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BIOMASS PRODUCTION, TOTAL PROTEIN, CHLOROPHYLLS, LIPIDS AND FATTY ACIDS OF FRESHWATER GREEN AND BLUE–GREEN ALGAE UNDER DIFFERENT NITROGEN REGIMES*

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Key Word Index—Blue–green algae; green algae; biomass; mass culture; lipids; fatty acids; protein; influence of nitrogen.

Abstract—Two green algae (*Chlorella vulgaris* and *Scenedesmus obliquus*) and four blue–green algae (*Anacystis nidulans, Microcystis aeruginosa, Oscillatoria rubescens and Spirulina platensis*) were grown in 81 batch cultures at different nitrogen levels. In all the algae increasing N levels led to an increase in the biomass (from 8 to 450 mg/l), in protein content (from 8 to 54 %) and in chlorophyll. At low N levels, the green algae contained a high percentage of total lipids (45 % of the biomass). More than 70 % of these were neutral lipids such as triacylglycerols (containing mainly 16 : 0 and 18 : 1 fatty acids) and trace amounts of hydrocarbons. At high N levels, the percentage of total lipids dropped to about 20 % of the dry weight. In the latter case the predominant lipids were polar lipids containing polyunsaturated C_{16} and C_{18} fatty acids. The blue–green algae, however, did not show any significant changes in their fatty acid and lipid compositions, when the nitrogen concentrations in the nutrient medium were varied. Thus the green but not the blue–green algae can be manipulated in mass cultures to yield a biomass with desired fatty acid and lipid compositions. The data may indicate a hitherto unrecognized distinction between prokaryotic and eukaryotic organisms.

INTRODUCTION

Microalgae grown in mass cultures are often considered as a supplemental source for food or for conversion to other useful compounds such as alcohol and methane (biogas, 'energy conversion') [1–11]. This is based upon the fact that various algae (e.g. species of *Chlorella, Scenedesmus* and *Spirulina*) exhibit high yields of about 10–40 g dry biomass per m^2 and per day [1, 6, 9, 12–14], provided that they are grown in monoculture over a long period. Furthermore, many microalgae have high protein contents (*ca* 30–65 %) [1, 9, 15] and contain a wide variety of other constituents [16–21] of potential pharmaceutical use.

However, in present day conditions of algal mass culture, there exist several fundamental problems: (1) Frequently the algae are grown in open ponds, where the algae can be attacked and destroyed by parasites [22, 23]; (2) Some of the more widely used algae (*Chlorella, Scenedesmus*) are unicellular. These organisms are so small (4–10 μm) that effective filtration is almost impossible. The separation of the algae by various other methods is 'highly energy-intensive' [6]; and (3) *Chlorella* and *Scenedesmus* appear to have a rather stable cell wall with a high cellulose content [24] rendering the cells difficult to digest [3]. At present, the most promising alga seems to be *Spirulina* (Cyanophyceae) which is filamentous (*ca* 500 μm) and easily digestable [3]. This alga, however, has the disadvantage that it requires an expensive alkaline nutrient medium (pH 8.5–11!) containing large amounts (*ca* 18 %) of $NaHCO_3$–Na_2CO_3 [25].

In a research program at our institute experiments are presently being carried out to grow freshwater and marine microalgae in mass cultures (8 l.–200 l.). In order to avoid the above problems the algae are grown under axenic conditions in closed tanks. The nutrient media are freed from bacteria either by sterilization or by sterile filtration. Filamentous algae are preferably employed since they can be harvested more easily. Wherever possible, those algae expected to furnish high biomass and protein yields and also producing pharmaceutically relevant constituents are used. The most promising algae will later be grown in still larger cultivation tanks.

This paper deals with the formation of biomass, proteins, chlorophylls, lipids and fatty acids in several freshwater green and blue–green microalgae. *Chlorella vulgaris, Scenedesmus obliquus* and *Spirulina platensis* were included in our studies, because they are presently the most commonly used algae in mass culture. These organisms serve as a standard for comparison with the other algae. According to the literature mass cultures of *Chlorella* and *Scenedesmus* species have daily yields of about 12–35 g of dry weight per m^2 and protein contents as high as 54 and 43 %, respectively. *Spirulina* species yield 10–12 g/m^2/day with protein contents of about 57 % [1, 3, 9, 26].

All the algae investigated here were grown in inorganic nutrient media containing different concentrations of nitrogen (NH_4Cl, KNO_3). Nitrogen is known to have a strong influence on the metabolism of lipids and fatty acids in various algae. As described earlier, nitrogen deficiency (for example in ageing cultures) leads to an accumulation of fats in diatoms [27–31]. The compositions of these fats (for example, ether-extractable material) has not always been reported. Variations in the

*Part 1 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.

nitrogen contents of the nutrient media also cause changes in the compositions of lipid-like substances such as algal carotenoids [32–36]. Our recent studies involving various freshwater and marine algae (microscopic and macroscopic algae of the Chloro-, Rhodo-, Phaeo- and Euglenophyceae) [37–43] revealed that in all the metabolism of fatty acids and lipids is influenced by the N contents of the nutrient medium. It was observed that at low N levels (< 0.001–0.003% NH_4Cl or KNO_3) these algae have a tendency to synthesize neutral lipids (triacylglycerols, wax esters) and fatty acids with a low degree of unsaturation (especially 16:0, 16:1 and 18:1). At higher N levels (> 0.003% NH_4Cl or KNO_3) the algae predominantly synthesize polar lipids such as monogalactosyl diacylglycerol, diagalactosyl diacylglycerol, sulfoquinovosyl diacylglycerol and phosphatidyl glycerol containing polyunsaturated C_{16} and C_{18} fatty acids.

