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Growth of and Omega-3 Fatty Acid Production by *Phaeodactylum tricornutum* under Different Culture Conditions

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Detailed studies were carried out on the effects of nitrogen source, phosphate, sodium chloride, growth factors, precursors, CO_2, temperature, initial pH, and inoculum size on biomass and eicosapentaenoic acid (EPA) production by *Phaeodactylum tricornutum*. The EPA content of total fatty acids increased with increasing concentrations of nitrate and urea. Sodium chloride was not required for growth or EPA production. While vitamins B1 and B12 did not affect growth significantly, EPA yield was increased by 65% by B12 supplementation. Maximum EPA production occurred when the air gassing supply was supplemented with 1% CO_2. Optimum culture temperature and initial pH for EPA production were 21.5 to 23°C and 7.6, respectively. EPA yields of up to 133 mg/liter of culture were observed. EPA constituted up to 30 to 40% of total fatty acids.

Recognition of the effects of omega-3 fatty acids, eicosapentaenoic acid (EPA) and docohexaenoic acid (DHA), in human health resulting from the pioneering work of Dyerberg et al. (14) created the momentum for extensive nutritional and pharmacological studies on the effects of these substances in human physiology. Observed beneficial physiological effects suggest that omega-3 fatty acids have potential uses for prevention or treatment of medical disorders in three areas: heart and circulatory (13, 29), inflammatory (24, 36), and cancer (6, 34). While many of the effects of omega-3 fatty acids were noted in animal or human subjects fed on diets of fish oils, which contain mixtures of EPA and DHA and other fatty acids, some evidence indicated that EPA and DHA have different physiological impacts (12, 37).

Although the current conventional sources of omega-3 fatty acids are fish and fish oils, the acids originate in marine microorganisms upon which the fish feed (1, 43). Supply of omega-3 fatty acids from fish is unlikely to meet future requirements and, in any case, separation of EPA from DHA in fish oils is difficult to achieve on a processing scale (15, 17). Consequently, alternative sources of EPA and DHA are being sought, especially from algae (5, 30).

TABLE 1. Effect of nitrate, ammonia, and urea on fatty acid profile and EPA production by *P. tricornutum*

Medium	Cell dry wt (g/liter)	Culture final pH	Fatty acid production (mg/g of cell dry wt)					Total fatty acid production		EPA production	
			16:0	16:1	18:1	20:5	Others[a]	mg/g of cell dry wt	mg/liter of culture broth	% of total fatty acids	mg/liter of culture broth
Without Tris											
Control	0.4	6.8	89.1	140.0	17.4	29.4	39.4	315.3	156.2	9.3	11.9
Nitrate	2.3	7.6	11.0	29.1	2.2	31.8	37.1	111.3	252.1	28.6	72.1
NH_4^+	—[b]	—	—	—	—	—	—	—	—	—	—
Urea	2.6	5.7	12.9	31.6	3.0	35.7	39.4	122.7	310.8	29.1	92.1
With Tris											
Control	0.6	7.4	43.3	76.1	12.8	18.9	30.3	181.5	101.2	10.5	10.6
Nitrate	2.6	8.1	9.6	28.9	1.2	24.6	33.5	97.8	251.2	25.2	63.2
NH_4^+	1.7	5.3	32.4	53.6	13.4	15.9	42.3	157.7	275.0	10.1	27.7
Urea	2.8	7.4	11.6	25.7	2.7	37.8	41.3	119.1	337.8	31.8	107.3

[a] Including 14:0, 18:0, 18:2, 18:3, 20:4, 22:6, and some other unidentified fatty acids.
[b] Culture died after 3 days of cultivation.

