THE EFFECT OF ENVIRONMENTAL CONDITIONS ON THE FATTY ACID COMPOSITION OF MICROALGAE AND CYANOBACTERIA

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ABSTRACT

A range of freshwater algae (Chlorella vulgaris 211/8K, Chlorella vulgaris 211/11c, Ankistrodesmus antarcticus 202/25, Scenedesmus obliquus 276/3A, Cyanidium caldarium 1355/4), marine and brackish algae (Nannochloris atomus 251/4B, Nannochloropsis oculata 849/1, Isochrysis galbana 927/1, Isochrysis sp. 927/14) and cyanobacteria (Anabaena flos-aquae 1403/13A, Anabaena variabilis 1403/12, Synechococcus sp. 1479/5, Synechococcus sp. PCC 7943) were grown in batch culture at initial nitrogen levels of 5, 25, 50 and 500 mg NO_3-N l^{-1} or NH_4-N l^{-1} (C. caldarium only) at three growth temperatures. Cultures were harvested in exponential and stationary growth phases. Protein, carbohydrate, lipid and fatty acid contents were determined.

All the algae and cyanobacteria investigated exhibited changes in cellular content of protein, carbohydrate and lipid in relation to changes in temperature, nitrogen availability and growth phase. C. vulgaris 211/8K, C. vulgaris 211/11c, N. atomus, and the cyanobacteria all exhibited a major shift to carbohydrate accumulation at stationary phase and with decrease in growth temperature, with the exception of the cyanobacteria which did not exhibit a uniform response to temperature. Ank. antarcticus and S. obliquus exhibited major shifts to lipid accumulation with decrease in temperature and at stationary growth phase. Protein contents of the cyanobacteria increased at stationary phase in contrast to the decrease at stationary phase observed in the freshwater, marine and brackish algae. Carbohydrate, protein and lipid contents were all found to depend on previous nitrate availability in the cultures.

The marine and brackish species showed a much broader range of fatty acids (C12 - C22) than the freshwater algae (predominantly C16 and C18) and cyanobacteria (predominantly C14, C16, C18). Quantitative changes in individual fatty acids rather than qualitative changes were found with temperature changes and growth phase. The degree of unsaturation decreased with decrease in temperature in the marine and brackish species in contrast to the increase in unsaturation observed with the freshwater algae and cyanobacteria.

Based on the results of the laboratory work, six algae and cyanobacteria - C. vulgaris 211/8K, S. obliquus, N. atomus, Isochrysis sp., A. flos-aquae, Synechococcus sp. PCC 7943 - were grown outdoors in a slurry based minipond system. All the species chosen grew successfully in algal treated slurry, with preferential uptake of ammonium-N before nitrite-N and nitrate-N. The algae behaved similarly outdoors in defined media and algal treated slurry to the laboratory based growth in relation to cellular content changes.

Manipulation of specific cell constituents in a slurry based system would improve the economics of algal wastewater treatment, the resultant biomass having economic potential. The interest in algal fatty acid content manipulation would probably only be in the aquaculture field, and not from medical or health food areas due to health hazards associated with sewage. Carbohydrate accumulating algae would also be of interest to the aquaculture field. The current high cost of production of algal feeds has spurred the search for alternative algae production, and it is suggested that growth in slurry with nitrogen depletion to optimise lipid, carbohydrate or specific component fatty acid production maybe an alternative.

CONTENTS

CONTENTS (cont)

CONTENTS (cont)

CONTENTS (cont)

LIST OF PLATES

LIST OF TABLES

LIST OF TABLES (cont)

LIST OF TABLES (cont)

LIST OF TABLES (cont)

LIST OF FIGURES

LIST OF FIGURES (cont)

LIST OF FIGURES (cont)

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1. INTRODUCTION

1.1 ALGAL AND CYANOBACTERIAL FATTY ACIDS

Microalgae and cyanobacteria can contain significant quantities of lipids (Table 1) which exhibit a wide range of constituent fatty acids (Table 2). This area has been the subject of a number of reviews, some concerned with all microalgal groups (Cobelas and Lechado, 1989; Borowitzka, 1988; Wood, 1974) and others with selected groups (Cyanophyceae, Nichols, 1973; marine algae, Pohl and Zurheide, 1979). Virtually all the acids found are straight chain molecules containing an even number of carbon atoms in the range C14 - C22, saturated and polyunsaturated, nearly all in the cis configuration.

1.2 COMMERCIAL EXPLOITATION

Although a review of the literature shows microalgae to be a potentially new biological source of fatty acids, commercially they have remained relatively unexploited although interest has increased over the last decade. Occasional reports of commercial exploitation have appeared in the literature (Anon, 1986; Anon, 1988), mainly concerning Omega-3 fatty acids specifically EPA (Cyanotech Corporation, 1988). Patents have also appeared, again in relation to EPA (The Nisshin Oil Mills Ltd, 1986; Suntory Limited, 1988).

The interest in algal fatty acids comes from three main areas: medical, the food industry and the exploitation of mass cultured algae (aquaculture feeds and high value products).

The medical interest stems from observations that societies with diets containing a high fish oil content exhibit a low incidence of cardiovascular disease (Carroll, 1986). Much work has focused on the lipid portion of the diet because of earlier evidence that dietary fat can significantly influence serum cholesterol levels and artherosclerosis. Recent studies have homed in on polyunsaturated fatty acids in fish oils.

Table 1: Summary of the Range of Lipid Levels Reported in Various Micro-algae and the Distribution of These Lipids (after Borowitzka, M A 1988)

Algal Class	Total lipids (% dry weight)	% Of Total Lipid			Hydrocarbon (% dry weight)
		Neutral lipid	Glyco-lipid	Phospho-lipid	
Cyanophyceae	2-23	11-68	12-41	16-50	0.005-0.6
Chrysophyceae	12-72				
Prymnesiophyceae	5-48				
Cryptophyceae	3-17				0.0035
Xanthophyceae	6-16	44	17	39	2.8
Rhodophyceae		41-58	42-59		
Dinophyceae	5-36				0.004-0.2
Bacillariophyceae	1-39	14-60	13-44	10-47	0.2-0.7
Chlorophyceae	1-70	21-66	6-62	17-53	0.03-1.0 (39.0)[a]
Euglenophyceae	17				

[a]High value for Botryococcus braunii

Table 2: Relative Composition of Fatty Acids of Micro-algae[a] (after Borowitzka, M A 1988)

Fatty Acid	Doublebond position	Algal class[b]									
		1	2	3	4	5	6	7	8	9	10
14:0		4	2	1	2	2	1	2	2	1	2
16:0		6	2	2	3	3	3	4	3	3	2
16:1	9	5	3	3	2	4	1	3	5	2	1
16:2	6,9	2	1	1	Tr.	Tr.	-	1	1	1	1
16:2	9,12	(5)[c]	1	1	1		-	1	1	1	-
16:3	6,9,12		2	2	1	Tr.	-	2	2	2	-
16:4	6,9,12,15				1	Tr.	-		1	2	2
17:1		1									
18:0		3	1	1	1	1	2	1	1	1	1
18:1	9	4	2	3	1	2	3	2	1	5	1
18:2	9,12	4	3	2	2	1	2	1	1	4	2
18:3	6,9,12	3	2	Tr.	1	Tr.	1	1	1	3	-
18:3	9,12,15	4	1	2	3		1	1		4	4
18:4	6,9,12,15	3	1	2	5	1	-	2	1	2	-
20:0					1	Tr.		1		1	
20:1	11	1	1		2					2	
20:2	8,11		Tr.	Tr.		-		1	1	1	
20:3	8,11,14		1	1		1	1		1	1	
20:4	5,8,11,14		1	1	1	2	4		1	1	1
20:4	8,11,14,17		1	1	1			1	1		
20:5	5,8,11,13,17		3	3	2	3	2	2	3	1	
22:0										1	
22:1	11			1				1	1	1	
22:5	4,7,10,13,16									1	
22:5	7,10,13,16,19			2	1	1		1	Tr.	1	
22:6	4,7,10,13,16,19			1		1		3			

References Based on Wood (1974); Hinchcliffe & Riley (1972); Nichols et al. (1984); Becker & Venkatamaran (1982); Piorreck et al. (1984); Tornabene et al. (1985); Ben-Amotz et al. (1985); Oren et al. (1985)

[a]Values in table are: blank -not reported; Tr. - trace; - -absent; 1 - up to 10% of total fatty acids; 2 - up to 20% etc.

[b]The classes are (with number of strains examined in square brackets): (1) Cyanophycea [53]; (2) Chrysophyceae [6]; (3) Haptophyceae [5]; (4) Cryptophyceae [10]; (5) Xanthophyceae [2]; (6) Rhodophyceae [23]; (7) Dinophyceae [8]; (8) Bacillariophyceae [13]; (9) Chlorophyceae [21]; (10) Euglenophyceae [3].

[c]Present in only a few strains examined.

x 3-scale 4 nuts?

Polyunsaturated fish oils are very effective at lowering serum triglyceride and serum cholesterol levels (Herold and Kinsella, 1986). In addition, inhibition effects on blood clotting (Dyerberg, 1982; Dyerberg, 1986; Herold and Kinsella, 1986) reduce the risk of thrombosis, a major factor in heart attacks and strokes.

The reduction in serum triglycerides and cholesterol may be due to decreased production of very low density lipoproteins by the liver, possibly as a result of a decrease in triglyceride synthesis (Dyerberg, 1986; Norum & Drevon, 1986). The effects on blood clotting are most likely related to alterations in the production of different prostanoids from polyunsaturated fatty acids (Dyerberg, 1986; Norum & Drevon, 1986; Bunting et al, 1983).

The main polyunsaturated fatty acid in vegetable oil is linoleic acid (18:2(n-6)) which is converted to arachidonic acid (20:4(n-6)) in the body. Arachidonic acid is converted by blood platelets into thromboxane TXA_2, which causes constriction of blood vessels and aggregation of platelets leading to blood clotting. Arachidonic acid is also converted in blood vessel walls to prostacyclin PGI_2 which relaxes blood vessels and prevents aggregation of platelets. Balanced formation of these substances is thought to permit clotting to occur following wounding but prevent clotting during normal physiological conditions (Bunting et al, 1983).

Fish oils contain little linoleic acid, the main PUFA'S are eicosapentaenoic acid (EPA; 20:5(n-3)) and docosahexaenoic acid (22: 6:(n-3)). EPA is similar in structure to arachidonic acid and is thus a potential substrate for conversion to thromboxane TXA_3 and prostacyclin PGI_3. Since TXA_3 does not aggregate platelets as effectively as TXA_2, but PGI_3 is as effective as PGI_2, it could alter blood clotting abilities. EPA, however, is not a good substrate for TXA_3 synthesis but may compete with arachidonic acid to decrease TXA_2 synthesis whilst increasing or at least altering normal synthesis of prostacyclin (Dyerberg, 1986; Norum & Drevon, 1986; Bunting et al, 1983).

PUFA'S also serve as substrates for the formation of leukotrienes. Leukotrienes derived from EPA differ in their biological properties to arachidonic acid derivatives and this may also explain some beneficial effects of fish oils (Dyerberg, 1986).

Polyunsaturated fish oils have also been investigated with respect to other chronic diseases including cancer, hypertension, multiple sclerosis and rheumatoid arthritis (Carroll, 1986). Recent reviews in this area include coronary heart disease (Ballard - Barbash and Callaway, 1987; Gurr, 1992), chronic diseases (Simopoulos, 1991) and medical importance of gamma-linolenic acid (Horrobin, 1992).

This research has also stimulated interest in the food industry for health foods and dietary supplements. Polyunsaturated fatty acids traditionally consumed as components of fish oil are not synthesised de novo but are acquired from consumed phytoplankton. Therefore, there is considerable interest in algal derived polyunsaturated fatty acids. Potential benefits include the absence of less desirable fatty acids, absence of fish odour and security of supply. Also, because algae can be grown under controlled conditions, it may be possible to achieve a more consistent formulation of the product than with fish oils which vary with season and the environment (Anon, 1988).

Microalgae especially marine species can contain appreciable amounts of essential fatty acids such as linoleic (18:2(n-6)), γ - linolenic (18:3(n-6)), eicosapentaenoic (EPA, 20: 5(n-3)) and arachidonic (20: 4(n-6)) acids. These fatty acids are an essential component of the diet of humans and animals and are becoming important feed additives in aquaculture.

Mass culture of algae for biomass production has been very successful, however in the past the main focus was on single cell protein, but more recently many other potential applications have been advanced including waste water treatment, production of extractable products, and aquaculture feeds (Shelef and Soeder, 1980). Commercial exploitation of algal mass culture has been restricted to specific high value products eg β-carotene, specific algal species

eg Spirulina, Chlorella for the health food market and production of algae as aquaculture feed (Shelef and Soeder, 1980). Mass culture for waste treatment has also been successful, however the cost effectiveness of the system limits its use. However, the identification of high value products from algae would stimulate commercial interest in these waste treatment systems.

1.2.1 Algal Wastewater Treatment

The interest with respect to algal fatty acids and wastewater treatment arose from work carried out at The Scottish Agriculture College, (Auchincruive, Ayr) on the aerobic and photosynthetic treatment of animal slurries (Fallowfield et al, 1992).

The introduction of more intensive livestock husbandry techniques has led to collection of animal faeces and urine in the form of liquid slurry which ideally would be returned to the land. However, its volume poses problems in storage and disposal specifically in certain areas of the United Kingdom and large areas of Europe, where slurry production exceeds land area available for optimum application (Williams, 1988). Animal slurries are strong effluents and chronic pollution can result from: (i) the high Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) of these slurries deoxygenating receiving waters (ii) run off and leaching of nitrogen and phosphorus from land leading to eutrophication of surface waters (iii) atmospheric pollution caused by odour from the storage, handling and land application of slurry.

Although, it is clear that legislation for environmental protection is moving towards controlling the release of nutrients into the environment, and this will have a profound effect on alternative wastewater treatment systems, the economics of the system will improve if suitable algal products are found.

The system at the Scottish Agriculture College is shown schematically in Fig 1. Aerobic treatment is effective at removing high concentrations of the carbonaceous pollutants which comprise the BOD and COD of the waste and can control the final form of nitrogen within the treated animal slurry.

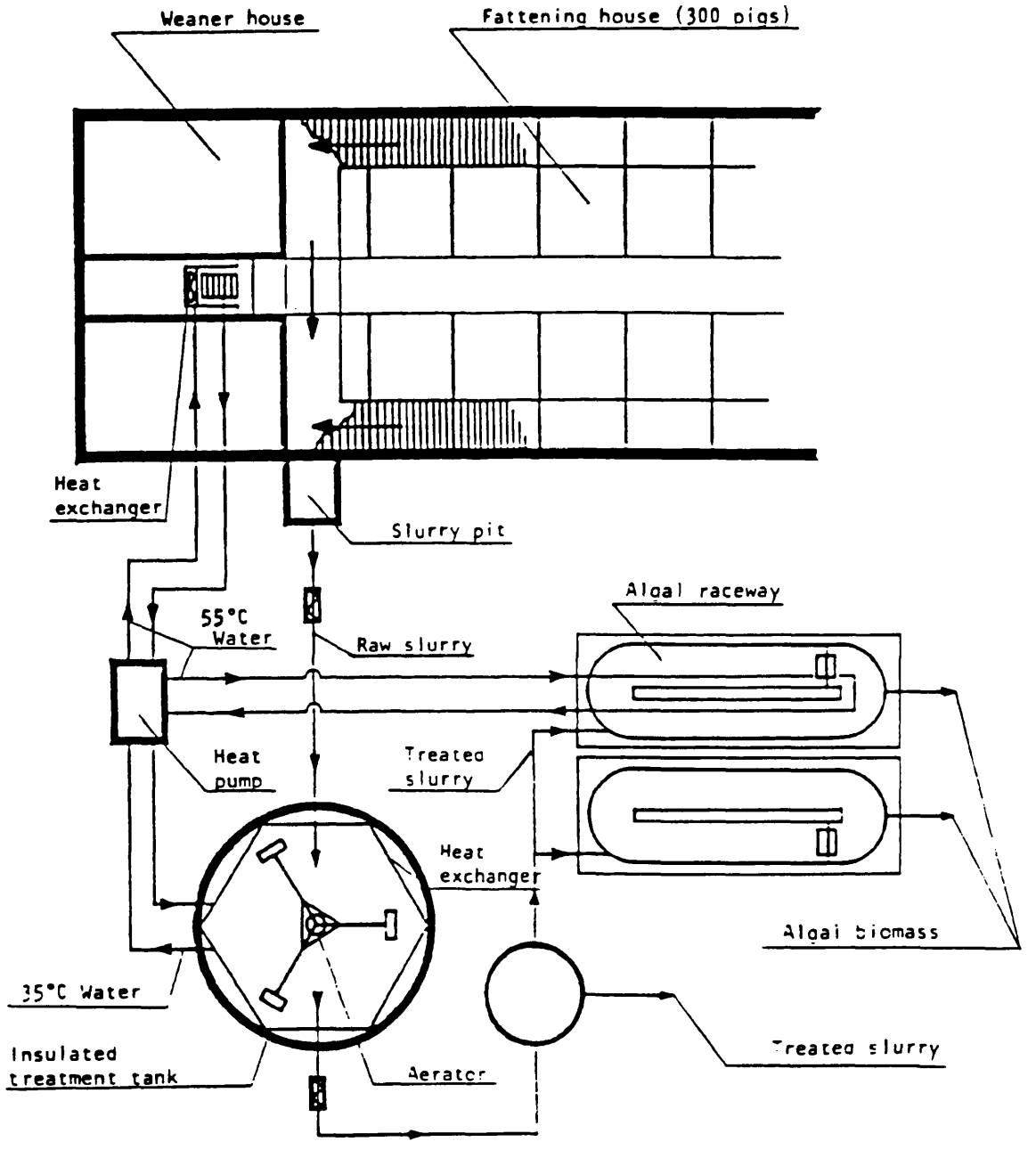


Fig 1: The Brickrow Farm Unit Piggery with integrated aerobic reactor and high rate algal ponds for the treatment of piggery wastes (after Fallowfield, 1992)

The treated waste contains residual BOD, nitrogen and phosphorus. The solid phase is relatively immobile, it is the liquid phase that is most likely to cause pollution problems after land spreading.

The high rate algal pond cannot be used to treat animal slurries without some pretreatment because high BOD/COD levels exceed the oxygenation capacity of the ponds. However, the aerobically treated, low solids content, liquid phase is suitable. The controlled growth of microalgae exploits the residual BOD as carbon source and removes significant quantities of the major inorganic pollutants, phosphorus and nitrogen prior to discharge.

The potential uses for algal biomass have been extensively reviewed (Shelef and Soeder, 1980; Borowitzka and Borowitzka, 1988; Lembi and Waaland, 1988; Cresswell, Reas and Shah, 1989). Product reclamation is a desirable objective for any treatment system, and although costs do enter into the equation, uses for the resultant algal biomass would eliminate the disposal problem.

Therefore, one of the objectives of this research project was to investigate whether laboratory observations could be emulated outdoors in a slurry based system.

1.3 MANIPULATION OF ALGAL FATTY ACIDS

Developments in the exploitation of algal fatty acids are likely to be a result of manipulations of physiological conditions to maximise the production of commercially important fatty acids. Environmental factors which affect composition include temperature, light, autotrophy/heterotrophy, nutrient limitation, growth phase, salinity and O_2/CO_2 environment (Table 3), (Cobelas, 1989).

Sato and Murata (1980) concluded that temperature was one of the most important environmental factors influencing the fatty acid composition of algae. The degree of unsaturation is usually found to be inversely correlated with

Table 3: Effect of some environmental factors on lipid (% of dry weight) of a range of micro-algae (after Borowitzka, M A 1988)

Environmental Variable	Organism	Variable	% lipid	Reference
Light intensity	Spirulina platensis	10 - 40 klx	4.2-6.2	Albitskaya et al. (1974)
	Phaeodactylum tricornutum	2.1 - 21 klx	20.8-22.8	Orcutt & Patterson (1975)
Temperature	Ochromonas danica	15°C - 30°C12	39.9-53.3	Aaronson (1973)
	Chlorella minutissima	20°C - 25°C	14.5-23.2	Seto et al. (1984)
Salinity	Botryococcus braunii	0 - 6%	36-51	Dubinsky et al. (1978)
CO_2 supply	Scenedesmus acutus	Low - high	10-12	Becker & Venkatamaran (1982)
Nutrition	Porphyridium cruentum	Autotrophic vs heterotrophic	3.8-11.4	Antia, Desai & Romilly (1970)
Senescence	Chlorella vulgaris	Young - old	22-28	Collyer & Fogg (1955)
	Scenedesmus obliquus	Young - old	19-32	Piorreck et al. (1984)
	Botryococcus braunii	Young - old	23-34	Belcher (1968)
	Euglena gracilis	Young - old	24-65	Piorreck et al. (1984)
	Navicula pelliculosa	Young - old	14.5-19	Piorreck et al. (1984)
	Phaeodactylum tricornutum	Young - old	24-29	Piorreck et al. (1984)
	Thallassiosira pseudonana	Young - old	7.8-21.6	Fisher & Schwarzenbach (1978)
	Anacystis nidulans	Young - old	14.4-10.2	Piorreck et al. (1984)
	Microcystis aeruginosa	Young - old	19.0-16.5	Piorreck et al. (1984)
	Desert blue-green no. 92	Young - old	9-26	Dubinsky et al. (1978)
	Chrysophyte F1	Log - stationary	26.6-58.5	Barclay et al. (1985)
	Amphora sp.	Log - stationary	11.7-25.7	Barclay et al. (1985)

growth temperature ie lower growth temperatures favouring unsaturation (Ackman et al, 1968; Lynch and Thompson, 1984). Lynch and Thompson (1984) suggest that acclimation to low temperature enhances acyl chain desaturation as a means of modifying membrane properties in response to low temperature stress.

Increased irradiance has been shown to enhance the formation of polyunsaturated fatty acids in Euglena gracilis and Chlorella vulgaris (Nichols, 1965; Constantopoulos and Bloch, 1967).

Materassi et al (1980) stated that photo heterotrophic growth on glucose resulted in an increase in the unsaturation of lipids in Scenedesmus. Barg (1943; cited Borowitzka, 1988) reported an increase in 'fat' accumulation in a range of freshwater and marine diatoms when cultured on glucose enriched media.

Probably the most studied limiting nutrient affecting fatty acid composition has been nitrogen. Most microalgal cells grown under nitrogen limitation have enhanced lipid levels (Table 4), however cyanobacteria appear to be little or non-affected. Nitrogen deficiency does not however appear to affect the level of unsaturation in a regular manner, different researchers reporting different responses (Cobelas, 1989). Other nutrient deficiencies may also lead to increased cell lipid content eg diatoms under silica limitation (Roessler, 1987; Shifrin and Chisholm, 1981).

Shaw (1966) stated that generally increasing culture age increased the saturated to unsaturated fatty acids ratio with some exceptions. However, it is often difficult to separate true ageing effects on microalgal lipids from nutrient deficiency effects since in batch cultures the age of a given culture is associated with nutrient conditions. However, interaction of growth conditions and culture age upon lipid content is best illustrated by the results of Piorreck and co-workers (Piorreck and Pohl, 1984; Piorreck et al, 1984) who found changes did occur amongst the green algae studied but not with the cyanobacteria investigated.

Table 4: Effects of N-limitation on the level of lipids in a range of micro-algae (after Borowitzka, M A 1988)

Alga	Lipid content (% dry weight)		Reference (refer to Borowitzka, 1988)
	+N	-N	
Cyanophyceae			
Spirulina platensis	21.8	11.2	1
Anacystis nidulans	14.8	14.3	1
Chlorophyceae			
Ankistrodesmus sp.	18.3	40.3	2
Botryococcus braunii	44.5	54.2	2
Chlamydomonas applanata	18.2	32.8	12
Chlorella pyrenoidosa	13.4	29.2	12
	10.0	70.0	6
	14.4	35.8	3
	20.0	86.0	11
Chlorella vulgaris (NH_4)	11.8	52.8	1
C. vulgaris (NO_3)	21.8	57.9	1
C. luteoviridis	17.5	28.8	10
C. capsulata	11.7	11.4	10
Dunaliella primolecta	23.1	16.6	5
D. salina (UTEX 200)	25.3	9.2	2
Nannochloris sp.	20.8	35.5	2
	20.2	47.8	12
Oocystis polymorpha	12.6	34.7	3
Ourococcus sp.	27.0	49.5	12
Scenedesmus obliquus (NH_4)	22.4	34.6	1
Tetraselmis suecica	23.4	14.6	5
Bacillariophyceae			
Cyclotella cryptica	23.0	36.8	12
Nitzschia palea	22.2	39.5	3
Phaeodactylum tricornutum	20.0	24.0	4
Skeletonema costatum	23.8	30.3	3
Thalassiosira weissflogii	22.2	24.0	12
Chrysophyceae			
Isochrysis sp. (UTEX 2307)	7.1	26.0	2
Isochrysis galbana	23.0	23.1	10
Monallanthus salina	40.8	72.2	3
Prymnesiophyceae			
Hymenomonas carterae	20.0	14.3	12
Cryptophyceae			
Cryptomonas rufescens	12.2	16.8	8
Eustigmatophyceae			
Monodus subterraneus	20.0	40.0	9
Rhodophyceae			
Porphyridium cruentum	98	176	7

Beach and Holz (1973) found a NaCl dependent inverse relationship between 18:1 and 22:6(n-3) in the triacylglycerols of a marine dinoflagellate Crypthecodinium cohnii. 18:1 fatty acid was high at high salinity (5.0% w/v NaCl) and 22:6(n-3) was high at low salinity (0.3% w/v NaCl). Seto et al (1984) also found fatty acid composition in Chlorella minutissima to be affected by salinity. As the concentration of salinity increased the percentage of 20:5(n-3) increased, whereas 16:0, 18:1(n-9) and 18:2(n-6) decreased.

Little work has been performed on the effects of O_2/CO_2 concentration. Hulanicka et al (1964) reported an increase in 18: 3(n-3) and 16:4 (n-3) under increased CO_2 tension in Euglena.

It was decided within this research project to investigate temperature, N-limitation and growth phase using a multifactorial approach, especially with respect to the outdoor slurry based experiments where light, heterotrophy, O_2 and CO_2 would be too expensive to control, and the removal of nutrients priority.

It was also very evident from the literature that different algal taxa responded differently to the various environmental factors. The fatty acid composition of green algae would seem to be more greatly affected by environmental changes than cyanobacteria. Therefore, green algae were chosen as an initial group for study, together with nitrogen-fixing and non-nitrogen fixing cyanobacteria and a range of brackish and marine species noted for their use in aquaculture.

1.4 OBJECTIVES

(i) To develop and utilise suitable growth systems for an investigation of the effects of environmental factors on algal growth.

(ii) To investigate and develop suitable methods for the analysis of algal growth.

(iii) To investigate the effects of nitrogen, temperature and phase of growth on the composition of algae from different algal taxa.

(iv) To utilise the results of the laboratory based investigation (iii) to

investigate outdoor algal growth utilising animal waste, specifically the formation of algal fatty acids as an alternative economic product for this system.

2. MATERIALS AND METHODS

2.1 ALGAE AND CYANOBACTERIA

All cultures were obtained from the culture collection of Algae and Protozoa (The Ferry House, Ambleside, Cumbria) with the exception of Synechococcus sp PCC 7943 (supplied by Peter Rowell, Dept. Biological Sciences, University of Dundee) and Cyanidium caldarium CCAP 1355/4 (supplied by Tom Ford, Royal Holloway and Bedford New College, University of London).

Four freshwater green algae:

(i) Chlorella vulgaris CCAP 211/8K
Sorokin's high temperature strain.

(ii) Chlorella vulgaris CCAP 211/11c
Previously studied for use in piggery waste treatment.

(iii) Scenedesmus obliquus CCAP 276/3A
A colonial green algae, widely used in physiological studies.

(iv) Ankistrodesmus antarcticus CCAP 202/25
An isolate of "green ice" in the Antarctic.

Four freshwater cyanobacteria:

(i) Anabaena flos-aquae CCAP 1403/13A

(ii) Anabaena variabilis CCAP 1403/12

(iii) Synechococcus sp CCAP 1479/5

(iv) Synechococcus sp PCC 7943

Two nitrogen fixing cyanobacteria ((i) and (ii) above) and two non nitrogen fixers ((iii) and (iv) above).

One brackish green algae:

(i) Nannochloris atomus CCAP 251/4B

Three marine chrysophytes:

(i) Nannochloropsis oculata CCAP 849/1

(ii) Isochrysis galbana CCAP 927/1

(iii) Isochrysis sp CCAP 927/14

The brackish and marine species were noted for their use in marine aquaculture.

Cyanidium caldarium CCAP 1355/4

A thermotolerant alga, which has been proposed as a "bridge alga" between Cyanophyta and Rhodophyta (Klein, 1970; Fredrick, 1976).

2.1.1 Algal and Cyanobacterial Stock Cultures

All strains were routinely subcultured on a monthly basis and incubated at room temperature on an orbital shaker (120 r.p.m.) at an irradiance of 70μmol $m^{-2}s^{-1}$.

All freshwater species were grown in ASM (Gorham et al, 1964):

ASM

ASM Stock Solutions

			gl^{-1}
No. 1	K_2HPO_4	.....	1.74
No. 2	$FeCl_3$	.....	0.032
	Ethylene diamine tetra acetic acid (or sodium salt) (EDTA).		
		.....	0.74
No.3	$MgCl_2$	.....	1.9
	$MgSO_4.7H_2O$	.....	4.9
	$CaCl_2.2H_2O$	.....	1.47
	NaCl	.....	5.85
Nitrate stock.	$NaNO_3$	.....	30.35

X line-up

			gl^{-1}
Trace elements stock.	$NaMO_4.2H_2O$	.....	0.504
	$CoCl_2.6H_2O$	.....	0.08
	$ZnSO_4.1OH_2O$	.....	0.088
	$MnCl_2.4H_2O$	.....	0.72

10ml. No. 1, 10 ml. No. 2, 10 ml. No. 3, 10 ml. Nitrate stock and 1 ml. Trace elements stock were mixed and made up to 1 litre with distilled water. pH was adjusted to 7.5 if necessary with dilute HCl or dilute NaOH. The media was then autoclaved for 15 mins at 121°C.

Marine and brackish species in **F/2** (Thompson et al, 1988):

F/2

F/2 Stock Solutions

	gl^{-1}
$NaNO_3$	0.075
$NaH_2PO_4.7H_2O$	0.00565
trace elements	1 ml stock solution
vitamin mix	1 ml stock solution
synthetic sea salt	33.6

Trace elements stock solution

	gl^{-1}
Na_2EDTA	4.36
$FeCl_3.6H_2O$	3.15
$CuSO_4.5H_2O$	0.01
$ZnSO_4.7H_2O$	0.022
$CoCl_2.6H_2O$	0.01
$MnCl_2.4H_2O$	0.18
$Na_2Mo0_4.2H_2O$	0.006

vitamin mix stock solution

	mg l^{-1}
vitamin B_{12}	0.5
vitamin B_1 (thiamine HC1)	100
biotin	0.5

The media was made up to 1 litre with distilled water, pH adjusted to 8.0 and autoclaved at 121°C/15 mins.

C. caldarium was grown in a specific media:

Culture Medium for C. caldarium

Stock Solutions

		g l^{-1}
1.	$(NH_4)_2SO_4$	150
2.	KH_2PO_4	30
3.	$MgSO_4.7H_2O$	30
4.	$CaCl_2.2H_2O$	2
5.	T.E.S. - Cyanidium	1.0ml
6.	*Fe-EDTA	0.5ml
7.	*H_2SO_4 - concentrated	1.0ml

Fe-EDTA

	g l^{-1}
Ethylene diamine tetra-acetic acid	33.4
$FeSO_4.7H_2O$	24.9

Heat to dissolve EDTA then add $FeSO_4$.

Aerate 1-2 hours.

T.E.S. - Cyanidium

	gl^{-1}
H_3BO_3	0.5
$MnCl_2.4H_2O$	0.43
$ZnSO_4.7H_2O$	0.05
$CuSO_4.5H_2O$	0.02
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.009
$CoCl_2.6H_2O$	0.008
NH_4VO_3	0.0045

Mix 10ml Soln. No. 1,2,3,4 and make up to 1 litre with distilled water. Add soln. 5, soln 6, soln 7 in volumes stated per litre. Final pH after autoclaving should be ≈ pH 1.8.

2.1.2 Experimental Media

2.1.2.1 Nitrogen limitation experiments

The sodium nitrate content of ASM and F/2 basal medium was adjusted to give concentrations of 5, 25, 50 and 500 mg NO_3-N l^{-1}. The level of ammonium sulphate was altered similarly for the experiments with C. caldarium.

2.1.2.2 Outdoor minipond experiments.

The level of nitrogen for ASM and F/2 was adjusted to 25mg NO_3-N l^{-1}.

The slurry based media was processed from the output from two high rate algal ponds, one run in April 1989 and the other in October 1989, the latter only being used for one experiment during October. The input and output values for BOD, COD, nitrogen and phosphate levels are given in Table 5.

Slurry supernatent from the ponds was allowed to sediment overnight. The supernatent was then centrifuged on a continuous centrifuge (Griffin Christ Junior 15000, 8,500 - 9,000 r.p.m.), once on maximum flow and then on

Table 5: Slurry Supernatent Inputs and Outputs for High Rate Algal Ponds (Fallowfield, H J pers comm, 1989)

	BOD mgl^{-1}	COD mgl^{-1}	NO_3-N mgl^{-1}	NO_2-N mgl^{-1}	NH_4-N mgl^{-1}	Urea mgl^{-1}	Soluble Phosphate mgl^{-1}	Total Phosphate mgl^{-1}
April 1989								
HRAP Input	>164	5400	0.09	0.1095	NA	34.00	15.18	8.38
HRAP Output	NA	110	0.5475	0.3840	NA	3.57	6.27	7.78
October 1989								
HRAP Input	8160	20800	7.3525	0.026	9.733	4.779	2.1	5.79
HRAP Output	8	90	18.8175	0.7055	22.541	0	6.1	6.62

restricted flow (12.5 l hr^{-1}) which removed 75% of particulate material determined by dry weight. The resulting slurry liquor was then autoclaved at 5lbs/10 mins and stored at 2°C.

The organic nitrogen (2.3.8), ammonia (2.3.7), nitrate and nitrite (2.3.6) levels of the slurry liquor were determined before each experiment to account for any loss during storage, specifically ammonia. Once the total nitrogen level was known, the nitrate-nitrogen level was spiked with sodium nitrate to give 25mg NO_3-N l^{-1} in addition to any other nitrogen source available i.e. nitrite and ammonium. For marine and brackish species usually grown in F/2, 33.6 g l^{-1} synthetica sea salt was added to the spiked slurry liquor in addition to antiforming agent.

2.1.3 Experimental Inoculum

2.1.3.1 Nitrogen limitation experiments

A range of inoculum levels (10^2 to 10^5 cells ml^{-1}) was investigated for the four green algae, using the Batch culture System (2.2.1).

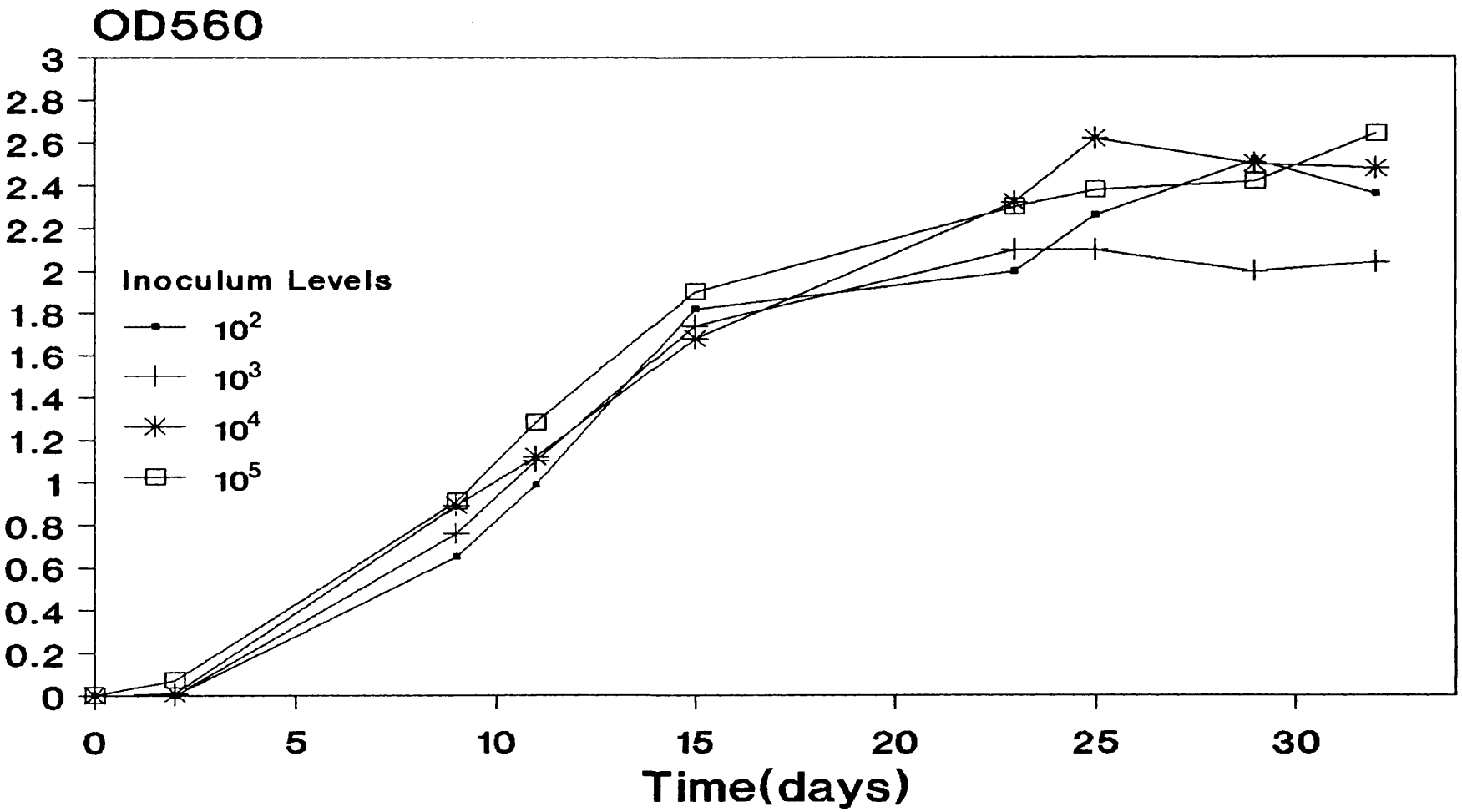
The algae were cultured in ASM at 50 mg NO_3-N l^{-1} (stated recipe level) at 30°C. The cultures were found to grow similarly irrespective of initial inoculum level (Fig 2). Therefore an inoculum level of 10^4 cells ml^{-1} was chosen for all experiments.

10 day stock cultures (2.1.1) were found to provide a suitable inoculum, usually 1ml, to give an initial cell number of 10^4 cells ml^{-1} (2.3.1).

2.1.3.2 Outdoor Minipond experiments

Algal species were cultured in 2 litre volumes of media (ASM or F/2) with stirring and sparging (200 ml min^{-1}), at an average irradiance of 230µmol $m^{-2}s^{-1}$, at room temperature. 10-15 day cultures were usually found to contain 10^7 cells ml^{-1} (2.3.1) and were used to inoculate the miniponds to give initial concentrations of 10^5 cells ml^{-1}.

FIG 2 GROWTH CURVES FOR 211/11c AT FOUR INOCULUM LEVELS



2.2 ALGAL EXPERIMENTAL CULTURE SYSTEMS

2.2.1 Batch Culture System

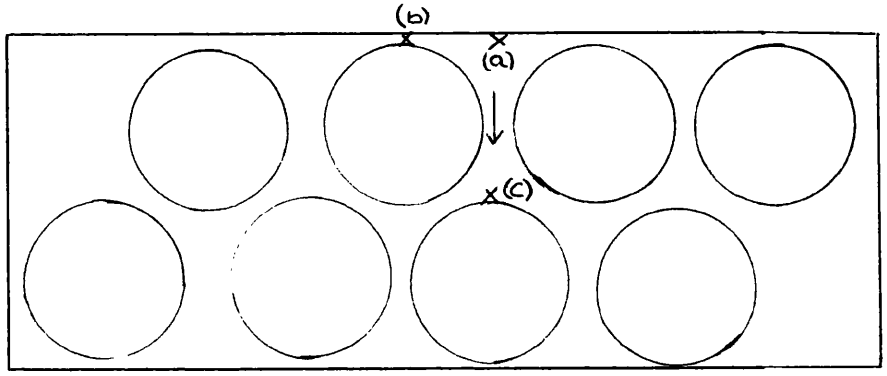
This system was developed for the nitrogen limitation experiments (Section 4). Four systems were designed and constructed, each capable of culturing up to 7.2 litres (8 x 900ml) of algae under controlled temperature, light and aeration conditions.

2.2.1.1 System description and development

The Batch Culture System is shown in Plates 1-4.

The system consisted of a glass tank (Clearseal, 30" x 15" x 12") within which a series of eight one litre pyrex bottles (BDH, 215/0180/05) were placed in a staggered pattern (Plate 2). A suitable light regime was provided by two fluorescent tubes either side of each tank (30", 30W, Aquaglo), the light environment for algal growth being optimised by the staggered bottle pattern which gave minimum shading for maximum number of culture bottles. Bottle holders (Plate 2) and lead collars were used to maintain bottle positions in the tanks.

The light regime was measured as an average of eight readings for tank glass surface (a), bottle surface (b) and incident bottle surface (c) using a PAR Quantum sensor (Skye Instruments, SKP200 measuring unit and SKP 215 Quantum sensor). This maintained light levels between 60 - 130 μmol $m^{-2}s^{-1}$.



Staggered bottle pattern showing position of quantum sensor (X)

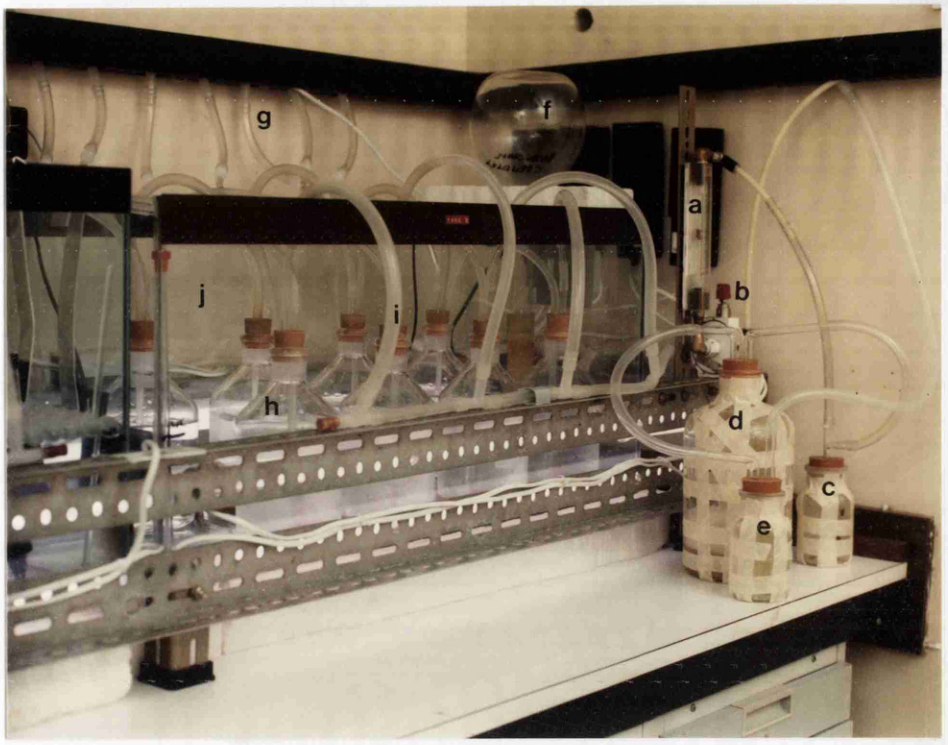


Plate 1: Batch Culture System: Front View
(a) pressure gauge (b) inlet air filter (c) water trap
(d) humidifier (e) water trap (f) gravity water feeder
(g) inlet air supply (h) culture vessel (i) outlet manifold
(j) glasstank

Plate 2: Batch Culture System: Top View (Empty)
(a) temperature sensor (b) heater (c) mixer
(d) staggered bottle holders

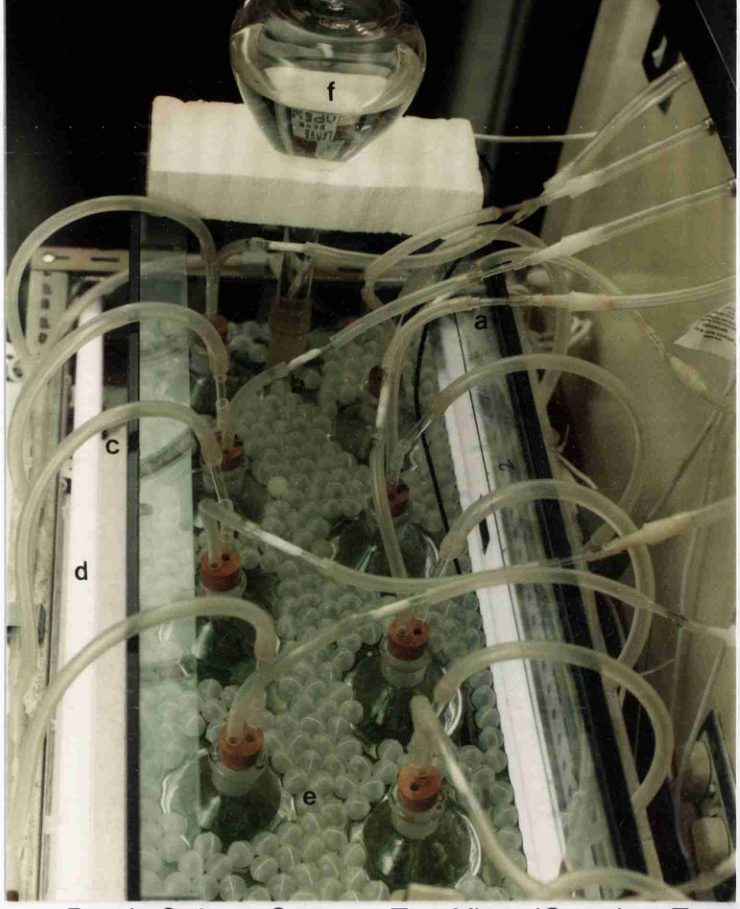


Plate 3: Batch Culture System: Top View (Ongoing Experiment)
(a) inlet capillary glass tube (b) inlet air filter (c) outlet manifold
(d) fluorescent light (e) surface spheres (f) gravity water feeder

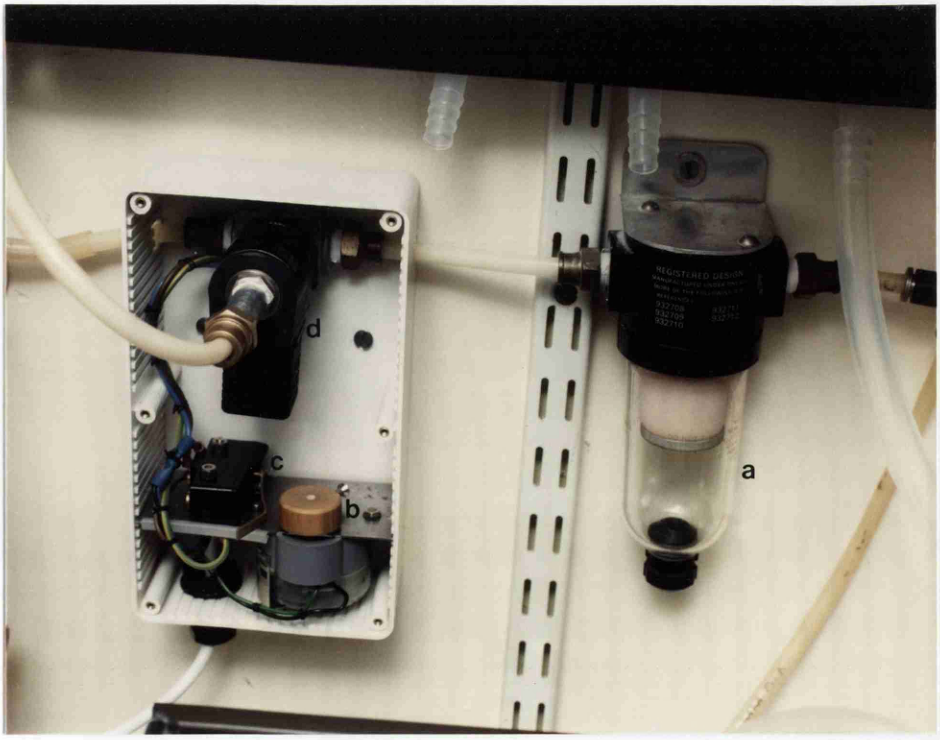


Plate 4: Batch Culture System: Air Interrupt System
(a) air filter (b) eccentric cam (1 rev/30 mins)
(c) microswitch (d) 3 part solenoid valve

The surrounding water temperature was controlled by thermostatically controlled heaters (Nova-Uno Regal Toughened Glass 200W heater, Nova-Uno Solid State thermostat "Hi Range" model) to ± 0.5°C. Measurement of the temperature at various positions within the tank demonstrated that mixing provided (Aquaclear Powerhead 400 mixer) was sufficient to maintain constant temperatures throughout the tanks. 17°C was obtained using a cooled, heat exchanger (water condensers with tap water at approx. 14°C) running against the lowest heater setting, placed inside the tanks. Temperature was monitored by the use of a chart recorder with manual calibration.

Gravity water feeders (2 litre volumetric flasks, upside down) together with plastic spheres maintained water levels over a two to three day period reducing maintenance of the system (Plate 3).

The system of operation consisted of a main inlet air filter, pressure gauge and humidifier leading to a split air line between two tanks (Plate 1). The incoming air supply was humidified to reduce water loss from the cultures which may result from a dry air stream. Capillary glass tubing (Plate 3(a)) was used on the inlet to each bottle to give a high constant back pressure to each bottle to improve and equalise sparging between bottles. An autoclavable glass fibre filter (Plate 3(b)) was also placed before each bottle. Glass spargers were replaced with open glass tubing to provide better mixing within the bottles and reduce sedimentation. Individual outlet filters which became wet and reduced gas flow were replaced by an autoclavable outlet manifold (Plates 1 & 3) designed to enable condensate to be drained to a common water trap before the outlet filter. However, the air outlet tubes directly above the bottles were not being cleared of condensate by air pressure alone causing cessation of sparging. An AIR INTERRUPT SYSTEM (AIS, Plate 4) positioned on the air line from the compressor was developed to overcome this problem. The AIS stopped the air supply for approximately two minutes every thirty minutes to allow condensate in the outlet to drain back into the respective culture bottle. This had two advantages in that sparging was maintained throughout the experimental period and the loss of water from the bottles was reduced from a mean of 3.3 ml day^{-1} to 1.9 ml day^{-1} following its introduction. 2 ml day^{-1} of

sterile distilled water was added to each culture bottle during experimental work to maintain constant volumes in relation to OD_{560} and dry weight. Incorporation of the AIS did not affect algal growth when compared to the growth before its introduction (Figs 3 and 4).

2.2.2 Outdoor Minipond System

This system was developed for the outdoor algal growth experiments (Section 5) and was designed to allow growth under natural outdoor conditions of temperature and light, the only controllable factor being nitrogen level. Four miniponds were constructed, each capable of culturing 16 litres of algae.

2.2.2.1 System description

Each minipond consisted of a Nalgene tray (43 x 51 x 12 cm, BDH 406/0355/02) covered with a raised clear perspex lid (92 x 61 cm) attached by six screws and angled to allow rainwater to run off (Plate 5). Mixing was provided by an aquarium mixer (Aquaclear Powerhead 201).

The system was incubated under ambient light and temperature conditions.

2.2.3 Continuous Culture System

The development of fatty acid methodology (Section 3) required the production of a large quantity of "standard" algal biomass and this was obtained by the use of a continuous culture system.

2.2.3.1 System description

The continuous culture system consisted of a 15 litre glass culture vessel, with a top plate containing entry ports for media, air, heater, acid/alkali, stirrer, pH probe, and condenser, and a bottom plate with an overflow port, sample port and air inlet sparger (Plates 6 & 7). pH, temperature, mixing, aeration, light and dilution rate could all be controlled (Plate 6).

FIG 3 GROWTH CURVES FOR C.vulgaris 211/8K AT 10^4 INOCULUM

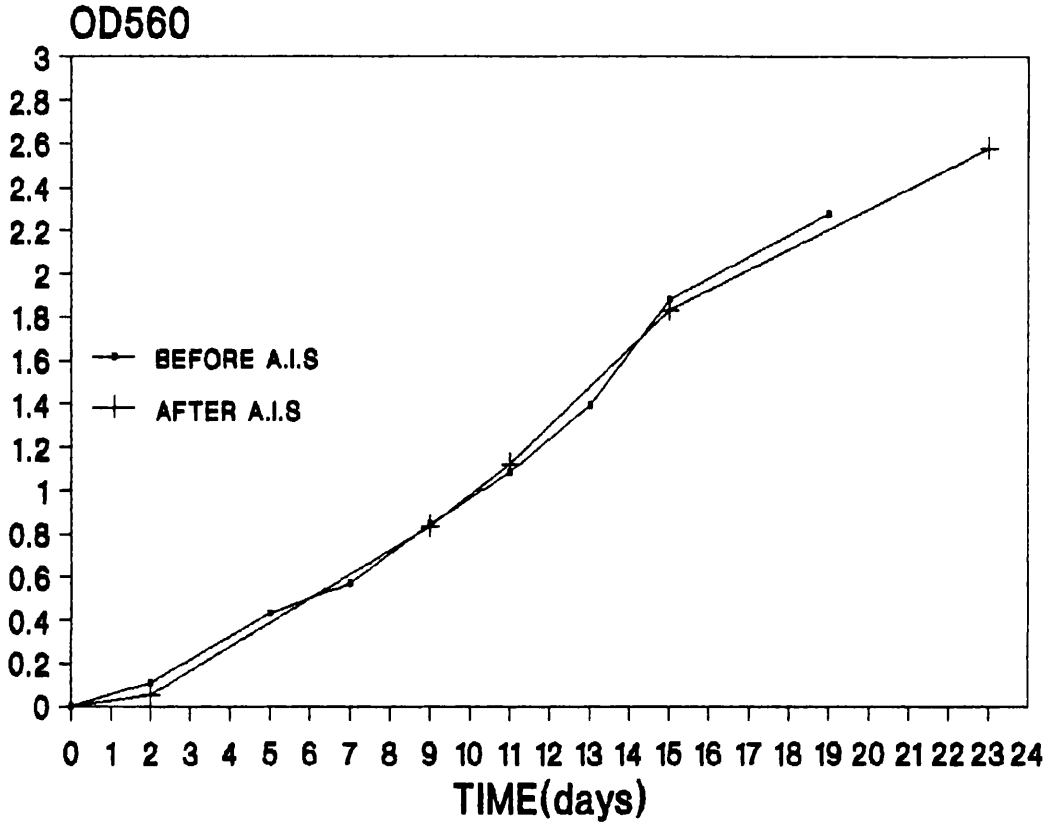
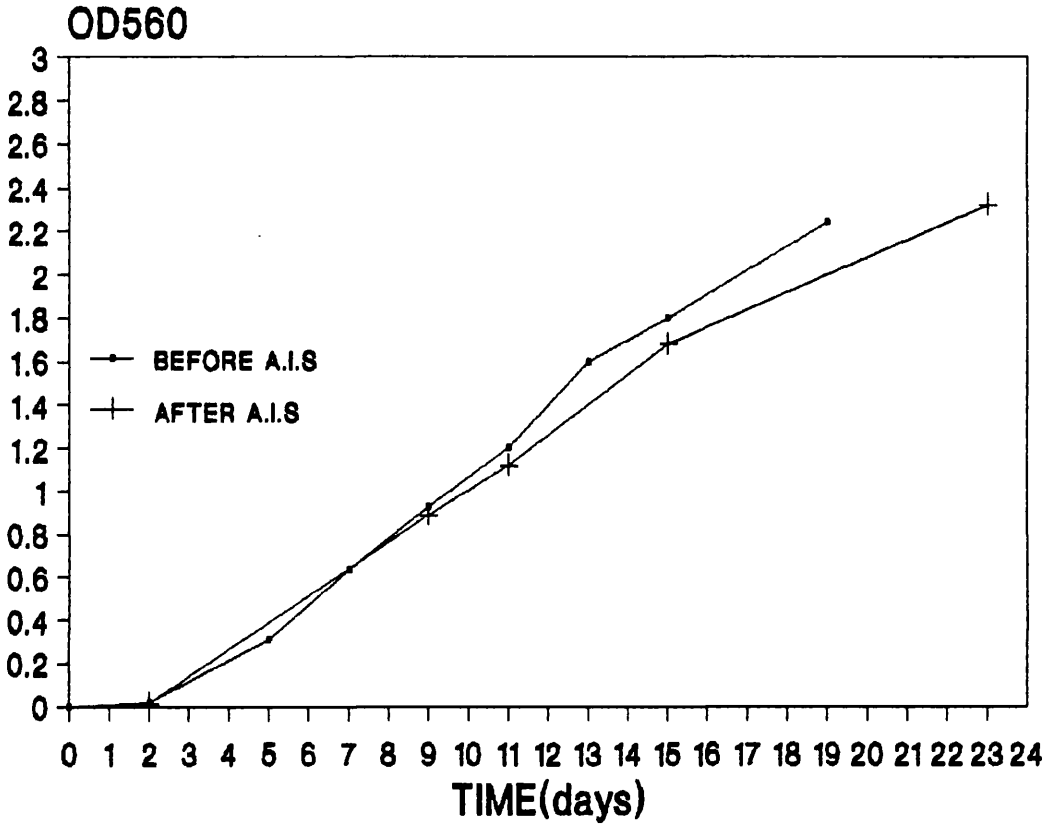


FIG 4 GROWTH CURVES FOR C.vulgaris 211/11c AT 10^4 INOCULUM



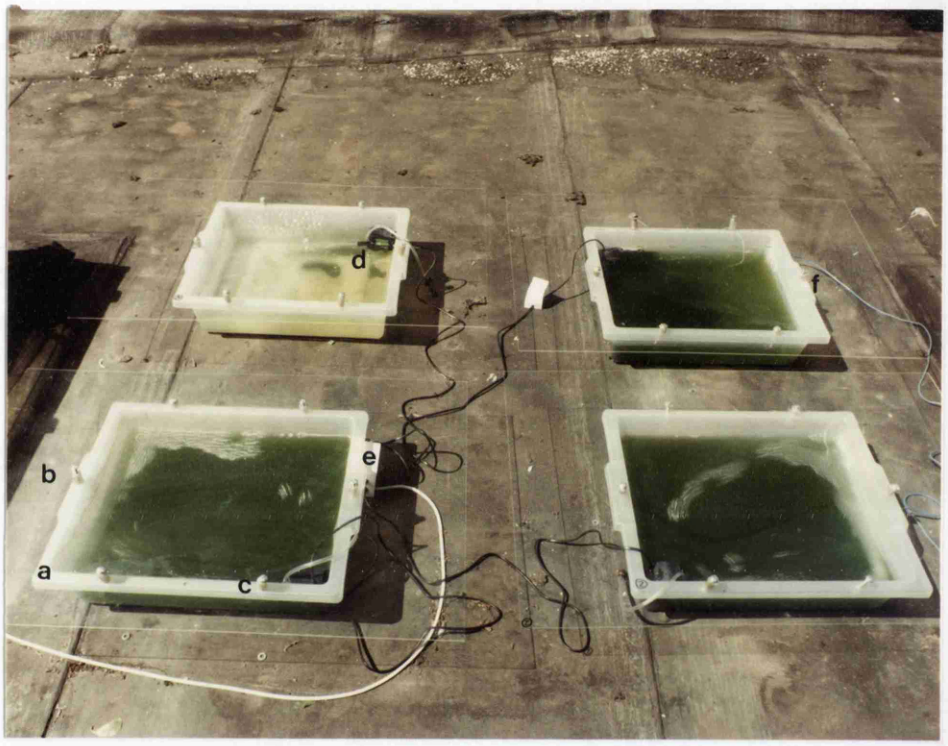


Plate 5: **Outdoor Miniponds:**
(a) polypropylene tray (b) perspex lid (c) bolt (d) mixer
(e) mixer control (f) temperature probe

Plate 6: Continuous Culture System
(a) feed reservoir (b) overflow (c) cooler
(d) feed pump (e) stirrer motor (f) temperature, pH and stirrer speed controllers.

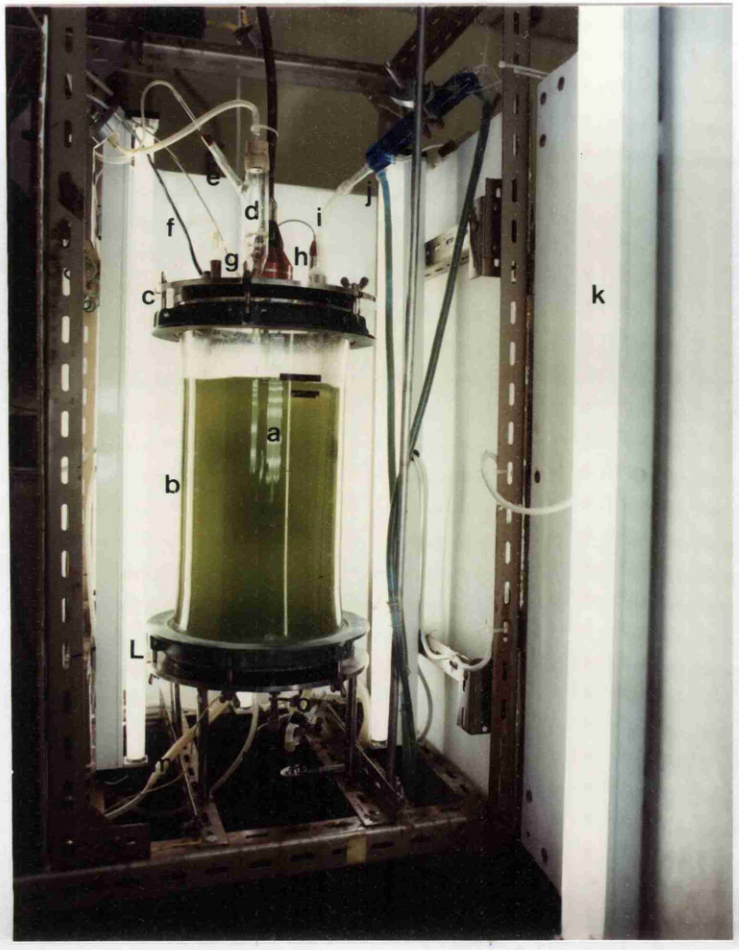


Plate 7: Continuous Culture Vessel
(a) 15 litres algal culture (b) glass culture vessel
(c) top plate (d) feed inlet (e) air inlet
(f) heater port (g) acid/alkali port (h) stirrer
(i) pH probe (j) condenser (k) 'aquaglo' 30W light source (x 4)
(l) bottom plate (m) overflow (n) air inlet sparger (o) sample port

2.2.3.2 Chemostatic culture production of algal biomass

The culture vessel was autoclaved empty with all ports sealed with cotton wool bungs (121°C/15mins), and associated inlet and outlet equipment eg feed inlet, overflow vessel were also autoclaved where required. Media (20 litres) was autoclaved for 121°C/30 mins.

The system was assembled aseptically, and the culture vessel was filled with sterile media (ASM) and allowed to equilibriate overnight at a controlled temperature (30°C), with continuous stirring (132 rpm) and sparging with air (2 litre min^{-1}). Following equilibration, the media was inoculated with 100ml of a 10 day stock culture (2.1.1). The algae were then cultured for 7-10 days at 30°C and an irradiance of 200μmol.$m^{-2}s^{-1}$ without an applied dilution rate. The pH was monitored continuously and pH 7.5 maintained by the automatic addition of 1N HCl or NaOH.

Once the culture had grown to a suitable cell density for harvesting, a dilution rate was applied by the constant addition of fresh sterile ASM growth media. The rate of addition was controlled by a peristaltic pump. Constant culture volume was maintained by an upright overflow pipe within the vessel. The spent media was collected aseptically in a darkened, chilled (5°C) vessel. The dilution rate was measured daily by calculation from the rate of culture overflow using the equation:-

$$\text{Dilution Rate } (d^{-1}) = \frac{\text{Volume of culture vessel (litres)}}{\text{Volume of overflow/day (litres)}}$$

OD_{560} (2.3.2) was monitored daily and once the culture had attained steady state the algae in the overflow vessel was harvested every 3-4 days eg C. vulgaris 211/11c cultured at a dilution rate of $0.2d^{-1}$, reached steady state at an approx. OD_{560} 0.75-0.86 and was maintained for a period of 28 days during which 13.4g dry weight was harvested (Fig 5). Similar yields were obtained for three other strains of green algae - C. vulgaris 211/8K, S. obliquus 276/3A, Ank. antarcticus 202/25 - used for the development of analytical methods for fatty acid determination.

FIG 5 CONTINUOUS CULTURE SYSTEM:
C.vulgaris 211/11c Growth Curve

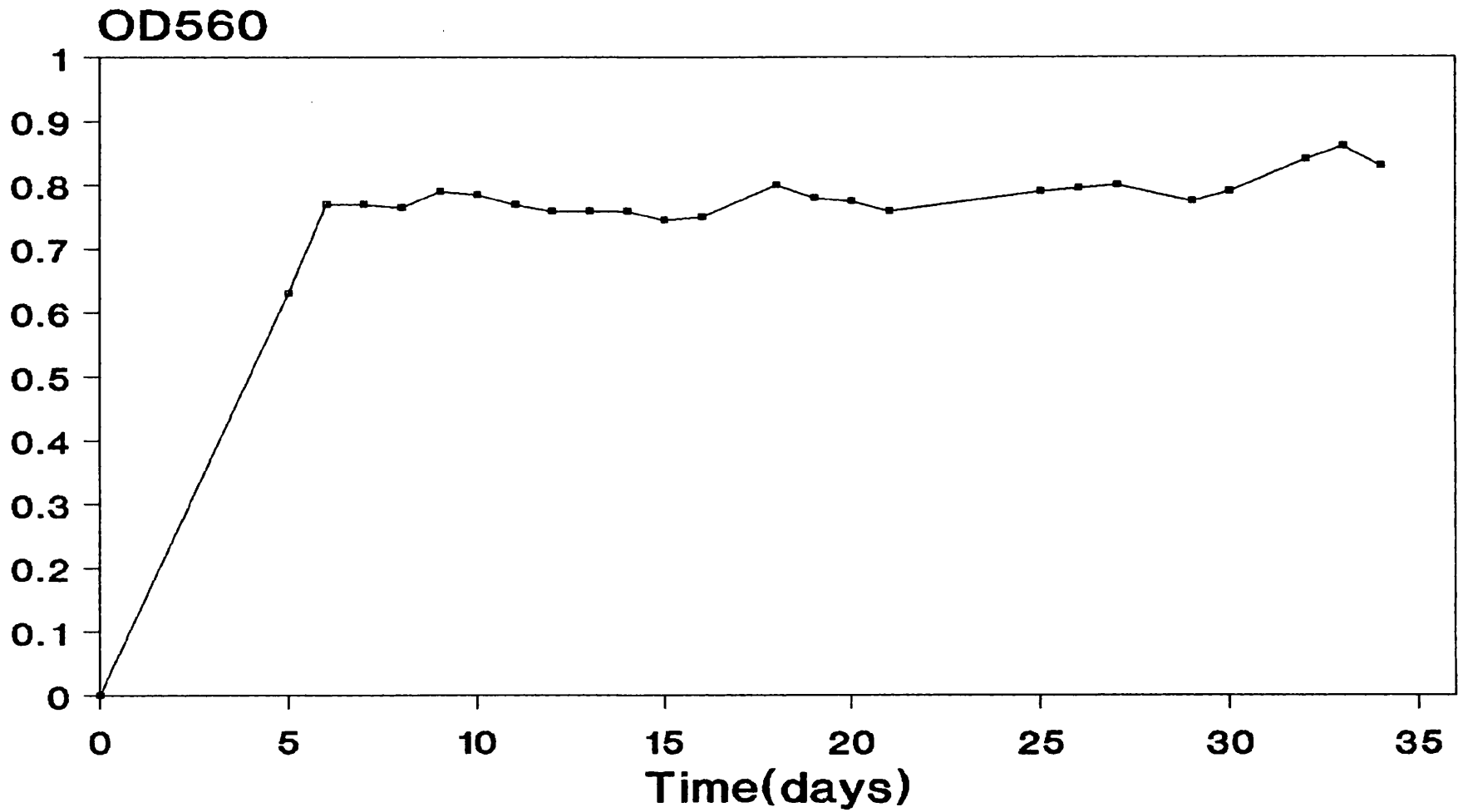


Table 6: Continuous Culture of four green algae used for development of fatty acid methodology.

Algae	OD_{560} Range (steady state)	Average dilution rate (d^{-1})	Harvest (g)
C. vulgaris 211/8K	0.72-0.78	0.21	10.64
C. vulgaris 211/11c	0.75-0.86	0.20	13.4
S. obliquus 276/3A	0.46-0.66	0.18	13.65
A. antarcticus 202/25	0.52-0.66	0.25	4.74

The algal material was freeze dried (2.3.9) and stored under nitrogen at -20°C.

2.3 STANDARD ANALYTICAL METHODS

2.3.1 Cell Numbers

Cell counts were performed using an Improved Neubauer counting chamber at 40x. magnification on a Leitz Microscope.

2.3.2 Optical Density

Optical density at 560nm (OD_{560}) was measured on duplicate samples using a Pye Unicam SP 1800 Spectrophotometer, read against a distilled water blank for greens and cyanobacteria and F/2 media blank for brackish and marine species.

2.3.3 Dry Weight

Duplicate samples of known volume (5ml) were filtered through pre dried (105°, 12 hours) weighed Whatman GF/C filters (2.5cm). The filters were dried

overnight at 105°C, allowed to cool in a dessicator, weighed and dry weight calculated by difference.

2.3.4 Ash Free dry weight

Duplicate samples of freeze dried algae were weighed into predried, preweighed crucibles and heated at 550°C for two hours. Crucibles were then transferred to a dessicator, cooled and reweighed. The residue weight was divided by initial dry weight, and multiplied by 100 to give %age ash of algal material.

2.3.5 pH

pH of the cultures was measured using a Gallenkamp pH stick.

2.3.6 Nitrate/Nitrite determination

Culture nitrate levels were determined from culture filtrate obtained from filtering through GF/C Whatman filters (2.5cm). The range of nitrate levels necessitated the use of two analytical methods. A nitrate specific electrode (Model 92-07, ORION) was used for determining high concentrations (10-100mg NO_3-N l^{-1}), but a nitrate reduction method (nitrate analysed as nitrite following reduction with spongy cadmium in the presence of borax and ammonium chloride) was used for lower NO_3-N levels (APHA, 1975; modified for a 5ml sample volume). Due to the presence of salt in the F/2 media all analysis for marine and brackish species was carried out using the nitrate reduction method only, as the nitrate probe could not be used. Duplicate samples were analysed.

2.3.7 Ammonia determination

Two methods for ammonia determination were used. For samples resulting from growth of C. caldarium, and samples from outdoor minipond culture, ammonia determination was carried out using a Urea nitrogen kit (Sigma Diagnostics Urea Nitrogen Procedure No 640). The method utilized part of the

procedure relating to the reaction of ammonia with alkaline hypochlorite and phenol in the presence of a catalyst (sodium nitroprusside) to form indophenol. The concentration of ammonia is directly proportional to the absorbance of indophenol, measured spectrophotometrically at 570nm. Ammonium sulphate at 150mg/100ml was used for the generation of a calibration curve in the range 0-75mg NH_4-N l^{-1}. Duplicate samples were analysed.

For slurry samples, determination of ammonia was carried out by steam distillation using a semi-micro method, a modification of the American Public Health Association Method (1971).

2.3.8 Organic Nitrogen

Duplicate samples were analysed using the Kjeldahl method for the determination of organic nitrogen using Zirconium dioxide and cupric sulphate as catalyst (Glowa, (1974)).

2.3.9 Harvesting and freeze drying

Algae were harvested by continuous centrifugation using a Griffin-Christ centrifuge (Junior 15000, 7,000 r.p.m.). This was found to remove at least 98% of biomass. The supernatent remaining in the rotor was emptied carefully and the residue scrapped into universal bottles with distilled water washings or F/2 media depending on the culture harvested, and centrifuged in an MSE benchtop chilspin (4,000 r.p.m./5 mins). The supernatent was discarded and the remaining biomass pellet freeze dried (Virtis consol 12 freeze drier). The freeze dried algae were placed under a nitrogen atmosphere, sealed and stored at -20°C.

2.3.10 Carbohydrate Determination

Carbohydrate analysis of the freeze dried algal material was carried out using the Anthrone Method (Herbert et al, 1971) using a glucose standard (0-80µg carbohydrate). Four replicates per sample were analysed.

2.3.11 Protein Determination

Protein analysis of the freeze dried algal material was carried out using the Coomassie Blue Dye binding method (Bradford, 1976), using a bovine serum albumin standard (0 - 100µg protein). Four replicates per sample were analysed.

2.3.12 Chlorophyll Determination

Duplicate 5ml samples were filtered through Whatman G/FC filters (2.5cm) and the filters placed in McCartney bottles containing 5ml 3:1 DMSO (Dimethyl sulfoxide) : 90% acetone. The bottles were stored at 2°C overnight in the dark. Each sample was then pipetted into a fresh McCartney bottle and made up to 5ml with 3:1 DMSO : 90% Acetone. Samples were then centrifuged (4,000 r.p.m./5 mins) and the optical density determined at 630, 647 and 664nm (Unicam SP1800 UV spectrophotometer). Chlorophyll a, b and c levels were calculated using the trichromatic equations of Jeffrey and Humphrey (1975) for 90% acetone.

2.3.13 Photosynthetic/Dark Respiration Rates

These were determined as oxygen evolution or consumption (Plates 8 and 9) using a Clark type polarographic oxygen electrode as described by Dubinsky et al (1987).

The oxygen electrode system encloses the electrode in a flat sided chamber giving a well defined light climate for measurement of the rate of change of concentration of dissolved oxygen under controlled temperature conditions. The electrode was calibrated at zero oxygen (using a saturated solution of sodium sulphite) and 100% oxygen (distilled water, sparged) and then the rate of change in concentration of dissolved oxygen with photosynthesis or dark respiration of an algal culture present in the chamber was recorded on a chart recorder. Photosynthetic and dark respiration rates were calculated using the zero oxygen and 100% oxygen readings and the solubility of oxygen at the experimental temperature.

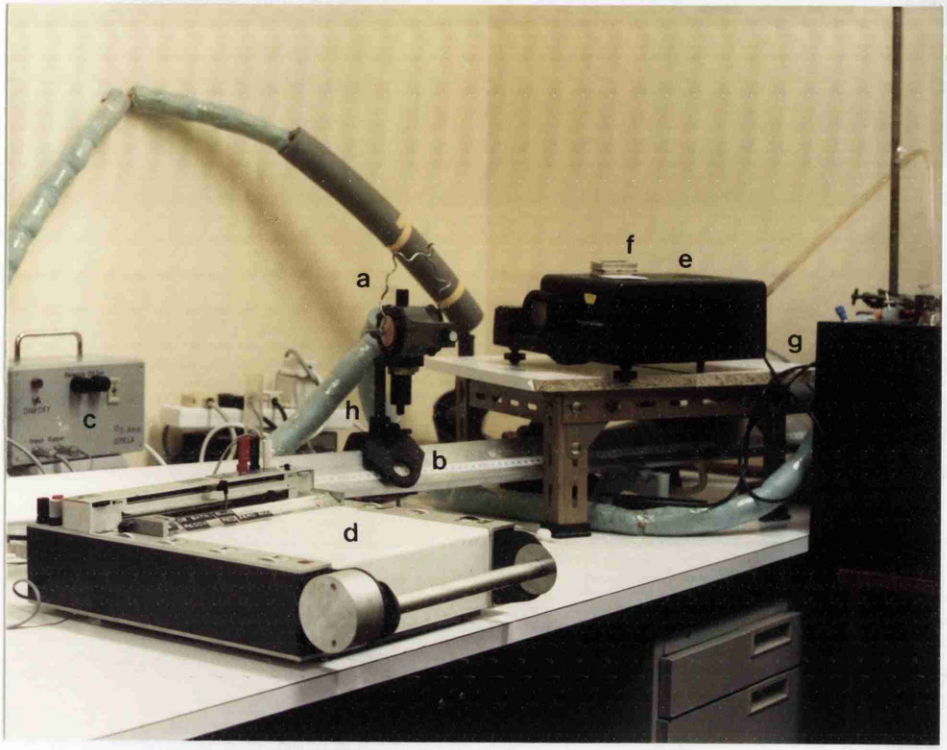


Plate 8: Oxygen Electrode Bench Experimental Set Up:
(a) oxygen electrode assembly with ear muffs
(b) optical bench (c) O_2 Amplifier (OTELA)
(d) chart recorder (e) slide projector light source
(f) neutral density filters (g) temperature regulated water bath
(h) inlet/outlet water supply

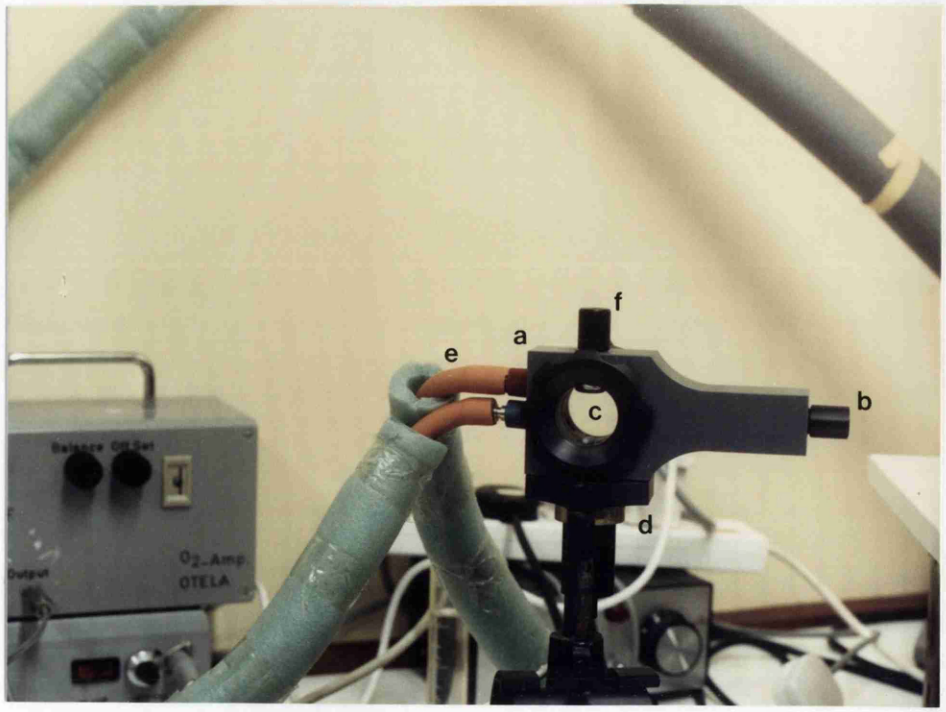


Plate 9: Oxygen Electrode Assembly:
(a) measurement chamber (cuvette)
(b) oxygen electrode port (c) magnetic flea
(d) magnetic stirrer (e) inlet/outlet water jacket
(f) filler cap (g) blanking plug

2.4 STATISTICAL ANALYSIS

Carbohydrate, Protein, Lipid and unsaturated fatty acid percentage totals data for all experiments was analysed using standard analysis of variance procedures in GENSTAT. In some cases, the data design was not orthogonal due to missing values. Only 'main effects' and 'first order interactions' were fitted, all other interaction terms were pooled with the residual error term. Where 'main effects' temperature, algae, phase, nitrogen - were significant (0.1% or $p < 0.001$ and 1% or $p < 0.01$ only), means were calculated and comparisons made. Calculation of means eg temperature means for carbohydrate - mean carbohydrate for all algae under analysis over all nitrogen levels in all phases at the three temperatures, 17°C, 30°C and 40°C.

3. FATTY ACID METHODOLOGY

3.1 INVESTIGATION OF FATTY ACID EXTRACTION/METHYLATION METHODS

A survey of the published literature available (Table 7) concluded that two extraction methods were predominantly applied to algae. These are the methods of Folch et al (1957) and Bligh and Dyer (1959). The methylation methods were, however, numerous with no universal method applied to algae.

The extraction method of Folch et al (1957) appeared to be most applicable to algal biomass, with the modification of Ways and Hanahon (1964) (Fig 6), due to the small sample size which might be expected as a result of some of the experimental treatments.

An initial investigation of extraction (sample ground with sharp sand and lipids extracted in 40°C/60°C Bpt petroleum ether) and methylation (Fig 7) used by the company sponsoring the work (Croda Universal Ltd) for seed oils was found to give very low levels of algal lipid (C. vulgaris 211/8K, 2-4% cellular lipid). Direct methylation alone of the algal material gave a higher lipid level (≈ 8%) which appeared to be more comparable to previously published values. The lower values initially found suggested the extraction method may not have been disrupting the cells sufficiently giving incomplete extraction.

The 'croda' methylation (Fig 7) was considered to be too severe for algae (Christie, pers. comm, 1987) and an alternative using 1% H_2SO_4 in methanol (Fig 8) was suggested (Christie, pers. comm, 1987; Christie, (1989a)). It was also noted that direct methylation was becoming more popular when applied to plant material and it had become more commonly reported in the literature (eg Browse et al 1986).

Therefore, it was decided to compare the extraction and methylation methods given in relation to estimated lipid contents of algal material.

Table 7: Literature Review on Methods of Algal Lipid Extraction & Fatty Acid Esterification

(NB: Ext - Extraction, ME - Methylation; For References given in method column see original paper given in Author column)

AUTHORS	ALGAE		METHOD
Ackman et al (1968)	12 marine unicellular algae	Ext: Me:	Bligh & Dyer (1959) BF_3 - Methanol
Ackman et al (1970)	Halosphaera viridis	Ext: Me:	Bligh & Dyer (1959) BF_3 - Methanol
Adams et al (1971)	Cyanidium caldarium	Ext: Me:	Soxhlet, chloroform Morrison & Smith (1964)
Ahern et al (1983)	Porphyridium cruentum	Ext: Me:	Schwarzenbach & Fisher (1978) MeOH: Benzene:conc. H_2SO_4 (20:10:1)
Anderson et al (1978a)	Diatom	Ext: Me:	Mod. Bligh & Dyer (1959) as in Tornabene et al (1974) or Kates (1972). 2.5% MeOH-HCl+H_2O, Pet. ether.
Anderson et al (1978b)	Nitzschia alba	Ext: Me:	Mod. Bligh & Dyer (1959) re. Kates (1972) 2.5% MeOH-HCl
Appleby et al (1971)	Greens & Cyanobacteria	Ext:	Folch et al (1957)
Araki et al (1986)	Red Alga	Ext: Me:	Bligh & Dyer (1959) 5% HCL in MeOH/90°C/2h
Beach & Holz (1973)	Dinoflagellate	Ext: Me:	Folch et al (1957) MeOH-KOH,HCl, ethyl ether
Beach et al (1974)	Crypthecodinium cohni		see, Beach & Holz (1973)

Table 7 (cont)

Ben-Amotz et al (1985)	Various microalgae	Ext: Me:	Mod. Bligh & Dyer (1959) re. Kates (1972) Mild alk.methanolysis (Tornabene & Ogg, 1971)
Berkaloff & Kader (1975)	Protosyphon botryoides	Ext: Me:	Bligh & Dyer (1959) Metclafe et al (1966)
Bishop et al (1986)	Cyanobacteria	Ext: Me:	Bishop et al (1980) BF_3- MEOH
Bryce et al (1972)	Anabaena cylindrica	Ext:	Folch et al (1957)
Cho & Thompson (1986)	Dunaliella salina	Ext:	Bligh & Dyer (1959)
Chu & Dupuy (1980)	Greens incl. Chlorella	Ext: Me:	Bligh & Dyer (1959) BF_3 -MEOH (14%v/v)
Constantopoulos & Bloch (1967)	Euglena gracilis	Ext:	Folch et al (1957)
Constantopoulos, (1970)	Euglena gracilis	Ext: Me:	Folch et al (1957) Diazomethane
Coombs et al (1967)	Navicula pelliculosa	Ext:	Folch et al (1957)
Czerpak (1983)	Anabaena, Chlorella, Scenedesmus	Ext: Me:	Ethyl ether/benzene (Matuch et al, 1972) 0.4N EtOH Na methoxide
Datz & Dohler (1981)	Synechococcus	Ext: Me:	EtOH/$CHCl_3$ (2:1 v/v) (Tevini, 1971) Muller & Goke (1973)

Table 7 (cont)

Dickson et al (1969)	Chlorella	Ext:	Folch et al (1957) in Soxhlet
		Me:	BF_3-MeOH
Dohler & Datz (1980)	Anacystis nidulans	see, Datz & Dohler (1981)	
Douglas et al (1969)	Botryococcus braunii	Ext:	ether
		Me:	MeOH: Benzene:conc. H_2SO_4
Dubinsky & Aaronson (1979)	Various algae	Ext:	improved Folch et al (1957) + 2-3 drops HCl
Eichenberger (1976)	Chlamydomonas reinhardii	Ext:	$CHCl_3$:MeOH(1:1)/70°C/ 30s, Et_2O
		Me:	3% MeOH-HCl, 1h
Erwin & Bloch (1963)	Various algae	Ext:	Ethyl ether
		Me:	Goldfine & Bloch (1961)
Evans & Kates (1984)	Dunaliella	Ext:	Hot isopropanol (Kates, 1972)
Evans et al (1982)	Dunaliella	see; Evans & Kates (1984)	
Fork et al (1979)	Synechococcus	Ext:	Folch et al (1957)
		Me:	3% HCl-MeOH, 90°C/2h
Fredrickson et al (1986)	Lake community	Ext:	Mod. Bligh & Dyer (1959)
Fried et al (1982)	Dunaliella	Ext:	Bligh & Dyer (1959)
		Me:	Diazomethane (Schlenk & Gellerman, 1969)
Gennity et al (1985)	Red algae	Ext:	Folch et al (1957) + sonication
		Me:	BF_3 (Bottino, 1975)
		:	H_2SO_4 (Woods & Lee, 1983)

Table 7 (cont)

Graff, et al (1970)	Scenedesmus obliquus	Ext: Me:	Folch et al (1957) BF3-MeOH/80°C/30 min
Harrington & Holz (1968)	Gyrodinium cohnii	Ext: Me:	ethyl ether Diazomethane
Henderson (1987)	Crypthecodinium cohnii	Ext: Me:	CCl_3: isopropanol (2:1 v/v) Christie (1982)
Holton, et al (1968)	Cyanobacteria	Ext:	Nichols, Harris & James (1965)
Iwamoto et al (1955)	Chlorella	Ext:	Ether
Jamieson & Reid (1972)	Marine algae	Ext:	Jamieson & Reid (1969)
Jamieson & Reid (1976)	Ulothrix aequalis	see Jamieson & Reid (1972)	
Janero & Barnett (1981)	Chlamydomonas reinhardtii	Me:	0.5N N Na-methoxide
Joseph (1975)	Dinoflagellate	Ext: Me:	Bligh & Dyer (1959) BF_3-MeOH
Kalacheva & Trubachev (1981)	Synechcoccus elongatus	Ext:	Isopropanol- CCl_3
Kates & Volcani (1966)	Diatoms	Ext: Me:	Mod. Bligh & Dyer (1959) Diazomethane
Kawaguchi et al (1987)	Phaeodactylum tricornutum	Ext:	Bligh & Dyer (1959)
Kenyon (1972)	Cyanobacteria	Me:	BF_3-MeOH

Table 7 (cont)

Klyachko-Gurvich (1974)	Chlorella pyrenoidosa	Ext: Vereshchagin & Klyachko-Gurvich (1965)
Korn (1964)	Euglena gracilis	Ext: Folch et al (1957) Me: 0.5 N MeOH-NaOH (Morgan et al, 1963)
Kost et al (1984)	Porphyridium cruentum	Ext: Folch et al (1957)
Lee & Loeblich (1971)	Marine & freshwater algae	Ext: Mod. Bligh & Dyer (1959) Me: 5% H_2SO_4/60°C/2 h
Lee & Picard (1982)	Unicellular algal biomass	Ext & Me: 3% HCl-MeOH/boil/3h Pet ether extraction of ME's
Lynch & Thompson (1982)	Dunaliella	Ext: Bligh & Dyer (1959) Me: BF_3-MeOH
Lynch & Thompson (1984)	Dunaliella	see Lynch & Thompson (1982)
Matucha et al (1972)	Chlorella vulgaris	Ext: Hot 96% MeOH then EtOH: diethyl ether (3:1) Me: MeOH-HCl
Metzger et al (1982)	10 sp green algae	Ext: Folch et al (1957)
Meyer et al (1979)	Platymonas (algal symbiont)	Ext: Bligh & Dyer (1959) Me: Schlenk & Gellerman (1960)
Miyazaki (1983)	Phytoplankton	Ext: Bligh & Dyer (1959) Me: 4% HCl-MeOH/95°C/4 h
Moseley & Thompson (1980)	Volvox carteri	Ext: Bligh & Dyer (1959)
Nagashima et al (1986)	Cyanidium caldarium	Ext: EtOAc Me: 5% HCl-MeOH/100°C/3h

Table 7 (cont)

Nichols (1965)	Chlorella vulgaris	Ext: Me:	Folch et al (1957) 3% H_2SO_4-MeOH
Nichols (1968)	Cyanobacteria & green algae	Ext: Me:	Folch et al (1957) MeOH-benzene-Conc H_2SO_4/90 mins
Nichols & Appleby (1969)	Algae incl. Euglena gracilis	Ext:	Folch et al (1957)
Nichols & Wood (1968)	Spirulina platensis	Ext: Me:	Folch et al (1957) MeOH-benzene-Conc H_2SO_4 (20:10:1 v/v)/90 min
Nichols et al (1965)	Chlorella vulgaris	Ext: Me:	Folch et al (1957) MeOH-benzene-Conc H_2SO_4 (20:10:1 v/v)/90 min.
Nichols et al (1967)	Chlorella vulgaris	see Nichols et al (1965)	
Nichols et al (1984)	Marine dinoflagellates	Ext: Me:	Folch et al (1957) Nichols et al (1982)
Nichols et al (1986)	Nitzschia cylindrus	Ext: Me:	Mod. Bligh & Dyer (1959) mild alk. hydrolysis; hexane (White et al, 1979)
Norman & Thompson (1985)	Dunaliella	Ext: Me:	Bligh & Dyer (1959) BF_3-MeOH
Norman et al (1985)	Dunaliella	Ext:	Bligh & Dyer (1959)
Nyberg & Kosimies -Soininen (1984)	Porphyridium purpureum	Ext:	Folch et al (1957)
Ono et al (1983)	Anacystis nidulans Anacystis variabilis	Ext:	Folch et al (1957)
Orcutt et al (1986)	Algal mats	Me:	White et al (1979)

Table 7 (cont)

Oren et al (1985)	Cyanobacteria	Ext:	Bligh & Dyer (1959)
		Me:	Diazomethane (Schlenk & Gellerman, 1960)
Paoletti et al (1976a & b)	Cyanobacteria & green algae	Ext:	Sand grinding; 2:1 $CHCl_3$: MeOH in Paquot apparatus
Parker et al (1967)	11 Cyanobacteria	Ext:	MeOH: CCl_3; sonication
		Me:	BF_3
Pettitt & Harwood (1987)	Red marine algae	Ext:	boiling in isopropanol
Pillsbury (1985)	5 phytoplankton	Ext:	Microsoxhlet; Mod. Shifrin & Chisholm (1981)
		Me:	BF_3-MeOH
Piorreck & Pohl (1984)	Green algae & cyanobacteria	Ext:	Sand grinding; Folch et al (1957)
		Me:	NaOMe
Piorreck et al (1984)	Green algae	see Piorreck & Pohl (1984)	
Podojil et al (1978)	Green algae	Ext:	Light pet/soxhlet/48 h EtOH-KOH, 2.5% H_2SO_4 ME's into diethyl ether
		Me:	BF_3-MeOH (14% v/v)
Potts et al (1987)	Nostoc commune	Ext:	Mod. Bligh & Dyer (1959) re. Gucket et al (1985)
		Me:	Mild alk.methanolysis
Reitz et al (1967)	Chlorella pyrenoidosa	Ext:	H_2O-MeOH (1:1); sonication
		Me:	Diazomethane
Rezanka & Podojil (1984)	Chlorella kessleri	Me:	BF_3-MeOH
Rezanka et al (1983)	Greens & cyanobacteria	Ext:	$CHCl_3$:MeOH (1:1v/v) soxhlet
		Me:	BF_3 MeOH (14% v/v)
Rezanka et al (1985)	Chlorella kessleri	see Rezanka et al (1983)	

Table 7 (cont)

Roessler (1987)	Diatoms	Ext:	60°C/1h each with 1. 50% MeOH/H_2O 2. MeOH (x2) 3. 50% MeOH/$CHCl_3$ (x2) Phase sep. Bligh & Dyer (1959)
Rosenberg (1963)	Euglena gracilis	Me:	Rosenberg & Chargaff (1958)
Rosenberg (1967)	Euglena gracilis	Me:	Metcalfe & Schmutz (1961)
Rosenberg & Gouaux (1967)	Euglena gracilis	Ext:	Folch et al (1957) in Waring Blender
Rosenberg & Pecker (1964)	Euglena gracilis	Ext: Me:	Rosenberg (1963) Hydroxamic acid reaction
Sargent et al (1985)	Phytoplankton	Ext: Me:	Folch et al (1957) + BHT (0.05%) acid catalysed
Sato & Murata (1980)	Anabaena variabilis	Ext: Me:	Folch et al (1957) 3% HCl in MeOH
Sato & Murata (1982a & b)	Anabaena variabilis	Ext:	Bligh & Dyer (1959) 3% HCl in MeOH
Sato et al (1979)	Anabaena variabilis Anabaena nidulans	Ext: Me:	Bligh & Dyer (1959) 3% HCl in MeOH (95%°C/2h) hexane
Sato et al (1987)	Chattonella antigua	Ext:	Bligh & Dyer (1959)
Schneider et al (1970)	11 cyanobacteria & green algae	Me:	BF_3
Seto et al (1984)	Chlorella minutissima	Ext: Me:	Homogenised (H_2O) then x2 Hexane: isopropanol (3:2) BF_3-MeOH
Sheffer et al (1986)	Dunaliella salina	Ext: Me:	Bligh & Dyer (1959) Diazomethane (Schlenk & Gellermen, 1960)

Table 7 (cont)

Shifrin & Chisholm (1981)	Phytoplankton	Ext:	Bligh & Dyer (1959) with microsoxhlet
Smith & Harwood (1984 a&b)	Fucus serratus	Ext:	a. Hot isopropanol 80°C/30 min b. Hot propan-2-ol 70°C/30 min sand grinding
		Me:	a. 2.5% H_2SO_4 in MeOH 70°C/2h b. as a.
Strain et al (1986)	Waste grown algal biomass	Ext:	Folch et al (1957) + sonication
Suen et al (1987)	Nannochloropsis sp.	Ext:	MeOH: $CHCl_3$: H_2O (10:5:4v/v); Tornabene (1985)
		Me:	2.5% MeOH-HCl
Tornabene et al (1983)	Neochloris oleoabundans	Ext:	Mod. Bligh & Dyer (1959) re. Kates et al (1964)
		Me:	2.5% MeOH-HCl
Usmanghani et al (1987)	Brown Seaweed	Ext:	x3 EtOH
Volkman et al (1980)	Biddulphia sinensis (marine diatom)	Ext:	$CHCl_3$: MeOH; sonication
		Me:	BF_3-MeOH
Volkman et al (1981)	4 marine Haptophyceae	Ext:	MeOH then Folch et al (1987) + sonication
		Me:	BF_3-MeOH (14%)
Vonshak et al (1985)	Porphyridium	Ext:	Bligh and Dyer (1959)
		Me:	MeOH-HCl
Williams (1965)	Marine planktonic algae	Ext:	$CHCl_3$
		Me:	MeOH: benzene: Conc.H_2SO_4 (10:1:1)

Table 7 (cont)

Wright et al (1980)	6 spp. green algae	Ext: Me:	Folch et al (1957); soxhlet 10% BCl_3 in MeOH
Zepke et al (1978)	Cyanobacteria	Ext:	boiling $CHCL_3$: MeOH: isopropanol (1:1:1)

Fig 6: Folch extraction

(Ref: Folch et al (1957), with modification of Ways and Hanahan (1964) from Christie (1982))

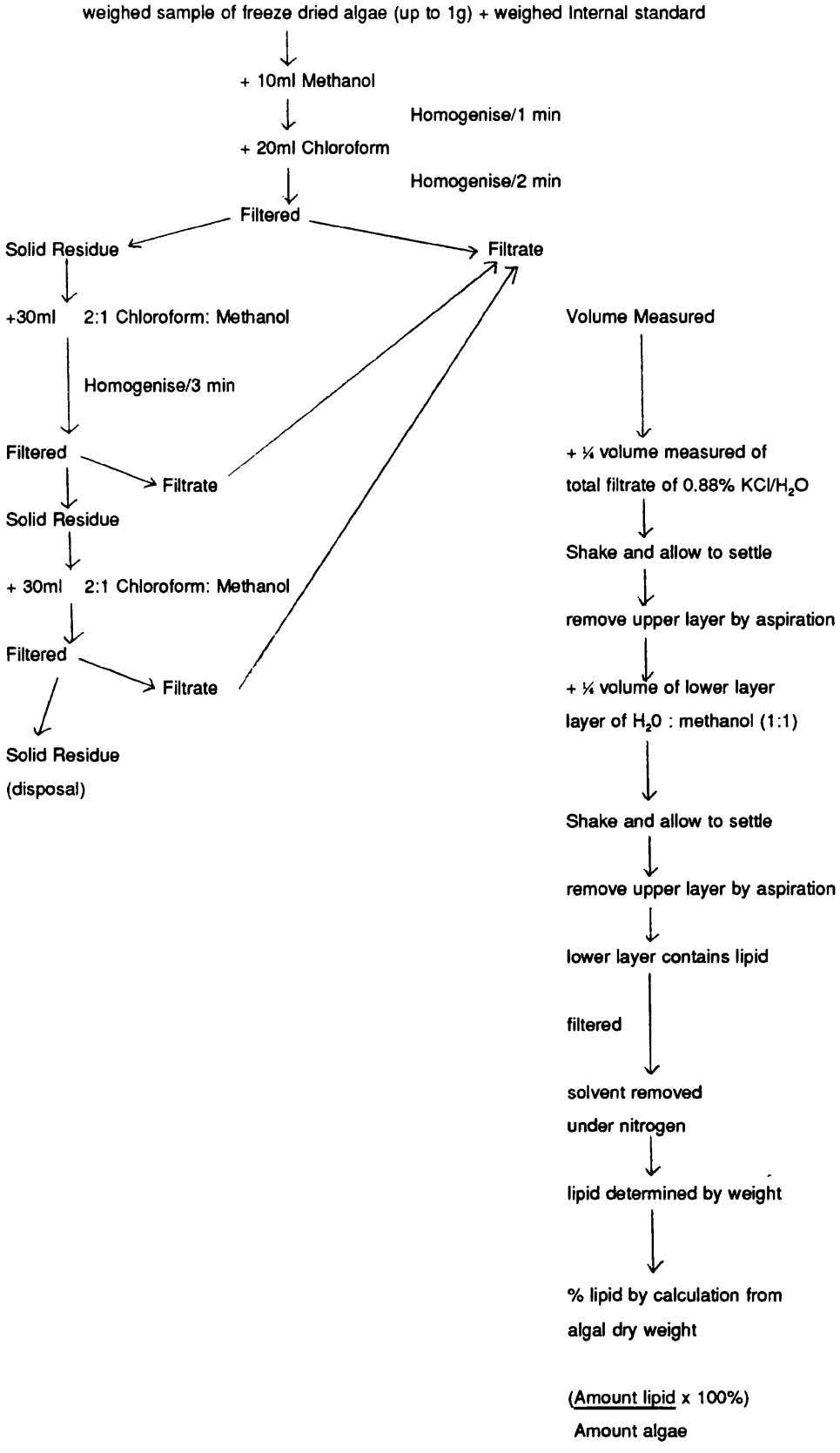


Fig 7: 'CRODA' Methylation

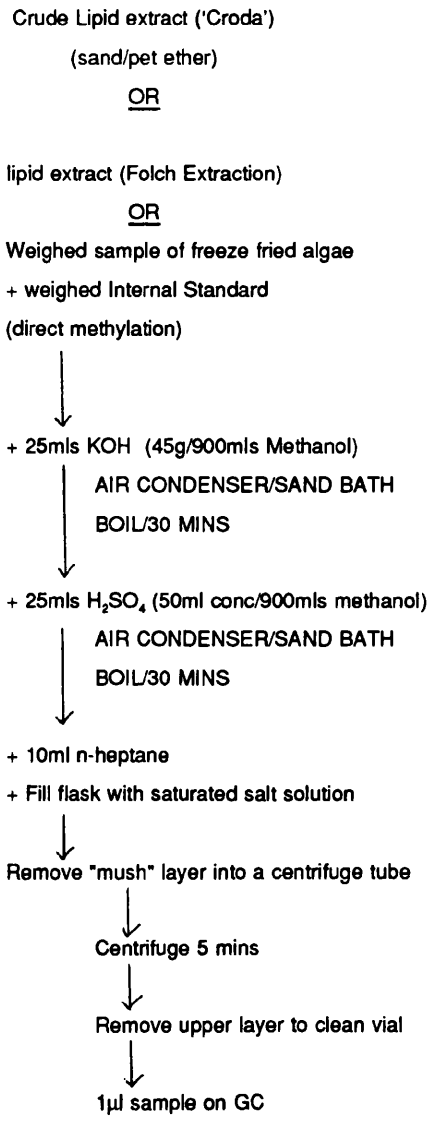
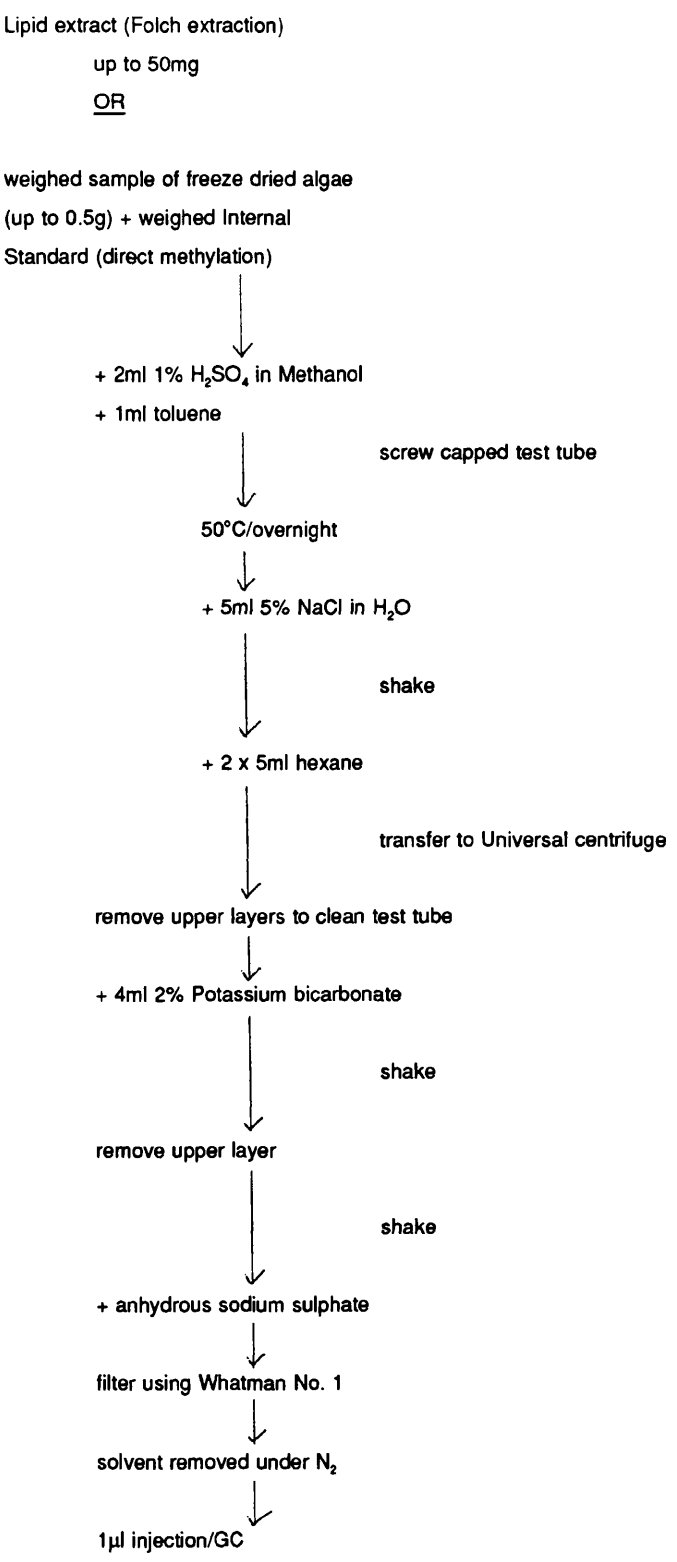


Fig 8: **'Christie' Methylation**

(Christie, pers comm (1987); Christie (1989a))



3.1.1 Comparison of Methodologies

The 'standard' algal biomass of the four freshwater green algae grown under continuous culture (2.2.3.2) was utilised. A comparison was made of estimated lipid contents determined:

i) gravimetrically using the Folch extraction procedure (Fig 6)

ii) that calculated from the sum of the methyl esters, prepared from the extracted lipids, methylated using either the 'Croda' (Fig 7) or 'Christie' (Fig 8) methods.

iii) direct methylation of the algal material by both methylation methods ('Croda' and 'Christie').