RESULTS

Growth phases and biomass production (Tables 1 and 2)

In Tables 1 and 2, the various algae are compared in biomass production at different growth phases. In all, an increase in the nitrogen concentrations of the nutrient medium led to an increase in the biomass (green algae: 20–400 mg/l; blue–green algae: 8–450 mg/l). The greatest increase in biomass production occurred between 0.003 and 0.01% KNO_3. The same effect was observed in *Chlorella vulgaris* and *Scenedesmus obliquus*, when they were grown with NH_4Cl instead of KNO_3. Because of the lower molecular weight of NH_4Cl, the greatest increase lay between 0.001 and 0.003% NH_4Cl (Table 2). When the green algae were grown with NH_4Cl, the growth phases were shorter than with KNO_3. At low N concentrations (up to 0.003% NH_4Cl or KNO_3) they grew better with NH_4Cl. At higher N concentrations, however, greater growth was associated with KNO_3.

The blue–green algae, *Anacystis nidulans* and *Spirulina platensis*, had productivities (average daily biomass production) similar to those of the green algae when grown with comparable nitrate concentrations. On the other hand, *Microcystis aeruginosa* and *Oscillatoria rubescens* were relatively unproductive due to their extremely long growth phases.

Total protein (Table 3)

In all the algae, the total protein increased with increasing nitrogen concentrations. At comparable nitrogen levels measurements were always higher in the blue–green algae (18–54%) than in the green algae (8–34%).

Chlorophyll content (Table 4)

Increasing N concentrations in the nutrient medium led to a big increase in chlorophyll content (% of dry weight) in the green algae. The most significant increases were observed between 0.001 and 0.003% NH_4Cl or 0.003 and 0.01% KNO_3. In contrast to the green algae the blue–green algae generally produced smaller amounts of chlorophyll. With increasing N concentrations these quantities increased more slowly than for the green algae and reached relatively high values only at very high N concentrations ($\geq$ 0.03% KNO_3).

Table 1. Growth phases (in days) of the algae grown at different concentrations of nitrogen (NH_4Cl/KNO_3) in the nutrient medium

	N-source	0.0003%	0.001%	0.003%	0.01%	0.03%	0.1%
Chlorella vulgaris	NH_4Cl	13	15	16	21	23	—
Scenedesmus obliquus	NH_4Cl	10	14	15	16	17	—
Chlorella vulgaris	KNO_3	16	29	30	38	59	36
Scenedesmus obliquus	KNO_3	15	20	40	42	51	72
Anacystis nidulans	KNO_3	15	15	15	15	29	45
Microcystis aeruginosa	KNO_3	41	42	55	76	82	84
Oscillatoria rubescens	KNO_3	*	*	79	99	106	134
Spirulina platensis	KNO_3	*	22	29	33	33	35

*, The alga could not be grown at this nitrate concentration.

Table 2. Dry weight (biomass; mg/l) of the algae grown at different concentrations of nitrogen (NH_4Cl/KNO_3) in the nutrient medium

	N-source	0.0003%	0.001%	0.003%	0.01%	0.03%	0.1%
Chlorella vulgaris	NH_4Cl	38	95	160	190	205	—
Scenedesmus obliquus	NH_4Cl	33	103	165	196	186	—
Chlorella vulgaris	KNO_3	17	57	77	212	293	287
Scenedesmus obliquus	KNO_3	18	61	168	243	264	403
Anacystis nidulans	KNO_3	8	23	32	240	405	453
Microcystis aeruginosa	KNO_3	11	30	81	208	206	136
Oscillatoria rubescens	KNO_3	*	*	50	166	327	289
Spirulina platensis	KNO_3	*	65	154	314	358	235

*, The alga could not be grown at this nitrate concentration.

Table 3. Total protein (% dry weight) of the algae grown at different concentrations of nitrogen (NH_4Cl/KNO_3) in the nutrient medium

	N-source	0.0003%	0.001%	0.003%	0.01%	0.03%	0.1%
Chlorella vulgaris	NH_4Cl	7.79	11.1	19.9	28.9	31.2	—
Scenedesmus obliquus	NH_4Cl	9.36	9.43	22.0	33.2	34.4	—
Chlorella vulgaris	KNO_3	12.6	6.75	14.5	30.7	31.1	32.2
Scenedesmus obliquus	KNO_3	8.19	9.00	8.81	34.0	32.1	32.9
Anacystis nidulans	KNO_3	21.2	18.3	33.4	33.9	39.7	46.3
Microcystis aeruginosa	KNO_3	28.1	27.6	23.5	24.9	46.5	50.1
Oscillatoria rubescens	KNO_3	*	*	28.0	35.6	53.8	48.6
Spirulina platensis	KNO_3	*	25.8	26.6	33.4	52.1	47.4

*, The alga could not be grown at this nitrate concentration.