Following an extensive screening of algae obtained from culture collections and newly isolated cultures, we identified *Phaeodactylum tricornutum* UTEX 640 as a high EPA-producing strain. *P. tricornutum* belongs to the *Bacillariophyceae* (diatoms), formerly classified as *Nitzschia closterium* (27). Our strain was the only freshwater strain of the species held in the University of Texas at Austin Collection (UTEX). The most notable characteristics of this strain were its ability to produce a high proportion of its fatty acids as EPA and its capacity to grow to high cell densities. From a downstream processing perspective, this strain has an addi-

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TABLE 2. Effect of nitrate concentration on fatty acid and EPA production by *P. tricornutum*

Nitrate concn (g/liter)	Culture dry wt (g/liter)	Culture final pH	Fatty acid production (mg/g of cell dry wt)					Total fatty acid production		EPA production	
			16:0	16:1	18:1	20:5	Others	mg/g of cell dry wt	mg/liter of culture broth	% of total fatty acids	mg/liter of culture broth
0.25	1.4	7.1	40.8	66.0	6.0	14.3	28.9	156.0	221.0	9.1	20.2
0.50	2.1	7.2	27.6	49.8	4.4	10.2	25.4	117.4	251.1	8.7	21.8
0.75	2.5	7.4	15.4	36.2	4.0	16.7	25.6	97.9	241.0	17.1	41.1
1.00	2.7	7.6	10.5	25.7	2.5	31.1	36.7	106.8	287.1	29.5	84.9
1.50	2.6	7.6	9.6	23.8	2.1	34.5	37.4	107.4	279.2	32.1	89.7

tional advantage in that it produces little DHA. Thus, the potential problem of separating DHA from EPA does not arise.

Studies were carried out on the effects of a variety of nutritional and physical factors on growth and EPA production by *P. tricornutum* UTEX 640, and the results are presented in this report.

MATERIALS AND METHODS

Organism. *P. tricornutum* UTEX 640 was obtained from the Culture Collection of Algae at the University of Texas at Austin. The culture was maintained by subculturing every 2 months on slant agar containing the Mann and Myers (28) medium and kept at 20°C with provision of light from a fluorescent lamp.

Culture conditions. *P. tricornutum* was grown in Mann and Myers (28) medium in test tubes (25 by 250 mm; 100-ml capacity) with a working volume of 75 ml. Culture tubes were closed by double-bored rubber stoppers for gas inlet and outlet where glass-wool filters were connected to minimize contaminations. Sterilized medium was inoculated with a 7-day-old inoculum to reach an initial optical density at 600 nm of 0.5 in a spectrophotometer with a 1-cm light path (or as otherwise stated). The culture tubes were incubated in a water bath maintained at 20°C. Air, supplemented with 5% CO_2, was bubbled at the rate of 75 ml/min (approximately 1 vol/vol/min). Light, adjusted to ca. 4,000 lx, was supplied from double fluorescent lamps (GRO-LUX, Sylvania, F40712-GRO-WS). The photoperiod was set at 16 h of light to 8 h of dark simulating the natural light cycle. Cultures were maintained for 7 days or as otherwise stated and then were analyzed for cell mass, lipid content, and fatty acid composition. All culture experiments and treatments were repeated at least twice, and excellent reproducibility was demonstrated.

Determination of cell mass. Fully grown cultures (50-ml volumes) were filtered through 0.8-μm membrane filters and washed twice with 50 ml of saline solution. Cells were dried at 60°C to constant weight.

Extraction and analysis of lipids. Lipid components were extracted by modified method of Bligh and Dyer (4). Lipid extracts were transmethylated by the method described by Holub and Skeaff (21). The methyl esters were analyzed in a gas chromatograph (Shimadzu GC-14A) equipped with a flame ionization detector and connected to an integrator (Shimadzu Chromatopac C-R6A). The column was a fused silica megabore, 30 m long and 0.52-mm inside diameter, coated with 1-μm thickness of 25% cyanopropyl–25% phenyl–50% methyl polysiloxane (Durabon 225; Chromatographic Specialities, Brockville, Ontario, Canada). Analysis conditions were 210°C column temperature, 250°C injection and detector temperatures, helium as carrier gas, and pentanoic acid (C15:1) as internal standard.

