampled for biomass, total protein, chlorophylls, lipids and fatty acids. With increasing time and decreasing nitrogen concentrations, the biomass of all of the algae increased, whereas the total protein and chlorophyll content dropped. Green and blue–green algae, however, behaved differently in their lipid metabolism. In the green algae the total lipid and fatty acid content as well as the composition of these compounds changed considerably during one growth phase and was dependent on the nitrogen concentration in the media at any given day of growth. More specifically, during the initial stages of growth the green algae produced larger amounts of polar lipids and polyunsaturated C_{16} and C_{18} fatty acids. Towards the end of growth, however, these patterns changed in that the main lipids of the green algae were neutral with mainly saturated fatty acids (mostly 18:1 and 16:0). Such changes did not occur in the blue–green algae. These differences between prokaryotic and eukaryotic algae can possibly be explained by the 'endosymbiont theory'.

INTRODUCTION

The data of the preceding publication [1] indicated that changes in nitrogen concentration (NH_4Cl/KNO_3) of the nutrient media have a considerable influence on the formation of lipids and fatty acids in green algae but very little effect on these compounds in blue–green algae. When (non-N_2 fixing) algae are grown in batch cultures, the nitrogen concentrations in the nutrient medium necessarily decrease during a particular growth phase† due to the algal uptake of nitrogen. This leads to the question of whether or not the algal lipids and fatty acids are affected by these changes in the nitrogen levels. Two green algae (*Chlorella vulgaris* and *Scenedesmus obliquus*) and two blue–green algae (*Anacystis nidulans*—'Synechococcus') and *Microcystis aeruginosa* have therefore been investigated at different stages (days) during growth.

RESULTS

Dry weight (biomass) and content of total protein and chlorophylls (see Tables 1–4)

The duration of the growth phase was 22 days for *Anacystis nidulans* and *Scenedesmus obliquus*, 28 days for *Chlorella vulgaris* and 46 days for *Microcystis aeruginosa*. During each growth phase, the nitrogen content in the nutrient media decreased from an initial 99 μmol (0.001 % KNO_3) to 4–24 μmol N/l. The biomass of the algae increased to 54 and 71 mg/l in the green algae and to 18 and 34 mg/l in the blue–green algae (for the green algae the first and second values always refer to *Chlorella vulgaris* and *Scenedesmus obliquus*, respectively, and for the blue–green algae, to *Anacystis nidulans* und *Microcystis aeruginosa*, respectively). The protein contents diminished about twice as much in the green algae (39.9–9.19 and 33.7–7.44 %) as in the blue–green algae (36.1–19.1 and 30.6–15.8 %). Similar results were obtained for the chlorophylls: With increasing duration of growth the contents of chlorophylls in the biomass decreased about twice as much in the green algae (from 3.94 to 0.45 and from 3.66 to 0.57 %, i.e. by factors of about 9 and 6) as in the blue–green algae (from 0.96 to 0.24 and from 0.45 to 0.24 %, i.e. by factors of about 4 and 2).

Lipids

The contents of total lipids in the green algae increased fairly constantly and approximately doubled during one phase of growth, reaching final values of 64.5 % of the dry weight in *Chlorella vulgaris* and of 47.1 % in *Scenedesmus obliquus* (see Tables 1 and 2). Furthermore, a big change could be observed in the compositions of the total lipids, whereby during the first days of growth the predominant lipids in the green algae were polar and included monogalactosyl diacylglycerol, digalactosyl diacylglycerol, sulfoquinovosyl diacylglycerol, phosphatidyl glycerol, phosphatidyl choline and traces of phosphatidyl ethanolamine and phosphatidyl inositol. However, after 8–11 days for *Chlorella vulgaris* and 11 days for *Scenedesmus obliquus* there appeared neutral lipids (mostly triacylgly-

*Part 2 in the series "Preparatory Experiments for the Axenic Mass Culture of Microalgae".

Abbreviations: DGDG, digalactosyl diacylglycerol; MGDG, monogalactosyl diacylglycerol; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; PI, phosphatidyl inositol; SQDG, sulfoquinovosyl diacylglycerol.

†The term 'growth phase' represents the period from the moment of inoculation of the algae to the beginning of the stationary phase of algal growth.