Table 4. Chlorophyll content (% dry weight) of the algae grown at different concentrations of nitrogen (NH_4Cl/KNO_3) in the nutrient medium

	N-source	0.0003%	0.001%	0.003%	0.01%	0.03%	0.1%
Chlorella vulgaris	NH_4Cl	0.29	0.60	1.38	1.29	0.96	—
Scenedesmus obliquus	NH_4Cl	0.37	0.61	3.27	3.45	3.43	—
Chlorella vulgaris	KNO_3	0.32	0.53	1.21	3.83	3.65	3.63
Scenedesmus obliquus	KNO_3	0.67	0.82	0.73	4.27	4.36	3.48
Anacystis nidulans	KNO_3	0.25	0.27	0.29	0.28	0.90	0.92
Microcystis aeruginosa	KNO_3	0.21	0.30	0.29	0.40	0.71	0.95
Oscillatoria rubescenes	KNO_3	*	*	0.41	0.50	0.74	0.61
Spirulina platensis	KNO_3	*	0.17	0.33	0.37	0.31	0.50

*, The alga could not be grown at this nitrate concentration.

Total lipids (Table 5)

At low nitrogen concentrations all of the green algae contained relatively large amounts of total lipids (33–63% of the dry weight). These amounts decreased significantly with increasing nitrogen concentrations. The largest decrease in total lipids was observed between 0.001 and 0.003% NH_4Cl or between 0.003 and 0.01% KNO_3 (higher molecular weight!). Unlike the green algae, the blue–green algae contained about the same quantities of total lipis (12–18% of the dry wight), which remained remarkably constant at all nitrogen concentrations. In *Microcystis aeruginosa* and *Spirulina platensis*, there was a slight increase in total lipids at the highest nitrogen concentration (0.1% KNO_3).

Neutral and polar lipids (Table 6)

In the green algae neutral lipids (mostly triacylglycerols, pigments and trace amounts of hydrocabons) predominated within the total lipids when the algae were grown at low N levels (0.0003–0.001% NH_4Cl or 0.0003–0.003% KNO_3). The triacylglycerols and hydrocarbons were identified by TLC with reference substances in various solvent systems. The relation of the neutral and polar lipid fractions (NLF : PLF; see Table 6) was about 1 : 0.1 to 1 : 0.5 at low N levels. This relation changed dramatically when the nitrogen concentrations increased. More specifically, at higher N concentrations (and most significantly between 0.001 and 0.003% NH_4Cl or 0.003 and 0.01% KNO_3) the percentage of neutral lipids decreased and that of the polar lipids increased to a final ratio of about 1 : 2 or even 1 : 3.

The polar lipids were composed of monogalactosyl diacylglycerol, diagalactosyl diacylgylcerol, sulfoquinovosyl diacylglycerol, phosphatidyl glycerol, phosphatidly ethanolamine, phosphatidyl inositol, and phosphatidyl choline. TLC of the lipids of *Chlorella vulgaris* and of *Scenedesmus obliquus* grown with NH_4Cl or KNO_3 yielded the same basic results. It is remarkable that the decrease in neutral lipids was greater than the concomitant increase in polar lipids. This explains the overall reduction of total lipid content in the green algae (Table 5). In contrast to the green algae, the lipid compositions of the blue–green algae remained constant at all nitrogen concentrations. No changes could be observed by means of TLC. In *Anacystis nidulans*, *Microcystis aeruginosa* and *Oscillatoria rubescens* the polar lipids clearly predominated. In *Spirulina platensis* the two lipid fractions were approximately equally distributed. The polar lipids of the blue–green algae were composed of monogalactosyl diacylglycerol, digalactosyl diacylglycerol, sulfoquinovosyl diacylglycerol and phosphatidyl glycerol. Moreover, the TLC findings revealed that the neutral fraction of the lipids in all of the blue–green algae consisted almost entirely of pigments; triacylglycerols and hydrocarbons could not be detected.

Fatty acids in the neutral and polar lipids (Table 7)

As was previously mentioned (see Table 6), the neutral lipids of the blue–green algae were pigments, and there

Table 5. Total lipids (% dry weight) of the algae grown at different concentrations of nitrogen (NH_4Cl/KNO_3) in the nutrient medium

	N-source	0.0003%	0.001%	0.003%	0.01%	0.03%	0.1%
Chlorella vulgaris	NH_4Cl	52.8	41.8	20.2	14.1	11.8	—
Scenedesmus obliquus	NH_4Cl	34.6	33.1	21.7	23.0	22.4	—
Chlorella vulgaris	KNO_3	57.9	62.9	42.7	22.0	21.8	22.6
Scenedesmus obliquus	KNO_3	45.6	44.3	50.1	26.9	29.8	21.2
Anacystis nidulans	KNO_3	14.3	12.7	13.6	12.0	15.4	14.8
Microcystis aeruginosa	KNO_3	17.7	13.7	13.7	13.8	18.2	23.4
Oscillatoria rubescens	KNO_3	*	*	12.7	12.0	11.3	12.8
Spirulina platensis	KNO_3	*	11.2	9.1	12.6	15.5	21.8

*, The alga could not be grown at this nitrate concentration.