RESULTS

P. tricornutum can use a variety of nitrogen sources, including ammonium, nitrate, urea, and other organic sources (35). The effect of nitrogen source on growth and fatty acid production by *P. tricornutum* was investigated with sodium nitrate, ammonium chloride, and urea as sole nitrogen sources in the medium of Mann and Myers (28) with and without inclusion of 1 g of Tris per liter. Nitrogen sources were incorporated into the medium at equimolar concentrations (11.8 mmol of nitrogen per liter). The results are presented in Table 1. High biomass production was observed with urea or nitrate as nitrogen source. pH in ammonium-containing cultures tended to drop as a result of ammonium ion assimilation. The lower biomass values observed in media containing ammonia were attributed to the reduced pH. Assimilation of nitrate which is reduced to ammonium ion tended to cause the culture pH to rise. Incorporation of Tris into the medium provided a buffering effect against the tendencies of nitrate and ammonium to cause shifts in culture pH. In cultures containing nitrate or urea, 92 to 107 mg of EPA was produced per liter of culture broth, representing 29 to 32% of cellular total fatty acid content. The effects of nitrate concentration on fatty acid production and EPA synthesis by *P. tricornutum* are presented in Table 2. Highest algal growth was observed at

TABLE 3. Effect of urea concentration on fatty acid and EPA production by *P. tricornutum*

Urea concn (g/liter)	Culture dry wt (g/liter)	Culture final pH	Fatty acid production (mg/g of cell dry wt)					Total fatty acid production		EPA production	
			16:0	16:1	18:1	20:5	Others	mg/g of cell dry wt	mg/liter of culture broth	% of total fatty acids	mg/liter of culture broth
0.35	2.4	7.3	33.6	66.4	7.9	21.8	30.8	160.5	388.6	13.6	52.7
0.7	3.0	7.2	13.4	31.4	3.5	32.2	37.1	117.5	351.8	27.3	96.4
1.4	3.1	7.5	11.4	26.0	2.2	38.0	39.5	117.1	362.3	32.4	117.5
3.5	2.7	8.0	9.9	26.9	2.5	31.7	33.3	104.3	282.1	30.1	85.8
7.0	1.9	8.2	8.7	18.2	1.8	18.0	24.1	70.8	137.8	25.0	34.9

TABLE 4. Effect of phosphate concentration on production of fatty acids and EPA by *P. tricornutum*

Phosphate concn (g/liter)	Concn as phosphorus (mg/liter)	Culture dry wt (g/liter)	Culture final pH	Fatty acid production (mg/g of cell dry wt)					Total fatty acid production		EPA production	
				16:0	16:1	18:1	20:5	Others	mg/g of cell dry wt	mg/liter of culture broth	% of total fatty acids	mg/liter of culture broth
0.05	8.9	3.0	7.6	15.3	39.4	4.0	22.6	30.7	112.0	330.8	20.1	66.7
0.075	13.3	3.0	7.7	14.7	35.4	2.7	21.1	29.6	103.4	312.5	20.1	63.7
0.1	17.8	3.1	7.5	13.3	34.5	3.3	32.3	35.8	119.2	368.8	27.1	100.0
0.25	44.5	3.2	7.6	12.9	31.7	2.7	31.0	33.9	112.3	359.0	27.6	99.4
0.5	88.9	3.2	7.6	13.3	31.0	2.5	32.4	34.7	113.8	364.7	28.4	103.8

nitrate concentrations of 1.0 to 1.5 g/liter, with the latter value resulting in highest EPA production per liter of culture. In related studies, it was observed that nitrate levels in excess of 1.5 g/liter reduced biomass dry weight and EPA production. The effects of urea concentration on fatty acid synthesis and EPA production were also investigated. The results are presented in Table 3. A urea concentration of 1.4 g/liter resulted in highest biomass and EPA production: 117.5 mg of EPA was produced per liter of culture broth in this case, which represented 32% of the total cellular fatty acids. In Tables 1, 2, and 3, highest total fatty acid content of cells was always observed at the lowest nitrogen concentration.

The effect of phosphate on production of fatty acids and EPA was investigated by varying the concentration of K_2HPO_4 in the growth medium. Cultures containing >0.5 g of K_2HPO_4 per liter were unable to grow. The biomass data and fatty acid profiles observed following cultivation with phosphate concentrations of 0.05 to 0.5 g/liter (8.9 to 88.9 mg of phosphate per liter) are presented in Table 4. While phosphate concentration in this range had little effect on biomass production, optimal EPA levels were observed with phosphate levels of 0.1 to 0.5 g/liter (17.8 to 88.9 mg of phosphate per liter).

Sodium chloride concentrations ranging from the low levels observed in fresh water to those occurring in seawater were incorporated into the culture medium to test the effect on growth and fatty acid production (Table 5). While a slight decrease in biomass production was noted with increasing sodium chloride concentration, EPA level as a percentage of total cellular fatty acids and per unit volume of culture broth was substantially reduced at salt concentrations above 5 g/liter.