The percentage conversion of cod liver oil and algal lipid to fatty acid methyl esters by both methylation methods was also determined. Fatty acid analysis was carried out by GC using a WCOT Fused Silica CP-Sil 88 column (50m - 0.25mm ID) with a 5M deactivated fused silica precolumn (Chrompack Cat No. 7488).

GC: United Technologies Packard Model 439 with Hewlett-Packard 3390A Integrator

GC Conditions: Maximum column temperature 226°C

Detector temperature 300°C

Injector temperature 240°C

Oven - initial 160°C

- final 225°C

- rise (°C/min) 5

Time initial (min) 2

Time final (min) 25

Stability time (min) 1

He Carrier Gas

Head pressure 125 Kpa

Split ration 100:1

Flow 0.43 ml/min

Total run time 40 min

Identification was by comparison to known authenticated standards, and quantitation by the use of an internal standard (C22:0). BHT was added to all solvents to prevent oxidation, and all solvents used were either Analar or HPLC grade.

3.1.2 Results

The results are shown in Table 8. Gravimetric determination recorded the highest lipid contents in comparison with extraction and methylation and direct methylation with the exception of direct methylation of C. vulgaris 211/11c. However, the results of percentage conversion of oil to methyl esters suggested that some of this material was none lipid, and also that not all the lipid present may have been converted. The conversion was expected to be less that for cod liver oil as algal lipids are complex and sterols, glycolipids, phospholipids and tocopherols which are water soluble may be lost. The differences in percentage conversion may be due to differences in content of lipid in the various species. Also, the presence of other compounds eg pigments, would affect the result. Further work including HPLC and GC-MS confirmed that not all material was lipid.

Table 8: Comparison of Extraction and Methylation Procedures

Sample	(1) Folch Ext. % Lipid		(2) Conversion Oil to ME's		(3) Folch & Methylation % Lipid		(4) Direct Methylation % Lipid	
	T-T	Flask	Chrt	Croda	Chrt	Croda	Chrt	Croda
CLO			88.0	74.4				
			89.4	74.4				
			83.0	80.6				
			81.0	79.6				
			x 85.3	77.2				
C. vulgaris 211/8k								
	11.2	11.8	66.0	66.0	7.4	7.75	10.7	8.6
			64.0	64.0	7.5	7.56	10.7	8.7
	13.7	11.6	66.0	64.0	9.1	7.37	10.1	10.7
			67.0	64.0	8.8	7.37	10.4	10.7
x	12.4	11.7	66.0	64.5	8.2	7.5	10.5	9.7
C. vulgaris 211/11c								
	7.9	8.4	67.0	54.0	5.3	4.5	9.6	10.1
			65.0	51.0	5.2	4.3	9.6	9.9
	8.5	8.9	66.0	58.0	5.6	5.2	9.7	10.5
			68.0	56.0	5.7	5.0	9.3	10.5
x	8.2	8.7	66.5	55.0	5.5	4.8	9.6	10.2
S. acutus								
	12.2	9.6	44.0	53.0	5.45	5.1	8.0	7.5
			46.0	54.0	5.6	5.2	8.2	7.6
	13.9	11.0	41.0	48.0	5.7	5.4	8.2	7.4
			40.0	48.0	5.6	5.4	8.0	7.6
x	13.0	10.3	43.0	51.0	5.6	5.3	8.1	7.5
A. antarticus								
	19.6	16.3	51.0	55.0	10.1	9.0	14.7	10.9
			50.0	55.0	9.8	9.0	14.8	11.2
	19.9	15.1	51.0	67.0	10.2	10.2	11.5	10.5
			51.0	65.0	10.1	9.9	11.6	10.6
x	19.7	15.7	51.0	61.0	10.1	9.5	13.1	10.8

Notes
T-T: Folch using test tubes for onward Christie methylation
Flask: ------ Folch using a round bottomed flask for onward Croda methylation
Chrt - Christie methylation method
Croda - Croda methylation method
x - mean of determinations
CLO - cod liver oil

The results of the comparative experiment, however, did demonstrate that for green algae, both direct methylation methods (calibrated with internal standard) gave higher percentage lipid contents than any combination of extraction and methylation. Furthermore, with the exception of C. vulgaris 211/11c it appeared that the 'Christie' methylation method gave consistently higher lipid contents than the 'Croda' methylation method. This may be due to the presence of KOH in the 'Croda' method which provides a source of water for hydrolysis which is irreversible and this may have resulted in esterification not going to completion.

Fatty acid methyl ester profiles for the four algae exhibited no significant peak differences qualitatively between extraction/methylation and direct methylation methods (eg Figs 9 and 10). Quantitative differences favoured the direct methylation methods. Therefore it was decided to use the 'Christie' methylation method as a direct methylation method for all analysis of algal biomass.

3.1.3 Further Method Development

3.1.3.1 Florisil

Water soluble materials eg pigments were removed in the direct methylation method with washing of the sample, however, lipid soluble materials eg phytol, carotenoids, sterols would remain. Therefore, a further step was added to the method after methylation and extraction of the methyl esters in the form of a clean up step. Adsorption chromatography using a short column of Florisil (60-100US MESH, FSA) in a pasteur pipette plugged with glass wool, and elution with hexane-diethyl ether (95:5 v/v) allowed samples to be cleaned up leaving impurities on the column.

3.1.3.2 Sample Size

During this experimental work, an average of 0.2g of algal material had been used per sample. A further investigation of sample size for the four

Fig 9: G.C. Chromatogram : C. vulgaris 211/8K

a. FOLCH EXT^{N} AND CHRISTIE METH b. DIRECT CHRISTIE METH

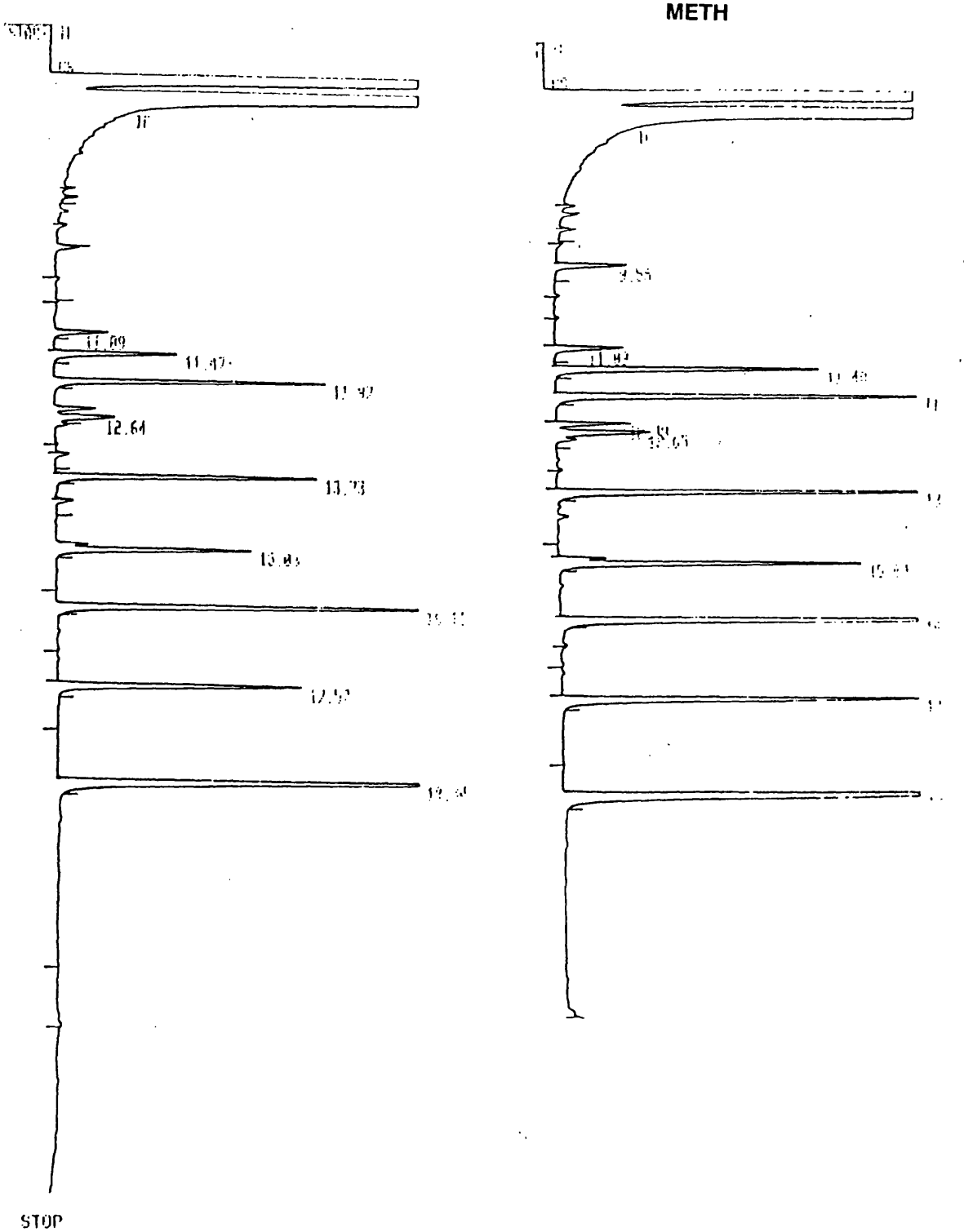
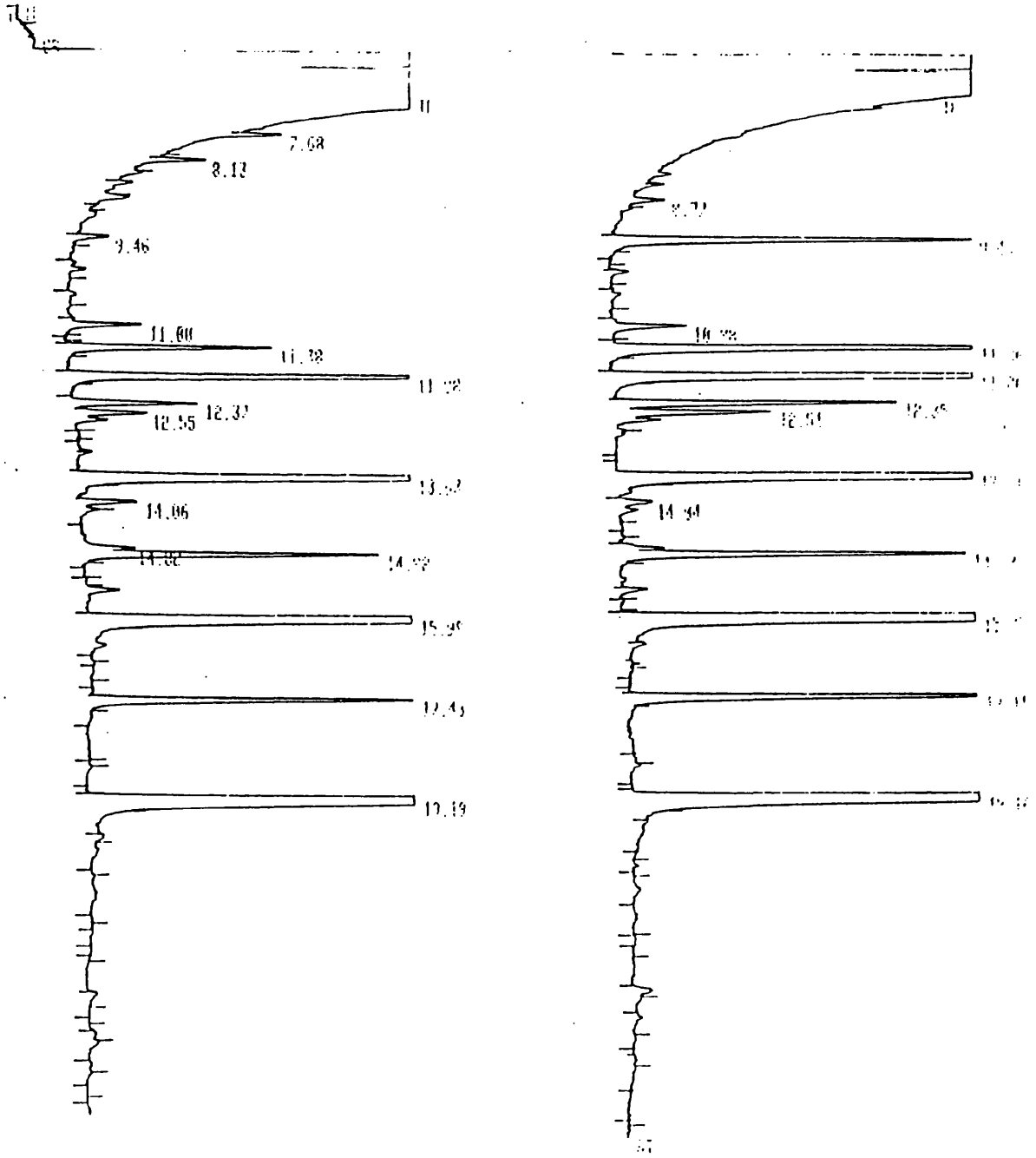


Fig 10: G.C. Chromatogram : C. vulgaris 211/11C

a. FOLCH EXT^{N} AND CHRISTIE METH b. DIRECT CHRISTIE METH



freshwater green algae led to the finding that the method was suitable for 0.1 - 0.25g algal material. Results are given for Ankistrodesmus antarcticus:

Amount of algal material (g)	% of Lipid (Sum of ME's)
0.2392	11.9
0.2367	11.2
0.2067	11.2
0.2087	11.4
0.1562	11.3
0.1405	11.6
0.1068	11.8
0.1030	11.5
0.0585	10.4
0.0601	11.0

3.1.4 Direct Methylation Method

A flow chart of the direct methylation method used for all samples is given (Fig 11) together with accompanying visual clarification (Plate 10). Duplicate samples were analysed by GC (3.2.2), each of the samples being injected twice to obtain an average for each fatty acid. Once all component fatty acids had been identified (3.2) their values were added together and given as a percentage of the total algal material calculated in relation to the known amount of Internal standard.

$$\frac{\text{Total Area \% Fatty Acid ME}}{\text{Area \% Internal standard}} \times \text{Amount (g) Internal standard} = \text{Lipid (g)}$$

$$\frac{\text{Lipid (g)}}{\text{Original weight of algae (g)}} \times 100 = \text{\% lipid in algal material (sum of fatty acid ME's)}$$

Fig 11 FATTY ACID ANALYSIS

(DIRECT EXTRACTION/METHYLATION)

≈.1 ≈.2g ALGAE + ≈.01 INTERNAL STANDARD
(C22:0 M.E.)

+ 1ml TOLUENE

+ 2ml 1% H_2SO_4/METHANOL (+BHT) (A)

↓ MIX, 50°C/OVERNIGHT

+ 5ml 5% NaCl in H_2O (B)

↓ MIX

+ 5ml HEXANE - BHT (C)

↓ CHILSPIN 4,000 rpm/5min

REMOVE + RETAIN UPPER LAYER

↓

REPEAT HEXANE - BHT EXTRACTION

+ COMBINE RETAINED UPPER LAYERS (D)

↓

+ 4ml 2% POTASSIUM BICARBONATE (E)

↓

REMOVE & RETAIN UPPER LAYER

↓

+ ANHYDROUS SODIUM SULPHATE (F)

↓

FILTER/WHATMAN No 1 (G)

↓

REDUCE TO DRYNESS/N_2

↓

+ 2ml HEXANE/10% DIETHYL ETHER

↓

ONTO FLORISIL COLUMN (H)

↓

SAMPLE COLLECTED AND REDUCED TO DRYNESS/N_2

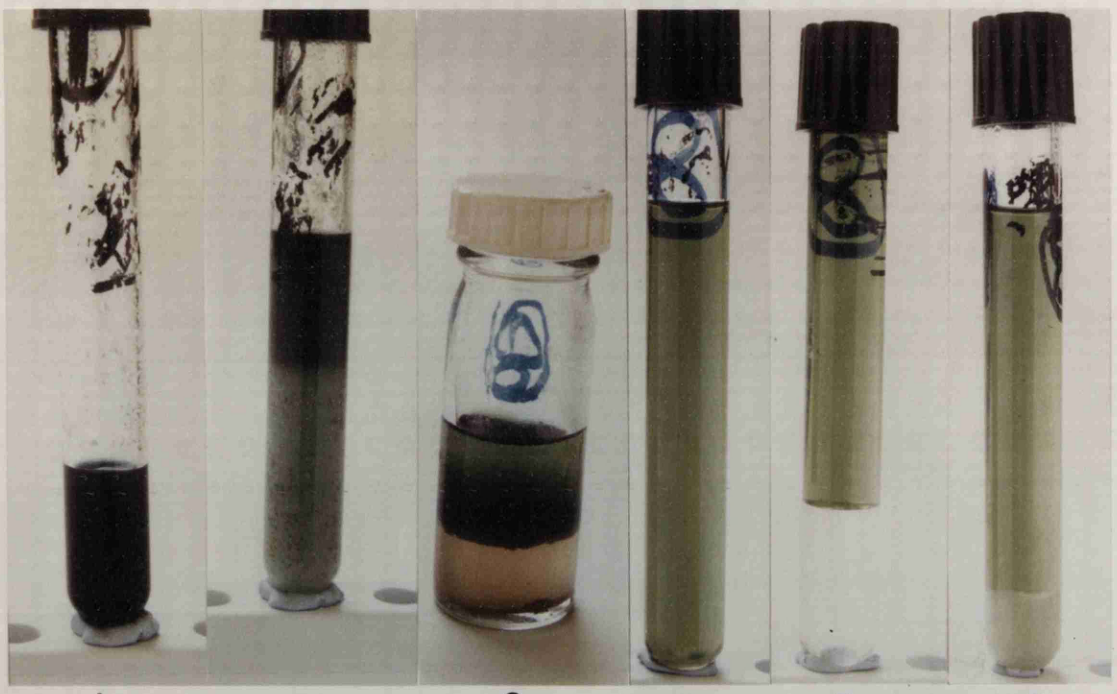
↓

+0.5ml HEXANE (J)

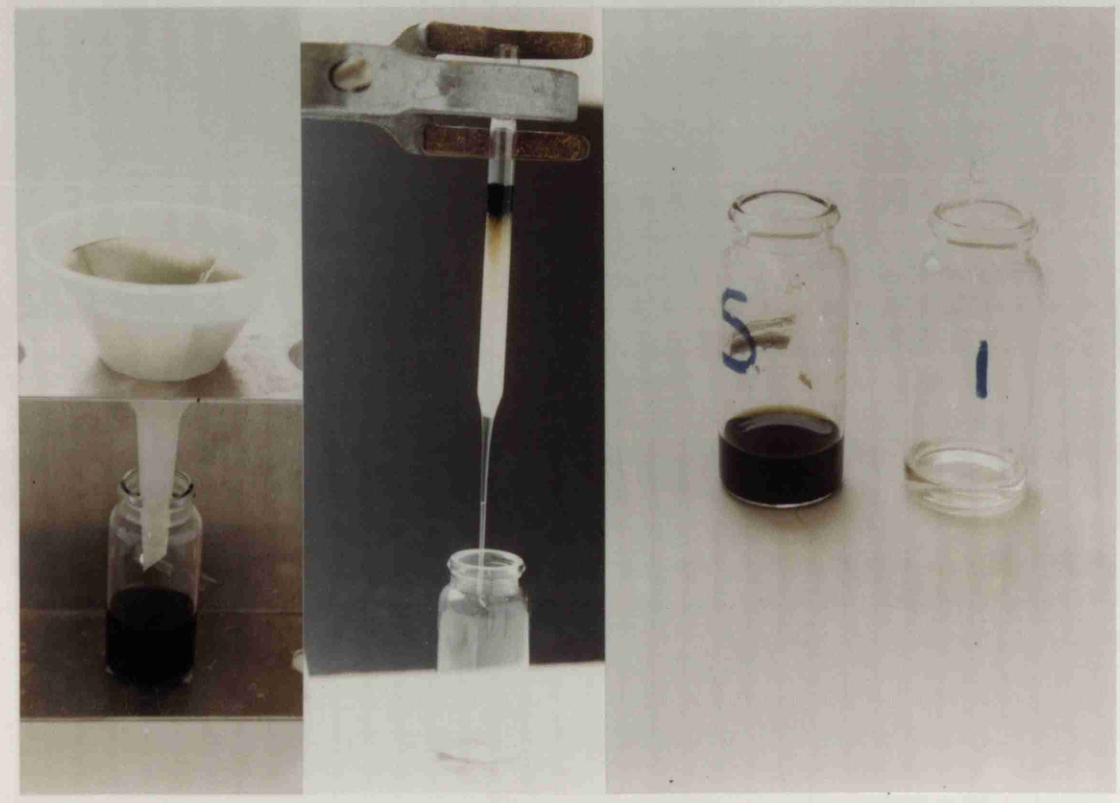
↓

GC ANALYSIS

PLATE 10 FATTY ACID METHODOLOGY SCHEMATIC



A B C D E F



G H J

3.2 IDENTIFICATION AND CONFIRMATION OF FATTY ACID METHYL ESTERS

3.2.1 Known Standards

Comparison to known standards was used for identification, the following standards being utilised:

Sigma: Lipid standard (189-6), Lipid standard (189-11), Linolenic acid methyl ester (L2626), γ-linolenic acid methyl ester (L6503), Palmitic acid methyl ester (P0750), 11,14 - Eicodienoic acid methyl ester (E7877), 8,11,14 - Eicotrienoic acid methyl ester (E3511).

Greyhound: Lipid mixtures (GHF07), (GHPUFA) and (GHME64).

Also, esterified cod liver oil, the identification of the peaks already known.

3.2.2 Columns and Conditions

Biomass generated from the nitrogen limitation experiments with the four freshwater green algae was analysed by GC using a CP-Sil-88 column under the same conditions as stated in Section 3.1.1. All other algal biomass (nitrogen limitation experiments: cyanobacteria, brackish and marine species and C. caldarium; outdoor minipond experiments: all strains investigated) were analysed by GC using a WCOT fused silica CP-Wax-52 CB column (25m - 0.25nm ID) with a 5m deactivated fused silica precolumn (Chrompack, Cat No. 7713).

GC: United Technologies Packard Model 439 with Hewlett-Packard 3390A Integrator.

GC Conditions: Maximum column temperature 275°C
Detector temperature 300°C
Injector temperature 240°C
Oven - initial 160°C
- final 225°C
- rise (°C/min) 5
Time initial (min) 2
Time final (min) 20
Stability time (min) 1
He Carrier Gas
Head Pressure 100Kpa
Split ratio 100:1
Flow 0.43 ml/min
Total run time 35 min

The CP-Wax-52-CB column was found to give a better separation of fatty acid methyl esters, specifically of C16:3 and C18:1 which coeluted with the CP-Sil-88 column. The samples which were previously analysed using the CP-Sil-88 column were also analysed by GC using a fused silica Silar 5CP Column to quantify the C16:3 and C18:1 components (Christie (1987)).
GC: Carlo Erba Model 4130.

GC Conditions: Maximum column temperature 275°C
Detector temperature 300°C
Injection temperature 60°C
Injector temperature 260°C
Oven - initial 60°C
- final 195°C
- rise (°C/min) 4
Time initial (min) 3
Time final (min) 17
Hydrogen Carrier Gas
Flow 2 ml/min
This was not continued when the column was changed to a CP-Wax-52-CB.

3.2.3 HPLC and Bond Elut

A method was also available to separate fatty acid methyl esters by their degree of unsaturation (Christie,1987). GC samples were dried down under a stream of nitrogen and redissolved in a few drops of dichloroethane.

Samples were then analysed by HPLC using a silver loaded Nucleosil-5SA column (Christie, 1987). A solvent gradient was applied and an analytical run carried out to obtain retention times of peaks. This was then utilized on a preparatory run to obtain samples for GC analysis via a stream splitter.

HPLC Equipment:

Spectra Physics Model 8700 Solvent delivery system
(Spectra Physics Ltd)

ACS Model 750/14 Mass Detector
(Applied Chromotography Systems)

Silver impregnated Nucleosil 5SA Column
(4.6 x 250mm) (HPLC Technology)

Stream Splitter (10:1)

Spectra Physics Integrator SP4270 (Spectra Physics Ltd)

Solvent gradient system used:

	Time (mins)
100% A	0
100% B	40

Solvent A 50% dichloromethane
50% dichloromethane

Solvent B 100ml dichloromethane
100ml dichloroethane
10ml methanol
10ml acetonitrile

10µl injection volume

Fatty acid methyl esters with zero to six double bonds could be resolved (Fig 12) and fractions (1-7) analysed by GC (Christie (1987) for conditions) (Fig 13).

This methodology was initially used for identification of fatty acid methyl esters of the four freshwater green algae and then it was routinely used for confirmation of fatty acid methyl esters until an alternative approach using a Bond Elut System was developed (Christie, per comm. (1988); Christie, 1989b). Again silver ion chromatography was utilized, with silver loaded Bond Elut SCX solid phase extraction columns being used (Analytichem International).

The Silver Loaded Bond Elut SCX Column was prepared as follows:

A solution of 20mg silver nitrate in 0.25ml acetonitrile-water 10:1 (v/v) was allowed to percolate through a Bond Elut SCX column. The column was wrapped in aluminium foil to the top of the bed. The column was washed with acetonitrile (5ml), acetone (5ml) and dichloromethane (10ml). A pipette bulb was found to help by applying slight pressure.

Fig 12: H.P.L.C. CHROMATOGRAM OF A. antarcticus

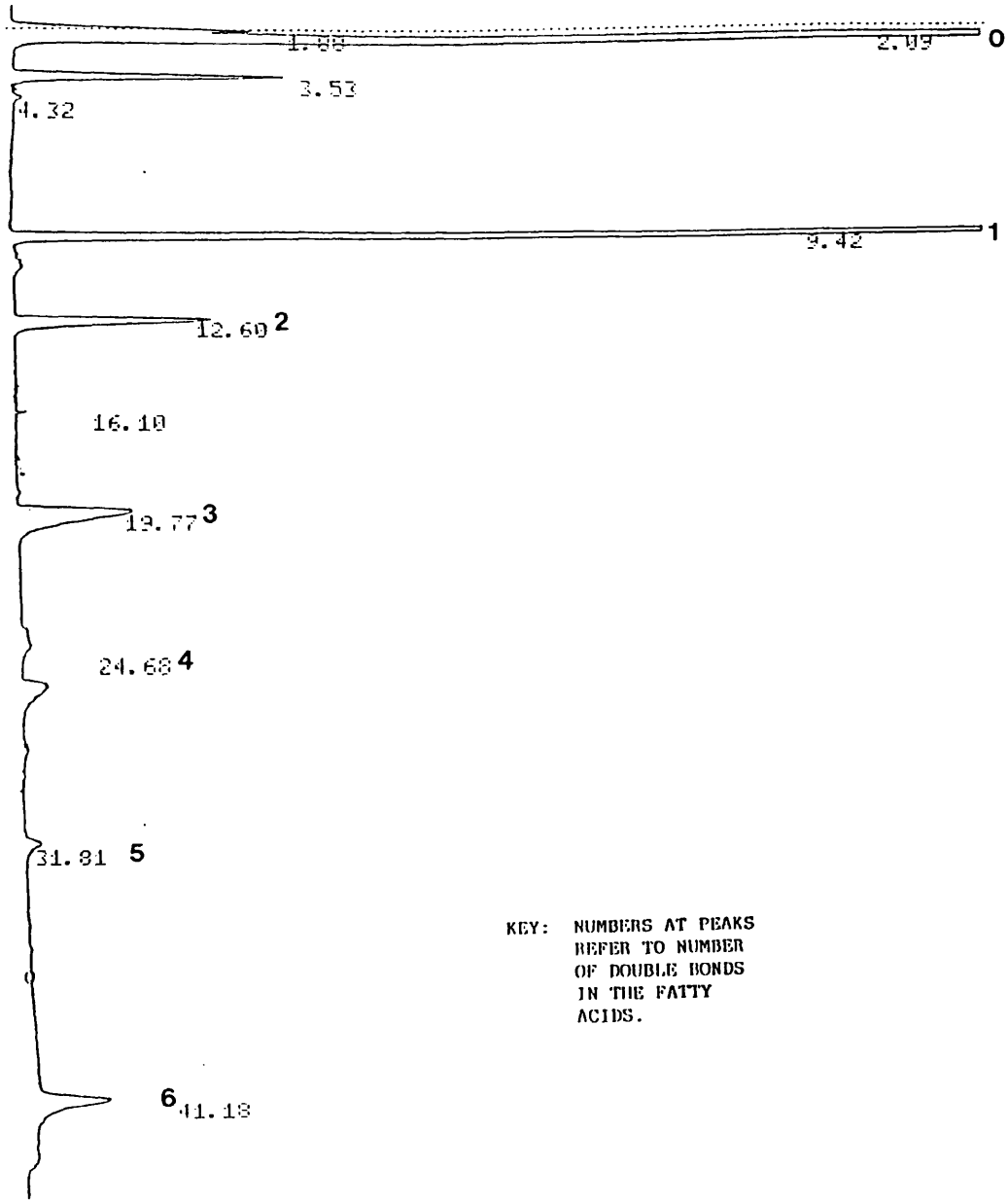
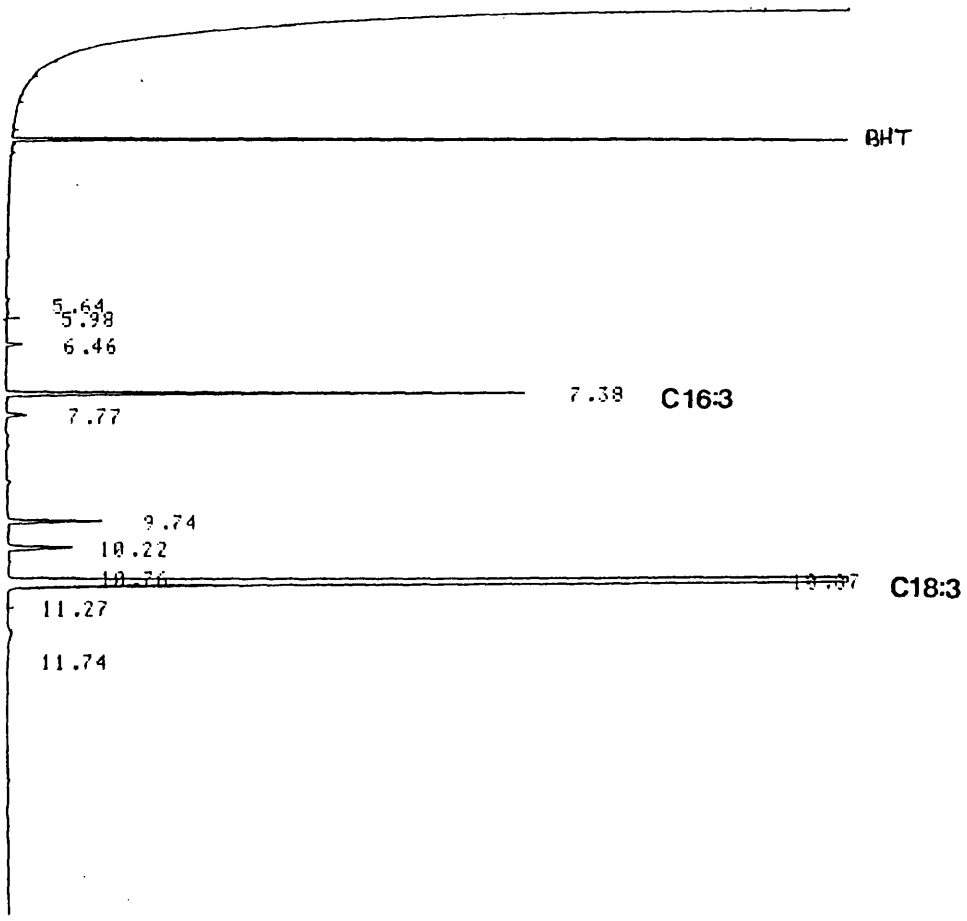


Fig 13: G.C. CHROMATOGRAM : FRACTION 3 FROM A. antarcticus



Elution depending on unsaturation was achieved using a series of solvent mixtures (Christie, 1989b). 0.5mg sample in 50µl dichloromethane was added to the column. Solvent mixtures were used as follows:

Saturated Fatty Acids - 5ml dichloromethane
Monoenes - 4.5ml dichloromethane + 0.5ml acetone
Dienes - 5ml acetone
Trienes - 9.7ml acetone + 0.3ml acetonitrile
Tetraenes - 9.4ml acetone + 0.6ml acetonitrile
Pentaenes - 4.4ml acetone + 0.6ml acetonitrile
Hexaenes - 3ml acetone + 2ml acetonitrile

For reuse (within 1 day), 10ml dichloromethane removed traces of acetonitrile from the column.

Analysis of fractions was by GC using a CP-Wax-52CB column (for GC and conditions, section 3.2.2). This method was equally successful as HPLC and quicker.

3.2.4 Gas Chromatography - Mass Spectrometry

Initial work on confirmation of identification of fatty acid methyl esters by GC and HPLC was carried out by the use of GC-MS for the four freshwater green algae, and was only used sporadically for further work if unusual peaks were found. HPLC and Bond Elut fractions were converted to picolinyl esters and analysed according to Christie et al (1986).

3.2.4.1 Picolinyl ester preparation

Fractions were dried down under a stream of N_2 at 50°C. 2ml of 1M KOH in 90% ethanol was added and samples left overnight at room temperature. 5ml of water and 2.2ml 1M HCl was added and mixed thoroughly. Fatty acids were extracted with 4ml ether: hexane (1:1) and centrifuged (1,500 rpm/2 mins), the ether hexane layer being removed. This was repeated.

8ml extract was washed with 2ml water and again centrifuged. The upper layer was removed and reduced to dryness under a stream of nitrogen at 50°C. 0.5ml trifluoroacetic anhydride was added and samples left for 30 mins at 50°C. The excess reagent was then blown off with nitrogen. 0.2ml HMP-DMAP reagent was added (0.5ml 3-hydroxymethyl pyridine, 100mg 4-dimethyl amino pyridine in 5.0 ml dichloromethane). Samples were left for 3 hours at room temperature. Solvent was then removed under a stream of N_2. 8ml hexane and 4ml water was added, and sample vortexed, centrifuged and separated. 4ml of water was further added and sample vortexed, centrifuged and separated. This was repeated. Solvent was then removed in a stream of nitrogen. 1ml ether and a few mgs of Bond Elut NH2 were added. The sample was left for 10 minutes then shaken and centrifuged. The solvent layer was carefully decanted and evaporated. The residue was redissolved in 1ml hexane (storage) or 0.2 - 0.3ml if used immediately.

4. NITROGEN LIMITATION EXPERIMENTS

4.1 INTRODUCTION

An investigation was carried out into the effects of nitrate depletion, temperature shift and growth phase on the biochemical content of a range of algae.

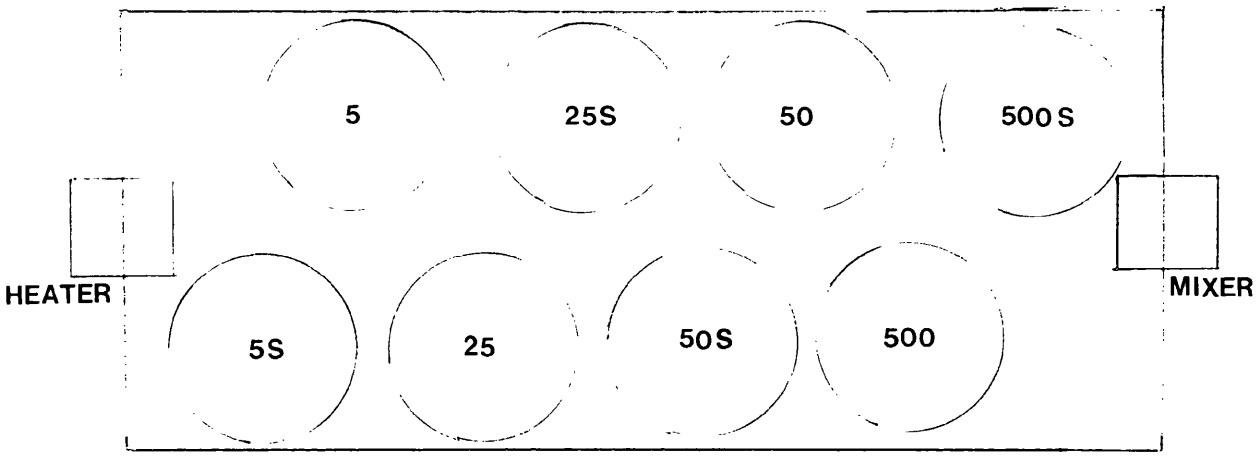
A multifactorial approach was used such that the three variables could be combined to investigate interactions as well as single effects. Four nitrate levels, 5, 25, 50 and 500mg NO_3-N l^{-1} or mg NH_4-N l^{-1} (2.1.2.1), three temperatures (17, 30 and 40°C) and two growth phases (exponential and stationary) were investigated utilizing thirteen different strains of algae (2.1) in a series of experiments using the Batch Culture System (2.2.1).

4.2 EXPERIMENTAL DESIGN

In order to obtain aseptic experimental conditions, the Batch Culture System was broken down into suitable components for autoclaving: (i) individual growth bottles (8 per tank) were filled with media (900 ml), sealed with a cotton wool bung and autoclaved (121°C/15 mins) (ii) inlet and outlet bottle tubing and bung (per bottle) were wrapped separately and autoclaved (121°C/15 mins) (iii) The outlet manifold was wrapped and autoclaved (121°C/15 mins).

After autoclaving and cooling, the system was assembled aseptically.

A set bottle pattern was chosen and maintained for every experiment to allow comparison of results. Two bottles at each of the four nitrogen levels, 5, 25, 50, 500 were placed in a tank per algal or cyanobacterial species. This allowed for up to four algal species to be grown simultaneously at the same temperature.



After assembly the heaters, mixers, lights and air supply (4.5 litres min^{-1} per 16 bottles) were switched on and the system allowed to equilibriate overnight at the required experimental temperature (17°C, 30°C or 40°C).

A suitable inoculum, usually 1ml, was then aseptically pipetted into each culture bottle to give initial cell numbers of 10^4 cells ml^{-1} for each species (2.1.3.1). An initial sample was taken after inoculation of all bottles, from the bottles chosen for sampling (bottles marked (S) on previous diagram), and thereafter every 3-4 days, deemed to be suitable from previous work on cell inoculum levels where a rough time scale for the growth of the four green algae was 30 days. A 25ml sample was found to be sufficient for all analysis.

OD_{560} (2.3.2), dry weight (2.3.3), pH (2.3.5) nitrate/nitrite (2.3.6) and ammonia (2.3.7) determinations were carried out on each sample. For nitrogen fixing cyanobacterial species, observations were made using a Leitz microscope for the presence of heterocysts.

A suitable harvesting regime was determined from the results of OD_{560}, dry weight and nitrate/nitrite levels, enabling exponential and stationary growth phases to be identified. Nitrogen depletion (< 0.1 mg NO_3-N l^{-1}) usually preceded stationary phase.

Harvesting at 5 mg NO_3/NH_4-N l^{-1} occurred only in stationary phase (2 bottles) for all species although the cyanobacterial nitrogen fixers were left after nitrogen depletion to observe changes in biochemical composition due to nitrogen fixation. Harvesting at 25mg NO_3/NH_4-N l^{-1} and 50mg NO_3/NH_4-N l^{-1} occurred in exponential and stationary phases (1 bottle per phase), again the cyanobacterial nitrogen fixers being left after nitrogen depletion. At 500 mg NO_3/NH_4-N l^{-1} all bottles were harvested together (2 bottles) in exponential phase once 5, 25 and 50 mg NO_3/NH_4-N l^{-1} cultures were harvested as these cultures were never found to reach nitrogen depletion. The harvested material was freeze dried (2.3.9) and carbohydrate (2.3.10), protein (2.3.11), and fatty acid content (3.1.4) determined.

For determination of photosynthetic and dark respiration rates (2.3.13), cultures were grown under similar irradiance, temperature and inoculum levels, but only at 25mg NO_3/NH_4-Nl $^{-1}$ using the Batch Culture System. Samples were taken at the same time as previous harvesting times for the nitrogen limitation experiments. OD_{560} (2.3.2), dry weight (2.3.3), nitrate (2.3.6) and ammonia (2.3.7) presence was also determined.

4.3 RESULTS AND STATISTICAL ANALYSIS

For all results in this section, 5, 25, 50 and 500 refer to initial nitrogen levels of 5, 25, 50 and 500mg NO_3-N l^{-1} or mg NH_4-N l^{-1} for C. caldarium results. Letters after 5, 25, 50, 500 ie E, S, S/LE and A refer to Exponential, Stationary, Stationary/Late Exponential growth phases and After nitrogen depletion respectively. For example, 50S, refers to 50mg NO_3-N l^{-1} initial nitrogen level -stationary phase.

4.3.1 Freshwater Green Algae

4.3.1.1 Growth and Nitrate Results

C. vulgaris 211/8K and C. vulgaris 211/11c were both found to grow at the three experimental temperatures whereas Ank. antarcticus 202/25 and S.

obliquus 276/3A did not grow at 40°C. Results for OD_{560} against time (C vulgaris 211/8K - Figs 14-16; C. vulgaris 211/11c - Figs 17-19; Ank. antarcticus 202/25 - Figs 20 and 21; S. obliquus 276/3A - Figs 22 and 23) and dry weight against time (C. vulgaris 211/11c - Figs 24-26; C. vulgaris 211/8K - Figs 27-29; Ank. antarcticus 202/25 - Figs 30 and 31; S. obliquus 276/3A - Figs 32 and 33) show all strains grew significantly faster at 30°C and 40°C than at 17°C, irrespective of initial nitrogen level. All cultures, with the exception of those at 500mg NO_3-N l^{-1}, went into stationary phase. Results of nitrate depletion against time (C. vulgaris 211/8K - Figs 34-36; C. vulgaris 211/11c - Figs 37-39; Ank. antarcticus 202/25 - Figs 40 and 41; S. obliquus 276/3A - Figs 42 and 43) show that nitrate levels were very low (< 1.0 mg NO_3-N l^{-1}), with the exception of S. obliquus 50s at 17°C (1.6mg NO_3-N l^{-1}) and Ank. antarcticus 50s at 17°C (3.4mg NO_3-N l^{-1}), or depleted at stationary phase harvest (Tables 9,10,11,12). Depletion or low levels of nitrate usually preceeded stationary phase. With all strains, depletion of nitrate occurred in the order 5 -> 25 -> 50 mg NO_3-N l^{-1}. None of the cultures grown at 500mg NO_3-N l^{-1} ever attained nitrogen depletion. pH results (C. vulgaris 211/8K - Figs 44-46; C. vulgaris 211/11c - Figs 47-49; Ank. antarcticus Figs 50 and 51; S. obliquus - Figs 52 and 53) all exhibited an increase in pH with exponential growth and decrease with later growth in the pH range 6-11.

FIG14 C.vulgaris 211/8K 17°C
OD 560 vs Time

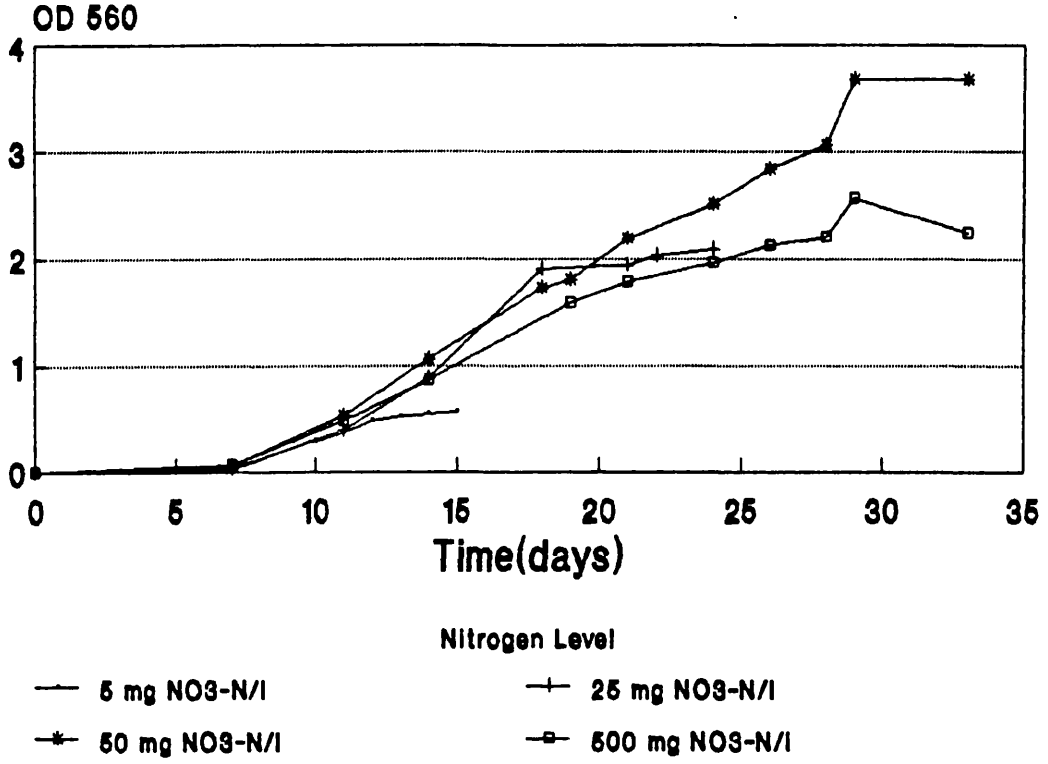


FIG15 C.vulgaris 211/8K 30°C
OD 560 vs Time

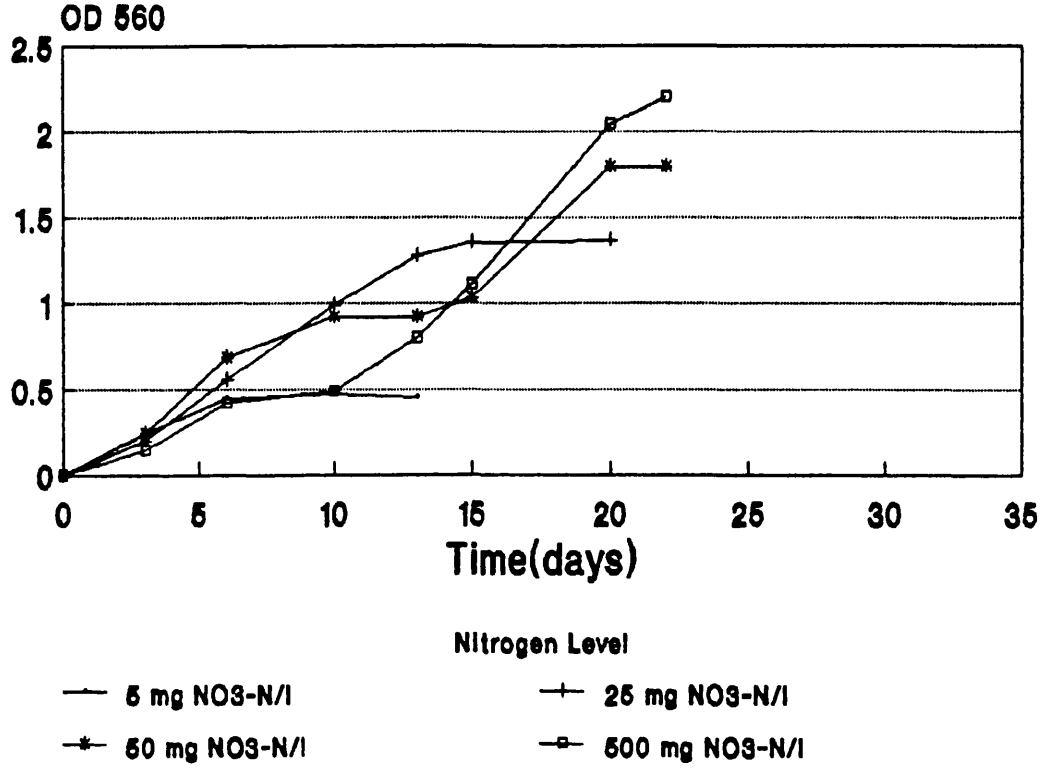


FIG16 C.vulgaris 211/8K 40°C
OD 560 vs Time

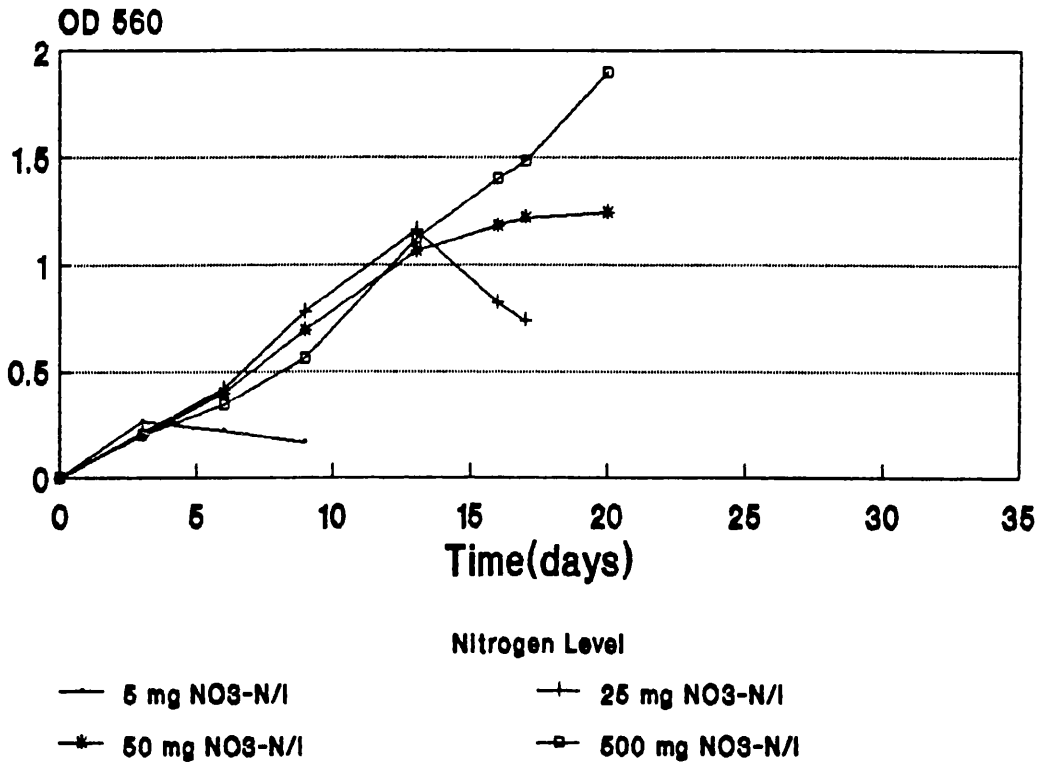


FIG17 C.vulgaris 211/11c 17°C
OD 560 vs Time

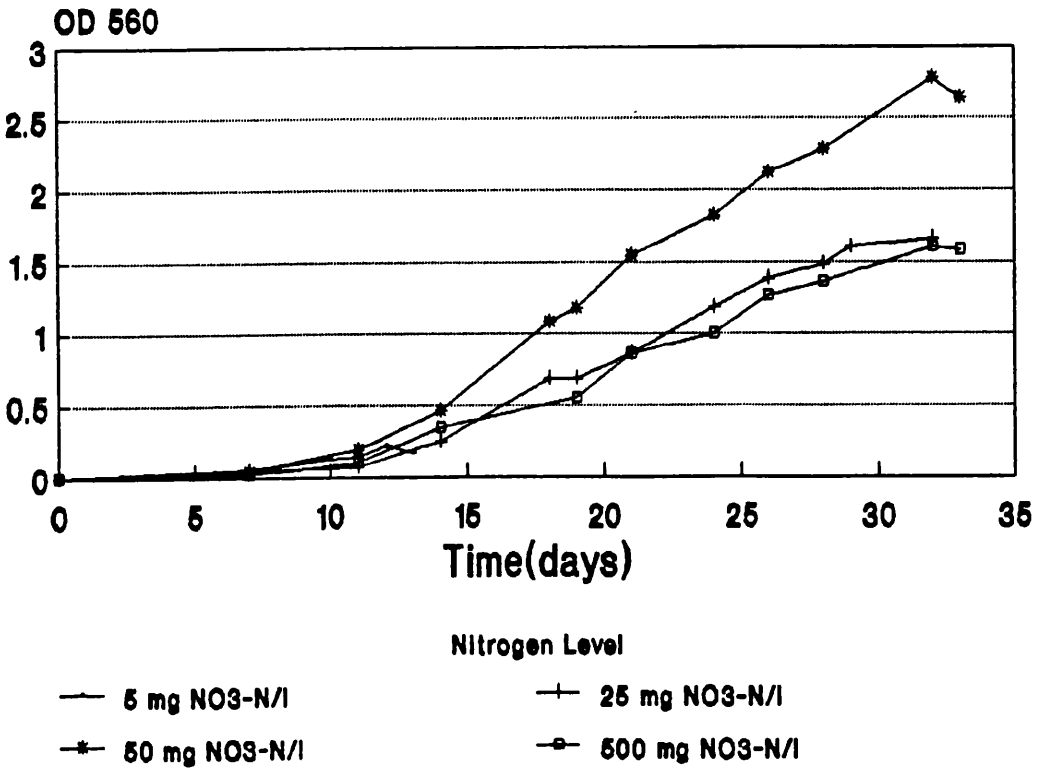


FIG18 C.vulgaris 211/11c 30°C
OD 560 vs Time

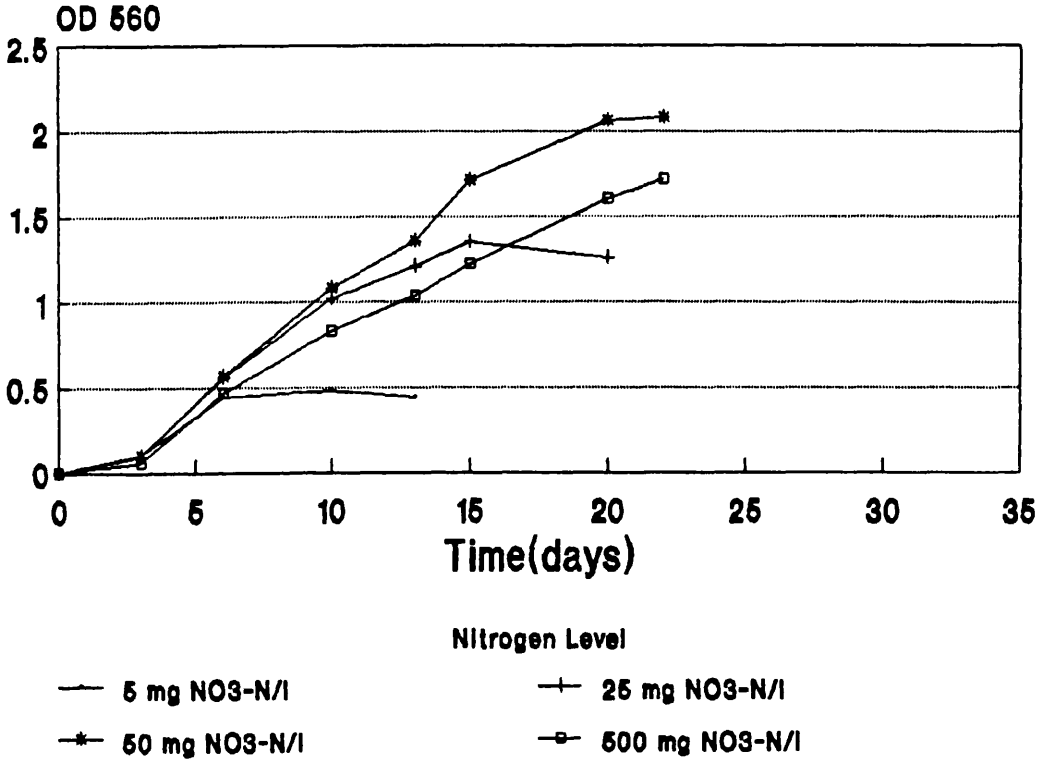


FIG19 C.vulgaris 211/11c 40°C
OD 560 vs Time

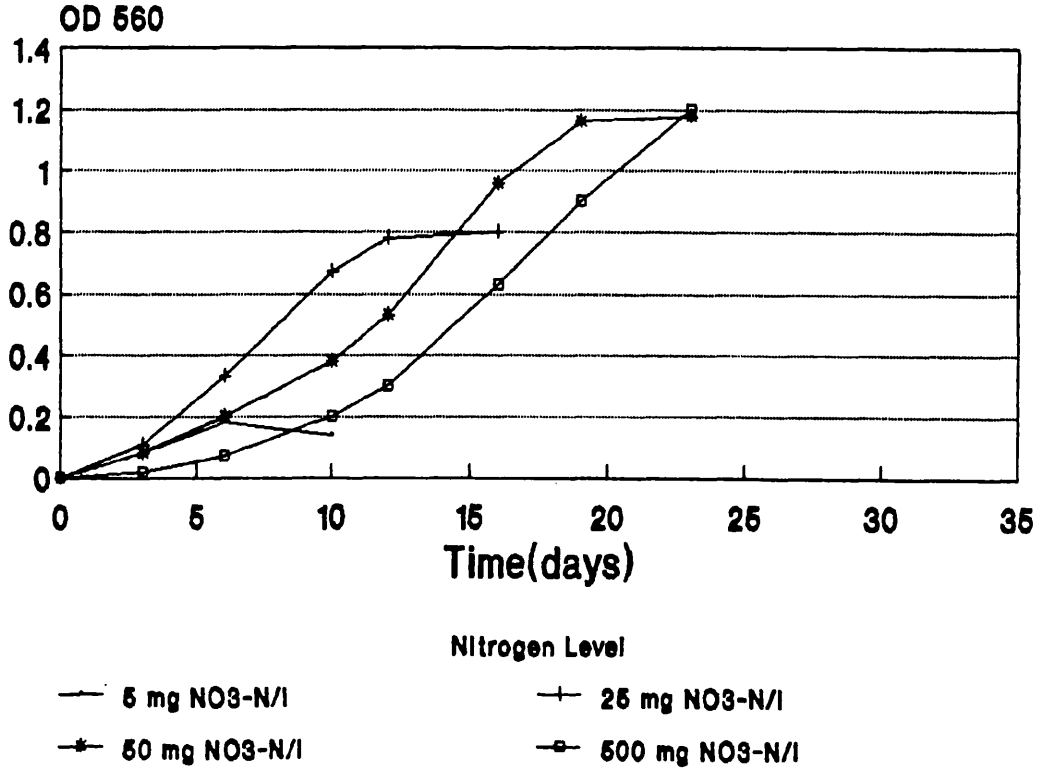


FIG 20 Ank.antarcticus 202/25 17°C
OD 560 vs Time

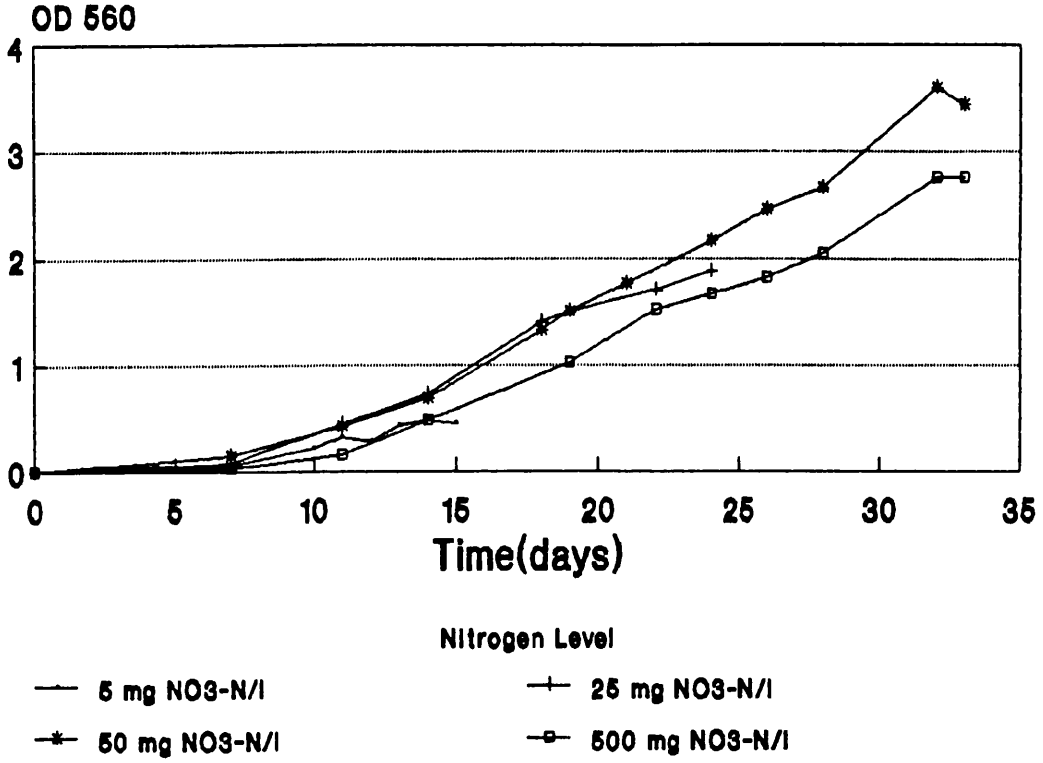


FIG 21 Ank.antarcticus 202/25 30°C
OD 560 vs Time

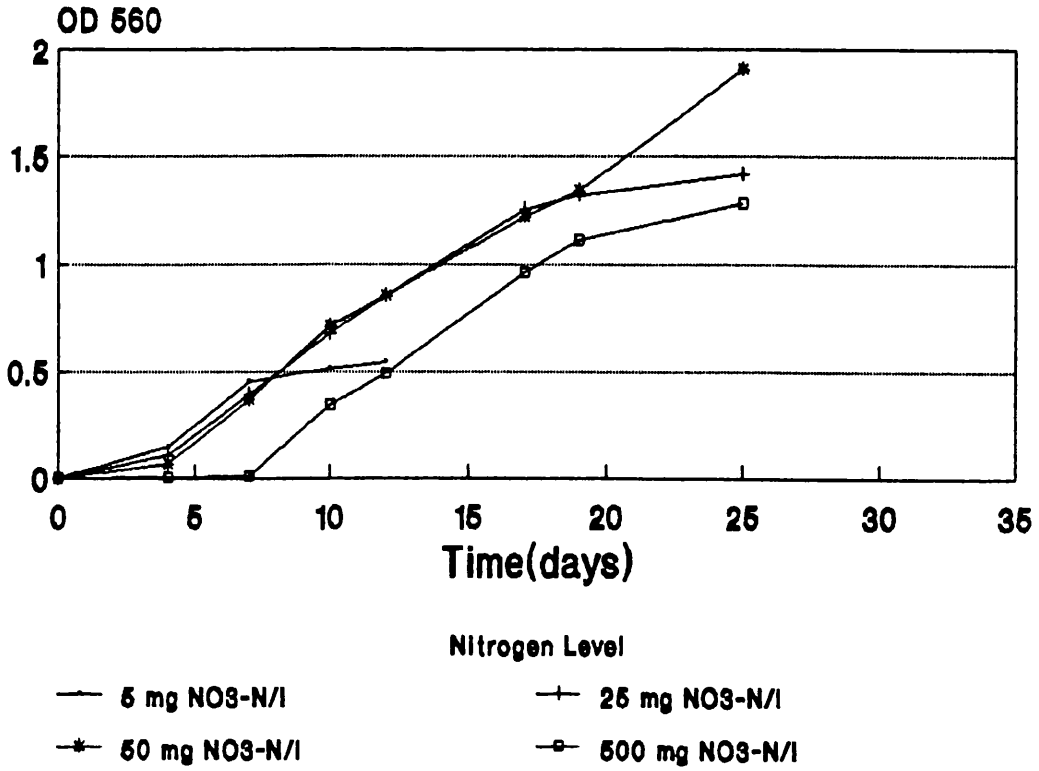


FIG22 S.obliquus 276/3A 17°C
OD 560 vs Time

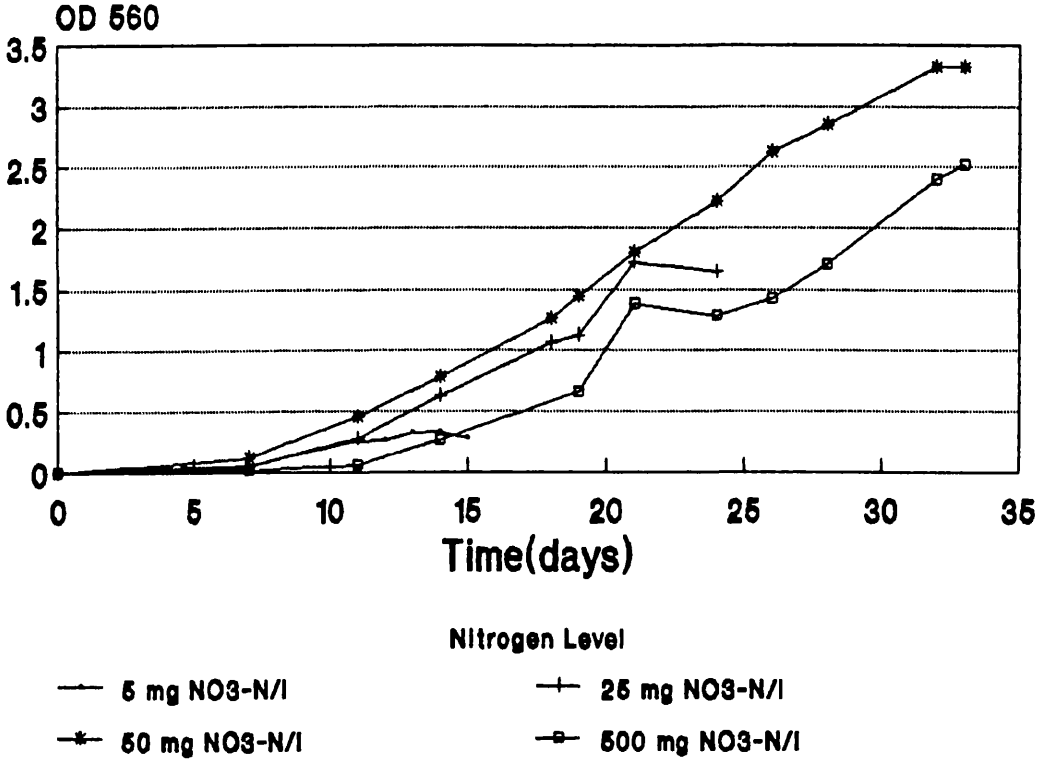


FIG23 S.obliquus 276/3A 30°C
OD 560 vs Time

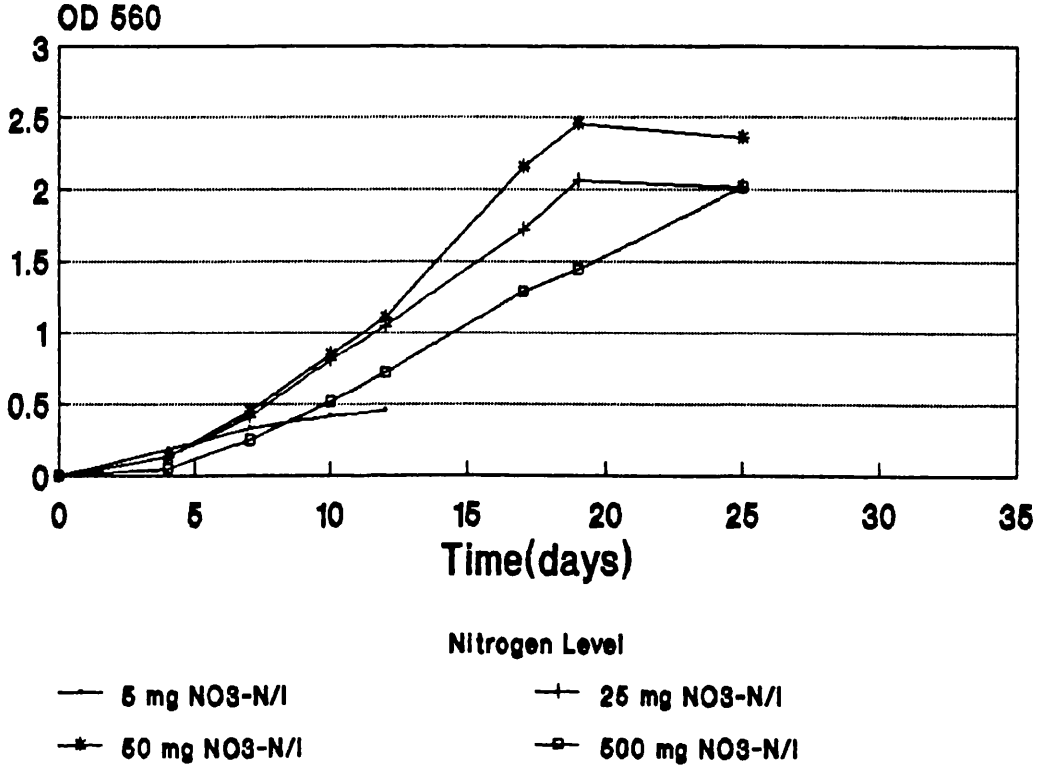


FIG24 C.vulgaris 211/11c 17°C
DRY WEIGHT vs Time

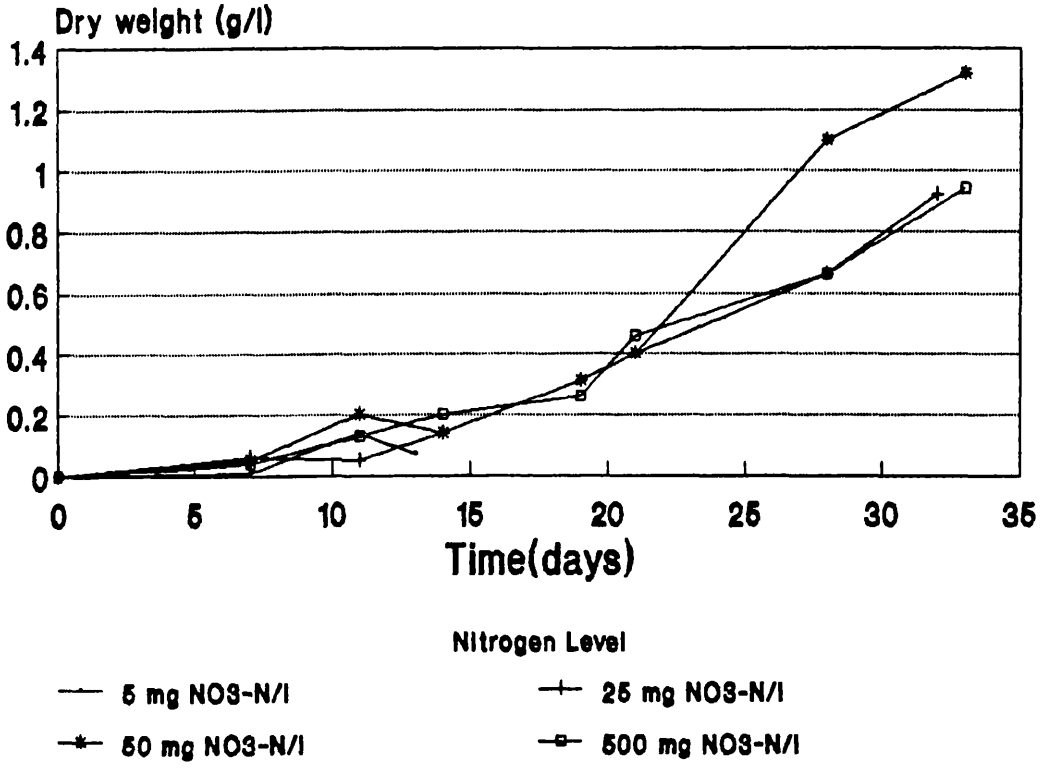


FIG25 C.vulgaris 211/11c 30°C
DRY WEIGHT vs Time

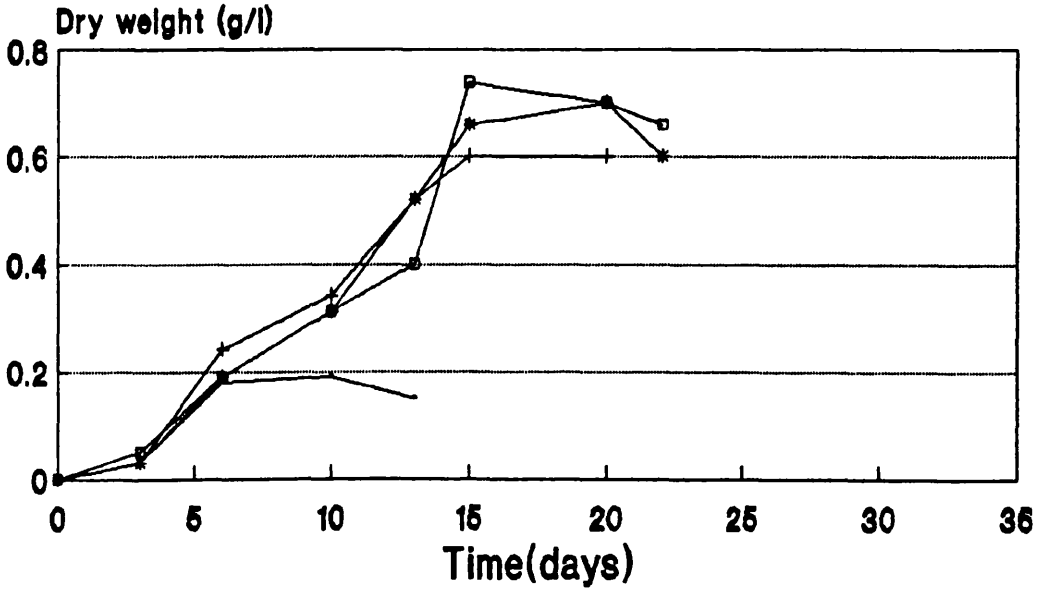


FIG26 C.vulgaris 211/11c 40°C
DRY WEIGHT vs Time

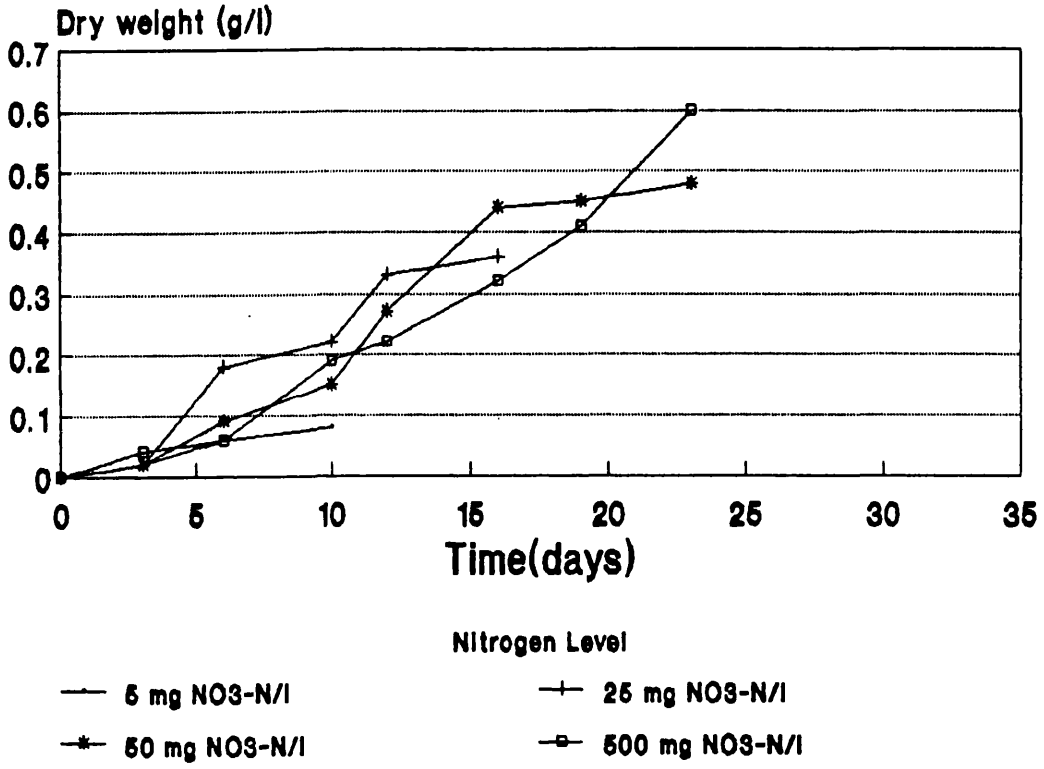


FIG27 C.vulgaris 211/8K 17°C
DRY WEIGHT vs Time

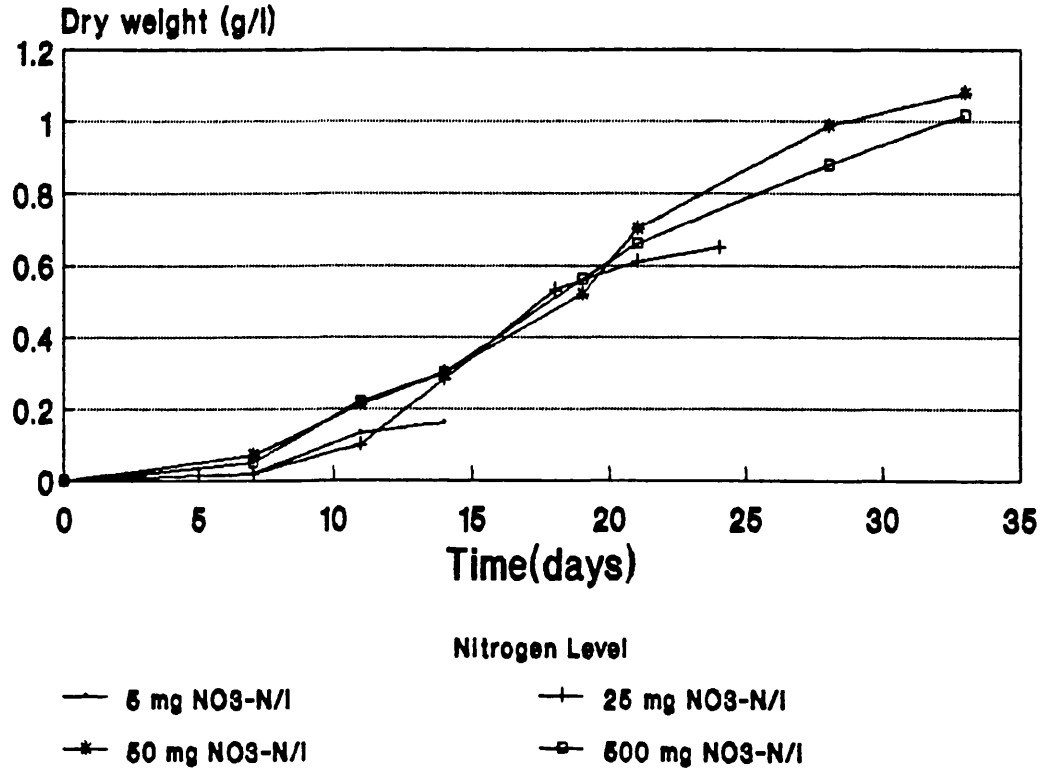


FIG 28 C.vulgaris 211/8K 30°C
DRY WEIGHT vs Time

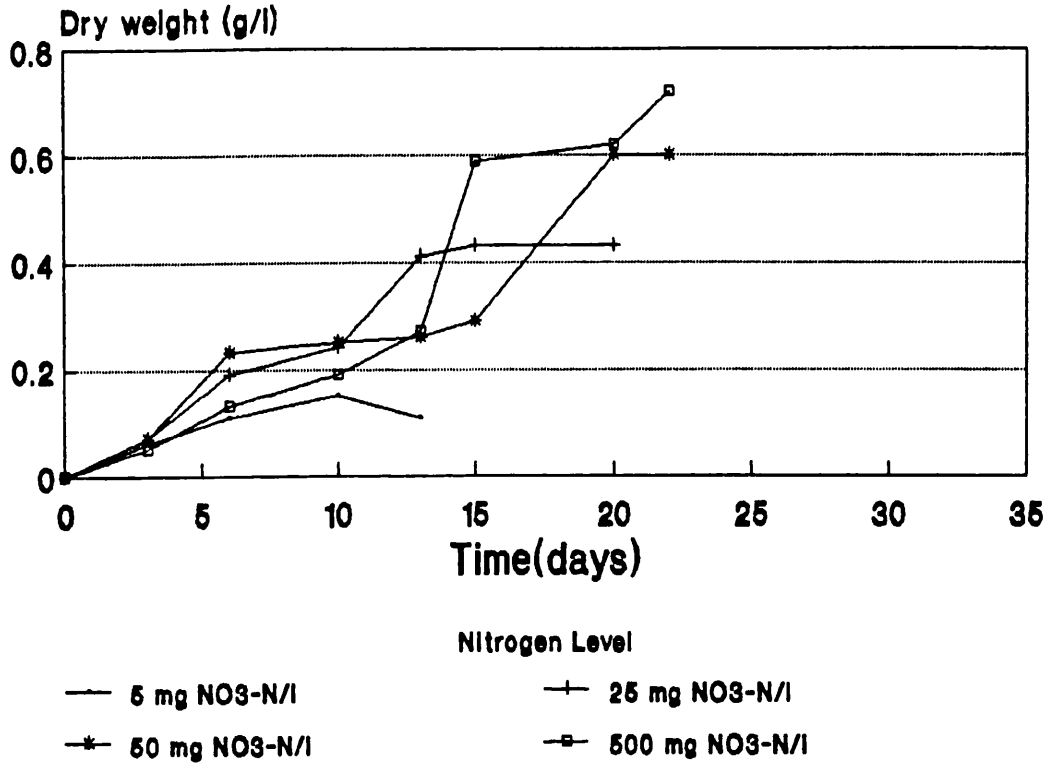


FIG 29 C.vulgaris 211/8K 40°C
DRY WEIGHT vs Time

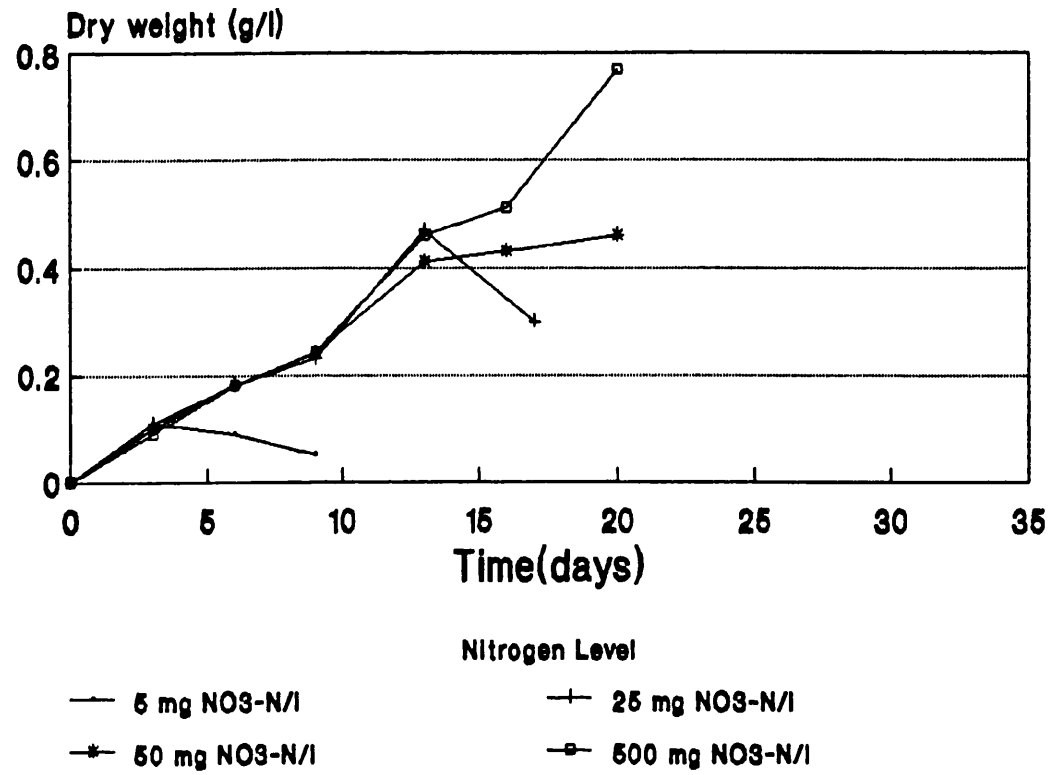


FIG30 Ank.antarcticus 202/25 17°C
DRY WEIGHT vs Time

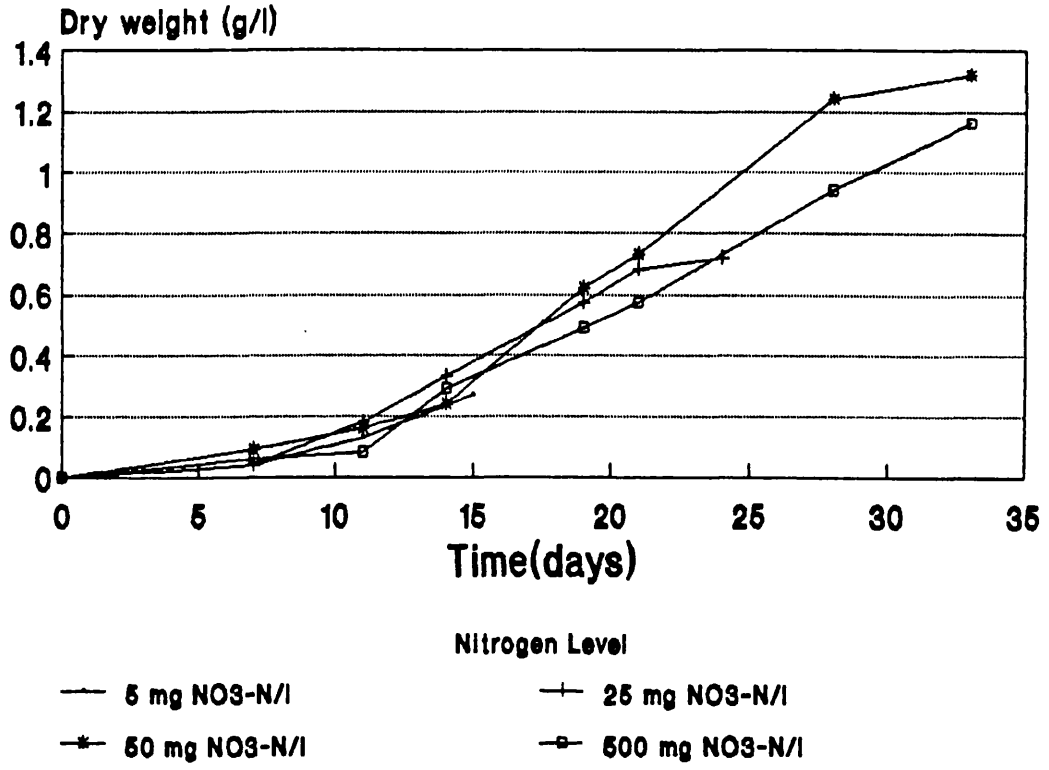


FIG31 Ank.antarcticus 202/25 30°C
DRY WEIGHT vs Time

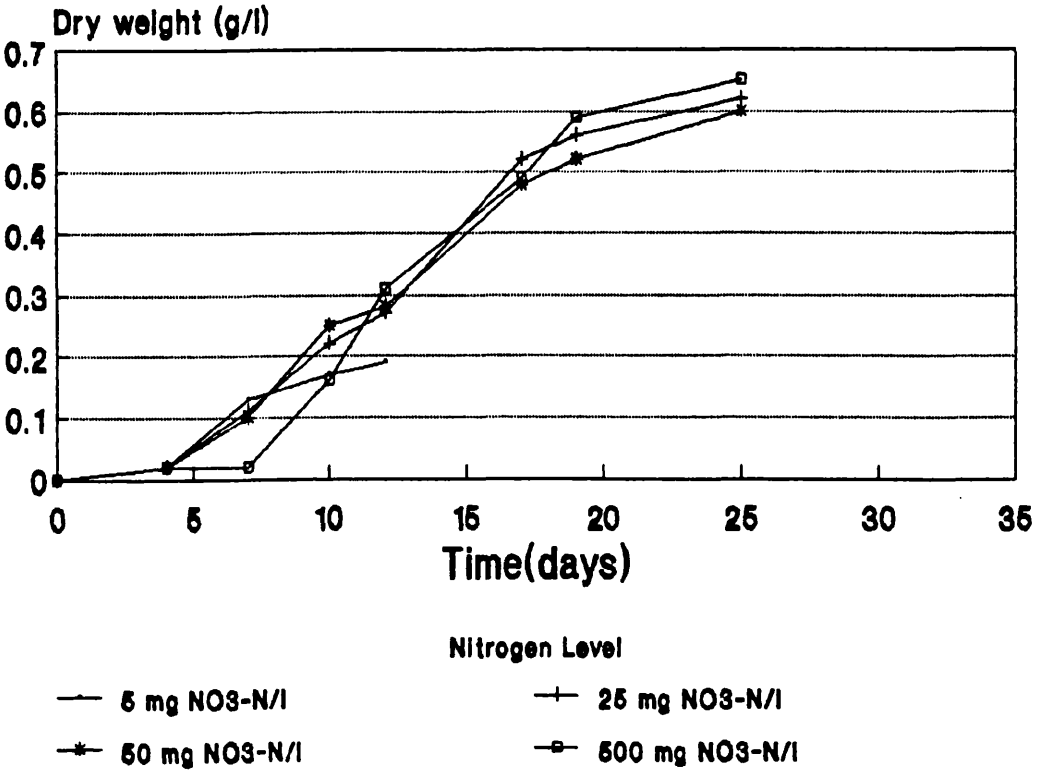


FIG 32 S.obliquus 276/3A 17°C
DRY WEIGHT vs Time

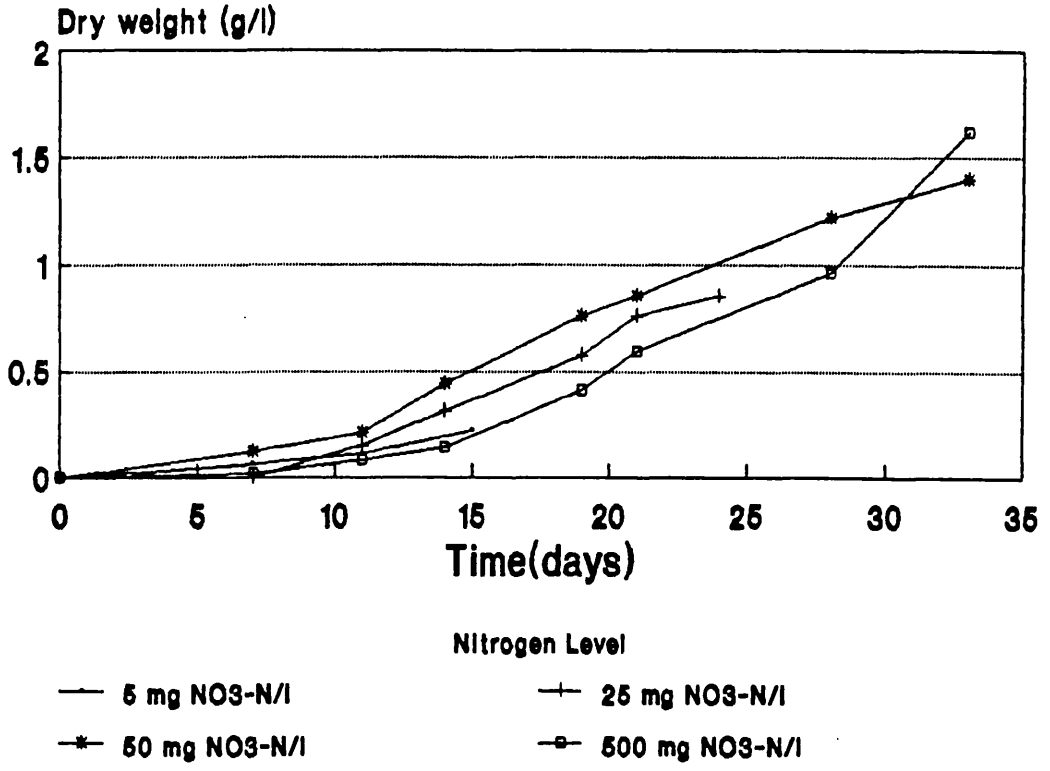


FIG 33 S.obliquus 276/3A 30°C
DRY WEIGHT vs Time

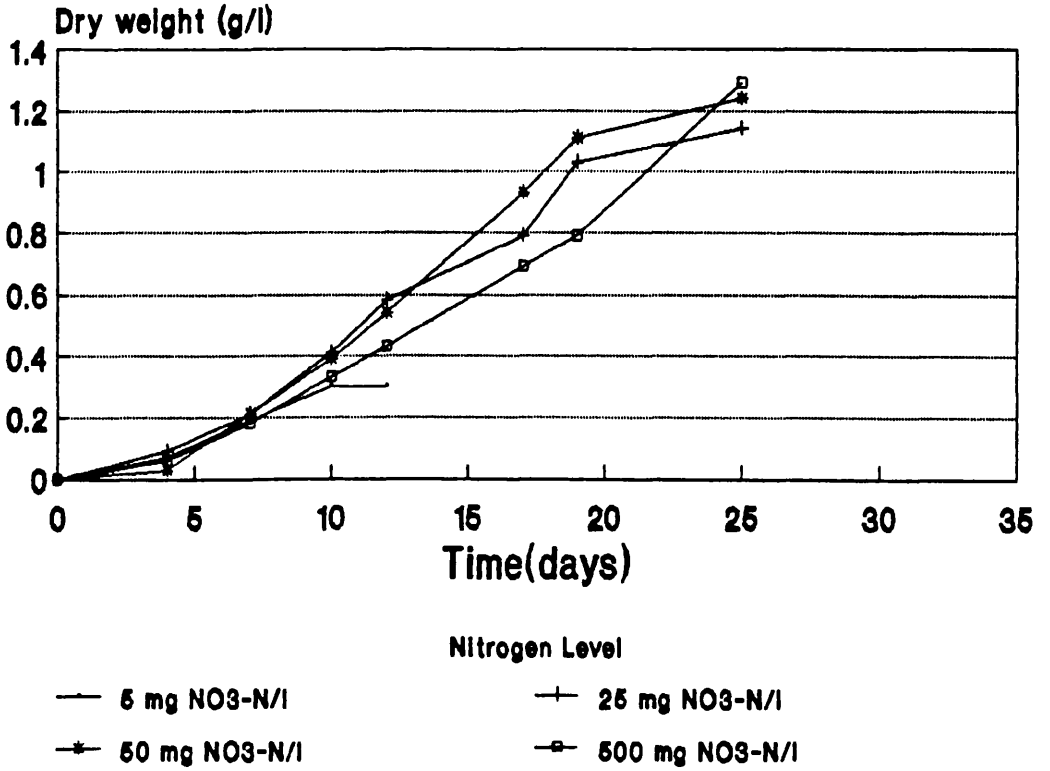
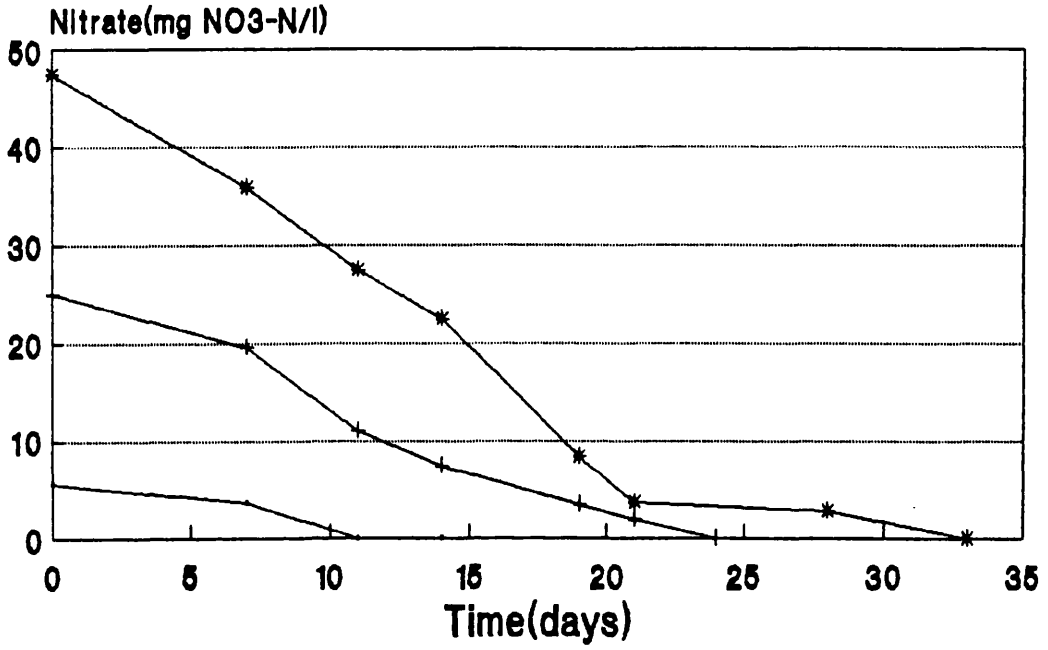