Table 6. Neutral and polar lipid fractions (% dry weight) of the algae grown at different concentrations of nitrogen (NH_4Cl/KNO_3) in the nutrient medium

	N-source		0.0003%	0.001%	0.003%	0.01%	0.03%	0.1%
Chlor. vulg.	NH_4Cl	N	45.8	28.9	7.6	2.5	2.8	—
		P	7.0	11.8	12.2	11.2	8.5	—
		N:P	1:0.1	1:0.5	1:2	1:4	1:3	—
Scened. obliq.	NH_4Cl	N	24.2	24.8	5.6	5.5	6.4	—
		P	10.1	7.9	15.6	17.4	15.6	—
		N:P	1:0.5	1:0.5	1:3	1:3	1:3	—
Chlor. vulg.	KNO_3	N	43.7	51.8	27.6	7.0	6.5	7.2
		P	9.6	10.7	14.4	13.9	14.9	15.4
		N:P	1:0.2	1:0.2	1:0.5	1:2	1:2	1:2
Scened. obliq.	KNO_3	N	36.2	34.1	39.9	5.4	10.8	7.7
		P	9.3	10.2	10.2	21.5	18.7	13.1
		N:P	1:0.3	1:0.3	1:0.3	1:4	1:2	1:2
Anac. nid.	KNO_3	N	2.9	2.4	2.6	1.7	2.4	2.7
		P	11.4	10.3	11.0	9.6	12.5	11.8
		N:P	1:4	1:4	1:4	1:5	1:5	1:4
Micr. aerug.	KNO_3	N	4.3	2.9	3.6	3.3	3.8	5.1
		P	12.4	10.4	10.1	10.5	14.4	18.3
		N:P	1:3	1:4	1:3	1:3	1:4	1:4
Oscill. rub.	KNO_3	N	*	*	5.2	3.1	3.1	3.9
		P	—	—	9.6	7.0	8.2	9.9
		N:P	—	—	1:2	1:2	1:3	1:3
Spir. plat.	KNO_3	N	*	5.3	4.3	6.8	6.4	13.1
		P	—	5.9	4.8	5.9	5.0	8.7
		N:P	—	1:1	1:1	1:1	1:1	1:1

*, The alga could not be grown at this nitrate concentration.
N = neutral lipid fraction; P = polar lipid fraction.

were no appreciable amounts of triacylglycerols and hydrocarbons. The neutral lipid fractions of the green algae, however, were comprised of pigments, trace amounts of hydrocarbons, and larger quantities of triacylglycerols not separable from the pigments by column chromatography. In order to obtain more precise information about the actual lipid content, the contents of fatty acids in both lipid fractions were determined quantitatively (Table 7). For the green algae at low N concentrations ($\leq 0.001\%$ $NH_4Cl/\leq 0.003\%$ KNO_3), most of the fatty acids were derived from the neutral fractions. At high N levels ($\geq 0.003\%$ $NH_4Cl/\geq 0.01\%$ KNO_3), however, the major portion of the fatty acids was found in the polar fractions. The blue–green algae showed a distinctly different picture: for all concentrations of nitrogen in the nutrient medium, the fatty acids were mostly found in the polar fractions and only a small proportion in the neutral fractions. The fatty acids in the neutral fractions were possibly derived from sterol esters, which reportedly occur in blue–green algae in small quantities [44–47]. Thus the data of Table 7 indicate that the lipid changes recorded in Table 6 are due to changes within the acyl bound lipids.

Fatty acids compositions of the green algae (Table 8)

These organisms have a great variety of fatty acids ranging from saturated fatty acids (12:0–18:0) to polyunsaturated C_{16}–C_{18} fatty acids. The percentage of these

Table 7. Fatty acids (% of the neutral and polar lipid fractions) of the algae grown at different concentrations of nitrogen (NH_4Cl/KNO_3) in the nutrient medium

	N-source		0.0003%	0.001%	0.003%	0.01%	0.03%	0.1%
Chlor. vulg.	NH_4Cl	FA in N	56.3	65.0	44.2	14.4	14.6	—
		FA in P	9.2	10.8	17.9	11.8	10.4	—
Scened. obliq.	NH_4Cl	FA in N	72.0	67.9	8.0	8.7	7.1	—
		FA in P	20.6	27.3	29.7	29.1	33.6	—
Chlor. vulg.	KNO_3	FA in N	60.0	53.7	67.1	7.8	10.3	8.1
		FA in P	15.9	14.1	17.9	26.8	22.6	24.0
Scened. obliq.	KNO_3	Fa in N	64.4	66.1	70.9	13.5	14.8	17.9
		FA in P	21.8	22.0	27.9	30.6	33.0	21.1
Anac. nid.	KNO_3	FA in N	7.5	5.8	5.3	2.1	2.5	2.8
		FA in P	15.5	13.1	24.8	15.6	24.9	20.6
Micr. aerug.	KNO_3	FA in N	5.8	6.2	2.3	2.8	1.4	2.3
		FA in P	13.4	23.0	20.7	14.6	9.7	6.4
Oscill. rub.	KNO_3	FA in N	*	*	4.5	3.6	4.9	4.5
		FA in P	—	—	17.1	21.7	18.6	13.2
Spir. plat.	KNO_3	FA in N	*	5.8	4.0	2.2	3.5	1.5
		FA in P	—	11.5	16.7	26.0	14.9	9.0