Table 1. Dry weight (biomass), total protein, chlorophhyll content, total lipids and total fatty acids during one growth phase of *Chlorella vulgaris*

	Days						
	5	8	11	14	18	22	28
N-concentration in the nutrient medium(μmol N/l)	94.7	44.1	18.7	18.3	18.5	19.0	17.8
Dry weight (biomass; mg/l)	3.3	12	29	38	50	53	54
Total protein (% dry wt)	31.3	39.6	16.9	14.1	12.1	9.31	9.19
Chlorophyll content (% dry wt)	3.94	3.85	2.03	1.54	0.80	0.56	0.45
Total lipids (% dry wt)	24.4	29.5	39.6	45.3	57.2	56.6	64.5
Total fatty acids (% total lipds)	25.2	30.5	46.8	52.3	63.1	59.9	64.9

N-concentration at the beginning of growth: 99 μmol N/l ≅ 0.001 % KNO_3.

Table 2. Dry weight (biomass), total protein, chlorophyll content, total lipids and total fatty acids during one growth phase of *Scenedesmus obliquus*

	Days					
	5	8	11	14	18	22
N-concentration in the nutrient medium (μmol N/l)	78.7	49.8	23.6	5.31	4.04	4.10
Dry weight (biomass; mg/l)	2.1	10	21	42	57	71
Total protein (% dry wt)	33.6	33.7	18.7	10.2	8.50	7.44
Chlorophyll content (% dry wt)	3.66	5.03	2.35	1.08	0.78	0.57
Total lipids (% dry wt)	25.7	26.2	24.1	36.9	42.2	47.1
Total fatty acids (% total lipids)	27.1	30.7	38.5	59.1	63.8	64.4

N-concentration at the beginning of growth: 99 μmol N/l ≅ 0.001 % KNO_3.

Table 3. Dry weight (biomass), total protein, chlorophyll content, total lipids, and total fatty acids during one growth phase of *Anacystis nidulans*

	Days					
	6	8	10	13	16	22
N-concentration in the nutrient medium (μmol N/l)	91.2	35.5	17.0	19.1	18.1	18.3
Dry weight (biomass; mg/l)	1.8	4.9	15	22	20	18
Total protein (% dry wt)	28.1	36.1	29.3	17.9	17.9	19.1
Chlorophyll content (% dry wt)	0.96	0.86	0.62	0.36	0.33	0.24
Total lipids (% dry wt)	14.4	16.8	13.3	11.1	9.33	10.2
Total fatty acids (% total lipids)	24.5	29.7	27.6	29.5	31.5	27.2

N-concentration at the beginning of growth: 99 μmol N/ ≅ 0.001 % KNO_3.

Table 4. Dry weight (biomass), total protein, chlorophyll content, total lipids, and total fatty acids during one growth phase of *Microcystis aeruginosa*

	Days				
	15	21	26	35	46
N-concentration in the nutrient medium (μmol N/l)	93.3	53.7	30.5	28.5	25.3
Dry weight (biomass; mg/l)	3.4	9.8	17	30	34
Total protein (% dry wt)	29.2	30.6	30.9	13.2	15.8
Chlorophyll content (% dry wt)	0.45	0.74	0.61	0.27	0.24
Chlorophyll content (% dry wt)	0.45	0.74	0.61	0.27	0.24
Total lipids (% dry wt)	19.0	20.3	23.4	18.2	16.5
Total fatty acids (% total lipids)	13.2	17.9	15.5	11.9	13.7

N-concentration at the beginning of growth: 99 μmol N/l ≅ 0.001 % KNO_3.

cerols and trace amounts of hydrocarbons). These lipid changes are shown in Fig. 1 (*Chlorella vulgaris*). The results obtained for the other green alga, *Scenedesmus obliquus*, were practically identical. Towards the end of the growth phase the main lipids of both species of green algae were neutral.

On the other hand, the nitrogen content in the nutrient medium did not affect lipid formation during one growth phase in the blue–green algae. The percentage of total lipids was always comparably low and remained more or less constant. In *Anacystis nidulans* they constituted about 9–17% of the dry weight and in *Microcystis aeruginosa* about 17–23% (Tables 3 and 4). Only polar lipids were present (Fig. 2, *Anacystis nidulans*). The results obtained with *Microcystis aeruginosa* were identical.