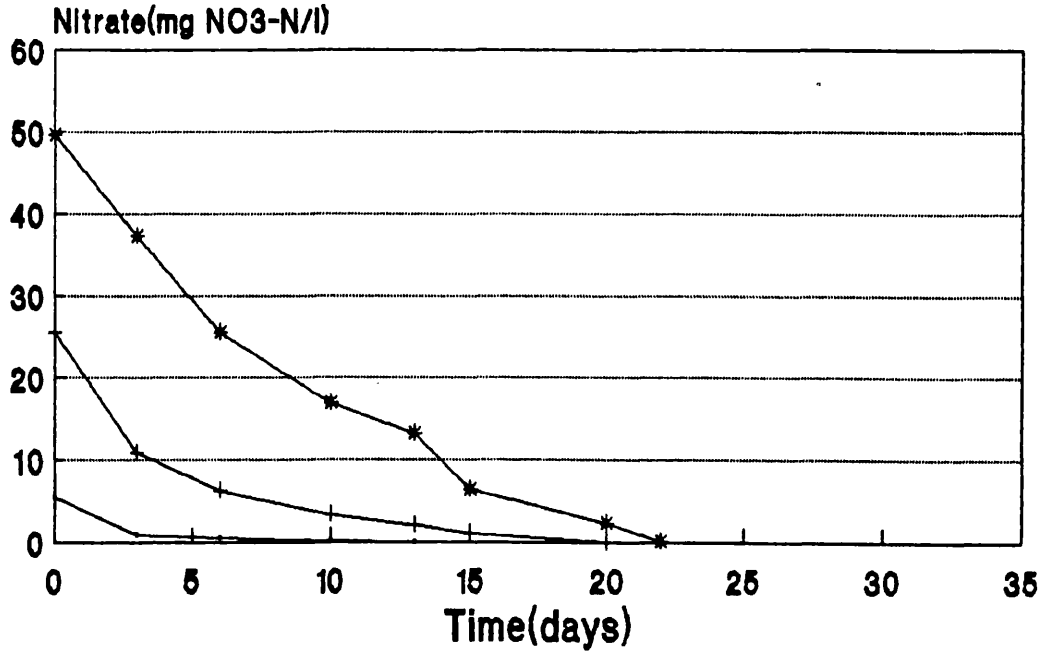
FIG34 C.vulgaris 211/8K 17°C
NITRATE vs Time



Nitrogen Level

5 mg NO3-N/l 25 mg NO3-N/l 50 mg NO3-N/l

FIG35 C.vulgaris 211/8K 30°C
NITRATE vs Time



Nitrogen Level

5 mg NO3-N/l 25 mg NO3-N/l 50 mg NO3-N/l

FIG36 C.vulgaris 211/8K 40°C
NITRATE vs Time

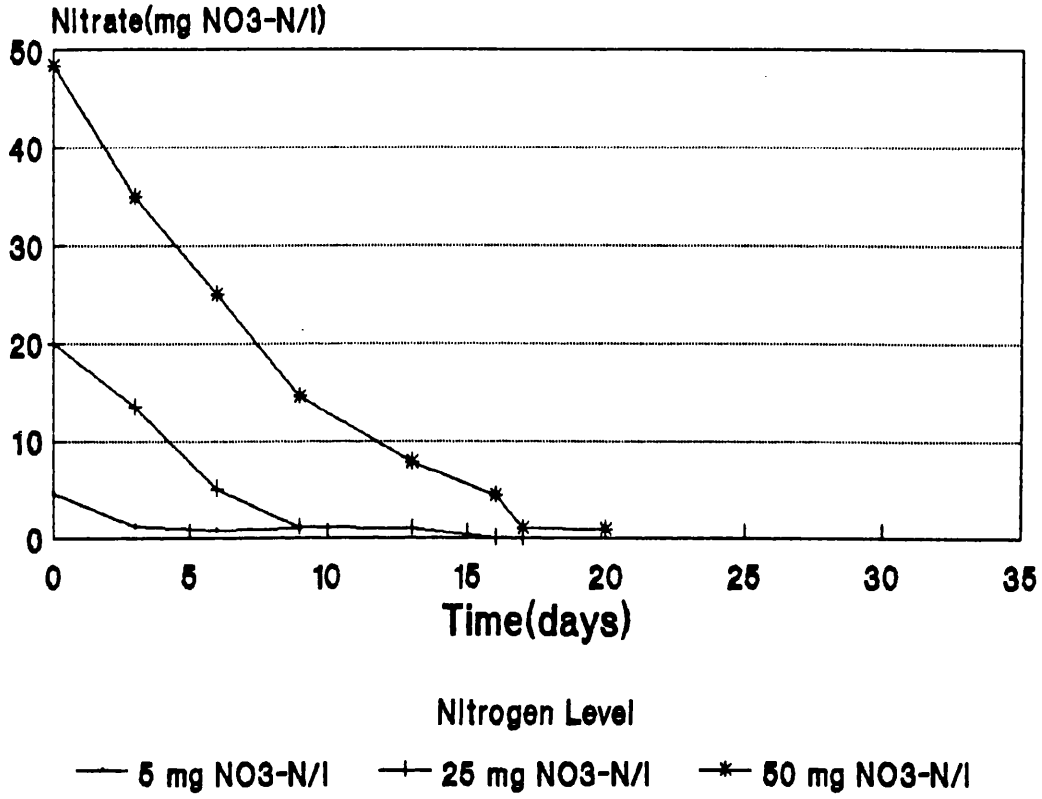


FIG37 C.vulgaris 211/11c 17°C
NITRATE vs Time

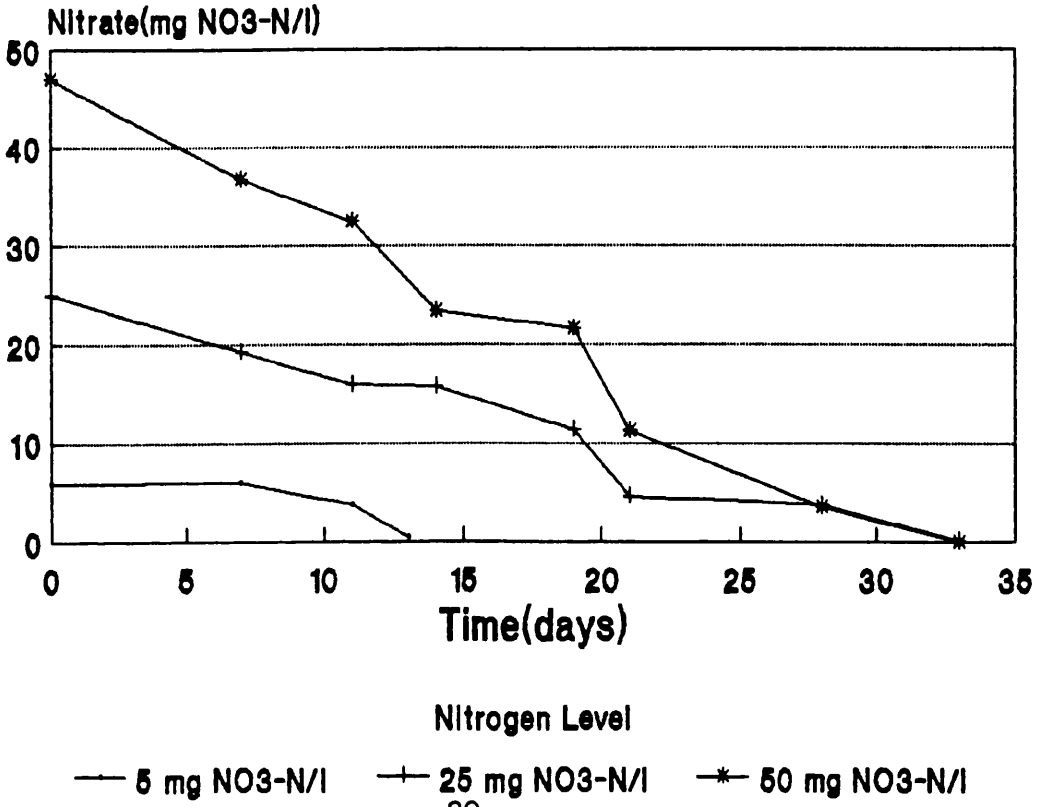


FIG38 C.vulgaris 211/11c 30 C
NITRATE vs Time

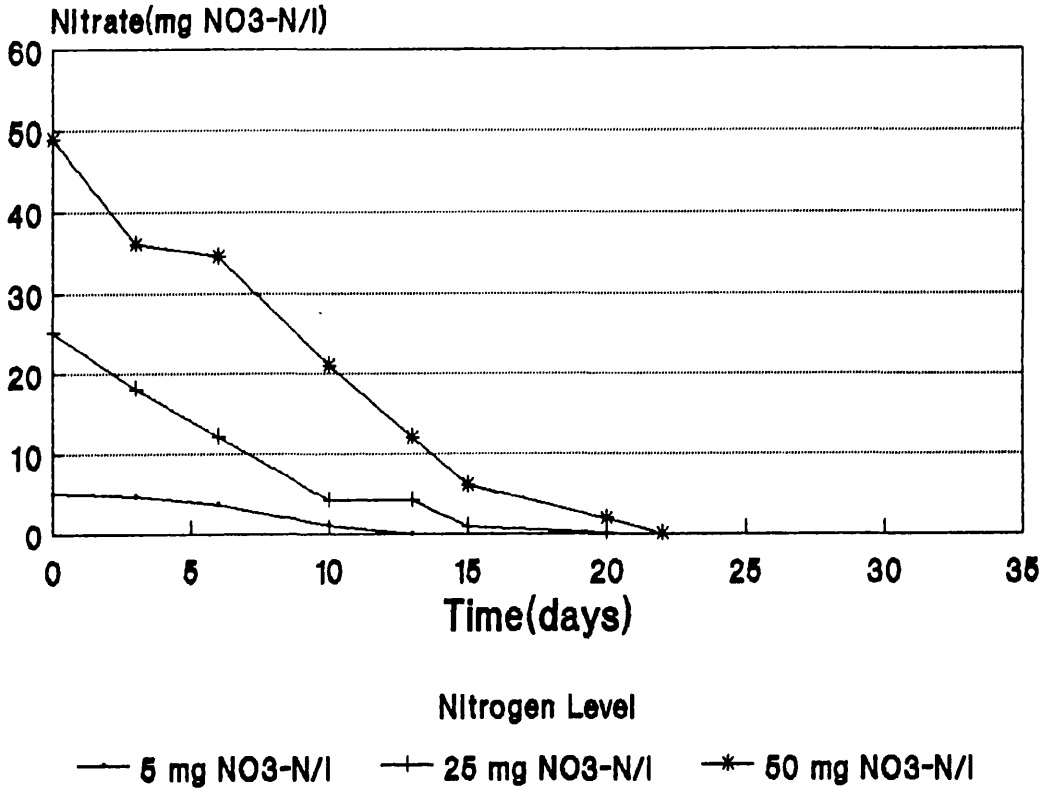


FIG39 C.vulgaris 211/11c 40 C
NITRATE vs Time

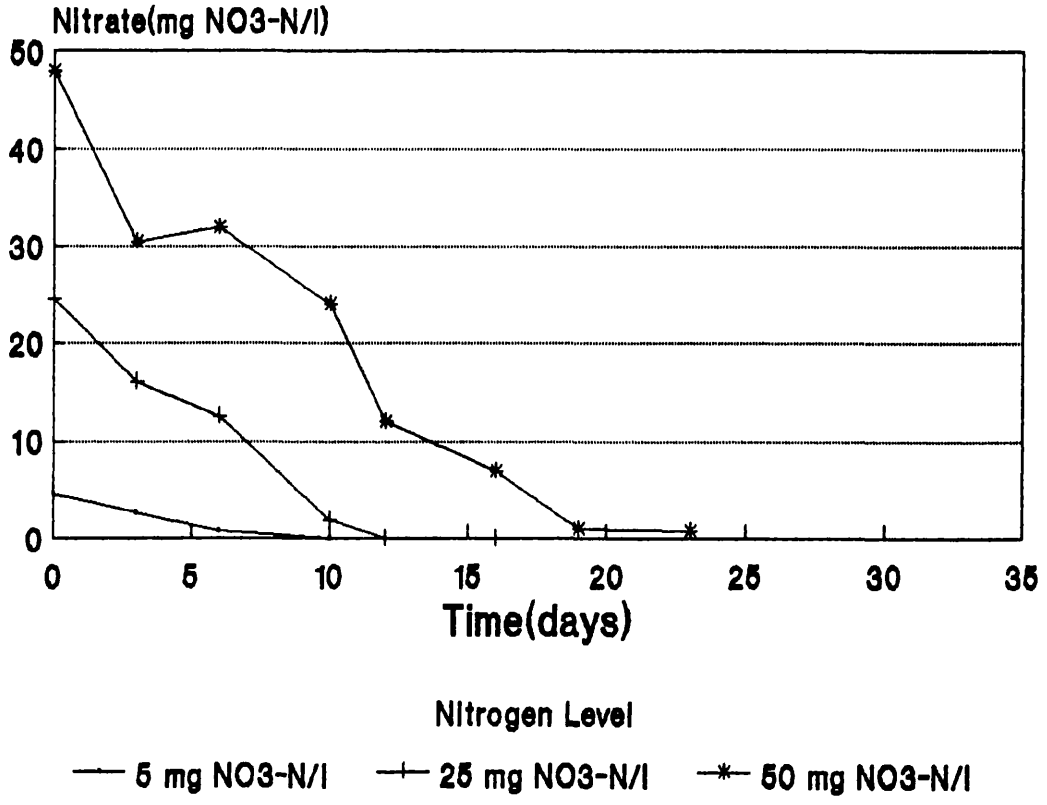
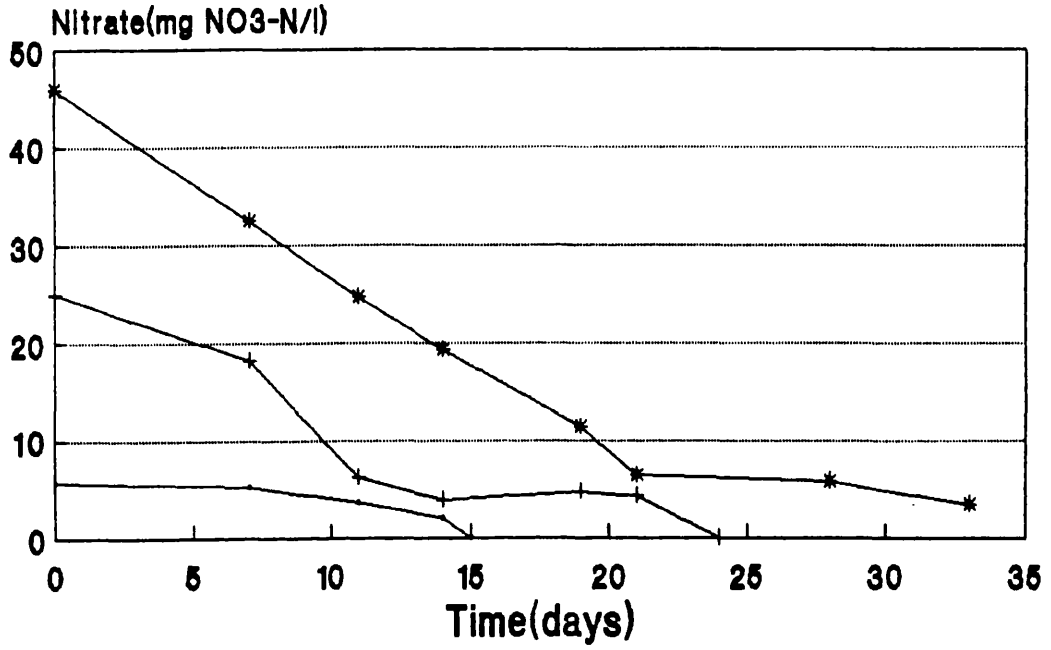


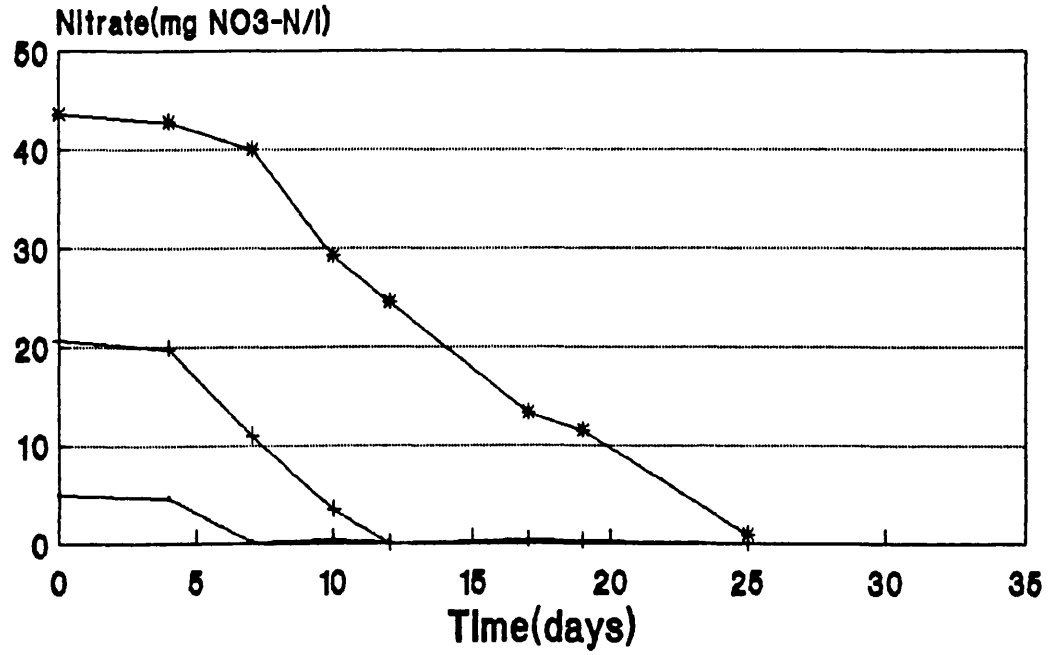
FIG40 Ank.antarcticus 202/25 17°C
NITRATE vs Time



Nitrogen Level

5 mg NO3-N/l 25 mg NO3-N/l 50 mg NO3-N/l

FIG41 Ank.antarcticus 202/25 30°C
NITRATE vs Time



Nitrogen Level

5 mg NO3-N/l 25 mg NO3-N/l 50 mg NO3-N/l

FIG40 Ank.antarcticus 202/25 17°C
NITRATE vs Time

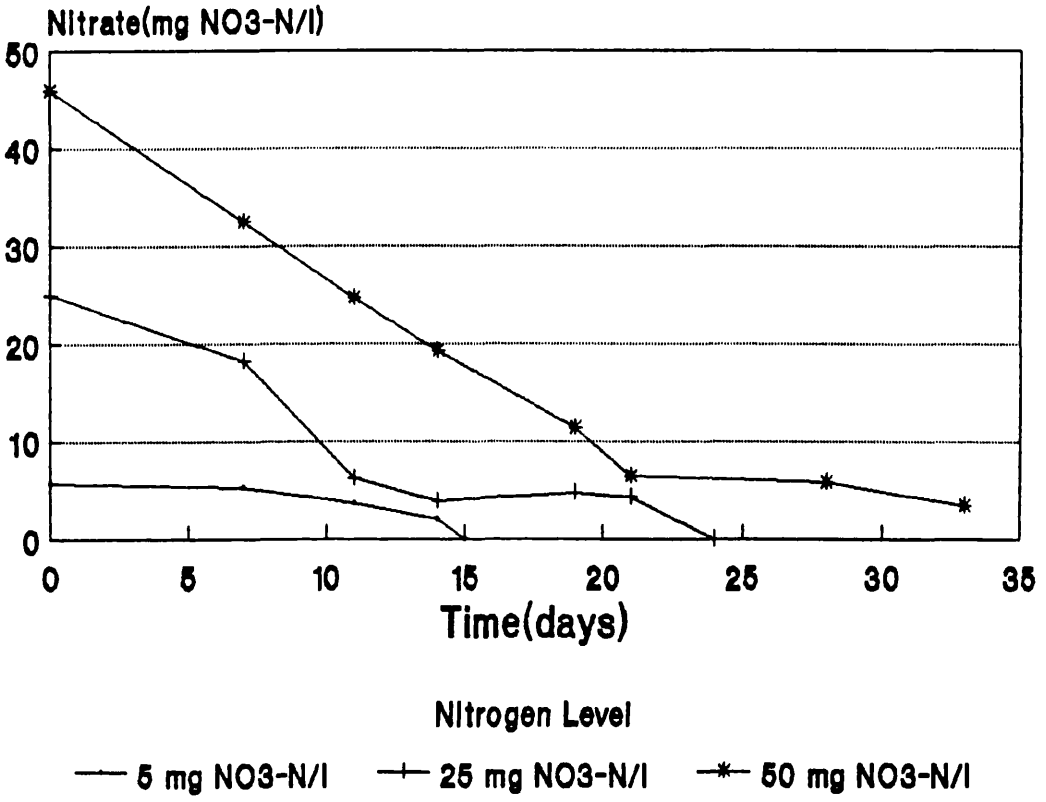


FIG41 Ank.antarcticus 202/25 30°C
NITRATE vs Time

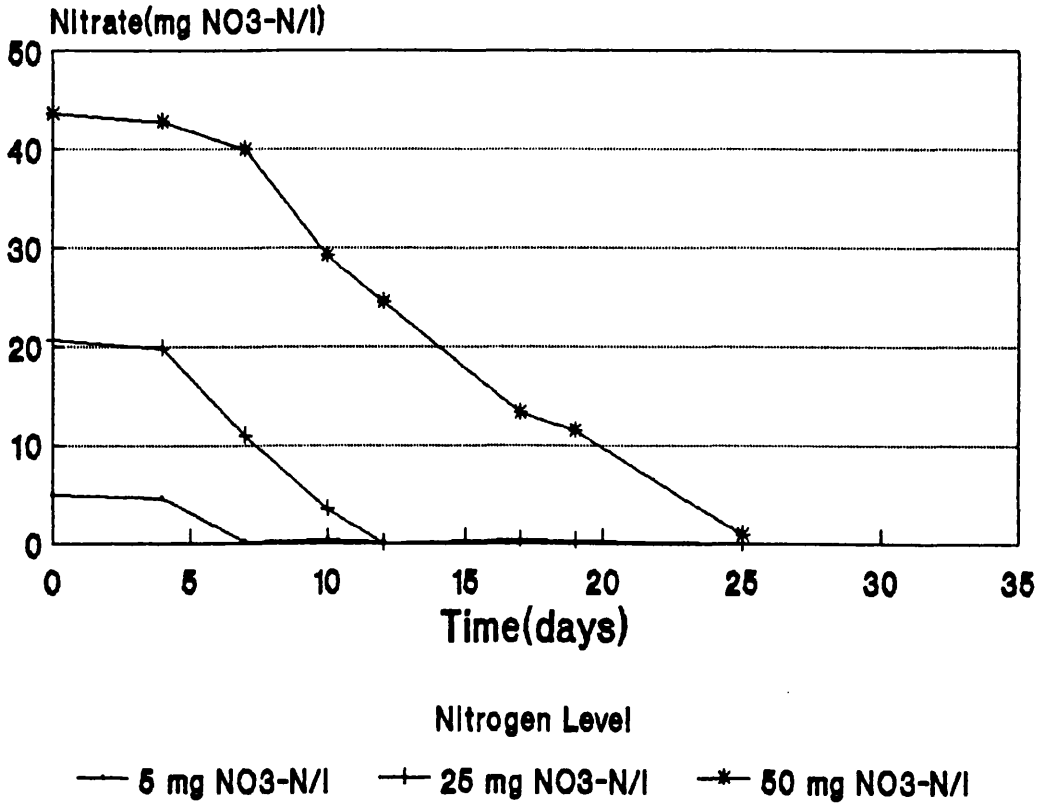


FIG42 S.obliquus 276/3A 17°C
NITRATE vs Time

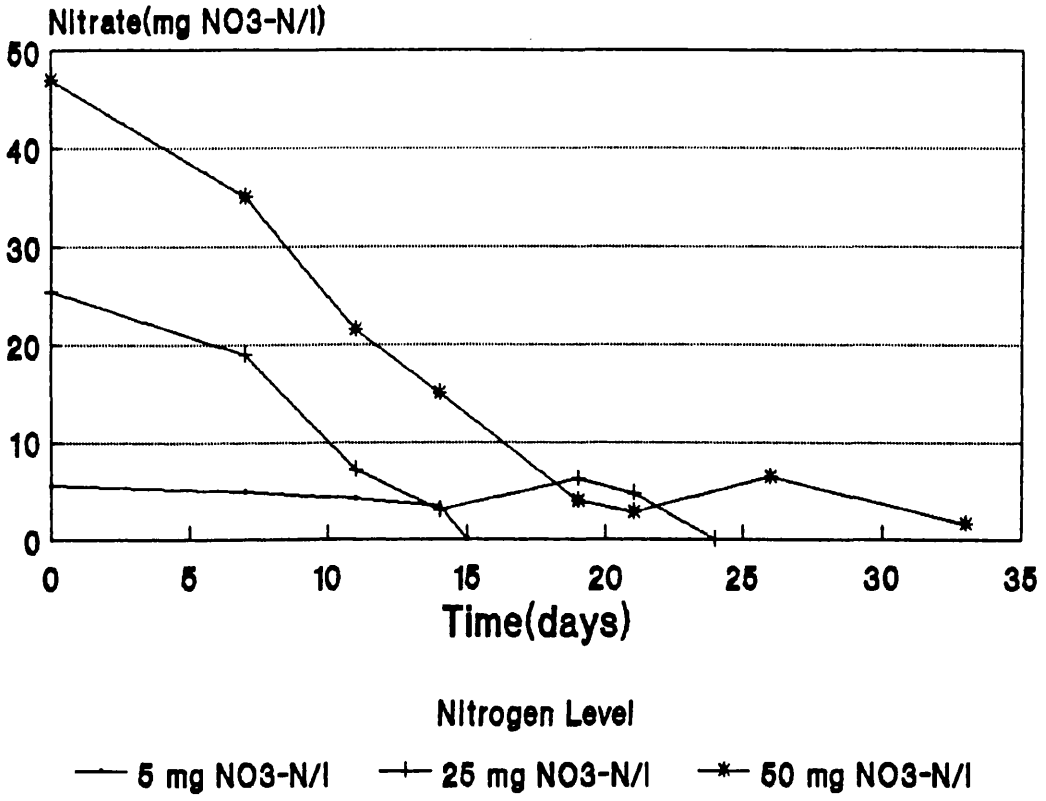


FIG43 S.obliquus 276/3A 30°C
NITRATE vs Time

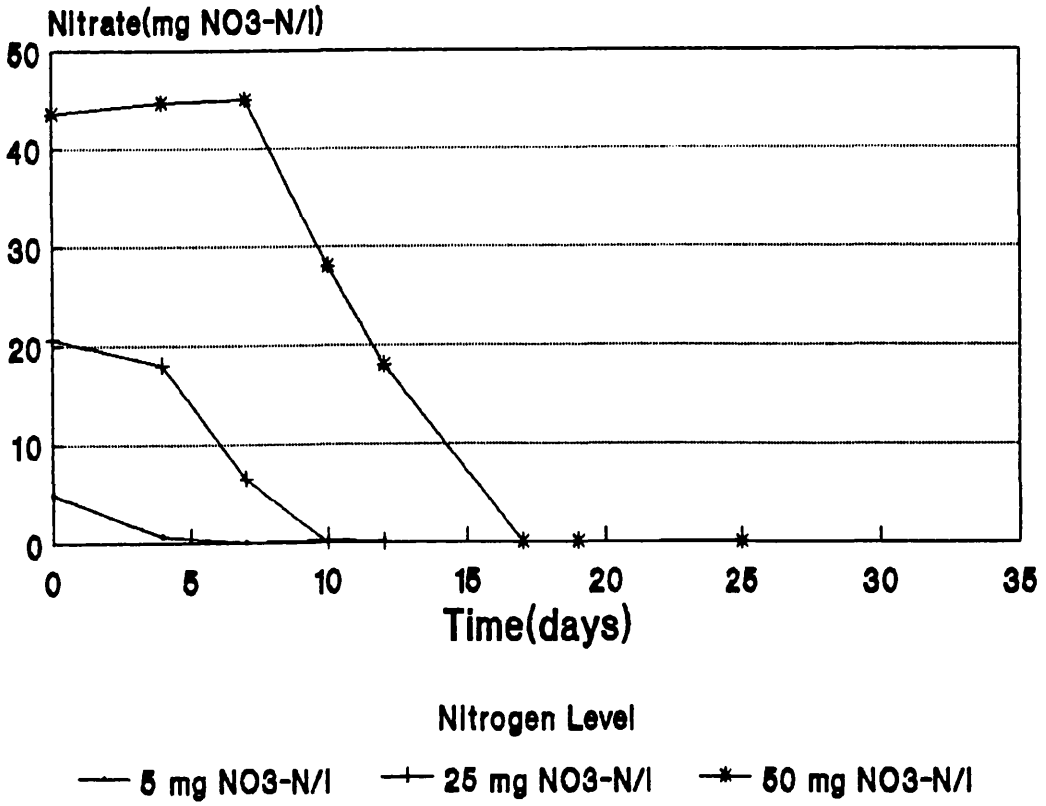


FIG44 C.vulgaris 211/8K 17°C
pH vs Time

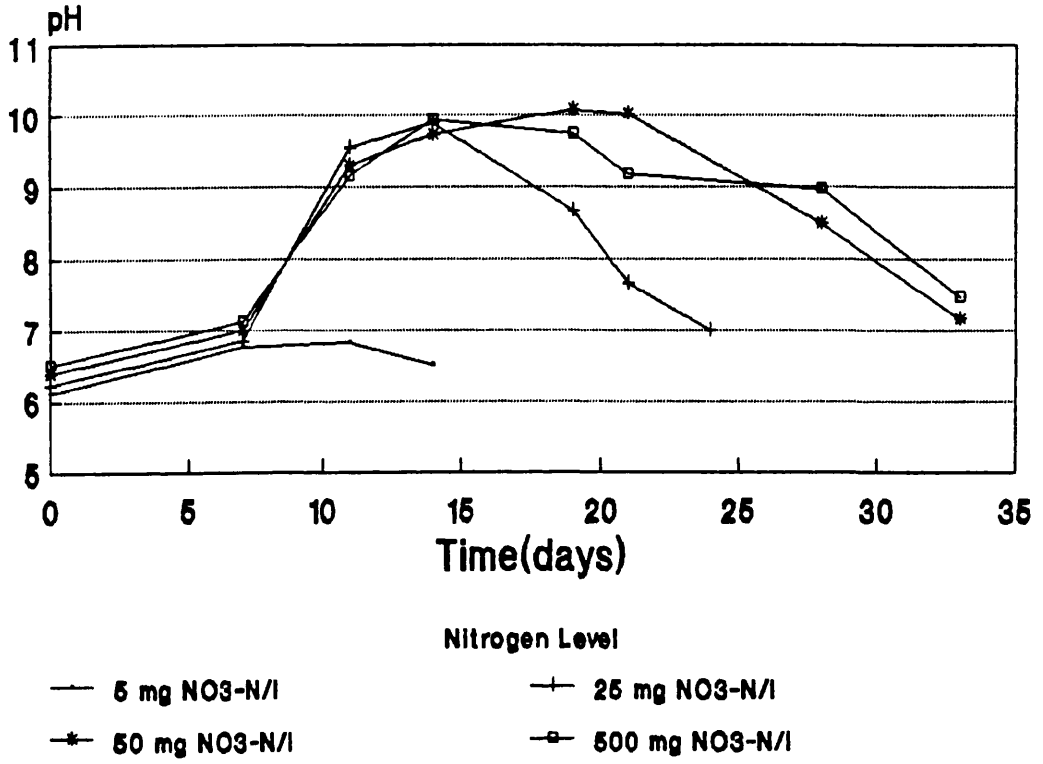


FIG45 C.vulgaris 211/8K 30°C
pH vs Time

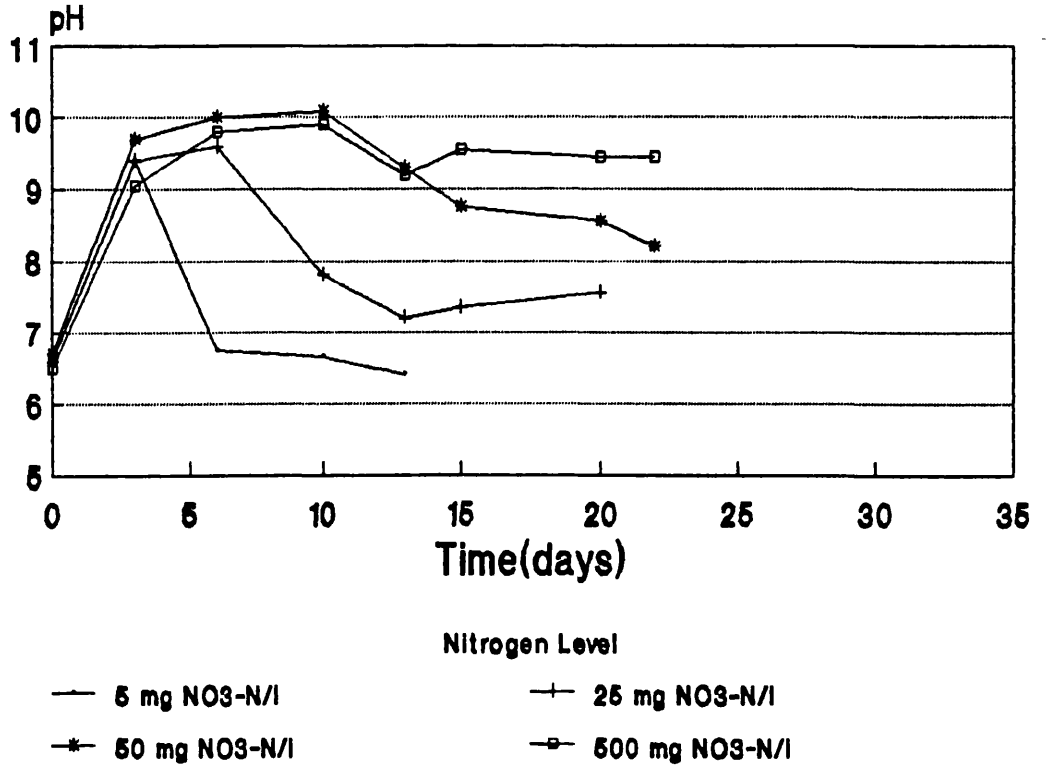


FIG46 C.vulgaris 211/8K 40°C
pH vs Time

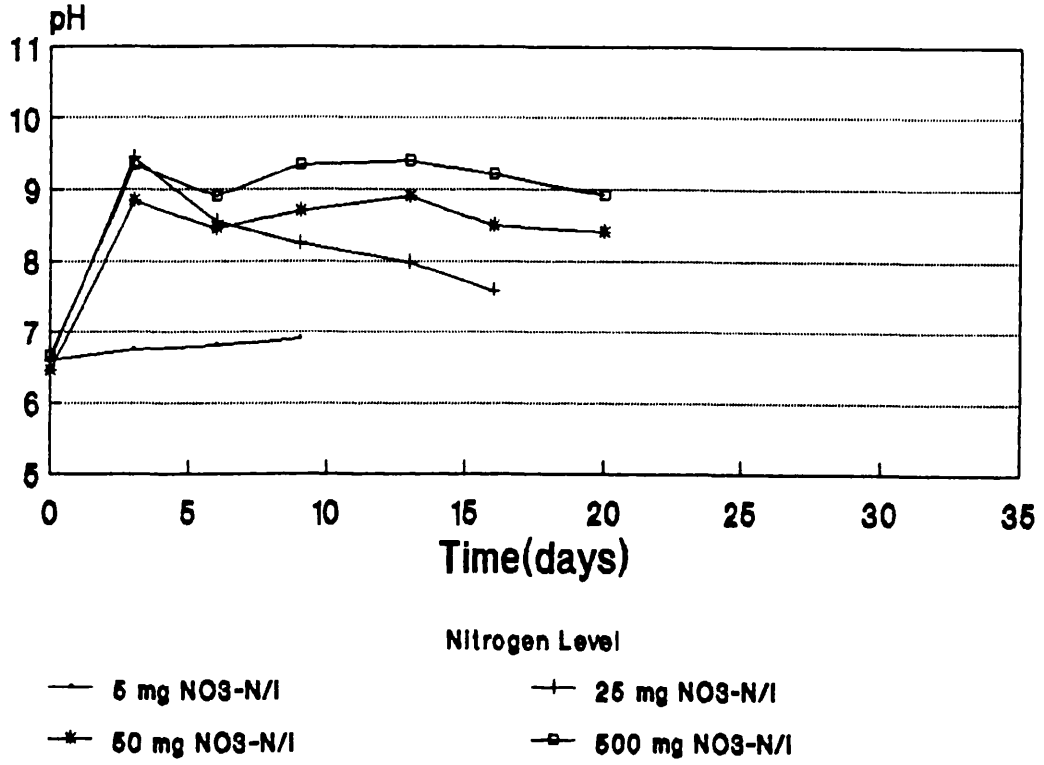


FIG47 C.vulgaris 211/11c 17°C
pH vs Time

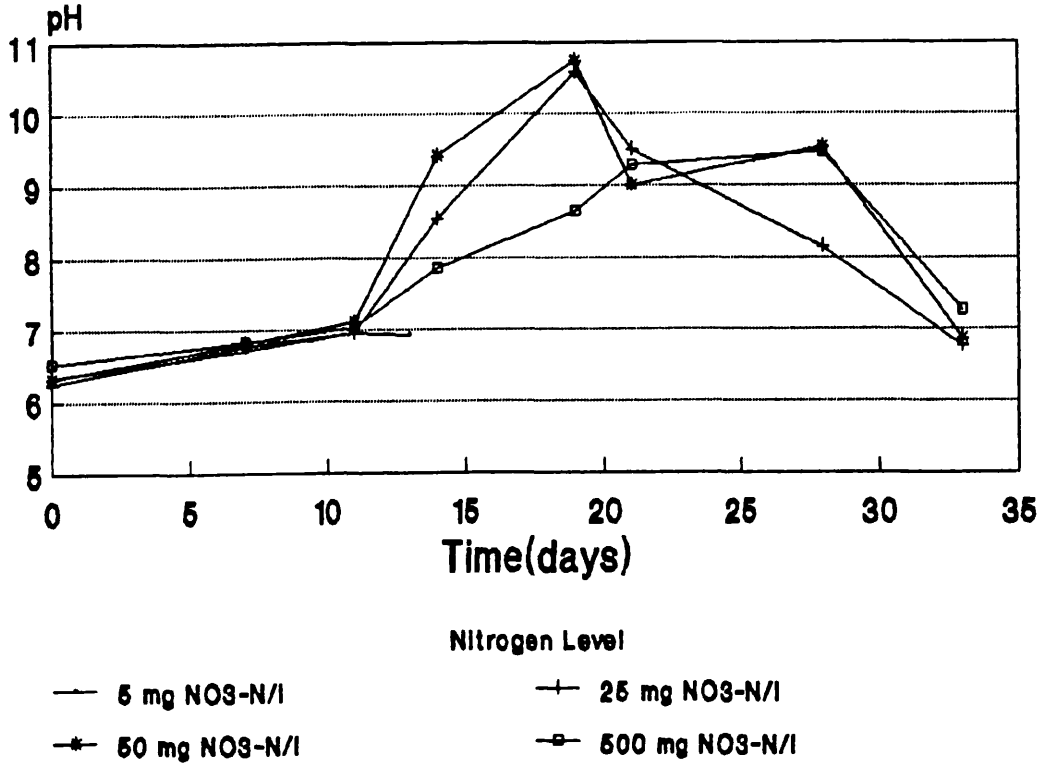


FIG48 C.vulgaris 211/11c 30°C
pH vs Time

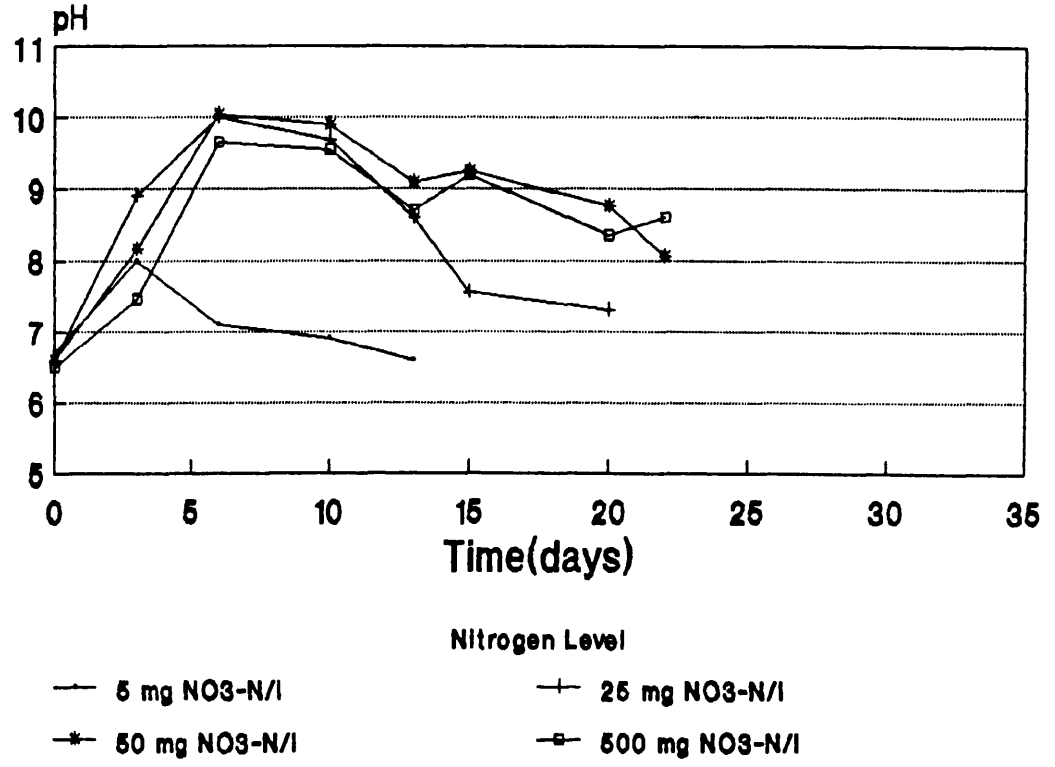


FIG49 C.vulgaris 211/11c 40°C
pH vs Time

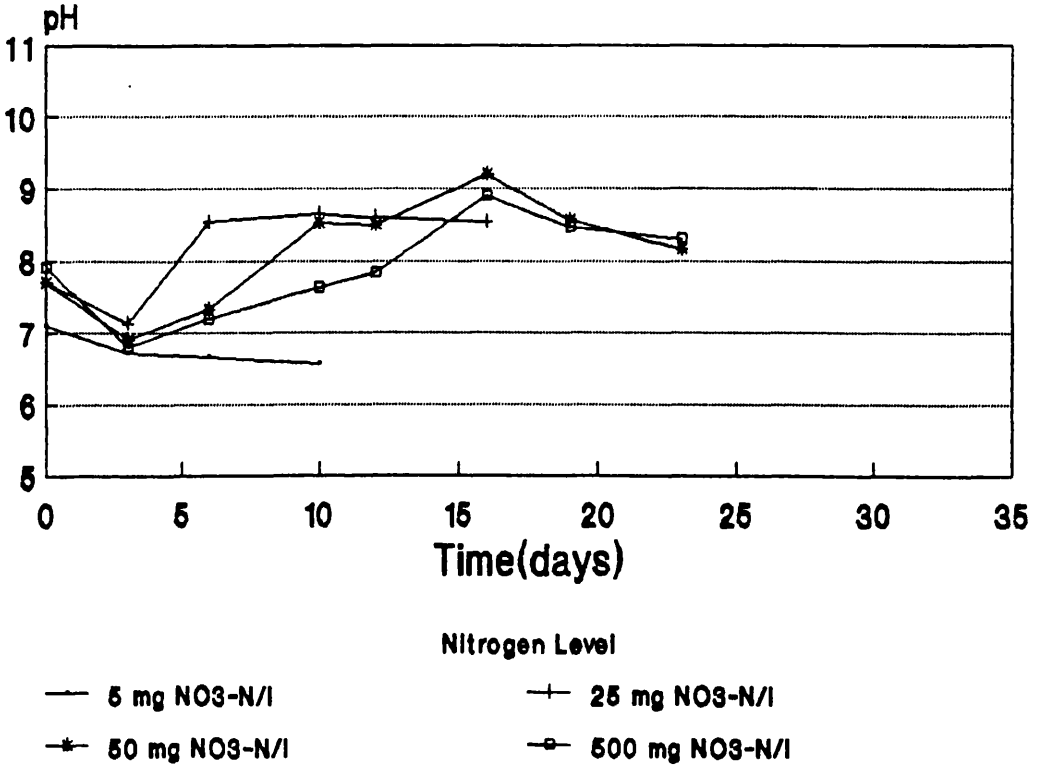


FIG50 Ank.antarcticus 202/25 17°C
pH vs Time

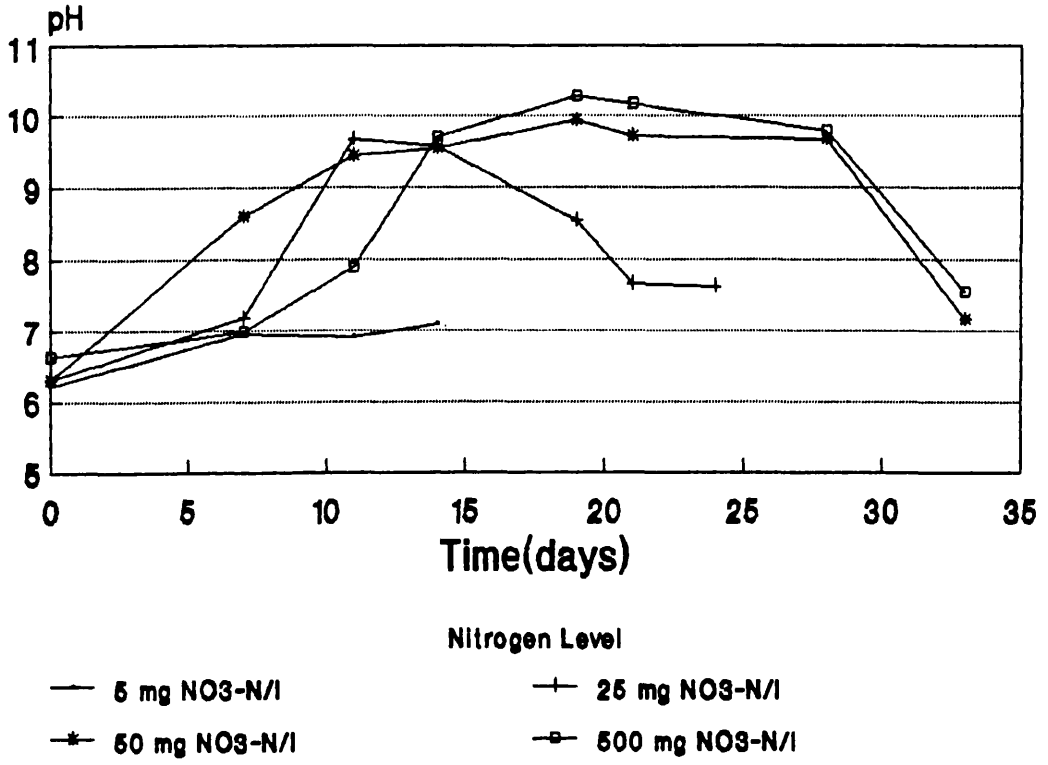


FIG51 Ank.antarcticus 202/25 30°C
pH vs Time

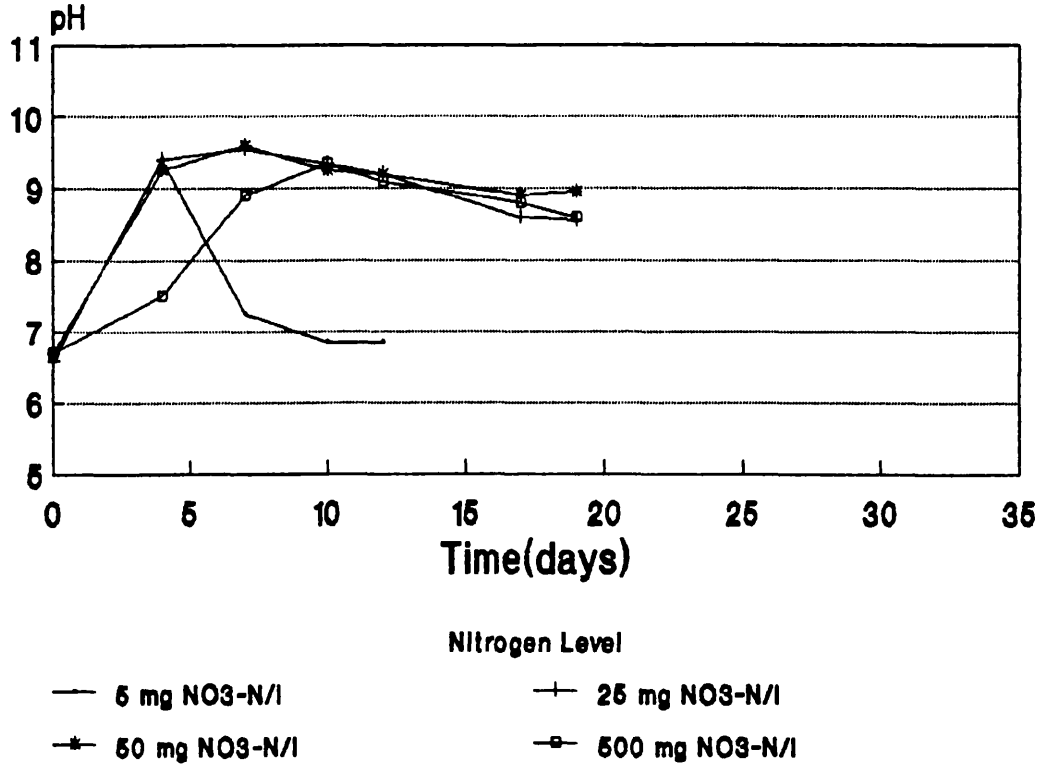


FIG52 S.obliquus 276/3A 17°C
pH vs Time

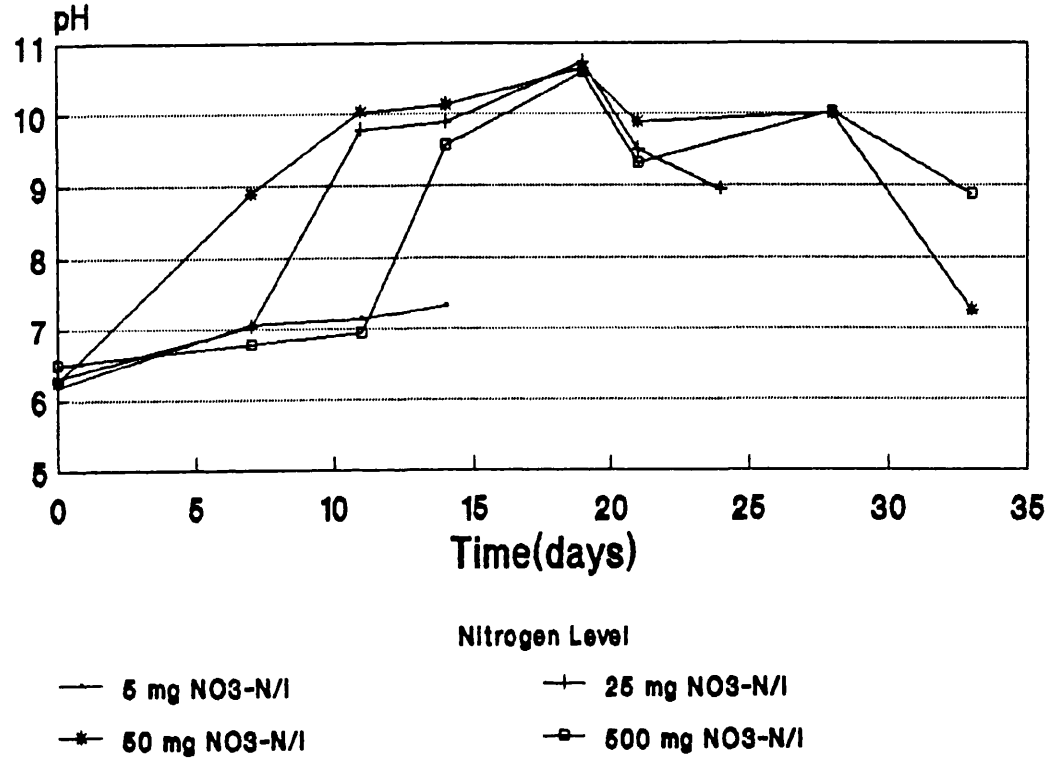


FIG53 S.obliquus 276/3A 30°C
pH vs Time

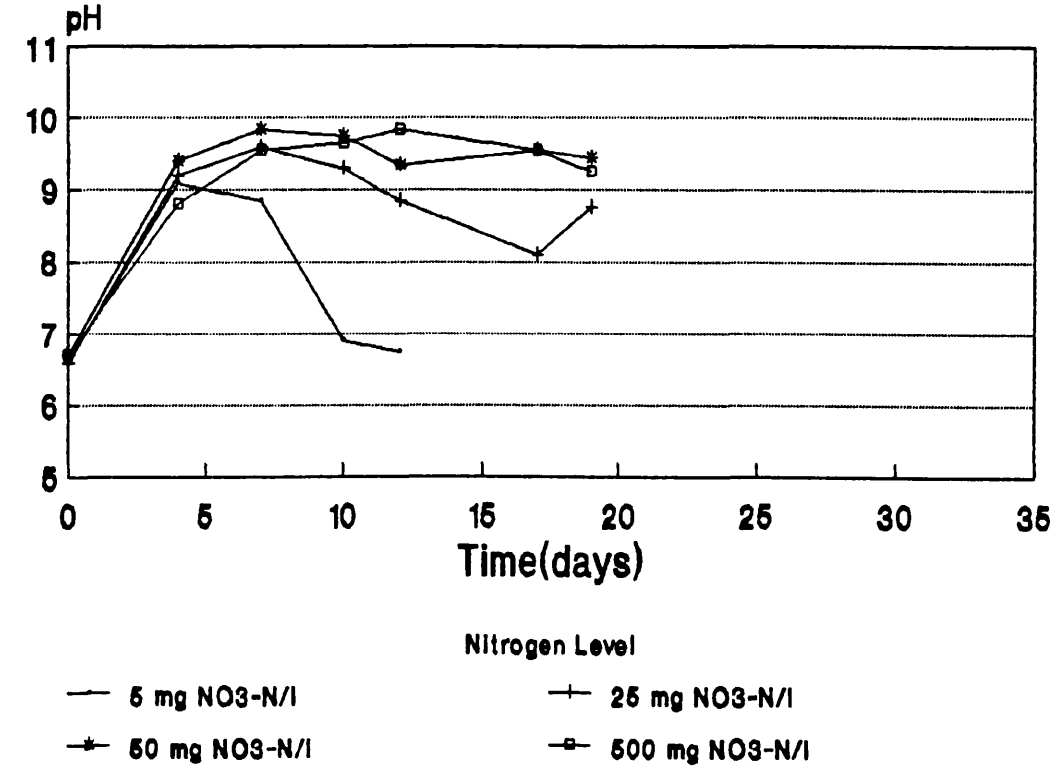


Table 9: Harvest Parameters for C. vulgaris 211/8K

Temperature/ Initial N Level		Phase	Time (Days)	OD_{560}	Dry wt (gl^{-1})	NO_3-N (mgl^{-1})
17°C	5	S	15	0.57	0.16	0.02
	25	E	14	0.88	0.28	7.4
	25	S	24	2.08	0.65	0.02
	50	E	14	1.05	0.30	22.5
	50	S	33	3.68	1.08	0
	500	E	33	2.24	1.02	432
30°C	5	S	13	0.45	0.11	0
	25	E	13	1.28	0.41	2.1
	25	S	20	1.37	0.43	0
	50	E	15	1.03	0.29	6.4
	50	S	22	1.80	0.60	0.09
	500	E	22	2.20	0.72	330
40°C	5	S	9	0.17	0.05	0.95
	25	E	9	0.78	0.23	1.1
	25	S	17	0.74	0.30	0.03
	50	E	13	1.06	0.41	7.8
	50	S	20	1.24	0.46	0.90
	500	E	20	1.09	0.77	295

E = Exponential Phase
S = Stationary Phase

Table 10: Harvest Parameters for C. vulgaris 211/11c

Temperature/ Initial N Level		Phase	Time (Days)	OD_{560}	Dry wt (gl^{-1})	NO_3-N (mgl^{-1})
17°C	5	S	13	0.17	0.07	0.49
	25	E	19	0.68	0.31	11.3
	25	S	32	1.66	0.92	0.09
	50	E	14	0.46	0.14	23.4
	50	S	33	2.64	1.32	0
	500	E	33	1.58	0.94	423
30°C	5	S	13	0.44	0.15	0
	25	E	13	1.21	0.52	4.2
	25	S	20	1.26	0.60	0.09
	50	E	15	1.71	0.66	0.9
	50	S	22	2.08	0.60	0.05
	500	E	22	1.72	0.66	345
40°C	5	S	10	0.14	0.08	0.01
	25	E	10	0.67	0.22	1.9
	25	S	16	0.80	0.36	0.02
	50	E	12	0.53	0.27	12.0
	50	S	23	1.18	0.48	0.80
	500	E	23	1.20	0.60	385

E = Exponential Phase
S = Stationary Phase

Table 11: Harvest Parameters for Ank. antarcticus 202/25

Temperature/ Initial N Level		Phase	Time (Days)	$0D_{560}$	Dry wt (gl^{-1})	NO_3-N (mgl^{-1})
17°C	5	S	15	0.45	0.27	0
	25	E	11	0.44	0.33	6.2
	25	S	24	1.88	0.72	0.03
	50	E	14	0.68	0.24	19.3
	50	S	33	3.44	1.32	3.4
	500	E	33	2.76	1.16	419
30°C	5	S	12	0.54	0.19	0.07
	25	E	10	0.68	0.22	3.4
	25	S	25	1.42	0.62	0
	50	E	12	0.85	0.28	24.5
	50	S	25	1.91	0.60	0.9
	500	E	25	1.28	0.65	398

Table 12: Harvest Parameters for S. obliquus 276/3A

Temperature/ Initial N Level		Phase	Time (Days)	$0D_{560}$	Dry wt (gl^{-1})	NO_3-N (mgl^{-1})
17°C	5	S	15	0.28	0.22	0.01
	25	E	11	0.28	0.15	7.2
	25	S	24	1.64	0.85	0.02
	50	E	11	0.45	0.21	21.5
	50	S	33	3.32	1.40	1.6
	500	E	33	2.52	1.62	397
30°C	5	S	12	0.46	0.30	0.14
	25	E	10	0.81	0.41	0.02
	25	S	25	2.02	1.14	0.09
	50	E	12	1.10	0.54	18
	50	S	25	2.36	1.24	0
	500	E	25	2.02	1.29	345

E = Exponential Phase S = Stationary Phase

4.3.1.2 Carbohydrate, Protein and Lipid Results

The results for carbohydrate, protein, lipid analyses are given in Tables 13, 14, 15, 16 for C. vulgaris 211/8K, C. vulgaris 211/11c, Ank. antarcticus 202/25 and S. obliquus 276/3A respectively.

C. vulgaris 211/8K increased lipid content with decrease in temperature (average lipid content at 17°C - 9.42%, 30°C - 9.18%, 40°C - 5.64%), increased protein content from 17°C to 30°C but decreased from 30°C to 40°C (average protein content at 17°C - 7.57%, 30°C - 8.92%, 40°C - 5.65%), and increased carbohydrate content with decrease in temperature (average carbohydrate content at 17°C - 40.95%, 30°C - 28.65%, 40°C - 18.88%). The major shift was the accumulation of carbohydrate with decreasing temperature. With respect to growth phase, C. vulgaris 211/8K increased lipid, decreased protein and accumulated carbohydrate in the stationary phase compared to exponential phase (Figs 54 and 55). These changes were independent of initial nitrogen level, but were in response to nitrate depletion or very low nitrate levels.

C. vulgaris 211/11c increased lipid content from 17°C (average, 4.67%) to 30°C (average, 7.38%) and decreased at 40°C (average, 4.97%). Protein content decreased from 17°C (average, 8.87%) to 30°C (7.57%) and then increased at 40°C (average, 7.88%). Carbohydrate accumulated with decrease in temperature (average carbohydrate content at 17°C - 56.43%, 30°C - 38.63%, 40°C - 31.15%). The accumulation of carbohydrate exhibited was common to both strains of C. vulgaris. With respect to growth phase, C. vulgaris 211/11c increased lipid, decreased protein and increased carbohydrate at stationary phase at 17°C and 30°C (Figs 56 and 57). At 40°C, the reverse appeared to occur.

Ank. antarcticus 202/25 decreased lipid content from 17°C to 30°C (average lipid content at 17°C - 18.43%, 30°C - 12.69%), decreased protein from 17°C to 30°C (average protein content at 17°C - 8.25%, 30°C - 5.47%) and slightly increased carbohydrate (average 17°C - 26.85%, 30°C - 27.70%). With respect to growth phase, lipid increased, protein decreased and carbohydrate increased with stationary phase (Figs 58 and 59).

S. obliquus 276/3A decreased lipid content from 17°C to 30°C (average lipid content 17°C - 17.13%, 30°C - 12.12%), slightly increased protein content (average protein content at 17°C - 9.47%, 30°C - 10.67%) and decreased carbohydrate content (average carbohydrate content at 17°C - 31.20%, 30°C - 25.48%). With respect to growth phase, lipid increased, protein decreased and carbohydrate increased with stationary phase (Figs 60 and 61).

4.3.1.3 Statistical Analysis of Carbohydrate, Lipid and Protein Results

Statistical analysis of the carbohydrate results for the four green algae show all the 'main effects' - temperature, algal species, nitrogen level and growth phase - to be significant at 0.1% ($p < 0.001$). Carbohydrate means at the three temperatures are 17°C - 38.86%, 30°C - 30.12% and 40°C - 18.35%, which are all significantly different and confirm carbohydrate accumulation at the lower temperatures. The phase means are exponential - 26.18% and stationary - 33.03%, a significant difference which confirms accumulation in stationary phase. The respective nitrogen means are '5' (mg NO_3-N l^{-1}) - 33.61%, '25' - 30.46%, '50' - 28.19% and '500' - 23.74%. All levels have significantly higher means than at '500' which confirms carbohydrate accumulation more readily occurs at lower nitrogen levels and with nitrate depletion. The means for the species, C. vulgaris 211/8K - 29.49%, C. vulgaris 211/11c - 42.07%, Ank antarcticus - 22.97%, S. obliquus - 21.90%, show significant differences between the species which divide into three groups with C. vulgaris 211/11c markedly different to the other three species, and C. vulgaris 211/8K significantly different from Ank. antarcticus and S. obliquus which themselves are not significantly different. There was one significant interaction term between temperature and algae (1% or $p < 0.01$). Further analysis (multiple range testing) confirmed that the interaction term resulted from C. vulgaris 211/8K and 211/11c exhibiting different behaviour to the other two species in that the decrease in carbohydrate from 17°C to 30°C was significant for these species but not significant for Ank antarcticus 202/25 and S. obliquus 276/3A. Therefore there appeared to be a difference in the behaviour amongst the four algae between C. vulgaris 211/8K and 211/11c and Ank. antarticus and S.

obliquus with respect to carbohydrate.

Statistical analysis of the protein results showed all the 'main effects' - temperature, algal species, nitrogen, phase - to be significiant at 0.1% ($P < 0.001$). Protein means at the three temperatures (17°C - 8.74%, 30°C - 8.16%, 40°C - 6.88%) were only shown to be significantly different at the highest temperature. This only relates to C. vulgaris 211/8K and 211/11c which grow at 40°C. A first order interaction between temperature and algae was also significant (1% or $p<0.01$). The interaction term was found to be due to a significant decrease in protein at 40°C for C. vulgaris 211/8K only, which suggested different behaviour of this strain compared to the others possibly due to its high temperature nature. Phase means were 8.95% for exponential and 6.77% for stationary, a significant decrease in stationary phase confirming protein decreased at stationary growth phase. The means for nitrogen, '5' (mg NO_3-N l^{-1}) - 6.25%, '25' - 8.24%, '50' - 9.51%, '500' - 8.20%, show '5' significantly lower than '25' and '25' significantly lower than '50', which suggests protein content is dependent on previous nitrate availability in the culture. Species means, C. vulgaris 211/8K - 7.38%, C. vulgaris 211/11c - 8.11%, Ank antarticus - 6.37% and S. obliquus - 9.58% - showed a significant difference between Ank. antarcticus and the other species.

Statistical analysis of the lipid results showed all the 'main effects' again to be significant at 0.1% ($p < 0.001$). Mean lipid content at the three temperatures (17°C - 12.41%, 30°C - 10.34%, 40°C - 9.02%) showed a significant decrease with increased temperature. Phase means were exponential - 7.81% and stationary - 13.37%, a significant increase from exponential to stationay phase. A significant interaction term between temperature and phase (1% or $p < 0.01$) when further investigated showed the mean lipid content was not significantly different over temperature in exponential phase, but that the interaction was due to the significant decrease in lipid levels with increasing temperature in stationary phase only. Nitrogen means ('5' (mg NO_3-N l^{-1}) - 15.71%, '25' - 11.17%, '50' - 8.78%, '500' - 7.95%) showed a significant reduction from '5' to '25' to '50', which implied that lipid content, like protein content, is dependent on previous nitrate availability. Species means (C. vulgaris 211/8K - 8.08%, C.

vulgaris 211/11c - 5.67%, Ank. antarticus - 13.84%, S. obliquus - 14.77%) exhibited a significant division into two groups with C. vulgaris 211/8K and 211/11c significantly different to Ank. antarcticus and S. obliquus. A significant interaction term was identified between algae and phase which showed that lipid levels for the exponential and stationary phases were only significantly different for Ank. antarcticus and S. obliquus. This confirmed a difference in behaviour between the algae in relation to lipid accumulation.

Therefore, it would appear that the four green algae were behaving differently. Although changes in carbohydrate, lipid and protein content were observed for all genera, statistical analysis showed that increases in carbohydrate with decreasing temperature were only significant for C. vulgaris 211/8K and 211/11c. Protein changes at higher temperatures were only significant for C. vulgaris 211/8K (high temperature strain) and at lower temperatures for Ank. antarcticus. Lipid accumulation was temperature and phase dependent. Furthermore significant changes in lipid content only occurred in Ank. antarcticus and S. obliquus.

4.3.1.4 Fatty Acid Results

The fatty acid profiles for all four species exhibited a predominance of C16 and C18 fatty acids (Tables 13, 14, 15, 16).

C. vulgaris 211/8K showed little difference qualitatively in fatty acids between temperature and growth phase, however quantitative differences were apparent (Table 13). The major fatty acids found in C. vulgaris 211/8K were 16:0, 16:2, 16:3, 18:2(n-6) and 18:3(n-3). 16:0 and 16:2 fatty acids increased with increasing temperature and 16:3, 18:1 and 18:3(n-3) decreased. Changes were also found with phase (Table 13). The average percentage total of unsaturated fatty acids (% UNFA) at the three temperatures for C. vulgaris 211/8K (17°C - 75.97%, 30°C - 71.30%, 40°C - 70.68%) showed an overall decrease in unsaturation with increasing temperature. The percentage unsaturation also decreased from exponential to stationary phase at all three temperatures (Table 13).

C. vulgaris 211/11c showed a major qualitative change at 17°C with the appearance of 16:4 fatty acid (Table 14). Quantitatively, changes occurred with all fatty acids with temperature and growth phase. The major fatty acids found in C. vulgaris 211/11c were 16:0, 18:2(n-6) and 18:3(n-3). 16:0, 16:2 and 18:2(n-6) fatty acids increased with increasing temperature and 16:3, 16:4, 18:1 and 18:3 (n-3) all decreased. Changes were also found with phase (Table 14). Again, the average percentage total of unsaturated fatty acids (mean % UNFA) at the three temperatures (17°C - 77.40%, 30°C - 73.55%, 40° - 65.05%) showed an overall decrease in unsaturation with increasing temperature. The percentage unsaturation also decreased from exponential to stationary phase with few exceptions (Table 14).

Ankistrodesmus antarcticus 202/25 exhibited minor qualitative changes with the appearance of 20:0 and 20:1 fatty acids at the lower temperature (Table 15). Quantitatively, changes occurred with temperature and growth phase. The major fatty acids found were 16:0, 18:1, 18:3(n-3). 16:4 and 18:4 fatty acids were also present. 16:0 and 18:4 fatty acids increased with increasing temperature and 18:1 decreased. Changes were also found between growth phases (Table 15). The average %UNFA at 17°C - 80.60% and 30°C - 71.15% showed a decrease with increasing temperature. The percentage unsaturation also decreased from exponential to stationary phase (Table 15).

S. obliquus 276/3A exhibited minor qualitative changes, similar to those of Ank. antarcticus (Table 16). It was also observed that 16:4 and 18:4 fatty acids were prevalent in S. obliquus and Ank. antarcticus at both temperatures, unlike C. vulgaris 211/8K where these fatty acids did not occur and C. vulgaris 211/11c where 16:4 fatty acid was only found at 17°C. Quantitative changes were again seen with temperature and growth phase for S. obliquus. The major fatty acids found were 16:0, 18:1 and 18:3(n-3). 16:0 and 18:2(n-6) increased with increasing temperature and 18:3 (n-3) decreased. Changes were also found between growth phases (Table 16). Unsaturation decreased with temperature, and decreased between exponential and stationary phase with the exception of 25E/S at 30°C (Table 16).

4.3.1.5 Statistical Analysis of the Percentage Unsaturation Results

Statistical analysis of the unsaturated fatty acid contents (% UNFA) gave significant results for all 'main effects' (temperature, algal species, nitrogen and phase) at 0.1% or $p < 0.001$. The means for temperature, (17°C - 79.22%, 30°C - 72.93%, 40°C - 69.39%) showed a significant decrease as the growth temperature increased. Phase means (exponential - 74.58%, stationary - 73.12%) showed a significant small decrease from exponential to stationary phase. Nitrogen means ('5' (mg NO_3-N l^{-1}) - 68.27%, '25' - 74.37%, '50' - 75.21%, '500' - 74.65%) showed only a significant result with '5' compared to other initial nitrogen levels. Species means (C. vulgaris 211/8K - 72.65%, C. vulgaris 211/11c - 72.00%, Ankistrodesmus antarcticus 77.08%, S. obliquus - 73.65%) showed a significant difference between Ank. antarcticus and the other three algae.

Overall, the degree of fatty acid unsaturation decreased with increasing culture temperature and attainment of stationary growth phase.

4.3.1.6 Gross Photosynthetic and Dark Respiration Rates

The results are given in Table 17 for the four green algae grown at 25mg NO_3-N l^{-1}. The cultures were harvested at the same times as for the nitrogen limitation experiments (see Tables 9-12).

Gross photosynthetic and dark respiration rates decreased from exponential to stationary phase at all temperatures, and increased with temperature for all species.

Table 13: Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) for C.vulgaris 211/8K

	17°C						30°C						40°C					
Fatty Acid	5S	25E	25S	50E	50S	500E	5S	25E	25S	50E	50S	500E	5S	25E	25S	50E	50S	500E
12:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14:0	.16	.17	.24	.12	.28	.19	.51	.47	.67	.17	.50	.48	.51	-	.42	.64	.30	.25
14:1(n-5)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16:0	20.71	21.26	22.37	20.31	22.89	21.76	27.50	26.29	29.23	21.18	26.98	22.90	38.96	25.69	28.44	25.42	24.65	25.80
16:1(n-7)	2.53	3.11	1.77	3.60	2.19	3.54	2.13	2.32	1.75	3.46	3.25	6.11	3.14	4.89	2.13	5.14	3.76	3.13
16:2	3.64	5.44	3.90	5.08	5.52	7.29	4.32	7.06	4.48	10.31	8.06	10.62	12.42	15.97	13.57	17.68	20.53	21.48
16:3(n-6)	12.66	13.03	11.88	14.39	9.32	9.39	9.01	8.19	8.16	8.10	6.36	7.03	6.56	4.59	6.34	6.28	4.04	3.32
16:4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18:0	2.23	1.17	2.24	0.96	2.70	1.17	2.77	1.95	3.02	0.56	2.36	.70	1.47	-	0.95	-	0.38	0.42
18:1(n-9+ n-7)	11.03	6.99	7.31	5.86	8.44	10.15	6.25	5.36	5.87	3.97	8.47	8.37	1.19	2.69	1.92	2.03	3.04	3.12
18:2(n-6)	24.45	25.44	29.44	23.98	32.87	29.43	29.60	30.82	29.76	33.40	30.86	30.44	22.50	34.25	30.60	28.36	32.19	34.54
18:3(n-3)	21.96	22.52	20.28	25.16	15.30	16.76	17.24	16.72	16.11	18.20	12.40	13.27	13.10	11.80	15.18	14.39	10.76	7.46
18:3(n-6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18:4(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:0	.44	.81	.59	.56	.48	.29	.64	.64	.81	.43	.74	-	-	-	.31	-	.24	.38
20:1(n-9)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:2(n-6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:3(n-6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:4(n-6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:4(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:5(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:0(IS)	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS
22:1(n-9)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:5(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:6(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
% Lipid	8.66	8.06	11.43	7.99	13.06	7.35	14.37	9.70	11.16	6.11	7.82	5.92	5.55	7.46	8.19	5.32	3.86	3.49
% SAFA	23.54	23.41	25.44	21.95	26.35	23.41	31.42	29.35	33.73	22.34	30.58	24.08	40.94	25.69	30.12	26.06	25.57	26.85
%UNFA	76.47	76.53	74.58	78.07	73.64	76.56	68.55	70.47	66.13	77.44	69.40	75.84	58.91	74.19	69.74	73.88	74.32	73.05
UNFA/SAFA	3.25	3.27	2.93	3.56	2.79	3.27	2.18	2.40	1.96	3.47	2.27	3.15	1.44	2.89	2.32	2.83	2.91	2.72
% Protein	6.50	11.20	4.70	12.10	6.10	4.80	ND	9.00	6.80	12.40	9.20	7.50	6.20	8.10	5.50	4.90	5.30	3.90
%Carbohydrate	56.30	38.80	52.10	29.30	47.60	21.60	ND	32.80	33.30	14.80	31.10	19.30	21.60	14.10	30.40	16.00	16.20	15.00

Note: (i) IS = Internal Standard; S = Stationary Phase; E = Exponential Phase; SAFA = Saturated Fatty Acids; UNFA = Unsaturated Fatty Acids

(ii) For systematic names of fatty acids see Appendix 1

FIG54 %Carbohydrate, Protein and Lipid
C.vulgaris 211/8k Exponential Phase

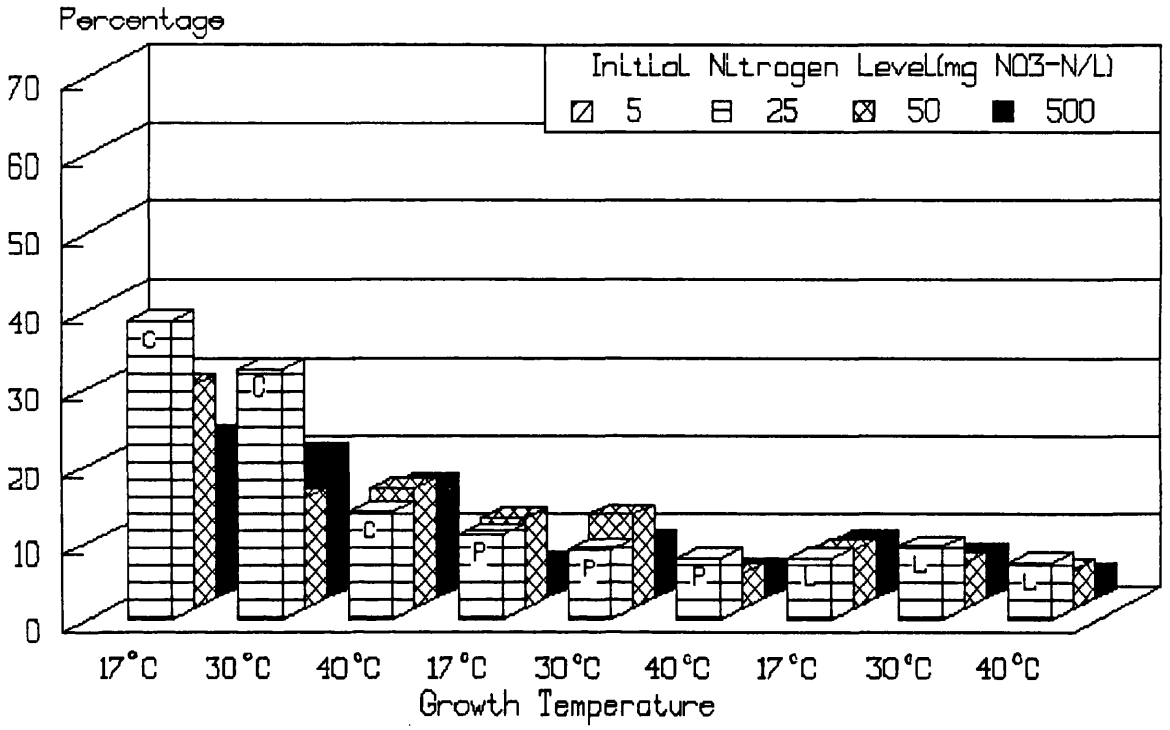


FIG55 %Carbohydrate, Protein and Lipid
C.vulgaris 211/8k Stationary Phase

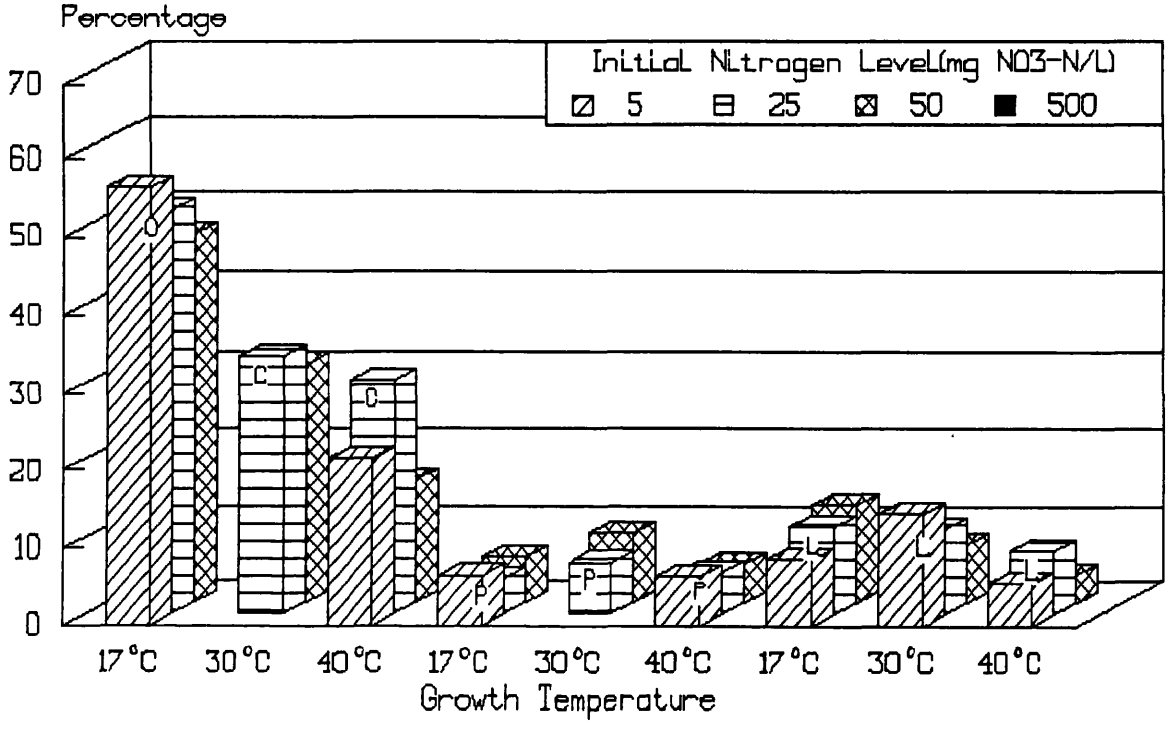


Table 14: Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) for C. vulgaris 211/11C

	17°C						30°C						40°C					
Fatty Acid	5S	25E	25S	50E	50S	500E	5S	25E	25S	50E	50S	500E	5S	25E	25S	50E	50S	500E
12:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14:0	.77	.43	.40	.32	.35	.18	.51	.69	.50	.26	.42	.28	-	.27	.40	.29	.31	.35
14:1(n-5)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16:0	28.52	18.77	20.04	17.37	18.54	17.53	27.53	25.40	25.05	21.22	22.96	21.29	49.02	29.15	30.33	30.91	28.87	28.01
16:1(n-7)	4.34	3.83	2.04	4.88	1.59	3.08	1.11	1.39	1.11	1.28	1.45	1.69	2.65	2.81	2.11	2.22	2.61	1.82
16:2	2.40	3.25	2.85	3.28	6.62	5.14	8.81	15.12	13.41	19.30	16.62	16.98	6.96	14.23	13.30	16.75	18.32	16.75
16:3(n-6)	5.42	7.99	11.42	10.11	12.45	12.11	5.58	4.95	5.61	4.75	5.18	5.91	1.95	5.85	6.08	4.39	4.04	4.10
16:4	8.01	7.75	4.54	8.39	3.15	5.04	0.34	-	-	-	-	-	-	-	-	-	-	-
17:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18:0	1.36	1.17	1.23	0.83	1.84	1.24	2.07	2.15	2.11	1.14	2.54	1.31	2.17	1.33	1.10	1.14	1.04	0.92
18:1(n-9+ n-7)	9.18	6.37	6.07	3.09	6.00	4.78	5.27	1.82	2.08	0.97	3.79	2.27	2.25	0.65	1.34	0.76	1.25	1.57
18:2(n-6)	12.89	18.65	20.72	17.92	21.83	17.79	37.63	40.78	40.92	43.28	39.79	40.87	24.51	35.19	34.31	35.41	37.70	37.81
18:3(n-3)	23.62	31.80	30.40	33.46	27.36	32.78	10.77	7.53	8.96	7.69	7.24	9.04	7.69	10.14	10.38	8.03	5.84	8.57
18:3(n-6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18:4(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:0	3.41	-	.30	.30	.26	.29	.35	-	.16	-	-	-	2.24	0.38	0.52	-	-	-
20:1(n-9)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:2(n-6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:3(n-6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:4(n-6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:4(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:5(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:0(IS)	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS
22:1(n-9)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:5(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:6(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
% Lipid	0.74	4.10	5.49	4.76	7.34	5.60	11.05	5.96	8.15	6.55	7.03	5.56	2.21	6.17	5.26	6.58	5.22	4.37
% SAFA	34.06	20.37	21.97	18.82	20.99	19.24	30.46	28.24	27.82	22.62	25.92	22.88	53.97	31.13	32.35	32.34	30.22	29.28
%UNFA	65.86	79.64	78.04	81.13	79.00	80.72	69.51	71.59	72.09	77.27	74.07	76.76	46.01	68.87	67.52	67.56	69.76	70.62
UNFA/SAFA	1.93	3.91	3.55	4.31	3.76	4.20	2.28	2.53	2.59	3.42	2.86	3.35	0.85	2.21	2.09	2.09	2.31	2.41
% Protein	6.30	12.10	7.50	10.20	8.30	8.80	9.00	8.50	5.40	8.10	6.10	8.30	6.40	8.10	11.10	3.60	9.60	8.50
%Carbohydrate	63.50	60.30	67.30	48.80	54.00	44.70	41.80	37.80	43.20	32.30	43.40	33.30	33.60	33.40	35.40	31.70	25.00	27.80

Note: (i) IS = Internal Standard; S = Stationary Phase; E = Exponential Phase; SAFA = Saturated Fatty Acids; UNFA = Unsaturated Fatty Acids

(ii) For systematic names of fatty acids see Appendix 1

FIG56 %Carbohydrate, Protein and Lipid
C.vulgaris 211/11c Exponential Phase

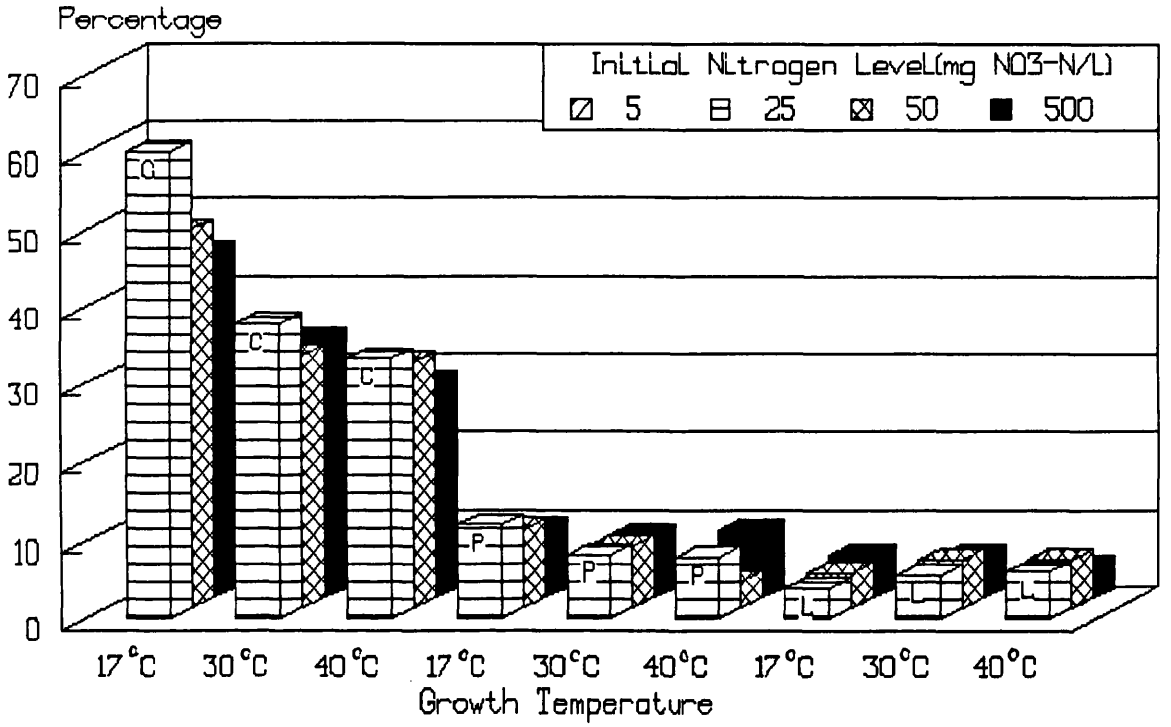


FIG57 %Carbohydrate, Protein and Lipid
C.vulgaris 211/11c Stationary Phase

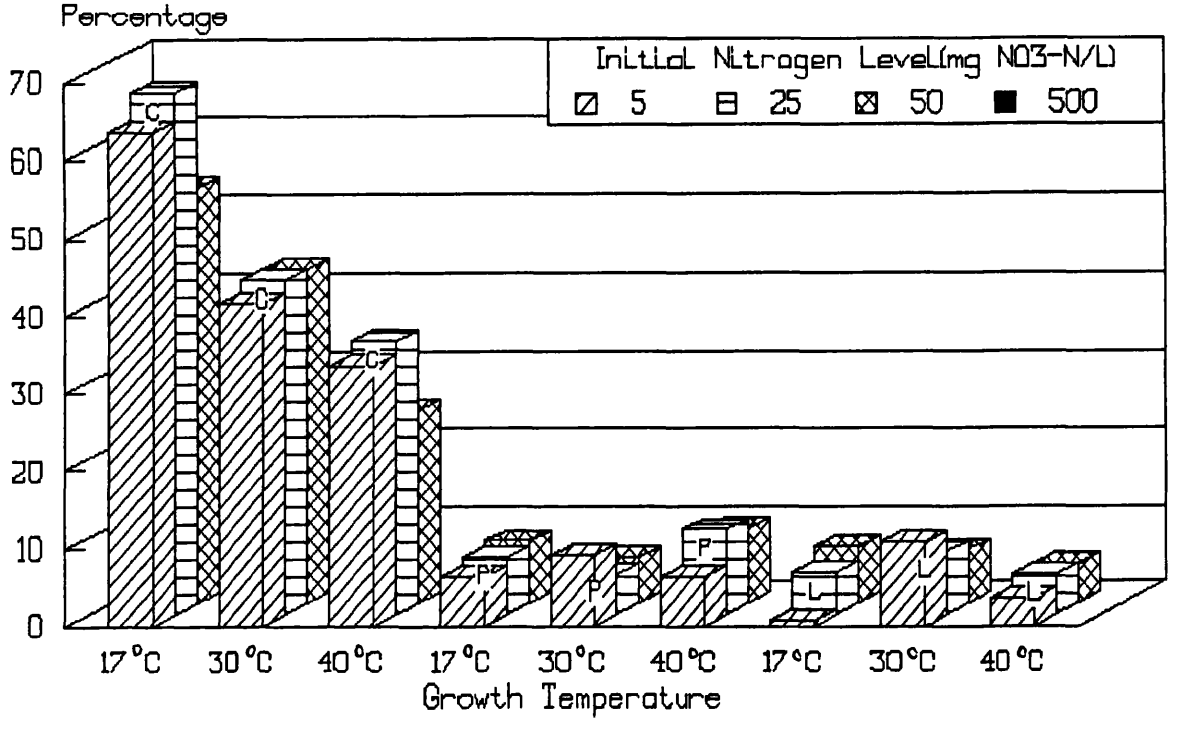


Table 15: Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) of Ank. antarcticus 202/25