*, The alga could not be grown at this nitrate concentration.
FA = fatty acids; N = neutral lipid fraction; P = polar lipid fraction.

fatty acids changed markedly when the N concentrations were varied. At low N concentrations of NH_4Cl (0.0003–0.001%) or KNO_3 (0.0003–0.003%—higher molecular weight!) the green algae mainly produced the 18:1 and 16:0 fatty acids and only minor portions of polyunsaturated fatty acids (16:2, 16:3, 18:2 and 18:3). At high N concentrations ($\geq 0.003\%$ $NH_4Cl/\geq 0.01\%$ KNO_3) the algae produced substantially lower amounts of 18:1 (and to some extent 16:0) but larger proportions of the polyunsaturated fatty acids.

Fatty acid compositions of the blue–green algae (Table 9)

In contrast to the green algae, the fatty acid compositions of the blue–green algae did not change significantly when the N concentrations in the nutrient medium varied. The main fatty acids in *Microcystis aeruginosa* were 16:0, 18:3 (γ) and 18:2; in *Oscillatoria rubescens* 18:3 (α) and 16:0; in *Spirulina platensis* 16:0, 18:2 and 18:3 (γ); and in *Anacystis nidulans* 16:1, 16:0 and 18:1. In the latter alga the contents of 18:1 and 16:1 varied slightly (about 5–10%). A decrease of the 16:1 was always accompanied by a corresponding equivalent increase of the 18:1, and vice versa (see also the next paper).

DISCUSSION

By varying the concentration of nitrogen in the nutrient medium, all the algae investigated here can be manipulated with respect to their biomass production and protein content. In our experiments the green algae yielded a maximum protein content of about 34%, which is lower than the high values (up to 55%) reported by other authors [3, 10, 12, 15]. In order to obtain such high protein contents the green algae must presumably be grown at still greater N concentrations than we used (maximum 0.1% KNO_3). According to our results, blue–green algae appear to be useful for mass culture, since they have similar yields but higher protein contents than green algae. Furthermore, they are more easily digestable [3] than green algae, which is probably attributable to the type of cell wall (peptidoglycan) and lack of cellulose [48]. Filamentous blue–green algae (*Spirulina*, *Oscillatoria* and numerous other species) have the further advantage of being easily harvested due to their considerable size (up to 500 μm), compared to green algae (3–12 μm).

Most of the lipids and fatty acids of the algae here investigated are principally known from previous publications. In almost all of these reports, however, no special attention has been paid to the influence of the nitrogen levels in the nutrient media. The main lipids of *Chlorella* are MGDG, DGDG, SQDG, PG, PC, PE and PI [49, 50]. The alga has also been reported to produce triacylglycerols [51, 52], hydrocarbons [53] and sterols [54–58]. The main fatty acids of *Chlorella* are 16:0–16:3 and 18:1–18:3 [59–63]. Otsuka *et al.* [52] described small amounts of 16:4 in a monoacylglycerol fraction of *Chlorella ellipsoidea*. *Scenedesmus obliquus* exhibits quite smilar lipid and fatty acid patterns, along with the additional 16:4 and 18:4 fatty acids [51, 53, 58, 60, 64, 65]. Saturated and unsaturated C_{20} and C_{22} fatty acids have been found in small quantities [60].

Blue–green algae have rather simple lipid and fatty acid compositions. The major lipids are MGDG, DGDG, SQDG and PG [49, 66]. Some *Oscillatoria* strains have been reported to produce trigalactosyl diacylglycerol [67]. There is also a report on the presence of triacylglycerols in *Spirulina platensis* [68], which, however, could not be confirmed by our investigations. According to the literature the following fatty acids have been detected: *Anacystis nidulans*: 16:0, 16:1 and 18:1 with traces of 17:0, 17:1 and 20:0 [49, 69–71]; *Microcystis aeruginosa*: 16:0, 16:1, 18:2 and 18:3 (γ) (72); *Oscillatoria strains*: 16:0, 16:1, 18:1, 18:2 and 18:3 (α) [73–75]; *Spirulina* strains: 16:0, 16:1, 18:1, 18:2, 18:3 (γ) and traces of 18:3 (α [63, 68, 75].