The effect of other medium components on patterns of growth and fatty acid production were also investigated. Magnesium sulfate concentrations in the range of 0.5 to 5.0 g/liter promoted optimum biomass production, and EPA levels did not vary significantly over this concentration range. Studies were carried out on the influence of vitamins B_1 and B_{12} on fatty acid production patterns. Neither vitamin was essential for growth and B_1 did not enhance EPA production (Table 6). However, supplementation with B_{12} enhanced EPA productivity. Supplementation with 100 ng of vitamin B_{12} per liter produced a 65% increase in yield of EPA per liter of culture broth compared with the control. Studies on the effects of supplementation of the medium with silica are summarized in Table 7. Although the organism is a diatom, silica concentrations in the range of 0 to 100 mg/liter had little effect on growth. Higher silica concentrations appeared to be toxic, resulting in reduced growth. Indeed, it was noted that cells from media having higher silica levels had a yellowish color rather than the typical dark brown appearance, suggesting abnormal growth. Increasing silica concentrations reduced EPA productivity per unit volume of culture broth. EPA yields per liter of culture broths in media supplemented with 100 to 500 mg of silica per liter were <50% of the values observed with the control.

Oleic acid was incorporated into the culture medium as a possible precursor for synthesis of highly unsaturated fatty acids. The effects of oleic acid concentration on growth and cell fatty acid composition are summarized in Table 8. Oleic acids at concentrations of 2 g/liter were found to inhibit cell growth and synthesis of EPA.

The effect of culture gassing with carbon dioxide was investigated by supplementation of air with CO_2 in the range of 0 to 15% (vol/vol). The results are presented in Table 9. CO_2, apart from having any other physiological effect on the cells, caused a variation in culture pH. Maximum biomass and EPA production per unit volume of culture was ob-

TABLE 5. Effect of sodium chloride concentration on EPA production by *P. tricornutum*

Sodium chloride concn (g/liter)	Culture dry wt (g/liter)	Culture final pH	Total fatty acid production		EPA production		
			mg/g of cell dry wt	mg/liter of culture broth	% of total fatty acids	mg/g of cell dry wt	mg/liter of culture broth
0.0	3.1	7.5	108.0	334.4	39.0	33.4	103.4
1.0	3.1	7.5	104.3	326.1	38.5	32.6	101.9
5.0	3.0	7.4	107.1	319.6	37.6	31.5	93.8
12.0	2.9	7.3	88.2	256.3	29.8	62.3	21.4
24.0	2.8	7.2	83.8	230.4	24.3	17.1	46.9

TABLE 6. Effect of vitamins B_1 and B_{12} on EPA production by *P. tricornutum*

Vitamin	Concn per liter	Culture dry wt (g/liter)	Culture final pH	Total fatty acid production		EPA production		
				mg/g of cell dry wt	mg/liter of culture broth	% of total fatty acids	mg/g of cell dry wt	mg/liter of culture broth
Control	0.0	2.7	7.7	81.2	216.2	23.2	21.6	57.4
B_1	1 μg	2.6	7.8	84.4	215.0	23.1	22.3	56.7
	10 μg	2.0	7.7	85.6	225.7	23.6	23.0	60.7
B_{12}	10 mg	2.7	7.6	95.0	257.2	26.6	28.5	77.4
	10 mg	2.8	7.6	107.1	301.3	27.9	33.4	94.0

TABLE 7. Effect of silica concentration on growth and fatty acid and EPA production by *P. tricornutum*

Silica concn (mg/liter)	Culture dry wt (g/liter)	Culture final pH	Total fatty acid production		EPA production		
			mg/g of cell dry wt	mg/liter of culture broth	% of total fatty acids	mg/g of cell dry wt	mg/liter of culture broth
0	2.7	7.5	95.7	256.0	25.0	27.1	72.5
10	2.6	7.5	84.9	223.4	24.7	23.7	62.3
50	2.6	7.6	83.0	212.6	24.1	22.8	58.3
100	2.4	7.6	64.6	155.2	21.9	16.4	39.3
500	1.8	7.8	70.5	128.8	21.8	19.2	35.0