	17°C						30°C					
Fatty Acid	5S	25E	25S	50E	50S	500E	5S	25E	25S	50E	50S	500E
12:0	-	-	-	-	-	-	-	-	-	-	-	-
14:0	.15	.20	.16	.23	.19	.11	.22	.47	.37	.48	.54	.35
14:1(n-5)	-	-	-	-	-	-	-	-	-	-	-	-
15:0	-	-	-	-	-	-	-	-	-	-	-	-
16:0	16.72	15.14	16.62	14.46	15.34	16.08	20.28	22.90	23.59	22.30	25.24	21.98
16:1(n-7)	0.94	3.35	1.03	2.41	0.32	1.15	1.29	5.29	1.04	4.83	1.93	3.58
16:2	0.77	0.82	0.83	0.95	1.49	1.80	1.56	0.65	2.26	1.46	1.98	1.88
16:3(n-6)	2.37	1.54	2.47	2.17	1.20	0.77	1.32	2.01	1.06	2.00	1.13	1.03
16:4	3.82	10.88	4.72	8.66	5.01	5.50	2.80	7.16	5.35	8.20	7.47	3.46
17:0	-	-	-	-	-	-	-	-	-	-	-	-
18:0	1.35	0.65	1.51	0.74	1.05	0.97	2.18	-	1.35	0.64	0.45	1.05
18:1(n-9+(n-7)	54.17	33.27	49.15	38.57	49.59	48.94	48.78	31.44	34.41	26.50	25.23	46.64
18:2(n-6)	3.57	3.50	4.18	3.45	5.63	6.73	6.26	5.56	7.98	4.33	7.07	5.50
18:3(n-3)	13.14	25.29	14.92	22.29	14.00	13.09	11.18	17.95	15.17	20.51	17.19	9.15
18:3(n-6)	-	-	-	-	-	-	-	-	-	-	-	-
18:4(n-3)	2.08	4.93	3.66	5.22	5.58	4.51	3.13	6.49	7.30	8.62	11.17	5.23
19:0	-	-	-	-	-	-	-	-	-	-	-	-
20:0	.18	.21	TR	.26	TR	-	0.15	-	-	-	-	-
20:1(n-9)	.70	.18	.65	.50	.61	.37	.78	-	-	-	-	-
20:2(n-6)	-	-	-	-	-	-	-	-	-	-	-	-
20:3(n-6)	-	-	-	-	-	-	-	-	-	-	-	-
20:4(n-6)	-	-	-	-	-	-	-	-	-	-	-	-
20:4(n-3)	-	-	-	-	-	-	-	-	-	-	-	-
20:5(n-3)	-	-	-	-	-	-	-	-	-	-	-	-
21:0	-	-	-	-	-	-	-	-	-	-	-	-
22:0(IS)	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS
22:1(n-9)	-	-	-	-	-	-	-	-	-	-	-	-
22:5(n-3)	-	-	-	-	-	-	-	-	-	-	-	-
22:6(n-3)	-	-	-	-	-	-	-	-	-	-	-	-
24:0	-	-	-	-	-	-	-	-	-	-	-	-
% Lipid	23.10	11.70	21.69	13.05	16.11	17.12	22.07	6.55	15.27	6.82	9.21	12.78
% SAFA	18.40	16.20	18.29	15.69	16.58	17.16	22.83	23.37	25.31	23.42	26.23	23.38
%UNFA	81.56	83.76	81.61	84.22	83.43	82.86	77.10	76.55	74.57	76.45	73.17	76.47
UNFA/SAFA	4.43	5.17	4.46	5.37	5.03	4.83	3.38	3.28	2.94	3.26	2.79	3.27
% Protein	7.70	11.10	7.60	11.20	8.40	10.80	5.80	13.50	7.10	14.70	12.40	10.50
%Carbohydrate	34.00	23.00	33.00	21.60	45.10	30.50	32.60	21.80	33.60	24.20	24.10	16.60

Note: (i) Ank. antarcticus did not grow at 4o° C

(ii) IS = Internal Standard; S - Stationary Phase; E = Exponential Phase; SAFA = Saturated Fatty Acids; UNFA = Unsaturated Fatty Acids; TR = Trace <0.1%

(iii) For systematic names of fatty acids see Appendix 1

FIG58 %Carbohydrate, Protein and Lipid
Ank. antarcticus 202/25 Exponential Phase

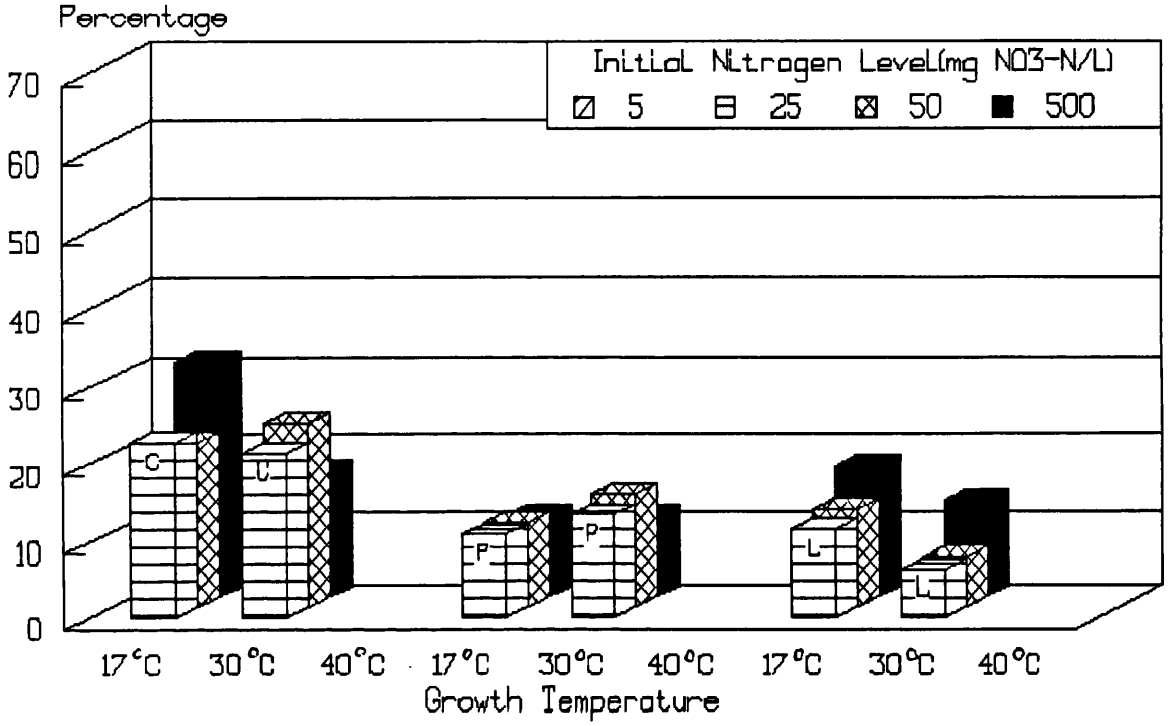


FIG59 %Carbohydrate, Protein and Lipid
Ank. antarcticus 202/25 Stationary Phase

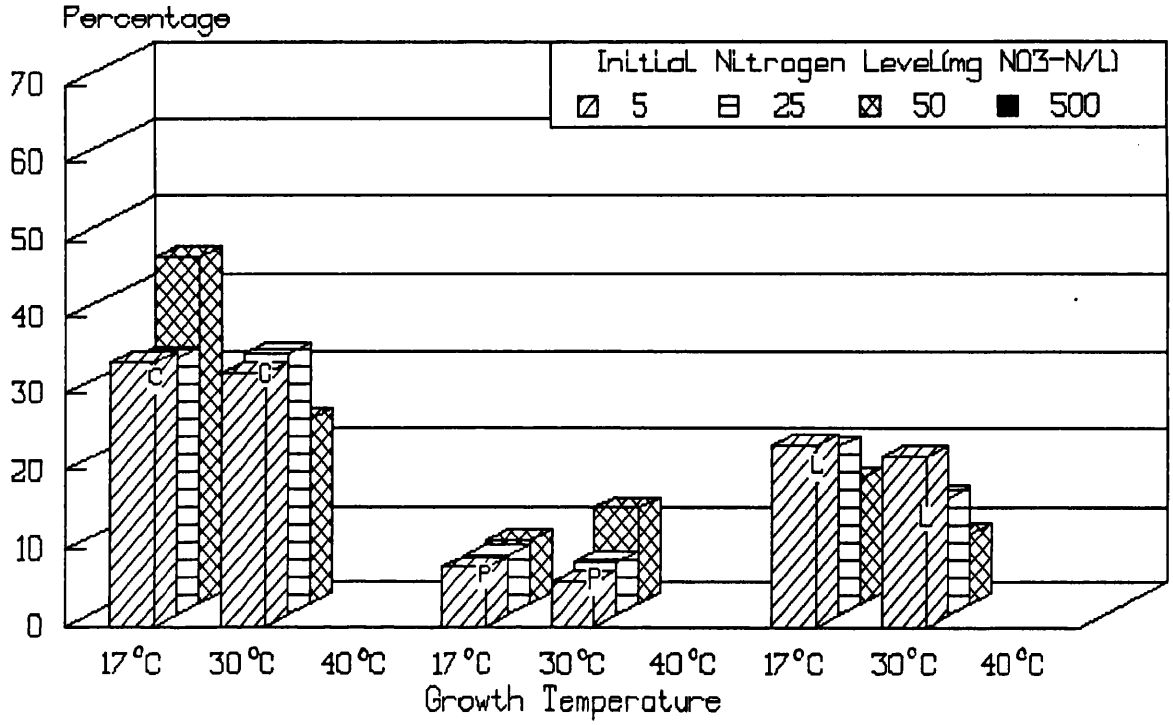


Table 16: Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) for S. obliquus 276/3A

	17°C						30°C					
Fatty Acid	5S	25E	25S	50E	50S	500E	5S	25E	25S	50E	50S	500E
12:0	-	-	-	-	-	-	-	-	-	-	-	-
14:0	.13	.18	.15	.19	.14	.16	.12	.39	.22	.32	.20	.26
14:1(n-5)	-	-	-	-	-	-	-	-	-	-	-	-
15:0	-	-	-	-	-	-	-	-	-	-	-	-
16:0	19.64	15.97	19.18	16.00	17.50	17.59	21.63	30.06	24.14	27.67	27.17	27.48
16:1(n-7)	1.64	2.98	1.66	2.96	1.78	2.88	1.56	4.45	2.04	3.22	3.50	3.54
16:2	1.41	0.39	1.06	1.16	1.26	0.79	2.30	1.30	2.20	2.98	2.53	1.15
16:3(n-6)	1.26	2.44	1.89	1.91	1.87	2.88	1.00	2.58	0.90	3.33	1.55	1.87
16:4	2.02	14.40	1.78	14.01	1.48	6.80	1.43	4.29	0.77	3.96	0.95	1.49
17:0	-	-	-	-	-	-	-	-	-	-	-	-
18:0	1.46	0.38	1.59	0.42	1.67	0.79	2.42	0.80	2.66	0.96	2.58	1.27
18:1(n-9+ n-7)	55.70	17.94	55.22	18.80	55.66	33.45	49.34	33.02	49.61	29.59	42.00	42.43
18:2(n-6)	4.89	6.32	4.90	6.08	6.42	10.18	11.53	12.05	12.20	13.73	13.47	12.13
18:3(n-3)	10.18	34.60	10.58	33.74	9.29	18.78	7.12	9.14	4.15	9.68	3.66	6.37
18:3(n-6)	-	-	-	-	-	-	-	-	-	-	-	-
18:4(n-3)	1.13	3.90	1.50	3.99	2.28	3.96	1.32	1.84	1.01	2.07	0.87	1.84
19:0	-	-	-	-	-	-	-	-	-	-	-	-
20:0	.16	.49	.14	.75	.17	1.27	-	-	-	1.26	0.75	-
20:1(n-9)	.31	TR	.27	TR	.43	.19	-	-	-	1.20	0.66	
20:2(n-6)	-	-	-	-	-	-	-	-	-	-	-	-
20:3(n-6)	-	-	-	-	-	-	-	-	-	-	-	-
20:4(n-6)	-	-	-	-	-	-	-	-	-	-	-	-
20:4(n-3)	-	-	-	-	-	-	-	-	-	-	-	-
20:5(n-3)	-	-	-	-	-	-	-	-	-	-	-	-
21:0	-	-	-	-	-	-	-	-	-	-	-	-
22:0(IS)	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS
22:1(n-9)	-	-	-	-	-	-	-	-	-	-	-	-
22:5(n-3)	-	-	-	-	-	-	-	-	-	-	-	-
22:6(n-3)	-	-	-	-	-	-	-	-	-	-	-	-
24:0	-	-	-	-	-	-	-	-	-	-	-	-
% Lipid	28.83	7.80	32.40	7.21	25.50	8.86	29.70	4.96	20.93	5.23	9.11	6.19
% SAFA	21.39	17.02	21.06	17.36	19.48	19.81	24.17	31.25	27.02	30.21	30.70	29.01
%UNFA	78.54	82.92	78.86	82.65	80.47	79.91	75.60	68.67	72.88	69.76	69.19	70.82
UNFA/SAFA	3.67	4.87	3.74	4.76	4.13	4.03	3.13	2.20	2.70	2.31	2.25	2.44
% Protein	5.70	11.70	5.10	13.40	4.50	9.10	1.50	6.90	3.60	7.60	7.40	5.80
%Carbohydrate	29.90	22.30	24.30	20.20	39.30	25.10	31.30	22.70	33.60	25.30	32.30	21.00

Note: (i) S obilquus 273/3A did not grow at 40°C

(ii) IS = Internal Standard; S = Stationary Phase; E = Exponential Phase; TR = Trace <0.1%; SAFA = Saturated Fatty Acids; UNFA = Unsaturated Fatty Acids

(iii) For systematic names of fatty acids see Appendix 1

FIG60 %Carbohydrate, Protein and Lipid
S. obliquus 276/3A Exponential Phase

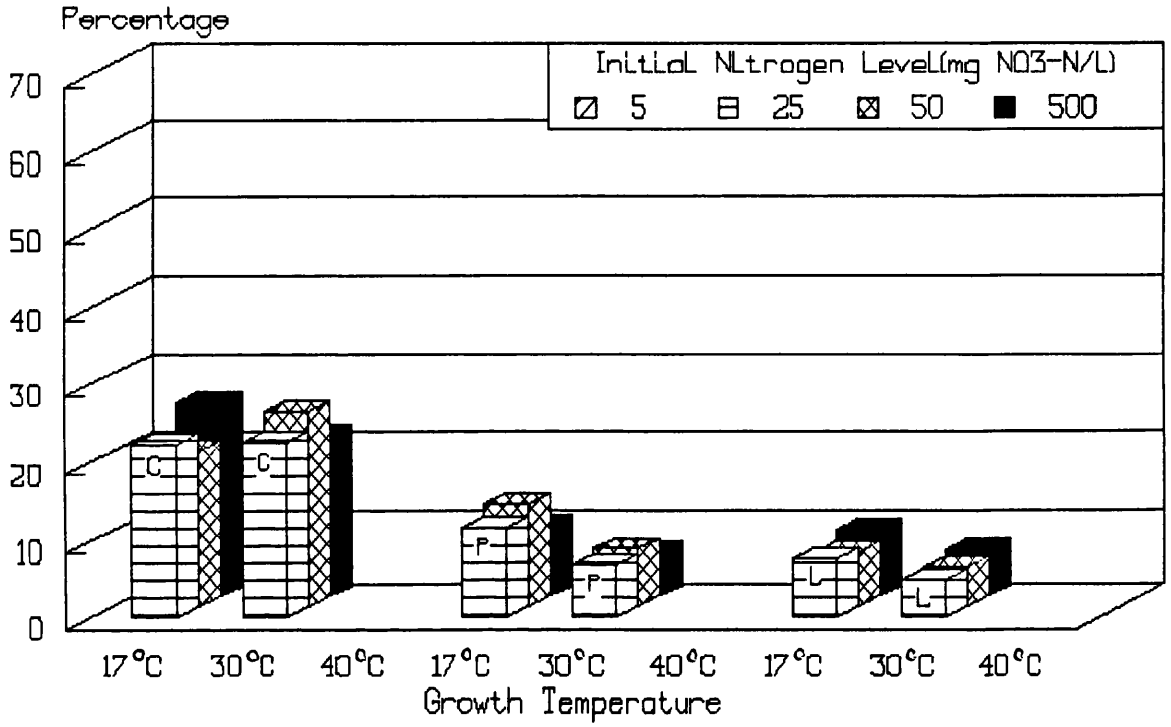


FIG61 %Carbohydrate, Protein and Lipid
S. obliquus 276/3A Stationary Phase

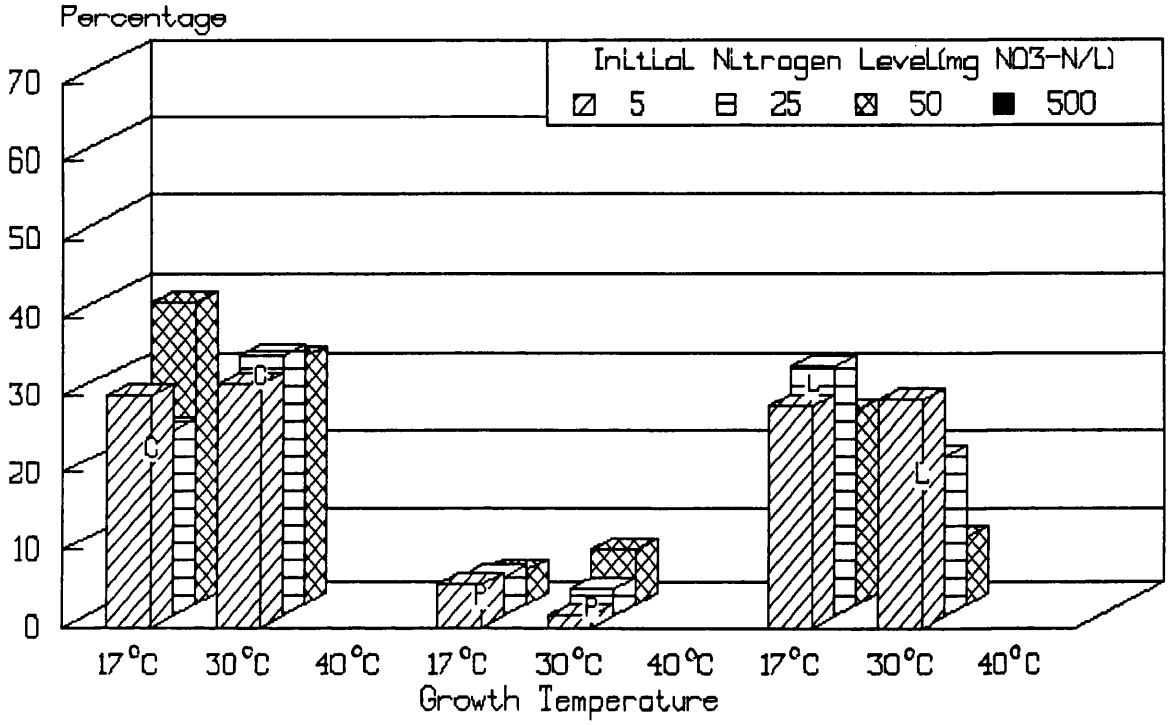


Table 17: Photosynthetic and Dark Respiration Rates for the Four Freshwater Green Algae

Organism	Time (Days)	OD_{560}	Dry Wt (gl^{-1})	Nitrogen Present (+ or -)	Dark Respiration Rate $mgO_2gDM^{-1}h^{-1}$	Gross Photosynthesis Rate $mgO_2g\ DM^{-1}h^{-1}$
17°C						
C. vulgaris 211/8K	14	1.24	0.35	+	5.69	17.09
	24	1.60	0.55	-	2.55	6.47
C. vulgaris 211/11C	19	1.26	0.36	+	7.91	23.33
	32	2.00	0.98	-	2.47	3.46
Ank. antarcticus 202/25	11	0.49	0.18	+	9.56	24.56
	24	1.29	0.56	-	2.04	12.25
S. obliquus 276/3A	11	0.69	0.35	+	5.60	25.97
	24	2.00	0.98	-	1.30	8.03
30°C						
C. vulgaris 211/8K	13	0.68	0.24	+	12.46	17.17
	20	1.47	0.46	-	1.96	2.93
C. vulgaris 211/11C	13	1.08	0.32	+	5.91	23.63
	20	1.30	0.55	-	10.15	11.45
Ank. antarcticus 202/25	10	0.73	0.21	+	14.33	60.71
	25	1.98	0.78	-	8.27	19.81
S. obliquus 276/3A	10	0.49	0.2	+	8.85	73.45
	25	1.80	0.65	-	9.23	37.62
40°C						
C. vulgaris 211/8K	9	0.52	0.10	+	9.50	59.40
	17	0.62	0.22	-	7.59	39.22
C. vulgaris 211/11C	10	0.38	0.10	+	9.50	90.50
	16	0.85	0.36	-	2.00	10.44

4.3.2 Brackish and Marine species

4.3.2.1 Growth and Nitrate Results

N. atomus 251/4B and Isochrysis sp. 927/14 were found to grow at two of the experimental temperatures, 17°C and 30°C, but N. oculata 849/1 and Isochrysis galbana 927/4 only grew at 17°C. Results for OD_{560} against time (N. atomus 251/4B - Figs 62 and 63; N. oculata 849/1 - Fig 64; Isochrysis sp 927/14 - Figs 65 and 66; Isochrysis galbana - Fig 67) and dry weight against time (N. atomus 251/4B - Figs 68 and 69; N. oculata 849/1 - Fig 70; Isochrysis sp 927/14 - Figs 71 and 72; Isochrysis galbana - Fig 73) show N. atomus and Isochrysis sp grew significantly faster at 30°C than at 17°C, and that all the algae were slow growing at 17°C.

Time considerations restricted the harvesting of cultures to a maximum of 45 days at 17°C, at which time 50mg NO_3-N l^{-1} cultures still had significant residual nitrate levels (Tables 18, 19, 20, 21). However, the growth rate was slowing down (Figs 62, 64, 65, 67) which suggested stationary phase had been reached or was approaching and these cultures were therefore designated S/LE to signify stationary/late exponential growth phase. All cultures at 5 and 25mg NO_3-N l^{-1}, at 17°C, achieved nitrate depletion or very low nitrate levels (≤ 0.25mg NO_3-N l^{-1}) at stationary phase harvest with the exception of N. oculata (Table 19).

At 30°C, harvesting at 26 days gave rise to a similar problem in that nitrate levels were still significantly high (Tables 18 and 20). Similarly, growth curves (Figs 63, 66, 69, 72) suggested stationary phase had been reached but these were again designated S/LE to signify stationary/late exponential growth phase.

Results of nitrate utilization against time (N. atomus - Figs 74 and 75; N. oculata - Fig 76; Isochrysis sp - Figs 77 and 78; Isochrysis galbana - Fig 79) showed that nitrate depletion occured in the order 5 -> 25 -> 50mg NO_3-N l^{-1}. Cultures at 500mg NO_3-N l^{-1} never attained nitrogen depletion.

pH results (N. atomus - Figs 80 and 81; N. oculata - Fig 82; Isochrysis sp - Figs 83 and 84; Isochrysis galbana - Fig 85) showed a rise and fall in pH with growth but the pH change was not as dramatic as for as the four fresh water green algae (Figs 44-53).

FIG62 N.atomus 251/4B 17°C
OD 560 vs Time

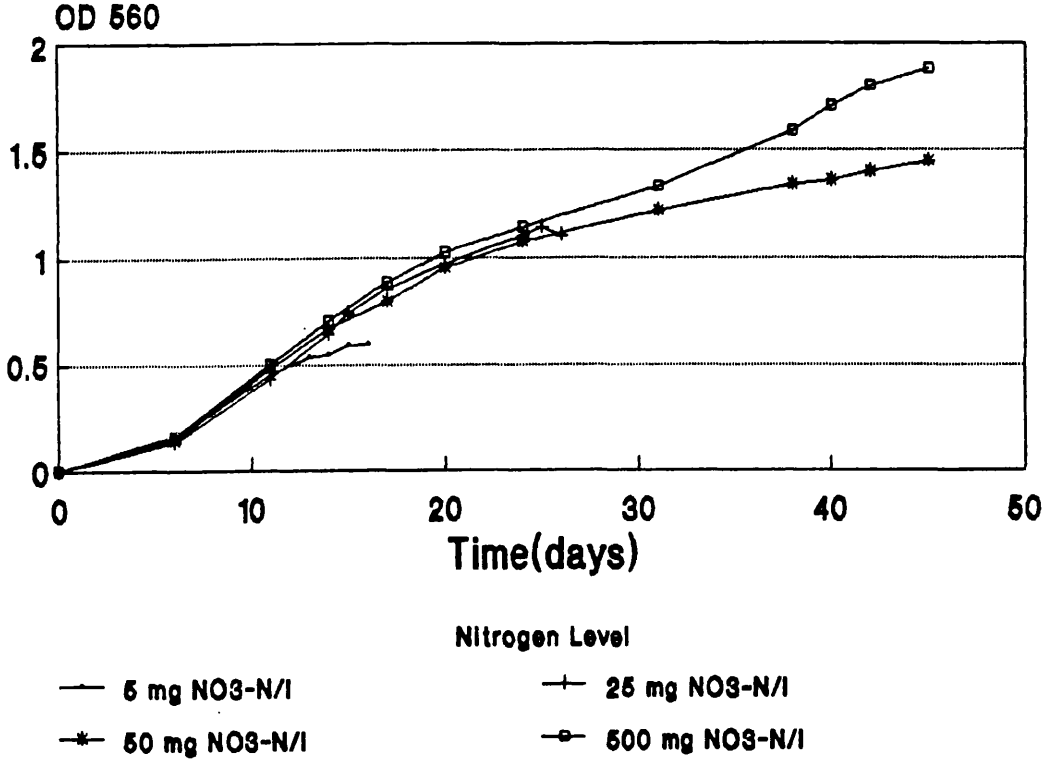


FIG63 N.atomus 251/4B 30°C
OD 560 vs Time

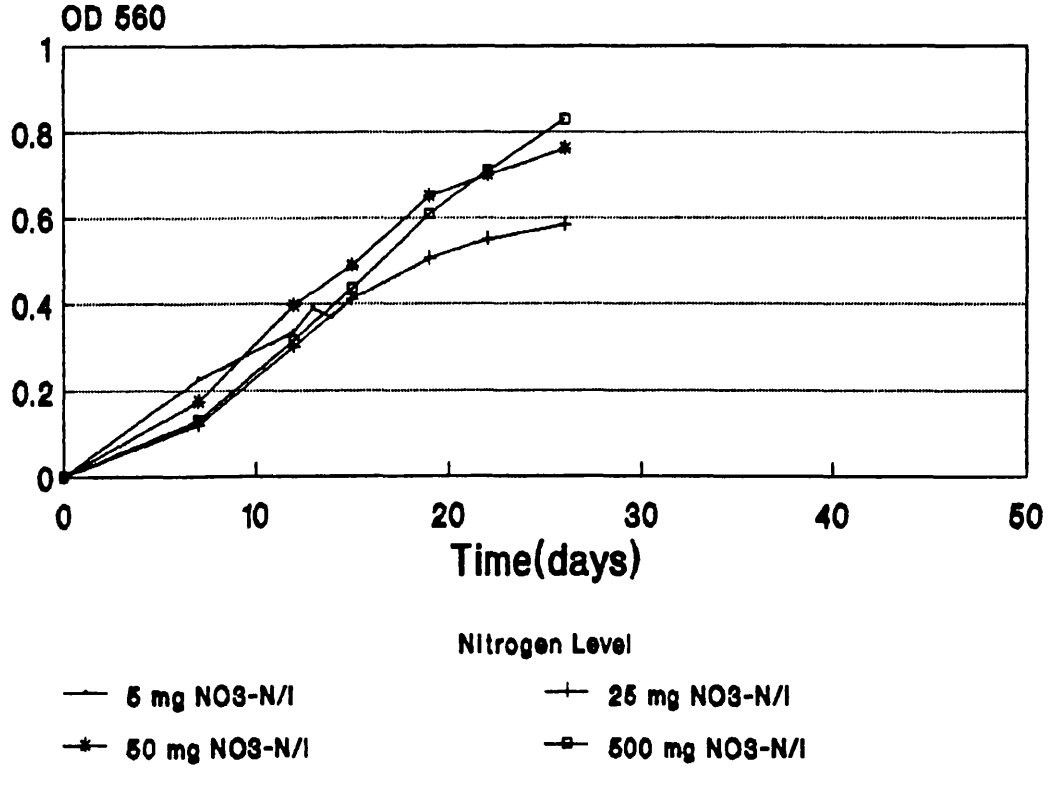


FIG64 N.oculata 849/1 17°C
OD 560 vs Time

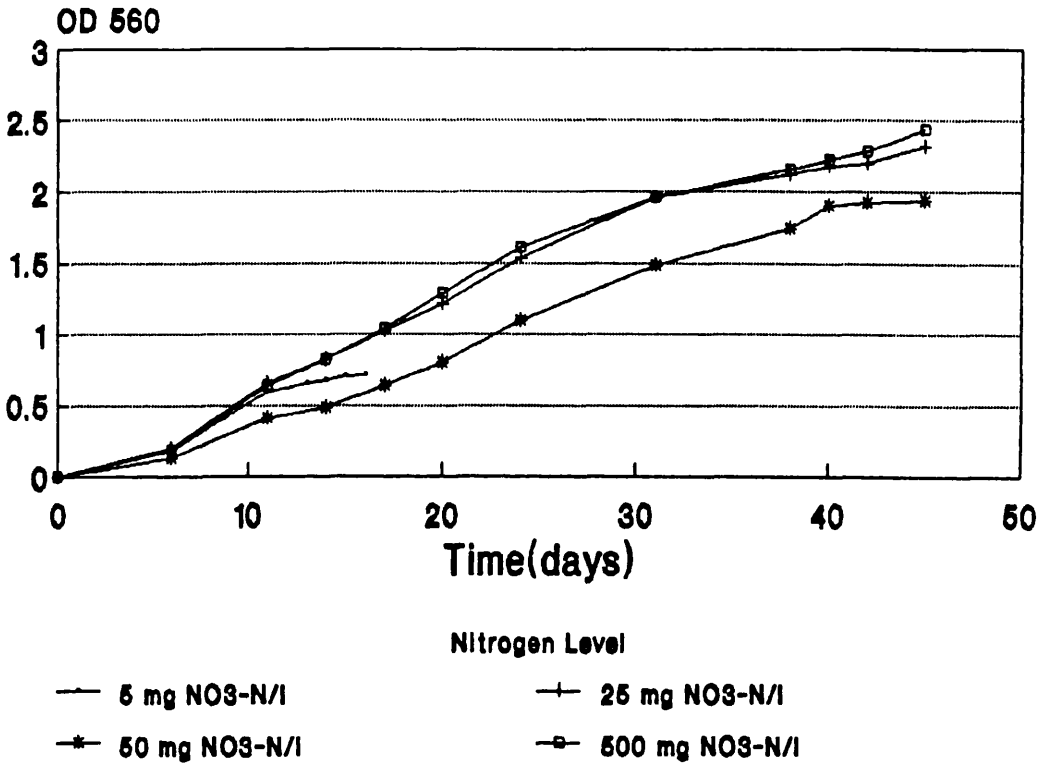


FIG65 Isochrysis sp. 927/14 17°C
OD 560 vs Time

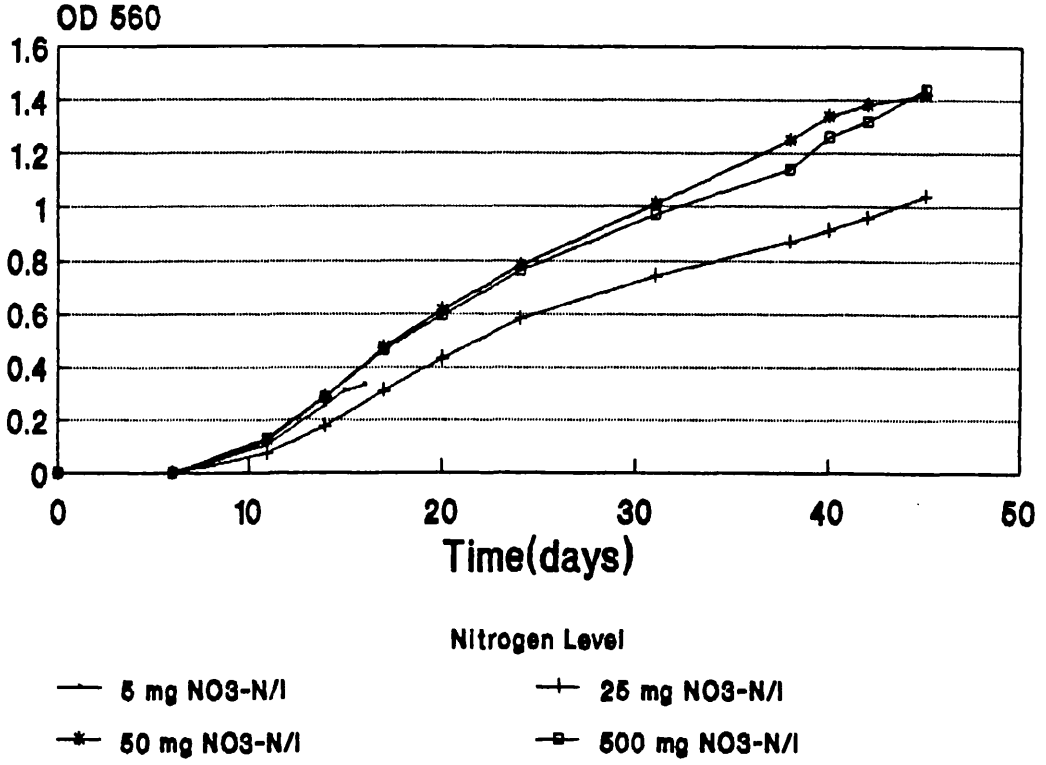


FIG66 Isochrysis sp. 927/14 30°C
OD 560 vs Time

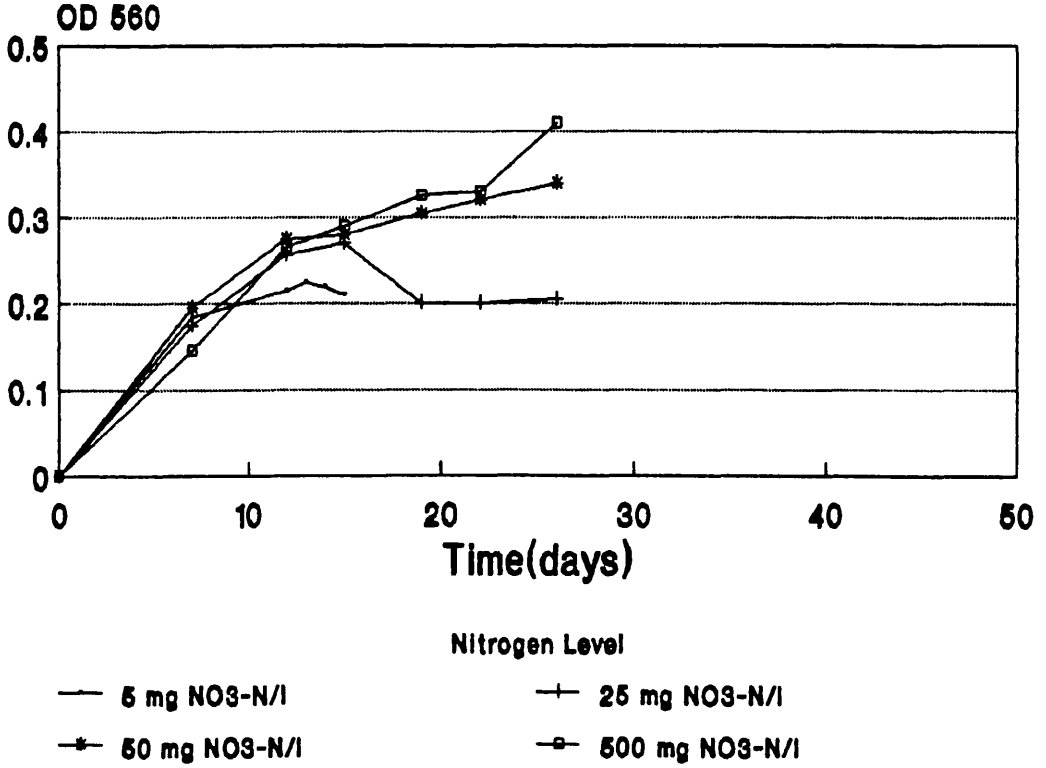


FIG67 Isochrysis galbana 927/1 17°C
OD 560 vs Time

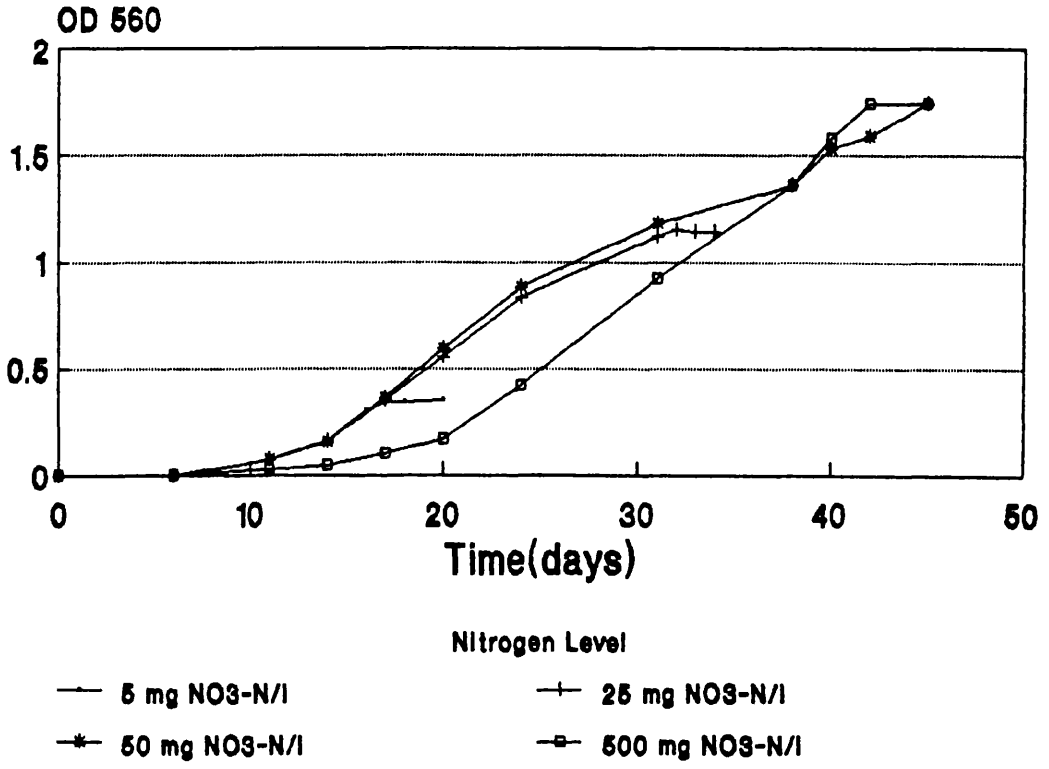


FIG68 N.atomus 251/4B 17°C
DRY WEIGHT vs Time

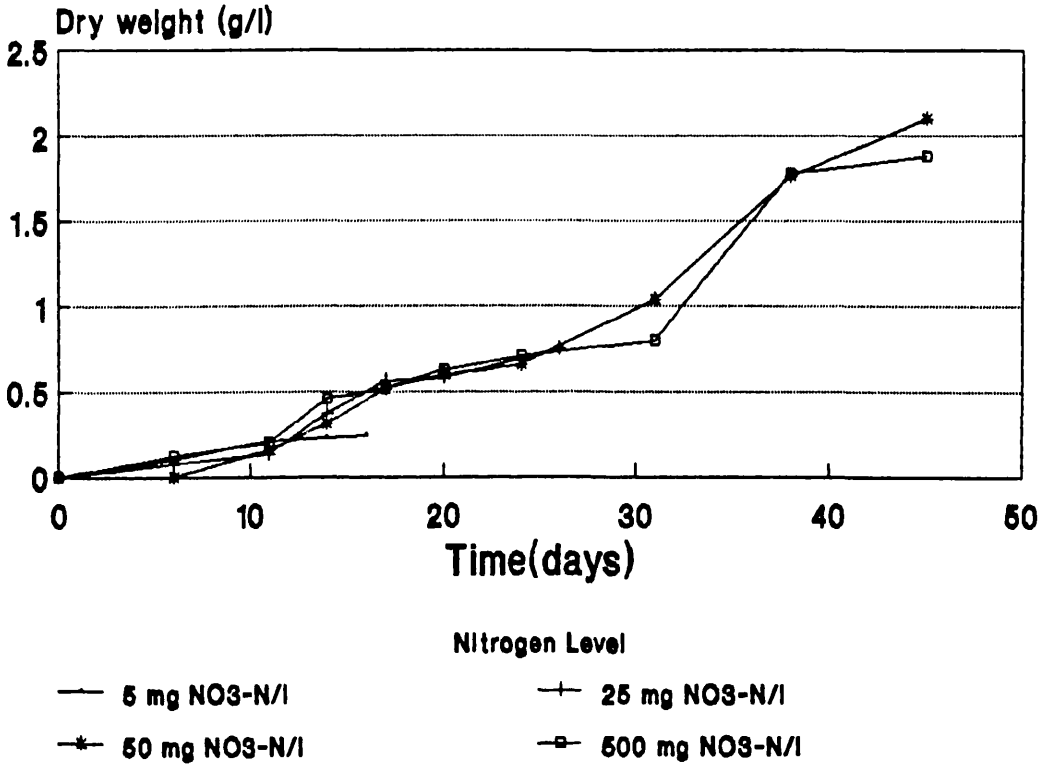


FIG69 N.atomus 251/4B 30°C
DRY WEIGHT vs Time

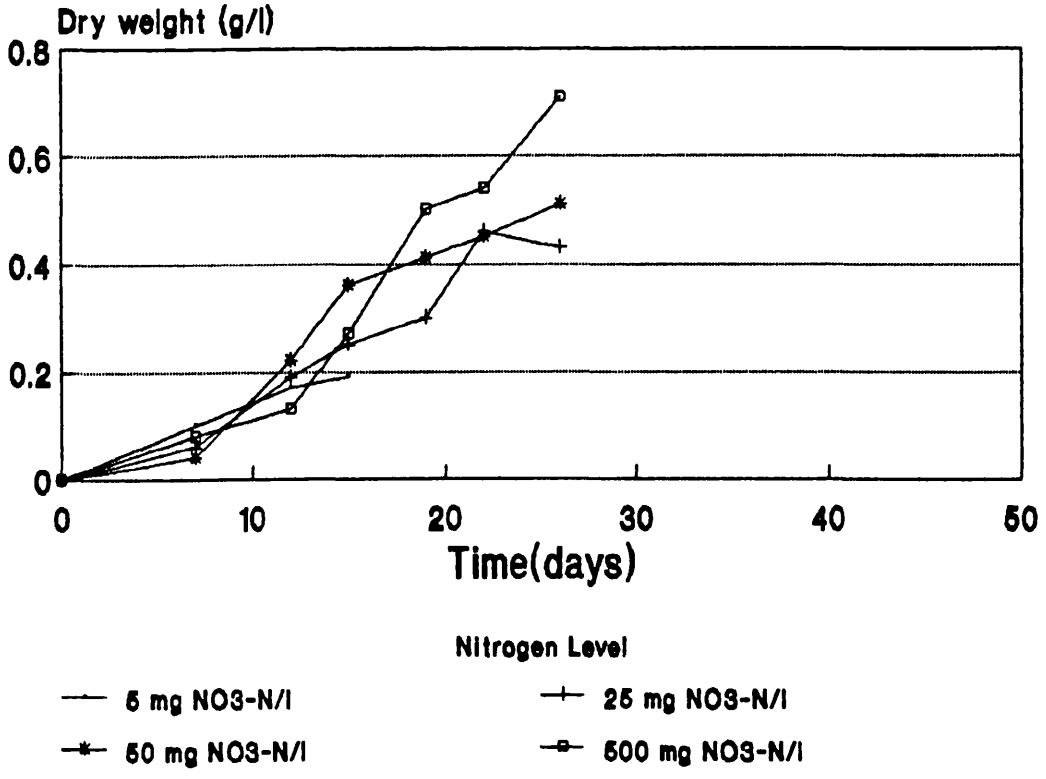


FIG70 N.oculata 849/1 17°C
DRY WEIGHT vs Time

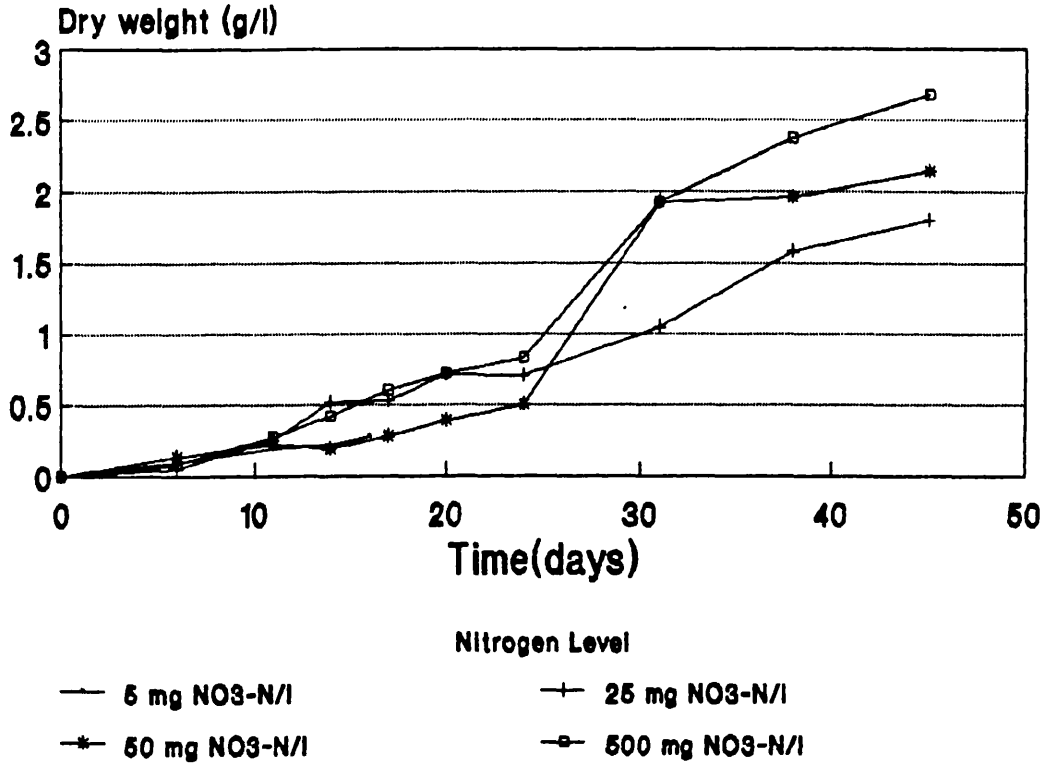


FIG71 Isochrysis sp. 927/14 17°C
DRY WEIGHT vs Time

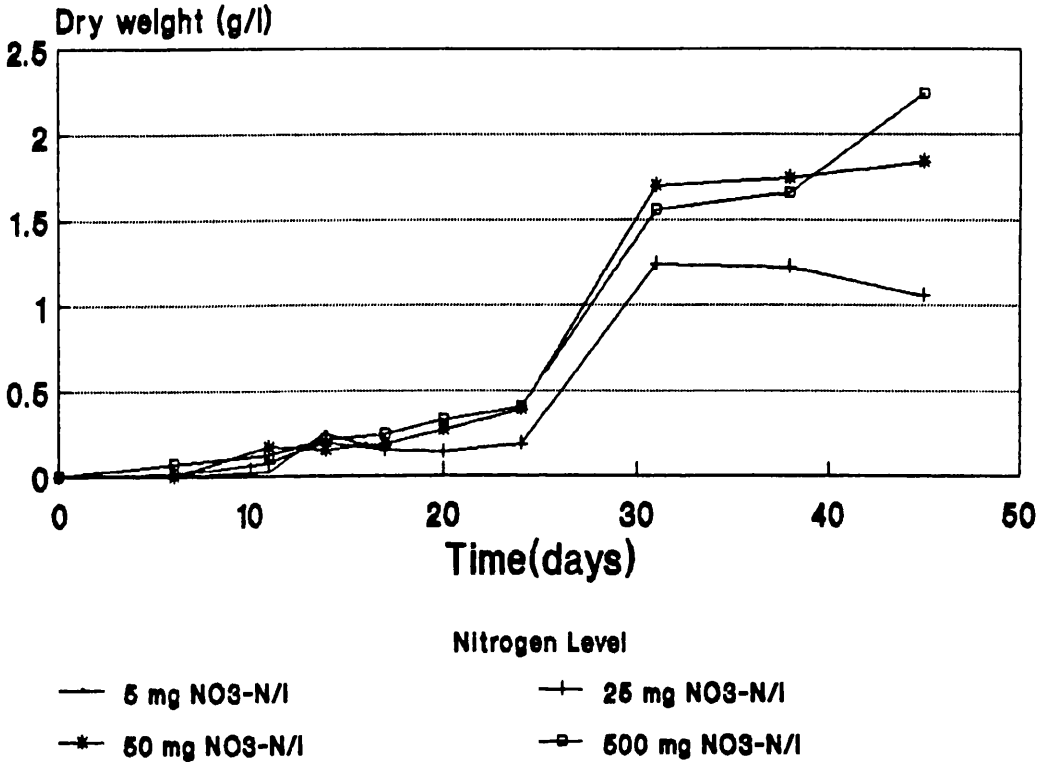


FIG72 Isochrysis sp. 927/14 30°C
DRY WEIGHT vs Time

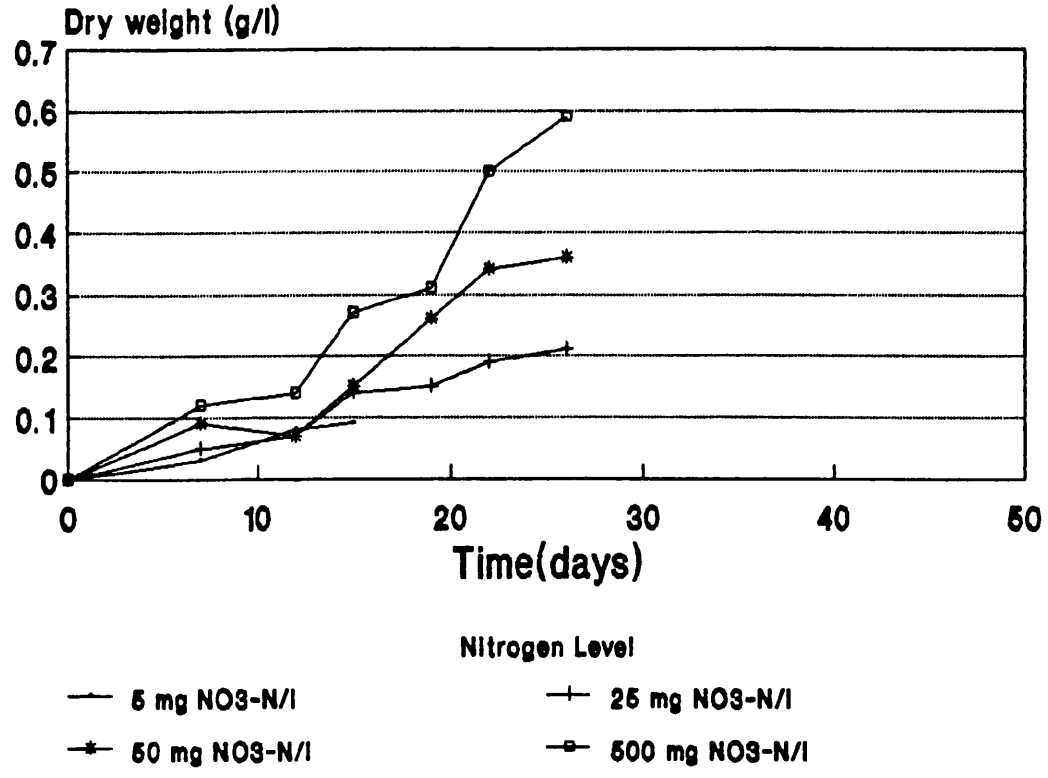


FIG73 Isochrysis galbana 927/1 17°C
DRY WEIGHT vs Time

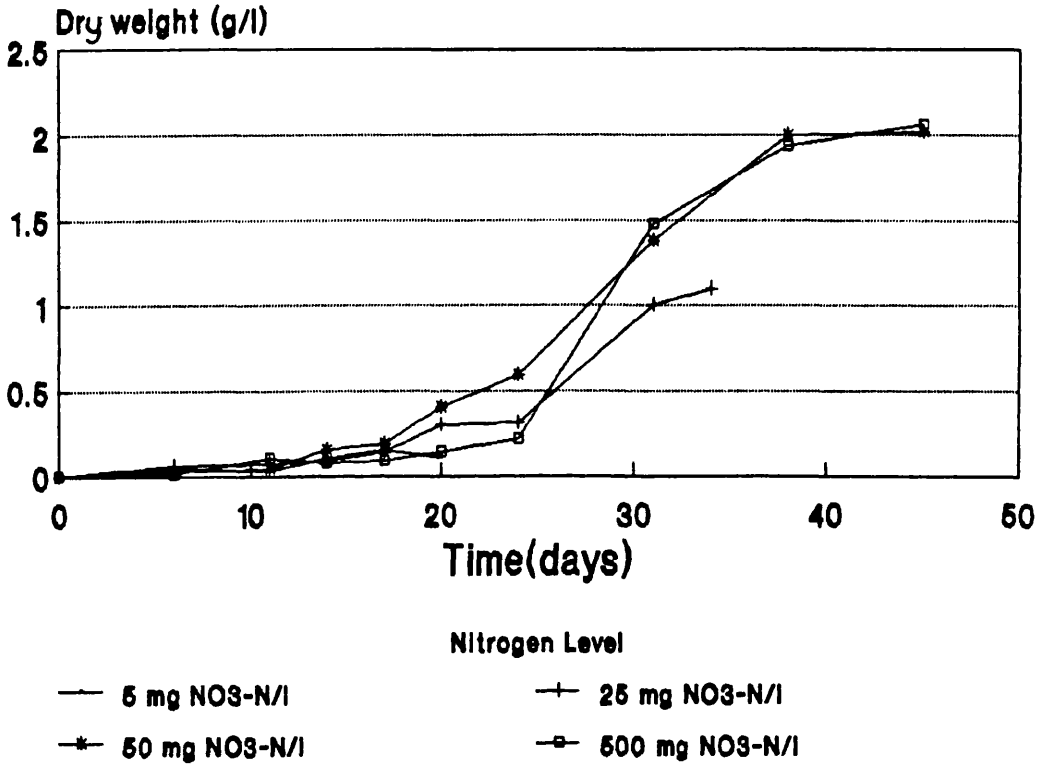


FIG74 N.atomus 251/4B 17°C
NITRATE vs Time

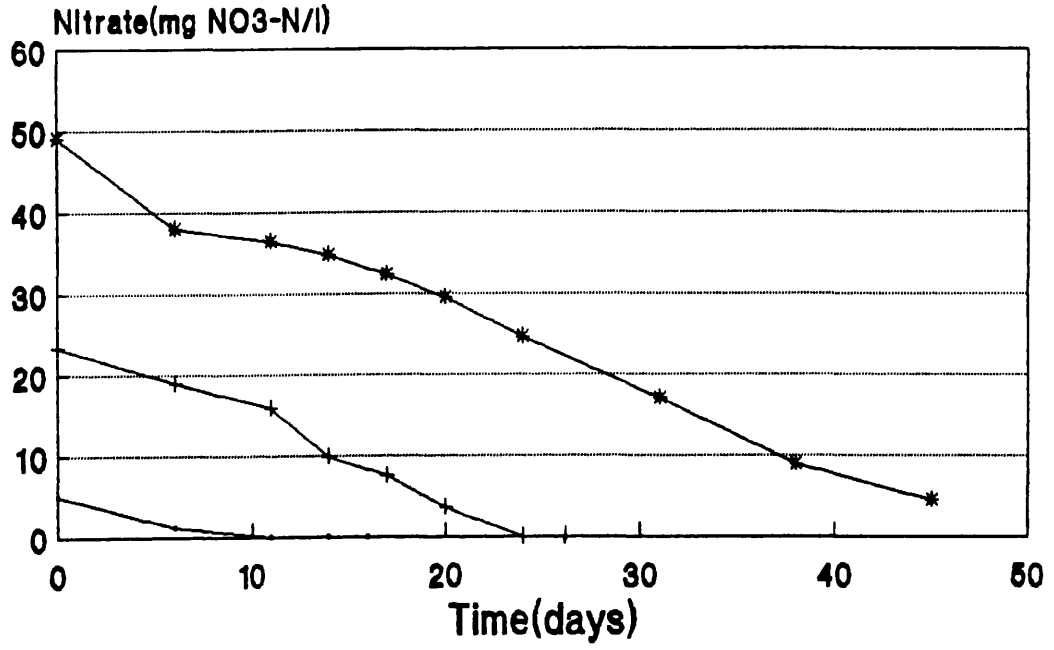


FIG75 N.atomus 251/4B 30°C
NITRATE vs Time

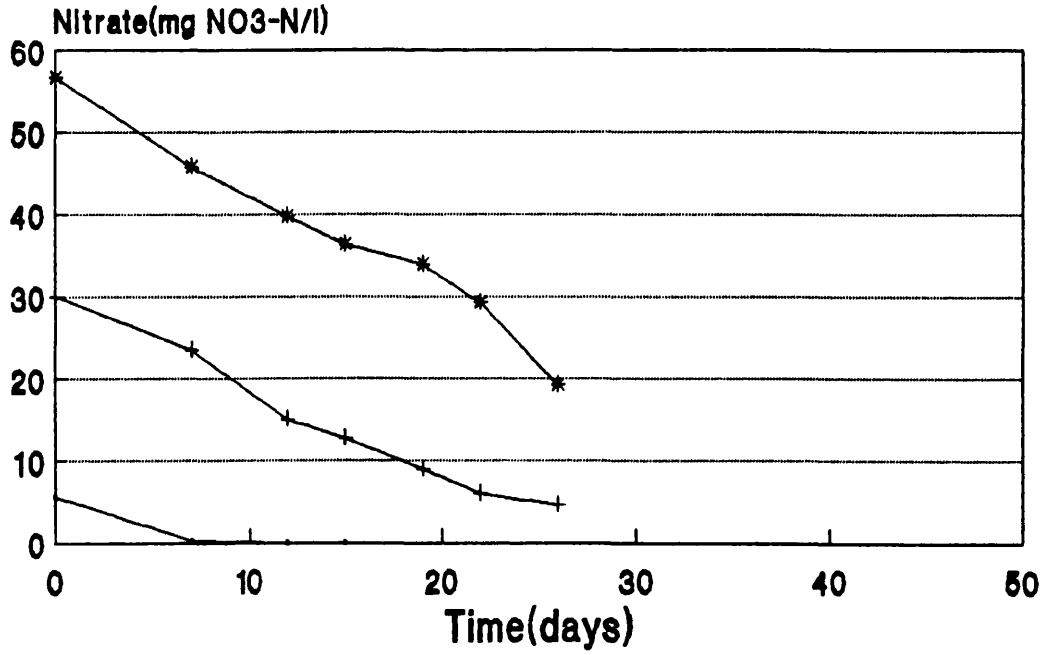


FIG76 N.oculata 849/1 17°C
NITRATE vs Time

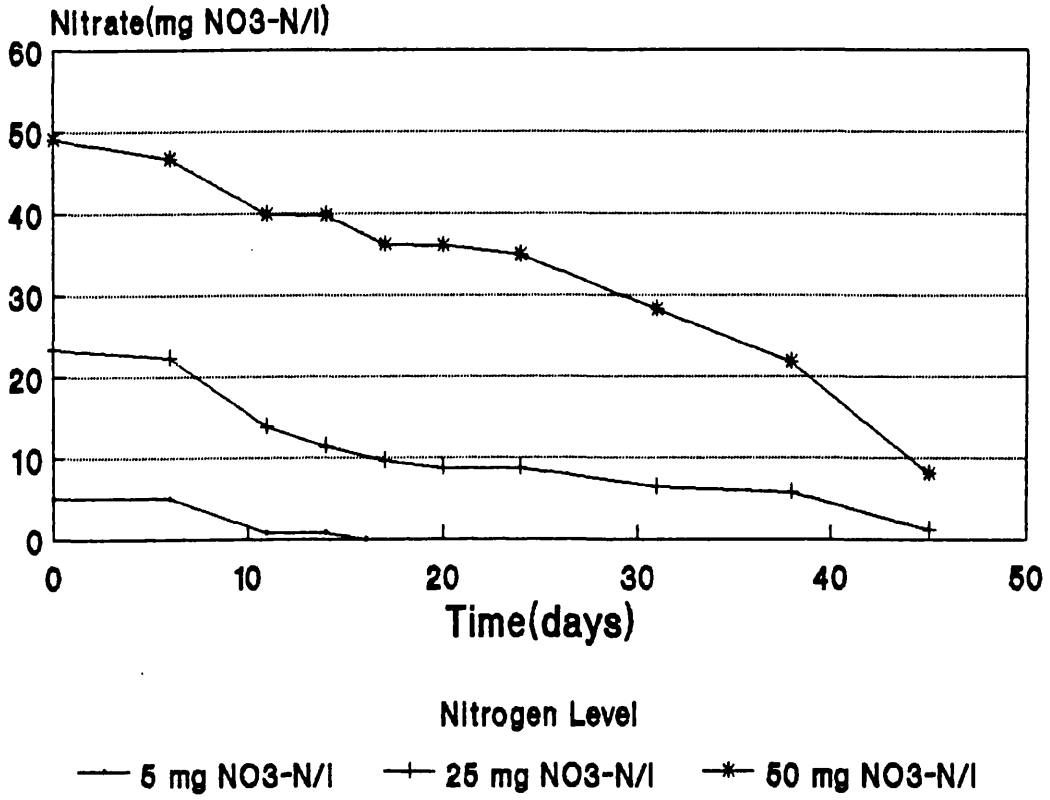


FIG77 Isochrysis sp. 927/14 17°C
NITRATE vs Time

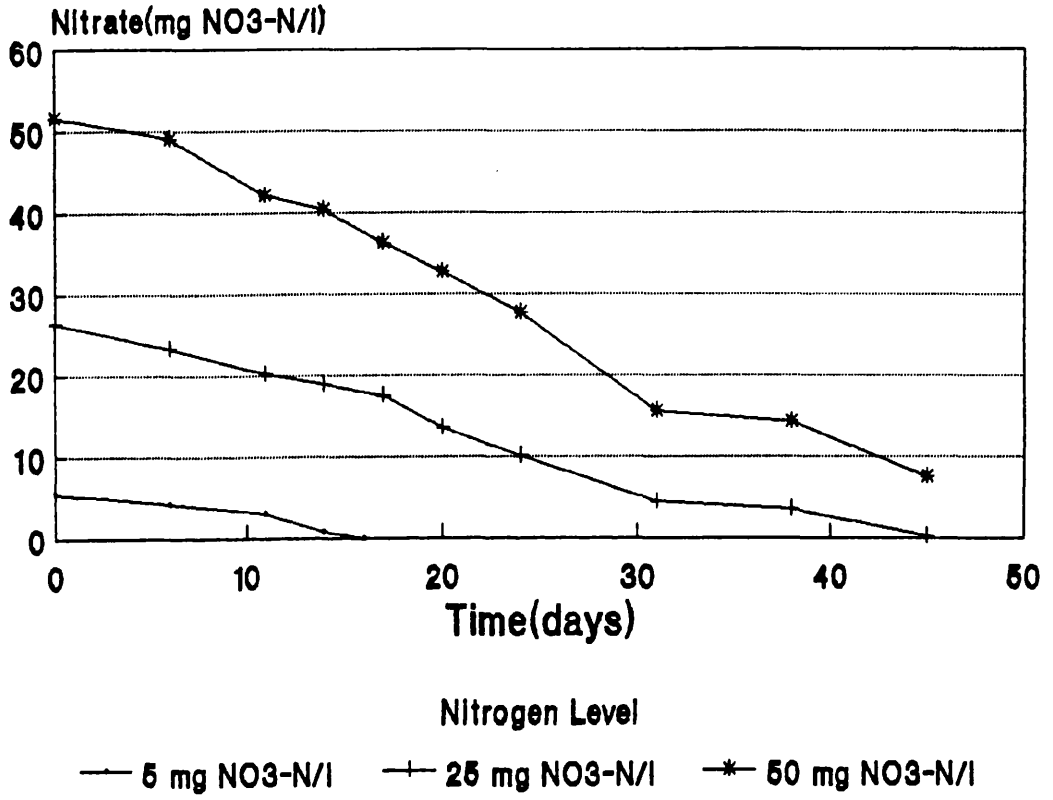


FIG78 Isochrysis sp. 927/14 30°C
NITRATE vs Time

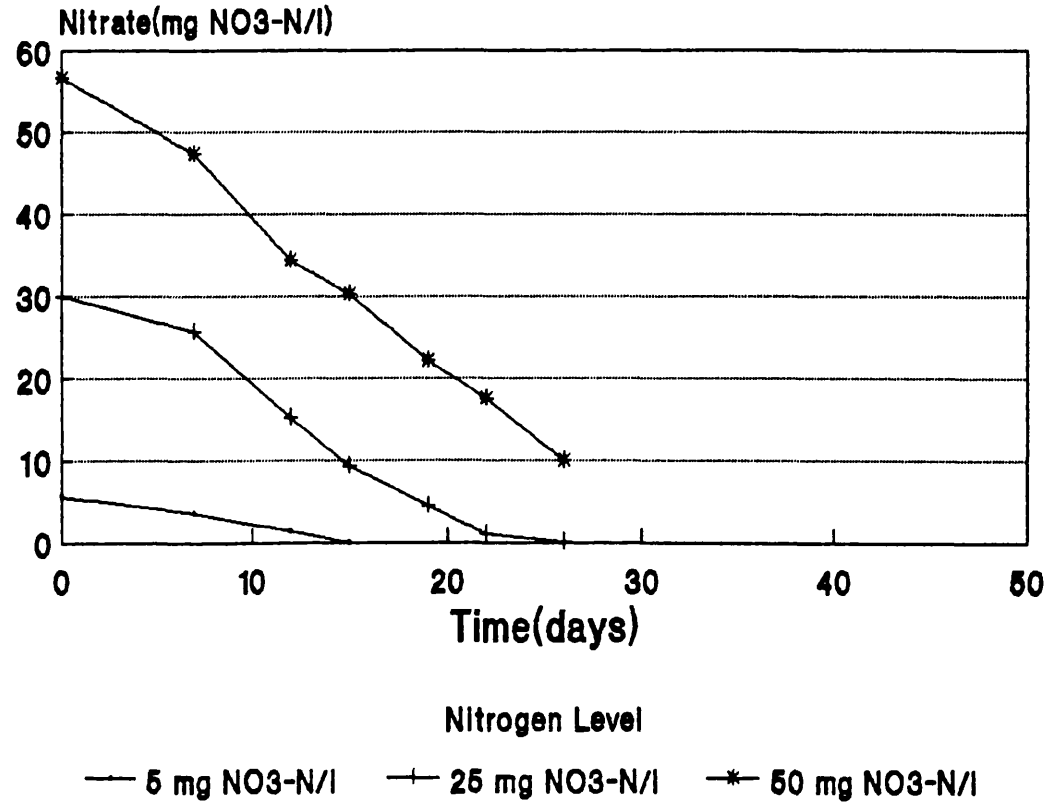


FIG79 Isochrysis galbana 927/1 17°C
NITRATE vs Time

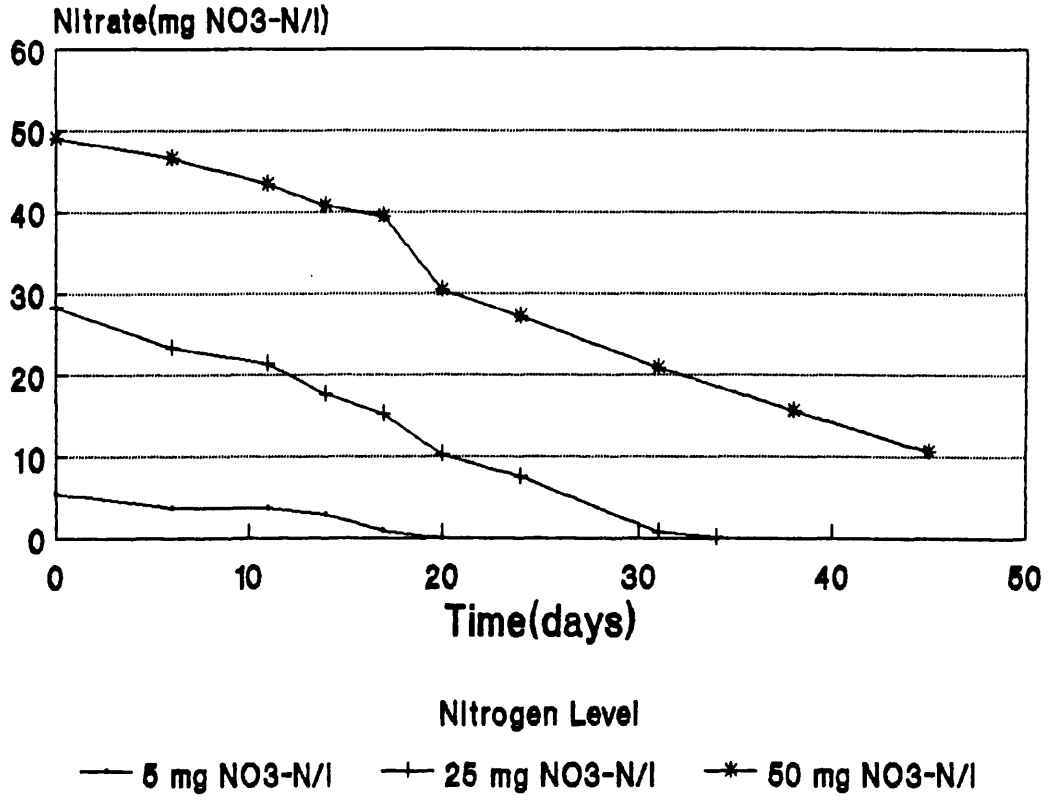


FIG80 N.atomus 251/4B 17°C
pH vs Time

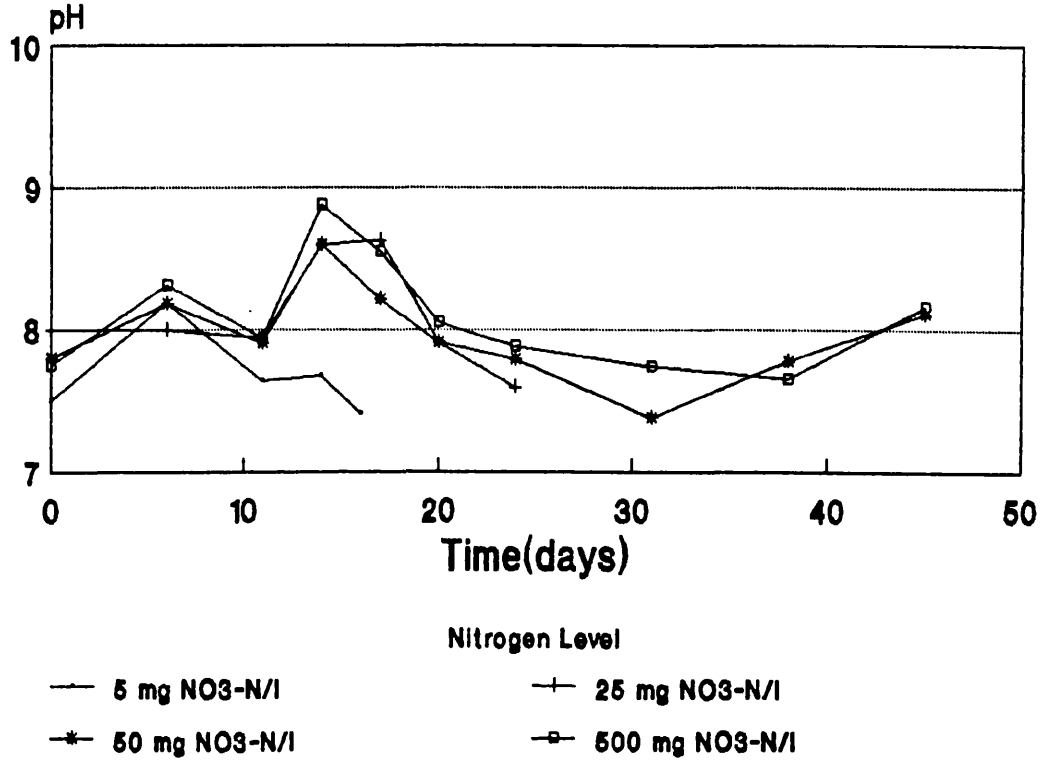


FIG81 N.atomus 251/4B 30°C
pH vs Time

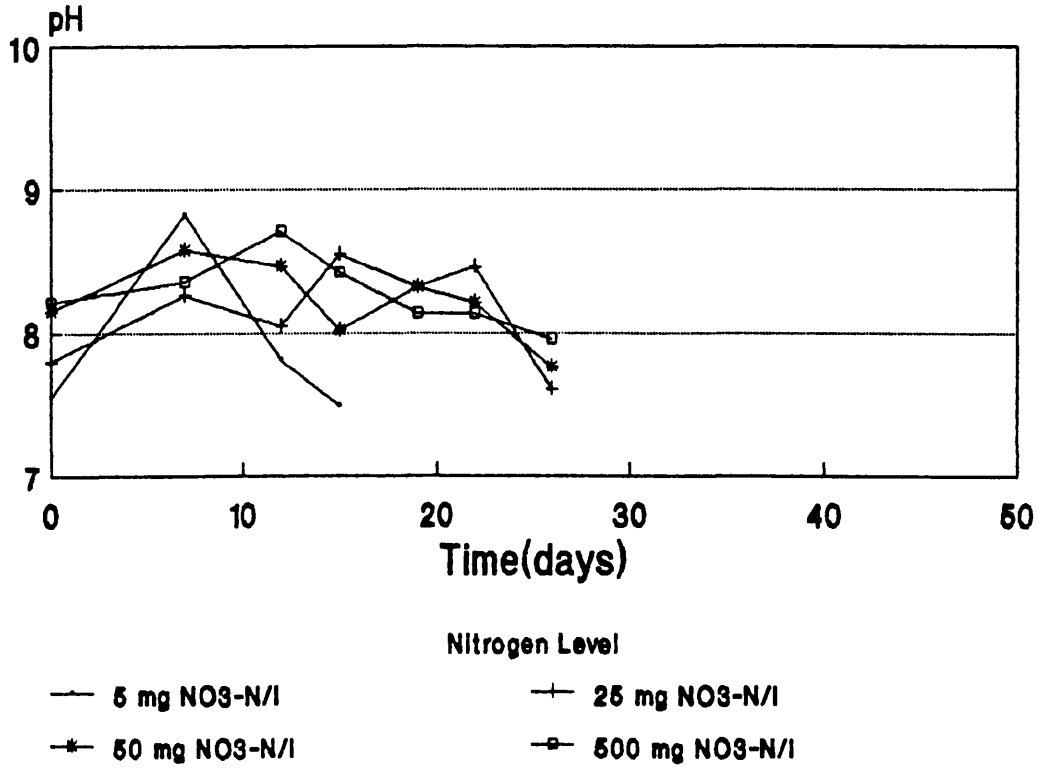


FIG82 N.oculata 849/1 17°C
pH vs Time

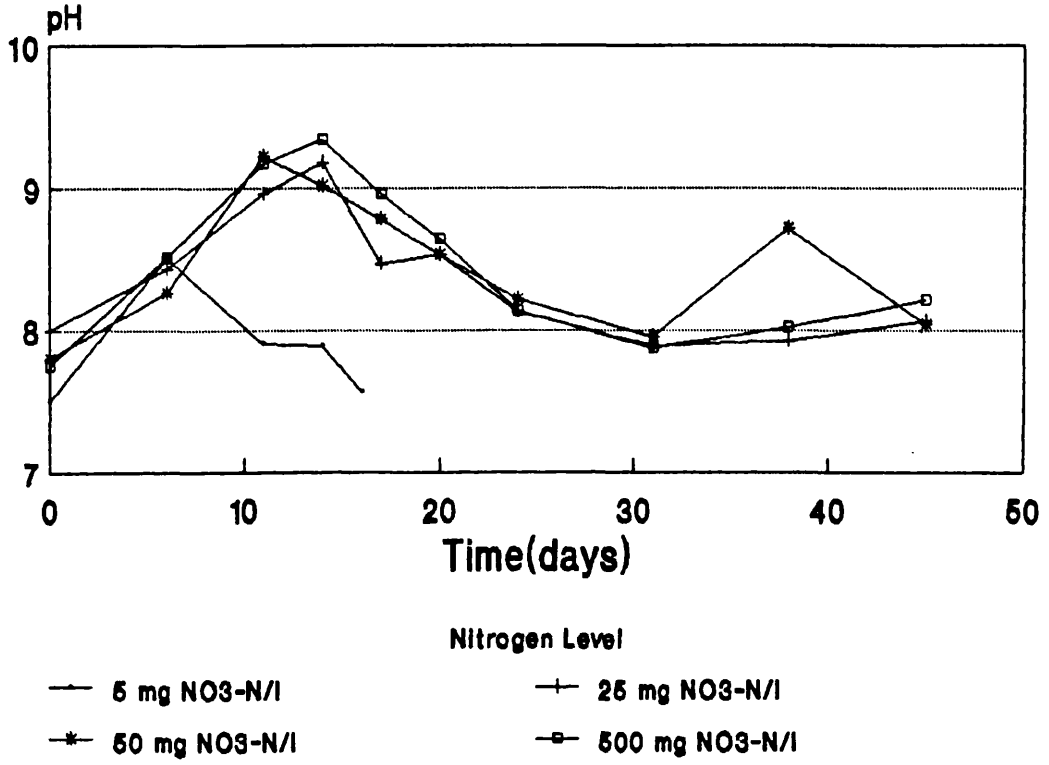


FIG83 Isochrysis sp. 927/14 17°C
pH vs Time

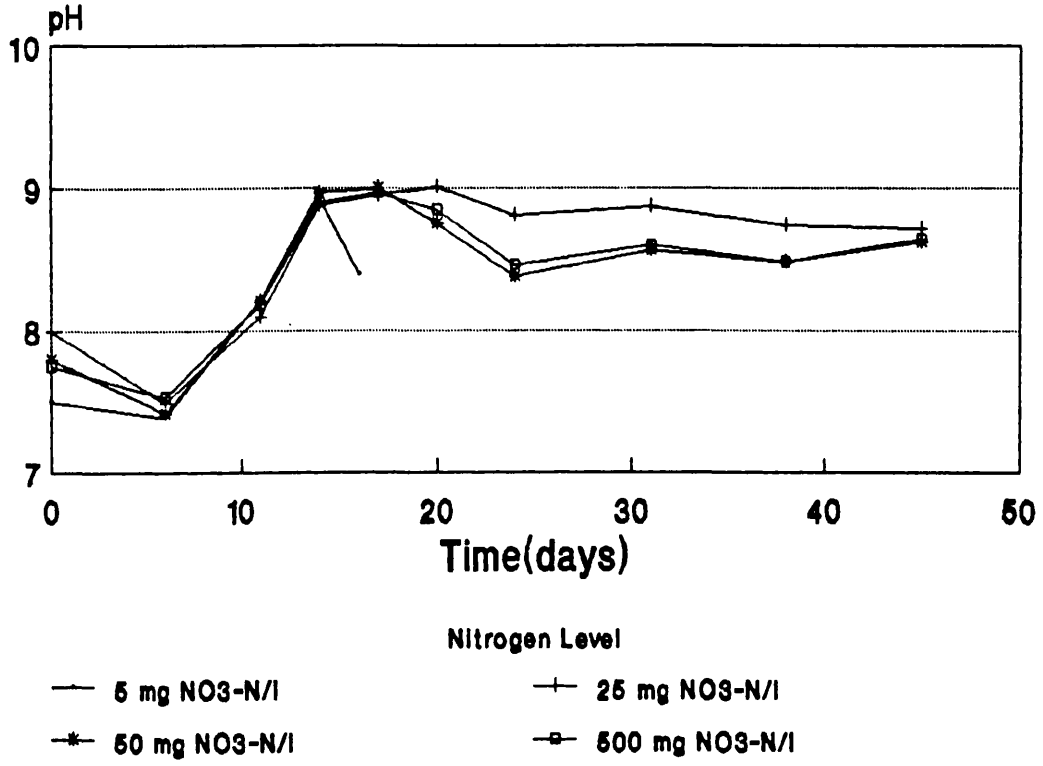


FIG84 Isochrysis sp. 927/14 30°C
pH vs Time

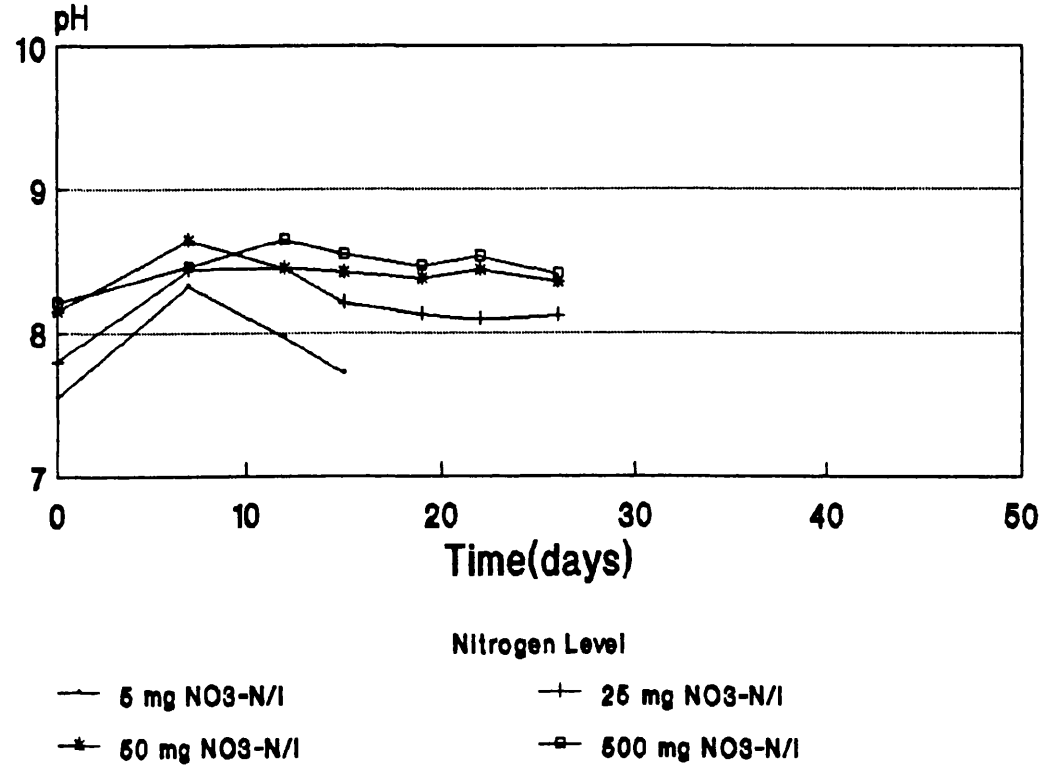


FIG85 Isochrysis galbana 927/1 17°C
pH vs Time

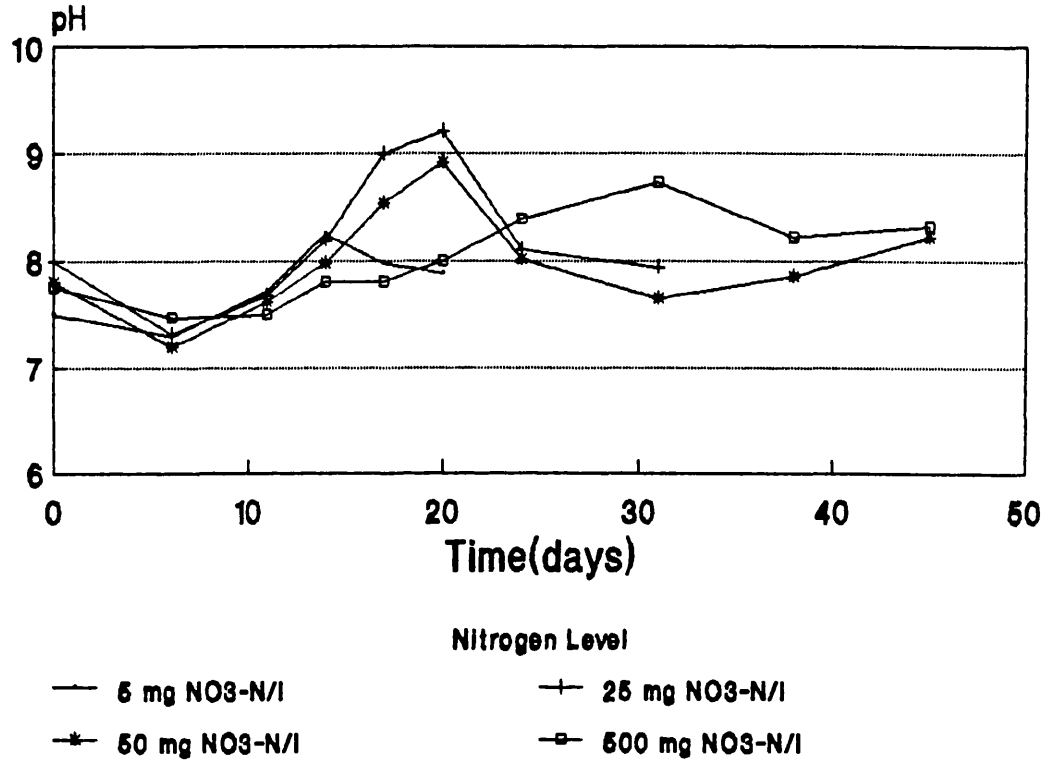


Table 18: Harvest Parameters for N. atomus 251/4B

Temperature/ Initial N Level		Phase	Time (Days)	$0D_{560}$	Dry wt (gl^{-1})	NO_3-N (mgl^{-1})
17°C	5	S	16	0.59	0.24	0
	25	E	15	0.74	0.37	9.94
	25	S	26	1.10	0.75	0
	50	E	20	0.97	0.60	29.57
	50	S/LE	45	1.45	2.10	4.51
	500	E	45	1.88	1.88	393
30°C	5	S	15	0.41	0.19	0
	25	E	15	0.41	0.25	12.66
	25	S/LE	26	0.59	0.43	4.69
	50	E	15	0.49	0.36	36.29
	50	S/LE	26	0.76	0.51	19.30
	500	E	26	0.83	0.71	431

Table 19: Harvest Parameters for N. oculata 849/1

Temperature/ Initial N Level		Phase	Time (Days)	$0D_{560}$	Dry wt (gl^{-1})	NO_3-N (mgl^{-1})
17°C	5	S	16	0.71	0.28	0
	25	E	20	1.21	0.71	8.76
	25	S/LE	45	2.32	1.80	1.20
	50	E	24	1.09	0.50	34.91
	50	S/LE	45	1.94	2.14	8.09
	500	E	45	2.44	2.68	376

E = Exponential Phase
S = Stationary Phase
S/LE = Stationary/Late Exponential Phase

Table 20: Harvest Parameters for Isochrysis sp. 927/14

Temperature/ Initial N Level		Phase	Time (Days)	$0D_{560}$	Dry wt (gl^{-1})	NO_3-N (mgl^{-1})
17°C	5	S	16	0.33	0.18	0
	25	E	24	0.58	0.19	10.09
	25	S	45	1.04	1.06	0.25
	50	E	24	0.78	0.39	27.72
	50	S/LE	45	1.42	1.84	7.6
	500	E	45	1.44	2.24	382
30°C	5	S	15	0.21	0.09	0.07
	25	E	15	0.27	0.14	9.44
	25	S	26	0.21	0.21	0.09
	50	E	22	0.32	0.19	17.55
	50	S/LE	26	0.34	0.36	10.06
	500	E	26	0.41	0.59	441

Table 21: Harvest Parameters for Isochrysis galbana 927/1

Temperature/ Initial N Level		Phase	Time (Days)	$0D_{560}$	Dry wt (gl^{-1})	NO_3-N (mgl^{-1})
17°C	5	S	20	0.35	0.11	0
	25	E	20	0.55	0.30	10.25
	25	S	34	1.14	1.10	0.09
	50	E	24	0.88	0.59	27.19
	50	S/LE	45	1.74	2.02	10.65
	500	E	45	1.74	2.06	401

E = Exponential Phase
S = Stationary Phase
S/LE = Stationary/Late Exponential Phase

4.3.2.2 Carbohydrate, Protein and Lipid Results

The results for carbohydrate, protein and lipid analyses are given in Tables 22, 23, 24, 25 for N. atomus, N. oculata, Isochrysis sp. and Isochrysis galbana respectively. It should be noted that the levels of lipid, protein and carbohydrate may be underestimated due to the fact that they are based on dry weight. The presence of salt in the F/2 media, used for cultivation of the marine and brackish species, would increase dry weight, and consequently reduce the percentage composition of cell constituents. Ash free dry weight was not determined for many samples due to lack of availability of algal material, and not reported where determined due to lack of duplication of samples.

N. atomus increased lipid content with a decrease in culture temperature (average lipid content at 17°C - 6.86%, 30°C - 3.32%), increased carbohydrate with decrease in growth temperature (average carbohydrate content at 17°C - 18.57%, 30°C - 15.14%) and increased protein with decrease in temperature (average protein content at 17°C - 5.62%, 30°C - 1.22%). Regarding growth phase, N. atomus accumulated lipid in stationary phase but only at 17°C (Table 22). Protein did not significantly change between phases. Carbohydrate decreased in stationary phase at 17°C with the exception of 5s, and increased in stationary phase at 30°C (Figs 86 and 87).

N. oculata did not grow at 30°C and therefore, a comparison across temperature was not possible. With respect to growth phase, N. oculata increased lipid content at 5s (with nitrogen depletion), which was not observed at 25 and 50 mg NO_3-N l^{-1} S/LE, suggesting the absence/presence of nitrate may be a trigger for lipid changes (Figs 88 and 89). Protein decreased slightly in stationary or stationary/late exponential phase (Figs 88 and 89). Carbohydrate results mirror the results for lipid and suggest the absence/presence of nitrate as a trigger for carbohydrate changes (Figs 88 and 89).

Isochrysis sp increased lipid content with decrease in growth temperature (average lipid content 17°C - 7.23%, 30°C - 1.96%), decreased protein slightly with decrease in temperature (average protein content at 17°C - 1.37%, 30°C - 1.57%) and increased carbohydrate slightly with a decrease in temperature (average carbohydrate content at 17°C - 5.85%, 30°C - 5.07%). Lipid content increased in stationary phase at 17°C and decreased at 30°C (Figs 90 and 91). Protein and carbohydrate contents did not change significantly between phases (Figs 90 and 91).

Isochrysis galbana did not grow at 30°C and can only be compared across growth phase. Lipid content decreased in stationary phase, 50 S/LE did not exhibit a decrease which may be due to nitrate availability (Figs 92 and 93). Protein decreased with stationary and stationary/late exponential phase (Figs 92 and 93). Carbohydrate exhibited a similar pattern to that of lipid content (Figs 92 and 93).

4.3.2.3 Statistical Analysis of Carbohydrate, Protein and Lipid Results

Statistical analysis of the lipid results for the four marine and brackish species found all the 'main effects', temperature, algal species, nitrogen level and phase were significant at 0.1% ($p < 0.001$). Lipid means at the two temperatures (17°C - 8.59%, 30°C - 4.19%) showed a significant reduction with increased temperature confirming lipid content increased at the lower culture temperature for N. atomus and Isochrysis sp. Nitrogen means ('5' (mg NO_3-N l^{-1}) - 11.22%, '25' - 7.34%, '50' - 4.74%, '500' - 2.96%) divide into three groups, with '5' significantly different to '25' both significantly different to '50' and '500'. Nitrogen history of the culture appeared to affect the level of lipid for all species. Phase means (exponential - 7.63%, stationary/late exponential - 5.15%) showed a significant reduction from exponential to stationary/late exponential phase. Species means (N atomus - 5.09%, N. oculata - 12.30%, Isochrysis sp -4.59%, Isochrysis galbana - 3.59%) showed a significant difference between N. oculata and the other algae.

Statistical analysis of the protein results gave significant 'main effects' for temperature (0.1% or $p < 0.001$), algae (0.1% or $p < 0.001$) and phase (1% or $p < 0.01$) only. Temperature means (17°C - 2.81%, 30°C - 0.72%) showed a significant reduction in protein content at the higher temperature. Species means (N. atomus - 3.42%, N. oculata - 0.26%, Isochrysis sp. - 1.47%, Isochrysis galbana - 1.91%) showed a significant difference between N. atomus, N. oculata and the two Isochrysis species which were themselves not significantly different. Phase means (2.24% - exponential, 1.29% - stationary) confirmed a significant reduction in protein content at stationary phase.

Statistical analysis of the carbohydrate results gave only two significant effects, algal species (0.1% or $p < 0.001$) and nitrogen (1% or $p < 0.01$). Species means (N. atomus - 16.85%, N. oculata - 6.74%, Isochrysis sp - 5.46%, Isochrysis galbana - 3.74%) showed a significant difference in behaviour between N. atomus and the other 3 algae, which were not significantly different to each other. The respective nitrogen means ('5' (mg NO_3-N l^{-1}) - 12.76%, '25' - 8.15%, '50' - 6.22%, '500' - 7.70%) showed the value at the lowest nitrogen level to be significantly higher than at the other three initial nitrogen levels, which themselves were not significantly different. This may be due to the the fact that cultures at 5mg NO_3-N l^{-1} were left for a period after depletion, whereas other cultures at 25, and 50mg NO_3-N l^{-1} were not and therefore, carbohydrate increases may be enforced by prolonged nitrogen depletion.

Therefore the four brackish and marine algae were behaving differently with respect to changes in cellular constituents. Statistical analysis was limited by the lack of results at the two higher temperatures. However, the results indicated that lipid accumulation again was temperature and phase dependent and N. oculata behaved differently to the other algae. A difference in behaviour was also found between N. atomus, N. oculata and the two Isochrysis species for protein content, and between N. atomus and the other algae for carbohydrate contents.

4.3.2.4 Fatty Acid Results

The fatty acid profiles for all four species exhibited a range of constituent fatty acids from C12 to C22 (Tables 22, 23, 24, 25), a much broader range than that found in the freshwater green algae and cyanobacteria studied.

N. atomus exhibited little difference qualitatively in fatty acids between temperatures and growth phases (Table 22), however, quantitative differences were found. The major fatty acids in N. atomus were 16:0 and 18:1(n-9). Changes were observed with temperature and growth phase, especially in 18:1(n-9), 18:2(n-6) and 18:3(n-3). The overall level of unsaturation (sum of individual fatty acid changes) increased from 17°C to 30°C (average % UNFA, 17°C - 66.05%, 30°C - 69.34%). The % UNFA at 17°C increased from exponential to stationary/late exponential phase, and decreased from exponential to stationary/late exponential phase at 30°C. The presence of 20:5(n-3) and 22:6(n-3) fatty acids was noted, and these fatty acids were present in the range 0.48 - 2.75% and 0.33 - 2.34% respectively, depending on temperature and growth phase.

N. oculata exhibited few qualitative changes between growth phases (Table 23), however quantitative differences were apparent. The major fatty acids in N.oculata were 16:0, 16:1 and 18:1(n-9). The % UNFA increased from exponential to stationary/late exponential phase. 22:6(n-3) was not present in all samples and when identified, it was at extremely low levels. However, 20:5(n-3) was present in the range 5.97 - 9.96% depending on phase of growth.