According to the studies by Kenyon *et al.* [72, 75] there

Table 8. Fatty acid compositions (% total fatty acids) of *Chlorella vulgaris* and *Scenedesmus obliquus* grown with varying concentrations of NH_4Cl or KNO_3, respectively

Alga	Fatty acid	% NH_4Cl or KNO_3 in the nutrient medium					
		0.0003	0.001	0.003	0.01	0.03	0.1
Chlorella vulgaris (grown with NH_4Cl)	12:0	tr	tr	0.1	0.3	0.3	
	14:0	0.2	0.2	0.2	0.4	0.5	
	14:1	tr	0.1	0.1	0.2	0.3	
	16:0	18.9	18.7	20.3	21.7	22.6	
	16:1	1.2	1.0	1.3	2.6	2.7	
	16:2	1.3	2.1	7.8	11.8	11.7	
	16:3	7.8	9.0	10.6	7.2	5.9	
	18:0	2.1	2.5	tr	tr	tr	
	18:1	50.8	44.8	24.6	11.1	12.7	
	18:2	6.5	8.0	13.3	22.8	23.4	
	18:3 (α)	10.9	13.4	21.2	21.4	20.0	
Scenedesmus obliquus (grown with NH_4Cl)	12:0	tr	tr	0.1	0.4	0.6	
	14:0	0.3	0.3	0.4	0.3	0.5	
	14:1	tr	0.1	0.2	0.2	0.2	
	16:0	23.9	23.0	19.4	16.6	15.9	
	16:1	1.6	2.0	2.2	5.7	4.3	
	16:2	1.4	1.3	0.9	2.1	1.4	
	16:3	3.9	4.2	4.7	9.2	5.0	
	16:4	2.0	2.3	15.1	11.2	12.5	
	18:0	3.1	2.9	0.4	0.5	0.4	
	18:1	48.5	48.6	12.3	16.4	11.7	
	18:2	8.1	8.2	14.2	15.6	15.4	
	18:3 (α)	5.5	6.2	22.6	16.2	21.9	
	18:3 (γ)	0.3	0.3	2.1	2.1	3.5	
	18:4	0.8	0.8	2.5	1.9	2.6	
Chlorella vulgaris (*grown with* KNO_3)	12:0	0.1	0.1	0.3	2.7	2.7	2.4
	14:0	0.1	0.4	0.2	1.4	0.6	0.5
	14:1	0.1	tr	0.1	0.4	0.4	0.5
	16:0	17.8	19.1	16.5	16.3	15.2	16.3
	16:1	1.3	2.2	1.2	1.5	3.2	2.9
	16:2	2.0	2.5	4.5	11.4	14.9	14.7
	16:3	6.6	6.7	8.5	14.2	10.0	9.6
	18:0	1.4	2.1	1.6	0.1	0.3	0.3
	18:1	48.6	44.4	35.8	5.6	5.6	6.4
	18:2	7.8	9.1	11.6	16.8	19.6	22.0
	18:3 (α)	14.1	13.5	19.6	29.7	27.5	24.3
Scenedesmus obliquus (grown with KNO_3)	12:0	tr	tr	0.3	tr	tr	tr
	14:0	0.3	0.2	0.3	0.8	0.9	1.0
	14:1	tr	0.1	tr	tr	tr	0.6
	16:0	23.1	23.5	25.0	13.6	13.5	12.7
	16:1	2.7	3.3	2.2	8.7	7.3	7.6
	16:2	2.1	2.1	1.1	3.2	4.6	3.8
	16:3	1.7	2.8	2.2	7.4	13.2	15.7
	16:4	2.5	2.9	2.5	10.0	7.7	8.0
	18:0	1.8	2.0	2.7	tr	tr	tr
	18:1	48.4	43.6	44.2	21.4	11.5	12.0
	18:2	8.2	10.2	10.4	8.8	18.8	15.3
	18:3 (α)	7.7	7.8	7.0	19.4	16.0	17.9
	18:3 (γ)	0.2	0.6	0.8	3.6	3.7	3.1
	18:4	0.9	1.0	1.3	3.1	2.6	2.4

tr, Trace amount.

are often several strains within individual species of unicellular and filamentous blue–green algae, which differ in their fatty acid compositions. Several blue–green algae are reported to contain small amounts of hydrocarbons (0.02–0.15% of the dry weight) [46, 76–81] and of sterols [44, 46, 47]. Our results concerning the algal lipids and fatty acids are in good agreement with the lit. data.

The effect of nitrogen described in this paper had

Table 9. Fatty acid compositions (% total fatty acids) of *Anacystis nidulans*, *Microcystis aeruginosa*, *Oscillatoria rubescens* and *Spirulina platensis*, grown with varying concentrations of KNO_3