TABLE 9. Effect of CO_2 supplementation on growth and on production of fatty acid and EPA by *P. tricornutum*

CO_2 concn (mg/liter)	Culture dry wt (g/liter)	Culture final pH	Total fatty acid production		EPA production		
			mg/g of cell dry wt	mg/liter of culture broth	% of total fatty acids	mg/g of cell dry wt	mg/liter of culture broth
Control	0.5	9.3	81.1	42.5	27.3	22.1	11.6
1.0	2.5	7.7	113.1	278.3	31.4	35.5	87.5
3.0	1.8	7.0	120.6	214.2	25.2	30.4	54.0
5.0	1.1	6.8	180.2	188.4	25.8	46.5	48.6
10.0	0.5	6.6	168.5	88.4	18.7	31.5	16.5
15.0	0.3	6.3	129.4	39.1	19.4	25.1	7.6

served when the air was supplemented with 1% (vol/vol) CO_2.

The effect of temperature on production of biomass and EPA is illustrated in Fig. 1. Highest biomass and EPA production was observed at temperatures of 21.5 to 23°C.

The effect of initial pH on growth and fatty acid production were investigated, and the results are presented in Table 10. Good growth was observed in cultures having initial pH values of between 6.4 and 8.4, and EPA content in cells from these cultures ranged from 28.7 to 34.2 mg/g of dried cells. Optimum EPA production per unit volume of cell culture occurred when the initial pH was 7.6.

The relationship between inoculum size and biomass yield and fatty acid profiles were determined after a 7-day incubation. The inoculum size was varied to produce cultures having initial optical densities at 600 nm (1-cm light path) ranging from 0.1 to 2.0. While the increases in biomass dry weight with increased inoculum size were to be expected, the patterns of fatty acid production reveal interesting trends (Fig. 2). Total fatty acid and EPA contents per gram of dried cells from the culture with the highest inoculum were 55 and 114% higher, respectively, than the corresponding values for cells from the culture broth with the lowest inoculum.

DISCUSSION

Nitrogen content of the medium has been reported to affect the proportion of saturated to unsaturated fatty acids in many microorganisms. Under nitrogen stress, *Botryococcus braunii*, *Dunaliella bardawil*, and *Dunaliella salina* produced a higher percentage of EPA (3). In contrast, the proportion of polyunsaturated fatty acids in the freshwater algae *Scenedesmus* and *Chlorella* increased at high nitrogen concentrations (33), which is consistent with our studies. It was noted that the lipid content of *P. tricornutum* increased at low or limiting nitrogen levels (19, 27, 42).

The phosphorus requirements for optimal growth differ considerably from species to species. Studies on the optimal phosphate concentrations for the growth of diatoms and green algae under defined laboratory conditions revealed that concentrations below 50 μg of P per liter were limiting, those of about 20 mg/liter were inhibitory, and 0.1 to 2 mg/liter was optimum (9).

Seto et al. (38) showed that *Chlorella minutissima*, a marine alga, had maximum cell growth in media containing 0.2% NaCl and slightly lower extractable lipid, but a higher percentage of total fatty acid. As with *P. tricornutum*, the fatty acid composition was significantly affected by NaCl concentrations. As the concentration of NaCl in the medium increased (from 0 to 1%), the percentage of EPA of the cells increased.

Specific vitamin requirements are common among diatoms for vitamin B_{12} (16) and less common for thiamine (26). As was observed with *P. tricornutum* UTEX 640, the marine species of *P. tricornutum* was found to synthesize B_{12} and B_1 (8).

Silicate deprivation in cultures of the diatom *Cyclotella cryptica* yielded slightly increased total lipid contents but suppressed biosynthesis of polyunsaturated fatty acids (39). In comparison with other diatoms, *P. tricornutum* is weakly silicified and enough silicon dissolved from glass vessels in alkaline culture media fulfils its meager requirements (27).