Isochrysis sp. again exhibited few qualitative changes in fatty acid content between temperatures and growth phases, but quantitative differences were apparent. The major fatty acids found in Isochrysis sp. were 14:0, 16:0, 18:1(n-9), 18:4(n-3) and 22:6(n-3), 20:5(n-3) also present in low quantities. 14:0 and 16:0 fatty acids increased with increasing temperature and 18:1(n-9), 18:4 and 22:6(n-3) fatty acids decreased. Changes were also observed with phase (Table 24). The overall degree of unsaturation (sum of individual fatty acid

changes) decreased from 17°C to 30°C (average %UNFA, 17°C - 66.95%, 30°C - 54.16%), the opposite of N. atomus. % UNFA increased from exponential to stationary/late exponential phase at 17°C and 30°C. The presence of 20:5(n-3) and 22:6(n-3) was noted, and these fatty acids were present in the range 0.55 - 0.91% and 4.70 - 17.45% respectively.

Isochrysis galbana showed a similar fatty acid profile to Isochrysis sp.. Quantitative differences rather than qualitative differences were again found between growth phases with respect to individual fatty acids (Table 25). The major fatty acids in Isochrysis galbana were 14:0, 16:0, 18:1(n-9) and 22:6(n-3). The % UNFA increased from exponential to stationary/late exponential phase. The presence of 20:5(n-3) and 22:6(n-3) was again noted, and ranged from 0.43 - 0.65% and 6.06 - 20.90% respectively, dependent on growth phase.

4.3.2.5 Statistical Analysis of the Percentage Unsaturation Results

Statistical analysis of the total unsaturated fatty acid contents (% UNFA) gave significant results for the effect of temperature (0.1% or $p < 0.001$), algal species (0.1% or $p < 0.001$), and phase (1.0% or $p < 0.01$) and a significant first order interaction for temperature and algae (0.1% or $p < 0.001$). Temperature means (17°C - 65.25%, 30°C - 60.51%) showed a significant reduction at the higher temperature, however a significant interaction term between algae and temperature showed that this was only the case for Isochrysis sp, N.atomus actually increased the percentage unsaturation at the higher temperature. Species means (N. atomus - 67.69%, N. oculata - 63.83%, Isochrysis sp - 60.56%, Isochrysis galbana - 59.43%) divided into three groups, comprising N. atomus, N. oculata and the two Isochrysis species. Phase means (exponential - 60.95%, stationary/late exponential - 64.80%) showed a significant increase from exponential to stationary/late exponential phase.

Quantitative changes In fatty acid content rather than qualitative changes were the result of changes of culture temperature or growth phase. 20:5(n-3) and 22:6(n-3) fatty acids were found to be present in all species, with the highest

levels of 20:5(n-3) and 22:6 (n-3) found in N. oculata and Isochrysis galbana respectively.

4.3.2.6 Gross Photosynthetic and Dark Respiration Rates

The results are given in Table 26 for the marine and brackish species grown at 25mg NO_3-N l^{-1}. All cultures were harvested at the same times as for the nitrogen limitation experiments (see Tables 18, 19, 20 and 21).

Gross photosynthetic and dark respiration rates decreased from exponential to stationary/late exponential phase at all temperatures, and increased with culture temperature for N. atomus and Isochrysis sp..

Table 22: Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) for N. atomus 251/4B

	17°C						30°C					
Fatty Acid	5S	25E	25S	50E	50S/LE	500E	5S	25E	25S/LE	50E	50S/LE	500E
12:0	.39	1.27	.19	.23	.38	.35	.26	.32	.38	.38	.43	.39
14:0	1.07	1.62	1.02	1.29	1.25	1.17	1.05	1.23	1.33	1.18	1.42	1.23
14:1(n-5)	-	-	-	-	-	-	-	-	-	-	-	-
15:0	.31	2.46	0.44	1.77	.27	.34	.26	.24	.38	.71	1.50	.79
16:0	30.48	35.23	25.48	30.23	23.99	23.35	23.89	24.43	24.76	24.66	25.57	26.88
16:1(n-7)	3.04	4.36	0.63	3.95	.94	.99	.78	.84	.67	.97	.72	.56
16:2	.27	1.80	2.54	2.08	2.55	3.44	2.25	2.65	2.79	4.92	3.40	4.22
16:3(n-6)	1.01	5.38	6.91	7.99	4.87	6.18	2.65	2.78	3.32	4.22	4.39	5.11
16:4	-	-	-	-	TR	-	-	-	TR	-	-	-
17:0	.26	0.75	0.19	-	TR	.16	.24	.19	.23	.30	1.14	.22
18:0	2.01	1.77	1.44	2.13	1.55	1.41	2.19	2.26	2.58	1.79	3.24	2.53
18:1(n-9)	52.30	21.19	31.89	22.42	36.78	33.80	42.19	39.51	34.29	26.54	23.39	24.46
18:1(n-7)	.54	.66	0.58	-	.60	.76	.78	.63	.59	.99	.93	.73
18:2(n-6)	2.64	6.02	11.15	7.74	13.71	12.96	10.33	12.26	15.32	15.78	13.96	16.01
18:3(n-3)	2.41	6.78	10.80	8.84	8.74	9.50	5.74	5.88	6.88	8.46	8.63	9.49
18:3(n-6)	-	-	0.10	.73	TR	-	.16	.24	.19	.35	1.06	-
18:4(n-3)	.14	.75	0.82	.86	.26	.47	.27	.33	.22	.58	-	.51
19:0	-	-	-	-	-	-	-	-	-	-	-	-
20:0	.67	2.63	.14	.41	-	-	.49	.49	TR	.81	1.31	.11
20:1(n-9)	.27	.24	.33	.64	.32	.31	.42	.33	.35	.50	.38	.35
20:2(n-6)	TR	-	.25	.69	.39	.33	.22	.22	.33	.35	.72	.49
20:3(n-6)	.14	.78	.28	.86	.15	.23	.76	.71	.56	.95	1.70	.77
20:4(n-6)	-	-	.12	-	TR	.13	.34	.29	.33	.42	-	.25
20:4(n-3)	.14	.42	.58	.71	.20	.26	.48	.46	.28	.48	-	.23
20:5(n-3)	.48	1.02	2.52	2.75	2.14	2.41	2.68	2.39	2.44	2.74	2.31	2.51
21:0	TR	.73	-	.46	TR	-	TR	-	-	TR	-	-
22:0(IS)	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS
22:1(n-9)	.24	-	.36	-	.14	-	.26	.24	.27	-	-	.26
22:5(n-3)	.22	2.08	.29	1.44	.16	.26	.18	.15	.22	.62	1.44	.26
22:6(n-3)	.79	2.03	.70	1.73	.40	.33	1.05	0.94	1.11	1.33	2.34	1.71
24:0	-	-	-	-	-	-	-	-	-	-	-	
% Lipid	14.88	1.19	3.99	1.51	14.53	5.03	4.86	7.38	4.45	1.81	0.49	0.93
% SAFA	35.19	46.46	28.90	36.52	27.44	26.78	28.38	29.16	29.66	29.83	34.61	32.15
%UNFA	64.63	53.51	70.85	63.43	72.35	72.36	71.54	70.85	70.16	70.20	65.37	67.92
UNFA/SAFA	1.84	1.15	2.45	1.74	2.64	2.70	2.52	2.43	2.36	2.35	1.89	2.11
% Protein	3.10	7.80	5.80	4.70	5.80	6.50	0.76	1.50	2.69	1.23	0.61	2.02
%Carbohydrate	37.70	27.10	9.80	15.90	11.10	9.80	22.21	14.94	16.88	11.24	11.32	14.25

Note: (i) N. atomus 251/4B did not grow at 40°C

(ii) IS = Internal Standard; S = Stationary Phase; E = Exponential Phase; SAFA = Saturated Fatty Acids; UNFA = Unsaturated Fatty Acids; TR = trace <0.1%; S/LE = Stationary/Late Exponential Phase

(iii) For systematic names of fatty acids see Appendix 1

FIG86 %Carbohydrate, Protein and Lipid
N. atomus 251/4B Exponential Phase

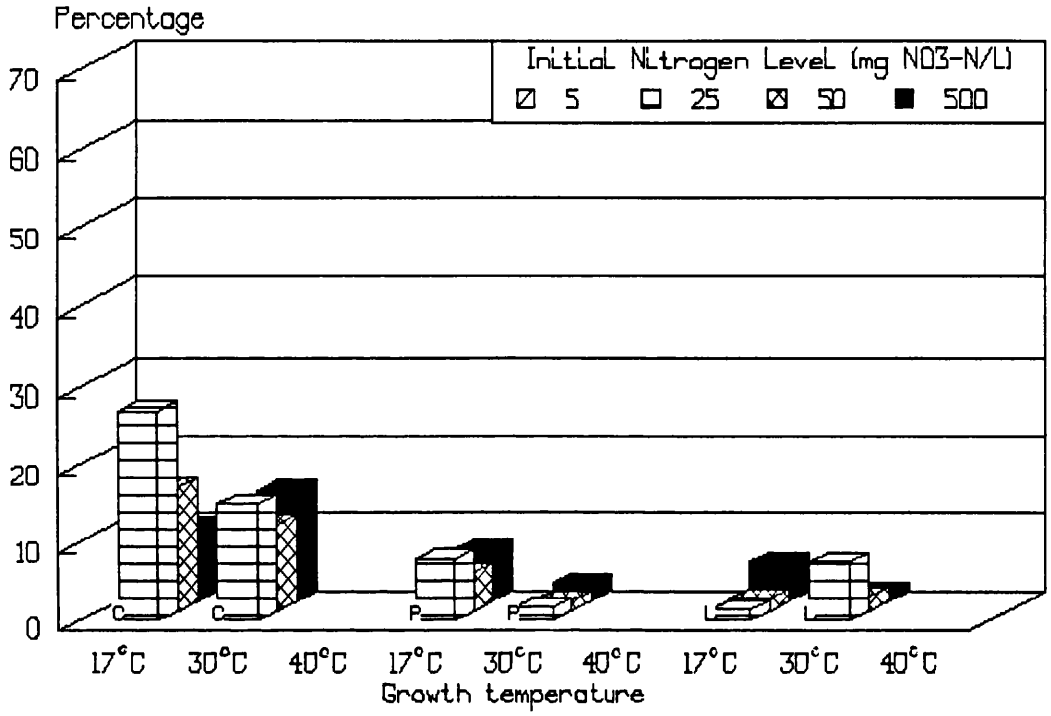


FIG87 %Carbohydrate, Protein and Lipid
N. atomus 251/4B Stationary Phase

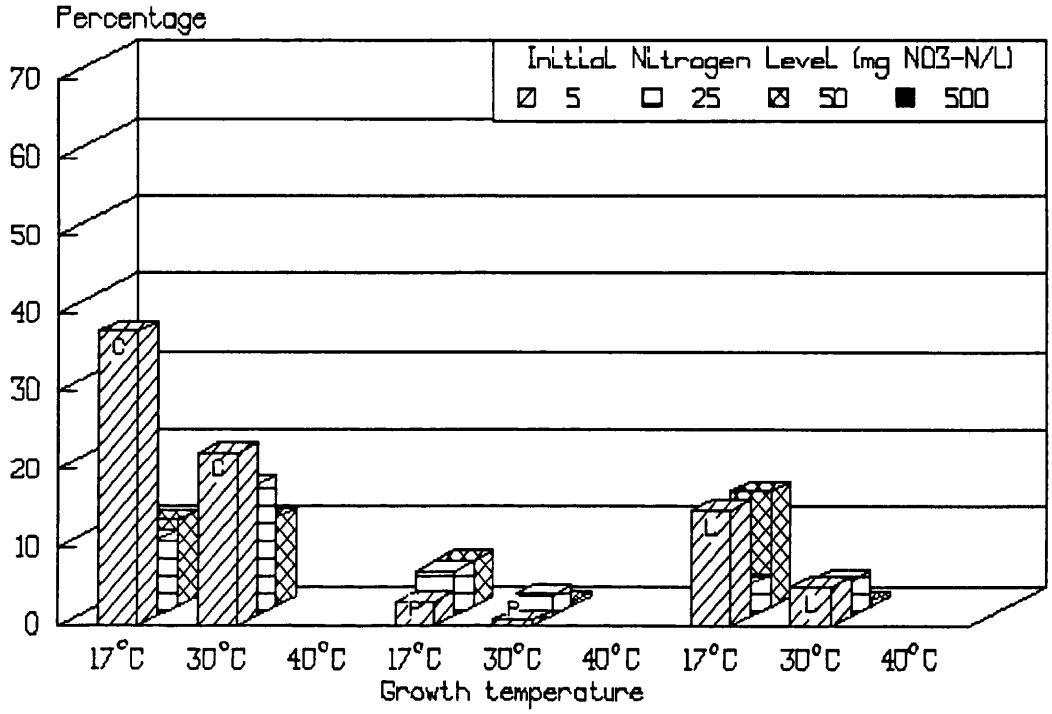


Table 23: Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) for N. oculata 849/1

	17°C					
Fatty Acid	5S	25E	25S/LE	50E	50S/LE	500E
12:0	.25	.20	.20	.22	.16	.19
14:0	4.89	4.39	4.09	4.45	3.37	3.13
14:1(n-5)	-	-	-	-	-	-
15:0	.49	.55	.49	.44	.48	.46
16:0	31.69	28.96	25.72	27.28	25.15	22.81
16:1(n-7)	33.37	30.98	33.73	32.52	32.83	34.17
16:2	TR	TR	.10	TR	TR	TR
16:3(n-6)	.18	.14	.24	.15	.20	.26
16:4	TR	.18	.13	.14	.16	.30
17:0	.26	.45	.24	.36	.30	.29
18:0	.99	2.39	1.42	2.18	1.61	1.55
18:1(n-9)	13.78	12.99	18.54	14.02	18.67	17.43
18:1(n-7)	.47	.34	.48	.36	.57	.55
18:2(n-6)	1.37	4.81	4.94	4.95	5.02	5.44
18:3(n-3)	TR	.29	.44	.37	.44	.48
18:3(n-6)	.23	.32	.33	.32	.34	.46
18:4(n-3)	TR	TR	-	TR	TR	TR
19:0	-	-	-	-	-	-
20:0	.28	.10	-	TR	.17	.12
20:1(n-9)	TR	TR	-	TR	TR	TR
20:2(n-6)	.38	.11	.15	.33	.35	.75
20:3(n-6)	1.05	.51	.42	.48	.66	.48
20:4(n-6)	1.81	1.59	2.24	1.67	2.42	2.85
20:4(n-3)	.26	.24	0.14	.19	.15	.31
20:5(n-3)	7.87	9.96	5.97	9.28	6.63	7.70
21:0	-	-	-	-	-	-
22:0(IS)	IS	IS	IS	IS	IS	IS
22:1(n-9)	-	-	-	-	-	TR
22:5(n-3)	.11	.19	-	TR	-	.13
22:6(n-3)	-	TR	-	TR	-	.25
24:0	-	-	-	-	-	-
% Lipid	31.98	14.51	7.80	12.48	10.76	9.48
% SAFA	38.85	37.04	32.16	34.93	31.24	28.55
%UNFA	60.88	62.65	67.88	64.78	68.44	71.56
UNFA/SAFA	1.57	1.69	2.11	1.85	2.19	2.51
% Protein	0.30	1.60	0.80	2.10	1.00	2.00
%Carbohydrate	15.40	9.80	1.90	9.90	1.70	7.90

Note: (i) N oculata 849/1 did not grow at 30°c or 40°C

(ii) IS = Internal Standard; S = Stationary Phase; E = Exponential Phase; SAFA = Saturated Fatty Acids; UNFA = Unsaturated Fatty Acids; S/LE = Stationary/Late Exponential Phase; TR = Trace = <0.1%

(iii) For systematic names of fatty acids see Appendix 1

FIG88 %Carbohydrate, Protein and Lipid
N. oculata 849/1 Exponential Phase

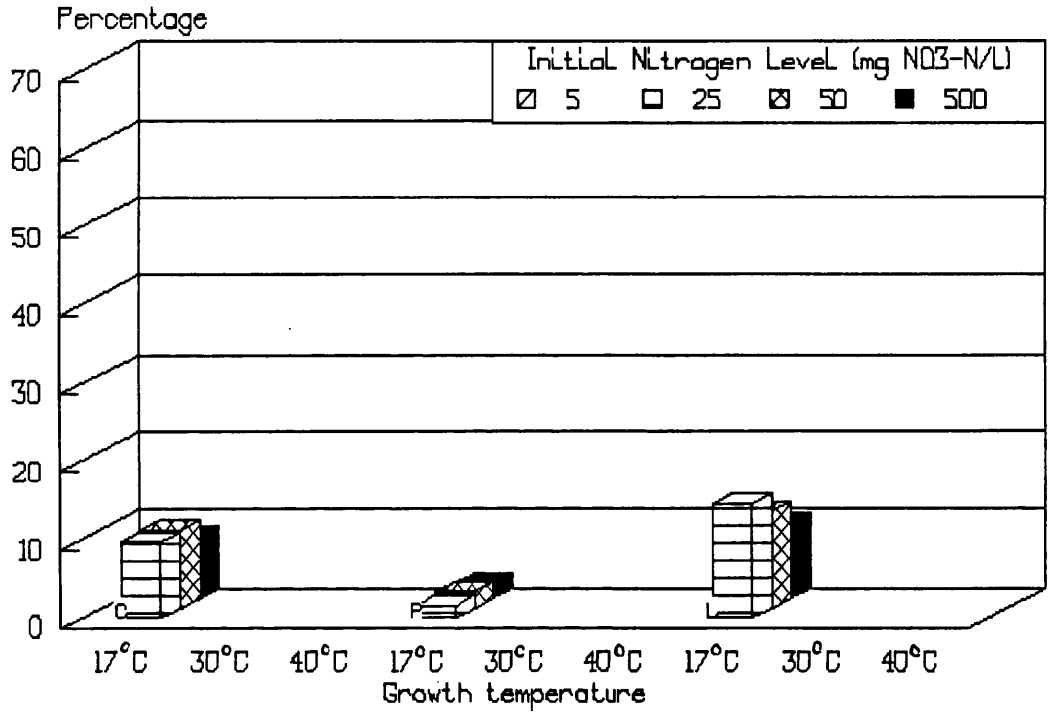


FIG89 %Carbohydrate, Protein and Lipid
N. oculata 849/1 Stationary Phase

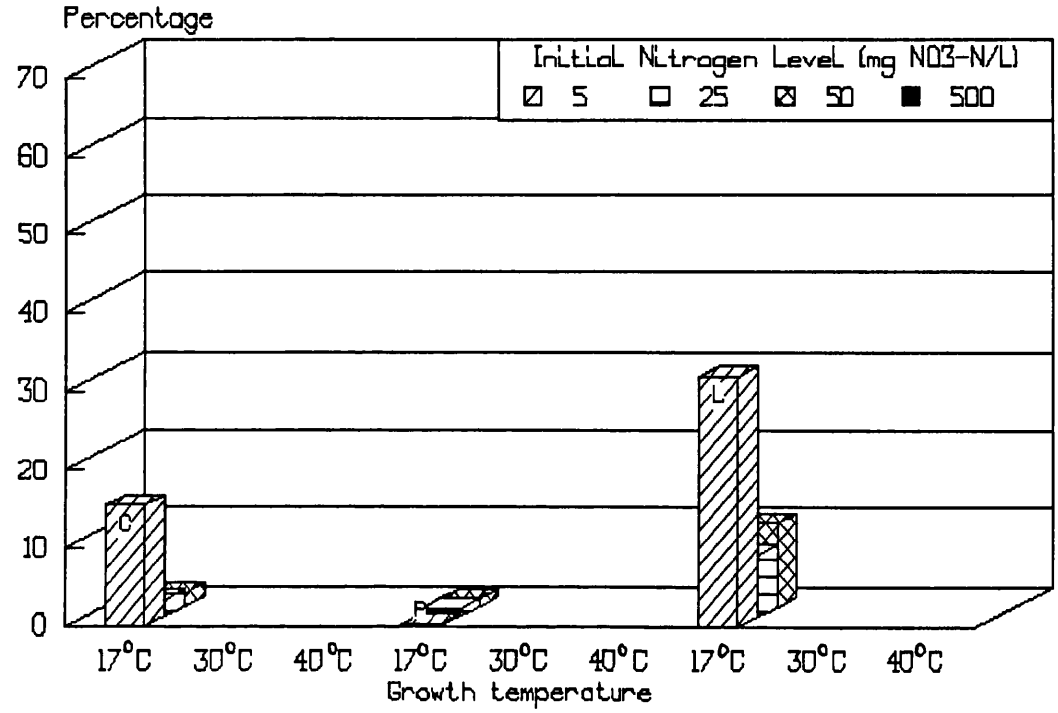


Table 24: Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) of Isochrysis sp. 927/14

	17°C						30°C					
Fatty Acid	5S	25E	25S	50E	50S/LE	500E	5S	25E	25S	50E	50S/LE	500E
12:0	.18	TR	-	-	TR	-	-	-	-	-	-	-
14:0	24.42	18.93	16.32	20.85	15.65	16.02	20.69	23.00	24.61	25.51	23.38	20.17
14:1(n-5)	-	TR	-	.12	-	-	-	-	-	-	-	-
15:0	.62	.54	.58	.68	.40	.47	.88	1.25	1.28	1.62	1.36	3.77
16:0	19.73	11.96	12.43	11.76	11.11	10.48	18.40	18.11	21.34	19.40	18.97	19.74
16:1(n-7)	3.03	3.25	3.97	3.67	4.23	4.78	3.52	4.60	5.10	5.06	5.22	4.18
16:2	-	.23	.28	.28	.30	.42	.20	.33	-	.26	.28	.34
16:3(n-6)	-	TR	.30	.20	.30	.26	.34	.25	-	.29	.19	-
16:4	-	.87	.12	-	1.11	1.77	.87	-	-	-	-	-
17:0	-	.28	-	-	.11	.13	.26	.42	.52	.59	.77	-
18:0	.49	.41	.49	.31	.31	.27	.76	.50	.95	.86	.51	2.96
18:1(n-9)	31.37	20.92	27.37	21.37	28.99	24.92	21.26	16.51	17.49	16.57	15.78	14.00
18:1(n-7)	-	-	.19	-	.10	.22	1.01	1.35	2.60	1.19	2.81	2.33
18:2(n-6)	4.43	3.10	3.40	2.56	3.10	2.56	2.71	2.34	3.31	2.50	2.30	3.23
18:3(n-3)	3.38	4.96	4.41	4.97	4.30	5.21	2.91	3.21	2.96	4.01	3.64	3.92
18:3(n-6)	-	.40	TR	.10	TR	TR	.32	-	-	-	.77	-
18:4(n-3)	5.49	14.56	14.75	15.79	15.22	17.65	9.81	12.15	8.93	11.05	10.59	11.15
19:0	-	-	-	-	-	-	-	-	-	-	-	-
20:0	1.60	.20	-	-	-	TR	-	.24	-	-	.38	1.84
20:1(n-9)	-	-	TR	-	.18	TR	.28	-	-	-	-	-
20:2(n-6)	-	TR	.11	-	.38	.23	-	-	-	-	-	-
20:3(n-6)	-	.21	.13	-	.19	.16	.14	.37	-	-	.52	-
20:4(n-6)	-	TR	.21	-	.14	.12	-	-	-	-	-	-
20:4(n-3)	-	-	TR	-	TR	-	-	-	-	-	-	-
20:5(n-3)	.55	.90	.91	.73	.83	.63	.78	.76	.91	.65	.67	-
21:0	-	-	-	-	-	-	-	-	-	-	-	-
22:0(IS)	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS
22:1(n-9)	-	.40	.21	.20	.46	.37	.47	.41	-	-	-	-
22:5(n-3)	-	.27	.20	.17	.12	.14	.46	.76	-	.63	.71	1.97
22:6(n-3)	4.70	17.45	13.55	16.28	12.33	12.97	13.95	13.43	10.00	9.79	11.17	10.41
24:0	-	-	-	-	-	-	-	-	-	-	-	-
% Lipid	12.35	5.23	6.91	5.85	7.49	5.55	3.42	3.12	1.56	2.03	1.33	0.27
% SAFA	47.04	32.32	29.82	33.60	27.58	27.37	40.99	43.52	48.70	47.98	45.37	48.48
%UNFA	52.95	67.52	70.11	66.44	72.28	72.41	59.03	56.47	51.30	52.00	54.65	51.53
UNFA/SAFA	1.13	2.09	2.35	1.98	2.62	2.65	1.44	1.30	1.05	1.08	1.20	1.07
% Protein	1.40	1.50	0.40	1.30	1.30	2.30	1.80	1.58	1.72	1.16	1.61	1.58
%Carbohydrate	7.30	5.10	4.10	4.70	4.70	9.20	7.47	4.58	4.80	5.08	4.59	3.89

Note: (i) Isochrysis sp. 972/14 did not grow at 40°C

(ii) IS = Internal standard; S = Stationary Phase; E = Exponential Phase; SAFA = Saturated Fatty Acids; UNFA = Unsaturated Fatty Acids; TR = Trace <0.1%; S/LE = Stationary/Late Exponential Phase

(iii) For systematic names of fatty acids see Appendix 1

FIG**90** %Carbohydrate, Protein and Lipid
Isochrysis sp. 927/14 Exponential Phase

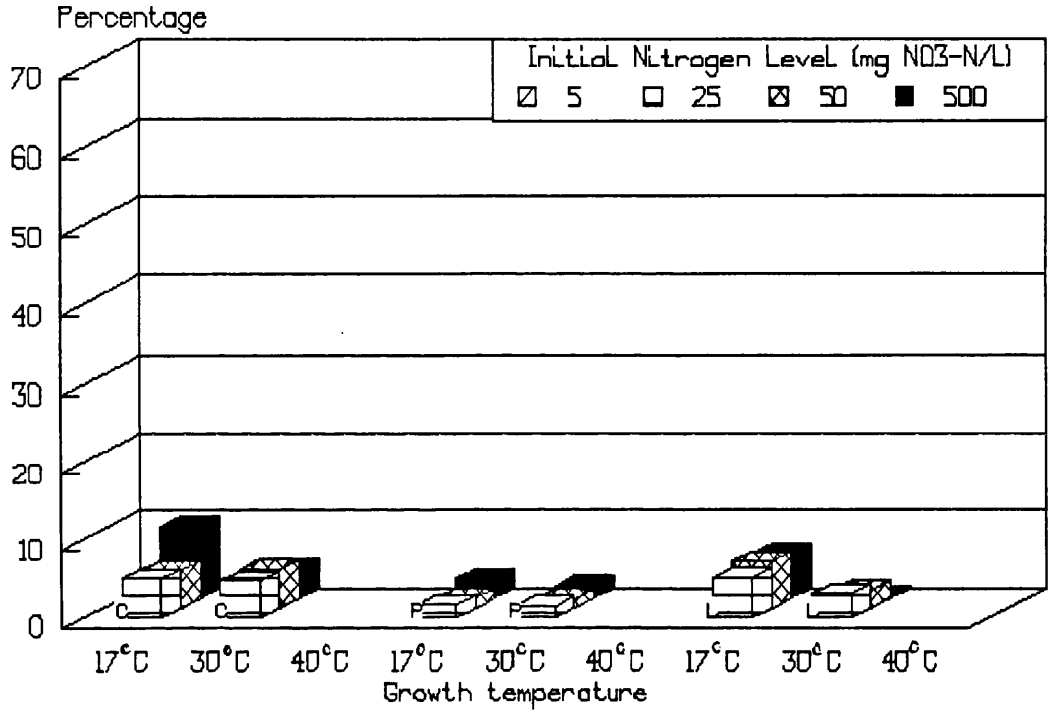


FIG**91** %Carbohydrate, Protein and Lipid
Isochrysis sp. 927/14 Stationary Phase

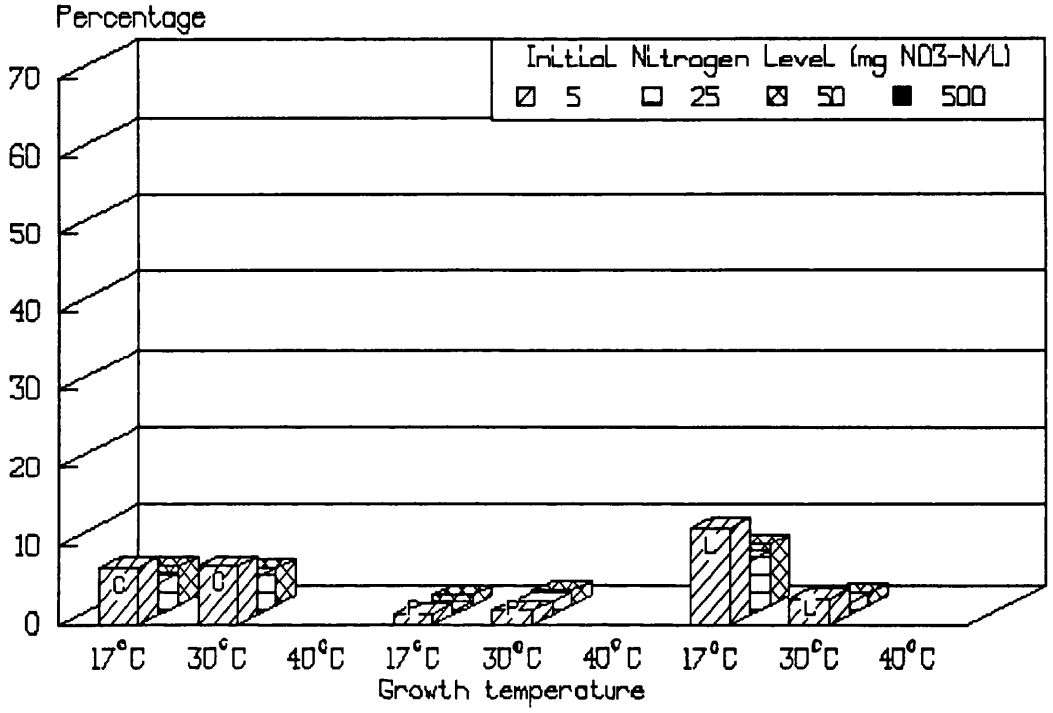


Table 25: Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) for I galbana 927/1

	17°C					
Fatty Acid	5S	25E	25S	50E	50S/LE	500E
12:0	-	-	-	-	-	-
14:0	20.32	29.61	23.23	23.44	21.98	23.45
14:1(n-5)	-	-	-	-	-	-
15:0	.69	.81	.41	.83	.41	1.32
16:0	16.18	15.16	12.80	10.91	11.71	11.97
16:1(n-7)	1.41	2.32	2.14	2.54	2.49	2.34
16:2	-	-	TR	-	.15	-
16:3(n-6)	-	-	.32	.24	.17	4.51
16:4	-	-	-	-	.27	-
17:0	.40	-	.13	-	TR	-
18:0	1.82	.66	.57	.53	.48	.71
18:1(n-9)	31.20	23.13	22.09	21.83	25.31	16.45
18:1(n-7)	1.72	1.78	2.31	1.83	2.35	3.79
18:2(n-6)	6.59	4.14	6.58	4.71	4.50	3.13
18:3(n-3)	3.14	4.77	4.63	6.79	4.37	4.90
18:3(n-6)	.44	.22	-	-	-	-
18:4(n-3)	3.77	6.18	7.58	9.56	8.17	5.32
19:0	-	-	-	-	-	-
20:0	.42	-	TR	.31	-	-
20:1(n-9)	2.81	-	-	-	TR	-
20:2(n-6)	.74	-	.54	.39	.38	-
20:3(n-6)	.40	-	.13	.23	.51	-
20:4(n-6)	-	-	TR	-	-	-
20:4(n-3)	-	-	-	-	-	-
20:5(n-3)	.43	.46	.51	.65	.52	.27
21:0	-	-	-	-	-	-
22:0(IS)	IS	IS	IS	IS	IS	IS
22:1(n-9)	.48	-	.18	.31	.51	-
22:5(n-3)	1.00	-	.34	.69	.35	.97
22:6(n-3)	6.06	10.76	15.52	14.07	15.65	20.90
24:0	-	-	-	-	-	-
% Lipid	4.63	13.24	6.68	3.69	5.34	1.16
% SAFA	39.83	46.24	37.14	36.02	34.58	37.45
%UNFA	60.19	53.76	62.87	63.84	65.70	62.58
UNFA/SAFA	1.51	1.16	1.69	1.77	1.90	1.67
% Protein	2.20	6.80	0.50	2.60	0.50	5.10
%Carbohdyrate	5.90	8.10	4.10	2.10	3.70	4.70

Note: (i) I. galbana 927/1 did not grow at 30°C and 40°C

(ii) IS = Internal Standard; S = Stationary Phase; E = Exponential Phase; SAFA = Saturated Fatty Acids; UNFA = Unsaturated Fatty Acids; TR = Trace <0.1%; S/LE = Stationary/Late Exponential Phase

(iii) For systematic names of fatty acids see Appendix 1

FIG 92 %Carbohydrate, Protein and Lipid
Isochrysis galbana 927/1 Exponential Phase

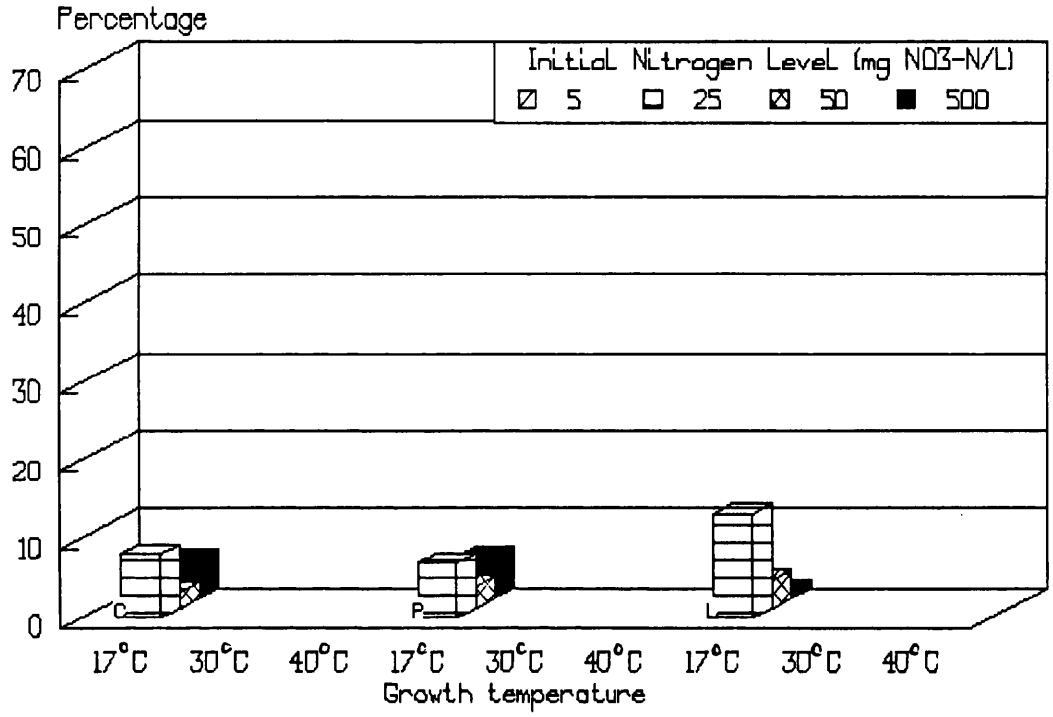


FIG 93 %Carbohydrate, Protein and Lipid
Isochrysis galbana 927/1 Stationary Phase

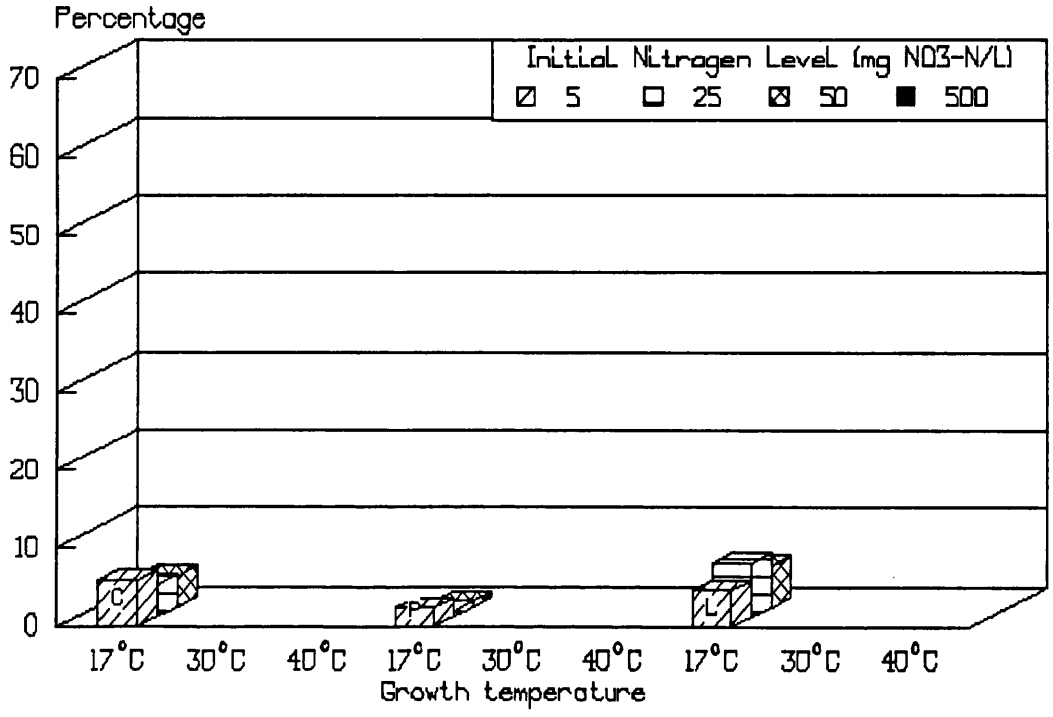


Table 26: Photosynthetic and Dark Respiration Rates for the Marine and Brackish Species

Organism	Time (Days)	OD_{560}	Dry Wt (gl^{-1})	Nitrogen Present (+ or -)	Dark Respiration Rate $mgO_2gDM^{-1}h^{-1}$	Gross Photosynthesis Rate $mgO_2g\ DM^{-1}h^{-1}$
17°C						
N. atomus 251/4B	15	0.62	0.49	+	2.88	12.78
	26	0.86	0.71	-	0.94	6.42
N. oculata 849/1	20	1.12	0.96	+	1.88	6.56
	45	2.12	1.73	TR	0.76	2.27
Isochrysis sp. 927/14	24	0.40	0.56	+	2.71	3.86
	45	1.53	1.61	TR	0.82	1.04
Isochrysis galbara 927/1	20	0.58	0.36	+	11.25	12.50
	34	1.29	1.00	TR	1.13	2.10
30°C						
N. atomus 251/4B	15	0.43	0.30	+	3.87	16.03
	26	0.89	0.75	+	4.53	13.60
Isochrysis sp. 927/14	15	0.27	0.20	+	8.45	12.90
	26	0.52	0.58	+	2.81	3.74

TR = Trace

4.3.3 Cyanobacteria

4.3.3.1 Growth and Nitrate Results

The two Synechococcus species required lower irradiances for growth (43 -55 μmol m^{-2} s^{-1}). Synechococcus sp PCC 7943 grew at the three experimental temperatures, however problems were encountered at 17°C with Synechococcus sp 1479/5. The cultures at the lower nitrate levels were turning white overnight after a few days growth, a problem not observed at 30°C. It was concluded that a combination of lower nitrate concentration and lower temperature was the cause of the problem. Synechococcus sp 1479/5 did not grow at 40°C.

Results for OD_{560} against time and dry weight against time for the two Synechococcus species are given in Figs 94 - 103. Growth was significantly slower at 17°C than at 30°C and 40°C for Synechococcus sp PCC 7943. All cultures of Synechococcus sp PCC 7943, with the exception of those at 500 mg NO_3-N l^{-1}, went into stationary phase (Figs 96-98). At 17°C, stationary phase was not preceded by nitrogen depletion (Fig 106). Synechococcus sp 1479/5 did not grow well at 17°C and at the three lower nitrogen levels cultures were harvested at 6 days. From OD_{560} (Fig 94) and dry weight results (Fig 99), it was concluded that these cultures were dying. Nitrate was not depleted with the exception of the lowest nitrogen level (Fig 104). Cultures at 30°C went into stationary phase (Fig 95) accompanied by nitrate depletion (Fig 105), with the exception of the 500mg NO_3-N l^{-1} culture. Results for nitrate depletion with time are given in Figs 104 - 108, and show depletion in the order 5 -> 25 -> 50 mg NO_3-N l^{-1} for both Synechococcus species. Harvest parameters are given in Tables 27 and 28.

pH results for the two Synechococcus species showed a rise and fall in pH with growth (Figs 109-113). The pH results for Synechococcus sp 1479/5 at 17°C (Fig 109) showed a lack of change in pH at the lower nitrate levels compared to the culture at 500mg NO_2-N l^{-1}. This confirmed that growth never really commenced, and cultures were dying.

A. flos-aquae 1403/13A grew at 17°C, 30°C and 40°C. A. varabilis 1403/12 only grew at 17°C and 30°C. As these two cyanobacteria were nitrogen fixing cyanobacteria, some of the cultures were allowed to continue growing past nitrate depletion (designated A in Tables 29 and 30) and others were harvested (designated S/LE in Tables 29 and 30). Results for OD_{560} are given in Figs 114-116 for A. flos-aquae and Figs 117 and 118 for A. variabilis. Dry weight results are shown in Figs 119-121 for A. flos-aquae and Figs 122 and 123 for A. variabilis. Growth at 17°C was slower than at 30°C and 40°C for both species. Cultures did not enter stationary phase after nitrate depletion. Results for nitrate depletion are given in Figs 124-126 for A. flos-aquae and Figs 127 and 128 for A. variabilis. All cultures, with the exception of 500mg NO_3-N l^{-1}, initial nitrogen level achieved nitrate depletion in the order 5 -> 25 -> 50 mg NO_3-N l^{-1}. In some cases nitrate was found to reappear at very low levels which may have been due to cell loss by death. Harvest parameters are given in Tables 29 and 30 for A. flos-aquae and A. variabilis respectively.

pH increased and then decreased with growth, with some fluctuations after nitrate depletion in cultures allowed to continue growing with nitrogen fixation (Figs 129-133). The pH changes observed with growth were found to be similar to that of the green algae.

Heterocysts counts (percentage composition of cells) at 17°C and 30°C for both nitrogen fixers are given in Tables 31 and 32. Heterocysts were found to increase with nitrate depletion at 5, 25 and 50mg NO_3-N l^{-1} but not at 500mg NO_3 l^{-1}. Low levels of nitrate and nitrate depletion induced heterocyst formation in these two strains as many other workers have found.