Fatty acids

In the green algae, the quantity (% of the total lipids) and composition of the fatty acids during one growth phase changed dramatically with the decreasing nitrogen levels of the nutrient medium (all of the other ions were present in sufficient quantities); there was an increase in total fatty acids from about 25% to 65% of total lipids with increasing duration of growth (Tables 1 and 2). There was also a considerable change in the composition of the green algal fatty acids: At high N levels (more than about

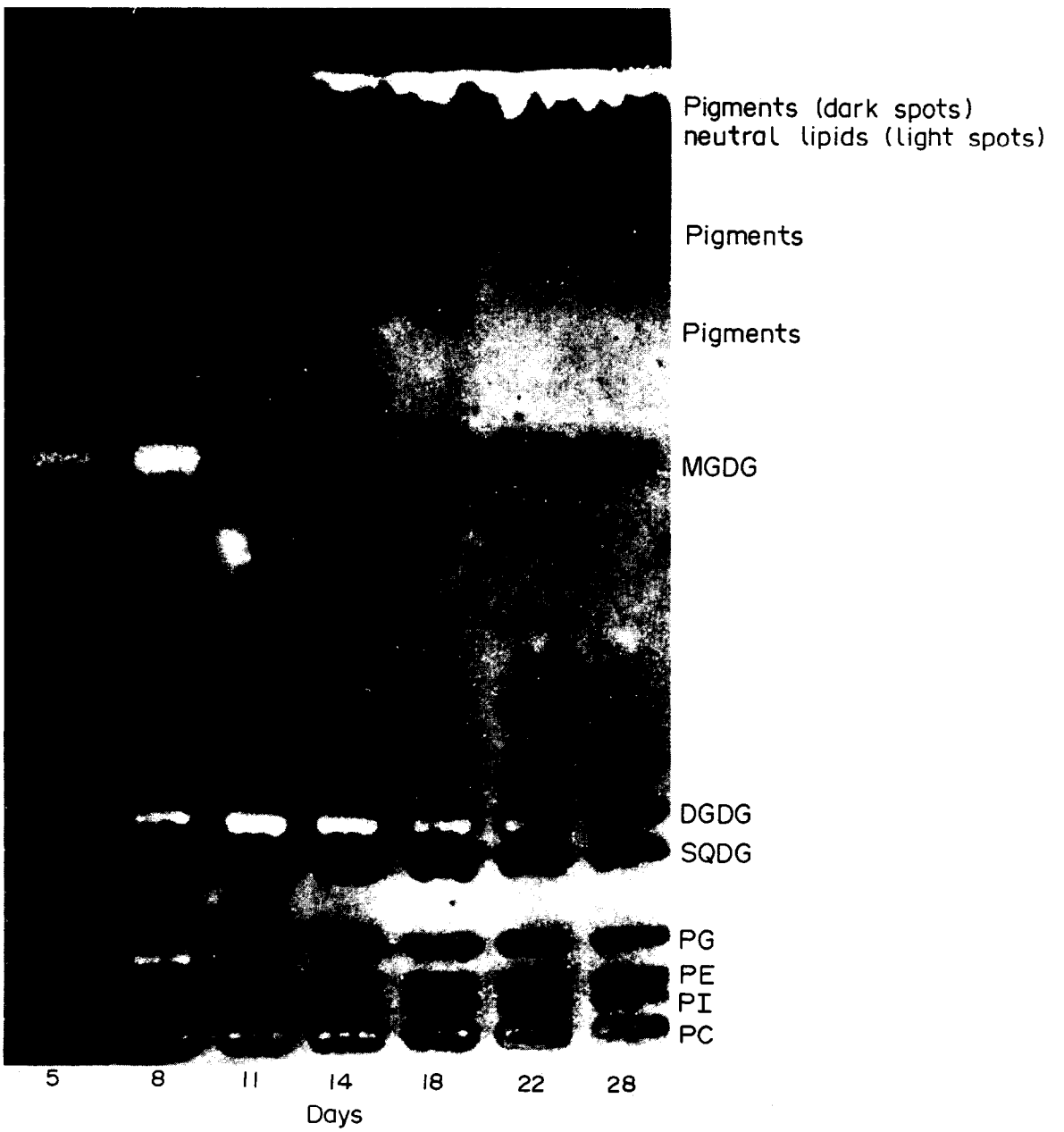

Fig. 1. TLC of the total lipids (0.3 mg each) of the green alga *Chlorella vulgaris* during one growth phase; grown at 0.001% KNO_3 in the nutrient medium. Solvent system Me_2CO–C_6H_6–H_2O (91:30:8) [2].