Alga	Fatty acid	% KNO_3 in the nutrient medium					
		0.0003	0.001	0.003	0.01	0.03	0.1
Anacystis nidulans	12:0	0.3	0.2	0.2	0.1	0.1	tr
	14:0	1.9	1.4	1.5	0.7	0.5	0.6
	14:1	1.3	0.9	1.8	0.9	1.4	1.6
	16:0	46.9	42.3	42.8	37.7	37.8	33.2
	16:1	39.2	34.3	43.2	38.5	45.5	50.6
	18:0	1.9	2.9	0.9	1.9	1.4	0.6
	18:1	8.2	14.2	8.1	16.4	11.4	11.7
	20:0	tr	0.7	tr	0.1	0.1	tr
Microcystis aeruginosa	12:0	tr	tr	tr	tr	tr	tr
	14:0	0.9	0.4	0.3	0.4	0.3	0.5
	14:1	0.2	0.2	0.1	0.1	tr	0.1
	16:0	47.2	48.1	50.2	48.0	47.4	46.5
	16:1	3.7	3.0	3.6	4.1	5.5	5.8
	18:0	1.3	1.4	0.8	1.0	0.6	0.7
	18:1	3.3	2.3	1.5	2.8	2.2	2.0
	18:2	8.8	7.9	8.3	12.8	12.7	8.8
	18:3 (γ)	32.6	33.2	32.6	28.4	29.4	33.3
Oscillatoria rubescens	12:0	+	+	0.6	0.1	0.1	0.2
	14:0			0.3	2.0	0.1	0.1
	14:1			0.1	tr	tr	tr
	16:0			27.4	26.2	23.2	24.3
	16:1			13.8	15.2	13.0	14.3
	16:2			0.2	0.2	0.9	0.3
	16:0			2.5	1.4	1.8	1.2
	18:1			2.4	1.9	3.1	3.3
	18:2			9.0	11.2	9.2	12.3
	18:3 (α)			43.8	43.6	48.5	43.9
Spirulina platensis	10:0	+	1.1	3.9	1.6	1.8	0.8
	12:0		tr	tr	tr	tr	tr
	14:0		0.5	07	0.4	0.3	0.5
	14:1		0.6	0.5	0.4	0.2	1.8
	15:0		tr	tr	tr	tr	tr
	16:0		38.5	36.0	34.7	37.5	36.5
	16:1		9.9	9.5	9.0	11.6	8.7
	16:2		0.6	0.4	0.2	0.2	0.4
	18:0		1.2	0.5	0.4	0.3	1.1
	18:1		3.9	5.2	3.9	3.4	7.7
	18:2		19.5	20.8	24.2	26.3	23.0
	18:3(γ)		24.2	22.2	24.9	18.3	18.7

tr, Trace amount.
+, The alga could not be grown at this nitrate concentration.

already been reported in our previous work on lipids and fatty acids in freshwater microalgae of the Chlorophyceae (several species of *Chlorella*, and *Bracteacoccusminor*) [37] and Euglenophyceae (*Euglena gracilis*) [38–40] as well as in macroscopic marine algae of the Chlorophyceae (*Enteromorpha linza*) [43], Phaeophyceae (*Fucus vesiculosus*) [41, 43] and Rhodophyceae (*Phycodrys sinuosa*) [41, 42].

Richardson *et al.* [82] grew two unicellular algae (*Chlorella sorokiniana* and *Oocystis polymorpha*) at rather high N concentrations (*ca* 3000–20000 μmol N/l) and did not observe modifications in the lipids and fatty acids. Our investigations indicate that such modifications occur only at lower N concentrations (*ca* 300–600 μmol N/l).

According to Kanazawa *et al.* [83, 84] the primary effect of increased concentrations of nitrogen (ammonia) on photosynthesizing cells of *Chlorella vulgaris* appears to be the increased formation of amino acids at the expense of sucrose synthesis. Similar results have been obtained with isolated mesophyll cells of *Papaver somniferum* [85] and with leaf discs of *Medicago sativa* [86].

However, at present there exists no direct explanation for the nitrogen effect on the metabolism of lipids and fatty acids in algae. The polar lipids and polyunsaturated fatty acids, which increase at higher nitrogen concentrations, are mostly located in the chloroplasts. The nitrogen effect is possibly a secondary one: chloroplasts are reported to have high protein contents [96, 97]. We recently

analysed cells of *Chlorella vulgaris* grown with 0.03% KNO_3 and discovered that the chloroplasts contained 40% protein, while the whole cells had an average of only 27% protein (paper in preparation). If one considers that chloroplasts lose stromal proteins during the isolation procedure, the actual chloroplast protein value should be even greater. With decreasing nitrogen levels the chlorophyll contents of the cells drop, indicating a rapid reduction or even breakdown of the whole chloroplast apparatus. During this process the chloroplast lipids and fatty acids also appear to be catabolized. The mechanisms of this process are as yet unknown. In contrast to all of the algae so far examined, the blue-green algae did not reveal any significant changes in the percentage and composition of their lipids and fatty acids, when they were grown at different concentrations of nitrogen. Moreover, they produced no detectable amounts of triacylglycerols. In essence, our results indicate that by varying the nitrogen concentrations of the nutrient media in mass culture experiments, blue-green algae can be manipulated with regard to their biomass production and protein contents but not with respect to the contents and composition of their lipids and fatty acids.

As described above, the regulation of N concentrations can control the metabolism of lipids and fatty acids in various green, red and brown algae. All these algae are eukaryotes. There was, however, no effect of nitrogen on the metabolism of lipids and fatty acids in blue-green algae, which are prokaryotes. Hence, our data may be the first indication of a hitherto unrecognized distinction between prokaryotic and eukaryotic organisms in their lipid and fatty acid metabolism. This will be studied more thoroughly in further investigations.

EXPERIMENTAL

Organisms. Chlorella vulgaris Beijerinck and *Scenedesmus obliquus* (Turp.) Krüger were obtained from the Pflanzenphysiologisches Institut, University of Göttingen, West Germany. Acquired from the Centre of Algae and Protozoa, Cambridge, Great Britain were *Anacystis nidulans* (Richt.) Dr., *Microcystis aeruginosa* Kützing and *Spirulina platensis* (Nordstedt) Geitler, while *Oscillatoria rubescens* DC. was generously donated by Dr. E. Meffert, Max Planck-Institut für Limnologie, Plön, West Germany [87].