FIG94 Synechococcus sp.1479/5 17°C
OD 560 vs Time

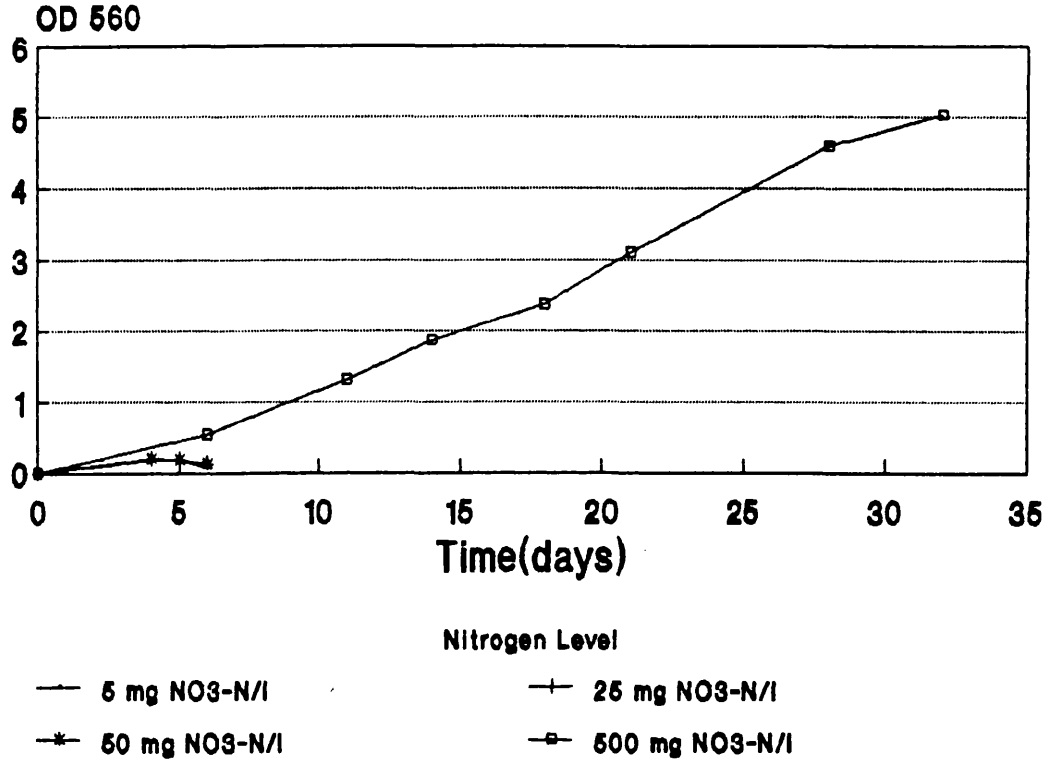


FIG95 Synechococcus sp.1479/5 30°C
OD 560 vs Time

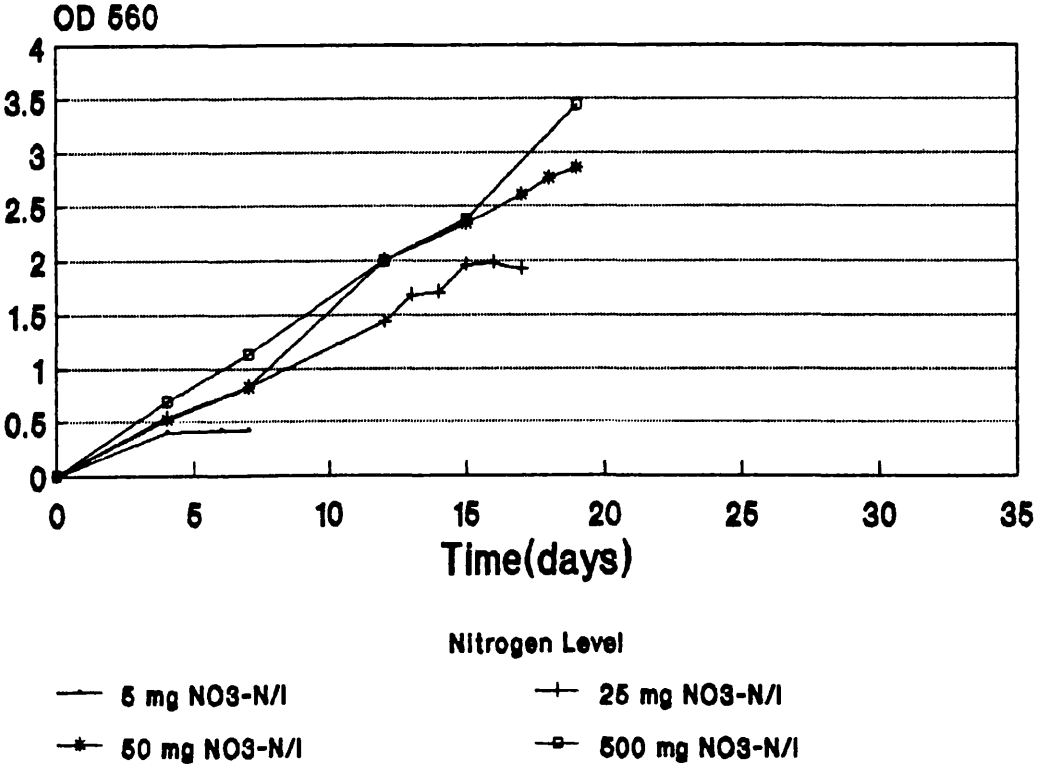


FIG 96 Synechococcus sp.PCC 7943 17°C
OD 560 vs Time

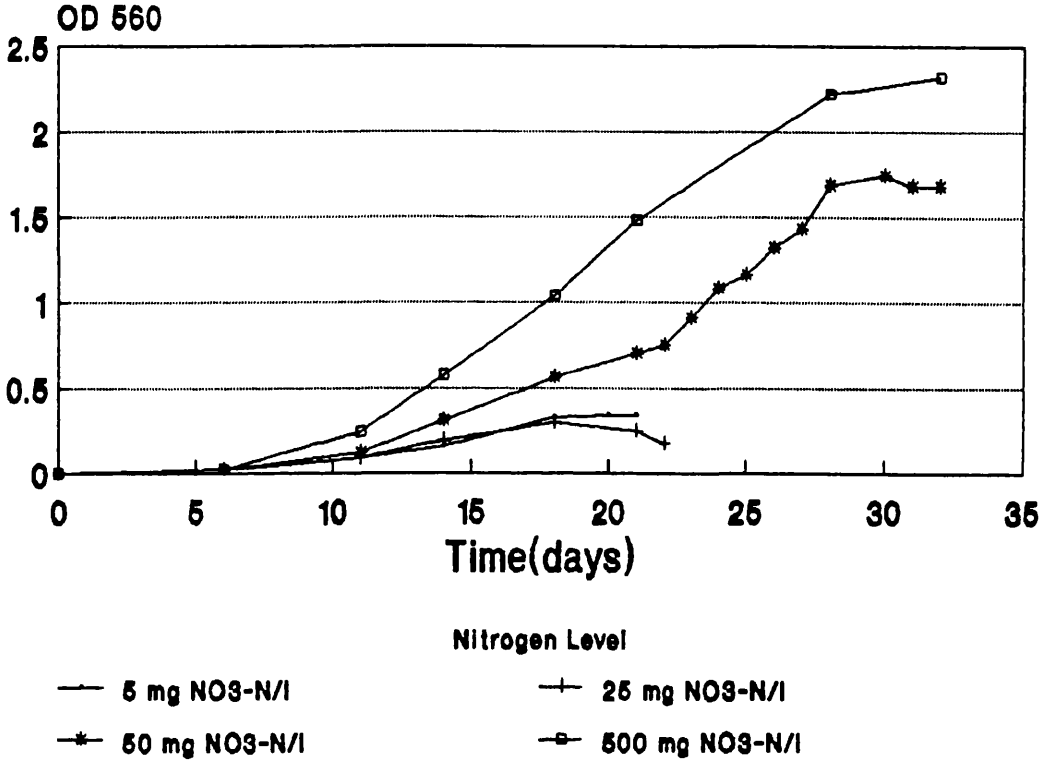


FIG 97 Synechococcus sp.PCC 7943 30°C
OD 560 vs Time

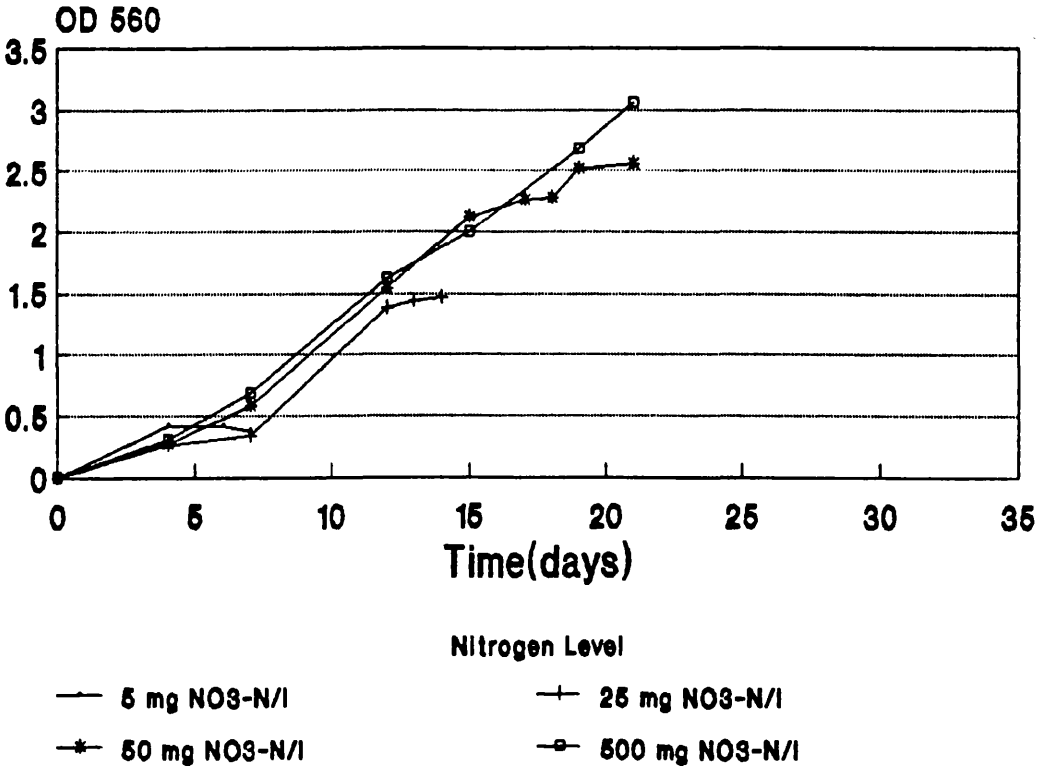


FIG 98 Synechococcus sp. PCC 7943 40°C
OD 560 vs Time

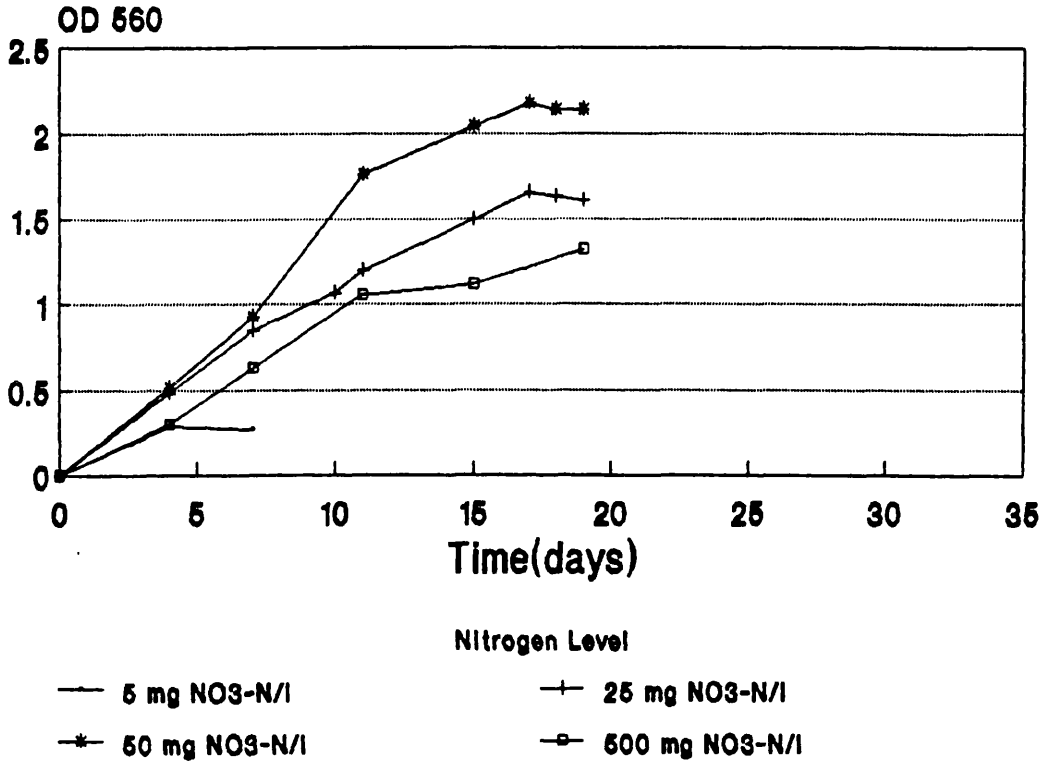


FIG 99 Synechococcus sp.1479/5 17°C
DRY WEIGHT vs Time

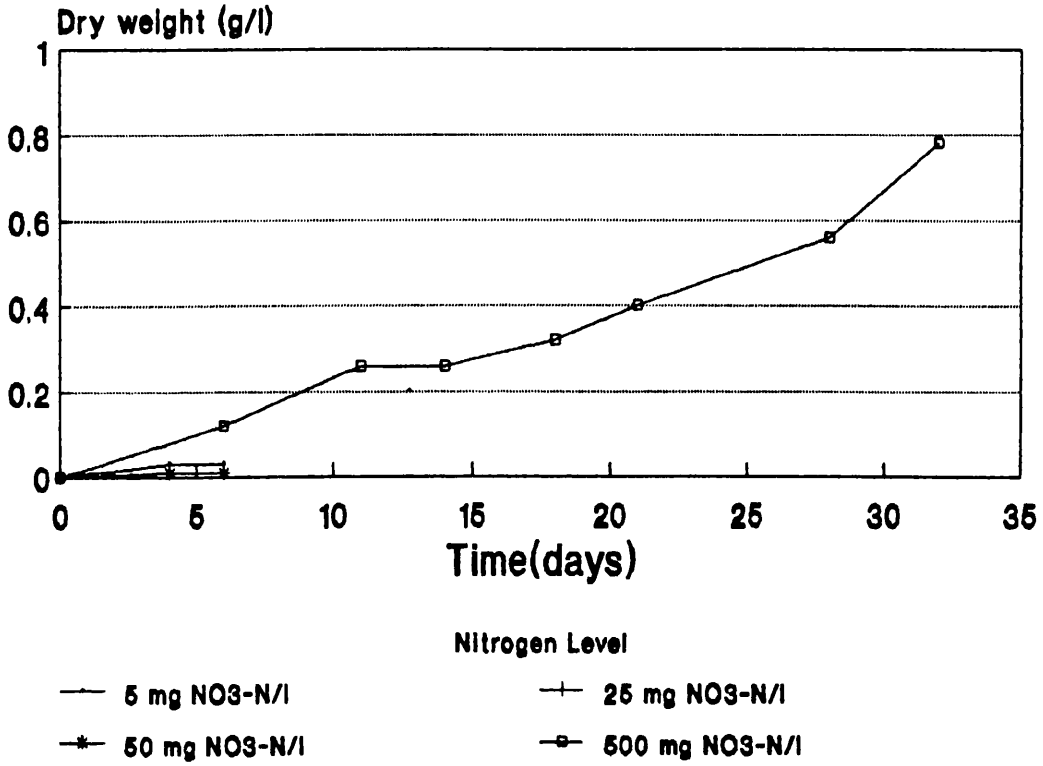


FIG 100 Synechococcus sp.1479/5 30°C
DRY WEIGHT vs Time

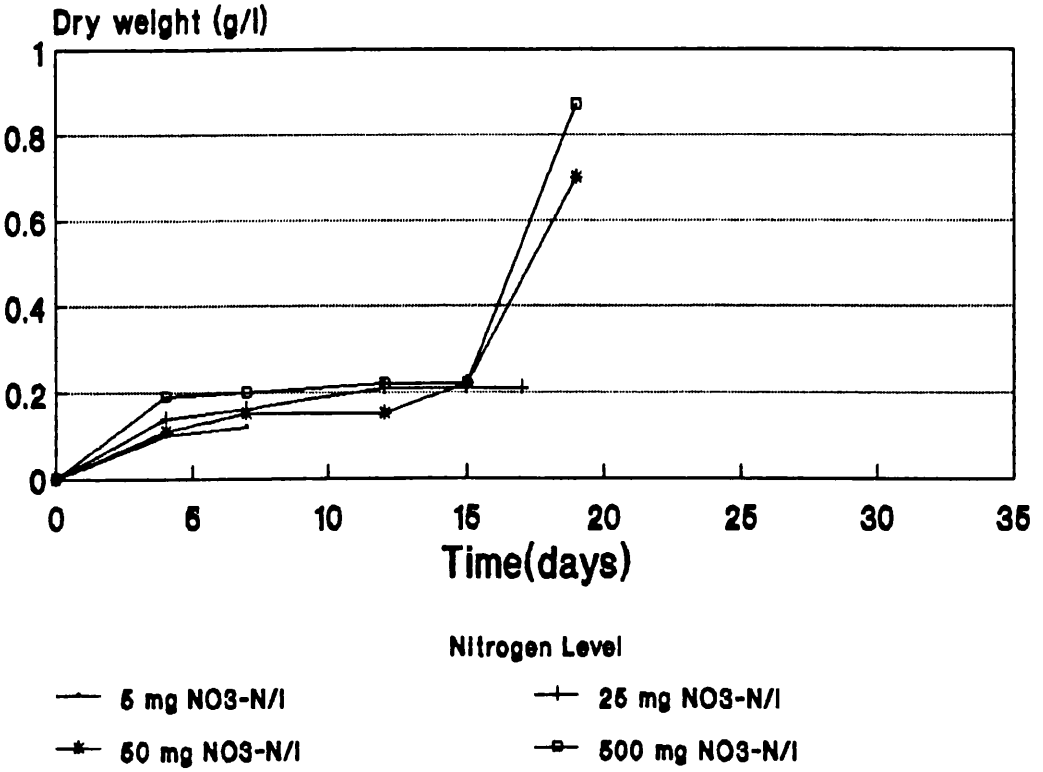


FIG 101 Synechococcus sp.PCC 7943 17°C
DRY WEIGHT vs Time

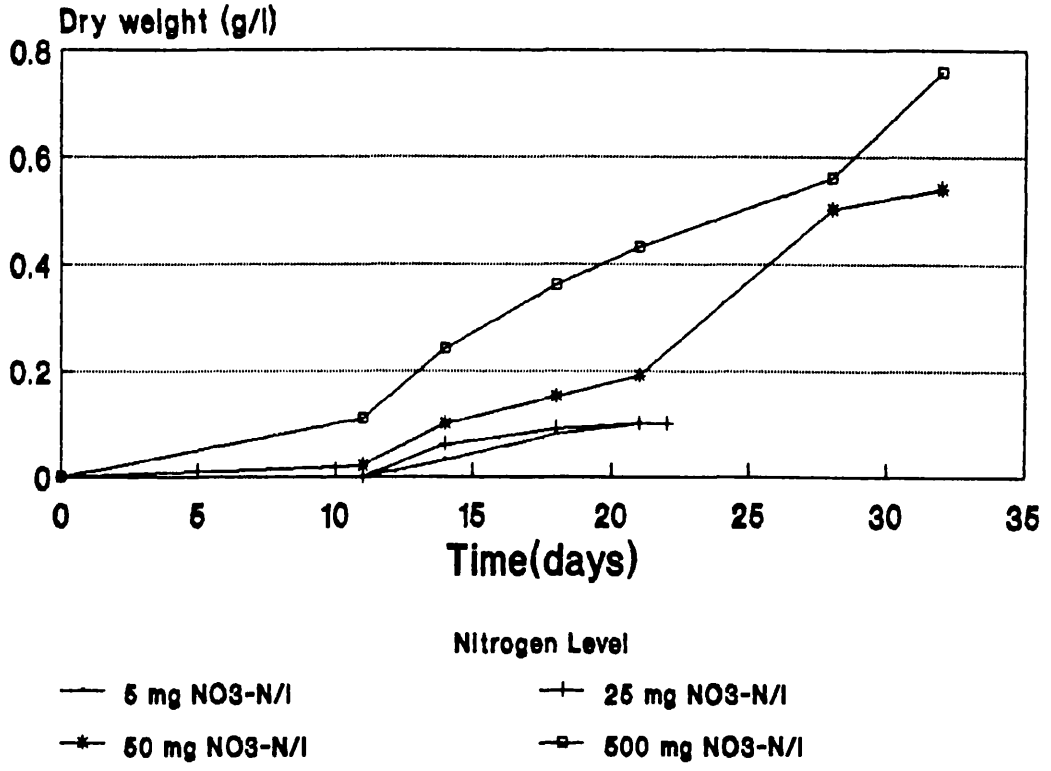


FIG 102 Synechococcus sp.PCC 7943 30°C
DRY WEIGHT vs Time

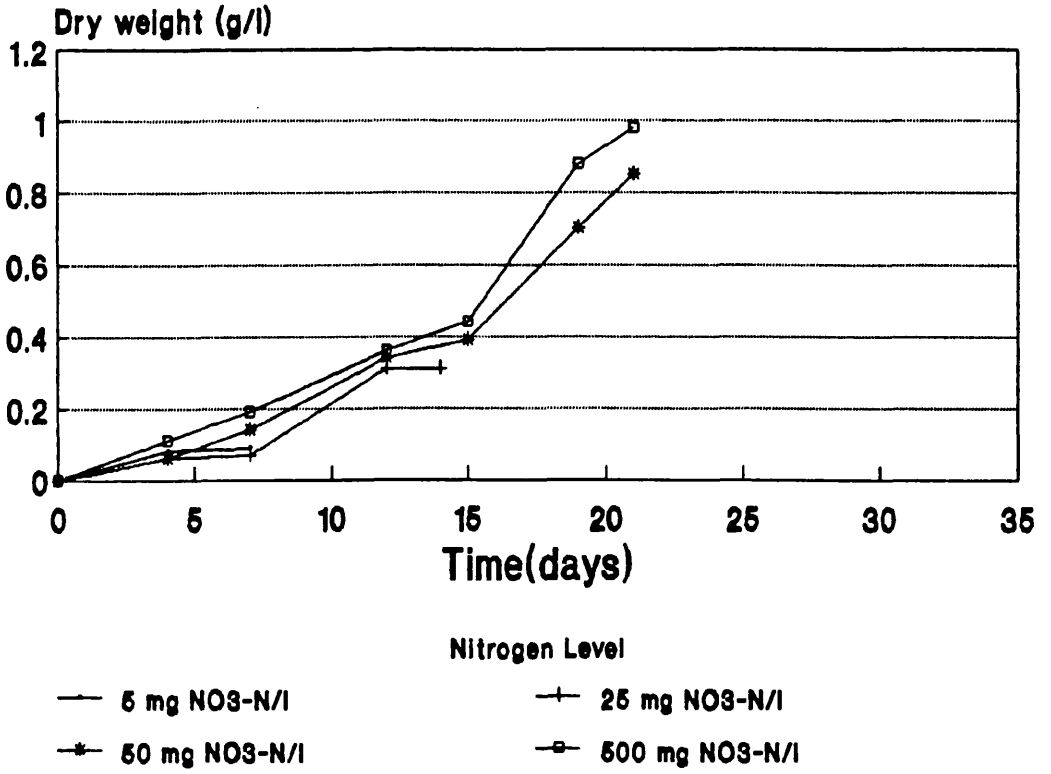


FIG 103 Synechococcus sp.PCC 7943 40°C
DRY WEIGHT vs Time

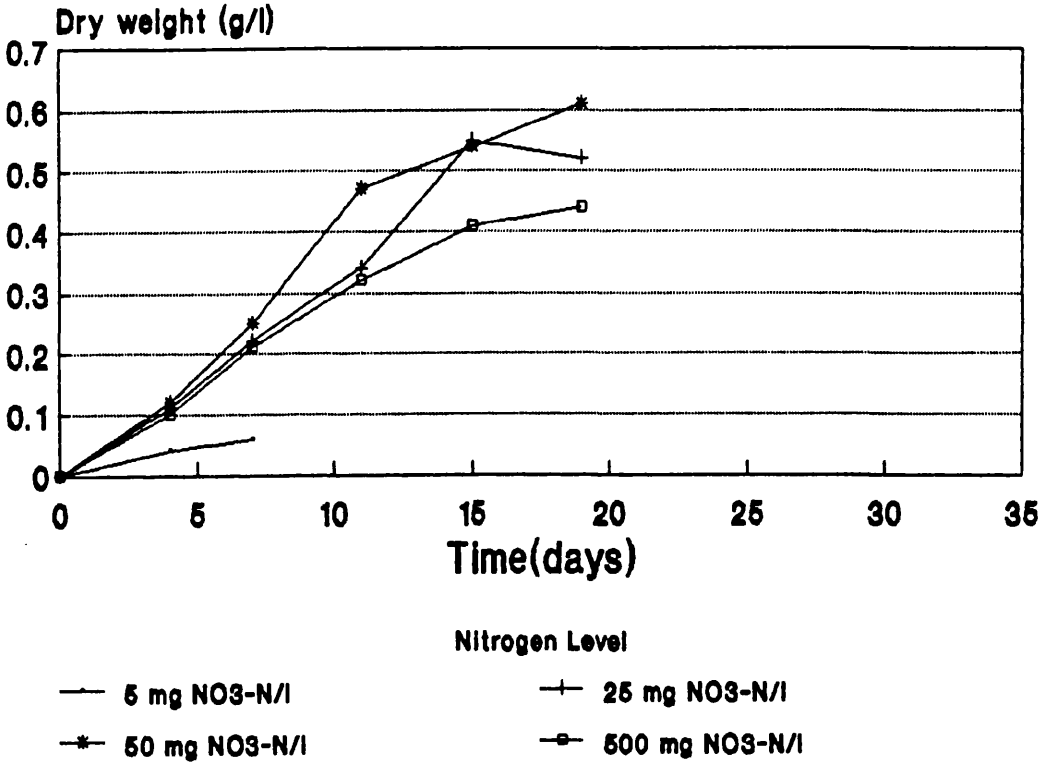


FIG 104 Synechococcus sp.1479/5 17°C
NITRATE vs Time

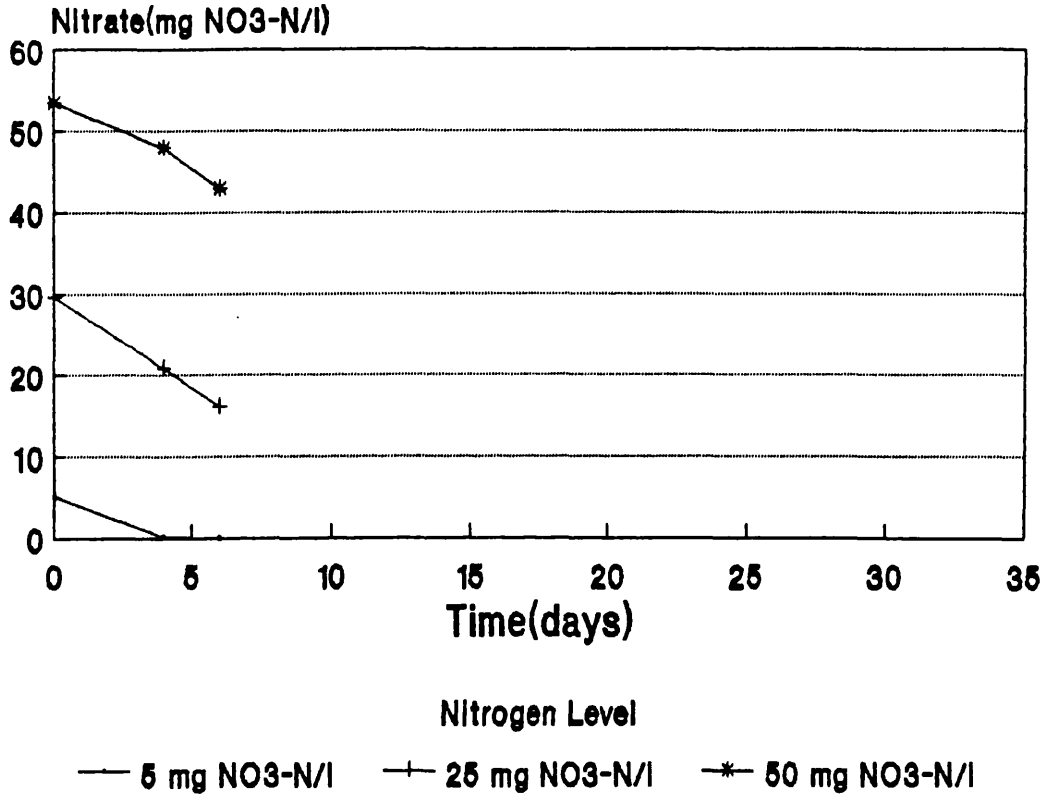


FIG 105 Synechococcus sp.1479/5 30°C
NITRATE vs Time

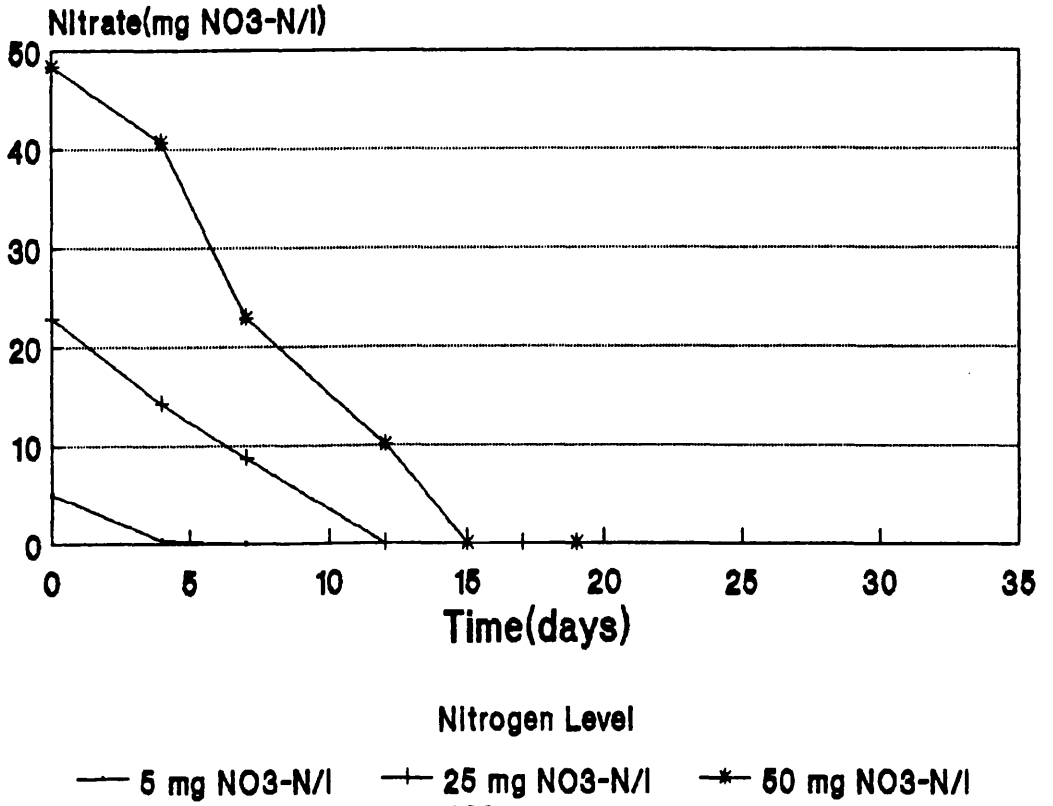


FIG 106 Synechococcus sp.PCC 7943 17°C
NITRATE vs Time

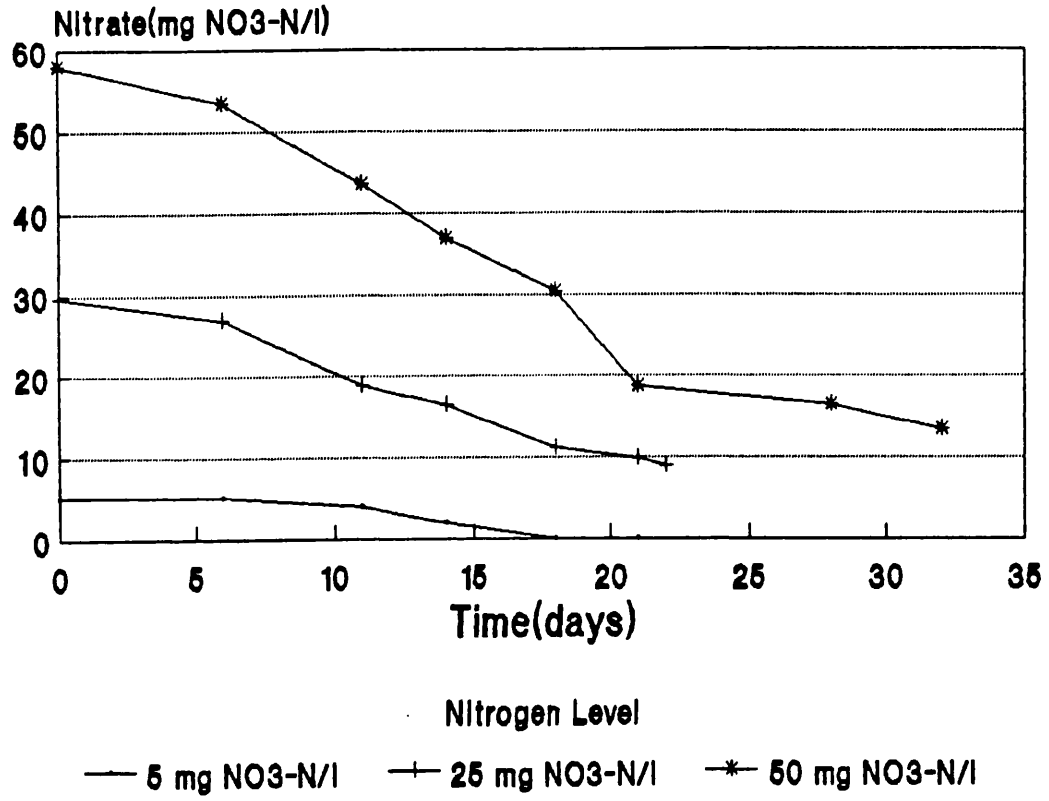


FIG 107 Synechococcus sp.PCC 7943 30°C
NITRATE vs Time

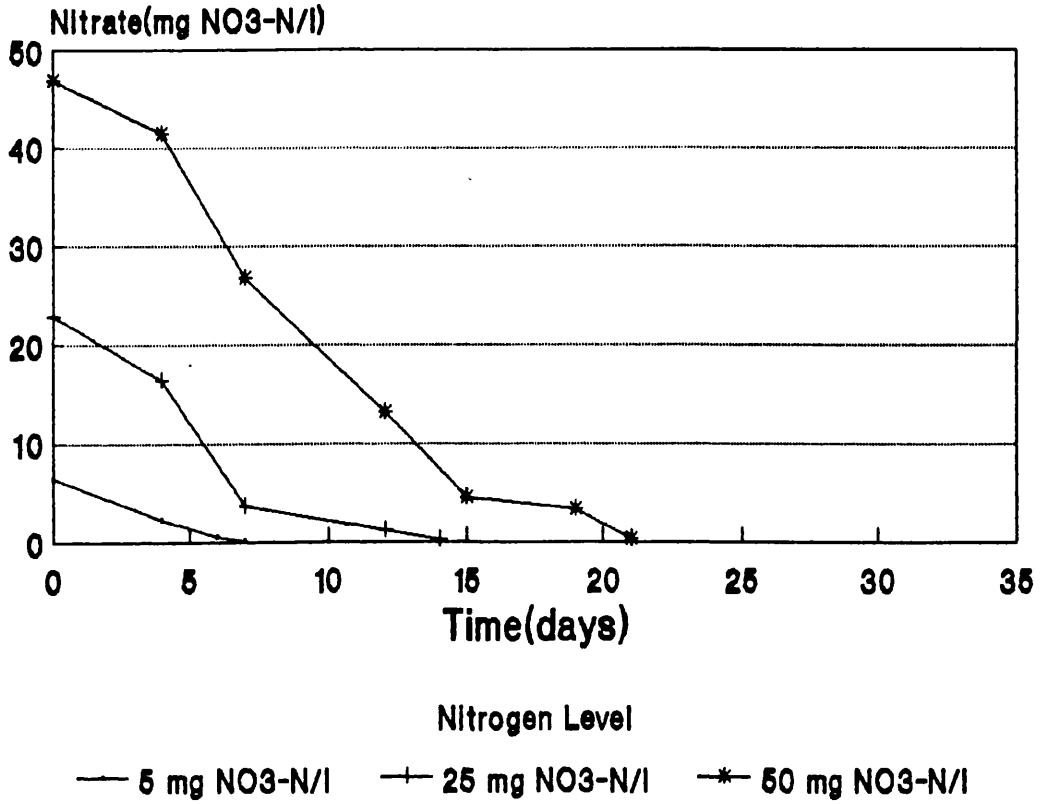


FIG 108 Synechococcus sp.PCC 7943 40°C
NITRATE vs Time

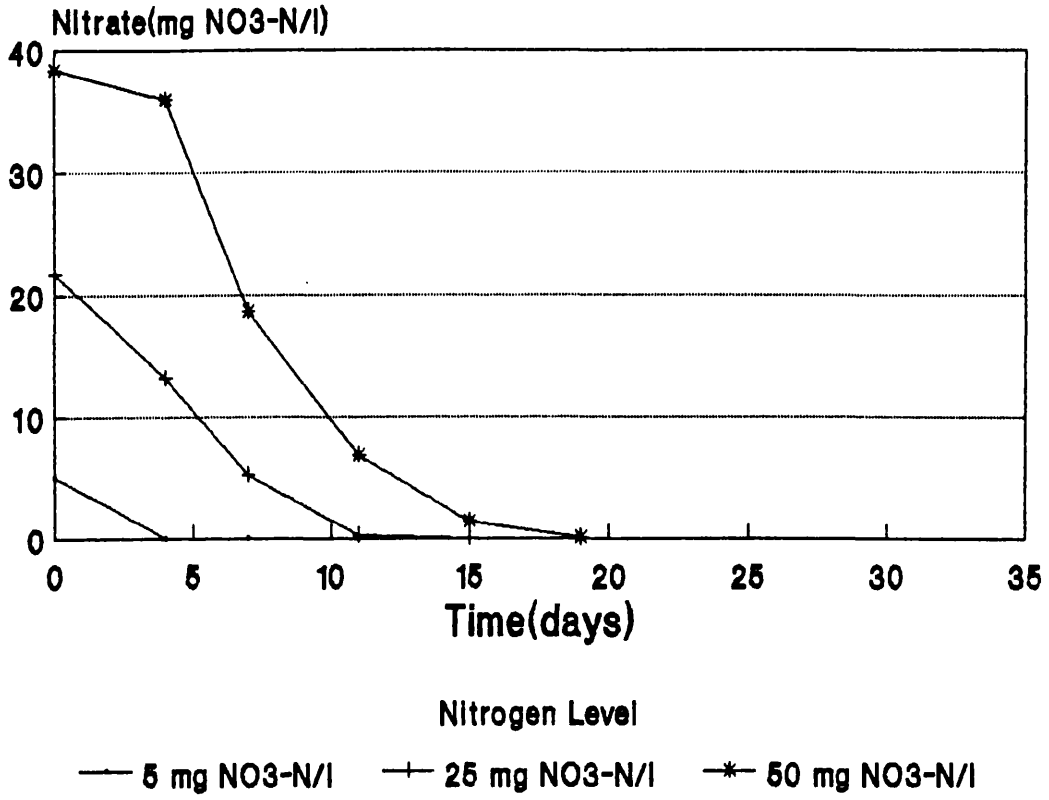


FIG 109 Synechococcus sp.1479/5 17°C
pH vs Time

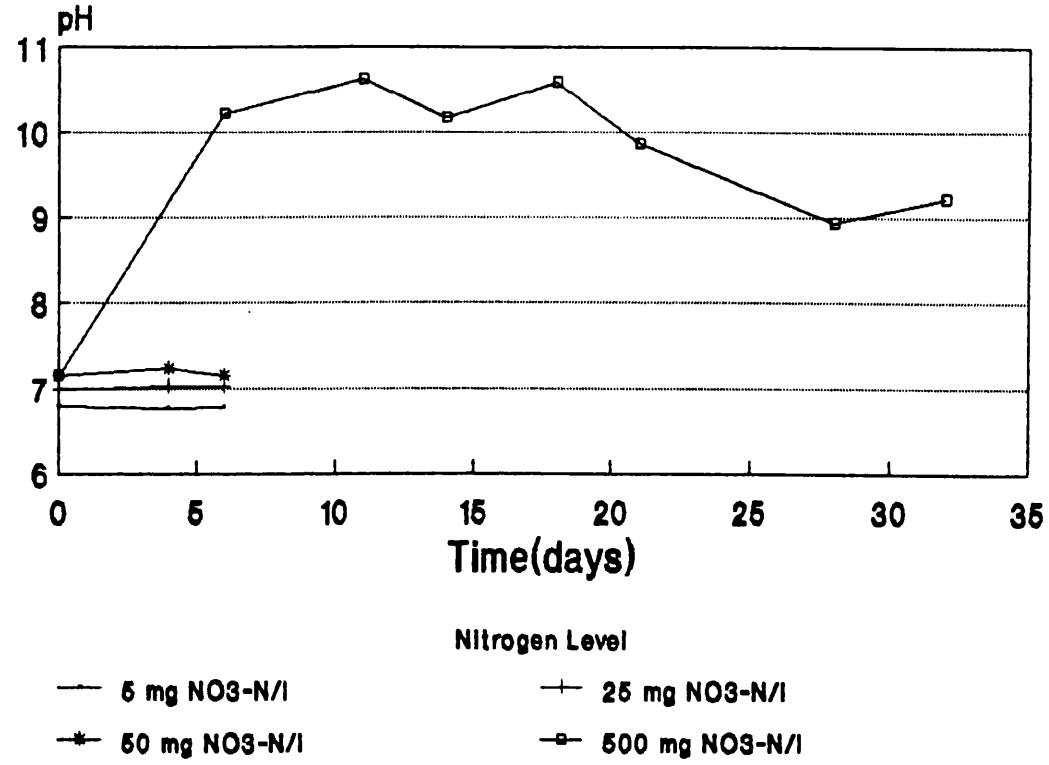


FIG 110 Synechococcus sp.1479/5 30°C
pH vs Time

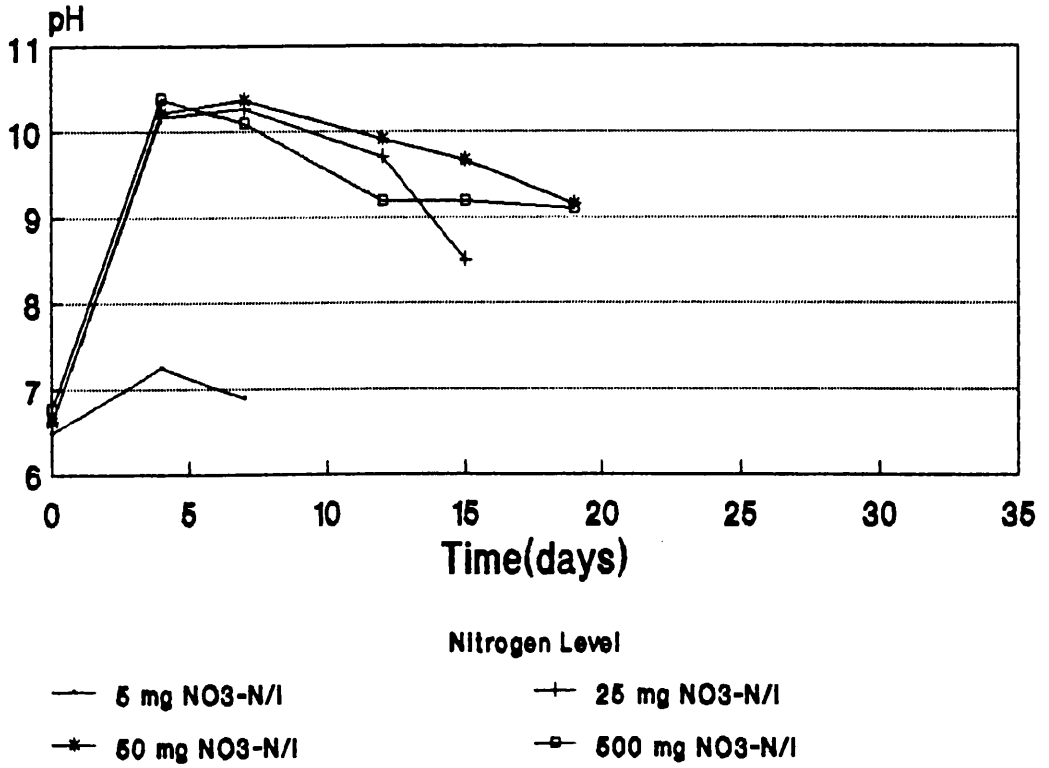


FIG 111 Synechococcus sp.PCC 7943 17°C
pH vs Time

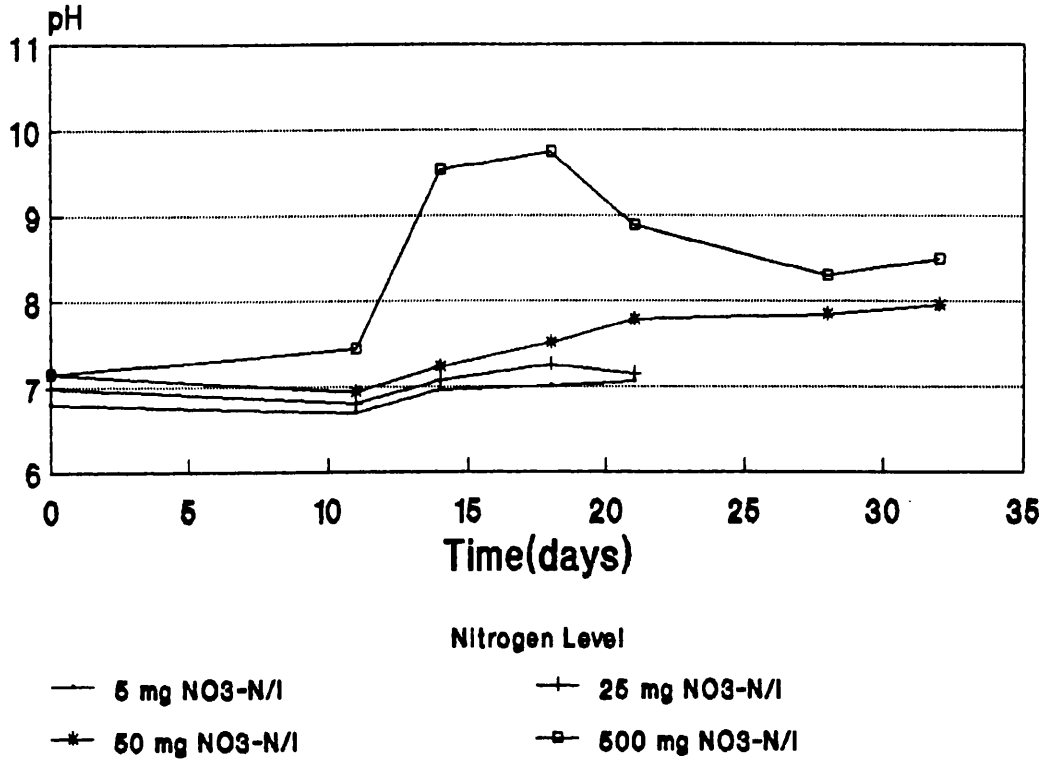


FIG 112 Synechococcus sp.PCC 7943 30°C
pH vs Time

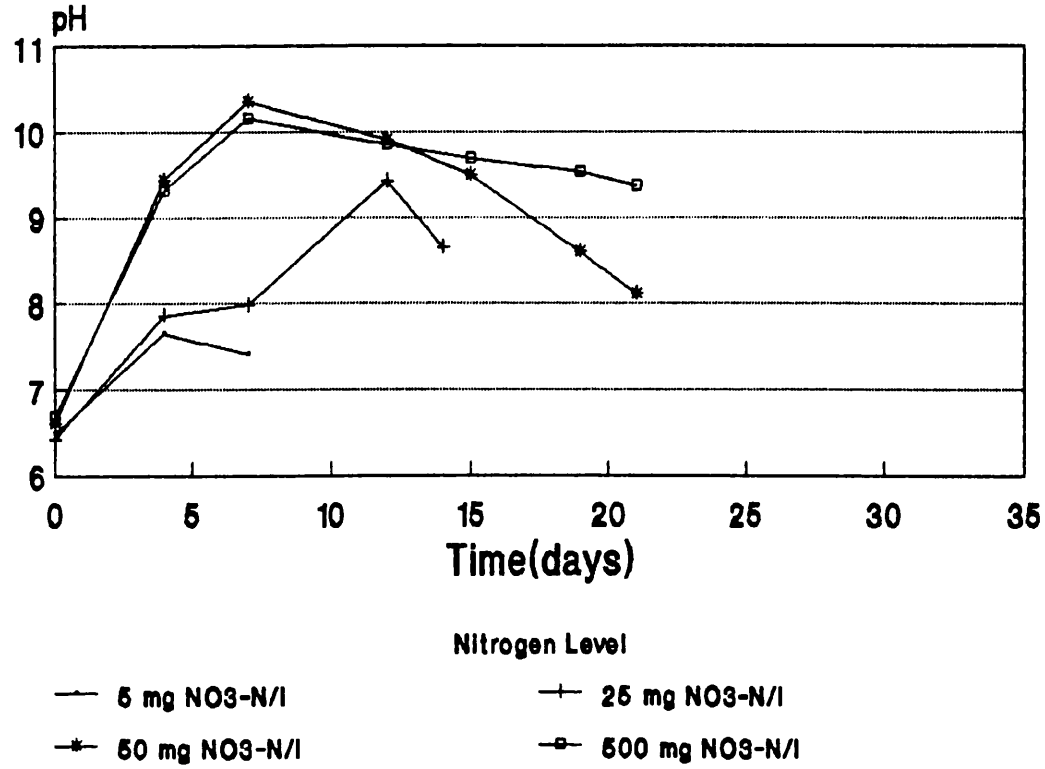


FIG 113 Synechococcus sp.PCC 7943 40°C
pH vs Time

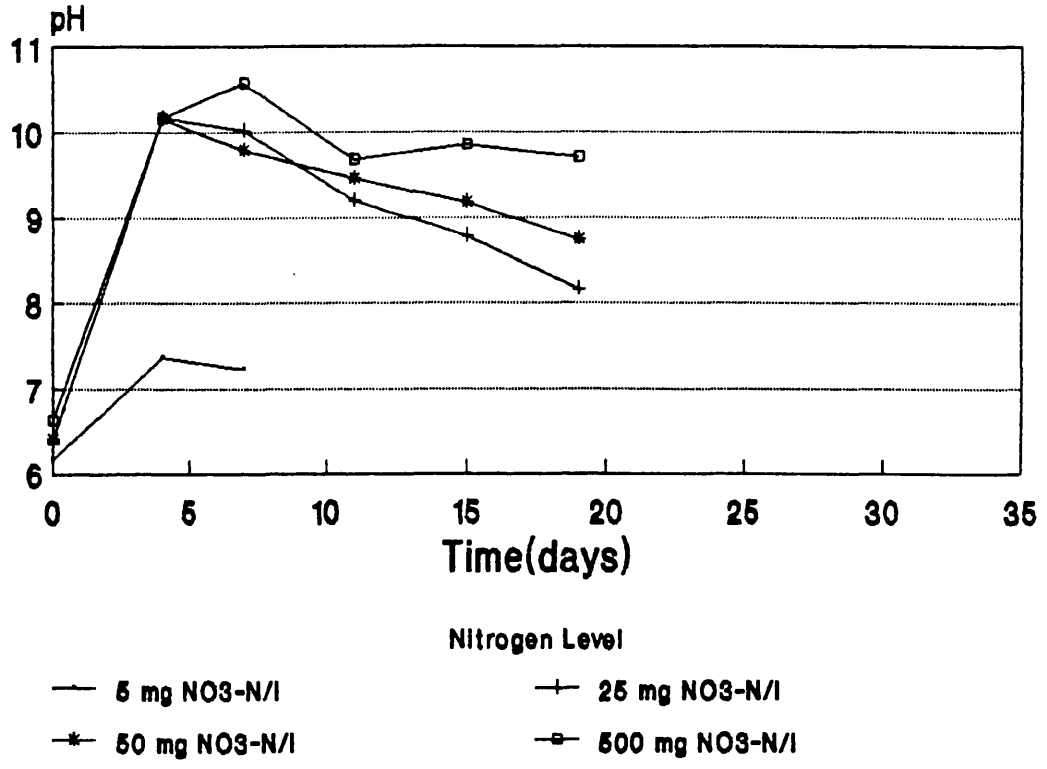


FIG 114 A.flos-aquae 1403/13A 17°C
OD 560 vs Time

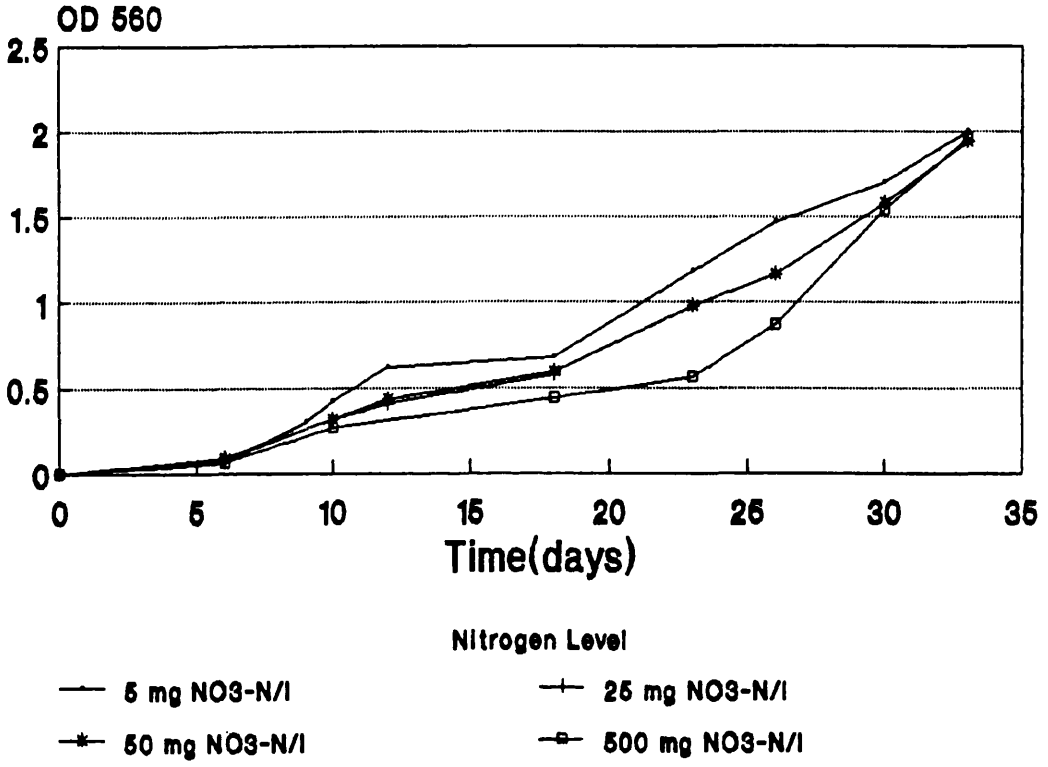


FIG 115 A.flos-aquae 1403/13A 30°C
OD 560 vs Time

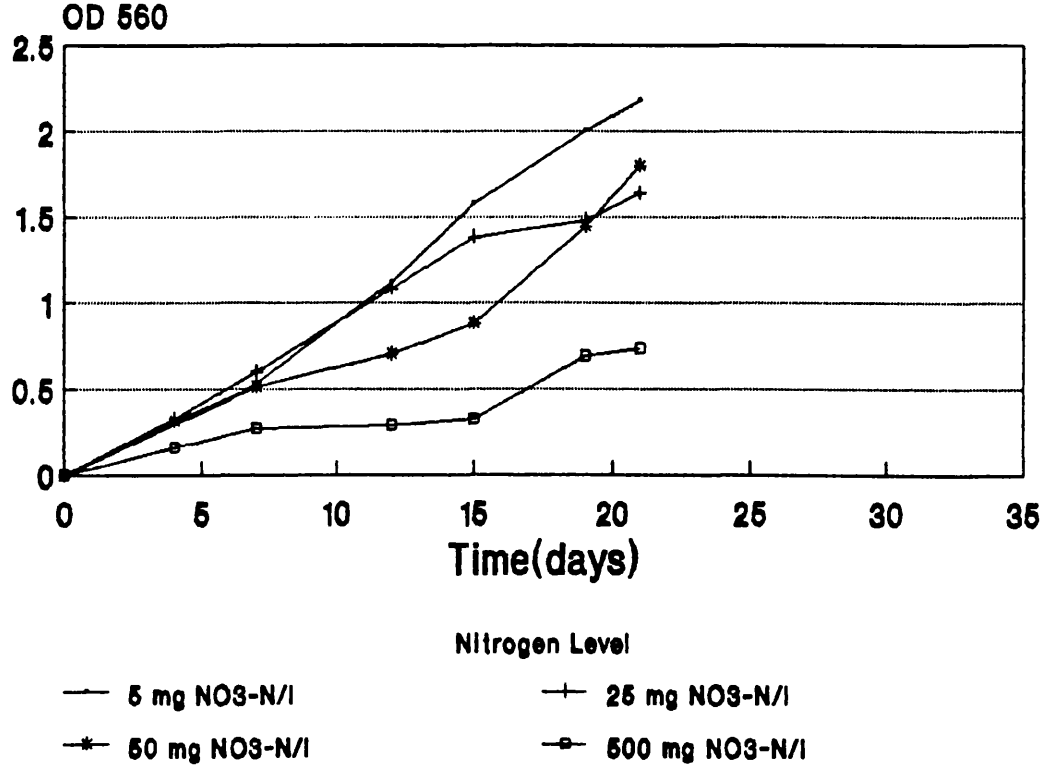


FIG 116 A.flos-aquae 1403/13A 40°C
OD 560 vs Time

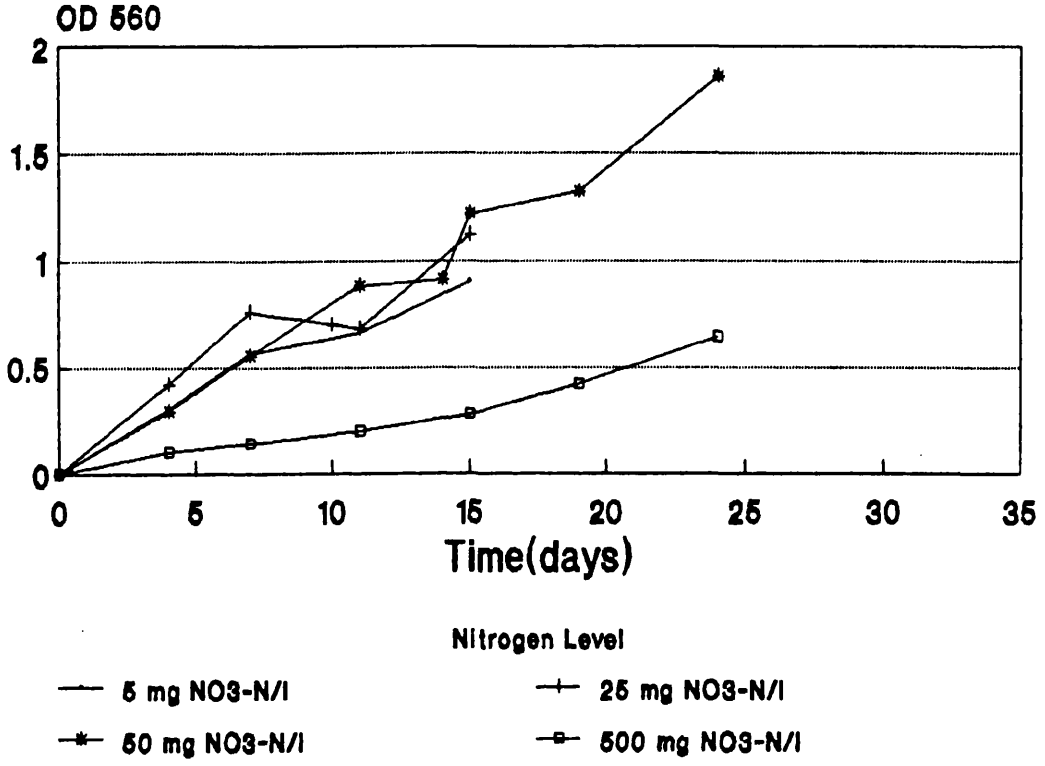


FIG 117 A.variabilis 1403/12 17°C
OD 560 vs Time

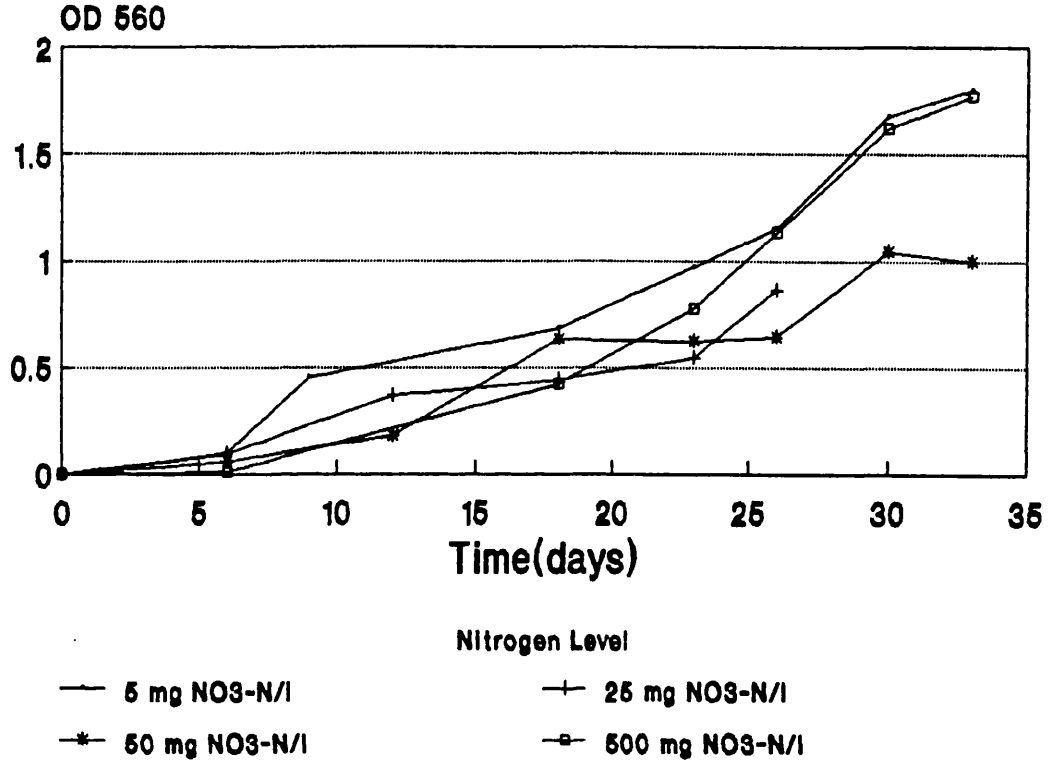


FIG 118 A.variabiiis 1403/12 30°C
OD 560 vs Time

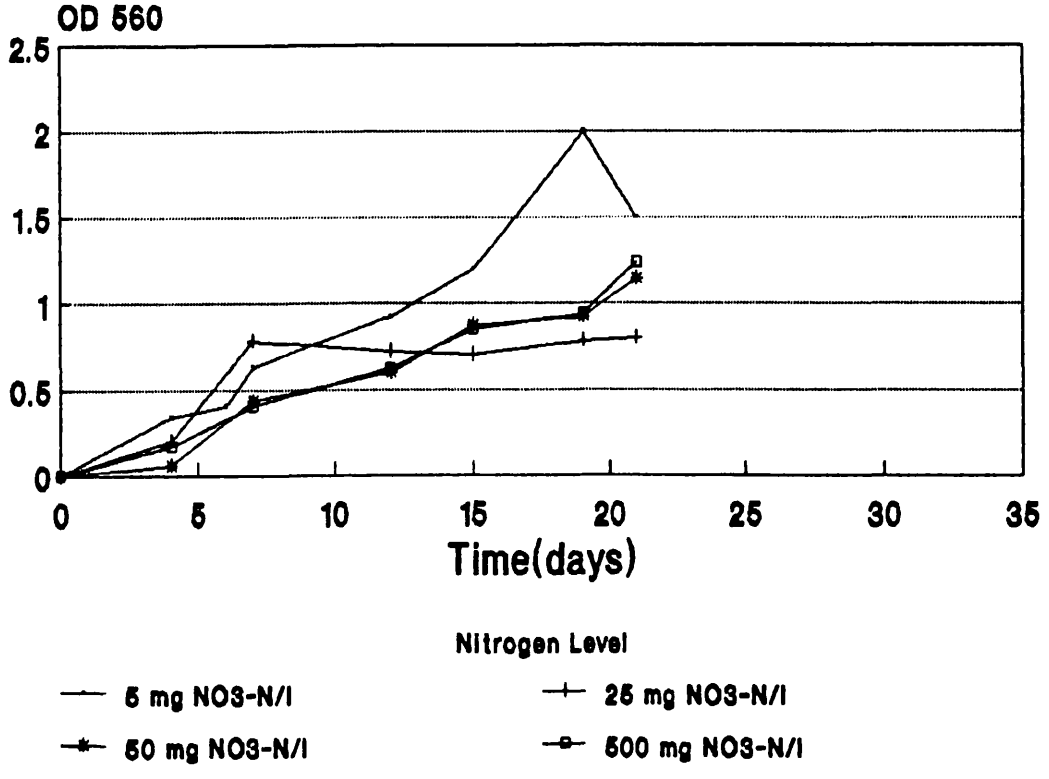


FIG 119 A.flos-aquae 1403/13A 17°C
DRY WEIGHT vs Time

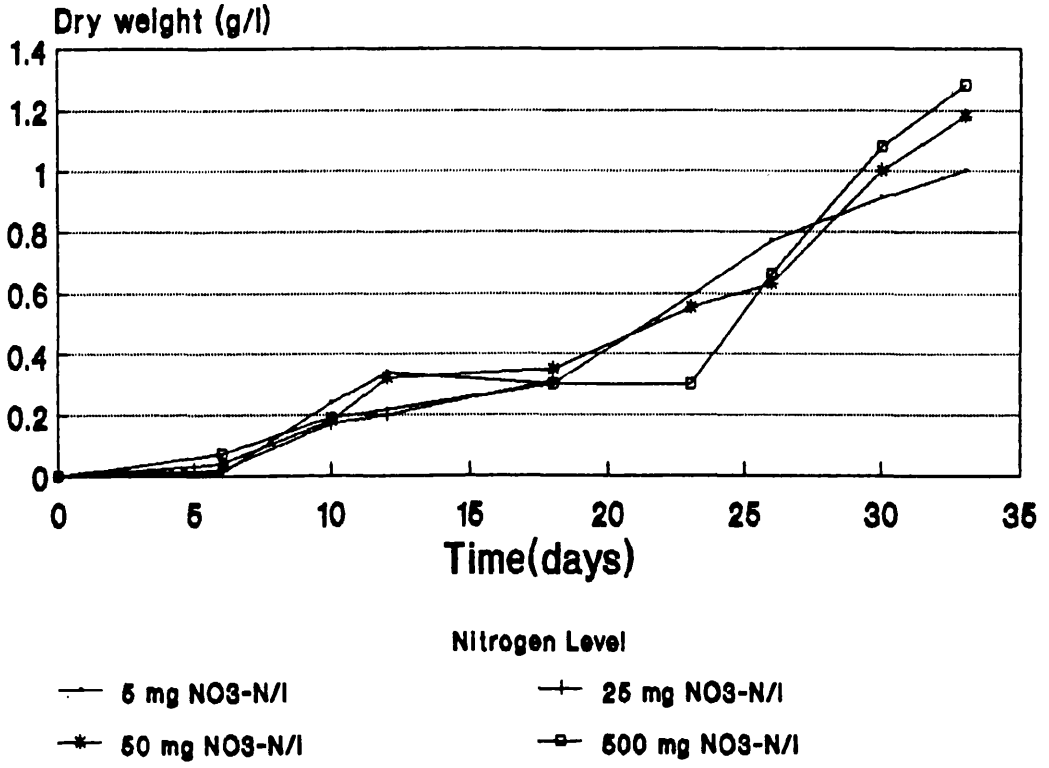


FIG 120 A.flos-aquae 1403/13A 30°C
DRY WEIGHT vs Time

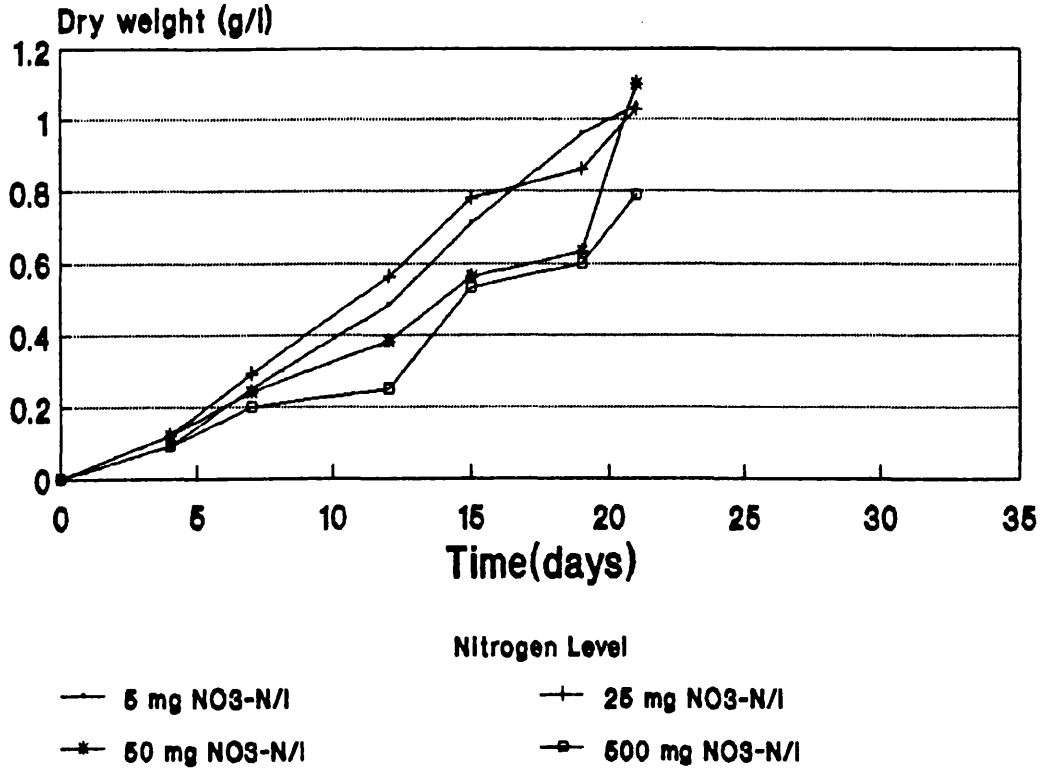


FIG 121 A.flos-aquae 1403/13A 40°C
DRY WEIGHT vs Time

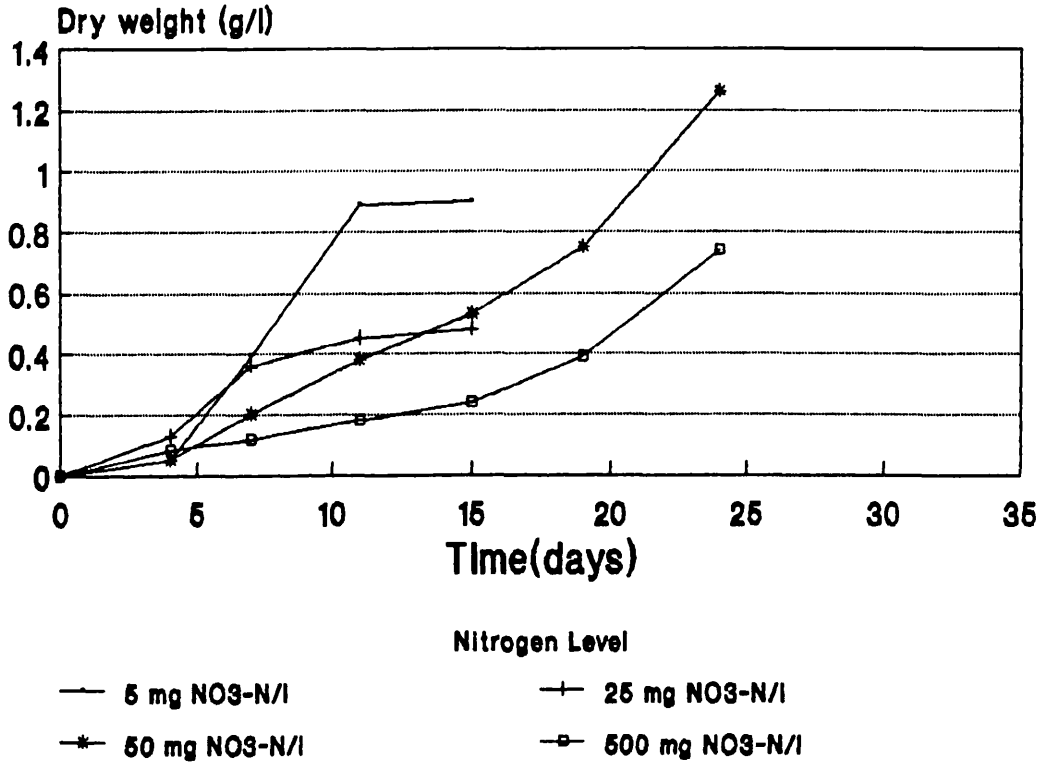


FIG 122 A.variabilis 1403/12 17°C
DRY WEIGHT vs Time

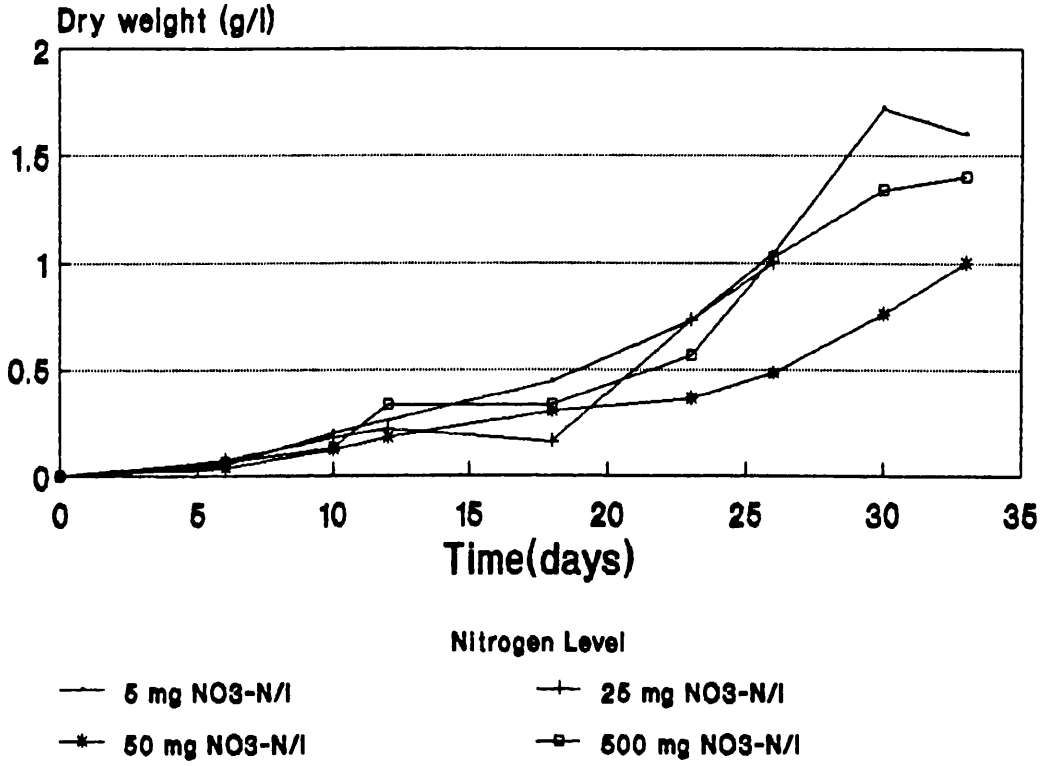


FIG 123 A.variabilis 1403/12 30°C
DRY WEIGHT vs Time

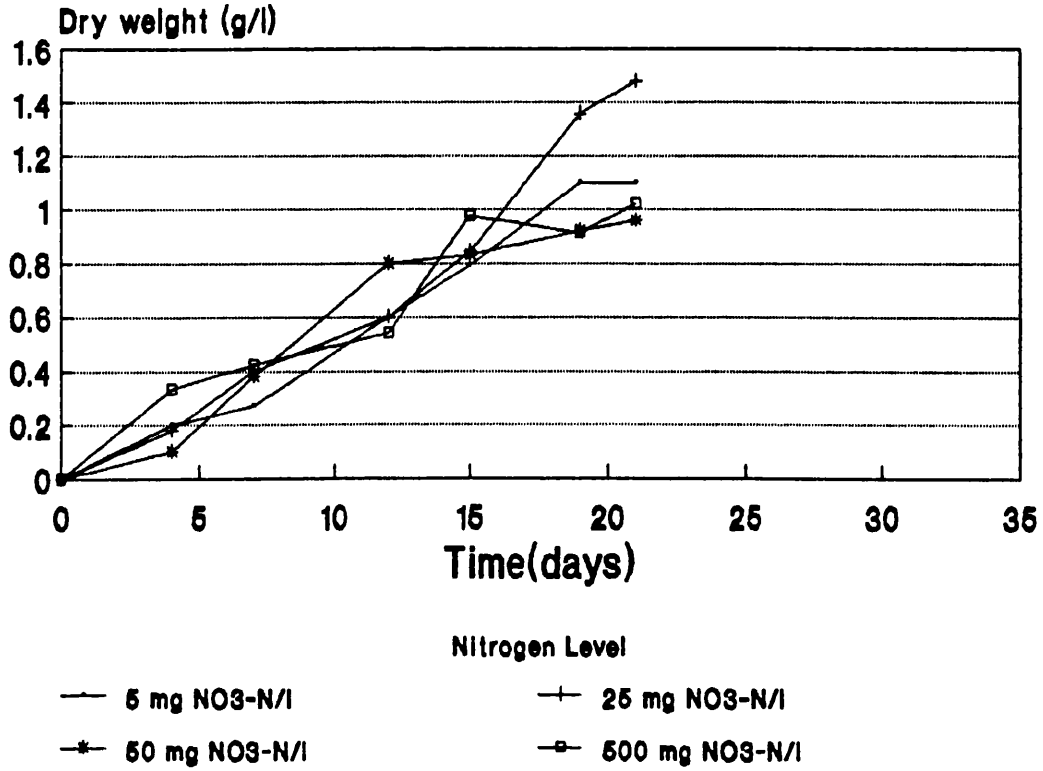


FIG 124 A.flos-aquae 1403/13A 17°C
NITRATE vs Time

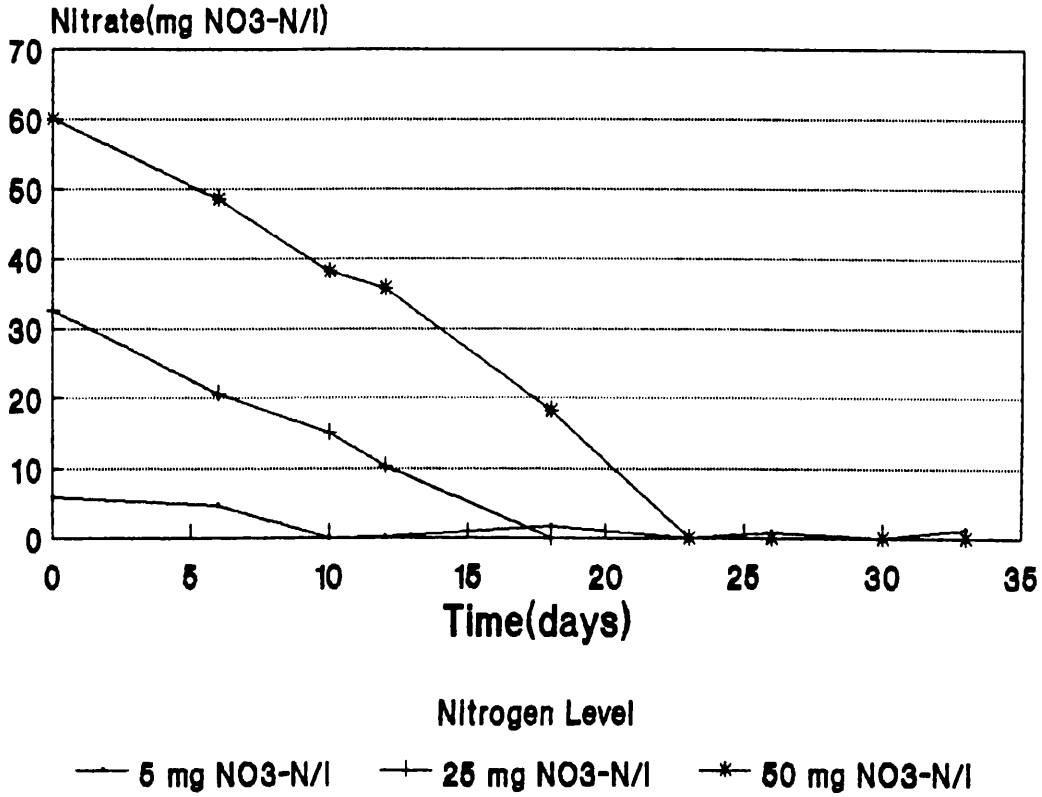


FIG 125 A.flos-aquae 1403/13A 30°C
NITRATE vs Time

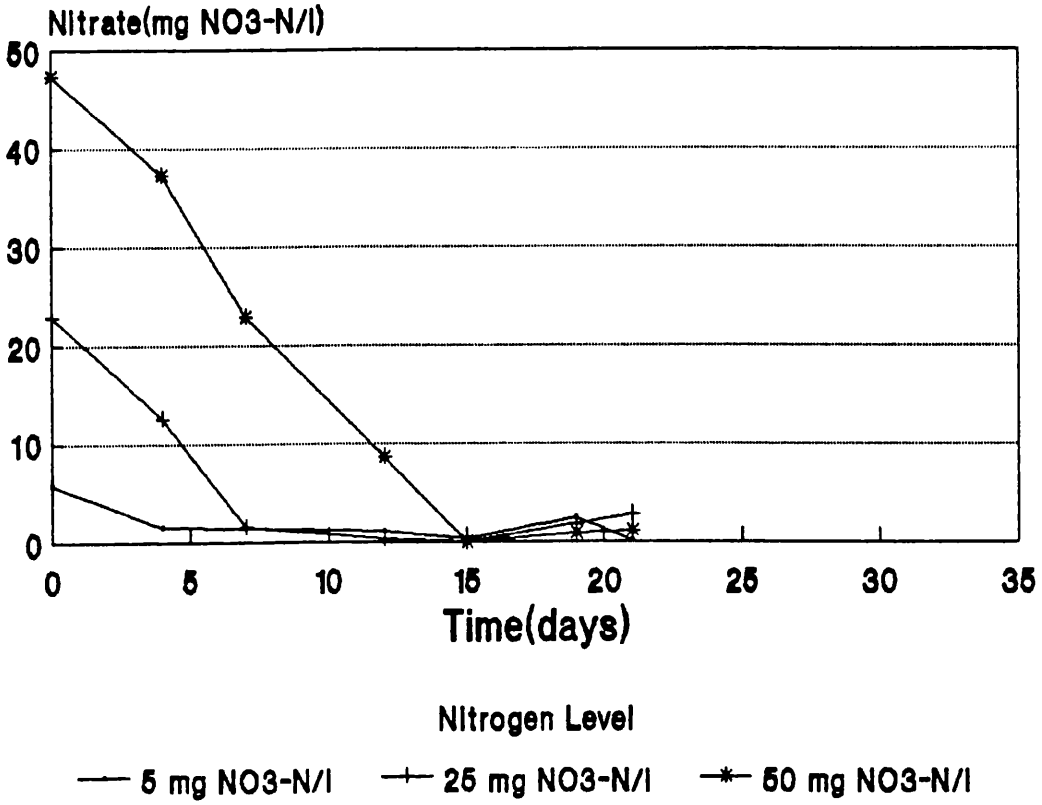


FIG 126 A.flos-aquae 1403/13A 40°C
NITRATE vs Time

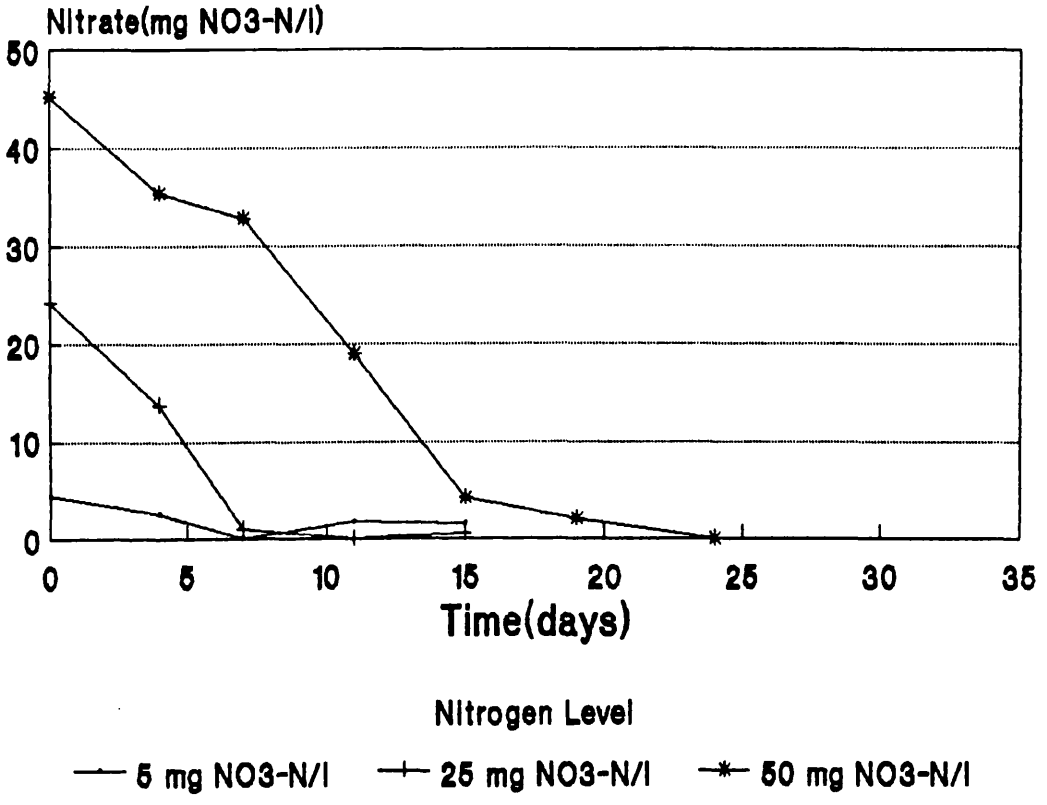


FIG 127 A.variabilis 1403/12 17°C
NITRATE vs Time

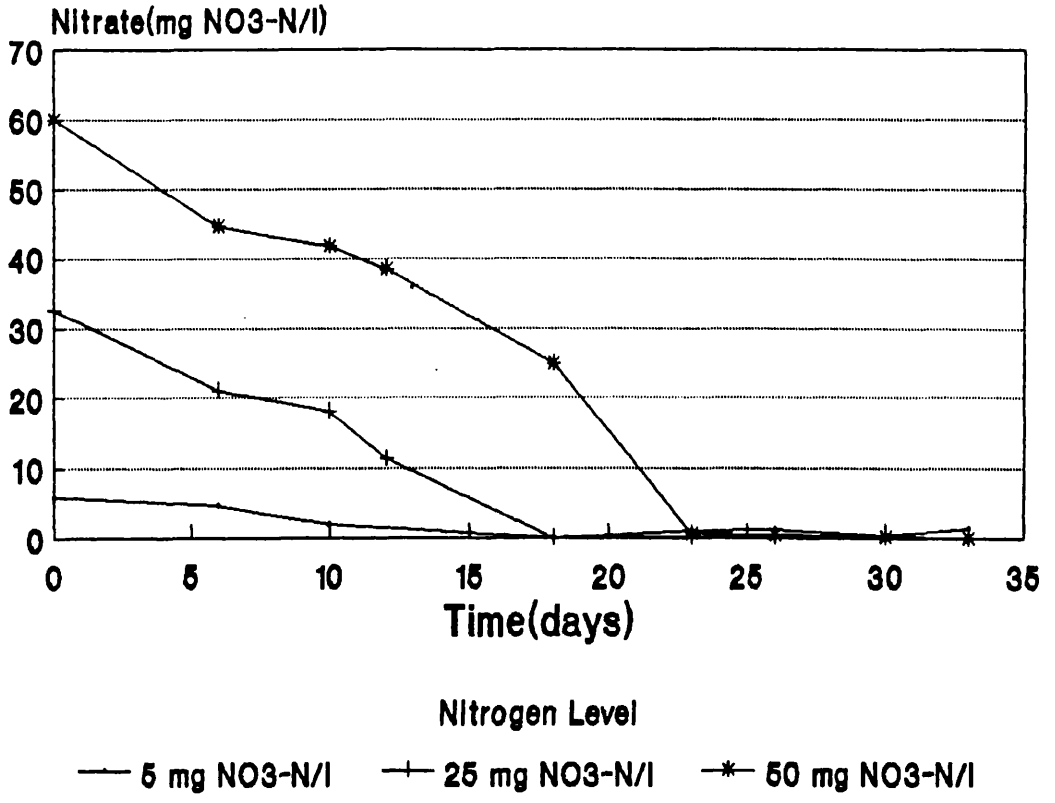


FIG 128 A.variabilis 1403/12 30°C
NITRATE vs Time

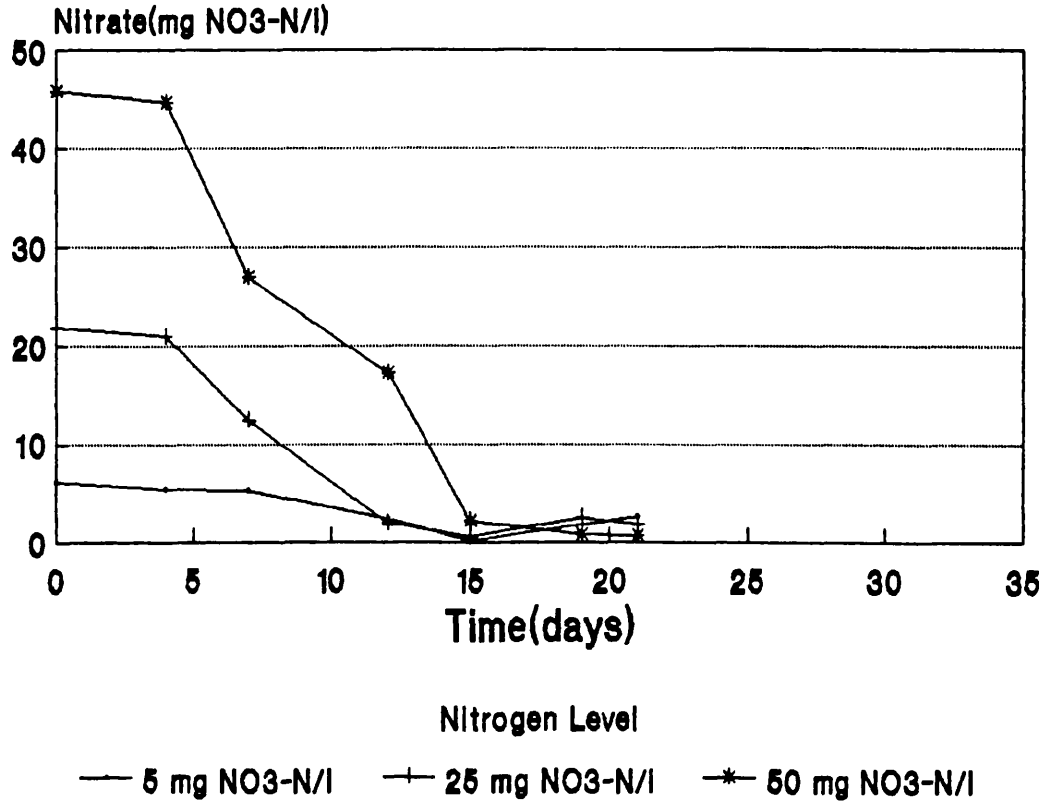


FIG 129 A.flos-aquae 1403/13A 17°C
pH vs Time

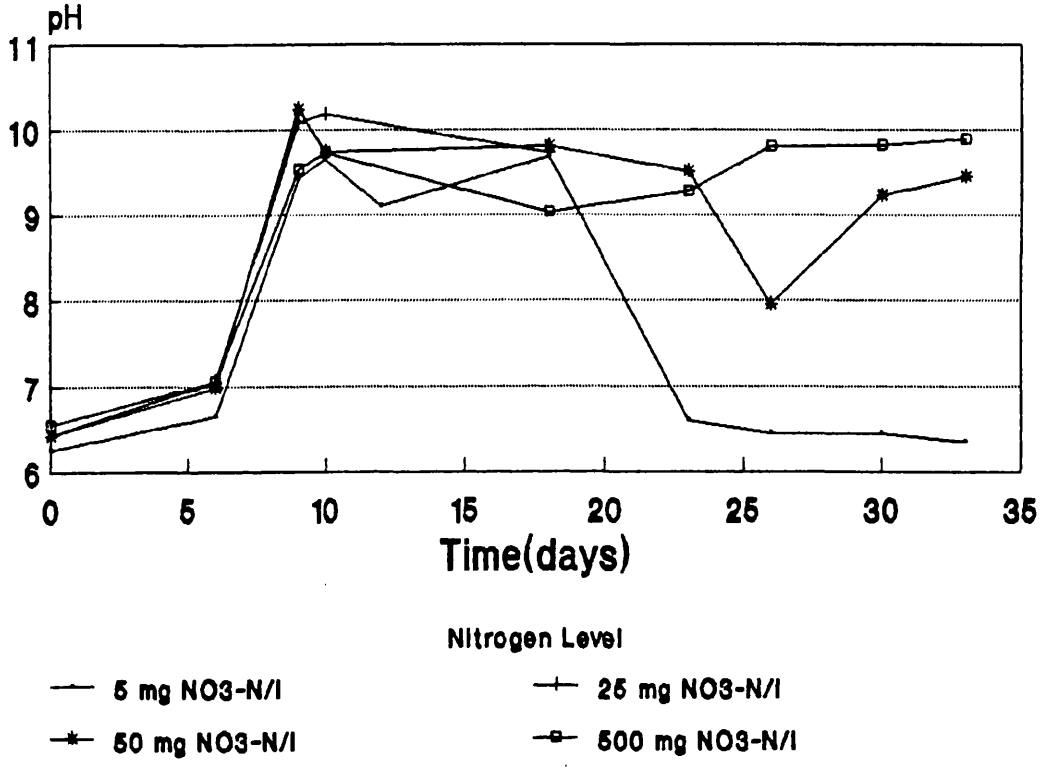


FIG 130 A.flos-aquae 1403/13A 30°C
pH vs Time

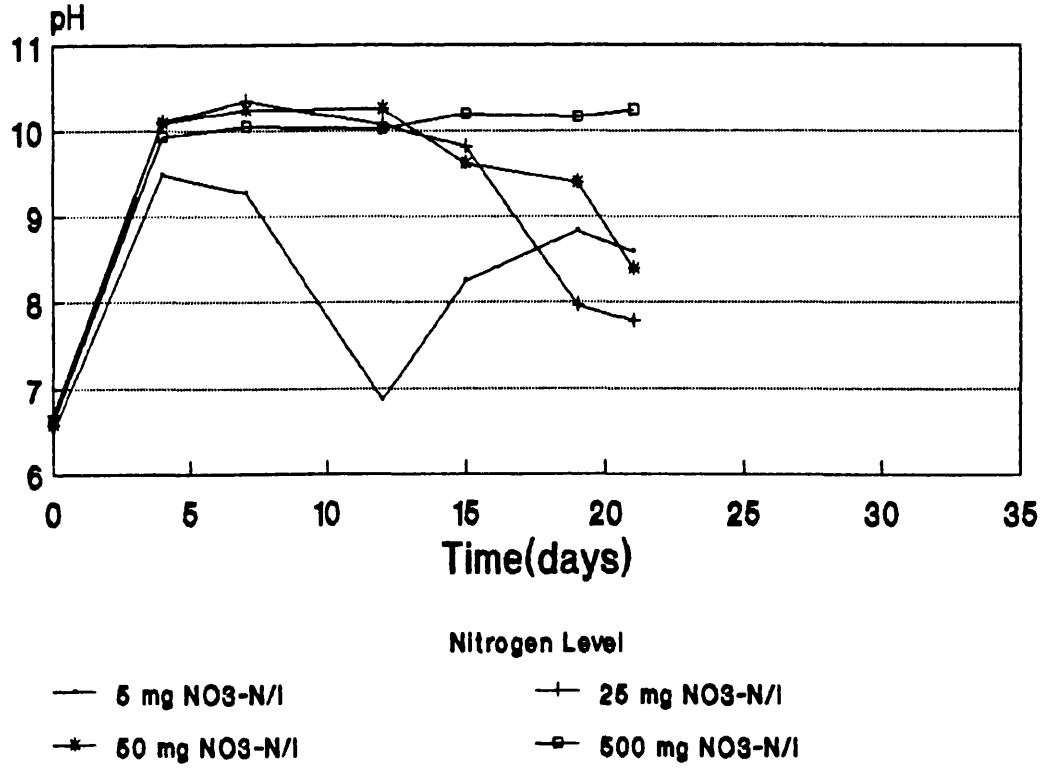


FIG 131 A.flos-aquae 1403/13A 40°C
pH vs Time

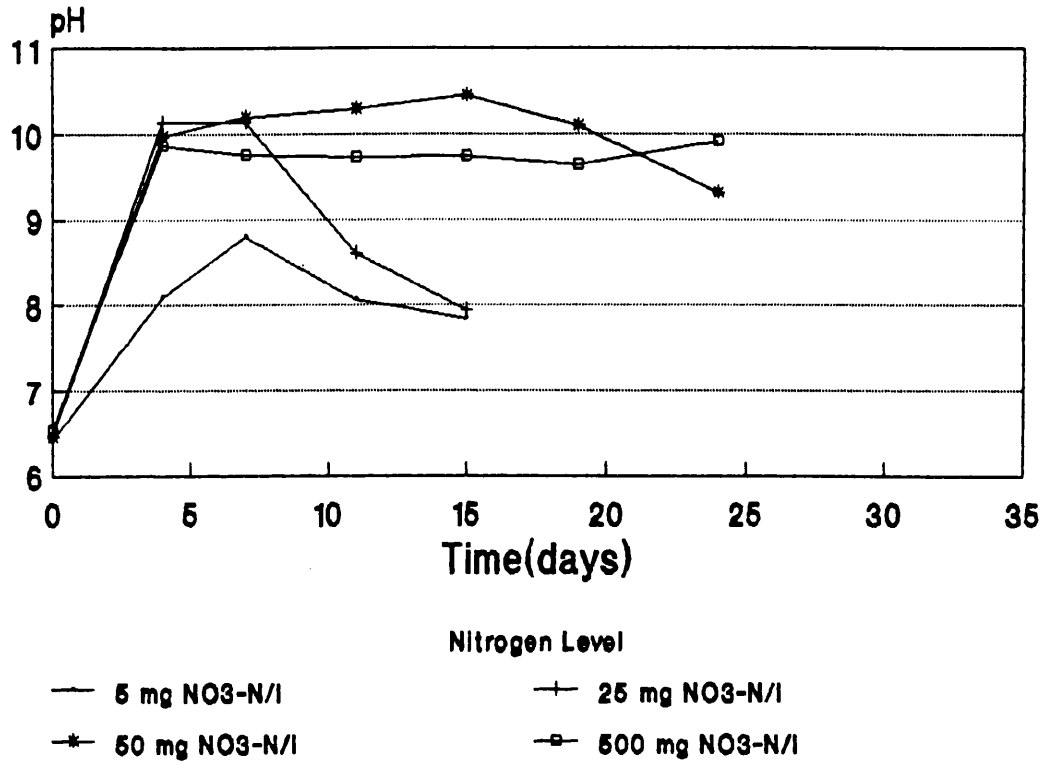


FIG 132 A.variabilis 1403/12 17°C
pH vs Time

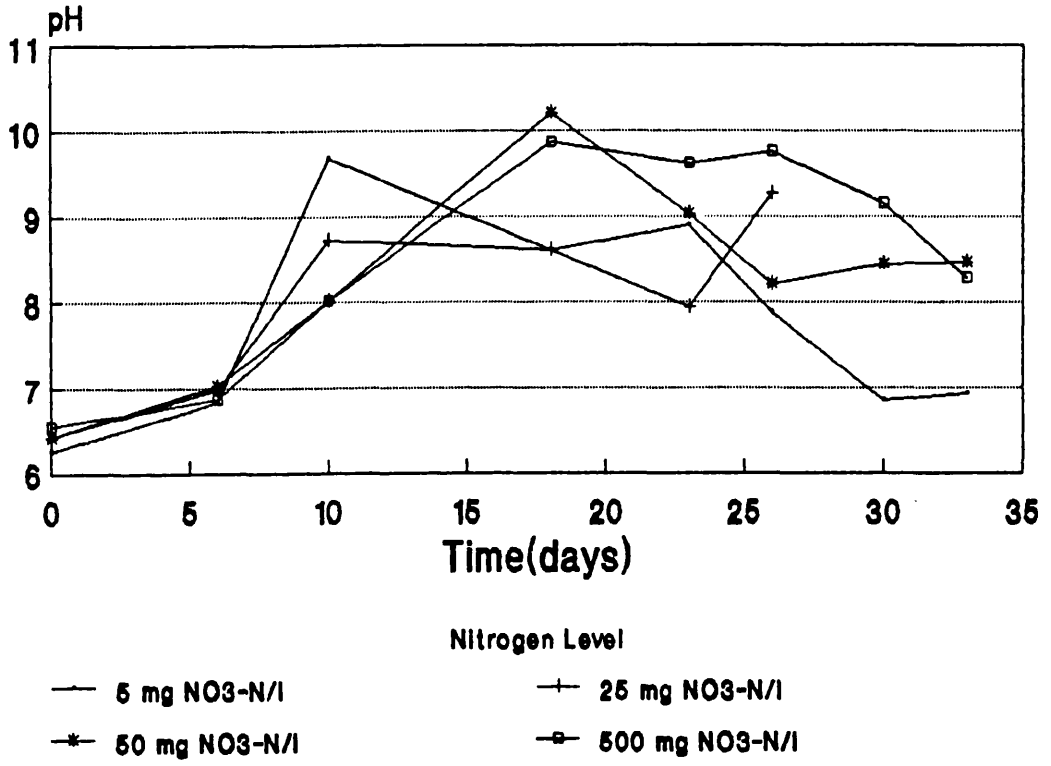


FIG 133 A.variabilis 1403/12 30°C
pH vs Time

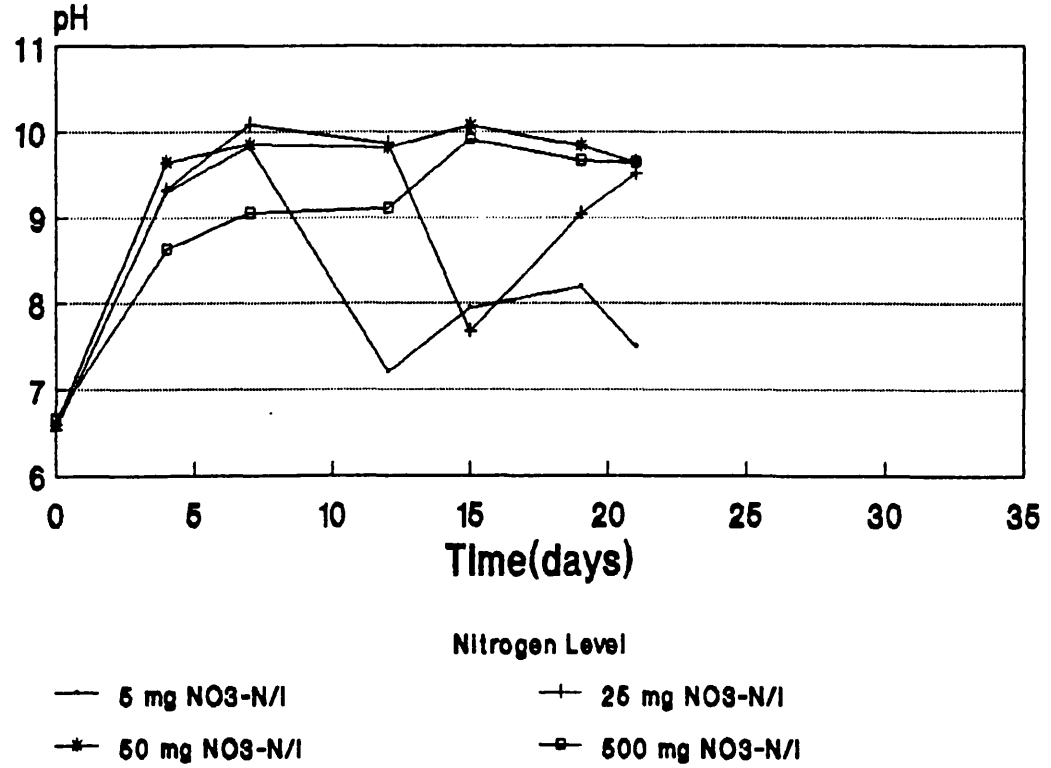


Table 27: Harvest Parameters for Synechococcus sp. 1479/5

Temperature/ Initial N Level		Phase	Time (Days)	$0D_{560}$	Dry wt (gl^{-1})	NO_3-N (mgl^{-1})
17°C	5	S	6	0.09	0.03	0
	25	S	6	0.13	0.01	16.16
	50	S	6	0.13	0.01	43.05
	500	E	32	5.04	0.78	382
30°C	5	S	7	0.42	0.12	0
	25	E	7	0.82	0.16	8.72
	25	S	17	1.92	0.21	0
	50	E	7	0.82	0.15	22.90
	50	S	19	2.86	0.70	0
	500	E	19	3.44	0.87	332

E = Exponential Phase
S = Stationary Phase

Table 28: Harvest Parameters for Synechococcus sp. PCC 7943

Temperature/ Initial N Level		Phase	Time (Days)	OD_{560}	Dry wt (gl^{-1})	NO_3-N (mgl^{-1})
17°C	5	S	21	0.34	0.10	0.02
	25	E	21	0.25	0.10	9.89
	25	S	22	0.18	0.10	8.99
	50	E	21	0.7	0.19	18.74
	50	S	32	1.68	0.54	13.54
	500	E	32	1.68	0.76	403
30°C	5	S	7	0.38	0.09	0.09
	25	E	7	0.34	0.07	3.70
	25	S	14	1.46	0.31	0.21
	50	E	7	0.58	0.14	26.73
	50	S	21	2.56	0.85	0.35
	500	E	21	3.06	0.98	381
40°C	5	S	7	0.27	0.06	0
	25	E	7	0.85	0.22	5.26
	25	S	19	1.61	0.52	0
	50	E	7	0.93	0.25	18.64
	50	S	19	2.14	0.61	0.09
	500	E	19	1.32	0.44	417

E = Exponential Phase
S = Stationary Phase

Table 29: Harvest Parameters for A. flos-aquae 1403/13A

Temperature/ Initial N Level		Phase	Time (Days)	$0D_{560}$	Dry wt (gl^{-1})	NO_3-N (mgl^{-1})
17°C	5	A	33	2.00	1.00	1.18
	25	E	12	0.41	0.20	10.28
	25	S/LE	18	0.58	0.31	0
	50	E	12	0.43	0.32	35.84
	50	A	33	1.94	1.18	0
	500	E	33	1.96	1.28	420
30°C	5	A	21	2.18	1.04	0.09
	25	E	7	0.60	0.29	1.50
	25	A	21	1.64	1.03	2.75
	50	E	7	0.51	0.24	22.90
	50	A	21	1.8	1.10	1.12
	500	E	21	0.73	0.79	393
40°C	5	A	15	0.90	0.90	1.55
	25	E	7	0.76	0.36	0.95
	25	A	15	1.12	0.48	0.56
	50	E	15	0.91	0.53	4.15
	50	S/LE	24	1.86	1.26	0
	500	E	24	0.64	0.74	328

E = Exponential Phase
S/LE = Stationary/Late Exponential Phase

A denotes after nitrogen depletion, cultures being left under nitrogen depletion for a period before harvesting, for observation of heterocysts due to nitrogen fixation.

Table 30: Harvest Parameters for A. variabilis 1403/12

Temperature/ Initial N Level		Phase	Time (Days)	$0D_{560}$	Dry wt (gl^{-1})	NO_3-N (mgl^{-1})
17°C	5	A	33	1.80	1.60	1.36
	25	E	12	0.37	0.22	11.47
	25	A	26	0.86	1.00	0.15
	50	E	12	0.18	0.18	38.52
	50	S/LE	33	1.00	1.00	0
	500	E	33	1.77	1.40	400
30°C	5	A	21	1.50	1.10	2.56
	25	E	7	0.78	0.40	12.44
	25	A	21	0.80	1.48	1.78
	50	E	7	0.43	0.38	26.95
	50	S/LE	21	1.14	0.96	0.73
	500	E	21	1.24	1.02	403

E = Exponential Phase
S/LE = Stationary Phase/Late Exponential Phase
A - denotes after nitrogen depletion, cultures being left under nitrogen depletion for a period before harvesting, for observation of heterocysts due to nitrogen fixation.

Table 31: Heterocysts (Percentage Composition of Cells) for A. flos-aquae 1403/13A at 17°C and 30°C

Time (days)	17°C				30°C			
	5	25	50	500	5	25	50	500
0	0	0	0	0	0	0	0	0
7	ND	ND	ND	ND	7.4	0	0	0
12	5.5	1.8	2.1	0.6	9.2	8.1	0.5	0
18	8.3	4.0	4.6	1.3	ND	ND	ND	ND
21	ND	ND	ND	ND	11.2	8.1	9.9	0.7
23	5.7	ND	2.1	2.3	ND	ND	ND	ND
26	8.5	ND	3.8	0.6	ND	ND	ND	ND
30	9.6	ND	8.3	0.7	ND	ND	ND	ND
33	13.0	ND	6.5	O	ND	ND	ND	ND

Table 32: Heterocysts (Percentage Composition of Cells) for A. variabilis 1403/12 at 17°C and 30°C

Time (days)	17°C				30°C			
	5	25	50	500	5	25	50	500
0	3.3	3.3	3.3	3.3	5.3	5.3	5.3	5.3
7	ND	ND	ND	ND	5.1	2.6	1.1	0.7
12	3.5	2.7	3.3	2.5	6.9	3.1	0.9	0
18	7.3	3.0	2.4	0.6	ND	ND	ND	ND
21	ND	ND	ND	ND	8.9	8.3	3.0	0
23	7.0	4.8	3.0	0.8	ND	ND	ND	ND
26	9.0	5.3	3.9	3.1	ND	ND	ND	ND
30	7.1	ND	3.2	0.6	ND	ND	ND	ND
33	4.7	ND	5.1	O	ND	ND	ND	ND

ND = Not Determined

4.3.3.2 Carbohydrate, Protein and Lipid Results

The results for carbohydrate, protein and lipid analyses are given in Tables 33, 34, 35 and 36 for Synechococcus sp. 1479/5, Synechococcus sp. PCC 7943, A. flos-aquae and A. variabilis respectively.

Problems with growth at 17°C for Synechococcus sp. 1479/5 makes comparison across temperatures difficult. However, from the results available, Synechococcus sp. 1479/5 increased protein content (average protein content at 17°C - 9.71%, 30° - 12.87%) and carbohydrate content (average carbohydrate content at 17°C - 12.33%, 30°C - 24.12%) with increase in temperature, but lipid content remained similar at both temperatures. With respect to growth phase, the results at 30°C indicated lipid and protein contents increased slightly at stationary phase, with carbohydrate increasing significantly (Figs 134 and 135). The major change in cellular constituents for Synechococcus 1479/5 was accumulation of carbohydrate at stationary phase but this was only observable at 30°C.

Synechococcus PCC 7943 maintained similar protein contents (average protein content at 17°C - 12.42%, 30°C - 14.96%, 40°C - 13.39%) and lipid contents (average lipid content at 17°C - 4.38%, 30°C - 4.92%, 40°C - 5.42%) with increasing culutre temperature. However, the average carbohydrate content decreased significantly (average carbohydrate content at 17°C - 33.45%, 30°C - 29.02%, 40°C - 23.34%) with increasing temperature. With respect to growth phase, lipid content decreased slightly in stationary phase, but protein content did not exhibit a regular pattern of increase or decrease in different phases but was temperature dependent. Carbohydrate increased with stationary phase at all temperatures. The major change therefore appeared to be carbohydrate accumulation in stationary phase but it was slightly greater at the lower temperature for Synechococcus PCC 7943 (Figs 136 and 137).

A. flos-aquae showed increased protein content with increasing temperature (average protein content at 17°C - 7.33%, 30°C - 8.09%, 40°C - 10.67%) but maintained lipid content at similar levels irrespective of temperature.

Carbohydrate content decreased significantly with increasing temperature (average carbohydrate content at 17°C - 29.07%, 30°C - 15.21%, 40°C - 11.42%). Regarding growth phase, lipid content did not exhibit a regular pattern of change with growth phase but protein increased at stationary phase. Carbohydrate accumulated at stationary phase at all temperatures (Figs 138 and 139). Although A. flos-aquae has the ability to fix nitrogen, it still appears to behave similarly to the two non-nitrogen fixers (Synechococcus species) in accumulation of carbohydrate.

A. variabilis maintained lipid content (average lipid content at 17°C - 2.44%, 30°C - 3.40%), protein content (average protein content at 17°C - 13.30%, 30°C - 14.40%) and carbohydrate content (average carbohydrate content at 17°C - 19.30%, 30°C - 21.05%) at similar levels from 17°C to 30°C. Regarding growth phase, lipid content did not exhibit a regular pattern of change with phase but protein increased at stationary phase (Figs 140 and 141). Carbohydrate accumulated at stationary phase (Figs 140 and 141), although contents were similar at 17°C and 30°C. A. variabilis, like A. flos-aquae, behaved similarly to the two non-nitrogen fixing cyanobacteria.

4.3.3.3 Statistical Analysis of Carbohydrate, Protein and Lipid Results

Statistical analysis of all the lipid contents for all cyanobacteria investigated gave two 'main effects', algae and nitrogen as significant (0.1% or $p < 0.001$). Species means (Synechococcus sp. 1479/5 - 4.16%, Synechococcus PCC 7943 - 4.91%, A. flos-aquae - 3.68%, A. variabilis -3.07%) exhibited a significant difference between Synechococcus sp. 1479/5 and Synechococcus PCC 7943 and the two Anaebaenas in lipid content. This is an interesting division between non-nitrogen fixing and nitrogen fixing cyanobacteria in relation to lipid content. Nitrogen means ('5' (mg NO_3-N l^{-1}) - 4.28%, '25' - 4.26%, '50' - 4.44%, '500' - 2.03%) only showed a significant decrease from '50' to '500' with the other three initial nitrogen levels not being significantly different. Therefore, nitrate depletion affects the lipid content in the cyanobacteria studied.

Statistical analysis of the protein results showed four significant 'main effects', temperature (1% or $p < 0.01$), algal species (0.1% or $p < 0.001$), nitrogen (0.1% or $p < 0.001$) and phase (1% or $p < 0.01$). Temperature means (17°C - 10.52%, 30°C - 12.58°C, 40°C - 12.88%) were not significantly different at the higher temperature, but there was a significant reduction at the lowest temperature. Species means (Synechococcus sp. 1479/5 - 11.39%, Synechococcus sp. PCC 7943 - 13.59%, A. flos-aquae - 8.70%, A. variabilis - 14.29%) showed significant differences between species. Nitrogen means ('5' (mg NO_3-N l^{-1}) - 11.62%, '25' - 10.10%, '50' - 12.29%, '500' - 15.57%) showed a significant higher value at '500' compared to the other initial nitrogen levels, demonstrating that nitrogen history affects protein content. Phase means (exponential - 10.84%, stationary - 13.15%) showed a significant increase in stationary phase.

Statistical analysis of the carbohydrate results showed significant results for the four 'main effects' (0.1% or $p < 0.001$). Temperature means (17°C - 22.99%, 30°C - 22.35%, 40°C - 14.86%) show a significant reduction at the highest temperature, confirming carbohydrate accumulation at lower temperatures. Species means (Synechococcus sp. 1479/5 - 14.52%, Synechococcus PCC 7943 - 28.61%, A. flos-aquae - 19.57%, A. variabilis - 17.57%) showed that the mean for A. variabilis was not significantly different from A. flos-aquae but was significantly different from Synechococcus sp. PCC 7943. The mean for Synechococcus sp. PCC 7943 was significantly greater than the other three means. Therefore there were differences between Synechococcus sp. 1479/5 and Synechococcus sp. PCC 7943 and between the non-nitrogen fixing and nitrogen fixing cyanobacteria in the amount of carbohydrate accumulated. Nitrogen means ('5' (mg NO_3-N l^{-1}) - 30.81%, '25' - 20.17%, '50' - 17.86%, '500' - 13.54%) showed significant differences between '5', '25' and '50' and '500', initial nitrogen level was affecting the amount of carbohydrate accumulated. Phase means were exponential - 14.76% and stationary - 25.38%, a significant increase at stationary phase.

Therefore, changes occurred in protein, lipid and carbohydrate contents for all cyanobacteria with temperature changes and growth phases, but the major

shifts were in carbohydrate accumulation at stationary phase which was especially noticeable for Synechococcus sp. PCC 7943.

4.3.3.4 Fatty Acid Results

Fatty Acid results are given in Tables 33, 34, 35 and 36 for Synechococcus sp. 1479/5, Synechococcus sp. PCC 7943, A. flos-aquae and A. variabilis respectively. The fatty acid profiles for all four species showed a predominance of C16 and C18 fatty acids, but also included significant quantities of C14 fatty acids especially in the two Synechococcus species.

Synechococcus sp. 1479/5 showed only minor quantitative changes in individual fatty acids with growth phase at 30°C resulting in almost identical levels of unsaturation between phases (Table 33). The major fatty acids found in Synechococcus sp. 1479/5 were 14:0, 16:0 and 16:1. With increasing temperature, 14:0 and 16:0 appeared to increase whilst 18:1(n-7) decreased. % UNFA decreased significantly with increased temperature (average % UNFA at 17°C - 63.50%, 30°C - 43.50%).

Synechococcus sp. PCC 7943 showed quantitative changes in individual fatty acids with growth phase and temperature (Table 34). The major fatty acids found in Synechococcus sp. PCC 7943 were 16:0 and 16:1. With increasing temperature, 16:0, 18:0, 18:1(n-9) and 18:1(n-7) fatty acids increased and 14:0, 14:1 and 16:1 decreased. % UNFA decreased in stationary phase at 17°C and 40°C and increased in stationary phase at 30°C. Average % UNFA decreased significantly with increased temperature (average % UNFA at 17°C - 50.71%, 30°C - 50.14%, 40°C - 45.22%).

A. flos-aquae also exhibited quantitative changes in individual fatty acids with growth phase and temperature (Table 35). The major fatty acids found in A. flos-aquae 1403/13A were 16:0, 16:1, 18:1, 18:2(n-6) and 18:3(n-3). Increasing temperature increased 16:0, 18:1(n-9) and 18:2(n-6), and decreased 18:3(n-3). % UNFA increased in stationary phase at all three temperatures (Table 33). Average % UNFA decreased significantly with increased temperature (Average

% UNFA at 17°C - 69.30%, 30°C - 65.73%, 40°C - 59.96%). A. flos aquae although exhibiting a similar fatty acid profile to the two Synechococcus species also had significant quantities of polyunsaturated fatty acids in the C18 range notably 18:2(n-6) and 18:3(n-3).

A. variabilis showed quantitative changes in individual fatty acids with temperature and growth phase (Table 36). Major fatty acids were found to be 16:0, 18:2(n-6) and 18:3(n-3). Increases were found in 16:0 and 18:2(n-6) with increasing temperature and decreases in 16:3 and 18:3(n-3). % UNFA decreased in stationary phase at 17°C, and increased at stationary phase at 30°C. Average %UNFA decreased significantly with increased temperature (average %UNFA at 17°C - 64.48%, 30°C - 60.78%). A. variabilis exhibited a similar fatty acid profile to A. flos-aquae, with significant quantities of 18:2(n-6) and 18:3(n-3) fatty acids.

4.3.3.5 Statistical Analysis of Percentage Unsaturation Results

Statistical analysis only gave two significant 'main effects' at 0.1% significance ($p < 0.001$), temperature and algal species. Temperature means (17°C - 62.00%, 30°C - 55.04%, 40°C - 52.14%) showed that the reduction in unsaturation as the temperature increased was significant for all species of cyanobacteria investigated. Algal species means (Synechococcus sp. 1479/5 - 51.38%, Synechococcus sp. PCC 7954 - 48.69%, A. flos-aquae - 64.99%, A. variabilis - 60.50%) showed a significant difference between the two Synechococcus species and the two Anaebaenas. Synechococcus sp. PCC 7954 also had a significantly lower value for unsaturation than Synechococcus sp. 1479/5.

4.3.3.6 Gross Photosynthetic and Dark Respiration Rates

The results are given in Table 37 for the cyanobacteria species grown at 25mg NO_3-N l^{-1}. The cultures were harvested at the same time as for the nitrogen limitation experiments.

Gross Photosynthetic and dark respiration rates decreased with increased temperature from 17°C to 30°C with the exception of Synechococcus sp. PCC 7943.

At 40°C, A. flos-aquae increased its photosynthetic and dark respiration rates from 30°C, whereas Synechococcus sp. PCC 7943 decreased its rates. All species decreased their gross photosynthetic and dark respiration rates from exponential to stationary/late exponential phases (and after nitrogen depletion) with the exception of Synechococcus sp. 1479/5 at 30°C.

Table 33: Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) for Synechococcus sp. 1479/5

	17°C				30°C					
Fatty Acid	5S	25S	50S	500E	5S	25E	25S	50E	50S	500E
12:0	.19	.27	.28	.18	.22	.17	.23	.15	.10	-
14:0	19.29	9.95	10.41	15.86	19.97	20.02	21.07	20.30	19.87	8.69
14:1(n-5)	.74	.27	.32	.52	.49	.43	.49	.50	.39	-
15:0	2.08	1.82	2.45	.73	1.87	.43	1.10	.47	1.15	3.64
16:0	20.99	17.26	17.65	25.25	29.51	31.17	30.89	31.00	31.23	24.59
16:1(n-7)	46.72	32.69	33.68	46.50	37.46	35.42	34.66	37.95	36.07	32.49
16:2	-	-	-	-	-	-	-	-	-	-
16:3(n-6)	.66	.55	.42	.80	.60	.23	.27	.29	.42	-
16:4	-	.35	-	-	-	-	-	-	-	-
17:0	1.02	.50	1.01	.86	1.45	.44	1.56	.35	1.14	3.06
18:0	1.60	.90	1.29	.98	1.37	4.02	1.86	3.38	1.89	4.38
18:1(n-9)	.78	3.48	2.55	.67	.64	1.60	1.26	1.22	1.00	1.75
18:1(n-7)	4.12	29.85	27.99	7.39	6.05	5.02	6.12	4.11	6.43	7.67
18:2(n-6)	-	.37	-	-	-	-	-	-	-	-
18:3(n-3)	-	-	-	-	-	-	-	-	-	-
18:3(n-6)	.34	.42	.59	-	-	-	-	-	-	-
18:4(n-3)	-	-	-	-	-	-	-	-	-	-
19:0	-	-	-	-	-	-	-	-	-	-
20:0	1.46	1.32	1.35	.25	.36	1.04	.56	.30	.31	3.72
20:1(n-9)	-	-	-	-	-	-	-	-	-	-
20:2(n-6)	-	-	-	-	-	-	-	-	-	-
20:3(n-6)	-	-	-	-	-	-	-	-	-	-
20:4(n-6)	-	-	-	-	-	-	-	-	-	-
20:4(n-3)	-	-	-	-	-	-	-	-	-	-
20:5(n-3)	-	-	-	-	-	-	-	-	-	-
21:0	-	-	-	-	-	-	-	-	-	-
22:0(IS)	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS
22:1(n-9)	-	-	-	-	-	-	-	-	-	
22:5(n-3)	-	-	-	-	-	-	-	-	-	
22:6(n-3)	-	-	-	-	-	-	-	-	-	
24:0	-	-	-	-	-	-	-	-	-	
% Lipid	4.06	3.75	3.91	4.29	5.13	4.71	6.33	4.43	4.66	0.67
% SAFA	46.63	32.02	34.44	44.11	54.75	57.28	57.27	55.95	55.69	58.08
%UNFA	53.36	67.98	65.55	55.88	45.24	42.70	42.80	44.07	44.31	41.91
UNFA/SAFA	1.41	2.12	1.90	1.27	0.83	0.75	0.75	0.79	0.80	0.72
% Protein	9.42	6.51	7.32	15.59	8.24	13.47	12.43	13.21	15.07	14.81
%Carbohydrate	17.33	6.34	6.95	18.73	37.80	20.30	33.67	17.41	26.88	8.68

Note: (i) Synechococcus sp. 1479/5 did not grow at 40°C

(ii) IS = Internal Standard; S = Stationary Phase; E = Exponential Phase; SAFA = Saturated Fatty Acids; UNFA = Unsaturated Fatty Acids

(iii) For systematic names of fatty acids see Appendix 1

FIG134 %Carbohydrate, Protein and Lipid
Synechococcus sp. 1479/5 Exponential Phase

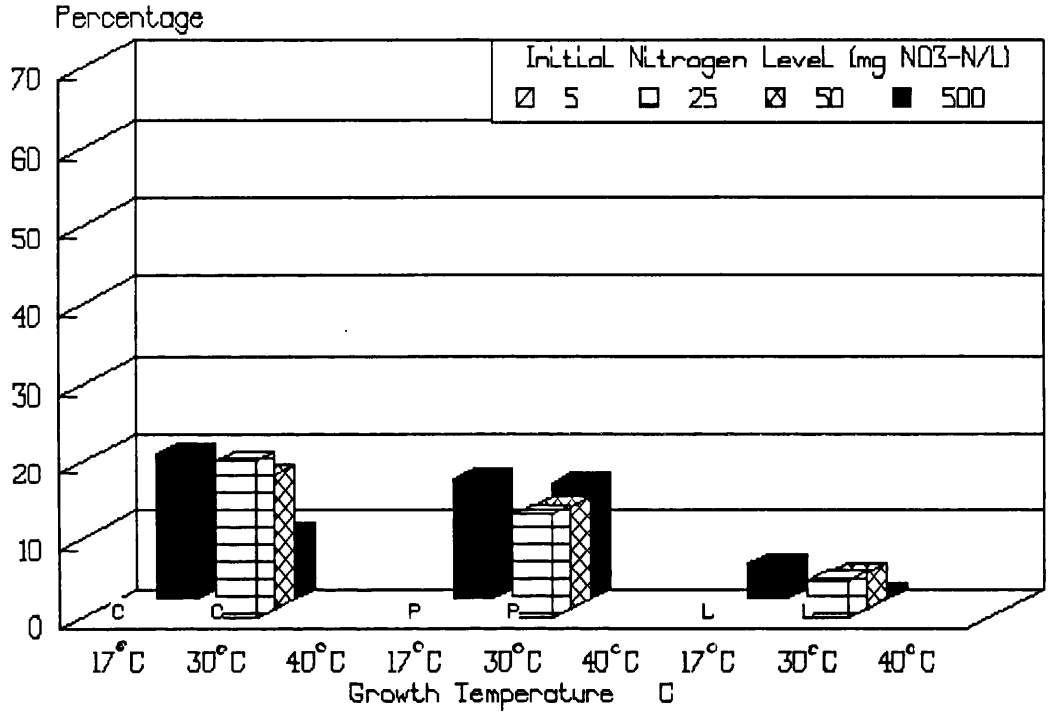


FIG135 %Carbohydrate, Protein and Lipid
Synechococcus sp. 1479/5 Stationary Phase

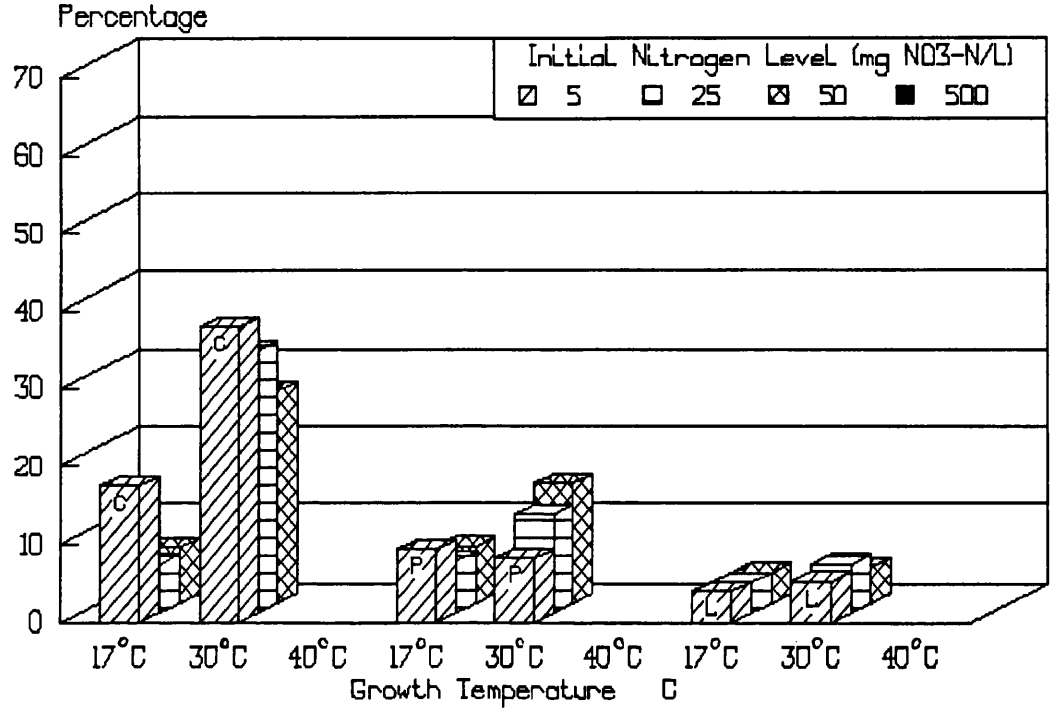


Table34 : Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) for Synechococcus sp. PCC 7943

	17°C						30°C						40°C					
Fatty Acid	5S	25E	25S	50E	50S	500E	5S	25E	25S	50E	50S	500E	5S	25E	25S	50E	50S	500E
12:0	.13	.47	.65	.20	.23	TR	.23	.25	-	.57	TR	TR	.39	-	-	-	-	.14
14:0	2.25	3.21	5.76	3.26	2.13	1.68	1.31	2.22	1.09	2.33	.89	1.15	2.38	1.71	.62	1.51	1.00	1.35
14:1(n-5)	2.90	5.50	4.36	7.79	2.05	4.20	1.50	2.30	.93	2.40	1.00	1.90	-	1.08	.20	.83	.38	.84
15:0	.86	1.49	2.39	.36	.48	.44	.30	.51	.42	.90	.40	0.40	.79	.51	.31	.33	.41	.51
16:0	39.47	40.69	47.32	45.49	47.08	42.09	43.80	54.91	40.33	49.66	41.80	41.67	49.96	52.47	52.98	46.12	51.46	44.51
16:1(n-7)	46.71	41.03	35.18	40.92	41.13	45.90	36.91	33.48	40.62	37.31	39.44	43.79	22.80	34.98	21.68	34.66	24.51	31.43
16:2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16:3(n-6)	.50	.35	.33	.31	.44	.28	.40	.46	.53	.35	.79	1.06	.28	.45	.61	.53	1.24	.90
16:4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17:0	.39	.21	.79	.11	.23	.34	.20	.42	.32	.42	.32	.43	1.13	-	.31	-	.42	.43
18:0	.97	.50	1.48	.33	1.31	.66	2.33	1.76	2.49	1.45	1.80	.87	5.38	1.27	3.46	.51	2.04	1.30
18:1(n-9)	.56	.75	.31	.19	1.03	.80	5.14	1.28	3.07	1.36	4.08	2.49	5.79	3.37	9.30	3.34	6.07	3.65
18:1(n-7)	4.97	4.03	1.00	1.03	3.90	3.04	7.71	2.12	10.19	3.09	9.25	5.79	7.37	3.75	9.64	8.99	12.17	14.50
18:2(n-6)	.12	.42	.10	-	-	-	-	-	-	-	-	-	1.02	-	.21	-	-	-
18:3(n-3)	.18	.62	-	-	-	-	.10	-	-	-	-	-	1.72	.30	-	.56	-	-
18:3(n-6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.17	.36	.14	.32
18:4(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:0	-	.27	-	-	-	-	-	.28	-	.15	.13	.39	1.00	.10	.31	2.25	.16	.10
20:1(n-9)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:2(n-6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:3(n-6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.19	-	-	-
20:4(n-6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:4(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:5(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:0(IS)	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS
22:1(n-9)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:5(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:6(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
% Lipid	4.47	3.65	4.74	5.47	3.25	4.67	6.43	4.96	4.46	5.31	4.34	4.03	5.37	5.84	4.48	8.16	6.44	2.24
% SAFA	44.07	46.84	58.39	49.75	51.46	45.21	48.17	60.35	44.65	55.48	45.34	44.91	61.03	56.06	57.99	50.72	55.49	48.34
%UNFA	55.94	52.70	41.28	50.24	48.55	54.22	51.76	39.64	55.34	44.51	54.56	55.03	38.98	43.93	42.00	49.27	44.51	51.64
UNFA/SAFA	1.27	1.13	0.71	1.01	0.94	1.20	1.07	0.66	1.24	0.80	1.20	1.23	0.64	0.78	0.72	0.97	0.80	1.07
% Protein	11.32	6.14	9.05	14.63	14.08	19.28	10.81	20.40	9.69	15.81	15.36	17.70	9.12	8.71	9.79	14.04	20.48	18.23
%Carbohydrate	47.24	18.19	31.89	30.38	44.86	28.14	39.50	22.42	45.92	15.88	36.65	13.80	43.98	13.20	44.19	11.44	19.20	8.04

Note: (i) IS = Internal Standard; S = Stationary phase; E = Exponential phase; SAFA = Saturated fatty acids; UNFA = Unsaturated fatty acids
(ii) For systematic names of fatty acids see Appendix 1

FIG136 %Carbohydrate, Protein and Lipid
Synechococcus sp. PCC7943 Exponential Phase

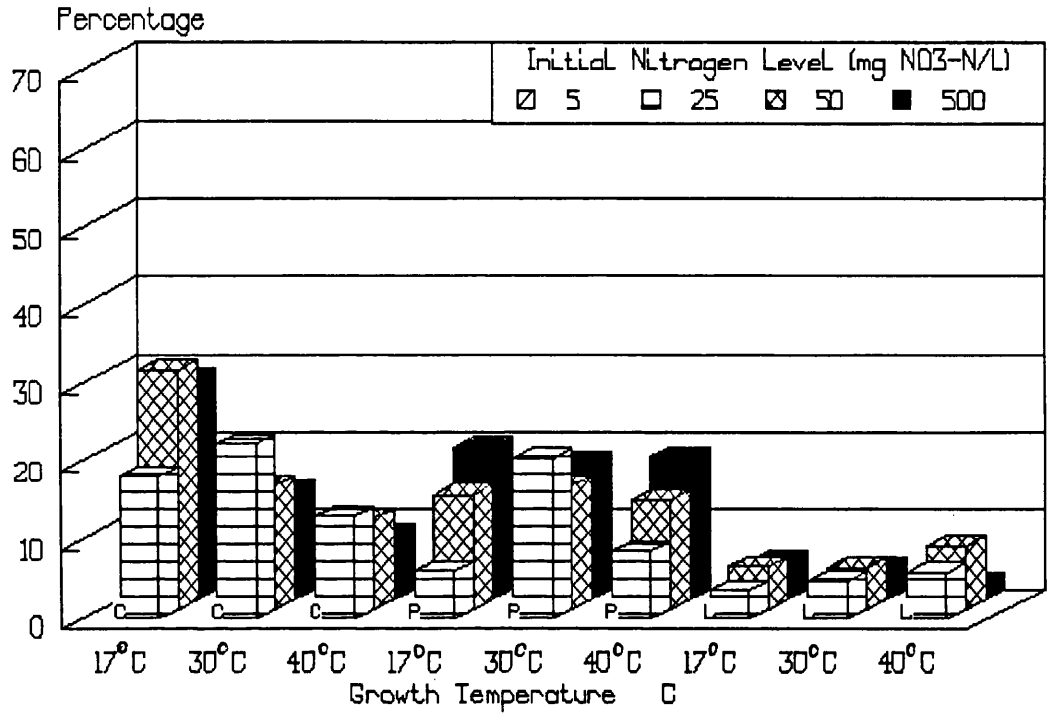


FIG137 %Carbohydrate, Protein and Lipid
Synechococcus sp. PCC7943 Stationary Phase

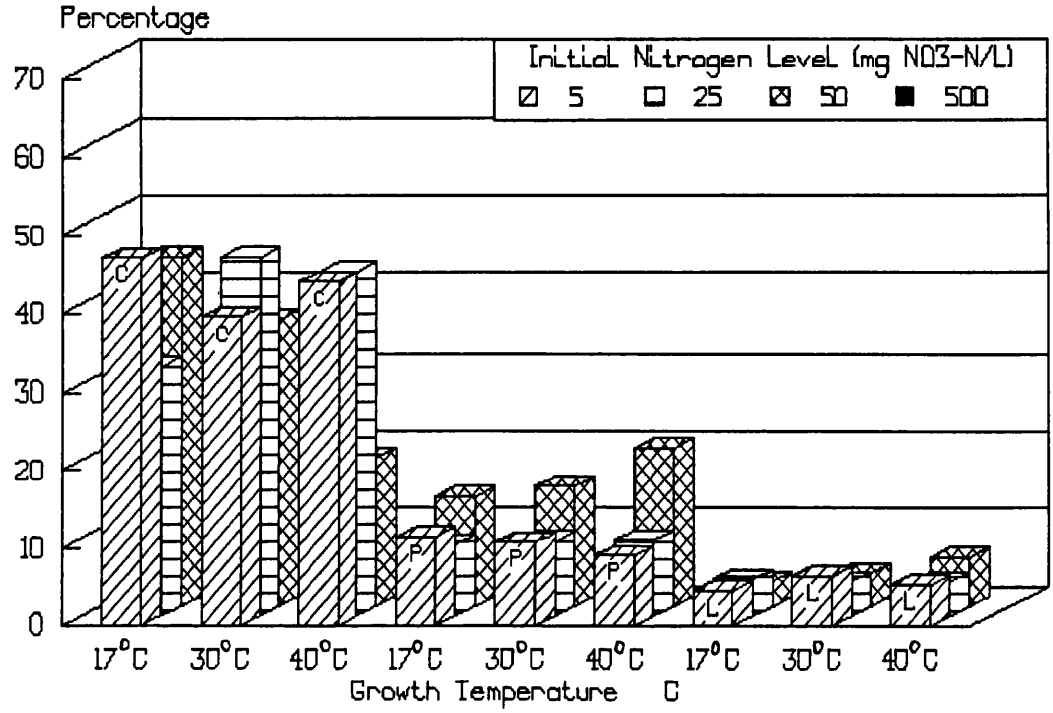


Table35: Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) for A. flos-aquae 1403/13A

	17°C						30°C						40°C					
Fatty Acid	5A	25E	25S/LE	50E	50A	500E	5A	25E	25A	50E	50A	500E	5A	25E	25A	50E	50S/LE	500E
12:0	-	-	-	-	-	-	.11	-	TR	.31	.18	TR	-	-	-	-	-	.36
14:0	.29	.60	.31	-	1.13	.33	.64	.54	.52	4.73	.90	.54	.84	.91	.94	1.20	.95	.85
14:1(n-5)	-	-	-	-	-	-	.10	-	.11	-	.43	TR	.19	.14	.19	-	.22	-
15:0	.67	.60	.56	1.79	.75	.47	.31	.88	.29	.70	.40	.30	.22	.34	.21	.36	.26	2.07
16:0	24.80	28.68	26.95	30.55	25.65	24.97	30.74	33.46	28.35	30.43	26.56	27.88	36.00	35.66	34.63	38.95	33.03	27.17
16:1(n-7)	19.84	19.29	20.25	19.91	17.39	17.17	19.67	19.25	20.15	17.42	16.85	17.96	18.90	18.11	18.78	17.43	16.86	12.24
16:2	-	-	-	-	.15	.25	TR	-	-	-	-	.15	.41	.83	.44	.44	.23	-
16:3(n-6)	.98	-	.62	-	.44	.38	1.43	1.51	1.26	1.61	1.21	.81	.57	.55	.59	1.08	.87	.86
16:4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17:0	.72	-	.60	-	.50	.34	.51	.91	.49	.90	.60	.43	.32	.47	.37	.79	.49	1.94
18:0	1.96	1.63	1.70	1.16	2.46	2.49	1.65	1.93	1.96	2.02	2.02	2.03	2.15	2.10	2.23	1.13	3.28	5.16
18:1(n-9)	5.58	2.20	1.67	2.63	4.37	8.41	10.73	7.18	10.60	8.72	9.83	7.84	15.62	8.22	13.41	12.16	16.96	22.66
18:1(n-7)	3.89	3.61	5.65	3.30	4.30	5.15	3.07	2.79	6.05	2.86	9.71	8.53	3.39	3.46	4.56	5.64	7.48	4.68
18:2(n-6)	11.63	5.50	5.89	5.90	11.30	16.01	16.26	14.91	18.34	16.06	18.61	19.42	18.42	23.04	20.56	15.63	18.10	15.27
18:3(n-3)	28.89	37.89	35.81	34.76	30.88	24.01	14.56	16.63	11.61	13.69	12.29	13.09	2.77	4.86	2.85	1.99	1.05	1.04
18:3(n-6)	-	-	-	-	-	-	TR	-	TR	-	.10	.14	.20	.43	.24	.81	.22	.81
18:4(n-3)-	-	-	-	-	-	-	-	-	-	-	TR	-	-	-	-	-	-	-
19:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:0	.49	-	-	-	.44	.11	.16	-	.10	.55	.15	.27	-	.49	-	1.51	-	2.95
20:1(n-9)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:2(n-6)	-	-	-	-	-	-	-	-	-	-	.16	.25	-	-	-	-	-	
20:3(n-6)	-	-	-	-	-	-	-	-	-	-	-	.20	-	.39	-	.96	-	1.93
20:4(n-6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:4(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:5(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:0(IS)	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS
22:1(n-9)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:5(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:6(n-3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
% Lipid	2.53	3.42	3.43	3.01	2.75	2.67	2.56	6.85	3.07	5.06	3.21	5.10	6.01	3.77	4.60	5.48	2.42	0.26
% SAFA	28.93	31.51	30.12	33.50	30.93	28.71	34.16	37.72	31.71	39.64	30.81	31.45	39.53	39.97	38.38	43.94	38.01	40.50
%UNFA	70.81	68.48	69.89	66.50	68.83	71.38	65.82	62.27	68.12	60.36	69.19	68.39	60.47	60.03	61.62	56.14	61.99	59.49
UNFA/SAFA	2.45	2.17	2.32	1.99	2.23	2.49	1.93	1.65	2.15	1.52	2.25	2.17	1.53	1.50	1.61	1.28	1.63	1.47
% Protein	12.07	6.54	5.08	3.15	7.04	10.12	10.28	4.56	8.30	3.25	12.44	9.73	15.14	7.30	8.62	10.66	10.98	11.33
%Carbohydrate	27.50	25.23	35.07	31.55	34.20	20.89	13.14	13.09	19.08	12.06	21.48	12.42	19.81	6.97	17.51	9.64	19.22	13.36

Note: (i) IS = Internal Standard; S = Stationary Phase; E = Exponential Phase; A = After Nitrogen Depletion; S/LE = Stationary/Late Exponential; SAFA = Saturated Fatty Acids; UNFA = Unsaturated fatty acids; TR = Trace < 0.1%;

(ii) For systematic names of fatty acids see Appendix 1

FIG138 %Carbohydrate, Protein and Lipid
A.flos-aquae 1403/13A Exponential Phase

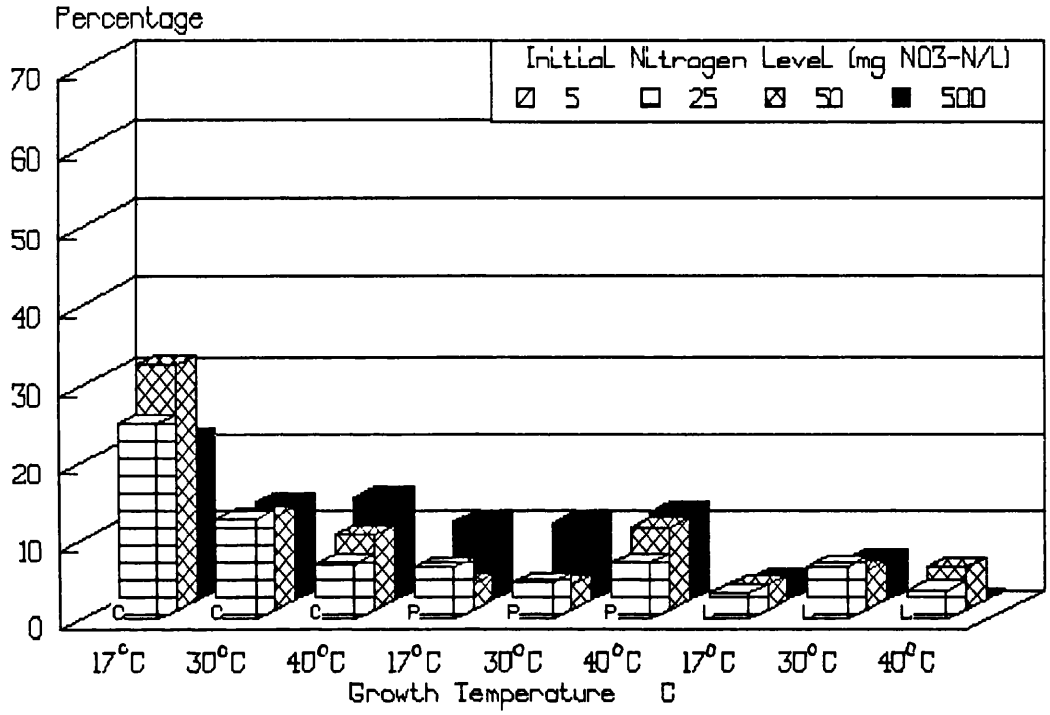


FIG139 %Carbohydrate, Protein and Lipid
A.flos-aquae 1403/13A Stationary Phase

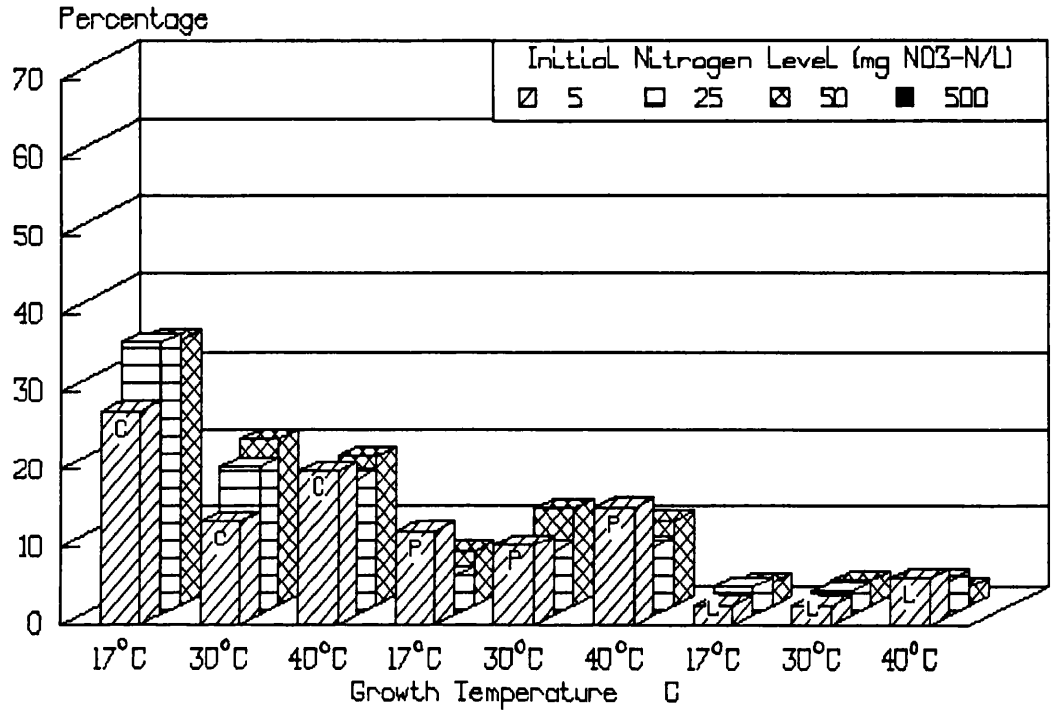


Table36: Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) for A. variabilis 1403/12

	17°C						30°C					
Fatty Acid	5A	25E	25A	50E	50S/LE	500E	5A	25E	25A	50E	50S/LE	500E
12:0	.38	.16	.24	.28	.46	.31	.21	.20	.22	.23	.34	.44
14:0	.64	.45	.41	.49	.82	.43	.51	.58	.58	.84	.48	.49
14:1(n-5)	-	-	-	-	-	-	-	-	-	-	-	-
15:0	1.37	.27	.35	.76	1.02	.63	.38	.52	.52	.73	.49	.60
16:0	26.31	30.31	29.01	31.70	30.30	37.67	30.10	37.42	32.33	41.31	32.69	34.05
16:1(n-7)	4.16	3.68	3.99	3.81	4.01	4.36	4.01	3.17	3.66	4.22	3.28	3.62
16:2	5.53	2.36	3.85	2.88	4.25	4.19	5.65	5.32	4.95	5.58	5.75	3.70
16:3(n-6)	5.31	10.02	7.24	10.96	5.52	3.65	1.92	3.02	1.91	2.66	2.11	1.43
16:4	-	-	-	-	-	-	-	-	-	-	-	-
17:0	.48	.43	.29	.34	.73	.66	.33	.41	.49	-	.76	.69
18:0	1.87	.86	1.88	.95	3.10	3.31	2.16	1.59	2.54	1.70	2.75	2.72
18:1(n-9)	3.90	.93	2.32	1.30	3.39	4.12	8.21	4.58	6.31	5.41	5.09	8.04
18:1(n-7)	1.65	1.25	3.47	.99	4.50	3.40	1.63	.79	2.57	.93	1.33	5.26
18:2(n-6)	19.77	5.88	12.82	6.07	14.77	16.83	28.60	22.36	27.36	19.22	29.50	26.33
18:3(n-3)	28.16	42.86	33.73	39.01	25.66	19.46	15.34	19.43	15.53	16.81	14.17	11.78
18:3(n-6)	.11	.23	-	-	.52	-	.15	-	.18	-	.12	-
18:4(n-3)	-	-	-	-	-	-	-	-	-	-	-	-
19:0	-	-	-	-	-	-	-	-	-	-	-	-
20:0	.37	.30	.41	.48	.89	.97	.13	.61	.24	.35	.69	.85
20:1(n-9)	-	-	-	-	-	-	-	-	-	-	-	-
20:2(n-6)	-	-	-	-	-	-	.46	-	.31	-	.46	-
20:3(n-6)	-	-	-	-	-	-	TR	-	.29	-	-	-
20:4(n-6)	-	-	-	-	-	-	.15	-	-	-	-	-
20:4(n-3)	-	-	-	-	-	-	-	-	-	-	-	-
20:5(n-3)	-	-	-	-	-	-	-	-	-	-	-	-
21:0	-	-	-	-	-	-	-	-	-	-	-	-
22:0(IS)	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS
22:1(n-9)	-	-	-	-	-	-	-	-	-	-	-	-
22:5(n-3)	-	-	-	-	-	-	-	-	-	-	-	-
22:6(n-3)	-	-	-	-	-	-	-	-	-	-	-	-
24:0	-	-	-	-	-	-	-	-	-	-	-	-
% Lipid	2.02	2.31	2.54	4.16	2.50	1.08	2.53	6.45	2.75	4.15	3.13	1.39
% SAFA	31.42	32.78	32.59	35.00	37.32	43.98	33.82	41.33	36.92	45.16	38.20	39.84
%UNFA	68.59	67.21	67.42	65.02	62.62	56.01	66.12	58.67	63.07	54.83	61.81	60.16
UNFA/SAFA	2.18	2.05	2.07	1.86	1.68	1.27	1.96	1.42	1.71	1.21	1.62	1.51
% Protein	13.16	7.78	15.11	8.80	17.65	17.27	14.98	13.38	16.50	9.60	13.44	18.50
%Carbohydrate	30.21	13.26	20.61	14.09	20.57	17.06	33.64	17.95	28.20	13.47	22.79	10.26

Note: (i) A. variabilis 1403/12 did not grow at 40°C

(ii) IS = Internal Standard; S = Stationary Phase; E = Exponential Phase; A = After Nitrogen Depletion; S/LE = Stationary/Late Exponential Phase; SAFA = Saturated Fatty Acids; UNFA = Unsaturated Fatty Acids

(iii) For systematic names of fatty acids see Appendix 1

FIG140 %Carbohydrate, Protein and Lipid
A.variabilis 1403/12 Exponential Phase

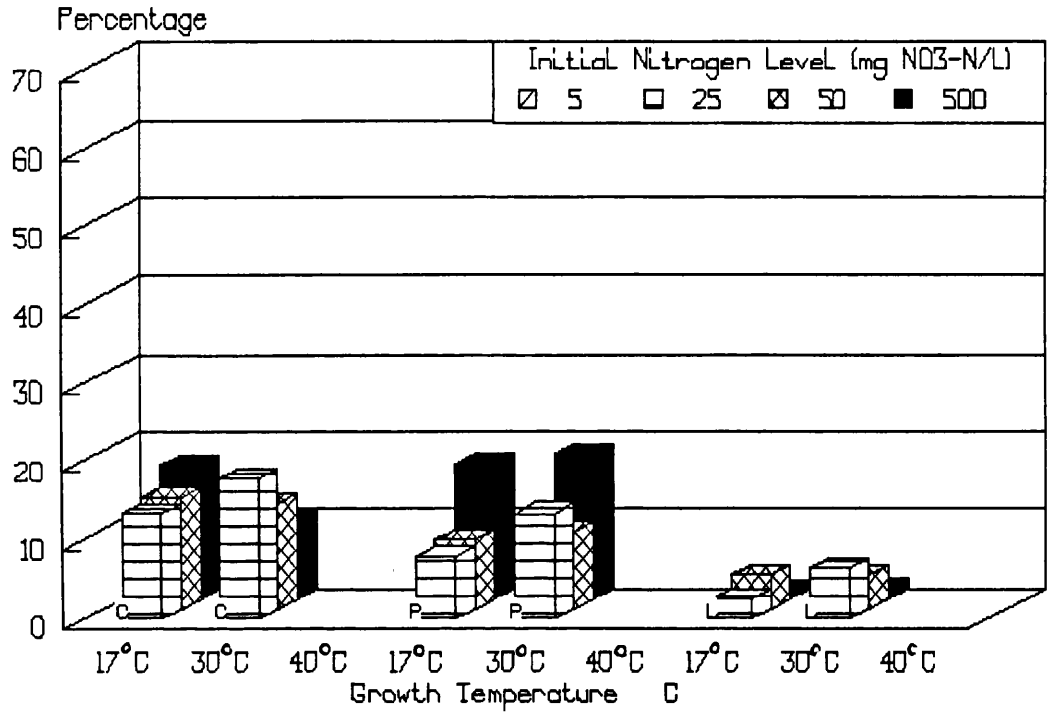


FIG141 %Carbohydrate, Protein and Lipid
A.variabilis 1403/12 Stationary Phase

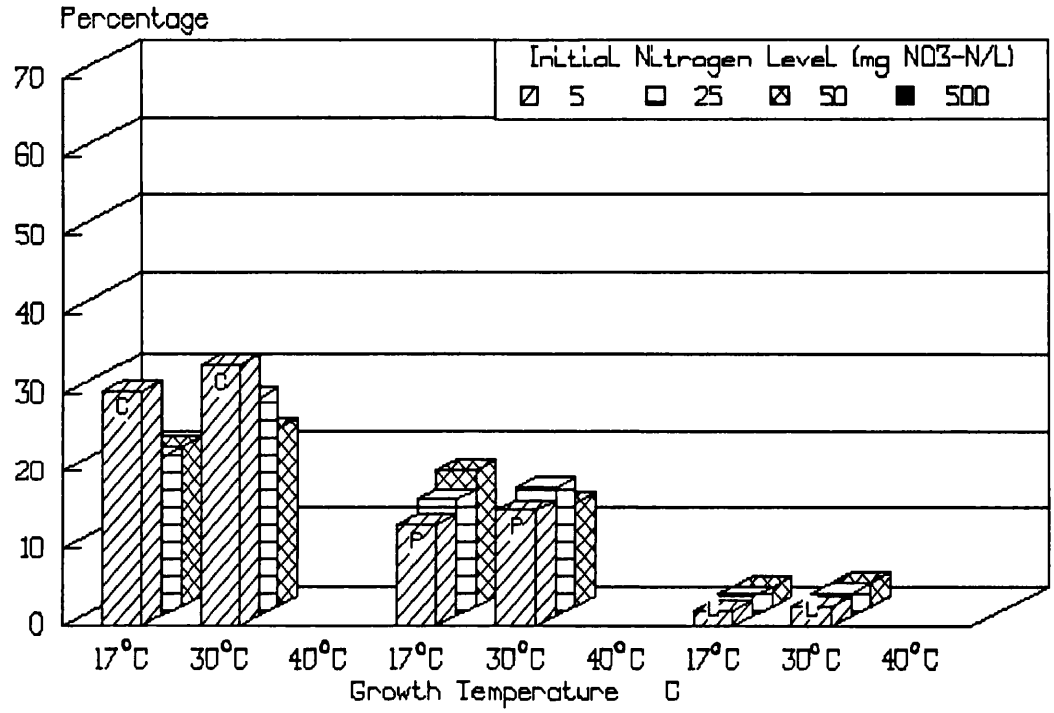


Table 37: Photosynthetic and Dark Respiration Rates for the Four Cyanobacterial Species

Organism	Time (Days)	OD_{560}	Dry Wt (gl^{-1})	Nitrogen Present (+ or -)	Dark Respiration Rate $mgO_2gDM^{-1}h^{-1}$	Gross Photosynthesis Rate $mgO_2g\ DM^{-1}h^{-1}$
17°C						
A. flos aquae 1403/13A	12	0.28	0.12	+	14.42	36.50
	18	0.64	0.36	-	6.25	12.50
A. variabilis 1403/12	12	0.33	0.18	+	8.33	38.40
	26	0.74	0.90	TR	1.43	4.30
Synechococcus sp 1479/5	6	0.16	0.01	+	53.00	118.00
Synechococcus sp PCC 7943	21	0.30	0.1	+	13.10	26.20
	22	0.35	0.12	+	12.80	26.00
30°C						
A. flos aquae 1403/13A	7	0.62	0.33	+	3.70	25.58
	21	1.80	1.10	-	1.80	7.36
A. variabilis 1403/12	7	0.49	0.30	+	4.40	14.80
	21	0.70	0.98	-	2.76	4.50
Synechococcus sp 1479/5	7	0.56	0.12	+	8.17	75.17
	17	1.20	0.20	-	19.15	102.80
Synechococcus sp PCC 7943	7	0.94	0.22	+	13.41	55.14
	14	1.41	0.33	-	9.15	29.75
40°C						
A. flos aquae 1403/13A	7	0.50	0.23	+	2.65	44.78
	15	0.86	0.50	-	3.16	11.08
Synechococcus sp PCC 7943	7	0.48	0.17	+	9.00	33.00
	19	1.20	0.48	-	3.19	11.69

TR = Trace

4.3.4 C. caldarium

4.3.4.1 Growth and Ammonium Results

C. caldarium only grew at 30°C and 40°C. Results for OD_{560} against time (Figs 142 and 143) and dry weight against time (Figs 144 and 145) show C. caldarium in exponential phase at the time of harvest at both temperatures (32 days, Table 28). This was the result of an error in making up the media which contained approximately twice the stated experimental amount of ammonium sulphate. This should be noted when looking at the figures and tables of results for C. caldarium. Results of ammonium uptake against time (Figs 146 and 147) show that NH_4-N depletion did not occur at either temperature. Results of pH against time, demonstrated the ability of C. caldarium to grow at acidic pH (pH 2.0-2.5)(Figs 148 and 149).