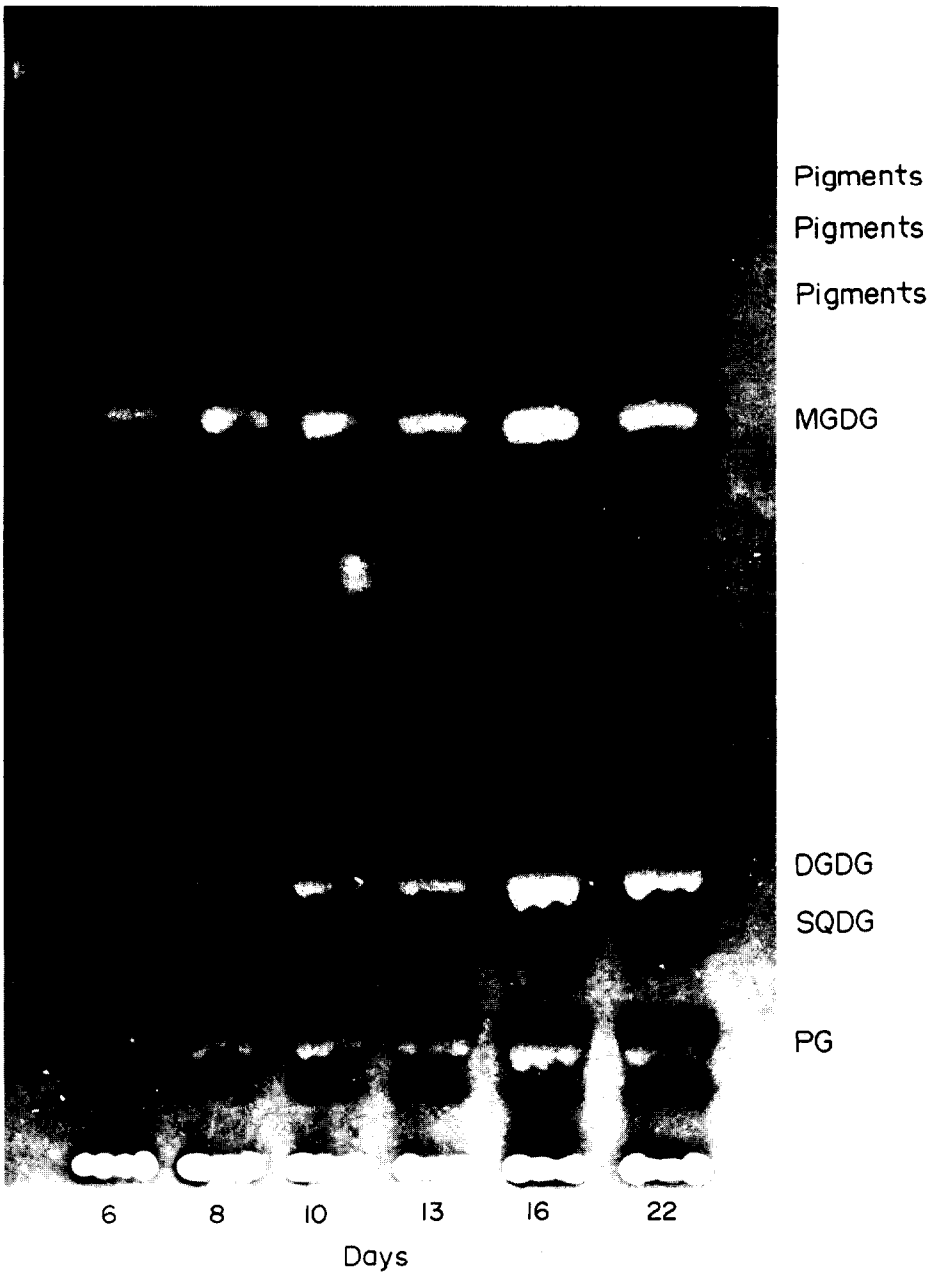

Fig. 2. TLC of the total lipids (0.3 mg each) of the blue–green alga *Anacystis nidulans* during one growth phase; grown at 0.001 % KNO_3 in the nutrient medium. Solvent system: see legend of Fig. 1.