The reduction in EPA content as a proportion of total fatty acids in media containing increasing oleic acid concentrations is primarily attributed to the cells' increased content of 16:0 and 16:1 fatty acids. Media containing free fatty acids have been reported to suppress biosynthesis of other fatty acids of microorganisms (25). However, addition of oleic,

TABLE 8. Effect of oleic acid on growth and on composition of fatty acid and EPA of *P. tricornutum*

Oleic acid concn (g/liter)	Culture dry wt (g/liter)	Culture final pH	Fatty acid production (mg/g of cell dry wt)					Total fatty acid production		EPA production	
			16:0	16:1	18:1	20:5	Others	mg/g of cell dry wt	mg/liter of culture broth	% of total fatty acids	mg/liter of culture broth
Control	2.6	7.7	9.3	21.7	2.5	28.0	33.0	94.5	246.0	29.7	72.9
0.5	2.6	7.8	10.3	24.1	2.8	31.4	35.6	104.2	271.4	30.1	81.7
1.0	2.6	8.0	10.4	26.5	3.0	28.2	33.8	102.2	262.2	27.6	72.4
5.0	2.2	7.9	18.1	45.8	7.1	30.0	35.9	136.8	306.7	21.9	67.2
10.0	1.6	7.8	42.7	77.3	30.7	21.7	44.9	217.2	355.2	10.0	35.4

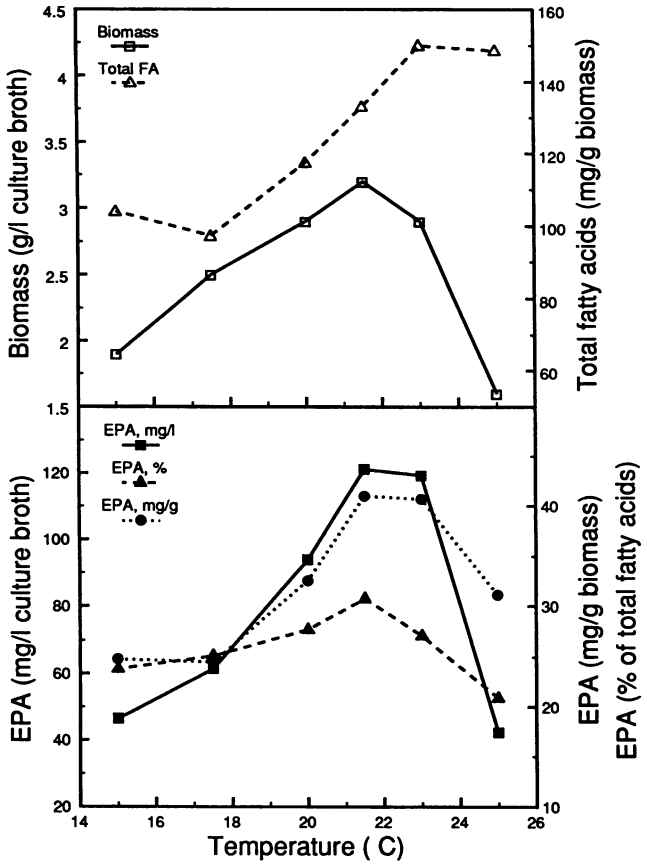

FIG. 1. Effect of temperature on growth and production of EPA and total fatty acids (FA) by *P. tricornutum*.