FIG 142 C.caldarium 1355/4 30°C
OD 560 vs Time

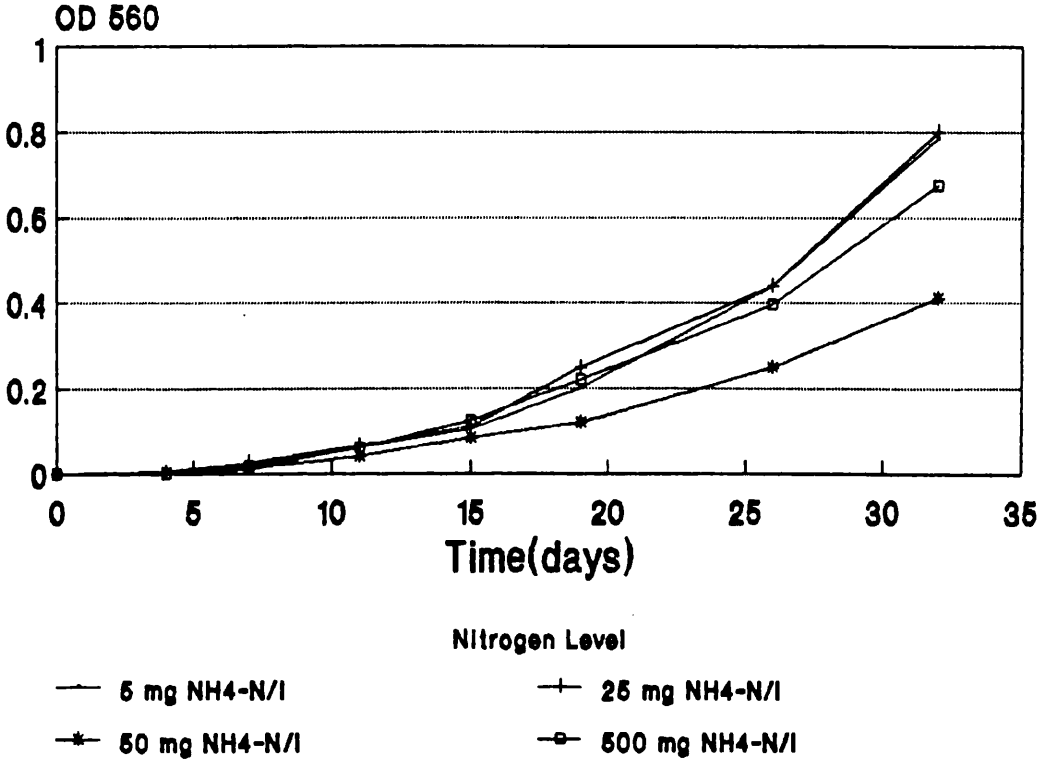


FIG 143 C.caldarium 1355/4 40°C
OD 560 vs Time

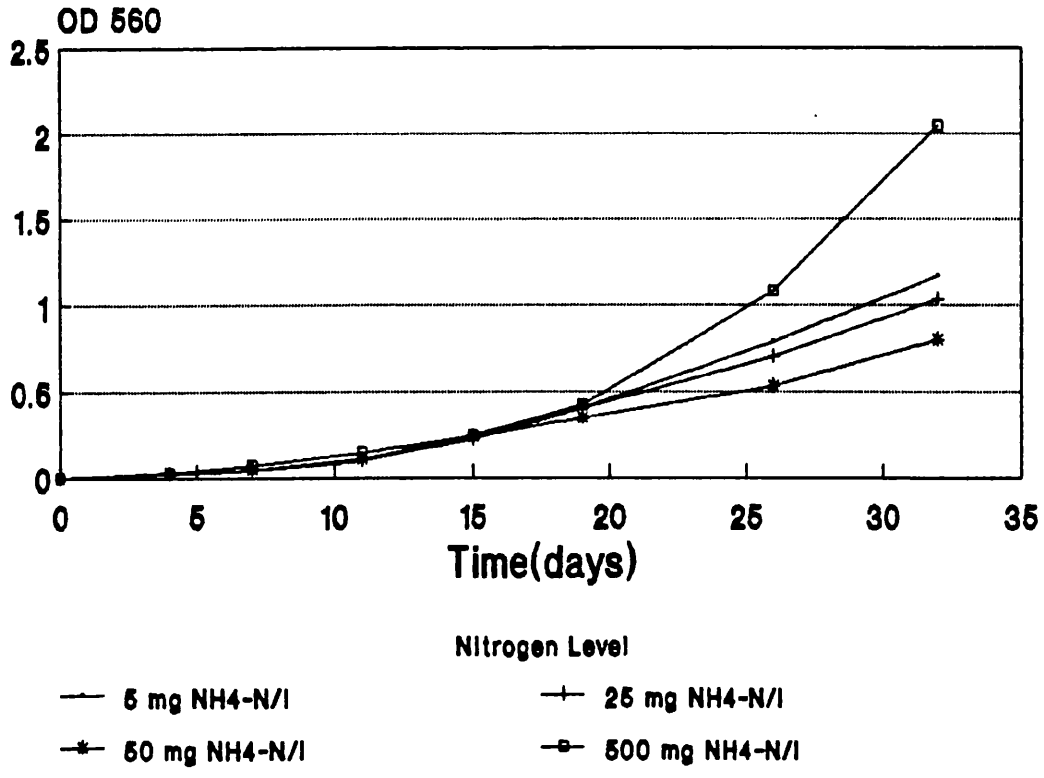


FIG 144 C.caldarium 1355/4 30°C
DRY WEIGHT vs Time

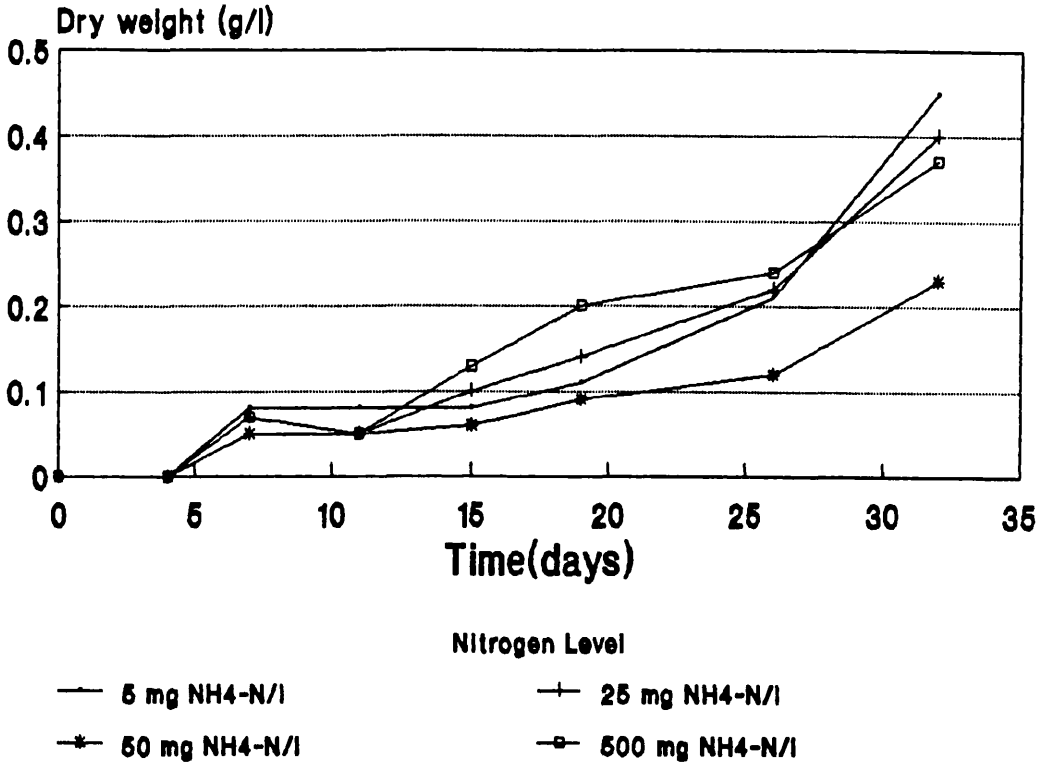


FIG 145 C.caldarium 1355/4 40°C
DRY WEIGHT vs Time

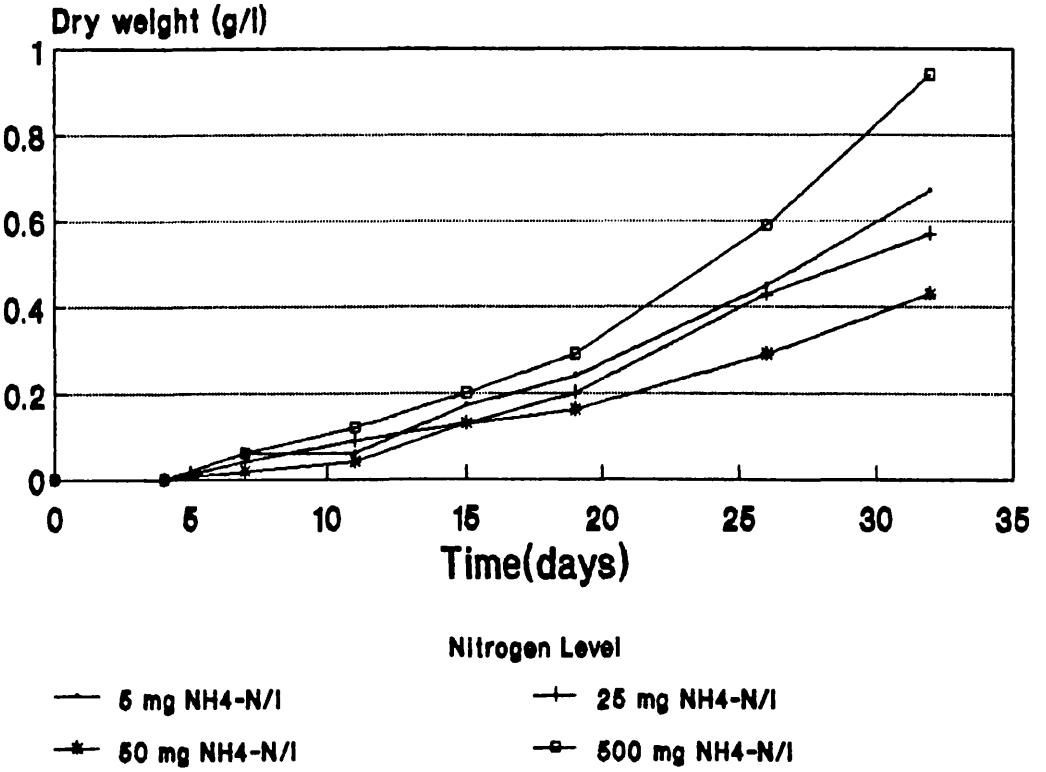
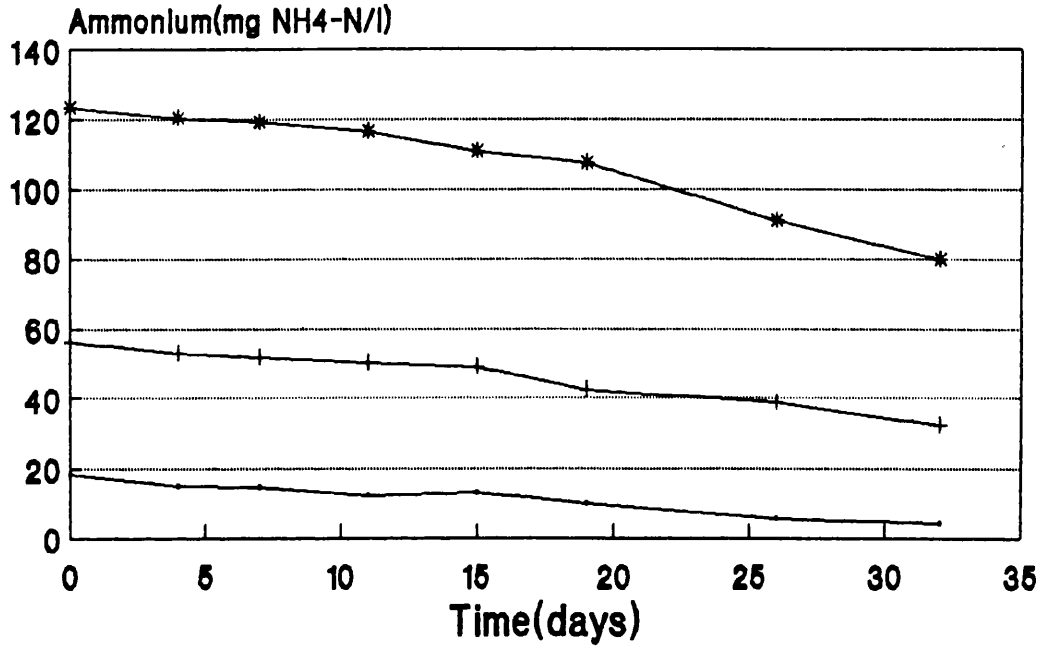


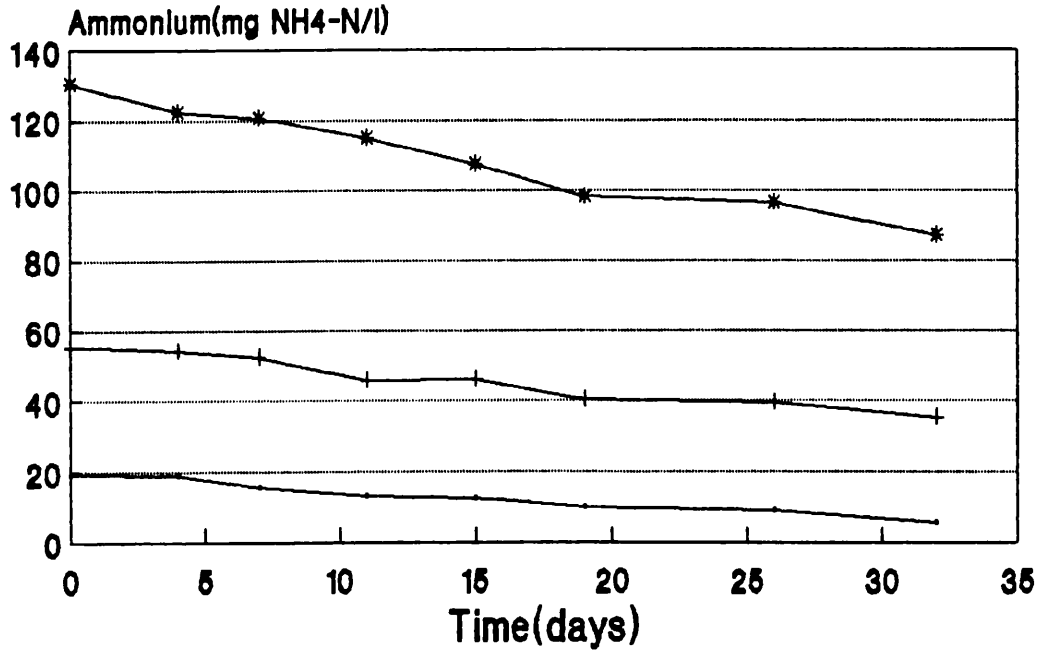
FIG 146 C.caldarium 1355/4 30°C
AMMONIUM vs Time



Nitrogen Level

5 mg NH4-N/l 25 mg NH4-N/l 50 mg NH4-N/l

FIG 147 C.caldarium 1355/4 40°C
AMMONIUM vs Time



Nitrogen Level

5 mg NH4-N/l 25 mg NH4-N/l 50 mg NH4-N/l

FIG 148 C.caldarium 1355/4 30°C
pH vs Time

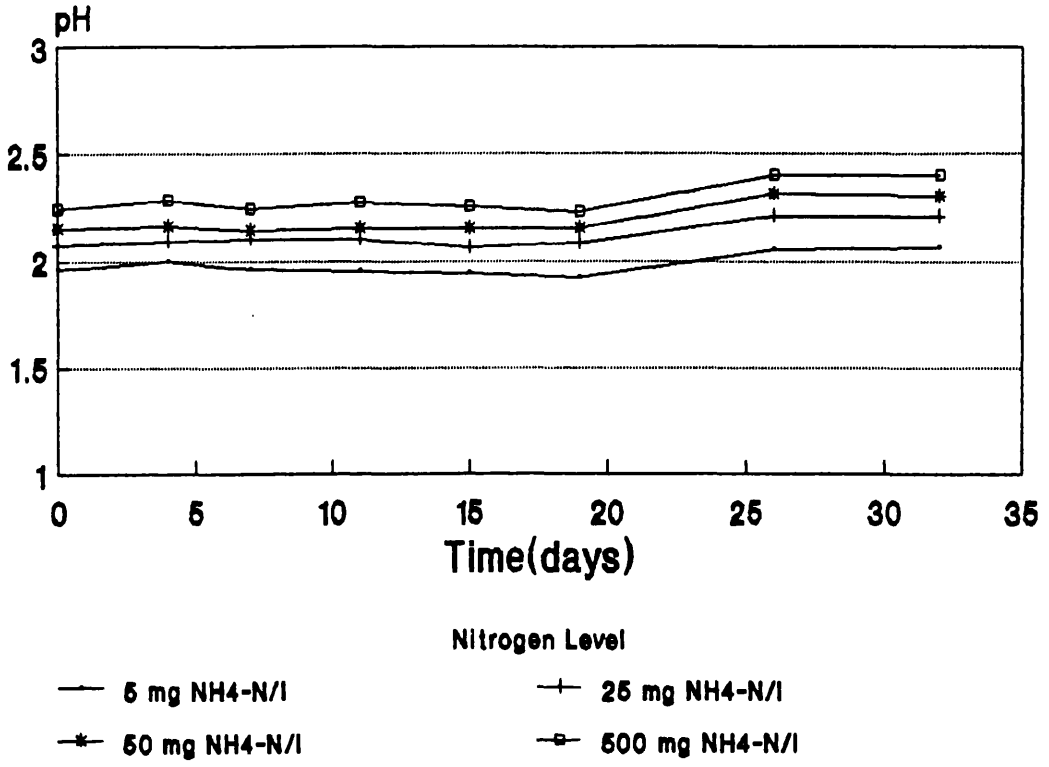


FIG 149 C.caldarium 1355/4 40°C
pH vs Time

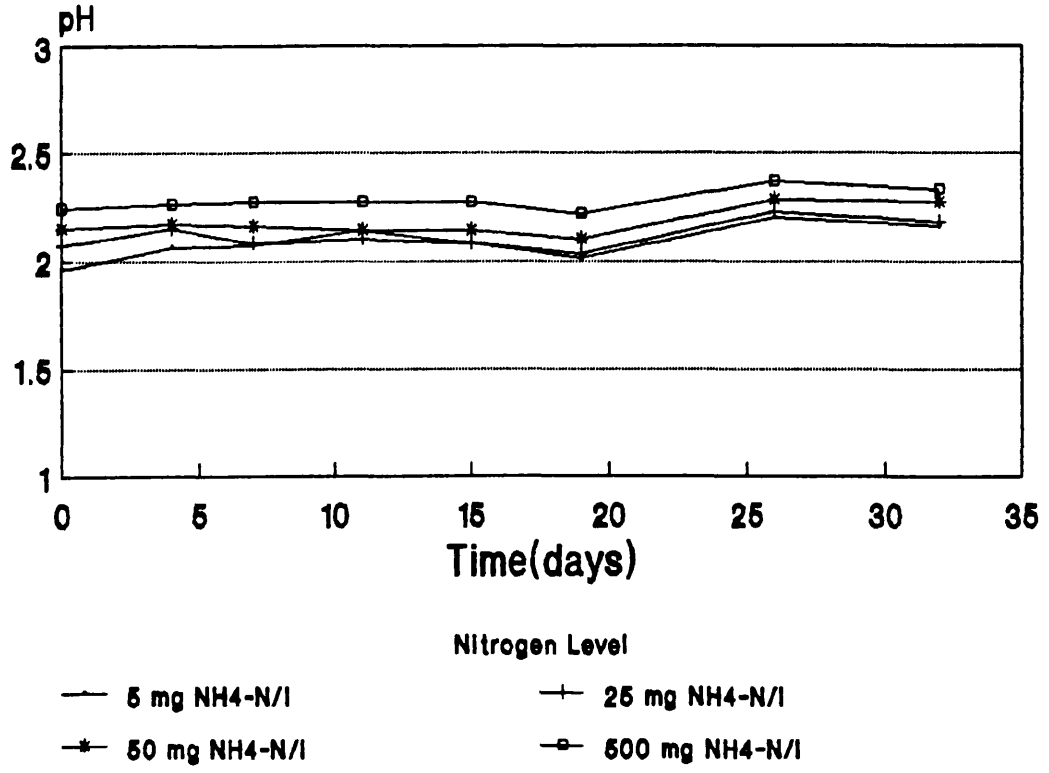


Table 38: Harvest Parameters for C. caldarium 1355/4

Temperature/ Initial N Level		Phase	Time (Days)	$0D_{560}$	Dry wt (gl^{-1})	NH_4-N (mgl^{-1})
30°C	5	E	32	0.79	0.45	4.33
	25	E	32	0.80	0.40	32.50
	50	E	32	0.41	0.23	79.88
	500	E	32	0.68	0.37	988
40°C	5	E	32	1.17	0.67	5.66
	25	E	32	1.03	0.57	35.44
	50	E	32	0.80	0.43	87.23
	500	E	32	2.04	0.94	1008

E = Exponential Phase
S = Stationary Phase

4.3.4.2 Carbohydrate, Protein and Lipid Results

The results are given in Table 39. The lipid and protein contents of C. caldarium in exponential phase at all nitrogen levels were not significantly different with increase in temperature. A similar result was observed for carbohydrate with the exception of the lowest ammonium-N level, where carbohydrate content was significantly higher (Fig 150).

4.3.4.3 Statistical Analysis of the Carbohydrate, Protein and Lipid Results

Statistical analysis of the carbohydrate results gave a significant result at 0.1% ($p < 0.001$) for the effects of nitrogen. Nitrogen means ('5' (mg NO_3-N l^{-1}) - 28.35%, '25' - 10.02%, '50' - 7.70%, '500' - 6.80%) divide into three significantly different groups consisting of '5', '25' and ('50' & '500') with decreasing carbohydrate values. This may suggest that nitrogen stress results in carbohydrate accumulation for C. caldarium. Statistical analysis of the protein and lipid results gave no significant effects.

4.3.4.3 Fatty Acid Results

The results are given in Table 39. C. caldarium had predominantly C18 fatty acids, with minor levels of C16 and C20 fatty acids, with the exception of 16:0. Quantitative differences in fatty acids were found between temperatures. Increasing temperature increased 16:0, 18:1(n-9), 18:2(n-6) and decreased 18:3(n-3). Mean %UNFA (30°C - 64.91%, 40°C - 59.24%) appeared to show a significant decrease at the highest temperature. Statistical analysis confirmed this result (1% significance or $p < 0.01$).

4.3.4.4 Gross Photosynthetic and Dark Respiration Rates

The results for C. caldarium are given in Table 40. These cultures were grown at a level of 25mg NH_4-N l^{-1}. A decrease in gross photosynthetic and dark respiration rates with NH_4-N depletion suggested the cultures had entered

stationary phase at 32 days. However, the results at 16 days would be comparable to the results of the nitrogen limitation experiments due to the error in media composition. Gross photosynthetic rate decreased from 30°C to 40°C but dark respiration rate increased.

Table 39: Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) for C. caldarium 1355/4

	30°C				40°C			
Fatty Acid	5E	25E	50E	500E	5E	25E	50E	500E
12:0	-	-	-	-	-	-	-	-
14:0	.38	.43	.44	.49	.37	.66	.61	.53
14:1(n-5)	-	-	-	-	-	-	-	-
15:0	.66	.71	.70	.76	.66	.78	.75	.71
16:0	28.90	30.67	30.58	31.38	32.67	39.54	38.78	35.76
16:1(n-7)	.50	.51	.55	.59	.83	1.17	1.15	1.27
16:2	-	-	-	-	-	.15	-	-
16:3(n-6)	.45	.52	.48	.48	.56	.57	.59	.54
16:4	-	-	-	-	-	-	-	-
17:0	.51	.47	.51	.43	.48	.38	.39	.34
18:0	4.07	2.51	2.81	2.56	3.16	1.57	2.05	1.82
18:1(n-9)	20.30	15.43	15.73	16.75	27.53	20.97	20.69	22.40
18:1(n-7)	.43	.43	.44	.52	.58	.57	.63	.81
18:2(n-6)	9.76	11.48	10.17	11.04	20.43	20.27	20.86	22.93
18:3(n-3)	32.91	35.63	36.74	33.78	11.58	12.57	12.75	11.65
18:3(n-6)	.16	.10	.15	.14	.15	.12	.11	.15
18:4(n-3)	-	-	-	-	-	-	-	-
19:0	-	-	-	-	-	-	-	-
20:0	.12	TR	TR	TR	.23	.24	.39	.17
20:1(n-9)	.48	.36	.38	.44	.28	.14	-	.23
20:2(n-6)	.26	.25	.25	.29	.35	.30	.26	.34
20:3(n-6)	.10	.11	-	.25	.13	-	-	.16
20:4(n-6)	-	-	-	-	-	-	-	.20
20:4(n-3)	-	.30	-	-	-	-	-	-
20:5(n-3)	-	-	-	-	-	-	-	-
21:0	-	-	-	-	-	-	-	-
22:0(IS)	IS	IS	IS	IS	IS	IS	IS	IS
22:1(n-9)	-	-	-	-	-	-	-	-
22:5(n-3)	-	-	-	-	-	-	-	-
22:6(n-3)	-	-	-	-	-	-	-	-
24:0	-	-	-	-	-	-	-	-
% Lipid	4.63	5.17	5.61	5.68	4.33	6.31	6.31	4.57
% SAFA	34.64	34.79	35.04	35.62	37.57	43.17	42.97	39.33
%UNFA	65.35	65.12	64.89	64.28	62.42	56.83	57.04	60.68
UNFA/SAFA	1.89	1.87	1.85	1.80	1.66	1.32	1.33	1.54
% Protein	4.93	5.44	5.75	8.61	4.67	5.36	5.89	6.52
%Carbohydrate	28.21	9.59	6.94	5.95	28.50	10.46	8.45	7.64

Note: (i) C. caldarium 1355/4 did not grow at 17°C

(ii) IS = Internal Standard; S - Stationary Phase; E = Exponential Phase; SAFA = Saturated Fatty Acids; UNFA = Unsaturated Fatty Acids; TR = ,0.1% Trace

(iii) For systematic names of fatty acids see Appendix 1

FIG150 %Carbohydrate, Protein and Lipid
C.caldarium 1355/4 Exponential Phase

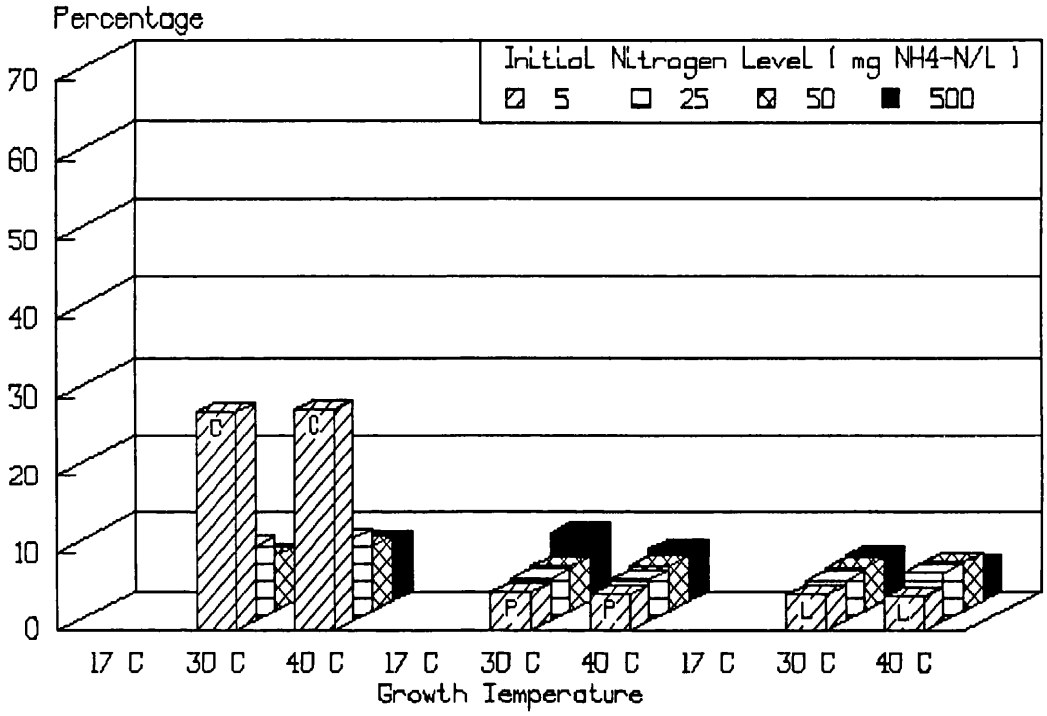


Table 40: Photosynthetic and Dark Respiration Rates for C. caldarium 1355/4

Organism	Time (Days)	OD_{560}	Dry Wt (gl^{-1})	Nitrogen Present (+ or -)	Dark Respiration Rate $mgO_2gDM^{-1}h^{-1}$	Gross Photosynthesis Rate $mgO_2g\ DM^{-1}h^{-1}$
30°C						
C. caldarium	16	0.39	0.16	+	3.31	63.69
	32	1.54	0.82	-	3.23	5.96
40°C						
C. caldarium	16	0.54	0.22	+	16.82	58.82
	32	2.10	0.76	-	3.55	15.39

4.3.5 Algae and Cyanobacteria chosen for Outdoor Minipond Experiments

The following cultures were chosen for comparative work in the outdoor minipond system:

(a) C. vulgaris 211/8K and S. obliquus 276/3A.
These two species were chosen because of their differing behaviour with respect to major shifts in biochemical composition, C. vulgaris 211/8K accumulating carbohydrate and S. obliquus 276/3A accumulating lipid. Also, differing fatty acid profiles in respect of the presence of 16:4 and 18:4 polyunsaturated fatty acids in S. obliquus 276/3A, which were not found in C. vulgaris 211/8K.

(b) N. atomus 251/4B and Isochrysis sp. 927/14.
These two species were able to grow at 17°C and 30°C and it was considered that the temperature variation outdoors would not exceed their growth range. The other two species investigated would not grow at 30°C. In addition, lipid accumulation at the lower temperature was found to be statistically significant for these two species. N. atomus, a green alga, had a higher carbohydrate content than the other three species, a similar property to the freshwater green algae. N. atomus and Isochrysis sp. also contained both 20:5 and 22:6 fatty acids.

(c) A. flos-aquae 1403/13A and Synechococcus sp. PCC 7943.
Both species grew at 17°C, 30°C and 40°C, an advantage with variable outdoor temperatures, also, Synechococcus sp. 1479/5 did not grow well at 17°C (section 4.3.3.1). Obviously, one species was a nitrogen fixer (A. flos-aquae). Levels of carbohydrate accumulation were greater with Synechococcus sp. PCC 7943 and A. flos-aquae. Lipid levels although appearing very similar between all cyanobacteria, were statistically shown to be significantly different between the two Synechococcus species and the two Anabaenas. In addition, A. flos-aquae along with A. variabilis exhibited a greater range of fatty acids specifically more polyunsaturated fatty acids than the Synechococcus species.

For all algae and cyanobacteria investigated, cellular constituents were found to vary depending on the level of available nitrogen and therefore, for

comparison of growth in defined medium (ASM or F/2) to growth in algal treated slurry, an approximate level of 25mg NO_3-N l^{-1} was made available in addition to any low levels of nitrate and ammonium available in the slurry supernatant.

5. OUTDOOR MINIPOND EXPERIMENTS

5.1 INTRODUCTION

Following the results obtained in the nitrogen limitation experiments (section 4.3), an investigation was carried out into whether these results could be emulated in an outdoor slurry based system.

Experiments were carried out using six strains of algae and cyanobacteria - C. vulgaris 211/8K, S. obliquus 276/3A, N. atomus 251/4B, Isochrysis sp. 927/14, A.flos-aquae 1403/13A and Synechococcus PCC 7943 - comparing growth in defined culturing media against a slurry based media (nitrate level 25mg NO_3-N l^{-1}), at different times of the year thus allowing for different ambient temperature and light conditions in the outdoor minipond systems (2.2.2).

5.2 EXPERIMENTAL DESIGN

The trays and perspex lids were thoroughly cleaned using disinfectant (Tepo) and dried. The pumps were allowed to run for a few hours in disinfectant and then with distilled water, to prevent cross contamination between experiments. The miniponds were then assembled in a rooftop location. Media was poured into each of the miniponds (two miniponds per algal or cyanobacterial species, one defined culturing media (ASM or F/2), one slurry based media, 2.1.2.2) and inoculum pipetted aseptically directly in front of the mixer unit (2.1.3.2). The volume of media used was adjusted to take account of algal inoculum volume to a final volume of 16 litres. The lids were secured and the pumps were switched on and mixing checked. The temperature was continuously monitored by the use of temperature probes in two of the miniponds connected to a manually calibrated chart recorder. Minimum and maximum temperatures per day were recorded. Ambient light conditions were monitored and recorded by a nearby weather station.

The experimental time scale ranged from 20-30 days and sampling was carried out daily if possible. A 50ml sample was taken initially after inoculation and thereafter at the same time every day. OD_{560} (2.3.2), dry weight (2.3.3), pH (2.3.5) nitrate/nitrite (2.3.6) and ammonia (2.3.7) were determined for all

samples. In addition organic nitrogen (2.3.8) was determined at the beginning and the end of each experiment. For cyanobacterial species, observations were made using a Leitz microscope for the presence of heterocysts. Exponential and stationary phases were determined from OD_{560} and dry weight. At exponential phase only, a suitable volume (3-5 litres) was removed for harvesting (2.3.9), and the supernatent was returned to the minipond. At stationary phase, all material was harvested. Due to the replacement of supernatent and the addition of sterile distilled water (to maintain minipond levels), samples were taken before and after these times eg before and after exponential harvesting. Chlorophyll analysis (2.3.12) was also carried out on the initial sample and samples taken at exponential and stationary phase harvesting. Harvested biomass was freeze dried (2.3.9) and carbohydrate (2.3.10), protein (2.3.11) and fatty acid content (3.1.4) determined.

5.3 RESULTS AND STATISTICAL ANALYSIS

5.3.1 C. vulgaris 211/8K and S. obliquus 276/3A

Two experiments were carried out, one from 2/5/89 to 22/5/89 (20 days, Expt. 1) and one from 21/9/89 to 15/10/89 (24 days, Expt. 2). Minimum and maximum daily temperatures and daily irradiances are given in Tables 41 and 42. Average minimum temperatures were similar for both experimental runs (11.3°C (Expt 1) and 10.7°C (Expt. 2)) but maximum temperatures differed by 5.9°C (22.5°C (Expt. 1) and 16.6°C (Expt. 2)). Irradiance was significantly lower on most days for the second experiment (21/9/89 - 15/10/89).

5.3.1.1 Growth, Nitrogen and Chlorophyll results

In both experiments, C. vulgaris 211/8K failed to grow in defined medium (ASM), although growth occured in the algal treated slurry (TS). Microscopical examination showed both C. vulgaris 211/8K and S. obliquus to be unialgal in both media systems.

OD_{560} against time (Figs 151 and 152) and dry weight against time (Figs 153

and 154) show similar patterns of growth for both species in each experiment. However, for S. obliquus, growth in algal treated slurry (TS) appeared better than in ASM in the first experiment and vice versa for the second experiment. This may have been due to irradiance, with higher levels in experiment 1 able to provide good growth in the slurry supernatent. C. vulgaris grew better in the first experiment also, probably due to increased maximum temperature and higher irradiances.

Results of nitrate and nitrite depletion are shown in Figs 155 - 158. Depletion of nitrite and nitrate was achieved by all cultures in both experiments with the exception of C. vulgaris in algal treated slurry in the second experiment (1.34 mg NO_3-N l^{-1} and 0.33 mg NO_2-N l^{-1} remaining). Ammonium (Expt. 1 - initial level 5.52mg NH_4-N l^{-1}, Expt. 2 - initial level 8.89mg NH_4-N l^{-1}) had depleted in all cases before depletion of other nitrogen sources in the order ammonium -> nitrite -> nitrate (7 days and 6 days respectively for experiments one and two). In all cultures, growth reached stationary phase. Total Organic nitrogen was found to decrease with growth in slurry supernatent but increase with growth in defined media for both C. vulgaris 211/8K (TS only) and S. obliquus. Harvest parameters are given in Table 43. Chlorophyll a, b and c all increased over time and from exponential to stationary phase (Table 43). pH against time showed an increase and then decrease in pH with growth (Figs 159 and 160).

FIG 151 OD560 in Defined media(ASM) & Algal Treated Slurry(TS) for C.vulgaris 211/8K & S.obliquus 276/3A (2/5-22/5/89)

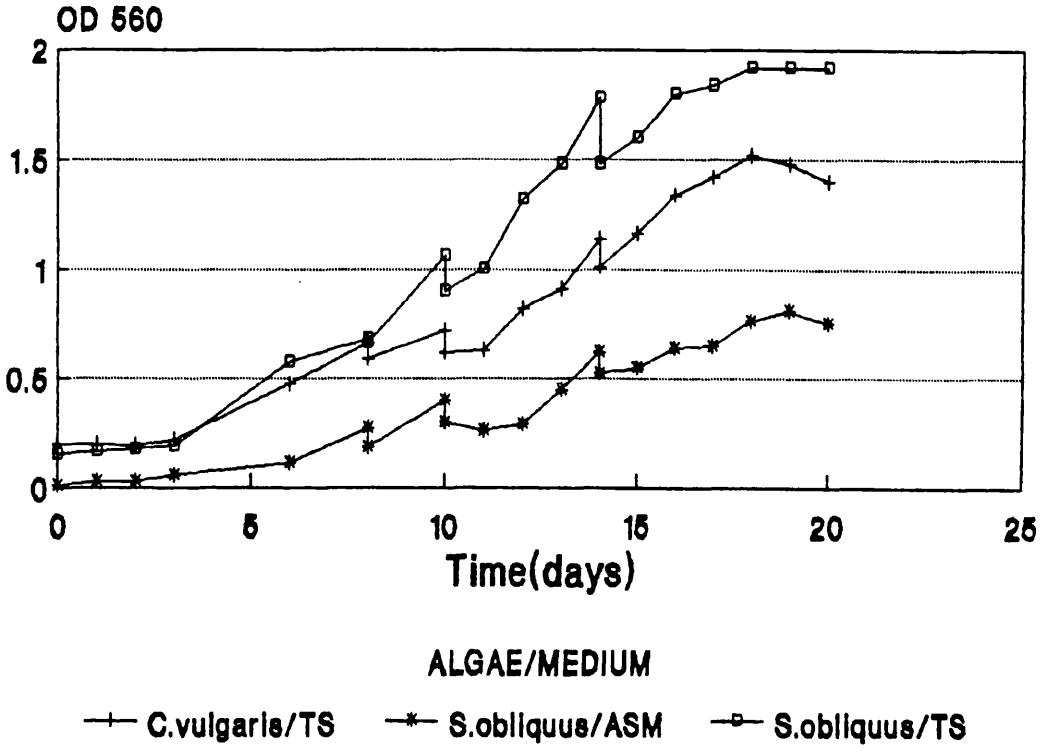


FIG 152 OD560 in Defined Medium(ASM) & Treated Slurry(TS) for C.vulgaris 211/8K & S.obliquus 276/3A (21/9-15/10/89)

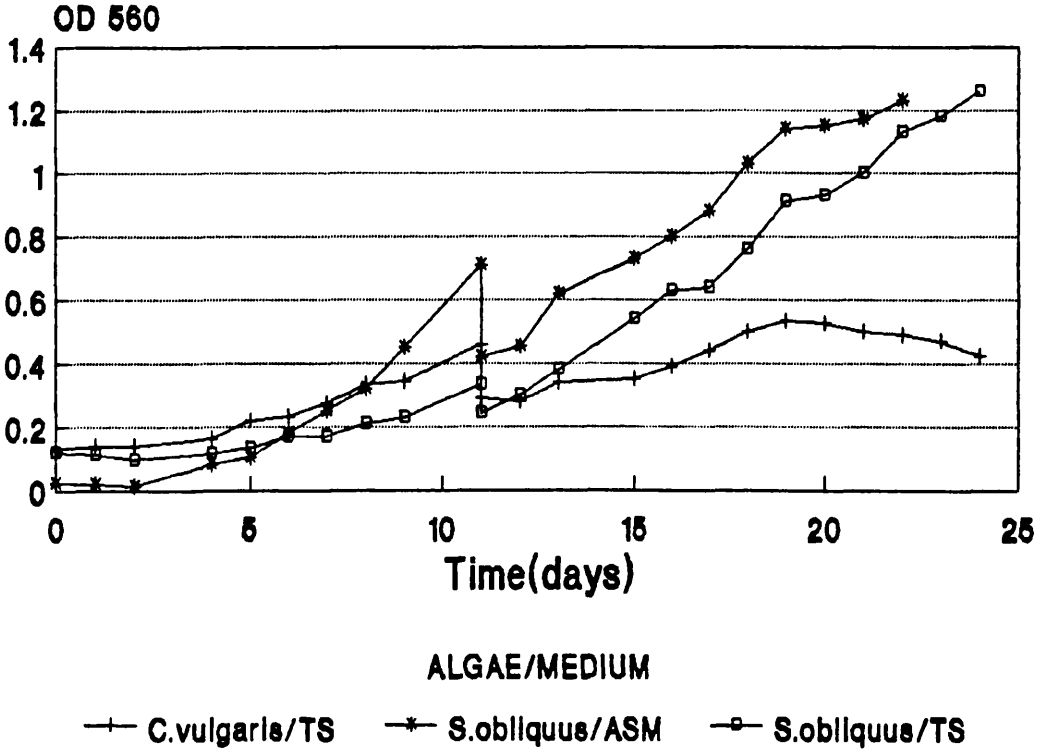


FIG 153 DRY WEIGHT in Defined medium(ASM) &Algal Treated Slurry(TS) for C.vulgaris 211/8K & S.obliquus 276/3A (2/5-22/5/89)

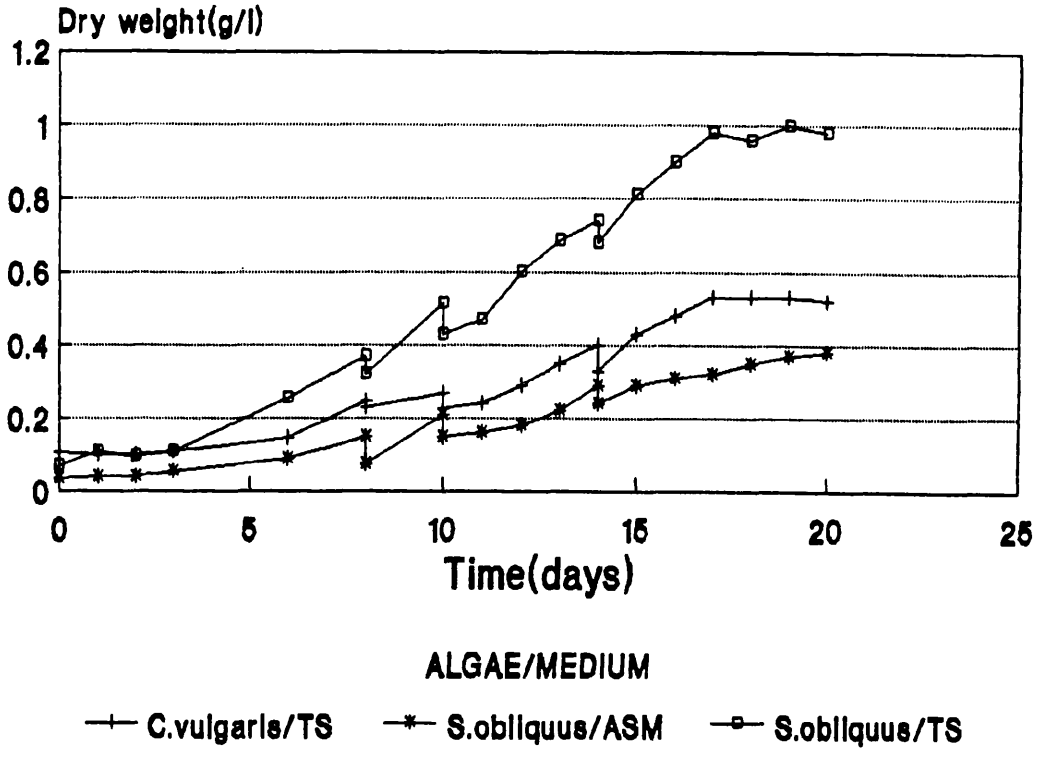


FIG 154 DRY WEIGHT: Defined Medium(ASM)& Treated Slurry(TS) for C.vulgaris 211/8K & S.obliquus 276/3A (21/9-15/10/89)

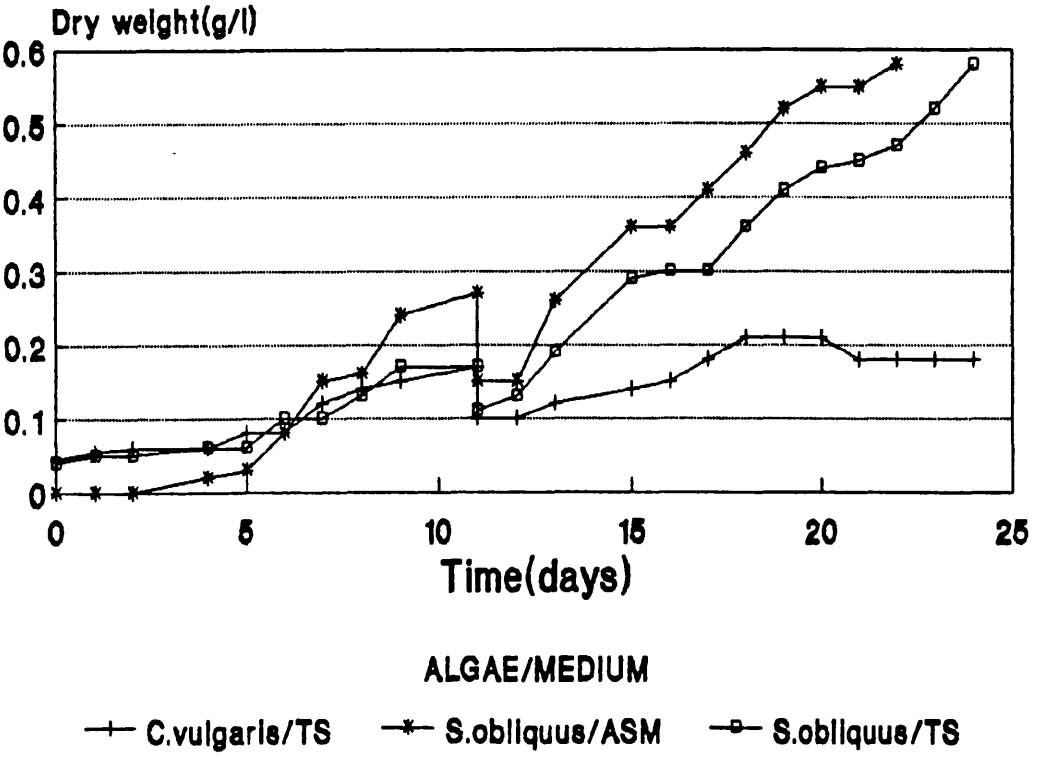


FIG 155 NITRATE in Defined medium(ASM) & Algal Treated Slurry(TS) for C.vulgaris 211/8K & S.obliquus 276/3A (2/5-22/5/89)

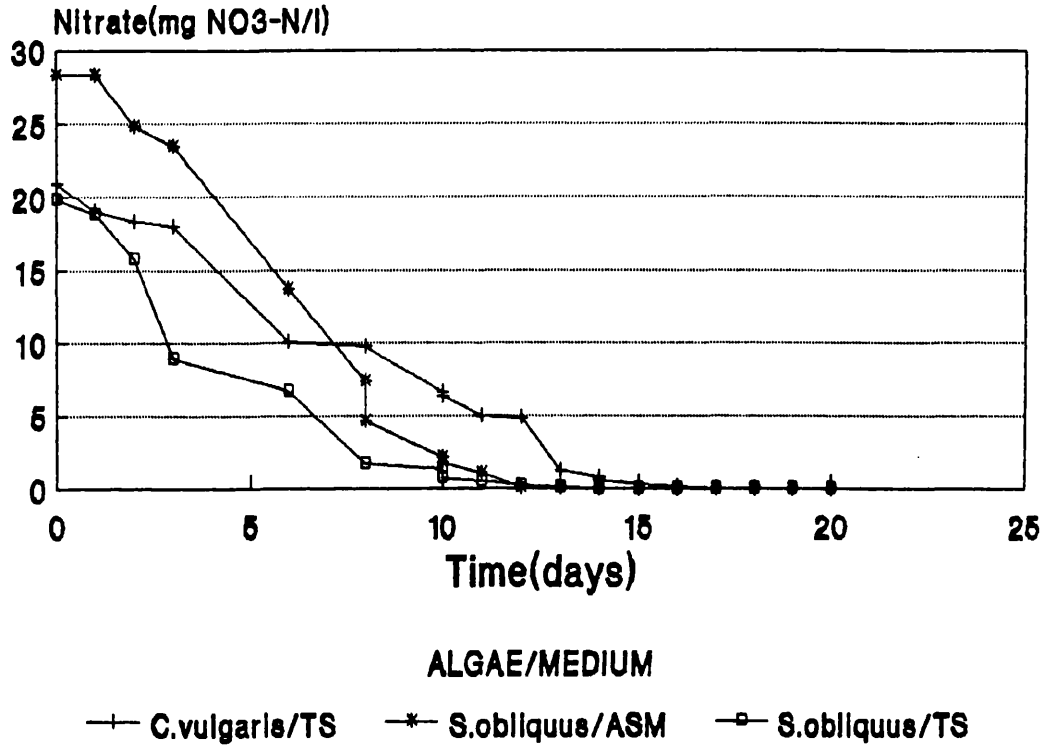


FIG 156 NITRITE in Defined Medium(ASM) & Algal Treated Slurry(TS) for C.vulgaris 211/8K & S.obliquus 276/3A (2/5-22/5/89)

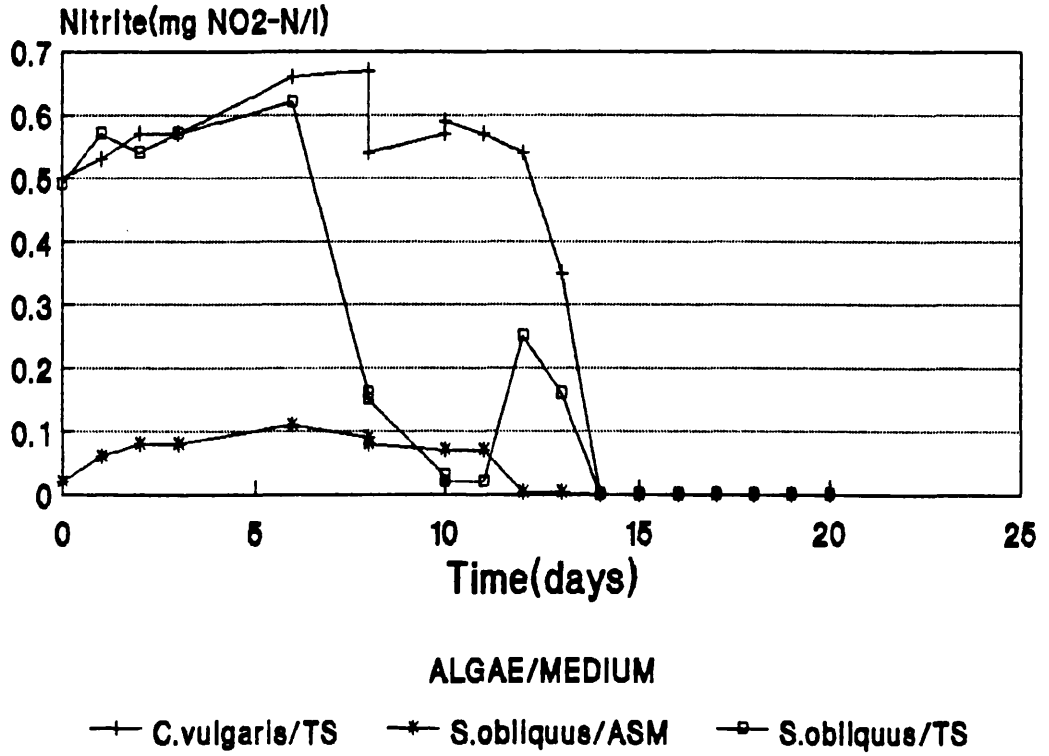


FIG 157 NITRATE in Defined Medium(ASM)& Treated Slurry(TS) for C.vulgaris 211/8K & S.obliquus 276/3A (21/9-15/10/89)

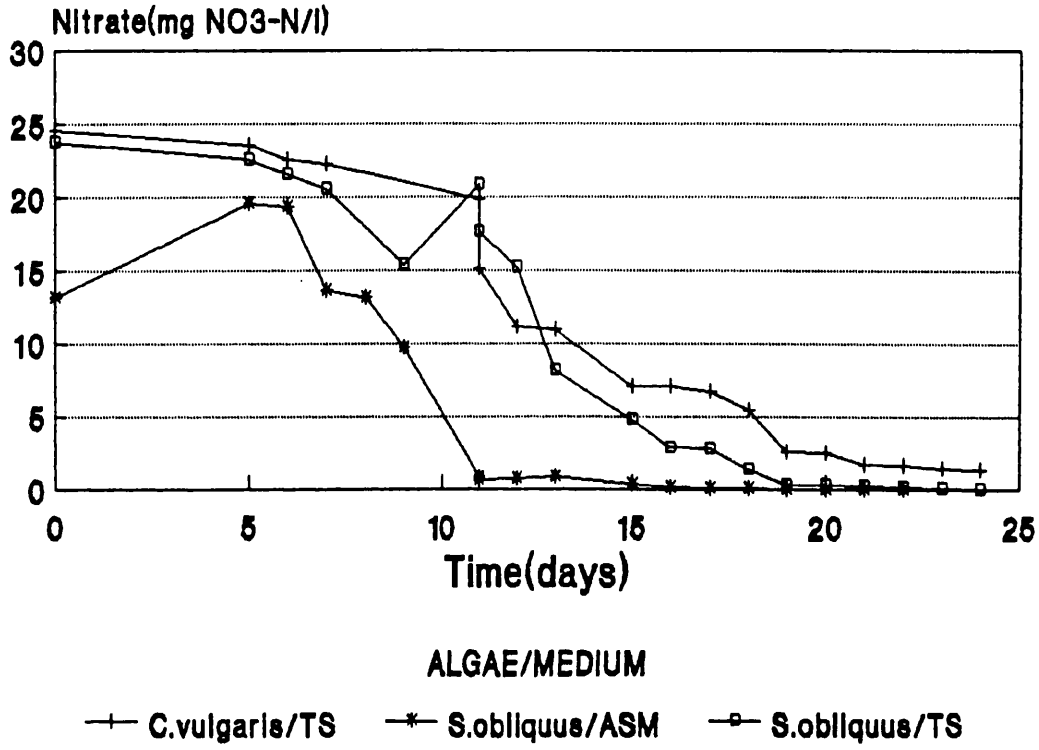


FIG 158 NITRITE in Defined Medium(ASM)& Treated Slurry(TS) for C.vulgaris 211/8K & S.obliquus 276/3A (21/9-15/10/89)

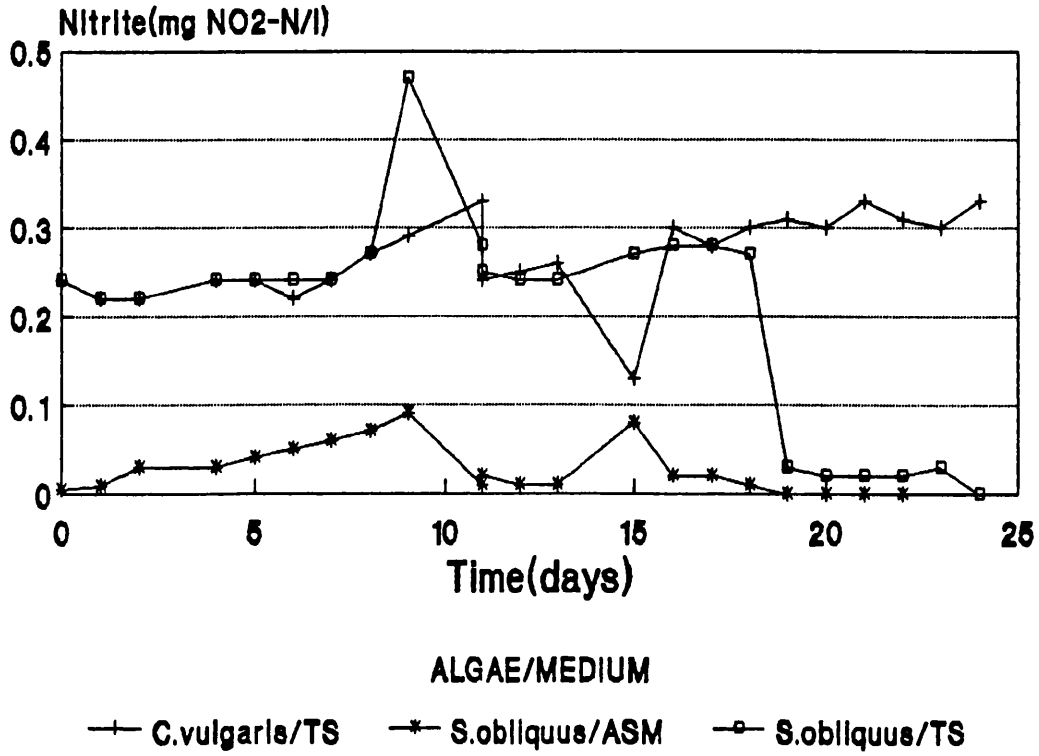


FIG 159 PH in Defined Medium(ASM) and Algal Treated Slurry(TS) for C.vulgaris 211/8K & S.obliquus 276/3A (2/5-22/5/89)

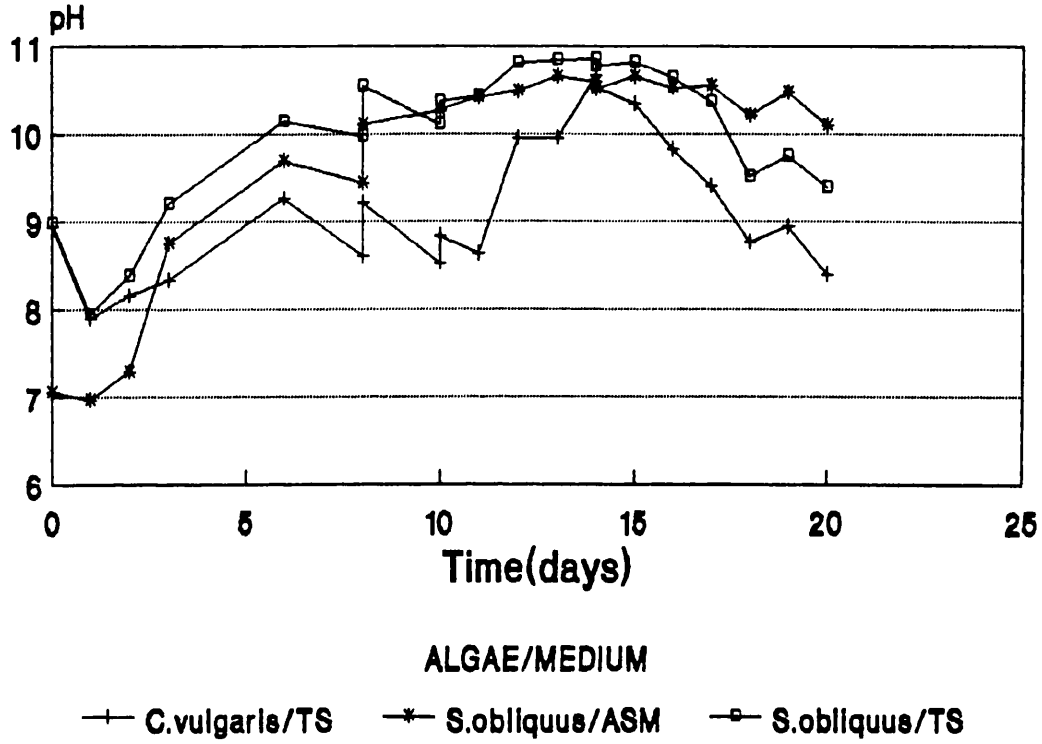


FIG 160 PH in Defined Medium(ASM) & Algal Treated Slurry(TS) for C.vulgaris 211/8K & S.obliquus 276/3A (21/9-15/10/89)

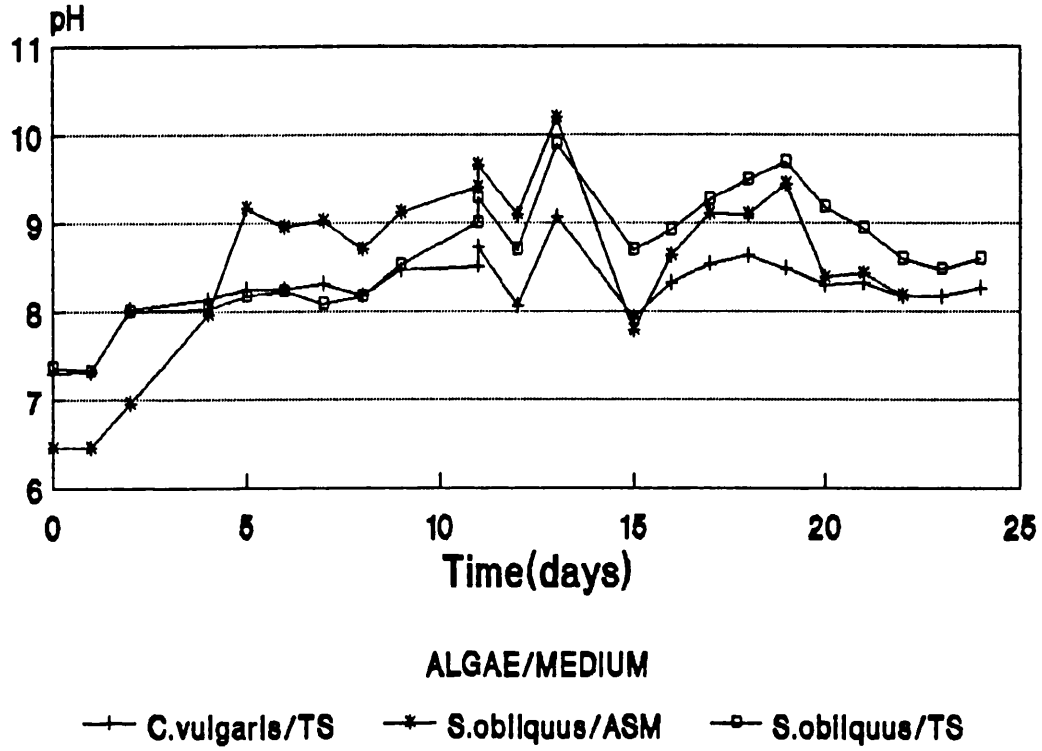


Table 41: Minimum and Maximum Daily Temperatures and Irradiance Levels for Outdoor Minipond Experiment Dates 2/5/89(0)-22/5/89(20)

Time (Days)	Minimum (°C)	Maximum (°C)	Irradiance ($Cal/cm^2/d$)
			337.1
0	NA	NA	260.1
1	10	18	699.5
2	10.5	23	680.6
3	9.5	21.5	709.9
4	12	NA	633.2
5	14	26	202.3
6	9.5	17.5	708.6
7	10	24	654.4
8	9.5	24	288.2
9	9	15	317.7
10	9.5	14	680.4
11	9	25	417.8
12	11.5	20	517.7
13	9.5	23	712.2
14	9.5	26.5	629.6
15	11.5	25	200.6
16	13	19	369.5
17	12.5	22	677.6
18	15.5	32	654.6
19	15.5	30	721.7
20	14.5	NA	
Average (°C)	11.3	22.5	

NA = Not Available

Table 42: Minimum and Maximum Daily Temperatures and Irradiance Levels for Outdoor Minipond Experiment Dates 21/9/89(0)-15/10/89(24)

Time (Days)	Minimum (°C)	Maximum (°C)	Irradiance ($Cal/cm^2/d$)
0	10	17	115.1
1	9	16	54.9
2	9	17	166.4
3	9	17	317.8
4	9	17	143.2
5	11	16	76.3
6	11	19	147.7
7	9	17	204.9
8	10	18	314.5
9	8	18	166.1
10	9	15	239.3
11	10	15	311.8
12	13	17	55.4
13	11	16	124.2
14	12	16	241.7
15	12	16	119.3
16	12	17	96.9
17	13	16	128.7
18	13	17	87.4
19	14	17	88.7
20	10	17	75.9
21	10	17	49.7
22	11	15	158.5
23	11	15	185.6
24	11	NA	73.3
Average (°C)	10.7	16.6	

NA = Not Available

Table 43: Harvest Parameters for Outdoor Minipond Experiments (Including Chlorophyll) for C. vulgaris 211/8K and S. obliquus 276/3A

Organism	Media	Phase	Time (Days)	OD_{560}	Dry Wt (gl^{-1})	NO_3-N (mgl^{-1})	NO_2-N (mgl^{-1})	NH_4-N (mgl^{-1})	Chlorophyll ($ugml^{-1}$)		
									a	b	c
2/5/89-22/5/89											
C. vulgaris	TS		0						0.45	0.25	0.17
C. vulgaris	TS	E	10	0.72	0.27	6.65	0.57	0	1.24	0.20	0.28
C. vulgaris	TS	S	20	1.40	0.52	0	0	ND	3.31	0.93	0.70
S. obliquus	ASM		0						0.28	0.13	0.14
S. obliquus	ASM	E	10	0.40	0.21	2.18	0.07	0	3.12	0.46	0.38
S. obliquus	ASM	S	20	0.75	0.38	0	0	ND	3.28	0.63	0.42
S. obliquus	TS		0						-	-	-
S. obliquus	TS	E	10	1.06	0.52	1.34	0.03	0	1.12	0.21	0.39
S. obliquus	TS	S	20	1.92	0.98	0	0	ND	2.63	0.58	0.17
21/9/89-15/10/89											
C. vulgaris	TS		0						0	0	0
C. vulgaris	TS	E	11	0.46	0.17	19.83	0.33	0	1.92	0.28	0.22
C. vulgaris	TS	S	24	0.43	0.18	1.34	0.33	ND	2.14	0.59	0.50
S. obliquus	ASM		0						0	0	0
S. obliquus	ASM	E	11	0.71	0.27	0.86	0.01	0	3.03	0.24	0.24
S. obliquus	ASM	S	22	1.23	0.58	0	0	ND	6.89	0.88	0.41
S. obliquus	TS		0						0	0	0
S. obliquus	TS	E	11	0.34	0.17	20.82	0.28	0	2.93	0.49	0.29
S. obliquus	TS	S	24	1.26	0.58	0	0	ND	6.95	0.75	0.56

ND = Not Determined; E = Exponential Phase; S = Stationary Phase

5.3.1.2 Carbohydrate, Protein and Lipid Results

Value for carbohydrate, protein and lipid contents are given in Table 44. C. vulgaris 211/8K, in algal treated slurry only, accumulated carbohydrate, slightly increased protein content and increased lipid content at stationary phase in the first experiment (Fig 161). In the second experiment, carbohydrate decreased, protein increased and lipid slightly decreased in stationary phase (Fig 162). The difference observed in lipid and carbohydrate content between experiments may have been due to the fact that nitrate and nitrite had not depleted in the second experiment (Table 43).

S. obliquus showed similar results in ASM to algal treated slurry for carbohydrate, accumulation occuring in stationary phase in both experiments (Figs 163 and 164). Protein changes were also similar in both media (ASM and TS) in both experiments, decreasing at stationary phase. Lipid accumulation occured at stationary phase in both media in the second experiment, however, it only accumulated in stationary phase in algal treated slurry in the first experiment.

5.3.1.3 Statistical Analysis of the Carbohydrate, Protein and Lipid Results

Statistical analysis of the carbohydrate results only gave one significant effect for growth phase (1% or $p < 0.01$). Phase means (exponential - 18.8% and stationary - 31.6%) showed a significant increase from exponential to stationary phase confirming carbohydrate accumulation at stationary phase. Temperature, algae and media were not found to be significant.

Statistical analysis of the protein results gave one significant 'main effect' of temperature (1% or $p < 0.01$). Temperature means (expt. 1 - 4.66%, expt. 2 - 10.27%), showed a significant reduction in protein content for expt. 1 (minimum temp 11.3°C, maximum 22.5°C).

Statistical analysis of the lipid results gave no significant main effects or first order interactions.

5.3.1.4 Fatty Acid Results

For C. vulgaris 211/8K, quantitative differences in individual fatty acids were observed between experiments, and between growth phases for each experiment (Table 44). The major fatty acids were 16:0, 16:3, 18:2(n-6) and 18:3(n-3) in both experiments. Individual fatty acid changes culminated in % UNFA decreasing at stationary phase in the first experiment and increasing slightly in stationary phase in the second experiment.

For S. obliquus, quantitative differences were observed between experiments and between growth phases in both experiments (Table 44). Similar fatty acids profiles were found with growth in both media. %UNFA decreased at stationary phase with the exception of growth in algal treated slurry in the second experiment.

Statistical analysis of the percentage unsaturation results gave no significant main effects or first order interactions.

Table 44: Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) for C. vulgaris 211/8K and S obliquus 276/3A in Defined Medium (ASM) and Algal Treated Slurry (TS)

	2/5/89-22/5/89								21/9/89-15/10/89							
	C. vulgaris				S. obliquus				C. vulgaris				S. obliquus			
	ASM		TS		ASM		TS		ASM		TS		ASM		TS	
Fatty Acid	E	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S
12:0	NG	NG	TR	TR	-	-	-	-	NG	NG	.13	.12	-	-	-	-
14:0	NG	NG	.17	.17	.44	.25	.29	.23	NG	NG	.23	.24	.48	.26	.70	.34
14:1(n-5)	NG	NG	-	-	-	-	-	-	NG	NG	-	-	-	-	-	-
15:0	NG	NG	.19	.22	.22	.12	.19	TR	NG	NG	.39	.35	.37	TR	.61	.22
16:0	NG	NG	19.69	24.09	16.45	20.98	17.49	18.63	NG	NG	19.70	19.25	19.69	20.32	24.59	16.56
16:1(n-7)	NG	NG	.69	.29	.39	.22	.24	.14	NG	NG	1.53	1.01	TR	.14	2.31	.46
16:2	NG	NG	1.72	2.30	.53	.36	.96	.87	NG	NG	2.63	4.27	1.20	.95	1.17	2.94
16:3(n-6)	NG	NG	22.23	13.16	3.11	2.30	3.35	2.59	NG	NG	21.24	20.14	4.05	2.31	2.09	3.01
16:4	NG	NG	-	.43	19.07	6.81	12.46	4.33	NG	NG	-	-	17.62	5.09	13.83	14.32
17:0	NG	NG	.48	.14	.16	TR	.20	TR	NG	NG	.53	.54	-	TR	.52	.19
18:0	NG	NG	.81	1.95	.26	1.25	.85	1.70	NG	NG	1.33	1.00	.51	1.60	.90	.60
18:1(n-9)	NG	NG	2.03	11.07	10.84	34.11	24.48	48.43	NG	NG	2.70	3.28	12.79	45.90	14.60	22.83
18:1(n-7)	NG	NG	.46	.64	.95	1.74	.97	-	NG	NG	1.03	.90	1.27	1.21	2.25	1.59
18:2(n-6)	NG	NG	11.39	19.41	4.62	6.41	5.07	5.32	NG	NG	13.44	16.44	6.01	4.96	5.15	6.02
18:3(n-3)	NG	NG	40.14	25.48	39.46	21.66	30.19	15.70	NG	NG	34.26	31.66	31.61	15.05	29.22	27.20
18:3(n-6)	NG	NG	-	-	.12	.21	.20	TR	NG	NG	.25	TR	.32	.12	.49	.29
18:4(n-3)	NG	NG	.12	.15	2.72	2.65	2.24	1.44	NG	NG	-	-	2.34	1.52	1.54	2.51
19:0	NG	NG	-	-	-	-	-	-	NG	NG	-	-	-	-	-	-
20:0	NG	NG	-	TR	TR	TR	.14	TR	NG	NG	.62	.39	-	-	-	-
20:1(n-9)	NG	NG	-	.10	TR	.30	.24	.17	NG	NG	-	-	.25	.43	-	.27
20:2(n-6)	NG	NG	TR	.10	-	-	-	-	NG	NG	-	.31	-	-	-	-
20:3(n-6)	NG	NG	TR	TR	-	TR	TR	-	NG	NG	-	-	.73	-	-	-
20:4(n-6)	NG	NG	TR	TR	.11	TR	-	TR	NG	NG	-	-	.74	-	-	-
20:4(n-3)	NG	NG	-	-	.42	.37	.40	.11	NG	NG	-	-	-	-	-	.64
20:5(n-3)	NG	NG	-	-	-	-	-	-	NG	NG	-	-	-	-	-	-
21:0	NG	NG	-	-	-	-	-	-	NG	NG	-	-	-	-	-	-
22:0(IS)	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS
22:1(n-9)	NG	NG	-	-	-	-	-	-	NG	NG	-	-	-	-	-	-
22:5(n-3)	NG	NG	-	-	-	-	-	-	NG	NG	-	-	-	-	-	-
22:6(n-3)	NG	NG	-	-	-	-	-	-	NG	NG	-	-	-	-	-	-
24:0	NG	NG	-	-	-	-	-	-	NG	NG	-	-	-	-	-	-
% Lipid	NA	NA	6.64	10.28	9.47	9.19	10.54	23.49	NA	NA	7.43	6.25	6.36	19.98	3.94	10.43
% SAFA	NA	NA	21.34	26.57	17.53	22.60	19.16	20.56	NA	NA	22.93	21.90	21.05	22.18	27.32	17.91
%UNFA	NA	NA	78.78	73.13	82.34	77.14	80.80	79.10	NA	NA	77.08	78.01	78.93	77.88	72.65	82.08
UNFA/SAFA	NA	NA	3.69	2.75	4.70	3.41	4.22	3.85	NA	NA	3.36	3.56	3.75	3.51	2.66	4.58
% Protein	NA	NA	3.16	4.08	8.00	3.02	4.28	2.73	NA	NA	8.01	14.58	14.31	4.63	9.78	7.62
%Carbohydrate	NA	NA	23.08	43.17	15.90	28.78	23.64	33.48	NA	NA	19.21	16.29	15.67	39.29	15.11	28.56

Note: (i) C. vulgaris 211/8K did not grow outdoors in ASM

(ii) NG = No growth; NA = Not available; IS = Internal Standard; S = Stationary phase; E = Exponential phase; SAFA = Saturated fatty acids; UNFA = Unsaturated fatty acids; TR = Trace <0.1%

(iii) For systematic names of fatty acids see Appendix 1

FIG 161
Comparison of Growth in Defined Medium (ASM)
and Algal Treated Slurry (TS)
C. vulgaris 211/8k
(2:5:89 - 22:5:89)

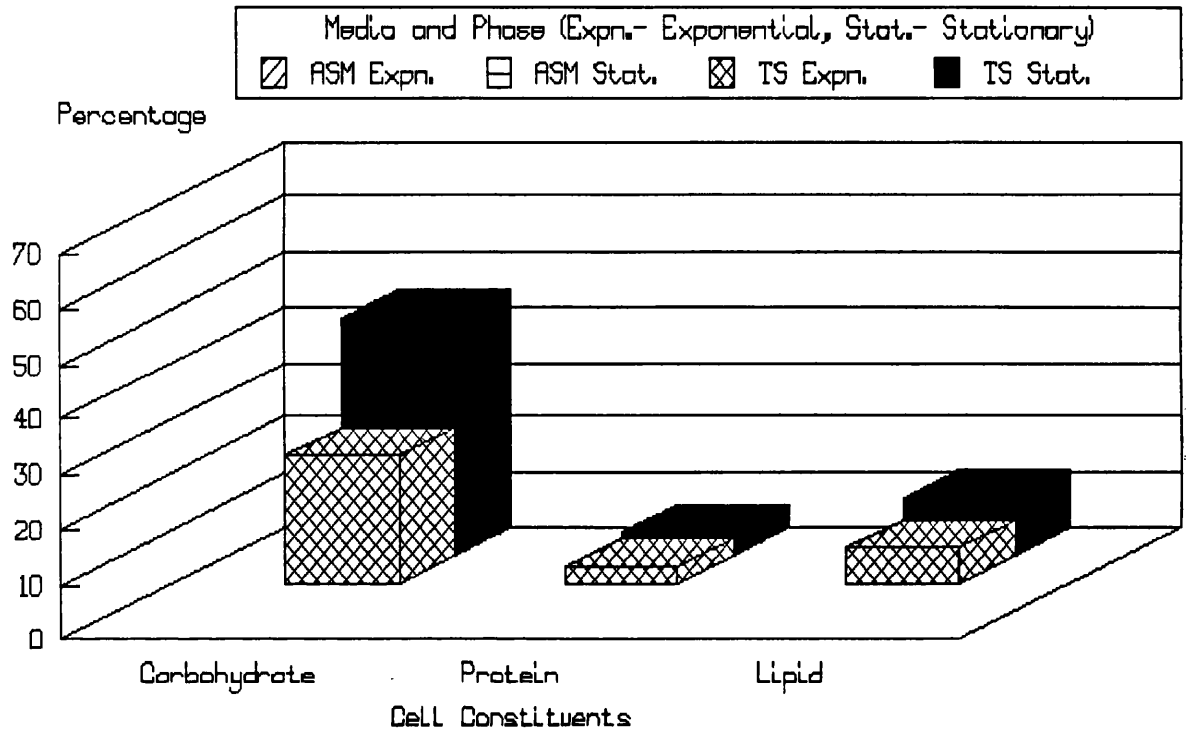


FIG 162
Comparison of Growth in Defined Medium (ASM)
and Algal Treated Slurry (TS)
C. vulgaris 211/8k
(21:9:89 - 15:10:89)

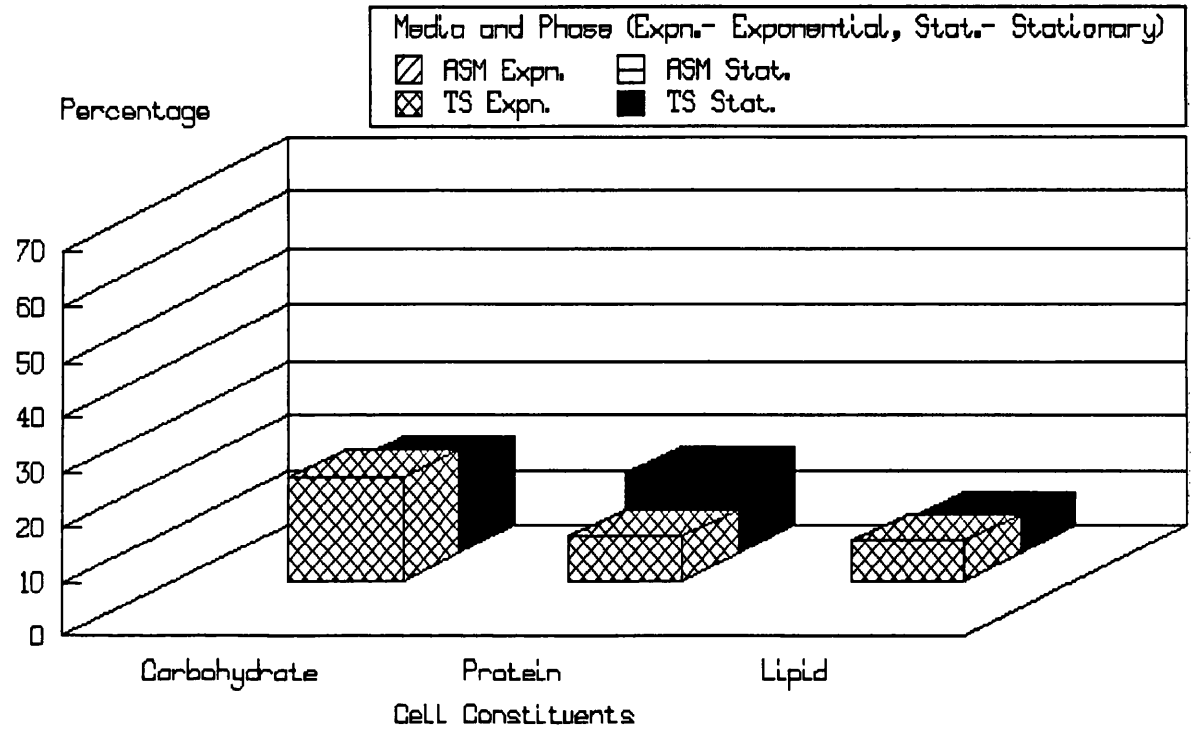


FIG 163

Comparison of Growth in Defined Medium (ASM) and Algal Treated Slurry (TS)
S. obliquus 276/3A
(2:5:89 - 22:5:89)

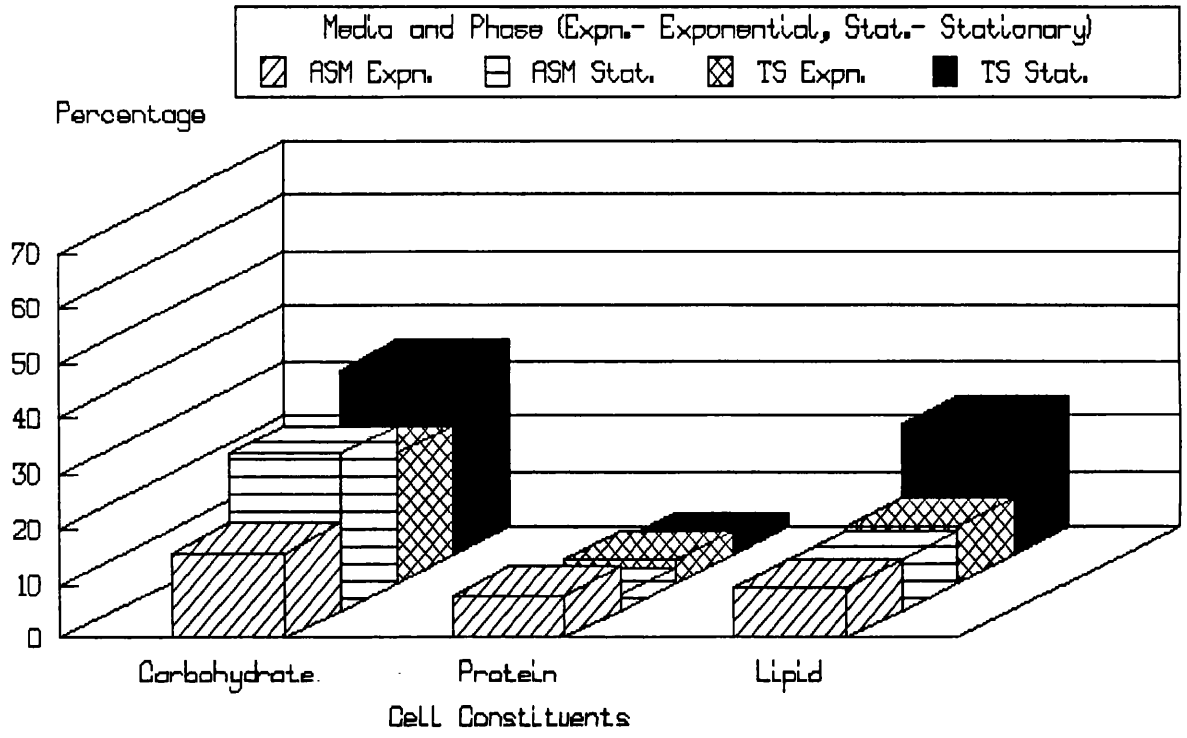
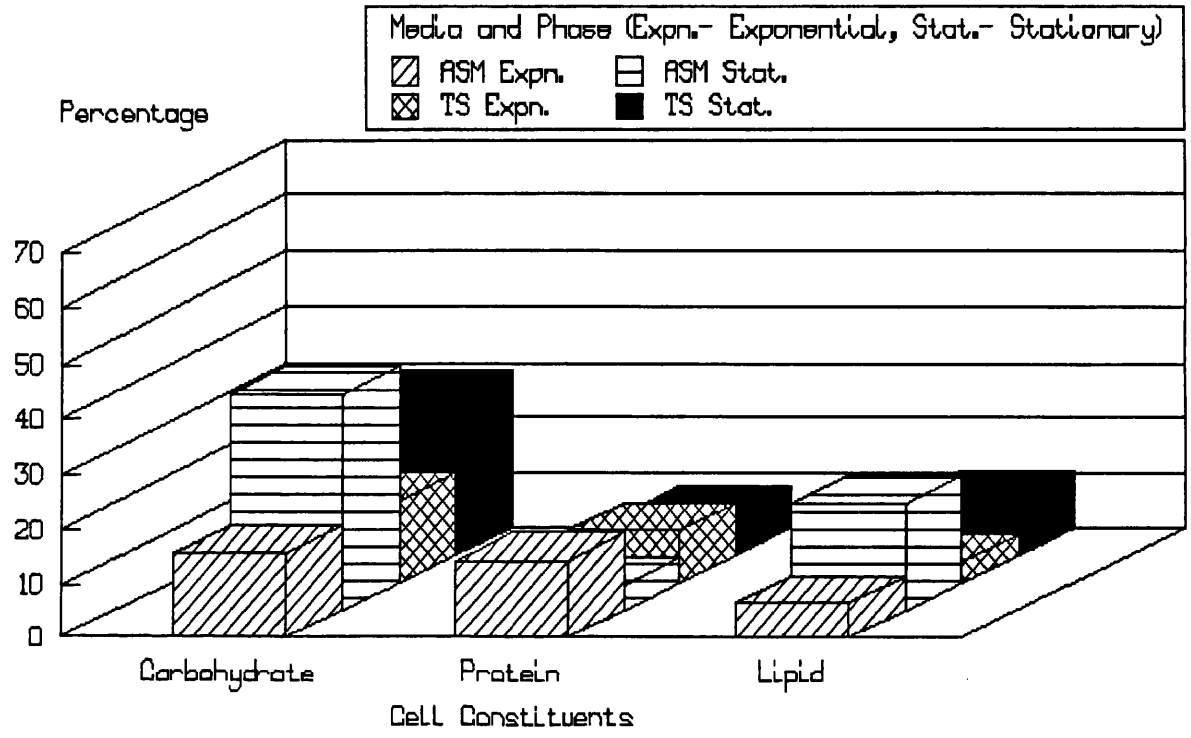


FIG 164

Comparison of Growth in Defined Medium (ASM) and Algal Treated Slurry (TS)
S. obliquus 276/3A
(21:9:89 - 15:10:89)



5.3.2 N. atomus and Isochrysis sp.

Two experiments were carried out, one from 2/6/89 to 25/6/89 (23 days, Expt. 1) and 20/10/89 - 21/11/89 (32 days, Expt. 2). Minimum and maximum daily temperatures and daily irradiance are given in Tables 45 and 46. Average minimum and maximum temperatures were greatly reduced in the second experiment (Table 46). Irradiance was also significantly lower on most days for the second experiment (20/10/89 - 21/11/89 - Table 46).