Nutrient media. (a) For *Chlorella vulgaris, Scenedesmus obliquus, Anacystis nidulans* and *Microcystis aeruginosa* the medium applied was the same as that described previously [88] with slight modifications and containing varying concns of NH_4Cl or KNO_3. NaCl: 0.3 g/l; $MgSO_4 \cdot 7H_2O$: 0.2 g/l; $CaCl_2 \cdot 2H_2O$: 0.04 g/l; $K_2HPO_4 \cdot 3H_2O$: 0.2 g/l; trace elements: $MnCl_2 \cdot H_2O$: 1.5 mg/l; H_3BO_3: 0.1 mg/l; $ZnSO_4 \cdot 7H_2O$: 0.1 mg/l; $CoSO_4 \cdot 7H_2O$: 0.1 mg/l; $Na_2MoO_4 \cdot 2H_2O$: 0.1 mg/l; $CuSO_4 \cdot 5H_2O$: 0.01 mg/l; $FeCl_3 \cdot 6H_2O$: 4.0 mg/l; $Na_2EDTA \cdot H_2O$: 5.5 mg/l; NH_4Cl: 0.003–0.3 g/l; KNO_3: 0.003–1 g/l.

(b) *Oscillatoria rubescen* was grown in a modified medium according to Staub [89]: $CaCl_2 \cdot 2H_2O$: 45 mg/l; K_2HPO_4: 31 mg/l; $MgSO_4 \cdot 7H_2O$: 25 mg/l; Na_2CO_3: 21 mg/l; trace elements as for the green algae (see above); KNO_3: 0.03–1 g/l.

(c) *Spirulina platenis* was grown in the medium prescried by Ogawa and Terui [25] with varying amounts of KNO_3 (0.01–1 g/l).

Growth conditons. All allgae were grown under bacteria-free conditions and continuous aeration in 8 l. batch cultures at light intensities of about 800 lux and 22°. Growth of the algae was monitored by spectral measurements of the algal suspensions at 440 nm. After reaching the stationary phase of growth the algae were centrifuged, freeze-dried, weighed and stored under nitrogen at −24°.

Lipid extraction. The dried algae were ground with sea sand and extraction proceded three times (2 hr each with $CHCl_3$–MeOH, 2:1) under N_2 and during continual shaking at room temp. The major portion of the solvent was removed through evaporation under N_2 at 40°. The lipids were then transfered in $CHCl_3$ to preweighed test tubes, concd in N_2 and dried to a constant weight in a vacuum desiccator. The total lipid conglomerate was dissolved in $CHCl_3$ to obtain a standard lipid solution of exactly 10 mg total lipid per 1.0 ml solvent.

Thin layer chromatography. Precoated plates (silica gel 60-Merck) were used for qualitative investigations. For quantitative analyses the plates (20 × 20 cm) were prepared in the laboratory (35.0 g TLC-silica gel 60H-Merck/80.0 ml distilled H_2O; thickness: 0.25 mm). After activation for 2 hr at 120°, the plates were stored over P_2O_5. Solvent systems: (a) Me_2CO–C_6H_6–H_2O* (91:30:8) [90] (TLC of the total lipids and the polar lipids); (b) $CHCl_3$–MeOH–H_2O (65:25:4; polar lipids); (c) hexane–C_6H_6–Et_2O (80:20:2; neutral lipids); (d) hexane–Et_2O–HOAc (90:25:2; neutral lipids). The lipids were identified by cochromatography with reference samples. After the completion of TLC the lipids were sprayed with an alkaline soln of Rhodamin 6 G (equal vols of 0.006% Rhodamin 6 G in H_2O and of 8% NaOH) [91] and visualized in $UV_{365\,nm}$.

Column chromatography. Separation of the total lipids into neutral and polar lipids was achieved through CC [92]. A glass column (30 cm × 1.5 cm inner diameter) was filled with a suspension of silica gel 60 (Merck) and Hyflo-supercel (Macherey & Nagel) (1:1) in $CHCl_3$ [93]. About 50 mg of the total lipid sample were transferred to the column and elution of the neutral fraction was first performed with $CHCl_3$. This fraction also contained all of the pigments. Subsequently, MeOH was used to obtain the polar fraction.

Preparation of the fatty acid methyl esters. The fatty acid methyl esters of the total, neutral and polar lipids were obtained by transmethylation with NaOMe under N_2 [94]. Free fatty acids were not present. For GC, 17:0, 19:0 or 20:0 fatty acid methyl esters were added as internal standards.

Gas chromatography. Flame ionisation detector (FID). Column: stainless steel capillary column (~ 50 m) with Silar 10C. Column temp.: 175°. Injector and detector temp.: 250°. Flow rate: 1 ml N_2/min. Split ratio: 1:150.

Determination of total protein. The content of total N_2 in the freeze-dried algae was determined according to the Kjeldahl method. Protein content was calculated using a factor of 6.25.

Determination of chlorophylls. The determination of the chlorophylls content of the total lipids was carried out according to Arnon [95].

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*Because the solvent system contained benzene, TLC was conducted under a hood. The separations were somewhat less distinct when toluene or xylene was substituted for benzene.

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