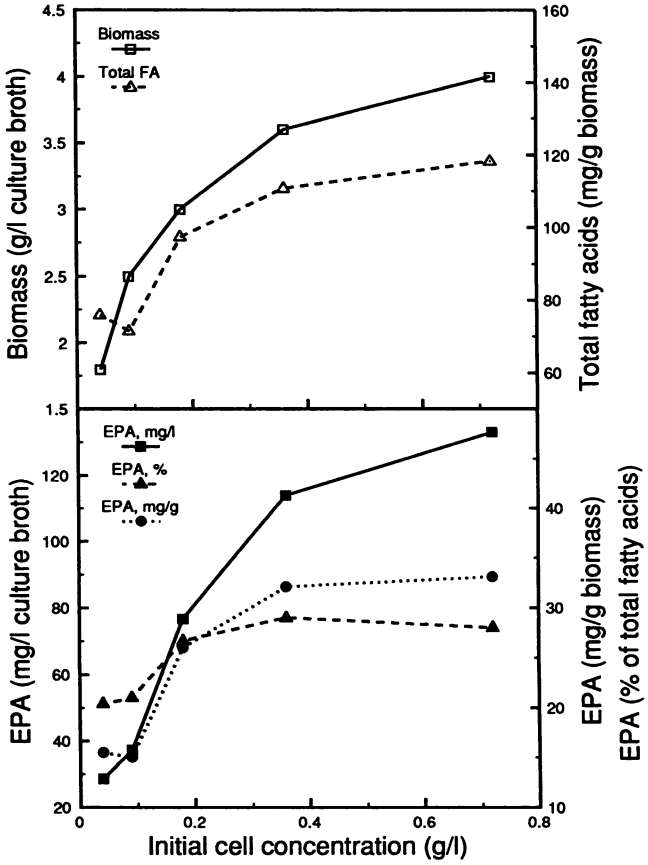

FIG. 2. Effect of initial cell concentration on growth and production of EPA and total fatty acids (FA) by *P. tricornutum*.

linoleic, or linolenic acid to cultures of *Euglena gracilis* enhanced production of polyunsaturated fatty acids, especially arachidonic acid and EPA (31).

The increased lipid content of *P. tricornutum* cells observed when the CO_2 concentration was raised in the air supply has been noted with autotrophically grown cells of *Chlorella fusea* (11).

Psychrophilic microorganisms with optimum growth temperatures below 20°C generally contain more highly unsaturated fatty acids than mesophiles, and thermophiles contain negligible amounts of polyunsaturated fatty acids (22, 41). Increased synthesis of unsaturated fatty acids at lower temperatures has been observed not only in eucaryotic algae (2, 32, 38), but also in bacteria (10, 22) and blue-green algae (20, 23), yeasts (7), and fungi (18, 40, 41). Seto et al. (38) observed that *Chlorella minutissima* only produced large amounts of EPA when incubated at low temperatures and suggested that the enzyme activities involved in desaturation and chain elongation might be highly thermolabile. The ability of strains to produce EPA only at low temperatures when growth rates are low is a disadvantage from a process productivity point of view. The capacity of *P. tricornutum* to produce maximum biomass and maximum EPA (as a percentage of total fatty acids and per unit dry cell weight) at the same relatively high optimum temperature is extremely advantageous from a processing perspective.

TABLE 10. Effect of initial pH on growth and EPA production by *P. tricornutum*

Initial pH	Cell dry wt (g/liter)	Culture final pH	Total fatty acid production		EPA production		
			mg/g of cell dry wt	mg/liter of culture broth	% of total fatty acids	mg/g of cell dry wt	mg/liter of culture broth
6.0	2.1	6.1	117.8	245.5	20.2	23.8	49.6
6.4	2.2	6.3	124.7	277.4	24.8	31.0	68.9
6.8	2.5	6.5	120.8	299.3	26.2	31.6	78.4
7.2	2.5	6.7	123.2	307.5	25.4	31.2	78.0
7.6	2.7	7.1	128.0	348.7	26.7	34.2	93.1
8.0	2.7	7.4	105.1	286.5	27.3	28.7	78.2
8.4	2.5	7.8	117.4	301.3	28.4	33.3	85.4
8.8	0.8	8.2	157.0	121.5	19.8	31.1	24.1

A comparison of data for optimal production of EPA by *P. tricornutum* UTEX 640 and the marine alga *Chlorella minutissima*, patented for EPA production by Nisshin Oil Mills, Japan, is presented in Table 11. Much higher biomass concentrations were observed with *P. tricornutum*, which resulted in an enhanced productivity of 19.0 mg of EPA produced per liter of culture per day.

The various factors noted above as having a positive influence on EPA productivity will now be taken into account in studies aimed at overall optimization culture conditions for EPA production by *P. tricornutum* UTEX 640.

TABLE 11. Comparison of EPA productivity of culture of *P. tricornutum* UTEX 640 and *Chlorella minutissima*

Cultures of:	Culture days	Dry cell concn (mg/liter)	Total fatty acids (mg/g of cell dry wt)	EPA (% of total fatty acids)	EPA yield (mg/liter of culture broth)	EPA productivity (mg/liter/day)
Chlorella minutissima[a]	4	379	79.7	39.9	12.05	3.01
P. tricornutum[b]	7	4,000	118.3	28.0	132.9	19.0

[a] Data from Seto et al. (38).
[b] Data from Fig. 2, with initial cell concentration of 0.72 g/liter.

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