5.3.2.1 Growth, Nitrogen and Chlorophyl Results

Isochrysis sp. did not grow in either media (F/2 or TS) in the second experiment, and this was assumed to be due to either low temperature, low irradiance levels or both.

Results for OD_{560} (Figs 165 and 166) and dry weight (Figs 167 and 168) show similar growth patterns for both species in each experiment. None of the cultures appeared to be in stationary phase but in late exponential growth at harvest and therefore, they were designated S/LE (Table 47).

Results of nitrate and nitrite utilization are shown in Figs 169 - 172. At all S/LE harvests, low levels of nitrate were still present (Table 47). In the first experiment, ammonium (initial level 8.37mg NH_4-N l^{-1}) had depleted in the algal treated slurry by 7 days for both species and nitrite (initial level 0.51mg NO_2N l^{-1} in slurry supernatent, 0.01mg NO_2-N l^{-1} in F/2) had depleted in all cultures at 19 days (Fig 170). In the second experiment, ammonium (initial level 14.51mg NH_4-N l^{-1}) had depleted at 15 days, but low levels of nitrite were still present at harvest (S/LE - 32 days) (Table 47). Depletion was in the order NH_4-N -> NO_2-N -> NO_3-N. Total organic nitrogen decreased with growth in slurry supernatent but increased with growth in defined media.

Harvest parameters are given in Table 47. Chlorophyll levels, a, b, c all increased with time and from exponential to stationary/late exponential phase, with the exception of N. atomus in both media in the first experiment, where

chlorophyll a decreased and chlorophylls b and c increased (Table 47). pH against time exhibited an increase and then decrease with growth (Figs 173 and 174).

FIG 165 OD560: Defined Medium(F/2)&Algal Treated Slurry(TS) for N.atomus 251/4B and Isochrysis sp.927/14 (2/6-25/6/89)

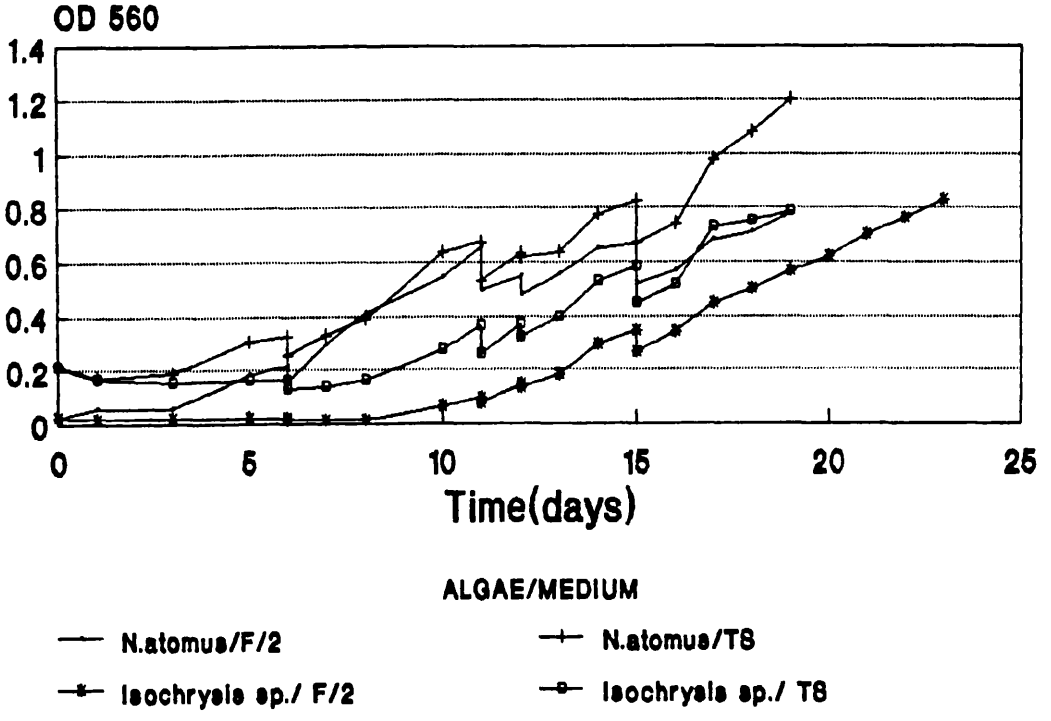


FIG 166 OD560 in Defined Medium(F/2) & Algal Treated Slurry(TS) for N.atomus 251/4B (20/10-21/11/89)

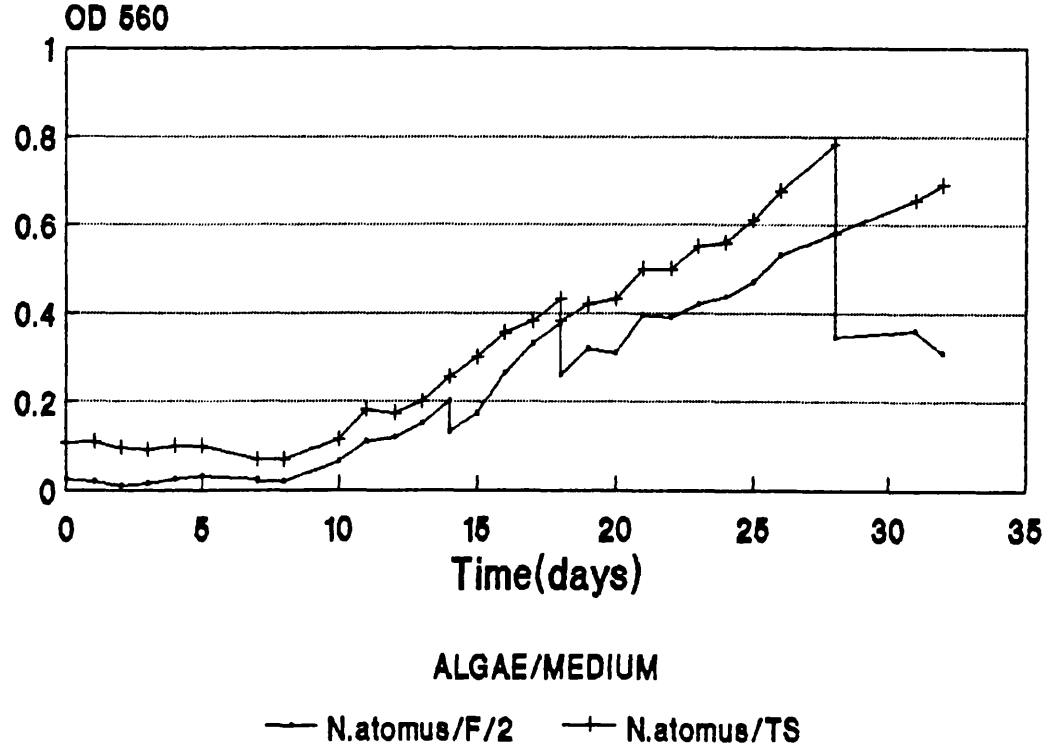


FIG 167 DRY WEIGHT:Defined Medium(F/2)& Treated Slurry(TS) for N.atomus 251/4B and Isochrysis sp.927/14 (2/6-25/6/89)

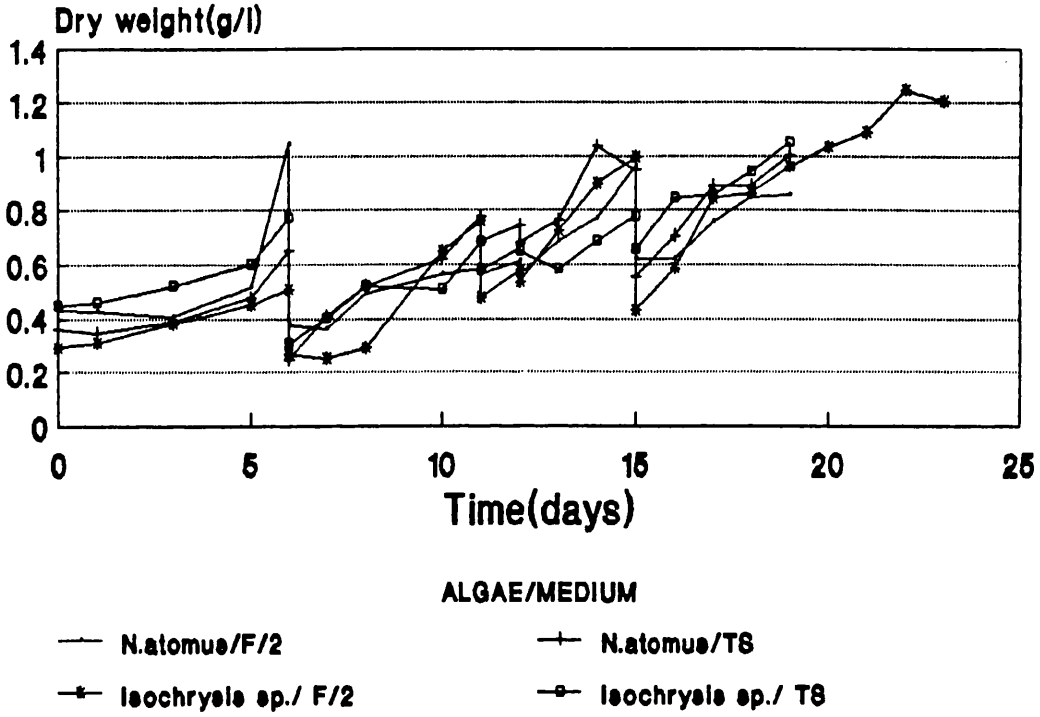


FIG 168 DRY WEIGHT in Defined Medium(F/2) & Algal Treated Slurry(TS) for N.atomus 251/4B (20/10-21/11/89)

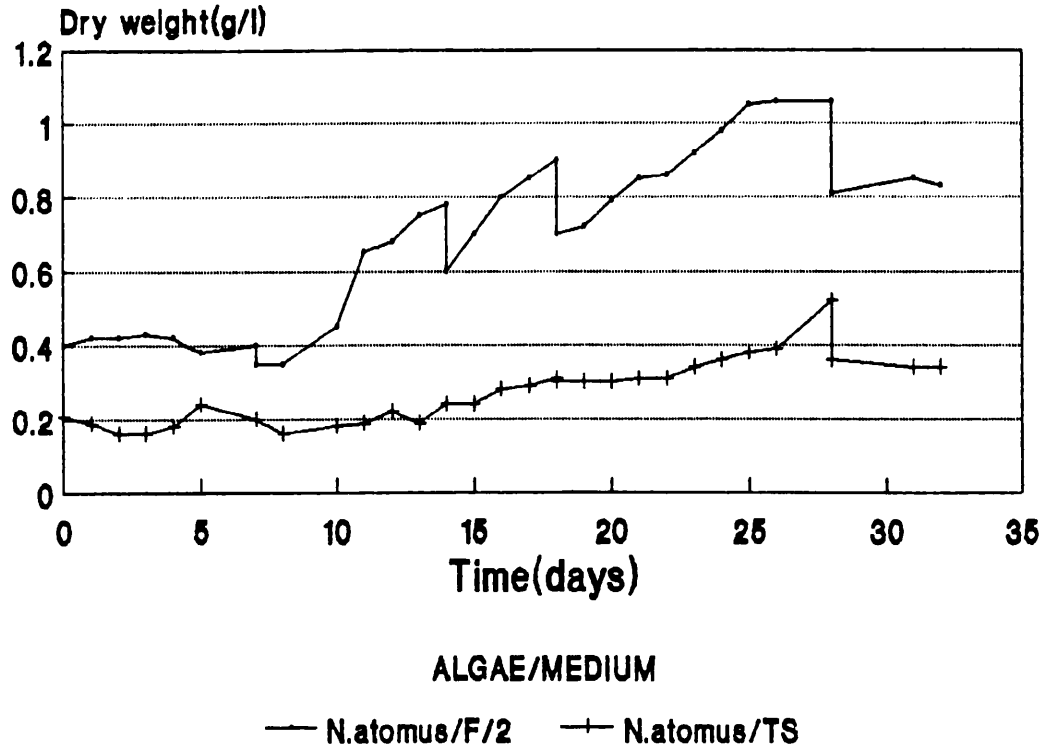


FIG 169 NITRATE in Defined Medium(F/2)& Treated Slurry(TS) for N.atomus 251/4B and Isochrysis sp.927/14 (2/6-25/6/89)

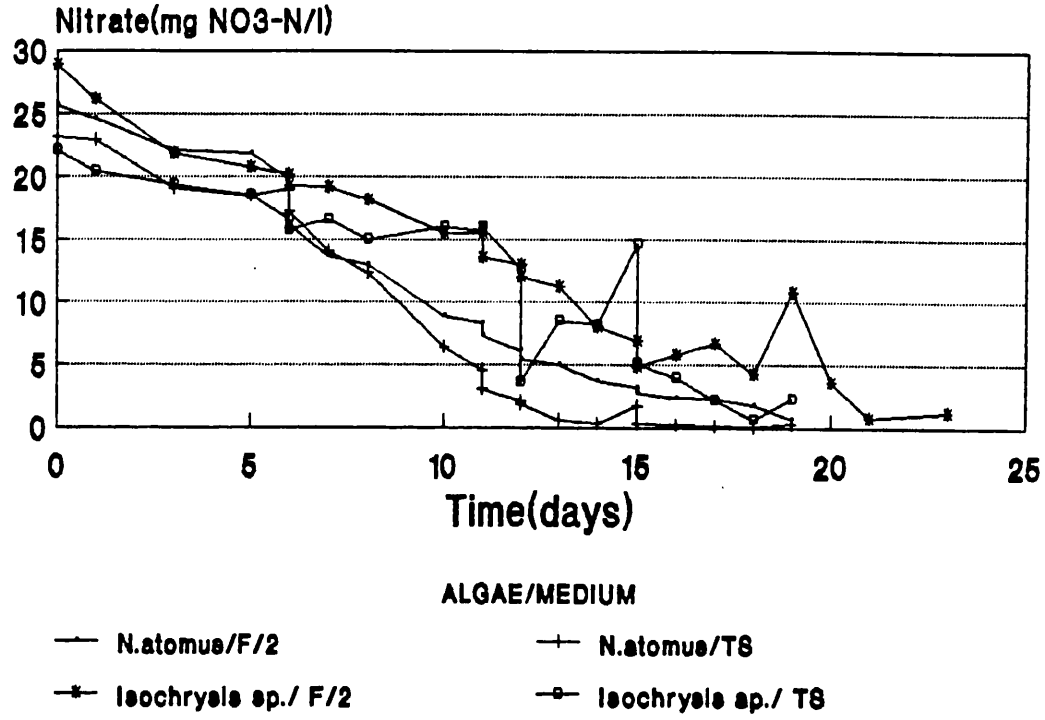


FIG 170 NITRITE in Defined Medium(F/2)& Treated Slurry(TS) for N.atomus 251/4B and Isochrysis sp.927/14 (2/6-25/6/89)

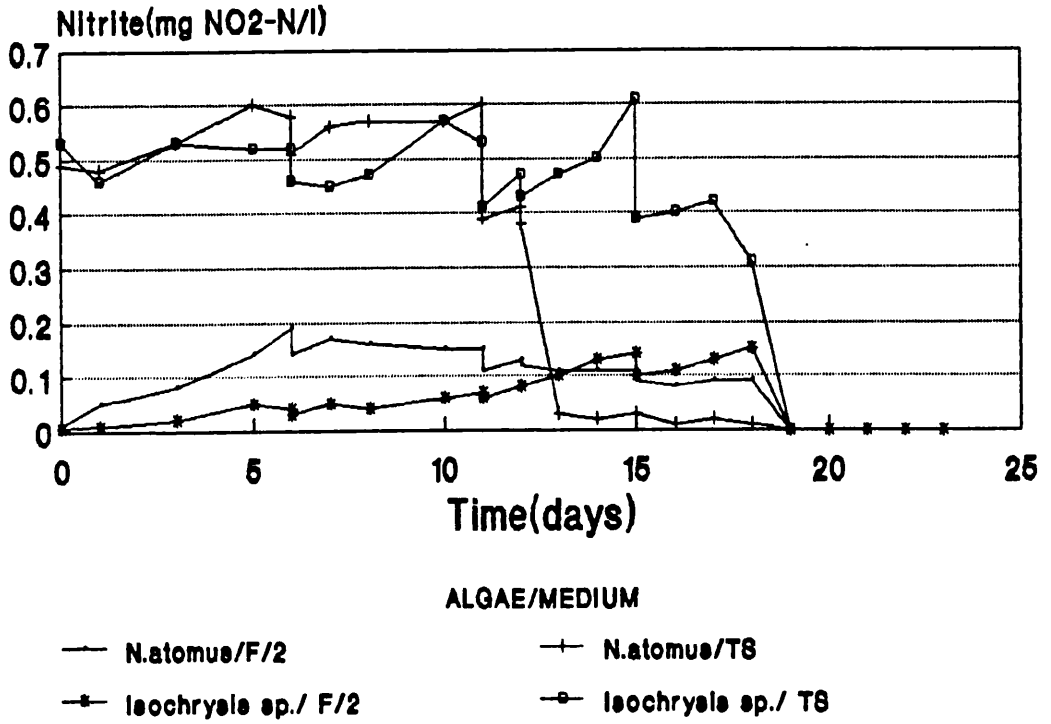


FIG 171 NITRATE in Defined Medium(F/2) & Algal Treated Slurry(TS) for N.atomus 251/4B (20/10-21/11/89)

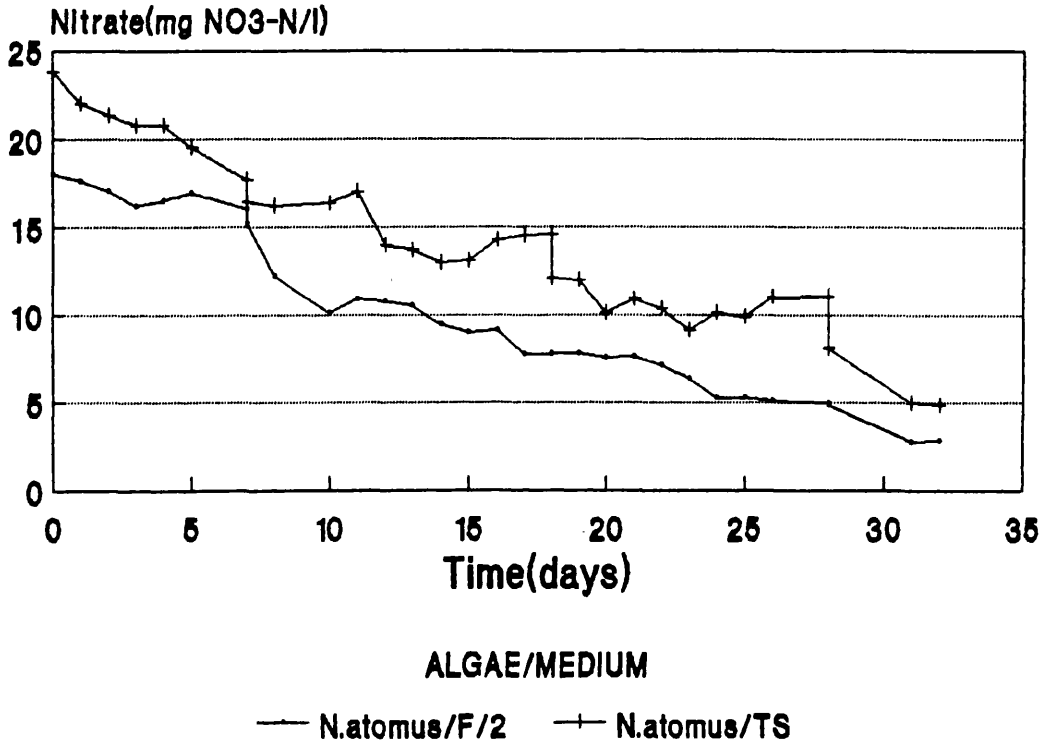


FIG 172 NITRITE in Defined Medium(F/2) &Algal Treated Slurry(TS) for N.atomus 251/4B (20/10-21/11/89)

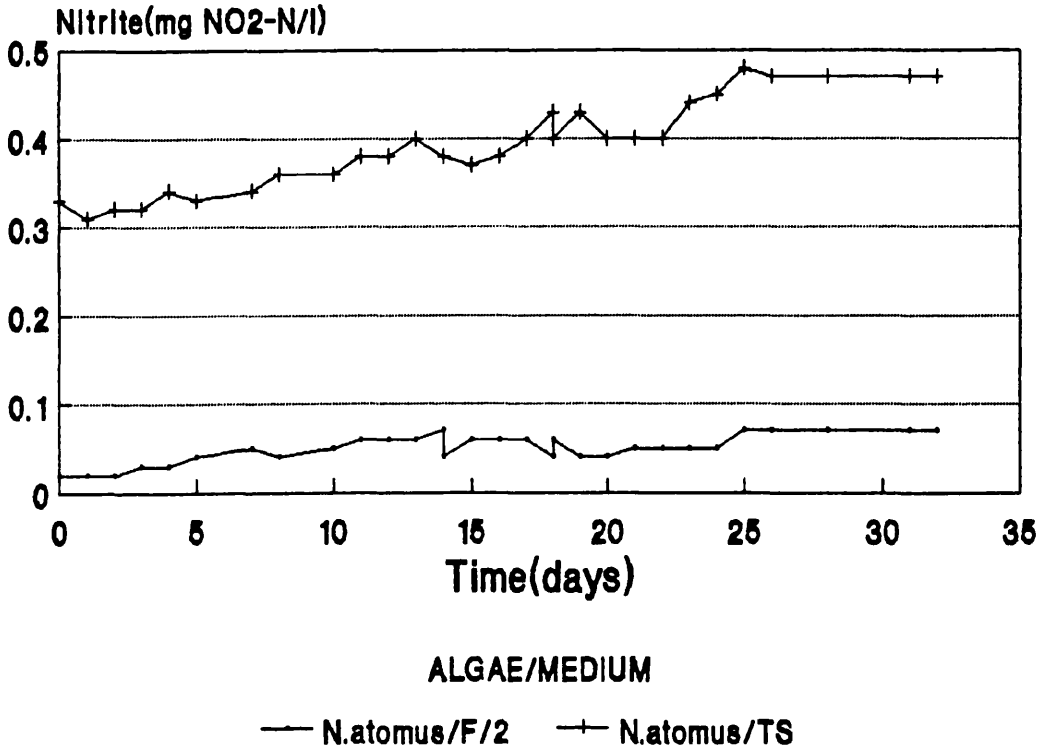


FIG 173 PH In Defined Medium(F/2)&Algal Treated Slurry(TS) for N.atomus 251/4B and Isochrysis sp.927/14 (2/6-25/6/89)

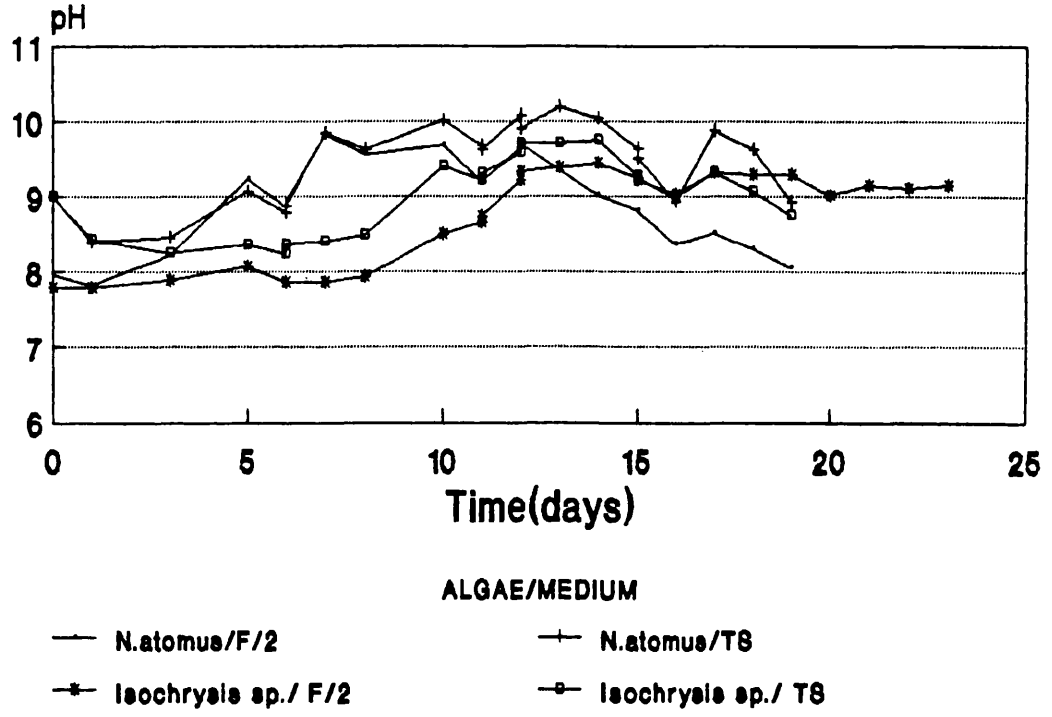


FIG 174 PH In Defined Medium(F/2) & Algal Treated Slurry(TS) for N.atomus 251/4B (20/10-21/11/89)

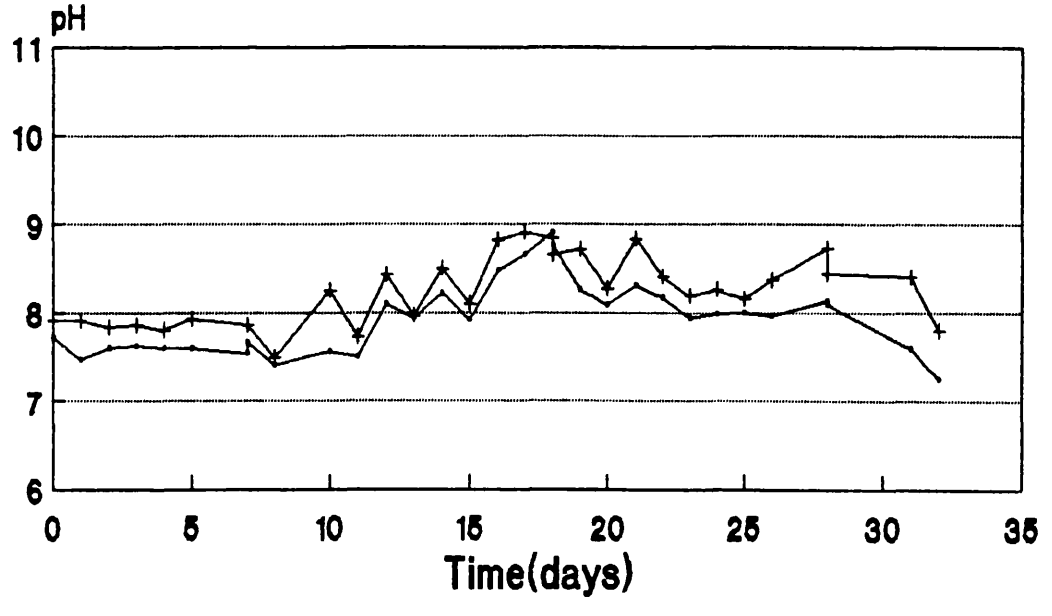


Table 45: Minimum and Maximum Daily Temperatures and Irradiance Levels for Outdoor Minipond Experiment Dates 2/6/89(0)-25/6/89(23)

Time (Days)	Minimum (°C)	Maximum (°C)	Irradiance ($Cal/cm^2/d$)
0	12	20	550.9
1	13	22	666.9
2	10	25.5	765.9
3	12	19	484.8
4	11	19	426.7
5	11	25	750.3
6	11	25.5	717.8
7	12	20.5	404.9
8	14.5	21	333.8
9	14	20	364.8
10	18	23	284.1
11	18	24.5	294.5
12	15.5	30	815.6
13	15.5	26	626.6
14	15.5	24.5	655.5
15	16	29.5	747.6
16	16	28	729.3
17	18.5	29.5	674.1
18	14	28	673.4
19	14	20	753.2
20	14	26	618.7
21	14	18	305.9
22	15	21	410.5
23	15	NA	247.8
Average (°C)	14.2	23.7	

NA = Not Available

Table 46: Minimum and Maximum Daily Temperatures and Irradiance Levels for Outdoor Minipond Experiment Dates 20/10/89(0)-21/11/89(32)

Time (Days)	Minimum (°C)	Maximum (°C)	Irradiance ($Cal/cm^2/d$)
0	8	14	154.6
1	9	13	132.8
2	9	16	118.6
3	10	15	186.4
4	8	14	20.7
5	9	13	27.9
6	8	14	106.6
7	8	13	8.89
8	11	18	91.6
9	10	14	63.4
10	11	15	152.6
11	8	16	128.5
12	9	15	125.5
13	8	14	128.7
14	7	12	86.3
15	6	12	76.8
16	6	9.5	120.8
17	6	13	80.6
18	7	14	60.90
19	7	11	NA
20	7	13	NA
21	8	14	NA
22	8	12	73.9
23	8	10	11.8
24	8	14	75.2
25	7	16	NA
26	4	14	NA
27	6	11	NA
28	4	13	NA
29	9	13	NA
30	10	12	NA
31	10.5	16	NA
32	9.5	NA	NA
Average (°C)	8	13.6	

NA = Not Available

Table 47: Harvest Parameters for Outdoor Minipond Experiments (Including Chlorophyll) with N. atomus 251/4B and Isochrysis sp. 927/14

Organism	Media	Phase	Time (Days)	OD_{560}	Dry Wt (gl^{-1})	NO_3-N (mgl^{-1})	NO_2-N (mgl^{-1})	NH_4-N (mgl^{-1})	Chlorophyll ($ugml^{-1}$)		
									a	b	c
2/6/89-25/6/89											
N. atomus	F/2		0						0	0	0
N. atomus	F/2	E	12	0.55	0.61	6.11	0.13	0	1.58	0.21	0.12
N. atomus	F/2	S/LE	19	1.78	0.86	0.68	0	ND	1.33	0.44	0.36
N. atomus	TS		0						0	0	0
N. atomus	TS	E	12	0.63	0.75	2.11	0.41	0	2.63	0.21	0.02
N. atomus	TS	S/LE	19	1.20	1.00	0.27	0	ND	2.31	0.23	0.23
Isochrysis sp	F/2		0						0	0	0
Isochrysis sp	F/2	E	12	0.15	0.58	13.06	0.08	0	0.26	0	0
Isochrysis sp	F/2	S/LE	23	0.83	1.21	1.21	0	ND	2.24	0.30	0.76
Isochrysis sp	TS		0						0	0	0
Isochrysis sp	TS	E	12	0.37	0.66	12.72	0.47	0	1.09	0.23	0.44
Isochrysis sp	TS	S/LE	19	0.79	1.05	2.3	0	ND	2.51	0.49	0.74
20/10/89-21/11/89											
N. atomus	F/2		0						0	0	0
N. atomus	F/2	E	18	0.38	0.90	7.71	0.04	0	2.04	0.13	0.24
N. atomus	F/2	S/LE	32	0.31	0.83	2.86	0.07	ND	2.67	0.44	0.39
N. atomus	TS		0						0	0	0
N. atomus	TS	E	18	0.43	0.31	14.59	0.43	0	4.28	0.31	0.40
N. atomus	TS	S/LE	32	0.69	0.34	4.89	0.47	ND	9.29	1.21	0.50

ND = Not Determined; E = Exponential Phase; S/LE = Stationary/Late Exponential Phase

5.3.2.2 Carbohydrate, Protein and Lipid Results

Results for carbohydrate, protein and lipid contents are given in Table 48. Isochrysis sp. increased its carbohydrate content, and slightly increased its protein content in stationary phase (Fig 175) in both F/2 and slurry supernatent. Lipid content increased slightly in stationary phase in F/2, but decreased slightly in algal treated slurry (Fig 175).

N. atomus increased its carbohydrate content in stationary phase in both media for the first experiment, but showed decreased carbohydrate content in stationary phase in both media for the second experiment (Fig 176 and 177). This may be irradiance or temperature related, both variables significantly lower in the second experiment. There was little variation in protein content between growth phases, but protein levels increased in the second experiment. Lipid levels increased in stationary phase in the second experiment, but decreased in the first experiment (Figs 176 and 177).

5.3.2.3 Statistical Analysis of the Carbohydrate, Protein and Lipid Results

Statistical analysis of the carbohydrate results gave only one significant effect, the 'main effect' of algae (0.1% or $p < 0.001$). Species means (N. atomus - 21.51%, Isochrysis sp. - 6.23%) show N. atomus having a significantly higher level of carbohydrate than Isochrysis sp.. There were no significant temperature, phase or media effects.

Statistical analysis of the protein results gave two significant effects, the 'main effects' of temperature and algae (both 0.1% or $p < 0.001$). Temperature means (Expt. 1 - 3.60%, Expt. 2 - 9.40%) showed a significant increased in protein content for the second experiment (lower minimum and maximum temperatures). Species means (N. atomus - 8.10%, Isochrysis sp. - 4.91%) showed a significantly higher value for N. atomus. However, it should be noted that both of these results may well have been affected by the lack of data for growth of Isochrysis sp. in the second experiment.

Statistical analysis of the lipid results gave no significant main effects or first order interactions.

5.3.2.4 Fatty Acid Results

Fatty acid results are given in Table 48.

For Isochrysis sp., quantitative differences in fatty acids were found between growth phases, but similar qualitative and quantitative profiles were found between media (F/2 and TS). The major fatty acids identified were 14:0, 16:0, 18:1(n-9), 18:4(n-3) and 22:6(n-3) under cultivation in defined media or algal treated slurry. Individual fatty acid changes culminated in %UNFA decreasing with stationary phase in algal treated slurry, but increasing in stationary phase for F/2 media.

For N. atomus, quantitative differences in fatty acids were found between growth phases, but similar qualitative profiles were observed in the two media (F/2 and TS). The major fatty acids identified were 16:0, 18:1(n-9), 18:3(n-3) and 18:4(n-3) in both media investigated. Individual fatty acid changes culminated in %UNFA not exhibiting a regular pattern of change with respect to phase, media or experiment. Statistical analysis of %UNFA results gave no significant main effects or first order interactions.

Table 48: Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) for N. atomus 251/4B and Isochrysis sp. 927/14 in Defined Medium F/2 and Algal Treate Slurry (TS)

	2/6/89-25/6/89								20/10/89-21/11/89							
	N. atomus				Isochrysis sp				N. atomus				Isochrysis sp			
	F/2		TS		F/2		TS		F/2		TS		F/2		TS	
Fatty Acid	E	S/LE	E	S/LE	E	S/LE	E	S/LE	E	S/LE	E	S/LE	E	S	E	S
12:0	.53	.29	.13	-	-	-	-	-	.41	.58	.29	.14	NG	NG	NG	NG
14:0	1.26	.97	.88	1.18	23.95	18.20	21.39	18.91	.95	1.60	.83	.12	NG	NG	NG	NG
14:1(n-5)	-	-	-	-	-	-	-	-	-	-	-	-	NG	NG	NG	NG
15:0	.29	.34	1.55	3.45	.46	.61	.41	.79	.19	.28	.23	.18	NG	NG	NG	NG
16:0	25.30	25.75	19.40	20.51	13.08	11.91	11.74	15.91	20.27	37.51	14.92	11.25	NG	NG	NG	NG
16:1(n-7)	.52	.55	.95	2.32	3.33	5.24	3.79	2.93	.16	.65	.46	.43	NG	NG	NG	NG
16:2	2.54	3.28	2.47	2.51	.26	.32	.29	.15	1.05	1.27	1.58	1.60	NG	NG	NG	NG
16:3(n-6)	14.17	9.78	8.44	6.40	-	.19	TR	.17	21.34	5.80	22.76	24.83	NG	NG	NG	NG
16:4	.11	TR	-	-	.13	TR	.14	-	TR	.16	-	TR	NG	NG	NG	NG
17:0	.14	.17	.30	-	-	.10	.15	.25	.21	.20	-	.18	NG	NG	NG	NG
18:0	.80	.90	1.59	1.08	.51	.16	.28	.26	.55	1.63	.70	.28	NG	NG	NG	NG
18:1	14.53	28.23	4.62	15.64	14.05	10.09	12.62	15.48	4.58	26.81	2.72	1.21	NG	NG	NG	NG
18:1	.43	.67	-	-	.48	.30	TR	-	.40	1.14	.79	.79	NG	NG	NG	NG
18:2(n-6)	8.15	10.60	10.83	12.18	4.51	2.41	4.04	2.36	3.54	6.56	3.34	3.08	NG	NG	NG	NG
18:3(n-3)	20.08	11.95	30.83	22.74	7.08	7.99	7.95	7.98	27.02	8.86	31.91	36.35	NG	NG	NG	NG
18:3(n-6)	.13	.14	.14	-	.15	.11	.42	.26	.18	TR	.34	.11	NG	NG	NG	NG
18:4(n-3)	2.39	1.11	5.03	3.67	18.49	23.15	21.47	13.79	7.21	.79	7.48	7.45	NG	NG	NG	NG
19:0	-	-	-	-	-	-	-	-	-	-	-	TR	NG	NG	NG	NG
20:0	TR	TR	.79	-	-	.11	.11	-	.13	.68	.23	.11	NG	NG	NG	NG
20:1(n-9)	.11	.21	-	-	-	-	-	TR	-	.15	-	.10	NG	NG	NG	NG
20:2(n-6)	TR	.15	-	-	.14	.18	.16	.20	-	.12	-	TR	NG	NG	NG	NG
20:3(n-6)	.40	.27	.74	1.14	.26	TR	.14	TR	.49	.14	.14	.40	NG	NG	NG	NG
20:4(n-6)	.11	TR	-	-	.26	.13	.24	.10	.51	.59	.44	.61	NG	NG	NG	NG
20:4(n-3)	1.86	.91	2.55	-	-	.12	-	-	3.37	.34	3.29	3.43	NG	NG	NG	NG
20:5(n-3)	5.06	2.83	4.34	4.67	-	.74	.88	.58	5.69	2.12	5.72	5.86	NG	NG	NG	NG
21:0	-	-	.59	-	-	-	-	-	.14	TR	.31	.15	NG	NG	NG	NG
22:0(IS)	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	NG	NG	NG	NG
22:1(n-9)	TR	TR	-	-	-	.16	-	-	-	-	-	-	NG	NG	NG	NG
22:5(n-3)	.19	.25	1.97	1.03	.48	.23	.27	.29	.40	.34	-	.28	NG	NG	NG	NG
22:6(n-3)	.72	.42	1.90	1.54	12.37	17.41	13.34	19.54	1.13	1.53	.91	.80	NG	NG	NG	NG
24:0	-	-	-	-	-	-	-	-	-	-	-	-	NG	NG	NG	NG
% Lipid	7.67	6.45	0.73	0.23	5.75	6.03	7.25	1.86	7.30	8.99	5.81	6.13	NA	NA	NA	NA
% SAFA	28.32	28.42	25.23	26.22	38.00	31.09	34.08	36.12	22.85	42.48	17.51	12.41	NA	NA	NA	NA
%UNFA	71.50	71.35	74.81	73.84	61.99	68.77	65.75	63.83	77.07	57.37	81.88	87.33	NA	NA	NA	NA
UNFA/SAFA	2.52	2.51	2.97	2.82	1.63	2.21	1.93	1.77	3.37	1.35	4.68	7.04	NA	NA	NA	NA
% Protein	5.22	5.25	6.00	4.31	1.25	2.21	2.11	2.46	9.33	12.05	11.80	10.82	NA	NA	NA	NA
%Carbohydrate	20.20	22.30	19.00	26.17	5.69	8.86	3.87	8.06	27.31	19.74	19.74	17.66	NA	NA	NA	NA

Note: (i) Isochrysis sp. 927/14 did not grow in the second experiment (20/10/89-21/11/89)
(ii) NG = No growth; NA = Not available; IS = Internal Standard; S = Stationary phase; E = Exponential phase; S/LE = Stationary/Late Exponential Phase; SAFA = Saturated fatty acids; UNFA = Unsaturated fatty acids; TR = Trace <0.1%
(iii) For systematic names of fatty acids see Appendix 1

FIG 175

Comparison of Growth in Defined Medium (F/2)
and Algal Treated Slurry (TS)
Isochrysis sp. 927/14
(2:6:89 - 25:6:89)

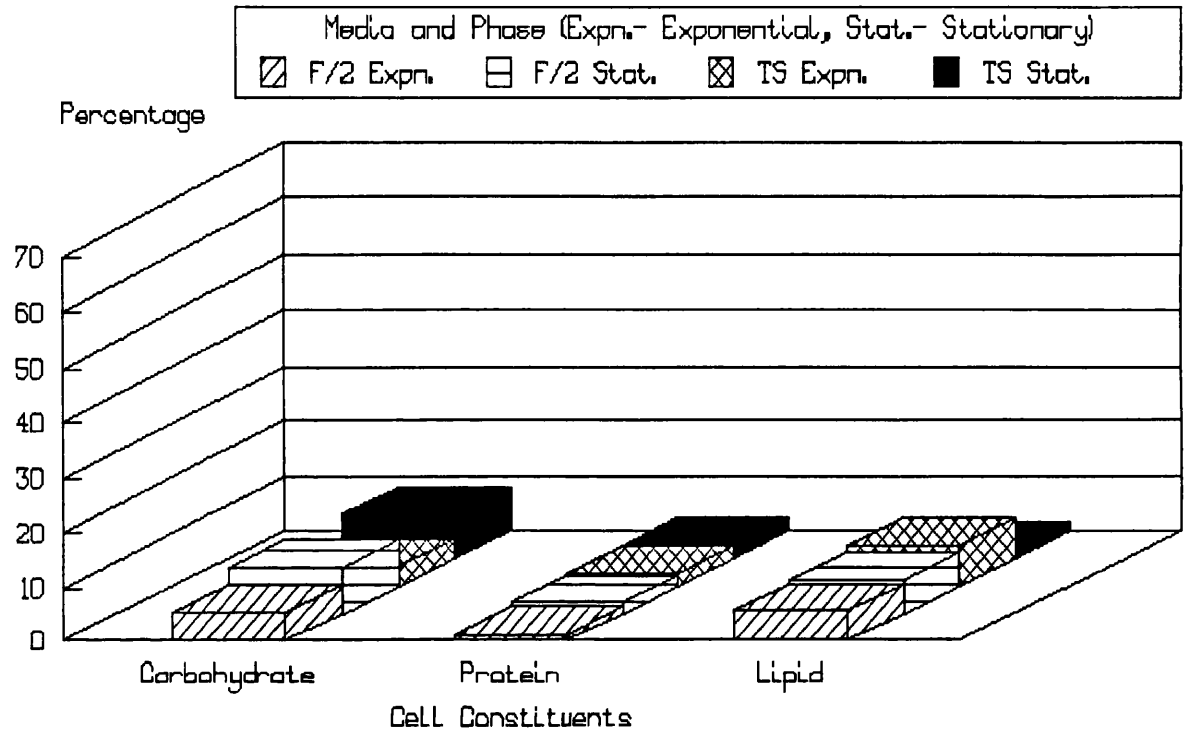


FIG 176

Comparison of Growth in Defined Medium (F/2) and Algal Treated Slurry (TS)

N. atomus 251/4B

(2:6:89 - 25:6:89)

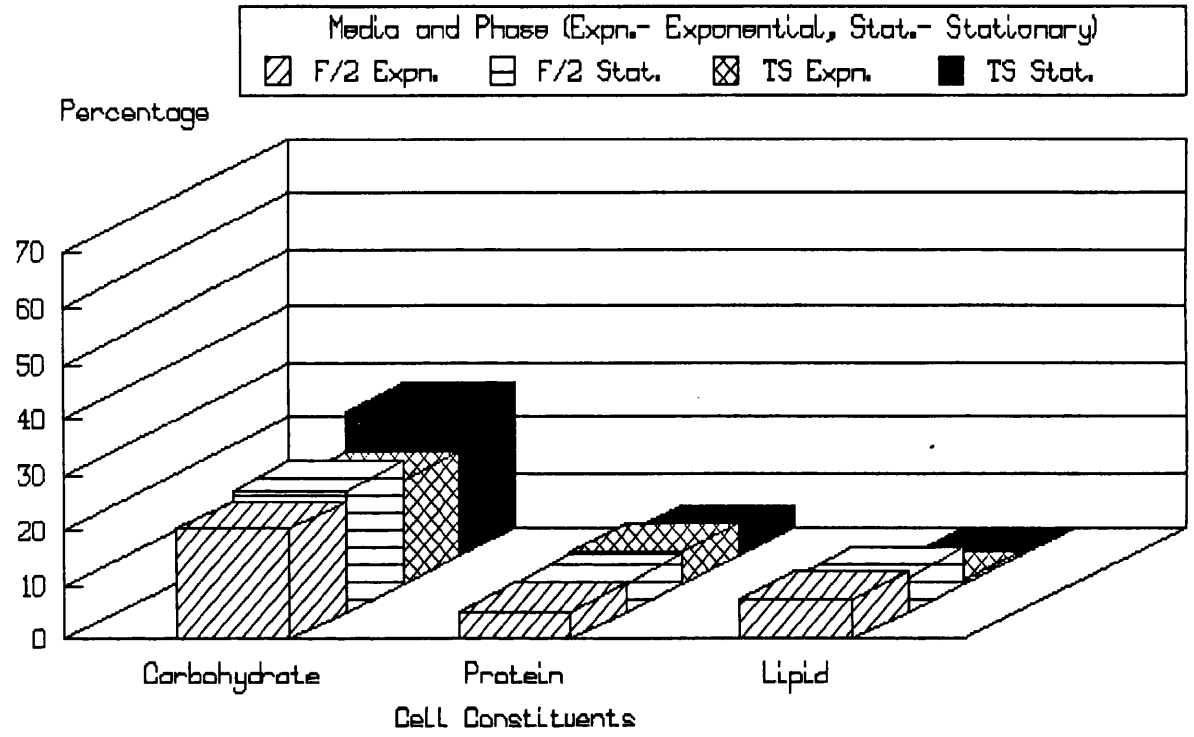
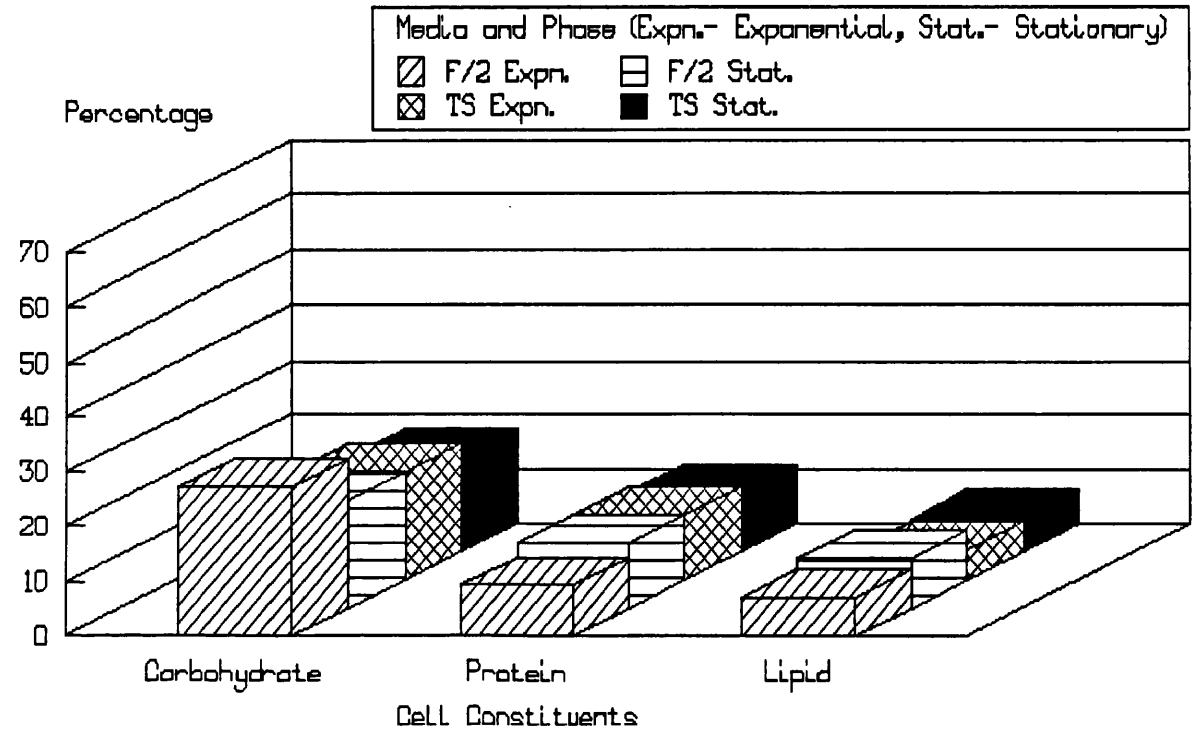


FIG 177

Comparison of Growth in Defined Medium (F/2) and Algal Treated Slurry (TS)

N. atomus 251/4B

(20:10:89 - 21:11:89)



5.3.3 A. flos-aquae and Synechococcus sp. PCC 7943

Only one experiment, comparing the growth of A.flos-aquae and Synechococcus sp. PCC 7943 in defined media (ASM) and algal treated slurry, was carried out due to time limitations and outdoor conditions. These species were grown between 26/6/89 to 20/7/89 (24 days). Minimum and maximum daily temperatures and daily irradiance are given in Table 49.

5.3.3.1 Growth, Nitrogen and Chlorophyll Results

Growth curves of OD_{560} against time (Fig 178) and dry weight against time (Fig 179) show Synechococcus sp. in ASM to lag behind growth in algal treated slurry, and behind growth of A. flos-aquae. All cultures were in late exponential/stationary phase at harvest and so were designated S/LE. A.flos-aquae, an organism capable of nitrogen fixation, was harvested at nitrogen depletion. Results for nitrate and nitrite utilization (Figs 180 and 181) show nitrate and nitrite depletion at S/LE harvests with the exception of A. flos-aquae which had a very low level of nitrate remaining (0.43mg NO_3-N l^{-1}, Table 50). Ammonium (initial level 7.39mg NH_4-N l^{-1}) depleted in the algal slurry by 8 days for A. flos-aquae, and 11 days for Synechococcus sp.. This was followed by nitrite and nitrate depletion. Total organic nitrogen decreased with growth in the algal treated slurry, but increased with growth in defined media for both cultures. Harvest parameters are given in Table 50. Chlorophylls a, b and c increased with A. flos-aquae in ASM and decreased in algal treated slurry for both A. flos-aquae and Synechococcus sp.. pH increased throughout the culture period for both algae (Figure 182).

FIG 178 OD560:Defined media(ASM)&Treated Slurry(TS) for A.flos-aquae 1403/13A and Synechococcus sp.PCC7943 (26/6-20/7/89)

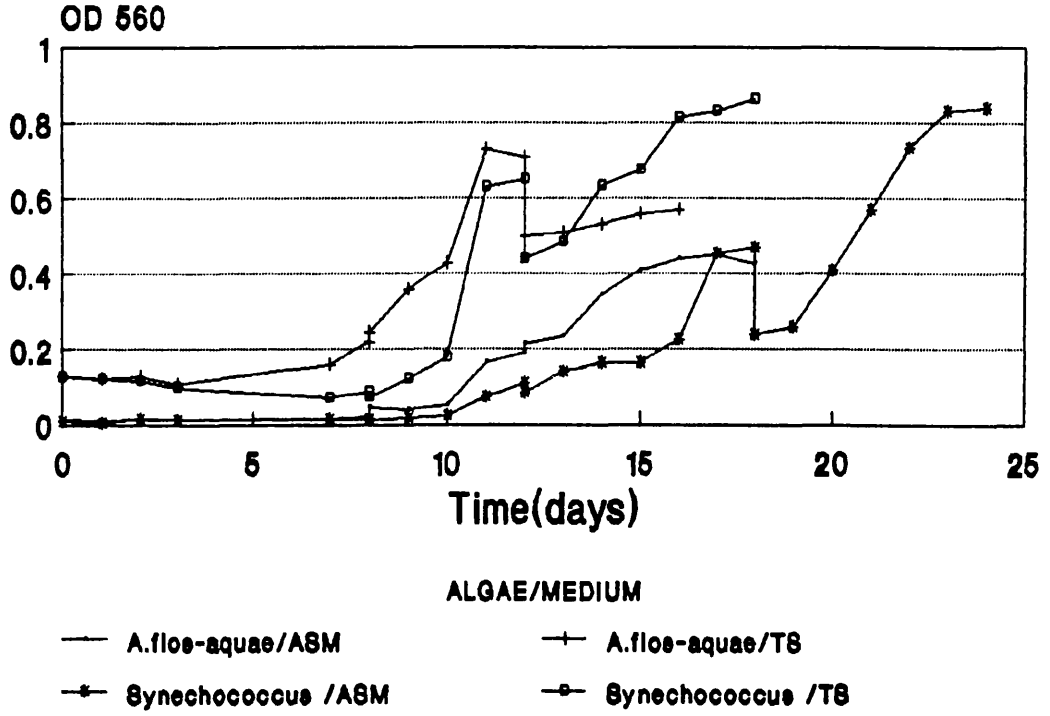


FIG 179 DRY WEIGHT:Defined medium&Treated Slurry(TS) for A.flos-aquae 1403/13A and Synechococcus sp.PCC7943 (26/6-20/7/89)

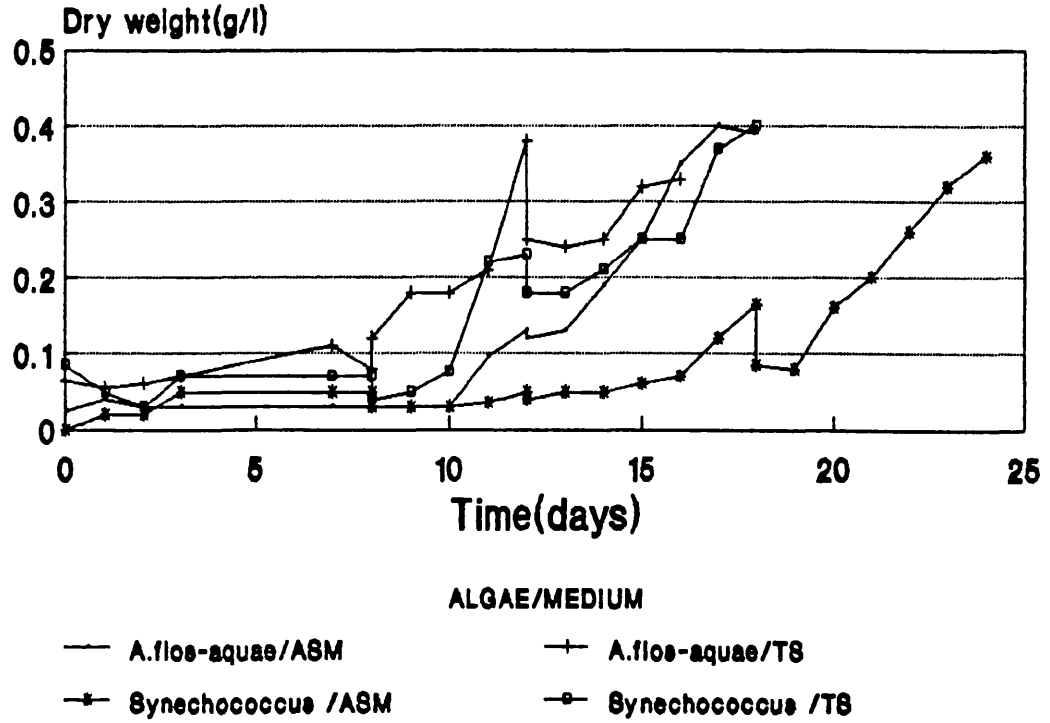


FIG 180 NITRATE:Defined Medium & Treated Slurry(TS) for A.flos-aquae 1403/13A and Synechococcus sp.PCC7943 (26/6-20/7/89)

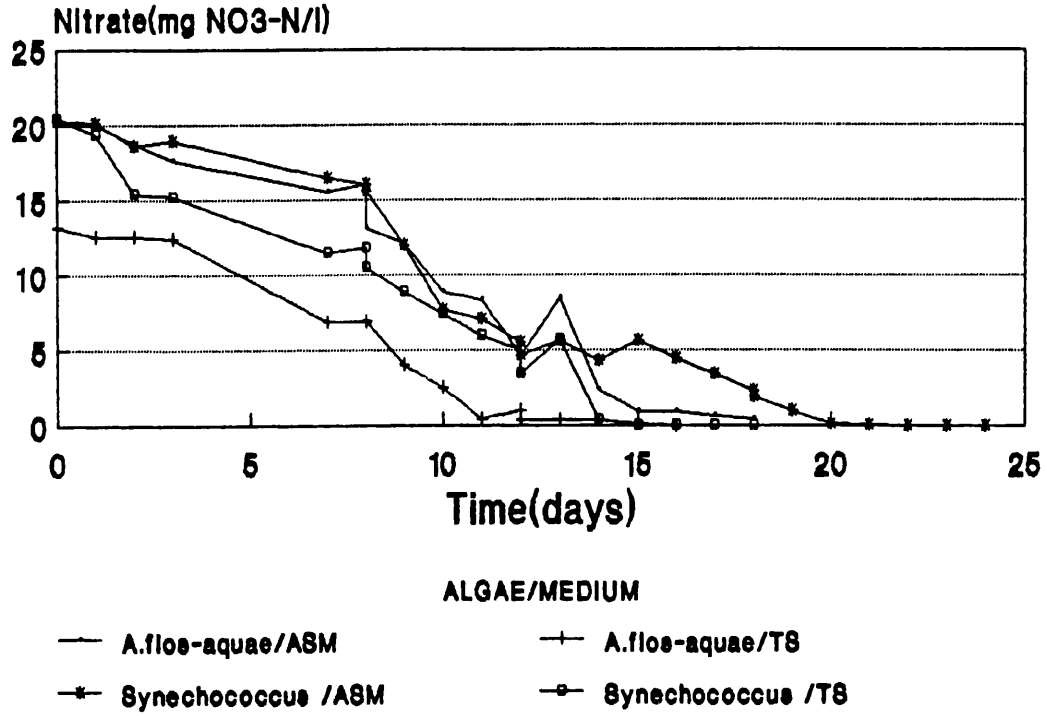


FIG 181 NITRITE: Defined Medium & Treated Slurry(TS) for A.flos-aquae 1403/13A and Synechococcus sp.PCC7943 (26/6-20/7/89)

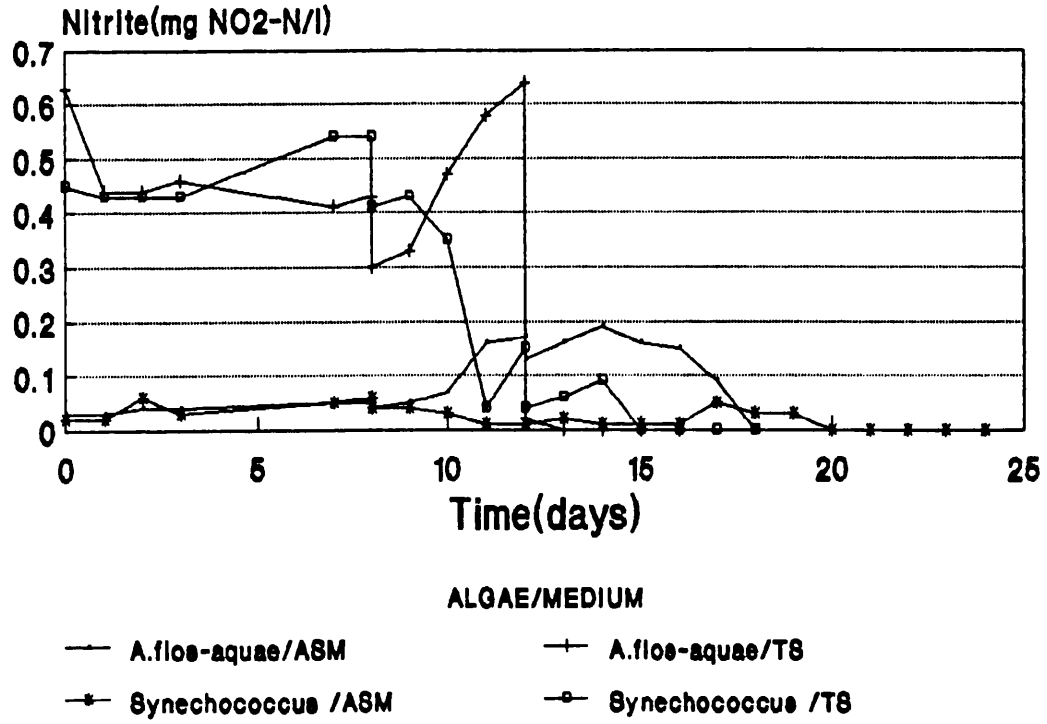


FIG 182 PH in Defined Medium(ASM)&Treated Slurry(TS) for A.flos-aquae 1403/13A and Synechococcus sp.PCC7943 (26/6-20/7/89)

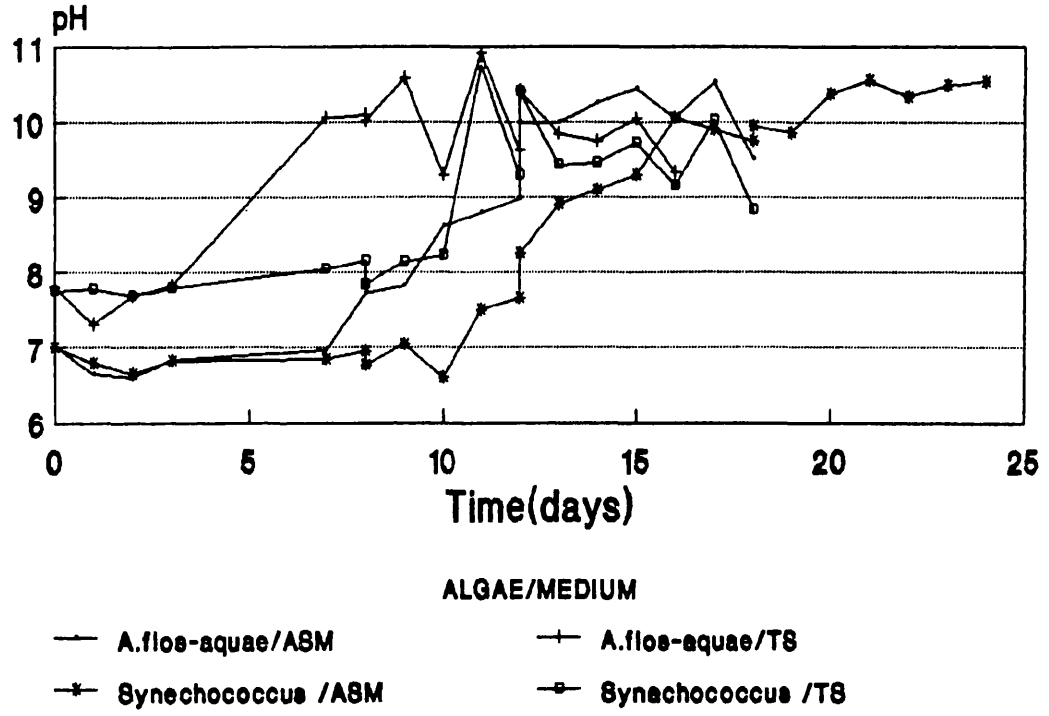


Table 49: Minimum and Maximum Daily Temperatures and Irradiance Levels for Outdoor Minipond Experiment Dates 26/6/89(0)-20/7/89(24)

Time (Days)	Minimum (°C)	Maximum (°C)	Irradiance ($Cal/cm^2/d$)
0	12	22	497.3
1	14	24	599.9
2	13	18	396.4
3	12	24	702.1
4	14	24	176.5
5	12	22	381.5
6	11	24	689.8
7	14	28	744.7
8	14	28	778.6
9	16	30	761.3
10	17	30	693.1
11	16	30	614.2
12	16	29	661.2
13	17	30	610.3
14	15	22	380.5
15	15	30	607.4
16	16	27	NA
17	14	30	787.7
18	15	26	619.1
19	17	29	722.7
20	17	30	685.9
21	15	27	623.9
22	16	27	750.0
23	17	31	665.8
24	20	NA	482.8
Average (°C)	15	26.8	

NA = Not Available

Table 50: Harvest Parameters for Outdoor Minipond Experiments (Including Chlorophyll) with A. flos-aquae 1403/13A and Synechococcus sp. PCC 7943

Organism	Media	Phase	Time (Days)	OD_{560}	Dry Wt (gl^{-1})	NO_3-N (mgl^{-1})	NO_2-N (mgl^{-1})	NH_4-N (mgl^{-1})	Chlorophyll ($ugml^{-1}$)		
									a	b	c
26/6/89-20/7/89											
Aflos-aquae	ASM		0						0	0	0
Aflos-aquae	ASM	E	15	0.41	0.25	0.96	0.16	0	2.10	0.03	0.38
Aflos-aquae	ASM	S/LE	18	0.43	0.39	0.43	0	ND	2.76	0.20	0.29
Aflos-aquae	TS		0						0	0	0
Aflos-aquae	TS	E	12	0.71	0.38	0.99	0.64	0	3.51	0	0.75
Aflos-aquae	TS	S/LE	16	0.57	0.33	0	0	ND	2.76	0.30	0.40
Synechococcus sp	ASM		0						0	0	0
Synechococcus sp	ASM	E	18	0.47	0.17	2.36	0.03	0	NA	NA	NA
Synechococcus sp	ASM	S/LE	24	0.84	0.36	0	0	ND	3.49	0.55	0.28
Synechococcus sp	TS		0						0	0	0
Synechococcus sp	TS	E	12	0.65	0.23	5.03	0.15	0	1.98	0.08	0.66
Synechococcus sp	TS	S/LE	18	0.86	0.40	0	0	ND	1.72	0.15	0.21

ND = Not Determined; NA = Not Available; E = Exponential Phase; S/LE = Stationary/Late Exponential Phase

5.3.3.2 Carbohydrate, Protein and Lipid Results

Results are given in Table 51. A. flos-aquae accumulated carbohydrate, decreased protein and decreased lipid contents at stationary phase in both ASM and algal treated slurry (Fig 183).

Synechococcus sp. accumulated carbohydrate and decreased protein content in both ASM and algal treated slurry (Fig 184). Lipid increased at stationary phase when grown in ASM and decreased at stationary phase when grown in algal treated slurry.

5.3.3.3 Statistical Analysis of the Carbohydrate, Protein and Lipid Results

Statistical analysis of the carbohydrate results gave two significant 'main effects' of phase and algae (0.1% or $P < 0.001$ and 1% or $p < 0.01$ respectively). Phase means (exponential - 15.2%, stationary - 37.6%) showed a significant increase in carbohydrate content from exponential to stationary phase. Species means (A. flos-aquae - 23.5%, Synechococcus sp. - 29.3%) showed that Synechococcus sp. had a significantly higher level of carbohydrate than A. flos-aquae.

Statistical analysis of the protein results gave no significant main effects or interactions, and this was also found for the lipid results.

5.3.3.4 Fatty Acid Results

These are given in Table 51. For both A. flos-aquae and Synechococcus sp., quantitative changes in individual fatty acids were found between phases. Similar fatty acid profiles were observed for A. flos-aquae in both media (ASM and TS), however, Synechococcus sp. showed qualitative differences, specifically the presence of 16:4 in ASM. Individual fatty acid changes culminated in %UNFA increasing in stationary phase for both A. flos-aquae and Synechococcus sp..

Statistical analysis of the percentage unsaturation results gave no significant main effects or first order interactions.

Table 51: Protein, Carbohydrate, Lipid and Fatty Acid Content (Percentage Composition) for A. flos-aquae 1403/13A and Synechococcus sp. PCC 7943 in Defined Medium (ASM) and Algal Treated Slurry (TS)

	26/6/89-20/7/89							
	A. flos aquae				Synechococcus sp.			
	ASM		TS		ASM		TS	
Fatty Acid	E	S/LE	E	S/LE	E	S/LE	E	S/LE
12:0	.15	-	-	-	.17	-	.13	-
14:0	1.55	.32	.35	.32	1.31	.35	1.59	.37
14:1(n-5)	4.60	-	-	.11	2.41	-	5.24	-
15:0	.38	.12	.33	.30	.32	.15	.55	.57
16:0	41.60	34.17	34.14	30.66	27.78	22.97	45.01	35.82
16:1(n-7)	40.84	17.75	17.95	17.11	12.70	.34	42.93	16.04
16:2	-	-	-	.11	.19	.62	-	-
16:3(n-6)	.64	-	-	-	2.40	2.62	.33	.67
16:4	.96	-	-	-	12.27	7.88	TR	-
17:0	.18	.19	.42	.44	.27	.13	.35	.38
18:0	.66	1.52	2.39	2.78	.65	1.37	.69	1.54
18:1(n-9)	1.62	2.48	4.20	4.01	6.55	31.97	.87	2.38
18:1(n-7)	2.92	2.42	1.79	3.74	.94	1.14	1.30	1.79
18:2(n-6)	.77	7.99	11.19	12.20	3.63	6.68	.14	5.49
18:3(n-3)	2.28	32.86	26.79	27.87	25.99	20.95	.16	32.37
18:3(n-6)	.21	.19	.45	.37	.30	.26	.18	.48
18:4(n-3)	.40	-	-	-	1.85	2.59	.11	-
19:0	.23	-	-	-	.25	-	-	-
20:0	-	-	-	-	-	-	.32	2.11
20:1(n-9)	-	-	-	-	-	-	-	-
20:2(n-6)	-	-	-	-	-	-	-	-
20:3(n-6)	-	-	-	-	-	-	-	-
20:4(n-6)	-	-	-	-	-	-	-	-
20:4(n-3)	-	-	-	-	-	-	-	-
20:5(n-3)	-	-	-	-	-	-	-	-
21:0	-	-	-	-	-	-	-	-
22:0(IS)	IS	IS	IS	IS	IS	IS	IS	IS
22:1(n-9)	-	-	-	-	-	-	-	-
22:5(n-3)	-	-	-	-	-	-	-	-
22:6(n-3)	-	-	-	-	-	-	-	-
24:0	-	-	-	-	-	-	-	-
% Lipid	9.46	4.64	5.51	4.01	8.34	13.77	4.95	2.26
% SAFA	44.75	36.32	37.63	34.50	30.75	24.97	48.64	40.79
%UNFA	55.24	63.69	62.37	65.52	69.23	75.05	51.26	59.22
UNFA/SAFA	1.23	1.75	1.66	1.90	2.25	3.01	1.05	1.45
% Protein	6.59	4.68	6.24	5.66	15.48	5.39	18.72	15.65
%Carbohydrate	9.03	32.87	14.27	37.94	18.64	36.45	18.68	43.26

Note (i) IS = Internal Standard; S = Stationary Phase; E = Exponential Phase; S/LE = Stationary/Late Exponential Phase; SAFA = Saturated Fatty Acids; UNFA = Unsaturated Fatty Acids

(i) For systematic names of fatty acids see Appendix 1

FIG 183
Comparison of Growth in Defined Medium (ASM)
and Algal Treated Slurry (TS)
A.flos-aquae 1403/13A
(26:6:89 - 20:7:89)

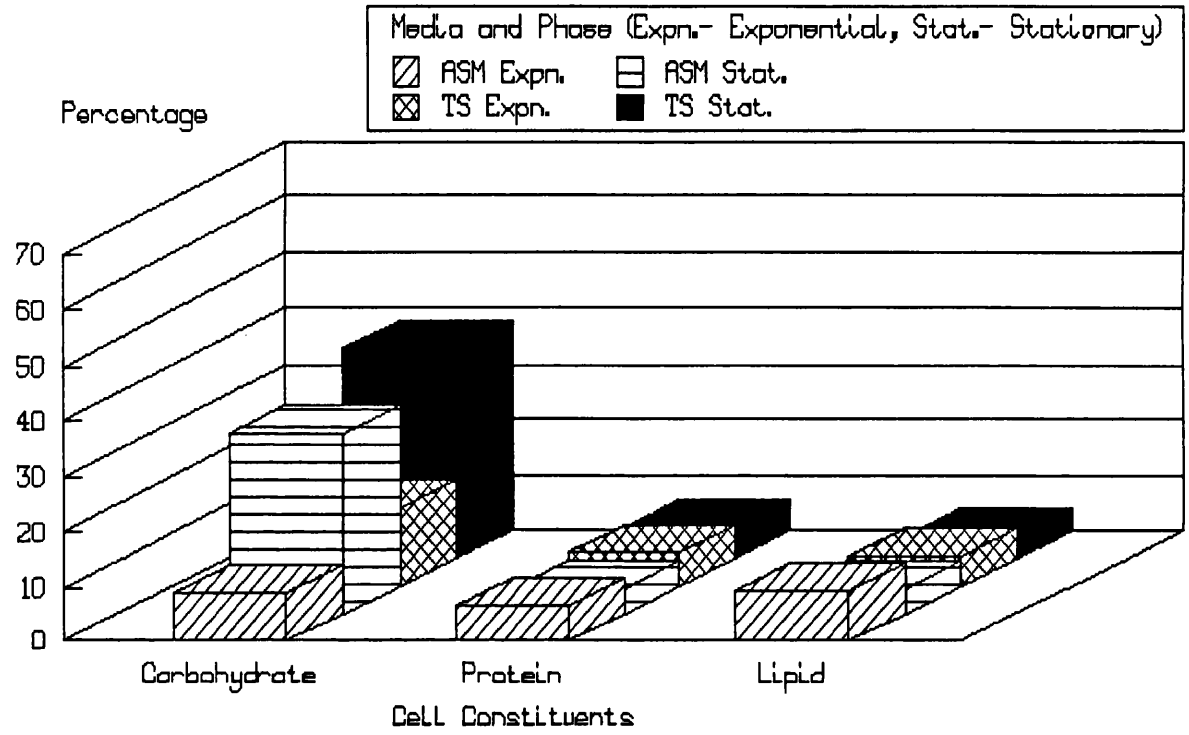
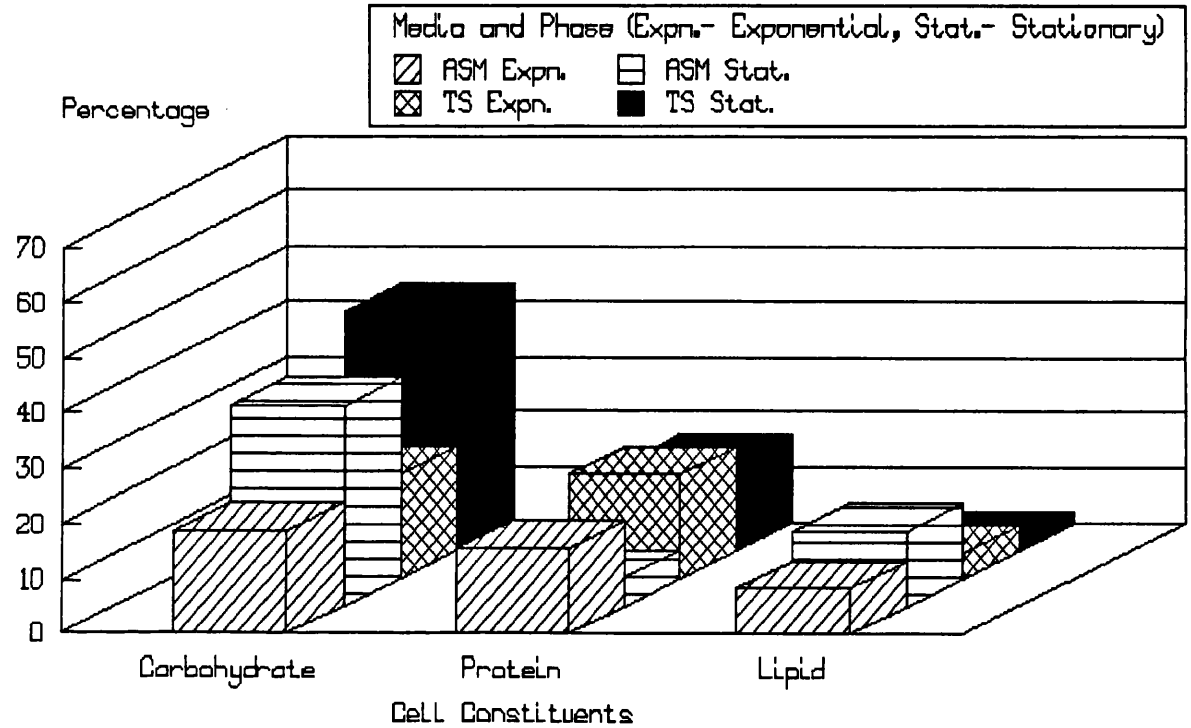


FIG 184
Comparison of Growth in Defined Medium (ASM)
and Algal Treated Slurry (TS)
Synechococcus sp. PCC7943
(26:6:89 - 20:7:89)



6. DISCUSSION AND CONCLUSIONS

6.1 DISCUSSION AND CONCLUSIONS

All the algae investigated exhibited different behaviour with respect to the cellular content of carbohydrate, protein and lipid in relation to changes in temperature, nitrogen availability and growth phase.

The difference between the four green algae was the major shift to carbohydrate accumulation with decrease in temperature and at stationary phase exhibited by C. vulgaris 211/8K and 211/11c, and the shift to lipid accumulation under similar conditions exhibited by Ank. antarcticus and S. obliquus. Statistical analysis confirmed this difference in behaviour between the four green algae, and also that previous nitrate availability affected the amount of carbohydrate or lipid accumulated.

The protein content of the four green algae decreased in stationary phase but statistical analysis showed the only significant decrease with temperature was at 40°C for C. vulgaris 211/8K. This difference in behaviour exhibited by C. vulgaris 211/8K may be due to the fact that it is a high temperature strain. Protein content was also found to be dependent on previous nitrate availability for the four green algae.

The fatty acid content of the four green algae was dependent on temperature and growth phase. Quantitative changes in individual fatty acids rather than qualitative changes were exhibited by all four green algae, although there appeared to be no regular pattern of change exhibited by any individual fatty acid between the algae studied with the exception of 16:0, which increased with increased temperature in all four green algae. The degree of unsaturation decreased with increased temperature and at stationary phase.

The four cyanobacteria studied all exhibited a major shift to carbohydrate accumulation at stationary phase similar to the green algae. However, the strains studied did not appear to exhibit a uniform response to temperature, Synechococcus sp. 1479/5 increased carbohydrate with increased temperature and Synechococcus sp. PCC 7943 and A.flos-aquae decreased carbohydrate

with increased temperature. All four cyanobacteria exhibited carbohydrate accumulation although the two Synechococcus species were non nitrogen fixing cyanobacteria and the two Anaebaenas were nitrogen fixing cyanobacteria. Since nitrate availability affected carbohydrate accumulation in the cyanobacteria studied, it appears that fixed nitrogen may not affect the partitioning of assimilated carbon into carbohydrate at stationary phase in the nitrogen fixing cyanobacteria.

The two Synechococcus species and the two Anaebaenas also exhibited differences in behaviour with respect to changes in lipid content. Previous nitrate availability also affected the lipid content of the four cyanobacteria.

Protein content of the four cyanobacteria increased at stationary phase in contrast to the decrease at stationary phase observed in the green algae studied. This may be due to nitrogen fixation providing a source of nitrogen for protein production in the nitrogen fixing cyanobacteria but this does not explain the same response in the non-nitrogen fixing cyanobacteria.

The cyanobacteria studied exhibited a similar range of fatty acids to that in the green algae studied with quantitative differences rather than qualitative differences shown in response to temperature changes and growth phase. As with the green algae, there appeared to be no regular pattern of change exhibited by any individual fatty acid in response to temperature change or growth phase between the genera studied. The degree of unsaturation decreased with increased temperature similar to the green algae.

Of the four marine and brackish algae, N. atomus, a brackish green algae, appeared to behave similarly to the freshwater green algae with increased carbohydrate and lipid content with decreased temperature and at stationary growth phase. Statistical analysis of the carbohydrate results for the four marine and brackish species showed a difference in behaviour of N. atomus to the other three marine and brackish algae studied. Previous nitrate availability again affected carbohydrate accumulation. Lipid accumulation with decreased temperature was confirmed statistically for N. atomus and Isochrysis sp., and

previous nitrate availability again affected lipid content, however lipid content appeared to decrease at stationary phase in contrast to the increase at stationary phase exhibited by the green algae. Protein decreased with increased temperature and at stationary phase.

The four marine and brackish species studied exhibited a much broader range of fatty acids than the green algae and cyanobacteria. As with the other genera studied quantitative changes in fatty acids rather than qualitative changes were exhibited with temperature changes and growth phase. Again, as with the other genera studied there was no regular pattern of change in individual fatty acids between the four marine and brackish species in response to temperature changes and growth phase. However, the level of unsaturation appeared to increase with increased temperature in contrast to the decrease observed with the green algae and cyanobacteria.

C. vulgaris 211/8K, C. vulgaris 211/11c, N. atomus and the cyanobacteria studied all exhibited a major shift to carbohydrate accumulation in stationary phase. Levels of carbohydrate accumulated were significant - C. vulgaris 211/8K - up to 56%; C. vulgaris 211/11c - up to 67%; N. atomus - up to 37%; Synechococcus sp. 1479/5 - up to 37%; Synechococcus sp. PCC 7943 - up to 48%; A. flos-aquae - up to 35%; A. variabilis - up to 33% of cell dry weight.

Carbohydrate accumulation in C. vulgaris has been observed by other workers under conditions of nitrogen limitation with carbohydrate approaching 50% of the total dry weight of cells (Pirt and Pirt, 1977). This area has been further researched by Behrens et al (1989) who observed nitrogen sufficient cultures of C. vulgaris contained approx. 20% of their dry weight as starch, whereas under nitrogen limitation, the starch content comprised up to 55% of the dry weight. El-Fouly et al (1985) also found carbohydrate accumulation in C. vulgaris as a result of nitrogen starvation in laboratory and in growth outdoors.

Carbohydrate accumulation under nitrogen limitation has also been found in Ankistrodesmus sp., Isochrysis sp., Nannochloris sp. (Ben-Amotz et al, 1985) and Isochrysis galbana (Utting, 1985). The two Isochrysis species studied in

this project did not exhibit a significant increase in carbohydrate content however due to the experimental timescale they did not achieve nitrate depletion.

Conditions of nitrogen starvation have also been shown by Gibson (1978) to induce carbohydrate accumulation in Oscillatoria redekei. Foy and Smith (1980) suggest carbohydrate synthesis may be induced by nitrogen starvation causing an accumulation of metabolic intermediates which stimulate glycogen synthesis in cyanobacteria. The accumulation of carbohydrate in certain algal species investigated in this project and not in others cannot be explained by differences in dark respiration rates at stationary phase under nitrogen depletion. Dark respiration rates were found to be similar between algae exhibiting carbohydrate accumulation and those accumulating lipid. It is therefore suggested that the effect of nitrogen depletion is on the enzymic activity of carbohydrate biosynthesis.

Ank. antarcticus and S. obliquus exhibited significant shifts in cellular content to lipid accumulation with decrease in temperature and at stationary phase (nitrogen depletion) (up to 23% total lipid for Ank. antarcticus and up to 32% total lipid for S. obliquus), but also increased their carbohydrate content. N. atomus behaved similarly.

Lipid content of N. oculata and the two Isochrysis species did not exhibit major changes but this may be due to the slow growth of these cultures, nitrate depletion not occuring within the experimental timescale for most cultures. However N. oculata and Isochrysis sp. cultures at 5mg NO_3-N l^{-1} initial N did increase their lipid contents with nitrogen depletion and therefore under nitrogen depletion they may infact accumulate lipid. The cyanobacteria studied did not significantly change their lipid content with change in temperature or growth phase.

Lipid accumulation appeared to be triggered by nitrate depletion, and previous nitrate availability affected the amount of lipid accumulated. Spoehr and Milner (1949) first showed that nitrogen deficiency induced an increase in lipid content

of Chlorella pyrenoidosa. This has also been observed in Isochrysis galbana (Utting, 1985; Kaplan, 1986; Sukenik and Wahnon, 1991), Ankistrodesmus sp., Isochrysis sp., Nannochloris sp. (Ben-Amotz et al, 1985), Nannochloropsis sp. (Suen et al, 1987), C. vulgaris and S. acutus (El-Fouly et al, 1985).

The comparative study by Shifrin and Chisolm (1981) indicated that the lipid contents of fifteen chlorophycean strains grown under nitrogen-deficient conditions increased to 130-320% of the values observed for exponential phase. Piorreck et al (1984) found that green algae but not cyanobacteria could be manipulated with respect to fatty acid and lipid compositions by nitrogen limitation. Therefore, the differences observed between the algae and cyanobacteria investigated in this study probably indicate fundamental metabolic differences in these strains and is comparable to the findings of Piorreck et al (1984).

The biochemical basis of lipid accumulation under nitrogen-deficient conditions has not been thoroughly investigated. Lipid accumulation can probably be attributed in part to the fact that storage lipids and most membrane lipids do not contain nitrogen and therefore continue to be synthesised in nitrogen deficient cells while the synthesis of nitrogen containing compounds eg proteins is curtailed (Roessler, 1990).

Changes in levels of enzyme activity are also evident because the ratio of storage lipids to membrane lipids greatly increases in nitrogen-starved algae (Roessler, 1990). Roessler (1988) has studied the enzymic activity with respect to silicon deficiency in diatoms. Experiments indicated that the activity of Acetyl-CoA carboxylase, which may catalyse the rate limiting step of fatty acid biosynthesis, doubled within four hours after the onset of silicon deficiency and that this increase could be blocked by adding protein synthesis inhibitors. These results suggest that an increased cellular level of Acetyl-CoA carboxylase may be induced by silicon deficiency, which may contribute to a higher capacity for lipid synthesis. A similar situation of inducement by nitrogen deficiency may exist in other groups of algae. Sukenik and Livne (1991) have investigated the variation in lipid and fatty acid content in relation to Acetyl-CoA

carboxylase in Isochrysis galbana under nitrogen limitation. Their results suggest that Acetyl-CoA carboxylase plays an important role in the regulation of flow of photosynthetic assimilated carbon into lipids in I. galbana.

Temperature has a major effect on the types of lipids produced by microalgae. The general trend towards increasing the degree of fatty acid unsaturation with decreasing temperature observed in higher plants and other organisms (Raison, 1986) also occurs in algae, thereby optimising membrane function over a range of temperatures. Patterson et al (1970) observed a greater degree of fatty acid unsaturation in Chlorella sorokiniana cells grown at 22°C relative to cells grown at 38°C. Thompson et al (1992) found a significant inverse relationship between percentage of PUFA's and temperature for eight species of marine phytoplankton.

Lipids provide the essential property of fluidity in membranes. Some investigators have tried to relate changes in fatty acid unsaturation to the growth temperature - dependent shift of temperature for the thermotrophic phase transition of membrane lipids (Sato et al (1979); Wada et al (1990)). Sato et al (1979) also found the degree of unsaturation of fatty acids to be inversely related to temperature in A. variabilis and A. nidulans. The increase in unsaturation of fatty acids on lowering the growth temperature predict a downward shift of temperature for the transition between the liquid-crystalline and phase seperation states of the membrane liquids. Lynch and Thompson (1984) suggest acclimation to low temperatures enhances acyl chain desaturation as a means of modifying membrane properties in response to low temperature.

Results from this study show an increase in unsaturation with decrease in temperature to occur with the green algae and the cyanobacteria but not with the marine and brackish species.

The effects of temperature on the total lipid content of microalgae have only been reported for a few species and a general trend has not became apparent. For example, the lipid content of Ochromonas danica increased from 39% to

53% as the temperature was raised from 15°C to 30°C (Aaronson 1973) but the lipid content of C. sorokiniana did not change in response to alterations in growth temperature (Patterson 1970). However, Cobelas (1989) states that temperature effects on lipid content depend upon the temperature optima of the microalgae involved, and as a rule cryo- and mesophilic algae show increasing lipid content as temperature increases. This was not found in this study where the algae exhibiting lipid accumulation exhibited increased lipid with decrease in temperature.

The green algae and the marine and brackish species studied exhibited decreased protein contents at stationary or stationary/late exponential phase, but the cyanobacteria studied showed increased protein contents. Ben-Amotz et al (1985) found decreased protein contents with concurrent increased carbohydrate and lipid contents under nitrogen deficiency in Ankistrodesmus sp., Isochrysis sp. and Nannochloris sp.. Utting (1985) found a similar result for Isochrysis galbana, and also found a correlation existed between protein and lipid contents, and protein and carbohydrate contents in this organism. Carbohydrate has been found to act as an intermediate reserve in some algae (Marker, 1965) because time is required after nitrogen becomes limiting for enzymes essential for lipid synthesis to be produced (Fogg, 1956). Consequently where protein decreased both lipid and carbohydrate might be expected to increase.

The fatty acid compositions of the algae investigated were generally in agreement with the known distribution of fatty acids in algae and cyanobacteria stated in the literature (Wood, 1974; Borowitzka, 1988). All algae studied exhibited quantitative changes in fatty acids with temperature changes and growth phase. No regular pattern of change was observed with respect to individual fatty acids between the different algae investigated. Other workers have also found changes in fatty acids with nitrogen-starvation (Piorreck et al (1984); Ben-Amotz et al (1985); Sukenik and Wahnon (1991)) and growth phase (Piorreck and Pohl, 1984).

The most interesting and wide ranging fatty acid compositions were observed in the marine and brackish species studied, especially due to the presence of 20:5(n-3) and 22:6(n-3). However, the higher levels of lipid, in comparison, were found in the green algae. It should be noted, however, that the compositions for the marine and brackish species are based on dry weight and maybe underestimates of the true values.

A comparison of the nitrogen limitation experiments and the outdoor minipond experiments showed all the algae chosen for growth outdoors grew successfully in algal treated slurry, with all the algae preferentially using ammonium-N before nitrite and nitrate. Similar results were found outdoors to the laboratory work, in both defined media and algal treated slurry. For C. vulgaris 211/8K carbohydrate accumulation was exhibited in stationary growth phase in the first outdoor experiment but not in the second. This may have been due to the fact that nitrogen depletion did not occur in the second experiment. S. obliquus exhibited lipid and carbohydrate increases in stationary phase in both defined media (ASM) and algal treated slurry (TS). Similar fatty acid compositions were obtained between media (ASM and TS) and were comparable to fatty acid results from the laboratory work for both green algae.

The two cyanobacteria also exhibited carbohydrate accumulation in stationary phase in defined media and algal treated slurry comparable to the laboratory results. Again, depletion of ammonium-N occured before nitrite-N and nitrate-N. Similar fatty acids compositions were observed between media (ASM and TS) and were similar to those observed in the laboratory work for both cyanobacteria.

N. atomus accumulated carbohydrate at stationary phase in the first experiment but did not increase carbohydrate at stationary phase in the second experiment. This may have been due to lack of nitrate depletion in the second experiment. Isochrysis sp. did not grow in the second experiment which was attributed to the low temperature, but did grow successfully in the first experiment. Comparable growth of Isochrysis sp. was found in defined medium and algal treated slurry with increased carbohydrate at stationary phase in both media

(F/2 and TS). Similar fatty acids compositions were obtained between media which were comparable to results obtained in the laboratory for both N. atomus and Isochrysis sp.. N. atomus contained significant quantities of 20:5(n-3) and Isochrysis sp. contained significant quantities of 22:6(n-3).

It would appear, therefore that the algae studied behaved similarly outdoors in defined media and algal treated slurry to the laboratory based growth. Temperature and light conditions would appear to be important, however, and although light conditions outdoors could not be altered without cost, temperature could be altered with the use of the heat generated via the aerobic treatment of piggery waste (Fig 1).

Many workers have also shown successful growth of C. vulgaris in pig slurry (Barlow, et al (1975); Allen and Garrett (1976); Boersma, 1975; De Pauw and De Leenheer (1979); Matusiak (1976); Strain et al (1986); and Scenedesmus obliquus in pig slurry (de la Noüe and Bassères (1989); De Pauw and De Leenheer (1979); Martin et al (1985); Nair et al (1981)). There are few reports with other species of algae. Pouliot (1989) investigated growth of cyanobacteria in domestic wastewater. The main area of interest with respect to sewage grown algae appears to be single cell protein (Boersma (1975); Becker (1981)).

This work has shown that the behaviour of the algae studied in the laboratory system can be emulated in an outdoor system using algal treated slurry. Therefore, manipulation of cellular content can be achieved in a slurry based system which would allow for optimisation of specific cell constituents. Interest in algal fatty acids would probably only be in the aquaculture field, with interest in developing specific algae with high contents of polyunsaturated fatty acids, and not from the medical or health food areas due to health hazards associated with growth on sewage.

At present, microalgae feeds have limited applications in aquaculture. The most prevalent use is in small scale indoor microalgae production units which produce microalgal culture for hatchery and nursery operation in shellfish and

finfish aquaculture (either fed directly or for raising plankton feeds) (De Pauw & Persoone, 1988). The raising of marine finfish and freshwater salmonids requires feeds containing large amounts of lipids (in particular the omega-3 fatty acids). This is mostly provided by fish meals, but since omega-3 fatty acids in fish oils derive from marine phytoplankton, production of marine microalgae, particularly of biomass high in omega-3 fatty acids would be of interest.

Selected strains of microalgae serve as preferred food for bivalve larvae, seed and adults. The specific microalgae considered highly desirable include Isochrysis galbana and Dunaliella salina and related species. They are generally characterised by elevated contents of omega-3 fatty acids.

Laboratory studies have shown major changes in the fatty acid composition can result from modification of culture conditions but little attention has been given to this in hatcheries. These variations can be exploited to maximise the nutritional quality of the algae.

Several marine fish and molluscs commonly grown in commercial aquaculture facilities have exhibited improved growth when fed algae containing high levels of EPA and DHA (Langdon and Waldock, 1981; Pillsbury, 1985). This is apparently due to the inability of juveniles to produce adequate levels of these essential fatty acids (Kanazawa et al, 1979; Waldock and Holland, 1984). The environmental conditions under which feed algae are grown affect the growth rate of cultured bivalves (Enright et al, 1986), apparently because of the different levels of long chain polyunsaturated fatty acids in the algae.

The other avenue of interest suggested by the results of this study is optimisation of carbohydrate accumulation. Interest has been shown in the use of 'high energy' genera for aquaculture feed (Solar Aquafarms Inc, Fallowfield pers. comm. 1989). This area has been reviewed by Brown et al (1989). Carbohydrate accumulation in cyanobacteria may then be of interest due to the fact that they would be easier to harvest due to their filementous growth. Nutritional deficiencies in a diet can be avoided by the use of mixed algal diets. For example, green algae can be used to provide a high carbohydrate content and their lack of C20 and C22 - polyunsaturated fatty acids can be met by

another species of the same size rich in these compounds.

The current high cost of production of algal feeds has spurred the search for alternative algae production techniques (Benemann, 1992). Growth in slurry with nitrogen depletion to optimise lipid, carbohydrate or specific component fatty acid production maybe an alternative.

7. FURTHER WORK

7. FURTHER WORK

(I) Research into the enzymology of lipid and carbohydrate biosynthesis in the algae studied under conditions of nitrate sufficiency and depletion. Acetyl-CoA carboxylase appears to be important with respect to lipid biosynthesis (Sukenik and Livne, 1991).

(II) Scale up of the growth of sewage grown algae using the high rate algal ponds to investigate whether the behaviour of the algae studied can be emulated on a larger scale.

(III) The use of genetic manipulation in fatty acid metabolism manipulation.

(IV) Investigation of the mathematical model suggested by Sattur and Karanth (1989a, 1989b, 1989c), who have developed a mathematical model for predicting microbial lipid production based on the carbon/nitrogen ratio.

APPENDIX

APPENDIX

Fatty Acids

Shorthand Designation	Systematic name
12:0	Dodecanoic
14:0	Tetradecanoic
14:1 (n-5)	cis-9-tetradecenoic
15:0	Pentadecanoic
16:0	Hexadecanoic
16:1 (n-7)	cis-9-hexadecenoic
16:2	cis- hexadecadienoic (position of double bonds unknown)
16:3 (n-6)	cis-6,9,12-hexadecatrienoic
16:4	cis- hexadecatetraenoic (position of double bonds unknown)
17:0	Heptadecanoic
18:0	Octadecanoic
18:1 (n-9)	cis-9-octadecenoic
18:1 (n-7)	cis-11-octadecenoic
18:2 (n-6)	cis-9,12-octadecadienoic
18:3 (n-3)	cis-9,12,15-octadecatrienoic
18:3 (n-6)	cis-6,9,12-octadecatrienoic
18:4 (n-3)	cis-6,9,12,15-octadecatetraenoic
19:0	Nonadecanoic
20:0	Eicosanoic
20:1 (n-9)	cis-11-eicosenoic
20:2 (n-6)	cis-11,14-eicosadienoic
20:3 (n-6)	cis-8,11,14-eicosatrienoic
20:4 (n-6)	cis-5,8,11,14-eicosatetraenoic
20:4 (n-3)	cis-8,11,14,17-eicosatetraenoic
20:5 (n-3)	cis-5,8,11,14,17-eicosapentaenoic
21:0	Heneicosanoic
22:0 (Internal standard)	Docosanoic
22:1 (n-9)	cis-13-docosenoic
22:5 (n-3)	cis,-7,10,13,16,19-docosapentaenoic
22:6 (n-3)	cis -4,7,10,13,16,19-docosahexaenoic
24:0	Tetracosanoic

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