44 μmol N/l), polyunsaturated C_{16} und C_{18} fatty acids predominated. Decreasing N levels induced a loss in polyunsaturated fatty acids and a simultaneous increase in less unsaturated fatty acids, especially 18:1. Figure 3 shows the fatty acid compositions of *Scenedesmus obliquus*. Similar results were obtained with the other green alga, *Chlorella vulgaris*. During one growth phase (22 days) the chief fatty acids in this alga were as follows: 12:0 (2.6–0.2 %), 14:0 (0.8–0.2 %), 14:1 (0.6–0.1 %), 16:0 (constant—*ca* 18 %), 16:1 (1.7–1.4 %), 16:2 (8.8–2.2 %), 16:3 (16.1–6.9 %), 18:0 (0.5–1.5 %), 18:1 (4.4–45.6 %), 18:2 (13.1–8.9 %) and 18:3 (α) (33.6–15.4 %). These fatty acid alterations in both green algae coincided with the previously mentioned change from polar to neutral lipids (e.g. in *Chlorella vulgaris* following 11 days of growth, see Fig. 1).

The total fatty acids of the blue–green algae, however, remained relatively constant during one phase of growth. The decreasing N levels in the nutrient medium did not produce any significant effects. In *Microcystis aeruginosa* the total fatty acids constituted about 12–18 % of the total lipids (see Table 4) with the 16:0 and 18:3 (γ) fatty acids as the main compounds (see Fig. 4). The fatty acid composition remained fairly constant throughout the entire

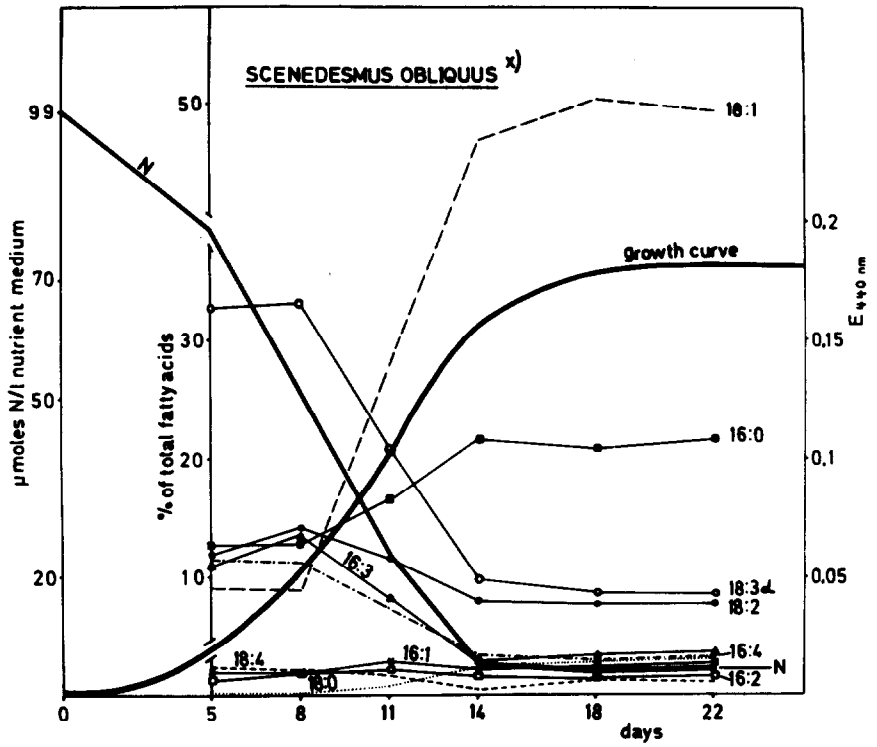

Fig. 3. Composition of the total fatty acids of *Scenedesmus obliquus* during one growth phase (initial N-concentration: 99 µmol N/l ≙ 0.001 % KNO_3). (×) The following fatty acids made up less than 1 % of the total fatty acids and were omitted: 12:0, 14:0 and 14:1.

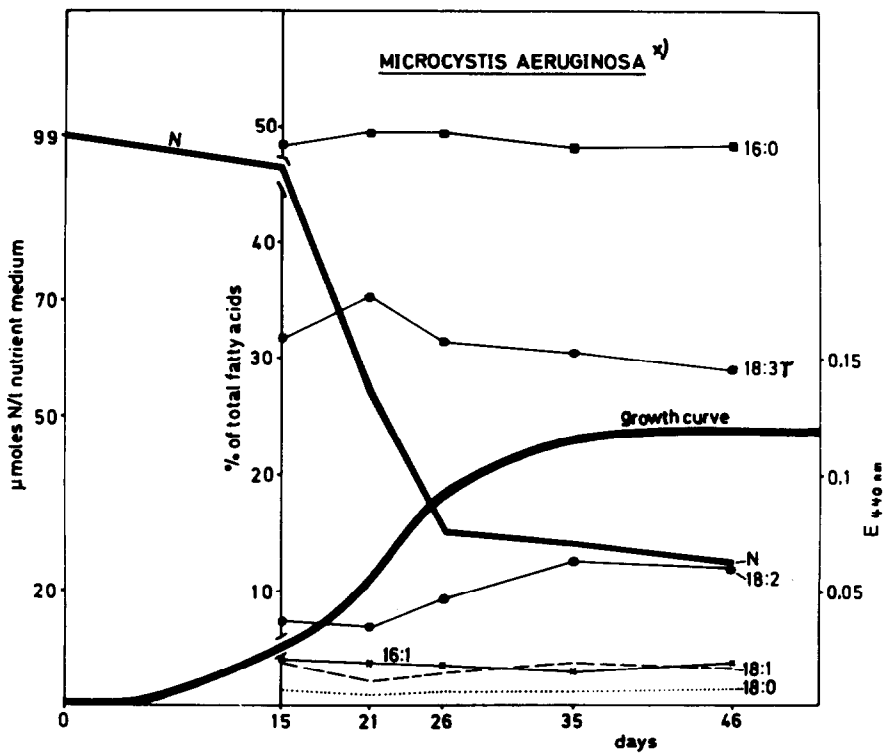

Fig. 4. Composition of the total fatty acids of *Microcystis aeruginosa* during one growth phase (initial N-concentration: 99 µmol N/l ≙ 0.001 % KNO_3). (×) The following fatty acids made up less than 1 % of the total fatty acids and were omitted: 12:0, 14:0 and 14:1.

growth phase. Similar results were obtained with *Anacystis nidulans*. During one growth phase (22 days) the main fatty acids here were: 12:0 (1.35–0.7%), 14:0 (1.45–1%), 16:0 (42.4–41.8%). 16:1 (48.1–34.9%), 18:0 (1.2–2.7%) and 18:1 (5.5–17.6%). As described earlier [1] the 16:1 and 18:1 fatty acids of *Anacystis nidulans* behaved in an inverse manner, whereby they respectively decreased and increased by *ca* 13%. These two fatty acids together always comprised about 53% of the total fatty acids. This behaviour of the 16:1 and 18:1 components cannot as yet be explained. The remaining fatty acids of *Anacystis nidulans* were relatively constant throughout the entire growth phase.

DISCUSSION

In the experiments described here the algae were grown at a relatively low nitrogen concentration (0.001% KNO_3). This was a deliberate manoeuvre towards clearly demonstrating the changes in algal lipid and fatty acid patterns. The green and blue–green algae practically paralleled one another in the formation of biomass, total protein and chlorophylls during one growth phase. With increasing time of growth (and with decreasing nitrogen levels in the nutrient medium) there resulted an increase in the biomass of all of the algae. Furthermore, the total protein and chlorophyll contents of both algal types decreased although apparently more considerably in the green algae.

In contrast to this parallel behaviour, the green and blue–green algae differed markedly in lipid metabolism during one growth phase. In the green algae, the content of lipids and fatty acids increased. Simultaneously, a redistribution from polar to neutral lipids and from polyunsaturated to saturated (16:0) and monounsaturated fatty acids (18:1) occurred. An alteration similar to that for the above unicellular green algae was previously observed by Wettern [3] in a filamentous and branched green alga, *Fritschiella tuberosa*. In earlier publications [4–7] an accumulation of 'fats' (ether extractable materials) was described for ageing cultures of diatoms. Those fats probably represented triacylglycerols, which developed for the same reason (nitrogen deficiency) as in our green algae. In contrast to the above organisms, the blue–green algae, *Anacystis nidulans* and *Microcystis aeruginosa*, did not alter in their lipid contents.

This difference in behaviour may be explained simply because blue–green algae are prokaryotes and green algae are eukaryotes. According to the 'endosymbiont theory', the chloroplasts of eukaryotic organisms arose from independent (free living) Cyanophyceae that invaded host cells and established symbiotic relationships with them [8–11]. Hence, blue–green algae and chloroplasts might be considered to be 'equivalent'. This is, for instance, exemplified by the lipids and fatty acids. Both the blue–green algae and chloroplasts are known to contain the same kind of polar lipids and polyunsaturated fatty acids but little or no neutral lipids such as triacylglycerols [12–30]. The latter are localized in the cytoplasm of eukaryotic algae [3, unpublished results].

Nitrogen deficiency leads to a strong decrease in the chlorophylls of the green algae, indicating a reduction in the protein-rich [31, 32] chloroplast apparatus as well as in the polar lipids and polyunsaturated fatty acids localized in the chloroplast membranes. So the percentage of cytoplasmic neutral lipids and fatty acids (which exhibit a low degree of unsaturation) increases in eukaryotic algae when the nitrogen concentrations in the nutrient medium decrease. At present it is not known whether this increase in the neutral lipids is only relative, or whether there is also de novo biosynthesis. Blue–green algae possess no physiological equivalent to the cytoplasm found in eukaryotic cells. Thus, the entirety of the blue–green algal cell including its components seems to be reduced rather uniformly when the nitrogen concentration in the medium decreases. This may lead to a diminution of the individual components (for example lipids and fatty acids) without major changes in their compositions. Finally, one crucial point should be reiterated. Our findings clearly indicate that the nitrogen content of the medium plays an extremely important role with regard to the formation of lipids and fatty acids in eukaryotic algae.

EXPERIMENTAL

Organisms. Chlorella vulgaris Beijerinck and *Scenedesmus obliquus* (Turp.) Krüger were obtained from the Pflanzenphysiologisches Institut, University of Göttingen, West Germany; *Anacystis nidulans* (Richt.) Dr. and *Microcystis aeruginosa* Kützing from the Centre of Algae and Protozoa, Cambridge, Great Britain.

Nutrient medium. The same medium (containing 0.001% KNO_3) as described in the previous paper [1] was applied.

Growth conditions. At light intensities of about 800 lux and at 22° all algae were grown in batch cultures under axenic conditions and continuous aeration. The algal suspensions (8–32 l., depending on the suspension densities) were centrifuged, and the algae were subsequently freeze-dried and weighed. Lipid extraction, TLC and lipid detection, preparation of the fatty acid methyl esters and determination of protein and chlorophylls were carried out as described previously [1].

Gas chromatography. Flame ionization detector (FID); glass columns (~2.5 m × 3 mm): (1) Packed with DEGS (5% on Chromosorb B, 80–100 mesh). Column temp: 160°. Flow rate: 15 ml N_2/min. Injector and detector temp: 250°. (2) Packed with Silar 10 C (15% on Gas-Chrom). Column temp: 160°. Flow rate: 15 ml N_2/min, Injector and detector temp: 250°.

Determination of the nitrogen in the nutrient medium. After centrifugation of the algae 25 ml of the supernatant were heated for 1 hr with *ca* 200 mg of Devarda alloy to reduce NO_3^- to NH_4^+. The nitrogen content was then determined according to the Kjeldahl method.

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